Chemical Constituents from Fruits of *Sapium indicum*

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Master of Science Thesis in Organic Chemistry

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Thesis Title	Chemical Constituents from Fruits of *Sapium indicum*
Author	Miss. Parinuch Chumkaew
Major Program	Organic Chemistry
Academic Year	2001

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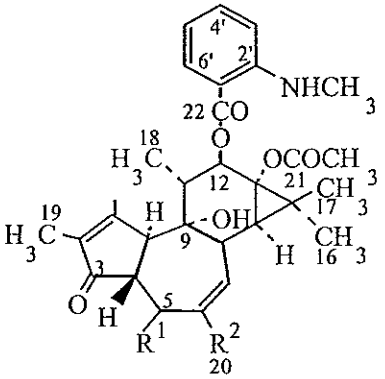
ชื่อวิทยานิพนธ์	องค์ประกอบทางเคมีจากผลสมอทะเล (*Sapium indicum*)
ผู้เขียน	นางสาวปรินุช ชุมแก้ว
สาขาวิชา	เคมีอินทรีย์
ปีการศึกษา	2544

บทคัดย่อ

ส่วนสกัดหยาบเฮกเซนและไดคลอโรมีเทนของผลสมอทะเล นำมาแยกและทำให้บริสุทธิ์ด้วยวิธีทางโครมาโทกราฟี สามารถแยกสารใหม่ได้จำนวน 2 สาร ได้แก่ 12-(N-methylaminobenzoyl)-4β,5,20-trideoxyphorbol-13-acetate (**PSI7**) และ 12-(N-methylaminobenzoyl)-4α,5,20-trideoxyphorbol-13-acetate (**PSI8**) และสารที่ทราบโครงสร้างแล้วจำนวน 8 สาร ซึ่งเป็นสารประเภท phorbol esters จำนวน 7 สาร **PSI1, PSI2, PSI3, PSI4, PSI5, PSI6** และ **PSI9** และสารประเภท oleanane triterpene จำนวน 1 สาร **PSI10**

โครงสร้างของสารทุกตัว วิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปี โดยเฉพาะ 1D และ 2D NMR สเปกโทรสโกปี นอกจากนี้ยังยืนยันโครงสร้างของสารที่ทราบโครงสร้าง โดยการเปรียบเทียบข้อมูลทางสเปกตรัมกับข้อมูลที่มีรายงานแล้ว



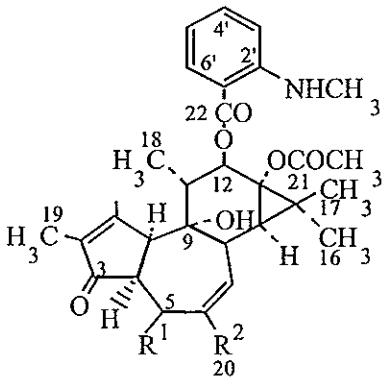
PSI1: $R^1 = H, R^2 = CH_2OH$

PSI3: $R^1 = OH, R^2 = CH_3$

PSI5: $R^1 = OH, R^2 = CH_2OH$

PSI7: $R^1 = H, R^2 = CH_3$

PSI9: $R^1 = H, R^2 = CHO$

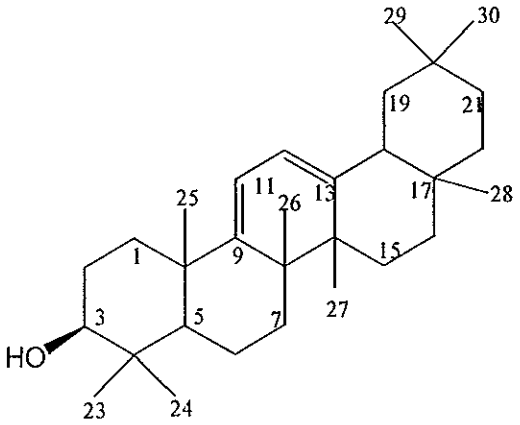


PSI2: $R^1 = H$, $R^2 = CH_2OH$

PSI4: $R^1 = OH$, $R^2 = CH_3$

PSI6: $R^1 = OH$, $R^2 = CH_2OH$

PSI8: $R^1 = H$, $R^2 = CH_3$



PSI10: 9(11),12-Oleanadien-3β-ol

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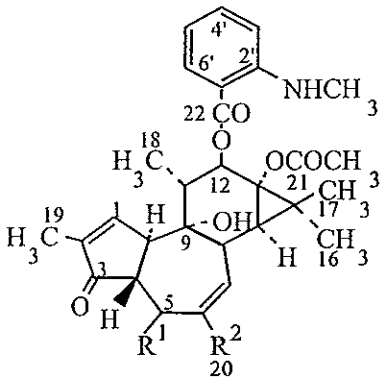
ABSTRACT

The crude hexane and dichloromethane extract from fruits of *Sapium indicum*, upon chromatographic separation, yielded two new compounds: 12-(N-methylaminobenzoyl)-4β,5,20-trideoxyphorbol-13-acetate (**PSI7**) and 12-(N-methylaminobenzoyl)-4α,5,20-trideoxyphorbol-13-acetate (**PSI8**) together with eight known compounds: seven phorbol esters (**PSI1, PSI2, PSI3, PSI4, PSI5, PSI6** and **PSI9**) and one oleanane triterpene (**PSI10**).

The structures of all compounds were elucidated by analysis of spectroscopic data especially 1D and 2D NMR. The structures of known compounds were also confirmed by comparison of the spectroscopic data with those reported in the literature.



PSI1: $R^1 = H, R^2 = CH_2OH$

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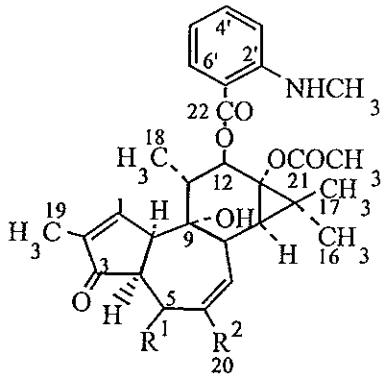

PSI2: $R^1 = H, R^2 = CH_2OH$

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PSI6: $R^1 = OH, R^2 = CH_2OH$

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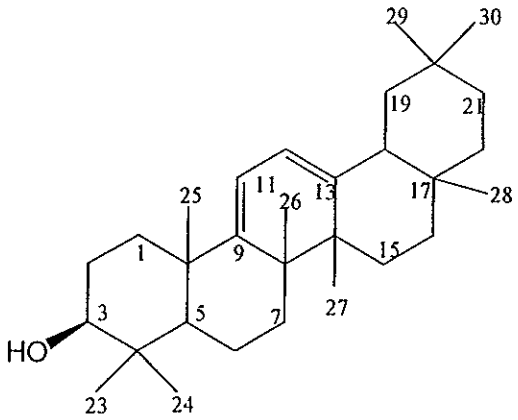

PSI10: 9(11),12-Oleanadien-3β-ol

ACKNOWLEDGEMENT

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I would like to extend my appreciation to Mr. Bunsit Watanathai for recording some 60 MHz NMR spectral data and Miss Dusanee Longjae for recording 500 MHz NMR spectral data.

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Parinuch Chumkaew

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ABBREVIATIONS AND SYMBOL

s	=	*singlet*
d	=	*doublet*
t	=	*triplet*
m	=	*multiplet*
dd	=	*doublet of doublet*
dt	=	*doublet of triplet*
br s	=	*broad singlet*
br t	=	*broad triplet*
g	=	gram
kg	=	kilogram
mg	=	milligram
mL	=	milliliter
nm	=	nanometer
cm^3	=	cubic centimeter
ft.	=	feet
in.	=	inch
%	=	percent
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
ε	=	molar extinction coefficient

ABBREVIATIONS AND SYMBOL (continued)

calc.	=	calculated
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
PLC	=	Preparative Thin Layer Chromatography
TMS	=	tetramethylsilane
$CDCl_3$	=	deuterochloroform

CHAPTER 1

INTRODUCTION

1.1 Introduction

Sapium indicum willd, a mangrove plant belonging to the Euphorbiaceae family, local names in Thailand; Samo thale (สมอทะเล) in middle part region, Kue-ro (กือเราะ), Khue-rak (คือรัก), Ku-ra (กุรา) and Ku-la (กุลา) in Malay-Peninsular (เต็ม, 2523). The family Euphorbiaceae contain about 250 genera and 4500 species. In Thailand only 53 genera and 241 species are found, from *Sapium* genera only 3 species are found; *S. baccatum, S. discolor* and *S. indicum* (ฮารง, 2527)

Figure 1 *Sapium indicum* willd.
(วุฒิ, 2540 และ เอื้อมพร, 2541)

Sapium indicum is a tree, 60 ft. high, generally much less, with short stock trunk up to 2 ft. thick, not buttressed : barks dark grey, becoming shallow and closely ridged and fissured : crown bushy, not very spreading, with upright limbs and slender, drooping or trailing, twigs strung with hanging fruits, the sprays of old trees weeping to the ground : twigs often reddish on the upperside, rather ziz-zag especially in the opening bud : twigs and leaves glabrous : young leaves in pale green sprays : old leaves yellow with reddish stalks : latex copious. Leaves-blade 3-5 in., narrowly elliptic or lanceolate, tapered gradually to a point, finely toothed or notched, the side upcurled, dark glossy green above, pale or yellowish beneath, not glaucous : stalks 13 mm. long, short, often pink on the upperside : with a minute yellow gland at the edge of the blade on either side of the base. Flower-spikes 2-5 in. long, singly from the leaves-axils or appearing terminal : female flowers solitary at the base of the spike, the style with 3 long arms, and the remainder of the spike set with male flowers, or the whole of the spike male. Fruits 1 in. wide, rather large, round, hard green then brownish grey, slightly rough, stalked, with 3 sepals at the base, containing 3 seeds, each with a light brown, woody shell, the latex copious in unripe fruits. Seeds 0.5 in. long, ellipsoid, slightly compressed; testa pale, polish. (Hooker, 1885)

1.2 Chemical constituents from Sapium genus

Informations from NAPRALERT database developed by University of Illinois at Chicago reported constituents from 12 species of the *Sapium* genus (*S. aucuparium, S. baccatum, S. cornutum, S. discolor, S. eugeniaefolium, S. indicum, S. insigne, S. japonicum, S. lateriflorum, S. pachstachys, S. rigidifolium* and *S. sebiferum*). These compounds are presented in **Table 1.**

Table 1 Compounds from plants of the *Sapium* genus

1 = Alicyclic **2 = Alkaloid** **3 = Benzenoid**

4 = Carbohydrate **5 = Coumarin** **6 = Diterpene**

7 = Flavonol **8 = Lipid** **9 = Steroid**

10 = Tannin **11 = Triterpene**

Scientific name	part	Compound	Structure	Bibliography
S. aucuparium	barks	β-D-fructose	**4a**	Saleh, *et al.*,
		β-D-glucose	**4b**	1969
	leaves	ellagic acid	**5a**	
		sucrose	**4c**	
S. baccatum	barks	aleuritolic acid	**11a**	Saha, *et al.*,
		3-O-acetyl aleuritolic acid	**11b**	1990
		baccatin	**11e**	
		β-sitosterol	**9a**	
		taraxerol	**11z**	
		taraxerone	**11aa**	
	leaves	bukittinggine	**2a**	Dayar, *et al.*, 1990
S. cornutum	barks	cucurbitacin B	**11f**	Tessier, *et al.*,
		cucurbitacin E	**11g**	1975
		cucurbitacin J	**11h**	
		cucurbitacin K	**11i**	

Table 1 (Continued)

Scientific name	Part	Compound	Structure	Bibliography
S. discolor	leaves	ellagic acid	**5a**	Hui, *et al.*, 1968
		lupeol	**11p**	
		β-sitosterol	**9a**	
		taraxerol	**11z**	
S. eugeniaefo-lium	leaves	α-amyrin	**11d**	Ahmad, *et al.*, 1991
		β-amyrin	**11c**	
		ellagic acid	**5a**	
		kaemperol	**7c**	
		β-sitosterol	**9a**	
	stem-barks	hopanoic acid	**11o**	Rusia, *et al.*, 1988
		moretenone	**11v**	
		urs-20(30)-ene-3-ol-28-oic acid	**11ac**	
S. indicum	fruits	4α-deoxyphorboldehyde	**6a**	Taylor, *et al.*, 1981
		4β-deoxyphorboldehyde	**6b**	
		4α-deoxy-13, 20-diacetoxy-5-hydroxy	**6c**	
		4β-deoxy-13, 20-diacetoxy-5-hydroxy	**6d**	
		4α-deoxy-12, 13, 20-triacetoxy	**6o**	

Table 1 (Continued)

Scientific name	part	Compound	Structure	Bibliography
S. indicum	fruits	4α-deoxy-13, 20-diacetoxy--12-O-(2-methylaminobenzoyl)	**6e**	Edwards, *et al.*, 1983
		4β-deoxy-13, 20-diacetoxy--12-O-(2-methylaminobenzoyl)	**6f**	
		4α-deoxy-5-hydroxy-12-O-(2-methylaminobenzoyl)	**6z**	
		4α-deoxy-5-hydroxy--12-O-(deca-2, 4, 6- trienoyl)	**6k**	
		4β-deoxy-5-hydroxy--12-O-(deca-2, 4, 6- trienoyl)	**6l**	
		4β-deoxy-12-O-(2-methyl-aminobenzoyl)-phorbaldehyde	**6b**	
		4α-deoxy-20-hydroxy-12-O-(deca-2,4,6-trienoyl)	**6m**	
		4β-deoxy-20-hydroxy12-O-(deca-2,4,6-trienoyl)	**6n**	
		4α-deoxy-12-O-(2-methyl-aminobenzoyl)-phorbaldehyde	**6a**	

Table 1 (Continued)

Scientific name	Part	Compound	Structure	Bibliography
S. indicum	fruits	4α, 20-dideoxy-5-hydroxy-12-O-(2-methylamino-benzoyl)	**6z**	Edwards, *et al.*, 1983
		4α, 20-dideoxy-5-hydroxy-12-O-(deca-2, 4, 6-trienoyl)	**6g**	
		4β, 20-dideoxy-5-hydroxy-12-O-(deca-2, 4, 6-trienoyl)	**6h**	
		4α, 20-dideoxy-5-hydroxy-12-O-tetradecanoyl	**6i**	
		4β, 20-dideoxy-5-hydroxy-12-O-tetradecanoyl	**6j**	
		sapatoxin A	**6af**	Taylor, *et al.*, 1982
		sapatoxin B	**6ag**	
		sapatoxin C	**6ah**	
		4α-sapinine	**6v**	
		sapintoxin A	**6u**	Taylor, *et al.*, 1981
		sapintoxin B	**6w**	
		sapintoxin C	**6y**	
		sapintoxin D	**6aa**	

Table 1 (Continued)

Scientific name	Part	Compound	Structure	Bibliography
S. indicum	fruits	sapintoxins A	**6ab**	Taylor, *et al.*,
		sapintoxins B	**6ac**	1981
		sapintoxins C	**6ad**	
		sapintoxins D	**6ae**	
	leaves	ellagic acid	**5a**	Kiamuddin, *et*
		gallic acid	**3a**	*al.*, 1979
		lupeol	**11p**	
		β-sitosterol	**9a**	
S. insigne	leaves	12-O-hexanoyl	**6ar**	Taylor, *et al.*,
		16-hydroxy-12-O-(deca-cis-2-cis-4-dienoyl)	**6aq**	1983
		16-hydroxy-12-O-dodeca-noyl	**6as**	
		16-hydroxy-12-O-hexanoyl	**6at**	
	roots	cycloart-23-en-25-ol	**11j**	Srivastava, *et* *al.*, 1985
		3-hydroxy-5, 7, 8-tri methyloxy	**7b**	Saxena, *et al.*, 1986

Table 1 (Continued)

Scientific name	Part	Compound	Structure	Bibliography
S. insigne	seeds	β-sitosterol	**9a**	Mukharya and
		taraxasterol	**11y**	Ansari, 1986
S. japonicum	leaves	afzelin	**7a**	Matsuda,
		ellagic acid	**5a**	1966
		furosine	**10a**	
		gallic acid	**3a**	
		kaempferol	**7c**	
		quercetin	**7d**	
		isoquercetrin	**7e**	
		rutin	**7f**	
		shikimic acid	**1a**	
		trifolin	**7g**	
		12-O-n-deca-2, 4, 6-trienoyl	**6k**	
S. lateriflorum	leaves	phorbol	**6s**	Taylor, *et al.*, 1981
S. pachstachys	leaves	cycloartanone	**11l**	Siems, *et al.*,
		cycloartenol	**11k**	1993
		lupenone	**11r**	
S. rigidifolium	barks	rigidol	**6t**	Siems, *et al.*,
		stigmasterol	**9b**	1993
		β-sitosterol	**9a**	

Table 1 (Continued)

Scientific name	Part	Compound	Structure	Bibliography
S. rigidifolium	leaves	aleuritolic acid	**11a**	Siems, *et al.*,
		β-amyrin	**11c**	1993
		kaur-16-ene	**6p**	
		kaurane-16-alpha-17-diol	**6q**	
		kauranoic acid	**6r**	
		lupenone	**11r**	
		lupeol	**11p**	
S. sebiferum	leaves	aleuritolic acid	**11a**	Pradhan, *et al.*,
		3,4-di-O-methylellagic acid	**5b**	1984
		ellagic acid	**5a**	
		friedelan-3-one	**11n**	
		friedelin	**11m**	Chen, *et al.*,
				1991
		furosine	**10a**	Neera, *et al.*,
		gallic acid	**3a**	1992
		gallic acid ethyl ester	**3c**	
		gallic acid methyl ester	**3b**	
		galloyl-β-d-glucose	-	
		galloyl--d-glucose	**10b**	Chen, *et al.*,
		12-O-hexanoyl	**6ar**	1991
		kaempferol	**7c**	

Table 1 (Continued)

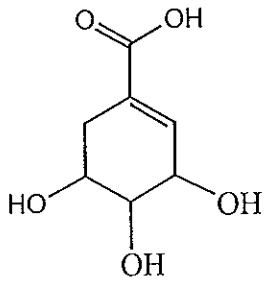
Scientific name	Part	Compound	Structure	Bibliography
S. sebiferum	leaves	loliolide	**8c**	Liu, *et al.*,
		quercetin	**7d**	1988
		isoquercetrin	**7e**	
		sebiferenic acid	**11w**	Pradhan, *et*
		sebiferic acid	**11x**	*al.*, 1984
		shikimic acid	**1a**	Matsuda, *et*
		β-sitosterol	**9a**	*al.*, 1966
		stigmasterol	**9b**	
	roots	sapium factor s-1	**6ai**	Seip, *et al.*,
		sapium factor s-2	**6aj**	1983
		sapium factor s-3	**6ak**	
		sapium factor s-4	**6al**	
		sapium factor s-5	**6am**	
		sapium factor s-6	**6an**	
		sapium factor s-7	**6ao**	
		sapium factor s-8	**6ap**	
	seeds	deca-2, 4-dienoic acid	-	
		deca-4, 6-dienoic acid	-	
		linoleic acid	**8a**	
		linolenic acid	**8b**	

Table 1 (Continued)

Scientific name	Part	Compound	Structure	Bibliography
S. sebiferum	seeds	oleic acid	-	
		sapintoxin A	**6c**	Brooks, *et al.*,
		sapintoxin c	**6g**	1987
		tocotrienol	**11ab**	
	stem-barks	lup-20(29)-en-3-β-17-β-diol	**11t**	
		lup-20(29)-en-3-β-ol-28-oic acid	**11s**	
		lupeol	**11p**	Srivastara, *et*
		moretenol	**11u**	*al.*, 1981
		moretenone	**11v**	
		stigmasterol	**9b**	

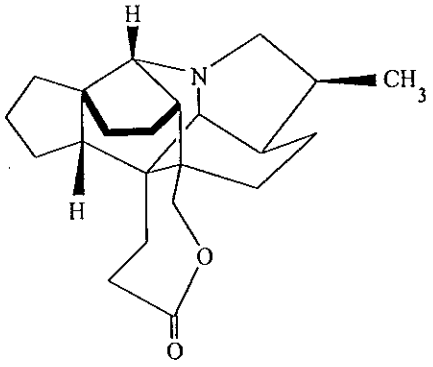
Structures of compounds from Sapium genus

1. Alicyclic



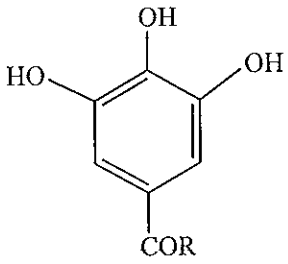
1a : shikimic acid

2. Alkaloid



2a : bukittinggine

3. Benzenoid

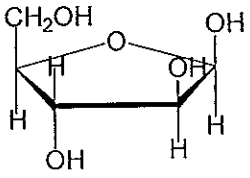


3a : R = OH; gallic acid

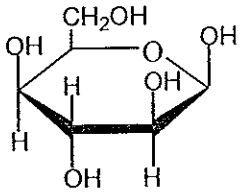
3b : R = OCH_3; gallic acid methyl ester

3c : R = OCH_2CH_3; gallic acid ethyl ester

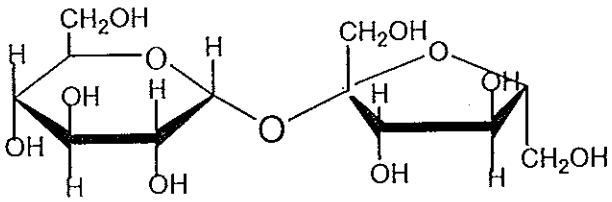
4. Carbohydrate



4a : β-D-fructose

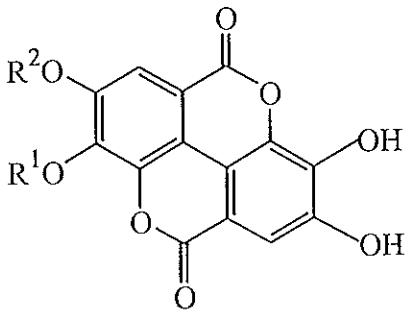


4b : β-D-glucose



4c : sucrose

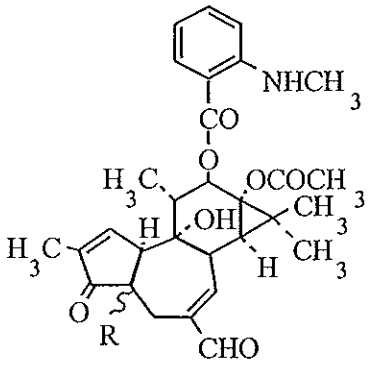
5. Coumarin



5a : $R^1 = R^2 = H$; ellagic acid

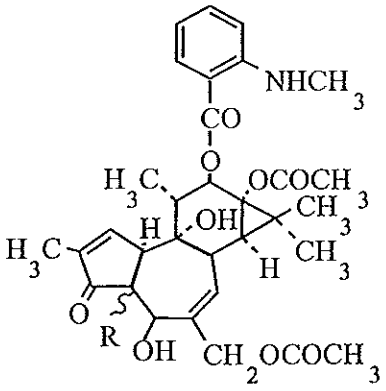
5b : $R^1 = R^2 = CH_3$; 3,4-di-O-methylellagic acid

6. Diterpene



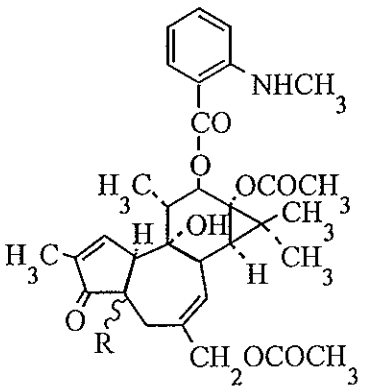
6a : R = αH; 4α-deoxyphorbaldehyde

6b : R = βH; 4β-deoxyphorbaldehyde



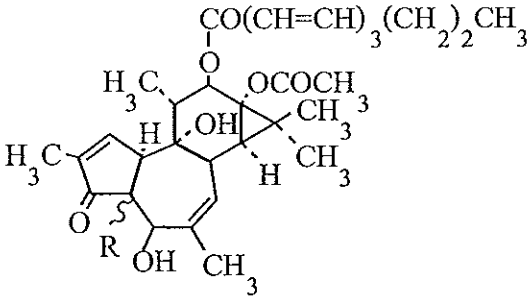
6c : R = αH; 4α-deoxy-13,20-diacetoxy-5-hydroxy

6d : R = βH; 4β-deoxy-13,20-diacetoxy-5-hydroxy



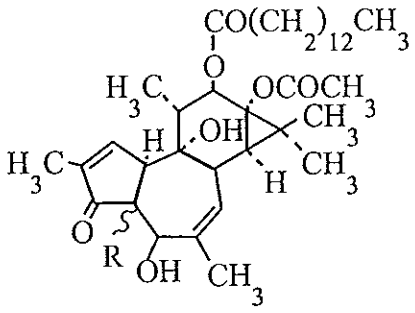
6e : R = αH; 4α-deoxy-13,20-diacetoxy-12-O-(2-methylaminobenzoyl)

6f : R = βH; 4β-deoxy-13,20-diacetoxy-12-O-(2-methylaminobenzoyl)



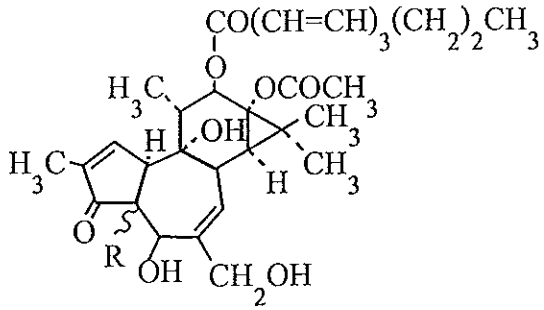
6g : R = αH; 4α,20-dideoxy-5-hydroxy-12-O-(deca-2,4,6-trienoyl)

6h : R = βH; 4β,20-dideoxy-5-hydroxy-12-O-(deca-2,4,6-trienoyl)



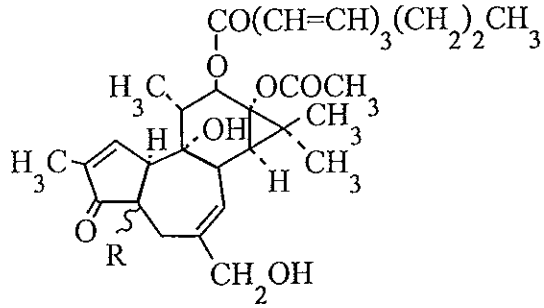
6i : R = αH; 4α,20-dideoxy-5-hydroxy-12-O-(tetradecanoyl)

6j : R = βH; 4β,20-dideoxy-5-hydroxy-12-O-(tetradecanoyl)



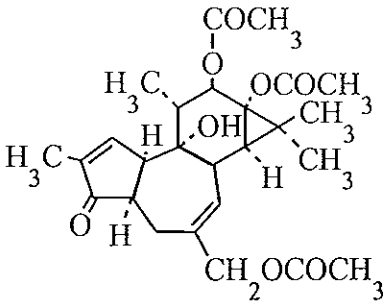
6k : R = αH; 4α-deoxy-5-hydroxy-12-O-(deca-2,4,6-trienoyl)

6l : R = βH; 4β-deoxy-5-hydroxy-12-O-(deca-2,4,6-trienoyl)

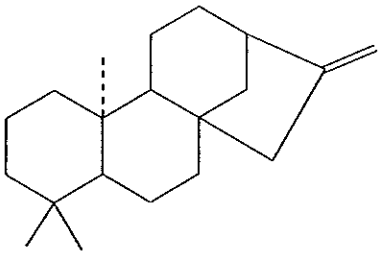


6m : R = αH; 4α-deoxy-20-hydroxy-12-O-(deca-2,4,6-trienoyl)

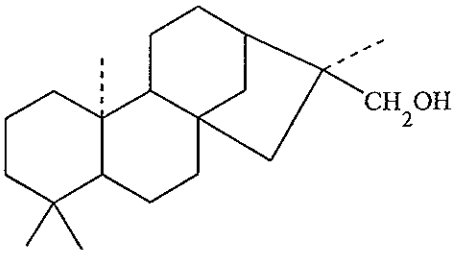
6n : R = βH; 4β-deoxy-20-hydroxy-12-O-(deca-2,4,6-trienoyl)



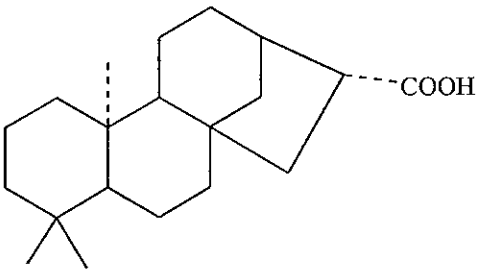
6o : 4α-deoxy-12,13,20-triacetoxy



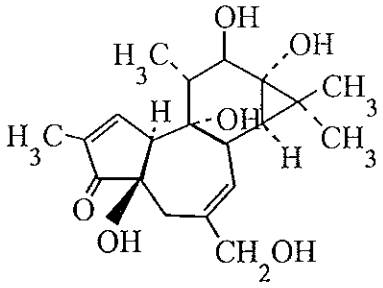
6p : kaur-16-ene



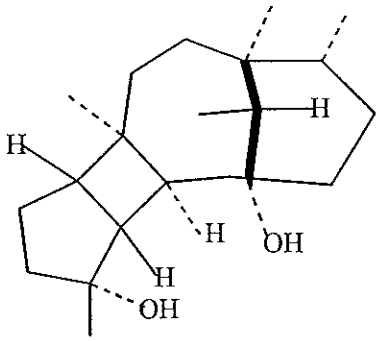
6q : kaurne-16α-17-diol



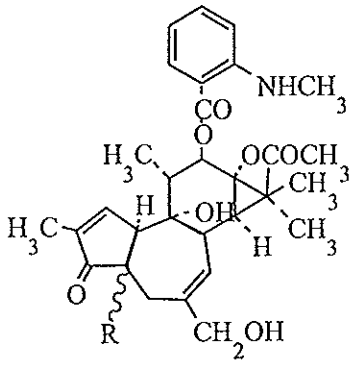
6r : kauranoic acid



6s : phorbol

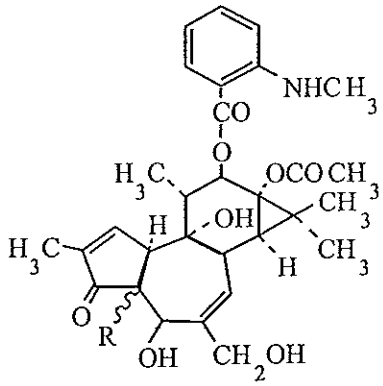


6t : rigidol



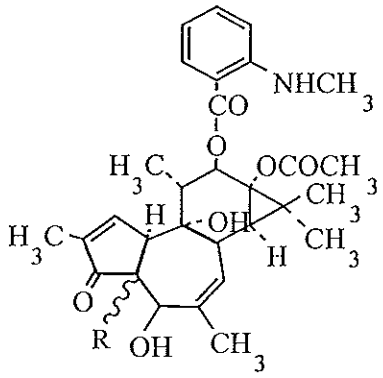
6u : R = βH; sapintoxin A

6v : R = αH; 4α- sapinine



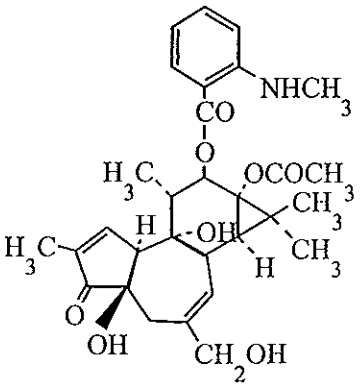
6w : R = βH; sapintoxin B

6x : R = αH; 4α-deoxy-5- hydroxy-12-O-(2-methylaminobenzoyl)

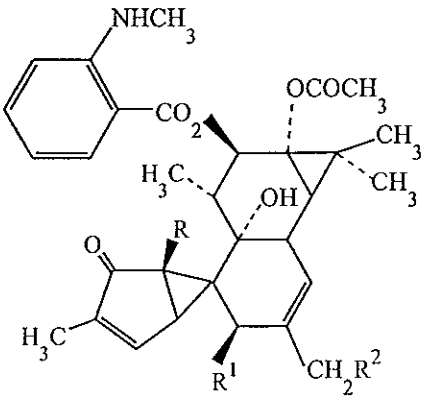


6y : R = βH; sapintoxin C

6z : R = αH; 4α,20-dideoxy-5-hydroxy-12-O-(2-methylaminobenzoyl)



6aa : sapintoxin D

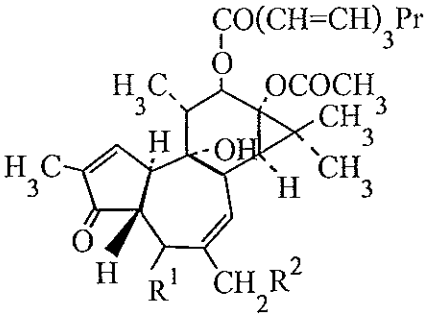


6ab : R = R^1 = H, R^2 = OH; sapintoxins A

6ac : R = H, R^1 = R^2 = OH; sapintoxins B

6ad : R = R^2 = H, R^1 = OH; sapintoxins C

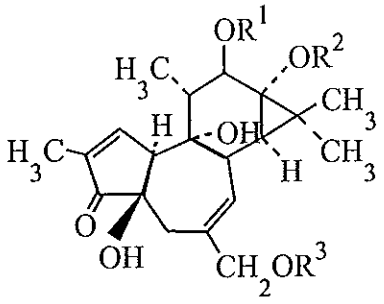
6ae : R = R^2 = OH, R^1 = H; sapintoxins D



6af : $R^1 = H$, $R^2 = OH$; sapatoxins A

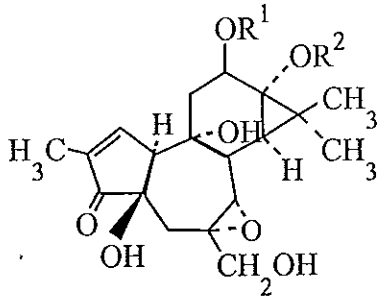
6ag : $R^1 = OH$, $R^2 = OH$; sapatoxins B

6ah : $R^1 = OH$, $R^2 = H$; sapatoxins C

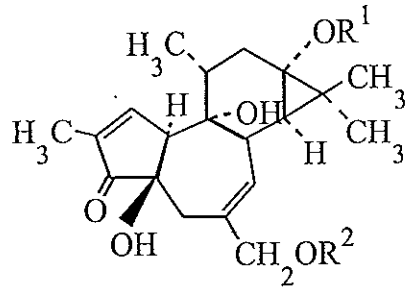


6ai : $R^1 = CO(CH_2)_{14}CH_3$, $R^2 = COCH_3$, $R^3 = H$;
sapium factor s-1

6aj : $R^1 = COC_{17}H_{33}$, $R^2 = COCH_3$, $R^3 = H$;
sapium factor s-2



6ak : $R^1 = CO(CH_2)_{14}CH_3$, $R^2 = COCH_3$;
sapium factor s-3



6al : $R^1 = CO(CH_2)_{14}CH_3$, $R^2 = H$;
sapium factor s-4

6am : $R^1 = CO(CH_2)_{12}CH_3$, $R^2 = H$;
sapium factor s-5

6an : $R^1 = COC_{13}H_{25}$, $R^2 = H$;

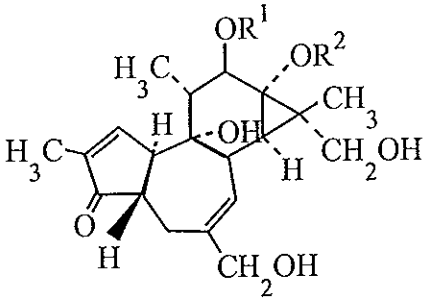
sapium factor s-6

6ao : $R^1 = COC_{13}H_{23}$, $R^2 = H$;

sapium factor s-7

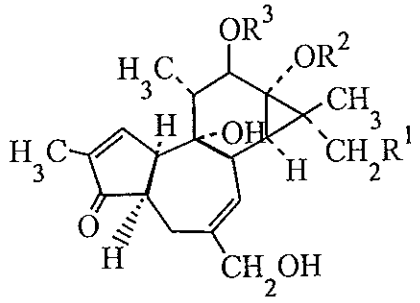
6ap : $R^1 = COC_{13}H_{21}$, $R^2 = H$;

sapium factor s-8



6aq : $R^1 = COCH{=}CHCH{=}CH(CH_2)_4CH_3$

$R^2 = COCH_3$; 16-hydroxy-12-O-(deca-cis-2-cis-4-dienoyl)



6ar : $R^1 = H$, $R^2 = COCH_3$, $R^3 = CO(CH_2)_4CH_3$;

12-O-hexanoyl

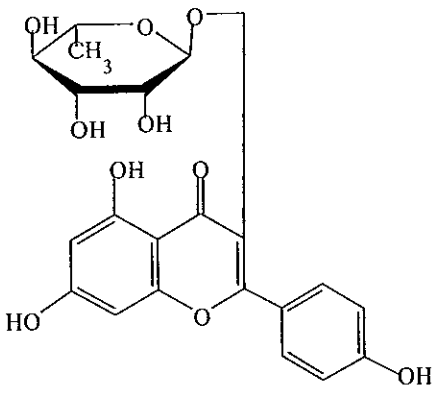
6as : $R^1 = OH$, $R^2 = COCH_3$, $R^3{=}CO(CH_2)_4CH_3$;

16-hydroxy-12-O-hexanoyl

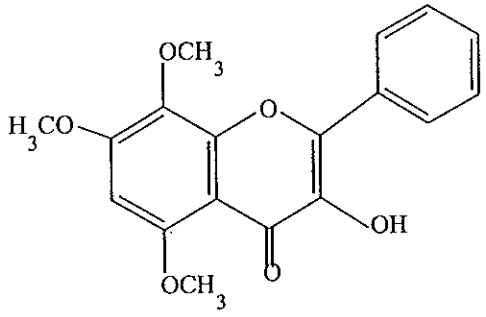
6at : $R^1 = OH$, $R^2 = COCH_3$, $R^3{=}CO(CH_2)_{10}CH_3$;

16-hydroxy-12-O-dodecanoyl

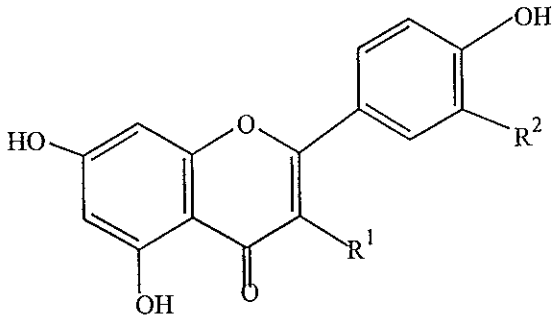
7. Flavonol



7a : afzelin



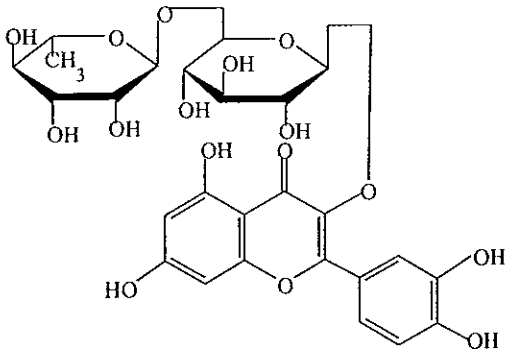
7b : 3-hydroxy-5, 7, 8-trimethyloxy



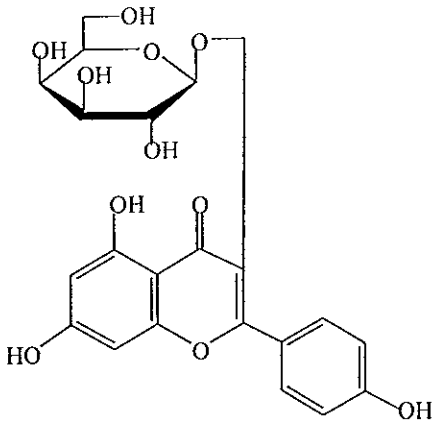
7c : R^1 = OH, R^2 = H; kaempferol

7d : R^1 = R^2 = OH; quercetin

7e : R^1 = glucosyl, R^2 = OH; isoquercetrin



7f : rutin

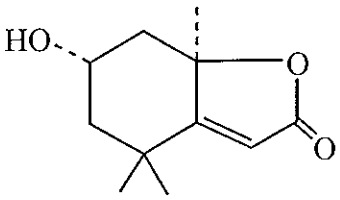


7g : trifolin

8. Lipid

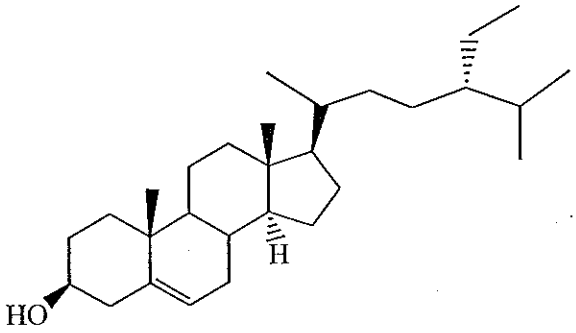
$CH_3(CH_2CH{=}CH)_2(CH_2)_7COOH$ **8a :** linoleic acid

$CH_3(CH_2CH{=}CH)_3(CH_2)_7COOH$ **8b :** linolenic acid

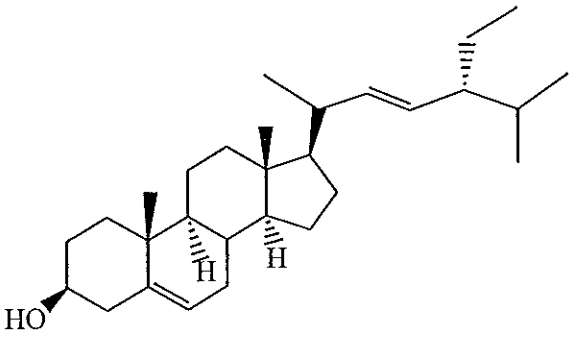


8c : loliolide

9. Steroid

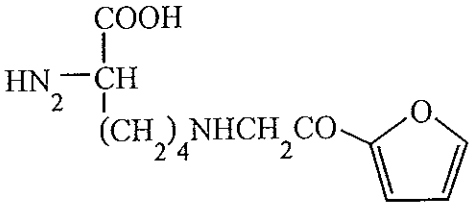


9a : β-sitosterol

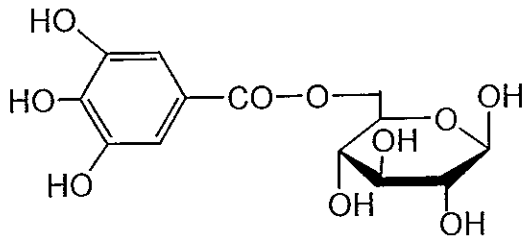


9b : stigmaserol

10. Tannin

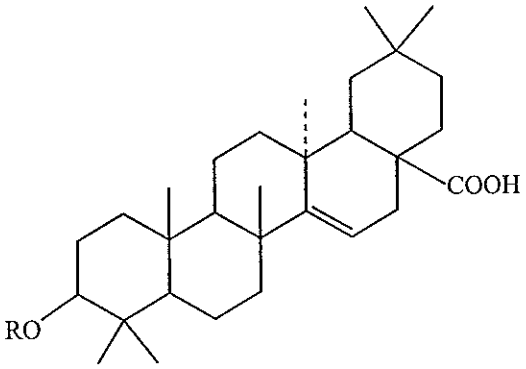


10a : furosine



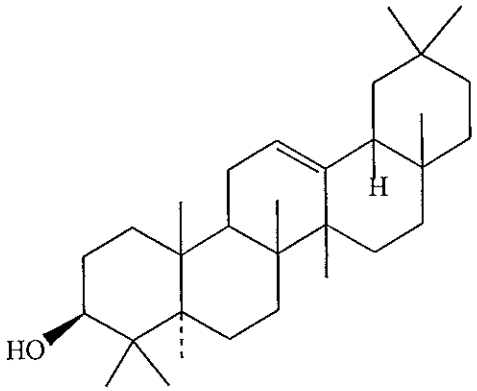
10b : galloyl-D-glucose

11. Triterpene

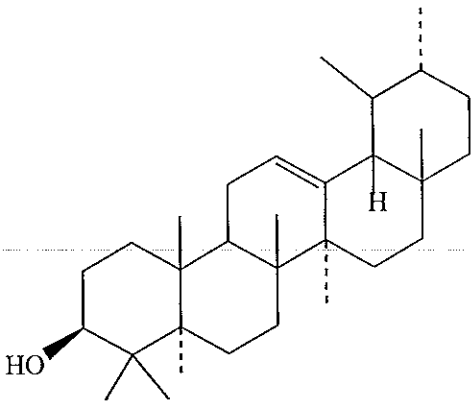


11a : R = H; aleuritolic acid

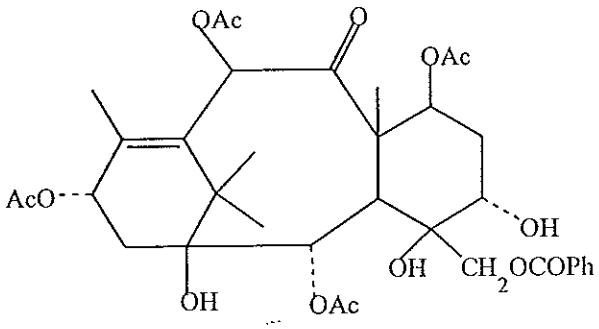
11b : R = $COCH_3$; 3-O-acetyl aleuritolic acid



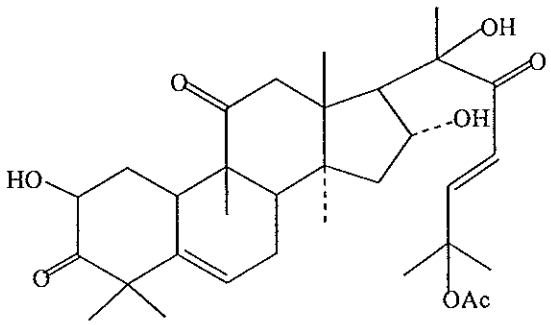
11c : β-amyrin



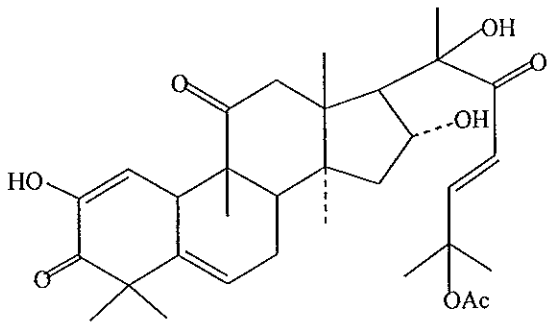
11d : α-amyrin



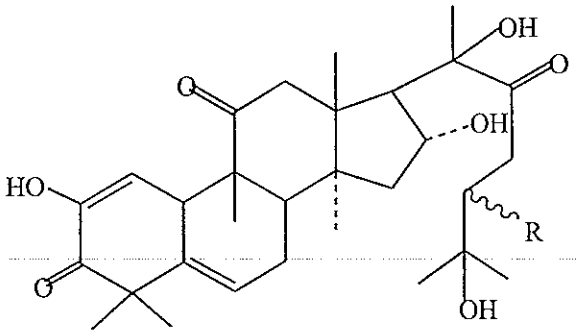
11e : baccatin



11f : cucurbitacin B

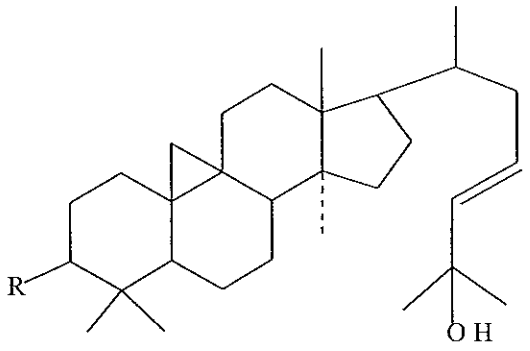


11g : cucurbitacin E



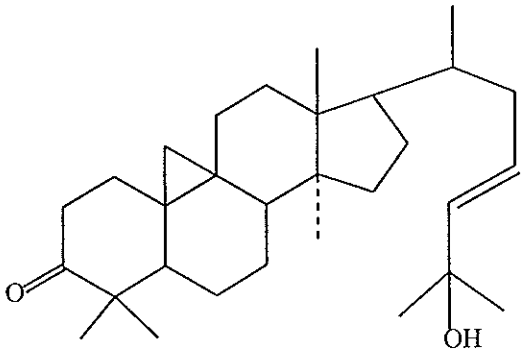
11h : R = β-OH; cucurbitacin J

11i : R = α-OH; cucurbitacin K

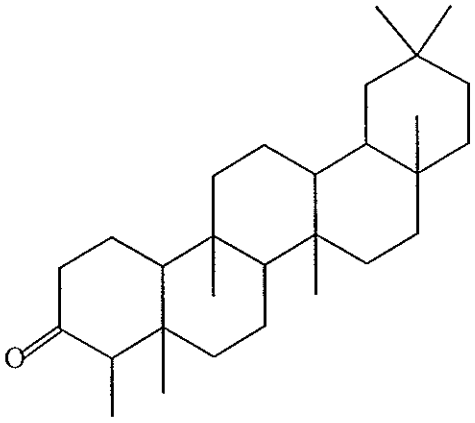


11j : R = OAc; 3-O-acetyl cycloart-23-en-25-ol

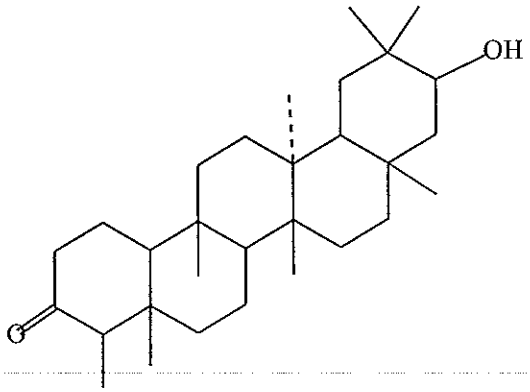
11k : R = OH; cycloartenol



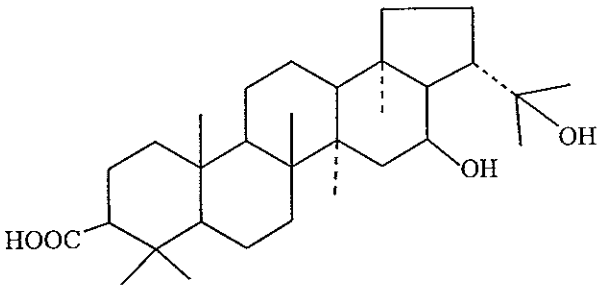
11l : cycloartenone



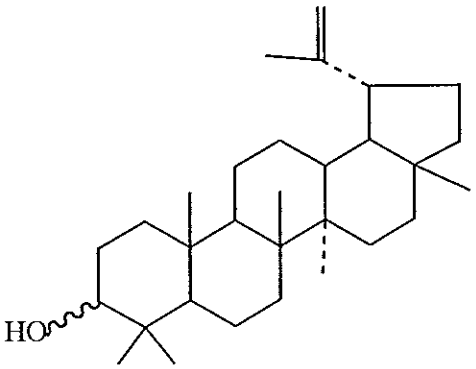
11m : friedelin



11n : friedelan-3-one

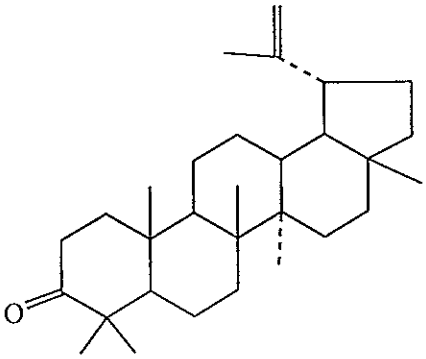


11o : hopanoic acid

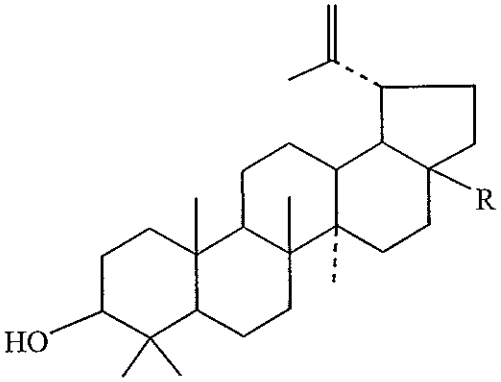


11p : lupeol

11q : 3-epi lupeol

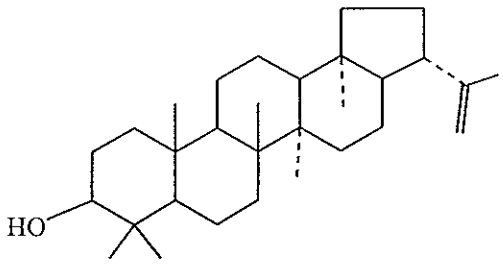


11r : lupenone

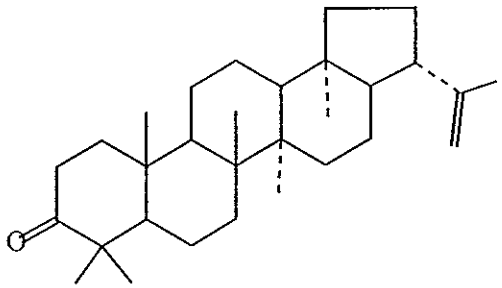


11s : lup-20(29)-en-3β-ol-28-oic acid

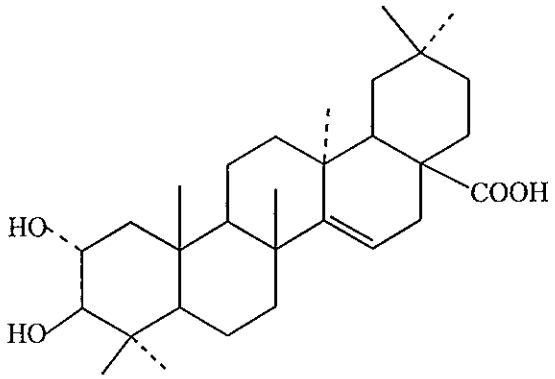
11t : lup-20(29)-en-3β-ol-17β-diol



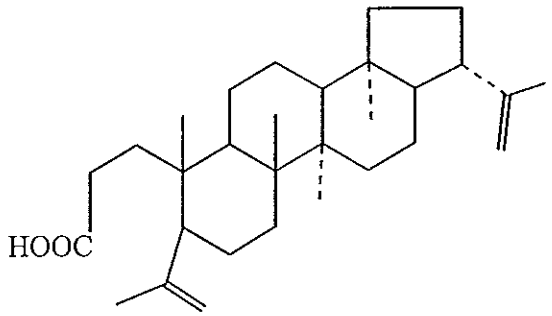
11u : moretenol



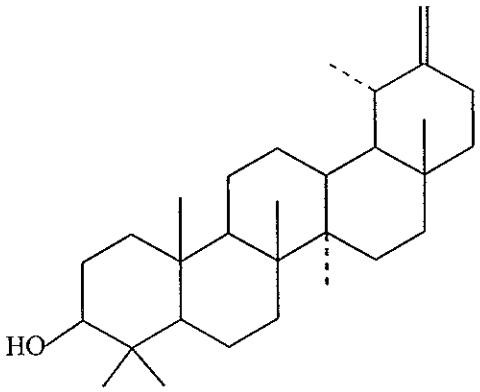
11v : moretenone



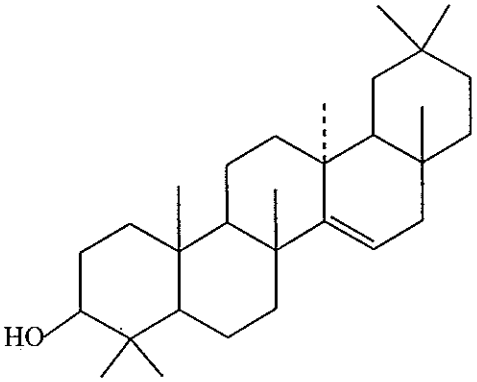
11w : sebiferenic acid



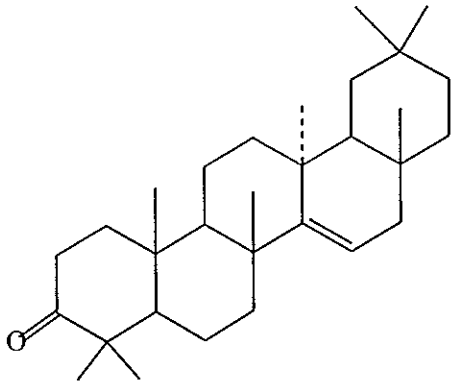
11x : sebiferic acid



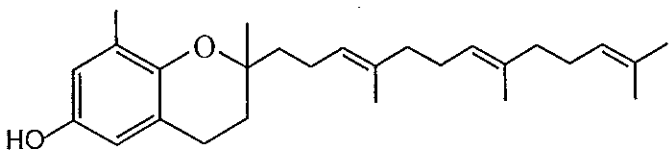
11y : taraxasterol



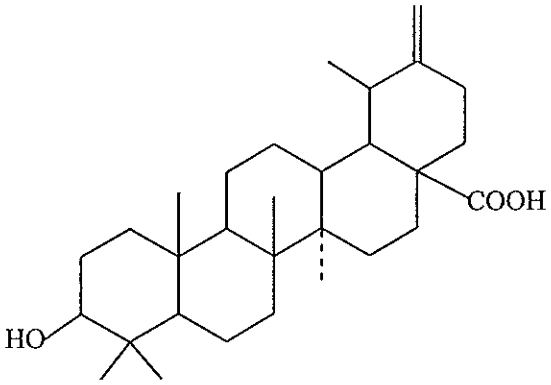
11z : taraxerol



11aa : taraxerone



11ab : tocotrienol



11ac : urs-20(30)-ene-3-ol-28-oic acid

This research involved isolation, purification and structure elucidation of fruits of *sapium indicum*

CHAPTER 2

EXPERIMENTAL

2.1 Chemicals and Instruments

Ultraviolet spectra (UV) were measured with UV-160A spectrophotometer (SHIMADZU) and principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in chloroform solution. Infrared spectra (IR) were obtained on a Perkin-Elmer 1750 FT-IR spectrophotometer and recorded in wavenumber (cm^{-1}). ^{1}H and ^{13}C-Nuclear magnetic resonance spectra (^{1}H and ^{13}C NMR) were recorded on a Varian UNITY INOVA 500 MHz in deuterochloroform solution with tetramethylsilane (TMS) as an internal standard. Spectra were recorded as chemical shift parameter (δ) value in ppm down field from TMS (δ 0.00). Optical rotation was measured in chloroform solution with sodium D line (590 nm) on an AUTOPOL^{R} II automatic polarimeter. The solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except for diethyl ether and ethyl acetate which were analytical grade reagents. Plates of silica gel GF_{254}, 20 x 20 cm, thickness 1.25 nm, activated at 110 ^{o}C for 3 hours were utilized in the case of preparative TLC. Pre-coated plates of silica gel 60 GF_{254} were used for analytical purposes. Quick column chromatography was performed on silica gel 60 GF_{254} (Merck). Column chromatography was performed by using silica gel (Merck) type 100 (70-230 mesh ASTM).

2.2 Plant material

The fruits of *Sapium indicum* were collected in Songkhla Province, Thailand in May 2000 and identified by Department of Biology, Faculty of Science, Prince of Songkla University, Hat-Yai, Thailand.

2.3 Isolation of Compounds from Fruits

Air-dried fruits of *Sapium indicum* (Euphorbiaceae) 3.8 kg were extracted twice with hexane (2x15 L), dichloromethane (2x12 L), and methanol (2x10 L) at room temperature, seven days for each solvent. The first investigation was a preliminary study to separate and purify the major components as well as to find out the suitable purification process. The objectives of the second and third were to increase the amount of minor components and to purify other minor components which could not be separated in the first investigation.

The First Investigation

The hexane extract was partially concentrated under reduced pressure to give yellow solid (2 g) which was filtered and the filtrate was further evaporated to dryness to afford a yellow gum (17 g). The yellow solid (2 g) was partially purified by column chromatography on silica gel (50 g) and eluted with a step gradient of hexane/ ethyl acetate (from 0% to 100% ethyl acetate) followed by ethyl acetate/ methanol (from 10% to 100% methanol). Solvents were removed by evaporation under reduced pressure. Fractions were checked on TLC using UV light and vanillin-H_2SO_4 spray

reagent. Fractions with similar TLC chromatograms were combined to afford eight fractions, as shown in **Table 2.**

Table 2 Fractions obtained from yellow solid by column chromatography

Fraction	Physical appearance	Weight (mg)
F1	orange-yellow viscous-liquid	60.2
F2	orange-yellow viscous-liquid	29.8
F3	brown-yellow viscous-liquid	35.4
F4	brown-yellow viscous-liquid	64.2
F5	brown-yellow viscous-liquid	20.0
F6	brown-yellow viscous-liquid	52.4
F7	brown-yellow viscous-liquid	600.8
F8	brown-yellow viscous-liquid	45.5

Fraction F7 was further purified by column chromatography on silica gel. Elution was conducted with a step gradient of chloroform/ ethyl acetate (from 0% to 100% ethyl acetate) followed by ethyl acetate/ methanol (from 10% to 100% methanol). Fractions with similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford five subfractions, as shown in **Table 3.**

Table 3 Subfractions obtained from fraction 7 by column chromatography

Fraction	Physical appearance	Weight (mg)
f7.1	yellow viscous-liquid	115.2
f7.2	yellow viscous-liquid	202.6
f7.3	yellow viscous-liquid	57.0
f7.4	yellow viscous-liquid	98.0
f7.5	yellow viscous-liquid	75.5

Subfraction f7.2 was further purified by preparative TLC on silica gel developing with 30% ethyl acetate in hexane to give a pure compound as a yellow viscous-liquid (140 mg), R_f 0.16 (20% hexane : ether). It was named **PSI1.**

$[\alpha]_D^{28}$	$+18.5^{\circ}$	(c = 5.4×10^{-3} g/ 10 cm^3, $CHCl_3$)
UV ($CHCl_3$) λ_{max} nm (log ε)		252.0 (5.25), 360.0 (4.94)
FT-IR (neat) ν_{cm-1}		3389 (O-H stretching), 1720, 1687 (C=O stretching)
^{1}H NMR ($CDCl_3$)(500MHz)		7.81 (*dd*, *J* = 1.5, 8.5 Hz, H-6'), 7.56 (*br s*, H-1), 7.40 (*dt*, *J* = 1.5, 8.5, 8.5 Hz, H-4'), 6.69 (*dd*, *J* = 1.5, 8.5 Hz, H-3'), 6.59 (*dt*, *J* = 1.5, 8.5, 8.5 Hz, H-5'), 5.65 (*d*, *J* = 10 Hz, H–12), 5.55 (*m*, H-7), 4.02 (*AB*, *J* = 13.5 Hz, 2H-20), 3.27 (*m*, H-10), 2.93 (*s*, $C\underline{H}_3N$), 2.86 (*dd*, *J* = 9.5, 18 Hz, Hβ-5), 2.52 (*dt*, *J* = 5, 9.5, 9.5 Hz, H-4), 2.44 (*br t*, H-

2.52 (*dt*, J = 5, 9.5, 9.5 Hz, H-4), 2.44 (*br t*, H-8), 2.17 (*dd*, J = 10, 18 Hz, H_{α}-5), 2.12 (*s*, $C\underline{H}_3CO$), 1.73 (*s*, 3H-19), 1.73 (*m*, superimposed with 3H-19, H-11), 1.32 (*s*, 3H-17), 1.19 (*s*, 3H-16), 1.12 (*d*, J = 5 Hz, H-14), 0.96 (*d*, J = 6.5 Hz, 3H-18).

^{13}C NMR ($CDCl_3$)(125MHz)		209.5, 173.7, 168.1, 159.5, 152.3, 142.1, 136.4, 134.9, 131.3, 126.5, 114.4, 110.8, 109.4, 77.8, 76.2, 67.4, 65.5, 54.1, 44.2, 42.6, 42.1, 35.7, 29.7, 29.6, 25.7, 23.7, 21.1, 16.8, 15.0, 10.1
DEPT 135°	CH_3:	29.7, 23.7, 21.1, 16.8, 15.0, 10.1
	CH_2:	67.4, 29.6
	CH:	159.5, 134.9, 131.3, 126.5, 114.4, 110.8, 76.2, 54.1, 44.2, 42.6, 42.1, 35.7
DEPT 90°	CH:	159.5, 134.9, 131.3, 126.5, 114.4, 110.8, 76.2, 54.1, 44.2, 42.6, 42.1, 35.7

Subfractions f7.3 and f7.4 were further purified by preparative TLC on silica gel developing with 30% ethyl acetate in hexane to give a pure compound as a yellow viscous-liquid (23.2 mg), R_f 0.21 (20% hexane : ether) It was named **PSI2.**

$[\alpha]_D^{28}$ -52.6° ($c = 5.7 \times 10^{-3}$ g/ 10 cm^3, $CHCl_3$)

UV ($CHCl_3$) λ_{max} nm (log ε)		251.5 (5.23), 360.5 (4.91)
FT-IR (neat) ν_{cm-1}		3388 (O-H stretching), 1721, 1687 (C=O stretching)
^{1}H NMR ($CDCl_3$)(500 MHz)		7.88 (*dd*, J = 1.5, 8 Hz, H-6'), 7.42 (*dt*, J = 1.5, 8, 8 Hz, H-4'), 7.08 (*s*, H-1), 6.70 (*d*, J = 8.5 Hz, H-3'), 6.62 (*t*, J = 8 Hz, H-5'), 5.72 (*d*, J = 10.5 Hz, H-12), 5.14 (*br s*, H-7), 4.01 (*AB*, J = 12.5 Hz, 2H-20), 3.54 (*m*, H-10), 3.45 (*dd*, J = 3.5, 16 Hz, H$_\beta$-5), 2.93 (*br s*, C$\underline{H}_3$N), 2.80 (*m*, H-4), 2.50 (*dd*, J = 5, 16 Hz, H$_\alpha$-5), 2.08 (*s*, C$\underline{H}_3$CO), 2.03 (*m*, H-8), 1.87 (*m*, H-11), 1.80 (*s*, 3H-19), 1.33 (*s*, 3H-17), 1.17 (*s*, 3H-16), 1.12 (*d*, J = 6.5 Hz, 3H-18), 0.84 (*d*, J = 5 Hz, H-14).
^{13}C NMR ($CDCl_3$)(125 MHz)		213.8, 174.1, 168.6, 156.5, 152.8, 143.7, 137.5, 135.2, 131.6, 126.7, 114.5, 111.0, 109.7, 78.1, 74.9, 69.2, 65.3, 49.4, 47.3, 43.3, 40.5, 36.9, 29.2, 24.9, 23.8, 20.7, 16.2, 11.5, 10.0
DEPT 135°	CH_3:	29.2, 24.9, 20.7, 16.2, 11.5, 10.0
	CH_2:	69.2, 24.9
	CH:	156.5, 135.2, 131.6, 126.7, 114.5, 111.0, 74.9, 49.4, 47.3, 43.3, 40.5, 36.9

DEPT 90°	CH:	156.5, 135.2, 131.6, 126.7, 114.5, 111.0, 74.9, 49.4, 47.3, 43.3, 40.5, 36.9

Fraction F6 was further purified by preparative TLC on silica gel developing with 30% ethyl acetate in hexane to give a pure compound as a yellow viscous-liquid (6.6 mg), R_f 0.24 (20% hexane : ether). It was named **PSI3.**

$[\alpha]_D^{28}$	+36.4°	(c = 5.5x10^{-3} g/ 10 cm^3, $CHCl_3$)
UV ($CHCl_3$) λ_{max} nm (log ε)		253.5 (5.06), 360.0 (4.74)
FT-IR (neat) ν_{cm-1}		3388 (O-H stretching), 1721, 1687 (C=O stretching)
^{1}H NMR ($CDCl_3$)(500 MHz)		7.82 (*dd*, *J* = 1.5, 8 Hz, H-6'), 7.70 (*br s*, H-1), 7.42 (*dt*, *J* = 1.5, 8, 8 Hz, H-4'), 6.71 (*d*, *J* = 8.5 Hz, H-3'), 6.59 (*dt*, *J* = 1.5, 8, 8 Hz, H-5'), 5.65 (*d*, *J* = 9.5 Hz, H–12), 5.33 (*m*, H-7), 4.86 (*br d*, *J* = 3.5 Hz, H-5), 3.54 (*m*, H-10), 2.93 (*s*, $C\underline{H}_3N$), 2.63 (*t*, *J* = 4.5 Hz, H-4), 2.34 (*br s*, H-8), 2.13 (*s*, $C\underline{H}_3CO$), 1.88 (*s*, 3H-20), 1.75 (*m*, 3H-19), 1.66 (*m*, H-11), 1.29 (*s*, 3H-17), 1.20 (*s*, 3H-16), 1.09 (*d*, *J* = 5.5 Hz, H-14), 0.97 (*d*, *J* = 6.5 Hz, 3H-18).

