Indian Journal of Chemistry
Vol.36B, September 1997, pp.787 - 792

Phytoecdysones from *Tinospora cordifolia:* Structural elucidation of Ecdysterone and makisterone A by 2D NMR spectroscopy

V D Gangan[*†], P Pradhan & A T Sipahimalani
Bio-Organic Division, Bhabha Atomic Research Centre, Bombay 400 085, India

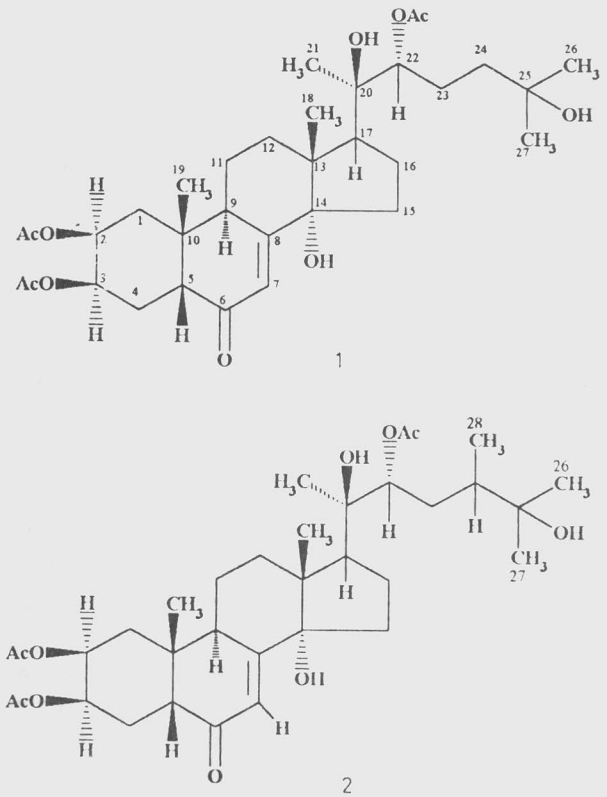
Received 10 July 1996; accepted 14 November 1996

The ***n***-BuOH fraction of the methanolic extract of *T. cordifolia* stems, has been acetylated and subjected to exhaustive chromatography (column, radial and preparative TLC). Two phytoecdysones, viz. ecdysterone **1** and makisterone A **2** have been isolated as their polyacetates. Their structures have been elucidated by extensive 1D and 2D NMR studies.

Tinospora cordifolia Miers (Menispermaceae), an important medicinal plant, cultivated throughout Indian subcontinent has been used, through centuries in Ayurvedic preparations, for treatment of various ailments[1,2]. More recently, Thatte and Dahanukar[3] have shown that the aqueous extracts of the plant were associated with the stimulation of phagocytic and bactericidal capacity of neutrophils and macrophages.

Chemical investigations of the plant have indicated the presence of several diterpene furan lactones[4], phenolic lignan[5], etc. Our previous studies, with the polar fraction of the plant, dealt with the isolation and characterisation of two phenylpropane glycosides[6], five norditerpene furan glycosides[7,8] and two diterpene furan glycosides[9]. This paper describes the isolation and characterisation of two phytoecdysones, viz. ecdysterone **1** and makisterone A **2** which were obtained as the minor components. Since **1** and **2** were required for bio-evaluation, their structures were determined essentially by non-destructive procedures, viz. high resolution 1D and 2D NMR spectroscopy. Although known from several plant sources[10], this constitutes the first report about their occurrence in any species of *Tinosporaceae* family and for their detailed characterisation based on 2D NMR techniques. All the proton and carbon resonances were assigned unequivocally using 2D NMR spectroscopy.

Following the procedure mentioned in our earlier publication[7], two phytoecdysones were isolated as their polyacetates (**1a, 1b** and **2a**) from the butanol fraction of the methanolic extract of the stem of the plant.



