Chemical Constituents from the *Citrus reticulata* Blanco

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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 *Citrus reticulata* Blanco
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Major Program	Organic Chemistry

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The Graduate School, Prince of Songkla University, has approved this thesis as fulfillment of the requirements for the Doctor of Philosophy Degree in Organic Chemistry.

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(Assoc. Prof. Dr. Teerapol Srichana)

Dean of Graduate School

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

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Uraiwan Phetkul .....Signature

(Miss. Uraiwan Phetkul)

Candidate

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

Uraiwan Phetkul .....Signature

(Miss Uraiwan Phetkul)

Candidate

ชื่อวิทยานิพนธ์ องค์ประกอบทางเคมีจากส้มจุก
ผู้เขียน นางสาวอุไรวรรณ เพ็ชรกูล
สาขาวิชา เคมีอินทรีย์
ปีการศึกษา 2556

บทคัดย่อ

การศึกษาองค์ประกอบทางเคมีของส่วนสกัดหยาบไดคลอโรมีเทนจากเปลือกกิ่งส้มจุกสามารถแยกสารองค์ประกอบได้ 15 สาร วิเคราะห์โครงสร้างของสารเหล่านี้ด้วยข้อมูลทางสเปกโทรสโกปีและเปรียบเทียบข้อมูลกับสารที่มีรายงานการวิจัยแล้ว พบว่าเป็นสารกลุ่มอะคริโดน (acridones) 6 สาร คือ 5-hydroxynoracronycine **CR2**, citracridone-I **CR6**, citrusinine-I **CR8**, citramine **CR9**, 2-methoxycitpressine **CR10** และ citracridone-III **CR14**, สารกลุ่มเดปไซด์ (depsides) 3 สาร คือ atranorin **CR1**, gustastatin **CR4**, 2-hydroxy-4-methoxy-6-(2-oxoheptyl)-2'-methoxy-4'-hydroxy-6-hetyl)-phenyl ester **CR12**, สารกลุ่มฟลาโวนอยด์ (flavonoids) 2 สาร คือ citflavanone **CR3**, citrusinol **CR7** สารกลุ่มคูมาริน (coumarin) 1 สาร คือ scopoletin **CR11** สารกลุ่มไอโซคูมาริน (isocoumarin) 1 สาร คือ 8-hydroxy-6-methoxy-pentylisocoumarin **CR5** สารกลุ่มลิโมนอยด์1 สาร limonin **CR13** และสารกลุ่มอนุพันธ์เบนซีน 1 สาร คือ 4-hydroxybenzoic acid **CR15** การทำเมทิลเลชัน (methylation) ของส่วนขั้วสูงจากส่วนสกัดหยาบไดคลอโรมีเทนจากส่วนเปลือกกิ่งสามารถแยกสารองค์ประกอบได้ 5 สาร พบสารกลุ่มลิโมนอยด์ 1 สาร คือ limonin **CR13**, สารกลุ่มอนุพันธ์รีซอไซคลิก resorcyclic derivative 2 สาร คือ methyl-2-hydroxy-4-methoxy-6-(2-oxoheptyl)-benzoate **CR16**, methyl 2,4-dimethoxy-6-heptyl-benzoate **CR17**, สารกลุ่มไอโซคูมาริน (isocoumarin) 1 สาร คือ 6,8-dimethoxypentyliso-coumarin **CR18** และสารกลุ่มอะคริโดน (acridones) 1 สาร คือ citracridone-II **CR19** สาร **CR12** และ **CR16** เป็นสารที่ยังไม่มีรายงานการวิจัย สาร **CR1, CR2, CR3, CR4, CR5, CR6, CR7, CR8, CR9, CR10, CR11, CR14, CR15, CR17, CR18, CR19** เป็นสารที่เคยมีการรายงานจากพืชชนิดอื่นแต่เป็นสารที่แยกได้ครั้งแรกจากส้มจุก

การศึกษาองค์ประกอบทางเคมีของส่วนสกัดหยาบไดคลอโรมีเทนและอะซีโตนจากส่วนเปลือกผลสามารถแยกสารองค์ประกอบได้ 11 สาร วิเคราะห์โครงสร้างของสารเหล่านี้ด้วยข้อมูลทางสเปกโทรสโกปีและเปรียบเทียบข้อมูลกับสารที่มีรายงานการวิจัยแล้ว พบว่าเป็นสารกลุ่มฟลาโวนอยด์ (flavonoids) 6 สาร คือ 5-demethoxynobiletin **CR20**, tangeretin **CR21**, nobiletin **CR22**, 5,7,8,4′-tetramethoxyflavone **CR23**, natsudaidain **CR24**, 5,7,4′-trihydroxy-

8,3′-dimethoxy-flavone **CR26**, สารกลุ่มฟลาโวนอยด์ ไกลโคไซด์ (flavonoid glycosides) 3 สาร คือ hesperidin **CR298**, naringin **CR29**, rutin **CR30** สารกลุ่มคูมารินไกลโคไซด์ (isocoumarin glycosides) 1 สาร คือ 8,3′-β-glucosyloxy-2′-hydroxy-3′-methylbutyl-7-methoxy-coumarin **CR27** และอนุพันธ์เบนซีน 1 สาร (benzene derivative) คือ 3,4-dihydroxy benzoic acid **CR25** การทำเมทิลเลชันของส่วนขั้วสูงจากส่วนสกัดอะซีโตนจากเปลือกผล สามารถแยกสารองค์ประกอบได้ 2 สาร เป็นสารกลุ่มฟลาโวนอยด์ (flavonoids) คือ naringenin trimethyl ether **CR31** และ 2,3-dihydro-5-hydroxy-4′,7-dimethoxyflavanone **CR32** สาร **CR25, CR26 และ CR27** เป็นสารที่เคยมีการรายงานจากพืชชนิดอื่นแต่เป็นสารที่แยกได้ครั้งแรกจากส้มจุก

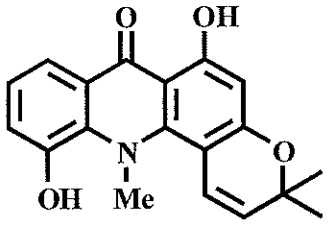
การศึกษาองค์ประกอบทางเคมีของส่วนสกัดหยาบไดคลอโรมีเทนและอะซีโตนจากส่วนใบสามารถแยกสารองค์ประกอบได้ 11 สาร วิเคราะห์โครงสร้างของสารเหล่านี้ด้วยข้อมูลทางสเปกโทรสโกปีและเปรียบเทียบข้อมูลกับสารที่มีรายงานการวิจัยแล้ว พบว่าเป็นสารกลุ่มฟลาโวนอยด์ (flavonoids) 4 สาร คือ 5-demethoxynobiletin **CR20**, tangeretin **CR21**, nobiletin **CR22**, 5,7,8,4′-tetramethoxyflavone **CR23**, 5, 7, 8, 3, 4′-pentamethoxyflavone **CR34** และ sudachitin **CR35**, สารกลุ่มคูมาริน (isocoumarin) 3 สาร คือ marmin **CR36**, crenulatin **CR38** และ isoimperatorin **CR39** และอนุพันธ์เบนซีน 1 สาร (benzene derivative) คือ 4-hydroxy benzaldehyde **CR37** และไตรเทอปีนนอยด์ (triterpenoid) 1 สาร คือ betulinic acid **CR33** สาร **CR35, CR36, CR37 และ CR38** เป็นสารที่เคยมีการรายงานจากพืชชนิดอื่นแต่เป็นสารที่แยกได้ครั้งแรกจากส้มจุก

การศึกษาองค์ประกอบทางเคมีของส่วนสกัดหยาบอะซีโตนจากส่วนเนื้อไม้ สามารถแยกสารองค์ประกอบได้ 6 สาร พบว่าเป็นสารกลุ่มอะคริโดน (acridones) 2 สาร citramine **CR9** และ1,3,5-trihydroxy-2,4-dimethoxy-10-methyl-10H-acridin-9-one **CR43**, กลุ่มลิโมนอยด์ 2 สาร คือ limonin **CR13** และ limonexic acid **CR42**, อนุพันธ์เบนซีน (benzene derivatives) 1 สาร คือ valencic acid **CR40** และสารกลุ่มคูมาเรท เอสเทอร์ (coumarate esters) 1 สาร คือ *p*-hydroxyphenylethyl-*p*-coumarate **CR41** สาร **CR43** เป็นสารที่ยังไม่มีรายงานการวิจัย สาร **CR40 และ CR41** เป็นสารที่เคยมีการรายงานจากพืชชนิดอื่นแต่เป็นสารที่แยกได้ครั้งแรกจากส้มจุก

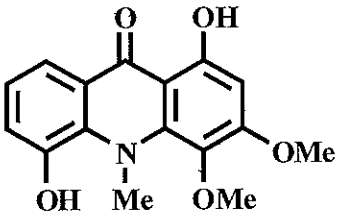
การศึกษาฤทธิ์ต้านจุลินทรีย์ พบว่าสาร **CR3** แสดงฤทธิ์ยับยั้งการเจริญของ *S. aureus* ATCC25923 และ methicillin-resistant *S. aureus* (MRSA) SK1 ด้วยค่าความเข้มข้นเท่ากับ 64 μg/mL. ในขณะที่ สาร **CR1-CR4, CR6, CR7, CR9, CR13, CR20, CR21** ไม่แสดง

ฤทธิ์ยับยั้งการเจริญของ *S. aureus* ATCC25923, MRSA SK1 and *E. coli* ATCC25922, *P. aeruginosa* ATCC27853 and *C. neoformans* ATCC90113, *C. albicans* NCPF3153, *C. neoforman* and *M. gypseum* ที่ความเข้มข้นเท่ากับ 200 μg/mL.

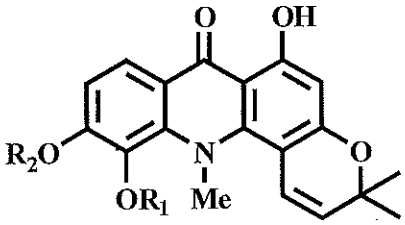
การศึกษาฤทธิ์ความเป็นพิษต่อเซลล์มะเร็ง T47D (Human ductal breast epithelial tumor cell line), AU565 (human breast adenocarcinoma cell line), SK-BR-3 (Human breast adenocarcinoma cell line)และA431 (human epidermoid carcinoma) พบว่า สาร **CR1**, **CR5**, **CR6**, **CR7**, **CR12** และ **CR36** แสดงฤทธิ์ปานกลางกับเซลล์มะเร็งดังกล่าว ในขณะที่สาร **CR2**, **CR3**, **CR8**, **CR9**, **CR14**, **CR20-CR22** และ **CR37** ไม่แสดงฤทธิ์ต้านเซลล์มะเร็งที่ความเข้มข้นเท่ากับ 100 μg/mL



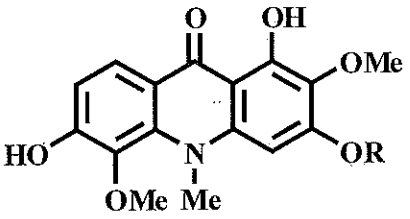
CR2



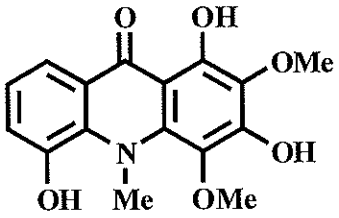
CR8



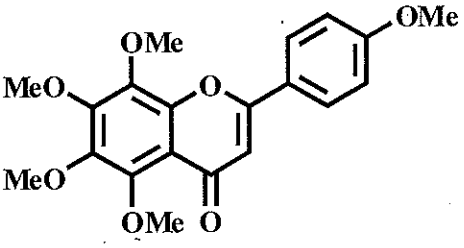
CR6: R_1 = Me, R_2 = H
CR14: R_1 = H, R_2 = H
CR19: R_1 = Me, R_2 = Me



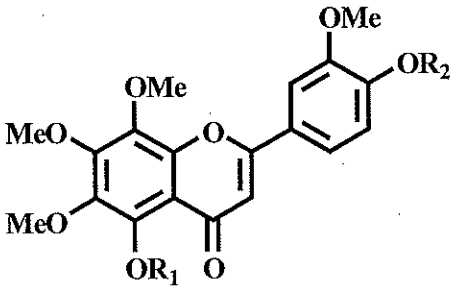
CR9: R = H
CR10: R = Me



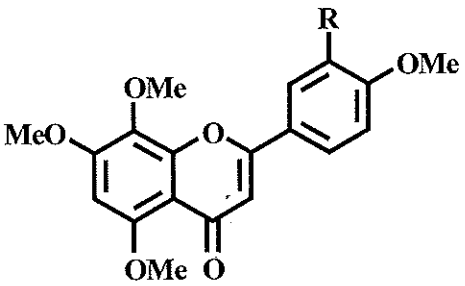
CR43



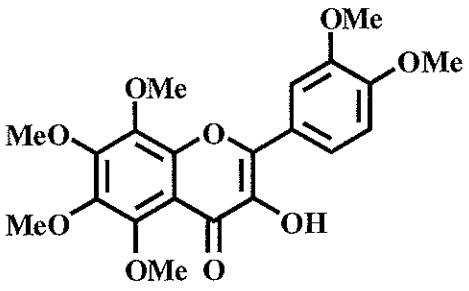
CR21



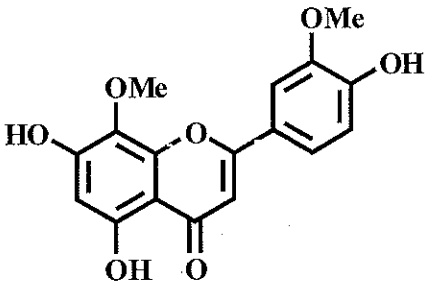
CR20: R_1 = H, R_2 = Me
CR22: R_1 = Me, R_2 = Me
CR35: R_1 = H, R_2 = H



CR23: R = H
CR34: R = OMe



CR24



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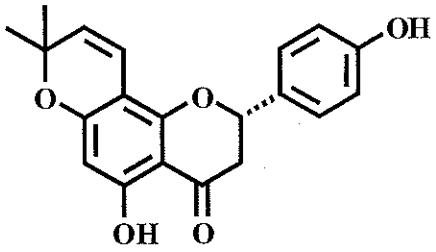

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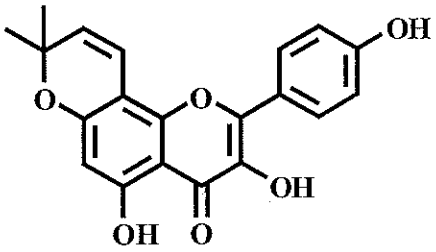
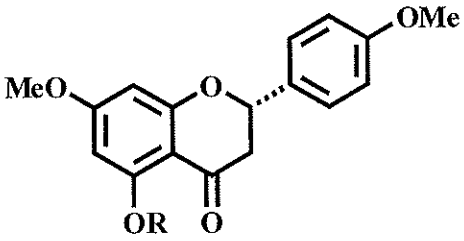
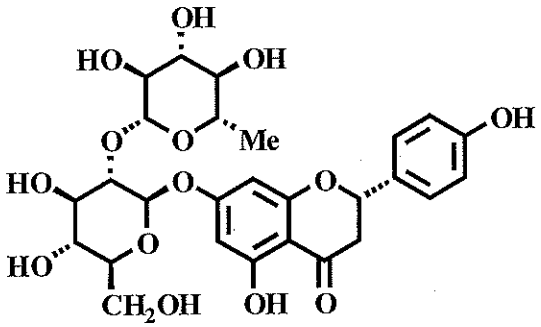


CR7



CR31: R = Me

CR32: R = H



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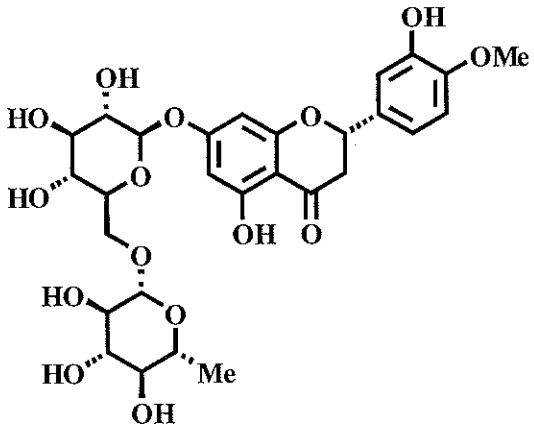

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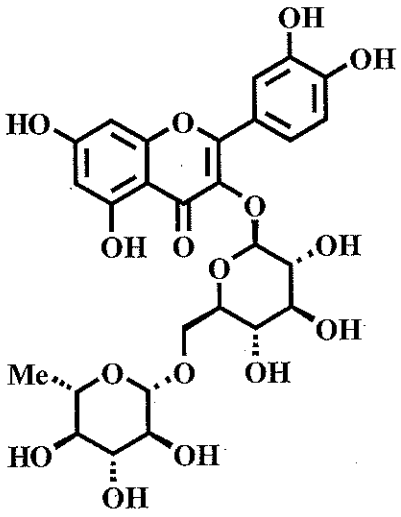

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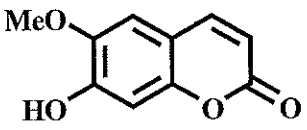

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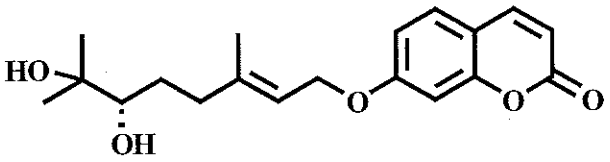

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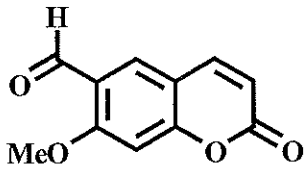

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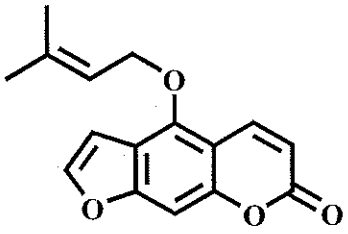

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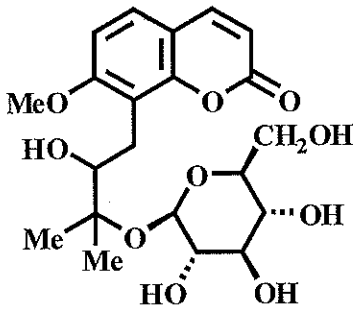

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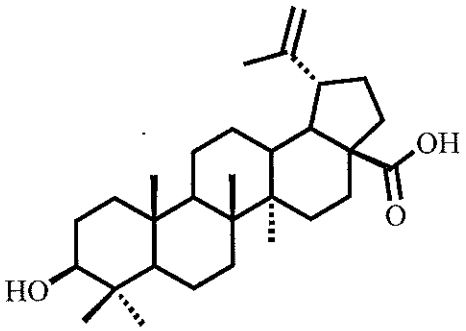

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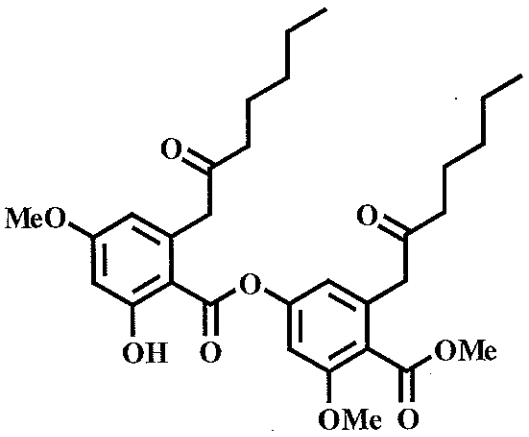

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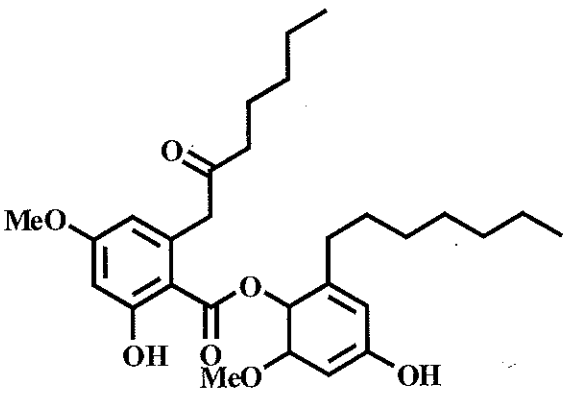

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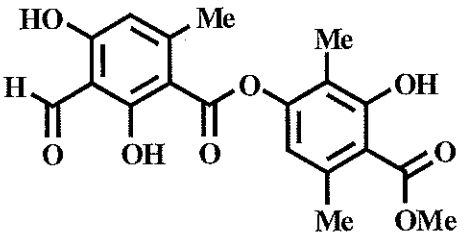

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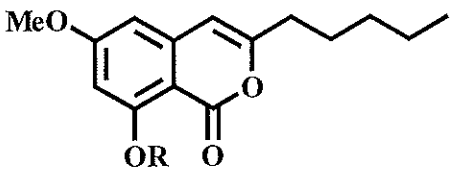
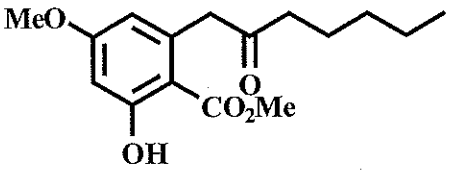


CR5: R = H

CR18: R = Me



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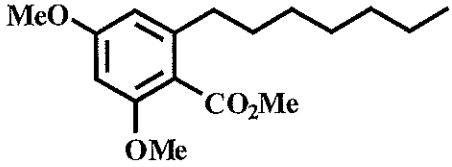

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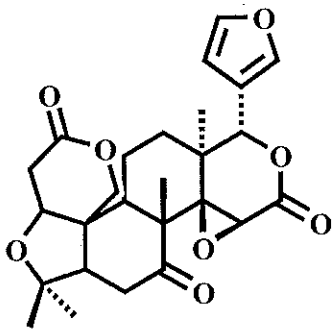

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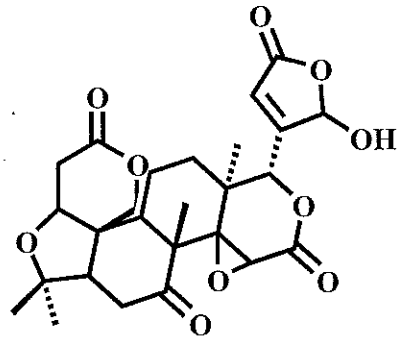

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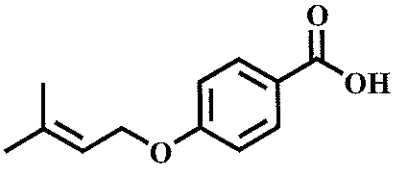

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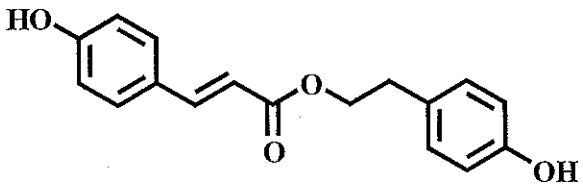

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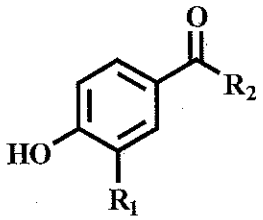


CR15: R_1 = H, R_2 = OH

CR25: R_1 = OH, R_2 = OH

CR37: R_1 = H, R_2 = H

Thesis Title Chemical Constituents from *Citrus reticulata* Blanco

Author Miss Uraiwan Phetkul

Major Program Organic Chemistry

Academic Year 2013

ABSTRACT

Chemical investigation of the dichloromethane extract of the branch barks of *Citrus reticulata* Blanco, resulted in the isolation of fourteen compounds. They were determined on the basis of spectroscopic analyses and by comparison of their spectroscopic data to those reported in the literatures. They are six acridones: 5-hydroxynoracronycine **CR2**, citracridone-I **CR6**, citrusinine-I **CR8**, citramine **CR9**, 2-methoxycitpressine **CR10**, citracridone-III **CR14**, three depsides: atranorin **CR1**, gustastatin **CR4**, 2-hydroxy-4-methoxy-6-(2-oxoheptyl)-2'-methoxy-4'-hydroxy-6-hetyl)-phenyl ester **CR12**, two flavonoids: citflavanone **CR3**, citrusinol **CR7**, one coumarin: scopoletin **CR11**, one isocoumarin: 8-hydroxy-6-methoxy-pentylisocoumarin **CR5**, one limonoid: limonin **CR13** and one benzene derivative: 4-hydroxybenzoic acid **CR15**. Methylation of the high polarity fractions from the branch barks gave five known compounds: They are one limonoid: limonin **CR13**, two resorcyclic derivatives: methyl-2-hydroxy-4-methoxy-6-(2-oxoheptyl)-benzoate **CR16**, methyl-2,4-dimethoxy-6-heptylbenzoate **CR17**, one isocoumarin: 6,8-dimethoxy-pentylisocoumarin **CR18** and one acridone: citracridone-II **CR19**. Compounds **CR11** and **CR16** are new naturally occurrence compounds. Compounds **CR1, CR2, CR3, CR4, CR5, CR6, CR7, CR8, CR9, CR10, CR11, CR14, CR15, CR17, CR18** and **CR19** were previously reported but they were the first isolated from *C. reticulata*.

Chemical investigation of the dichloromethane and acetone extracts of the peels of *C. reticulata* Blanco, resulted in the isolation of eleven compounds. They are six flavonoids: 5-demethoxynobiletin **CR20**, tangeretin **CR21**, nobiletin **CR22**, 5,7,8,4′-tetra -methoxyflavone **CR23**, natsudaidain **CR24,** 5,7,4′-trihydroxy-8,3′-dimethoxy-flavone **CR26**, three flavonoid glycosides: hesperidin **CR28**,

naringin **CR29**, rutin **CR30,** one isocoumarin glycoside: 8,3′-*β*-glucosyloxy-2′-hydroxy-3′-methylbutyl-7-methoxycoumarin **CR27** and one benzene derivative: 3, 4-dihydroxy benzoic acid **CR25**. Methylation of the high polarity fractions from the peels gave two known compounds: naringenin trimethyl ether **CR31** and 2,3-dihydro-5-hydroxy-4′,7-dimethoxy-flavanone **CR32**. Compounds **CR25, CR26** and **CR27** were previously reported but they were the first isolated from *C. reticulata.*

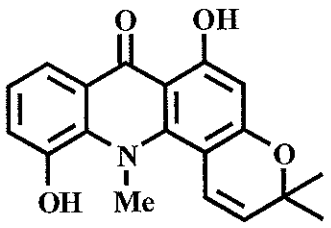
Chemical investigation of the dichloromethane and acetone extracts of the leaves of *C. reticulata* Blanco resulted in the isolation of eleven compounds. They are four flavonoids: 5-demethoxynobiletin **CR20**, tangeretin **CR21**, nobiletin **CR22**, 5,7,8,4′-tetramethoxyflavone **CR23**, 5,7,8,3′,4′-pentamethoxyflavone **CR34** and sudachitin **CR35**, three coumarin: marmin **CR36**, crenulatin **CR38** and isoimperatorin **CR39**, one benzene derivative: 4-hydroxy benzaldehyde **CR37** and one triterpenoid: betulinic acid **CR33**. **CR36, CR37** and **CR38** were previously reported but they were the first isolated from *C. reticulata.*

Chemical investigation of the acetone extract of the woods of *C. reticulata* Blanco, resulted in the isolation of six compounds. They are two acridones: citramine **CR9** and 1,3,5-trihydroxy-2,4-dimethoxy-10-methyl-10H-acridin-9-one **CR43**, two limonoids: limonin **CR13**, limonexic acid **CR42**, one benzene derivatives; valencic acid **CR40** and one coumarate ester: *p*-hydroxyphenylethyl-*p*-coumarate **CR41**. Compound **CR43** is new naturally occurrence compound. **CR9, CR40** and **CR41** were previously reported but they are the first isolated from *C. reticulata.*

Compound **CR3** inhibited the growth of *S. aureus* ATCC25923 and MRSA SK1with MIC values of 64 and 64 *μ*g/mL whereas compounds **CR1-CR4, CR6, CR7, CR9, CR13, CR20, CR21** had no effect on *S. aureus* ATCC25923, methicillin-resistant *S. aureus* (MRSA) SK1 and *E. coli* ATCC25922, *P. aeruginosa* ATCC27853 and *M. gypseum, C. neoformans* ATCC90113, *C. neoforman,* and *C. albicans* NCPF3153 up to a dose of 200 *μ*g/mL.

Compounds **CR1, CR5, CR6, CR7, CR13** and **CR36** affected the growth of cell lines T47D (Human ductal breast epithelial tumor cell line), AU565 (human breast adenocarcinoma cell line), SK-BR-3 (Human breast adenocarcinoma

cell line) and A431 (human epidermoid carcinoma) with IC50 values of less than 100 μg/mL. Compounds **CR1, CR5, CR6, CR7, CR12** and **CR36** showed moderate cytotoxicity against the tested cancer cell lines. Compounds **CR2, CR3, CR8, CR9, CR14, CR20-CR22** and **CR37** showed no cytotoxicity against the tested cancer cell lines up to the final concentration of 100 μg/mL.



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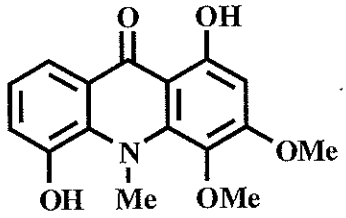

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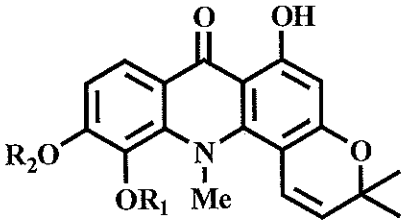

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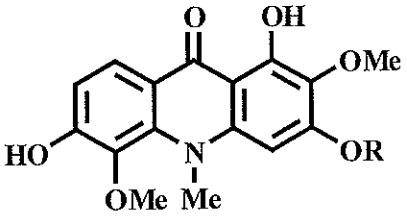

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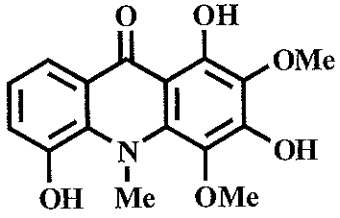

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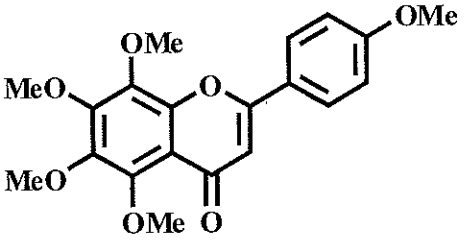

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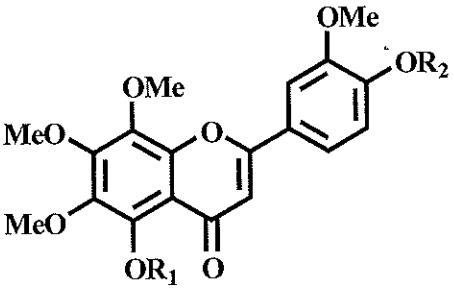

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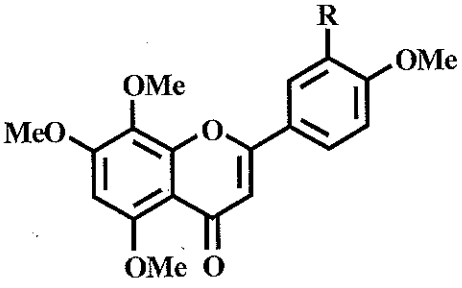

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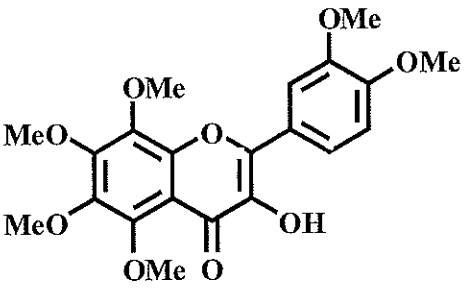

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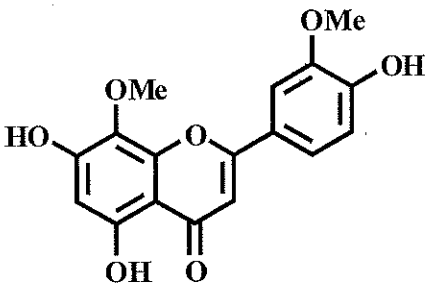

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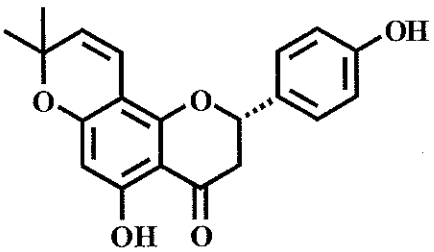

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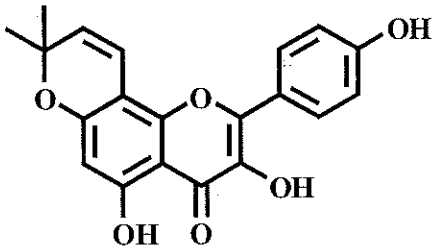

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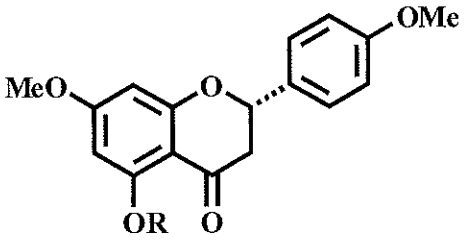

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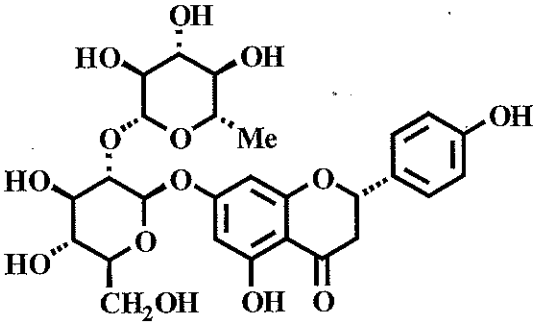

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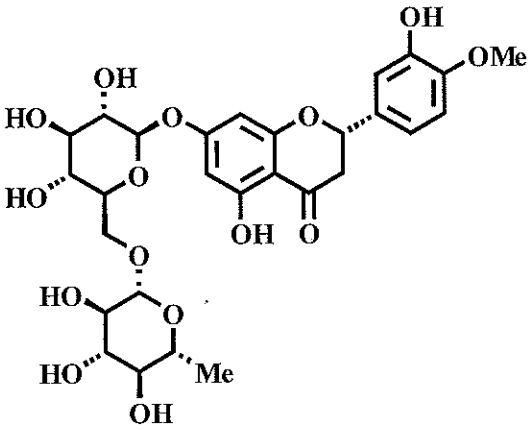

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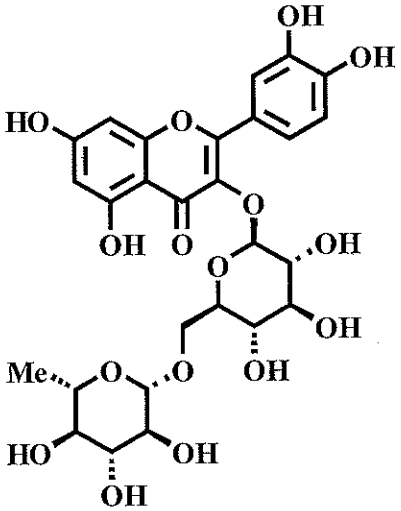

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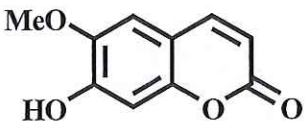

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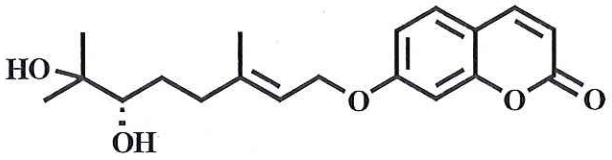

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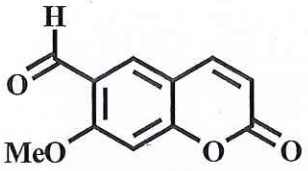

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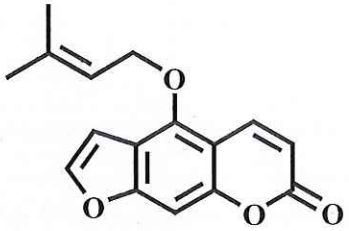

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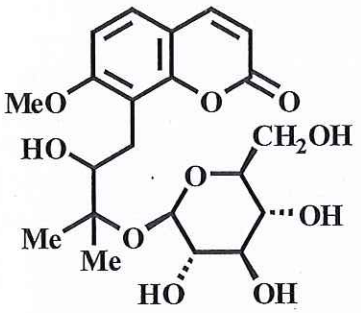

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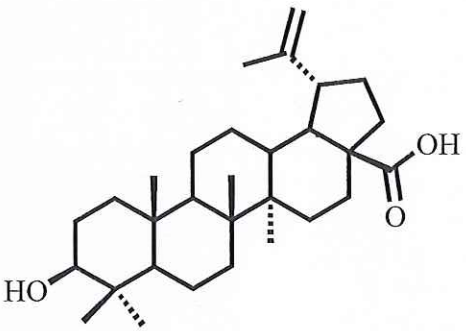

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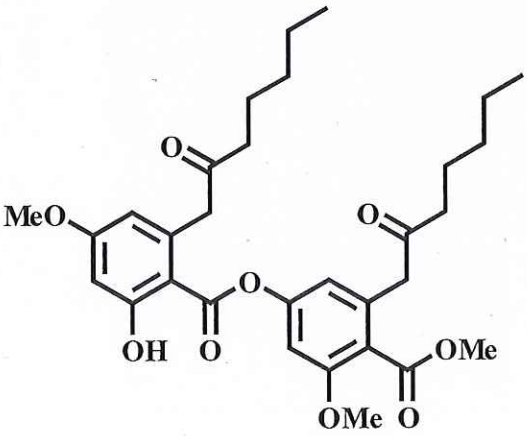

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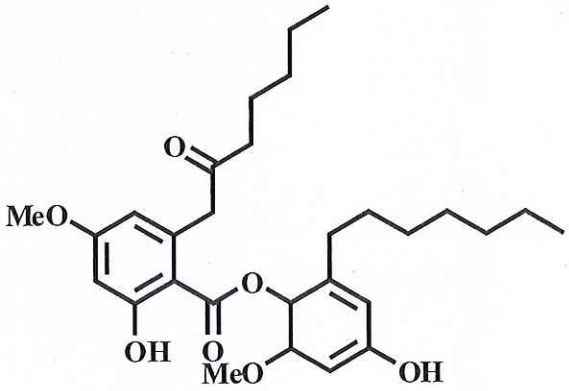

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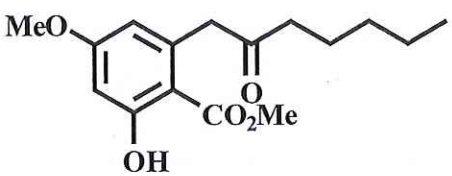

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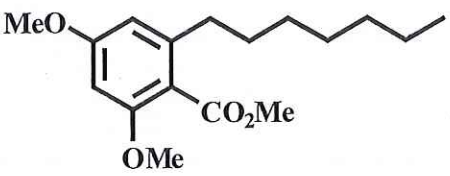

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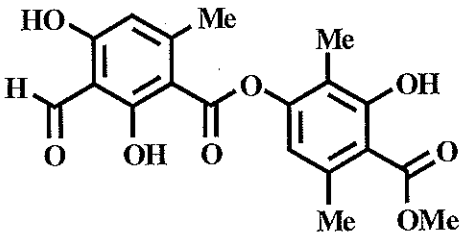

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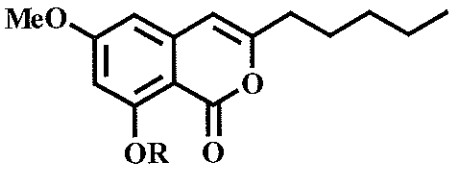

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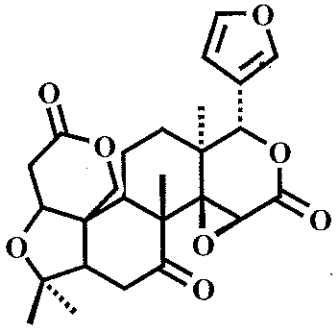

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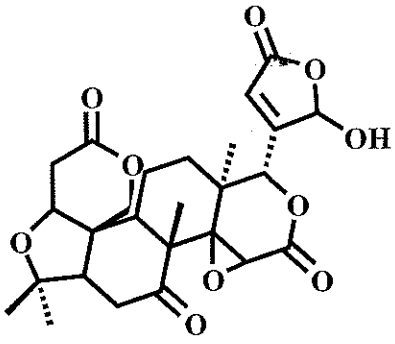

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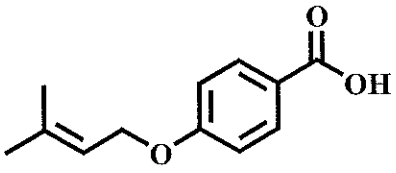

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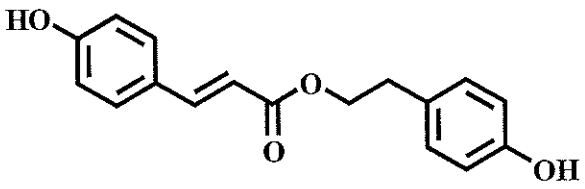

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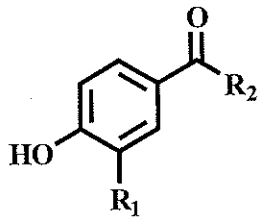

CR15: R_1 = H, R_2 = OH

CR25: R_1 = OH, R_2 = OH

CR37: R_1 = H, R_2 = H

ACKNOWLEDGEMENTS

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My sincere thanks are expressed to Associate Professor Dr. Souwalak Phongpaichit, Department of Microbiology, Faculty of Science, Prince of Songkla University for antibacterial activities testing, to Associate Professor Dr. Ramida Watanapokasin, Department of Biochemistry, Faculty of Medicine, Srinkharinwirot University for cytotoxic activities testing and to Mr. J. Wai, Department of Biology, Faculty of Science, Prince of Songkla University for plant identification.

I would like to acknowledge my sincere thanks to the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant NO. PHD/0129/2552) and PSU Ph.D. Scholarship for a scholarship and financial support. The Graduate School, Prince of Songkla University is gratefully acknowledged for partial financial support.

I would like to thank Department of Chemistry, Faculty of Science, Prince of Songkla University for making available the facilities used in this research.

Finally, none of this would have been possible without love and encouragement of my family. I thank them for their kindness and valuable advice. Everything will always keep in my mind.

Uraiwan Phetkul

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LIST OF ABBREVIATIONS AND SYMBOLS

s	=	singlet
d	=	doublet
t	=	triplet
m	=	multiplet
dd	=	doublet of doublets
dt	=	doublet of triplets
br	=	broad
br s	=	broad singlet
g	=	gram
kg	=	kilogram
mg	=	milligram
ml	=	milliliter
μg	=	microgram
%	=	percent
nm	=	nanometer
m.p.	=	melting point
cm^{-1}	=	reciprocal centimeter (wave number)
δ	=	chemical shift relative to TMS
J	=	coupling constant
λ_{max}	=	maximum wavelength
ν	=	absorption frequencies
ε	=	molar extinction coefficient
^{o}C	=	degree celcius
MHz	=	Megahertz
ppm	=	part per million
IR	=	Infrared
UV	=	Ultraviolet
NMR	=	Nuclear Magnetic Resonance
2D NMR	=	Two Dimentional Nuclear Magnetic Resonance

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

COSY	=	Correlated Spectroscopy
DEPT	=	Distortionless Enhancement by Polarization Transfer
HMBC	=	Heteronuclear Multiple Bond Correlation
HMQC	=	Heteronuclear Multiple Quantum Coherence
TMS	=	tetramethylsilane
Acetone-d_6	=	deuteroacetone
DMSO-d_6	=	deuterodimethyl sulphoxide
$CDCl_3$	=	deuterochloroform
MeOH	=	methanol
CH_2Cl_2	=	dichloromethane
TLC	=	thin layer chromatography

CHAPTER 1
INTRODUCTION

1.1 Introduction

Nowadays natural products from plants were extensively used, such as ingredients for medicines, cosmetics or supplementary food. Medicinal properties of each plant depend on its chemical constituents. Therefore, the research on the study of chemical constituents from plants is primarily necessary.

Citrus is a common term and is a genus of flowering plants in the family Rutaceae, originating in tropical and subtropical Southeast Asia. *Citrus* is an important crop mainly used in the food industries for production of fresh juice. The main by-product of its processing is fruit peels, which represent troughly one half of the fruit mass. *Citrus* species have been shown to possess many constituents which have important effects on the human health viz. vitamin C, carotenoids (β-carotene), flavonoids, limonoids, coumarins, acridone alkaloids, high quality soluble fiber, vitamin-B complex and related nutrients.

The flavonoids in *Citrus* were reported for pharmacological and biological activities such as antioxidant, antiviral, antiallergic, cardioprotective, and anticarcinogenic effects (Tripoli *et al.*, 2007). Polymethoxyflavones were reported to exhibit anticancer effects against human cancer cell lines (Li *et al.*, 2007; Du *et al.*, 2010; Hamdal *et al.*, 2011), anti-inflammatory and antifungal (Li *et al.*, 2012). Methanolic extracts from peel and tissues of *C. reticulata* var. Ponkan, *C. reticulata* var. Page and *C. reticulata* var. Clementine contained phenolic compounds and flavonoids. These compounds were determined for a DPPH radical scavenging assay, they were found to exhibit good antioxidants activity (Ghasemi, Ghasemi & Ebrahimzadeh, 2009).

Citrus reticulata (Neck orange) is widely growing in the southern part of Thailand. Literature survey also reveal that *C.reticulata* fruit peels were widely used by the ancients for treatment of different kinds of diseases. We are therefore study the chemical constituents in the woods, leaves, peels and branch barks of *C.*

reticulata Blanco and evaluated for their antioxidant, antibacterial and cytotoxic activities.

Genus *Citrus*:

The genus *Citrus* is undoubtedly the most important genus in the family Rutaceae. *Citrus* is native to the tropics of Asia, South China, Vietnam, Philippines, Thailand, India, and the Malaysian Peninsula. *Citrus* growing regions include Mediterranean, subtropical, semitropical, and tropic zones.

1.2 Review of Literatures

1.2.1 The Chemical Constituents from the Genus *Citrus*

Seventeen species of genus *Citrus* (Rutaceae) have been found in Thailand, they are *C. aurantifolia* (Christm.) Swingle, *C. aurantium* L var. *aurantium, C. halimii* B.C. Stone, *C. hystrix* DC., *C. ichangensis* Swingle, *C.japonica* Thunb, *C. latipes* Swingle, *C. limon* (L.) Burm.f., *C.macroptera* Mont., *C. madurensis* Lour.,*C. maxima* Merr., *C.medica* Linn., *C. medica* L. var. *sarcodactylis.* Swing, *C. nobilis* Lour., *C. reticulata* Blanco, *C. semperflorens* Lush. and *C.sinensis* (L.) Osbeck.

Various types of secondary metabolites from this genus have been reported, including acridone alkaloids, coumarins, flavonoids and limonoids. The chemical constituents isolated from the *Citrus* genus were summarized in **Table 1** (The literature survey from SciFinder Scholar database: 1961-2013).

Table 1 Compounds isolated from the plants of *Citrus* genus in Thailand

Scientific name/ Investigated part/ Compounds	Structures	Bibliography
C. aurantifolia		
Peels		
Bergapten	**B5**	Nagwa *et al.*, 2010
Bergaptol	**B6**	
Psoralene	**B11**	
Isopimpinellin	**B12**	
Imperatorin	**B13**	
Leave		
Isobergapten	**B7**	Nagwa *et al.*, 2010
Angelicin	**B8**	
***C. hystrix* DC**		
Fruit		
Oxypeucedanin	**B4**	Murakami *et al.*, 1999
Bergamottin	**B9**	
9-[(6',7'-Dihydroxy-3',7'-dimethyl-2-octe-nyl)oxy]psoralen	**B10**	
***C. japonica* Thunb**		
Seed		
Limonin	**D1**	Hasegawa *et al.*, 1998
Ichangensin	**D5**	
Calamin	**D8**	
Limonyl acetate	**D9**	
Limonin-17-β-*D*-glucopyranoside	**D11**	

Table 1 continued

Scientific name/ Investigated part/ Compounds	Structures	Bibliography
C. limon Burm. f.		
Peels		
5,7,2'-Trimethoxyflavanone	**A16**	Ryo *et al.*, 1931
7,2'-Dimethoxyflavanone	**A17**	
Ichangin 4-*β*-glucopyranoside	**D12**	Yoshiharu *et al.*, 1990
Nomilinic acid 4-*β*-glucopyranoside	**D13**	
Fruit		
6-*C*-*β*-Glucosyldiosmin	**A18**	Miyake *et al.*, 1997
6,8-Di-*C*-*β*-glucosyldiosmin	**A19**	
Homoeriodictyol-7-*O*-rutinoside	**A25**	
Limonflavonyl lactone A	**A1**	Sultana *et al.*, 2008
Limonflavonyl lactone B	**A2**	
C. maxima		
Stem bark		
5-Hydroxynoracronycine	**C1**	Teng *et al.*, 2005
Citracridone-III	**C3**	
Citrusinine-I	**C4**	
Glycocitrine-I	**C5**	
Grandisine-I	**C6**	
Natsucitrine-II	**C7**	
5-Hydroxynoracronycine alcohol	**C8**	

Table 1 continued

Scientific name/ Investigated part/ Compounds	Structures	Bibliography
C. medica* L. var. *sarcodactylis		
3,5,6-Trihydroxy-4',7-dimethoxyflavone	**A11**	He *et al.*, 1985
3,5,6-Trihydroxy-3',4',7-trimethoxy-flavone	**A12**	
Scopoletin	**B14**	
6,7-Dimethoxycoumarin	**B15**	
Citflavone	**A4**	Yin *et al.*, 2004
Diosmetin	**A5**	
Diosmin	**A20**	
7-Hydroxy-5-methoxycoumarin	**B19**	
Limettin	**B20**	
Umbelliferone	**B21**	
Limonin	**D1**	
Obacunone	**D2**	
Nomilin	**D3**	
***C. mitis* Blanco**		
Citromitin	**A9**	Sastry *et al.*, 1961
5-Demethylcitromitin,	**A10**	
C. nobilis		
Root bark		
2,2-Dimethylpyranoflavanol	**A3**	Wu *et al.*, 1987
Xanthyletin	**B1**	
Xanthoxyletin	**B2**	
Nordentatin	**B3**	
Crenyllatin	**B16**	

Table 1 Continued

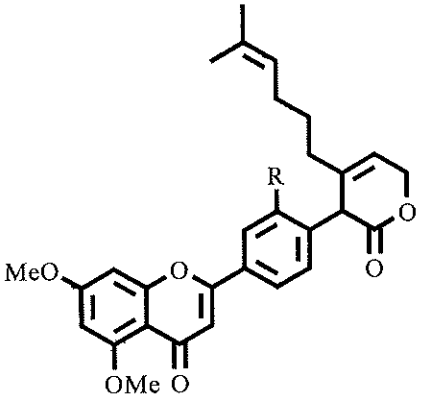
Scientific name/ Investigated part/ Compounds	Structures	Bibliography
Suberosin	**B17**	
Seed	**B18**	Bui *et al.*, 2004
Suberenol	**C1**	
5-Hydroxynoracronycine	**C2**	
Citrusinine-I	**C4**	
Citracridone-I	**C9**	
Citropone	**D1**	
Limonin	**D2**	
Obacunone	**D3**	
Nomilin	**D4**	
Deacetyl nomilin	**D7**	
Limonexic acid	**D9**	
Citrobilin	**D10**	
Ichangensin		
***C. reticulata* Blanco**		
Seed		
Limonin	**D1**	Jayaprakash et al.,
Obacunone	**D2**	1997/ Khalil *et al.*,
Nomilin	**D3**	2003
Deacetylnomilin	**D4**	
Ichangin	**D5**	
Isolimonexic acid methyl ether	**D6**	
Peels		
Isosinensetin	**A6**	Du et al., 2010/ Wang
Tangeretin	**A7**	*et al.*, 2005

Table 1 Continued

Scientific name/ Investigated part/ Compounds	Structures	Bibliography
Tetramethyl-*o*-isoscutellarein	**A8**	
Sinensetin	**A13**	
Nobiletin	**A14**	
Narirutin	**A22**	
Hesperidin	**A21**	
Didymin	**A23**	
5-demethylnobiletin,	**A24**	
6,7-dimethylesculetin	**A15**	
Stigmasterol	**E1**	
β-sitosterol	**E2**	
Cholesterol	**E3**	
Campesterol	**E4**	

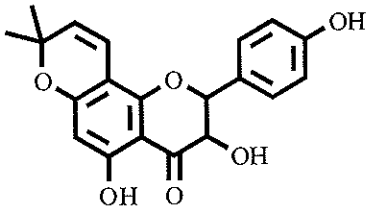
Flavonoids

The chemical structure of flavonoids is composed of two aromatic rings, which are connected through a pyrone ring. Flavonoids are a large class of naturally occurring aromatic secondary metabolites. Citrus plants contain a wide range of flavonoid constituents. Flavonoids that were found in this genus were **polymethoxyflavanoids**: e.g, sinensetin, nobilitin, 5-demethylnobiletin; **flavanones**: e.g. hesperitin, eriodictyol, naringinin, **flavones**: e.g. diosmetin, luteolin; **flavonols**; e.g. 1-limocitrin, 2-limocitrol, isolimocitrol, **flavanols**: 2,2-dimethylpyranoflavanol, **flavanone glycosides**: hesperidin, narirutin, eriocitrin, didymin, homoeriodictyol-7-*O*-rutinoside, **flavone glycosides**: diosmin, **flavonol glycosides**: rutin, quercetin-3-*O*-rutinoside-7-*O*-glucoside

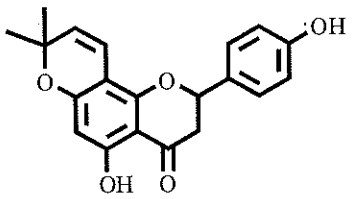


A1: R = H: Limonflavonyl lactone A

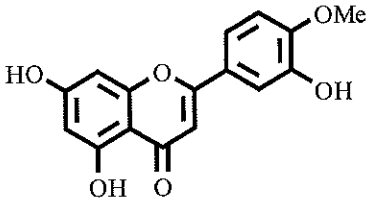
A2: R = OMe: Limonflavonyl lactone B



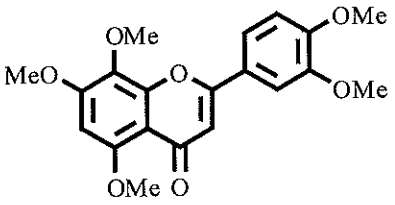
A3: 2,2-Dimethylpyrano flavanol



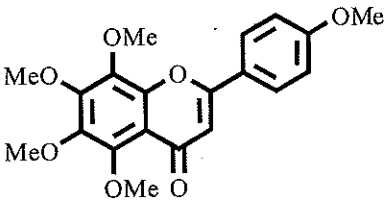
A4: Citflavanone



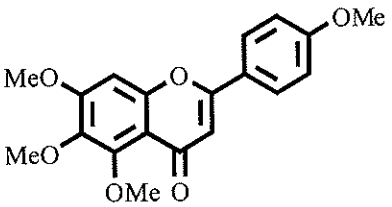
A5: Diosmetin



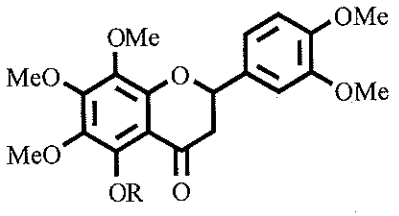
A6: Isosinensetin



A7: Tangeretin

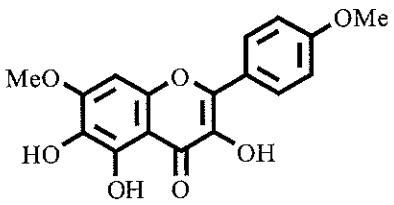


A8: Tetramethylscutellarein

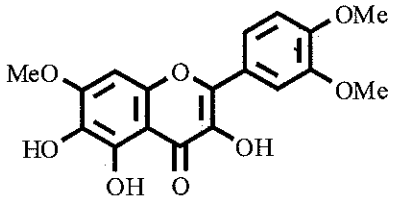


A9: R = Me: Citromitin

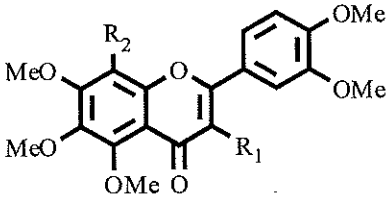
A10: R = H: 5-Demethylcitromitin



A11: 3,5,6-Trihydroxy-4',7-dimethoxy flavone



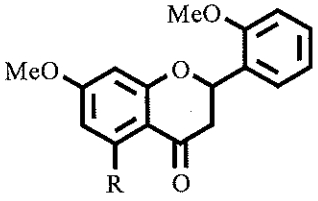
A12: 3,5,6-Trihydroxy-3',4',7 trimethoxy flavone



A13: R_1 = H, R_2 = H: Sinensetin

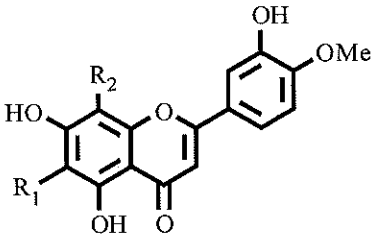
A14: R_1 = OMe, R_2 = OMe: Nobilitin

A15: R_1 = H, R_2 = OMe:5-Demethyl-nobiletin



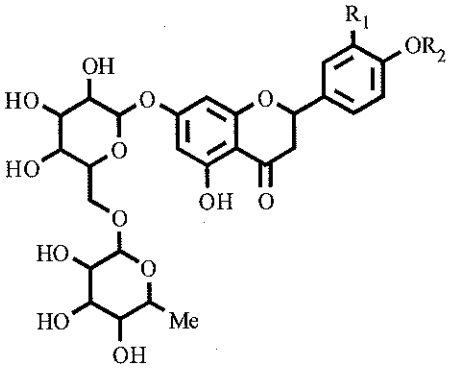
A16: R = OMe: 5,7,2'-Trimethoxy-flavone

A17: R = H: 7,2'-Dimethoxyflavanone:

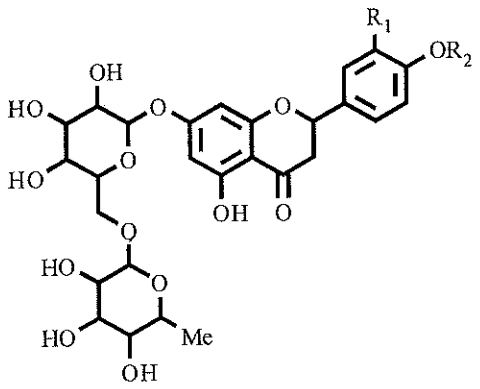


A18: R_1 = glucose, R_2 = H: 6-*C*-*β*-Glucosyldiosmin:

A19: 6R_1 = glucose, R_2 = glucose: 6,8-Di-*C*-*β*-glucosyldiosmin



A20: Diosmin



A21: R_1 = OH, R_2 = Me: Hesperidin

A22: R_1 = H, R_2 = H: Narirutin

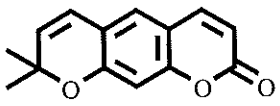
A23: R_1 = H, R_2 = Me: Didymin

A24: R_1 = Me, R_2 = H: Homoeriodictyol-7-*O*-rutinoside

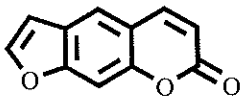
Coumarins

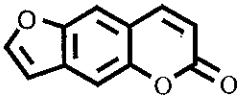
Coumarins one of the major classes of oxygen heterocyclic compounds in *Citrus* genus. Pyranocoumarins, furanocoumarins and simple coumarins were reported from this genus. They have been found in the different parts of *Citrus* plants

1. ***Pyranocoumarins*** are coumarins which contain a pyran ring fused at C-7 or C-8. The pyranocoumarins which occur in fruits and roots of Rutaceae families.



