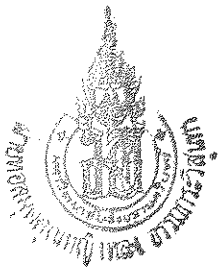

Chemical Constituents from *Derris scandens* and Antioxidation Properties

Suwanna Deachathai

Master of Science Thesis in Organic Chemistry

Prince of Songkla University

2001

Thesis Title	Chemical Constituents from *Derris scandens* and Antioxidation Properties
Author	Miss Suwanna Deachathai
Major Program	Organic Chemistry
Academic Year	2001

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The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirment for the Master of Science degree in Organic Chemistry.

.....

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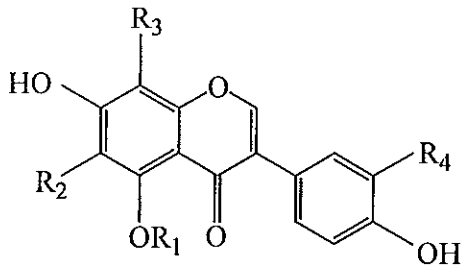
ชื่อวิทยานิพนธ์	องค์ประกอบทางเคมีของเถาวัลย์เปรียงและสมบัติต้านปฏิกิริยาออกซิเดชัน
ผู้เขียน	นางสาวสุวรรณา เดชาทัย
สาขาวิชา	เคมีอินทรีย์
ปีการศึกษา	2544

บทคัดย่อ

การสกัดและแยกสารจากลำต้นเถาวัลย์เปรียง (*Derris scandens* Benth.) ด้วยเมธานอลได้สารที่ยังไม่มีรายงานคือ 4′,5-dihydroxy-2″,2″-dimethylchromeno [6,7:5″,6″]isoflavone-3′-carboxaldehyde (**DS3**) 4′,5-dihydroxy-3′-prenyl-2″,2″-dimethylchromeno[7,8:6″,5″]isoflavone (**DS16**) และ 3,3′-dihydroxy-5-methoxy-5′,6′-methylenedioxybenzil (**DS18**) และสารที่เคยมีรายงานแล้วคือ isoflavone 10 สาร (**DS2 DS6 DS7 DS9 DS10 DS11 DS12 DS13 DS22** และ **DS23**) pterocarpan 2 สาร (**DS4** และ **DS20**) steroid 1 สาร (**DS15**) และ coumestan 1 สาร (**DS19**) ส่วนการสกัดและแยกสารด้วยอะซิโทนได้ isoflavone 2 สาร คือ **DS24** และ **DS25** สารประกอบ **DS2 DS4 DS6 DS7 DS10** และ **DS20** เป็นสารที่มีรายงานในพืชชนิดนี้ ในขณะที่ **DS9 DS11 DS12 DS13 DS19 DS23 DS24** และ **DS25** มีรายงานในพืชชนิดอื่น โครงสร้างของสารประกอบเหล่านี้วิเคราะห์โดยใช้เทคนิคทางสเปกโทรสโกปี UV IR NMR และ MS

DS6 DS7 และ **DS12** สามารถต้านปฏิกิริยาออกซิเดชันต่ออนุมูลอิสระ 1,1-diphenyl-2-picrylhydrazyl (DPPH) ได้ดีที่สุดด้วยค่า IC_{50} 3.63 8.75 และ 2.75 ไมโครโมลาร์ **DS6** และ **DS12** ออกฤทธิ์ดีกว่า butylated hydroxytoluene (BHT) ซึ่งเป็นสารมาตรฐาน ส่วนสารประกอบอื่น ๆ แสดงฤทธิ์ได้ดีปานกลาง

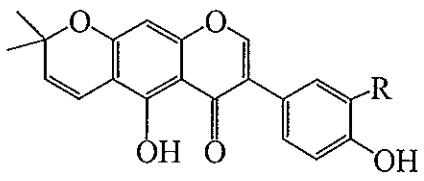


DS2 : $R_1 = R_3 = H$, $R_2 = R_4$ = isoprenyl : 4′,5,7-trihydroxy-6,3′-diprenylisoflavone

DS6 : R_1 = Me, $R_2 = R_3$ = isoprenyl, $R_4 = H$

4′,7-dihydroxy-5-methoxy-6,8-diprenylisoflavone

DS11 : $R_1 = R_2 = R_4 = H$, R_3 = isoprenyl : 4′,5,7-trihydroxy-8-prenylisoflavone

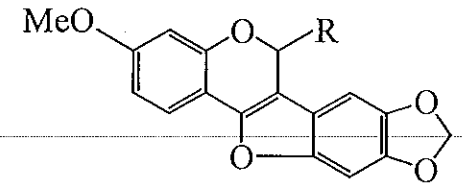


DS3 : R = CHO

4′,5-dihydroxy-2″,2″-dimethylchromeno[6,7:5″,6″]isoflavone-3′-carboxaldehyde

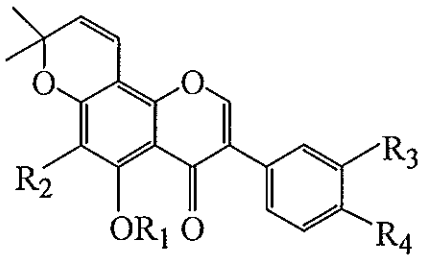
DS10 : R = isoprenyl

4′,5-dihydroxy-3′-prenyl-2″,2″- dimethylchromeno[6,7:5″,6″]isoflavone



DS4 : $R = H_2$: 3-methoxy-8,9-methylenedioxy-6a,11a-dehydropterocarpan

DS19 : R = O : 7-methoxy-11,12-methylenedioxycoumestan



DS7 : R_1 = OMe, R_2 = isoprenyl, R_3 = H, R_4 = OH

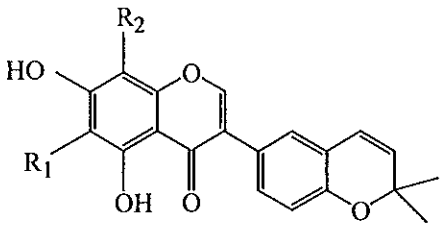
4′-hydroxy-5-methoxy-6-prenyl-2‴,2‴-dimethylchromeno[7,8:6‴,5‴]isoflavone

DS13 : R_1 = OH, R_2 = H, R_3+ R_4 = OCH_2O

5-hydroxy-3′,4′-methylenedioxy-2″,2″-dimethylchromeno[7,8:6″,5″]isoflavone

DS16 : R_1 = OH, R_2 = H, R_3 = isoprenyl, R_4 = OH

4′,5-dihydroxy-3-prenyl-2″,2″-dimethylchromeno[7,8:6″,5″]isoflavone

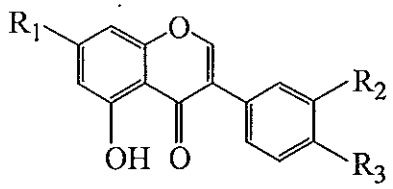


DS9 : R_1= isoprenyl, R_2= H

5,7-dihydroxy-6-prenyl-2‴,2‴-dimethylchromeno[3′,4′:5‴,6‴]isoflavone

DS23 : R_1= H, R_2= isoprenyl

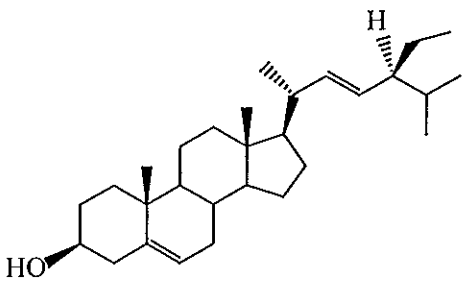
5,7-dihydroxy-8-prenyl-2‴,2‴- dimethylchromeno[3′,4′:5‴,6‴]isoflavone



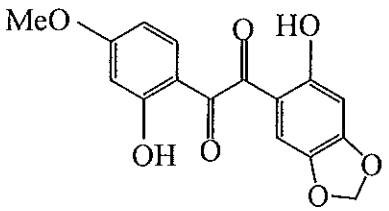
DS12 : R_1= OMe, R_2= R_3= OH : 3′,4′,5-trihydroxy-7-methoxyisoflavone

DS24 : R_1 = R_3 = OH, R_2 = OMe : 4′,5,7-trihydroxy-3′-methoxyisoflavone

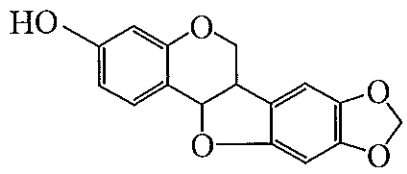
DS25 : R_1 = R_3 = OH, R_2 = H : 4′,5,7-trihydroxyisoflavone



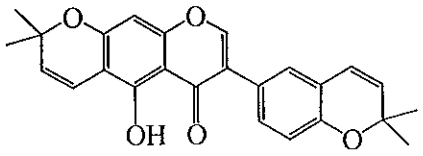
DS15 : stigmasterol



DS18 : 3,3′-dihydroxy-5-methoxy-5′,6′-methylenedioxybenzil



DS20 : (-)-3-hydroxy-8,9-methylenedioxy-6a,11a-dihydropterocarpan



DS22

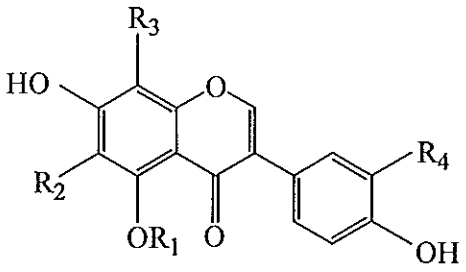
5-hydroxy-2″,2″-dimethylchromeno[6,7:5″,6″]-2″,2″-dimethylchromeno[3′,4′:5‴,6‴]isoflavone

Thesis Title	Chemical Constituents from *Derris scandens* and Antioxidation Properties
Author	Miss Suwanna Deachathai
Major Program	Organic Chemistry
Academic Year	2001

ABSTRACT

Extraction of the stems of *Derris scandens* Benth. with methanol yielded three new compounds, 4′,5-dihydroxy-3′-aldehyde-2″,2″-dimethylchromeno [6,7:5″,6″]isoflavone (**DS3**), 4′,5-dihydroxy-3′-prenyl-2″,2″-dimethylchromeno [7,8:6″,5″]isoflavone (**DS16**) and 3,3′-dihydroxy-5-methoxy-5′,6′-methylenedioxybenzil (**DS18**) together with ten known isoflavones (**DS2**, **DS6**, **DS7**, **DS9**, **DS10**, **DS11**, **DS12**, **DS13**, **DS22** and **DS23**), two pterocarpans (**DS4** and **DS20**), a steroid (**DS15**) and a coumestan (**DS19**). Two known isoflavone were obtained from acetone extract (**DS24** and **DS25**). **DS2**, **DS4**, **DS6**, **DS7**, **DS10** and **DS20** were already known from *D. scandens*, while **DS9**, **DS11**, **DS12**, **DS13**, **DS19**, **DS23**, **DS24** and **DS25** have not yet been found in this plant. Their structures were determined on the basis of UV, IR, NMR and MS data.

DS6, **DS7** and **DS12** were found to show potent antioxidative activity (IC_{50} 3.63, 8.75 and 2.75 μM) by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay. **DS6** and **DS12** showed higher activity than that of butylated hydroxytoluene (BHT) and the other showed moderate activity.

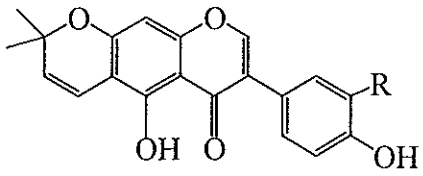


DS2 : $R_1 = R_3 = H$, $R_2 = R_4$ = isoprenyl : 4′,5,7-trihydroxy-6,3′-diprenylisoflavone

DS6 : R_1 = Me, $R_2 = R_3$ = isoprenyl, $R_4 = H$

4′,7-dihydroxy-5-methoxy-6,8-diprenylisoflavone

DS11 : $R_1 = R_2 = R_4 = H$, R_3 = isoprenyl : 4′,5,7-trihydroxy-8-prenylisoflavone

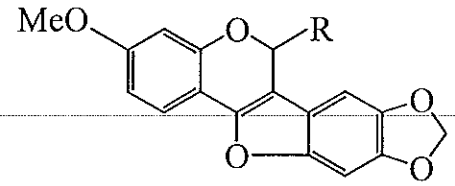


DS3 : R = CHO

4′,5-dihydroxy-2″,2″-dimethylchromeno[6,7:5″,6″]isoflavone-3′-carboxaldehyde

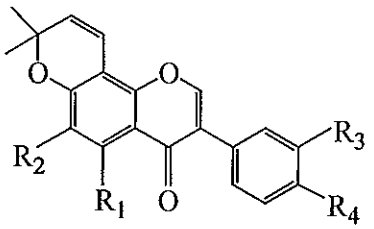
DS10 : R = isoprenyl

4′,5-dihydroxy-3′-prenyl-2″,2″- dimethylchromeno[6,7:5″,6″]isoflavone



DS4 : $R = H_2$: 3-methoxy-8,9-methylenedioxy-6a,11a-dehydropterocarpan

DS19 : R = O : 7-methoxy-11,12-methylenedioxycoumestan



DS7 : R_1 = OMe, R_2 = isoprenyl, R_3 = H, R_4 = OH

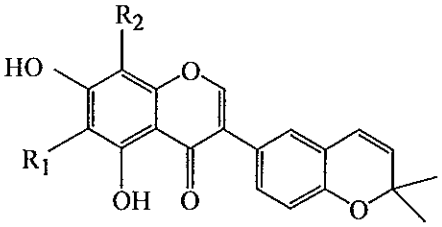
4′-hydroxy-5-methoxy-6-prenyl-2‴,2‴-dimethylchromeno[7,8:6‴,5‴]isoflavone

DS13 : R_1 = OH, R_2 = H, R_3+ R_4 = OCH_2O

5-hydroxy-3′,4′-methylenedioxy-2″,2″-dimethylchromeno[7,8:6″,5″]isoflavone

DS16 : R_1 = OH, R_2 = H, R_3 = isoprenyl, R_4 = OH

4′,5-dihydroxy-3-prenyl-2″,2″-dimethylchromeno[7,8:6″,5″]isoflavone

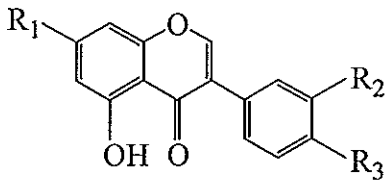


DS9 : R_1= isoprenyl, R_2= H

5,7-dihydroxy-6-prenyl-2‴,2‴-dimethylchromeno[3′,4′:5‴,6‴]isoflavone

DS23 : R_1= H, R_2= isoprenyl

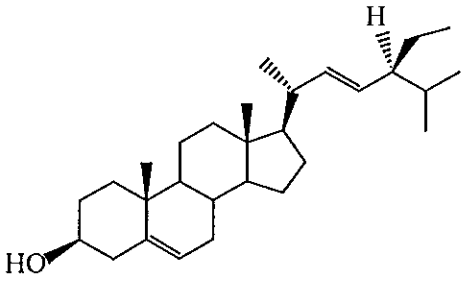
5,7-dihydroxy-8-prenyl-2‴,2‴- dimethylchromeno[3′,4′:5‴,6‴]isoflavone



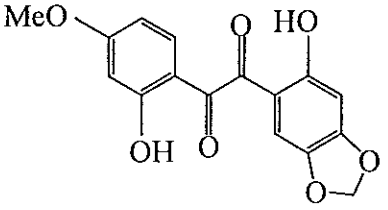
DS12 : R_1= OMe, R_2= R_3= OH : 3′,4′,5-trihydroxy-7-methoxyisoflavone

DS24 : R_1 = R_3 = OH, R_2 = OMe : 4′,5,7-trihydroxy-3′-methoxyisoflavone

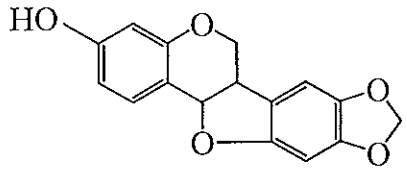
DS25 : R_1 = R_3 = OH, R_2 = H : 4′,5,7-trihydroxyisoflavone



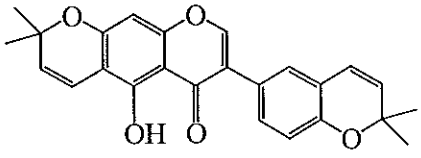
DS15 : stigmasterol



DS18 : 3,3′-dihydroxy-5-methoxy-5′,6′-methylenedioxybenzil



DS20 : (-)-3-hydroxy-8,9-methylenedioxy-6a,11a-dihydropterocarpan



DS22

5-hydroxy-2″,2″-dimethylchromeno[6,7:5″,6″]-2″,2″-dimethylchromeno[3′,4′:5‴,6‴]isoflavone

ACKNOWLEDGEMENT

I wish to express my deepest and sincere gratitude to my supervisor, Assistant Professor Dr. Wilawan Mahabusarakam, for her valuable instructions, expert guidance, excellent suggestions and kindness which are more than I can describe here. Everything will always be in my mind.

My sincere thanks are expressed to Dr. Kanda Panthong my co-advisor, for her kindness and valuable advice. Special thanks are addressed to Professor Dr. Walter C. Taylor, Department of Organic Chemistry, the University of Sydney, Australia for his guidance and providing MS spectra. In addition, I am also very grateful to Assistant Professor Dr. Chaweewan Jansakul for providing the raw material.

I would like to express my appreciation to the staffs of the Department of Chemistry, Faculty of Science, Prince of Songkla University for making this thesis possible and to Mr. Bunsit Watanathai for recording some spectral data.

This research was made possible by a scholarship from Postgraduate Education and Research Program in Chemistry, funded by The Royal Thai Government (PERCH) and the Graduate School, Prince of Songkla University for supporting materials.

Finally, none of this would have been possible without love and encouragement of my family and friends. I thank them for their understanding during all of the times when I could not be with them and their steady love that supports me.

Suwanna Deachathai

CONTENTS

CONTENTS (continued)

CONTENTS (continued)

LISTS OF TABLES

LISTS OF TABLES (continued)

LISTS OF ILLUSTRATIONS

LISTS OF ILLUSTRATIONS (continued)

LISTS OF ILLUSTRATIONS (continued)

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LISTS OF ILLUSTRATIONS (continued)

ABBREVIATIONS AND SYMBOLS

s	=	*singlet*
d	=	*doublet*
t	=	*triplet*
m	=	*multiplet*
dd	=	*doublet of doublet*
br s	=	*broad singlet*
g	=	gram
kg	=	kilogram
mg	=	miligram
μg	=	microgram
mM	=	millimolar
mL	=	milliliter
h	=	hour
min	=	minute
%	=	percent
nm	=	nanometer
cm^3	=	cubic centimeter
m.p.	=	melting point
cm^{-1}	=	reciprocal centimeter (wave number)
δ	=	chemical shift relative to TMS
J	=	coupling constant
$[\alpha]_D$	=	specific rotation
λ_{max}	=	maximum wavelength
ν	=	absorption frequencies

ABBREVIATIONS AND SYMBOLS (continued)

ε	=	molar extinction coefficient
m/z	=	a value of mass divided by charge
^{o}C	=	degree celcius
MHz	=	Megahertz
ppm	=	part per million
c	=	concentration
EIMS	=	Electron Impact Mass Spectra
IR	=	Infrared
UV	=	Ultraviolet-Visible
MS	=	Mass Spectroscopy
NMR	=	Nuclear Magnetic Resonance
2D NMR	=	Two Dimentional Nuclear Magnetic Resonance
COSY	=	Correlated Spectroscopy
DEPT	=	Distortionless Enhancement by Polarization Transfer
HMBC	=	Heteronuclear Multiple Bond Correlation
HMQC	=	Heteronuclear Multiple Quantum Coherence
NOE	=	Nuclear Overhauser Effect Spectroscopy
CC	=	Column Chromatography
PLC	=	Preparative Thin Layer Chromatography
TMS	=	tetramethylsilane
DMSO	=	dimethyl sulphoxide
$CDCl_3$	=	deuterochloroform
CD_3OD	=	deuteromethanol

CHAPTER 1

INTRODUCTION

1.1 Introduction

Derris scandens Benth., a plant belonging to the Leguminosae family, local names in Thailand:"Thao-Wan-Priang" (เถาวัลย์เปรียง) in middle part region, "Yan-Mho" (ย่านเหมาะ) in Nakhon Si Thammarat, "Khruea-Khao-Nang" (เครือเขาหนัง) and "Thao-Taa-Plaa" (เถาตาปลา) in Nakhon Ratchasima (เที่ยงบูรณธรรม, 2542).

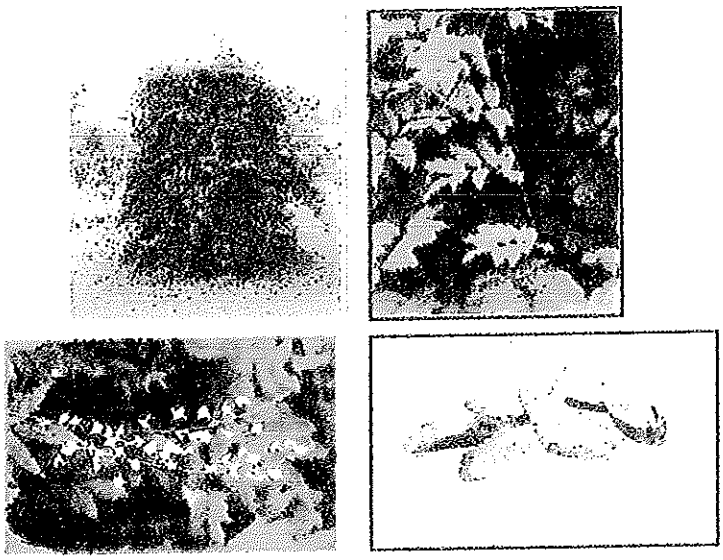


Figure 1 *Derris scandens* Benth.

(ภาควิชาเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล)

D. scandens is a woody vine. Leaves odd pinanately compound, about 15 cm long; leaflets 3-5 pairs, oblong - ovate, 2-5 cm long, 1-2 cm wide, apex broadly

acuminate, usually retuse, base acute; petiolule short : inflorescence racemose, axillary. Flowers papilionaceous form; calyx 5, dull-purple, somewhat oblique, 2-3 mm long; corolla 5, white to pale pink, irregular, 1 cm long; stamen 10; ovary superior, 1-celled. Fruit flattened pod, lanceolate or oblong-lanceolate, narrow, spindled at both ends, about 4-6 cm long, and 1-2 cm wide; seeds 1-3 (Faculty of Pharmacy Mahidol University, 1986). About 40 species are abundant in tropical countries in the world (Merrill, 1968).

1.2 Chemical constituents from Derris species

According to NAPRALERT database, Science direct, Chemical Abstracts and Dictionary of Natural Products, several types of compounds have been reported to be present in *Derris* genus, such as alkaloid, anthraquinone, aurone, auronol, chalcone, coumarin, coumaronochromone, flavan, flavanone, flavone, isoflavan, isoflavone, pterocarpan, saponin, steroid, stilbene and triterpene. Table 1 shows the chemical constituents isolated from *Derris* species.

Table 1 Compounds isolated from *Derris* species

a : Alkaloid **b** : Anthraquinone **c** : Aurone and Auronol

d : Chalcone **e** : Coumarin **f** : Coumaronochromone

g : Flavan and Isoflavan **h** : Flavanone **i** : Flavone

j : Isoflavone **k** : Pterocarpan **l** : Steroid

m : Miscellaneous

Scientific name	Compound	Bibliography
D. amazonica		
- aerial parts	(6aS, 11aS)-Dimethylhomopterocarpin **1k** Lupenone **2l** Lupeol **3l** (3S)-2′-*O*-methyl vestitol **4g** *β*-Sitosterol **5l**	Braz Filho, *et al.*, 1975
- roots	Rotenone **6j**	Moretti and Grenand, 1982
D. araripensis		
- roots	3,6-Dimethoxy-6″,6″-dimethylchromeno [7,8:2″,3″]flavone **7i** 3,6-Dimethoxy-3′,4′-methylenedioxy-6″,6″-dimethylchromeno[7,8:2″,3″]flavone **8i** 3′,4′-Methylenedioxy-5,6-dimethoxyfurano [7,8:2″,3″]flavone **9i** 3,4-Methylenedioxy-2′-hydroxy-5′,6′-dimethoxyfurano[3′,4′:2″,3″] dihydrochalcone **10d**	Do Nascimento and Mors, 1981

Table 1 (continued)

Scientific name	Compound	Bibliography
	3′,4′-Methylenedioxy-5-hydroxy-6-methoxyfurano[7,8:2″,3″]flavanone **11h** 3′,4′-Methylenedioxy-3,5,6-trimethoxyfurano[7,8:2″,3″]flavone **12i** 3′,4′-Methylenedioxy-3,5,6-trimethoxyfurano[7,8:2″,3″]flavanonol **13h** 3,4,5,6-Tetramethoxyfurano[7,8:2″,3″]flavan **14g** 3,5,6-Trimethoxyfurano[7,8:2″,3″]flavone **15i**	
D. brevipes - stems	Damnacanthal **16b** Rotenone **6j** β-Sitosterol **5l**	Desai, *et al.*, 1977
D. elliptica - leaves	2S-Carboxy-4R,5S-dihydroxypiperidine **17a** 2S-Carboxy-4S,5S-dihydroxypiperidine **18a** 2,5-Dihydroxymethyl-3,4-dihydroxypyrrolidine **19a**	Marlier, *et al.*, 1976 Welter, *et al.*, 1976
- roots	Deguelin **20j** 6a,12a-Dehydrorotenone **21j** Elliptinol **22j** Elliptone **23j** Tephrosin **24j**	Kodama, *et al.*, 1980 Ahmed, *et al.*, 1989
	Tubaic acid **25m**	Obara, *et al.*,

Table 1 (continued)

Scientific name	Compound	Bibliography
	β-Tubaic acid **26m** (+)-Maackiain **27k** (-)-Maackiain **27k** Rotenone **6j** α-Toxicarol **28j**	1976 Obara and Matsubara, 1981 Crombie, *et al.*, 1968
D. ferruginea -	6a,12a-Dehydrorotenone **21j**	Crombie, *et al.*, 1968
D. floribunda - roots	Derricidin **29d** 3,4-Dihydroxylonchocarpin **30d** 5,7-Dihydroxy-6-prenylflavanone **31h** 3,5-Dimethoxy-4-prenylstilbene **32m** Isobavachromene **33d** Isocordoin **34d** Lonchocarpin **35d** 3,4′,5-Trimethoxy-4-prenylstilbene **36m**	Braz Filho, *et al.*, 1975
D. glabrescens - seeds	Derrusnin **37e** Glabrescin **38e** Glabrescione A **39j** Glabrescione B **40j**	Delle Monache, *et al.*, 1977

Table 1 (continued)

Scientific name	Compound	Bibliography
D. laxiflora		
- twigs and leaves	3′,4′,5,7-Tetrahydroxy-6,8-diprenylflavanone **41h**	Kim, *et al.*, 1995
	Hiravanone **42h**	
	Laxiflorin **43h**	
	Lonchocarpol A **44h**	
	Lupinifolin **45h**	
- roots	β-Amyrin **46l**	Lin, *et al.*, 1991
	Flemichapparin B **47k**	
	Isolaxifolin **48i**	
	Laxifolin **49i**	
	Lupeol **3l**	
	Lupinifolin **45h**	
	3′-Methoxylupinifolin **50h**	
	Prunetin **51j**	
	Derrichalcone **52d**	Lin, *et al.*, 1991; 1992
	Derriflavanone **53h** or **54h**	
	Epi-derriflavanone **54h** or **53h**	
	Laxichalcone **55d**	
D. malaccensis		
- roots	Rotenone **6j**	Yoxopeus, 1952
	Malaccol **56j**	Falshaw, *et al.*, 1966
	Sumatrol **57j**	

Table 1 (continued)

Scientific name	Compound	Bibliography
D. mollis		
- roots	Betulinic acid **58l**	Lyra, *et al.*,
	3,4′-Dimethoxyfurano[7,8:4″,5″]flavone **59i**	1979
	Karanjin **60i**	
	Lanceolatin B **61i**	
	Lupeol **3l**	
	4′-Methoxyfurano[7,8:4″,5″]flavone **62i**	
	Pongaglabrone **63i**	
	Pongapin **64i**	
D. negrensis		
- entire plant	6a,12a-Dehydrorotenone **21j**	Vasconcelos and
	Rotenone **6j**	Maia, 1976
D. nicou		
-	Rotenone **6j**	Mors, *et al.*,1973
D. oblonga		
- roots	6a,12a-Dehydro-α-toxicarol **65j**	Lin and Kuo,
	6a,12a-Dehydro-β-toxicarol **66j**	1993
	Derricarpin **67k**	
	Oblongin **68f**	Lin and Kuo,
	Oblonginol **69f**	1993
	12-Deoxo-12α-acetoxyelliptone **70j**	Lin, *et al.*, 1993
	Villosol **71j**	Lin and Kuo,
		1995
	β-Amyrin **46l**	Lin and Kuo,

Table 1 (continued)

Scientific name	Compound	Bibliography
	Daidzein **72j**	1995
	6a,12a-Dehydrodeguelin **73j**	
	6a,12a-Dehydrorotenone **21j**	
	Emodin **74b**	
	Formononetin **75j**	
	6-Hydroxy-6a,12a-dehydro-α-toxicarol **76j**	
	12a-Hydroxyrotenone **77j**	
	11-Hydroxytephrosin **78j**	
	Lupenone **2l**	
	Lupeol **3l**	
	Maackiain **27k**	
	8-Methoxycoumestrol **79e**	
	6-Oxo-6a,12a-dehydro-α-toxicarol **80j**	
	Physcion **81b**	
	Sucrose **82m**	
	Sumatrol **57j**	
	Tephrosin **24j**	
	Toxicarolisoflavone **83j**	
D. obtusa		
- root barks	Derriobtusone A **84c**	Do Nascimento,
	Derriobtusone B **85c**	*et al.*, 1976
	3,6-Dimethoxy-6″,6 ″-dimethylchromeno [7,8:2″,3″]flavone **7i**	
	Furano[6,7:2″,3″]aurone **86c**	

Table 1 (continued)

Scientific name	Compound	Bibliography
	1-Heptacosanol **87m** 5-Hydroxy-6″,6″-dimethylchromeno [7,8:2″,3″]flavone **88i** 4-Hydroxyfurano[6,7:2″,3″]aurone **89c** 4-Methoxyfurano[6,7:2″,3″]aurone **90c** 3,4-Methylenedioxy-5′-hydroxy-2′-methoxyfurano[3′,4′:2″,3″]chalcone **91d** 3′,4′-Methylenedioxyfurano[6,7:2″,3″]aurone **92c** β-Sitosterol **5l**	
D. rariflora - wood	5,7-Dihydroxy-6-prenylflavanone **31h** 3,5-Dimethoxy-4-prenylstilbene **93m** 5-Hydroxy-7-methoxy-6-prenylflavanone **94h** β-Sitosterol **5l**	Braz Filho, *et al.*, 1975
- roots	Rotenone **6j**	Braz Filho, *et al.*, 1975
D. reticulata - stems	Dereticulatin **95h** 2‴,3‴-Epoxylupinifolin **96h** Lupinifolin **45h**	Mahidol, *et al.*, 1997
D. robusta - fruits	4′-Hydroxy-3′,5,6′,7-tetramethoxyflavone **97i** 6-Hydroxy-2′,4′,7-trimethoxyisoflavone **98j**	Gupta, *et al.*, 1998

Table 1 (continued)

Scientific name	Compound	Bibliography
	23-Hydroxyoctacos-5-en-3-one **99m**	Gupta, *et al.*,
	Octacosan-3-one **100m**	1999
- seeds	Robustigenin **101j**	Chibber and Sharma, 1979
	Rubone **102d**	Chibber, *et al.*, 1979
	5-Hydroxy-7-methoxyisoflavone **103j**	Chibber and Sharma, 1979
	Robustigenin-5-*O*-methyl ether **104j**	Chibber and Sharma, 1979
	Derrugenin **105j**	Chibber and Sharma, 1979 Tsukayama, *et al.*, 1980
	Daucosterol **106l**	Chibber and
	Derrusnin **37e**	Sharma, 1980
	O,*O*-Dimethylalpinumisoflavone **107j**	
	Robustin methyl ether **108e**	
	Robustone **109j**	
	Robustone methyl ether **110j**	
	β-Sitosterol **5l**	
	Derrone **111j**	Chibber and Sharma, 1980

Table 1 (continued)

Scientific name	Compound	Bibliography
	Derrone-4′-*O*- methyl ether **112j**	Chibber, *et al.*, 1981
- roots	Robustic acid **113e**	Johnson and Pelter, 1966
	Robustic acid methyl ether **114e**	
	Robustin **115e**	
	Derrubone **116j**	East, *et al.*, 1969
	Derrusnin **37e**	
	Derrustone **117j**	
	Robustic acid **113e**	
	Robustic acid methyl ether **114e**	
	Robustin **115e**	
	Robustin methyl ether **108e**	
	Robustone **109j**	
	Robustone methyl ether **110j**	
D. scandens		
- stems	4,4′-Di-*O*-methyl scandenin **118e**	Rao, *et al.*, 1994
	3′-*γ*,*γ*-Dimethylallylwighteone **119j**	
	Eturunagarone **120j**	
	Robustic acid **113e**	
	Scandenone **121j**	
	Scandinone **122j**	
	4′,5,7-Trihydroxy-6,8-diprenylisoflavone **123j**	
	Derrisisoflavone A **124j**	Sekine, *et al.*, 1999
	Derrisisoflavone B **125j**	

Table 1 (continued)

Scientific name	Compound	Bibliography
	Derrisisoflavone C **126j**	
	Derrisisoflavone D **127j**	
	Derrisisoflavone E **128j**	
	Derrisisoflavone F **129j**	
	Erysenegalensein E **130j**	
	Lupalbigenin **131j**	
	Lupinisoflavone G **132j**	
	Lupinisol A **133j**	
	Scandinone **122j**	
	4′,5,7-Trihydroxy-6,8-diprenylisoflavone **123j**	
	Derriscanoside A **134j**	Dianpeng, *et al.*,
	Derriscanoside B **135j**	1999
	Daidzein-7-*O*-rhamnosyl(1→6)glucoside **136j**	Suwannaroj,
	Genistein-7-*O*-rhamnosyl(1→6)glucoside **137j**	*et al.*, 2000
	Orobol-7-*O*-rhamnosyl(1→6)glucoside **138j**	
- roots	Osajin **139j**	Pelter and
	Scandenone **121j**	Stainton, 1966
	Scandinone **122j**	
	Lonchocarpic acid **140e**	Johnson, *et al.*,
	Scandenin **141e**	1966
	Chandalone **142j**	Falshaw, *et al.*,
	Lonchocarpenin **143e**	1969
	Lupeol **31**	Sengupta, *et al.*,
	Scandenin **141e**	1971

Table 1 (continued)

Scientific name	Compound	Bibliography
D. sericea		
- roots	Derricin **144d**	Do Nascimento
	Lonchocarpin **35d**	and Mors, 1970
	Isolonchocarpin **145h**	
	Derricidin **29d**	Do Nascimento
	Derricin **144d**	and Mors, 1972
D. species		
- roots	6a,12a-Dehydrodeguelin **73j**	Clark and
	Dehydrotoxicarol **146l**	Keenan, 1933
	Alpinumisoflavone **147j**	Rocha and
	Alpinumisoflavone-4′-methyl ether **148j**	Zoghbi, 1982
	4′-*O*-γ,γ-Dimethylallylalpinumisoflavone **149j**	
-	α-Amyrin **150l**	Zoghbi, *et al.*,
	Coumestrol **151e**	1988
	α-Toxicarol **28j**	
D. spruceana		
- roots	Deguelin **20j**	Menichini, *et al.*,
	12a-Hydroxyrotenone **77j**	1982
	Rotenone **6j**	
	Tephrosin **24j**	
	2,4-Dimethoxy-2″,2″-dimethylchromeno	Garcia, *et al.*,
	[3′,4′:6″,5″]stilbene **93m**	1986

Table 1 (continued)

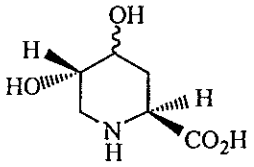
Scientific name	Compound	Bibliography
	3′,4′-Methylenedioxy-5-hydroxy-2″,2″-dimethylchromeno[7,8:6″,5″]isoflavone **152j** 3′,4′-Methylenedioxy-3-phenyl-4,5-dimethoxy-2″,2″-dimethylchromeno[7,8:6″,5″] coumarin **153e** 3′,4′-Methylenedioxy-3-phenyl-4-hydroxy-5-methoxy-2″,2″-dimethylchromeno [7,8:6″,5″]coumarin **154e** Scandenin **141e** Sitosterol **5l**	
D. trifoliata - leaves	α-Amyrin **150l** β-Amyrin **46l** Campesterol **155l** Cholesterol **156l** β-Sitosterol **5l** Stigmast-7-en-3-β-ol **157l** Stigmasterol **158l**	Ghosh, *et al.*, 1985
	Quercetin-3-*O*-β-neohesperidoside **159i** Rhamnetin-3-*O*-β-neohesperidoside **160i**	Nair and Seetharaman, 1986
-	1-Hexacosanol **161m** Lupeol **3l**	Sudachan, 1967

Table 1 (continued)

Scientific name	Compound	Bibliography
	β-Sitosterol **5l** Stigmasterol **158l**	
D. uliginosa - roots	Rotenone **6j** 6a,12a-Dehydrorotenone **21j** Lupeol **3l**	Milsum, 1938; Petard, 1951; Gaudin and Vacherat, 1938 Bose, *et al.*, 1976
D. urucu - roots	6a,12a-Dehydrorotenone **21j** Flemichapparin B **47k** 12a-Hydroxyrotenone **77j** Rotenone **6j** Tephrosin **24j** Derrissaponin **162m**	Braz Filho, *et al.*, 1975 Parente and Mors, 1980

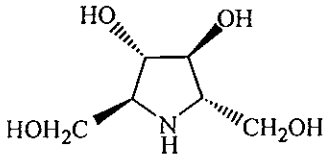
Structures of compounds from Derris species

a. Alkaloid



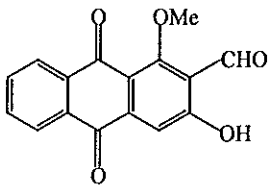
17a : 2S-Carboxy-4R,5S-dihydroxypiperidine

18a : 2S-Carboxy-4S,5S-dihydroxypiperidine

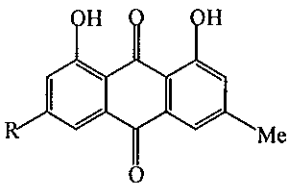


19a : 2,5-Dihydroxymethyl-3,4-dihydroxypyrrolidine

b. Anthraquinone



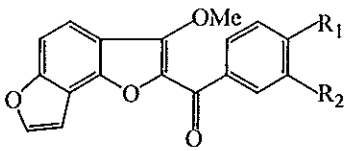
16b : Damnacanthal



74b : R = OH : Emodin

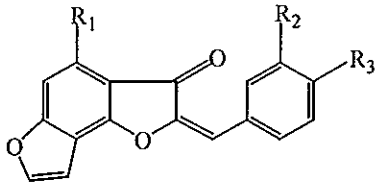
81b : R = OMe : Physcion

c. Aurone and Auronol



84c : $R_1 = R_2 = H$: Derriobtusone A

85c : $R_1 + R_2 = OCH_2O$: Derriobtusone B



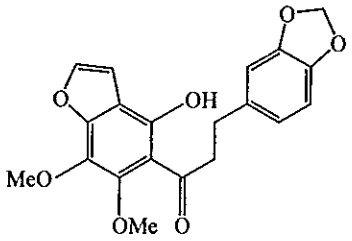
86c : R_1= R_2= R_3= H : Furano[6,7:2″,3″]aurone

89c : R_1= OH, R_2= R_3= H : 4-Hydroxyfurano[6,7:2″,3″]aurone

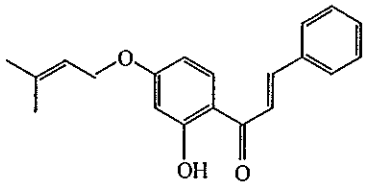
90c : R_1= OMe, R_2= R_3= H : 4-Methoxyfurano[6,7:2″,3″]aurone

92c : R_1= H, R_2+ R_3= OCH_2O : 3′,4′-Methylenedioxyfurano[6,7:2″,3″] aurone

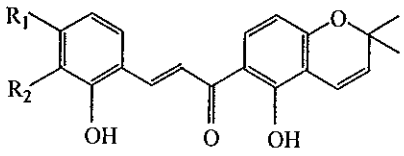
d. Chalcone



10d : 3,4-Methylenedioxy-2′-hydroxy-5′,6′-dimethoxyfurano [3′,4′:2″,3″] dihydrochalcone



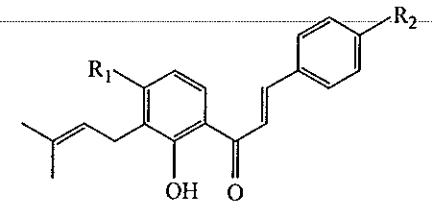
29d : Derricidin



30d : R_1= R_2= OH : 3,4-Dihydroxylonchocapin

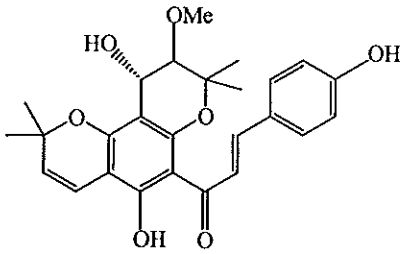
33d : R_1= OH, R_2= H : Isobavachromene

35d : R_1= R_2= H : Lonchocarpin

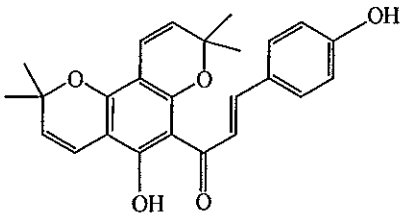


34d : R_1= OH, R_2= H : Isocordoin

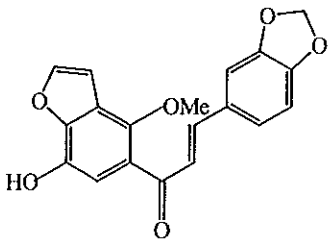
144d : R_1= OMe, R_2= H : Derricin



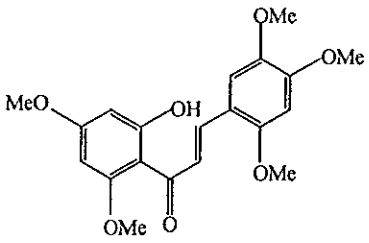
52d : Derrichalcone



55d : Laxichalcone

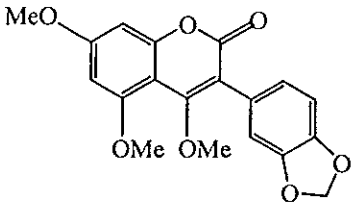


91d : 3,4-Methylenedioxy-5′-hydroxy-2′-methoxyfurano[3′,4′:2″,3″]chalcone

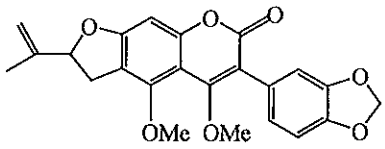


102d : Rubone

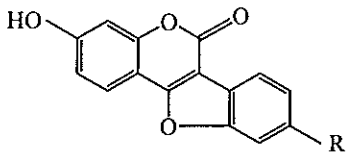
e. Coumarin



37e : Derrusnin

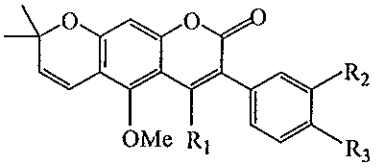


38e : Glabrescin



79e : R = OMe : 8-Methoxycoumestrol

151e : R = OH : Coumestrol

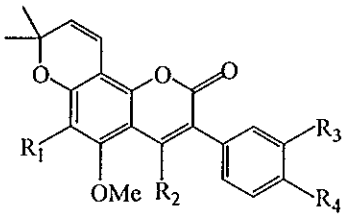


108e : R_1= OMe, R_2+ R_3= OCH_2O :
Robustin methyl ether

113e : R_1= OH, R_2= H, R_3= OMe : Robustic acid

114e : R_1= R_3 = OMe, R_2= H :
Robustic acid methyl ether

115e : R_1= OH, R_2+ R_3= OCH_2O : Robustin

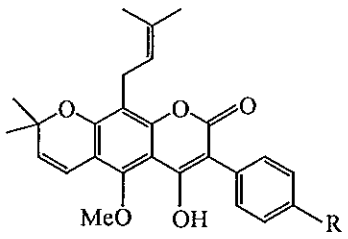


118e : R_1= isoprenyl, R_2= R_4= OMe, R_3= H :
4,4′-Di-*O*-methyl scandenin

141e : R_1= isoprenyl, R_2= R_4= OH, R_3= H :
Scandenin

153e : R_1= H, R_2= OMe, R_3+ R_4= OCH_2O :
3′,4′-Methylenedioxy-3-phenyl-4,5-dimethoxy-2″,2″-dimethylchromeno [7,8:6″,5″]coumarin

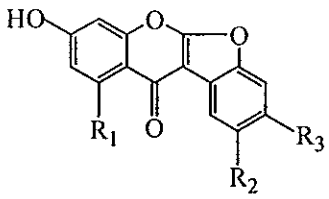
154e : R_1= H, R_2= OH, R_3+ R_4= OCH_2O :
3′,4′-Methylenedioxy-3-phenyl-4-hydroxy-5- methoxy-2″,2″-dimethylchromeno[7,8:6″,5″]coumarin



140e : R = OH : Lonchocarpic acid

143e : R = OMe : Lonchocarpenin

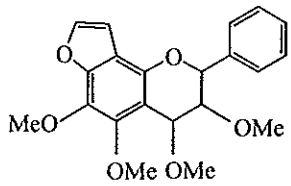
f. Coumaronochromone



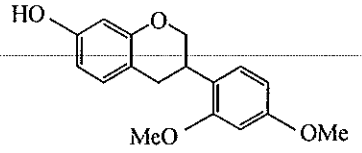
68f : R_1= H, R_2= OMe, R_3= OH : Oblongin

69f : R_1= R_2= OH, R_3= OMe : Oblonginol

g. Flavan and Isoflavan

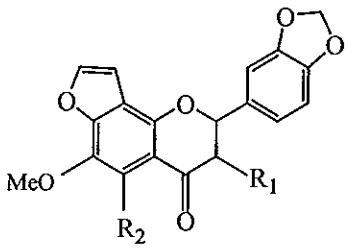


14g : 3,4,5,6-Tetramethoxyfurano[7,8:2″,3″] flavan



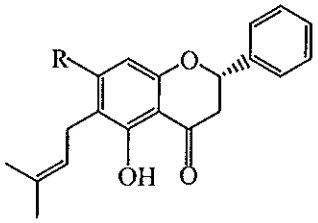
4g : (3S)-2′-*O*-methyl vestitol

h. Flavanone



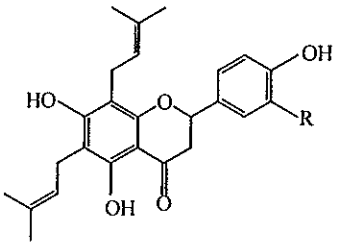
11h : R_1= H, R_2= OH : 3′,4′-Methylenedioxy-5-hydroxy-6-methoxyfurano[7,8:2″,3″] flavanone

13h : R_1= R_2= OMe : 3′,4′-Methylenedioxy-3,5,6- trimethoxyfurano[7,8:2″,3″] flavanonol



31h : R= OH : 5,7-Dihydroxy-6-prenylflavanone

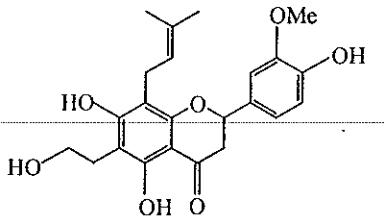
94h : R= OH : 5-Hydroxy-7-methoxy-6-prenylflavanone



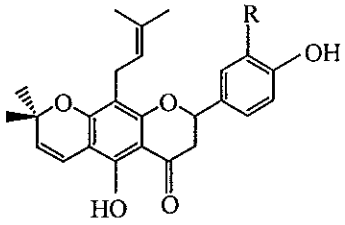
41h : R= OH : 3′,4′,5,7-Tetrahydroxy-6,8-diprenylflavanone

42h : R= OMe : Hiravanone

44h : R= H : Lonchocarpol A

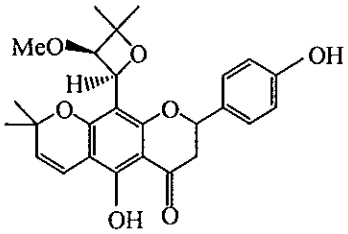


43h : Laxiflorin

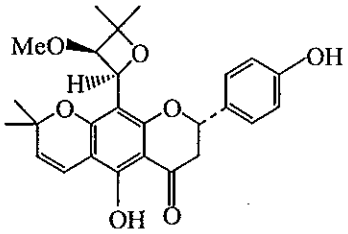


45h : R = H : Lupinifolin

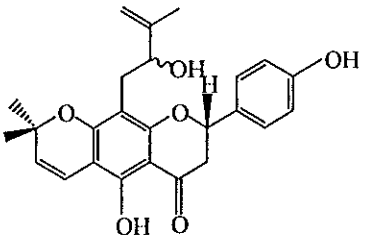
50h : R = OMe : 3′-Methoxylupinifolin



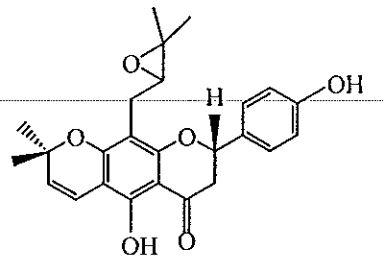
53h : Derriflavanone



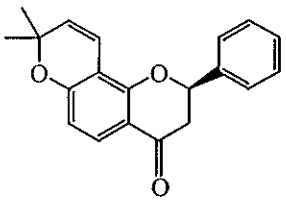
54h : Derriflavanone



95h : Dereticulatin

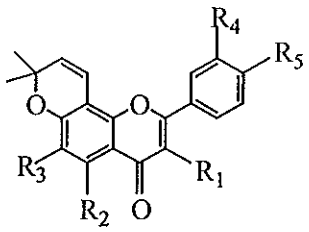


96h : 2‴,3‴-Epoxylupinifolin



145h : Isolonchocarpin

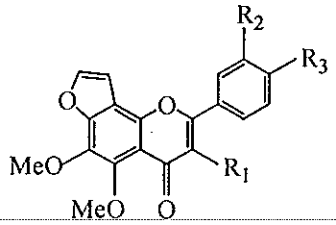
i. Flavone



7i : R_1= R_3= OMe, R_2= R_4= R_5= H :
3,6-Dimethoxy- 6″,6″-dimethylchromeno [7,8:2″,3″]flavone

8i : R_2= H, R_1= R_3= OMe, R_4+ R_5= OCH_2O :
3,6-Dimethoxy-3′,4′-methylenedioxy-6″,6″-dimethylchromeno[7,8:2″,3″]flavone

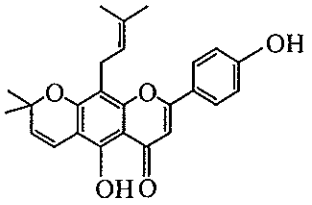
88i : R_2= OH, R_1= R_3= R_4= R_5= H :
5-Hydroxy-6″,6″-dimethylchromeno [7,8:2″,3″]flavone



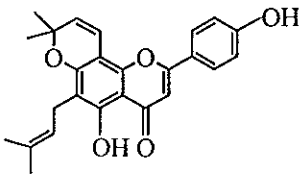
9i : R_1= H, R_2+ R_3= OCH_2O :
3′,4′-Methylenedioxy-5,6-dimethoxyfurano [7,8:2″,3″]flavone

12i : R_1= OMe, R_2+ R_3= OCH_2O :
3′,4′-Methylenedioxy-3,5,6-trimethoxyfurano [7,8:2″,3″]flavone

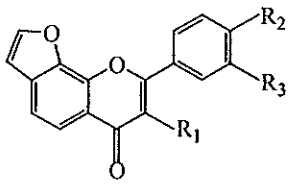
15i : R_1= OMe, R_2= R_3= H :
3,5,6-Trimethoxyfurano[7,8:2″,3″]flavone



48i : Isolaxifolin



49i : Laxifolin



59i : R_1= R_2= OMe, R_3= H : 3,4′-Dimethoxyfurano [7,8:4″,5″]flavone

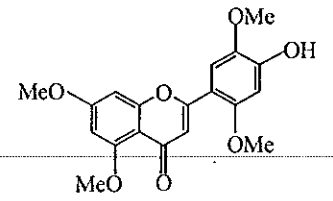
60i : R_1= OMe, R_2= R_3= H : Karanjin

61i : R_1= R_2= R_3= H : Lanceolatin B

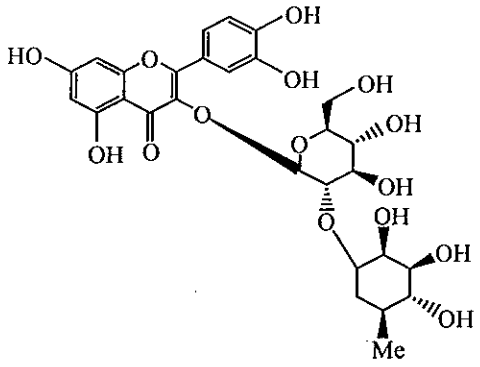
62i : R_1= R_3= H, R_2= OMe : 4′-Methoxyfurano[7,8:4″,5″]flavone

63i : R_1= H, R_2+ R_3= OCH_2O : Pongaglabrone

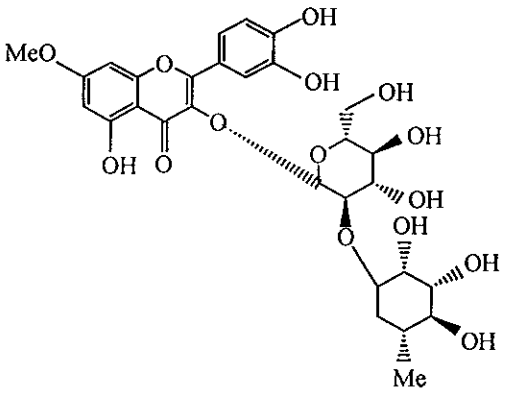
64i : R_1= OMe, R_2+ R_3= OCH_2O : Pongapin



97i : 4′-Hydroxy-3′,5,6′,7-tetramethoxyflavone

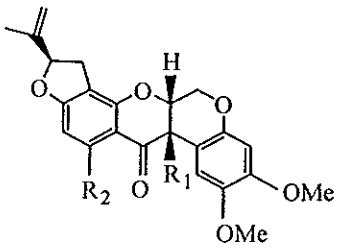


159i : Quercetin-3-*O*-β-neohesperidoside



160i : Rhamnetin-3-*O*-β-neohesperidoside

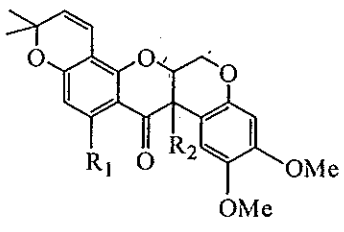
j. Isoflavone



6j : R_1= R_2= H : Rotenone

57j : R_1= H, R_2= OH : Sumatrol

77j : R_1= OH, R_2= H :
12a-Hydroxyrotenone

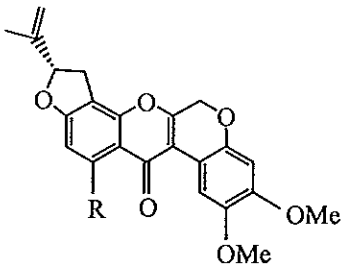


20j : R_1= R_2= H : Deguelin

24j : R_1= H, R_2= OH : Tephrosin

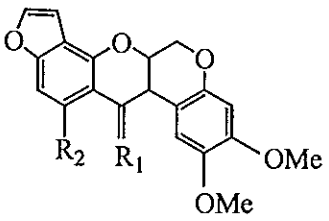
28j : R_1= OH, R_2= H : α-Toxicarol

78j : R_1= R_2= OH : 11-Hydroxytephrosin



21j : R= H : 6a,12a-Dehydrorotenone

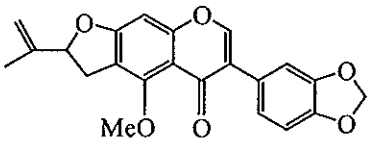
71j : R= OH : Villosol



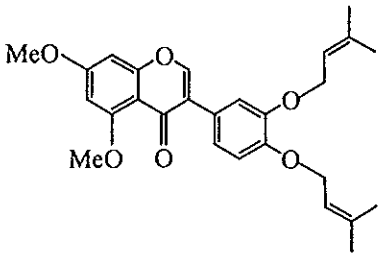
22j : R_1= H, α- OH, R_2= H : Elliptinol

23j : R_1= O, R_2= H : Elliptone

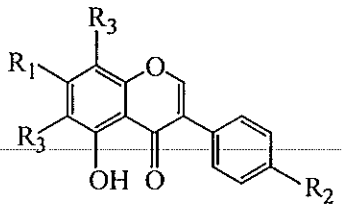
56j : R_1= O, R_2= OH : Malaccol



39j : Glabrescione A



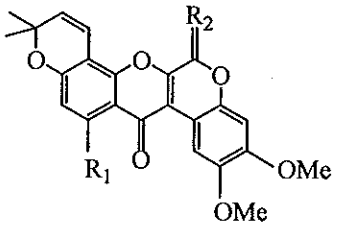
40j : Glabrescione B



51j : R_1= OMe, R_2= OH, R_3= H : Prunetin

103j : R_1= OMe, R_2= R_3= H :
5-Hydroxy-7-methoxyisoflavone

123j : R_1= R_2= OH, R_3= isoprenyl :
4′,5,7-Trihydroxy-6,8-diprenylisoflavone



65j : R_1= OH, R_2= H_2 :

6a,12a-Dehydro-α-toxicarol

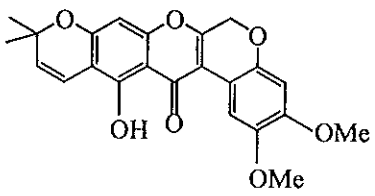
73j : R_1= H, R_2= H_2 : 6a,12a-Dehydrodeguelin

76j : R_1= OH, R_2= H, OH :

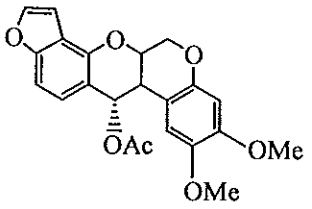
6-Hydroxy-6a,12a-dehydro-α-toxicarol

80j : R_1= OH, R_2= O :

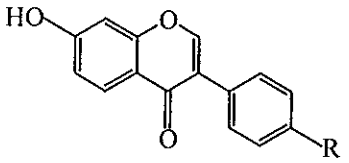
6-Oxo-6a,12a-dehydro-α-toxicarol



66j : 6a,12a-Dehydro-β-toxicarol

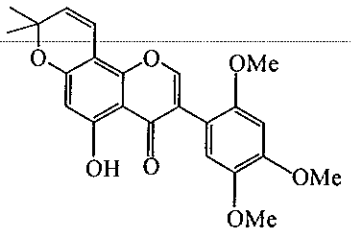


70j : 12-Deoxo-12α-acetoxyelliptone

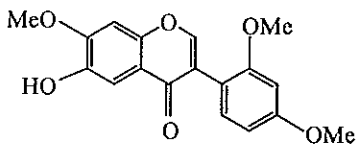


72j : R= OH : Daidzein

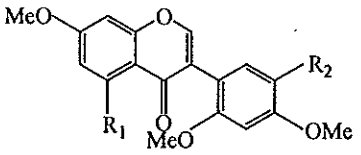
75j : R= OMe : Formononetin



83j : Toxicarolisoflavone



98j : 6-Hydroxy-2′,4′,7-trimethoxyisoflavone



101j : R_1= OH, R_2= OMe : Robustigenin

104j : R_1= R_2= OMe :

Robustigenin-5-*O*-methyl ether

105j : R_1= R_2= OH : Derrugenin

107j : R_1= R_4= OMe, R_2= R_3= OH :

O,*O*-Dimethylalpinumisoflavone

109j : R_1= OH, R_2= H, R_3+ R_4= OCH_2O :

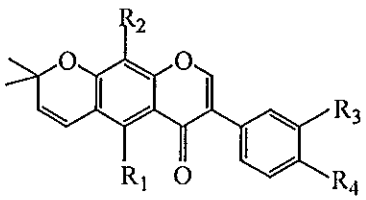
Robustone

110j : R_1= OMe, R_2= H, R_3+ R_4= OCH_2O :

Robustone methyl ether

120j : R_1= R_4= OH, R_2= $(CH_2)_2C(CH_3)_2OCH_3$,

R_3= H : Eturunagarone



121j : R_1= R_4= OH, R_2= isoprenyl, R_3= H :

Scandenone

142j : R_1= R_4= OH, R_2= H, R_3= isoprenyl :

Chandalone

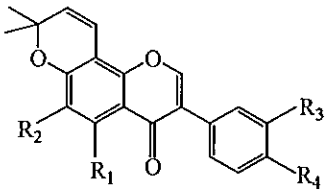
147j : R_1= R_4= OH, R_2= R_3= H : Alpinumisoflavone

148j : R_1= OH, R_2= R_3= H, R_4= OMe :

Alpinumisoflavone-4′-methyl ether

149j : R_1= OH, R_2= R_3= H, R_4= $OCH_2CH=C(CH_3)_2$:

4′-*O*-*γ*,*γ*- Dimethylallylalpinumisoflavone



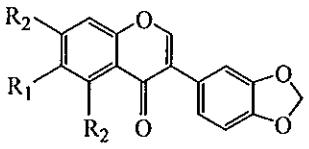
111j : R_1= R_4= OH, R_2= R_3= H : Derrone

112j : R_1= OH, R_2= R_3= H, R_4= OMe : Derrone-4′-*O*-methyl ether

122j : R_1= OMe, R_2= isoprenyl, R_3= H, R_4= OH : Scandinone

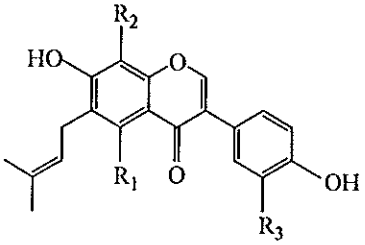
139j : R_1= R_4= OH, R_2= isoprenyl, R_3= H : Osajin

152j : R_1= OH, R_2= H, R_3+ R_4= OCH_2O : 3′,4′-Methylenedioxy-5-hydroxy-2″,2″-dimethylchromeno[7,8:6″,5″]isoflavone



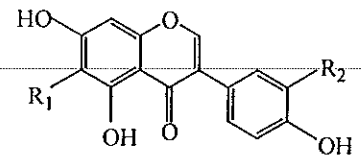
116j : R_1= isoprenyl, R_2= OH : Derrubone

117j : R_1= H, R_2= OMe : Derrustone



119j : R_1= OH, R_2= H, R_3= isoprenyl : 3′-γ,γ-Dimethylallylwighteone

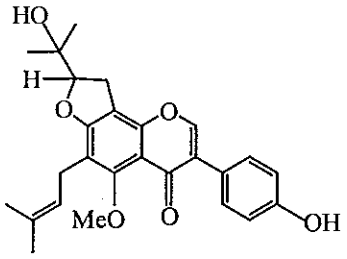
124j : R_1= OMe, R_2= isoprenyl, R_3= H : Derrisisoflavone A



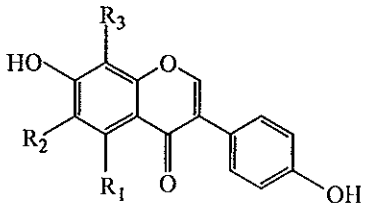
125j : R_1= isoprenyl, R_2= $CH_2CH(OH)C(CH_2)CH_3$: Derrisisoflavone B

131j : R_1= R_2= isoprenyl : Lupalbigenin

133j : R_1= $CH_2CH(OH)C(CH_2)CH_3$, R_2= isoprenyl : Lupinisol A



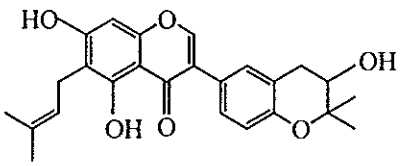
126j : Derrisisoflavone C



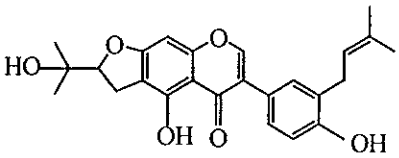
127j : R_1= OMe, R_2= $CH_2CH(OH)C(CH_2)CH_3$,
R_3= isoprenyl : Derrisisoflavone D

128j : R_1= OMe, R_2= isoprenyl,
R_3= $CH_2CH(OH)C(CH_2)CH_3$:
Derrisisoflavone E

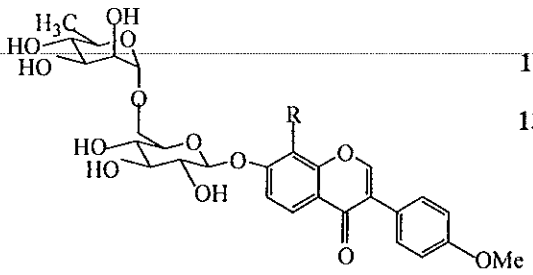
130j : R_1= OH, R_2= isoprenyl,
R_3= $CH_2CH(OH)C(CH_2)CH_3$:
Erysenegalensein E



129j : Derrisisoflavone F

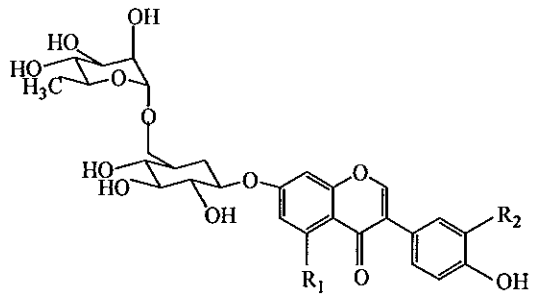


132j : Lupinisoflavone G



134j : R = OH : Derriscanoside A

135j : R = OMe : Derriscanoside B

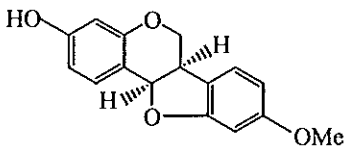


136j : R_1= R_2= H : Daidzein-7-*O*-rhamnosyl(1→6)glucoside

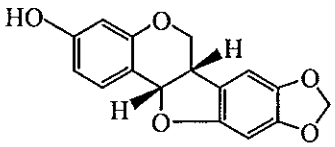
137j : R_1= OH, R_2= H : Genistein-7-*O*- rhamnosyl(1→6)glucoside

138j : R_1= OH, R_2= OH : Orobol-7-*O*-rhamnosyl(1→6)glucoside

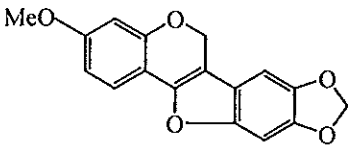
k. Pterocarpan



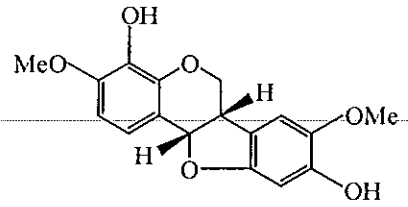
1k : (6aS, 11aS)-Dimethylhomopterocarpin



27k : Maackiain

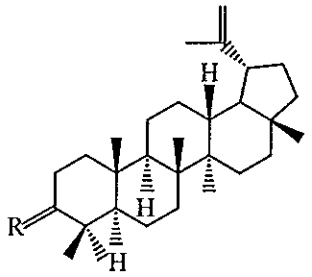


47k : Flemichapparin B



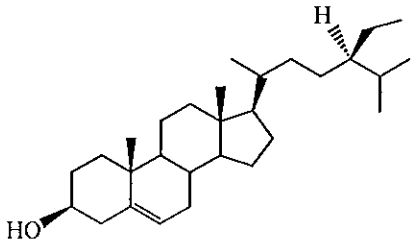
67k : Derricarpin

I. Steroid

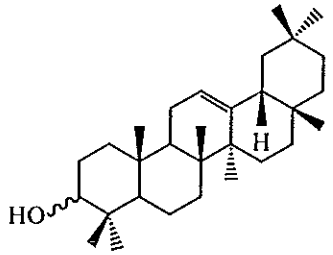


2l : R = O : Lupenone

3l : R = β-OH, H : Lupeol

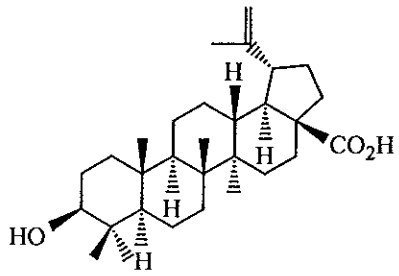


5l : β-Sitosterol

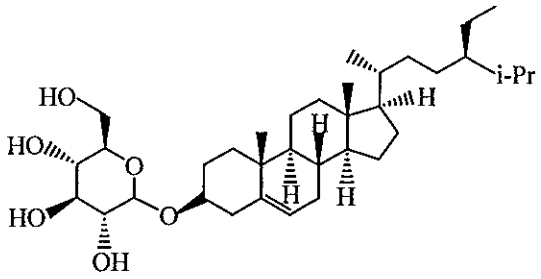


46l : β-3-OH : β-Amyrin

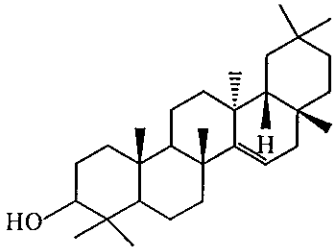
150l : α-3-OH : α-Amyrin



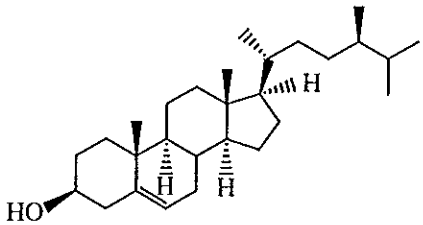
58l : Betulinic acid



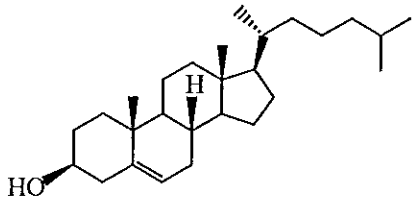
106l : Daucosterol



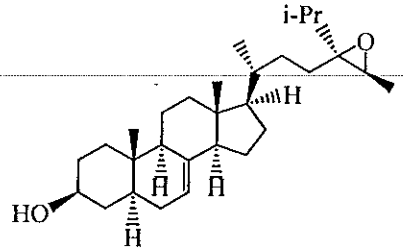
146l : Dehydrotoxicarol



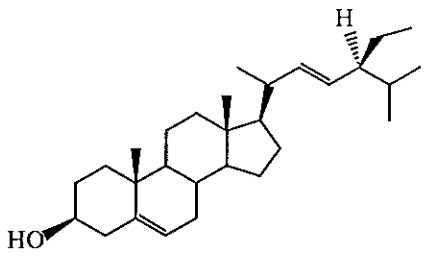
155l : Campesterol



156l : Cholesterol

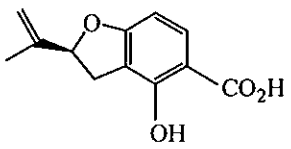


157l : Stigmast-7-en-3-βol

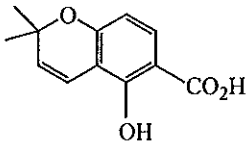


158l : Stigmasterol

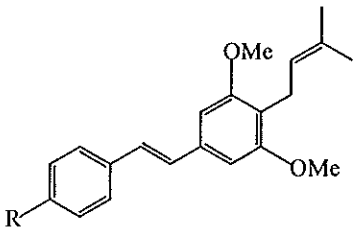
m. Miscellaneous



25m : Tubaic acid

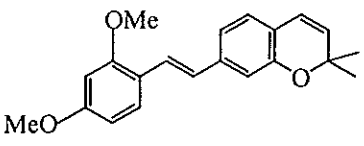


26m : β-Tubaic acid

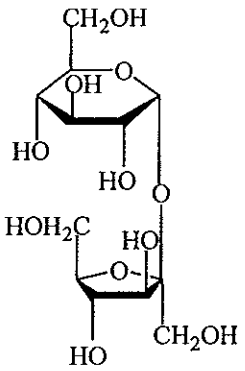


32m : 3,5-Dimethoxy-4-prenylstilbene

36m : 3,4′,5-Trimethoxy-4-prenylstilbene



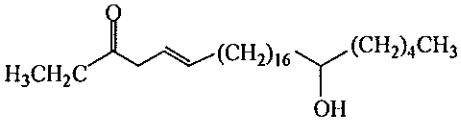
93m : 3,5-Dimethoxy-4-prenylstilbene



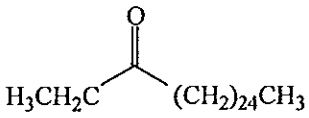
82m : Sucrose

$H_3C(CH_2)_{25}CH_2OH$

87m : 1-Heptacosanol



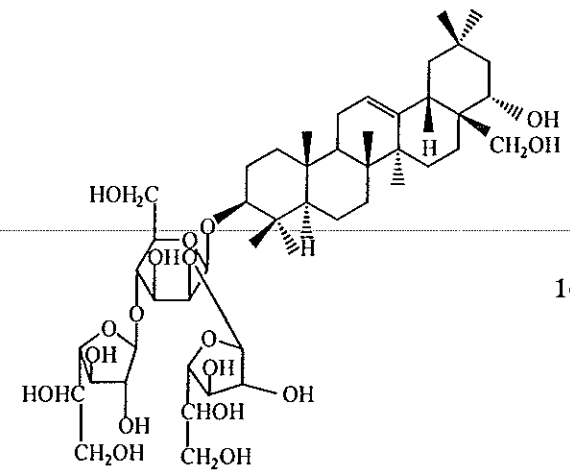
99m : 23-Hydroxyoctacos-5-en-3-one



100m : Octacosan-3-one

$H_3C(CH_2)_{24}CH_2OH$

161m : 1-Hexacosanol



162m : Derrissaponin

1.3 Biological activities from Derris species

D. scandens Benth. was known as an Asian medicinal plants and folk medicines. In Thailand, its dried stem is used for expectorant, antitussive, diuretic, antidysentery and for the treatment of muscle ache and pain, while the root is used as a fish poison (Chavalittumrong, *et al.*, 1999). It was recently found that warangalone, robustic acid, 8-γ,γ-dimethylallylwighteone, and 3′-γ,γ-dimethylallylwighteone are selective and potent inhibitors of rat liver cyclic AMP-dependent protein kinase catalylic subunit (cAK) (Wang, *et al.*, 1997). On the other hand, *n*-butanol extract had hypotensive activity in the rats (Jansakul, *et al.*, 1997) while 50 % ethanolic extract showed marked *in vitro* immunomodulating activity in mouse splenic lymphocytes (Chuthaputti and Chavalittumrong, 1998). In addition, the hydroalcoholic extract exhibited *in vitro* immunomodulating activity on human immunocompetent and immunocompromised peripheral blood mononuclear cells (PBMC) (Sriwanthana and Chavalittumrong, 2001).

However, the importance of *Derris* plants in traditional medicine throughout the tropical world is apparent from print-out of the NAPRALERT database. The significant biological activities of the extracts of *Derris* species are summarized in Table 2 and the important ethnomedical applications are summarized in Table 3.

Table 2 Biological activities of *Derris* species

Scientific name	Type of biological activity	Bibliography
D. araripensis		
- dried stem barks	Mollouscicidal activity	Pinheiro and Rouquayrol, 1974
D. elliptica		
- dried entire plant	Cytotoxic activity	Thai Farmer Bank, 1982
- dried leaves	Antibacterial activity	Blech, *et al.*, 1992
- fresh barks	Fish poison	Kulakkattolickal, 1987
- dried roots	Fish poison	Mc Cullough, *et al.*, 1980
	Mollouscicidal activity	
	Toxicity assessment	Haag, *et al.*, 1943
	Insecticide activity	Shin-Foon, 1985
	Mollouscicidal activity	Maimi and Morallo-Rejesus, 1980
	Antifungal activity	Soytong, *et al.*, 1985
	Antiyeast activity	
	Fungal stimulant	
	Histaminergic effect	Mokkhasmit, *et al.*, 1971
	Hypotensive activity	
	Toxic effect (general)	Mokkhasmit, *et al.*, 1971
- roots	Insecticide activity	Tattersfield and Potter, 1940
		Yamaguchi, *et al.*, 1950

Table 2 (continued)

Scientific name	Type of biological activity	Bibliography
	Fish poison	Gaudin and Vacherat, 1938
		Tooby, *et al.*, 1975
-	Insect repellent activity	Morimoto, *et al.*, 1999
	Antiimplantation effect	Matsui, *et al.*, 1971
	Anticrustacean activity	Rahmani, *et al.*, 1992
	Antitumor activity	
D. fordii		
-	Insecticide activity	Chin, *et al.*, 1944
- dried roots	Insecticide activity	Tattersfield, *et al.*, 1948
D. heptaphylla		
- dried aerial parts	Analgesic activity	Bhakuni, *et al.*, 1988
	Antibacterial activity	
	Anticonvulsant activity	
	Antifungal activity	
	Antiprotozoan activity	
	Antiviral activity	
	Antiyeast activity	
	Cytotoxic activity	
	Diuretic activity	
	Hypothermic activity	
	Spasmolytic activity	
	Toxicity assessment (quantitative)	

Table 2 (continued)

Scientific name	Type of biological activity	Bibliography
D. laxiflora		
- dried leaves + twigs	Protein tyrosine kinase inhibition	Kim, *et al.*, 1995
D. malaccensis		
- dried leaves	Antimutagenic activity	Ishii, *et al.*, 1984
- fresh roots	Antinematodal activity	Alen, *et al.*, 2000
D. negrensis		
- dried roots	Glutamate-transferase inhibition	Vianna, *et al.*, 1979
	Succinate oxidase inhibition	
	Toxic effect (general)	
	Toxic assessment (quantitative)	
D. nicou		
-	Cytotoxic activity	Sampaio, *et al.*, 1984
D. obtusa		
- dried leaves	Molluscicidal activity	Pinheiro De Sousa and Rouquayrol, 1974
D. scandens		
- aerial parts	Abortifacient effect	Dhawan, *et al.*, 1977
	Analgesic activity	
	Antibacterial activity	
	Anticonvulsant activity	
	Antifungal activity	
	Antiimplantation effect	
	Antiinflammatory activity	

Table 2 (continued)

Scientific name	Type of biological activity	Bibliography
	Antispasmodic activity	
	Antiyeast activity	
	Barbiturate potentiation	
	Diuretic activity	
	Hypoglycemic activity	
	Hypothermic activity	
	Semen coagulation	
	Spermicidal effect	
	Toxicity assessment (quantitative)	
- dried stems	Histaminergic effect	Mokkhasmit, *et al.*, 1971
	Hypotensive activity	
	Smooth muscle stimulant activity	
	Leukotriene B-4 production	Hoult, *et al.*, 1997
	inhibition	
	Thromboxane B-2 synthesis	
	inhibition	
	Toxic effect (general)	Mokkhasmit, *et al.*, 1971
D. trifoliata		
- entire plant	Cytotoxic activity	Anon, 1976
D. uliginosa		
- roots	Insecticide activity	Bose, *et al.*, 1976
	Fish poison	Gaudin and Vacherat,
	Toxic effect (general)	1938

Table 3 Ethnomedical applications of *Derris* species

Scientific name	Ethnomedical application	Bibliography
D. amazonica		
- dried roots	Fish poison	Moretti and Grenand, 1982
D. elliptica		
- dried roots	Blood purification	Mokkhasmit, *et al.*, 1971
	Leprosy	Wasuwat, 1967
	Antipyretic	Mokkhasmit, 1971
- fresh roots	Treat the sting of a stonefish	Holdsworth, 1990
- roots	Abortifacient	Rutter, 1929
		Quisumbing, 1951
		Gimlette, 1939
		Burkill, 1966
	Insecticide	Nayar, 1955
	Fish poison	Gaudin and Vacherat, 1938
		Pickard and Cox, 1986
D. ferruginea		
- dried roots	Insecticide	Nayar, 1955
D. indica		
- fresh barks + leaves	Contusions	Sabnis and Bedi, 1983
	Relax pulled muscles	
- dried pods	Waist thread	Ramachandran and Nair, 1981
- dried seeds	Fish poison	Joshi, 1986

Table 3 (continued)

Scientific name	Ethnomedical application	Bibliography
D. malaccensis		
- dried roots	Fish poison	Pickard and Cox, 1986
	Leprosy	Wasuwat, 1967
D. pterocarpus		
- dried leaves	Fish poison	Moretti and Grenand, 1982
D. robusta		
- fresh roots	Root juice is mixed with the juice of sida acuta and used for sore throat	Alam, 1992
D. scandens		
- dried entire plant	Fish poison	Kapoor and Kapoor, 1980
- fresh roots	After childbirth if the mother has scanty or no milk secretion, the root crushed with or without water and the juice given orally increases milk secretion	Bennet, 1978
- dried stems	Rheumatism	Hoult, *et al.*, 1997
	Analgesic	Mokkhasmit, *et al.*, 1971
	Cathartic	Wasuwat, 1967
	Antipyretic	Mokkhasmit, *et al.*, 1971

Table 3 (continued)

Scientific name	Ethnomedical application	Bibliography
D. species		
- roots west	Insecticide	Ayensu, 1978
- fresh roots	Poison antidote, a vomit inducer	Holdsworth, 1974
D. spruceana		
- dried leaves	Fish poison	Moretti and Grenand, 1982
D. trifoliata		
- dried entire plant	Antispasmodic Counter-irritant Stimulant	Nair and Seetharaman, 1986
- dried roots	Fish poison	Pickard and Cox, 1986
D. uliginosa		
- dried roots	Fish poison	Pickard and Cox, 1986
- roots	Fish poison	Gaudin and Vacherat, 1938

Recently, natural antioxidants have attracted attention because some synthetic antioxidants have been found to be carcinogenic and harmful to lungs and liver (Yamasaki, *et al.*, 1994). Reactive oxygen species such as hydroxyl (OH•), peroxyl radicals (ROO•) and the superoxide anion (O_2•-) are constantly produced as a result of metabolic reactions in living systems (Wang, *et al.*, 1999). Living systems are protected from oxidative damage by these reactive species by enzymes such as superoxide dismutase and glutathione peroxidase and by antioxidant compounds such as ascorbic acid, tocopherols and carotenoids (Wang, *et al.*, 1999). However, when free-radical production exceeds the antioxidant capacity of the organism, these radical species attack lipids, proteins and DNA, thus damaging structural integrity and function of cell membranes, enzymes and genetic material (Wang, *et al.*, 1999). A growing body of evidence indicates that various pathological conditions, including cardiovascular disease, arthritis, various cancers and Alzheimer's disease, are associated, at least in part, with the damaging effects of uncontrolled free radical production (Wang, *et al.*, 1999).

Natural antioxidants occur in all higher plants and in all parts of the plant (wood, bark, stems, pods, leaves, fruits, roots, flowers and seeds). These are usually phenolic or polyphenolic compounds (Kim, *et al.*, 1997). Flavonoids have received the most attention and much is known about the structural requirements for antioxidant activity. Based on NAPRALERT database, the antioxidation activity of chemical constituents isolated from *Derris* species has not been investigated.

The stems of *D. scandens* has been used in Thai folk medicine in the form of decoction, thus it is of interest to investigate chemical constituents. Furthermore the stems of *D. scandens* has been previously reported to consist of phenolic constituents

(Table 1), it thus prompted us to evaluate the antioxidation activity of the chemical constituents in this plant. The results are detailed in the subsequent sections.

