**Chemical Constituents from the Roots of *Caesalpinia mimosoides* and *Caesalpinia pulcherrima* and their Anti-inflammatory activity**

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**A Thesis Submitted in Fulfillment of the Requirements for the Degree of**
**Doctor of Philosophy in Organic Chemistry**
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**Thesis Title**    Chemical Constituents from the Roots of *Caesalpinia mimosoides* and *Caesalpinia pulcherrima* and their Anti-inflammatory activity

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**Major Program**    Organic Chemistry

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This is to certify that the work here submitted is the result of the candidate's own investigation. Due acknowledgement has been made of any assistance received.

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I hereby certify that this work has not already been accepted in substance for any degree, and is not being concurrently submitted in candidature for any degree.


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|---|---|
| **ชื่อวิทยานิพนธ์** | องค์ประกอบทางเคมีจากรากของผักปู่ย่าและหางนกยูงไทยและ<br>ฤทธิ์ต้านการอักเสบ |
| **ผู้เขียน** | นางสาวอรพรรณ ยอดสะอึ |
| **สาขาวิชา** | เคมีอินทรีย์ |
| **ปีการศึกษา** | 2555 |

**บทคัดย่อ**

*ส่วนที่ 1 การศึกษาองค์ประกอบทางเคมีจากส่วนรากต้นผักปู่ย่า*

ส่วนสกัดหยาบไดคลอโรมีเทนและอะซิโตนจากรากของต้นผักปู่ย่าได้แสดงฤทธิ์การต้านไนตริกออกไซด์ที่ดีมาก ที่ค่า $IC_{50}$ 11.0 และ 21.6 µg/ml ตามลำดับ จึงนำมาสู่การการศึกษาองค์ประกอบทางเคมีจากรากต้นผักปู่ย่า สามารถแยกสารใหม่เป็นสารกลุ่มไดเทอร์พีนได้ 4 สาร คือ mimosol A-D (**CM1-CM4**), สารกลุ่มไดเมอร์ไดเทอร์พีน 1 สาร คือ mimosol E (**CM9**) และ สารกลุ่ม dibenzo[b,d]furans จำนวน 2 สาร คือ mimosol F, G (**CM10, CM11**) นอกจากนี้ยังสามารถแยกสารประกอบที่มีการรายงานแล้ว 11 สาร ซึ่งแบ่งเป็นสารกลุ่มไดเทอร์พีน 4 สาร [taepeenin A (**CM5**), taepeenin D (**CM6**), nortaepeenin A (**CM7**) และ taepeenin L (**CM8**)] สารกลุ่มโฮโมไอโซฟลาโวน 3 สาร [(*E*)-7-hydroxy-3-(4-methoxybenzyl)chroman-4-one (**CM12**), (*E*)-7,8-dihydroxy-3-(4-methoxybenzyl)chroman-4-one (**CM13**) และ (*E*)-7-hydroxy-8-methoxy-3-(4-methoxybenzyl)chroman-4-one (**CM14**)] สารกลุ่มฟีนิลโพรพานอยด์ 3 สาร [tetracosyl caffeate (**CM15**) resveratrol (**CM16**) และ bergenin (**CM17**)] และ สารประกอบเซสควิเทอร์พีน 1 สาร [(+)-

pterocarpol　(**CM18**)]　โครงสร้างของสารประกอบเหล่านี้ วิเคราะห์ด้วยข้อมูลสเปกโทรสโกปี

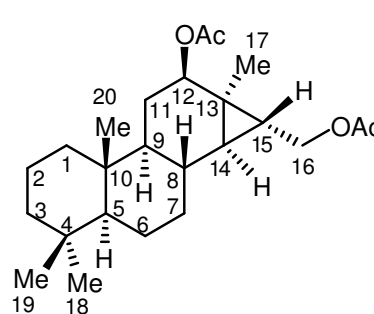และเปรียบเทียบข้อมูลกับที่มีรายงานมาก่อนหน้านี้

　　　　สารทั้งหมดได้ถูกนำไปทดสอบฤทธิ์ต้านการอักเสบ โดยการยับยั้งไลโพโพลีแซค

คาไรด์ (LPS) ซึ่งเป็นตัวเหนี่ยวนำให้เกิดไนตริคออกไซด์ (NO) ใน RAW264.7 เซลล์โมเดล

และเนื่องจากว่าสารประกอบ **CM4, CM6, CM8** และ **CM12-CM14** แสดงฤทธิ์ยับยั้ง NO ใน

ระดับที่ดีมาก จึงได้นำสารดังกล่าวมาทดสอบฤทธิ์การต้าน TNF-$\alpha$ ต่อไป ผลที่ได้พบว่าสาร **CM4**

แสดงฤทธิ์การต้าน NO และ TNF-$\alpha$ ในระดับที่ดีมากด้วยค่า IC$_{50}$ = 3.0 and 6.5 μM ตามลำดับ
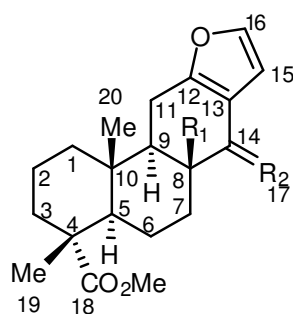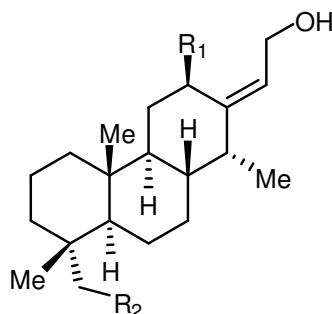


**CM1:** R$_1$ = OH; R$_2$ = CH$_2$　　**CM2:** R$_1$ = OH; R$_2$ = H　　　**CM4**

**CM7:** R$_1$ = H; R$_2$ = O　　　　**CM3:** R$_1$ = H; R$_2$ = OH

　　　　　　　　　　　　　　　**CM8:** R$_1$ = R$_2$ = H
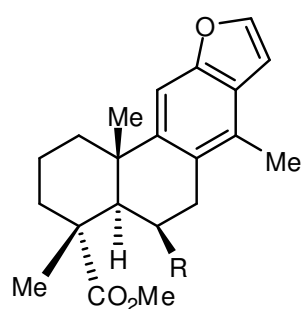


**CM5:** R = H
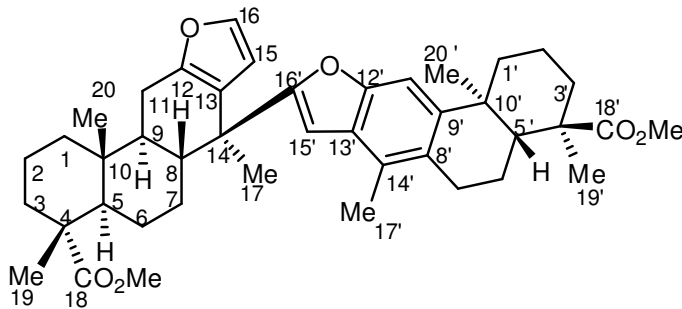
**CM6:** R = OAc　　　　　　　　　　　**CM9**
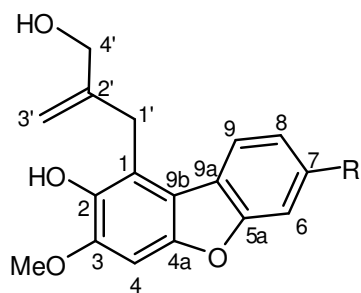
**CM10:** R = OH

**CM11:** R = OMe
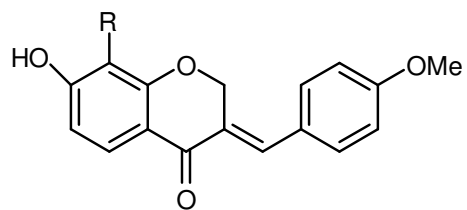
**CM12:** R = H

**CM13:** R = OH

**CM14:** R = OMe
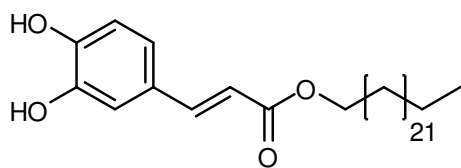
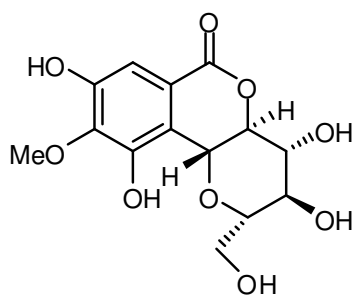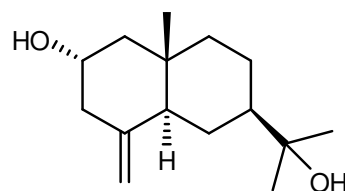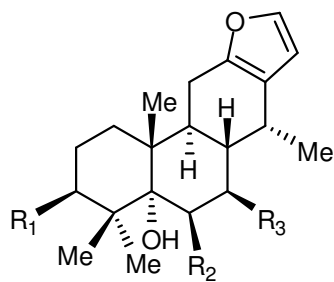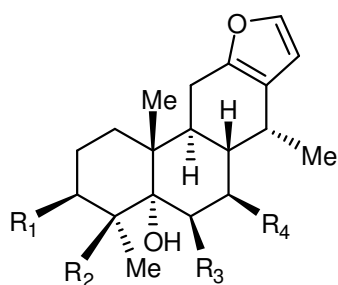**CM15**
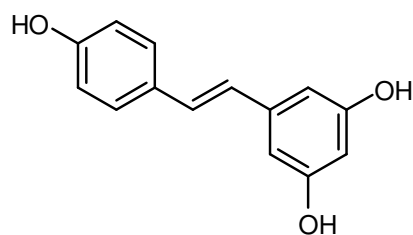
**CM16**

**CM17**

**CM18**

*ส่วนที่ 2 การศึกษาองค์ประกอบทางเคมีจากส่วนรากต้นหางนกยูงไทย*

การแยกสารในส่วนสกัดไดคลอโรมีเทนของส่วนรากจากต้นหางนกยูงไทยได้สาร ใหม่ในกลุ่มของไดเทอร์พีน 15 สาร คือ pulcherrins D–R (**CP1**-**CP15**) รวมทั้งสารที่มีการ รายงานมาแล้ว 11 สาร คือ vouacapen-5$\alpha$-ol (**CP16**), isovouacapenol C (**CP17**), 6$\beta$-cinnamoyl-7$\beta$-hydroxyvouacapen-5$\alpha$-ol (**CP18**), pulcherrin A (**CP19**), pulcherrin B (**CP20**), pulcherrimin C (**CP21**), pulcherrimin A (**CP22**), pulcherrimin E (**CP23**), pulcherrin C (**CP24**), pulcherrimin B (**CP25**) และ 8,9,11,14-didehydrovouacapen-5$\alpha$-ol (**CP26**) โครงสร้างสารประกอบทั้งหมดวิเคราะห์โดยใช้วิธีทางสเปกโทรสโกปีและเปรียบเทียบ กับข้อมูลที่มีรายงานมาแล้ว สำหรับโครงสร้างสารประกอบ **CP16** และ **CP17** ยืนยันโครงสร้าง ด้วยเทคนิคการเลี้ยวเบนของรังสีเอกซ์บนผลึกเดี่ยว

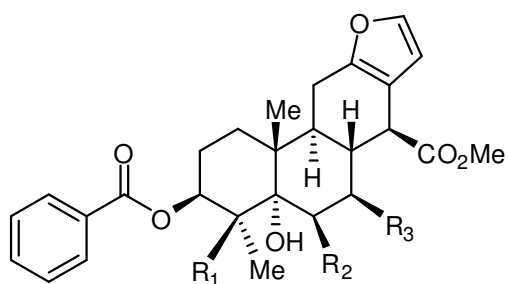สารทั้งหมดได้ถูกนำไปทดสอบฤทธิ์ต้านการอักเสบ โดยการยับยั้งไลโพโพลีแซค คาไรด์ (LPS) ซึ่งเป็นตัวเหนี่ยวนำให้เกิดไนตริคออกไซด์ (NO) ใน RAW264.7 เซลล์โมเดล พบว่าสารประกอบ **CP8, CP9, CP11**-**CP15** และ **CP17**-**CP26** แสดงฤทธิ์ยับยั้งไนตริคออก ไซด์ (NO) ที่เป็นสาเหตุของการเกิดการอักเสบอยู่ในระดับที่ดีมากด้วยค่า IC$_{50}$ 2.9-12.5 $\mu$M ซึ่งดีกว่ายามาตรฐานคือ indomethacin (IC$_{50}$ = 14.5 $\mu$M)
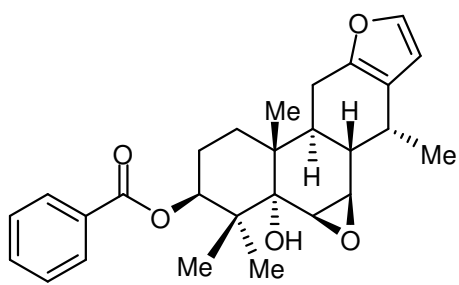
|        | R$_1$   | R$_2$          | R$_3$          |
|--------|---------|----------------|----------------|
| **CP1**  | H       | H              | OAc            |
| **CP2**  | H       | OH             | OAc            |
| **CP3**  | H       | OAc            | OH             |
| **CP4**  | H       | OH             | OCOPh          |
| **CP5**  | OCOPh   | H              | H              |
| **CP6**  | H       | OCOPh          | H              |
| **CP7**  | H       | OCOCH=$\overset{E}{\text{C}}$HPh | H |
| **CP16** | H       | H              | H              |
| **CP17** | H       | OCOPh          | OH             |
| **CP18** | H       | OCOCH=CHPh $\overset{E}{}$ | OH |
| **CP19** | H       | OH             | OCOCH=CHPh $\overset{E}{}$ |
| **CP20** | OCOPh   | H              | OH             |

|        | $R_1$   | $R_2$                 | $R_3$  | $R_4$  |
|--------|---------|-----------------------|--------|--------|
| **CP8**  | H       | CHO                   | OCOPh  | OH     |
| **CP9**  | H       | $CH_2OCOPh$           | OH     | H      |
| **CP10** | H       | $CO_2H$               | OCOPh  | H      |
| **CP11** | OCOPh   | $CO_2H$               | OCOPh  | H      |
| **CP21** | H       | $CO_2H$               | OCOPh  | OCOPh  |
| **CP22** | OH      | $CO_2H$               | OCOPh  | OCOPh  |
| **CP23** | OCOPh   | $CO_2H$               | OCOPh  | OAc    |



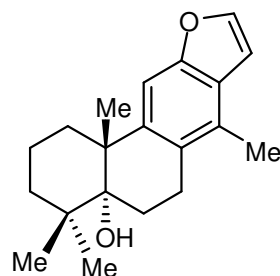|        | $R_1$      | $R_2$  | $R_3$  |
|--------|------------|--------|--------|
| **CP12** | Me         | OAc    | OH     |
| **CP13** | $CH_2OAc$  | OH     | OAc    |
| **CP24** | Me         | OH     | OAc    |

**CP14**

**CP15**

**CP25**

**CP26**

**Thesis Title**    Chemical Constituents from the Roots of *Caesalpinia mimosoides* and *Caesalpinia pulcherrima* and their Anti-inflammatory activity

**Author**    Miss Orapun  Yodsaoue

**Major Program**    Organic Chemistry

**Academic Year**    2012

## ABSTRACT

*Part I Chemical Investigation of the Roots of C. mimosoides*

The crude $CH_2Cl_2$ and acetone extracts showed potent NO inhibitory activity with $IC_{50}$ values of 11.0 and 21.6 μg/ml, respectively. Further separation and purification led to the isolation of four diterpenes, named mimosol A–D (**CM1**–**CM4**), a dimer, named mimosol E (**CM9**) and two dibenzo[b,d]furans, named mimosol F, G (**CM10**, **CM11**), along with eleven known compounds including four diterpenes [taepeenin A (**CM5**), taepeenin D (**CM6**), nortaepeenin A (**CM7**) and taepeenin L (**CM8**)], three homoisoflavones [(*E*)-7-hydroxy-3-(4-methoxybenzyl)chroman-4-one (**CM12**), (*E*)-7,8-dihydroxy-3-(4-methoxybenzyl)chroman-4-one (**CM13**) and (*E*)-7-hydroxy-8-methoxy-3-(4-methoxybenzyl)chroman-4-one (**CM14**)], three phenylpropanols [tetracosyl caffeate (**CM15**), resveratrol (**CM16**) and bergenin (**CM17**)] and a sesquiterpene [(+)-pterocarpol (**CM18**)]. Their structures were elucidated by analysis of their spectroscopic data and comparison with literature data.

The anti-inflmmatory activity of all compounds were evaluated for inhibitory activity against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 macrophage cell line of which compounds **CM4**, **CM6**, **CM8**, and **CM12**–**CM14** showed strong NO-inhibitory activity. These compounds were also tested for the inhibitory effect on LPS-induced tumor necrosis factor-alpha (TNF-*α*) release in RAW264.7 cells. The results indicated that **CM4** possessed potent inhibitory activity for both tests with $IC_{50}$ values of 3.0 and 6.5 μM, respectively.
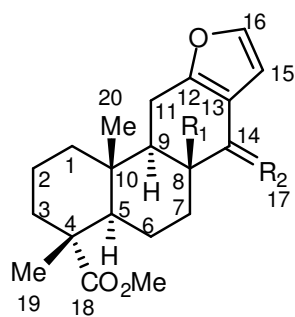
**CM1:** $R_1$ = OH; $R_2$ = $CH_2$

**CM7:** $R_1$ = H; $R_2$ = O
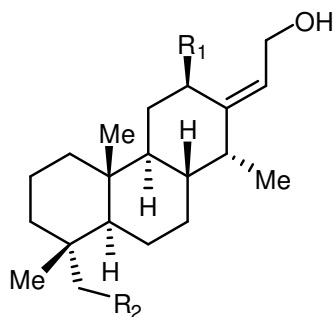
**CM2:** $R_1$ = OH; $R_2$ = H

**CM3:** $R_1$ = H; $R_2$ = OH

**CM8:** $R_1$ = $R_2$ = H
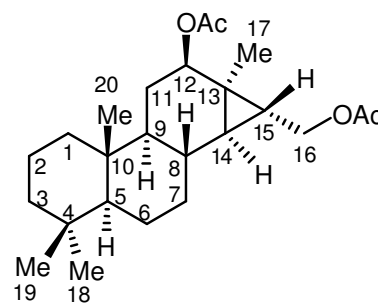
**CM4**

**CM5:** R = H

**CM6:** R = OAc

**CM9**
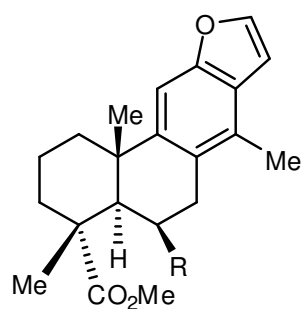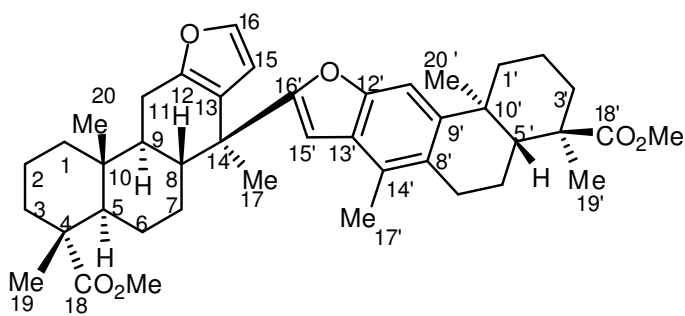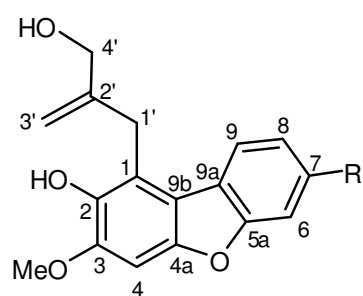
**CM10:** R = OH

**CM11:** R = OMe
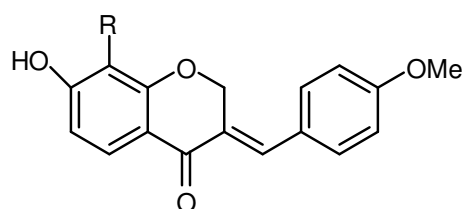
**CM12:** R = H

**CM13:** R = OH

**CM14:** R = OMe
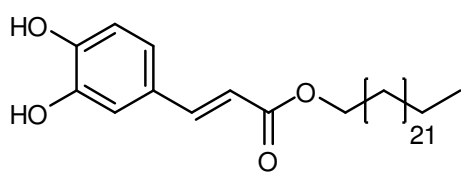
**CM15**
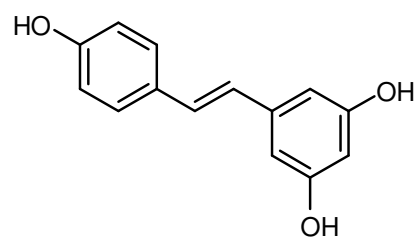
**CM16**
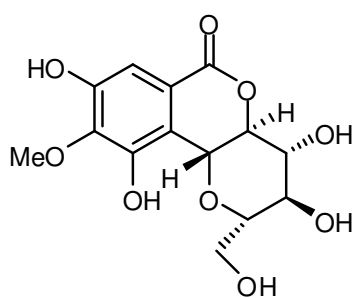
**CM17**
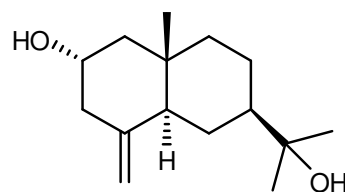
**CM18**

*Part II Chemical Investigation of the Roots of C. pulcherrima*

The $CH_2Cl_2$ extract from the roots of *C. pulcherrima* was separated to afford 15 new diterpenes, named pulcherrin D–R (**CP1**–**CP15**) together with eleven known compounds (**CP16**–**CP26**). The known compounds were identified as vouacapen-5$\alpha$-ol (**CP16**), isovouacapenol C (**CP17**), 6$\beta$-cinnamoyl-7$\beta$-hydroxyvouacapen-5$\alpha$-ol (**CP18**), pulcherrin A (**CP19**), pulcherrin B (**CP20**), pulcherrimin C (**CP21**), pulcherrimin A (**CP22**), pulcherrimin E (**CP23**), pulcherrin C (**CP24**), pulcherrimin B (**CP25**) and 8,9,11,14-didehydrovouacapen-5$\alpha$-ol (**CP26**). All compounds were identified by spectroscopic data and comparison with those reported in the literatures. Moreover, the structures of compounds **CP16** and **CP17** were also confirmed by X-ray diffraction analysis.
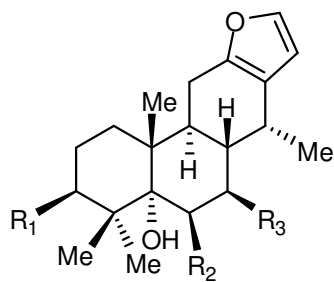
The anti-inflammatory activity of all isolated compounds were investigated with the lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW264.7 macrophage cell line. Compounds **CP8**, **CP9**, **CP11**–**CP15** and **CP17**–**CP26** showed potent NO inhibitory activity with $IC_{50}$ values in the range of 2.9-12.5 μM better than that of the positive control (indomethacin $IC_{50}$ = 14.5 μM).

|        | $R_1$ | $R_2$             | $R_3$             |
|--------|-------|-------------------|-------------------|
| **CP1**  | H     | H                 | OAc               |
| **CP2**  | H     | OH                | OAc               |
| **CP3**  | H     | OAc               | OH                |
| **CP4**  | H     | OH                | OCOPh             |
| **CP5**  | OCOPh | H                 | H                 |
| **CP6**  | H     | OCOPh             | H                 |
| **CP7**  | H     | OCOCH=CHPh *E*    | H                 |
| **CP16** | H     | H                 | H                 |
| **CP17** | H     | OCOPh             | OH                |
| **CP18** | H     | OCOCH=CHPh *E*    | OH                |
| **CP19** | H     | OH                | OCOCH=CHPh *E*    |
| **CP20** | OCOPh | H                 | OH                |

|        | $R_1$  | $R_2$                | $R_3$  | $R_4$  |
|--------|--------|----------------------|--------|--------|
| **CP8**  | H      | CHO                  | OCOPh  | OH     |
| **CP9**  | H      | CH$_2$OCOPh          | OH     | H      |
| **CP10** | H      | CO$_2$H              | OCOPh  | H      |
| **CP11** | OCOPh  | CO$_2$H              | OCOPh  | H      |
| **CP21** | H      | CO$_2$H              | OCOPh  | OCOPh  |
| **CP22** | OH     | CO$_2$H              | OCOPh  | OCOPh  |
| **CP23** | OCOPh  | CO$_2$H              | OCOPh  | OAc    |



|        | $R_1$       | $R_2$  | $R_3$  |
|--------|-------------|--------|--------|
| **CP12** | Me          | OAc    | OH     |
| **CP13** | CH$_2$OAc   | OH     | OAc    |
| **CP24** | Me          | OH     | OAc    |

**CP14**



**CP15**



**CP25**



**CP26**

# ACKNOWLEDGEMENT

Orapun Yodsaoue

# CONTENTS

# CONTENTS (Continued)

# CONTENTS (Continued)

# CONTENTS (Continued)

# LIST OF TABLES

# LIST OF TABLES (Continued)

# LIST OF TABLES (Continued)

# LIST OF TABLES (Continued)

# LIST OF TABLES (Continued)

# LIST OF ILLUSTRATIONS

# LIST OF ILLUSTRATIONS (Continued)

# LIST OF ILLUSTRATIONS (Continued)

# LIST OF ILLUSTRATIONS (Continued)

# LIST OF ILLUSTRATIONS (Continued)

# LISTS OF SCHEMES

# ABBREVIATIONS AND SYMBOLS

| | | |
|---|---|---|
| *s* | = | *singlet* |
| *d* | = | *doublet* |
| *t* | = | *triplet* |
| *q* | = | *quartet* |
| *m* | = | *multiplet* |
| *dd* | = | *doublet of doublet* |
| *dt* | = | *doublet of triplet* |
| *br s* | = | *broad singlet* |
| $R_f$ | = | Retention factor |
| g | = | Gram |
| nm | = | Nanometer |
| mp | = | melting point |
| $cm^{-1}$ | = | reciprocol centimeter (wavenumber) |
| $\delta$ | = | chemical shift relative to TMS |
| $J$ | = | coupling constant |
| $[\alpha]_D$ | = | specific rotation |
| $\lambda_{max}$ | = | maximum wavelength |
| $\nu$ | = | absorption frequencies |
| $\varepsilon$ | = | molar extinction coefficient |
| m/z | = | a value of mass divided by charge |
| °C | = | degree celcius |
| MHz | = | Megahertz |
| ppm | = | part per million |
| *c* | = | Concentration |
| IR | = | Infrared |
| UV-VIS | = | Ultraviolet-Visible |
| MS | = | Mass Spectroscopy |

# ABBREVIATIONS AND SYMBOLS (continued)

| | | |
|---|---|---|
| NMR | = | Nuclear Magnetic Resonance |
| 2D NMR | = | Two Dimensional Nuclear Magnetic Resonance |
| COSY | = | Correlation Spectroscopy |
| DEPT | = | Distortionless Enhancement by Polarization Transfer |
| HMBC | = | Heteronuclear Multiple Bond Correlation |
| HMQC | = | Heteronuclear Multiple Quantum Coherence |
| NOE | = | Nuclear Overhauser Effect Spectroscopy |
| CC | = | Column Chromatography |
| QCC | = | Quick Column Chromatography |
| PLC | = | Preparative Thin Layer Chromatography |
| TMS | = | Tetramethylsilane |
| $CDCl_3$ | = | Deuterochloroform |
| $CD_3OD$ | = | Deuteromethanol |
| DMSO-$d_6$ | = | Deuterodimethylsulfoxide |
| NO | = | Nitric Oxide |
| LPS | = | Lipopolysaccharide |
| μM | = | micro molar |
| μg | = | micro gram |
| $IC_{50}$ | = | the half maximal inhibitory concentration |

# CHAPTER 1
# INTRODUCTION

## 1.1 General introduction

Natural products represent a rich source of biologically active compounds and are examples of molecular diversity. Recognized potential in drug discovery and development in the areas of human diseases, animal diseases and plant diseases of new drugs originated from natural sources: plant, animal, microbial or mineral origin.

## 1.2 The cassane-type diterpenes

Diterpenoids are group of geranylgeranyl diphosphate which derived from farnesyl diphosphate and isopentenyl-diphosphate. Diterpenes can be found from many sources such as groups of organisms including terrestrial fungi, lichens, marine origins, and insects, but the majority of diterpenes are from plants. Diterpenoids exhibit diverse biological properties, such as anti-inflammatory, cytotoxic, antifeedant, platelet-aggregation, and antimicrobial effects.

Despite being relatively the smallest class of terpenoid compounds, the chemical structures of diterpenes are otherwise widely varied, ranging in term of numbers of carbocyclic systems from non-cyclic to as high as tetra-carbocyclic core skeletons. Among various groups of diterpenes, the cassanes, which are the focus of this investigation, is possibly the most common and most extensively studied class.

Chemically, cassanes-type diterpenes possess tricarbocyclic core skeleton, occasionally with a furanoid extension attached to ring C, which was called "furanocassane-type diterpenes". These compounds are found most exclusively in the plants, especially of the genus *Caesalpinia*.

Cassane skeleton                                Furanocassane skeleton
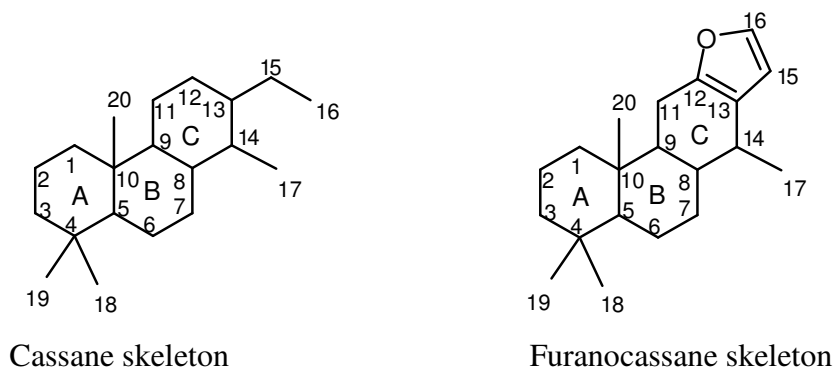
## 1.3 Biological activity of cassane

To date, there have been up to more than 100 naturally occurring furanocassane-type diterpenes reported. These can be classified into two categories, namely furanocassane and non-furanocassane. The biological activities of this class of compounds were reported such as DNA repair-deficient yeast mutant (Patil et al., 1997), antiviral (Jiang,et al., 2002a; Jiang, et al., 2002b), antitubercular (Promsawan et al., 2003), antimalarial (Linn et al., 2005; Kalauni et al., 2006; Pudhom et al., 2007), anitrypanosomal (Torres-Mendoza et al., 2004), antibacterial (Dickson et al., 2007), antioxidant (Dickson et al., 2007) and cytotoxic activities (McPherson et al., 1986; Yadav et al., 2009).

## 1.4 *Caesalpinia* genus

*Caesalpinia* belongs to the Leguminosae-Caesalpinioideae family. This family contains about 150 genera with 2,200 species. In Thailand only 20 genera with 113 species are found, from *Caesalpinia* genus only 19 species are found (Smitinand, T. 2001).

**Table 1.** Species of *Caesalpinia* genus in Thailand

1. *Caesalpinia andamanica* (Prain) Hattink
2. *Caesalpinia bonduc* (L.) Roxb.
3. *Caesalpinia coriaria* (Jacq.) Willd.
4. *Caesalpinia crista* L.
5. *Caesalpinia cucullata* Roxb.

**Table 1** (continued)

6. *Caesalpinia decapetala* (Roth) Alston

7. *Caesalpinia digyna* Rottl.

8. *Caesalpinia enneaphylla* Roxb.

9. *Caesalpinia furfuracea* (Prain) Hattink

10. *Caesalpinia godefroyana* O. Kuntze

11. *Caesalpinia hymenocarpa* (Prain) Hattink

12. *Caesalpinia major* (Medik.) Dandy & Exell

13. *Caesalpinia mimosoides* Lamk.

14. *Caesalpinia minax* Hance

15. *Caesalpinia parviflora* Prain

16. *Caesalpinia pubescens* (Desf.) Hatt.

17. *Caesalpinia pulcherrima* (L.) Swartz

18. *Caesalpinia sappan* L.

19. *Caesalpinia tortuosa* Roxb.

Plants from several species of this genus have shown diverse biological activities such as *C. pulcherrima* exhibit antitubercular (Promsawan et al., 2003), *C. crista* exhibits antimalarial (Linn et al., 2005; Kalauni et al., 2006), *C. benthamiana* exhibits antibacterial and antioxidant (Dickson et al., 2007), and *C. bonduc* exhibits antimalarial and cytotoxic activities (Pudhom et al., 2007).

## 1.4.1 *Caesalpinia mimosoides* Lamk.

*Caesalpinia mimosoides* Lamk. has many local Thai names such as "Phak pu ya (ผักปู่ย่า)" and Cha-Luead (ช้าเลือด), the origin of *C. mimosoides* is not certain, but is thought to be in the region from India, Burma, Laos, Vietnam and China. *C. mimosoides* has been found in old clearings, scrub areas, and mixed deciduous forests in northern and north-eastern Thailand. *C. mimosoides* is erect or climbing shrub, densely hispid and bristly on all parts. Stipules have awl shaped, 7-15 mm long, but caducous. The leaves are bipinnate, rachis 25-40 cm long; bearing 10-30

pairs of pinnae, each with 10-20 pairs of leaflets, leaflets are ovate-oblong, opposite, 10 mm long and 4 mm wide. Inflorescence has large terminal panicle. The flowers are broadly obovate petals, 1.5-2 cm long, 1.2-1.5 cm wide which appear bright yellow. Pods are flat and glabrescent, 20-30 cm long, 1-1.5 cm wide. Seeds are ovate and flat, 1.15 cm long, 5-6 mm wide, light brown. The young shoots and leaves are locally consumed as fresh vegetables and appetizers. The young shoots and flowers have also been used as a carminative and to relieve dizziness and fainting. This plant has been reported to exhibit antimicrobial (Chanwitheesuk et al., 2007) and antioxidant activities (Chanwitheesuk et al., 2005).



**Figure 1**  Different parts of *Caesalpinia mimosoides* Lamk.
**a)** Tree  **b)** Stem  **c)** Leaves  **d)** Flowers  **e)** Fruits  **f)** Seeds

### 1.4.2 *Caesalpinia pulcherrima* **Swartz.**

*Caesalpinia pulcherrima* Swartz. is known locally as "Hang Nok Yung Thai (หางนกยูงไทย)". Other common names for this species are Poinciana, Peacock Flower, Red Bird of Paradise, Mexican Bird of Paradise, Dwarf Poinciana, Pride of Barbados, and flamboyan-de-jardin. *C. pulcherrima* is a large perennial shrub or small tree, 1-3 m tall that is widely distributed in tropical areas and has been used as ornamental plant. (Smitinand, 2001) The leaves are bipinnate, 20-40 cm long, bearing 3-10 pairs of pinnae, each with 6-10 pair of leaflets 15-25 mm long and 10-15 mm broad. The flowers are borne in recemes up to 2 cm long which appear yellow, pink, off-white and red with yellow margins. This plant is a striking ornamental plant, widely grown in tropical gardens. It is also the national flower of the Caribbean island of Barbados, and is depicted on the Queen's personal Barbadian flag.
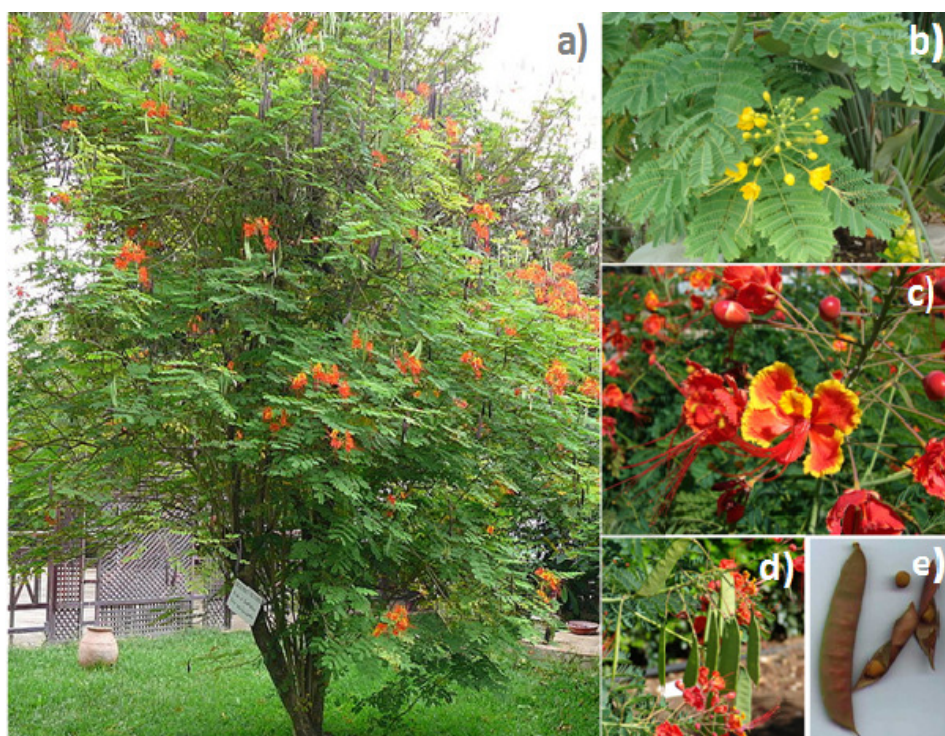


**Figure 2** Different parts of *Caesalpinia pulcherrima*
**a)** Tree  **b)** Leaves  **c)** Flowers  **d)** Fruits  **e)** Seeds

**1.5 Review of literatures**

Chemical constituents isolated from 19 species of the genus *Caesalpinia* were summarized by Sarot Cheenpracha in 2007 (Cheenpracha, 2007). Information from NAPRALERT database developed by University of Illinois at Chicago and Chemical Abstracts of the year 2007 reported additional constituents from three new species of the *Caesalpinia* genus and they could be classified into groups, such as benzenoids, coumarins, diterpenes, flavonoids, flavonols, flavones, flavonones, sesquiterpenes, steroids and triterpenes. These compounds are presented in Table 2.

**Table 2.** Compounds from plants of *Caesalpinia* genus

| **a** : Benzenoids | **b** : Chalcones | **c** : Diterpenes | **d** : Flavonoids |
|---|---|---|---|
| **e :** Iridoids | **f** : Quinones | **g** : Phenylpropanoids | |
| **h** : Sesquiterpenes | **I** : Steroids | **j** : Triterpenes | |

| Scientific Name | Investigated part | Compound | Bibliography |
|---|---|---|---|
| *C. benthamiana* | Root bark | Benthaminin 1, **14c** <br> Benthaminin 2, **15c** <br> Deoxycaesaldekarin C, **44c** | Dickson et al., 2007 |
| *C. bonduc* | Part not Specified | Caesalpinolide A, **39c** <br> Caesalpinolide B, **41c** <br> 6*β*-Acetoxy-17-methylvoucapane-8(14),9(11)-diene, **12c** <br> 17-Methylvouacapane-8(14),9(11)-diene, **13c** <br> Caesalpinolide D, **40c** <br> Caesalpinolide C, **42c** <br> Caesalpinolide E, **43c** | Yadav et al., 2007 <br> Yadav et al., 2009 |

**Table 2** (Continued)

| Scientific name | Investigated part | Compound | Bibliography |
|---|---|---|---|
| *C. bonduc* | Part not Specified | Friedelin, **113j**<br>Lupeol, **114j** | Yadav et al., 2009 |
| | Bark | Caesaldekarin J, **21c**<br>17-Hydroxycampesta-4,6-dien-3-one, **109i**<br>13,14-*seco*-Stigmasta-5,14-dien-3$\alpha$-ol, **110i**<br>13,14-*seco*-Stigmasta-9(11),14-dien-3$\alpha$-ol, **111i**<br>Pipataline, **105g** | Udenigwe et al., 2007 |
| | | Caesalpinianone, **75d**<br>6-*O*-Methylcaesalpinianone, **76d**<br>Hematoxylol, **88d**<br>Stereochenol A, **103f**<br>4′-*O*-Acetylloganic acid, **101e**<br>6′-*O*-Acetylloganic acid, **102e**<br>2-*O*-$\beta$-*D*-Glucosyloxy-4-methoxybenzenepropanoic acid, **104g** | Ata et al., 2009 |
| | Kernels | 2-Acetoxycaesaldekarin E, **11c**<br>Bonducellpin B, **16c**<br>Bonducellpin C, **17c**<br>Bonducellpin E, **18c**<br>Bonducellpin F, **19c**<br>Bonducellpin G, **20c**<br>Caesalmin B, **22c**<br>Caesalmin D, **26c**<br>Caesalmin E, **27c** | Pudhom et al., 2007 |

**Table 2** (Continued)

| Scientific name | Investigated part | Compound | Bibliography |
|---|---|---|---|
| *C. bonduc* | Kernels | $\alpha$-Caesalpin, **28c** | Pudhom et al., 2007 |
| | | $\varepsilon$-Caesalpin, **29c** | |
| | | Caesalpinin C, **31c** | |
| | | 14(17)-Dehydrocaesalpin F, **32c** | |
| | | Caesalpinin I, **33c** | |
| | | Caesalpinin K, **34c** | |
| | Seeds | Neocaesalpin W, **51c** | Wu et al., 2007 |
| | | β-Amyrin, **112j** | |
| *C. crista* | Seeds | Caesaljapin, **23c** | Yang et al., 2009 |
| | | Caesaljapin B, **24c** | |
| | | Caesaljapin C, **25c** | |
| | | Caesalpinilinn, **30c** | |
| | | Caesalpinista A, **36c** | |
| | | Caesalpinista B, **37c** | |
| | | Deoxycaesaldekarin C, **38c** | |
| *C. ferrea* | Stem | Pauferrol A, **10b** | Nozaki et al., 2007 |
| *C. magnifoliolata* | Seeds | Caesalmins D, **26c** | Yin et al., 2008 |
| | | Caesalmins E, **27c** | |
| | | Magnicaesalpin, **45c** | |
| | | Neocaesalpin L, **46c** | |
| | | Neocaesalpin O, **47c** | |
| *C. millettii* HOOK. *Et* ARN | Stems | Bonducellin, **80d** | Chen and Yang, 2007 |
| | | Eucomin, **85d** | |
| | | Intricatinol, **86d** | |
| | | 8-Methoxybonducellin, **87d** | |
| | | 8-Methoxyisobonducellin, **90d** | |

**Table 2** (Continued)

| Scientific name | Investigated part | Compound | Bibliography |
|---|---|---|---|
| *C. millettii* HOOK. *Et* ARN | Stems | Tamarixetin 3-*O*-(6″-*O*-*E*-caffeoyl)-*β*-D-alactopyranoside, **99d** | Chen and Yang, 2007 |
| *C. mimosoides* | Part not specified | Gallic acid, **6a** | Chanwitheesuk et al. 2007 |
| *C.paraguariensis* | Stem bark | Ellagic acid, **4a** <br> 3-*O*-Metilellagic acid, **5a** | Sgariglia et al., 2011 |
| *C. pulcherrima* | Aerial parts | (3*E*)-3-(1,3-Benzodioxol-5-yl methylene)-2,3-dihydro-7-hydroxy-4*H*-1-benzopyran-4-one, **70d** <br> (3*E*)-3-(1,3-Benzodioxol-5-yl methylene)2,3-dihydro-7-methoxy-4*H*-1-benzopyran-4-one, **71d** <br> (3*E*)-2,3-Dihydro-3-[(3,4-dimethoxyphenyl)methylene]-7-methoxy-4*H*-1benzopyran-4-one, **77d** <br> (3*E*)-2,3-Dihydro-6,7dimethoxy-3[(3-hydroxy-4-methoxyphenyl)methylene]-4*H*1-benzopyran-4-one, **78d** <br> (3*E*)2,3-Dihydro-7-hydroxy-3-[(3-hydroxy-4-methoxyphenyl)methylene]-4*H*-1-benzopyran-4-one, **79d** <br> Bonducellin, **80d** <br> Sappanone A, **81d** <br> 7-*O*-Methyl bonducellin, **82d** <br> 2-Methoxybonducellin, **89d** | Das et al., 2009 |

**Table 2** (Continued)

| Scientific name | Investigated part | Compound | Bibliography |
|---|---|---|---|
| *C. pulcherrima* | Stems | Neocaesalpin P, **48c** | Pranithanchai et al., 2009 |
| | | Neocaesalpin Q, **49c** | |
| | | Neocaesalpin R, **50c** | |
| | | Pulcherrin A, **63c** | |
| | | Pulcherrin B, **64c** | |
| | | Isovouacapenol C, **65c** | |
| | | 6$\beta$-Cinnamoyl-7$\beta$-hydroxyvoua-capen-5$\alpha$-ol, **66c** | |
| | | Pulcherrimin E, **67c** | |
| | | Pulcherrimin C, **68c** | |
| | | Pulcherrin C, **69c** | |
| | | Bonducellin, **80d** | |
| | | $\alpha$-Cadinol, **106h** | |
| | | 7-Hydroxycadalene, **107h** | |
| | | Teucladiol, **108h** | |
| *C. sappan* | Heartwood | Protosappanin A, **92d** | Fu et al., 2008 |
| | | Sappanchalcone, **86d** | |
| | | Sappanone B, **98d** | |
| | | 3'-Deoxy-4-*O*-methylepisappa-nol, **84d** | |
| | | (8*S*,8'*S*)-Bisdihydrosiringenin, **1a** | |
| | | Brazilein, **74d** | |
| | | 3-Deoxysappanchalcone, **9b** | |
| | | (+)-Lyoniresinol, **7a** | |
| | | 3-Deoxysappanone B, **83d** | |
| | | Protosappanin B, **93d** | |
| | | Isoprotosappanin B, **25c** | |

**Table 2** (Continued)

| Scientific name | Investigated part | Compound | Bibliography |
|---|---|---|---|
| *C. sappan* | Heartwood | 3'-*O*-Methylbrazilin, **73d**<br>Brazilin, **72d** | Fu et al., 2008 |
| | | Caesappanin A, **2a**<br>Caesappanin B, **3a** | Shu et al., 2011 |
| | | 7,3',4'-Trihydroxy-3-benzyl-2*H*-chromene, **100d**<br>4-*O*-Methylsappanol, **91d** | Zhao et al., 2008 |
| | | Brazilin, **72d**<br>Sappanchalcone, **8b**<br>Protosappanin A, **92d**<br>Protosappanin B, **93d**<br>Protosappanin C, **94d**<br>Protosappanin D, **96d**<br>Protosappanin E, **97d** | Washiyama et al., 2009 |
| | Seeds | Phanginin A, **52c**<br>Phanginin B, **53c**<br>Phanginin C, **54c**<br>Phanginin D, **55c**<br>Phanginin E, **56c**<br>Phanginin F, **57c**<br>Phanginin G, **58c**<br>Phanginin H, **59c**<br>Phanginin I, **60c**<br>Phanginin J, **61c**<br>Phanginin K, **62c** | Yodsaoue et al., 2008 |
| | Part not specified | Sappanchalcone, **8b**<br>3′-Deoxy-4-*O*-ethylepisappanol, **84d** | Moon et al., 2010 |

**a: Benzenoids**
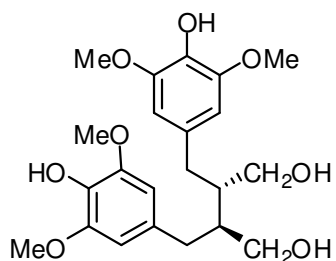
**1a**: (+)-(8*S*,8'*S*)-Bisdihydrosiringenin
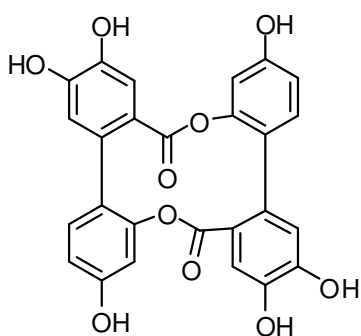
**2a**: Caesappanin A
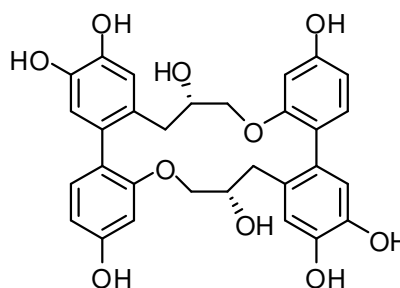
**3a**: Caesappanin B
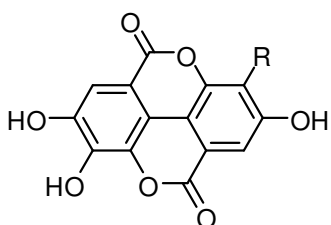
**4a**: R = OH; Ellagic acid

**6a**: R = H; Gallic acid
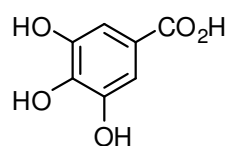
**5a**: R = OMe; 3-*O*-Metilellagic acid
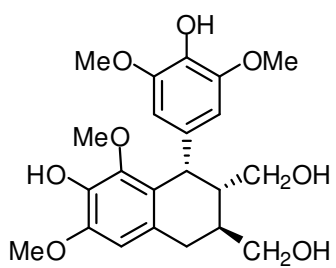
**7a:** (+)-Lyoniresinol

**b: Chalcones**
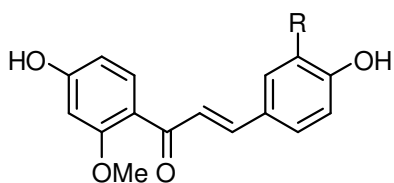


**8b**: R = OH; Sappanchalcone

**9b**: R = H; 3-Deoxyappanchalcone
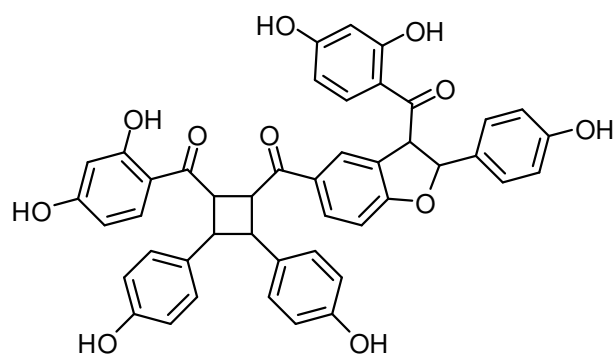
**10b**: Pauferrol A

**c: Diterpenes**
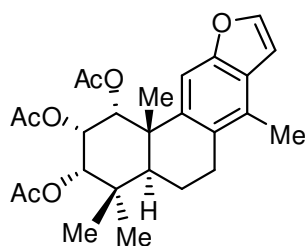


**11c**: 2-Acetoxycaesaldekarin E
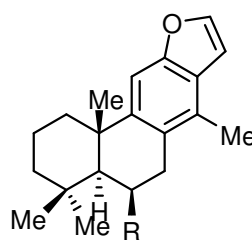
**12c**: R = OAc; 6β-Acetoxy-17-methylvoucapane-8(14),9(11)-diene

**13c**: R = H; 17-Methylvouacapane-8(14),9(11)-diene
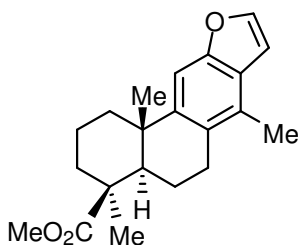


**14c**: Benthaminin 1
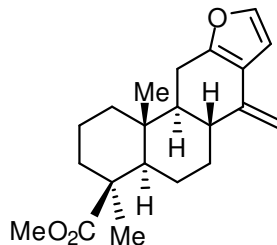
**15c**: Benthaminin 2
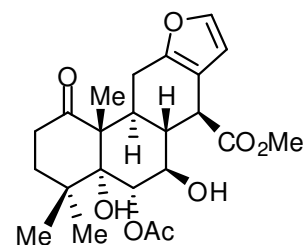
**16c**: Bonducellpin B
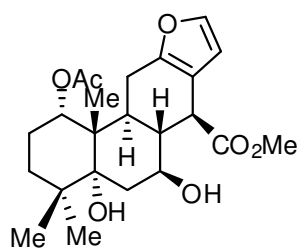
**17c**: Bonducellpin C
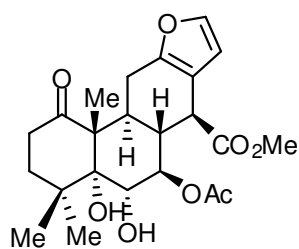
**18c**: Bonducellpin E
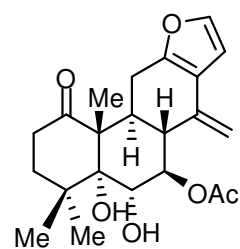
**19c**: Bonducellpin F
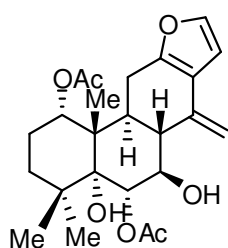
**20c**: Bonducellpin G
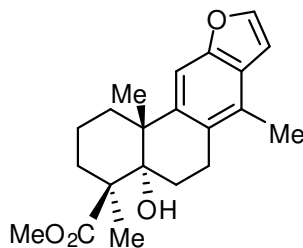
**21c**: Caesaldekarin J
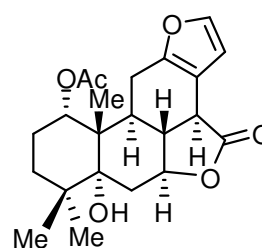
**22c**: Caesalmin B
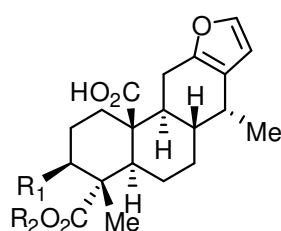
**23c**: R$_1$ = H, R$_2$ = Me; Caesaljapin

**24c**: R$_1$ = R$_2$ = H; Caesaljapin B

**25c**: R$_1$ = OAc, R$_2$ = Me; Caesaljapin C
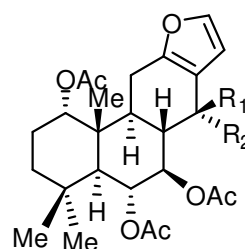
**26c**: R$_1$ = Me, R$_2$ = OH; Caesalmin D

**27c**: R$_1$ = OH, R$_2$ = Me; Caesalmin E
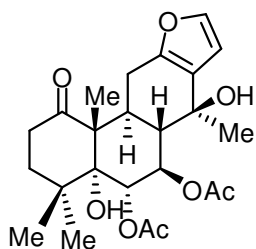
**28c**: $\alpha$-Caesalpin
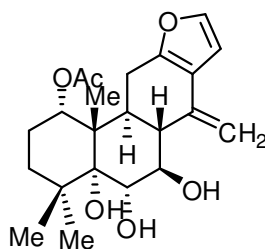
**29c**: $\varepsilon$-Caesalpin
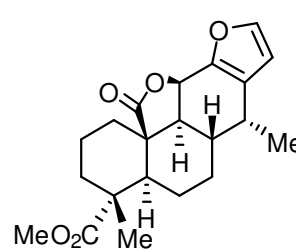
**30c**: Caesalpinilinn
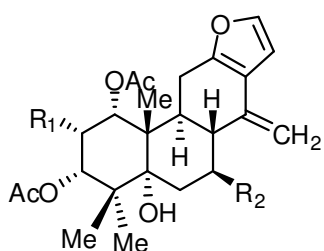
**31c**: $R_1 = R_2 = OH$; Caesalpinin C

**32c**: $R_1 = OAc$, $R_2 = H$; 14(17)-Dehydrocaesalpin F
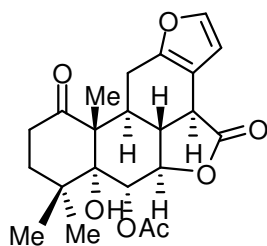
**33c**: Caesalpinin I
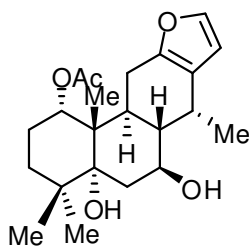
**34c**: Caesalpinin K
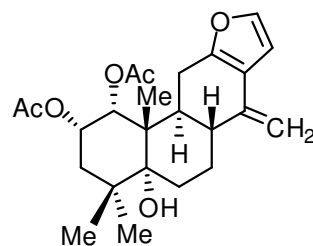
**35c**: Caesalpinin P
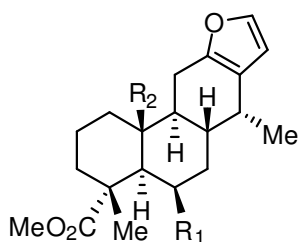
**36c**: $R_1 = OH$, $R_2 = CH_2OH$; Caesalpinista A

**37c**: $R_1 = OH$, $R_2 = CH_2OAc$; Caesalpinista B

**38c**: $R_1 = H$, $R_2 = Me$; Deoxycaesaldekarin C
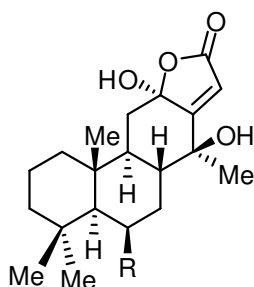
**39c**: R = OAc; Caesalpinolide A

**40c**: R = H; Caesalpinolide D
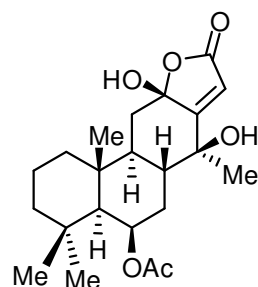
**41c**: Caesalpinolide B
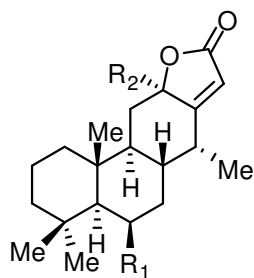
**42c**: $R_1 = R_2 = OH$; Caesalpinolide C

**43c**: $R_1 = OAc$, $R_2 = OH$; Caesalpinolide E
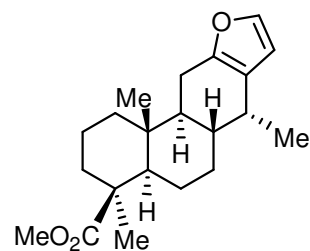
**44c**: Deoxycaesaldekarin C
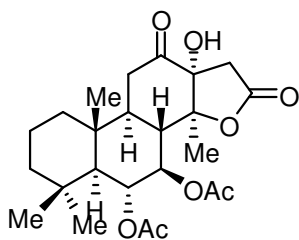
**45c**: Magnicaesalpin
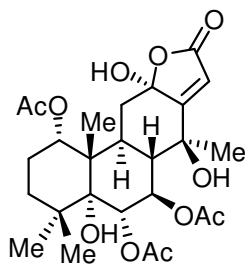
**46c**: Neocaesalpin L
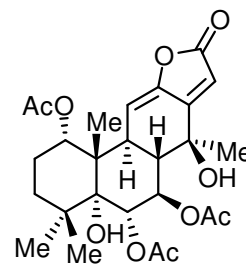
**47c**: Neocaesalpin O
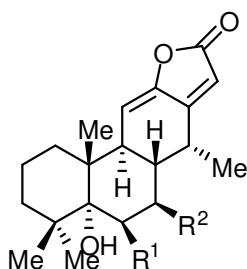
**48c**: $R_1$ = OCOCH=CHPh, $R_2$ = OH; Neocaesalpin P

**49c**: $R_1$ = OCOPh, $R_2$ = H; Neocaesalpin Q

**50c**: $R_1$ = OCOPh, $R_2$ = OH; Neocaesalpin R
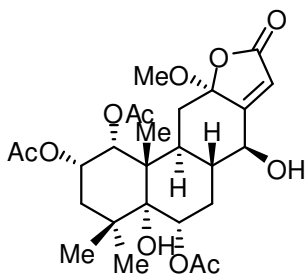
**51c**: Neocaesalpin W
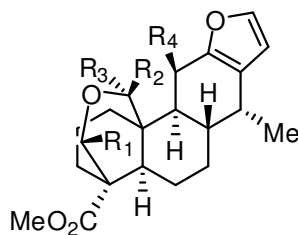
**52c**: $R_1 = R_3 = R_4$ = H, $R_2$ = OH; Phanginin A

**53c**: $R_1$ = OH, $R_2 = R_3 = R_4$ = H; Phanginin B

**54c**: $R_1 = R_2 = R_4$ = H, $R_3$ = OMe; Phanginin C

**55c**: $R_1$ = OMe, $R_2 = R_3 = R_4$ = H; Phanginin D

**56c**: $R_1$ = =O, $R_2 = R_3 = R_4$ = H; Phanginin E

**57c**: $R_1 = R_2$ = H, $R_3 = R_4$ = OH; Phanginin F
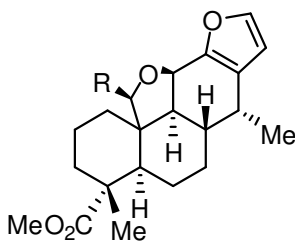
**58c**: R = OH; Phanginin G

**59c**: R = H; Phanginin H
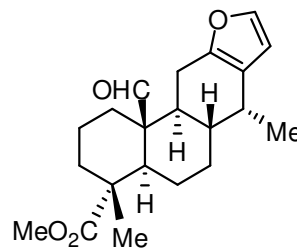
**60c**: R = Me; Phanginin I

**61c**: R = CHO; Phanginin J

**62c**: R = $CO_2$Me; Phanginin K
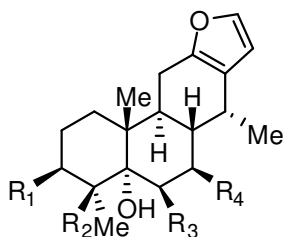
**63c**: R$_1$ = H, R$_2$ = Me, R$_3$ = OH, R$_4$ = OCOCH=CHPh; Pulcherrin A

**64c**: R$_1$ = OCOPh, R$_2$ = Me, R$_3$ = H, R$_4$ = OH; Pulcherrin B

**65c**: R$_1$ = H, R$_2$ = Me, R$_3$ = OCOPh, R$_4$ = OH; Isovouacapenol C

**66c**: R$_1$ = H, R$_2$ = Me, R$_3$ = OCOCH=CHPh, R$_4$ = OH; 6$\beta$-Cinnamoyl-7$\beta$-hydroxy-vouacapen-5$\alpha$-ol

**67c**: R$_1$ = OCOPh, R$_2$ = CO$_2$H, R$_3$ = OCOPh, R$_4$ = OAc; Pulcherrimin E

**68c**: R$_1$ = H, R$_2$ = CO$_2$H, R$_3$ = OCOPh, R$_4$ = OCOPh; Pulcherrimin C
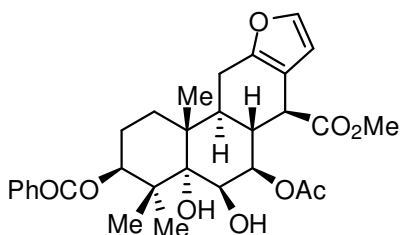


**69c**: Pulcherrin C

**d : Flavonoids**
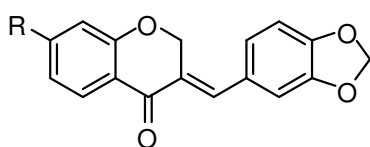


**70d**: R = OH; (3$E$)-3-(1,3-Benzodioxol-5-ylmethylene)-2,3-dihydro-7-hydroxy-4$H$-1-benzopyran-4-one

**71d**: R = OMe; (3$E$)-3-(1,3-Benzodioxol-5-ylmethylene)-2,3-dihydro-7-methoxy-4$H$-1-benzopyran-4-one
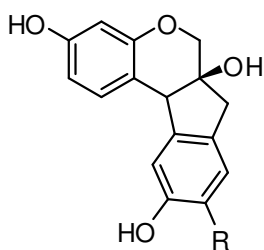


**72d**: R = OH; Brazilin

**73d**: R = OMe;  3'-$O$-Methylbrazilin
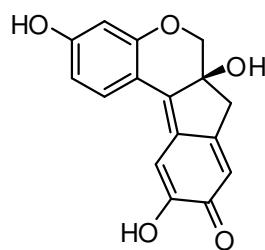


**74d**: Brazilein
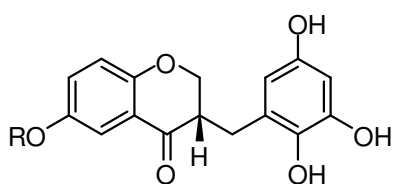
**75d**: R = H; Caesalpinianone

**76d**: R = CH₃; 6-*O*-Methylcaesalpinianone
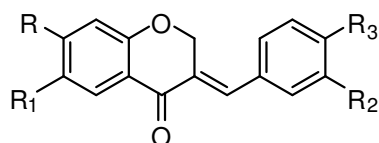
**77d**: R = R₂ = R₃ = OMe, R₁ = H; (3*E*)-2,3-Dihydro-3-[(3,4-dimethoxyphenyl)methylene]-7-methoxy-4*H*-1benzopyran-4-one

**78d**: R = R₁ = R₃ = OMe, R₂ = OH; (3*E*)-2,3-Dihydro-6,7-dimethoxy-3[(3-hydroxy-4-methoxyphenyl)methylene]-4*H*1-benzopyran-4-one

**79d**: R = R₂ = OH, R₁ = H, R₃ = OMe; (3*E*)2,3-Dihydro-7-hydroxy-3-[(3-hydroxy-4-methoxyphenyl)methylene]-4*H*-1-benzopyran-4-one

**80d**: R = OH, R₁ = R₂= H, R₃ = OMe; Bonducellin

**81d**: R = R₂ = R₃ = OH, R₁ = H; Sappanone A

**82d**: R = R₃ = OMe, R₁ = R₂ = H; 7-*O*-Methylbonducellin
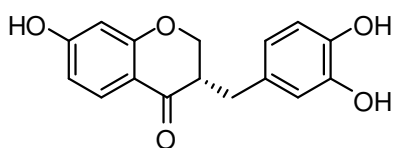
**83d**: 3-Deoxysappanone B
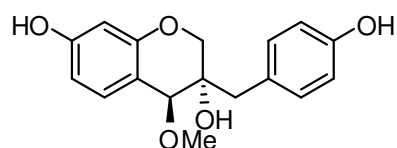
**84d**: 3′-Deoxy-4-*O*-methylepisappanol
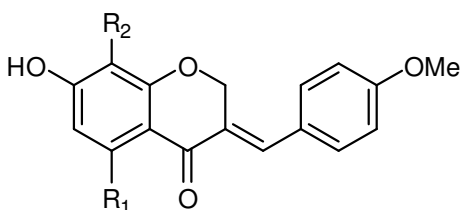
**85d**: R₁ = OH, R₂ = H; Eucomin

**86d**: R₁ = H, R₂ = OH; Intricatinol

**87d**: R₁ = H, R₂ = OMe; 8-Methoxybonducellin
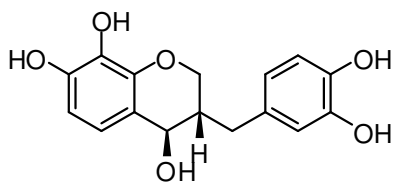
**88d**: Hematoxylol
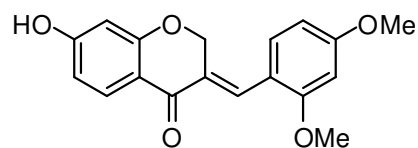
**89d**: 2-Methoxybonducellin
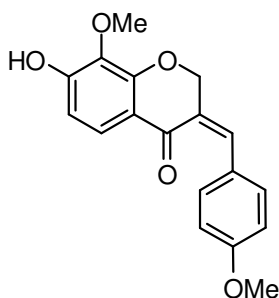
**90d**: 8-Methoxyisobonducellin
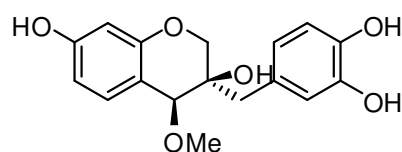
**91d**: 4-*O*-Methylsappanol
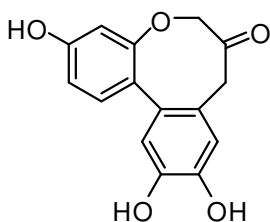
**92d**: Protosappanin A
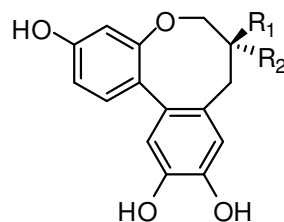
**93d**: R$_1$ = CH$_2$OH, R$_2$ = OH; Protosappanin B

**94d**: R$_1$ = CHO, R$_2$ = OH; Protosappanin C

**95d**: R$_1$ = OH, R$_2$ = CH$_2$OH; Isoprotosappanin B
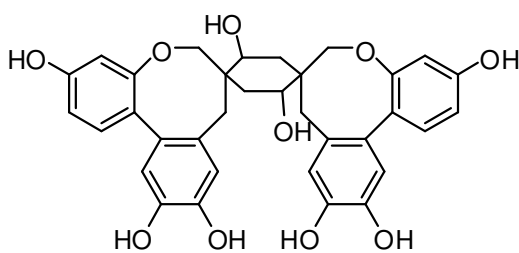
**96d**: Protosappanin D
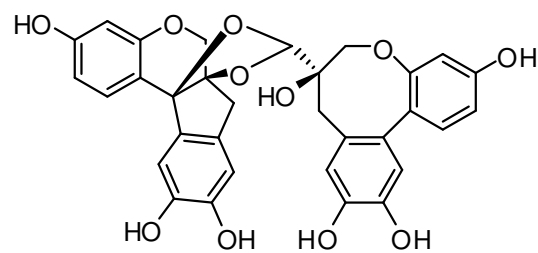
**97d**: Protosappanin E
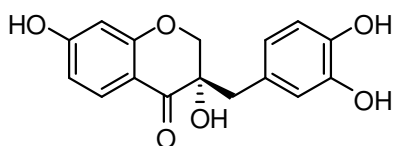
**98d**: Sappanone B
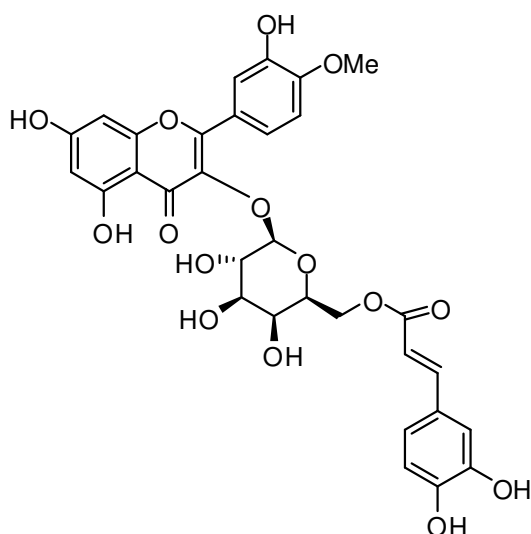
**99d**: Tamarixetin 3-*O*-(6″-*O*-*E*-caffeoyl)-*β*-*D*-alactopyranoside
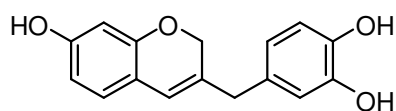
**100d**: 7,3′,4′-Trihydroxy-3-benzyl-2*H*-chromene

**e: Iridoids**
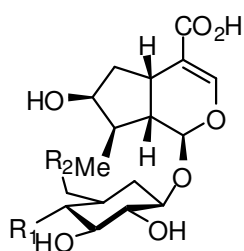
**101e**: R$_1$ = OAc, R$_2$ = OH; 4′-*O*-Acetylloganic acid

**102e**: R$_1$ = OH, R$_2$ = OAc; 6′-*O*-Acetylloganic acid

**f: Quinones**
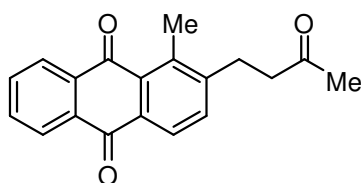
**103f**: Stereochenol A

## g: Phenylpropanoids
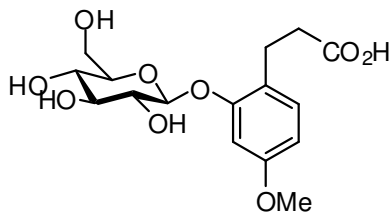
**104g**: 2-*O-β-D*-Glucosyloxy-4-methoxybenzenepropanoic acid
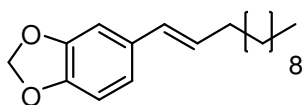
**105g**: Pipataline

## h: Sesquiterpenes
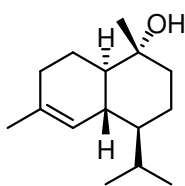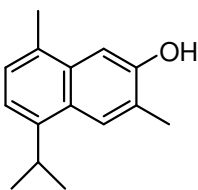
**106h**: *α*-Cadinol          **107h**: 7-Hydroxycadalene          **108h**: Teucladiol
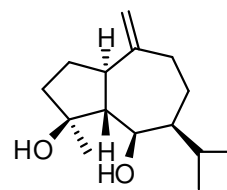
## i: Steroids
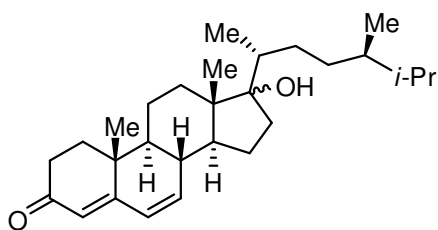
**109i**: 17-Hydroxycampesta-4,6-dien-3-one
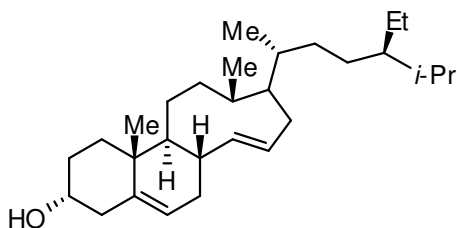
**110i**: 13,14-*seco*-Stigmasta-5,14-dien-3*α*-ol
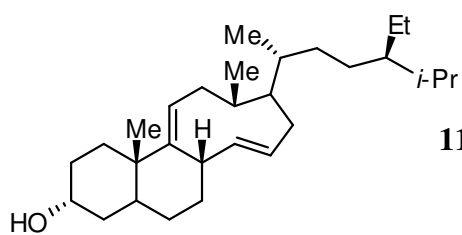
**111i**: 13,14-*seco*-Stigmasta-9(11),14-dien-3$\alpha$-ol

## j: Triterpenes
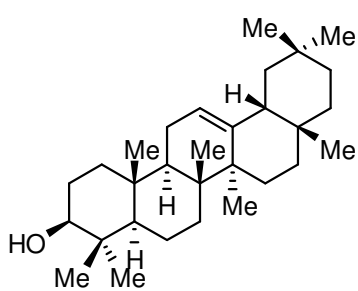
**112j**: β-Amyrin
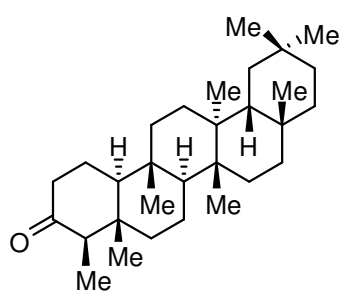
**113j**: Friedelin
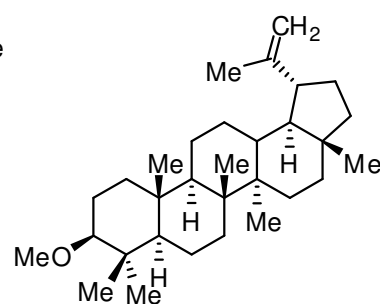
**114j**: Lupeol

**1.6 Objectives of this study**

1. Isolation of chemical constituents from the roots of *Caesalpinia mimosoides* Lamk. and *Caesalpinia pulcherrima* Swartz.
2. Structures elucidation of pure compounds by spectroscopic techniques such as UV, IR, NMR, MS
3. Anti-inflammatory activity evaluation of the isolated pure compounds

# CHAPTER 2
# EXPERIMENTAL

## 2.1 Instruments and chemicals

Melting point was recorded in °C on a Fisher-Johns melting point apparatus. Infrared spectra were recorded using FTS FT-IR spectrophotometer and major bands ($\nu$) were recorded in wave number ($cm^{-1}$). Ultraviolet (UV) absorption spectra were recorded using a SPECORD S 100 (Analytikjena) and UV-160A spectrophotometer (SHIMADZU) and principle bands ($\lambda_{max}$) were recorded as wavelengths (nm) and log $\varepsilon$ in chloroform and methanol solution. Nuclear magnetic resonance spectra were recorded using 300 MHz Bruker FTNMR Ultra Shield$^{TM}$. Spectra were recorded in deuterochloroform, deuteroacetone, deuteromethanol and deuterodimethyl sulphoxide solution and were recorded as $\delta$ value in ppm downfield from TMS (internal standard $\delta$ 0.00). The EI-MS was performed using a MAT 95 XL. Single-crystal X-ray diffraction measurements were collected using SMART 1-K CCD diffractometer with monochromated Mo-K$\alpha$ radiation ($\lambda = 0.71073$ Å) using $\omega$-scan mode and SHELXTL for structure solution and refinement. Optical rotation was measured in chloroform and/or methanol solution with sodium D line (590 nm) on an AUTOPOL$^{R}$ II automatic polarimeter. Solvent for extraction and chromatography were distilled at their boiling point ranges prior to use. Quick column chromatography was performed on silica gel 60 GF$_{254}$ (Merck). Column chromatography was performed on silica gel (Merck) type 100 (0.063 – 0.200).

## 2.2 Plants material

### 2.2.1 The roots of *C. mimosoides* Lamk.

The roots of *C. mimosoides* Lamk. was collected from Khonkaen province, north-eastern part of Thailand in October 2006. Botanical identification was achieved through comparison with a voucher specimen number QBG33200 in the herbarium collection of Queen Sirikit Garden, Mae Rim District, Chiang Mai, Thailand.

### 2.2.2 The roots of *C. pulcherrima* Swartz.

The roots of *C. pulcherrima* (L.) Swartz. was collected from Songkhla province, Thailand in October 2005. Identification was made by Assoc. Prof. Dr. Kitichate Sridith, Department of Biology, Faculty of Science, Prince of Songkla University and a specimen (No. SC51) deposited at Prince of Songkla University Herbarium.

## 2.3 Plants extraction

### 2.3.1 The extraction of the roots of *C. mimosoides*

The air-dried roots (1.7 Kg) of *C. mimosoides* were extracted with dichloromethane and acetone successively (each 2 x 10 L, for 5 days) at room temp. The crude extracts were evaporated under reduced pressure to afford brownish dichloromethane (40.3 g) and acetone extracts (47.1 g), respectively.

Air-dried roots of *C. mimosoides*
(1.7 kg)

Extraction with $CH_2Cl_2$ and Acetone

Crude $CH_2Cl_2$ extract
(40.3 g)

Crude acetone extract
(47.1 g)
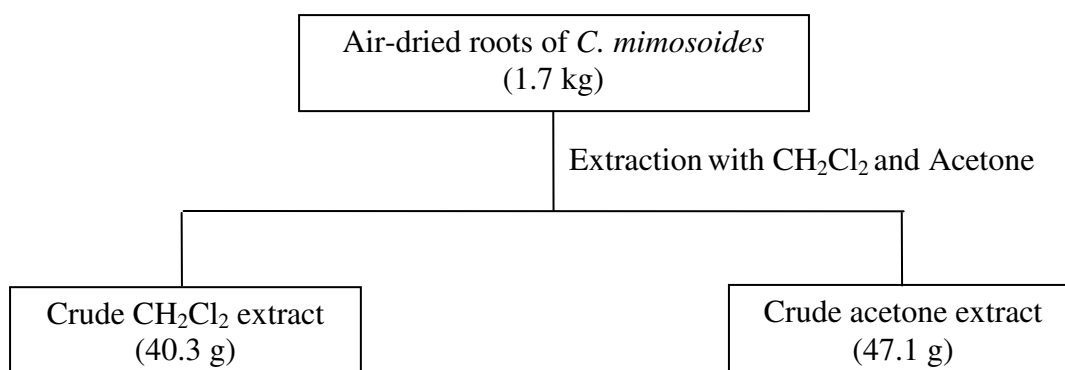
**Scheme 1** Extraction of the roots of *C. mimosoides*

### 2.3.2 The extraction of the roots of *C. pulcherrima*

Air-dried roots (6.3 kg) of *C. pulcherrima* was extracted with $CH_2Cl_2$ (each 2 × 10 L, for 5 days) at room temperature. The crude extract was evaporated under reduced pressure to afford brownish $CH_2Cl_2$ (75.3 g).

```
┌─────────────────────────────────┐
│  Air-dried roots of C. pulcherrima │
│            (6.3 kg)              │
└─────────────────────────────────┘
              │  Extraction with CH₂Cl₂
┌─────────────────────────────────┐
│     Crude CH₂Cl₂ extract         │
│            (75.3 g)             │
└─────────────────────────────────┘
```
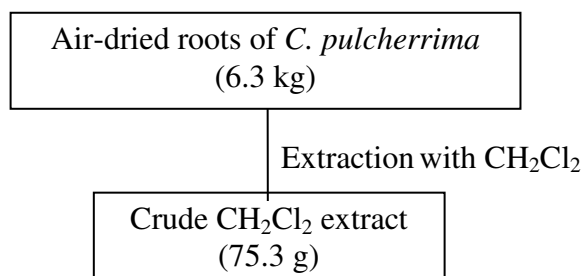
**Scheme 2** Extraction of the roots of *C. pulcherrima*

## 2.4 Isolation and Chemical Investigation

### 2.4.1 Investigation of the crude methylene chloride extract from the roots of *C. mimosoides*
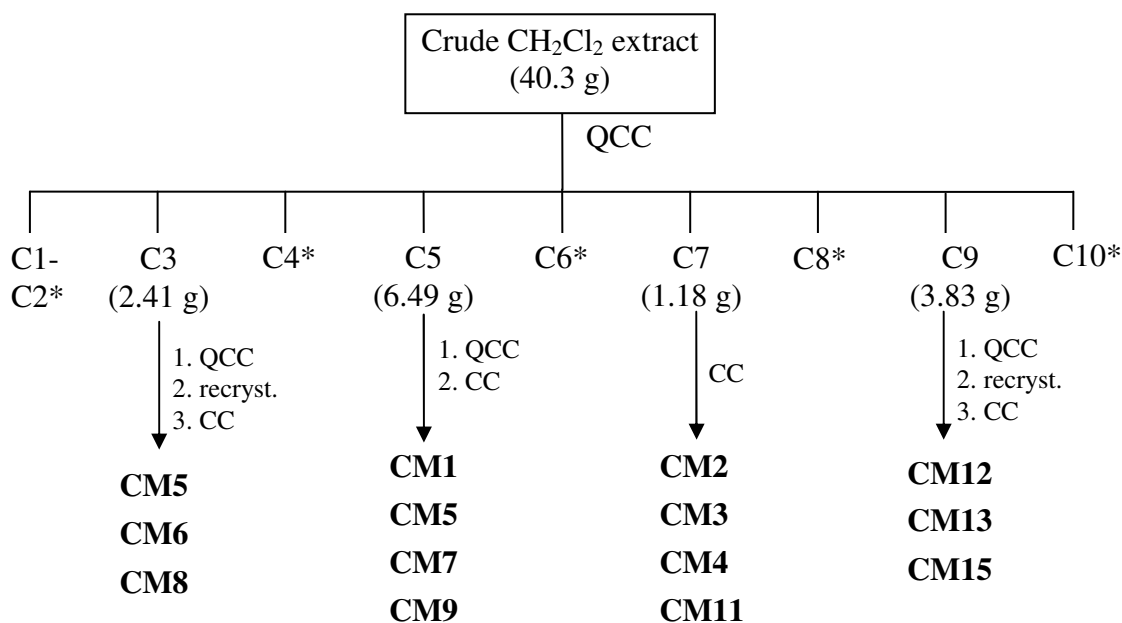


\* Not further investigated

**Scheme 3** Isolation of compounds **CM1-CM9**, **CM11-CM13** and **CM15** from the crude $CH_2Cl_2$ of the roots of *C. mimosoides*

The crude $CH_2Cl_2$ extract was further purified by QCC using hexane as eluent and increasing polarity with $CH_2Cl_2$, acetone and MeOH successively to give ten fractions (C1-C10).

Fraction C3 (2.41 g) was subjected to QCC with EtOAc-hexane (1:19, v/v) to afford five subfractions (C3a-C3e). Subfraction C3b (851.6 mg) was recrystallized from $CH_2Cl_2$ to give **CM6** (680.3 mg) and the mother liquor (160.5 mg) which was further subjected to CC with EtOAc-hexane (1:49, v/v) to give **CM5** (40.6 mg) and **CM8** (7.2 mg). Subfraction C3c (793.7 mg) was separated by CC eluting with EtOAc-hexane (1:19, v/v) to give **CM6** (15.6 mg) and **CM8** (35.2 mg).

Fraction C5 (6.49 g) was subjected to QCC using hexane as eluent and increasing polarity with EtOAc to afford six subfractions (C5a-C5f). Subfraction C5c (793.7 mg) was separated by CC with acetone-hexane (1:49, v/v) to give **CM5** (460.4 mg), **CM9** (10.2 mg) and **CM1** (50.8 mg). Subfraction C5e (742.8 mg) was purified by CC with acetone-hexane (1:19, v/v) to give **CM7** (10.4 mg).

Fraction C7 (1.18 g) was subjected to CC with acetone-hexane (1:9, v/v) to afford six subfractions (C7a-C7f). Subfraction C7c (350.8 mg) was purified by CC with acetone-hexane (3:17, v/v) to give **CM3** (102.4 mg), **CM2** (7.4 mg) and **CM4** (7.0 mg). Subfraction C7e (110.0 mg) was separated by CC with $CH_2Cl_2$ to give **CM11** (10.3 mg).

Fraction C9 (3.83 g) was subjected to QCC using hexane as eluent and increasing polarity with acetone to afford six subfractions (C9a-C9f). Subfraction C9b (384.7 mg) was recrystallized from $CH_2Cl_2$ to give **CM15** (230.3 mg). Subfraction C9d (793.7 mg) was purified by CC with acetone-$CH_2Cl_2$ (1:49, v/v) to give **CM12** (13.3 mg). Subfraction C9e (220.5 mg) was separated by CC with acetone-$CH_2Cl_2$ (1:19, v/v) to give **CM13** (10.3 mg).

**2.4.2 Investigation of the crude acetone extract from the roots of *C. mimosoides***
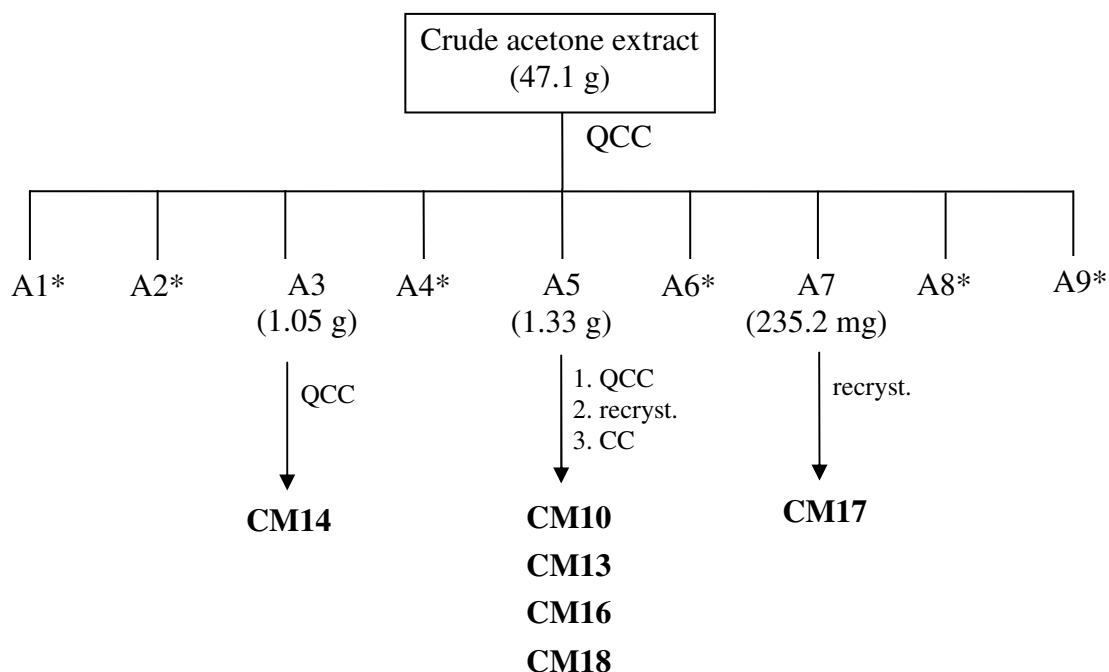


* Not further investigated

**Scheme 4** Isolation of compounds **CM10**, **CM13**, **CM14** and **CM16-CM18** from the crude acetone of the roots of *C. mimosoides*

The crude acetone (47.1 g) extract was fractionated by QCC using hexane as eluent and increasing polarity with $CH_2Cl_2$, acetone and MeOH successively to give nine fractions (A1-A9, Scheme 4).

Fraction A3 (1.05 g) was subjected to QCC with acetone-$CH_2Cl_2$ (1:49, v/v) to give **CM14** (35.3 mg).

Fraction A5 (1.33 g) was separated by QCC with acetone-$CH_2Cl_2$ (1:49, v/v) to afford seven subfractions (A5a-A5g). Subfraction A5c (88.1 mg) was recrystallized from $CH_2Cl_2$ to give **CM13** (15.3 mg). Subfraction A5e (350.0 mg) was purified by CC with MeOH-$CH_2Cl_2$ (1:24, v/v) to afford **CM18** (70.3 mg) and **CM16** (51.3 mg). Subfraction A5f (80.0 mg) was separated by CC with EtOAc-$CH_2Cl_2$ (1:9, v/v) to give **CM10** (10.3 mg).

Fraction A7 (235.2 mg) was recrystallized from MeOH to give **CM17** (150.3 mg).

### *Compound CM1*

White solid; mp 214-216 °C; $[\alpha]_D^{27}$ −41.4° (*c* 0.76, CHCl$_3$); UV (CH$_3$OH) $\lambda_{max}$ (log $\varepsilon$) 210 (4.95), 235 (4.65) nm; IR (neat) $\upsilon_{max}$ 3429 (O-H), 2930 (C-H), 1718 (C=O) cm$^{-1}$; HREIMS: *m/z* 344.1997 [M]$^+$ (calcd for C$_{21}$H$_{28}$O$_4$, 344.1988); $^1$H NMR (acetone-$d_6$, 300 MHz) and $^{13}$C NMR (acetone-$d_6$, 75 MHz), see Table 3.

### *Compound CM2*

White solid; mp 143-145 °C; $[\alpha]_D^{27}$ −40.6° (*c* 0.30, CHCl$_3$); UV (CHCl$_3$) $\lambda_{max}$ (log $\varepsilon$) 225 (3.47) nm; IR (neat) $\upsilon_{max}$ 3397 (O-H), 2928 (C-H) cm$^{-1}$; HREIMS: *m/z* 288.2460 [M-H$_2$O]$^+$ (calcd for C$_{20}$H$_{32}$O$_4$, 288.2453); $^1$H NMR (CDCl$_3$, 300 MHz) and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 4.

### *Compound CM3*

White solid; mp 102-103 °C; $[\alpha]_D^{27}$ −37.1° (*c* 1.10, CH$_3$OH); UV (CHCl$_3$) $\lambda_{max}$ (log $\varepsilon$) 223 (3.45) nm; IR (neat) $\upsilon_{max}$ 3364 (O-H), 2930 (C-H) cm$^{-1}$; HREIMS: *m/z* 306.2545 [M]$^+$ (calcd for C$_{20}$H$_{34}$O$_2$, 306.2559); $^1$H NMR (CDCl$_3$, 300 MHz) and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 5.

### *Compound CM4*

Viscous oil; $[\alpha]_D^{27}$ +24.16° (*c* 1.02, CHCl$_3$); IR (neat) $\upsilon_{max}$ 1735 (C=O), 1243 cm$^{-1}$; HREIMS: *m/z* 390.2780 [M]$^+$ (calcd for C$_{24}$H$_{38}$O$_4$, 390.2770); $^1$H NMR (CDCl$_3$, 300 MHz) and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 6.

### *Compound CM5*

White solid; mp 155-156 °C; $[\alpha]_D^{27}$ +101.9° (*c* 0.77 in CHCl$_3$); UV (CHCl$_3$) $\lambda_{max}$ (log $\varepsilon$): 253 (3.55), 281 (2.90), 292 (2.87) nm; IR (neat) $\upsilon_{max}$: 2930 (C-H), 1720 (C=O) cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 7.

### *Compound CM6*

White solid; mp 116-117 °C; $[\alpha]_D^{27}$ −41.1° (*c* 0.02 in CHCl$_3$); UV (CHCl$_3$) $\lambda_{max}$ (log $\varepsilon$): 254 (4.79), 281 (4.47), 290 (4.40) nm; IR (neat) $\upsilon_{max}$: 2927 (C-H), 1735 (C=O) cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 9.

*Compound CM7*

Viscous oil; $[\alpha]_D^{27}$ –3.90º (*c* 0.58 in CHCl$_3$); UV (CH$_3$OH) $\lambda_{max}$ (log $\varepsilon$): 253 (4.65) nm; IR (neat) $\upsilon_{max}$: 2927 (C-H), 1728 (C=O) cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 11.

*Compound CM8*

White solid; mp 124-126 °C; $[\alpha]_D^{27}$ +25.4º (*c* 0.67 in CHCl$_3$); UV (CH$_3$OH) $\lambda_{max}$ (log $\varepsilon$): 243 (3.22) nm; IR (neat) $\upsilon_{max}$: 3359 (O-H), 2935 (C-H) cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 13.