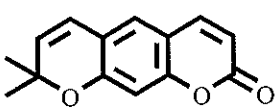
2. ***Furanocoumarins***, or ***furocoumarins*** are organic chemical compounds produced by a variety of plants. The chemical structure of furanocoumarins consists of a furan ring fused with coumarin. About 6 *Furanocoumarins* were found in *Citrus* genus. Begaptol and bergapten are the most common linear furanocoumarins.



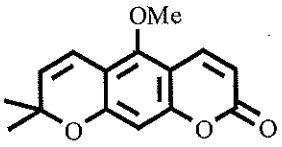


3. *Simple coumarin*

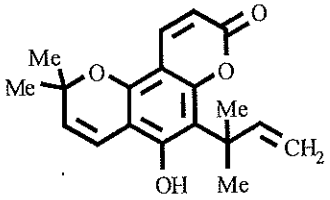
There are about 8 *simple coumarins* found in *Citrus* genus. scopoletin, umbelliferone, crenyllatin and 6,7-dimethoxycoumarin are the most common *simple coumarin*.



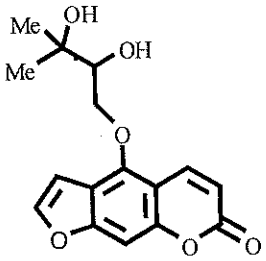
B1: Xanthyletin



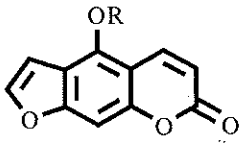
B2: Xanthoxyletin



B3: Nordentatin

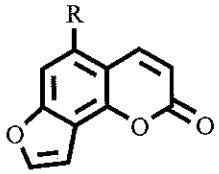


B4: Oxypeucedanin



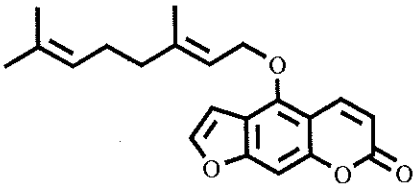
B5: R = Me : Bergapten

B6: R = H : Begaptol

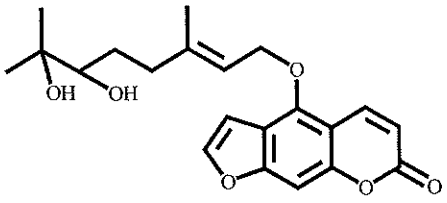


B7: R = OMe: Isobergapten

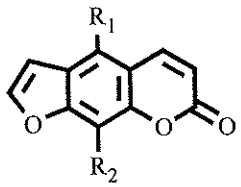
B8: R = H : Angelicin



B9: Bergamotin



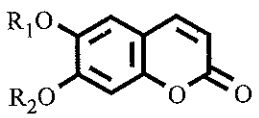
B10: 5-[(6'-7'-dihydroxy-3', 7'-dimethyl-2octenyl)-oxyl]psoralen



B11 : R_1, R_2 = H: Psoralene

B12 : R_1 = OMe , R_2 = OMe : Isopimpinellin

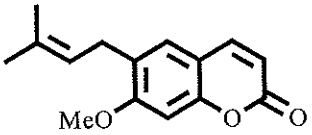
B13 : R_1 = O-isoprene, R_2 = H : Imperatorin



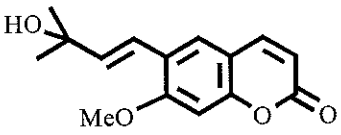
B14: R_1 = OMe, R_2 = H: Scopoletin

B15: R_1 = OMe, R_2 = Me: 6,7-Dimethoxycoumarin

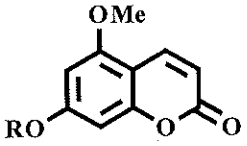
B16: R_1 = CHO, R_2 = Me: Crenyllatin



B17: Suberosin

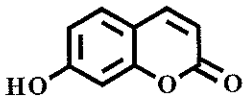


B18: Suberenol



B19: R = H: 7-Hydroxy-5-methoxy-coumarin

B20: R = Me: Limetti

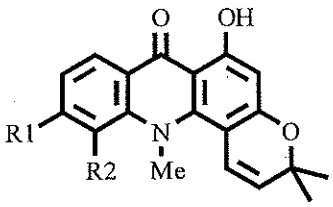


B21: Umbelliferone

Acridones

Acridones has carbonyl group at 9th position and nitrogen at 10th position. It is oxidized product of acridine. Acridones is also known by the name of 9(10H)-acridinon. There are about 9 acridone alkaloids found from this genus.

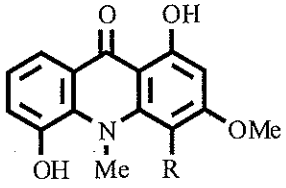
Citracridone I, citrusinine I and 5- hydroxynoracronycine have been isolated from *Citrus limon*. From *Citrus reticulata* only one alkaloid named 9(10H)-acridinone was isolated.



C1: R_1 = H, R_2 = OH: 5-Hydroxy-noracronycine

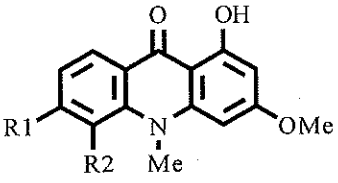
C2: R_1 = OH, R_2 = OMe : Citracridone-I

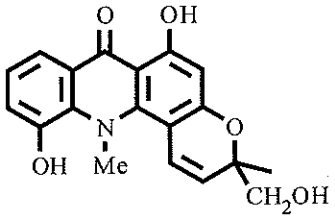
C3: R_1 = OH, R_2 = OH : Citracridone-III



C4: R= OMe : Citrusinine-I

C5: R= prenyl : Glycocitrine-I

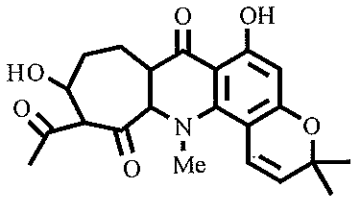




C6: R_1 = OMe, R_2 = OH: Grandisine-I

C7: R_1 = OH, R_2 = OMe: Natsucitrine-II

C8: 5-Hydroxynoracronycine alcohol



C9: Citropone A

Limonoids

Limonoids are classed as oxygenated tetracyclic triterpene derivatives, which are widely distributed in plants from the Rutaceae. The chemical structure is composed of 4 six-membered rings and a furan ring. Limonoids were isolated from tissues, and seeds of all Citrus species. They were found as 2 classes.

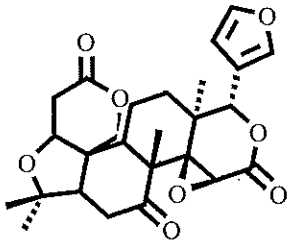
Limonoid aglycones

About 11 limonoid aglycones were found in *Citrus* genus, such as limonin, nomilin, obacunone, ichangin, deacetylnomilin. Limonin was the first characterized compound of this group. It has been known as a constituent of *Citrus* since 1841. It contains a furan ring attached to the D-ring, at C-17, as well as oxygen-

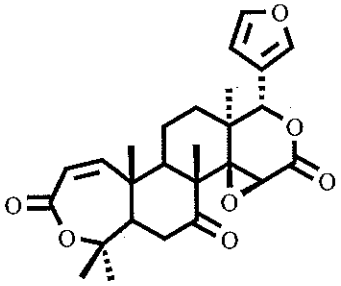
containing functional groups at C-3, C-4, C-7, C-16, and C-17. Limonin contains a C-14, 15-epoxide group.

Limonoid glycoside

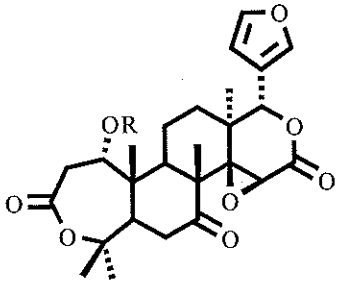
About 3 limonoid glycosides were reported in this genus such as limonin-17-β-D-glucopyranoside, ichangin 4-β-glucopyranoside, nomilinic acid 4-β-glucopyranoside.



D1: Limonin

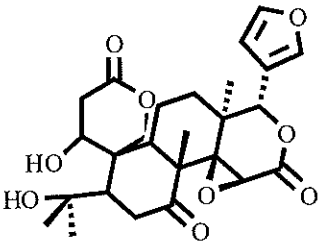


D2: Obacunone

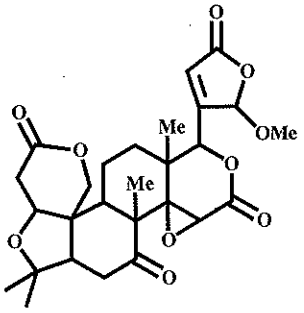


D3: R = Ac: Nomilin

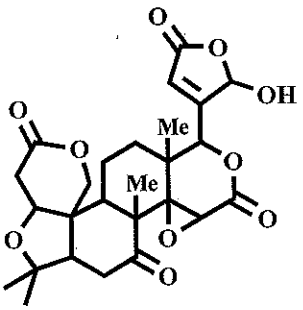
D4: R = H : Deacetylnomilin



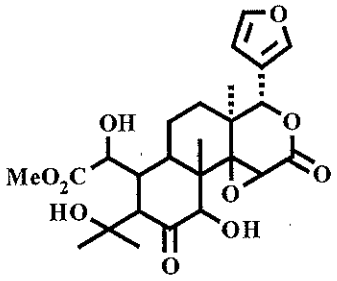
D5: Ichangin



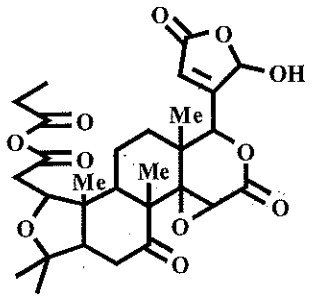
D6: Isolimonexic acid methyl ether



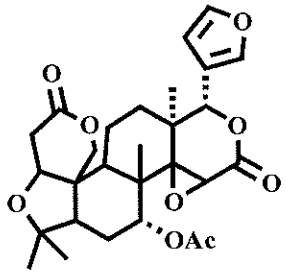
D7: Limonexic acid



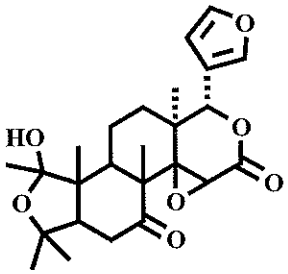
D8: Calamin



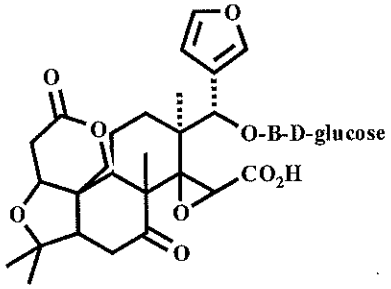
D9: Citrobilin



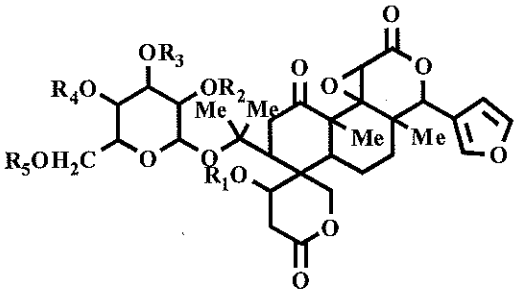
D9: Limonyl acetate



D10: Ichangensin



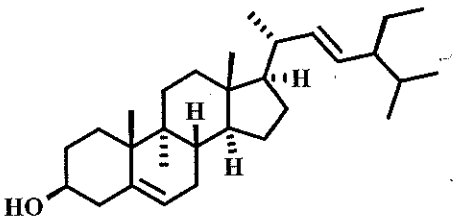
D11: Limonin-17-β-D-glucopyranoside



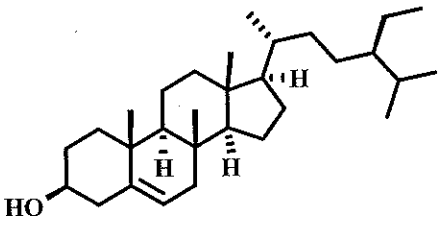
D12: R_1, R_2, R_3, R_4, R_5 = H : Ichangin 4-β-glucopyranoside

D13: R_1, R_2, R_3, R_4, R_5 = Ac : Nomilinic acid 4-β-glucopyranoside

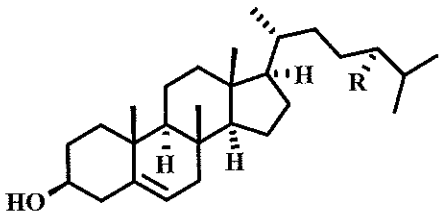
Triterpenoids



E1: Stigmasterol



E2: β-Sitosterol



E3: R = H : Cholesterol

E4: R = Me : Campesterol

1.2.2 The biological activity of some compounds from *Citrus*

Acridones, coumarins, flavonoids and limonoids in *Citrus* have been reported for various pharmacological and biological activities, such as antioxidant, antiviral, antibacterial, antifungal and cytotoxic activities (Tripoli *et al.*, 2007, Teng *et al.*, 2005).

5-hydroxynoracronycinealcohol, 5-hydroxynoracronycine, glycocitrune-I, citrusinine I and citracridone III displayed cytotoxic activity against two tumor cells (HepG2 and KB) with inhibitory concentration, $IC_{50} < 50$ μM (Teng *et al.*, 2005).

Sinensetin showed activity against the growth of human ovarian cancer cell line (HO8910), lung, colon, breast ER^-, breast ER^+, prostate and melanoma with IC_{50} value of 12.5, 13.7, 9.5, 3.9, 5.5, 16.5 and 10.8 μM, respectively (Du *et al.*, 2010, Manthey, A.J and Guthrie, N., 2002).

Nobilitin inhibited the growth of human ovarian cancer cell line (HO8910), lung, colon, breast ER-, breast ER^+, prostate and melanoma with IC_{50} values of 16.8, 3.5, 4.7, 1.2, 2.9, 1.0 and 0.50 μM, respectively (Manthey, A.J and Guthrie, N., 2002).

5-Demethylnobiletin exhibited activity against *Aspergillus niger* with MIC values 0.1 mg/mL (Liu *et al.*, 2012).

Tangeretin also showed cytotoxic to six cancer cell lines: lung, colon, breast ER-, breast ER+, prostate and melanoma with IC_{50} value of 3.2, 1.6, 1.3, 0.34, 0.54 and 0.27 μM, respectively (Manthey, A.J and Guthrie, N., 2002).

Isosinensetin showed antiproliferative activity against breast cancer cell line (MCF-7) with IC_{50} 15.1 μM, and ovarian cancer cell line (HO8910) with IC_{50} 31.1 μM (Du *et al.*, 2010).

Hesperitin exhibited cytotoxicity against the five cancer cell lines; lung, colon, breast ER+, prostate and melanoma with IC_{50} >200 μM (Manthey, A.J and Guthrie, N., 2002).

Naringenin exhibited cytotoxicity activity against the five cancer cell lines; lung, colon, breast ER+, prostate and melanoma with IC_{50} >200, >200, 180, >200 and >158 μM, respectively (Manthey, A.J and Guthrie, N., 2002)

Limonin and nomilin were tested for their ability to inhibit proliferation of MDAMB-435 estrogen receptor-negative human breast cancer cells, by the incorporation of [3H] thymidine. Nomilin was the most effective with IC_{50} value of 0.4 μg/ml, and limonin (12.5 μg/ml) (Guthrie *et al.*, 1997).

Nobilitin and tangeretin showed exhibitory activity against *Aspergillus niger* with MIC values of 0.8 and 0.4 mg/mL, respectively (Liu *et al.*, 2012).

Xanthyletin inhibited the growth of *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Salmonella typhi*at with $IC_{100} \leq 100$ μg/ml and *Bordetella bronchiseptica* with $IC_{100} \leq 50$ μg/mL. It also was active against MDR *A. baumannii* JVC 1053 with MIC values of 100 μg/mL, while limonin showed inhibitory activity with the MIC value of 50μg/mL (Panthong *et al.*, 2013).

Scopoletin and glycocitrine-I inhibited the growth of *Bordetella bronchiseptica* at $IC_{100} \leq 100$ μg/mL (Wu *et al.*, 1988).

5-hydroxynoracronycine showed anti-HIV-1 protease activity with an IC_{50} value of 93.1 μM and it also exhibited DPPH scavenging activity with IC_{50} value of 0.19 mg/mL, respectively (Panthong *et al.*, 2013).

Citflavanone, auraptene, xanthyletin, 7,8-dihydrofurocoumarin, 6,8-dimethoxycoumarin, limonin showed anti-HIV-1 activities with EC_{50} value of 30.1, 59.7, 41.2, 336.3, 102.9 and >340.4 μM, respectively (Feng *et al.*, 2010).

1.2.3 The Chemical Constituents of *Citrus reticulata* Blanco

About twenty pure compounds were isolated from *C. reticulata* Blanco They are eleven polymethoxyflavonoids: nobiletin, ponkanetin, 5-demethylnobiletin, 6,7-dimethylesculetin, 4,5,7,8-tetramethoxyflavone, 3,5,6,8,4'-pentamethoxy flavones, 3,5,6,8,3',4'-hexamethoxyflavones, isosinensetin, sinensetin, nobiletin and tetramethyl-*o*-scutellarein; six limonoids: limonin, deacetylnomilin, obacunone, deacetylnomilin, ichangin and isolimonexic acid methyl ether; four triterpenoids: cholesterol, campesterol, stigmasterol, and β-sitosterol.

1.2.4 The biological activity of crude extracts and pure compounds of *Citrus reticulata*

Antibacterial properties:

In 2005, Li and co-worker reported that an ethanol extract of *C. reticulata* inhibited the growth of *Helicobacter pylori* with MIC 40 μg/mL (Li *et al.*, 2005).

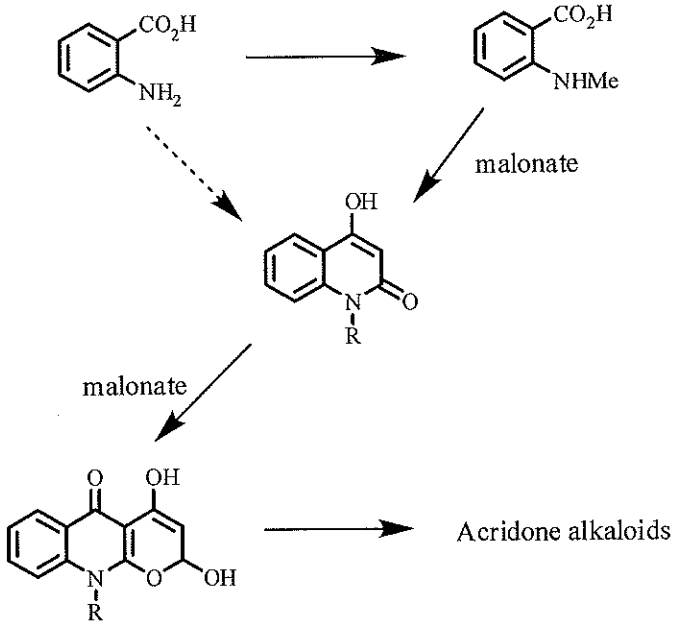
Anticancer activity:

In 1993, Sugiyama and co-worker reported that flavones extracted from the fruit peel of *C. reticulata* induced differentiation in mouse myeloid leukemia cells (M1), and the cells exhibited phagocytic activity *in vitro* (Sugiyama *et al.*, 1993).

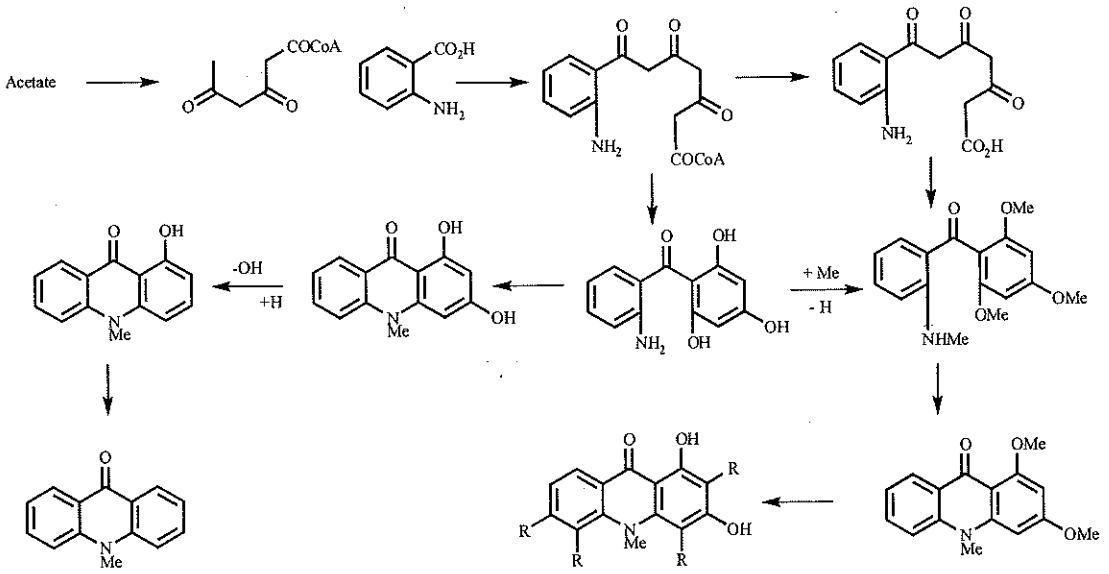
The limonoids extracted from *C. reticulata* exhibited inhibitory activity against human breast cancer cell lines (MCF-7), but did not inhibit leukemia (HL-60), ovary (SKOV-3), cervix (HeLa), stomach (NCI-SNU-1), or liver (Hep G2) cancer cells lines. (Tian *et a.*, 2001).

1.2.5 Biosynthesis of acridone alkaloids

It has been hypothesized that acridone alkaloids are biosynthesized via two different pathways illustrated bellows. Biosynthesis of acridone alkaloids is given in **scheme 1** and **scheme 2**.

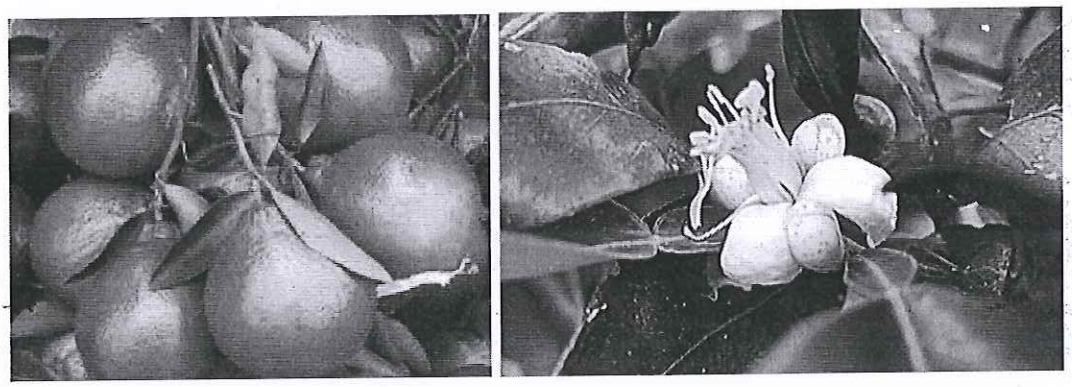


Scheme 1. Biosynthesis of acridone alkaloids via quinonone pathway (Groger, D and Johne, S.,1968)



Scheme 2. Biosynthesis of acridone alkaloids via aminobenzophenone pathway (Khatoon, T., 1995).

1.2.6 Description of *Citrus reticulata*



C. reticulata locally known as "neck orange" belongs to the family Rutaceae. It is a small to medium-sized, shrubby tree that grows up to 5-8 meters tall, with spiny shoots and alternately arranged evergreen leaves with an entire margin. The flowers are solitary or in small corymbs, each flower 2-4 cm diameter, with five (rarely four) white petals. The flowers bloom in January to February. The fruit is a type of berry, with a yellowish-green peel. Its flowers, fruit peels and leaves have a sweet smell.

The objectives

Acridones, flavonoids, coumarins and limonoids are compounds that have been reported for the interesting biological activity and could be found in *Citrus*. There has not been any comprehensive study on the chemistry of *Citrus reticulata* Blanco except for its fruit. Based upon this lack of investigation and the diversity and biological activity of compounds from related species was considered to be important to study other part of *Citrus reticulata* Blanco. The second objective is to investigation the chemistry of the peels from *C. reticulata* Blanco in Thailand as it is anticipated that the constituents present will be different from those previously reported.

CHAPTER 2

EXPERIMENTAL

2.1 General Method

Column chromatography was performed by using silica gel 100 (70-230 Mesh ASTM, Merck), silica gel 60 RP-18 (40-63 μm, Merck) or Sephadex^{TM} LH-20 (Amersham Biosciences, Sweden). Quick column chromatography (QCC) was performed on silica gel 60H (230-400 Mesh ASTM, Merck). For thin-layer chromatography (TLC), aluminum sheets of silica gel 60 GF_{254} (20×20 cm, layer thickness 0.2 mm, Merck) were used for analytical purposes and the compounds were visualized under ultraviolet light. Solvents for extraction and chromatography were distilled at their boiling ranges prior to use. Melting points were recorded in °C on a digital Electrothermal Melting Point Apparatus (Electrothermal 9100). Ultraviolet spectra were measured with UV-160A spectrophotometer (SHIMADZU). Principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in ethanol solution. Infrared spectra (IR) were obtained on a FTS165 FT-IR spectrophotometer, and were recorded in wave number (cm^{-1}). ^{1}H and ^{13}C-Nuclear magnetic resonance spectra were recorded on a FT-NMR Bruker Ultra Shield^{TM} 300 MHz or 500 MHz spectrometer at Department of Chemistry, Faculty of Science, Prince of Songkla University and on a Varian Inova 600 MHz spectrometer, Griffith University. Spectra were recorded in $CDCl_3$, Me_2CO and DMSO-d_6 and were recorded as δ value in ppm downfield from TMS (internal standard δ 0.00). The FAB-MS and HRFABMS mass spectra were obtained using a MAT 95 XL mass spectrometer; low and high resolution mass spectra were recorded on a MAT 95 XL at Scientific Equipment Center, Prince of Songkla University. HPLC separations were achieved using a Rainin Microsorb C18 semipreparative column (3 μm, 10 mm × 50 mm) and Diol-120-NP column (150x20 mm), Griffith University.

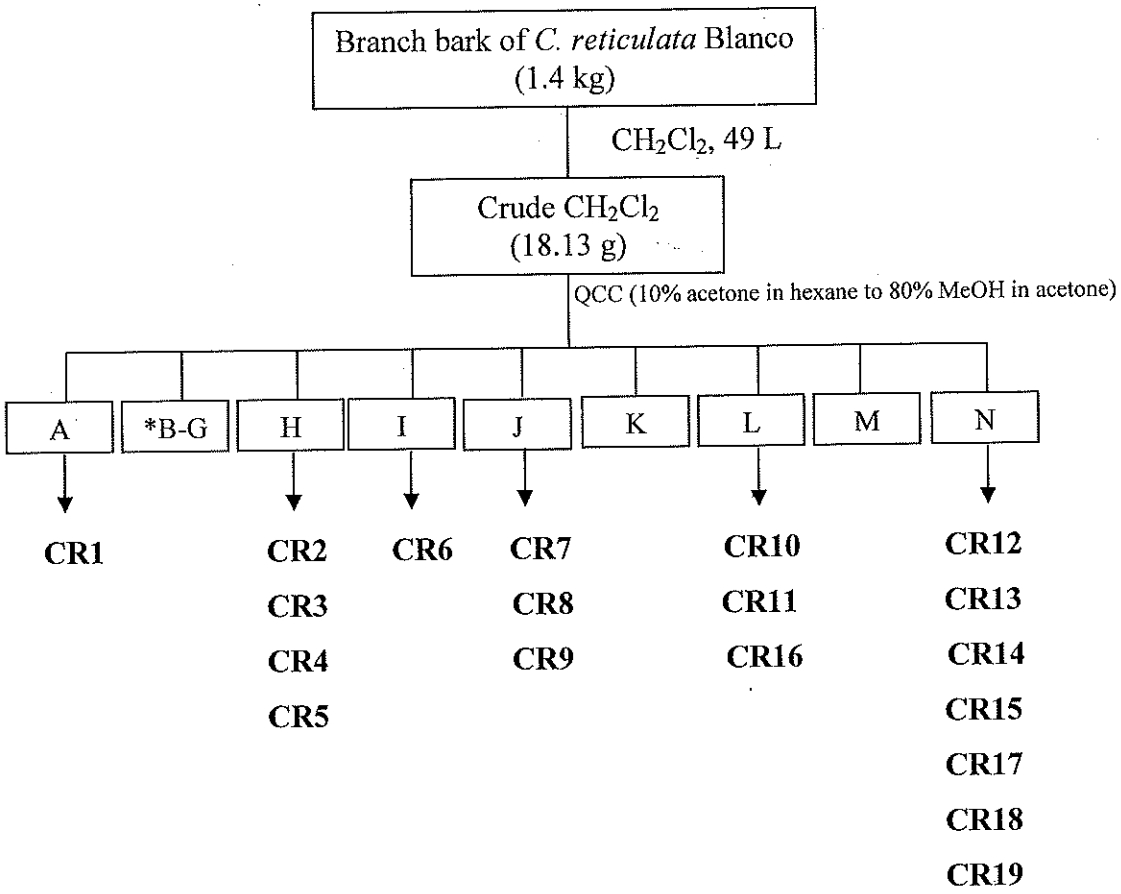
2.2 Plant Material

The branch bark, leaves, peels and woods of *C. reticulata* Blanco were collected from Sadao district, Songkhla province in the Southern part of Thailand, in April 2011. Identification of the plants was made by J. Wai, Department of Biology, Faculty of Science, Prince of Songkla University. A voucher specimen U.Phetkul 1 (PSU) has been deposited in the Herbarium of Department of Biology, Faculty of Science, Prince of Songkla University, Thailand.

2.3 Extraction and isolation

2.3.1 Extraction and isolation from the branch bark

Dried branch bark of *C. reticulata* Blanco (1.4 kg) were chopped and immersed in CH_2Cl_2 (7.5 L) at room temperature. Removal of the solvent, the dark-brown gum (18.13 g) was obtained. The extract was subjected to a QCC (a gradient of 10% acetone in hexane to 80% MeOH in acetone) to give 14 fractions (**A-N**). The process of extraction was shown in **Scheme 3.**



* No further investigation

Scheme 3. Isolation of compounds **CR1-CR19** of dichloromethane extract from the branch bark

Table 2. Physical characteristic and weight of the fractions of dichloromethane extract of the branch bark

Fractions	Weight (g)	Appearance
A	0.6243	orange gel mixed with white solid
B	0.6243	orange gel
C	2.0041	orange gel
D	1.8970	orange gel
E	1.2315	orange gel
F	3.4880	orange gel
G	3.5605	brown viscous liquid
H	0.7142	brown viscous liquid
I	0.5272	brown viscous liquid
J	0.9663	brown viscous liquid
K	1.3525	brown viscous liquid
L	0.8902	brown viscous liquid
M	0.6206	brown viscous liquid
N	2.9120	brown viscous liquid

Isolation of CR1

Fraction **A** (624.3 mg) was added with hexane to give a colorless needle **CR 1** (7.0 mg).

Isolation of CR2, CR3, CR4 and CR5

Fraction **H** (714.2 mg) was further separated by CC (20% to 50% acetone in hexane) to give sixteen fractions (**H1-H16**). The solid which formed in fraction **H5** were collected by filtration to give an orange solid **CR2** (17.0 mg). Fraction **H10** (100.35 mg) was further separated by CC (20% to70% acetone in hexane) to give fractions **H10A-H10R**. Subfraction **H10G** (11.12 mg) was chromatographed on silica gel (20% to 50% acetone in hexane) to give a yellow solid **CR3** (2.3 mg). Subfraction **H10P** (8.12 mg) was chromatographed on silica gel (20% acetone in hexane) to give amorphous powders **CR4** (2.1 mg) and **CR5** (1.7 mg).

Isolation of CR6

Fraction **I** (527.2 mg) was further separated by CC (20% acetone in hexane) to give ten fractions (**I1-I1O**). Fraction **I8** (100.35 mg) was further separated by CC (20% acetone in hexane) to give an orange solid **CR6** (3.7 mg).

Isolation of CR7, CR8 and CR9

Fraction **J** (966.3 mg) was further separated by CC (20% to 80% acetone in hexane) to give fractions **JA-JO**. Fraction **JF** was further purified by CC (20% to 50% acetone in hexane) to afford subfraction **JFA-JFO**. Subfraction **JFL** (141.62 mg) was separated by (20% to 50% acetone in hexane) to give a yellow solid **CR7** (11.7 mg). Subfraction **JFM** (88.19 mg) was chromatographed on silica gel (20% to 50% acetone in hexane) solvent systems to give yellow solids **CR8** (2.3 mg) and **CR9** (0.7 mg).

Isolation of CR10 and CR11

Chromatography of fraction **L** (890.2 mg) on Sephadex LH-20 column (20% CH_2Cl_2 in MeOH) gave fraction **LA-LF**. Fraction **LC** was further purified by CC (30% acetone in hexane) to give subfraction **LCA-LCF**. Subfraction **LCD** was further purified by CC (30 to 50% acetone in hexane) to give a yellow solid **CR10** (0.8 mg) and a white solid **CR11** (2.0 mg).

Isolation of CR12, CR13, CR14 and CR15

Fraction **N** (2.9 g) was subjected on Sephadex LH-20 column (20% CH_2Cl_2 in MeOH) to give fraction **NA-NI**. Purification of fraction **NB** by CC (2% MeOH in CH_2Cl_2) provide as amorphous powder **CR12** (18.0 mg), a yellow solid **CR13** (2.3 mg), an oange solid **CR14** (2.1 mg) and a colorless gum **CR15** (0.8 mg).

Isolation of CR16

Methylation of fraction **LE** (58.95 mg) with MeI (2.0 ml) and K_2CO_3 (10.0 mg) in Me_2CO (1.0 ml) for 8 h and purified by CC (20% acetone in hexane) gave 9 fractions. Fraction **LEG** (15.713 mg) was purified by PTLC (20% acetone in hexane) to give and amorphous powder **CR16** (1.5 mg).

Isolation of CR13, CR17, CR18 and CR19

Fraction **ND-NI** were combined (**NDI**, 559.13 mg) and was methylated with MeI (4.0 ml) and K_2CO_3 (10.0 mg) in Me_2CO (2.0 ml) and further purification on Sephadex LH-20 column and eluted with (20% CH_2Cl_2 in MeOH) to give 3

fractions (**NDIA- NDIC**). Fraction **NDIC** was further purified by PTLC (20% acetone in hexane) to give white solids **CR13** (1.2 mg), amorphous powders **CR17** (0.8 mg), **CR18** (1.1 mg) and yellow solid **CR19** (0.9 mg).

CR1: atranorin

^{1}H-NMR 300 MHz ($CDCl_3$+DMSO-d_6) Table 8; Page 48

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) Table 8; Page 48

CR2:5-hydroxynoracronycine, m.p. 261-263 °C

UV λ_{max} (MeOH) (log ε): 212 (4.03), 267 (4.32), 283 (4.31), 414 (3.34) nm

IR (Neat) ν (cm^{-1}): 3446 (O-H stretching), 1636 (C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$+DMSO-d_6) Table 11; Page 57

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) Table 11; Page 57

CR3: citflavanone

^{1}H-NMR 300 MHz ($CDCl_3$) Table 19; Page 74

^{13}C NMR 75 MHz ($CDCl_3$) Table 19; Page 74

CR4: gustastatin

^{1}H-NMR 300 MHz ($CDCl_3$) Table 9; Page 51

^{13}C NMR 75 MHz ($CDCl_3$) Table 9; Page 51

CR5: 8-hydroxy-6-methoxy-pentylisocoumarin

^{1}H-NMR 300 MHz ($CDCl_3$) Table 39; Page 118

^{13}C NMR 75 MHz ($CDCl_3$) Table 39; Page 118

CR6: citracridone-I, m.p. 274-276 °C

UV λ_{max} (MeOH) (logε): 205 (3.54), 269 (3.92), 338 (3.19), 392 (2.40) nm

IR (neat) ν (cm^{-1}): 3405 (O-H stretching), 1626 (C=O stretching),

1604 (C=C stretching)

^{1}H-NMR 300 MHz ($CDCl_3$+DMSO-d_6) Table 12; Page 60

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) Table 12; Page 60

CR7: citrusinol

m.p. 253-254 °C

UV λ_{max}(MeOH) (log ε): 222 (3.25), 241 (3.20), 248 (3.14), 267 (3.09), 331(3.02) nm

IR (neat) ν (cm^{-1}): 3550 (O-H stretching) and 1620 (C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$+DMSO-d_6) Table 20; Page 76

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) Table 20; Page 76

CR8: citrusinine-I, m.p. 206-207°C

UV λ_{max} (MeOH) (logε): 203 (3.80), 221 (3.74), 263 (4.19), 319 (3.71), 416 (3.27) nm

IR (Neat) ν (cm^{-1}): 3386 (O-H stretching), 1633 (C=O stretching) 1604 (aromatic)

^{1}H-NMR 300 MHz ($CDCl_3$+DMSO-d_6) Table 13; Page 62

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) Table 13; Page 62

CR9: citramine, m.p. 226-228 °C

UV λ_{max} (MeOH) (log ε): 214 (3.15), 271 (3.08), 331 (2.45), 386 (2.06) nm

IR (Neat) ν (cm^{-1}): 3418 (O-H stretching), 1651(C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$+DMSO-d_6) Table 14; Page 64

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) Table 14; Page 64

CR10: 2-methoxycitpressine

^{1}H-NMR 300 MHz ($CDCl_3$) Table 15; Page 66

^{13}C NMR 75 MHz ($CDCl_3$) Table 15; Page 66

CR11: scopoletin

UV (MeOH) λ_{max} (log ε): 222 (5.02), 249 (4.87), 268 (4.84) and 311 (4.82) nm

IR (neat) ν (cm^{-1}): 3323 (O-H stretching) and 1704 (C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$) Table 34; Page 107

^{13}C NMR 75 MHz ($CDCl_3$) Table 34; Page 107

CR12: 2-hydroxy-4-methoxy-6-(2-oxoheptyl)-2'-methoxy-4'-hydroxy-6-(hetyl)-phenyl ester, m.p. 125-127 °C,

HRFABMS at $([M+1]^+$ *m/z*501.2848 for $C_{29}H_{40}O_7$ (calcd501. 2870). UV λ_{max} ($CHCl_3$) (log ε): 265.0 (3.52), 286 (3.25), 311(3.88) and 400 (1.09) nm

IR (Neat) ν (cm^{-1}): 1651 (C=O stretching).

^{1}H-NMR 300 MHz ($CDCl_3$) Table 10; Page 55

^{13}C NMR 75 MHz ($CDCl_3$) Table 10; Page 55

CR13: limonin

m.p. 285-286 °C

IR (neat) ν (cm^{-1}): 1730, 1709 (C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$+ DMDO-d_6) Table 41; Page 122

^{13}C NMR 75 MHz ($CDCl_3$ + DMDO-d_6) Table 41; Page 122

CR14: citracridone-III

m.p. 274-276 °C

UV λ_{max} nm (MeOH) (log ε): 225 (3.87), 281 (3.77), 343 (3.74) and 386 (3.53) nm

IR (neat) ν (cm^{-1}): 3368 (O-H stretching), 1655 (C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$+DMSO-d_6) Table 16; Page 68

^{13}C NMR 75 MHz ($CDCl_3$+DMSO-d_6) Table 16; Page 68

CR15:4-hydroxybenzoic acid

UV λ_{max} (MeOH) (log ε): 253 (2.54) nm

IR (neat) ν (cm^{-1}): 3372 (O-H stretching), 1650 (C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$) Table 44; Page 129

CR16: methyl-2-hydroxy-4-methoxy-6-(2-oxoheptyl)-benzoate

HRFABMS m/z: HR-EIMS at m/z 294.1472 for $C_{16}H_{22}O_5$ (calcd for 294.1462).

UV λ_{max} (MeOH) (log ε): 266.5 (3.61) nm

IR (neat) ν (cm^{-1}): 1765 (C=O stretching).

^{1}H-NMR 300 MHz ($CDCl_3$) Table 45; Page 131

^{13}C NMR 75 MHz ($CDCl_3$) Table 45; Page 131

CR17: methyl 2,4-dimethoxy-6-heptylbenzoate

^{1}H-NMR 300 MHz ($CDCl_3$) Table 46; Page 132

CR18: 6,8-dimethoxypentylisocoumarin

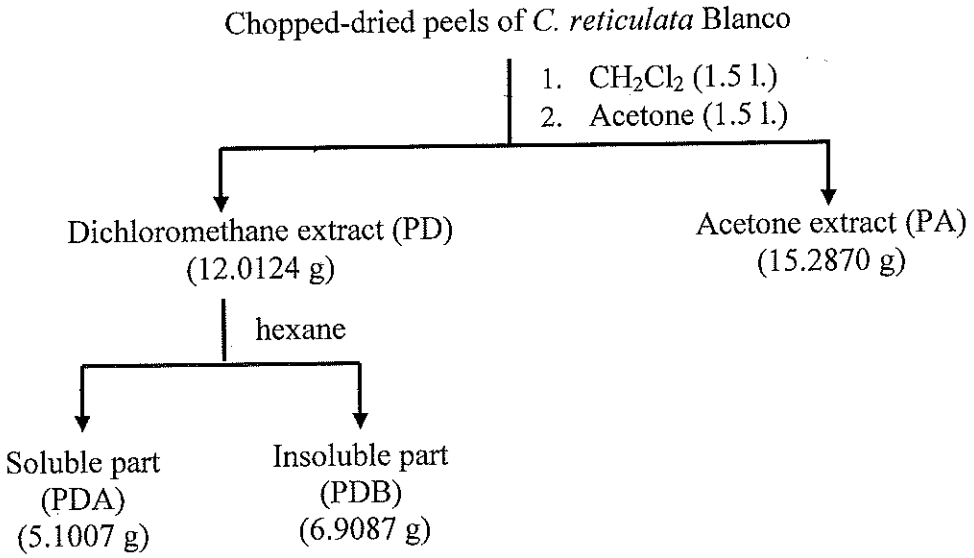
^{1}H-NMR 300 MHz ($CDCl_3$) Table 40; Page 119

CR19: citracridone-II

^{1}H-NMR 300 MHz ($CDCl_3$) Table 17; Page 69

2.3.2 Extraction and isolation from the Peels

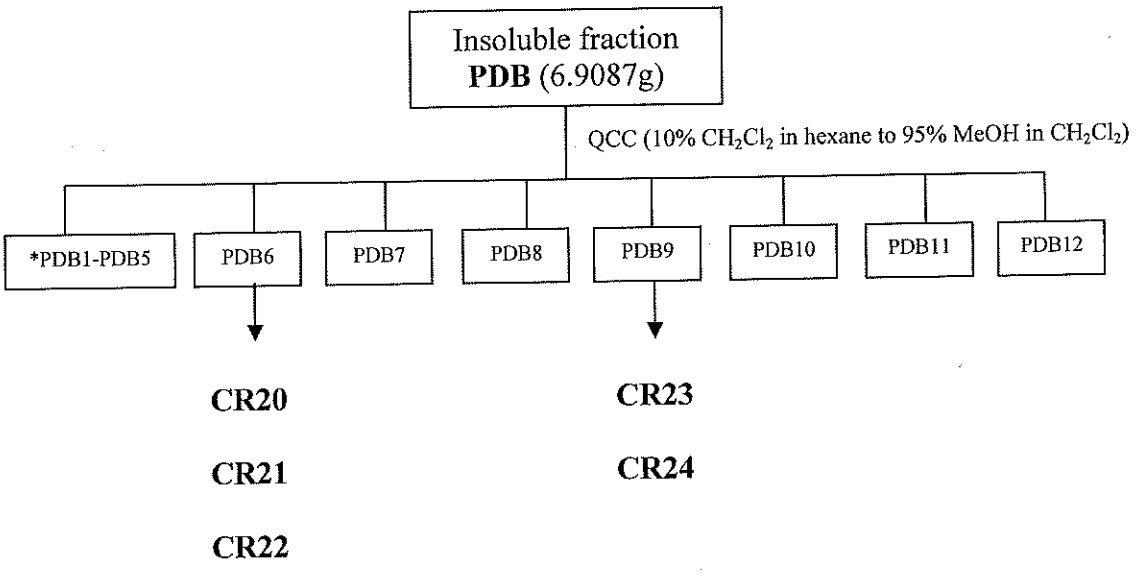
Chopped-dried fruit peels (893.9 g) of *C. reticulata* (Neck orange) were successively immersed in CH_2Cl_2 (1.5 liters) and Me_2CO (1.5 liters) at room temperature (3 days x 2 times). After removal of solvent, the dark-brown viscous CH_2Cl_2 extract (12.0124 g) and Me_2CO extract (15.287 g) were obtained, respectively. The CH_2Cl_2 extract was dissolved in hexane to give soluble (5.1007g) and insoluble (6.9087 g) fractions. The process of extraction was shown in **Scheme 4**.



Scheme 4. Extraction of crude extracts from the peels of *C. reticulata* Blanco

2.3.2.1 Purification of insoluble fraction PDB

The CH_2Cl_2 insoluble fraction **PDB** (6.9087 g) was separated by a QCC over silica gel 60H using a gradient of 10% CH_2Cl_2 in hexane to 95% MeOH in CH_2Cl_2 as eluents. On the basis of their TLC characteristics, the fractions which contained the same major components were combined to give 12 fractions (**A-L**) **(Table 2)**. Further purification of subfractions gave five pure compounds in **Scheme 5**.



* No further investigation

Scheme 5. Isolation of compounds **CR20-CR24** from insoluble fraction **PDB**

Table 3. Physical characteristic and weight of the fractions from hexane insoluble fraction of dichloromethane extract from the peels

Fractions	Weight (g)	Appearance
PDB1	0.3336	yellow viscous liquid
PDB2	0.4859	yellow viscous liquid
PDB3	0.6191	orange viscous liquid
PDB4	0.3654	green viscous liquid
PDB5	0.2542	brown viscous liquid
PDB6	2.7178	brown viscous liquid
PDB7	0.4546	green viscous
PDB8	0.2754	dark green viscous
PDB9	0.8860	dark green viscous
PDB10	0.2134	green solid viscous
PDB11	0.0587	dark green viscous
PDB12	0.0312	dark brown solid

Isolation of CR20, CR21 and CR22

Fraction **PDB6** (2.7178 mg) was further separated by CC (20% acetone in hexane) to give ten fractions (**PDB6A- PDB6LJ**). The solid which formed in fraction **PDB6E** were collected by filtration to give a yellow solid **CR20** (2.1 mg). Fraction **PDB6H** (100.35 mg) was further separated by CC (20% to 50 % acetone in hexane) to give yellow solids **CR21** (2.3 mg) and **CR22** (4.1 mg).

Isolation of CR23 and CR24

Fraction **PDB9** (886.00 mg) was further separated by CC (20% to 80% acetone in hexane) to give sixteen fractions (**PDB9A-PDB9N**). Subfraction **PDB9G** (30.59 g) was further separated by CC (30% to 80% acetone in hexane) to give a yellow solid **CR23** (2.1 mg). Fraction **PDB10** (70.1 mg) was purified by CC (30% to 80% acetone in hexane) to give fractions **PDB10**A-**PDB10**K. Fraction **PDB10**F was further purified by CC (30% acetone in hexane) afforded a yellow solids **CR24** (1.0 mg).

CR20: 5-demethoxynobiletin

UV λ_{max} (MeOH) (log ε): 204 (5.39), 254 (4.95), 283 (5.05), 329 (5.09) nm

IR (KBr) ν (cm^{-1}): 3428 (O-H stretching), 1697 (C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$) Table 21; Page 78

^{13}C NMR 75 MHz ($CDCl_3$) Table 21; Page 78

CR21: tangeretin

UV λ_{max} (MeOH) (log ε): 271 (5.27) and 332 (5.43) nm

IR (KBr) ν (cm^{-1}): 1647 (C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$) Table 22; Page 80

^{13}C NMR 75 MHz ($CDCl_3$) Table 22; Page 80

CR22: nobiletin

UV λ_{max} (MeOH) (log ε): 248 (5.41), 271 (5.38) and 332 (5.52) nm

IR (KBr) ν (cm^{-1}): 1647 (C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$) Table 23; Page 82

^{13}C NMR 75 MHz ($CDCl_3$) Table 23; Page 82

CR23: 5,7,8,4'-trimethoxyflavone

UV λ_{max} (MeOH) (log ε): 270 (3.33), 311 (3.23) nm

IR (KBr) ν (cm^{-1}): 1636 (C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$) Table 24; Page 84

^{13}C NMR 75 MHz ($CDCl_3$) Table 24; Page 84

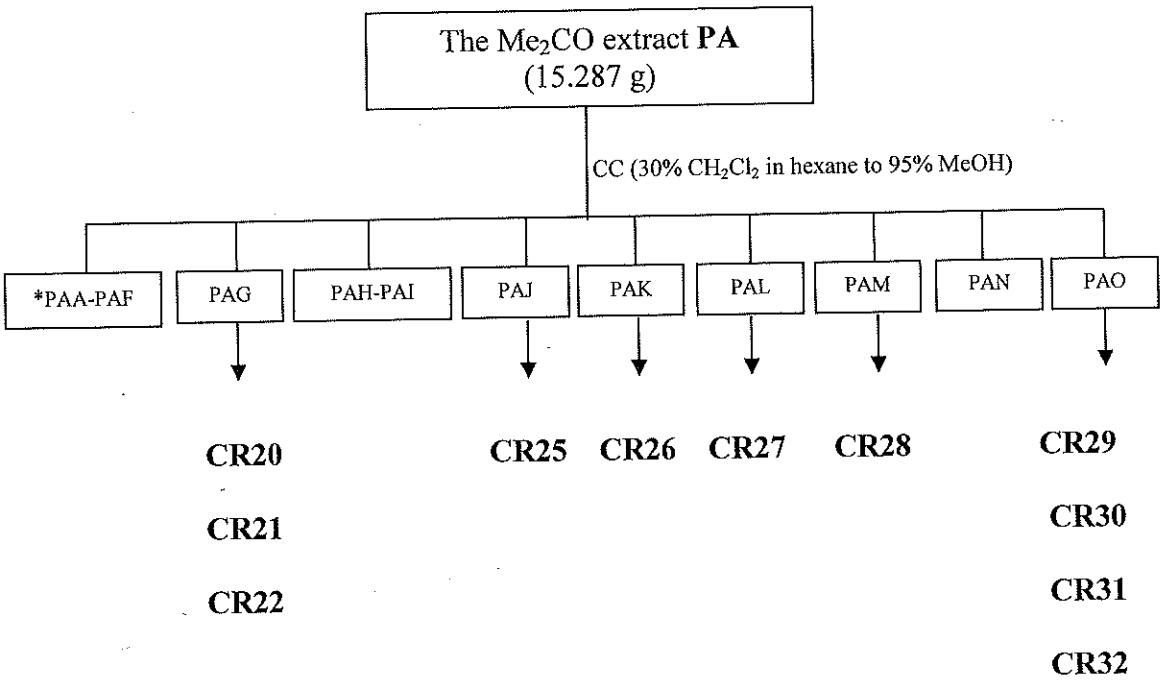
CR24: natsudaidain

^{1}H-NMR 300 MHz ($CDCl_3$) Table 25; Page 86

^{13}C NMR 75 MHz ($CDCl_3$) Table 25; Page 86

2.3.2.2 Purification of acetone extract

The Me_2CO extract **PA** was separated by a CC over diol using gradient solvent systems of a gradient of 30% CH_2Cl_2 in hexane to 95% MeOH as eluents. On the basis of their TLC characteristics, the fractions which contained the same major components were combined) to give 15 fractions **(PAA-PAO) (Table 4)**. Further purification of subfractions gave 11 pure compounds in **Scheme 6**.



