

PHYTOCHEMICAL STUDY OF TABEBUIA PENTAPHYLLA HEMSL
CULTIVATED IN EGYPT

D.W.Bishay; A.M.Abdel-Baky; S.A.Ross and Z.Z.Ibrahim.

Pharmacognosy Dept., Faculty of Pharmacy, Assiut University
Assiut, Egypt

ABSTRACT

Preliminary phytochemical screening of Tabebuia pentaphylla Hemsl. revealed the presence of sterols and/or triterpenes, flavonoids and iridoids. From the hexane extract of the leaves α -amyrin, β -sitosterols, betulin, betulinic acid, oleanolic acid and a long chain alcohol were isolated. While kaempferol, quercetin, kaempferol-3-O-diglucoside and quercetin-3-O-diglucoside were isolated from the ethyl acetate extract. Specioside (6-O-coumaryl catalpol) and oleanolic acid were isolated from ethyl acetate extract of the bark. The cytotoxicity tests of some isolated compounds were carried out on cell cultures of P-388 and KB cells. The results obtained showed some positive significances.

INTRODUCTION

Tabebuia pentaphylla Hemsl. is a large tree belonging to Family Bignoniaceae. The plant is used as an antipyretic, hypnotic and diuretic¹.

Some Tabebuia species have been investigated by many authors and proved to contain quinones²⁻¹¹, flavonoids anthocyanins¹²⁻¹⁷ sterols and triterpenes¹⁸⁻²¹, iridoids¹² and oxygen heterocyclic compounds (tectol)²².

EXPERIMENTAL

The plant material consisting of the leaves young shoots and stems bark was collected from trees cultivated in Aswan Botanic Island, during the flowering stage in May 1983. The plant was kindly identified by Agr. Engineer I. Aly Mousa, the director of the Botanic Island. Melting points were determined using a Kofler hot-stage instrument and are uncorrected, $^1\text{H-NMR}$ spectra were recorded in CDCl_3 , with Varian XL series 300 Hz. Mass spectra were measured using a Varian MAT-112S double-focusing spectrometer, operating at 70-ev., $^{13}\text{C-NMR}$ spectra were recorded in CDCl_3 with a Nicolet NT-360 instrument operating at 90 MHz. Perkin-Elmer infrared spectrophotometer 720 was used for recording infra-red spectra and UV was measured on Perkin-Elmer model 550 spectrophotometer Thomson THN 80 eV.

Extraction :

- a) One kg. of the powdered leaves of Tabebuia pentaphylla Hemsl. was extracted with 70 % ethanol. The semisolid residue obtained after concentrating the extract was digested with warm distilled water, filtered and the filtrate was successively extracted with hexane, chloroform and ethyl acetate.
- b) A half kg. of the powdered stem bark was successively extracted with hexane, chloroform and ethyl acetate. Each fraction was separately concentrated to a dry residue and then kept dry for further investigation.

Investigation of hexane extract of the leaves (Fraction A):

A part of (Fraction A) was investigated on silica gel G plates using pet. ether-ethyl acetate (8:2) and 50 % methanolic H_2SO_4 spray. Nine dark reddish brown spots having hR_f values 81, 68, 52, 50, 47, 40, 38, 30 and 25 were revealed.

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Column chromatography of (Fraction A) :

Twenty grams of fraction A was transferred onto a silica gel column eluted with hexane and then with mixtures of hexane and ethyl acetate in an increasing polarity. Fractions 100 ml. each were concentrated and screened by TLC. Similar fractions were collected, concentrated and crystallised in different solvents to give compounds a, b, c, d, e and f.

Compound a :

White flakes, mp. 82-85 °C (methanol). IR, (ν cm⁻¹) 3340 (OH), ¹H-NMR, (300 MHz, CDCl₃) δ 3.64 (OH); 1.563, 1.25 and 0.879 for \equiv CH, =CH₂ and -CH₃ respectively. MS showed [M⁺] at m/z 524 and predominant ions at m/z : 448 (rel. int. at 6.83 %), 420 (4.92); 153 (9.99); 139 (15); 111 (55.4); 97 (97.4); 83 (100); 69 (49); 57 (79.75) and 43 (20.11) respectively.

Acetate, mp. 67-69 °C, IR, (ν cm⁻¹) 1740 (C=C). Compound a proved to be a long chain branched alcohol.

Compound b :

White needles, soluble in benzene ether and methanol mp. 184-186 °C. It gave violet colour with Liebermann-Burchard's test²³.

This compound was identified as α -amyrin by co-chromatography and by mixed melting point with an authentic samples.