*Compound CM9*

White solid; mp 141-142 °C; $[\alpha]_D^{27}$ +132.8º (*c* 0.37, CHCl$_3$); UV (CHCl$_3$) $\lambda_{max}$ (log $\varepsilon$) 258 (5.28), 283 (4.93), 293 (4.89) nm; IR (neat) $\upsilon_{max}$ 2929 (C-H), 1720 (C=O) cm$^{-1}$; HREIMS: *m/z* 654.3948 [M]$^+$ (calcd for C$_{42}$H$_{54}$O$_6$, 654.3920); $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Tables 15 and 16.

*Compound CM10*

White solid; mp 215-216 °C; UV (CH$_3$OH) $\lambda_{max}$ (log $\varepsilon$) 207 (5.33), 222 (5.35), 250 (5.12), 309 (5.12), 318 (5.11) nm; IR (neat) $\upsilon_{max}$ 3386 (O-H), 2927 (C-H) cm$^{-1}$; HREIMS: *m/z* 300.1011 [M]$^+$ (calcd for C$_{17}$H$_{16}$O$_5$, 300.1007); $^1$H NMR (acetone-$d_6$, 300 MHz), and $^{13}$C NMR (acetone-$d_6$, 75 MHz), see Table 17.

*Compound CM11*

White solid; mp 152-153 °C; UV (CH$_3$OH) $\lambda_{max}$ (log $\varepsilon$) 209 (5.28), 222 (5.32), 250 (5.11), 258 (5.08), 308 (5.10), 315 (5.07) nm; IR (neat) $\upsilon_{max}$ 3419 (O-H), 2927 (C-H) cm$^{-1}$; HREIMS: *m/z* 314.1118 [M]$^+$ (calcd for C$_{18}$H$_{18}$O$_5$, 314.1154); $^1$H NMR (acetone-$d_6$, 300 MHz), and $^{13}$C NMR (acetone-$d_6$, 75 MHz), see Table 18.

*Compound CM12*

Yellow solid; mp 178-180 °C; UV (CH$_3$OH) $\lambda_{max}$ (log $\varepsilon$): 209 (5.43), 232 (5.24), 317 (5.23), 357 (5.29) nm; IR (neat) $\upsilon_{max}$: 3367 (O-H), 2927 (C-H), 1700 (C=O), 1605 (C=C) cm$^{-1}$; $^1$H NMR (acetone-$d_6$, 300 MHz), and $^{13}$C NMR (acetone-$d_6$, 75 MHz), see Table 19.

### *Compound CM13*

Yellow solid; mp 191-192 °C; UV (CH$_3$OH) $\lambda_{max}$ (log $\varepsilon$): 211 (5.62), 350 (5.58) nm; IR (neat) $\upsilon_{max}$: 3367 (O-H), 2928 (C-H), 1697 (C=O), 1603 (C=C) cm$^{-1}$; $^1$H NMR (acetone-$d_6$, 300 MHz), and $^{13}$C NMR (acetone-$d_6$, 75 MHz), see Table 21.

### *Compound CM14*

Yellow solid; mp 103-105 °C; UV (CH$_3$OH) $\lambda_{max}$ (log $\varepsilon$): 210 (5.40), 327 (5.23), 350 (5.24)  nm; IR (neat) $\upsilon_{max}$: 3376 (O-H), 2928 (C-H), 1699 (C=O), 1598 (C=C) cm$^{-1}$; $^1$H NMR (acetone-$d_6$, 300 MHz), and $^{13}$C NMR (acetone-$d_6$, 75 MHz), see Table 23.

### *Compound CM15*

White solid; mp 85-86 °C; UV (CH$_3$OH) $\lambda_{max}$ (log $\varepsilon$): 205 (5.28), 243 (4.92), 298 (4.98), 330 (5.09)  nm; IR (neat) $\upsilon_{max}$: 3367 (O-H), 2918 (C-H), 1700 (C=O) cm$^{-1}$; $^1$H NMR (acetone-$d_6$, 300 MHz), and $^{13}$C NMR (acetone-$d_6$, 75 MHz), see Table 25.

### *Compound CM16*

White solid; mp 250-251 °C; UV (CH$_3$OH) $\lambda_{max}$ (log $\varepsilon$): 216 (6.17), 305 (6.23), 318 (6.21) nm; IR (neat) $\upsilon_{max}$: 3360 (O-H), 2925 (C-H), 1607 (C=C) cm$^{-1}$; $^1$H NMR (acetone-$d_6$, 300 MHz), and $^{13}$C NMR (acetone-$d_6$, 75 MHz), see Table 27.

### *Compound CM17*

White solid; mp 154-156 °C; $[\alpha]_D^{27}$ –53.1º (*c* 1.71 in CH$_3$OH); UV (CH$_3$OH) $\lambda_{max}$ (log $\varepsilon$): 219 (4.48), 275 (5.02), 311 (4.90)  nm; IR (neat) $\upsilon_{max}$: 3381 (O-H), 2925 (C-H), 1699 (C=O) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 300 MHz), and $^{13}$C NMR (DMSO-$d_6$, 75 MHz), see Table 29.

### *Compound CM18*

White solid; mp 99-100 °C; $[\alpha]_D^{27}$ +45.6º (*c* 0.24 in CH$_3$OH); IR (neat) $\upsilon_{max}$: 3365 (O-H), 2934 (C-H) cm$^{-1}$; $^1$H NMR (acetone-$d_6$, 300 MHz), and $^{13}$C NMR (acetone-$d_6$, 75 MHz), see Table 31.

**2.4.3 Investigation of the crude methylene chloride extract from the roots of *C. pulcherrima***
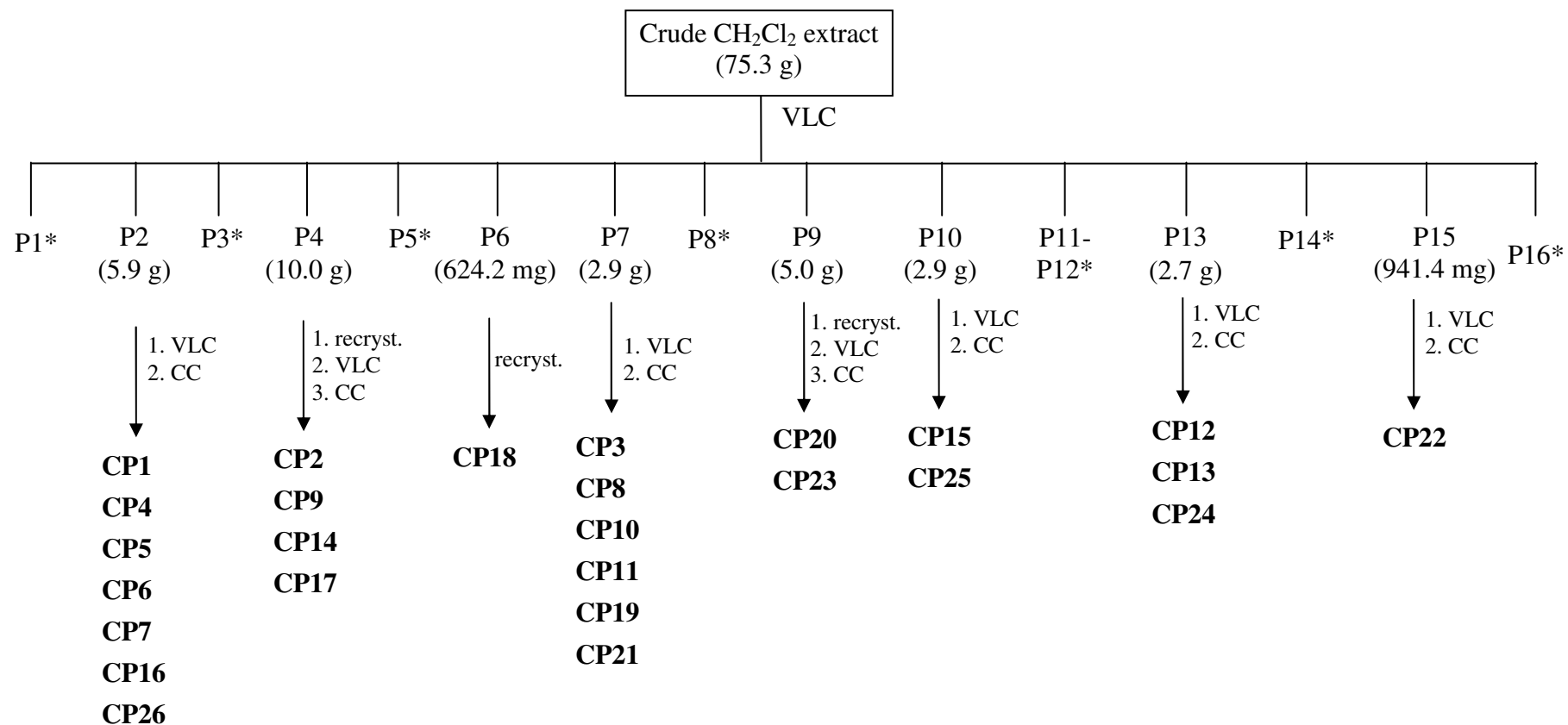


**Scheme 5.** Isolation of compounds **CP1–CP26** from *C. pulcherrima*

The crude $CH_2Cl_2$ extract was further purified by VLC using hexane as eluent and increasing polarity with EtOAc and MeOH to give sixteen fractions (P1–P16).

Fraction P2 (5.9 g) was further purified by VLC with hexane–$CH_2Cl_2$ (1:4, v/v) to give **CP26** (90.5 mg), **CP16** (50.2 mg), **CP6** (275.2 mg), **CP7** (90.0 mg) and **CP5** (28.1 mg) and a mixture of two compounds (158.0 mg) which was further separated by CC with acetone–hexane (1:9, v/v) to give **CP4** (15.0 mg) and **CP1** (10.3 mg).

Fraction P4 (10.0 g) was recrystallized from $CH_2Cl_2$ to give **CP17** (2.54 g), and the mother liquor (7.5 g) was further subjected to VLC with hexane as eluent and increasing polarity with $CH_2Cl_2$ and EtOAc to afford six subfractions (P4a–P4f). Subfraction P4c (183.4 mg) was purified by CC with acetone–hexane (1:9, v/v) to give **CP2** (5.8 mg). Subfraction P4d (584.9 mg) was separated by CC with $CH_2Cl_2$–hexane (7:3, v/v) to yield **CP14** (5.2 mg) and **CP9** (10.0 mg).

Repeated recrystallization from $CH_2Cl_2$ of fraction P6 (624.2 mg) yielded **CP18** (138.0 mg).

Fraction P7 (2.9 g) was separated by VLC with hexane as eluent and increasing polarity with $CH_2Cl_2$ and EtOAc to give nine subfractions (P7a–P7i) and **CP21** (355.0 mg). Each subfraction was further separated by CC with acetone–hexane (1:4, v/v) to afford **CP19** (50.0 mg) from subfraction P7b (80.0 mg), **CP8** (18.2 mg) and **CP11** (25.0 mg) from subfraction P7e (401.4 mg), **CP3** (3.0 mg) from subfraction P7f (273.5 mg) and finally **CP10** (25.0 mg) from subfraction P7g (117.0 mg).

Fraction P9 (5.0 g) was recrystallized from $CH_2Cl_2$ to give **CP23** (1.24 g), with the mother liquor (3.8 g) further subjected to VLC with EtOAc–$CH_2Cl_2$ (3:7, v/v) to afford five subfractions (P9a–P9e). Subfraction P9c (256.4 mg) was purified by CC with acetone–$CH_2Cl_2$ (3:7, v/v) to yield **CP20** (70.0 mg).

Fraction P10 (2.9 g) was further purified by VLC and eluted with a gradient of $CH_2Cl_2$–EtOAc (1:4 to 1:1, v/v) to give seven subfractions (P10a–P10g). Purification of subfraction P10c (283.2 mg) by CC with acetone–$CH_2Cl_2$ (2:3, v/v) afforded **CP15** (7.0 mg) while **CP25** (13.0 mg) was purified from subfraction P10f (124.7 mg) by CC with $CH_2Cl_2$–acetone (1:9, v/v).

Fraction P13 (2.7 g) was further purified by VLC with acetone–$CH_2Cl_2$ (1:4, v/v) to give six subfractions (P13a–P13f). Subfraction P13c (151.7 mg) was separated by CC with EtOAc–hexane (2:3, v/v) to yield **CP13** (10.0 mg) and **CP24** (90.0 mg). Subfraction P13e (197.8 mg) was isolated by CC with EtOAc–$CH_2Cl_2$ (1:9, v/v) to give **CP12** (10.0 mg).

Finally, **CP22** (12.5 mg) was isolated from fraction P15 (941.4 mg) by VLC ($CH_2Cl_2$ to MeOH–$CH_2Cl_2$, 3:7, v/v) and followed by CC with EtOAc–$CH_2Cl_2$ (1:19, v/v).

### *Compound CP1*

Viscous oil; $[\alpha]_D^{25}$ +23.7 (*c* 0.27, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 216 (3.88) nm; IR (neat) $\upsilon_{max}$ 3453 (O–H), 2931 (C–H), 1723 (C=O) cm$^{-1}$; HREIMS: *m/z* 360.2301 [M]$^+$ (calcd for $C_{22}H_{32}O_4$, 360.2301); $^1$H NMR ($CDCl_3$, 300 MHz), and $^{13}$C NMR ($CDCl_3$, 75 MHz), see Table 35.

### *Compound CP2*

Viscous oil; $[\alpha]_D^{25}$ +36.1 (*c* 0.20, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 214 (3.84) nm; IR (neat) $\upsilon_{max}$ 3455 (O–H), 2920 (C–H), 1723 (C=O) cm$^{-1}$; HREIMS: *m/z* 376.2250 [M]$^+$ (calcd for $C_{22}H_{32}O_5$, 376.2250); $^1$H NMR ($CDCl_3$, 300 MHz), and $^{13}$C NMR ($CDCl_3$, 75 MHz), see Table 36.

### *Compound CP3*

Viscous oil; $[\alpha]_D^{25}$ +67.4 (*c* 0.08, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 215 (3.78) nm; IR (neat) $\upsilon_{max}$ 3425 (O–H), 2929 (C–H), 1734 (C=O) cm$^{-1}$; HREIMS: *m/z* 376.2252 [M]$^+$ (calcd for $C_{22}H_{32}O_5$, 376.2250); $^1$H NMR ($CDCl_3$, 300 MHz), and $^{13}$C NMR ($CDCl_3$, 75 MHz), see Table 37.

### *Compound CP4*

White solid; mp 126–128 °C; $[\alpha]_D^{25}$ +57.1 (*c* 0.18, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 225 (3.26) nm; IR (neat) $\upsilon_{max}$ 3494 (O–H), 2932 (C–H), 1710 (C=O) cm$^{-1}$; HREIMS: *m/z* 438.2410 [M]$^+$ (calcd for $C_{27}H_{34}O_5$, 438.2406); $^1$H NMR ($CDCl_3$, 300 MHz), and $^{13}$C NMR ($CDCl_3$, 75 MHz), see Table 38. The physical and spectral data of **CP4** from the synthesis (Roach et al., 2003): white solid; mp 125–127 °C; $[\alpha]_D^{25}$ +23.7 (c 0.27, $CHCl_3$).

*Compound CP5*

White solid; mp 195–196 °C; $[\alpha]_D^{25}$ +106.5 (*c* 0.25, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 228 (3.96) nm; IR (neat) $v_{max}$ 3525 (O–H), 2929 (C–H), 1700 (C=O) cm$^{-1}$; HREIMS: *m/z* 422.2454 [M]$^+$ (calcd for C$_{27}$H$_{34}$O$_4$, 422.2457); $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 39.

*Compound CP6*

White solid; mp 131–133 °C; $[\alpha]_D^{25}$ +28.8 (*c* 0.18, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 226 (3.87) nm; IR (neat) $v_{max}$ 3549 (O–H), 2934 (C–H), 1708 (C=O) cm$^{-1}$; HREIMS: *m/z* 422.2459 [M]$^+$ (calcd for C$_{27}$H$_{34}$O$_4$, 422.2457); $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 40.

*Compound CP7*

White solid; mp 135–136 °C; $[\alpha]_D^{25}$ +52.8 (*c* 0.17, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 216 (3.65), 275 (3.67) nm; IR (neat) $v_{max}$ 3516 (O–H), 2933 (C–H), 1709 (C=O) cm$^{-1}$; HREIMS: *m/z* 448.2617 [M]$^+$ (calcd for C$_{29}$H$_{36}$O$_4$, 448.2614); $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 41.

*Compound CP8*

Viscous oil; $[\alpha]_D^{25}$ +20.3 (*c* 0.21, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 225 (3.94) nm; IR (neat) $v_{max}$ 3471 (O–H), 2935 (C–H), 1710 (C=O) cm$^{-1}$; HREIMS: *m/z* 452.2198 [M]$^+$ (calcd for C$_{27}$H$_{32}$O$_6$, 452.2199); $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 42.

*Compound CP9*

Viscous oil; $[\alpha]_D^{25}$ +64.1 (*c* 0.07, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 227 (3.82) nm; IR (neat) $v_{max}$ 3471 (O–H), 2927 (C–H), 1720 (C=O) cm$^{-1}$; HREIMS: *m/z* 438.2405 [M]$^+$ (calcd for C$_{27}$H$_{34}$O$_5$, 438.2406); $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 43.

*Compound CP10*

Viscous oil; $[\alpha]_D^{25}$ +19.7 (*c* 0.20, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 225 (3.90) nm; IR (neat) $v_{max}$ 3508 (O–H), 2931 (C–H), 1707 (C=O) cm$^{-1}$; HREIMS: *m/z* 452.2196 [M]$^+$ (calcd for C$_{27}$H$_{32}$O$_6$, 452.2199); $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 44.

### *Compound CP11*

Viscous oil; $[\alpha]_D^{25}$ +23.6 (*c* 0.14, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 226 (3.96) nm; IR (neat) $\upsilon_{max}$ 3508 (O–H), 2934 (C–H), 1704 (C=O) cm$^{-1}$; HREIMS: *m/z* 572.2411 [M]$^+$ (calcd for C$_{34}$H$_{36}$O$_8$, 572.2410); $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 45.

### *Compound CP12*

Viscous oil; $[\alpha]_D^{25}$ +26.7 (*c* 0.30, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 228 (3.98) nm; IR (neat) $\upsilon_{max}$ 3470 (O–H), 2930 (C–H), 1720 (C=O) cm$^{-1}$; HREIMS: *m/z* 540.2361 [M]$^+$ (calcd for C$_{30}$H$_{38}$O$_9$, 540.2359); $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 46.

### *Compound CP13*

Viscous oil; $[\alpha]_D^{25}$ +54.7 (*c* 0.18, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 227 (3.89) nm; IR (neat) $\upsilon_{max}$ 3468 (O–H), 2927 (C–H), 1716 (C=O) cm$^{-1}$; HREIMS: *m/z* 598.2423 [M]$^+$ (calcd for C$_{32}$H$_{38}$O$_{11}$, 598.2414); $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 47.

### *Compound CP14*

Viscous oil; $[\alpha]_D^{25}$ +48.8 (*c* 0.17, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 226 (3.92) nm; IR (neat) $\upsilon_{max}$ 3436 (O–H), 2930 (C–H), 1713 (C=O) cm$^{-1}$; HREIMS: *m/z* 436.2250 [M]$^+$ (calcd for C$_{27}$H$_{32}$O$_5$, 436.2250); $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 48.

### *Compound CP15*

Viscous oil; $[\alpha]_D^{25}$ +83.8 (*c* 0.20, CHCl$_3$); IR (neat) $\upsilon_{max}$ 3372 (O–H), 2926 (C–H), 1688 (C=O) cm$^{-1}$; HREIMS: *m/z* 304.2405 [M]$^+$ (calcd for C$_{20}$H$_{32}$O$_2$, 304.2402); $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 49.

### *Compound CP16*

White solid; mp 98-100 ºC; $[\alpha]_D^{25}$ +80.9º (*c* 0.26, CHCl$_3$); (CHCl$_3$) $\lambda_{max}$ (log $\varepsilon$) 225 (3.47) nm; IR (neat) $\upsilon_{max}$ 3574 (O-H), 2931 (C-H) cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 50.

*Compound CP17*

White solid; mp 116-118 °C; $[\alpha]_D^{25}$ +60.0º (*c* 0.28, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 227 (3.37) nm; IR (neat) $\upsilon_{max}$ 3515 (O-H), 2936 (C-H), 1708 (C=O) cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 52.

*Compound CP18*

White solid; mp 220-222 °C; $[\alpha]_D^{25}$ +59.9º (*c* 0.13, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 217 (3.63), 273 (3.45) nm; IR (neat) $\upsilon_{max}$ 3458 (O-H), 2932 (C-H), 1704 (C=O) cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 54.

*Compound CP19*

Viscous oil; $[\alpha]_D^{25}$ +41.5º (*c* 0.08, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 216 (3.66), 276 (3.53) nm; IR (neat) $\upsilon_{max}$ 3493 (O-H), 2931 (C-H), 1712 (C=O) cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 56.

*Compound CP20*

White solid; mp 161-163 °C; $[\alpha]_D^{25}$ +71.5º (*c* 0.21, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 225 (3.52) nm; IR (neat) $\upsilon_{max}$ 3470 (O-H), 2936 (C-H), 1713 (C=O) cm$^{-1}$; $^1$H NMR (acetone-$d_6$, 300 MHz), and $^{13}$C NMR (acetone-$d_6$, 75 MHz), see Table 58.

*Compound CP21*

White solid; mp 140-142 °C; $[\alpha]_D^{25}$ +72.2º (*c* 1.84, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 227 (3.45) nm; IR (neat) $\upsilon_{max}$ 3486 (O-H), 2935 (C-H), 1714 (C=O) cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 60.

*Compound CP22*

White solid; mp 193-195 °C; $[\alpha]_D^{25}$ +78.1º (*c* 0.03, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 228 (3.44) nm; IR (neat) $\upsilon_{max}$ 3483 (O-H), 2932 (C-H), 1711 (C=O) cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 62.

*Compound CP23*

White solid; mp 220-221 °C; $[\alpha]_D^{25}$ +58.5º (*c* 0.11, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 224 (3.43) nm; IR (neat) $\upsilon_{max}$ 3454 (O-H), 2957 (C-H), 1725 (C=O) cm$^{-1}$; $^1$H NMR (acetone-$d_6$, 300 MHz), and $^{13}$C NMR (acetone-$d_6$, 75 MHz), see Table 64.

*Compound CP24*

Viscous oil; $[\alpha]_D^{25}$ +73.9° (*c* 0.07, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 228 (3.49) nm; IR (neat) $\upsilon_{max}$ 3534 (O-H), 2932 (C-H), 1721 (C=O) cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 66.

*Compound CP25*

Viscous oil; $[\alpha]_D^{25}$ +177.1° (*c* 0.11, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 229 (3.57) nm; IR (neat) $\upsilon_{max}$ 3456 (O-H), 2931 (C-H), 1728 (C=O) cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 68.

*Compound CP26*

Viscous oil; $[\alpha]_D^{25}$ +60.5° (*c* 0.18, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 218 (3.46), 283 (3.37), 289 (3.30) nm; IR (neat) $\upsilon_{max}$ 3402 (O-H), 2918 (C-H) cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz), and $^{13}$C NMR (CDCl$_3$, 75 MHz), see Table 70.

**2.5  Bioassay**

**2.5.1 Anti-inflammatory activity assay**

**2.5.1.1 Inhibitory effects of compounds on LPS-induced NO production from RAW264.7 cells**

Inhibitory effects on NO production by murine macrophage-like RAW264.7 cells were evaluated using a modified method from that previously reported (Banskota et al., 2003).  Briefly, the RAW264.7 cell line [purchased from Cell Lines Service (CLS)] was cultured in Rosewell Park Memorial Institute (RPMI) medium supplemented with 0.1% sodium bicarbonate and 2 mM glutamine, penicillin G (100 units/ml), streptomycin (100 µg/ml) and 10% fetal calf serum (FCS).  The cells were harvested with trypsin-ethylenediaminetetraacetic acid (EDTA) and diluted to a suspension in a fresh medium.  The cells were seeded in 96-well plates with 1 x $10^5$ cells/well and allowed to adhere for 1 h at 37°C in a humidified atmosphere containing 5% $CO_2$.  After that the medium was replaced with a fresh medium containing 200 µg/ml of LPS together with the test samples at various concentrations and was then incubated for 48 h.  NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent.  Cytotoxicity was determined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) colorimetric method.  Briefly, after 48 h incubation with the test samples, MTT solution (10 µl, 5 mg/ml in phosphate buffer saline (PBS)) was added to the wells.  After 4 h incubation, the medium was removed, and isopropanol containing 0.04 M HCl was then added to dissolve the formazan production in the cells.  The optical density of the formazan solution was measured with a microplate reader at 570 nm.   The test compounds were considered to be cytotoxic when the optical density of the sample-treated group was less than 80% of that in the control (vehicle-treated) group.  L-NA and caffeic acid phenethylester (CAPE) were used as positive controls. The stock solution of each test sample was dissolved in DMSO, and the solution was added to the medium RPMI (final DMSO is 1%).  Inhibition (%) was calculated using the following equation and $IC_{50}$ values were determined graphically (n = 4):

$$\text{Inhibition } (\%) = \frac{A - B}{A - C} \times 100$$

*A-C* : NO$_2^-$ concentration (µM) [*A* : LPS (+), sample (-); *B* : LPS (+), sample(+); *C* : LPS (-), sample (-)].

**2.5.1.2 Inhibitory effects of compounds on LPS-induced TNF-α release from RAW264.7 cells**

Briefly, the RAW264.7 cell line was cultured in RPMI medium supplemented with 0.1% sodium bicarbonate and 2 mM glutamine, penicillin G (100 units/ml), streptomycin (100 µg/ml) and 10% FCS. The cells were harvested with trypsin-EDTA and diluted to a suspension in a fresh medium. The cells were seeded in 96-well plates with 1.0 x 10$^5$ cells/well and allowed to adhere for 1 h at 37°C in a humidified atmosphere containing 5% CO$_2$. After that the medium was replaced with a fresh medium containing 200 µg/ml of LPS together with the test samples at various concentrations and was then incubated for 48 h. The supernatant was transferred into 96 well ELISA plate and then TNF-α concentrations were determined using commercial ELISA kit. The test samples were dissolved in DMSO, and the solution was added to RPMI. The inhibition on TNF-α production was calculated and IC$_{50}$ values were determined graphically.

**2.5.1.3 Statistical analysis**

The results were expressed as mean ± standard error means (S.E.M) of four determinations at each concentration for each sample. The IC$_{50}$ values were calculated using the Microsoft Excel program. Statistical significance was calculated by one-way analysis of variance (ANOVA), followed by Dunnett's test.

# CHAPTER 3
# RESULTS AND DISCUSSION

## 3.1 Structural elucidation of compounds from the roots of *C. mimosoides*

The air-dried roots of *C. mimosoides* were extracted with $CH_2Cl_2$ and acetone successively. The crude $CH_2Cl_2$ and acetone extracts showed potent NO inhibitory activity with $IC_{50}$ values of 11.0 and 21.6 μg/ml, respectively. Further separation and purification led to the isolation of seven new compounds of four new diterpenes, named mimosol A-D (**CM1-CM4**), a dimer, named mimosol E (**CM9**) and two dibenzo[b,d]furans, named mimosol F, G (**CM10, CM11**). The known compounds were identified by analysis of their spectroscopic data and comparison with literature data to be taepeenin A (**CM5**), taepeenin D (**CM6**), nortaepeenin A (**CM7**) (Cheenpracha et al., 2005), taepeenin L (**CM8**) (Cheenpracha et al., 2006), (*E*)-7-hydroxy-3-(4-methoxybenzyl)chroman-4-one (**CM12**), (*E*)-7,8-dihydroxy-3-(4-methoxybenzyl)chroman-4-one (**CM13**), (*E*)-7-hydroxy-8-methoxy-3-(4-methoxyben zyl)chroman-4-one (**CM14**) (Chen and Yang, 2007), tetracosyl caffeate (**CM15**) (Tanaka et al., 1998), resveratorol (**CM16**) (Miyaichi et al., 2006), bergenin (**CM17**) (Wang et al., 2005) and (+)-pterocarpol (**CM18**) (Nasini and Piozzi, 1981). Their structures were determined using 1D and 2D NMR spectroscopic data. All carbons were assigned by ^{13}C NMR, HMQC and HMBC data.

### 3.1.1 Compound CM1
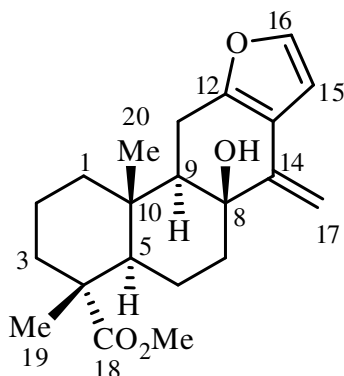


Compound **CM1** was obtained as a white solid and had the molecular formula $C_{21}H_{28}O_4$ as determined by HREIMS. The IR spectrum exhibited absorptions for hydroxyl (3429 cm$^{-1}$) and carbonyl ester (1718 cm$^{-1}$) functional groups. The UV spectrum had absorption bands at $\lambda_{max}$ 210 and 235 nm. In addition, compound **CM1** gave a red-pink colour on Ehrlich test indicating a furan chromophore (Kuroda et al., 2004). The $^1$H and $^{13}$C NMR (Table 3, Figures 5 and 6) spectroscopic data of **CM1** showed characteristic of a 2,3-furanocassane framework (Cheenpracha et al., 2005, 2006; McPherson et al., 1986; Patil et al., 1997; Pranithanchai et al., 2009; Ragasa et al., 2002; Roach et al., 2003; Yodsaoue et al., 2008). The presence of a 2,3-disubstituted furan ring was deduced from the resonances at $\delta_H$ 6.53 and 7.35 (each d, $J$ = 2.1 Hz) and $\delta_C$ 106.9 (C-15), 118.8 (C-13), 141.3 (C-16) and 151.7 (C-12). The $^{13}$C NMR spectroscopic data displayed 21 carbons including those of an oxyquaternary carbon at $\delta$ 70.9 (C-8), an exocyclic double bond at $\delta$ 103.0 (C-17) and 142.5 (C-14), and an ester carbonyl carbon at $\delta$ 178.3 (C-18). The $^1$H NMR spectroscopic data displayed peaks for two tertiary methyl groups at $\delta$ 0.62 (Me-20) and 1.10 (Me-19), and a OMe at $\delta$ 3.65 (OMe-18). The signals of terminal olefinic methylene protons at $\delta$ 5.11 and 5.14 (each s, 2H-17) whose HMBC correlations with the carbons at $\delta$ 70.9 (C-8), 118.8 (C-13), 142.5 (C-14) and 151.7 (C-12) together with that of the methine proton at $\delta$ 1.90 (d, $J$ = 7.5 Hz, H-9) with the carbons at $\delta$ 13.8 (C-20), 19.1 (C-11), 37.5 (C-7), 38.0 (C-10), 70.9 (C-8), 142.5 (C-14) and 151.7 (C-12), suggested the location of the exocyclic double bond at C-14 and OH at C-8, respectively. In addition, the methyl protons at $\delta$ 0.62 (Me-20) showed NOESY cross-peaks with the methyl protons at $\delta$ 1.10 (Me-19) and 2.96 (H$_{ax}$-11) which was in

agreement with the trans/anti/trans ring junction (A/B/C) of a cassane framework, suggesting a $\beta$-orientation of OH-8. Therefore, **CM1** was determined to be 8$\beta$-hydroxy-14(17)-ene-18$\alpha$-methoxycarbonyl-18-norvouacapene, a new compound (Yodsaoue et al., 2010) and was named as mimosol A.
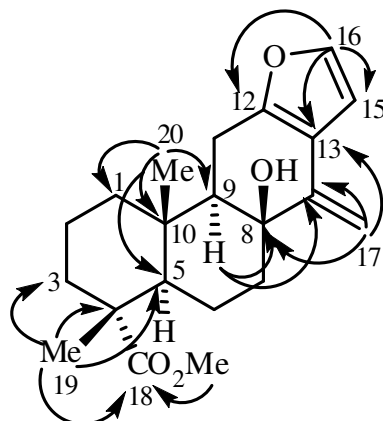


Selective HMBC correlations of **CM1**

**Table 3** ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **CM1**

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|----------|------------------------------|------------|------|------|
| 1 | 1.05 (m) | 39.0 | CH$_2$ | 20 |
|   | 1.82 (m) |  |  |  |
| 2 | 1.14 (m) | 17.6 | CH$_2$ | 3, 4 |
|   | 1.48 (m) |  |  |  |
| 3 | 1.50 (m) | 36.5 | CH$_2$ | 5, 19 |
|   | 1.75 (m) |  |  |  |
| 4 | - | 47.3 | C | - |
| 5 | 1.93 (dd, *J* = 12.3, 2.7) | 51.2 | CH | 4, 10, 19, 20 |
| 6 | 1.55 (m) | 22.8 | CH$_2$ | 8 |
|   | 1.60 (m) |  |  |  |
| 7 | 1.62 (dt, *J* = 12.9, 3.6) | 37.5 | CH$_2$ | 8, 14 |
|   | 2.57 (td, *J* = 12.9, 3.0) |  |  |  |
| 8 | - | 70.9 | C | - |

**Table 3** (continued)

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 9 | 1.90 (br d, *J* = 7.5) | 56.4 | CH | 7, 8, 10, 11, 12, 14, 20 |
| 10 | - | 38.0 | C | - |
| 11 | 2.69 (br d, *J* = 17.7) | 19.1 | $CH_2$ | 8, 9, 10, 12, 13 |
| | 2.96 (dd, *J* = 17.7, 7.5) | | | |
| 12 | - | 151.7 | C | - |
| 13 | - | 118.8 | C | - |
| 14 | - | 142.5 | C | - |
| 15 | 6.53 (d, *J* = 2.1) | 106.9 | CH | 12, 13 |
| 16 | 7.35 (d, *J* = 2.1) | 141.3 | CH | 12, 13, 15 |
| 17 | 5.11 (br s) | 103.0 | $CH_2$ | 8, 12, 13, 14 |
| | 5.14 (br s) | | | |
| 18 | - | 178.3 | C | - |
| 19 | 1.10 (s) | 16.2 | $CH_3$ | 3, 4, 5, 18 |
| 20 | 0.62 (s) | 13.8 | $CH_3$ | 1, 5, 9, 10 |
| 18-OMe | 3.65 (s) | 51.2 | $CH_3$ | 18 |

### 3.1.2 Compound CM2
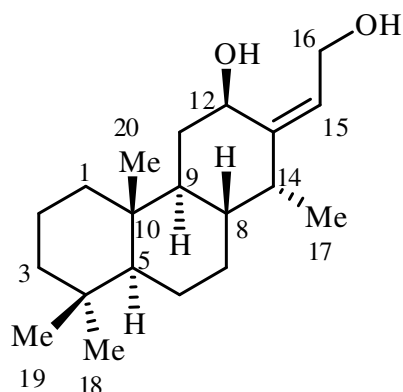


Compound **CM2** was assigned a molecular formula $C_{20}H_{32}O$ $[M-H_2O]^+$ on the basis of a molecular ion at *m/z* 288.2460 by HREIMS. Its IR spectrum displayed a hydroxyl stretching at 3397 $cm^{-1}$. The $^1H$ NMR spectral data (Table 4, Figure 14) showed the signals of three tertiary methyl groups at $\delta_H$ 0.83 (Me-19), 0.83 (Me-20) and 0.86 (Me-18), and a Me doublet at $\delta_H$ 0.95 (d, *J* = 7.2 Hz, Me-17). An olefinic proton at $\delta$ 5.54 (t, *J* = 6.6 Hz, H-15) and oxymethylene protons at $\delta$ 4.21 (d, *J* = 6.6 Hz, 2H-16) connecting to a carbon at $\delta_C$ 58.5 were displayed. An oxymethine proton at $\delta_H$ 4.43 (dd, *J* = 10.2, 4.8 Hz, H-12: $\delta_C$ 70.7) displayed *J* values consistent with axial orientation. The $^{13}C$ NMR spectrum (Table 4, Figure 15) and DEPT experiments displayed 20 carbons, two of these were $sp^2$ carbons at $\delta$ 118.8 and 152.0. From the HMBC experiments, the Me-17 at $\delta$ 0.95 showed long-range correlations to the carbons at $\delta$ 40.4 (C-8), 45.8 (C-14) and 152.0 (C-13). An olefinic proton at $\delta$ 5.54 (H-15) also showed long-range correlations to the carbons at $\delta$ 45.8 (C-14), 70.7 (C-12) and 152.0 (C-13). An oxymethine proton at $\delta$ 4.43 (H-12) gave cross-peaks with the carbons at $\delta$ 37.2 (C-11), 58.5 (C-16), 118.8 (C-15) and 152.0 (C-13) and oxymethylene protons at $\delta$ 4.21 (2H-16) with the carbons at $\delta$ 118.8 (C-15) and 152.0 (C-13). These results suggested that a hydroxyl group was located at C-12 and the =CHCH_2OH substitutent was at C-13. From the NOESY cross-peaks, the oxymethine proton at $\delta$ 4.43 (H-12) showed a cross-peak with the methyl protons at $\delta$ 0.95 (Me-17) confirming of their axial orientations. An olefinic proton at $\delta$ 5.54 (H-15) displayed a cross-peak with a methine proton at $\delta$ 2.27 (H-14), thus indicating *Z*-configuration of the double bond. Therefore, **CM2** was elucidated as 12$\beta$,16-

dihydroxycass-13(15)(*Z*)-ene, a new compound (Yodsaoue et al., 2010) and was named as mimosol B.
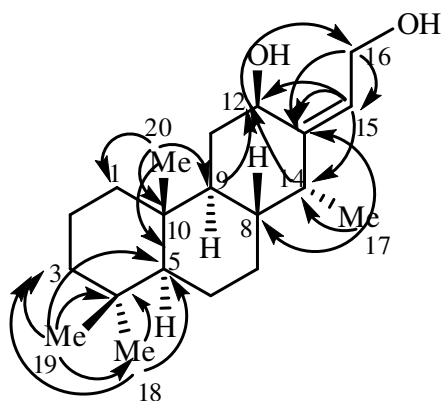


Selective HMBC correlations of **CM2**

**Table 4** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CM2**

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 0.93 (m) | 39.6 | CH$_2$ | 2, 3, 20 |
| | 1.70 (br d, *J* = 12.6) | | | |
| 2 | 1.38 (m) | 18.9 | CH$_2$ | 3, 4, 10 |
| | 1.56 (m) | | | |
| 3 | 1.14 (dt, *J* = 12.9, 3.9) | 42.1 | CH$_2$ | 2, 5, 19 |
| | 1.43 (m) | | | |
| 4 | - | 33.2 | C | - |
| 5 | 0.80 (m) | 55.1 | CH | 1, 3, 9, 10, 18, 19, 20 |
| 6 | 1.27 (m) | 21.6 | CH$_2$ | 7 |
| | 1.58 (m) | | | |
| 7 | 1.20 (m) | 31.0 | CH$_2$ | 6, 7 |
| | 1.54 (m) | | | |
| 8 | 1.53 (m) | 40.4 | CH | 6 |
| 9 | 1.20 (m) | 47.5 | CH | 6, 10, 11, 12 |
| 10 | - | 36.8 | C | - |

**Table 4**  (continued)

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 11 | 1.23 (m) | 37.2 | $CH_2$ | 8, 9, 10, 12, 13 |
|  | 1.97 (ddd, *J* = 15.6, 8.1, 4.8) |  |  |  |
| 12 | 4.43 (dd, *J* = 10.2, 4.8) | 70.7 | CH | 11, 13, 15, 16 |
| 13 | - | 152.0 | C | - |
| 14 | 2.27 (dq, *J* = 7.2, 4.5) | 45.8 | CH | 8, 9, 12, 13, 15, 17 |
| 15 | 5.54 (t, *J* = 6.6) | 118.8 | CH | 12, 13, 14 |
| 16 | 4.21 (d, *J* = 6.6) | 58.5 | $CH_2$ | 13, 15 |
| 17 | 0.95 (d, *J* = 7.2) | 14.9 | $CH_3$ | 8, 13, 14 |
| 18 | 0.86 (s) | 33.6 | $CH_3$ | 3, 4, 5, 19 |
| 19 | 0.83 (s) | 22.0 | $CH_3$ | 3, 4, 5, 18 |
| 20 | 0.83 (s) | 14.2 | $CH_3$ | 1, 5, 9, 10 |

### 3.1.3 Compound CM3
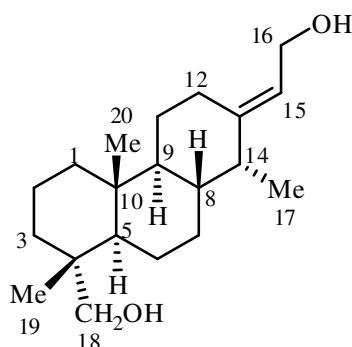


The molecular formula of compound **CM3** was found to be $C_{20}H_{34}O_2$ ([M]$^+$, *m/z* 306.2545), by HREIMS. The $^1H$ and $^{13}C$ (Table 5, Figures 20 and 21) NMR spectroscopic data of **CM3** were comparable with those of **CM2**, except that a methyl singlet at $\delta_H$ 0.86: $\delta_C$ 33.6 (Me-18) and an oxymethine proton at $\delta_H$ 4.43 (H-12) in **CM2** were replaced by oxymethylene protons 2H-18 at $\delta_H$ 3.02 and 3.33 (each d, *J* = 10.8 Hz: $\delta_C$ 72.1) and methylene protons 2H-12 at $\delta$ 1.80 and 2.34, respectively in **CM3**. The oxymethylene protons at $\delta$ 3.02 and 3.33 (2H-18) showed HMBC correlations with the carbons at $\delta$ 17.9 (C-19), 35.4 (C-3), 37.6 (C-4) and 48.3 (C-5), confirming of their attachment at C-4. The relative stereochemistry of **CM3** was assigned by NOESY experiment in which oxymethylene protons 2H-18 ($\delta$ 3.02, 3.33) showed cross-peaks with $\delta$ 0.70 (Me-19), 1.08 (H$_{ax}$-5), 1.20 (H$_{eq}$-3) and 1.43 (H$_{eq}$-6), indicating its equatorial orientation. An *E*-configuration of the double bond was suggested by a cross-peak of an olefinic proton at $\delta$ 5.29 (br t, *J* = 6.9 Hz, H-15) with a methine proton at $\delta$ 2.12 (m, H-14). Therefore, compound **CM3** was assigned as 16,18-dihydroxycass-13(15)(*E*)-ene, a new compound (Yodsaoue et al., 2010) and was named as mimosol C. Although **CM3** was previously reported as a product of semi-synthesis (Leal et al., 2003), this work establishes the diterpene as a bona fide natural product.
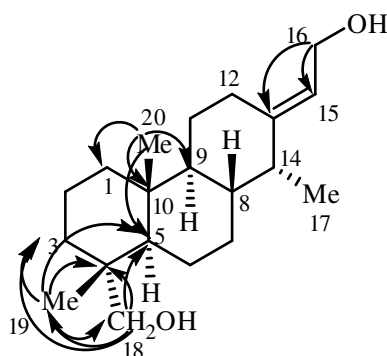


Selective HMBC correlations of **CM3**

**Table 5** ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **CM3**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|----------|------------------------------|------------|------|------|
| 1 | 0.87 (m) | 39.1 | $CH_2$ | 2, 5, 10 |
|   | 1.63 (m) | | | |
| 2 | 1.45 (m) | 18.2 | $CH_2$ | 4, 10 |
|   | 1.53 (m) | | | |
| 3 | 1.20 (m) | 35.4 | $CH_2$ | 4, 5, 18 |
|   | 1.33 (td, $J$ = 12.9, 4.2) | | | |
| 4 | - | 37.6 | C | - |
| 5 | 1.08 (m) | 48.3 | CH | 6, 10, 18, 19, 20 |
| 6 | 1.22 (m) | 21.4 | $CH_2$ | 7 |
|   | 1.43 (m) | | | |
| 7 | 1.23 (m) | 31.4 | $CH_2$ | 6, 9 |
|   | 1.40 (m) | | | |
| 8 | 1.49 (m) | 40.5 | CH | - |
| 9 | 1.09 (m) | 48.3 | CH | 7, 10, 11, 20 |
| 10 | - | 36.8 | C | - |
| 11 | 0.87 (m) | 26.6 | $CH_2$ | 8, 9, 10, 12, 13 |
|   | 1.71 (m) | | | |
| 12 | 1.80 (td, $J$ = 13.5, 4.2) | 23.6 | $CH_2$ | 9, 11, 13, 14, 15 |
|   | 2.34 (dt, $J$ = 13.5, 3.0) | | | |
| 13 | - | 149.8 | C | - |
| 14 | 2.12 (m) | 44.3 | CH | 8, 9, 12, 13, 15, 17 |
| 15 | 5.29 (br t, $J$ = 6.9) | 118.7 | CH | 12, 14, 16 |
| 16 | 4.04 (d, $J$ = 6.9) | 58.6 | $CH_2$ | 13, 15 |
| 17 | 0.87 (d, $J$ = 7.2) | 14.1 | $CH_3$ | 8, 13, 14 |
| 18 | 3.02 (d, $J$ = 10.8) | 72.1 | $CH_2$ | 3, 4, 5, 19 |
|   | 3.33 (d, $J$ = 10.8) | | | |
| 19 | 0.70 (s) | 17.9 | $CH_3$ | 3, 5, 4, 18 |
| 20 | 0.76 (s) | 14.7 | $CH_3$ | 1, 5, 9, 10 |

### 3.1.4 Compound CM4
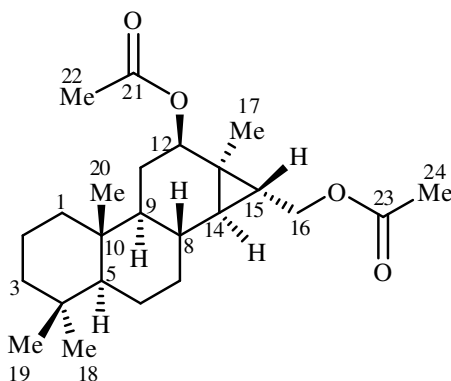


The empirical formula of compound **CM4** was deduced as $C_{24}H_{38}O_4$ from an exact mass measurement ([M]$^+$ $m/z$ 390.2780) by HREIMS. The carbonyl ester functionality was shown in IR absorption at 1735 cm$^{-1}$. The $^{13}$C NMR (Table 6, Figure 23) and DEPT spectra exhibited peaks for 24 carbons; two of these were ester carbonyls at $\delta$ 171.1 (C-23) and 171.2 (C-21), an oxymethine at $\delta$ 78.4 (C-12) and an oxymethylene carbon at $\delta$ 65.2 (C-16). The $^1$H NMR spectral data (Table 6, Figure 22) showed six singlet signals of four aliphatic methyl groups at $\delta$ 0.71 (Me-20), 0.78 (Me-19), 0.84 (Me-18) and 1.10 (Me-17) and two acetoxy methyl groups at $\delta$ 2.04 (Me-22: $\delta_C$ 21.2) and 2.06 (Me-24: $\delta_C$ 21.1). The presence of a cyclopropane ring was deduced from the $^1$H NMR, COSY and HMQC spectra that exhibited two signals at $\delta_H$ 0.46 (dd, $J$ = 5.1, 1.2 Hz, H-14: $\delta_C$ 34.0) and 1.18 (m, H-15: $\delta_C$ 25.5). From the HMBC experiments, the oxymethine proton H-12 ($\delta_H$ 5.09, dd, $J$ = 13.2, 6.3 Hz: $\delta_C$ 78.4) showed long-range correlations to the carbons at $\delta$ 19.3 (C-17), 25.4 (C-11), 25.5 (C-15) and 171.2 (C-21). The oxymethylene protons 2H-16 at $\delta$ 3.86 (dd, $J$ = 11.7, 8.4 Hz) and 4.28 (dd, $J$ = 11.7, 6.9 Hz) showed long-range correlations to the carbons at $\delta$ 24.3 (C-13), 25.5 (C-15), 34.0 (C-14) and 171.1 (C-23). These data suggested that two OAc groups were attached at C-12 and C-16 whereas C-13, C-14 and C-15 formed a cyclopropane ring. The observed HMBC correlations between a singlet methyl group at $\delta$ 1.10 (Me-17) with the carbons at $\delta$ 24.3 (C-13), 25.5 (C-15), 34.0 (C-14) and 78.4 (C-12), confirmed its location at C-13. The large $J$ value for H-12 ($J$ = 13.2 Hz) indicated its axial orientation. In the NOESY spectrum, the oxymethine proton at $\delta$ 5.09 (H-12) correlated with the methyl protons at $\delta$ 1.10 (Me-

17) and 0.64 (H-9) and a methine proton at $\delta$ 0.46 (H-14) displayed a cross-peak with the methyl protons at $\delta$ 1.10 (Me-17) and oxymethylene protons at $\delta$ 3.86 and 4.28 (2H-16) but no correlation with H-15. These data supported $\alpha$-orientation of H-12, H-14, and Me-17, hence suggesting a *cis* cyclopropyl ring with an $\alpha$-acetoxymethyl side chain. Thus, **CM4** was assigned as 12$\beta$,16$\alpha$-diacetoxy-14$\beta$,15-cyclopimarane, a new compound (Yodsaoue et al., 2010) and was named as mimosol D.
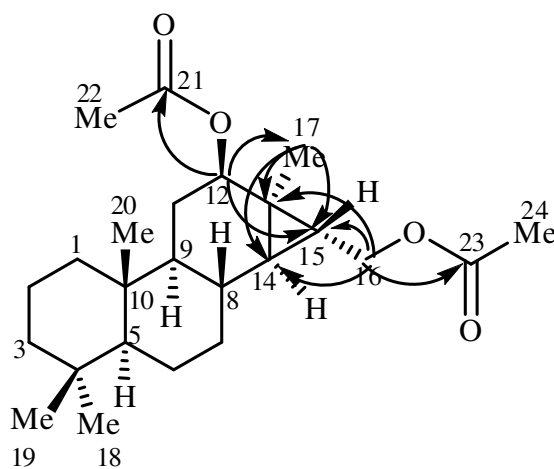


Selected and HMBC correlations for compound **CM4**
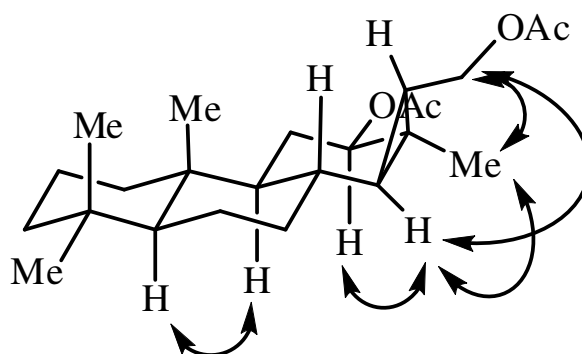


Selected NOESY cross-peaks for compound **CM4**

**Table 6** ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **CM4**

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 0.85 (m) | 38.6 | $CH_2$ | 5, 10 |
|   | 1.62 (m) |  |  |  |
| 2 | 1.42 (m) | 18.6 | $CH_2$ | 1, 3, 4 |
|   | 1.54 (m) |  |  |  |
| 3 | 1.14 (m) | 42.1 | $CH_2$ | 1, 4, 18, 19 |
|   | 1.39 (m) |  |  |  |
| 4 | - | 33.2 | C | - |
| 5 | 0.86 (m) | 55.0 | CH | 3, 4, 6, 18, 19 |
| 6 | 1.27 (m) | 22.1 | $CH_2$ | 5, 8, 10 |
|   | 1.63 (m) |  |  |  |
| 7 | 1.19 (m) | 35.9 | $CH_2$ | - |
|   | 1.92 (m) |  |  |  |
| 8 | 1.39 (m) | 36.2 | CH | - |
| 9 | 0.64 (m) | 53.1 | CH | 12, 11, 14, 20 |
| 10 | - | 36.8 | C | - |
| 11 | 0.62 (m) | 25.4 | $CH_2$ | 8, 9, 10, 12, 13 |
|   | 1.80 (m) |  |  |  |
| 12 | 5.09 (dd, *J* = 13.2, 6.3) | 78.4 | CH | 11, 15, 17, 21 |
| 13 | - | 24.3 | C | - |
| 14 | 0.46 (dd, *J* = 5.1, 1.2) | 34.0 | CH | 7, 8, 9, 12, 15, 16, 17 |
| 15 | 1.18 (m) | 25.5 | CH | 8, 12, 14, 17 |
| 16 | 3.86 (dd, *J* = 11.7, 8.4) | 65.2 | $CH_2$ | 13, 14, 15, 23 |
|   | 4.28 (dd, *J* = 11.7, 6.9) |  |  |  |
| 17 | 1.10 (s) | 19.3 | $CH_3$ | 12, 13, 14, 15 |
| 18 | 0.84 (s) | 33.4 | $CH_3$ | 3, 4, 5, 19 |
| 19 | 0.78 (s) | 21.6 | $CH_3$ | 3, 4, 5, 18 |
| 20 | 0.71 (s) | 14.3 | $CH_3$ | 1, 5, 9, 10 |
| 21 | - | 171.2 | C | - |
| 22 | 2.04 (s) | 21.2 | $CH_3$ | 21 |
| 23 | - | 171.1 | C | - |
| 24 | 2.06 (s) | 21.1 | $CH_3$ | 23 |

### 3.1.5 Compound CM5
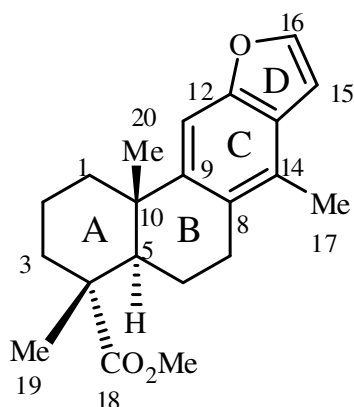


Compound **CM5** was isolated as a white solid, mp 155-156 °C, $[\alpha]_D^{27}$ + 101.9° (*c* 0.77 in CHCl₃). The IR (1720 and 771 cm⁻¹) and UV ($\lambda_{max}$ 253, 281, 292 nm) absorption bands were characteristic of ester carbonyl and benzofuran moieties, respectively. The ¹H and ¹³C NMR spectral data (Table 7, Figures 24 and 25) of compound **CM5** revealed that this compound had the same A and B rings as compound **CM1**. The difference was found in ring C which was aromatic in compound **CM5**. This was supported by the presence of one aromatic proton at $\delta$ 7.31 (br s, H-11) and the aromatic methyl at δ 2.33 (s, Me-17) confirming the presence of trisubstituted benzofuran moiety in compound **CM5**. The observed HMBC correlations between an aromatic proton at $\delta$ 7.31 (H-11) with the carbons at $\delta$ 27.5 (C-7), 37.8 (C-10), 125.4 (C-13), 127.5 (C-8), 147.2 (C-9) and 153.5 (C-12) and of an aromatic methyl at $\delta$ 2.33 (s, Me-17) with the carbons at $\delta$ 105.0 (C-15), 125.4 (C-13), 127.5 (C-8), 128.2 (C-14), 147.2 (C-9) and 153.5 (C-12), suggested the attachment of a methyl at C-14. Thus, compound **CM5** was identified as taepeenin A (Cheenpracha et al., 2005).
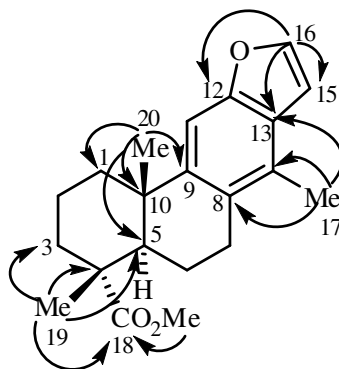


Selective HMBC correlations of **CM5**

**Table 7** ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **CM5**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.50 (m) | 38.9 | $CH_2$ | 2, 3, 5, 9, 10, 20 |
|  | 2.37 (m) |  |  |  |
| 2 | 1.73 (m) | 18.7 | $CH_2$ | 1, 3, 10 |
|  | 1.80 (m) |  |  |  |
| 3 | 1.66 (m) | 36.6 | $CH_2$ | 1, 2, 4, 5, 18, 19 |
|  | 1.78 (m) |  |  |  |
| 4 | - | 47.7 | C | - |
| 5 | 2.26 (dd, $J$ = 12.6, 2.1) | 44.4 | CH | 1, 3, 4, 7, 9, 10, 18, 19, 20 |
| 6 | 1.56 (m) | 21.8 | $CH_2$ | 5, 7, 10 |
|  | 1.91 (m) |  |  |  |
| 7 | 2.82 (m) | 27.5 | $CH_2$ | 5, 6, 8, 9, 13, 14 |
| 8 | - | 127.5 | C | - |
| 9 | - | 147.2 | C | - |
| 10 | - | 37.8 | C | - |
| 11 | 7.31 (br s) | 104.3 | CH | 7, 8, 9, 10, 12, 13 |
| 12 | - | 153.5 | C | - |
| 13 | - | 125.4 | C | - |
| 14 | - | 128.2 | C | - |
| 15 | 6.71 (dd, $J$ = 2.4, 0.9) | 105.0 | CH | 12, 13, 16 |
| 16 | 7.51 (d, $J$ = 2.4) | 144.2 | CH | 12, 13, 15 |
| 17 | 2.33 (s) | 16.0 | $CH_3$ | 8, 9, 12, 13, 14, 15 |
| 18 | - | 179.2 | C | - |
| 19 | 1.30 (s) | 16.6 | $CH_3$ | 3, 4, 5, 18 |
| 20 | 1.26 (s) | 25.6 | $CH_3$ | 1, 5, 9, 10 |
| 19-OMe | 3.67 (s) | 52.0 | $OCH_3$ | 18 |

**Table 8** Comparison of ^{1}H and ^{13}C NMR spectra data between compounds **CM5** (recorded in CDCl$_3$, 300 Hz) and taepeenin A (**R**, recorded in CDCl$_3$, 300 Hz)

| Position | CM5 $\delta_H$ (mult., *J*, Hz) | R $\delta_H$ (mult., *J*, Hz) | CM5 $\delta_C$ | R $\delta_C$ |
|---|---|---|---|---|
| 1 | 1.50 (m) | 1.55 (m) | 38.9 | 38.9 |
|  | 2.37 (m) | 2.38 (m) |  |  |
| 2 | 1.73 (m) | 1.74 (m) | 18.7 | 18.8 |
|  | 1.80 (m) | 1.81 (m) |  |  |
| 3 | 1.66 (m) | 1.68 (m) | 36.6 | 36.6 |
|  | 1.78 (m) | 1.80 (m) |  |  |
| 4 | - | - | 47.7 | 47.7 |
| 5 | 2.26 (dd, *J* = 12.6, 2.1) | 2.27 (dd, *J* = 12.9, 2.4) | 44.4 | 44.4 |
| 6 | 1.56 (m) | 1.55 (m) | 21.8 | 21.8 |
|  | 1.91 (m) | 1.95 (m) |  |  |
| 7 | 2.82 (m) | 2.83 (m) | 27.5 | 27.6 |
| 8 | - | - | 127.5 | 127.5 |
| 9 | - | - | 147.2 | 147.2 |
| 10 | - | - | 37.8 | 37.8 |
| 11 | 7.31 (br s) | 7.32 (br s) | 104.3 | 104.3 |
| 12 | - | - | 153.5 | 153.6 |
| 13 | - | - | 125.4 | 125.4 |
| 14 | - | - | 128.2 | 128.3 |
| 15 | 6.71 (dd, *J* = 2.4, 0.9) | 6.72 (dd, *J* = 2.1, 0.9) | 105.0 | 105.0 |
| 16 | 7.51 (d, *J* = 2.4) | 7.53 (d, *J* = 2.1) | 144.2 | 144.2 |
| 17 | 2.33 (s) | 2.35 (s) | 16.0 | 15.9 |
| 18 | - | - | 179.2 | 179.2 |
| 19 | 1.30 (s) | 1.31 (s) | 16.6 | 16.6 |
| 20 | 1.26 (s) | 1.27 (s) | 25.6 | 25.6 |
| 19-OMe | 3.67 (s) | 3.70 (s) | 52.0 | 52.0 |

### 3.1.6 Compound CM6
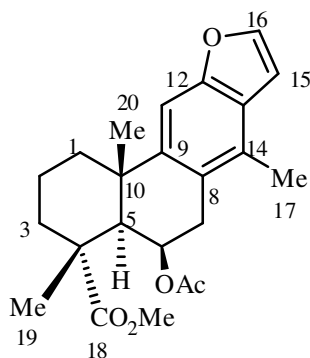


Compound **CM6** was obtained as a white solid, mp 116-117 °C, $[\alpha]_D^{27}$ −41.1° (*c* 0.02 in CHCl$_3$). The UV and IR spectrum showed absorption bands similar to those of **CM5**. The $^1$H and $^{13}$C NMR spectral data (Table 9, Figures 26 and 27) of compound **CM6** were closely related to those of compound **CM5**, except for the presence of an additional acetyl group ($\delta_H$ 2.00 and $\delta_C$ 170.7, 21.7). The $^1$H NMR spectral data exhibited a signal due to an oxymethine proton at $\delta$ 5.30 (dt, *J* = 5.4, 1.5 Hz) for H-6 which was connected to an oxymethine carbon at $\delta$ 70.7 (C-6) in the HMQC spectrum. This proton signal showed HMBC correlations with the carbons at $\delta$ 34.8 (C-7), 38.0 (C-10), 46.2 (C-5), 48.0 (C-4), 123.8 (C-8), and 170.7 (O*C*OMe) confirming the location of the OAc group at C-6. The $\alpha$-orientations of both protons at C-5 and C-6 were determined from the results of small coupling constants of protons H-5 ($\delta$ 2.50, br s) and H-6 ($\delta$ 5.30, dt, *J* = 5.4, 1.5 Hz) and the observed cross-peaks between these protons and 7-H$_\alpha$ ($\delta$ 3.12) from NOESY experiments. This result suggested that H-5 and H-6 should be $\alpha$-axial and $\alpha$-equatorial oriented, respectively. Thus, compound **CM6** was determined as taepeenin D (Cheenpracha et al., 2005).
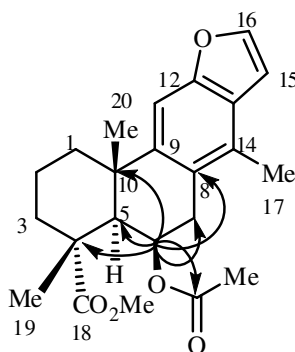


Selective HMBC correlations of **CM6**

**Table 9** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CM6**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.58 (m) | 42.1 | CH$_2$ | 2 |
|  | 2.31 (m) |  |  |  |
| 2 | 1.76 (m) | 19.0 | CH$_2$ | 1 |
|  | 1.92 (m) |  |  |  |
| 3 | 1.72 (m) | 38.4 | CH$_2$ | 1, 2, 4, 5, 18, 19 |
|  | 1.80 (m) |  |  |  |
| 4 | - | 48.0 | C | - |
| 5 | 2.50 (br s) | 46.2 | CH | 1, 3, 4, 7, 9, 10, 18, 19, 20 |
| 6 | 5.30 (dt, $J$ = 5.4, 1.5) | 70.7 | CH | 4, 5, 7, 8, 10, 21 |
| 7 | 2.96 (br d, $J$ = 18.0) | 34.8 | CH$_2$ | 5, 6, 8, 9, 14 |
|  | 3.12 (dd, $J$ = 18.0, 5.4) |  |  |  |
| 8 | - | 123.8 | C | - |
| 9 | - | 145.5 | C | - |
| 10 | - | 38.0 | C | - |
| 11 | 7.38 (br s) | 105.0 | CH | 8, 9, 10, 12, 13, 14 |
| 12 | - | 153.8 | C | - |
| 13 | - | 125.8 | C | - |
| 14 | - | 128.6 | C | - |
| 15 | 6.73 (dd, $J$ = 2.1, 0.9) | 105.0 | CH | 12, 13 |
| 16 | 7.54 (d, $J$ = 2.1) | 144.5 | CH | 12, 13, 15 |
| 17 | 2.33 (s) | 16.0 | CH$_3$ | 8, 9, 13, 14, 15 |
| 18 | - | 178.6 | C | - |
| 19 | 1.45 (s) | 18.1 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 1.64 (s) | 27.5 | CH$_3$ | 1, 5, 9, 10 |
| 19-OMe | 3.71 (s) | 52.2 | CH$_3$ | 18 |
| 21 | - | 170.7 | C | - |
| 22 | 2.00 (s) | 21.7 | CH$_3$ | 6, 21 |

**Table 10** Comparison of $^1$H and $^{13}$C NMR spectra data between compounds **CM6** (recorded in CDCl$_3$, 300 Hz) and taepeenin D (**R**, recorded in CDCl$_3$, 300 Hz)

| Position | CM6 $\delta_H$ (mult., *J*, Hz) | R $\delta_H$ (mult., *J*, Hz) | CM6 $\delta_C$ | R $\delta_C$ |
|---|---|---|---|---|
| 1 | 1.58 (m) | 1.58 (m) | 42.1 | 42.1 |
| | 2.31 (m) | 2.31 (m) | | |
| 2 | 1.76 (m) | 1.76 (m) | 19.0 | 19.0 |
| | 1.92 (m) | 1.92 (m) | | |
| 3 | 1.72 (m) | 1.67 (m) | 38.4 | 38.4 |
| | 1.80 (m) | 1.79 (m) | | |
| 4 | - | - | 48.0 | 48.0 |
| 5 | 2.50 (br s) | 2.50 (br s) | 46.2 | 46.1 |
| 6 | 5.30 (dt, *J* = 5.4, 1.5) | 5.30 (dt, *J* = 5.7, 1.5) | 70.7 | 70.7 |
| 7 | 2.96 (br d, *J* = 18.0) | 2.96 (br d, *J* = 18.3) | 34.8 | 34.8 |
| | 3.12 (dd, *J* = 18.0, 5.4) | 3.12 (dd, *J* = 18.3, 5.4) | | |
| 8 | - | - | 123.8 | 123.8 |
| 9 | - | - | 145.5 | 145.5 |
| 10 | - | - | 38.0 | 38.0 |
| 11 | 7.38 (br s) | 7.38 (br s) | 105.0 | 105.0 |
| 12 | - | - | 153.8 | 153.8 |
| 13 | - | - | 125.8 | 125.8 |
| 14 | - | - | 128.6 | 128.7 |
| 15 | 6.73 (dd, *J* = 2.1, 0.9) | 6.73 (dd, *J* = 2.4, 0.9) | 105.0 | 105.0 |
| 16 | 7.54 (d, *J* = 2.1) | 7.54 (d, *J* = 2.4) | 144.5 | 144.5 |
| 17 | 2.33 (s) | 2.33 (s) | 16.0 | 16.1 |
| 18 | - | - | 178.6 | 178.6 |
| 19 | 1.45 (s) | 1.45 (s) | 18.1 | 18.1 |
| 20 | 1.64 (s) | 1.64 (s) | 27.5 | 27.6 |
| 19-OMe | 3.71 (s) | 3.71 (s) | 52.2 | 52.3 |
| 21 | - | - | 170.7 | 170.7 |
| 22 | 2.00 (s) | 2.00 (s) | 21.7 | 21.7 |

### 3.1.7 Compound CM7
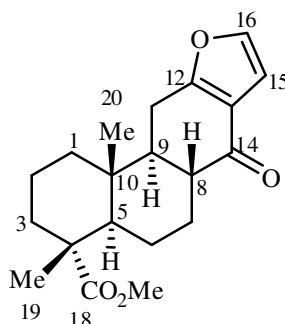


Compound **CM7** was obtained as viscous oil, $[\alpha]_D^{27}$ – 3.90° (*c* 0.58 in CHCl$_3$). The IR (1728 cm$^{-1}$) spectrum displayed absorption band of carbonyl ester. The $^{13}$C NMR (Table 11, Figure 29) and DEPT spectra exhibited 20 carbons, two of these were conjugated carbonyl ($\delta$ 195.8) and an ester carbonyl ($\delta$ 178.9). Excluding the signal due to the methoxy substituent, **CM7** contained only 19 carbons in the main carbon framework, suggesting it to be a norditerpene. The NMR data (Table 11, Figure 28) of **CM7** displayed similarities with **CM1**, except that the signals of an exocyclic double bond at $\delta_H$ 5.14, 5.11 (s, 2H-17); $\delta_C$ 103.0 and 142.5 (C-14) was replaced by a carbonyl carbon at $\delta$ 195.8 (C-14). An oxyquaternary carbon signal at $\delta$ 70.9 (C-8) of **CM1** was replaced by the methine proton signal at $\delta_H$ 2.31 (td, *J* = 12.0, 4.2 Hz, H-8); $\delta_C$ 45.1. The latter proton showed HMBC correlations with the carbons at $\delta$ 26.8 (C-7), 53.0 (C-9) and 195.8 (C-14). The methine proton H-9 ($\delta_H$ 1.88 (td, *J* = 12.0, 5.4 Hz); $\delta_C$ 53.0) showed HMBC correlations with carbons at $\delta$ 14.8 (C-20), 22.9 (C-11), 36.9 (C-10), 45.1 (C-8), 49.0 (C-5), and 195.8 (C-14). These data suggested the location of conjugated carbonyl at C-14. Thus on the basis of its spectroscopic data and comparison with previously reported data of nortaepeenin A (Cheenpracha et al., 2005), compound **CM7** was assigned as nortaepeenin A.
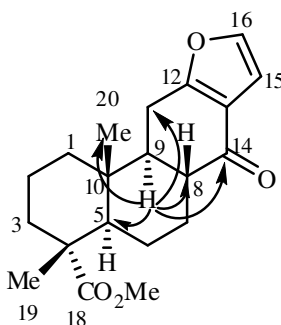


Selective HMBC correlations of **CM7**

**Table 11** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CM7**

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.15 (m) | 37.9 | CH$_2$ | 2, 3, 10, 20 |
| | 1.72 (m) | | | |
| 2 | 1.62 (m) | 17.9 | CH$_2$ | 3, 4 |
| | 1.70 (m) | | | |
| 3 | 1.61 (m) | 36.6 | CH$_2$ | 2, 5 |
| 4 | - | 47.4 | C | - |
| 5 | 1.78 (dd, *J* = 12.6, 2.4) | 49.0 | CH | 3, 4, 10, 18, 19, 20 |
| 6 | 1.29 (m) | 23.5 | CH$_2$ | 5 |
| | 1.49 (m) | | | |
| 7 | 1.30 (m) | 26.8 | CH$_2$ | 8, 9 |
| | 2.47 (m) | | | |
| 8 | 2.31 (td, *J* = 12.0, 4.2) | 45.1 | CH | 7, 9, 14 |
| 9 | 1.88 (td, *J* = 12.0, 5.4) | 53.0 | CH | 5, 8, 10, 11, 14, 20 |
| 10 | - | 36.9 | C | - |
| 11 | 2.66 (dd, *J* = 17.1, 12.0) | 22.9 | CH$_2$ | 8, 9, 10, 12, 13 |
| | 2.89 (dd, *J* = 17.1, 5.4) | | | |
| 12 | - | 166.4 | C | - |
| 13 | - | 119.9 | C | - |
| 14 | - | 195.8 | C | - |
| 15 | 6.73 (d, *J* = 1.8) | 106.7 | CH | 12, 13, 16 |
| 16 | 7.30 (d, *J* = 1.8) | 142.8 | CH | 12, 13, 15 |
| 17 | - | - | - | - |
| 18 | - | 178.9 | C | - |
| 19 | 1.21 (s) | 16.8 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 1.01 (s) | 14.8 | CH$_3$ | 1, 5, 9, 10 |
| 21 | 3.66 (s) | 52.0 | CH$_3$ | 18 |

**Table 12** Comparison of $^1$H and $^{13}$C NMR spectra data between compounds **CM7** (recorded in CDCl$_3$, 300 Hz) and nortaepeenin A (**R**, recorded in CDCl$_3$, 300 Hz)

| Position | CM7 δ$_H$ (mult., *J*, Hz) | R δ$_H$ (mult., *J*, Hz) | CM7 δ$_C$ | R δ$_C$ |
|---|---|---|---|---|
| 1 | 1.15 (m) | 1.14 (m) | 37.9 | 37.9 |
|   | 1.72 (m) | 1.74 (m) | | |
| 2 | 1.62 (m) | 1.69 (m) | 17.9 | 17.9 |
|   | 1.70 (m) | | | |
| 3 | 1.61 (m) | 1.62 (m) | 36.6 | 36.7 |
| 4 | - | - | 47.4 | 47.3 |
| 5 | 1.78 (dd, *J* = 12.6, 2.4) | 1.78 (dd, *J* = 12.3, 2.4) | 49.0 | 49.0 |
| 6 | 1.29 (m) | 1.29 (m) | 23.5 | 23.5 |
|   | 1.49 (m) | 1.49 (m) | | |
| 7 | 1.30 (m) | 1.31 (m) | 26.8 | 26.8 |
|   | 2.47 (m) | 2.46 (m) | | |
| 8 | 2.31 (td, *J* = 12.0, 4.2) | 2.31 (td, *J* = 12.0, 4.2) | 45.1 | 45.0 |
| 9 | 1.88 (td, *J* = 12.0, 5.4) | 1.88 (td, *J* = 12.0, 5.1) | 53.0 | 52.9 |
| 10 | - | - | 36.9 | 36.9 |
| 11 | 2.66 (dd, *J* = 17.1, 12.0) | 2.66 (dd, *J* = 17.1, 12.0) | 22.9 | 22.8 |
|   | 2.89 (dd, *J* = 17.1, 5.4) | 2. 90 (dd, *J* = 17.1, 5.1) | | |
| 12 | - | - | 166.4 | 166.3 |
| 13 | - | - | 119.9 | 119.8 |
| 14 | - | - | 195.8 | 195.7 |
| 15 | 6.73 (d, *J* = 1.8) | 6.63 (d, *J* = 1.8) | 106.7 | 106.5 |
| 16 | 7.30 (d, *J* = 1.8) | 7.30 (d, *J* = 1.8) | 142.8 | 142.8 |
| 17 | - | - | - | - |
| 18 | - | - | 178.9 | 178.9 |
| 19 | 1.21 (s) | 1.21 (s) | 16.8 | 16.8 |
| 20 | 1.01 (s) | 1.01 (s) | 14.8 | 14.8 |
| 21 | 3.66 (s) | 3.65 (s) | 52.0 | 52.0 |

### 3.1.8 Compound CM8
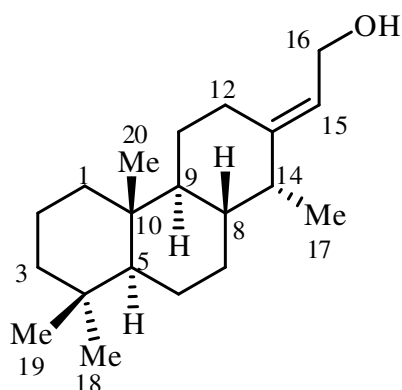


Compound **CM8** was obtained as a white solid, mp 124-126 °C, $[\alpha]_D^{27}$ + 25.4° (*c* 0.67 in CHCl$_3$). The IR spectrum displayed the absorbance of hydroxyl (3359 cm$^{-1}$) group. The $^1$H and $^{13}$C NMR spectral data (Table 13, Figures 30 and 31) of **CM8** showed characteristics similar to those of **CM2,** except that the signal of an oxymethine proton at $\delta$ 4.43 (dd, *J* = 10.2, 4.8 Hz, H-11); $\delta_C$ 70.7 in **CM2** was replaced by those of the methylene protons at $\delta$ 0.97 and 1.75 (each m) $\delta_C$ 26.6. This finding was supported by HMBC spectrum, in which an olefinic proton of H-15 at $\delta$ 5.37 (t, *J* = 6.6 Hz) was correlated with the carbons at $\delta$ 26.6 (C-12), 44.3 (C-14) and 58.6 (C-16). Thus, compound **CM8** was determined as taepeenin L (Cheenpracha et al., 2005).
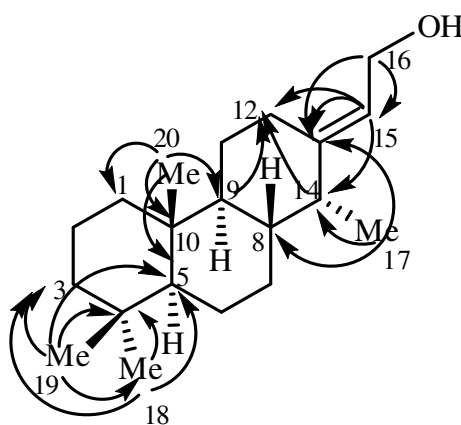


Selective HMBC correlations of **CM8**

**Table 13** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CM8**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 0.94 (m) | 39.6 | CH$_2$ | 2, 5, 9, 10, 20 |
|   | 1.70 (m) | | | |
| 2 | 1.40 (m) | 18.9 | CH$_2$ | 1, 3, 4, 10 |
|   | 1.56 (m) | | | |
| 3 | 1.14 (m) | 42.2 | CH$_2$ | 1, 2, 4, 5, 18, 19 |
|   | 1.43 (m) | | | |
| 4 | - | 33.2 | C | - |
| 5 | 0.81 (m) | 55.3 | CH | 1, 3, 4, 9, 10, 18, 19, 20 |
| 6 | 1.25 (m) | 21.7 | CH$_2$ | 4, 5, 7, 8, 10 |
|   | 1.58 (m) | | | |
| 7 | 1.27 (m) | 31.7 | CH$_2$ | 5, 6, 8, 9, 14 |
|   | 1.47 (m) | | | |
| 8 | 1.52 (m) | 40.6 | CH | 7, 9, 10, 13, 14, 17 |
| 9 | 1.11 (m) | 48.4 | CH | 1, 5, 7, 8, 10, 12, 20 |
| 10 | - | 37.0 | C | - |
| 11 | 1.85 (qd, $J$ = 13.5, 4.2) | 23.7 | CH$_2$ | 9, 12, 13, 15 |
|    | 2.43 (br d, $J$ = 13.5) | | | |
| 12 | 0.97 (m) | 26.6 | CH$_2$ | 9, 11, 13, 14, 15 |
|    | 1.75 (m) | | | |
| 13 | - | 149.9 | C | - |
| 14 | 2.19 (qn, $J$ = 7.2) | 44.3 | CH | 9, 13, 14, 15 |
| 15 | 5.37 (t, $J$ = 6.6) | 118.8 | CH | 11, 14, 16 |
| 16 | 4.14 (d, $J$ = 6.6) | 58.6 | CH$_2$ | 13, 15 |
| 17 | 0.95 (d, $J$ = 7.2) | 14.4 | CH$_3$ | 8, 13, 14 |
| 18 | 0.86 (s) | 22.1 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 0.82 (s) | 33.7 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 0.79 (s) | 14.2 | CH$_3$ | 1, 5, 9, 10 |

**Table 14** Comparison of ^{1}H and ^{13}C NMR spectra data between compounds **CM8**
(recorded in CDCl$_3$, 300 Hz) and taepeenin L (**R**, recorded in CDCl$_3$, 300 Hz)

| Position | CM8 | R | CM8 | R |
|---|---|---|---|---|
| | $\delta_H$ (mult., *J*, Hz) | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | $\delta_C$ |
| 1 | 0.94 (m) | 0.86–1.00 m | 39.6 | 39.7 |
| | 1.70 (m) | 1.65–1.77 (m) | | |
| 2 | 1.40 (m) | 1.36–1.41 (m) | 18.9 | 18.9 |
| | 1.56 (m) | 1.44–1.58 (m) | | |
| 3 | 1.14 (m) | 1.09–1.20 (m) | 42.2 | 42.2 |
| | 1.43 (m) | 1.35–1.47 (m) | | |
| 4 | - | - | 33.2 | 33.2 |
| 5 | 0.81 (m) | 0.78–0.88 (m) | 55.3 | 55.8 |
| 6 | 1.25 (m) | 1.20–1.35 (m) | 21.7 | 21.7 |
| | 1.58 (m) | 1.58–1.67 (m) | | |
| 7 | 1.27 (m) | 1.46–1.54 (m) | 31.7 | 31.7 |
| | 1.47 (m) | | | |
| 8 | 1.52 (m) | 1.50–1.57 (m) | 40.6 | 40.7 |
| 9 | 1.11 (m) | 1.08–1.19 (m) | 48.4 | 48.4 |
| 10 | - | - | 37.0 | 37.0 |
| 11 | 1.85 (qd, *J* = 13.5, 4.2) | 0.90–1.01 (m) | 26.6 | 26.6 |
| | 2.43 (br d, *J* = 13.5) | 1.71–1.82 (m) | | |
| 12 | 0.97 (m) | 1.84–1.94 (m) | 23.7 | 23.7 |
| | 1.75 (m) | 2.39–2.50 (m) | | |
| 13 | - | - | 149.9 | 151.0 |
| 14 | 2.19 (qn, *J* = 7.2) | 2.17–2.24 (m) | 44.3 | 44.3 |
| 15 | 5.37 (t, *J* = 6.6) | 5.37 (td, *J* = 7.2, 1.5) | 118.8 | 118.7 |
| 16 | 4.14 (d, *J* = 6.6) | 4.12 (d, *J* = 7.2) | 58.6 | 58.7 |
| 17 | 0.95 (d, *J* = 7.2) | 0.95 (d, *J* = 7.2) | 14.4 | 14.4 |
| 18 | 0.86 (s) | 0.86 (s) | 33.7 | 33.7 |
| 19 | 0.82 (s) | 0.82 (s) | 22.1 | 22.1 |
| 20 | 0.79 (s) | 0.79 (s) | 14.2 | 14.2 |

### 3.1.9 Compound CM9



Fragment A

Fragment B
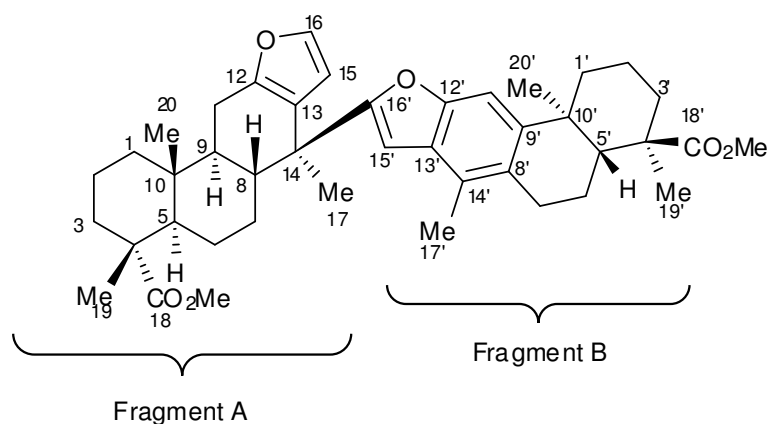
Compound **CM9** was isolated as a white solid. It showed $[M]^+$ at *m/z* 654.3948 ($C_{42}H_{54}O_6$) in the HREIMS spectrum. The UV spectrum ($\lambda_{max}$ 258, 283 and 293 nm) suggested the presence of a benzofuran chromophore (Lyder et al., 1998). The IR spectrum of **CM9** displayed the absorbance of a carbonyl ester (1720 cm$^{-1}$) group. The $^{13}$C NMR (Tables 15 and 16, Figure 33) and DEPT spectroscopic data displayed 42 carbons; twelve of these were sp$^2$ carbons attributable to 4 methine and 8 quaternary carbons. The $^1$H NMR data (Tables 15 and 16, Figure 32) showed two fragments, A and B, both being cassane-type diterpenes. The $^1$H and $^{13}$C-NMR and HMBC data established the dimeric structure of compound **CM9** that was closely related to taepeenin J previously isolated from *C. crista* (Cheenpracha et al., 2006). The differences were shown as the disappearance of two methyl singlets at $\delta_H$ 0.95 (Me-18) and 0.75 (Me-18') in taepeenin J and the appearance of two methyl ester at $\delta_H$ 3.66 (OMe-18) and 3.57 (OMe-18') in **CM9**, together with the presence of two ester carbonyl carbons at $\delta_C$ 179.2 (C-18) and 179.1 (C-18'). The locations of two methyl ester groups at C-4 and C-4' were confirmed by their HMBC correlations: in fragment A the methyl ester protons at $\delta$ 3.66 (OMe-18) correlated with the carbonyl carbon at $\delta$ 179.2 (C-18) and a singlet methyl at $\delta$ 1.18 (Me-19) correlated with the carbons at $\delta$ 36.5 (C-3), 47.3 (C-4), 49.1 (C-5) and the carbonyl carbon at $\delta$ 179.2 (C-18) whereas in fragment B methyl ester protons at $\delta$ 3.57 (OMe-18') correlated with the carbonyl carbon at $\delta$ 179.1 (C-18') and a singlet methyl at $\delta$ 1.29 (Me-19') correlated with the carbons at $\delta$ 36.6 (C-3'), 44.3 (C-5'), 47.7 (C-4') and the carbonyl carbon at $\delta$ 179.1 (C-18'). The connectivity between the two fragments at C-14 (fragment A) and C-16' (fragment B) was supported by HMBC correlations. The methyl protons at $\delta$ 1.64

(Me-17) exhibited the cross-peaks with the carbons at $\delta$ 40.1 (C-14), 44.0 (C-8), 121.9 (C-13) and 162.2 (C-16') whereas an aromatic proton at $\delta$ 6.12 (H-15') correlated with the carbon at $\delta$ 40.1 (C-14). The NOESY cross-peaks of the aromatic proton at $\delta$ 6.12 (H-15') with the methyl protons at $\delta$ 1.64 (Me-17) and 2.28 (Me-17') and of a methine proton at $\delta$ 1.90 (H-9) with the methyl protons (Me-17) supported the $\beta$-equatorial orientation of fragment B at C-14. Thus, **CM9** was deduced to be 14$\beta$-(8'(14'),9'(11')-diene-18'$\alpha$-methoxycarbonyl-18'-norvouacapen-16'-yl)-18$\alpha$-methoxycarbonyl-18-norvouacapene, a new compound (Yodsaoue et al., 2010) and was named as mimosol E.
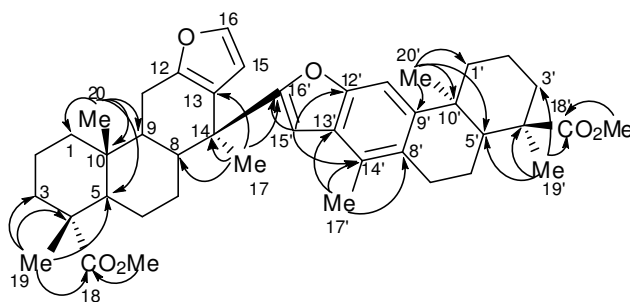


Selective HMBC correlations of **CM9**
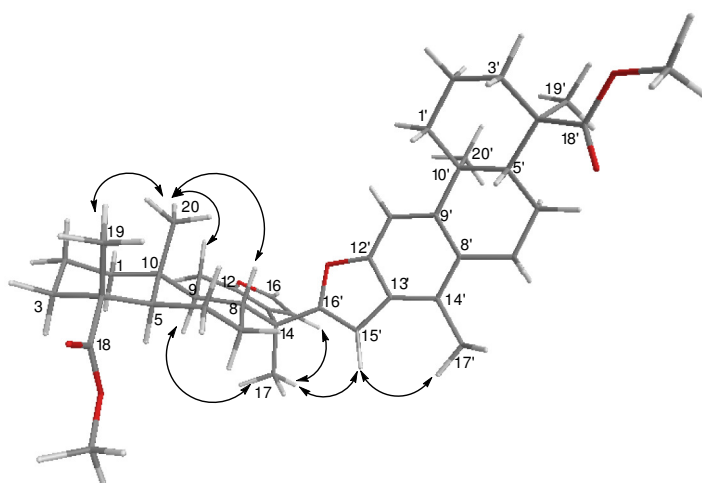


Selective NOESY cross-peaks of **CM9**

**Table 15** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CM9**

(Fragment A)

| Position | δ$_H$ (mult., *J*, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.08-1.12 (m) | 38.0 | CH$_2$ | 20 |
| | 1.76-1.80 (m) | | | |
| 2 | 1.50-1.62 (m) | 17.9 | CH$_2$ | - |
| | 1.72-1.82 (m) | | | |
| 3 | 1.75-1.80 (m) | 36.5 | CH$_2$ | 5, 18, 19 |
| | 1.82-1.90 (m) | | | |
| 4 | - | 47.3 | C | - |
| 5 | 1.61 (m) | 49.1 | CH | 1, 4, 6, 10, 18, 19, 20 |
| 6 | 1.28-1.31(m) | 23.9 | CH$_2$ | 4, 10 |
| | 1.45-1.51 (m) | | | |
| 7 | 1.97-2.02 (m) | 28.3 | CH$_2$ | - |
| | 2.04-2.08 (m) | | | |
| 8 | 1.65 (m) | 44.0 | CH | 17 |
| 9 | 1.90 (m) | 47.7 | CH | 5, 8, 20 |
| 10 | - | 37.4 | C | - |
| 11 | 2.49 (dd, *J* = 15.3, 10.2) | 21.8 | CH$_2$ | 8, 9, 10, 13 |
| | 2.79 (dd, *J* = 15.3, 7.2) | | | |
| 12 | - | 150.1 | C | - |
| 13 | - | 121.9 | C | - |
| 14 | - | 40.1 | C | - |
| 15 | 6.08 (d, *J* = 1.8) | 108.5 | CH | 12, 13 |
| 16 | 7.23 (br s) | 140.7 | C | 12, 13, 15 |
| 17 | 1.64 (s) | 24.7 | CH$_3$ | 14, 8, 13, 16' |
| 18 | - | 179.2 | C | - |
| 19 | 1.18 (s) | 17.0 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 0.94 (s) | 14.5 | CH$_3$ | 1, 5, 9, 10 |
| 18-OMe | 3.66 (s) | 51.9 | CH$_3$ | 18 |

**Table 16** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CM9**

(Fragment B)

| Position | δ$_H$ (mult., *J*, Hz) | δ$_C$ | DEPT | HMBC |
|----------|------------------------|-------|------|------|
| 1' | 1.48-1.55 (m) | 38.9 | CH$_2$ | 20' |
| | 2.20-2.35 (m) | | | |
| 2' | 1.62-1.70 (m) | 18.7 | CH$_2$ | - |
| | 1.72-1.82 (m) | | | |
| 3' | 1.44-1.58 (m) | 36.6 | CH$_2$ | 5', 18', 19' |
| | 1.60-1.68 (m) | | | |
| 4' | - | 47.7 | C | - |
| 5' | 2.26 (m) | 44.3 | CH | 4', 6', 7', 9', 10', 18', 19', 20' |
| 6' | 1.50-1.60 (m) | 21.9 | CH$_2$ | 4' |
| | 1.62-1.78 (m) | | | |
| 7' | 2.80-2.97 (m) | 27.5 | CH$_2$ | 5' |
| | 2.60-2.72 (m) | | | |
| 8' | - | 127.4 | C | - |
| 9' | - | 146.1 | C | - |
| 10' | - | 37.7 | C | - |
| 11' | 7.23 (s) | 104.3 | CH | 8', 10', 12', 13' |
| 12' | - | 153.4 | C | - |
| 13' | - | 126.5 | C | - |
| 14' | - | 127.1 | C | - |
| 15' | 6.12 (s) | 102.4 | CH | 12', 14', 16' |
| 16' | - | 162.2 | C | - |
| 17' | 2.28 (s) | 15.9 | CH$_3$ | 8', 13', 14' |
| 18' | - | 179.1 | C | - |
| 19' | 1.29 (s) | 16.6 | CH$_3$ | 3', 4', 5', 18' |
| 20' | 1.28 (s) | 25.5 | CH$_3$ | 1', 5', 9', 10' |
| 18'-OMe | 3.57 (s) | 51.9 | CH$_3$ | 18' |

### 3.1.10 Compound CM10
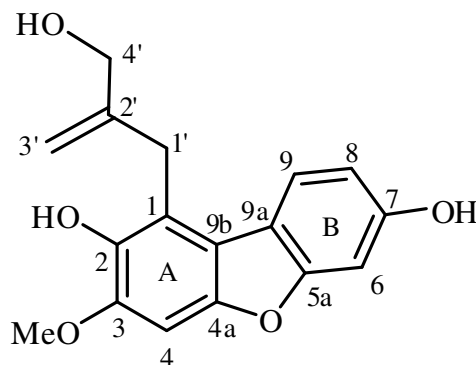


The molecular formula of **CM10** was established as $C_{17}H_{16}O_5$ ([M]$^+$, *m/z* 300.1011), based on the HREIMS mass spectrum. The UV spectrum showed absorption maxima at $\lambda_{max}$ 207, 222, 250, 309 and 318 nm. The IR spectrum displayed the absorbance of a hydroxyl stretching frequency (3386 cm$^{-1}$). The $^{13}$C NMR (Table 17 , Figure 37) and DEPT spectral data showed 17 carbons, suggesting twelve aromatic carbons identified as four protonated ($\delta$ 93.4, 97.9, 110.9, 122.2), and eight non-protonated, of which five oxygenated ($\delta$ 140.9, 146.5, 149.6, 156.3, 157.6) and three non-oxygenated ($\delta$ 116.2, 117.1, 117.9) carbons. Two low-field signals at $\delta$ 108.4 and 147.0 representing two carbons of a disubstituted double bonds were observed. These data allowed the formulation of a dibenzofuran ring which contained twelve aromatic carbons and an oxygen atom, whose structure was consistent with ten degrees of unsaturation calculated for this compound. The $^1$H NMR spectrum (Table 17, Figure 36) displayed the presence of two sets of downfield resonances. One of them was shown at $\delta$ 7.14 (1H, s, H-4) suggesting the presence of a 1,2,3,5,6-pentasubstituted benzene ring (ring A) and another as the proton resonances at $\delta$ 6.81 (1H, dd, *J* = 7.8, 2.1 Hz, H-8), 6.97 (1H, d, *J* = 2.1 Hz, H-6) and 7.76 (1H, d, *J* = 7.8 Hz, H-9) indicating the presence of a 1,2,4-trisubstituted benzene ring (ring B). The presence of a biphenyl linkage between C-9a and C-9b (forming a furan skeleton) (Qu et al., 2007) was determined by the HMBC correlation, in which an aromatic proton H-9 at $\delta$ 7.76 showed correlations with the carbons at $\delta$ 97.9 (C-6), 110.9 (C-8), 116.2 (C-9b) and 157.6 (C-5a) whereas an aromatic proton H-4 ($\delta$ 7.14) showed correlations with the carbons at $\delta$ 116.2 (C-9b), 140.9 (C-2), 146.5 (C-3) and 149.6 (C-4a). In

addition, the methylene protons at $\delta$ 3.87 (2H-1′) showed HMBC correlations with the carbons at $\delta$ 65.3 (C-4′), 108.4 (C-3′), 116.2 (C-9b), 117.9 (C-1), 140.9 (C-2) and 147.0 (C-2′), suggesting its location at C-1. A methoxyl group ($\delta$ 3.96) was assigned at C-3 due to its HMBC correlation to the carbons at $\delta$ 146.5 (C-3). The signals of the terminal olefinic methylene protons at $\delta_H$ 4.53 and 4.99 (each m, 2H-3′: $\delta_C$ 108.4) exhibited a COSY cross-peak with the methylene protons at $\delta$ 3.87 (br s, 2H-1′: $\delta_C$ 29.6) and oxymethylene protons at $\delta$ 4.22 (br s, 2H-4′: $\delta_C$ 65.3). Moreover, NOESY cross-peaks were observed between the methoxyl protons and the aromatic proton H-4 ($\delta$ 7.14, s) and between methylene protons 2H-1' ($\delta$ 3.87) and the aromatic proton H-9 ($\delta$ 7.76). Therefore, **CM10** was elucidated as 1-(2-(hydroxymethyl)allyl)-3-methoxydibenzo[b,d]furan-2,7-diol, a new compound (Yodsaoue et al., 2010) and was named as mimosol F.
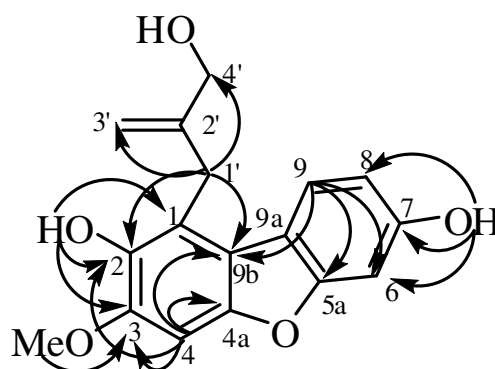


Selective HMBC correlations of **CM10**

**Table 17** $^{1}$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CM10**

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|----------|------------------------------|------------|------|------|
| 1 | - | 117.9 | C | - |
| 2 | - | 140.9 | C | - |
| 3 | - | 146.5 | C | - |
| 4 | 7.14 (s) | 93.4 | CH | 2, 3, 4a, 9b |
| 4a | - | 149.6 | C | - |
| 5a | - | 157.6 | C | - |
| 6 | 6.97 (d, *J* = 2.1) | 97.9 | CH | 5a, 7, 8, 9a |
| 7 | - | 156.3 | C | - |
| 8 | 6.81 (dd, *J* = 7.8, 2.1) | 110.9 | CH | 6, 9a |
| 9 | 7.76 (d, *J* = 7.8) | 122.2 | CH | 5a, 6, 9b |
| 9a | - | 117.1 | C | - |
| 9b | - | 116.2 | C | - |
| 1′ | 3.87 (br s) | 29.6 | CH$_2$ | 1, 2, 2′, 3′, 4′, 9b |
| 2′ | - | 147.0 | C | - |
| 3′ | 4.53 (m) | 108.4 | CH$_2$ | 1′, 2′, 4′ |
|    | 4.99 (m) |  |  |  |
| 4′ | 4.22 (br s) | 65.3 | CH$_2$ | 1′, 2′, 3′ |
| 3-OMe | 3.96 (s) | 55.9 | CH$_3$ | 3 |
| 2-OH | 8.71 (s) | - | - | 1, 2, 3 |
| 7-OH | 7.37 (s) | - | - | 6, 7, 8 |

### 3.1.11 Compound CM11
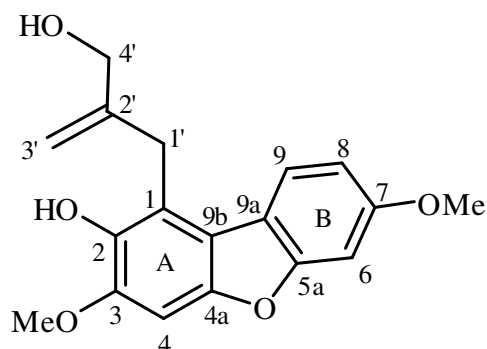


Compound **CM11** had a molecular formula $C_{18}H_{18}O_5$, ([M]$^+$ *m/z* 314.1118), based on HREIMS which was 14 mass units more than that of **CM10**, suggesting the addition of a Me group. The $^1H$ and $^{13}C$ NMR spectra (Table 18, Figures 43 and 44) of **CM11** displayed characteristics similar to those of **CM10**, except for the presence of an additional methoxyl group at $\delta_H$ 3.87 (s) in **CM11** whose HMBC correlation with the carbon at $\delta$ 158.7 (C-7) and NOESY cross-peak with the protons at $\delta$ 6.88 (dd, *J* = 7.8, 2.4 Hz, H-8) and 7.12 (d, *J* = 2.4 Hz, H-6) suggested the location of an OMe group at C-7. Thus, **CM11** was deduced to be 1-(2-(hydroxymethyl)allyl)-3,7-dimethoxydibenzo[b,d]furan-2-ol, a new compound (Yodsaoue et al., 2010) and was named as mimosol G.