CHAPTER 2

EXPERIMENTAL

2.1 General method

Melting points were recorded in °C and were measured on a digital Electrothermal 9100 Melting Point Apparatus. Infrared spectra were recorded by using FTS 165 FT-IR spectrometer. Major bands (λ_{max}) were recorded in wave number (cm^{-1}). Ultraviolet (UV) absorption spectra were recorded using UV-160A spectrometer (SHIMADZU). Principal bands (λ_{max}) were recorded as wavelengths (nm) and log ε in methanol solution. The high resolution 500 MHz ^{1}H and ^{13}C NMR spectra were performed on a FTNMR, Varian UNITY INOVA spectrometer at Central Instrument Facilities, Prince of Songkla University. Spectra were recorded in deuterochloroform tetradeutero-methanol or hexadeutero-dimethyl sulphoxide solution and were recorded as δ value in ppm down field from TMS (internal standard δ0.00). Optical rotation was measured in methanol solution with sodium D line (590 nm) on an AUTOPOL^{R} II automatic polarimeter. High resolutiuon mass spectra were recorded on an AEI-MS9 at University of Sydney, Australia. Solvents for extraction and chromatography were distilled at their boiling point ranges prior to use. Solvents for crystallization were analytical grade reagent. Pre-coated TLC aluminum sheets of silica gel 60 PF_{254} (20x20 cm, layer thickness 0.2 mm) were use for analytical purposes and the compounds were visualized under ultraviolet light and / or anisaldehyde - sulfuric acid reagent. Plates of silica gel PF_{245}, 20 x 50 cm, thickness 1.25 nm,

activated at 110 °C for 3 h were utilized in the case of preparative TLC. Quick column chromatography was performed on silica gel 60 H (Merck). Column chromatography was performed by using silica gel (Merck) type 100 (70-230 mesh ASTM). DPPH (Fluka) were used for antioxidation activity testing and the absorbance were measured by spectronic 21 (MILTON ROY).

2.2 Plant material

Stems of *Derris scandens* Benth. were collected from Phang-nga province in the Southern part of Thailand. The voucher specimen was identified by Professor Puangpen Sirirugsa and has been deposited at Prince of Songkhla University Herbarium, Biology Department, Faculty of Science, Prince of Songkhla University, Thailand.

2.3 Extraction and Isolation

Chopped dry stems of *Derris scandens* (6.5 kg) was immersed at room temperature in acetone (5 day) and methanol (3 day), respectively. After evaporation, the viscous crude acetone extract (331.3 g) and crude methanolic extract (363.5g) were obtained.

The crude methanolic extract (363.5 g) was separated into two fractions, by dissolving in the mixture of ethyl acetate and water, ethyl acetate soluble fraction (251.5 g)and water soluble fraction were obtained. The residual after removal of ethyl acetate was further dissolved in dichloromethane, the soluble (Crude I, 168.8 g) and insoluble (Crude II, 82.6 g) portions were obtained. The process of extraction was shown in Figure 2.

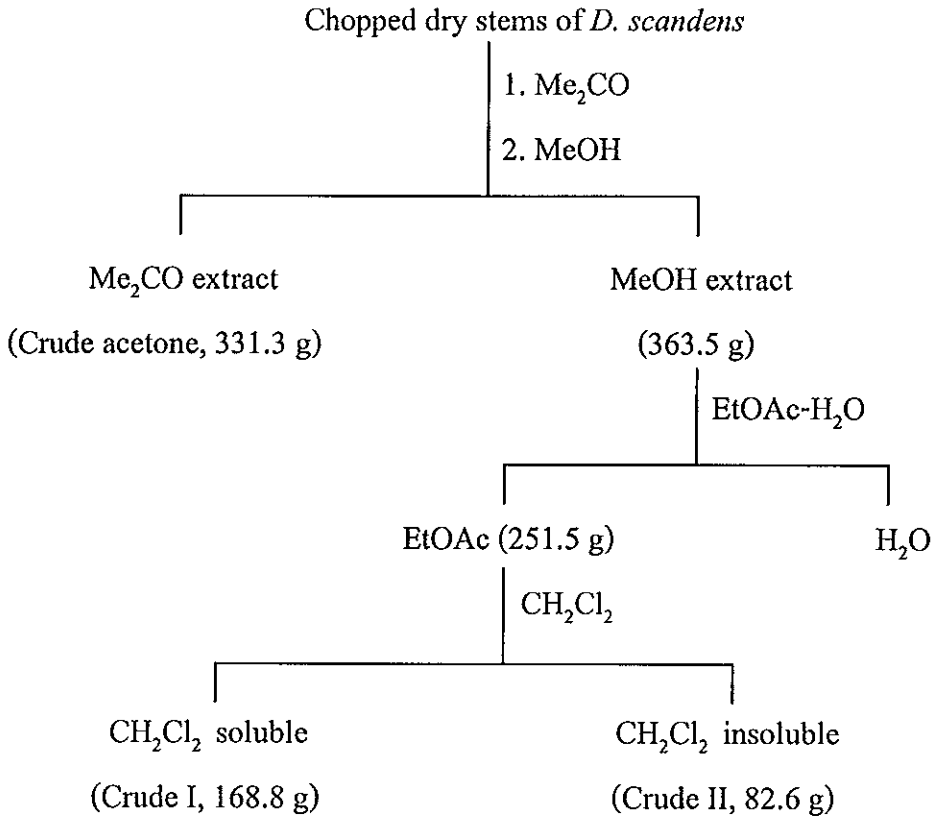


Figure 2 Extraction of Crude I, II and Crude acetone from stems of *D. scandens*

2.3.1 Purification of Crude I

A portion of Crude I (30 g) was subjected to quick column chromatography using silica gel as the stationary phase and eluted with hexane, hexane-benzene, dichloromethane, dichloromethane-chloroform, chloroform and then with chloroform-acetone. On the basis of their TLC characteristic, the collected fractions (250 mL each) which contained the same major components were combined, fractions A1-A10 were obtained (Table 4). The selected fraction were further purified to obtain eight pure compounds as shown in Figure 3.

Table 4 Physical characteristic and weight of fractions obtained from QCC

Fraction	Weight (g)	Physical characteristic
A1	6.658	white solid mixed with deep yellow viscous liquid
A2	1.242	yellow viscous liquid
A3	2.997	yellow solid mixed with deep yellow viscous liquid
A4	0.032	yellow solid
A5	0.240	yellow viscous liquid
A6	2.330	deep yellow viscous liquid
A7	2.146	yellow solid mixed with deep yellow viscous liquid
A8	7.760	deep yellow viscous liquid
A9	0.670	brown viscous liquid
A10	3.039	brown viscous liquid

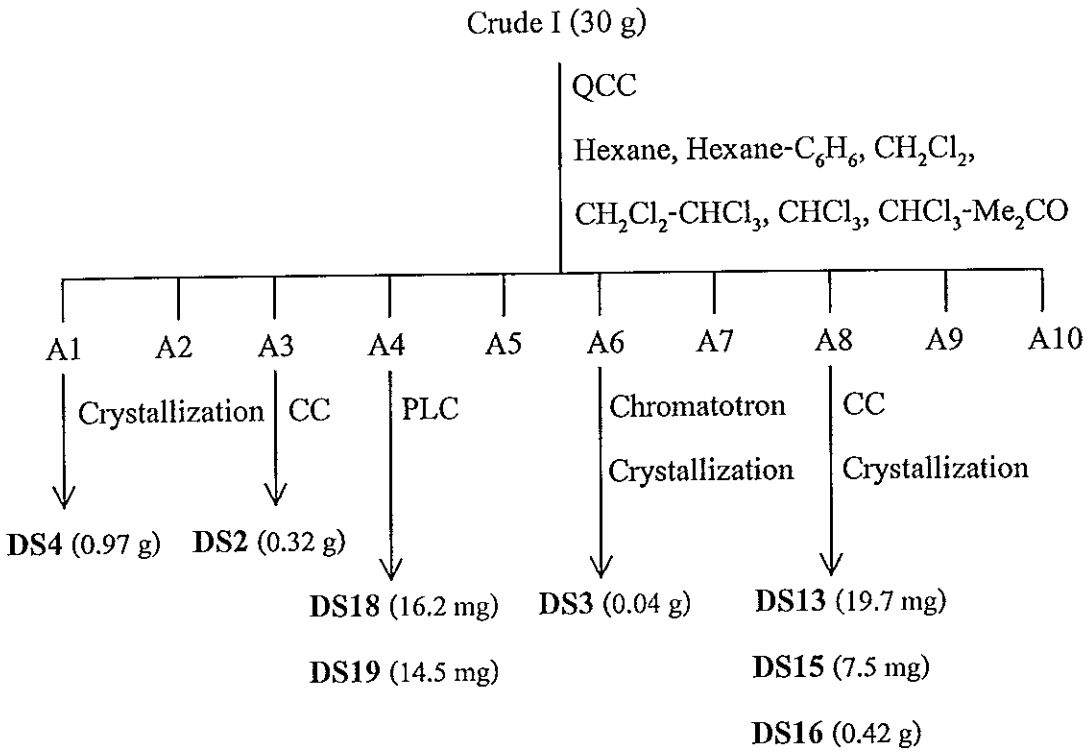


Figure 3 Isolation of compound **DS2-4, 13, 15-16** and **18-19** from Crude I

Isolation of compound DS4

Fraction A1 was recrystallized in hexane to give a white solid of **DS4** (0.97 g).

Melting point : 92-93 °C

UV (CH_3OH) λ_{max} nm (log ε) : 356.5 (4.50), 337.5 (4.52), 216.0 (4.94)

IR (KBr) ν (cm^{-1}) : 1609, 1571 (C=C stretching)

^{1}H NMR ($CDCl_3$) (δ ppm) : 7.36 (1H, *d*, *J* = 8.3 Hz), 7.01 (1H, *s*), 6.71 (1H, *s*), 6.53 (1H, *dd*, *J* = 8.3, 1.9 Hz), 6.50 (1H, *d*, *J* = 1.9 Hz), 5.90 (2H, *s*), 5.50 (2H, *s*), 3.80 (3H, *s*)

^{13}C NMR ($CDCl_3$) (δppm) : 160.15, 154.93, 150.48, 147.71, 145.63, 144.74, 120.91, 119.16, 109.89, 107.21, 106.34, 102.49, 101.39, 97.24, 94.06, 65.56, 55.50

DEPT (135 °) ($CDCl_3$) : 55.50 (CH_3); 101.39, 65.56 (CH_2); 120.91, 107.21, 102.49, 97.24, 94.06 (CH); 160.15, 154.93, 150.48, 147.71, 145.63, 144.74, 119.16, 109.89, 106.34 (C)

EIMS m/z (% relative intensity) : 296 ($[M]^+$,100), 281 (19), 252 (9), 225 (6), 148 (17), 139 (15), 119 (12), 87 (6), 69 (6), 63 (9)

Isolation of compound DS2

Fraction A3 was rechromatographed on column chromatography and eluted with 50% hexane-dichloromethane. The major component of this fraction, **DS2**, was obtained and recrystallized in the mixed solvent of dichloromethane and hexane. The yellow solid of **DS2** were collected (0.32 g).

Melting point : 149-150 °C

UV (CH_3OH) λ_{max} nm (logε) : 266.5 (4.55), 217.0 (4.50)

IR (KBr) ν (cm^{-1}) : 3255 (O-H stretching), 1655 (C=O stretching)

^{1}H NMR ($CDCl_3$) (δ ppm) : 13.22 (1H, *s*), 7.82 (1H, *s*), 7.23 (1H, *d*, *J* = 1.9 Hz), 7.22 (1H, *d*, *J* = 6.4 Hz), 6.83 (1H, *dd*, *J* = 6.4, 1.9 Hz), 6.36 (1H, *s*), 5.33 (1H, *t*-

like, J = 7.0 Hz), 5.28 (1H, *t-like*, J = 7.0 Hz), 3.45 (2H, *d*, J = 7.0 Hz), 3.37 (2H, *d*, J = 7.0 Hz), 1.83 (3H, *s*), 1.77 (6H, *s*), 1.76 (3H, *s*)

^{13}C NMR ($CDCl_3$) (δ ppm) : 181.04, 161.40, 159.60, 156.15, 154.69, 152.65, 136.01, 135.05, 130.60, 128.23, 127.11, 123.66, 123.08, 121.53, 121.06, 115.95, 110.03, 105.87, 93.99, 29.83, 25.83, 25.79, 21.50, 17.92

DEPT (135 °) ($CDCl_3$) : 25.79, 17.92 (CH_3); 29.83, 21.50 (CH_2); 152.65, 130.60, 128.23, 121.53, 121.06, 115.95, 93.99 (CH); 161.40, 159.60, 156.15, 154.69, 136.01, 135.05, 127.11, 123.66, 123.08, 110.03, 105.87 (C); 181.04 (C=O)

EIMS m/z (% relative intensity) : 406 ($[M]^+$, 94), 389 (19), 363 (85), 351 (100), 321 (11), 308 (25), 295 (28), 283 (18), 167 (10), 149 (7), 131 (7), 115 (7), 91 (7), 69 (34)

Isolation of compound DS18 and DS19

Fraction A4 was further purified by preparative TLC on silica gel plates and eluted with 50% hexane-dichloromethane (3 elutions) to give two isolated bands.

The component from the first band was recrystallized in benzene to afford yellow solid of **DS18** (16.2 mg).

Melting point : 132-133 °C

UV (CH_3OH) λ_{max} nm (log ε) : 282.8 (3.99), 258.6 (3.62), 212.0 (4.13)

IR (KBr) ν (cm^{-1}) : 3433 (O-H stretching), 1624 (C=O stretching)

^{1}H NMR ($CDCl_3$) (δppm) : 12.22 (1H, *s*), 11.81 (1H, *s*), 7.40 (1H, *d*, J = 8.4 Hz), 6.81 (1H, *s*), 6.53 (1H, *s*), 6.51 (1H, *d*, J = 2.2 Hz), 6.45 (1H, *dd*, J = 8.8, 2.2 Hz), 5.99 (2H, *s*), 3.87 (3H, *s*)

^{13}C NMR ($CDCl_3$) (δ ppm) : 194.84, 194.40, 168.34, 167.40, 164.84, 156.86, 141.63, 134.32, 111.02, 109.58, 109.25, 108.02, 102.54, 101.31, 99.11, 55.72

DEPT (135 °) ($CDCl_3$) : 55.72 (CH_3); 102.54 (CH_2); 134.32, 109.25, 108.02, 101.31, 99.11 (CH); 168.34, 167.40, 164.84, 156.86, 141.63, 111.02, 109.58 (C);

194.84, 194.40 (C=O)

EIMS m/z (% relative intensity) : 316 ($[M]^+$, 15), 298 (16), 165 (55), 151 (100), 149 (9), 137 (5), 95 (10), 79 (5)

The compound from the second band, **DS19**, was obtained as a yellow solid (14.5 mg).

Melting point : 251-252 °C

UV (CH_3OH) λ_{max} nm (log ε) : 347.4 (4.05), 245.2 (3.86), 213.0 (4.20)

IR (KBr) ν (cm^{-1}) : 1627 (C=O stretching)

^1H NMR ($CDCl_3$) (δ ppm) : 7.85 (1H, *d*, *J* = 7.2 Hz), 7.47 (1H, *s*), 7.12 (1H, *s*), 6.98 (1H, *dd*, *J* = 7.2, 2.7 Hz), 6.96 (1H, *d*, *J* = 2.7 Hz), 6.07 (2H, *s*), 3.91 (3H, *s*)

^{13}C NMR ($CDCl_3$) (δ ppm) : 162.95, 160.47, 158.94, 155.30, 150.99, 147.79, 146.52, 122.54, 117.33, 113.22, 106.37, 104.19, 102.10, 101.59, 100.32, 94.10, 55.69

DEPT (135 °) ($CDCl_3$) : 55.69 (CH_3); 102.10 (CH_2); 122.54, 113.22, 101.59, 100.32, 94.10 (CH); 162.95, 160.47, 155.30, 150.99, 147.79, 146.52, 117.33, 106.37, 104.19 (C); 158.94 (C=O)

EIMS m/z (% relative intensity) : 310 ($[M]^+$, 100), 295 (52), 239 (14), 183 (5), 155 (5)

Isolation of compound DS3

Fraction A6 was rechromatographed on silica gel column using dichloromethane as the eluent to give **DS3** as a yellow solid (0.04 g).

Melting point : 79-80 °C

UV (CH_3OH) λ_{max} nm (log ε) : 279.0 (4.42), 231.0 (4.22)

IR (KBr) ν (cm^{-1}) : 3450 (O-H stretching), 1656 (C=O stretching)

^1H NMR ($CDCl_3$) (δ ppm) : 12.98 (1H, *s*), 11.11 (1H, *s*), 9.98 (1H, *s*), 7.89 (1H, *s*), 7.83 (1H, *d*, *J* = 1.8 Hz), 7.68 (1H, *dd*, *J* = 9.1, 1.8 Hz), 7.09 (1H, *d*, *J* = 9.1 Hz), 6.74 (1H, *d*, *J* = 9.8 Hz), 6.36 (1H, *s*), 5.65 (1H, *d*, *J* = 9.8 Hz), 1.49

(6H, *s*)

^{13}C NMR ($CDCl_3$) (δppm) : 196.71, 180.71, 161.90, 160.06, 157.51, 157.08, 152.89, 137.45, 134.47, 128.60, 122.89, 122.49, 120.82, 118.31, 115.59, 106.21, 106.04, 95.26, 78.45, 28.59

DEPT (135 °) ($CDCl_3$) : 28.59 (CH_3); 152.89, 137.45, 134.47, 128.60, 118.31, 115.59, 95.26 (CH); 161.90, 160.06, 157.51, 157.08, 122.89, 122.49, 120.82, 106.21, 106.04, 110.03, 78.45 (C); 196.71, 180.71 (C=O)

EIMS m/z (% relative intensity) : 364 (20), 349 ($[M]^+$, 100), 300 (6), 271 (7), 255 (11), 213 (7), 174 (7), 135 (11), 97 (15), 83 (22), 69 (25), 55.0 (37)

Isolation of compound DS13, DS15 and DS16

Fraction A8 was rechromatographed on column chromatography and eluted with the mixed solvent of hexane-dichloromethane to give nine fractions (A8.1-A8.9).

Fraction A8.7 was rechromatographed on column chromatography and eluted with 20% dichloromethane-hexane. Two components, **DS13** and **DS15**, were obtained and crystallized in dichloromethane-hexane mixture. The yellow solid of **DS13** were collected (19.7 mg) and the white solid of **DS15** were obtained (7.5 mg).

DS13

Melting point : 123-124 °C

UV (CH_3OH) λ_{max} nm (log ε) : 267.5 (4.45), 217.0 (4.22)

IR (KBr) ν (cm^{-1}) : 3447 (O-H stretching), 1656 (C=O stretching)

^{1}H NMR ($CDCl_3$) (δ ppm) : 12.85 (1H, *s*), 7.88 (1H, *s*), 7.05 (1H, *d*, *J* = 3.8 Hz), 6.95 (1H, *dd*, *J* = 7.5, 3.8 Hz), 6.88 (1H, *d*, *J* = 7.5 Hz), 6.68 (1H, *d*, *J* = 10.0 Hz), 6.29 (1H, *s*), 6.00 (2H, *s*), 5.59 (1H, *d*, *J* = 10.0 Hz), 1.47 (6H, *s*)

^{13}C NMR ($CDCl_3$) (δ ppm) : 180.73, 162.28, 159.61, 152.43, 147.91, 147.80, 130.81, 127.47, 124.32, 123.74, 122.42, 114.51, 109.61, 108.48, 105.98, 101.23, 101.08, 100.39, 78.07, 28.18

DEPT (135 °) ($CDCl_3$) : 28.18 (CH_3); 101.23 (CH_2); 152.43, 127.47, 122.42, 114.51, 109.61, 108.48, 100.39 (CH); 162.28, 159.61, 147.91, 147.80, 130.81, 124.32, 123.74, 105.98, 101.08, 78.07 (C); 180.73 (C=O)

EIMS m/z (% relative intensity) : 364 ($[M]^+$, 40), 349 (100), 300 (6), 271 (16), 255 (10), 229 (10), 203 (16), 174 (18), 147 (10), 124 (16), 95 (18), 69 (22), 55 (36)

DS15

Melting point : 156-157 °C

Liebermann-Burchard ($CHCl_3$) : green colour (steroid)

Optical rotation : $[\alpha]_D^{28}$ -55.48 ° (c = 1.5 x 10^{-2} g/100cm^3, CH_3OH)

UV (CH_3OH) λ_{max} nm (log ε) : 208.5 (3.71)

IR (KBr) ν (cm^{-1}) : 3433, 3309 (O-H stretching), 2959, 2869 (C-H stretching)

^{1}H NMR ($CDCl_3$) (δ ppm) : 5.36-5.33 (1H, *m*), 5.16 (1H, *dd*), 5.02 (1H, *dd*), 3.56-3.48 (1H, *m*), 1.02 (3H, *s*), 1.05 (3H, *s*), 0.86 (3H, *s*), 0.82 (3H, *s*), 0.80 (3H, *s*), 0.69 (3H, *s*)

EIMS m/z (% relative intensity) : 412 ($[M]^+$, 35), 351 (21), 300 (27), 271 (47), 255 (69), 253 (40), 213 (64), 199 (47), 185 (40), 173 (40), 159 (82), 145 (100), 133 (67), 119 (51), 105 (93), 91 (96), 79 (79), 67 (46), 55 (38)

Fraction A8.8 was crystallized in the mixed solvent of hexane-dichloromethane (3:1). Compound **DS16** was obtained as a yellow solid (0.42 g).

Melting point : 115-116 °C

UV (CH_3OH) λ_{max} nm (log ε) : 283.5 (4.65), 249.5 (4.00)

IR (KBr) ν (cm^{-1}) : 3450 (O-H stretching), 1653 (C=O stretching)

^{1}H NMR ($CDCl_3$) (δ ppm) : 13.18 (1H, *s*), 7.80 (1H, *s*), 7.22 (1H, *d*, J = 2.1 Hz), 7.20 (1H, *dd*, J = 7.7, 2.1 Hz), 6.77 (1H, *d*, J = 7.7 Hz), 6.74 (1H, *d*, J = 9.8 Hz),

6.34 (1H, *s*), 5.88 (1H, *br s*), 5.63 (1H, *d*, *J* = 9.8 Hz), 5.34 (1H, *t-like*, *J* = 7.4 Hz), 3.37 (2H, *d*, *J* = 7.4 Hz), 1.77 (6H, *s*), 1.48 (6H, *s*)

^{13}C NMR ($CDCl_3$) (δppm) : 181.03, 159.47, 157.28, 156.81, 154.65, 152.59, 134.45, 130.40, 128.10, 128.03, 127.42, 123.78, 122.69, 121.69, 115.84, 115.44, 106.07, 105.51, 94.81, 78.00, 29.45, 28.25, 25.72, 17.83

DEPT (135 °) ($CDCl_3$) : 28.25, 25.72, 17.83 (CH_3); 29.45 (CH_2); 152.59, 130.40, 128.10, 128.03, 121.69, 115.84, 115.44, 94.81 (CH); 159.47, 157.28, 156.81, 154.65, 134.45, 127.42, 123.78, 122.69, 106.07, 105.51, 78.00 (C); 181.03 (C=O)

EIMS m/z (% relative intensity) : 404 ($[M]^+$, 23), 389 (100), 333 (14), 321 (14), 32 (7), 305 (5), 203 (9)

Acetylation of compound DS16

Compound **DS16** (46.5 mg) was acetylated with acetic anhydride in pyridine at room temperature overnight. The reaction mixture was worked up by pouring into ice water and then extracted with dichloromethane. The lower layer was separated and washed with 10% hydrochloric acid then water. The organic fraction was dried over anhydrous sodium sulfate and evaporated. The residue was purified on preparative TLC and eluted with dichloromethane to give derivative **DS16(A)** as a yellow viscous liquid (81.0 mg).

^{1}H NMR ($CDCl_3$) (δ ppm) : 13.10 (1H, *s*), 7.83 (1H, *s*), 7.39 (1H, *d*, *J* = 2.0 Hz), 7.37 (1H, *dd*, *J* = 8.0, 2.0 Hz), 7.09 (1H, *d*, *J* = 8.0 Hz), 6.73 (1H, *d*, *J* = 9.6 Hz), 6.33 (1H, *s*), 5.62 (1H, *d*, *J* = 9.6 Hz), 5.24 (1H, *t-like*, *J* = 6.4 Hz), 3.28 (1H, *d*, *J* = 6.4 Hz), 2.31 (3H, *s*), 1.73 (3H, *s*), 1.70 (3H, *s*), 1.47 (6H, *s*)

2.3.2 Purification of Crude II

Crude II (82.6 g) was fractionated by quick column chromatography using hexane, hexane-benzene, benzene, benzene-dichloromethane, dichloromethane-chloroform, chloroform-acetone and methanol as the eluents. The fractions containing similar components were combined into eleven fractions (B1-B11) (Table 5) and selected fractions were further purified to afford **DS6-7, 9-12, 20** and **22-23** (Figure 4).

Table 5 Physical characteristic and weight of fractions obtained from QCC

Fraction	Weight (g)	Physical characteristic
B1	0.817	pale yellow viscous liquid
B2	1.053	colourless solid mixed with yellow viscous liquid
B3	0.926	white solid mixed with orange viscous liquid
B4	7.882	yellow viscous solid
B5	16.855	brown viscous liquid
B6	3.142	yellow solid mixed with yellow viscous liquid
B7	4.677	white solid mixed with yellow viscous liquid
B8	5.290	white solid mixed with yellow viscous liquid
B9	9.961	brown viscous liquid
B10	1.152	white solid mixed with brown viscous liquid
B11	4.742	yellow solid mixed with brown viscous liquid

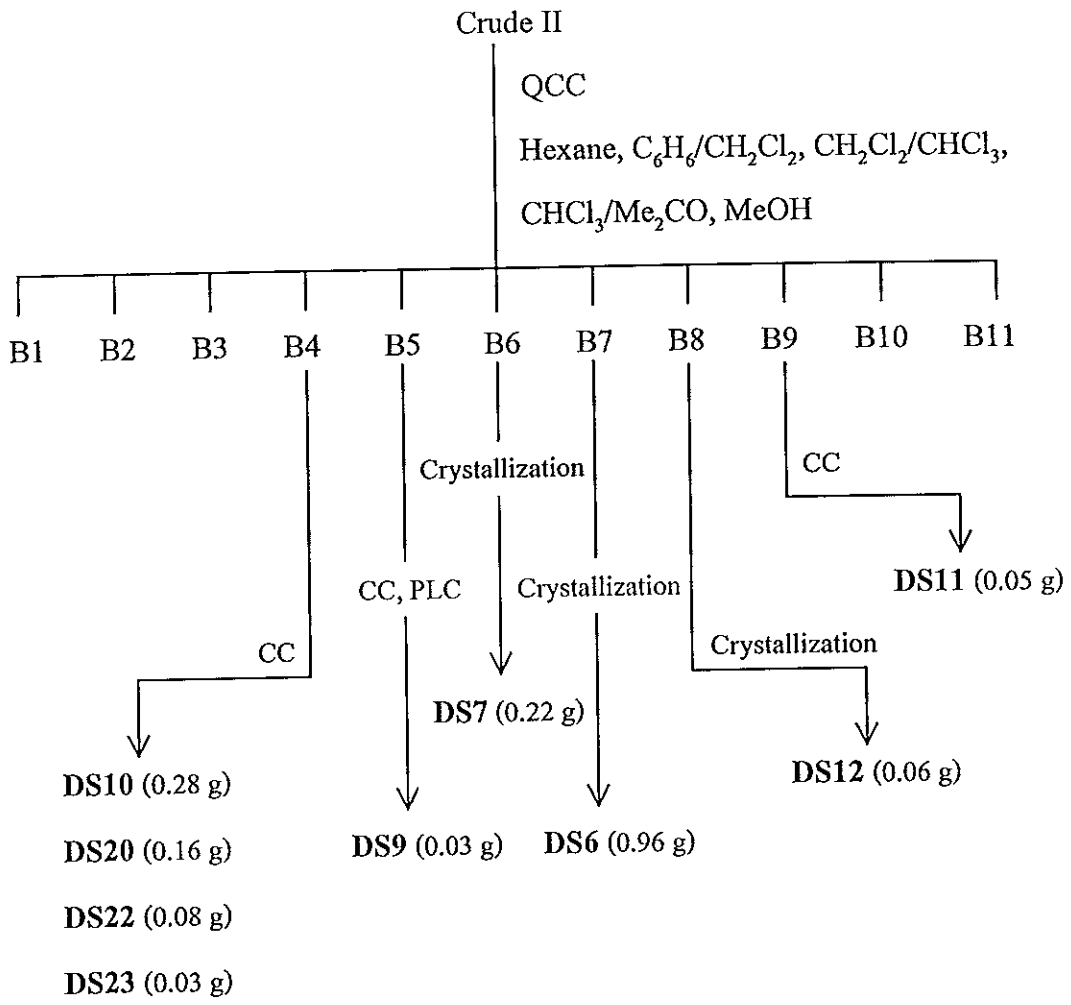


Figure 4 Isolation of compound **DS6-7, 9-12, 20** and **22-23** from Crude II

Isolation of compound DS22, DS23, DS10 and DS20

Fraction B4 was separated on column chromatography, eluted with benzene, dichloromethane, acetone and then methanol to give twelve fractions (B4.1-B4.12).

Fraction B4.2 was further purified on preparative TLC, using chloroform as an eluent. The substance from the major band was crystallized in benzene to give yellow solid of **DS22** (0.08 g).

Melting point : 69-70 °C

UV (CH_3OH) λ_{max} nm (log ε) : 282.2 (4.58)

IR (neat) ν (cm^{-1}) : 3351 (O-H stretching), 1742 (C=O stretching)

^{1}H NMR ($CDCl_3$) (δppm) : 13.18 (1H, *s*), 7.82 (1H, *s*), 7.23 (1H, *dd*, *J* = 2.3, 8.0 Hz), 7.17 (1H, *d*, *J* = 2.3 Hz), 6.83 (1H, *d*, *J* = 8.0 Hz), 6.73 (1H, *d*, *J* = 9.6 Hz), 6.35 (1H, *d*, *J* = 9.6 Hz), 6.33 (1H, *s*), 5.64 (1H, *d*, *J* = 9.6 Hz), 5.62 (1H, *d*, *J* = 9.6 Hz), 1.46 (6H, *s*), 1.44 (6H, *s*)

Fraction B4.4 was rechromatographed on column chromatography and eluted with dichloromethane, dichloromethane-acetone and methanol. The major component, **DS23**, was obtained and recrystallized in dichloromethane-hexane mixture. The yellow solid of **DS23** were collected (0.03 g).

Melting point : 106-107 °C

UV (CH_3OH) λ_{max} nm (log ε) : 267.2 (4.67)

IR (neat) ν (cm^{-1}) : 3351 (O-H stretching), 1742 (C=O stretching)

^{1}H NMR ($CDCl_3$) (δ ppm) : 13.27 (1H, *s*), 7.85 (1H, *s*), 7.25 (1H, *dd*, *J* = 8.3, 2.3 Hz), 7.19 (1H, *d*, *J* = 2.3 Hz), 6.85 (1H, *d*, *J* = 8.3 Hz), 6.33 (1H, *s*), 6.30 (1H, *d*, *J* = 9.0 Hz), 5.65 (1H, *d*, *J* = 9.0 Hz), 5.30 (1H, *t-like*, *J* = 6.8 Hz), 3.48 (2H, *br d*, *J* = 6.8 Hz), 1.86 (3H, *s*), 1.79 (3H, *s*), 1.46 (6H, *s*)

EIMS m/z (% relative intensity) : 404 ($[M]^+$, 24), 389 (100), 363 (15), 351 (13), 333 (30), 319 (11), 307 (13), 187 (7), 167 (18)

Faction B4.6 which contained **DS10** was crystallized in the mixed solvent of hexane and dichloromethane (1:1). Compound **DS10** was obtained as a pale yellow solid (0.28 g).

Melting point : 63-65 °C

UV (CH_3OH) λ_{max} (nm) (log ε) : 282.0 (4.56), 226.0 (4.32)

IR (KBr) ν (cm^{-1}) : 3343 (O-H stretching), 1653 (C=O stretching)

^{1}H NMR ($CDCl_3$) (δ ppm) : 13.18 (1H, *s*), 7.81 (1H, *s*), 7.25 (2H, *m*), 6.85 (1H, *d*, *J* = 9.1 Hz), 6.74 (1H, *d*, *J* = 9.8 Hz), 6.33 (1H, *s*), 5.62 (1H, *d*, *J* = 3.0 Hz), 5.35 (1H , *t*, *J* = 2.2 Hz), 3.39 (2H, *d*, *J* = 2.2 Hz), 1.79 (3H, *s*), 1.78 (3H, *s*), 1.47 (6H, *s*)

^{13}C NMR ($CDCl_3$+ DMSO - d_6) (δ ppm) : 181.00, 159.50, 157.32, 156.93, 154.75, 152.55, 134.99, 130.56, 128.20, 128.14, 127.16, 123.74, 122.97, 121.58, 115.93, 115.52, 106.13, 105.56, 94.84, 78.03, 29.81, 28.34, 25.84, 17.97

DEPT (135 °) ($CDCl_3$+ DMSO-d_6) : 28.34, 25.84, 17.97 (CH_3); 29.81 (CH_2); 152.55, 130.56, 128.20, 128.14, 121.58, 115.93, 115.52, 94.84 (CH); 159.50, 157.32, 156.93, 154.75, 134.99, 127.16, 123.74, 122.97, 106.13, 105.56, 78.03 (C); 181.00 (C=O)

EIMS m/z (% relative intensity) : 404 ($[M]^+$, 35), 389 (100), 333 (4), 255 (4), 203 (5), 167 (6)

Crystallization of faction B4.8 in benzene gave a brown solid of **DS20** (0.16g).

Melting point : 121-122 °C

Optical rotation : $[\alpha]_D^{28}$ -29.03 ° (c = 1.4 x 10^{-2} g/100 cm^3, CH_3OH)

UV (CH_3OH) λ_{max} (nm) (log ε) : 310.6 (3.82), 260.8 (2.69), 212.2 (4.20)

IR (KBr) ν (cm^{-1}) : 3430 (O-H stretching), 1630, 1597 (C=C stretching)

^{1}H NMR ($CDCl_3$) (δppm) : 7.36 (1H, *d*, *J* = 8.0 Hz), 6.72 (1H, *s*), 6.54 (1H, *dd*, *J* =

8.0, 2.0 Hz), 6.43 (1H, *s*), 6.41 (1H, *d*, 2.0 Hz), 5.92 (1H, *d*, *J* = 1.0 Hz), 5.89 (1H, *d*, *J* = 1.0 Hz), 5.47 (1H, *d*, *J* = 7.0 Hz), 4.95 (1H, *br s*), 4.22 (1H, *dd*, *J* = 11.2, 4.8 Hz), 3.65 (1H, *t*, *J* = 11.2 Hz), 3.47 (1H, *m*)

^{13}C NMR ($CDCl_3$) (δppm) : 157.19, 156.84, 154.39, 148.25, 141.84, 132.18, 117.87, 112.61, 109.67, 104.62, 103.55, 101.15, 93.66, 78.18, 66.11, 39.65

DEPT (135 °) ($CDCl_3$) : 101.15, 66.11 (CH_2); 132.18, 109.67, 104.62, 103.55, 93.66, 78.18, 39.65 (CH); 157.19, 156.84, 154.39, 148.25, 141.84, 117.87, 112.61 (C)

EIMS m/z (% relative intensity) : 284 ($[M]^+$, 100), 267 (17), 241 (5), 197 (5), 175 (11), 162 (20), 151 (14), 134 (12), 115 (7)

Isolation of compound DS9

Fraction B5 was rechromatographed on column chromatography eluting with benzene, dichloromethane and then acetone to afford thirteen fractions. The fourth fraction was purified on preparative TLC, eluting with chloroform. The yellow solid of **DS9** was obtained (0.03 g).

Melting point : 97-98 °C

UV (CH_3OH) λ_{max} (nm) (log ε) : 266.5 (4.51)

IR (KBr) ν (cm^{-1}) : 3239 (O-H stretching), 1645 (C=O stretching)

^{1}H NMR ($CDCl_3$) (δppm) : 13.25 (1H, *br s*), 7.84 (1H, *s*), 7.23 (1H, *dd*, *J* = 8.0, 1.6 Hz), 7.17 (1H, *d*, *J* = 1.6 Hz), 6.84 (1H, *d*, *J* = 8.0 Hz), 6.38 (1H, *s*), 6.36 (1H, *d*, *J* = 9.6 Hz), 5.64 (1H, *d*, *J* = 9.6 Hz), 5.29 (1H, *t*, *J* = 7.2 Hz), 3.47 (2H, *d*, *J* = 7.2 Hz), 1.84 (3H, *s*), 1.78 (3H, *s*), 1.45 (6H, *s*)

^{13}C NMR ($CDCl_3$+ DMSO - d_6) (δppm) : 180.93, 161.40, 159.68, 156.16, 153.27, 152.55, 136.10, 131.05, 129.54, 126.94, 123.48, 123.14, 122.11, 121.32, 121.06, 116.50, 109.96, 105.89, 94.00, 76.56, 28.07, 25.80, 21.51, 17.80

DEPT (135 °) ($CDCl_3$+ DMSO-d_6) : 28.07, 25.80, 17.80 (CH_3); 21.51 (CH_2); 152.55,

131.05, 129.54, 126.94, 122.11, 121.32, 116.05, 94.00 (CH); 161.40, 159.68, 156.16, 153.27, 136.10, 123.48, 123.14, 121.06, 109.96, 105.89, 76.56 (C); 180.93 (C=O)

EIMS m/z (% relative intensity) : 404 ($[M]^+$, 34), 389 (100), 363 (12), 349 (12), 333 (10), 319 (9), 307 (9), 187 (5), 167 (17), 115 (6), 69 (8)

Isolation of compound DS7

Crystallization of fraction B6 in benzene gave a pale yellow solid of **DS7** (0.22 g).

Melting point : 209-210 °C

UV (CH_3OH) λ_{max} (nm) (log ε) : 262.5 (4.74)

IR (KBr) ν (cm^{-1}) : 3279 (O-H stretching), 1630 (C=O stretching)

^{1}H NMR ($CDCl_3$) (δ ppm) : 7.83 (1H, *s*), 7.34 (2H, *d*, *J* = 6.4 Hz), 6.85 (2H, *d*, *J* = 6.4 Hz), 6.79 (1H, *d*, *J* = 10.2 Hz), 5.67 (1H, *d*, *J* = 10.2 Hz), 5.19 (1H, *t-like*, *J* = 7.0 Hz), 3.8 (3H, *s*), 3.41 (2H, *d*, *J* = 7.0 Hz), 1.83 (3H, *s*), 1.70 (3H, *s*), 1.50 (6H, *s*)

^{13}C NMR ($CDCl_3$) (δ ppm) : 175.91, 158.33, 156.31, 156.18, 152.44, 150.54, 131.65, 130.76, 129.05, 126.09, 124.14, 122.85, 122.07, 115.94, 115.46, 112.95, 106.05, 78.05, 62.66, 28.37, 25.99, 22.49, 18.21

DEPT (135 °) ($CDCl_3$) : 62.66, 28.37, 25.99, 18.21 (CH_3); 22.49 (CH_2); 150.54, 130.76, 129.05, 122.85, 115.94, 115.46 (CH); 158.33, 156.31, 156.18, 152.44, 131.65, 126.09, 124.14, 122.07, 112.95, 106.05, 78.05 (C); 175.91 (C=O)

EIMS m/z (% relative intensity) : 418 ($[M]^+$, 56), 403 (100), 387 (26), 375 (23), 361 (16), 349 (62), 319 (6), 285 (10), 227 (6), 215 (8), 209 (9), 194 (12), 167 (9), 118 (11), 77 (8)

Isolation of compound DS6

Crystallization of fraction B7 in benzene gave a yellow solid of **DS6** (0.96 g).

Melting point : 166-167 °C.

UV (CH_3OH) λ_{max} (nm) (log ε) : 342.0 (4.24), 234.5 (4.59)

IR (KBr) ν (cm^{-1}) : 3365 (O-H stretching), 1631 (C=O stretching)

^{1}H NMR ($CDCl_3$) (δ ppm) : 7.81 (1H, *s*), 7.30 (2H, *d*, *J* = 8.6 Hz), 6.83 (2H, *d*, *J* = 8.6 Hz), 5.19 (1H, *t-like*, *J* = 6.8 Hz), 5.16 (1H, *t-like*, *J* = 6.8 Hz), 3.76 (3H, *s*), 3.49 (2H, *d*, *J* = 6.8 Hz), 3.45 (2H, *d*, *J* = 6.8 Hz), 1.79 (3H, *s*), 1.78 (3H, *s*), 1.69 (3H, *s*), 1.68 (3H, *s*)

^{13}C NMR ($CDCl_3$) (δ ppm) : 176.32, 158.10, 156.91, 156.24, 155.19, 150.81, 134.88, 134.10, 130.51, 128.40, 125.58, 123.40, 121.84, 121.27, 119.85, 115.50, 112.90, 111.80, 62.30, 25.81, 25.79, 22.78, 22.33, 17.99, 17.93

DEPT (135 °) ($CDCl_3$) : 62.30, 25.81, 25.79, 17.99, 17.93 (CH_3); 22.78, 22.33 (CH_2); 150.81, 130.51, 128.40, 121.84, 121.27, 115.50 (CH); 158.10, 156.91, 156.24, 155.19, 134.88, 134.10, 125.58, 123.40, 119.85, 112.90, 111.80 (C); 176.32 (C=O)

EIMS m/z (% relative intensity) : 420 ($[M]^+$,100), 405 (58), 389 (34), 377 (21), 363 (37), 349 (92), 331 (32), 321 (42), 309 (26), 295 (97), 182 (8), 167 (18), 91 (12), 77 (12)

Isolation of compound DS12

Crystallization of fraction B8 in benzene gave a white solid of **DS12** (0.06 g).

Melting point : 230-231 °C

UV (CH_3OH) λ_{max} (nm) (log ε) : 259.5 (4.50), 214.0 (4.39)

IR (KBr) ν (cm^{-1}) : 3468 (O-H stretching), 1665 (C=O stretching)

^{1}H NMR ($CDCl_3$) (δ ppm) : 12.72 (1H, *s*), 9.05 (1H, *s*), 8.72 (1H, *s*), 7.99 (1H, *s*), 7.05 (1H, *d*, *J* = 8.5 Hz), 6.55 (1H, *d*, *J* = 2.9 Hz), 6.46 (1H, *d*, *J* = 8.5,

2.9 Hz), 6.45 (1H, *d*, *J* = 2.9 Hz), 6.38 (1H, *d*, *J* = 2.9 Hz), 3.89 (3H, *s*)

^{13}C NMR ($CDCl_3$) (δ ppm) : 182.51, 166.54, 162.70, 159.43, 158.17, 157.50, 155.22, 130.75, 123.50, 111.46, 108.82, 106.34, 105.67, 98.84, 92.35, 55.59

DEPT (135 °) ($CDCl_3$) : 55.59 (CH_3); 155.22, 130.75, 108.82, 106.34, 98.84, 92.35 (CH); 166.54, 162.70, 159.43, 158.17, 157.50, 123.50, 111.46, 105.67 (C); 182.51 (C=O)

EIMS m/z (% relative intensity) : 300 ($[M]^+$, 100), 283 (28), 271 (8), 231 (7), 167 (81), 161 (6), 150 (13), 138 (15), 134 (41), 123 (7), 105 (15), 95 (20), 78 (14), 69 (24)

Isolation of compound DS11

Fraction B9 was dissolved in benzene. Yellow solid was obtained from the benzene soluble portion. Further purification of yellow solid by column chromatography gave a pure pale yellow solid of **DS11** (0.05 g).

Melting point : 130-131 °C

UV (CH_3OH) λ_{max} (nm) (log ε) : 264.5 (4.43), 213.0 (4.23)

IR (KBr) ν (cm^{-1}) : 3397 (O-H stretching), 1656 (C=O stretching)

^{1}H NMR ($CDCl_3$) (δppm) : 12.83 (1H, *s*), 7.91 (1H, *s*), 7.40 (2H, *d*, *J* = 1.8 Hz), 6.90 (2H, *d*, *J* = 1.8 Hz), 6.33 (1H, s), 5.24 (1H, *t*, *J* = 5.9 Hz), 3.48 (2H, *d*, *J* = 5.9 Hz), 1.83 (3H, *s*), 1.74 (3H, *s*)

^{13}C NMR ($CDCl_3$) (δ ppm) : 181.21, 160.78, 160.67, 155.81, 155.01, 152.59, 135.34, 130.33, 123.27, 123.16, 120.99, 115.54, 106.32, 105.02, 99.68, 25.79, 21.60, 17.90

DEPT (135 °) ($CDCl_3$) : 25.79, 17.90 (CH_3); 21.60 (CH_2); 152.59, 130.33, 120.99, 115.54, 99.68 (CH); 160.78, 160.67, 155.81, 155.01, 135.34, 123.27, 123.16, 106.32, 105.02 (C); 181.21 (C=O)

EIMS m/z (% relative intensity) : 338 ($[M]^+$, 87), 323 (100), 300 (17), 283 (36), 270

(46), 254 (5), 165 (11), 142 (10), 118 (11), 69 (10)

2.3.3 Purification of Crude acetone

A portion of crude acetone (15.3 g) was separated into two fractions by dissolving in the mixture of hexane-dichloromethane (1:1). The soluble portion (9.5 g) was dissolved in dichloromethane and partitioned with 5% disodiumtetraborate. The aqueous layer was acidified with 5% hydrochloric acid then partitioned with dichloromethane. The lower layer was washed with water and dried over anhydrous sodium sulfate. The residue after removal of the solvent (4.2 g) was subjected to column chromatography on silica gel, eluting with dichloromethane, dichloromethane-chloroform, chloroform and chloroform-acetone. The fractions containing similar components were combined to give seven fractions (C1-C7) (Table 6). Fraction C3 and C6 were further purified to obtain **DS24** and **DS25** (Figure 5).

Table 6 Physical characteristic and weight of fractions obtained from CC

Fraction	Weight (g)	Physical characteristic
C1	0.23	pale yellow viscous liquid
C2	0.15	colourless solid mixed with yellow viscous liquid
C3	1.02	white solid mixed with orange viscous liquid
C4	0.48	yellow viscous solid
C5	0.53	brown viscous liquid
C6	1.14	yellow solid mixed with yellow viscous liquid
C7	0.37	yellow viscous liquid

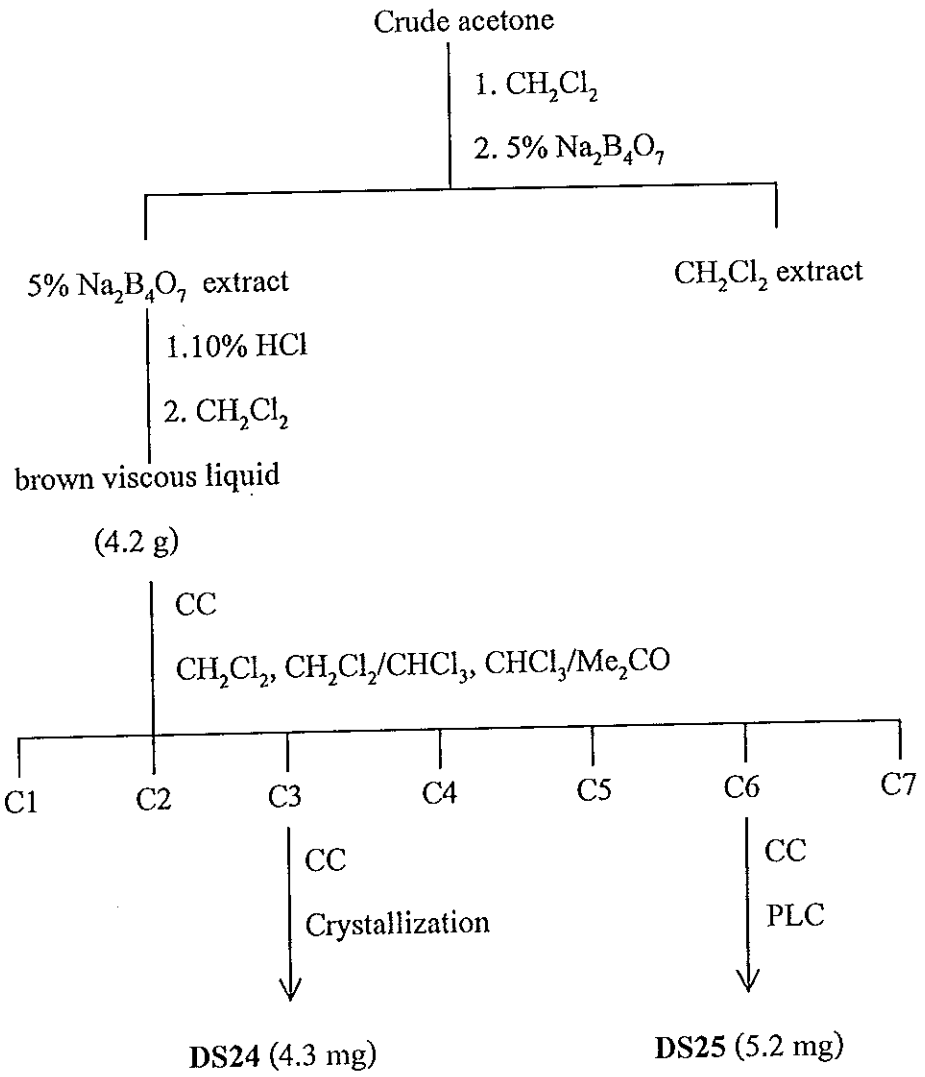


Figure 5 Isolation of compound **DS24** and **DS25** from Crude acetone

Isolation of compound DS24

Fraction C3 was rechromatographed on column chromatography and eluted with dichloromethane-chloroform (1:1). **DS24** was obtained and recrystallized in dichloromethane. The pale yellow solid of **DS24** were collected (4.3 mg).

Melting point : 124-125 °C

UV (CH_3OH) λ_{max} (nm) (log ε) : 260.8 (4.54)

IR (neat) ν (cm^{-1}) : 3351 (O-H stretching)

^{1}H NMR ($CDCl_3$ + CD_3OD) (δ ppm) : 12.83 (1H, *s*), 7.84 (1H, *s*), 7.11 (1H, *d*, *J* = 1.5 Hz), 6.96 (1H, *d*, *J* = 7.6 Hz), 6.92 (1H, *dd*, *J* = 7.6, 1.5 Hz), 6.35 (1H, *d*, *J* = 1.7 Hz), 6.28 (1H, *d*, *J* = 1.7 Hz), 3.93 (3H, *s*), 3.40 (1H, *s*)

EIMS m/z (% relative intensity) : 300 ($[M]^+$, 100), 285 (14), 271 (9), 257 (19), 253 (16), 229 (26), 153 (16), 149 (32), 133 (11), 120 (9), 105 (10)

Isolation of compound DS25

Fraction C6 was separated on column chromatography and eluted with chloroform. The selected fraction was further purified by preparative TLC, using 2 % acetone-chloroform as an eluent (4 elutions). A yellow solid (5.2 mg) of **DS25** was obtained from the major band.

Melting point : 268-269 °C

UV (CH_3OH) λ_{max} (nm) (log ε) : 261.8 (4.37)

IR (neat) ν (cm^{-1}) : 3351 (O-H stretching)

^{1}H NMR ($CDCl_3$ + CD_3OD) (δ ppm) : 12.83 (1H, *s*), 7.82 (1H *s*), 7.35 (2H, *d*, *J* = 8.0 Hz), 6.89 (2H, *d*, *J* = 8.0 Hz), 6.34 (1H, *d*, *J* = 2.6 Hz), 6.28 (1H, *d*, *J* = 2.6 Hz)

EIMS m/z (% relative intensity) : 270 ($[M]^+$, 100), 210 (8), 153 (34), 124 (14), 118 (9), 89 (9)

2.4 Estimation of the antioxidative activity

The antioxidative activity of the compounds isolated from *Derris scandens* were determine by DPPH assay.

2.4.1 Screening on the free radical scavenging activity

A 0.61 mM of sample in absolute ethanol (50 μL) was mixed with 0.05 mM DPPH ethanolic solution (3 mL) in a cuvette and the solution was incubated at 37 °C. The absorbances were measured every 20 minutes at 517 nm against 0.05 mM DPPH. Measurements were performed at least triplicate. The degree of loss of color implied the activity. The residual absorbance at 517 nm were shown in Table 7.

Table 7 The absorption of the samples solutions (10 μM)

Sample	Average absorbances (517 nm)			
	20 min	40 min	60 min	80 min
DPPH	0.560	0.560	0.560	0.550
DS2	0.530	0.500	0.460	0.460
DS3	0.550	0.530	0.510	0.505
DS4	0.550	0.550	0.540	0.540
DS6	0.400	0.360	0.250	0.240
DS7	0.430	0.380	0.320	0.315
DS9	0.550	0.540	0.520	0.510
DS10	0.530	0.510	0.490	0.490
DS11	0.550	0.530	0.500	0.495
DS12	0.410	0.350	0.230	0.220
DS13	0.550	0.520	0.510	0.510
DS16	0.540	0.500	0.470	0.470
DS18	0.550	0.540	0.540	0.540
DS19	0.540	0.520	0.480	0.480
DS20	0.550	0.550	0.540	0.540
BHT	0.430	0.350	0.290	0.285
Ascorbic acid	0.280	0.220	0.180	0.180

2.4.2 *Evaluation of inhibitory concentration (IC_{50})*

Three compounds, **DS6, DS7** and **DS12** which show the highest activity were selected for further study. The solution of DPPH (0.05 mM, 3 mL) and the sample were mixed to give the final concentration of 10.00, 8.75, 7.50, 6.25, 5.00, 3.75, 2.50 and 1.25 μM. The absorbances were measured after incubated at 45 and 60 minutes at 517 nm. The results were shown in Table 8.

The absorbance of the solution at each time period was plotted against the concentration. The concentration that needed to decrease the absorption of DPPH solution to 0.27 (the absorbance of 0.025 mM DPPH) were the IC_{50}.

Table 8 The absorption of the samples solutions at 45 and 60 minutes

Final concentration (μM)	Average absorbance (517 nm)									
	DS6		**DS7**		**DS12**		BHT		Ascorbic acid	
	45 min	60 min	45 min	60 min	45 min	60 min	45 min	60 min	45 min	60 min
1.25	0.345	0.335	0.350	0.330	0.325	0.310	0.325	0.310	0.340	0.310
2.50	0.310	0.300	0.345	0.315	0.300	0.290	0.310	0.295	0.275	0.250
3.75	0.270	0.265	0.330	0.310	0.265	0.250	0.300	0.290	0.235	0.225
5.00	0.265	0.260	0.300	0.295	0.255	0.245	0.295	0.280	0.225	0.215
6.25	0.260	0.250	0.295	0.280	0.250	0.240	0.285	0.275	0.225	0.215
7.50	0.250	0.240	0.285	0.275	0.250	0.240	0.275	0.265	0.220	0.215
8.75	0.250	0.240	0.275	0.270	0.245	0.235	0.265	0.260	0.220	0.215
10.00	0.250	0.240	0.275	0.270	0.245	0.230	0.265	0.260	0.220	0.215

CHAPTER 3

RESULTS AND DISCUSSION

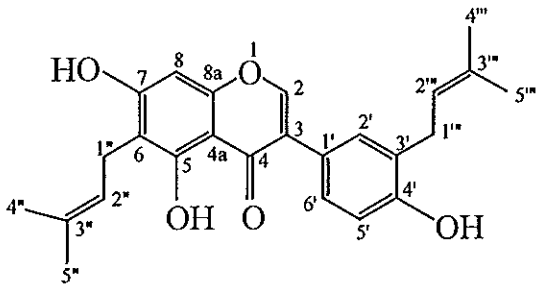
3.1 Structural determination

The stems of *Derris scandens* collected from Phang-nga province in Thailand was dried, chopped and extracted with acetone and methanol. The crude methanolic extract was separated into two fractions, ethyl acetate soluble fraction and water soluble fraction. The residual after removal of ethyl acetate was further dissolved in dichloromethane. The soluble portion was purified by column chromatography and/or chromatotron and/or crystallization and/or preperative TLC to obtain eight compounds; two new isoflavones (**DS3** and **DS16**), two isoflavones (**DS2** and **DS13**), pterocarpan (**DS4**), steroid (**DS15**), coumestan (**DS19**) and one new benzil (**DS18**).

The insoluble portion was fractionated by quick column chromatography on silica gel, eluted with hexane, hexane-benzene, benzene, benzene-dichloromethane, dichloromethane-chloroform, chloroform-acetone and methanol to give eleven fractions. Selected fractions were further purified by column chromatography and/or crystallization and/or preperative TLC to obtain nine compounds; eight isoflavones (**DS6**, **DS7**, **DS9**, **DS10**, **DS11**, **DS12**, **DS22** and **DS23**) and pterocarpan (**DS20**).

The crude acetone extract was dissolved in hexane-dichloromethane (1:1). The soluble portion was extracted with 5% disodiumtetraborate and dichloromethane. The 5% disodiumtetraborate fraction after acidification was subjected to column chromatography over silica gel using dichloromethane, dichloromethane-chloroform, chloroform and chloroform-acetone as eluents to give nine fractions. Selected fractions were further purified by column chromatography and/or crystallization and/or preperative TLC to obtain two isoflavone (**DS24** and **DS25**).

DS2 : 4′,5,7-Trihydroxy-3′,6-diprenylisoflavone (lupalbigenin)

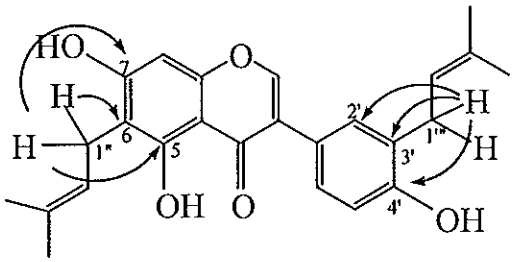


Compound **DS2** was isolated as a yellow solid, m.p.149-150°C (Ref.120°C). Its molecular formula of $C_{25}H_{26}O_5$ were established on the basis of mass spectrum (M^+ m/z 406). The UV spectrum showed maxima absorptions at 266.5 and 217.0 nm, which is a typical absorption of isoflavone. The IR spectrum showed the stretching of hydroxyl group at 3255 cm^{-1} and carbonyl group at 1655 cm^{-1}.

The ^{1}H NMR spectrum (Table 9) showed a sharp *singlet* signal of a chelated hydroxy group 5-OH at δ13.22, a *singlet* signal of vinylic proton H-2 at δ7.82, a *singlet* signal of isolated aromatic proton H-8 at δ6.36. The resonances of ABM pattern were shown at δ7.23, 7.22 and 6.83, and were assigned to be the resonances of aromatic proton H-2′, H-5′ and H-6′, respectively. In addition two sets of signals due to two prenyl side chain were observed and were assigned to attach to the parent structure at C-6 and C-3′. The first prenyl side chain showed the two *singlet* signals of *gem*-dimethyl protons at δ 1.83 and 1.76, and a *doublet* signal of the benzylic methylene protons at δ3.45 which coupled to an olefinic methine proton (H-2″) at δ 5.28, whereas the second prenyl side chain showed a *singlet* signals of *gem*-dimethyl protons at δ1.77, and a *doublet* signal of the benzylic methylene protons at δ3.37 of which coupled to an olefinic methine proton (H-2‴) at δ5.33. The ^{13}C NMR and the DEPT spectra suggested that **DS2** contained four methyl carbons [δ17.92 (6H), 25.79 and 25.83), two methylene carbons (δ21.50 and 29.83), seven methine carbons (δ

93.99, 115.95, 121.06, 121.53, 128.23, 130.60 and 152.65), eleven quaternary carbons (δ161.40, 159.60, 156.15, 154.69, 136.01, 135.05, 127.11, 123.66, 123.08, 110.03 and 105.87) and a carbonyl carbon (δ 181.04).

HMBC correlation (Table 9) showed the correlation of H-1$'''$ (δ3.37) of isoprenyl group to C-2$'$ (δ130.60), C-3$'$ (δ127.11) and C-4$'$ (δ154.69), whereas H-2$'$ (δ7.23) correlated to C-1$'''$ (δ29.83) and C-4$'$ (δ154.69), its therefore confirmed that one isoprenyl side chain was at C-3$'$. In addition, the correlation of H-1$''$ (δ3.45) to C-5 (δ159.60), C-6 (δ110.03) and C-7 (δ161.40) supported that another isoprenyl group was at C-6. **DS2** was therefore assigned to be 4$'$,5,7-trihydroxy-3$'$,6-diprenylisoflavone which was known as lupalbigenin.



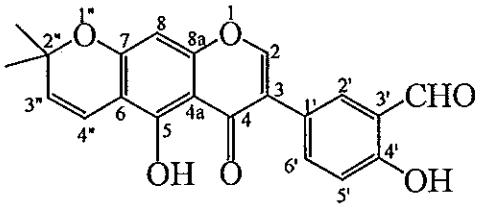
Major HMBC correlations of **DS2**

Table 9 The NMR spectral data of **DS2**

Position	δ_C*	δ_H, *mult*, *J* (Hz)	HMBC
2	152.65 (CH)	7.82 (1H, *s*)	C-3, C-4, C-9, C-1′
3	123.66 (C)		
4	181.04 (C=O)		
4a	105.87 (C)		
5	159.60 (C)		
6	110.03 (C)		
7	161.40 (C)		
8	93.99 (CH)	6.36 (1H, *s*)	C-4, C-6, C-7, C-9, C-10
8a	156.15 (C)		
1′	123.08 (C)		
2′	130.60 (CH)	7.23 (1H, *d*, 1.9)	C-4′, C-6′, C-1‴
3′	127.11 (C)		
4′	154.69 (C)		
5′	115.95 (CH)	7.22 (1H, *d*, 6.4)	C-1′, C-3′, C-4′
6′	128.23 (CH)	6.83 (1H, *dd*, 6.4, 1.9)	C-2′
1″	21.50 (CH_2)	3.45 (2H, *d*, 7.0)	C-5, C-6, C-7, C-2″, C-3″
2″	121.06 (CH)	5.28 (1H, *t-like*, 7.0)	C-1″, C-4″, C-5″
3″	136.01 (C)		
4″	17.92 (CH_3)	1.83 (3H, *s*)	C-2″, C-3″
5″	25.83 (CH_3)	1.76 (3H, *s*)	C-3″, C-4″
1‴	29.83 (CH_2)	3.37 (2H, *d*, 7.0)	C-2′, C-3′, C-4′, C-2‴, C-3‴
2‴	121.53 (CH)	5.33 (1H, *t-like*, 7.0)	C-4‴, C-5‴
3‴	135.05 (C)		
4‴	17.92 (CH_3)	1.77 (6H, *s*)	C-2‴, C-3‴, C-4‴, C-5‴
5‴	25.79 (CH_3)	1.77 (6H, *s*)	C-2‴, C-3‴, C-4‴, C-5‴
5-OH		13.22 (1H, *s*)	C-5, C-6, C-10

* Carbon type was deduced from DEPT experiments.

DS3 : 4′,5-Dihydroxy-2″,2″-dimethylchromeno[6,7:5″,6″]isoflavone -3′-carboxaldehyde

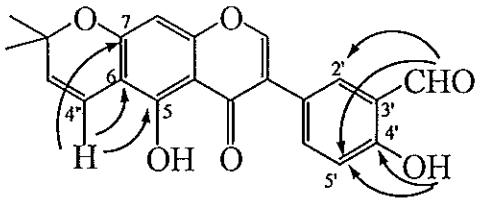


Compound **DS3** was isolated as a yellow solid, m.p. 79-80 °C. Its molecular ion of 364 corresponded to $C_{21}H_{16}O_6$. The UV spectrum showed maxima absorptions at 279.0 and 231.0 nm. The IR spectrum showed the broad band of O-H stretching (3450 cm^{-1}) and the sharp band of C=O stretching (1656 cm^{-1}).

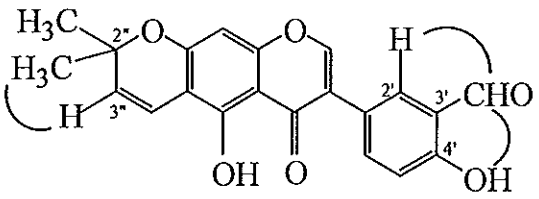
The ^1H NMR spectrum of **DS3** (Table 10) showed the signals of a chelated phenolic hydroxy group 5-OH at δ12.98, a nonchelated hydroxy group at δ11.11, a formyl proton at δ9.98 and a noncoupling olefinic proton H-2 at δ7.89. Furthermore, a *singlet* of aromatic proton dued to proton H-8 at δ 6.36 was observed. The resonances of ABM type of aromatic protons were present at δ7.83 (*d*), 7.09 (*d*) and 7.68 (*dd*) and were assigned to be H-2′, H-5′ and H-6′, respectively. The signals of methyl groups and two *doublets* of two geminal olefinic protons were observed at δ 1.49 (6H), 6.74 and 5.65, these protons were established to be part of chromene ring. The ^{13}C and DEPT experiment suggested that **DS3** consisted of two carbonyl carbons (δ 196.71 and 180.71), two methyl carbons (δ 28.59), seven methine carbons (δ 152.88, 137.45, 134.47, 128.60, 118.31, 115.59 and 95.26) and ten quaternary carbons (δ161.90, 160.06, 157.51, 157.08, 122.89, 122.49, 120.82, 106.21, 106.04 and 78.45).

The substitution patterns were assigned and confirmed by HMBC. The formyl proton (CHO) was found to be correlated to C-5′ (δ118.31), C-4′ (δ161.90), C-3′ (δ120.82) and C-2′ (δ134.47), accordingly the formyl group was placed at C-

3′. This assignment was confirmed by NOE experiment, irradiation at H-2′ (δ7.83) enhanced the signal of CHO (δ9.98) and irradiation at CHO (δ9.98) resulted in the enhancement of signals of H-2′ (δ7.83) and 4′-OH (δ11.11). The connection of the chromene ring to the parent structure was indicated by the data from HMBC as follow; H-4″ showed the correlation to C-5, C-6 and C-7, whereas H-3″ correlated to C-6. Therefore, **DS3** was assigned to be 4′,5-dihydroxy-2″,2″-dimethylchromeno [6,7:5″,6″]isoflavone-3′-carboxaldehyde which is a new natural occurring compound.



Major HMBC correlations of **DS3**



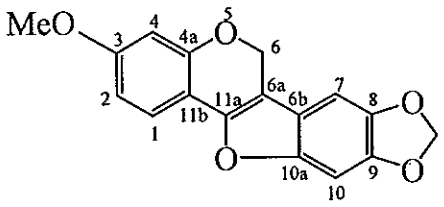
NOE of **DS3**

Table 10 The NMR spectral data of **DS3**

Position	δ_C*	δ_H, *mult*, *J* (Hz)	HMBC
2	152.89 (CH)	7.89 (1H, *s*)	C-3, C-8a, C-1′
3	122.49 (C)		
4	180.71 (C)		
4a	106.04 (C)		
5	157.08 (C)		
6	106.21 (C)		
7	160.06 (C)		
8	95.26 (CH)	6.36 (1H, *s*)	C-6, C-7, C-8a
8a	157.51 (C)		
1′	122.89 (C)		
2′	134.47 (CH)	7.83 (1H, *d*, 1.8)	C-3, C-4′, C-6′
3′	120.82 (C)		
4′	161.90 (C)		
5′	118.31 (CH)	7.09 (1H, *d*, 9.1)	C-1′, C-3′, C-4′
6′	137.45 (CH)	7.68 (1H, *dd*, 9.1, 1.8)	C-3, C-2′, C-4′
2″	78.45 (C)		
3″	128.60 (CH)	5.65 (1H, *d*, 9.8)	C-6, C-2″, $(\underline{C}H_3)_2$-2″
4″	115.59 (CH)	6.74 (1H, *d*, 9.8)	C-5, C-6, C-7, C-2″
5-OH		12.98 (1H, *s*)	C-5, C-6, C-7
3′- CHO		9.98 (1H, *s*)	C-2′, C-3′, C-4′, C-5′
4′-OH		11.11 (1H, *s*)	C-3′, C-4′, C-5′, C-6′
2″- Me_2	28.59 (CH_3)	1.49 (6H, *s*)	C-2″, C-3″, $(\underline{C}H_3)_2$-2″

* Carbon type was deduced from DEPT experiments.

DS4 : 3-Methoxy-8,9-methylenedioxy-6a,11a-dehydropterocarpan (flemichapparin B)

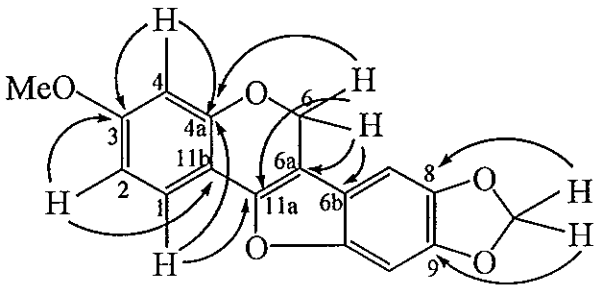


Compound **DS4** is a white solid, m.p. 92-93 °C. The molecular formula was determined as $C_{17}H_{12}O_5$ by EIMS (M^+ m/z 296). The UV spectrum showed maxima absorptions at 356.5, 337.5 and 216.0 nm. The IR spectrum exhibited no absorption band of carbonyl group.

The ^{1}H NMR spectra (Table 11) exhibited two *singlets* of two isolated aromatic proton H-7 and H-10 at δ 7.01 and 6.71 and ABM type aromatic proton signals of H-1, H-2 and H-4 at δ 7.36, 6.53 and 6.50, respectively. A signal of methoxy group was shown at δ3.80 and was found to correlate to C-3 on HMBC, thus it was located at C-3. Two *singlets* of two protons each at δ 5.50 and 5.90 were assigned to be the resonances of a methylene protons CH_2-6 and a methylenedioxy protons (OCH_2O). The proton pattern suggested that this compound was a derivative of dehydropterocarpan. The DEPT experiments indicated the carbon resonances of a methoxy carbon at δ 55.50, two methylene carbons at δ 101.39 and 65.56, five methine carbons at δ 120.91, 107.21, 102.49, 97.24 and 94.06, and nine quaternary carbons at δ 160.15, 154.93, 150.48, 147.71, 145.63, 144.74, 119.16, 109.89 and 106.34.

The substitution pattern in this compound was supported by its HMBC spectral data (Table 11). HMBC correlations of methylenedioxy protons (OCH_2O) at δ 5.90 to C-8 (δ145.63) and C-9 (δ144.74) suggested that the methylenedioxy unit was connected to parent structure at C-8 and C-9. Proton CH_2-6 showed correlation to C-4a (δ 154.93), C-6a (δ 119.16), C-6b (δ 106.34), C-11a (δ 147.71) and C-11b (δ

109.89), this confirmed the location of CH_2 to be at C-6. Thus compound **DS4** is indicated to be 3-methoxy-8,9-methylenedioxy-6a,11a-dehydropterocarpan. This compound was known as flemichapparin B.



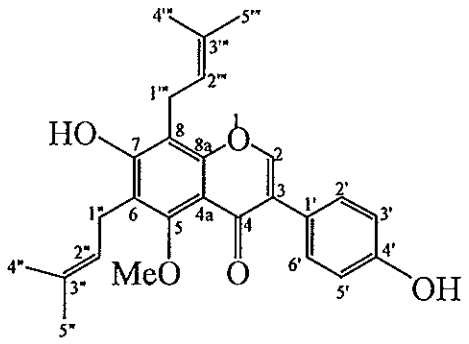
Major HMBC correlations of **DS4**

Table 11 The NMR spectral data of **DS4**

Position	δ_C*	δ_H, *mult*, *J* (Hz)	HMBC
1	120.91 (CH)	7.36 (1H, *d*, 8.3)	C-3, C-4a, C-11a
2	107.21 (CH)	6.53 (1H, *dd*, 8.3, 1.9)	C-3, C-4, C-11b
3	160.15 (C)		
4	102.49 (CH)	6.50 (1H, *d*, 1.9)	C-2, C-3, C-4a, C-11b
4a	154.93 (C)		
6	65.56 (CH_2)	5.50 (2H, *s*)	C-4a, C-6a, C-6b, C-11a, C-11b
6a	119.16 (C)		
6b	106.34 (C)		
7	94.06 (CH)	7.01 (1H, *s*)	C-6a, C-8, C-9, C-10, C-10a
8	145.63 (C)		
9	144.74 (C)		
10	97.24 (CH)	6.71 (1H, *s*)	C-6b, C-7, C-8, C-9, C-10a
10a	150.48 (C)		
11a	147.71 (C)		
11b	109.89 (C)		
3-OMe	55.50 (CH_3)	3.80 (3H, *s*)	C-3
OCH_2O	101.39 (CH_2)	5.90 (2H, *s*)	C-8, C-9

* Carbon type was deduced from DEPT experiments.

DS6 : 4′,7-Dihydroxy-5-methoxy-6,8-diprenylisoflavone (derrisisoflavone A)

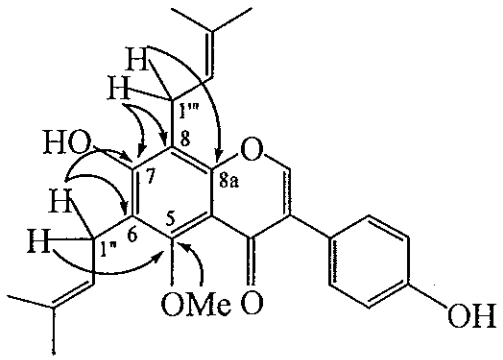


Compound **DS6** was obtained as a yellow solid, m.p. 166-167 °C. Its molecular formula of $C_{26}H_{28}O_5$ were established on the basis of mass spectrum (M^+ m/z 420). The UV spectrum showed maxima absorptions at 342.0 and 234.5 nm, which was a typical absorption of isoflavones. The IR spectrum showed the stretching of hydroxyl group (3365 cm^{-1}) and carbonyl group (1631 cm^{-1}).

The ^1H NMR spectrum (Table 12) showed the presence of the characteristic signal of H-2 of an isoflavone (δ 7.81), a methoxy group (δ 3.76), four aromatic protons which coupled to each other as AA′BB′ type at δ7.30 (2H, *d*, *J* = 8.6 Hz) and 6.83 (2H, *d*, *J* = 8.6 Hz), and two prenyl groups. The proton signals of a prenyl group appeared as follow: the *gem*-dimethyl protons (CH_3-4″,5″) at δ1.79 (*s*) and 1.69 (*s*), benzylic methylene protons (CH_2-1″) at δ3.45 (*d*), an olefinic methine proton (CH-2″) at δ5.16 (*t*). The signals of the second prenyl group appeared as follow: the *gem*-dimethyl protons (CH_3-4‴,5‴) at δ 1.78 (*s*) and 1.68 (*s*), the benzylic methylene protons (CH_2-1‴) at δ3.49 (*d*), an olefinic methine proton (CH-2‴) at δ 5.19. The ^{13}C NMR showed the signals of 26 carbon atoms (Table 12). Analysis of the DEPT spectra indicated the presence of a methoxy carbon (δ62.30), a carbonyl carbon (δ 176.32), four methyl carbons (δ 25.81, 25.79, 17.99 and 17.93), two methylene carbons (δ22.78 and 22.33), six methine carbons (δ150.81, 130.51, 128.40, 121.84,

121.27 and 115.50) and eleven quaternary carbons (δ158.10, 156.91, 156.24, 155.19, 134.88, 134.10, 125.58, 123.40, 119.85, 112.90 and 111.80).

In the HMBC spectrum, correlation between H-1$''$ (δ3.45) with C-5 (δ 156.24), C-6 (δ119.85) and C-7 (δ158.10) were present, it suggested the position of a prenyl unit to be at C-6 in A ring. Correlation between H-1$'''$ with C-7 (δ158.10), C-8 (δ111.80) and C-8a (δ155.19), indicated that the second prenyl unit was at C-8. Moreover, the methoxy hydrogens at δ 3.76 (OMe-5) was found to show the correlation with the carbon signal at C-5 (δ156.24), this showed that the position of methoxy group is at C-5. By NOE experiments, irradiation at OMe-5 (δ3.76) gave enhancement of the signals of H-1$''$ (δ3.45) and H-2$''$ (δ5.16), and irradiation of H-1$''$ (δ3.45) and H-2$''$ (δ5.16) resulted in the enhancement of the signal at OMe-5 (δ 3.76), thus the position of methoxy group at C-5 in A ring was confirmed. **DS6** was considered to be 4$'$,7-dihydroxy-5-methoxy-6,8-diprenylisoflavone.



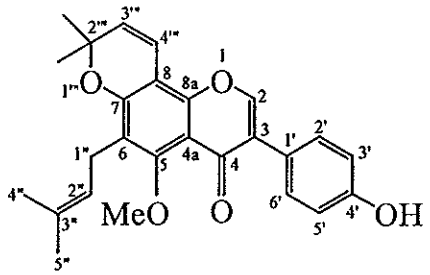
Major HMBC corelations of **DS6**

Table 12 The NMR spectral data of **DS6**

Position	δ_C*	δ_H, *mult*, *J* (Hz)	HMBC
2	150.81 (CH)	7.81 (1H, *s*)	C-3, C-4, C-9
3	125.58 (C)		
4	176.32 (C=O)		
4a	112.90 (C)		
5	156.24 (C)		
6	119.85 (C)		
7	158.10 (C)		
8	111.80 (C)		
8a	155.19 (C)		
1′	123.40 (C)		
2′	128.40 (CH)	7.30 (2H, *d*, 8.6)	C-3, C-4′, C-6′
3′	115.50 (CH)	6.83 (2H, *d*, 8.6)	C-1′, C-4′, C-5′
4′	156.91 (C)		
5′	115.50 (CH)	6.83 (2H, *d*, 8.6)	C-1′, C-4′, C-5′
6′	130.51 (CH)	7.30 (2H, *d*, 8.6)	C-3, C-4′, C-6′
1″	22.78 (CH_2)	3.45 (2H, *d*, 6.8)	C-5, C-6, C-7, C-3″
2″	121.84 (CH)	5.16 (1H, *t-like*, 6.8)	
3″	134.88 (C)		
4″	17.99 (CH_3)	1.79 (3H, *s*)	C-2″, C-3″
5″	25.81 (CH_3)	1.69 (3H, *s*)	C-2″, C-3″
1‴	22.33 (CH_2)	3.49 (2H, *d*, 6.8)	C-7, C-8, C-9, C-3‴, C-2‴
2‴	121.27 (CH)	5.19 (1H, *t-like*, 6.8)	
3‴	134.10 (C)		
4‴	17.93 (CH_3)	1.78 (3H, *s*)	C-2‴, C-3‴
5‴	25.79 (CH_3)	1.68 (3H, *s*)	C-2‴, C-3‴
5-OMe	62.30 (CH_3)	3.76 (3H, *s*)	C-5

* Carbon type was deduced from DEPT experiments.

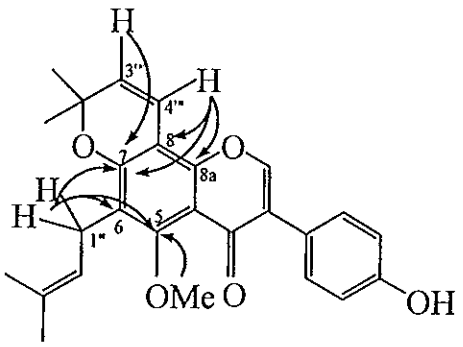
DS7 : 4′-Hydroxy-5-methoxy-6-prenyl-2‴,2‴-dimethylchromeno[7,8:6‴,5‴] isoflavone (scandinone)



Compound **DS7** is a pale yellow solid, m.p. 209-210 °C. EIMS showed the molecular ion of 418 which corresponded to $C_{26}H_{26}O_5$. An isoflavone nucleus was elucidated from the UV spectra (λ_{max} 262.5 nm). **DS7** exhibited IR absorption band at 3279 cm^{-1} and 1630 cm^{-1} which indicated the presence of hydroxyl group and carbonyl group, respectively.

The ^{1}H NMR spectrum (Table 13) revealed a characteristic signal of an isoflavone derivative of which H-2 resonated at δ7.83. The AA′BB′ type signals of B ring exhibited at δ 7.34 (2H, *d*, *J* = 6.4 Hz) and 6.85 (2H, *d*, *J* = 6.4 Hz) assignable to H-2′,6′ and H-3′,5′, respectively. The typical signals due to a 3,3-dimethylallyl group were observed at δ 3.41, 5.19, 1.83 and 1.70. In addition, the *doublet* signals of two olefinic protons at δ5.67 (H-3‴) and 6.79 (H-4‴), and two methyl groups at δ1.50 which corresponded to part of 2,2-dimethyl chromene ring were detected. A *singlet* signal of methoxy group was shown at δ 3.89. Accordingly compound **DS7** was considered to be a 6,7,8-trisubstituted methoxyisoflavone. The ^{13}C NMR spectrum and the DEPT experiments showed a signal of carbonyl carbon (δ175.91), eleven signals of quaternary carbon (δ 158.33, 156.31, 156.18, 152.44, 131.65, 126.09, 124.14, 122.07, 112.95, 106.05 and 78.05), six signals of methine carbon (δ150.54, 130.76, 129.05, 122.85, 115.94 and 115.46), a signal of methylene carbon (δ22.49) and four signals of methyl carbon (δ62.66, 28.37, 25.99 and 18.21).

The structure and the arrangement of the substituents were deduced by the HMBC experiments. Correlations between H-4‴ to C-7, C-8 and C-8a, confirmed the connection of chromene ring. The correlation of C-5 to OCH_3-5 showed that methoxy group was at C-5. The remaining isoprenyl unit was confirmed to be *ortho* to chromene ring and methoxy group by the correlations between H-1″ to C-5, C-6 and C-7. Accordingly, **DS7** was confirmed to be 4′-hydroxy-5-methoxy-6-prenyl-2‴,2‴-dimethylchromeno[7,8:6‴,5‴]isoflavone.



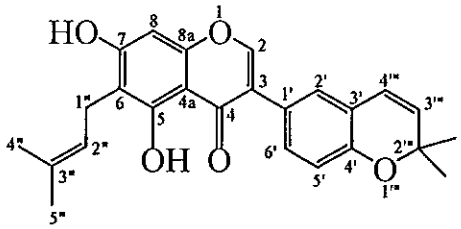
Major HMBC correlations of **DS7**

Table 13 The NMR spectral data of **DS7**

Position	δ_C*	δ_H, *mult*, *J* (Hz)	HMBC
2	150.54 (CH)	7.83 (1H, *s*)	C-3, C-4, C-8a, C-1′
3	126.09 (C)		
4	175.91 (C=O)		
4a	112.95 (C)		
5	158.33 (C)		
6	122.07 (C)		
7	156.18 (C)		
8	106.05 (C)		
8a	152.44 (C)		
1′	124.14 (C)		
2′,6′	130.76 (CH)	7.34 (2H, *d*, 6.4)	C-3, C-2′,6′, C-4′
3′,5′	115.94 (CH)	6.85 (2H, *d*, 6.4)	C-1′, C-3′,5′, C-4′
4′	156.31 (C)		
1″	22.49 (CH_2)	3.41 (2H, *d*, 7.0)	C-5, C-6, C-7, C-2″, C-3″
2″	122.85 (CH)	5.19 (1H, *t-like*, 7.0)	
3″	131.65 (C)		
4″	18.21 (CH_3)	1.83 (3H, *s*)	C-5″
5″	25.99 (CH_3)	1.70 (3H, *s*)	C-4″
2‴	78.05 (C)		
3‴	129.05 (CH)	5.67 (1H, *d*, 10.2)	C-8, C-2‴, ($\underline{C}H_3$)$_2$-2‴
4‴	115.46 (CH)	6.79 (1H, *d*, 10.2)	C-7, C-8, C-8a, C-2‴
2‴-Me_2	28.37 (CH_3)	1.50 (6H, *s*)	C-2‴, C-3‴, ($\underline{C}H_3$)$_2$-2‴
5-OMe	62.66 (CH_3)	3.89 (3H, *s*)	C-5

* Carbon type was deduced from DEPT experiments.

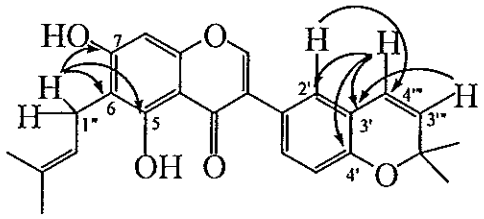
DS9 : 5,7-Dihydroxy-6-prenyl-2‴,2‴-dimethylchromeno[3′,4′:5‴,6‴]isoflavone (isochandalone)



Compound **DS9** was isolated as a yellow solid, m.p. 97-98 °C. Its molecular formula was $C_{25}H_{24}O_5$ as indicated by mass spectrum (M^+ *m/z* 404). The UV spectrum showed maxima absorption at 266.5 nm. The IR spectrum showed absorption band of O-H stretching at 3239 cm^{-1} and C=O stretching at 1645 cm^{-1}.