Compound c :

White needles, mp. 135-137 °C (Methanol). This substance was identified as β -sitosterol by co-chromatography and mixed melting point with an authentic samples. On spotting on TLC argentized silica gel G (system : Pet. ether-CHCl₃-HOAc, 75:25:0.5), only one spot appear after spraying with SbCl₃ in CHCl₃.

Compound d :

White needles, mp. 245-248 °C (MeOH). IR (ν cm⁻¹) 3420 (OH), 1640 (C=C) and 880. ¹H-NMR (300 MHz, CDCl₃) δ 4.664 (1H, d., J=2.4 Hz), 4.533 (1H, d., J=1.95 Hz), 4.273 (1H, d., J=5 Hz), 4.230 (1H, t.) and six singlets at δ 1.63, 0.975, 0.930, 0.873, 0.765 and 0.653 (3H each).

The singlets at δ 4.66 and 4.533 (1H each) and signal at δ 1.631 (3H, s.) suggested terminal methylene moiety and one olefinic methyl group²⁴.

MS showed [M⁺] at m/z 442 for C₃₀H₅₀O₂; 427 (rel. int. 8 %) for C₂₉H₄₇O₂ (M-CH₃) and 424 (32 %) for C₃₀H₄₈O (M-H₂O)²⁵⁻²⁶.

The above mentioned physical, chemical and spectral data of compound d, are similar to those reported for triterpene alcohol betulin²²⁻²³.

Compound e :

White needles, mp. 276-277 °C (methanol). IR (ν cm⁻¹) 3460 (OH), 3010, 2920, 2890 (C-H), 1640 (C=C) and 880. ¹H-NMR (300 MHz, DMSO) δ 12.10 (1H), 4.68 (1H, d., J=1 Hz), 4.562 (1H, s.), 4.280 (1H, d., J=1 Hz), five signals at δ 1.642, 0.929, 0.758, 0.645 (each of 3H) and 0.864 (6H, s.) for six methyl groups. Signals at δ 12.1 for carboxyl and at δ 4.28 for hydroxyl protons disappeared on addition of D₂O. The two signals at δ 4.562 and 4.684 (1H each) and singlet at δ 1.64 suggest terminal methylene and one olefinic methyl groups²⁴.

MS showed [M⁺] at m/z 456 corresponding to C₃₀H₄₈O₃, with a predominant ions at m/z 438 (15.3%) for C₃₀H₄₆O₂ (M-H₂O), 248 (23), 220 (10) 207 (52.7), 189 (81.8), 188 (14.6) and 43 (100).

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Acetate, white needles mp. 261-263 °C. IR ($\nu_{\text{cm}^{-1}}$) 3450 (OH) 1730 (C=O), 1690 (C=O), 1637 (C=C), 1250 (CHCOO⁻). MS [M⁺] at m/z 498.

From the above data it is concluded that compound e is betulinic acid. This was also confirmed by co-chromatography and mixed melting point with an authentic sample.

Compound f:-

White needles, mp. 279-280 °C. IR ($\nu_{\text{cm}^{-1}}$) 3400 (OH), 1690 (C=O), 1645 (C=C). ¹H-NMR (300 MHz, CDCl₃) δ 5.29 (1H), 3.23 (1H, m.) and seven singlets at δ 1.25, 1.14, 0.99, 0.932, 0.915, 0.779 and 0.769 for seven methyl groups. MS showed [M⁺] at m/z 456 corresponding to C₃₀H₄₈O₃ with a predominant ions at m/z 423 for C₂₉H₄₃O₂ (M-CH₃-H₂O). Acetate white crystals mp. 269-270 °C. IR ($\nu_{\text{cm}^{-1}}$) 3420 (OH), 1725 (C=O), 1705 (C=O), 1650 (C=C).

The physicochemical, spectral and co-chromatographic investigation with an authentic oleanolic acid showed that compound F was superimposed.

Investigation of chloroform extract of the leaves:-

The chloroform extract when examined by TLC (System: chloroform-MeOH, 9:1), revealed the presence of seven spots and was kept for further investigation.

Investigation of ethyl acetate extract of the leaves (Fraction B):-

TLC investigation of ethyl acetate (Fraction B), using cellulose plates and chloroform-methanol-water (75:23:2), revealed five flavonoidal spots with R_f values 63, 60, 44, 28 and 18 as revealed with AlCl₃ spray reagent²². Column chromatographic fractionation of (fraction B) on silica gel column was performed eluting

with ethyl acetate and then with mixture of ethyl acetate-methanol in an increasing polarities. The fractions collected (25 ml each) were screened by TLC on cellulose plates as mentioned above.

Four flavonoidal compounds were obtained F_1 , F_2 , F_3 and F_4 . Compound F_1 was in trace amount and was identified as kaempferol by co-chromatography with an authentic sample.