Selective HMBC correlations of **CM11**

**Table 18** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CM11**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|----------|------------------------------|------------|------|------|
| 1 | - | 118.0 | C | - |
| 2 | - | 141.1 | C | - |
| 3 | - | 146.8 | C | - |
| 4 | 7.17 (s) | 93.4 | CH | 1, 2, 3, 4a, 9b |
| 4a | - | 149.8 | C | - |
| 5a | - | 157.5 | C | - |
| 6 | 7.12 (d, $J$ = 2.4) | 96.2 | CH | 7, 8, 9a |
| 7 | - | 158.7 | C | - |
| 8 | 6.88 (dd, $J$ = 7.8, 2.4) | 110.2 | CH | 6, 7 |
| 9 | 7.83 (d, $J$ = 7.8) | 122.1 | CH | 6, 8, 9a, 9b |
| 9a | - | 118.0 | C | - |
| 9b | - | 116.0 | C | - |
| 1′ | 3.88 (br s) | 29.6 | CH$_2$ | 1, 2, 2′, 3′, 4′, 9b |
| 2′ | - | 147.0 | C | - |
| 3′ | 4.52 (m) | 108.4 | CH$_2$ | 1′, 4′ |
|  | 5.00 (sext, $J$ = 1.8) |  |  |  |
| 4′ | 4.22 (br s) | 65.3 | CH$_2$ | 1′, 2′, 3′ |
| 3-OMe | 3.97 (s) | 55.9 | CH$_3$ | 3 |
| 7-OMe | 3.87 (s) | 55.1 | CH$_3$ | 7 |
| 2-OH | 7.42 (s) | - | - | 1, 2, 3 |

### 3.1.12 Compound CM12
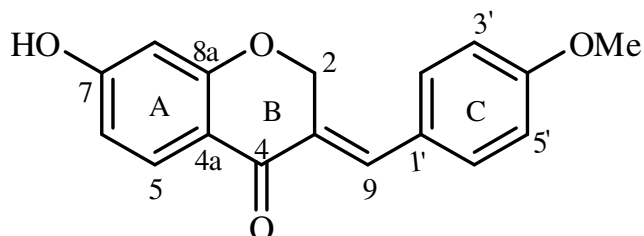


**Compound CM12** was obtained as a yellow solid**,** mp: 178-180 °C. The UV absorption bands at $\lambda_{max}$ 209, 232, 317 and 357 nm supported the presence of conjugated-carbonyl chromophore in the structure. The IR spectrum showed absorption bands of hydroxyl group (3367 cm$^{-1}$), and C=O stretching (1700 cm$^{-1}$). The $^{13}$C NMR and DEPT spectral data (Table 19, Figure 46) indicated the presence of 18 carbons including 14 aromatic carbons, one carbonyl carbon, one aliphatic carbon and one methoxyl carbon. The $^1$H NMR spectral data (Table 19, Figure 45) displayed the oxymethylene protons at $\delta$ 5.40 (2H, d, $J$ = 1.8 Hz) and one olefinic proton at $\delta$ 7.71 (br s) which was identified as $\beta$-unsaturated proton. The aromatic proton signals at $\delta$ 6.40 (d, $J$ = 2.1 Hz), 6.62 (dd, $J$ = 8.7, 2.1 Hz) and 7.83 (d, $J$ = 8.7 Hz) suggested the presence of a 1,2,4-trisubstituted benzene ring whereas the other proton signals at $\delta$ 7.05 (2H, br d, $J$ = 8.7 Hz), 7.40 (2H, br d, $J$ = 8.7 Hz) confirmed a 1,4-disubstituted benzene ring.

The structure of **CM12** was confirmed by HMBC correlations. The oxymethylene protons at $\delta$ 5.40 (2H-2) showed correlations with the carbons at $\delta$ 129.3 (C-3), 135.3 (C-9), 163.0 (C-8a) and 179.7 (C-4) and an aromatic proton at $\delta$ 7.83 (H-5) showed correlation with the carbons at $\delta$ 102.6 (C-8), 163.0 (C-8a), 164.3 (C-7) and 179.7 (C-4). The correlation of an olefinic proton at $\delta$ 7.71 (H-9) with the carbons at $\delta$ 132.0 (C-2′, 6′), 129.3 (C-3) and 179.7 (C-4) confirmed the location of the 1,4-disubstituted benzene ring at C-9. In addition, the $^1$H NMR spectrum displayed the presence of a methoxyl group at $\delta$ 3.87 which showed correlation with the carbon at $\delta$ 160.8 (C-4') whose location was assigned at C-4' of the 1,4-disubstituted benzene

ring. Therefore, **CM12** was identified as (*E*)-7-hydroxy-3-(4-methoxybenzyl-chroman-4-one) (Chen and Yang, 2007).
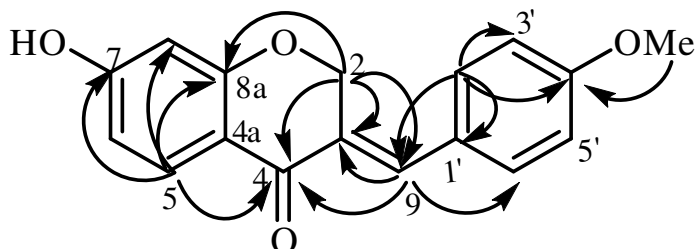


Selective HMBC correlations of **CM12**

**Table 19** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CM12**

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 2 | 5.40 (d, *J* = 1.8) | 67.8 | $CH_2$ | 3, 4, 9, 8a |
| 3 | - | 129.3 | C | - |
| 4 | - | 179.7 | C | - |
| 4a | - | 115.2 | C | - |
| 5 | 7.83 (d, *J* = 8.7) | 126.5 | CH | 4, 7, 8, 8a |
| 6 | 6.62 (dd, *J* = 8.7, 2.1) | 110.9 | CH | 4a, 7, 8 |
| 7 | - | 164.3 | C | - |
| 8 | 6.40 (d, *J* = 2.1) | 102.6 | CH | 4a, 6, 7 |
| 8a | - | 163.0 | C | - |
| 9 | 7.71 (br s) | 135.3 | CH | 3, 4, 2′, 6′ |
| 1′ | - | 127.0 | C | - |
| 2′ | 7.40 (d, *J* = 8.7) | 132.0 | CH | 9, 3′, 4′, 5′, 6′ |
| 3′ | 7.05 (d, *J* = 8.7) | 114.2 | CH | 1′, 4′, 5′ |
| 4′ | - | 160.8 | C | - |
| 5′ | 7.05 (d, *J* = 8.7) | 114.2 | CH | 1′, 3′, 4′ |
| 6′ | 7.40 (d, *J* = 8.7) | 132.0 | CH | 9, 2′, 3′, 4′, 5′ |
| 4′-OMe | 3.87 (s) | 54.9 | $CH_3$ | 4′ |

**Table 20** Comparison of $^1$H and $^{13}$C NMR spectra data between compounds **CM12** (recorded in acetone-$d_6$, 300 MHz) and (*E*)-7-hydroxy-3-(4-methoxybenzyl-chroman-4-one) (**R**, recorded in DMSO-$d_6$, 500 Hz)

| Position | CM12 $\delta_H$ (mult., *J*, Hz) | R $\delta_H$ (mult., *J*, Hz) | CM12 $\delta_C$ | R $\delta_C$ |
|---|---|---|---|---|
| 2 | 5.40 (d, *J* = 1.8) | 5.35 (d, *J* = 1.5) | 67.8 | 67.52 |
| 3 | - | - | 129.3 | 128.79 |
| 4 | - | - | 179.7 | 179.47 |
| 4a | - | - | 115.2 | 114.23 |
| 5 | 7.83 (d, *J* = 8.7) | 7.72 (d, *J* = 8.0) | 126.5 | 129.37 |
| 6 | 6.62 (dd, *J* = 8.7, 2.1) | 6.52 (dd, *J* = 8.5, 2.0) | 110.9 | 111.09 |
| 7 | - | - | 164.3 | 164.57 |
| 8 | 6.40 (d, *J* = 2.1) | 6.31 (d, *J* = 2.0) | 102.6 | 102.39 |
| 8a | - | - | 163.0 | 162.44 |
| 9 | 7.71 (br s) | 7.62 (s) | 135.3 | 135.19 |
| 1′ | - | - | 127.0 | 126.48 |
| 2′ | 7.40 (d, *J* = 8.7) | 7.39 (d, *J* = 8.5) | 132.0 | 132.24 |
| 3′ | 7.05 (d, *J* = 8.7) | 7.03 (d, *J* = 8.5) | 114.2 | 114.28 |
| 4′ | - | - | 160.8 | 160.24 |
| 5′ | 7.05 (d, *J* = 8.7) | 7.03 (d, *J* = 8.5) | 114.2 | 114.28 |
| 6′ | 7.40 (d, *J* = 8.7) | 7.3.9 (d, *J* = 8.5) | 132.0 | 132.24 |
| 4′-OMe | 3.87 (s) | 3.81 (s) | 54.9 | 55.31 |

### 3.1.13 Compound CM13
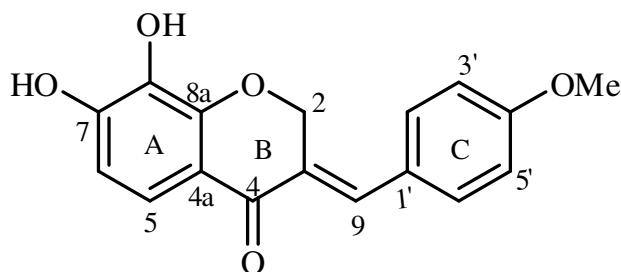


Compound **CM13** was isolated as a yellow solid, mp 191-192 °C. The absorption bands of UV and IR spectrum were similar to compound **CM12**. The $^1$H and $^{13}$C NMR spectra (Table 21, Figures 47 and 48) of compound **CM13** were comparable with those of compound **CM12**. The difference was shown as the disappearance of the signals of a 1,2,4-trisubstituted benzene ring in **CM12** but the appearance of a 1,2,3,4-tetrasubstitutated benzene ring as signals of *ortho*-coupled aromatic protons at $\delta$ 6.54 (d, $J$ = 8.4 Hz, H-6) and 7.41 (d, $J$ = 8.4 Hz, H-5). Thus, **CM13** was identified as (*E*)-7,8-dihydroxy-3-(4-methoxybenzyl-chroman-4-one) (Chen and Yang, 2007).
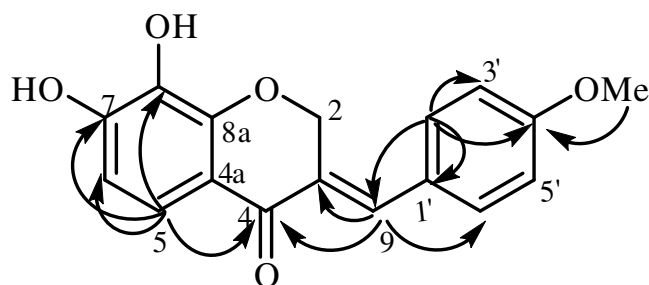


Selective HMBC correlations of **CM13**

**Table 21** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CM13**

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 2 | 5.43 (d, *J* = 1.8) | 68.1 | CH$_2$ | 3, 4, 9, 8a, 1′ |
| 3 | - | 129.6 | C | - |
| 4 | - | 181.1 | C | - |
| 4a | - | 115.8 | C | - |
| 5 | 7.41 (d, *J* = 8.4) | 118.9 | CH | 4, 6, 8, 7 |
| 6 | 6.54 (d, *J* = 8.4) | 110.2 | CH | 4a, 7, 8 |
| 7 | - | 151.6 | C | - |
| 8 | - | 132.6 | C | - |
| 8a | - | 150.1 | C | - |
| 9 | 7.72 (s) | 135.5 | CH | 3, 4, 1′, 2′, 6′ |
| 1′ | - | 127.1 | C | - |
| 2′ | 7.41 (d, *J* = 9.0) | 132.0 | CH | 9, 1′, 3′, 4′, 5′, 6′ |
| 3′ | 7.06 (d, *J* = 9.0) | 114.2 | CH | 1′, 4′, 5′ |
| 4′ | - | 160.8 | C | - |
| 5′ | 7.06 (d, *J* = 9.0) | 114.2 | CH | 1′, 3′, 4′ |
| 6′ | 7.41 (d, *J* = 9.0) | 132.0 | CH | 9, 1′, 2′, 3′, 4′, 5′ |
| 4′-OMe | 3.88 (s) | 54.9 | CH$_3$ | 4′ |

**Table 22** Comparison of ^{1}H and ^{13}C NMR spectra data between compounds **CM13** (recorded in *acetone-d₆*, 300 MHz) and (*E*)-7,8-dihydroxy-3-(4-methoxybenzylchroman-4-one) (**R**, recorded in DMSO-*d₆*, 500 Hz)

| Position | CM13 $\delta_H$ (mult., *J*, Hz) | R $\delta_H$ (mult., *J*, Hz) | CM13 $\delta_C$ | R $\delta_C$ |
|---|---|---|---|---|
| 2 | 5.43 (d, *J* = 1.8) | 5.35 (d, *J* = 1.5) | 68.1 | 67.78 |
| 3 | - | - | 129.6 | 129.13 |
| 4 | - | - | 181.1 | 180.15 |
| 4a | - | - | 115.8 | 115.08 |
| 5 | 7.41 (d, *J* = 8.4) | 7.25 (d, *J* = 9) | 118.9 | 118.35 |
| 6 | 6.54 (d, *J* = 8.4) | 6.55 (d, *J* = 9) | 110.2 | 110.43 |
| 7 | - | - | 151.6 | 152.34 |
| 8 | - | - | 132.6 | 132.69 |
| 8a | - | - | 150.1 | 150.34 |
| 9 | 7.72 (s) | 7.63 (s) | 135.5 | 135.17 |
| 1′ | - | - | 127.1 | 126.54 |
| 2′ | 7.41 (d, *J* = 9.0) | 7.41 (d, *J* = 9.0) | 132.0 | 132.12 |
| 3′ | 7.06 (d, *J* = 9.0) | 7.05 (d, *J* = 9.0) | 114.2 | 114.29 |
| 4′ | - | - | 160.8 | 160.23 |
| 5′ | 7.06 (d, *J* = 9.0) | 7.05 (d, *J* = 9.0) | 114.2 | 132.12 |
| 6′ | 7.41 (d, *J* = 9.0) | 7.41 (d, *J* = 9.0) | 132.0 | 114.29 |
| 4′-OMe | 3.88 (s) | 3.81 (s) | 54.9 | 55.32 |

**3.1.14 Compound CM14**
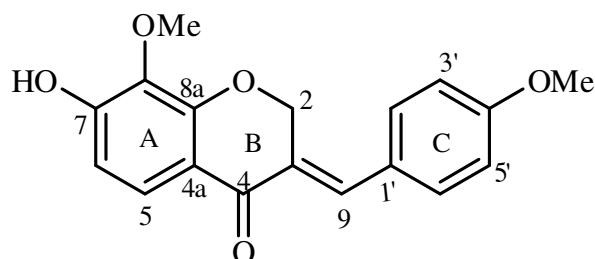


Compound **CM14** was isolated as a yellow solid, mp 103-105 °C. The absorption bands of UV and IR spectra were similar to **CM13**. The $^1$H NMR spectral data (Table 23, Figure 49) of **CM14** and **CM13** showed structure similarity, except for appearance of a methoxyl signal at $\delta_H$ 3.93 (s) in **CM14**. In the NOESY spectrum, the methoxyl signal at $\delta_H$ 3.93 (8-OMe) did not showed a cross-peak with the aromatic proton at $\delta$ 6.70 (d, $J$ = 9.0 Hz, H-6), thus establishing a methoxyl group at C-8. Therefore, **CM14** was identified as (*E*)-7-hydroxy-8-methoxy-3-(4-methoxybenzyl-chroman-4-one) (Chen and Yang, 2007).
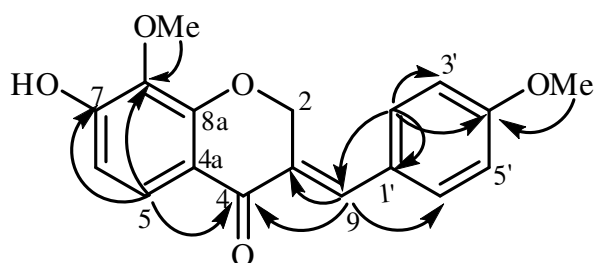


Selective HMBC correlation of **CM14**

**Table 23** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CM14**

| Position | δ$_H$ (mult., *J*, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 2 | 5.43 (d, *J* = 1.8) | 68.3 | CH$_2$ | 3, 4, 8a, 9 |
| 3 | - | 127.1 | C | - |
| 4 | - | 181.0 | C | - |
| 4a | - | 116.7 | C | - |
| 5 | 7.74 (d, *J* = 9.0) | 124.2 | CH | 4, 8, 7 |
| 6 | 6.70 (d, *J* = 9.0) | 109.8 | CH | 4a, 7, 8 |
| 7 | - | 155.0 | C | - |
| 8 | - | 134.3 | C | - |
| 8a | - | 154.1 | C | - |
| 9 | 7.83 (s) | 137.1 | CH | 3, 4, 2′, 6′ |
| 1′ | - | 127.1 | C | - |
| 2′ | 7.27 (d, *J* = 8.7) | 131.9 | CH | 9, 3′, 4′, 5′, 6′ |
| 3′ | 6.97 (d, *J* = 8.7) | 114.3 | CH | 1′, 2′, 4′, 5′, 6′ |
| 4′ | - | 160.7 | C | - |
| 5′ | 6.97 (d, *J* = 8.7) | 114.3 | CH | 1′, 2′, 3′, 4′, 6′ |
| 6′ | 7.28 (d, *J* = 8.7) | 131. 6 | CH | 4, 9, 2′, 3′, 5′ |
| 4′-OMe | 3.87 (s) | 55.4 | CH$_3$ | 4′ |
| 8-OMe | 3.93 (s) | 61.3 | CH$_3$ | 8 |

**Table 24** Comparison of $^1$H and $^{13}$C NMR spectra data between compounds **CM14** (recorded in acetone-$d_6$, 300 MHz) and (*E*)-7-hydroxy-8-methoxy-3-(4-methoxybenzyl-chroman-4-one) (**R**, recorded in DMSO-$d_6$, 500 Hz)

| Position | CM14 $\delta_H$ (mult., *J*, Hz) | R $\delta_H$ (mult., *J*, Hz) | CM14 $\delta_C$ | R $\delta_C$ |
|---|---|---|---|---|
| 2 | 5.43 (d, *J* = 1.8) | 5.41 (s) | 68.3 | 67.78 |
| 3 | - | - | 127.1 | 128.63 |
| 4 | - | - | 181.0 | 179.77 |
| 4a | - | - | 116.7 | 115.27 |
| 5 | 7.74 (d, *J* = 9.0) | 7.49 (d, *J* = 9.5) | 124.2 | 123.06 |
| 6 | 6.70 (d, *J* = 9.0) | 6.61 (d, *J* = 9.5) | 109.8 | 111.04 |
| 7 | - | - | 155.0 | 156.94 |
| 8 | - | - | 134.3 | 135.03 |
| 8a | - | - | 154.1 | 155.10 |
| 9 | 7.83 (s) | 7.64 (s) | 137.1 | 135.46 |
| 1′ | - | - | 127.1 | 126.45 |
| 2′ | 7.27 (d, *J* = 8.7) | 7.41 (d, *J* = 8.5) | 131.9 | 132.24 |
| 3′ | 6.97 (d, *J* = 8.7) | 7.04 (d, *J* = 8.5) | 114.3 | 114.29 |
| 4′ | - | - | 160.7 | 160.31 |
| 5′ | 6.97 (d, *J* = 8.7) | 7.04 (d, *J* = 8.5) | 114.3 | 114.29 |
| 6′ | 7.28 (d, *J* = 8.7) | 7.41 (d, *J* = 8.5) | 131. 6 | 132.24 |
| 4′-OMe | 3.87 (s) | 3.70 (s) | 55.4 | 55.33 |
| 8-OMe | 3.93 (s) | 3.81 (s) | 61.3 | 60.28 |

### 3.1.15 Compound CM15
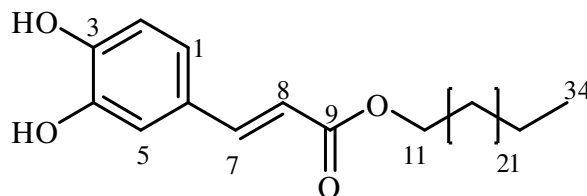


Compound **CM15** was obtained as a white solid, mp 85-86 °C. The UV ($\lambda_{max}$ 205, 243, 298 and 330 nm) and IR (3367, 1700 cm$^{-1}$) absorption bands supported the presence of conjugated carbonyl and hydroxyl group in the structure.

The $^1$H NMR spectral data (Table 25, Figure 51) displayed two olefinic protons at $\delta$ 6.28 and 7.55 (each 1H, d, $J$ = 15.6 Hz) which were identified as *trans*-double bond at H-8 and H-7, respectively. The aromatic proton signals at $\delta$ 6.87 (d, $J$ = 8.4 Hz), 7.03 (dd, $J$ = 8.4, 1.8 Hz) and 7.16 (d, $J$ = 1.8 Hz) were assigned as a 1,2,4-trisubstitued benzene ring. In addition, the $^1$H NMR spectrum displayed the presence of oxymethylene protons at $\delta$ 4.15 (t, $J$ = 6.6 Hz), a methyl signal at $\delta$ 0.88 (t, $J$ = 6.6 Hz) and a long chain group at $\delta$ 1.29 (m, 42H). In the COSY experiment, oxymethylene protons at $\delta$ 4.15 showed cross-peak with the methylene protons at $\delta$ 1.68 (m) and the methyl signal at $\delta$ 0.88 showed cross-peak with the methylene protons at $\delta$ 1.29 (m). The EIMS yielded a quasi-molecular ion at $m/z$ 516 consistent with the molecular formula $C_{33}H_{56}O_4$, which showed a major fragment ion at $m/z$ 179 indicating the caffeic acid fragment. Subtraction of molecular mass of these moieties (179 units) from M$^+$ (516 units) gives us the remaining unaccounted 337 units, which suitably fits for a long saturated $-(CH_2)_{23}CH_3$.

The structure of **CM15** was confirmed by HMBC correlations. The proton signal at $\delta$ 7.55 (H-7) showed correlations with the carbons at $\delta$ 114.3 (C-5), 114.8 (C-8), 126.8 (C-1), 126.8 (C-6) and 166.6 (C-9) and oxymethylene protons at $\delta$ 4.15 (2H-11) showed correlations with the carbons at $\delta$ 28.5 (C-12) and 166.6 (C-9), suggesting that the 1,2,4-trisubstitued benzene ring was connected to C-7. In addition H-7 showed a cross-peak with the proton at $\delta$ 7.16 (H-5) in the NOESY experiment. Thus, **CM15** was identified as tetracosyl caffeate (Tanaka et al., 1998).
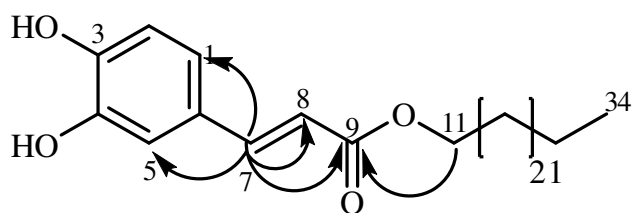
Selective HMBC correlations of **CM15**

**Table 25** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CM15**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|----------|------------------------------|-----------|----------|----------------|
| 1 | 7.03 (dd, $J$ = 8.4, 1.8) | 126.8 | CH | 3, 5, 7 |
| 2 | 6.87 (d, $J$ = 8.4) | 115.5 | CH | 3, 4, 6 |
| 3 | - | 147.8 | C | - |
| 4 | - | 145.4 | C | - |
| 5 | 7.16 (d, $J$ = 1.8) | 114.3 | CH | 1, 3, 5, 7 |
| 6 | - | 126.8 | C | - |
| 7 | 7.55 (d, $J$ = 15.6) | 144.0 | CH | 1, 5, 6, 8, 9 |
| 8 | 6.28 (d, $J$ = 15.6) | 114.8 | CH | 6, 7, 9 |
| 9 | - | 166.6 | C | - |
| 10 | - | | - | - |
| 11 | 4.15 (t, $J$ = 6.6) | 63.8 | CH$_2$ | 9, 12, 13 |
| 12 | 1.68 (m) | 28.5 | CH$_2$ | 11 |
| 13-33 | 1.29 (m) | 22.5-31.8 | CH$_2$ | - |
| 34 | 0.88 (t, $J$ = 6.6) | 13.5 | CH$_3$ | - |

**Table 26** Comparison of ^{1}H NMR spectral data between compounds **CM15** (recorded in *acetone-d₆*, 300 MHz) and tetracosyl caffeate (**R**, recorded in *acetone-d₆*)

| Position | CM15 δ$_H$ (mult., *J*, Hz) | R δ$_H$ (mult., *J*, Hz) |
|---|---|---|
| 1 | 7.03 (dd, *J* = 8.4, 1.8) | 7.03 (dd, *J* = 8.0, 1.0) |
| 2 | 6.87 (d, *J* = 8.4) | 6.86 (d, *J* = 8.0) |
| 3 | - | - |
| 4 | - | - |
| 5 | 7.16 (d, *J* = 1.8) | 7.15 (d, *J* = 1.0) |
| 6 | - | - |
| 7 | 7.55 (d, *J* = 15.6) | 7.55 (d, *J* = 16.0) |
| 8 | 6.28 (d, *J* = 15.6) | 6.27 (d, *J* = 16.0) |
| 9 | - | - |
| 10 | - | - |
| 11 | 4.15 (t, *J* = 6.6) | 4.14 (t, *J* = 7.0) |
| 12 | 1.68 (m) | 1.68 (m) |
| 13-33 | 1.29 (m) | 1.3 (m) |
| 34 | 0.88 (t, *J* = 6.6) | 0.89 (t, *J* = 6.6) |

### 3.1.16 Compound CM16
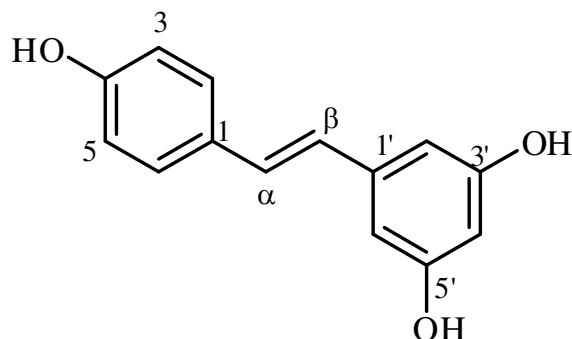


Compound **CM16** was obtained as a white solid, mp 250-251 °C. The UV absortion bands at $\lambda_{max}$ 216, 305, 318 nm supported the presence of a conjugated chromophore in the structure. The IR spectrum showed absorption bands of hydroxyl group (3360 cm$^{-1}$), and aromatic stretching (1607 cm$^{-1}$).

The $^{13}$C NMR and DEPT spectral data (Table 27, Figure 54) exhibited 14 carbons, including nine methines ($\delta$ 101.8, 104.8 (2C), 115.5 (2C), 126.0, 128.2 (2C), 129.1) and five quaternary carbons ($\delta$ 129.9, 140.0, 157.3, 158.7 (2C)). The $^1$H NMR spectral data (Table 27, Figure 53) displayed the presence of a 1,4-disubstituted benzene ring at $\delta$ 7.42, 6.85 (each 2H, dd, $J$ = 8.4, 1.8 Hz ), and a 1,3,5-trisustituted benzene ring at $\delta$ 6.29 (1H, t, $J$ = 2.1 Hz) and 6.56 (2H, t, $J$ = 2.1 Hz). In addition, the proton signals at $\delta$ 6.90 and 7.03 (each, 1H, d, $J$ = 16.5 Hz) were deduced as a *trans* double bond at C-$\alpha$ and C-$\beta$, respectively. The locations of a 1,4-disubstituted and a 1,3,5-trisustituted benzene ring were confirmed by HMBC correlations of an olefinic proton at $\delta$ 7.03 (H-$\alpha$) with the carbons at $\delta$ 126.0 (C-$\beta$), 128.2 (C-2, 6) and 140.0 (C-1'), and the olefinic proton at $\delta$ 6.90 (H-$\beta$) with the carbons at $\delta$ 104.8 (C-2', 6'), 129.1 (C-$\alpha$), 129.9 (C-1) and 140.0 (C-1'). Thus, compound **CM16** was identified as *trans*-resveratrol (Guiso et al., 2002).
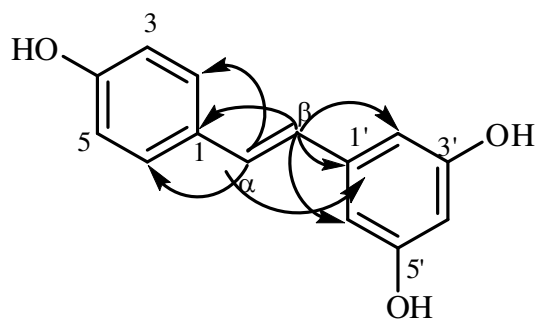
Selective HMBC correlations of **CM16**
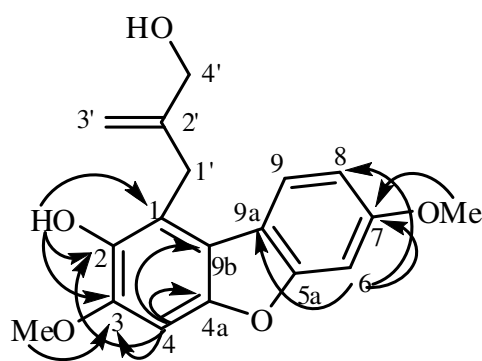
**Table 27** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CM16**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| α | 7.03 (d, $J$ = 16.5) | 129.1 | CH | 2, 6, 1', β |
| 1 | - | 129.9 | C | - |
| 2 | 7.42 (dd, $J$ = 8.4, 1.8) | 128.2 | CH | 3, 4, 5, 6, α |
| 3 | 6.85 (dd, $J$ = 8.4, 1.8) | 115.5 | CH | 1, 4, 5 |
| 4 | - | 157.3 | C | - |
| 5 | 6.85 (dd, $J$ = 8.4, 1.8) | 115.5 | CH | 1, 3, 4 |
| 6 | 7.42 (dd, $J$ = 8.4, 1.8) | 128.2 | CH | 2, 3, 4, 5, α |
| β | 6.90 (d, $J$ = 16.5) | 126.0 | CH | α, 1, 1', 2', 6' |
| 1' | - | 140.0 | C | - |
| 2' | 6.56 (t, $J$ = 2.1) | 104.8 | CH | 3', 4', 5', 6' |
| 3' | - | 158.7 | C | - |
| 4' | 6.29 (t, $J$ = 2.1) | 101.8 | CH | 2', 3', 5', 6' |
| 5' | - | 158.7 | C | - |
| 6' | 6.56 (t, $J$ = 2.1) | 104.8 | CH | 2', 3', 4', 5' |

**Table 28** Comparison of $^1$H and $^{13}$C NMR spectra data between compounds **CM16** (recorded in acetone-$d_6$, 300 MHz) and *trans*-resveratrol (**R**, recorded in acetone-$d_6$)

| Position | CM16 δ$_H$ (mult., *J*, Hz) | R δ$_H$ (mult., *J*, Hz) | CM16 δ$_C$ | R δ$_C$ |
|---|---|---|---|---|
| α | 7.03 (d, *J* = 16.5) | 6.98 (d, *J* = 16.2) | 129.1 | 128.9 |
| 1 | - | - | 129.9 | 129.8 |
| 2 | 7.42 (dd, *J* = 8.4, 1.8) | 7.39 (d, *J* = 8.1) | 128.2 | 128.5 |
| 3 | 6.85 (dd, *J* = 8.4, 1.8) | 6.82 (d, *J* = 8.1) | 115.5 | 116.2 |
| 4 | - | - | 157.3 | 158.1 |
| 5 | 6.85 (dd, *J* = 8.4, 1.8) | 6.82 (d, *J* = 8.1) | 115.5 | 116.2 |
| 6 | 7.42 (dd, *J* = 8.4, 1.8) | 7.39 (d, *J* = 8.1) | 128.2 | 128.5 |
| β | 6.90 (d, *J* = 16.5) | 6.87 (d, *J* = 16.2) | 126.0 | 126.6 |
| 1′ | - | - | 140.0 | 140.6 |
| 2′ | 6.56 (t, *J* = 2.1) | 6.52 (t, *J* = 2.1) | 104.8 | 105.5 |
| 3′ | - | - | 158.7 | 159.3 |
| 4′ | 6.29 (t, *J* = 2.1) | 6.24 (t, *J* = 2.1) | 101.8 | 102.5 |
| 5′ | - | - | 158.7 | 159.3 |
| 6′ | 6.56 (t, *J* = 2.1) | 6.52 (t, *J* = 2.1) | 104.8 | 105.5 |

### 3.1.17 Compound CM17
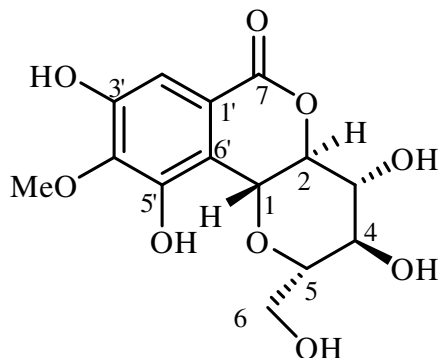


Compound **CM17** was isolated as a white solid, mp 154-156 °C, $[\alpha]_D^{27}$ – 53.1° (*c* 1.71 in CH₃OH). The UV spectrum displayed maximum absorption at $\lambda_{max}$ 219, 275, 311 nm, suggesting the presence of conjugation in the molecule. The IR spectrum suggested hydroxyl (3381 cm⁻¹) and carbonyl (1699 cm⁻¹) functionalities. The ¹³C and DEPT NMR spectral data (Table 29, Figure 56) indicated the presence of 14 carbons including six aromatic, five oxymetine, one oxymethylene, one methoxyl and one carbonyl carbons. The ¹H NMR spectral data (Table 29, Figure 55) displayed the presence of characteristic signal of sugar moiety. The oxymetine proton at $\delta$ 4.95 (d, *J* = 9.0 Hz, H-1), was inferred to β-configuration of sugar moiety based on the value of the coupling constant. Other proton signal of sugar moiety were resonances at $\delta$ 3.47 (t, *J* = 9.0 Hz, H-4), 3.69 (m, H-5), 3.74 (dd, *J* = 9.0, 6.6 Hz, H$_a$-6), 3.85 (t, *J* = 9.0 Hz, H-3), 4.05 (dd, *J* = 9.0, 6.6 Hz, H$_b$-6) and 4.08 (t, *J* = 9.0 Hz, H-2) and the large vicinal coupling constants ($J_{ax,ax}$ = 9.0 Hz), confirming the *β-C*-glucoside ring . The proton signal displayed the presence of a one proton singlet at $\delta$ 7.09 which was assigned as H-2′. From the HMBC experiments, the aromatic proton at $\delta$ 7.09 (H-2′) showed correlations with the carbons at $\delta$ 72.8 (C-1), 116.0 (C-6′), 118.0 (C-1′), 140.9 (C-4′), 150.9 (C-3′) and 164.6 (C-7), the oxymethine proton at $\delta$ 4.08 (H-2) with the carbons at $\delta$ 72.8 (C-1), 74.2 (C-3), 116.0 (C-6′) and 164.6 (C-7), and the oxymetine proton at $\delta$ 4.95 (H-1) with the carbons at $\delta$ 74.2 (C-3), 79.9 (C-2), 81.5 (C-5), 116.0 (C-6′), 118.0 (C-1′), 140.9 (C-4′) and 148.0 (C-5′). These data suggested an aryl *β-C-*

glucoside and an aryl δ-lactone ring. Therefore, compound **CM17** was identified as bergenin (Wang et al., 2005).
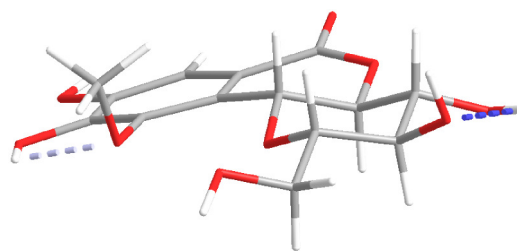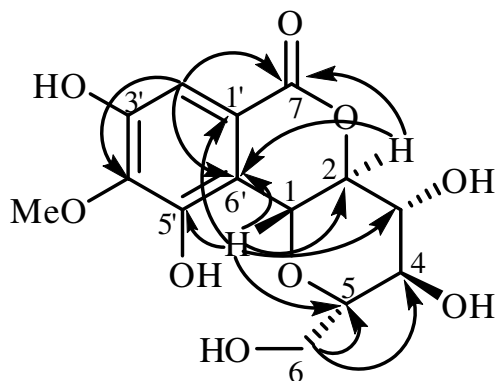


Selective HMBC correlations of **CM17**          Conformation of **CM17**

**Table 29** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CM17**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 4.95 (d, $J$ = 9.0) | 72.8 | CH | 2, 3, 5, 1′, 4′, 5′, 6′ |
| 2 | 4.08 (t, $J$ = 9.0) | 79.9 | CH | 1, 3, 7, 6′ |
| 3 | 3.85 (t, $J$ = 9.0) | 74.2 | CH | 1, 2, 5 |
| 4 | 3.47 (t, $J$ = 9.0) | 70.4 | CH | 5, 6 |
| 5 | 3.69 (m) | 81.5 | CH | 4 |
| 6 | 3.74 (dd, $J$ = 9.0, 6.6) | 61.2 | CH$_2$ | 4, 5 |
|  | 4.05 (dd, $J$ = 9.0, 6.6) |  |  |  |
| 7 | - | 164.6 | C | - |
| 1′ | - | 118.0 | C | - |
| 2′ | 7.09 (s) | 109.8 | CH | 1, 7, 1′, 3′, 4′, 6′ |
| 3′ | - | 150.9 | C | - |
| 4′ | - | 140.9 | C | - |
| 5′ | - | 148.0 | C | - |
| 6′ | - | 116.0 | C | - |
| 4′-OMe | 3.91 (s) | 59.2 | CH$_3$ | 4′ |

**Table 30** Comparison of $^1$H and $^{13}$C NMR spectra data between compounds **CM17** (recorded in DMSO-$d_6$, 300 Hz) and bergenin (**R**, recorded in DMSO-$d_6$, 500 Hz)

| Position | CM17 $\delta_H$ (mult., *J*, Hz) | R $\delta_H$ (mult., *J*, Hz) | CM17 $\delta_C$ | R $\delta_C$ |
|---|---|---|---|---|
| 1 | 4.95 (d, *J* = 9.0) | 4.95 (d, *J* = 10.4) | 72.8 | 72.2 |
| 2 | 4.08 (t, *J* = 9.0) | 3.98 (t, *J* = 9.9) | 79.9 | 79.8 |
| 3 | 3.85 (t, *J* = 9.0) | 3.65 (m) | 74.2 | 73.7 |
| 4 | 3.47 (t, *J* = 9.0) | 3.21 (m) | 70.4 | 70.7 |
| 5 | 3.69 (m) | 3.56 (t, *J* = 7.6) | 81.5 | 81.7 |
| 6 | 3.74 (dd, *J* = 9.0, 6.6) | 3.44 (m) | 61.2 | 61.1 |
|  | 4.05 (dd, *J* = 9.0, 6.6) |  |  |  |
| 7 | - | - | 164.6 | 163.3 |
| 1′ | - | - | 118.0 | 118.0 |
| 2′ | 7.09 (s) | 6.98 (s) | 109.8 | 109.5 |
| 3′ | - | - | 150.9 | 150.9 |
| 4′ | - | - | 140.9 | 140.6 |
| 5′ | - | - | 148.0 | 148.0 |
| 6′ | - | - | 116.0 | 116.0 |
| 4′-OMe | 3.91 (s) | 3.77 (s) | 59.2 | 59.8 |

### 3.1.18 Compound CM18
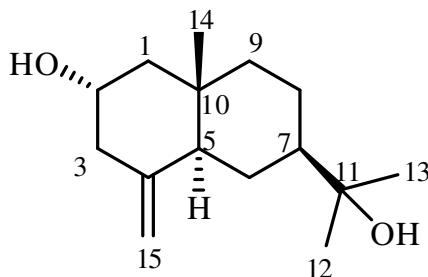


Compound **CM18** was isolated as a white solid, mp 99-100 °C, $[\alpha]_D^{27}$ + 45.6° (*c* 0.24 in CH$_3$OH). The IR spectrum showed absorption band of hydroxyl group (3365 cm$^{-1}$). The $^{13}$C NMR and DEPT spectra (Table 31, Figure 58) exhibited 15 carbons, attributable to three methyl, six methylene, three methine and three quaternary carbons indicating a sesquiterpenoid skeleton. Two low-field signals at $\delta$ 106.0 and 149.1 representing two carbons of an exocyclic double bond and the signals at $\delta$ 66.9 and 71.1 indicated the presence of two oxygenated carbons in the molecule. The $^1$H NMR spectrum (Table 31, Figure 57) displayed the presence of three singlet signals at $\delta$ 0.70 (3H, s, Me-14) and 1.16 (6H, s, Me-12 and 13), a set of methylene protons at $\delta$ 4.54 and 4.78 (each dd, *J* = 3.3, 1.8 Hz, 2H-15) and an oxymethine proton at $\delta$ 3.78 (m, H-2). On the basis of HMBC experiment, the correlations of an olefinic protons at $\delta$ 4.54 and 4.78 (2H-15) with the carbons at $\delta$ 46.6 (C-3), 49.1 (C-5) and 149.1(C-4) and of methyl protons at $\delta$ 0.70 (Me-14) with the carbons at $\delta$ 34.8 (C-10), 40.9 (C-9), 49.1 (C-5) and 51.1 (C-1) confirmed the structure of **CM18**. The relative stereochemistry of **CM18** was analyzed by NOESY correlations, the methyl protons at $\delta$ 0.70 (Me-14) showed a cross-peak with the oxymethine proton at $\delta$ 3.78 (m, H-2).

The optical rotation of **CM18** is dextrorotatory ($[\alpha]_D^{27}$ + 45.6°), the same as (+)-ptercarpol (lit. $[\alpha]_D^{27}$ + 30.6°) (Nasini and Piozzi, 1981) suggesting the same configuration at C-2, C-5, C-7 and C-10. Thus **CM18** was assigned as (+)-ptercarpol (Nasini and Piozzi, 1981).
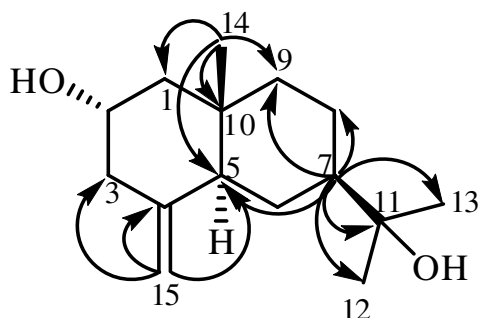
Selective HMBC correlation of **CM18**

**Table 31** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CM18**

| Position | δ$_H$ (mult., *J*, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.23 (m) | 51.1 | CH$_2$ | 3, 2, 5, 9, 10, 14 |
| | 1.55 (m) | | | |
| 2 | 3.78 (m) | 66.9 | CH | - |
| 3 | 1.94 (t, *J* = 11.7) | 46.6 | CH$_2$ | 1, 2, 4, 5, 15 |
| | 2.59 (ddd, *J* = 11.7, 4.8, 1.8) | | | |
| 4 | - | 149.1 | C | - |
| 5 | 1.75 (m) | 49.1 | CH | 3, 4, 7, 10, 14, 15 |
| 6 | 1.18 (m) | 29.6 | CH$_2$ | 5, 7, 8, 10 |
| | 1.72 (m) | | | |
| 7 | 1.39 (m) | 49.4 | CH | 8, 9, 11, 13, 14 |
| 8 | 1.32 (m) | 21.8 | CH$_2$ | 9, 10 |
| | 1.65 (m) | | | |
| 9ax | 1.19 (m) | 40.9 | CH$_2$ | 1, 5, 7, 8, 10, 14 |
| eq | 1.55 (dt, *J* = 11.7, 3.3) | | | |
| 10 | - | 34.8 | C | - |
| 11 | - | 71.1 | C | - |
| 12 | 1.16 (s) | 26.5 | CH$_3$ | 7, 11, 13 |
| 13 | 1.16 (s) | 26.9 | CH$_3$ | 7, 11, 12 |
| 14 | 0.70 (s) | 16.7 | CH$_3$ | 1, 5, 9, 10 |
| 15 | 4.54 (dd, *J* = 3.3, 1.8) | 106.0 | CH$_2$ | 3, 4, 5 |
| | 4.78 (dd, *J* = 3.3, 1.8) | | | |

**Table 32** Comparison of $^1$H and $^{13}$C NMR spectral data between compounds **CM18** (recorded in acetone-$d_6$, 300 Hz) and (+)-ptercarpol (**R**, recorded in CDCl$_3$, 400 Hz)

| Position | CM18 $\delta_H$ (mult., *J*, Hz) | CM18 $\delta_C$ | R $\delta_C$ |
|---|---|---|---|
| 1 | 1.23 (m) | 51.1 | 51.03 |
|  | 1.55 (m) |  |  |
| 2 | 3.78 (m) | 66.9 | 67.89 |
| 3 | 1.94 (t, *J* = 11.7) | 46.6 | 46.55 |
|  | 2.59 (ddd, *J* = 11.7, 4.8, 1.8) |  |  |
| 4 | - | 149.1 | 148.21 |
| 5 | 1.75 (m) | 49.1 | 49.41 |
| 6 | 1.18 (m) | 29.6 | 40.88 |
|  | 1.72 (m) |  |  |
| 7 | 1.39 (m) | 49.4 | 49.23 |
| 8 | 1.32 (m) | 21.8 | 24.69 |
|  | 1.65 (m) |  |  |
| 9ax | 1.19 (m) | 40.9 | 22.00 |
| eq | 1.55 (dt, *J* = 11.7, 3.3) |  |  |
| 10 | - | 34.8 | 35.25 |
| 11 | - | 71.1 | 72.82 |
| 12 | 1.16 (s) | 26.9 | 27.36 |
| 13 | 1.16 (s) | 26.5 | 27.12 |
| 14 | 0.70 (s) | 16.7 | 17.25 |
| 15 | 4.54 (dd, *J* = 3.3, 1.8) | 106.0 | 107.97 |
|  | 4.78 (dd, *J* = 3.3, 1.8) |  |  |

**3.2 Anti-inflammatory of compounds CM1-CM18 from the roots of**

   *C. mimosoides*

The $CH_2Cl_2$ and acetone extracts exhibited potent inhibitory activity against LPS-induced NO production in RAW264.7 cell lines with $IC_{50}$ values of 11.0 and 21.6 µg/ml, respectively. Therefore all isolated compounds were evaluated for their anti-NO activity whose results were shown in Table 33. Compound **CM4** ($IC_{50}$ = 3.0 µM) possessed the highest activity, followed by compounds **CM13**, **CM12**, **CM14**, **CM8** and **CM6** ($IC_{50}$ = 3.9, 4.4, 5.6, 7.1 and 8.2 µM, respectively), whereas other compounds exhibited moderate and mild activities. The inhibitory activities of all compounds were much stronger than that of NO synthase inhibitor (L-nitroarginine (L-NA), $IC_{50}$ = 61.8 µM) except compound **CM17** showed weaker activity ($IC_{50}$ = 83.0 µM). Compounds **CM4**, **CM12** and **CM13** also showed higher inhibitory activity than caffeic acid phenethylester (CAPE) ($IC_{50}$ = 5.6 µM). Structure–activity relationships of these classes of diterpenes (**CM1–CM9**) for anti-inflammatory activity are suggested as follows: (i) the acetoxyl group on the molecule was necessary for increasing the activity: compound **CM6** with the acetoxyl group was strongly active ($IC_{50}$ = 8.2 µM), whereas compound **CM5** was much less active ($IC_{50}$ = 56.8 µM). (ii) One hydroxyl substituent gave higher activity than two hydroxyls as shown in compound **CM8** ($IC_{50}$ = 7.1 µM) vs. compounds **CM2** and **CM3** ($IC_{50}$ = 19.3 and 15.4 µM), respectively. This result implied that a hydroxyl substitution at other positions besides C-16 decreased the activity. Compounds **CM4, CM6**, **CM8** and **CM12–CM14** were also tested for the inhibitory effect on LPS-induced TNF-α release in RAW264.7 cells (Table 34). The results revealed that **CM4** and **CM12** possessed the most potent activity against TNF-α release with $IC_{50}$ values of 6.5 and 9.5 µM, respectively, whereas, compounds **CM6**, **CM8**, **CM13,** and **CM14** exhibited moderate activity with $IC_{50}$ values of 38.8, 35.2, 11.4, and 14.6 µM, respectively. From the present study, compound **CM4** was a new compound that showed strong inhibition on both NO and TNF-α releases.

**Table 33** Inhibitory effects on NO production of compounds **CM1**–**CM18** from
*C. mimosoides*

| No | % Inhibition at various concentrations (μM) | | | | | | IC$_{50}$ |
|---|---|---|---|---|---|---|---|
| | **0** | **1** | **3** | **10** | **30** | **100** | **(μM)** |
| **CM1** | 0.0 ± 6.9 | - | - | 36.7 ± 3.3** | 68.4 ± 2.1** | 99.8 ± 2.3** | 15.9 |
| **CM2** | 0.0 ± 5.6 | - | - | 35.3 ± 2.8** | 54.0 ± 2.9** | 101.9 ± 2.3** | 19.3 |
| **CM3** | 0.0 ± 5.6 | - | - | 32.8 ± 2.3** | 78.1 ± 4.4** | 99.4 ± 3.2[b]** | 15.4 |
| **CM4** | 0.0 ± 5.6 | 19.4 ± 3.1 | 49.3 ± 2.5** | 83.7 ± 0.9** | 89.3 ± 3.1[b]** | 100.2 ± 2.5[b]** | 3.0 |
| **CM5** | 0.0 ± 9.6 | - | - | 5.1 ± 1.2 | 25.6 ± 2.4 | 68.9 ± 2.6** | 56.8 |
| **CM6** | 0.0 ± 9.6 | - | 25.0 ± 2.3* | 60.4 ± 4.1** | 75.8 ± 2.5** | 100.3 ± 3.5[b]** | 8.2 |
| **CM7** | 0.0 ± 6.9 | - | - | 40.5 ± 3.6** | 75.0 ± 2.1** | 98.1 ± 3.3** | 13.2 |
| **CM8** | 0.0 ± 6.9 | - | 27.1 ± 4.4* | 51.9 ± 2.9** | 98.0 ± 3.5[b]** | 100.0 ± 1.9[b]** | 7.1 |
| **CM9** | 0.0 ± 4.2 | - | - | 23.3 ± 2.1** | 60.9 ± 2.0** | 94.3 ± 3.5** | 22.8 |
| **CM10** | 0.0 ± 4.4 | - | - | 31.2 ± 1.2 | 49.6 ± 3.7 ** | 81.0 ± 3.6** | 25.9 |
| **CM11** | 0.0 ± 4.4 | - | - | 4.8 ± 2.9 | 28.5 ± 2.5* | 67.0 ± 4.4** | 57.2 |
| **CM12** | 0.0 ± 4.9 | - | 39.5 ± 2.4* | 71.0 ± 3.8** | 95.0 ± 1.7** | 99.4 ± 3.4[b]** | 4.4 |
| **CM13** | 0.0 ± 4.9 | - | 43.4 ± 2.1** | 72.1 ± 2.0** | 97.5 ± 2.5** | 100.5 ± 2.4[b]** | 3.9 |
| **CM14** | 0.0 ± 4.4 | - | 35.4 ± 2.5* | 63.0 ± 0.7** | 85.8 ± 1.5** | 101.7 ± 3.0** | 5.6 |
| **CM15** | 0.0 ± 4.9 | - | - | 22.0 ± 3.1** | 68.5 ± 2.2[b]** | 99.7 ± 2.1[b]** | 20.8 |
| **CM16** | 0.0 ± 4.2 | - | - | 28.4 ± 3.1 | 60.2 ± 1.5** | 94.6 ± 2.3** | 21.1 |
| **CM17** | 0.0 ± 4.2 | - | - | 2.4 ± 1.5 | 22.3 ± 2.6** | 56.2 ± 3.9** | 83.0 |
| **CM18** | 0.0 ± 3.5 | - | - | 15.4 ± 1.8 | 42.8 ± 2.6** | 91.8 ± 2.0** | 31.0 |
| L–NA | 0.0 ± 9.9 | 11.7 ± 4.6 | 20.2 ± 5.9 | 34.7 ± 1.8 * | 71.6 ± 2.6** | | 61.8 |
| CAPE | 0.0 ± 9.9 | 30.7 ± 3.2* | 68.6 ± 3.4** | 98.7 ± 1.2[b]** | 98.9 ± 2.1[b]** | | 5.6 |

[a]Each value represents mean ± S.E.M. of four determinations.

Statistical significance, * *p*<0.05, ** *p*<0.01

[b]Cytotoxic effect was observed.

**Table 34** Inhibition on TNF–α production of compounds **CM4**, **CM6**, **CM8**, and **CM12**–**CM14** isolated from *C. mimosoides*

| No | % Inhibition at various concentrations (μM) | | | | | $IC_{50}$ |
|---|---|---|---|---|---|---|
| | **0** | **3** | **10** | **30** | **100** | **(μM)** |
| **CM4** | $0.0 \pm 5.7$ | $36.6 \pm 0.7$ | $60.0 \pm 1.0^{**}$ | $71.2 \pm 0.9^{**}$ | $95.2 \pm 1.5^{b**}$ | 6.5 |
| **CM6** | $0.0 \pm 5.7$ | - | $21.2 \pm 1.4$ | $37.7 \pm 2.0^{*}$ | $75.3 \pm 2.1^{**}$ | 38.8 |
| **CM8** | $0.0 \pm 5.7$ | - | $8.2 \pm 1.0^{**}$ | $42.3 \pm 1.0^{**}$ | $86.7 \pm 1.8^{**}$ | 35.2 |
| **CM12** | $0.0 \pm 5.6$ | - | $50.0 \pm 3.3^{**}$ | $72.1 \pm 2.4^{**}$ | $95.4 \pm 1.2^{b**}$ | 9.5 |
| **CM13** | $0.0 \pm 5.6$ | - | $48.5 \pm 2.5^{**}$ | $68.5 \pm 1.5^{**}$ | $99.7 \pm 0.9^{b**}$ | 11.4 |
| **CM14** | $0.0 \pm 5.7$ | - | $43.6 \pm 2.3^{**}$ | $62.1 \pm 2.0^{**}$ | $98.4 \pm 2.8^{b**}$ | 14.6 |

[a]Each value represents mean ± S.E.M. of four determinations.

[b]Cytotoxic effect was observed.

Statistical significance, * $p<0.05$, ** $p<0.01$

**3.3 Structural elucidation of compounds from the roots of *C. pulcherrima***

The $CH_2Cl_2$ extract from the roots of *C. pulcherrima* was subjected to vacuum liquid chromatography and column chromatography over silica gel to afford 15 new diterpenes (**CP1–CP15**) together with eleven known compounds (**CP16–CP26**). The known compounds were identified as vouacapen-5$\alpha$-ol (**CP16**) (McPherson et al., 1986), isovouacapenol C (**CP17**) (Ragasa et al., 2002), 6$\beta$-cinnamoyl-7$\beta$-hydroxyvouacapen-5$\alpha$-ol (**CP18**) (McPherson et al., 1986), pulcherrin A (**CP19**) (Pranithanchai et al., 2009), pulcherrin B (**CP20**) (Pranithanchai et al., 2009), pulcherrimin C (**CP21**) (Patil et al., 1997), pulcherrimin A (**CP22**) (Patil et al., 1997), pulcherrimin E (**CP23**) (Roach et al., 2003), pulcherrin C (**CP24**) (Pranithanchai et al., 2009), pulcherrimin B (**CP25**) (Patil et al., 1997) and 8,9,11,14-didehydrovouacapen-5$\alpha$-ol (**CP26**) (McPherson et al., 1986) by comparison of their spectroscopic data with those reported in the literatures and comparison with the authentic samples. Compounds **CP1–CP14** showed characteristic of the 2,3-disubstituted furan by the Ehrlich reagent (Kuroda et al., 2004) and the UV absorptions (Cheenpracha et al., 2005). The IR spectrum of all new compounds showed the presence of as ester carbonyl (1700–1777 cm$^{-1}$) and hydroxyl (3549–3425 cm$^{-1}$) functionalities.

### 3.3.1 Compound CP1
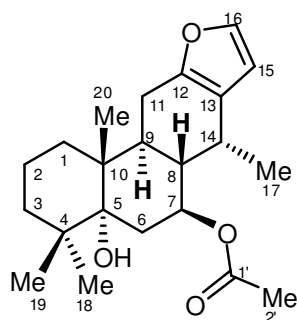


Compound **CP1** had the molecular formula $C_{22}H_{32}O_4$ ([M]$^+$ *m/z* 360.2301) based on HREIMS. The presence of a 2,3-furanocassane framework was inferred from the $^1$H and $^{13}$C NMR spectral data (Table 35, Figures 61 and 62). The $^1$H NMR spectrum showed four singlet signals of three aliphatic methyl groups at $\delta$ 0.89 (Me-18), 1.00 (Me-19), and 1.04 (Me-20) and an acetoxy methyl group at $\delta$ 2.00 (OCO*CH$_3$*) and a doublet signal of a secondary methyl group at $\delta$ 0.94 (*J* = 6.9 Hz, Me-17). The signal of a 2,3-disubstituted furan ring was evident from resonances at $\delta$ 6.12 and 7.16 (each d, *J* = 1.8 Hz, H-15 and H-16, respectively). The $^{13}$C NMR spectroscopic data displayed 22 carbons including those of an ester carbonyl carbon at $\delta$ 170.7 (O*C*OCH$_3$). An oxymethine proton was displayed at $\delta$ 5.22 (td, *J* = 11.1, 6.0 Hz, H-7; $\delta_C$ 72.3) whose coupling constants suggested its axial orientation. This proton also showed HMBC correlations to the carbons at $\delta$ 27.6 (C-14), 31.5 (C-6), 39.8 (C-8) and 170.7 (O*C*OCH$_3$) which suggested the location of the OAc group at C-7. In the NOESY spectrum, the correlations between the oxymethine proton at $\delta$ 5.22 (H-7) and the protons at $\delta$ 0.94 (Me-17), 2.01 (H-6$\alpha$) and 2.46 (H-9) placed them on the same side of the molecule. An OH group was placed at C-5 ($\delta$ 77.9) and assumed to be $\alpha$-oriented by biogenetic pathway and comparison with the previously isolated furanoditerpenoids from this plant (McPherson et al., 1986, Patil et al., 1997, Ragasa et al., 2002, Promsawan et al., 2003, Pranithanchai et al., 2009, Che et al., 1986, Das et al., 2010, Ragasa et al., 2003). From these data, **CP1** was deduced to be 7$\beta$-acetoxyvouacapen-5$\alpha$-ol, a new compound (Yodsaoue et al., 2011) and named as pulcherrin D.
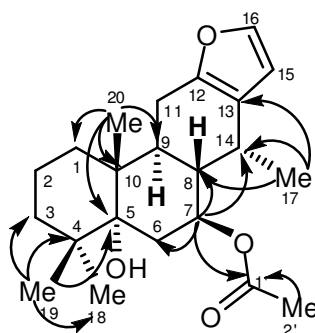
Selective HMBC correlations of **CP1**

**Table 35** ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **CP1**

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|----------|------------------------------|------------|------|------|
| 1 | 1.32 (m) | 32.3 | CH$_2$ | 3, 10, 20 |
| | 1.40 (m) | | | |
| 2 | 1.51 (m) | 18.1 | CH$_2$ | 4, 10 |
| | 1.58 (m) | | | |
| 3 | 1.11 (br d, *J* = 8.4) | 35.8 | CH$_2$ | 1, 4, 5, 18, 19 |
| | 1.57 (m) | | | |
| 4 | - | 38.5 | C | - |
| 5 | - | 77.9 | C | - |
| 6eq | 2.01 (dd, *J* = 12.9, 6.0) | 31.5 | CH$_2$ | 4, 5, 7, 8, 10 |
| ax | 1.64 (dd, *J* = 12.9, 11.1) | | | |
| 7 | 5.22 (td, *J* = 11.1, 6.0) | 72.3 | CH | 6, 8, 14, 1' |
| 8 | 1.87 (td, *J* = 11.1, 4.8) | 39.8 | CH | 6, 7, 9, 11, 14, 17 |
| 9 | 2.46 (m) | 36.8 | CH | 1, 8, 10, 11, 12, 14, 20 |
| 10 | - | 40.9 | C | - |
| 11 | 2.32 (m) | 22.4 | CH$_2$ | 8, 9, 10, 12, 13 |
| | 2.46 (m) | | | |
| 12 | - | 149.3 | C | - |
| 13 | - | 121.8 | C | - |

**Table 35** (continued)

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 14 | 2.75 (qd, *J* = 6.9, 4.8) | 27.6 | CH | 8, 9, 12, 13, 15, 17 |
| 15 | 6.12 (d, *J* = 1.8) | 109.6 | CH | 12, 13, 16 |
| 16 | 7.16 (d, *J* = 1.8) | 140.5 | CH | 12, 13, 15 |
| 17 | 0.94 (d, *J* = 6.9) | 17.1 | CH$_3$ | 8, 13, 14 |
| 18 | 0.89 (s) | 28.0 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 1.00 (s) | 24.7 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 1.04 (s) | 17.4 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 170.7 | C | - |
| 2' | 2.00 (s) | 21.3 | CH$_3$ | 1' |

**3.3.2 Compound CP2**
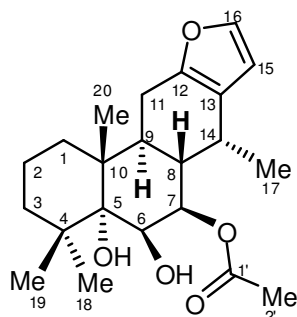


Compound **CP2** had the molecular formula $C_{22}H_{32}O_5$ ([M]$^+$ *m/z* 376.2250) inferred from HREIMS. The $^1H$ and $^{13}C$ NMR spectral data (Table 36, Figures 68 and 69) of **CP2** were closely related to those of **CP1**. The only difference was found as replacement of the methylene protons at $\delta$ 1.64 and 2.01 (2H-6) in **CP1** with an oxymethine proton at $\delta$ 4.15 (d, $J$ = 3.9 Hz; $\delta_C$ 71.3) in **CP2.** The HMBC correlations of the latter proton with the carbons at $\delta$ 35.0 (C-8), 39.3 (C-4), 40.6 (C-10), 74.8 (C-7) and 77.7 (C-5) suggested its location at C-6 whose α-orientation was suggested by its NOESY cross-peaks with Me-18 ($\delta$ 0.95) and H-7 ($\delta$ 5.38) and the small vicinal coupling constants ($J_{7ax,6eq}$ = 3.9 Hz). Therefore, **CP2** was 6$\beta$-hydroxy-7$\beta$-acetoxyvouacapen-5$\alpha$-ol, a new compound (Yodsaoue et al., 2011) and named as pulcherrin E.
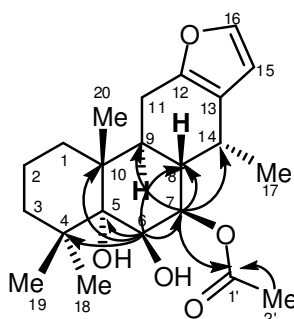


Selective HMBC correlations of **CP2**

**Table 36** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP2**

| Position | δ$_H$ (mult., *J*, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.28 (m) | 35.2 | CH$_2$ | 2, 20 |
|   | 1.44 (m) | | | |
| 2 | 1.36 (m) | 18.1 | CH$_2$ | 10 |
|   | 1.63 (m) | | | |
| 3 | 1.08 (m) | 37.5 | CH$_2$ | 1, 5, 18, 19 |
|   | 1.56 (m) | | | |
| 4 | - | 39.3 | C | - |
| 5 | - | 77.7 | C | - |
| 6 | 4.15 (d, *J* = 3.9) | 71.3 | CH | 4, 5, 7, 8, 10 |
| 7 | 5.38 (dd, *J* = 11.4, 3.9) | 74.8 | CH | 8, 9, 14, 1' |
| 8 | 2.11 (m) | 35.0 | CH | 7, 9, 11, 14, 17 |
| 9 | 2.42 (m) | 37.2 | CH | 8, 10, 11, 12, 14, 20 |
| 10 | - | 40.6 | C | - |
| 11 | 2.41 (m) | 21.7 | CH$_2$ | 8, 9, 10, 12, 13 |
|    | 2.45 (m) | | | |
| 12 | - | 149.4 | C | - |
| 13 | - | 121.6 | C | - |
| 14 | 2.72 (qd, *J* = 6.9, 5.1) | 27.8 | CH | 8, 9, 12, 13, 15, 17 |
| 15 | 6.12 (d, *J* = 2.1) | 109.5 | CH | 12, 13, 16 |
| 16 | 7.16 (d, *J* = 2.1) | 140.5 | CH | 12, 13, 15 |
| 17 | 0.92 (d, *J* = 6.9) | 17.3 | CH$_3$ | 8, 13, 14 |
| 18 | 0.95 (s) | 27.6 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 1.38 (s) | 25.5 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 1.29 (s) | 17.2 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 170.1 | C | - |
| 2' | 2.08 (s) | 21.2 | CH$_3$ | 7, 1' |

### 3.3.3 Compound CP3
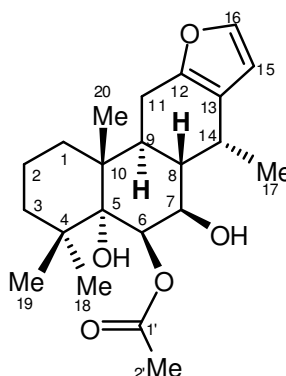


Compound **CP3** had the same molecular formula $C_{22}H_{32}O_5$ as **CP2**. The $^1H$ and $^{13}C$ NMR spectral data (Table 37, Figures 70 and 71) of **CP3** were closely related to those of **CP2** which differed only in the chemical shifts of positions 6 and 7. The oxymethine proton H-6 of **CP3** appeared at $\delta_H$ 5.48 ($\delta_C$ 73.4) more downfield than that of **CP2** ($\delta_H$ 4.15; $\delta_C$ 71.3) as a result of the deshielding effect of the OAc group while H-7 of **CP3** resonanced at $\delta_H$ 4.31 ($\delta_C$ 69.1), higher field than that of **CP2** ($\delta_H$ 5.38; $\delta_C$ 74.8). The HMBC correlations of an oxymethine proton at $\delta$ 5.48 (H-6) with the carbons at $\delta$ 37.7 (C-8), 39.1 (C-4), 41.2 (C-10), 69.1 (C-7), 77.2 (C-5) and 171.4 (O$\underline{C}$OCH$_3$) and of an oxymethine proton at $\delta$ 4.31 (H-7) with the carbons at $\delta$ 27.3 (C-14), 37.7 (C-8) and 73.4 (C-6) confirmed the locations of the OAc group at C-6 and OH at C-7, respectively. The NOESY cross-peaks of H-6/H-7/H-9 and H-7/H-6/H-17 confirmed the $\alpha$-orientations of H-6 and H-7. Thus, **CP3** was assigned to be 6$\beta$-acetoxy-7$\beta$-hydroxyvouacapen-5$\alpha$-ol, a new compound (Yodsaoue et al., 2011) and was named as pulcherrin F.



Selective HMBC correlations of **CP3**

**Table 37** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP3**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.43 (m) | 35.0 | CH$_2$ | 3, 5, 10, 20 |
|  | 1.50 (m) |  |  |  |
| 2 | 1.46 (m) | 18.0 | CH$_2$ | 4, 10 |
|  | 1.69 (m) |  |  |  |
| 3 | 1.15 (m) | 37.8 | CH$_2$ | 4, 19 |
|  | 1.65 (m) |  |  |  |
| 4 | - | 39.1 | C | - |
| 5 | - | 77.2 | C | - |
| 6 | 5.48 (d, $J$ = 4.2) | 73.4 | CH | 4, 5, 7, 8, 10, 1' |
| 7 | 4.31 (dd, $J$ = 10.8, 4.2) | 69.1 | CH | 6, 8, 14 |
| 8 | 1.93 (ddd, $J$ = 12.0, 10.8, 5.1) | 37.7 | CH | 7, 9, 11, 14, 17 |
| 9 | 2.36 (br dd, $J$ = 12.0, 8.7) | 37.1 | CH | 8, 10, 11, 20 |
| 10 | - | 41.2 | C | - |
| 11 | 2.47 (m) | 21.6 | CH$_2$ | 8, 9, 12, 13 |
|  | 2.51 (m) |  |  |  |
| 12 | - | 149.2 | C | - |
| 13 | - | 121.9 | C | - |
| 14 | 3.02 (qd, $J$ = 6.9, 5.1) | 27.3 | CH | 8, 9, 12, 13, 17 |
| 15 | 6.21 (d, $J$ = 1.8) | 109.7 | CH | 12, 13, 16 |
| 16 | 7.23 (d, $J$ = 1.8) | 140.5 | CH | 12, 13, 15 |
| 17 | 1.07 (d, $J$ = 6.9) | 17.1 | CH$_3$ | 8, 13, 14 |
| 18 | 1.04 (s) | 27.7 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 1.21 (s) | 25.3 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 1.34 (s) | 17.0 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 171.4 | C | - |
| 2' | 2.12 (s) | 21.7 | CH$_3$ | 1' |

### 3.3.4 Compound CP4
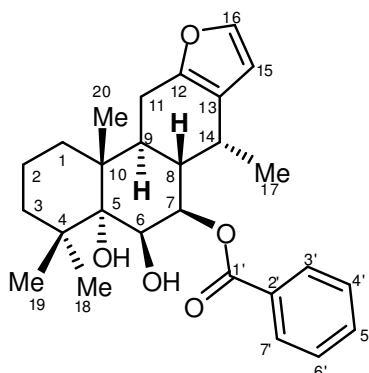


The molecular weight of compound **CP4**, $C_{27}H_{34}O_5$, was assigned at *m/z* 438.2410 [M]$^+$ by HREIMS. The NMR spectra (Table 38, Figures 72 and 73) of **CP4** displayed characteristic similar to those of **CP2** except for the replacement of an acetoxy group at $\delta$ 2.08 in **CP2** with a benzoyloxy group at $\delta$ 7.40 (br t, *J* = 7.5 Hz; H-4', H- 6'), 7.53 (tt, *J* = 7.5, 1.2 Hz; H-5') and 8.02 (br d, *J* = 7.5 Hz; H-3', H-7') in **CP4**. This evidence was confirmed by HMBC correlations of an oxymethine proton at $\delta$ 5.62 (dd, *J* = 10.8, 3.9 Hz; H-7) to the carbons at $\delta$ 27.7 (C-14), 35.2 (C-8) and 165.6 (C-1'), and of H-6 ($\delta$ 4.31) with the carbons at $\delta$ 35.2 (C-8), 39.3 (C-4), 40.7 (C-10), 75.6 (C-7) and 77.8 (C-5). An oxymethine proton H-6 was deduced to be equatorially oriented by a small vicinal coupling constant ($J_{6eq,7ax}$ = 3.9 Hz), whereas H-7 was an axial proton by  the large vicinal coupling constant ($J_{7ax,8ax}$ = 10.8 Hz). It was further supported by NOESY cross-peaks of H-7 with Me-17, H-9 and H-6. Thus, **CP4** was assigned to be 6$\beta$-hydroxy-7$\beta$-benzoyloxyvouacapen-5$\alpha$-ol, a new compound (Yodsaoue et al., 2011) and was named as pulcherrin G. This compound was first isolated from natural product, however it was previously obtained from the partial synthesis (Roach et al., 2003).



Selective HMBC correlations of **CP4**

**Table 38** $^{1}$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP4**

| Position | δ$_H$ (mult., *J*, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.34 (m) | 35.2 | CH$_2$ | 2, 10, 20 |
| | 1.50 (m) | | | |
| 2 | 1.42 (m) | 18.2 | CH$_2$ | 1, 4 |
| | 1.64 (m) | | | |
| 3 | 1.10 (m) | 37.5 | CH$_2$ | 1, 4, 5, 19 |
| | 1.63 (m) | | | |
| 4 | - | 39.3 | C | - |
| 5 | - | 77.8 | C | - |
| 6 | 4.31 (d, *J* = 3.9) | 71.4 | CH | 4, 5, 7, 8, 10 |
| 7 | 5.62 (dd, *J* = 10.8, 3.9) | 75.6 | CH | 8, 14, 1' |
| 8 | 2.33 (ddd, *J* = 12.0, 10.8, 4.8) | 35.2 | CH | 7, 9, 11, 14, 17 |
| 9 | 2.49 (m) | 37.3 | CH | 1, 10, 11, 12, 20 |
| 10 | - | 40.7 | C | - |
| 11 | 2.48 (m) | 21.8 | CH$_2$ | 8, 9, 10, 12, 13 |
| 12 | - | 149.5 | C | - |
| 13 | - | 121.6 | C | - |
| 14 | 2.82 (qd, *J* = 6.9, 4.8) | 27.7 | CH | 8, 9, 12, 13, 15, 17 |
| 15 | 6.10 (d, *J* = 1.8) | 109.5 | CH | 12, 13, 16 |
| 16 | 7.16 (d, *J* = 1.8) | 140.6 | CH | 12, 13, 15 |
| 17 | 0.94 (d, *J* = 6.9) | 17.4 | CH$_3$ | 8, 13, 14 |
| 18 | 0.96 (s) | 27.8 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 1.39 (s) | 25.5 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 1.34 (s) | 17.3 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 165.6 | C | - |
| 2' | - | 130.0 | C | - |
| 3'/7' | 8.02 (br d, *J* = 7.5) | 129.7 | CH | 1', 2', 5' |
| 4'/6' | 7.40 (br t, *J* = 7.5) | 128.6 | CH | 1', 2' |
| 5' | 7.53 (tt, *J* = 7.5, 1.2) | 133.3 | CH | 3', 7' |

### 3.3.5 Compound CP5
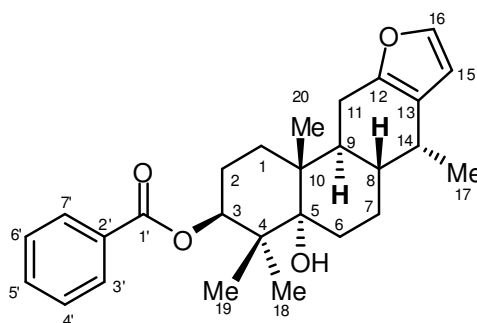


Compound **CP5** had the molecular formula $C_{27}H_{34}O_4$ by HREIMS. The $^1H$ and $^{13}C$ NMR spectra (Table 39, Figures 74 and 75) were comparable to those of **CP1** except that the signals of an acetoxy group in **CP1** was replaced by those of a benzoyloxy group in **CP5** shown as the resonances at $\delta$ 7.37 (t, $J$ = 7.2 Hz; H-4', H-6'), 7.48 (tt, $J$ = 7.2, 1.5 Hz; H-5') and 7.98 (dt, $J$ = 7.2, 1.5 Hz; H-3', H-7'). The correlations of an oxymethine proton at $\delta$ 5.29 (H-3) with the carbons at $\delta$ 19.6 (C-19), 23.1 (C-18), 23.8 (C-2), 43.5 (C-4) and 166.2 (C-1') in the HMBC spectrum placed the benzoyloxy group at C-3. The relative stereochemistry of H-3 was assigned to be axially oriented by the large and small vicinal coupling constants ($J_{3ax,2ax}$ = 11.4 Hz, $J_{3ax,2eq}$ = 4.8 Hz). In the NOESY spectrum, the benzoyloxy protons at $\delta$ 7.98 (H-3', H-7') displayed a cross-peak with the methyl protons at $\delta$ 1.17 (Me-19), confirming the $\beta$-orientation of the benzoyloxy group. Thus, **CP5** was assigned to be 3$\beta$-benzoyloxyvouacapen-5$\alpha$-ol, a new compound (Yodsaoue et al., 2011) and was named as pulcherrin H.
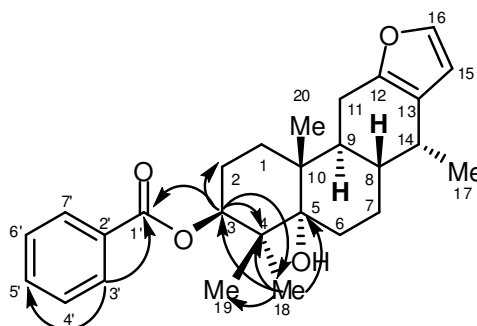


Selective HMBC correlations of **CP5**

**Table 39** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP5**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1eq | 1.40 (td, $J$ = 8.4, 2.7) | 31.2 | CH$_2$ | 2, 3, 5, 9, 10, 20 |
| ax | 1.74 (m) | | | |
| 2 | 1.75 (m) | 23.8 | CH$_2$ | 1, 3, 4, 10 |
| | 1.84 (m) | | | |
| 3 | 5.29 (dd, $J$ = 11.4, 4.8) | 77.8 | CH | 2, 4, 18, 19, 1' |
| 4 | - | 43.5 | C | - |
| 5 | - | 78.6 | C | - |
| 6 ax | 1.55 (br d, $J$ = 12.0) | 26.1 | CH$_2$ | 4, 5, 7, 8, 10 |
| eq | 1.85 (m) | | | |
| 7 | 1.46 (m) | 24.1 | CH$_2$ | 5, 6, 8, 9, 14 |
| | 1.70 (m) | | | |
| 8 | 1.74 (m) | 34.3 | CH | 6, 7, 10, 14, 17 |
| 9 | 2.30 (m) | 37.6 | CH | 8, 10, 11, 12, 20 |
| 10 | - | 41.0 | C | - |
| 11 | 2.32 (m) | 22.4 | CH$_2$ | 8, 9, 10, 12, 13 |
| | 2.43 (m) | | | |
| 12 | - | 149.4 | C | - |
| 13 | - | 122.6 | C | - |
| 14 | 2.55 (qd, $J$ = 6.9, 3.9) | 31.3 | CH | 8, 9, 12, 13, 17 |
| 15 | 6.11 (d, $J$ = 1.5) | 109.5 | CH | 12, 13, 16 |
| 16 | 7.15 (d, $J$ = 1.5) | 140.4 | CH | 12, 13, 15 |
| 17 | 0.95 (d, $J$ = 6.9) | 17.5 | CH$_3$ | 8, 13, 14 |
| 18 | 0.97 (s) | 23.1 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 1.17 (s) | 19.6 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 1.05 (s) | 17.2 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 166.2 | C | - |
| 2' | - | 131.0 | C | - |
| 3'/7' | 7.98 (dt, $J$ = 7.2, 1.5) | 129.5 | CH | 1', 5' |
| 4'/6' | 7.37 (t, $J$ = 7.2) | 128.3 | CH | 1', 2' |
| 5' | 7.48 (tt, $J$ = 7.2, 1.5) | 132.7 | CH | 3', 7' |

**3.3.6 Compound CP6**
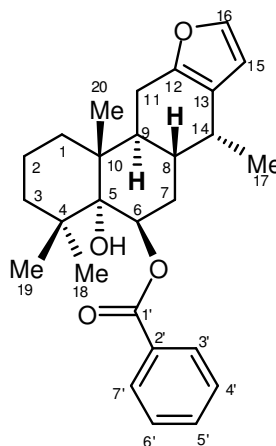


Compound **CP6** showed the molecular ion peak at *m/z* 422.2459 [M]$^+$ by HREIMS corresponding to a molecular formula of $C_{27}H_{34}O_4$. The $^1H$ and $^{13}C$ NMR spectroscopic data (Table 40, Figures 76 and 77) were closely related to those of **CP5** except for the arrangement of a benzoyloxy group whose location in **CP6** was at C-6 whereas that of **CP5** at C-3. The observed HMBC correlations of a proton at $\delta$ 5.47 (H-6) with the carbons at $\delta$ 30.7 (C-8), 31.6 (C-7), 39.0 (C-4), 41.3 (C-10), 76.4 (C-5), and the carbonyl carbon of a benzoyloxy group at $\delta$ 165.8 (C-1') supported the assignment. The small vicinal coupling constants ($J_{6eq,7ax,}$ = 2.7 Hz and $J_{6eq,7eq}$ = 2.7 Hz) suggested the relative stereochemistry of H-6 to be equatorially oriented. In the NOESY spectrum, an oxymethine proton at $\delta$ 5.47 (H-6) showed cross-peaks with the methyl protons at $\delta$ 0.93 (Me-18) and the aromatic protons at $\delta$ 7.95 (H-3', H-7') correlated with the methyl protons at $\delta$ 1.44 (Me-20), confirming a $\beta$-orientation of a benzoyloxy group. Thus, **CP6** was assigned to be 6$\beta$-benzoyloxyvouacapen-5$\alpha$-ol, a new compound (Yodsaoue et al., 2011) and was named as pulcherrin I.



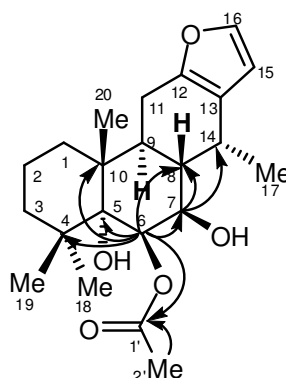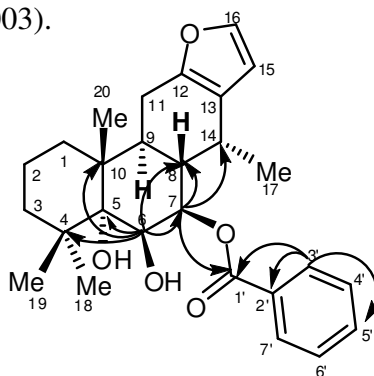Selective HMBC correlations of **CP6**

**Table 40** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP6**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.38 (m) | 34.9 | CH$_2$ | 2, 3, 5, 9, 10 |
| | 1.56 (m) | | | |
| 2 | 1.40 (m) | 18.3 | CH$_2$ | 1, 3, 4, 10 |
| | 1.64 (m) | | | |
| 3 | 1.02 (br d, $J$ = 8.4) | 38.1 | CH$_2$ | 1, 2, 4, 5, 18, 19 |
| | 1.64 (m) | | | |
| 4 | - | 39.0 | C | - |
| 5 | - | 76.4 | C | - |
| 6 | 5.47 (t, $J$ = 2.7) | 72.8 | CH | 4, 5, 7, 8, 10, 1' |
| 7ax | 1.53 (ddd, $J$ = 14.4, 3.9, 2.7) | 31.6 | CH$_2$ | 6, 8, 9, 5 |
| eq | 2.23 (td, $J$ = 14.4, 2.7) | | | |
| 8 | 1.98 (m) | 30.7 | CH | 7, 9, 11, 14, 17 |
| 9 | 2.35 (m) | 38.0 | CH | 1, 7, 8, 10, 11, 12, 20 |
| 10 | - | 41.3 | C | - |
| 11 | 2.34 (m) | 21.9 | CH$_2$ | 8, 9, 10, 12, 13 |
| | 2.43 (m) | | | |
| 12 | - | 149.5 | C | - |
| 13 | - | 122.4 | C | - |
| 14 | 2.45 (m) | 31.2 | CH | 7, 8, 9, 12, 13, 15, 17 |
| 15 | 6.06 (d, $J$ = 1.8) | 109.5 | CH | 12, 13, 14, 16 |
| 16 | 7.11 (d, $J$ = 1.8) | 140.4 | CH | 12, 13, 15 |
| 17 | 0.90 (d, $J$ = 7.2) | 17.6 | CH$_3$ | 8, 13, 14 |
| 18 | 0.93 (s) | 27.8 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 1.13 (s) | 26.0 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 1.44 (s) | 17.2 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 165.8 | C | - |
| 2' | - | 130.6 | C | - |
| 3'/7' | 7.95 (br d, $J$ = 7.2) | 129.7 | CH | 1', 5' |
| 4'/6' | 7.33 (br t, $J$ = 7.2) | 128.6 | CH | 1', 5' |
| 5' | 7.45 (br t, $J$ = 7.2) | 133.1 | CH | 3', 4,' 6', 7' |

### 3.3.7 Compound CP7
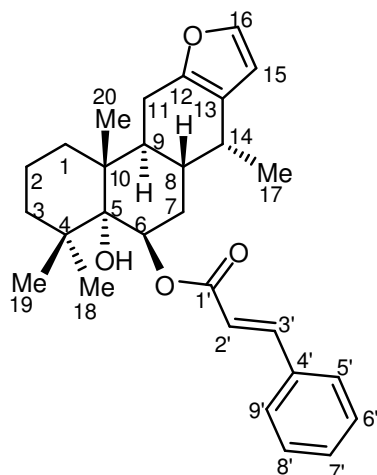


Compound **CP7** showed the molecular formula $C_{29}H_{36}O_4$ ($[M]^+$ *m/z* 448.2617) by HREIMS. The $^1H$ and $^{13}C$ NMR spectroscopic data (Table 41, Figures 78 and 79) were closely related to those of **CP6** except for the replacement of a benzoyloxy group at $\delta$ 7.33 (br t, *J* = 7.2 Hz; H-4', H-6'), 7.45 (br t, *J* = 7.2 Hz; H-5') and 7.95 (br d, *J* = 7.2 Hz; H-3', H-7') in **CP6** with a *trans*-cinnamoyloxy moiety in **CP7** at $\delta$ 6.33 and 7.60 (each d, *J* = 15.9 Hz, H-2' and H-3', respectively) and 7.29-7.44 (m, H-5' to H-9'). The HMBC correlation of H-6 ($\delta$ 5.31) to the carbonyl carbon of the cinnamoyloxy group at $\delta$ 166.0 (C-1') suggested the location of the *trans*-cinnamoyloxy side chain at C-6. Thus, **CP7** was assigned to be 6$\beta$-cinnamoyloxyvouacapen-5$\alpha$-ol, a new compound (Yodsaoue et al., 2011) and was named as pulcherrin J.