The ^1H NMR (Table 14) revealed the presence of a prenyl side chain; the signals of *gem*-dimethyl protons at δ 1.84 and 1.78, the signals due to benzylic methylene protons (CH_2-1″) at δ3.47 of which coupled to an olefinic methine proton (CH-2″) at δ5.29. A *singlet* signal corresponding to an aromatic proton H-8 and a *singlet* signal of olefinic proton H-2 were present at δ6.38 and 7.84, respectively. The ABM type of three aromatic protons resonated at δ7.17, 6.84 and 7.23 were observed and assigned to be H-2′, H-5′ and H-6′, respectively. Two methyl groups resonated as a *singlet* at δ1.45 and two *cis*-olefinic protons as two *doublets* at δ6.53 and 5.64 were observed, it was implied to have dimethylchromene ring in **DS9**. A chelated C-5 hydroxy group signal was detected at δ13.25. The ^{13}C NMR spectrum and the DEPT spectra indicated the existence of four methyl carbons [δ28.07 (2C), 25.80 and 17.80], a methylene carbon (δ 21.51), eight methine carbons (δ 152.55, 131.05, 129.54, 126.94, 122.11, 121.32, 116.50 and 94.00), eleven quaternary carbons (δ 161.40, 159.68, 156.16, 153.27, 136.10, 123.48, 123.14, 121.06, 109.96, 105.89 and 76.56) and a carbonyl carbon (δ180.93).

The location of the prenyl unit was deduced to be at C-6 by the result of the 2D HMBC correlations of H-1″ to C-5 (δ 159.68), C-6 (δ 109.96) and C-7 (δ 161.40). The carbon signals at C-2′ (δ126.94), C-3′ (δ121.06) and C-4′ (δ153.27) showed correlation to H-4‴ (δ6.36) and C-3′ (δ121.06) and C-4‴ (δ122.11) to H-3‴ (δ5.64) and H-2′ (δ7.17), respectively, these correlations confirmed the presence of dimethylchromene ring and suggested that this unit fused to aromatic nucleus at C-3′ and C-4′. **DS9** was identified to be 5,7-dihydroxy-6-prenyl-2‴,2‴-dimethylchromeno[3′,4′:5‴,6‴]isoflavone.



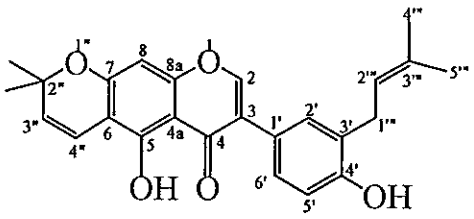
Major HMBC correlations of **DS9**

Table 14 The NMR spectral data of **DS9**

Position	δ_C*	δ_H, *mult*, *J* (Hz)	HMBC
2	152.55 (CH)	7.84 (1H, *s*)	C-3, C-4, C-8a, C-1′
3	123.14 (C)		
4	180.93 (C=O)		
4a	105.89 (C)		
5	159.68 (C)		C-4a, C-5, C-6
6	109.96 (C)		
7	161.40 (C)		
8	94.00 (CH)	6.38 (1H, *s*)	C-4, C-4a, C-6, C-7, C-8a
8a	156.16 (C)		
1′	123.48 (C)		
2′	126.94 (CH)	7.17 (1H, *d*, 1.6)	C-3, C-1′, C-4′, C-6′, C-4‴
3′	121.06 (C)		
4′	153.27 (C)		
5′	116.50 (CH)	6.84 (1H, *d*, 8.0)	C-3′, C-4′
6′	129.54 (CH)	7.23 (1H, *dd*, 8.0, 1.6)	C-3, C-1′, C-2′, C-4′
1″	21.51 (CH_2)	3.47 (2H, *d*, 7.2)	C-5, C-6, C-7, C-2″, C-3″
2″	121.32 (CH)	5.29 (1H, *t*, 7.2)	C-1″, C-4″, C-5″
3″	136.10 (C)		
4″	17.80 (CH_3)	1.84 (3H, *s*)	C-2″, C-3″, C-5″
5″	25.80 (CH_3)	1.78 (3H, *s*)	C-2″, C-3″
2‴	76.56 (C)		
3‴	131.05 (CH)	5.64 (1H, *d*, 9.6)	C-3′, C-2‴
4‴	122.11 (CH)	6.36 (1H, *d*, 9.6)	C-2′, C-3′, C-4′
5-OH		13.25 (1H, *br s*)	C-4a, C-5, C-6
2‴-Me_2	28.07 (CH_3)	1.45 (6H, *s*)	C-2‴, C-3‴, ($\underline{C}H_3$)$_2$-2‴

* Carbon type was deduced from DEPT experiments.

DS10 : 5,4′-Dihydroxy-3′-prenyl-2″,2″-dimethylchromeno[6,7:5″,6″]isoflavone (chandalone)

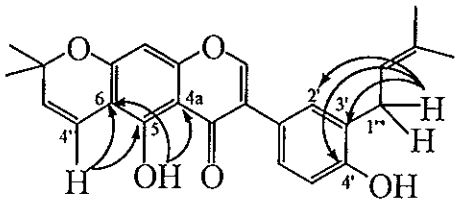


Compound **DS10** is a pale yellow solid, m.p. 63-65 °C and its molecular formula was determined to be $C_{25}H_{24}O_5$ from EIMS (M^+ *m/z* 404). In the UV spectrum, strong absorptions at 282.0 and 226.0 nm were detected. The IR spectrum exhibited absorption band of hydroxy group at 3343 cm^{-1}, conjugated carbonyl group at 1653 cm^{-1}.

The ^1H NMR spectrum (Table 15) demonstrated the resonance of a chelated hydroxy group at δ 13.18 and two signals of H-2, H-8 at δ 7.81 and 6.33. The two vicinal olefinic protons which formed an AB *quartet* signals at δ 5.62 and 6.74, and the two methyl groups of chromene ring appeared as a *singlet* signal at δ 1.47 (6H). In addition, a set of signals of prenyl side chain appeared in the spectrum as follow: the methylene protons resonated at δ 3.39, a *triplet* signal of olefinic proton resonated at δ 5.35 and two *singlet* signals of two methyl groups resonated at δ 1.79 and 1.78. The ^{13}C NMR and DEPT experiments indicated the presence of a carbonyl carbon (δ 181.00), three methyl carbons (δ 28.34, 25.84 and 17.97), a methylene carbon (δ 29.81), eight methine carbons (δ 152.55, 130.56, 128.20, 128.14, 121.58, 115.93, 115.52 and 94.84) and eleven quaternary carbons (δ 159.52, 157.32, 156.93, 154.75, 134.99, 127.16, 123.74, 122.97, 106.13, 105.56 and 78.03).

The location of prenyl side chain at C-3′ was determined from HMBC correlations; methylene protons (CH_2-1‴) at δ 3.39 correlated to C-2′ (δ 130.56), C-

3′ (δ 127.16) and C-4′ (δ 154.75). Correlation of H-4″ (δ 6.74) to C-5 (δ 159.50) and C-6 (δ 156.93), correlation of OH-5 (δ 13.18) to C-4a (δ 106.13) and C-6 (δ 156.93) and correlation of H-8 (δ 6.33) to C-7 (δ 105.56) and C-8a (δ 157.32), identified the position of dimethylchromene ring to be at C-6 and C-7. 5,4′-dihydroxy-3′-phenyl]-2″,2″-dimethylchromeno[6,7:5″,6″]isoflavone then was proposed.



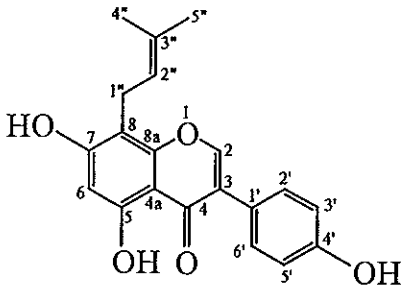
Major HMBC correlations of **DS10**

Table 15 The NMR spectral data of **DS10**

Position	δ_C*	δ_H, *mult*, *J* (Hz)	HMBC
2	152.55 (CH)	7.81 (1H, *s*)	C-3, C-4, C-8a
3	123.74 (C)		
4	181.00 (C=O)		
4a	106.13 (C)		
5	159.50 (C)		
6	156.93 (C)		
7	105.56 (C)		
8	94.84 (CH)	6.33 (1H, *s*)	C-4, C-4a, C-5, C-7, C-8a
8a	157.32 (C)		
1′	122.97 (C)		
2′	130.56 (CH)	7.25 (2H, *m*)	C-2′, C-4′, C-6′, C-1‴
3′	127.16 (C)		
4′	154.75 (C)		
5′	115.93 (CH)	6.85 (1H, *d*, 9.1)	C-3, C-3′, C-4′
6′	128.20 (CH)	7.25 (2H, *m*)	C-2′, C-4′, C-6′, C-1‴
2″	78.03 (C)		
3″	128.14 (CH)	5.62 (1H, *d*, 3.0)	C-2″
4″	115.52 (CH)	6.74 (1H, *d*, 9.8)	C-5, C-6, C-2″
1‴	29.81 (CH_2)	3.39 (2H, *d*, 2.2)	C-2′, C-3′, C-4′, C-2‴, C-3‴
2‴	121.58 (CH)	5.35 (1H, *t*, 2.2)	C-4‴, C-5‴
3‴	134.99 (C)		
4‴	17.97 (CH_3)	1.78 (3H, *s*)	C-2‴, C-5‴
5‴	25.84 (CH_3)	1.79 (3H, *s*)	C-3‴, C-4‴
5-OH		13.18 (1H, *s*)	C-4a, C-6, C-7
2″-Me_2	28.34 (CH_3)	1.47 (6H, *s*)	($\underline{C}H_3$)$_2$-2″, C-2″, C-3″

* Carbon type was deduced from DEPT experiments.

DS11 : 4′,5,7-Trihydroxy-8-prenylisoflavone (lupiwighteone)

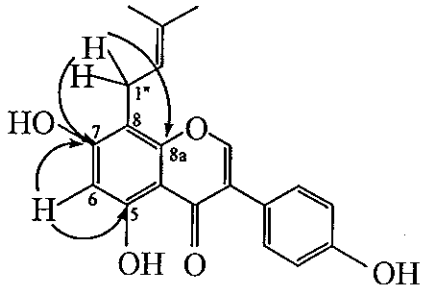


Compound **DS11** was isolated as a pale yellow solid, m.p. 130-131 °C. A molecular of $C_{20}H_{18}O_5$ was assigned (M^+ *m/z* 338). The UV spectra (λ_{max} 264.5 and 213.0 nm) suggested an isoflavone skeleton. The presence of hydroxyl group (3397 cm^{-1}) and carbonyl carbon (1656 cm^{-1}) was proposed from the IR.

The ^{1}H NMR spectrum (Table 16) revealed the presence of a prenyl group of which *gem*-dimethyl protons resonated at δ 1.74 and 1.83, the benzylic methylene protons and an olefinic methine proton resonated at δ3.48 and 5.24. A series of AA′ BB′ type signals which was assigned to be signals of H-2′,6′ and H-3′,5′ of the B ring were observed at δ7.40 and 6.90. In addition, the ^{1}H NMR showed the signal of a chelated phenolic hydroxyl group at δ12.83 and two *singlet* signals of proton H-2 at δ 7.91, H-6 at δ 6.33 as followed. The aromatic was proposed from proton H-6 ^2J correlation with C-5 and C-7 (δ_C 160.78 and 160.67), and ^3J correlation with C-8 (δ_C 106.32) on HMBC. The ^{13}C NMR signals from DEPT spectra showed resonances of a carbonyl carbon, nine quaternary carbons, seven methine carbons, a methylene carbon and two methyl carbons.

In addition, the proof for the position of the prenyl group was obtained from the result of ^3J cross peaks of the methylene proton H-1″ to C-7 and C-8a (δ_C 160.67 and 155.01). Data from the above HMBC correlation together with the value of ^{13}C chemical shift allowed for the construction of the A ring bearing free aromatic proton,

hydroxyl group and a prenyl group at C-6, C-7 and C-8, respectively. Final proof for the proposed structure confirmed the positions 2′, 3′, 5′and 6′ of the B ring unoccupied. Therefore **DS11** was 4′,5,7-trihydroxy-8-prenylisoflavone.



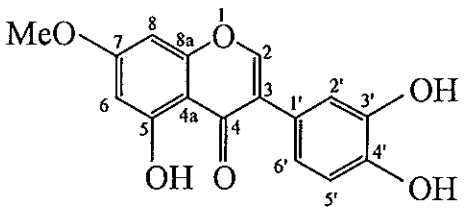
Major HMBC correlations of **DS11**

Table 16 The NMR spectral data of **DS11**

Position	δ_C*	δ_H, *mult*, *J* (Hz)	HMBC
2	152.59 (CH)	7.91 (1H, *s*)	C-3, C-4, C-8a, C-1′
3	123.27 (C)		
4	181.21 (C=O)		
4a	105.02 (C)		
5	160.78 (C)		
6	99.68 (CH)	6.33 (1H, *s*)	C-4a, C-5, C-7, C-8
7	160.67 (C)		
8	106.32 (C)		
8a	155.01 (C)		
1′	123.16 (C)		
2′,6′	130.33 (CH)	7.40 (2H, *d*, 1.8)	C-3, C-1′, C-2′,6′, C-4′
3′,5′	115.54 (CH)	6.90 (2H, *d*, 1.8)	C-3, C-1′, C-3′, C-4′
4′	155.81 (C)		
1″	21.60 (CH_2)	3.48 (2H, *d*, 5.9)	C-4a, C-7, C-8a, C-2″, C-3″
2″	120.99 (CH)	5.24 (1H, *t*, 5.9)	
3″	135.34 (C)		
4″	17.90 (CH_3)	1.83 (3H, *s*)	C-2″, C-3″, C-5″
5″	25.79 (CH_3)	1.74 (3H, *s*)	C-2″, C-3″, C-4″
5-OH		12.83 (1H, *s*)	C-5, C-6, C-7

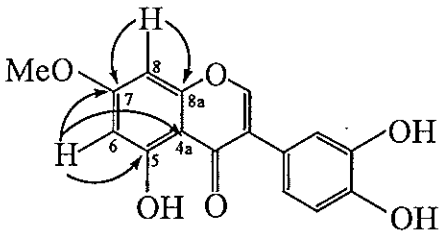
* Carbon type was deduced from DEPT experiments.

DS12 : 3′,4′,5-Trihydroxy-7-methoxyisoflavone (santal)



Compound **DS12** was isolated as a white solid, m.p. 230-231 °C and exhibited molecular ion of 300 which corresponded to $C_{16}H_{12}O_6$. The UV spectrum showed the maxima absorptions at 259.5 and 214.0 nm. The presence of a carbonyl group (1665 cm^{-1}) and the hydroxy group (3468 cm^{-1}) were suggested in the IR spectrum.

The ^{1}H NMR spectrum (Table 17), showed a sharp *singlet* signal of a chelated hydroxyl group at δ12.72 and two *singlet* signals of two hydroxyl group at δ 9.05 and δ 8.72. A typical *singlet* signal of vinylic proton H-2 of isoflavone was observed at δ7.99. A *meta* coupling signal of aromatic protons were present at δ6.38 and δ6.45 and were deduced to be signal of H-6 and H-8 from the correlation of H-6 (δ6.38) to C-4a (δ105.67), C-5 (δ162.70), C-7 (δ166.54) and C-8 (δ92.35), and between H-8 (δ6.45) to C-4a (δ105.67), C-6 (δ98.84), C-7 (δ166.54) and C-8a (δ 92.35). The ABM pattern of three aromatic protons were resonated at δ6.55, 7.05 and δ 6.46 and they were assigned to be H-2′, H-5′ and H-6′, respectively. In addition, spectral data showed that this compound had a methoxy group resonating at δ 3.89 and was located at C-7, this deduction was suggested from the correlation of OCH_3-7 (δ3.89) to C-7 (δ166.54). The ^{13}C NMR spectrum showed 16 signals (Table 17). The DEPT spectra suggested a carbonyl carbon, a methoxy carbon, six methine carbons and eight quaternary carbons. This compound were 3′,4′,5-trihydroxy-7-methoxyisoflavone and was found to have the same structure as santal.



Major HMBC correlations of **DS12**

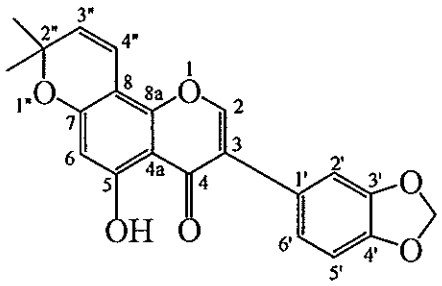
Table 17 The NMR spectral data of **DS12**

Position	δ_C*	δ_H, *mult*, *J* (Hz)	HMBC
2	155.22 (CH)	7.99 (1H, *s*)	C-3, C-4, C-8a, C-1′
3	111.46 (C)		
4	182.51 (C=O)		
4a	105.67 (C)		
5	162.70 (C)		
6	98.84 (CH)	6.38 (1H, *d*, 2.9)	C-4a, C-5, C-7, C-8
7	166.54 (C)		
8	92.35 (CH)	6.45 (1H, *d*, 2.9)	C-4a, C-6, C-7, C-8a
8a	158.17 (C)		
1′	123.50 (C)		
2′	106.34 (CH)	6.55 (1H, *d*, 2.9)	C-3, C-3′, C-4′, C-6′
3′	157.50 (C)		
4′	159.43 (C)		
5′	130.75 (CH)	7.05 (1H, *d*, 8.5)	C-1′, C-3′, C-4′
6′	108.82 (CH)	6.46 (1H, *dd*, 8.5, 2.9)	C-3, C-2′
5-OH		12.72 (1H, *s*)	C-4a, C-5, C-6
7-OMe	55.59 (CH_3)	3.89 (3H, *s*)	C-7
3′-OH		9.05† (1H, *s*)	
4′-OH		8.72† (1H, *s*)	

* Carbon type was deduced from DEPT experiments.

† Assignment may be interchangeable.

DS13 : 5-Hydroxy-3′,4′-methylenedioxy-2″,2″-dimethylchromeno[7,8:6″,5″] isoflavone (isorobustone)

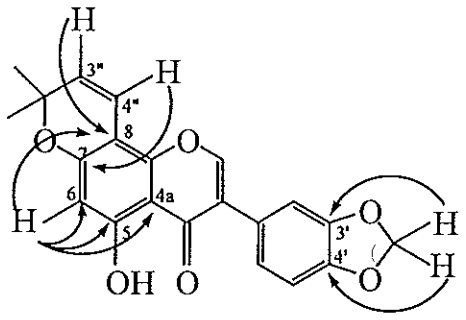


Compound **DS13** is a yellow solid, m.p. 123-124 °C. Its molecular formula of $C_{21}H_{16}O_6$ was established on the basis of mass spectrum (M^+ m/z 364). An isoflavone nucleus was elucidated from the UV (λ_{max} 267.5 and 217.0 nm) spectra. The IR spectra showed absorption bands of hydroxy (3447 cm^{-1}) and conjugated carbonyl (1656 cm^{-1}) functionalities.

The ^1H NMR spectrum (Table 18) revealed a signal of an isoflavone derivative, which the characteristic signal of H-2 was at δ 7.88, and a chelated hydroxyl group was at δ 12.85. The ABM type signal was exhibited at δ 7.05, 6.88 and 6.95 and was assigned to be H-2′, H-5′ and H-6′, respectively, of B ring. A typical signals of methylenedioxy (OCH_2O) were observed at δ 6.00 and were assigned to attach to B ring at C-3′ and C-4′. An AB quartet (δ 5.59 and 6.68) and the two methyl groups appearing as a *singlet* signal (δ 1.47, 6H) were proposed to be part of chromene ring. In addition, the *singlet* signal at δ 6.29 was shown and assigned to be uncoupled aromatic proton H-6. The ^{13}C NMR spectrum and DEPT experiments showed the resonances of ten quaternary carbons, seven methine carbons, a methylenedioxy carbon, two methyl carbons and a carbonyl carbon.

Supporting for the relative structure shown in **DS13** was provided by HMBC (Table 18). ^3J correlations of the methylenedioxy protons at δ 6.00 to C-3′ (δ 147.80)

and C-4′ (δ147.91) confirmed that a methylenedioxy ring was fused to positions C-3′ and C-4′ of the B ring. The *singlet* aromatic proton H-6 (δ6.29) was placed at C-6 (δ 100.39) according to the correlation between its proton with C-4a (δ105.98), C-5 (δ 162.28), C-7 (δ159.61) and C-8 (δ101.08). Furthermore, HMBC correlation of the olefinic proton H-4″ (δ 6.68) to C-7 (δ 159.61) indicated the attachment of a chromene ring to positions C-7 and C-8 of the A ring. The proof of the structure showed that **DS13** was 5-hydroxy-3′,4′-methylenedioxy-2″,2″-dimethylchromeno [7,8:6″,5″]isoflavone



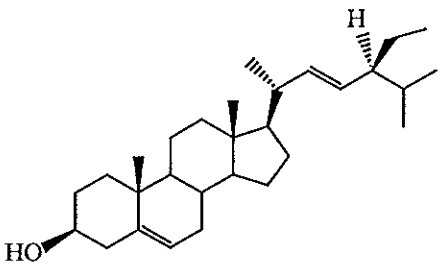
Major HMBC correlations of **DS13**

Table 18 The NMR spectral data of **DS13**

Position	δ_C*	δ_H, *mult*, *J* (Hz)	HMBC
2	152.43 (CH)	7.88 (1H, *s*)	C-2, C-3, C-4
3	123.74 (C)		
4	180.73 (C)		
4a	105.98 (C)		
5	162.28 (C)		
6	100.39 (CH)	6.29 (1H, *s*)	C-4a, C-5, C-7, C-8
7	159.61 (C)		
8	101.08 (C)		
8a	130.81 (C)		
1′	124.32 (C)		
2′	109.61 (CH)	7.05 (1H, *d*, 3.8)	C-3′, C-4′, C-6′
3′	147.80 (C)		
4′	147.91 (C)		
5′	108.48 (CH)	6.88 (1H, *d*, 7.5)	C-1′, C-3′, C-4′
6′	122.42 (CH)	6.95 (1H, *dd*, 7.5, 3.8)	C-3, C-2′, C-3′, C-4′
2″	78.07 (C)		
3″	127.47 (CH)	5.59 (1H, *d*, 10.0)	C-8, C-2″
4″	114.51 (CH)	6.68 (1H, *d*, 10.0)	C-7, C-2″
5-OH		12.85 (1H, *s*)	C-4a, C-5, C-6
2″-Me_2	28.18 (CH_3)	1.47 (6H, *s*)	($\underline{C}H_3$)$_2$-2″, C-2″, C-3″
OCH_2O	101.23 (CH_2)	6.00 (2H, *s*)	C-3′, C-4′

* Carbon type was deduced from DEPT experiments.

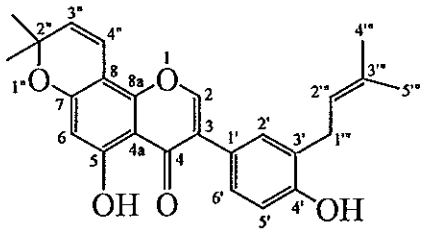
DS15 : Stigmasterol



Compound **DS15** was obtained as a white solid, m.p. 156-157 °C, $[\alpha]_D^{28}$ -55.48 ° (c = 1.5 x 10^{-2}g/100cm^3, CH_3OH). Its molecular formula $C_{29}H_{48}O$ was established by EIMS spectrum (m/z 412). The UV spectrum exhibited the maxima absorption at 208.5 nm. In IR spectrum, the absorption band of O-H stretching (3433 and 3309 cm^{-1}) and C-H stretching (2959 and 2869 cm^{-1}) were shown.

The ^{1}H NMR spectrum contained an oxymethine proton signal at δ 3.56-3.48, three olefinic protons at δ 5.36-5.33 (*m*), 5.16 (*dd*) and 5.02 (*dd*) and six methyl groups at δ 1.02, 1.05, 0.86, 0.82, 0.80 and 0.69. The ^{1}H NMR data, mass spectral data, optical rotation value and melting point were corresponded to the previous reported data of stigmasterol. Therefore, **DS15** was assigned to be stigmasterol.

DS16 : 4′,5-Dihydroxy-3′-prenyl-2″,2″-dimethylchromeno[7, 8 : 6″,5″]isoflavone



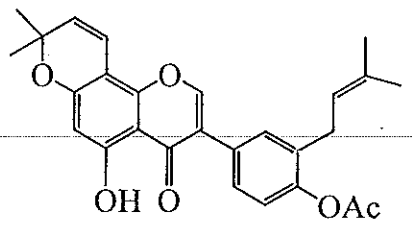
Compound **DS16** was obtained as a yellow solid, m.p. 115-116 °C. Its molecular formula $C_{25}H_{24}O_5$ was established by EIMS spectrum (M^+ m/z 404). The UV spectrum exhibited the maxima absorptions at 283.5 and 249.5 nm. In IR spectrum, absorption band of O-H stretching and C=O stretching were shown at 3450 and 1653 cm^{-1}, respectively. The data suggested that **DS16** was isoflavone.

The ^1H NMR spectrum (Table 19) contained two *singlet* signals of hydroxy proton, the one at δ13.18 was a chelated hydroxy group (OH-5) and the broad signal at δ5.88 was assigned to be OH-4′. The characteristic H-2 of isoflavone structure was shown as a sharp *singlet* signal at δ7.80. The ABM system signals at δ7.22 (*d*, *J* = 2.1 Hz), 7.20 (*dd*, *J* = 7.7 and 2.1 Hz) and 6.77 (*d*, *J* = 7.7 Hz) attributed to a 2′,5′,6′-trisubstituted benzene ring was observed. The set of olefinic proton signals at δ6.74 and 5.63, and a *singlet* signal of two methyl groups at δ1.48 (6H) which was the part of chromene ring were detected. In addition, the methylene protons as a *doublet* signal (δ3.37), the olefinic proton as a *triplet* signal (δ5.34) and two methyl groups as a *singlet* signal (δ 1.77) of the prenyl side chain appeared in the spectrum. Two hydroxyl groups were present, the one resonating at low field (δ13.18) was nearby the carbonyl group and another one was placed at C-4′. The *singlet* signal of aromatic proton at δ6.34 was assigned to locate at C-6. According to the data, **DS16** was proposed to be 4′,5-dihydroxy-3′-prenyl-2″,2″-dimethylchromeno[7, 8 : 6″,5″] isoflavone. The ^{13}C NMR spectrum and DEPT experimentals (Table 19) showed the

presence of four methyl carbons [δ28.25 (2C), 25.72 and 17.83], a methylene carbon (δ29.45), eight methine carbons (δ152.59, 130.40, 128.10, 128.03, 121.69, 115.84, 115.44 and 94.81), eleven quaternary carbons (δ 159.47, 157.28, 156.81, 154.65, 134.45, 127.42, 123.78, 122.69, 106.07, 105.51 and 78.00) and a carbonyl carbon (δ 181.03).

The structure of this compound was supported by HMBC spectrum (Table 19). The H-4'' (δ6.74) showed correlation to C-4a (δ106.07), C-7 (δ156.81), C-8 (δ 105.51) and C-8a (δ157.28). Not only H-4'' but also H-3'' (δ5.63) had correlation with C-8 (δ105.51), this result suggested that the chromene moiety was connected to the 7,8-positions. Correlation between H-6 (δ 6.34) to C-4a (δ 106.07), C-5 (δ 159.47), C-7 (δ156.81) and C-8 (δ105.51) enabled us to deduce the location of the proton to be at the C-6 position. The structure of ring B was assigned based on the ^{1}H NMR data and comparable to **DS10** as well as NOE experiments. The NOE studies displayed enhancement of the H-5' (δ 6.77), H-1''' (δ 3.37) and H-2''' (δ 5.34) signals upon irradiation of the hydroxy proton at C-4', it thus confirmed the position of OH and isoprenyl moiety of B ring.

A resonance of acetyl group was shown in the NMR spectrum of acetylated product, **DS16(A)** (Table 19) and the down field shifts of H-2' (δ7.39), H-5' (δ7.09) and H-6' (δ7.37) were observed. The results supported the position of a hydroxyl group at ring B.



DS16(A)

Table 19 The NMR spectral data of **DS16** and **DS16(A)**

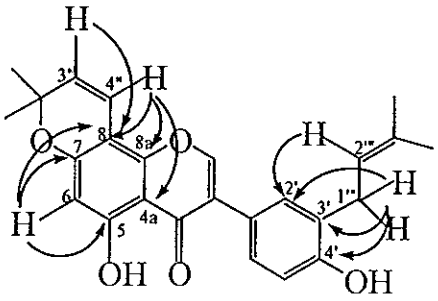
Position	δ_C*	δ_H, *mult*, *J* (Hz) **DS16**	δ_H, *mult*, *J* (Hz) **DS16(A)**†	HMBC
2	152.59 (CH)	7.80 (1H, *s*)	7.83 (1H, *s*)	C-3, C-4, C-8a
3	123.78 (C)			
4	181.03 (C=O)			
4a	106.07 (C)			
5	159.47 (C)			
6	94.81 (CH)	6.34 (1H, *s*)	6.33 (1H, *s*)	C-4a, C-5, C-7, C-8
7	156.81 (C)			
8	105.51 (C)			
8a	157.28 (C)			
1′	122.69 (C)			
2′	130.40 (CH)	7.22 (1H, *d*, 2.1)	7.39 (1H, *d*, 2.0)	C-3, C-4′, C-6′
3′	127.42 (C)			
4′	154.65 (C)			
5′	115.84 (CH)	6.77 (1H, *d*, 7.7)	7.09 (1H, *d*, 8.0)	C-1′, C-3′, C-4′
6′	128.03 (CH)	7.20 (1H, *dd*, 7.7, 2.1)	7.37 (1H, *dd*, 8.0, 2.0)	C-3, C-2′, C-4′
2″	78.00 (C)			
3″	128.10 (CH)	5.63 (1H, *d*, 9.8)	6.73 (1H, *d*, 9.6)	C-8, C-2″
4″	115.44 (CH)	6.74 (1H, *d*, 9.8)	5.62 (1H, *d*, 9.6)	C-4a, C-7, C-8, C-8a, C-2″
1‴	29.45 (CH_2)	3.37 (2H, *d*, 7.4)	3.28 (2H, *d*, 6.4)	C-2′, C-3′, C-4′, C-2‴, C-3‴
2‴	121.69 (CH)	5.34 (1H, *t-like*, 7.4)	5.24 (1H, *t-like*, 6.4)	C-2′, C-1‴, C-4‴
3‴	134.45 (C)			
4‴	17.83 (CH_3)	1.77 (6H, *s*)	1.73 (3H, *s*)	C-2‴, C-3‴, C-4‴, C-5‴

Table 19 (continued)

Position	δ_C*	δ_H, *mult*, *J* (Hz) **DS16**	δ_H, *mult*, *J* (Hz) **DS16(A)**†	HMBC
5‴	25.72 (CH_3)	1.77 (6H, *s*)	1.70 (3H, *s*)	C-2‴, C-3‴, C-4‴, C-5‴
5-OH		13.18 (1H, *s*)	13.10 (1H, *s*)	C-4a, C-5, C-7
4′-OH		5.88 (1H, *br s*)		
4′-OAc			2.31 (3H, *s*)	
2″-Me_2	28.25 (CH_3)	1.48 (6H, *s*)	1.47 (6H, *s*)	C-2″, ($\underline{C}H_3$)$_2$-2″, C-3″, C-4″

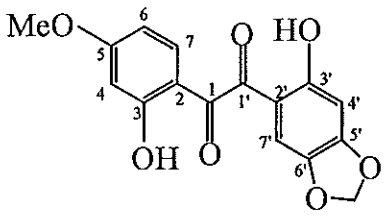
* Carbon type was deduced from DEPT experiments.

† δ_C and HMBC unrecorded.



Major HMBC correlations of **DS16**

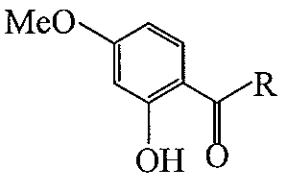
DS18 : 3,3′-Dihydroxy-5-methoxy -5′,6′-methylenedioxybenzil



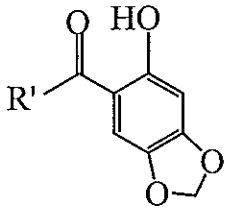
Compound **DS18** is a yellow solid, m.p. 132-133 °C. The molecular formula was determined as $C_{16}H_{12}O_7$ by EIMS (M^+ m/z 316). The UV spectrum showed maxima absorption at 282.8, 258.6 and 212.0 nm. The IR spectrum exhibited O-H stretching at 3433 cm^{-1} and C=O stretching at 1624 cm^{-1}. ^{13}C NMR spectrum indicated that there were two carbonyl carbons in the structure (δ194.84 and 194.40).

The ^1H NMR spectrum (Table 20) showed a *singlet* signal of methoxy group at δ3.87 and a *singlet* resonance of methylenedioxy group at δ5.99. Two signals of hydroxy proton which formed hydrogen bonding to carbonyl group were displayed at δ12.22 and 11.81. Three signals at δ7.40, 6.51 and 6.45 appeared as ABM type, this suggested that there was 1,3,5-trisubstituted benzene ring in the structure. Two more *singlets* of aromatic proton were observed at δ6.81 and 6.53, it implied that **DS18** contained one more aromatic ring with 1, 2, 4, 5-tetrasubstituted. The ^{13}C NMR spectrum and DEPT experiments indicated the carbon resonances of a methoxy carbon at δ55.72, a methylene carbon at δ102.54, five methine carbons at δ134.32, 109.25, 108.02, 101.31 and 99.11, and seven quaternary carbons at δ168.34, 167.40, 164.84, 156.86, 141.63, 111.02 and 109.58 and two carbonyl carbons at δ194.84 and 194.40. The type of carbon corresponded to the carbons in subunit A and B, indicated that two subunit were linked to each other by the two carbonyl groups. According to substitution pattern in aromatic ring, subunit A and B were assigned and were

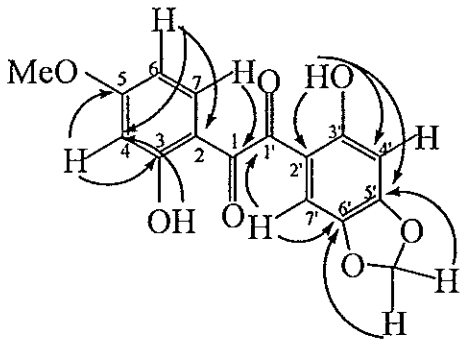
confirmed by HMBC. Subsequently, **DS18** was identified to be 3,3′-dihydroxy-5-methoxy -5′,6′-methylenedioxybenzil which is a new natural occurring compound.



subunit A



subunit B



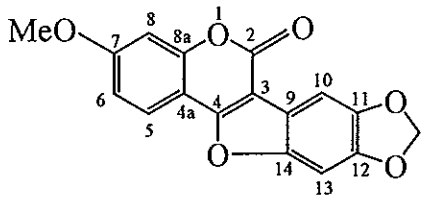
Major HMBC correlations of **DS18**

Table 20 The NMR spectral data of **DS18**

Position	δ_C*	δ_H, *mult*, *J* (Hz)	HMBC
1	194.84 (C=O)		
2	111.02 (C)		
3	167.40 (C)		
4	101.31 (CH)	6.51 (1H, *d*, 2.2)	C-2, C-3, C-5
5	168.34 (C)		
6	109.25 (CH)	6.45 (1H, *dd*, 8.8, 2.2)	C-2, C-4
7	134.32 (CH)	7.40 (1H, *d*, 8.4)	C-1, C-3, C-5
1′	194.40 (C=O)		
2′	109.58 (C)		
3′	164.84 (C)	12.22 (1H, *s*)	C-2′, C-3′, C-4′, C-5′
4′	99.11 (CH)	6.53 (1H, *s*)	C-2′, C-3′, C-5′, C-6′
5′	156.86 (C)		
6′	141.63 (C)		
7′	108.02 (CH)	6.81 (1H, *s*)	C-1′, C-3′, C-5′, C-6′
3-OH		11.81 (1H, *s*)	C-3, C-4
5-OMe	55.72 (CH_3)	3.87 (3H, *s*)	C-5
3′-OH		12.22 (1H, *s*)	C-2′, C-3′, C-4′, C-5′
OCH_2O	102.54 (CH_2)	5.99 (2H, *s*)	C-1, C-2

* Carbon type was deduced from DEPT experiments.

DS19 : 7-Methoxy-11,12-methylenedioxycoumestan (flemichapparin C)

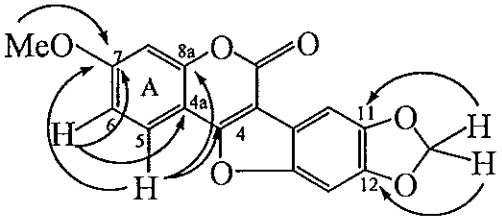


Compound **DS19** was obtained as a yellow solid, m.p. 251-252 °C and had a molecular formula $C_{17}H_{10}O_6$, established by EIMS spectrum (M^+ m/z 310). Its UV spectrum showed the maxima absorptions at 347.4, 245.2 and 213.0 nm. The IR spectrum showed the stretching of C=O at 1627 cm^{-1}.

The ^1H NMR spectrum (Table 21) showed the presence of ABM system signals at δ7.85 (*d*, *J* = 7.2 Hz), 6.98 (*dd*, *J* = 7.2 and 2.7 Hz) and 6.96 (*d*, *J* = 2.7 Hz) which were attributable to aromatic proton H-5, H-6 and H-8 in A ring. A methoxy group was shown in the spectrum and was located at C-7. Enhancements of H-6 (δ 6.98) and H-8 (δ 6.96) on the NOE experiments were observed on irradiation of methoxy protons at δ3.91. The result suggested that the methoxy group was *ortho* to H-6 and H-8. Two isolated aromatic protons resonated at δ 7.47 and 7.12 were assigned to be at the *para* position H-10 and H-13 of the second aromatic ring. The *singlet* signal at δ 6.07 (2H, *s*) was implied to have methylenedioxy group. The methylenedioxy proton (δ6.07) exhibited correlation with C-11 (δ147.79) and C-12 (δ 146.52) on HMBC, it was therefore indicated that the methylenedioxy was connected to aromatic carbon C-11 and C-12. The characteristic signal of H-2 of an isoflavone and a chelated hydroxy signal were not observed, thus **DS19** was elucidated as coumestan derivative, 7-methoxy-11,12-methylenedioxycoumestan. The ^{13}C NMR spectrum and DEPT experiments (Table 21) indicated the presence of a methyl carbon (δ55.69), a methylene carbon (δ102.10), five methine carbons (δ122.54, 113.22,

101.59, 100.32 and 94.10), nine quaternary carbons (δ162.95, 160.47, 155.30, 150.99, 147.79, 146.52, 117.33, 106.37 and 104.19) and a carbonyl carbon (δ158.94).

The attribution of ring A was resulted from the ^{13}C NMR and HMBC data as shown below.



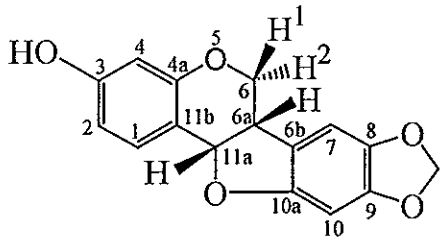
Major HMBC correlations of **DS19**

Table 21 The NMR spectral data of **DS19**

Position	δ_C*	δ_H, *mult*, *J* (Hz)	HMBC
2	158.94 (C=O)		
3	104.19 (C)		
4	155.30 (C)		
4a	106.37 (C)		
5	122.54 (CH)	7.85 (1H, *d*, 7.2)	C-4, C-7, C-8a
6	113.22 (CH)	6.98 (1H, *dd*, 7.2, 2.7)	C-4, C-4a, C-6, C-7
7	162.95 (C)		
8	101.59 (CH)	6.96 (1H, *d*, 2.7)	C-4a
8a	160.47 (C)		
9	117.33 (C)		
10	100.32 (CH)	7.47 (1H, *s*)	C-11, C-12, C-14
11	147.79 (C)		
12	146.52 (C)		
13	94.10 (CH)	7.12 (1H, *s*)	C-9, C-11, C-12, C-14
14	150.99 (C)		
7-OMe	55.69 (CH_3)	3.91 (3H, *s*)	C-7
OCH_2O	102.10 (CH_2)	6.07 (2H, *s*)	C-11, C-12

* Carbon type was deduced from DEPT experiments.

DS20 : (-)-3-Hydroxy-8,9-methylenedioxy-6a,11a-dihydropterocarpan ((-)-maackiain)



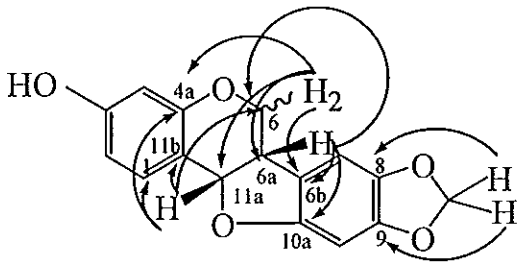
Compound **DS20** is a brown solid, m.p. 121-122 °C, $[\alpha]_D^{28}$ -29.03° (c = 1.4 x 10^{-2} g/100 cm^3, CH_3OH) and its molecular formula was determined to be $C_{16}H_{12}O_5$, as indicated by EIMS (M^+ m/z 284). The strong absorptions at 310.6, 260.8 and 212.2 nm were detected on UV spectrum. The IR spectrum exhibited absorption band of O-H stretching (3430 cm^{-1}), C=C stretching (1630, 1597 cm^{-1}) and no carbonyl group.

The ^{1}H NMR (Table 22) showed the existence of two *singlet* signals of aromatic protons H-7 and H-10 at δ6.72 and 6.43, and ABM type of aromatic proton which were assigned to H-1, H-2 and H-4 at δ7.36, 6.54 and 6.41. Two *doublet* signals at δ4.22 and 3.65, a *multiplet* signal at δ3.47 were found to couple to each other. Moreover a *multiplet* signal at δ3.47 was further coupled to a *doublet* signal at δ5.47. Thus these signals were suggested to be signals of H^1-6, H^2-6, H-6a and H-11a, respectively. The remaining signals appearing as two *singlets* with very small coupling constant (1 Hz) at δ 5.92 and δ 5.89 were proposed to be two protons of methylenedioxy ring. Accordingly, **DS20** was deduced to be dihydropterocarpan derivative. The ^{13}C NMR spectrum and the DEPT spectra indicated the existence of two methylene carbons (δ 101.15 and 66.11), seven methine carbons (δ 132.18, 109.67, 104.62, 103.55, 93.66, 78.18 and 39.65) and seven quaternary carbons (δ 157.19, 156.84, 154.39, 148.25, 141.84, 117.87 and 112.61).

In the HMBC experiments; correlations between H^1,H^2-6 (δ4.22 and 3.65) to C-4a (δ 156.84), C-6b (δ 117.87) and C-11a (δ 78.18), confirmed the position of

methyleneoxy protons. The remaining of proton H-6a (δ3.47) was confirmed by the correlations to C-6 (δ66.11), C-6b (δ117.87) and C-11a (δ78.18), whereas H-11a (δ 5.47) showed the correlation to C-1 (δ132.18), C-4a (δ156.84) and C-6 (δ66.11). In addition, the correlations of methylenedioxy (OCH_2O) to C-8 (δ148.25) and C-9 (δ 141.84) supported that methylenedioxy ring was fused to aromatic nucleus at C-8 and C-9.

Stereochemistry of H-6a and H-11a were assigned by NOE experiments. H^1-6 (δ4.22) and H-11a (δ5.47) signals were enhanced upon irradiation at H-6a (δ3.47). It was therefore proposed that H^1-6, H-6a and H-11a were *cis*. According to the NMR assignment and specific rotation, **DS20** was considered to be (-)-3-hydroxy-8,9-methylenedioxy-6a,11a-dihydropterocarpan which corresponded to (-)-maackiain.



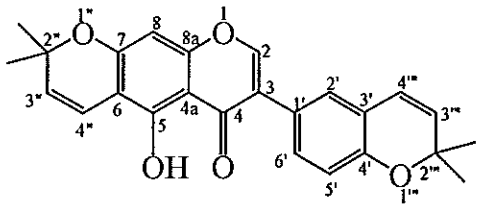
Major HMBC correlations of **DS20**

Table 22 The NMR spectral data of **DS20**

Position	δ_C*	δ_H, *mult*, *J* (Hz)	HMBC
1	132.18 (CH)	7.36 (1H, *d*, 8.0)	C-3, C-4a, C-11a
2	109.67 (CH)	6.54 (1H, *dd*, 8.0, 2.0)	C-4, C-4a, C-11b
3	157.19 (C)	4.95 (1H, *br s*)	C-2, C-4, C-4a
4	103.55 (CH)	6.41 (1H, *d*, 2.0)	C-2, C-3, C-4a, C-11b
4a	156.84 (C)		
6	66.11 (CH_2)	4.22 (1H, *dd*, 11.2, 4.8)[1]	C-4a, C-6a, C-6b, C-11a
		3.65 (1H, *t*, 11.2)[2]	C-4a, C-6a, C-6b, C-11a
6a	39.65 (CH)	3.47 (1H, *m*)	C-6, C-6b, C-10a
6b	117.87 (C)		
7	104.62 (CH)	6.72 (1H, *s*)	C-6a, C-8, C-9, C-10, C-10a
8	148.25 (C)		
9	141.84 (C)		
10	93.66 (CH)	6.43 (1H, *s*)	C-6b, C-8, C-9, C-10a
10a	154.39 (C)		
11a	78.18 (CH)	5.47 (1H, *d*, 7.0)	C-1, C-4a, C-6, C-11b
11b	112.61 (C)		
3-OH		4.95 (1H, *br s*)	C-2, C-4, C-4a
OCH_2O	101.15 (CH_2)	5.92 (1H, *d*, 1.0)	C-8, C-9
		5.89 (1H, *d*, 1.0)	

* Carbon type was deduced from DEPT experiments.

DS22 : 5-Hydroxy-2″,2″-dimethylchromeno[6,7:5″,6″]-2‴,2‴-dimethylchromeno [3′,4′:5‴,6‴]isoflavone



Compound **DS22** was isolated as a yellow solid, m.p. 69-70 °C. The UV spectrum showed maximum absorption at 282.2 nm. The presence of hydroxy group (3351 cm^{-1}) and a carbonyl group (1742 cm^{-1}) were suggested in the IR spectrum.

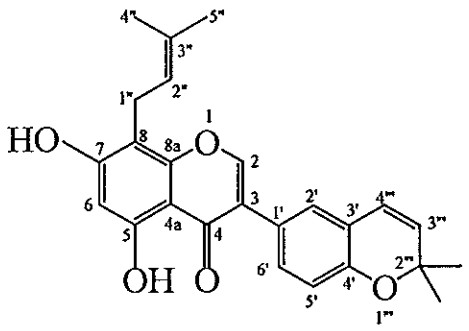
The ^{1}H NMR spectrum (Table 23) showed a *singlet* signal of a chelated hydroxyl group at δ13.18, a *singlet* signal of vinylic proton H-2 at δ7.82, a *singlet* aromatic proton signal H-8 at δ6.33 (1H, *s*). The ABM pattern of three aromatic protons were existed at δ7.17 (1H, *d*, *J* = 2.3 Hz), 6.83 (1H, *d*, *J* = 8.0 Hz) and 7.23 (1H, *dd*, *J* = 2.3 and 8.0 Hz) and were assigned to be the resonances of H-2′, H-5′ and H-6′, respectively. Two sets of the proton resonance of 2,2 dimethyl chromene ring were detected. Two olefinic protons of the first chromene rings showed two *doublet* signals at δ6.73 (1H, *J* = 9.6 Hz) and 5.64 (1H, *J* = 9.6 Hz), whereas the second chromene ring showed two *doublet* signals of two olefinic protons at δ6.35 (1H, *J* = 9.6 Hz), and 5.62 (1H, *J* = 9.6 Hz). Four methyl groups of two chromene rings were exibited two *singlet* signals at δ1.46 (6H) and 1.44 (6H). Base on ^{1}H NMR spectral data, **DS22** was proposed to be 5-hydroxy-2″,2″-dimethylchromeno[6,7:5″,6″]-2‴,2‴-dimethylchromeno[3′,4′:5‴,6‴]isoflavone.

Table 23 The ^{1}H NMR spectral data of **DS22**

Position	δ_H , *mult* , *J* (Hz)
2	7.82 (1H, *s*)
5-OH	13.18 (1H, *s*)
6	6.33 (1H, *s*)
2′	7.17 (1H, *d*, 2.3)
5′	6.83 (1H, *d*, 8.0)
6′	7.23 (1H, *dd*, 8.0, 2.3)
3″	5.64† (1H, *d*, 9.6)
4″	6.73†† (1H, *d*, 9.6)
2″-Me_2	1.46††† (6H, *s*)
3‴	5.62† (1H, *d*, 9.6)
4‴	6.35†† (1H, *d*, 9.6)
2‴-Me_2	1.44††† (6H, *s*)

†, ††, ††† Assignment may be interchangeable.

DS23 : 5,7-Dihydroxy-8-prenyl-2″,2″-dimethylchromeno[3′,4′:5‴,6‴]isoflavone (ulexone A)



Compound **DS23** was obtained as a yellow solid, m.p. 106-107 °C (Ref. 108-110 °C). A molecular formula of $C_{25}H_{24}O_5$ was assigned ($[M]^+$ m/z 404). The UV spectra (λ_{max} 267.2 nm) suggested an isoflavone skeleton. In IR spectrum, the absorption band of O-H stretching (3351 cm^{-1}) and C=O stretching (1742 cm^{-1}) were shown.

The ^1H NMR spectrum (Table 24) exhibited a sharp *singlet* signal of a chelated hydroxy group at δ13.27 and a *singlet* resonance of olefinic proton at δ7.85. These two signals implied that **DS23** was an isoflavone containing 5-OH and olefinic proton H-2. The A ring was proposed to be pentasubstituted benzene ring according to an isolated resonance of aromatic proton at δ6.33. The signals at δ7.19 (1H, *d*, *J* = 2.3 Hz), 6.85 (1H, *d*, *J* = 8.3 Hz) and 7.25 (1H, *dd*, *J* = 2.3, 8.3 Hz) were attributed to H-2′, H-5′ and H-6′ of ring B. The proton signals of prenyl side chain were shown as follow: two *singlet* signals of *gem*-dimethyl protons resonated at δ1.86 and 1.79, the signals due to benzylic methylene protons (δ 3.48) which coupled to an olefinic methine proton (δ5.30). Two *doublets* resonances of vinylic protons were observed at δ6.30 and 5.65, furthermore, the resonance of two methyl groups existed at δ1.46 (6H, *s*). These data suggested that **DS23** contained 2,2-dimethylchromene ring. 5,7-

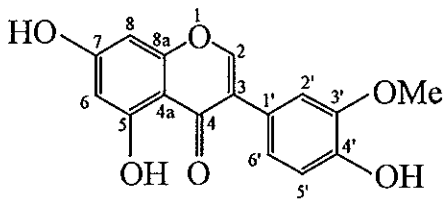
dihydroxy-8-prenyl-2″,2″-dimethylchromeno[3′,4′:5‴,6‴]isoflavone or ulexone A was assigned for **DS23.**

Table 24 The ^{1}H NMR spectral data of **DS23**

Position	δ_H , *mult* , *J* (Hz)
2	7.85 (1H, *s*)
5-OH	13.27 (1H, *s*)
6	6.33 (1H, *s*)
2′	7.19 (1H, *d*, 2.3)
5′	6.85 (1H, *d*, 8.3)
6′	7.25 (1H, *dd*, 8.3, 2.3)
1″	3.48 (2H, *br d*, 6.8)
2″	5.30 (1H, *t-like*, 6.8)
4″	1.86† (3H, *s*)
5″	1.79† (3H, *s*)
2‴-Me_2	1.46† (6H, *s*)
3‴	5.65$^{\dagger\,\dagger}$ (1H, *d*, 9.0)
4‴	6.30$^{\dagger\,\dagger}$ (1H, *d*, 9.0)

†, †† Assignment may be interchangeable.

DS24 : 4′,5,7-Trihydroxy-3′-methoxyisoflavone (3′-methylorobol)



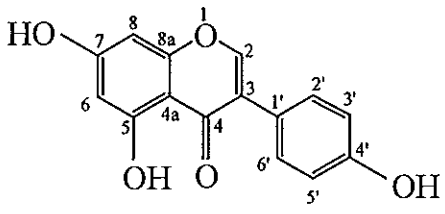
Compound **DS24** is a pale yellow solid, m.p. 124-125 °C. EIMS showed the molecular ion of 300 which corresponded to $C_{16}H_{12}O_6$. An isoflavone nucleus was elucidated from the UV spectrum (λ_{max} 260.8 nm). In IR spectrum, the absorption band of O-H stretching (3351 cm^{-1}) were shown.

The ^1H NMR spectrum (Table 25) showed the signal of an isoflavone derivative, of which the characteristic signal of H-2 was at δ 7.84, and a signal of a chelated hydroxyl group was at δ 12.83. Two *doublets* of aromatic protons present at δ 6.35 and 6.28 were the signal of H-8 and H-6. The ABM type of aromatic protons resonated at δ 7.11, 6.96 and 6.92 were deduced for H-2′, H-5′ and H-6′, respectively. In addition, the signals of a methoxy group and a hydroxy group were observed at δ 3.93 and 3.40, respectively. NOE experiment, irradiation at OMe (δ 3.93) enhanced the signal of H-2′ (δ 7.11) and irradiation at H-2′ (δ 7.11) resulted in the enhancement of the signals of OMe (δ 3.93) and H-2 (δ 7.84). The results suggested that the methoxy group was at C-3′. **DS24** was therefore assigned to be 4′,5,7-trihydroxy-3′-methoxyisoflavone which was known as 3′-methylorobol.

Table 25 The ^{1}H NMR spectral data of **DS24**

Position	δ_{H} , *mult* , *J* (Hz)
2	7.84 (1H, *s*)
5-OH	12.83 (1H, *s*)
6	6.28 (1H, *d*, 1.7)
8	6.35 (1H, *d*, 1.7)
2′	7.11 (1H, *d*, 1.5)
3′-OMe	3.93 (3H, *s*)
4′-OH	3.40 (1H, *s*)
5′	6.96 (1H, *d*, 7.6)
6′	6.92 (1H, *dd*, 7.6, 1.5)

DS25 : 4′,5,7-Trihydroxyisoflavone (genistein)



Compound **DS25** is a yellow solid, m.p. 268-269 °C (Ref. 270 °C). EIMS showed the molecular ion of 270 which corresponded to $C_{15}H_{10}O_5$. An isoflavone nucleus was elucidated from the UV spectra (λ_{max} 261.8 nm). **DS25** exhibited IR absorption band at 3351 cm^{-1} indicated the presence of hydroxyl group.

The ^1H NMR spectrum (Table 26) revealed a signal of an isoflavone derivative, of which the characteristic signal of H-2 was at δ7.82, and a sharp *singlet* signal of a chelated hydroxyl group at δ12.83. A series of AA′BB′ type signals which was assigned to be signals of H-2′,6′ and H-3′,5′ of the B ring, were observed at δ 7.35 (2H) and 6.89 (2H), respectively. Two *doublets* with the same coupling constant (J = 2.6 Hz) of two *meta* proton of the A ring were existed at δ 6.34 and 6.28. Therefore **DS25** was expected to be 4′,5,7-trihydroxyisoflavone. This compound was known as genistein.

Table 26 The ^{1}H NMR spectral data of **DS25**

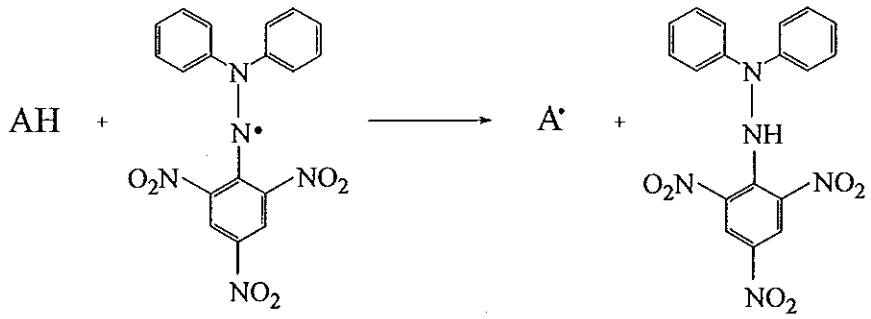
Position	δ_H , *mult* , *J* (Hz)
2	7.82 (1H, *s*)
5-OH	12.83 (1H, *s*)
6	6.28 (1H, *d*, 2.6)
8	6.34 (1H, *d*, 2.6)
2′,6′	7.35 (2H, *d*, 8.0)
3′,5′	6.89 (2H, *d*, 8.0)

3.2 Evaluation of antioxidation activity

Phenolic compounds were known to be the antioxidant with an excellent hydrogen or electron donors (Shahidi, *et al.*, 1992). Most of compounds isolated from *D. scandens* were isoflavone which contained free phenolic hydroxy functionality. It was therefore of considerable interest in the studies of antioxidative activity.

The *in vitro* assay system which was used to evaluate the activity was the free radical scavenging activity of the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Tamura, *et al.*, 1990)

DPPH is a free radical which shows a maximum absorption at 517 nm. When DPPH accepts an electron or hydrogen radical, it becomes a more stable compound and the absorption vanishes.



To determine the scavenging activity, the **DS** samples were tested at the final concentration of 10 μM. The scavenging activity were monitored by following the decrease of the absorbance at 517 nm with time.

Radical scavenging properties of compounds from *D. scandens* were evaluated against the DPPH radical. BHT and ascorbic acid were used as reference compounds. These compounds were all active in this assay. **DS6**, **DS7** and **DS12** exhibited strongest activity. While **DS6** and **DS12** showed higher activity than that of BHT. However their potency remained less than that of ascorbic acid. The results were shown in Figure 6.

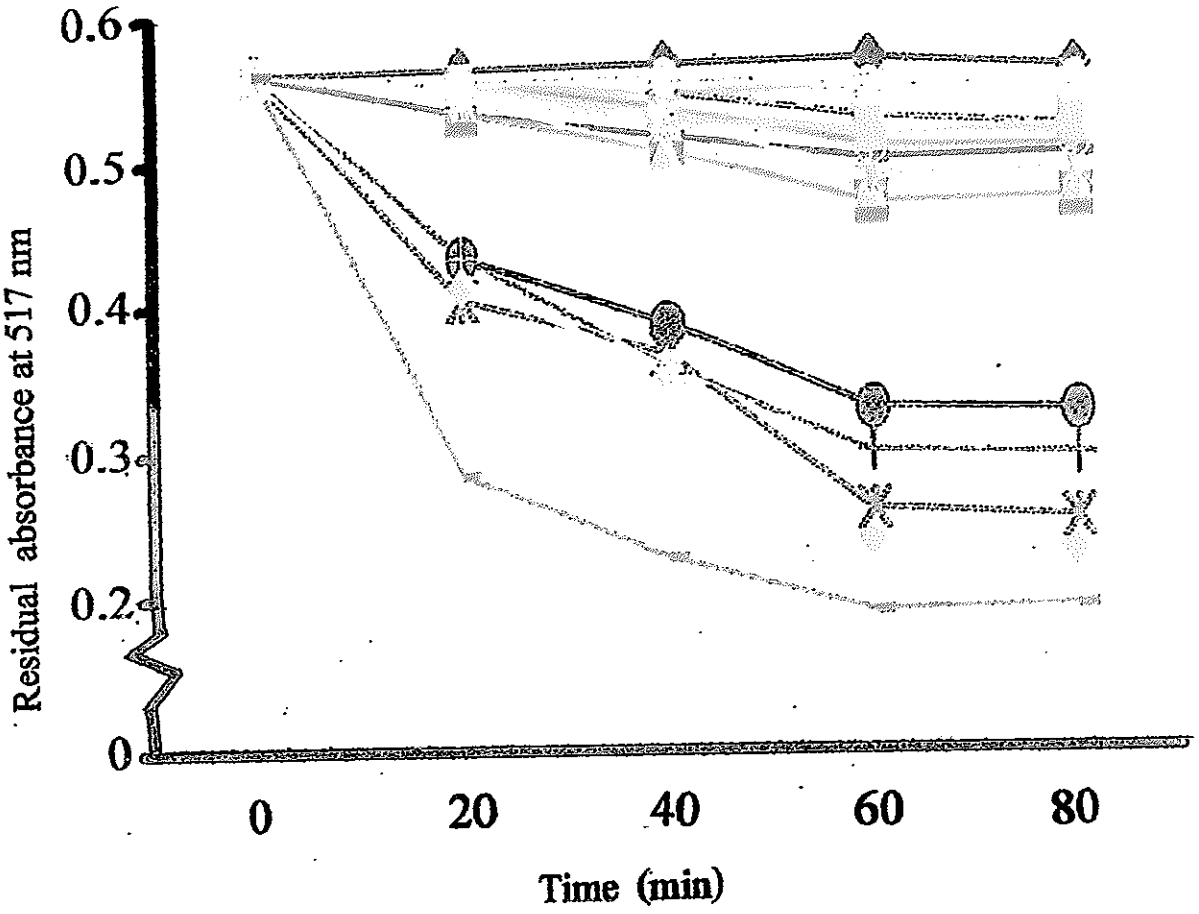


Figure 6 Scavenging activity of compounds from *D. scandens*

DS2 DS3 DS4 DS6 DS7 DS9 DS10 DS11 DS12 DS13 DS16 DS18 DS19 DS20 BHT and Ascorbic acid On DPPH. Control oxidation is represented by .

The assessment of the antioxidation activity was extended for the three most active ones, **DS6**, **DS7** and **DS12**. The antioxidation effect of these compounds was evaluated as the concentration required to scavenge 50% DPPH free radical (IC_{50}). **DS6** and **DS7** exhibited the activity with IC_{50} 3.63 and 8.75 μM, whereas **DS12** had IC_{50} 2.75 μM at 60 min (Table 27). In comparison to BHT, **DS6** and **DS12** seemed to be a better hydrogen radical donor than BHT by DPPH.

Table 27 IC_{50} values for the antioxidation activity

	DS6	**DS7**	**DS12**	BHT	Ascorbic acid
IC_{50} (μM, 45 min)	4.63	8.75	3.75	7.88	2.63
IC_{50} (μM, 60 min)	3.63	8.75	2.75	6.88	2.00