* No further investigation

Scheme 6. Isolation of compounds **CR20-CR23, CR25-CR32** from acetone extract

Table 4. Physical characteristic and weight of the fractions from hexane insoluble fraction of acetone extract from the peels

Fractions	Weight (g)	Appearance
PAA	0.6712	orange viscous gel
PAB	0.8009	yellow viscous liquid
PAC	0.2499	yellow viscous liquid
PAD	0.4145	dark brown viscous liquid
PAE	0.4298	dark green viscous liquid
PAF	0.4865	green viscous liquid
PAG	0.8732	yellow green viscous liquid
PAH	0.7812	brown green viscous liquid
PAI	1.2359	dark brown viscous liquid
PAJ	1.3990	brown viscous liquid
PAK	0.8792	brown viscous liquid
PAL	1.4550	brown viscous liquid
PAM	0.8309	black solid
PAN	0.2011	black solid
PAO	1.9890	black solid

Isolation of CR20, CR21 and CR22

Fraction **PAG** (873.2 mg) was further separated by CC (30% CH_2Cl_2 in hexane-2% to 95% CH_2Cl_2 in MeOH) to give sixteen fractions (**PAGA-PAGP**). Fraction **PAGH** were collected by filtration to give a yellow solids **CR20** (2.1 mg). Fraction **PAGH10**, **PAGH11**were combined which was further separated by CC (20% acetone in hexane) to give yellow solids **CR21** (2.3 mg) and **CR22** (4.1 mg).

Isolation of CR25

Fraction **PAJ** (1.339 g) was subjected on CC (20% to 95% acetone in hexane) to give ten fractions (**PAJA-PAJJ**). Fraction **PAJF** was purified on CC (40% to 95% acetone in hexane) to provide a brown gum **CR25** (2.5mg).

Isolation of CR26

Fraction **PAK** (879.2 mg) was purified by CC (20% to 95% acetone in hexane) to afford twelve fractions (**PAKA-PAKL**). Fraction **PAKI** was purified on CC (40% -to 95% acetone in hexane) to provide a yellow solid **CR26** (1.2 mg).

Isolation of CR27

Chromatography of fraction **PAL** (1.455 mg) on reverse phase column (50% H_2O in MeOH) gave a colorless solid **CR27** (2.0 mg).

Isolation of CR28

Chromatography of fraction **PAM** (1.989 g) on reverse phase column (50% H_2O in MeOH) provided a yellow solid **CR28** (1.3 mg).

Isolation of CR29 and CR30

Chromatography of fraction **PAO** (2.756 g) on a reverse phase column (50% H_2O in MeOH) gave six fractions (**PAOA-PAOF**). Yellow solids **CR29** and **CR30** (2.0 mg) were obtained from fraction **PAOC** and **PAOD**, respectively.

Isolation of CR31 and **CR32**

Methylation of fraction **PAOF** (238.9 mg) with MeI (4.0 ml) and K_2CO_3 (20.0 mg) in Me_2CO (4.0 ml) for 8 h and purified by PTLC (20% acetone in hexane) to give amorphous powders **CR31** (0.9 mg) and **CR32** (1.3 mg).

CR25: 3, 4-dihydroxy benzoic acid

UV λ_{max} (MeOH) nm (log ε): 268 (3.52), 276 (3.05) nm

IR (neat) ν (cm^{-1}): 3564 (O-H stretching), 1678 (C=O stretching)

^{1}H-NMR 300 MHz (DMSO-d_6) Table 47; Page 134

^{13}C NMR 75 MHz (DMSO-d_6) Table 47; Page 134

CR26: 5,7,4'-trihydroxy-3',8-dimethoxyflavone

^{1}H-NMR 300 MHz ($CDCl_3$+ DMSO-d_6) Table 26; Page 88

^{13}C NMR 75 MHz ($CDCl_3$+ DMSO-d_6) Table 26; Page 88

CR27: 8,3'-β-glucosyloxy-2'-hydroxy-3'-methylbutyl-7-methoxy-coumarin

^{1}H-NMR 500 MHz ($CDCl_3$+DMSO-d_6) Table 35; Page 109

^{13}C NMR 125 MHz ($CDCl_3$+DMSO-d_6) Table 35; Page 109

CR28: hesperidin

^{1}H-NMR 500 MHz ($CDCl_3$+DMSO-d_6) Table 31; Page 98

^{13}C NMR 125 MHz ($CDCl_3$+DMSO-d_6) Table 31; Page 98

CR29: naringin

^{1}H-NMR 300 MHz (DMSO-d_6) Table 32; Page 101

^{13}C NMR 75 MHz (DMSO-d_6) Table 32; Page 101

CR30: rutin

^{1}H-NMR 500 MHz ($CDCl_3$) Table 33; Page 104

^{13}C NMR 125 MHz ($CDCl_3$) Table 33; Page 104

CR31: naringenin trimethyl ether

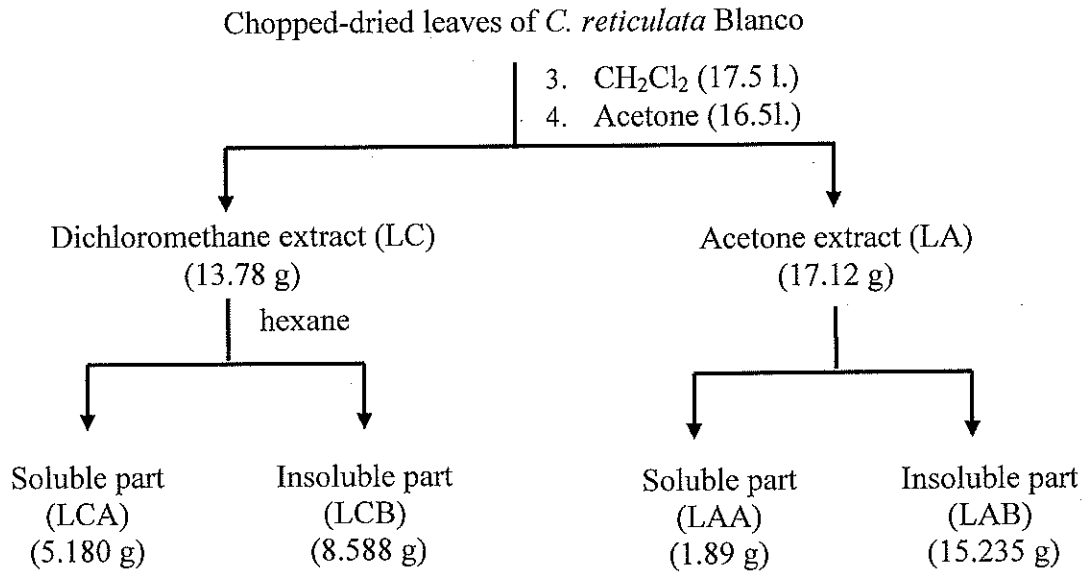
^{1}H-NMR 300 MHz ($CDCl_3$) Table 27; Page 90

CR32: 2,3-dihydro-5-hydroxy-4′,7-dimethoxyflavanone

^{1}H-NMR 300 MHz ($CDCl_3$) Table 28; Page 92

2.3.3 Extraction and isolation from the leaves

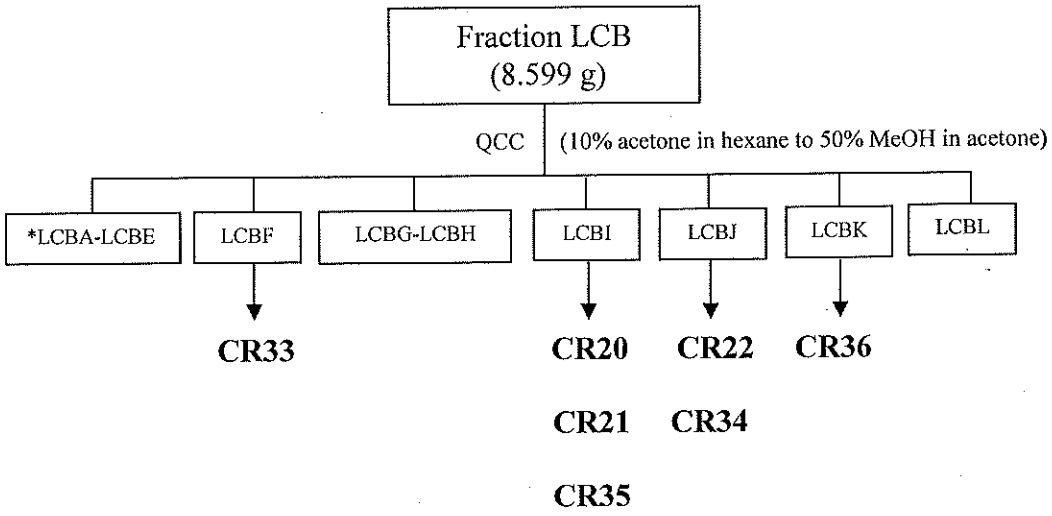
Chopped-dried leaves (1.4 kg) of *C.reticulata* (Neck orange) were successively immersed in CH_2Cl_2 (14.0 liters) and Me_2CO (14.0 liters) at room temperature (3 days x 2 times). After removal of solvent, the dark-brown viscous CH_2Cl_2 extract (13.788 g) and Me_2CO extract (17.12 g) were obtained, respectively. The CH_2Cl_2 extract was dissolved in hexane to give soluble (5.180 g) and insoluble (8.599 g) fractions. The Me_2CO extract was further fractionated in hexane to give soluble (1.89 g) and insoluble (15.235 g) fractions in **Scheme 7**.



Scheme 7. Extraction of crude extracts from the leaves of *C. reticulata* Blanco

2.3.1.1 Purification of insoluble fractions LCB

The CH_2Cl_2 insoluble fractions LC (8.599 g) was separated by a QCC over silica gel 60H using gradient solvent systems of a gradient of 10% acetone in hexane to 50% MeOH in acetone as eluents. On the basis of their TLC characteristics, the fractions which contained the same major components were combined to give 12 fractions (**LCBA-LCBL**) in **Scheme 8**.



* No further investigation *

Scheme 8. Isolation of compounds **CR20-CR22, CR33-CR36** from fraction **LCB**

Table 5. Physical characteristic and weight of the fractions from hexane insoluble fraction of dichloromethane extract from the leaves

Fractions	Weight (g)	Appearance
LCBA	0.5609	yellow viscous liquid
LCBB	0.4642	yellow viscous liquid
LCBC	0.5368	orange viscous liquid
LCBD	0.6077	orange viscous liquid
LCBE	1.131	dark green viscous solid
LCBF	0.8763	dark brown viscous solid
LCBG	0.5448	dark green solid
LCBH	0.6189	dark green solid
LCBI	0.5412	dark green solid
LCBJ	0.5568	dark green solid
LCBK	0.9873	dark green solid
LCBL	0.6123	dark green solid

Isolation of CR33

Fraction **LCBF** (876.3 mg) was purified by CC (20% to 50% acetone in hexane) to afford a white solids **33** (2.1 mg).

Isolation of CR20 and CR21

Fraction **LCBI** (541.2 mg) was purified by CC (20% to 50% acetone in hexane) to afford yellow solids **CR20** (1.9 mg) and **CR21** (2.1mg).

Isolation of CR22, CR34 and CR35

Fraction **LCBJ** (556.8 mg) was purified on Sephadex LH-20 column (20% MeOH in CH_2Cl_2) to provide fractions **LCBJA-LCBJE**. Fraction **LCBJC** was further purified by CC (20% acetone in hexane) afforded yellow solids **CR22** (2.4 mg), **CR34** (1.1 mg) and **CR35** (0.9 mg).

Isolation of CR36

Fraction **LCBK** (987.3 mg) was purified on Sephadex LH-20 column (20% MeOH in CH_2Cl_2) to give fraction **LCBKA-LCBKF**. Fraction **LCBKD** was further purified by CC (20% to 50% acetone in hexane) to afford a white solid **CR36** (3.8 mg).

CR33: betulinic acid

^{1}H-NMR 300 MHz ($CDCl_3$) Table 43; Page 128

CR34: 5, 7, 8, 3', 4'-pentamethoxyflavone

UV λ_{max} (MeOH) (log ε): 248 (3.07), 271 (3.13), 339 (3.16) nm

IR (KBr) ν (cm^{-1}): 1636 (C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$) Table 29; Page 94

^{13}C NMR 75 MHz ($CDCl_3$) Table 29; Page 94

CR35: sudachitin

^{1}H-NMR 300 MHz ($CDCl_3$) Table 30; Page 96

^{13}C NMR 75 MHz ($CDCl_3$) Table 30; Page 96

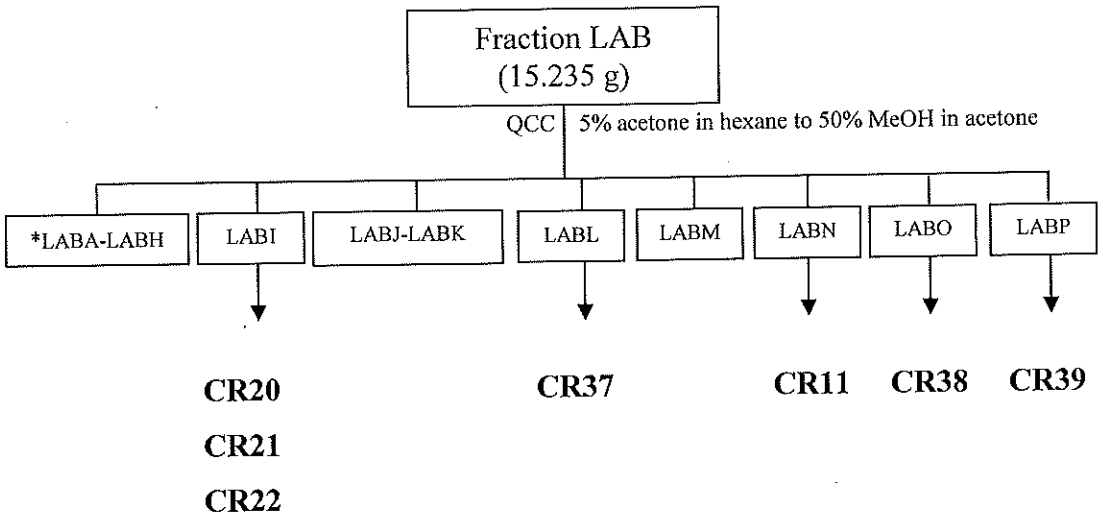
CR36: marmin

^{1}H-NMR 300 MHz ($CDCl_3$) Table 36; Page 112

^{13}C NMR 75 MHz ($CDCl_3$) Table 36; Page 112

2.3.1.1 Purification of insoluble fractions LAB

The Me_2CO insoluble fraction **LAB** (15.235 g) was separated by QCC using gradient solvent systems of a gradient of 5% acetone in hexane to 50% MeOH in acetone. On the basis of their TLC characteristics, the fractions which contained the same major components were combined to give 16 fractions (**LABA-LABP**) in **Scheme 9**.



* No further investigation

Scheme 9. Isolation of compounds **CR11, CR20-CR22, CR37-CR39** from fraction **LAB**

Table 6. Physical characteristic and weight of the fractions from acetone extract from the leave

Fractions	Weight (g)	Appearance
LABA	0.5609	yellow viscous liqiud
LABB	0.6642	yellow viscous liqiud
LABC	0.5368	orange viscous liqiud
LABD	0.6077	orange viscous liquid
LABE	0.7131	dark green viscous solid
LABF	0.5763	dark brown viscous solid
LABG	0.5448	dark green solid
LABH	0.6732	dark green solid
LABI	0.9788	dark green solid
LABJ	1.339	dark green solid
LABK	0.4532	dark green solid
LABL	0.9702	dark green solid
LABM	0.4532	dark green solid
LABN	0.2444	dark green solid
LABO	2.0776	dark green solid
LABP	2.3726	dark green solid

Isolation of CR20, CR21 and CR22

Fraction **LABI** (978.8 mg) was purified by CC (40% to 100% CH_2Cl_2 in hexane) to afford yellow solids of **CR20** (1.8 mg), **CR21** (2.1) and **CR22** (3.2).

Isolation of CR37

Fraction **LABL** (970.2 mg) was purified by CC (80% to 95% CH_2Cl_2 in hexane) to give fraction **LABLA-LABLK**. Fraction **LALG** was further purified by CC (80% to 95% CH_2Cl_2 in hexane) afforded a colorless solids **CR37** (1.3 mg).

Isolation of CR11

Fraction **LABN** (244.4 mg) was purified on Sephadex LH-20 column (20% MeOH in CH_2Cl_2) to give fraction **LABNA-LABND**. Fraction **LABNC** was

further purified by CC (1 to 3% MeOH in CH_2Cl_2) to provide a yellow solid **CR11** (0.7 mg).

Isolation of CR38

Fraction **LABO** (2.0776 g) was purified on Sephadex LH-20 using 20% MeOH in $CH_2Cl_2$to give fraction **LABOA-LABOE**. Fraction **LABOD** was further purified by CC (40% to 95% acetone in hexane) to give a white solid **CR38** (1.5 mg).

Isolation of CR39

Fraction **LABP** (2.3726mg) was purified on Sephadex LH-20 column using 20% MeOH in CH_2Cl_2 to give fraction **LABPA-LABPG**. Fraction **LAPE** was further purified by CC (50% acetone in hexane) to afford a white solid **CR39** (2.3 mg).

CR37: 4-Hydroxybenzaldehyde

m.p. 224-226 °C

UV λ_{max} nm (MeOH) (log ε): 257 (3.68), 275 (3.04) nm

IR (neat) ν (cm^{-1}): 3209 (O-H stretching), 1674 (C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$) Table 48; Page 135

CR38: crenulatin

m.p. 252-254 °C

UVλ_{max} (MeOH) (log ε): 208 (3.72), 215 (3.75), 256 (3.92), 310 (3.62), 330 (3.66) nm

IR (Neat) ν (cm^{-1}):1680 (C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$) Table 37; Page 114

^{13}C NMR 75 MHz ($CDCl_3$) Table 37; Page 114

CR39: isoimperatorin

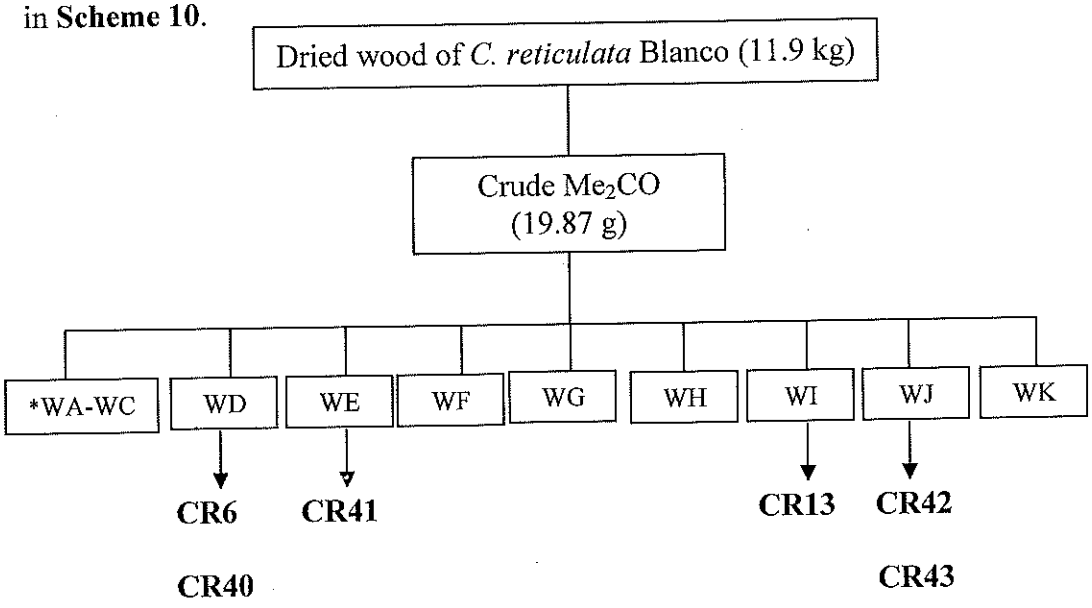
^{1}H-NMR 300 MHz ($CDCl_3$) Table 38; Page 116

^{13}C NMR 75 MHz ($CDCl_3$) Table 38; Page 116

2.3.4 Extraction and isolation from the wood

Dried wood of *C. reticulata* Blanco (11.9 kg) was chopped and immersed in Me_2CO at room temperature (5 days). After evaporation of the solvent, a dark brown gum (19.87 g) was obtained. The extract was subjected to QCC using hexane-Me_2CO, Me_2CO and Me_2CO-MeOH as eluents. Based on their TLC

characteristics, the eluted fractions were combined to give 11 fractions (**WA-WK**) in **Scheme 10**.



* No further investigation

Scheme 10. Isolation of compounds **CR6, CR13, CR40-CR43** from acetone extract from the wood.

Table 7. Physical characteristic and weight of the fractions from acetone extract from the wood

Fractions	Weight (g)	Appearance
WA	0.1179	orange gel mixed with white solid
WB	0.6243	orange gel
WC	2.0041	orange gel
WD	0.8912	orange gel
WE	0.7395	orange gel
WF	3.4880	orange gel
WG	3.5605	brown viscous liquid
WH	0.8816	brown viscous liquid
WI	0.7575	brown viscous liquid
WJ	1.2406	brown viscous liquid
WK	1.2525	brown viscous liquid

Isolation of CR6 and **CR40**

Fraction WD (891.2mg) was further subjected to a column of Sephadex LH-20 (10% MeOH in CH_2Cl_2) to give 14 fractions (**WDA-WDN**). Fraction **WDE** (82.7mg) was further purified by CC (20% Me_2CO in hexane) to give a white solid of **CR40** (1.8 mg) and a yellow solid of **CR6** (0.9 mg).

Isolation of CR41

Fraction **WE** (739.5mg) was applied to a Sephadex LH-20 column (10% MeOH in CH_2Cl_2) to give 14 fractions (**WEA-WEN**). Fraction **WEH** (37.2mg) was further purified by CC (10% EtOAc in hexane) to provide a white solid of **CR41** (3.5 mg).

Isolation of CR42 and **CR43**

Fraction **WJ** (1240.6mg) was further purified by Sephadex LH-20 and (20% MeOH in CH_2Cl_2) to give five fractions (**WJA-WJO**). Fraction **WJC** (517.8mg) was dissolved in CH_2Cl_2 to give a white solid **CR42** (4.7mg) and a filtrate (513.1mg) which was further purified by CC (2% MeOH in CH_2Cl_2) to give a yellow solid **CR43** (3.2 mg).

Isolation of CR13

Fractions **WI** and **WK** were combined (2.109 g) and further purified by HPLC using a gradient of 100% H_2O-100% MeOH over a 60-min period to give a white solid **CR13** (5.2 mg).

CR40: valencic acid

^{1}H-NMR 300 MHz ($CDCl_3$) Table 49; Page 137

^{13}C NMR 75 MHz ($CDCl_3$) Table 49; Page 137

CR41: *p*-hydroxyphenylethyl-*p*-coumarate

^{1}H-NMR 300 MHz ($CDCl_3$) Table 50; Page 139

^{13}C NMR 75 MHz ($CDCl_3$) Table 50; Page 139

CR42: limonexic acid

m.p. 285-286 °C

IR (neat) ν (cm^{-1}): 3233 (O-H stretching), 1743 (C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$) Table 42; Page 125

^{13}C NMR 75 MHz ($CDCl_3$) Table 42; Page 125

CR43:1, 3, 5-trihydroxy-2,4-dimethoxy-10-methyl-10H-acridin-9-one

HR-EIMS m/z: HR-EIMS at m/z 317.0972 for $C_{16}H_{15}NO_6$ (calcd for 317.0958)

EIMS m/z (% relative intensity): 317 (M^+, 62), 301 (100), 273 (23)

UV λ_{max} (MeOH) (log ε): 206(3.61), 269 (2.32), 336 (2.10)

IR (neat) ν (cm^{-1}): 3418 (O-H stretching) and 1651 (C=O stretching)

^{1}H-NMR 300 MHz ($CDCl_3$) Table 18; Page 72

^{13}C NMR 75 MHz ($CDCl_3$) Table 18; Page 72

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Structure elucidation of compounds from *Citrus reticulata* Blanco

The crude dichloromethane extract from the branch bark of *Citrus reticulata* Blanco, yielded one new compound and fourteen known compounds: atranorin **CR1**, 5-hydroxynoracronycine **CR2**, citflavanone **CR3**, gustastatin **CR4**, 8-hydroxy-6-methoxypentylisocoumarin **CR5**, citracridone-I **CR6**, citrusinol **CR7**, citrusinine-I **CR8**, citramine **CR9**, 2-methoxycitpressine **CR10**, scopoletin **CR11**, 2-hydroxy-4-methoxy-6-(2-oxoheptyl)-2'-methoxy-4'-hydroxy-6-(hetyl)phenyl ester **CR12**, limonin **CR13** and citracridone-III **CR14** and 4-hydroxybenzoic acid **CR15**. Methylation of the high polarity fractions from the branch barks gave one new methylated compound and four known compounds: limonin **CR13**, methyl-2-hydroxy-4-methoxy-6-(2-oxoheptyl)benzoate **CR16**, methyl 2,4-dimethoxy-6-heptyl-benzoate **CR17**, 6,8-dimethoxypentylisocoumarin **CR18** and citracridone-II **CR19**.

The crude dichloromethane and crude acetone extracts from the peels of *Citrus reticulata* Blanco, yielded eleven known compounds: 5-demethoxynobiletin **CR20**, tangeretin **CR21**, nobiletin **CR22**, 5,7,8,4'-tetramethoxyflavone **CR23**, natsudaidain **CR24**, 3,4-dihydroxy benzoic acid **CR25**, 5,7,4'-trihydroxy-3',8-dimethoxyflavone **CR26**, 8,3'-β-glucosyloxy-2'-hydroxy-3'-methylbutyl-7-methoxy-coumarin **CR27**, hesperidin **CR28**, naringin **CR29**, rutin **CR30**. Methylation of the high polarity fractions from the peels gave two known compounds: naringenin trimethyl ether **CR31** and 2,3-dihydro-5-hydroxy-4',7-dimethoxyflavanone **CR32**.

The crude dichloromethane and crude acetone extract of the leaves of *Citrus reticulata* Blanco, yielded ten known compounds; 5-demethoxynobiletin **CR20**, tangeretin **CR21**, nobiletin **CR22**, betulinic acid **CR33**, 5,7,8,3',4'-penta-methoxyflavone **CR34**, sudachitin **CR35**, marmin **CR36**, 4-hydroxybenzaldehyde **CR37**, crenulatin **CR38**, isoimperatorin **CR39**.

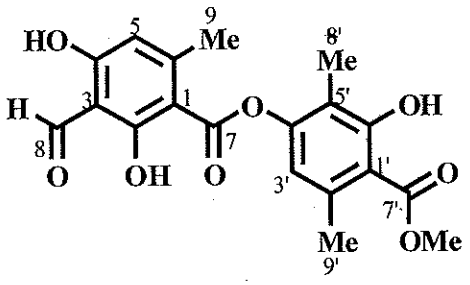
The crude acetone extract of the wood of *Citrus reticulata* Blanco, yielded one new compounds and five known compounds: citrusinine-I **CR8**, limonin

CR13,valencic acid **CR40**, *p*-hydroxyphenylethyl-*p*-coumarate **CR41**, limonexic acid **CR42** and 1,3,5-trihydroxy-2,4-dimethoxy-10-methyl-10H-acridin-9-one **CR43**.

Their structures were elucidated mainly by 1D and 2D NMR spectroscopic data: ^{1}H, ^{13}C NMR, DEPT 135°, DEPT 90°, HMQC, HMBC and COSY. The physical data of the known compounds were also compared with the reported values.

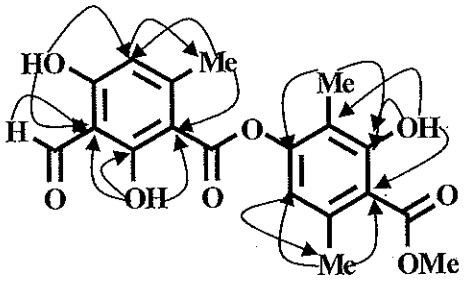
3.1.1 Depsides

CR1: Atranorin



CR1 was isolated a colorless needle. The ^{1}H NMR spectrum showed the resonances of aldehydic proton at δ 10.37, four methyl groups at δ 2.07, 2.53, 2.67 and 3.97, two *singlet* aromatic protons at δ 6.41 (H-5) and δ 6.50 (H-3'), and three hydrogen bonded hydroxy groups at δ 12.49 (2-OH), δ 12.54 (4-OH) and δ 11.94 (6'-OH). The ^{13}C NMR spectrum displayed three carbonyl carbons including one carbonyl aldehyde group (δ 193.8), two carbonyl ester groups (δ 169.7 and δ 172.2). The HMBC correlations of 2-OH to C-1 (δ 102.8), C-2 (δ 169.1), C-3 (δ108.5); 4-OH to C-3 (δ 108.5) and C-5 (δ 112.8) and formyl protons H-8 to C-3 (δ 108.5) indicated that 2-OH, 4-OH, formyl were on ring A.While the HMBC correlations of 6'-OH to C-1′ (δ 110.3), C-5′ (δ 116.7), C-6′ (δ 162.8), 8′-Me (δ 2.07) to C-5', and 9′-Me (δ 2.53) to C-2' (δ 139.9) and C-1' indicated that 6'-OH, 8′-Me and 9′-Me were on ring B. The formyl group was assigned *ortho* to 2-OH and 4-OH as a result from HMBC correlations of these three protons to C-3 (δ 108.5). Aromatic protons H-5 (δ 6.31) showed correlation to C-9 and of 9-Me (δ 2.67) to C-5 (δ 112.8) indicating that H-5 was *ortho* to 9-Me. In addition, the 9-Me (δ 2.67) was assigned to the position *ortho* to the ester group (δ 169.7), and *meta* to 2-OH according to the HMBC correlation of 9-Me and 2-OH to C-1(δ 102.8). The location of the methyl groups 9′-Me (δ 2.53) and hydrogen bonded hydroxy group 6′-OH (δ 11.94) were located at the positon *ortho* to the ester group (δ 172.2) due to since protons showed correlations to C-1′ (δ 110.3).The chemical shift value of δ 2.07 allowed it to be assigned to the methyl group (8′-Me). It was located *ortho* to 6′-OH according to the HMBC correlation of

the both protons to C-6′ (162.8). Aromatic proton H-3' (δ 6.50) showed correlation to C-9′ and of 9′-Me (δ 2.53) to C-3' (δ 115.9) confirming H-3' was *ortho* to 9'-Me. Thus, these data allowed to deduce the structure of **CR1**, a natural product classified as a depside which was known as atranorin (Quilhot *et al*,, 1975).



Major HMBC correlations of **CR1**

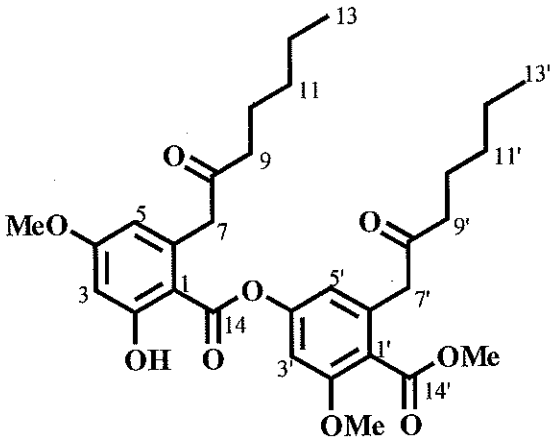
Table 8. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR1** ($CDCl_3$+DMSO-d_6)

Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
1	-	102.8 (C)	-
2	-	169.1 (C)	-
3	-	108.5 (C)	-
4	-	167.5 (C)	-
5	6.41 (*s*)	112.8 (CH)	C-1, C-3, C-4, C-9
6	-	152.4 (C)	-
7	-	169.7 (C=O)	-
8	10.37 (*s*)	193.8 (CH)	C-1, C-2, C-3
9	2.67 (*s*)	25.6 (CH_3)	C-1, C-5, C-6
1′	-	110.3 (C)	-
2′	-	139.9 (C)	-
3′	6.50 (*s*)	115.9 (CH)	C-1′, C-5′, C-4′, C-9′

Table 8. ^{1}H,^{13}C NMR and HMBC spectroscopic data of **CR1** ($CDCl_3$+DMSO-d_6) continued

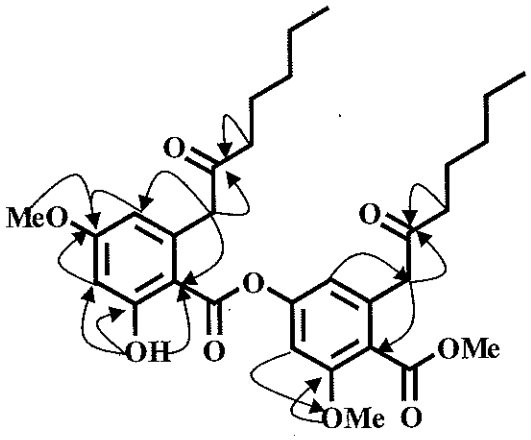
Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
4′	-	152.0 (C)	-
5′	-	116.7 (C)	-
6′	-	162.8 (C)	-
7′	-	172.2 (C=O)	-
8′	2.07 (*s*)	19.4 (CH_3)	C-4′, C-5′, C-6′
9′	2.53 (*s*)	24.0 (CH_3)	C-1′, C-2′, C-3′
7′CO_2Me	3.97 (*s*)	52.3 (CH_3)	C-7′
2-OH	12.49 (*s*)	-	C-1, C-2
4-OH	12.54 (*s*)	-	C-3, C-4, C-5
6′-OH	11.94 (*s*)	-	C-1′, C-5′, C-6′

CR4:Gustastatin



CR4 was isolated as amorphous powder. The ^{13}C and DEPT 135 NMR resonances could be ascribed to a 1,2,3,5-tetrasubstituted aromatic ring containing resonances at δ 104.3 (C-1), 166.5 (C-2), 100.1 (C-3), 164.9 (C-4), 113.4 (C-5), and 138.9 (C-6) and to a 1,2,3,5- tetrasubstituted aromatic ring with carbons resonating at δ 121.8 (C-1′), 158.4 (C-2′), 104.5 (C-3′), 151.3 (C-4′), 116.2 (C-5′), and 135.4 (C-6′), respectively. Additional signals were two carbonyl ester resonances at δ 169.1 (C-14) and 167.5 (C-14′), and three methoxyl carbons resonances at δ 55.4 (4-OMe), 56.3 (2′-OMe) and 52.3 (1′CO_2Me). The ^1H NMR (**Table 9**) spectrum showed the resonances of a chelated hydroxyl group at δ 11.28, a *meta*-aromatic proton at δ 6.46 (*d*, 2.1, H-3) and 6.29 (*d*, H-5), methoxy proton at δ 3.82. The COSY correlations of H-9 (2.44, *t*) to H-10 (1.56, *m*), H-10 to H-11 (1.31, *m*), H-11 to H-12 (1.27, *m*) and H-12 to H-13 (0.86, *t*), together with the HMBC correlations of H-9 (δ 2.37) and H-7 (δ 4.07, *s*) to the carbonyl carbon C-8 (δ 207.4) indicated the presence of a 2-oxoheptyl side chain. The location of the 2-oxoheptyl groups at C-6 was confirmed by HMBC correlations of H-7 with C-1, C-5, C-6. The methoxy proton, H-3 and H-5 both showed a correlation to C-4 confirming that methoxy group was attached at C-4. The chelated hydroxyl group was placed at C-2 (δ 166.5), a *peri* position to the ester carbonyl carbon, since had HMBC cross peaks with C-1, C-2 and C-3. The 1,2,3,5-tetrasubstituted aromatic system was shown at δ 6.59 (*d*, 2.1 Hz, H-3') and 6.57 (*d*, 2.1 Hz, H-5'), two methoxy protons at δ 3.87 (14'CO_2Me) and 3.84 (2'-OMe). The COSY correlations of H-9' (2.41, *t*) to H-10' (1.53, *m*), H-10' to H-11' (1.21, *m*), H-

11' to H-12' (1.27, *m*) and H-12' to H-13' (0.83, *t*), together with the HMBC correlations of H-9' (δ 2.37) and H-7' (δ 3.70, *s*) to the carbonyl carbon C-8' (δ 206.5) indicated the presence of a 2-oxoheptyl side chain. The second hepta-2-one side chain was placed *ortho* to C-5' due to HMBC correlations of H-7 ' to C-5'. The methoxy proton (δ 3.84, 2'-OMe) and H-3' showed correlations to C-2' (158.4) confirmed that the methoxy protons was *ortho* to H-3'. Proton H-7' further showed correlation to a quaternary carbon that resonated at δ 121.8 which was the carbon that bonded to CO_2Me. The ring B was then assigned to form an ester bond at C-4'. From the above mentioned data and comparing these data with the those previously reported, it was clear that the **CR4** was gustastatin (Pettit *et al.*, 2004).



Major HMBC correlation of **CR4**

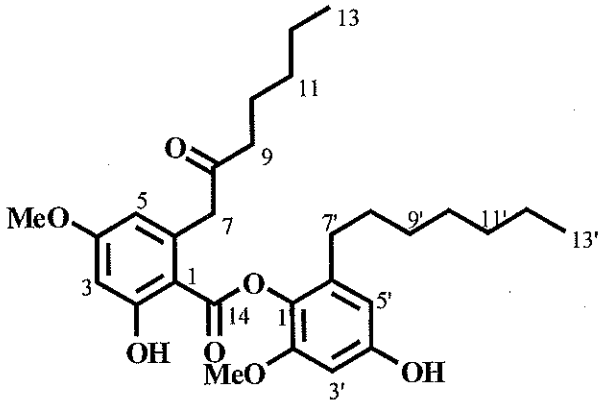
Table 9. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR4** ($CDCl_3$)

Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
1	-	104.3 (C)	-
2	-	166.5 (C)	-
3	6.46 (*d*, *J* = 3.0 Hz)	100.1 (CH)	C-1, C-4, C-5
4	-	164.9 (C)	-
5	6.29 (*d*, *J* = 3.0 Hz)	113.4 (CH)	C-1, C-3, C-4
6	-	138.9 (C)	-
7	4.07 (*s*)	51.2 (CH_2)	C-1, C-5, C-6, C-8

Table 11. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR4** ($CDCl_3$) (continued)

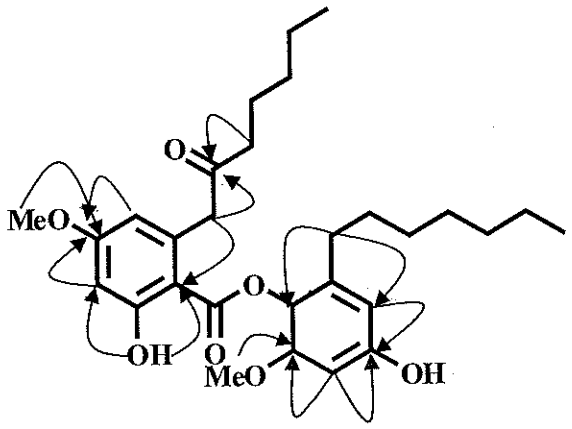
Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
8	-	207.4 (C=O)	-
9	2.44 (*t*, *J* = 7.5 Hz)	42.5 (CH_2)	C-8, C-11
10	1.56 (*m*)	31.3 (CH_2)	C-12
11	1.21 (*m*)	23.4 (CH_2)	C-9, C-11
12	1.27 (*m*)	22.4 (CH_2)	C-10
13	0.86 (*t*, *J* = 6.9 Hz)	13.9 (CH_3)	C-11
14	-	169.1 (C=O)	-
1'	-	121.8 (C)	-
2'	-	158.4 (C)	-
3'	6.57 (*d*, *J* = 2.1 Hz)	104.5 (CH)	C-2', C-4', C-5'
4'	-	151.3 (C)	-
5'	6.59 (*d*, *J* = 2.1 Hz)	116.2 (CH)	C-3', C-4', C-6', C-7'
6'	-	135.4 (C)	-
7'	3.70 (*s*)	47.5 (CH_2)	C-1', C-5', C-6'
8'	-	206.5 (C=O)	C-10'
9'	2.41 (*t*, *J* = 7.5 Hz)	42.2 (CH_2)	C-7', C-11'
10'	1.53 (*m*)	31.3 (CH_2)	C-8', C-12
11'	1.21 (*m*)	23.3 (CH_2)	C-9', C-13'
12'	1.27 (*m*)	22.4 (CH_2)	C-10'
13'	0.83 (*t*, *J* = 6.9 Hz)	13.8 (CH_3)	C-11'
14'	-	167.5 (C=O)	C-14'
2-OH	11.28 (*s*)	-	C-1, C-2, C-3
CO_2Me	3.87 (*s*)	55.4 (CH_3)	-
4-OMe	3.82 (*s*)	55.4 (CH_3)	C-4
2'-OMe	3.84 (*s*)	56.3 (CH_3)	C-2'

CR12: 2-hydroxy-4-methoxy-6-(2-oxoheptyl)-2'-methoxy-4'-hydroxy-6-(hetyl)-phenyl ester



CR12 was obtained as an amorphous powder, m.p. 125-127 °C. Its UV spectrum showed absorption peaks at 265.0, 286, 311 and 400 nm. The IR spectrum showed the absorption band of C=O stretching at 1726.0 cm^{-1}. A molecular ion in the FAB-Ms at *m/z* 501.2848 corresponded to a molecular formula of $C_{29}H_{40}O_7$. The ^{13}C NMR spectrum (**Table 10**) showed the resonances of four methine aromatic carbons: δ 100.1 (C-3), 113.4 (C-5), 103.2 (C-3′), 115.5 (C-5′), three quaternary aromatic carbons: δ 104.4 (C-1), 139.0 (C-6), 119.5 (C-6′), five oxy aromatic carbons 166.6 (C-2), 169.1 (C-4), 158.0 (C-4′), 151.6 (C-2′), 145.4 (C-1′), two methoxyl carbons: δ 55.5 (4-OMe), 56.4 (2′-OMe), a carbonyl ester carbon: δ 164.9 (C-14) and a carbonyl ketone: δ 207.2 (C-8). The ^1H NMR spectrum showed doublet resonances of *meta*-aromatic protons H-3 and H-5 at δ 6.45 and δ 6.28, and *meta*-aromatic protons H-3' and H-5' at δ 6.52 and δ 6.58, that indicated the presence of two 1,2,3,5-tetrasubstituted benzene rings. The COSY correlations of H-9 (2.37, *t*) to H-10 (1.42, *m*), H-10 to H-11 (1.20, *m*), H-11 to H-12 (1.10, *m*) and H-12 to H-13 (0.86, *t*), together with the HMBC correlations of H-9 (δ 2.37) and H-7 (δ 4.06, *s*) to the carbonyl carbon C-8 (δ 207.2) indicated the presence of an 2-oxoheptyl side chain. The proton H-7 and a hydrogen bonded hydroxyl group resonating at δ 11.27 showed a HMBC correlation to C-1 (δ 104.4), indicated that the hydroxyl group (2-OH) and 2-oxoheptyl side chain were *ortho* to the carbonyl ester. A methoxyl group at δ 3.82 was 4-OMe due to H-3, H-5 and 4-OMe showing HMBC correlations to C-4. A heptyl side chain was deduced from the COSY correlations of

H-7' (δ 2.75, *t*) to H-8' (1.53, *m*), H-8' to H-9' (1.42, *m*), H-9' to H-10' (1.35, *m*), H-10' to H-11' (1.20, *m*), H-11' to H-12' (1.10, *m*) and H-12' to H-13' (0.82, *t*), and it was attached at C-6' due to the H-7' showing HMBC correlation to C-5' (δ 115.5) and C-1' (δ 145.4). The remaining methoxyl group (δ 3.84, 2'-OMe) was assigned at C-2' according to the HMBC correlations of the methoxyl group and H-3' (δ 6.52) to C- 2' (δ 151.6). The evidence that H-7' was further correlated to the oxy carbon at δ 145.4, while the H-3' and H-5' was correlated to the oxy carbons at δ 145.4 and δ 158.0, together with the interpretation of the molecular formula of $C_{29}H_{40}O_7$ ($[M+1]^+$ *m/z* 501.2848) implied the presence of a hydroxyl group and an ester bond formed between two aromatic rings. Two possible structures could be proposed with the ester bond attached at either the C-1' or C-4' and the hydroxyl group attached at either the C-4' or C-1', respectively. A comparison of the ^{13}C chemical shifts published for alkenylresorcinols; 4-hydroxy-2-methoxy-6-[(8*Z*)-pentadec-8-en-1-yl]phenyl acetate, 4-hydroxy-2-methoxy-6-pentadecylphenyl acetate and 1-*O*-methyl-6-acetoxy-5-(pentadec-10*Z*-enyl)resorcinol demonstrated that the carbons with an ester bond *ortho* to the alkyl and alkoxy groups consistently resonated at least 10 ppm further up field compared with the hydroxyl group meta to the alkyl and alkoxyl groups. Accordingly, the ester bond was assigned to attach at the C-1' (δ 145.4) and the hydroxyl group was at C-4' (δ 158.0) (Al-Mekhlafi *et al.*, 2012; Bao *et al.*, 2010). Therefore 2-hydroxy-4-methoxy-6-(2-oxoheptyl)-2'-methoxy-4'-hydroxy-6-(hetyl)-phenyl ester was assigned for **CR12**. It is a new depside



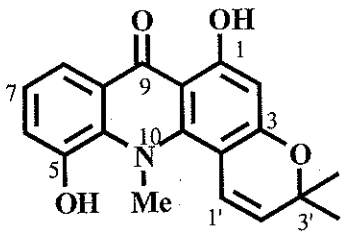
Major HMBC correlations of **CR12**

Table 10. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR12** ($CDCl_3$)

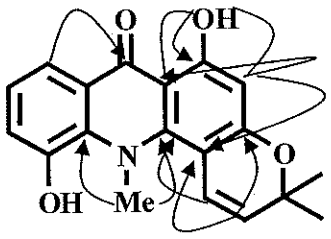
Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
1	-	104.4 (C)	-
2	11.27 (*s*)	166.6 (C)	C-1, C-2, C-3
3	6.45 (*d*, *J* = 3.0 Hz)	100.1(CH)	C-1, C-4, C-5
4	-	169.1 (C)	-
5	6.28 (*d*, *J* = 3.0 Hz)	113.4 (CH)	C-1, C-3, C-4
6	-	139.0 (C)	-
7	4.04 (*s*)	51.4 (CH_2)	C-1, C-5, C-6, C-8
8	-	207.2 (C=O)	-
9	2.37 (*t*, *J* = 7.5 Hz)	42.5 (CH_2)	C-8, C-11
10	1.42 (*m*)	23.3 (CH_2)	C-12
11	1.20 (*m*)	31.7 (CH_2)	C-9, C-11
12	1.10 (*m*)	29.7 (CH_2)	C-10
13	0.86 (*t*, *J* = 6.9 Hz)	13.9 (CH_3)	C-11
14	-	164.9 (C)	-
1'	-	145.4 (C)	-
2'	-	158.0 (C)	-
3'	6.52 (*d*, *J* = 2.1 Hz)	103.2 (CH)	C-2', C-4', C-5'
4'		151.6 (C)	
5'	6.58 (*d*, *J* = 2.1 Hz)	115.5 (CH)	C-3', C-4', C-6', C-7'
6'		119.5 (C)	
7'	2.75 (*t*, *J* = 7.5 Hz)	33.9 (CH_2)	C-1', C-5', C-6'
8'	1.53 (*m*)	29.7 (CH_2)	C-10'
9'	1.42 (*m*)	23.3 (CH_2)	C-'7, C-11'
10'	1.35 (*m*)	31.7 (CH_2)	C-8', C-12
11'	1.20 (*m*)	29.7 (CH_2)	C-9', C-13'
12'	1.10 (*m*)	22.4 (CH_2)	C-10'
13'	0.82 (*t*, *J* = 6.9 Hz)	13.8 (CH_3)	C-11'
4-OMe	3.82 (*s*)	55.5 (CH_3)	C-4
2'-OMe	3.84 (*s*)	56.4 (CH_3)	C-2'

3.1.2 Acridones

CR2: 5-hydroxynoracronycine



CR2 was obtained as an orange solid. The UV spectrum showed maximum absorption bands at 212, 267, 283 and 414 nm. Its IR spectrum showed the stretching of hydroxyl (3446 cm^{-1}) and chelated carbonyl groups (1636 cm^{-1}). Its spectrum **(Table 11)** showed the characteristic signal of a chelated hydroxy proton (1-OH) and *N*-methyl proton of acridone skeleton at δ 14.43 and δ 3.81, respectively. The spectrum further showed the resonances of a singlet aromatic proton (H-2) at δ 6.13, 1,2,3-tri-substituted aromatic protons at δ 7.14 (*t*, 7.8, H-7), δ 7.26 (*d*, 7.8, H-6) and δ7.75 (*d*, 7.8, H-8). The singlet aromatic proton (H-2) was placed *ortho* to the chelated hydroxyl protons (1-OH), and chromene ring according to HMBC correlations of 1-OH to C-2 (δ 102.2), H-2 to C-9a (δ 111.7), C-4 (δ 107.0). The 2, 2-dimethyl chromene ring was suggested from the resonances of methyl protons at δ 1.51 (H-4'/H-5') and *cis*-olefinic protons at δ 6.68 (*d*, 9.8, H-1') and δ 5.56 (*d*, 9.8, H-2'). It was placed at C-3 and C-4 according to the HMBC correlations of H-2' to C-4 (δ 107.0), H-1' to C-3 (δ 165.9) and C-4a (δ 152.5). Proton H-8 was confirmed to be *peri* to a carbonyl group since HMBC it showed correlations to the carbonyl carbon at C-9 (δ 186.6). Consequently, a hydroxy proton that resonated at δ 9.98 was placed at C-5. It was thus identified as 1,5-dihydroxy-10,3',3'-trimethyl-10,3'-dihydro-3*H*-pyrano-[2,3-*c*]acridin-9-one which was identical to 5-hydroxynoracronycine (Wu *et al.*, 1983).



Major HMBC correlations of **CR2**

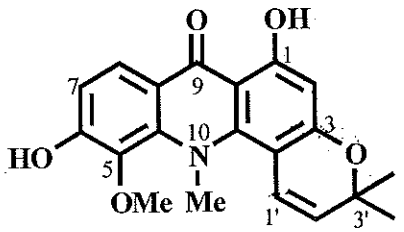
Table 11. ^{1}H,^{13}C NMR and HMBC spectroscopic data of **CR2** ($CDCl_3$+DMSO-d_6)

Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
1	-	169.1 (C)	-
2	6.13 (*s*)	102.2 (CH)	C-1, C-4, C-9[a]
3	-	165.9 (C)	-
4	-	107.0 (C)	-
5	-	152.5 (C)	-
6	7.26 (*d*, *J* = 7.8 Hz)	124.9 (CH)	C-5, C-10a, C-8
7	7.14 (*t*, *J* = 7.8 Hz)	128.1 (CH)	C-5, C-8a
8	7.75 (*d*, *J* = 7.8 Hz)	120.7 (CH)	C-10a, C-9
9	-	186.6 (C=O)	-
4a	-	152.5 (C)	-
8a	-	129.5 (C)	-
9a	-	111.7 (C)	-
10a	-	141.8 (C)	-

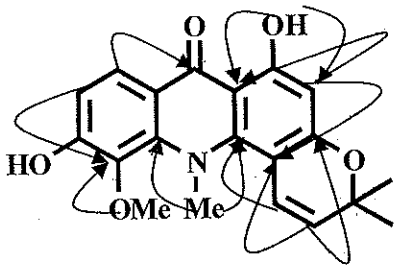
Table 11. ^{1}H,^{13}C NMR and HMBC spectroscopic data of **CR2** ($CDCl_3$+DMSO-d_6) (continued)

Position	δ_H **(multiplicity, *J*)**	δ_C **(C-Type)**	**HMBC**
1′	6.68 (*d*, *J* = 9.8 Hz)	125.8 (CH)	C-3, C-4a, C-3′
2′	5.56 (*d*, *J* = 9.8 Hz)	128.4 (CH)	C-3, C-4, C-3′
3′	-	81.4 (C)	-
4′/5′	1.51 (*s*)	31.8 (CH_3)	C-2′, C-3′, C-5′
1-OH	14.43 (*s*)	-	C-1, C-2, C-9a
5-OH	9.98 (*s*)	-	-
N-Me	3.81 (*s*)	53.4 (CH_3)	C-4a, C-10a

CR6: citracridone-I



Compound **CR6** was isolated as an orange solid. The ^{1}H NMR and HMBC spectroscopic data (**Table 12**) indicated that **CR6** had signals very similars to those of **CR2** including: chelated hydroxy proton 1-OH (δ 14.40), *N*-methyl proton (δ 3.81), aromatic proton H-2 (δ 6.27) and 2,2-dimethyl chromene ring (H-4'/H-5', δ 1.52; H-1', δ 6.54; H-2', δ 5.58) at C-3/C-4. The spectrum further showed doublet resonances of *ortho*-aromatic protons at δ 6.99 (H-7) and δ 8.07 (H-8) and methoxyl signal at δ 3.90 (5-OMe). The down field aromatic proton (H-8) was confirmed to be *peri* to a carbonyl group due to the HMBC correlations of it to C-9 (δ 181.5). The methoxyl group (δ 3.90) was placed at C-5 according to the HMBC correlations from the methoxyl proton and H-7 to C-5 (δ 135.8). The hydroxyl group was placed at C-6 to complete the structure. Therefore **CR6** was assigned as 1,6-dihydroxy-5-methoxy-10,3',3'-trimethylpyrano[2,3-*c*]-acridin-9-one which was known as citracridone-I (Wu *et al.*, 1983).