Selective HMBC correlations of **CP7**

**Table 41** ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **CP7**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.35 (m) | 34.8 | $CH_2$ | 2, 3, 5, 9, 10, 20 |
| | 1.49 (m) | | | |
| 2 | 1.39 (m) | 18.2 | $CH_2$ | 4 |
| | 1.65 (m) | | | |
| 3 | 1.05 (br d, $J$ = 9.3) | 38.1 | $CH_2$ | 1, 4, 5, 19 |
| | 1.65 (m) | | | |
| 4 | - | 39.0 | C | - |
| 5 | - | 76.3 | C | - |
| 6 | 5.31 (dd, $J$ = 3.0, 2.4) | 72.3 | CH | 1', 4, 5, 8, 10 |
| 7ax | 1.50 (dt, $J$ = 13.8, 2.4) | 31.5 | $CH_2$ | 6, 8, 9, 14 |
| eq | 2.18 (td, $J$ = 13.8, 3.0) | | | |
| 8 | 1.98 (m) | 30.6 | CH | 7, 9, 11, 14, 17 |
| 9 | 2.35 (m) | 38.0 | CH | 1, 7, 8, 10, 11, 12, 14, 20 |
| 10 | - | 41.4 | C | - |
| 11 | 2.36 (m) | 21.8 | $CH_2$ | 8, 9, 10, 12, 13 |
| | 2.44 (m) | | | |
| 12 | - | 149.5 | C | - |
| 13 | - | 122.4 | C | - |
| 14 | 2.49 (m) | 31.1 | CH | 8, 9, 12, 13, 15, 17 |
| 15 | 6.09 (d, $J$ = 1.8) | 109.5 | CH | 12, 13, 16 |
| 16 | 7.14 (d, $J$ = 1.8) | 140.4 | CH | 12, 13, 15 |
| 17 | 0.92 (d, $J$ = 6.6) | 17.6 | $CH_3$ | 8, 13, 14 |
| 18 | 0.94 (s) | 27.7 | $CH_3$ | 3, 4, 5, 19 |
| 19 | 1.17 (s) | 25.9 | $CH_3$ | 3, 4, 5, 18 |
| 20 | 1.37 (s) | 16.9 | $CH_3$ | 1, 5, 9, 10 |
| 1' | - | 166.0 | C | - |
| 2' | 6.33 (d, $J$ = 15.9) | 118.6 | CH | 1', 3', 4', 5', 9' |
| 3' | 7.60 (d, $J$ = 15.9) | 145.2 | CH | 1', 2', 4', 5', 9' |
| 4' | - | 134.3 | C | - |
| 5'/9' | 7.44 (m) | 128.6 | CH | 3', 4', 7' |
| 6'/8' | 7.29 (m) | 129.7 | CH | 4' |
| 7' | 7.29 (m) | 130.4 | CH | 5', 9' |

### 3.3.8 Compound CP8
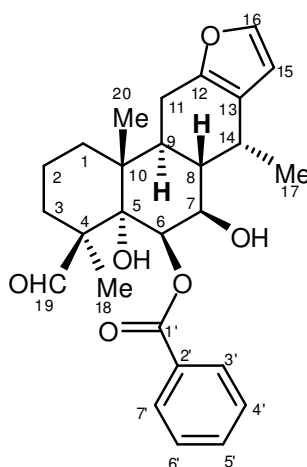


Compound **CP8** had the molecular formula $C_{27}H_{32}O_6$ ([M]$^+$ *m/z* 452.2198), based on HREIMS. The $^1$H and $^{13}$C NMR spectral data (Table 42, Figures 80 and 81) were related to those of **CP6**. The major differences were the replacement of the $^1$H NMR signals of Me-19 at $\delta$ 1.13 and the methylene protons at $\delta$ 1.53 (ddd, *J* = 14.4, 3.9, 2.7 Hz; $H_{eq}$-7) and 2.23 (td, *J* = 14.4, 2.7 Hz; $H_{ax}$-7) of **CP6** with an aldehydic proton at $\delta$ 9.65 (d, *J* = 1.2 Hz; H-19) and an oxymethine proton at $\delta$ 4.33 (dd, *J* = 11.1, 4.2 Hz; H-7), respectively in **CP8**. The HMBC correlations of an oxymethine proton at $\delta$ 4.33 (H-7) with the carbons at $\delta$ 27.2 (C-14), 37.7 (C-8), and 73.8 (C-6), of an aldehydic proton at $\delta$ 9.65 (H-19) with the carbons at $\delta$ 29.1 (C-3), 55.8 (C-4), and 78.6 (C-5) and of the methyl protons at $\delta$ 1.10 (Me-18) with the carbons at $\delta$ 29.1 (C-3), 55.8 (C-4), 78.6 (C-5) and 202.3 (C-19) confirmed the attachments of an OH and an aldehyde groups at C-7 and C-4, respectively. In the NOESY spectrum, the aldehydic proton at $\delta$ 9.65 (H-19) displayed a cross-peak with the methyl protons at $\delta$ 1.18 (Me-20) indicating a $\beta$-orientation. The large and small coupling constants ($J_{7ax,8ax}$ = 11.1 Hz, $J_{7ax,6eq}$ = 4.2 Hz) of H-7 and its NOESY cross-peaks with H-6, H-9 and Me-17 confirmed an α-axial orientation. Thus, **CP8** was deduced to be 6$\beta$-benzoyloxy-7$\beta$-hydroxy-19-formylvouacapen-5$\alpha$-ol, a new compound (Yodsaoue et al., 2011) and was named as pulcherrin K.
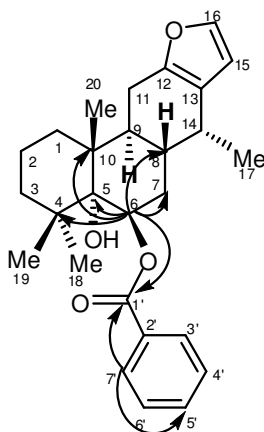
Selective HMBC correlations of **CP8**

**Table 42** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP8**

| Position | δ$_H$ (mult., *J*, Hz) | δ$_C$ | DEPT | HMBC |
|----------|------------------------|-------|------|------|
| 1 | 1.47 (m) | 34.2 | CH$_2$ | 2, 3, 5, 9, 10, 20 |
|   | 1.53 (m) | | | |
| 2 | 1.45 (m) | 17.8 | CH$_2$ | 3, 4 |
|   | 1.65 (m) | | | |
| 3 | 1.40 (m) | 29.1 | CH$_2$ | 2, 4, 5, 19 |
|   | 1.90 (m) | | | |
| 4 | - | 55.8 | C | - |
| 5 | - | 78.6 | C | - |
| 6 | 5.92 (d, *J* = 4.2) | 73.8 | CH | 4, 5, 7, 8, 10, 1' |
| 7 | 4.33 (dd, *J* = 11.1, 4.2) | 69.0 | CH | 6, 8, 14 |
| 8 | 1.99 (td, *J* = 11.1, 5.1) | 37.7 | CH | 6, 7, 9, 11, 14, 17 |
| 9 | 2.27 (m) | 36.7 | CH | 8, 10, 11, 20 |
| 10 | - | 41.2 | C | - |
| 11 | 2.48 (m) | 22.2 | CH$_2$ | 8, 9, 10, 12, 13 |
|    | 2.55 (m) | | | |
| 12 | - | 148.8 | C | - |

**Table 42** (continued)

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|----------|------------------------------|------------|------|------|
| 13 | - | 121.8 | C | - |
| 14 | 2.96 (qd, *J* = 6.9, 5.1) | 27.2 | CH | 8, 9, 12, 13, 15, 17 |
| 15 | 6.12 (d, *J* = 1.8) | 109.6 | CH | 12, 13, 16 |
| 16 | 7.17 (d, *J* = 1.8) | 140.7 | CH | 12, 13, 15 |
| 17 | 0.97 (d, *J* = 6.9) | 17.0 | CH$_3$ | 8, 13, 14 |
| 18 | 1.10 (s) | 19.1 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 9.65 (d, *J* = 1.2) | 202.3 | CH | 3, 4, 5 |
| 20 | 1.18 (s) | 17.0 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 167.3 | C | - |
| 2' | - | 129.2 | C | - |
| 3'/7' | 7.92 (d, *J* = 7.2) | 129.9 | CH | 1', 5' |
| 4'/6' | 7.38 (t, *J* = 7.2) | 128.8 | CH | 1', 2' |
| 5' | 7.52 (br t, *J* = 7.2 ) | 133.8 | CH | 3', 7' |

**3.3.9 Compound CP9**
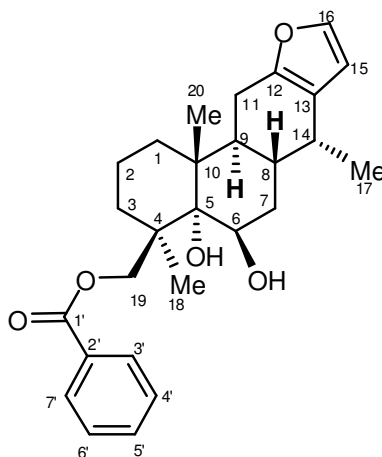


Compound **CP9** was deduced as $C_{27}H_{34}O_5$ from an exact mass measurement ([M]$^+$ *m/z* 438.2405) by HREIMS. The $^1$H and $^{13}$C NMR spectral data (Table 43, Figures 82 and 83) of **CP9** were comparable to those of **CP6**. The difference was shown as the replacement of a singlet methyl at $\delta$ 1.13 (Me-19) in **CP6** with an oxymethylene proton signals at $\delta$ 4.88 and 5.01 (each d, *J* = 11.4 Hz; 2H-19) in **CP9**. In addition the oxymethine proton H-6 in **CP9** appeared at $\delta$ 4.17 (t, *J* = 3.6 Hz), more highfield than that of **CP6** ($\delta$ 5.47, t, *J* = 2.7 Hz) indicating the OH group at C-6 instead of a benzoyloxy group as in **CP6**. The HMBC correlations of the oxymethylene proton signals at $\delta$ 4.88 and 5.01 (2H-19) with the carbons at $\delta$ 20.8 (C-18), 31.8 (C-3), 44.0 (C-4), 76.7 (C-5), and 166.6 (C-1') suggested the attachment of a benzoyloxy group at C-19. In the NOESY spectrum, the cross-peaks of the oxymethylene protons at $\delta$ 4.88 and 5.01 (2H-19) with the methyl protons at $\delta$ 1.31 (Me-20), and of an oxymethine proton at $\delta$ 4.17 (H-6) with the methyl protons at $\delta$ 1.11 (Me-18) indicated an oxymethylene protons to be *β*-oriented and H-6 as *α*-oriented, respectively. Therefore, **CP9** was assigned as 6*β*-hydroxy-19-benzoyloxyvouacapen-5*α*-ol, a new compound (Yodsaoue et al., 2011) and was named as pulcherrin L.
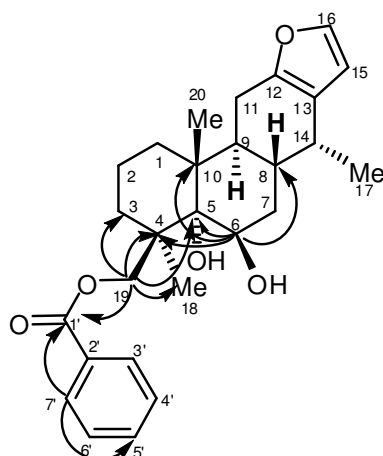
Selective HMBC correlations of **CP9**

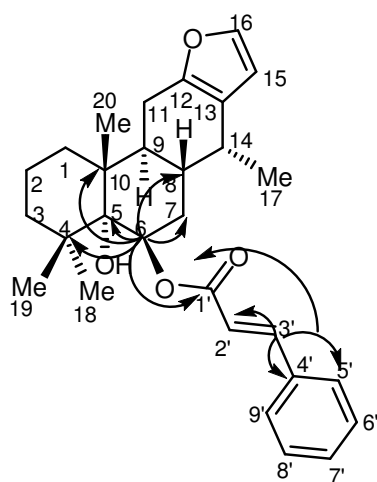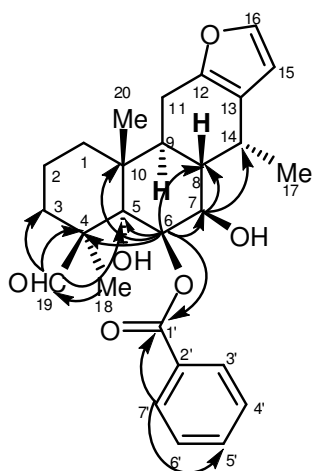**Table 43** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP9**

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.34 (m) | 34.6 | CH$_2$ | 9, 10, 20 |
|  | 1.42 (m) |  |  |  |
| 2 | 1.45 (m) | 17.9 | CH$_2$ | 3, 4, 10 |
|  | 1.68 (m) |  |  |  |
| 3 | 1.48 (m) | 31.8 | CH$_2$ | 4, 19 |
|  | 1.66 (m) |  |  |  |
| 4 | - | 44.0 | C | - |
| 5 | - | 76.7 | C | - |
| 6 | 4.17 (t, *J* = 3.6) | 71.0 | CH | 4, 5, 8, 10 |
| 7 | 1.41 (m) | 35.4 | CH$_2$ | 6, 8, 9, 14 |
|  | 2.19 (dt, *J* = 13.5, 3.6) |  |  |  |
| 8 | 2.07 (m) | 29.8 | CH | 7, 9, 14, 17 |
| 9 | 2.28 (m) | 38.6 | CH | 1, 8, 10, 11, 20 |
| 10 | - | 41.1 | C | - |
| 11 | 2.42 (m) | 21.9 | CH$_2$ | 8, 9, 10, 12, 13 |
| 12 | - | 149.4 | C | - |

**Table 43** (continued)

| Position | δ$_H$ (mult., *J*, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 13 | - | 122.5 | C | - |
| 14 | 2. 54 (qd, *J* = 7.2, 5.4) | 31.2 | CH | 8, 9, 12, 13, 17 |
| 15 | 6.12 (d, *J* = 1.8) | 109.5 | CH | 12, 13, 16 |
| 16 | 7.16 (d, *J* = 1.8) | 140.4 | CH | 12, 13, 15 |
| 17 | 0.94 (d, *J* = 7.2) | 17.7 | CH$_3$ | 8, 13, 14 |
| 18 | 1.11 (s) | 20.8 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 4.88 (d, *J* = 11.4) | 68.2 | CH$_2$ | 3, 4, 5, 18, 1' |
|  | 5.01 (d, *J* = 11.4) |  |  |  |
| 20 | 1.31 (s) | 16.2 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 166.6 | C | - |
| 2' | - | 130.5 | C | - |
| 3'/7' | 7.97 (br d, *J* = 7.5) | 129.5 | CH | 1', 5' |
| 4'/6' | 7.38 (t, *J* = 7.5) | 128.5 | CH | 2' |
| 5' | 7.55 (br t, *J* = 7.5) | 132.9 | CH | 3', 7' |

### 3.3.10 Compound CP10
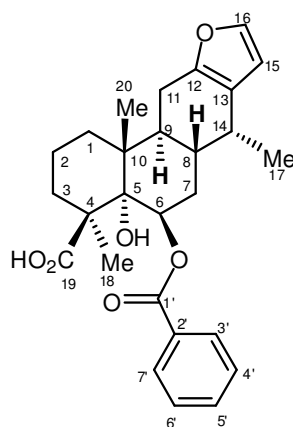


Compound **CP10** showed the molecular ion $[M]^+$ at *m/z* 452.2196 by HREIMS spectrum in agreement with the formula $C_{27}H_{32}O_6$. The $^1H$ and $^{13}C$ NMR spectral data (Table 44, Figures 84 and 85) of **CP10** showed characteristics similar to those of **CP6** except for the disappearance of a methyl singlet at $\delta_H$ 1.13 (Me-19; $\delta_C$ 26.0) and the appearance of a carboxyl carbon at $\delta_C$ 181.9 in **CP10**. This finding was supported by HMBC spectrum in which the methyl protons at $\delta$ 0.97 (Me-18) were correlated with the carbons at $\delta$ 34.1 (C-3), 48.4 (C-4), 76.5 (C-5) and 181.9 (C-19). The relative stereochemistry of **CP10** was assigned by NOESY experiment, in which Me-18 ($\delta$ 0.97) showed a cross-peak with $\delta$ 5.45 (H-6) whereas the benzoyloxy protons H-3'/H-7' ($\delta$ 7.84) with $\delta$ 1.32 (Me-20). Therefore, **CP10** was assigned as 6$\beta$-benzoyloxy-19-carboxyvouacapen-5$\alpha$-ol, a new compound (Yodsaoue et al., 2011) and was named as pulcherrin M.
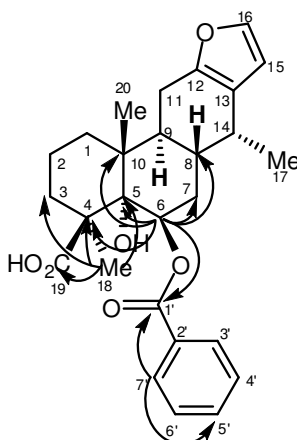


Selective HMBC correlations of **CP10**

**Table 44** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP10**

| Position | δ$_H$ (mult., *J*, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.47 (m) | 34.7 | CH$_2$ | 3, 5, 10, 20 |
| | 1.73 (m) | | | |
| 2 | 1.42 (m) | 18.7 | CH$_2$ | 4 |
| | 1.64 (m) | | | |
| 3 | 1.38 (m) | 34.1 | CH$_2$ | 2, 4, 5, 18, 19 |
| | 1.75 (br d, *J* = 13.8) | | | |
| 4 | - | 48.4 | C | - |
| 5 | - | 76.5 | C | - |
| 6 | 5.45 (t, *J* = 2.7) | 70.7 | CH | 4, 5, 7, 8, 10, 1' |
| 7 | 1.58 (dt, *J* = 14.1, 2.7) | 30.8 | CH$_2$ | 5, 6, 8, 9, 14 |
| | 2.14 (m) | | | |
| 8 | 1.98 (m) | 30.7 | CH | 7, 9, 14, 17 |
| 9 | 2.18 (m) | 38.0 | CH | 7, 8, 10, 11, 14, 20 |
| 10 | - | 41.7 | C | - |
| 11 | 2.40 (m) | 22.2 | CH$_2$ | 8, 9, 10, 12, 13 |
| | 2.50 (m) | | | |
| 12 | - | 149.3 | C | - |
| 13 | - | 122.2 | C | - |
| 14 | 2.47 (m) | 31.0 | CH | 9, 12, 13, 15, 17 |
| 15 | 6.08 (d, *J* = 1.5) | 109.5 | CH | 12, 13, 16 |
| 16 | 7.13 (d, *J* = 1.5) | 140.5 | CH | 12, 13, 15 |
| 17 | 0.92 (d, *J* = 6.9) | 17.5 | CH$_3$ | 8, 13, 14 |
| 18 | 0.97 (s) | 24.2 | CH$_3$ | 3, 4, 5, 19 |
| 19 | - | 181.9 | C | - |
| 20 | 1.32 (s) | 17.6 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 165.7 | C | - |
| 2' | - | 130.6 | C | - |
| 3'/7' | 7.84 (br d, *J* = 7.2) | 129.5 | CH | 1', 5' |
| 4'/6' | 7.32 (t, *J* = 7.2) | 128.4 | CH | 1', 2' |
| 5' | 7.43 (tt, *J* = 7.2, 1.2) | 132.8 | CH | 3', 7' |

### 3.3.11 Compound CP11
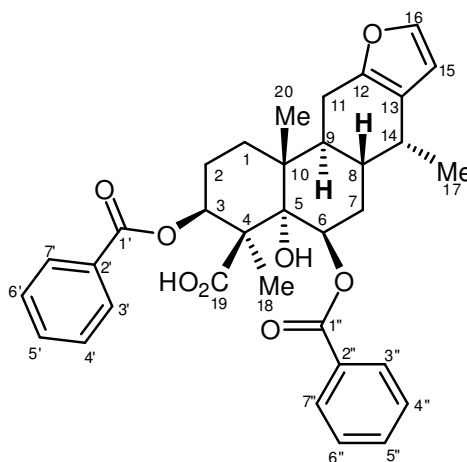


The molecular weight of compound **CP11**, $C_{34}H_{36}O_8$ ([M]$^+$ was assigned at *m/z* 572.2411) by HREIMS. The NMR spectroscopic data (Table 45, Figures 86 and 87) of **CP11** displayed similarities with pulcherrin M (**CP10**) except for the presence of an additional monosubstituted benzene ring in the range $\delta$ 7.18-7.85 and an oxymethine proton at $\delta$ 5.28 (dd, *J* = 12.0, 4.5 Hz; H-3) in **CP11**. The latter proton was attached to the oxymethine carbon at $\delta$ 77.7 in the HMQC spectrum and showed HMBC correlations to the carbons at $\delta$ 19.9 (C-18), 24.3 (C-2), 53.3 (C-4) 166.1 (C-1') and 177.4 (C-19), confirming the location of a benzoyloxy group at C-3. The stereochemistry of H-3 as $\alpha$-axial oriented was determined from the results of the large and small coupling constants ($J_{3ax,2ax}$ = 12.0 Hz, $J_{3ax,2eq}$ = 4.5 Hz) and by the observed cross-peak with Me-18 ($\delta$ 1.22) in the NOESY experiment. Thus, **CP11** was 3$\beta$,6$\beta$-dibenzoyloxy-19-carboxyvouacapen-5$\alpha$-ol, a new compound (Yodsaoue et al., 2011) and was named as pulcherrin N.

Selective HMBC correlations of **CP11**

**Table 45** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP11**

| Position | δ$_H$ (mult., *J*, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.56 (m) | 33.1 | CH$_2$ | 3, 10, 20 |
| | 1.89 (m) | | | |
| 2 | 1.83 (m) | 24.3 | CH$_2$ | 1, 3, 4, 10 |
| | 2.54 (m) | | | |
| 3 | 5.28 (dd, *J* = 12.0, 4.5) | 77.7 | CH | 2, 4, 18, 19, 1' |
| 4 | - | 53.3 | C | - |
| 5 | - | 78.5 | C | - |
| 6 | 5.57 (br s) | 70.9 | CH | 4, 5, 7, 8, 10, 1" |
| 7 | 1.67 (m) | 30.4 | CH$_2$ | 5 |
| | 2.18 (m) | | | |
| 8 | 2.04 (br t, *J* = 11.4) | 30.5 | CH | 7, 10, 14 |
| 9 | 2.35 (td, *J* = 11.4, 8.7) | 37.8 | CH | 7, 8, 10, 11, 14, 20 |
| 10 | - | 41.8 | C | - |
| 11 | 2.51 (m) | 22.2 | CH$_2$ | 8, 9, 10, 12, 13 |
| | 2.56 (m) | | | |
| 12 | - | 149.1 | C | - |
| 13 | - | 122.2 | C | - |
| 14 | 2.50 (m) | 30.9 | CH | 8, 9, 12, 13, 17 |

**Table 45** (continued)

| Position | δ_H (mult., *J*, Hz) | δ_C | DEPT | HMBC |
|----------|---------------------|-----|------|------|
| 15 | 6.10 (d, *J* = 1.8) | 109.5 | CH | 12, 13, 16 |
| 16 | 7.16 (d, *J* = 1.8) | 140.6 | CH | 12, 13, 15 |
| 17 | 0.94 (d, *J* = 6.9) | 17.6 | CH$_3$ | 8, 13, 14 |
| 18 | 1.22 (s) | 19.9 | CH$_3$ | 3, 4, 5, 19 |
| 19 | - | 177.4 | C | - |
| 20 | 1.55 (s) | 16.7 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 166.1 | C | - |
| 2' | - | 130.1 | C | - |
| 3'/7' | 7.85 (d, *J* = 7.5) | 129.6 | CH | 1', 5' |
| 4'/6' | 7.27 (t, *J* = 7.5) | 128.3 | CH | 2' |
| 5' | 7.36 (br t, *J* = 7.5) | 133.2 | CH | 3', 7' |
| 1" | - | 165.8 | C | - |
| 2" | - | 130.2 | C | - |
| 3"/7" | 7.85 (d, *J* = 7.5) | 129.4 | CH | 1", 5" |
| 4"/6" | 7.18 (t, *J* = 7.5) | 128.5 | CH | 2" |
| 5" | 7.43 (br t, *J* = 7.5) | 133.1 | CH | 3", 7" |

### 3.3.12 Compound CP12
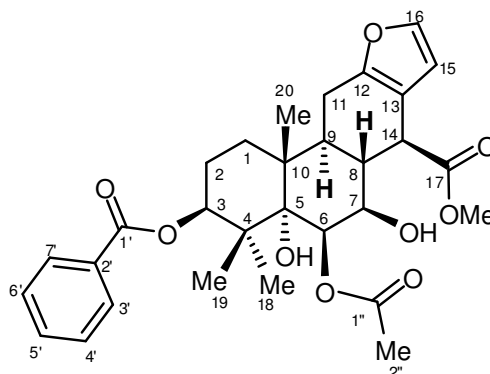


Compound **CP12** with the molecular formula $C_{30}H_{38}O_9$ by HERIMS showed comparable $^1H$ and $^{13}C$ NMR spectral data (Table 46, Figures 88 and 89) with those of **CP3** except for the appearance of the additional signals of an oxymethine proton at $\delta_H$ 5.24 (H-3; $\delta_C$ 76.7) and a benzoyloxy group ($\delta_H$ 7.37-7.95; $\delta_C$ 128.4, 129.5, 130.6, 133.0, 166.1) in **CP12** whose location of the latter at C-3 was supported by the HMBC correlations of H-3 with the carbons at $\delta$ 19.2 (C-19), 22.7 (C-18), 43.9 (C-4) and 166.1 (C-1'). In addition the methyl doublet at $\delta_H$ 1.07 (Me-17; $\delta_C$ 17.1) in **CP3** was replaced with a singlet signal of a methyl ester at C-17 ($\delta_H$ 3.68; $\delta_C$ 52.2) and an ester carbonyl at $\delta_C$ 175.9 in **CP12**. The location of an CO$_2$Me group was confirmed by HMBC spectrum, in which the methine proton H-14 ($\delta$ 3.38) showed the correlations with the ester carbonyl carbon at $\delta$ 175.9. The large vicinal coupling constant of H-3 ($J_{3ax,2ax}$ = 10.8 Hz) and H-14 ($J_{14ax,8ax}$ = 8.4 Hz) suggested the relative stereochemistry of H-3 and H-14 to be α-axially oriented. In the NOESY spectrum, the hydroxyl proton at C-5 ($\delta$ 2.01) showed cross-peaks with H-3, H-6, H-7, H-9 and Me-18 whereas the methine proton H-14 ($\delta$ 3.38) displayed cross-peaks with H-7 and H-9 but not with H-8 supporting a benzoyloxy and CO$_2$Me group as $\beta$-oriented. Thus, **CP12** was deduced to be 3$\beta$-benzoyloxy-6$\beta$-acetoxy-7$\beta$-hydroxy-14$\beta$-methoxycarbo nylvouacapen-5$\alpha$-ol, a new compound (Yodsaoue et al., 2011) and was named as pulcherrin O.
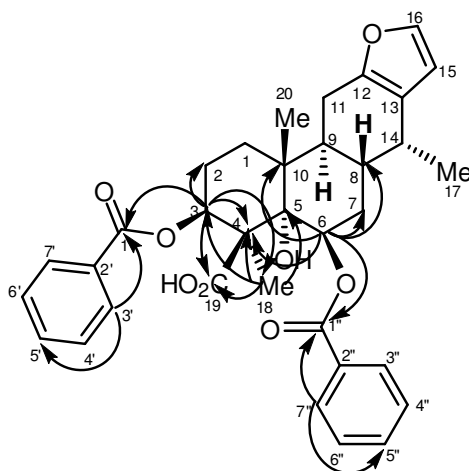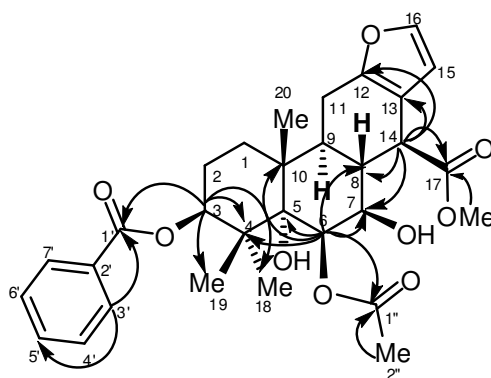
Selective HMBC correlations of **CP12**

**Table 46** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP12**

| Position | δ$_H$ (mult., $J$, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.45 (m) | 32.6 | CH$_2$ | 2, 3, 5, 9, 10, 20 |
| | 1.78 (m) | | | |
| 2 | 1.75 (m) | 23.9 | CH$_2$ | 3, 4, 10 |
| | 1.86 (m) | | | |
| 3 | 5.24 (dd, $J$ = 10.8, 5.7) | 76.7 | CH | 4, 18, 19, 1' |
| 4 | - | 43.9 | C | - |
| 5 | - | 78.8 | C | - |
| 6 | 5.42 (d, $J$ = 4.2) | 73.4 | CH | O$\underline{C}$OMe$_3$, 4, 5, 7, 8, 10 |
| 7 | 4.05 (dd, $J$ = 10.2, 4.2) | 74.0 | CH | - |
| 8 | 2.38 (ddd, $J$ = 10.5, 9.9, 8.1) | 37.6 | CH | 6, 7, 9, 10, 17 |
| 9 | 2.30 (m) | 41.2 | CH | 1, 8, 10, 11, 20 |
| 10 | - | 41.1 | C | - |
| 11 | 2.49 (m) | 21.5 | CH$_2$ | 8, 9, 10, 12, 13 |
| 12 | - | 150.7 | C | - |
| 13 | - | 113.1 | C | - |
| 14 | 3.38 (d, $J$ = 8.1) | 45.6 | CH | 7, 8, 12, 13, 17 |
| 15 | 6.13 (d, $J$ = 1.8) | 108.8 | CH | 12, 13, 16 |

**Table 46** (continued)

| Position | δ<sub>H</sub> (mult., *J*, Hz) | δ<sub>C</sub> | DEPT | HMBC |
|---|---|---|---|---|
| 16 | 7.17 (d, *J* = 1.8) | 141.2 | CH | 12, 13, 15 |
| 17 | - | 175.9 | C | - |
| 18 | 1.03 (s) | 22.7 | $CH_3$ | 3, 4, 5, 19 |
| 19 | 1.26 (s) | 19.2 | $CH_3$ | 3, 4, 5, 18 |
| 20 | 1.40 (s) | 16.5 | $CH_3$ | 1, 5, 9, 10 |
| 17-OMe | 3.68 (s) | 52.2 | $CH_3$ | 17 |
| O*C*OCH$_3$ | | 170.8 | C | - |
| OCO*CH$_3$* | 2.10 (s) | 21.7 | $CH_3$ | O*C*OMe$_3$ |
| 1' | - | 166.1 | C | - |
| 2' | - | 130.6 | C | - |
| 3'/7' | 7.95 (br d, *J* = 7.2) | 129.5 | CH | 1', 5' |
| 4'/6' | 7.37 (t, *J* = 7.2) | 128.4 | CH | 1' |
| 5' | 7.92 (tt, *J* = 7.2, 2.1) | 133.0 | CH | 2', 7' |
| 5-OH | 2.01 (br s) | - | - | 5, 6, 10 |

### 3.3.13 Compound CP13
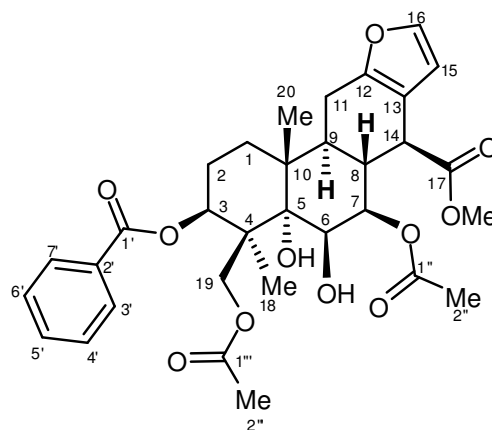


The molecular formula of compound **CP13** was determined to be $C_{32}H_{38}O_{11}$ ([M ]$^+$ *m/z* 598.2423) by HREIMS. The $^1H$ and $^{13}C$ NMR spectral data (Table 47, Figures 90 and 91) of **CP13** were similar to those of **CP12**. The differences were shown as a replacement of a singlet at $\delta$ 1.26 (Me-19) in **CP12** with an oxymethylene protons at $\delta$ 4.63 and 5.39 (each, d, *J* = 12.0 Hz; 2H-19) and an acetyl group ($\delta_H$ 1.98: $\delta_C$ 21.0 and $\delta_C$ 171.6) in **CP13**, whose position was supported by the HMBC correlations of oxymethylene protons at $\delta$ 4.63 and 5.39 (2H-19) with the carbons at $\delta$ 15.2 (C-18), 48.2 (C-4), 76.7 (C-3), 79.0 (C-5), and 171.6 (O$\underline{C}$OCH$_3$). Furthermore the HMBC correlations of an oxymethine proton at $\delta$ 5.19 (H-7) with the carbons at $\delta$ 34.3 (C-8), 45.4 (C-14) and 170.7 (O$\underline{C}$OCH$_3$) and of an oxymethine proton at $\delta$ 4.16 (H-6) with carbons at $\delta$ 34.3 (C-8), 40.9 (C-10), 78.2 (C-7), and 79.0 (C-5) implied the locations of an OAc group and an OH at C-7 and C-6, respectively. The relative stereochemistry of **CP13** was analyzed by NOESY experiment, in which the oxymethylene protons (2H-19) showed a cross-peak with the methyl protons at $\delta$ 1.35 (Me-20). Therefore, **CP13** was 3$\beta$-benzoyloxy-6$\beta$-hydroxy-7$\beta$,19-diacetoxy-14$\beta$-methoxycarbonylvouacapen-5$\alpha$-ol, a new compound (Yodsaoue et al., 2011) and was named as pulcherrin P.
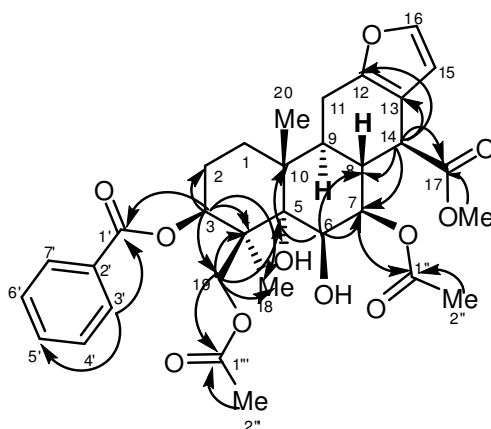
Selective HMBC correlations of **CP13**

**Table 47** ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **CP13**

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.41 (m) | 32.3 | CH$_2$ | 3, 10, 20 |
|   | 1.82 (m) | | | |
| 2 | 1.80 (m) | 23.9 | CH$_2$ | 3 |
| 3 | 5.31 (dd, *J* = 10.8, 4.8) | 76.7 | CH | 2, 4, 18, 19, 1' |
| 4 | - | 48.2 | C | - |
| 5 | - | 79.0 | C | 5, 6, 10 |
| 6 | 4.16 (d, *J* = 3.3) | 71.3 | CH | 5, 7, 8, 10 |
| 7 | 5.19 (dd, *J* = 11.1, 3.3) | 78.2 | CH | O<u>C</u>OMe$_3$, 8, 14 |
| 8 | 2.76 (ddd, *J* = 11.1, 9.0, 8.4) | 34.3 | CH | 7, 9, 14, 17 |
| 9 | 2.32 (ddd, *J* = 9.0, 7.5, 4.8) | 41.5 | CH | 8, 10, 11, 20 |
| 10 | - | 40.9 | C | - |
| 11 | 2.51 (m) | 21.4 | CH$_2$ | 8, 9, 12, 13 |
| 12 | - | 150.5 | C | - |
| 13 | - | 112.8 | C | - |
| 14 | 3.29 (d, *J* = 8.4) | 45.4 | CH | 7, 8, 12, 13, 17 |
| 15 | 6.07 (d, *J* = 1.8) | 108.3 | CH | 12, 13, 16 |

**Table 47** (continued)

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 16 | 7.17 (d, $J$ = 1.8) | 144.4 | CH | 12, 13, 15 |
| 17 | - | 174.6 | C | - |
| 18 | 1.06 (s) | 15.2 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 4.63 (d, $J$ = 12.0) | 64.0 | CH$_2$ | 3, 4, 5, 18, 19-O_C_OMe$_3$ |
|  | 5.39 (d, $J$ = 12.0) |  |  |  |
| 20 | 1.35 (s) | 15.7 | CH$_3$ | 1, 5, 9, 10 |
| 17-OMe | 3.68 (s) | 52.1 | CH$_3$ | 17 |
| O_C_OCH$_3$ | - | 170.7 | C | - |
| OCO_CH_$_3$ | 2.00 (s) | 21.0 | CH$_3$ | 7-O_C_OMe$_3$ |
| 19-O_C_OCH$_3$ | - | 171.6 | C | - |
| 19-OCO_CH_$_3$ | 1.98 (s) | 21.0 | CH$_3$ | 19-O_C_OMe$_3$ |
| 1' | - | 166.1 | C | - |
| 2' | - | 130.4 | C | - |
| 3'/7' | 8.03 (br d, $J$ = 7.8) | 129.7 | CH | 1', 5' |
| 4'/6' | 7.38 (t, $J$ = 7.8) | 128.3 | CH | 2' |
| 5' | 7.50 (br t, $J$ = 7.8) | 133.0 | CH | 3', 7' |
| 5-OH | 2.21 (s) | - | - | 5, 6, 10 |

### 3.3.14 Compound CP14
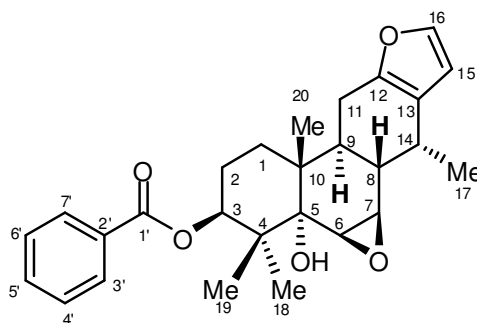


Compound **CP14** showed the molecular ion $[M]^+$ at *m/z* 436.2250 by HREIMS spectrum in agreement with the formula $C_{27}H_{32}O_5$. The $^1H$ and $^{13}C$ NMR spectral data (Table 14, Figures 92 and 93) of **CP14** showed characteristics similar to those of **CP5** except for the presence of a 1,2-disubstituted epoxide ring resonanced as two oxymethine protons at $\delta_H$ 3.25 and 3.01 (each d, *J* = 4.2 Hz; $\delta_C$ 55.0, 54.0, respectively) instead of 2 sets of methylene protons as in **CP5**. The signal at $\delta_H$ 3.25 was deduced to be an oxymetine proton H-6 from its HMBC correlations with the carbons at $\delta$ 39.1 (C-10), 43.1 (C-4), 54.0 (C-7) and 77.2 (C-5), and the other proton as H-7 ($\delta_H$ 3.01) from its HMBC correlations with the carbons at $\delta$ 31.0 (C-14), 35.3 (C-9), 35.6 (C-8) and 55.0 (C-6), whose data suggested an epoxide ring between C-6 and C-7. The relative stereochemistry of **CP14** was determined on the basis of coupling constants and the results of NOESY experiments. The large *J* values for H-6 and H-7 (*J* = 4.2 Hz) suggested a *cis* epoxide ring. From the NOESY correlations, an oxymethine proton at $\delta$ 3.25 (H-6) showed cross-peaks with the protons at $\delta$ 1.16 (Me-18) and 3.01 (H-7), and an oxymethine proton at $\delta$ 3.01 (H-7) with the methyl protons at $\delta$ 1.11 (Me-17) indicating that this *cis* epoxide ring should be $\beta$-oriented. Thus, **CP14** was assigned as 3$\beta$-benzoyloxy-6$\beta$,7$\beta$-epoxyvouacapen-5$\alpha$-ol, a new compound (Yodsaoue et al., 2011) and was named as pulcherrin Q.
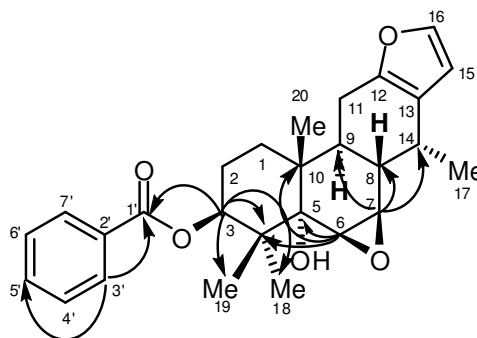
Selective HMBC correlations of **CP14**

**Table 48** ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **CP14**

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.28 (m) | 31.7 | CH$_2$ | 2, 3, 5, 10, 20 |
|   | 1.74 (m) | | | |
| 2 | 1.75 (m) | 23.7 | CH$_2$ | 1, 3, 4, 10 |
|   | 1.89 (m) | | | |
| 3 | 5.23 (dd, *J* = 11.7, 4.5) | 76.9 | CH | 4, 18, 19, 1' |
| 4 | - | 43.1 | C | - |
| 5 | - | 77.2 | C | - |
| 6 | 3.25 (d, *J* = 4.2) | 55.0 | CH | 4, 5, 7, 10 |
| 7 | 3.01 (d, *J* = 4.2) | 54.0 | CH | 6, 8, 9, 14 |
| 8 | 2.24 (m) | 35.6 | CH | 9, 11, 14 |
| 9 | 2.32 (m) | 35.3 | CH | 7, 8, 10, 11, 12, 20 |
| 10 | - | 39.1 | C | - |
| 11 | 2.28 (m) | 23.6 | CH$_2$ | 8, 9, 12, 13 |
|    | 2.41 (m) | | | |
| 12 | - | 149.8 | C | - |
| 13 | - | 122.1 | C | - |
| 14 | 2.90 (qd, *J* = 6.9, 5.4) | 31.0 | CH | 8, 9, 12, 13, 17 |
| 15 | 6.15 (d, *J* = 1.8) | 109.3 | CH | 12, 13, 16 |
| 16 | 7.17 (d, *J* = 1.8) | 141.0 | CH | 12, 13, 15 |
| 17 | 1.11 (d, *J* = 6.9) | 17.1 | CH$_3$ | 8, 13, 14 |

**Table 48** (continued)

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|----------|------------------------------|------------|------|------|
| 18 | 1.16 (s) | 23.2 | $CH_3$ | 3, 4, 5, 19 |
| 19 | 1.34 (s) | 19.6 | $CH_3$ | 3, 4, 5, 18 |
| 20 | 1.24 (s) | 16.4 | $CH_3$ | 1, 5, 9, 10 |
| 1' | - | 166.2 | C | - |
| 2' | - | 130.8 | C | - |
| 3'/7' | 8.00 (br d, *J* = 7.5) | 129.6 | CH | 1', 5' |
| 4'/6' | 7.39 (t, *J* = 7.5) | 128.4 | CH | 2' |
| 5' | 7.51 (tt, *J* = 7.5, 1.5) | 132.9 | CH | 3', 7' |

### 3.3.15 Compound CP15
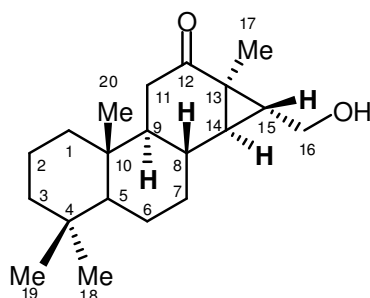


Compound **CP15** had the molecular formula $C_{20}H_{32}O_2$ ([M]$^+$ *m/z* 304.2405) based on HREIMS. The $^{13}C$ NMR (Table 49, Figure 95) and DEPT spectral data exhibited 20 carbons including a carbonyl at $\delta$ 211.8 (C-12) and an oxymethylene carbon at $\delta$ 62.3 (C-16). The $^1H$ NMR spectral data (Table 49, Figure 94) showed four aliphatic methyl groups at $\delta$ 0.71 (Me-20), 0.73 (Me-19), 0.78 (Me-18) and 1.17 (Me-17), and the oxymethylene protons at $\delta$ 3.47 (dd, *J* = 11.7, 8.1 Hz; H-16) and 3.73 (dd, *J* = 11.7, 5.7 Hz; H-16). The presence of a cyclopropane ring was deduced from the $^1H$ NMR, COSY and HMQC spectra that exhibited two signals at $\delta_H$ 0.94 (dd, *J* = 5.7, 1.5 Hz, H-14: $\delta_C$ 38.5) and 1.41 (m, H-15: $\delta_C$ 37.3). The observed HMBC correlations of a singlet methyl group at $\delta$ 1.17 (Me-17) with the carbons at $\delta$ 33.4 (C-13), 37.3 (C-15), 38.5 (C-14) and 211.8 (C-12), and of the oxymethylene protons at $\delta$ 3.47 and 3.73 (2H-16) with the carbons at $\delta$ 33.4 (C-13), 37.3 (C-15) and 38.5 (C-14) supported the assignments. These data suggested a carbonyl group at C-12 and an OH group at C-16 whereas C-13, C-14 and C-15 formed a cyclopropane ring. The NOESY cross-peaks of the proton signal at $\delta$ 1.79 (t, *J* = 14.1 Hz; H$_{ax}$-11) with the protons at $\delta$ 0.71 (Me-20), 1.41 (H-15) and 1.60 (H-8), of a methine proton at $\delta$ 0.94 (H-14) with the methyl protons at $\delta$ 1.17 (Me-17), 1.04 (H-9) and oxymethylene protons at $\delta$ 3.47 and 3.73 (2H-16) but no correlation with H-15 supported the $\alpha$-orientation of H-14, Me-17 and 2H-16 hence suggesting a *cis* cyclopropyl ring with an $\alpha$-hydroxy methyl side chain. The stereochemistry of compound **CP15** was implied by biogenetic pathway from the pimarane skeleton (Yodsaoue et al., 2010). Therefore, **CP15** was assigned as 13,14,15-cyclopropa-12-oxo-16-hydroxypimarane, a new compound (Yodsaoue et al., 2011) and was named as pulcherrin R.
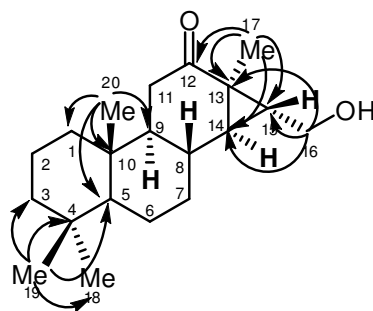
Selective HMBC correlations of **CP15**

**Table 49** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP15**

| Position | δ$_H$ (mult., *J*, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 0.80 (m) | 38.1 | CH$_2$ | 2, 3, 5, 20 |
| | 1.47 (m) | | | |
| 2 | 1.37 (m) | 18.6 | CH$_2$ | 1, 3, 4, 10 |
| | 1.48 (m) | | | |
| 3 | 0.99 (m) | 42.0 | CH$_2$ | 1, 2, 4, 5, 18, 19 |
| | 1.32 (m) | | | |
| 4 | - | 33.2 | C | - |
| 5 | 0.80 (dd, *J* = 10.8, 2.7) | 54.6 | CH | 7, 9, 10, 18, 19 |
| 6 | 1.20 (m) | 22.0 | CH$_2$ | 5, 7, 10 |
| | 1.59 (m) | | | |
| 7 | 1.19 (m) | 35.2 | CH$_2$ | 5, 6, 8, 9, 14 |
| | 1.98 (m) | | | |
| 8 | 1.60 (m) | 36.9 | CH | 7, 10, 11, 14, 15 |
| 9 | 1.04 (td, *J* = 14.1, 2.1) | 56.9 | CH | 1, 7, 8, 10, 11, 20 |
| 10 | - | 37.3 | C | - |
| 11eq | 1.79 (t, *J* = 14.1) | 36.4 | CH$_2$ | 8, 9, 12, 13 |
| ax | 2.13 (dd, *J* = 14.1, 2.1) | | | |

**Table 49** (continued)

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|----------|------------------------------|-----------|------|------|
| 12 | - | 211.8 | C | - |
| 13 | - | 33.4 | C | - |
| 14 | 0.94 (dd, *J* = 5.7, 1.5) | 38.5 | CH | 7, 9, 12, 13, 15, 16, 17 |
| 15 | 1.41 (m) | 37.3 | CH | 12, 13, 15, 16 |
| 16 | 3.47 (dd, *J* = 11.7, 8.1) | 62.3 | CH$_2$ | 13, 14, 15 |
|    | 3.73 (dd, *J* = 11.7, 5.7) | | | |
| 17 | 1.17 (s) | 14.1 | CH$_3$ | 12, 13, 14, 15 |
| 18 | 0.78 (s) | 33.4 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 0.73 (s) | 21.5 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 0.71 (s) | 14.1 | CH$_3$ | 1, 5, 9, 10 |

### 3.3.16 Compound CP16
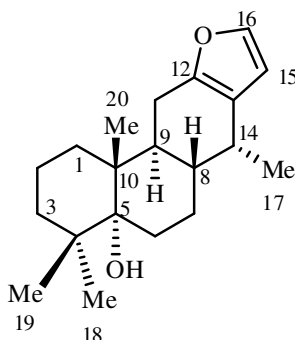


Compound **CP16** was isolated as a white solid, mp 98–100 °C, $[\alpha]_D^{27}$ + 80.9º (*c* 0.26 in CHCl₃). The IR spectrum displayed the absorbance of hydroxyl (3574 cm⁻¹) group.

The ¹H and ¹³C NMR spectral data (Table 50, Figures 96 and 97) of **CP16** showed characteristics similar to those of **CP1,** except that the signal of an oxymethine proton at *δ* 5.22 (td, *J* = 11.1, 6.0 Hz, H-7); $\delta_C$ 72.3 in **CP1** was replaced by those of the methylene protons at δ 1.35 and 1.77 (each m); $\delta_C$ 22.3. Moreover, **CP16** did not show the signal of an acetoxy methyl group at $\delta_H$ 2.00 (s, 7-OAc); $\delta_C$ 21.3 and 170.7. This finding was supported by HMBC spectrum, in which a methine proton H-9 at δ 2.30 (m) was correlated with the carbons at δ 17.1 (C-20), 22.3 (C-7), 24.4 (C-11), 32.5(C-1), 34.5 (C-8), 41.2 (C-10) and 149.8 (C-12). The X-ray structure of **CP16** established its stereochemistry. Thus, compound **CP16** was determined as vouacapen-5*α*-ol (McPherson et al., 1986).
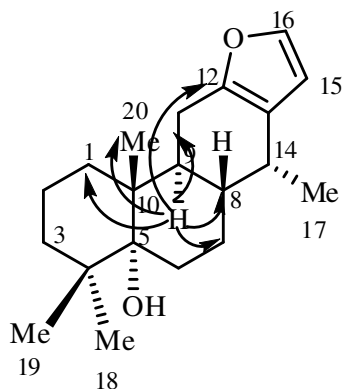


Selective HMBC correlations of **CP16**        X-ray structure of **CP16**
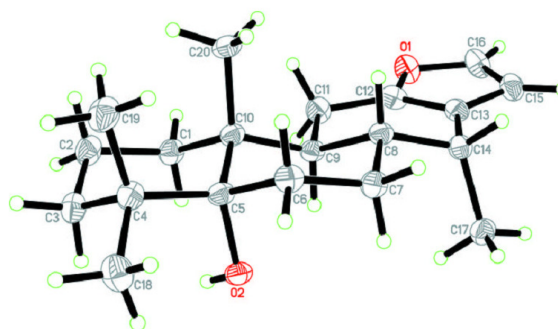
**Table 50** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP16**

| Position | δ$_H$ (mult., *J*, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.27 (m) | 32.5 | CH$_2$ | 2, 3, 5, 10, 20 |
|  | 1.37 (m) |  |  |  |
| 2 | 1.38 (m) | 18.2 | CH$_2$ | 1, 3, 4, 10 |
|  | 1.58 (m) |  |  |  |
| 3 | 1.10 (m) | 36.4 | CH$_2$ | 1, 2, 4, 5, 18, 19 |
|  | 1.60 (m) |  |  |  |
| 4 | - | 38.4 | C | - |
| 5 | - | 76.9 | C | - |
| 6 | 1.50 (m) | 25.7 | CH$_2$ | 5, 7, 8, 10 |
|  | 1.73 (m) |  |  |  |
| 7 | 1.35 (m) | 22.3 | CH$_2$ | 5, 6, 8, 9, 14 |
|  | 1.77 (m) |  |  |  |
| 8 | 1.75 (m) | 34.5 | CH | 7, 14 |
| 9 | 2.30 (m) | 37.6 | CH | 1, 7, 8, 10, 11, 12, 20 |
| 10 | - | 41.2 | C | - |
| 11 | 2.24 (m) | 24.4 | CH$_2$ | 8, 9, 10, 12, 13 |
|  | 2.40 (m) |  |  |  |
| 12 | - | 149.8 | C | - |
| 13 | - | 122.6 | C | - |
| 14 | 2.50 (qd, *J* = 6.9, 4.5) | 31.5 | CH | 8, 9, 12, 13, 15, 17 |
| 15 | 6.10 (d, *J* = 1.8) | 109.6 | CH | 12, 13, 16 |
| 16 | 7.13 (d, *J* = 1.8) | 140.3 | CH | 12, 13, 15 |
| 17 | 0.94 (d, *J* = 6.9) | 17.5 | CH$_3$ | 8, 13, 14 |
| 18 | 0.87 (s) | 28.0 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 1.00 (s) | 24.8 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 0.98 (s) | 17.1 | CH$_3$ | 1, 5, 9, 10 |

Table 51 Comparison of <sup>1</sup>H and <sup>13</sup>C NMR spectral data between compounds **CP16**
(recorded in CDCl<sub>3</sub>, 300 MHz) and vouacapen-5α-ol  (**R**, recorded in
CDCl<sub>3</sub>, 360 MHz)

| Position | CP16 δ$_H$ (mult., $J$, Hz) | R δ$_H$ (mult., $J$, Hz) | CP16 δ$_C$ | R δ$_C$ |
|---|---|---|---|---|
| 1 | 1.27 (m) 1.37 (m) | 1.18 (br s, $J$ = 14) | 32.5 | 32.5 |
| 2 | 1.38 (m) 1.58 (m) | | 18.2 | 18.2 |
| 3 | 1.10 (m) 1.60 (m) | | 36.4 | 36.4 |
| 4 | - | 1.15-1.85 (m) | 38.4 | 38.4 |
| 5 | - | | 76.9 | 76.8 |
| 6 | 1.50 (m) 1.73 (m) | | 25.7 | 25.7 |
| 7 | 1.35 (m) 1.77 (m) | | 22.3 | 22.3 |
| 8 | 1.75 (m) | | 34.5 | 34.5 |
| 9 | 2.30 (m) | 2.44 (br dd, $J$ = 10, 12) | 37.6 | 37.6 |
| 10 | - | - | 41.2 | 41.2 |
| 11 | 2.24 (m) 2.40 (m) | 2.35 (m) | 24.4 | 24.8 |
| 12 | - | - | 149.8 | 149.8 |
| 13 | - | - | 122.6 | 122.6 |
| 14 | 2.50 (qd, $J$ = 6.9, 4.5) | 2.58 (qd, $J$ = 7, 4) | 31.5 | 31.5 |
| 15 | 6.10 (d, $J$ = 1.8) | 6.18 (d, $J$ = 2) | 109.6 | 109.6 |
| 16 | 7.13 (d, $J$ = 1.8) | 7.23 (d, $J$ = 2) | 140.3 | 140.3 |
| 17 | 0.94 (d, $J$ = 6.9) | 1.01 (d, $J$ = 7) | 17.5 | 17.5 |
| 18 | 0.87 (s) | 1.05 (s) | 28.0 | 28.1 |
| 19 | 1.00 (s) | 1.07 (s) | 24.8 | 24.8 |
| 20 | 0.98 (s) | 0.94 (s) | 17.1 | 17.1 |

### 3.3.17 Compound CP17
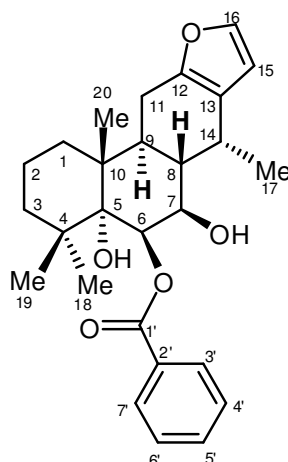


Compound **CP17** was obtained as a white solid, mp 116-118 °C $[\alpha]_D^{25}$ +60.0° (*c* 0.28, CHCl$_3$). The absorption bands of UV and IR spectrum were similar to **CP6**. The $^1$H and $^{13}$C NMR spectra (Table 52, Figures 98 and 99) of compound **CP17** were comparable with those of compound **CP6**. The only difference was found as replacement of the methylene protons at $\delta$ 1.53 and 2.23 (2H-7) in **CP6** with an oxymethine proton at $\delta$ 4.45 (dd, $J$ = 11.1, 3.9 Hz); $\delta_C$ 68.9 in **CP17**. The HMBC correlations of the latter proton with the carbons at $\delta$ 27.4 (C-14), 37.9 (C-8) and 74.4 (C-6) suggested its location at C-7 whose $\alpha$-orientation was suggested by the X-ray structure of **CP17** and the large vicinal coupling constants ($J_{7ax,8ax}$ = 11.1 Hz). Therefore, **CP17** was determined as isovouacapenol C (Ragasa et al., 2002).
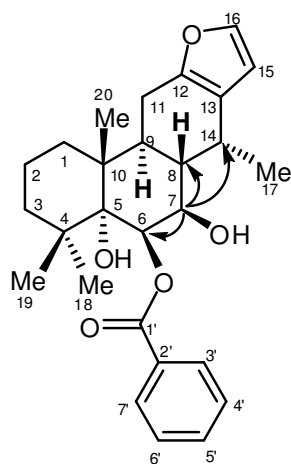


Selective HMBC correlations of **CP17**
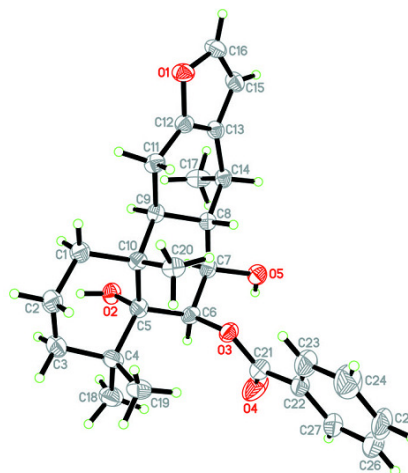


X-ray structure of **CP17**

**Table 52** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP17**

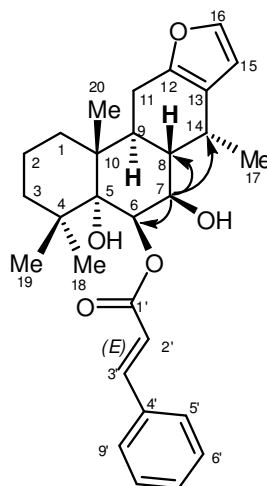| Position | δ$_H$ (mult., *J*, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.45 (m) | 34.9 | CH$_2$ | 5, 10, 20 |
|  | 1.65 (m) |  |  |  |
| 2 | 1.75 (m) | 18.3 | CH$_2$ | - |
|  | 1.82 (m) |  |  |  |
| 3 | 1.15 (m) | 37.8 | CH$_2$ | 1, 5, 19 |
|  | 1.78 (m) |  |  |  |
| 4 | - | 39.3 | C | - |
| 5 | - | 77.8 | C | - |
| 6 | 5.91 (d, *J* = 3.9) | 74.4 | CH | 4, 5, 7, 8, 10, 1' |
| 7 | 4.45 (dd, *J* = 11.1, 3.9) | 68.9 | CH | 6, 8, 14 |
| 8 | 2.07 (td, *J* = 11.1, 5.1) | 37.9 | CH | 7, 9, 11, 14, 17 |
| 9 | 2.53 (m) | 37.1 | CH | 8, 10, 11, 12, 20 |
| 10 | - | 41.0 | C | - |
| 11 | 2.50 (m) | 21.9 | CH$_2$ | 8, 9, 12, 13 |
|  | 2.61 (m) |  |  |  |
| 12 | - | 149.4 | C | - |
| 13 | - | 122.1 | C | - |
| 14 | 3.07 (qd, *J* = 6.6, 5.1) | 27.4 | CH | 8, 9, 12, 13, 15, 17 |
| 15 | 6.24 (d, *J* = 1.5) | 109.8 | CH | 12, 13, 16 |
| 16 | 7.28 (d, *J* = 1.5) | 140.5 | CH | 12, 13, 15 |
| 17 | 1.06 (d, *J* = 6.6) | 17.2 | CH$_3$ | 8, 13, 14 |
| 18 | 1.18 (s) | 27.9 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 1.20 (s) | 25.6 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 1.56 (s) | 17.6 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 167.5 | C | - |
| 2' | - | 130.1 | C | - |
| 3'/7' | 8.10 (d, *J* = 7.2) | 130.0 | CH | 1', 2', 5' |
| 4'/6' | 7.45 (t, *J* = 7.2) | 128.6 | CH | 1', 2' |
| 5' | 7.57 (t, *J* = 7.2) | 133.3 | CH | 3', 7' |
| 5-OH | 2.44 (br s) | - | - | 5, 6, 10 |

**Table 53** Comparison of ^{1}H and ^{13}C NMR spectral data between compounds **CP17** (recorded in CDCl$_3$, 300 MHz) and isovouacapenol C (**R**, recorded in CDCl$_3$, 400 MHz)

| Position | CP17 δ$_H$ (mult., *J*, Hz) | R δ$_H$ (mult., *J*, Hz) | CP17 δ$_C$ | R δ$_C$ |
|---|---|---|---|---|
| 1 | 1.45 (m) | 1.49 (m) | 34.9 | 35.1 |
|  | 1.65 (m) | 1.54 (m) |  |  |
| 2 | 1.75 (m) | 1.56 (m) | 18.3 | 18.1 |
|  | 1.82 (m) | 1.70 (m) |  |  |
| 3 | 1.15 (m) | 1.18 (m) | 37.8 | 37.8 |
|  | 1.78 (m) | 1.67 (m) |  |  |
| 4 | - | - | 39.3 | 39.3 |
| 5 | - | - | 77.8 | 77.9 |
| 6 | 5.91 (d, *J* = 3.9) | 5.81 (d, *J* = 4.1) | 74.4 | 74.0 |
| 7 | 4.45 (dd, *J* = 11.1, 3.9) | 4.41 (dd, *J* = 11.0, 4.1) | 68.9 | 69.3 |
| 8 | 2.07 (td, *J* = 11.1, 5.1) | 2.02 (m) | 37.9 | 38.1 |
| 9 | 2.53 (m) | 2.43 (m) | 37.1 | 37.2 |
| 10 | - | - | 41.0 | 41.0 |
| 11 | 2.50 (m) | 2.57 (m) | 21.9 | 21.8 |
|  | 2.61 (m) |  |  |  |
| 12 | - | - | 149.4 | 149.2 |
| 13 | - | - | 122.1 | 122.0 |
| 14 | 3.07 (qd, *J* = 6.6, 5.1) | 3.04 (m) | 27.4 | 27.3 |
| 15 | 6.24 (d, *J* = 1.5) | 6.20 (d, *J* = 1.9) | 109.8 | 109.7 |
| 16 | 7.28 (d, *J* = 1.5) | 7.24 (d, *J* = 1.9) | 140.5 | 140.5 |
| 17 | 1.06 (d, *J* = 6.6) | 1.09 (d, *J* = 6.8) | 17.2 | 17.1 |
| 18 | 1.18 (s) | 1.54 (s) | 27.9 | 17.6 |
| 19 | 1.20 (s) | 1.18 (s) | 25.6 | 25.5 |
| 20 | 1.56 (s) | 1.12 (s) | 17.6 | 27.3 |
| 1' | - | - | 167.5 | 167.2 |
| 2' | - | - | 130.1 | 130.0 |
| 3'/7' | 8.10 (d, *J* = 7.2) | 8.05 | 130.0 | 129.9 |
| 4'/6' | 7.45 (t, *J* = 7.2) | 7.45 | 128.6 | 128.6 |
| 5' | 7.57 (t, *J* = 7.2) | 7.57 | 133.3 | 133.2 |

### 3.3.18 Compound CP18
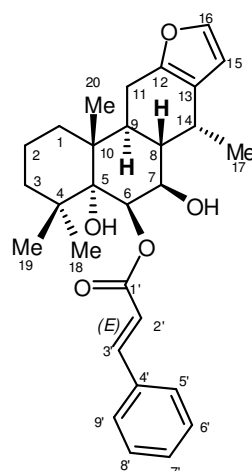


Compound **CP18** was isolated as a white solid; mp 220-222 °C; $[\alpha]_D^{25}$ +59.9° (*c* 0.13, CHCl$_3$). The absorption band for UV and IR spectrum were identical to **CP7**. The NMR spectroscopic data of **CP18** displayed similarities with **CP7**. The $^{13}$C NMR spectrum (Table 54, Figure 101) exhibited a couple of oxymethine carbons at $\delta$ 68.9 and 73.8, these being assigned to C-7 and C-6, respectively. The $^1$H NMR (Table 54, Figure 100) signal of H-7 was observed at $\delta$ 4.41 (dd, *J* = 11.1, 3.9 Hz), whose HMBC spectrum showed correlations to the carbons at $\delta$ 27.8 (C-14), 37.8 (C-8) and 73.8 (C-6). The relative stereochemistry of **CP18** was determined on the basis of coupling constants and the results of NOESY experiments. The large *J* values for H-7 and H-8 (*J* = 11.1 Hz) indicated that H-7 should be an axial proton. In addition, the oxymethine proton at $\delta$ 4.41 (H-7) showed a cross-peak with the protons at $\delta$ 1.10 (Me-17) and 2.48 (H-9$\alpha$) in the NOESY experiment confirming the $\alpha$-orientation of H-7. Thus, **CP18** was characterized as 6$\beta$-cinnamoyl-7$\beta$-hydroxyvouacapen-5$\alpha$-ol (McPherson et al., 1986).

Selective HMBC correlations of **CP18**

**Table 54** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP18**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.43 (m) | 34.9 | CH$_2$ | 5 |
|   | 1.63 (m) | | | |
| 2 | 1.65 (m) | 18.2 | CH$_2$ | 10 |
|   | 1.73 (m) | | | |
| 3 | 1.12 (m) | 37.8 | CH$_2$ | 4, 5, 19 |
|   | 1.73 (m) | | | |
| 4 | - | 39.3 | C | - |
| 5 | - | 77.7 | C | - |
| 6 | 5.71 (d, $J$ = 3.9) | 73.8 | CH | 1', 4, 5, 7, 8, 10 |
| 7 | 4.41 (dd, $J$ = 11.1, 3.9) | 68.9 | CH | 6, 8, 14 |
| 8 | 2.02 (dt, $J$ = 11.1, 5.1) | 37.8 | CH | 7, 9, 14, 17 |
| 9 | 2.48 (m) | 37.1 | CH | 10, 11, 12, 20 |
| 10 | - | 41.1 | C | - |
| 11 | 2.53 (m) | 21.8 | CH$_2$ | 9, 10, 12, 13 |
| 12 | - | 149.4 | C | - |
| 13 | - | 122.0 | C | - |

**Table 54** (continued)

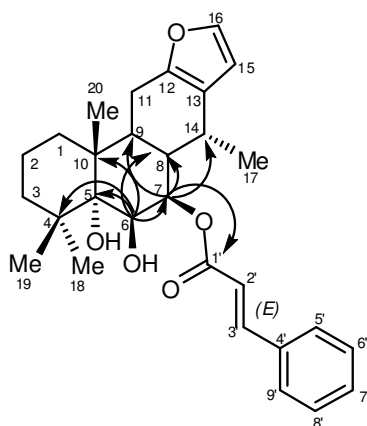| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 14 | 3.08 (qd, $J$ = 6.6, 5.1) | 27.8 | CH | 8, 9, 12, 13, 15, 17 |
| 15 | 6.21 (d, $J$ = 1.8) | 109.8 | CH | 12, 13, 16 |
| 16 | 7.25 (d, $J$ = 1.8) | 140.4 | CH | 12, 13, 15 |
| 17 | 1.10 (d, $J$ = 6.6) | 17.3 | CH$_3$ | 8, 13, 14 |
| 18 | 1.11 (s) | 27.9 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 1.21 (s) | 25.6 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 1.45 (s) | 17.3 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 167.5 | C | - |
| 2' | 6.47 (d, $J$ = 15.9) | 118.2 | CH | 1', 3', 4' |
| 3' | 7.72 (d, $J$ = 15.9) | 145.8 | CH | 1', 2', 4', 5', 9' |
| 4' | - | 134.2 | C | - |
| 5'/9' | 7.50 (m) | 128.3 | CH | 3', 4', 7' |
| 6'/8' | 7.37 (m) | 128.9 | CH | 5', 4' |
| 7' | 7.37 (m) | 130.6 | CH | 5', 9' |

**Table 55** Comparison of ^{1}H and ^{13}C NMR spectral data between compounds **CP18** (recorded in CDCl$_3$, 300 MHz) and 6$\beta$-cinnamoyl-7$\beta$-hydroxyvouacapen-5$\alpha$-ol (**R**, recorded in CDCl$_3$, 360 MHz)

| Position | CP18 $\delta_H$ (mult., $J$, Hz) | R $\delta_H$ (mult., $J$, Hz) | CP18 $\delta_C$ | R $\delta_C$ |
|---|---|---|---|---|
| 1 | 1.43 (m) | 1.17 (br d, $J = 12$) | 34.9 | 35.0 |
|  | 1.63 (m) | 1.68 (br d) |  |  |
| 2 | 1.65 (m) | 1.54 (br d) | 18.2 | 18.1 |
|  | 1.73 (m) | 1.65 (br d) |  |  |
| 3 | 1.12 (m) | 1.54 (br d) | 37.8 | 37.8 |
|  | 1.73 (m) | 1.76 (br d) |  |  |
| 4 | - | - | 39.3 | 39.3 |
| 5 | - | 1.80 (OH) | 77.7 | 76.8 |
| 6 | 5.71 (d, $J = 3.9$) | 5.65 (d, $J = 4$) | 73.8 | 73.6 |
| 7 | 4.41 (dd, $J = 11.1, 3.9$) | 4.38 (dd, $J = 11, 3.5$) | 68.9 | 69.2 |
| 8 | 2.02 (dt, $J = 11.1, 5.1$) | 1.98 (ddd, $J = 12, 11, 5$) | 37.8 | 37.9 |
| 9 | 2.48 (m) | 2.45 (dt, $J = 12, 9$) | 37.1 | 37.2 |
| 10 | - | - | 41.1 | 41.1 |
| 11 | 2.53 (m) | 2.54 (br d, $J = 9$) | 21.8 | 21.8 |
| 12 | - | - | 149.4 | 149.2 |
| 13 | - | - | 122.0 | 120.0 |
| 14 | 3.08 (qd, $J = 6.6, 5.1$) | 3.05 (qd, $J = 7, 6$) | 27.8 | 27.3 |
| 15 | 6.21 (d, $J = 1.8$) | 6.20 (d, $J = 2$) | 109.8 | 109.7 |
| 16 | 7.25 (d, $J = 1.8$) | 7.23 (d, $J = 2$) | 140.4 | 140.5 |
| 17 | 1.10 (d, $J = 6.6$) | 1.07 (d, $J = 7$) | 17.3 | 17.3 |
| 18 | 1.11 (s) | 1.21 (s) | 27.9 | 27.7 |
| 19 | 1.21 (s) | 1.45 (s) | 25.6 | 25.5 |
| 20 | 1.45 (s) | 1.09 (s) | 17.3 | 17.1 |
| 1' | - | - | 167.5 | 167.4 |
| 2' | 6.47 (d, $J = 15.9$) | 6.44 (d, $J = 16$) | 118.2 | 118.0 |
| 3' | 7.72 (d, $J = 15.9$) | 7.72 (d, $J = 16$) | 145.8 | 145.9 |
| 4' | - | - | 134.2 | 134.2 |
| 5'/9' | 7.50 (m) | 7.53 (m) | 128.3 | 128.9 |
| 6'/8' | 7.37 (m) | 7.38 (m) | 128.9 | 128.2 |
| 7' | 7.37 (m) | 7.38 (m) | 130.6 | 130.5 |

### 3.3.19 Compound CP19
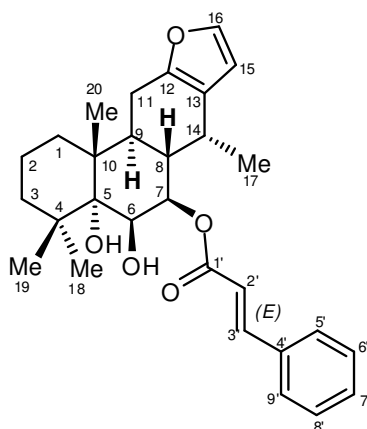


Compound **CP19** was purified as viscous oil; $[\alpha]_D^{25}$ +41.5° (*c* 0.08 in CHCl$_3$). The absorption band for UV and IR spectra were identical to **CP18**. The $^1$H and $^{13}$C NMR spectra (Table 56, Figures 102 and 103) of **CP19** were closely related to those of **CP18**, and differed only in the chemical shifts of positions 6 and 7. The oxymethine proton H-6 of **CP19** appeared at $\delta_H$ 4.24, higher field than that of **CP18** ($\delta_H$ 5.71) while H-7 of **CP19** resonanced at $\delta_H$ 5.50 more downfield than that of **CP18** ($\delta_H$ 4.41) as a result of the deshielding effect of the cinnamoyloxy group. The HMBC correlations of an oxymethine proton at $\delta$ 4.24 (H-6) with the carbons at $\delta$ 35.2 (C-8), 39.3 (C-4), 40.7 (C-10), 75.0 (C-7) and 77.8 (C-5) and of an oxymethine proton at $\delta$ 5.50 (H-7) with the carbons at $\delta$ 27.6 (C-14), 35.2 (C-8), 37.2 (C-9) and 166.1 (C-1') confirmed the locations of the OH at C-6 and the cinnamoyloxy group at C-7. Thus, **CP19** was assigned to be pulcherrin A (Pranithanchai et al., 2009).



Selective HMBC correlations of **CP19**

**Table 56** $^{1}$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP19**

| Position | δ$_H$ (mult., *J*, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.31 (m) | 35.2 | CH$_2$ | 5, 10, 20 |
|   | 1.47 (m) | | | |
| 2 | 1.40 (m) | 18.2 | CH$_2$ | 1 |
|   | 1.65 (m) | | | |
| 3 | 1.07 (m) | 37.5 | CH$_2$ | 1, 4, 5, 19 |
|   | 1.60 (m) | | | |
| 4 | - | 39.3 | C | - |
| 5 | - | 77.8 | C | - |
| 6 | 4.24 (d, *J* = 3.9) | 71.4 | CH | 4, 5, 7, 8, 10 |
| 7 | 5.50 (dd, *J* = 11.4, 3.9) | 75.0 | CH | 1', 8, 9, 14 |
| 8 | 2.23 (td, *J* = 11.4, 5.1) | 35.2 | CH | 7, 9, 11, 14, 17 |
| 9 | 2.39 (td, *J* = 11.4, 4.5) | 37.2 | CH | 1, 8, 10, 11, 12, 14, 20 |
| 10 | - | 40.7 | C | - |
| 11 | 2.45 (m) | 21.8 | CH$_2$ | 9, 8, 12, 13 |
| 12 | - | 149.5 | C | - |
| 13 | - | 121.6 | C | - |
| 14 | 2.78 (qd, *J* = 7.2, 5.1) | 27.6 | CH | 8, 9, 12, 13, 15, 17 |
| 15 | 6.11 (d, *J* = 1.2) | 109.5 | CH | 12, 13, 16 |
| 16 | 7.15 (d, *J* = 1.2) | 140.5 | CH | 12, 13, 15 |
| 17 | 0.94 (d, *J* = 7.2) | 17.4 | CH$_3$ | 8, 13, 14 |
| 18 | 0.96 (s) | 27.8 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 1.39 (s) | 25.5 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 1.31 (s) | 17.3 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 166.1 | C | - |
| 2' | 6.42 (d, *J* = 16.2) | 117.7 | CH | 1', 3', 4' |
| 3' | 7.68 (d, *J* = 16.2) | 145.6 | CH | 1', 2', 4', 5', 9' |
| 4' | - | 134.2 | C | - |
| 5'/9' | 7.47 (m) | 128.2 | CH | 3', 4', 7' |
| 6'/8' | 7.33 (m) | 129.0 | CH | 4', 8' |
| 7' | 7.34 (m) | 130.6 | CH | 5', 9' |

**Table 57** Comparison of $^1$H and $^{13}$C NMR spectral data between compounds **CP19** (recorded in CDCl$_3$, 300 MHz) and pulcherrin A (**R**, recorded in CDCl$_3$, 300 MHz)

| Position | CP19 δ$_H$ (mult., *J*, Hz) | R δ$_H$ (mult., *J*, Hz) | CP19 δ$_C$ | R δ$_C$ |
|---|---|---|---|---|
| 1 | 1.31 (m) | 1.43 (m) | 35.2 | 35.2 |
|   | 1.47 (m) | 1.54 (m) |   |   |
| 2 | 1.40 (m) | 1.50 (m) | 18.2 | 18.2 |
|   | 1.65 (m) | 1.67 (m) |   |   |
| 3 | 1.07 (m) | 1.17 (m) | 37.5 | 37.6 |
|   | 1.60 (m) | 1.67 (m) |   |   |
| 4 | - | - | 39.3 | 39.2 |
| 5 | - | - | 77.8 | 77.8 |
| 6 | 4.24 (d, *J* = 3.9) | 4.32 (dd, *J* = 3.6, 2.1) | 71.4 | 71.5 |
| 7 | 5.50 (dd, *J* = 11.4, 3.9) | 5.58 (dd, *J* = 11.1, 3.6) | 75.0 | 75.0 |
| 8 | 2.23 (td, *J* = 11.4, 5.1) | 2.31 (td, *J* = 11.1, 4.8) | 35.2 | 35.2 |
| 9 | 2.39 (td, *J* = 11.4, 4.5) | 2.49 (m) | 37.2 | 37.3 |
| 10 | - | - | 40.7 | 40.7 |
| 11 | 2.45 (m) | 2.53 (m) | 21.8 | 21.8 |
| 12 | - | - | 149.5 | 149.5 |
| 13 | - | - | 121.6 | 121.7 |
| 14 | 2.78 (qd, *J* = 7.2, 5.1) | 2.86 (qd, *J* = 6.9, 4.8) | 27.6 | 27.6 |
| 15 | 6.11 (d, *J* = 1.2) | 6.19 (d, *J* = 1.8) | 109.5 | 109.5 |
| 16 | 7.15 (d, *J* = 1.2) | 7.23 (d, *J* = 1.8) | 140.5 | 140.5 |
| 17 | 0.94 (d, *J* = 7.2) | 1.02 (d, *J* = 6.9) | 17.4 | 17.2 |
| 18 | 0.96 (s) | 1.47 (s) | 27.8 | 27.8 |
| 19 | 1.39 (s) | 1.04 (s) | 25.5 | 25.5 |
| 20 | 1.31 (s) | 1.39 (s) | 17.3 | 17.4 |
| 1' | - | - | 166.1 | 166.0 |
| 2' | 6.42 (d, *J* = 16.2) | 6.51 (d, *J* = 15.9) | 117.7 | 117.8 |
| 3' | 7.68 (d, *J* = 16.2) | 7.75 (d, *J* = 15.9) | 145.6 | 145.6 |
| 4' | - | - | 134.2 | 134.2 |
| 5'/9' | 7.47 (m) | 7.55 (m) | 128.2 | 128.2 |
| 6'/8' | 7.33 (m) | 7.41 (m) | 129.0 | 129.0 |
| 7' | 7.34 (m) | 7.41 (m) | 130.6 | 130.5 |

### 3.3.20 Compound CP20
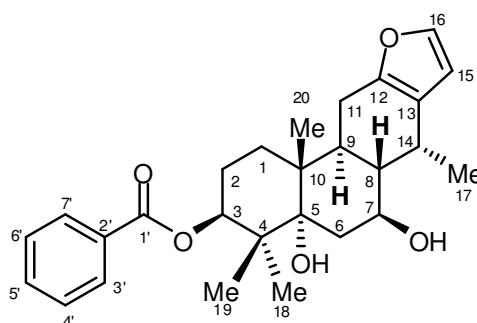


Compound **CP20** was isolated as a white solid; mp 161-163 °C; $[\alpha]_D^{25}$ +71.5° ($c$ 0.21 in CHCl$_3$). The $^1$H and $^{13}$C NMR spectra (Table 58, Figures 104 and 105) of **CP20** were comparable with those of **CP5**. The only difference was found as replacement of the methylene protons at $\delta$ 1.46 and 1.72 (2H-7) in **CP5** with an oxymethine proton at $\delta$ 4.10 (td, $J$ = 11.4, 5.1 Hz); $\delta_C$ 66.7 in **CP20**. The HMBC correlations of the latter proton with the carbons at $\delta$ 27.3 (C-14), 35.5 (C-6) and 42.7 (C-8) suggested its location at C-7 whose $\alpha$-orientation was suggested by its NOESY cross-peak with Me-17 ($\delta$ 1.06) and the large vicinal coupling constants ($J_{7ax,8ax}$ = 11.4 Hz). Therefore, **CP20** was pulcherrin B (Pranithanchai et al., 2009).
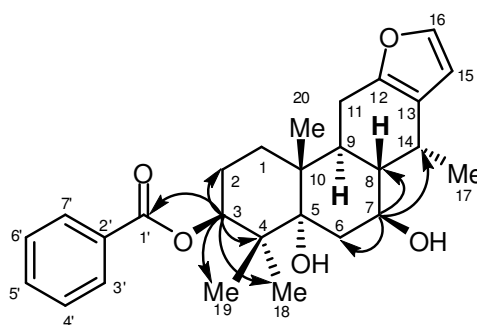


Selective HMBC correlations of **CP20**

**Table 58** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP20**

| Position | δ$_H$ (mult., $J$, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.50 (m) | 30.8 | CH$_2$ | 2, 3, 5, 10, 20 |
|  | 1.85 (m) | | | |
| 2 | 1.84 (m) | 23.8 | CH$_2$ | 1, 3, 4, 10 |
|  | 1.89 (m) | | | |
| 3 | 5.39 (dd, $J$ = 11.1, 5.4) | 77.7 | CH | 1', 2, 4, 18, 19 |
| 4 | - | 43.4 | C | - |
| 5 | - | 79.0 | C | - |
| 6 | 1.87 (m) | 35.5 | CH$_2$ | 4, 5, 7, 8, 10 |
|  | 2.13 (dd, $J$ = 11.4, 5.1) | | | |
| 7 | 4.10 (td, $J$ = 11.4, 5.1) | 66.7 | CH | 6, 8, 14 |
| 8 | 1.71 (td, $J$ = 11.4, 5.1) | 42.7 | CH | 6, 7, 9, 11, 14, 17 |
| 9 | 2.55 (m) | 36.6 | CH | 1, 8, 10, 11, 14, 20 |
| 10 | - | 40.8 | C | - |
| 11 | 2.40 (m) | 22.3 | CH$_2$ | 8, 9, 10, 12, 13 |
|  | 2.53 (m) | | | |
| 12 | - | 149.2 | C | - |
| 13 | - | 122.4 | C | - |
| 14 | 3.14 (qd, $J$ = 7.2, 5.1) | 27.3 | CH | 8, 9, 12, 13, 15, 17 |
| 15 | 6.25 (br s) | 109.7 | CH | 12, 13, 16 |
| 16 | 7.30 (br s) | 140.4 | CH | 12, 13, 15 |
| 17 | 1.06 (d, $J$ = 7.2) | 16.7 | CH$_3$ | 8, 13, 14 |
| 18 | 1.10 (s) | 22.9 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 1.26 (s) | 19.3 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 1.17 (s) | 16.8 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 165.7 | C | - |
| 2' | - | 131.2 | C | - |
| 3'/7' | 8.05 (d, $J$ = 8.1) | 129.3 | CH | 1', 4', 5', 6' |
| 4'/6' | 7.52 (t, $J$ = 8.1) | 128.5 | CH | 1', 2', 3', 7' |
| 5' | 7.64 (t, $J$ = 8.1) | 132.9 | CH | 3', 7' |
| 5-OH | 3.68 (s) | - | - | 5, 6, 10 |

**Table 59** Comparison of ${}^1$H and ${}^{13}$C NMR spectral data between compounds **CP20** (recorded in acetone-$d_6$, 300 MHz) and pulcherrin B (**R**, recorded in CDCl$_3$, 300 MHz)

| Position | CP20 δ$_H$ (mult., *J*, Hz) | R δ$_H$ (mult., *J*, Hz) | CP20 δ$_C$ | R δ$_C$ |
|---|---|---|---|---|
| 1 | 1.50 (m) | 1.51 (m) | 30.8 | 31.0 |
| | 1.85 (m) | 1.77 (m) | | |
| 2 | 1.84 (m) | 1.80 (m) | 23.8 | 23.8 |
| | 1.89 (m) | 1.92 (m) | | |
| 3 | 5.39 (dd, *J* = 11.1, 5.4) | 5.30 (dd, *J* = 11.5 , 5.0) | 77.7 | 77.3 |
| 4 | - | - | 43.4 | 43.5 |
| 5 | - | - | 79.0 | 79.9 |
| 6 | 1.87 (m) | 1.86 (dd, *J* = 13.0, 11.0) | 35.5 | 35.9 |
| | 2.13 (dd, *J* = 11.4, 5.1) | 2.05 (dd, *J* = 13.0, 5.5) | | |
| 7 | 4.10 (td, *J* = 11.4, 5.1) | 4.12 (dt, *J* = 11.0, 5.5) | 66.7 | 68.1 |
| 8 | 1.71 (td, *J* = 11.4, 5.1) | 1.74 (td, *J* = 11.0, 7.0) | 42.7 | 42.8 |
| 9 | 2.55 (m) | 2.46 (m) | 36.6 | 36.7 |
| 10 | - | - | 40.8 | 40.9 |
| 11 | 2.40 (m) | 2.43 (m) | 22.3 | 22.5 |
| | 2.53 (m) | 2.53 (dd, *J* = 13.5, 5.0) | | |
| 12 | - | - | 149.2 | 149.1 |
| 13 | - | - | 122.4 | 121.9 |
| 14 | 3.14 (qd, *J* = 7.2, 5.1) | 3.09 (quint, *J* = 7.0) | 27.3 | 27.4 |
| 15 | 6.25 (br s) | 6.22 (d, *J* = 2.0) | 109.7 | 109.7 |
| 16 | 7.30 (br s) | 7.25 (d, *J* = 2.0) | 140.4 | 140.7 |
| 17 | 1.06 (d, *J* = 7.2) | 1.10 (d, *J* = 7.0) | 16.7 | 17.1 |
| 18 | 1.10 (s) | 1.08 (s) | 22.9 | 23.1 |
| 19 | 1.26 (s) | 1.26 (s) | 19.3 | 19.7 |
| 20 | 1.17 (s) | 1.18 (s) | 16.8 | 17.5 |
| 1' | - | - | 165.7 | 166.2 |
| 2' | - | - | 131.2 | 130.8 |
| 3'/7' | 8.05 (d, *J* = 8.1) | 8.04 (dd, *J* = 7.5, 1.0) | 129.3 | 129.5 |
| 4'/6' | 7.52 (t, *J* = 8.1) | 7.45 (t, *J* = 7.5) | 128.5 | 128.4 |
| 5' | 7.64 (t, *J* = 8.1) | 7.57 (tt, *J* = 7.5, 1.0) | 132.9 | 140.7 |

### 3.3.21 Compound CP21
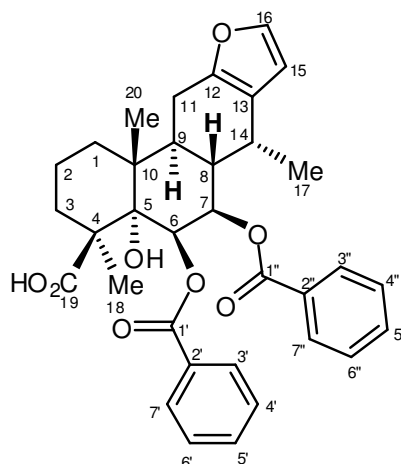


Compound **CP21** was isolated as a white solid; mp 140-142 °C; $[\alpha]_D^{25}$ +72.2° (*c* 1.84 in CHCl$_3$). The $^1$H NMR spectroscopic data (Table 60, Figure 106) of **CP21** displayed similarities with **CP10**, except for the presence of an additional monosubstituted benzene ring in the range $\delta$ 7.28-7.78 and an oxymethine proton at $\delta$ 5.75 (dd, *J* = 11.1, 3.6 Hz; H-7). The latter proton was attached to the oxymethine carbon at $\delta$ 72.5 in the HMQC spectrum and showed HMBC correlations with the carbons at $\delta$ 27.4 (C-14), 35.7 (C-8), 69.0 (C-6) and 166.3 (C-1″), confirming the location of a benzoate group at C-7. The stereochemistry of H-7 as $\alpha$-axial orientation was determined by the results of the large coupling constants ($J_{7ax,8ax}$ = 11.1 Hz) and by the observed cross-peak with Me-17 ($\delta$ 0.99) in the NOESY experiments. Thus, **CP21** was pulcherrimin C (Patil et al., 1997).
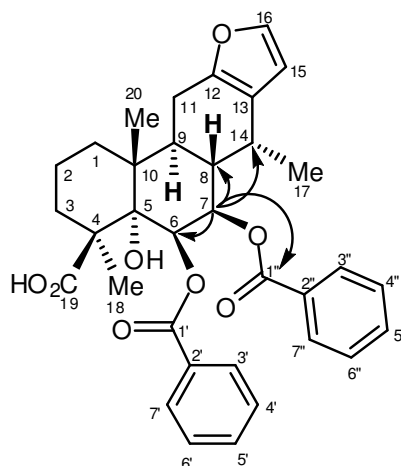


Selective HMBC correlations of **CP21**

**Table 60** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP21**

| Position | δ$_H$ (mult., *J*, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.50 (m) | 34.6 | CH$_2$ | 5, 9, 10, 20 |
|  | 1.73 (m) |  |  |  |
| 2 | 1.45 (m) | 18.7 | CH$_2$ | 10 |
|  | 1.93 (m) |  |  |  |
| 3 | 1.55 (m) | 33.5 | CH$_2$ | 1, 5, 18, 19 |
|  | 1.78 (m) |  |  |  |
| 4 | - | 49.0 | C | - |
| 5 | - | 77.8 | C | - |
| 6 | 6.05 (d, *J* = 3.6) | 69.0 | CH | 1', 4, 5, 7, 8, 10 |
| 7 | 5.75 (dd, *J* = 11.1, 3.6) | 72.5 | CH | 1", 6, 8, 14 |
| 8 | 2.43 (td, *J* = 11.1, 5.1) | 35.7 | CH | 7, 9, 17 |
| 9 | 2.53 (m) | 37.3 | CH | 8, 10, 11, 12, 20 |
| 10 | - | 41.6 | C | - |
| 11 | 2.56 (m) | 22.2 | CH$_2$ | 8, 9, 10, 13, 12 |
|  | 2.65 (m) |  |  |  |
| 12 | - | 149.1 | C | - |
| 13 | - | 121.3 | C | - |
| 14 | 2.85 (qd, *J* = 6.9, 5.1) | 27.4 | CH | 8, 9, 12, 13, 15, 17 |
| 15 | 6.13 (d, *J* = 1.8) | 109.5 | CH | 12, 13, 16 |
| 16 | 7.23 (d, *J* = 1.8) | 140.8 | CH | 12, 13, 15 |
| 17 | 0.99 (d, *J* = 6.9) | 17.1 | CH$_3$ | 8, 13, 14 |
| 18 | 1.13 (s) | 24.2 | CH$_3$ | 3, 4, 5,1 9 |
| 19 | - | 181.2 | C | - |
| 20 | 1.36 (s) | 17.8 | CH$_3$ | 1, 5, 9,1 0 |
| 1' | - | 165.7 | C | - |
| 2' | - | 130.5 | C | - |
| 3'/7' | 7.76 (br d, *J* = 7.5) | 129.6 | CH | 1', 5' |
| 4'/6' | 7.35 (t, *J* = 7.5) | 128.3 | CH | 1', 2' |

**Table 60** (continued)

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|----------|------------------------------|-----------|------|------|
| 5' | 7.49 (br t, $J$ = 7.5) | 132.6 | CH | 3', 7' |
| 1" | - | 166.3 | C | - |
| 2" | - | 129.9 | C | - |
| 3"/7" | 7.78 (br d, $J$ = 7.5) | 129.5 | CH | 1", 5" |
| 4"/6" | 7.28 (t, $J$ = 7.5) | 128.2 | CH | 1", 2" |
| 5" | 7.49 (br t, $J$ = 7.5) | 132.9 | CH | 3", 7" |

**Table 61** Comparison of ^{1}H and ^{13}C NMR spectral data between compounds **CP21** (recorded in CDCl$_3$, 300 MHz) and pulcherrimin C (**R**, recorded in CDCl$_3$, 400 MHz)

| Position | CP21 $\delta_H$ (mult., $J$, Hz) | R $\delta_H$ (mult., $J$, Hz) | CP21 $\delta_C$ | R $\delta_C$ |
|----------|-----------------------------------|-------------------------------|-----------------|--------------|
| 1 | 1.50 (m)<br>1.73 (m) | 1.53 (m)<br>1.70 (m) | 34.6 | 34.6 |
| 2 | 1.45 (m)<br>1.93 (m) | 1.44 (m)<br>1.93 (m) | 18.7 | 18.7 |
| 3 | 1.55 (m)<br>1.78 (m) | 1.55 (m)<br>1.76 (m) | 33.5 | 33.4 |
| 4 | - | - | 49.0 | 49.0 |
| 5 | - | - | 77.8 | 77.8 |
| 6 | 6.05 (d, $J$ = 3.6) | 6.05 (d, $J$ = 3.7) | 69.0 | 68.9 |
| 7 | 5.75 (dd, $J$ = 11.1, 3.6) | 5.76 (dd, $J$ = 11.1, 3.7) | 72.5 | 72.4 |
| 8 | 2.43 (td, $J$ = 11.1, 5.1) | 2.44 (ddd, $J$ = 12.0, 11.1, 5.0) | 35.7 | 35.6 |
| 9 | 2.53 (m) | 2.52 (m) | 37.3 | 37.3 |
| 10 | - | - | 41.6 | 41.5 |
| 11 | 2.56 (m)<br>2.65 (m) | 2.63 (m)<br>2.67 (m) | 22.2 | 22.2 |

**Table 61** (continued)

| Position | CP21<br>$\delta_H$ (mult., *J*, Hz) | R<br>$\delta_H$ (mult., *J*, Hz) | CP21<br>$\delta_C$ | R<br>$\delta_C$ |
|---|---|---|---|---|
| 12 | - | - | 149.1 | 149.1 |
| 13 | - | - | 121.3 | 121.4 |
| 14 | 2.85 (qd, *J* = 6.9, 5.1) | 2.86 (qd, *J* = 7.0, 5.0) | 27.4 | 27.4 |
| 15 | 6.13 (d, *J* = 1.8) | 6.14 (d, *J* = 1.8) | 109.5 | 109.5 |
| 16 | 7.23 (d, *J* = 1.8) | 7.24 (d, *J* = 1.8) | 140.8 | 140.8 |
| 17 | 0.99 (d, *J* = 6.9) | 1.00 (d, *J* = 7.0) | 17.1 | 17.1 |
| 18 | 1.13 (s) | 1.35 (s) | 24.2 | 17.8 |
| 19 | - | - | 181.2 | 181.6 |
| 20 | 1.36 (s) | 1.12 (s) | 17.8 | 24.2 |
| 1' | - | - | 165.7 | 165.6 |
| 2' | - | - | 130.5 | 130.5 |
| 3'/7' | 7.76 (br d, *J* = 7.5) | 7.76 (dd, *J* = 8.4, 1.3) | 129.6 | 129.5 |
| 4'/6' | 7.35 (t, *J* = 7.5) | 7.36 (dd, *J* = 8.4, 8.4) | 128.3 | 128.3 |
| 5' | 7.49 (br t, *J* = 7.5) | 7.50 (tm, *J* = 8.4) | 132.6 | 132.6 |
| 1" | - | - | 166.3 | 166.2 |
| 2" | - | - | 129.9 | 129.9 |
| 3"/7" | 7.78 (br d, *J* = 7.5) | 7.78 (dd, *J* = 8.4, 1.3) | 129.5 | 129.6 |
| 4"/6" | 7.28 (t, *J* = 7.5) | 7.28 (dd, *J* = 8.4, 8.4) | 128.2 | 128.1 |
| 5" | 7.49 (br t, *J* = 7.5) | 7.48 (tm, *J* = 8.4) | 132.9 | 132.9 |