APPENDIX

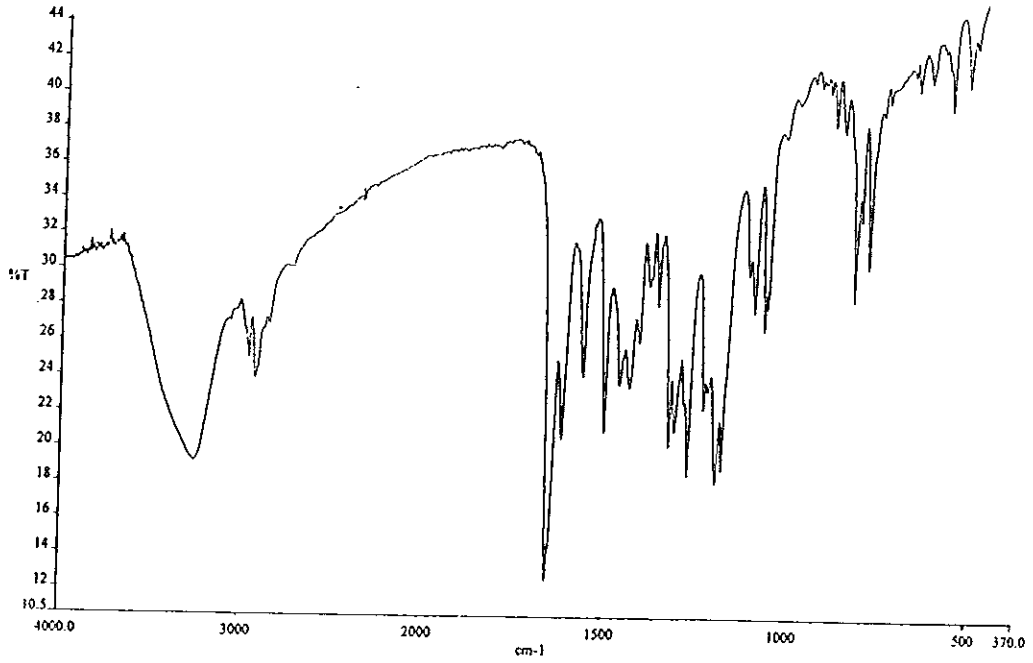


Figure 7 IR (KBr) spectrum of **DS2**

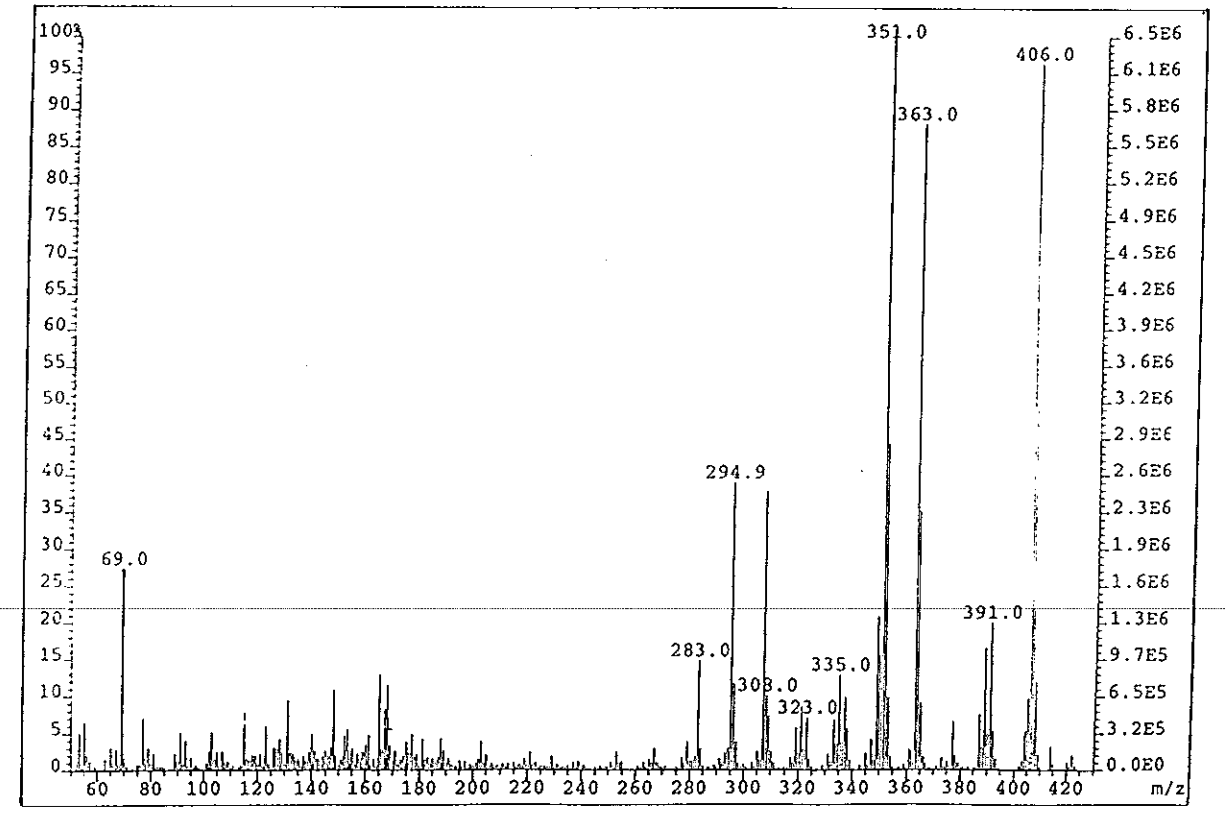


Figure 8 Mass spectrum of **DS2**

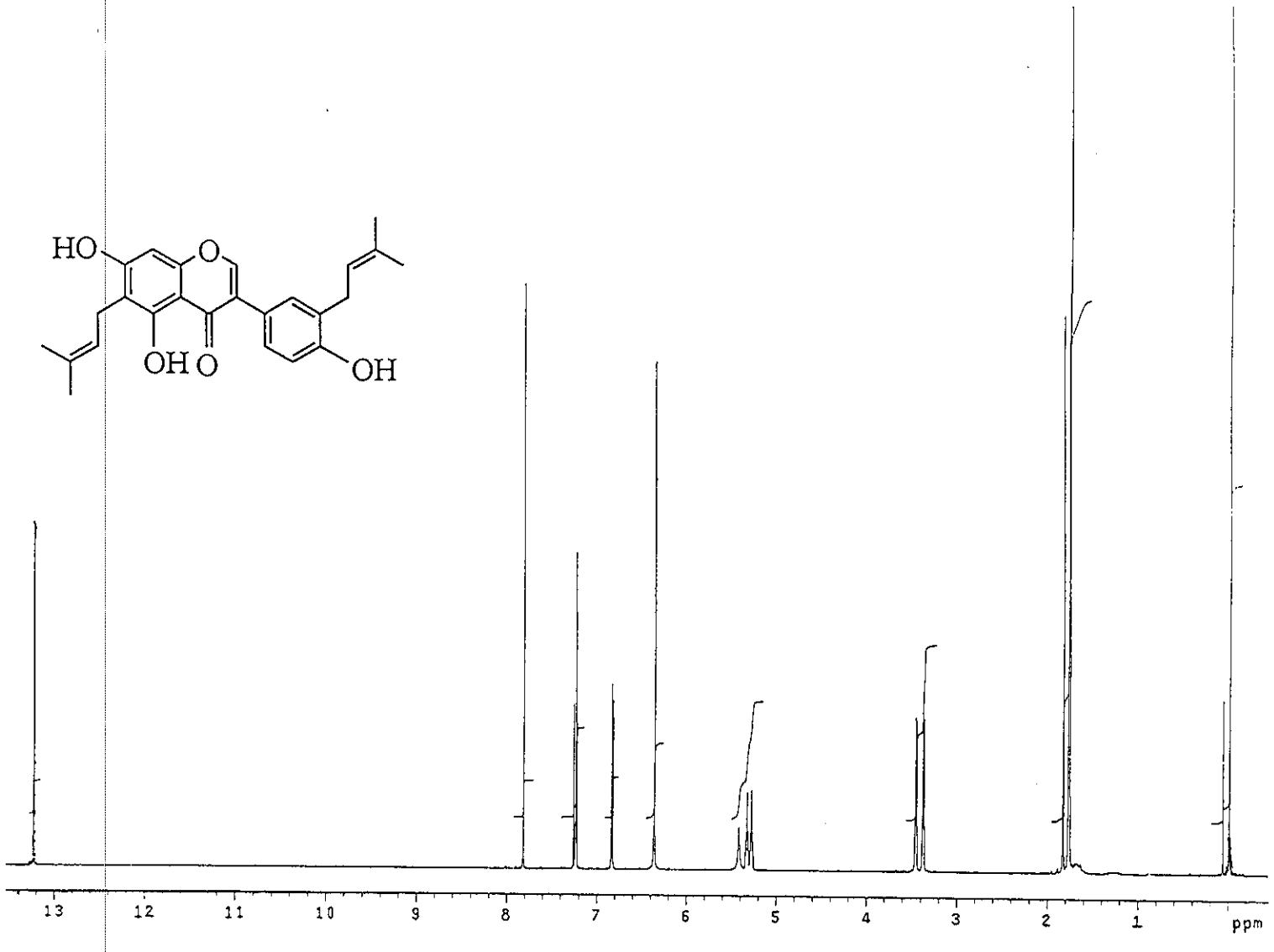


Figure 9 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS2**

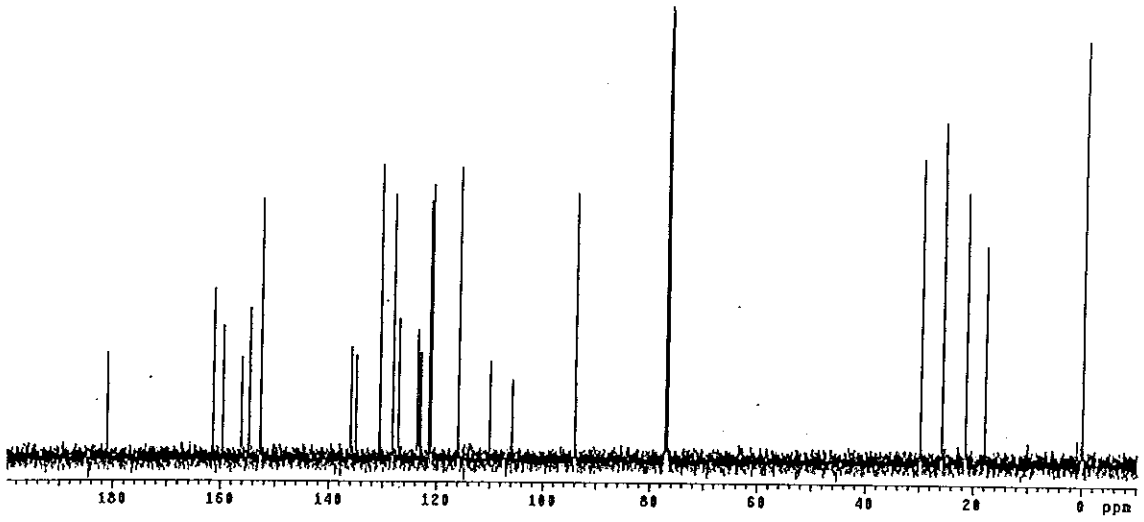


Figure 10 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **DS2**

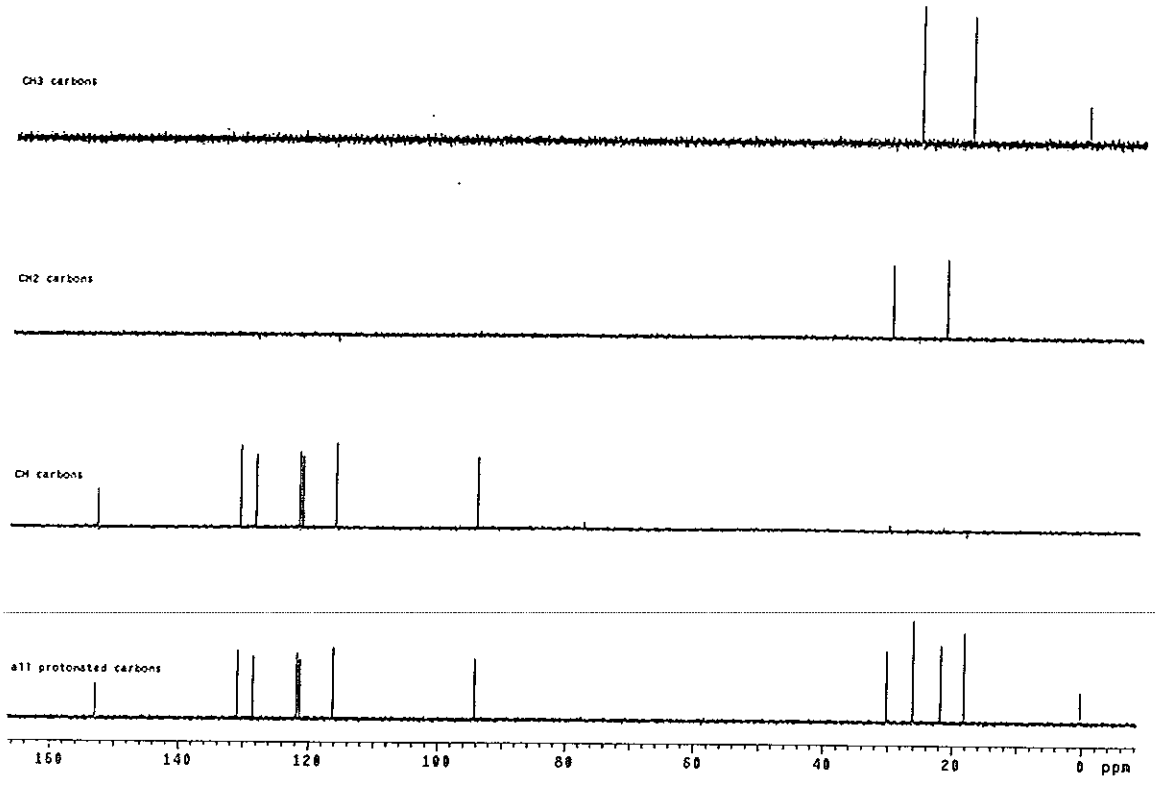


Figure 11 DEPT (135°) ($CDCl_3$) spectrum of **DS2**

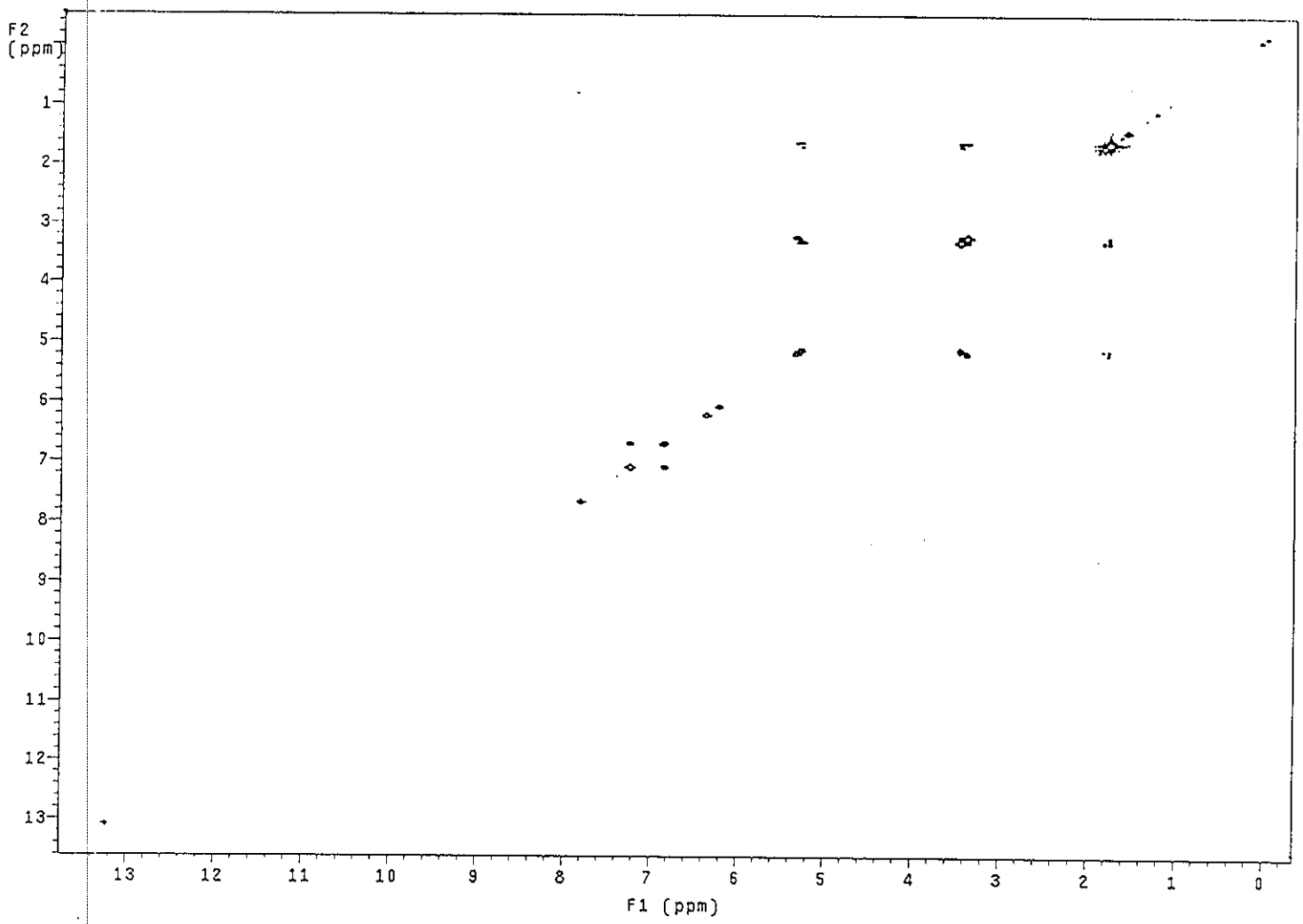


Figure 12 ^{1}H-^{1}H COSY spectrum of **DS2**

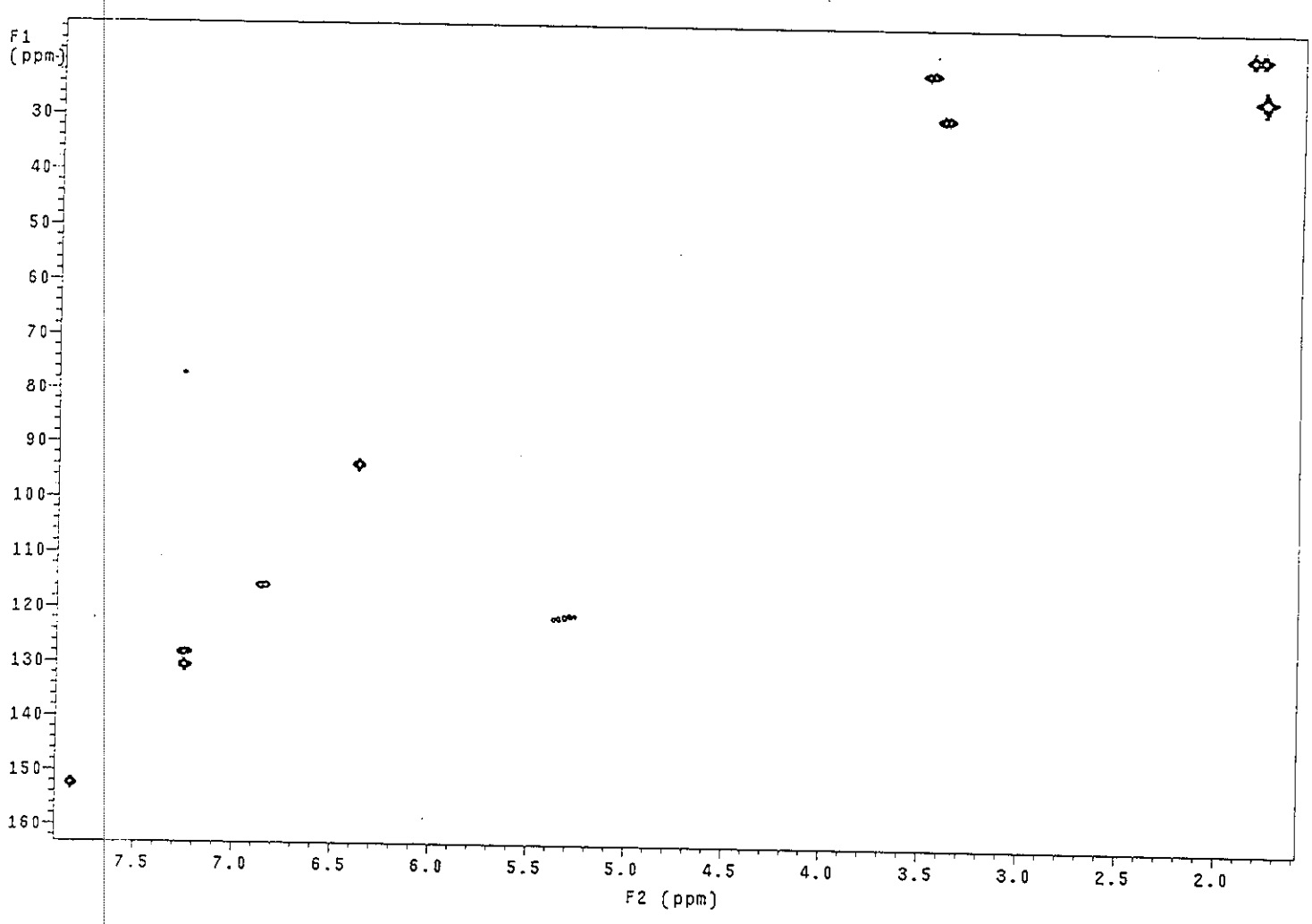


Figure 13 2D HMQC spectrum of **DS2**

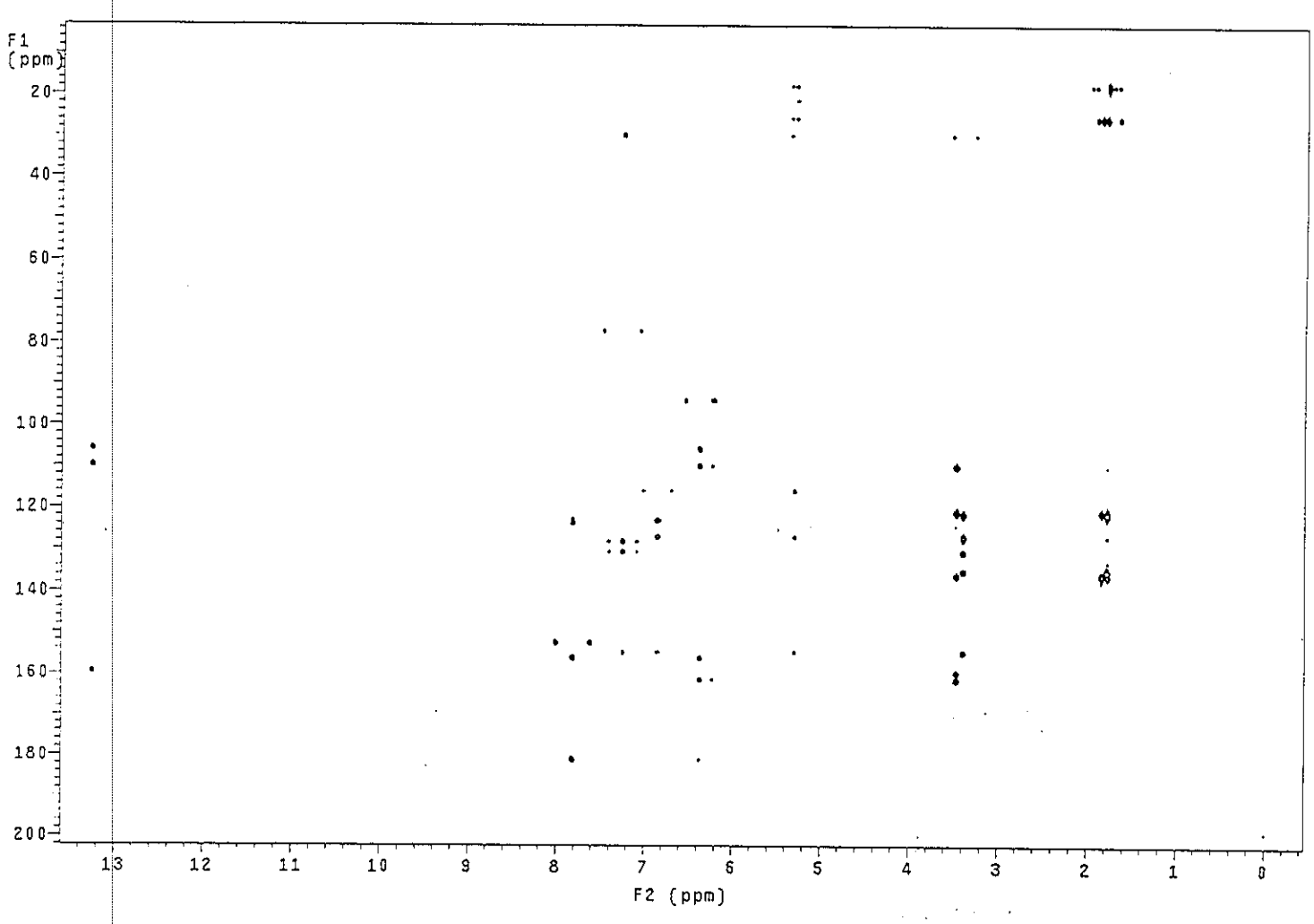


Figure 14 2D HMBC spectrum of **DS2**

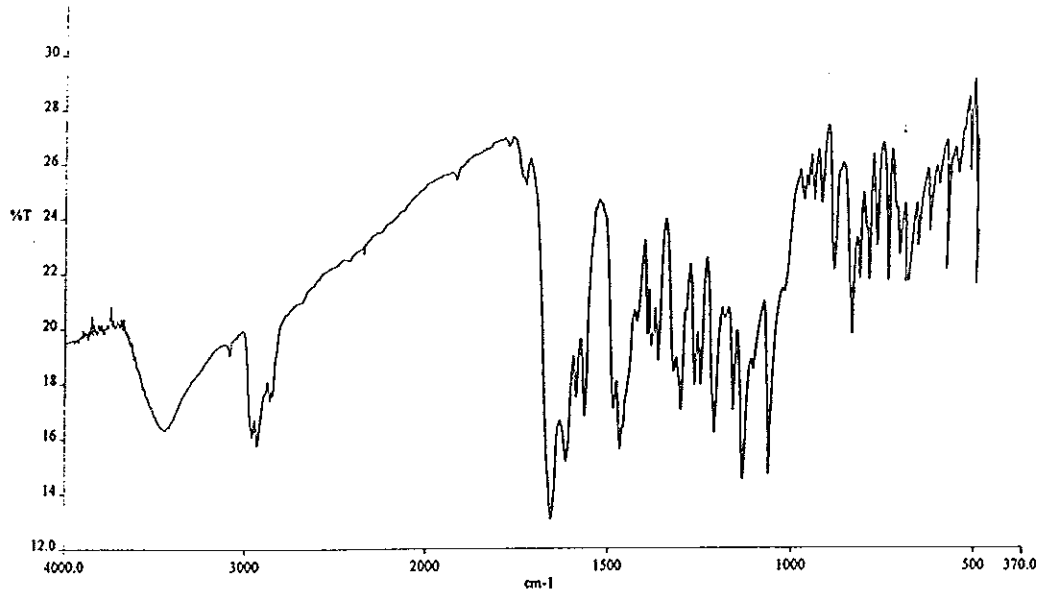


Figure 15 IR (KBr) spectrum of DS3

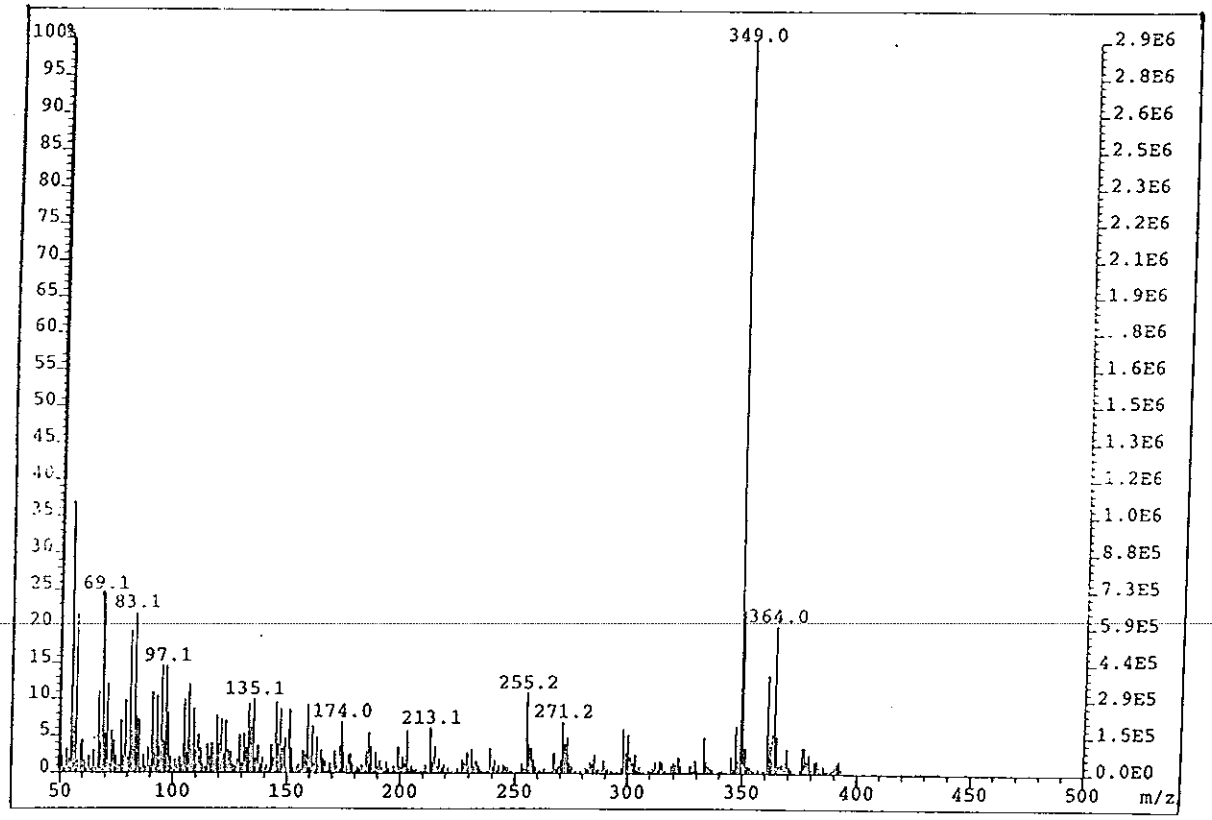


Figure 16 Mass spectrum of DS3

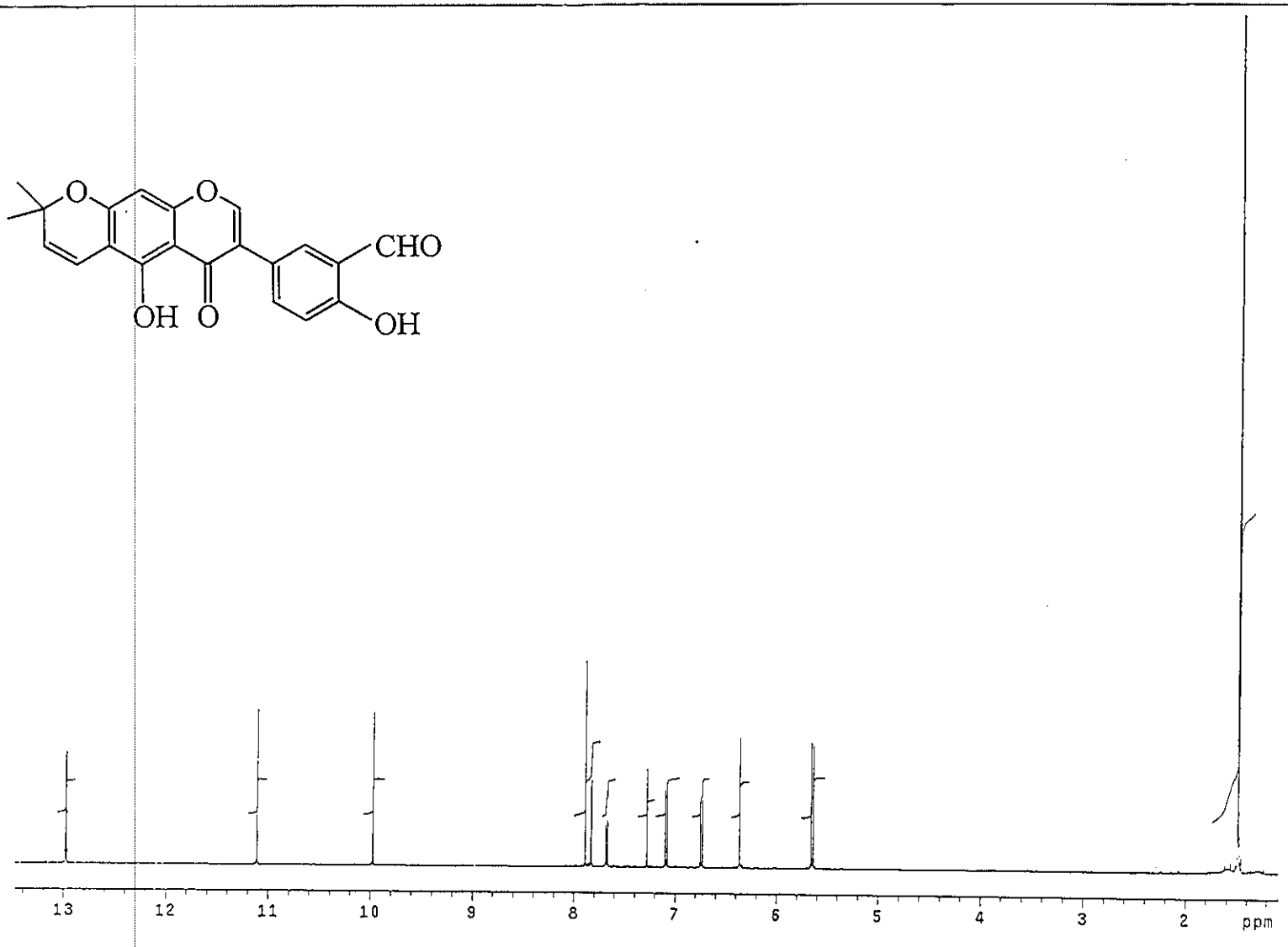


Figure 17 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS3**

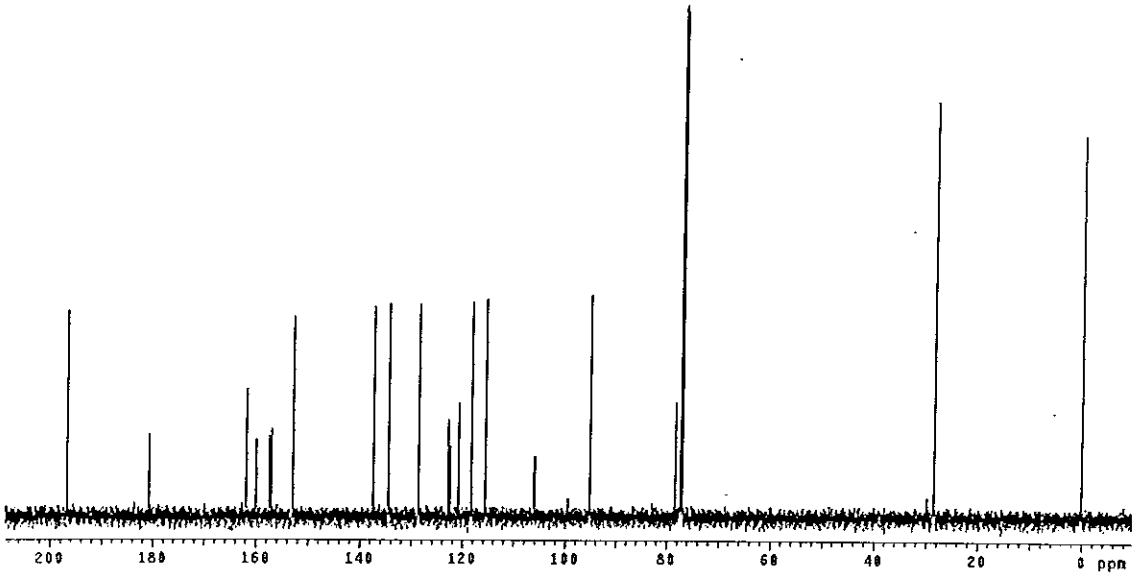


Figure 18 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **DS3**

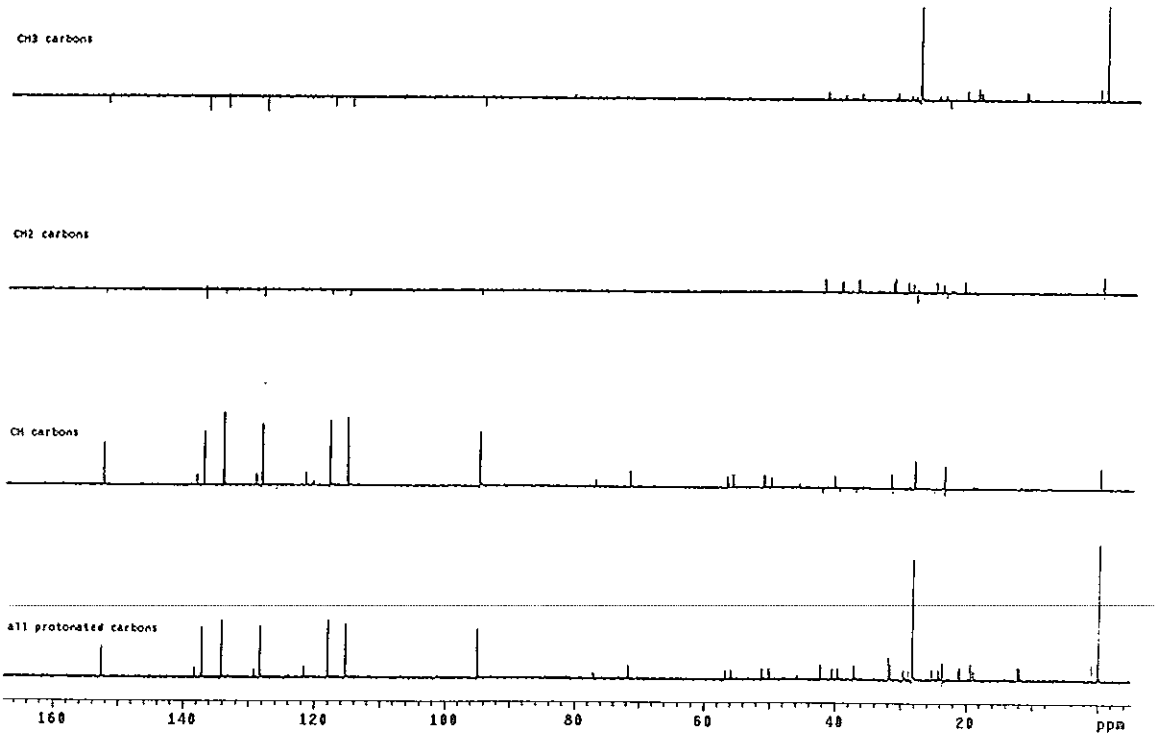


Figure 19 DEPT (135°) ($CDCl_3$) spectrum of **DS3**

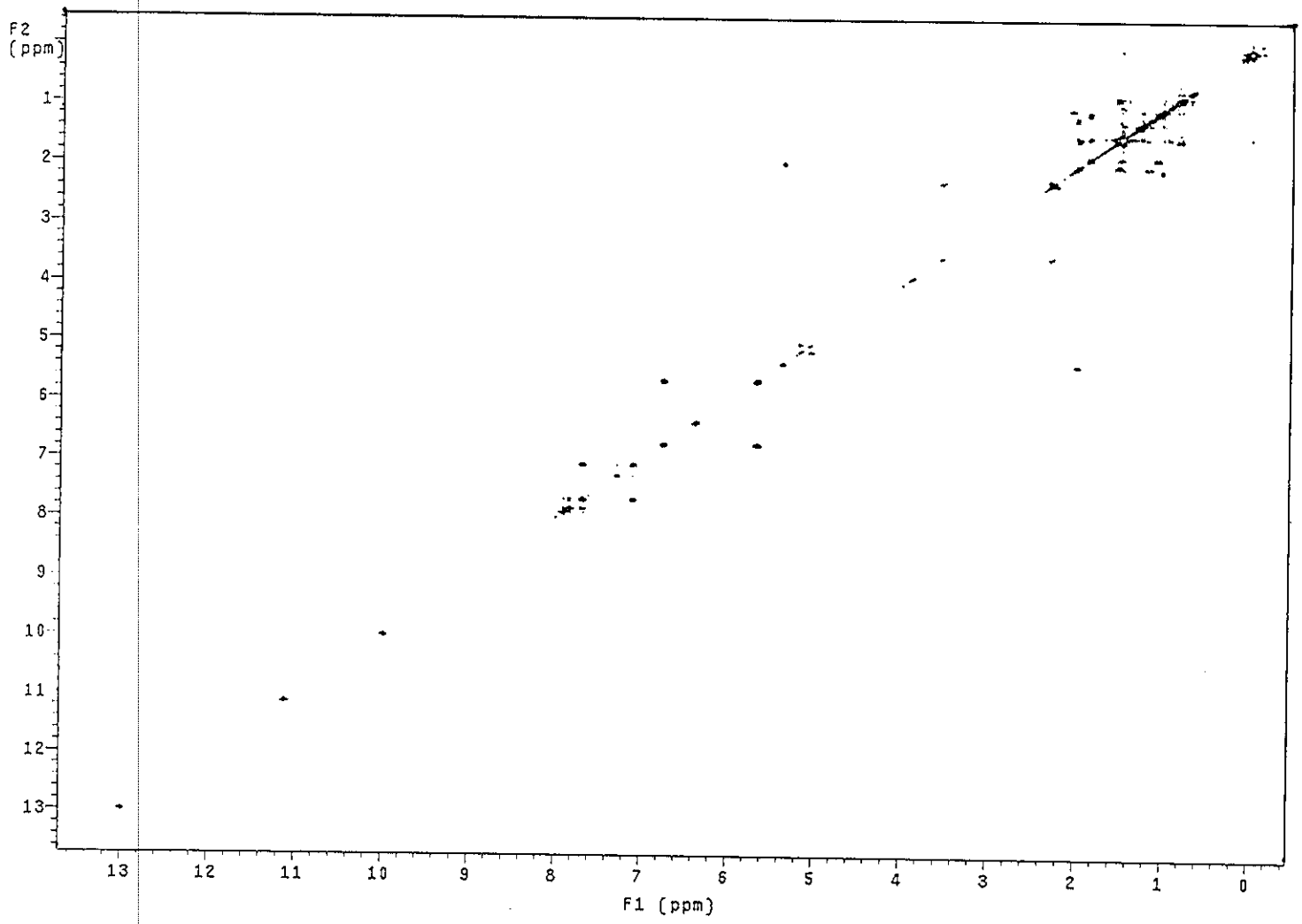


Figure 20 ^{1}H-^{1}H COSY spectrum of **DS3**

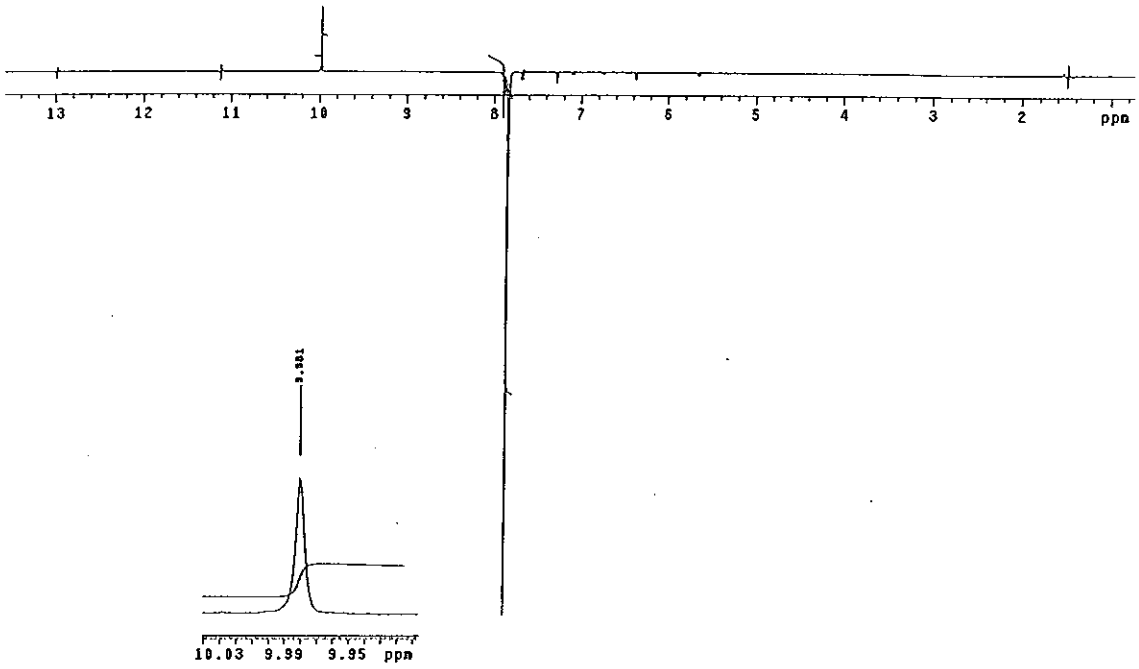


Figure 21 NOEDIFF spectrum of **DS3** after irradiation at δ_H 7.83

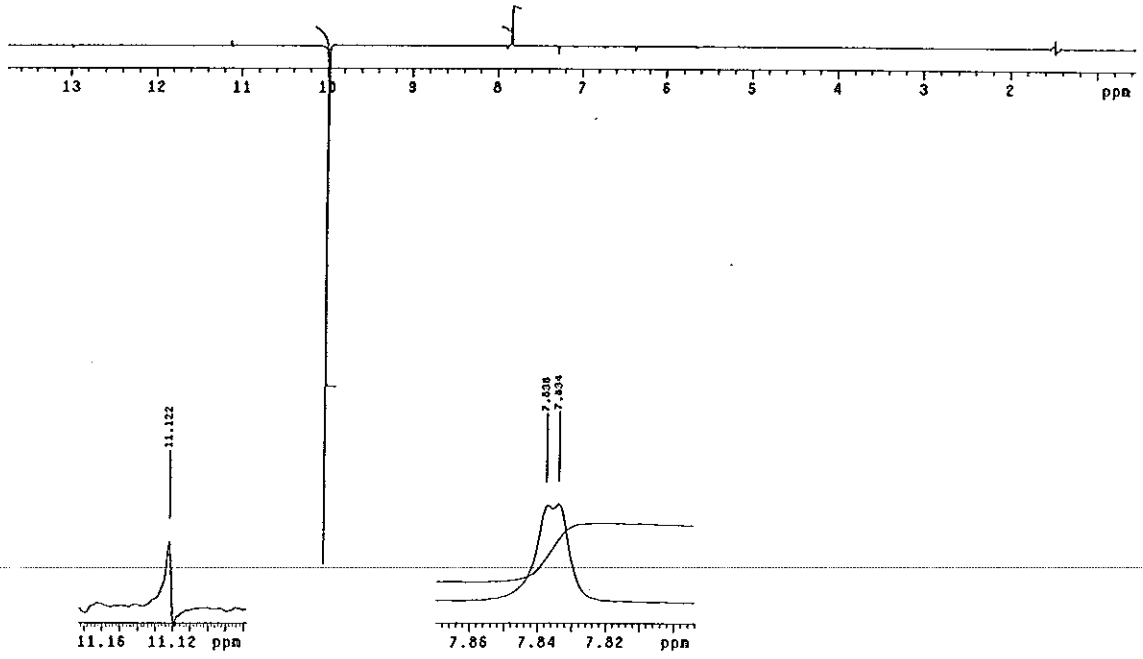


Figure 22 NOEDIFF spectrum of **DS3** after irradiation at δ_H 9.98

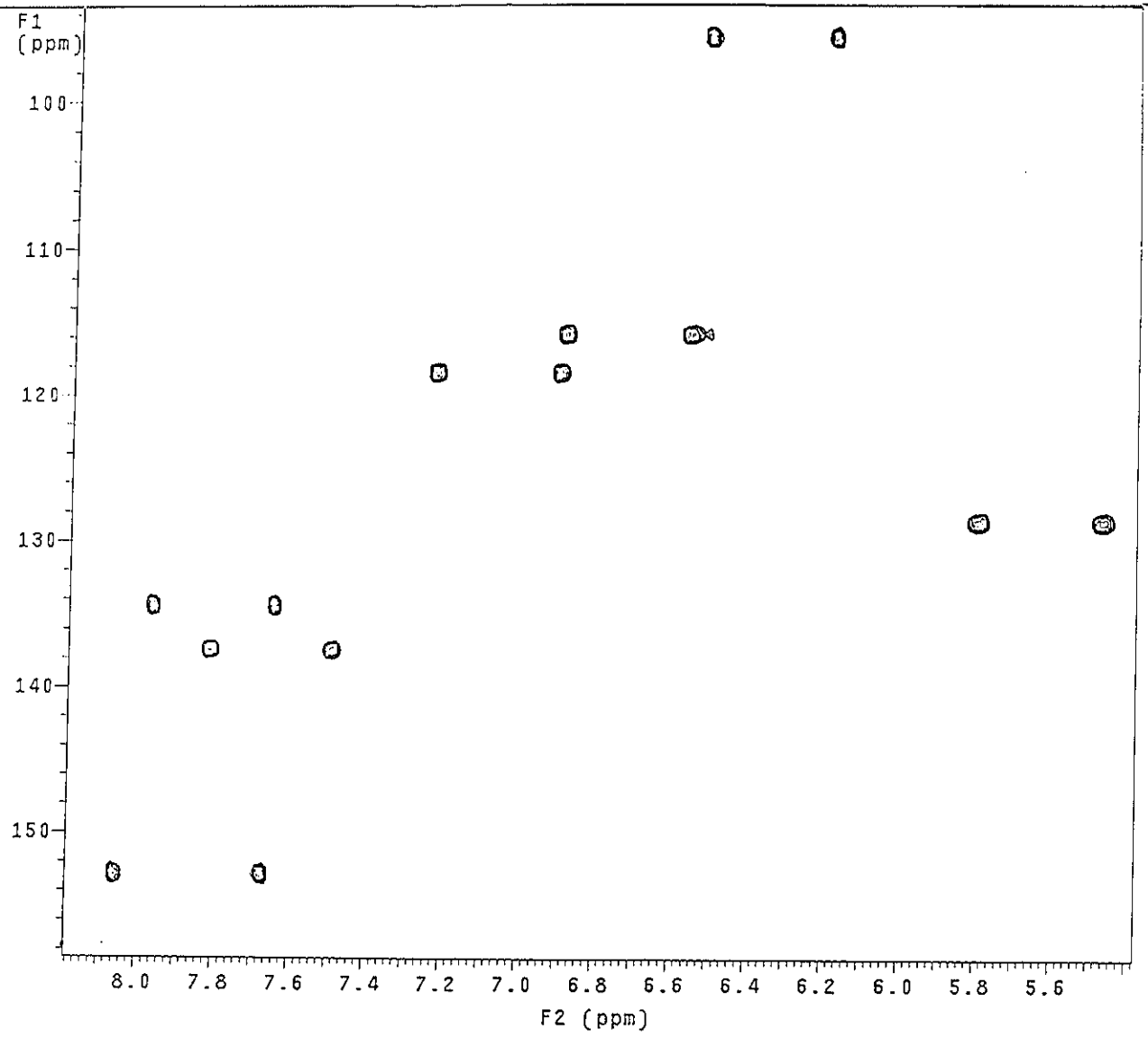


Figure 23 2D HMQC spectrum of **DS3**

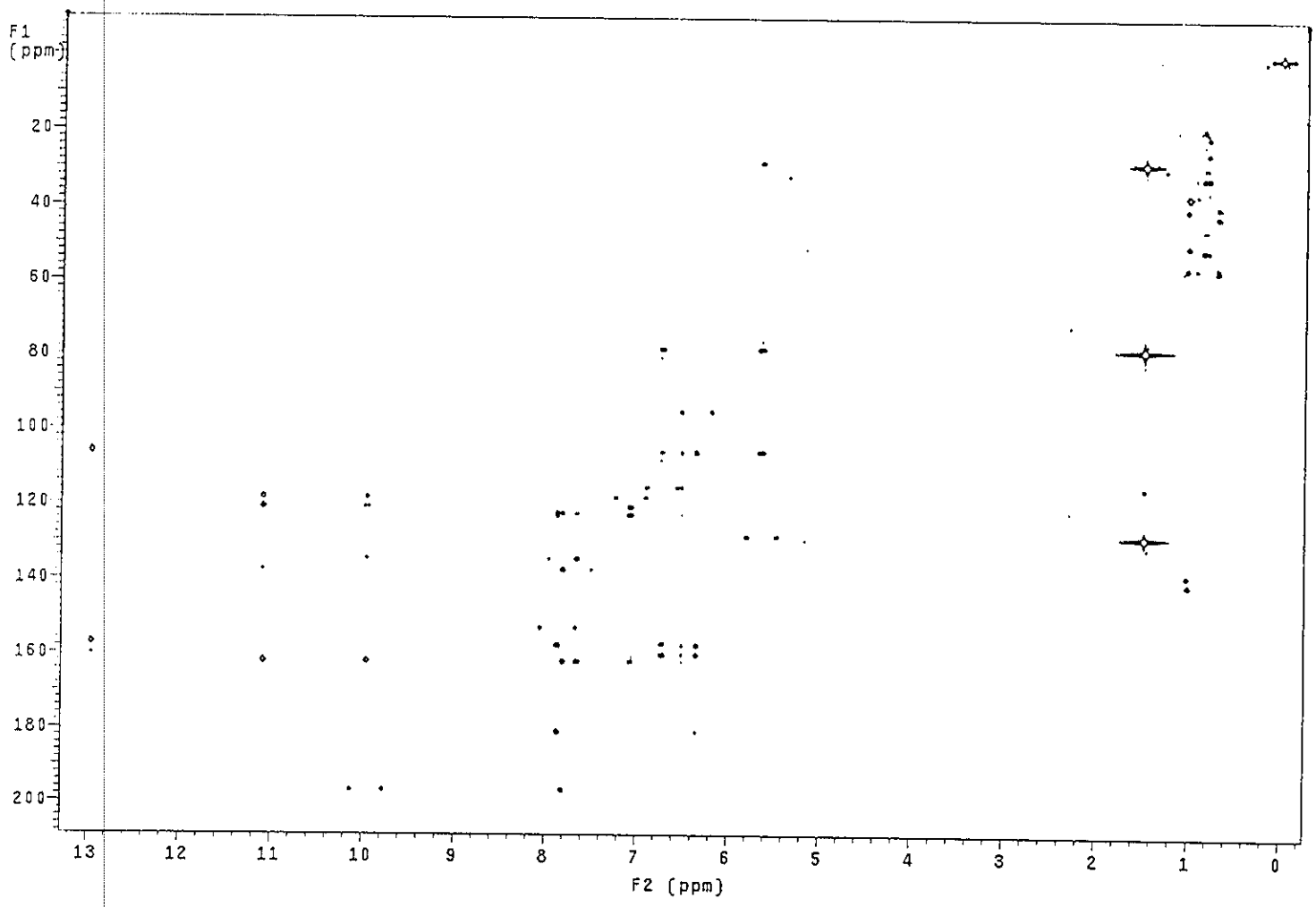


Figure 24 2D HMBC spectrum of **DS3**

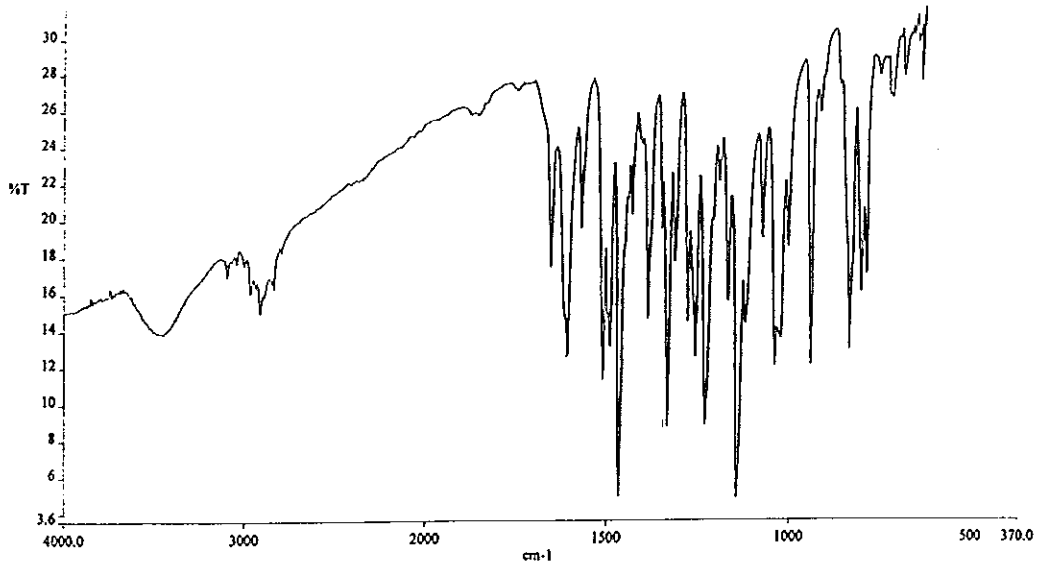


Figure 25 IR (KBr) spectrum of **DS4**

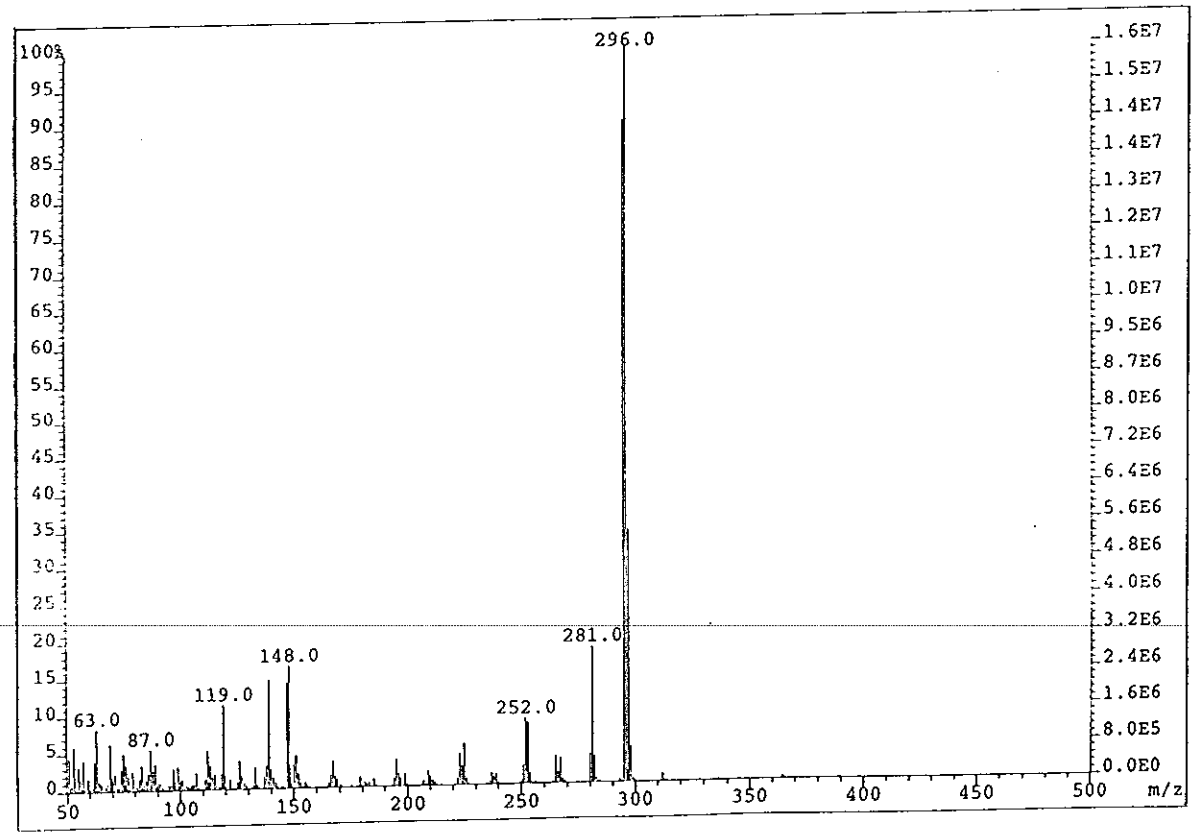


Figure 26 Mass spectrum of **DS4**

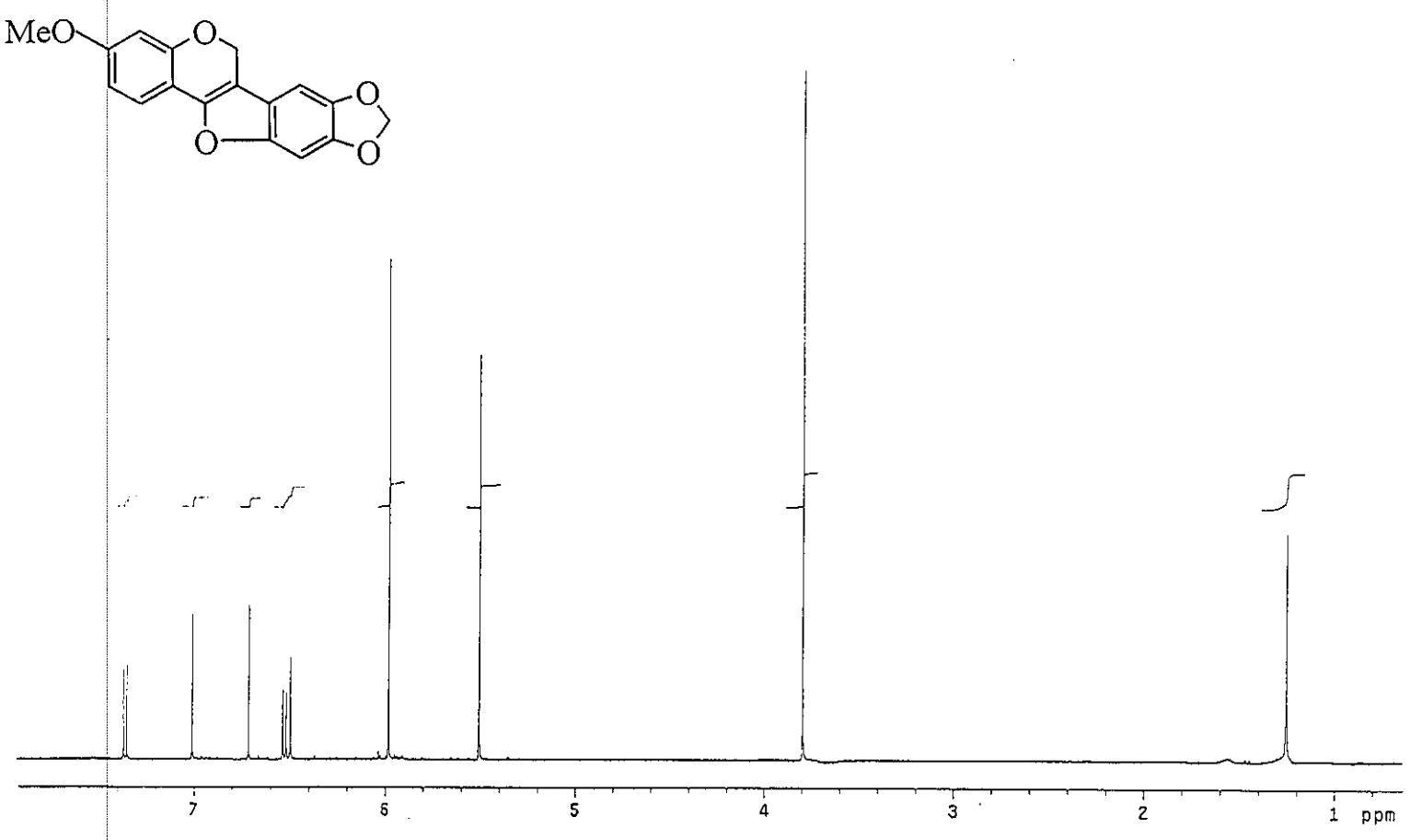


Figure 27 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS4**

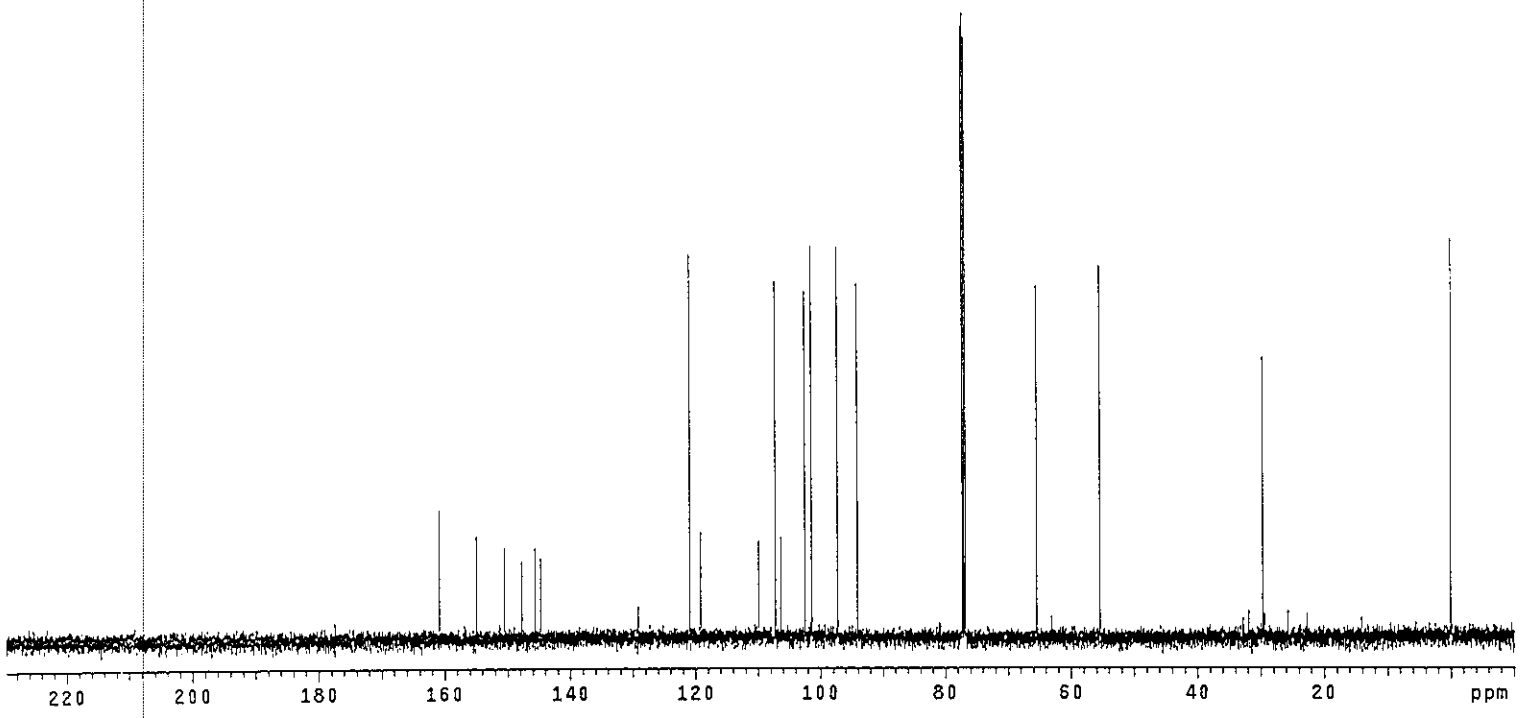
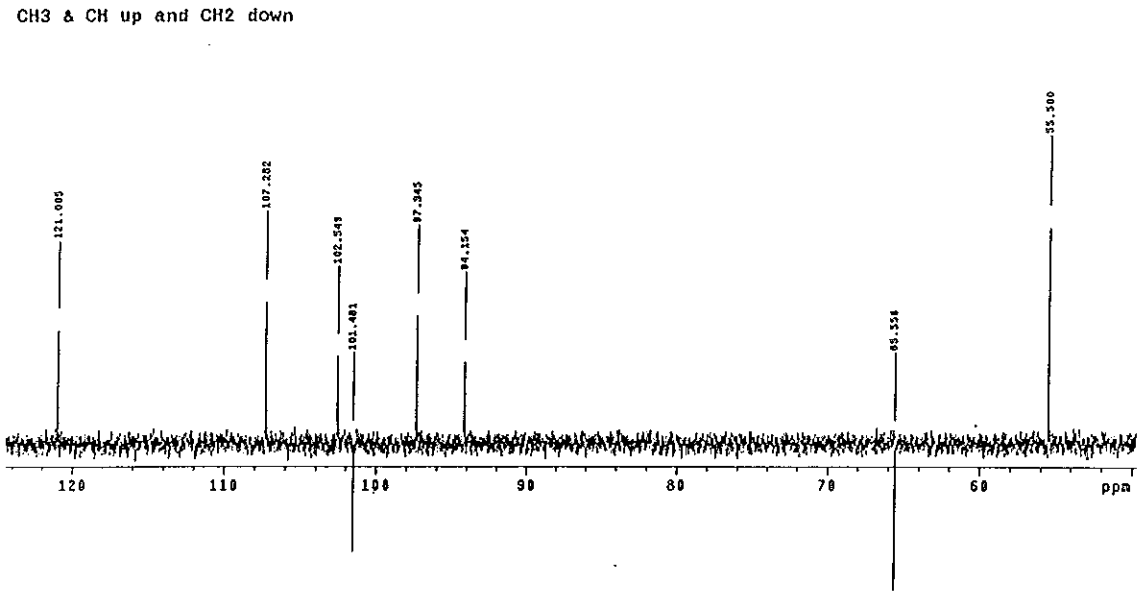