^{13}C NMR ($CDCl_3$)(125 MHz)		208.5, 173.6, 168.1, 162.6, 152.3, 140.6, 138.3, 134.9, 131.3, 127.3, 114.4, 110.8, 109.4, 78.3, 76.3, 71.1, 65.6, 51.5, 51.4, 43.0, 42.4, 36.5, 29.5, 25.8, 23.7, 21.7, 21.1, 16.9, 15.3, 10.1
DEPT 135°	CH_3:	29.5, 23.7, 21.7, 21.1, 16.9, 15.3, 10.1
	CH:	162.6, 134.9, 131.3, 127.3, 114.4, 110.8, 76.3, 71.1, 51.5, 51.4, 43.0, 42.4, 36.5
DEPT 90°	CH:	162.6, 134.9, 131.3, 127.3, 114.4, 110.8, 76.3, 71.1, 51.5, 51.4, 43.0, 42.4, 36.5

Fraction F4 was further purified by preparative TLC on silica gel developing with 50% ether in hexane to give a pure compound as a yellow viscous-liquid (4 mg), R_f 0.30 (20% hexane : ether). It was named **PSI9.**

$[\alpha]_D^{28}$	+55.6°	(c = 1.8×10^{-3} g/ 10 cm^3, $CHCl_3$)
UV ($CHCl_3$) λ_{max} nm (log ε)		252.5 (5.73), 360.5 (5.41)
FT-IR (neat) ν_{cm-1}		3387 (O-H stretching), 1724, 1687 (C=O stretching)

^{1}H NMR ($CDCl_3$)(500 MHz)		9.45 (*s*, C<u>H</u>O), 7.82 (*dd*, *J* = 2, 8 Hz, H-6'), 7.51 (*br s*, H-1), 7.42 (*dt*, *J* = 2, 8, 8 Hz, H-4'), 6.71 (*d*, *J* = 8 Hz, H-3'), 6.60 (*dt*, *J* = 2, 8, 8 Hz, H-5'), 6.57 (*br s*, H-7), 5.69 (*d*, *J* = 10 Hz, H-12), 3.10 (*m*, H-10), 2.94 (*s*, C<u>H</u>$_3$N), 2.76 (*d*, *J* = 11 Hz, 2H-5), 2.70 (*br t*, *J* = 6.5 Hz, H-8), 2.53 (*m*, H-4), 2.16 (*s*, C<u>H</u>$_3$CO), 1.88 (*m*, H-11), 1.73 (*m*, 3H-19), 1.33 (*s*, 3H-17), 1.24 (*s*, 3H-16), 1.25 (*d*, *J* = 4.5 Hz, H-14), 0.98 (*d*, *J* = 6.5 Hz, 3H-18).
^{13}C NMR ($CDCl_3$)(125 MHz)		208.4, 193.0, 173.9, 167.9, 158.4, 154.1, 152.3, 144.7, 136.9, 135.1, 131.2, 114.5, 110.9, 109.1, 78.4, 75.6, 65.1, 53.9, 43.8, 43.2, 42.8, 34.9, 29.6, 25.8, 24.9, 23.6, 21.1, 16.7, 15.1, 10.2
DEPT 135°	CH_3:	29.6, 23.6, 21.1, 16.7, 15.1, 10.2
	CH_2:	25.8
	CH:	193.0, 158.4, 154.1, 135.1, 131.2, 114.5, 110.9, 75.6, 53.89, 43.8, 43.2, 42.8, 34.9
DEPT 90°	CH:	193.0, 158.4, 154.1, 135.1, 131.2, 114.5, 110.9, 75.6, 53.89, 43.8, 43.2, 42.8, 34.9

Fraction F3 was further purified by preparative TLC on silica gel developing with 20% ether in hexane to give a pure compound as a yellow viscous-liquid (4.2 mg), R_f 0.53 (40% hexane : ether). It was named **PSI7.**

$[\alpha]_D^{28}$	$+45.5^{\circ}$	(c = 4.4×10^{-3} g/ 10 cm^3, $CHCl_3$)
UV ($CHCl_3$) λ_{max} nm (log ε)		253.5 (5.32), 360.0 (5.01)
FT-IR (neat) ν_{cm-1}		3388 (O-H stretching), 1724, 1687 (C=O stretching)
^{1}H NMR ($CDCl_3$)(500 MHz)		7.83 (*dd*, *J* = 1.5, 8 Hz, H-6'), 7.59 (*br s*, H-1), 7.42 (*dt*, *J* = 1.5, 8, 8 Hz, H-4'), 6.70 (*br d*, *J* = 8 Hz, H-3'), 6.60 (*dt*, *J* = 1.5, 8, 8 Hz, H-5'), 5.65 (*d*, *J* = 9.5 Hz, H–12), 5.25 (*m*, H-7), 3.32 (*m*, H-10), 2.94 (*s*, $C\underline{H}_3N$), 2.87 (*dd*, *J* = 9, 18.5 Hz, H_β-5), 2.49 (*m*, H-4), 2.40 (*m*, H-8), 2.13 (*s*, $C\underline{H}_3CO$), 2.04 (*dd*, *J* = 10.5, 18.5 Hz, H_α-5), 1.75 (*s*, 3H-20), 1.73 (*m*, 3H-19), 1.32 (*s*, 3H-17), 1.19 (*s*, 3H-16), 1.05 (*d*, *J* = 5.5 Hz, H-14), 0.95 (*d*, *J* = 6.5 Hz, 3H-18).

^{13}C NMR ($CDCl_3$)(125 MHz)		213.8, 173.7, 168.1, 160.0, 152.3, 139.0, 136.4, 134.9, 131.3, 125.7, 114.4, 110.8, 109.4, 77.9, 76.3, 65.6, 54.3, 44.6, 42.5, 42.2, 35.8, 34.0, 29.6, 25.7, 25.4, 23.7, 21.2, 16.9, 15.1, 10.2
DEPT 135°	CH_3:	29.6, 25.7, 23.7, 21.2, 16.9, 15.1, 10.2
	CH_2:	34.0
	CH:	160.0, 134.9, 131.3, 125.7, 114.4, 110.8, 76.3, 54.3, 44.6, 42.5, 42.2, 35.8
DEPT 90°	CH:	160.0, 134.9, 131.3, 125.7, 114.4, 110.8, 76.3, 54.3, 44.6, 42.5, 42.2, 35.8

A part of the yellow gum (17 g) was partially separated by quick column chromatography on silica gel (100 g) and eluted with a step gradient of hexane/ ethyl acetate (from 0% to 100% ethyl acetate) followed by ethyl acetate/ methanol (from 10% to 100% methanol). Solvent was removed by evaporation under reduced pressure. Fractions were checked on TLC using UV light and vanillin-H_2SO_4 spray reagent. Fractions with similar TLC chromatograms were combined to afford fifteen fractions, as shown in **Table 4.**

Table 4 Fractions obtained from yellow gum by column chromatography

Fraction	Physical appearance	Weight (g)
F1	orange viscous-liquid	0.154
F2	red-orange viscous-liquid	5.550
F3	orange-yellow viscous-liquid	1.608
F4	orange-yellow viscous-liquid	0849
F5	dark-green viscous-liquid	1.243
F6	yellow viscous-liquid	0.325
F7	yellow viscous-liquid	1.250
F8	yellow viscous-liquid	1.067
F9	yellow viscous-liquid	0.488
F10	yellow viscous-liquid	0.940
F11	yellow viscous-liquid	0.805
F12	yellow viscous-liquid	1.828
F13	yellow viscous-liquid	0.147
F14	yellow viscous-liquid	0.136
F15	yellow viscous-liquid	0.452

Fraction F6 was found to contain phorbol on the basis of its TLC characteristic and ^{1}H-NMR (60 MHz), so it was further purified by preparative TLC on silica gel developing with 30% ether in hexane to give a pure compound as a yellow viscous-liquid (9.3 mg), R_f 0.68 (20% hexane : ether). It was named **PSI8.**

$[\alpha]_D^{28}$	-57.9°	(c = 6.9x10^{-3} g/ 10 cm^3, $CHCl_3$)
UV ($CHCl_3$) λ_{max} nm (log ε)		247.5 (5.03), 357.0 (4.57)
FT-IR (neat) ν_{cm-1}		3388 (O-H stretching), 1721, 1687 (C=O stretching)
^{1}H NMR ($CDCl_3$)(500 MHz)		7.90 (*dd*, *J* = 1.5, 8 Hz, H-6'), 7.42 (*dt*, *J* = 1.5, 8, 8 Hz, H-4'), 7.05 (*s*, H-1), 6.70 (*dd*, *J* = 1.5, 8 Hz, H-3'), 6.61 (*dt*, *J* = 1.5, 8, 8 Hz, H-5'), 5.71 (*d*, *J* = 10.5 Hz, H–12), 4.83 (*br s*, H-7), 3.45 (*m*, H-10), 3.41 (*br d*, *J* = 15 Hz, Hβ-5), 2.93 (*d*, *J* = 4.5 Hz, C$\underline{H}_3$N), 2.70 (*m*, H-4), 2.37 (*dd*, *J* = 4.5, 15 Hz, Hα-5), 2.07 (*s*, C$\underline{H}_3$CO), 1.95 (*br s*, H-8), 1.84 (*m*, H-11), 1.80 (*m*, 3H-19), 1.74 (*s*, 3H-20), 1.32 (*s*, 3H-17), 1.15 (*s*, 3H-16), 1.09 (*d*, *J* = 6 Hz, 3H-18), 0.83 (*d*, *J* = 5 Hz, H-14).
^{13}C NMR ($CDCl_3$)(125 MHz)		212.6, 174.1, 168.7, 156.1, 152.8, 143.6, 135.2, 131.6, 124.5, 114.5, 111.0, 109.8, 78.1, 75.1, 65.4, 49.0, 46.9, 43.2, 40.6, 37.3, 29.7, 29.2, 28.5, 24.8, 23.8, 20.8, 16.2, 11.4, 10.0.

DEPT 135°	CH_3:	29.2, 28.5, 23.8, 20.8, 16.2, 11.4, 10.0
	CH_2:	29.7
	CH:	156.1, 135.2, 131.6, 124.5, 114.5, 111.0, 75.1, 49.0, 46.9, 43.2, 40.6, 37.3
DEPT 90°	CH:	156.1, 135.2, 131.6, 124.5, 114.5, 111.0, 75.1, 49.0, 46.9, 43.2, 40.6, 37.3

Fraction F10 was found to contain phorbol on the basis of its TLC characteristic and ^{1}H-NMR (60 MHz), so it was further purified by preparative TLC on silica gel developing with 50% ether in hexane to give a pure compound as a yellow viscous-liquid (10.4 mg), R_f 0.32 (20% hexane : ether). It was named **PSI4.**

$[\alpha]_D^{28}$	-33.3°	(c = 3.0x10^{-3} g/ 10 cm^3, $CHCl_3$)
UV ($CHCl_3$) λ_{max} nm (log ε)		247.5 (5.39), 359.5 (5.12)
FT-IR (neat) ν_{cm-1}		3388 (O-H stretching), 1721, 1687 (C=O stretching)
^{1}H NMR ($CDCl_3$)(500 MHz)		7.88 (*dd*, *J* = 1.5, 8 Hz, H-6'), 7.43 (*dt*, *J* = 1.5, 8, 8 Hz, H-4'), 7.07 (*br s*, H-1), 6.72 (*d*, *J* = 8 Hz, H-3'), 6.61 (*dt*, *J* = 1.5, 8, 8 Hz, H-5'), 5.70 (*d*, *J* = 10 Hz, H-12), 4.88 (*br s*, H-7), 4.45 (*br s*, H-5), 3.64 (*m*, H-10), 3.12 (*dd*, *J* = 4.5, 6.5 Hz, H-4), 2.94 (*d*, *J* = 4.5 Hz, C$\underline{H}_3$N), 2.10 (*s*, C$\underline{H}_3$CO),

		2.05 (*m*, H-8), 1.88 (*br s*, 3H-20), 1.85 (*m*, H-11), 1.81 (*br t*, J = 1.5 Hz, 3H-19), 1.31 (*s*, 3H-17), 1.18 (*s*, 3H-16), 1.12 (*d*, J = 6 Hz, 3H-18), 0.86 (*d*, J = 4.5 Hz, H-14).
^{13}C NMR ($CDCl_3$)(125 MHz)		207.5, 173.9, 168.0, 154.6, 152.4, 144.0, 137.7, 135.0, 131.3, 125.4, 114.3, 110.9, 109.3, 78.5, 74.0, 70.9, 65.3, 56.1, 47.8, 43.4, 40.1, 38.1, 29.6, 27.1, 25.3, 24.1, 21.1, 16.5, 11.7, 10.4
DEPT 135°	CH_3:	29.6, 27.1, 24.1, 21.1, 16.5, 11.7, 10.4
	CH:	154.6, 135.0, 131.3, 125.4, 114.3, 110.9, 74.0, 70.9, 56.1, 47.8, 43.4, 40.1, 38.1
DEPT 90°	CH:	154.6, 135.0, 131.3, 125.4, 114.3, 110.9, 74.0, 70.9, 56.1, 47.8, 43.4, 40.1, 38.1

Fraction F11 was further purified by preparative TLC on silica gel developing with 50% ether in hexane to give a mixture of the unseparable **PSI9** and **PSI9a** as a yellow viscous-liquid (2 mg), R_f 0.30 (20% hexane: ether). No further separation was conducted.

Fraction F15 was found to contain the compound **PSI6** as a major component by comparison of the TLC chromatogram.

Fraction F14 was found to contain phorbol on the basis of its TLC characteristic and ^{1}H-NMR (60 MHz), so it was further purified by preparative TLC on silica gel developing with 50% ether in hexane to give a pure compound as a yellow viscous-liquid (9.2 mg), R_f 0.13 (15% hexane : ether). It was named **PSI5.**

$[\alpha]_D^{28}$ +13.5°	(c = 7.4x10^{-3} g/ 10 cm^3, $CHCl_3$)
UV ($CHCl_3$) λ_{max} nm (log ε)	252.5 (5.11), 360.0 (4.79)
FT-IR (neat) V_{cm-1}	3388 (O-H stretching), 1724, 1687 (C=O stretching)
^{1}H NMR ($CDCl_3$)(500 MHz)	7.82 (*dd*, *J* = 1.5, 8 Hz, H-6'), 7.71 (*br s*, H-1), 7.42 (*dt*, *J* = 1.5, 8, 8 Hz, H-4'), 6.71 (*d*, *J* = 8 Hz, H-3'), 6.60 (*dt*, *J* = 1.5, 8, 8 Hz, H-5'), 5.65 (*d*, *J* = 10 Hz, H–12), 5.62 (*d*, *J* = 5 Hz, H-7), 5.20 (*d*, *J* = 4.5 Hz, H-5), 4.25 (*AB*, *J* = 13 Hz, 2H-20), 3.59 (*m*, H-10), 2.93 (*s*, $C\underline{H}_3N$), 2.64 (*t*, *J* = 4.5 Hz, H-4), 2.35 (*br t*, *J* = 5 Hz, H-8), 2.13 (*s*, $C\underline{H}_3CO$), 1.75 (*m*, 3H-19), 1.68 (*m*, H-11), 1.29 (*s*, 3H-17), 1.20 (*s*, 3H-16), 1.13 (*d*, *J* = 5 Hz, H-14), 0.97 (*d*, *J* = 6.5 Hz, 3H-18).

^{13}C NMR ($CDCl_3$)(125 MHz)		208.4, 174.0, 168.2, 162.7, 152.6, 143.1, 138.5, 135.2, 131.5, 130.3, 114.7, 111.1, 109.5, 78.6, 76.3, 71.1, 67.1, 65.7, 52.1, 51.4, 43.2, 42.5, 36.3, 29.8, 26.0, 23.9, 21.4, 17.1, 15.5, 10.3
DEPT 135°	CH_3:	29.8, 23.9, 21.4, 17.1, 15.5, 10.3
	CH_2:	67.1
	CH:	162.7, 135.2, 131.5, 130.3, 114.7, 111.1, 76.3, 71.1, 52.1, 51.4, 43.2, 42.5, 36.3
DEPT 90°	CH:	162.7, 135.2, 131.5, 130.3, 114.7, 111.1, 76.3, 71.1, 52.1, 51.4, 43.2, 42.5, 36.3

Fraction F9 was further purified by preparative TLC on silica gel developing with 30% ethyl acetate in hexane to give a pure compound as a yellow viscous-liquid (2.2 mg), R_f 0.35 (40% hexane : ether). It was named **PSI10.**

$[\alpha]_D^{28}$	+90.9°	(c = 2.2×10^{-3} g/ 10 cm^3, $CHCl_3$)
UV ($CHCl_3$) λ_{max} nm (log ε)		281.2 (5.31)
FT-IR (neat) ν_{cm-1}		3438 (O-H stretching)

^{1}H NMR ($CDCl_3$)(500 MHz)		5.58 (*d*, *J* = 6 Hz, H-11), 5.53 (*d*, *J* = 6 Hz, H-12), 3.24 (*dd*, *J* = 6, 11.5 Hz, H-3), 1.25 (*s*, 3H), 1.19 (*s*, 3H), 1.14 (*s*, 3H), 1.04 (*s*, 3H), 0.99 (*s*, 3H), 0.89 (*s*, 3H), 0.88 (*s*, 3H), 0.81 (*s*, 3H)
^{13}C NMR ($CDCl_3$)(125 MHz)		154.3, 147.1, 120.7, 115.7, 78.7, 51.1, 46.8, 45.5, 42.8, 40.6, 38.9, 38.7, 37.1, 37.0, 34.6, 33.2, 32.1, 32.2, 31.1, 28.7, 28.2, 27.8, 27.2, 25.6, 25.2, 23.7, 20.9, 20.1, 18.3, 15.6
DEPT 135°	CH_3:	33.2, 28.6, 28.3, 25.3, 23.7, 20.9, 20.1, 15.1
	CH_2:	46.9, 38.8, 37.2, 34.7, 32.2, 27.9, 27.3, 25.7, 18.4
	CH:	120.7, 115.8, 78.6, 51.2
DEPT 90°	CH:	120.7, 115.8, 78.6, 51.2

The Second Investigation

The dichloromethane extract was concentrated under reduced pressure to give a brown viscous-liquid (14 g) which was partially separated by quick column chromatography on silica gel (100 g) and eluted with a step gradient of hexane/ ethyl acetate (from 0% to 100% ethyl acetate) followed by ethyl acetate/ methanol (from 10% to 100% methanol). Solvent was removed by evaporation under reduced pressure. Fractions were checked on TLC using UV light and vanillin-H_2SO_4 spray reagent. Fractions with similar TLC chromatograms were combined to afford sixteen fractions, as shown in **Table 5.**

Table 5 Fractions obtained from crude dichloromethane by column chromatography

Fraction	Physical appearance	Weight (g)
F1	orange-yellow viscous-liquid	0.459
F2	orange-yellow viscous-liquid	0.802
F3	orange-yellow viscous-liquid	0955
F4	dark-green viscous-liquid	1.206
F5	dark-green viscous-liquid	0.452
F6	dark-green viscous-liquid	0.822
F7	yellow viscous-liquid	1.502
F8	yellow viscous-liquid	1.975

Table 5 (Continued)

Fraction	Physical appearance	Weight (g)
F9	yellow viscous-liquid	0.859
F10	yellow viscous-liquid	0.460
F11	yellow viscous-liquid	1.024
F12	yellow viscous-liquid	0.626
F13	yellow viscous-liquid	0.411
F14	yellow viscous-liquid	0.215
F15	yellow viscous-liquid	0.862
F16	yellow viscous-liquid	0.978

Fraction F7 was further purified by column chromatography on silica gel and eluted with a step gradient of hexane/ ethyl acetate (from 0% to 100% ethyl acetate) followed by ethyl acetate/ methanol (from 10% to 100% methanol). Fractions with similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford five subfractions, as shown in **Table 6.**

Table 6 Subfractions obtained from fraction F7 by column chromatography

Fraction	Physical appearance	Weight (mg)
f7.1	yellow viscous-liquid	326.6
f7.2	yellow viscous-liquid	117.2
f7.3	yellow viscous-liquid	492.1
f7.4	yellow viscous-liquid	92.5
f7.5	yellow viscous-liquid	455.7

Subfraction f7.3 was found to contain the compound **PSI1** as a major component by comparison of the TLC chromatogram.

Subfraction f7.5 was found to contain the compound **PSI2** as a major component by comparison of the TLC chromatogram.

Fraction F8 was further purified by column chromatography on silica gel and eluted with a step gradient of hexane/ ethyl acetate (from 0% to 100% ethyl acetate) followed by ethyl acetate/ methanol (from 10% to 100% methanol). Fractions with similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford ten subfractions, as shown in **Table 7.**

Table 7 Subfractions obtained from fraction F8 by column chromatography

Fraction	Physical appearance	Weight (mg)
f8.1	yellow viscous-liquid	36.8
f8.2	yellow viscous-liquid	112.2
f8.3	yellow viscous-liquid	240.0
f8.4	yellow viscous-liquid	138.2
f8.5	yellow viscous-liquid	48.0
f8.6	yellow viscous-liquid	25.6
f8.7	yellow viscous-liquid	128.4
f8.8	yellow viscous-liquid	150.2
f8.9	yellow viscous-liquid	128.9
f8.10	yellow viscous-liquid	245.1

Subfraction f8.2 were found to contain the compound **PSI1** and **PSI3** as two major components by comparison of the TLC chromatogram.

Subfraction f8.3 were found to contain the compound **PSI2** and **PSI3** as two major components by comparison of the TLC chromatogram.

Subfraction f8.4 was found to contain the compound **PSI3** as a major component by comparison of the TLC chromatogram.

Fraction F10 was further purified by column chromatography on silica gel and eluted with a step gradient of hexane/ ethyl acetate (from 0% to 100% ethyl acetate) followed by ethyl acetate/ methanol (from 10% to 100% methanol). Fractions with similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford ten subfractions, as shown in **Table 8.**

Table 8 Subfractions obtained from fraction F10 by column chromatography

Fraction	Physical appearance	Weight (mg)
f10.1	yellow viscous-liquid	21.5
f10.2	yellow viscous-liquid	49.0
f10.3	yellow viscous-liquid	110.8
f10.4	yellow viscous-liquid	39.8
f10.5	yellow viscous-liquid	35.2
f10.6	yellow viscous-liquid	25.8
f10.7	yellow viscous-liquid	40.6
f10.8	yellow viscous-liquid	75.0
f10.9	yellow viscous-liquid	50.2
f10.10	yellow viscous-liquid	11.8

Subfraction f10.3 was further purified by preparative TLC on silica gel developing with 40% dichloromethane in ethyl acetate to give a pure compound as a yellow viscous-liquid (9.8 mg), R_f 0.13 (40% hexane : ether). It was named **PSI6.**

$[\alpha]_D^{28}$	-31.9°	(c = 9.4x10^{-3} g/ 10 cm^3, $CHCl_3$)
UV ($CHCl_3$) λ_{max} nm (log ε)		252.5 (5.00), 361.0 (4.69)
FT-IR (neat) ν_{cm-1}		3387 (O-H stretching), 1721, 1687 (C=O stretching)
^{1}H NMR ($CDCl_3$)(500 MHz)		7.89 (*dd*, *J* = 1.5, 8 Hz, H-6'), 7.45 (*dt*, *J* = 1.5, 8, 8 Hz, H-4'), 7.11 (*br s*, H-1), 6.74 (*d*, *J* = 8 Hz, H-3'), 6.63 (*dt*, *J* = 1.5, 8, 8 Hz, H-5'), 5.72 (*d*, *J* = 10.5 Hz, H–12), 5.21 (*d*, *J* = 1.5 Hz, H-7), 4.57 (*br s*, H-5), 4.15 (*br s*, 2H-20), 3.71 (*m*, H-10), 3.21 (*m*, H-4), 2.95 (*s*, C$\underline{H}_3$N), 2.11 (*s*, C$\underline{H}_3$CO), 2.11 (*m*, superimposed with COC$\underline{H}_3$, H-8), 1.87 (*m*, H-11), 1.82 (*m*, 3H-19), 1.34 (*s*, 3H-17), 1.19 (*s*, 3H-16), 1.16 (*d*, *J* = 6.5 Hz, 3H-18), 0.92 (*d*, *J* = 4.5 Hz, H-14).
^{13}C NMR ($CDCl_3$)(125 MHz)		209.5, 173.7, 168.1, 159.5, 152.3, 142.1, 136.4, 134.9, 131.3, 126.5, 114.4, 110.8, 109.4, 77.8, 76.2, 67.4, 65.5, 54.1, 44.2, 42.6, 42.1, 35.7, 29.7, 29.6, 25.7, 23.7, 21.1, 16.8, 15.0, 10.1

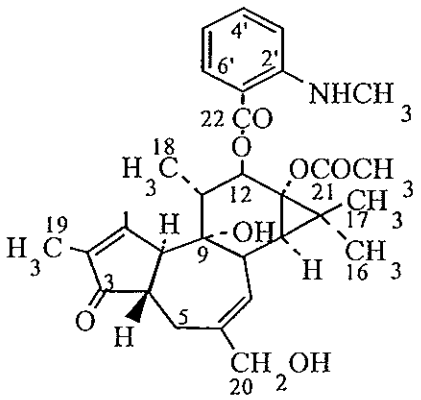
DEPT 135°	CH_3:	29.7, 23.7, 21.1, 16.8, 15.0, 10.1
	CH_2:	68.2
	CH:	159.5, 134.9, 131.3, 126.5, 114.4, 110.8, 76.2, 54.1, 44.2, 42.6, 42.1, 35.7
DEPT 90°	CH:	159.5, 134.9, 131.3, 126.5, 114.4, 110.8, 76.2, 54.1, 44.2, 42.6, 42.1, 35.7

CHAPTER 3

RESULTS AND DISCUSSION

The hexane and dichloromethane extract from fruits of *sapium indicum* were separated by column chromatography and/ or preparative TLC methods to obtain ten compounds; two new phorbol esters (**PSI7** and **PSI8**) together with eight known compounds; seven phorbol esters (**PSI1**, **PSI2**, **PSI3**, **PSI4**, **PSI5**, **PSI6** and **PSI9**) and one oleanane triterpene (**PSI10**). The structures of all compounds were elucidated using 1D, 2D NMR and another spectroscopic data. The structure elucidation of the known compounds were confirmed by comparison of their spectroscopic data, especially ^{1}H NMR spectral data with those reported in the literature. For structural elucidation, the ^{13}C NMR signals were assigned from DEPT, HMQC, HMBC, NOE and COSY spectra.

3.1 Compound PSI1: 12-(N-Methylaminobenzoyl)-4β, 5-dideoxyphorbol-13-acetate



Compound **PSI1** was obtained as a yellow viscous-liquid. The UV spectrum **(Figure 2)** showed maximum absorptions at 252.0 and 360.0 nm suggesting the presence of a conjugated enone and benzoyl chromophores. The IR spectrum **(Figure 3)** showed the stretching of hydroxy group at 3389 cm^{-1}, carbonyl group at 1720 and 1685 cm^{-1} and C=C at 1580 and 1520 cm^{-1}. It also gave a brown spot with vanillin-H_2SO_4 spray reagent, a positive test for diterpene compound.

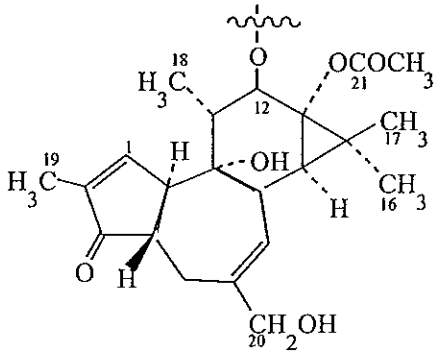
The ^{13}C NMR spectrum showed the signals of 30 carbon atoms **(Table 9)(Figure 5)**. Analysis of the DEPT spectra indicated the presence of three carbonyl carbons (δ209.5, 173.7 and 168.1), six methyl carbons (δ29.7, 23.7, 21.1, 16.8, 15.0 and 10.1), two methylene carbons (δ67.4 and 29.6), twelve methine carbons (δ159.5, 134.9, 131.3, 126.5, 114.4, 110.8, 76.2, 54.1, 44.2, 42.6, 42.1 and 35.7) and seven quaternary carbons (δ 152.3, 142.1, 136.4, 109.4, 77.8, 65.5 and 25.7).

The ^{1}H NMR spectrum **(Table 9)(Figure 4)** showed the characteristic signal of phorbol skeleton at δ7.56 (*br s*, H-1), 5.55 (*m*, H-7), 4.02 (*AB*, J = 13.5 Hz, 2H-20) and 1.12 (*d*, J = 5 Hz, H-14). The signals of methyl groups were also observed at δ 1.73 (*s*, 3H), 1.32 (*s*, 3H), 1.19 (*s*, 3H) and δ0.96 (*d*, J = 6.5 Hz) which were assigned

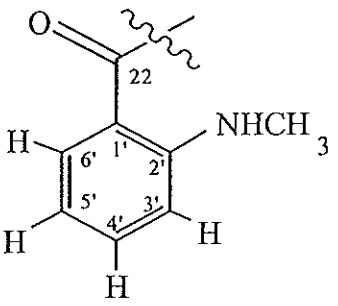
to C-19, C-17, C-16 and C-18, respectively. In addition, the proton signals of anthranilic acid moiety were present at δ7.81 (*dd*, *J* = 1.5, 8.5 Hz), 7.40 (*dt*, *J* = 1.5, 8.5, 8.5 Hz), 6.69 (*dd*, *J* = 1.5, 8.5 Hz) and 6.59 (*dt*, *J* = 1.5, 8.5, 8.5 Hz) together with the acetyl group at δ2.12 (*s*, 3H). The downfield chemical shift methyl signal at δ 2.93 (*s*, 3H) is probably due to attachment to highly electronegative atom such as N or O. The other signals were *doublet* at δ5.65 (*J* = 10 Hz, 1H), two *multiplets* at δ 3.27 and 1.73, a *doublet of triplet* at δ2.52 (*J* = 5, 9.5 Hz), a *broad triplet* at δ2.44 and two *doublet of doublet* signals of one proton each at δ2.86 (*J* = 9.5, 18 Hz) and 2.17 (*J* = 10, 18 Hz).

The substitution pattern in this compound was supported by its HMBC spectral data **(Table 10)(Figure 9).** HMBC correlations of olefinic proton H-1 at δ (7.56) were to the carbon signals at δ136.4, 209.5 and 54.1, which could be assigned as C-2, C-3 and C-10, respectively. The last signal showed ^{1}H-^{13}C direct coupling (HMQC) at δ3.27, so it was assigned to H-10. The methylene protons signal at δ4.02 (2H-20) showed correlations to C-5 (δ29.6), C-6 (δ142.1) and C-7 (δ126.5), this confirmed the location of methylene at C-20. The carbon signals at δ77.8, 42.6 and 76.2 showed correlation to 3H-18 (δ0.96). Only the last two signals showed proton signals (HMQC) at δ1.73 and 5.65, Hence they were assigned to H-11 and H-12, respectively and carbon signal at δ77.8 should be assigned to C-9. Correlations of H-14 (δ1.12) were to C-12 (δ76.2), C-13 (δ65.5), C-15 (δ25.7) and C-17 (δ16.8). The signal at δ2.44 showed correlations to C-6 (δ142.1), C-7 (δ126.5), C-9 (δ 77.8), C-14 (δ35.7) and C-15 (δ25.7) so it was assigned to H-8. The signal at δ2.52 assigned to H-4 was confirmed by the correlations to C-3 (δ209.5), C-5 (δ29.6), C-9 (δ77.8) and C-10 (δ54.1) Thus, the signals at δ2.86 and 2.17 (HMQC, δC-5 = 29.6) should be assigned to 2H-5. The H-5 (δ2.17) was further confirmed by the correlations to C-3 (δ209.5), C-4 (δ44.2), C-6 (δ142.1), C-7 (δ126.5) and C-20 (δ 67.4). The remaining proton H-12 (δ5.65) was confirmed by the correlations to C-11

(δ 42.6), C-13 (δ 65.5), C-15 (δ 25.7), C-18 (δ 15.0) and C-22 (δ 168.1), this information indicated clearly that an acyl to oxygen bond formation (ester formation) occurred between an aromatic carboxylic group and hydroxy group of phorbol skeleton at C-12 (δ 76.2). In addition, the downfield aromatic protons H-6' (δ 7.81) showed correlations to C-1' (δ 152.3), C-4' (δ 134.9) and C-22 (δ 168.1); H-4' (δ 7.40) showed correlations to C-1' (δ 152.3) and C-6' (δ 131.3); H-3' (δ 6.69) showed correlations to C-2' (δ 109.4), C-5' (δ 114.4) and C-22 (δ 168.1). The H-5' (δ 6.59) showed correlations to C-2' (δ 109.4) and C-3' (δ 110.8). The last signal at δ 2.93 was assigned to N-methyl by comparison to literature. Thus **PSI1** composed of phorbol acetate unit and anthranilic acid unit connected by ester linkage at C-12 of phorbol skeleton. The more detail informations were summarized in **Tables 9-13, Figures 2-10.**

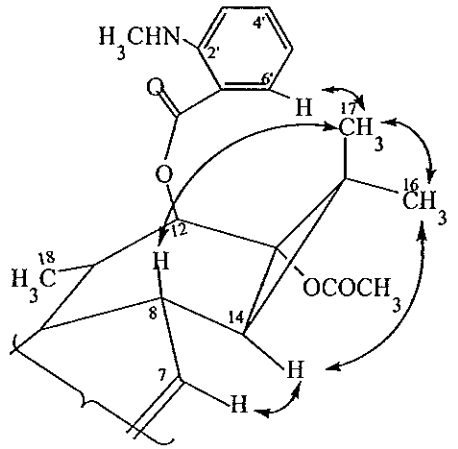


phorbol acetate unit

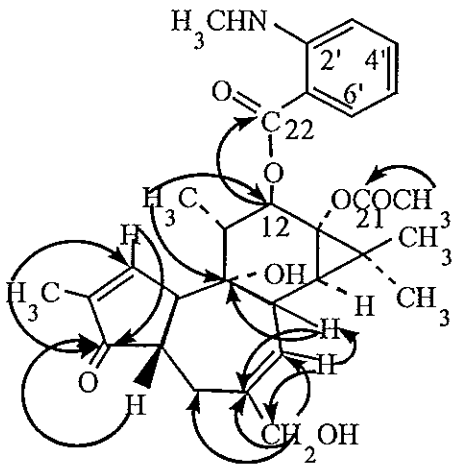


anthranilic acid unit

From NOE experiment, irradiation of 3H-17 (δ 1.32) showed enhancement of H-8 (δ 2.44), H-16 (δ 1.19) and H-6' (δ 7.81). Irradiation of 3H-16 (δ 1.19) showed enhancement of H-14 (δ 1.12). Irradiation of H-14 (δ 1.12) showed enhancement of H-7 (5.55).



NOE of PSI1



Selected HMBC Correlations of PSI1

Table 9 ^{1}H and ^{13}C NMR spectral data of compound **PSI1**

Position	δ_C	δ_H, mult, J(Hz)
1	159.5	7.56 (*br s*)
2	136.4	
3	209.5	
4	44.2	2.52 (*dt*, 5, 9.5)
5β	29.6	2.86 (*dd*, 9.5, 18)
5α	29.6	2.17 (*dd*, 10, 18)
6	142.1	
7	126.5	5.55 (*m*)
8	42.1	2.44 (*br t*)
9	77.8	
10	54.1	3.27 (*m*)
11	42.6	1.73 (*m*, superimposed with 3H-19)
12	76.2	5.65 (*d*, 10)
13	65.5	
14	35.7	1.12 (*d*, 5)
15	25.7	
16	23.7	1.19 (*s*)
17	16.8	1.32 (*s*)
18	15.0	0.96 (*d*, 6.5)
19	10.1	1.73 (*s*)
20	67.4	4.02 (*AB*, 13.5)
21	173.7	
22	168.1	

Table 9 (Continued)

Position	δC	δH, mult, *J*(Hz)
1'	152.3	
2'	109.4	
3'	110.8	6.69 (*dd*, 1.5, 8.5)
4'	134.9	7.40 (*dt*, 1.5, 8.5,8.5)
5'	114.4	6.59 (*dt*, 1.5,8.5,8.5)
6'	131.3	7.81 (*dd*, 1.5, 8.5)
NCH_3	29.7	2.93 (*s*)
$COCH_3$	21.1	2.12 (*s*)

Table 10 Major HMBC Correlations of compound **PSI1**

Position	δH, mult, *J*(Hz)	δC
1	7.56 (*br s*)	C-2 (136.4), C-3 (209.5), C-10 (54.1)
4	2.52 (*dt*, 5, 9.5)	C-5 (29.6), C-9 (77.8), C-10 (54.1)
5α	2.17 (*dd*, 10, 18)	C-3 (209.5), C-4 (44.2), C-6 (142.1), C-7 (126.5), C-20 (67.4)
7	5.55 (*m*)	C-8 (42.1), C-20 (67.4)
8	2.44 (*br t*)	C-6 (142.1), C-7 (126.5), C-9 (77.8), C-14 (35.7), C-15 (25.7)

Table 10 (Continued)

Position	δC	δH, mult, *J*(Hz)
11	1.73 (*m*, superimposed-with 3H-19)	C-12 (76.2), C-18 (15.0)
12	5.65 (*d*, 10)	C-11 (42.6), C-13 (65.5), C-15 (25.7), C-18 (15.0), C-22 (168.1)
14	1.12 (*d*, 5)	C-12 (76.2), C-13 (65.5), C-15 (25.7), C-17 (16.8)
16	1.19 (*s*)	C-13 (65.5), C-14 (35.7), C-15 (25.7), C-17 (16.8)
17	1.32 (*s*)	C-13 (65.5), C-14 (35.7), C-15 (25.7), C-16 (23.7)
18	0.96 (*d*, 6.5)	C-9 (77.8), C-11 (42.6), C-12 (76.2), C-13 (65.5)
19	1.73 (*s*)	C-1 (159.5), C-2 (136.4), C-3 (209.5)
20	4.02 (*AB*, 13.5)	C-5 (29.6), C-6 (142.1), C-7 (126.5)
3'	6.69 (*dd*, 1.5, 8.5)	C-2' (109.4), C-5' (114.4), C-22 (168.1)

Table 10 (Continued)

Position	δC	δH, mult, *J*(Hz)
4'	7.40 (*dt*, 1.5, 8.5)	C-1' (152.3), C-6' (131.3)
5'	6.59 (*dt*, 1.5,8.5)	C-2' (136.4), C-3' (209.5)
6'	7.81 (*dd*, 1.5, 8.5)	C-1' (152.3), C-4' (134.9), C-22 (168.1)
$COC\underline{H}_3$	2.12 (*s*)	C-21 (173.7)

Table 11 ^{13}C NMR and DEPT spectral data of compound **PSI1**

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
1	159.5	CH	159.5	159.5	159.5
2	136.4	C			
3	209.5	C=O			
4	44.2	CH	44.2	44.2	44.2
5	29.6	CH_2	29.6		29.6
6	142.1	C			
7	126.5	CH	126.5	126.5	126.5
8	42.1	CH	42.1	42.1	42.1
9	77.8	C			

Table 11 (Continued)

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
10	54.1	CH	54.1	54.1	54.1
11	42.6	CH	42.6	42.6	42.6
12	76.2	CH	76.2	76.2	76.2
13	65.5	C			
14	35.7	CH	35.7	35.7	35.7
15	25.7	C			
16	23.7	CH_3	23.7		23.7
17	16.8	CH_3	16.8		16.8
18	15.0	CH_3	15.0		15.0
19	10.1	CH_3	10.1		10.1
20	67.4	CH_2	67.4		67.4
21	173.7	C=O			
22	168.1	C=O			
1'	152.3	C			
2'	109.4	C			
3'	110.8	CH	110.8	110.8	110.8
4'	134.9	CH	134.9	134.9	134.9
5'	114.4	CH	114.4	114.4	114.4
6'	131.3	CH	131.3	131.3	131.3
$N\underline{C}H_3$	29.7	CH_3	29.7		29.7
$CO\underline{C}H_3$	21.1	CH_3	21.1		21.1

Table 12 COSY Correlations among some protons of compound **PSI1**

δH, mult, *J*(Hz)	**Proton Correlations with δH (ppm)**
H-1 (*br s*)	H-10, H-19
H-4 (*dt*, 5, 9.5)	H-5α, H-10
H-5α (*dd*, 10, 18)	H-4, H-5β
H-5β (*dd*, 9.5, 18)	H-4, H-5α
H-7 (*m*)	H-8, H-20
H-8 (*br t*)	H-14
H-11 (*m*, superimposed with 3H-19)	H-12, H-18
H-12 (*d*, 10)	H-11
H-3' (*dd*, 1.5, 8.5)	H-5'
H-4' (*dt*, 1.5, 8.5, 8.5)	H-3', H-5'
H-6' (*dd*, 1.5, 8.5)	H-4', H-5'

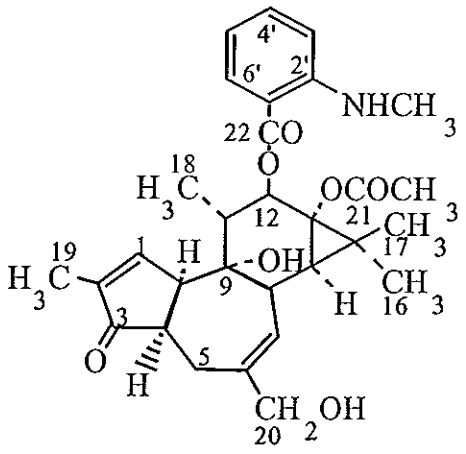
Comparison of ^{1}H NMR spectral data between compound **PSI1** and 12-(N-Methylaminobenzoyl)-4β, 5-dideoxyphorbol-13-acetate showed similarity **(Table 13).** Thus compound **PSI1** was confirmed as 12-(N-Methylaminobenzoyl)-4β,5-dideoxyphorbol-13-acetate which was previously isolated from fruits of *Sapium indicum* (Taylor, *et.al.*, 1981).

Table 13 Comparison of ^{1}H NMR spectrum between 4β,5-dideoxyphorbol and **PSI1**

Position	4β,5-dideoxyphorbol, δ_H (ppm) (Recorded in $CDCl_3$)	Compound PSI1, δ_H (ppm) (Recorded in $CDCl_3$)
1	7.57 (*s*)	7.56 (*br s*)
4	2.85 (*m*)	2.52 (*dt*, *J* = 5, 9.5 Hz)
5β	3.46 (*dd*, *J* = 3.1, 15.4 Hz)	2.86 (*dd*, *J* = 9.5, 18 Hz)
5α	2.51 (*dd*, *J* = 5.8, 15.4 Hz)	2.17 (*dd*, *J* = 10, 18 Hz)
7	5.59 (*d*, *J* = 3.68 Hz)	5.55 (*m*)
8	2.45 (*m*)	2.44 (*br t*)
10	3.28 (*m*)	3.27 (*m*)
11	2.18 (*m*)	1.73 (*m*, superimposed with 3H-19)
12	5.64 (*d*, *J* = 11.6 Hz)	5.65 (*d*, *J* = 10 Hz)
14	1.13 (*d*, *J* = 5.2 Hz)	1.12 (*d*, *J* = 5 Hz)
16	1.32 (*s*)	1.19 (*s*)*
17	1.19 (*s*)	1.32 (*s*)*
18	0.96 (*d*, *J* = 6.3 Hz)	0.96 (*d*, *J* = 6.5 Hz)
19	1.73	1.73 (*s*)
20	4.04 (*s*)	4.02 (*AB*, *J* = 13.5 Hz)
3'	6.69 (*d*, *J* = 8.1 Hz)	6.69 (*dd*, *J* = 1.5, 8.5 Hz)
4'	7.41 (*t*, *J* = 6.9 Hz)	7.40 (*dt*, *J* = 1.5, 8.5,8.5 Hz)
5'	6.59 (*t*, *J* = 8.1 Hz)	6.59 (*dt*, *J* = 1.5,8.5,8.5 Hz)
6'	7.82 (*dd*, *J* = 1.8, 8.1 Hz)	7.81 (*dd*, *J* = 1.5, 8.5 Hz)
NCH_3	2.94 (*d*, *J* = 5.1 Hz)	2.93 (*s*)
$COCH_3$	2.13 (*s*)	2.12 (*s*)

*The chemical shift was confirmed by NOE experiment.

3.2 Compound PSI2: 12-(N-Methylaminobenzoyl)-4α, 5-dideoxyphorbol-13-acetate



Compound **PSI2** was isolated as a yellow viscous-liquid. The IR spectrum **(Figure 12)** showed absorption bands which were ascribed to O-H stretching of hydroxy group (3388 cm^{-1}), C=O stretching of carbonyl group (1720 and 1687 cm^{-1}) and C=C stretching (1581 and 1520 cm^{-1}). The UV spectrum **(Figure 11)** showed maximum absorptions at 251.5 and 360.5 nm suggesting the presence of a conjugated enone and benzoyl chromophores. It also gave a brown spot with vanillin-H_2SO_4 spray reagent, a positive test for diterpene compound.

Comparison of spectral data of 4-epimer in the literature (Taylor, *et.al.*, 1981) indicated differences only at H-1 and H-14 chemical shifts. H-1 and H-14 proton chemical shift of 4α-epimer appeared at approx. 7.0 ppm and 0.8 ppm, respectively, whereas those of 4β-epimer appeared at approx. 7.6 ppm and 1.1 ppm, respectively.

The ^{1}H NMR spectral data of **PSI2** was similar to that of **PSI1 (Table 19)(Figure 13)** except proton chemical shift of **PSI2** appeared at δ 7.08 (*s*, H-1) as compare to that of **PSI1** at δ 7.56 (*br s*, H-1). The H-14 proton chemical shift of **PSI2** appeared at δ 0.84 (*d*, J = 5 Hz) compare to that of **PSI1** at δ 1.12 (*d*, J = 5 Hz). Therefore **PSI2** was suggested to be 12-(N-Methylaminobenzoyl)-4α,5-

dideoxyphorbol-13-acetate, 4α-epimer of **PSI1.** 1D and 2D NMR of **PSI2** were summarized in **Tables 14-19, Figures 11-18.**

Comparison of ^{1}H NMR spectral data between compound **PSI2** and 12-(N-Methylaminobenzoyl)-4α,5-dideoxyphorbol-13-acetate showed similarity. Thus compound **PSI2** was confirmed as 12-(N-Methylaminobenzoyl)-4α,5-dideoxy-phorbol-13-acetate which was previously isolated from fruits of *Sapium indicum* (Taylor, *et.al.*, 1981).

Table 14 Comparison of ^{1}H NMR spectrum between 4α,5-dideoxyphorbol and **PSI2**

Position	**4α,5-dideoxyphorbol, δH (ppm) (Recorded in $CDCl_3$)**	**Compound PSI2, δH (ppm) (Recorded in $CDCl_3$)**
1	7.09 (*s*)	7.08 (*s*)
4	2.85 (*m*)	2.80 (*m*)
5β	3.46 (*dd*, *J* = 3.1, 15.4 Hz)	3.45 (*dd*, *J* = 3.5, 16 Hz)
5α	2.51 (*dd*, *J* = 5.8, 15.4 Hz)	2.50 (*dd*, *J* = 5, 16 Hz)
7	5.15 (*s*)	5.14 (*br s*)
8	2.03 (*m*)	2.03 (*m*)
10	3.54 (*m*)	3.54 (*m*)
11	1.87 (*m*)	1.87 (*m*)
12	5.71 (*d*, *J* = 10.3 Hz)	5.72 (*d*, *J* = 10.5 Hz)
14	0.88 (*d*, *J* = 6.6 Hz)	0.84 (*d*, *J* = 5 Hz)
16	1.32 (*s*)	1.17 (*s*)*
17	1.19 (*s*)	1.33 (*s*)*

* The chemical shift was confirmed by NOE experiment of **PSI1.**

Table 14 (Continued)

Position	4α-deoxyphorbol, δH (ppm) (Recorded in $CDCl_3$)	Compound PSI2, δH (ppm) (Recorded in $CDCl_3$)
18	1.12 (*d*, *J* = 6.3 Hz)	1.12 (*d*, *J* = 6.5 Hz)
19	1.73 (*s*)	1.80 (*s*)
20	3.97 (*AB*, *J* = 28.6 Hz)	4.01 (*AB*, *J* = 12.5 Hz)
3'	6.69 (*d*, *J* = 8.1 Hz)	6.70 (*d*, *J* = 8 Hz)
4'	7.41 (*t*, *J* = 6.9 Hz)	7.42 (*dt*, *J* = 2, 8, 8 Hz)
5'	6.59 (*t*, *J* = 8.1 Hz)	6.62 (*t*, *J* = 8 Hz)
6'	7.82 (*dd*, *J* = 1.8, 8.1 Hz)	7.88 (*dd*, *J* = 1.5, 8 Hz)
$NC\underline{H}_3$	2.94 (*d*, *J* = 5.1 Hz)	2.93 (*br s*)
$COC\underline{H}_3$	2.13 (*s*)	2.08 (*s*)

Table 15 ^{1}H and ^{13}C NMR spectral data of compound **PSI2**

Position	δC	δH, mult, *J*(Hz)
1	156.5	7.08 (*s*)
2	137.5	
3	213.8	
4	49.4	2.80 (*m*)
5β	24.9	3.45 (*dd*, 3.5, 16)
5α	24.9	2.50 (*dd*, 5, 16)
6	143.7	
7	126.7	5.14 (*br s*)
8	40.5	2.03 (*m*)
9	78.1	
10	47.3	3.54 (*m*)

Table 15 (Continued)

Position	δ_C	δ_H, mult, J(Hz)
11	43.3	1.87 (*m*)
12	74.9	5.72 (*d*, 10.5)
13	65.3	
14	36.9	0.84 (*d*, 5)
15	24.9	
16	23.8	1.17 (*s*)
17	16.2	1.33 (*s*)
18	11.5	1.12 (*d*, 6.5)
19	10.0	1.80 (*s*)
20	69.2	4.01 (*AB*, 12.5)
21	174.1	
22	168.6	
1'	152.8	
2'	109.7	
3'	111.0	6.70 (*d*, 8)
4'	135.2	7.42 (*dt*, 2, 8, 8)
5'	114.5	6.62 (*t*, 8)
6'	131.6	7.88 (*dd*, 1.5, 8)
$NC\underline{H}_3$	29.2	2.93 (*br s*)
$COC\underline{H}_3$	20.7	2.08 (*s*)

Table 16 Major HMBC Correlations of compound **PSI2**

Position	δ_H, mult, *J*(Hz)	δ_C
1	7.08 (*s*)	C-4 (49.4), C-10 (47.3), C-19 (10.0)
4	2.80 (*m*)	C-3 (213.8), C-5 (24.9), C-6 (143.7), C-10 (47.3)
5β	3.45 (*dd*, 3.5, 16)	C-3 (213.8), C-4 (49.4), C-6 (143.7)
5α	2.50 (*dd*, 5, 16)	C-4 (49.4), C-6 (143.7), C-7 (126.7), C-10 (47.3), C-20 (69.2)
7	5.14 (*br s*)	C-5 (24.9), C-9, C-14, C-20
8	2.03 (*m*)	C-13 (65.3)
10	3.54 (*m*)	C-1 (156.5), C-2 (137.5), C-3 (213.8), C-4 (49.4), C-8 (40.5), C-9 (78.1)
11	1.87 (*m*)	C-12 (74.9), C-18 (11.5)
12	5.72 (*d*, 10.5)	C-11 (43.3), C-13 (65.3), C-15 (24.9), C-18 (11.5), C-22 (168.6)
14	0.84 (*d*, 5)	C-9 (78.1), C-12 (74.9), C-13 (65.3), C-15 (24.9), C-16 (23.8), C-17 (16.2)

Table 16 (Continued)

Position	δ_H, mult, *J*(Hz)	δ_C
16	1.17 (*s*)	C-13 (65.3), C-14 (36.9), C-15 (24.9), C-17 (16.2)
17	1.33 (*s*)	C-13 (65.3), C-14 (36.9), C-15 (24.9), C-16 (23.8)
18	1.12 (*d*, 6.5)	C-9 (78.1), C-11 (43.3), C-12 (74.9), C-13 (65.3)
19	1.80 (*s*)	C-1 (156.5), C-2 (137.5), C-3 (213.8)
20	4.01 (*AB*, 12.5)	C-5 (24.9), C-6 (143.7), C-7 (126.7)
3'	6.70 (*d*, 8)	C-3' (111.0), C-5' (114.5), C-22 (168.6)
4'	7.42 (*dt*, 2, 8, 8)	C-1' (152.8), C-6' (131.6)
5'	6.62 (*t*, 8)	C-1' (152.8), C-3' (111.0), C-4' (135.2), C-6' (131.6)
6'	7.88 (*dd*, 1.5, 8)	C-1' (152.8), C-4', C-22 (168.6)
$COCH_3$	2.08 (*s*)	C-21 (174.1)

Table 17 ^{13}C NMR and DEPT spectral data of compound **PSI2**

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
1	156.5	CH	156.5	156.5	156.5
2	137.5	C			
3	213.8	C=O			
4	49.4	CH	49.4	49.4	49.4
5	24.9	CH_2	24.9		24.9
6	143.7	C			
7	126.7	CH	126.7	126.7	126.7
8	40.5	CH	40.5	40.5	40.5
9	78.1	C			
10	47.3	CH	47.3	47.3	47.3
11	43.3	CH	43.3	43.3	43.3
12	74.9	CH	74.9	74.9	74.9
13	65.3	C			
14	36.9	CH	36.9	36.9	36.9
15	24.9	C			
16	23.8	CH_3	23.8		23.8
17	16.2	CH_3	16.2		16.2
18	11.5	CH_3	11.5		11.5
19	10.0	CH_3	10.0		10.0
20	69.2	CH_2	69.2		69.2
21	174.1	C=O			
22	168.6	C=O			

Table 17 (Continued)

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
1'	152.8	C			
2'	109.7	C			
3'	111.0	CH	111.0	111.0	111.0
4'	135.2	CH	135.2	135.2	135.2
5'	114.5	CH	114.5	114.5	114.5
6'	131.6	CH	131.6	131.6	131.6
$N\underline{C}H_3$	29.2	CH_3	29.2		29.2
$CO\underline{C}H_3$	20.7	CH_3	20.7		20.7

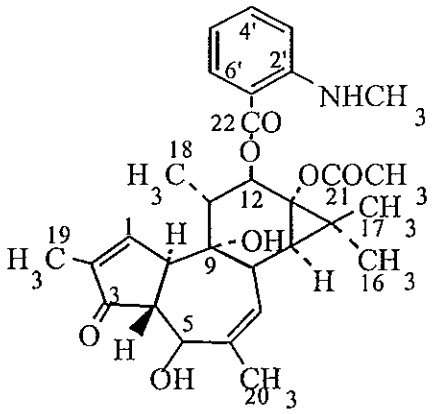
Table 18 COSY Correlations among some protons of compound **PSI2**

δH, mult, *J*(Hz)	Proton Correlations with δH (ppm)
H-1 (*s*)	H-10, H-19
H-4 (*m*)	H-5α, H-10
H-5β(*dd*, 3.5, 16)	H-4, H-5α
H-7 (*br s*)	H-5β, H-8
H-8 (*m*)	H-14
H-10 (*m*)	H-4, H-19
H-11 (*m*)	H-12, H-18
H-12 (*d*, 10.5)	H-11
H-4' (*dt*, 2, 8, 8)	H-3', H-5'
H-6' (*dd*, 1.5, 8)	H-5'

Table 19 Comparison of ^{1}H NMR spectrum between **PSI1** and **PSI2**

Position	Compound PSI1, δH (ppm) (Recorded in $CDCl_3$)	Compound PSI2, δH (ppm) (Recorded in $CDCl_3$)
1	7.56 (*br s*)	7.08 (*s*)
4	2.52 (*dt*, *J* = 5, 9.5 Hz)	2.80 (*m*)
5β	2.86 (*dd*, *J* = 9.5, 18 Hz)	3.45 (*dd*, *J* = 3.5, 16 Hz)
5α	2.17 (*dd*, *J* = 10, 18 Hz)	2.50 (*dd*, *J* = 5, 16 Hz)
7	5.55 (*m*)	5.14 (*br s*)
8	2.44 (*br t*)	2.03 (*m*)
10	3.27 (*m*)	3.54 (*m*)
11	1.73 (*m*, superimposed with 3H-19)	1.87 (*m*)
12	5.65 (*d*, *J* = 10 Hz)	5.72 (*d*, *J* = 10.5 Hz)
14	1.12 (*d*, *J* = 5 Hz)	0.84 (*d*, *J* = 5 Hz)
16	1.19 (*s*)	1.17 (*s*)
17	1.32 (*s*)	1.33 (*s*)
18	0.96 (*d*, *J* = 6.5 Hz)	1.12 (*d*, *J* = 6.5 Hz)
19	1.73 (*s*)	1.80 (*s*)
20	4.02 (*AB*, *J* = 13.5 Hz)	4.01 (*AB*, *J* = 12.5 Hz)
3'	6.69 (*dd*, *J* = 1.5, 8.5 Hz)	6.70 (*d*, *J* = 8 Hz)
4'	7.40 (*dt*, *J* = 1.5, 8.5,8.5 Hz)	7.42 (*dt*, *J* = 2, 8, 8 Hz)
5'	6.59 (*dt*, *J* = 1.5,8.5,8.5 Hz)	6.62 (*t*, *J* = 8 Hz)
6'	7.81 (*dd*, *J* = 1.5, 8.5 Hz)	7.88 (*dd*, *J* = 1.5, 8 Hz)
$NC\underline{H}_3$	2.93 (*s*)	2.93 (*br s*)
$COC\underline{H}_3$	2.12 (*s*)	2.08 (*s*)

3.3 Compound PSI3: 12-(N-Methylaminobenzoyl)-4β, 20-dideoxy-5-hydroxyphorbol-13-acetate

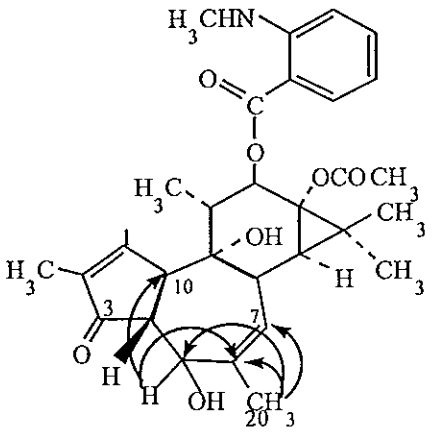


Compound **PSI3** was obtained as a yellow viscous-liquid. The IR spectrum **(Figure 20)** exhibited absorption bands at 3388 cm^{-1} for hydroxy group, 1721 and 1687 cm^{-1} for carbonyl group and 1581 and 1520 cm^{-1} for C=C stretching while the UV spectra **(Figure 19)** revealed the presence of a conjugated enone and benzoyl chromophores with maximum absorption bands at 252.0 and 360.0 nm. Additionally, it gave a brown spot with vanillin-H_2SO_4 spray reagent, a positive test for diterpene compound.

Compound **PSI3** showed similar bands in IR and UV spectrum with **PSI1**. Comparison of the ^1H NMR spectral data **(Table 25)(Figure 21)** of the two compounds revealed close structural similarity. The characteristic signal of phorbol skeleton appeared at δ 7.70 (*br s*, H-1), 5.65 (*d*, *J* = 4.5 Hz, H-12), 3.54 (*m*, H-10), 2.63 (*t*, *J* = 4.5 Hz, H-4), 2.34 (*br s*, H-8), 1.75 (*m*, 3H-19), 1.66 (*m*, H-11), 1.29 (*s*, 3H-17), 1.20 (*s*, 3H-16), 1.09 (*d*, *J* = 5.5 Hz, H-14) and 0.97 (*d*, *J* = 6.5 Hz, 3H-18). The moiety of anthranilic acid appeared at δ 7.82 (*dd*, *J* = 1.5, 8 Hz, H-6'), 7.42 (*dt*, *J* = 1.5, 8, 8 Hz, H-4'), 6.71 (*d*, *J* = 8 Hz, H-3') and 6.59 (*dt*, *J* = 1.5, 8, 8 Hz, H-5'). Spectrum of **PSI3** differed from **PSI1** at two positions : a down field signals at δ 4.87

(*br d*, J = 3.5 Hz, 1H) as compare to signal of **PSI1** (δ 2.86, *dd*, J = 9.5, 18 Hz and 2.17, *dd*, J = 10, 18 Hz, 2H-5) and a higher field signal of **PSI3** at δ 1.88 (*s*, 3H) as compare to signal of **PSI1** (δ 4.02, *AB*, J = 13.5 Hz, 2H-20). Therefore a signal of **PSI3** at δ 1.88 was suggested to be C-20 methyl protons and a downfield signal at δ 4.87 suggested a connection to hydroxy proton.

The HMBC correlation **(Table 22)(Figure 26)** confirmed the above assignment. The correlation of H-20 (δ 1.88) was to C-5 (δ 71.1), C-6 (δ 140.6) and C-7 (δ 127.3). The signal of C-5 (δ 71.1) showed ^{1}H-^{13}C direct coupling (HMQC) at δ 4.87, so the latter was assigned to H-5. The H-5 (δ 4.87) correlated to C-6 (δ 140.6) and C-10 (δ 51.5). The more detail informations were summarized in **Tables 21-26, Figures 19-26.**



Selected HMBC Correlations of PSI3

Comparison of ^{1}H NMR spectral data between compound **PSI3** and 12-(N-Methylaminobenzoyl)-4β,20-dideoxy-5-hydroxyphorbol-13-acetate showed similarity **(Table 20)**. Thus compound **PSI3** was confirmed as 12-(N-Methylaminobenzoyl)-4β,20-dideoxy-5-hydroxyphorbol-13-acetate which was previously isolated from fruits of *Sapium indicum* (Taylor, *et.al.*, 1981).