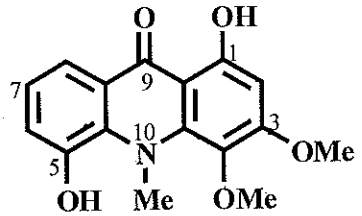


Major HMBC correlations of **CR6**

Table 12. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR6** ($CDCl_3$)

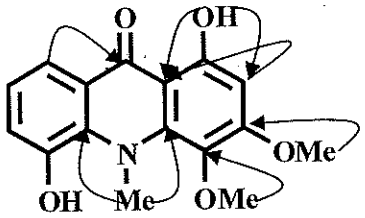
Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
1	-	164.7 (C)	-
2	6.27 (*s*)	98.7 (CH)	C-1, C-3, C-4, C-9a
3	-	161.1 (C)	-
4	-	102.5 (C)	-
5	-	135.8 (C)	-
6	-	154.4 (C)	-
7	6.99 (*d*, J = 8.7 Hz)	112.0 (CH)	C-5, C-8[a]
8	8.07 (*d*, J = 8.7 Hz)	123.4 (CH)	C-6, C-9, C-10[a]
9	-	181.5 (C=O)	-
4a	-	147.3 (C)	-
8a	-	118.5 (C)	-
9a	-	106.8 (C)	-
10a	-	141.5 (C)	-
1′	6.54 (*d*, J = 9.9 Hz)	120.4 (CH)	C-3, C-4a, C-3′
2′	5.58 (*d*, J = 9.9 Hz)	124.7 (CH)	C-4, C-3′, C-4′/5′
3′	-	77.0 (C)	-
4′/5′	1.52 (*s*)	27.2 (CH_3)	C-2′, C-3′
1-OH	14.23 (*s*)	-	C-1, C-2, C-9a
5-OMe	3.90 (*s*)	60.0 (CH_3)	C-5
N-Me	3.70 (*s*)	47.9 (CH_3)	C-4a, C-10a

CR8:Citrusinine-I



CR8 was obtained as a yellow solid. The UV spectrum showed maximum absorption bands at 203, 221, 263, 319, and 416 nm. Its IR spectrum showed the stretching of hydroxyl group (3386 cm^{-1}) and carbonyl groups (1633 cm^{-1}). Its ^1H NMR spectrum **(Table 13)** showed the resonances of a chelated hydroxy proton (δ 14.24, *s*, 1-OH), a hydroxy proton (δ 9.36, *brs*, 5-OH), an *N*-methyl protons (δ 3.84, *s*), tri-substituted aromatic protons (δ 7.24, *dd*, 7.8, 1.5, H-6; δ 7.11, *t*, 7.8, H-7; δ 7.82, *dd*, 7.8, 1.5, H-8), an aromatic proton (δ 6.36, *s*, H-2) and two methoxy protons at δ 3.96 (3-OMe) and δ 3.79 (4-OMe). Proton H-8 was confirmed at *peri* to a carbonyl group because it showed HMBC correlations to the carbonyl carbon C-9 (δ 182.3). A singlet aromatic proton at δ 6.36 (H-2) was placed *ortho* to a chelated hydroxyl protons (1-OH) according to HMBC correlations of 1-OH to carbon at δ 93.3 (C-2), and H-2 to the carbon at δ 106.2 (C-9a). One methoxyl group (δ 3.96) showed a correlation to the carbon at δ 159.4, another (δ 3.79) showed a correlation to a carbon at δ 125.0. The location of -OMe was assigned by comparison to the published data. The data published for 1,3,4-trioxygenated acridones demonstrates that C-3 consistently resonates downfield compared with C-4. The methoxyl group at δ 3.96 then was placed at C-3 (δ 159.4), while the methoxyl group at δ 3.79 was placed at C-4 (δ 125.0). The downfield shift of C-3 relative to C-4 can be explained by the electron withdrawing effect of the carbonyl group which predominantly influences the chemical shift of the aromatic carbon *para* to it, resulting in a lower electron density at C-3 relative to C-4 in acridones. C-4 is unfiled because it is *ortho* to both a OMe and a *N*-Me groups, and

para to a OH group. Therefore **CR8** was assigned as 1,5-dihydroxy-3,4-dimethoxy-10-methyl-9(10*H*)-acridinonewhich was known as citrusinine-I (Wu *et al.*, 1983).

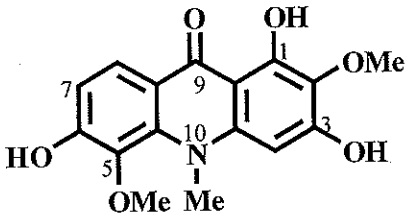


Major HMBC correlations of **CR8**

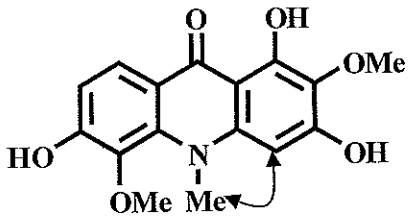
Table 13. ^{1}H,^{13}C NMR and HMBC spectroscopic data of **CR8** ($CDCl_3$+DMSO-d_6)

Position	δ_H **(multiplicity, *J*)**	δ_C **(C-Type)**	**HMBC**
1	-	160.2 (C)	-
2	6.36 (*s*)	93.3 (CH)	C-1, C-4, C-9a
3	-	159.4 (C)	-
4	-	125.0 (C)	-
5	-	148.0 (C)	-
6	7.24 (*dd*, *J* = 7.8, 1.5 Hz)	120.0 (CH)	C-8, C-10a
7	7.11 (*t*, *J* = 7.8 Hz)	122.4 (CH)	C-5, C-8a
8	7.82 (*dd*, *J* = 7.8, 1.5 Hz)	116.2 (CH)	C-6, C-9, C-10a
9	-	182.3 (C=O)	-
4a	-	137.0 (C)	-
8a	-	124.4 (C)	-
9a	-	106.2 (C)	-
10a	-	133.0 (C)	-
1-OH	14.24 (*s*)	-	C-1, C-2, C-9a
5-OH	9.36 (*s*)	-	C-5, C-10a
3-OMe	3.96 (*s*)	56.0 (CH_3)	C-3
4-OMe	3.79 (*s*)	60.2 (CH_3)	C-4
N-Me	3.84 (*s*)	46.0 (CH_3)	C-4a, C-10a

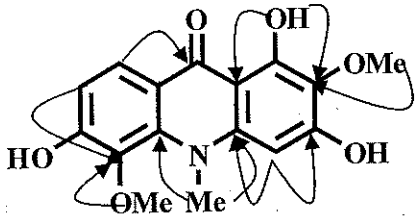
CR9: Citramine



CR9 was obtained as a yellow solid. The UV spectrum showed maximum absorption bands at 214, 271, 331 and 386 nm. The IR spectrum showed the absorption bands of hydroxyl group at 3418 cm^{-1} and chelated carbonyl group at 1651 cm^{-1}. It ^{1}H NMR spectrum (**Table 14**) indicated the presence of a chelated hydroxyl group (1-OH, δ 14.89), two hydroxyl groups (δ 9.08, 8.67), an *N*-methyl group (δ 3.99), *ortho*-coupled aromatic protons (H-7, δ 6.94. *d*, 9.0; H-8, δ 8.07, *d*, 9.0), an isolated aromatic proton (H-4, δ 6.42), and two methoxyl groups (5-OMe, δ 3.77; 2-OMe, δ 3.96). A methoxyl group 2-OMe (δ 3.96) was placed *ortho* to the chelated hydroxyl protons 1-OH according to HMBC correlations of 1-OH and methoxy protons 2-OMe (δ 3.96) to carbon C-2 (δ 128.7). A singlet aromatic proton at δ 6.42 (H-4) was placed at C-4, due to the NOE enhancement of the *N*-methyl signal by irradiation at the resonance of H-4, and the enhancement of H-4 by irradiation at the resonance of *N*-methyl signal. In addition a methoxyl group 5-OMe that resonated at δ 3.77 was placed at C-5 according to HMBC correlation from the methoxyl proton and H-7 to C-5 (δ 134.9). Finally, aromatic proton H-4 showed a correlation to the carbon at δ 156.5 which was assigned for the oxygenated aromatic carbons (C-3) thus completing assignment. Compound **CR9** therefore was identified as 1,3,6-trihydroxy-2,5-dimethoxy-10-methyl-10*H*-acridin-9-one which was identical to citramine (Takemura *et al.*, 1996).



NOE of **CR9**



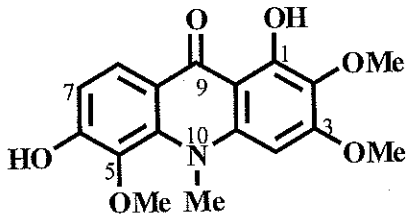
Major HMBC correlations of **CR9**

Table 14. ^{1}H,^{13}C NMR and HMBC spectroscopic data of **CR9** ($CDCl_3$+DMSO-d_6)

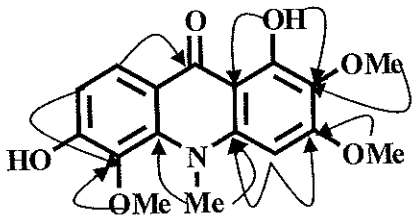
Position	δ_H **(multiplicity, *J*)**	δ_C **(C-Type)**	**HMBC**
1	-	155.5 (C)	-
2	-	128.7 (C)	-
3	-	156.5 (C)	-
4	6.42 (*s*)	91.1 (CH)	C-2, C-3, C-9a
5	-	134.9 (C)	-
6	-	155.7 (C)	-
7	6.94 (*d*, *J* = 9.0 Hz)	104.7 (CH)	C-5, C-8a
8	8.07 (*d*, *J* = 9.0 Hz)	122.9 (CH)	C-6, C-9, C-10a
9	-	180.4 (C=O)	-
4a	-	143.0 (C)	-
8a	-	116.3 (C)	-
9a	-	104.7 (C)	-
10a	-	138.6 (C)	-
1-OH	14.89 (*s*)	-	C-1, C-2, C-9a
3-OH*	8.67 (*s*)	-	-
6-OH*	9.08 (*s*)	-	-
2-OMe	3.96 (*s*)	61.1 (CH_3)	C-2
5-OMe	3.77 (*s*)	60.5 (CH_3)	C-5
N-Me	3.99 (*s*)	31.0 (CH_3)	C-4a, C-10a

*exchangeable position

CR10: 2-methoxycitpressine



CR10 was obtained as a yellow solid. The ^{1}H NMR, ^{13}C NMR, HMQC and HMBC spectrum (**Table 15**) of **CR10** indicated that **CR10** was an acridone which had a chelated hydroxyl group 1-OH (δ 14.48, *s*), a hydroxyl group 6-OH (δ 6.39, *s*), an *N*-methyl group (δ 4.04, *s*), *ortho*-coupled aromatic protons H-7 (δ 7.00, *d*, 9.0) and H-8, (δ 8.18, *d*, 9.0), an isolated aromatic proton H-4 (δ 6.31, *s*), and methoxyl groups 2-OMe (δ 3.93, *s*), 5-OMe (δ 3.78, *s*) as for **CR9** with the additional of 3-OMe at δ 4.03. This methoxyl group and H-4 showed correlation to C-3 (δ 159.4). **CR10** therefore was identified as 1,6-dihydroxy-2,3,5-trimethoxy-10-methyl-10*H*-acridin-9-onewhich was identical to 2-methoxycitpressine (Bowen and Patel, 1986).

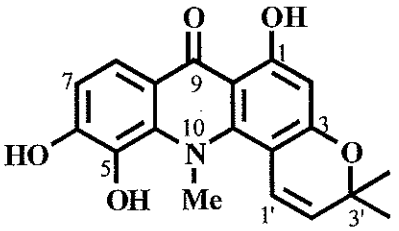


Major HMBC correlations of **CR10**

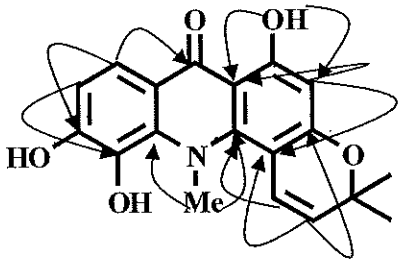
Table 15. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR10** ($CDCl_3$)

Position	**δ_H (multiplicity, *J*)**	**δ_C (C-Type)**	**HMBC**
1	-	155.5 (C)	-
2	-	130.4 (C)	-
3	-	159.4 (C)	-
4	6.31 (*s*)	91.1 (CH)	C-2, C-3, C-9a
5	-	134.1 (C)	-
6	-	154.2 (C)	-
7	7.00 (*d*, $J = 9.0$ Hz)	110.0 (CH)	C-5, C-8a
8	8.18 (*d*, $J = 9.0$ Hz)	122.9 (CH)	C-6, C-9, C-10a
9	-	180.4 (C=O)	-
4a	-	142.0 (C)	-
8a	-	116.3 (C)	-
9a	-	105.7 (C)	-
10a	-	137.0 (C)	-
1-OH	14.48 (*s*)	-	C-1, C-2, C-9a
6-OH	6.39 (*s*)	-	C-5, C-6, C-7
2-OMe	3.93 (*s*)	61.1 (CH_3)	C-2
3-OMe	4.03 (*s*)	60.1 (CH_3)	C-3
5-OMe	3.78 (*s*)	60.5 (CH_3)	C-5
N-Me	4.04 (*s*)	31.0 (CH_3)	C-4a, C-10a

CR14: Citracridone-III



CR14 was obtained as a orange solid. The UV spectrum showed absorption bands at λ_{max}225, 281, 343 and 386 nm. The IR spectrum showed the stretchings of hydroxyl (3368 cm^{-1}) and conjugated carbonyl group (1655 cm^{-1}). The ^{1}H NMR and HMBC spectroscopic data (**Table 16**) indicated the presence of a chelated hydroxyl proton (1-OH, δ 14.54), *N*-methyl (δ 3.81), *ortho*-coupled aromatic proton H-7 (δ 6.97) and H-8 (δ 7.85), a singlet aromatic proton (H-2, δ 6.19) and 2,2-dimethylchromene ring (H-4'/H-5', δ 1.51; H-1' δ 6.70; H-2', δ 5.55) as for **CR6**, with the absence of methoxyl group. Proton H-8 showed correlation to oxy-carbon resonating at δ 145.2 whereas H-7 correlated to an oxy-carbon resonating at δ 129.3 (C-5), suggested that the signals at δ 145.2 andδ 129.3 belonged to C-6 and C-5, respectively and the substituents at C-6 and C-5 were hydroxyl groups. **CR14** was therefore identified as 1,5,6-trihydroxy-10,3',3'-trimethyl-3,12-dihydro-3*H*-pyrano-[2,3-*c*]acridin-9-one which was known as citracridone-III (Teng*et al.*, 2005).

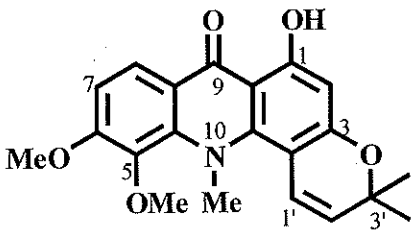


Major HMBC correlations of **CR14**

Table16. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR14** ($CDCl_3$+DMSO-d_6)

Position	δ_H (multiplicity)	δ_C (C-Type)	HMBC
1	-	158.8 (C)	-
2	6.19 (*s*)	97.5 (CH)	C-1, C-3, C-4, C-9a
3	-	156.1 (C)	-
4	-	101.8 (C)	-
5	-	129.3 (C)	-
6	-	145.2 (C)	-
7	6.97 (*d*, J = 9.0 Hz)	118.8 (CH)	C-5, C-6, C-8a
8	7.85 (*d*, J = 9.9 Hz)	111.8 (CH)	C-6, C-9, C10a
9	-	176.8 (C=O)	-
8a	-	113.1 (C)	-
4a	-	143.0 (C)	-
9a	-	107.2 (C)	-
10a	-	132.9 (C)	C-3, C-14a, C-3'
1'	6.70 (*d*, J = 9.0 Hz)	116.4 (CH)	C-1, C-2, C-9a
2'	5.55 (*d*, J = 9.0 Hz)	124.7 (CH)	C-3, C-4a
3'	-	77.0 (C)	C-3, C-4
4'/5	1.51 (*s*)	27.2 (CH_3)	C3'
1-OH	14.54 (*s*)	-	C-1, C-2, C-9a
N-Me'	3.81 (*s*)	-	C-4a, C-10a

CR19: Citracridone-II

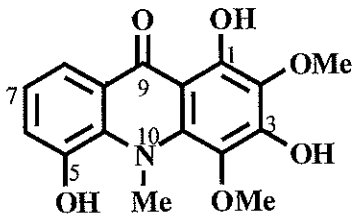


Compound **CR19** was obtained as a yellow solid. The ^{1}H NMR spectrum data (**Table 17**) indicated the presence of a chelated hydroxy proton 1-OH (δ 14.54), *N*-methyl (δ 3.70), *ortho*-coupled aromatic proton H-7 (δ 6.99) and H-8 (δ 8.17), a singlet aromatic proton H-2 (δ 6.27), 2,2-dimethylchromene ring (H-4'/ H-5',δ 1.52; H-1' δ 6.70; H-2',δ 5.55) and methoxy proton (δ 3.90) as for **CR6**, with the additional of a methoxyl signal at δ 3.86. From the above mentioned data and comparing these data with those previously reported, **CR19** was 6-hydroxy-10, 11-di-methoxy-3,3,12-trimethylpyrano[2,3-*c*]acridin-7-one. It was known as citracridone-II (Teng*et al.*, 2005).

Table 17. ^{1}H NMR spectrum data of **CR19** ($CDCl_3$)

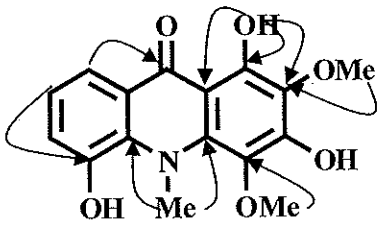
Position	**δ_H (multiplicity, *J*)**
2	6.27 (*s*)
7	6.99 (*d*, *J* = 8.7 Hz)
8	8.17 (*d*, *J* = 8.7 Hz)
1′	6.70 (*d*, *J* = 9.9 Hz)
2′	5.55 (*d*, *J* = 9.9 Hz)
4′/5′	1.52 (*s*)
1-OH	14.54 (*s*)
5-OMe	3.90 (*s*)
6-OMe	3.86 (*s*)
N-Me	3.70 (*s*)

CR43: 1,3,5-trihydroxy-2,4-dimethoxy-10-methyl-10H-acridin-9-one



CR43 was obtained as a yellow solid mp 220-223°. The UV spectrum showed maximum absorption bands at 206, 269 and 336 nm. Its IR spectrum showed O-H stretching at 3418 cm^{-1} and C=O stretching at 1651 cm^{-1}. A molecular ion in the HREI-MS at *m/z* 317.0958 corresponded to a molecular formula of $C_{16}H_{15}NO_6$.The ^1H NMR spectrum (**Table 18**) showed the resonances for an ABM spin system of a 1,2,3-trisubstituted benzene ring (δ 7.82, *d*, H-8; δ7.11, *t*, H-7; δ 7.22, *d*, H-6; 9.0), a hydrogen-bonded hydroxy proton (δ 14.31, 1-OH), two non-hydrogen-bonded hydroxy protons (δ 9.44, 5-OH and δ 8.53, 3-OH), two methoxyl groups (δ 3.98 and δ 3.83) and an N-CH_3 (δ3.84). The ^{13}C NMR spectrum showed the resonances of an *N*-methyl carbon CH_3 (δ 45.4), 2 methoxy carbons (δ 60.0 and δ 60.5), 1 carbonyl carbon (δ 182.4, C-9) and 12 aromatic carbons, including 5 oxygenated aromatic carbons (δ 151.3, C-1; δ 129.1, C-2; δ 150.8, C-3; δ 128.4, C-4 and δ 147.9, C-5), 3 methine aromatic carbons (δ 119.5, C-6; δ 122.3, C-7 and δ 116.1, C-8) and 4 additional non-protonated aromatic carbons (δ 138.2, C-4a; δ 124.1, C-8a; δ 105.3, C-9a and δ137.0, C-10a). In the HMBC experiment, the hydrogen-bonded phenolic 1-OH showed a weak $^4J_{CH}$ to the ketone carbon C-9 as well as to C-9a, C-1 and C-2. The aromatic doublet H-8 also correlated with C-9 indicating that both H-8 and 1-OH were *peri* to the carbonyl carbon. The *N*-Me carbon correlated with C-4a and C-10a. The phenol proton 5-OH also correlated with C-4a as well as with C-5 and C-6. These data suggested that **CR43** possessed an acridone skeleton with 5-OH *peri* to *N*-Me. One of the methoxy protons (δ 3.98) also corrected with C-2 indicating that it was *ortho* to the hydrogen-bonded phenol and thus directly attached to C-2. The remaining methoxy proton (δ 3.83) showed a correlation with the oxygenated aromatic carbon

resonating at δ 128.4, whereas the remaining unassigned oxygenated aromatic at δ 150.8 showed no correlation with any proton. The phenol proton resonating at δ 8.53 showed no HMBC correlations. Two possible structures could be proposed from the interpretation of these data with the third phenol attached at either C-3 or C-4 and the second methoxy group attached at either C-4 or C-3, respectively. A comparison of ^{13}C chemical shifts published for 1,2,3,4-tetraoxygenated acridones and 1,2,3,4-tetraoxygenated xanthones demonstrates that C-3 consistently resonates at least 10 ppm further downfield compared with C-4. The downfield shift of C-3 relative to C-4 can be explained by the electron withdrawing effect of the carbonyl group which predominantly influences the chemical shift of the aromatic carbon *para* to it resulting in a lower electron density at C-3 relative to C-4 in acridones and xanthones. When a direct comparison of the ^{13}C chemical shifts reported for melicopicine (Rasoanaivo et al. 1999), xanthones drimiopsin A and B (Mulholland et al. 2004), and 1,3-dihydroxy-2,4-dimethoxyxanthone (Tanaka &Takaishi 2006) with those observed for **CR43**, the chemical shifts for C-1 to C-4 were in close agreement and this suggested that the carbon at δ 128.4 could be assigned to C-4 and the carbon at δ 150.8 could be assigned to C-3. Therefore, it was thus identified as 1,3,5-trihydroxy-2,4-dimethoxy-10-methyl-10H-acridin-9-one. It is a new acridone.



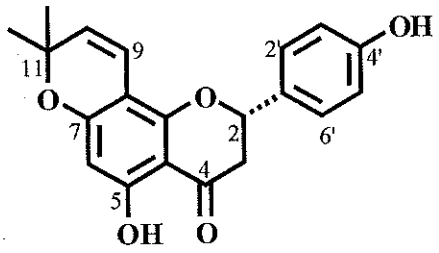
Major HMBC correlations of **CR43**

Table 18. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR43** ($CDCl_3$+DMSO-d_6)

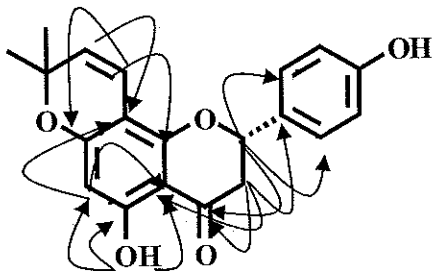
Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
1	-	146.7 (C)	-
2	-	123.9 (C)	-
3	-	146.2 (C)	-
4	-	124.5 (C)	-
5	-	143.3 (C)	-
6	7.22 (*d*, *J* = 9.0 Hz)	115.0 (CH)	C-5, C-8, C-10a
7	7.11 (*t*, *J* = 9.0 Hz)	117.8 (CH)	C-5, C-8a
8	7.82 (*d*, *J* = 9.0 Hz)	111.5 (CH)	C-6, C-9, C-10a
9	-	177.8 (C=O)	-
10	-	-	
4a	-	133.7 (C)	-
8a	-	119.5 (C)	-
9a	-	100.8 (C)	-
10a	-	132.4 (C)	-
1-OH	14.31 (*s*)	-	C-2, C-9a
3-OH	-	-	-
5-OH	9.44 (*s*)	-	C-5, C-6, C-10a
2-OMe	3.98 (*s*)	56.0 (CH_3)	C-2
4-OMe	3.83 (*s*)	55.5 (CH_3)	C-3, C-4, C-4a
N-Me	3.84 (*s*)	40.8 (CH_3)	C-4a, C-10a

3.1.3 Flavonoids

CR3: Citflavanone



CR3 was isolated as a yellow solid. The ^{1}H NMR spectrum (**Table 19**) showed characteristic signals of H-2, H-3$_{ax}$ and H-3$_{eq}$ of flavanones at δ 5.38 (*dd*, J = 12.9, 3.0 Hz), δ 3.06 (*dd*, J = 17.1, 12.9 Hz) and δ 2.84 (*dd*, J = 17.1, 3.0 Hz). This was confirmed by HMBC correlations of H-2 to C-4 (δ 197.6), C-2′/ 6′ (δ 127.6); H-3 to C-4 (δ 197.6), C-4a (δ 102.9), C-1′ (δ 130.9). The spectrum further showed signals of a chelated hydroxy group (5-OH, δ 12.03), a *para*-substituted B ring (δ 7.33, H-2'/H-6'; δ 6.90, H-3'/H-5'), a singlet aromatic proton (δ 6.00, H-6) and a 2,2-dimethyl-chromene ring (δ 1.49, *s*, H-12; δ 6.52, *d*, J = 9.9 Hz, H-9; δ 5.47, *d*, J = 9.9 Hz, H-10). A singlet aromatic proton at δ 6.00 was confirmed for H-6 due to the chelated hydroxyl proton showing correlations to C-6 (δ 97.7) and C-4a, and H-6 to C-4a. The HMBC correlation of H-6 and H-10 to C-8 (δ 103.8), and H-9 to C-7 (δ 159.5) confirmed the attachment of the chromene ring to C-7 and C-8 of the A ring. It was thus identified as 5-hydroxy-2-(4-hydroxyphenyl)-8,8-dimethyl-2,3-dihydro-pyrano-[2,3-*h*]chromen-4-one which was known as citflavanone (Wu, 1989).

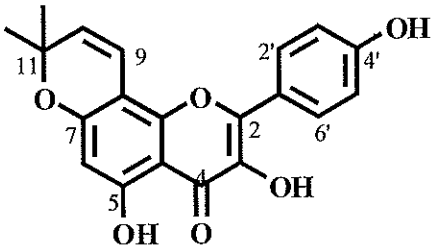


Major HMBC correlation of **CR3**

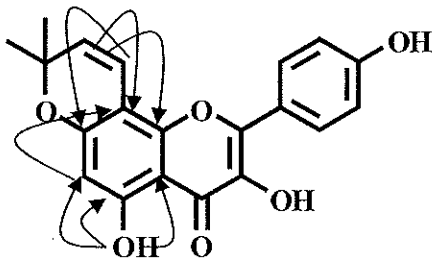
Table19. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR3** ($CDCl_3$+ DMSO-d_6)

Position	δ_H **(multiplicity, *J*)**	δ_C **(C-Type)**	**HMBC**
2	5.38 (*dd*, *J* = 12.9, 3.0 Hz)	79.0 (CH)	C-4, C-2', C-6'
3	3.06 (*dd*, *J* = 17.1, 12.9 Hz)	43.4 (CH_2)	C-4, C-1', C-4a
	2.84 (*dd*, *J* = 17.1, 3.0 Hz)		C-4, C-1', C-4a
4	-	197.6 (C=O)	-
4a	-	102.9 (C)	-
5	-	163.8 (C)	-
6	6.00 (*s*)	97.7 (CH)	C-4a, C-5, C-8
7	-	159.5 (C)	-
8	-	103.8 (C)	-
8a	-	150.9 (C)	-
9	6.52 (*d*, *J* = 9.9 Hz)	114.5 (CH)	C-7, C-8a, C-11
10	5.47 (*d*, *J* = 9.9 Hz)	127.5 (CH)	C-7, C-8, C-11
11	-	78.1 (C)	-
12	1.49 (*s*)	28.4 (CH_3)	C-10, C-11, C-12
1'	-	130.9 (C)	-
2'/6'	7.33 (*d*, *J* = 9.0 Hz)	127.6 (CH)	C-2, C-2', C-4', C-6'
3'/5'	6.90 (*d*, *J* = 9.0 Hz)	115.6 (CH)	C-1', C-3', C-4', C-5'
4'	-	159.3 (C)	-
5-OH	12.03 (*s*)	-	C-5, C-6, C-4a

CR7: Citrusinol



CR7 was obtained as a yellow solid. m.p. 253-254°C. The UV spectrum showed absorption bands at λ_{max} 222, 241, 248, 267 and 331 nm. The IR spectrum showed the stretching of hydroxyl (3550 cm^{-1}) and conjugated carbonyl group (1620 cm^{-1}). The ^1H NMR spectrum **(Table 20)** showed the presence of a *para*-substituted B ring (δ 8.22, H-2'/H-6'; δ 7.06, H-3'/H-5', J = 9.0 Hz), a chelated hydroxyl group (δ 12.30, 5-OH), a singlet aromatic proton (δ 6.25, H-6) and a 2,2-dimethylchromene ring (δ 1.49, *s*, H-12; δ 6.63, *d*, J = 9.9 Hz, H-9; δ 5.79, *d*, J = 9.9 Hz, H-10). A singlet aromatic proton at δ 6.25 was confirmed for H-6 due to the chelated hydroxyl proton showing correlations to C-6 (δ 98.8) and C-4a, and H-6 to C-4a (δ 101.2). The HMBC correlations of H-6 and H-10 to C-8 (δ 103.8), and H-9 to C-7 (δ 159.5) confirmed the attachment of the chromene ring to C-7 and C-8 of the A ring. Finally, the flavonol was proposed to complete, the structure with the carbon signal C-3 resonating at δ 136.0. Therefore **CR7** was assigned as 3,5-dihydroxy-2-(4-hydroxyphenyl)-8,8-dimethylpyrano[2,3-*h*]chromen-4-one which was known as citrusinol (Shang *et al.*, 2007).

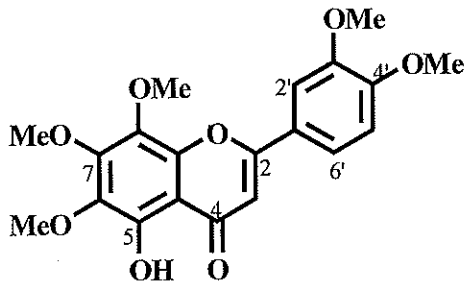


Major HMBC correlations of **CR7**

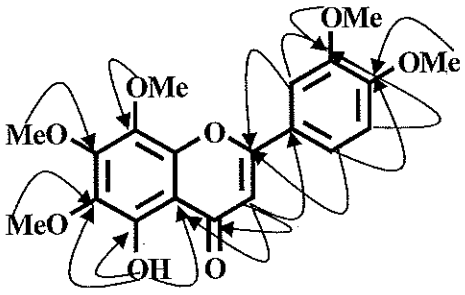
Table20. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR7** ($CDCl_3$ + DMSO-d_6)

Position	δ_H (multiplicity)	δ_C (C-Type)	HMBC
2	-	146.3 (C)	-
3	-	136.0 (C)	-
4	-	175.6 (C=O)	-
4a	-	101.2 (C)	-
5	-	160.8 (C)	-
6	6.25 (*s*)	98.8 (CH)	C-4a, C-5, C-8
7	-	159.5 (C)	-
8	-	103.8 (C)	-
8a	-	150.9 (C)	-
9	6.63 (*d*, *J* = 9.9 Hz)	114.5 (CH)	C-7, C-8a, C-11
10	5.79 (*d*, *J* = 9.9 Hz)	127.5 (CH)	C-7, C-8, C-11
11	-	78.1 (C)	-
12	1.49 (*s*)	28.4 (CH_3)	C-10, C-11, C-12
1'	-	122.5 (C)	-
2'/6'	8.22 (*d*, *J* = 9.0 Hz)	129.6 (CH)	C-2, C-2', C-4', C-6'
3'/5'	7.06 (*d*, *J* = 9.0 Hz)	115.6 (CH)	C-1', C-3', C-4', C-5'
4'	-	159.3 (C)	-
5-OH	12.30 (*s*)	-	C-5, C-6, C-4a

CR20: 5-demethoxynobiletin



CR20 was obtained as a yellow solid. The UV spectrum showed maximum absorption at λ_{max} 204, 254, 283 and 329 nm. Its IR spectrum showed the absorption bands of O-H stretching at 3428 cm^{-1}, C=O stretching at 1597 cm^{-1}. Its ^{1}H NMR spectrum (**Table 21**) showed signals of an olefinic proton H-3 (δ 6.60, *s*), a chelated hydroxyl group 5-OH (δ 12.52), five methoxyl groups (δ 3.94, 3.95, 3.96, 3.97, 4.10), and ABX signals of aromatic protons H-5' (δ 7.00, *d*, 8.7), H-2' (7.42, *d*, 2.4) and H-6' (7.59, *dd*, 8.7, 2.4). The ^{13}C NMR spectrum, showed the presence of 20 carbons, including 11 quaternary carbons (C), 4 methine carbons (CH) and 5 methoxy carbons (OMe). Correlation of H-3 (δ 6.60) to C-4 (δ 182.9), C-4a (δ 107.0), C-1' (δ 123.7), and H-2'/ H-6', H-3 to C-2 (δ 163.9) confirmed that the B ring was attached to C ring at C-2 (δ 163.9). The signal at δ 3.95 was assigned to a 4'-OMe as resulted from this proton and H-2'/H-6'showing correlations to C-4' (δ 152.5). The methoxyl group at δ 3.96 was *ortho* to chelated hydroxyl proton (5-OH) according to HMBC correlation of 5-OH to C-5, C-4a C-6 and the methoxyl group (δ 3.96) to C-6. The resonance at δ 3.97 and H-5' correlated to C-3' (δ 149.4), indicating that this resonance (δ 3.97) belonged to 3'-OMe. Therefore, **CR20** was assigned as 5-hydroxy-6,7,8,3',4'-pentamethoxyflavone which was known as 5-demethoxynobiletin (Li *et al.*, 2006).

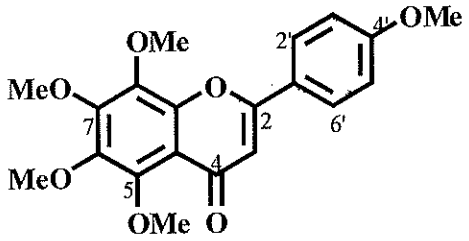


Major HMBC correlations of **CR20**

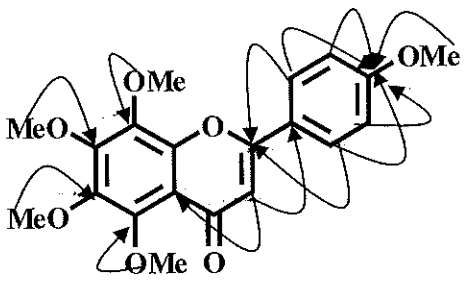
Table 21. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR20** ($CDCl_3$)

Positions	**δ_H (multiplicity, *J*)**	**δ_C (C-Type)**	**HMBC**
2	-	163.9 (C)	-
3	6.60 (*s*)	103.9 (CH)	C-2, C-4, C-4a, C-1'
4	-	182.9 (C=O)	-
4a	-	107.0 (C)	-
5	-	149.5 (C)	-
6	-	136.5 (C)	-
7	-	152.9 (C)	-
8	-	132.9 (C)	-
8a	-	145.7 (C)	-
1'	-	123.7 (C)	-
2'	7.42 (*d*, J = 2.4 Hz)	108.8 (CH)	C-2, C-4', C-6'
3'	-	149.4 (C)	-
4'	-	152.5 (C)	-
5'	7.00 (*d*, J = 8.7 Hz)	111.3 (CH)	C-1', C-3'
6'	7.59 (*dd*, J = 8.7, 2.4 Hz)	120.1 (CH)	C-2, C-2', C-4'
6-OMe	3.94 (*s*)	61.1 (CH_3)	C-6
7-OMe	4.10 (*s*)	61.7 (CH_3)	C-7
8-OMe	3.96 (*s*)	62.0 (CH_3)	C-8
3'-OMe	3.97 (*s*)	56.1 (CH_3)	C-3'
4'-OMe	3.95 (*s*)	56.0 (CH_3)	C-4'
5-OH	12.52 (*s*)	-	C-4a, C -5, C-6

CR21: Tangeretin



CR21 was obtained as a yellow solid. The UV spectrum showed maximum absorption at λ_{max} 271 and 332 nm. Its IR spectrum showed C=O stretching absorption band at 1647 cm^{-1}. Its ^{1}H-NMR spectrum (**Table 22**) showed signals of an olefinic proton of flavone (δ 6.61, H-3), four methoxyl groups (δ 4.11, δ 4.03, δ 3.86 and δ 3.96), and a *para*-substituted B ring (H-2'/H-6', δ 7.88, *d*, *J* = 9.0 Hz; H-3'/H-5', δ 7.01, *d*, *J* = 9.0 Hz). The ^{13}C-NMR spectrum showed the presence of 20 carbons, including 10 quaternary carbons (C), 5 methine carbons (CH) and 5 methoxy carbons (OMe). The HMBC correlations of methoxy protons at δ 3.86, H-2'/6' and H-3'/5' to C-4' (δ 163.3) suggested that the methoxyl signal at δ 3.86 was 4'-OMe. Due to the resonance effect by the carbonyl group (C-4), the chemical shift value of methoxy groups at δ 3.96 and δ 4.11 were assigned for 5-OMe and 7-OMe or exchangeable. The methoxy group at δ 4.11 is the most downfield of the methyl proton signals since it is desheilded by the carbonyl carbon. From the above mentioned data and comparing these data those the previously reported, it was clear that the **CR21** is 5,6,7,8,4'-pentamethoxy-flavone. **CR21** was known as tangeretin (Han *et al.*, 2010).



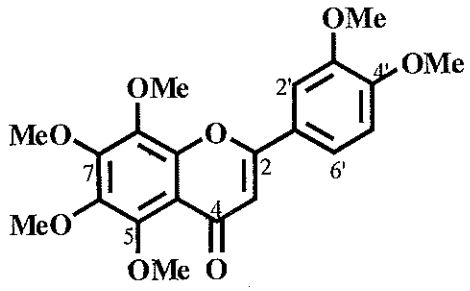
Major HMBC correlations of **CR21**

Table 22. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR21** ($CDCl_3$)

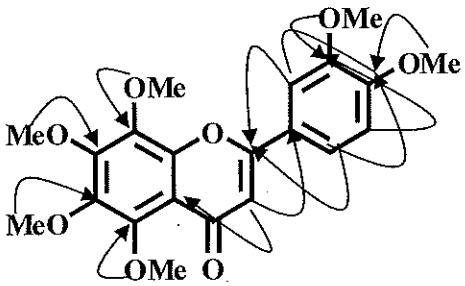
Positions	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
2	-	161.3 (C)	-
3	6.61 (*s*)	106.6 (CH)	C-2, C-4, C-4a, C-1'
4	-	177.3 (C=O)	-
4a	-	114.8 (C)	-
5	-	148.4 (C)	-
6	-	138.1 (C)	-
7	-	151.4 (C)	-
8	-	138.1 (C)	-
8a	-	147.7 (C)	-
1'	-	123.8 (C)	-
2'	7.88 (*d*, *J* = 9.0 Hz)	127.7 (CH)	C-2, C-4', C-6'
3'	7.01 (*d*, *J* = 9.0 Hz)	114.5 (CH)	C-1', C-3', C-4'
4'	-	162.3 (C)	-
5'	7.01 (*d*, *J* = 9.0 Hz)	114.5 (CH)	C-1', C-3', C-4'
6'	7.88 (*d*, *J* = 9.0 Hz)	127.7 (CH)	C-2, C-4', C-6'
5-OMe	3.96 (*s*)	62.2* (CH_3)	C-5
6-OMe	4.03 (*s*)	61.5* (CH_3)	C-6
7-OMe	4.11 (*s*)	62.0* (CH_3)	C-7
8-OMe	3.96 (*s*)	61.6* (CH_3)	C-8
4'-OMe	3.86 (*s*)	55.5 (CH_3)	C-4'

*exchangeable position

CR22: Nobiletin



CR22 was obtained as a yellow solid. The UV spectrum showed maximum absorptions at λ_{max} 248, 271 and 332 nm. The IR spectrum showed C=O stretching absorption band at 1647cm^{-1}. The ^{1}H-NMR spectrum (**Table 23**) showed signals of a characteristic flavone proton H-3 (δ 6.63, *s*), six methoxyl groups (δ 4.12, δ 4.05, 4.00 and δ 3.97 × 3) and aromatic protons H-5', H-2' and H-6' (δ 7.01, *d*, J = 8.7 Hz; δ 7.43, *d*, J =1.8 Hz; δ 7.58, *dd*, J = 8.7, 1.8 Hz), respectively as those of **CR20** with the absence of a singlet chelated hydroxyl signal and the presence of additional methoxyl group. The 5-OMe then was assigned. The ^{13}C NMR spectrum, showed the presence of 21 carbons, including 11 quaternary carbons (C), 4 methine carbons (CH) and 6 methoxy carbons (OMe). Due to resonance effect by C=O (C-4), the chemical shift value at 3.97 and 4.12 then were assigned for 5-OMe and 7-OMe or exchangeable. The methoxyl resonances at δ 4.00 Hz was assigned for 3'-OMe according to the HMBC correlations of its protons and H-5' to C-3' (δ 148.3). Whereas the one at δ 3.97 belonged to 4'-OMe in accordance with the HMBC correlations of its proton, H-2' and H-6' to C-4' (δ 151.9). From the above mentioned data and comparing these data with those previously reported, it was clear that the **CR22** is nobiletin (Han *et al.*, 2010).



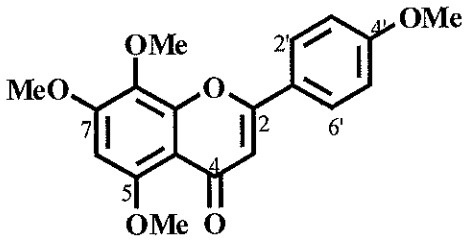
Major HMBC correlations of **CR22**

Table 23 ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR22** ($CDCl_3$)

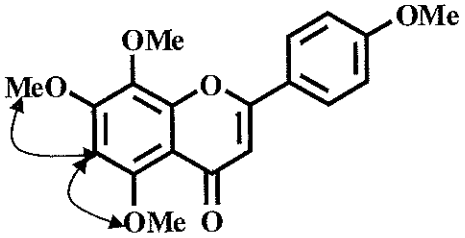
Positions	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
2	-	161.0 (C)	-
3	6.63 (*s*)	106.8 (CH)	C-2, C-4, C-4a, C-1'
4	-	177.3 (C=O)	-
4a	-	114.9 (C)	-
5	-	149.2 (C)	-
6	-	138.0 (C)	-
7	-	151.4 (C)	-
8	-	144.0 (C)	-
8a	-	147.7 (C)	-
1'	-	123.9 (C)	-
2'	7.43 (*d*, *J* = 1.8 Hz)	108.5 (CH)	C-2, C-4', C-6'
3'	-	148.3 (C)	-
4'	-	151.9 (C)	-
5'	7.01 (*d*, *J* = 8.7 Hz)	111.2 (CH)	C-1', C-3'
6'	7.58 (*dd*, *J* = 8.7, 1.8 Hz)	119.2 (CH)	C-2, C-2', C-4'
5-OMe	3.97 (*s*)	62.2* (CH_3)	C-5
6-OMe	4.05 (*s*)	61.6* (CH_3)	C-6
7-OMe	4.12 (*s*)	61.9* (CH_3)	C-7
8-OMe	3.97 (*s*)	61.8* (CH_3)	C-8
3'-OMe	4.00 (*s*)	55.7 (CH_3)	C-3'
4'-OMe	3.97 (*s*)	56.0 (CH_3)	C-4'

*exchangeable position

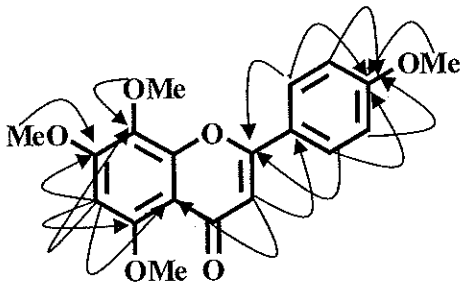
CR23: 5, 7, 8, 4'-tetramethoxyflavone



CR23 was obtained as a yellow solid. The UV spectrum showed maximum absorption at λ_{max} 270 and 311 nm. Its IR spectrum showed C=O stretching absorption bands at 1636 cm^{-1}. Its ^{1}HNMR spectrum (**Table 24**) showed signals for an olefinic proton H-3 (δ 6.61), a *para*-substituted B ring (δ 7.92, *d*, 9.0, H-2'/H-6'; δ 7.04, *d*, 9.0, H-3'/H-5'), four methoxyl groups 5-OMe (δ 3.98), 7-OMe (δ 4.02), 8-OMe (δ 3.96) and 4'-OMe (δ 3.89) as for **CR21**. The difference was the presence of singlet of aromatic at δ 6.47 instead of the methoxyl group. This signal was assigned for an aromatic proton H-6 due to the HMBC correlations of H-6 to C-4a (δ 107.0), C-5 (δ154.7), C-7 (δ 155.1) and C-8 (δ 129.0). The differential NOE technique by irradiation of the signal at δ 6.47 (H-6) affected the signal of methoxyl groups at δ 3.98 and δ 4.02, confirmed that H-6 was *ortho* to methoxyl groups at δ 3.98 and δ 4.02. The methoxyl group at C-8 (δ 129.0) affected by two *ortho* and one *para* donating groups resonated at highest field. The chemical shift values at δ 3.98 and 4.02 then were assigned for 5-OMe and 7-OMe or exchangeable. Thus **CR23** was assigned as 5,7,8,4'-tetramethoxyflavone (Iinuma *et al.*, 1980).



NOE of **CR23**



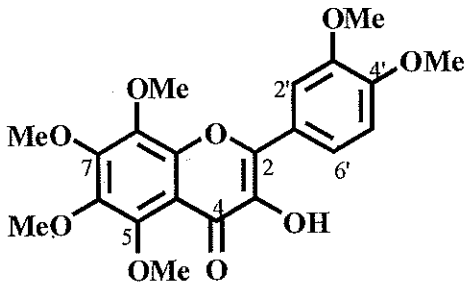
Major HMBC correlations of **CR23**

Table 24 ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR23** ($CDCl_3$)

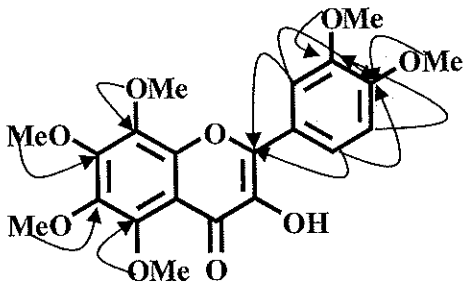
Positions	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
2	-	159.6 (C)	-
3	6.61 (*s*)	105.0 (CH)	C-2, C-4, C-4a, C-1'
4	-	176.9 (C=O)	-
4a	-	107.0 (C)	-
5	-	154.7 (C)	-
6	6.47 (*s*)	90.9 (CH)	C-4a, C-5, C-7, C-8
7	-	155.1 (C)	-
8	-	129.0 (C)	-
8a	-	150.3 (C)	-
1'	-	122.0 (C)	-
2'	7.92 (*d*, J = 9.0 Hz)	126.2 (CH)	C-2, C-4', C-6'
3'	7.04 (*d*, J = 9.0 Hz)	112.8 (C-H)	C-1', C-3', C-4'
4'	-	160.7 (C)	-
5'	7.04 (*d*, J = 9.0 Hz)	112.8 (CH)	C-1', C-3', C-4'
6'	7.92 (*d*, J = 9.0 Hz)	126.2 (CH)	C-2, C-4', C-6'
5-OMe	3.98* (*s*)	54.7 (CH_3)	C-5
7-OMe	4.02* (*s*)	54.7 (CH_3)	C-7
8-OMe	3.96 (*s*)	60.0 (CH_3)	C-8
4'-OMe	3.89 (*s*)	53.8 (CH_3)	C-4'

*exchangeable position

CR24: Natsudaidain



CR24 was obtained as a yellow solid. The ^{1}H-NMR spectrums (**Table 25**) was very similar to those of **CR22**. It showed the ABX signal of three aromatic protons H-5', H-2' and H-6' at δ 7.03 (*d*, 8.4), 7.89 (*d*, 1.8) and 7.92 (*dd*, 8.4, 1.8), respectively and six methoxyl groups at δ 3.95, 3.97, 3.99*x*2, 4.04, and 4.10. The ^{13}C NMR spectrum, showed the presence of 21 carbons, including 12 quaternary carbons (C), 3 methine carbons (CH) and 6 methoxy carbons (OMe). The hydroxyl group was placed at C-3 to fulfill structure. The HMBC correlation of H-2'/ H-6' to C-2 (δ 142.5) confirmed that the B ring combined to C ring at C-2. Due to resonance effect by the carbonyl group (C-4), the chemical shift values of methoxyl groups at δ 4.04 and δ 4.10 then were assigned for 5-OMe and 7-OMe or exchangeable. The methoxyl resonances at δ 3.99 was assigned for 3'-OMe according to the HMBC correlations of its protons and H-5' to C-3' (δ 148.9). Whereas the one at δ 3.97 belonged to 4'-OMe in according to the HMBC correlations of its proton, H-2' and H-6' to C-4' (δ 150.5). Therefore **CR24** was assigned as natsudaidain (Liu *et al.*, 2012)



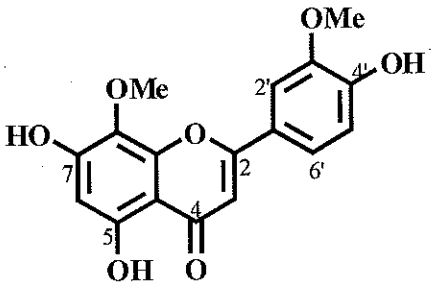
Major HMBC correlations of **CR24**

Table 25. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR24** ($CDCl_3$)

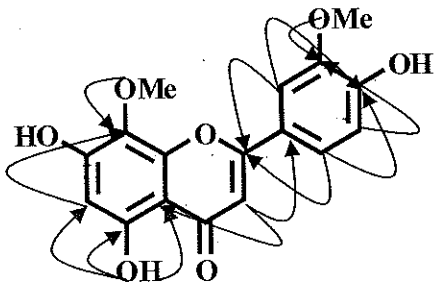
Positions	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
2	-	142.5 (C)	-
3	-	-	-
4	-	177.8 (C=O)	-
4a	-	108.8 (C)	-
5	-	147.7 (C)	-
6	-	137.7 (C)	-
7	-	151.6 (C)	-
8	-	143.3 (C)	-
8a	-	-	-
1'	-	124.5 (C)	-
2'	7.89 (*d*, *J* = 1.8 Hz)	110.4 (CH)	C-2, C-4', C-6'
3'	-	148.9 (C)	-
4'	-	150.5 (C)	-
5'	7.03 (*d*, *J* = 8.4 Hz)	111.2 (CH)	C-1', C-3', C-4'
6'	7.92 (*dd*, *J* = 8.4, 1.8 Hz)	121.0 (CH)	C-2, C-2', C-4'
5-OMe	3.99 (*s*)	62.2* (CH_3)	C-5
6-OMe	4.04 (*s*)	62.0* (CH_3)	C-6
7-OMe	4.10 (*s*)	61.9* (CH_3)	C-7
8-OMe	3.95 (*s*)	61.8* (CH_3)	C-8
3'-OMe	3.99 (*s*)	55.9 (CH_3)	C-3'
4'-OMe	3.97 (*s*)	55.8 (CH_3)	C-4'

*exchangeable position

CR26: 5,7,4'-trihydroxy-3',8-dimethoxyflavone



CR26 was obtained as a yellow solid. Its ^{1}H NMR spectrum (**Table 26**) showed signals of an olefinic proton H-3 (δ 6.81), a chelated hydroxyl group 5-OH (δ 12.60), an aromatic proton singlet H-6 (δ 6.20), three aromatic protons H-5', H-2' and H-6' in ABX spin system δ 7.03 (*d*, 8.4), 7.53 (*d*, 1.8) and 7.55 (*dd*, 8.4, 1.8), methoxyl resonances 8-OMe and 4'-OMe (δ 3.83 and δ 3.89). The aromatic proton at δ 6.20 was proposed to be at H-6 because HMBC correlations were observed from H-6 to C-4a (δ 103.5), C-5 (δ 156.9) and from 5-OH to C-6 (δ 99.2) and C-4a. The methoxy proton at δ 3.83 and the aromatic proton H-6 showed correlation to carbon at δ 128.3, H-6 also correlated to the oxygenated aromatic carbon at δ 157.0. Thus a methoxy proton at δ 3.83 was located at C-8. The location 3'-OMe was confirmed by the HMBC correlations of 3'-OMe, H-2' and H-5' to C-3' (δ 148.2). H-2' and H-6' showed correlation to the carbon at δ 151.2 confirmed that the hydroxyl group was substituted at C-4'. Thus **CR26** was assigned as 5,7,4'-trihydroxy-3',8-dimethoxy-flavone (Pukalskas, *et al.*, 2010).

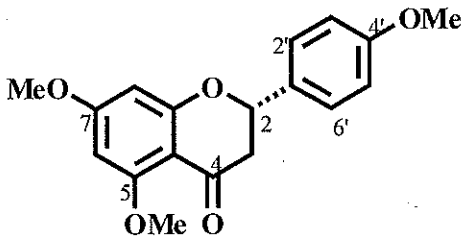


Major HMBC correlations of **CR26**

Table 26. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR26** ($CDCl_3$+DMSO-d_6)

Positions	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
2	-	159.6 (C)	-
3	6.81 (*s*)	103.5 (CH)	C-2, C-4, C-4a, C-1'
4	-	182.5 (C=O)	-
4a	-	103.5 (C)	-
5	-	156.9 (C)	-
6	6.20 (*s*)	99.2 (CH)	C-4a, C-5, C-7, C-8
7	-	157.0 (C)	-
8	-	128.3 (C)	-
8a	-	149.9 (C)	-
1'	-	121.9 (C)	-
2'	7.53 (*d*, *J* = 1.8 Hz)	116.2 (CH)	C-2, C-4', C-6'
3'	-	148.2 (C)	-
4'	-	151.2 (C)	-
5'	7.03 (*d*, *J* = 8.4 Hz)	110.2 (CH)	C-1', C-3', C-4'
6'	7.55 (*d*, *J* = 8.4, 1.8 Hz)	120.9 (CH)	C-2, C-4', C-6'
5-OH	12.60 (*s*)	-	C-5, C-4a, C-6
8-OMe	3.83 (*s*)	61.8 (CH_3)	C-8
3'-OMe	3.89 (*s*)	56.2 (CH_3)	C-3'

CR31: Naringenin trimethyl ether

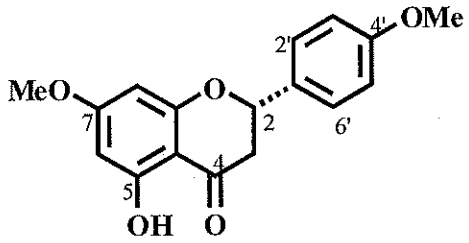


Compound **CR31** was isolated as a yellow solid. The ^{1}H NMR spectrum (**Table 27**) showed characteristic signals of H-2, H-3$_{ax}$ and H-3$_{eq}$ of flavanones at 5.38 (*dd*, 13.1, 2.9), δ 3.12 (*dd*, 17.1, 13.1) and δ 2.85 (*dd*, 17.1, 2.9). The spectrum further showed signals of three methoxy group (δ 3.91, 7-OMe, δ 3.96, 5-OMe, δ 3.89, 4′-OMe). The aromatic protons at δ 7.35 (*d*, 8.4 Hz) was assigned for H-2′ /H-6′ which were coupled with aromatic protons at δ 6.90, (*d*, 8.4, H-3′/H-5′). The remaining signal at δ 6.21 (*d*, 2.0) and δ 6.39 (*d*, 2.0) were assigned for H-6 and H-8, respectively. Comparison these data with the previously reported data, it was clear that the **CR31** was 2,3-dihydro-5-hydroxy-4',7-dimethoxyflavone . It was known as naringenin trimethyl ether (Rossi *et al.*, 1997)

Table 27. ^{1}H NMR spectrum data of **CR31** ($CDCl_3$)

Position	**δ_H (multiplicity, *J*)**
2	5.35 (*dd* , *J* = 13.1, 2.9 Hz)
3a	3.12 (*dd*, *J* = 17.1, 13.1 Hz)
3b	2.85 (*dd*, *J* = 17.1, 2.9 Hz)
6	6.21 (*d*, *J* = 2.0 Hz)
8	6.39 (*d*, *J* = 2.0 Hz)
2'	7.35 (*d*, *J* = 8.4 Hz)
3'	6.90 (*d*, *J* = 8.4 Hz)
5'	6.90 (*d*, *J* = 8.4 Hz)
6'	7.35 (*d*, *J* = 8.4 Hz)
5-OMe	3.96 (*s*)
7-OMe	3.91 (*s*)
4′-OMe	3.89 (*s*)

CR32: 2,3-dihydro-5-hydroxy-4′,7-dimethoxyflavanone

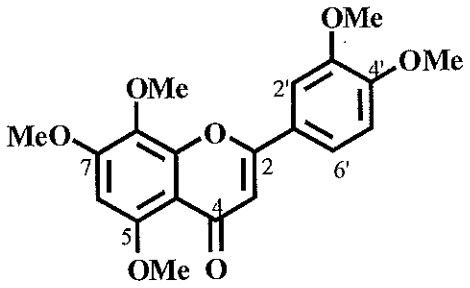


Compound **CR32** was a yellowish solid. The ^{1}H NMR spectrum (**Table 28**) of this compound was similar to those of **CR31** with the absence of a methoxyl group at δ 3.91 (*s*, 5-OMe) in **CR31**. The spectrum showed a chelated hydroxyl group (5-OH) at δ 12.27 a *para*-aromatic protons at δ 7.35 (*d*, 8.4 Hz, H-2′ / H-6′) and δ 6.90 (*d*, 8.4 Hz, H-3′/H-5′) and at δ 6.21 (*d*, 2.0, H-6) and δ 6.39 (*d*, 2.0, H-8). The remaining signals at δ 5.38 (*dd*, 13.1, 2.9), δ 3.12 (*dd*, 17.1, 13.1) and δ 2.85 (*dd*, 17.1, 2.9) corresponded to H-2, H-3_{ax} and H-3_{eq}, respectively. Comparison these data with the previously reported data, it was identical to 2,3-dihydro-5-hydroxy-4′,7-dimethoxyflavanone (Lam and Wrang, 1975).