Figure 28 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **DS4**



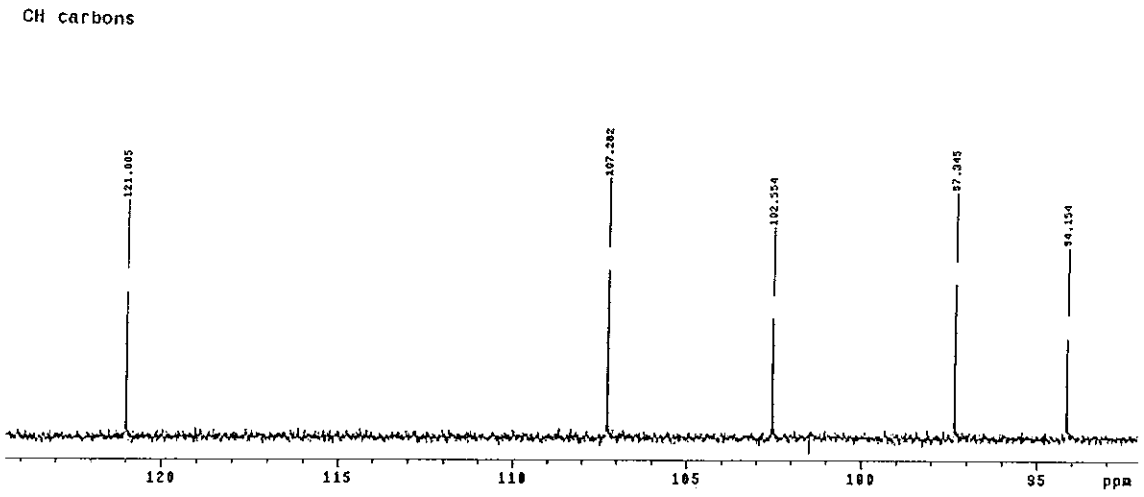


Figure 29 DEPT (135°) ($CDCl_3$) spectrum of **DS4**

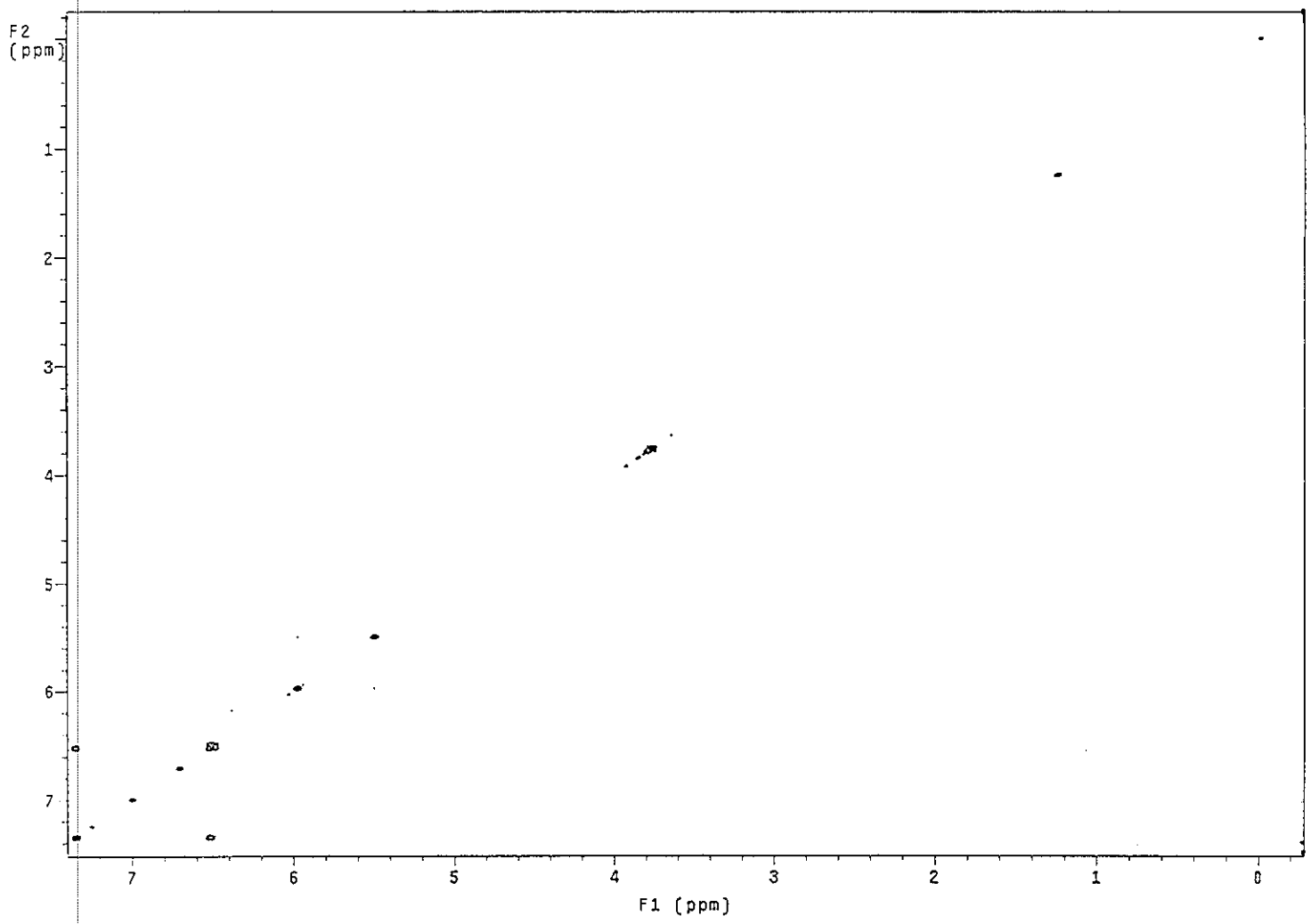


Figure 30 ^{1}H-^{1}H COSY spectrum of **DS4**

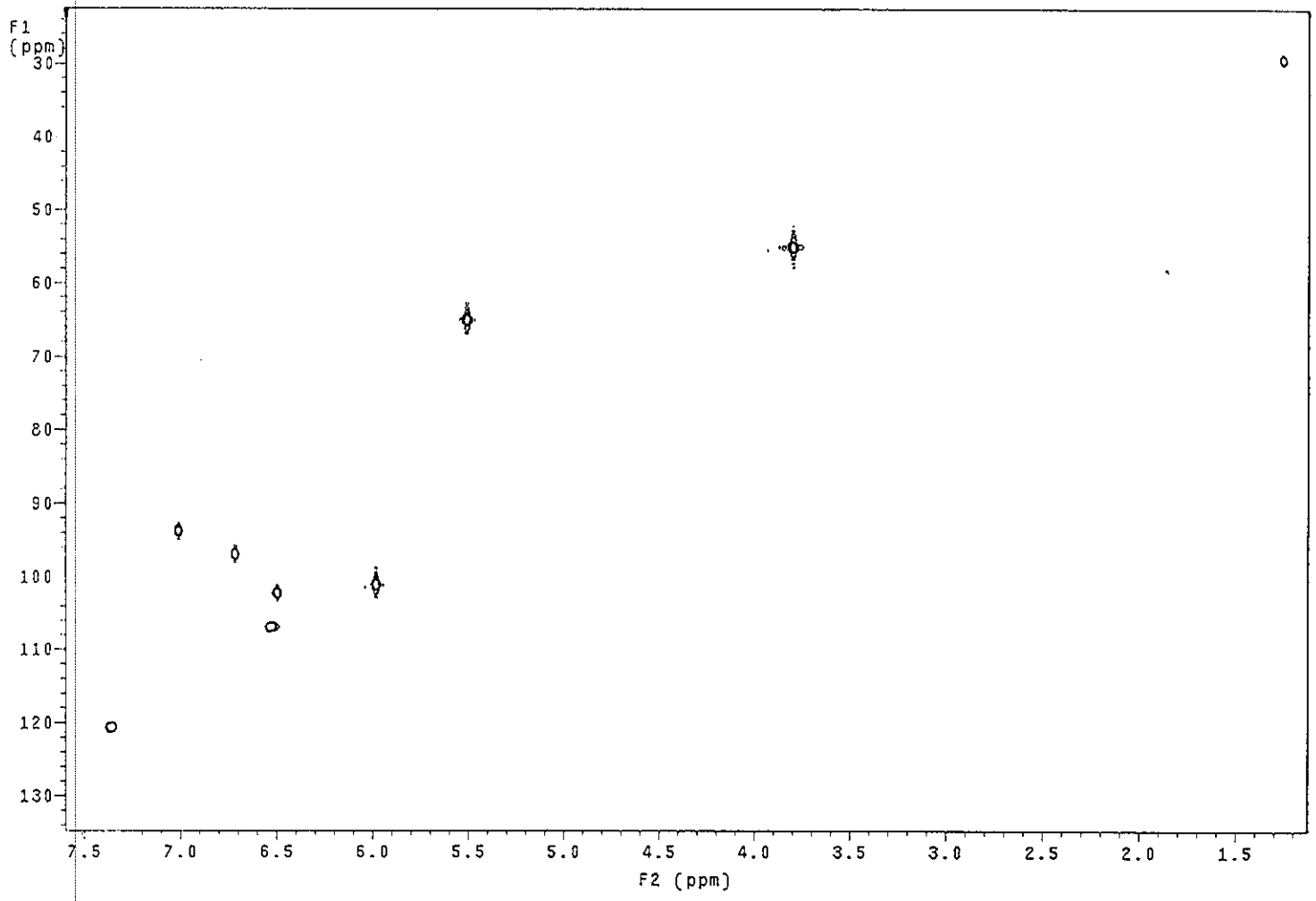


Figure 31 2D HMQC spectrum of **DS4**

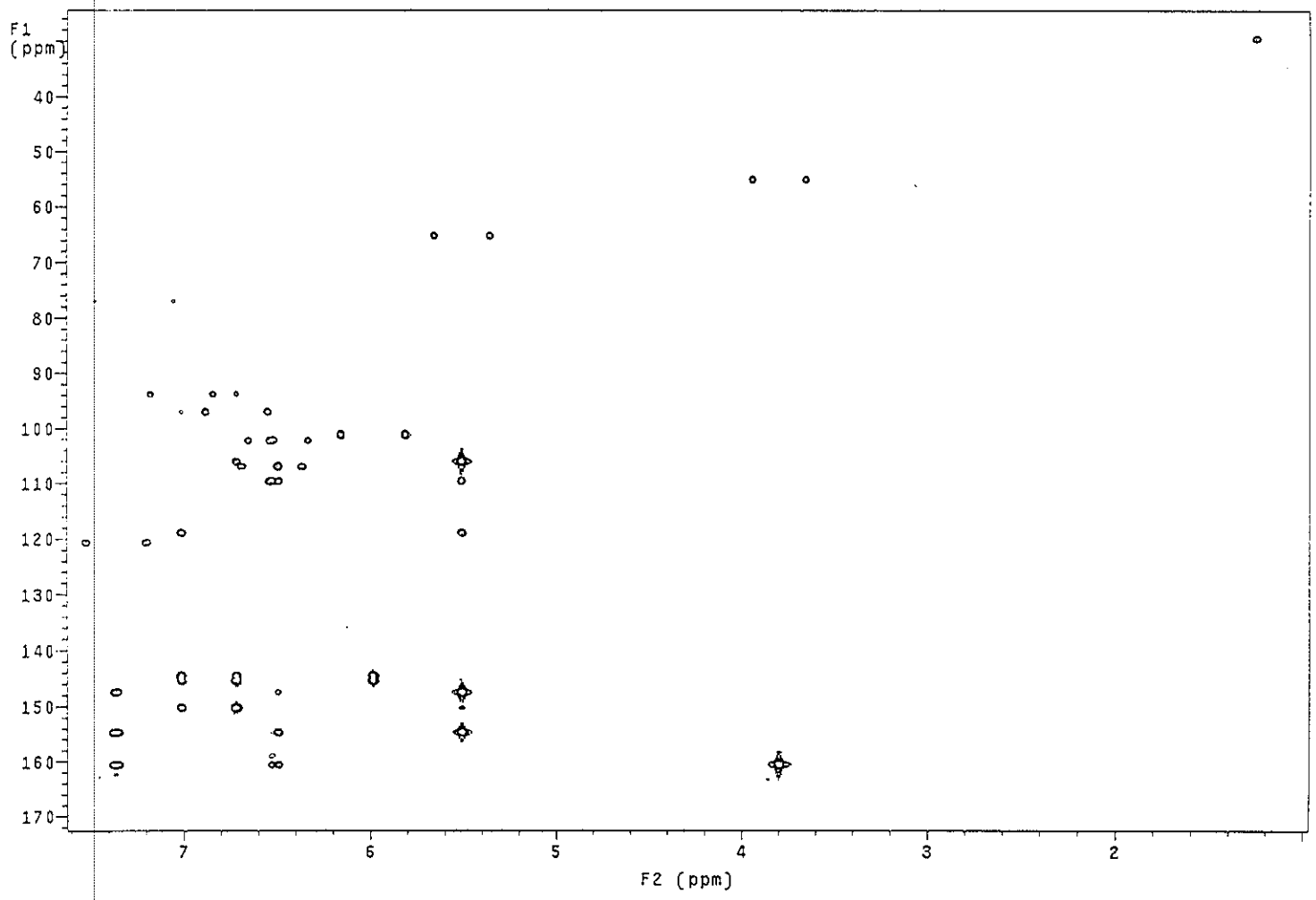


Figure 32 2D HMBC spectrum of **DS4**

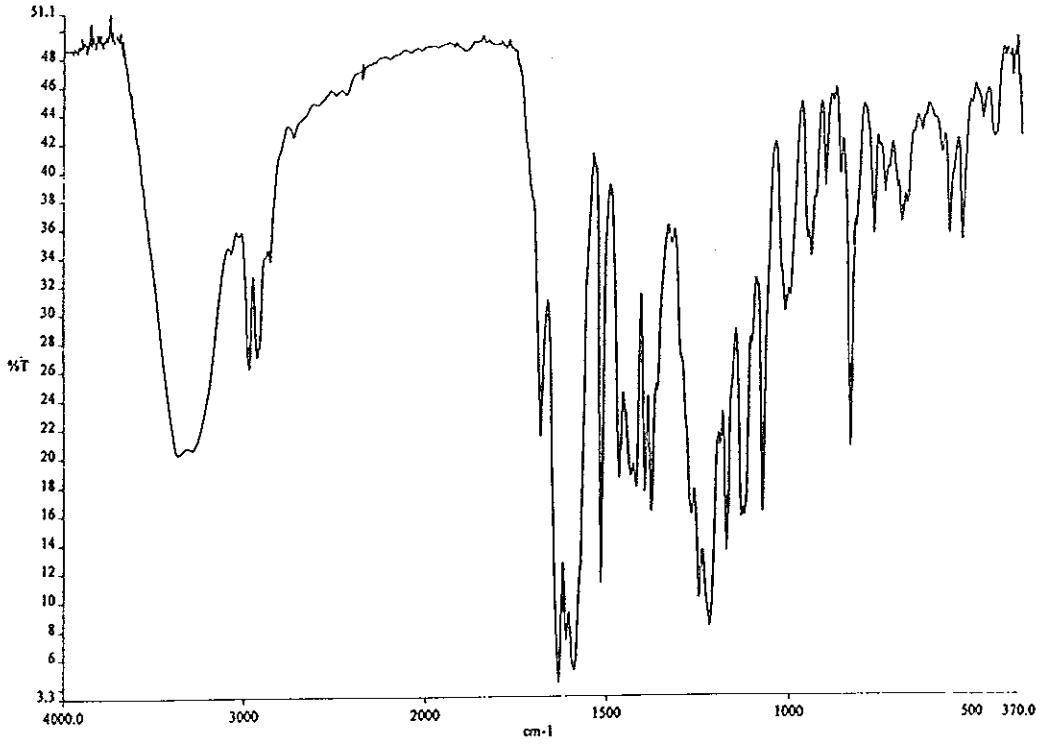


Figure 33 IR (KBr) spectrum of DS6

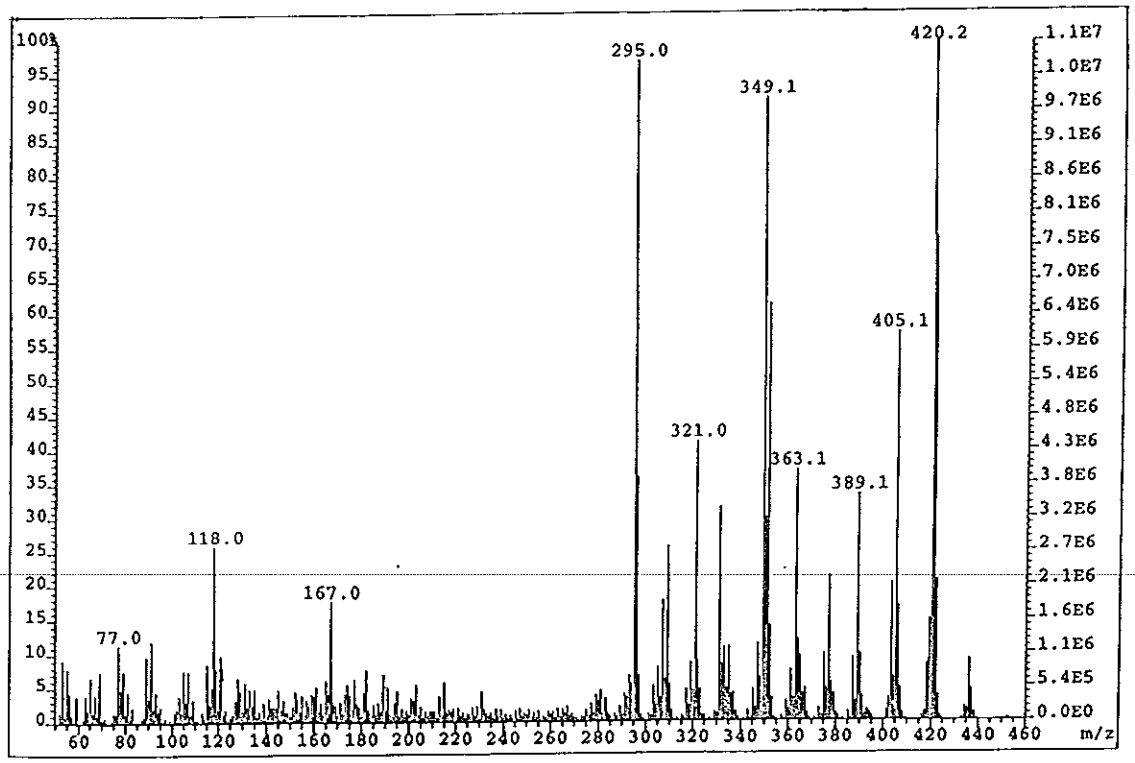


Figure 34 Mass spectrum of DS6

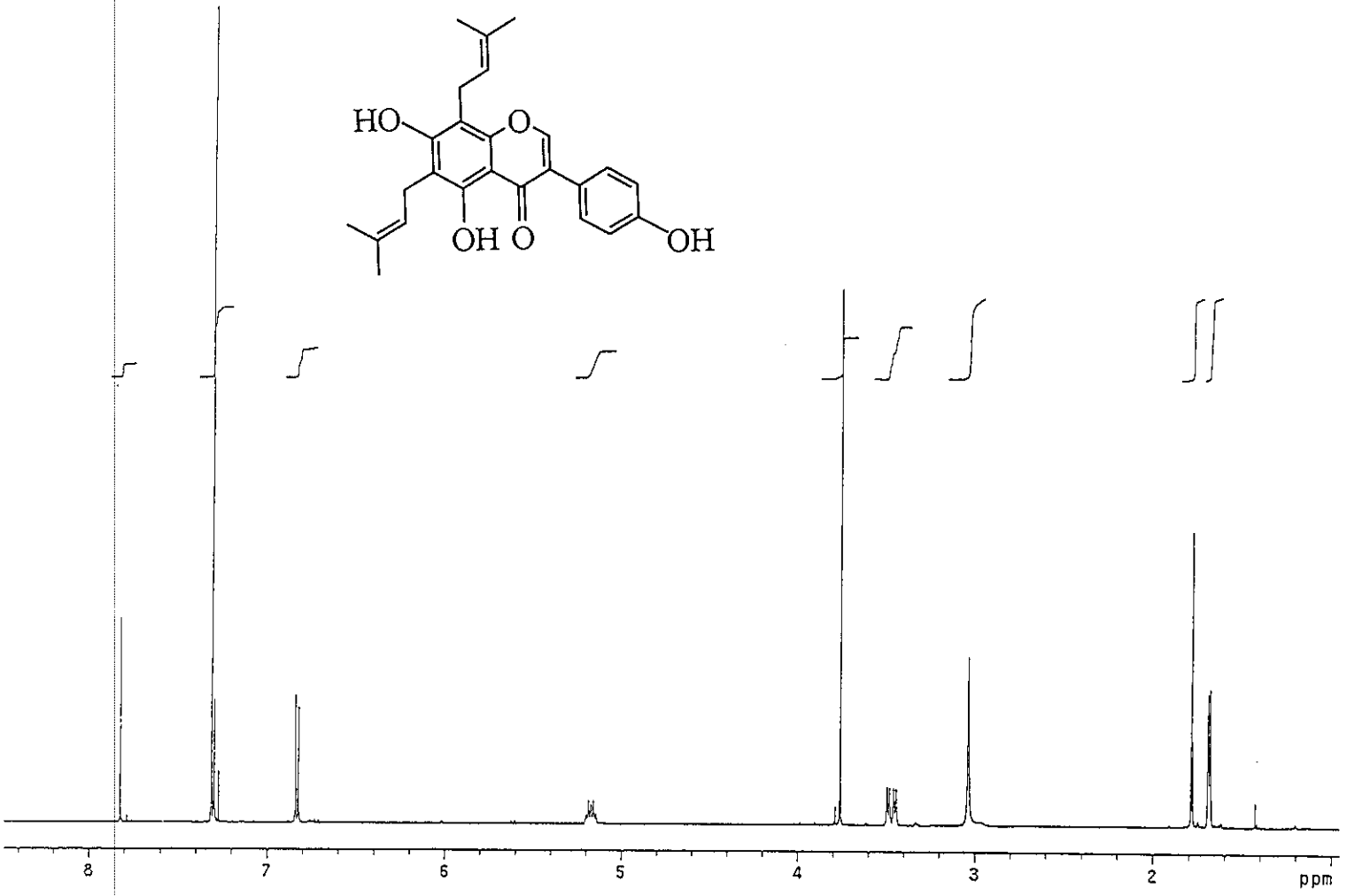


Figure 35 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS6**

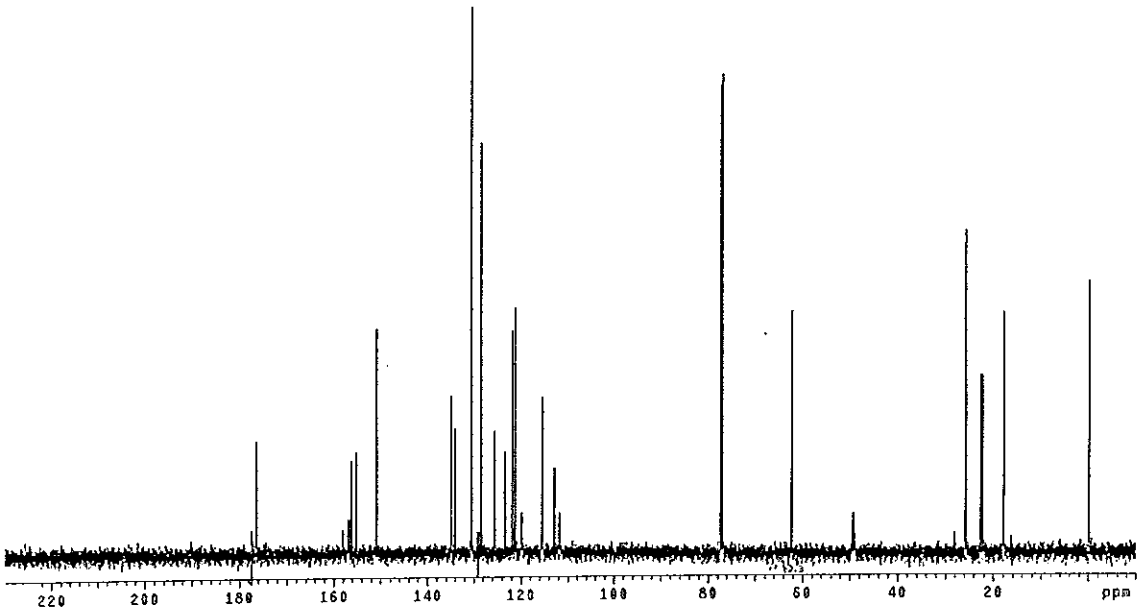


Figure 36 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **DS6**

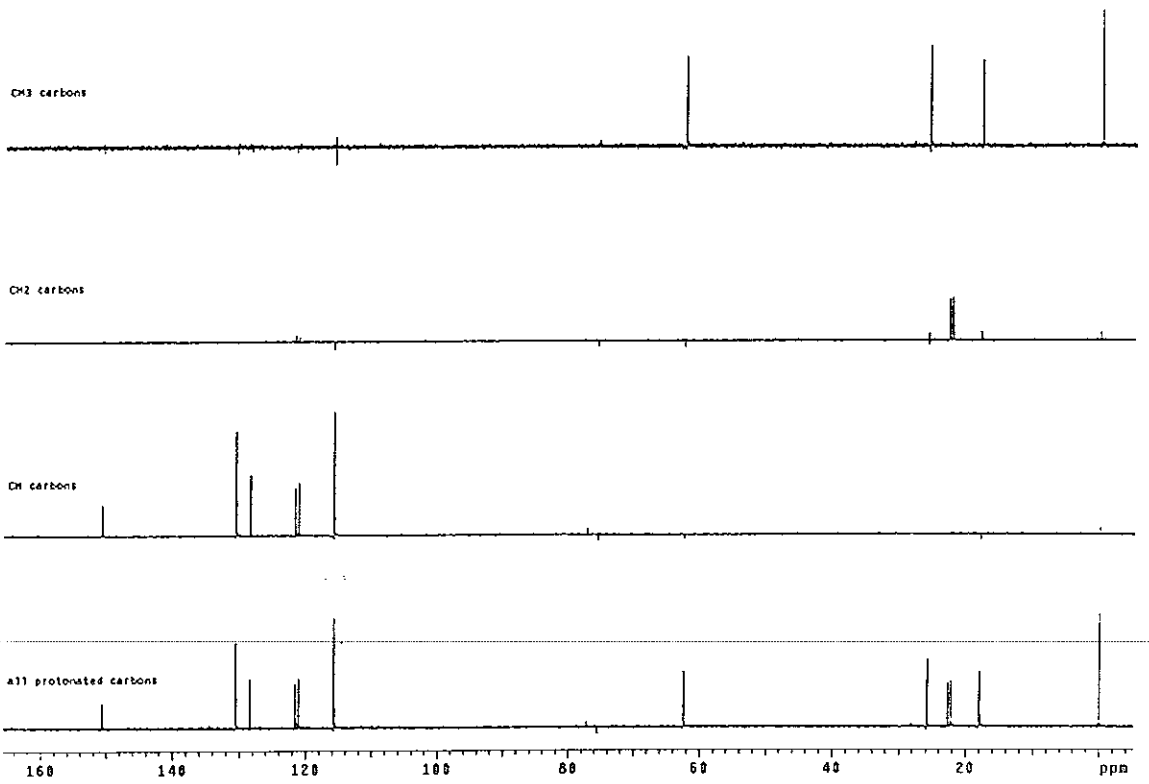


Figure 37 DEPT (135°) ($CDCl_3$) spectrum of **DS6**

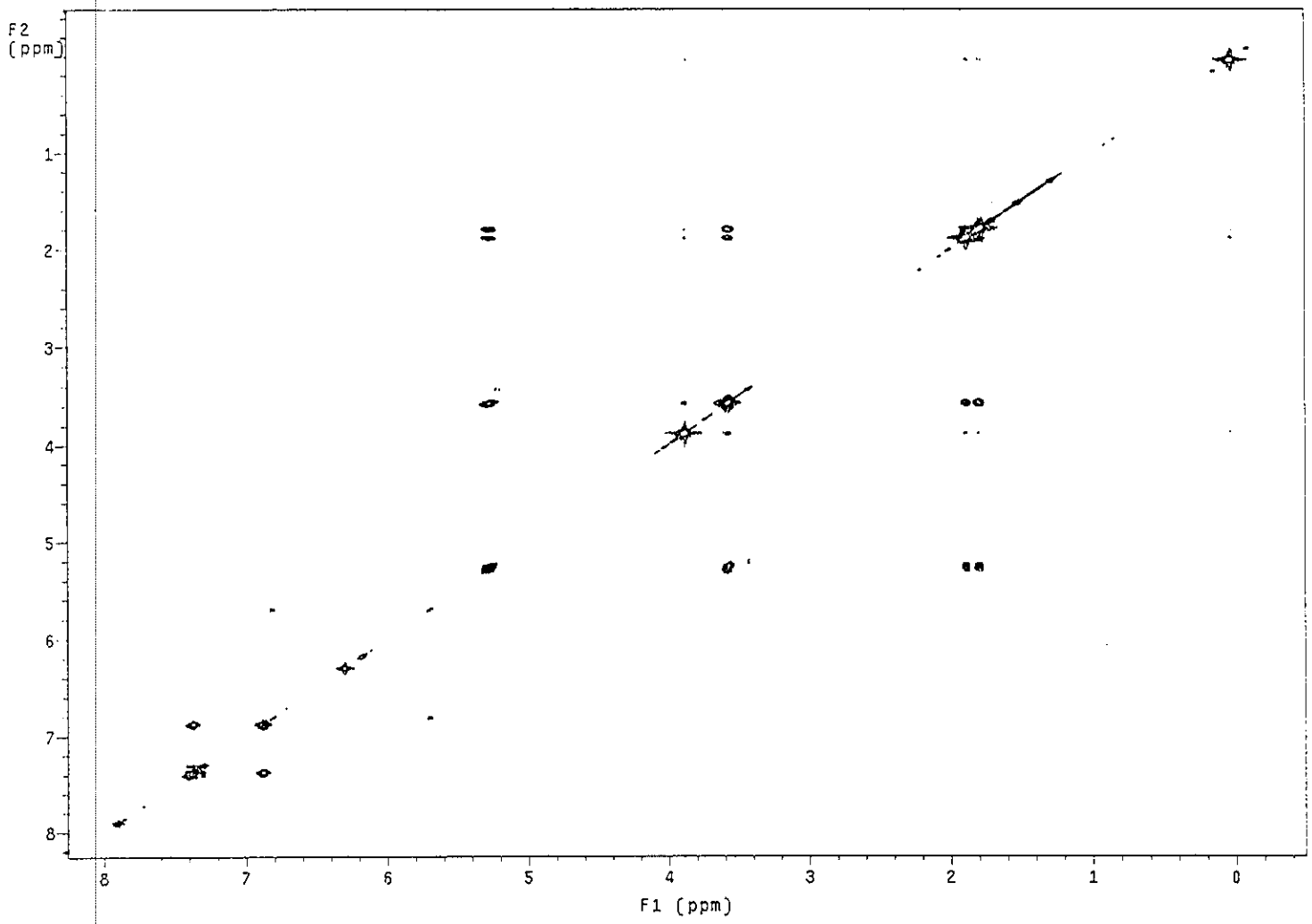


Figure 38 ^{1}H-^{1}H COSY spectrum of **DS6**

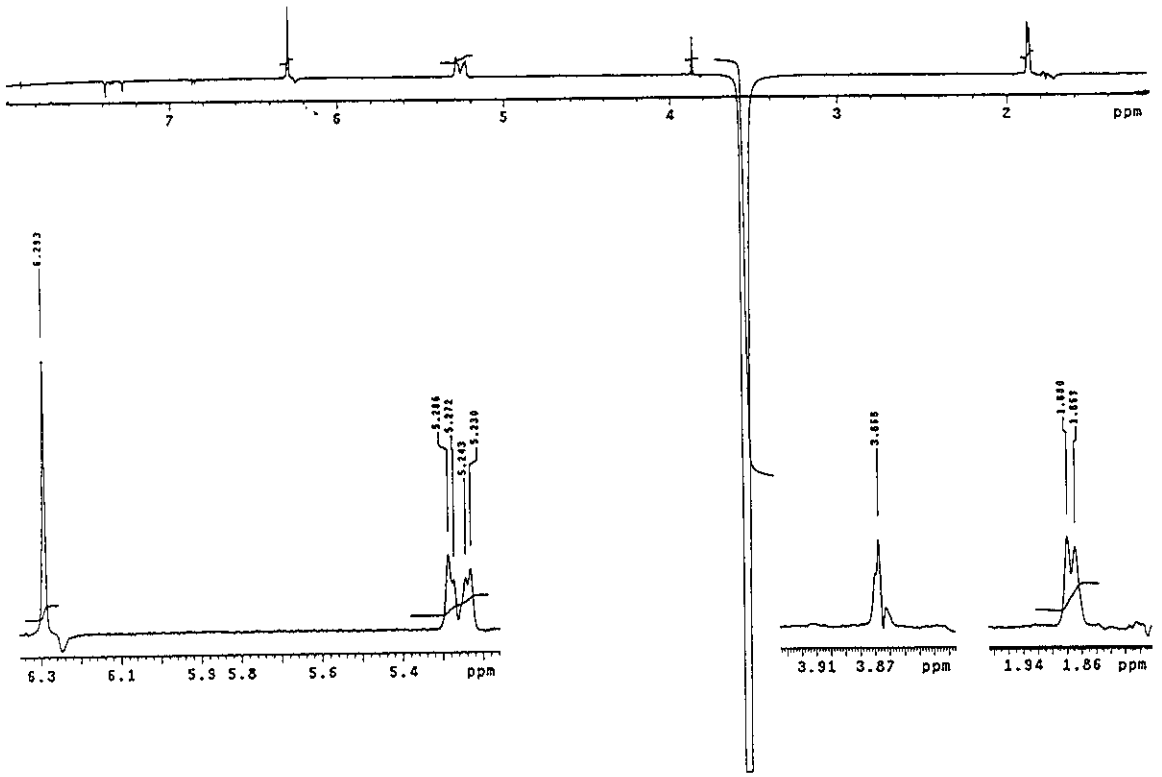


Figure 39 NOEDIFF spectrum of **DS6** after irradiation at δ_H 3.76

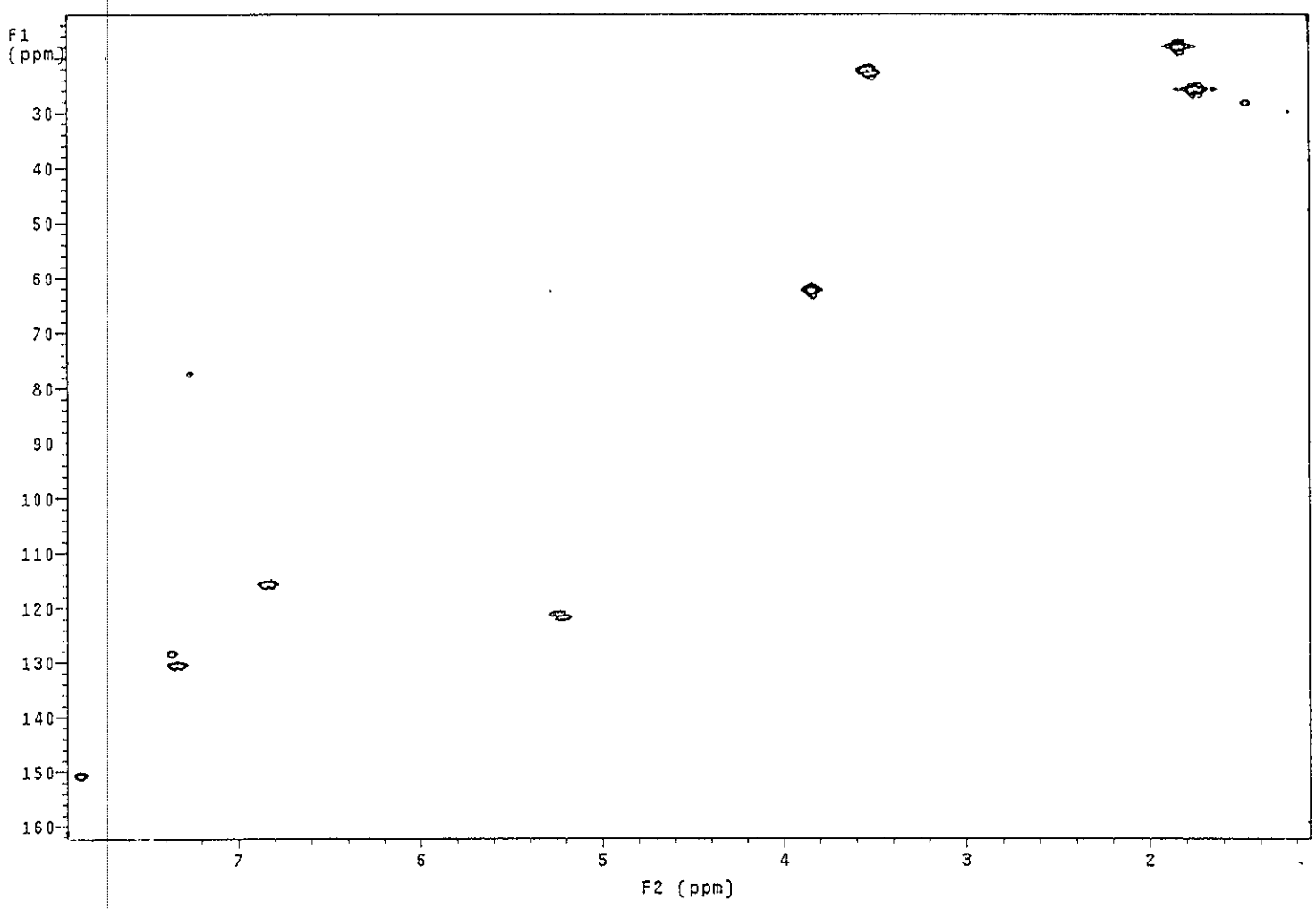


Figure 40 2D HMQC spectrum of **DS6**

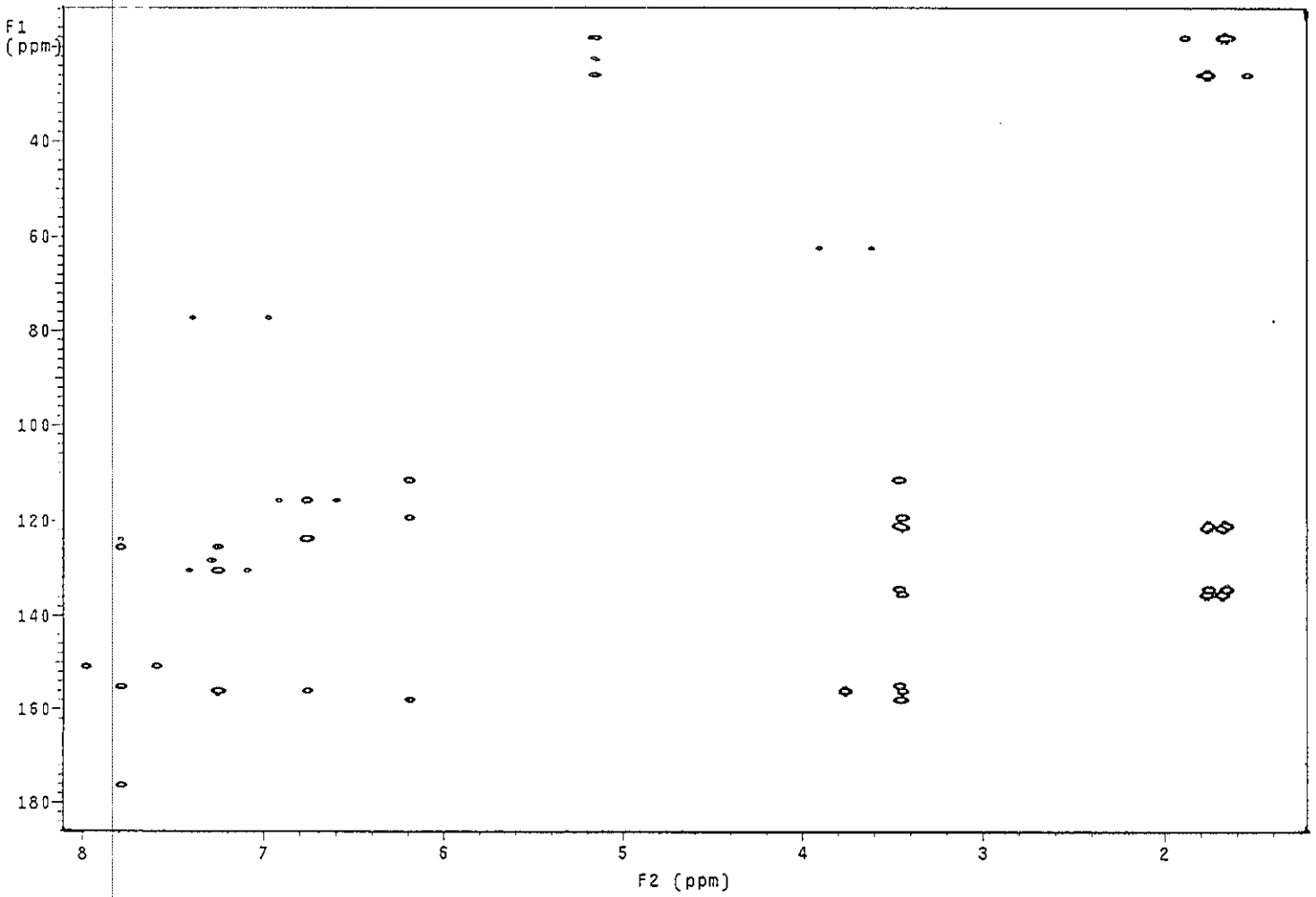


Figure 41 2D HMBC spectrum of **DS6**

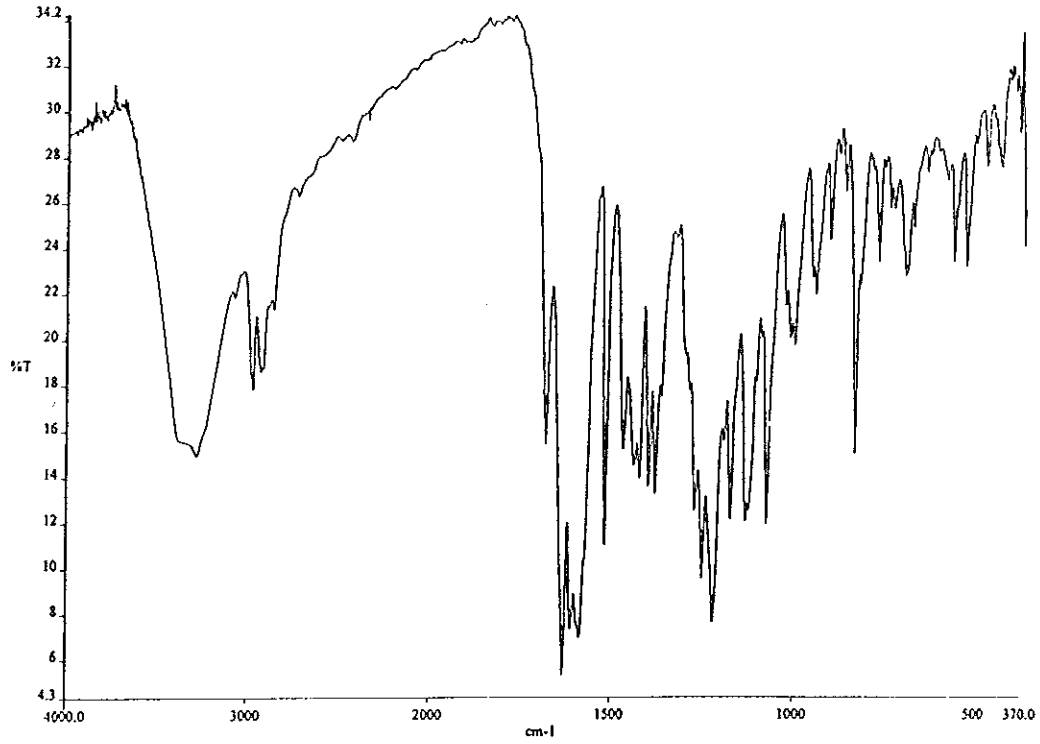


Figure 42 IR (KBr) spectrum of **DS7**

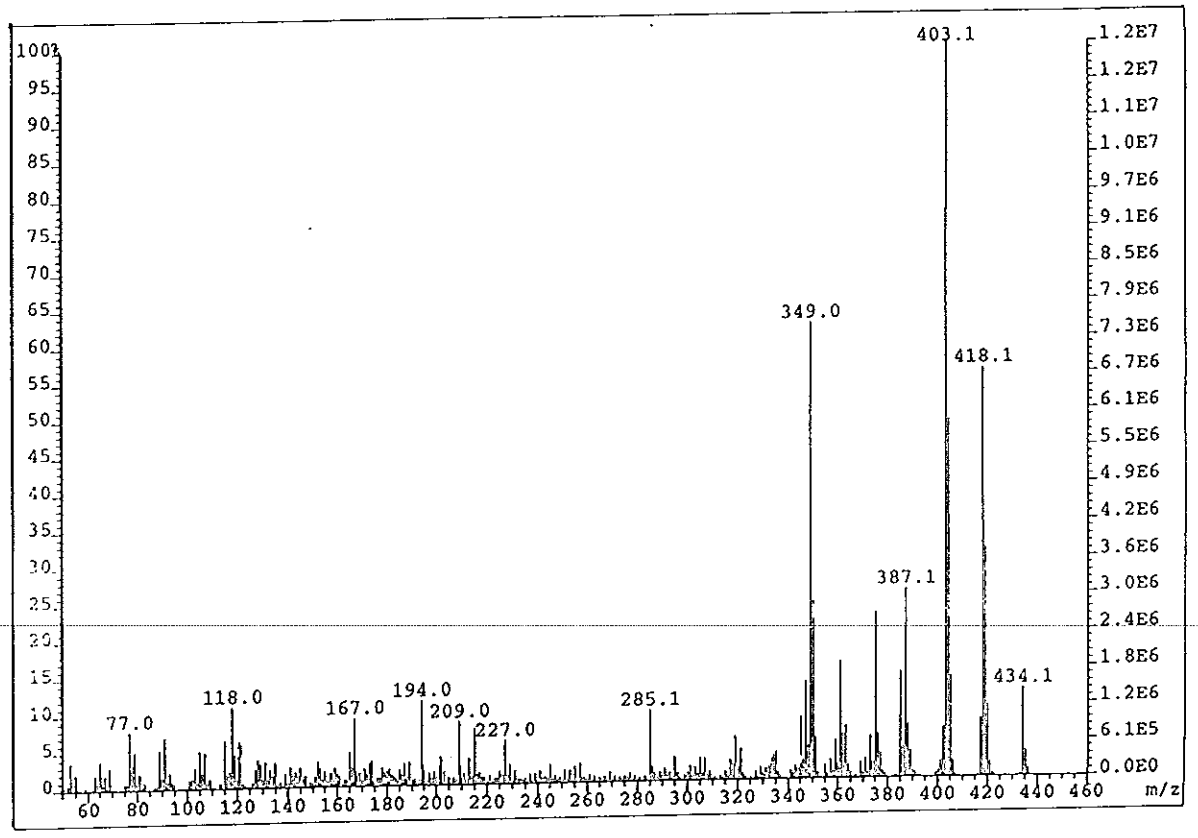


Figure 43 Mass spectrum of **DS7**

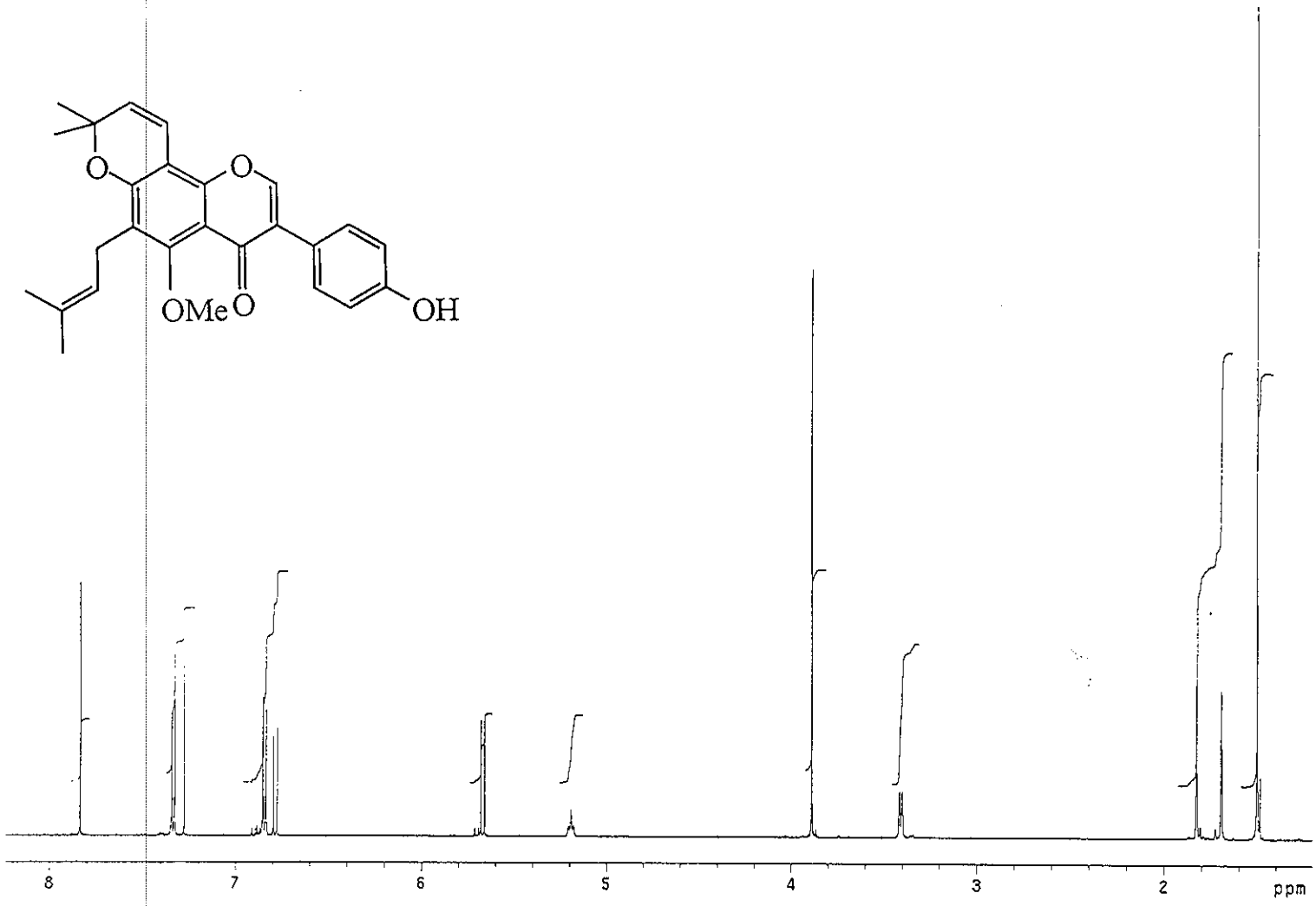


Figure 44 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS7**

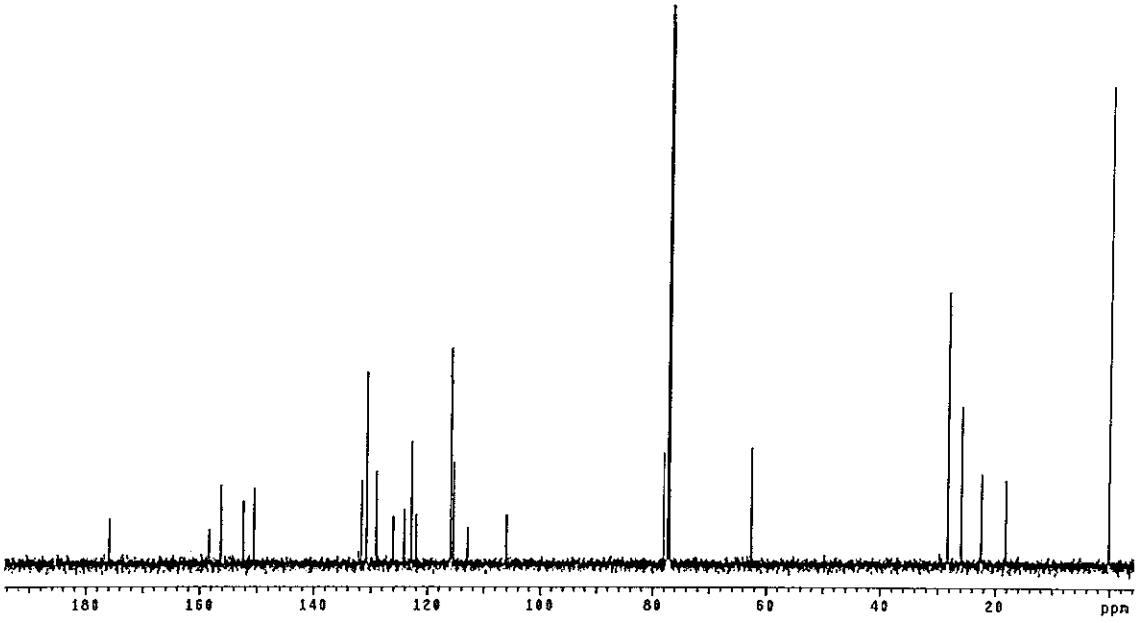


Figure 45 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **DS7**

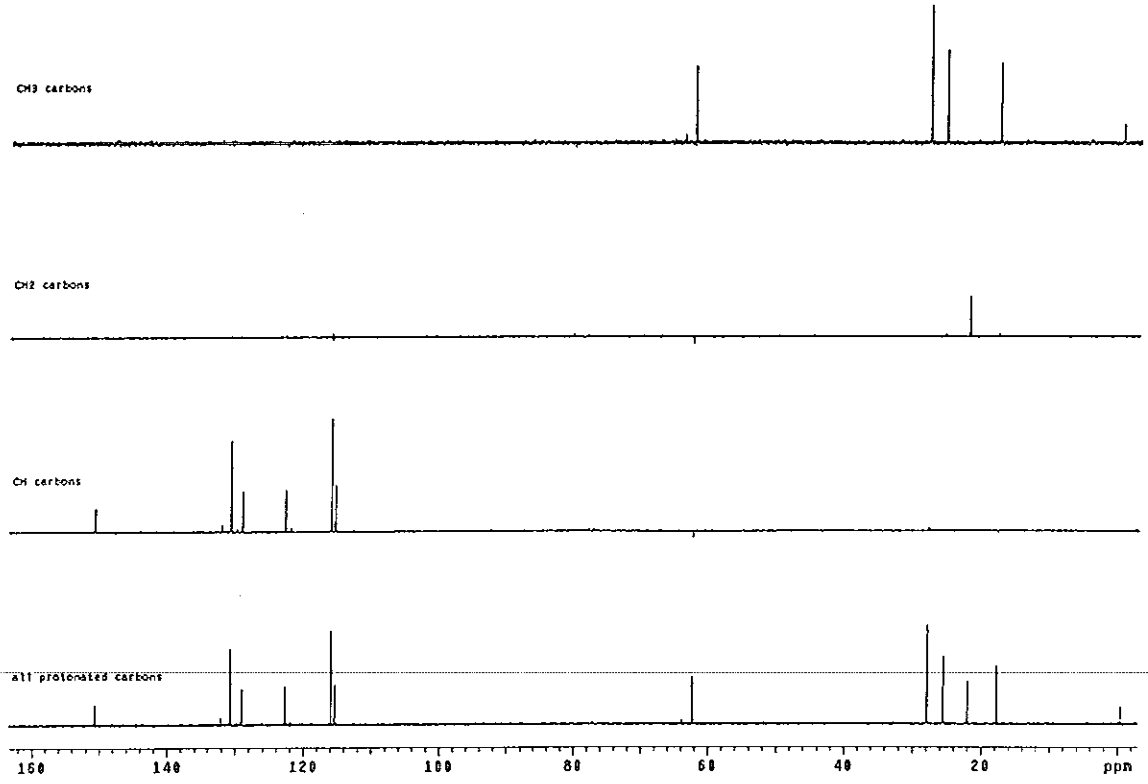


Figure 46 DEPT (135°) ($CDCl_3$) spectrum of **DS7**

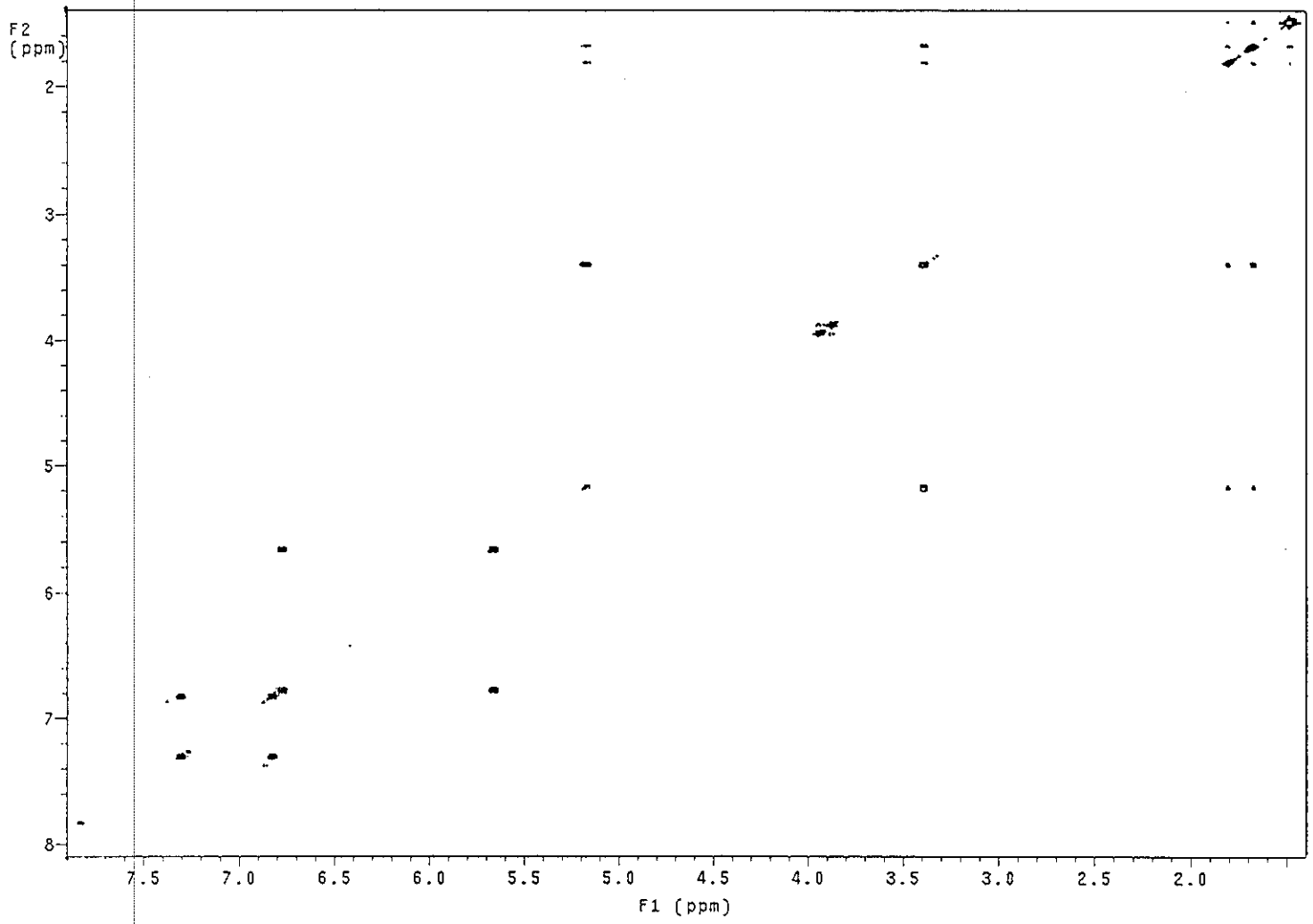


Figure 47 ^{1}H-^{1}H COSY spectrum of **DS7**

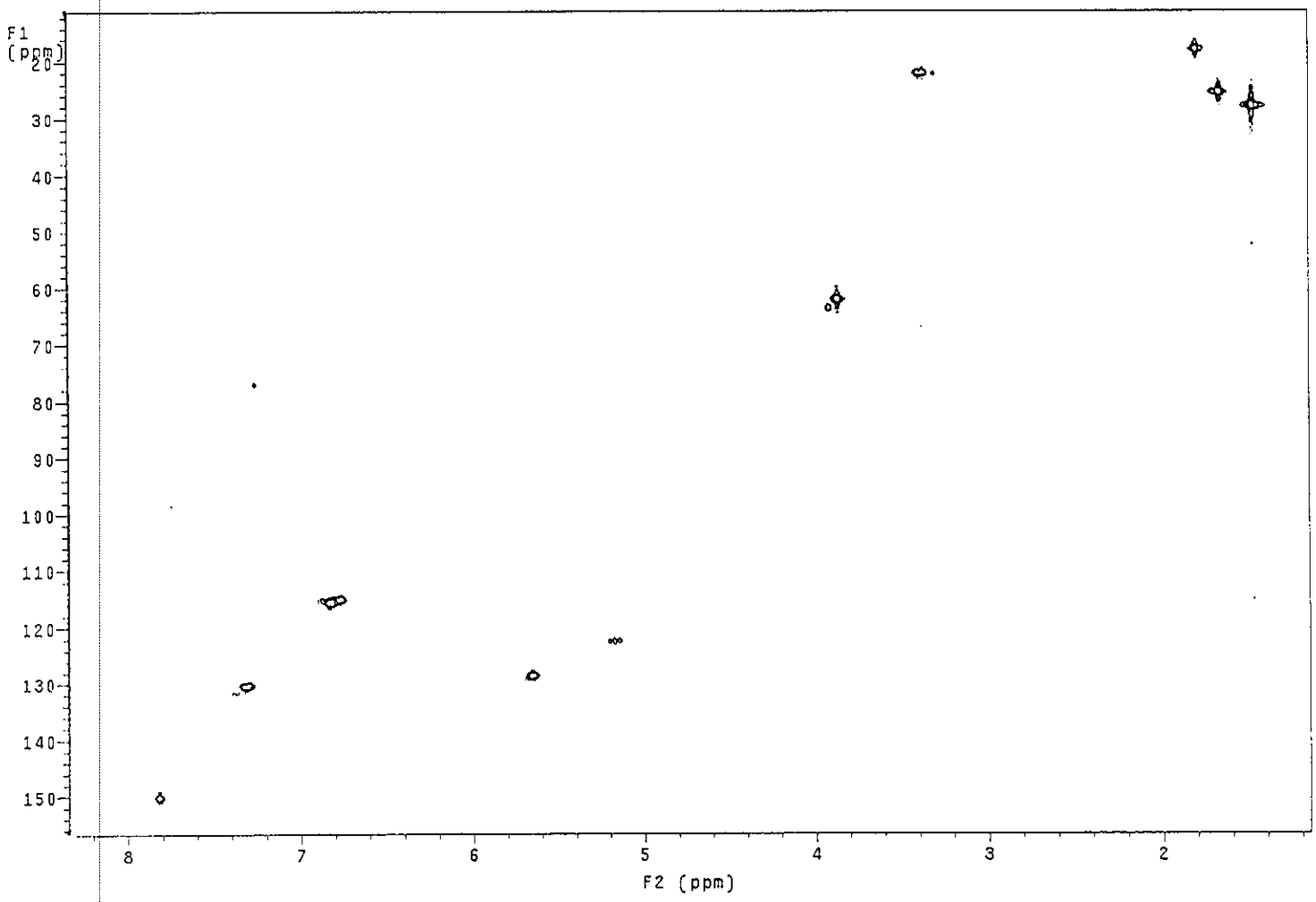


Figure 48 2D HMQC spectrum of **DS7**

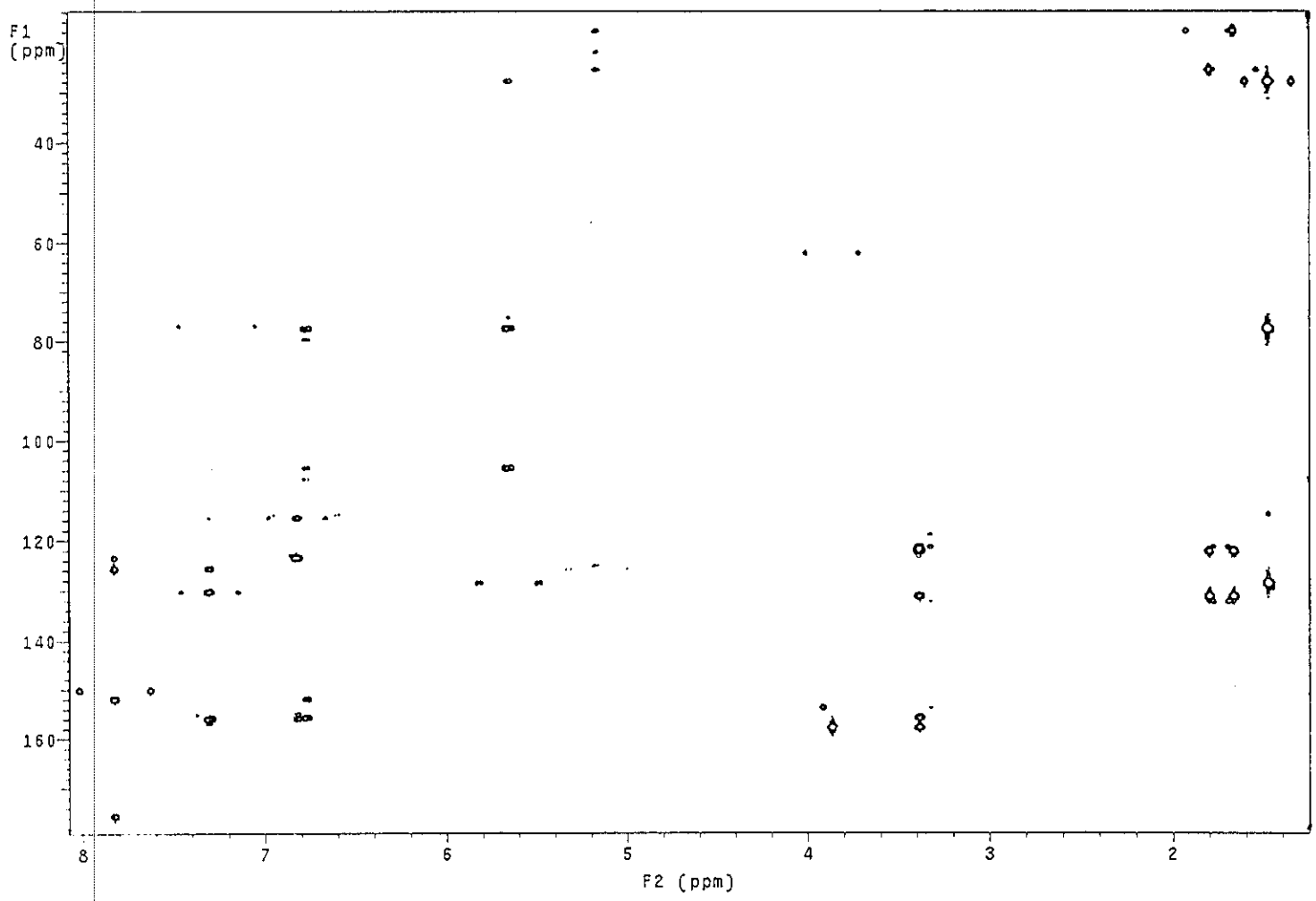


Figure 49 2D HMBC spectrum of **DS7**

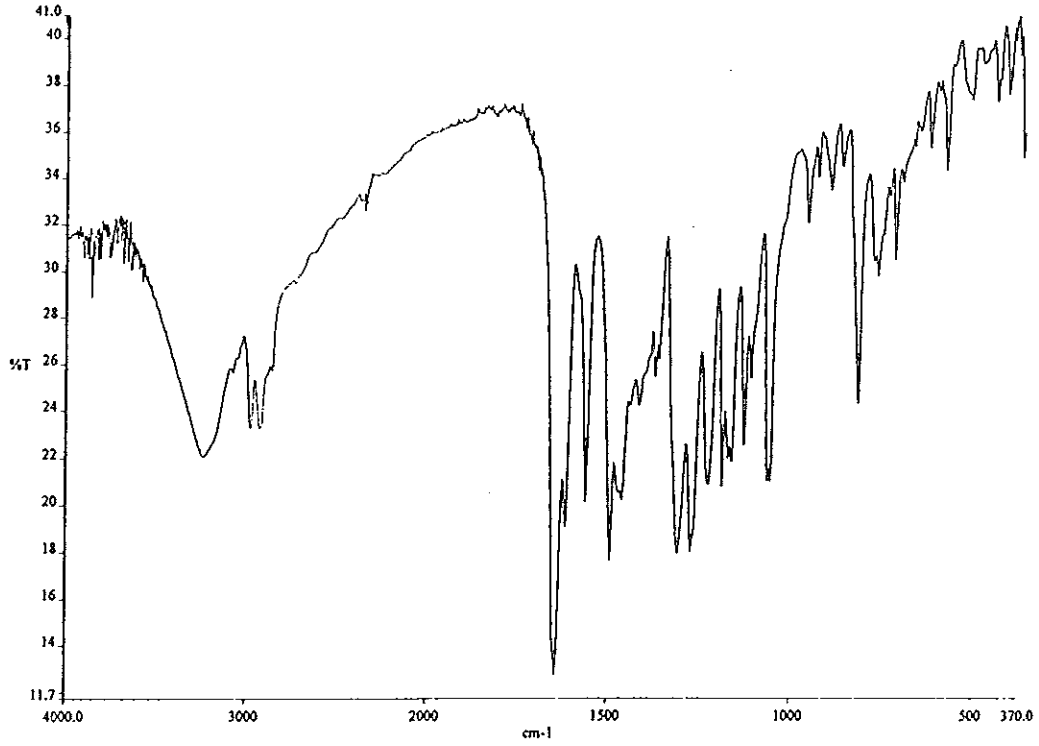


Figure 50 IR (KBr) spectrum of DS9

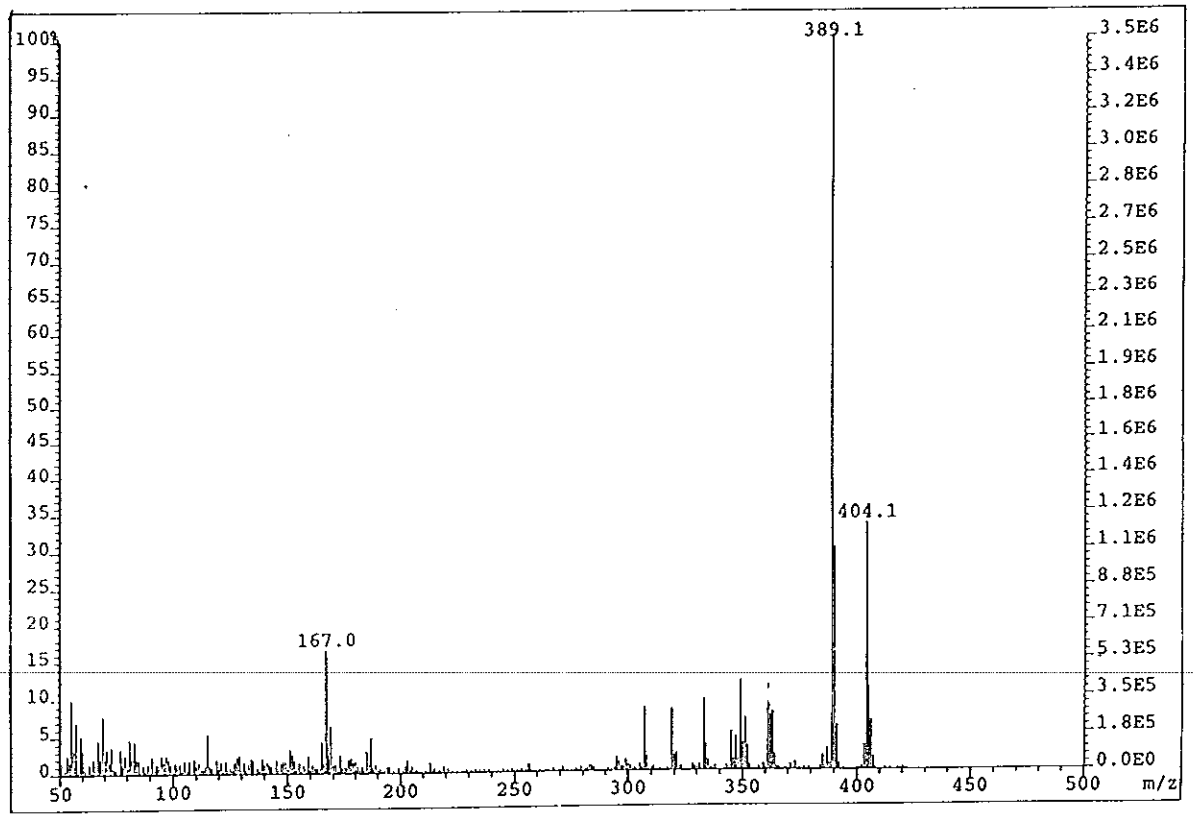


Figure 51 Mass spectrum of **DS9**

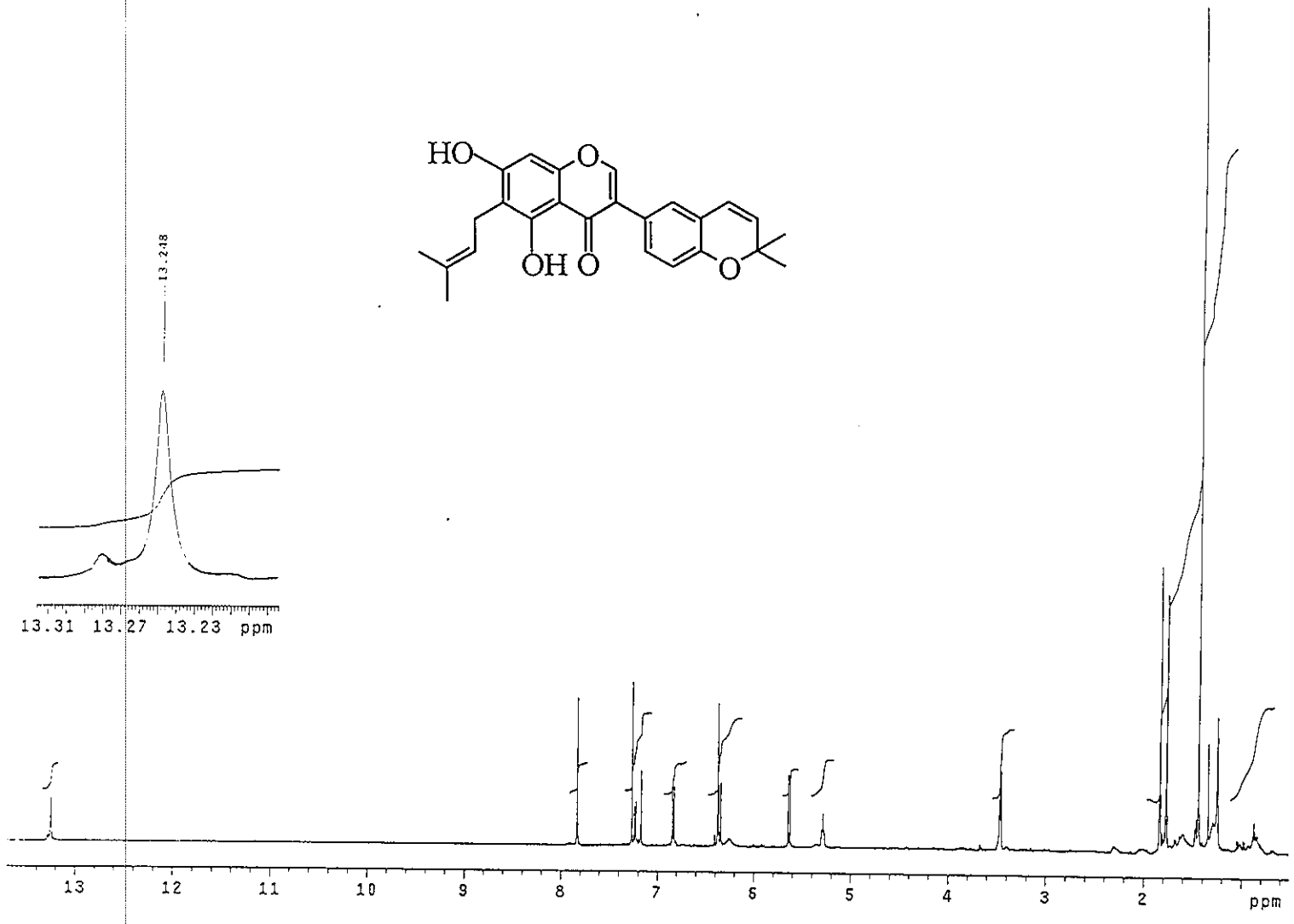


Figure 52 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS9**

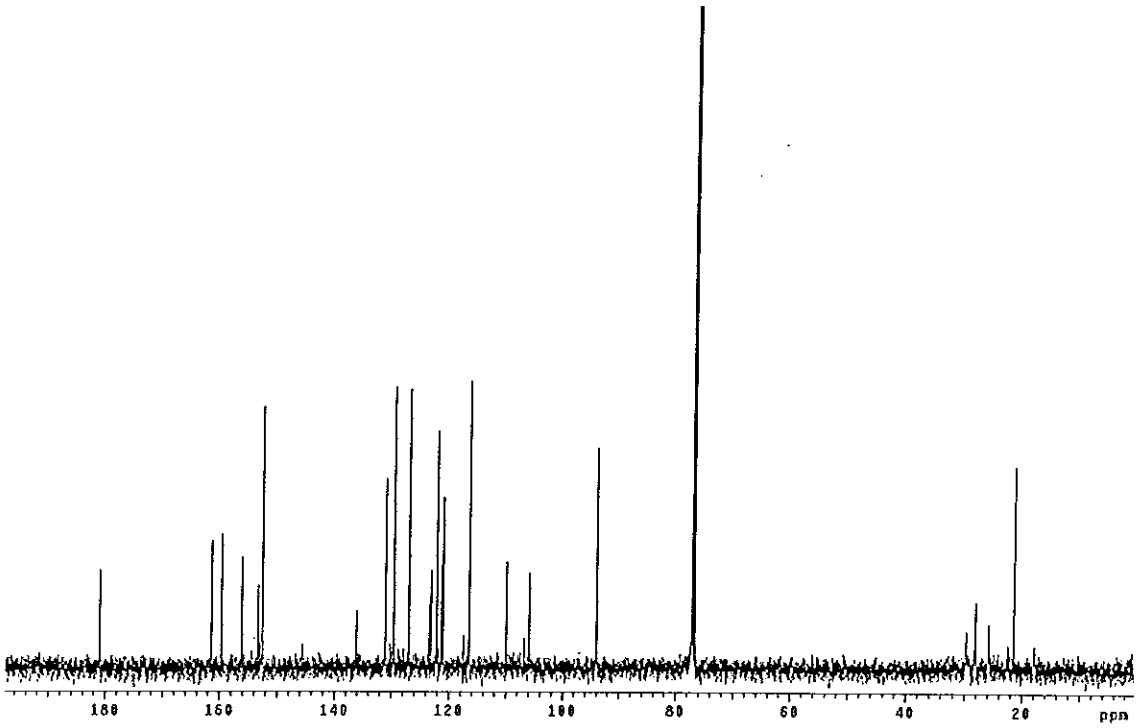


Figure 53 ^{13}C NMR (125 MHz) ($CDCl_3$ + DMSO-d_6) spectrum of **DS9**

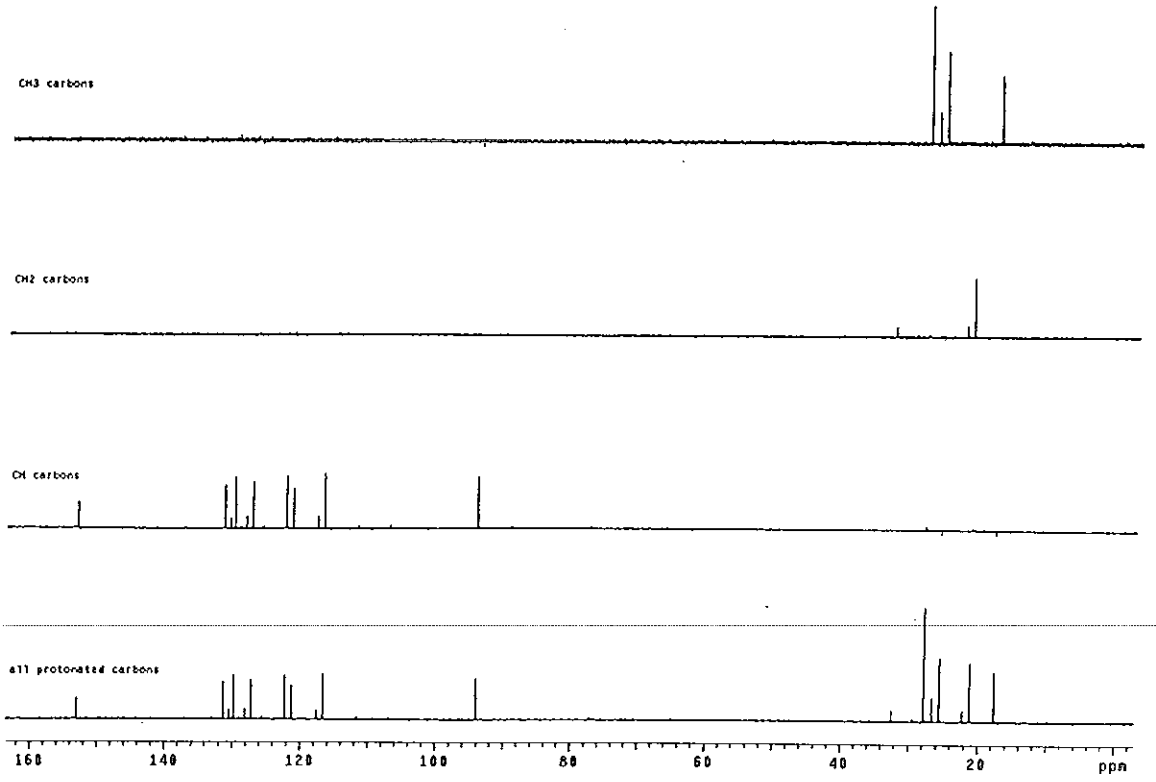


Figure 54 DEPT (135°) ($CDCl_3$ + DMSO-d_6) spectrum of **DS9**

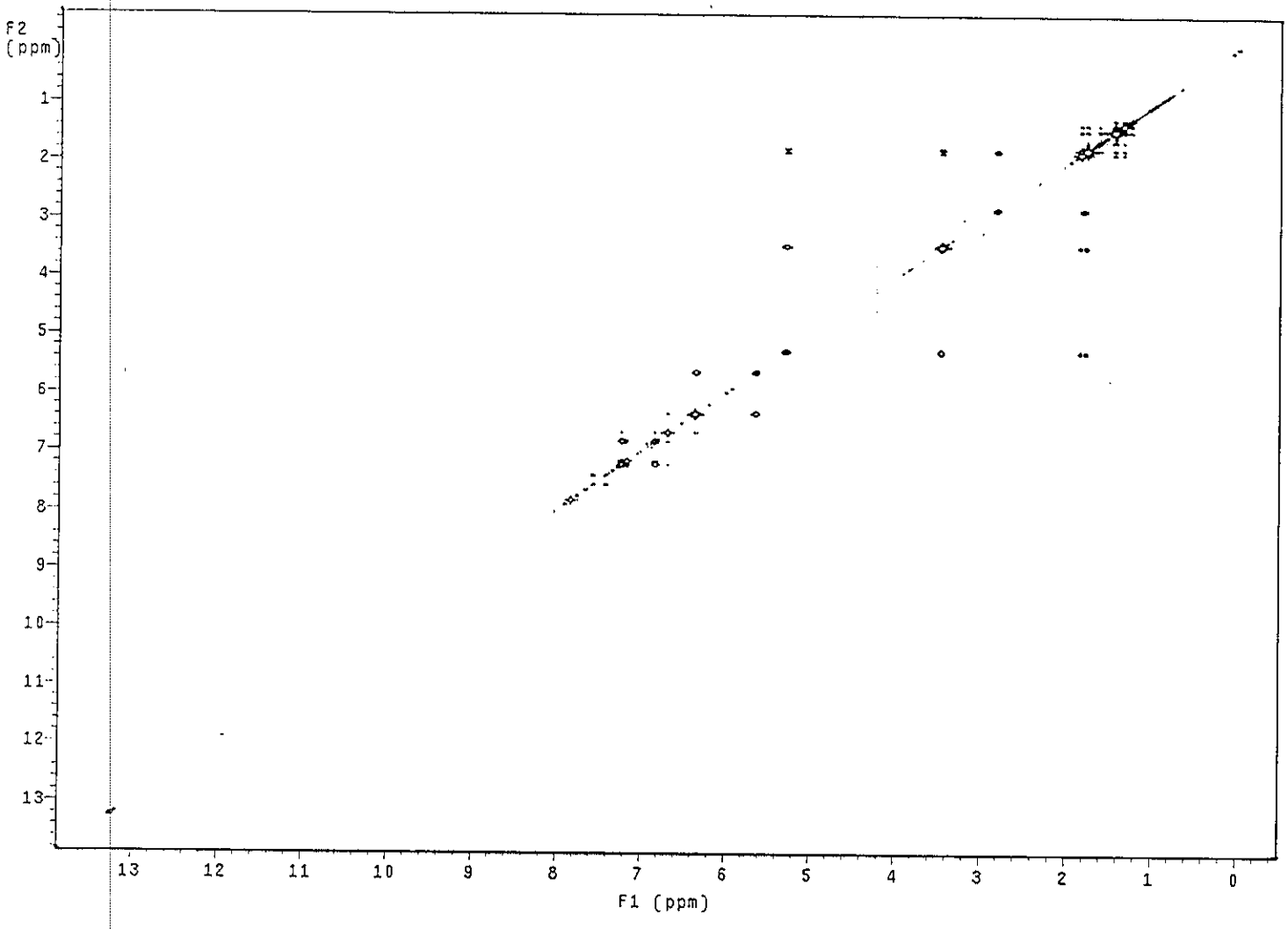


Figure 55 ^{1}H-^{1}H COSY spectrum of **DS9**

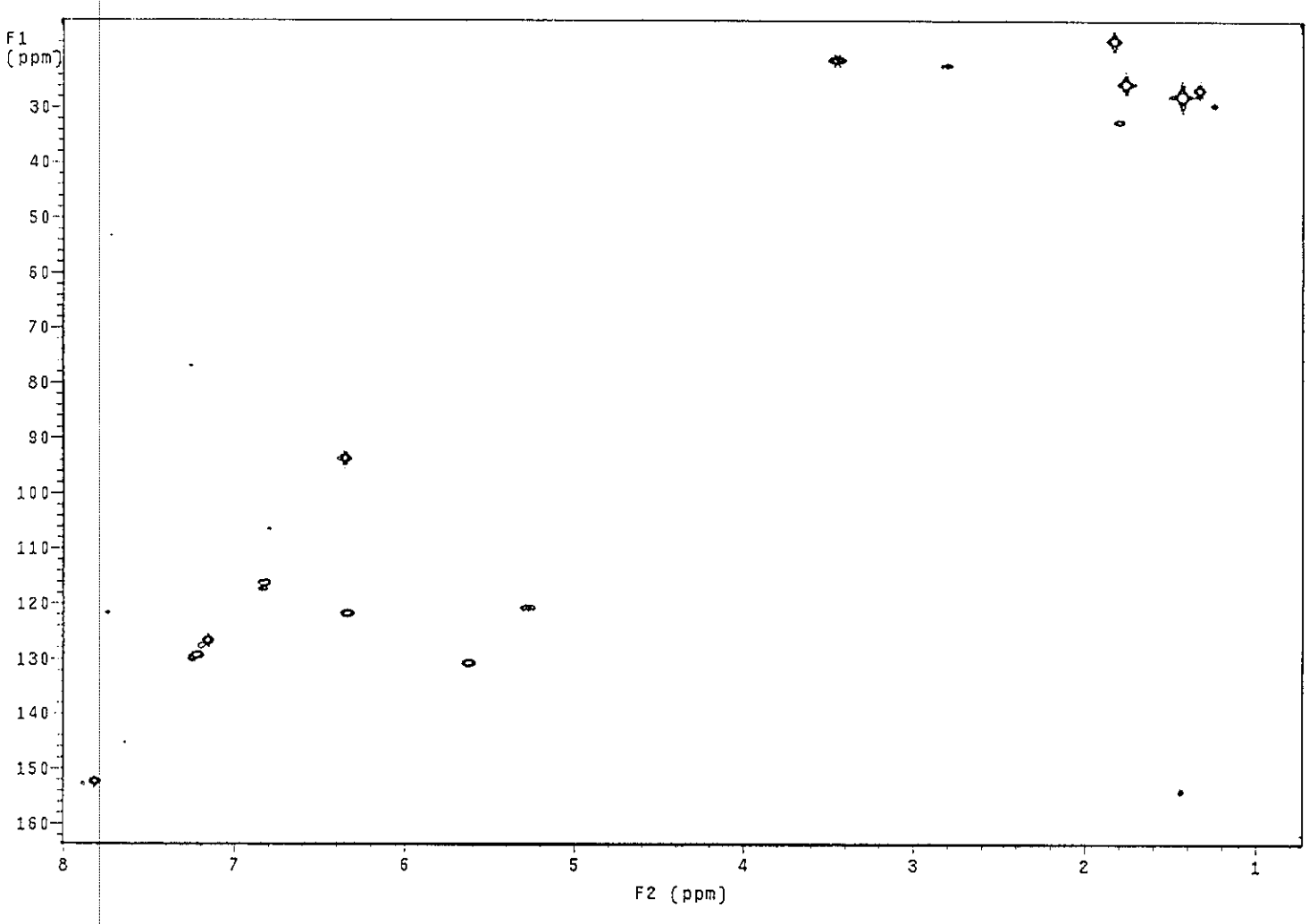


Figure 56 2D HMQC spectrum of **DS9**

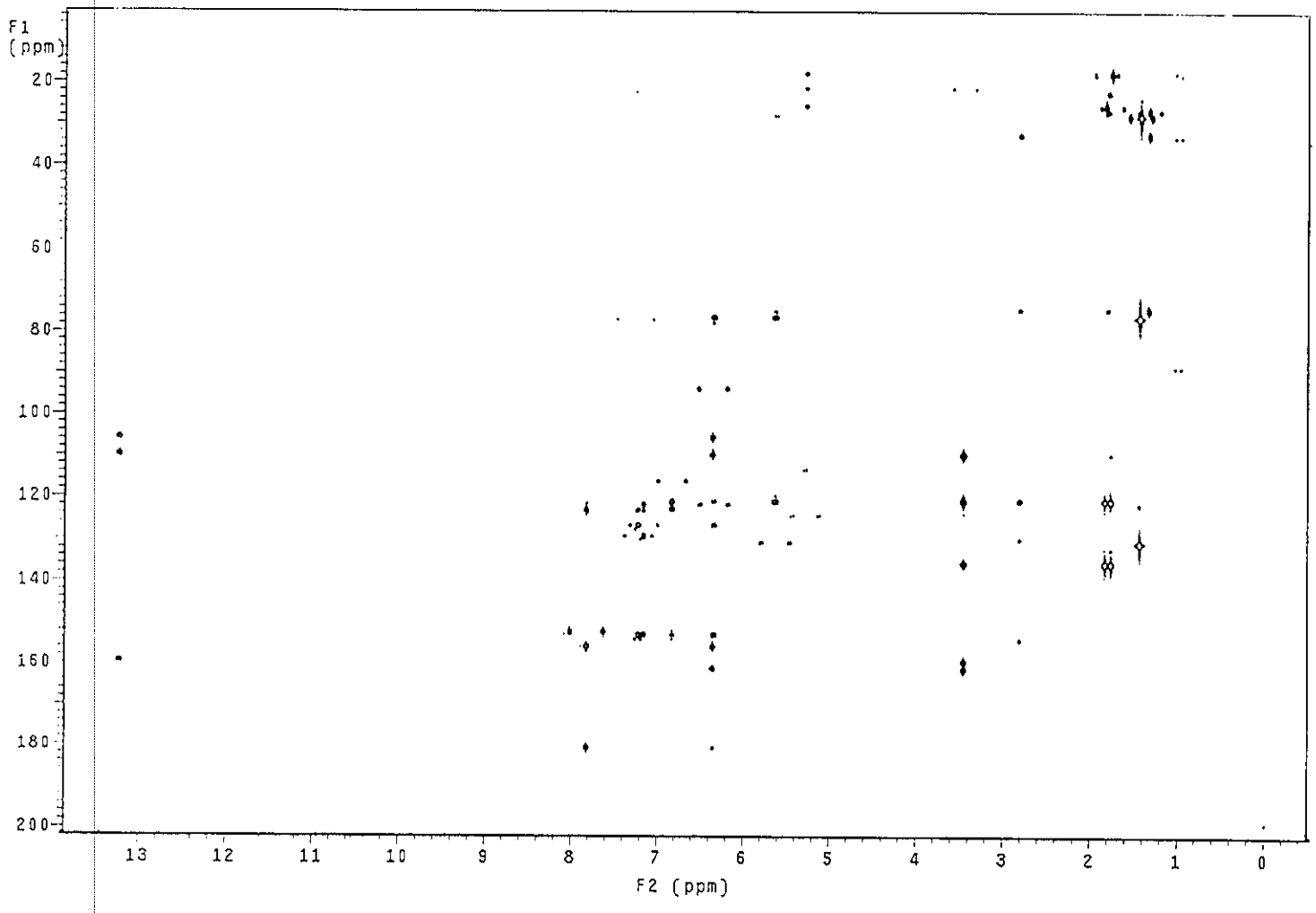


Figure 57 2D HMBC spectrum of **DS9**

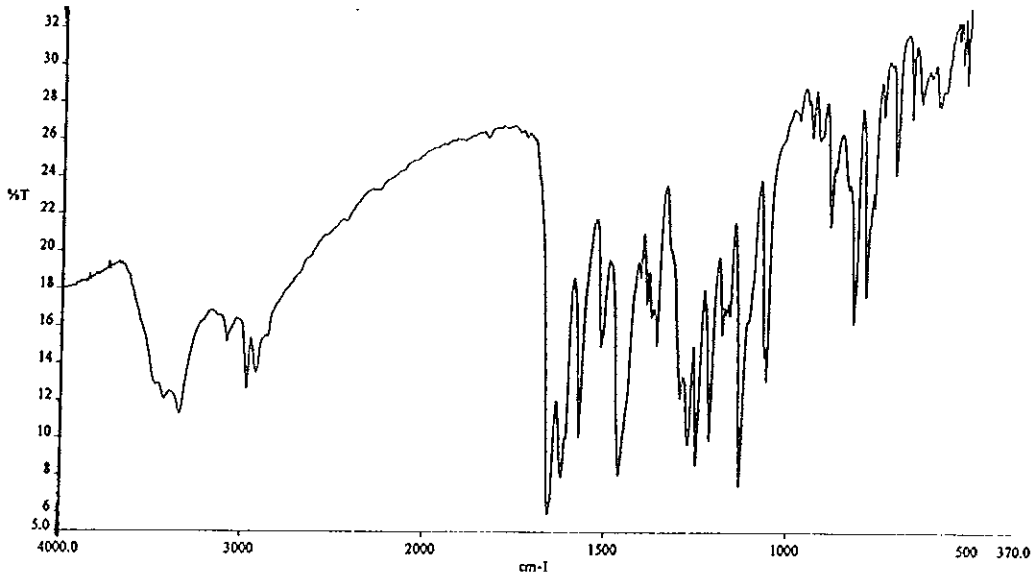


Figure 58 IR (KBr) spectrum of **DS10**

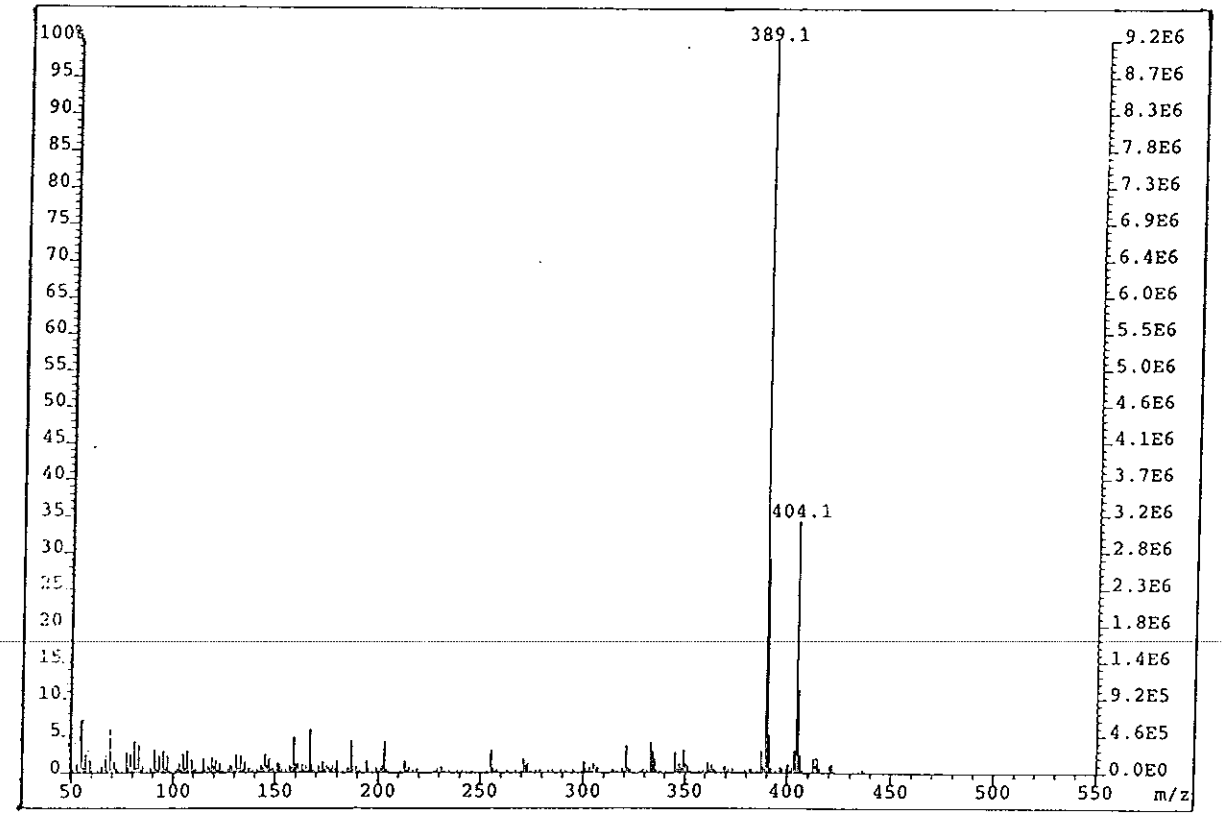


Figure 59 Mass spectrum of **DS10**