Table 20 Comparison of ^{1}H NMR spectrum between 4β,20-dideoxyphorbol and **PSI3**

Position	**4β,20-dideoxyphorbol, δH (ppm) (Recorded in $CDCl_3$)**	**Compound PSI3, δH (ppm) (Recorded in $CDCl_3$)**
1	7.70 (*s*)	7.70 (*br s*)
4	2.63 (*t*, *J* = 3.7 Hz)	2.63 (*t*, *J* = 4.5 Hz)
5	4.87 (*d*, *J* = 3.7Hz)	4.87 (*br d*, *J* = 3.5 Hz)
7	5.32 (*d*, *J* = 5.2 Hz)	5.33 (*m*)
8	2.34 (*m*)	2.34 (*br s*)
10	3.53 (*m*)	3.54 (*m*)
11	1.66 (*m*)	1.66 (*m*)
12	5.65 (*d*, *J* = 9.2 Hz)	5.65 (*d*, *J* = 9.5 Hz)
14	1.08 (*d*, *J* = 5.5 Hz)	1.09 (*d*, *J* = 5.5 Hz)
16	1.20 (*s*)	1.20 (*s*)
17	1.29 (*s*)	1.29 (*s*)
18	0.96 (*d*, *J* = 6.3 Hz)	0.97 (*d*, *J* = 6.5 Hz)
19	1.73 (*s*)	1.75 (*m*)
20	1.87 (*s*)	1.88 (*s*)
3'	6.69 (*d*, *J* = 8.1 Hz)	6.71 (*d*, *J* = 8 Hz)
4'	7.43 (*t*, *J* = 6.9 Hz)	7.42 (*dt*, *J* = 1.5, 8, 8 Hz)
5'	6.59 (*t*, *J* = 8.1 Hz)	6.59 (*dt*, *J* = 1.5, 8, 8 Hz)
6'	7.87 (*dd*, *J* = 2, 8.1 Hz)	7.82 (*dd*, *J* = 1.5, 8 Hz)
NCH_3	2.93 (*d*, *J* = 4.4 Hz)	2.93 (*s*)
$COCH_3$	2.13 (*s*)	2.13 (*s*)

Table 21 ^{1}H and ^{13}C NMR spectral data of compound **PSI3**

Position	δ_C	δ_H, mult, J(Hz)
1	162.6	7.70 (*br s*)
2	138.3	
3	208.5	
4	51.4	2.63 (*t*, 4.5)
5	71.1	4.87 (*br d*, 3.5)
6	140.6	
7	127.3	5.33 (*m*)
8	42.4	2.34 (*br s*)
9	78.3	
10	51.5	3.54 (*m*)
11	43.0	1.66 (*m*)
12	76.3	5.65 (*d*, 9.5)
13	65.6	
14	36.5	1.09 (*d*, 5.5)
15	25.8	
16	23.7	1.20 (*s*)
17	16.9	1.29 (*s*)
18	15.3	0.97 (*d*, 6.5)
19	10.1	1.75 (*m*)

Table 21 (Continued)

Position	δC	δH, mult, *J*(Hz)
20	21.7	1.88 (*s*)
21	173.6	
22	168.1	
1'	152.3	
2'	109.4	
3'	110.8	6.71 (*d*, 8)
4'	134.9	7.42 (*dt*, 1.5, 8, 8)
5'	114.4	6.59 (*dt*, 1.5,8, 8)
6'	131.3	7.82 (*dd*, 1.5, 8)
NCH_3	29.5	2.93 (*s*)
$COCH_3$	21.1	2.13 (*s*)

Table 22 Major HMBC correlations of compound **PSI3**

Position	δH, mult, *J*(Hz)	δC
1	7.70 (*br s*)	C-3 (208.5), C-4 (51.4), C-10 (51.5)
4	2.63 (*t*, 4.5)	C-3 (208.5), C-9 (78.3), C-10 (51.5)
5	4.87 (*br d*, 3.5)	C-6 (140.6), C-10 (51.5)
7	5.33 (*m*)	C-14 (36.5), C-20 (21.7)

Table 22 (Continued)

Position	δH, mult, *J*(Hz)	δC
8	2.34 (*br s*)	C-7 (127.3), C-14 (36.5), C-15 (25.8)
11	1.66 (*m*)	C-12 (76.3)
12	5.65 (*d*, 9.5)	C-11 (43.0), C-13 (65.6), C-15 (36.5), C-18 (15.3), C-22 (168.1)
14	1.09 (*d*, 5.5)	C-12 (76.3), C-13 (65.6), C-15 (25.8), C-16 (23.7)
16	1.20 (*s*)	C-13 (65.6), C-14 (36.5), C-15 (36.5), C-17 (16.9)
17	1.29 (*s*)	C-13 (65.6), C-14 (36.5), C-15 (36.5), C-16 (23.7)
18	0.97 (*d*, 6.5)	C-9 (78.3), C-11 (43.0), C-12 (76.3)
19	1.75 (*m*)	C-1 (162.6), C-2 (138.3), C-3 (208.5)
20	1.88 (*s*)	C-5 (71.1), C-6 (140.6), C-7 (127.3)
3'	6.71 (*d*, 8)	C-2' (109.4), C-5' (114.4)
4'	7.42 (*dt*, 1.5, 8, 8)	C-1' (152.3), C-6' (131.3)
5'	6.59 (*dt*, 1.5, 8, 8)	C-2' (109.4), C-3' (110.8

Table 22 (Continued)

Position	δH, mult, *J*(Hz)	δC
6'	7.82 (*dd*, 1.5, 8)	C-1' (152.3), C-4' (134.9), C-22 (168.1)
$COCH_3$	2.13 (*s*)	C-21 (173.6)

Table 23 ^{13}C NMR and DEPT spectral data of compound **PSI3**

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
1	162.6	CH	162.6	162.6	162.6
2	138.3	C			
3	208.5	C=O			
4	51.4	CH	51.4	51.4	51.4
5	71.1	CH	71.1	71.1	71.1
6	140.6	C			
7	127.3	CH	127.3	127.3	127.3
8	42.4	CH	42.4	42.4	42.4
9	78.3	C			
10	51.5	CH	51.5	51.5	51.5
11	43.0	CH	43.0	43.0	43.0
12	76.3	CH	76.3	76.3	76.3
13	65.6	C			
14	36.5	CH	36.5	36.5	36.5
15	25.8	C			

Table 23 (Continued)

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
16	23.7	CH_3	23.7		23.7
17	16.9	CH_3	16.9		16.9
18	15.3	CH_3	15.3		15.3
19	10.1	CH_3	10.1		10.1
20	21.7	CH_3	21.7		21.7
21	173.6	C=O			
22	168.1	C=O			
1'	152.3	C			
2'	109.4	C			
3'	110.8	CH	110.8	110.8	110.8
4'	134.9	CH	134.9	134.9	134.9
5'	114.4	CH	114.4	114.4	114.4
6'	131.3	CH	131.3	131.3	131.3
$N\underline{C}H_3$	29.5	CH_3	29.5		29.5
$CO\underline{C}H_3$	21.1	CH_3	21.1		21.1

Table 24 COSY correlations among some protons of compound **PSI3**

δH, mult, *J*(Hz)	**Proton correlations with δH (ppm)**
H-1 (*br s*)	H-10, H-19
H-4 (*t*, 4.5)	H-10
H-5 (*br d*, 3.5)	H-4, H-20
H-7 (*m*)	H-5, H-8, H-20
H-8 (*br s*)	H-14
H-11 (*m*)	H-12, H-18
H-3' (*d*, 8)	H-5'
H-4' (*dt*, 1.5, 8, 8)	H-3', H-5'
H-6' (*dd*, 1.5, 8)	H-4', H-5'

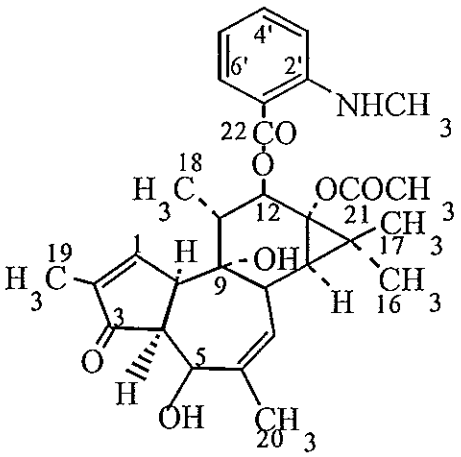
Table 25 Comparison of ^{1}H NMR spectrum between **PSI1** and **PSI3**

Position	**Compound PSI1, δH (ppm) (Recorded in $CDCl_3$)**	**Compound PSI3, δH (ppm) (Recorded in $CDCl_3$)**
1	7.56 (*br s*)	7.70 (*br s*)
4	2.52 (*dt*, *J* = 5, 9.5 Hz)	2.63 (*t*, *J* = 4.5 Hz)
5	2.86 (*dd*, *J* = 9.5, 18 Hz)	4.87 (*br d*, *J* = 3.5 Hz)
	2.17 (*dd*, *J* = 10, 18 Hz)	
7	5.55 (*m*)	5.33 (*m*)
8	2.44 (*br t*)	2.34 (*br s*)
10	3.27 (*m*)	3.54 (*m*)
11	1.73 (*m*, superimposed with 3H-19)	1.66 (*m*)
12	5.65 (*d*, *J* = 10 Hz)	5.65 (*d*, *J* = 9.5 Hz)

Table 25 (Continued)

Position	Compound PSI1, δH (ppm) (Recorded in $CDCl_3$)	Compound PSI3, δH (ppm) (Recorded in $CDCl_3$)
14	1.12 (*d*, *J* = 5 Hz)	1.09 (*d*, *J* = 5.5 Hz)
16	1.19 (*s*)	1.20 (*s*)
17	1.32 (*s*)	1.29 (*s*)
18	0.96 (*d*, *J* = 6.5 Hz)	0.97 (*d*, *J* = 6.5 Hz)
19	1.73 (*s*)	1.75 (*m*)
20	4.02 (*AB*, *J* = 13.5 Hz)	1.88 (*s*)
3'	6.69 (*dd*, *J* = 1.5, 8.5 Hz)	6.71 (*d*, *J* = 8 Hz)
4'	7.40 (*dt*, *J* = 1.5, 8.5,8.5 Hz)	7.42 (*dt*, *J* = 1.5, 8, 8 Hz)
5'	6.59 (*dt*, *J* = 1.5,8.5,8.5 Hz)	6.59 (*dt*, *J* = 1.5, 8, 8 Hz)
6'	7.81 (*dd*, *J* = 1.5, 8.5 Hz)	7.82 (*dd*, *J* = 1.5, 8 Hz)
$NC\underline{H}_3$	2.93 (*s*)	2.93 (*s*)
$COC\underline{H}_3$	2.12 (*s*)	2.13 (*s*)

3.4 Compound PSI4: 12-(N-Methylaminobenzoyl)-4α, 20-dideoxy-5-hydroxyphorbol-13-acetate



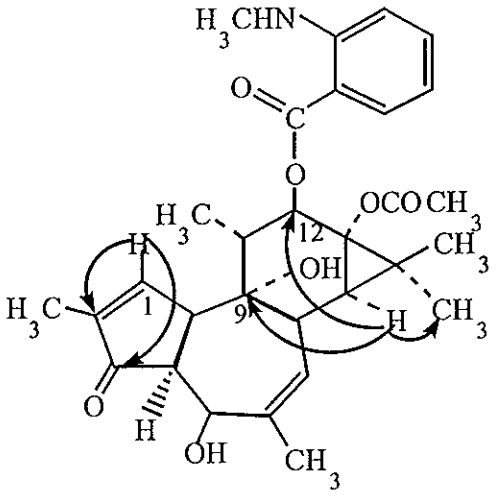
Compound **PSI4** was isolated as a yellow viscous-liquid. This compound showed the characteristic of diterpene by giving a brown spot with vanillin-H_2SO_4 spray reagent. The IR spectrum **(Figure 28)** exhibited an absorption band of hydroxy group (3388 cm^{-1}), carbonyl group (1721, 1687 cm^{-1}) and C=C stretching (1581, 1520 cm^{-1}) while the UV spectra **(Figure 27)** revealed the presence of a conjugated enone and benzoyl chromophores with maximum absorption bands at 247.5 and 359.5 nm.

The ^{13}C NMR spectrum showed the signals of 30 carbon atoms **(Table 27)(Figure 30)**. Analysis of the DEPT spectra indicated the presence of three carbonyl carbons (δ207.5, 173.9 and 168.0), seven methyl carbons (δ29.6, 27.1, 24.1, 21.1, 16.5, 11.7 and 10.4), thirteen methine carbons (δ154.6, 135.0, 131.3, 125.4, 114.3, 110.9, 74.0, 70.9, 56.1, 47.8, 43.4, 40.1 and 38.1) and seven quaternary carbons (δ152.4, 144.0, 137.7, 109.3, 78.5, 65.3 and 25.3).

Comparison of the ^1H NMR spectral data **(Table 31)(Figure 29)** of **PSI4** with **PSI3** revealed close structural similarity. Difference in the spectrum of **PSI4** was shown as the signals at δ3.12 (*dd*, J = 4.5, 6.5 Hz, 1H) which appeared at the lower field than the signal of **PSI3** (δ2.63, *t*, J = 4.5 Hz, 1H) which could be assign to H-4.

Two proton signals of **PSI4** (δ7.07, *br s*, 1H and 0.86 *d*, J = 4.5 Hz, 1H) appeared at the higher field than the signal of **PSI3** (δ7.70, *br s*, 1H and 1.09, *d*, J = 5.5 Hz, 1H) which should be assigned to H-1 and H-14, respectively. Thus the compound **PSI4** should be 4α-epimer of **PSI3**.

In the HMBC correlation of **PSI4 (Table 28)(Figure 34)**, the H-1 (δ7.07) was confirmed by the correlations to C-2 (δ144.0) and C-3 (δ207.5). The H-14 (δ 0.86) was found to correlate to C-9 (δ78.5), C-12 (74.0), C-13 (δ65.3), C-15 (δ 25.3), C-16 (δ24.1) and C-17 (δ16.5). The important data were shown in **Tables 26-31, Figures 27-34.**



Selected HMBC Correlations of PSI4

Comparison of ^{1}H NMR spectral data between compound **PSI4** and 12-(N-Methylaminobenzoyl)-4α,20-dideoxy-5-hydroxyphorbol-13-acetate showed similarity **(Table 26)**. Thus compound **PSI4** was confirmed as 12-(N-Methylaminobenzoyl)-4α,20-dideoxy-5-hydroxyphorbol-13-acetate which was previously isolated from fruits of *Sapium indicum* (Taylor, *et.al.*, 1981).

Table 26 Comparison of ^{1}H NMR spectrum between 4α,20-dideoxyphorbol and **PSI4**

Position	**4α,20-dideoxyphorbol, δH (ppm) (Recorded in $CDCl_3$)**	**Compound PSI4, δH (ppm) (Recorded in $CDCl_3$)**
1	7.17 (*s*)	7.07 (*br s*)
4	3.12 (*dd*, *J* = 4.6, 6.4 Hz)	3.12 (*dd*, *J* = 4.5, 6.5 Hz)
5	4.60 (*d*, *J* = 6.4 Hz)	4.45 (*br s*)
7	5.05 (*br s*)	4.88 (*br s*)
8	2.31 (*m*)	2.05 (*m*)
10	3.64 (*m*)	3.64 (*m*)
11	1.63 (*m*)	1.85 (*m*)
12	5.64 (*d*, *J* = 11.6 Hz)	5.70 (*d*, *J* = 10 Hz)
14	0.84 (*d*, *J* = 5.2 Hz)	0.86 (*d*, *J* = 4.5 Hz)
16	1.11 (*s*)	1.18 (*s*)
17	1.16 (*s*)	1.31 (*s*)
18	0.96 (*d*, *J* = 6.3 Hz)	1.12 (*d*, *J* = 6 Hz)
19	1.83 (*s*)	1.81 (*br t*, *J* = 1.5 Hz)
20	1.86 (*s*)	1.88 (*br s*)
3'	6.72 (*d*, *J* = 7.9 Hz)	6.72 (*d*, *J* = 8 Hz)
4'	7.43 (*t*, *J* = 6.9 Hz)	7.43 (*dt*, *J* = 1.5, 8, 8 Hz)
5'	6.61 (*t*, *J* = 8.1 Hz)	6.61 (*dt*, *J* = 1.5, 8, 8 Hz)
6'	7.87 (*dd*, *J* = 1.6, 8.1 Hz)	7.88 (*dd*, *J* = 1.5, 8 Hz)
$NC\underline{H}_3$	2.94 (*s*)	2.94 (*d*, *J* = 4.5 Hz)
$COC\underline{H}_3$	2.13 (*s*)	2.10 (*s*)

Table 27 ^{1}H and ^{13}C NMR spectral data of compound **PSI4**

Position	δC	δH, mult, *J*(Hz)
1	154.6	7.07 (*br s*)
2	144.0	
3	207.5	
4	56.1	3.12 (*dd*, 4.5, 6.5)
5	70.9	4.45 (*br s*)
6	137.7	
7	125.4	4.88 (*br s*)
8	40.1	2.05 (*m*)
9	78.5	
10	47.8	3.64 (*m*)
11	43.4	1.85 (*m*)
12	74.0	5.70 (*d*, 10)
13	65.3	
14	38.1	0.86 (*d*, 4.5)
15	25.3	
16	24.1	1.18 (*s*)
17	16.5	1.31 (*s*)
18	11.7	1.12 (*d*, 6)
19	10.4	1.81 (*br t*, 1.5)
20	27.1	1.88 (*br s*)

Table 27 (Continued)

Position	δC	δH, mult, *J*(Hz)
21	173.9	
22	168.0	
1'	152.4	
2'	109.3	
3'	110.9	6.72 (*d*, 8)
4'	135.0	7.43 (*dt*, 1.5, 8, 8)
5'	114.3	6.61 (*dt*, 1.5,8, 8)
6'	131.3	7.88 (*dd*, 1.5, 8)
$NC\underline{H}_3$	29.6	2.94 (*d*, 4.5)
$COC\underline{H}_3$	21.1	2.10 (*s*)

Table 28 Major HMBC correlations of compound **PSI4**

Position	δH, mult, *J*(Hz)	δC
1	7.07 (*br s*)	C-2 (144.0), C-3 (207.5)
4	3.12 (*dd*, 4.5, 6.5)	C-5 (70.9), C-9 (78.5), C-10 (47.8)
5	4.45 (*br s*)	C-6 (137.7)
7	4.88 (*br s*)	C-5 (70.9), C-8 (40.1), C-9 (78.5), C-14 (38.1), C-20 (27.1)

Table 28 (Continued)

Position	δH, mult, *J*(Hz)	δC
8	2.05 (*m*)	C-13 (65.3), C-14 (38.1), C-20 (27.1)
10	3.64 (*m*)	C-4 (56.1), C-8 (40.1), C-9 (78.5)
11	1.85 (*m*)	C-9 (78.5), C-10 (47.8), C-12 (74.0), C-18 (11.7)
12	5.70 (*d*, 10)	C-11 (43.4), C-13 (65.3), C-15 (25.3), C-18 (11.7), C-22 (168.0)
14	0.86 (*d*, 4.5)	C-9 (78.5), C-12 (74.0), C-13 (74.0), C-15 (25.3), C-16 (24.1), C-17 (16.5)
16	1.18 (*s*)	C-13 (74.0), C-14, C-15 (25.3), C-17 (16.5)
17	1.31 (*s*)	C-13 (74.0), C-14, C-15 (25.3), C-16 (24.1)
18	1.12 (*d*, 6)	C-9 (78.5), C-11 (43.4), C-12 (74.0), C-13 (74.0
19	1.81 (*br t*, 1.5)	C-1 (154.6), C-2 (144.0), C-3 (207.5)
20	1.88 (*br s*)	C-5 (70.9), C-6 (137.7), C-7 (125.4)
3'	6.72 (*d*, 8)	C-2' (109.3), C-5' (114.3)

Table 28 (Continued)

Position	δH, mult, *J*(Hz)	δC
4'	7.43 (*dt*, 1.5, 8, 8)	C-1' (152.4), C-6' (131.3)
5'	6.61 (*dt*, 1.5,8, 8)	C-1' (152.4), C-2' (109.3), C-3' (110.9), C-4' (135.0), C-6' (131.3)
6'	7.88 (*dd*, 1.5, 8)	C-1' (152.4), C-4' (135.0), C-22 (168.0)
$COC\underline{H}_3$	2.10 (*s*)	C-21 (173.9)

Table 29 ^{13}C NMR and DEPT spectral data of compound **PSI4**

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
1	154.6	CH	154.6	154.6	154.6
2	144.0	C			
3	207.5	C=O			
4	56.1	CH	56.1	56.1	56.1
5	70.9	CH	70.9	70.9	70.9
6	137.7	C			
7	125.4	CH	125.4	125.4	125.4
8	40.1	CH	40.1	40.1	40.1
9	78.5	C			
10	47.8	CH	47.8	47.8	47.8

Table 29 (Continued)

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
11	43.4	CH	43.4	43.4	43.4
12	74.0	CH	74.0	74.0	74.0
13	65.3	C			
14	38.1	CH	38.1	38.1	38.1
15	25.3	C			
16	24.1	CH_3	24.1		24.1
17	16.5	CH_3	16.5		16.5
18	11.7	CH_3	11.7		11.7
19	10.4	CH_3	10.4		10.4
20	27.1	CH_3	27.1		27.1
21	173.9	C=O			
22	168.0	C=O			
1'	152.4	C			
2'	109.3	C			
3'	110.9	CH	110.9	110.9	110.9
4'	135.0	CH	135.0	135.0	135.0
5'	114.3	CH	114.3	114.3	114.3
6'	131.3	CH	131.3	131.3	131.3
$N\underline{C}H_3$	29.6	CH_3	29.6		29.6
$CO\underline{C}H_3$	21.1	CH_3	21.1		21.1

Table 30 COSY correlations among some protons of compound **PSI4**

δH, mult, *J*(Hz)	**Proton Correlations with δH (ppm)**
H-1 (*br s*)	H-10, H-19
H-4 (*dd*, 4.5, 6.5)	H-5
H-7 (*br s*)	H-8, H-20
H-8 (*m*)	H-14
H-11 (*m*)	H-12, H-18
H-3' (*d*, 8)	H-5'
H-4' (*dt*, 1.5, 8, 8)	H-3', H-5'
H-6' (*dd*, 1.5, 8)	H-4', H-5'

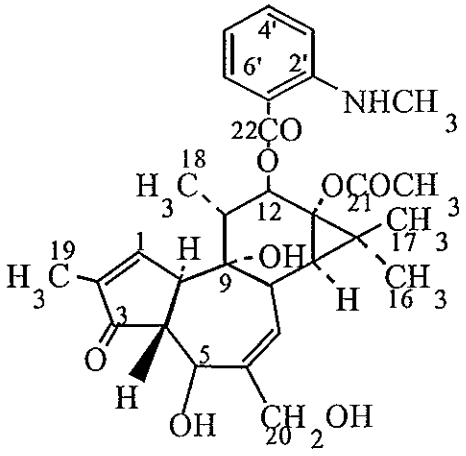
Table 31 Comparison of ^{1}H NMR spectral data between **PSI3** and **PSI4**

Position	**Compound PSI3, δH (ppm) (Recorded in $CDCl_3$)**	**Compound PSI4, δH (ppm) (Recorded in $CDCl_3$)**
1	7.70 (*br s*)	7.07 (*br s*)
4	2.63 (*t*, J = 4.5 Hz)	3.12 (*dd*, J = 4.5, 6.5 Hz)
5	4.87 (*br d*, J = 3.5 Hz)	4.45 (*br s*)
7	5.33 (*m*)	4.88 (*br s*)
8	2.34 (*br s*)	2.05 (*m*)
10	3.54 (*m*)	3.64 (*m*)
11	1.66 (*m*)	1.85 (*m*)
12	5.65 (*d*, J = 9.5 Hz)	5.70 (*d*, J = 10 Hz)
14	1.09 (*d*, J = 5.5 Hz)	0.86 (*d*, J = 4.5 Hz)
16	1.20 (*s*)	1.18 (*s*)

Table 31 (Continued)

Position	Compound PSI3, δH (ppm) (Recorded in $CDCl_3$)	Compound PSI4, δH (ppm) (Recorded in $CDCl_3$)
17	1.29 (*s*)	1.31 (*s*)
18	0.97 (*d*, *J* = 6.5 Hz)	1.12 (*d*, *J* = 6 Hz)
19	1.75 (*m*)	1.81 (*br t*, *J* = 1.5 Hz)
20	1.88 (*s*)	1.88 (*br s*)
3'	6.71 (*d*, *J* = 8 Hz)	6.72 (*d*, *J* = 8 Hz)
4'	7.42 (*dt*, *J* = 1.5, 8, 8 Hz)	7.43 (*dt*, *J* = 1.5, 8, 8 Hz)
5'	6.59 (*dt*, *J* = 1.5, 8, 8 Hz)	6.61 (*dt*, *J* = 1.5, 8, 8 Hz)
6'	7.82 (*dd*, *J* = 1.5, 8 Hz)	7.88 (*dd*, *J* = 1.5, 8 Hz)
NCH_3	2.93 (*s*)	2.94 (*d*, *J* = 4.5 Hz)
$COCH_3$	2.13 (*s*)	2.10 (*s*)

3.5 Compound PSI5: 12-(N-Methylaminobenzoyl)-4β-deoxy-5, 20-dihydroxyphorbol-13-acetate



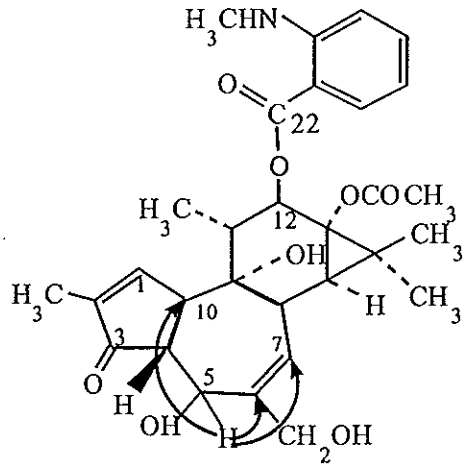
Compound **PSI5** was obtained as a yellow viscous-liquid. The UV spectrum **(Figure 35)** showed maximum absorptions at 252.5 and 360.0 nm suggesting the presence of a conjugated enone and benzoyl chromophores. The IR spectrum **(Figure 36)** showed the stretching of hydroxy group at 3388 cm^{-1}, carbonyl group at 1724 and 1687 cm^{-1} and C=C 1581 and 1520 cm^{-1}. It also gave a brown spot with vanillin-H_2SO_4 spray reagent, a positive test for diterpene compound.

The ^{13}C NMR spectrum showed the signals of 30 carbon atoms **(Table 33)(Figure 38)**. Analysis of the DEPT spectra indicated the presence of three carbonyl carbons (δ208.4, 174.0 and 168.2), six methyl carbons (δ29.8, 23.9, 21.4, 17.1, 15.5 and 10.3), one methylene carbon (δ67.1), thirteen methine carbons (δ162.7, 135.2, 131.5, 130.3, 114.7, 111.1, 76.3, 71.1, 52.1, 51.4, 43.2, 42.5 and 36.3) and seven quaternary carbons (δ152.6, 143.1, 138.5, 109.5, 78.6, 65.7 and 26.0).

The ^{1}H NMR spectral data of **PSI5** was similar to that of **PSI1** **(Table 37)(Figure 37)**. Difference in the spectrum of **PSI5** was shown as the signals at δ5.20 (*d*, J = 4.5 Hz, 1H) which appeared at the lower field than the signal of **PSI1** (δ2.86,

dd, J = 9.5, 18 Hz and δ2.17, *dd, J* = 10, 18 Hz, 2H-5). These observation indicated that C-5 of **PSI5** should be connected to the hydroxy proton.

In the HMBC correlation of compound **PSI5 (Table 34)(Figure 42)**, the H-5 (δ5.20) was confirmed by correlations to C-6 (δ143.1), C-7 (δ130.3) and C-10 (δ 52.1). 1D and 2D NMR of **PSI5** were summarized in **Tables 32-37, Figures 35-42.**



Selected HMBC Correlations of PSI5

Comparison of ^{1}H NMR spectral data between compound **PSI5** and 12-(N-Methylaminobenzoyl)-4β-deoxy-5,20-dihydroxyphorbol-13-acetate showed similarity **(Table 32)**. Thus compound **PSI5** was confirmed as 12-(N-Methylaminobenzoyl)-4β-deoxy-5,20-dihydroxyphorbol-13-acetate which was previously isolated from fruits of *Sapium indicum* (Taylor, *et.al.*, 1981).

Table 32 Comparison of ^{1}H NMR spectrum between 4β-deoxyphorbol and **PSI5**

Position	4β-deoxyphorbol, δH (ppm) (Recorded in $CDCl_3$)	Compound PSI5, δH (ppm) (Recorded in $CDCl_3$)
1	7.69 (*s*)	7.71 (*br s*)
4	2.63 (*t*, *J* = 4.4 Hz)	2.64 (*t*, *J* = 4.5 Hz)
5	5.14 (*br s*)	5.20 (*d*, *J* = 4.5 Hz)
7	5.63 (*br s*)	5.62 (*d*, *J* = 5 Hz)
8	2.36 (*m*)	2.35 (*br t*, *J* = 5 Hz)
10	3.55 (*m*)	3.59 (*m*)
11	1.74 (*m*)	1.68 (*m*)
12	5.62 (*d*, *J* = 9.7 Hz)	5.65 (*d*, *J* = 10 Hz)
14	1.10 (*d*, *J* = 5.5 Hz)	1.13 (*d*, *J* = 5 Hz)
16	1.20 (*s*)	1.20 (*s*)
17	1.29 (*s*)	1.29 (*s*)
18	0.96 (*d*, *J* = 6.2 Hz)	0.97 (*d*, *J* = 6 Hz)
19	1.75 (*s*)	1.75 (*m*)
20	4.23 (*br s*)	4.25 (*AB*, *J* = 13 Hz)
3'	6.68 (*d*, *J* = 8.8 Hz)	6.71 (*d*, *J* = 8 Hz)
4'	7.41 (*t*, *J* = 7.0 Hz)	7.42 (*dt*, *J* = 1.5, 8, 8 Hz)
5'	6.61 (*t*, *J* = 7.9 Hz)	6.60 (*dt*, *J* = 1.5, 8, 8 Hz)
6'	7.81 (*dd*, *J* = 1.4, 7.9 Hz)	7.82 (*dd*, *J* = 1.5, 8 Hz)
$NC\underline{H}_3$	2.93 (*d*, *J* = 4.9 Hz)	2.93 (*s*)
$COC\underline{H}_3$	2.13 (*s*)	2.13 (*s*)

Table 33 ^{1}H and ^{13}C NMR spectral data of compound **PSI5**

Position	δ_C	δ_H, mult, *J*(Hz)
1	162.7	7.71 (*br s*)
2	138.5	
3	208.4	
4	51.4	2.64 (*t*, 4.5)
5	71.1	5.20 (*d*, 4.5)
6	143.1	
7	130.3	5.62 (*d*, 5)
8	42.5	2.35 (*br t*, 5)
9	78.6	
10	52.1	3.59 (*m*)
11	43.2	1.68 (*m*)
12	76.3	5.65 (*d*, 10)
13	65.7	
14	36.3	1.13 (*d*, 5)
15	26.0	
16	23.9	1.20 (*s*)
17	17.1	1.29 (*s*)
18	15.5	0.97 (*d*, 6)
19	10.3	1.75 (*m*)
20	67.1	4.25 (*AB*, 13)

Table 33 (Continued)

Position	δC	δH, mult, *J*(Hz)
21	174.0	
22	168.2	
1'	152.6	
2'	109.5	
3'	111.1	6.71 (*d*, 8)
4'	135.2	7.42 (*dt*, 1.5, 8, 8)
5'	114.7	6.60 (*dt*, 1.5, 8, 8)
6'	131.5	7.82 (*dd*, 1.5, 8)
$NC\underline{H}_3$	29.8	2.93 (*s*)
$COC\underline{H}_3$	21.4	2.13 (*s*)

Table 34 Major HMBC correlations of compound **PSI5**

Position	δH, mult, *J*(Hz)	δC
1	7.71 (*br s*)	C-2 (138.5), C-3 (208.4), C-10 (52.1), C-19 (10.3)
4	2.64 (*t*, 4.5)	C-3 (208.4), C-5 (71.1), C-9 (78.6), C-10 (52.1)
5	5.20 (*d*, 4.5)	C-6 (143.1), C-7 (130.3), C-10 (52.1)
7	5.62 (*d*, 5)	C-5 (71.1), C-8 (42.5), C-9 (78.6), C-20 (67.1)

Table 34 (Continued)

Position	δH, mult, *J*(Hz)	δC
8	2.35 (*br t*, 5)	C-6 (143.1), C-7 (130.3), C-9 (78.6), C-11 (43.2), C-14 (36.3), C-15 (26.0)
10	3.59 (*m*)	C-1 (162.7), C-9 (78.6), C-11 (43.2)
11	1.68 (*m*)	C-12 (76.3), C-18 (15.5)
12	5.65 (*d*, 10)	C-11 (43.2), C-14 (36.3), C-15 (26.0), C-18 (15.5), C-22 (168.2)
14	1.13 (*d*, 5)	C-12 (76.3), C-13 (65.7), C-16 (23.9)
16	1.20 (*s*)	C-13 (65.7), C-14 (36.3), C-15 (23.9), C-17 (17.1)
17	1.29 (*s*)	C-13 (65.7), C-14 (36.3), C-15 (23.9), C-16 (23.9)
18	0.97 (*d*, 6)	C-9 (78.6), C-11 (43.2), C-12 (76.3)
19	1.75 (*m*)	C-1 (162.7), C-2 (138.5), C-3 (208.4), C-9 (78.6), C-10 (52.1)
20	4.25 (*AB*, 13)	C-5 (71.1), C-6 (143.1), C-7 (130.3)
3'	6.71 (*d*, 8)	C-2' (109.5), C-5' (114.7)

Table 34 (Continued)

Position	δH, mult, *J* (Hz)	δC
4'	7.42 (*dt*, 1.5, 8)	C-1' (152.6), C-6' (131.5)
5'	6.60 (*dt*, 1.5, 8)	C-1' (152.6), C-3' (111.1), C-4' (135.2), C-6' (131.5)
6'	7.82 (*dd*, 1.5, 8)	C-1' (152.6), C-4' (135.2), C-22 (168.2)
$COC\underline{H}_3$	2.13 (*s*)	C-21 (174.0)

Table 35 ^{13}C NMR and DEPT spectral data of compound **PSI5**

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
1	162.7	CH	162.7	162.7	162.7
2	138.5	C			
3	208.4	C=O			
4	51.4	CH	51.4	51.4	51.4
5	71.1	CH	71.1	71.1	71.1
6	143.1	C			
7	130.3	CH	130.3	130.3	130.3
8	42.5	CH	42.5	42.5	42.5
9	78.6	C			
10	52.1	CH	52.1	52.1	52.1

Table 35 (Continued)

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
11	43.2	CH	43.2	43.2	43.2
12	76.3	CH	76.3	76.3	76.3
13	65.7	C			
14	36.3	CH	36.3	36.3	36.3
15	26.0	C			
16	23.9	CH_3	23.9		23.9
17	17.1	CH_3	17.1		17.1
18	15.5	CH_3	15.5		15.5
19	10.3	CH_3	10.3		10.3
20	67.1	CH_2	67.1		67.1
21	174.0	C=O			
22	168.2	C=O			
1'	152.6	C	111.1	111.1	111.1
2'	109.5	C	135.2	135.2	135.2
3'	111.1	CH	114.7	114.7	114.7
4'	135.2	CH	131.5	131.5	131.5
5'	114.7	CH	29.8		29.8
6'	131.5	CH	21.4		21.4
$N\underline{C}H_3$	29.8	CH_3			
$CO\underline{C}H_3$	21.4	CH_3			

Table 36 COSY correlations among some protons of compound **PSI5**

δH, mult, *J*(Hz)	Proton correlations with δH (ppm)
H-5 (*br d*, 3.5)	H-4
H-8 (*br s*)	H-14
H-10 (*m*)	H-4, H-19
H-11 (*m*)	H-18
H-12 (*d*, 10)	H-11
H-4' (*dt*, 1.5, 8, 8)	H-3', H-5'
H-6' (*dd*, 1.5, 8)	H-4', H-5'

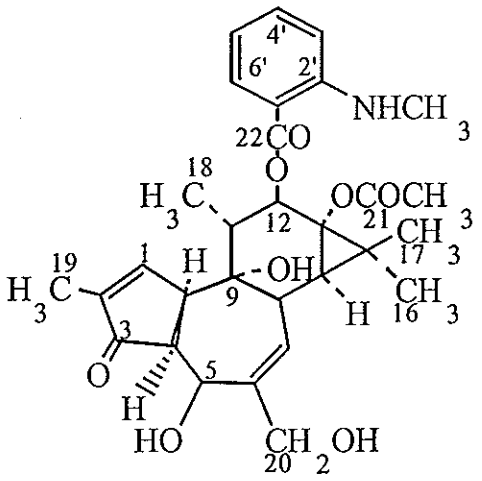
Table 37 Comparison of ^{1}H NMR spectral data between **PSI1** and **PSI5**

Position	Compound PSI1, δH (ppm) (Recorded in $CDCl_3$)	Compound PSI5, δH (ppm) (Recorded in $CDCl_3$)
1	7.56 (*br s*)	7.71 (*br s*)
4	2.52 (*dt*, *J* = 5, 9.5 Hz)	2.64 (*t*, *J* = 4.5 Hz)
5	2.86 (*dd*, *J* = 9.5, 18 Hz)	5.20 (*d*, *J* = 4.5 Hz)
	2.17 (*dd*, *J* = 10, 18 Hz)	
7	5.55 (*m*)	5.62 (*d*, *J* = 5 Hz)
8	2.44 (*br t*)	2.35 (*br t*, *J* = 5 Hz)
10	3.27 (*m*)	3.59 (*m*)
11	1.73 (*m*, superimposed with 3H-19)	1.68 (*m*)
12	5.65 (*d*, *J* = 10 Hz)	5.65 (*d*, *J* = 10 Hz)
14	1.12 (*d*, *J* = 5 Hz)	1.13 (*d*, *J* = 5 Hz)
16	1.19 (*s*)	1.20 (*s*)

Table 37 (Continued)

Position	Compound PSI1, δH (ppm) (Recorded in $CDCl_3$)	Compound PSI5, δH (ppm) (Recorded in $CDCl_3$)
17	1.32 (*s*)	1.29 (*s*)
18	0.96 (*d*, *J* = 6.5 Hz)	0.97 (*d*, *J* = 6 Hz)
19	1.73 (*s*)	1.75 (*m*)
20	4.02 (*AB*, *J* = 13.5 Hz)	4.25 (*AB*, *J* = 13 Hz)
3'	6.69 (*dd*, *J* = 1.5, 8.5 Hz)	6.71 (*d*, *J* = 8 Hz)
4'	7.40 (*dt*, *J* = 1.5, 8.5, 8.5 Hz)	7.42 (*dt*, *J* = 1.5, 8, 8 Hz)
5'	6.59 (*dt*, *J* = 1.5,8.5, 8.5 Hz)	6.60 (*dt*, *J* = 1.5, 8, 8 Hz)
6'	7.81 (*dd*, *J* = 1.5, 8.5 Hz)	7.82 (*dd*, *J* = 1.5, 8 Hz)
$NC\underline{H}_3$	2.93 (*s*)	2.93 (*s*)
$COC\underline{H}_3$	2.12 (*s*)	2.13 (*s*)

3.6 Compound PSI6: 12-(N-Methylaminobenzoyl)-4α-deoxy-5, 20-dihydroxyphorbol-13-acetate



Compound **PSI6** was isolated as a yellow viscous-liquid. This compound showed the characteristic of diterpene by visualizing as a brown spot with vanillin-H_2SO_4 spray reagent. The UV spectrum **(Figure 43)** showed maximum absorptions at 252.7 and 361.0 nm suggesting the presence of a conjugated enone and benzoyl chromophores. The IR spectrum **(Figure 44)** showed the stretching of hydroxy group at 3387 cm^{-1}, carbonyl group at 1721, 1687 cm^{-1} and C=C at 1581, 1520 cm^{-1}.

The ^{13}C NMR spectrum showed the signals of 30 carbon atoms **(Table 39)(Figure 46)**. Analysis of the DEPT spectra indicated the presence of three carbonyl carbons (δ207.9, 174.1 and 168.0), six methyl carbons (δ29.6, 24.0, 21.1, 16.6, 11.8 and 10.5), one methylene carbon (δ68.2), thirteen methine carbons (δ154.9, 135.1, 131.3, 126.8, 114.4, 110.9, 73.9, 67.3, 55.9, 47.9, 43.4, 40.1 and 37.7) and seven quaternary carbons (δ152.4, 144.2, 139.9, 109.2, 78.5, 65.3 and 25.5).

Compound **PSI6,** 4α-epimer of **PSI5**, showed similar bands in IR and UV spectrum with **PSI5**. Comparison of the ^1H NMR spectral data **(Table 43)(Figure 45)** of two compounds revealed close structural similarity. Spectrum of **PSI6** differed from

PSI5 at three positions : the signals at δ7.11 (*br s*, 1H), 3.21 (*m*, 1H) and 0.92 (*d*, *J* = 4.5 Hz, 1H) which could be assigned to H-1, H-4 and H-14.

The HMBC correlations of **PSI6 (Table 40)(Figure 50)** were the same as **PSI5 (Table 34).** Comparison of ^{1}H NMR spectral data between compound **PSI6** and 12-(N-Methylaminobenzoyl)-4α-deoxy-5,20-dihydroxyphorbol-13-acetate showed similarity **(Table 38).** Thus compound **PSI6** was confirmed as 12-(N-Methylaminobenzoyl)-4α-deoxy-5,20-dihydroxyphorbol-13-acetate which was previously isolated from fruits of *Sapium indicum* (Taylor, *et.al.*, 1981).

Table 38 Comparison of ^{1}H NMR spectrum between 4α-deoxyphorbol and **PSI6**

Position	**4α-deoxyphorbol, δH (ppm) (Recorded in $CDCl_3$)**	**Compound PSI6, δH (ppm) (Recorded in $CDCl_3$)**
1	7.19 (*s*)	7.11 (*br s*)
4	3.21 (*t*, *J* = 5.1 Hz)	3.21 (*m*)
5	4.79 (*d*, *J* = 3.7 Hz)	4.57 (*br s*)
7	5.37 (*d*, *J* = 3.2 Hz)	5.21 (*d*, *J* = 1.5 Hz)
8	2.33 (*t*, *J* = 6.6 Hz)	2.11 (*m*, superimposed with $COC\underline{H}_3$)
10	3.69 (*m*)	3.71 (*m*)
11	1.60 (*m*)	1.87 (*m*)
12	5.25 (*d*, *J* = 11.9 Hz)	5.72 (*d*, *J* = 10.5 Hz)
14	0.97 (*d*, *J* = 6.4 Hz)	0.92 (*d*, *J* = 4.5 Hz)
16	1.09 (*s*)	1.19 (*s*)
17	1.17 (*s*)	1.34 (*s*)
18	0.84 (*d*, *J* = 5.5 Hz)	1.16 (*d*, *J* = 6.5 Hz)

Table 38 (Continued)

Position	4α-deoxyphorbol, δH (ppm) (Recorded in $CDCl_3$)	Compound PSI6, δH (ppm) (Recorded in $CDCl_3$)
19	1.81 (*s*)	1.82 (*m*)
20	4.10 (*AB*, *J*= 16.9 Hz)	4.15 (*br s*)
3'	6.71 (*d*, *J* = 8.5 Hz)	6.74 (*d*, *J* = 8 Hz)
4'	7.43 (*t*, *J* = 8.5 Hz)	7.45 (*dt*, *J* = 1.5, 8, 8 Hz)
5'	6.60 (*t*, *J* = 7.8 Hz)	6.63 (*dt*, *J* = 1.5, 8, 8 Hz)
6'	7.86 (*dd*, *J* = 1.6, 7.8 Hz)	7.89 (*dd*, *J* = 1.5, 8 Hz)
$NC\underline{H}_3$	2.94 (*d*, *J* = 3.4 Hz)	2.95 (*s*)
$COC\underline{H}_3$	2.13 (*s*)	2.11 (*s*)

Table 39 ^{1}H and ^{13}C NMR spectral data of compound **PSI6**

Position	δC	δH, mult, *J*(Hz)
1	154.9	7.11 (*br s*)
2	144.2	
3	207.9	
4	55.9	3.21 (*m*)
5	67.3	4.57 (*br s*)
6	139.9	
7	126.8	5.21 (*d*, 1.5)
8	40.1	2.11 (*m*, superimposed with $COC\underline{H}_3$)

Table 39 (Continued)

Position	δC	δH, mult, *J*(Hz)
9	78.5	
10	47.9	3.71 (*m*)
11	43.4	1.87 (*m*)
12	73.9	5.72 (*d*, 10.5)
13	65.3	
14	37.7	0.92 (*d*, 4.5)
15	25.5	
16	24.0	1.19 (*s*)
17	16.6	1.34 (*s*)
18	11.8	1.16 (*d*, 6.5)
19	10.5	1.82 (*m*)
20	68.2	4.15 (*br s*)
21	174.1	
22	168.0	
1'	152.4	
2'	109.2	
3'	110.9	6.74 (*d*, 8)
4'	135.1	7.45 (*dt*, 1.5, 8, 8)
5'	114.4	6.63 (*dt*, 1.5, 8, 8)
6'	131.3	7.89 (*dd*, 1.5, 8)
$NC\underline{H}_3$	29.6	2.95 (*s*)
$COC\underline{H}_3$	21.1	2.11 (*s*)

Table 40 Major HMBC correlations of compound **PSI6**

Position	δH, mult, *J*(Hz)	δC
1	7.11 (*br s*)	C-2 (144.2), C-3 (207.9), C-4 (55.9), C-9 (78.5), C-10 (47.9), C-19 (10.5)
4	3.21 (*m*)	C-1 (154.9), C-3 (207.9), C-5 (67.3), C-6 (139.9), C-9 (78.5), C-10 (47.9)
5	4.57 (*br s*)	C-4 (55.9)
7	5.21 (*d*, 1.5)	C-5 (67.3), C-6 (139.9), C-8 (40.1), C-9 (78.5), C-14 (37.7), C-20 (68.2)
8	2.11 (*m*, superimposed with $COC\underline{H}_3$)	C-6 (139.9), C-7 (126.8), C-13 (65.3), C-14 (37.7), C-15 (25.5)
10	3.71 (*m*)	C-4 (55.9), C-8 (40.1), C-9 (78.5)
11	1.87 (*m*)	C-9 (78.5), C-12 (73.9), C-18 (11.8)
12	5.72 (*d*, 10.5)	C-11 (43.4), C-13 (65.3), C-15 (25.5), C-18 (11.8), C-22 (168.0)
14	0.92 (*d*, 4.5)	C-9 (78.5), C-12 (73.9), C-13 (65.3), C-15 (25.5), C-16 (24.0), C-17 (16.6)

Table 40 (Continued)

Position	δH, mult, *J*(Hz)	δC
16	1.19 (*s*)	C-13 (65.3), C-14 (37.7), C-15 (25.5), C-17 (16.6)
17	1.34 (*s*)	C-13 (65.3), C-14 (37.7), C-15 (25.5), C-16 (24.0)
18	1.16 (*d*, 6.5)	C-9 (78.5), C-11 (43.4), C-12 (73.9)
19	1.82 (*m*)	C-1 (154.9), C-2 (144.2), C-3 (207.9)
20	4.15 (*br s*)	C-5 (67.3), C-6 (139.9), C-7 (126.8), C-14 (37.7)
3'	6.74 (*d*, 8)	C-2' (109.2), C-5' (114.4)
4'	7.45 (*dt*, 1.5, 8, 8)	C-1' (152.4), C-6' (131.3)
5'	6.63 (*dt*, 1.5, 8, 8)	C-1' (152.4), C-2' (109.2), C-3' (110.9), C-4' (135.1), C-6' (131.3)
6'	7.89 (*dd*, 1.5, 8)	C-1' (152.4), C-4' (135.1), C-22 (168.0)
$COCH_3$	2.11 (*s*)	C-21 (174.1)

Table 41 ^{13}C NMR and DEPT spectral data of compound **PSI6**

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
1	154.9	CH	154.9	154.9	154.9
2	144.2	C			
3	207.9	C=O			
4	55.9	CH	55.9	55.9	55.9
5	67.3	CH	67.3	67.3	67.3
6	139.9	C			
7	126.8	CH	126.8	126.8	126.8
8	40.1	CH	40.1	40.1	40.1
9	78.5	C			
10	47.9	CH	47.9	47.9	47.9
11	43.4	CH	43.4	43.4	43.4
12	73.9	CH	73.9	73.9	73.9
13	65.3	C			
14	37.7	CH	37.7	37.7	37.7
15	25.5	C			
16	24.0	CH_3	24.0		24.0
17	16.6	CH_3	16.6		16.6
18	11.8	CH_3	11.8		11.8
19	10.5	CH_3	10.5		10.5
20	68.2	CH_2	68.2		68.2
21	174.1	C=O			
22	168.0	C=O			

Table 41 (Continued)

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
1'	152.4	C			
2'	109.2	C			
3'	110.9	CH	110.9	110.9	110.9
4'	135.1	CH	135.1	135.1	135.1
5'	114.4	CH	114.4	114.4	114.4
6'	131.3	CH	131.3	131.3	131.3
$N\underline{C}H_3$	29.6	CH_3	29.6		29.6
$CO\underline{C}H_3$	21.1	CH_3	21.1		21.1

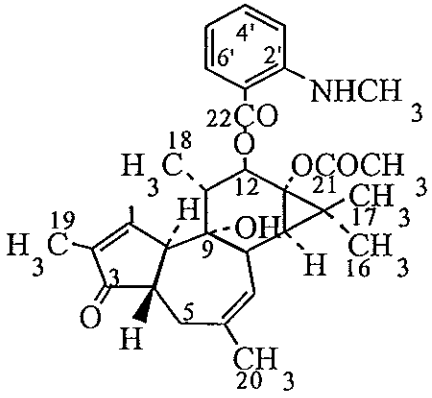
Table 42 COSY correlations among some protons of compound **PSI6**

δH, mult, *J*(Hz)	Proton Correlations with δH (ppm)
H-1 (*br s*)	H-10, H-19
H-5 (*br s*)	H-4
H-7 (*d*, 1.5)	H-8, H-20
H-8 (*m*, superimposed with $CO\underline{C}H_3$)	H-14
H-10 (*m*)	H-4
H-11 (*m*)	H-18
H-12 (*d*, 10.5)	H-11
H-3' (*d*, 8)	H-5'
H-4' (*dt*, 1.5, 8, 8)	H-3', H-5'
H-6' (*dd*, 1.5, 8)	H-4', H-5'

Table 43 Comparison of ^{1}H NMR spectral data between **PSI5** and **PSI6**

Position	Compound PSI5, δH (ppm) (Recorded in $CDCl_3$)	Compound PSI6, δH (ppm) (Recorded in $CDCl_3$)
1	7.71 (*br s*)	7.11 (*br s*)
4	2.64 (*t*, J = 4.5 Hz)	3.21 (*m*)
5	5.20 (*d*, J = 4.5 Hz)	4.57 (*br s*)
7	5.62 (*d*, J = 5 Hz)	5.21 (*d*, J = 1.5 Hz)
8	2.35 (*br t*, J = 5 Hz)	2.11 (*m*, superimposed with $COC\underline{H}_3$)
10	3.59 (*m*)	3.71 (*m*)
11	1.68 (*m*)	1.87 (*m*)
12	5.65 (*d*, J = 10 Hz)	5.72 (*d*, J = 10.5 Hz)
14	1.13 (*d*, J = 5 Hz)	0.92 (*d*, J = 4.5 Hz)
16	1.20 (*s*)	1.19 (*s*)
17	1.29 (*s*)	1.34 (*s*)
18	0.97 (*d*, J = 6 Hz)	1.16 (*d*, J = 6.5 Hz)
19	1.75 (*m*)	1.82 (*m*)
20	4.25 (*AB*, J = 13 Hz)	4.15 (*br s*)
3'	6.71 (*d*, J = 8 Hz)	6.74 (*d*, J = 8 Hz)
4'	7.42 (*dt*, J = 1.5, 8, 8 Hz)	7.45 (*dt*, J = 1.5, 8, 8 Hz)
5'	6.60 (*dt*, J = 1.5, 8, 8 Hz)	6.63 (*dt*, J = 1.5, 8, 8 Hz)
6'	7.82 (*dd*, J = 1.5, 8 Hz)	7.89 (*dd*, J = 1.5, 8 Hz)
$NC\underline{H}_3$	2.93 (*s*)	2.95 (*s*)
$COC\underline{H}_3$	2.13 (*s*)	2.11 (*s*)

3.7 Compound PSI7: 12-(N-Methylaminobenzoyl)-4β, 5, 20-trideoxyphorbol -13-acetate



Compound **PSI7** was obtained as a yellow viscous-liquid. The molecular formula was determined as $C_{30}H_{37}NO_6$ by EIMS (M^+ m/z 507). The IR spectrum **(Figure 52)** showed the stretching of hydroxy group at 3388 cm^{-1} and carbonyl group at 1724, 1687 cm^{-1} and C=C 1581, 1520 cm^{-1}. The UV spectrum **(Figure 51)** showed maximum absorptions at 253.5 and 360.0 nm suggesting the presence of a conjugated enone and benzoyl chromophores. It also gave a brown spot with vanillin-H_2SO_4 spray reagent, a positive test for diterpene compound.

The ^{13}C NMR spectrum showed the signals of 30 carbon atoms **(Table 44)(Figure 54)**. Analysis of the DEPT spectra indicated the presence of three carbonyl carbons (δ210.1, 173.7 and 168.1), seven methyl carbons (δ29.6, 25.7, 23.7, 21.2, 16.9, 15.1 and 10.2), one methylene carbon (δ34.0), twelve methine carbons (δ160.0, 134.9, 131.3, 125.7, 114.4, 110.8, 76.3, 54.3, 44.6, 42.5, 42.2 and 35.8) and seven quaternary carbons (δ152.3, 139.0, 136.4, 109.4, 77.9, 65.6 and 25.4).

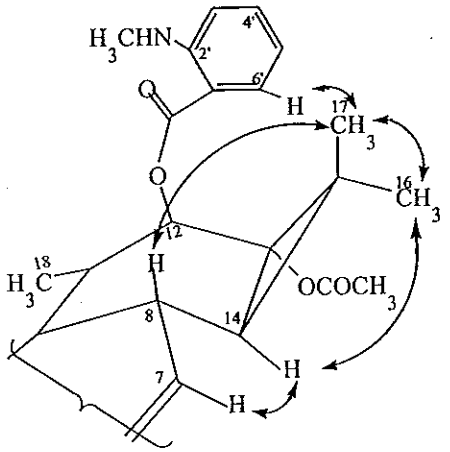
The ^1H NMR spectrum **(Table 44)(Figure 53)** showed the characteristic signals of phorbol skeleton at δ7.59 (*br s*, H-1), 5.25 (*m*, H-7), 1.05 (*d*, J = 5.5 Hz, H-14). The signals of methyl groups appeared at 1.75 (*s*, 3H), 1.73 (*s*, 3H), 1.32 (*s*, 3H),

1.19 (*s*, 3H) and 0.95 (*d*, J = 6.5 Hz, 3H), which were assigned to C-20, C-19, C-17, C-16 and C-18, respectively. In addition, the resonances of four aromatic protons were present at δ7.83 (*dd*, J = 1.5, 8 Hz, H-6'), 7.42 (*dt*, J = 1.5, 8, 8 Hz, H-4'), 6.70 (*br d*, J = 8 Hz, H-3') and 6.60 (*dt*, J = 1.5, 8, 8 Hz, H-5') together with the acetyl group at δ 2.13 (*s*, 3H). The downfield chemical shift methyl signal at δ2.94 (*d*, J = 4.5 Hz, 3H) is probably due to attachment to highly electronegative atom such as N or O. The other signals were *doublet* at δ5.65 (J = 9.5 Hz), a *doublet of triplet* at δ2.49 (J = 4.5, 10.5 Hz), a *broad triplet* at δ2.40, two *multiplets* at δ3.32 and 1.71 and two *doublet of doublet* at δ2.87 (J = 9, 18.5 Hz) and 2.04 (J = 10.5, 18.5 Hz).

The substitution pattern in this compound was supported by its HMBC spectral data **(Table 45)(Figure 58).** The H-1 (δ7.59) was found to correlate to δ 136.4, 210.1, 54.3, 44.6 and 10.2, which could be assigned as C-2, C-3, C-10, C-4 and C-19, respectively. Two signals at δ 54.3 and 44.6 showed ^{1}H-^{13}C direct coupling (HMQC) at δ3.32 and 2.49 which were assigned to H-10 and H-4, respectively. The H-10 showed correlations to C-1 (δ160.0) and C-9 (δ77.9). The methyl proton signal at δ 1.75 (3H-20) showed correlations to C-5 (δ 34.0), C-6 (δ 139.0) and C-7 (δ 125.7), thus confirmed the location of one methyl proton at C-20. The proton signal at δ2.87 and 2.04 (HMQC, δC-5 = 34.0) should be assigned to 2H-5. The H-5 (δ2.87) was further confirmed by the correlations to C-4 (δ 44.6), C-6 (δ 139.0), C-7 (δ 125.7) and C-10 (δ 54.3), whereas another signal at δ 2.04 (H-5) was found to correlate to C-4 (δ44.6), C-6 (δ139.0), C-7 (δ125.7) and C-20 (δ25.7). The carbon signals at δ77.9, 42.5 and 76.3 showed the correlation peaks with the 3H-18 (δ0.95). Only the last two signals showed proton signals (HMQC) at δ 1.71 and 5.65, hence they were assigned to H-11 and H-12, respectively. Correlations of H-12 were to C-11 (δ 42.5), C-13 (δ 65.6), C-15 (δ 25.4), C-18 (δ 15.1) and C-22 (δ 168.1) this information indicated that an acyl to oxygen bond formation (ester formation) occurred between an aromatic carboxylic group and hydroxy group of phorbol skeleton at C-12

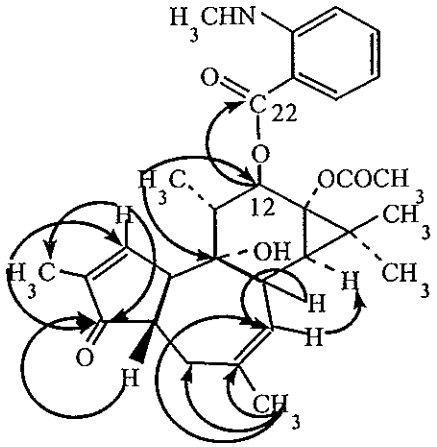
(δ76.3). The signal at δ2.40 showed correlations to C-7 (δ125.7), C-14 (δ35.8) and C-15 (δ25.4), so it was assigned to H-8. In addition, the downfield aromatic protons H-6' (δ 7.83) showed correlations to C-1' (δ 152.3), C-4' (δ 134.9) and C-22 (δ 168.1); H-4' (δ7.42) showed correlations to C-1' (δ152.3) and C-6' (δ131.3); H-3' (δ 6.70) showed correlations to C-2' (δ 109.4) and C-5' (δ 114.4). The H-5' (δ 6.60) showed correlations to C-3' (δ 110.8). The last signal at δ 2.94 was assigned to N-methyl. Thus **PSI7** composed of phorbol acetate unit and anthranilic acid unit connected by ester linkage at C-12 of phorbol skeleton. The more detail information were summarized in **Tables 44-47, Figures 51-60.**

From NOE experiment, irradiation of 3H-17 (δ 1.32) showed enhancement of H-8 (δ 2.04), H-16 (δ 1.19) and H-6' (δ 7.83). Irradiation of 3H-16 (δ 1.19) showed enhancement of H-14 (δ 1.05). Irradiation of H-14 (δ 1.05) showed enhancement of H-7 (5.25), as shown below.



NOE of PSI7

The spectral data indicated that **PSI7** was 12-(N-Methylaminobenzoyl)-4β, 5, 20-trideoxyphorbol-13-acetate which is a new naturally occurring compound.



Selected HMBC Correlations of PSI7

Table 44 ^{1}H and ^{13}C NMR spectral data of compound **PSI7**

Position	δC	δH, mult, *J*(Hz)
1	160.0	7.59 (*br s*)
2	136.4	
3	210.1	
4	44.6	2.49 (*dt*, 4.5, 10.5)
5β	34.0	2.87 (*dd*, 9, 18.5)
5α	34.0	2.04 (*dd*, 10.5, 18.5)
6	139.0	
7	125.7	5.25 (*m*)
8	42.2	2.40 (*br t*)
9	77.9	
10	54.3	3.32 (*m*)
11	42.5	1.71 (*m*)
12	76.3	5.65 (*d*, 9.5)
13	65.6	
14	35.8	1.05 (*d*, 5.5)
15	25.4	
16	23.7	1.19 (*s*)
17	16.9	1.32 (*s*)
18	15.1	0.95 (*d*, 6.5)
19	10.2	1.73 (*m*)
20	25.7	1.75 (*s*)
21	173.7	
22	168.1	

Table 44 (Continued)

Position	δC	δH, mult, *J*(Hz)
1'	152.3	
2'	109.4	
3'	110.8	6.70 (*br d*, 8)
4'	134.9	7.42 (*dt*, 1.5, 8, 8)
5'	114.4	6.60 (*dt*, 1.5, 8, 8)
6'	131.3	7.83 (*dd*, 1.5, 8)
$NC\underline{H}_3$	29.6	2.94 (*d*, 4.5)
$COC\underline{H}_3$	21.2	2.13 (*s*)

Table 45 Major HMBC correlations of compound **PSI7**

Position	δH, mult, *J*(Hz)	δC
1	7.59 (*br s*)	C-2 (136.4), C-3 (210.1), C-4 (44.6), C-10 (54.3), C-19 (10.2)
4	2.49 (*dt*, 4.5, 10.5)	C-3 (210.1)
5β	2.87 (*dd*, 9, 18.5)	C-4 (44.6), C-6 (139.0), C-7 (125.7), C-10 (54.3)
5α	2.04 (*dd*, 10.5, 18.5)	C-4 (44.6), C-6 (139.0), C-7 (125.7), C-20 (25.7)

Table 45 (Continued)

Position	δH, mult, *J*(Hz)	δC
7	5.25 (*m*)	C-14 (35.8)
8	2.40 (*br t*)	C-7 (125.7), C-14 (35.8), C-15 (25.4)
10	3.32 (*m*)	C-1 (160.0), C-9 (77.9)
11	1.71 (*m*)	C-12 (76.3)
12	5.65 (*d*, 9.5)	C-11 (42.5), C-13 (65.6), C-15 (25.4), C-18 (15.1), C-22 (168.1)
14	1.05 (*d*, 5.5)	C-13 (65.6), C-17 (16.9)
16	1.19 (*s*)	C-13 (65.6), C-14 (35.8), C-15 (25.4), C-17 (16.9)
17	1.32 (*s*)	C-13 (65.6), C-14 (35.8), C-15 (25.4), C-16 (23.7)
18	0.95 (*d*, 6.5)	C-9 (77.9), C-11 (42.5), C-12 (76.3)
19	1.73 (*m*)	C-1 (160.0), C-2 (136.4), C-3 (210.0)
20	1.75 (*s*)	C-5 (34.0), C-6 (139.0), C-7 (125.7)

Table 45 (Continued)

Position	δH, mult, *J*(Hz)	δC
3'	6.70 (*br d*, 8)	C-2' (109.4), C-5' (114.4)
4'	7.42 (*dt*, 1.5, 8, 8)	C-1' (152.3), C-6' (131.3)
5'	6.60 (*dt*, 1.5, 8, 8)	C-3' (110.8)
6'	7.83 (*dd*, 1.5, 8)	C-1' (152.3), C-4' (134.9), C-22 (168.1)
$COCH_3$	2.13 (*s*)	C-21 (173.7)

Table 46 ^{13}C NMR and DEPT spectral data of compound **PSI7**

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
1	160.0	CH	160.0	160.0	160.0
2	136.4	C			
3	210.1	C=O			
4	44.6	CH	44.6	44.6	44.6
5	34.0	CH_2	34.0		34.0
6	139.0	C			
7	125.7	CH	125.7	125.7	125.7
8	42.2	CH	42.2	42.2	42.2
9	77.9	C			

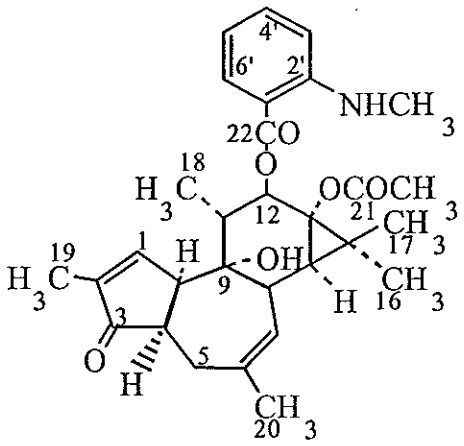
Table 46 (Continued)

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
10	54.3	CH	54.3	54.3	54.3
11	42.5	CH	42.5	42.5	42.5
12	76.3	CH	76.3	76.3	76.3
13	65.6	C			
14	35.8	CH	35.8	35.8	35.8
15	25.4	C			
16	23.7	CH_3	23.7		23.7
17	16.9	CH_3	16.9	110.8	16.9
18	15.1	CH_3	15.1	134.9	15.1
19	10.2	CH_3	10.2	114.4	10.2
20	25.7	CH_3	25.7	131.3	25.7
21	173.7	C=O			
22	168.1	C=O			
1'	152.3	C	110.8		110.8
2'	109.4	C	134.9		134.9
3'	110.8	CH	114.4		114.4
4'	134.9	CH	131.3		131.3
5'	114.4	CH	29.6		29.6
6'	131.3	CH	21.2		21.2
NCH_3	29.6	CH_3			
$COCH_3$	21.2	CH_3			

Table 47 COSY correlations among some protons of compound **PSI7**

δH, mult, *J*(Hz)	**Proton Correlations with δH (ppm)**
H-1 (*br s*)	H-19
H-4 (*dt*, 4.5, 10.5)	H-5α, H-5β
H-5β (*dd*, 9, 18.5)	H-4, H-5α
H-7 (*m*)	H-8, H-20
H-8 (*br t*)	H-7, H-14
H-10 (*m*)	H-4
H-11 (*m*)	H-12, H-18
H-12 (*d*, 9.5)	H-11
H-4' (*dt*, 1.5, 8, 8)	H-3', H-5'
H-6' (*dd*, 1.5, 8)	H-5'

3.8 Compound PSI8: 12-(N-Methylaminobenzoyl)-4α, 5, 20-trideoxyphorbol -13-acetate



Compound **PSI8** was isolated as a yellow viscous-liquid. The molecular formula was determined as $C_{30}H_{37}NO_6$ by EIMS (M^+ m/z 507). This compound showed a brown spot with vanillin-H_2SO_4 spray reagent indicating the diterpene characteristic. The IR spectrum **(Figure 62)** exhibited an absorptions band at 3388 cm^{-1} for hydroxy group, 1721 and 1687 cm^{-1} for carbonyl group and 1581 and 1520 cm^{-1} for C=C stretching while the UV spectra **(Figure 61)** revealed the presence of a conjugated enone and benzoyl chromophores with maximum absorption bands at 247.5 and 357.5 nm.

The ^{13}C NMR spectrum showed the signals of 30 carbon atoms **(Table 48)(Figure 64)**. Analysis of the DEPT spectra indicated the presence of three carbonyl carbons (δ212.6, 174.1 and 168.7), seven methyl carbons (δ29.2, 28.5, 23.8, 20.8, 16.2, 11.4 and 10.0), one methylene carbon (δ29.7), twelve methine carbons (δ156.1, 135.2, 131.6, 124.5, 114.5, 111.0, 75.1, 49.0, 46.9, 43.2, 40.6 and 37.3) and seven quaternary carbons (δ152.8, 143.6, 135.2, 109.8, 78.1, 65.4 and 24.8).

The ^{1}H NMR spectral data of **PSI8** was similar to that of **PSI7 (Table 52)(Figure 63)** except proton chemical shift of **PSI8** appeared at δ7.05 (*s*, 1H) and 0.83 (*d*, J = 5 Hz, 1H) which were assigned to H-1 and H-14 as compare to those of **PSI7** : H-1 (δ7.59) and H-14 (δ1.05). Thus PSI8 was suggested to be 4α-epimer of **PSI7**. Two signals of **PSI8** at δ3.41 (*br d*, J = 15 Hz, 1H) and 2.37 (*dd*, J = 4.5, 15 Hz, 1H) appeared at the lower field than the signal of **PSI7** at δ2.87 (*dd*, J = 9, 18.5 Hz, 1H) and 2.04 (*dd*, J = 10.5, 18.5 Hz, 1H) so it was assigned to 2H-5.

The HMBC correlations of **PSI8 (Table 49)(Figure 65)** were the same as **PSI7 (Table 45)**. The spectral data indicated that **PSI8** was 12-(N-Methylaminobenzoyl)-4α,5,20-trideoxyphorbol-13-acetate which is a new naturally occurring compound.

Table 48 ^{1}H and ^{13}C NMR spectral data of compound **PSI8**

Position	δ**C**	δ**H, mult, *J*(Hz)**
1	156.1	7.05 (*s*)
2	143.6	
3	212.6	
4	49.0	2.70 (*m*)
5β	29.7	3.41 (*br d, 15*)
5α	29.7	2.37 (*dd, 4.5, 15*)
6	135.2	
7	124.5	4.83 (*br s*)

Table 48 (Continued)

Position	δC	δH, mult, *J*(Hz)
8	40.6	1.95 (*br s*)
9	78.1	
10	46.9	3.45 (*m*)
11	43.2	1.84 (*m*)
12	75.1	5.71 (*d*, 10.5)
13	65.4	
14	37.3	0.83 (*d*, 5)
15	24.8	
16	23.8	1.15 (*s*)
17	16.2	1.32 (*s*)
18	11.4	1.09 (*d*, 6)
19	10.0	1.80 (*m*)
20	28.5	1.74 (*s*)
21	174.1	
22	168.7	
1'	152.8	
2'	109.8	
3'	111.0	6.70 (*dd*, 1.5, 8)
4'	135.2	7.42 (*dt*, 1.5, 8, 8)
5'	114.5	6.61 (*dt*, 1.5,8, 8)
6'	131.6	7.90 (*dd*, 1.5, 8)
$NC\underline{H}_3$	29.2	2.93 (*d*, 4.5)
$COC\underline{H}_3$	20.8	2.07 (*s*)

Table 49 Major HMBC correlations of compound **PSI8**

Position	δH, mult, *J*(Hz)	δC
1	7.05 (*s*)	C-3 (212.6), C-4 (49.0), C-9 (78.1), C-10 (46.9)
4	2.70 (*m*)	-
5β	3.41 (*br d, 15*)	-
5α	2.37 (*dd,* 4.5, 15)	C-4 (49.0), C-6 (135.2), C-7 (124.5), C-10 (46.9)
7	4.83 (*br s*)	C-5 (29.7), C-9 (78.1), C-20 (28.5)
8	1.95 (*br s*)	-
10	3.45 (*m*)	C-1 (156.1), C-8 (40.6), C-9 (78.1)
11	1.84 (*m*)	C-12 (75.1)
12	5.71 (*d*, 10.5)	C-11 (43.2), C-13 (65.4), C-15 (24.8), C-18 (11.4), C-22 (168.7)
14	0.83 (*d*, 5)	C-8 (40.6), C-13 (65.4), C-15 (24.8)
16	1.15 (*s*)	C-13 (65.4), C-14 (37.3), C-15 (24.8), C-17 (16.2)

Table 49 (Continued)

Position	δH, mult, *J*(Hz)	δC
17	1.32 (*s*)	C-13 (65.4), C-14 (37.3), C-15 (24.8), C-16 (23.8)
18	1.09 (*d*, 6)	C-9 (78.1), C-11 (43.2), C-12 (75.1)
19	1.80 (*m*)	C-1 (156.1), C-2 (143.6), C-3 (212.6)
20	1.74 (*s*)	C-6 (135.2), C-7 (124.5)
3'	6.70 (*dd*, 1.5, 8)	C-2' (109.8), C-5' (114.5)
4'	7.42 (*dt*, 1.5, 8, 8)	C-1' (152.8), C-6' (131.6)
5'	6.61 (*dt*, 1.5,8, 8)	C-3' (111.0)
6'	7.90 (*dd*, 1.5, 8)	C-1' (152.8), C-4' (135.2), C-22 (168.7)
$COC\underline{H}_3$	2.07 (*s*)	C-21 (174.1)

Table 50 ^{13}C NMR and DEPT spectral data of compound **PSI8**

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
1	156.1	CH	156.1	156.1	156.1
2	143.6	C			
3	212.6	C=O			
4	49.0	CH	49.0	49.0	49.0
5	29.7	CH_2	29.7		29.7
6	135.2	C			
7	124.5	CH	124.5	124.5	124.5
8	40.6	CH	40.6	40.6	40.6
9	78.1	C			
10	46.9	CH	46.9	46.9	46.9
11	43.2	CH	43.2	43.2	43.2
12	75.1	CH	75.1	75.1	75.1
13	65.4	C			
14	37.3	CH	37.3	37.3	37.3
15	24.8	C			
16	23.8	CH_3	23.8		23.8
17	16.2	CH_3	16.2		16.2
18	11.4	CH_3	11.4		11.4
19	10.0	CH_3	10.0		10.0
20	28.5	CH_3	28.5		28.5
21	174.1	C=O			
22	168.7	C=O			

Table 50 (Continued)

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
1'	152.8	C			
2'	109.8	C			
3'	111.0	CH	111.0	111.0	111.0
4'	135.2	CH	135.2	135.2	135.2
5'	114.5	CH	114.5	114.5	114.5
6'	131.6	CH	131.6	131.6	131.6
N$\underline{C}H_3$	29.2	CH_3	29.2		29.2
CO$\underline{C}H_3$	20.8	CH_3	20.8		20.8

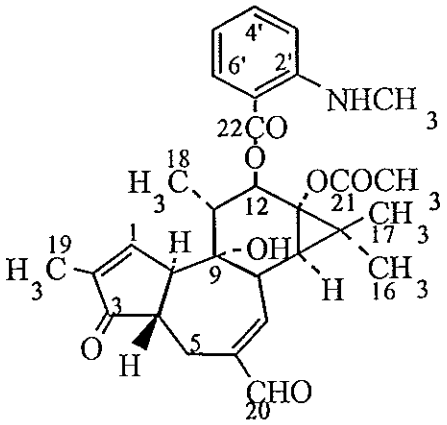
Table 51 COSY correlations among some protons of compound **PSI8**

δH, mult, *J*(Hz)	Proton Correlations with δH (ppm)
H-1 (*s*)	H-10
H-4 (*m*)	H-5α, H-10
H-5β(*br d*, 15)	H-5α, H-20
H-7 (*br s*)	H-8, H-20
H-8 (*m*)	H-14
H-10 (*m*)	H-4, H-11
H-11 (*m*)	H-12, H-18
H-12 (*d*, 10.5)	H-11
H-4' (*dt*, 1.5, 8, 8)	H-3', H-5'
H-6' (*dd*, 1.5, 8)	H-4', H-5'

Table 52 Comparison of ^{1}H NMR spectral data between **PSI7** and **PSI8**

Position	Compound PSI7, δH (ppm) (Recorded in $CDCl_3$)	Compound PSI8, δH (ppm) (Recorded in $CDCl_3$)
1	7.59 (*br s*)	7.05 (*s*)
4	2.49 (*dt*, 4.5, 10.5, 10.5)	2.70 (*m*)
5β	2.87 (*dd*, 9, 18.5)	3.41 (*br d, 15*)
5α	2.04 (*dd*, 10.5, 18.5)	2.37 (*dd, 4.5, 15*)
7	5.25 (*m*)	4.83 (*br s*)
8	2.40 (*br t*)	1.95 (*br s*)
10	3.32 (*m*)	3.45 (*m*)
11	1.71 (*m*)	1.84 (*m*)
12	5.65 (*d*, 9.5)	5.71 (*d*, 10.5)
14	1.05 (*d*, 5.5)	0.83 (*d*, 5)
16	1.19 (*s*)	1.15 (*s*)
17	1.32 (*s*)	1.32 (*s*)
18	0.95 (*d*, 6.5)	1.09 (*d*, 6)
19	1.73 (*m*)	1.80 (*m*)
20	1.75 (*s*)	1.74 (*s*)
3'	6.70 (*br d*, 8)	6.70 (*dd*, 1.5, 8)
4'	7.42 (*dt*, 1.5, 8, 8)	7.42 (*dt*, 1.5, 8, 8)
5'	6.60 (*dt*, 1.5, 8, 8)	6.61 (*dt*, 1.5,8, 8)
6'	7.83 (*dd*, 1.5, 8)	7.90 (*dd*, 1.5, 8)
NCH_3	2.94 (*d*, 4.5)	2.93 (*d*, 4.5)
$COCH_3$	2.13 (*s*)	2.07 (*s*)

3.9 Compound PSI9: 12-(N-Methylaminobenzoyl)-4β, 5-dideoxy-20-phorbaldehyde -13-acetate



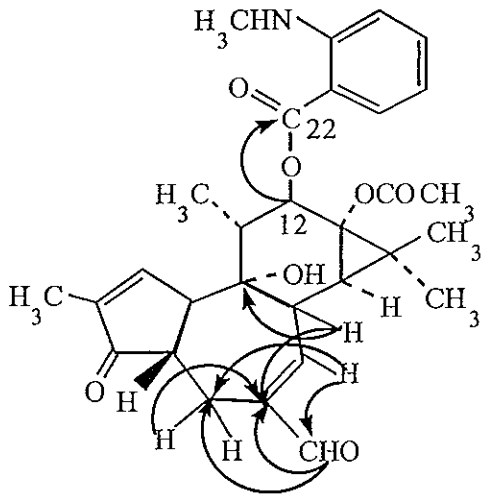
Compound **PSI9** was obtained as a yellow viscous-liquid. The UV spectrum **(Figure 69)** showed maximum absorptions at 252.5 and 360.0 nm suggesting the presence of a conjugated enone and benzoyl chromophores. The IR spectrum **(Figure 70)** showed the stretching of hydroxy group at 3388 cm^{-1}, carbonyl group at 1724 and 1687 cm^{-1} and C=C at 1581 and 1520 cm^{-1}. It also gave a brown spot with vanillin-H_2SO_4 spray reagent, a positive test for diterpene compound.