Compound F_2 :

Yellow needles mp. 316°C soluble in most organic solvents, insoluble in water. From the UV-spectrophotometric data with shift reagents (Table 1) and by co-chromatography with an sample it was concluded that this compound F_2 is quercetin.

Compound F_3 :

Yellow amorphous powder, mp. 233°C , soluble in water and methanol. The UV-spectrophotometric data (Table 1) proved that this substance is a flavonol glycoside.

Partial acid hydrolysis and complete acid hydrolysis indicated that it is a bioside giving kaempferol aglycone (AF_3) on complete acid hydrolysis (Table 2).

$^1\text{H-NMR}$ (300 MHz, DMSO) 7.98 (2H, d., $J = 8.6$ Hz) for H-2 and H-6, 6.87 (2H, d., $J = 8.6$ Hz) for H - 3 and H - 5, 6.1 (1H, bs.) for H - 8, 5.9 (1H, bs.) for H -6, 5.3 (1H, d.) , 3-3.5 (12H, m) for sugar protons.

The sugar moiety was identified as glucose. From the above data compound F_3 was found to be kaempferol-3, 7-0- diglucoside.

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Compound F₄ :

Amorphous yellow powder mp. 197 °C, soluble in water and methanol. UV-data (Table 1) showed that compound F₄ is a flavonol glycoside with a free OH at C-7. Mild and complete acid hydrolysis indicated that compound F₄ is a bioside, the sugar moiety was identified as glucose and the aglycone was identified as quercetin by UV data (table 2) and by co-chromatography with an authentic sample of quercetin. ¹H-NMR (300 MHz, DMSO) showed a δ at 7.56 (2H, m.) for H-2 and H-6, δ 6.83 (1H, d., J=9.0 Hz) for H-5, δ 6.388 (1H, d., J=2 Hz) for H-8, 6.188 (1H, d., J=2 Hz) for H-6, δ 5.463 (1H, d., J=7.3 Hz). δ 5.398 (1H, d.) δ 3-3.5 (12H, m.) for sugar protons.

From the above physicochemical and spectral data compound F₄ is identified as quercetin-3-O-diglucoside.

Investigation of the hexane and chloroform extracts of the bark :

Both the hexane and chloroform extracts (Fractions A & B) of the bark were examined by TLC in different solvent systems. Both contain a large number of spots and were kept for further investigation.

Investigation of ethyl acetate extract of the stem bark (Fraction C) :

TLC on silica gel G plates using chloroform-methanol-water (75:25:3) showed five brown spots R_f 97, 57.51.47 and 38 as visualized with 50 % alcoholic sulphuric acid as spray reagent.

Column Chromatography :

Twenty grams of fraction C was transferred on the top of silica gel column eluted with ethyl acetate and then with a mixture of ethyl acetate-methanol in increasing polarities.

Two compounds were isolated, TPB₁ and TPB₄.

TPB₁ was identified as oleanolic acid and was identical with compound F.

Compound TPB₄ :

White amorphous powder mp. 218°C(dec.). UV $\lambda_{\text{max}}^{\text{MeOH}}$: 222 sh. (Log ϵ 4.40), 243 (Log. 4.408), 302 (log 4.62) and 316 nm (Log. ϵ 4.690).

$\lambda_{\text{max}}^{\text{MeOH+NaOH}}$: 242 (Log. ϵ 4.408), 314 (Log ϵ 4.250) and 366nm (Log. ϵ 4.78) indicating a bathochromic shift (50).

IR ($\nu_{\text{cm}^{-1}}$) 3400-3450 (OH), 1705 (C=O), 1660, 1640 (C=C) for iridoids 1600, 1515 (aromatic system) 830 (p-substituted benzene).

¹H-NMR (300 MHz, DMSO) (Fig.1) and 2D-NMR (H-H) (Fig.2) showed δ 10.074 (1H,s.) for phenolic protons, 7.65 (1H,d., $J_{3,2} = 16$ Hz) for H-3, 7.6 and 6.81 (each d., $J=8.6$. A \bar{A} , B \bar{B} for H-5, H-9 and H-6, H-8), 6.48 (1H,d., $J_{2,3} = 16$ Hz) for H-2, 6.45 (1H,d., $J_{3,4} = 5.85$ Hz) for H-3, 6.43 (1H,dd.) for H-4, 5.17 (1H,d., $J_{1,2} = 9$ Hz) for H-1, 5.012 (1H,d., $J_{1,9} = 7.5$ Hz), 4.94 (1H,dd.) for H-6, 3.72 (1H,d., $J_{7,6} = 8.0$ Hz) for H-7, 3.06 (1H,m.) for H-5, 3.032 (1H,d., $J_{9,1} = 7.5$ Hz).