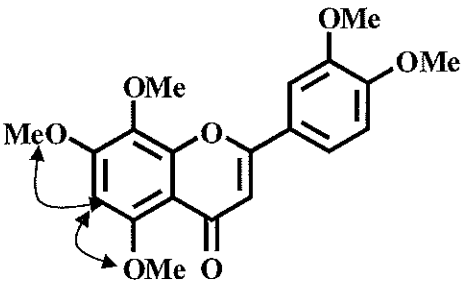
Table 28. ^{1}H NMR spectrum data of **CR32** ($CDCl_3$)

Position	δ_H **(multiplicity, *J*)**
2	5.38 (*dd* , *J* = 13.1, 2.9 Hz)
3a	3.12 (*dd*, *J* = 17.1, 13.1 Hz)
3b	2.85 (*dd*, *J* = 17.1, 2.9 Hz)
6	6.21 (*d*, *J* = 2.0 Hz)
8	6.39 (*d*, *J* = 2.0 Hz)
2'	7.35 (*d*, *J* = 8.4 Hz)
3'	6.90 (*d*, *J* = 8.4 Hz)
5'	6.90 (*d*, *J* = 8.4 Hz)
6'	7.35 (*d*, *J* = 8.4 Hz)
5-OH	12.27 (*s*)
7-OMe	3.91 (*s*)
4'-OMe	3.89 (*s*)

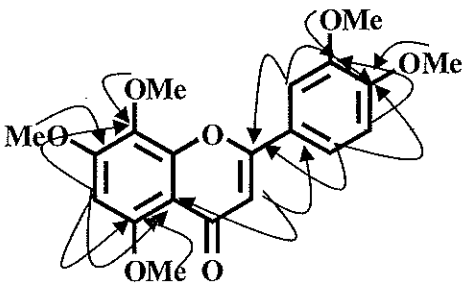
CR34: 5, 7, 8, 3', 4'-pentamethoxyflavone



CR34 was a yellow solid. The UV spectrum showed maximum absorption at λ_{max} 248, 271 and 339 nm. Its IR spectrum showed a C=O stretching absorption bands at 1636 cm^{-1}. The ^1H NMR spectrum (**Table 29**) showed resonances of a flavone proton H-3 at δ 6.62, aromatic proton H-6 at δ 6.44 (s), aromatic protons H-5', H-2' and H-6' of a spin system ABX with signals at δ 7.00 (*d*, J = 8.7), 7.42 (*d*, J = 1.8) and 7.59 (*dd*, J = 8.7, 1.8), and five methoxyl groups at δ 4.00 (5-OMe), δ 4.02. (7-OMe), δ 3.97 (8-OMe), δ 3.98 (3'-OMe), δ 3.97 (4'-OMe). The aromatic proton at δ 6.44 was assigned to H-4 according to the HMBC correlations of 5-OMe to C-5 (δ 156.3), 7-OMe to C-7 (δ 156.5) and H-6 to C-4a (δ 108.6), C-5 (δ 156.3) and C-7 (δ 156.5). Proton H-6 was further shown to be *ortho* to 5-OMe and 7-OMe due to NOE technique that irradiation of H-6 resonance affected the signal of 5-OMe and 7-OMe. Due to resonance effect by C=O (C-4), the chemical shift value of δ 4.00 and δ 4.02 then were assigned for 5-OMe and 7-OMe or exchangeable. The location of 3'-OMe and 4'-OMe were assigned by the HMBC correlations of 3'-OMe and H-5' to C-3' (δ 149.2), and 4'-OMe, H-2' and H-6' to C-4' (δ 151.8). **CR34** was identified as 5, 7, 8, 3', 4'-pentamethoxyflavone (Chen, J. *et al.*, **1997**). Its spectroscopic data were in agreement to those of previously reported.



NOE of **CR34**

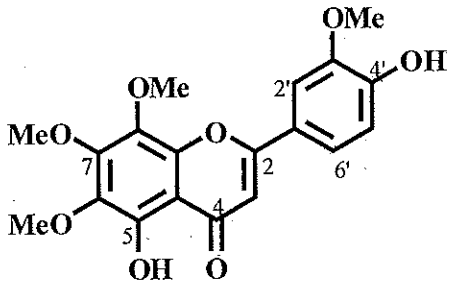


Major HMBC correlations of **CR34**

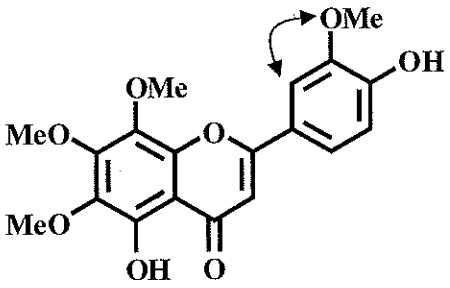
Table 29. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR34** ($CDCl_3$)

Positions	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
2	-	160.5 (C)	-
3	6.62 (*s*)	107.1 (CH)	C-2, C-4, C-4a, C-1′
4	-	177.8 (C=O)	-
4a	-	108.6 (C)	-
5	-	156.3 (C)	-
6	6.44 (*s*)	92.6 (CH)	C-4a, C-5, C-7, C-8
7	-	156.5 (C)	-
8	-	130.7 (C)	-
8a	-	151.9 (C)	-
1'	-	124.0 (C)	-
2'	7.42 (*d*, *J* =1.8 Hz)	108.6 (CH)	C-2, C-4', C-6'
3'	-	149.2 (C)	-
4'	-	151.8 (C)	-
5'	7.00 (*d*, *J* = 8.4 Hz)	111.2 (CH)	C-1', C-3', C-4'
6'	7.59 (*dd*, *J* = 8.4, 1.8 Hz)	119.6 (CH)	C-2, C-2', C-4'
5-OMe	4.00 (*s*)	56.6 (CH_3)	C-5
7-OMe	4.02 (*s*)	56.3 (CH_3)	C-7
8-OMe	3.97 (*s*)	61.5 (CH_3)	C-8
3'-OMe	3.98 (*s*)	56.0 (CH_3)	C-3'
4'-OMe	3.97 (*s*)	55.9 (CH_3)	C-4'

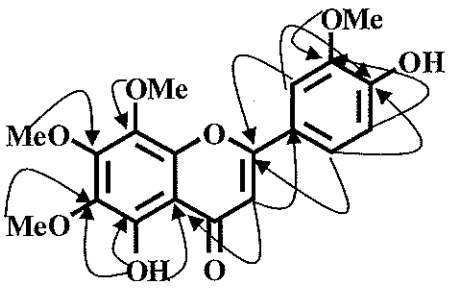
CR35: sudachitin



CR35 was obtained as a yellow solid. The ^{1}H-NMR spectrum (**Table 30**) showed signalsof olefinic proton H-3 (δ 6.60, *s*), chelated hydroxyl group (δ 12.52) and four methoxyl groups (δ 4.10, 4.02, 3.97, 3.95), aromatic protons H-5' (ABX signal, δ 7.06, *d*, 8.7), H-2' (7.42, *d*, 2.4) and H-6' (7.56, *dd*, 8.7, 2.4). The spectrum was similar to that of **CR20**, except for the absence of one methoxyl group. The HMQC and HMBC data were displayed in the same manner as for **CR20.** Therefore methoxy group at δ 4.11 and δ 4.02 were assigned for 7-OMe and 3'-OMe, respectively, whereas the methoxyl protons at δ 3.97 and 3.95 were for 6-OMe and 8-OMe, or exchangeable. Aromatic H-2' and H-6' showed correlation to the oxygenated aromatic carbon at δ 149.6. To complete the structure, a 4'-OH then was assigned. The NOE experiment also confirmed the assignment, irradiation at the resonance of H-2' (δ 7.42) enhanced the resonance of the methoxyl proton at δ 4.02 (3'-OMe), while irradiation at the resonance of H-5' (δ 7.06) was no effect to any proton. Thus **CR35** was assigned as 5, 4'-dihydroxy-6,7,8,3'-tetramethoxyflavone or sudachitin (Nakagawa *et al.*, 2006).



NOE of **CR34**



Major HMBC correlations of **CR35**

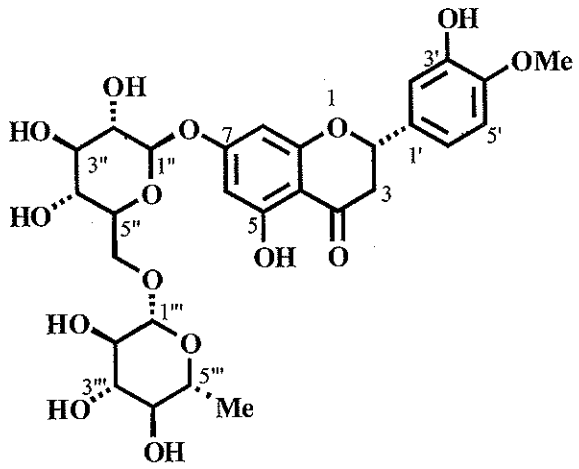
Table 30. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR35** ($CDCl_3$+DMSO-d_6)

Positions	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
2	-	164.2 (C)	-
3	6.60 (*s*)	104.0 (CH)	C-2, C-4, C-4a, C-1'
4	-	177.3 (C=O)	-
4a	-	114.9 (C)	-
5	-	-	-
6	-	133.0 (C)	-
7	-	153.1 (C)	-
8	-	136.0 (C)	-
8a	-	147.7 (C)	-
1'	-	123.9 (C)	-
2'	7.42 (*d*, *J* = 1.8 Hz)	108.5 (CH)	C-2, C-4', C-6'
3'	-	146.9 (C)	-
4'	-	149.6 (C)	-
5'	7.06 (*d*, *J* = 8.7 Hz)	114.3 (CH)	C-1', C-3'
6'	7.56 (*dd*, *J* = 8.7, 1.8 Hz)	119.2 (CH)	C-2, C-2', C-4'
5-OH	12.52 (*s*)	-	C-5, C-6, C-4a
6-OMe	3.97 (*s*)	61.9* (CH_3)	C-6
7-OMe	4.10 (*s*)	61.8 (CH_3)	C-7
8-OMe	3.95 (*s*)	60.9* (CH_3)	C-8
3'-OMe	4.02 (*s*)	56.0 (CH_3)	C-3'

*exchangeable position

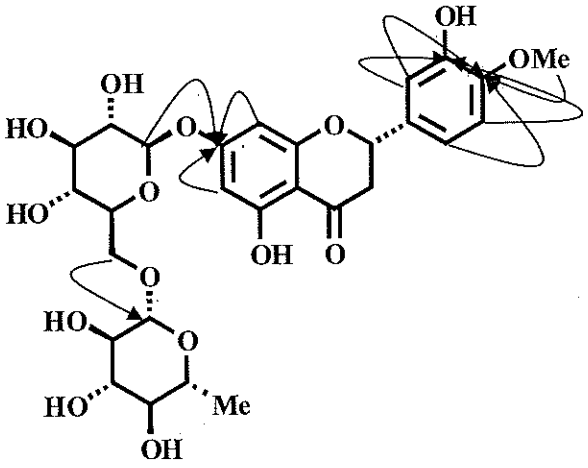
3.1.4 Flavooid glycosides

CR28: Hesperidin



CR28 was obtained as a yellow solid. The ^{1}H NMR spectrum (**Table 31**) showed characteristic signals of H-2, H-3$_{ax}$ and H-3$_{eq}$ of flavanones at δ 5.38 (*dd*, *J* = 12.9, 3.0 Hz), δ 3.06 (*dd*, *J* = 17.1, 12.9 Hz) and δ 2.84 (*dd*, *J* = 17.1, 3.0 Hz). The spectrum further showed signals of a chelated hydroxyl group at δ 12.65 (5-OH), methoxyl group at δ 3.86 (4'-OMe), an AB pattern with *meta* coupling constant (2.1) at δ 6.14 (H-6) and δ 6.17 (H-8), and a tri-substituted aromatic ring at δ 6.89 (*s*, H-5'), δ 7.00 (*s*, H-6') and δ 6.89 (*s*, H-2'). The location 4'-OMe was confirmed by the HMBC correlations of 4'-OMe, H-2' and H-6' to C-4' (δ 148.6). H-2' and H-5' showed correlation to the carbon at δ 147.1 confirmed that the hydroxyl group was substituted at C-3'. The spectrum also showed signals of a glucoside moiety at δ 4.90 (*d*, *J* = 10.0 Hz, glucosyl H-1''), δ 3.48 (2H, *m*, H-6''), δ 3.2-3.6 (*m*, glucosyl protons) and rhamnoside moiety at δ 4.50 (*br s*, rhamnosyl, H-1'''), δ 3.2-3.6 (*m*, rhamnosyl protons), and δ 1.19 (3H, *m*, rhamnosyl CH_3). The oxymethylene protons of the glucosyl moiety (H-6'') showed correlations to δ 105.9 (C-1''') indicating that C-1'''of the rhamnosyl was connected to C-6'' of glucose. The anomeric proton of the glucoside H-1'' (δ 4.90) and H-6/H-8 correlated with C-7 (δ 165.8) indicating that the sugar moieties were attached at C-7 of the flavanone. The ^{13}C-NMR spectrum, showed the resonaces of 28 carbons, including one carbonyl carbon, δ 197.7

(C-4), five oxy-aromatic carbons δ 165.8 (C-7), 163.7 (C-5), 163.1 (C-8a), 148.6 (C-4′), 147.1 (C-3′), two quaternary aromatic carbons δ 131.6 (C-1′), 103.9 (C-4a), five methane aromatic carbons δ 118.5 (C-6′), 114.8 (C-2′), 112.7 (C-5′), 97.0 (C-6), 96.2 (C-8), one oxy-methine carbon δ 79.1 (C-2), one methylene carbon δ 47.2 (C-3), and twelve carbons of sugar δ 105.9 (C-1‴), 104.9 (C-1″), 76.9 (C-5″), 76.1 (C-3″), 76.1 (C-5‴), 76.0 (C-2‴), 72.7 (C-4‴), 74.8 (C-2″), 70.9 (C-3‴), 68.9 (C-4″), 66.7 (C-6″), 18.5 (C-6‴). From the above mentioned data and comparing these data with the previously reported data, it was clear that the **CR28** was hesperidin (Hamdan *et al.*, 2011).



Major HMBC correlations of **CR28**

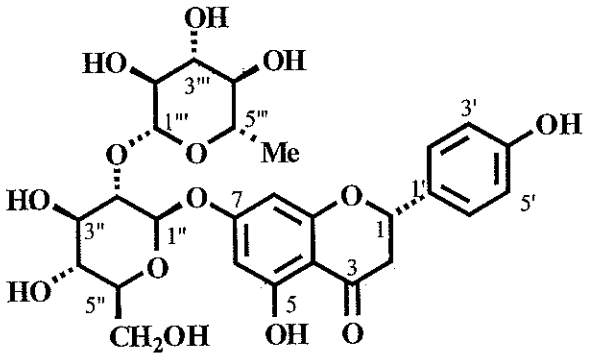
Table 31. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR28** ($CDCl_3$+ DMSO-d_6)

Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
2	5.38 (*dd*, *J* = 12.9, 3.0 Hz)	79.1 (CH)	C-4, C-1', C-2', C-6'
3	3.06 (*dd*, *J* = 17.1, 12.9 Hz)	42.7 (CH_2)	C-4, C-4a, C-1'
	2.84 (*dd*, *J* = 17.1, 3.0 Hz)	-	C-4, C-4a, C-1'
4	-	197.7 (C=O)	-
4a	-	103.9 (C)	-
5	-	163.7 (C)	-
6	6.14 (*d*, *J* = 2.1 Hz)	97.0 (CH)	C-5, C-7, C-8, C-4a
7	-	165.8 (C)	-
8	6.17 (*d*, *J* = 2.1 Hz)	92.6 (CH)	C-6, C-7, C-4a, C-8a

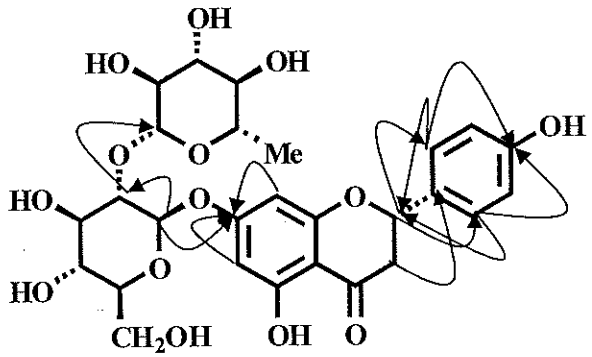
Table 31. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR28** ($CDCl_3$+ DMSO-d_6) (continued)

Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
8a	-	163.1 (C)	-
1'	-	131.6 (C)	-
2'	6.89 (*s*)	114.8 (CH)	C-2, C-4', C-6'
3'	-	147.1 (C)	-
4'	-	148.6 (C)	-
5'	6.89 (*s*)	112.7 (CH)	C-2, C-3', C-4'
6'	7.00 (*s*)	118.5 (CH)	C-2, C-4', C-6'
1''	4.90 (*d*, *J* = 10.0 Hz)	104.9 (CH)	C-7
2''	3.36 (*m*)	74.8 (CH)	-
3''	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	76.3 (CH)	-
4''	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	68.9 (CH)	-
5''	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	76.9 (CH)	-
6''	3.48 (*m*)	66.7 (CH_2)	C-1'''
1'''	4.70 (*brs*)	105.9 (CH)	-
2'''	3.59 (*m*)	76.0 (CH)	-
3'''	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	70.9 (CH)	-
4'''	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	72.7 (CH)	-
5'''	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	76.1 (CH)	-
6'''	1.19 (*d*, *J* = 6.6 Hz)	18.5 (CH_3)	C-5'''
5-OH	12.65 (*s*)	-	C-5, C-6, C-4a
4'-OMe	3.86 (*s*)	-	C-4'

CR29: Naringin



CR29 was obtained as a yellow solid. The ^{1}H NMR spectrum (**Table 32**) showed characteristic signals of H-2, H-3$_{ax}$ and H-3$_{eq}$ of flavanones at δ 5.50 (*dd*, J = 12.7, 2.7 Hz), δ 3.36 (*dd*, J = 17.2, 12.7 Hz) and δ 2.73 (*dd*, J = 17.2, 2.7 Hz), a chelated hydroxyl group 5-OH at δ 12.03, a *meta* aromatic protons H-6 at δ 6.11, H-8 at δ 6.10 (J = 2.1 Hz), a *para*-substituted aromatic protons H-2'/H-6 at δ 7.31, H-3'/H-5' at δ 6.80 . The spectrum showed signals for glucoside moiety at δ 5.10 (*d*, J = 9.0 Hz, glucosyl H-1''), non-equivalent oxy methylene proton at δ 3.63 and δ 3.39 (*m*, H-6'', glucosyl protons), 3.2-3.6 (*m*, glucosyl protons) and rhamnoside moiety at δ 5.07 (*d*, J = 3.0 Hz, rhamnosyl, H-1'''), 3.2-3.6 (*m*, rhamnosyl protons) and δ 1.14 (3H, *m*, rhamnosyl CH_3). According to the HMBC correlation of the anomeric proton H-1'' (δ 5.07) showed a correlation to δ 77.1 (C-2''), and H-2'' to δ 100.0 (C-1''') indicated that the glucoside and rhamnose was connect between C-1''' and C-2''. The sugar side chain was assigned at C-7 (165.8) due to the HMBC correlation of H-6, H-8 and anomeric proton of glucoside (δ 5.10, H-1'') make a correlation with C-7. From the above mentioned data and comparing these data with the previously reported data, it was clear that the **CR29** was naringin (Hamdan *et al.*, 2011).



Major HMBC correlations of **CR29**

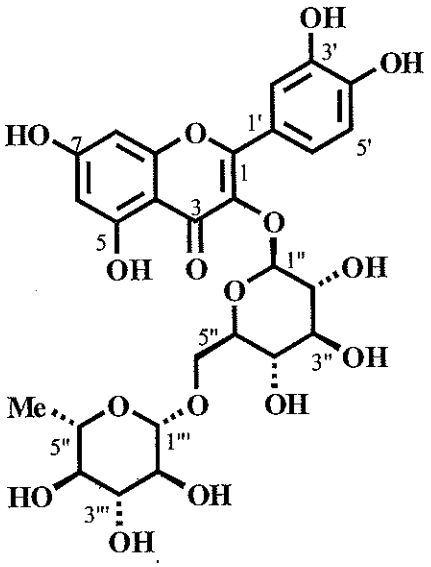
Table 32. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR29** (DMSO-d_6)

Position	δ_H (multiplicity, J)	δ_C (C-Type)	HMBC
2	5.50 (*dd*, J = 12.7, 2.7 Hz)	79.1 (CH)	C-4, C-1', C-2', C-6'
3	3.36 (*dd*, J = 17.2, 12.7 Hz)	42.7 (CH_2)	C-4, C-4a, C-1'
	2.73 (*dd*, J = 17.2, 2.7 Hz)		C-4, C-4a, C-1'
4	-	197.7 (C=O)	-
4a	-	103.9 (C)	-
5	-	163.7 (C)	-
6	6.11 (*d*, J = 2.1 Hz)	97.0 (CH)	C-5, C-7, C-8, C-4a
7	-	165.8 (C)	-
8	6.10 (*d*, J = 2.1 Hz)	103.8 (CH)	C-6, C-7, C-4a, C-8a
8a	-	163.1 (C)	-
1'	-	131.6 (C)	-
2'	7.31 (*d*, J = 8.4 Hz)	115.7 (CH)	C-2, C-4', C-6'
3'	6.80 (*d*, J = 8.4 Hz)	147.1 (C)	-
4'	-	148.6 (C)	-
5'	6.80 (*d*, J = 8.4 Hz)	112.7 (CH)	C-2, C-3', C-4'
6'	7.31 (*d*, J = 8.4 Hz)	118.5(CH)	C-2, C-4', C-6'
1''	5.10 (*d*, J = 9.0 Hz)	97.0 (CH)	C-2''
2''	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	77.1 (CH)	C-1'''

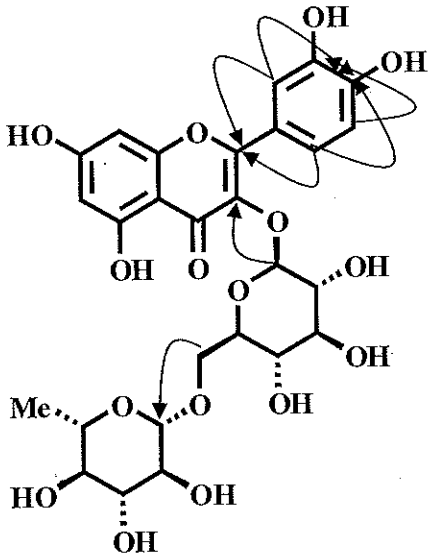
Table 32. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR29** (DMSO-d_6) (Continued)

Position	δ_H (multiplicity, J)	δ_C (C-Type)	HMBC
3″	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	76.1 (CH)	-
4″	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	68.9 (CH)	-
5″	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	76.9 (CH)	-
6″	3.63 (*m*) 3.39 (*m*)	60.8 (CH_2)	-
1‴	5.07 (*d*, J = 0.9 Hz)	100.0 (CH)	-
2‴	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	70.5 (CH)	C-2″
3‴	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	70.9 (CH)	-
4‴	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	72.7 (CH)	-
5‴	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	68.3 (CH)	-
6‴	1.14 (*d*, J = 6.6 Hz)	18.5 (CH_3)	C-5‴
5-OH	12.03 (*s*)	-	C-5, C-6, C-4a

CR30: Rutin



CR30 was obtained as a yellow solid. The ^{1}H NMR spectrum (**Table 33**) exhibited a sharp singlet of a chelated hydroxyl proton at δ 12.58 (5-OH), two aromatic protons in an AB pattern with *meta* coupling constant (2.1) at δ 6.21 (H-6) and δ 6.39 (H-8), and additional three aromatic protons in an ABX pattern at δ 6.87 (*d*, 8.4, H-5′), δ 7.49 (*dd*, 8.4, 2.4, H-6′) and δ 7.51 (*d*, J = 2.4, H-2′). These resonances corresponded to a quercitin moiety (Boligon, *et al.*, 2009). The ^{1}H NMR spectrum also showed signals for anomeric proton H-1″ and H-6″ of glucose moiety at δ 5.13 (*d*, 10.0 Hz) and 3.67 (*d*, 15.0 Hz), signals of anomeric proton H-1‴ and methyl of rhamnose moiety at δ 4.36 (*br s*) and δ 0.98 (*d*, 6.0 Hz). The protons in the sugar moiety resonated between 3.2-3.6 ppm. The glucosylation at C-3 (δ 133.8) of quercitin moiety was confirmed from the HMBC correlation which was seen between the anomeric proton signal of glucose H-1″ (δ 5.31) and C-3 (δ 133.8). In addition, a cross-peak between the δ 4.36 (H-1‴, rhamnose) and the carbon δ 67.1 (C-6" of the glucose) confirmed that the glycosylation of the glucose unit by the rhamnose took place on the C-6″. Comparing these data with the previously reported data, it was clear that the **CR30** was rutin. (Agrawal 1992; El-Sawi and Sleem, 2010)



Major HMBC correlations of **CR30**

Table 33. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR30** (DMSO-d_6)

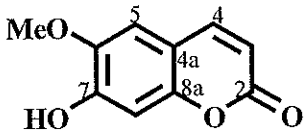
Position	δ_H (multiplicity, J)	δ_C (C-Type)	HMBC
2	-	156.9 (C)	-
3	-	133.8 (C)	-
4	-	177.8 (C=O)	-
4a	-	104.5 (C)	-
5	-	161.5 (C)	-
6	6.21 (*d*, J = 2.1 Hz)	99.1 (CH)	C-5, C-7, C-8, C-4a
7	-	165.8 (C)	-
8	6.39 (*d*, J = 2.1 Hz)	95.7 (CH)	C-6, C-7, C-4a, C-8a
8a	-	157.6 (C)	-
1'	-	121.5 (C)	-
2'	7.51 (*d*, J = 2.4 Hz)	116.7(CH)	C-4'
3'	-	147.1 (C)	-
4'	-	148.6 (C)	-
5'	6.87 (*d*, J = 8.4 Hz)	115.7 (CH)	C-2, C-4', C-6'
6'	7.49 (*dd*, J = 8.4, 2.4 Hz)	122.1 (CH)	C-2, C-3', C-4'
1''	5.13 (*d*, J = 10.0 Hz)	101.3 (CH)	C-3

Table 33. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR30** (DMSO-d_6) (continued)

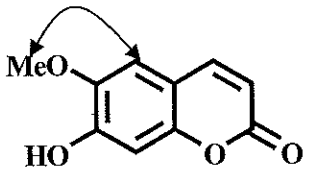
Position	δ_H (multiplicity, J)	δ_C (C-Type)	HMBC
2''	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	73.7 (CH)	-
3''	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	77.4 (CH)	-
4''	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	71.1 (CH)	-
5''	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	75.9 (CH)	-
6''	3.67 (*d*, J = 15 HZ)	67.1 (CH_2)	C-1'''
1'''	4.36 (*brs*)	101.5 (CH)	-
2'''	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	70.9 (CH)	-
3'''	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	70.7 (CH)	-
4'''	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	72.3 (CH)	-
5'''	3.2-3.6 (*m*, rhamnosyl and glucosyl protons)	68.7 (CH)	-
6'''	0.98 (*d*, J = 6.0 HZ)	18.2 (CH_3)	C-5'''
5-OH	12.58 (*s*)		C-5, C-6, C-4a

3.1.5 Coumarins

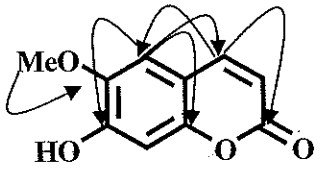
CR11: Scopoletin



CR11 was obtained as a white solid The UV spectrum showed maximum absorptions at λ_{max} 222, 249, 268 and 311 nm. Its IR spectrum showed the absorption bands of C=O stretching at 1704 $cm^{-1}$and O-H stretching at 3323 cm^{-1}. The ^{1}H-NMR spectrum (**Table 34**) indicated that it was a coumarin of which α, β-olefinic protons resonated at δ 6.29 and δ 7.62 (*d*, J = 9.3 Hz),respectively. Two singlet aromatic proton signals at δ 6.87 and δ 6.94 were assigned for H-5 and H-8. The HMBC correlations of H-4 to C-2, C-8a, C-5 and of H-5 to C-4, C-7 and C-8a confirmed the position of H-4 and H-5 at the C-4 and C-5, respectively. Furthermore, the spectrum showed two remaining singlets at δ 3.97 (6-OMe) and 6.18 (7-OH). The NOE experiment that irradiation at δ 3.97 (6-OMe) enhanced the resonance of H-5 suggested that the methoxyl group was at C-6 (δ 149.7). Accordingly, a hydroxyl groups were placed at C-7 (δ 143.2). Compound **CR11** therefore was identified as 7-hydroxy-6-methoxychromen-2-one which was identical to scopoletin (Mohamed *et al.*, 2009).



NOE of **CR11**

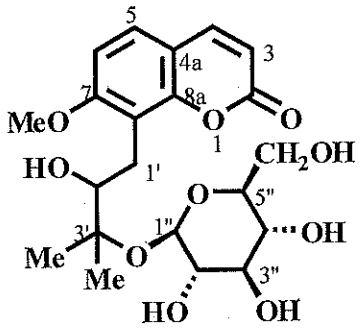


Major HMBC correlations of **CR11**

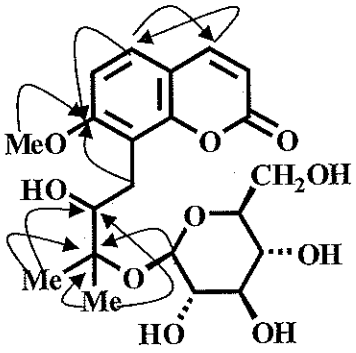
Table 34. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR11** ($CDCl_3$)

Positions	**δ_H (multiplicity, *J*)**	**δ_C (C-Type)**	**HMBC**
2	-	161.3 (C=O)	-
3	6.29 (*d*, *J* = 9.3 Hz)	113.5 (CH)	C-2, C-4a
4	7.62 (*d*, *J* = 9.3 Hz)	144.0 (CH)	C-2, C-5, C-8a
4a	-	111.5 (C)	-
5	6.87 (*s*)	107.5 (CH)	C-4, C-6, C-7
6	-	149.7 (C)	-
7	-	143.2 (C)	-
8	6.94 (*s*)	103.2 (CH)	C-4a, C-6, C-7
8a	-	150.3 (C)	-
6-OH	6.18 (*s*)	-	C-4, C-6, C-8
7-OMe	3.97 (*s*)	56.4 (CH_3)	C-7

CR27: 8,3′-β-glucosyloxy-2′-hydroxy-3′-methylbutyl-7-methoxy-coumarin



CR27 was obtained as colorless solid. The ^{1}H-NMR spectrum (**Table 35**) indicated that it was a coumarin of which α, β-olefinic protons resonated at δ 6.15 and δ 7.60 (*d*, J = 9.3 Hz), respectively. The *ortho* aromatic protons resonated at δ 7.28 (*d*, 8.4, H-5) and δ 6.80 (*dd*, 8.4, H-6). Methoxy protons resonated at δ 3.97. The HMBC correlations of H-4 to C-5 (δ 127.5) and H-5 to C-4 (δ 145.2) confirmed that H-5 was *peri* to the olefinic proton H-4. The methoxyl goup was located at C-7 according to the correlation of H-5 and methoxy protons (7-OMe) showed correlations to C-7 (δ 160.8). The presence of prenyl group was deduced from signals analysis of nonequivalent methylene protons H-1′ at δ 3.00 (*t*, J =12.0 Hz) and δ 2.90 (*d*, J =12.0 Hz), methine protons H-2′ at 3.60 (*d*, J =12.0 Hz) and two methyl protons H-4′/ H-5 at δ 1.21 and δ 1.23, and HMBC correlations from H-4′/ H-5′ to the C-2′ (δ 77.0). The oxycarbons C-2' and C-3' of the prenyl resonated at δ 77.0 and δ 81.0. The spectrum further showed the signal of a glucose moiety at 4.50 (*d*, J = 2.9 Hz, H-1″), 3.70 (2H, *m*, H-6″), 3.2-3.6 (4H, *m*, glucosyl protons). Protons H-1″ and H-4′/ H-5 showed correlation to C-3′ (δ 81.0) indicating that the glucose was connected at C-3′ of the isoprene unit. The side chain was placed at C-8 and *ortho* with 7-OMe according to the HMBC correlations of H-1′ to C-7 (δ 160.8). Therefore, **CR27** was assigned as 8,3′-β-glucosyloxy-2′-hydroxy-3′-methylbutyl-7-methoxy-coumarin (David *et al.*, 1987).



Major HMBC correlations of **CR27**

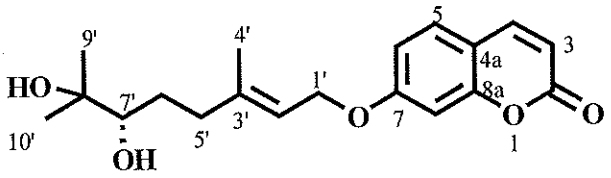
Table 35. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR27** ($CDCl_3$+DMSO-d_6)

Positions	δ_H **(multiplicity, *J*)**	δ_C **(C-type)**	**HMBC**
2	-	170.1 (C=O)	-
3	6.15 (*d*, *J* = 9.3 Hz)	113.5 (CH)	C-2, C-4a
4	7.60 (*d*, *J* = 9.3 Hz)	145.2(CH)	C-2, C-5, C-8a
4a	-	111.5 (C)	-
5	7.28 (*d*, *J* = 6.6 Hz)	127.7 (CH)	C-4, C-6, C-7
6	6.80 (*d*, *J* = 6.6 Hz)	149.7 (C)	C-4, C-5, C-7
7	-	160.8 (C)	-
8	-	116.4 (C)	-
8a	-	153.4 (C)	-
7-OMe	3.97 (*s*)	56.4 (CH_3)	C-7
1′	3.00 (*t*, *J* = 12.0 Hz)	25.4 (CH_2)	C-7, C-8
	2.90 (*d*, *J* = 12.0 Hz)		-
2′	3.60 (*d*, *J* = 12.0 Hz)	77.0 (CH)	C-8
3′	-	81.0 (C)	-
4′	1.21 (*s*)	23.0 (CH_3)	C-2′, C-3′
5′	1.23 (*s*)	24.1 (CH_3)	C-2′, C-3′
1″	4.50 (*d*, *J* = 2.9 Hz)	101.1 (CH)	C-3′
2″	3.2-3.6 (*m*, glucosyl protons)	71.4 (CH)	-

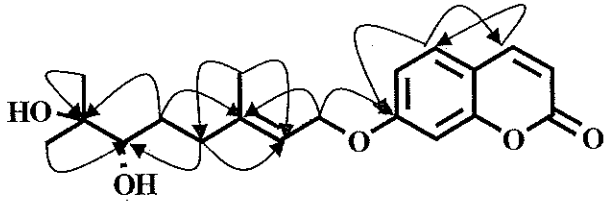
Table 35. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR27** ($CDCl_3$+DMSO-d_6) (continued)

Positions	**δ_H (multiplicity, *J*)**	**δ_C (C-Type)**	**HMBC**
3″	3.2-3.6 (*m*, glucosylprotons)	76.1(CH)	-
4″	3.2-3.6 (*m*, glucosylprotons)	68.9 (CH)	-
5″	3.2-3.6 (*m*, glucosylprotons)	76.9 (CH)	-
6″	3.70 (*m*, glucosylprotons)	66.7 (CH_2)	C-5″

CR36: marmin



CR36 was a white solid, $[\alpha]_D^{29}$ +28° (c 1.0, EtOH). The ^{13}C NMR spectrum showed α,β-unsaturated carbonyl signals at δ 113.4 (C-3), δ 143.5 (C-4) and δ 162.1 (C-2). The ^1H NMR spectrum (**Table 36**) showed the doublet resonances (J = 9.3 Hz) of α, β-olefinic protons at δ 6.29 (H-3) and δ 7.38 (H-4), respectively. The trisubstituted benzene ring was proposed from the ABX signal of aromatic protons H-5, H-6 and H-8 at δ 7.37 (*d*, 8.4), δ 6.86 (*dd*, 8.4, 2.1) and δ 6.84 (*d*, 2.1), respectively. The HMBC correlations of H-4 to C-5 (δ 129.8), and H-5 to C-4 (143.5) confirmed H-5 was *peri* to olefinic proton H-4. The presence of *O*-geranyl group was shown by the characteristic signal of oxy methylene protons H-1′ at δ 4.63 (*d*, J = 6.9 Hz), methine protons H-2′ at 5.54 (*t*, 6.9 Hz), two methelene protons at δ 1.70 (*m*, H-5′), 1.48 (*m*, H-6′) and three methyl protons at δ 1.68 (H-4′) and δ 1.24 (H-9′/ H-10′). The HMBC correlations of H-1′, H-4′, H-6′ to C-3′ (δ 141.9), and of H-6′, H-9′, H-10′ to C-8′ (73.7) indicated the presence of a geranyl side chain. The oxy-carbons C-7′ (δ 78.0) and C-8′ (δ 73.7) was assigned for diol rather than an oxirane ring because in general the carbon signals of oxirane ring were shown at δ 55-65 ppm. The HMBC correlations of H-1′, H-5 to C-7 (δ 161.2) suggested that the side chain was at C-7. Therefore, **CR36** then was identified as 7-[(6*R*)-6,7-dihydroxy-3,7-dimethyl-(2*E*)-2-octeyloxy]coumarin. Its spectroscopic data was in agreement with the previously reported data of marmin (Chen *et al.*, 1996).

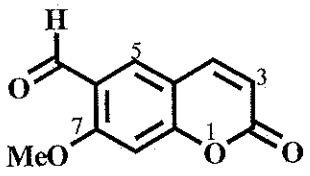


Major **HMBC** correlations of **CR36**

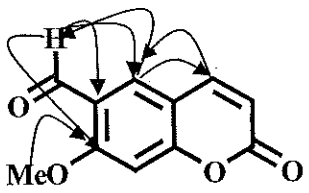
Table 36. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR36** ($CDCl_3$)

Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
2	-	162.1 (C=O)	-
3	7.38 (*d*, *J* = 9.3 Hz)	113.4 (CH)	C-2, C-4a
4	6.29 (*d*, *J* = 9.3 Hz)	143.5 (CH)	C-2, C-5, C-8a
5	7.37 (*d*, *J* = 8.4 Hz)	129.8 (CH)	C-4, C-7, C-8a
6	6.86 (*dd*, *J* = 8.4, 2.1 Hz)	100.0 (CH)	C-4a, C-6, C-7, C-8a
7	-	161.2 (C)	-
8	6.84 (*d*, *J* = 2.1 Hz)	104.0 (CH)	C-4a, C-6, C-7, C-8a
4a	-	112.5 (C)	-
8a	-	156.9 (C)	-
1'	4.63 (*d*, *J* = 6.9 Hz)	65.2(CH_2)	C-7, C-3'
2'	5.54 (*t*, *J* = 6.9 Hz)	118.4 (CH)	C-3', C-4', C-5'
3'	-	141.9 (C)	-
4'	1.68 (*s*)	16.3 (CH_3)	C-3', C-5', C-6'
5'	1.70 (*m*)	35.9 (CH_2)	C-3', C-4', C-7'
6'	1.48 (*m*)	29.7 (CH_2)	C-3', C-8'
7'	3.28 (*m*)	78.0 (CH)	C-5', C-8', C-9', C-10'
8'	-	73.7 (C)	-
9'/10'	1.24 (*s*)	25.7 (CH_3)	C-7', C-8'

CR38 : crenulatin



Compound **CR38** was a white solid. The ^{13}C NMR spectrum showed α,β-unsaturated carbonyl signals at δ 114.6 (C-3), δ 143.3 (C-4) and δ 159.8 (C-2). The ^{1}H NMR spectrum **(Table 37)** showed signals of α,β-olefinic protons at δ 6.33, (*d*, 9.0, H-3) and δ 7.70 (*d*, 9.0, H-4), singlet aromatic protons H-5 at δ 7.99 and H-8 at δ 6.89, a methoxyl group 7-OMe at δ 4.02, and a formyl group at δ 10.42 (6-CHO). The spectrum was similar to that of **CR11** except for the absence of hydroxy group, at C-6 which was replaced with a formyl group. The HMBC correlation of formyl proton to C-5 (δ 129.0), C-6 (δ 122.2), C-7 (δ 164.2), of H-5 to formyl carbonyl carbon (δ 187.8), and of 7-OMe to C-7 indicated that the formyl group was at C-6, and the 7-OMe was at C-7. Therefore, **CR38** was assigned as crenulatin (Wu *et al.*, 1983).

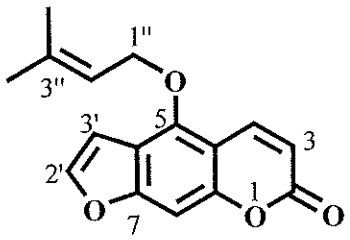


Major HMBC correlations of **CR38**

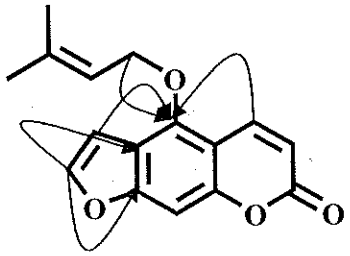
Table 36. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR38** ($CDCl_3$)

Position	**δ_H (multiplicity, *J*)**	**δ_C (C-Type)**	**HMBC**
2	-	159.8 (C=O)	-
3	6.33 (*d*, *J* = 9.0 Hz)	114.6 (CH)	C-2, C-4a
4	7.70 (*d*, *J* = 9.0 Hz)	143.3 (CH)	C-4a, C-5, C-8a
5	7.99 (*s*)	129.0 (CH)	C-4, C-8a, C-7, C-1'
6	-	122.2 (C)	-
7	-	164.2 (C)	-
8	6.89 (*s*)	99.9 (CH)	C-4a, C-6, C-8a
4a	-	112.5 (C)	-
8a	-	159.6 (C)	-
6-CHO	10.42 (*s*)	187.8 (CH)	C-5, C-6, C-7
7-OMe	4.02 (*s*)	56.4 (CH_3)	C-7

CR39: isoimperatorin



Compound **CR39** was obtained as a yellow solid. The ^{13}C NMR spectrum showed α,β-unsaturated carbonyl signals at δ 112.6 (C-3), δ 139.3 (C-4) and δ 161.4 (C-2). The ^{1}H-NMR spectrum (**Table 38**) showed signals of α, β-olefinic protons at δ 6.29 and δ 8.17 (*d*, J = 9.9 Hz), aromatic proton H-8 at δ 7.15. The presence of isoprene unit was shown by the characteristic signal of oxy methylene protons at δ 4.91 (*d*, 6.9, H-1″), methine protons at 5.52 (*t*, 6.9, H-2″) and two methyl protons at δ 1.89 (H-4″) and 1.70 (H-5″). It was assigned as an *O*-isoprene from the signal at δ 4.91 (*d*, 6.9, H-1″). The presence of a furan ring was proposed from the low field olefinic doublets protons H-2' at δ 7.04 and H-3′ at δ 7.61 (*d*, J = 2.4 Hz), together with the HMBC correlations of H-2' to C-6 (δ 116.2), C-7 (δ 158.4) of coumarin. The carbon C-2' was assigned attach to oxygen atom due to its low field chemical shift (δ 144.8). The isoprene side chain was placed at C-5 according to the HMBC correlations of H-3′, H-1″ and H-4 to C-5 (δ 149.7). Therefore, **CR39** was assigned isoimperatorin or 4-(3-methylbut-2-enoxy)furo[3,2-g]chromen-7-one (Intekhab, J and Aslam, M., 2009).



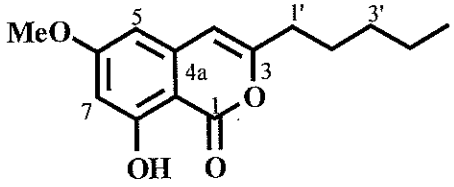
Major HMBC correlations of **CR39**

Table 38. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR39** ($CDCl_3$)

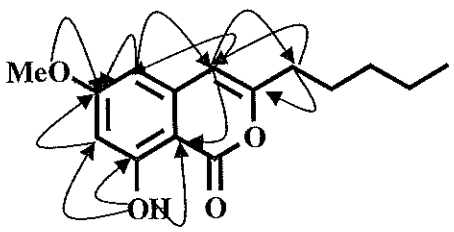
Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
2	-	161.4 (C=O)	-
3	6.29 (*d*, *J* = 9.9 Hz)	112.6 (CH)	C-4a, C-2
4	8.17 (*d*, *J* = 9.9 Hz)	139.3 (CH)	C-2, C-5, C-8a
5	-	149.7 (C)	-
6	-	116.2 (C)	-
7	-	158.4 (C)	-
8	7.15 (*s*)	93.9 (CH)	C-4a, C-6, C-7, C-8a
9	-	152.7 (C)	-
10	-	106.4 (C)	-
2'	7.61 (*d*, *J* = 2.4 Hz)	144.8 (CH)	C-6, C-7 , C-3'
3'	7.04 (*d*, *J* = 2.4 Hz)	105.0 (CH)	C-5, C-6, C-7 , C-2'
1"	4.91 (*d*, *J* = 6.9 Hz)	69.6 (CH_2)	C-5, C-3"
2"	5.52 (*t*, *J* = 6.9 Hz)	119.2 (CH)	C-4"
3"	-	139.0 (C)	-
4"	1.89 (*s*)	25.8 (CH_3)	C-3"
5"	1.70 (*s*)	18.2 (CH_3)	C-3"

3.1.6 Isocoumarins

CR5: 8-hydroxy-6-methoxy-pentylisocoumarin



CR5 was obtained as amorphous powder. The ^{1}H NMR spectrum (**Table 39**) showed the resonances of *meta*-aromatic protons at δ 6.45 (*d*, J = 2.0 Hz, H-7), 6.31 (*d*, J = 2.0 Hz, H-5), a hydrogen bonded hydroxy proton at δ 11.27 (8-OH), a methoxyl group at δ 3.86 (4-OMe), an olefinic proton at δ 6.17 (H-4); and a pentyl group. The pentyl group was deduced from the resonances at δ 2.48 (*t*, 7.6, H-1′), δ 1.69 (*m*, H-2′), δ 1.35 (4H, *m*, H-3′, 4′), δ 0.86 (H-5′). The olefinic proton H-4 and 8-OH showed a HMBC correlation to C-8a (δ 100.1), indicated that the hydroxyl group (8-OH) and H-4 were *ortho* to the carbonyl ester. A methoxyl group at δ 3.86 was assigned to 6-OMe due to H-5, H-7 and 6-OMe showing HMBC correlations to C-6. The proton H-7 was placed *ortho* to the chelated hydroxyl protons (8-OH) according to HMBC correlations of 8-OH to the carbon at δ 100.1 (C-8a). The HMBC correlations of H-4 to C-8a, C-5, C-1′ and of H-5 to C-4, C-7, C-8a confirmed the position of H-4 and H-5 respectively. The side chain was placed at C-3 according to the HMBC correlations of H-1′ and H-4 to C-3 (δ 158.0). From the above mentioned data and comparing these data with those previously reported, it was clear that **CR5** was 8-hydroxy-6-methoxypentyl-isocoumarin (Kijjoa, *et* al., 1991).

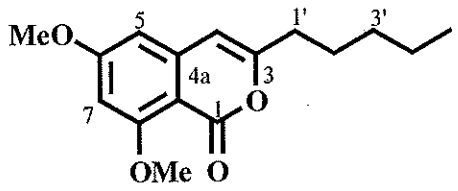


Major HMBC correlations of **CR5**

Table 39. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR5** ($CDCl_3$)

Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
1	-	166.7 (C=O)	-
2	-	-	-
3	-	158.0 (C)	-
4	6.17 (*s*)	103.8 (CH)	C-5, C-8a, C-1'
4a	-	139.4 (C)	-
5	6.31 (*d*, *J* = 2.0 Hz)	101.0 (CH)	C-4, C-4a, C-7, C-8a
6	-	166.4 (C)	-
7	6.45 (*d*, *J* = 2.0 Hz)	100.1 (CH)	C-5, C-8, C-8a
8	-	163.6 (C)	C-7, C-8, C-8a
8a	-	100.1 (C)	-
1'	2.48 (*t*, *J* = 7.6 Hz)	33.2 (CH_2)	C-4
2'	1.69 (*m*)	26.4 (CH_2)	C-3
3'	1.35 (*m*)	31.1 (CH_2)	C-1'
4'	1.35 (*m*)	22.3 (CH_2)	C-3', C-5'
5'	0.86 (*t*, *J* = 6.9 Hz)	13.9 (CH_3)	C-3', C-4'
6-OMe	3.86 (*s*)	60.2 (CH_3)	C-6
8-OH	11.27 (*s*)	-	C-8

CR18 : 6,8-dimethoxypentylisocoumarin



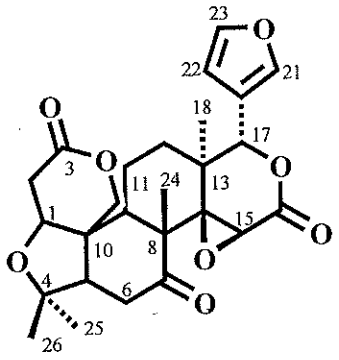
CR18 was obtained as amorphous powder. The ^{1}H NMR spectrum (**Table 40**) showed the resonances of *meta*-aromatic protons at δ 6.45 (*d*, J = 2.0 Hz, H-3), 6.31 (*d*, J = 2.0 Hz, H-5), a methoxyl group at δ 3.86 (4-OMe), an olefinic proton at δ 6.17, and protons of a pentyl group at δ 2.48 (*t*, 7.6, H-1′), 1.69 (*m*, H-2′), 1.35 (4H, *m*, H-3′, 4′), 0.86 (H-5′). This spectrum was very similar to that of **CR5** except for the absence of the chelated hydroxyl proton but the presence of a methoxyl group (δ 3.87). The DEPT 135° showed four methylene carbons at δ 33.2 (C-1'), 30.0 (C-2'), 31.1 (C-'), 26.5 (C-4') and a methyl carbon at δ 13.8 (C-5'). From the above mentioned data and comparing these data with those previously reported, it was clear that **CR18** was 6,8-dimethoxypentylisocoumarin (Kijjoa, *et al.*, 1991).

Table 40. ^{1}H NMR spectroscopic data **CR18** ($CDCl_3$)

Position	δ_H (multiplicity, J)
4	6.17 (*s*)
5	6.31 (*d*, J = 2.0 Hz)
7	6.45 (d, J = 2.0 Hz)
8	3.87 (*s*)
1'	2.48 (*t*, J = 7.6 Hz)
2'	1.69 (*m*)
3'	1.35 (*m*)
4'	1.35 (*m*)
5'	0.86 (*m*)
6-OMe	3.86 (*s*)
8-OMe	3.87 (*s*)

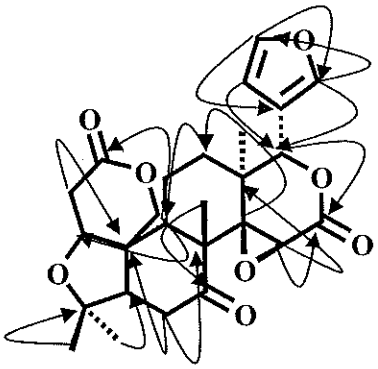
3.1.7 Limonoids and Triterpenoid

CR13: Limonin



CR13 was obtained as a white solid, m.p. 285-286°C, $[\alpha]^{27}_{D}$ -132.5° (*c* 0.10, Me_2CO), $[\alpha]^{27}_{D}$ -124.7° (*c* 0.12, Me_2CO, liturature). The IR spectrum of **CR13** showed stretching of carbonyl at 1730 and 1709 cm^{-1}. The ^1H NMR spectrum **(Table 41)** suggested the presence of a substituted furan from a singlet of H-21 at δ 7.42, and doublets of H-22 at δ 6.36 and H-23 at δ 7.45 associated with a coupling constant value of 1.5 Hz. It was further established that **CR13** was a limonoid with four of singlet methyl groups resonating at δ 1.17 (H-18), 1.08 (H-24), 1.25 (H-25), and1.16 (H-26).The presence of an epoxy lactone moiety of limonoid was revealed by the signals of a carbonyl carbon at δ 171.7 (C-16), an oxy carbon at δ 83.8 (C-17) and epoxy carbons at δ 58.6 (C-15) and δ 70.7 (C-14) together with the characteristic H-15 and H-17 singlet signal at δ 4.05 and 5.48, respectively. The HMBC correlation of H-17 to C-21 (δ 147.9) and C-22 (δ 114.5) suggested the attachment of the furan ring at C-17. Moreover, lactone moiety was indicated by the signals of a carbonyl carbon at δ 174.3 (C-3) and oxy carbon at δ 70.0 (C-19) along with signals of methylene protons at δ 2.30 and 2.70 (2 *x dd*, *J* = 15.0, 3.0 Hz, H-2α, H-2β), oxy-methylene protons at δ 4.50 and 4.82 (2 *x d*, *J* = 12.0 Hz, H-19α, H-19β) and an oxy methine proton at δ 4.09 (*brs*, H-1). The presence of a system of $-(CH_3)_2$-C-CH-CH_2-C=O in the molecules was inferred from an ABC pattern at δ 2.73 (*dd*, *J* = 12.0, 3.0 Hz, H-6α), 3.16 (*dd*, *J* = 12.0, 3.0 Hz, H-6β) and δ 2.44 (*dd*, *J* = 12.0, 3.0 Hz, H-5α)

as well as two methyl singlets at δ 1.25 (H-25) and δ 1.16 (H-26), The ^3J correlations of H-5 to the oxy-methine carbon C-1 (δ 84.9), H-6 to and the quaternary carbon C-10 (δ 50.6), H-26 to C-5 (δ 64.8) and to C-5 (δ 64.8) implied that -$(CH_3)_2$-C-CH-CH_2-C=O was linked to the lactone ring by C-5 to C-10. Methylene protons resonated as multiplet at δ 1.87, 1.78 were assigned for H-11α, H-11β which was coupled by methylene proton H-12α, H-12β (δ 1.51, δ 1.82, *m*,) and a methine proton H-9 (δ 2.58, *dd*, J = 9.0, 3.0 Hz). The ^3J correlation of H-11 to C-10 (δ 50.6), C-8 (δ 55.9) and C-13 (δ 44.5) together with ^3J correlation of H-12 to C-14 (δ 70.7) confirmed. The carbon signals of quaternary carbon, methine carbon, methylene carbon and methyl carbon were in agreement with the assigned structure. **CR13** was then identified as 7,16-dioxo-7,16-dideoxylimondiol. Its structure and spectroscopic data were in agreement to those of limonin (Khalil *et al.*, 2003).