The ^{13}C NMR spectrum showed the signals of 30 carbon atoms **(Table 53)(Figure 72)**. Analysis of the DEPT spectra indicated the presence of four carbonyl carbons (δ 208.4, 193.0, 173.9 and 167.9), six methyl carbons (δ 29.6, 23.6, 21.1, 16.7, 15.1 and 10.2), one methylene carbon (δ 25.8), twelve methine carbons (δ 158.4, 154.1, 135.1, 131.2, 114.5, 110.9, 75.6, 53.9, 43.8, 43.2, 42.8 and 34.9) and seven quaternary carbons (δ 152.3, 144.7, 136.9, 109.1, 78.4, 65.1 and 24.9).

Comparison of the ^{1}H NMR spectral data **(Table 57)(Figure 71)** of **PSI9** with **PSI1** revealed close structural similarity. Differences in the spectrum of **PSI9** and **PSI1** were shown as signals at δ 9.45 (*s*, 1H), 6.57 (*br s*, 1H) and 2.76 (*d*, J = 11 Hz, 2H), which were not observed in compound **PSI1**.

In the HMBC spectrum **(Table 54)(Figure 76)** the signals at δ2.70 (H-8) showed correlation to the carbon signal at δ25.8, 144.7, 154.1, 78.4 and 34.9, which could be assigned as C-5, C-6, C-7, C-9 and C-14, respectively. The signals at δ25.8 and 154.1 showed ^{1}H-^{13}C direct coupling (HMQC) at δ 2.76 and 6.57 and were assigned to 2H-5 and H-7. The 2H-5 was confirmed by the correlations to C-4 (δ 43.8), C-6 (δ144.7) and C-7 (δ154.1). The H-7 (δ6.57) was found to correlate to C-5 (δ25.8), C-14 (δ34.9) and C-20 (δ 193.0). Only the last carbon signal showed proton signal (HMQC) at δ 9.45, hence it was assigned as a formyl proton H-20. Correlations of H-20 (δ9.45) were to C-5 (δ25.8) and C-6 (δ144.7). Thus compound **PSI9** should be an oxidized form of **PSI1**.

The spectral data indicated that **PSI9** was 12-(N-Methylaminobenzoyl)-4β, 5-dideoxy-20-phorbaldehyde-13-acetate which was previously isolated from fruits of *Sapium indicum*. The spectrum of **PSI9** were similar with those in the literature (Taylor, *et.al.*, 1981).



Selected HMBC Correlations of PSI9

Table 53 ^{1}H and ^{13}C NMR spectral data of compound **PSI9**

Position	δC	δH, mult, *J*(Hz)
1	158.4	7.51 (*br s*)
2	136.9	
3	208.4	
4	43.8	2.53 (*m*)
5	25.8	2.76 (*d, 11*)
6	144.7	
7	154.1	6.57 (*br s*)
8	42.8	2.70 (*br t*, 6.5)
9	78.4	
10	53.9	3.10 (*m*)
11	43.2	1.80 (*m*)
12	75.6	5.69 (*d*, 10)
13	65.1	
14	34.9	1.25 (*d*, 4.5)
15	24.9	
16	23.6	1.24 (*s*)
17	16.7	1.33 (*s*)
18	15.1	0.98 (*d*, 6.5)
19	10.2	1.73 (*m*)
20	193.0	9.45 (*s*)
21	173.9	
22	167.9	

Table 53 (Continued)

Position	δC	δH, mult, *J*(Hz)
1'	152.3	
2'	109.1	
3'	110.9	6.71 (*d*, 8)
4'	135.1	7.42 (*dt*, 2, 8, 8)
5'	114.5	6.60 (*dt*, 2, 8, 8)
6'	131.2	7.82 (*dd*, 2, 8)
$NC\underline{H}_3$	29.6	2.94 (*d*, $J = 3$)
$COC\underline{H}_3$	21.1	2.16 (*s*)

Table 54 Major HMBC correlations of compound **PSI9**

Position	δH, mult, *J*(Hz)	δC
1	7.51 (*br s*)	C-9 (78.4)
5	2.76 (*d, 11*)	C-4 (43.8), C-6 (144.7), C-7 (154.1)
7	6.57 (*br s*)	C-5 (25.8), C-14 (34.9), C-20 (193.0)
8	2.70 (*br t*, 6.5)	C-5 (25.8), C-6 (144.7), C-7 (154.1), C-9 (78.4), C-14 (34.9)
12	5.69 (*d*, 10)	C-11 (43.2), C-13 (65.1), C-15 (24.9), C-22 (173.9)

Table 54 (Continued)

Position	δ_H, mult, J(Hz)	δ_C
14	1.25 (*d*, 4.5)	C-7 (154.1), C-13 (65.1), C-14 (34.9), C-16 (23.6)
16	1.24 (*s*)	C-13 (65.1), C-14 (34.9), C-15 (24.9), C-17 (16.7)
17	1.33 (*s*)	C-13 (65.1), C-14 (34.9), C-15 (24.9), C-16 (23.6)
18	0.98 (*d*, 6.5)	C-9 (78.4), C-11 (43.2), C-12 (75.6)
19	1.73 (*m*)	C-1 (158.4), C-2 (136.9)
20	9.45 (*s*)	C-5 (25.8), C-6 (144.7)
3'	6.71 (*d*, 8)	C-2' (109.1), C-5' (114.5)
4'	7.42 (*dt*, 2, 8, 8)	C-1' (152.3), C-6' (131.2)
5'	6.60 (*dt*, 2, 8, 8)	C-2' (109.1), C-3' (110.9)
6'	7.82 (*dd*, 2, 8)	C-1' (152.3), C-4' (135.1), C-22 (167.9)
$COCH_3$	2.16 (*s*)	C-21 (173.9)

Table 55 ^{13}C NMR and DEPT spectral data of compound **PSI9**

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
1	158.4	CH	158.4	158.4	158.4
2	136.9	C			
3	208.4	C=O			
4	43.8	CH	43.8	43.8	43.8
5	25.8	CH_2	25.8		25.8
6	144.7	C			
7	154.1	CH	154.1	154.1	154.1
8	42.8	CH	42.8	42.8	42.8
9	78.4	C			
10	53.9	CH	53.9	53.9	53.9
11	43.2	CH	43.2	43.2	43.2
12	75.6	CH	75.6	75.6	75.6
13	65.1	C			
14	34.9	CH	34.9	34.9	34.9
15	24.9	C			
16	23.6	CH_3	23.6		23.6
17	16.7	CH_3	16.7		16.7
18	15.1	CH_3	15.1		15.1
19	10.2	CH_3	10.2		10.2
20	193.0	CHO	193.0		193.0
21	173.9	C=O			
22	167.9	C=O			

Table 55 (Continued)

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
1'	152.3	C			
2'	109.1	C			
3'	110.9	CH	110.9	110.9	110.9
4'	135.1	CH	135.1	135.1	135.1
5'	114.5	CH	114.5	114.5	114.5
6'	131.2	CH	131.2	131.2	131.2
$N\underline{C}H_3$	29.6	CH_3	29.6		29.6
$CO\underline{C}H_3$	21.1	CH_3	21.1		21.1

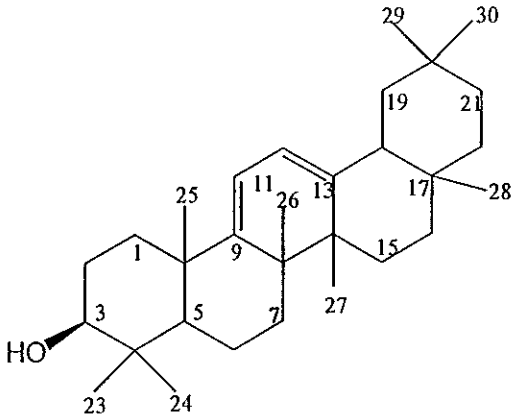
Table 56 COSY correlations among some protons of compound **PSI9**

δH, mult, *J*(Hz)	Proton Correlations with δH (ppm)
H-1 (*br s*)	H-19
H-4 (*m*)	H-10
H-5 (*d*, 11)	H-4
H-7 (*br s*)	H-8
H-8 (*br t*, 6.5)	H-14
H-12 (*d*, 10.5)	H-11
H-4' (*dt*, 2, 8, 8)	H-3', H-5'
H-6' (*dd*, 2, 8)	H-4', H-5'

Table 57 Comparison of ^{1}H NMR spectral data between **PSI1** and **PSI9**

Position	PSI1, δH (ppm) (Recorded in $CDCl_3$)	Compound PSI9, δH (ppm) (Recorded in $CDCl_3$)
1	7.56 (*br s*)	7.51 (*br s*)
4	2.52 (*dt*, *J* = 5, 9.5 Hz)	2.53 (*m*)
5	2.86 (*dd*, *J* = 9.5, 18 Hz)	2.76 (*d*, *J* = 11 Hz)
	2.17 (*dd*, *J* = 10, 18 Hz)	
7	5.55 (*m*)	6.57 (*br s*)
8	2.44 (*br t*)	2.70 (*br t*, *J* = 6.5 Hz)
10	3.27 (*m*)	3.10 (*m*)
11	1.73 (*m*, superimposed with 3H-19)	1.80 (*m*)
12	5.65 (*d*, *J* = 10 Hz)	5.69 (*d*, *J* = 10 Hz)
14	1.12 (*d*, *J* = 5 Hz)	1.25 (*d*, *J* = 4.5 Hz)
16	1.19 (*s*)	1.24 (*s*)
17	1.32 (*s*)	1.33 (*s*)
18	0.96 (*d*, *J* = 6.5 Hz)	0.98 (*d*, *J* = 6.5 Hz)
19	1.73 (*s*)	1.73 (*m*)
20	4.02 (*AB*, *J* = 13.5 Hz)	9.45 (*s*)
3'	6.69 (*dd*, *J* = 1.5, 8.5 Hz)	6.71 (*d*, *J* = 8 Hz)
4'	7.40 (*dt*, *J* = 1.5, 8.5, 8.5 Hz)	7.42 (*dt*, *J* = 2, 8, 8 Hz)
5'	6.59 (*dt*, *J* = 1.5,8.5, 8.5 Hz)	6.60 (*dt*, *J* = 2, 8, 8 Hz)
6'	7.81 (*dd*, *J* = 1.5, 8.5 Hz)	7.82 (*dd*, *J* = 2, 8 Hz)
$NC\underline{H}_3$	2.93 (*s*)	2.94 (*d*, *J* = 3 Hz)
$COC\underline{H}_3$	2.12 (*s*)	2.16 (*s*)

3.10 Compound PSI10: 9(11), 12-Oleanadien-3β-ol



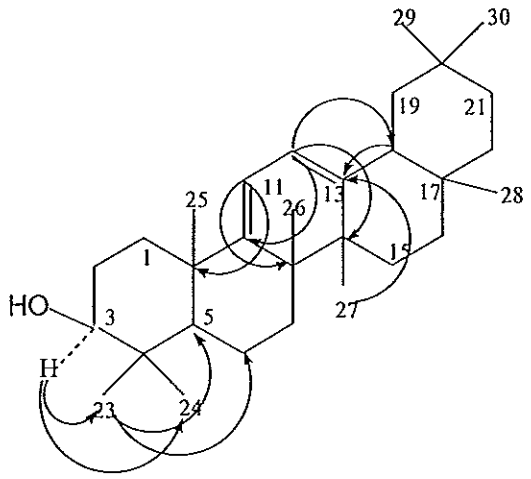
Compound **PSI10** was isolated as a yellow viscous-liquid. This compound showed the characteristic of triterpene by visualizing as a purple spot with vanillin-H_2SO_4 spray reagent. The IR spectrum **(Figure 78)** exhibited an absorption band at 3438 $cm^{-1}$for hydroxy group while the UV spectra **(Figure 77)** revealed the presence of a conjugated chromophore with a maximum absorption band at 281 nm, this value was possible for tetrasubstitued homoannular diene ($\lambda_{maxcalc}$ 273, by R.M. Silverstein and G.C. Bassler).

The ^{13}C NMR spectrum **(Table 58)(Figure 80)** together with DEPT and HMQC spectra exhibited 30 resonances for 30 carbon atoms; eight methyl carbons (δ 33.2, 28.7, 28.2, 25.2, 23.7, 20.9, 20.1 and 15.1) nine methylene carbons (δ46.8, 38.8, 37.2, 34.7, 32.2, 27.9, 27.3, 25.7 and 18.4) five methine carbons (δ120.6, 115.8, 78.7, 51.2 and 45.6) eight quaternary carbons (δ154.3, 147.1, 42.8, 40.7, 38.9, 37.0, 32.2 and 31.1).

The ^{1}H NMR spectrum **(Table 58)(Figure 79)** of **PSI10** indicated the presence of eight methyl protons at δ1.25 (*s*, 3H), 1.19 (*s*, 3H), 1.14 (*s*, 3H), 1.03 (*s*, 3H), 0.99 (*s*, 3H), 0.89 (*s*, 3H), 0.87 (*s*, 3H) and 0.81 (*s*, 3H) and one oxymethine

proton at δ3.24 (*dd*, J = 5, 11.5 Hz) was assigned to H-3. These signals were regarded as being due to a pentacyclic triterpene. The signals of two olefinic protons appeared at δ5.58 (*d*, J = 6 Hz) and 5.53 (*d*, J = 6 Hz).

In the ^{1}H-^{1}H COSY spectrum **(Figure 82)**, the correlation of the proton signal at δ5.58 was with that at δ5.53 and this suggested a vicinal conjugated diene. From HMBC correlation spectrum **(Figure 84)**, the proton signal at δ5.58 (*d*, J = 6 Hz) exhibited correlation peaks with C-8 (δ37.0) and C-10 (δ40.7) and proton signal at δ 5.53 (*d*, J = 6 Hz) showed correlations to C-9 (δ154.3), C-11 (δ115.8), C-14 (δ42.8) and C-18 (δ 45.6), thus those were assigned to H-11 and H-12, respectively. The oxymethine proton H-3 (δ3.24) showed cross peaks with C-23 (δ28.3) and C-24 (δ 15.1). The splitting pattern of H-3 as *doublet of doublet* with coupling constants of 6.0 and 11.5 Hz implied that the H-3 was in the axial α-position. Thus **PSI10** was assigned as 9(11), 12-Oleanadien-3β-ol (Tanaka, *et.al.*, 1988).



Selected HMBC Correlations of PSI10

Table 58 ^{1}H and ^{13}C NMR spectral data of compound **PSI10**

Position	δC	δH, mult, *J*(Hz)
1	38.8	1.44 (*m*, 1H), 1.33 (*m*, 1H)
2	27.9	1.64 (*m*, 2H)
3	78.7	3.24 (*dd*, *J* = 6, 11.5 Hz)
4	38.9	
5	51.2	0.85 (*d*, *J* = 1.5 Hz)
6	18.4	1.61 (*m*, 1H), 1.50 (*m*, 1H)
7	32.2	1.31 (*m*, 2H)
8	37.0	
9	154.3	
10	40.7	
11	115.8	5.58 (*d*, *J* = 6 Hz)
12	120.6	5.53 (*d*, *J* = 6 Hz)
13	147.1	
14	42.8	
15	25.7	1.05 (*m*, 2H)
16	27.2	1.98 (*m*, 2H)
17	32.2	
18	45.6	2.14 (*dd*, *J* = 3.5, 13.5 Hz)
19	46.8	1.64 (*m*, 1H), 1.08 (*m*, 1H)
20	31.1	
21	34.7	1.33 (*m*, 2H)
22	37.2	1.98 (*m*, 1H), 1.29 (*m*, 1H)
23	28.2	1.03 (*s*)

Table 58 (Continued)

Position	δC	δH, mult, *J*(Hz)
24	15.1	0.81 (*s*)
25	20.1	0.99 (*s*)
26	20.9	1.14 (*s*)
27	25.2	1.19 (*s*)
28	28.7	1.25 (*s*)
29	23.7	0.89 (*s*)
30	33.2	0.87 (*s*)

Table 59 Comparison of ^{13}C NMR spectral data between 9(11), 12-Oleanadien-3β-ol and **PSI10**

Position	9(11), 12-Oleanadien-3β-ol, δC (ppm) (Recorded in $CDCl_3$)	Compound PSI10, δC (ppm) (Recorded in $CDCl_3$)
1	38.7	38.8
2	27.8	27.9
3	78.5	78.6
4	38.9	38.9
5	51.1	51.2
6	18.3	18.4
7	32.1	32.2
8	37.0	37.0
9	154.3	154.3

Table 59 (Continued)

Position	9(11), 12-Oleanadien-3-ol, δC (ppm) (Recorded in $CDCl_3$)	Compound PSI10, δC (ppm) (Recorded in $CDCl_3$)
10	40.6	40.7
11	115.7	115.8
12	120.7	120.6
13	147.1	147.1
14	42.8	42.8
15	25.6	25.7
16	27.2	27.2
17	32.1	32.2
18	45.6	45.6
19	46.9	46.8
20	31.1	31.1
21	34.6	34.7
22	37.1	37.2
23	28.2	28.2
24	15.0	15.1
25	20.0	20.1
26	20.9	20.9
27	25.2	25.2
28	28.7	28.7
29	23.7	23.7
30	33.2	33.2

Table 60 ^{13}C NMR and DEPT spectral data of compound **PSI10**

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
1	38.8	CH_2	38.8		38.8
2	27.9	CH_2	27.9		27.9
3	78.6	CH	78.6	78.6	78.6
4	38.9	C			
5	51.2	CH	51.2	51.2	51.2
6	18.4	CH_2	18.4		18.4
7	32.2	CH_2	32.2		32.2
8	37.0	C			
9	154.3	C			
10	40.7	C			
11	115.8	CH	115.8	115.8	115.8
12	120.6	CH	120.6	120.6	120.6
13	147.1	C			
14	42.8	C			
15	25.7	CH_2	25.7		25.7
16	27.2	CH_2	27.2		27.2
17	32.2	C			
18	45.6	C			
19	46.8	CH_2	46.8		46.8
20	31.1	C			
21	34.7	CH_2	34.7		34.7
22	37.2	CH_2	37.2		37.2

Table 60 (Continued)

Position	δC	Type of carbon	DEPT-45°	DEPT-90°	DEPT-135°
23	28.2	CH_3	28.2		28.2
24	15.1	CH_3	15.1		15.1
25	20.1	CH_3	20.1		20.1
26	20.9	CH_3	20.9		20.9
27	25.2	CH_3	25.2		25.2
28	28.7	CH_3	28.7		28.7
29	23.7	CH_3	23.7		23.7
30	33.2	CH_3	33.2		33.2

3.11 *Biological activities*

The biological activity of compounds **PSI1, PSI3, PSI5, PSI6, PSI7, PSI8** and **PSI9** showed in vitro antituberculous activity against *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA) while no activity was observed with compounds **PSI2** and **PSI4.** The results were summarized in **Table 61.**

Table 61 Biological activities of compounds **PSI1-PSI9** from *Sapium indicum*

Compound	Antituberculous	
	Active/ Inactive	MIC* (μg/ml)
PSI1	Active	3.12
PSI2	Inactive	-
PSI3	Active	25
PSI4	Inactive	-
PSI5	Active	25
PSI6	Active	12.5
PSI7	Active	50
PSI8	Active	200
PSI9	Active	25

*MIC = Minimum Inhibitory Concentration

APPENDIX

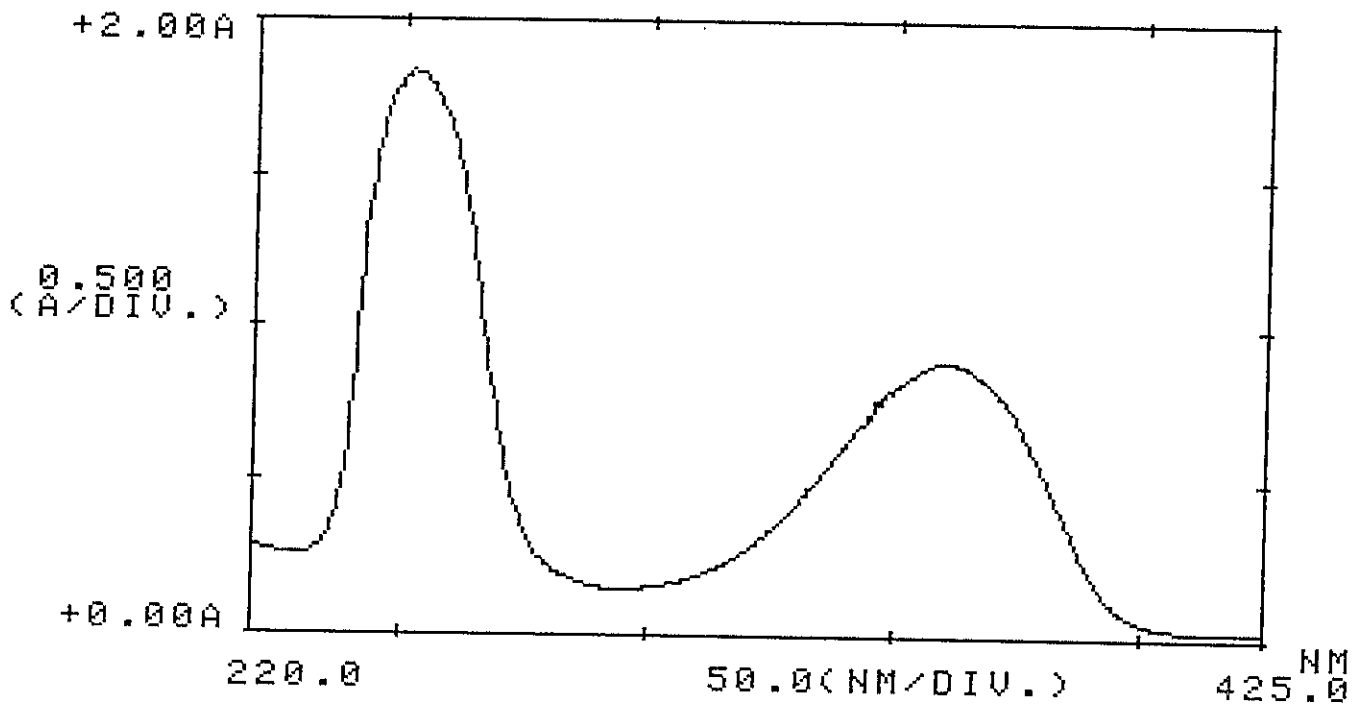


Figure 2 UV ($CHCl_3$) spectrum of compound **PSI1**

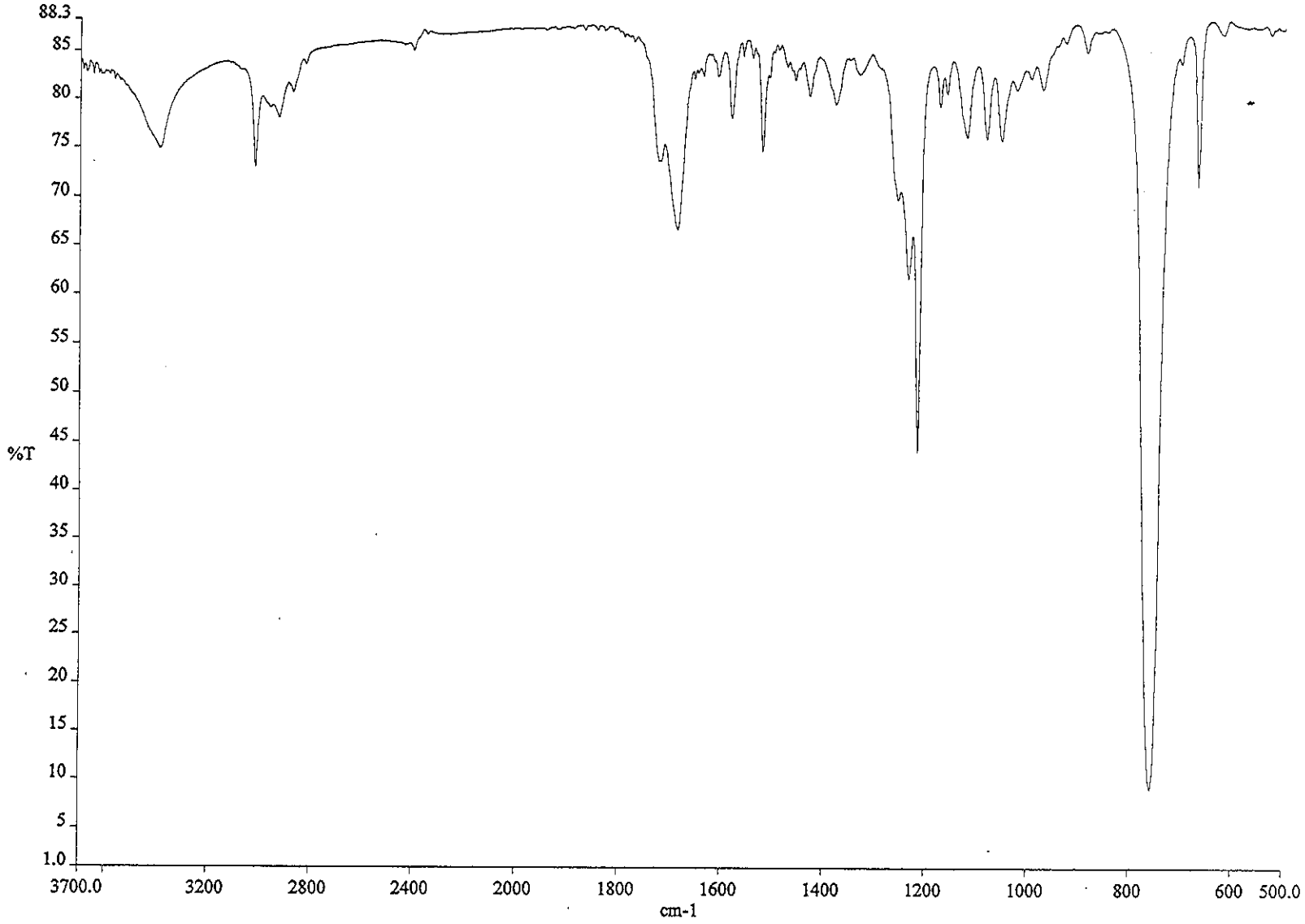


Figure 3 IR (neat) spectrum of compound **PSI1**

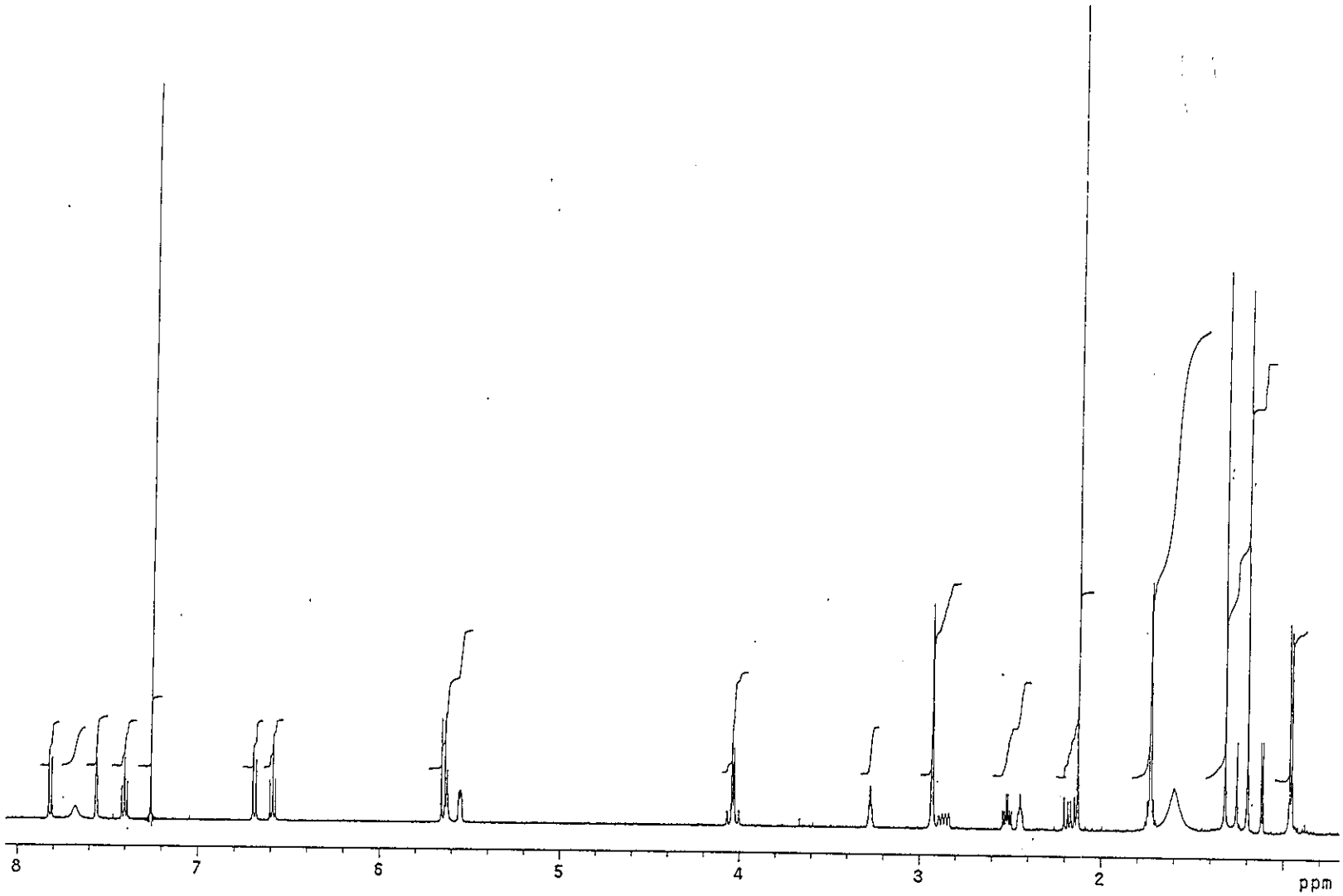


Figure 4 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **PSI1**

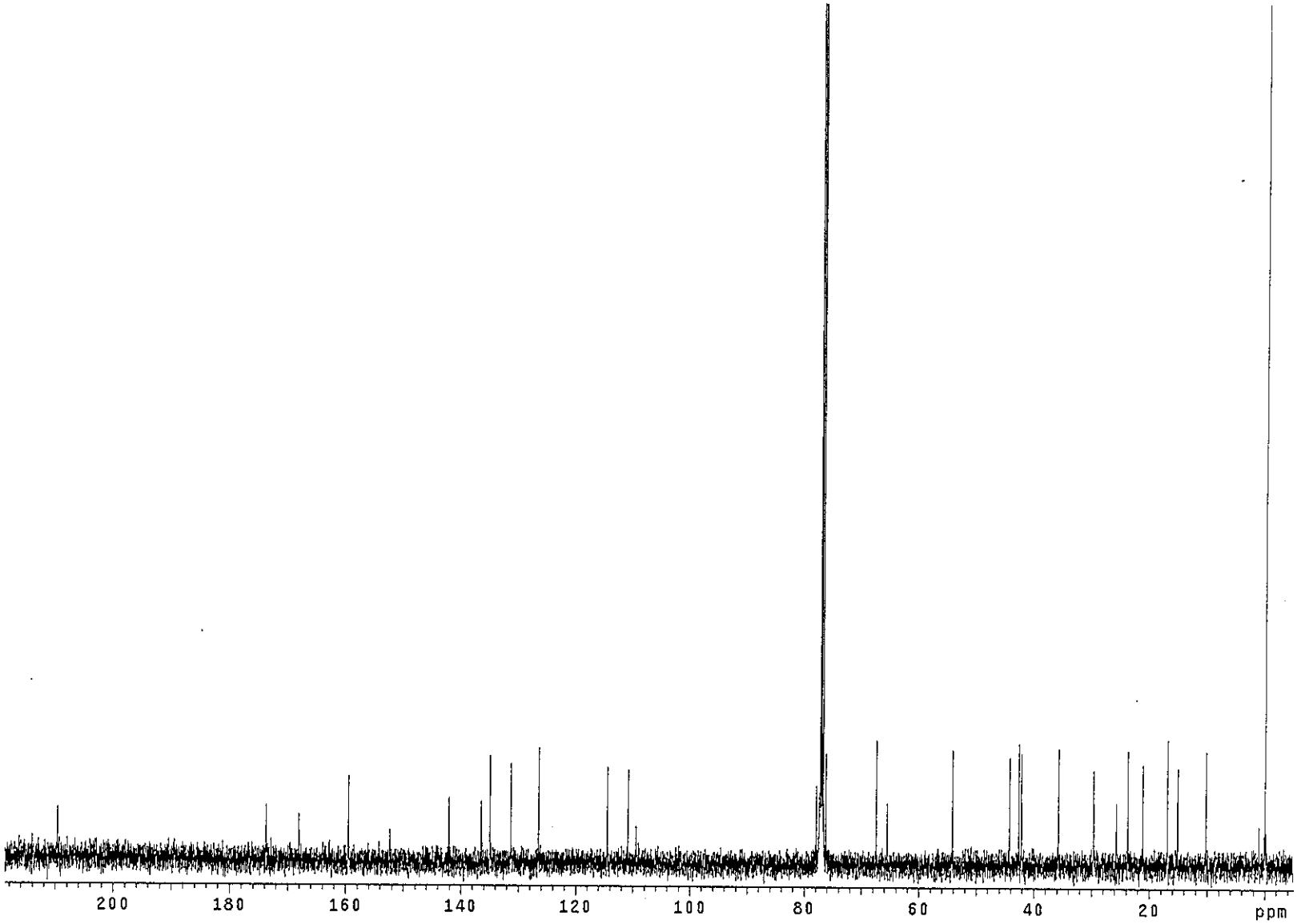


Figure 5 ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of compound **PSI1**

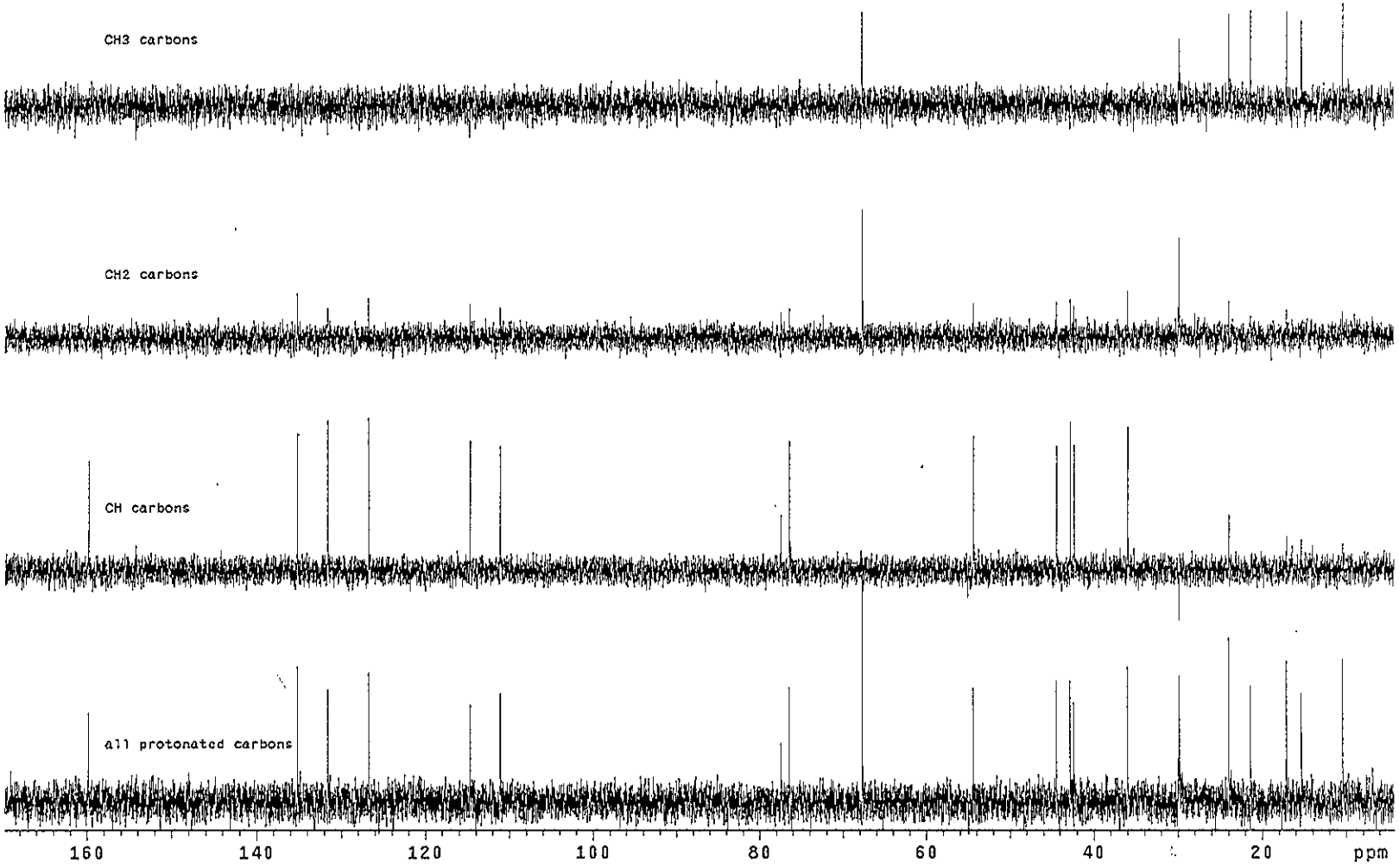


Figure 6 DEPT ($CDCl_3$) spectrum of compound **PSI1**

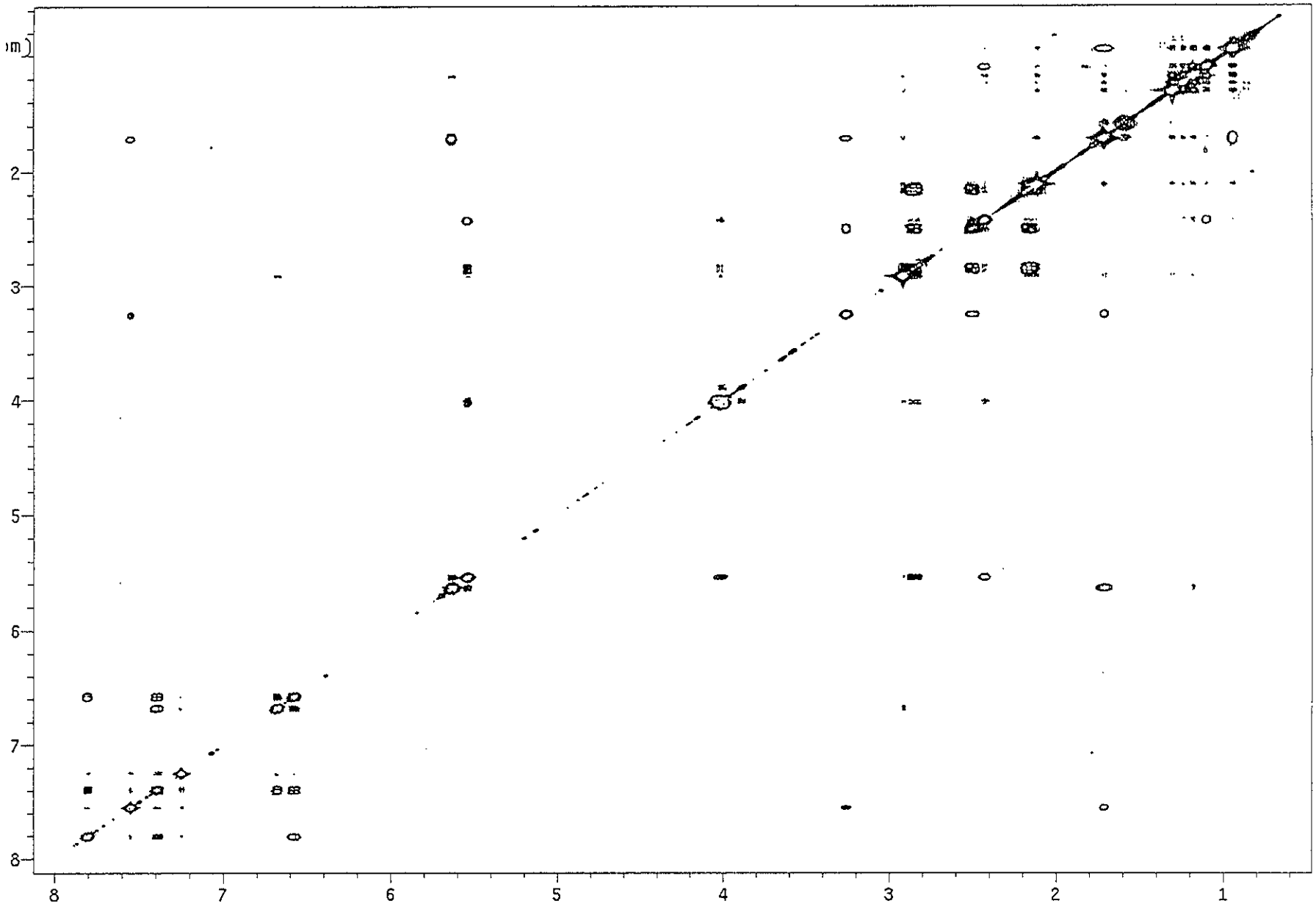


Figure 7 2D COSY spectrum of compound **PSI1**

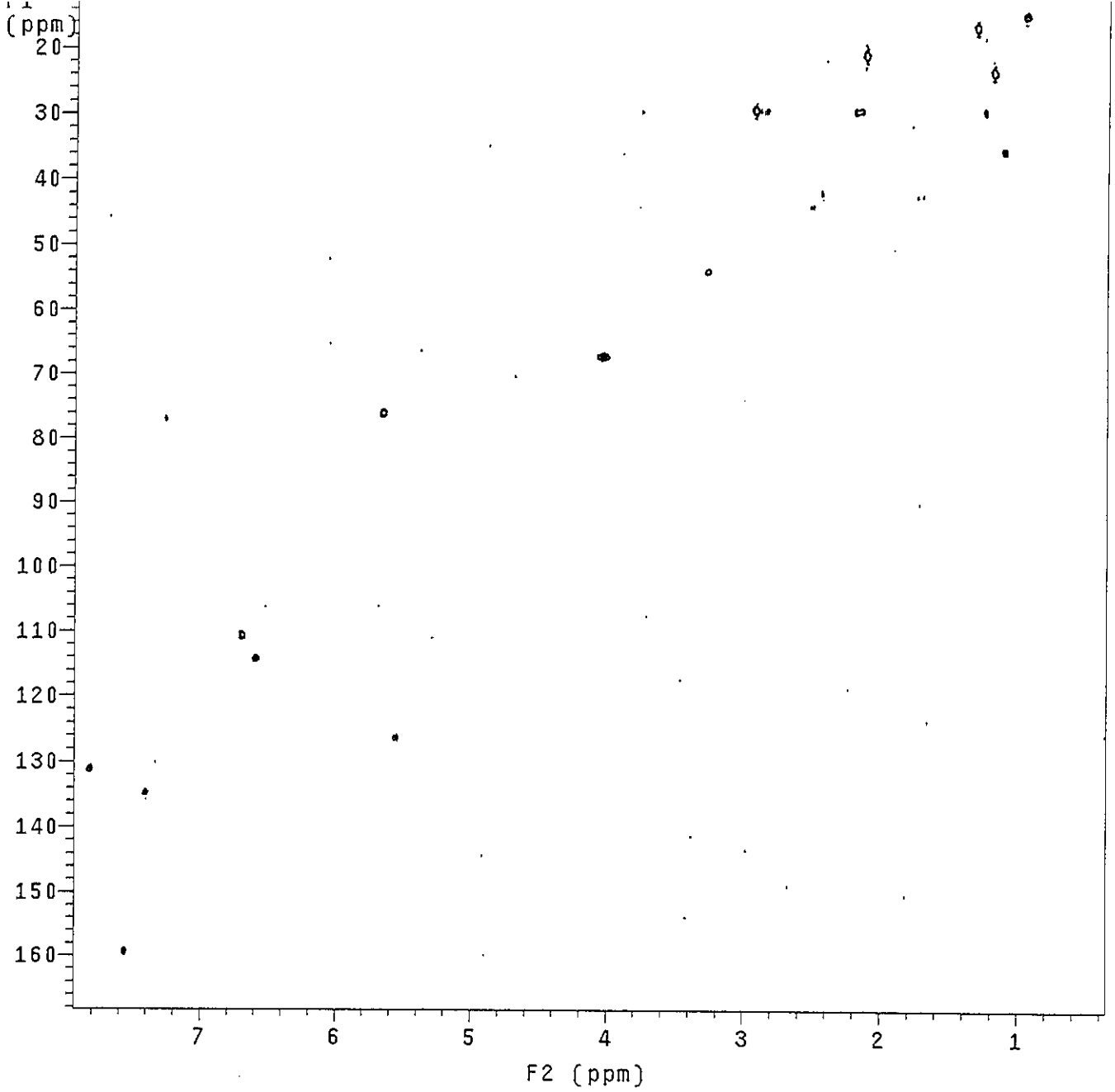


Figure 8 2D HMQC spectrum of compound **PSI1**

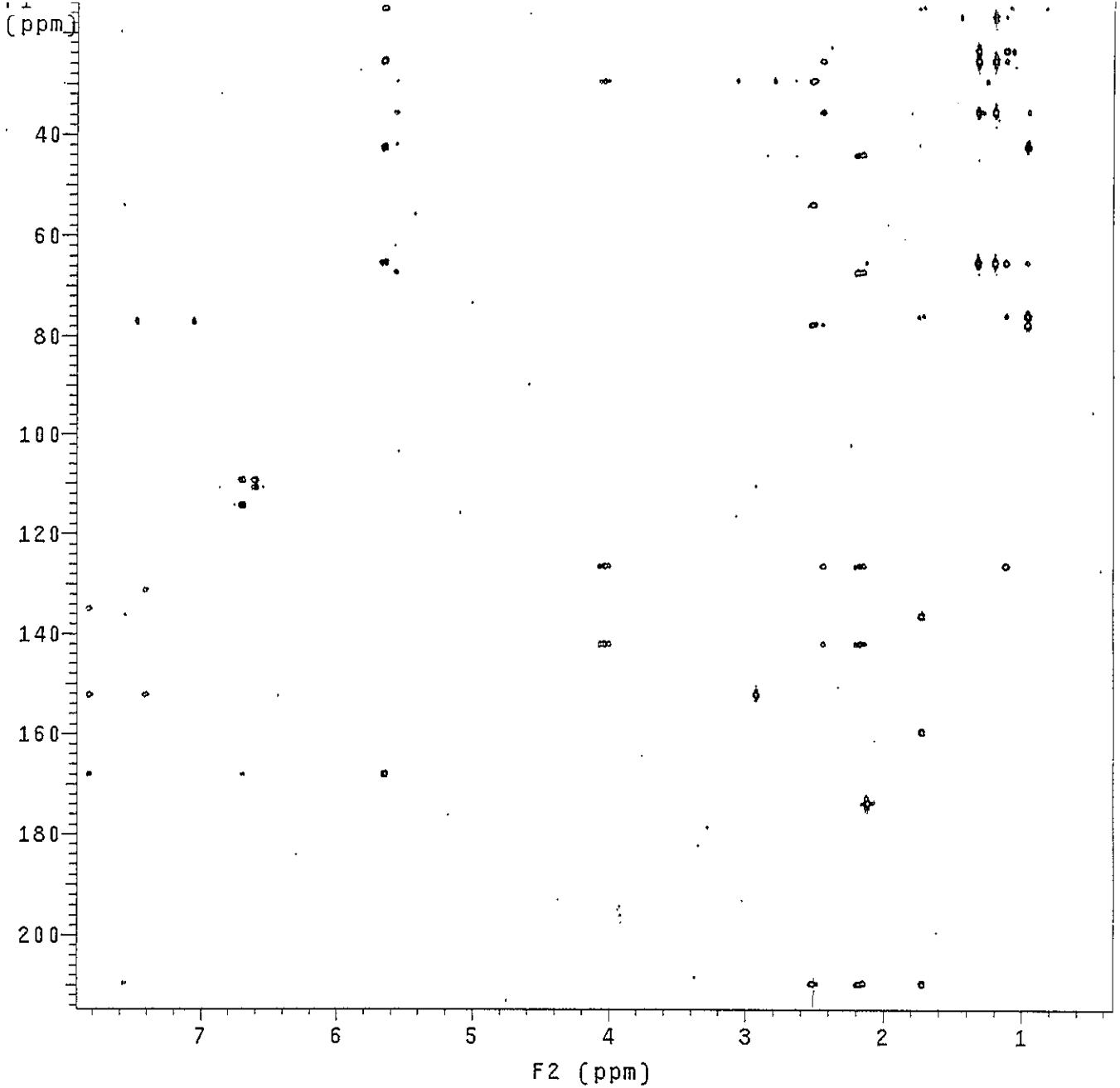


Figure 9 2D HMBC spectrum of compound **PSI1**

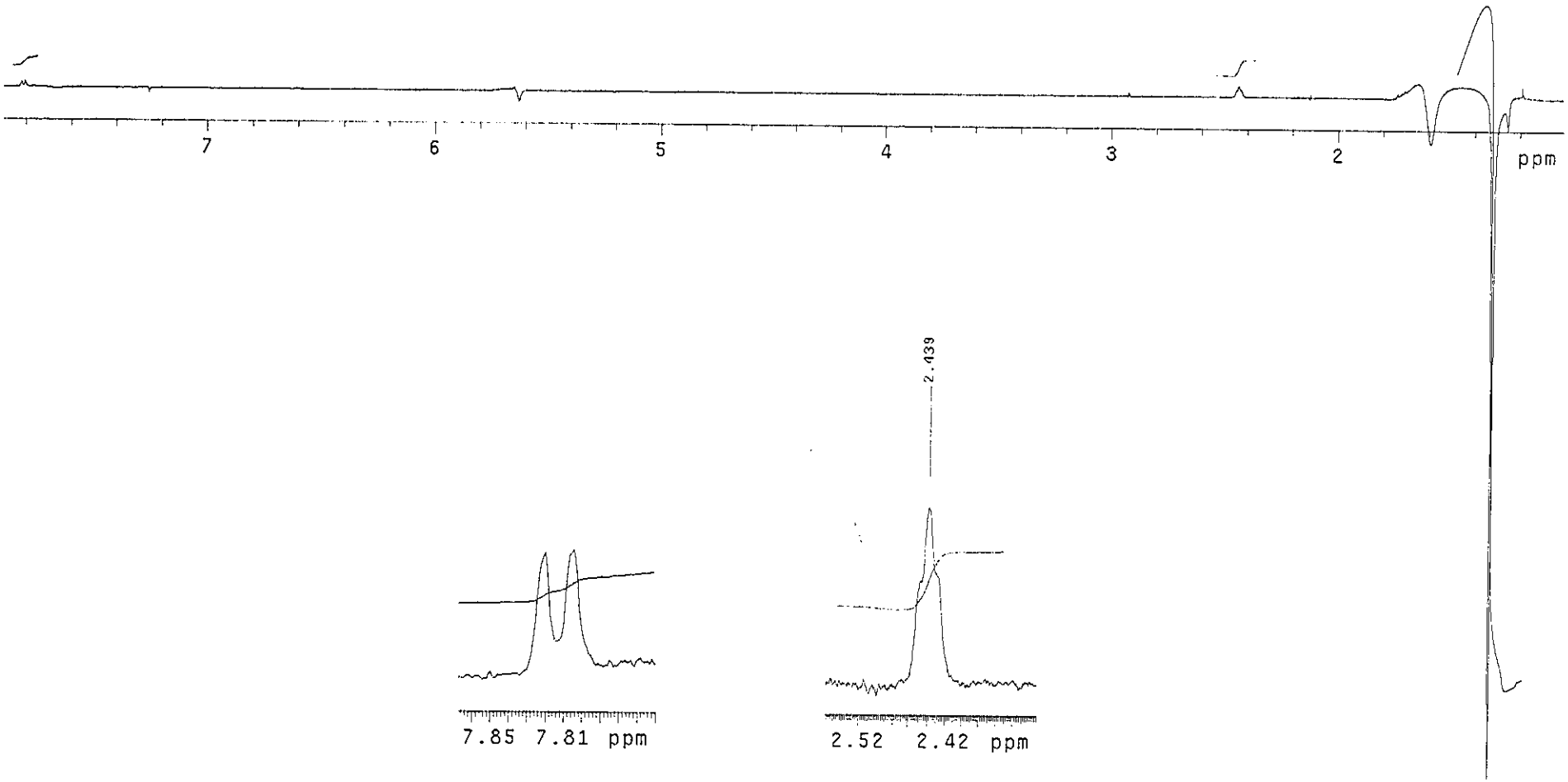


Figure 10 NOE spectrum of compound **PSI1** irradiated at δ1.32, (1)

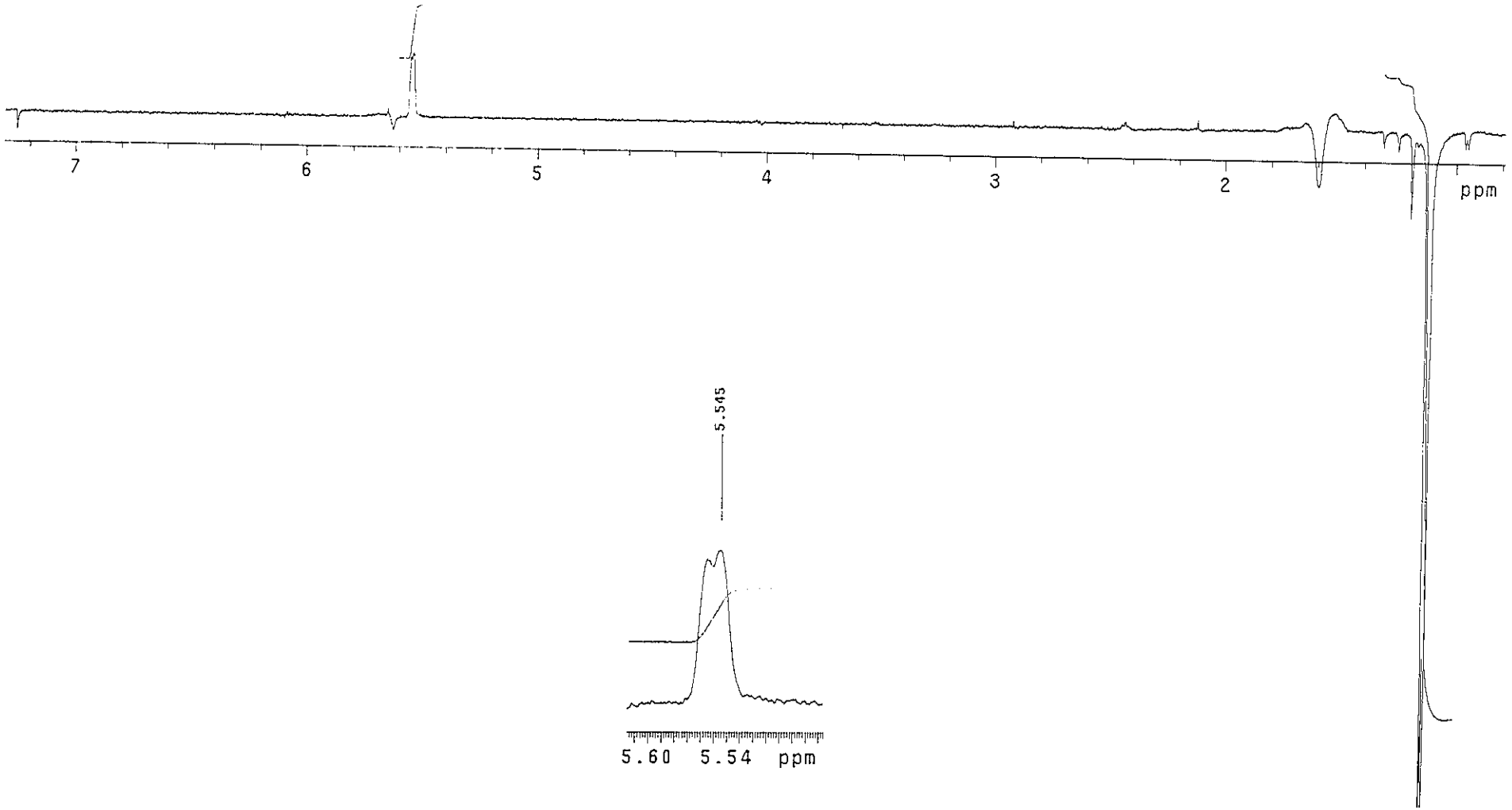


Figure 10 NOE spectrum of compound **PSI1** irradiated at δ1.12, (2)

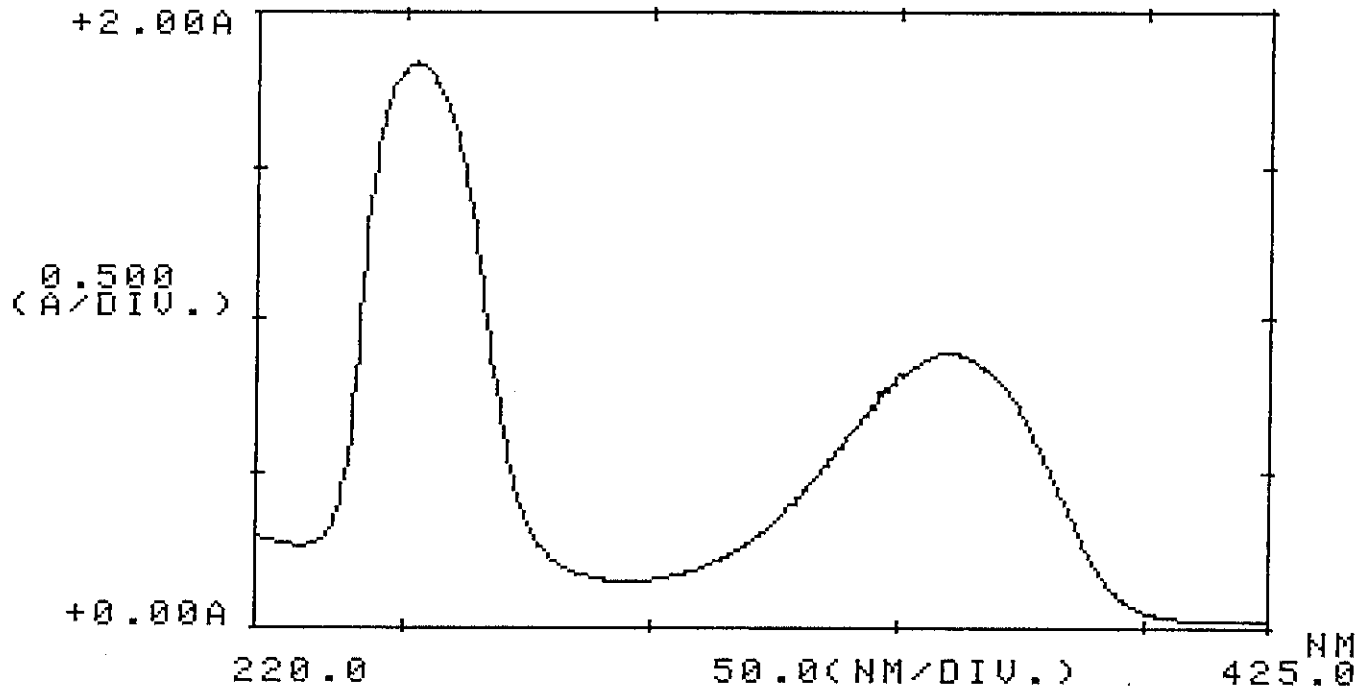


Figure 11 UV ($CHCl_3$) spectrum of compound **PSI2**

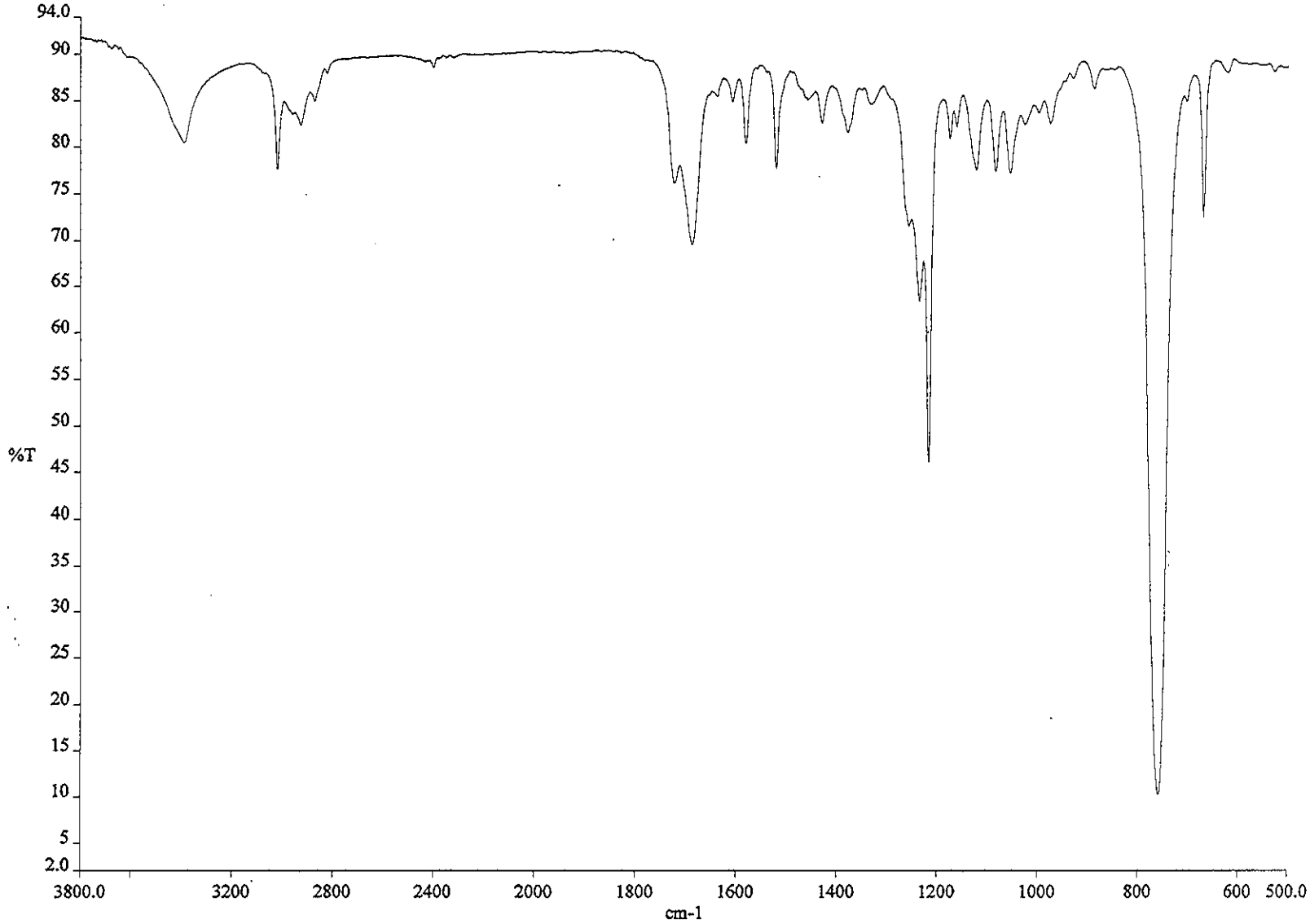


Figure 12 IR (Neat) spectrum of compound **PSI2**

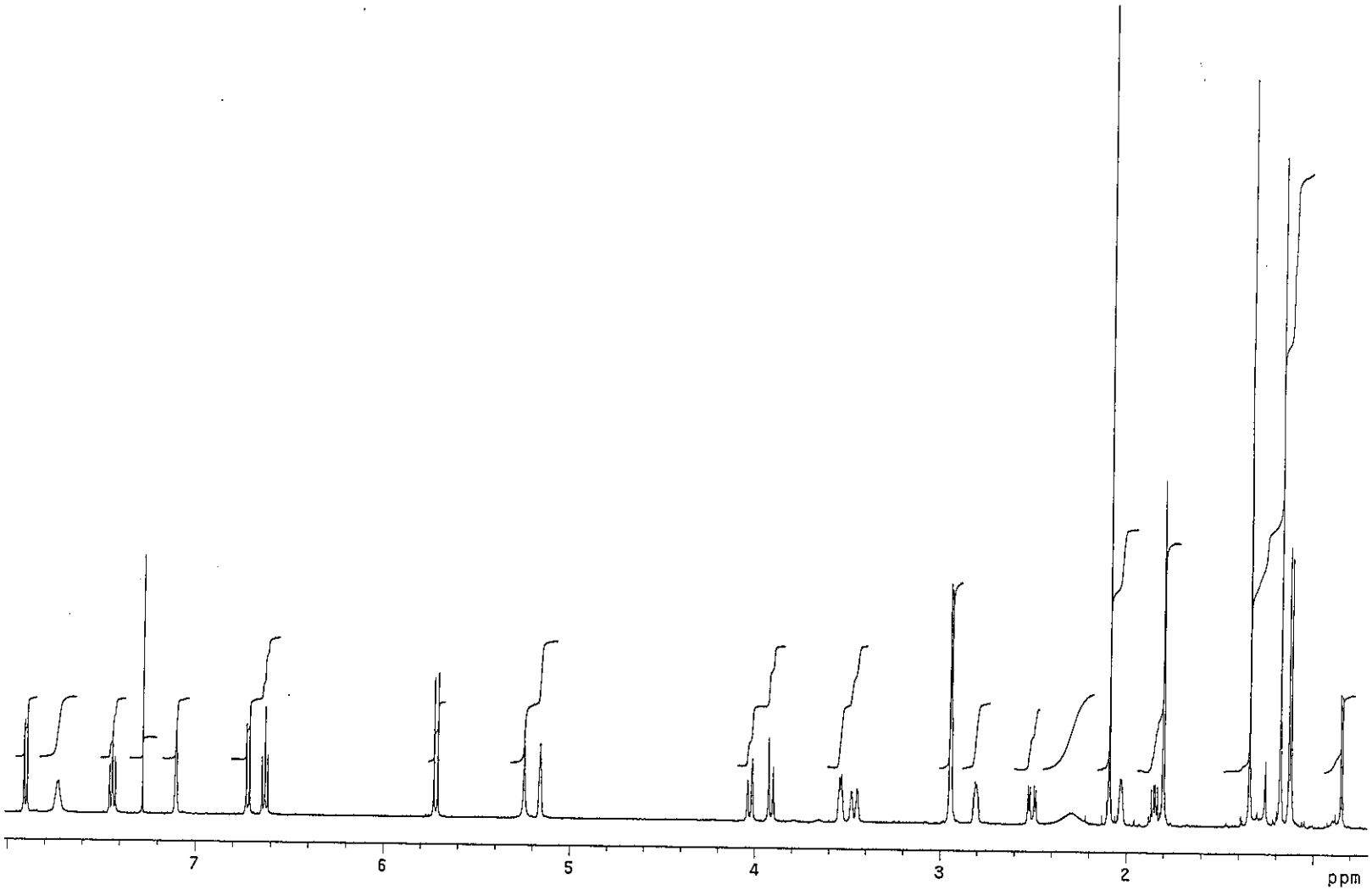


Figure 13 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **PSI2**

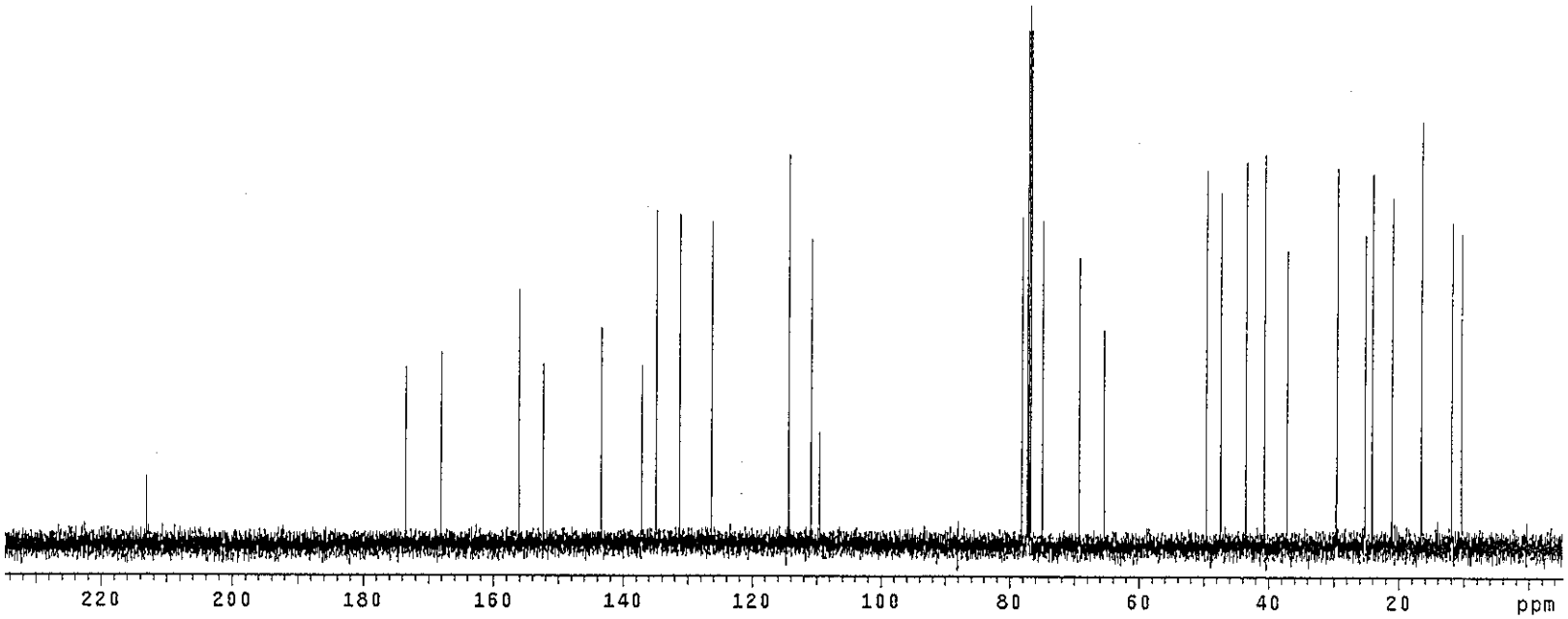


Figure 14 ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of compound **PSI2**