### 3.3.22 Compound CP22
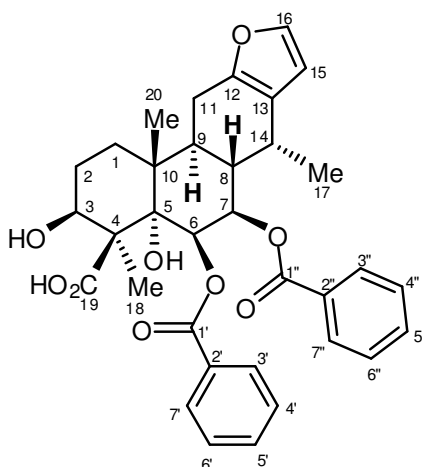


Compound **CP22** was obtained as a white solid**,** mp: 193-195 °C, $[\alpha]_D^{25}$ +78.1° (*c* 0.03, CHCl$_3$). The $^1$H and $^{13}$C NMR spectral data (Table 62, Figures 108 and 109) resembled those of **CP21**, except that the signal of the methylene protons of **CP21** at $\delta_H$ 1.55 and 1.78 ($\delta_C$ 33.5, C-3) was replaced by the oxymethine proton at $\delta_H$ 3.32 ($\delta_C$ 69.3). This finding was supported by HMBC spectrum that showed correlations to the carbons at $\delta$ 20.2 (C-18), 33.4 (C-1) 37.0 (C-9) 54.7 (C-4) and 178.1 (C-19). The stereochemistry of H-3 as $\alpha$-axial orientation was determined by the results of the large coupling constants ($J_{3ax,2ax}$ = 11.7 Hz) and by the observed cross-peak with Me-18 ($\delta$ 1.24) in the NOESY experiment. Thus **CP22** was characterized as pulcherrimin A (Patil et al., 1997).
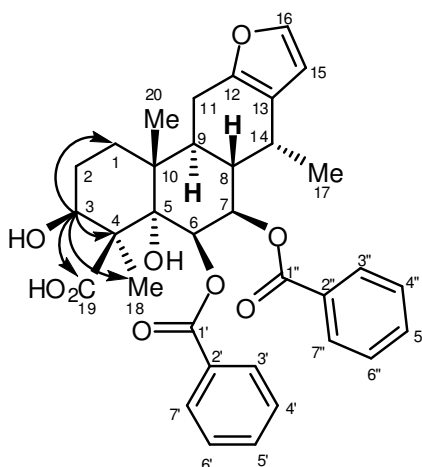


Selective HMBC correlations of **CP22**

**Table 62** ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **CP22**

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.45 (m) | 33.4 | CH$_2$ | 5, 10 |
| | 1.71 (br t, *J* = 10.2) | | | |
| 2 | 1.49 (m) | 27.6 | CH$_2$ | - |
| | 2.20 (m) | | | |
| 3 | 3.32 (dd, *J* = 11.7, 4.5) | 74.7 | CH | 1, 4, 9, 18, 19 |
| 4 | - | 54.7 | C | - |
| 5 | - | 79.4 | C | - |
| 6 | 6.03 (d, *J* = 3.6) | 69.3 | CH | 1', 4, 5, 7, 8, 10 |
| 7 | 5.64 (dd, *J* = 11.1, 3.6) | 72.4 | CH | 1'', 6, 8, 14 |
| 8 | 2.33 (td, *J* = 11.1, 4.8) | 35.4 | CH | 6, 9, 17 |
| 9 | 2.48 (m) | 37.0 | CH | 7, 8, 10, 11, 12, 20 |
| 10 | - | 41.4 | C | - |
| 11 | 2.45 (m) | 22.3 | CH$_2$ | 8, 9, 10, 12, 13 |
| | 2.55 (m) | | | |
| 12 | - | 149.0 | C | - |
| 13 | - | 121.3 | C | - |
| 14 | 2.78 (qd, *J* = 6.9, 4.8) | 27.3 | CH | 8, 9, 12, 13, 15, 17 |
| 15 | 6.06 (d, *J* = 1.8) | 109.4 | CH | 12, 13, 16 |
| 16 | 7.16 (d, *J* = 1.8) | 140.8 | CH | 12, 13, 15 |
| 17 | 0.91 (d, *J* = 6.9) | 17.1 | CH$_3$ | 8, 13, 14 |
| 18 | 1.24 (s) | 20.2 | CH$_3$ | 3, 4, 5, 19 |
| 19 | - | 178.1 | C | - |
| 20 | 1.34 (s) | 17.6 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 166.1 | C | - |
| 2' | - | 129.8 | C | - |
| 3'/7' | 7.61 (br d, *J* = 7.2) | 129.5 | CH | 1', 5' |
| 4'/6' | 7.24 (t, *J* = 7.2) | 128.2 | CH | 2' |
| 5' | 7.40 (br t, *J* = 7.2) | 132.9 | CH | 3', 7' |
| 1'' | - | 166.4 | C | - |
| 2'' | - | 130.2 | C | - |
| 3''/7'' | 7.76 (br d, *J* = 7.2) | 129.7 | CH | 1'', 5'' |
| 4''/6'' | 7.23 (t, *J* = 7.2) | 128.4 | CH | 2'' |
| 5'' | 7.44 (br t, *J* = 7.2) | 133.1 | CH | 3'', 7'' |

**Table 63** Comparison of $^1$H and $^{13}$C NMR spectral data between compounds **CP22** (recorded in CDCl$_3$, 300 MHz) and pulcherrimins A (**R**, recorded in CDCl$_3$, 400 MHz)

| Position | CP22 δ$_H$ (mult., *J*, Hz) | R δ$_H$ (mult., *J*, Hz) | CP22 δ$_C$ | R δ$_C$ |
|---|---|---|---|---|
| 1 | 1.45 (m) | 1.52 (ddd, *J* = 12.6, 3.3, 3.3) | 33.4 | 33.4 |
|  | 1.71 (br t, *J* = 10.2) | 1.80 (ddd, *J* = 12.6, 11.4, 3.3) |  |  |
| 2 | 1.49 (m) | 1.58 (m) | 27.6 | 27.5 |
|  | 2.20 (m) | 2.14 (ddd, *J* = 12.6, 11.4, 3.3) |  |  |
| 3 | 3.32 (dd, *J* = 11.7, 4.5) | 3.42 (dd, *J* = 12.2, 4.7) | 74.7 | 74.6 |
| 4 | - | - | 54.7 | 54.6 |
| 5 | - | - | 79.4 | 79.2 |
| 6 | 6.03 (d, *J* = 3.6) | 6.12 (d, *J* = 3.8) | 69.3 | 69.3 |
| 7 | 5.64 (dd, *J* = 11.1, 3.6) | 5.72 (dd, *J* = 11.4, 3.8) | 72.4 | 72.5 |
| 8 | 2.33 (td, *J* = 11.1, 4.8) | 2.42 (ddd, *J* = 11.8, 11.4, 5.0) | 35.4 | 35.4 |
| 9 | 2.48 (m) | 2.57 (m) | 37.0 | 36.9 |
| 10 | - | - | 41.4 | 41.3 |
| 11 | 2.45 (m) | 2.57 (m) | 22.3 | 22.3 |
|  | 2.55 (m) | 2.63 (m) |  |  |
| 12 | - | - | 149.0 | 149.0 |
| 13 | - | - | 121.3 | 121.3 |
| 14 | 2.78 (qd, *J* = 6.9, 4.8) | 2.86 (qd, *J* = 7.0, 5.0) | 27.3 | 27.3 |
| 15 | 6.06 (d, *J* = 1.8) | 6.13 (d, *J* = 1.8) | 109.4 | 109.4 |
| 16 | 7.16 (d, *J* = 1.8) | 7.23 (d, *J* = 1.8) | 140.8 | 140.7 |
| 17 | 0.91 (d, *J* = 6.9) | 1.00 (d, *J* = 7.0) | 17.1 | 17.0 |
| 18 | 1.24 (s) | 1.44 (s) | 20.2 | 17.7 |
| 19 | - | - | 178.1 | 177.9 |
| 20 | 1.34 (s) | 1.34 (s) | 17.6 | 20.2 |
| 1' | - | - | 166.1 | 166.3 |
| 2' | - | - | 129.8 | 129.8 |

**Table 63** (continued)

| Position | CP22 δ$_H$ (mult., *J*, Hz) | R δ$_H$ (mult., *J*, Hz) | CP22 δ$_C$ | R δ$_C$ |
|---|---|---|---|---|
| 3'/7' | 7.61 (br d, *J* = 7.2) | 7.70 (dd, *J* = 8.4, 1.3) | 129.5 | 129.5 |
| 4'/6' | 7.24 (t, *J* = 7.2) | 7.31 (dd, *J* = 8.4, 8.4) | 128.2 | 128.4 |
| 5' | 7.40 (br t, *J* = 7.2) | 7.49 (tm, *J* = 8.4) | 132.9 | 132.9 |
| 1" | - | - | 166.4 | 166.5 |
| 2" | - | - | 130.2 | 130.1 |
| 3"/7" | 7.76 (br d, *J* = 7.2) | 7.85 (dd, *J* = 8.4, 1.3) | 129.7 | 129.7 |
| 4"/6" | 7.23 (t, *J* = 7.2) | 7.35 (dd, *J* = 8.4, 8.4) | 128.4 | 128.2 |
| 5" | 7.44 (br t, *J* = 7.2) | 7.53 (tm, *J* = 8.4) | 133.1 | 133.1 |

**3.3.23 Compound CP23**
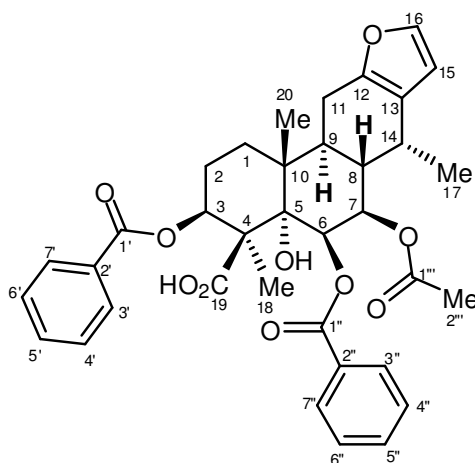


Compound **CP23** was purified as a white solid mp 220-221 °C; $[\alpha]_D^{25}$ +58.5° (*c* 0.11, CHCl$_3$). The $^1$H and $^{13}$C NMR spectra (Table 64, Figures 110 and 111) of **CP23** displayed characteristics similar to those of **CP11**, except for the presence of an additional acetoxy methyl group at $\delta_H$ 1.76 (s) and an oxymethine proton at $\delta_H$ 5.45 (td, *J* = 11.7, 5.1; $\delta_C$ 70.8) in **CP23**. The HMBC correlation of an oxymethine proton at $\delta$ 5.45 (H-7) with the carbons at $\delta$ 27.2 (C-14), 35.6 (C-8), 69.1 (C-6) and 170.1 (O$\underline{C}$OCH$_3$) and of the acetoxy methyl proton at $\delta$ 1.76 with the carbon at $\delta$ 170.1 (O$\underline{C}$OCH$_3$) confirmed the location of OAc group at C-7. The stereochemistry of H-7 as *α*-axial orientation was determined by the results of the large coupling constants ($J_{7ax,8ax}$ = 11.7 Hz) and by the observed cross-peaks with Me-17 ($\delta$ 0.85) and H-9 ($\delta$ 2.53) in the NOESY experiments. Thus, **CP23** was pulcherrimin E (Roach et al., 2003).
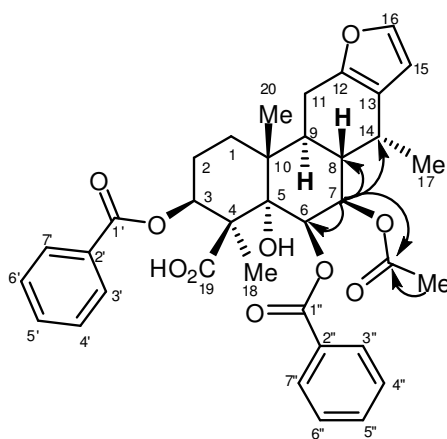


Selective HMBC correlations of **CP23**

**Table 64** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP23**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|----------|------------------------------|------------|------|------|
| 1 | 1.57 (m) | 33.0 | CH$_2$ | 2, 3, 9, 10, 20 |
|   | 1.96 (m) | | | |
| 2 | 1.77 (m) | 24.4 | CH$_2$ | 1, 3, 4, 10 |
|   | 2.71 (m) | | | |
| 3 | 5.30 (dd, $J$ = 12.3, 4.8) | 77.5 | CH | 2, 4, 18, 19, 1' |
| 4 | - | 53.6 | C | - |
| 5 | - | 79.1 | C | - |
| 6 | 5.96 (d, $J$ = 3.9) | 69.1 | CH | 1", 4, 5, 7, 8, 10 |
| 7 | 5.45 (td, $J$ = 11.7, 5.1) | 70.8 | CH | 1''', 6, 8, 14 |
| 8 | 2.12 (m) | 35.6 | CH | 7, 9, 11, 14, 17 |
| 9 | 2.53 (m) | 36.8 | CH | 1, 8, 10, 11, 12, 14, 20 |
| 10 | - | 41.5 | C | - |
| 11 | 2.51 (m) | 22.0 | CH$_2$ | 8, 9, 10, 12, 13 |
| 12 | - | 149.9 | C | - |
| 13 | - | 121.4 | C | - |
| 14 | 2.68 (m) | 27.2 | CH | 8, 9, 12, 13, 15, 17 |
| 15 | 6.11 (d, $J$ = 1.8) | 109.5 | CH | 12, 13, 16 |
| 16 | 7.18 (d, $J$ = 1.8) | 140.8 | CH | 12, 13, 15 |
| 17 | 0.85 (d, $J$ = 7.2) | 16.6 | CH$_3$ | 8, 13, 14 |
| 18 | 1.39 (s) | 20.1 | CH$_3$ | 3, 4, 5, 19 |
| 19 | - | 174.2 | C | - |
| 20 | 1.62 (s) | 16.9 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 165.5 | C | - |
| 2' | - | 130.8 | C | - |
| 3'/7' | 7.83 (br d, $J$ = 7.2) | 129.3 | CH | 1', 5' |
| 4'/6' | 7.36 (t, $J$ = 7.2) | 128.5 | CH | 2' |
| 5' | 7.46 (br t, $J$ = 7.2) | 133.0 | CH | 3', 7' |
| 1" | - | 166.0 | C | - |
| 2" | - | 130.6 | C | - |
| 3"/7" | 7.88 (br d, $J$ = 7.2) | 129.6 | CH | 1", 5" |

**Table 64** (continued)

| Position | δ_H (mult., *J*, Hz) | δ_C | DEPT | HMBC |
|----------|---------------------|-----|------|------|
| 4"/6" | 7.32 (t, *J* = 7.2) | 128.5 | CH | 2" |
| 5" | 7.49 (t, *J* = 7.2) | 132.8 | CH | 3", 7" |
| 1'" | - | 170.1 | C | - |
| 2'" | 1.76 (s) | 20.1 | CH_3 | 1'" |
| 5-OH | 5.06 (s) | - | - | 5, 6, 10 |

**Table 65** Comparison of $^1$H and $^{13}$C NMR spectral data between compounds **CP23** (recorded in acetone-$d_6$, 300 MHz) and Pulcherrimin E (**R**, recorded in CDCl$_3$, 400 MHz)

| Position | CP23 δ_H (mult., *J*, Hz) | R δ_H (mult., *J*, Hz) | CP23 δ_C | R δ_C |
|----------|--------------------------|------------------------|----------|-------|
| 1 | 1.57 (m) 1.96 (m) | 1.68 (dd, *J* = 13.2, 3.8) 2.02 (dd, *J* = 13.2, 3.8) | 33.0 | 32.9 |
| 2 | 1.77 (m) 2.71 (m) | 1.93 (m) 2.61 (m) | 24.4 | 24.3 |
| 3 | 5.30 (dd, *J* = 12.3, 4.8) | 5.33 (dd, *J* = 12.2, 4.9) | 77.5 | 77.0 |
| 4 | - | - | 53.6 | 53.4 |
| 5 | - | - | 79.1 | 79.4 |
| 6 | 5.96 (d, *J* = 3.9) | 5.95 (d, *J* = 4.0) | 69.1 | 69.0 |
| 7 | 5.45 (td, *J* = 11.7, 5.1) | 5.50 (dd, *J* = 11.7, 4.0) | 70.8 | 71.0 |
| 8 | 2.12 (m) | 2.29 (dt, *J* = 11.7, 5.0) | 35.6 | 35.2 |
| 9 | 2.53 (m) | 2.58 (m) | 36.8 | 36.9 |
| 10 | - | - | 41.5 | 41.6 |
| 11 | 2.51 (m) | 2.62 (m) 2.66 (m) | 22.0 | 22.2 |
| 12 | - | - | 149.9 | 148.7 |
| 13 | - | - | 121.4 | 121.4 |
| 14 | 2.68 (m) | 2.83 (dq, *J* = 7.0, 5.0) | 27.2 | 27.3 |
| 15 | 6.11 (d, *J* = 1.8) | 6.18 (d, *J* = 1.9) | 109.5 | 109.5 |

**Table 65** (continued)

| Position | CP23 δ_H (mult., *J*, Hz) | R δ_H (mult., *J*, Hz) | CP23 δ_C | R δ_C |
|---|---|---|---|---|
| 16 | 7.18 (d, *J* = 1.8) | 7.27 (d, *J* = 1.9) | 140.8 | 140.9 |
| 17 | 0.85 (d, *J* = 7.2) | 0.99 (d, *J* = 7.0) | 16.6 | 17.1 |
| 18 | 1.39 (s) | 1.62 (s) | 20.1 | 17.2 |
| 19 | - | - | 174.2 | 176.4 |
| 20 | 1.62 (s) | 1.28 (s) | 16.9 | 19.9 |
| 1' | - | - | 165.5 | 162.1 |
| 2' | - | - | 130.8 | 130.0 |
| 3'/7' | 7.83 (br d, *J* = 7.2) | 7.96 (dd, *J* = 8.5, 1.1) | 129.3 | 129.6 |
| 4'/6' | 7.36 (t, *J* = 7.2) | 7.39 (dd, *J* = 8.5, 8.5) | 128.5 | 128.5 |
| 5' | 7.46 (br t, *J* = 7.2) | 7.56 (tm, *J* = 8.5) | 133.0 | 133.2 |
| 1" | - | - | 166.0 | 162.1 |
| 2" | - | - | 130.6 | 130.2 |
| 3"/7" | 7.88 (br d, *J* = 7.2) | 7.91 (dd, *J* = 8.4, 1.3) | 129.6 | 129.4 |
| 4"/6" | 7.32 (t, *J* = 7.2) | 7.24 (dd, *J* = 8.4, 8.4) | 128.5 | 128.6 |
| 5" | 7.49 (t, *J* = 7.2) | 7.46 (tm, *J* = 8.4) | 132.8 | 133.1 |
| 1''' | - | - | 170.1 | 171.2 |
| 2''' | 1.76 (s) | 1.95 (s) | 20.1 | 20.9 |

### 3.3.24 Compound CP24
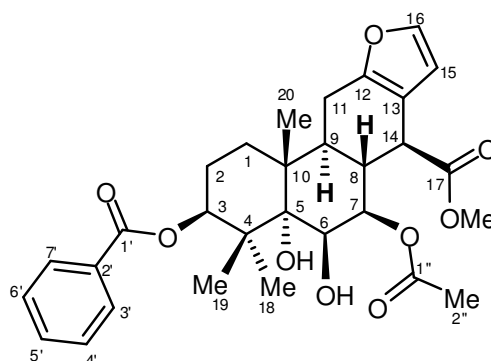


Compound **CP24** was isolated as viscous oil; $[\alpha]_D^{25}$ +73.9º (*c* 0.07, CHCl$_3$). The $^1$H and $^{13}$C NMR spectra (Table 66, Figures 112 and 113) were closely related to those of **CP13**. The differences were shown as a replacement of an oxymethylene protons at $\delta$ 4.63 and 5.39 (each, d, *J* = 12.0 Hz; 2H-19) and an acetyl group ($\delta_H$ 1.98: $\delta_C$ 21.0 and $\delta_C$ 171.6) in **CP13** with a methyl singlet at $\delta$ 1.58 (Me-19), whose HMBC spectrum showed correlations with the carbons at $\delta$ 22.5 (C-18), 44.2 (C-4), 77.3 (C-3) and 78.6 (C-5), confirming its location at C-4. Thus, **CP24** was assigned to be pulcherrin C (Pranithanchai et al., 2009).
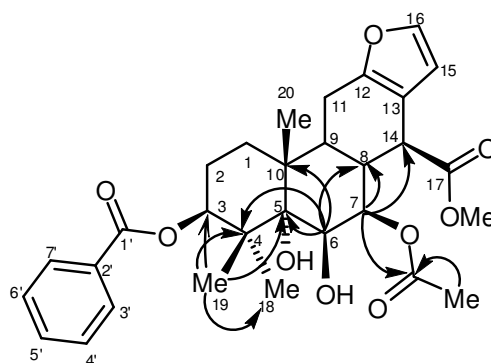


Selective HMBC correlations of **CP24**

**Table 66** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP24**

| Position | δ$_H$ (mult., $J$, Hz) | δ$_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.43 (m) | 32.6 | CH$_2$ | 3, 5, 10, 20 |
|  | 1.86 (m) |  |  |  |
| 2 | 1.40 (m) | 23.8 | CH$_2$ | 1, 3, 4, 10 |
|  | 1.88 (m) |  |  |  |
| 3 | 5.31 (dd, $J$ = 11.1, 3.6) | 77.3 | CH | 2, 4, 18, 19, 1' |
| 4 | - | 44.2 | C | - |
| 5 | - | 78.6 | C | - |
| 6 | 4.15 (d, $J$ = 3.3) | 71.6 | CH | 4, 5, 7, 8, 10 |
| 7 | 5.25 (dd, $J$ = 11.1, 3.3) | 79.2 | CH | 1", 8, 14 |
| 8 | 2.76 (td, $J$ = 11.1, 8.4) | 34.2 | CH | 7, 9, 11, 14, 17 |
| 9 | 2.40 (m) | 41.3 | CH | 8, 10, 11, 12, 20 |
| 10 | - | 40.9 | C | - |
| 11 | 2.51 (m) | 21.3 | CH$_2$ | 8, 9, 12, 13 |
| 12 | - | 150.9 | C | - |
| 13 | - | 112.6 | C | - |
| 14 | 3.35 (d, $J$ = 8.4) | 45.2 | CH | 7, 8, 12, 13, 17 |
| 15 | 6.11 (d, $J$ = 1.8) | 108.2 | CH | 12, 13, 16 |
| 16 | 7.21 (d, $J$ = 1.8) | 141.3 | CH | 12, 13, 15 |
| 17 | - | 175.2 | C | - |
| 18 | 1.06 (s) | 22.5 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 1.58 (s) | 19.6 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 1.48 (s) | 16.5 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 166.4 | C | - |
| 2' | - | 130.8 | C | - |
| 3'/7' | 8.03 (br d, $J$ = 7.5) | 129.5 | CH | 1', 5' |
| 3'/6' | 7.43 (t, $J$ = 7.5) | 128.3 | CH | 2' |
| 5' | 7.52 (br t, $J$ = 7.5) | 132.8 | CH | 3', 7' |
| 1" | - | 171.0 | C | - |
| 2" | 2.04 (s) | 20.9 | CH$_3$ | 1" |
| 17-OMe | 3.74 (s) | 52.1 | CH$_3$ | 17 |
| 5-OH | 2.93 (s) | - | - | 5, 10 |

**Table 67** Comparison of ^{1}H and ^{13}C NMR spectral data between compounds **CP24** (recorded in CDCl$_3$, 300 MHz) and pulcherrin C (**R**, recorded in CDCl$_3$, 300 MHz)

| Position | CP24 $\delta_H$ (mult., *J*, Hz) | R $\delta_H$ (mult., *J*, Hz) | CP24 $\delta_C$ | R $\delta_C$ |
|---|---|---|---|---|
| 1 | 1.43 (m) | 1.46 (m) | 32.6 | 32.7 |
|  | 1.86 (m) | 1.89 (m) |  |  |
| 2 | 1.40 (m) | 1.84 (m) | 23.8 | 23.9 |
|  | 1.88 (m) | 1.94 (m) |  |  |
| 3 | 5.31 (dd, *J* = 11.1, 3.6) | 5.31 (dd, *J* = 9.0, 6.0) | 77.3 | 78.8 |
| 4 | - | - | 44.2 | 44.2 |
| 5 | - | - | 78.6 | 77.0 |
| 6 | 4.15 (d, *J* = 3.3) | 4.15 (d, *J* = 3.3) | 71.6 | 72.3 |
| 7 | 5.25 (dd, *J* = 11.1, 3.3) | 5.33 (dd, *J* = 11.4, 3.3) | 79.2 | 78.8 |
| 8 | 2.76 (td, *J* = 11.1, 8.4) | 2.76 (td, *J* = 11.4, 8.7) | 34.2 | 34.3 |
| 9 | 2.40 (m) | 2.41 (m) | 41.3 | 41.3 |
| 10 | - | - | 40.9 | 40.9 |
| 11 | 2.51 (m) | 2.56 (br d, *J* = 8.1) | 21.3 | 21.3 |
| 12 | - | - | 150.9 | 150.8 |
| 13 | - | - | 112.6 | 112.7 |
| 14 | 3.35 (d, *J* = 8.4) | 3.38 (d, *J* = 8.7) | 45.2 | 45.1 |
| 15 | 6.11 (d, *J* = 1.8) | 6.13 (d, *J* = 1.5) | 108.2 | 108.3 |
| 16 | 7.21 (d, *J* = 1.8) | 7.24 (d, *J* = 1.5) | 141.3 | 141.4 |
| 17 | - | - | 175.2 | 174.9 |
| 18 | 1.06 (s) | 1.08 (s) | 22.5 | 22.6 |
| 19 | 1.58 (s) | 1.61 (s) | 19.6 | 19.6 |
| 20 | 1.48 (s) | 1.50 (s) | 16.5 | 16.6 |
| 1' | - | - | 166.4 | 166.2 |
| 2' | - | - | 130.8 | 130.8 |
| 3'/7' | 8.03 (br d, *J* = 7.5) | 8.05 (d, *J* = 7.5) | 129.5 | 129.6 |
| 3'/6' | 7.43 (t, *J* = 7.5) | 7.24 (t, *J* = 7.5) | 128.3 | 128.4 |
| 5' | 7.52 (br t, *J* = 7.5) | 7.57 (t, *J* = 7.5) | 132.8 | 132.9 |
| 1" | - | - | 171.0 | 170.2 |
| 2" | 2.04 (s) | 2.06 (s) | 20.9 | 21.0 |
| 17-OMe | 3.74 (s) | 3.75 (s) | 52.1 | 52.1 |

**3.3.25 Compound CP25**
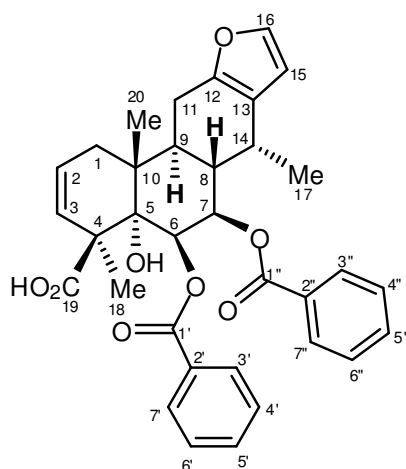


Compound **CP25** was isolated as viscous oil; $[\alpha]_D^{25}$ +177.1° (*c* 0.11 in CHCl₃). The ¹H NMR data of **CP25** (Table 68, Figure 114) were similar to those of **CP21**, except that compound **CP25** showed the presence of additional olefinic protons at *δ* 5.76 (br dd, *J* = 10.5, 6.0 Hz, H-2) and 5.16 (dd, *J* = 10.5, 1.5 Hz, H-3) instead of two methylene groups at C-2 (*δ*ₕ 1.45, 1.93) and C-3 (*δ*ₕ 1.55, 1.78) in **CP21**. This finding was supported by HMBC spectrum of **CP25**, in which the methyl protons at *δ* 1.03 (Me-18) were correlated with the carbons at *δ* 50.6 (C-4), 77.2 (C-5), 129.4 (C-3) and 179.3 (C-19), the olefinic proton at *δ* 5.76 (H-2) with the carbons at *δ* 36.6 (C-1), 40.8 (C-10) and 50.6 (C-4) and the olefinic proton at *δ* 5.16 (H-3) with the carbons at *δ* 36.6 (C-1), 50.6 (C-4) and 77.2 (C-5). From these data, compound **CP25** was identified as pulcherrimin B (Patil et al., 1997).
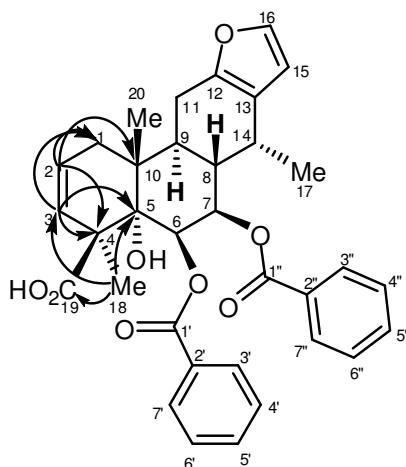


Selective HMBC correlations of **CP25**

**Table 68** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP25**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 2.01 (dd, $J$ = 17.4, 6.0) | 36.6 | CH$_2$ | 2, 3, 5, 10, 20 |
| | 2.27 (br d, $J$ = 17.4) | | | |
| 2 | 5.76 (br dd, $J$ = 10.5, 6.0) | 123.4 | CH | 1, 4, 10 |
| 3 | 5.16 (dd, $J$ = 10.5, 1.5) | 129.4 | CH | 1, 4, 5 |
| 4 | - | 50.6 | C | - |
| 5 | - | 77.2 | C | - |
| 6 | 5.94 (d, $J$ = 3.0) | 69.5 | CH | 1', 4, 5, 7, 8, 10 |
| 7 | 5.70 (dd, $J$ = 11.1, 3.0) | 72.2 | CH | 1", 6, 8, 14 |
| 8 | 2.38 (td, $J$ = 11.1, 4.8) | 35.5 | CH | 7, 9, 14, 17 |
| 9 | 2.51 (td, $J$ = 11.1, 6.6) | 37.2 | CH | 8, 10, 11, 12, 20 |
| 10 | - | 40.8 | C | - |
| 11 | 2.64 (m) | 22.2 | CH$_2$ | 8, 9, 12, 13 |
| 12 | - | 148.8 | C | - |
| 13 | - | 121.3 | C | - |
| 14 | 2.85 (qd, $J$ = 6.6, 4.8) | 27.4 | CH | 8, 9, 12, 13, 17 |
| 15 | 6.11 (d, $J$ = 1.8) | 109.4 | CH | 12, 13, 16 |
| 16 | 7.21 (d, $J$ = 1.8) | 140.8 | CH | 12, 13, 15 |
| 17 | 0.96 (d, $J$ = 6.6) | 17.0 | CH$_3$ | 8, 13, 14 |
| 18 | 1.03 (s) | 22.6 | CH$_3$ | 3, 4, 5, 19 |
| 19 | - | 179.3 | C | - |
| 20 | 1.53 (s) | 16.9 | CH$_3$ | 1, 5, 9, 10 |
| 1' | - | 165.1 | C | - |
| 2' | - | 130.3 | C | - |
| 3'/7' | 7.71 (br d, $J$ = 8.1) | 129.7 | CH | 1', 5' |
| 4'/6' | 7.37 (t, $J$ = 8.1) | 128.4 | CH | 1', 2' |
| 5' | 7.53 (br t, $J$ = 8.1) | 132.9 | CH | 3', 7' |
| 1" | - | 166.2 | C | - |

**Table 68** (continued)

| Position | δ_H (mult., *J*, Hz) | δ_C | DEPT | HMBC |
|----------|---------------------|------|------|------|
| 2" | - | 130.1 | C | - |
| 3"/7" | 7.83 (br d, *J* = 7.8) | 129.7 | CH | 1", 5" |
| 4"/6" | 7.34 (t, *J* = 7.8) | 128.1 | CH | 1", 2" |
| 5" | 7.53 (br t, *J* = 7.8) | 132.8 | CH | 3", 7" |

**Table 69** Comparison of $^1$H and $^{13}$C NMR spectral data between compounds **CP25** (recorded in CDCl$_3$, 300 MHz) and pulcherrimin B (**R**, recorded in CDCl$_3$, 400 MHz)

| Position | CP25 δ_H (mult., *J*, Hz) | R δ_H (mult., *J*, Hz) | CP25 δ_C | R δ_C |
|----------|--------------------------|------------------------|----------|-------|
| 1 | 2.01 (dd, *J* = 17.4, 6.0) | 2.07 (dd, *J* = 17.2, 5.8) | 36.6 | 36.8 |
|   | 2.27 (br d, *J* = 17.4) | 2.27 (dm, *J* = 17.2) | | |
| 2 | 5.76 (br dd, *J* = 10.5, 6.0) | 5.78 (dm, *J* = 10.7) | 123.4 | 123.5 |
| 3 | 5.16 (dd, *J* = 10.5, 1.5) | 5.22 (dm, *J* = 10.7) | 129.4 | 129.3 |
| 4 | - | - | 50.6 | 50.5 |
| 5 | - | - | 77.2 | 77.6 |
| 6 | 5.94 (d, *J* = 3.0) | 5.97 (d, *J* = 3.4) | 69.5 | 69.5 |
| 7 | 5.70 (dd, *J* = 11.1, 3.0) | 5.75 (dd, *J* = 11.3, 3.4) | 72.2 | 72.0 |
| 8 | 2.38 (td, *J* = 11.1, 4.8) | 2.41 (dd, *J* = 11.3, 11.2, 5.0) | 35.5 | 35.4 |
| 9 | 2.51 (td, *J* = 11.1, 6.6) | 2.53 (td, *J* = 11.4, 11.2, 6.5) | 37.2 | 37.3 |
| 10 | - | - | 40.8 | 40.8 |
| 11 | 2.64 (m) | 2.67 (m) | 22.2 | 22.2 |
| 12 | - | - | 148.8 | 148.7 |
| 13 | - | - | 121.3 | 121.3 |
| 14 | 2.85 (qd, *J* = 6.6, 4.8) | 2.88 (qd, *J* = 7.0, 5.0) | 27.4 | 27.4 |
| 15 | 6.11 (d, *J* = 1.8) | 6.12 (d, *J* = 1.8) | 109.4 | 109.4 |
| 16 | 7.21 (d, *J* = 1.8) | 7.22 (d, *J* = 1.8) | 140.8 | 140.8 |

**Table 69** (continued)

| Position | CP25<br>$\delta_H$ (mult., *J*, Hz) | R<br>$\delta_H$ (mult., *J*, Hz) | CP25<br>$\delta_C$ | R<br>$\delta_C$ |
|---|---|---|---|---|
| 17 | 0.96 (d, *J* = 6.6) | 0.98 (d, *J* = 7.0) | 17.0 | 17.1 |
| 18 | 1.03 (s) | 1.56 (s) | 22.6 | 16.9 |
| 19 | - | - | 179.3 | 177.8 |
| 20 | 1.53 (s) | 1.26 (s) | 16.9 | 22.7 |
| 1' | - | - | 165.1 | 165.0 |
| 2' | - | - | 130.3 | 130.2 |
| 3'/7' | 7.71 (br d, *J* = 8.1) | 7.72 (dd, *J* = 8.4, 1.3) | 129.7 | 129.6 |
| 4'/6' | 7.37 (t, *J* = 8.1) | 7.35 (dd, *J* = 8.4, 8.4) | 128.4 | 128.5 |
| 5' | 7.53 (br t, *J* = 8.1) | 7.55 (tm, *J* = 8.4) | 132.9 | 132.8 |
| 1" | - | - | 166.2 | 165.7 |
| 2" | - | - | 130.1 | 130.1 |
| 3"/7" | 7.83 (br d, *J* = 7.8) | 7.85 (dd, *J* = 8.4, 1.3) | 129.7 | 129.7 |
| 4"/6" | 7.34 (t, *J* = 7.8) | 7.35 (dd, *J* = 8.4, 8.4) | 128.1 | 128.1 |
| 5" | 7.53 (br t, *J* = 7.8) | 7.55 (tm, *J* = 8.4) | 132.8 | 132.9 |

### 3.3.26 Compound CP26
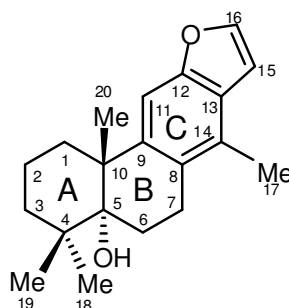


Compound **CP26** was obtained as viscous oil; $[\alpha]_D^{25}$ +60.5° (*c* 0.18 in CHCl$_3$). The IR (3402 cm$^{-1}$) and UV ($\lambda_{max}$ 253, 281, 292 nm) absorption bands were characteristic of hydroxyl and benzofuran moieties, respectively. Its $^1$H and $^{13}$C NMR spectral data (Table 70, Figures 116 and 117) revealed that **CP26** had the same A and B rings as **CP16**. The difference was found in ring C, which was aromatic in **CP26**. This was supported in the $^1$H NMR spectrum by the appearance of one aromatic proton at $\delta$ 7.25 (s, H-11) and one aromatic methyl group at $\delta$ 2.30 (Me-17) in **CP26** and the disappearance of methylene protons at $\delta$ 2.24 and 2.40 (each, m, H-11) and two methine protons at $\delta$ 1.75 (m, H-8) and 2.30 (m, H-9) in **CP16**. The HMBC spectrum showed correlations between an aromatic proton at $\delta$ 7.25 (s, H-11) with the carbons at $\delta$ 43.8 (C-10), 125.6 (C-13), 126.9 (C-8), 144.7 (C-9) and 153.9 (C-12) and of the methyl protons at $\delta$ 2.30 (Me-17) with the carbons at $\delta$ 125.6 (C-13), 126.9 (C-8) and 128.4 (C-14). From these data, compound **CP26** was identified as 8,9,11,14-didehydrovouacapen-5$\alpha$-ol (McPherson et al., 1986).
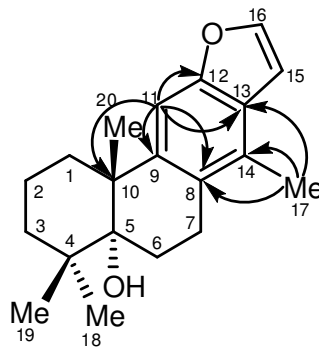


Selective HMBC correlations of **CP26**

**Table 70** $^1$H and $^{13}$C NMR, DEPT and HMBC spectral data of compound **CP26**

| Position | $\delta_H$ (mult., $J$, Hz) | $\delta_C$ | DEPT | HMBC |
|---|---|---|---|---|
| 1 | 1.98 (m) | 33.1 | CH$_2$ | 2, 3, 5, 9, 10 |
| 2 | 1.62 (m) | 18.9 | CH$_2$ | 1, 3, 4 |
|   | 1.76 (m) |  |  |  |
| 3 | 1.15 (m) | 36.3 | CH$_2$ | 1, 2, 4, 5, 18, 19 |
|   | 1.78 (m) |  |  |  |
| 4 | - | 38.0 | C | - |
| 5 | - | 75.9 | C | - |
| 6 | 1.96 (m) | 23.9 | CH$_2$ | 4, 5, 7, 8, 10 |
|   | 2.17 (m) |  |  |  |
| 7 | 2.82 (dd, $J$ = 9.0, 5.4) | 22.9 | CH$_2$ | 5, 6, 8, 9, 13, 14 |
| 8 | - | 126.9 | C | - |
| 9 | - | 144.7 | C | - |
| 10 | - | 43.8 | C | - |
| 11 | 7.25 (br s) | 105.1 | CH | 8, 9, 10, 12, 13 |
| 12 | - | 153.9 | C | - |
| 13 | - | 125.6 | C | - |
| 14 | - | 128.4 | C | - |
| 15 | 6.66 (d, $J$ = 2.1) | 105.0 | CH | 12, 13, 16 |
| 16 | 7.46 (d, $J$ = 2.1) | 144.2 | CH | 12, 13, 15 |
| 17 | 2.30 (s) | 15.9 | CH$_3$ | 8, 13, 14 |
| 18 | 0.98 (s) | 27.8 | CH$_3$ | 3, 4, 5, 19 |
| 19 | 1.08 (s) | 24.9 | CH$_3$ | 3, 4, 5, 18 |
| 20 | 1.27 (s) | 29.3 | CH$_3$ | 1, 5, 9, 10 |

**Table 71** Comparison of ${}^{1}$H and ${}^{13}$C NMR spectral data between compounds **CP26** (recorded in CDCl$_3$, 300 MHz) and 8,9,11,14-didehydrovouacapen-5$\alpha$-ol (**R**, recorded in CDCl$_3$, 360 MHz)

| Position | CP26 δ$_H$ (mult., *J*, Hz) | R δ$_H$ (mult., *J*, Hz) | CP26 δ$_C$ | R δ$_C$ |
|---|---|---|---|---|
| 1 | 1.98 (m) | 1.24 (br d, *J* = 13) | 33.1 | 33.0 |
|  |  | 1.98 (m) |  |  |
| 2 | 1.62 (m) | 1.70 (m) | 18.9 | 18.9 |
|  | 1.76 (m) | 1.83 (m) |  |  |
| 3 | 1.15 (m) | 1.83 (m) | 36.3 | 36.2 |
|  | 1.78 (m) | 2.04 (m) |  |  |
| 4 | - | - | 38.0 | 37.9 |
| 5 | - | - | 75.9 | 75.8 |
| 6 | 1.96 (m) | 2.05 (m) | 23.9 | 24.8 |
|  | 2.17 (m) | 2.22 (ddd, *J* = 14, 9, 9) |  |  |
| 7 | 2.82 (dd, *J* = 9.0, 5.4) | 2.89 (dd, *J* = 9, 5.5) | 22.9 | 23.8 |
| 8 | - | - | 126.9 | 125.4 |
| 9 | - | - | 144.7 | 144.5 |
| 10 | - | - | 43.8 | 43.7 |
| 11 | 7.25 (br s) | 7.32 (s) | 105.1 | 104.8 |
| 12 | - | - | 153.9 | 153.7 |
| 13 | - | - | 125.6 | 128.3 |
| 14 | - | - | 128.4 | 126.8 |
| 15 | 6.66 (d, *J* = 2.1) | 6.73 (d, *J* = 2) | 105.0 | 105.0 |
| 16 | 7.46 (d, *J* = 2.1) | 7.53 (d, *J* = 2) | 144.2 | 144.1 |
| 17 | 2.30 (s) | 2.38 (s) | 15.9 | 27.7 |
| 18 | 0.98 (s) | 1.05 (s) | 27.8 | 29.3 |
| 19 | 1.08 (s) | 1.15 (s) | 24.9 | 24.8 |
| 20 | 1.27 (s) | 1.34 (s) | 29.3 | 15.8 |
| 5-OH | - | 1.42 | - | - |

**3.4 Anti-inflammatory of compounds CP1-CP26 from the roots of *C. pulcherrima***

The $CH_2Cl_2$ extract from the roots of *C. pulcherrima* showed an inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 cell line with an $IC_{50}$ value of 6.1 μg/ml. Further separation and purification led to the isolation of 26 diterpenes (**CP1−CP26**) whose anti-inflammatory activities indicated that compound **CP14** was the most potent inhibitor of NO production (Table 72) with an $IC_{50}$ value of 2.9 μM and compounds **CP8**, **CP9**, **CP11−CP15**, and **CP17−CP26** significantly reduced LPS-stimulated NO production with the $IC_{50}$ values in the range of 3.4−12.5 μM better than that of the positive control, indomethacin ($IC_{50}$ = 14.5 μM), whereas other compounds exhibited weak activity. Compounds **CP18** ($IC_{50}$ = 5.3 μM) and **CP17** ($IC_{50}$ = 8.2 μM) showed much better activity than **CP3** ($IC_{50}$ = 59.7 μM) suggesting that the cinnamoyloxy and benzoyloxy groups at C-6 may increase the activity more than the acetoxy group. The substitution of a benzoyloxy group at C-3 (**CP11** and **CP23**, $IC_{50}$ = 4.2 and 6.0 μM) and C-7 (**CP21** and **CP22**, $IC_{50}$ = 6.0 and 5.2 μM) demonstrated significantly increase in NO inhibitory activity compared to that of **CP10** ($IC_{50}$ = 26.7 μM). The oxidation at C-19 of **CP10** ($IC_{50}$ = 26.7 μM) resulted in two-fold increase in activity against NO production compared to that of **CP6** ($IC_{50}$ = 47.5 μM). The oxidation at C-17 and the substitution of a benzoyloxy group at C-3 of **CP12** ($IC_{50}$ 4.2 μM) and **CP24** ($IC_{50}$ 6.5 μM) displayed significantly increase in the activity compared to that of **CP2** ($IC_{50}$ 46.1 μM) and **CP3** ($IC_{50}$ 59.7 μM).

**Table 72** Inhibitory effects on NO production[a] of compounds **CP1–CP26**

| No | % Inhibition at various concentrations ($\mu$M) | | | | | | $IC_{50}$ |
|---|---|---|---|---|---|---|---|
| | **0** | **1** | **3** | **10** | **30** | **100** | **($\mu$M)** |
| **CP1** | 0.0 ± 2.0 | - | - | 13.7 ± 1.6 | 32.1 ± 2.0** | 70.8 ± 2.2** | 48.5 |
| **CP2** | 0.0 ± 2.0 | - | - | 9.9 ± 3.4 | 37.3 ± 3.1** | 71.2 ± 1.9** | 46.1 |
| **CP3** | 0.0 ± 2.0 | - | - | -0.5 ± 3.6 | 24.1 ± 3.1** | 67.9 ± 4.1** | 59.7 |
| **CP4** | 0.0 ± 8.6 | - | - | 36.8 ± 1.1** | 39.0 ± 1.9** | 79.4 ± 1.2** | 43.2 |
| **CP5** | 0.0 ± 2.3 | - | - | 25.2 ± 1.7** | 44.2 ± 2.4** | 45.1 ± 2.2[b]** | >100 |
| **CP6** | 0.0 ± 2.3 | - | - | 8.3 ± 1.5 | 33.0 ± 1.3** | 72.8 ± 1.4[b]** | 47.5 |
| **CP7** | 0.0 ± 2.3 | - | - | 17.5 ± 2.4 | 47.6 ± 2.9** | 71.8 ± 2.0[b]** | 37.4 |
| **CP8** | 0.0 ± 4.8 | - | - | 53.2 ± 3.1** | 67.0 ± 2.1** | 104.3 ± 1.8[b]** | 10.2 |
| **CP9** | 0.0 ± 4.8 | - | - | 57.9 ± 2.6** | 82.4 ± 1.9** | 104.3 ± 2.0[b]** | 6.4 |
| **CP10** | 0.0 ± 2.0 | - | - | 15.6 ± 2.1 | 69.8 ± 2.0** | 76.4 ± 2.0[b]** | 26.7 |
| **CP11** | 0.0 ± 4.8 | 27.3 ± 2.1 | 34.8 ± 2.0* | 71.0 ± 3.8** | 95.0 ± 1.7** | 99.4 ± 3.4[b]** | 4.2 |
| **CP12** | 0.0 ± 8.2 | - | 38.3 ± 2.6* | 78.0 ± 4.2** | 97.8 ± 4.9[b]** | 105.4 ± 1.9[b]** | 4.2 |
| **CP13** | 0.0 ± 8.2 | - | 42.6 ± 1.8** | 77.4 ± 3.3** | 101.1 ± 5.0** | 104.8 ± 4.8[b]** | 3.4 |
| **CP14** | 0.0 ± 8.2 | - | 49.7 ± 2.4** | 81.2 ± 4.1** | 103.8 ± 4.7** | 104.9 ± 5.4[b]** | 2.9 |
| **CP15** | 0.0 ± 8.2 | - | 32.8 ± 2.1* | 67.7 ± 4.6** | 98.4 ± 3.8** | 100.5 ± 4.6[b]** | 5.4 |
| **CP16** | 0.0 ± 2.3 | - | - | 9.7 ± 2.0 | 35.9 ± 2.7** | 67.5 ± 0.9[b]** | 50.7 |
| **CP17** | 0.0 ± 8.6 | - | 29.6 ± 1.8 | 55.6 ± 1.3** | 71.7 ± 4.2** | 104.5 ± 1.6[b]** | 8.2 |
| **CP18** | 0.0 ± 8.6 | - | 38.5 ± 2.1* | 60.1 ± 0.4** | 88.3 ± 0.9** | 104.0 ± 0.9[b]** | 5.3 |
| **CP19** | 0.0 ± 8.6 | - | - | 46.2 ± 1.9** | 68.6 ± 3.1** | 105.4 ± 1.0[b]** | 12.5 |
| **CP20** | 0.0 ± 4.8 | - | - | 52.4 ± 2.3** | 76.8 ± 2.5** | 97.9 ± 5.0[b]** | 8.4 |
| **CP21** | 0.0 ± 9.3 | -2.3 ± 2.8 | 2.3 ± 2.0 | 100.0 ± 1.5[b]** | 102.0 ± 5.2[b]** | 108.7 ± 1.8[b]** | 6.0 |
| **CP22** | 0.0 ± 9.3 | - | 36.2 ± 2.2* | 64.7 ± 0.5** | 100.0 ± 2.0** | 106.0 ± 5.2[b]** | 5.2 |
| **CP23** | 0.0 ± 9.3 | 2.3 ± 3.2 | 13.0 ± 1.3 | 102.2 ± 4.2[b]** | 103.8 ± 5.0[b]** | 108.7 ± 1.5[b]** | 5.6 |
| **CP24** | 0.0 ± 9.3 | - | 28.2 ± 2.5 | 56.5 ± 4.3** | 103.3 ± 2.7** | 109.2 ± 2.9[b]** | 6.5 |
| **CP25** | 0.0 ± 8.2 | - | 39.5 ± 2.2* | 71.5 ± 3.3** | 105.4 ± 2.2** | 105.9 ± 3.1[b]** | 4.4 |
| **CP26** | 0.0 ± 8.2 | - | 35.4 ± 2.4* | 58.1 ± 3.7** | 71.0 ± 2.7** | 100.0 ± 3.2** | 7.0 |
| Indomethacin | 0.0 ± 4.2 | - | 15.5 ± 1.7 | 36.4 ± 2.3** | 60.9 ± 3.7** | 104.5 ± 1.7** | 14.5 |

[a]Each value represents mean ± S.E.M. of four determinations.

Statistical significance, * $p<0.05$, ** $p<0.01$

[b]Cytotoxic effect was observed.

## 3.5 Proposed biogenesis of cassane and cyclopimarane diterpenes

           The tricyclic diterpene structures of cassane and cyclopimarane diterpenes could be derived from common pimar-15-en-8-yl carbocation intermediate generated by cyclization of (+)-copalyl diphosphate (Devon and Scott, 1972; Ravn et al., 2002) as shown in Scheme 6. The $14 \rightarrow 8$ hydride shift of pimar-15-en-8-yl carbocation to form a pimar-15-en-14-yl carbocation followed by $13 \rightarrow 14$ methyl shift (pathway a) results in cass-15-en-13-yl carbocation which will give rise to a cassane-type diterpenes with the trans/anti/trans ring junction (A/B/C). On the other hand the addition of water to C-16 double bond of a homoallylic cation (pimar-15-en-14-yl carbocation) concomitant with ring closure (pathway b) results in 14,15-cyclopimaran-16-ol, a precursor of **CM4** and **CP15**.
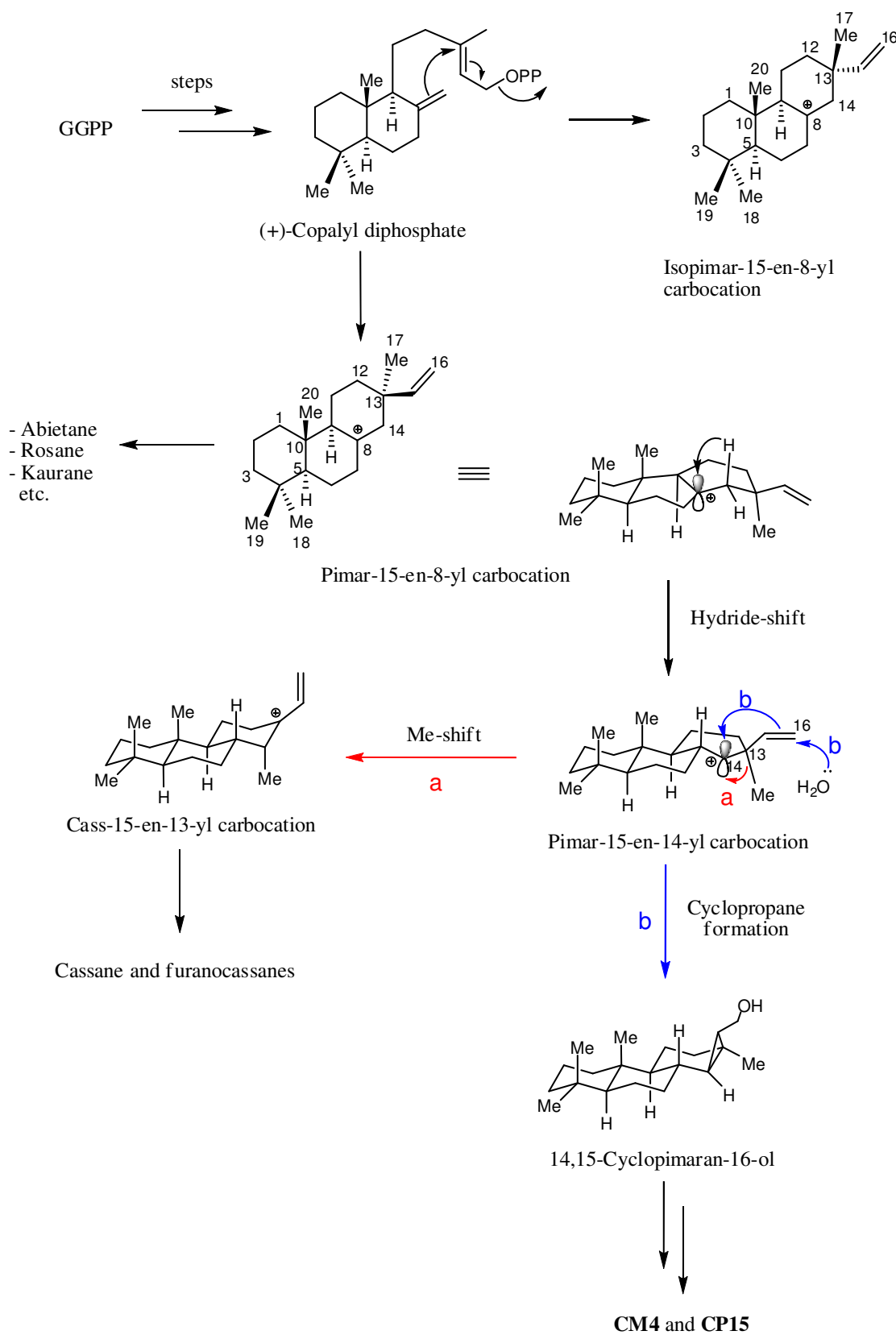
**Scheme 6** Plausible biosynthesis pathway of cassane and cyclopimarane diterpenes

# CHAPTER 4
# CONCLUSION

The bioassay guided separation of the crude $CH_2Cl_2$ and acetone extracts of *C. mimosoides* led to the isolation of seven new compounds together with eleven known compounds. The new compounds were identified as four diterpenes, named mimosol A–D (**CM1–CM4**), a dimer, named mimosol E (**CM9**) and two dibenzo[b,d]furans, named mimosol F, G (**CM10**, **CM11**). The known compounds were identified by analysis of their spectroscopic data and comparison with literature data to be taepeenin A (**CM5**), taepeenin D (**CM6**), nortaepeenin A (**CM7**), taepeenin L (**CM8**), (*E*)-7-hydroxy-3-(4-methoxybenzyl)chroman-4-one (**CM12**), (*E*)-7,8-dihydroxy-3-(4-methoxybenzyl)-chroman-4-one (**CM13**), (*E*)-7-hydroxy-8-methoxy-3-(4-methoxybenzyl)chroman-4-one (**CM14**), tetracosyl caffeate (**CM15**), resveratrol (**CM16**), bergenin (**CM17**) and (+)-pterocarpol (**CM18**).

The $CH_2Cl_2$ extract from *C. pulcherrima* was purified to afford 15 new diterpenes, named pulcherrin D–R (**CP1–CP15**) together with eleven known compounds (**CP16–CP26**). The known compounds were identified as vouacapen-5$\alpha$-ol (**CP16**), isovouacapenol C (**CP17**), 6$\beta$-cinnamoyl-7$\beta$-hydroxyvouacapen-5$\alpha$-ol (**CP18**), pulcherrin A (**CP19**), pulcherrin B (**CP20**), pulcherrimin C (**CP21**), pulcherrimins A (**CP22**), pulcherrimin E (**CP23**), pulcherrin C (**CP24**), pulcherrimin B (**CP25**) and 8,9,11,14-didehydrovouacapen-5$\alpha$-ol (**CP26**). Moreover, the structures of compounds **CP16** and **CP17** were also confirmed by X-ray diffraction analysis.

The anti-inflmmatory activity of all compounds were evaluated for inhibitory activity against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 cell line. Compounds from *C. mimosoides* **CM4**, **CM13**, **CM12**, **CM14**, **CM8** and **CM6** possessed high activity with $IC_{50}$ values of 3.0, 3.9, 4.4, 5.6, 7.1, and 8.2 μM, respectively. Compounds from *C. pulcherrima* **CP8**, **CP9**, **CP11–CP15** and **CP18–CP26** with $IC_{50}$ of 10.2, 6.4, 4.2, 4.2, 3.4, 2.9, 5.4, 5.3, 8.2, 6.0, 5.2, 5.6, 4.4 and 7.0 μM, respectively, whereas other compounds exhibited moderate and mild activities. In addition, compounds **CM4**, **CM6**, **CM8**, and **CM12**–

**CM14** were also tested for the inhibitory effect on LPS-induced tumor necrosis factor-alpha (TNF-$\alpha$) release in RAW264.7 cells. The results indicated that **CM4** possessed potent inhibitory activity for both tests with IC$_{50}$ values of 3.0 and 6.5 $\mu$M, respectively.

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**APPENDIX**
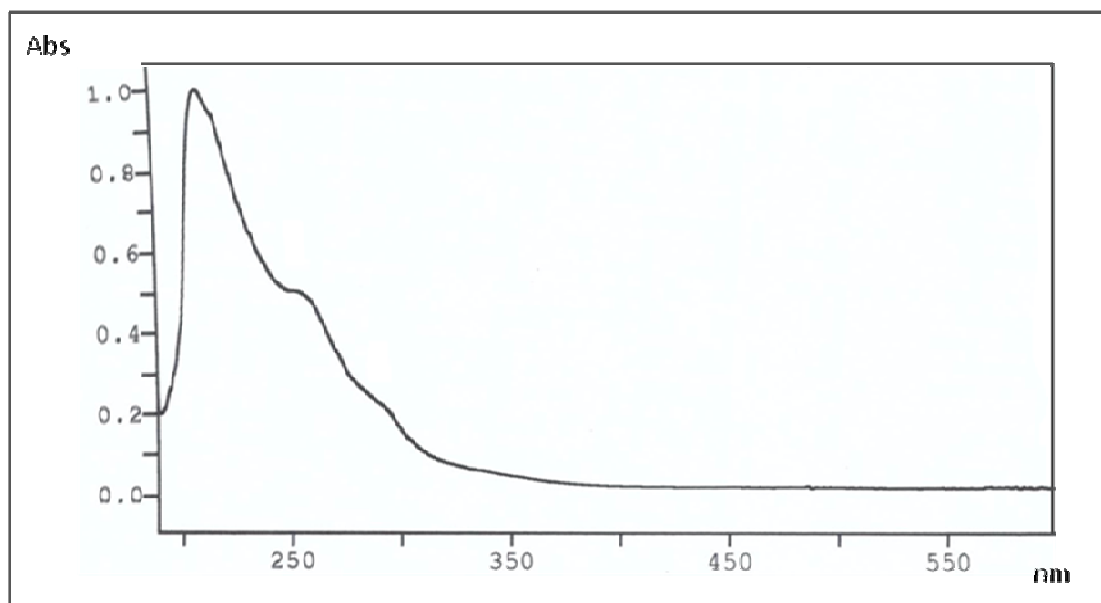
**Figure 3**  UV (MeOH) spectrum of compound **CM1**
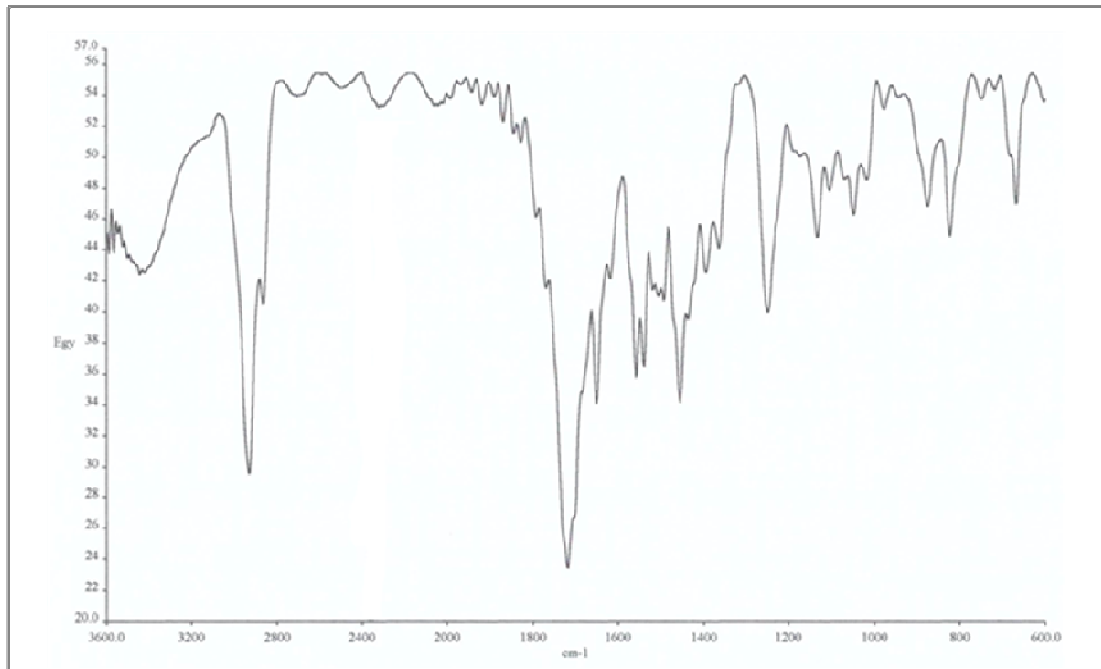


**Figure 4**  IR (neat) spectrum of compound **CM1**
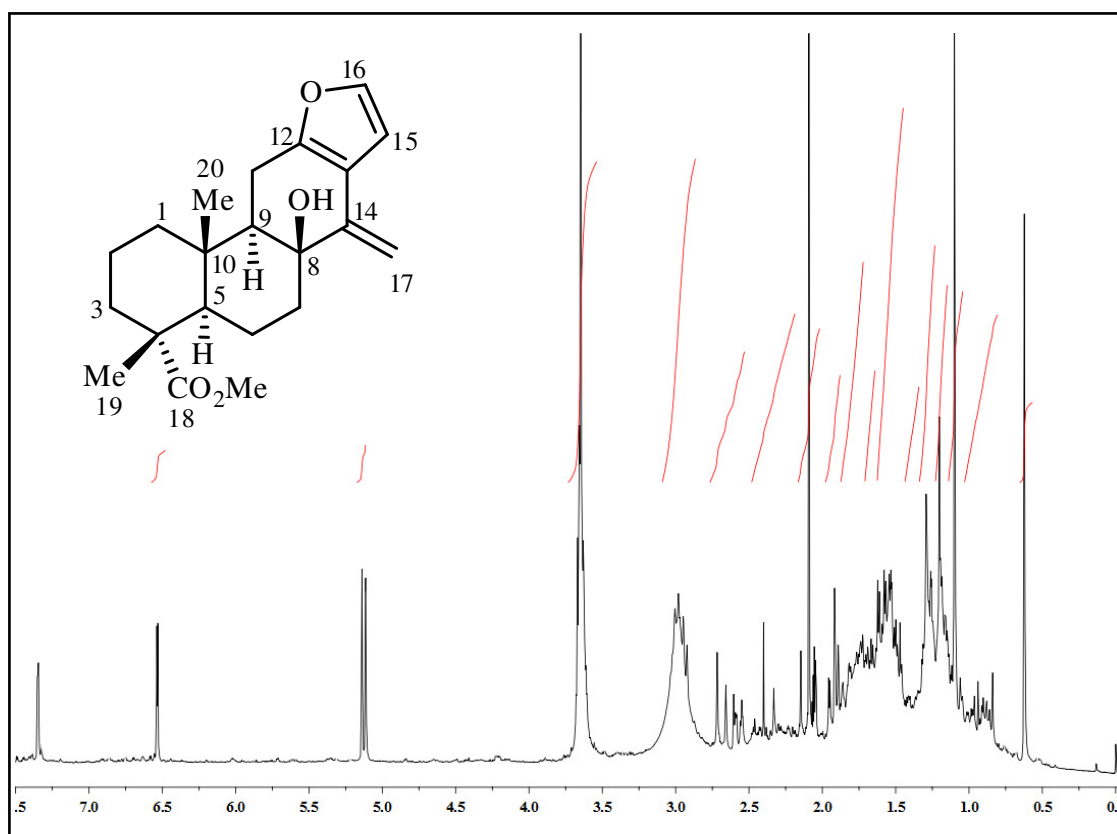
**Figure 5**   $^1$H NMR (300 MHz) (acetone-$d_6$) spectrum of compound **CM1**
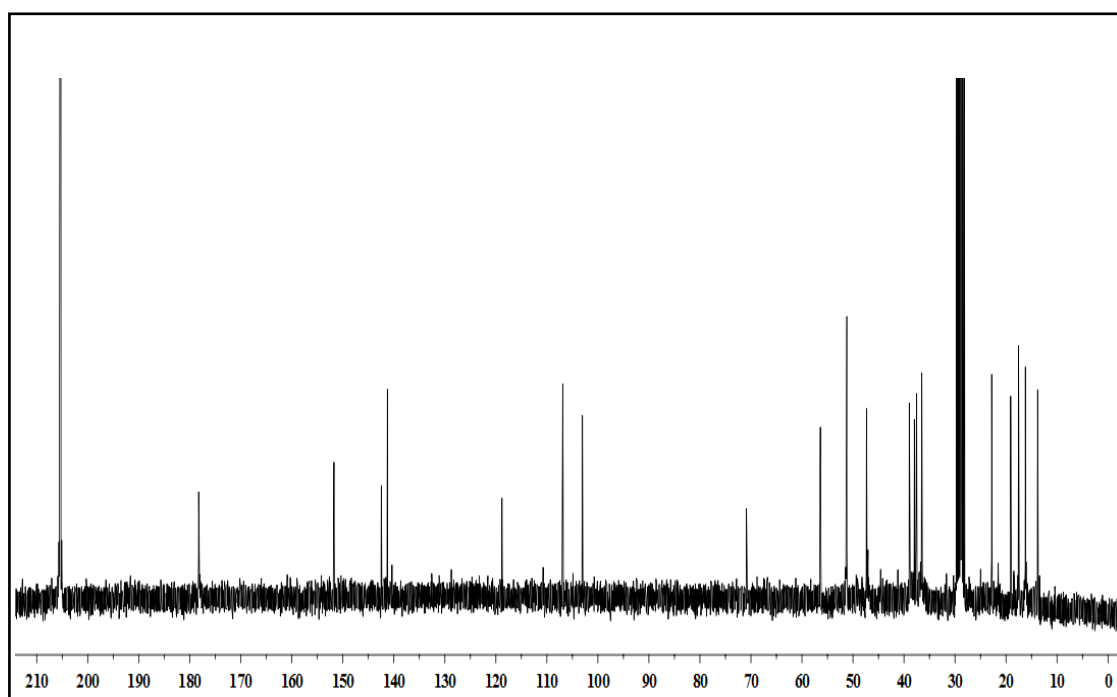


**Figure 6**   $^{13}$C NMR (75 MHz) (acetone-$d_6$) spectrum of compound **CM1**
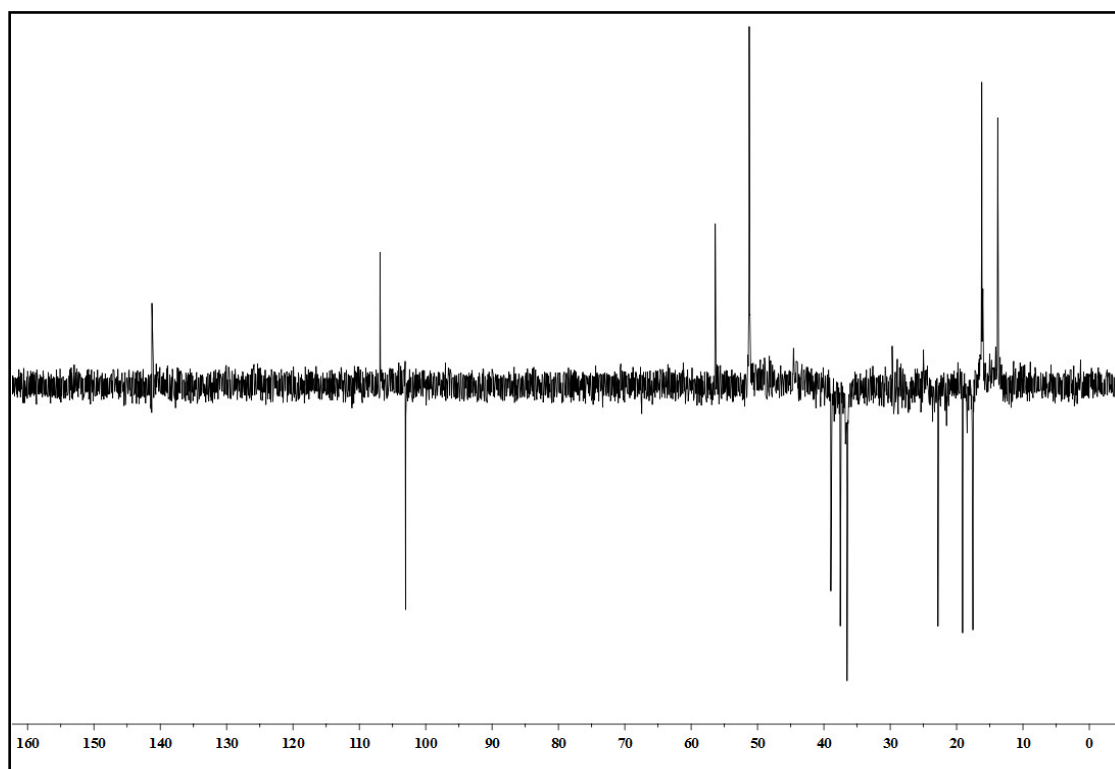
**Figure 7**   DEPT 135° (acetone-$d_6$) spectrum of compound **CM1**
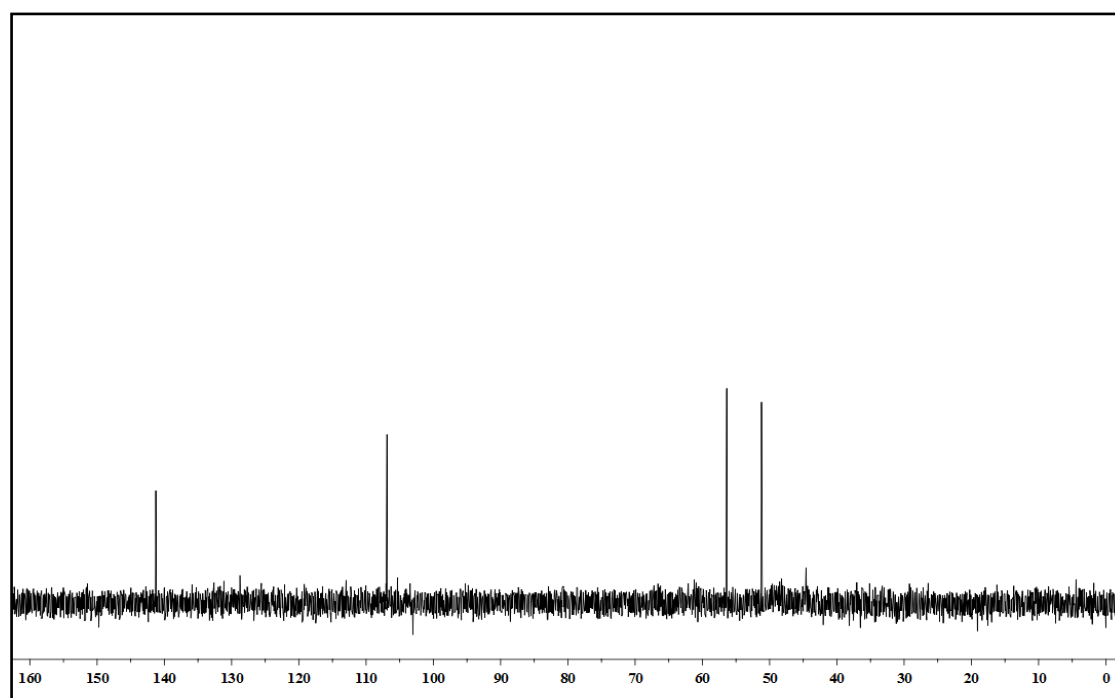


**Figure 8**   DEPT 90° (acetone-$d_6$) spectrum of compound **CM1**
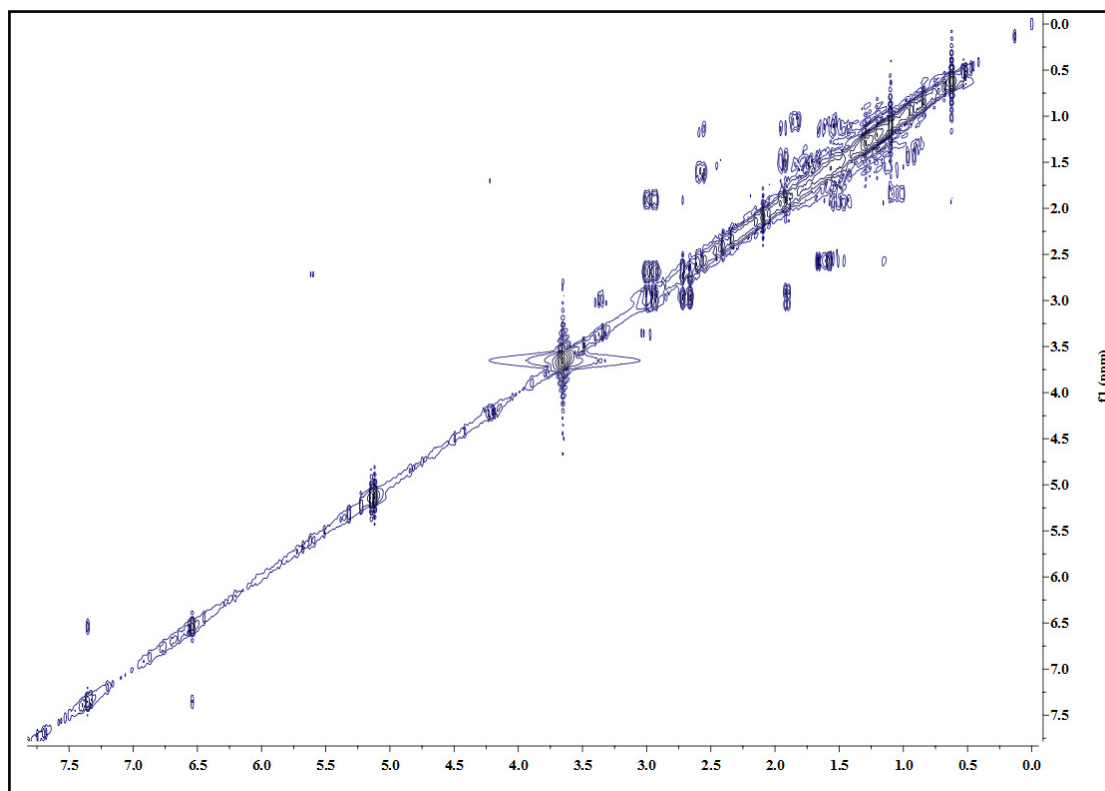
**Figure 9**    2D COSY (acetone-$d_6$) spectrum of compound **CM1**
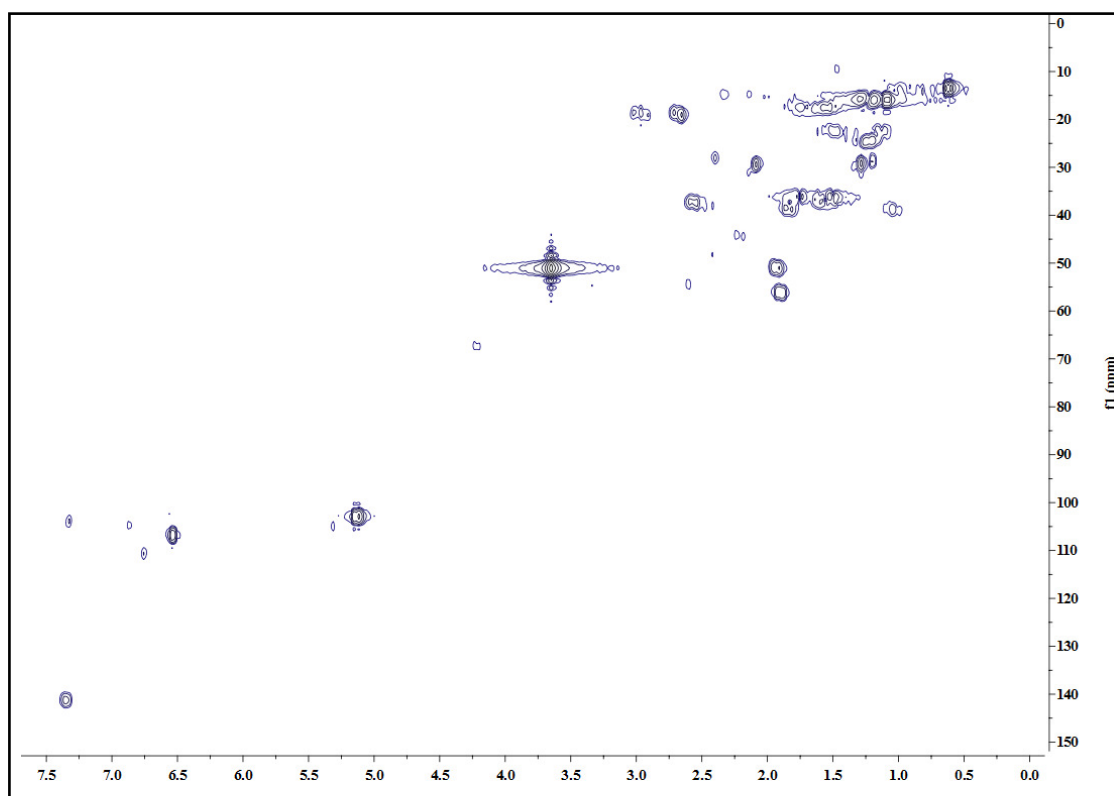


**Figure 10**    2D HMQC (acetone-$d_6$) spectrum of compound **CM1**
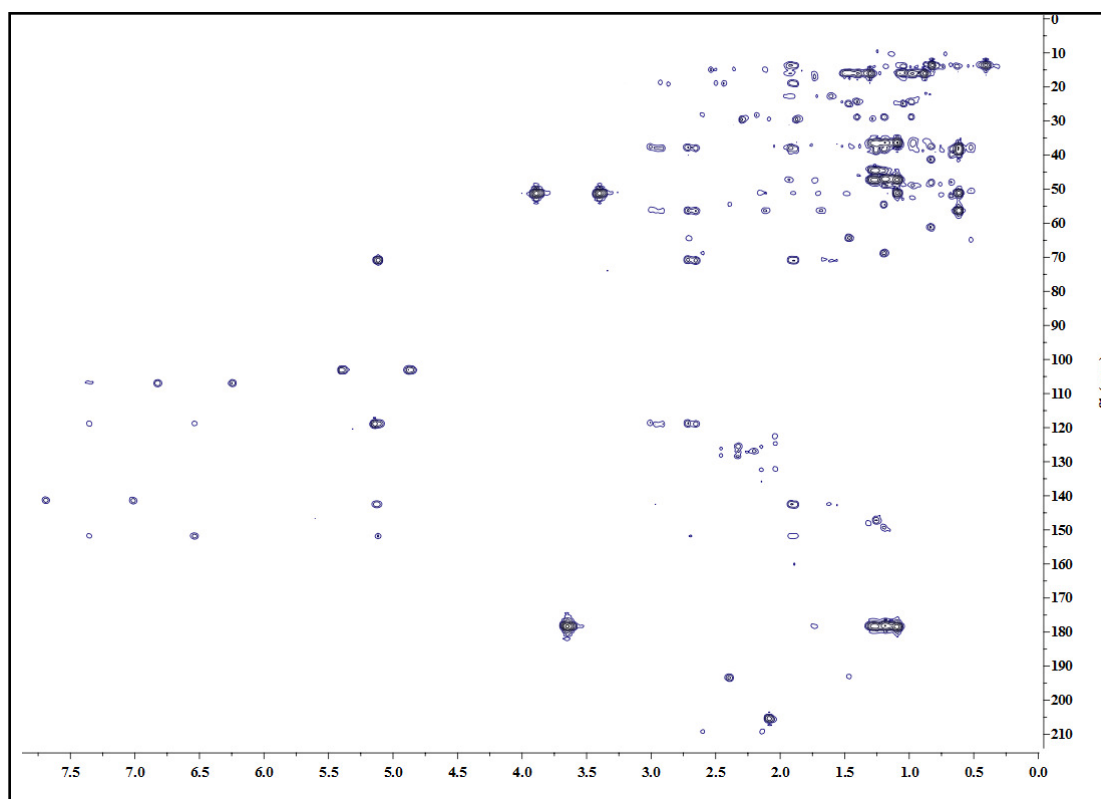
**Figure 11**    2D HMBC (acetone-$d_6$) spectrum of compound **CM1**
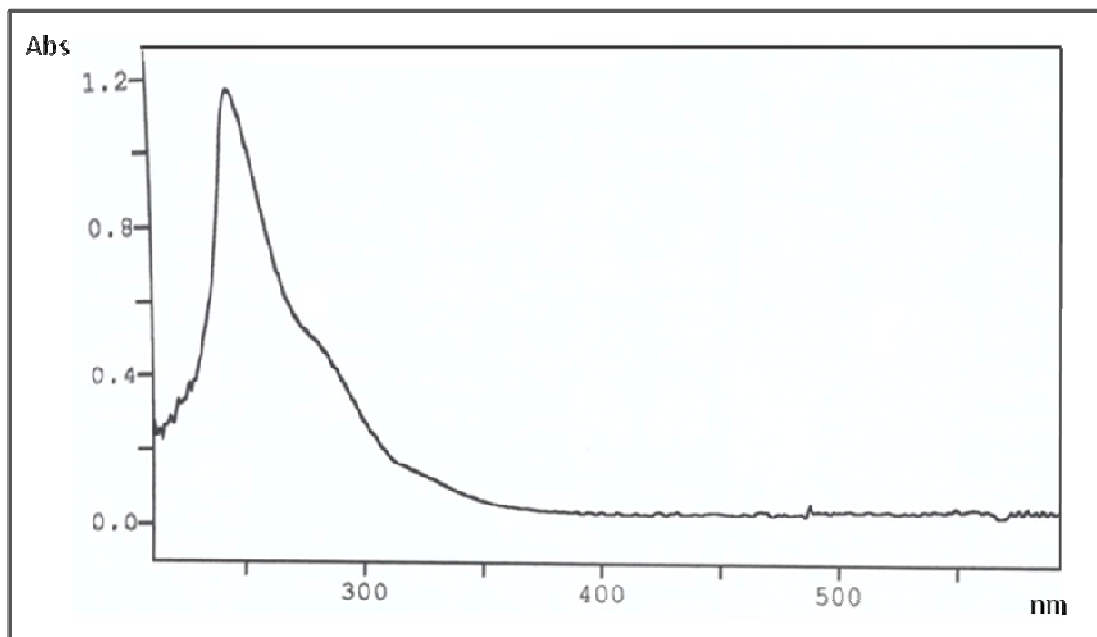
**Figure 12**    UV (MeOH) spectrum of compound **CM2**
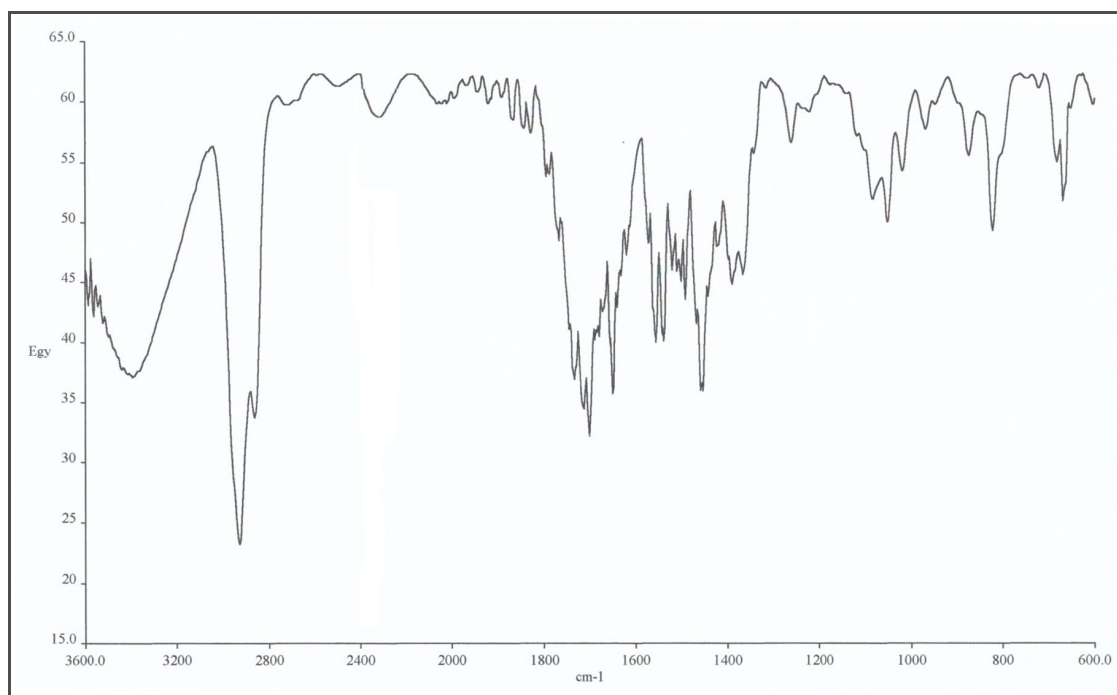


**Figure 13**    IR (neat) spectrum of compound **CM2**
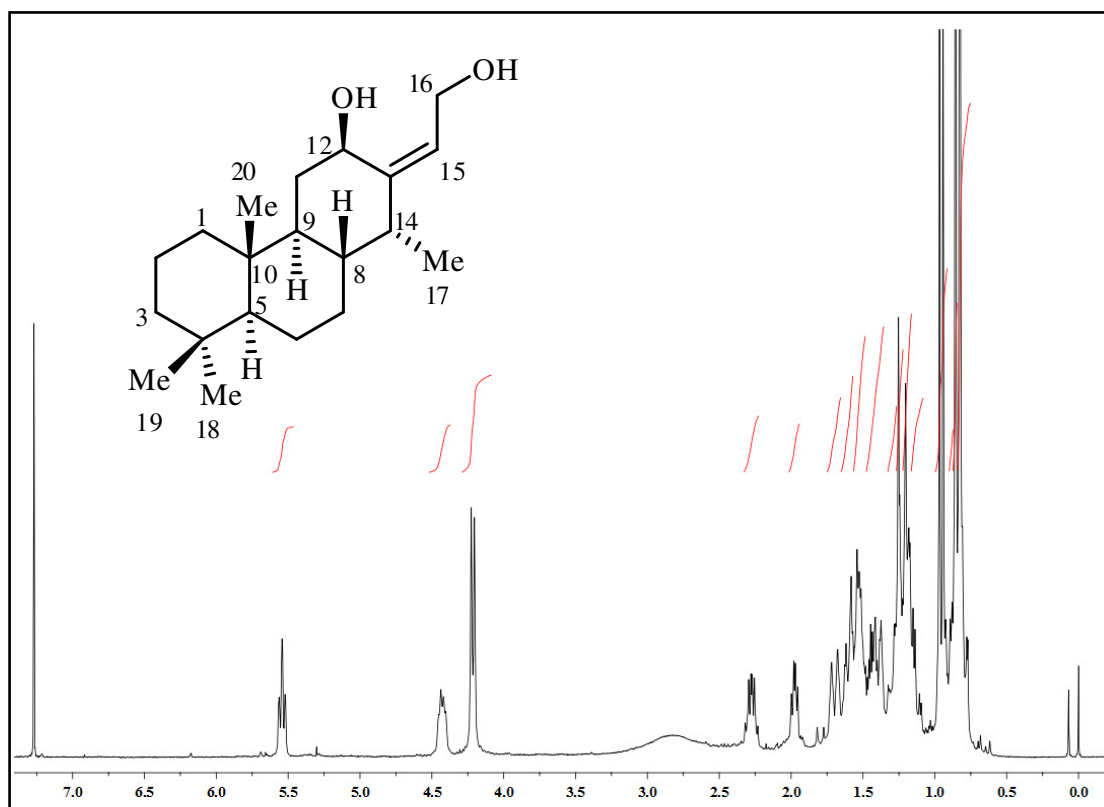
**Figure 14** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CM2**
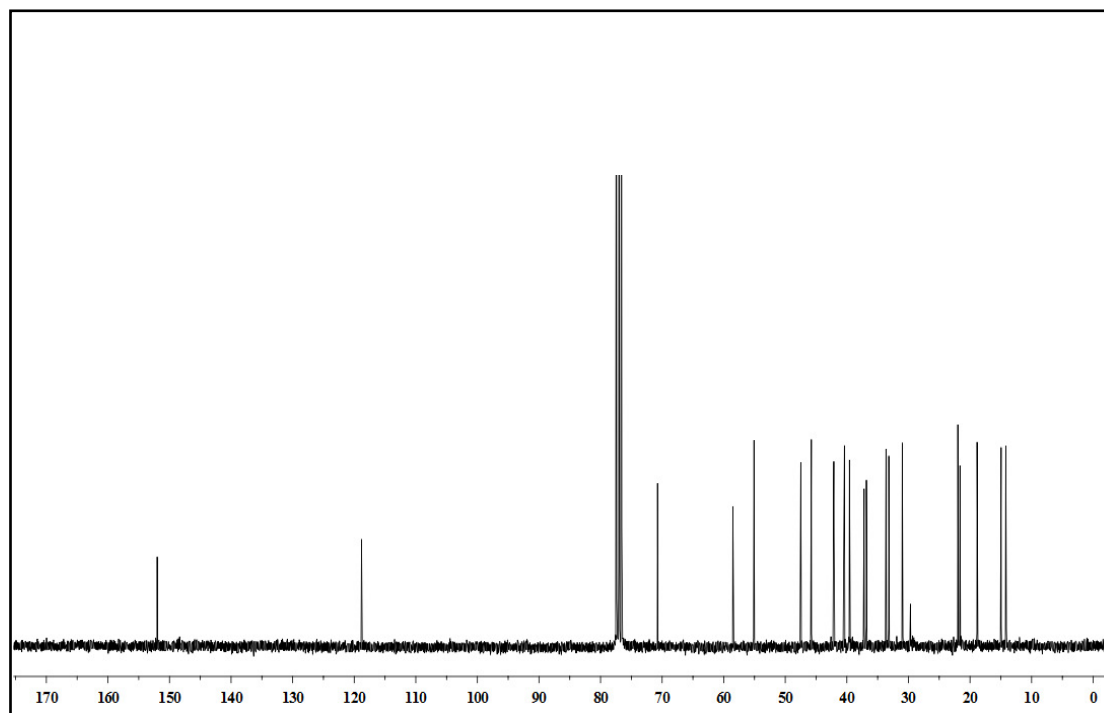