Major HMBC correlations of **CR13**

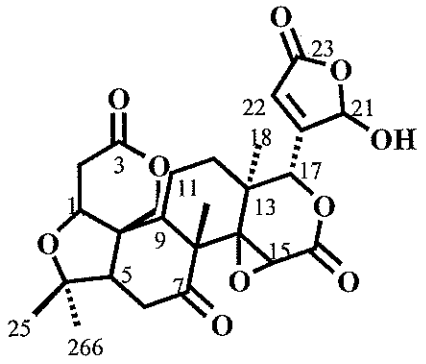
Table 41. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR13** ($CDCl_3$ DMSO-d_6)

Position	δ_H (multiplicity)	δ_C (C-Type)	HMBC
1	4.09 (*br s*)	84.9 (CH)	C-3
2	2.30 (*dd*, *J* = 15.0, 3.0 Hz)	41.1 (CH_2)	C-1, C-9
	2.70 (*dd*, *J* = 15.0, 3.0 Hz)		C-3
3	-	174.3 (C=O)	-
4	-	82.7 (C)	-
5	2.44 (*dd*, *J* = 12.0, 3.0 Hz)	64.8 (CH)	C-1, C-7, C-10
6	2.73 (*dd*, *J* = 12.0, 3.0 Hz)	42.7 (CH_2)	C-4, C-7
	3.16 (*dd*, *J* = 12.0, 3.0 Hz)		C-5, C-10
7	-	211.4 (C=O)	-
8	-	55.9 (C)	-
9	2.58 (*dd*, *J* = 9.0, 3.0 Hz)	52.6 (CH)	C-11
10	-	50.6 (C)	-
11	1.87 (*m*)	23.6 (CH_2)	C-10, C-13
	1.78 (*m*)		C-8
12	1.51 (*m*)	35.2 (CH_2)	C-11, C-12, C-14
	1.82 (*m*)		C-11, C-17
13	-	44.5 (C)	-
14	-	70.7 (C)	-
15	4.05 (*s*)	58.6 (CH)	C-14, C-16
16	-	171.7 (C)	-
17	5.48 (*s*)	83.8 (CH)	C-12, C-18, C-21, C-22
18	1.17 (*s*, 3H)	22.3 (CH_3)	C-12, C-14, C-13, C-17
19	4.50 (*d*, *J* = 12.0 Hz)	70.0 (CH_2)	C-1, C-5, C-10
	4.82 (*d*, *J* = 12.0 Hz)		C-1, C-5, C-3

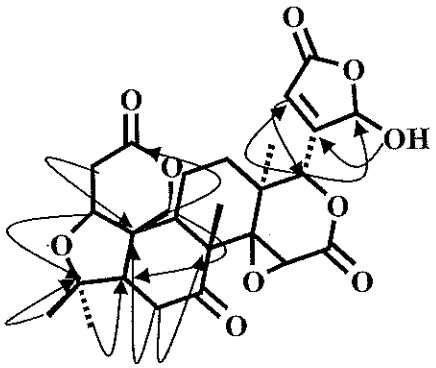
Table 41. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR13** ($CDCl_3$+DMSO-d_6) (continued)

Position	δ_H (multiplicity)	δ_C (C-Type)	HMBC
20	-	124.8 (C)	-
21	7.42 (*s*)	147.9 (CH)	C-20, C-22, C-23
22	6.36 (*d*, J = 1.5 Hz)	114.5 (CH)	C-20, C-21, C-23
23	7.45(*d*, J = 1.5 Hz)	145.8 (CH)	C-20, C-21
24	1.08 (*s*)	22.3 (CH_3)	C-9, C-14, C-7
25	1.25 (*s*)	34.8 (CH_3)	C-26
26	1.16 (*s*)	25.2 (CH_3)	C-25, C-4, C-5

CR42: limonexic acid



CR42 was obtained as white solid, the second limonoid isolated, has spectroscopic properties similar to those of limonin, **CR13**. The IR spectrum indicated the presence of carbonyl absorption at 1730 cm^{-1} and β-substituted furan at 875 cm^{-1}. The ^1H NMR spectrum **(Table 42)** suggested the presence of proton H-17 (δ 5.33) and H-15 (δ 3.85) of epoxy lactone, and four tertiary methyls (δ 1.04, 1.10, 1.15 and 1.32). Furthermore, the ^1H NMR spectrum showed signal of a system -O-CH-CH_2-C=O at δ 2.26 (*dd, J* = 14.8, 3.2, H-2_a), 2.60 (*dd, J* = 14.8, 3.2, H-2_b) and 4.12 (*s*, H-1). The signal of non-equivalent oxy-methylene protons were observed at δ 4.85 and 4.43 (1H each, *d*, *J* = 13.0 Hz, H-19). The ^1H NMR spectrum suggested the presence of a β-substituted furan at δ 5.98 (1H, *br s*, H-21), δ 6.26 (1H, *br s*, H-22) and δ 8.06 (1H, *br s*, 21-OH). The absence of signal at δ 8.06 when addition of a drop of D_2O confirmed that it was hydroxyl signal. The HMBC correlations of H-22 (δ 6.26) to the carbons at δ 78.9 (C-17), δ 98.8 (C-21) and δ 169.0 (C-23) and H-21 (δ 5.98) to the carbons at δ 122.7 (C-22), δ 169.0 (C-23) together with HMBC correlations of 21-OH at δ 8.06 to the carbons at δ 98.8 (C-21) and 165.4 (C-20) confirmed the position of H-22, H-21 and 21-OH, respectively. Based on these data, the structure of **CR42** was assigned as limonexic acid ($[\alpha]^{27}_D$ -139.0° (*c* 0.10, Me_2CO), $[\alpha]^{27}_D$ -127.0° (*c* 0.12, Me_2CO, liturature). (Khalil *et al.*, 2003).



Major HMBC correlations of **CR42**

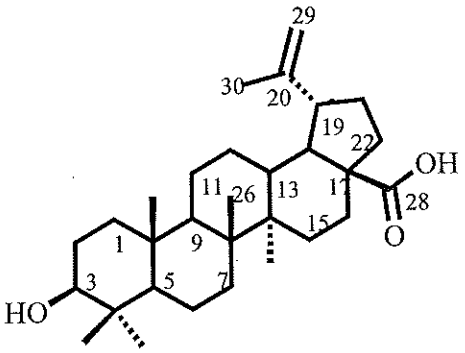
Table 42. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR42** ($CDCl_3$+DMSO-d_6)

Position	δ_H **(multiplicity)**	δ_C **(C-Type)**	**HMBC**
1	4.12 (*br s*)	80.0 (CH)	C-3
2	2.26 (*dd*, *J* = 14.8, 3.2 Hz)	36.0 (CH_2)	C-1, C-9
	2.60 (*dd*, *J* = 14.8, 3.2 Hz)		C-3
3	-	169.3 (C=O)	-
4	-	80.0 (C)	-
5	2.48 (*m*)	50.3 (CH)	C-1, C-7, C-10
6	2.79 (*m*)	36.3 (CH_2)	C-4, C-7
	3.02 (*t*, *J* = 15.0 Hz)		C-5, C-10
7	-	206.3 (C=O)	-
8	-	51.4 (C)	-
9	2.51 (*dd*, *J* = 10.0, 2.0 Hz)	48.2 (CH)	C-11
10	-	45.8 (C)	-
11	1.84 (*m*)	18.2 (CH_2)	C-10, C-13
	1.99 (*m*)		C-8

Table 42. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR42** ($CDCl_3$+DMSO-d_6) (continued)

Position	δ_H (multiplicity)	δ_C (C-Type)	HMBC
12	1.32 (*m*)	29.4 (CH_2)	C-11, C-12, C-14
	1.72 (*m*)		C-11, C-17
13	-	37.1 (C)	-
14	-	65.3 (C)	-
15	3.85 (*s*)	53.2 (CH)	C-14, C-16
16	-	165.5 (C)	-
17	5.33 (*s*)	78.9 (CH)	C-12, C-18, C-20, C-22
18	1.10 (*s*)	20.9 (CH_3)	C-12, C-14, C-13, C-17
19	4.43 (*d*, J = 13.0 Hz)	65.3 (CH_2)	C-1, C-5, C-10
	4.85 (*d*, J = 13.0 Hz)		C-1, C-5, C-3
20	-	165.4 (C)	-
21	5.98 (*br s*)	98.8 (CH)	C-22, C-23
22	6.26 (*br s*)	122.7 (CH)	C-17, C-21, C-23
23	-	169.0 (C=O)	-
24	1.15 (*s*)	20.9 (CH_3)	C-9, C-14, C-7
25	1.32 (*s*)	29.6 (CH_3)	C-26
26	1.04 (*s*)	21.1 (CH_3)	C-25, C-4, C-5
21-OH	8.06 (*br s*)	-	C-20, C-21

CR33: Betulinic acid



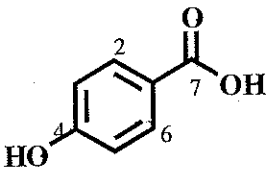
CR33 was obtained as a colorless crystal. It gave a purple vanillin-sulfuric acid test indicating a triterpene. It ^{1}H NMR spectrum (**Table 43**) exhibited characteristic of five methyl singlet signals (δ 0.75, δ 0.82, δ 0.92, δ 0.96, δ 0.98) a vinylic methyl (δ 1.70) and vinylic protons of isopropenyl moiety [δ 4.63 (*br s*), δ 4.76 (*br s*), and a typical lupane H_{β}-19 proton at δ 2.86 (*dt*). An oxymethine proton H-3 signal was shown at δ 3.18 (*dd*). This spectrum was found to be identical to that of betulinic acid (in appendix), the authentic sample in our laboratory, from *Melaleuca cajuputi*. It also showed the same TLC character as that of betulinic acid. Thus, **CR33** was as betulinic acid (Macias *et al.*, 1994).

Table 43. ^{1}H NMR spectrum data of **CR33** ($CDCl_3$)

Position	δ_H (multiplicity, *J*)	Position	δ_H (multiplicity, *J*)
1	0.95 (*m*), 1.70 (*m*)	16	1.43 (*m*), 2.23 (*m*)
2	1.57 (*m*), 1.62 (*m*)	17	-
3	3.19 (*dd*, J = 10.8, 5.4 Hz)	18	1.63 (*m*)
4	-	19	3.02 (*m*)
5	0.71 (*m*)	20	-
6	1.45 (*m*), 1.55 (*m*)	21	1.40 (*m*), 1.93 (*m*)
7	1.42 (*m*)	22	1.43 (*m*), 1.91 (*m*)
8	-	23	0.95 (*s*)
9	1.33 (*m*)	24	0.75 (*s*)
10	1.25 (*m*), 1.45 (*m*)	25	0.86 (*s*)
11	1.07 (*m*), 1.73 (*m*)	26	0.97 (*s*)
12	2.30 (*m*)	27	1.01 (*s*)
13	-	28	-
14	-	29	4.59 (*dd*, J = 2.2, 1.0 Hz) 4.71 (*d*, J = 2.2 Hz)
15	1.18 (*m*), 1.53 (*m*)	30	1.69 (*d*, J = 1.0 Hz)

3.1.8 Benzene derivatives, resorcyclic derivatives and coumarate ester

CR15: 4-hydroxybenzoic acid

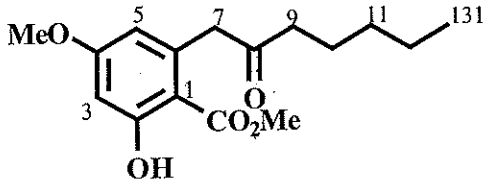


CR15 was obtained as a colorless gum. The ^{1}H NMR spectrum showed the presence of a *para*-disubstituted benzene [δ 7.93 (*d*, J = 8.7 Hz, 2H) and 6.85 (*d*, J= 8.7 Hz, 2H)]. This spectrum was found to be identical to that of 4-hydroxybenzoic acid (inappendix), the authentic sample in our laboratory, from *Arthocarpus elasticus* (Chithada, 2010). It also showed the same TLC character as that of 4-hydroxybenzoic acid. Thus, **CR15** was elucidated to be 4-hydroxybenzoic acid (Choi *et al.*, 2002).

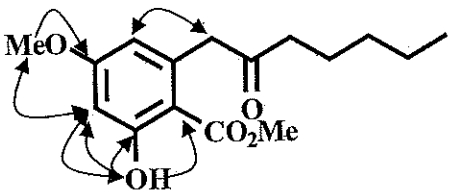
Table 44. ^{1}H NMR spectroscopic data of **CR15** ($CDCl_3$)

Positions	δ_H **(multiplicity,** J**)**
2,6	7.93 (*d*, J = 8.7 Hz)
3,5	6.85 (*d*, J = 8.7 Hz)

CR16: Methyl-2-hydroxy-4-methoxy-6-(2-oxoheptyl)-benzoate



CR16 was obtained as amorphous powder. The UV spectrum showed the maximum absorption at 266.5 nm. The IR spectrum showed a C=O stretching absorption band at 1725 cm^{-1}. A molecular ion in the FAB-Ms at m/z 294.1472 corresponded to a molecular formula of $C_{16}H_{22}O_5$. The ^1H NMR spectrum (**Table 45**) showed resonances of *meta*-aromatic protons at δ 6.45 (H-3), 6.28 (H-5), a hydrogen bonded hydroxy proton at δ 11.27 (2-OH), a methoxyl group at δ 3.84 (4-OMe), a methyl ester group at δ 3.83 (1-CO_2Me) and an 2-oxoheptyl group. The COSY and HMBC experiments indicated that the proton resonances at δ 4.06 (H-7), 2.37 (H-9), 1.42 (H-10), 1.20 (H-11), 1.10 (H-12), 0.86 (H-13) and the carbonyl carbon resonance at δ 207.2 (C-8) corresponded to a 2-oxoheptyl side chain as for **CR12**. The NOSEY correlations of H-7 to H-5, and 4-OMe to H-5 and H-3 allowed an assignment to a 2-oxoheptyl group *ortho* to H-5 while a 4-OMe was *ortho* to H-5 and H-3. The hydroxyl proton was assigned for a 2-OH according to HMBC correlation of the 2-OH to C-3 (δ 97.0). The chemical shift value of 2-OH (δ 11.27) revealed that it formed a hydrogen bond to the adjacent group. Consequently, the remaining methyl ester group was placed at C-1 (δ 107.0). Compound **CR16** was then assigned as methyl-2-hydroxy-4-methoxy-6-(2-oxoheptyl)-benzoate. It is a new resorcyclic derivative.

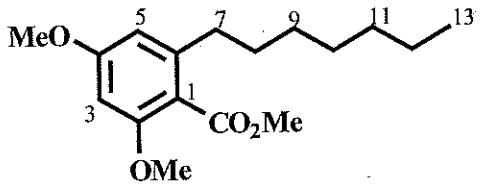


NOESY and HMBC correlations of **CR16**

Table 45. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR16** ($CDCl_3$)

Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
1	-	104.4 (C)	-
2	11.27 (*s*)	167.0 (C)	C-1,C-2, C-3
3	6.45 (*d*, *J* = 2.0 Hz)	97.0 (CH)	C-1, C-2, C-3, C-4
4	-	164.0 (C)	-
5	6.32 (*d*, *J* = 2.0 Hz)	110.0 (CH)	C-1, C-3, C-7
6	-	140.0 (C)	-
7	4.06 (*s*)	48.9 (CH_2)	C-1, C-5, C-6, C-8
8	-	205.0 (C=O)	-
9	2.37 (*t*, *J* = 7.5 Hz)	41.9 (CH_2)	-
10	1.42 (*m*)	-	-
11	1.20 (*m*)	29.7 (CH_2)	-
12	1.10 (*m*)	29.7 (CH_2)	-
13	0.86 (*t*, *J* = 6.9 Hz)	-	-
4-OMe	3.84 (*s*)	55.4 (CH_3)	C-4
CO_2Me	3.83(*s*)	62.0 (CH_3)	C-14

CR17: methyl 2,4-dimethoxy-6-heptyl-benzoate

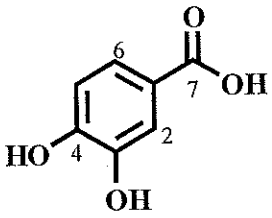


CR17 was obtained as amorphous powder. The ^{1}H NMR spectrum (**Table 46**) showed the resonances of *meta*-aromatic protons at δ 6.30 (*d*, 2.10, H-3), 6.28 (*d*, 2.10, H-5), two methoxyl group at δ 3.86 (2-OMe), δ 3.84 (4-OMe), a methyl ester group at δ 3.83 (1-CO_2Me), and a heptyl group. The heptyl group was deduced from the resonances at δ 2.47 (H-7), 1.69 (H-8), 1.42 (H-9), 1.20 (H-10/11), 1.10 (H-12), 0.86 (H-13). The DEPT 135° showed six methylene carbons δ 33.9, 30.4x2, 31.7x2, 22.4 and a methyl carbon 14.0. From the above mentioned data and comparing these data with those previously reported, it was clear that **CR17** was methyl 2,4-dimethoxy-6-heptyl-benzoate (Torger, B.,1965).

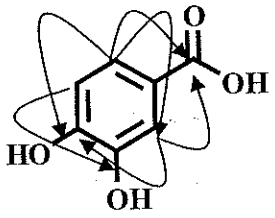
Table 46. ^{1}H NMR spectroscopic data of **CR17** ($CDCl_3$)

Position	**δ_H (multiplicity, *J*)**
3	6.30 (*d*, *J* = 2.0 Hz)
5	6.28 (*d*, *J* = 2.0 Hz)
7	2.47 (*t*, *J* = 7.6 Hz)
8	1.69 (*m*)
9	1.42 (*m*)
10/11	1.20 (*m*)
12	1.10 (*m*)
13	0.86 (*t*)
2-OMe	3.86 (*s*)
4-OMe	3.84 (*s*)
1-CO_2Me	3.83 (*s*)

CR25: 3, 4-dihydroxy benzoic acid



CR25 was obtained as brown gum. The UV spectrum showed maximum absorption at λ_{max} 268 and 276 nm. Its IR spectrum showed a C=O stretching absorption bands at 1678 cm^{-1} and O-H stretching at 3564. The ^{1}H NMR spectrum (**Table 47**) displayed signals for three aromatic protons of a 1,2,4-trisubstituted benzene (δ 7.49, *d*, J = 1.2 Hz; δ 6.84, *d*, J = 5.1 Hz; δ 7.56, *dd*, J = 5.1, 1.2 Hz). According to their multiplicity and coupling constants values, the aromatic proton resonating at δ 7.49, 6.84 and 7.56 were attributed to H-2, H-5 and H-6, respectively. The ^{13}C NMR spectrum showed signals assigned to a carbonyl carbon of a carboxylic acid at δ 168.6. The HMBC correlations of H-2 and H-6 to C-7 indicated that both aromatic protons were *ortho* to the carbonyl group. The aromatic proton H-5 correlated to C-1 and C-3, confirming the proton H-5 *meta* to carbonyl group. According to the carbon chemical shifts, the hydroxyl group then was substituted at C-4 (δ 150.4). Therefore, **CR25** was identified as 3, 4-dihydroxy benzoic acid (Yin *et al.*, 2004).

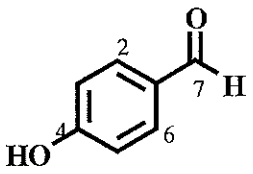


Major HMBC correlations of **CR25**

Table 47. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR25** (DMSO-d_6)

Positions	**δ_H (multiplicity, *J*)**	**δ_C (C-Type)**	**HMBC**
1	-	122.0 (C)	-
2	7.49 (*d*, J = 1.2 Hz)	112.3 (CH)	C-3, C-4, C-6, C-7
3	-	146.5 (C)	-
4	-	150.4 (C)	-
5	6.84 (*d*, J =5.1 Hz)	114.5 (CH)	C-1, C-3, C-4
6	7.56 (*dd*, J = 5.1, 1.2 Hz)	124.5 (CH)	C-2, C-4, C-7
7	-	168.6 (C=O)	-

CR37: 4-hydroxy benzaldehyde

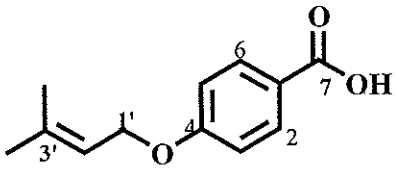


CR37 was obtained as a colorless gum. Its ^{1}H NMR spectrum (**Table 48**) showed characteristic signal of a *para*-disubstituted benzene at δ 7.81 (*d*, J = 8.4 Hz, H-2/H-6) and δ 6.96 (*d*, J = 8.4 Hz, H-3/H-5) and a formyl group at δ 9.88. This spectrum was found to be identical to that of 4-hydroxybenzaldehyde (in appendix), the authentic sample in our laboratory, from *Arthocarpus elasticus*. It also showed the same TLC character as that of 4-hydroxybenzaldehyde. Thus, **CR37** was 4-hydroxybenzaldehyde (Yin *et al.*, 2004).

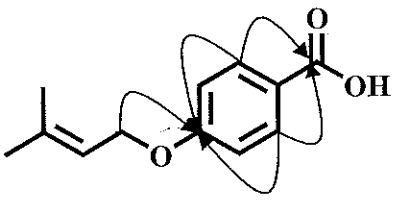
Table 48. ^{1}H NMR of **CR37** ($CDCl_3$)

Positions	**δ_H (multiplicity, J)**
2/6	7.81 (*d*, J = 8.4 Hz)
3/5	6.69 (*d*, J = 8.4 Hz)
7	9.88 (*s*)

CR40: valencic acid



CR40 was isolated as a white solid, m.p. 189-190°C. The ^{1}H NMR spectroscopic data **(Table 50)** of **CR40** showed the characteristic signals of a *para*-disubstituted benzene at δ 8.04 (2H, *d*, 8.9) and δ 6.94 (2H, *d*, 8.9) of H-2/H-6 and H-3/H-5 respectively. The substituent at C-4 was identified for an oxyprenyl group according to signals of methyl singlet signals at δ 1.75 and 1.80, a doublet of methylene protons at δ 4.57 (6.7 Hz, H-1') and a triplet of methine proton at δ 5.48 (6.7 Hz, H-2'). It was confirmed at C-4 due to HMBC correlations of H_2-1' (δ 4.57) to δ163.3 (C-4), 118.9 (C-2') and 138.8 (C-3'), whereas carboxyl group at C-1 was confirmed by HMBC correlations of H-6 (δ 8.04) to δ 171.6 (C-7), 121.6 (C-1), 114.3 (C-5) and 163.3 (C-4).The ^{13}C NMR spectral data **(Table** 47) exhibited 10 carbon signals including four aromatic carbons [δ 121.6 (C-1), 132.2 (C-2/C-6), 114.3 (C-3/C-5), 163.3 (C-4)] five carbons of oxyprenyl group [δ 18.2 (C-5'), 25.8 (C-4'), 65.0 (C-1'), 118.9 (C-2'), 138.8 (C-3')]. A signal of a carboxyl carbon was shown at δ 171.6 (C-7). Accordingly, the structure of **CR40** was assigned as valencic acid (Ito *et al.*, 1988).

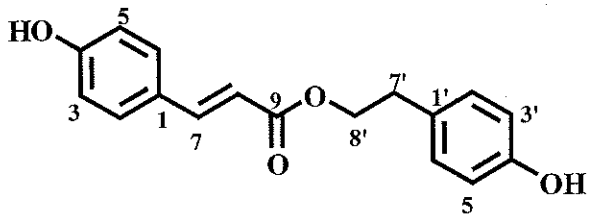


Selected HMBC correlations of **CR40**

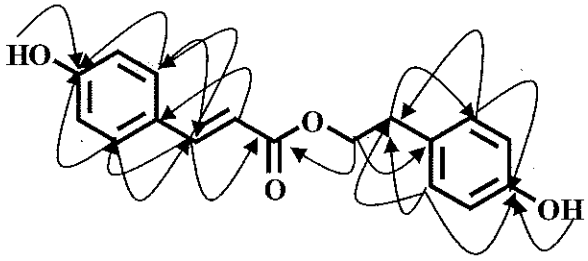
Table 49. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR40** ($CDCl_3$)

Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
1	-	121.6 (C)	-
2/6	8.04 (*d*, *J* = 8.9 Hz)	132.2 (CH)	C-1, C-4, C-5, C-7
3/5	6.94 (*d*, *J* = 8.9 Hz)	114.3 (CH)	C-1, C-2
4	-	163.3 (C)	-
7	-	171.6 (C=O)	-
1'	4.57 (*d*, *J* = 6.7 Hz)	65.0 (CH_2)	C-4, C-2', C-3'
2'	5.48 (*t*, *J* = 6.7 Hz)	118.9 (CH)	C-1', C-3', C-4', C-5'
3'	-	138.8 (C)	-
4'	1.80 (*s*)	25.8 (CH_3)	C-2', C-3', C-5'
5'	1.75 (*s*)	18.2 (CH_3)	C-2', C-3', C-4'

CR41: *p*-hydroxyphenylethyl-*p*-coumarate



CR41 was obtained as a white solid. The ^{13}C NMR spectrum showed carbons of α,β-unsaturated carbonyl ester at δ 144.4 (C-7), δ 123.2 (C-8) and δ 171.3 (C-9) The ^{1}H-NMR spectrum (**Table 50**) displayed α, β-olefinic protons at δ 7.45 (*d*, 15.6, C-7) and δ 6.35 (*d*, 15.6, C-8), hydroxyl protons at δ 8.85 (4-OH) and 9.45 (4'-OH). The spectrum further showed signals of aromatic protons for two of *para*-disubstituted benzene ring. The first *para*-disubstituted benzene resonated at δ 7.34 (*d*, 8.4, H-2/H-6), δ 6.81 (*d*, 8.4, H-3/H-5), while the second ring resonated at δ 7.91 (*d*, 8.7, H-2'/H-6') and δ 7.05 (*d*, 8.7, H-3'/H-5'). The HMBC correlation of H-3/H-5 and H-8 to C-1 (δ 131.2) confirmed that α, β-olefinic protons connected to C-1. The resonances at δ 3.45 (*t*, 6.9) was assigned oxy-methylene proton H-8' which was coupled by methylene protons H-7' (δ 2.75, *t*, 6.9). The aromatic proton H-3'/H-5' and oxy-methylene proton H-8' showed correlation to C-1' (δ 134.6), confirmed that -OCH_2CH_2- connected to C-1'. Furthermore, H-8 and H-8' correlated to C-9 (δ 171.3) which was suggested an α, β-olefinic protons and -OCH_2CH_2- connected by carbonyl ester group. Therefore, **CR41** was assigned as *p*-hydroxyphenylethyl-*p*-coumarate (Kaewamatawong *et al.*, 2007)



Major HMBC correlations of **CR41**

Table 50. ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **CR41** ($CDCl_3$)

Position	δ_H (multiplicity, *J*)	δ_C (C-Type)	HMBC
1	-	131.2 (C)	-
2/6	7.34 (*d*, *J* = 8.4 Hz)	134.0 (CH)	C-4, C-7
3/5	6.81 (*d*, *J* = 8.4 Hz)	120.6 (CH)	C-1, C-2, C-6
4	-	163.7 (C)	-
7	7.45 (*d*, *J* = 15.6 Hz)	144.4 (CH)	C-1, C-2, C-6, C-9
8	6.35 (*d*, *J* = 15.6 Hz)	123.2 (CH)	C-1, C-9
9	-	171.3 (C=O)	-
1'	-	134.6 (C)	-
2'/4'	7.91(*d*, *J* = 8.7 Hz)	134.4 (CH)	C-4', C-7'
3'/5'	7.05 (*d*, *J* = 8.7 Hz)	120.2 (CH)	C-1', C-2', C-6'
6'	-	160.3 (C)	-
7'	2.75 (*t*, *J* = 6.9 Hz)	39.6 (CH_2)	C-1', C-2', C-6', C-9
8'	3.45 (*t*, *J* = 6.9 Hz)	45.9 (CH_2)	C-1', C-9
4-OH	8.85 (*s*)	-	C-4
4'-OH	9.45 (*s*)	-	C-4'

3.2 Antimicrobial activities of some of the isolated compounds from *Citrus reticulata*

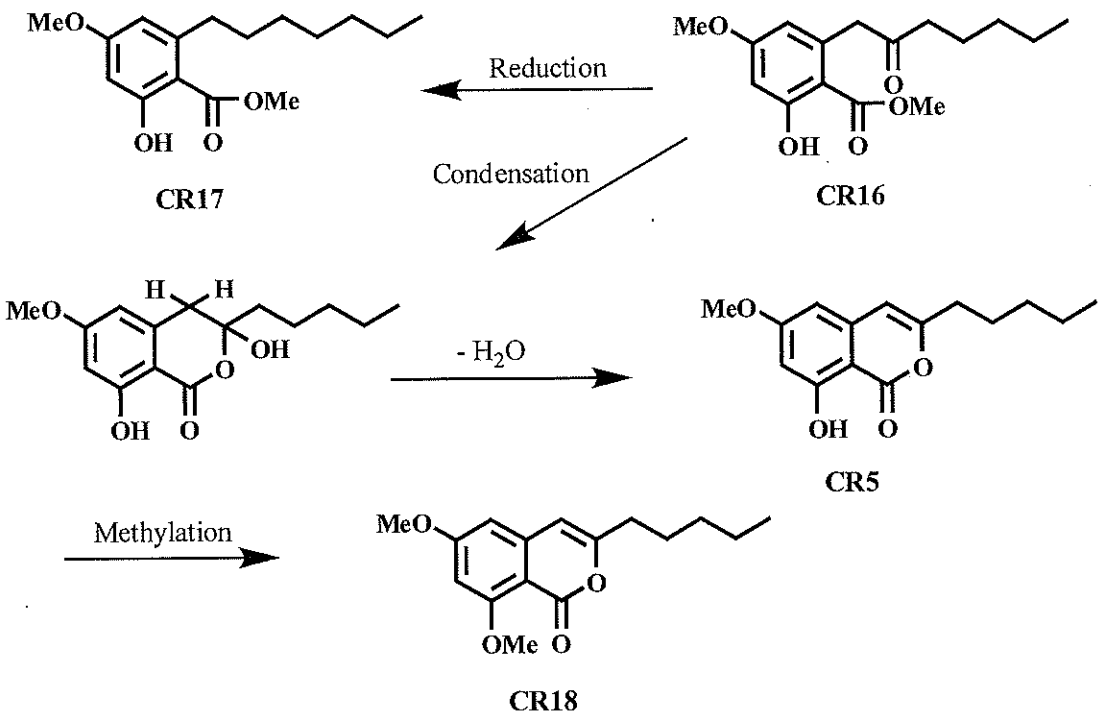
Compounds **CR1-CR4, CR6, CR7, CR9, CR13, CR20, CR21** and the extracts of the branch bark, peel, leaves and woods were tested for their antibacterial activity on *S. aureus* ATCC25923, methicillin-resistant *S. aureus* (MRSA) SK1 and *E. coli* ATCC25922, *P. aeruginosa* ATCC27853 and *C. neoformans* ATCC90113, antifungal activity on *C. albicans* NCPF3153, *C. neoforman* and *M. gypseum*. The extracts and pure compounds **CR1, CR2, CR4, CR6, CR7, CR9, CR13, R20, CR21** had no effect on these microorganisms up to a dose of 200 μg/mL. Only **CR3** inhibited the growth of *S. aureus* ATCC25923 and MRSA SK1with MIC values of 64 and 64 μg/mL.

3.3 Cytotoxic activities of some of the isolated compounds from *Citrus reticulata*

Compounds **CR1, CR2, CR3, CR5-CR9, CR13, CR14, CR20-CR22, CR36** and **CR37** were evaluated for cytotoxicity against cell lines A431, SKBR-3, T47D and AU565. Compounds **CR1, CR5, CR6, CR7, CR13** and **CR36** affected the growth of cell lines with IC_{50} values of less than 100 μg/mL. Compound **CR1** was toxic to SKBR-3 with an IC_{50} value of 90.32 μg/mL. Compound **CR5** inhibited the growth of T47D and SKBR-3 with IC_{50} values of 59.57, and 45.02 μg/mL, respectively. Compound **CR6** inhibited the growth of SKBR-3 with IC_{50} values of 48.54 μg/mL. Compound **CR7** affected the growth of AU565, T47D and SKBR-3 with IC_{50} values of 77.32, 66.20 and 39.39 μg/mL, respectively. Compound **CR12** inhibited the growth of A431 with an IC_{50} values of 83.47 μg/mL. Compound **CR36** affected the growth of T47D and SKBR-3 with IC_{50} values of 40.04, and 15.00 μg/mL, respectively. Compounds **CR2, CR3, CR8, CR9, CR14, CR20-CR22** and **CR37** showed no cytotoxicity against the tested cancer cell lines up to the final concentration of 100 μg/mL.

3.4 The structure relationship isocoumarins and resorcyclic derivatives

The biosynthetic pathway of **CR4, CR16, CR17 and CR18** can be proposed. Reduction of carbonyl carbon (C-8) of methyl-2-hydroxy-4-methoxy-6-(2-oxoheptyl)-benzoate **CR16** provide methyl 2,4-dimethoxy-6-heptyl-benzoate **CR17**. Condensation of **CR16** and then dehydration afford 8-hydroxy-6-methoxypentyl-isocoumarin **CR5**. Methylation at 8-OH position of **CR5** gave 6,8-dimethoxy-pentylisocoumarin **CR18**

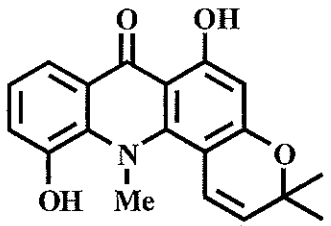


CHAPTER 4

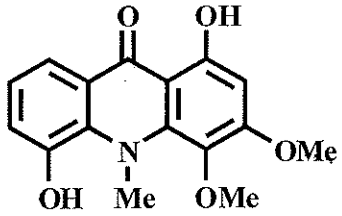
CONCLUSION

With the aim of studying of the chemical constituents of the branch bark, leaves, peels and wood of *Citrus reticulata* Blanco resulted in the isolation of forty three compounds. We found that the branch bark was a source of acridones (**CR2**, **CR6**, **CR8**, **CR9**, **CR10**, **CR14**, **CR19**). Acridones were also found in the woods (**CR8, CR43**). Polymethoxyflavonoids were found in the peels (**CR20-CR26**) and leaves (**CR20-CR22, CR34-CR35**). The high polar fractions from the peels contain flavonoids glycosides (**CR28, CR29, CR30**). Apart from those compounds, flavonol (**CR7**), flavanones (**CR3, CR31, CR32**), coumarins (**CR11**, **CR37**, **CR38**, **CR39**), coumarins glycoside (**CR27**) depsides (**CR1**, **CR4**, **CR12**), isocoumarins (**CR5**, **CR18**), resocyclic derivatives (**CR16**, **CR17**), limonoids (**CR13**, **CR42**), benzene derivatives (**CR15**, **CR24**, **CR37**, **CR40**), coumarate ester (**CR41**) and triterpenoid (**CR33**) were also isolated.

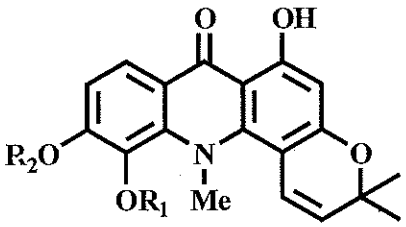
Acridones



CR2: 5-hydroxynoracronycine



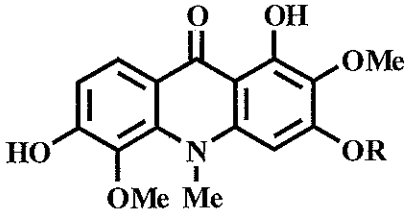
CR8: citrusinine-I



CR6: R_1 = Me, R_2 = H: citracridone-I

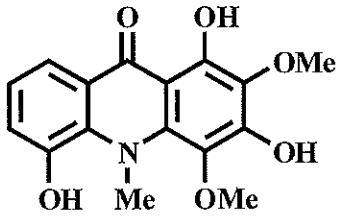
CR14: R_1 = H, R_2 = H: citracridone-III

CR19: R_1 = Me, R_2 = Me: citracridone-II



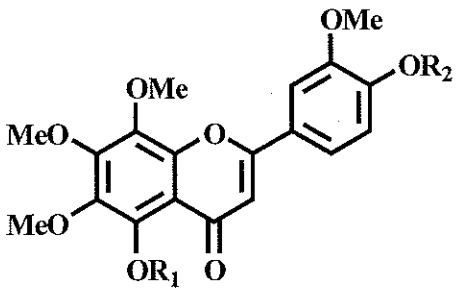
CR9: R = H: citramine

CR10: R = Me: 2-methoxycitpressine



CR43: 1,3,5-trihydroxy-2,4-dimethoxy-10-methyl-10H-acridin-9-one

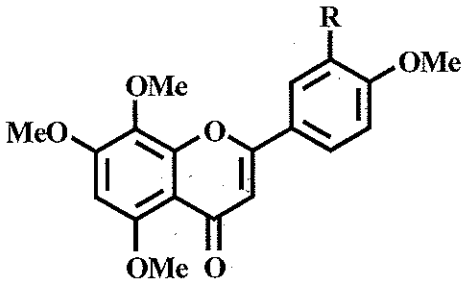
Flavonoids



CR20: R_1 = H, R_2 = Me: 5-demethoxynobiletin

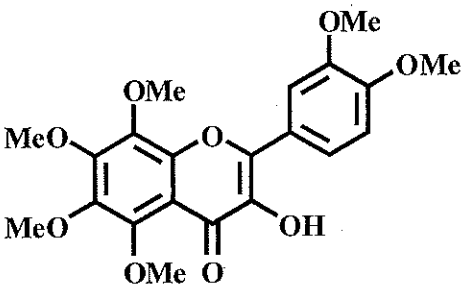
CR22: R_1 = Me, R_2 = Me: nobiletin

CR35: R_1 = H, R_2 = H: sudachitin

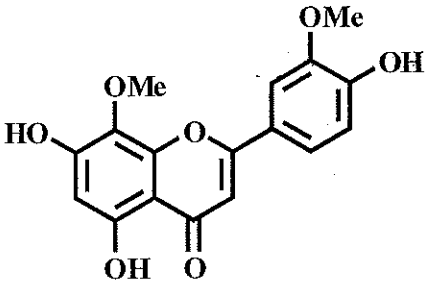


CR23: R = H: 5,7,8,4'-tetramethoxy-flavone

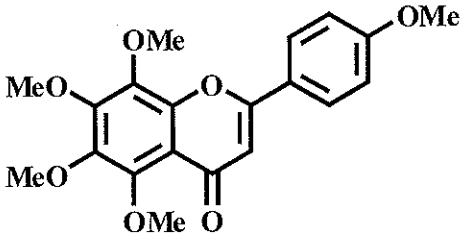
CR34: R = OMe: 5,7,8,3',4'-penta-methoxyflavone



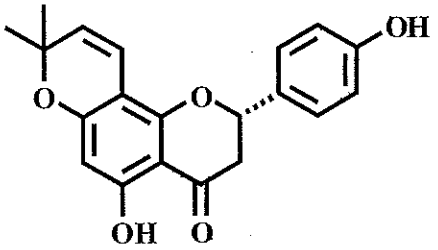
CR24: natsudaidain



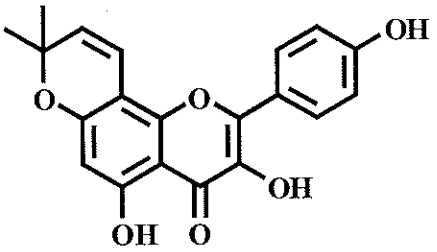
CR26: 5,7,4'-trihydroxy-3',8-dimethoxy-flavone



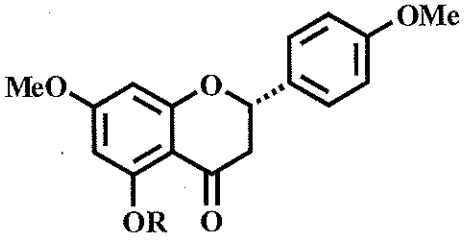
CR21: tangeretin



CR3: citflavanone



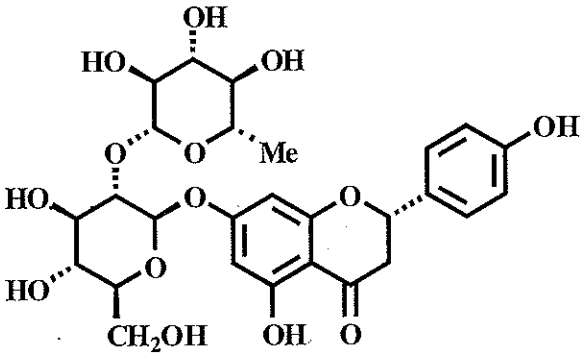
CR7: citrusinol



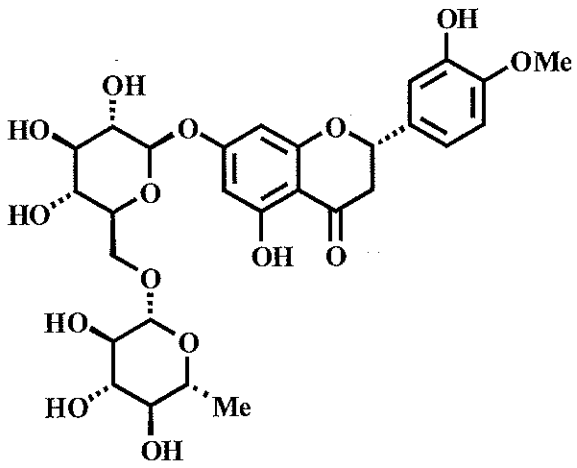
CR31: R = Me: naringenin trimethyl ether

CR32: R = H: 2,3-dihydro-5-hydroxy-4′,7-dimethoxy-flavanone

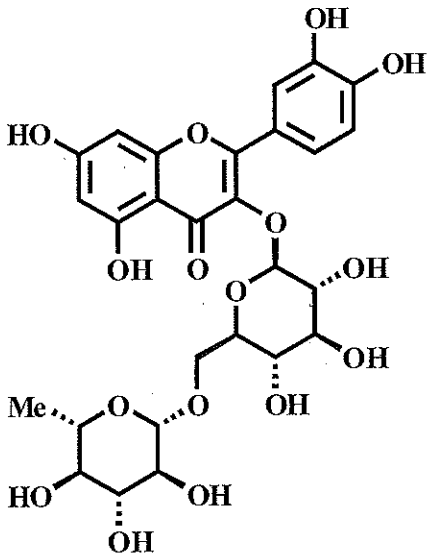
Flavonoids glycosides



CR29: naringin

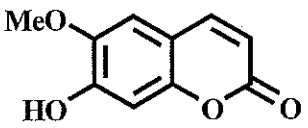


CR28: hesperidin

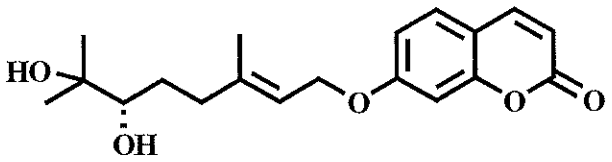


CR30: rutin

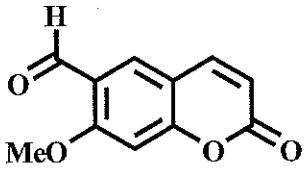
Coumarins



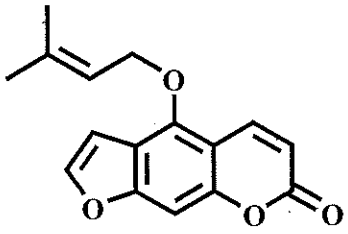
CR10: scopoletin



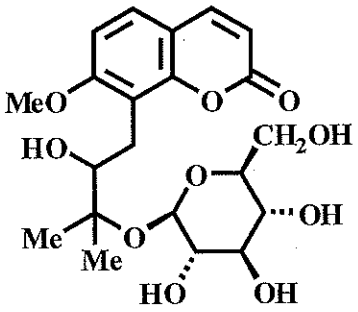
CR36: marmin



CR38: crenulatin

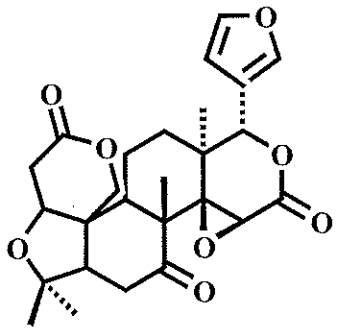


CR39: isoimperatorin

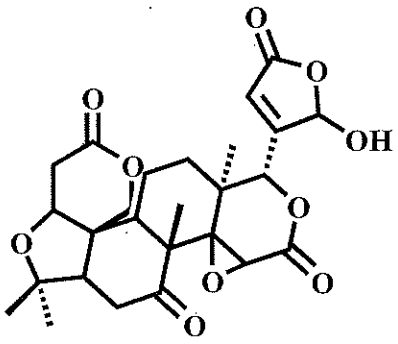


CR27: 8,3′-*β*-glucosyloxy-2′-hydroxy-3′-methylbutyl-7-methoxycoumarin

Limonoids

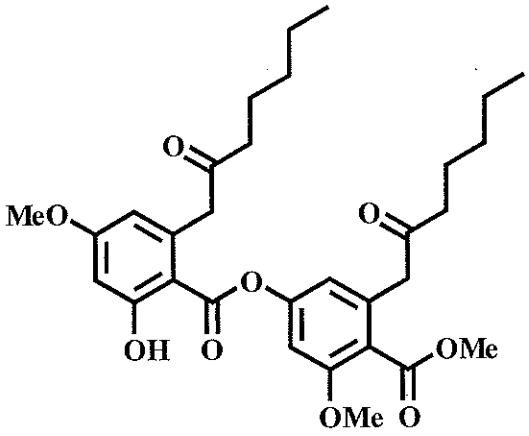


CR13: limonin

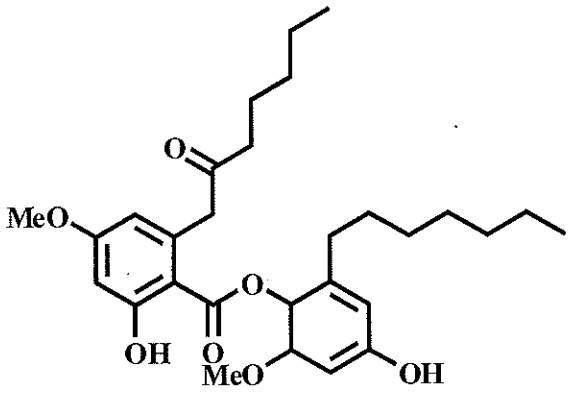


CR42: limonexic acid

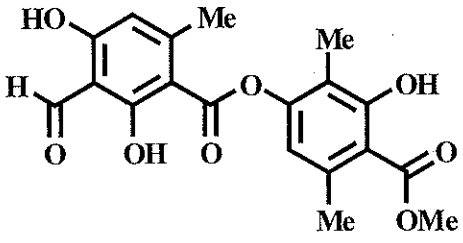
Depsides, isocoumarins and resorcyclic derivatives



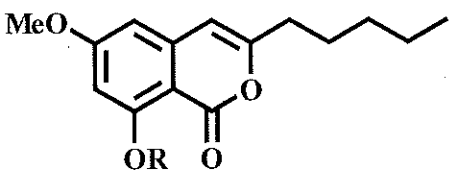
CR4: gustastatin



CR12: 7-hydroxy-4-methoxy-6-(2-oxo-heptyl)- 2'-methoxy-4'-hydroxy-6-(hetyl)-phenyl ester

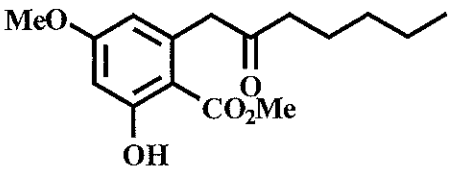


CR1: : atranorin

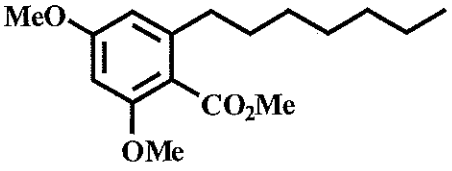


CR5: R = H: 8-hydroxy-6-methoxypentyl-isocoumarin

CR18: R = Me: 6,8-dimethoxypentyl-isocoumarin

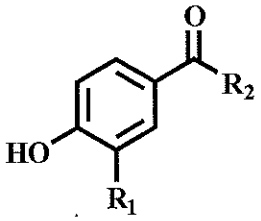


CR16: methyl-2-hydroxy-4-methoxy-6-(2-oxoheptyl)benzoate



CR17: methyl 2,4-dimethoxy-6-heptyl-benzoate

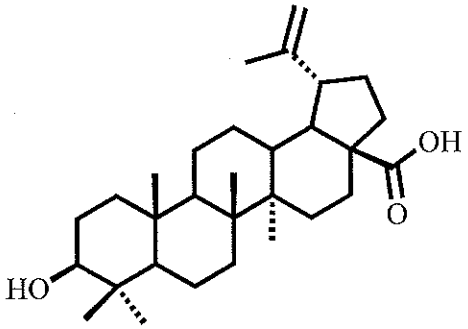
Benzene derivatives, coumarate esters and triterpeoid



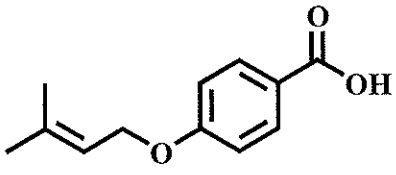
CR15: R_1 = H, R_2 = OH: 4-hydroxy-benzoic acid

CR25: R_1 = OH, R_2 = OH: 3,4-dihydroxybenzoic acid

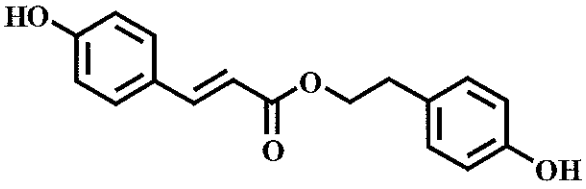
CR37: R_1 = H, R_2 = H: 4-hydroxy benzaldehyde



CR33: betulinic acid



CR40: valencic acid



CR41: *p*-hydroxyphenylethyl-*p*-coumarate

Biological activity of some pure compounds

Compounds **CR1-CR4, CR6, CR7, CR9, CR13, CR20, CR21** had no effect on *S. aureus* ATCC25923, methicillin-resistant *S. aureus* (MRSA) SK1 and *E. coli* ATCC25922, *P. aeruginosa* ATCC27853 and *C. neoformans* ATCC90113, antifungal activity on *C. albicans* NCPF3153, *C. neoforman* and *M. gypseum* up to a dose of 200 μg/mL. Only **CR3** inhibited the growth of *S. aureus* ATCC25923 and MRSA SK1with MIC values of 64 and 64 μg/mL. Compounds **CR1, CR5, CR6, CR7, CR13** and **CR36** affected the growth of cell lines A431, SKBR-3, T47D and AU565 with IC_{50} values of less than 100 μg/mL. Compounds **CR2, CR3, CR8, CR9, CR14, CR20-CR22** and **CR37** showed no cytotoxicity against the tested cancer cell lines up to the final concentration of 100 μg/mL.