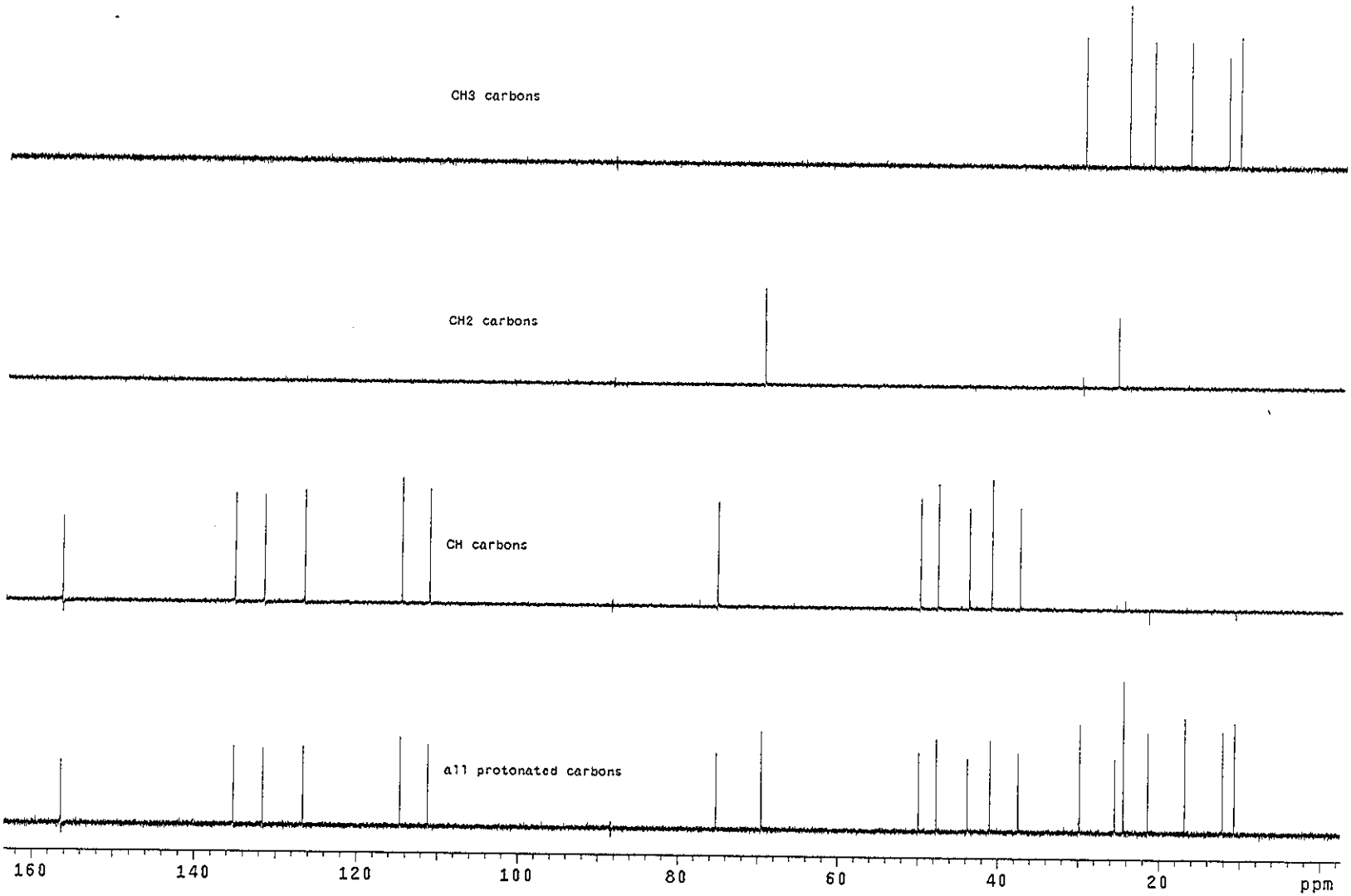


Figure 15 DEPT ($CDCl_3$) spectrum of compound **PSI2**

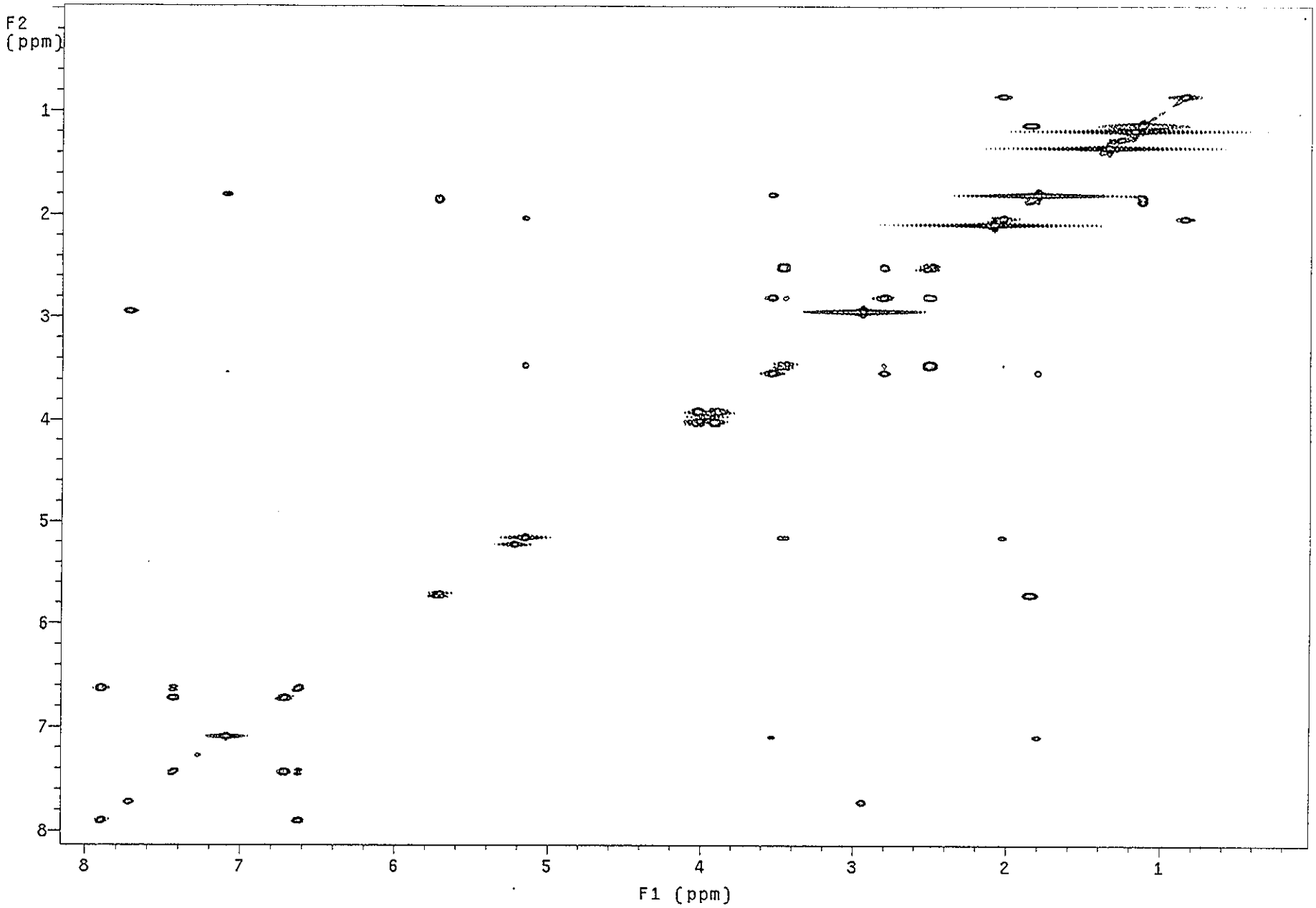


Figure 16 DEPT ($CDCl_3$) spectrum of compound **PSI2**

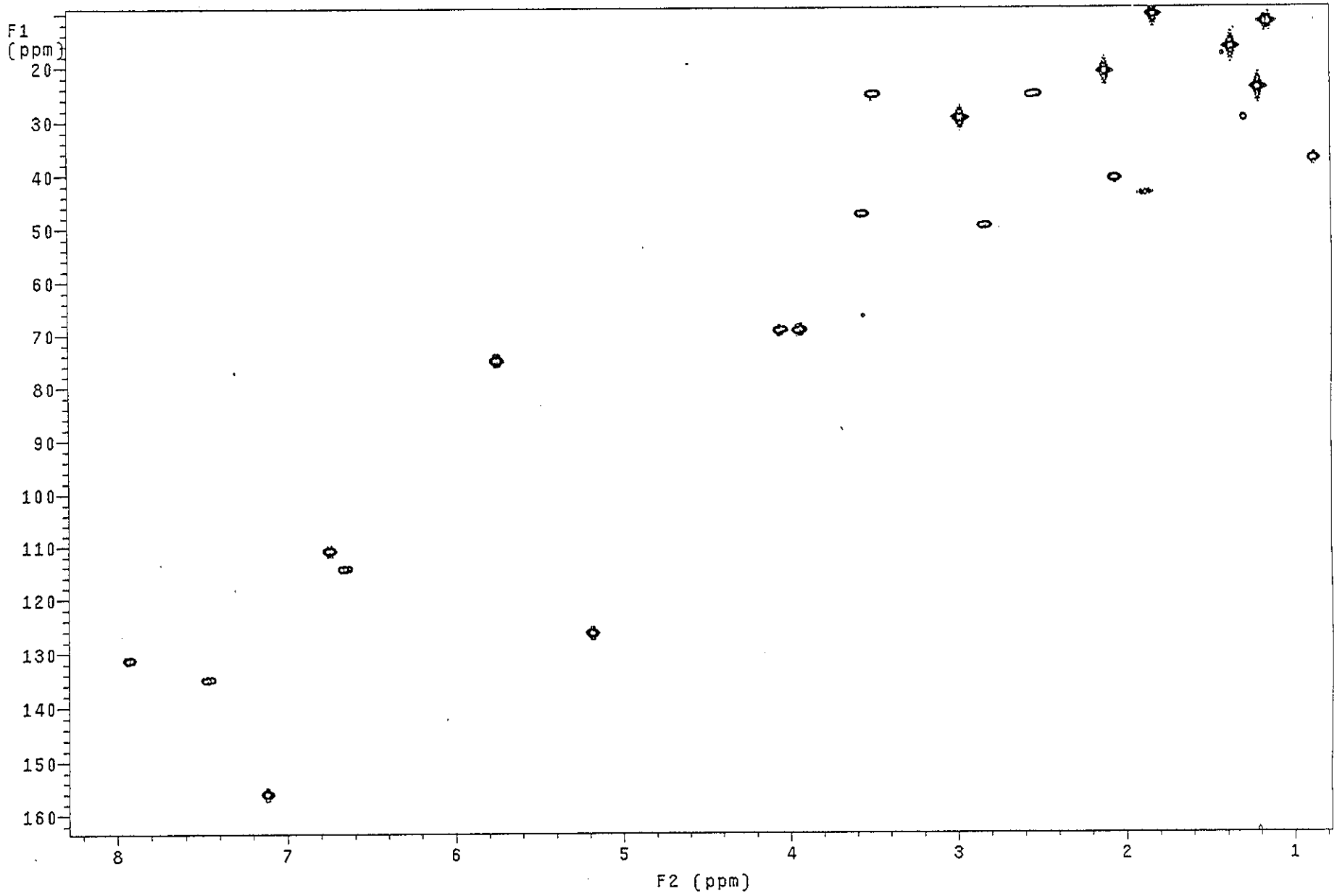


Figure 17 2D HMQC spectrum of compound **PSI2**

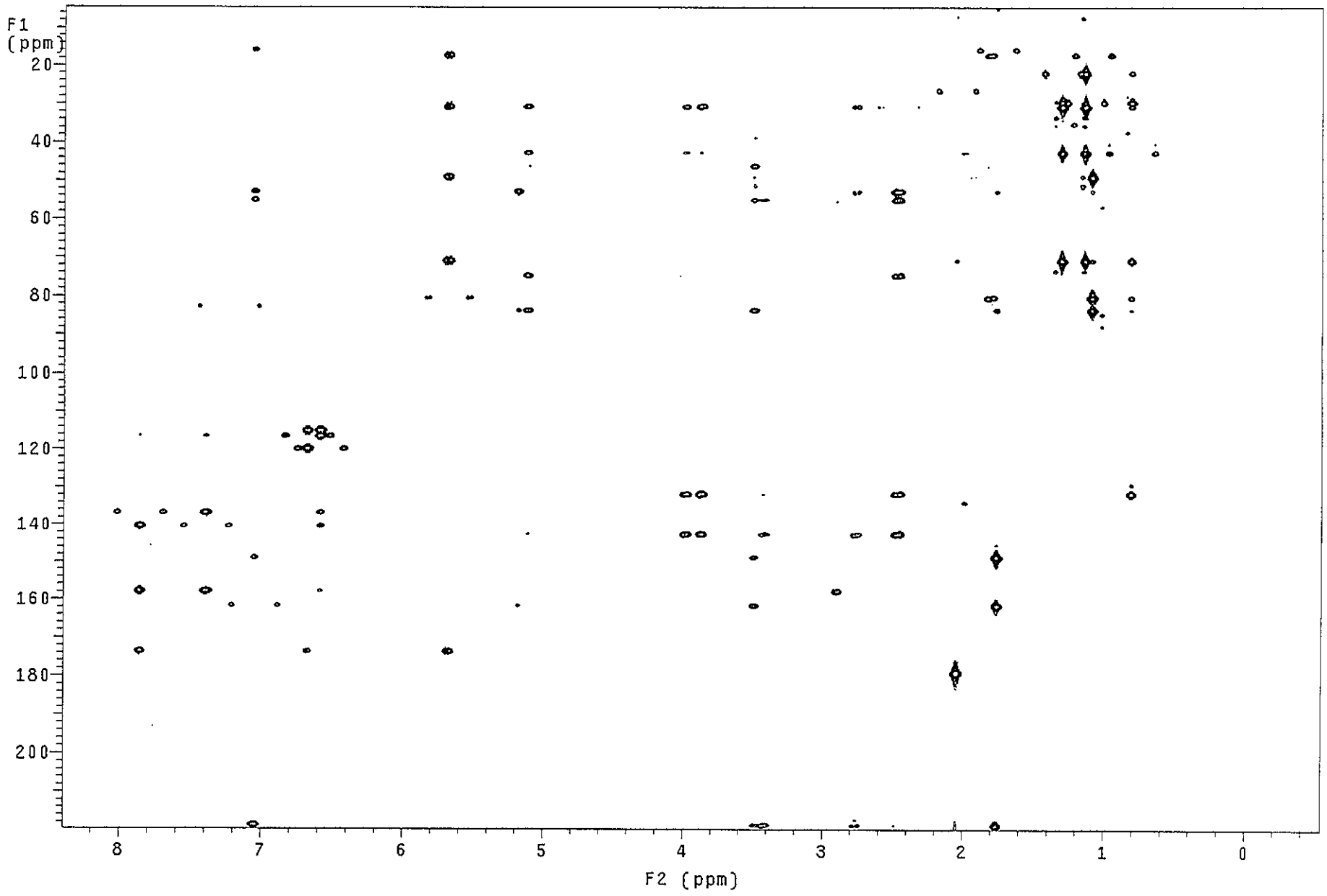


Figure 18 2D HMBC spectrum of compound **PSI2**

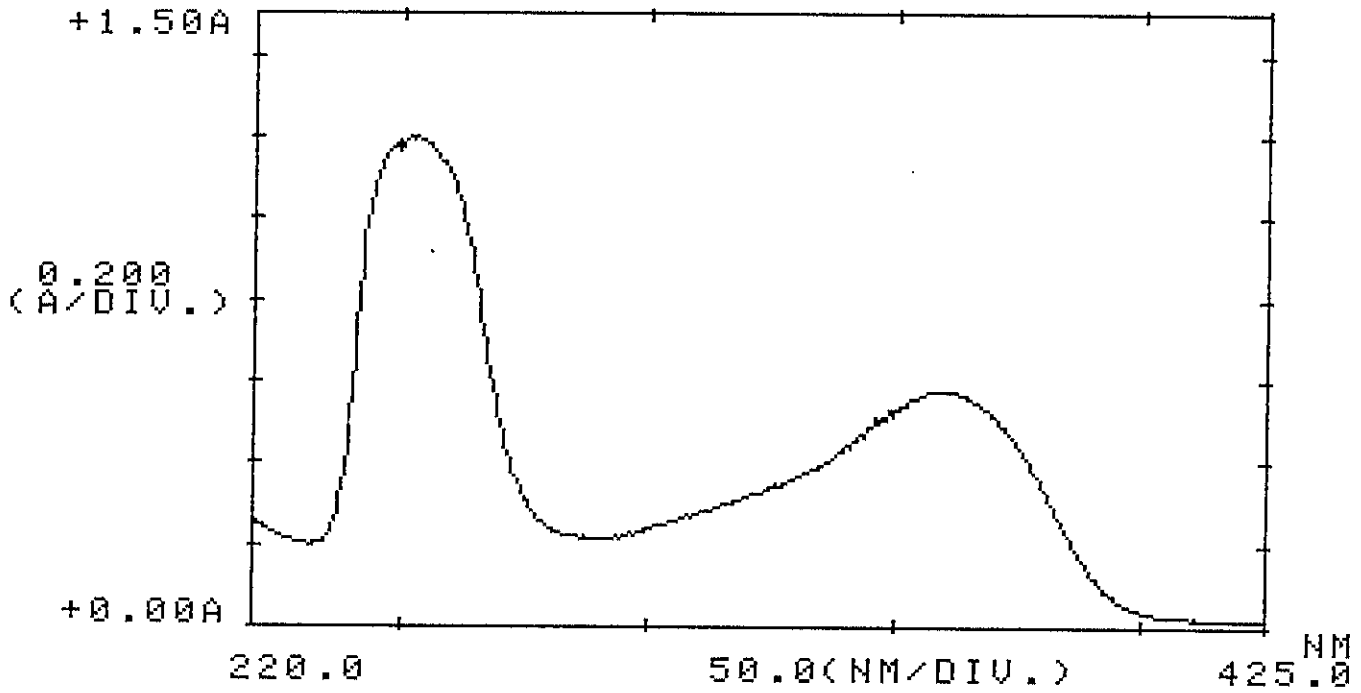


Figure 19 UV ($CHCl_3$) spectrum of compound **PSI3**

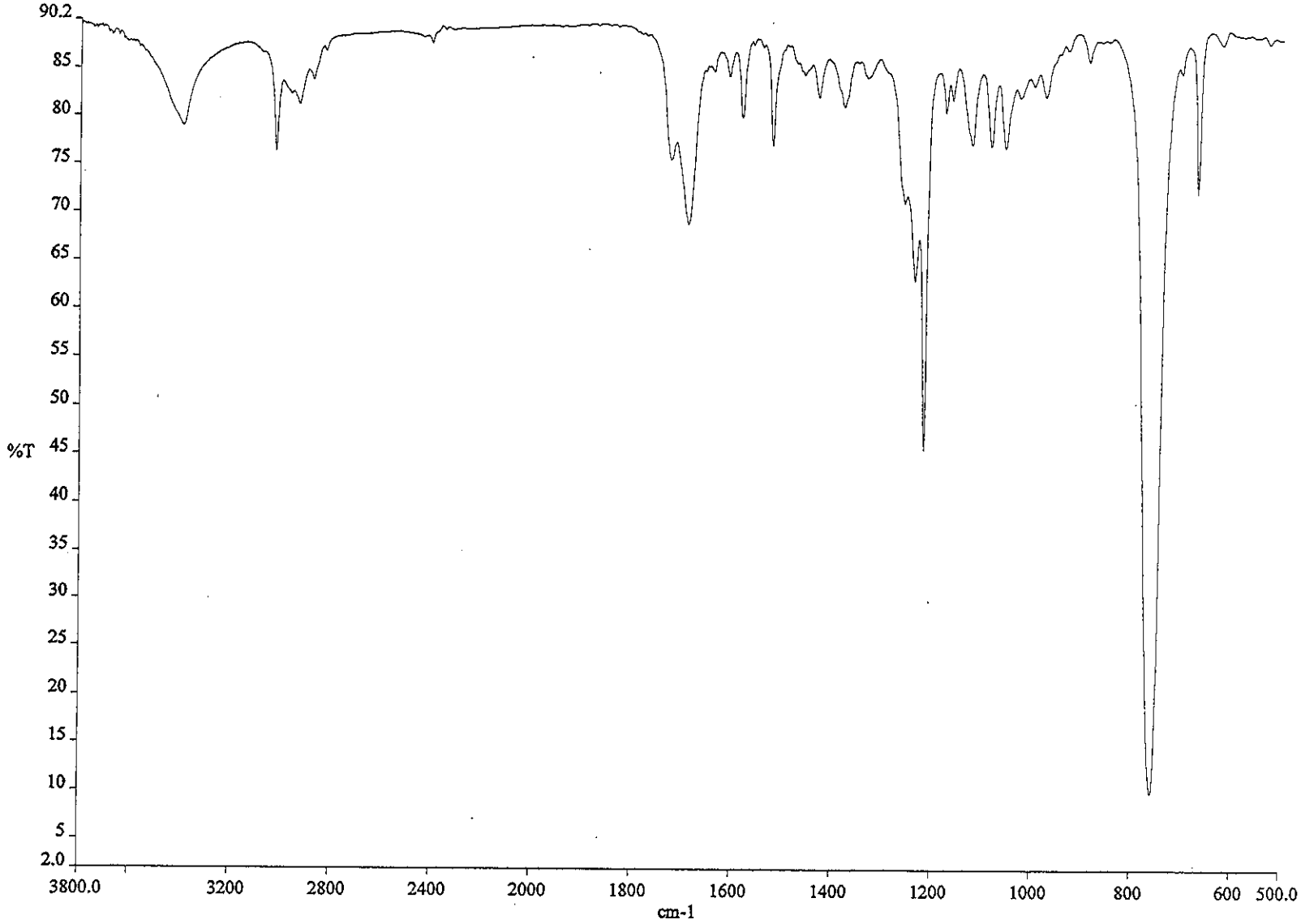


Figure 20 IR (Neat) spectrum of compound **PSI3**

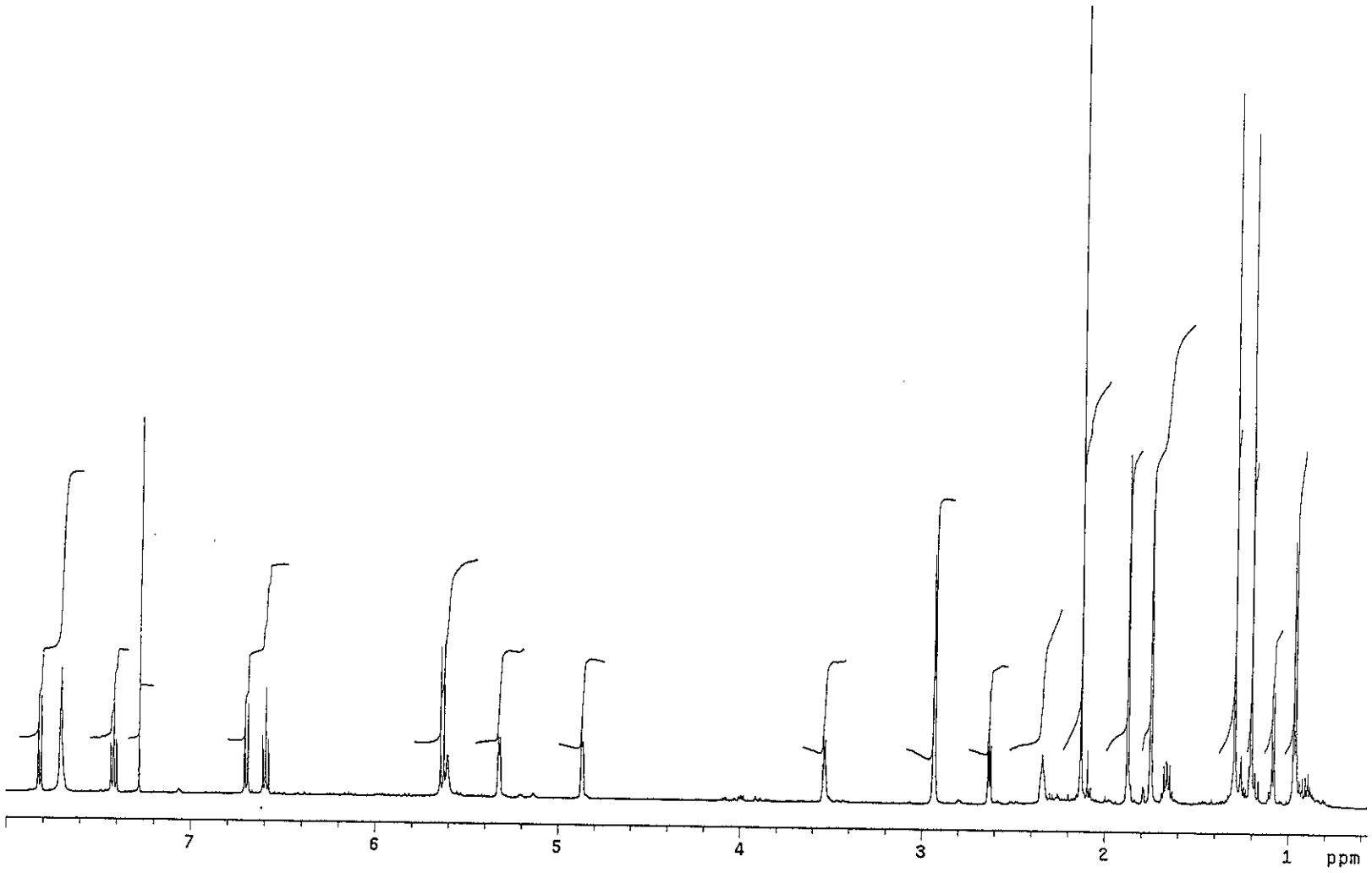


Figure 21 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **PSI3**

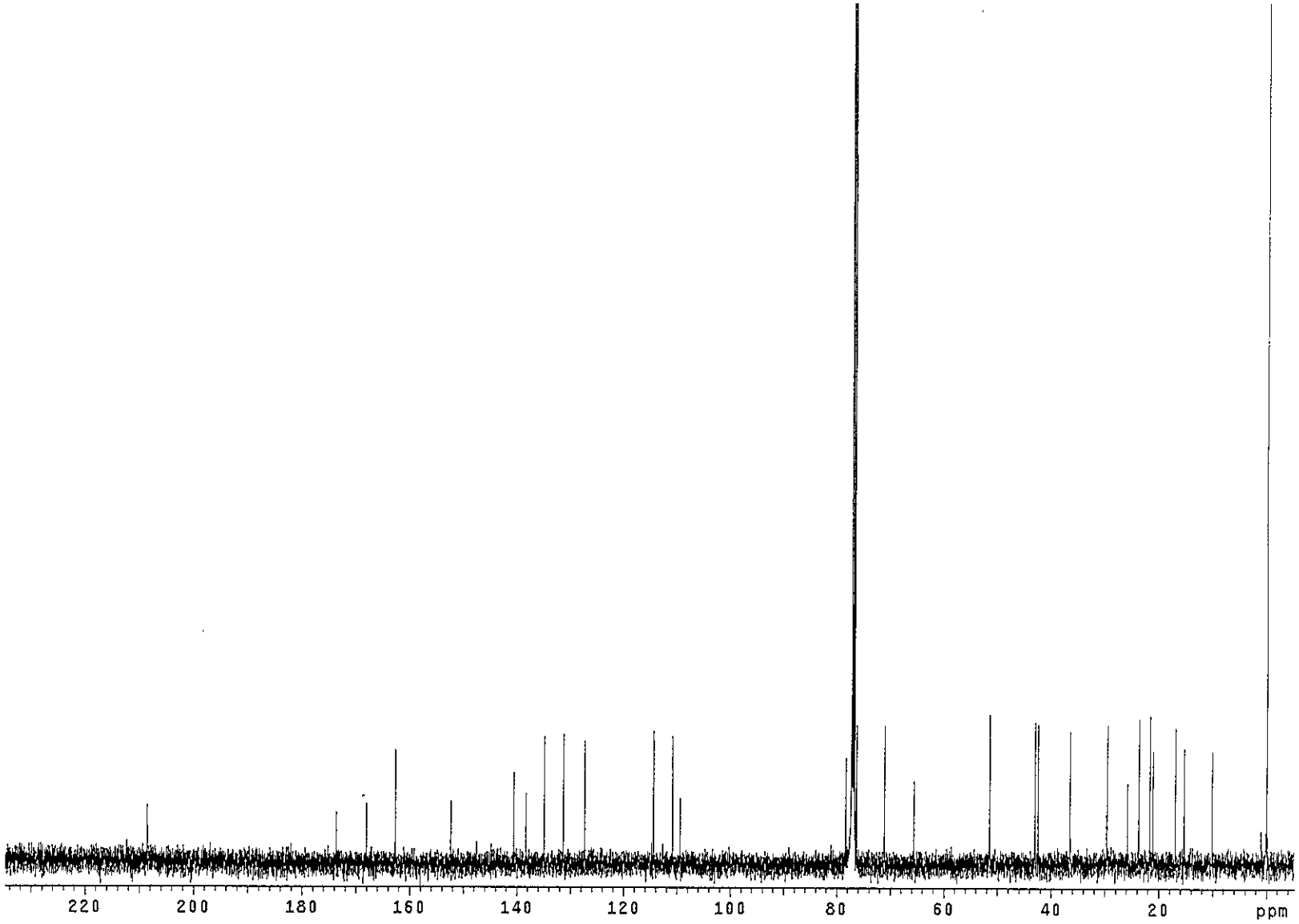


Figure 22 ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of compound **PSI3**

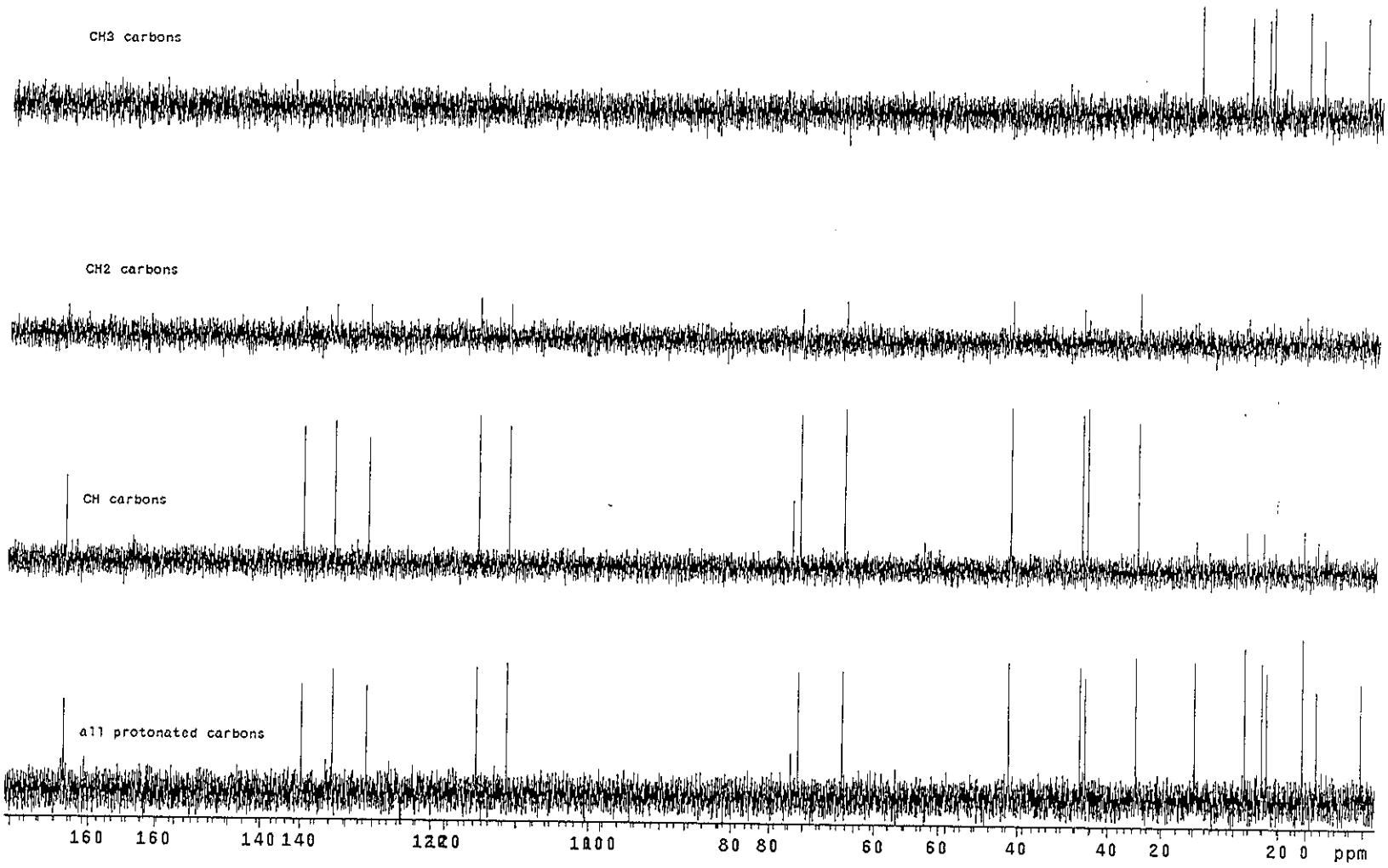


Figure 23 DEPT ($CDCl_3$) spectrum of compound **PSI3**

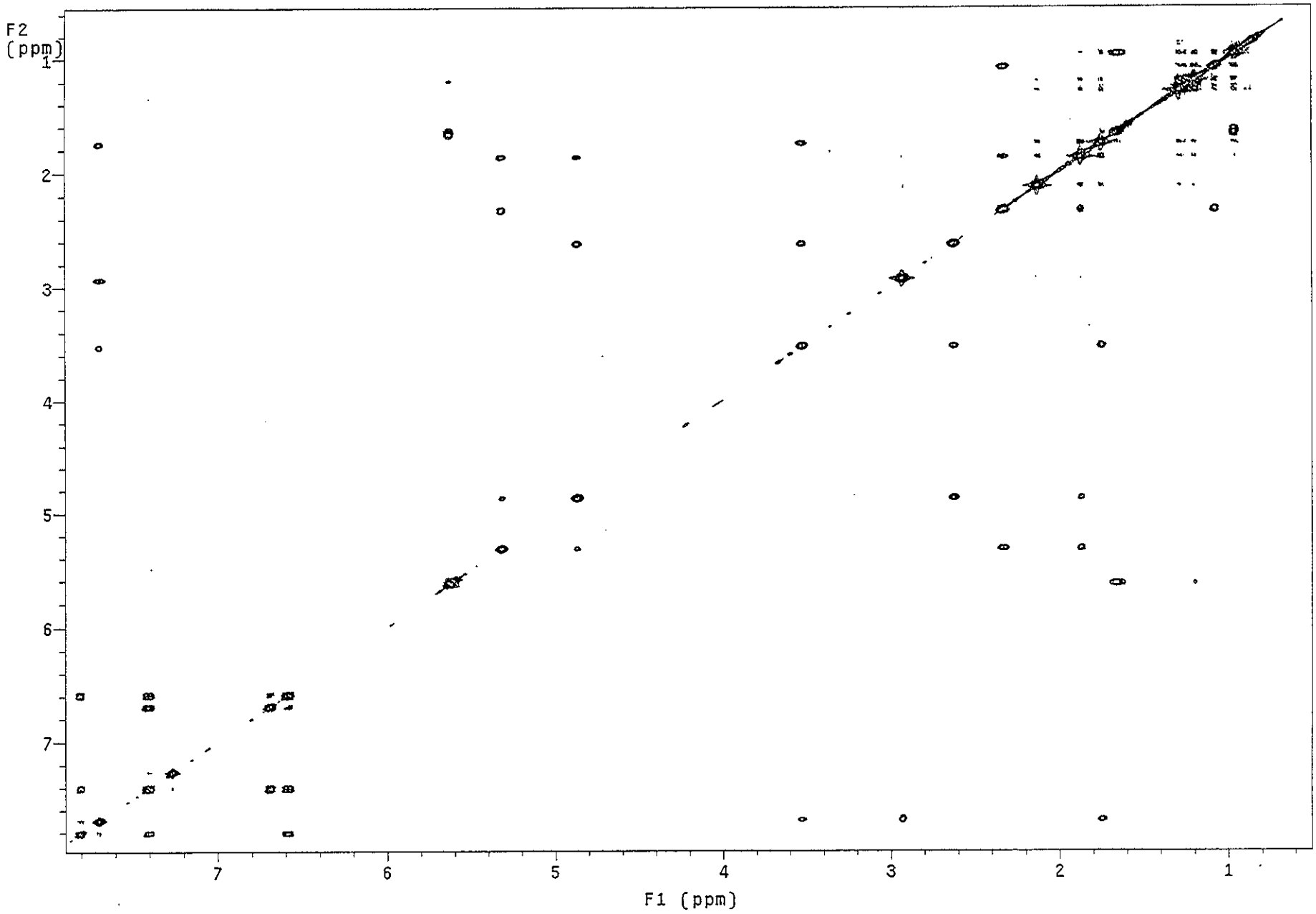


Figure 24 2D COSY spectrum of compound **PSI3**

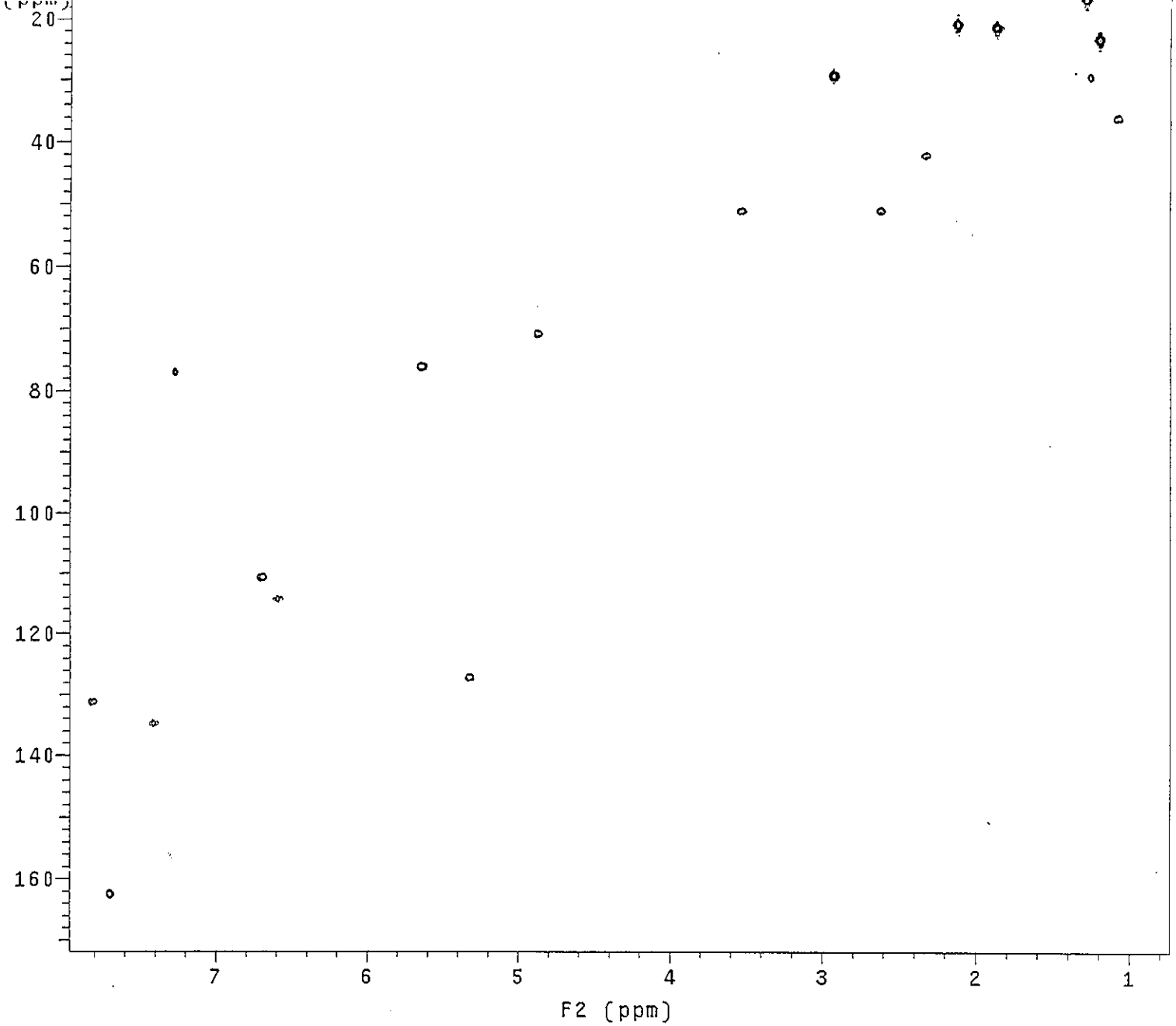


Figure 25 2D HMQC spectrum of compound **PSI3**

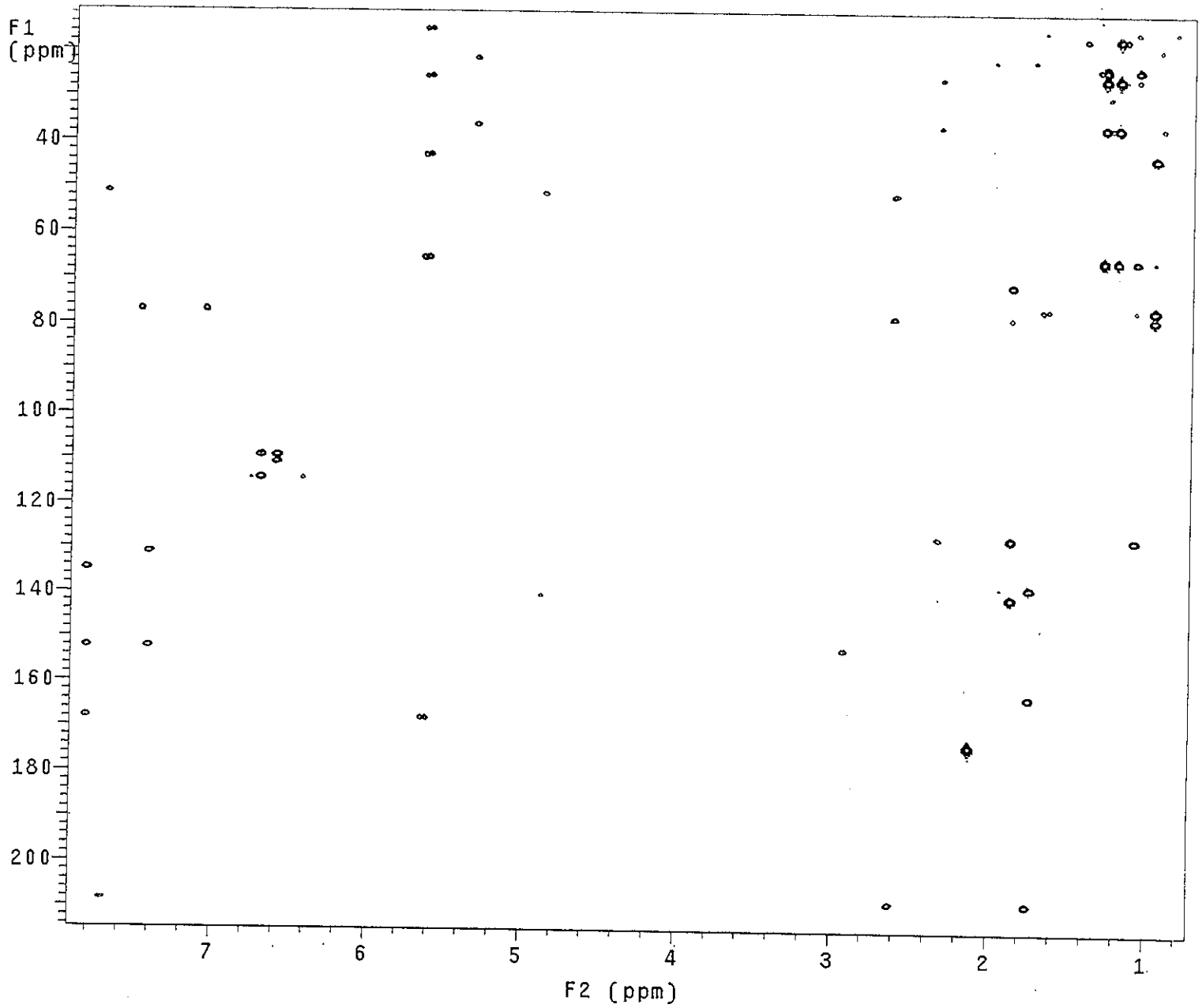


Figure 26 2D HMBC spectrum of compound **PSI3**

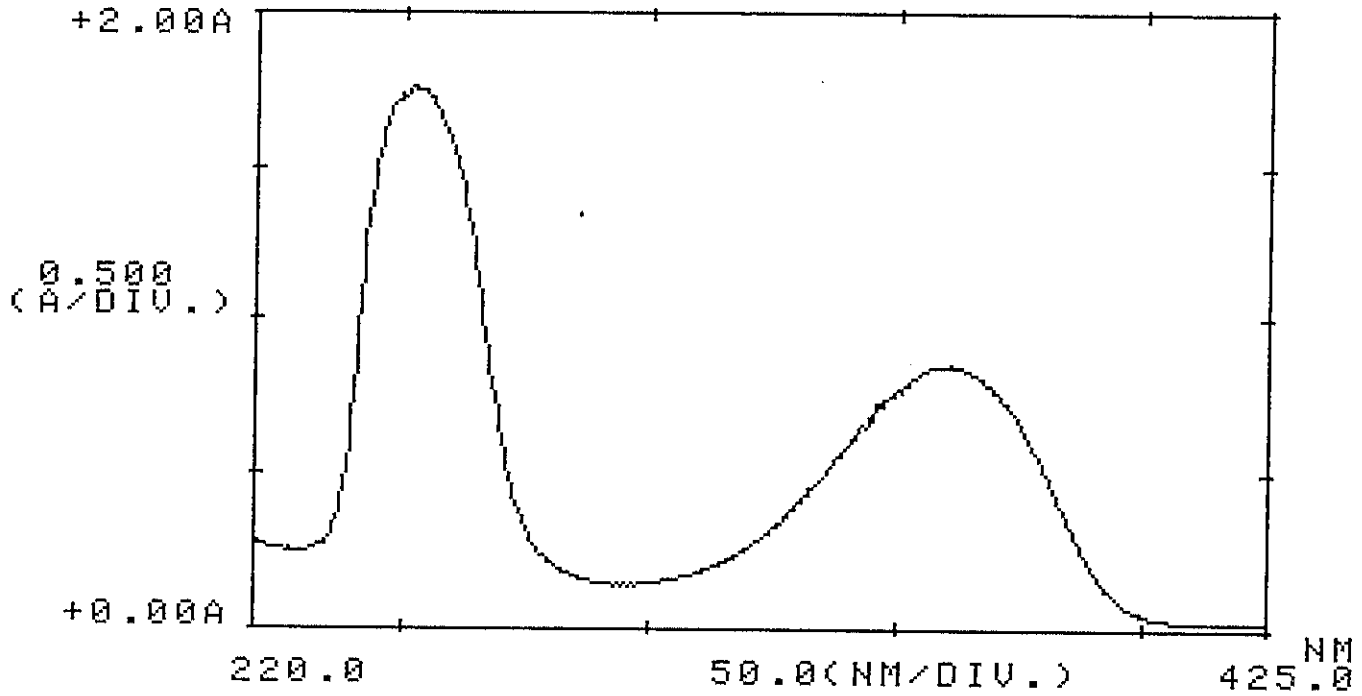


Figure 27 UV ($CHCl_3$) spectrum of compound **PSI4**

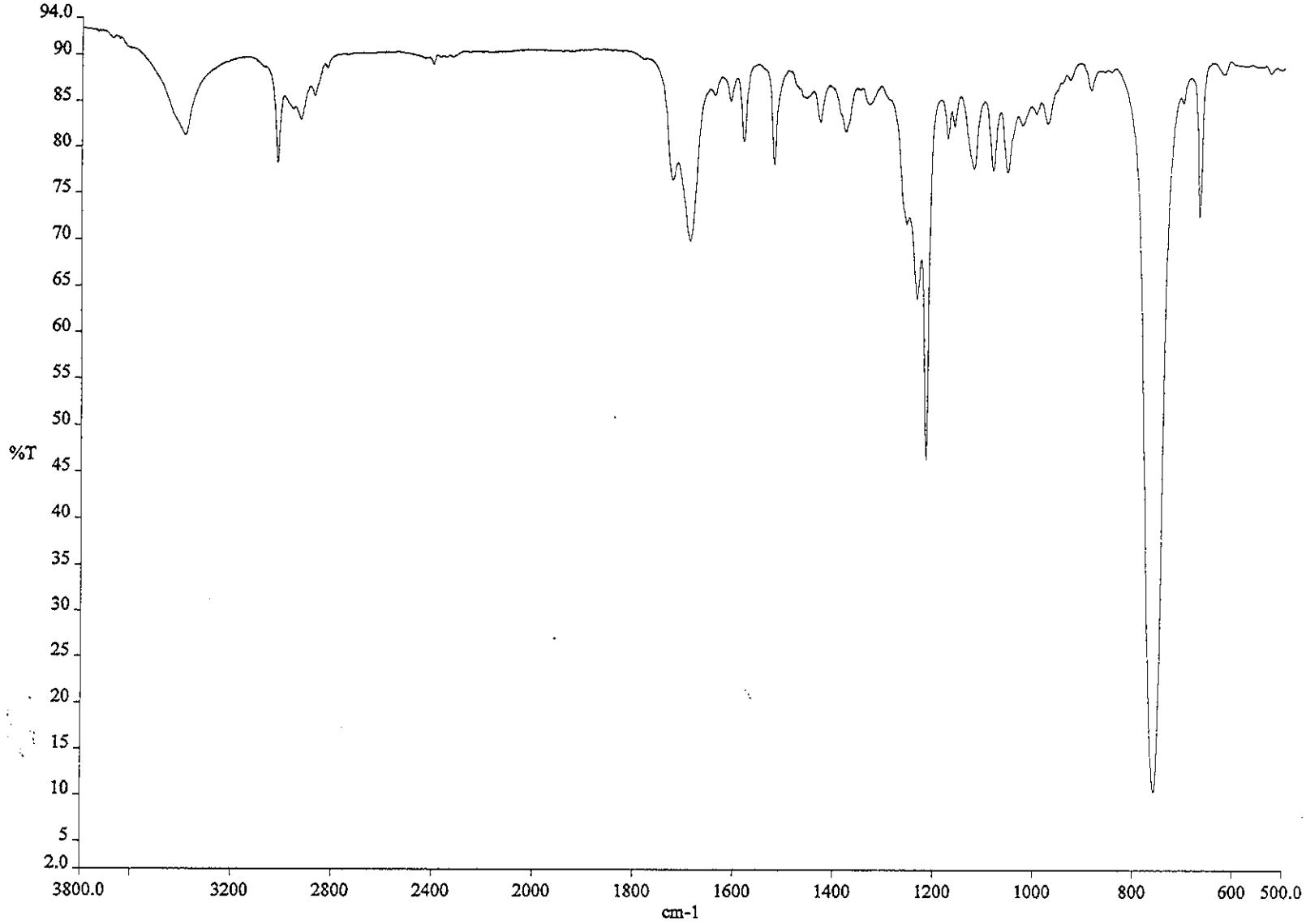


Figure 28 IR (Neat) spectrum of compound **PSI4**

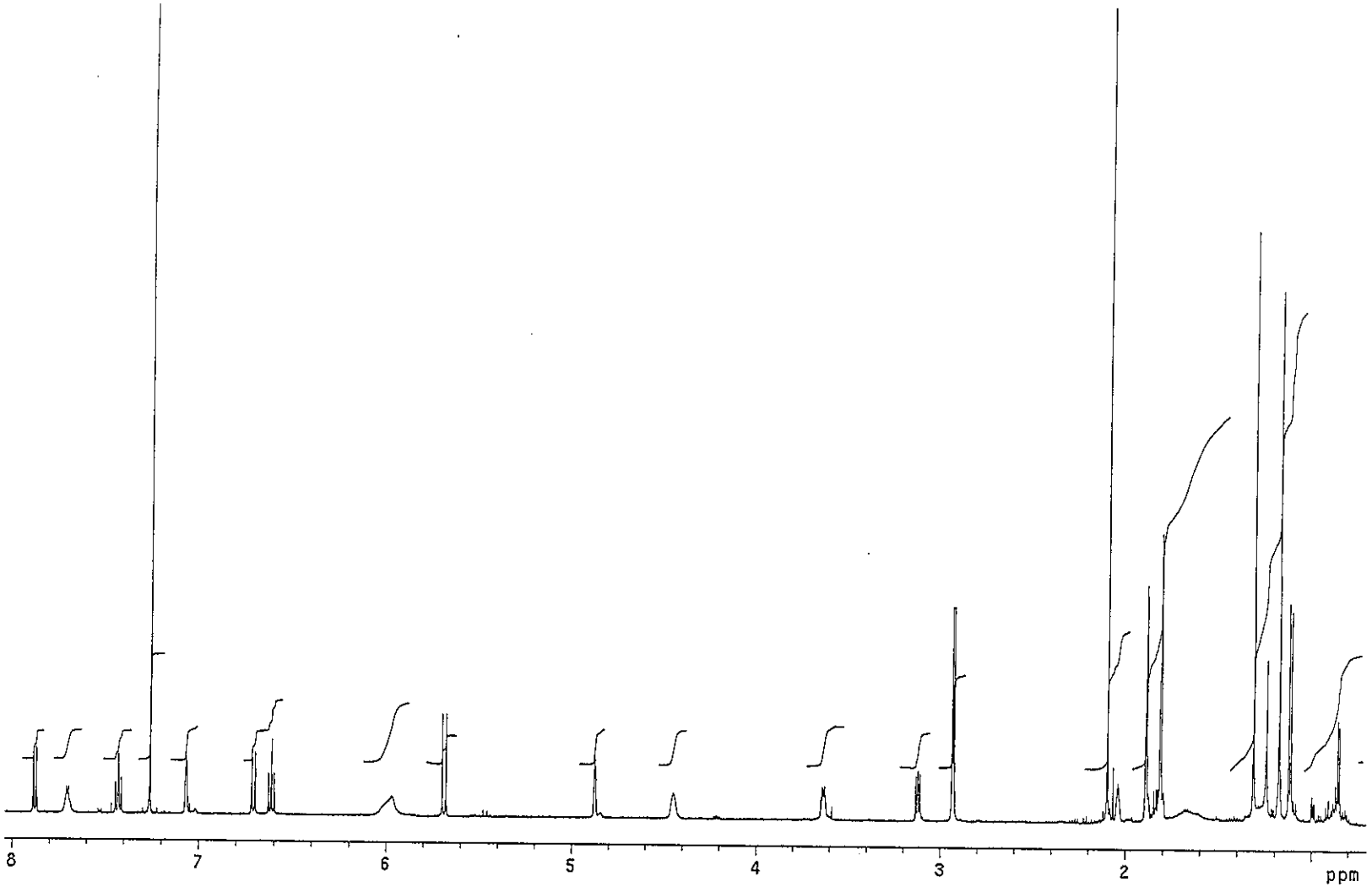


Figure 29 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **PSI4**

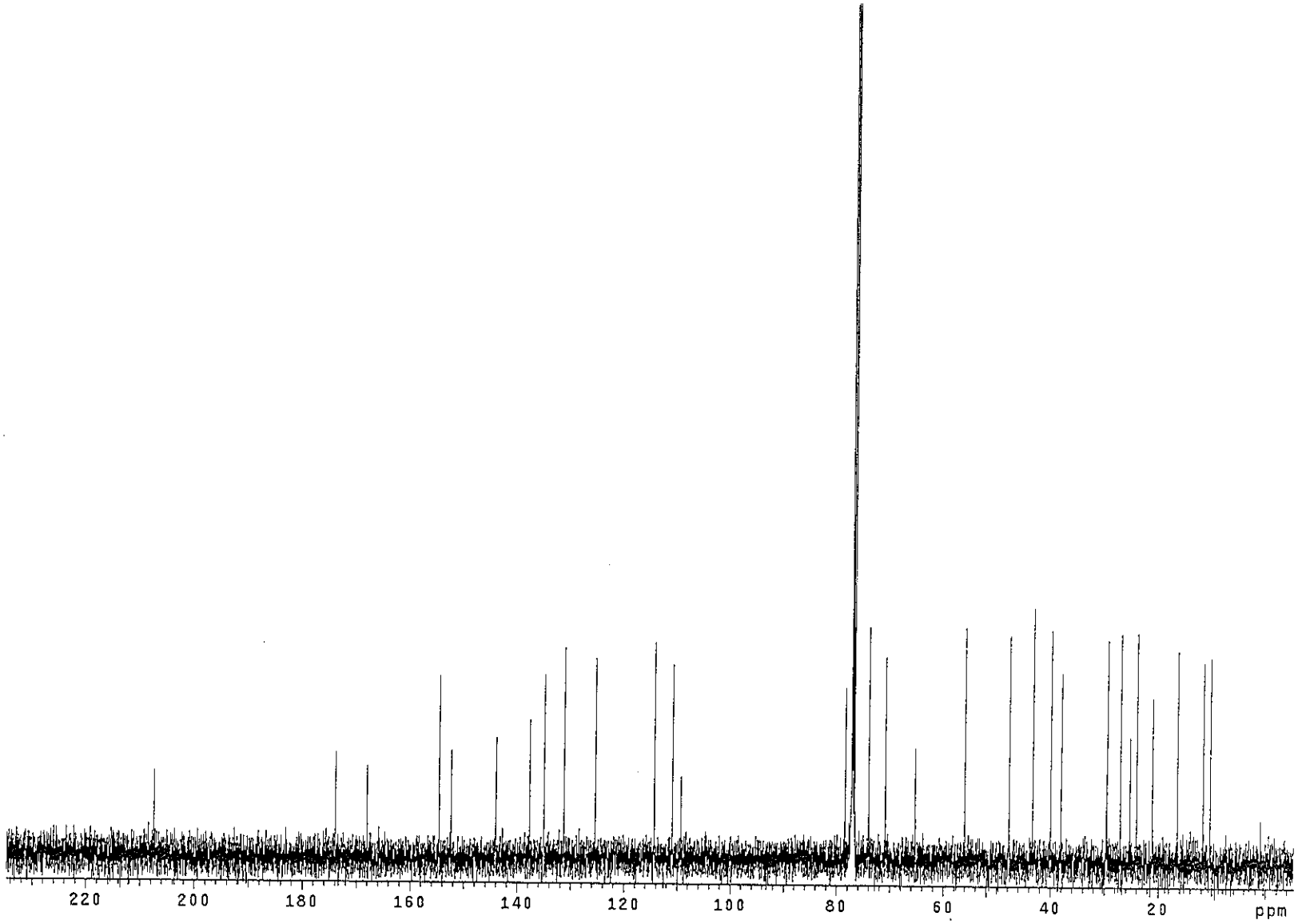


Figure 30 ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of compound **PSI4**