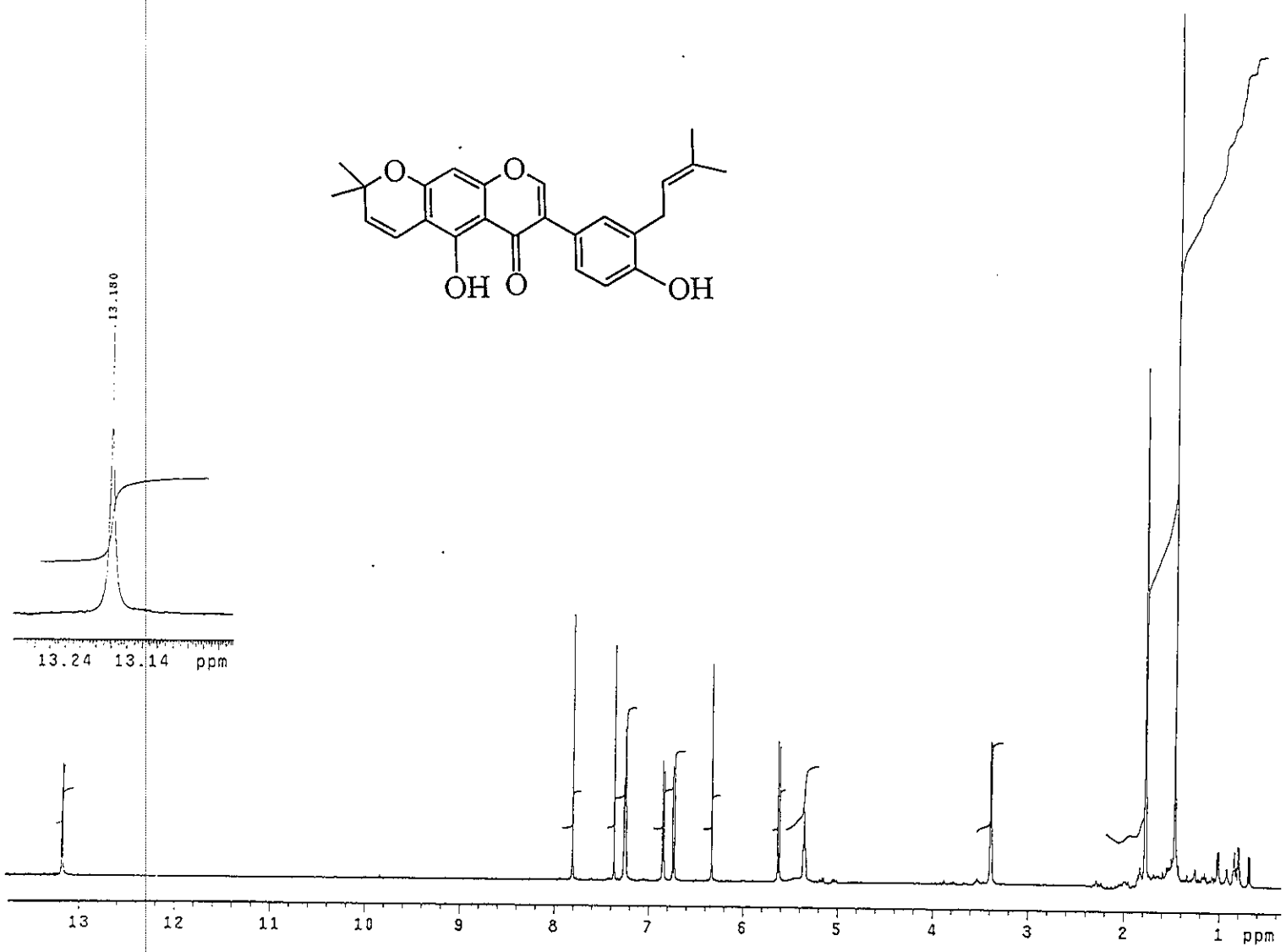


Figure 60 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS10**

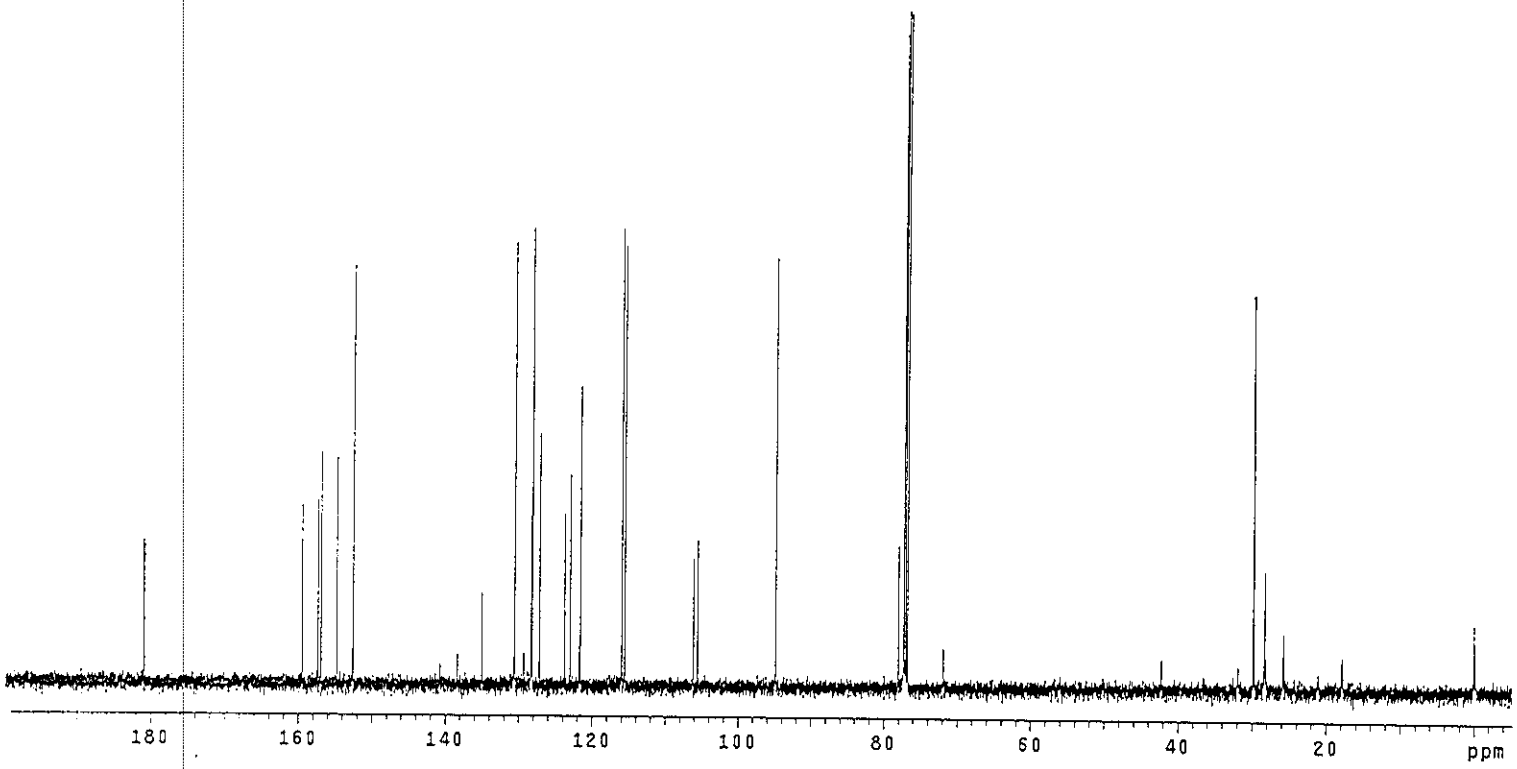


Figure 61 ^{13}C NMR (125 MHz) ($CDCl_3$ + DMSO-d_6) spectrum of **DS10**

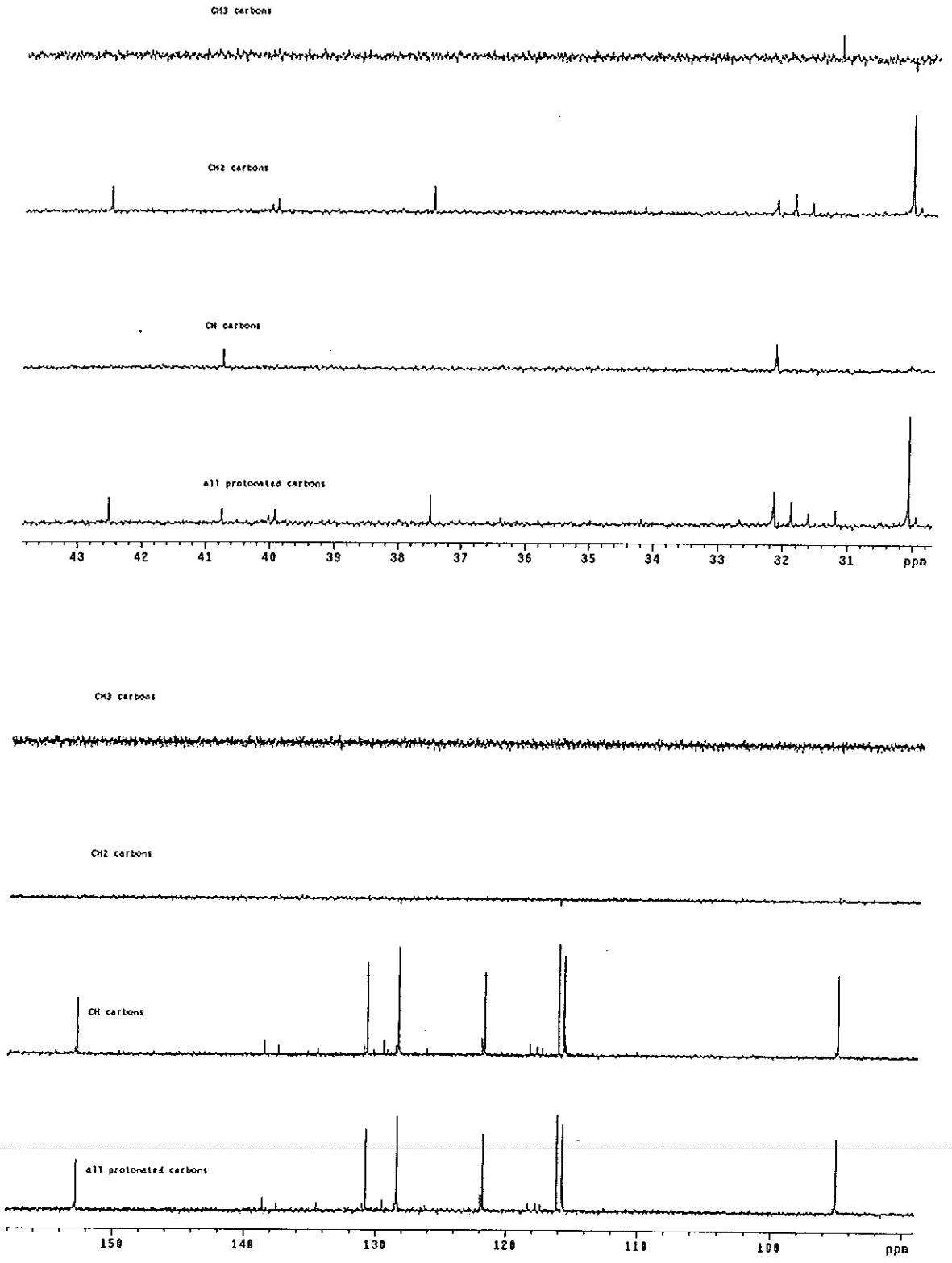


Figure 62 DEPT (135°) ($CDCl_3$ + DMSO-d_6) spectrum of **DS10**

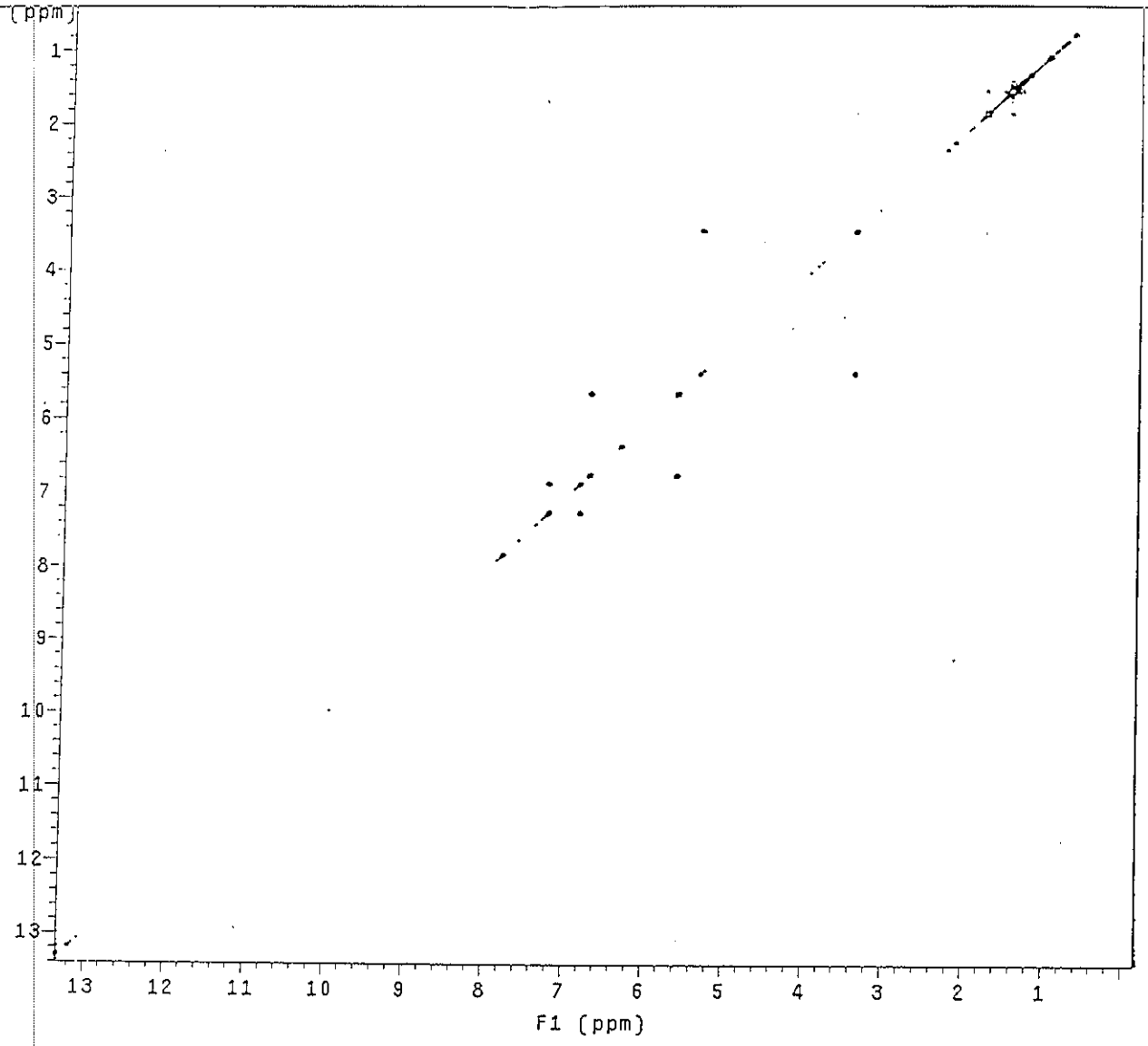


Figure 63 ^{1}H-^{1}H COSY spectrum of **DS10**

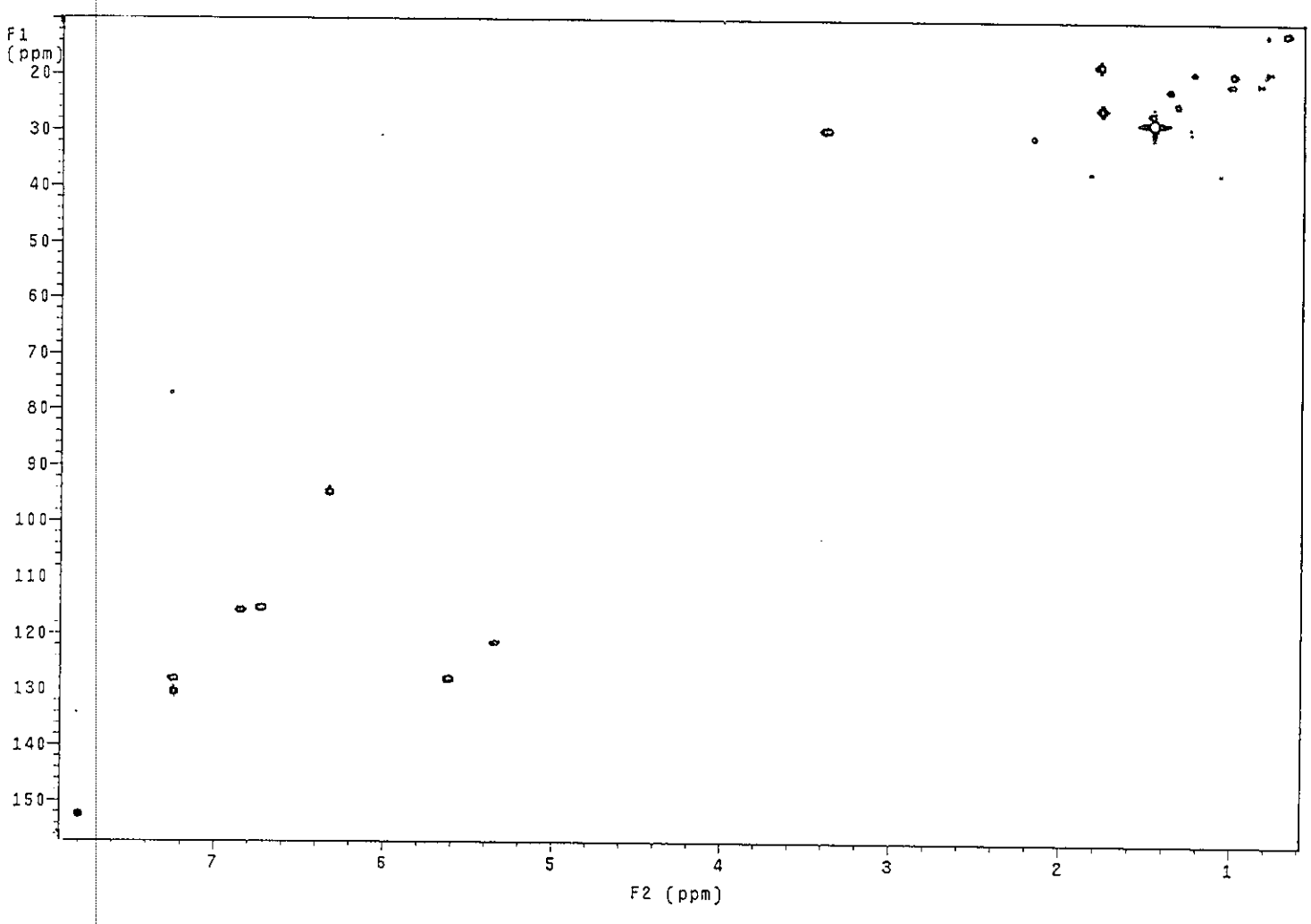


Figure 64 2D HMQC spectrum of **DS10**

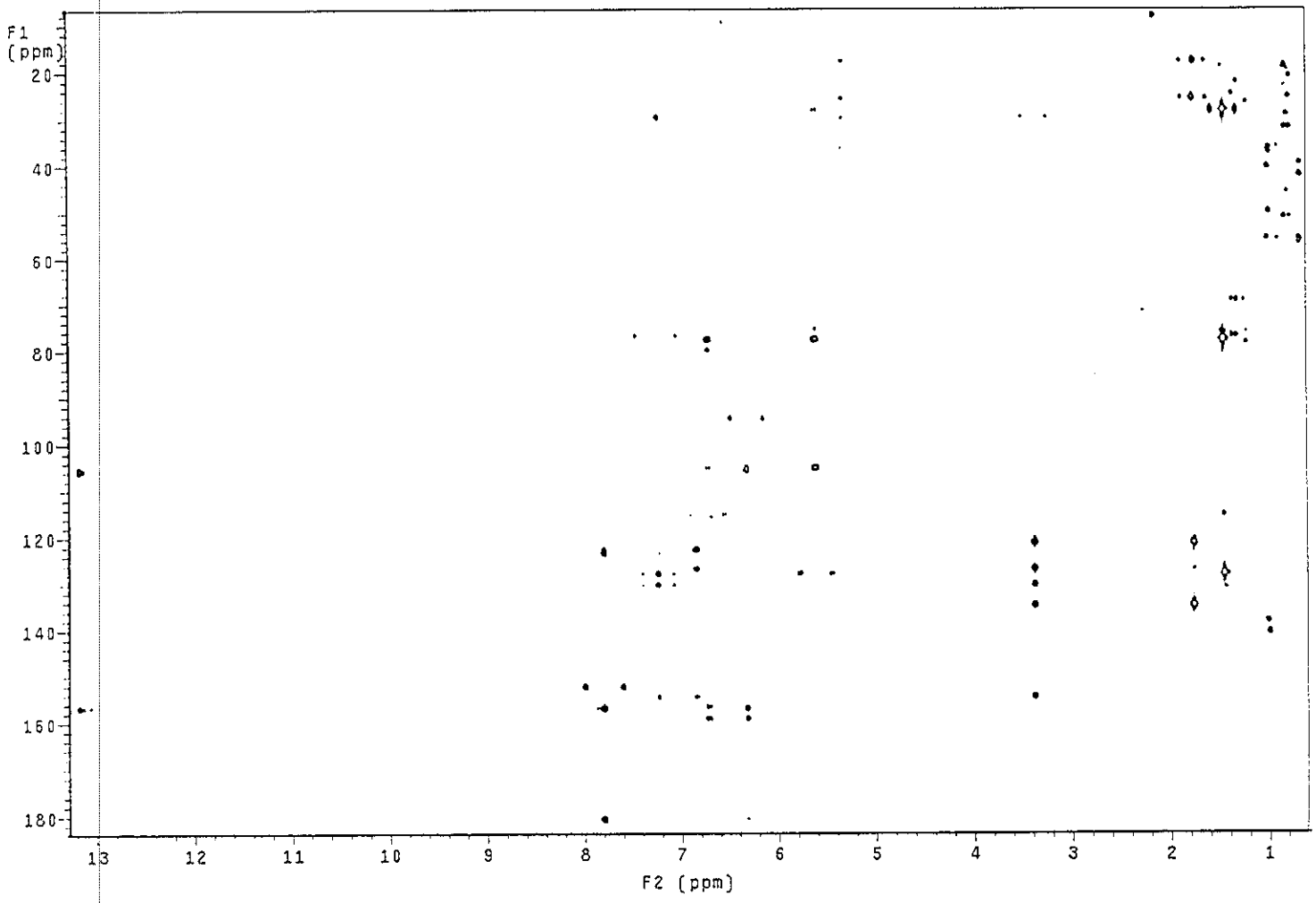


Figure 65 2D HMBC spectrum of **DS10**

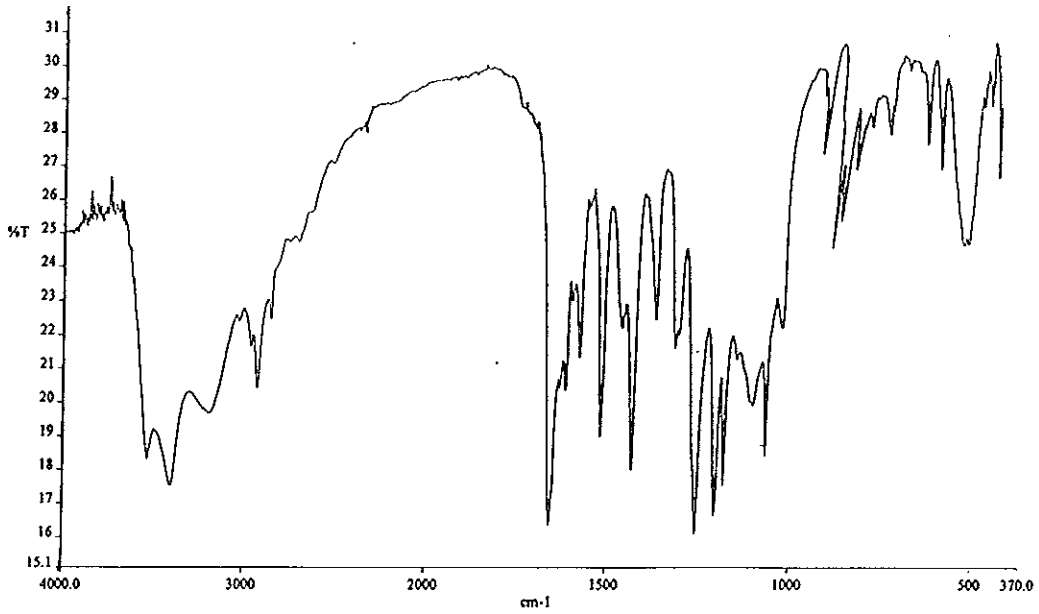


Figure 66 IR (KBr) spectrum of **DS11**

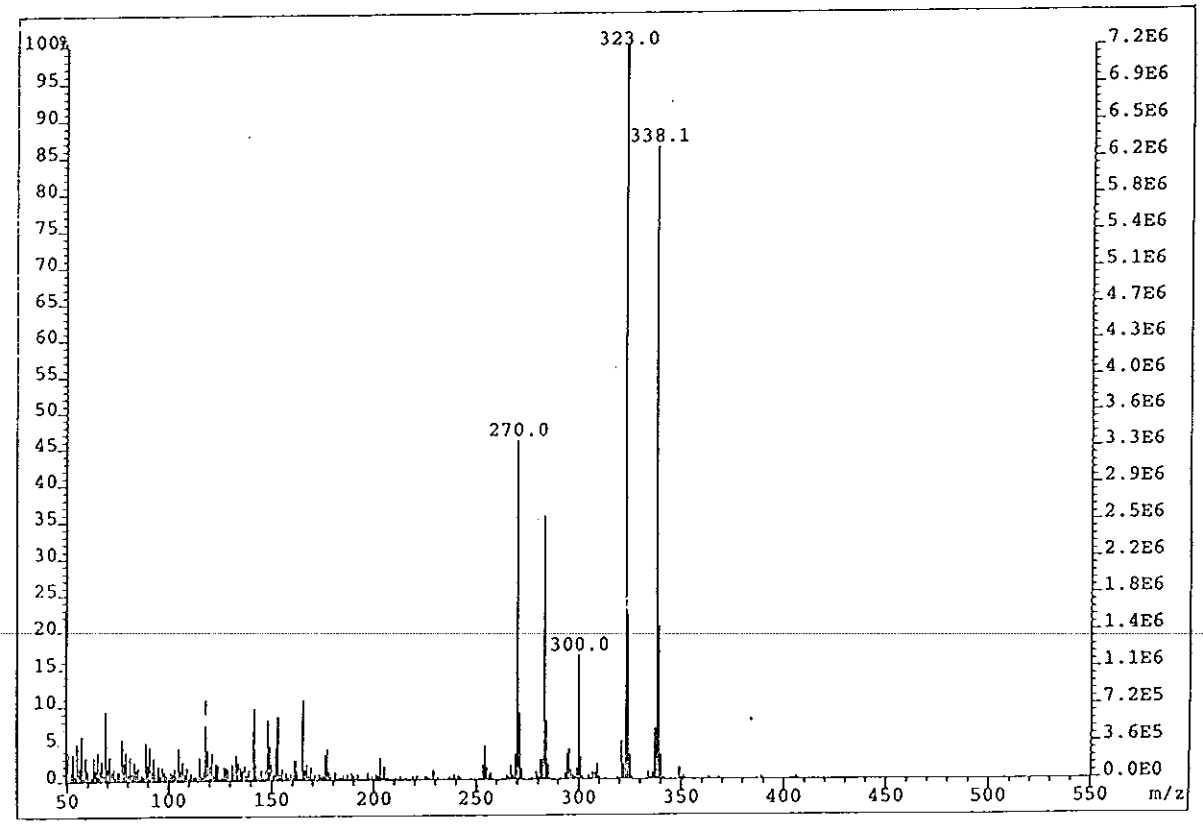


Figure 67 Mass spectrum of **DS11**

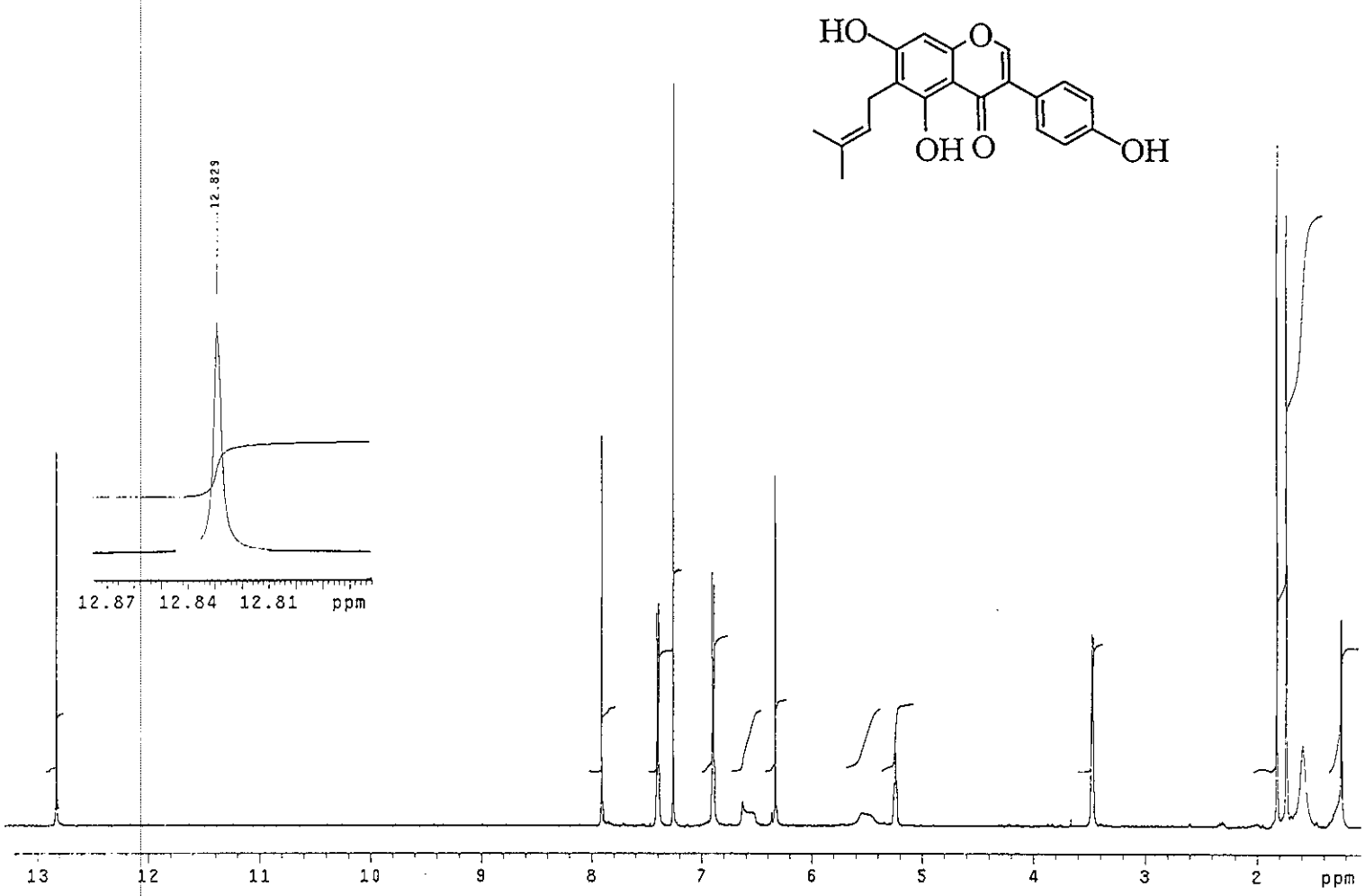


Figure 68 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS11**

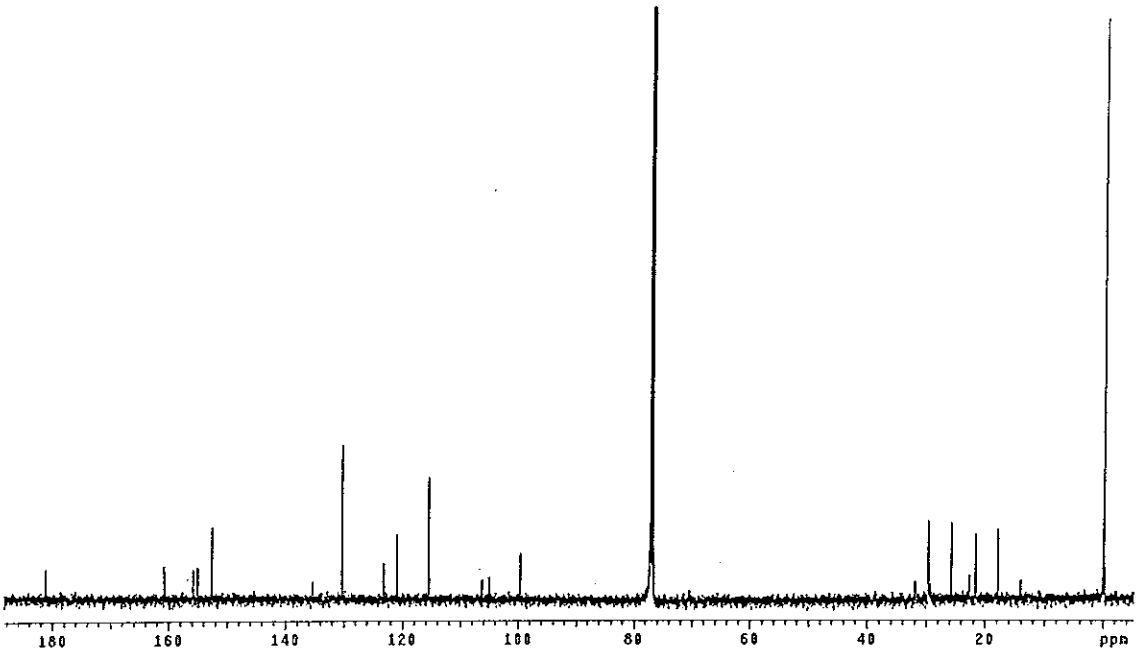


Figure 69 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **DS11**

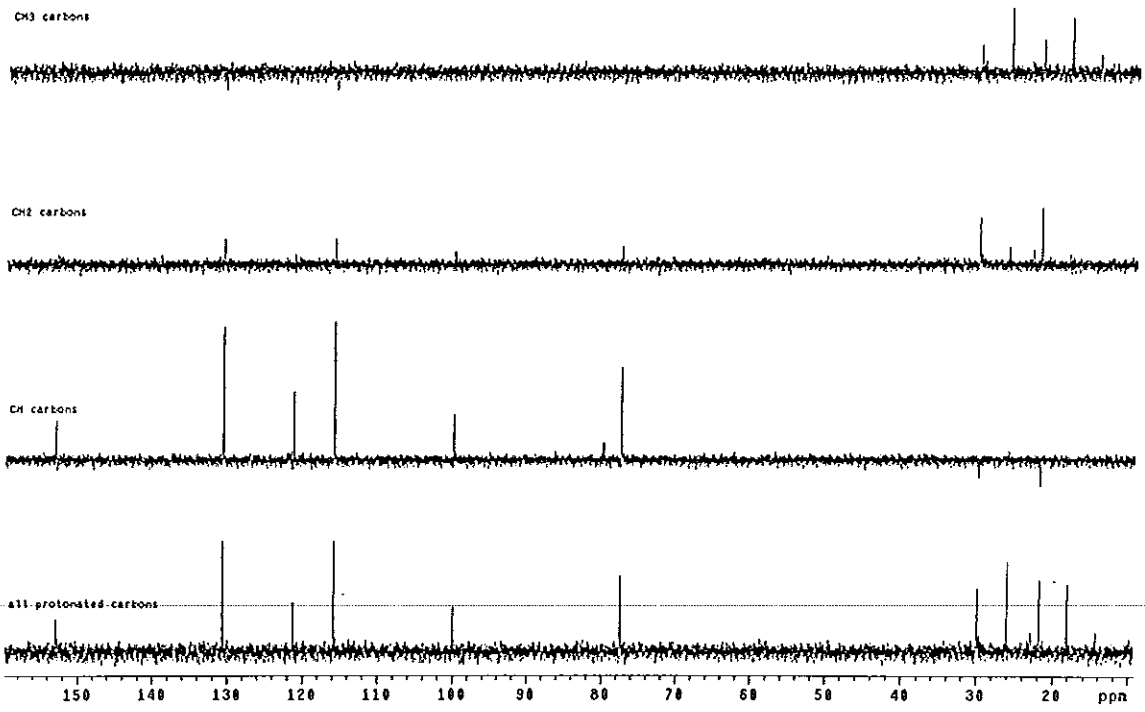


Figure 70 DEPT (135°) ($CDCl_3$) spectrum of **DS11**

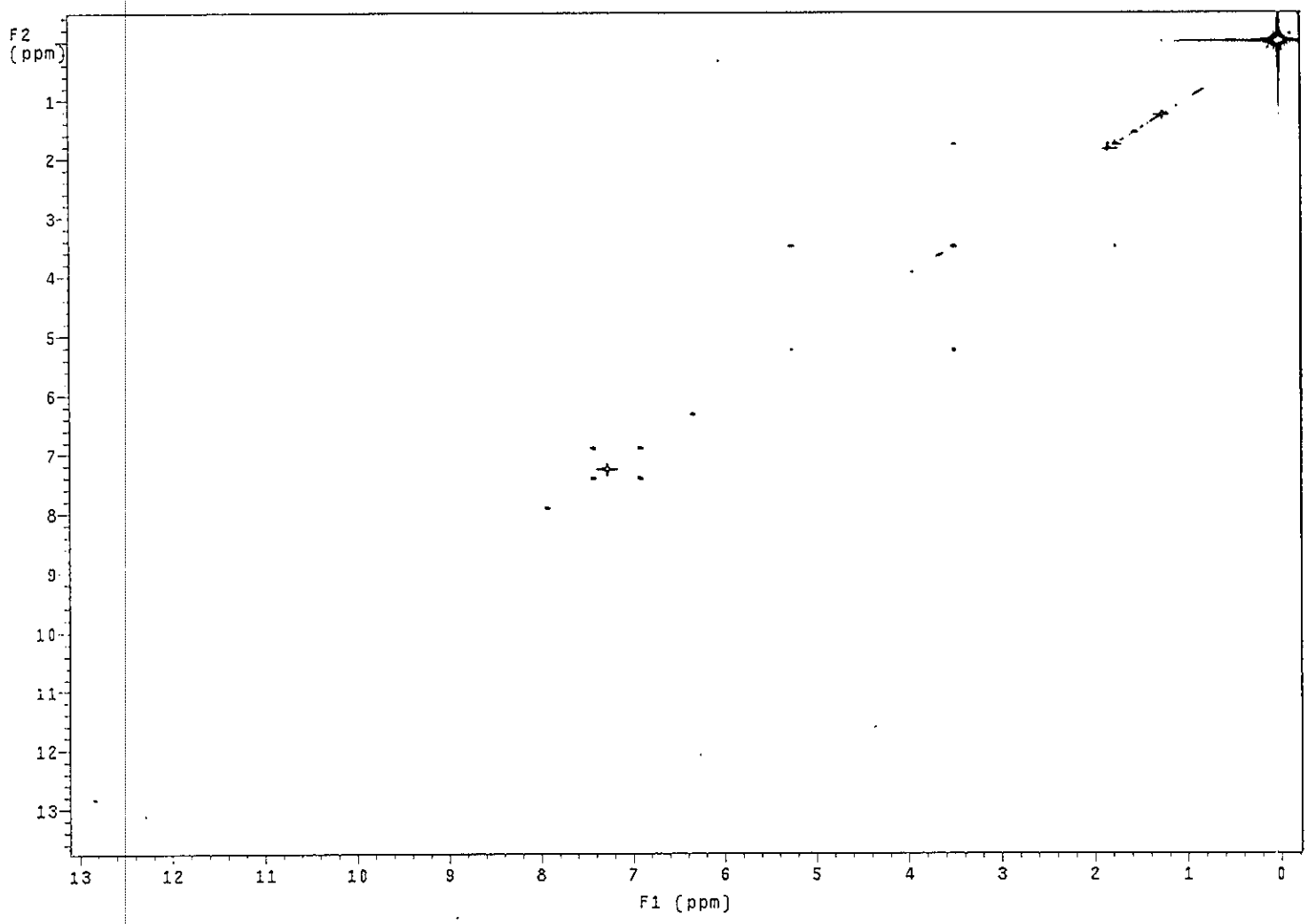


Figure 71 ^{1}H-^{1}H COSY spectrum of **DS11**

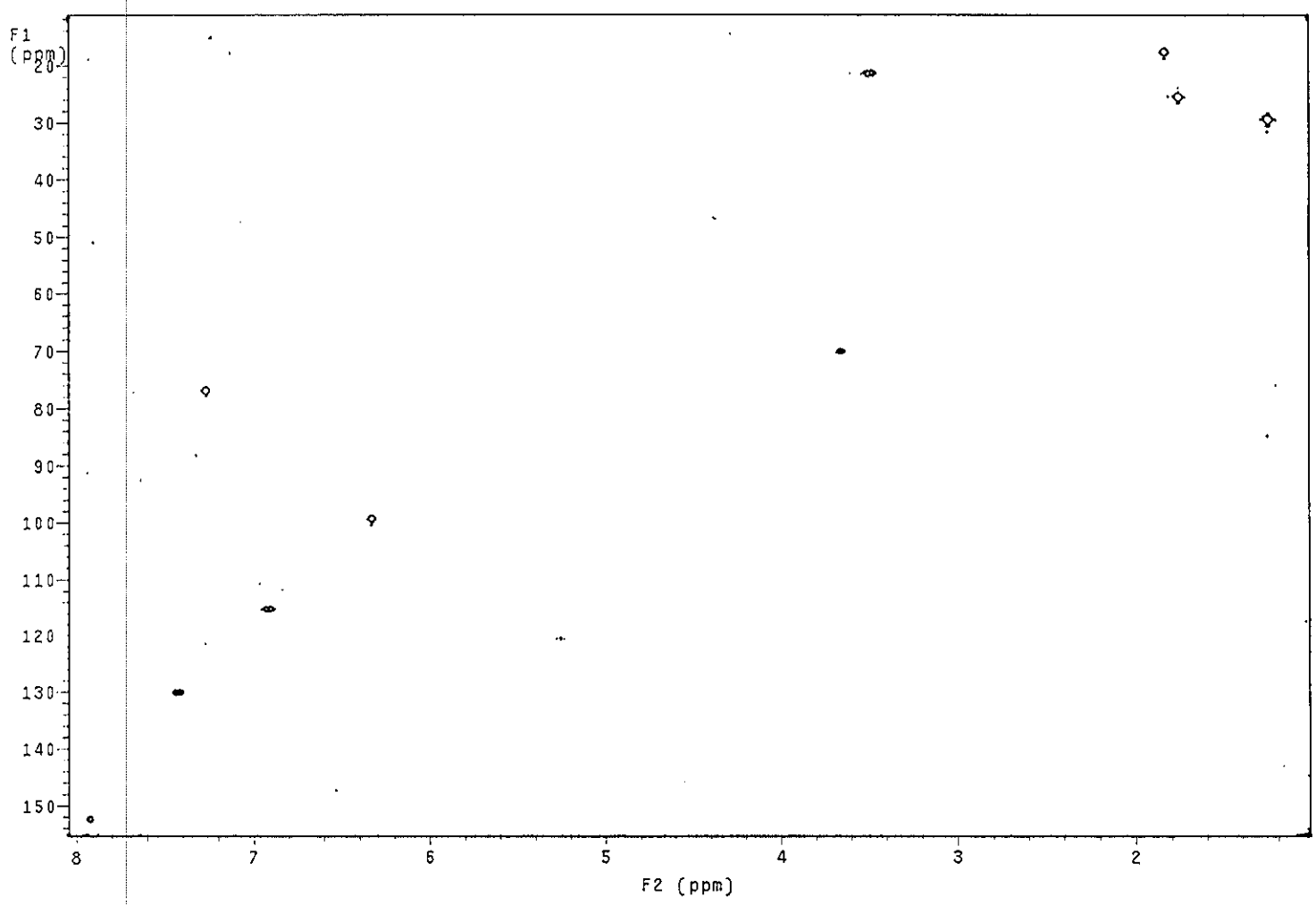


Figure 72 2D HMQC spectrum of **DS11**

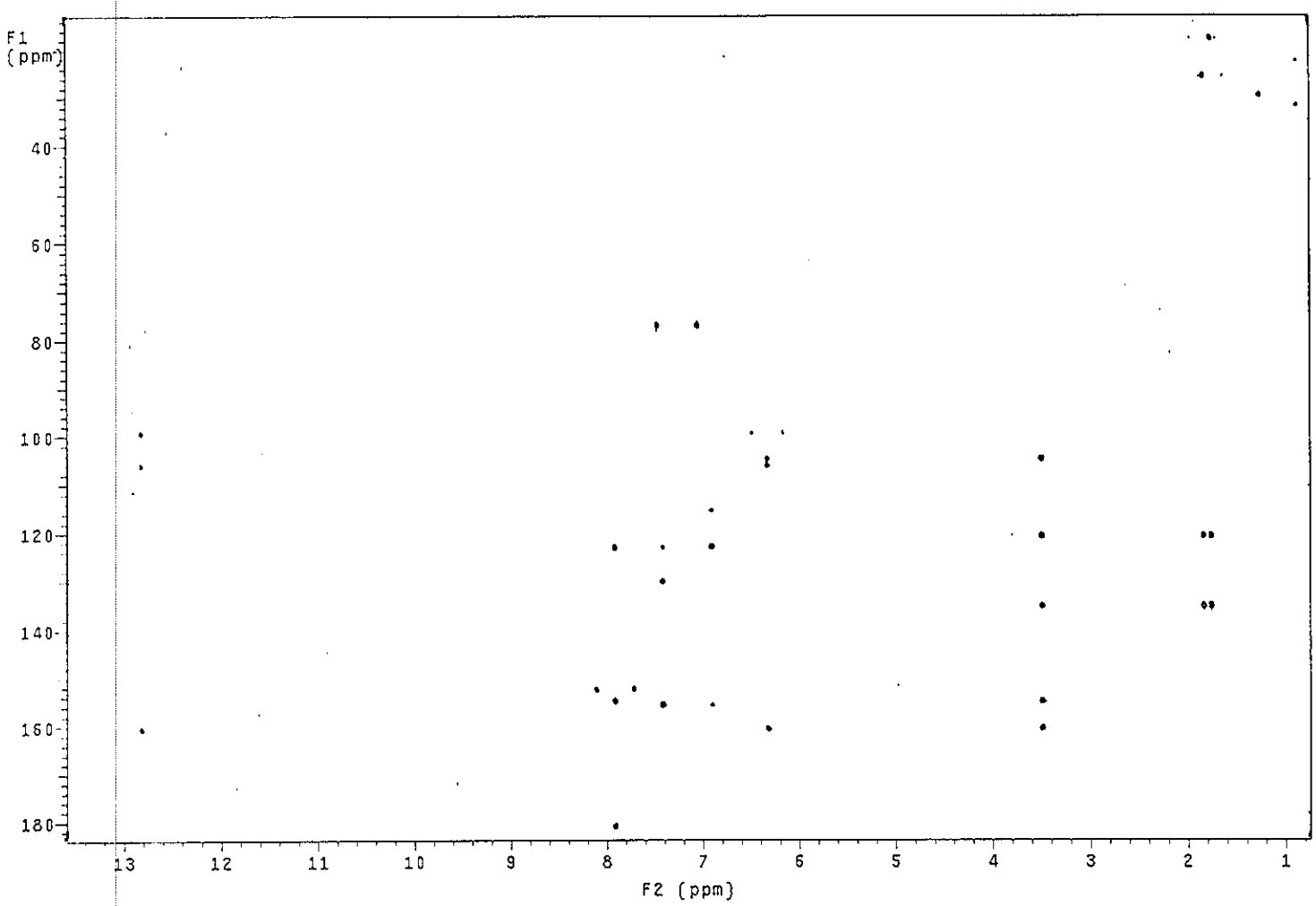


Figure 73 2D HMBC spectrum of **DS11**

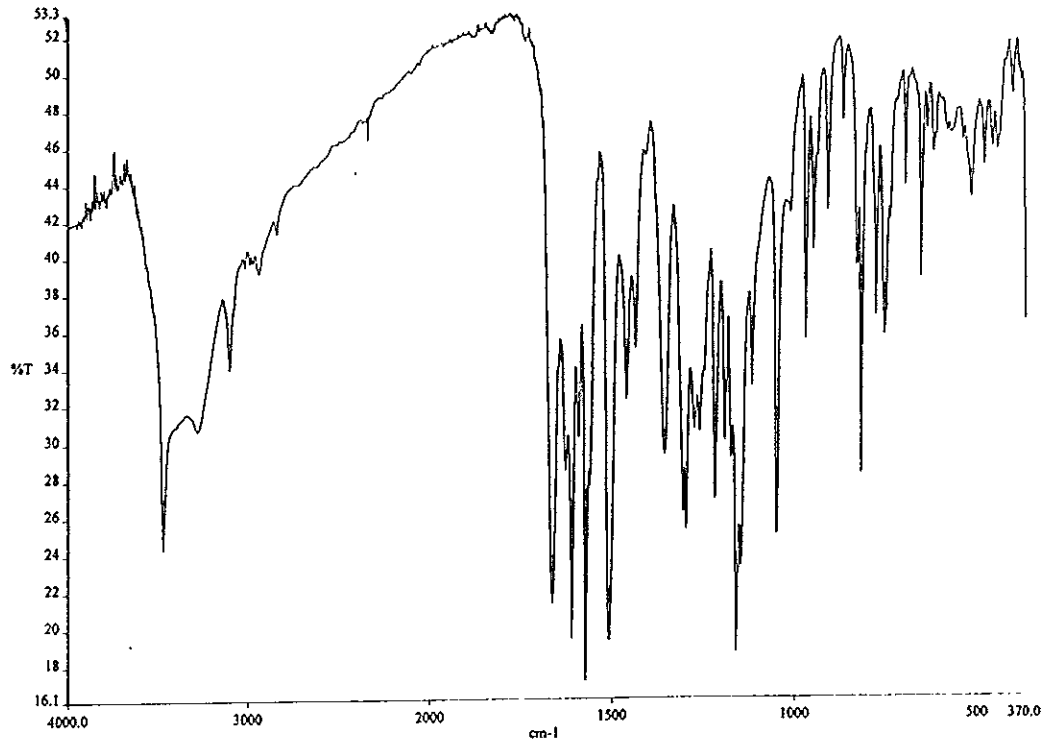


Figure 74 IR (KBr) spectrum of DS12

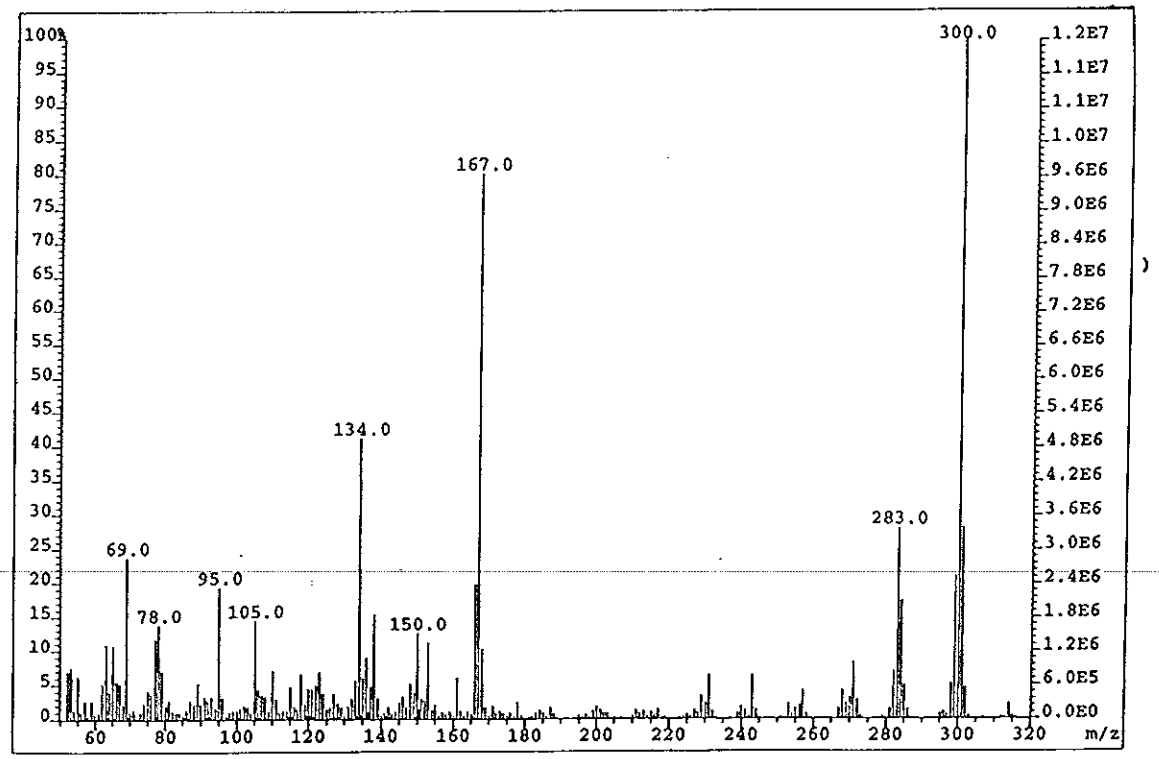


Figure 75 Mass spectrum of DS12

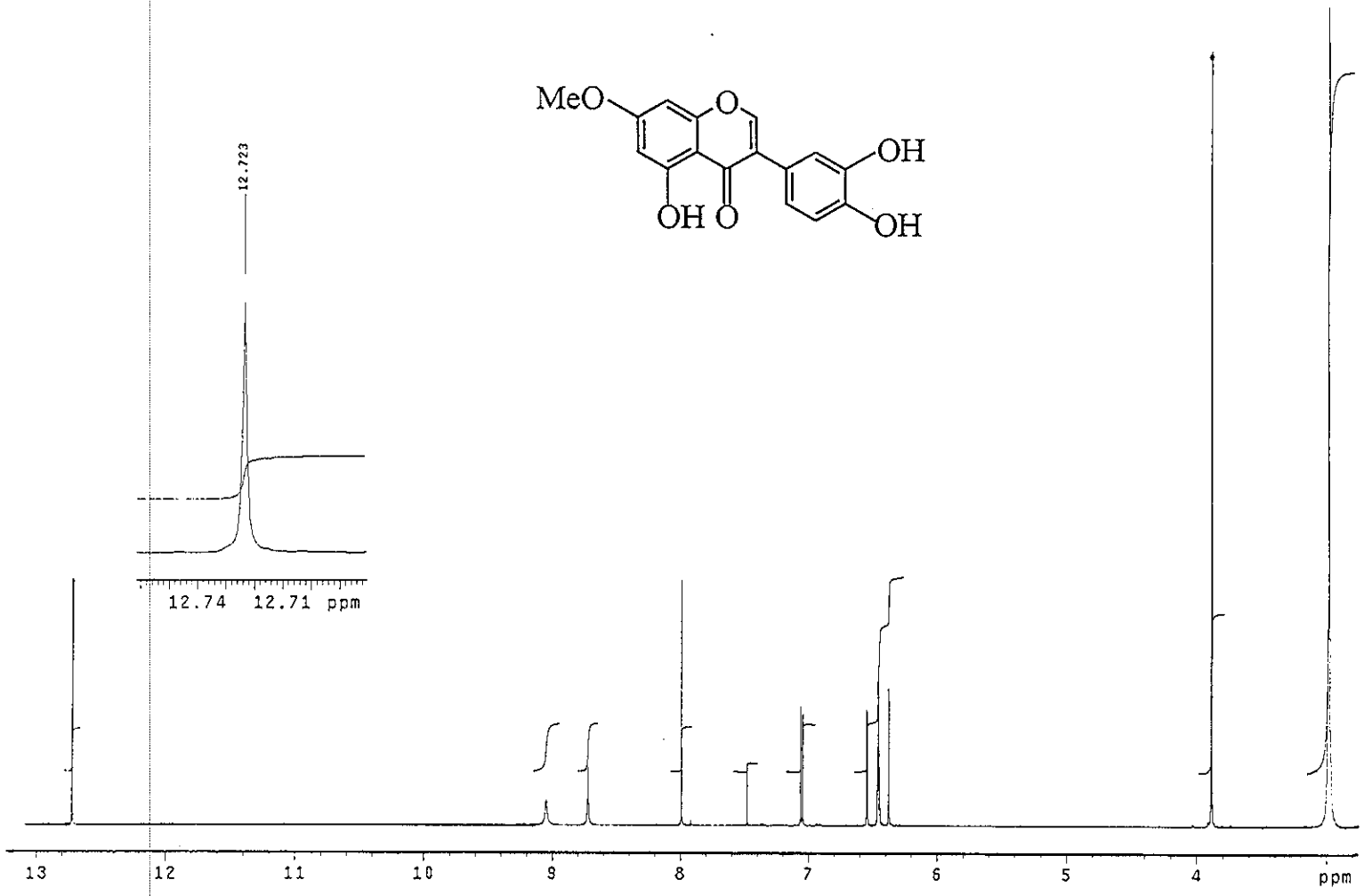


Figure 76 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS12**

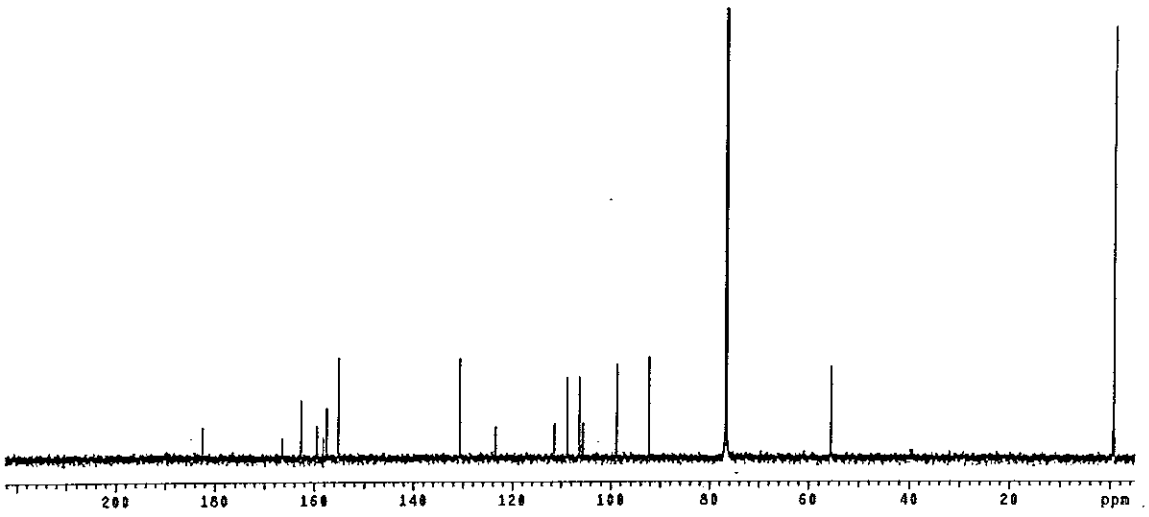


Figure 77 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **DS12**

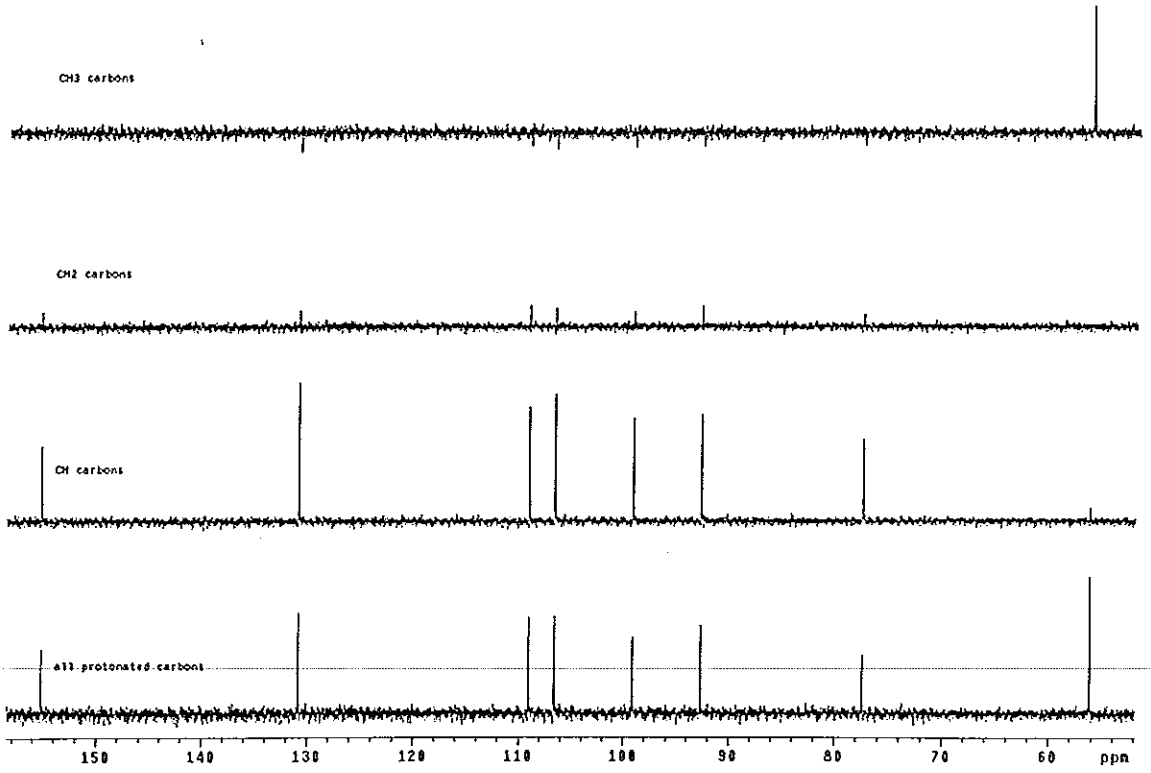


Figure 78 DEPT (135°) ($CDCl_3$) spectrum of **DS12**

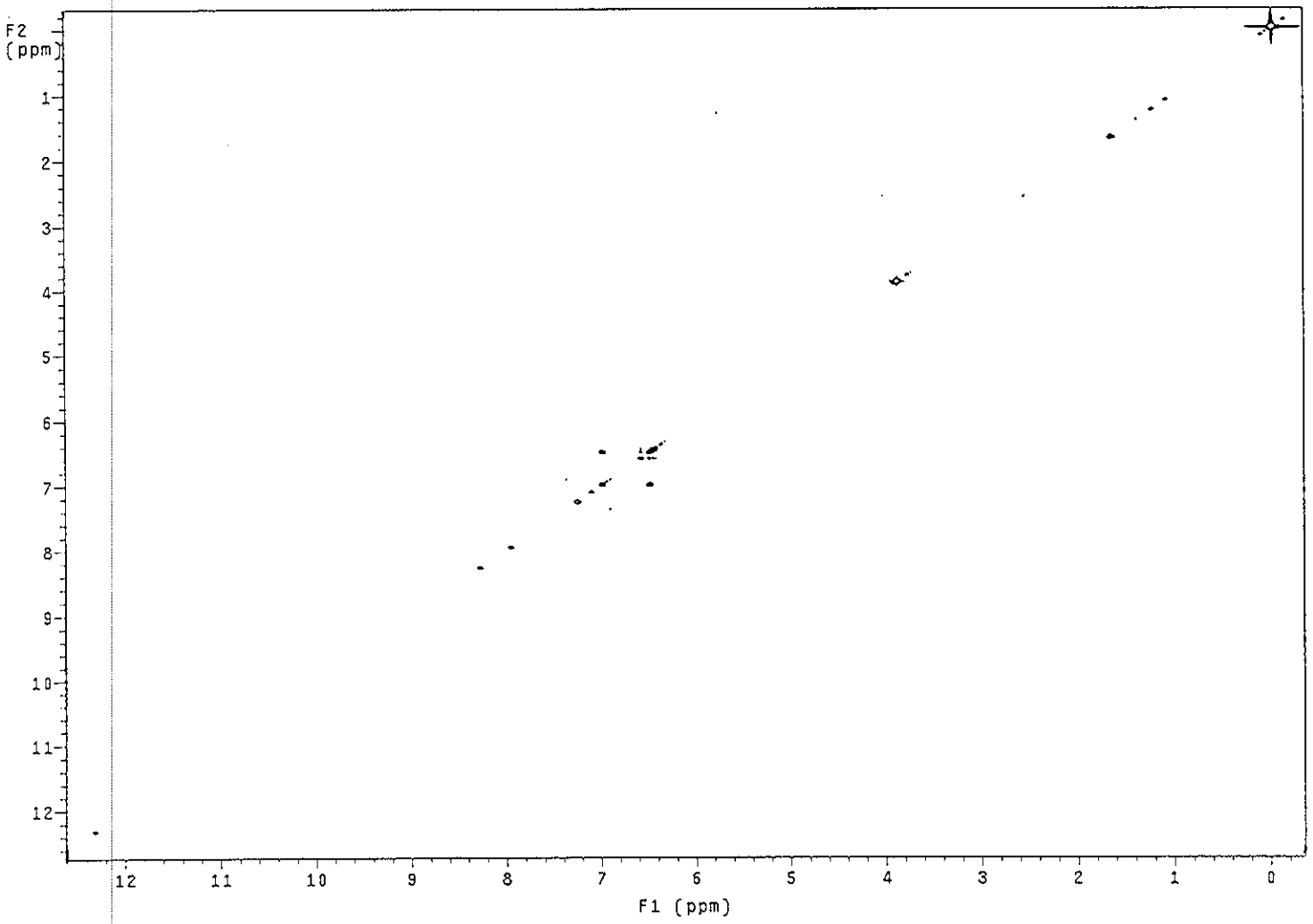


Figure 79 ^{1}H-^{1}H COSY spectrum of **DS12**

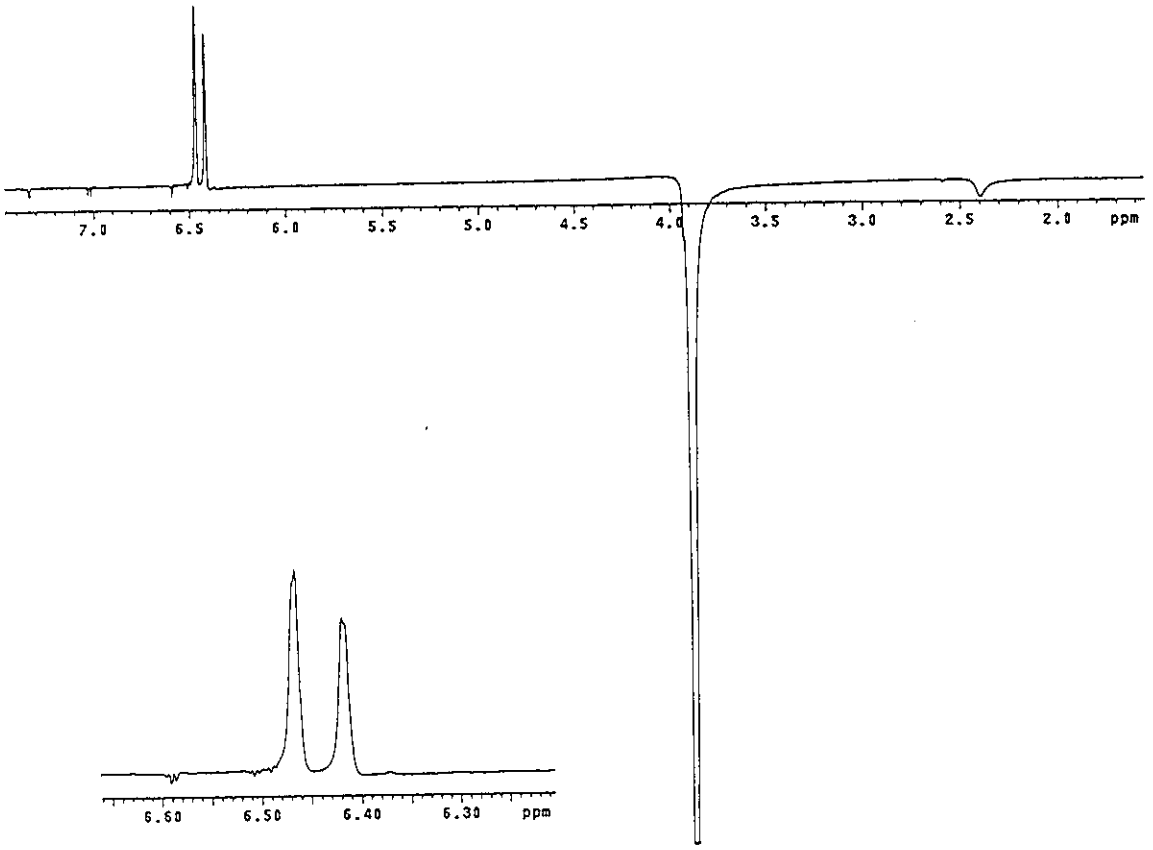


Figure 80 NOEDIFF spectrum of **DS12** after irradiation at δ_H 3.89

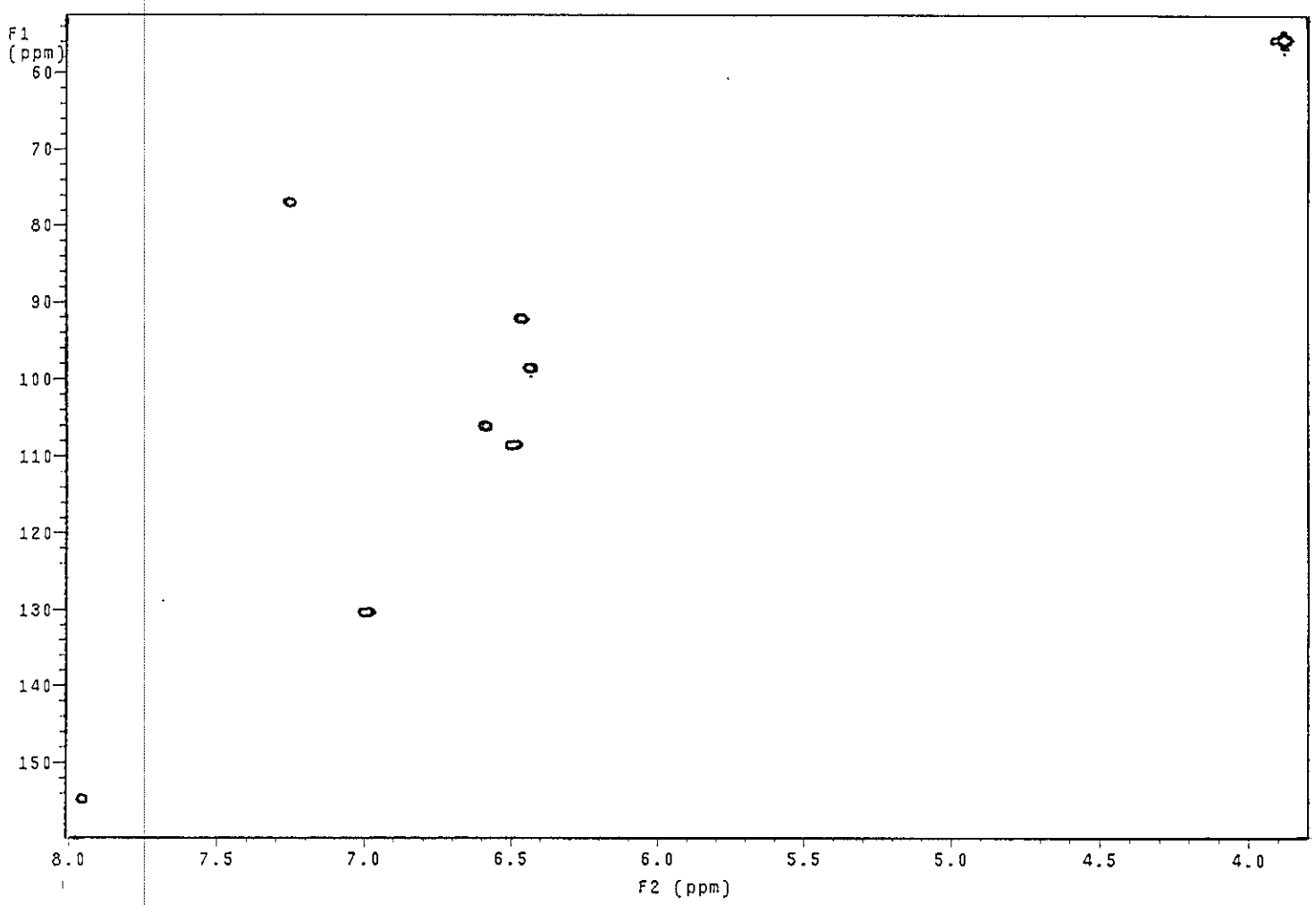


Figure 81 2D HMQC spectrum of **DS12**

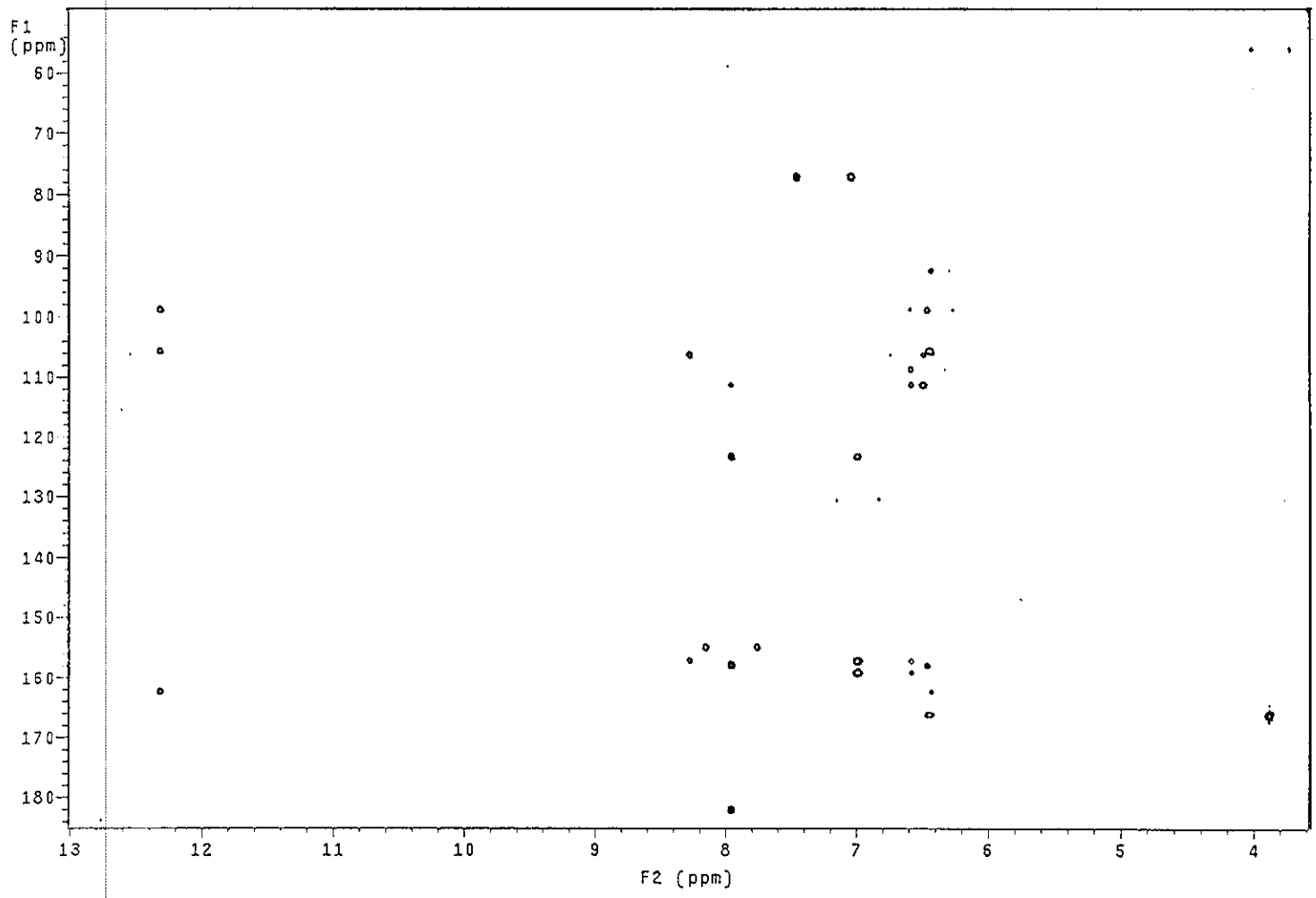


Figure 82 2D HMBC spectrum of **DS12**

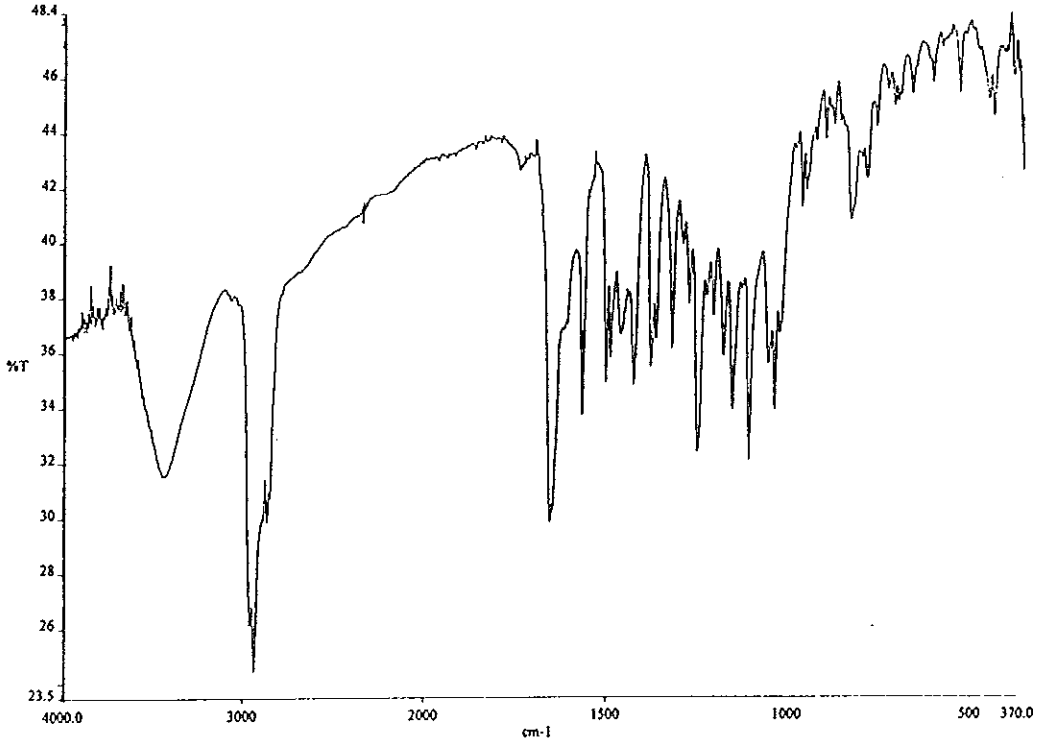


Figure 83 IR (KBr) spectrum of **DS13**

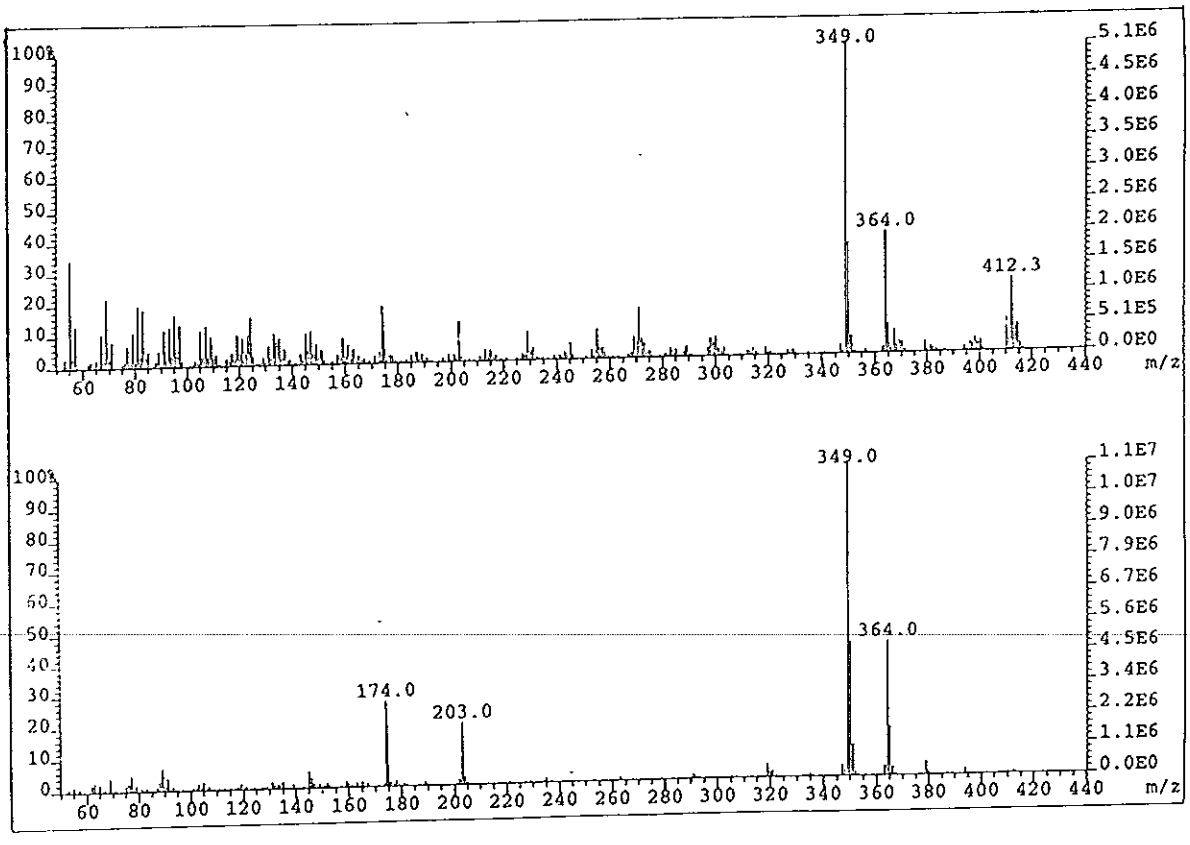


Figure 84 Mass spectrum of **DS13**

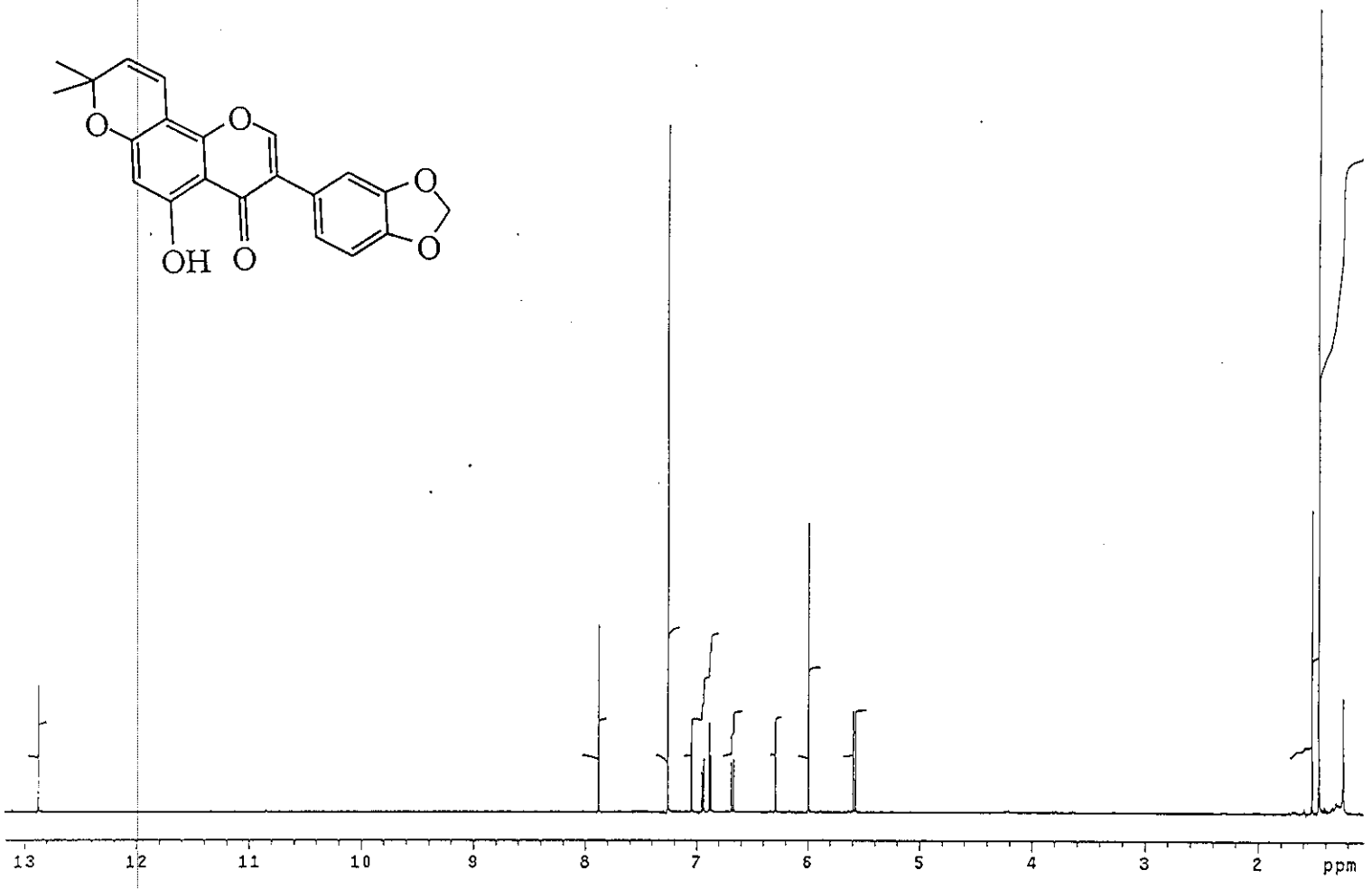


Figure 85 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS13**

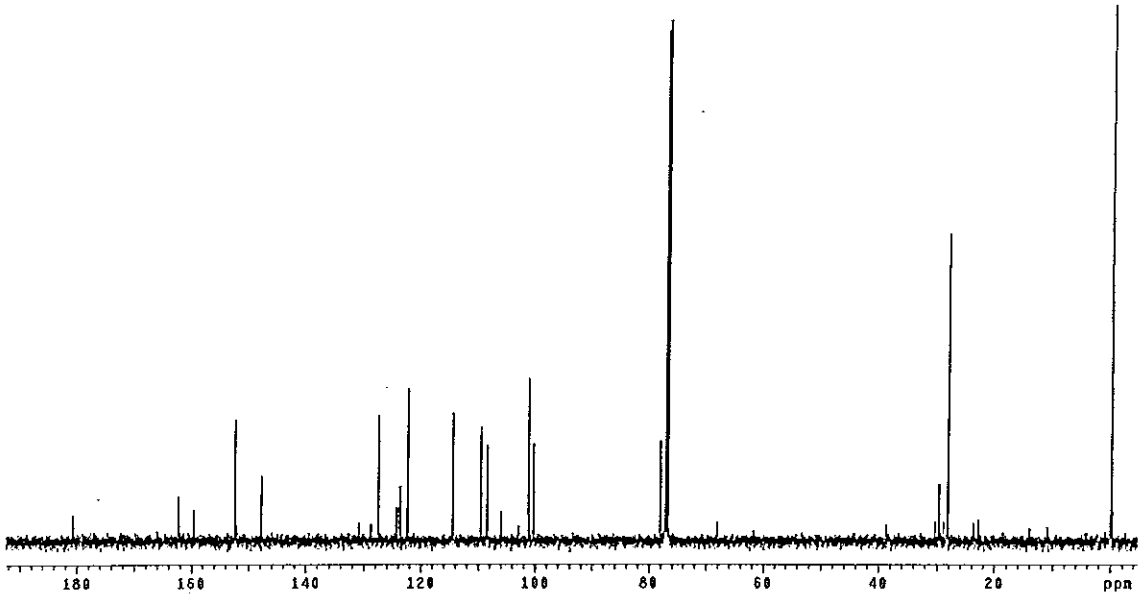


Figure 86 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **DS13**

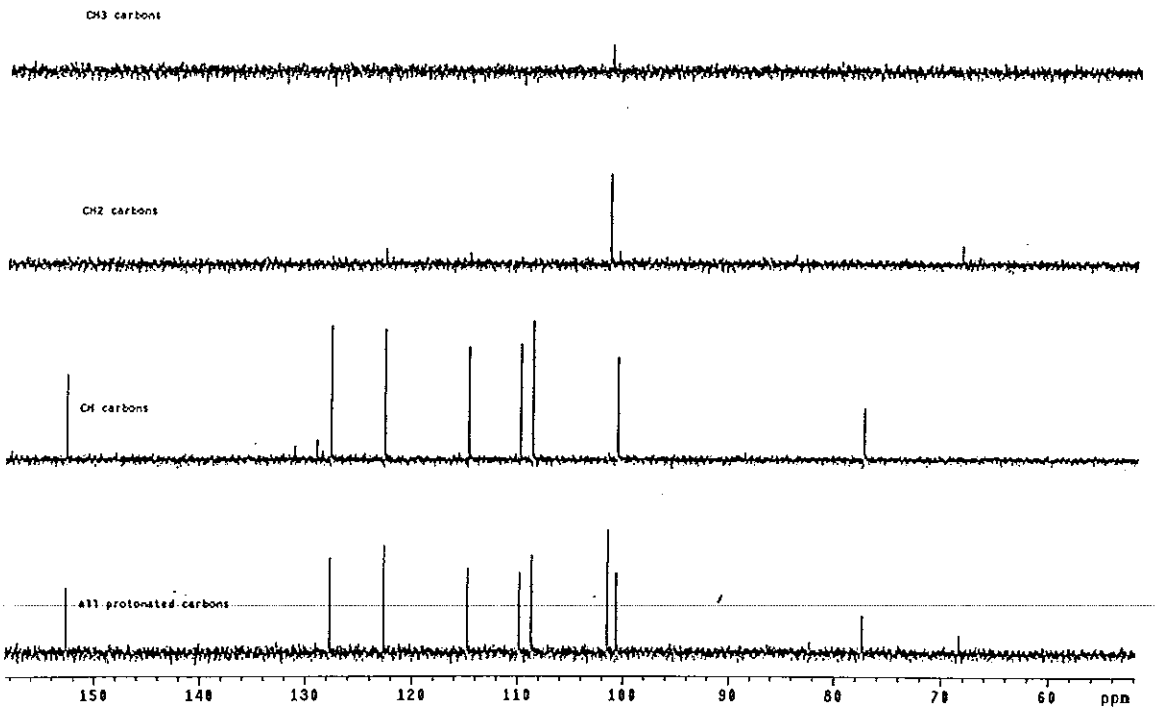


Figure 87 DEPT (135°) ($CDCl_3$) spectrum of **DS13**

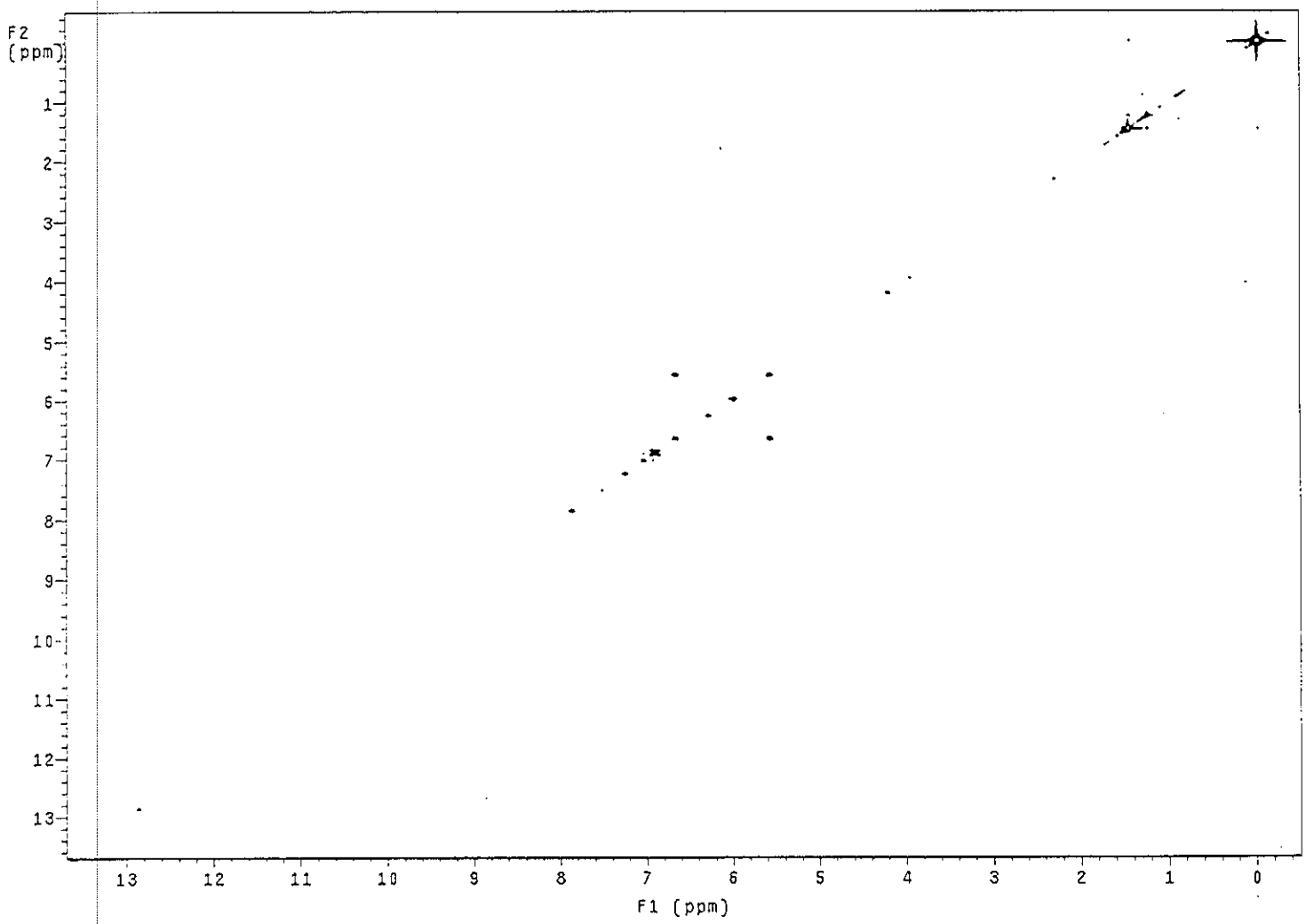


Figure 88 ^{1}H-^{1}H COSY spectrum of **DS13**

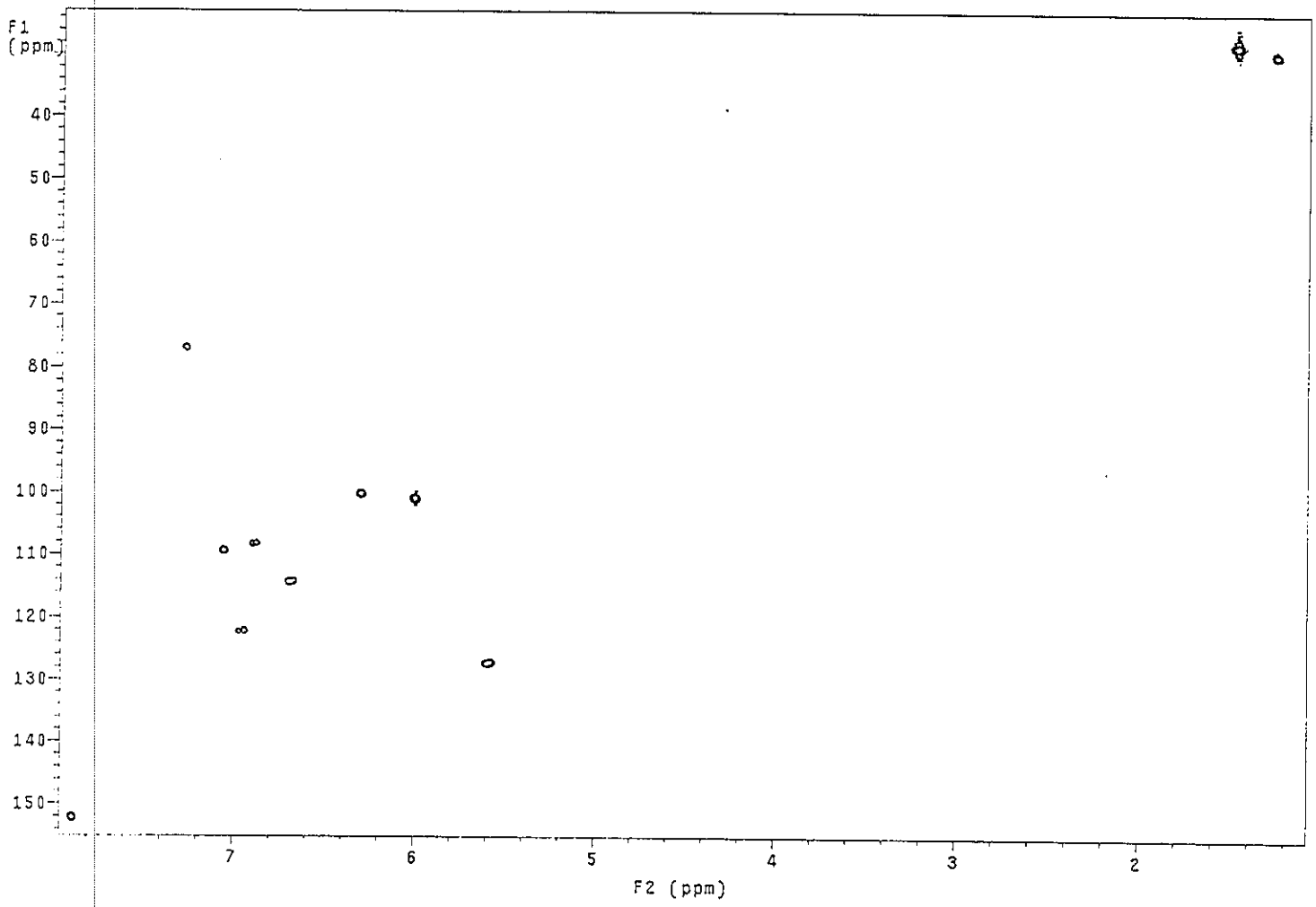


Figure 89 2D HMQC spectrum of **DS13**

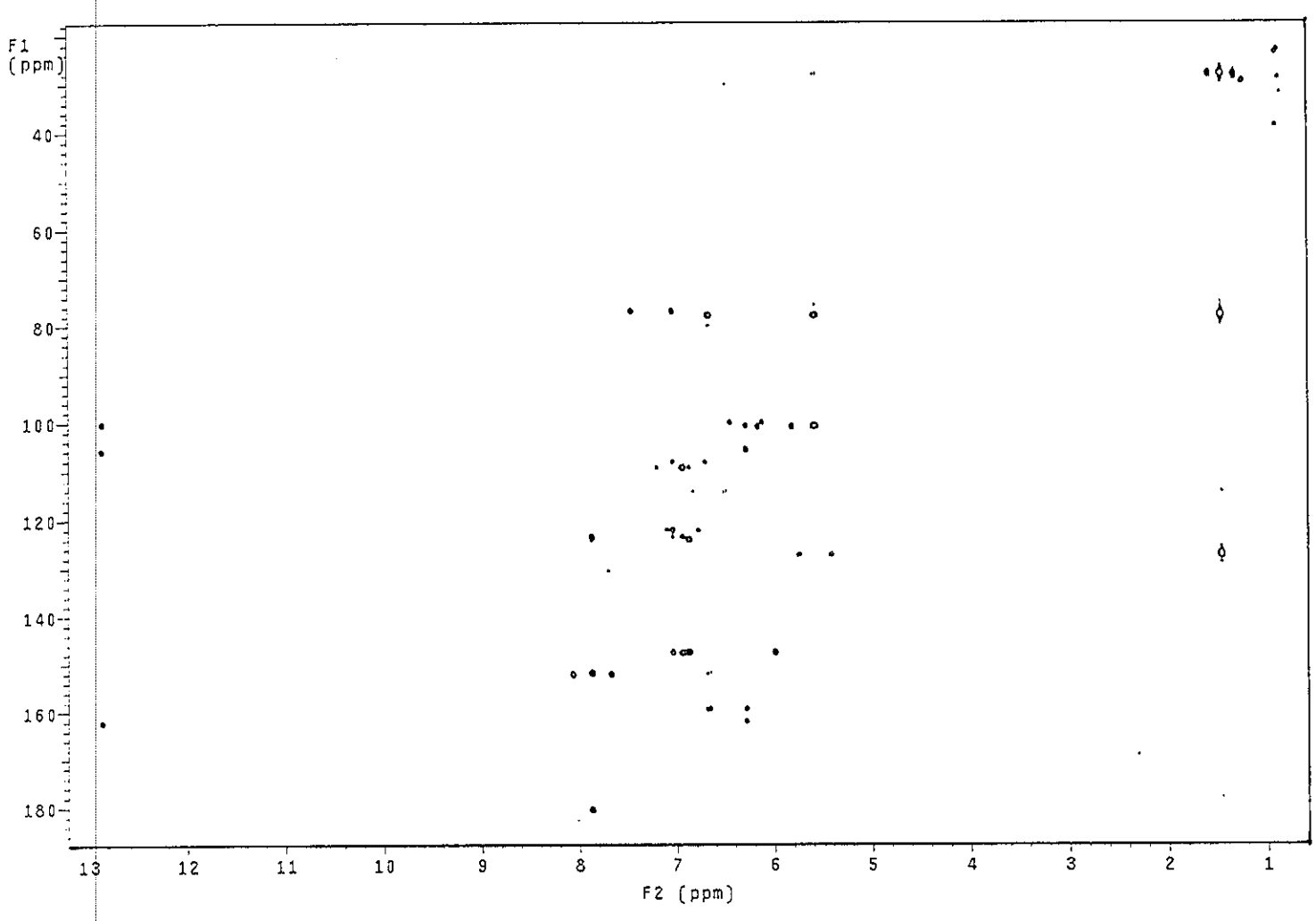