**Figure 15** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CM2**
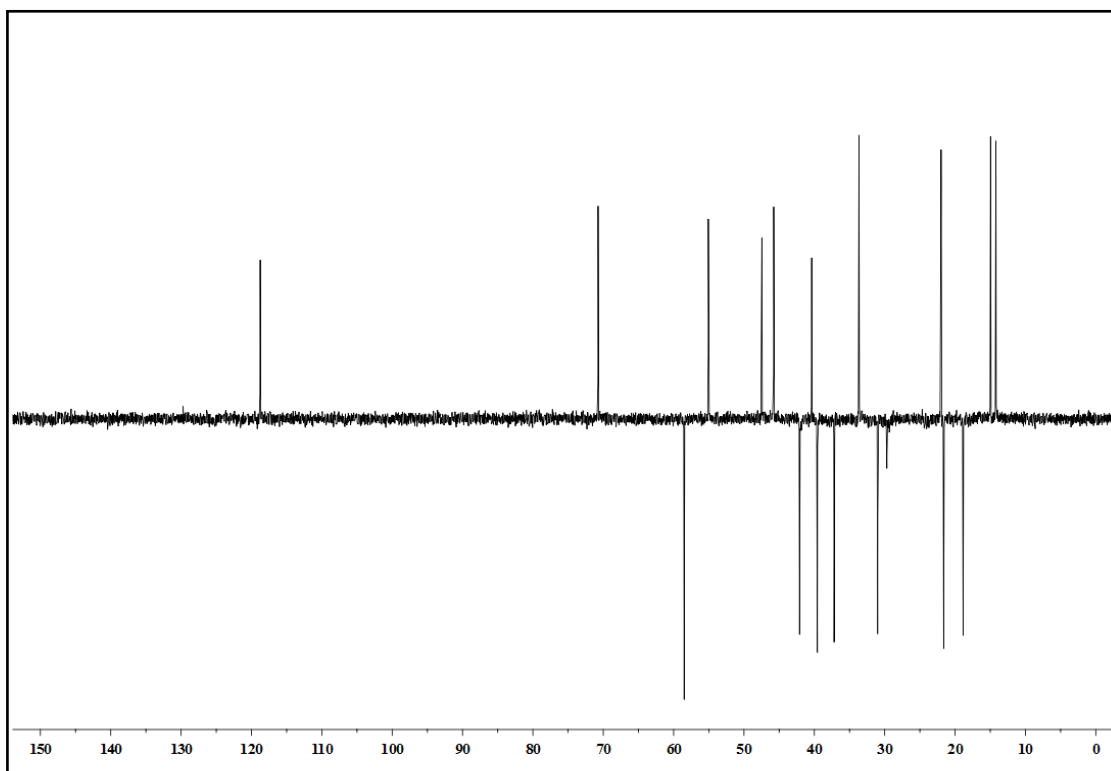
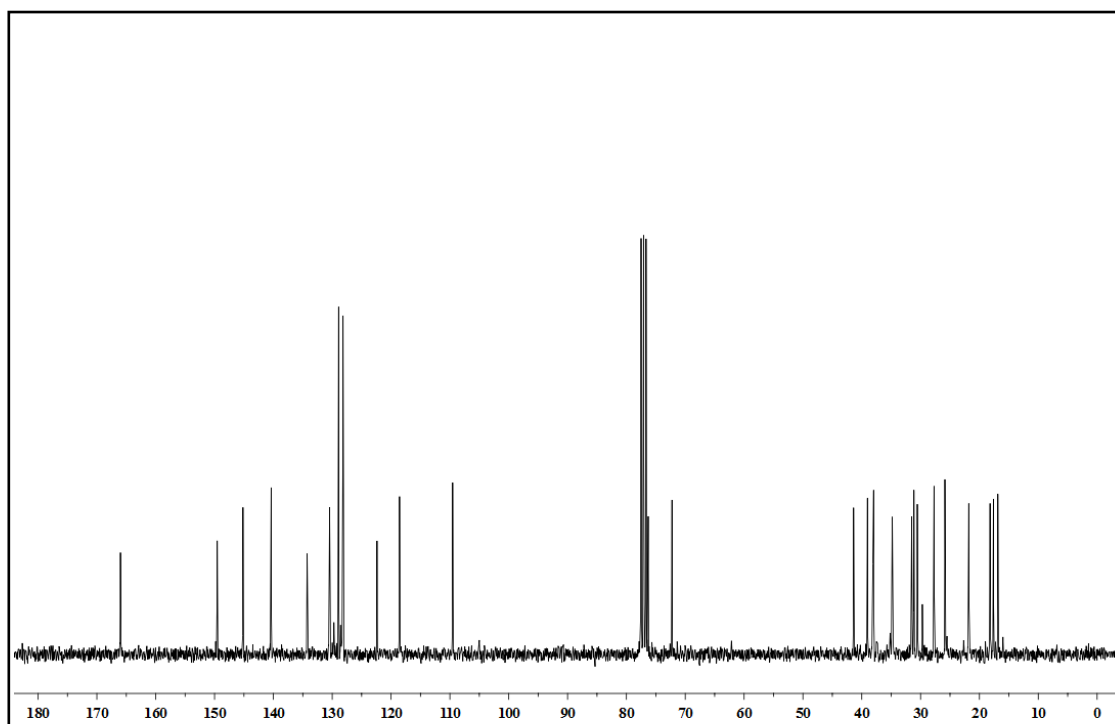**Figure 16** DEPT 135° (CDCl$_3$) spectrum of compound **CM2**
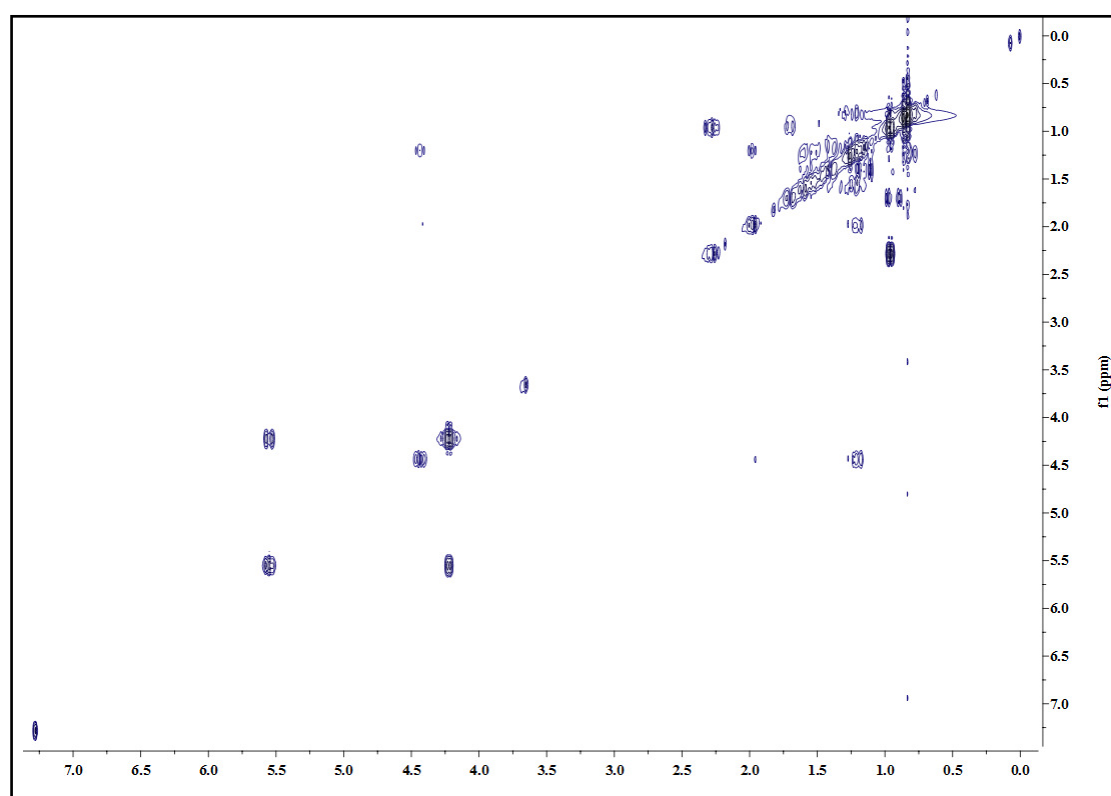


**Figure 17** 2D COSY (CDCl$_3$) spectrum of compound **CM2**
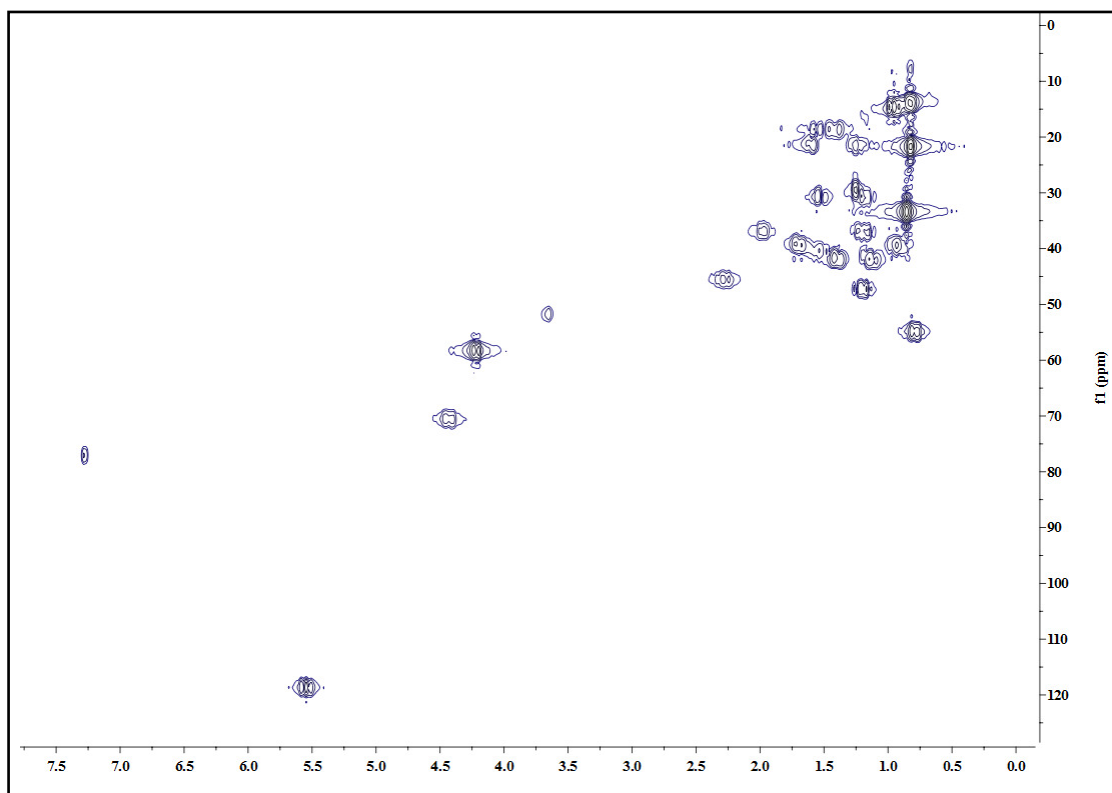
**Figure 18** 2D HMQC (CDCl$_3$) spectrum of compound **CM2**
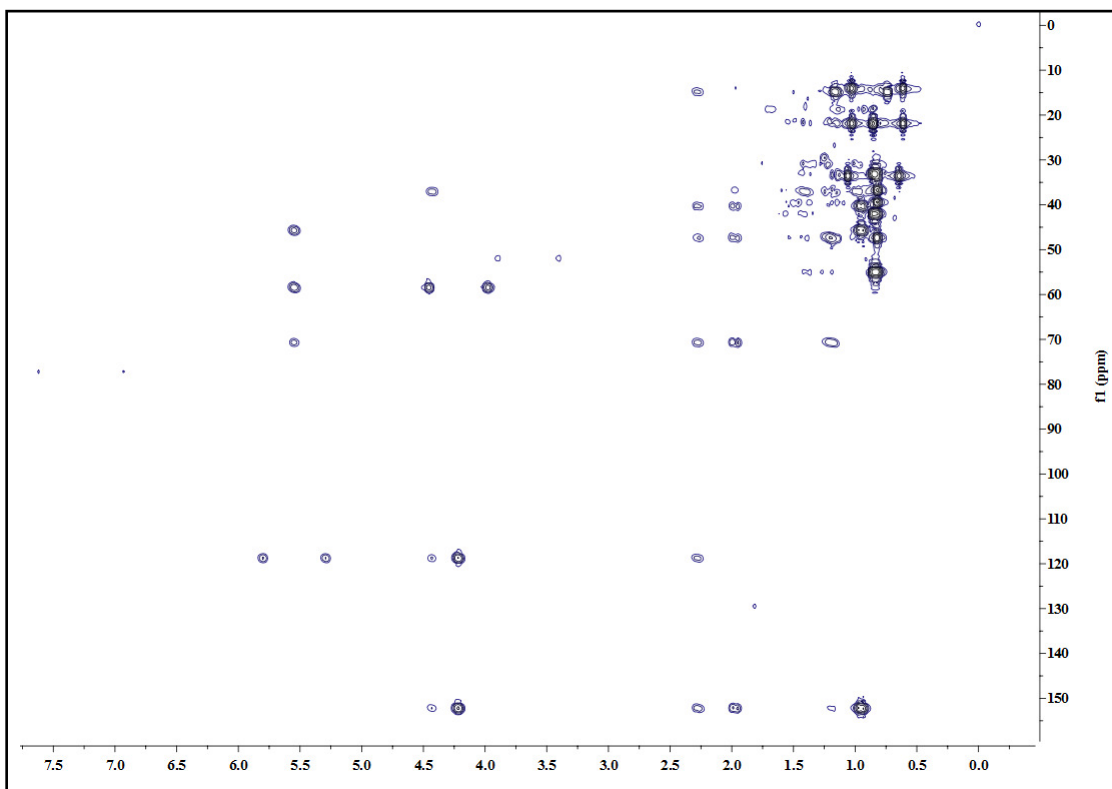


**Figure 19** 2D HMBC (CDCl$_3$) spectrum of compound **CM2**
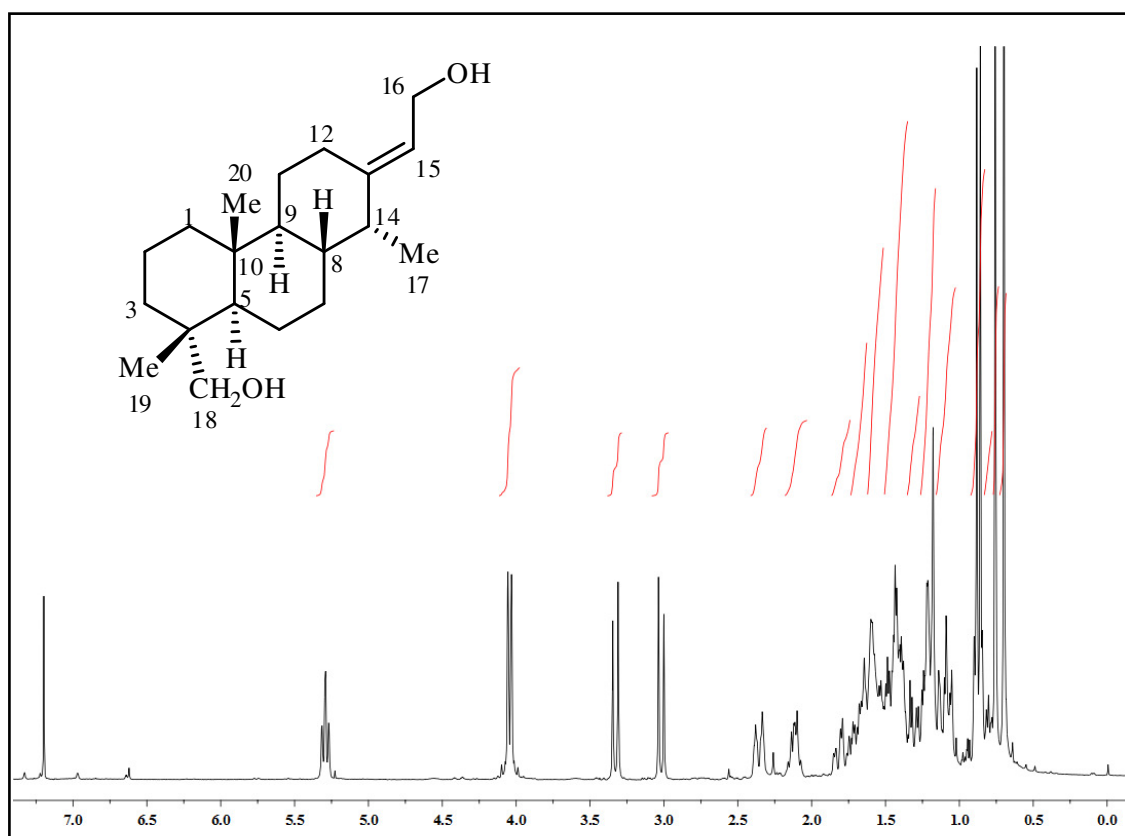
**Figure 20** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CM3**
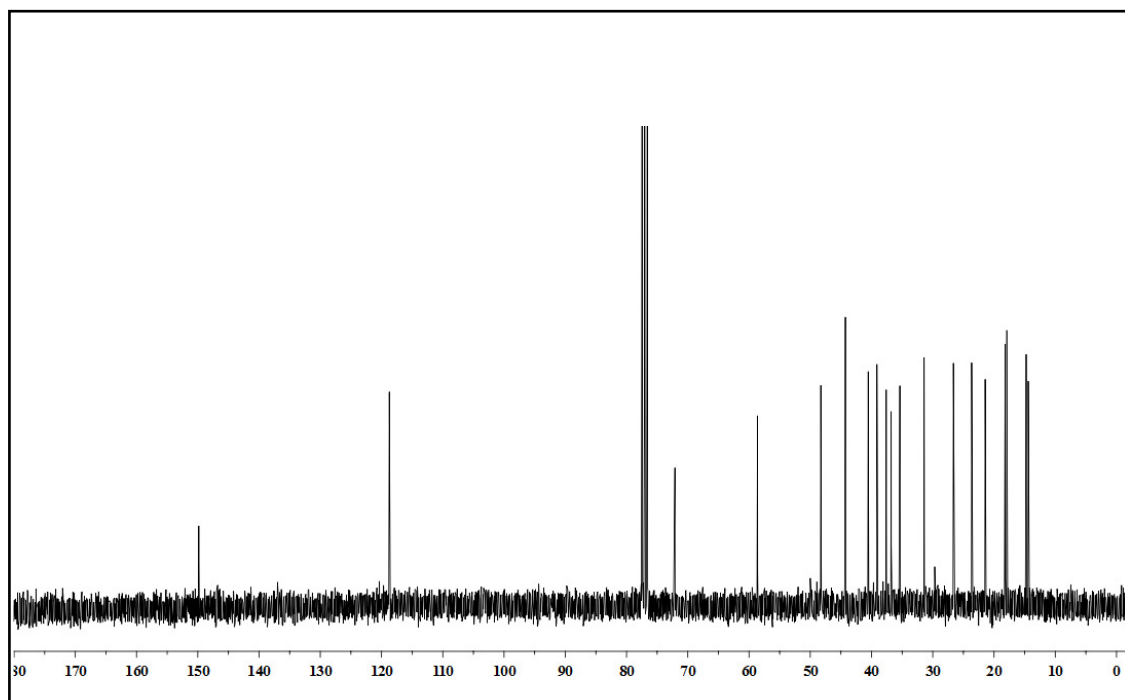


**Figure 21** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CM3**
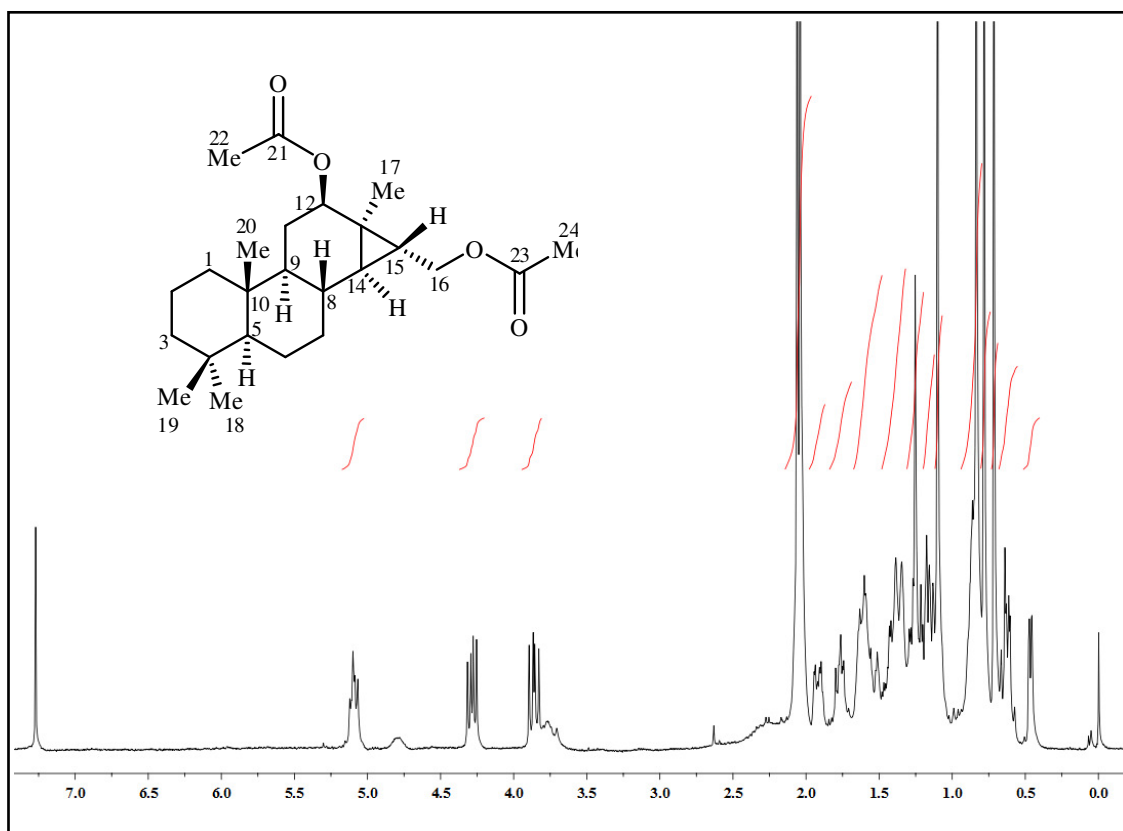
**Figure 22**  $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CM4**
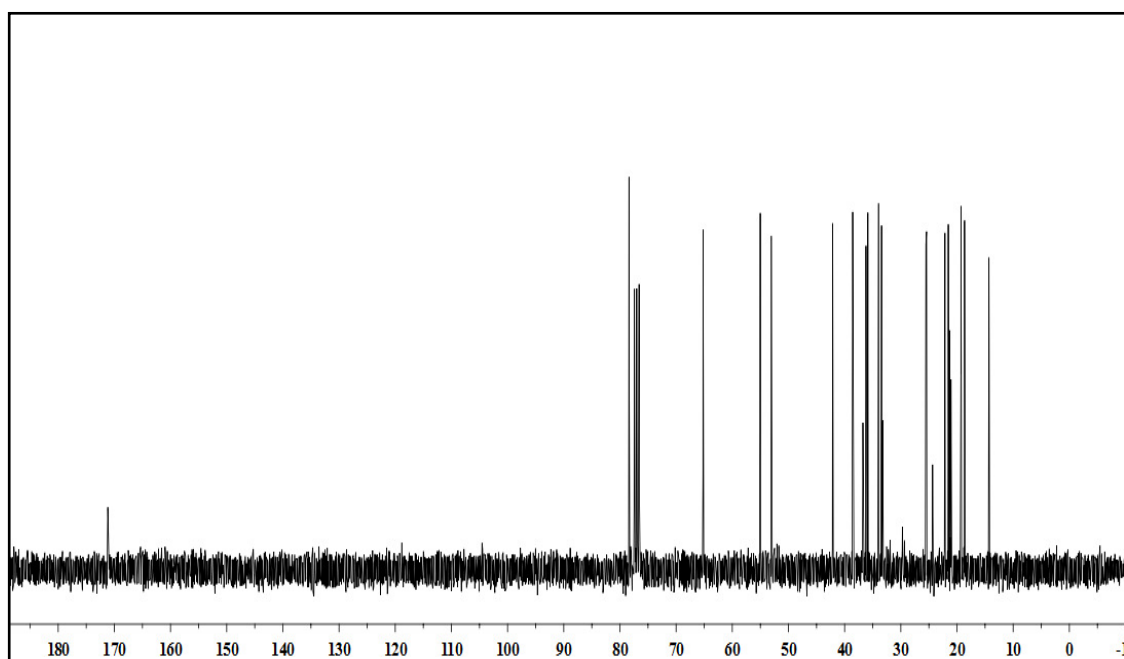


**Figure 23**  $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CM4**
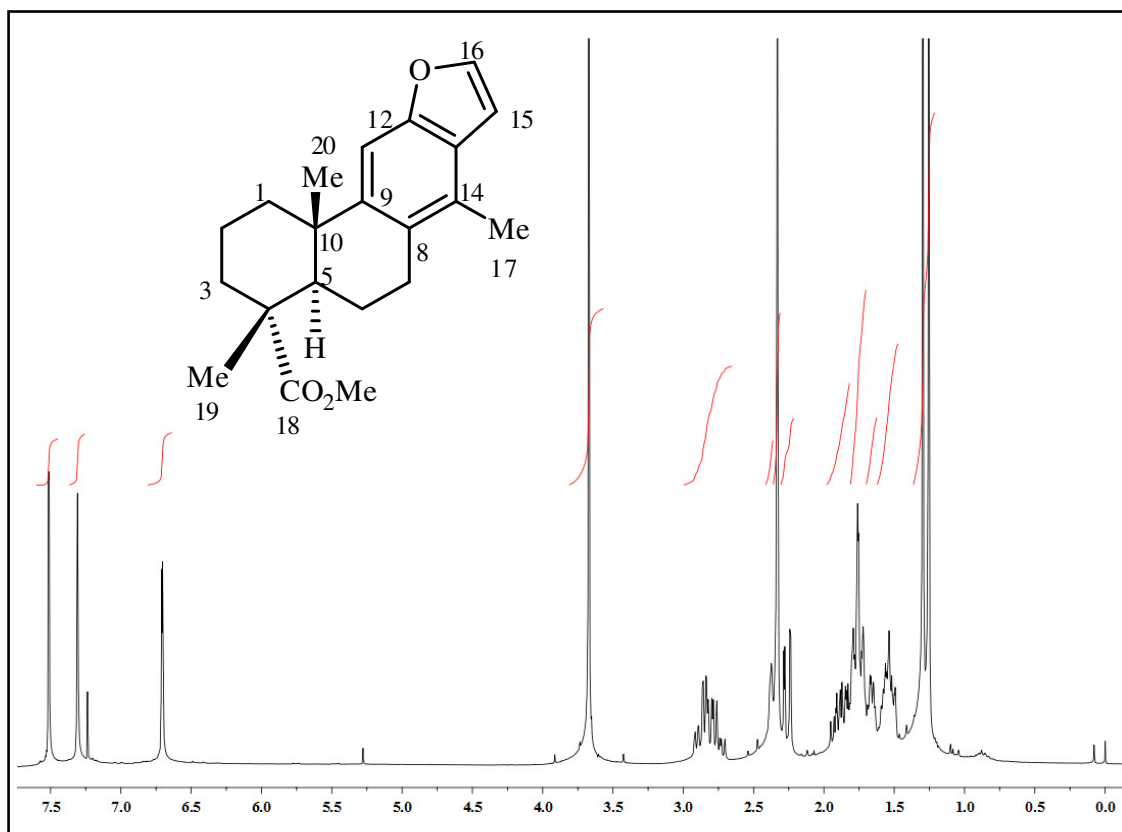
**Figure 24** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CM5**
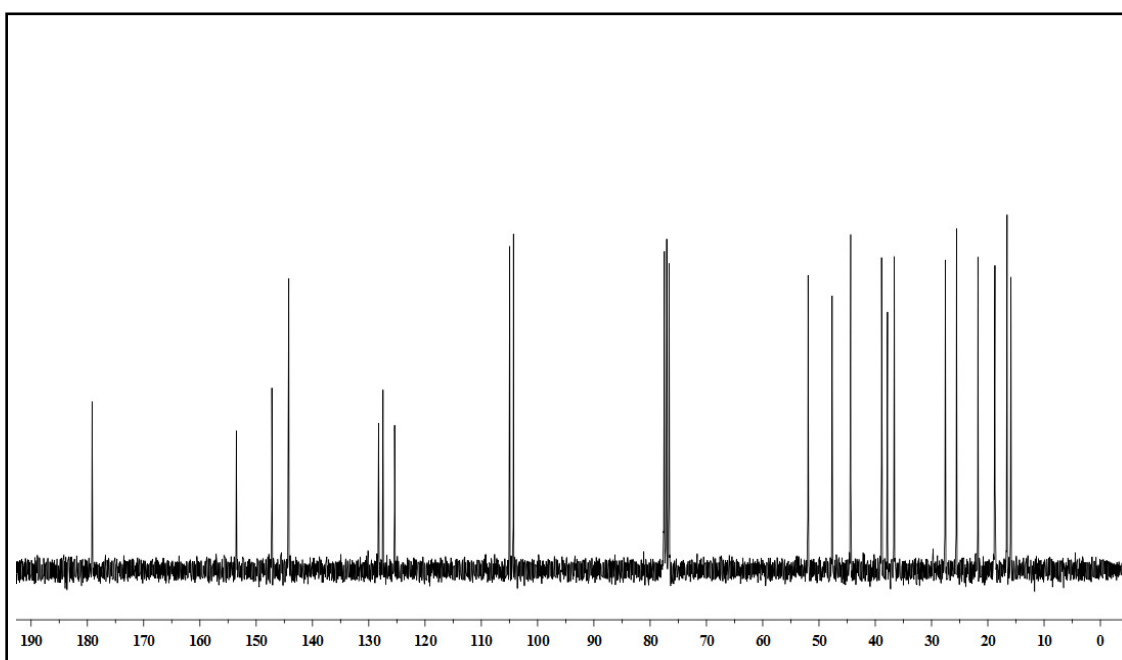


**Figure 25** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CM5**
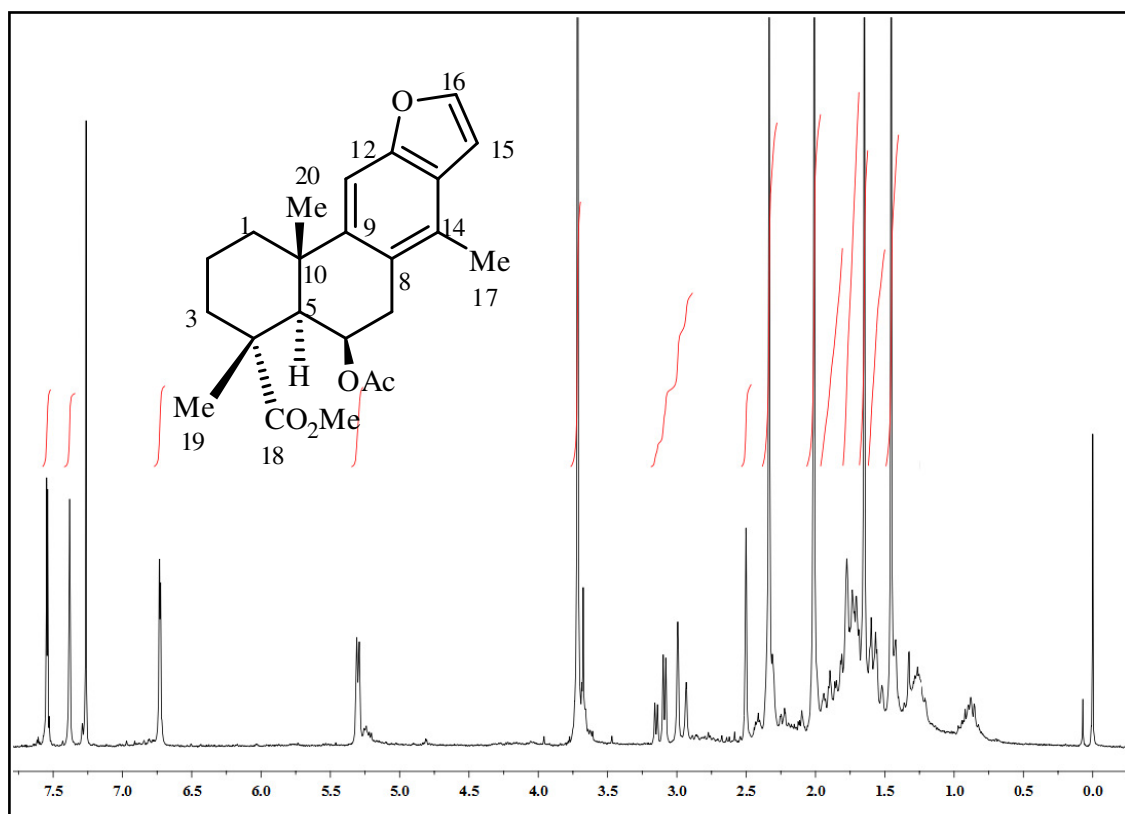
**Figure 26** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CM6**
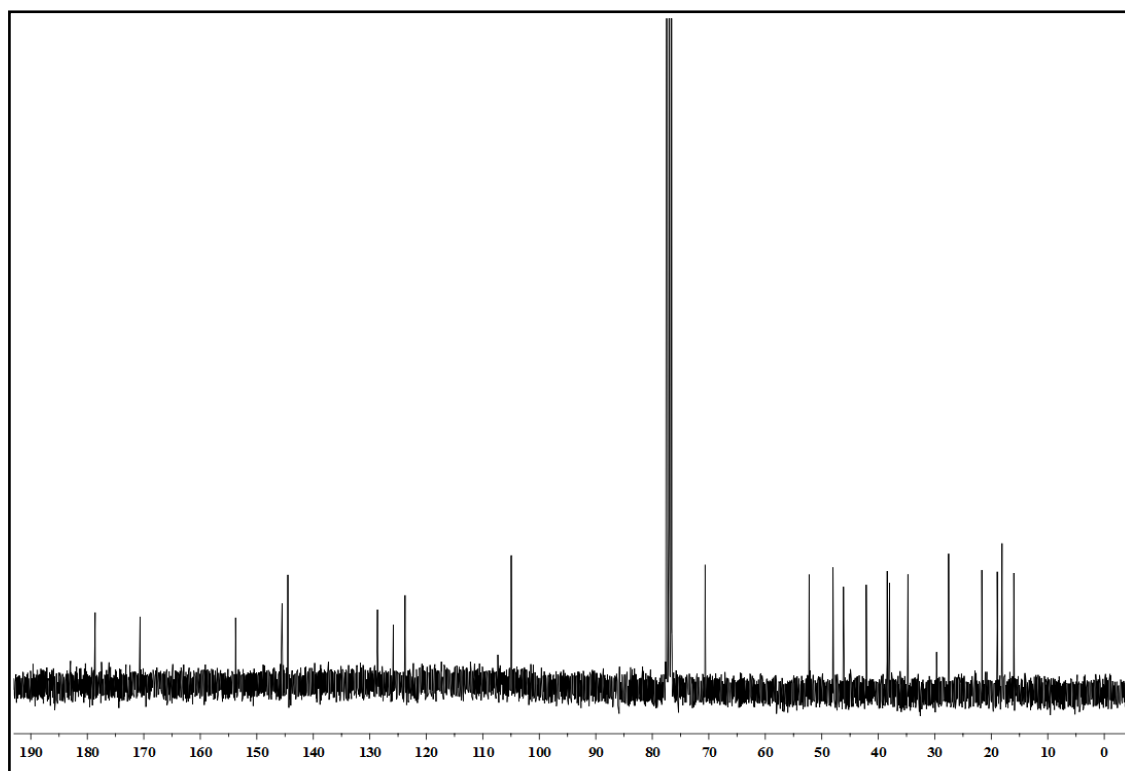


**Figure 27** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CM6**
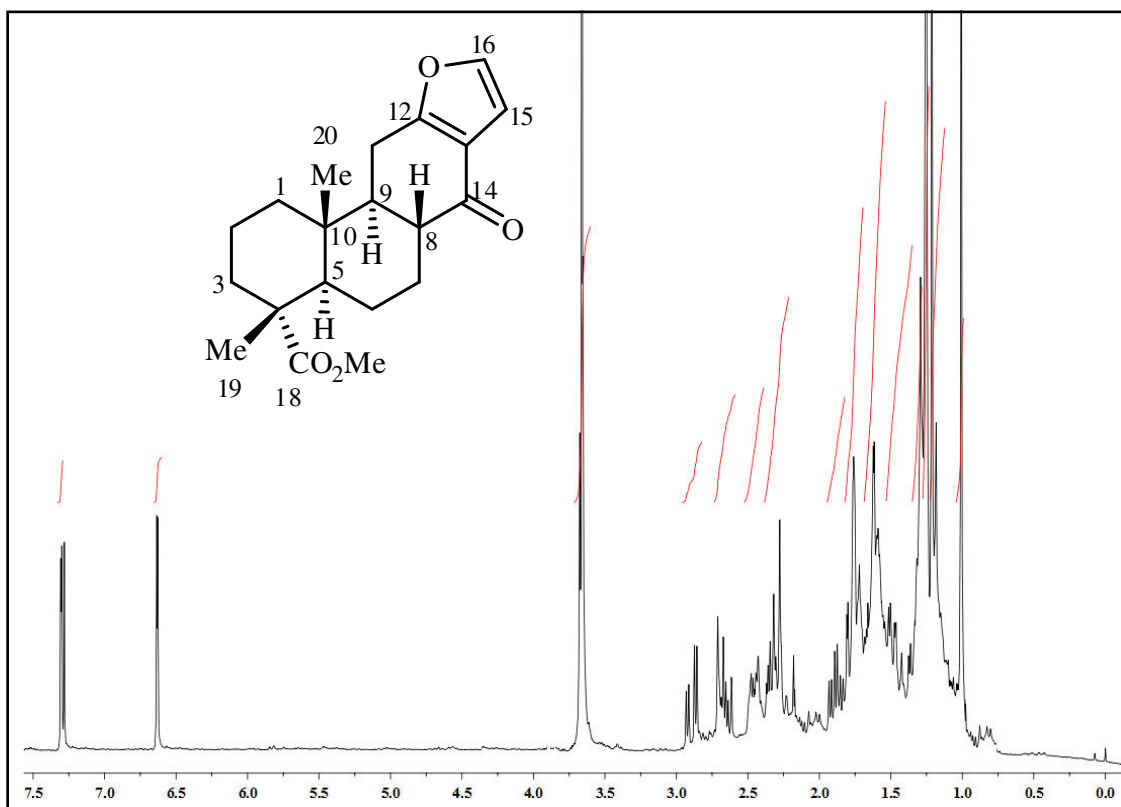
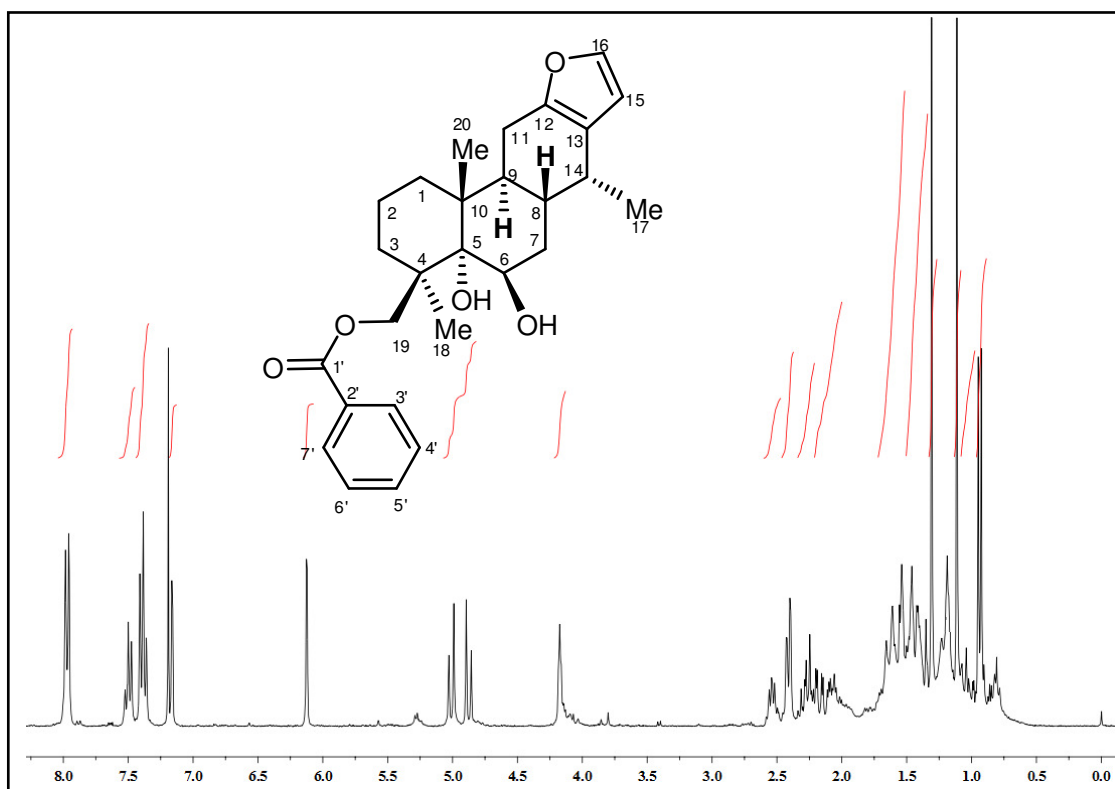**Figure 28** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CM7**
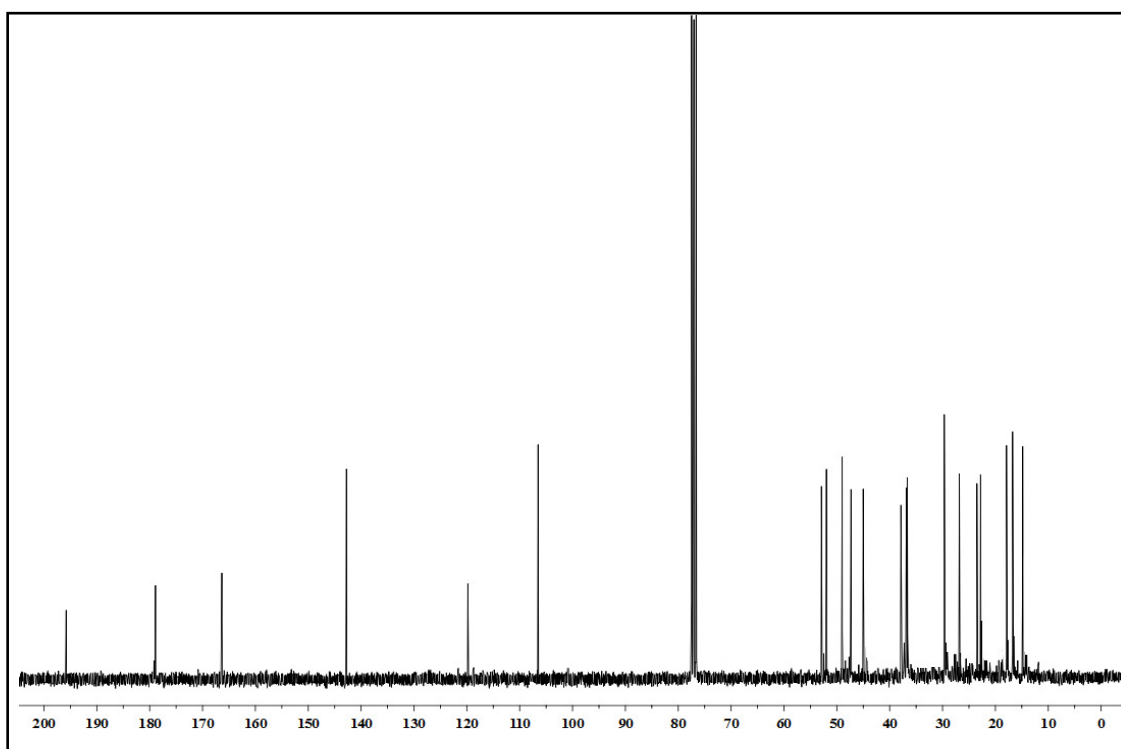


**Figure 29** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CM7**
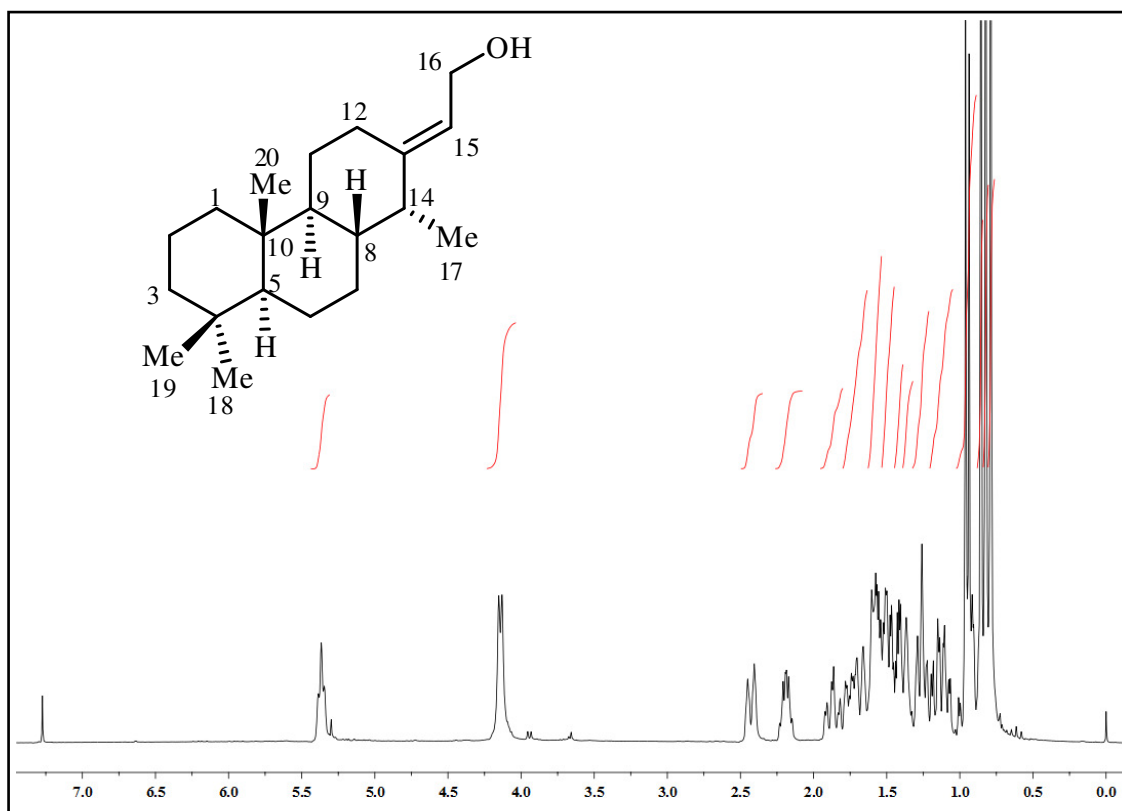
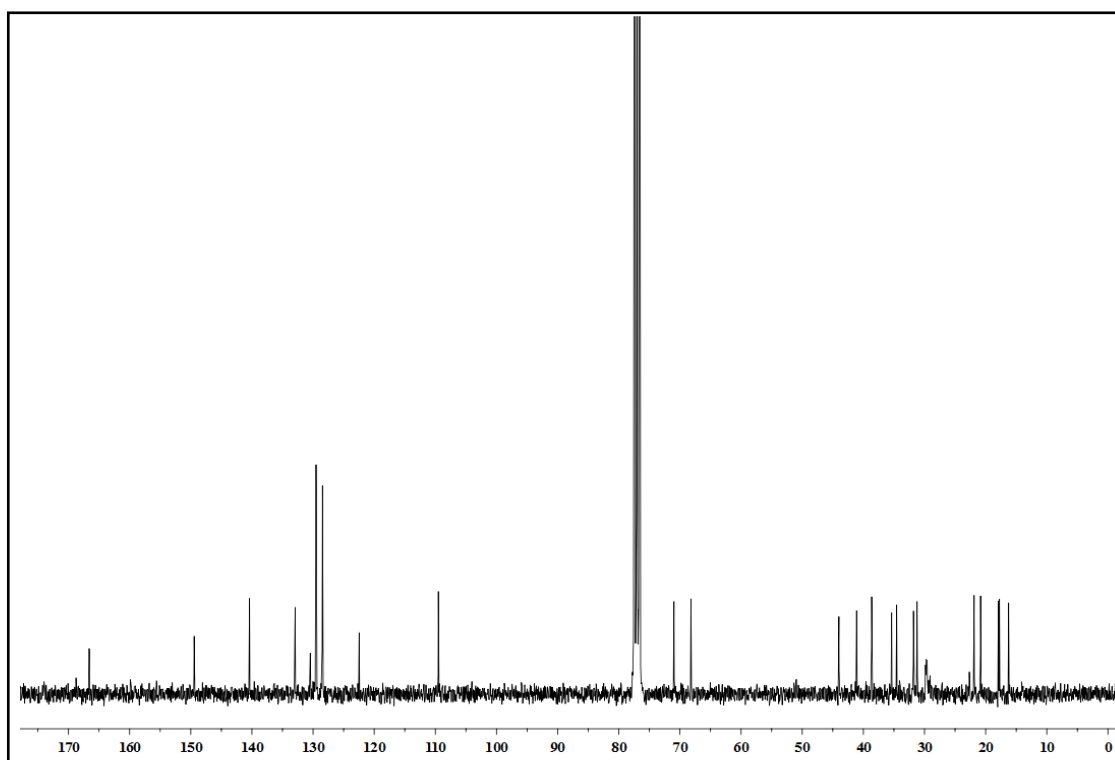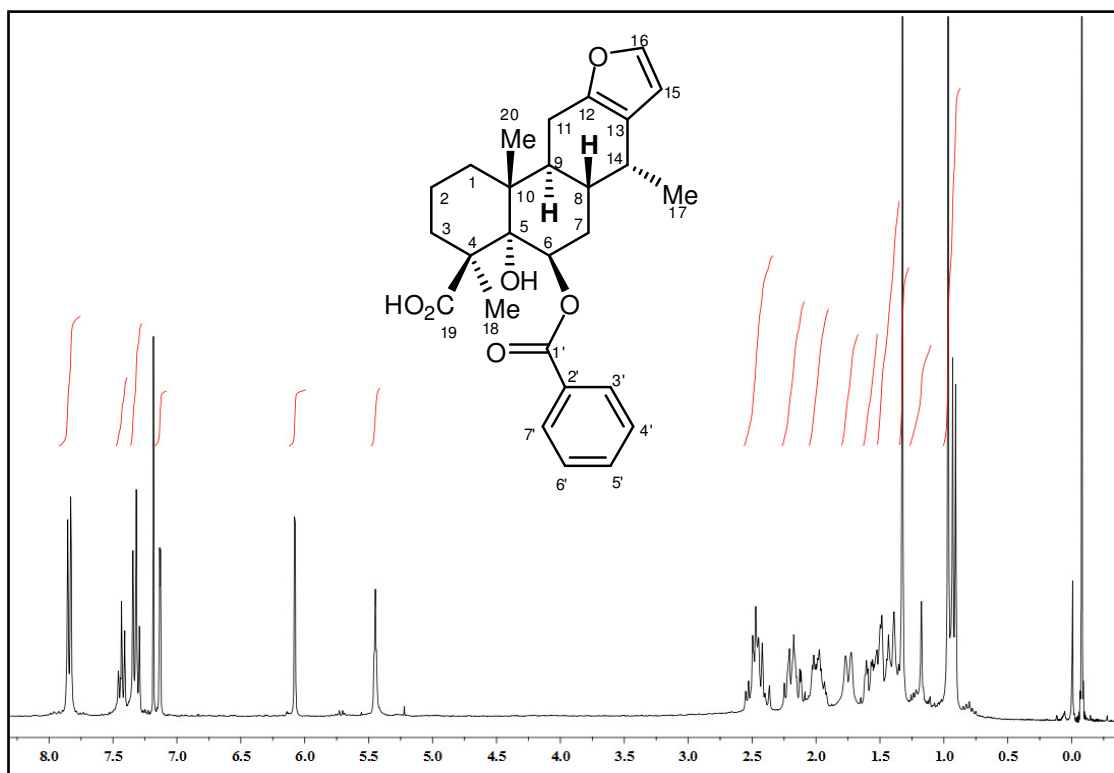**Figure 30** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CM8**
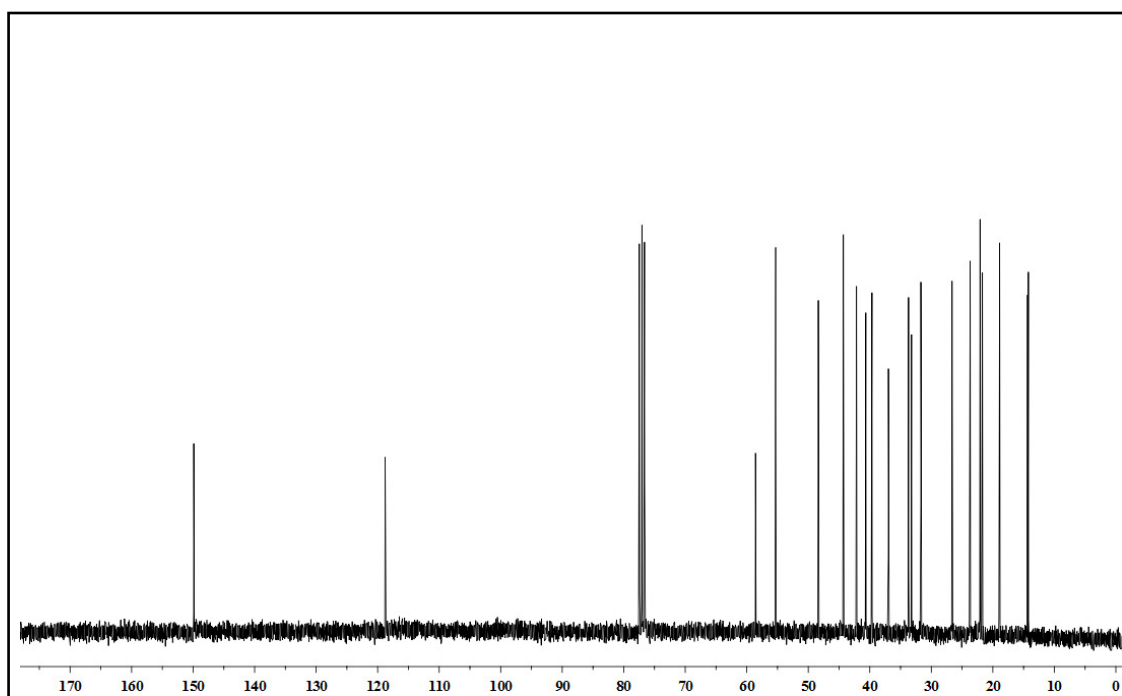


**Figure 31** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CM8**
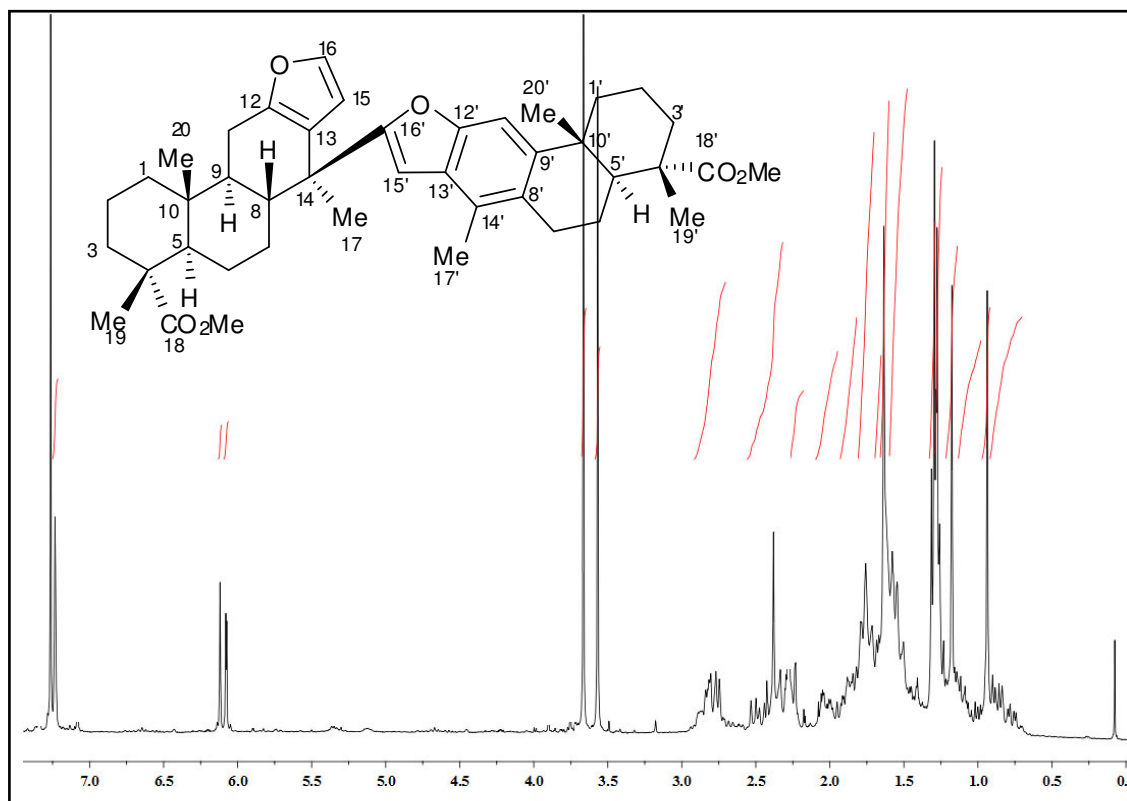
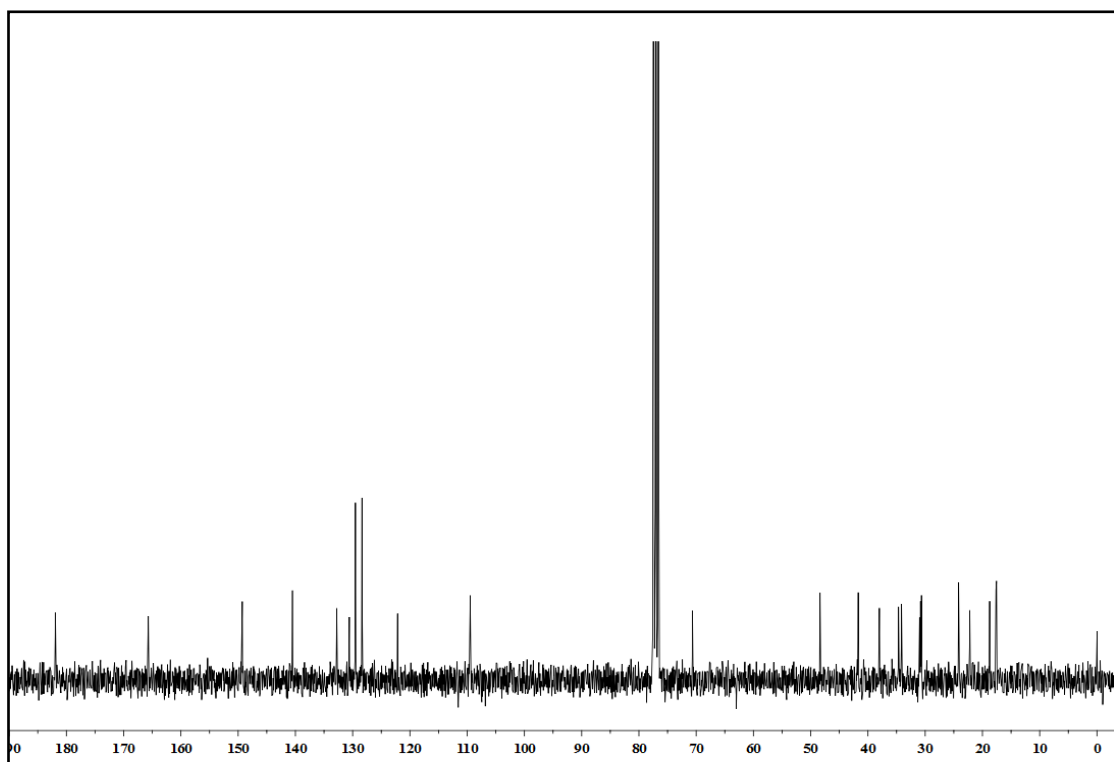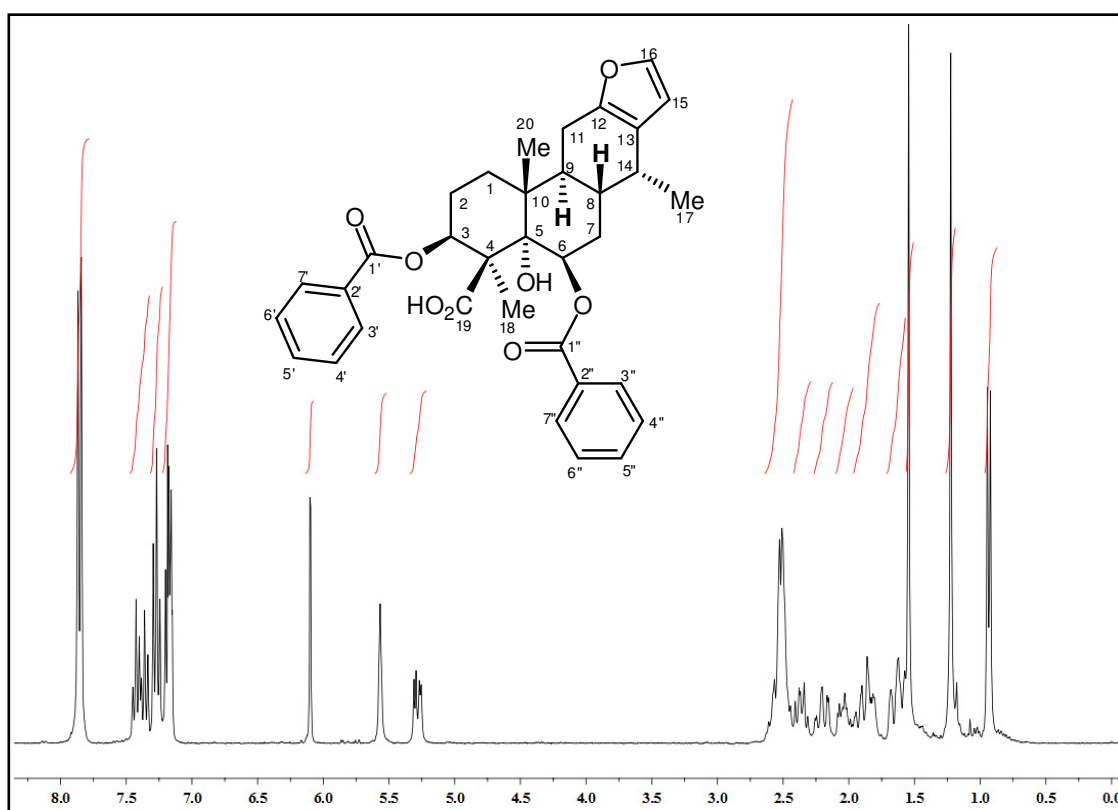**Figure 32**   $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CM9**
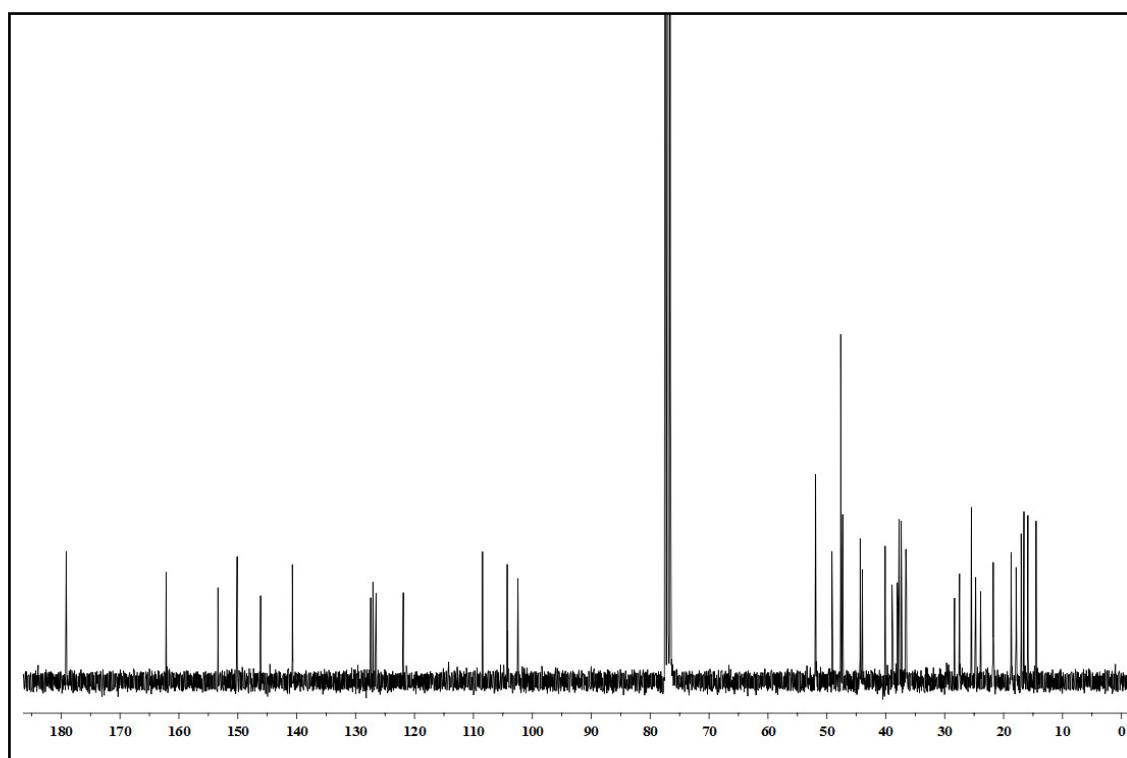


**Figure 33**   $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CM9**
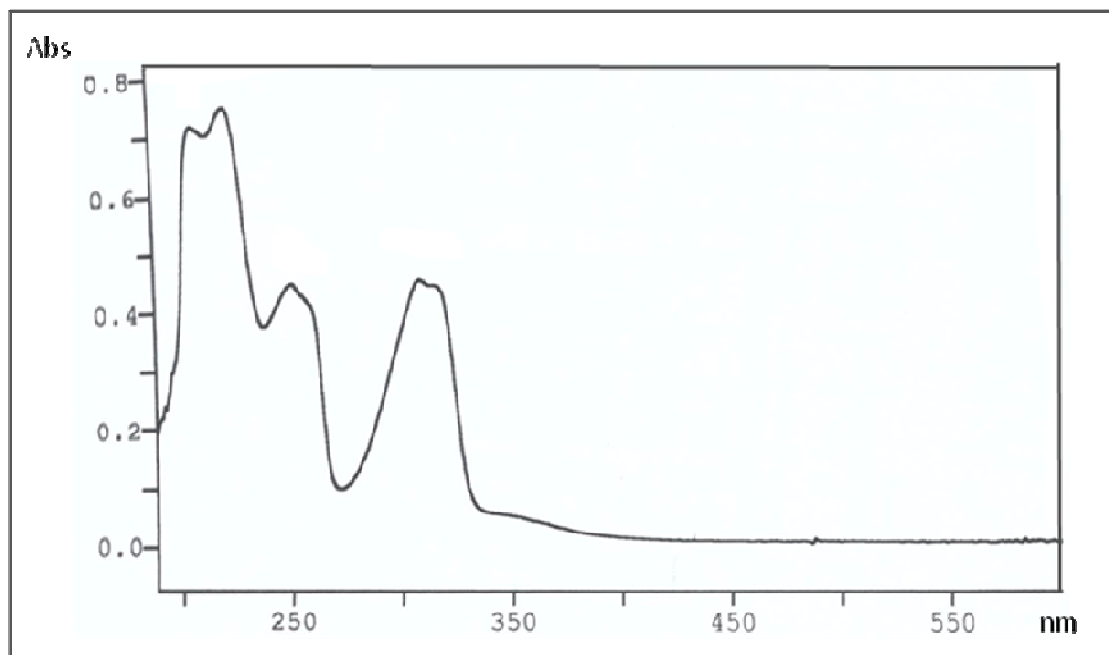
**Figure 34**  UV (MeOH) spectrum of compound **CM10**
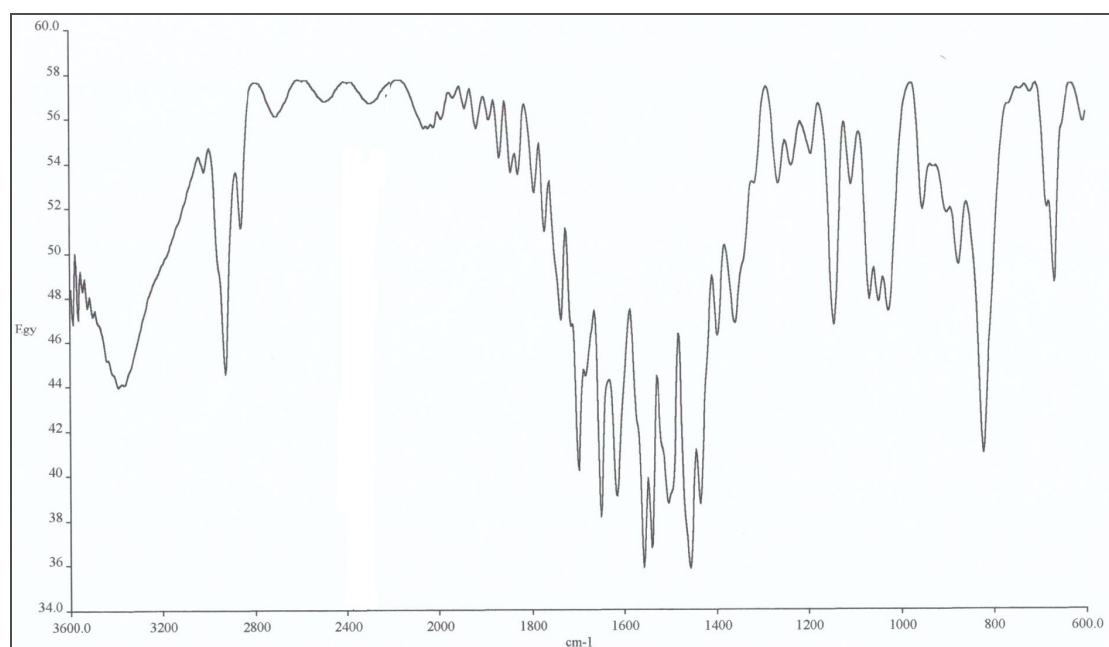


**Figure 35**  IR (neat) spectrum of compound **CM10**
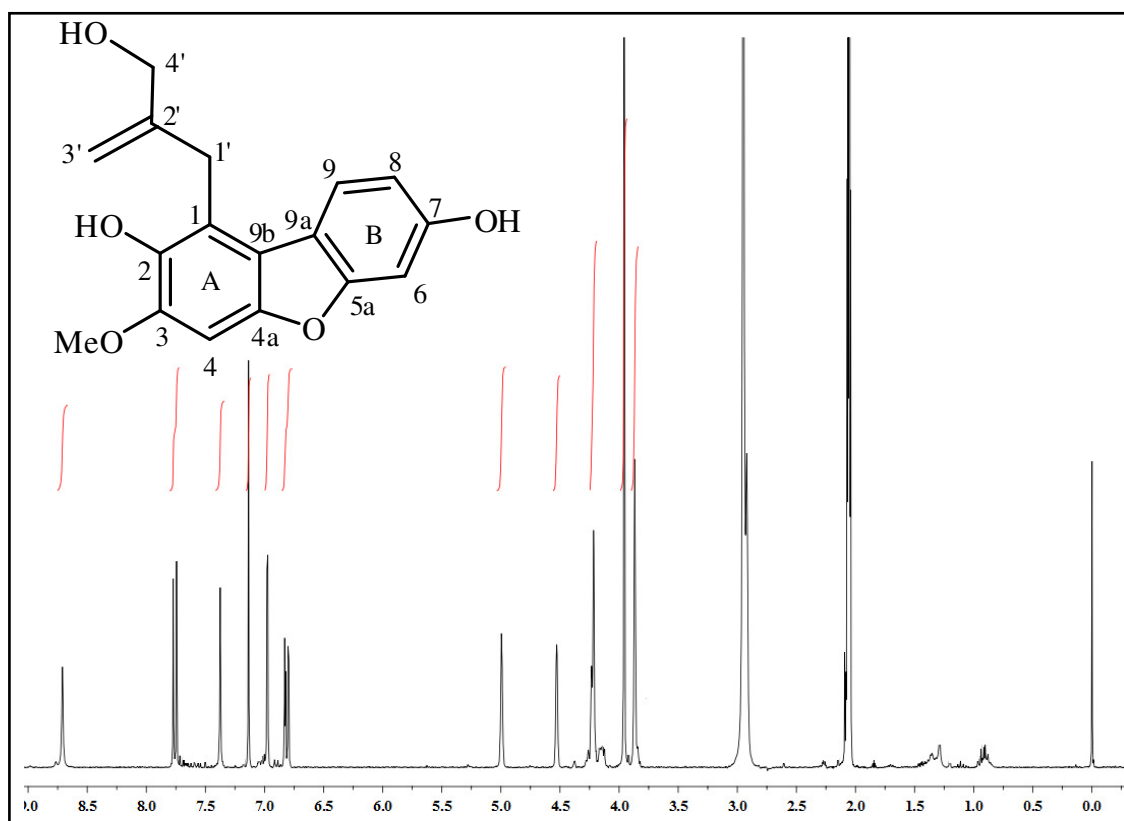
**Figure 36** $^1$H NMR (300 MHz) (acetone-$d_6$) spectrum of compound **CM10**
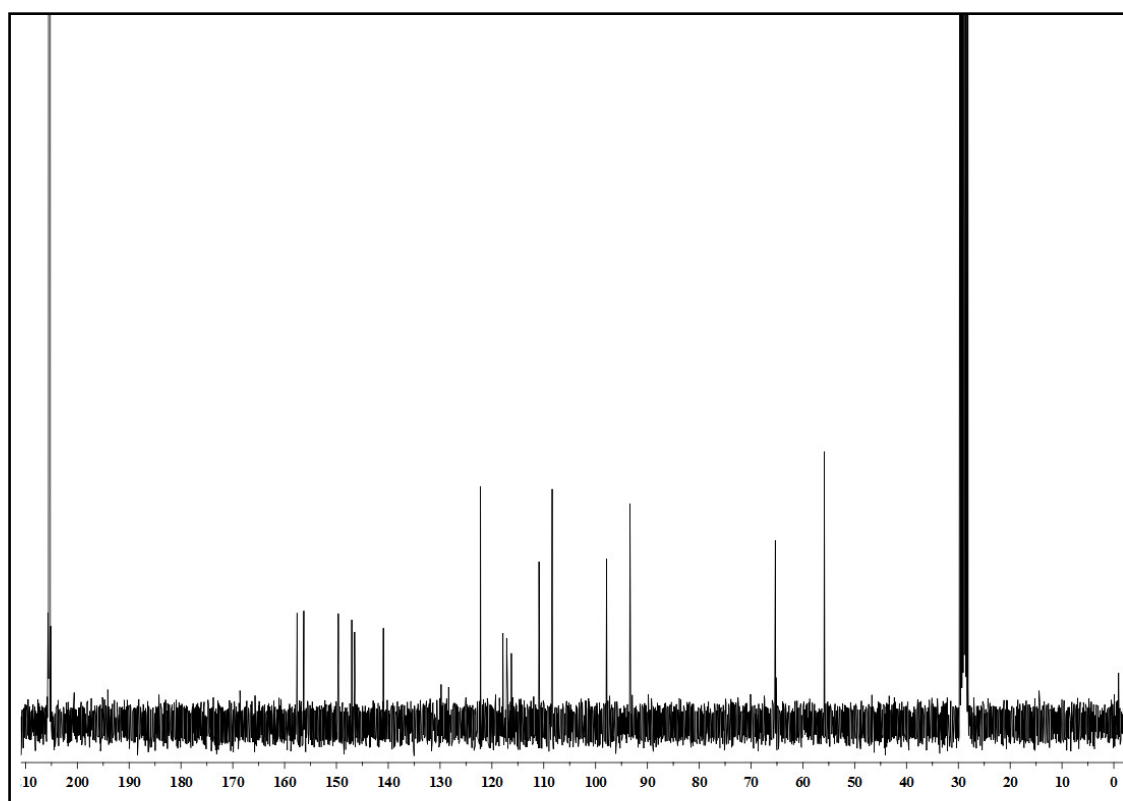


**Figure 37** $^{13}$C NMR (75 MHz) (acetone-$d_6$) spectrum of compound **CM10**
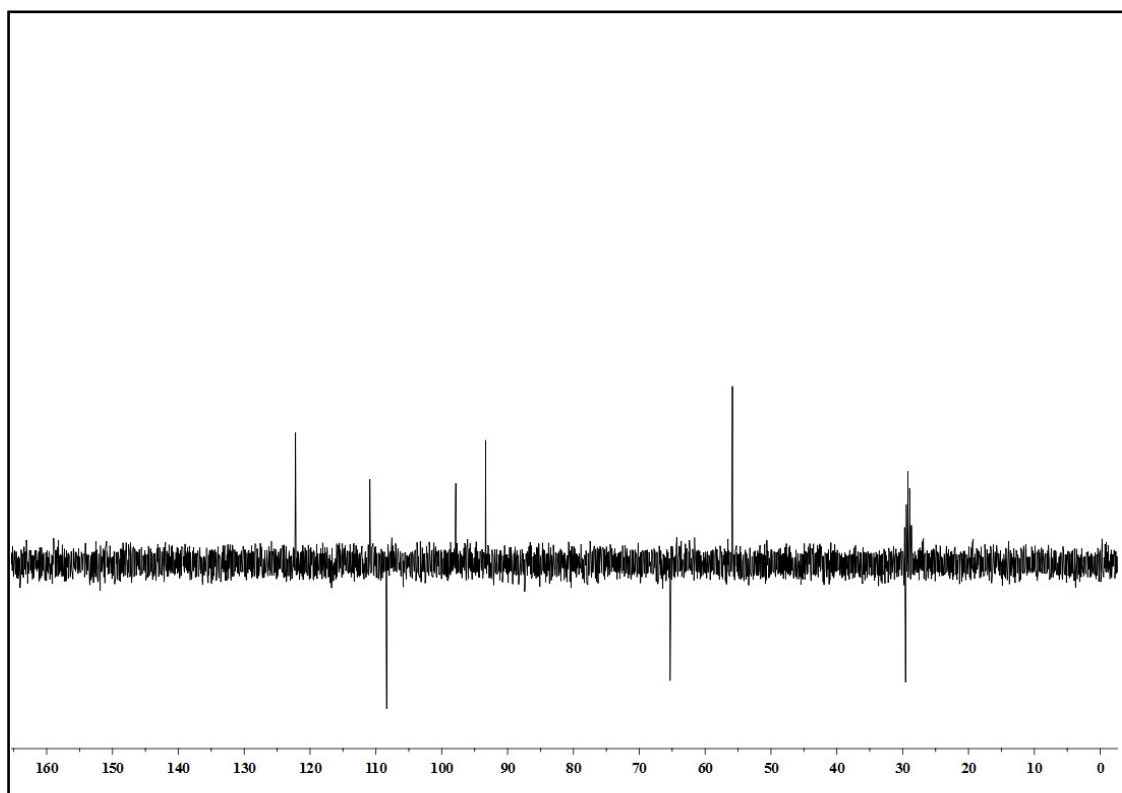
**Figure 38** DEPT 135˚ (acetone-$d_6$) spectrum of compound **CM10**
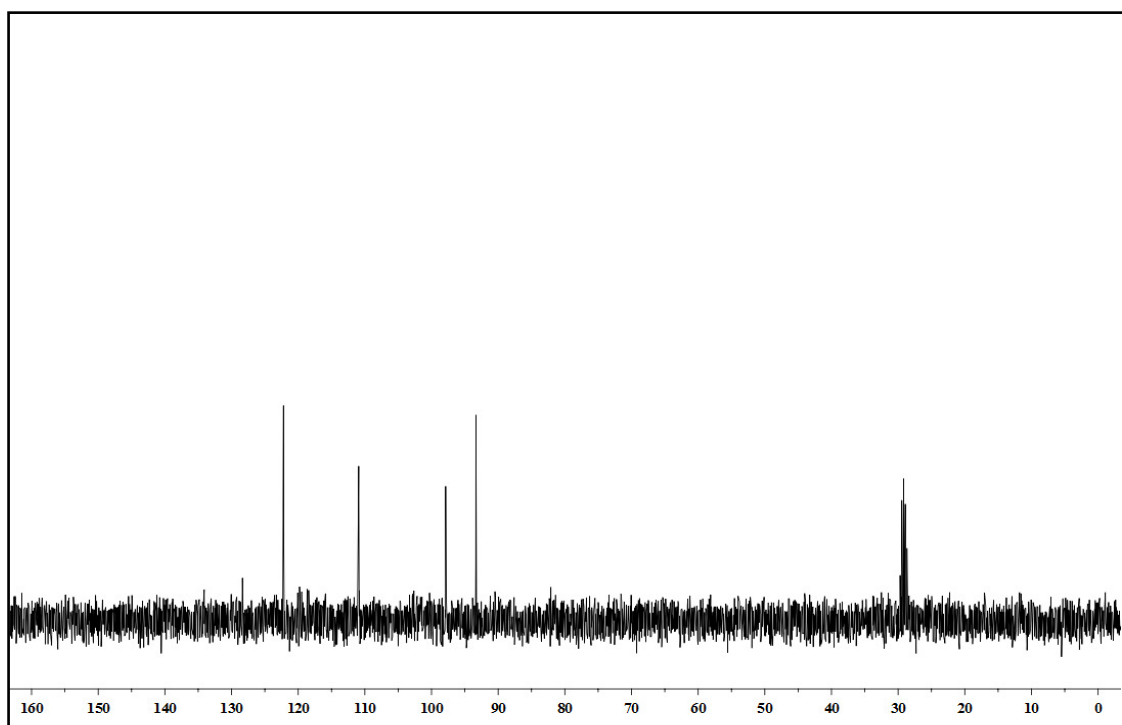


**Figure 39** DEPT 90˚ (acetone-$d_6$) spectrum of compound **CM10**
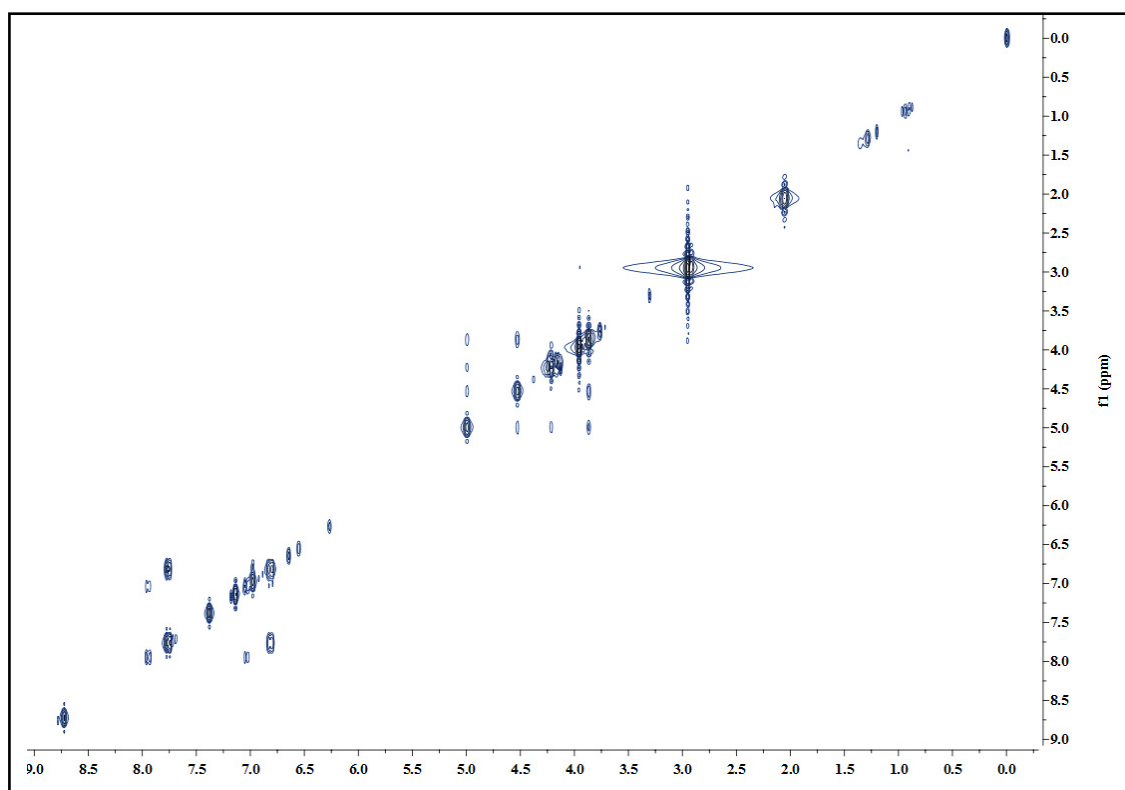
**Figure 40**    2D COSY (acetone-$d_6$) spectrum of compound **CM10**
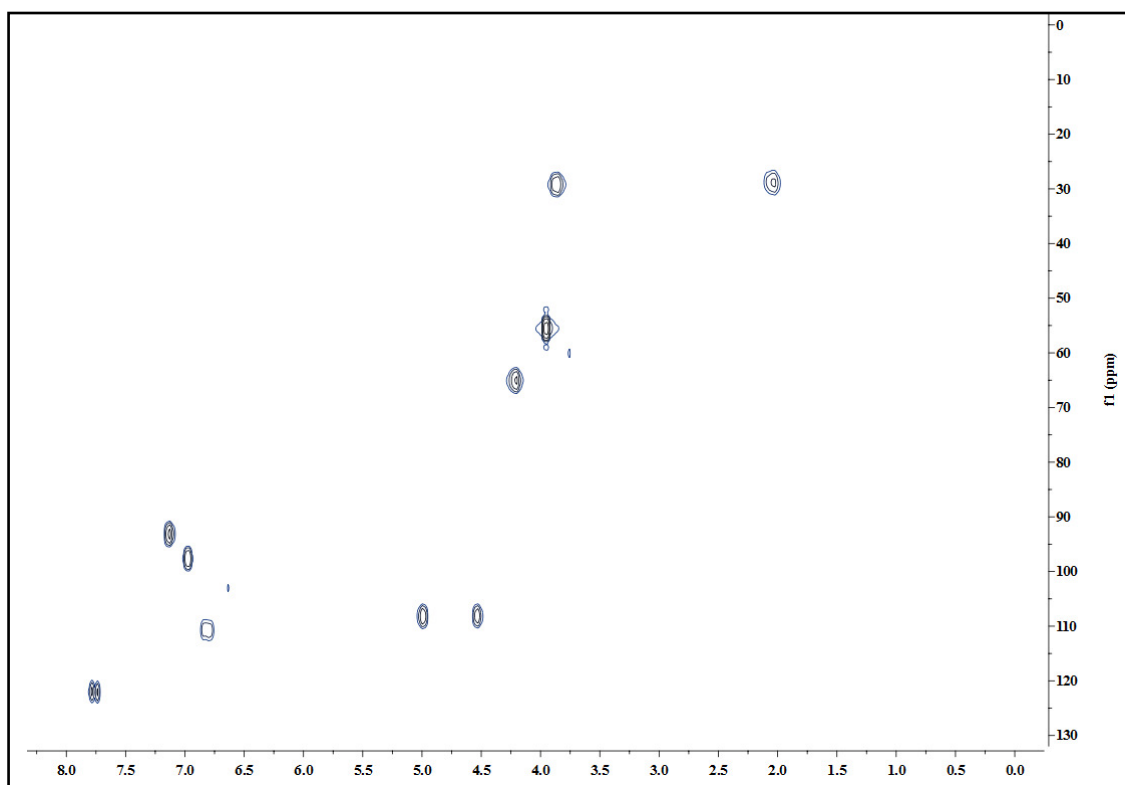


**Figure 41**   2D HMQC (acetone-$d_6$) spectrum of compound **CM10**
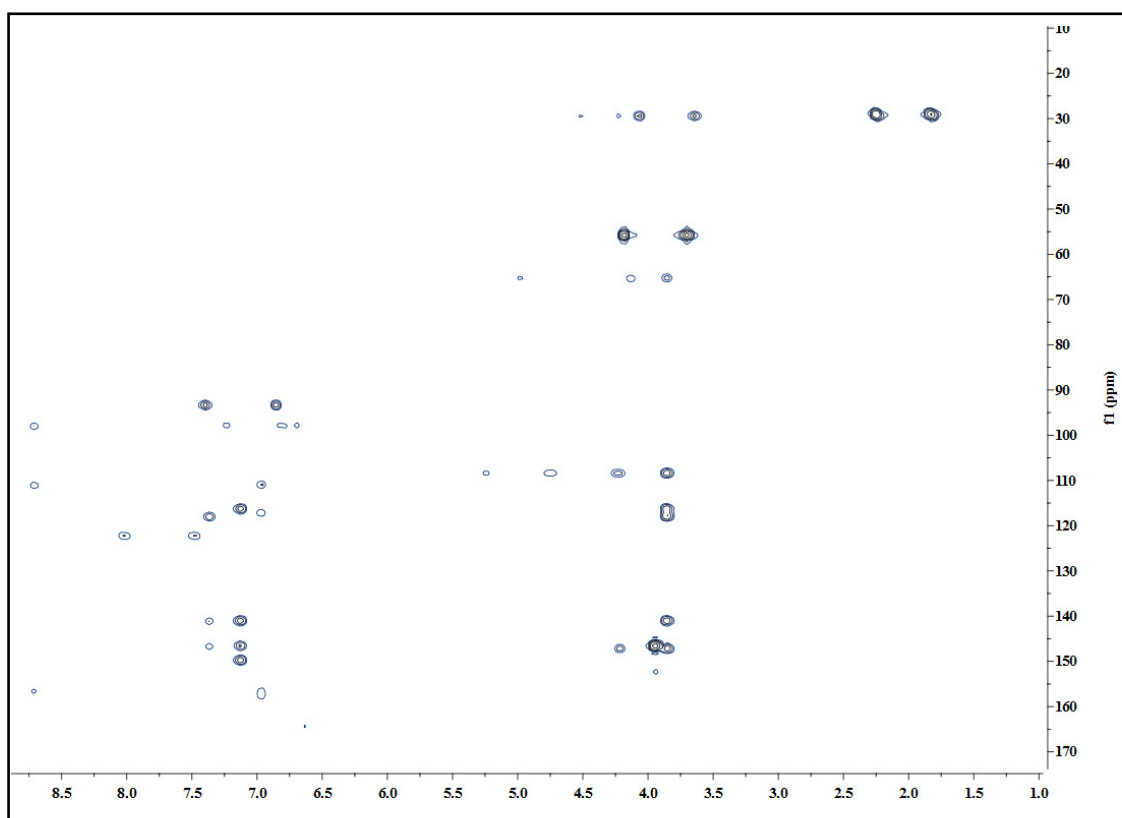
**Figure 42** 2D HMBC (acetone-*d*₆) spectrum of compound **CM10**
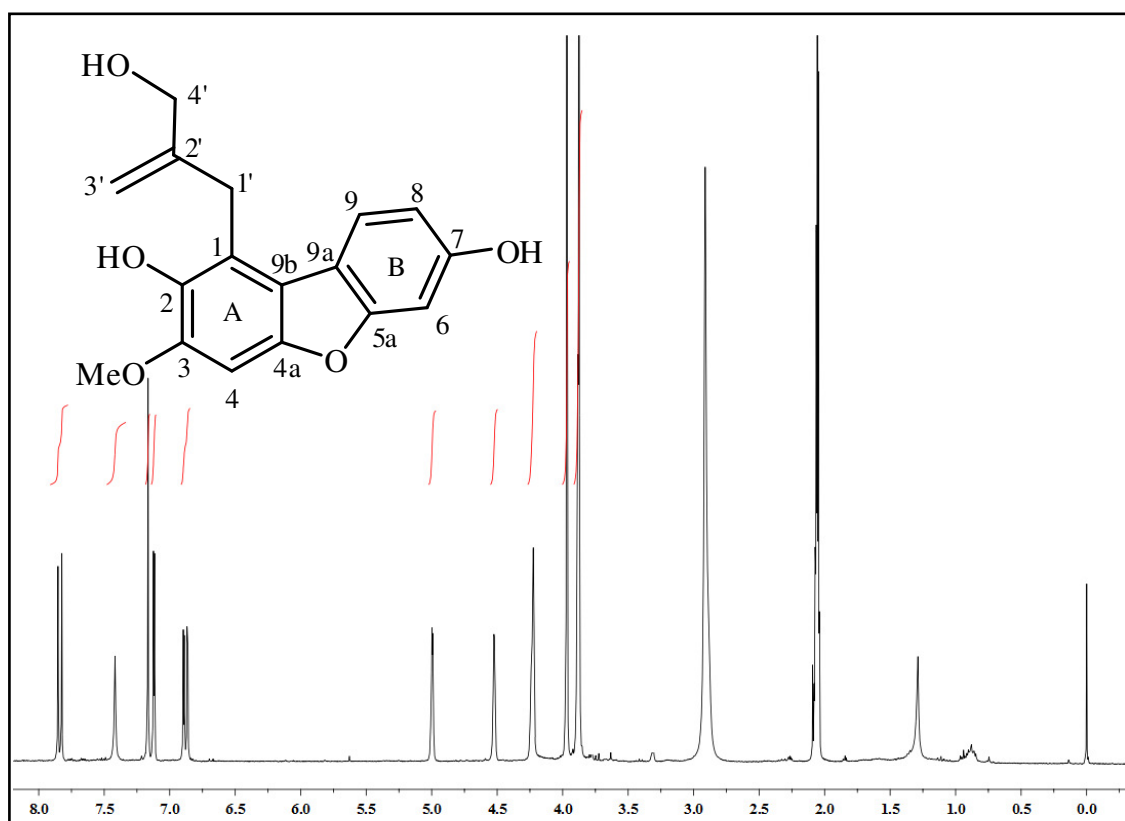
**Figure 43** $^1$H NMR (300 MHz) (acetone-$d_6$) spectrum of compound **CM11**
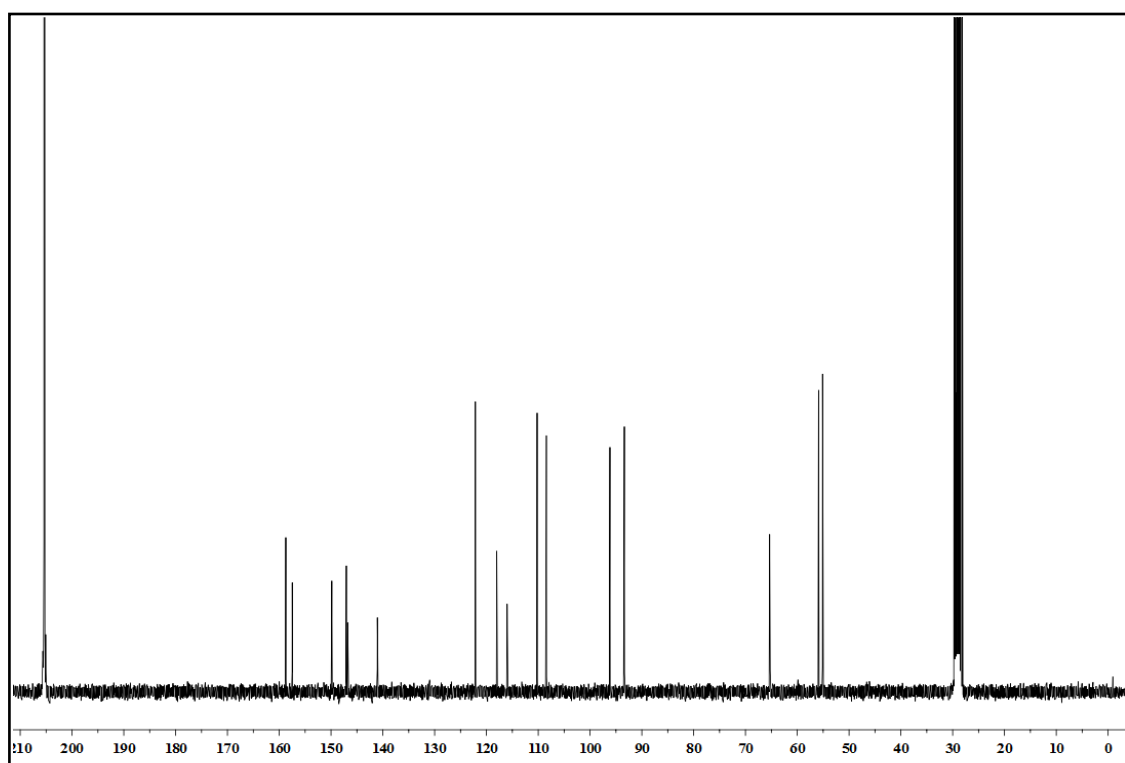