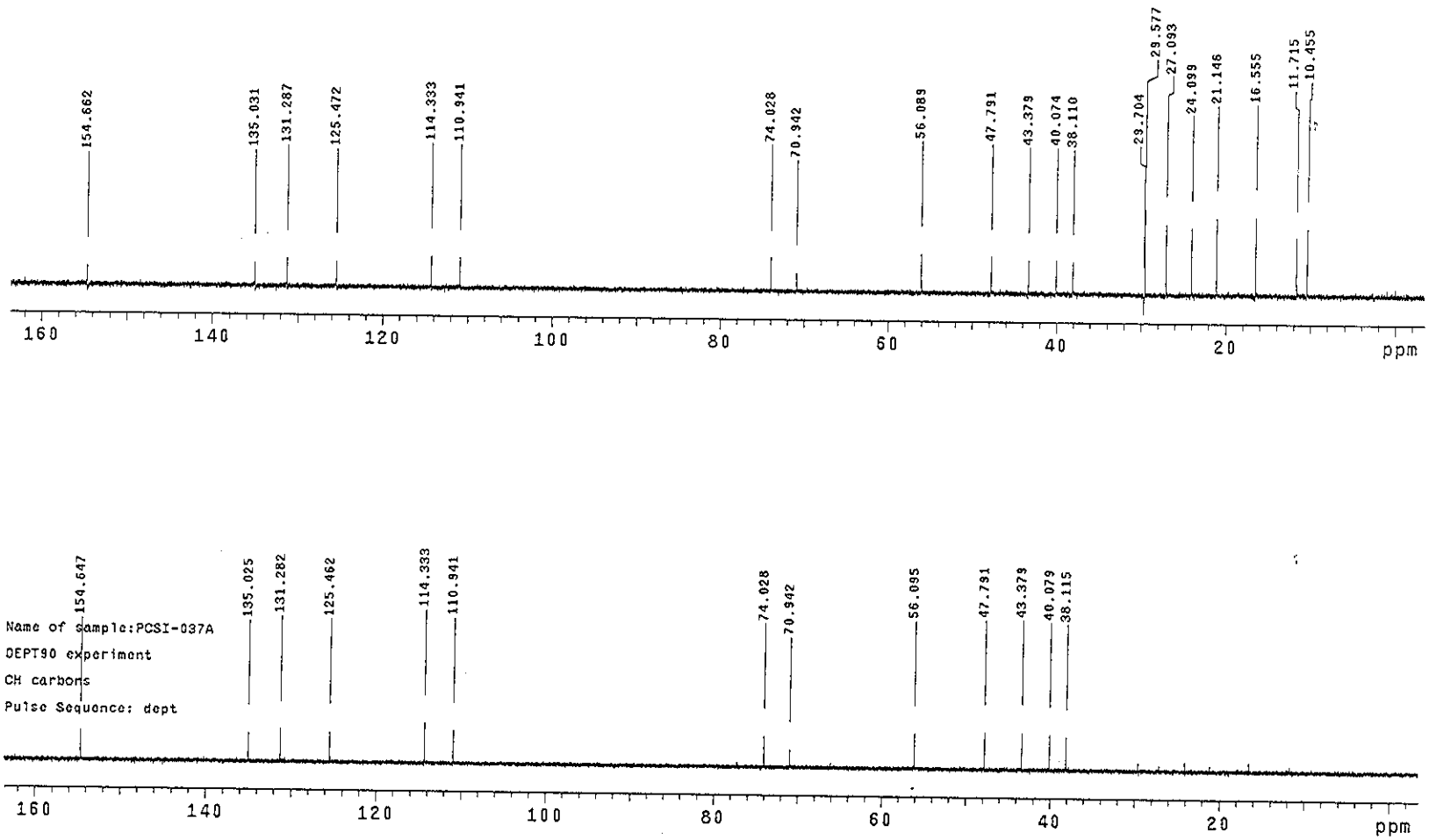


Figure 31 DEPT ($CDCl_3$) spectrum of compound **PSI4**

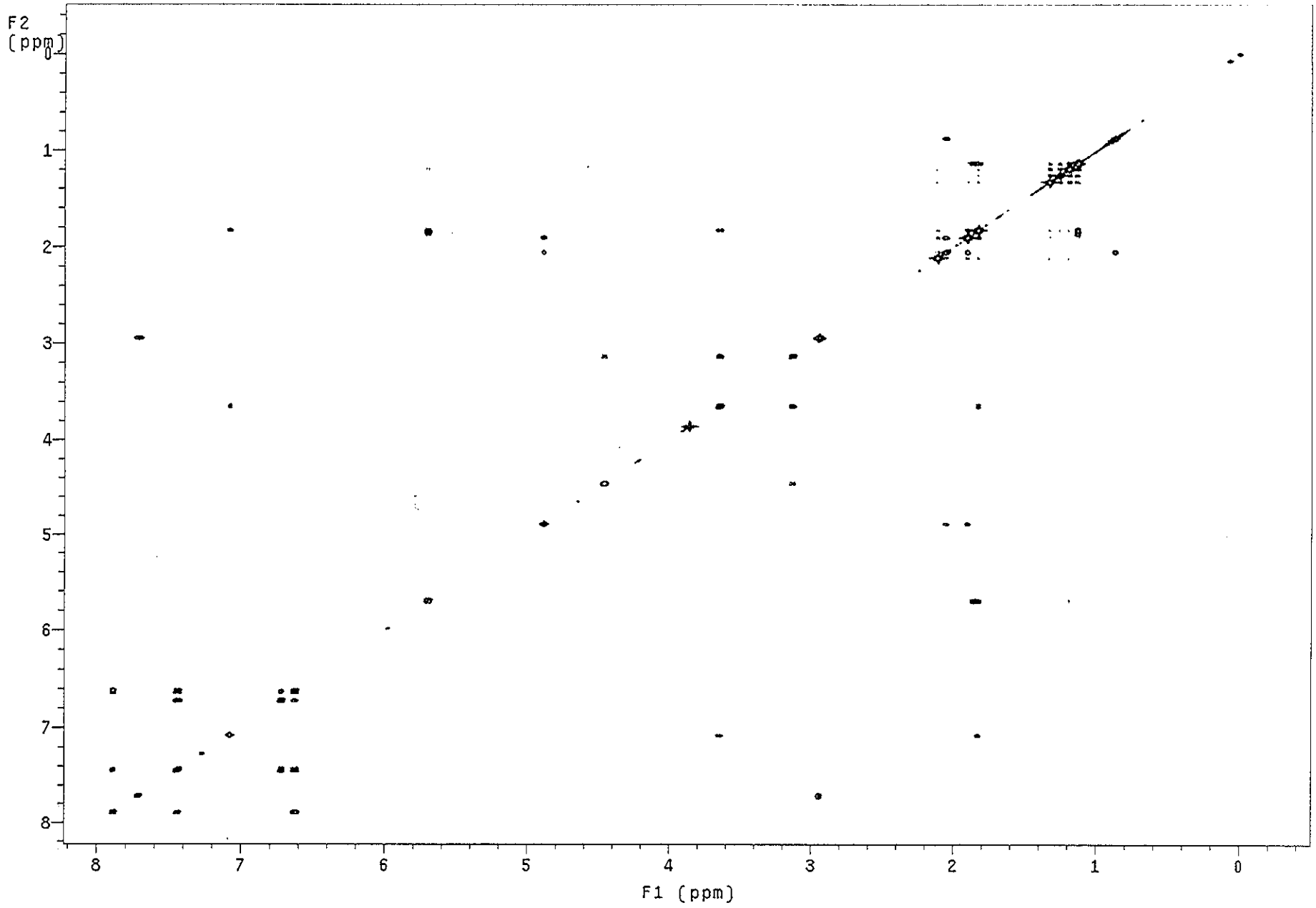


Figure 32 2D COSY spectrum of compound **PSI4**

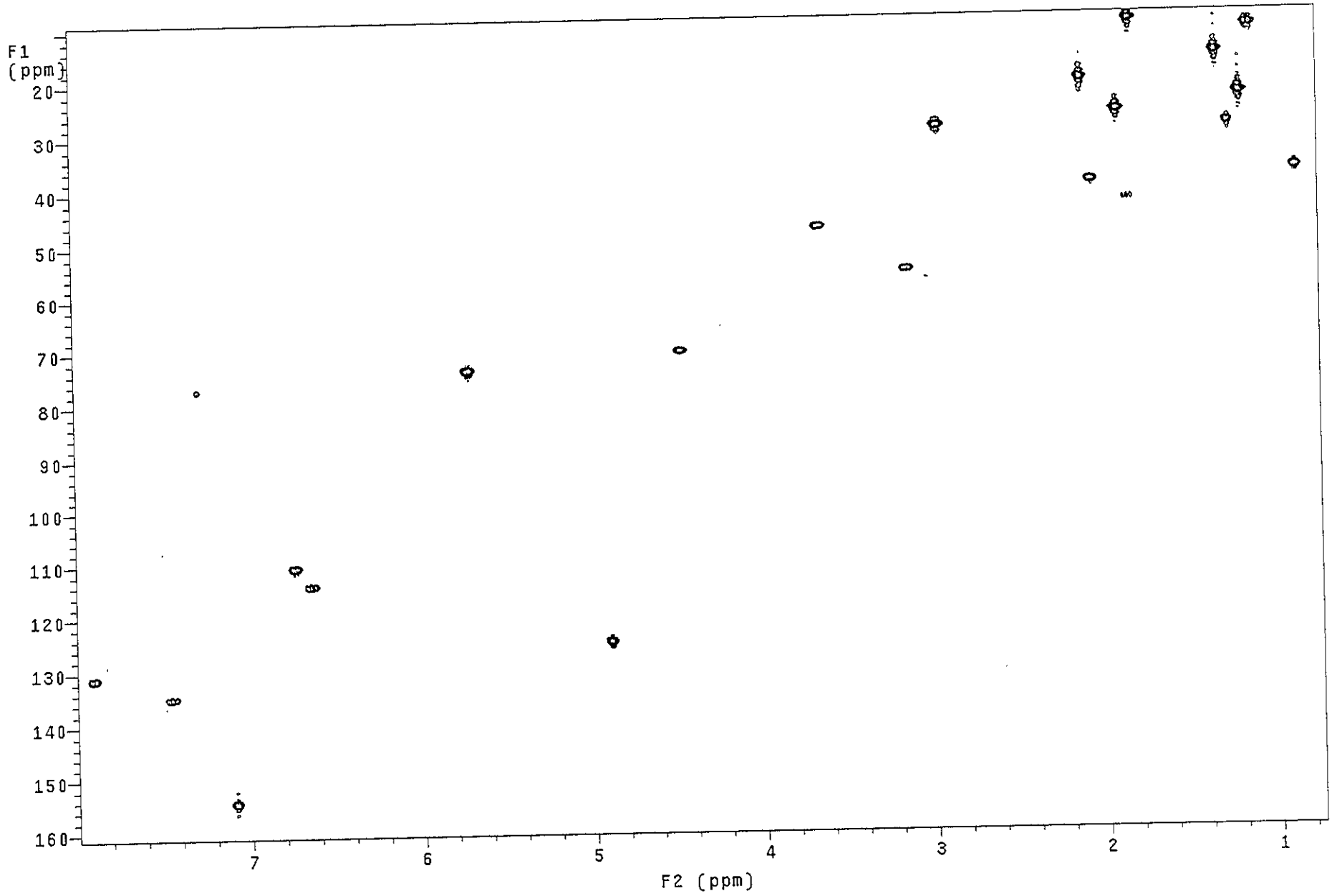


Figure 33 2D HMQC spectrum of compound **PSI4**

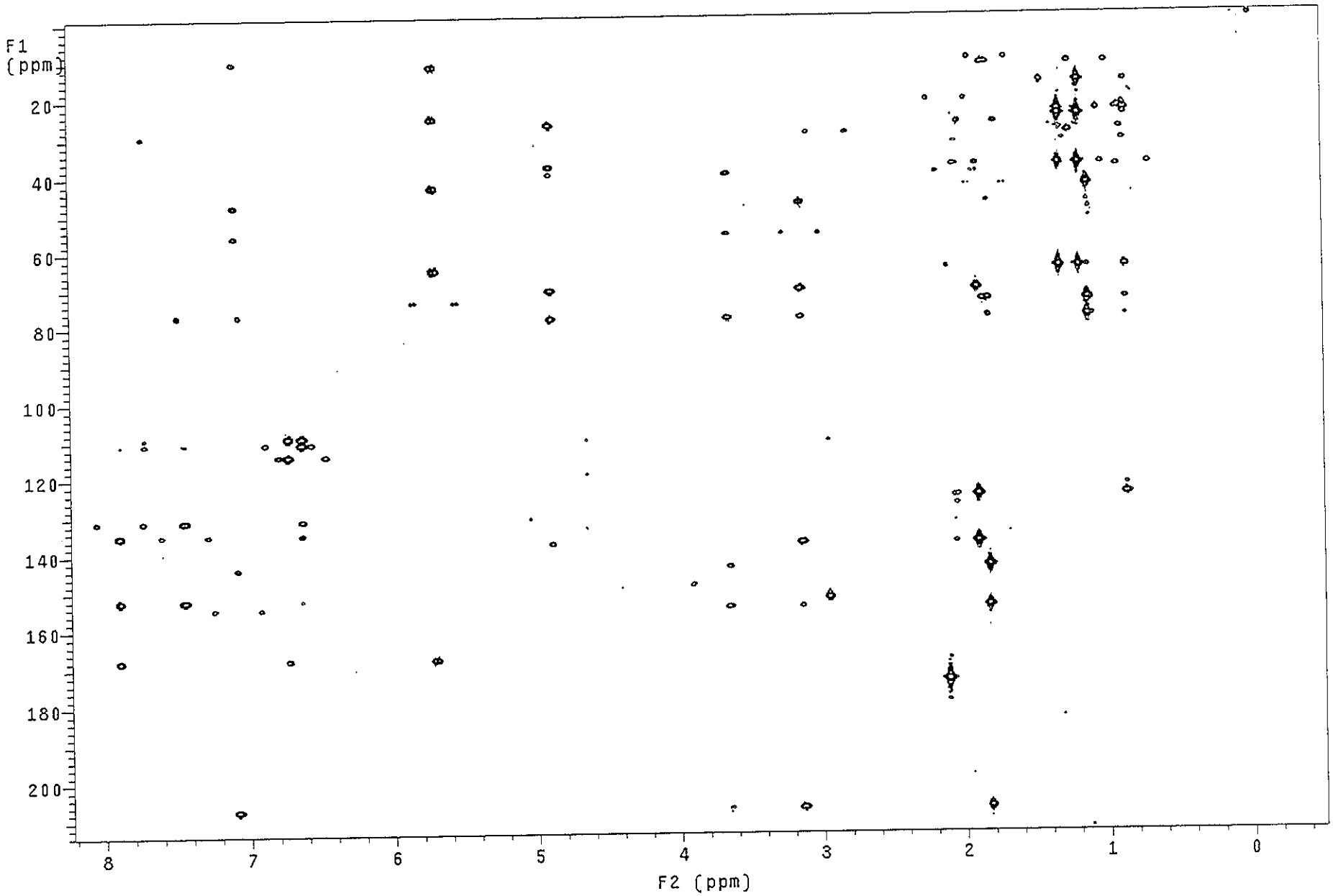


Figure 34 2D HMBC spectrum of compound **PSI4**

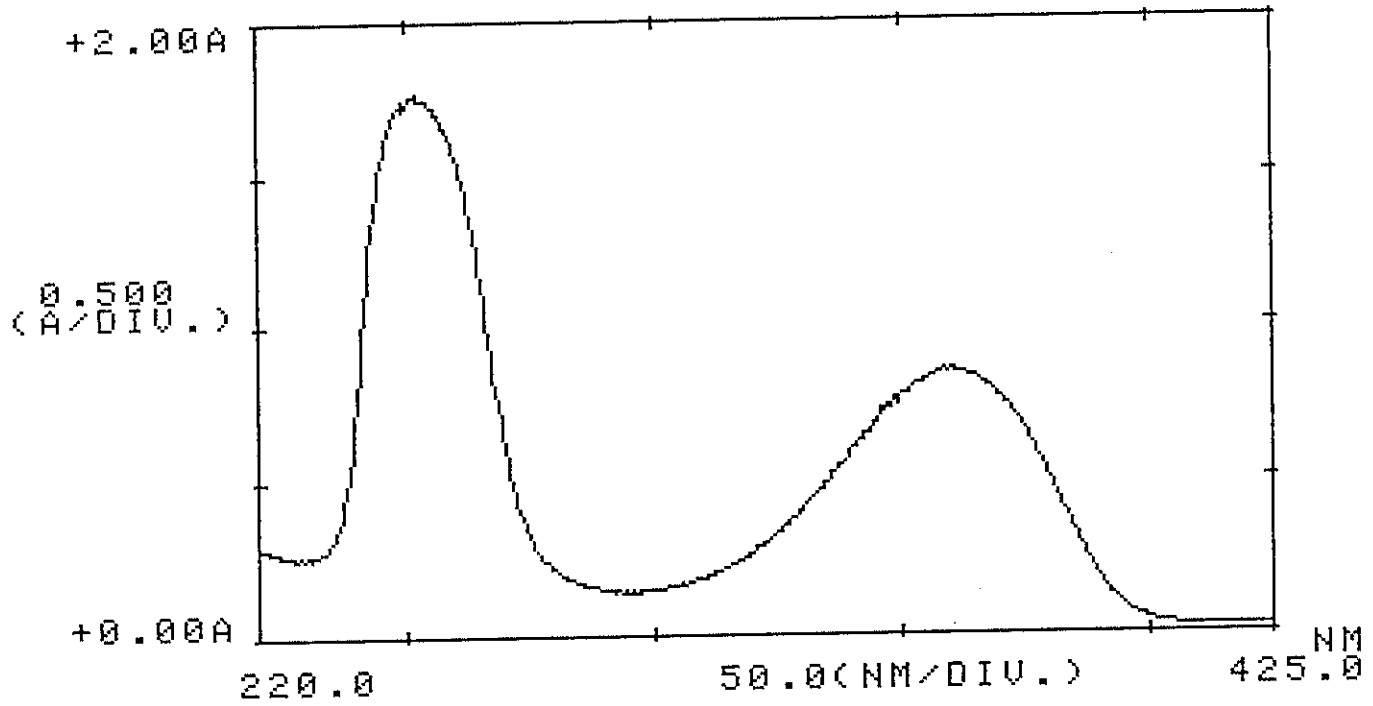


Figure 35 UV ($CHCl_3$) spectrum of compound **PSI5**

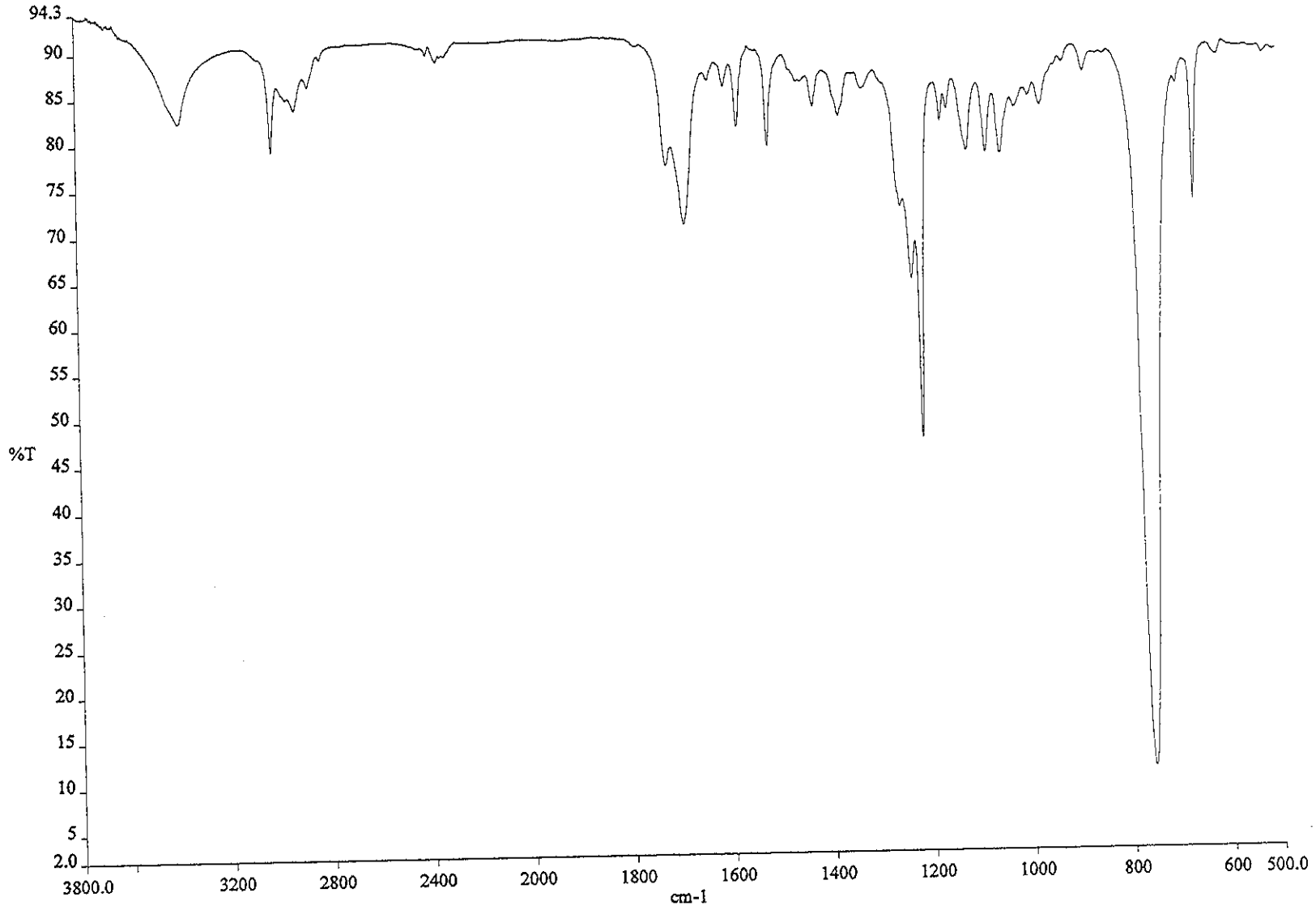


Figure 36 IR (Neat) spectrum of compound **PSI5**

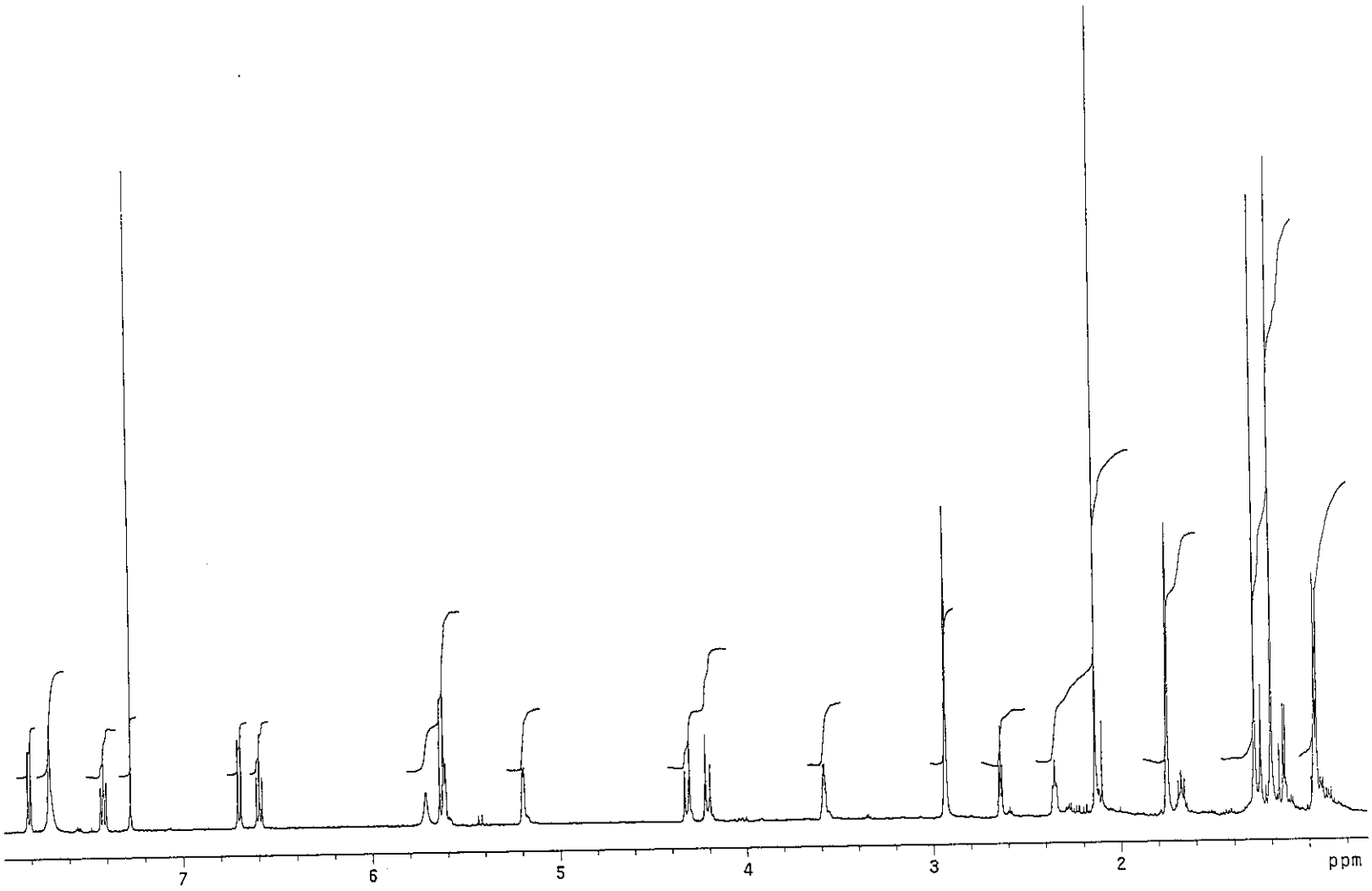


Figure 37 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **PSI5**

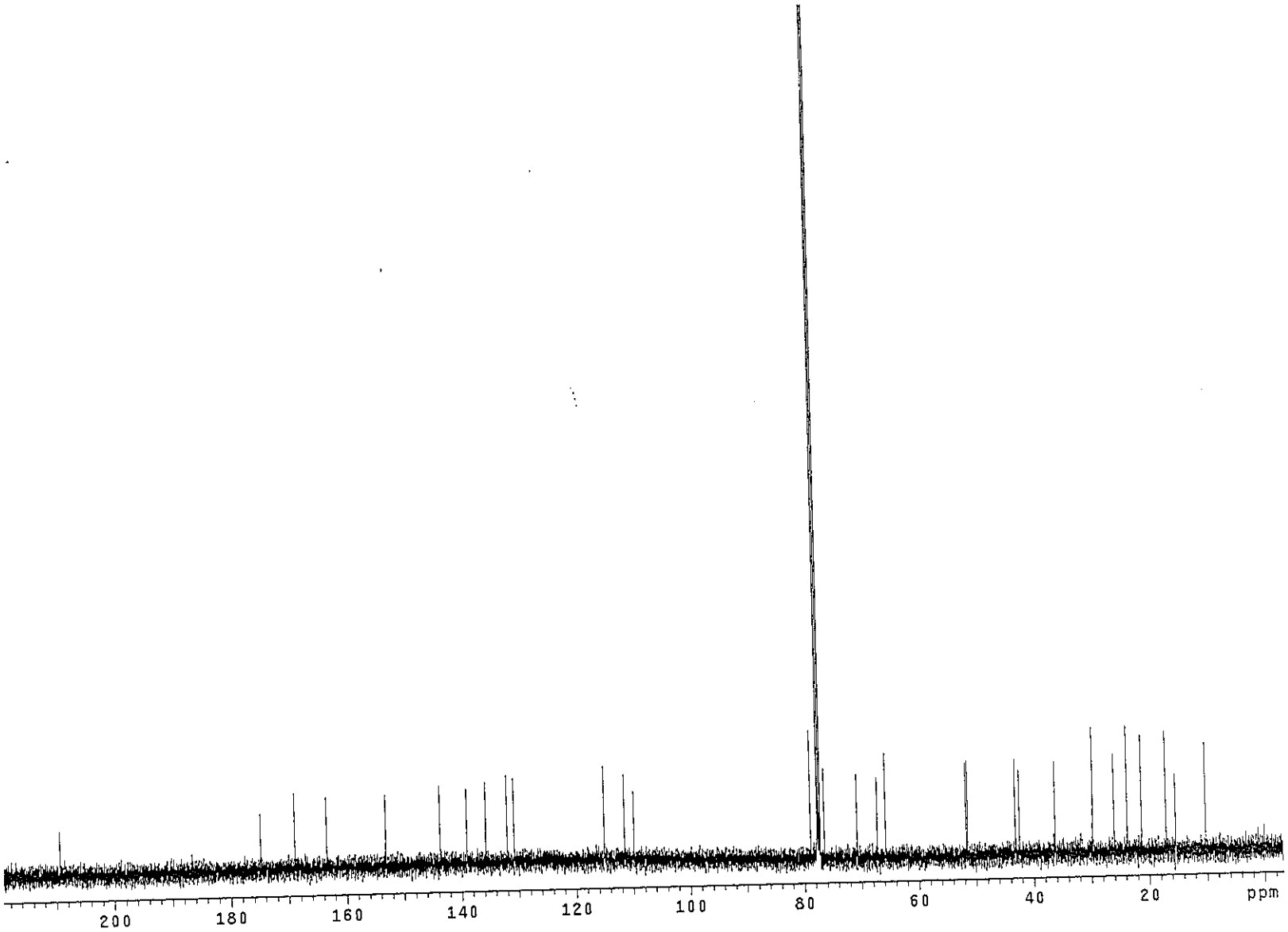


Figure 38 ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of compound **PSI5**

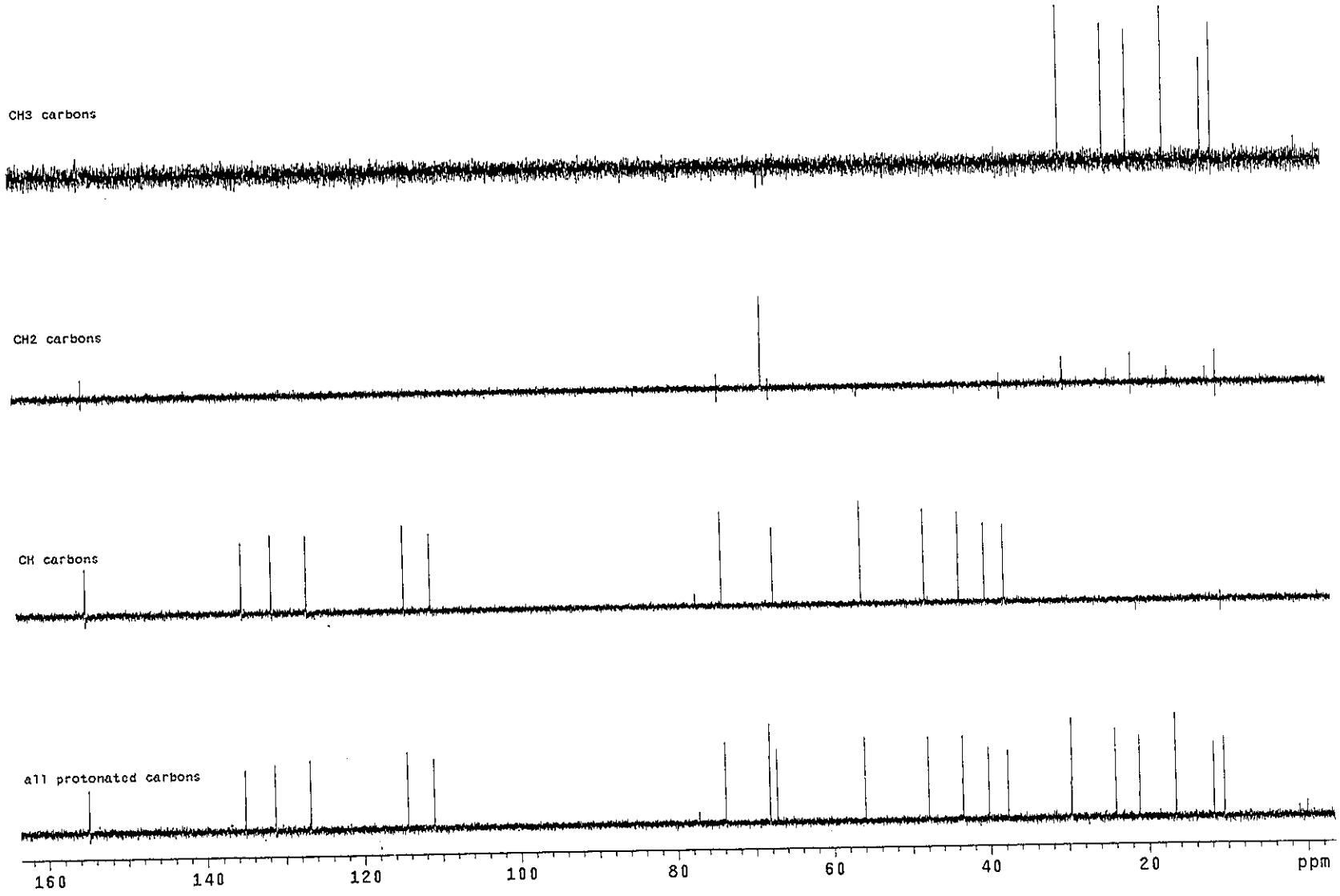


Figure 39 DEPT ($CDCl_3$) spectrum of compound **PSI5**

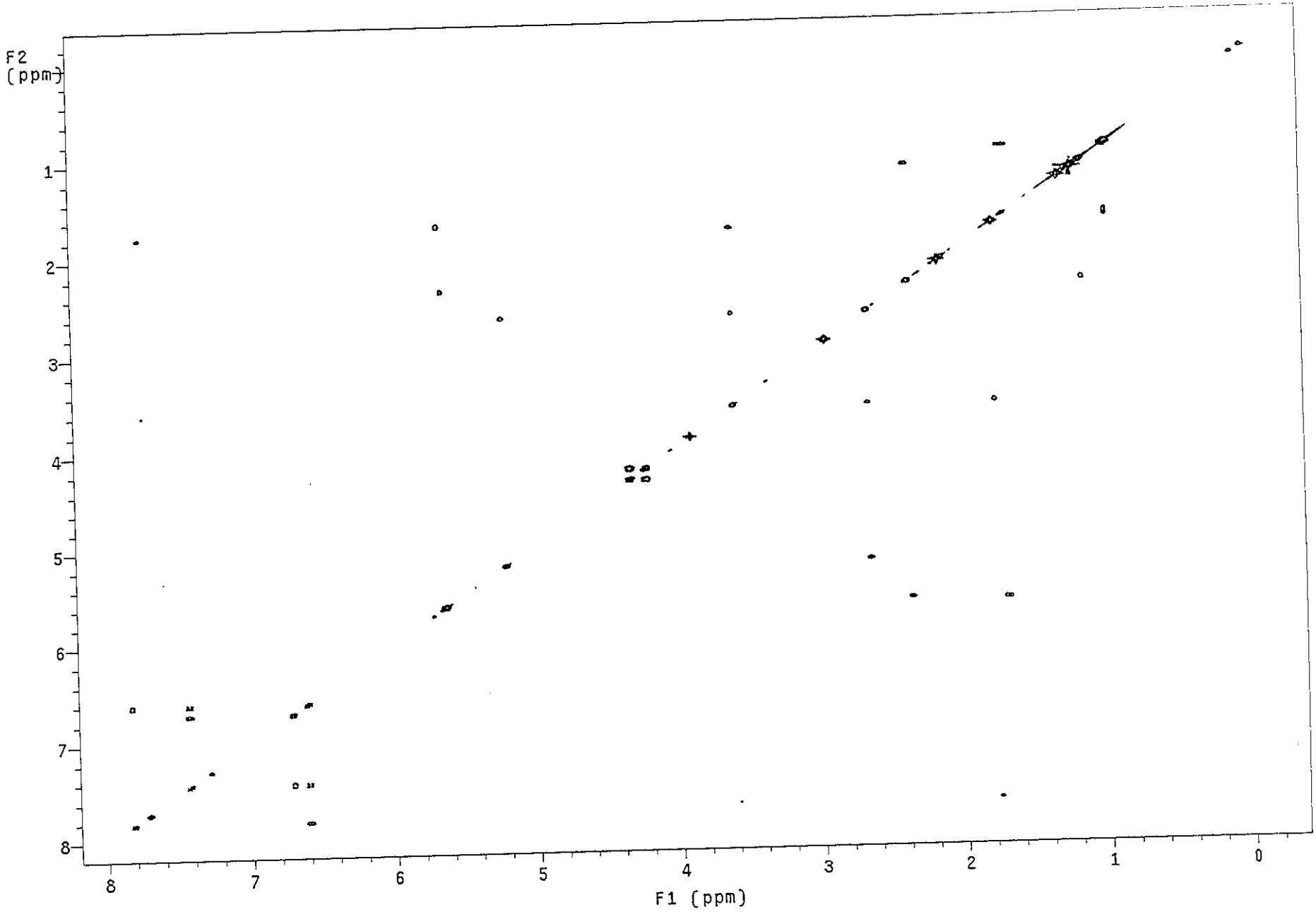


Figure 40 2D COSY spectrum of compound **PSI5**

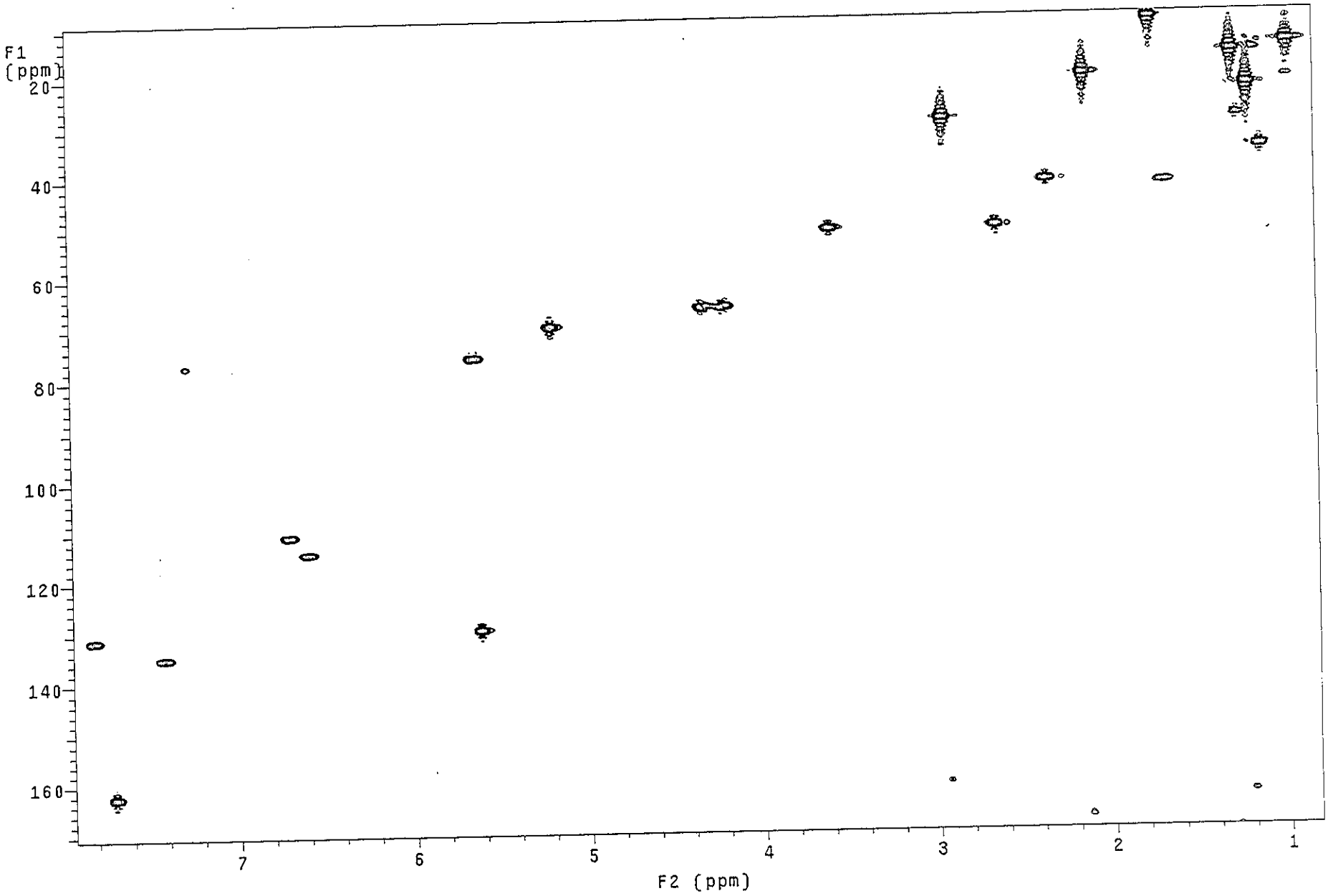


Figure 41 2D HMQC spectrum of compound **PSI5**

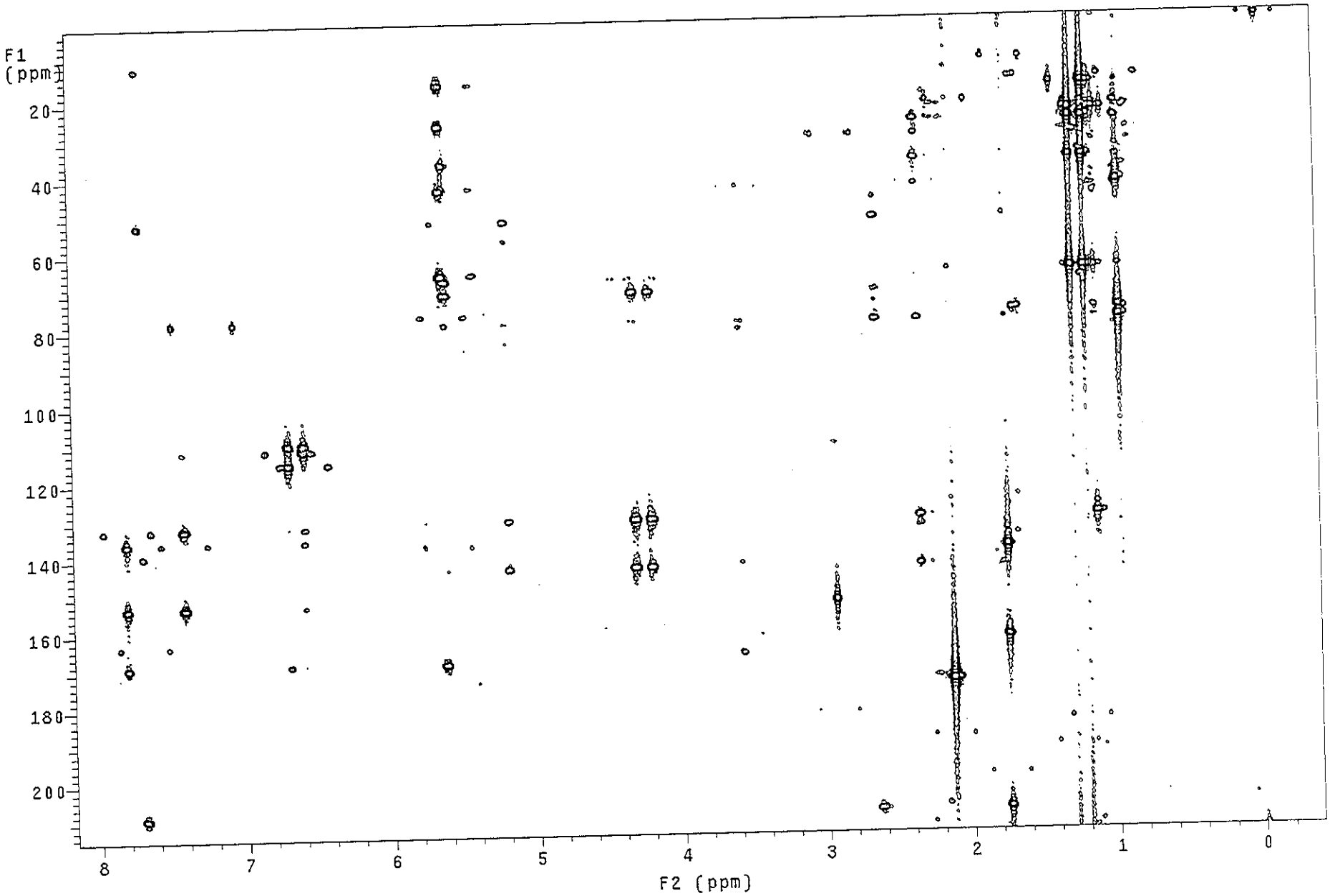


Figure 42 2D HMBC spectrum of compound **PSI5**

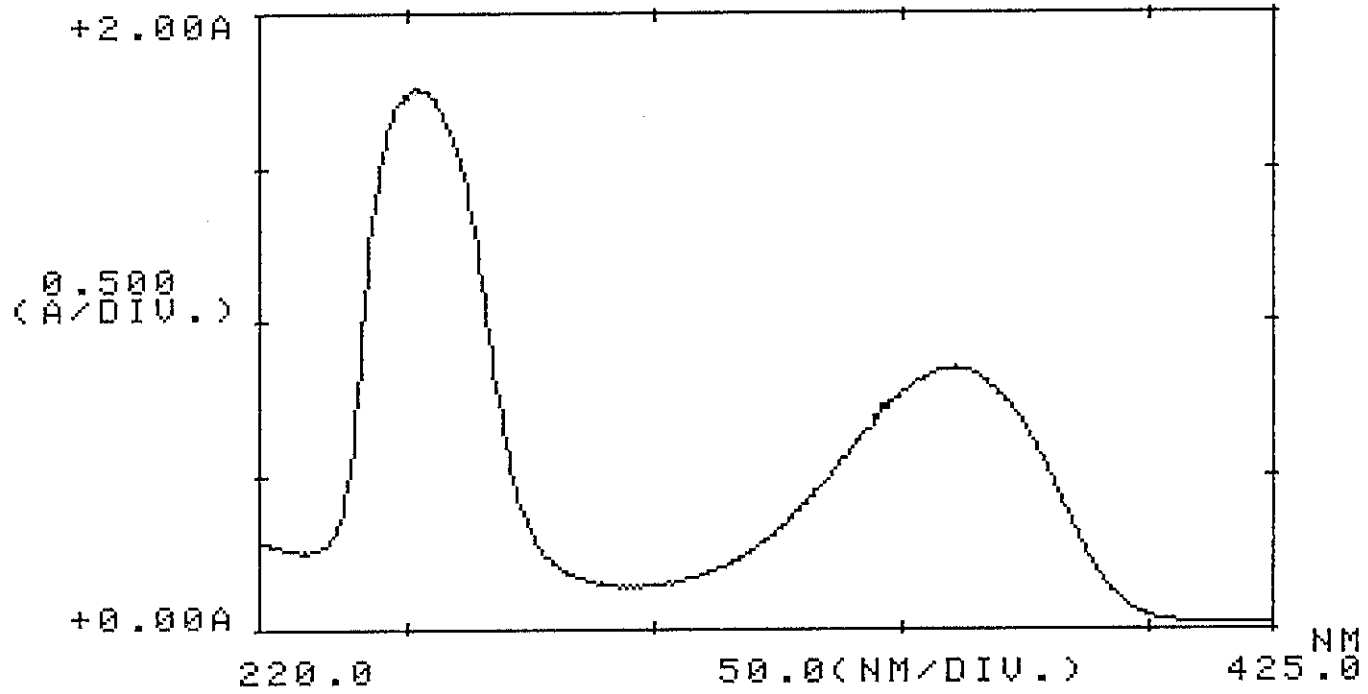


Figure 43 UV ($CHCl_3$) spectrum of compound **PSI6**

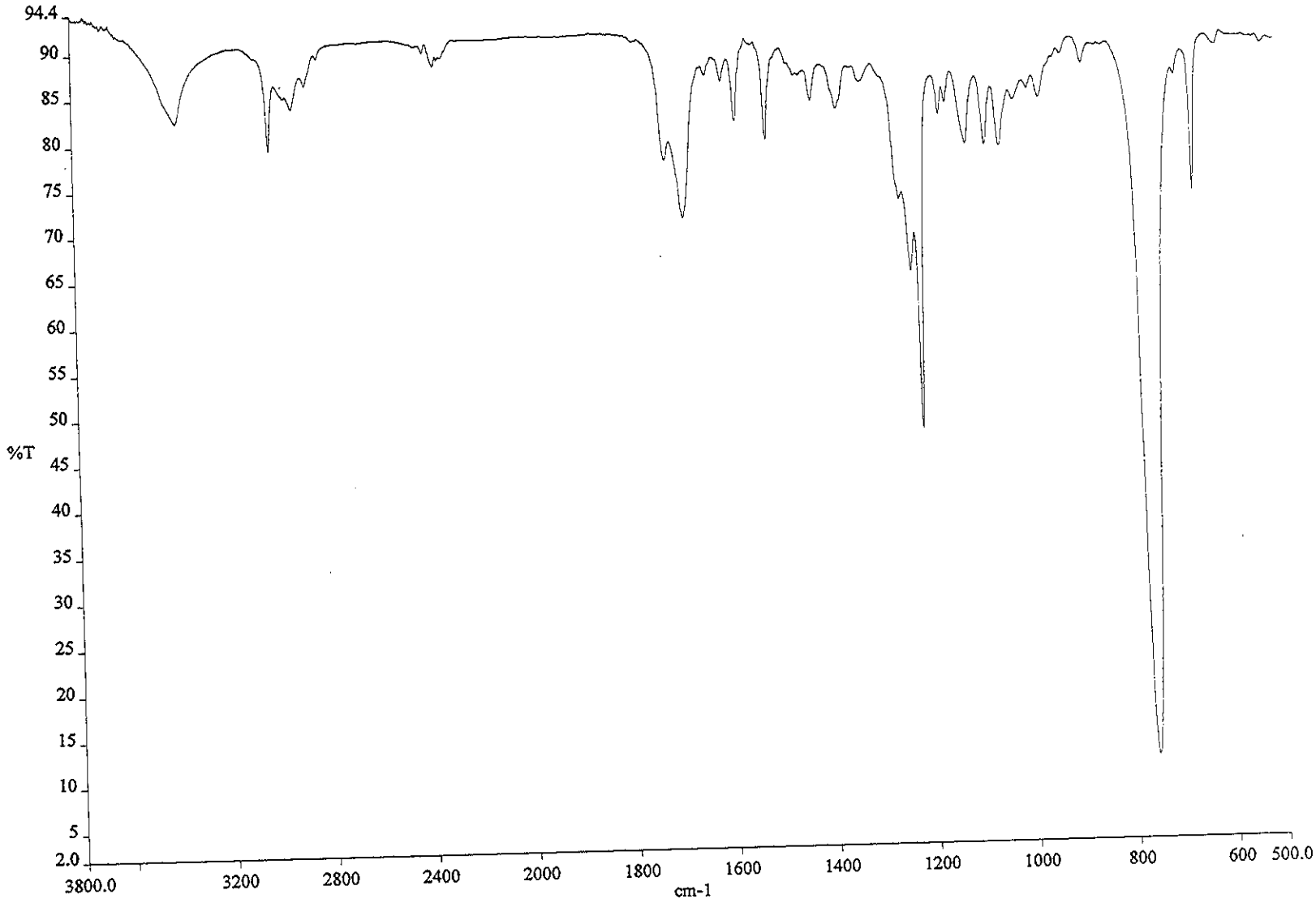


Figure 44 IR (Neat) spectrum of compound **PSI6**

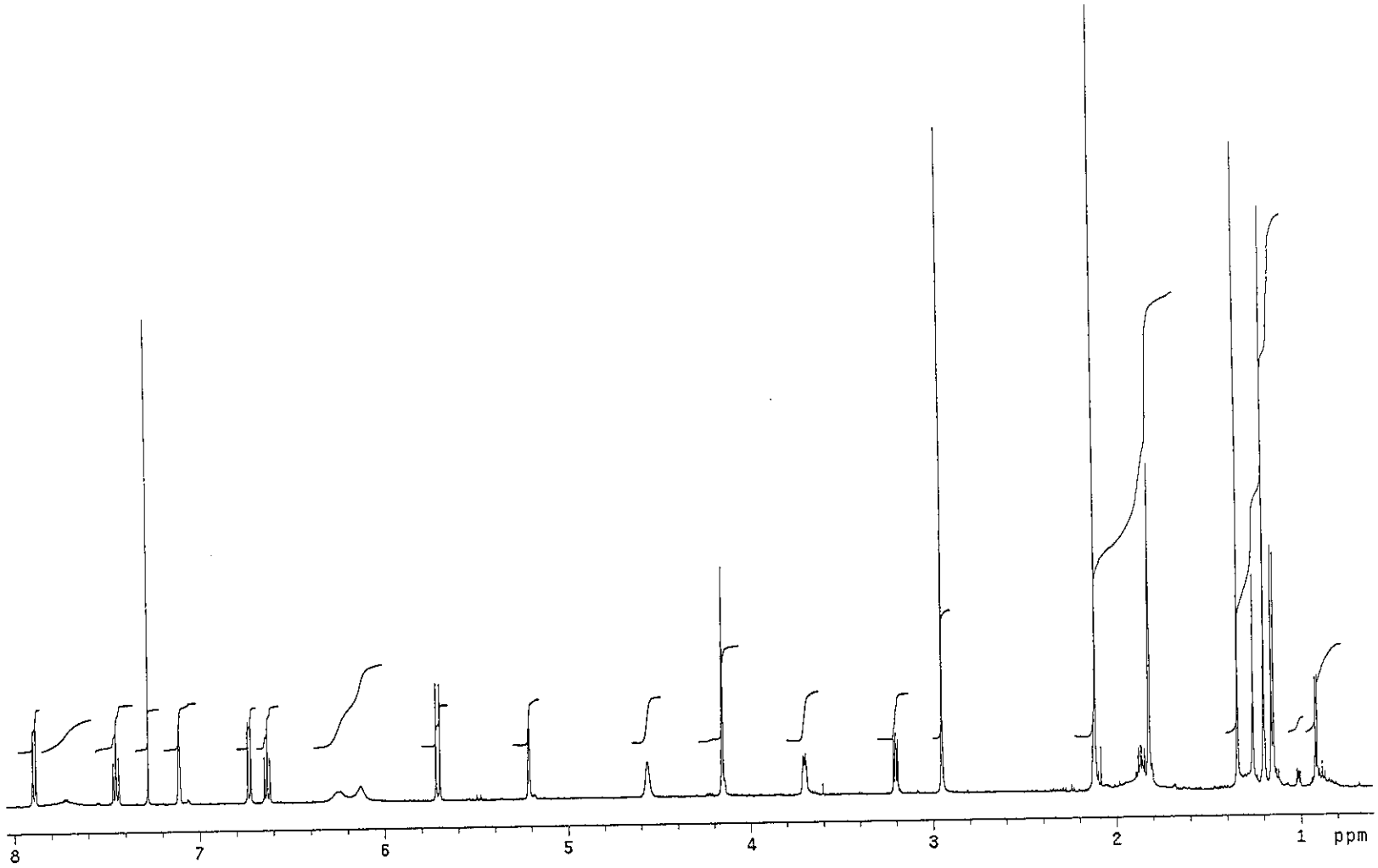


Figure 45 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **PSI6**

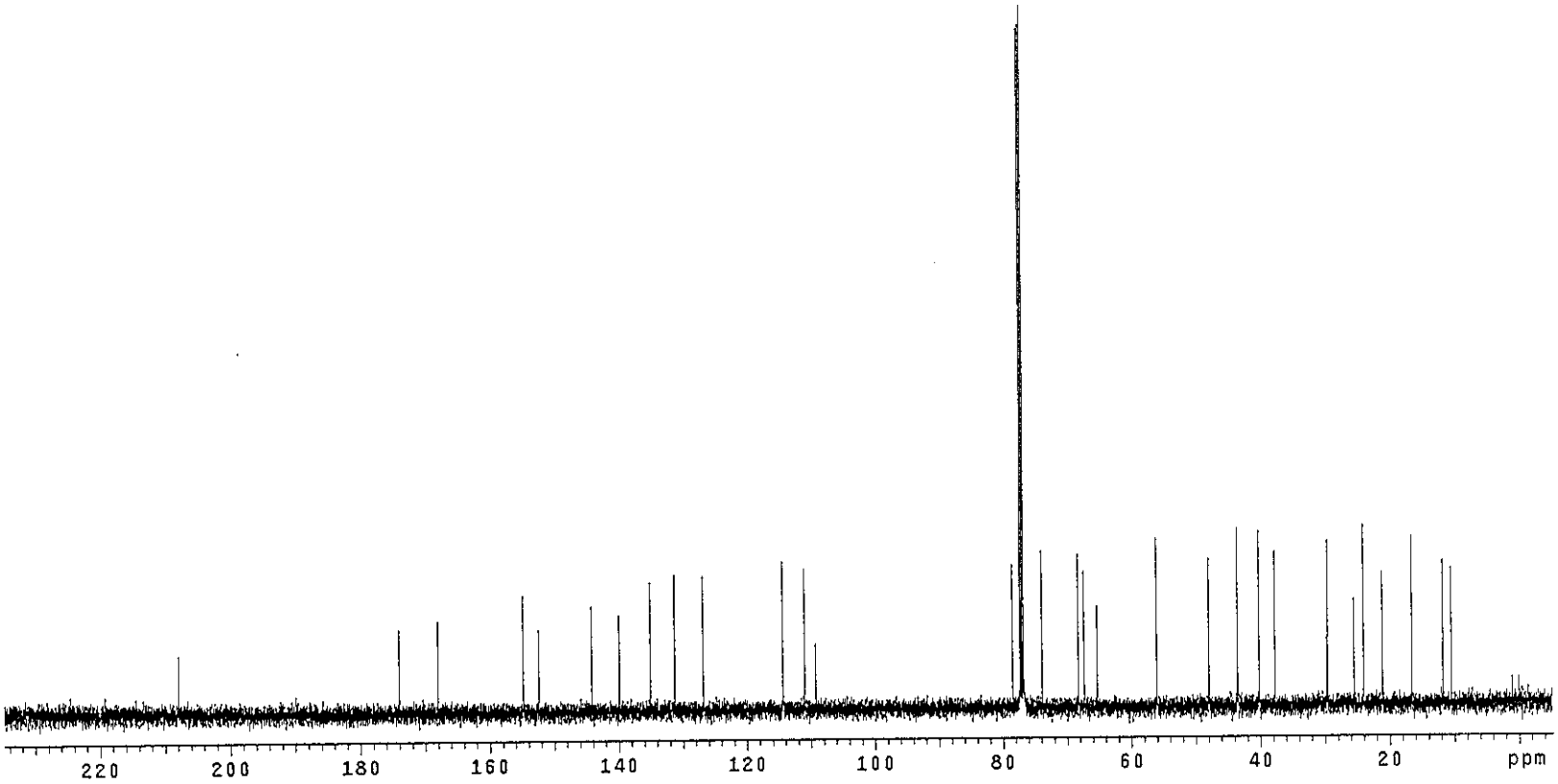


Figure 46 ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of compound **PSI6**