The sugar protons appear in region 4.608-5.182 and were confirmed by addition of D₂O. ¹³C-NMR is listed in (Table 3) as compared with that of specioside (P-coumaryl catalpol glucoside)¹², and is depicted in Fig.3.

MS showed [M⁺] at m/z 346 corresponding to aglycone and base peak at m/z 146 (Fig.4). Acid hydrolysis yielded glucose as a sugar moiety. Acetate of TPB₄ : needle crystals (MeOH) mp. 147°C, $\lambda_{\text{max}}^{\text{MeOH}}$: 226 and 284 nm. $\lambda_{\text{max}}^{\text{MeOH+NaOH}}$: 246, 366 (bathochromic shift 82 nm).

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IR ($\nu_{\text{cm}^{-1}}$) 1775 (broad band C=O groups) ; $^1\text{H-NMR}$ (300 MHz, CDCl_3) is listed in (Table 4) as compared with specioside acetate.

From physicochemical and spectral data of compound TPB_4 , it was proved to be identical with specioside (6-O-P-coumaryl catalpol glucoside) previously isolated from Tabebuia rosea DC.⁹

Cytotoxic activities of some isolated substances and their acetates :

In-vitro cytotoxic tests were carried out on some of the isolated substances and their acetates (Table 5) shows the effect of these substances on both P_{388} and KB cells (cell cultures).

From Table 5 it is indicated that betulinic acid showed an inhibitory effect on both P_{388} and KB cells, while its acetate showed inhibitory effect only on P_{388} cells, Specioside acetate had only moderate inhibitory effect on KB cells, while specioside showed no effect on both systems.

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Table 1:UV-Spectra of isolated flavonoids F_1 , F_2 and F_3 with
 different ionizing and complexing reagents.

Subs. Band		max											
No.		MeOH	+AlCl ₃	+AlCl ₃ +HCl	+NaOAc	+NaOAc+H ₃ BO ₃	+NaOMe						
		λ_{max}	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	
F_2	I	373	456	+83	430	+57	396	+23	392	+19	412	+39	
		302	330*		360,304		328,dec.		328				dec.
	II	257	273	+16	266	+9	273	+16	261	+4	255		
		270	303						288*		273	+16	
F_3	I	350	401	+51	397	+47	404	+54	406	+56	402	+52	
		314*	352		348		358		355		350		
	II	267	274	+7	275	+8	267	+3	266		270	+4	
F_4	I	362	432	+70	402	+40	390	+28	376	+14	412	+50	
		302*	335,306*		358		322*		302*				
	II	260,268*	267	+7	274,294*	+14	275	+15	265	+5	276	+16	

Table 2:UV-Spectra of the aglycones AF_3 and AF_4 with different
 ionizing and complexing reagents.

AF_3	I	372	432	+60	432	+60	390	+18	378	+6	412	+40	
		294*	350,307*		350,307*		332						334,dec.
	II	258	258	+7	272	+6	276	+9	263				
		267*							272	+5	276	+9	
AF_4	I	374	456	+82	426	+52	398	+24	288	+14	410	+36	
					362,304								333,dec.
	II	258	259	+1	266*	+9	276	+20	262	+4	255		
		272	271	+13	276	+16			288*		272*	+16	

*shoulder.

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Table 3: ^{13}C -NMR of substance TPB_4 as compared to that of specioside
(A) isolated from *Tabebuia rosea* DC.

Carbon atom	TPB_4	(A) ¹²
1	93.029	93.08 (d.)
3	141.168	141.08 (d.)
4	101.757	101.76 (d.)
5	35.135	35.23 (d.)
6	79.246	79.27 (d.)
7	58.278	58.35 (d.)
8	65.776	65.79 (s.)
9	42.822	42.82 (d.)
10	58.564	58.70 (t.)
1'	97.897	97.94 (d.)
2'	73.473	73.49 (d.)
3'	77.409	77.45 (d.)
4'	70.302	70.35 (d.)
5'	76.446	76.46 (d.)
6'	61.461	61.48 (t.)
1''	166.673	166.54 (s.)
2''	113.555	113.56 (d.)
3''	145.690	145.56 (d.)
4''	125.029	125.01 (s.)
5'', 9''	130.598	130.48 (d.)
6'', 8''	115.852	115.84 (d.)
7''	160.048	159.94 (s.)

Table 4: $^1\text{H-NMR}$ (300 MHz, CDCl_3) of acetate derivative of substance TPB₄ as compared to that of specioside acetate.