**Figure 44** $^{13}$C NMR (75 MHz) (acetone-$d_6$) spectrum of compound **CM11**
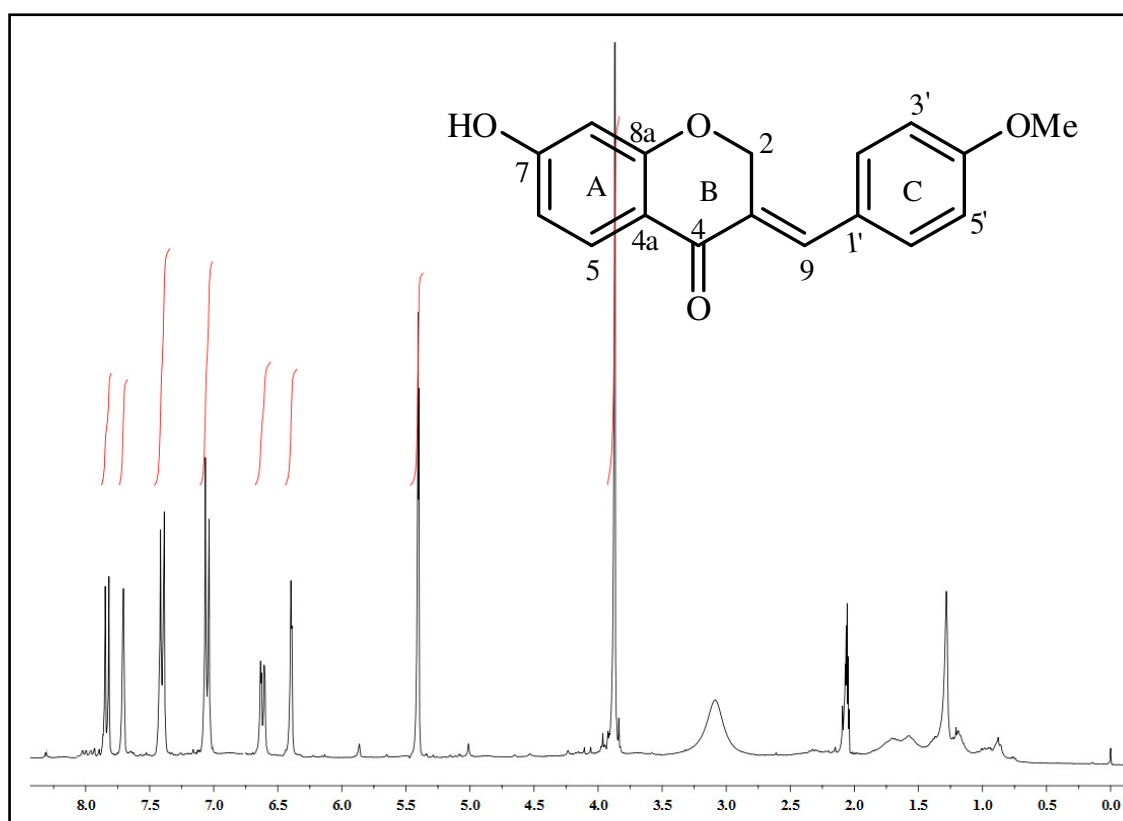
**Figure 45**  $^1$H NMR (300 MHz) (acetone-$d_6$) spectrum of compound **CM12**
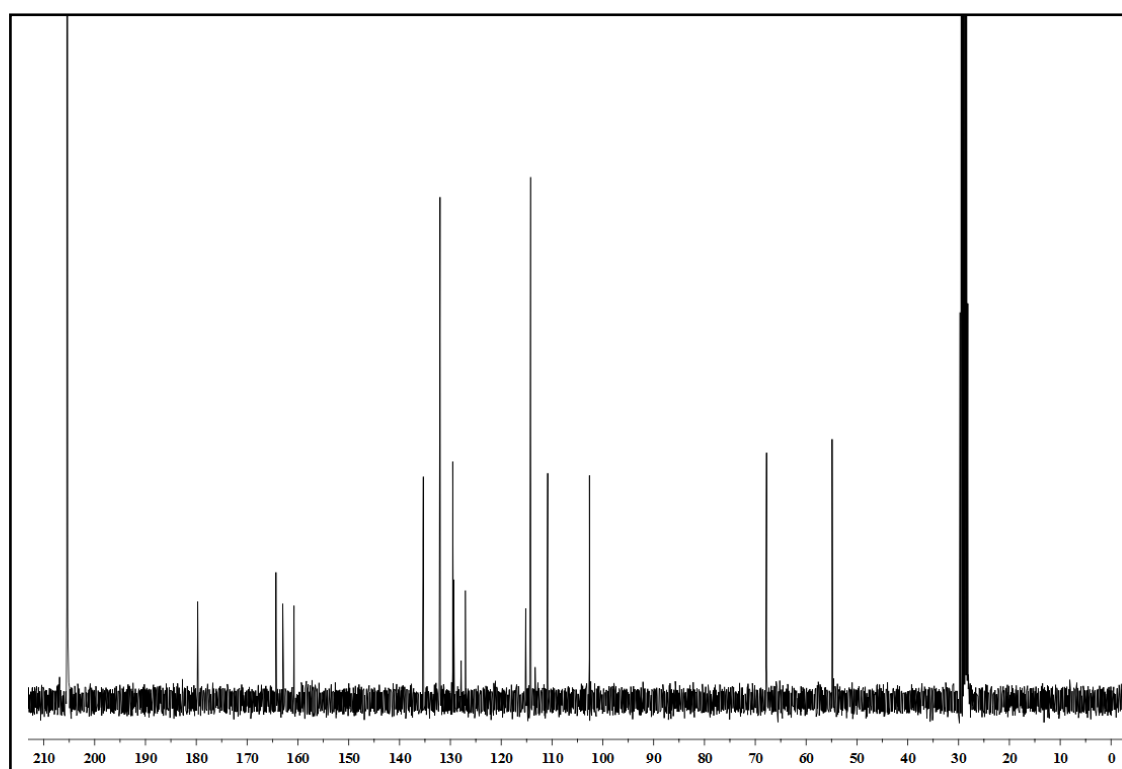


**Figure 46**  $^{13}$C NMR (75 MHz) (acetone-$d_6$) spectrum of compound **CM12**
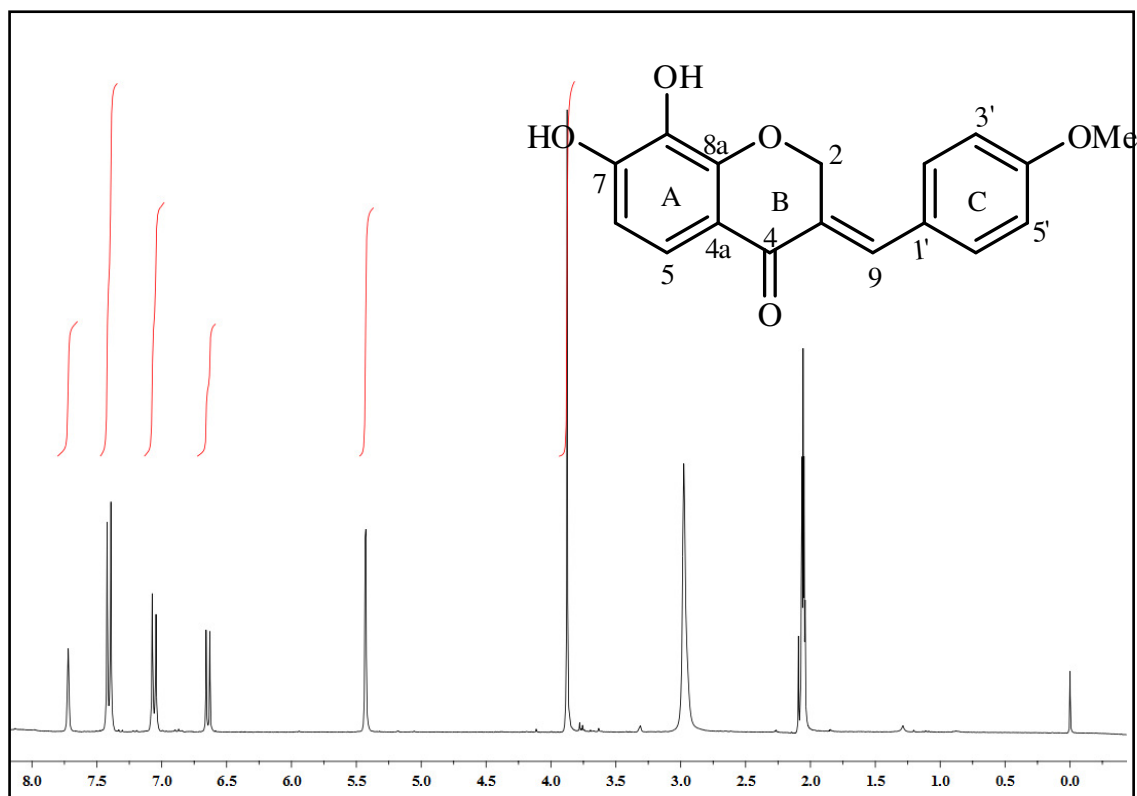
**Figure 47** $^1$H NMR (300 MHz) (acetone-$d_6$) spectrum of compound **CM13**
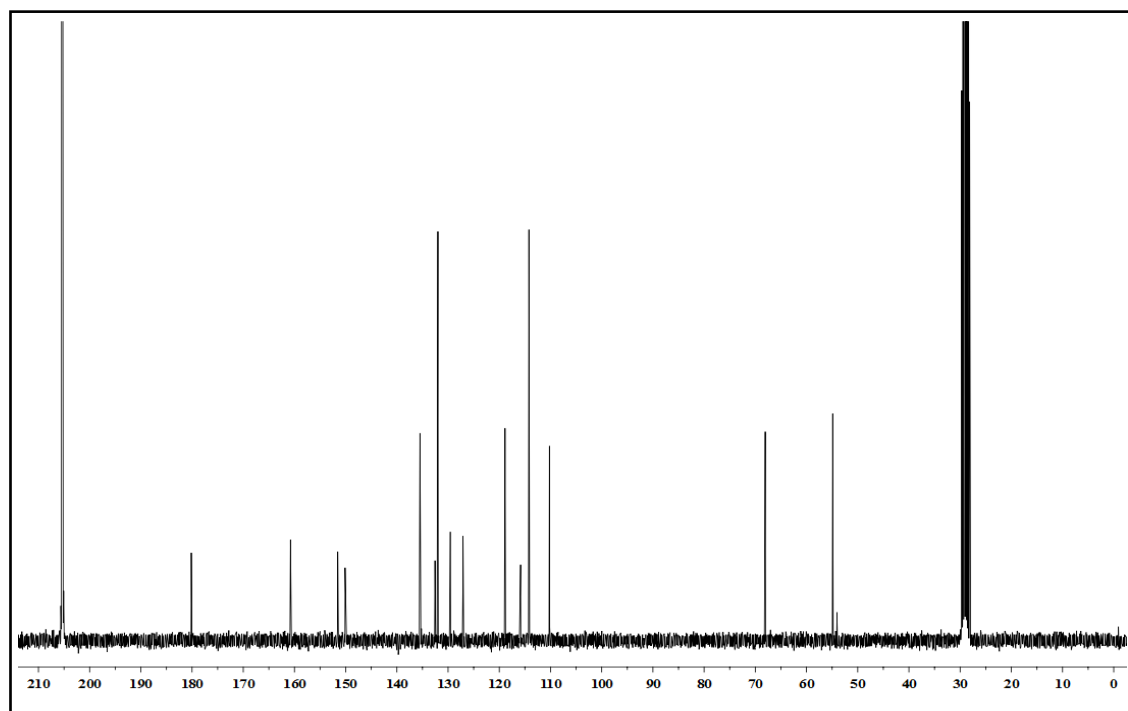


**Figure 48** $^{13}$C NMR (300 MHz) (acetone-$d_6$) spectrum of compound **CM13**
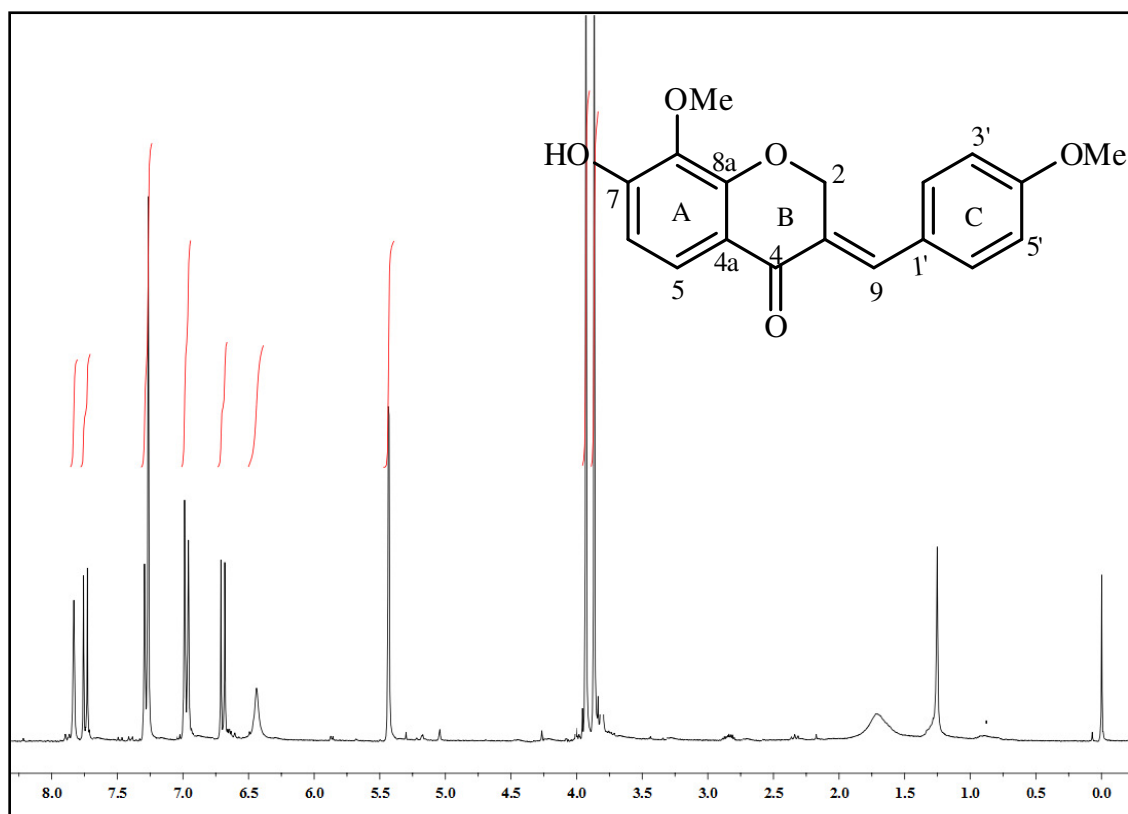
**Figure 49** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CM14**
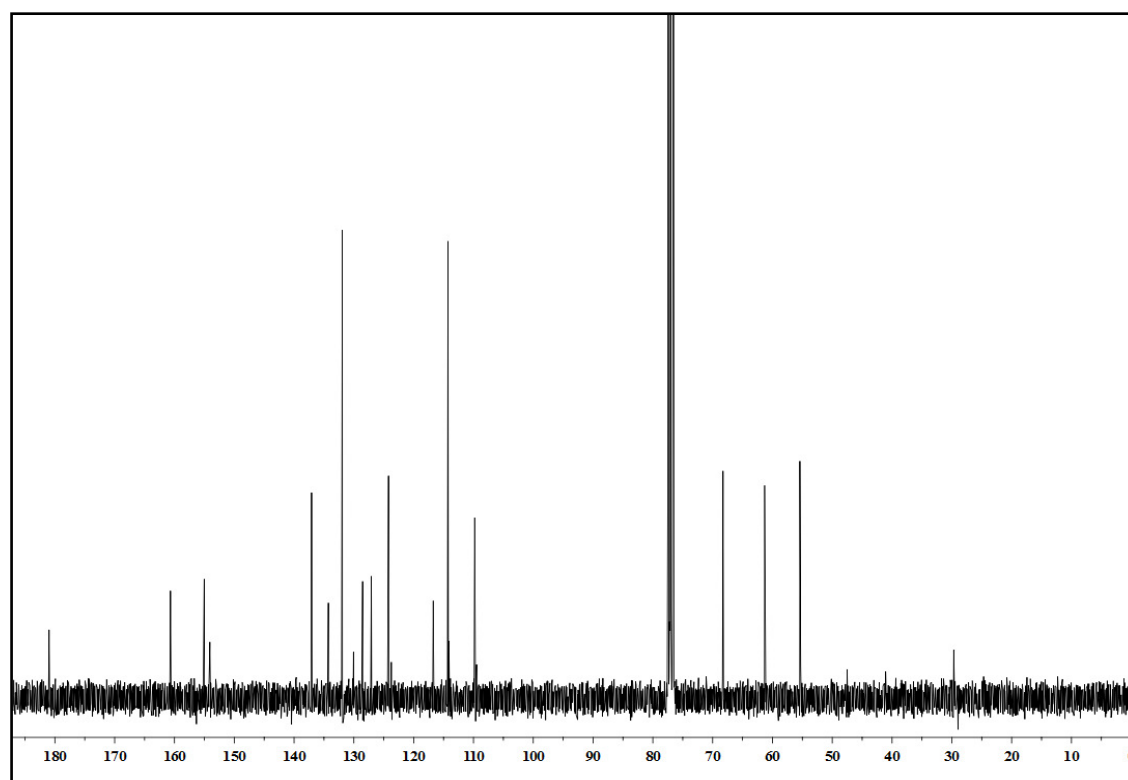


**Figure 50** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CM14**
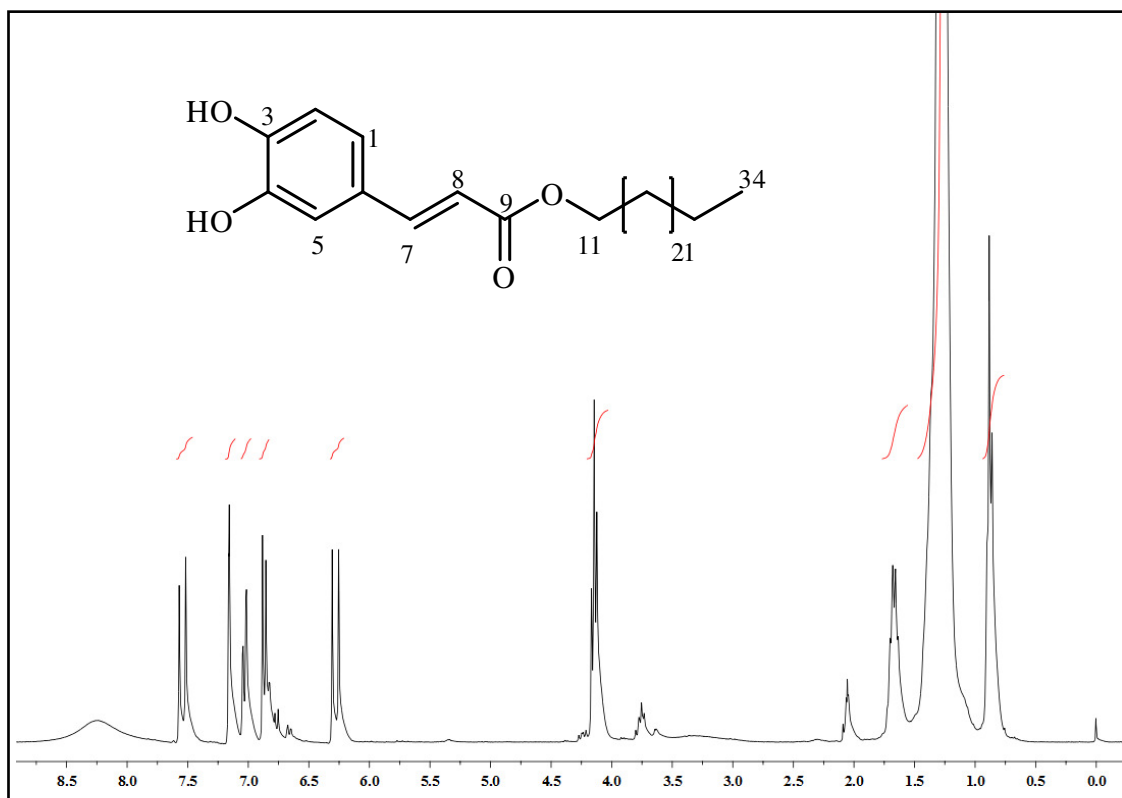
**Figure 51** ¹H NMR (300 MHz) (acetone-$d_6$) spectrum of compound **CM15**
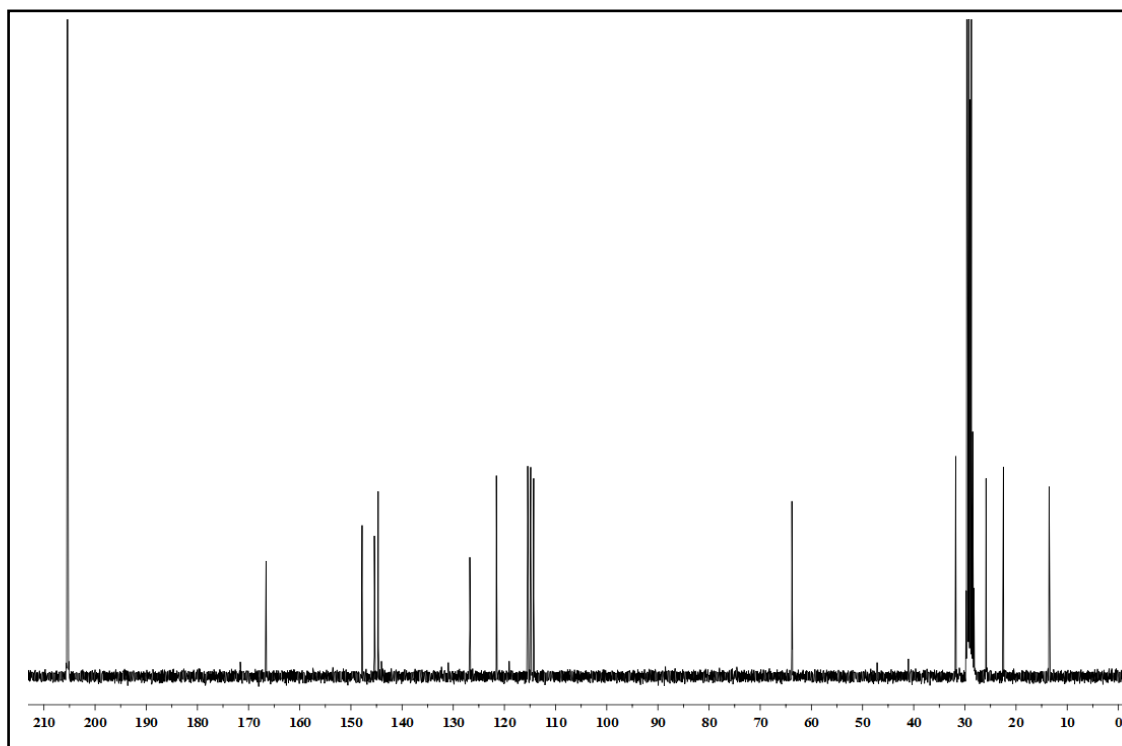


**Figure 52** ¹³C NMR (75 MHz) (acetone-$d_6$) spectrum of compound **CM15**
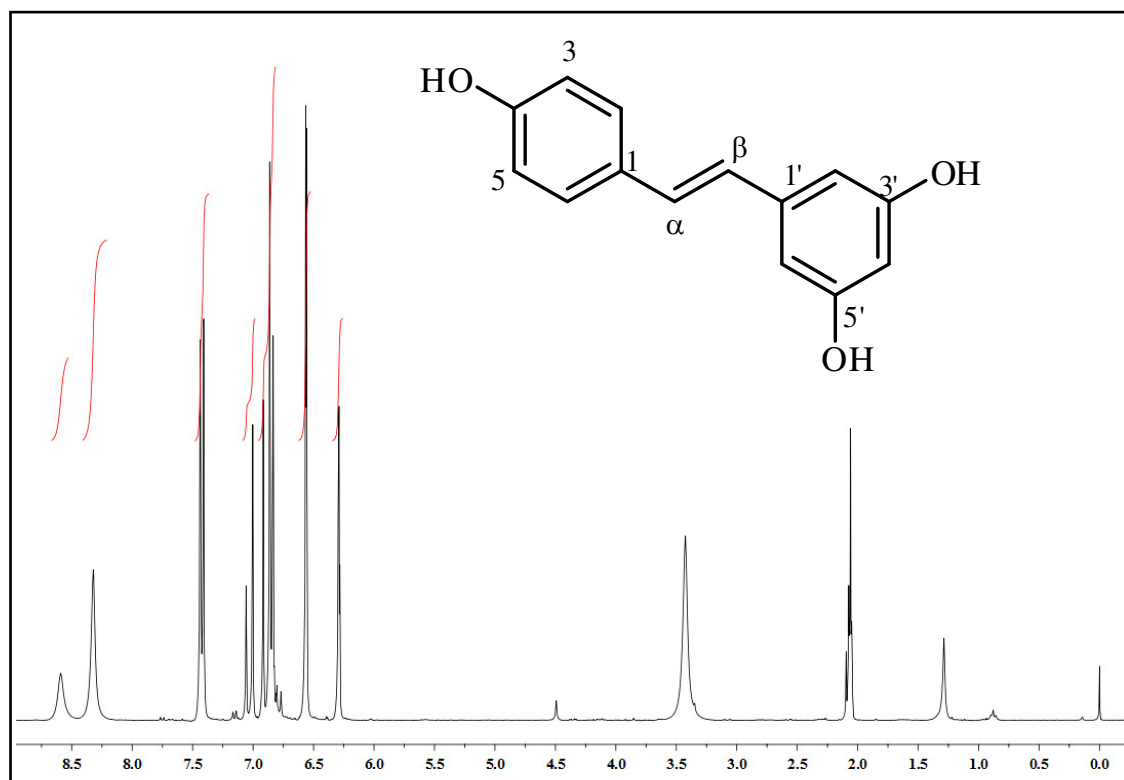
**Figure 53** $^1$H NMR (300 MHz) (acetone-$d_6$) spectrum of compound **CM16**
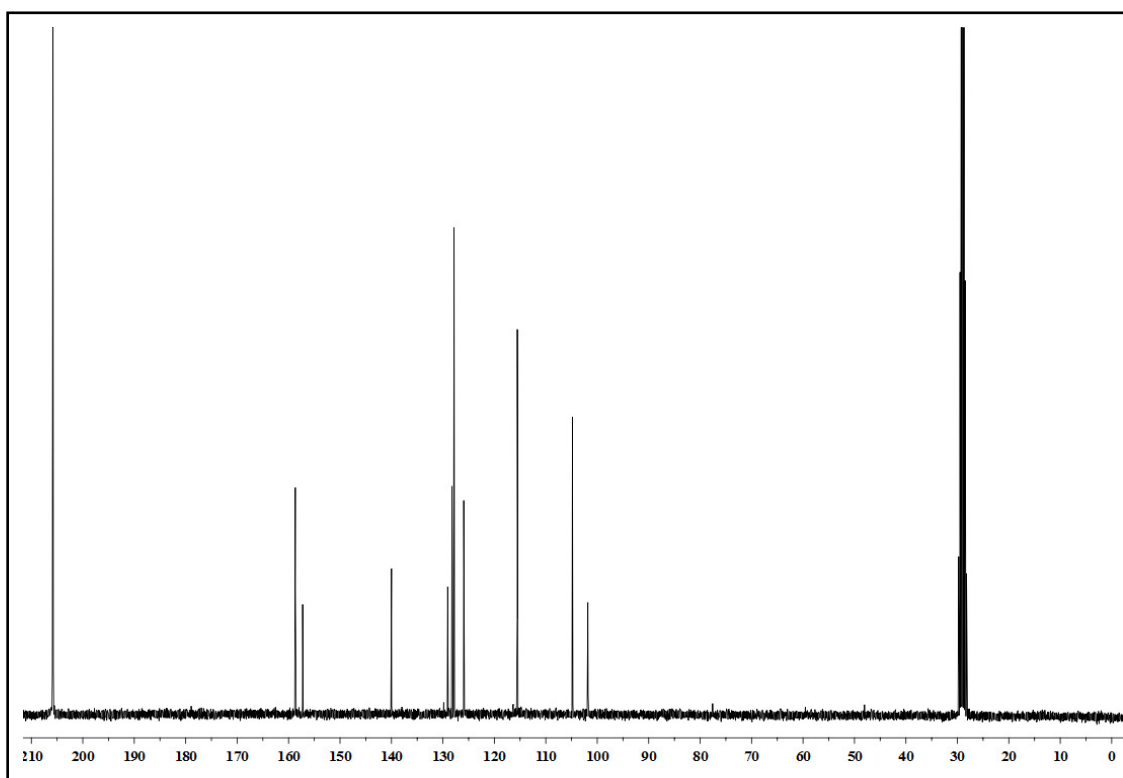


**Figure 54** $^{13}$C NMR (300 MHz) (acetone-$d_6$) spectrum of compound **CM16**
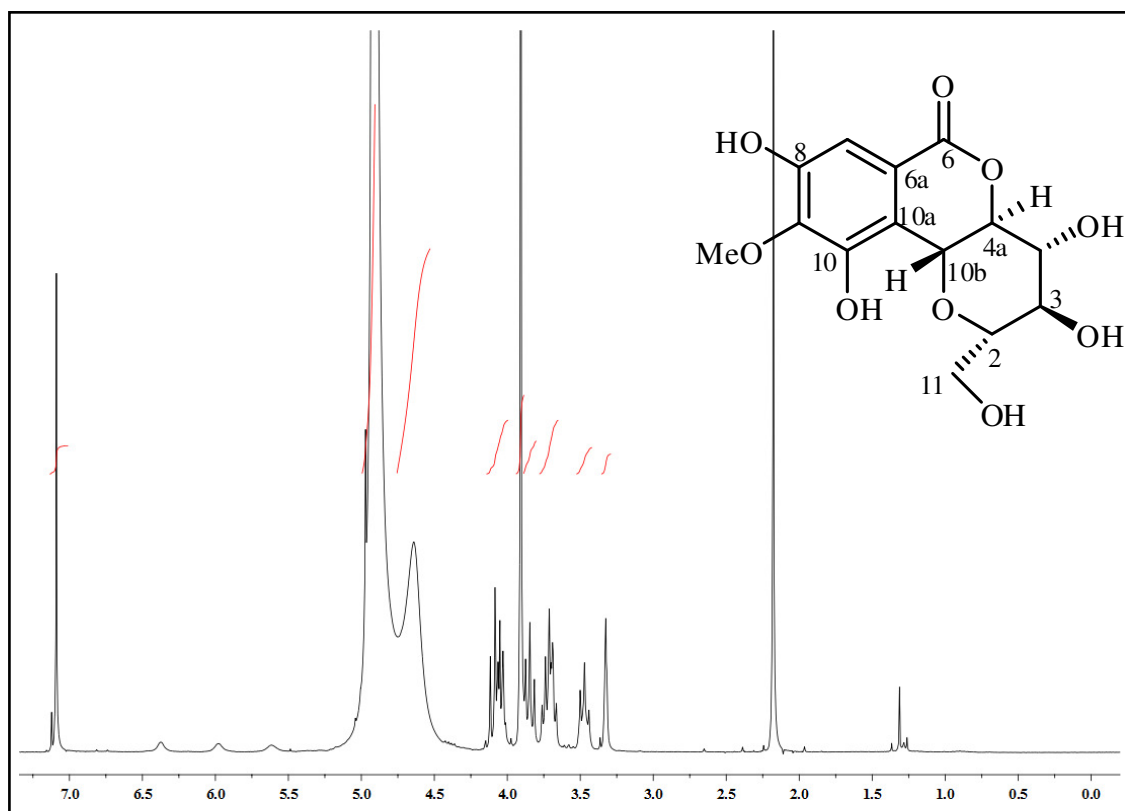
**Figure 55** $^1$H NMR (300 MHz) (CD$_3$OD) spectrum of compound **CM17**
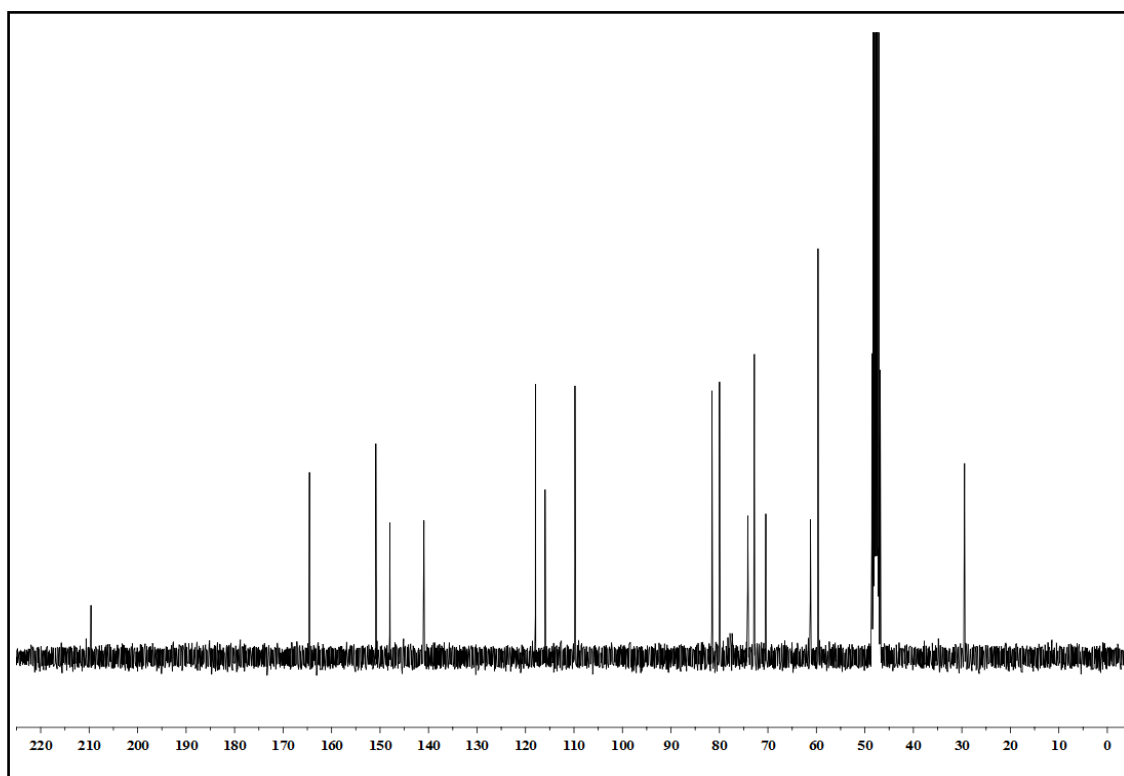


**Figure 56** $^{13}$C NMR (75 MHz) (CD$_3$OD) spectrum of compound **CM17**
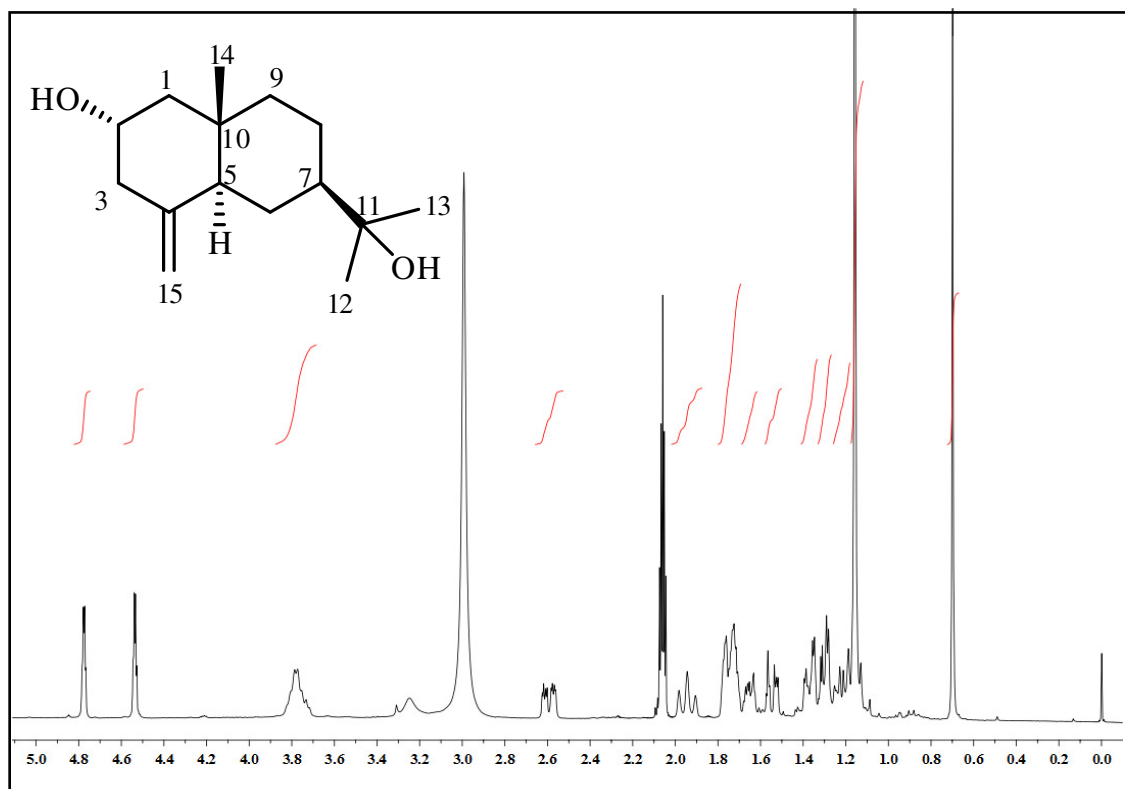
**Figure 57** $^1$H NMR (300 MHz) (acetone-$d_6$) spectrum of compound **CM18**
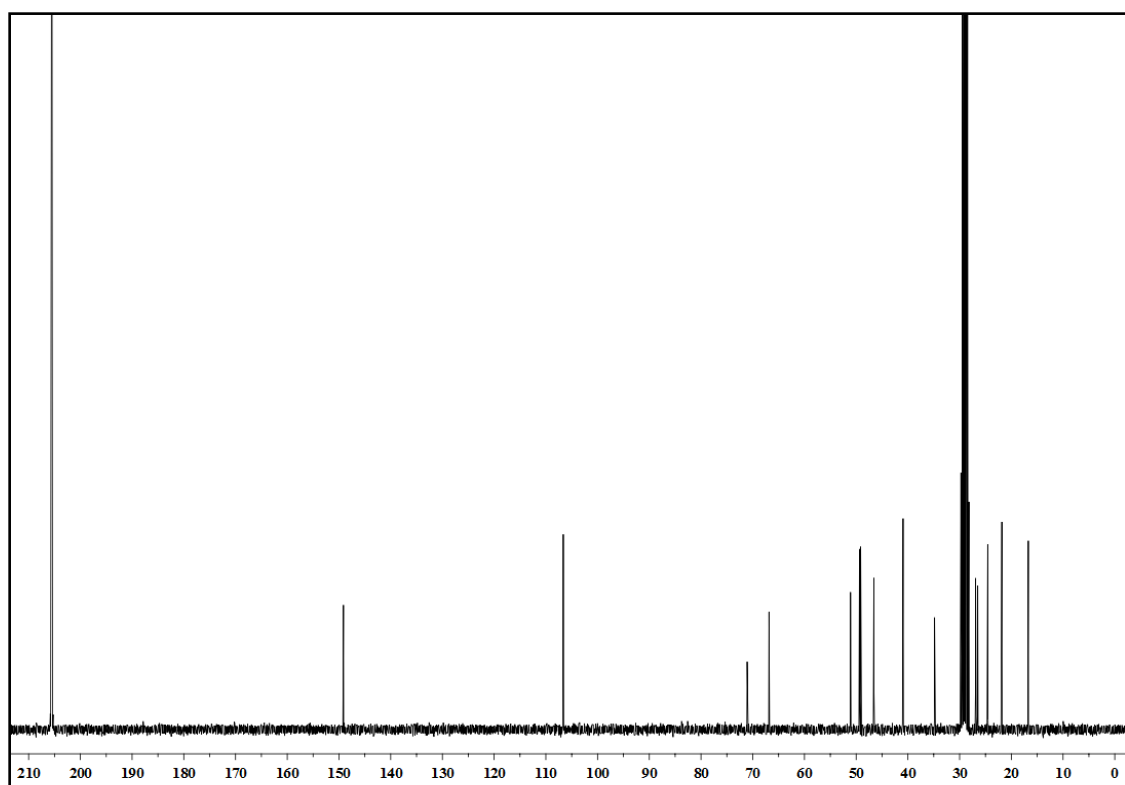


**Figure 58** $^{13}$C NMR (75 MHz) (acetone-$d_6$) spectrum of compound **CM18**

217
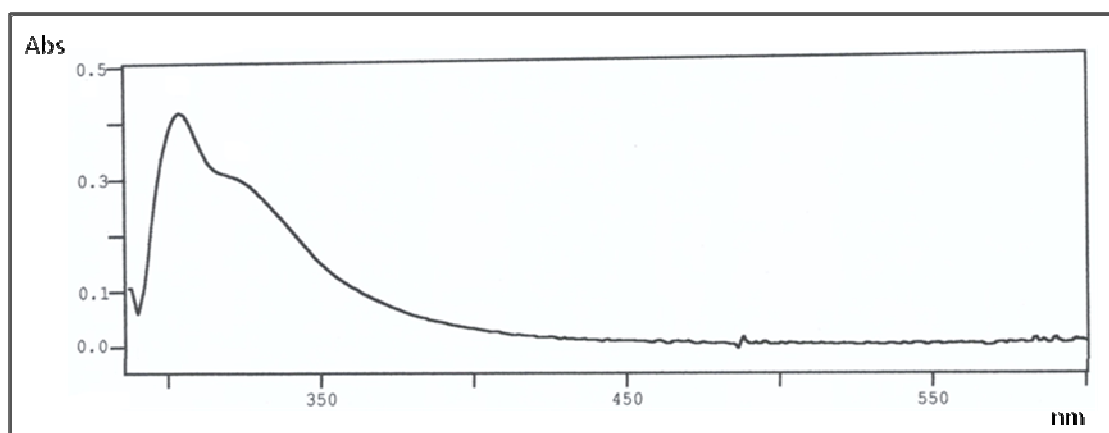


**Figure 59** UV (CHCl$_3$) spectrum of compound **CP1**
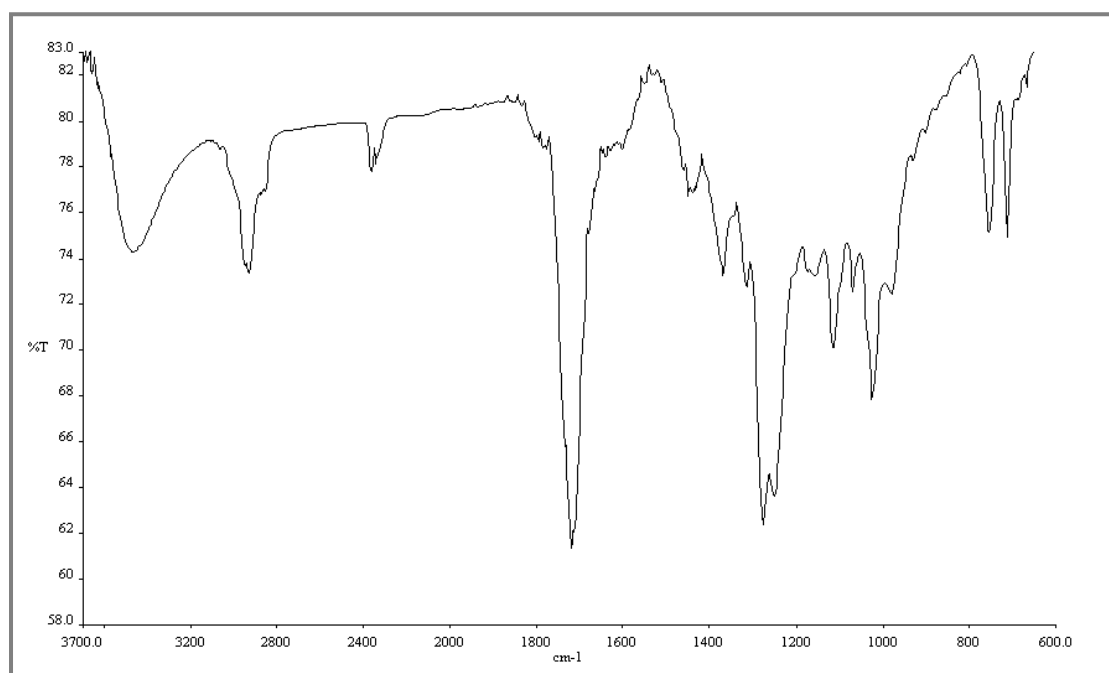


**Figure 60** IR (neat) spectrum of compound **CP1**
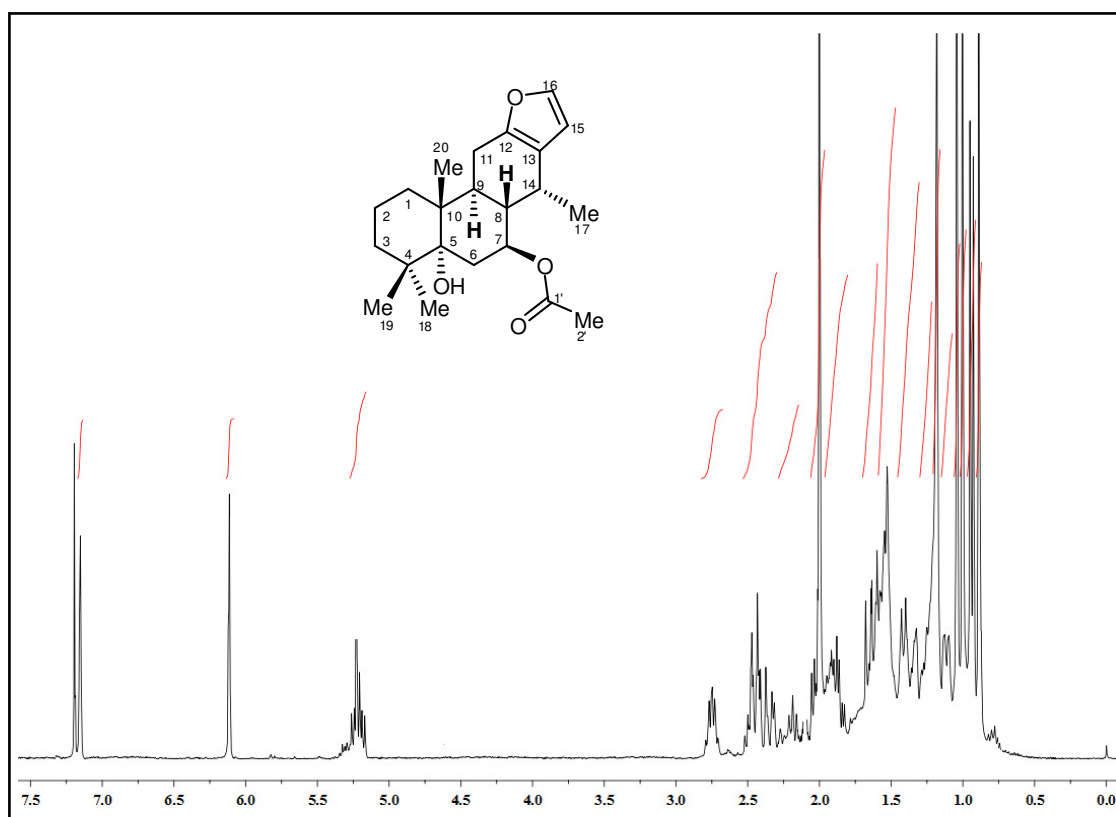
**Figure 61** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP1**
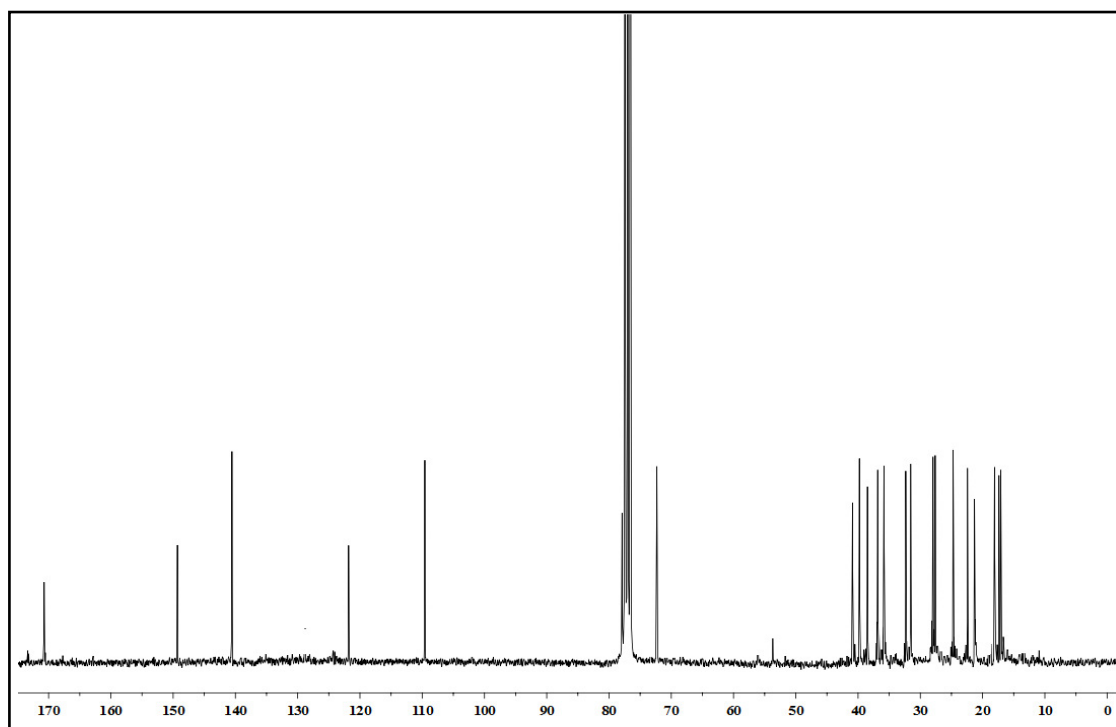


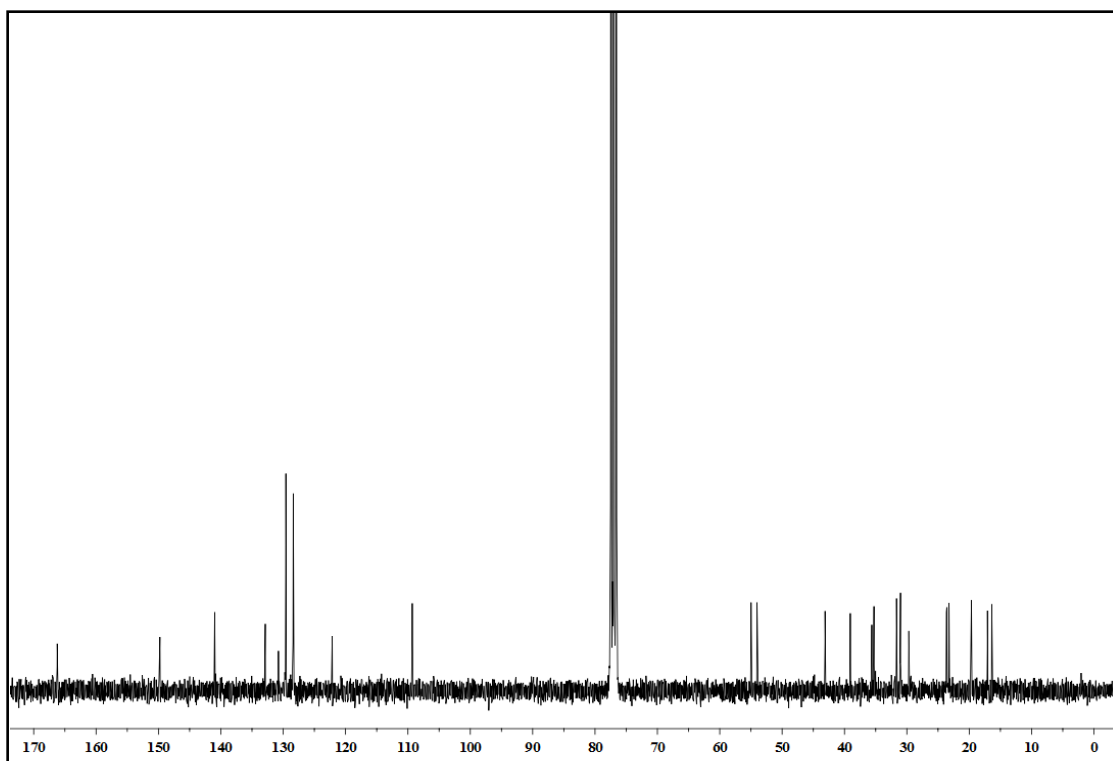**Figure 62** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP1**
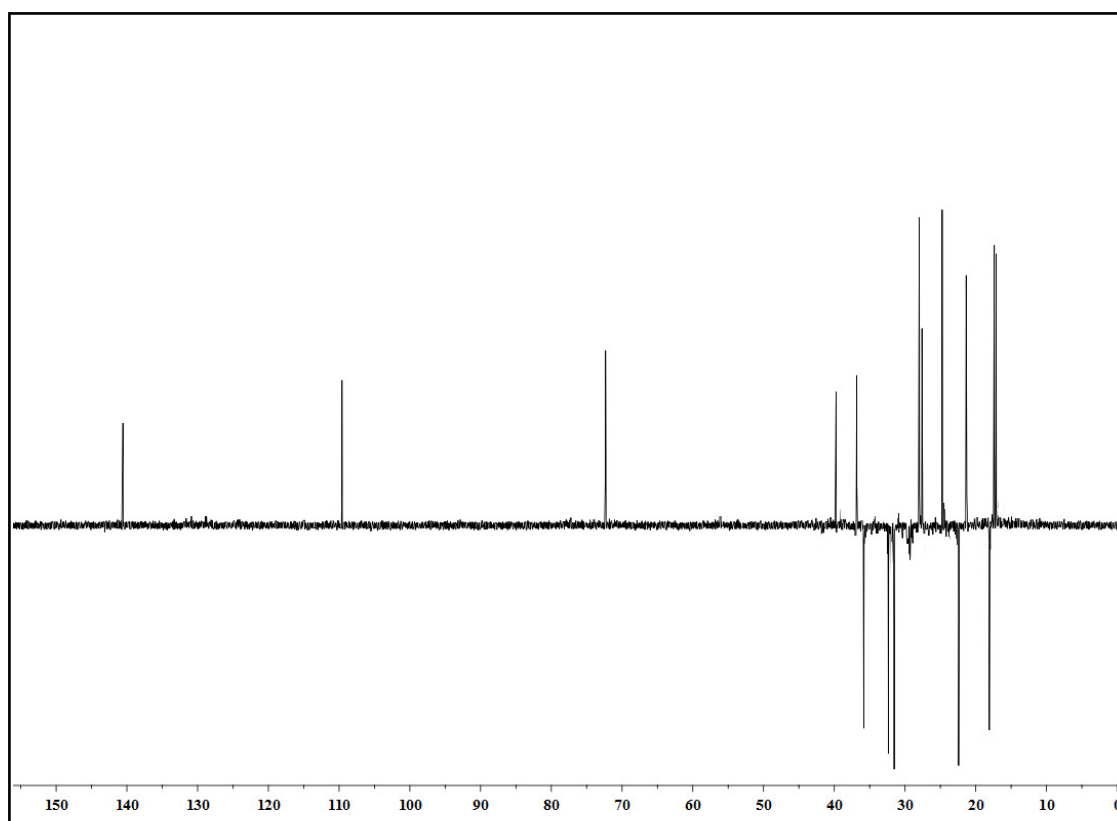
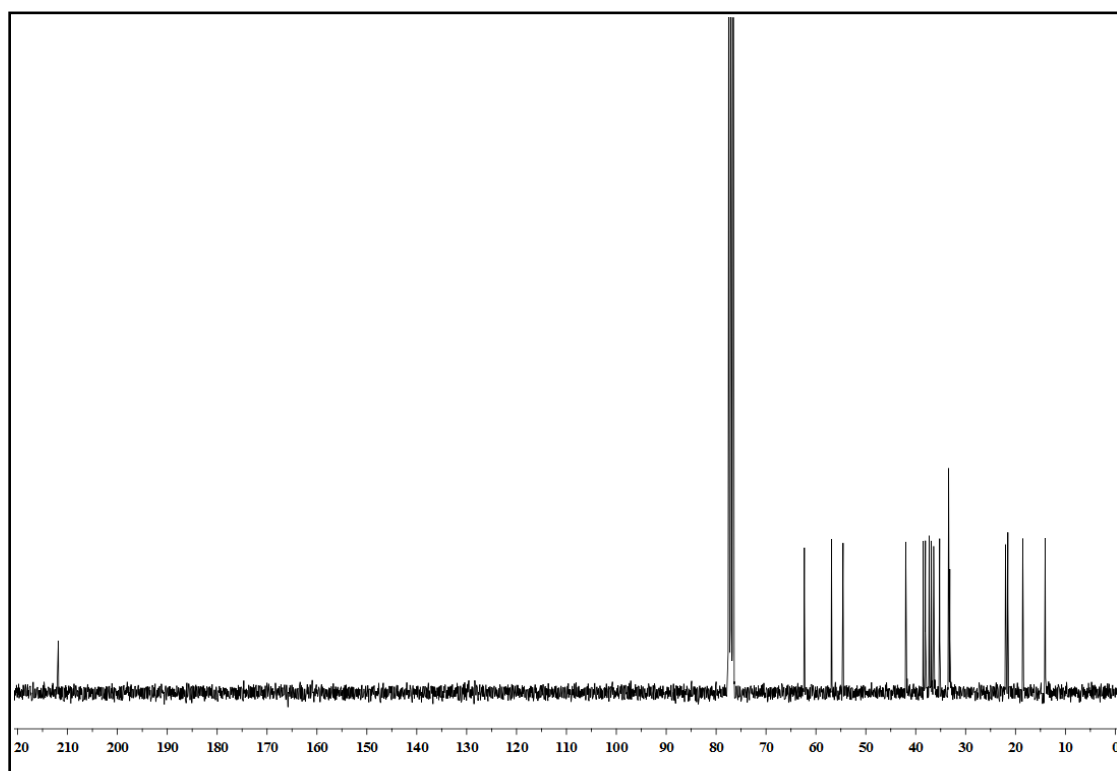**Figure 63** DEPT 135˚ (CDCl$_3$) spectrum of compound **CP1**
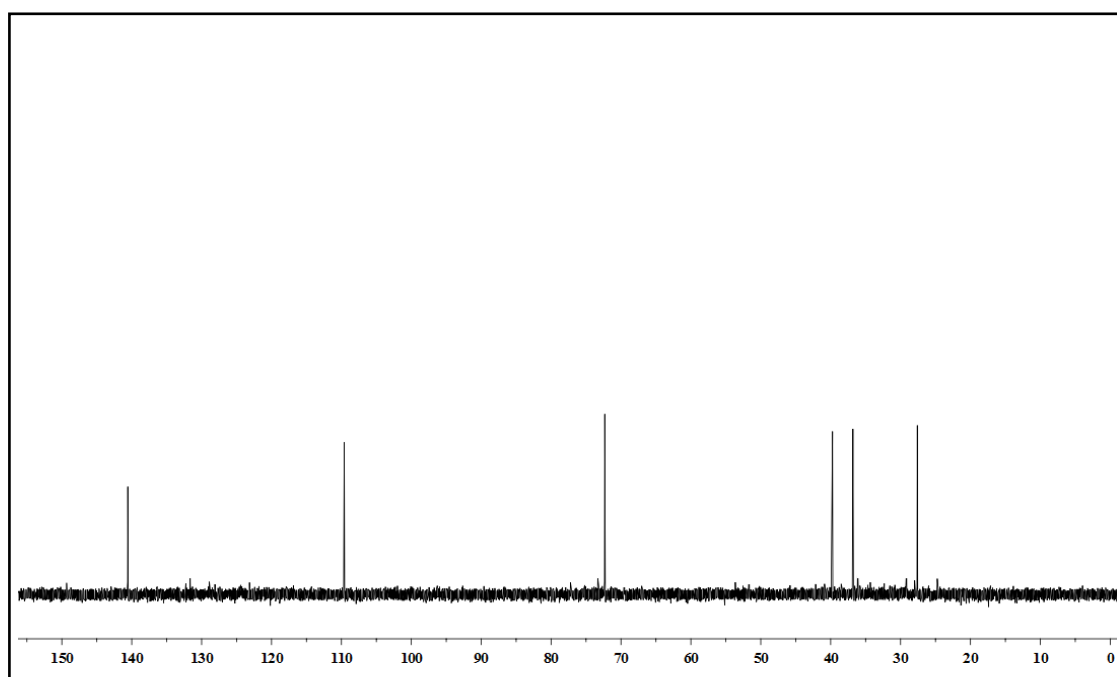


**Figure 64** DEPT 90˚ (CDCl$_3$) spectrum of compound **CP1**
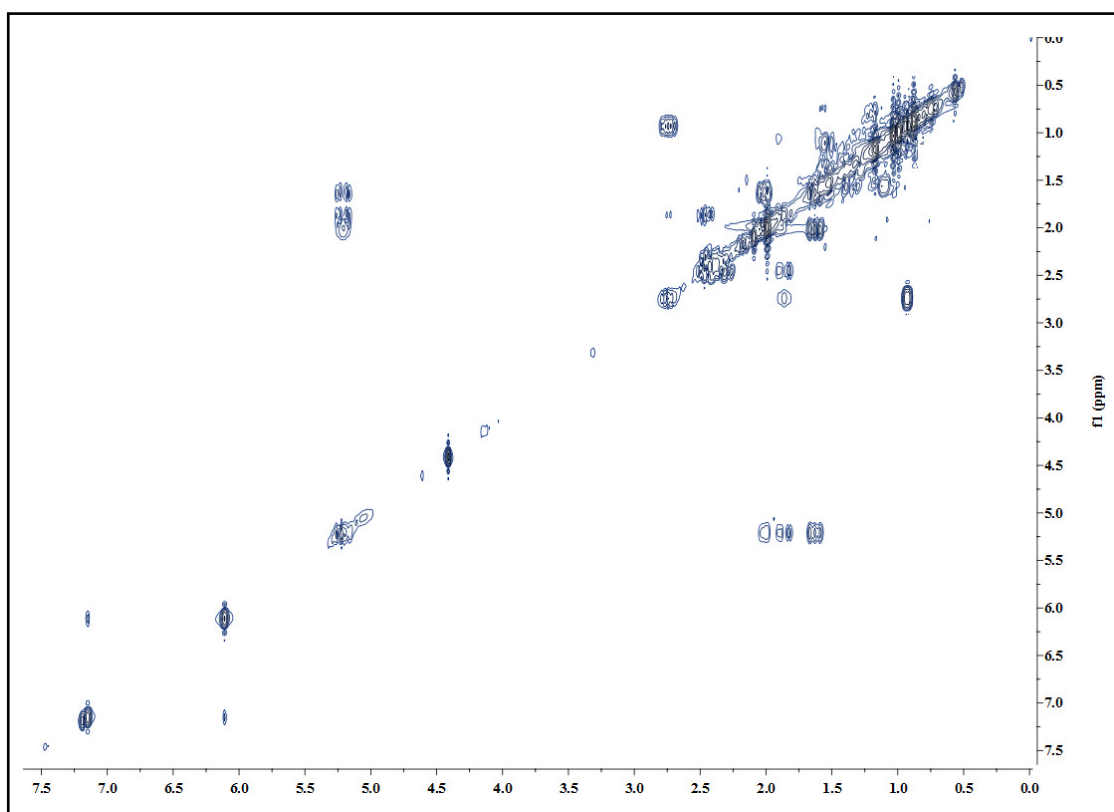
**Figure 65**    2D COSY (CDCl₃) spectrum of compound **CP1**
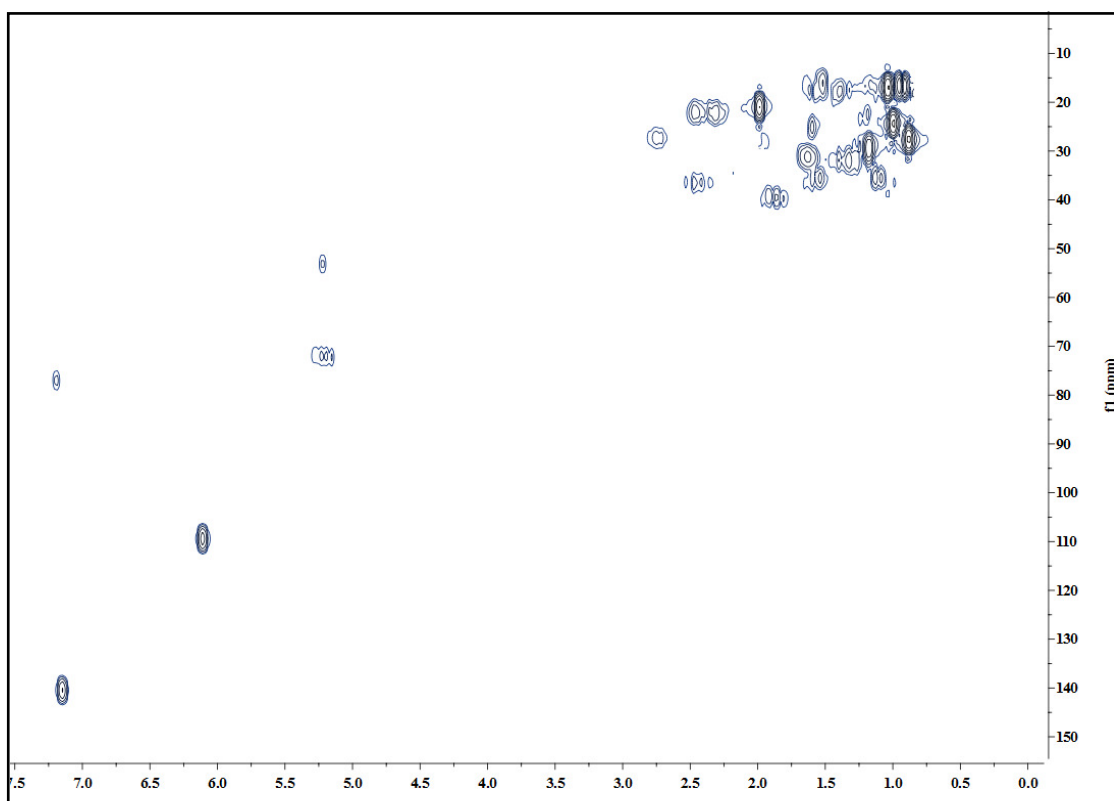


**Figure 66**    2D HMQC (CDCl₃) spectrum of compound **CP1**
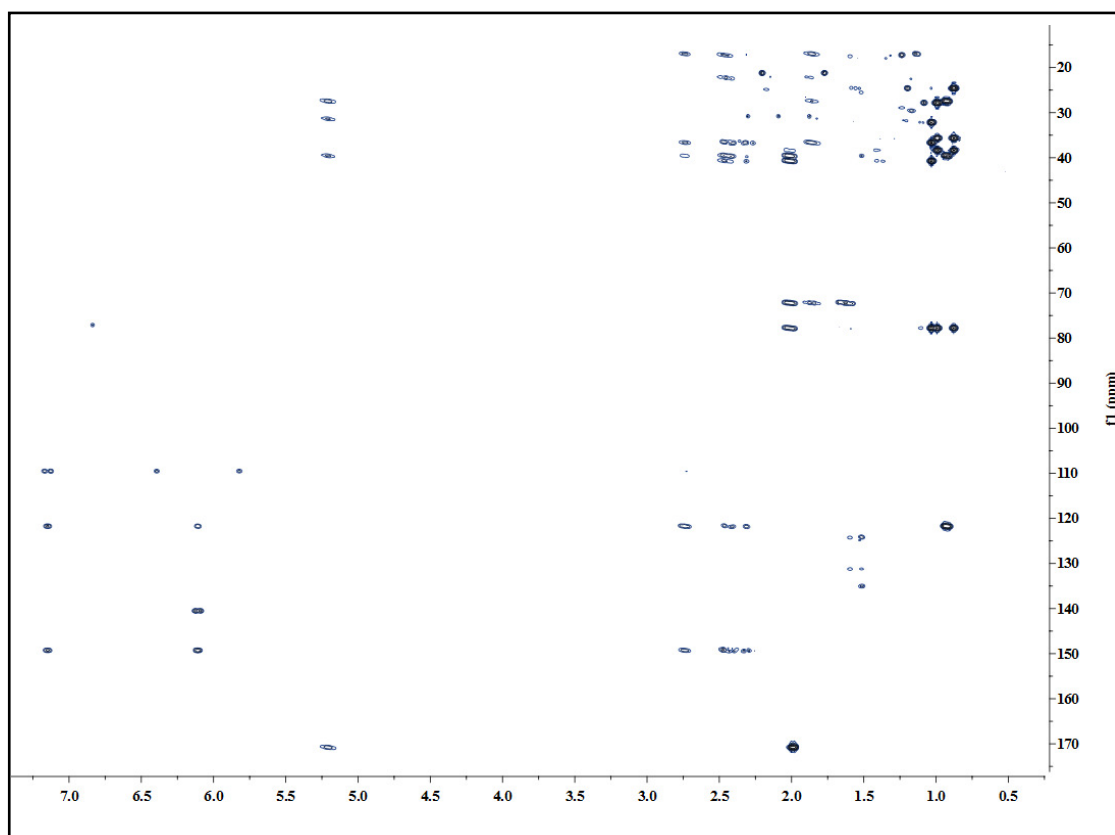
**Figure 67** 2D HMBC (CDCl$_3$) spectrum of compound **CP1**
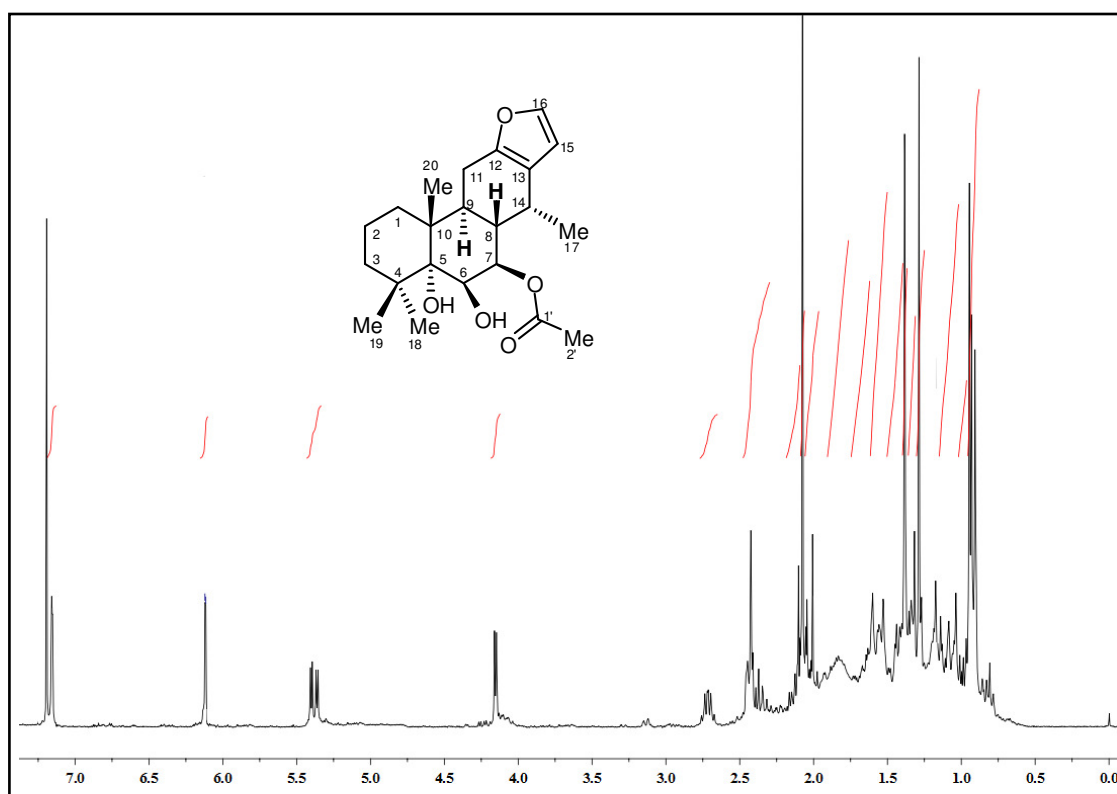
**Figure 68** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP2**
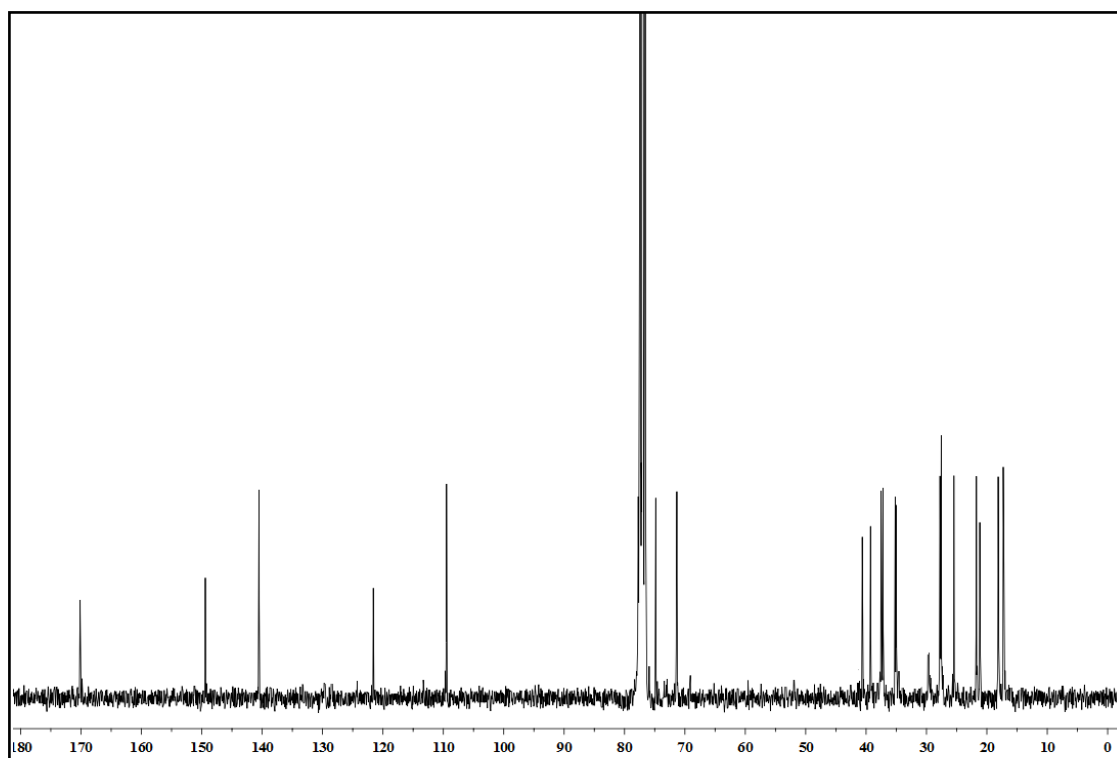


**Figure 69** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP2**
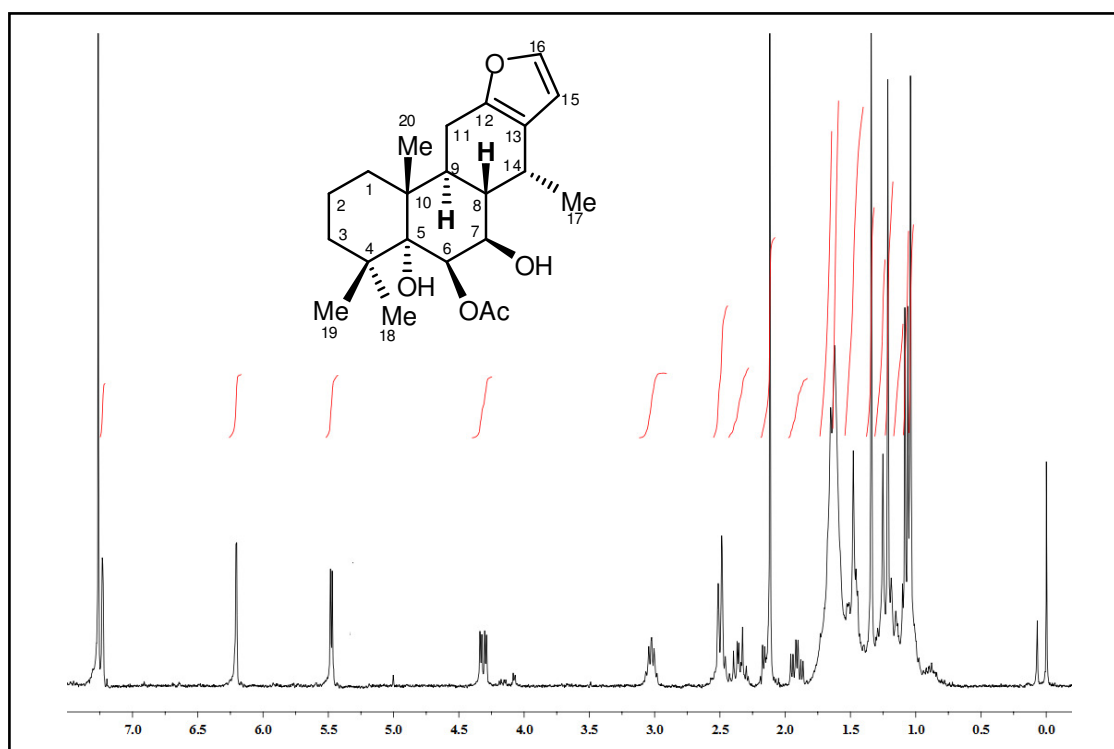
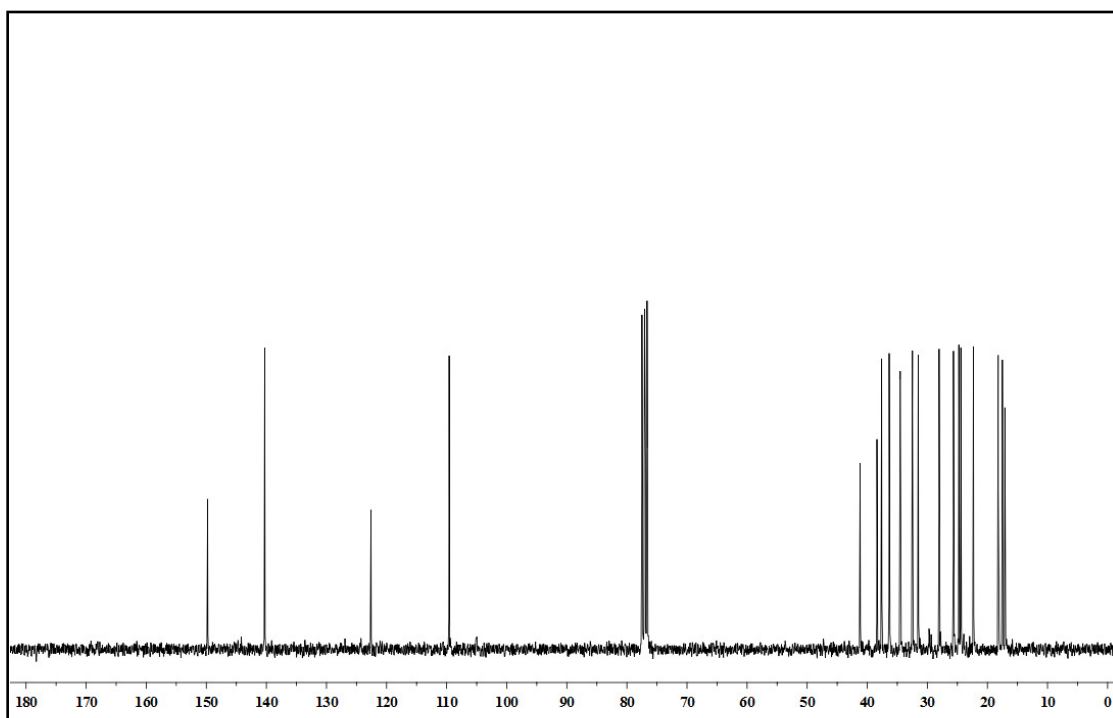**Figure 70** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP3**
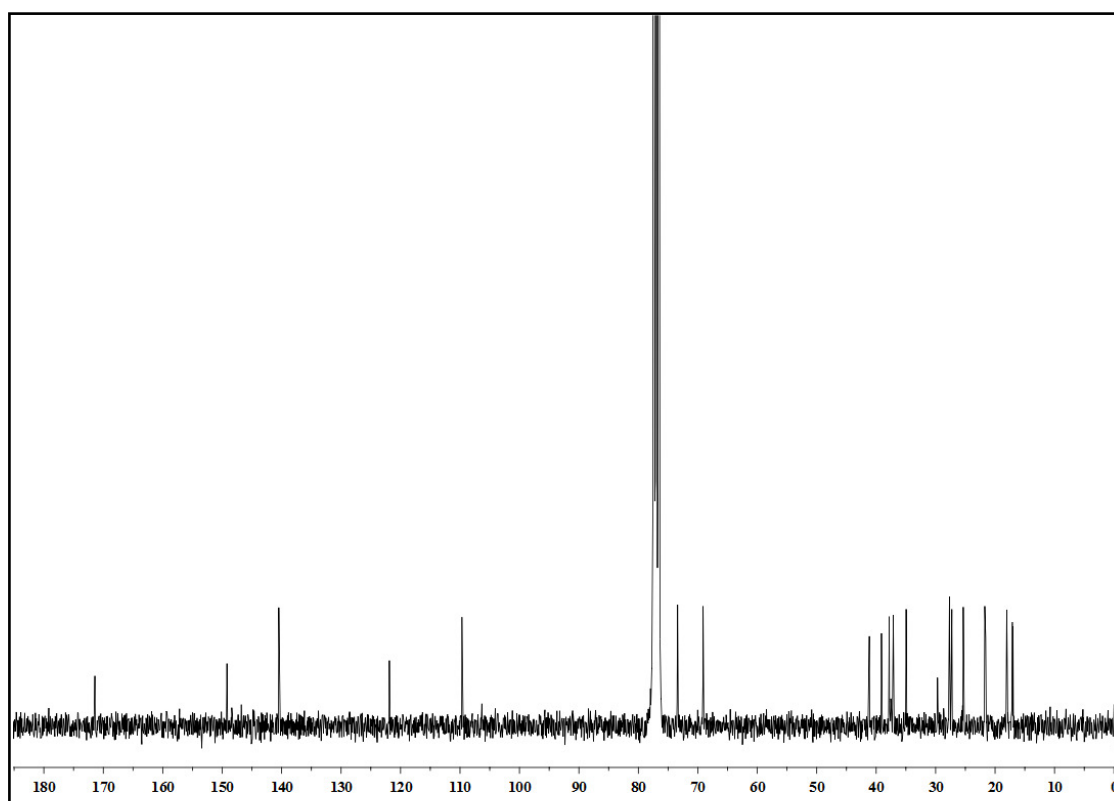


**Figure 71** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP3**
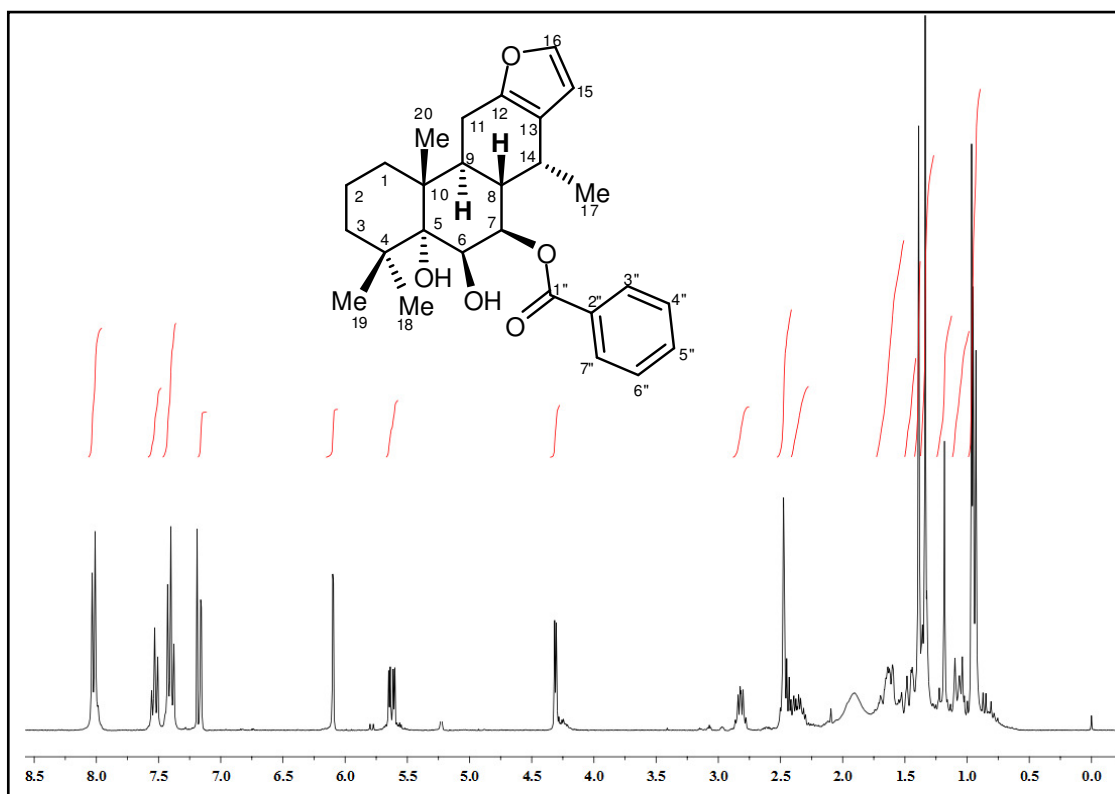
**Figure 72** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP4**
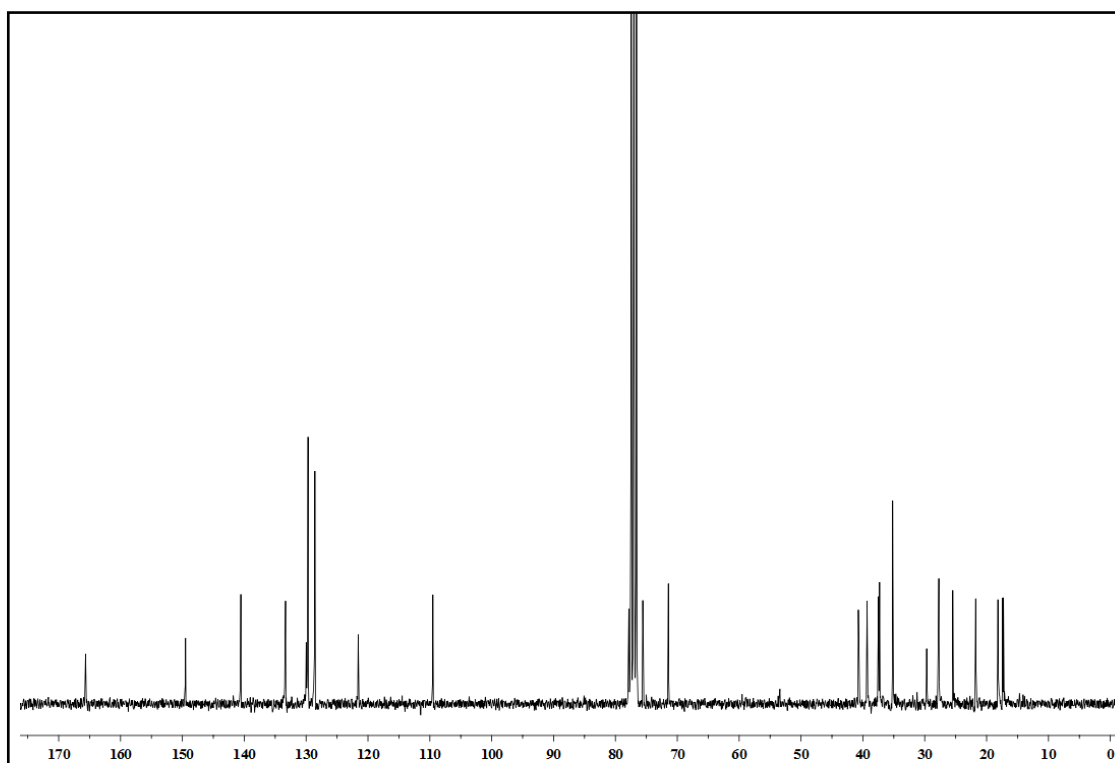