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APPENDIX

^{1}H NMR spectrum of compounds CR1-CR43

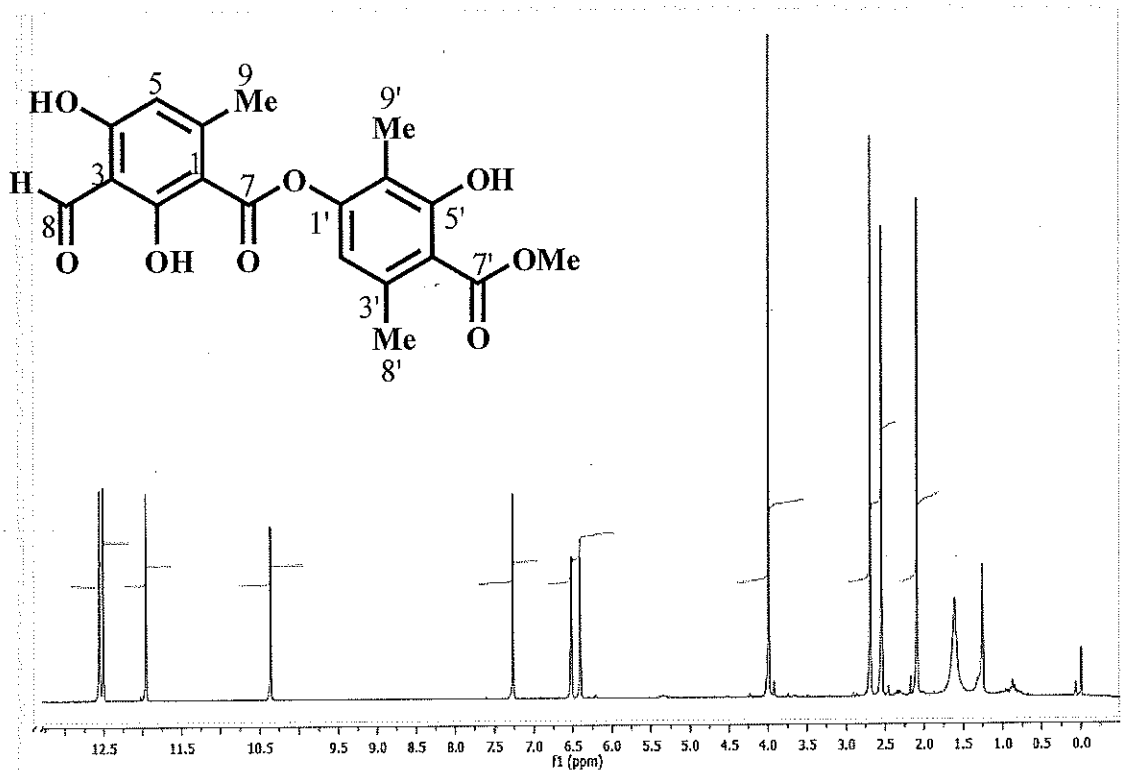


Figure A-1 ^{1}H NMR ($CDCl_3$+DMSO-d_6) spectrum of **CR1**

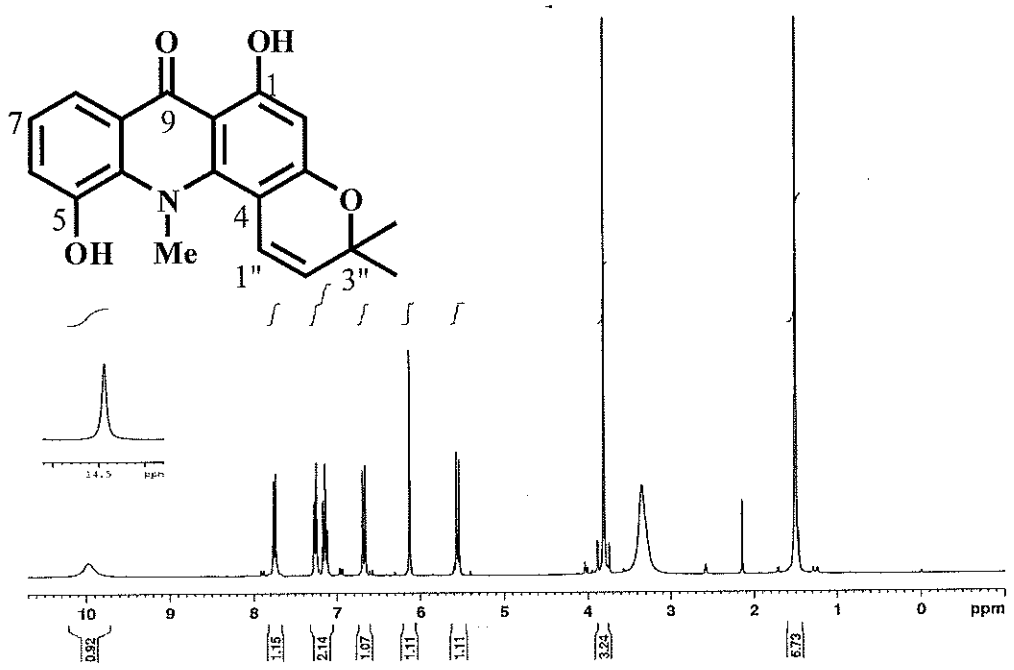


Figure A-2 ^{1}H NMR ($CDCl_3$+DMSO-d_6) spectrum of **CR2**

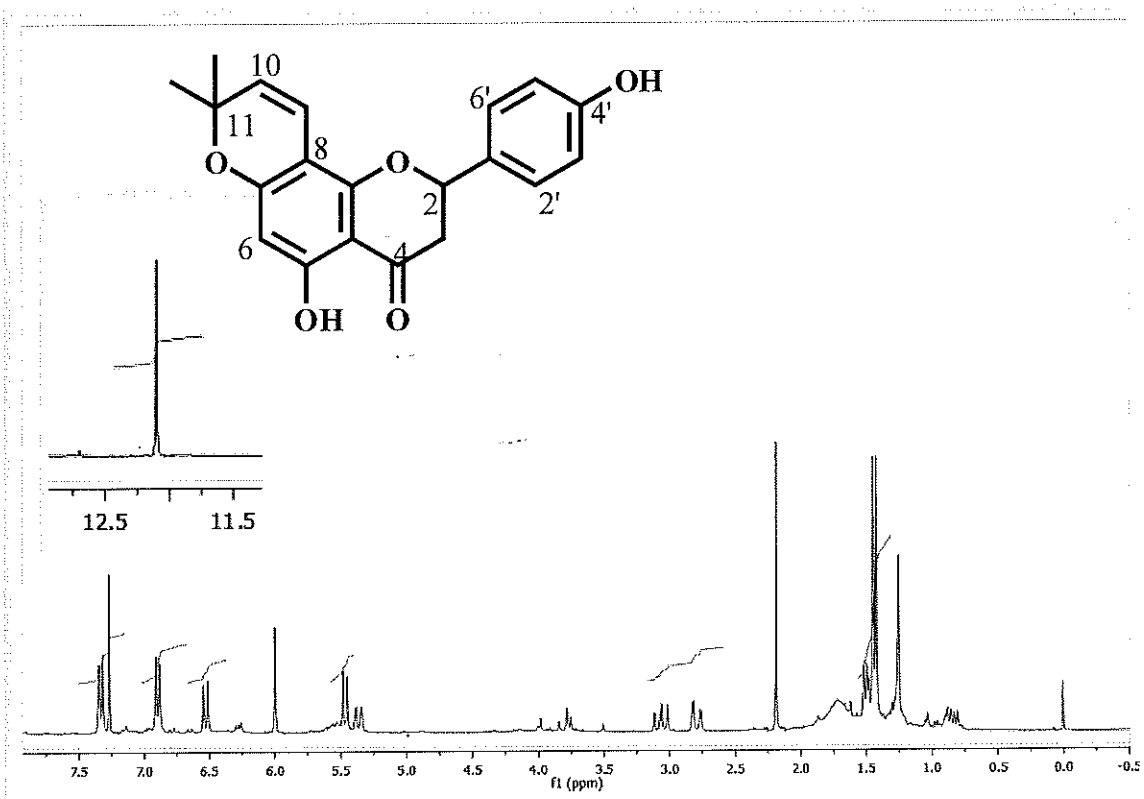
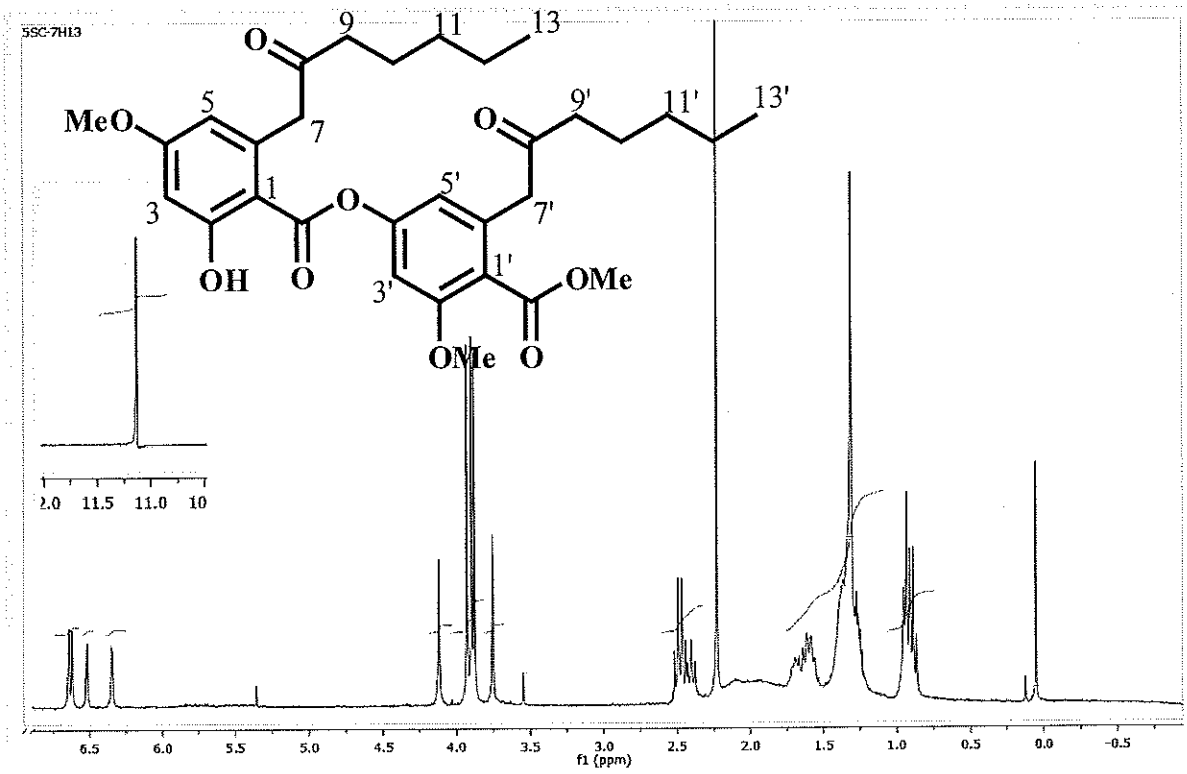


Figure A-3 ^{1}H NMR ($CDCl_3$) spectrum of **CR3**



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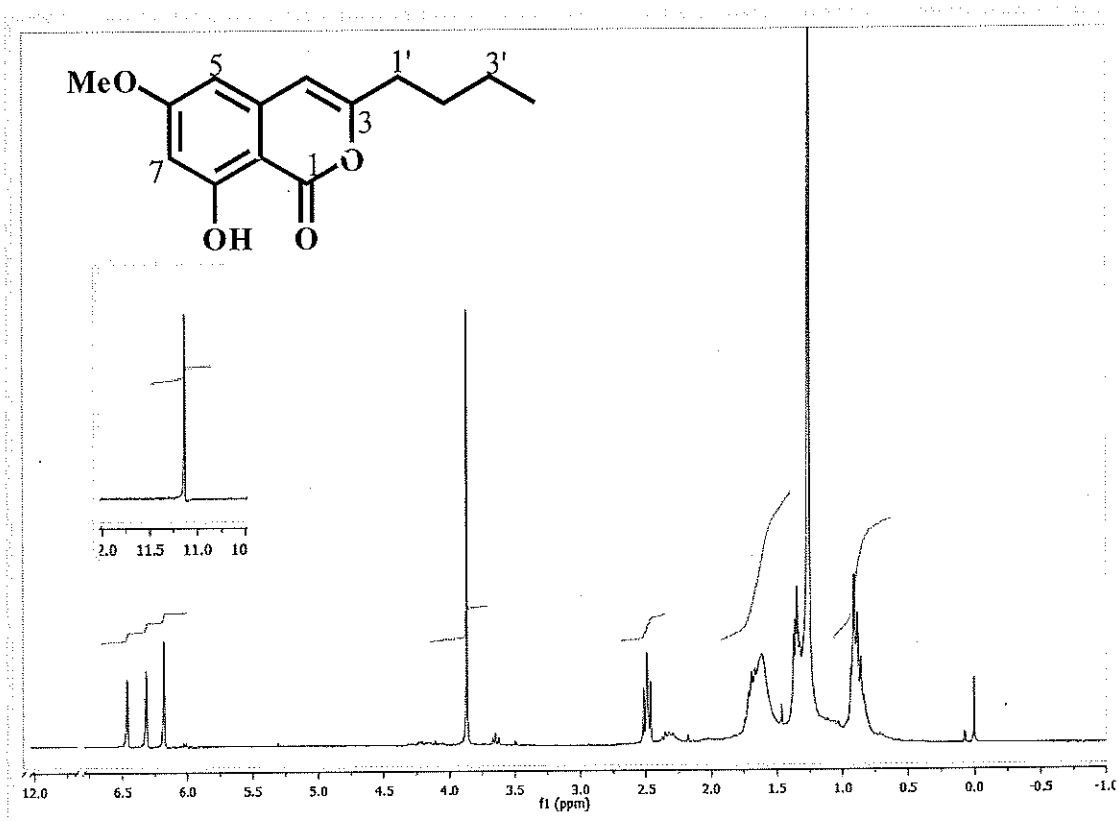

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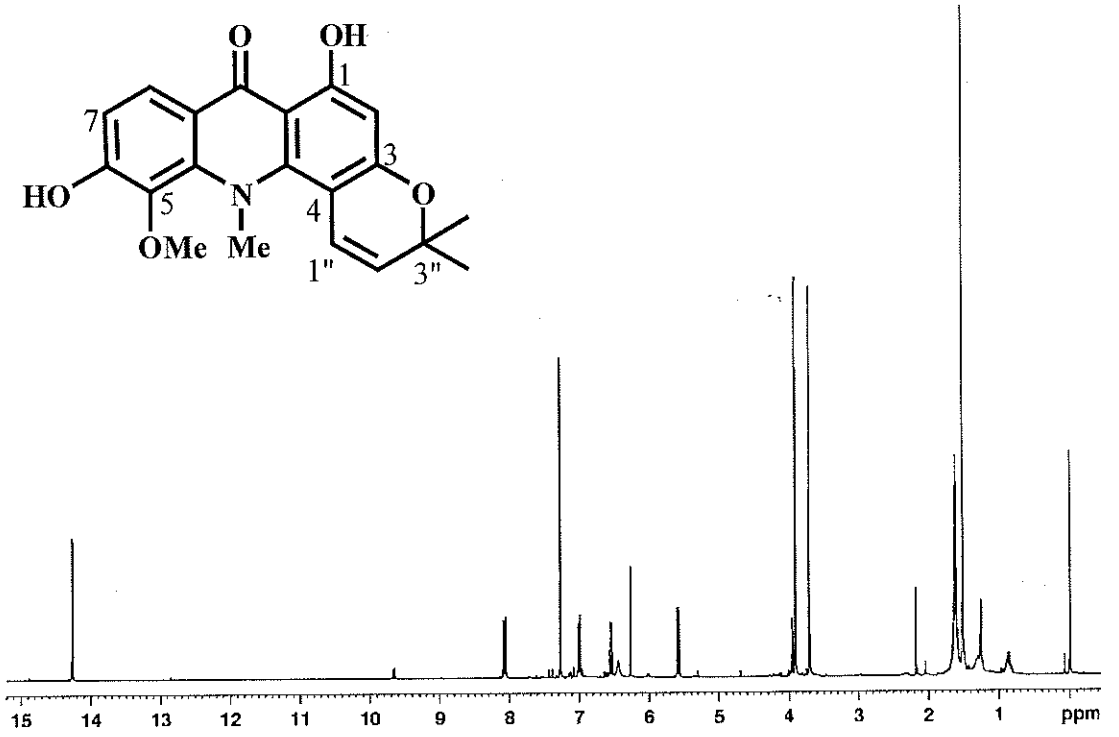

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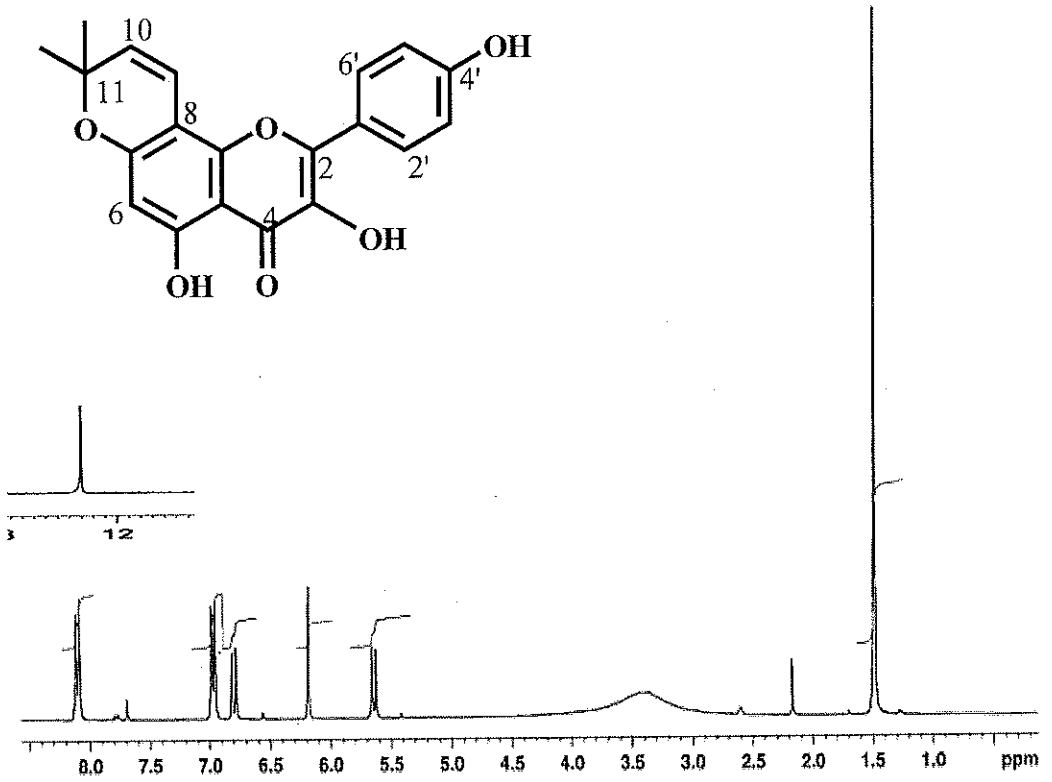

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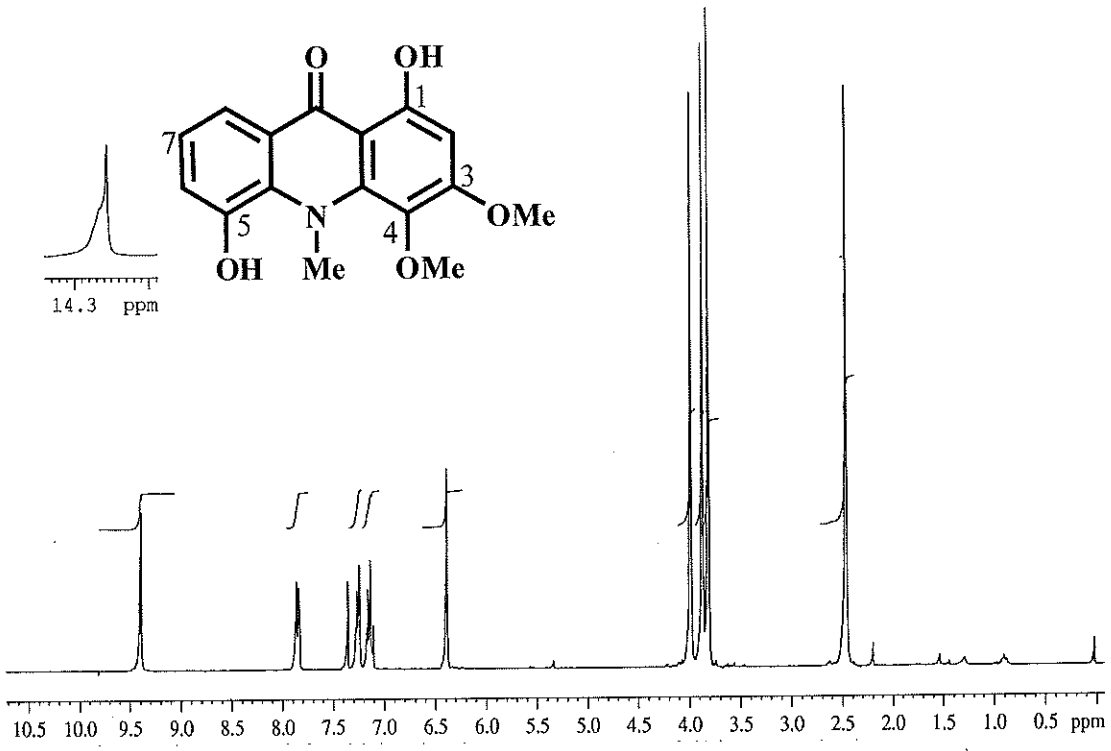


Figure A-8 ^{1}H NMR ($CDCl_3$+DMSO-d_6) spectrum of **CR8**

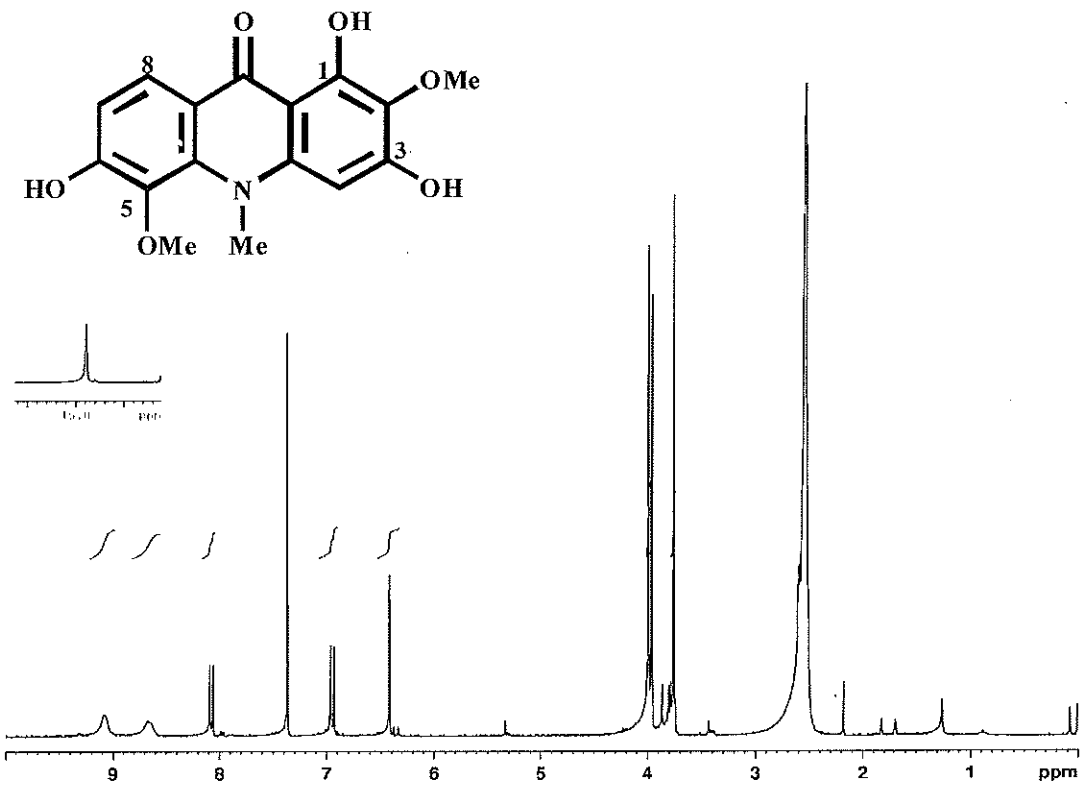


Figure A-9 ^{1}H NMR ($CDCl_3$+DMSO-d_6) spectrum of **CR9**

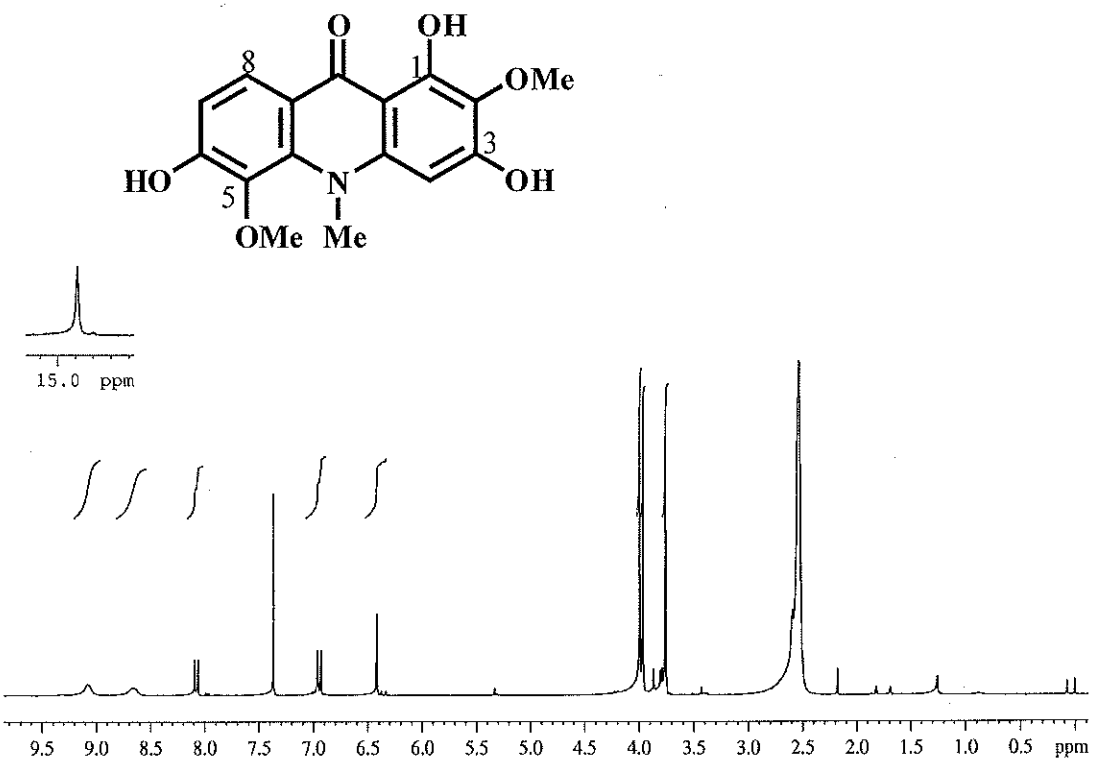


Figure A-10 ^{1}H NMR ($CDCl_3$) spectrum of **CR10**

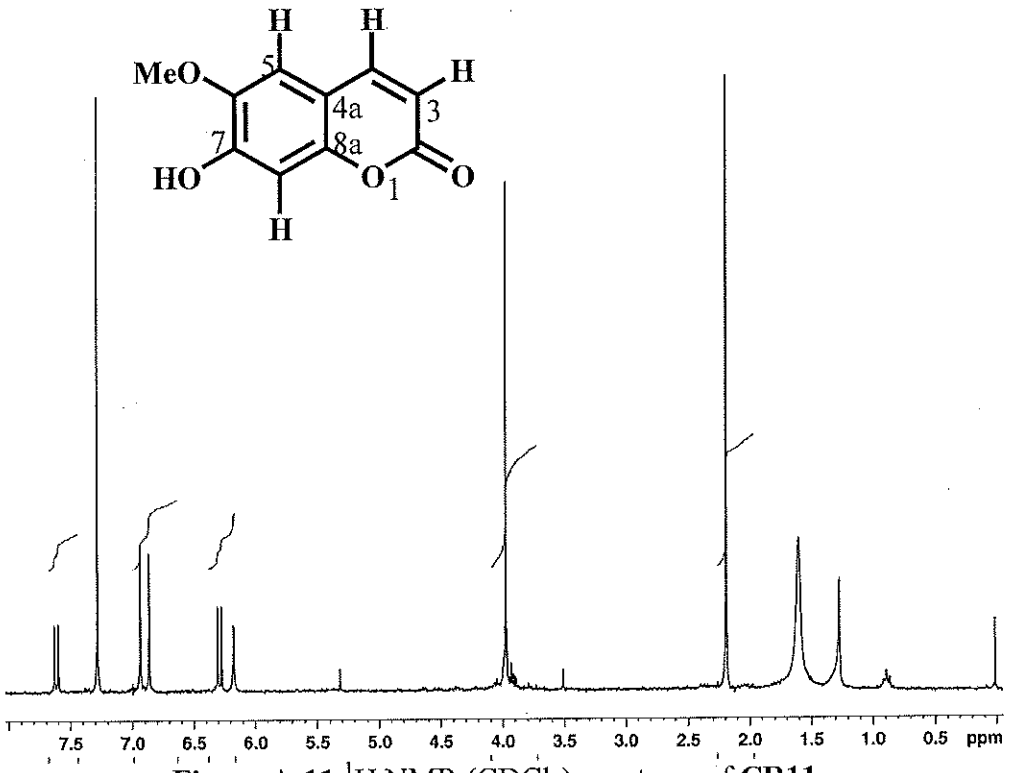


Figure A-11 ^{1}H NMR ($CDCl_3$) spectrum of **CR11**

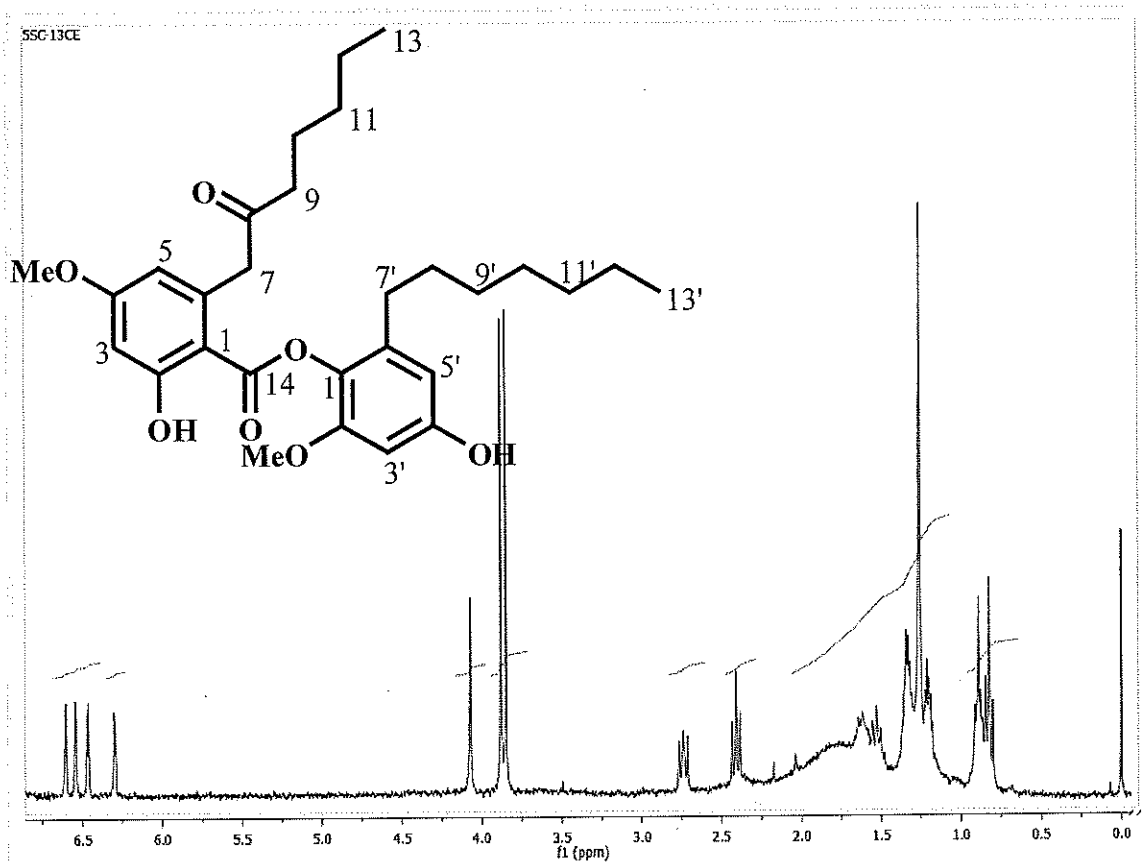


Figure A-12 ^{1}H NMR ($CDCl_3$) spectrum of **CR12**

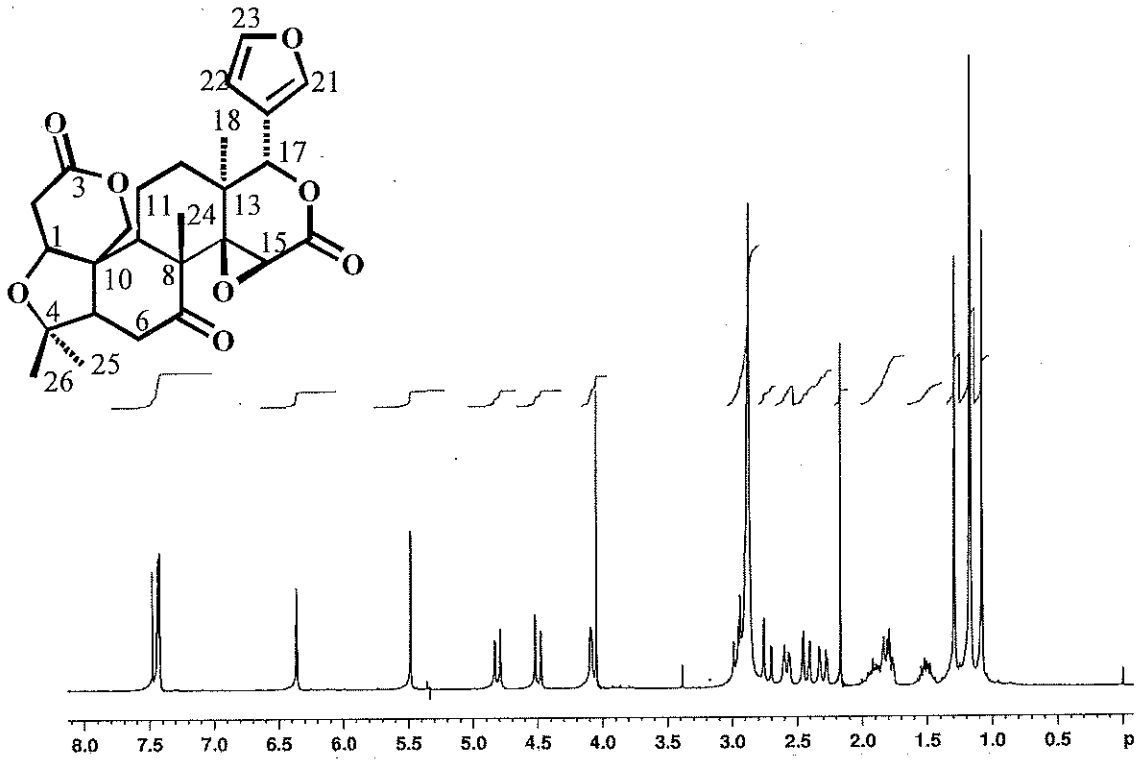


Figure A-13 ^{1}H NMR ($CDCl_3$+ DMDO-d_6) spectrum of **CR13**

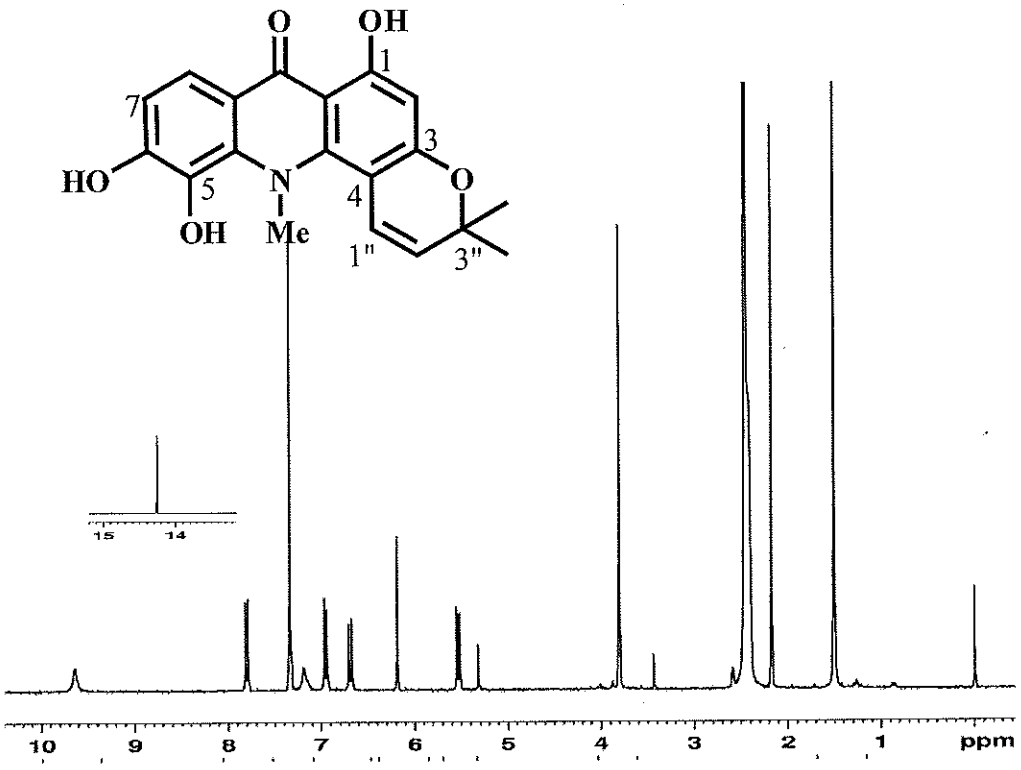


Figure A-14 ^{1}H NMR ($CDCl_3$+ DMDO-d_6) spectrum of **CR14**

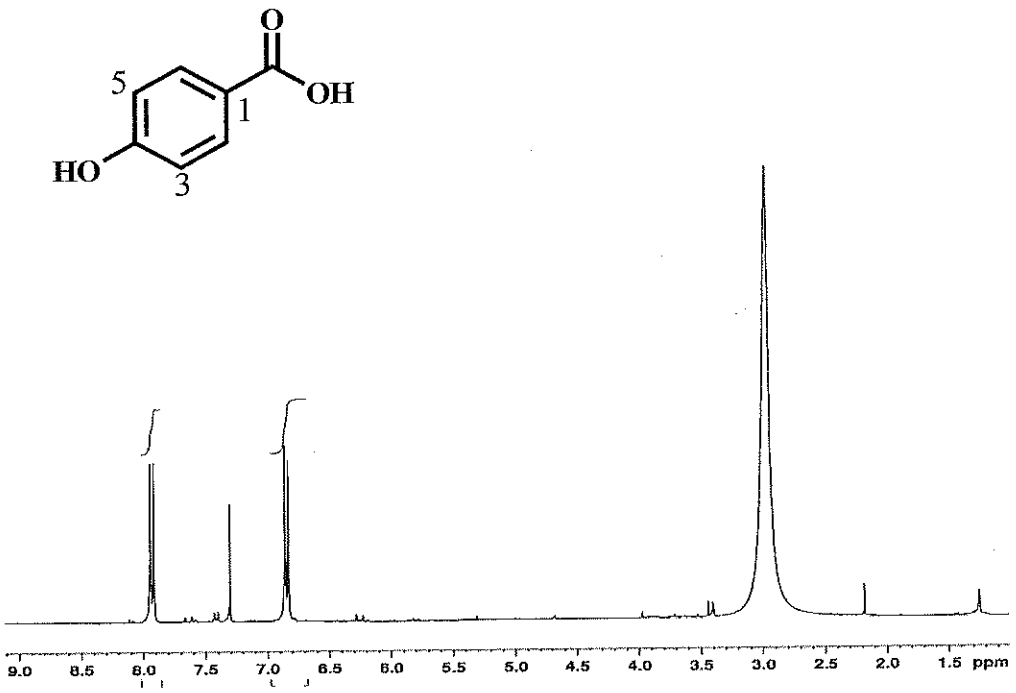


Figure A-15 ^{1}H NMR ($CDCl_3$) spectrum of **CR15**

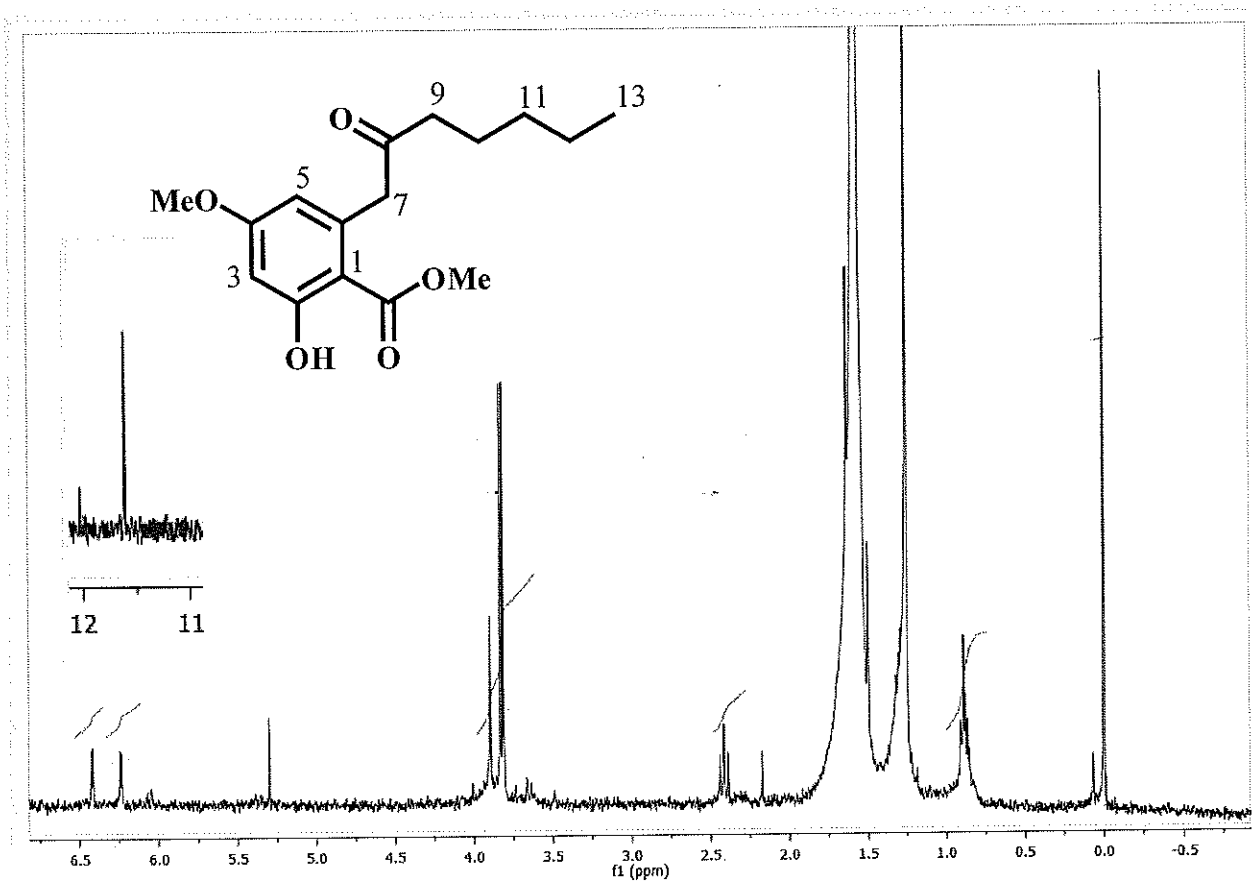


Figure A-16 ^{1}H NMR ($CDCl_3$) spectrum of **CR16**

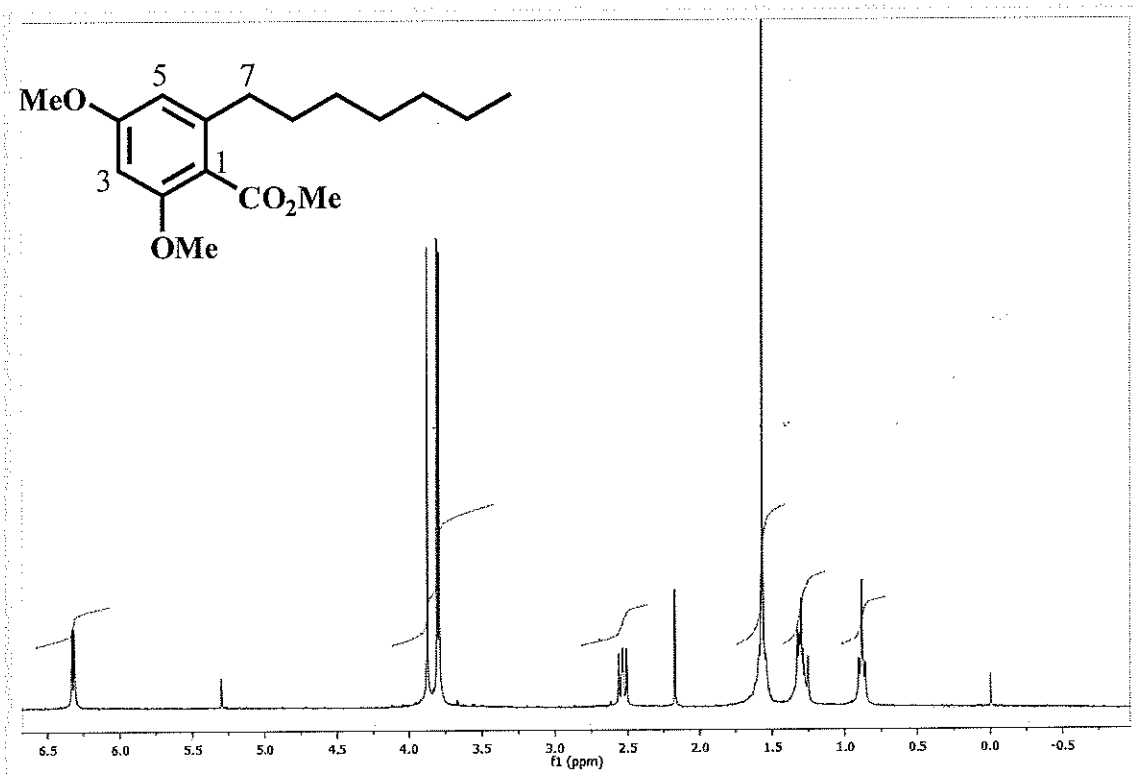


Figure A-17 ^{1}H NMR ($CDCl_3$) spectrum of **CR17**

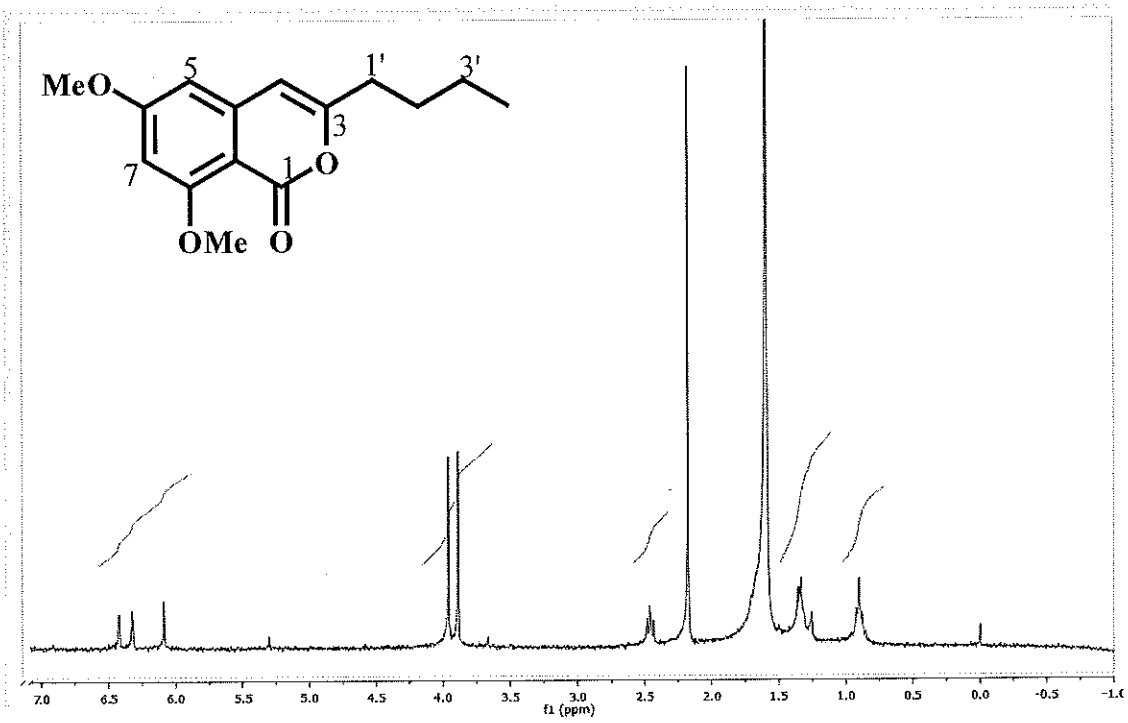
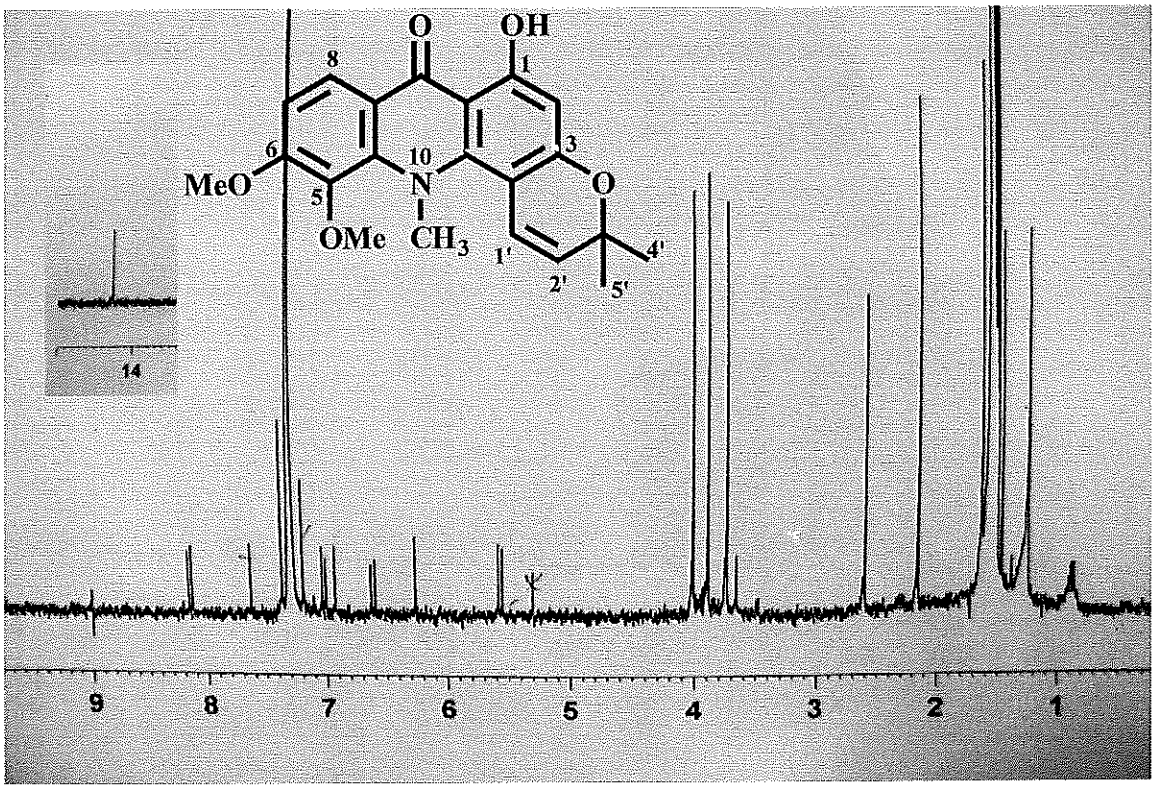


Figure A-18 ^{1}H NMR ($CDCl_3$) spectrum of **CR18**



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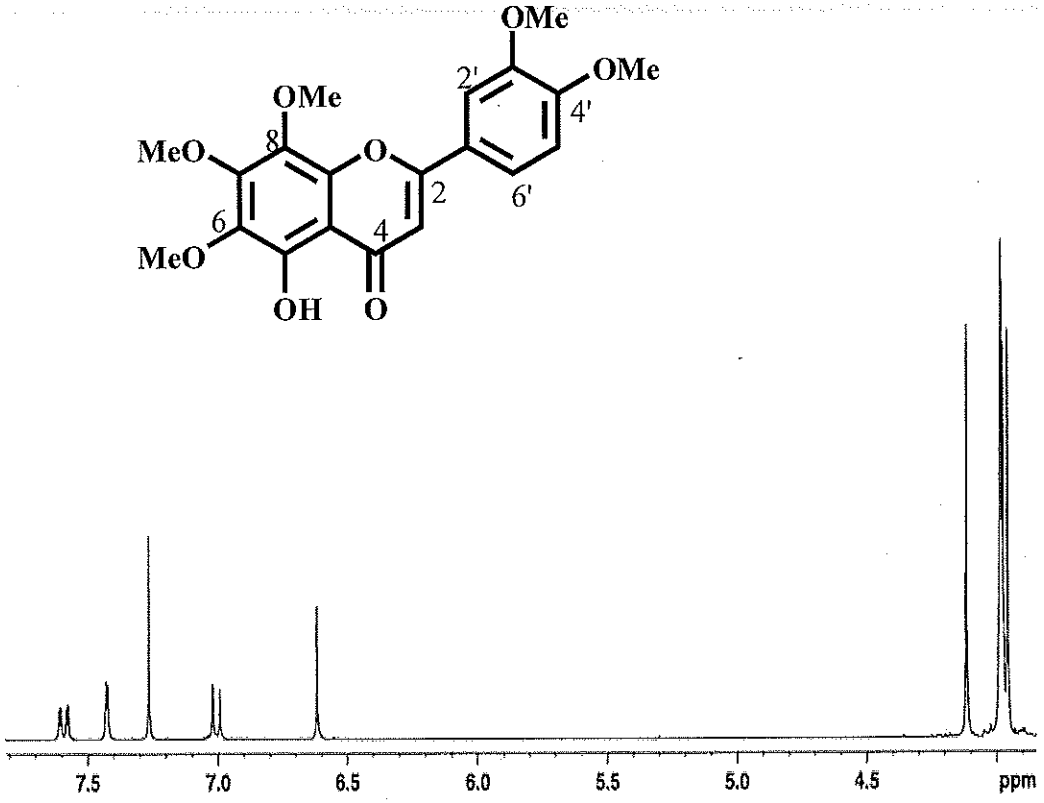