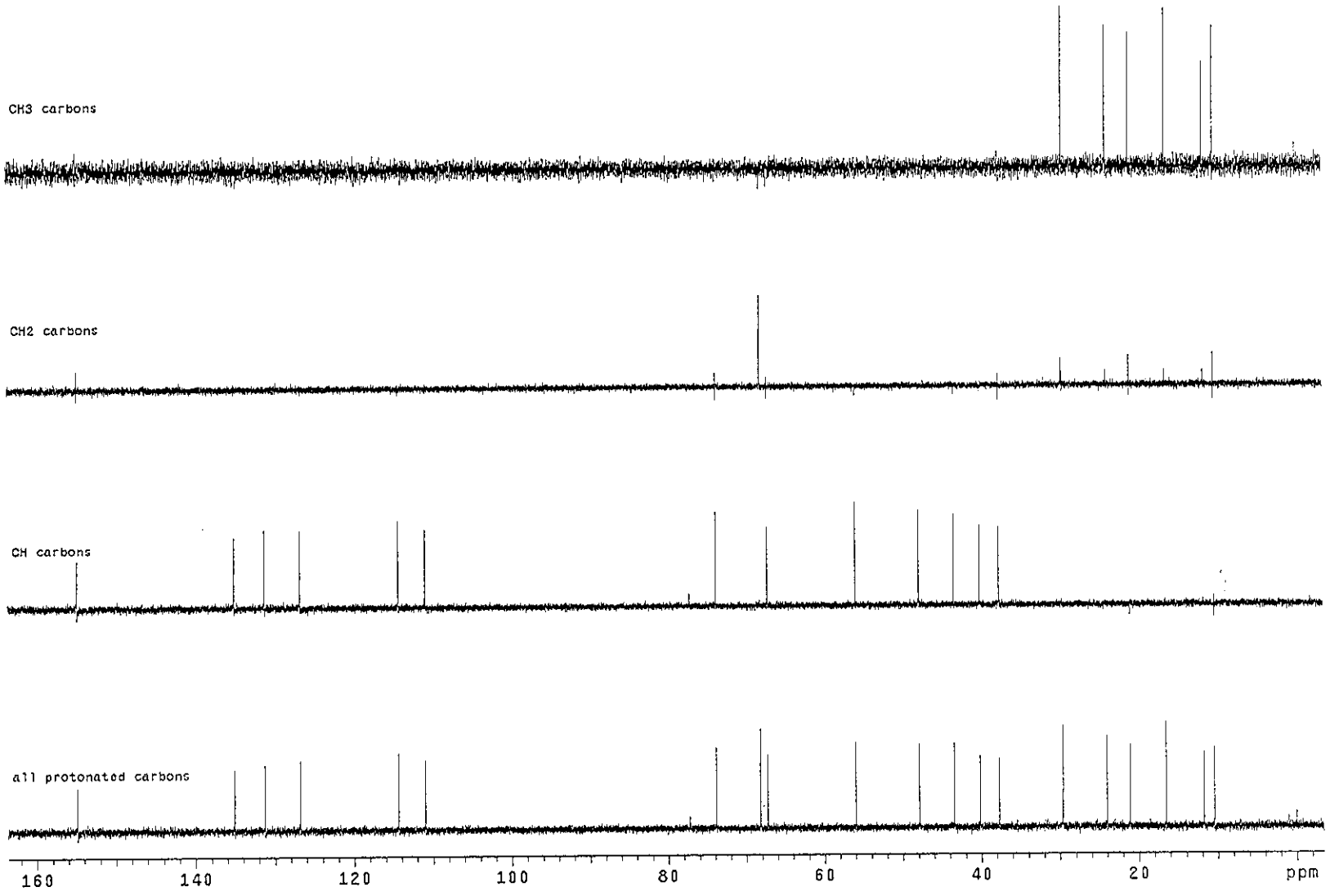


Figure 47 DEPT ($CDCl_3$) spectrum of compound **PSI6**

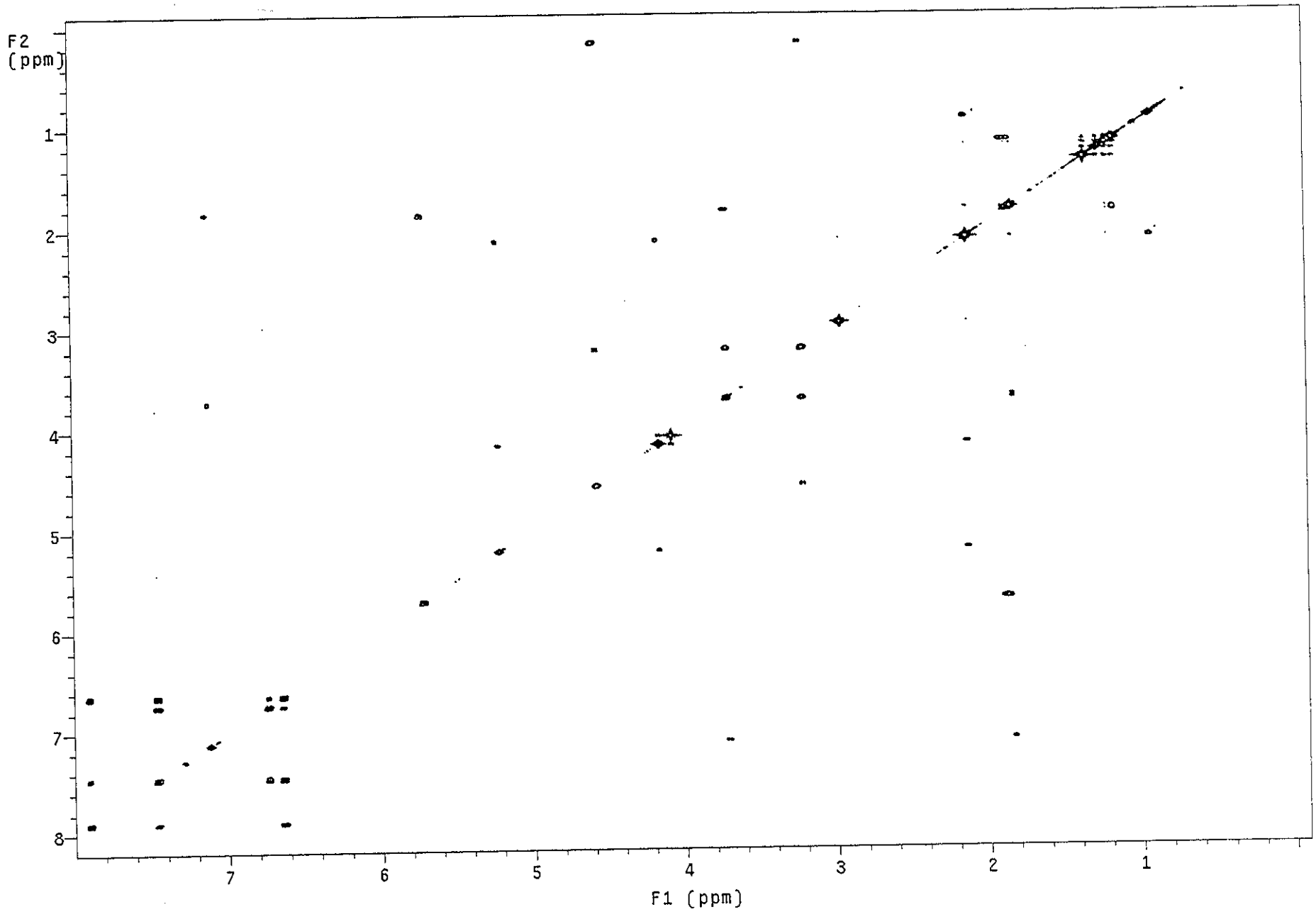


Figure 48 2D COSY spectrum of compound **PSI6**

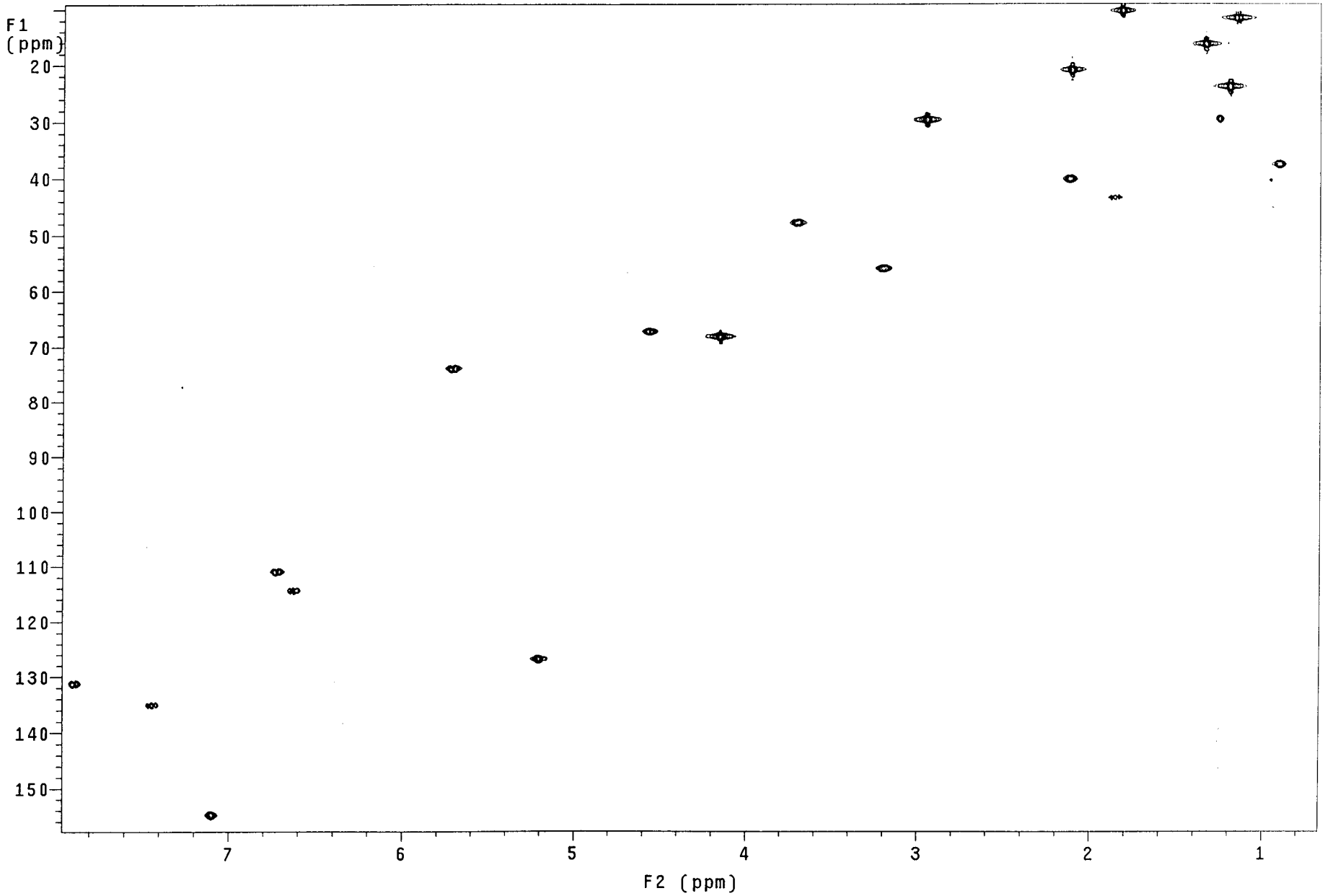


Figure 49 2D HMQC spectrum of compound **PSI6**

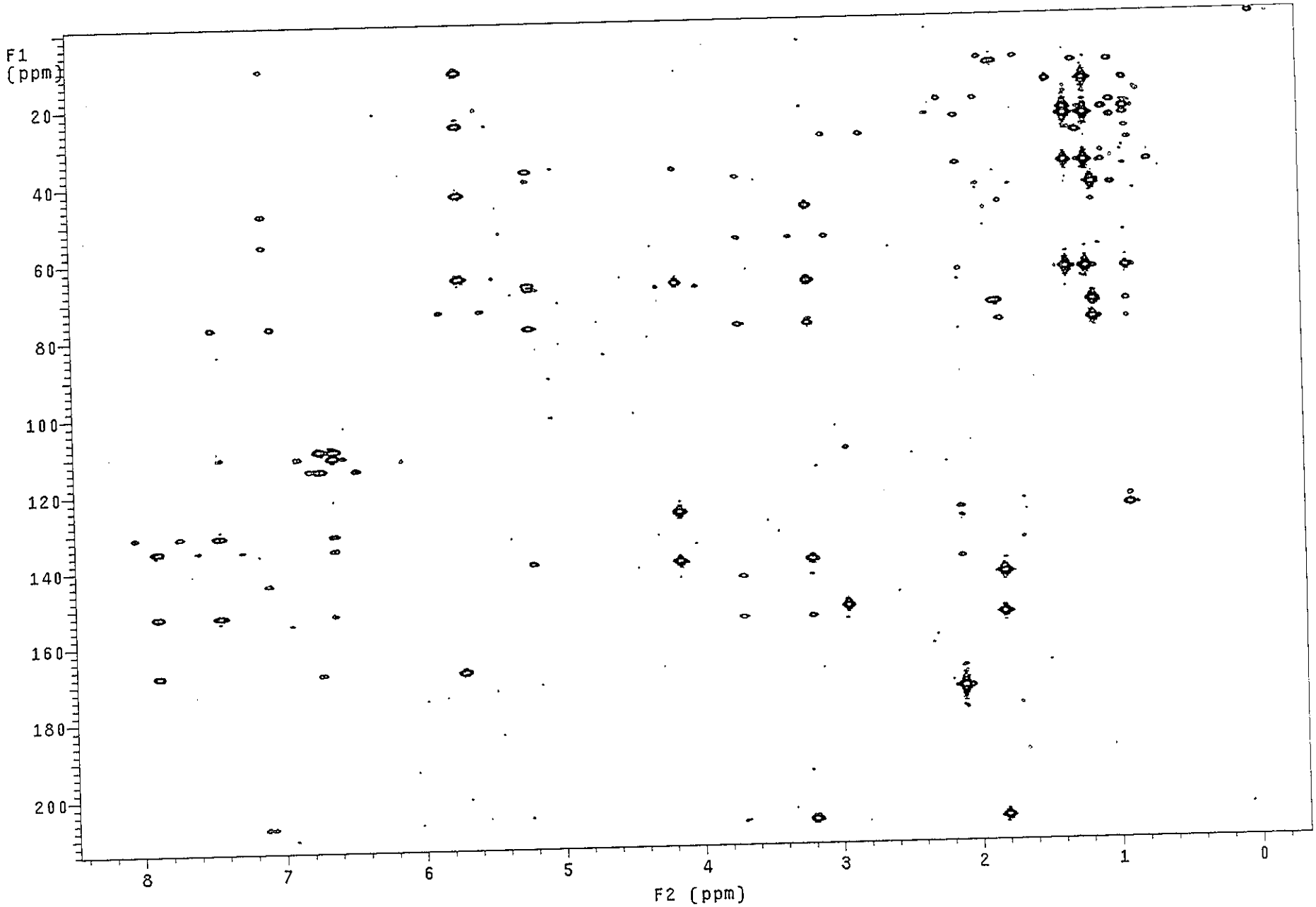


Figure 50 2D HMBC spectrum of compound **PSI6**

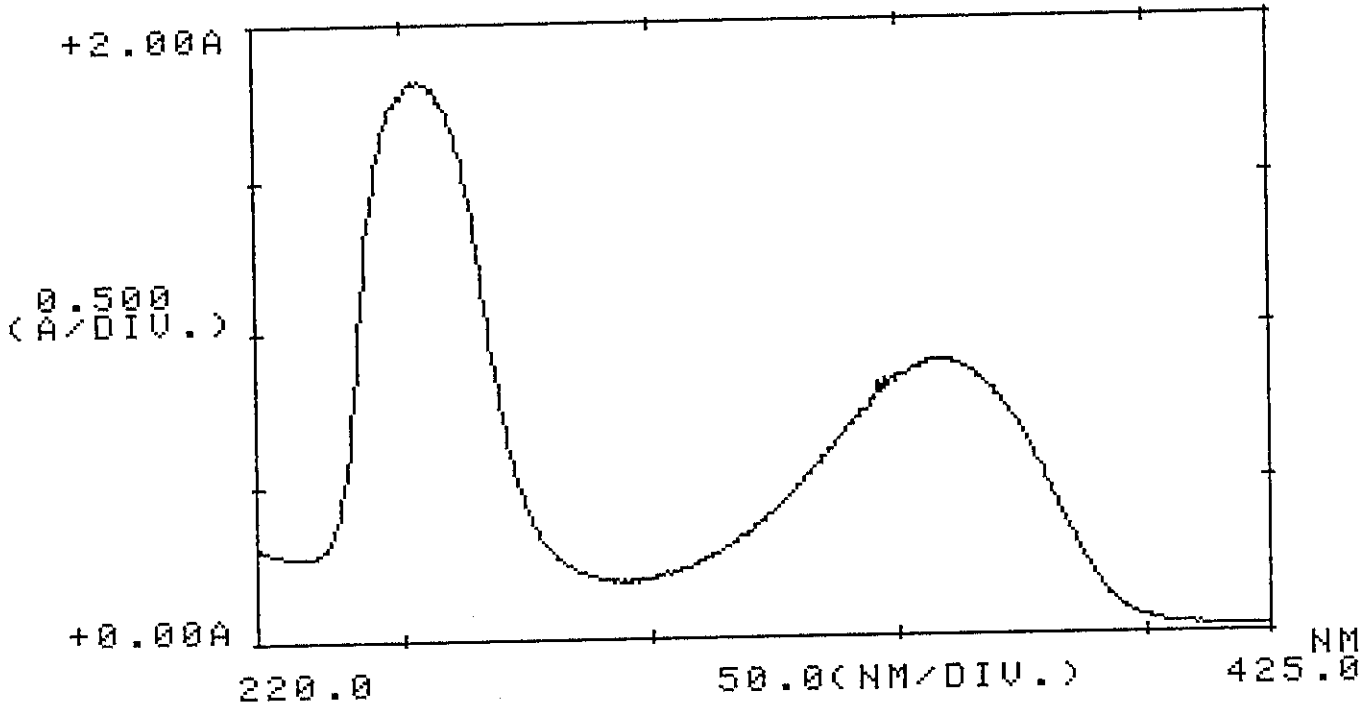


Figure 51 UV ($CHCl_3$) spectrum of compound **PSI7**

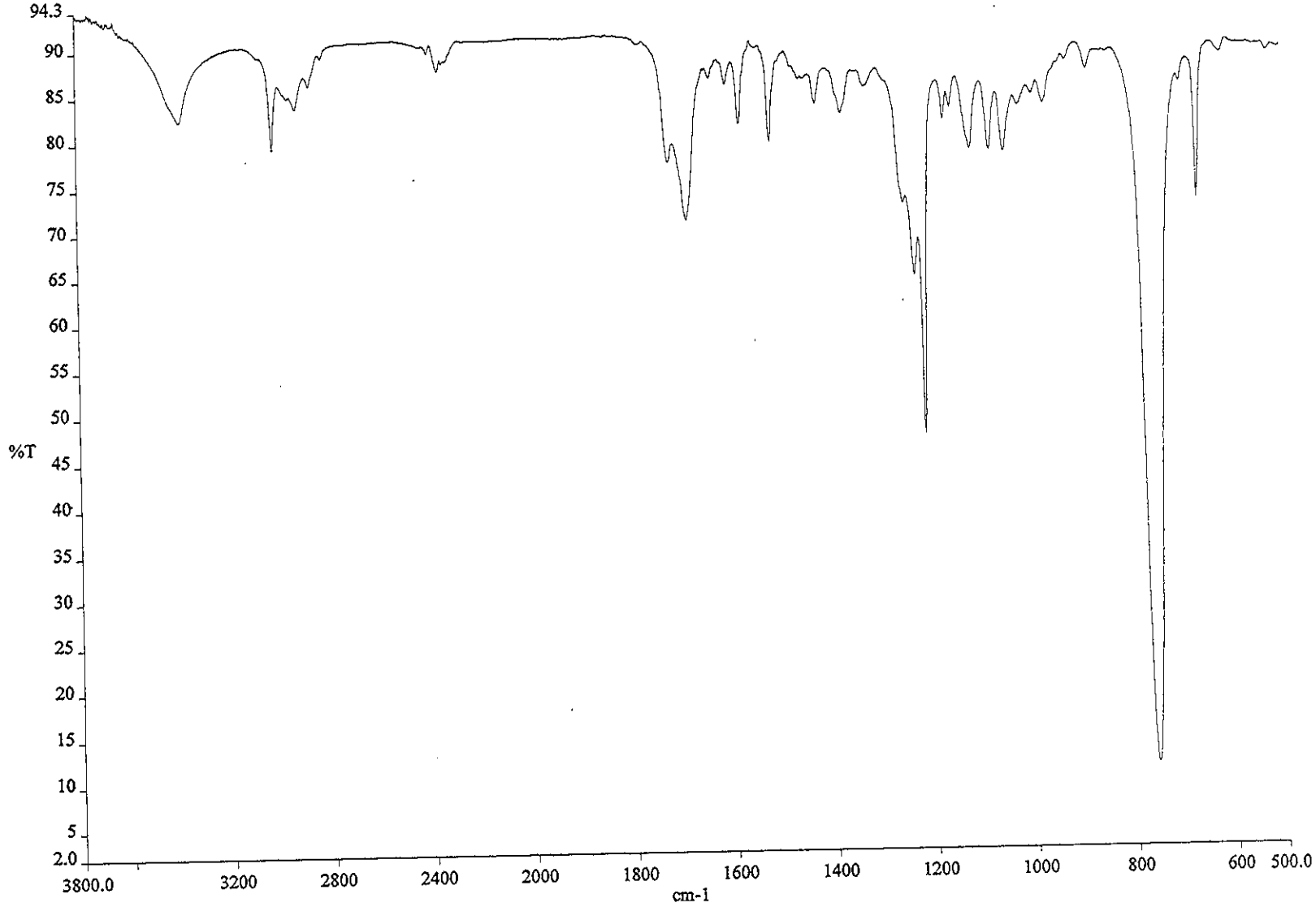


Figure 52 IR (Neat) spectrum of compound **PSI7**

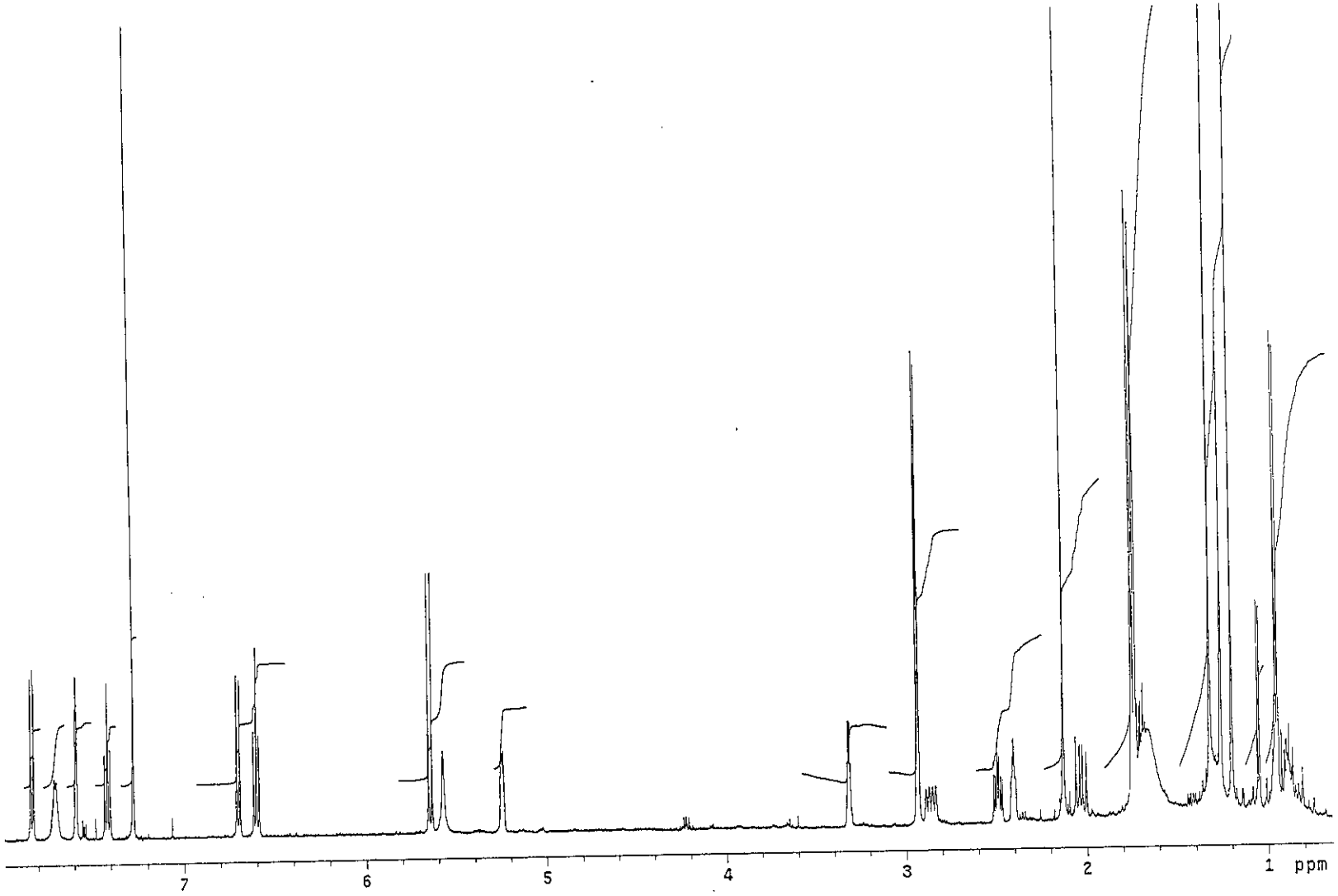


Figure 53 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **PSI7**

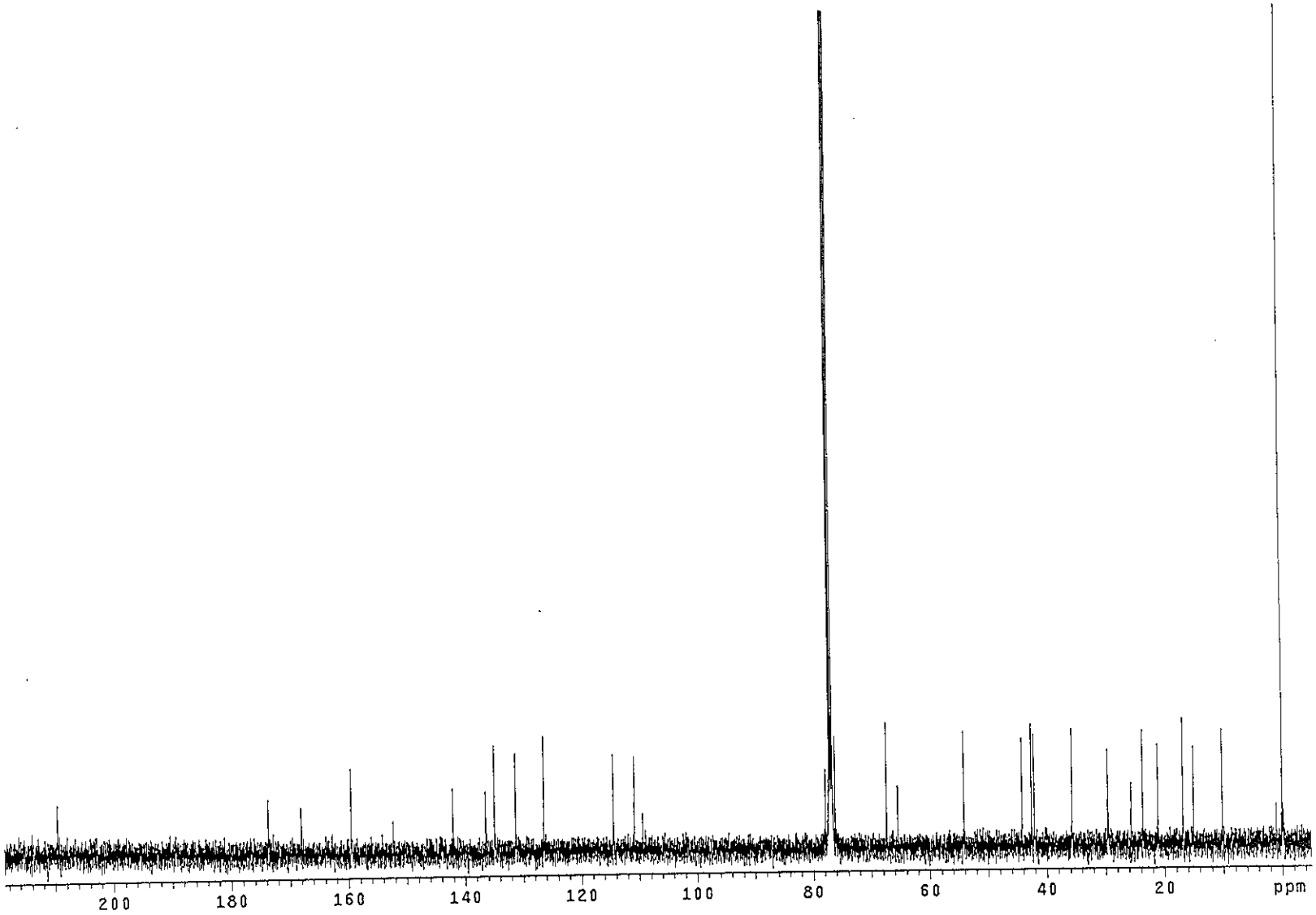


Figure 54 ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of compound **PSI7**

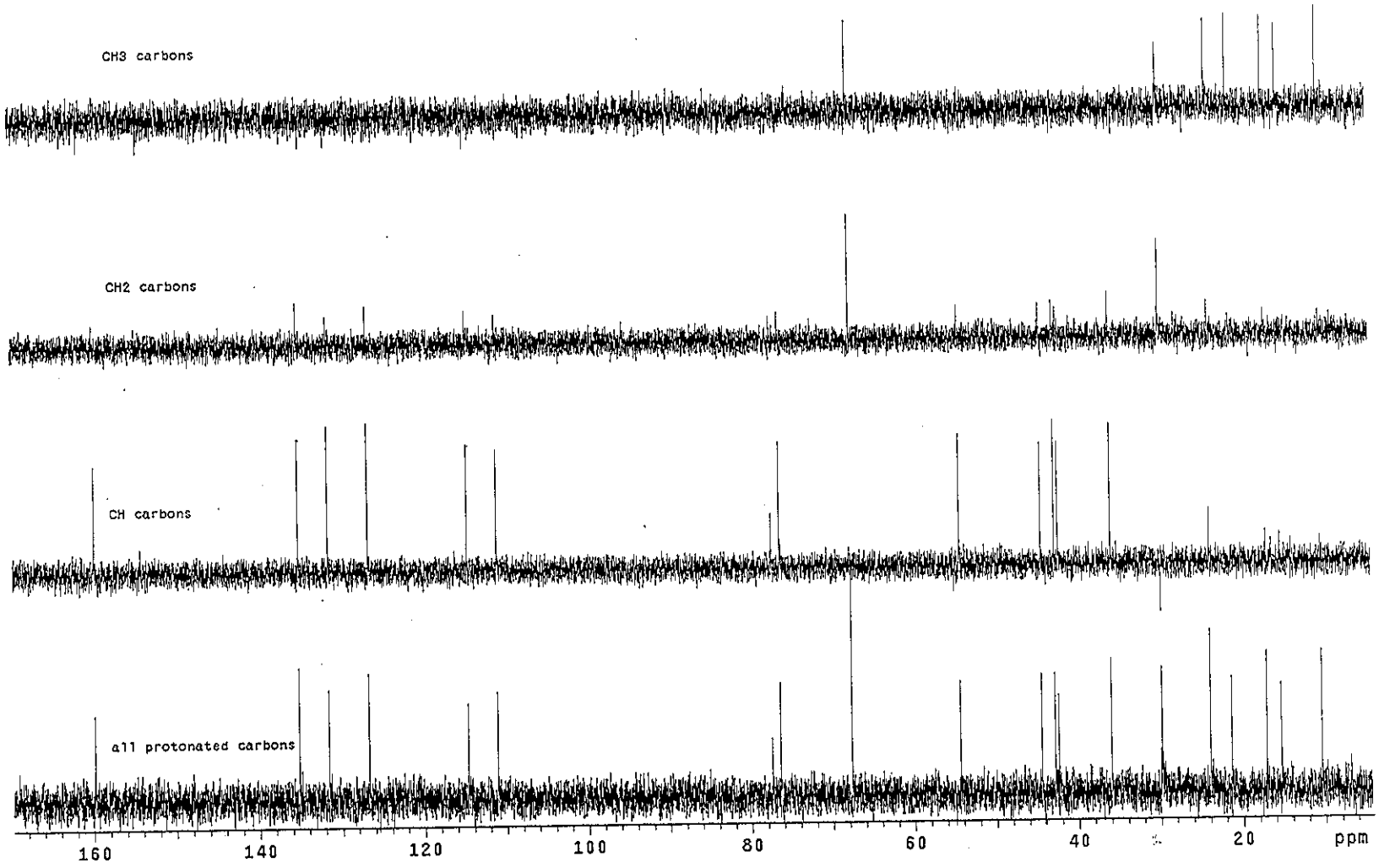


Figure 55 DEPT ($CDCl_3$) spectrum of compound **PSI7**

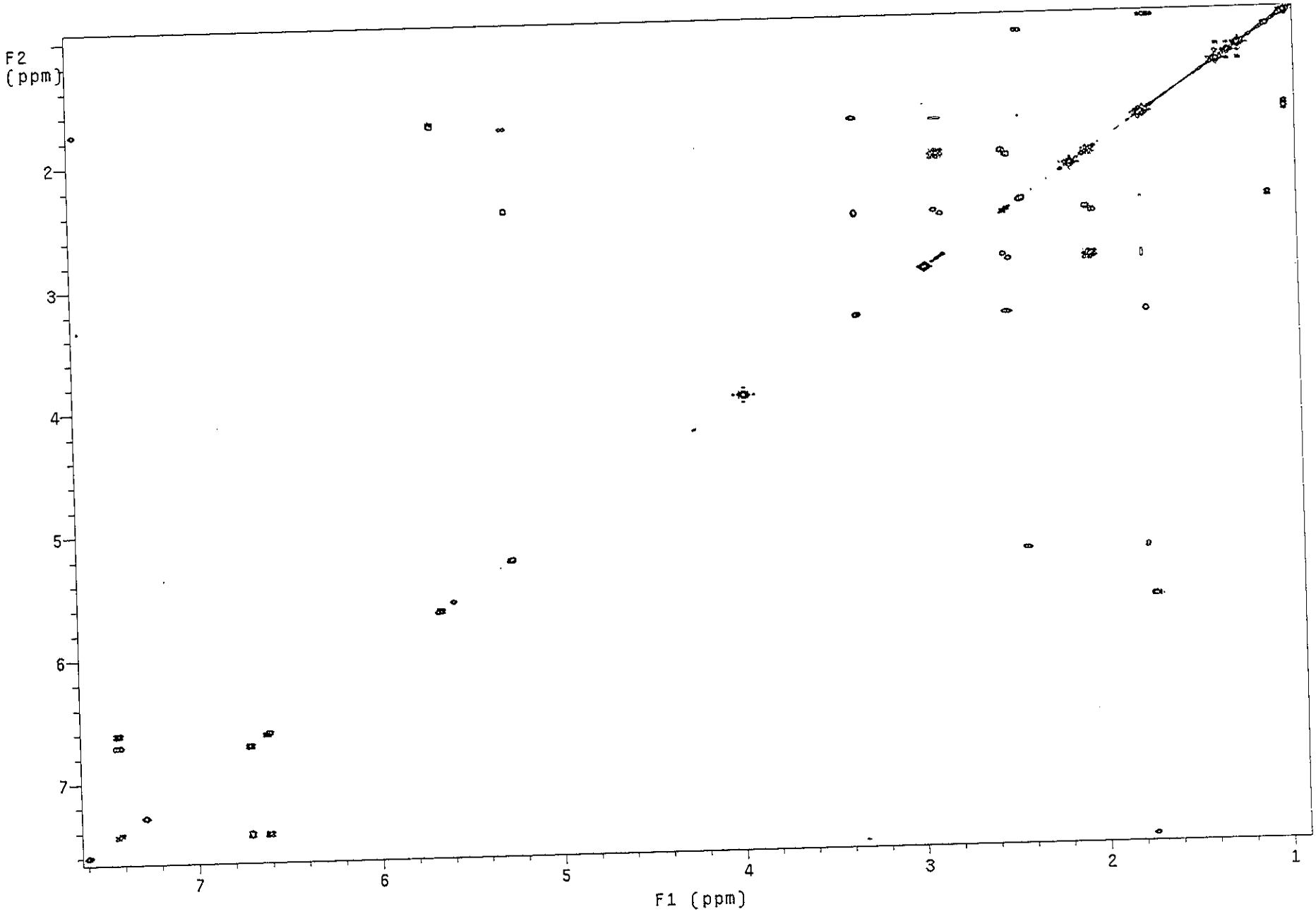


Figure 56 2D COSY spectrum of compound **PSI7**

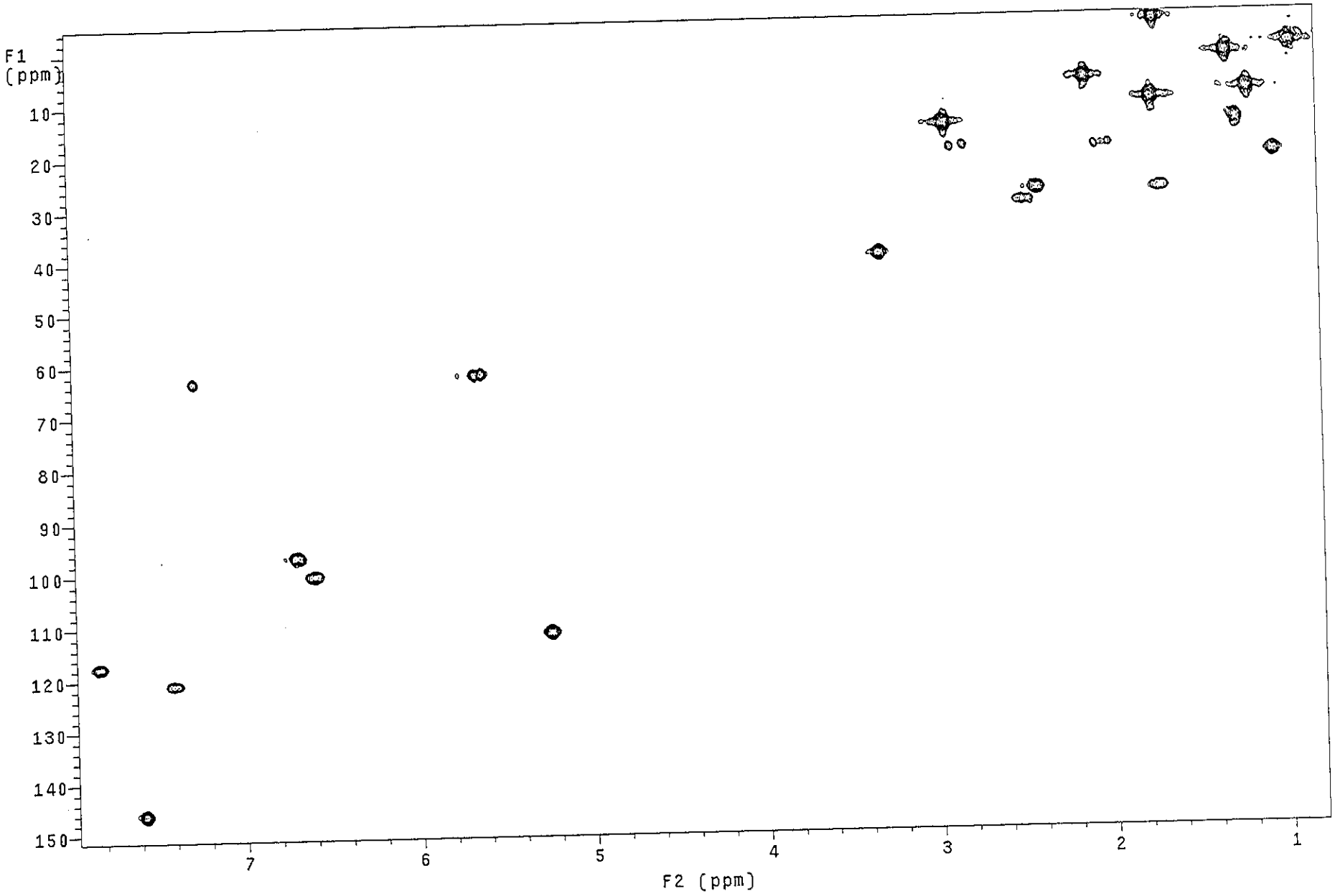


Figure 57 2D HMQC spectrum of compound **PSI7**

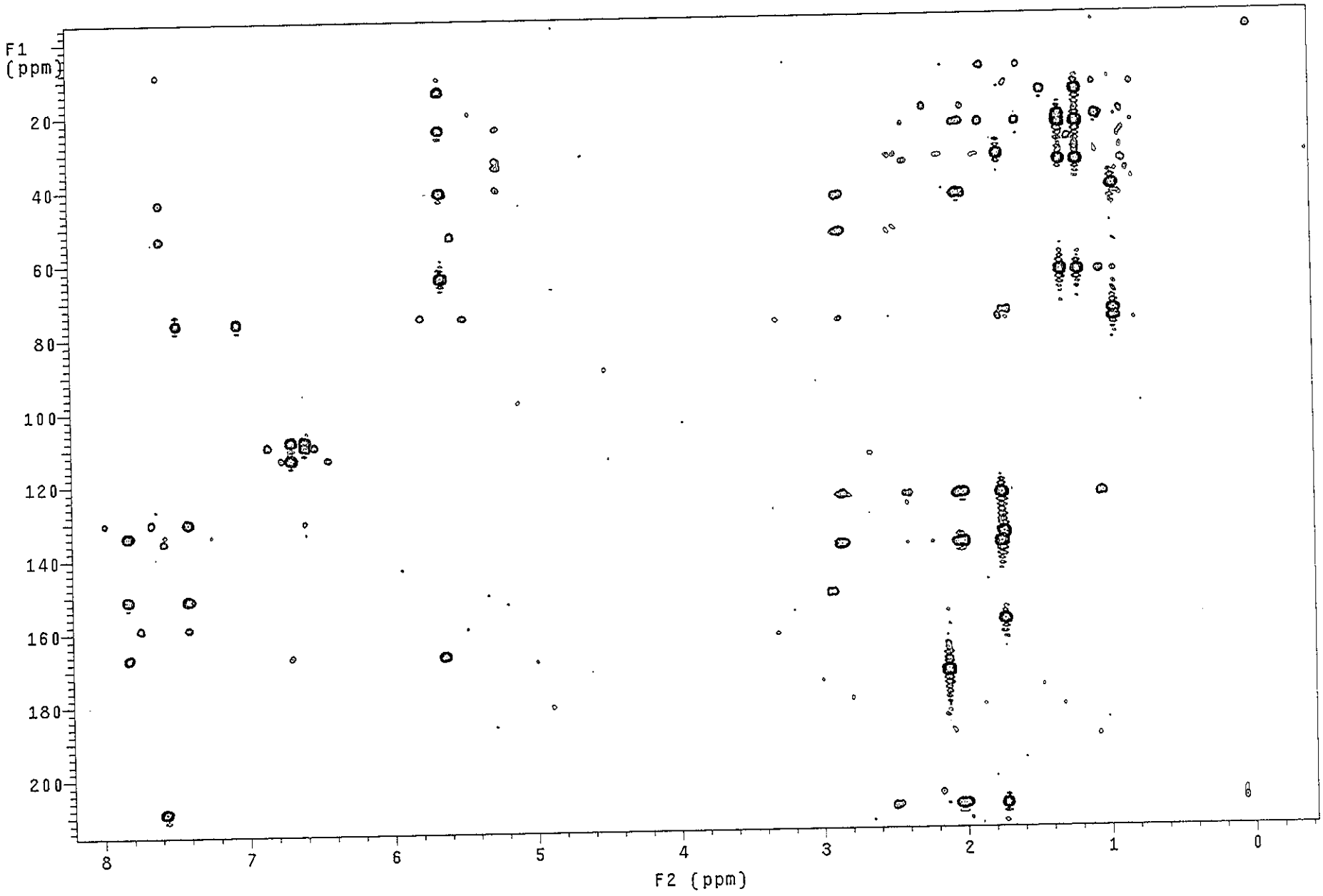


Figure 58 2D HMBC spectrum of compound **PSI7**

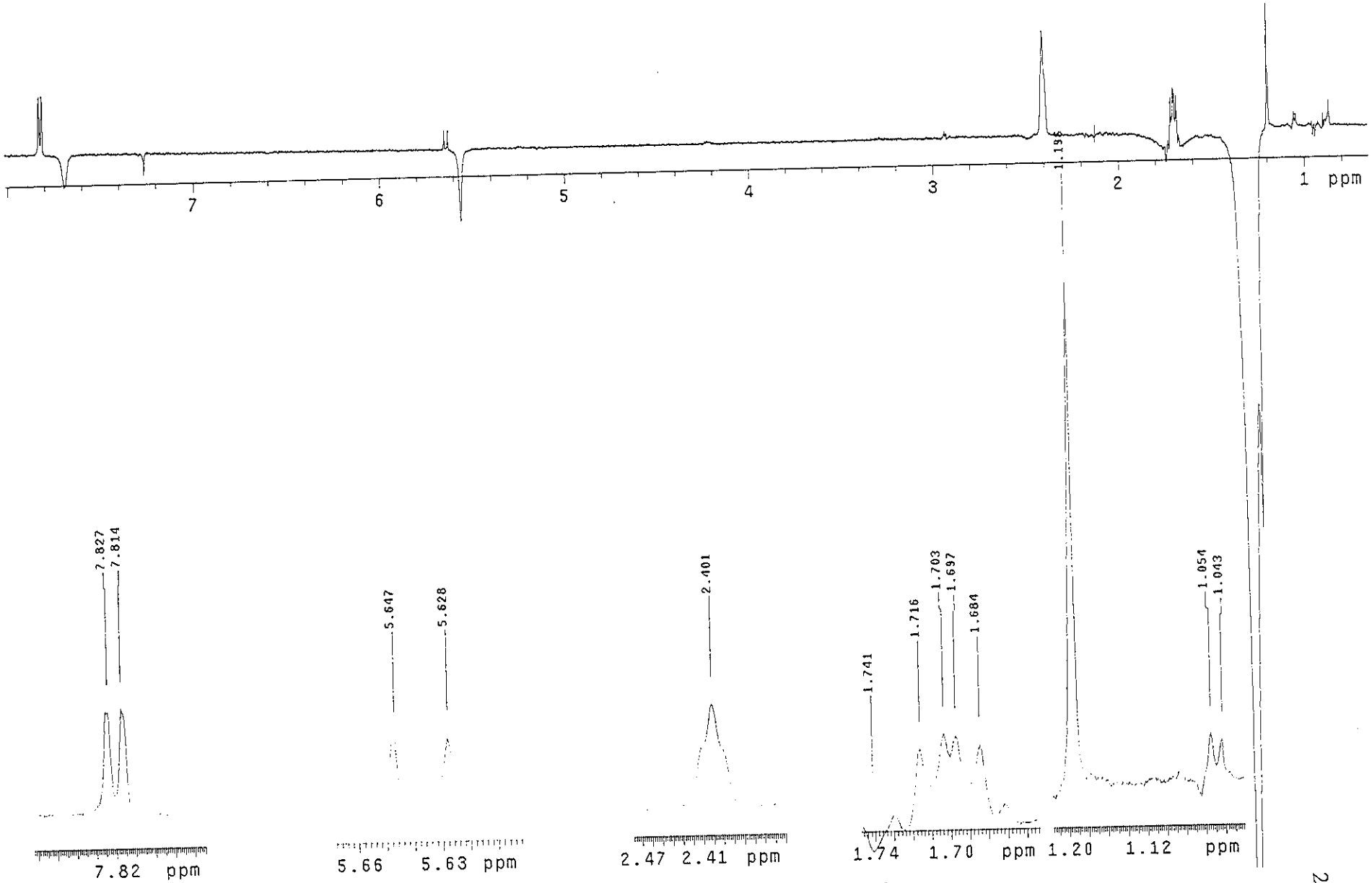


Figure 59 NOE spectrum of compound **PSI7** Irradiated at δ 1.32, (1)

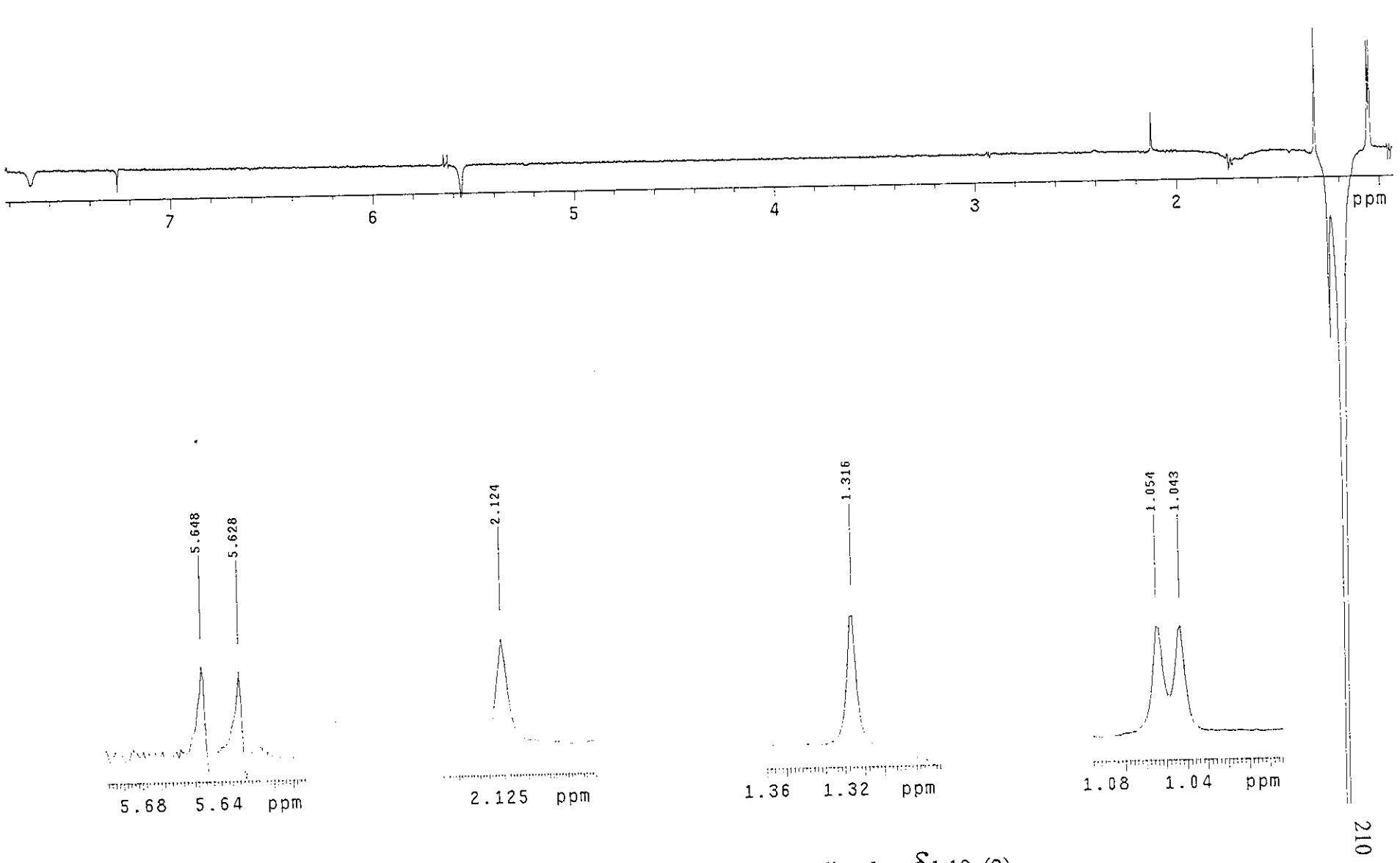


Figure 59 NOE spectrum of compound **PSI7** Irradiated at δ 1.19, (2)

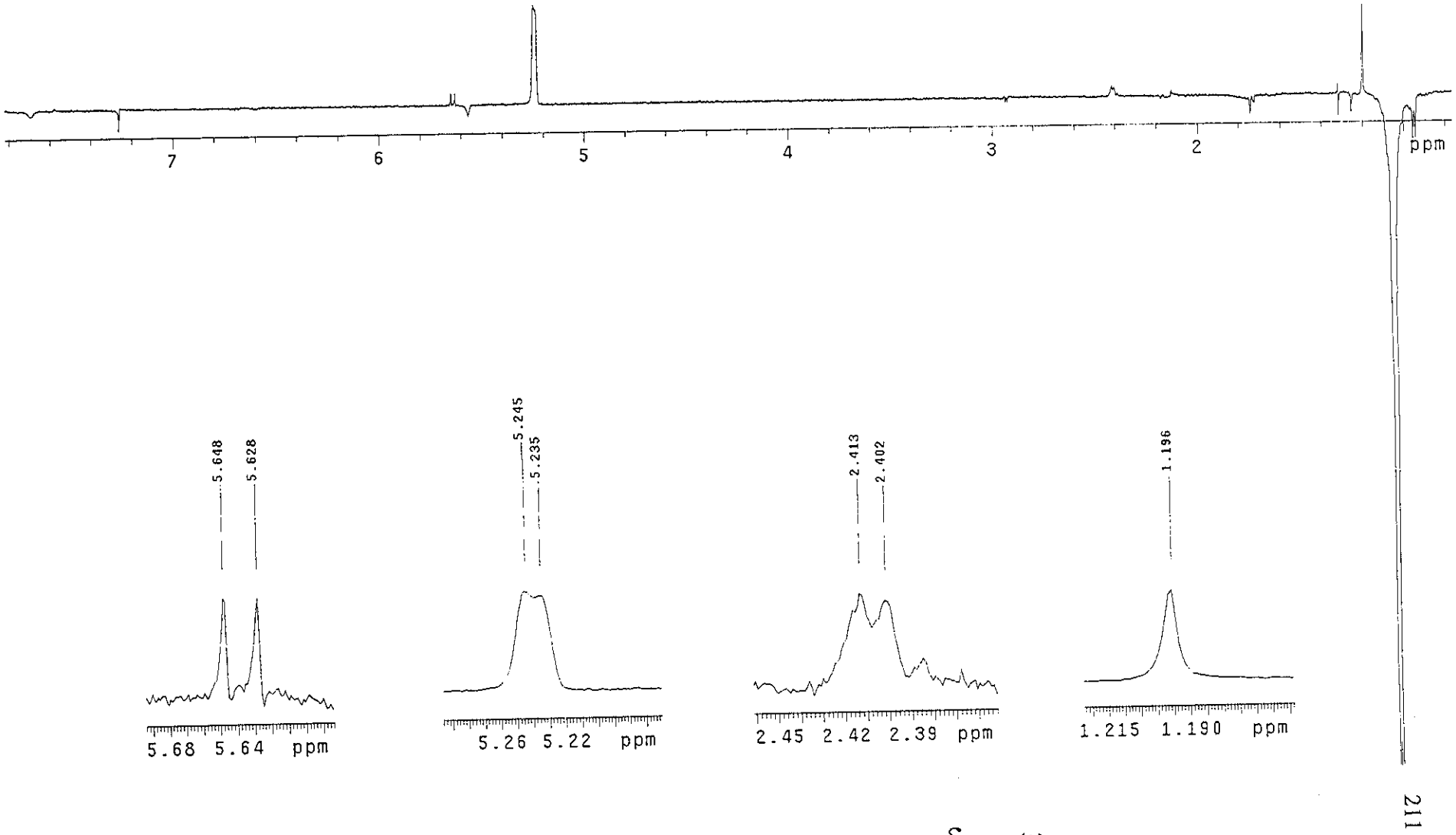


Figure 59 NOE spectrum of compound **PSI7** Irradiated at δ 1.05, (3)

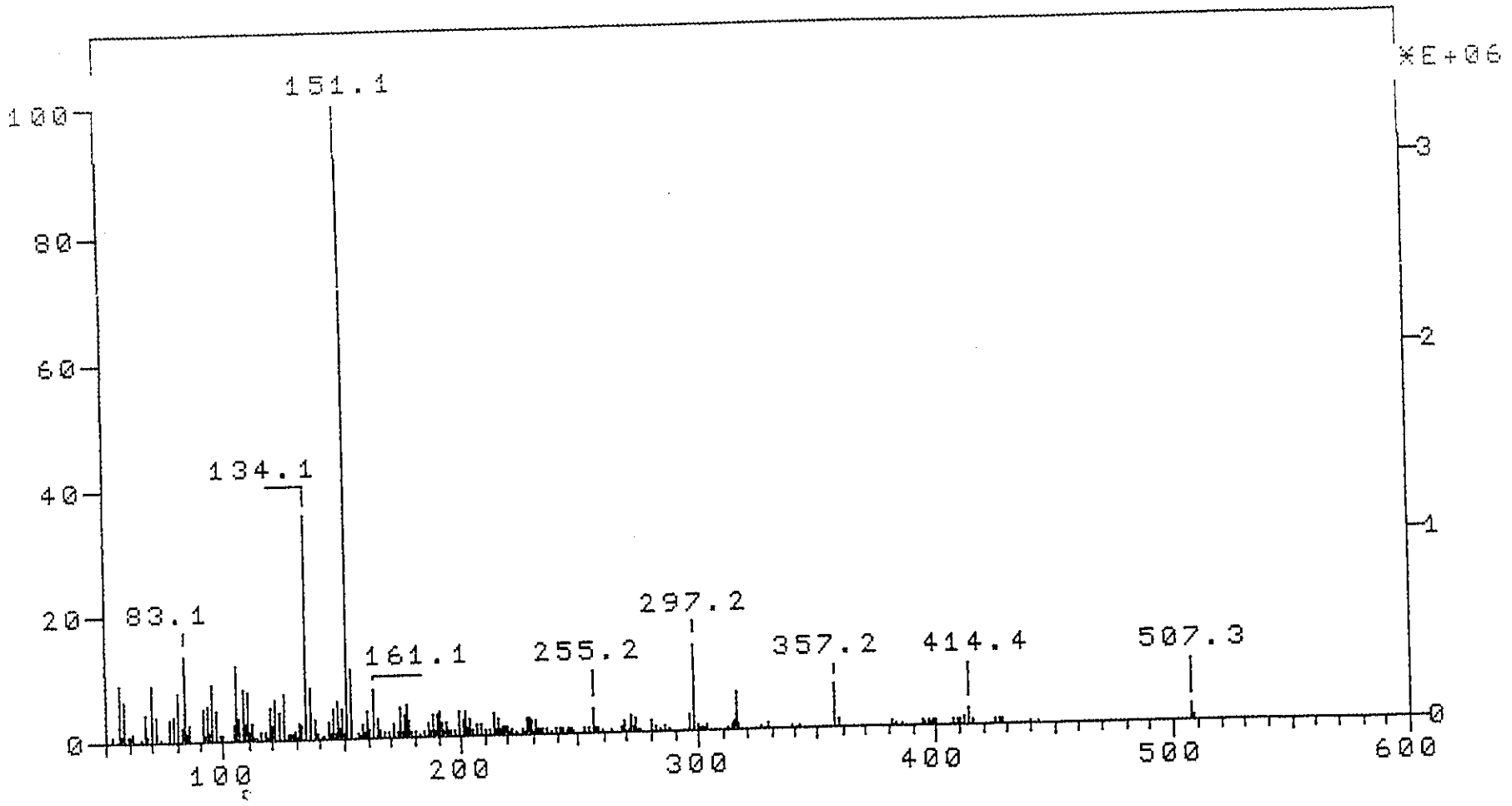


Figure 60 Mass spectrum of compound **PSI7**

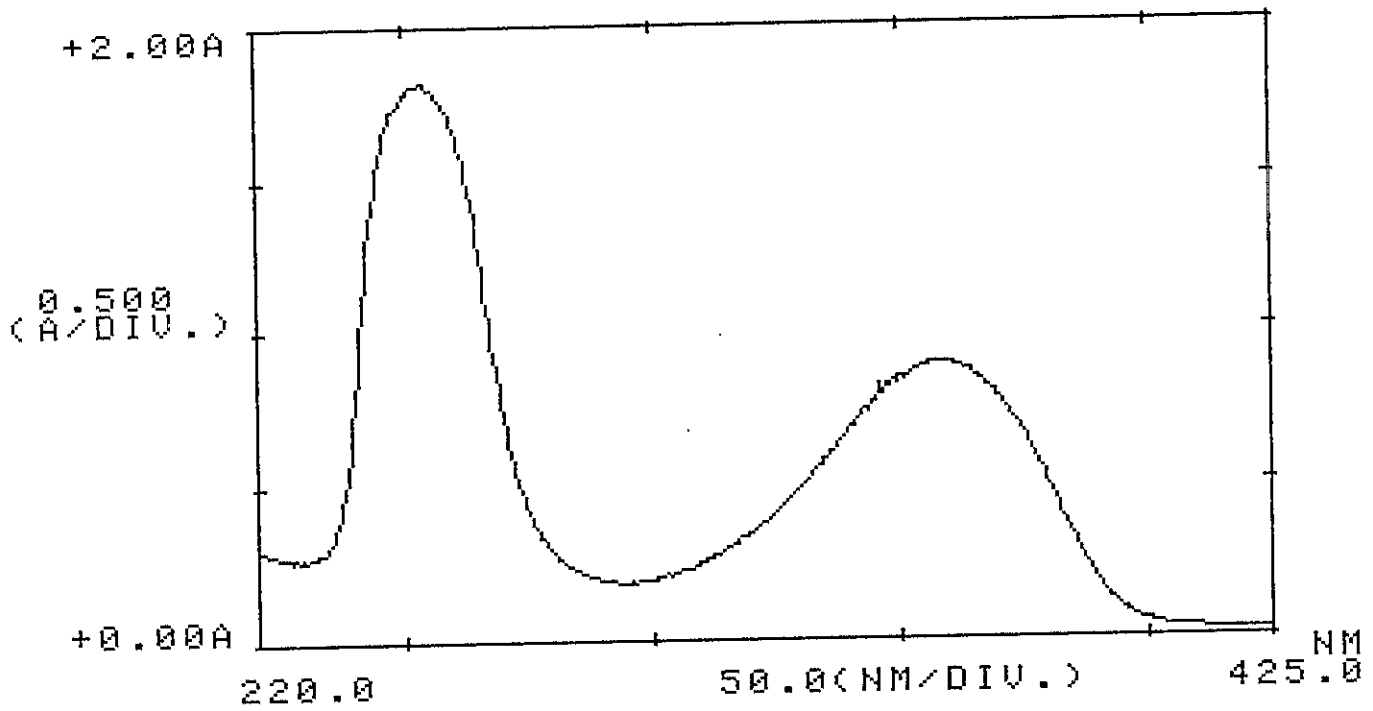


Figure 61 UV ($CHCl_3$) spectrum of compound **PSI8**

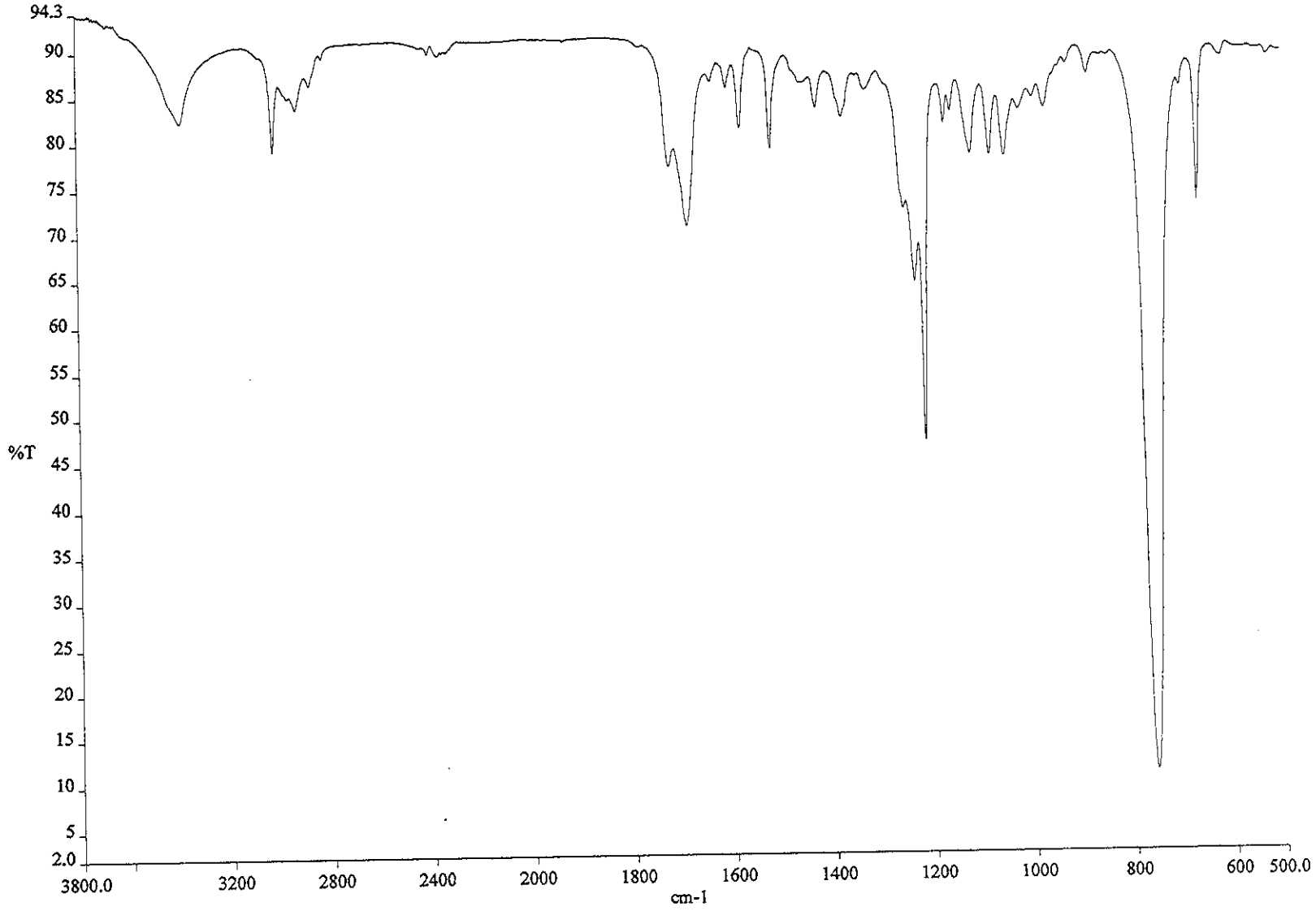


Figure 62 IR (Neat) spectrum of compound **PSI8**

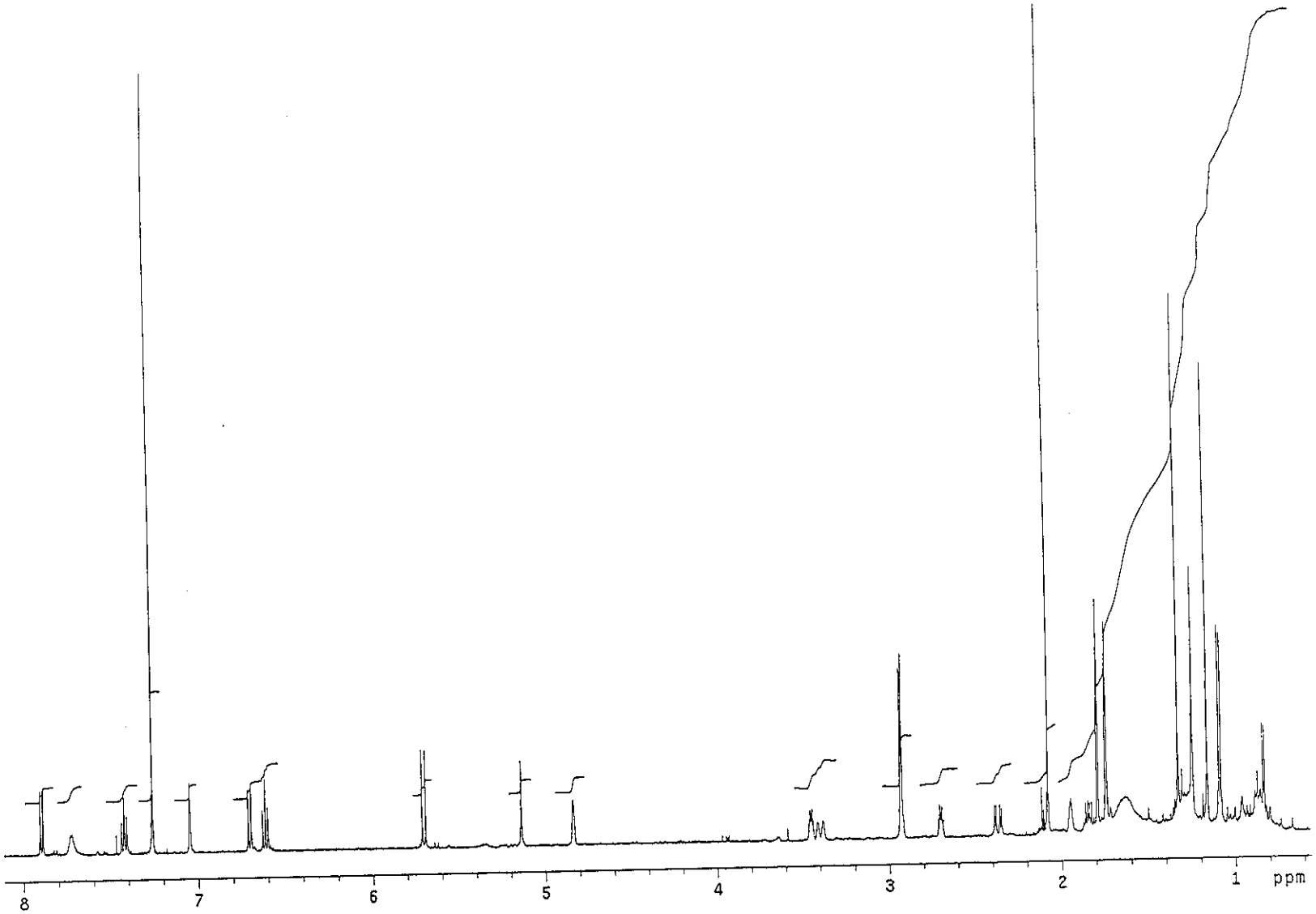


Figure 63 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **PSI8**

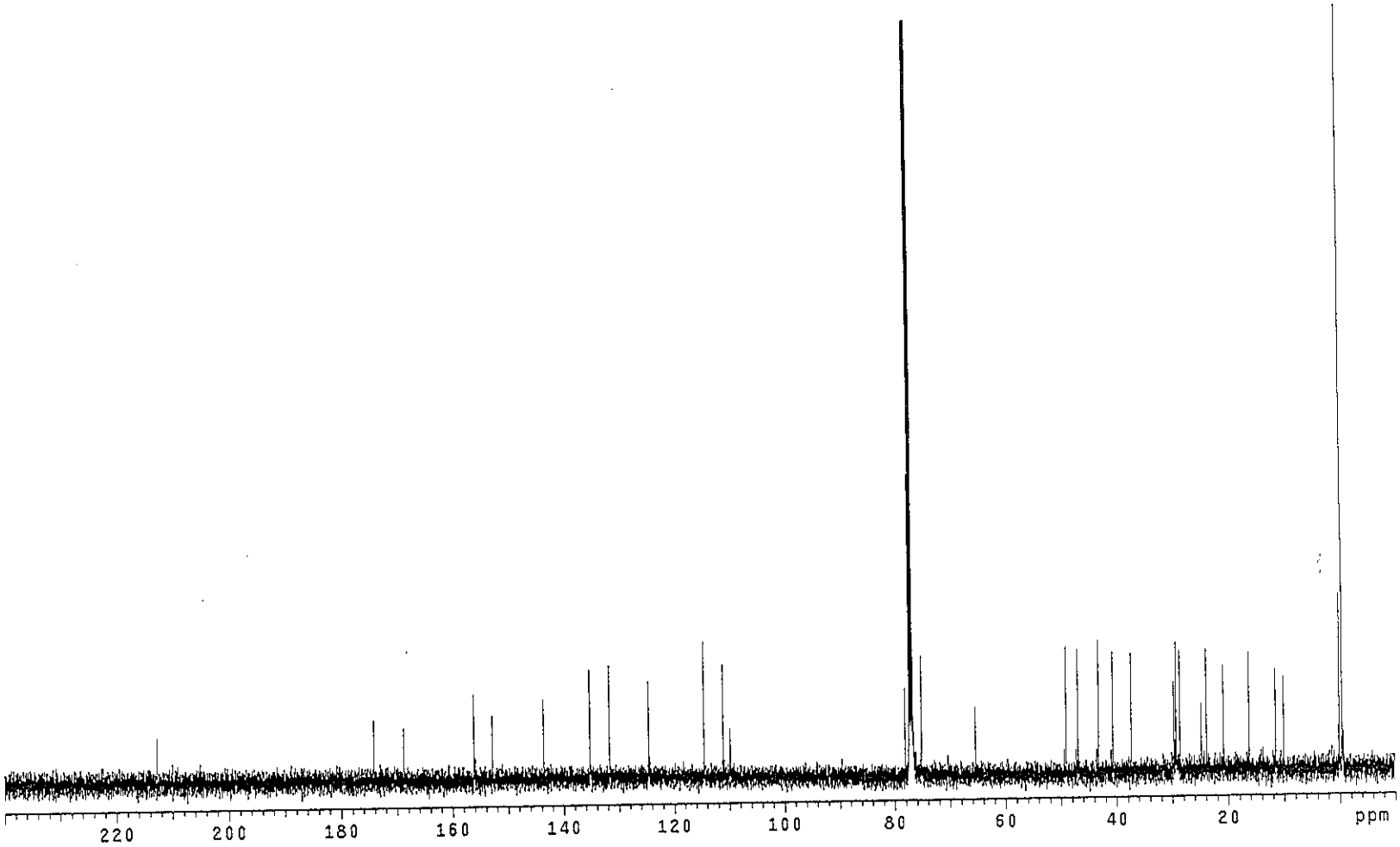


Figure 64 ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of compound **PSI8**