Figure 90 2D HMBC spectrum of **DS13**

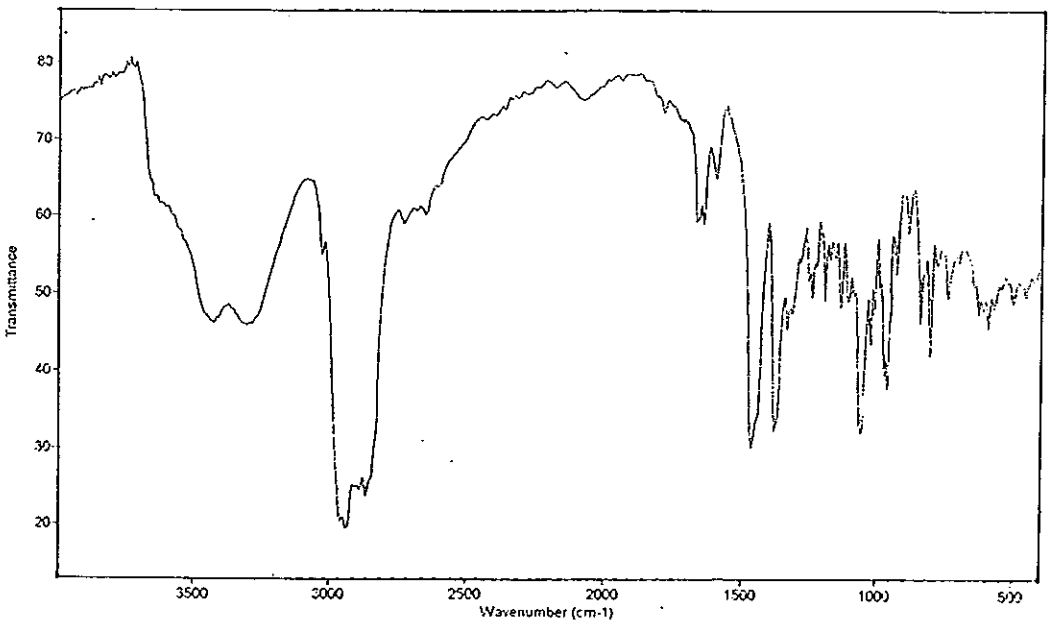


Figure 91 IR (KBr) spectrum of **DS15**

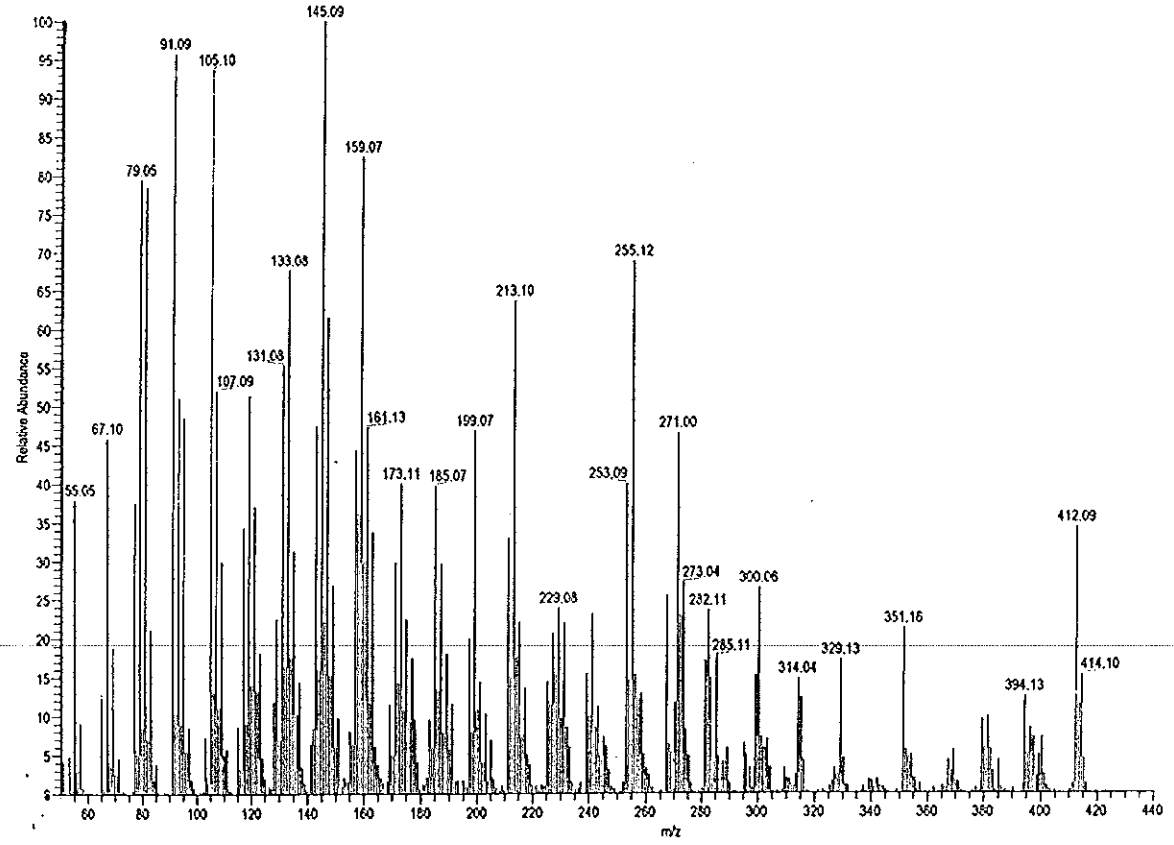


Figure 92 Mass spectrum of **DS15**

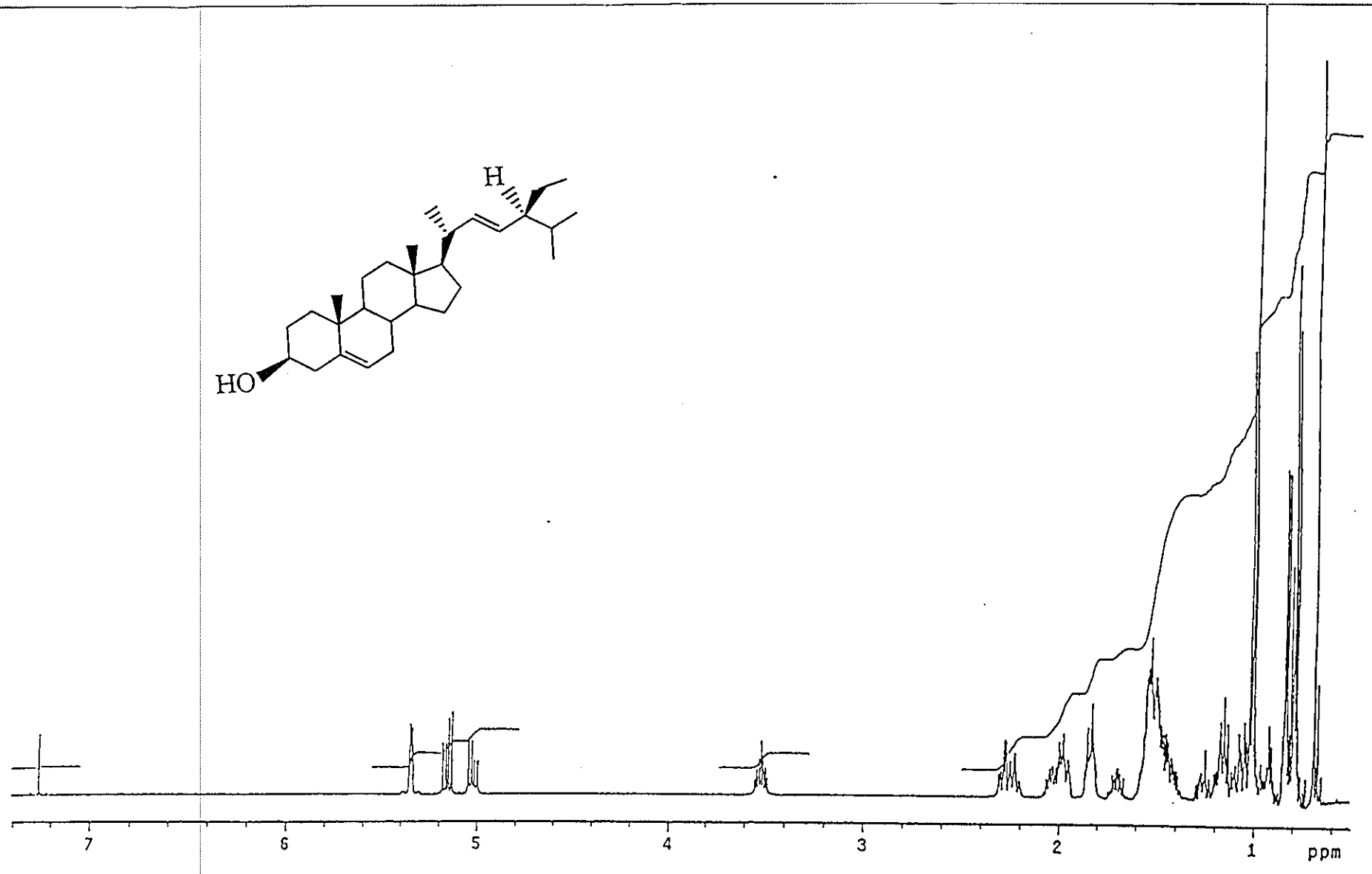


Figure 93 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS15**

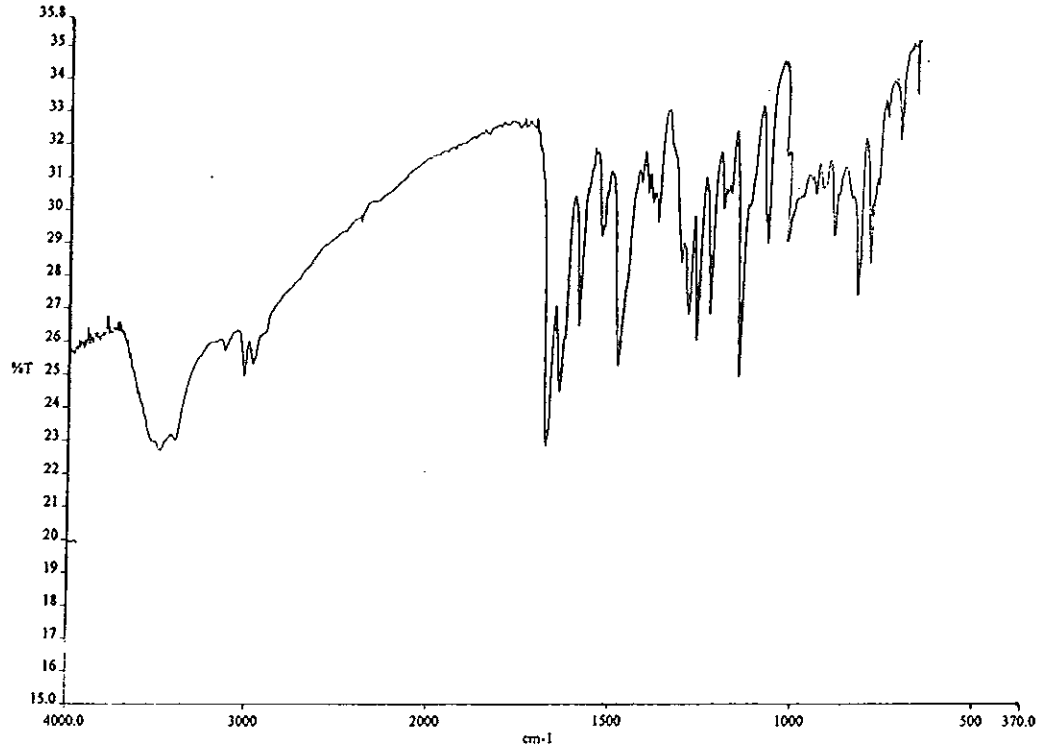


Figure 94 IR (KBr) spectrum of **DS16**

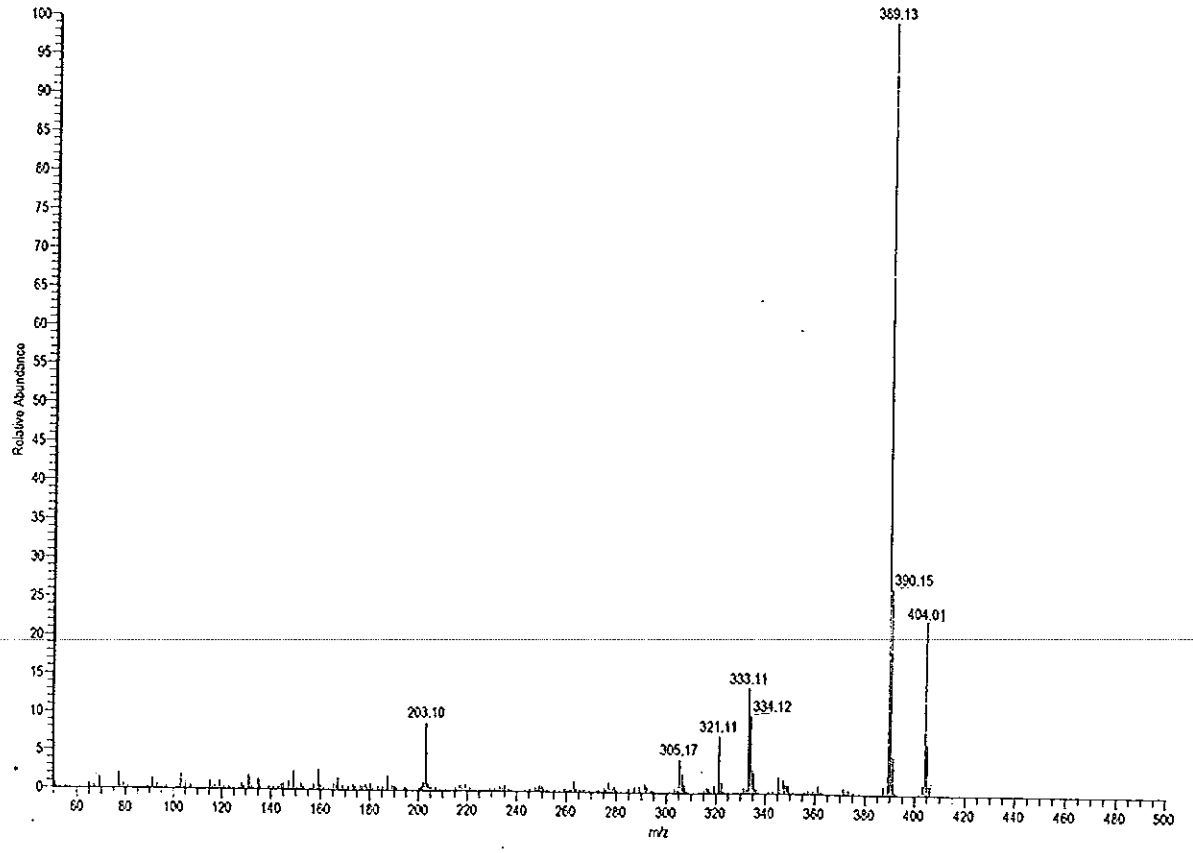


Figure 95 Mass spectrum of **DS16**

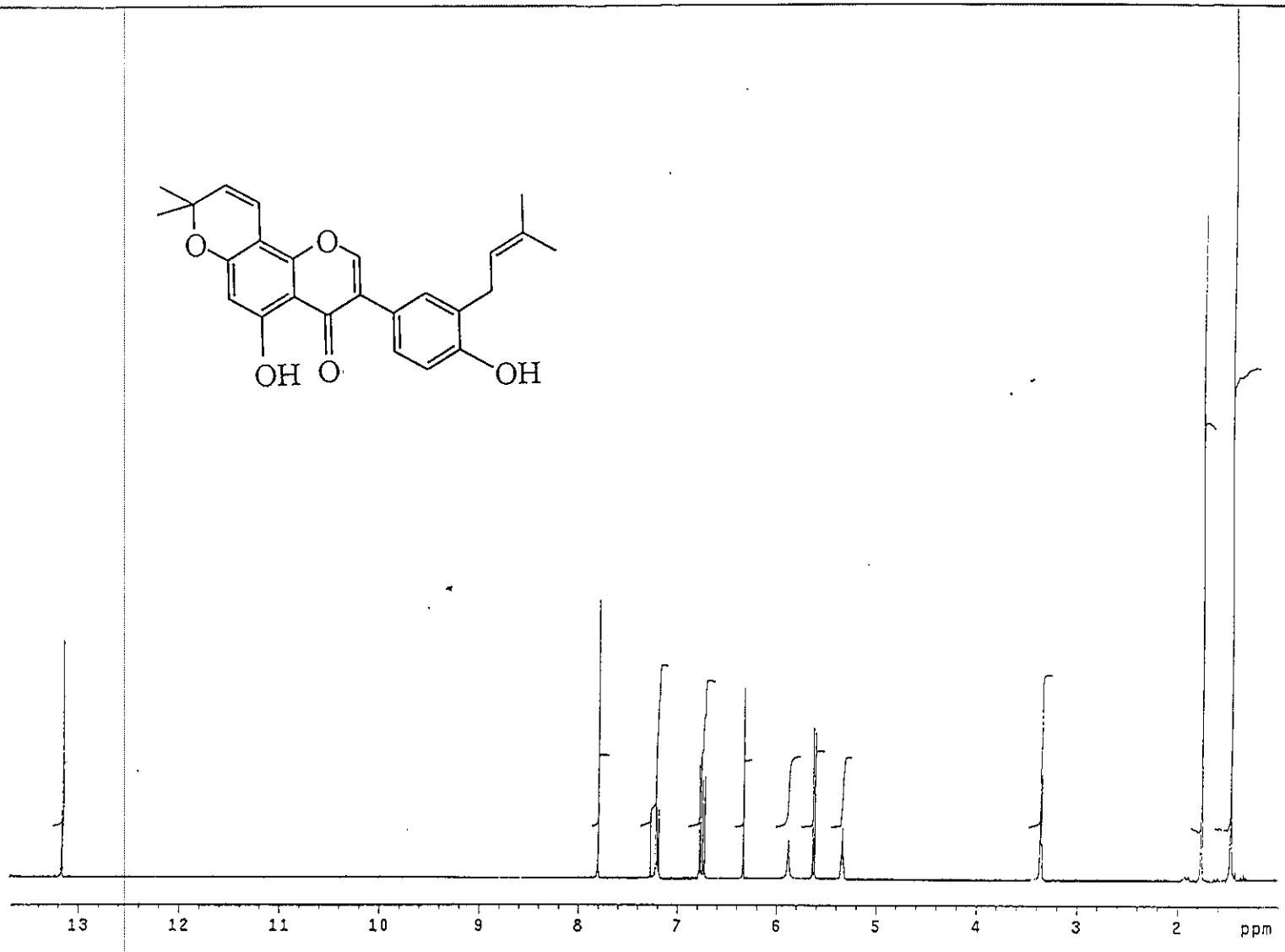


Figure 96 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS16**

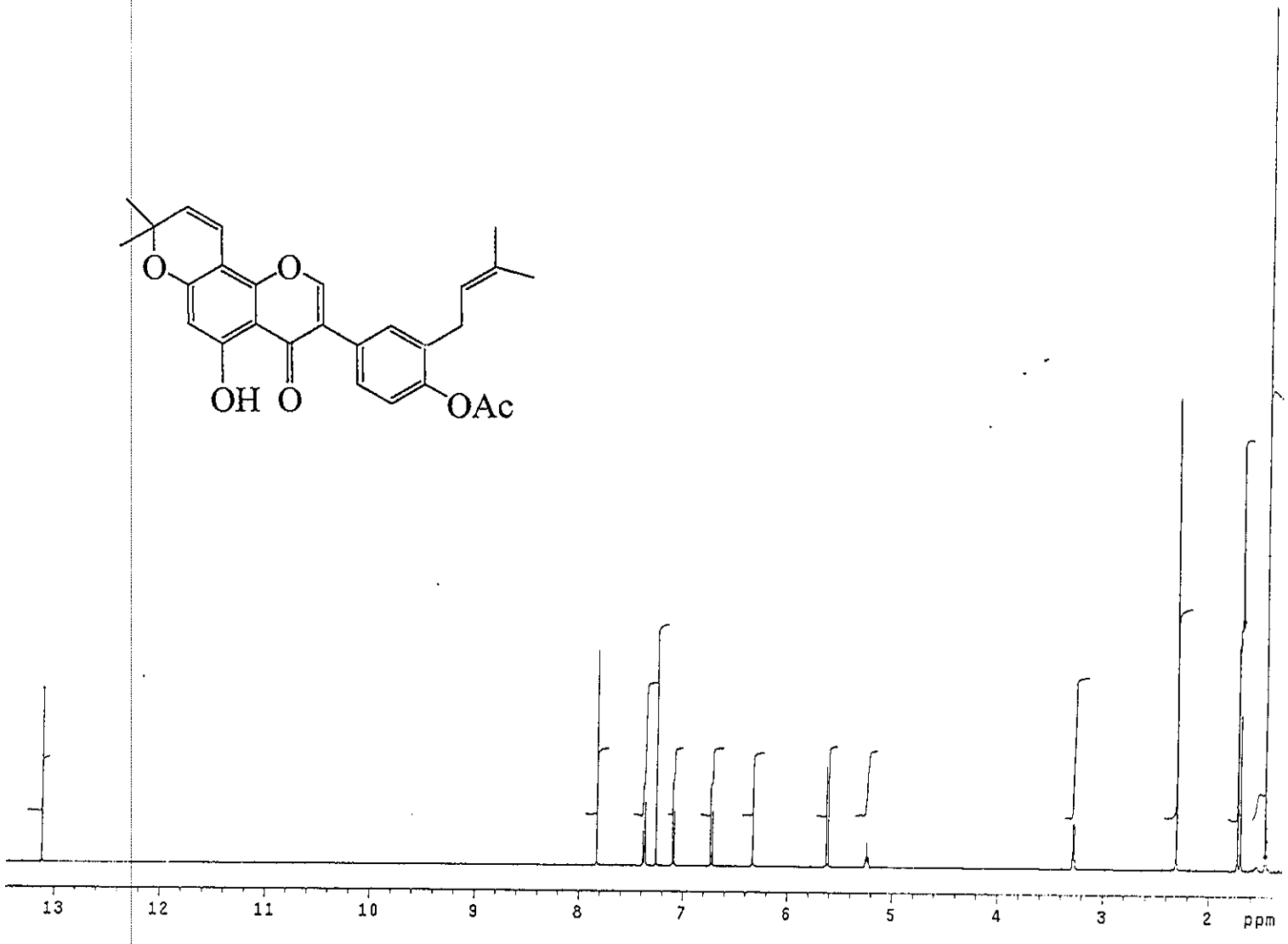


Figure 97 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS16(A)**

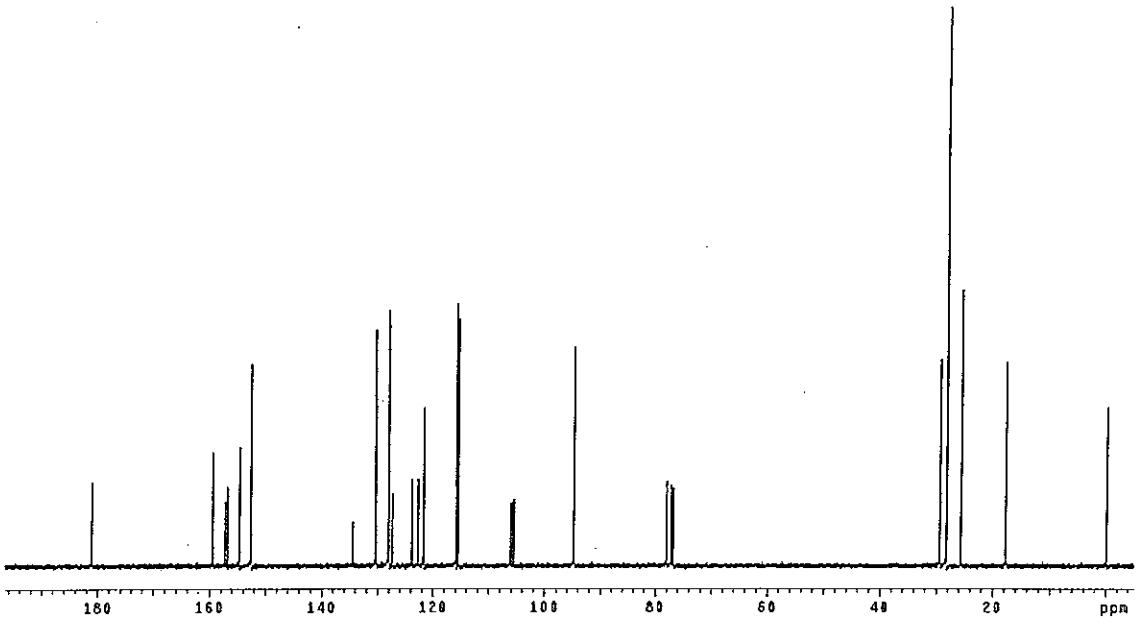


Figure 98 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **DS16**

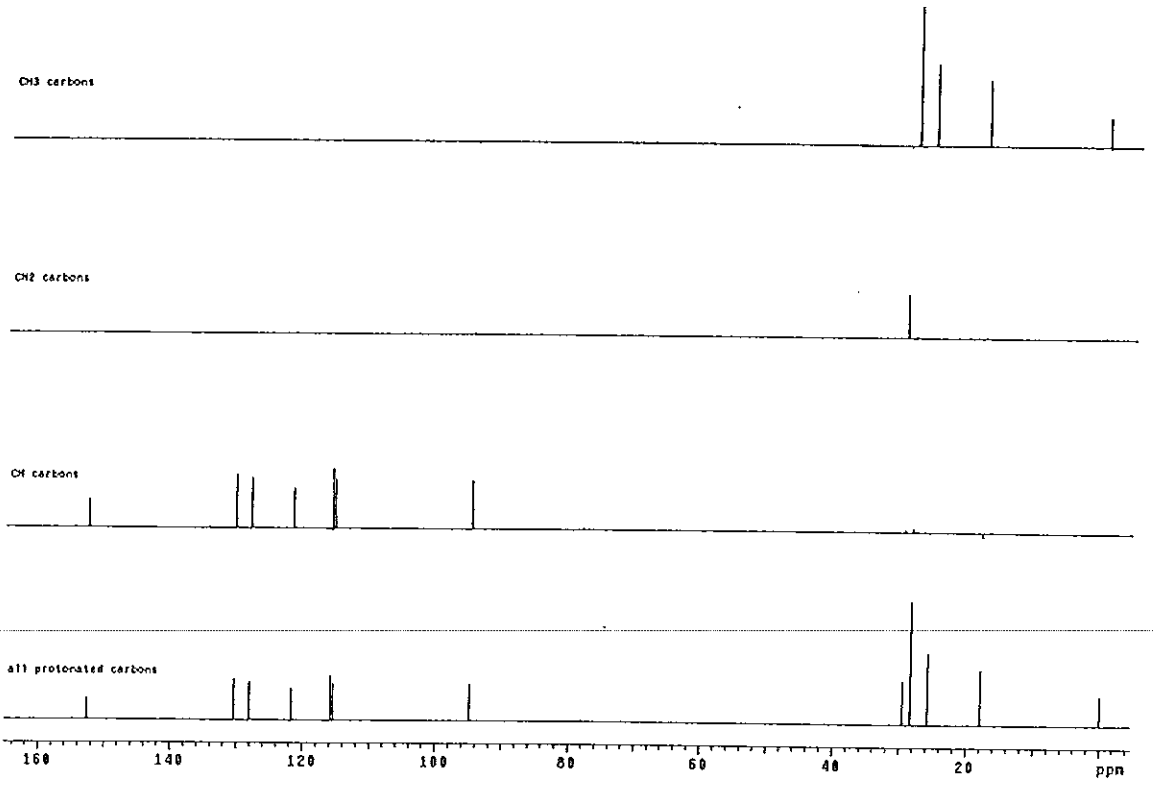


Figure 99 DEPT (135°) ($CDCl_3$) spectrum of **DS16**

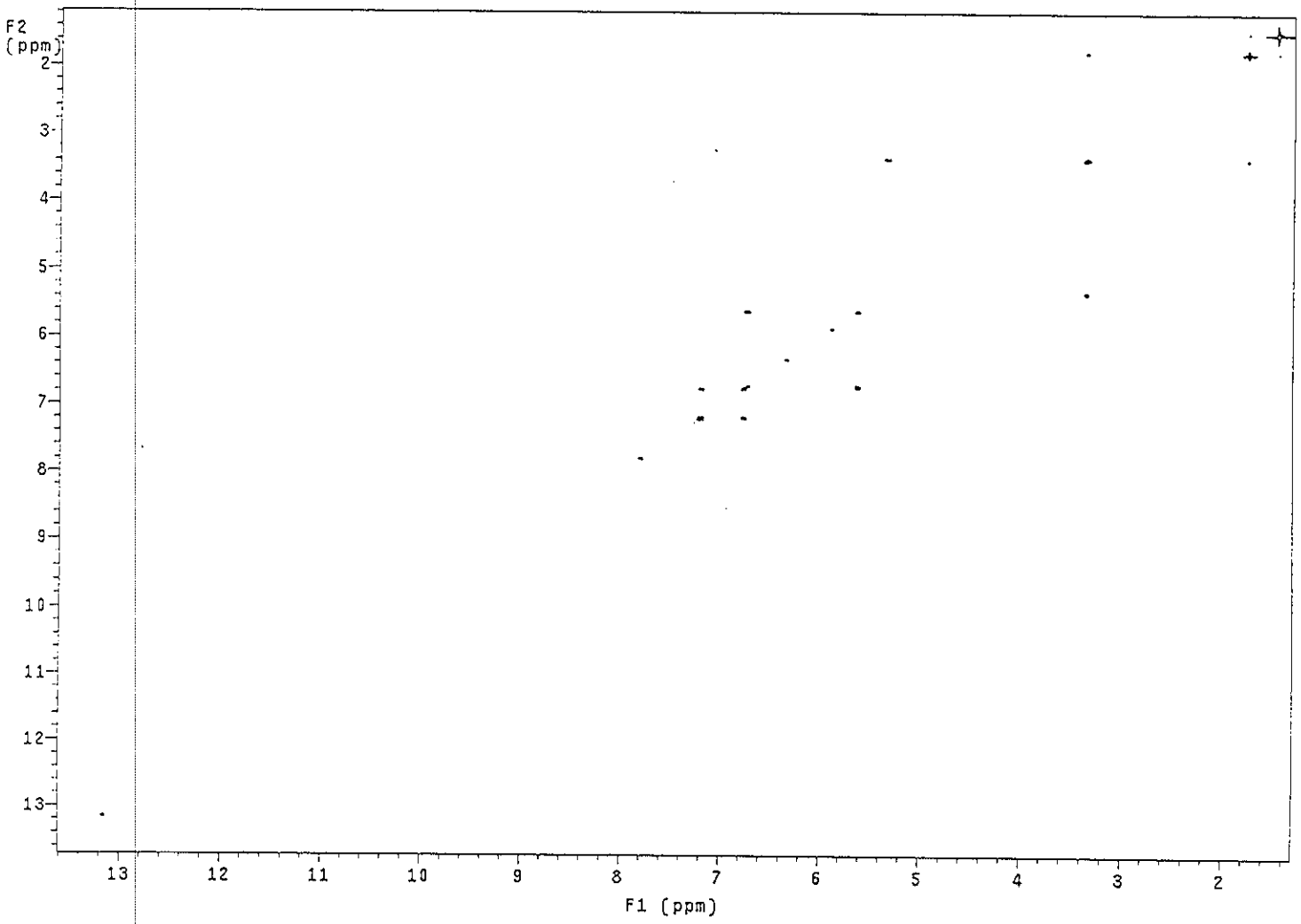


Figure 100 ^{1}H-^{1}H COSY spectrum of **DS16**

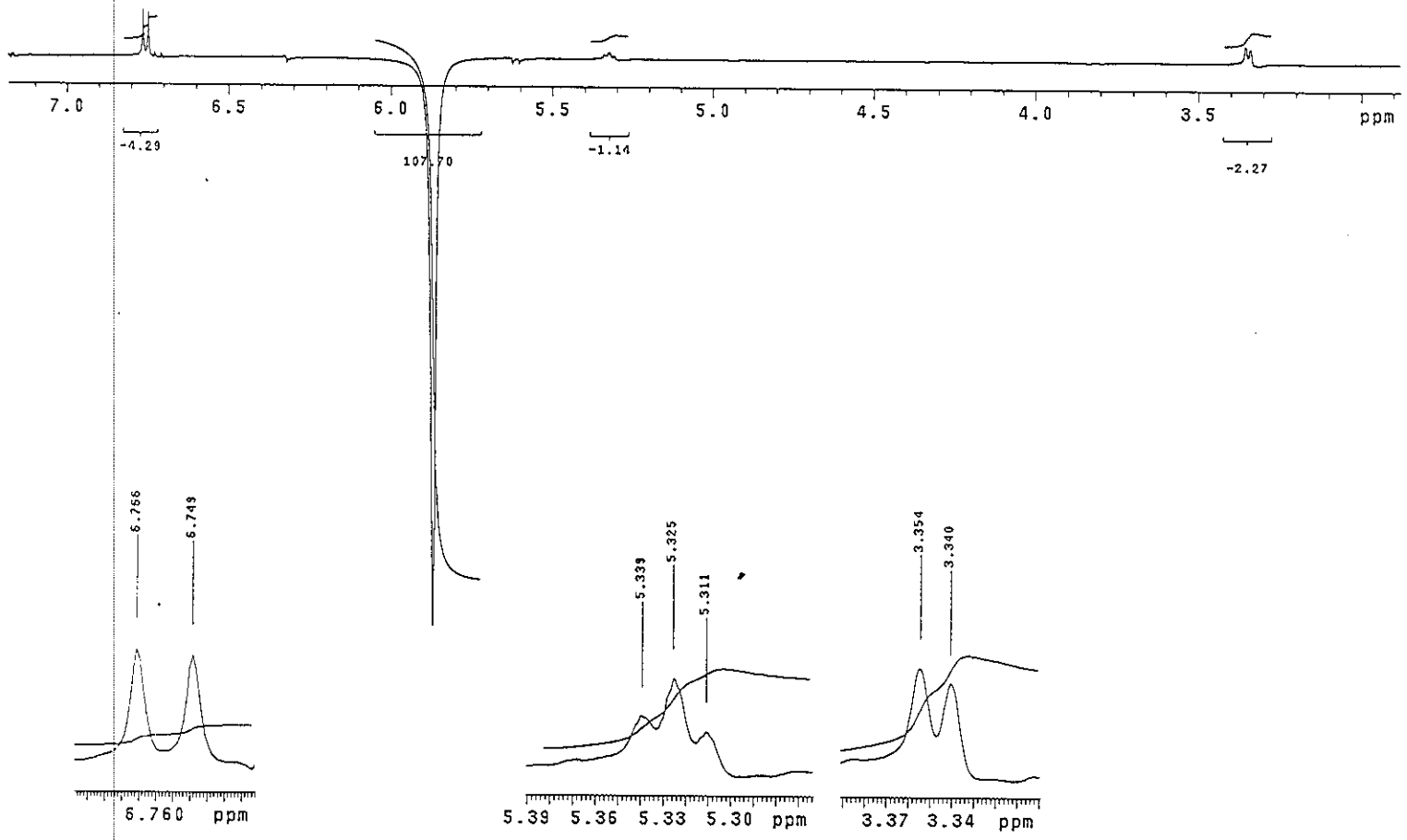


Figure 101 NOEDIFF spectrum of **DS16** after irradiation at δ_H 5.88

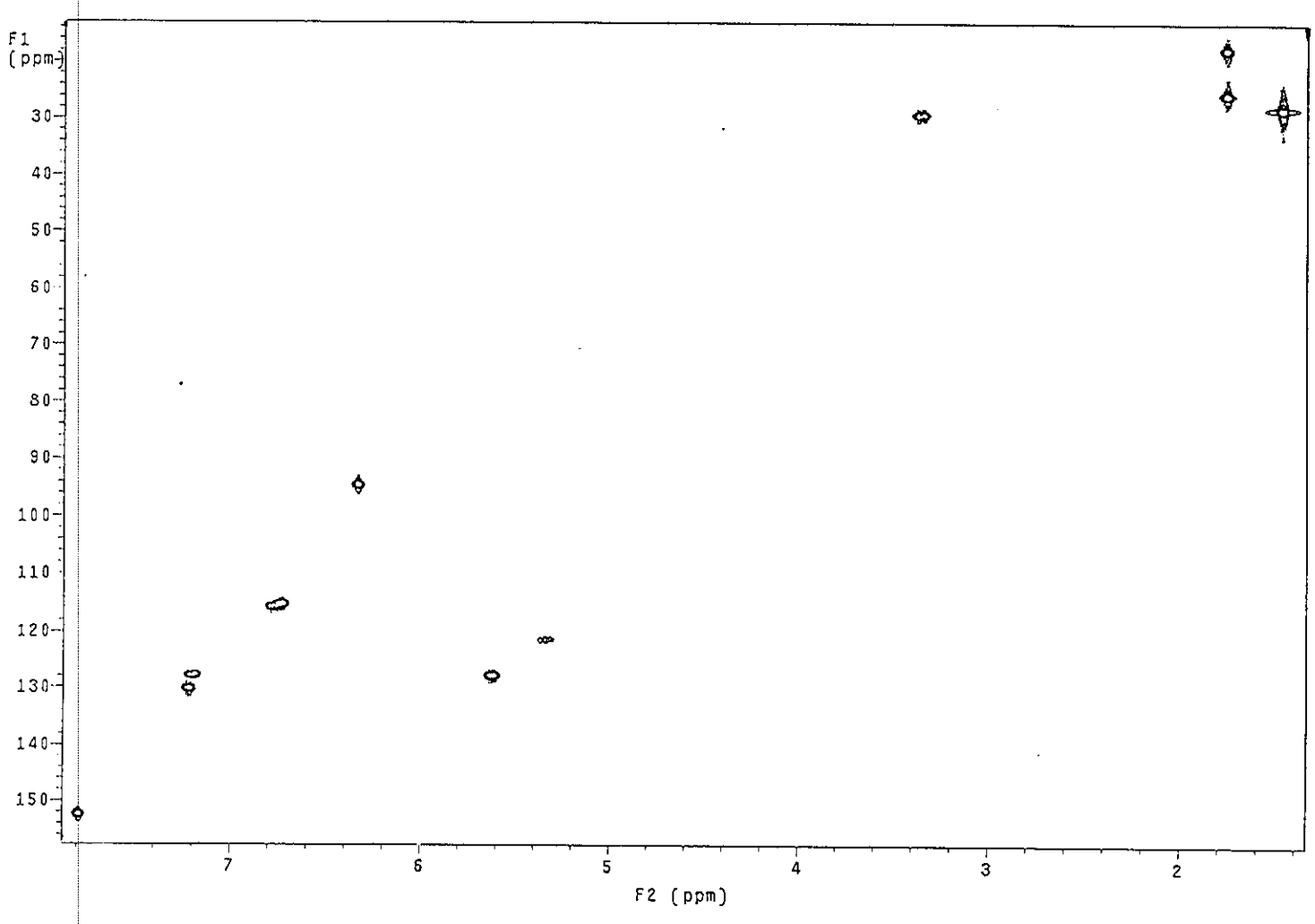


Figure 102 2D HMQC spectrum of **DS16**

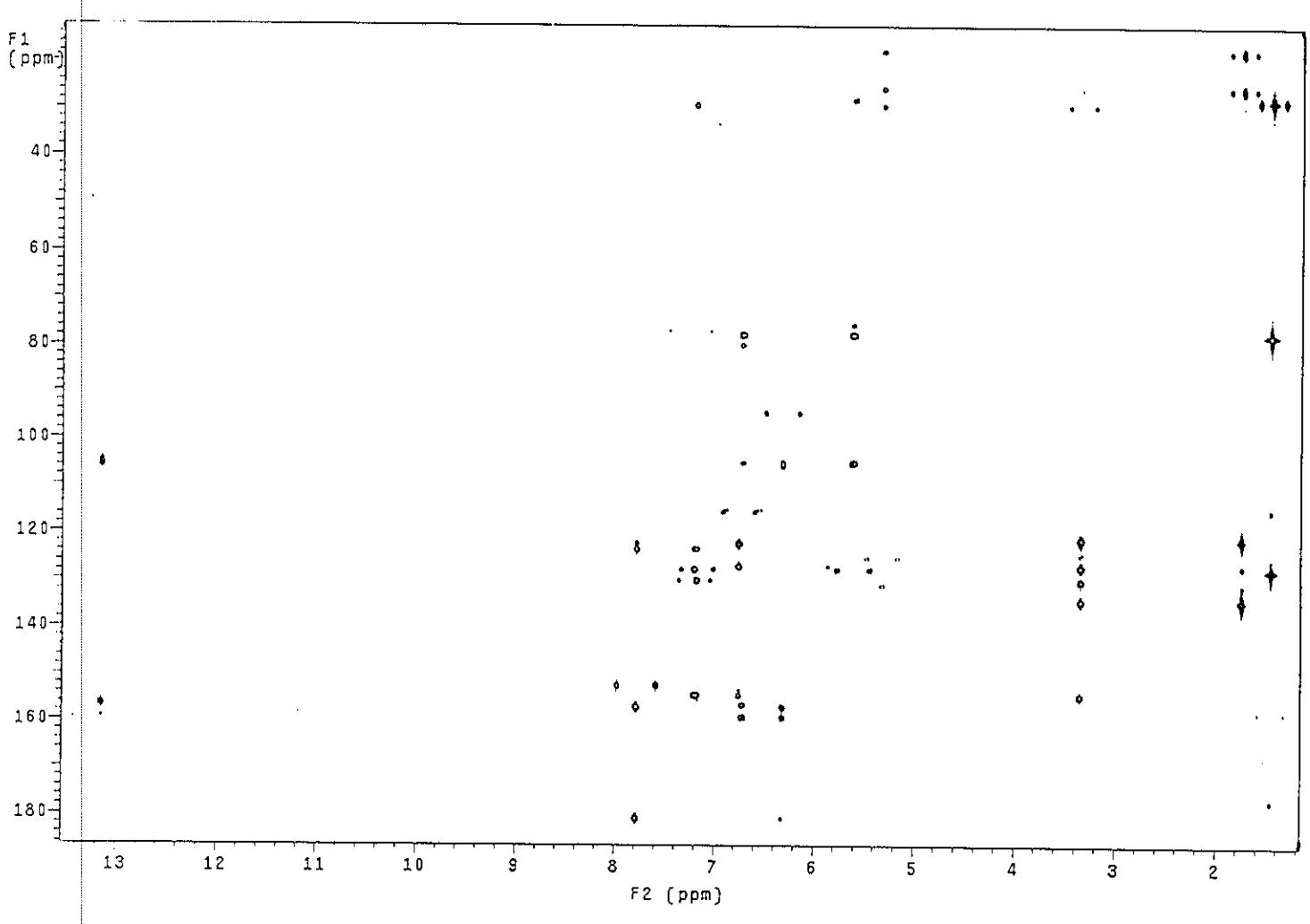


Figure 103 2D HMBC spectrum of **DS16**

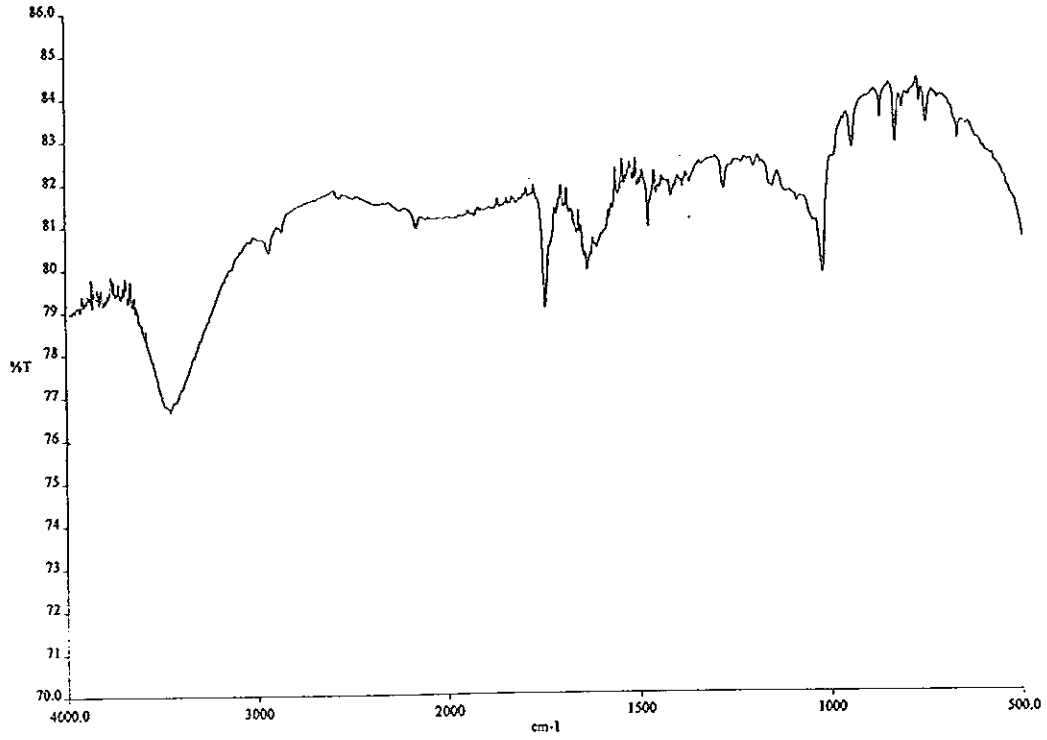


Figure 104 IR (KBr) spectrum of **DS18**

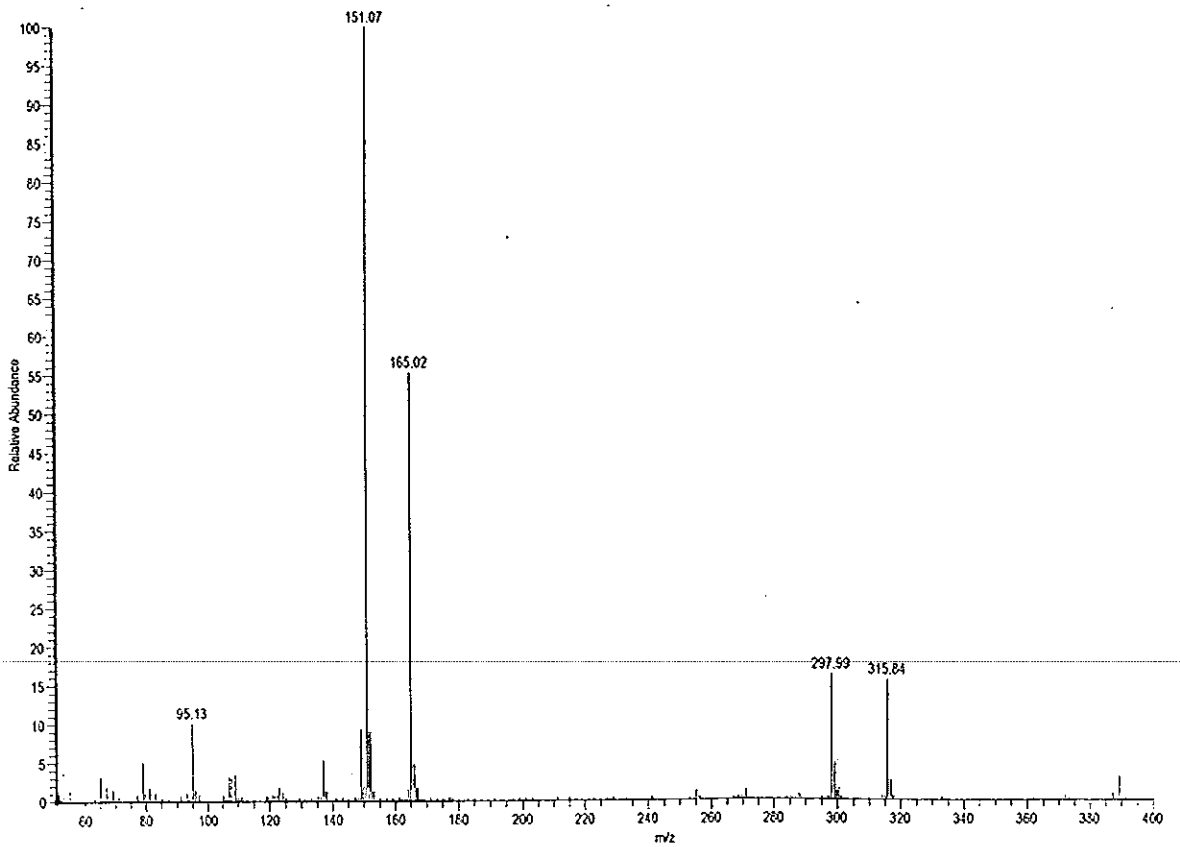


Figure 105 Mass spectrum of **DS18**

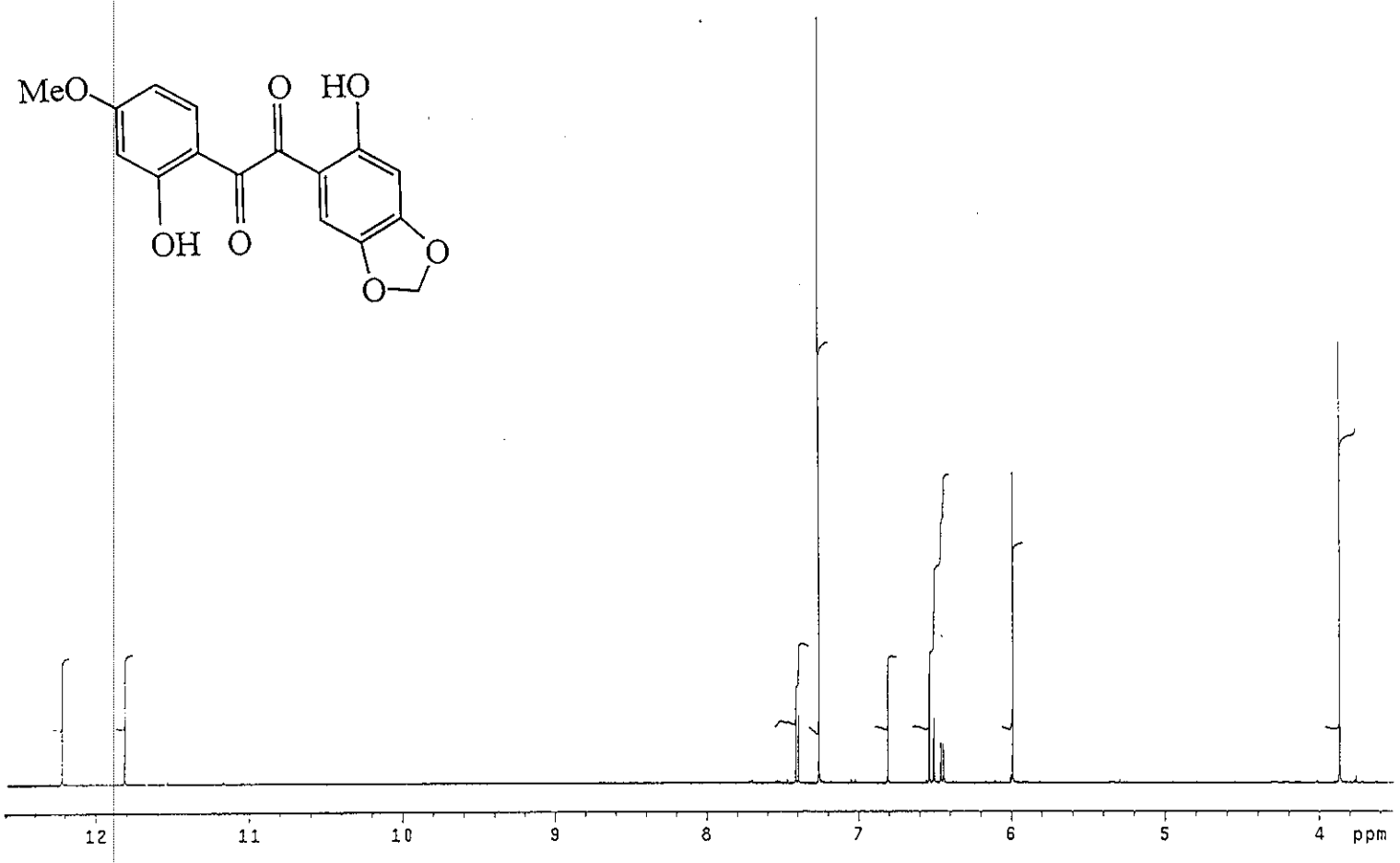


Figure 106 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS18**

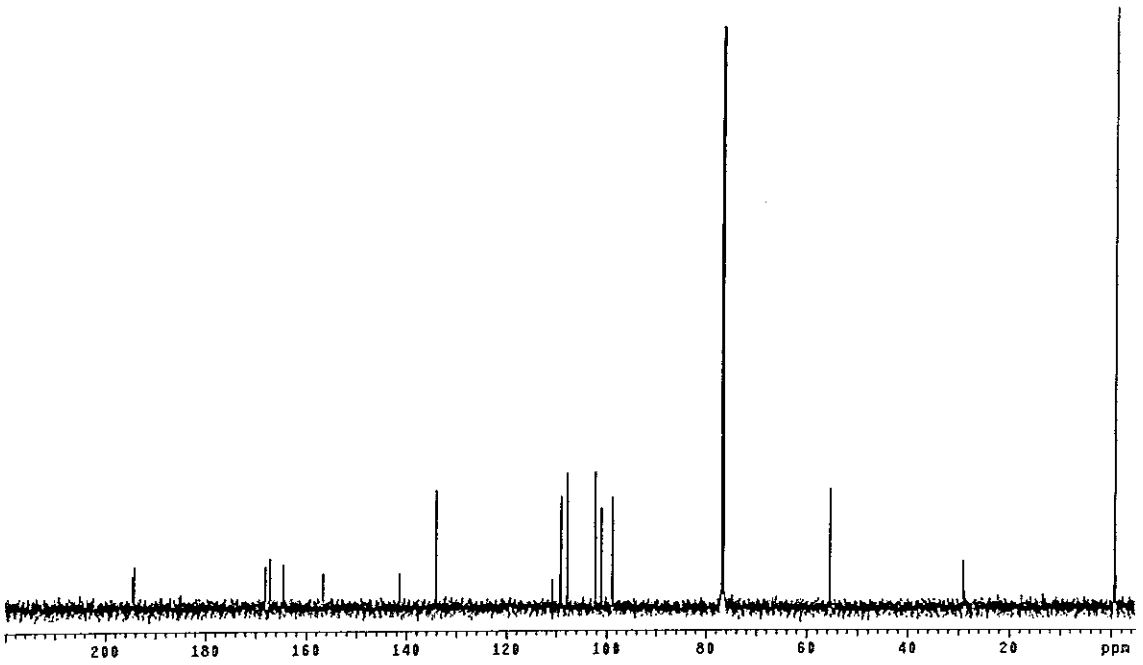


Figure 107 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **DS18**

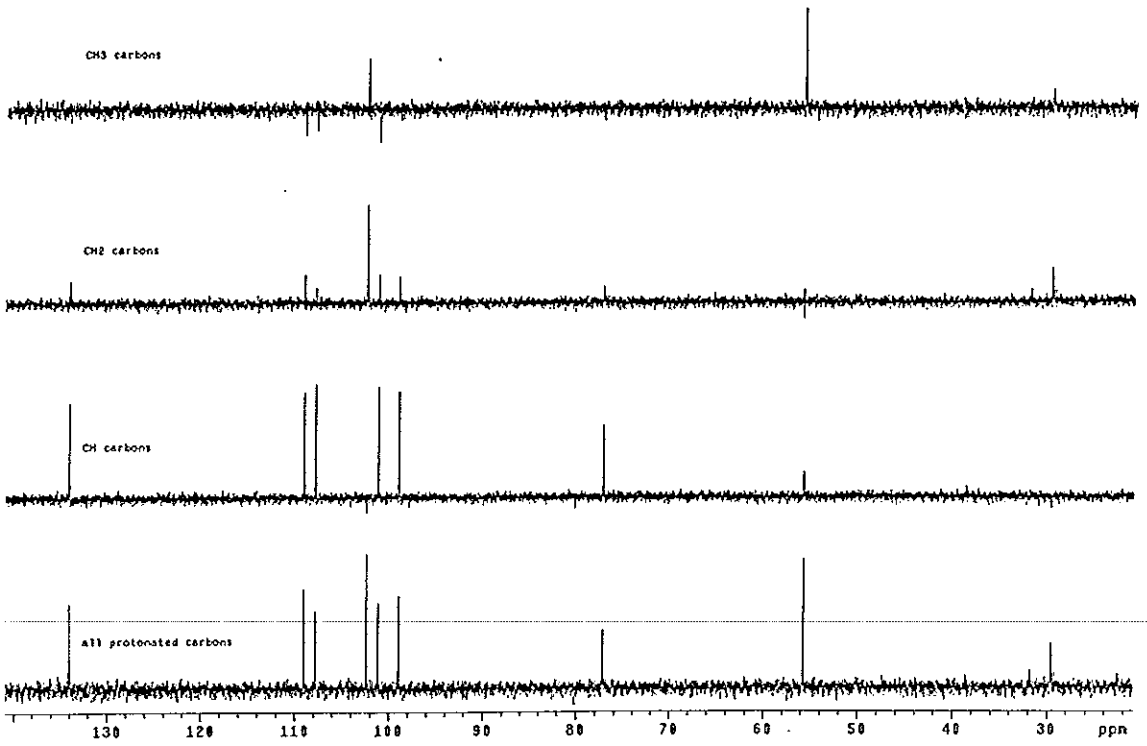


Figure 108 DEPT (135°) ($CDCl_3$) spectrum of **DS18**

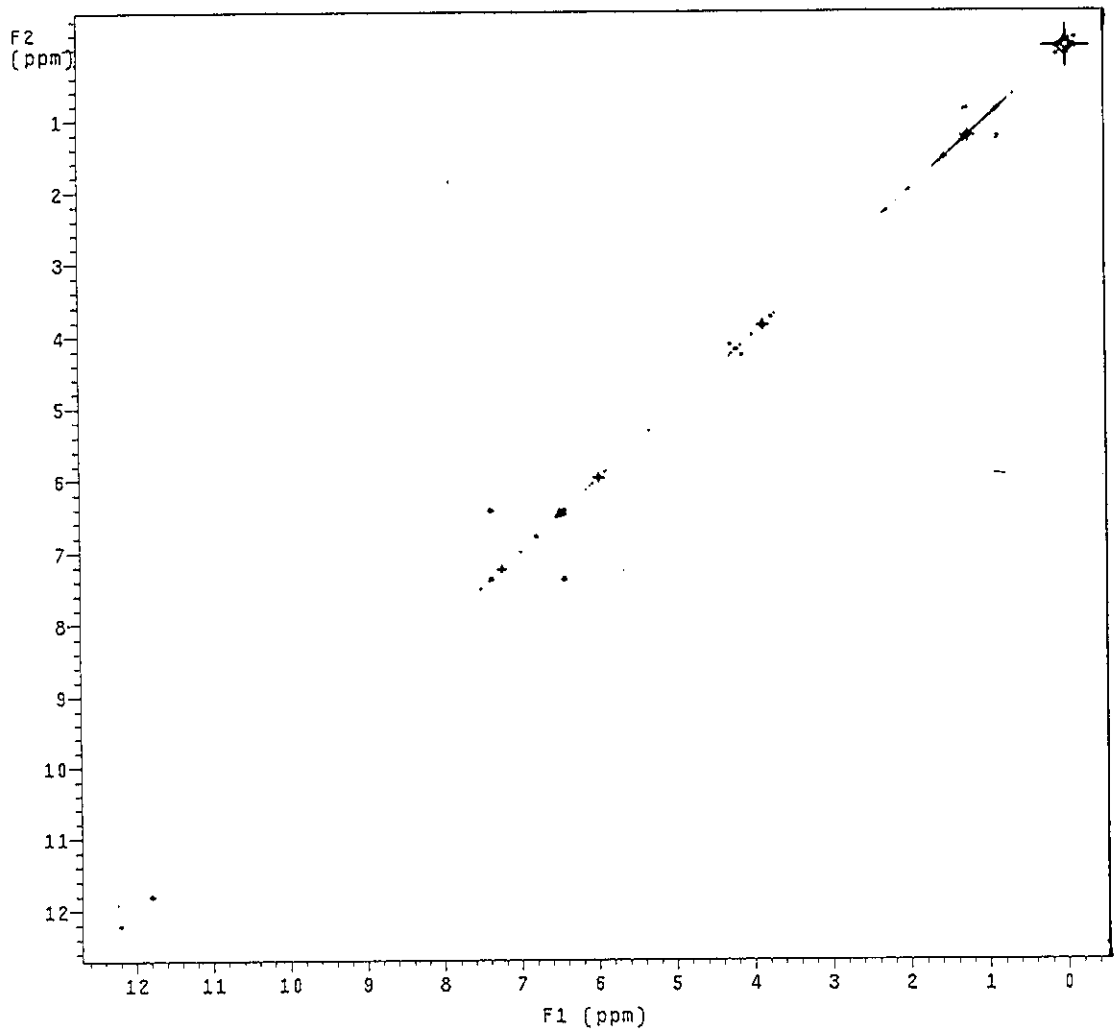


Figure 109 ^{1}H-^{1}H COSY spectrum of **DS18**

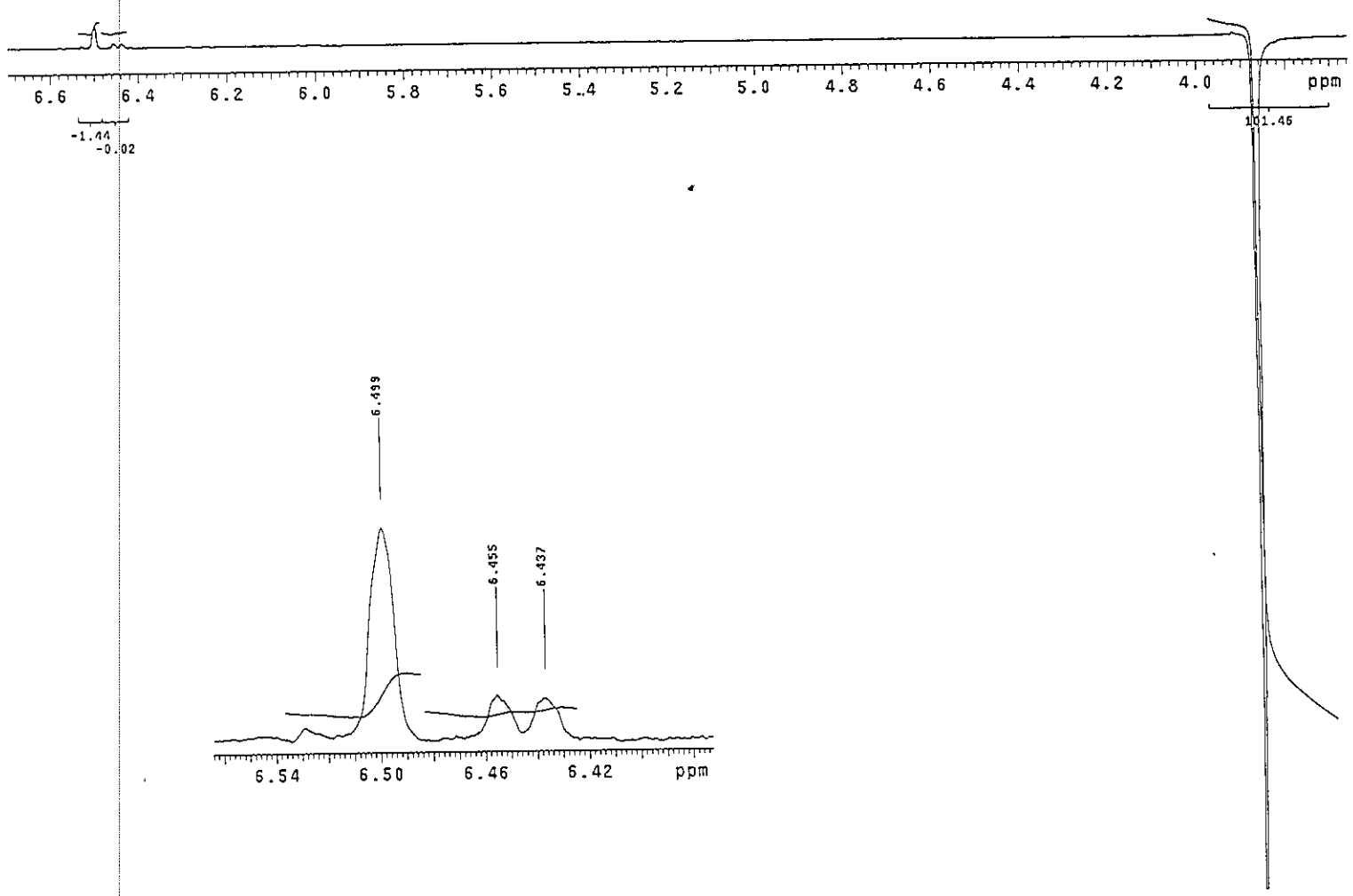


Figure 110 NOEDIFF spectrum of **DS18** after irradiation at δ_H 3.87

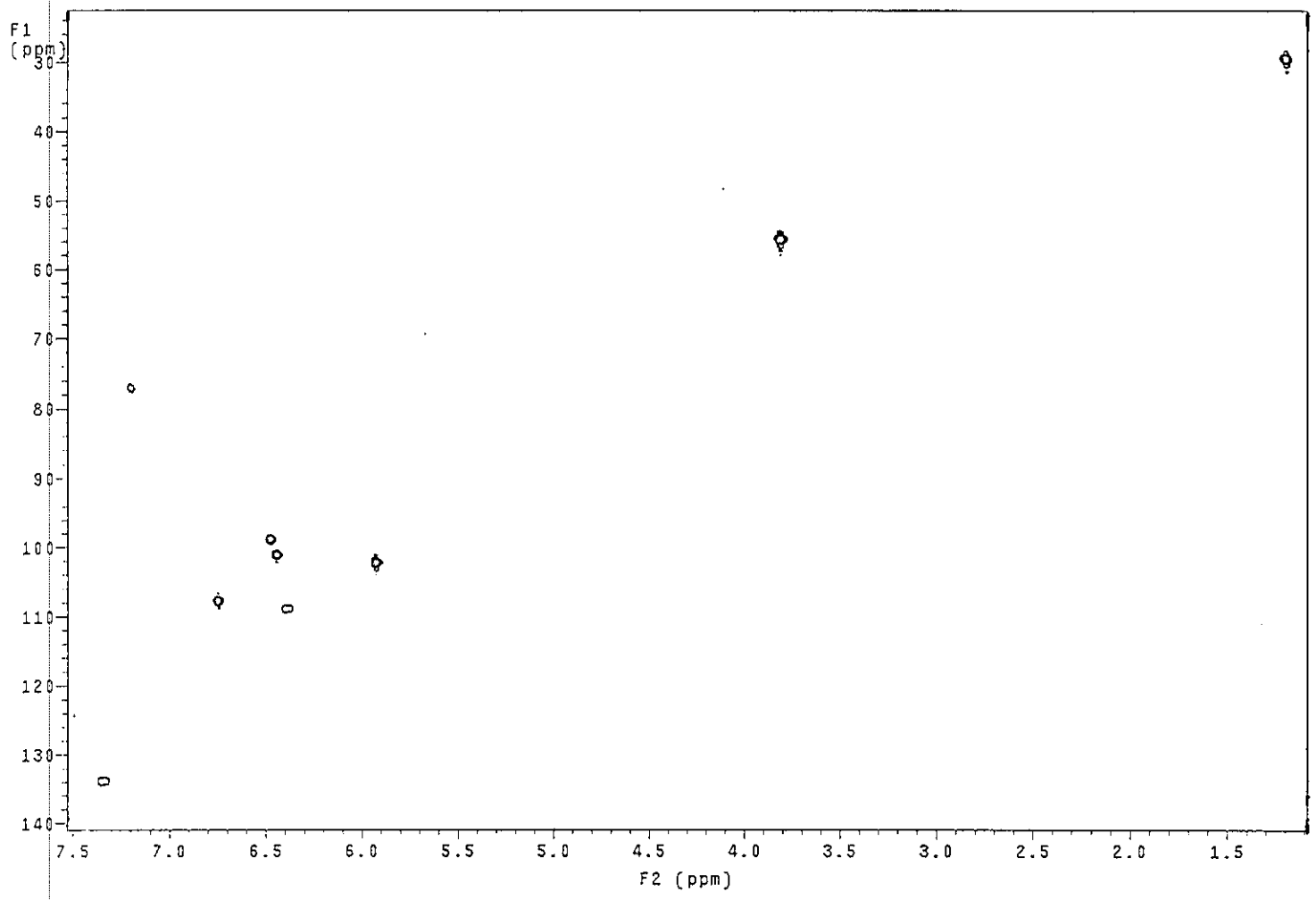


Figure 111 2D HMQC spectrum of **DS18**

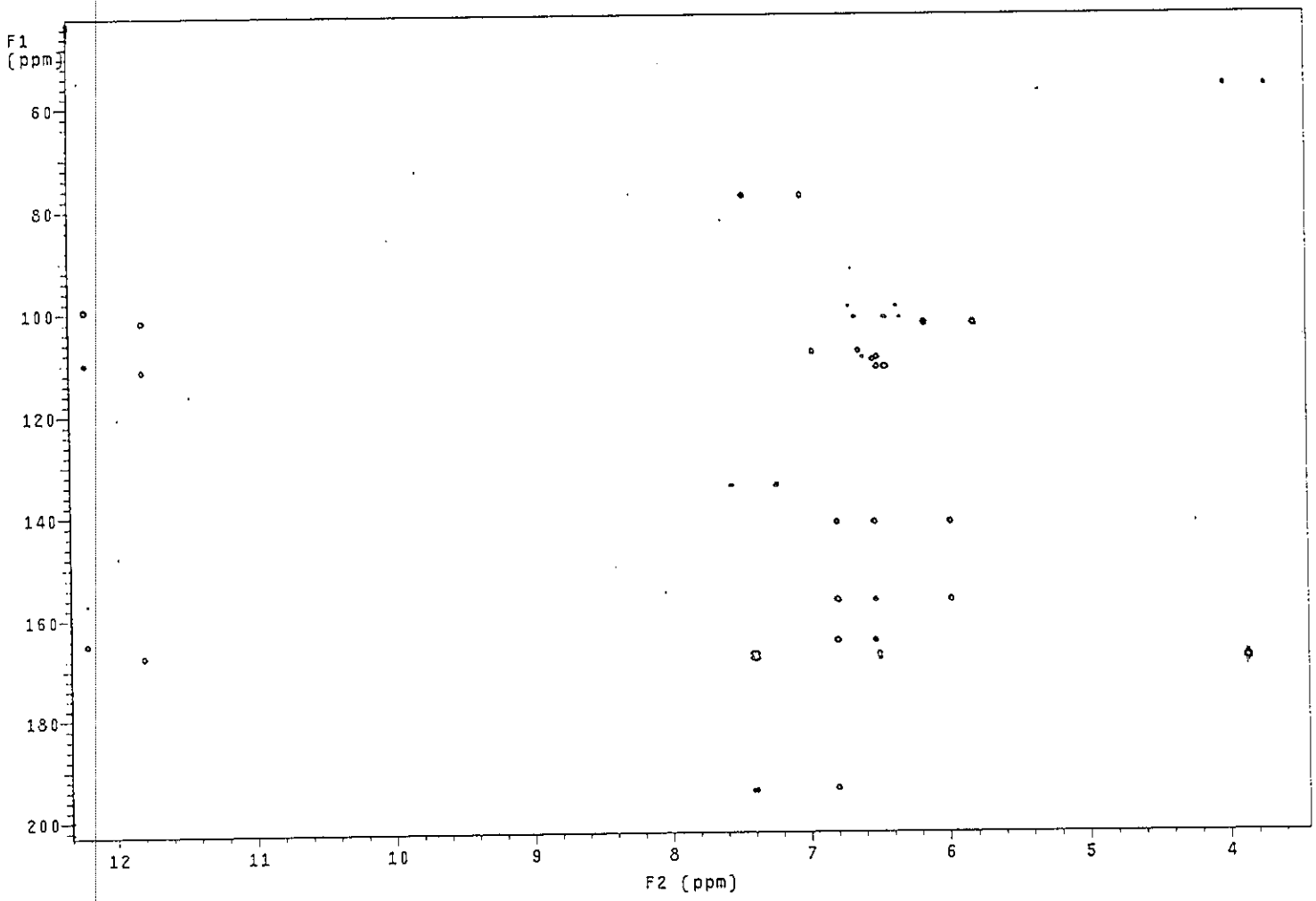


Figure 112 2D HMBC spectrum of **DS18**

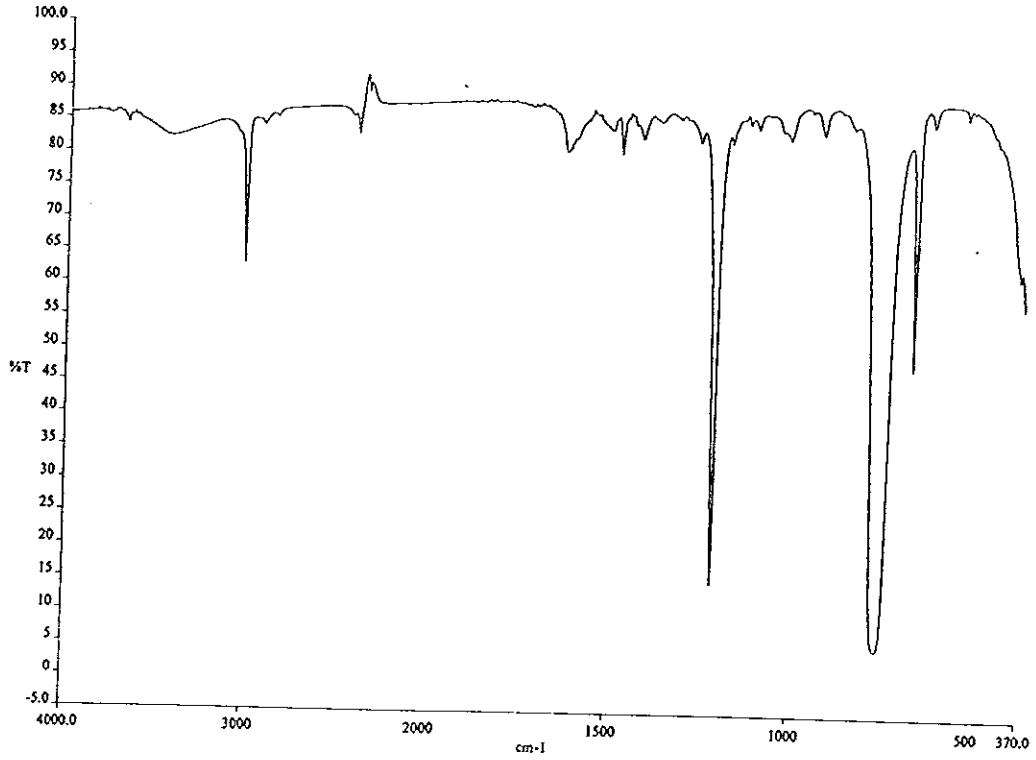


Figure 113 IR (KBr) spectrum of **DS19**

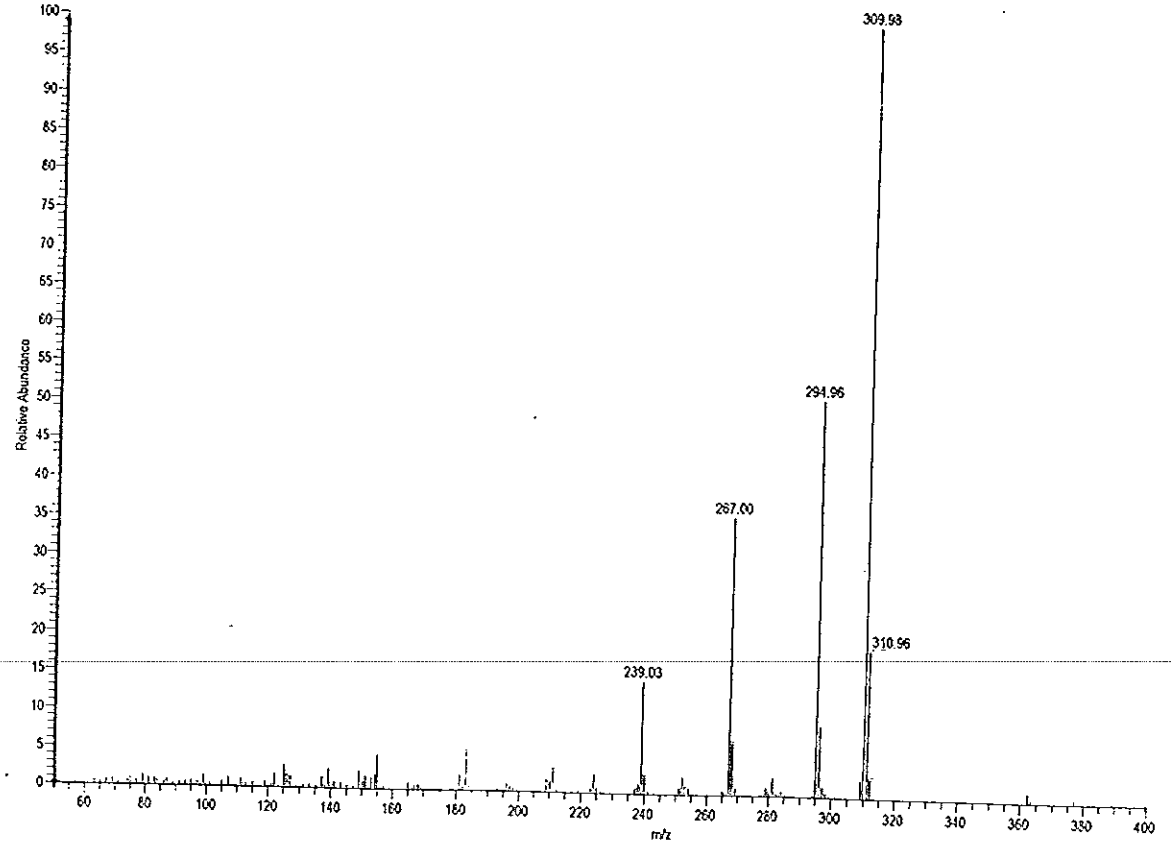


Figure 114 Mass spectrum of **DS19**

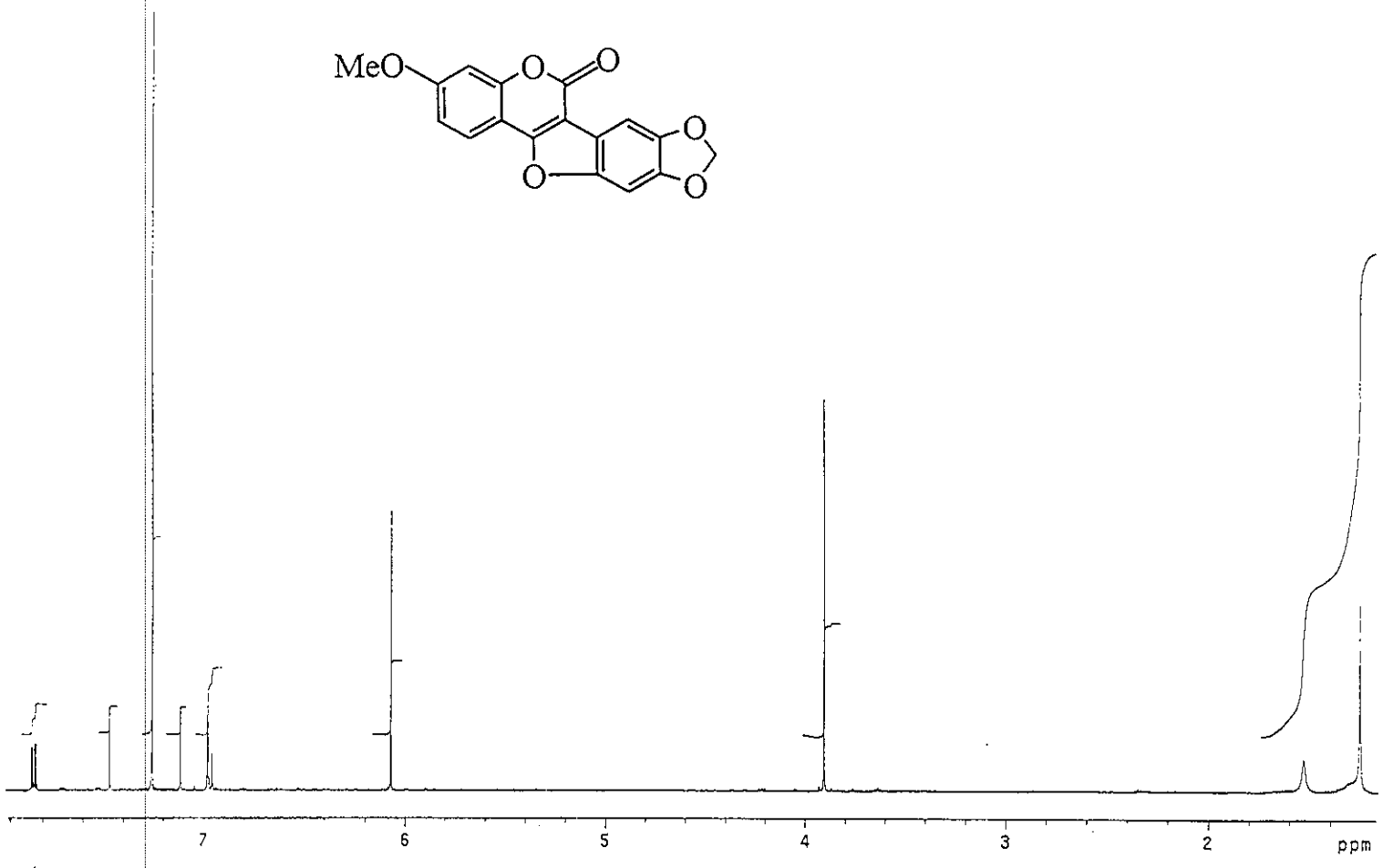


Figure 115 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS19**

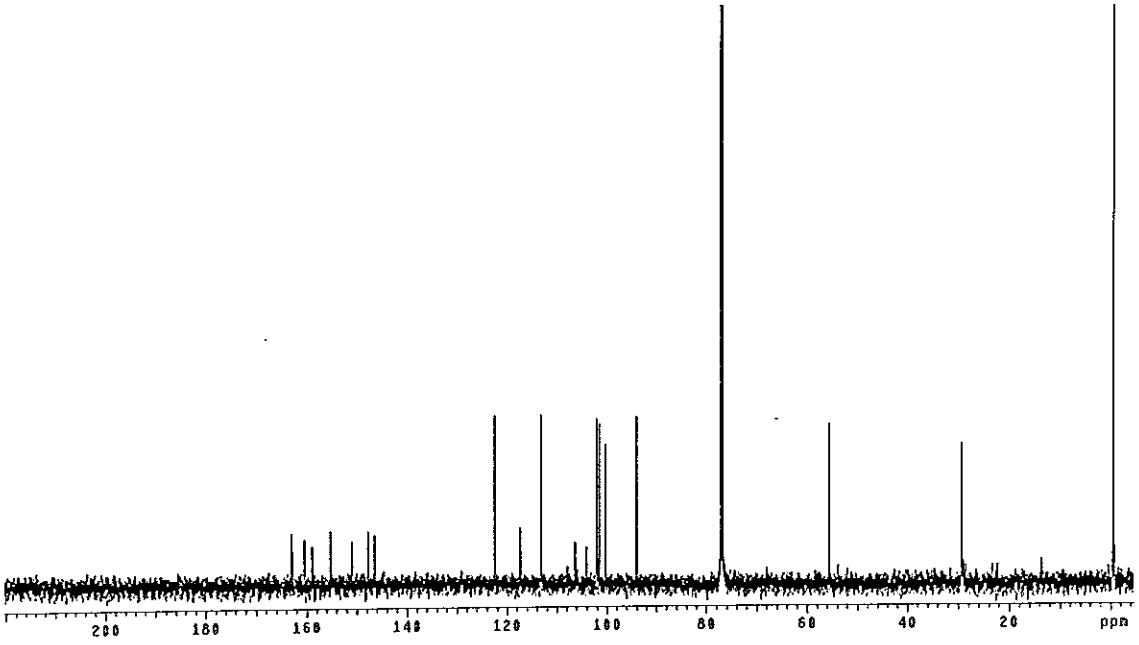


Figure 116 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **DS19**

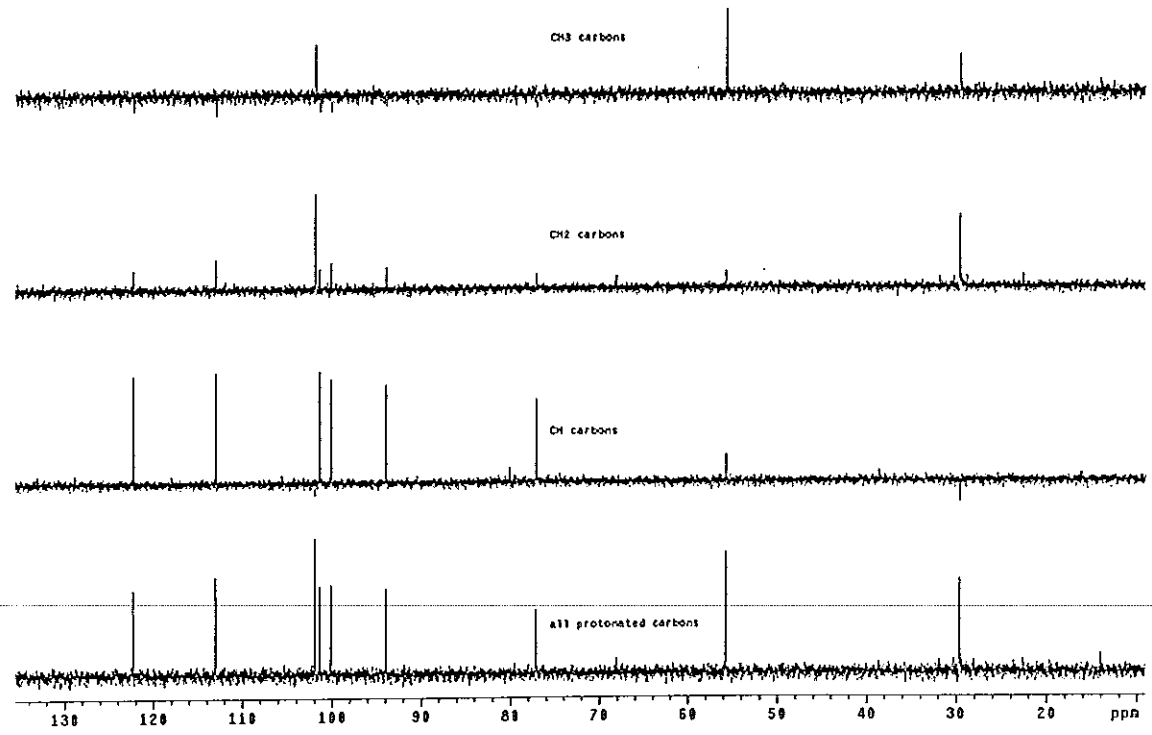


Figure 117 DEPT (135°) ($CDCl_3$) spectrum of **DS19**

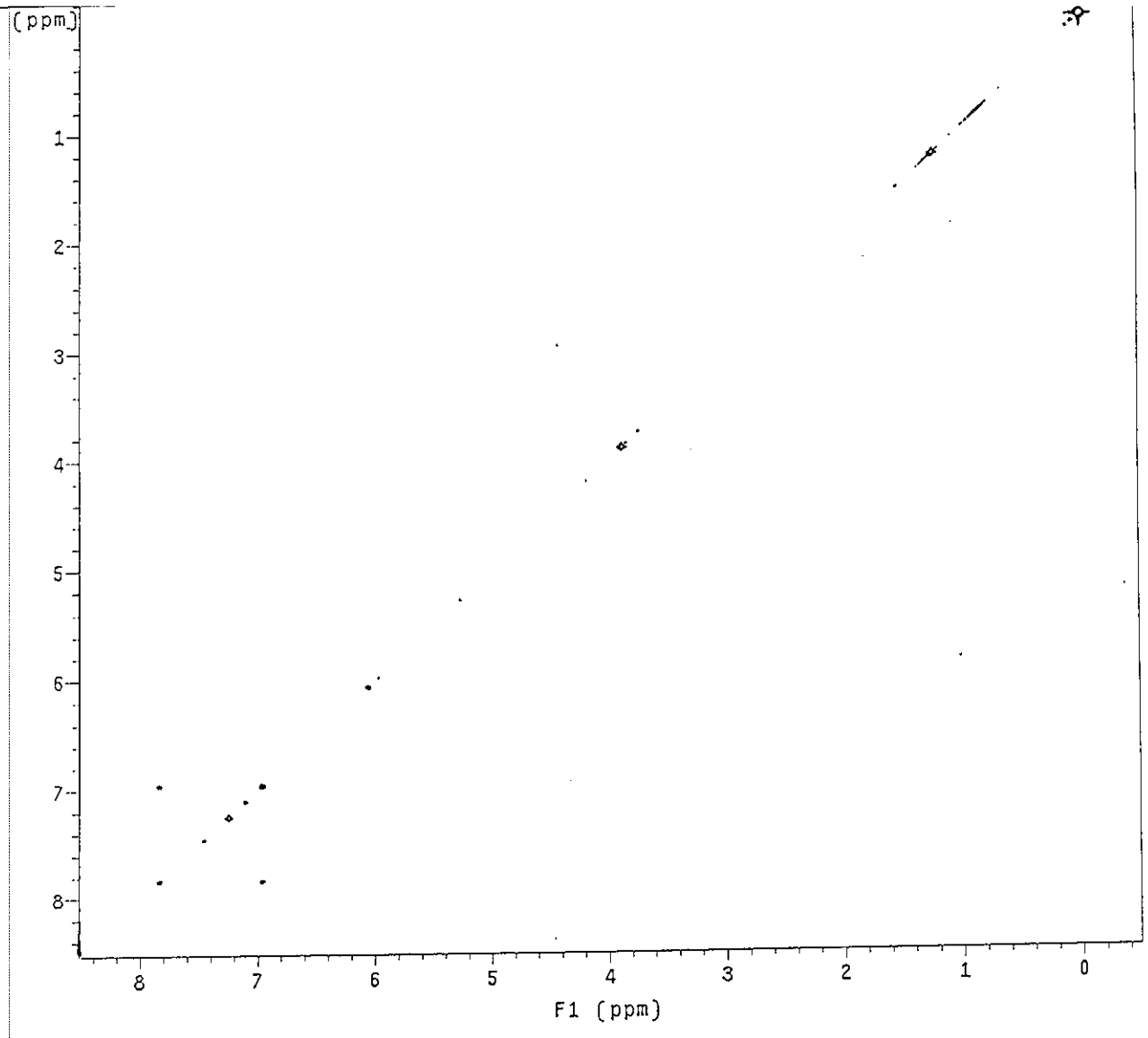


Figure 118 ^{1}H-^{1}H COSY spectrum of **DS19**

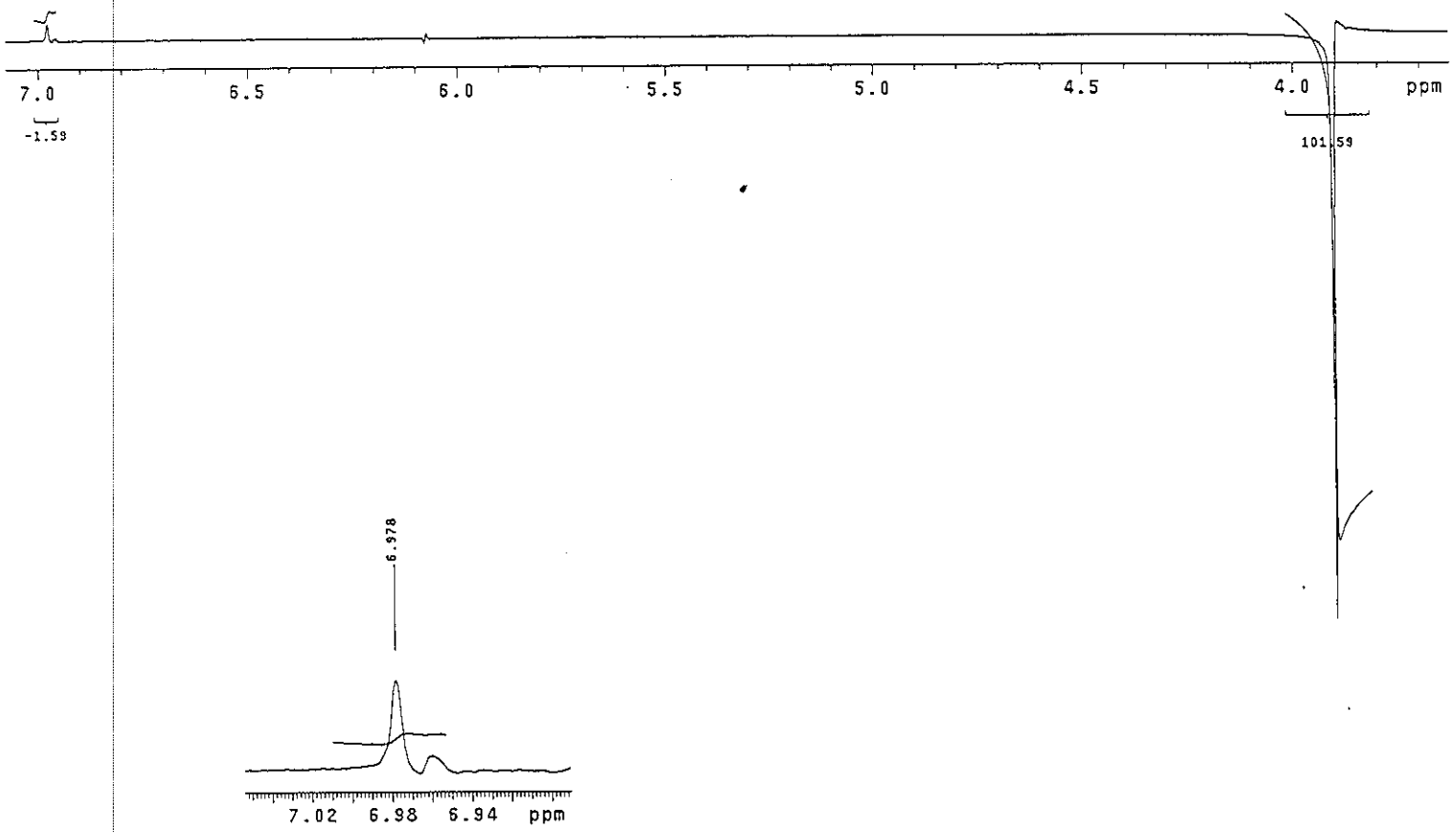


Figure 119 NOEDIFF spectrum of **DS19** after irradiation at δ_H 3.91

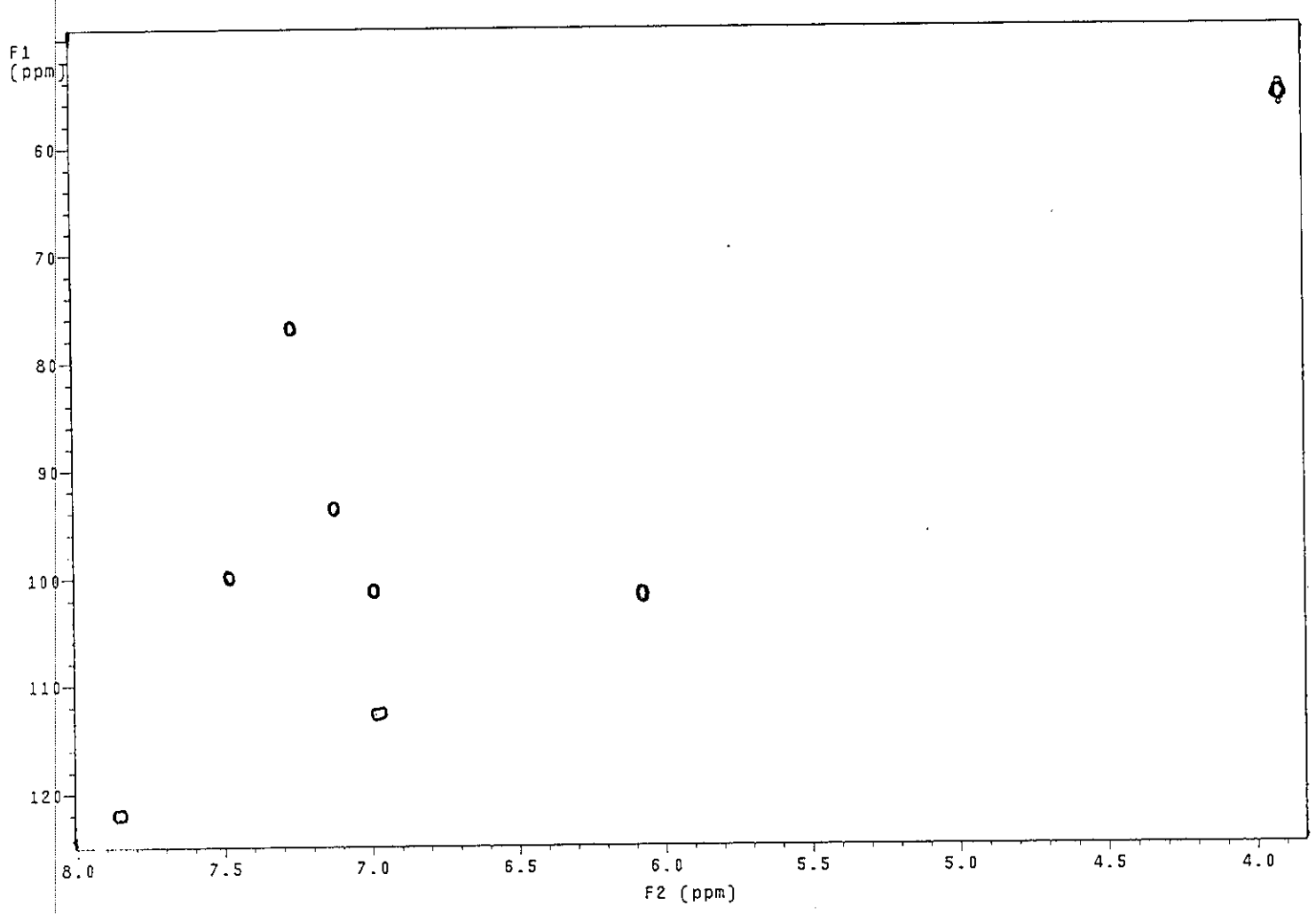


Figure 120 2D HMQC spectrum of **DS19**