Assigned proton	TPB ₄ acetate δ ppm	Specioside acetate ¹² δ ppm (90 MHz, CDCl_3)		
H-3''	7.72 1H, d., $J_{2'',3''}=16.0$ Hz	7.85 1H, d., $J_{2'',3''}=16.0$ Hz		
H-5'', H-9''	7.56 { AA' BB' system 2H each, $J=8.6$ Hz.	7.39 } AA' BB', 2H each 7.07 }		
H-6'', H-8''				
H-2''	6.45 d., $J_{2'',3''}=16.0$ Hz	6.5 d., $J=16.0$ Hz		
H-3	6.33 dd., $J_{3,4}=6.0$ Hz $J_{3,5}=1.54$ Hz	6.33 d., $J=7.0$ Hz		
H-1, H-4, H-6 } H-10B	4.84 (m., 8H)	4.8 (m., 8H)		
H-1', H-2' } H-3', H-4' }				
2H-6'	4.26 2H, complex	4.26 (C, 2H)		
H _A -10	3.98 (d., $J=12.69$ Hz)	4.01 (d., $J=13.5$ Hz, 1H)		
H-7, H-5'	3.725 (2H, m.)	3.75 (2H, m.)		
H-9, H-5	2.692 (2H, m.)	2.55 (2H, m.)		
	2.725	2.75		
aromatic ac.	2.323 (3H, s.)	2.3 (3H, s.)		
aliphatic acetate.	2.134 (3H, s.) } 2.130 (3H, s.) }	2.1 (6H, s.)		
			2.055 (3H, s.) } 2.044 (3H, s.) }	2.05 (6H, s.)
	2.023 (3H, s.)	2.00 (6H, s.)		

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Table 5: Cytotoxic activity of some isolated substances and
their acetate*

No.	Substances	LD ₅₀ ug/ml.	LD ₅₀ ug/ml
		P ₃₈₈	KB
1	a	> 25	> 25
2	d (Betulin)	> 21	> 25
3	(Betulinic acid)	5.5	6.5
4	(Acetate of betulinic acid)	4.5	25
5	(TPB ₄ , Specioside)	25	25
6	(TPB ₄ acetate, Specioside acetate)	9.5	17.9

*Average of three determinations.