**Figure 73** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP4**
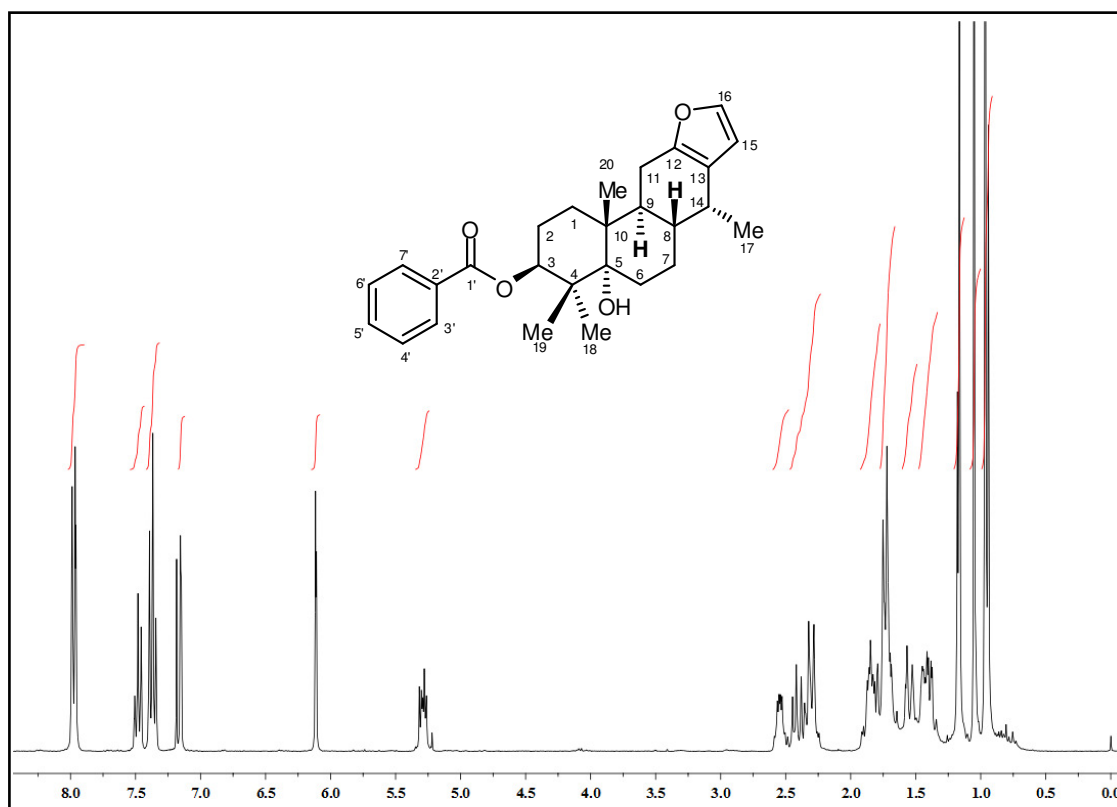
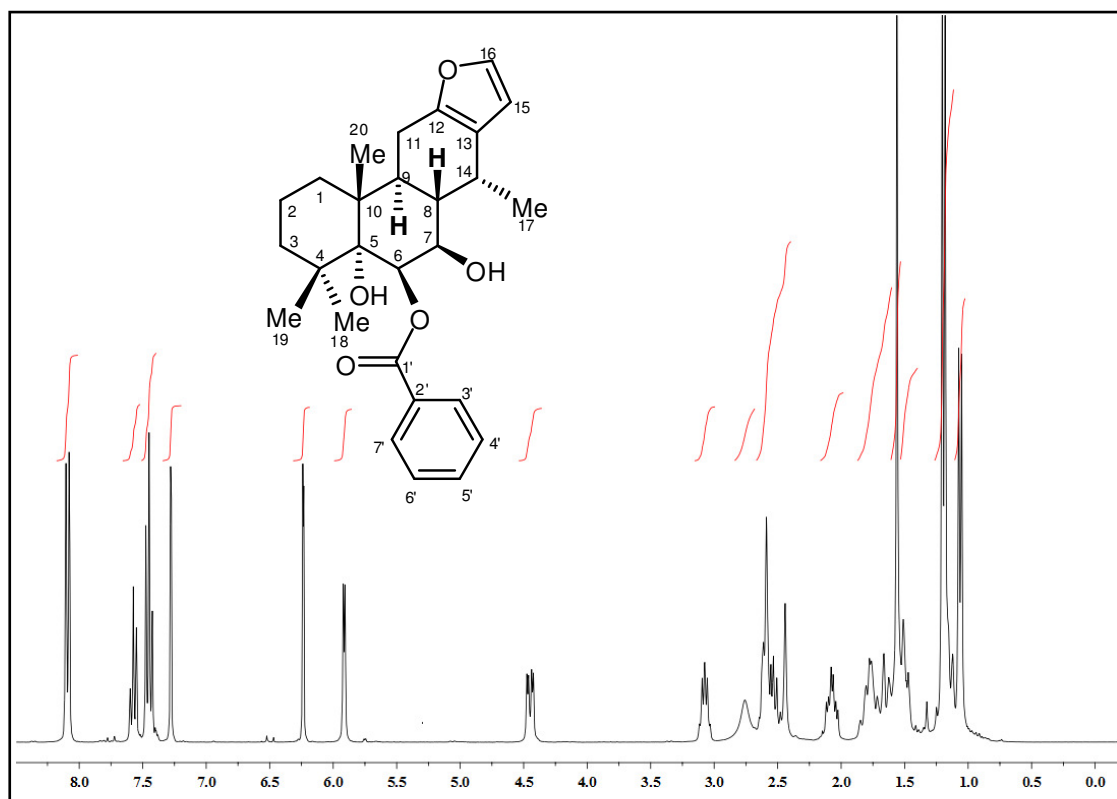**Figure 74** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP5**
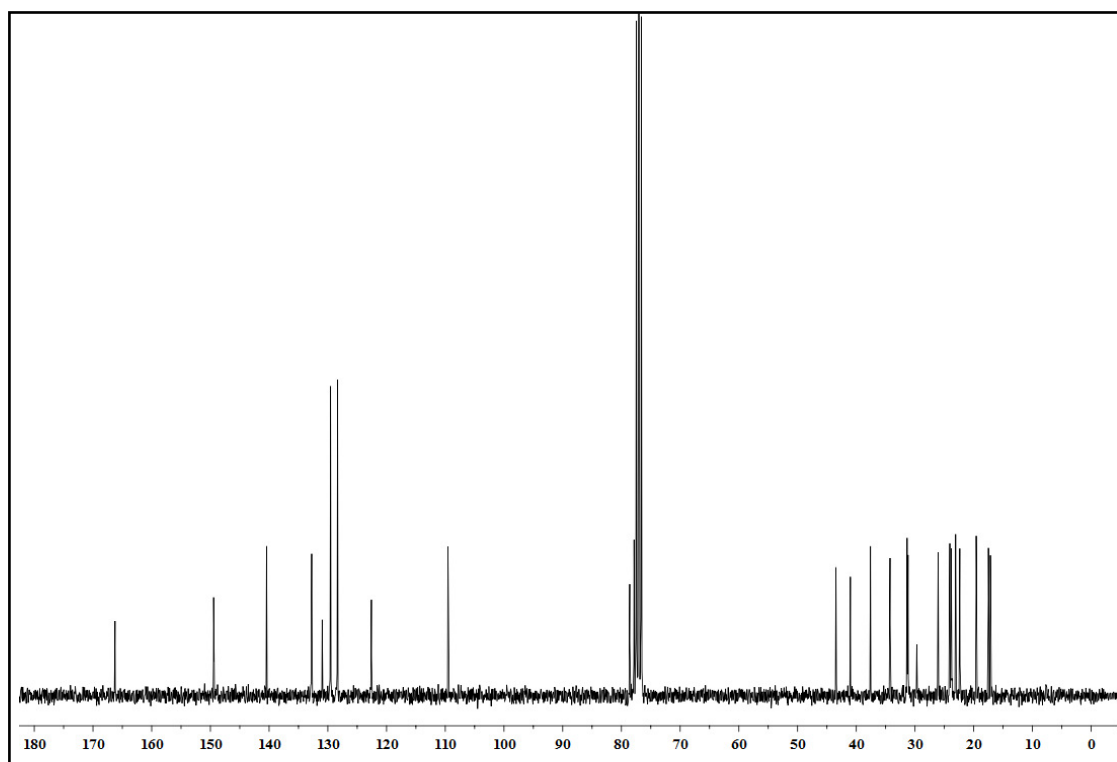


**Figure 75** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP5**
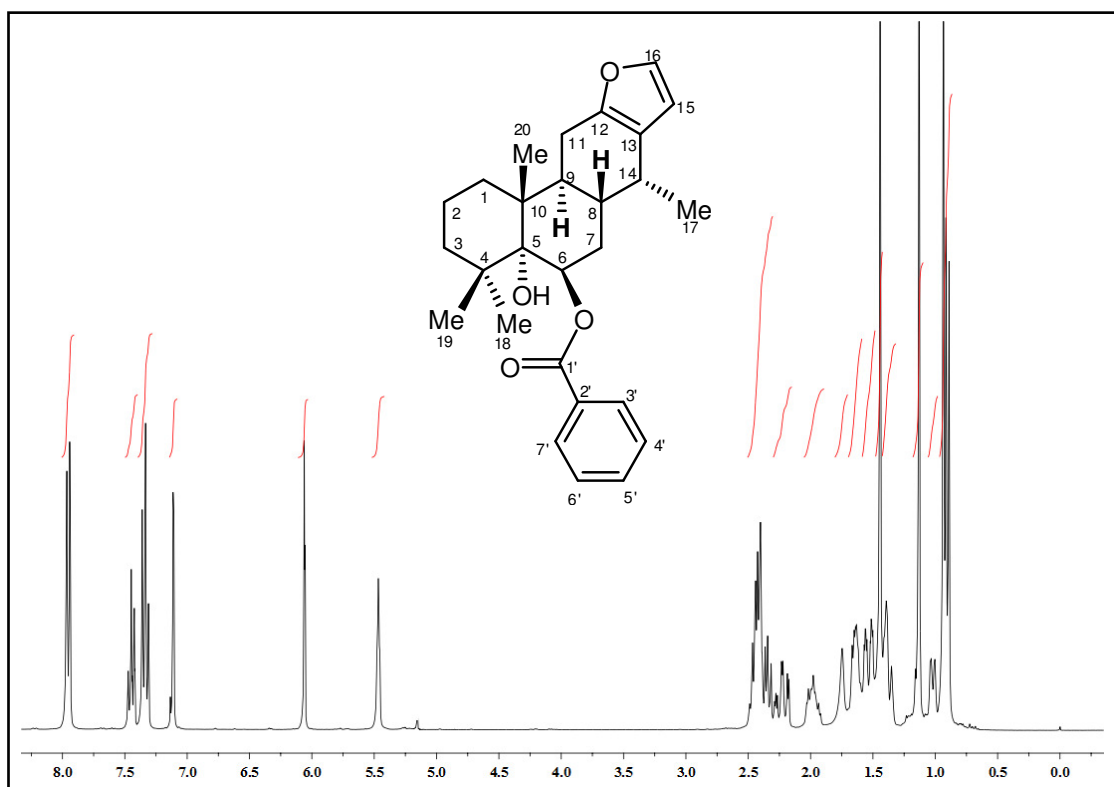
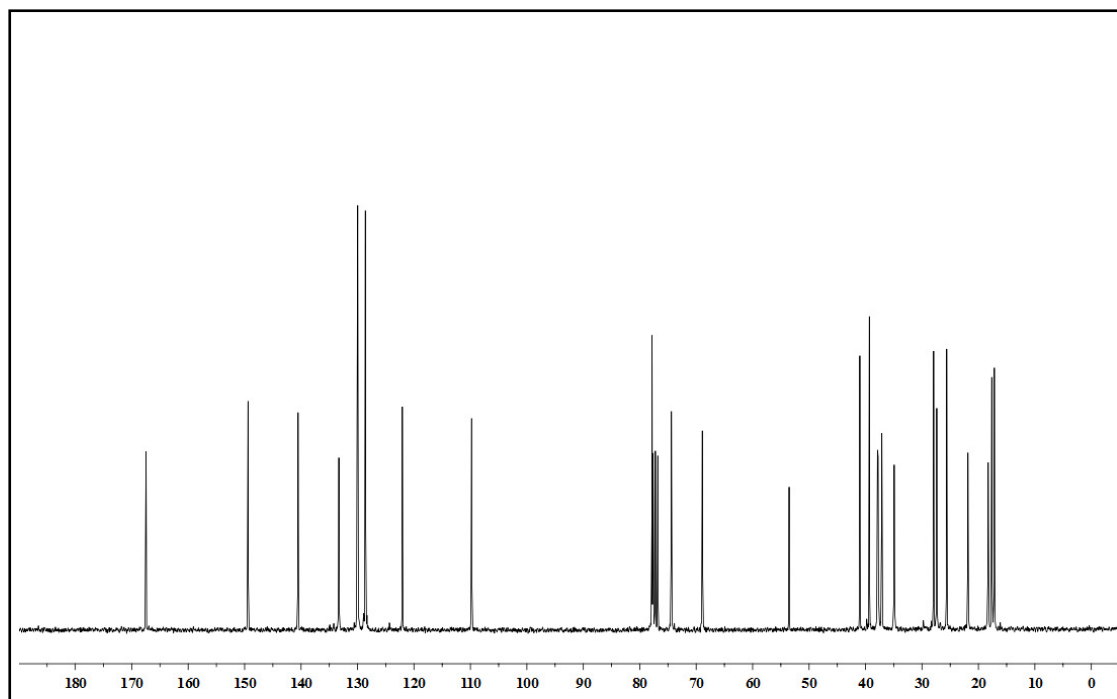**Figure 76** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP6**
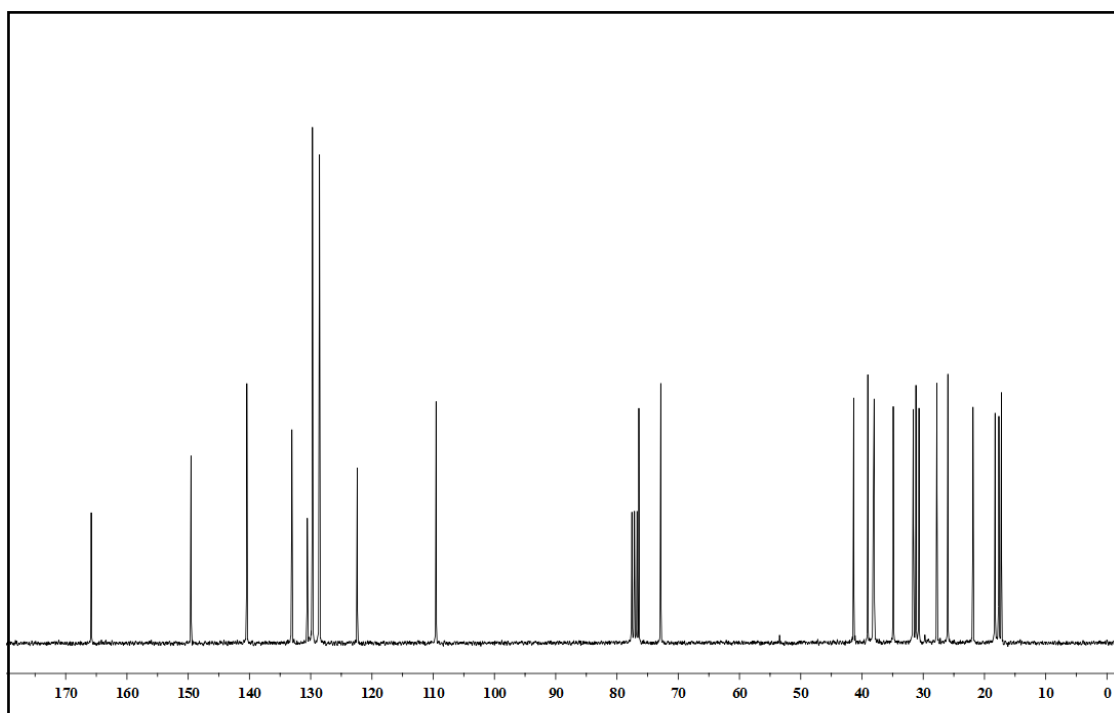


**Figure 77** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP6**
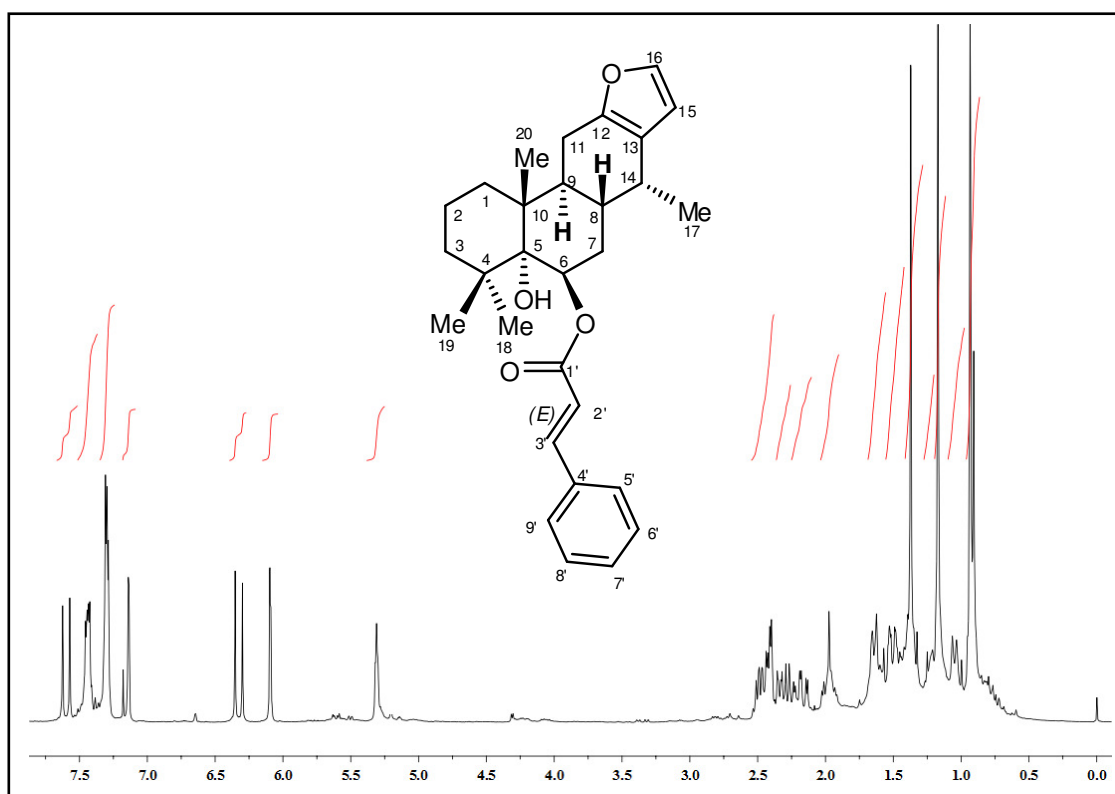
**Figure 78**  $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP7**



**Figure 79**  $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP7**
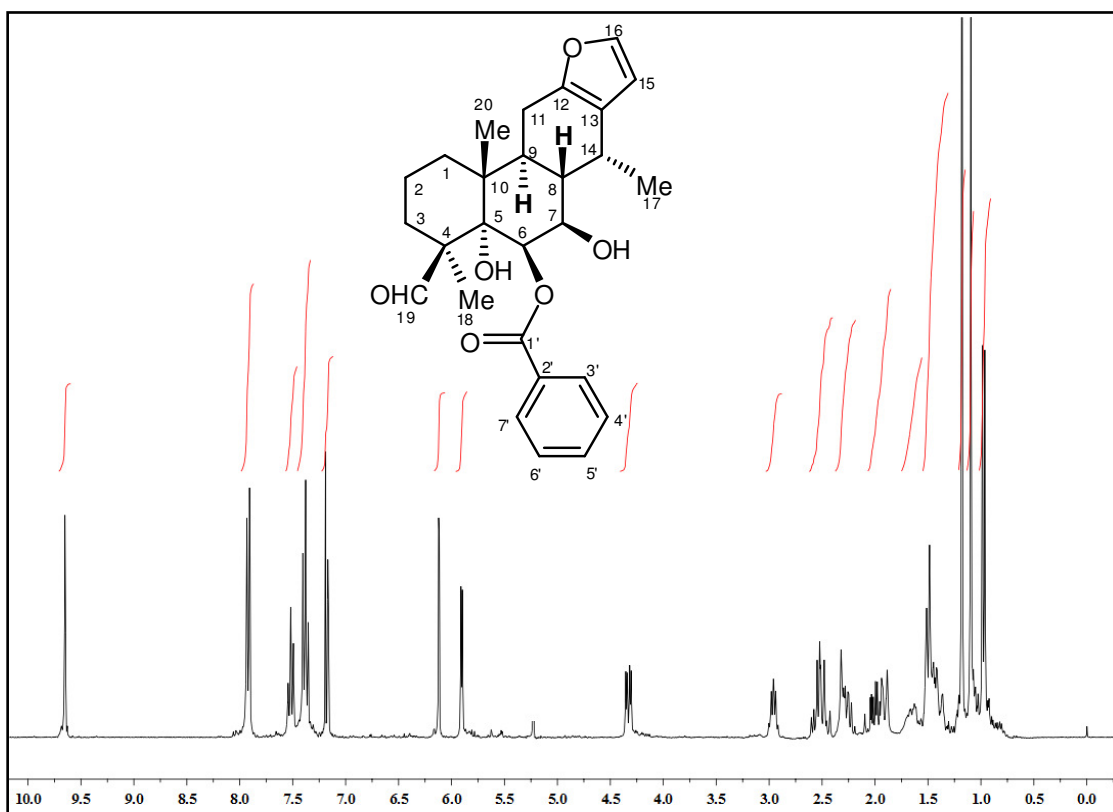
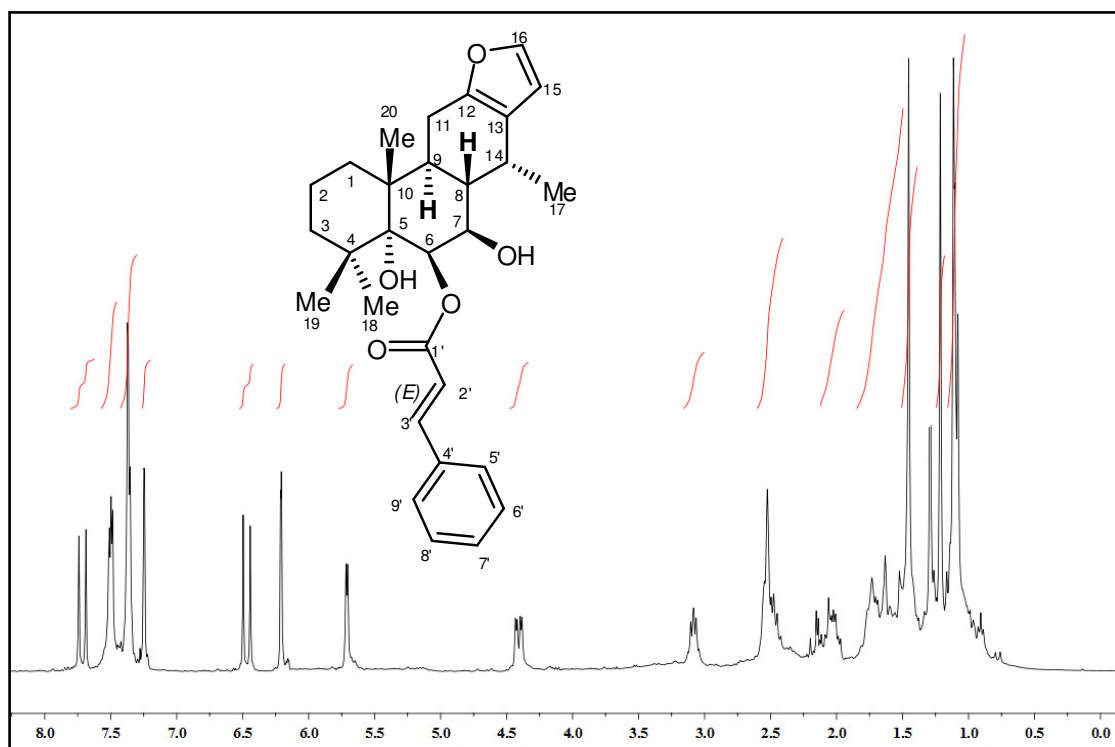**Figure 80** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP8**
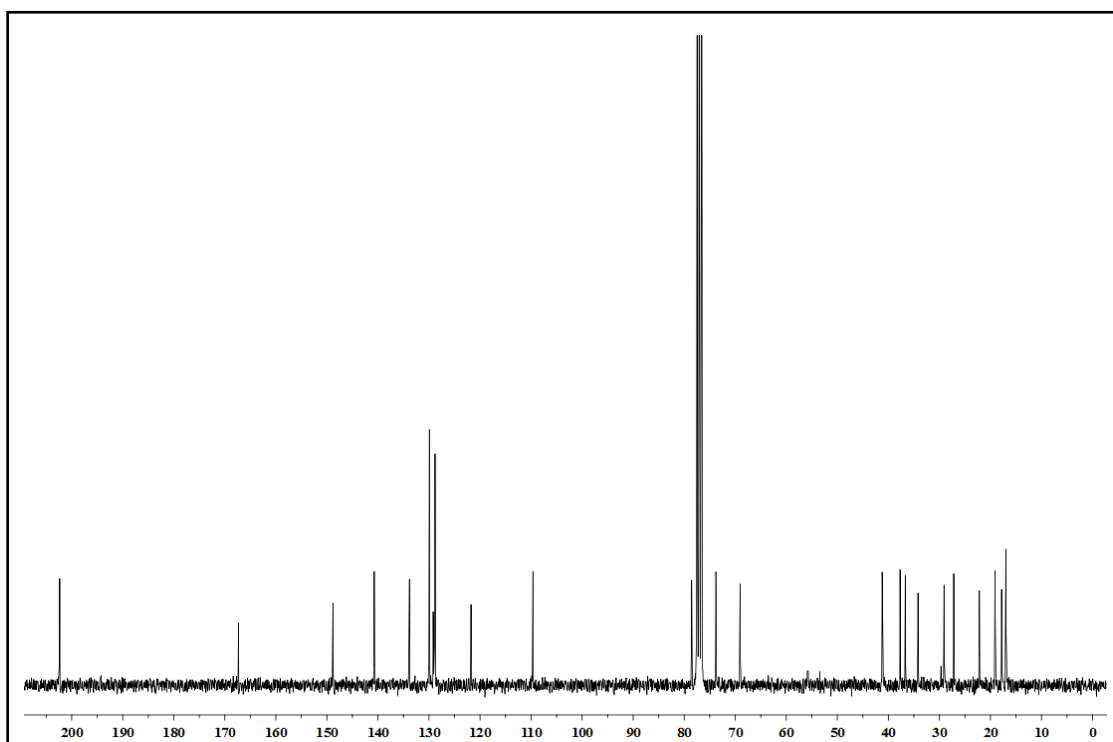


**Figure 81** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP8**

**Figure 82** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP9**



**Figure 83** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP9**

**Figure 84**  $^{1}$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP10**



**Figure 85**  $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP10**

**Figure 86** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP11**
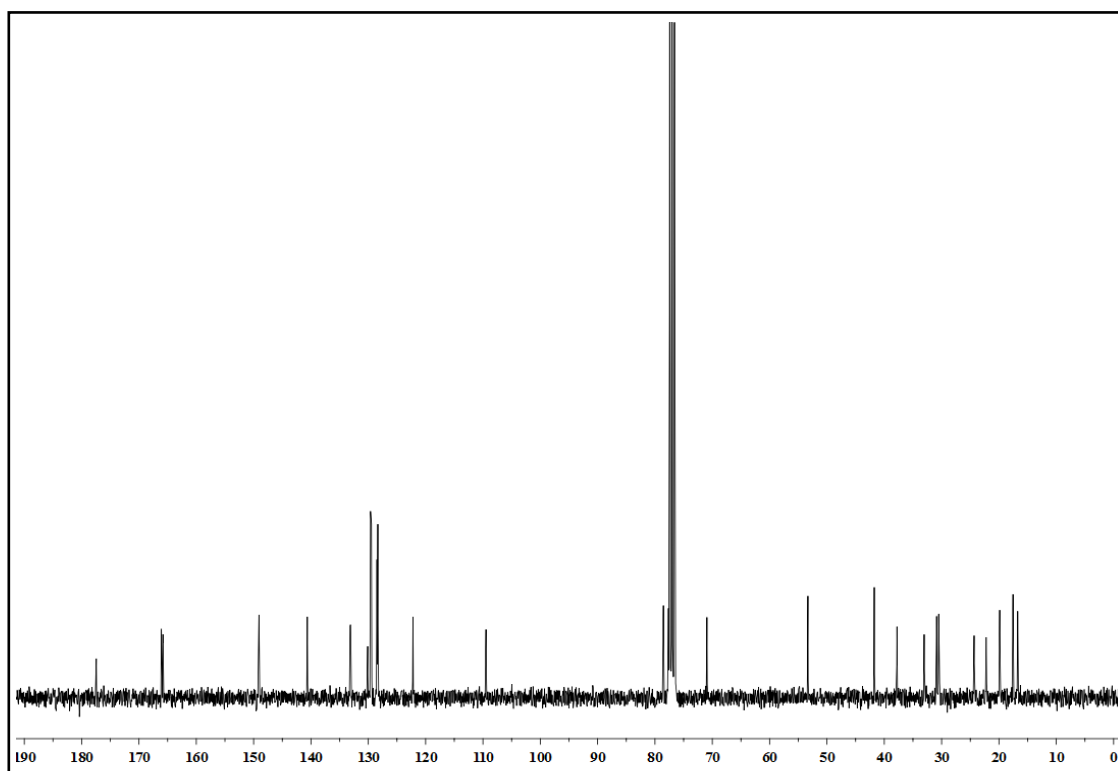


**Figure 87** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP11**
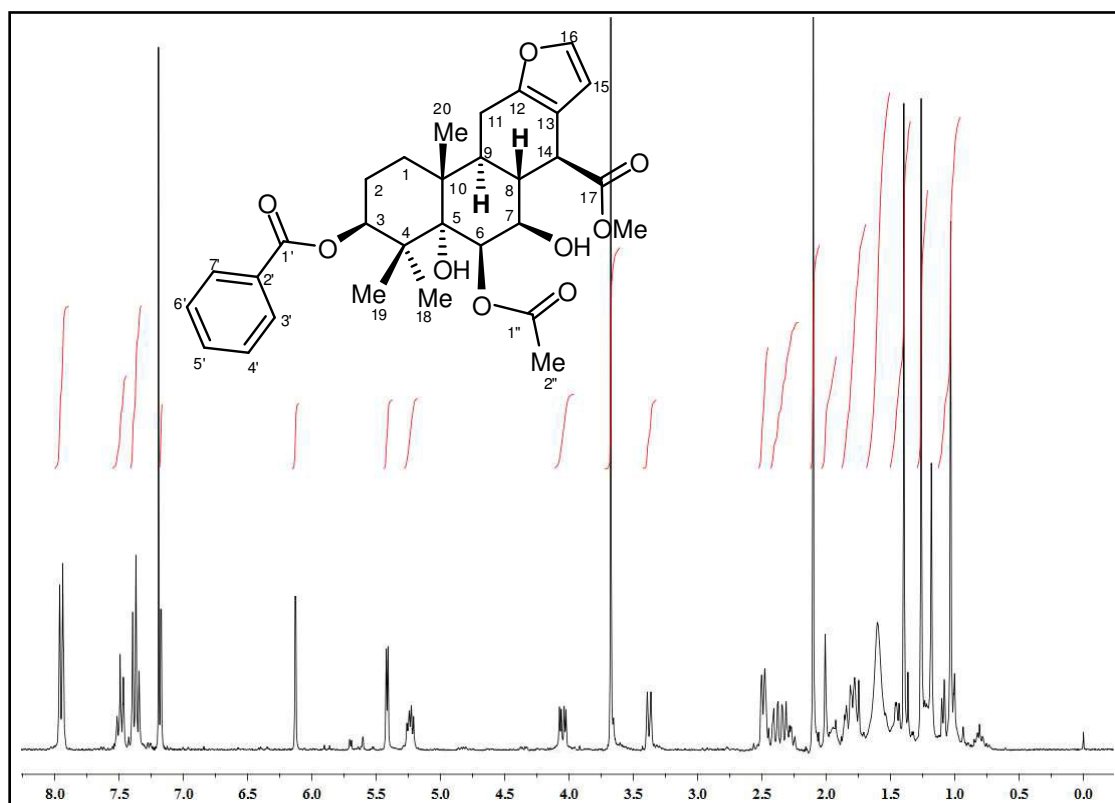
**Figure 88** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP12**
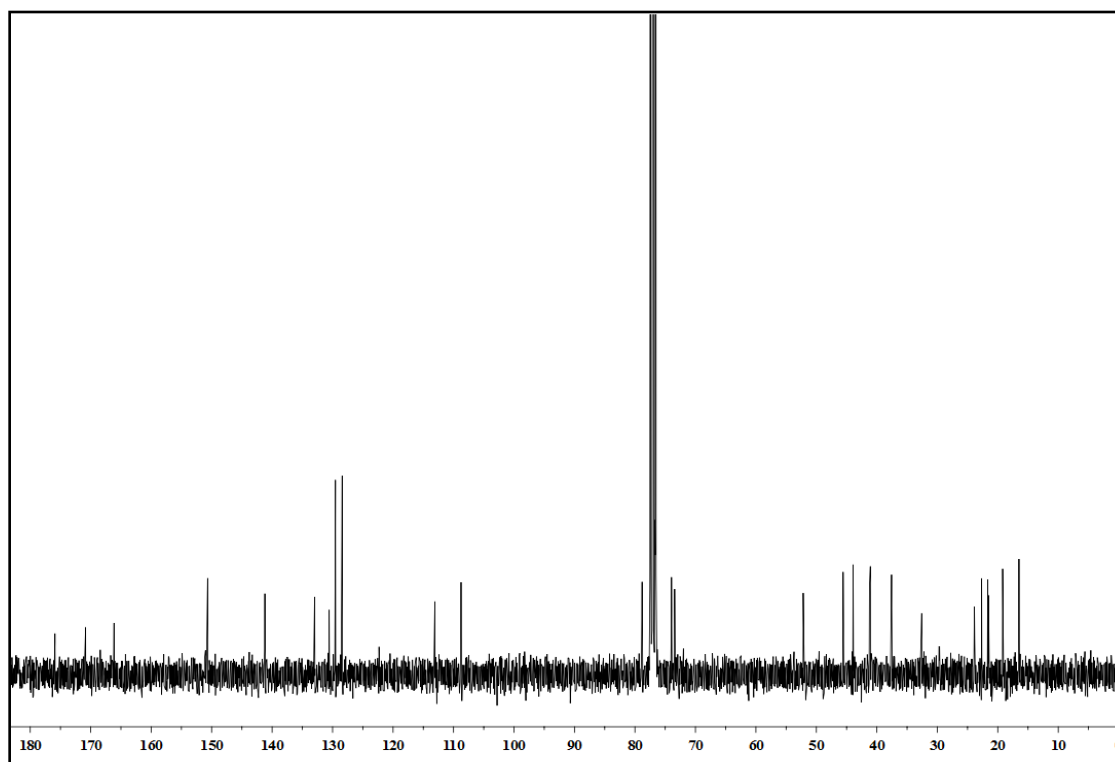


**Figure 89** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP12**
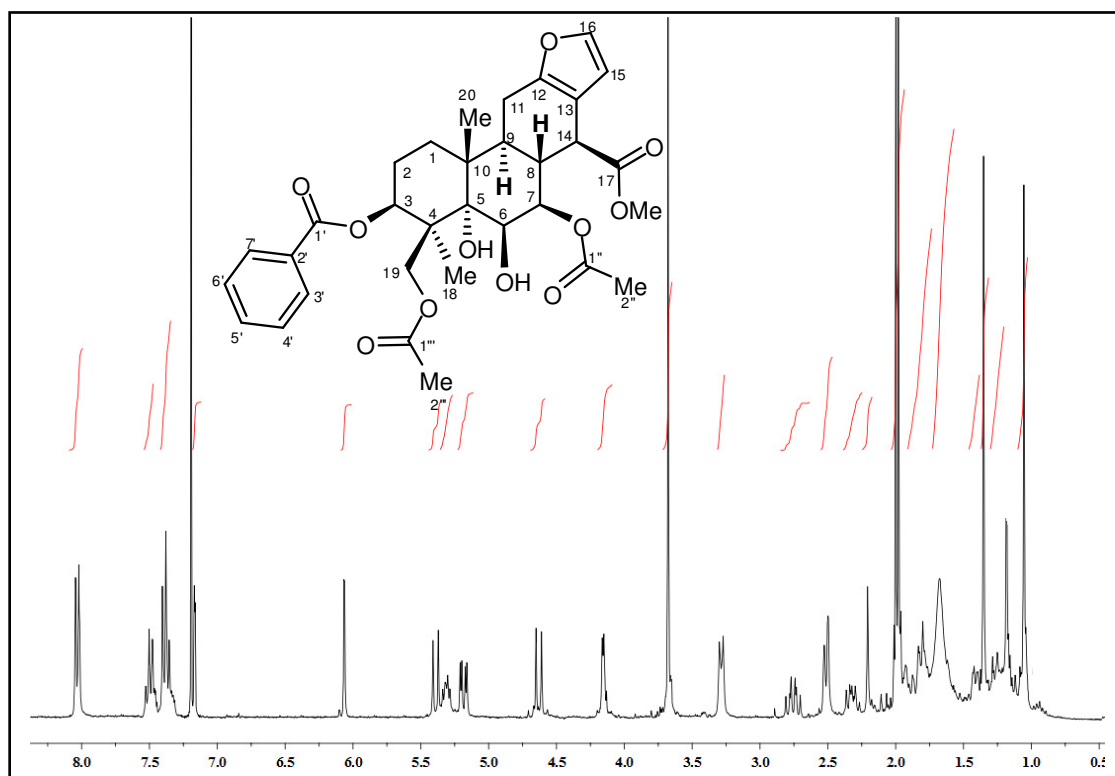
**Figure 90** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP13**
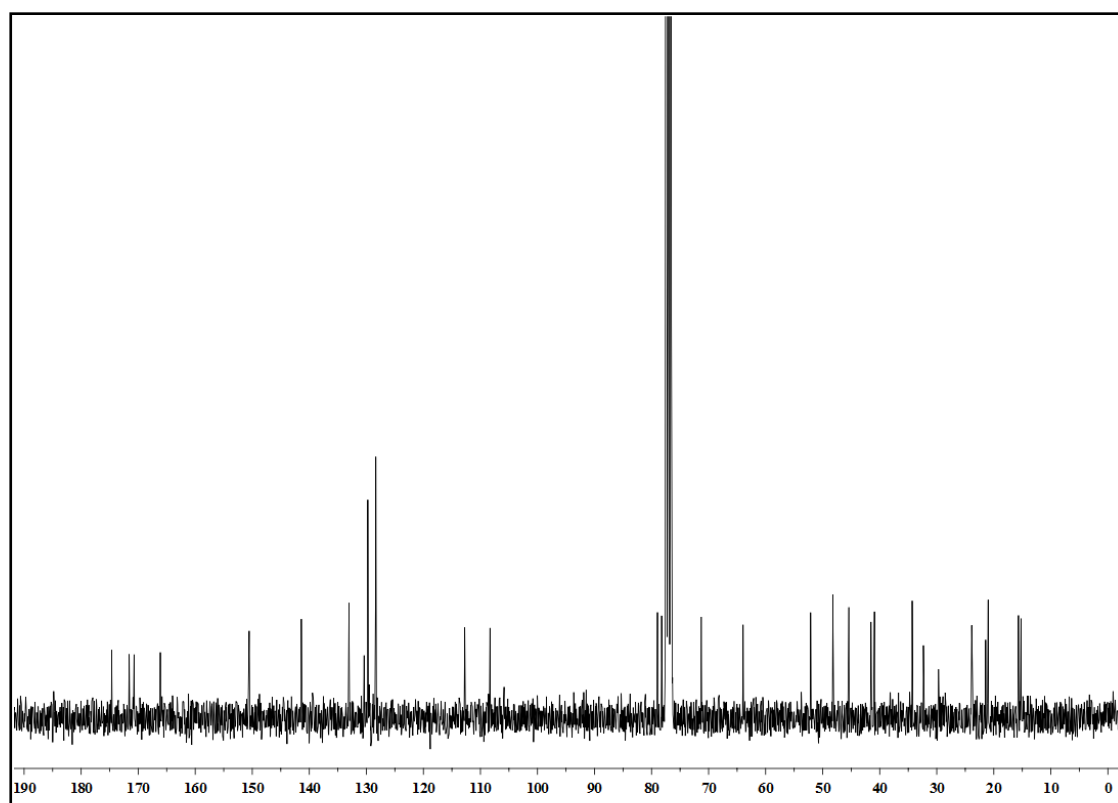


**Figure 91** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP13**
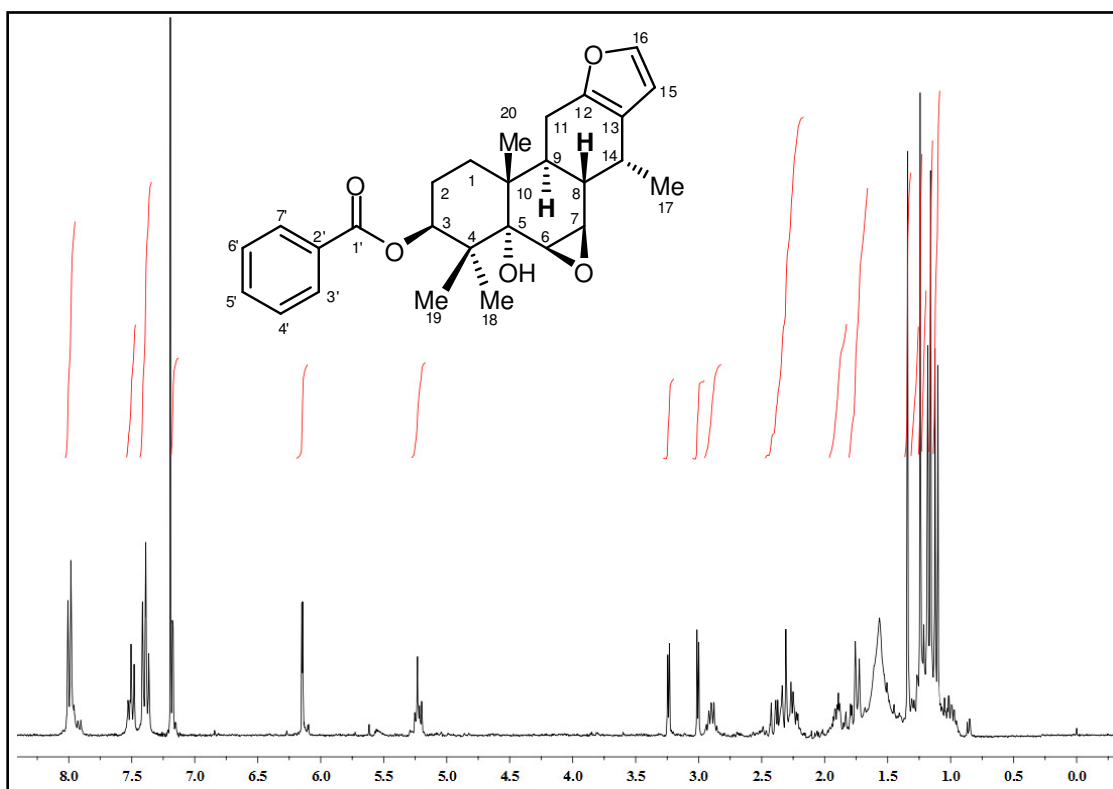
**Figure 92** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP14**



**Figure 93** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP14**
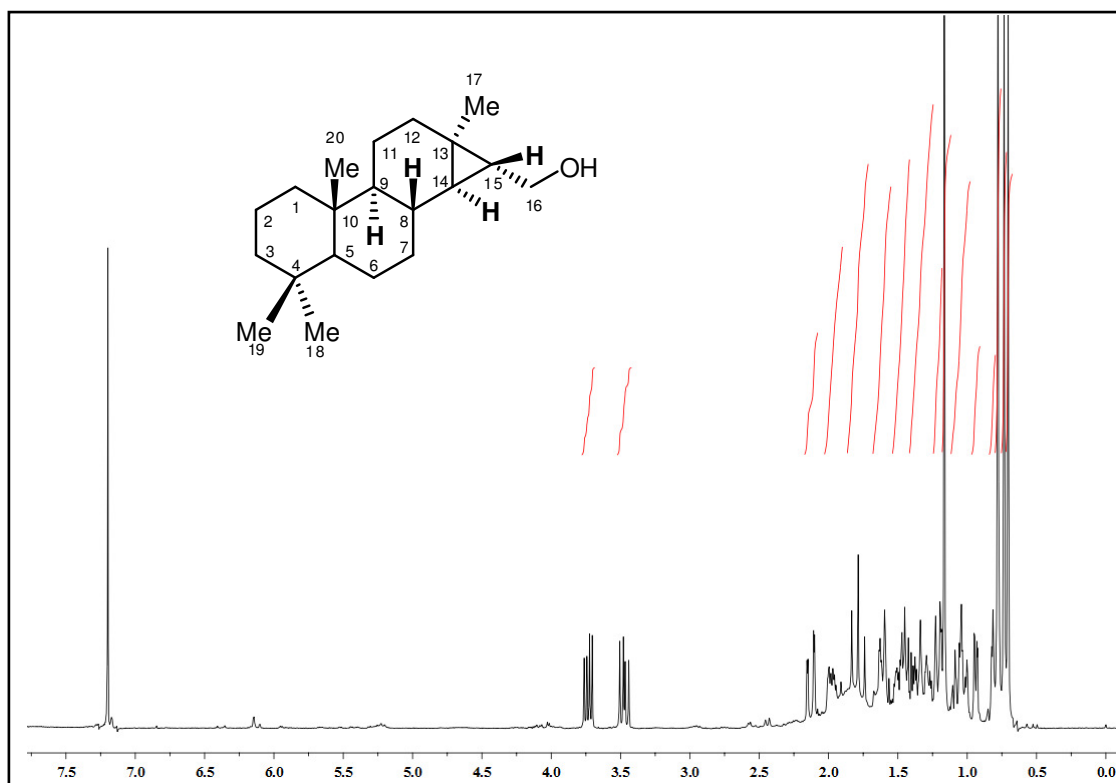
**Figure 94** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP15**



**Figure 95** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP15**
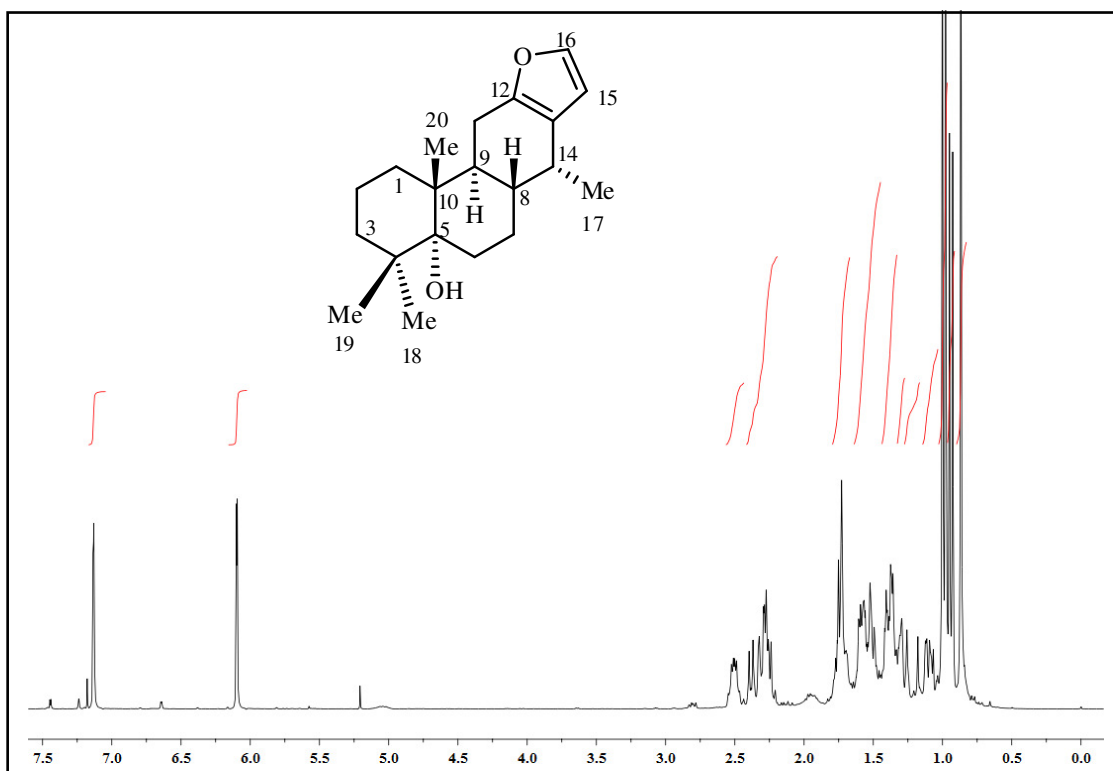
**Figure 96** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP16**



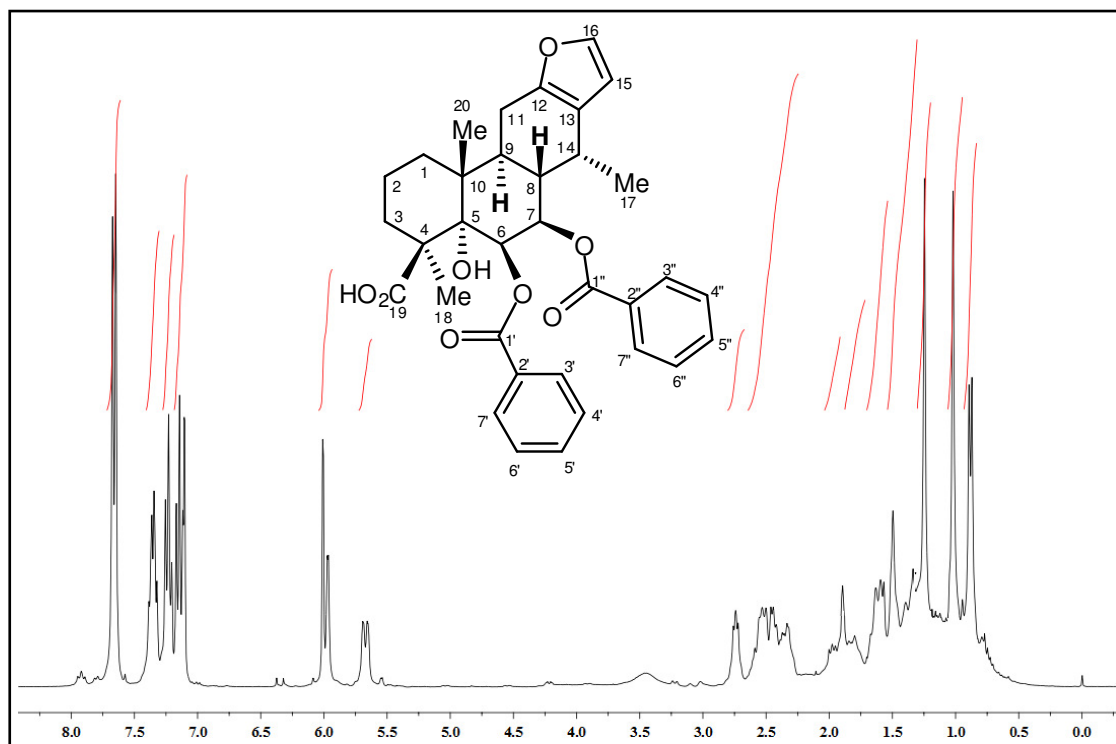**Figure 97** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP16**

**Figure 98** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP17**



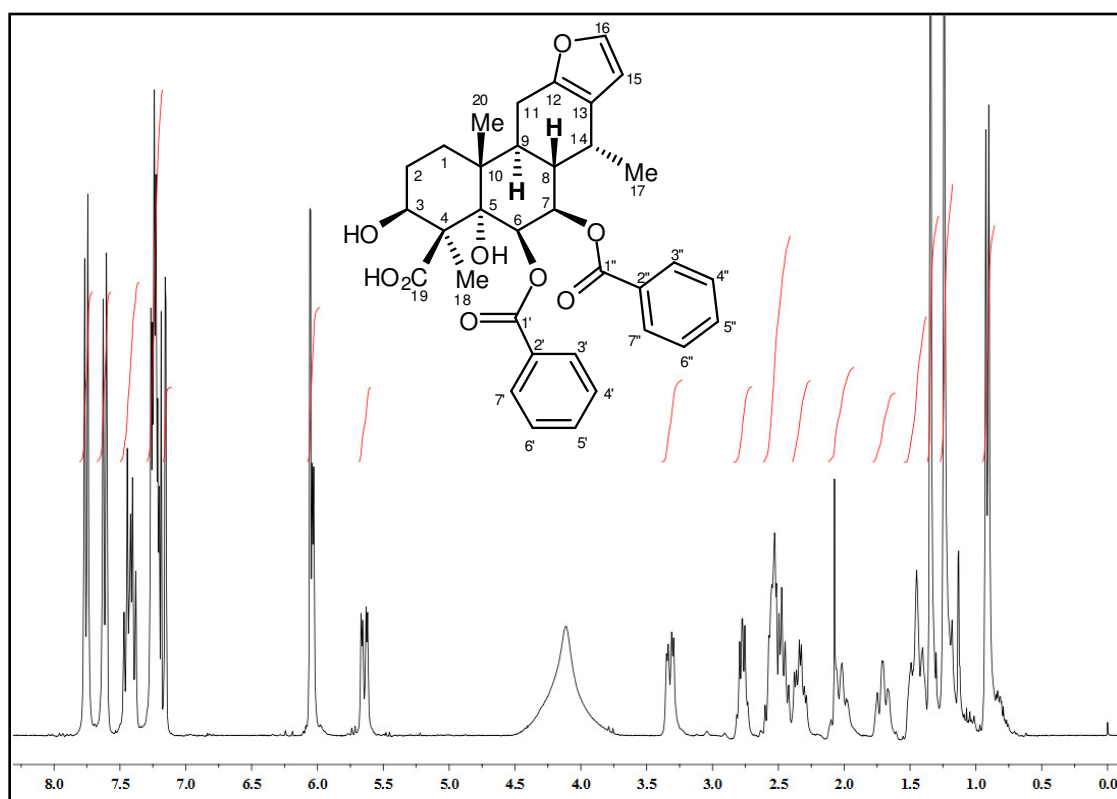**Figure 99** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP17**

**Figure 100**  $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP18**
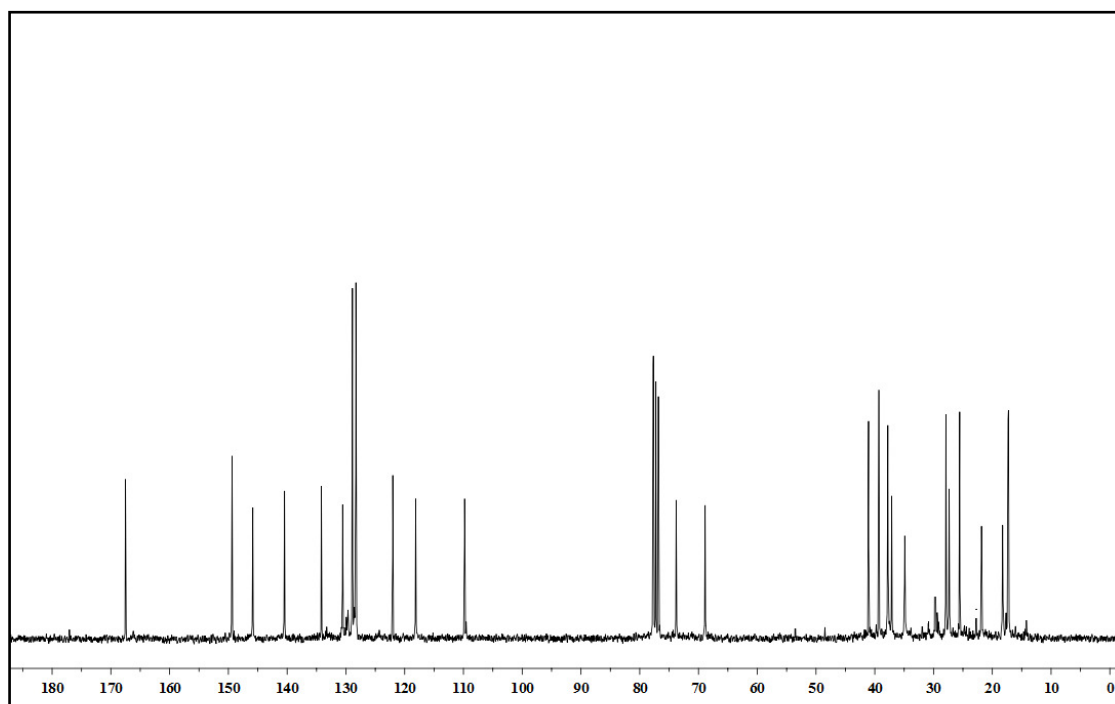


**Figure 101**  $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP18**
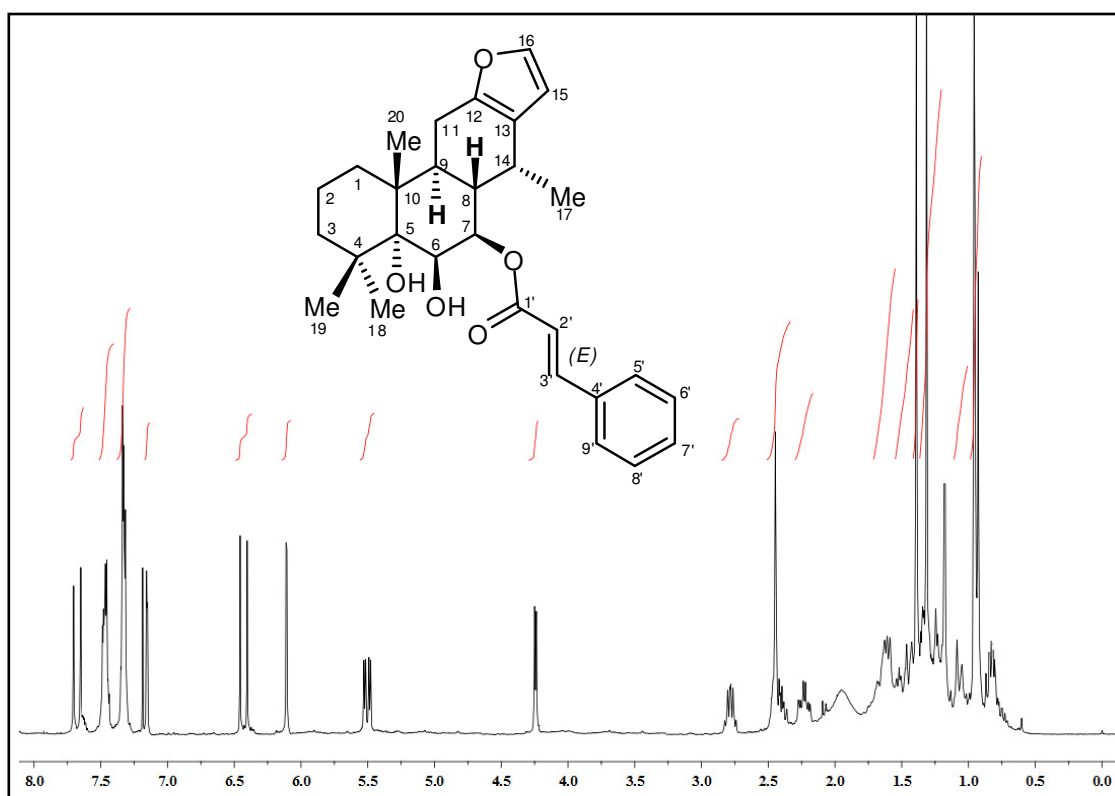
**Figure 102**  $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP19**
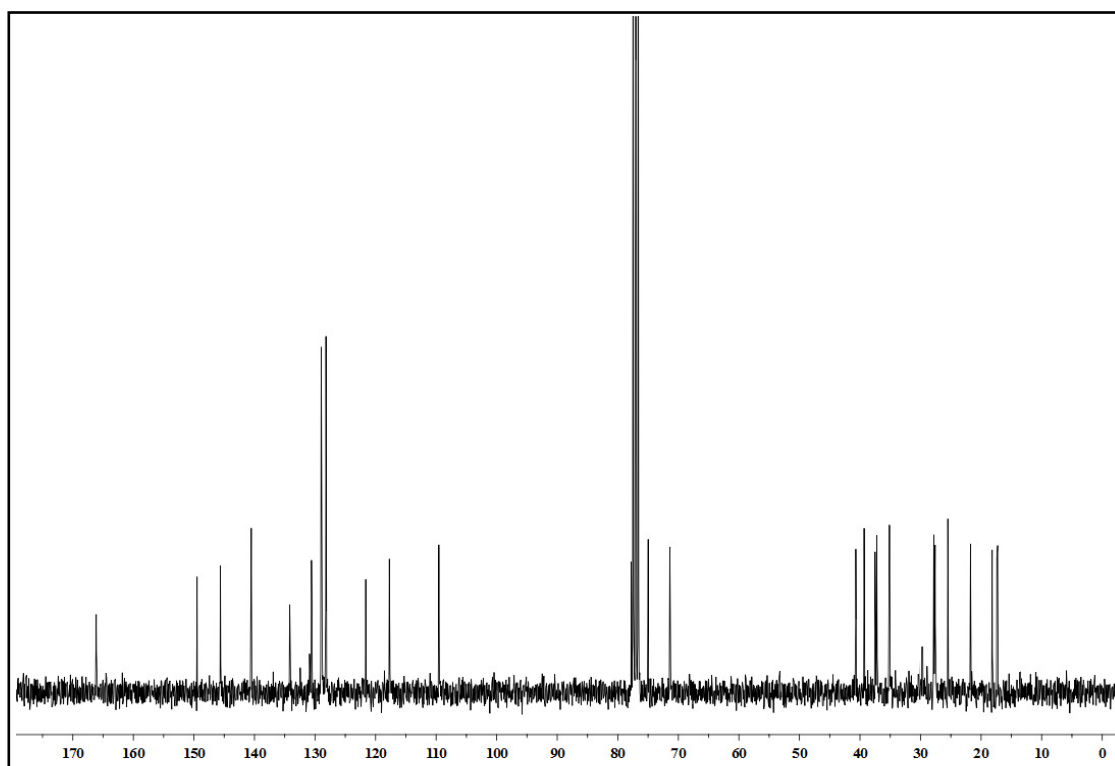


**Figure 103**  $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP19**
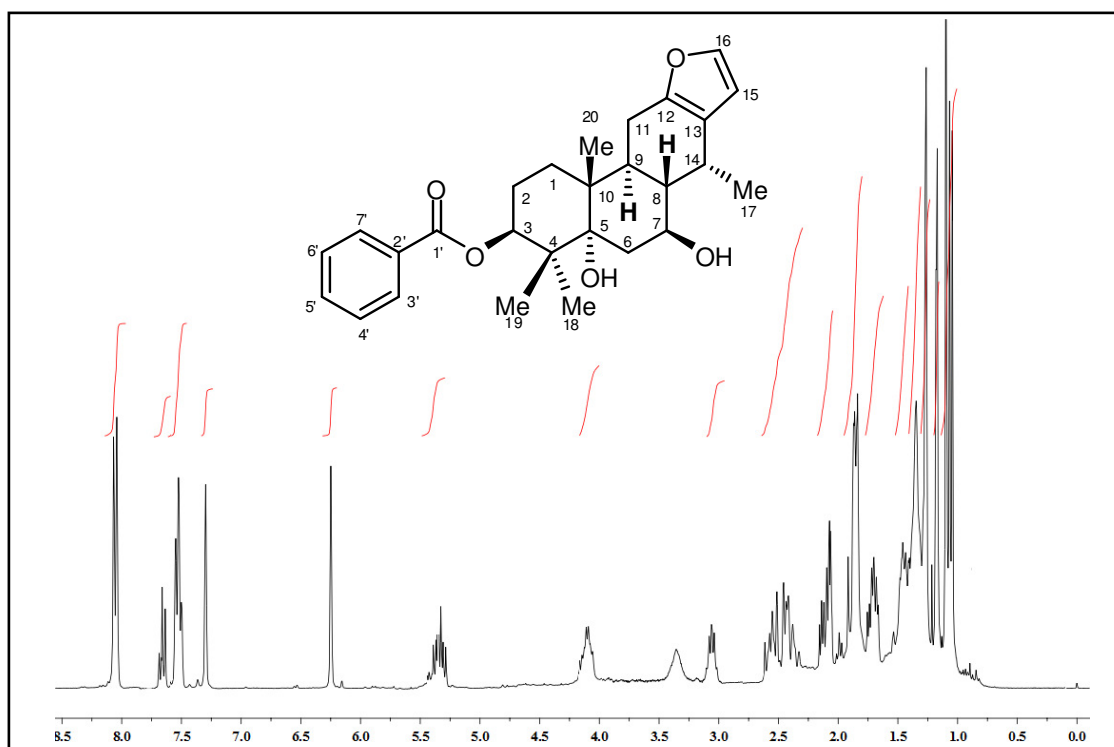
**Figure 104** $^1$H NMR (300 MHz) (acetone-$d_6$) spectrum of compound **CP20**
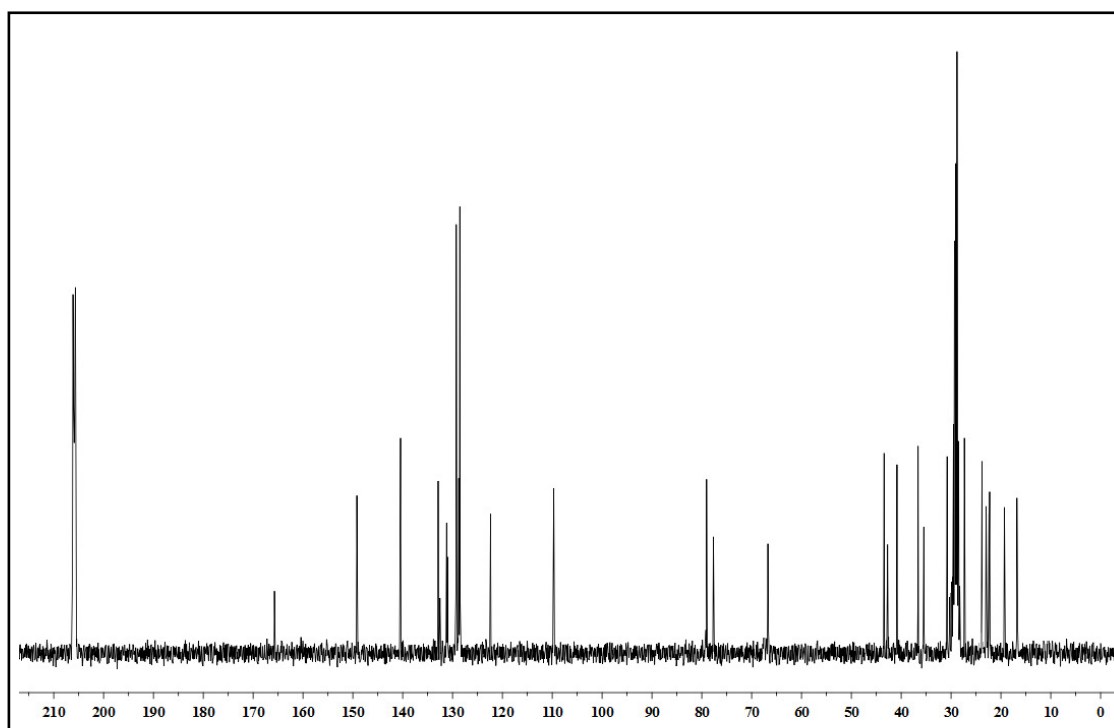


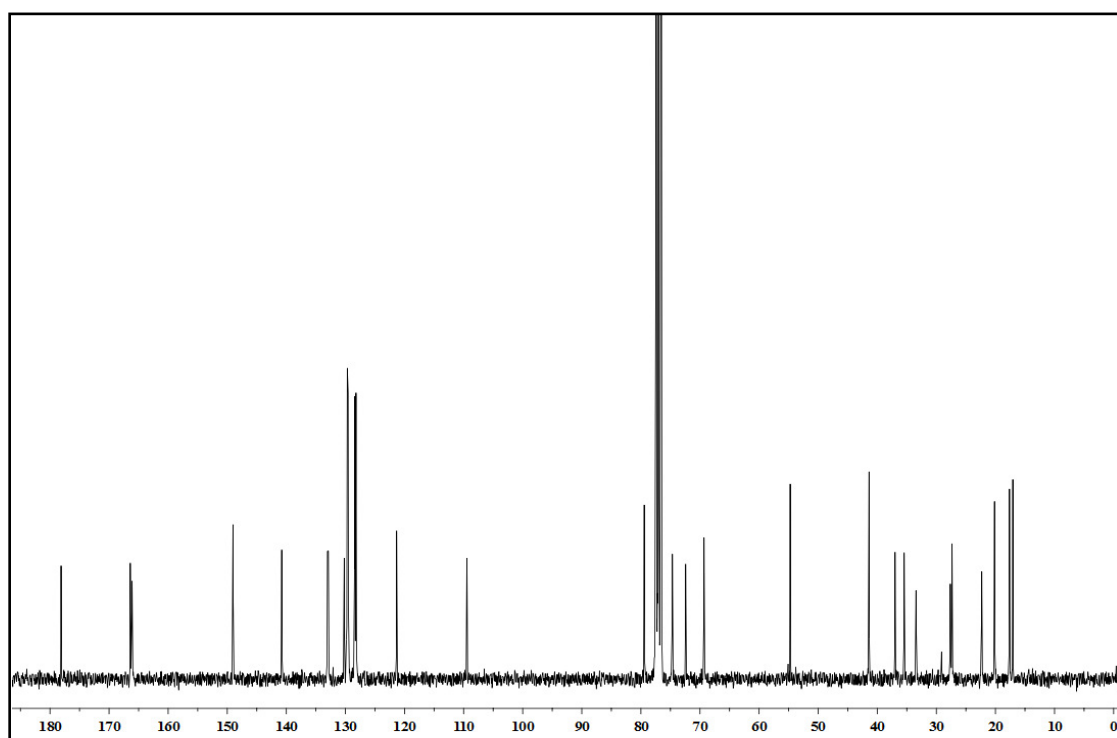**Figure 105** $^{13}$C NMR (75 MHz) (acetone-$d_6$) spectrum of compound **CP20**

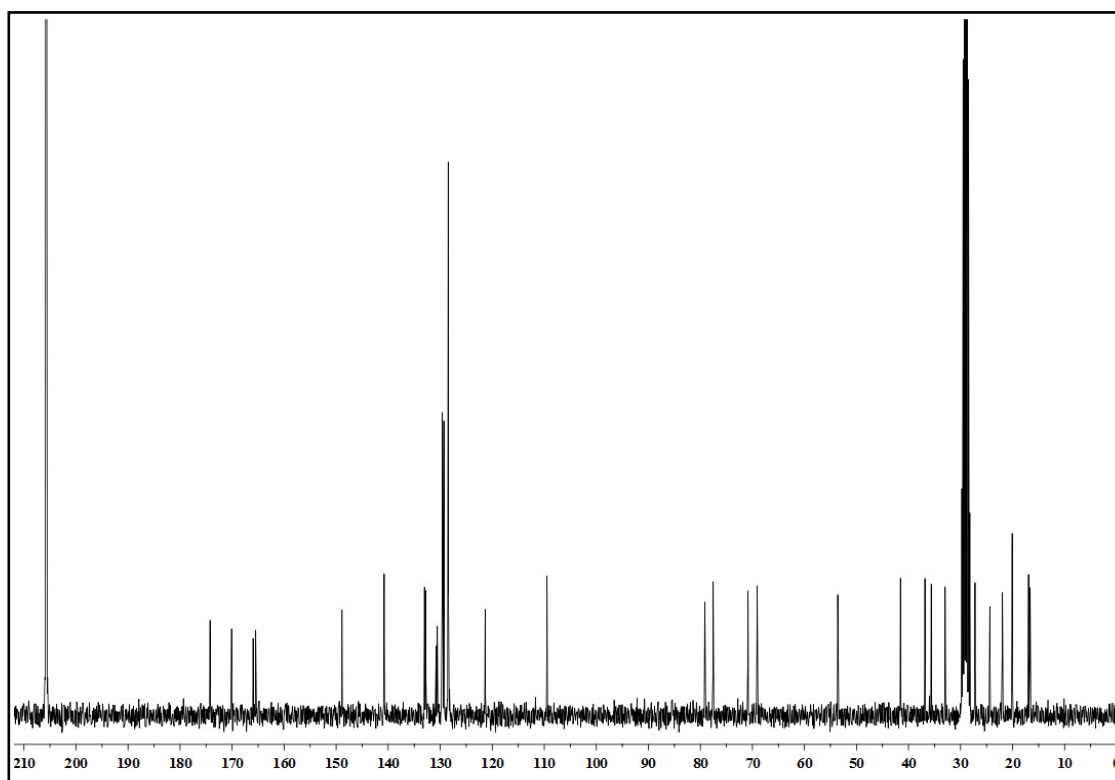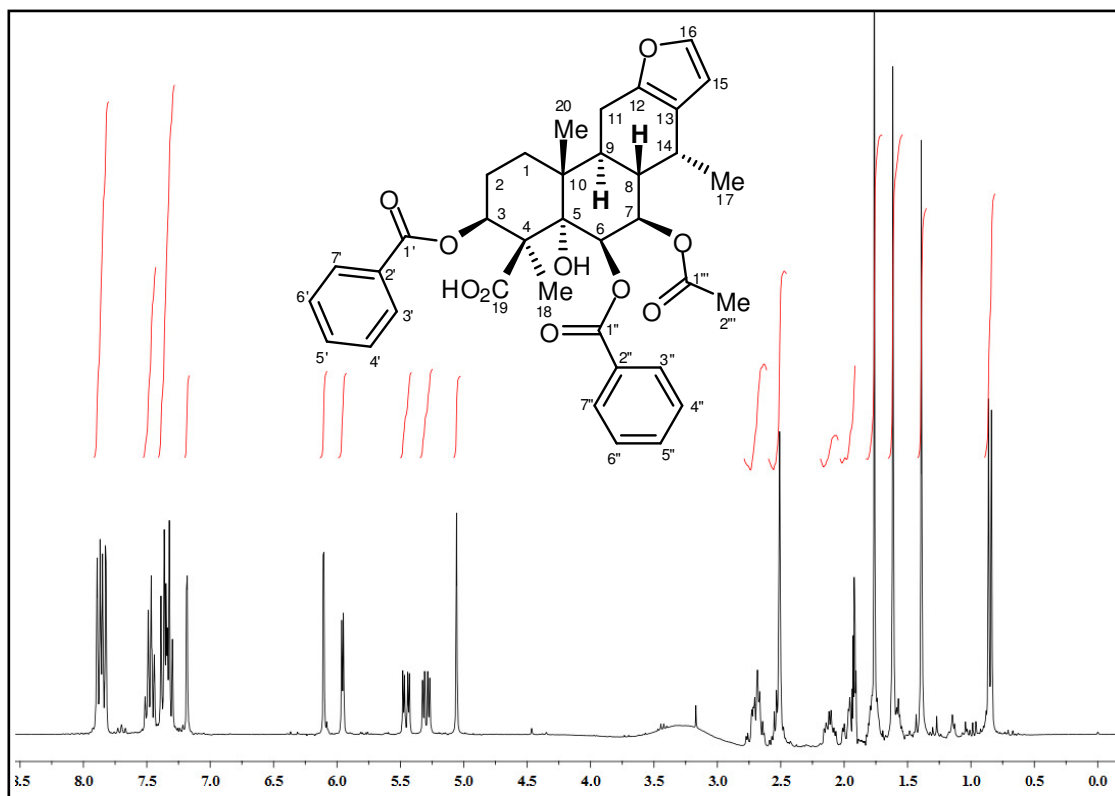**Figure 106** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP21**
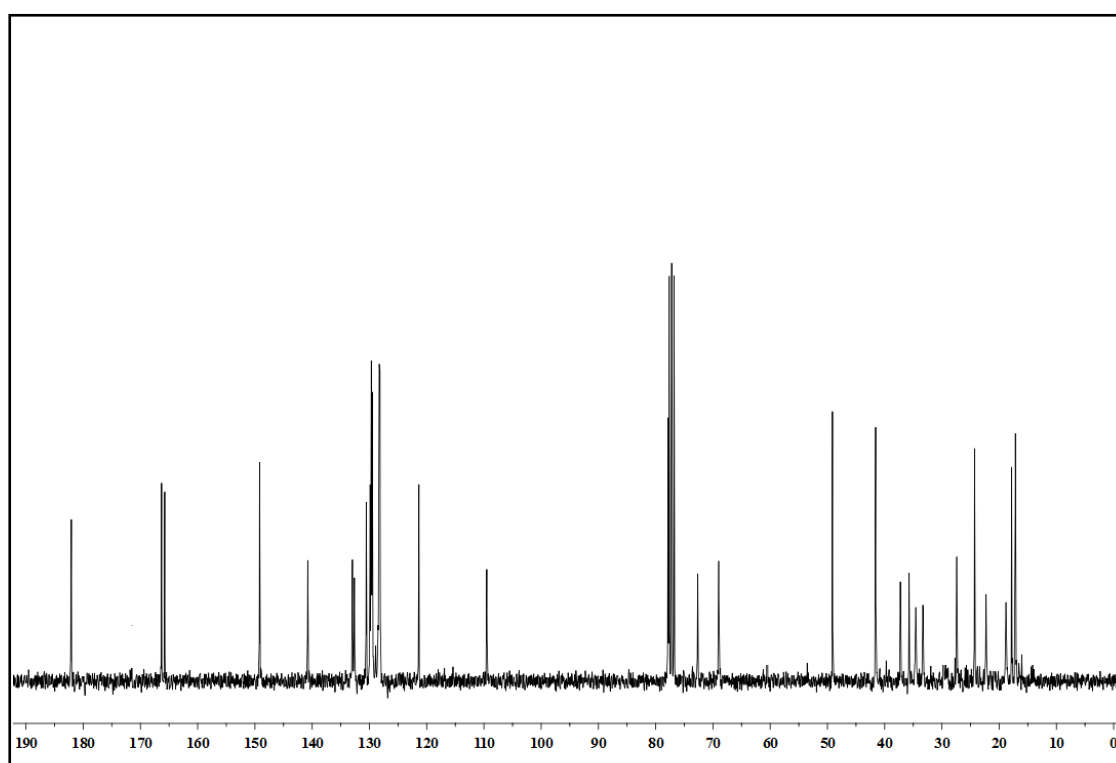


**Figure 107** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP21**

**Figure 108** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP22**



**Figure 109** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP22**

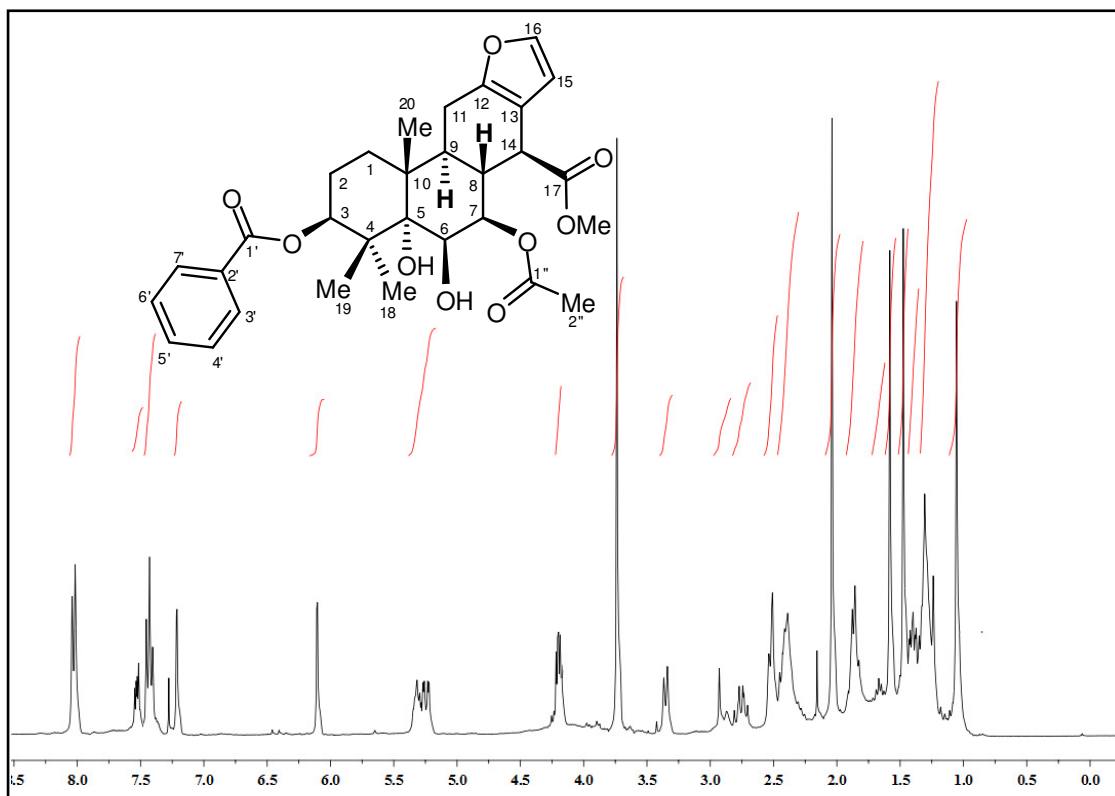**Figure 110** $^1$H NMR (300 MHz) (acetone-$d_6$) spectrum of compound **CP23**



**Figure 111** $^{13}$C NMR (75 MHz) (acetone-$d_6$) spectrum of compound **CP23**

**Figure 112** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP24**
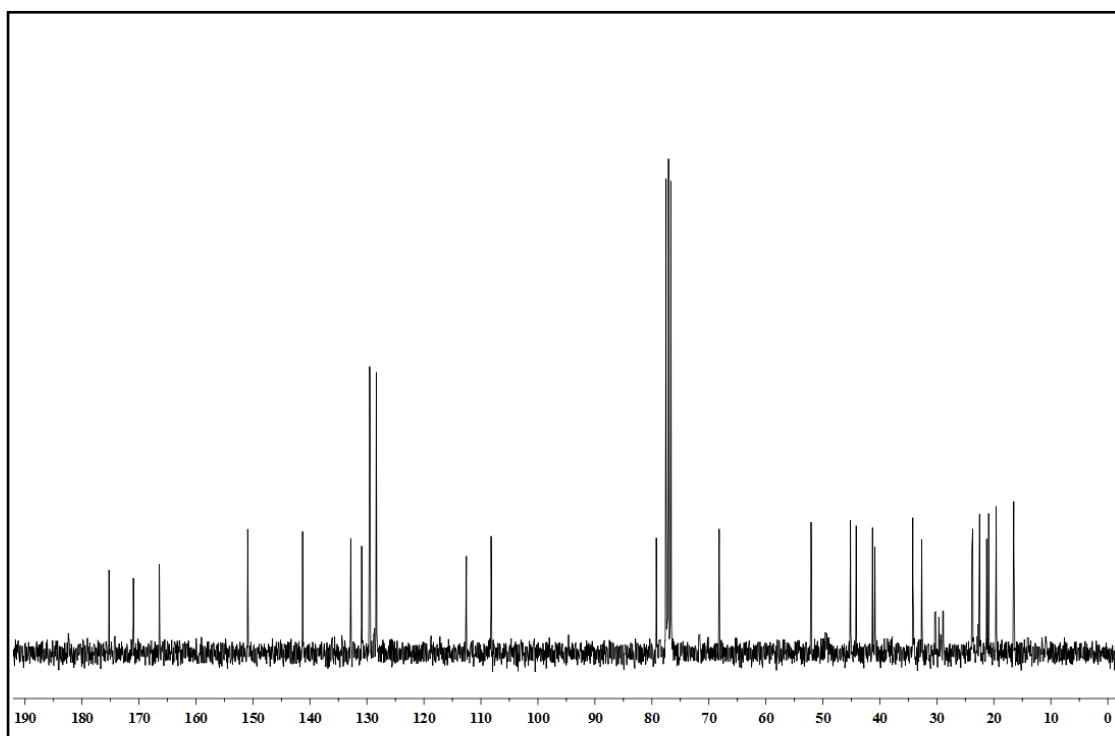


**Figure 113** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP24**
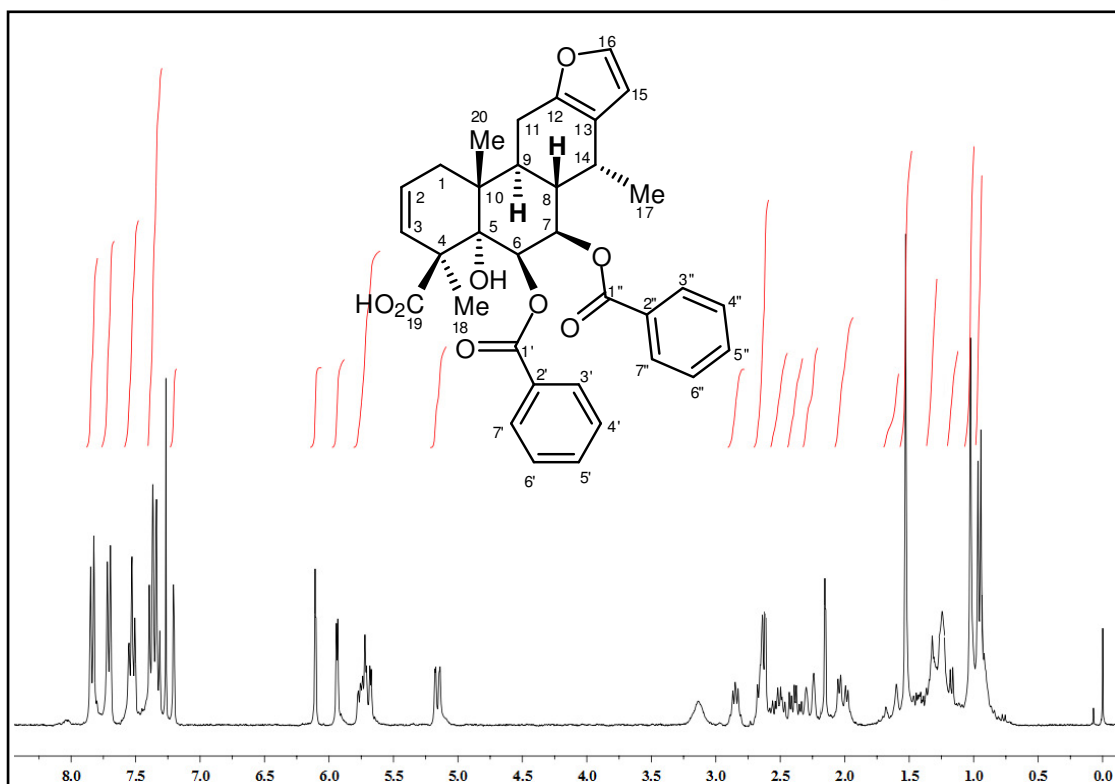
**Figure 114** $^1$H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CP25**
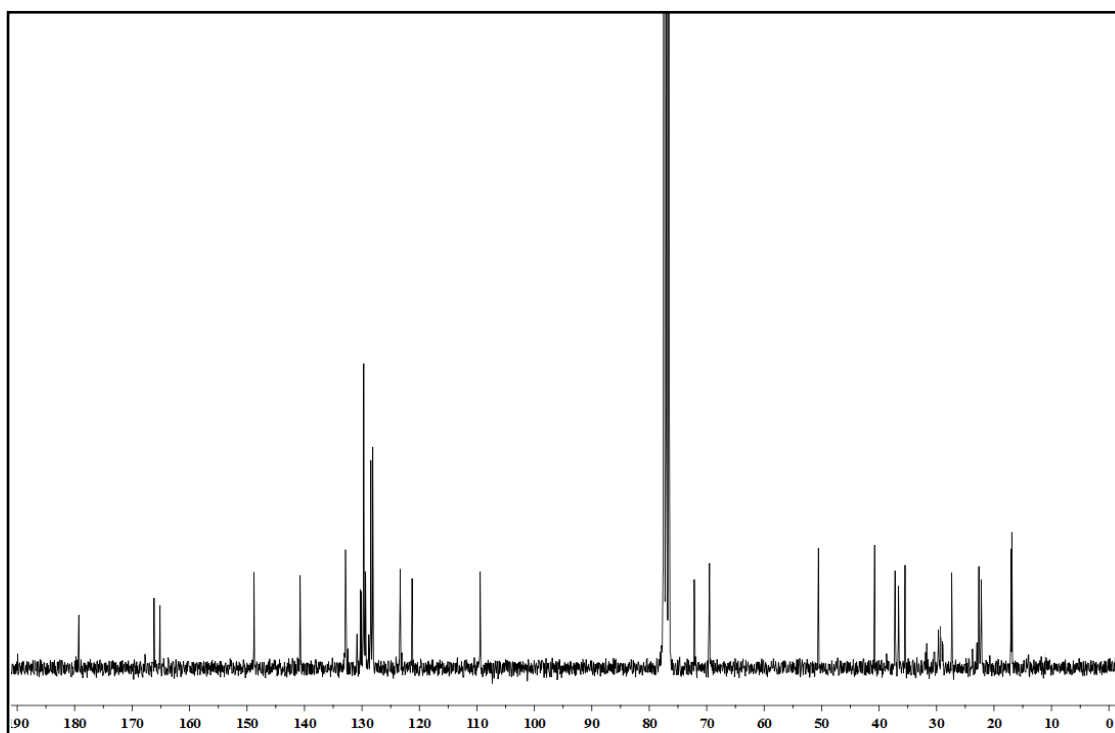


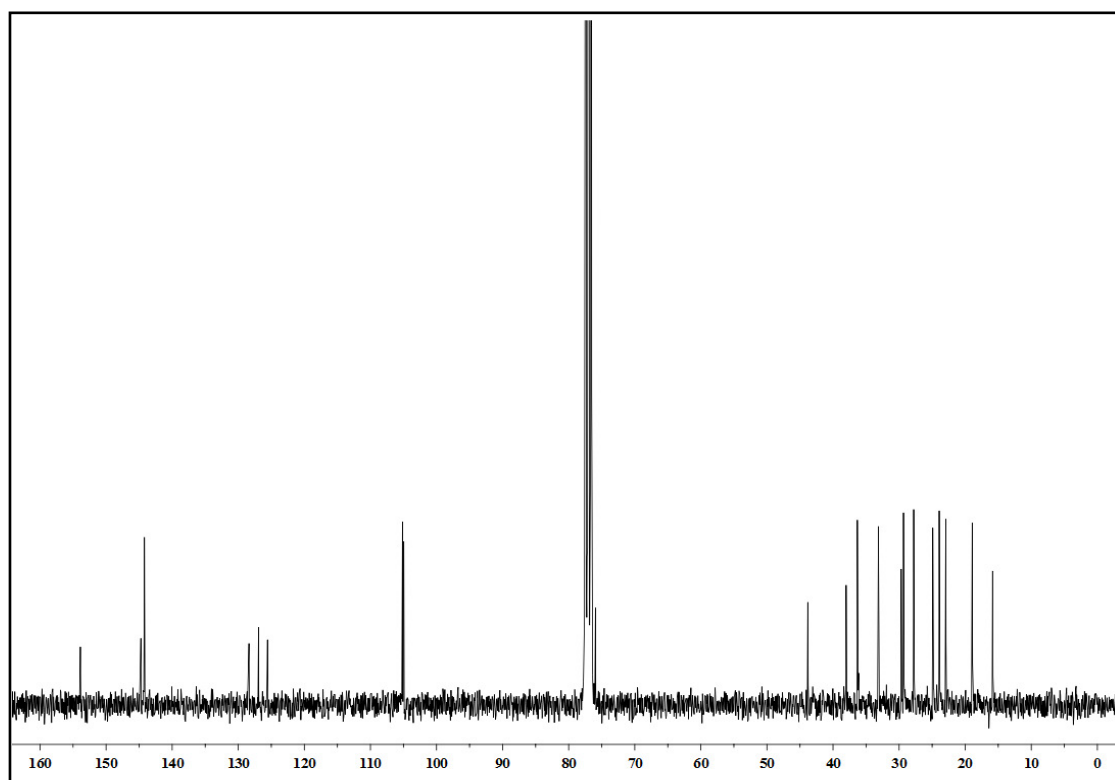**Figure 115** $^{13}$C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CP25**
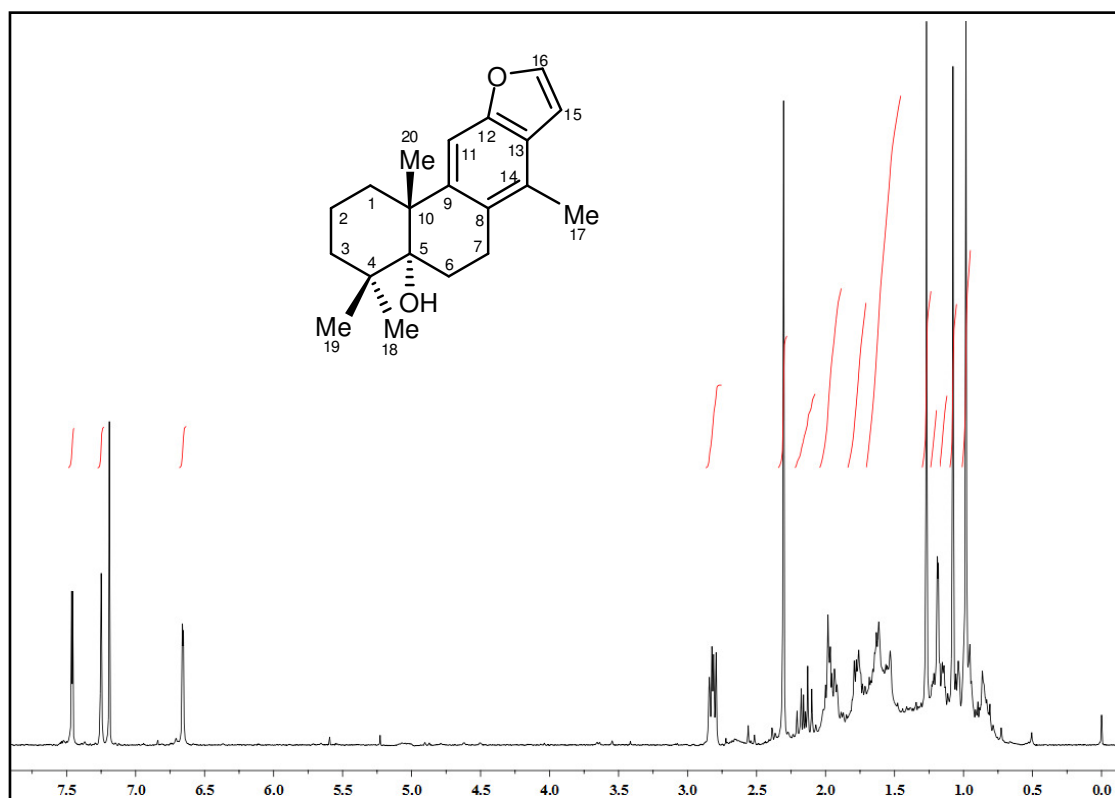
**Figure 116** ¹H NMR (300 MHz) (CDCl₃) spectrum of compound **CP26**



**Figure 117** ¹³C NMR (75 MHz) (CDCl₃) spectrum of compound **CP26**

# VITAE

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| Master of Science (Organic Chemistry) | Prince of Songkla University, Hat-Yai, Songkhla, Thailand | 2007 |

**Scholarship Awards during Enrolment**

Scholarship was awarded by the Office of the Higher Education Commission, Thailand for supporting by grant fund under the program Strategic Scholarships for Frontier Research Network for the Joint Ph.D. Program Thai Doctoral degree and the Prince of Songkla University.

**Lists of Publication and Proceeding**

**Publications**

**Yodsaoue, O.,** Karalai, C., Ponglimanont, C., Tewtrakul, S. and Chantrapromma, S. 2010. Potential anti-inflammatory diterpenoids from the roots of *Caesalpinia mimosoides* Lamk. Phytochemistry71, 1756–1764.

**Yodsaoue, O.,** Karalai, C., Ponglimanont, C., Tewtrakul, S. and Chantrapromma, S. 2011. Pulcherrins D–R, potential anti-inflammatory diterpenoids from the roots of *Caesalpinia pulcherrima*. Tetrahedron 67, 6838–6846.

Fun, H.-K., **Yodsaoue, O.,** Karalai, C. and Chantrapromma, S. 2010. Absolute configuration of isovouacapenol C. Acta Cryst. E66, o2059–o2060.

Fun, H.-K., **Yodsaoue, O**., Chantrapromma, S. and Karalai, C. 2010. Absolute configuration of vouacapen-5$\beta$-ol. Acta Cryst. E66, o2166–o2167

**Proceeding: International conferences**

**Yodsaoue, O.,** Karalai, C., Ponglimanont, C. and Tewtrakul, S. Anti-inflammatory constituents from the roots of *Caesalpinia mimosoides.*: PERCH-CIC Congress VI. Jomtein Palm Beach, Pattaya, Chonburi, Thailand. 3-6 May 2008. (Poster)

**Yodsaoue, O.,** Karalai, C., Ponglimanont, C., Tewtrakul, S. and Chantrapromma, S. Anti-inflammatory constituents from the roots of *Caesalpinia mimosoides*.: Commission on Higher Education Congress III: University Staff Development Consortium CHE-USDC Congress III. A-One The Royal Cruise Hotel, Pattaya, Chonburi, Thailand. 9-11 August 2010. (Poster)