Figure A-20 ^{1}H NMR ($CDCl_3$) spectrum of **CR20**

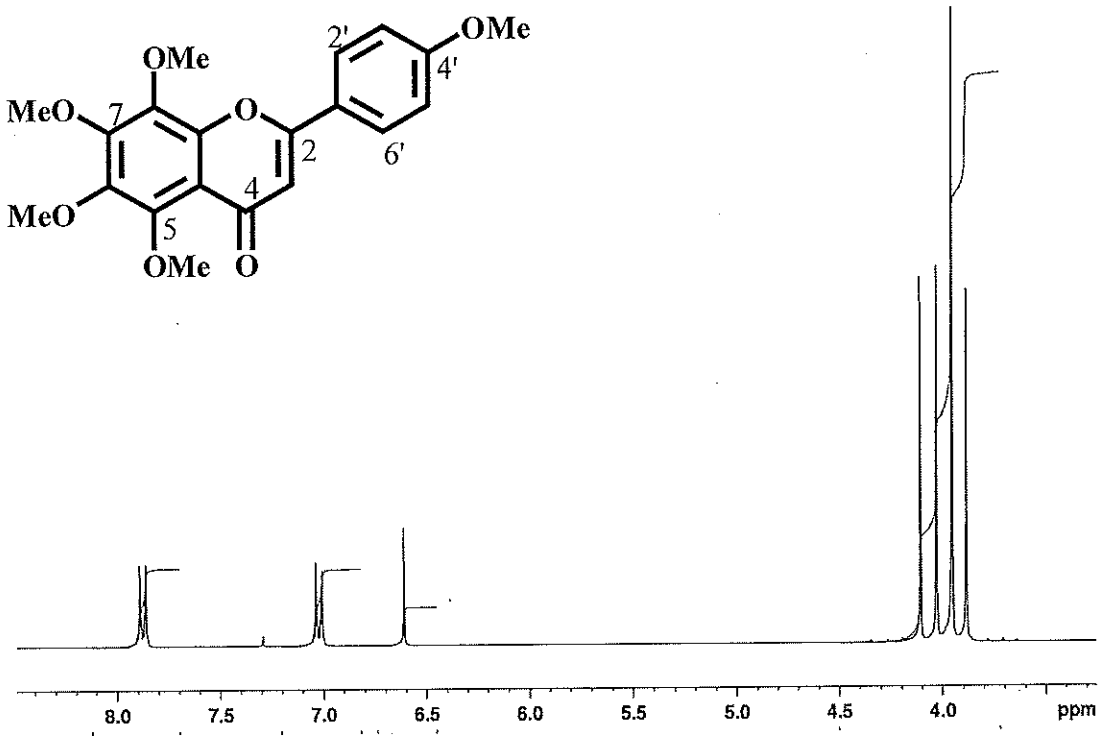

Figure A-21 ^{1}H NMR ($CDCl_3$) spectrum of **CR21**

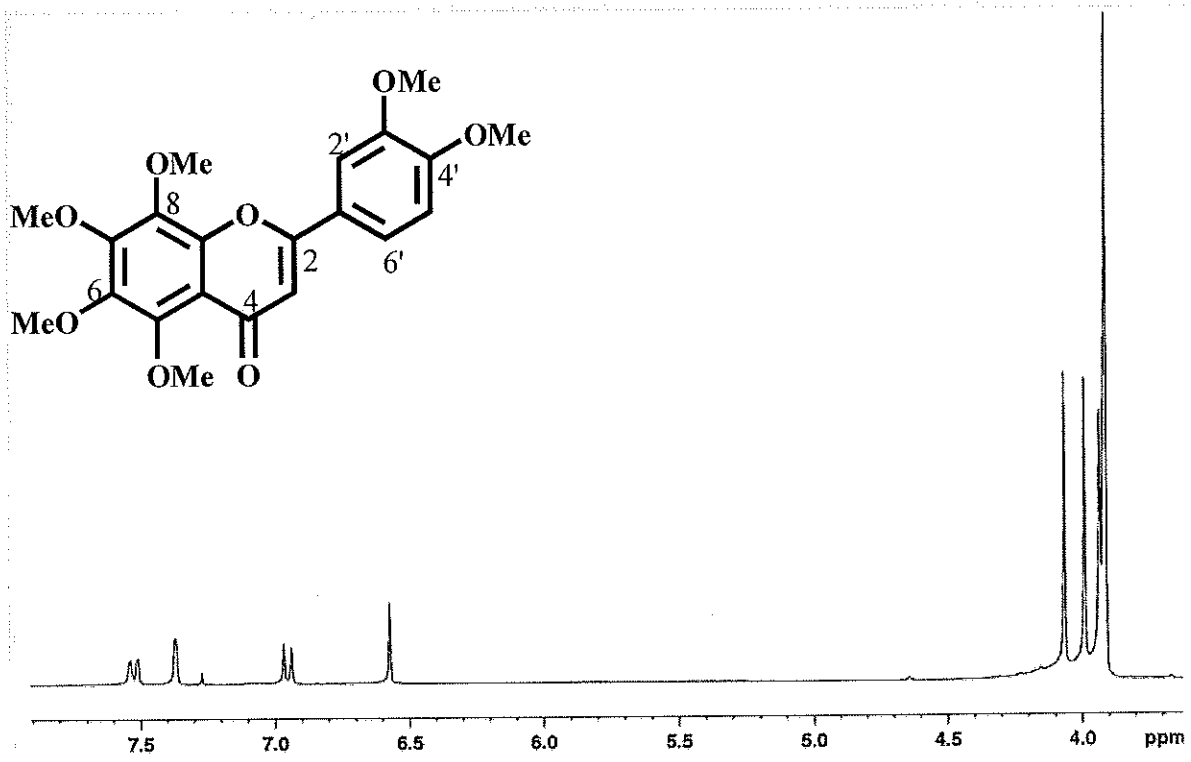

Figure A-22 ^{1}H NMR ($CDCl_3$) spectrum of **CR22**

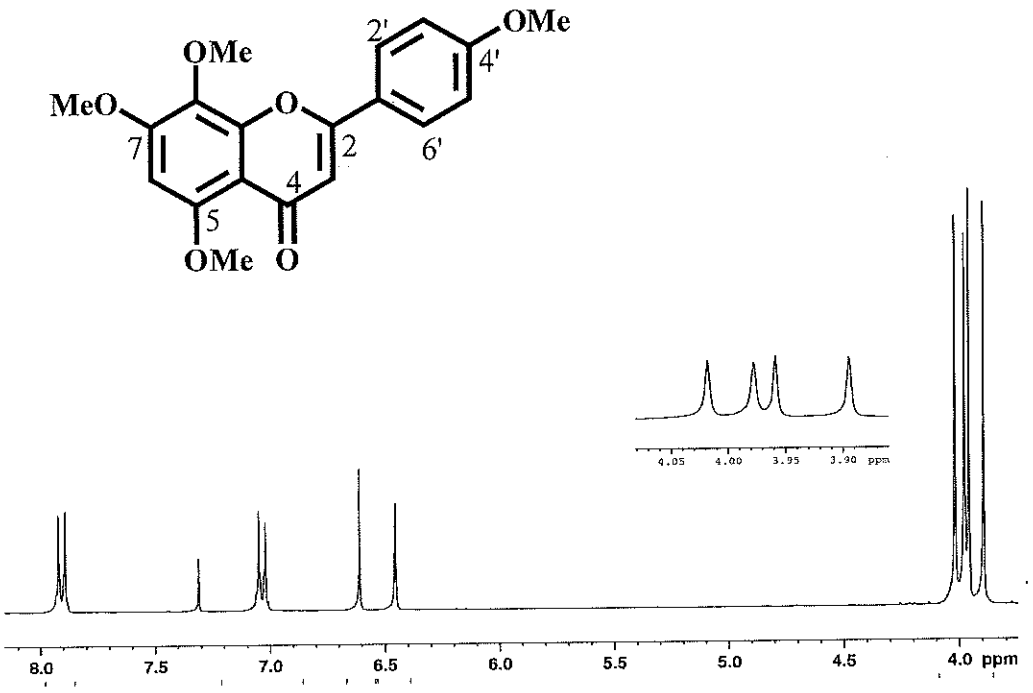

Figure A-23 ^{1}H NMR ($CDCl_3$) spectrum of **CR23**

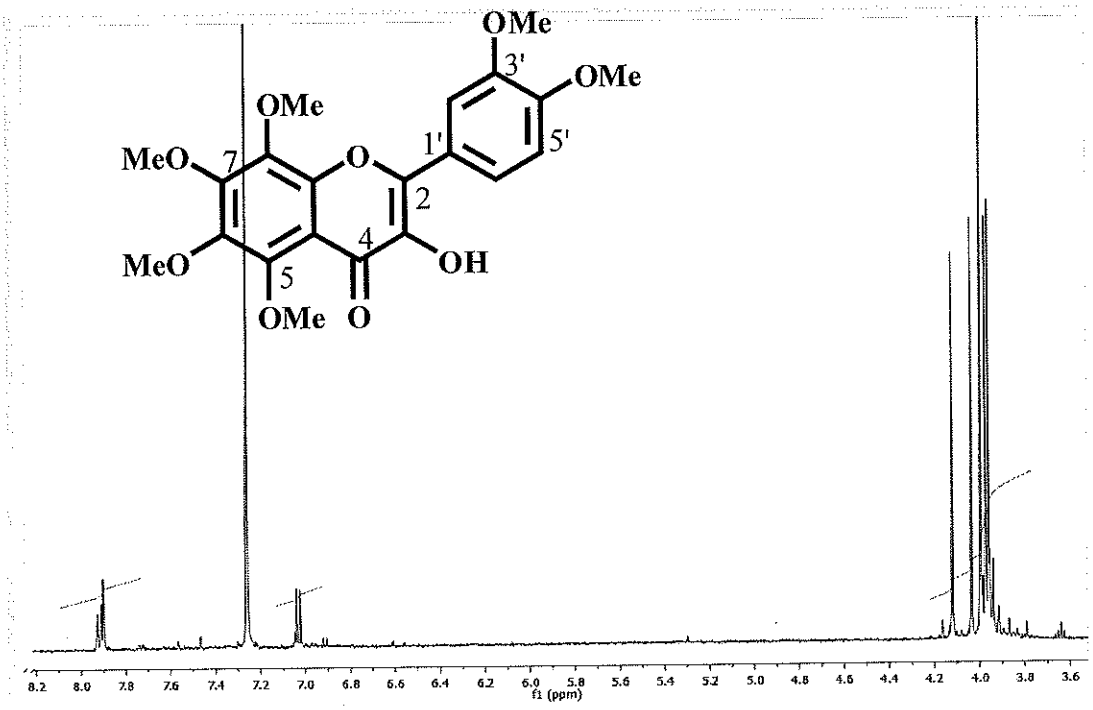

Figure A-24 ^{1}H NMR ($CDCl_3$) spectrum of **CR24**

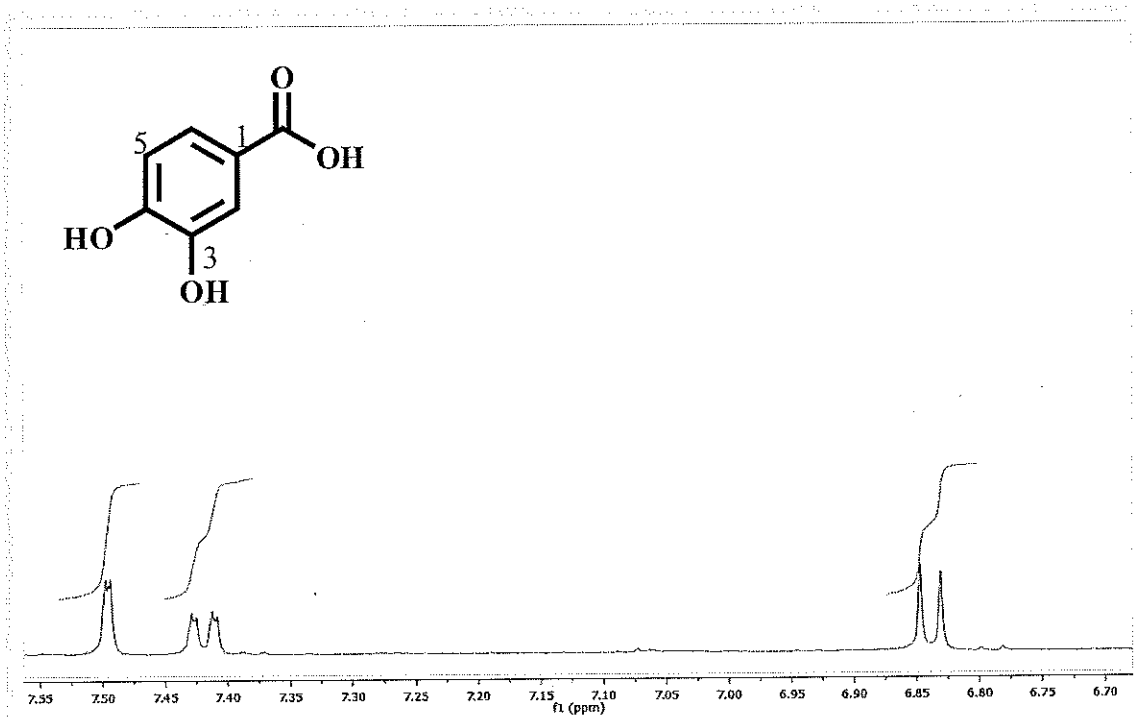

Figure A-25 ^{1}H NMR (DMDO-d_6) spectrum of **CR25**

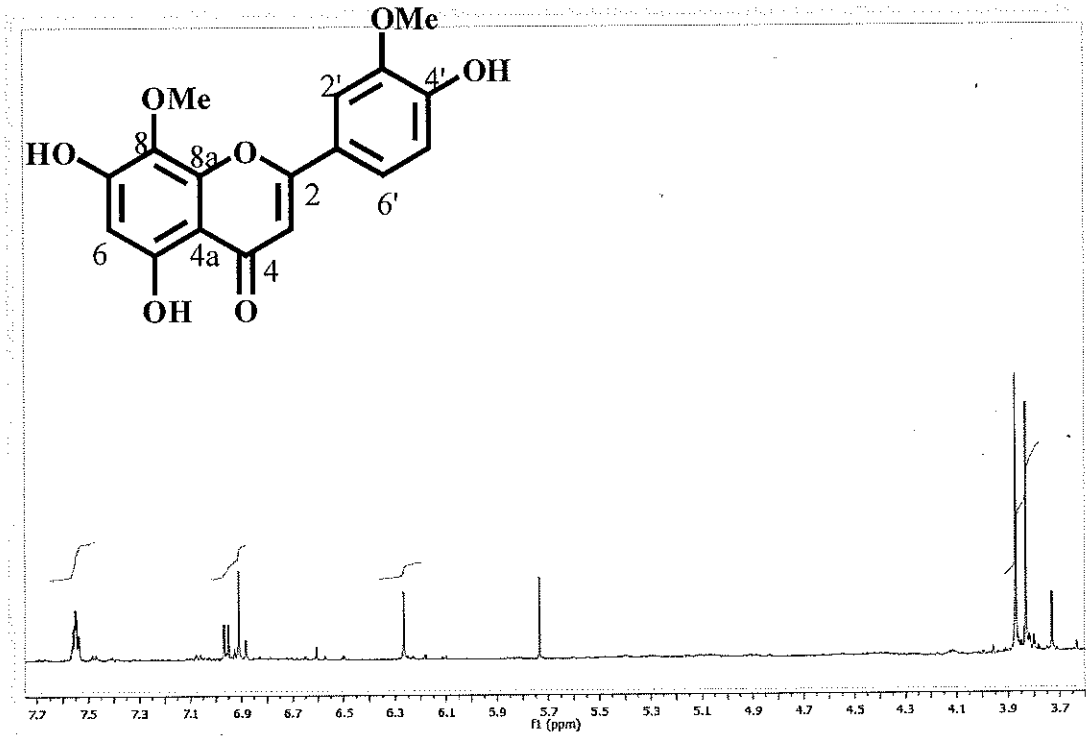

Figure A-26 ^{1}H NMR ($CDCl_3$+ DMDO-d_6) spectrum of **CR26**

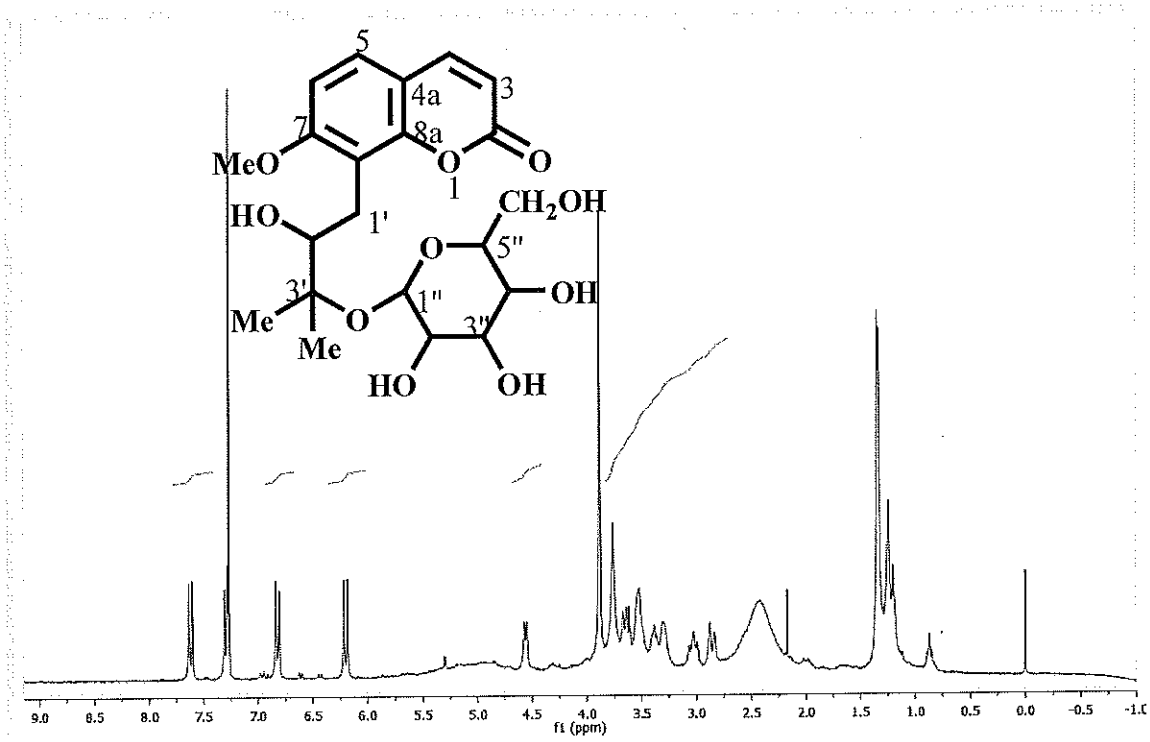

Figure A-27 ^{1}H NMR ($CDCl_3$+ DMDO-d_6) spectrum of **CR27**

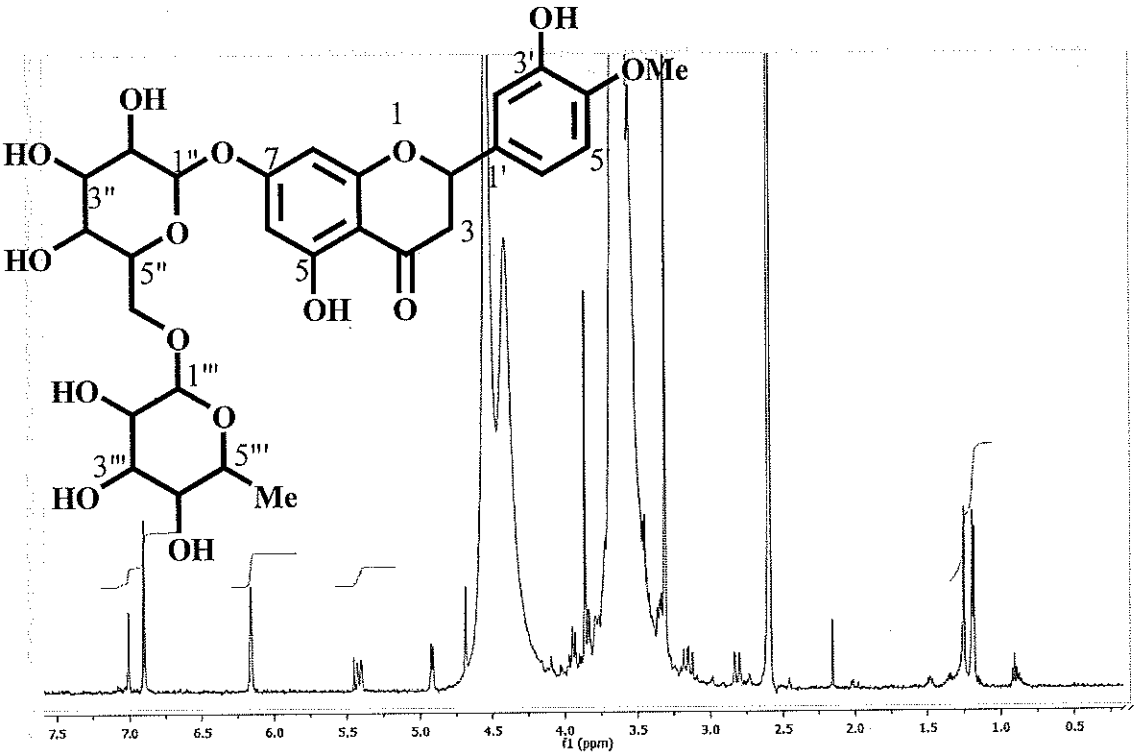

Figure A-28 ^{1}H NMR ($CDCl_3$+ DMDO-d_6) spectrum of **CR28**

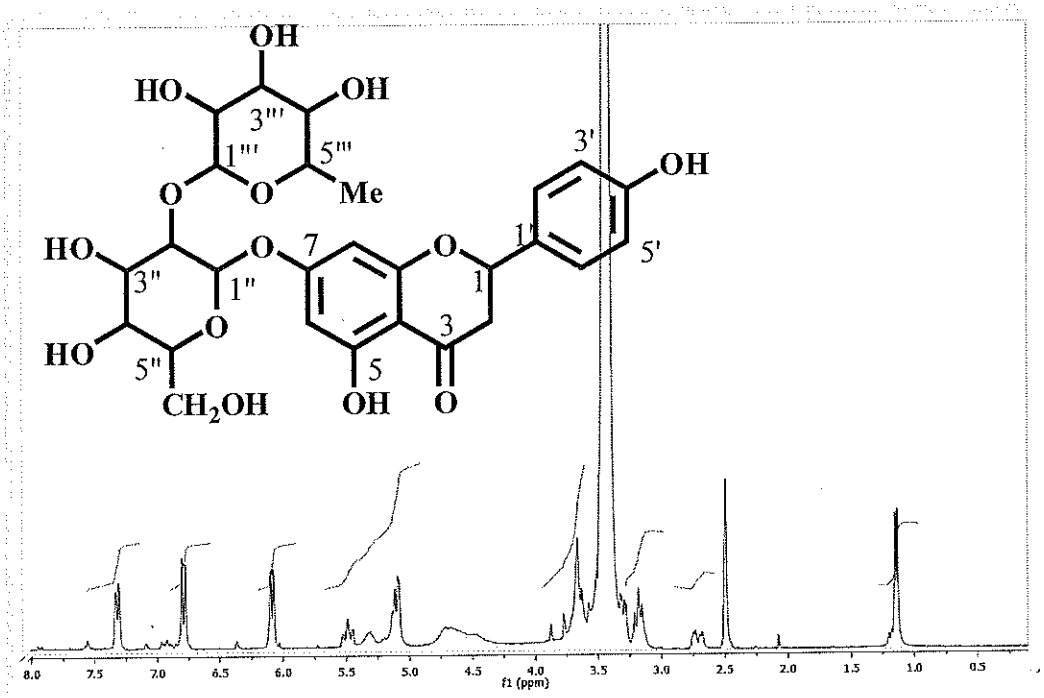

Figure A-29 ^{1}H NMR (DMDO-d_6) spectrum of **CR29**

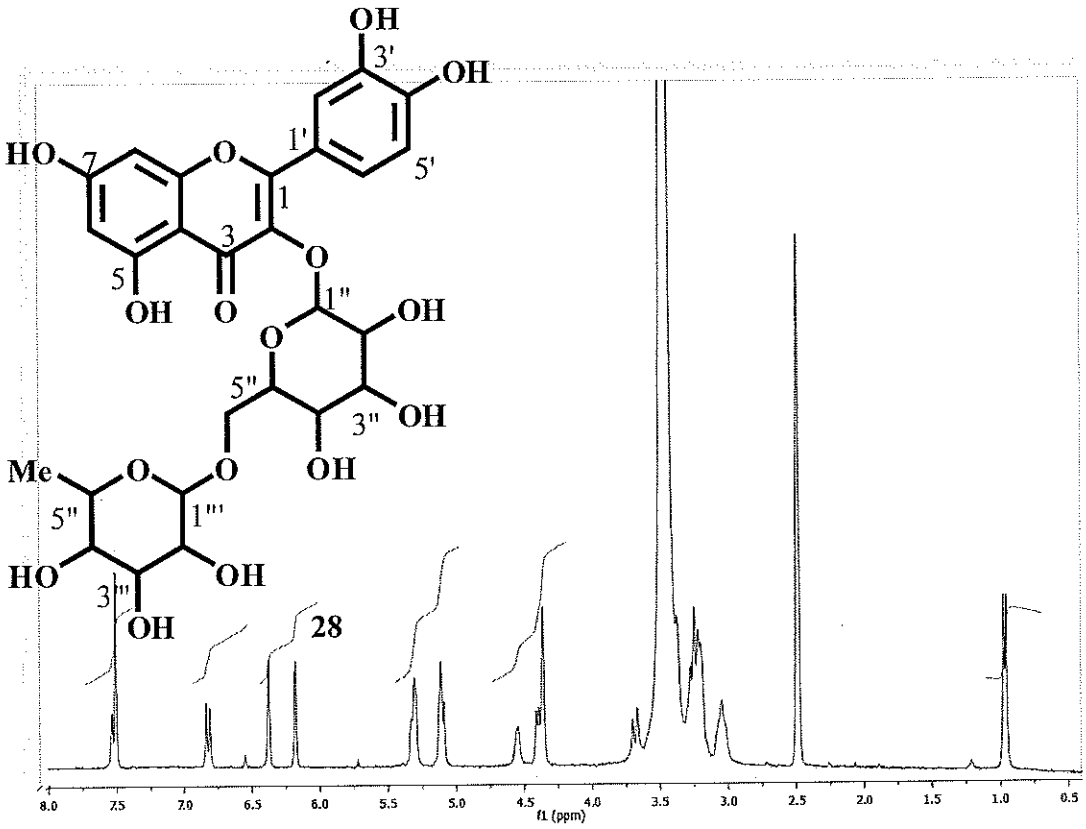

Figure A-30 ^{1}H NMR (DMDO-d_6) spectrum of **CR30**

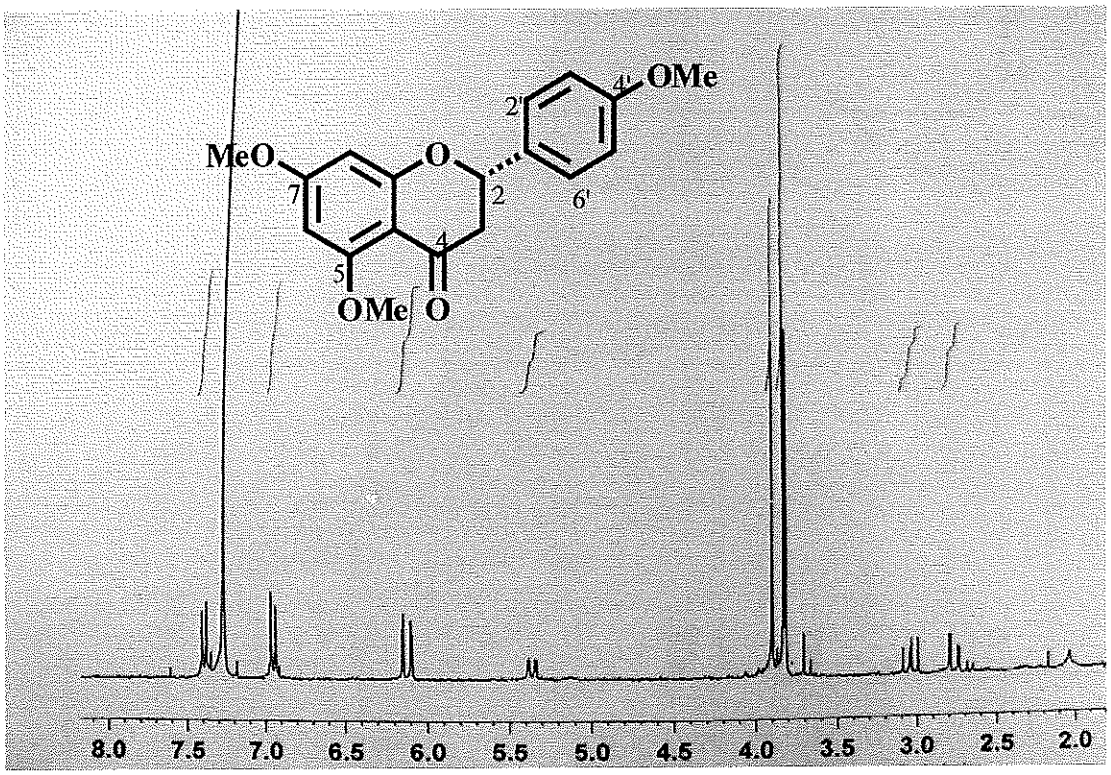

Figure A-31 ^{1}H NMR ($CDCl_3$) spectrum of **CR31**

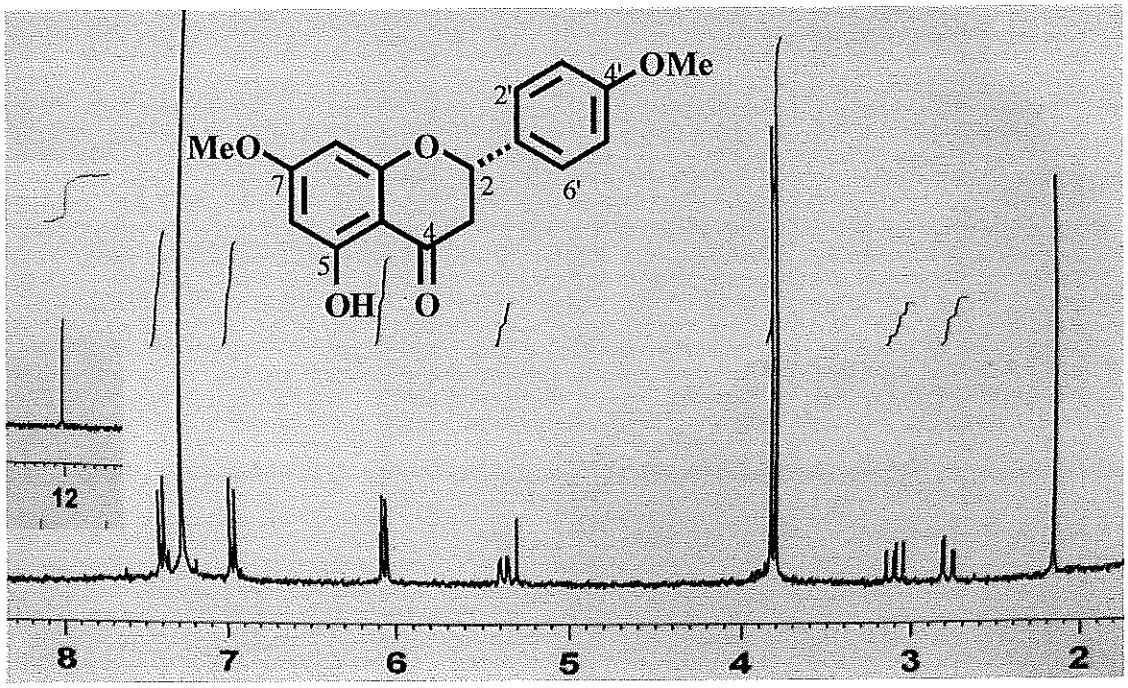

Figure A-32 ^{1}H NMR ($CDCl_3$) spectrum of **CR32**

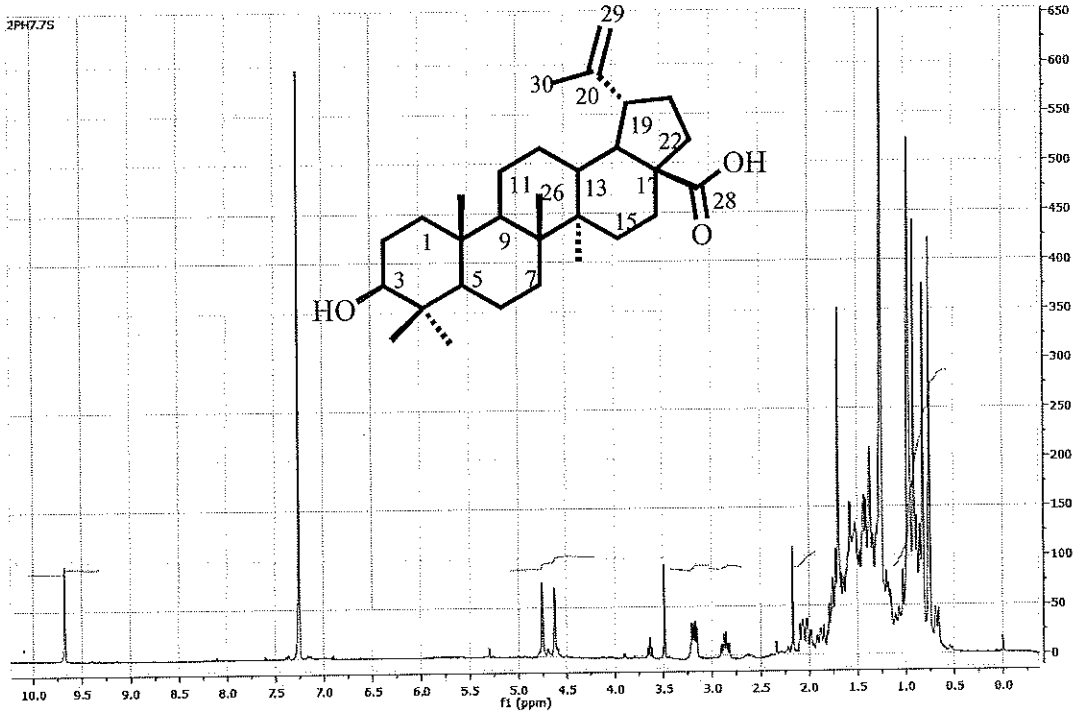

Figure A-33 ^{1}H NMR ($CDCl_3$) spectrum of **CR33**

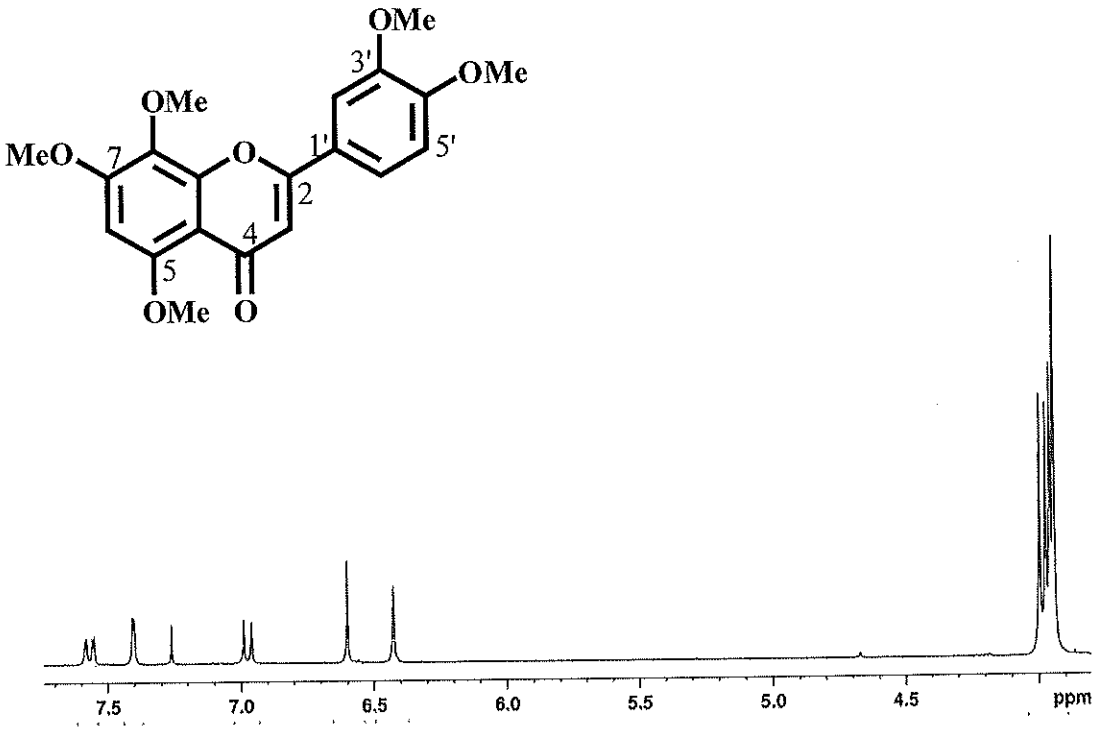

Figure A-34 ^{1}H NMR ($CDCl_3$) spectrum of **CR34**

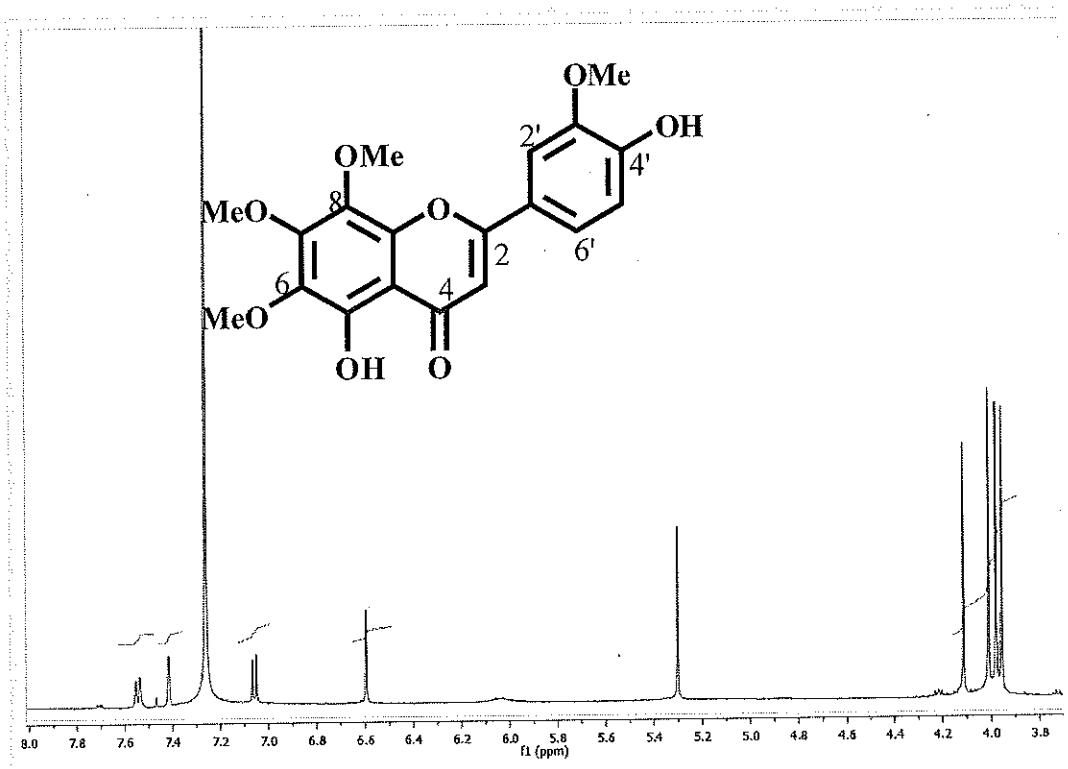

Figure A-35 ^{1}H NMR ($CDCl_3$) spectrum of **CR35**

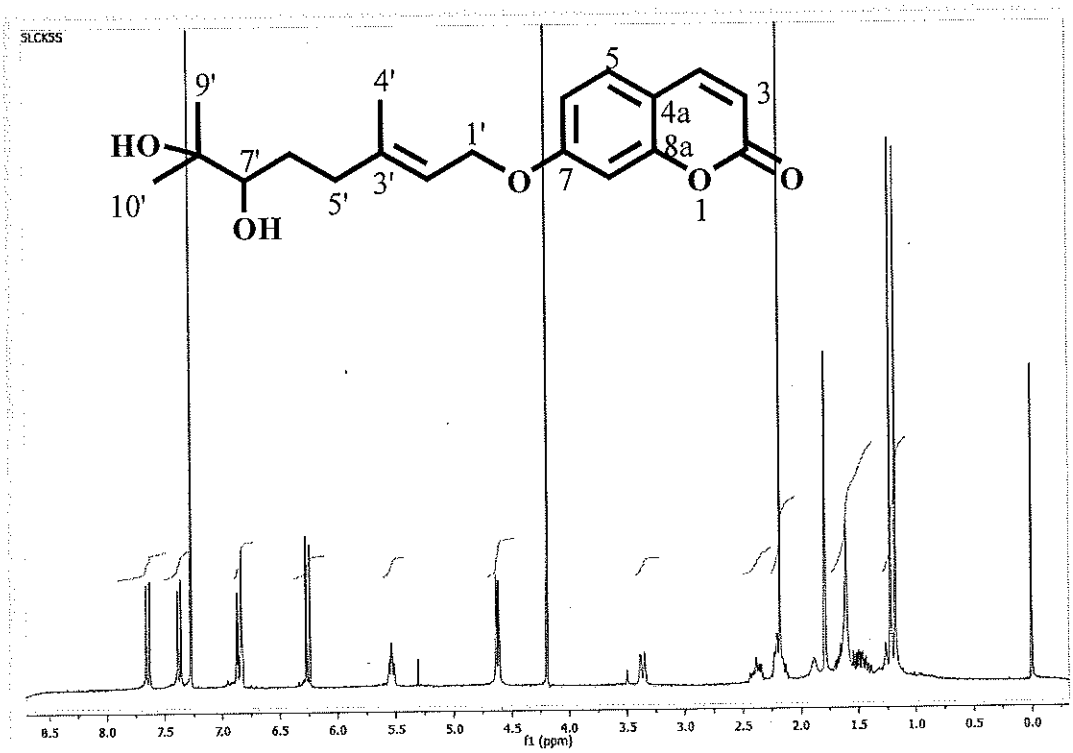

Figure A-36 ^{1}H NMR ($CDCl_3$) spectrum of **CR36**

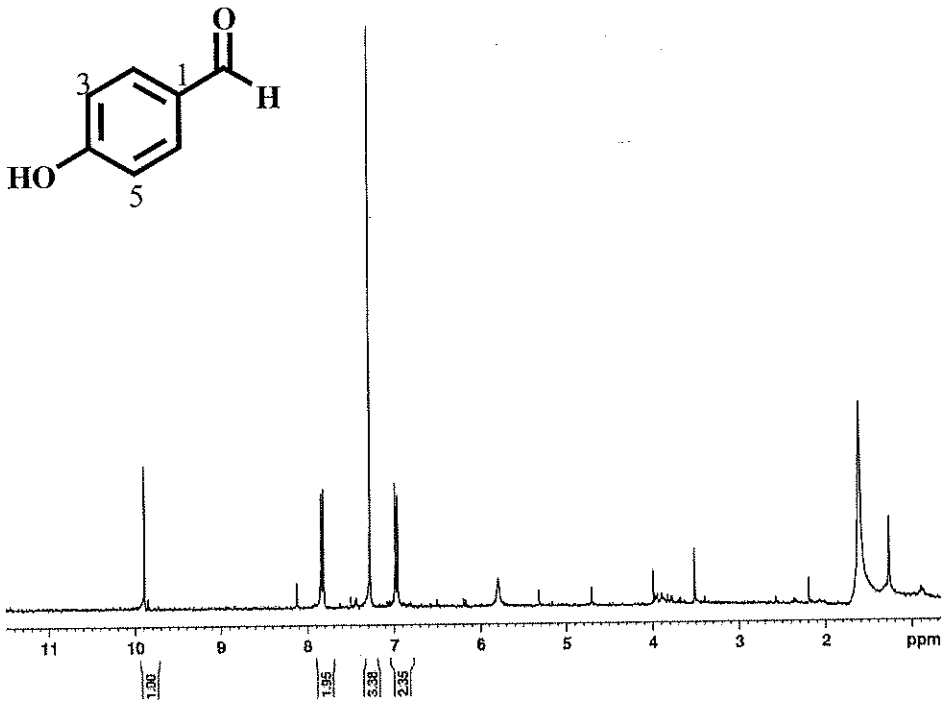

Figure A-37 ^{1}H NMR ($CDCl_3$) spectrum of **CR37**

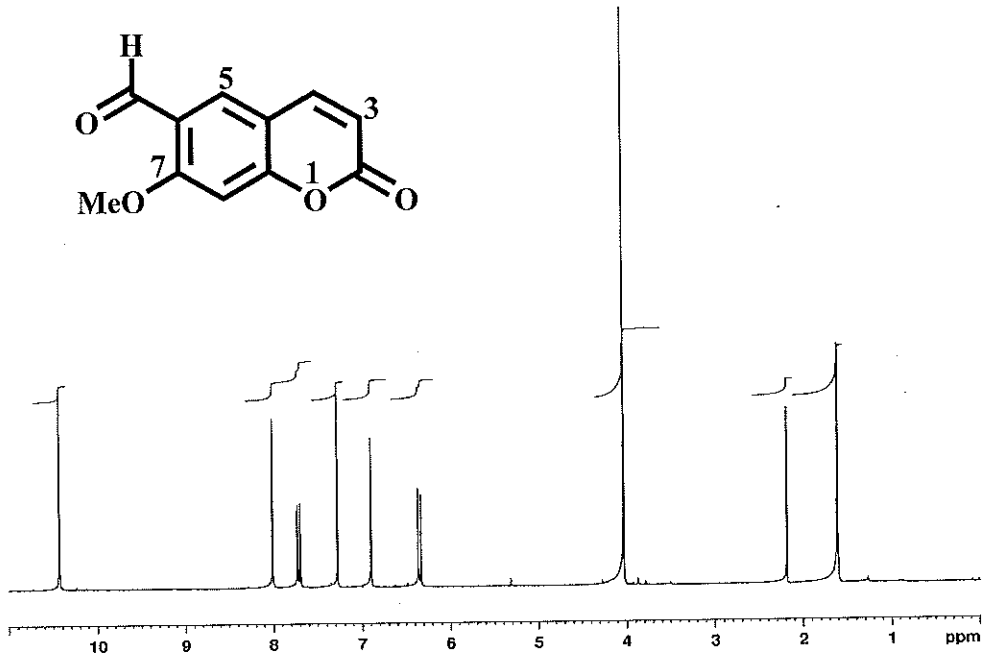

Figure A-38 ^{1}H NMR ($CDCl_3$) spectrum of **CR38**

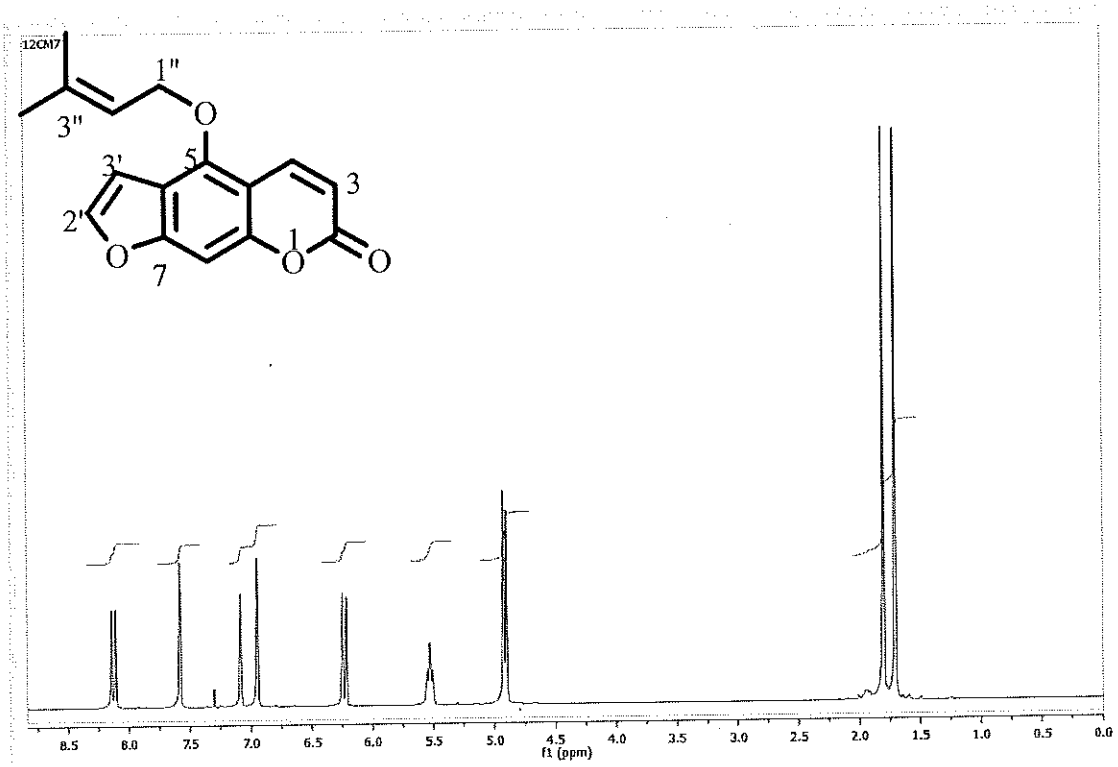

Figure A-39 ^{1}H NMR ($CDCl_3$) spectrum of **CR39**

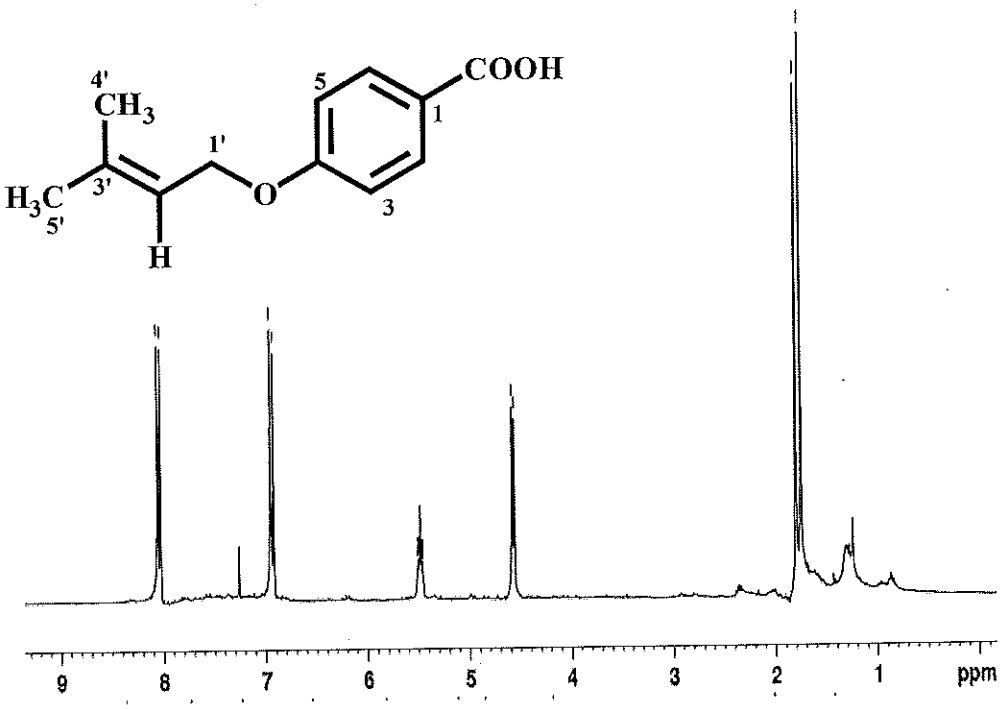

Figure A-40 ^{1}H NMR ($CDCl_3$) spectrum of **CR40**

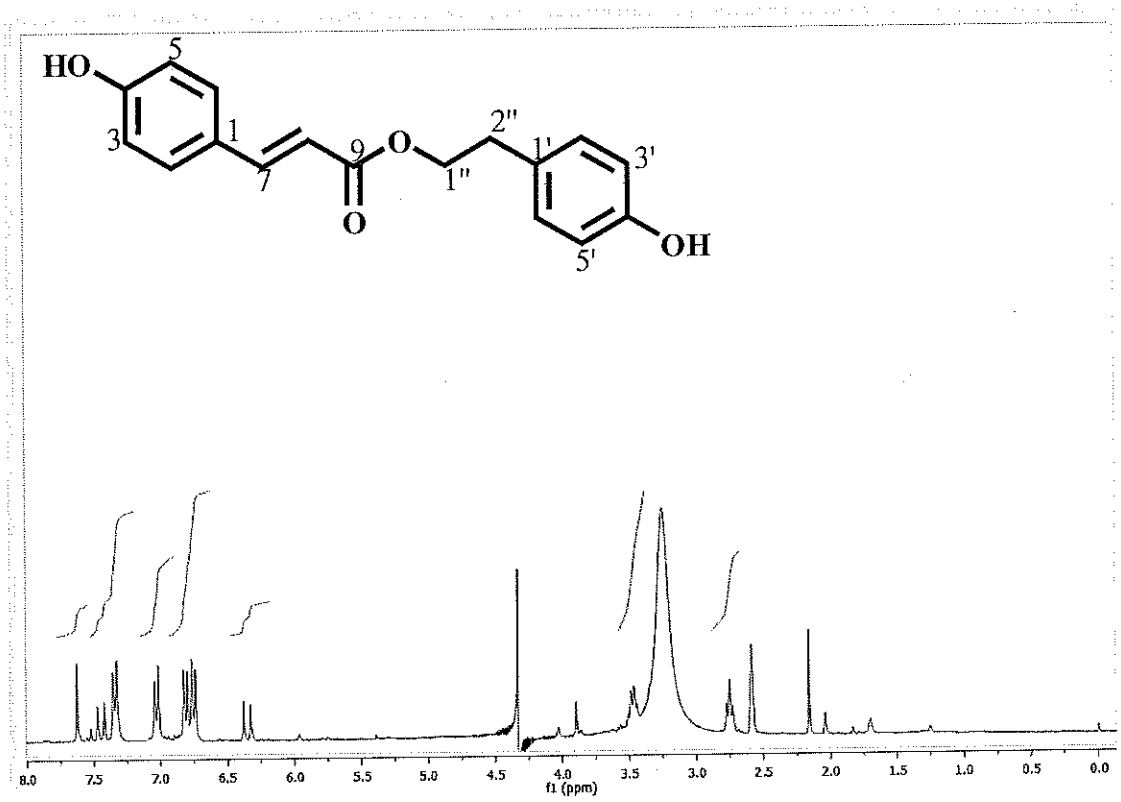

Figure A-41 ^{1}H NMR ($CDCl_3$) spectrum of **CR41**

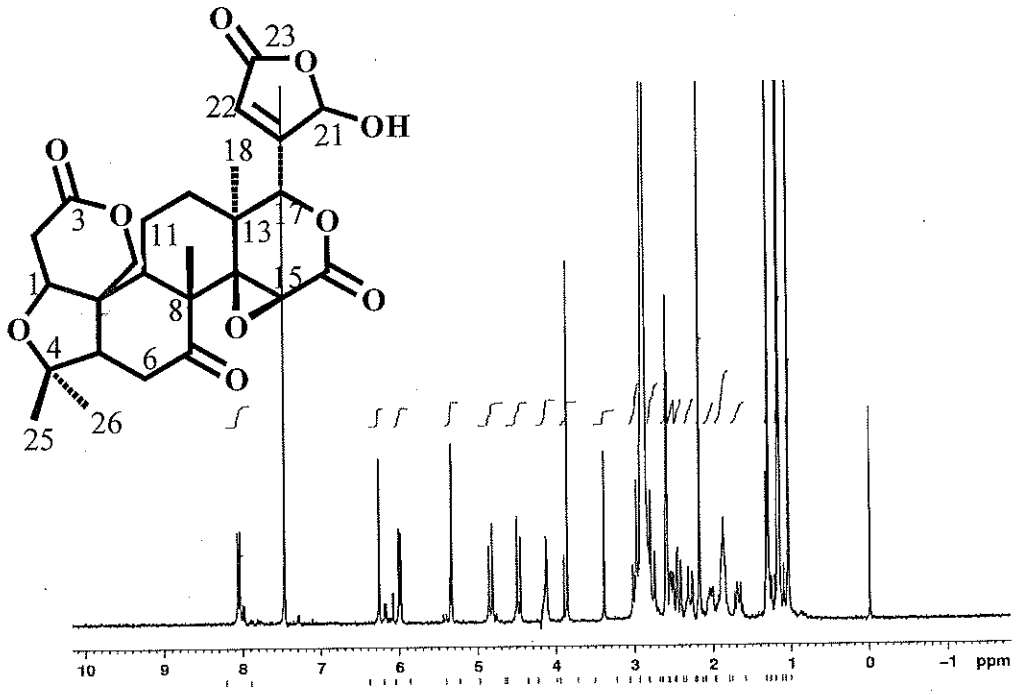

Figure A-42 ^{1}H NMR ($CDCl_{3+}$ DMSO.d_6) spectrum of **CR42**

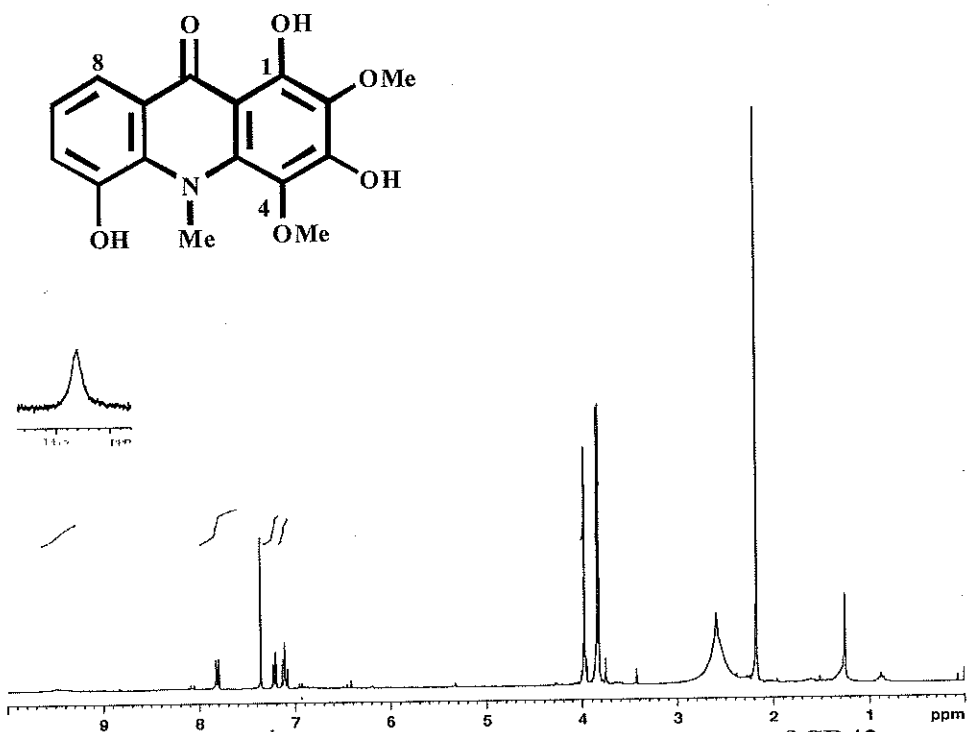

Figure A-43 ^{1}H NMR ($CDCl_3$+DMSO-d_6) spectrum of **CR43**

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2. Prince of Songkla scholar ship for Ph.D
3. Graduate School, Prince of Songkla University

List of Publications

1. Uraiwan Phetkul, Nutthakran Wanlaso, Wilawan Mahabusarakam, Souwalak Phongpaichit & Anthony R. Carroll, **2013**. New acridone from the wood of Citrus reticulata Blanco. *Natural Product Research*, 27(20), 1922-1926.
2. Uraiwan Phetkul, Souwalak Phongpaichit, Ramida Watanapokasin & Wilawan Mahabusarakam. **2014**. New depside from Citrus reticulata Blanco. *Natural Product Research*. Published online: 18 Mar 2014

List of Proceedings

1. Uraiwan Phekul, Wilawan Mahabusarakam. "Chemical Constituents from the Branch Bark of *Citrus reticulata* Blanco". 27TH International Symposium on the Chemistry of Natural Prod, Brisbane, Australia, July 10-15, 2011. (international; poster presentation)
2. Uraiwan Phekul, Wilawan Mahabusarakam. "Chemical Constituents from the Branch Bark of *Citrus reticulata* Blanco" RGJ-Ph.D. Congress XI. Jomtien Palm Beach Hotel & Resort Pattaya, Chonburi, April 6-8, 2012. (international; oral presentation)
3. Uraiwan Phekul, Wilawan Mahabusarakam. "Chemical Constituents from the Peels of *Citrus reticulata* Blanco" The 5th International Conference on Natural Products for Health and Beauty (NATPRO 5). Moevenpick Resort & Spa Karon Beach Phuket, Thailand, May 6-8, 2014. (international; poster presentation)