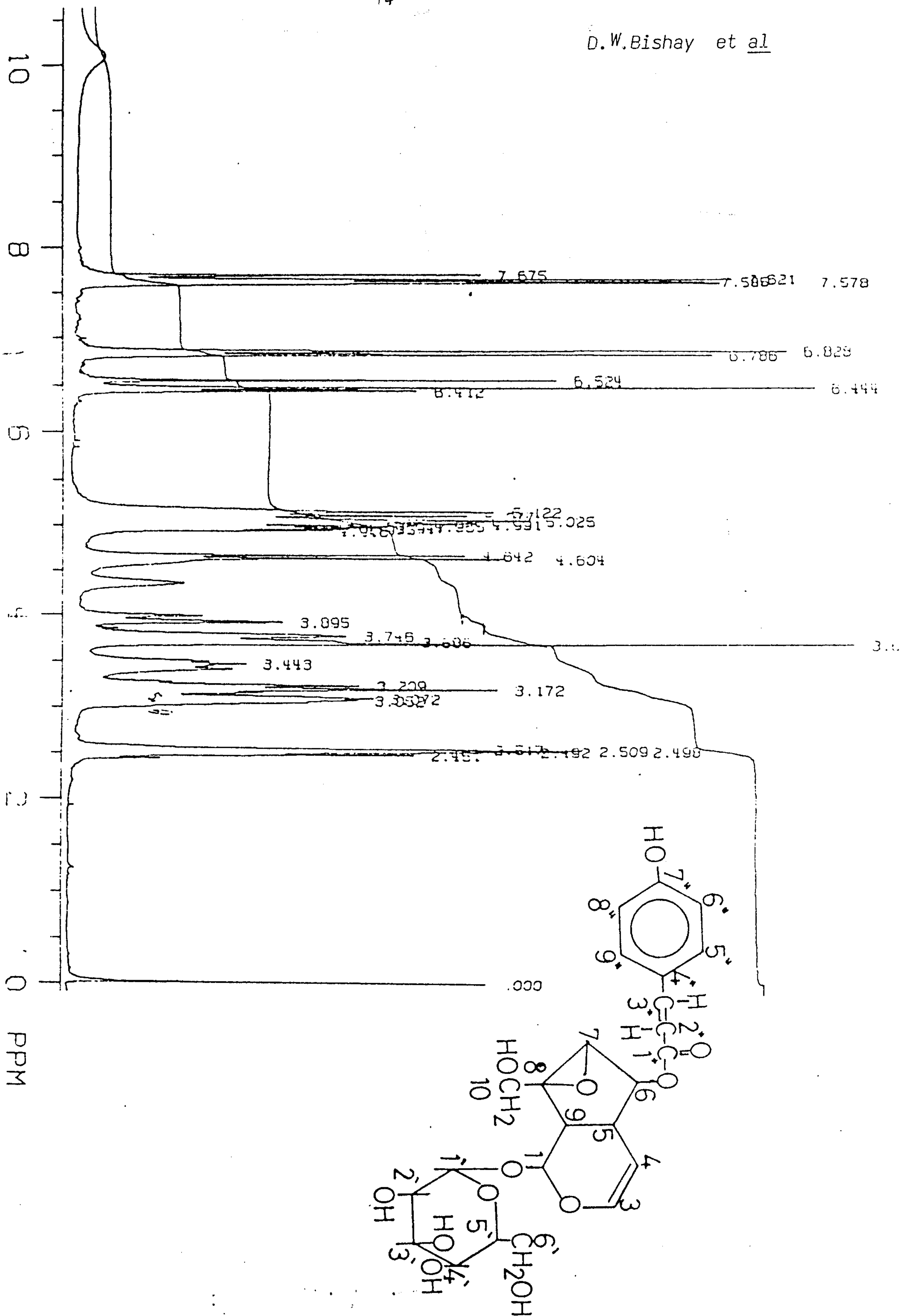


Fig.1 300 MHz ¹H-NMR of TPB₄

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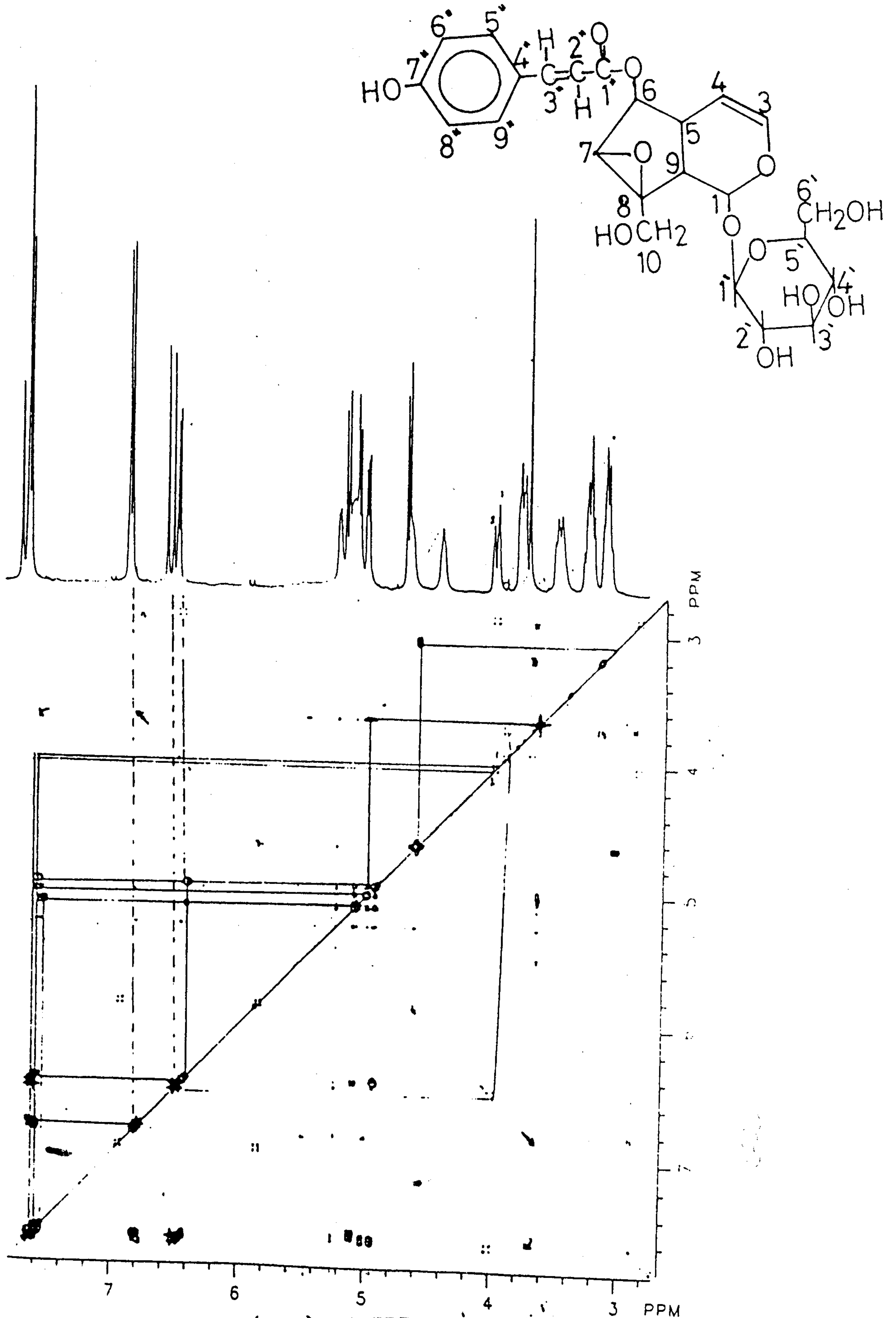