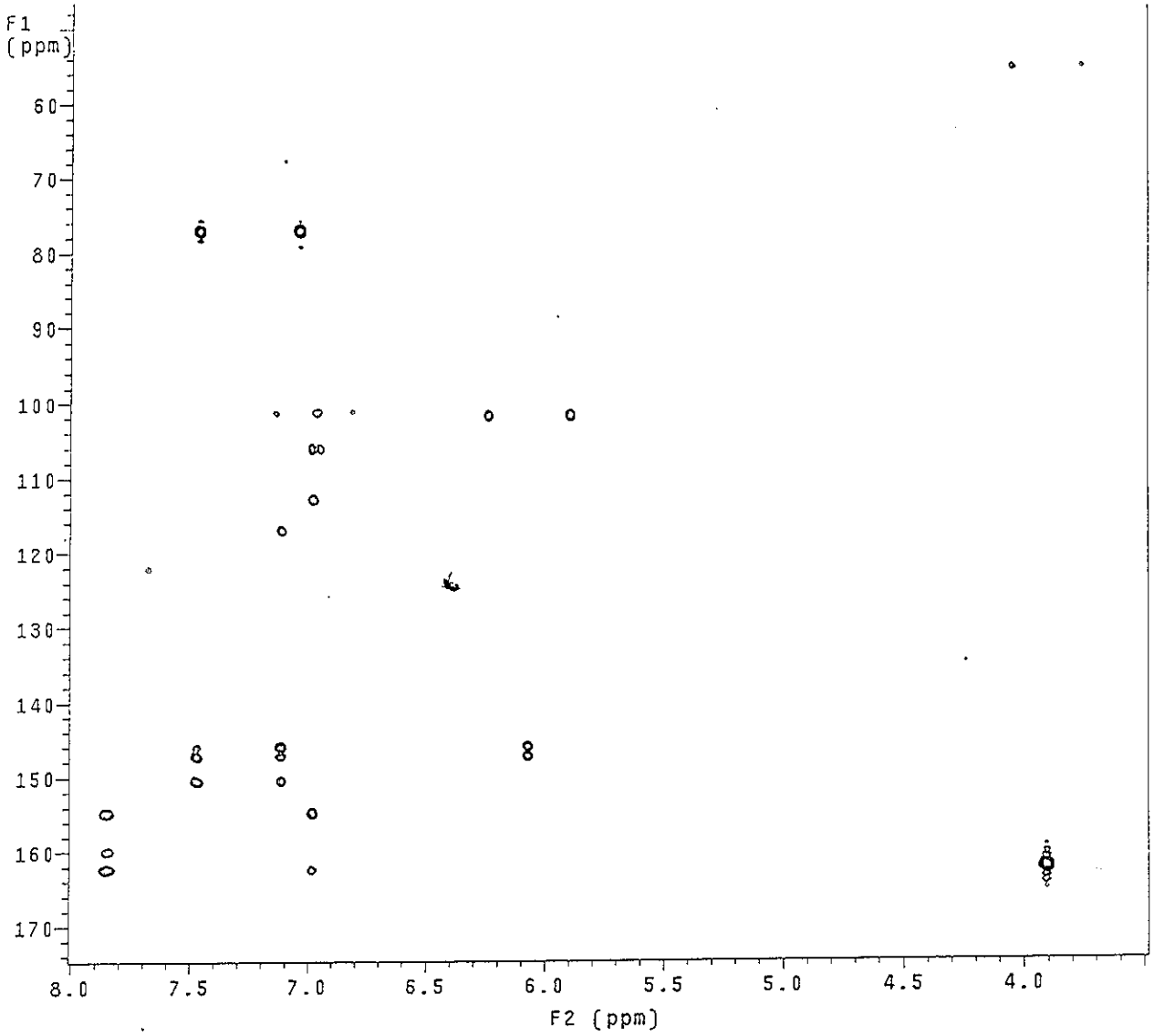


Figure 121 2D HMBC spectrum of **DS19**

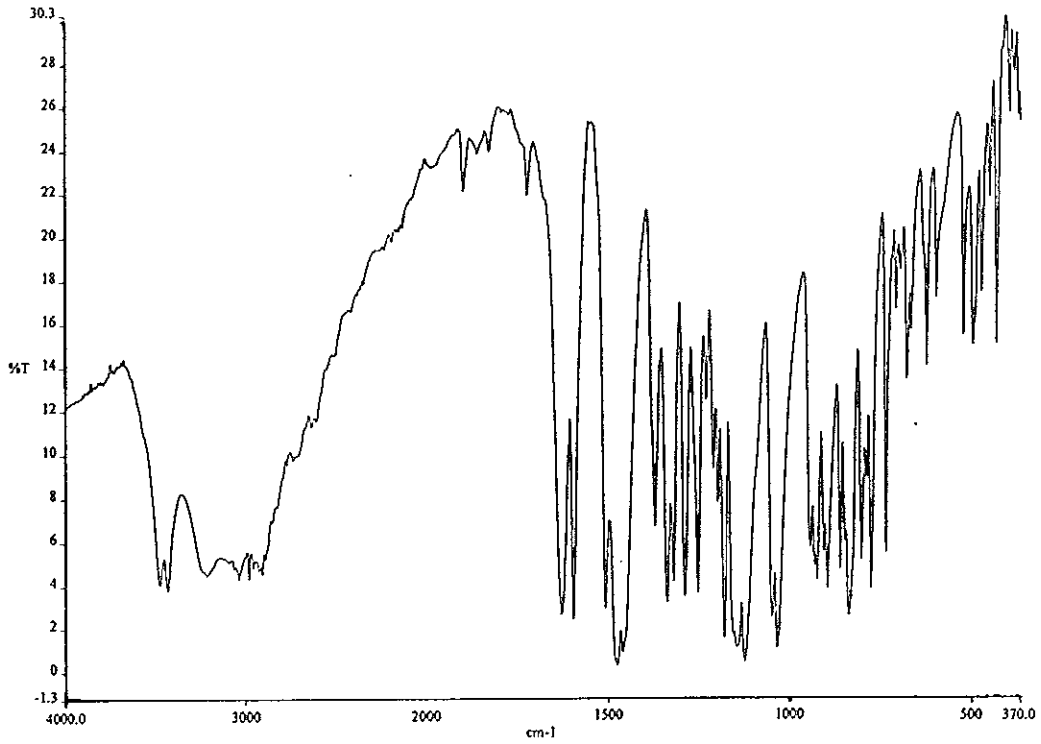


Figure 122 IR (KBr) spectrum of **DS20**

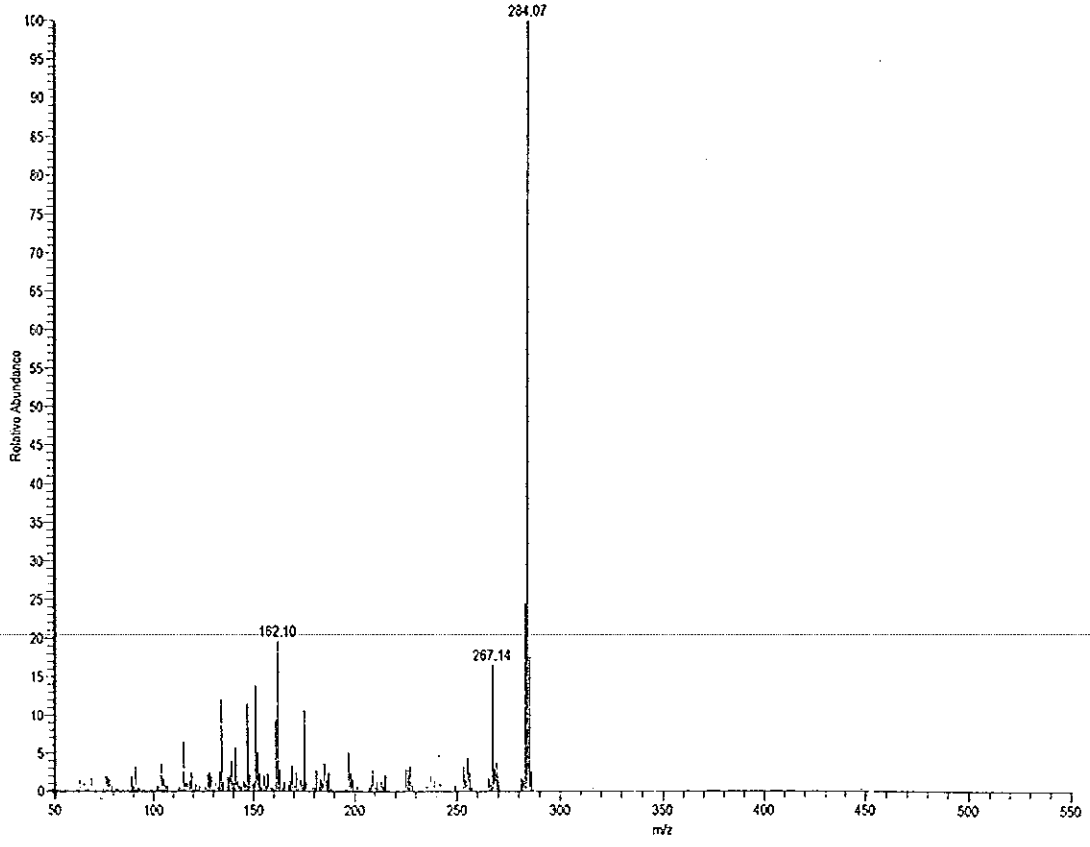


Figure 123 Mass spectrum of **DS20**

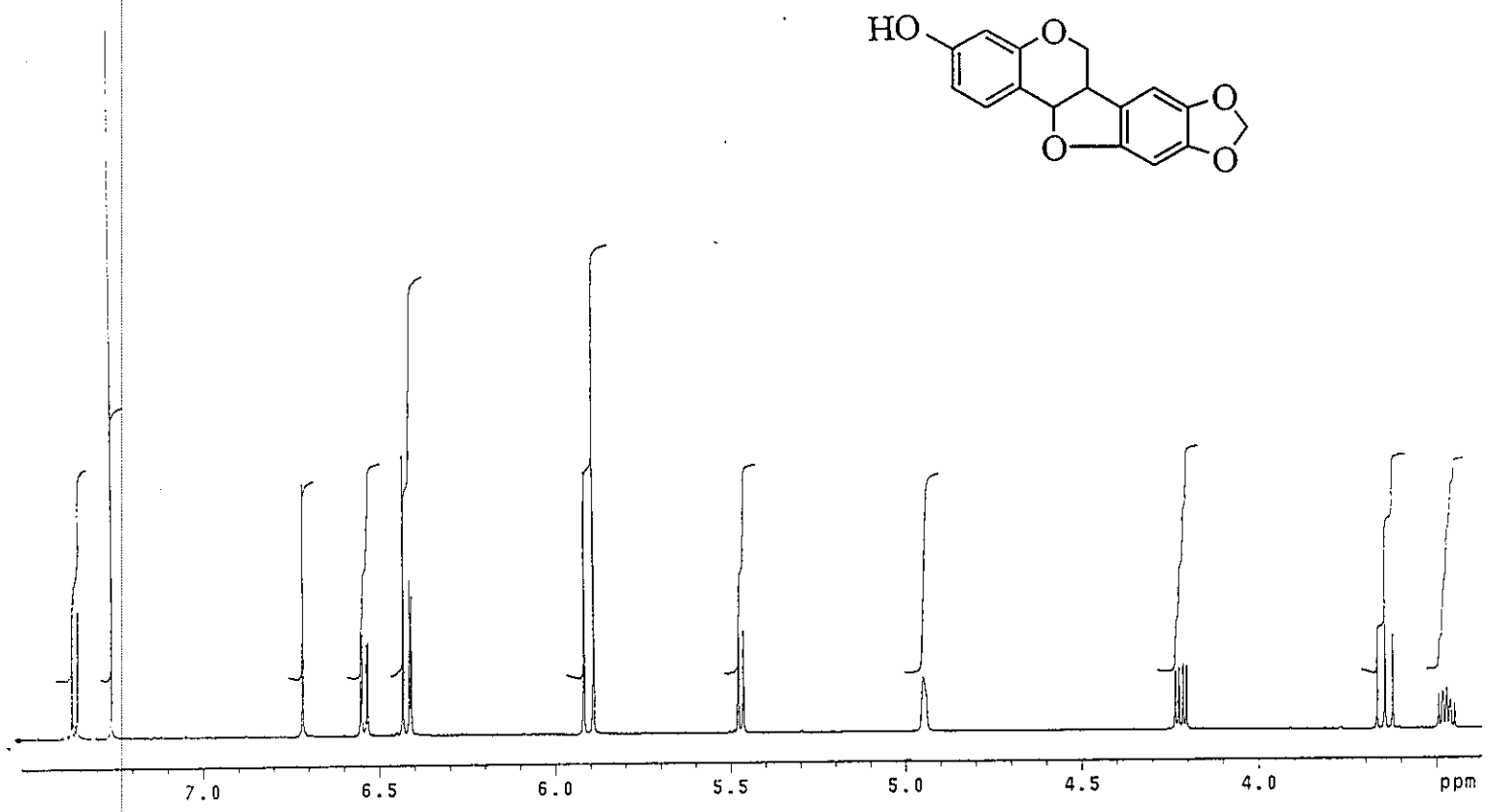


Figure 124 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS20**

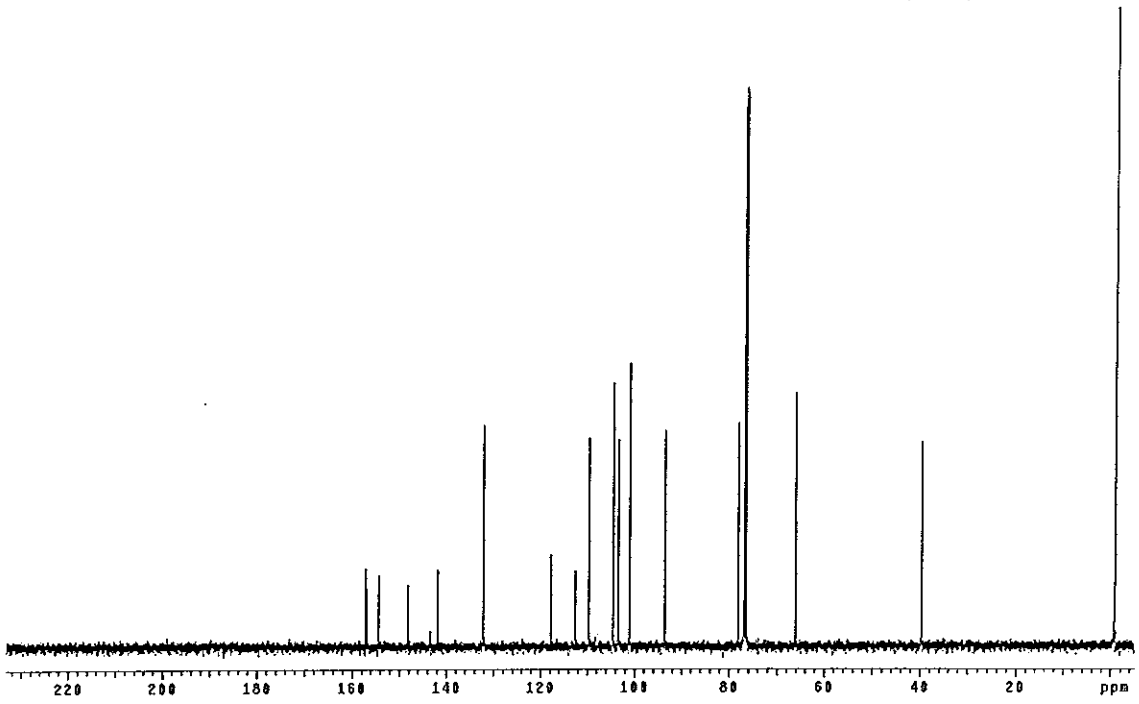


Figure 125 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **DS20**

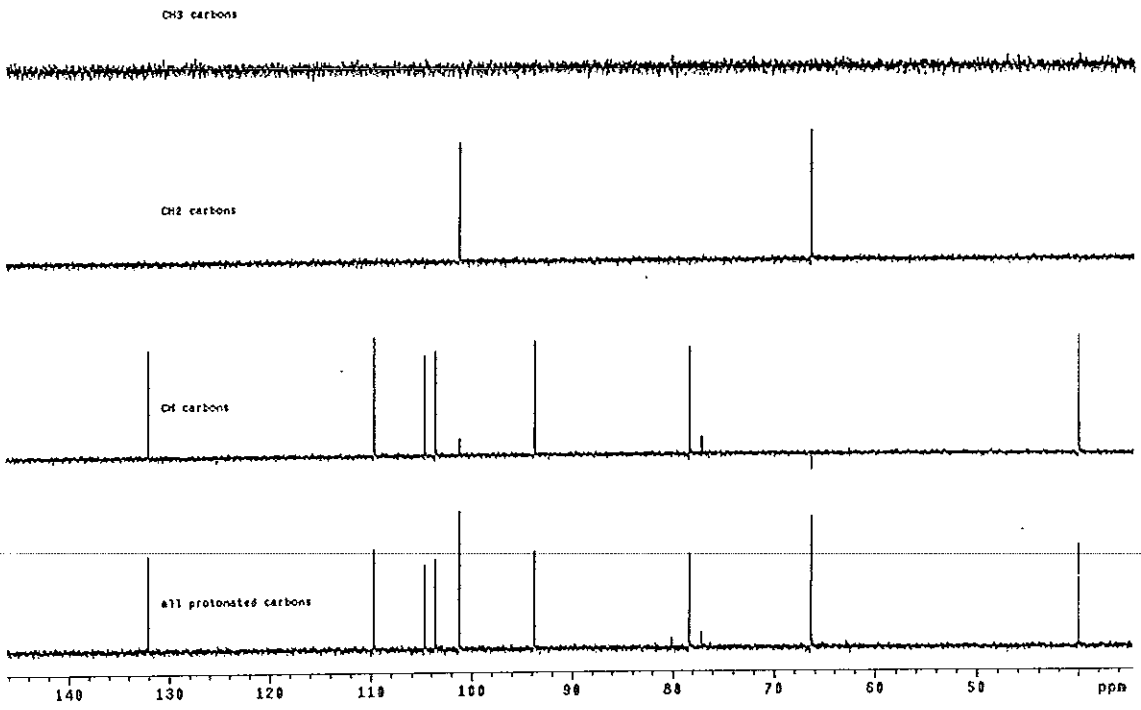


Figure 126 DEPT (135°) ($CDCl_3$) spectrum of **DS20**

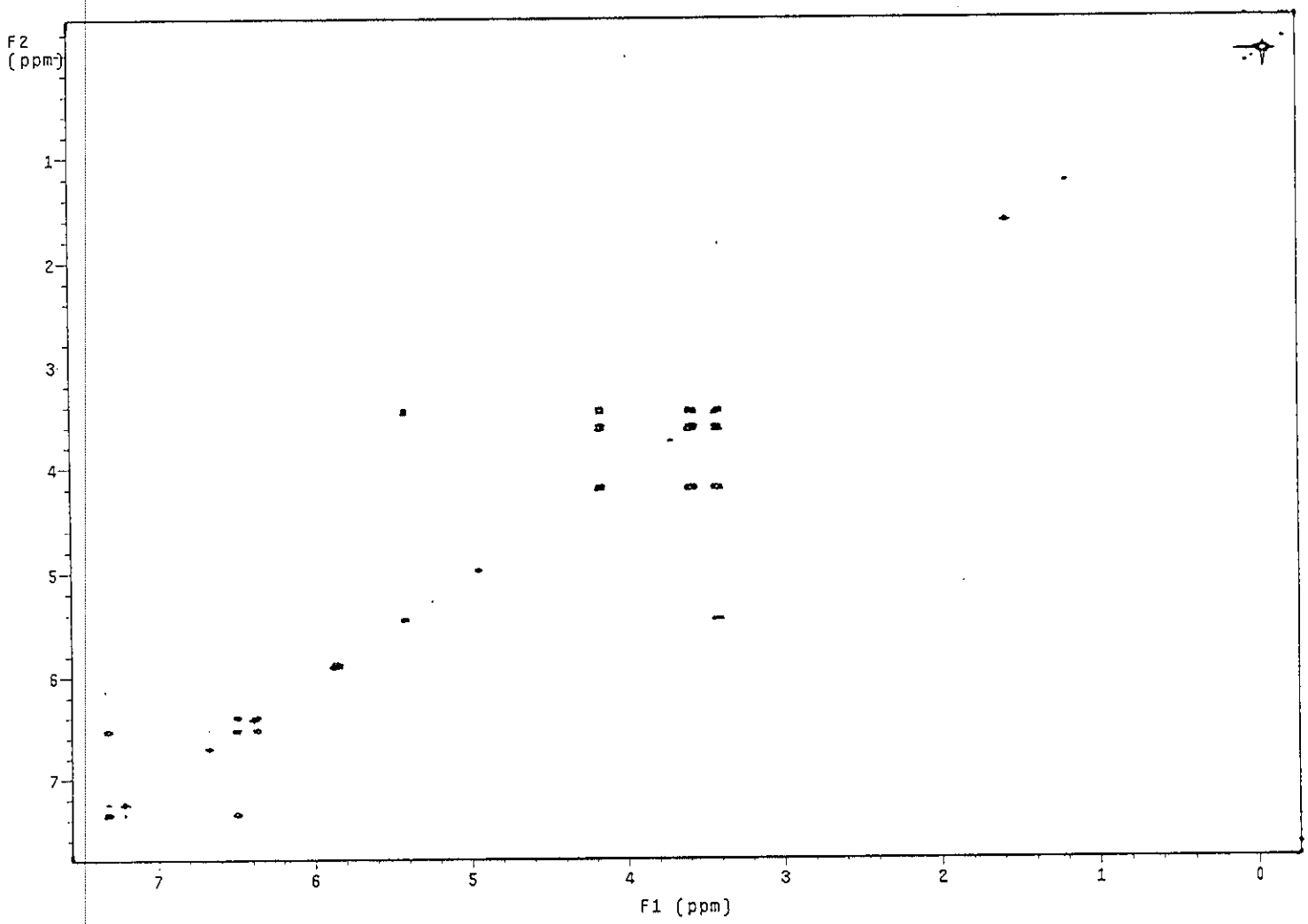


Figure 127 ^{1}H-^{1}H COSY spectrum of **DS20**

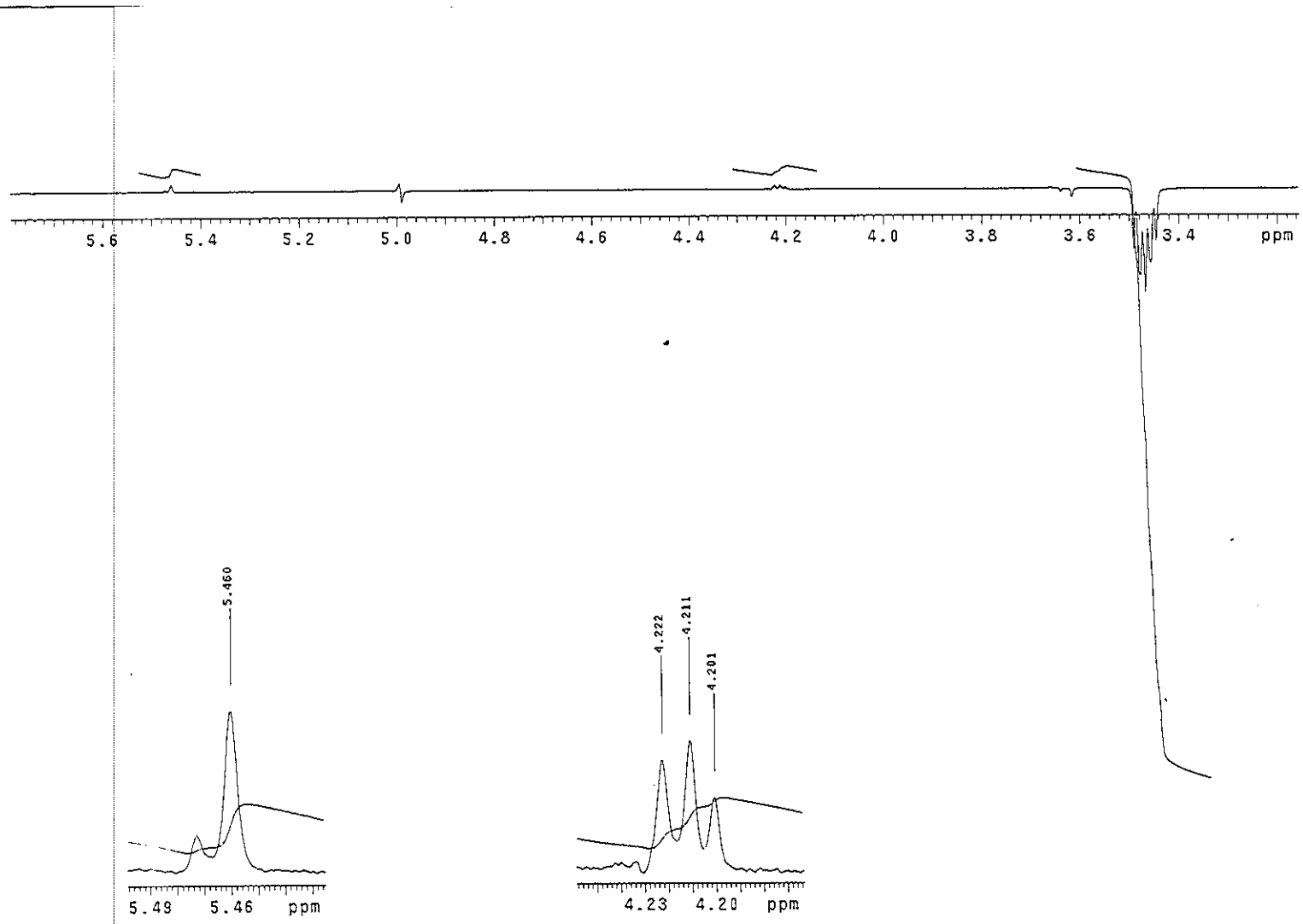


Figure 128 NOEDIFF spectrum of **DS20** after irradiation at δ_H 3.47

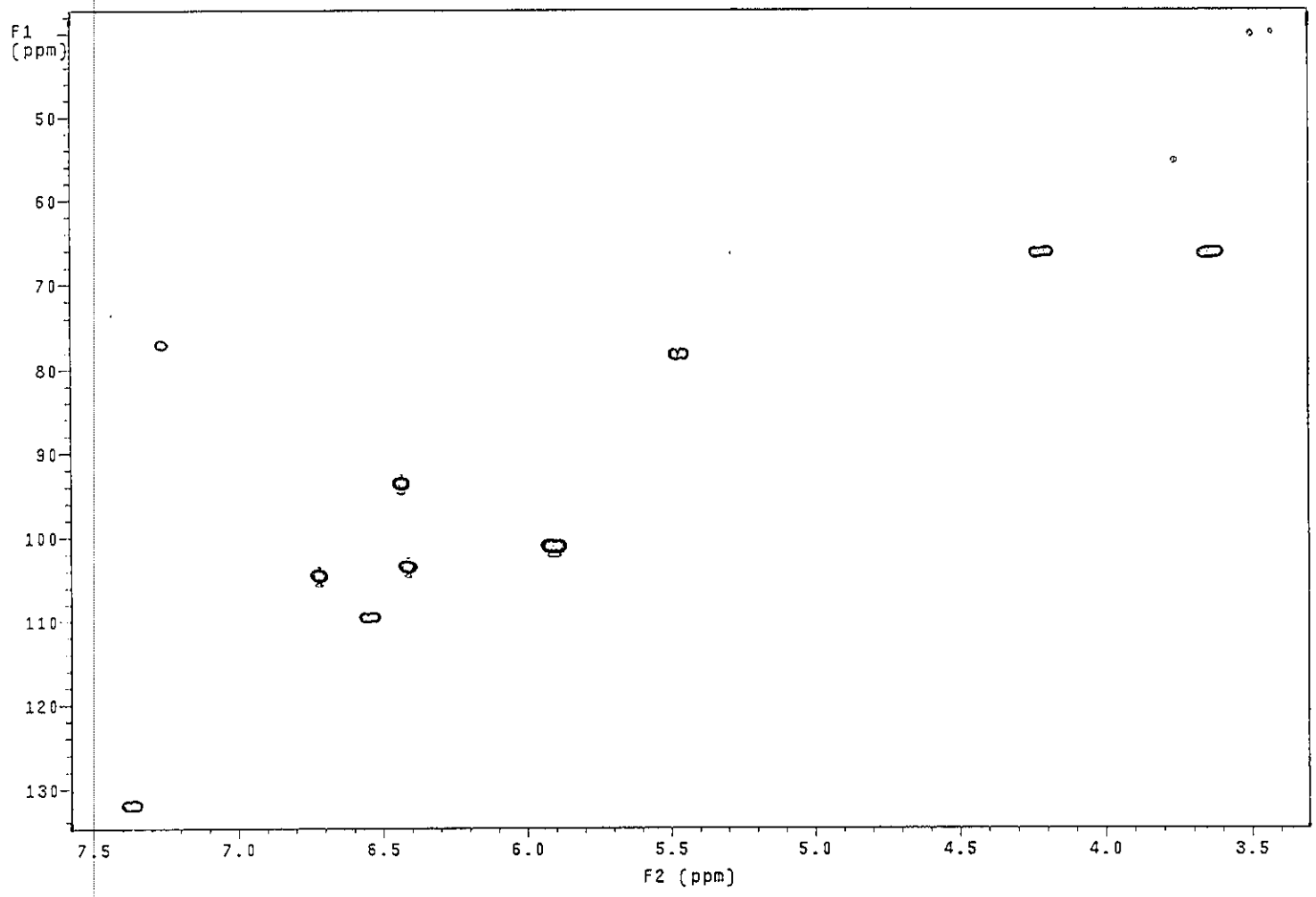


Figure 129 2D HMQC spectrum of **DS20**

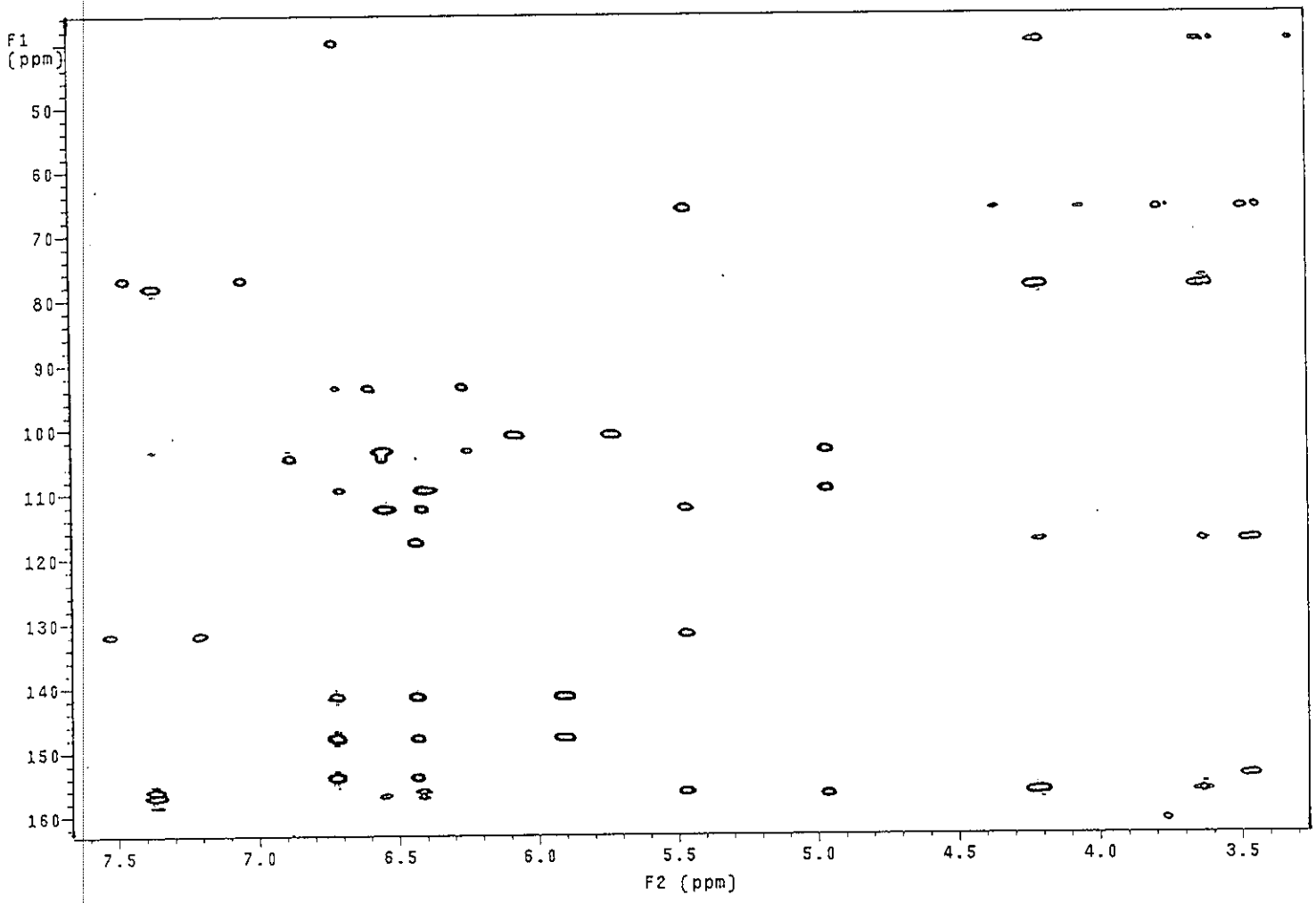


Figure 130 2D HMBC spectrum of **DS20**

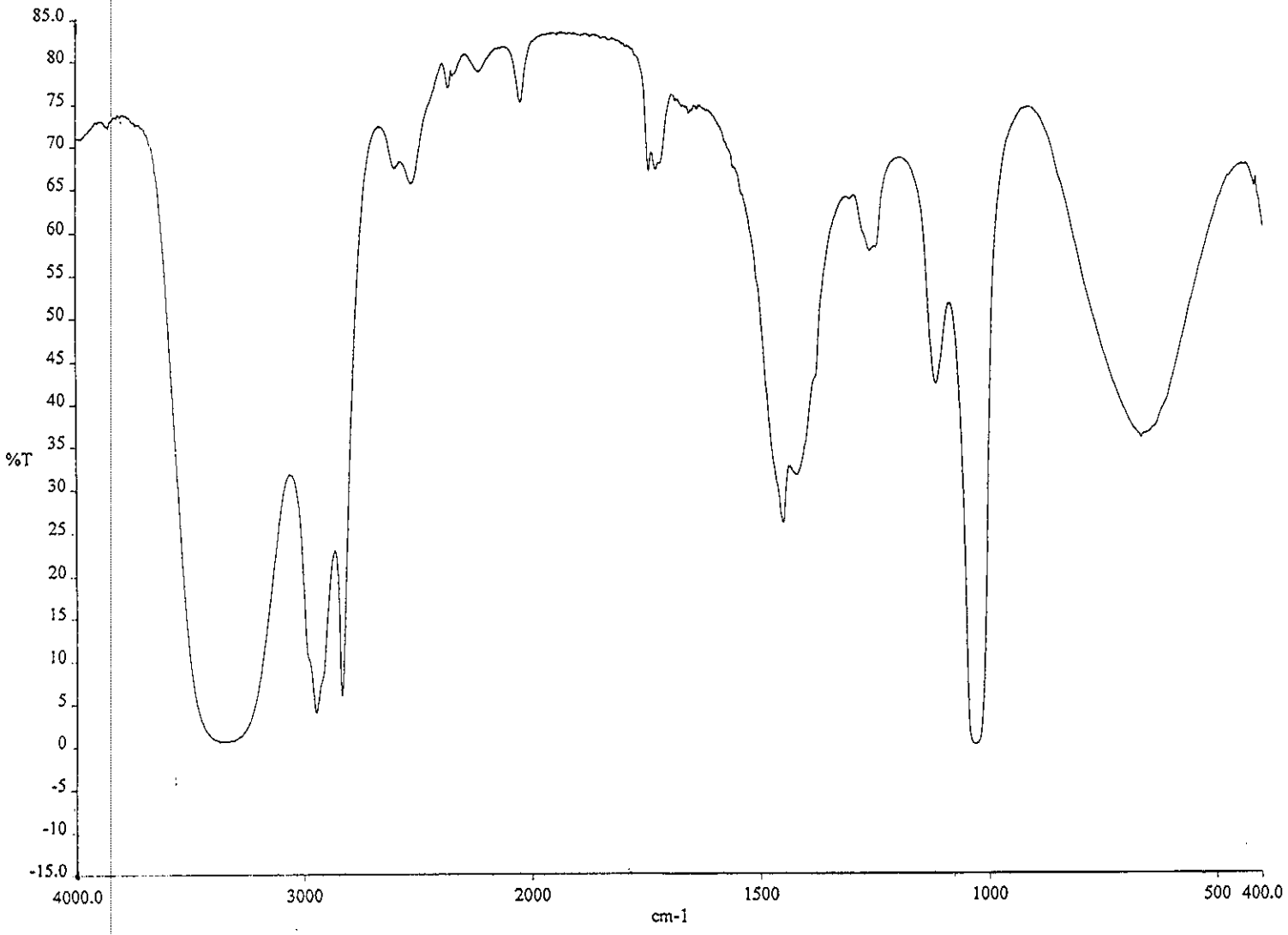


Figure 131 IR (neat) spectrum of **DS22**

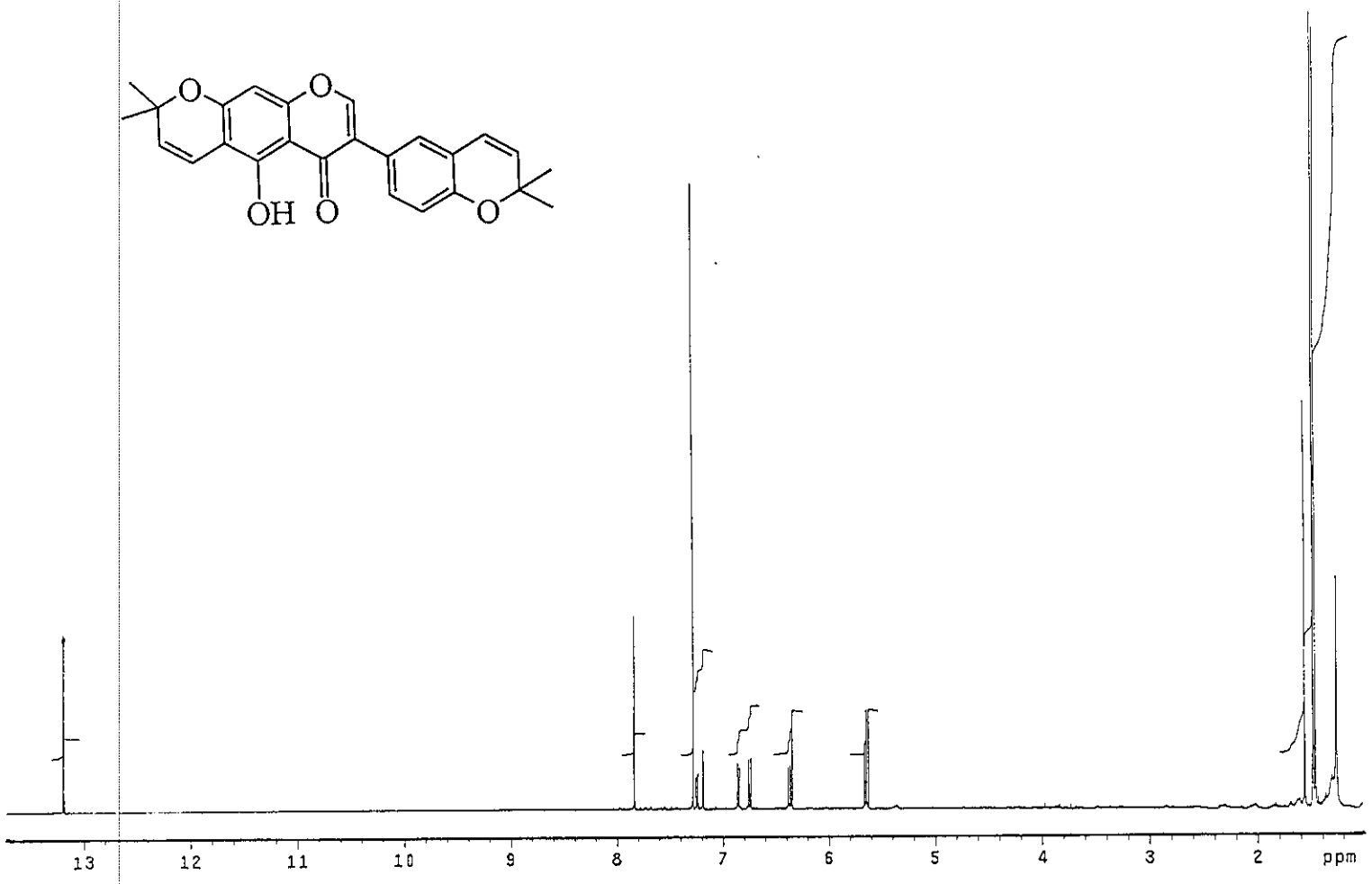


Figure 132 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS22**

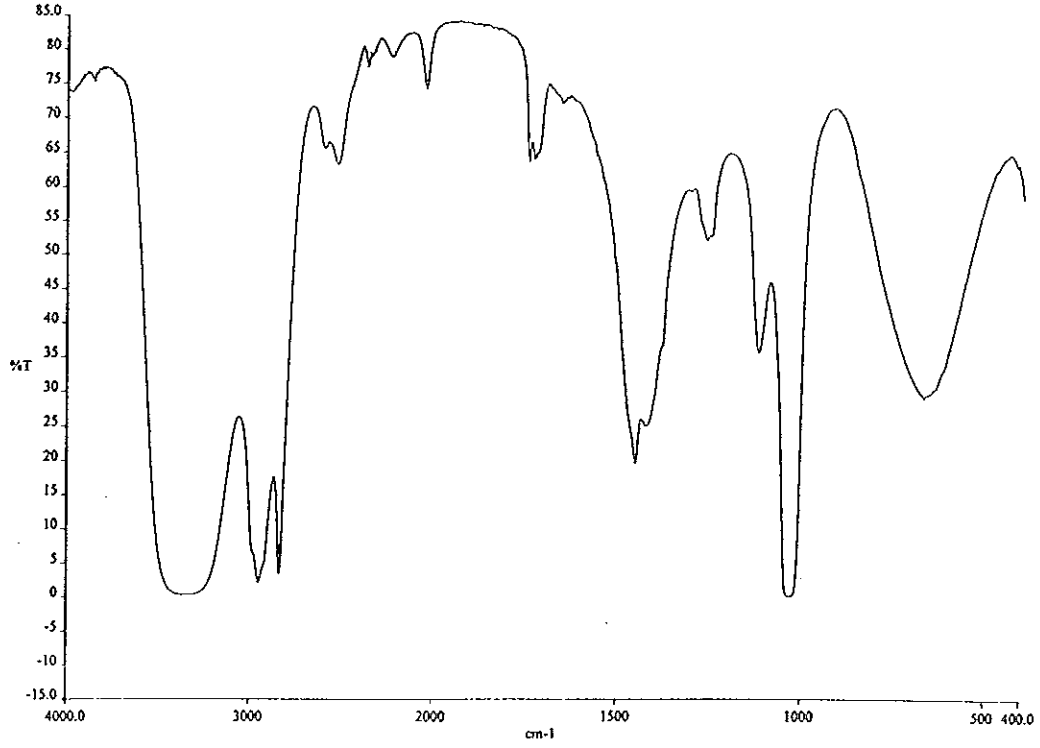


Figure 133 IR (neat) spectrum of **DS23**

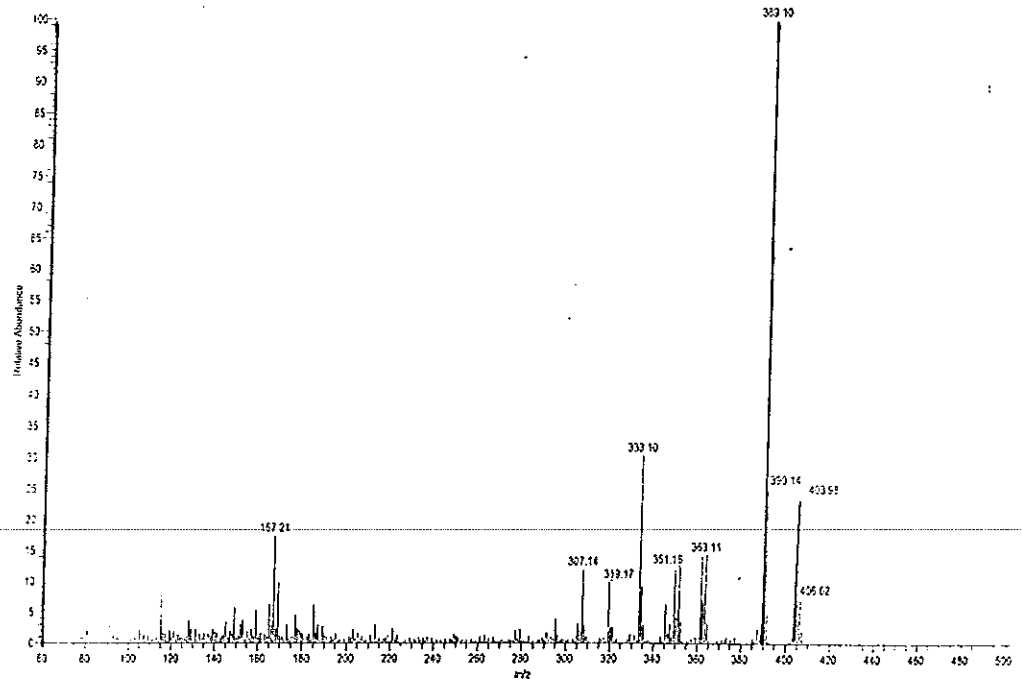


Figure 134 Mass spectrum of **DS23**

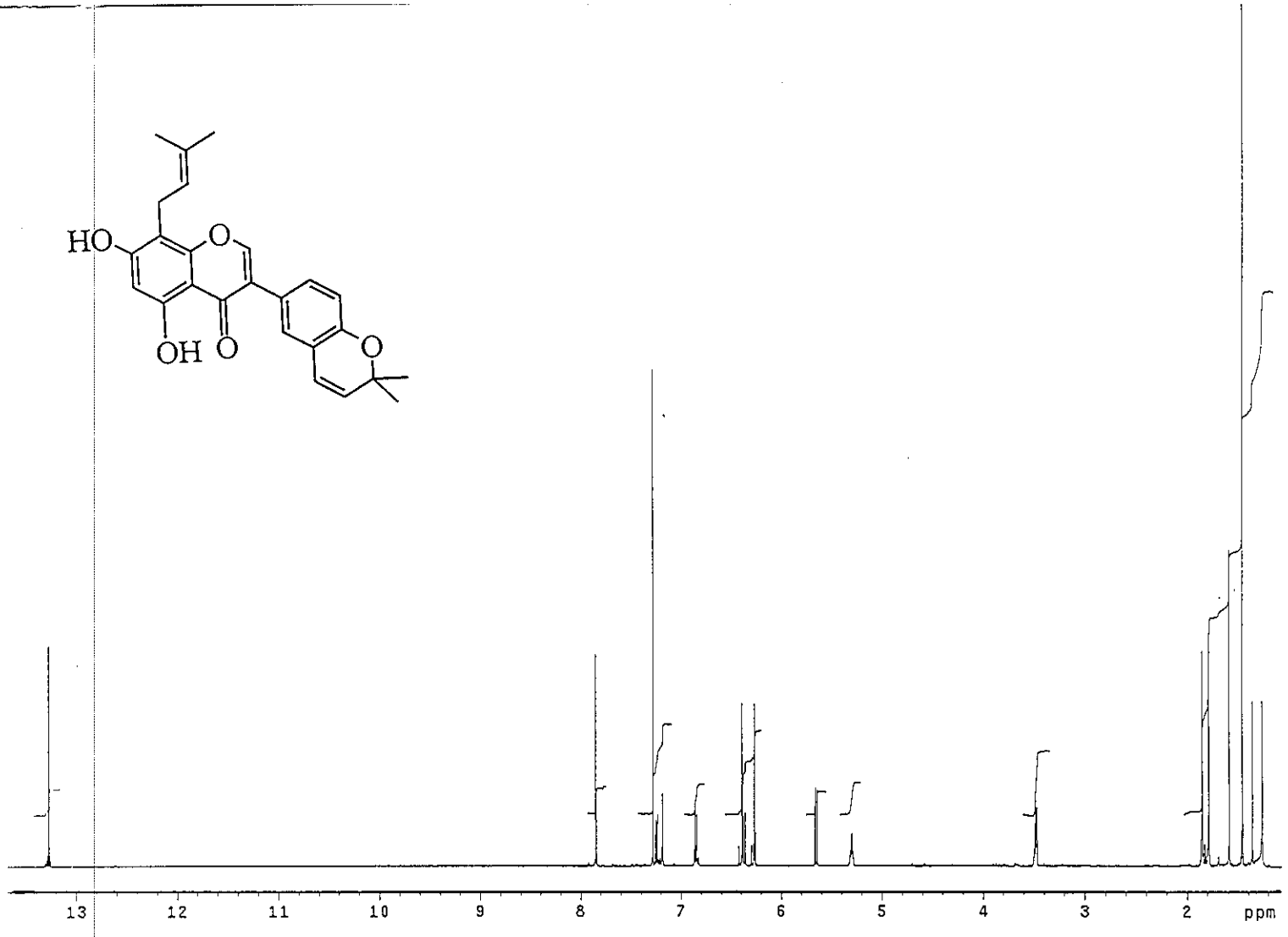


Figure 135 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS23**

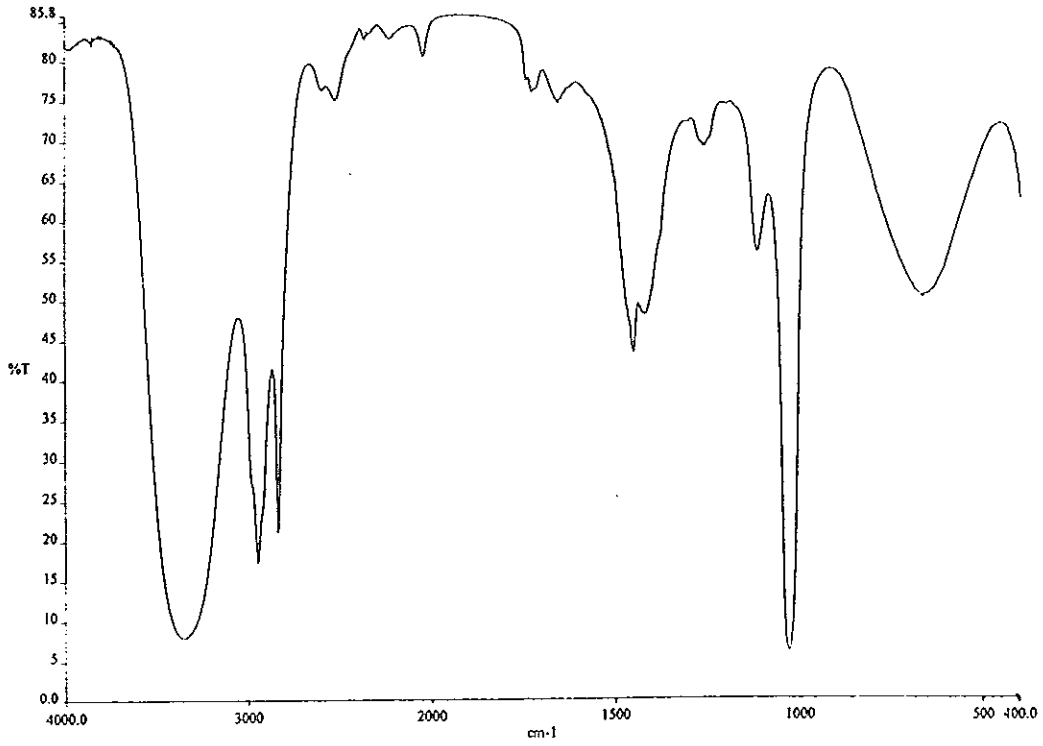


Figure 136 IR (neat) spectrum of **DS24**

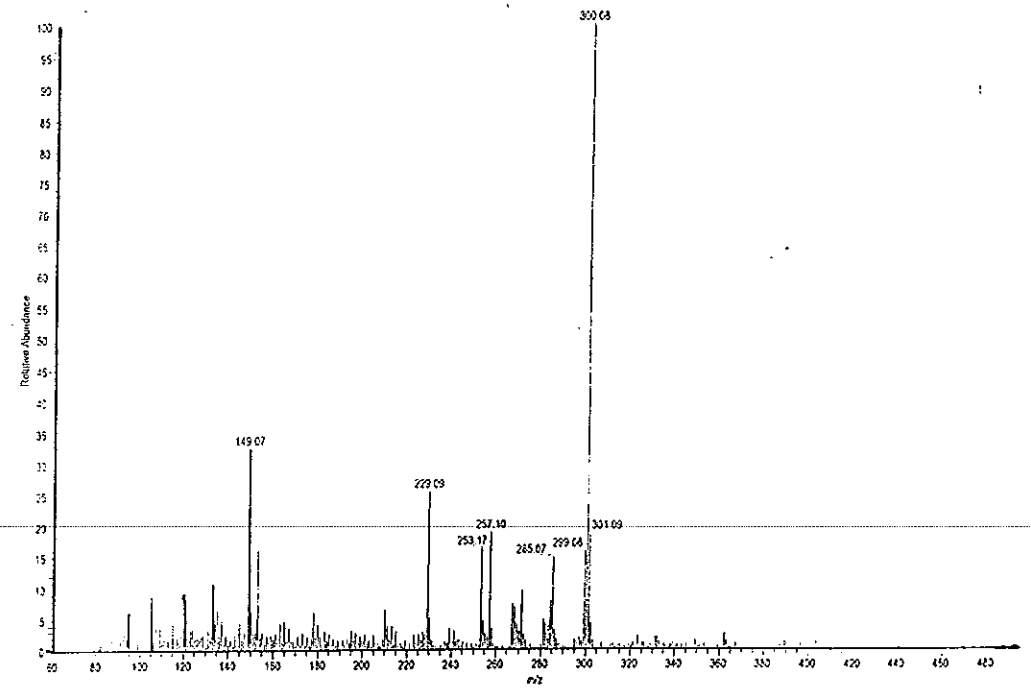


Figure 137 Mass spectrum of **DS24**

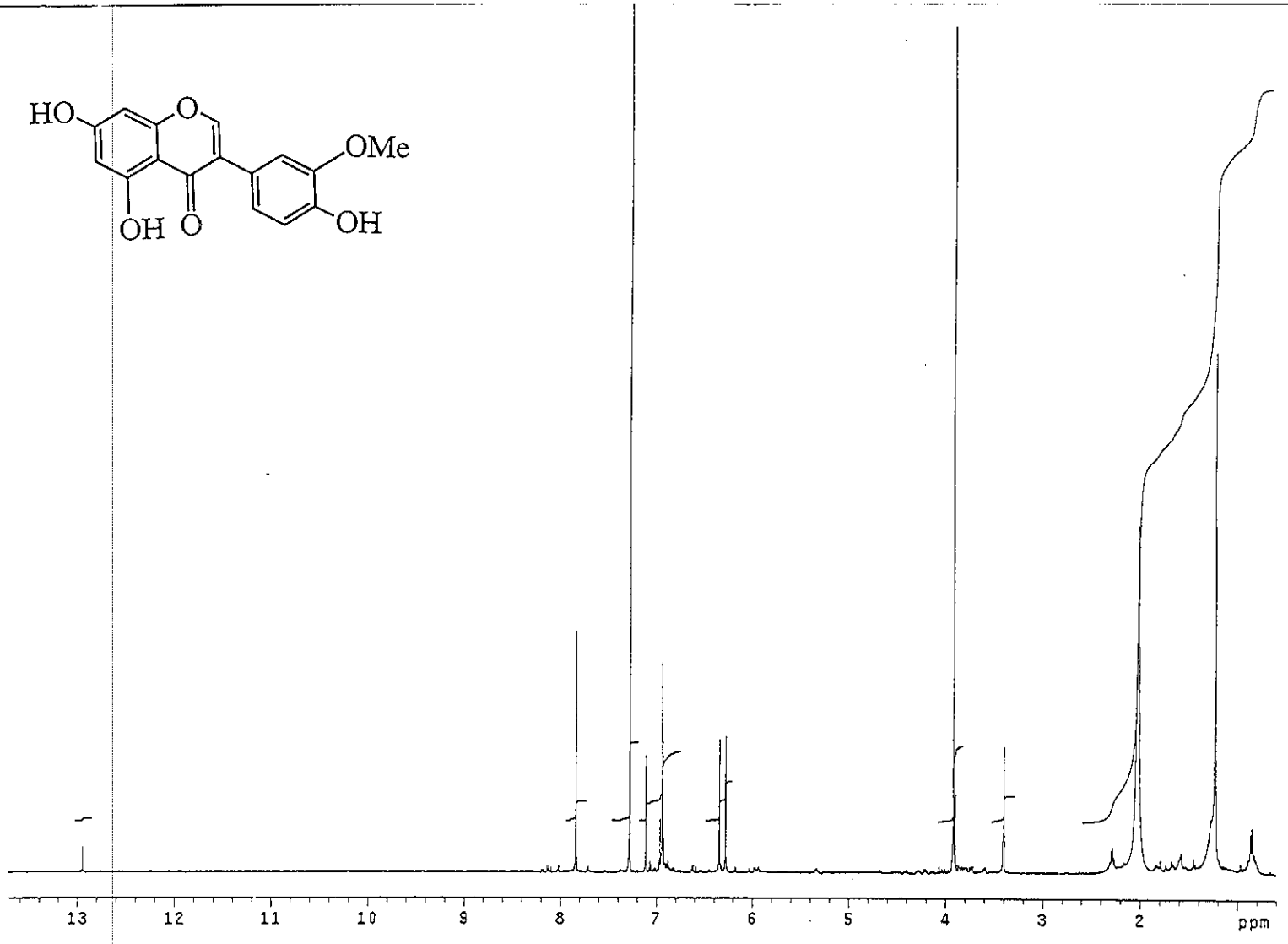


Figure 138 ^{1}H NMR (500 MHz) ($CDCl_3$ + CD_3OD) spectrum of **DS24**

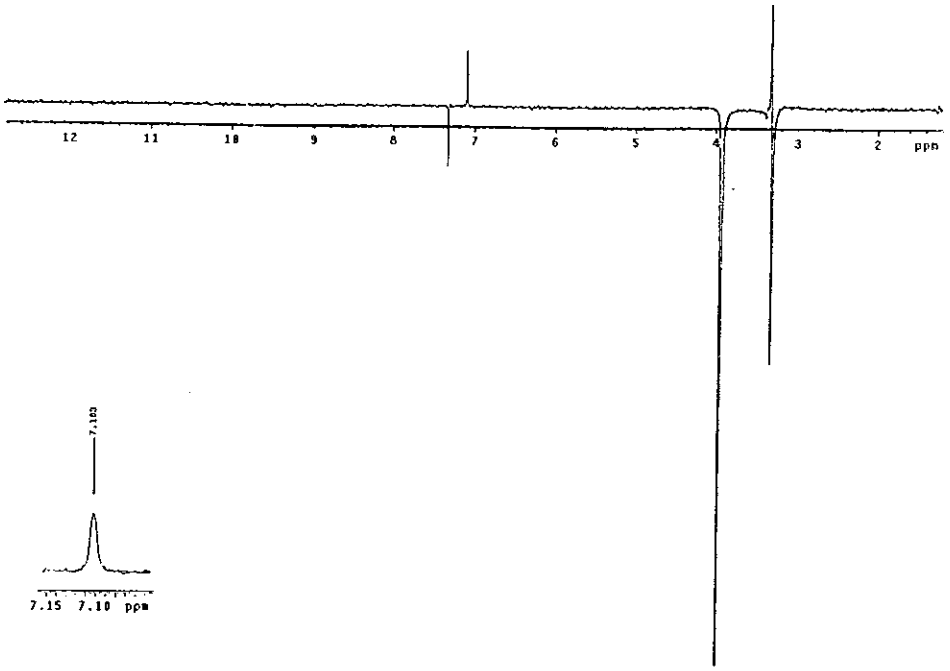


Figure 139 NOEDIFF spectrum of DS24 after irradiation at δ_H 3.93

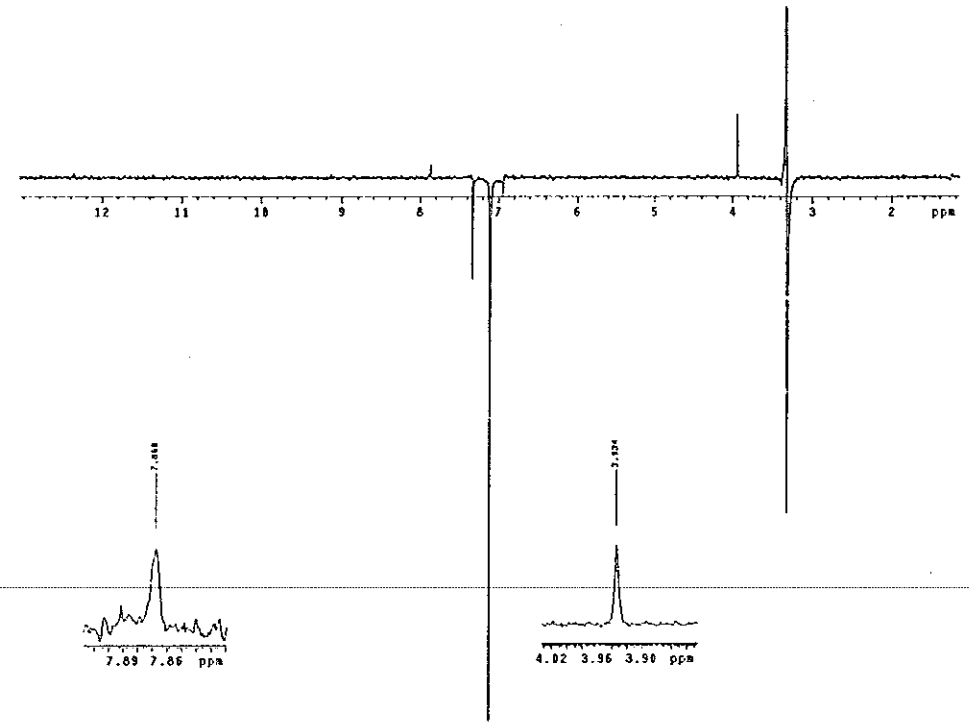


Figure 140 NOEDIFF spectrum of DS24 after irradiation at δ_H 7.11

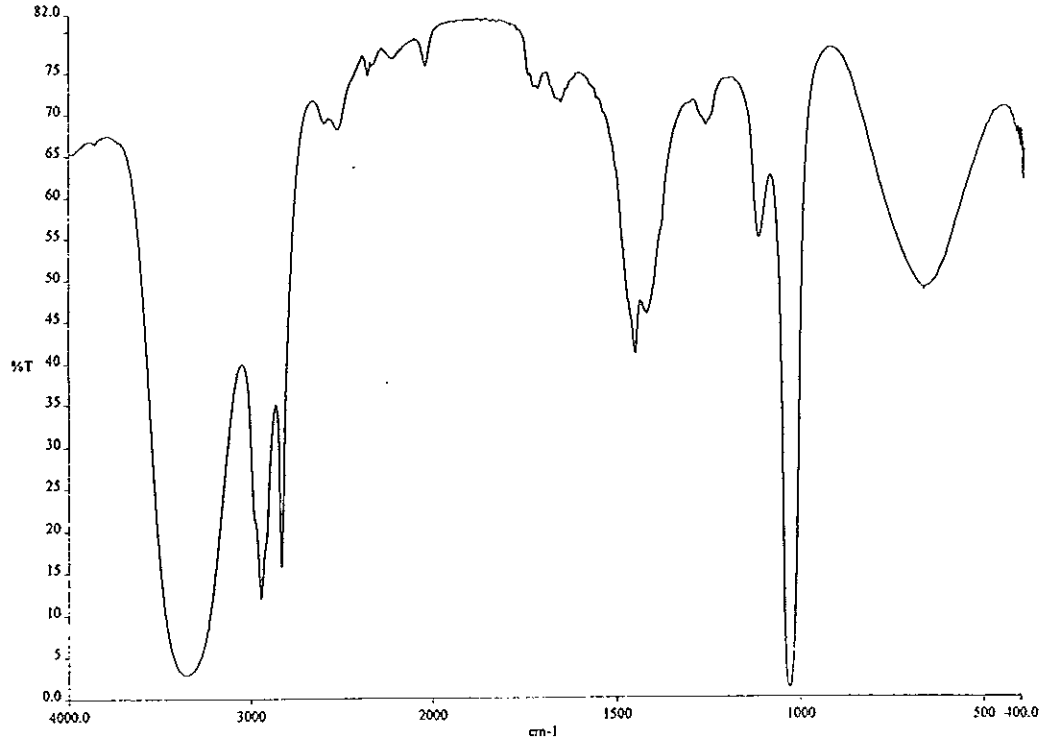


Figure 141 IR (neat) spectrum of DS25

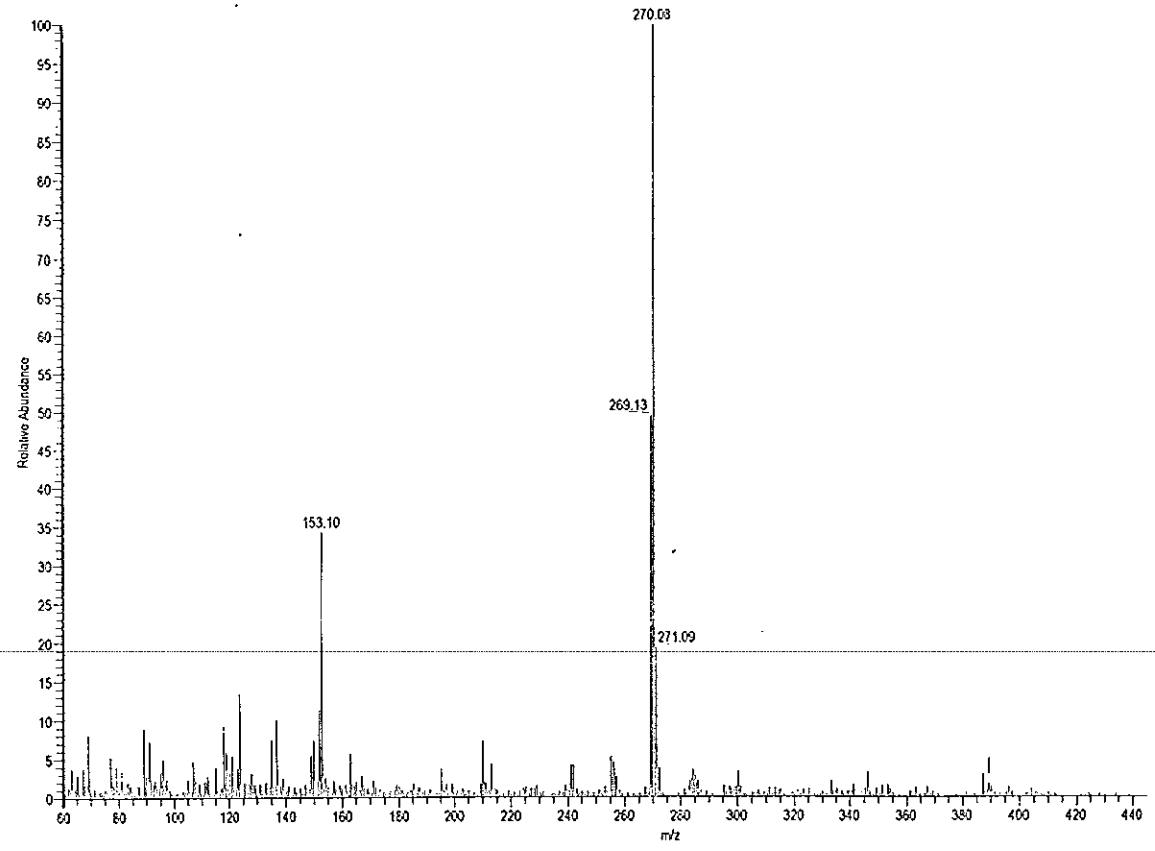


Figure 142 Mass spectrum of DS25

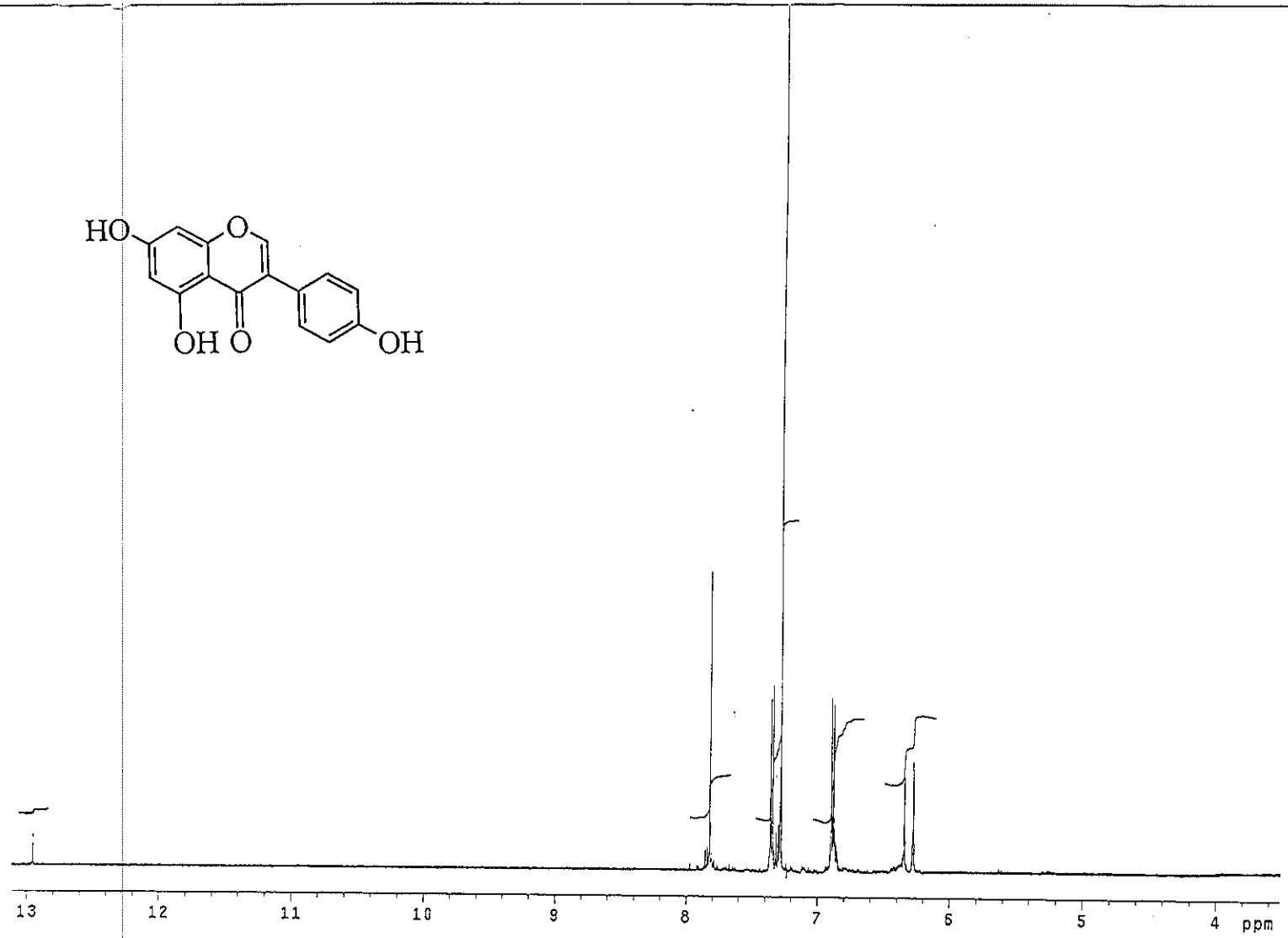


Figure 143 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DS25**

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ภาควิชาเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล. 2543. สารานุกรมสมุนไพร เล่ม1 สมุนไพรสวนสิรีรุกขชาติ. พิมพ์ครั้งที่ 3. หน้า 81. กรุงเทพฯ : อมรพริ้นติ้งแอนด์พับลิชชิ่ง จำกัด (มหาชน).

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VITAE

Name Miss Suwanna Deachathai

Birth Date 18 May 1976

Place of Birth Trang, Thailand

Educational Attainment

Degree	Name of Institution	Year of Graduation
B.Sc. (Chemistry)	Prince of Songkla University	1999

Scholarship Awards during Enrolment

The Higher Education Development Project : Postgraduate Education and Research Program in Chemistry, funded by The Royal Thai Government (PERCH).