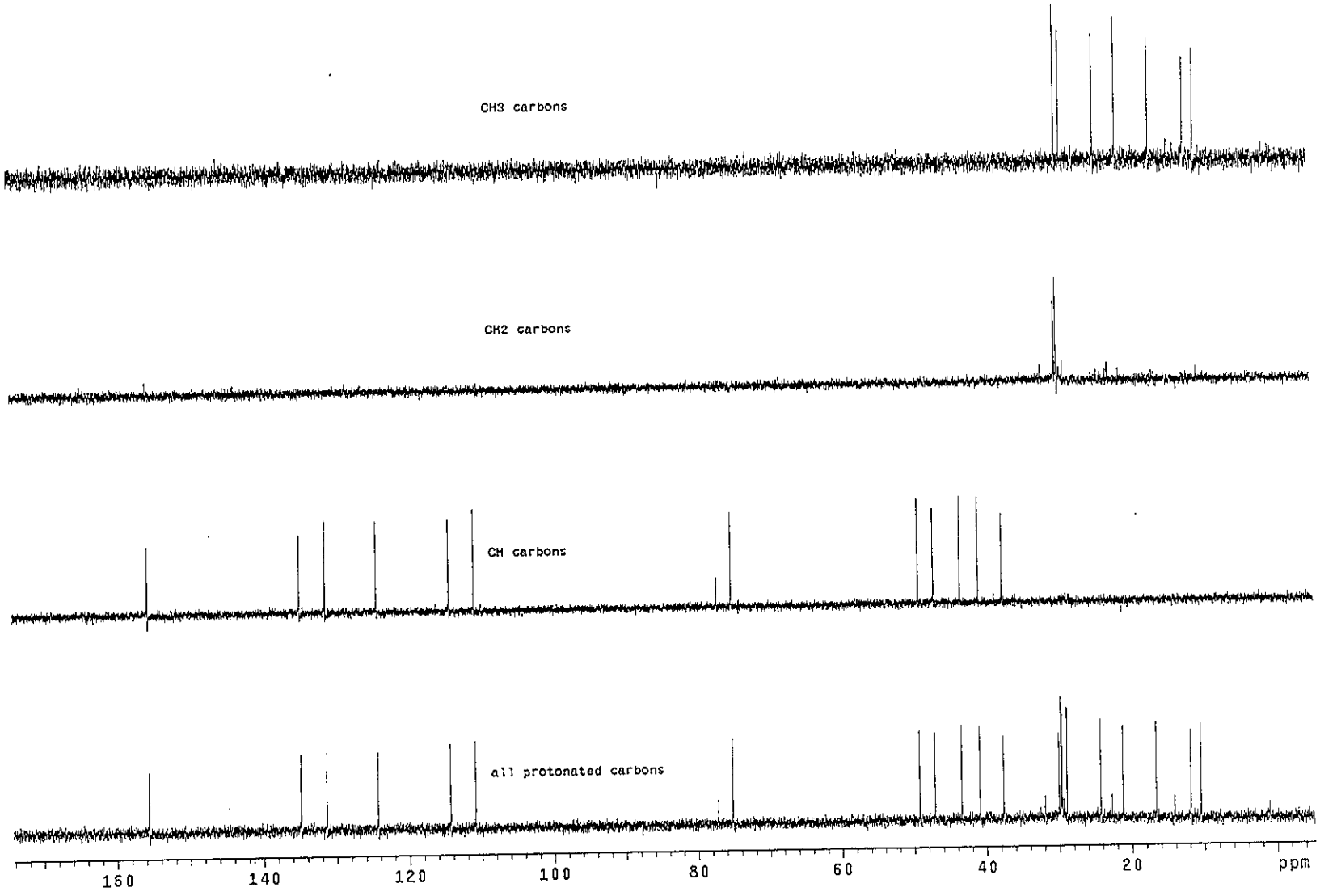


Figure 65 DEPT ($CDCl_3$) spectrum of compound **PSI8**

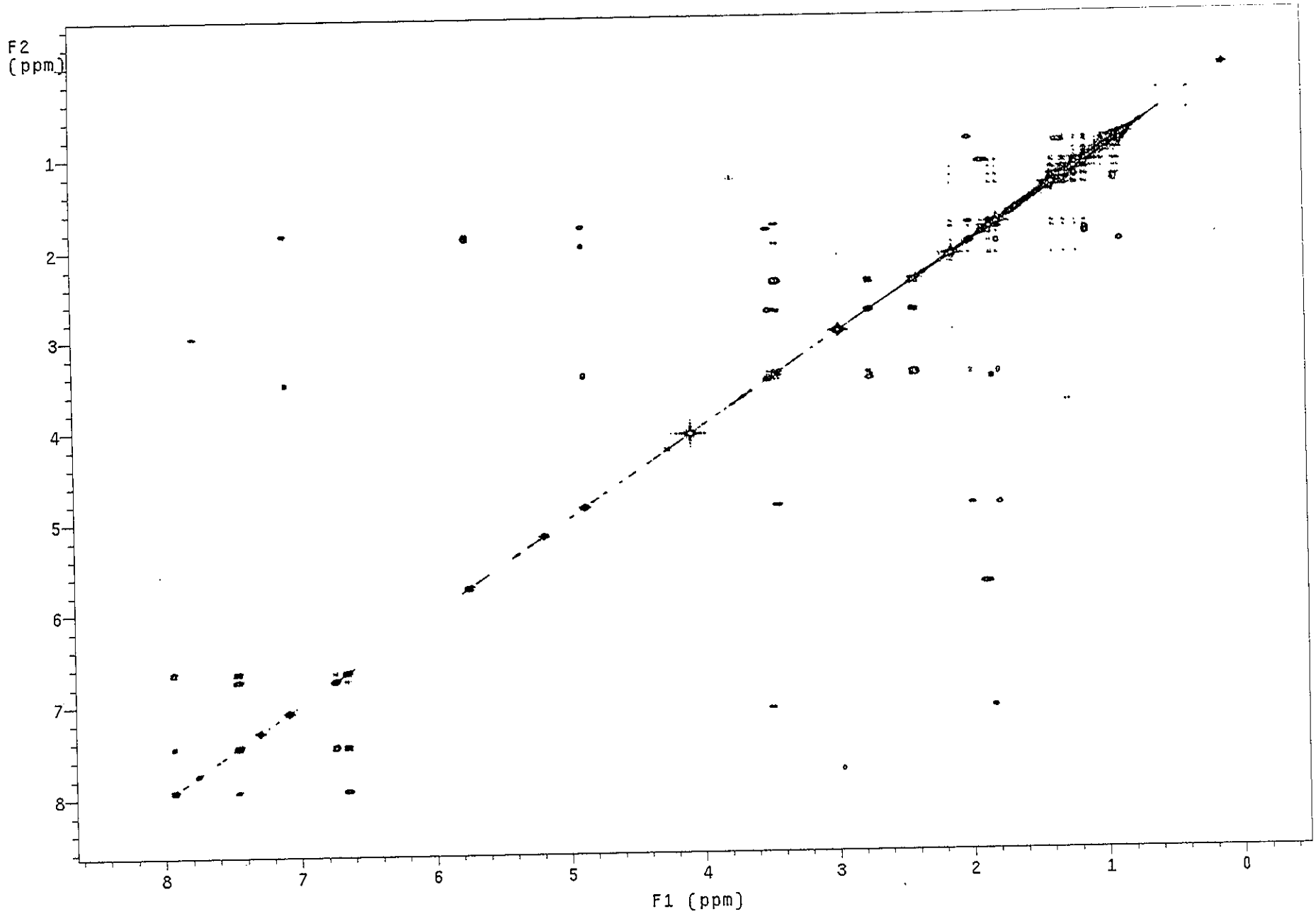


Figure 66 2D COSY spectrum of compound **PSI8**

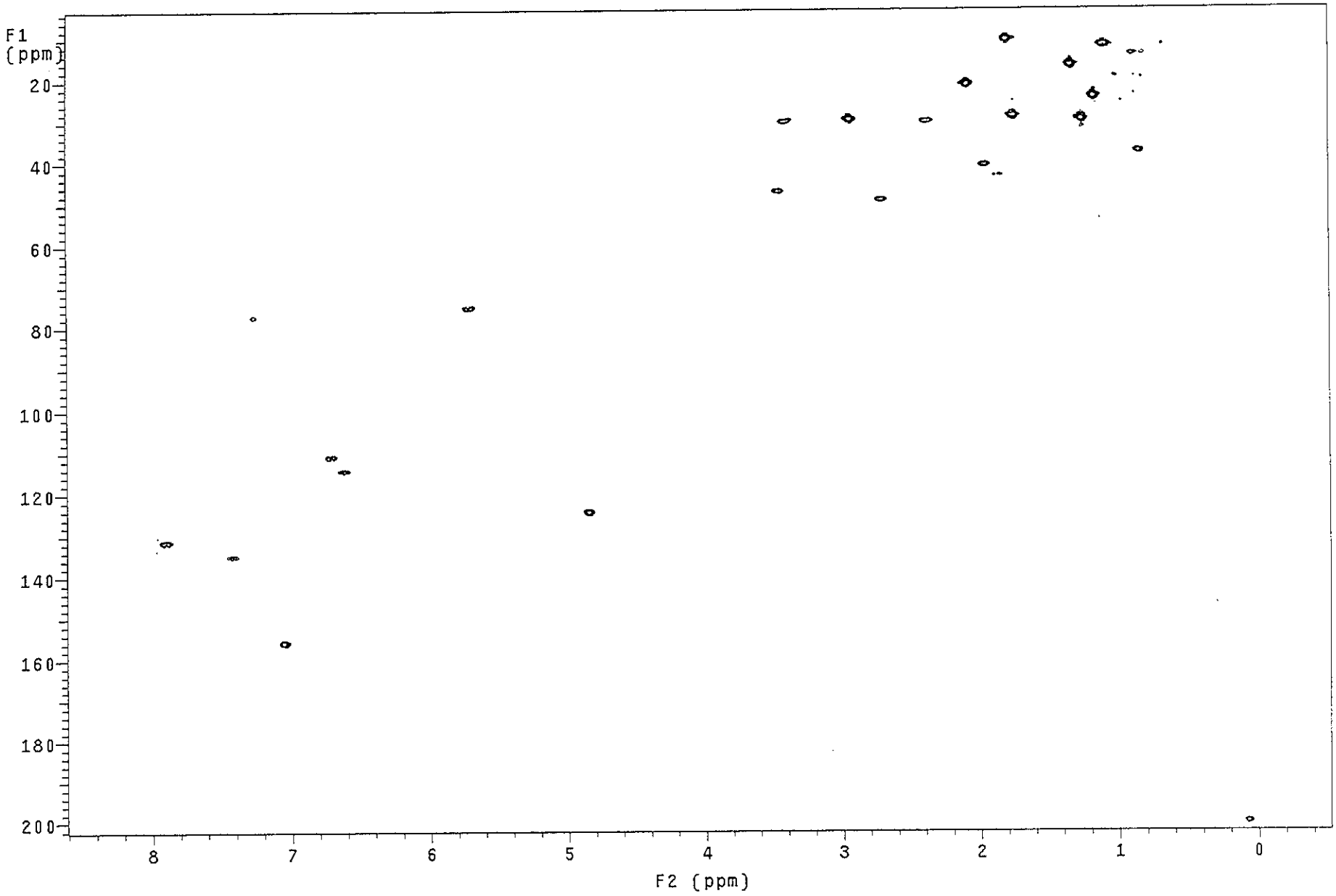


Figure 67 2D HMQC spectrum of compound **PSI8**

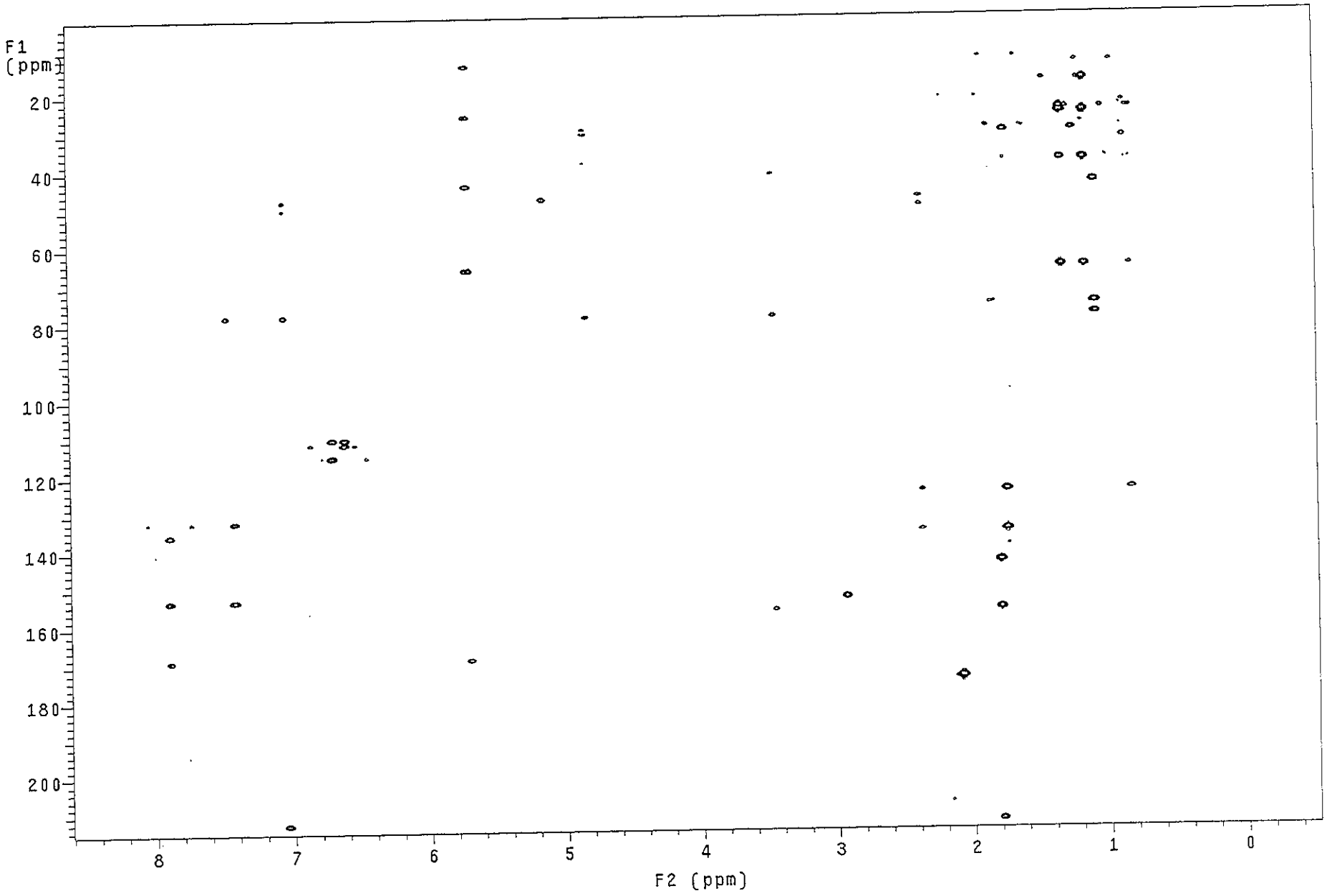


Figure 68 2D HMBC spectrum of compound **PSI8**

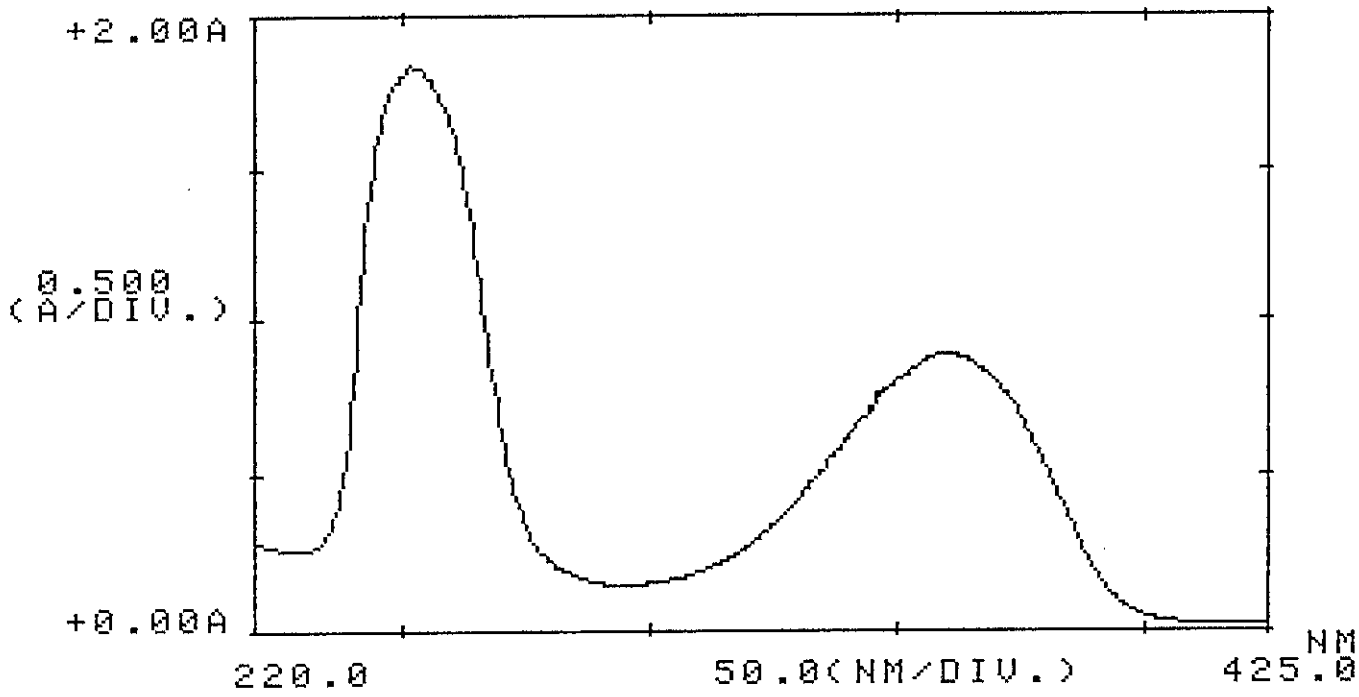


Figure 69 UV ($CHCl_3$) spectrum of compound **PSI9**

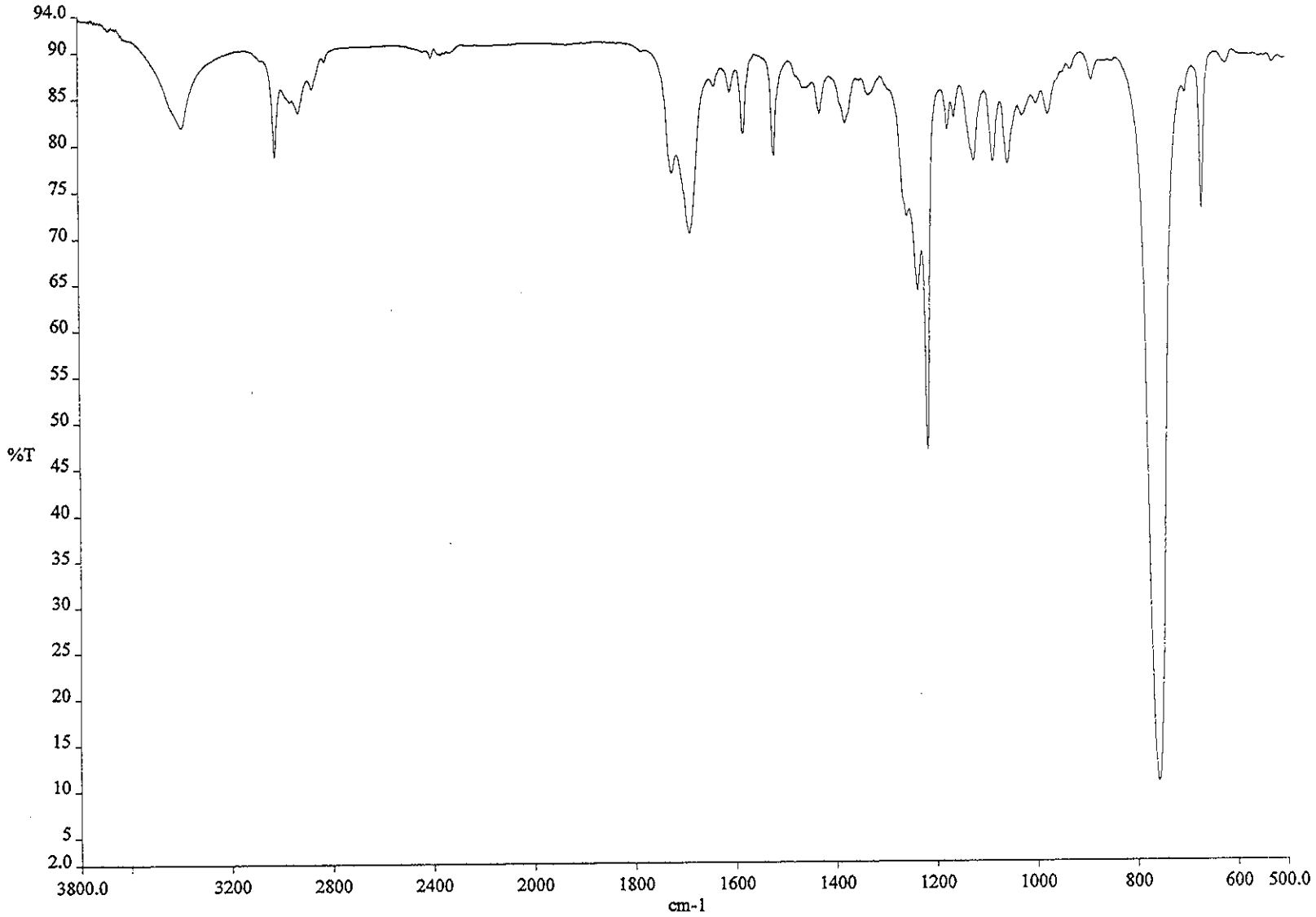


Figure 70 IR (Neat) spectrum of compound **PSI9**

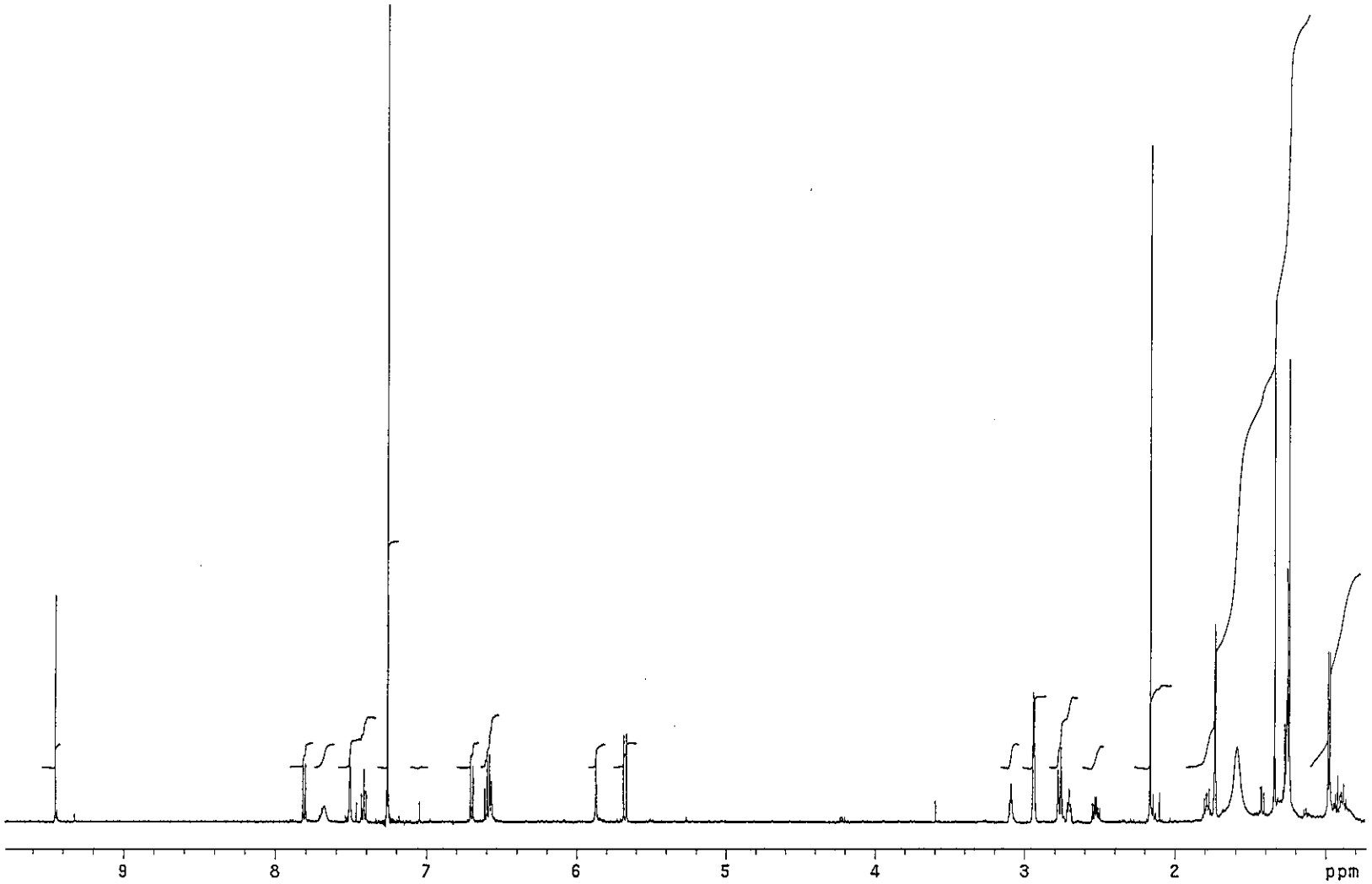


Figure 71 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **PSI9**

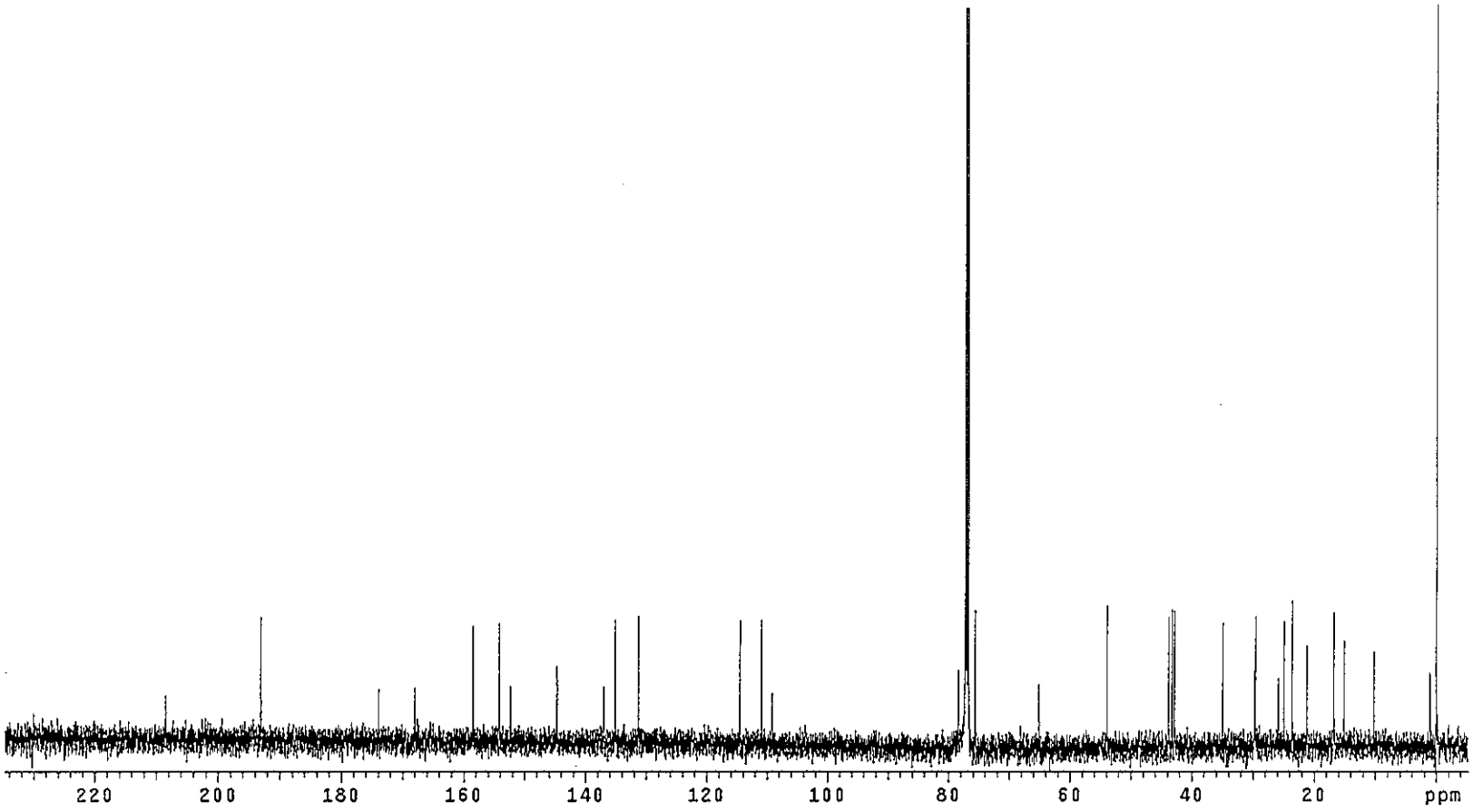


Figure 72 ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of compound **PSI9**

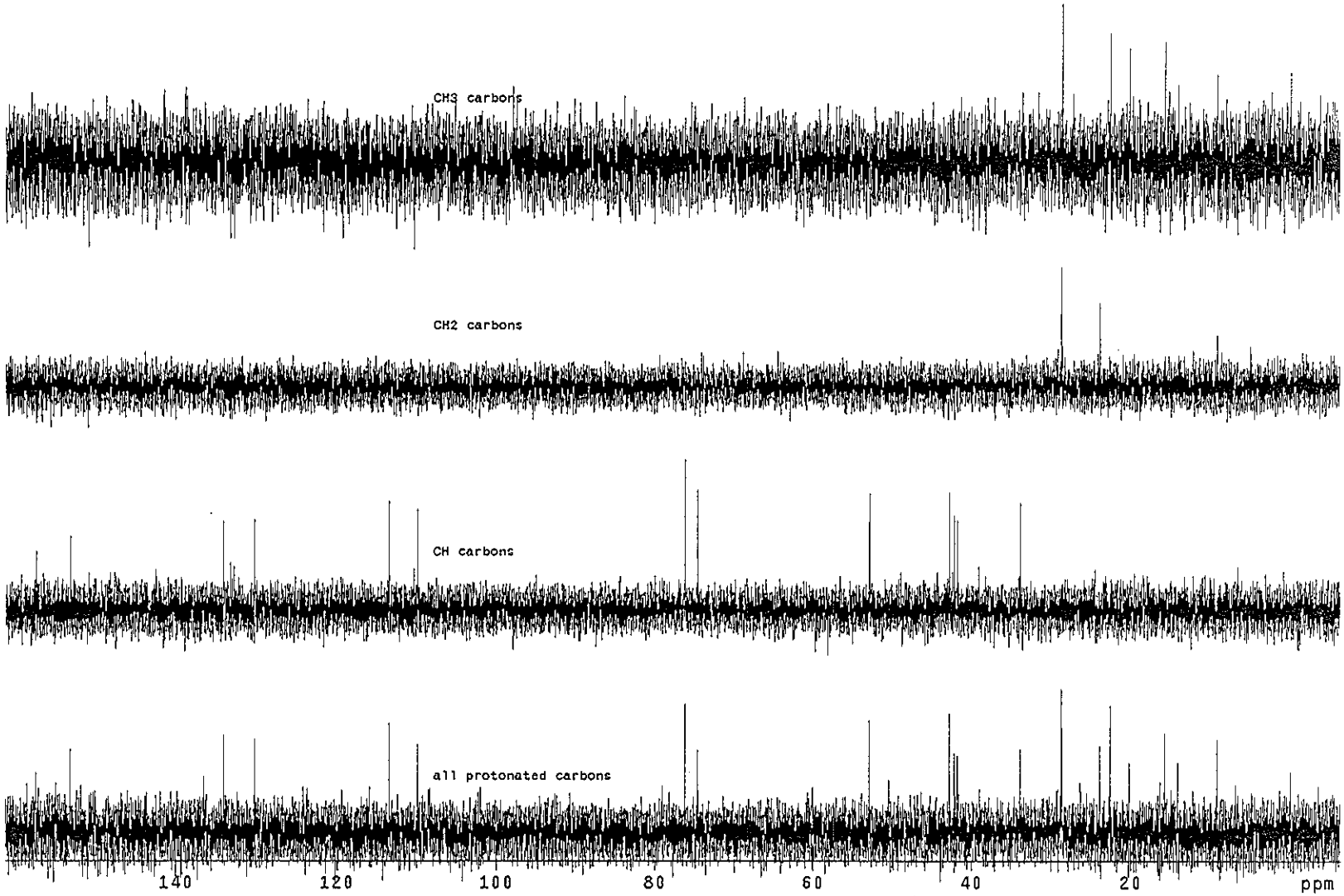


Figure 73 DEPT ($CDCl_3$) spectrum of compound **PSI9**

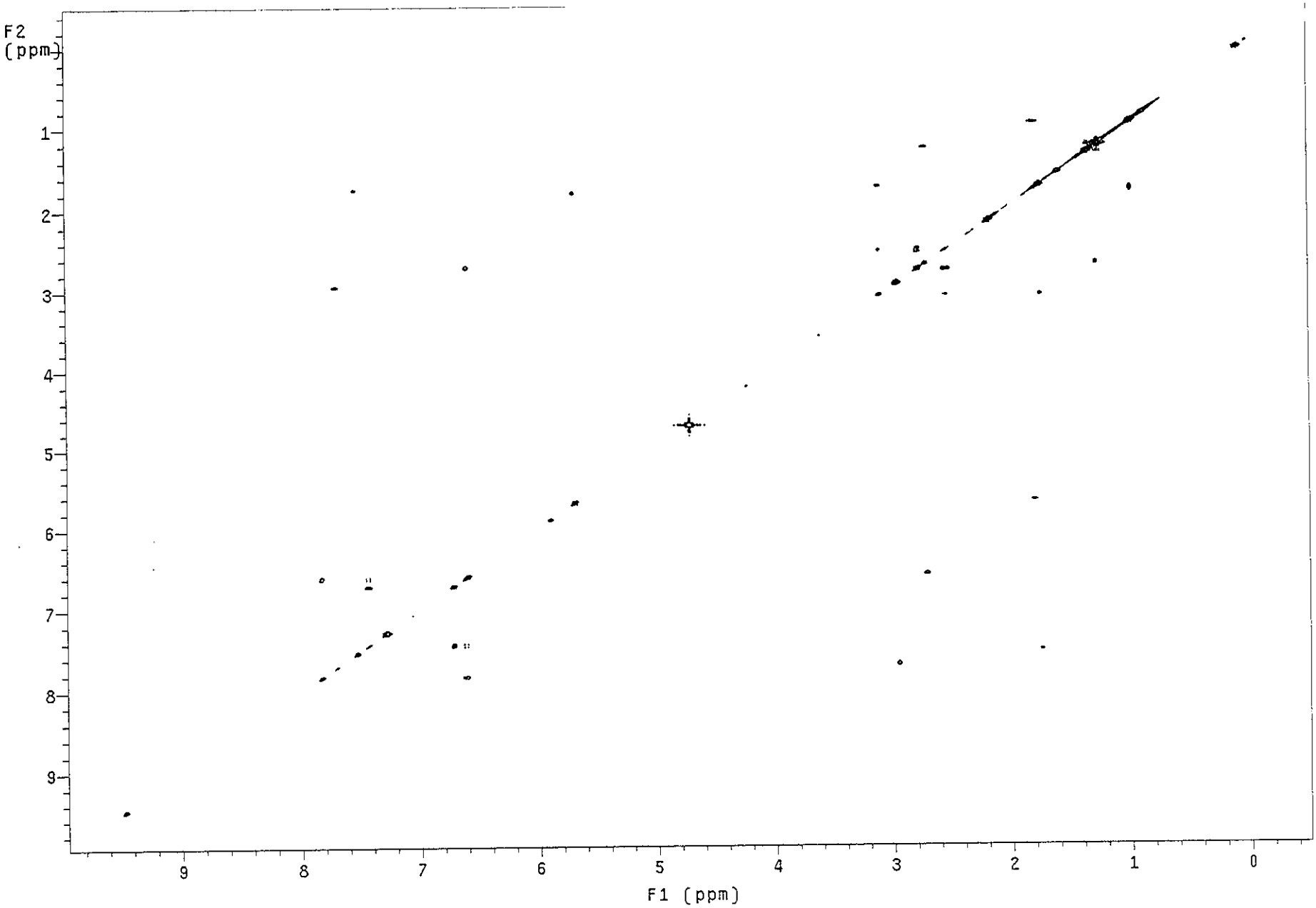


Figure 74 2D COSY spectrum of compound **PSI9**

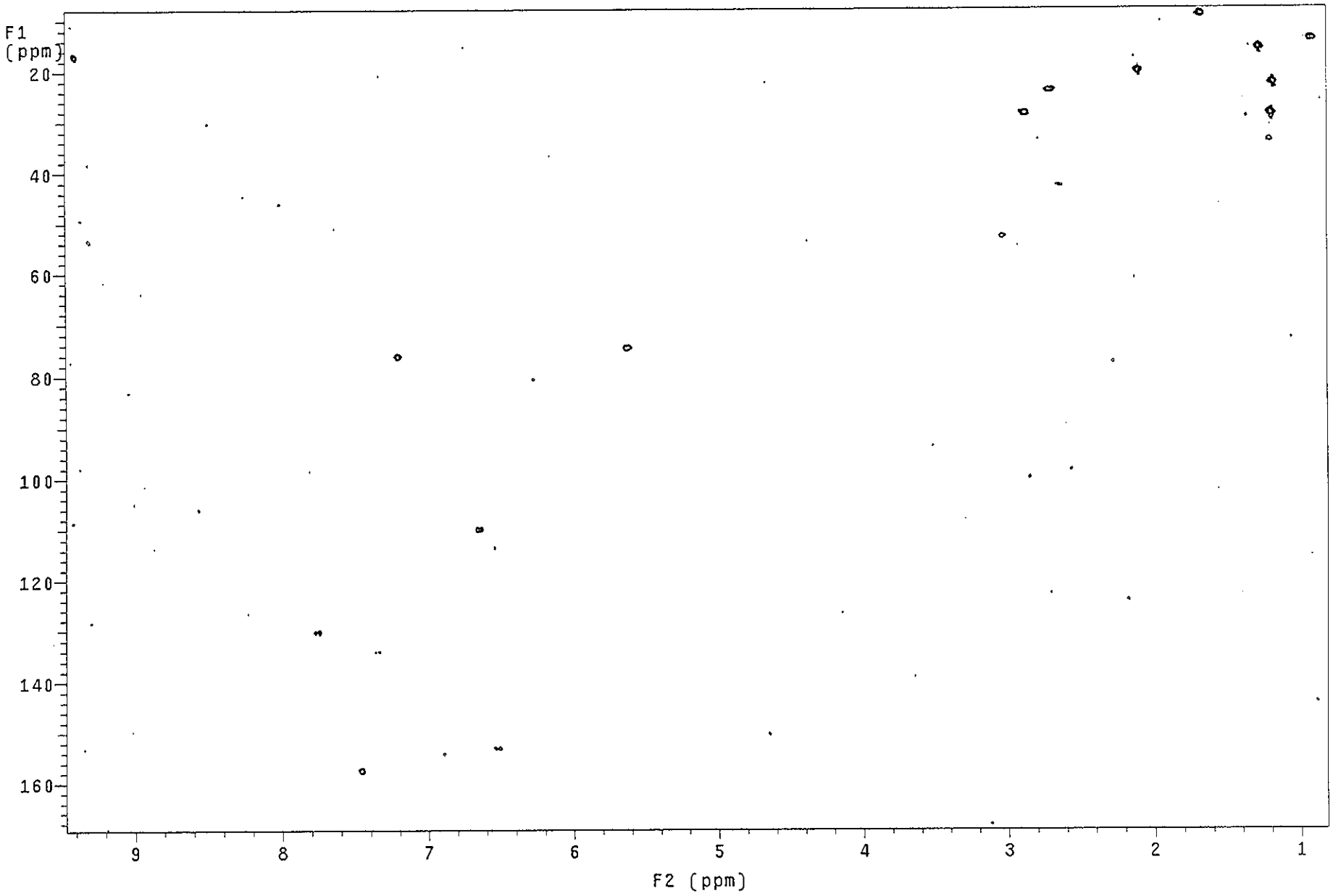


Figure 75 2D HMQC spectrum of compound **PSI9**

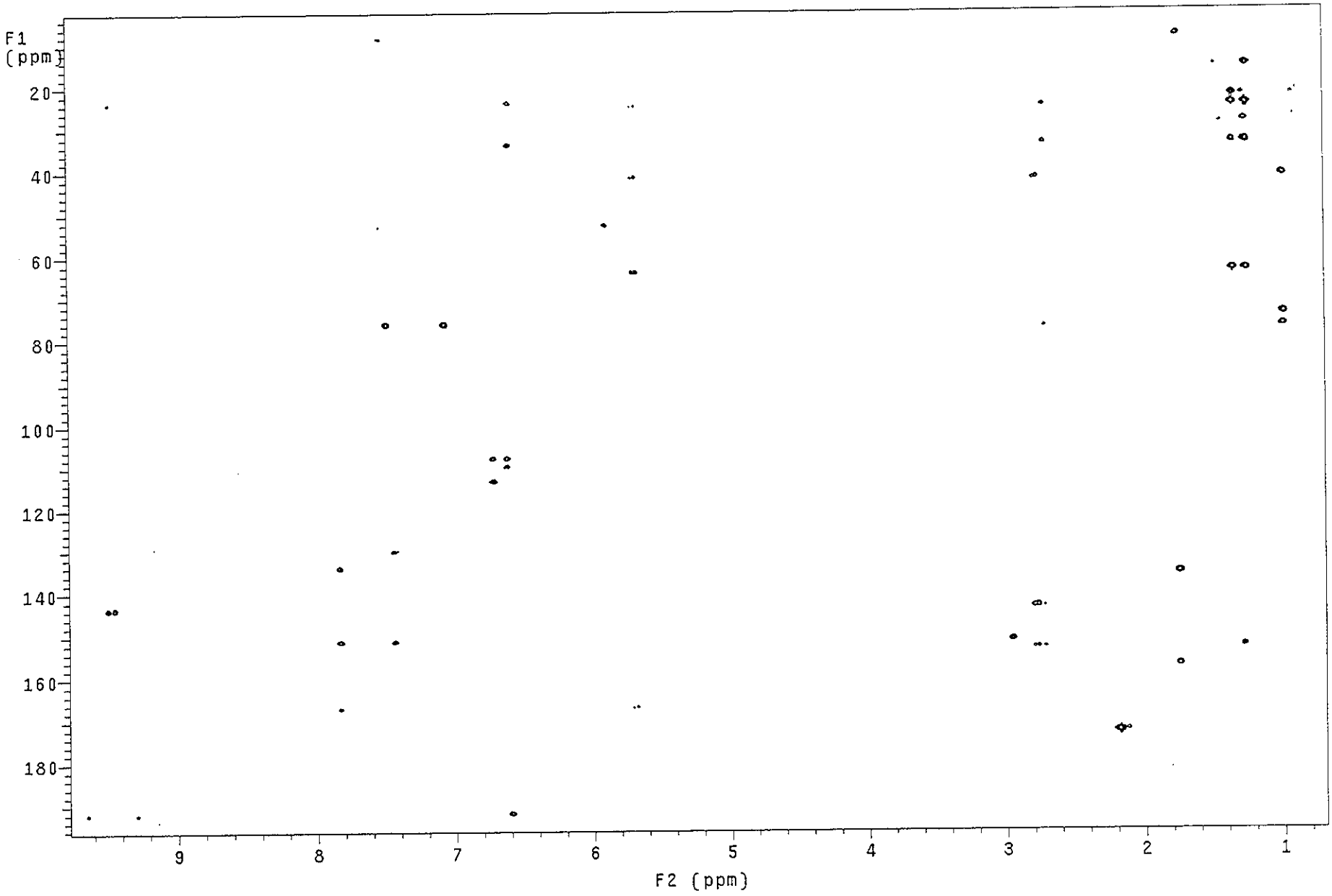


Figure 76 2D HMBC spectrum of compound **PSI9**

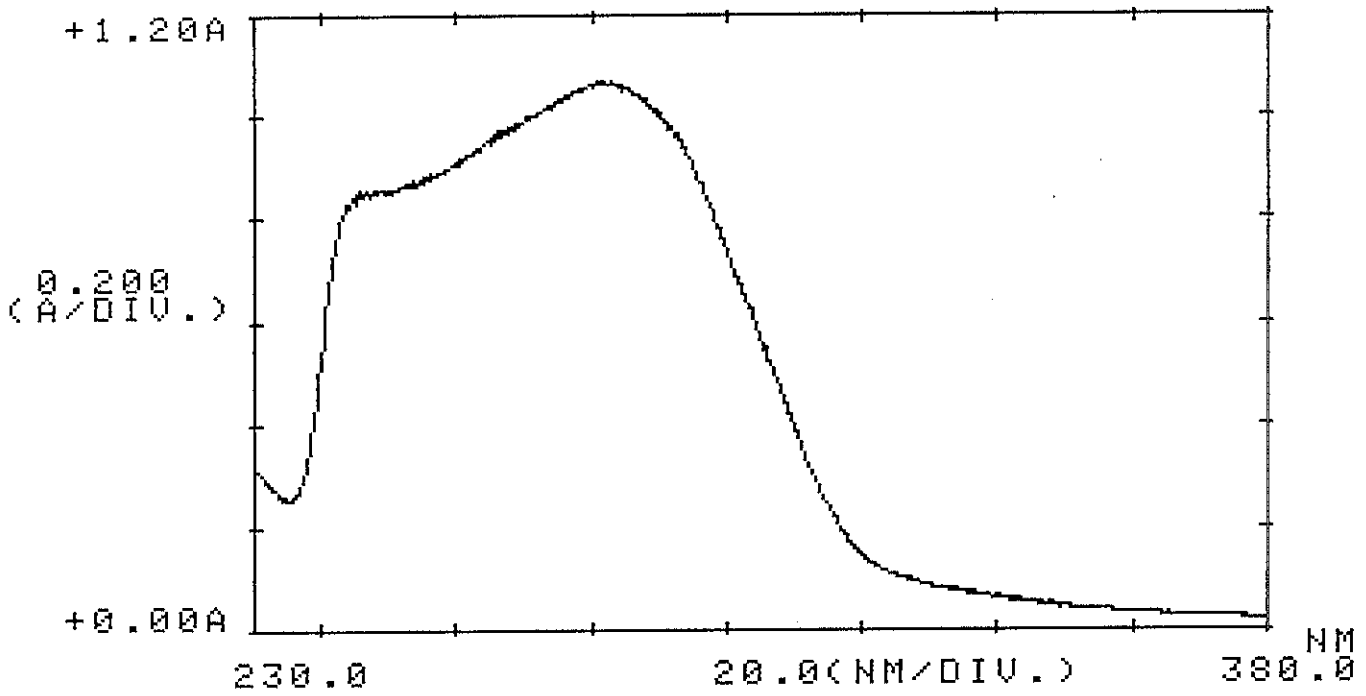


Figure 77 UV ($CHCl_3$) spectrum of compound **PSI10**

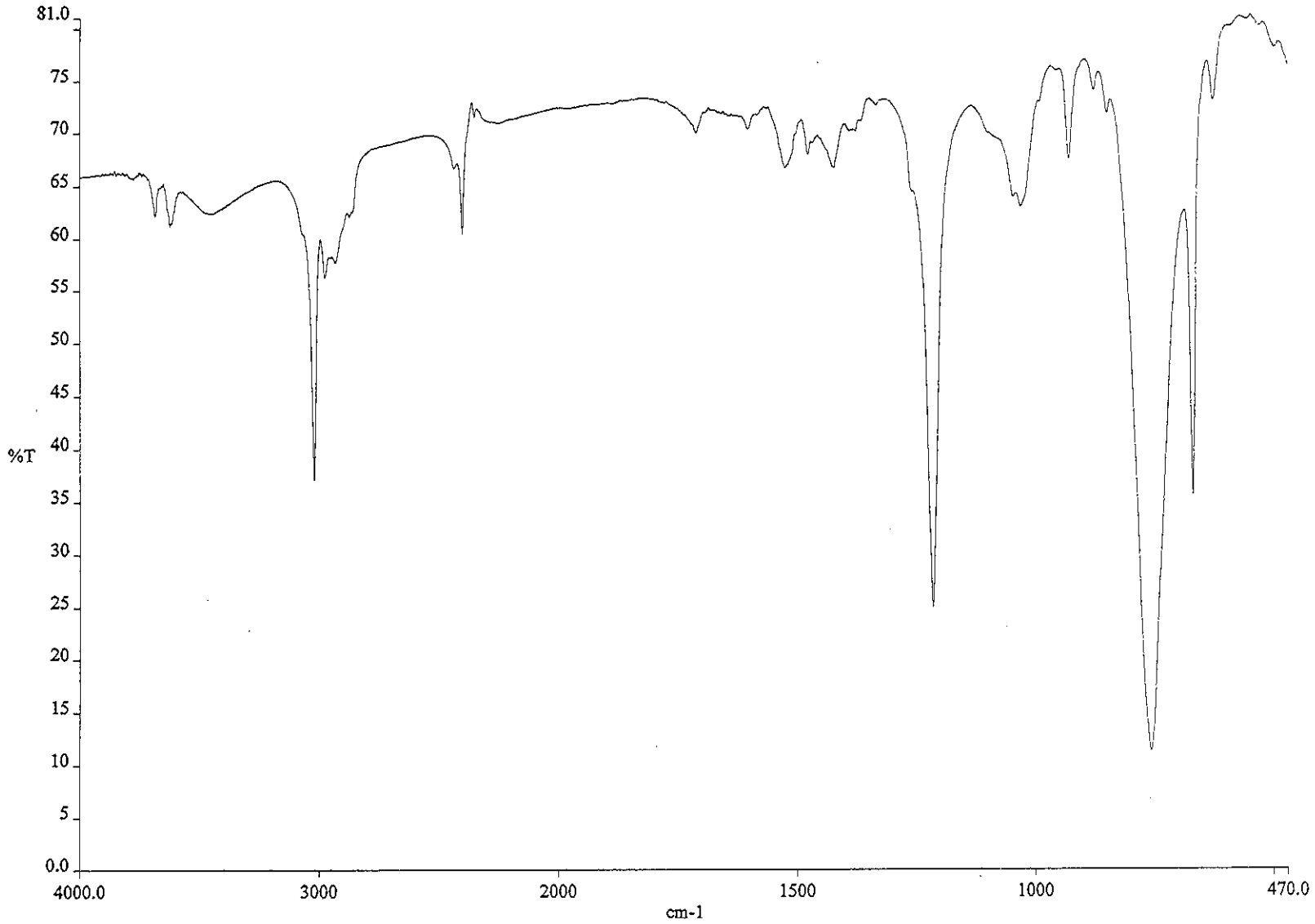


Figure 78 IR (Neat) spectrum of compound **PSI10**

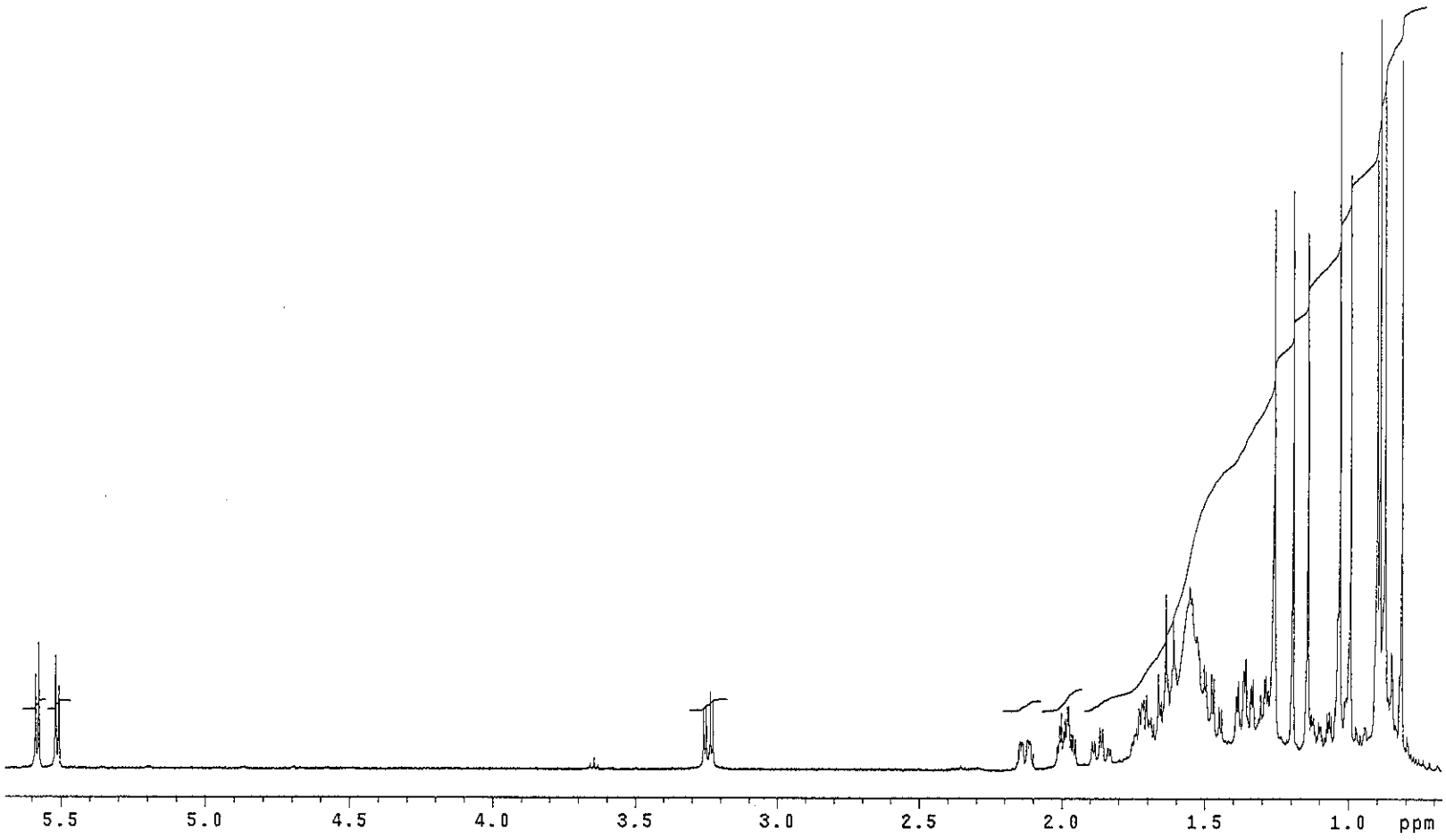


Figure 79 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **PSI10**

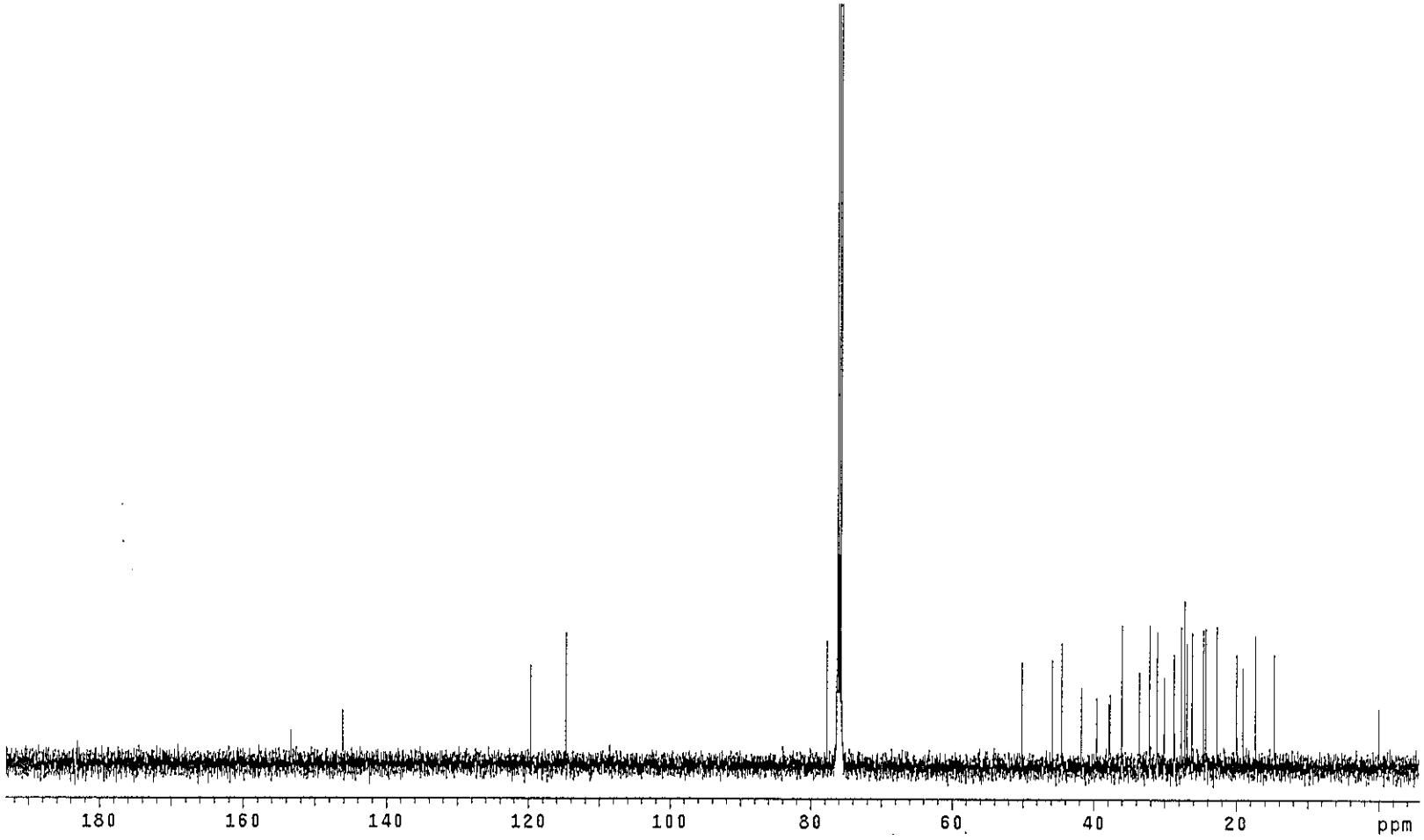


Figure 80 ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of compound **PSI10**

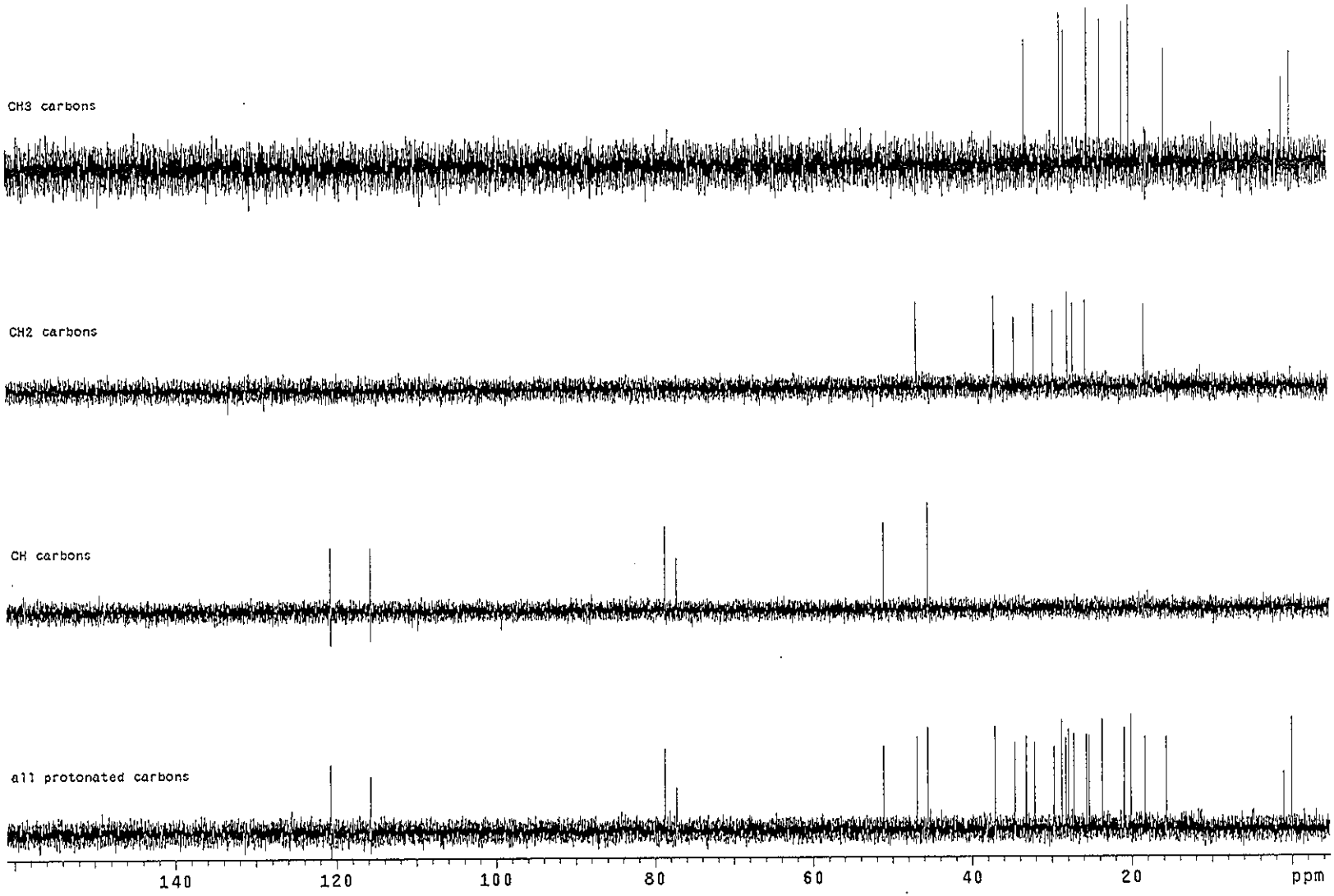


Figure 81 DEPT ($CDCl_3$) spectrum of compound **PSI10**

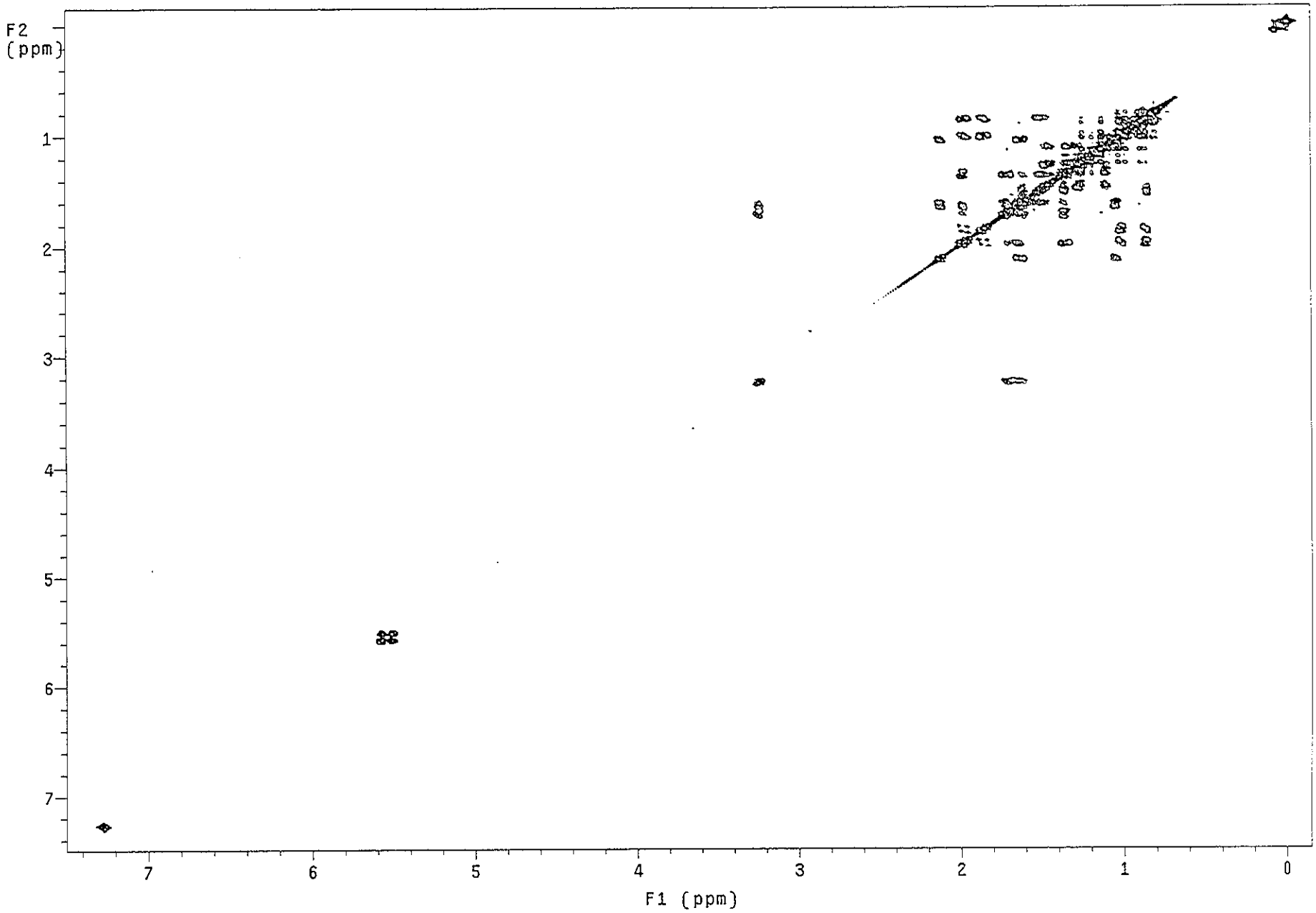


Figure 82 2D COSY spectrum of compound **PSI10**

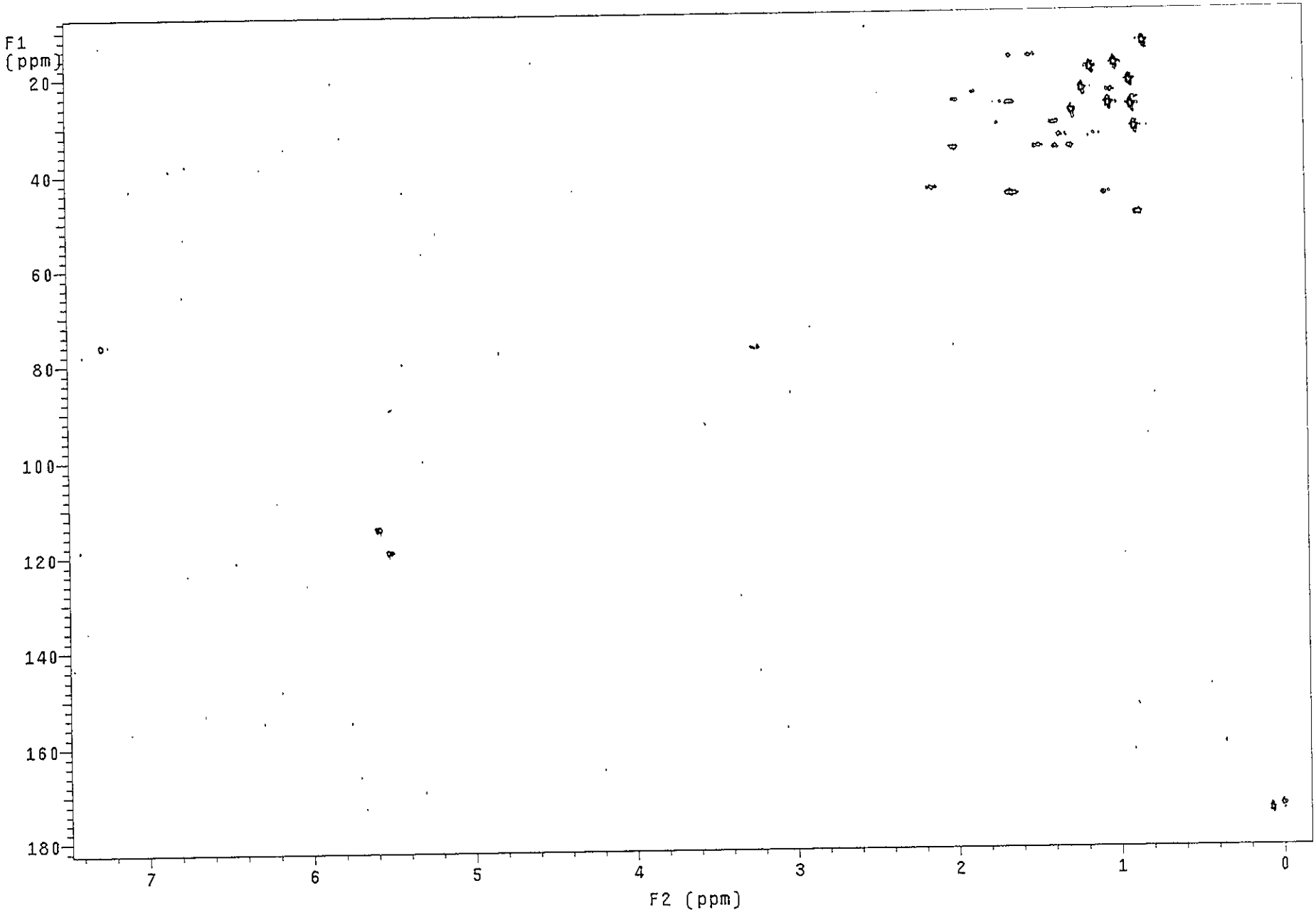


Figure 83 2D HMQC spectrum of compound **PSI10**

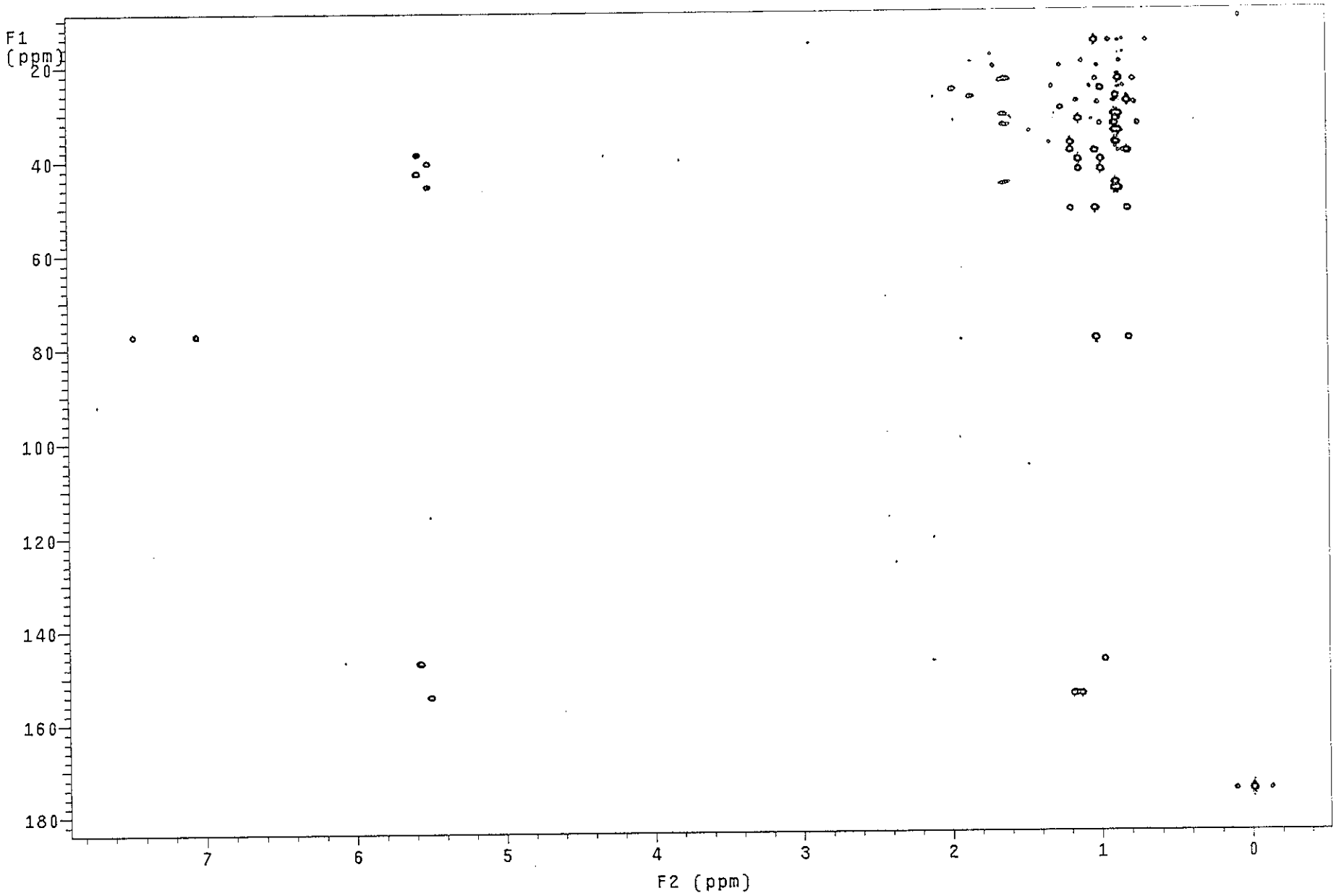


Figure 84 2D HMBC spectrum of compound **PSI10**

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