Fig.2 2D-NMR (H-H) of TPB₄

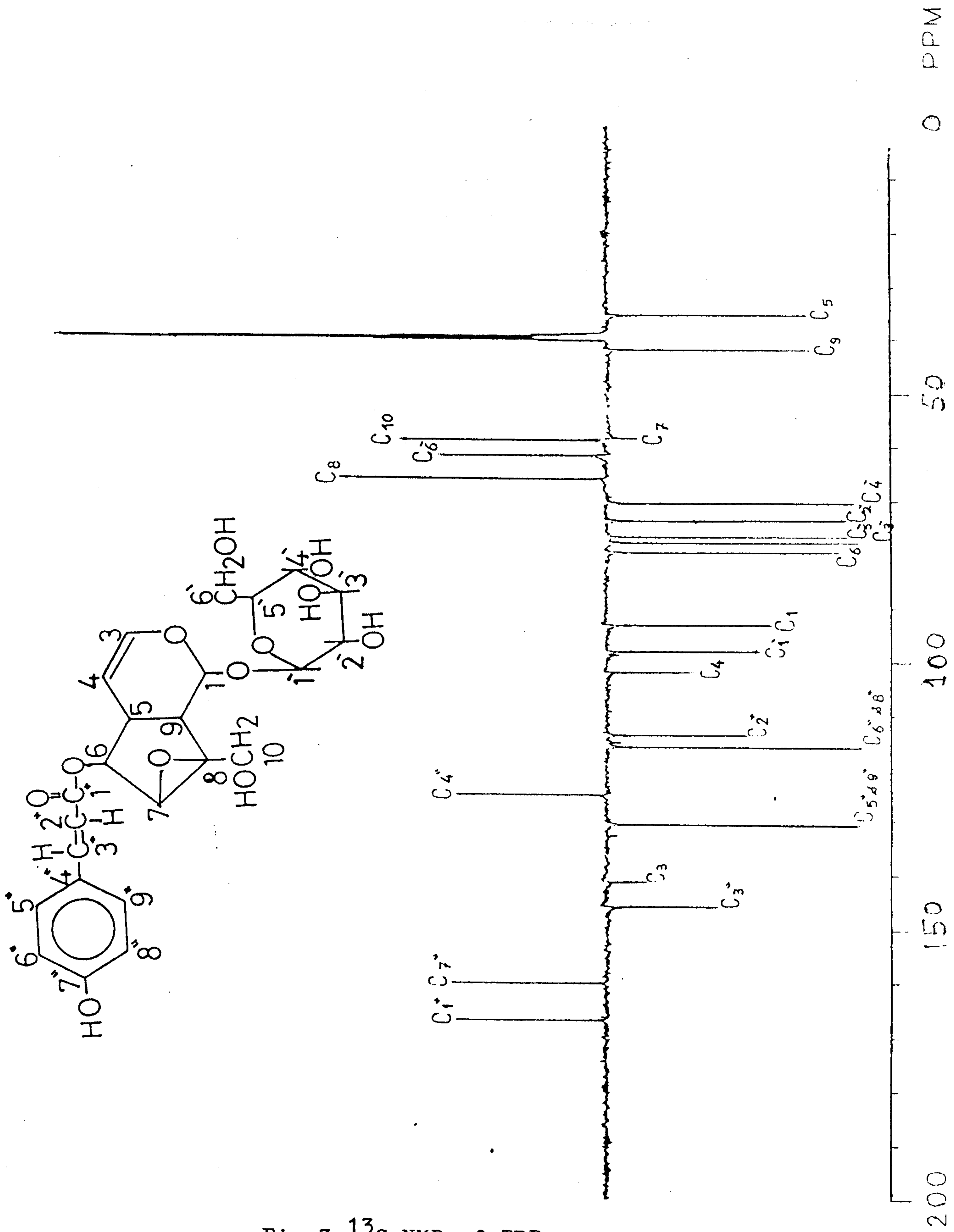
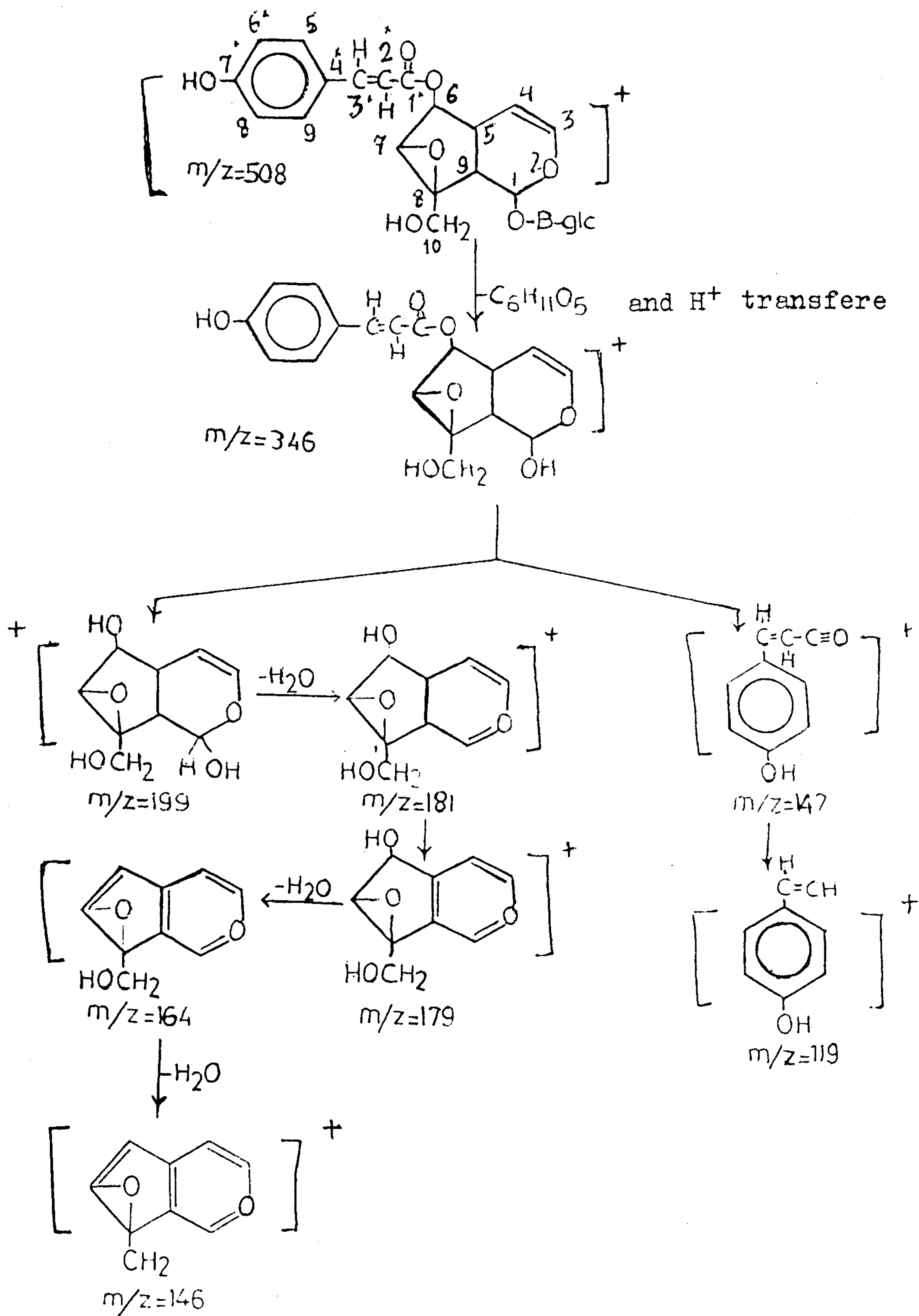


Fig.3 ¹³C-NMR of TPB₄

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Fig.4. Fragmentation pattern of specioside (TPB₄)



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دراسة الكيمياء العقاقيرية لنبات التابيبيا

بينتا فيلا هيمسل المنزرع فى مصر

داود ونيس بشاى - عفاف محمد عبدالباقى - سمير أنيس روس وزيدان زيد ابراهيم .

قسم العقاقير - كلية الصيدلة - جامعة أسيوط

تبين من المسح الكيمياءى الاولى لنبات التابيبيا بينتا فيلا هيمسل

وجود استيرولات و/او تربينات ثلاثية فلافونيدات وايرود ويدات .

من خلاصة البترول الايثيرى للاوراق تم فصل والتعرف على الفا- أميرين،

بيتا - سيتوستيرول - بيتيولين ، حمض البيتيولينيك وحمض الاوليانوليك .

ومن خلاصة الخللات الايثيليه للاوراق تم أيضا فصل والتعرف على

كامبيفيرول ، كوارستين ، كامبيفيرول ٣ - ٧ - أ ثنائى جليوكوزيد وكوارستين

٣ - أ ثنائى جليوكوزيد .

كذلك تم فصل والتعرف على السبيكيوزيد (٦ - أ - كوماريل كاتالبول)

وحمض الاوليانوليك من خلاصة الخللات الايثيليه للقلق .