Scheme I

Compounds **1a** {m.p. 145°, $[\alpha]^{26}_D$ + 63.8° (MeOH, *c* 0.170)}, **1b** {m.p. 199°, $[\alpha]^{26}_D$ + 74.1° (MeOH, *c* 0.130)} and **2a** {m.p. 208° $[\alpha]^{26}_D$ + 60.0° (MeOH, *c* 0.160)} were colourless solids and showed similr colour reactions with $SbCl_3$ spray. Their UV

Present address : Hindustan CIBA-GEIGY Research Centre, Chemical Development, Aarey Road, Goregaon (E), Bombay 400 063, India

spera showed an absorpion maxima at 241 nm (ε 4.1) indicating the possibility of α, β— unsaturated carbonyl system. The IR spectra exhibited a strong characteristic absorption for hydroxyl groups at 3250-3600 cm^{-1}, several strong absorptions in the carbonyl region (1660-1750 cm^{-1}) and bands around 1217-1267 cm^{-1} indicative of acetoxy carbonyls and the possibility of α, β unsaturated ketone system. The FAB MS $[Na]^+$ of the compounds **1a**, **1b** and **2a** showed molecular ions at 629 $[M+Na]^+$, 671 $[M+Na]^+$ and 643 $[M+Na]^+$, thus indicating molecular weights of 606 $[M]^+$, 648$[M]^+$ and 620 $[M]^+$ respectively. Thus compared to **1a**, **1b** possessed an additional acetyl group and **2a** an additional methyl group. The UV, IR and NMR (^1H, ^{13}C) spectral evidence and comparison of the spectral data[10,11], indicated the compounds **1a** and **1b** to be identical to ecdysterone 2,3,22- triacetate, ecdysterone 2,3,22, 25-tetraacetate and compound **2a** to makisterone A 2,3,22- triacetate. The assignments of the protons attached to carbons are based on ^{13}C-^1H HETCOR experiment and differentiation into number of methyls, methylenes, methines and quaternary carbons were inferred from DEPT experiment. The unambiguous assignments of various C and H positions, were achieved on the basis of 2D experiments involving ^{13}C-^1H COLOC, ^1H-^1H COSY and ^1H-^1H NOESY experiments.

Ecdysterone 2,3,22 - triacetate 1a. The resonance signal at δ_H 5.89 showed a long range COSY interaction with methine protons at δ_H 2.42, 3.12 ascertaining the position of former at C-7 (δ_c 121.5) and the latter at C-5 (δ_c 50.9), C-9 (δ_c 33.5), respectively. The two methylenes at C-1, C-4 and two methines at C-2, C-3 were fixed on the basis of a chain of spin-spin couplings observed in COSY spectrum between H-5 (δ_H2.42) ↔ H-4 (δ_H 1.82; δ_c 29.1) ↔ H-3 (δ_H5.38; δ_c 67.0) ↔ H-2 (δ_H 5.09; δ_c 68.5) ↔ $H_{a,b}$-1 (δHa1.52, δH 1.82; 33.9).The proton H-1 did not show further COSY cross peaks and was therefore adjacent to quaternary C-10 (δ_c 38.2). The deshielding of C-2 and C-3 signified that these could be attached to oxygen bonded functionalities and thus supporting the presence of two secondary hydroxyl groups at these positions. The position of an angular methyl group at C-19 was fixed on the basis of COLOC experiment as cross interactions were observed between C-10 (δ_c 38.2) ↔ H-19 methyl (δ_H 1.01; δ_c 23.8), C-1 (δ_c 33.9) ↔ H-19 and C-5 (δ_c 50.9) ↔ H-19.COSY experiment wcs also useful in establishing the positions of two methylenes at C-11, C-12 as scalr couplings were observed between H-9 (δ_H 3.12) ↔ $H_{a,b}$-11 (H_a 1.48, H_b 1.80; δ_c 20.4) and H_b-11 (δ_H 1.80)eq ↔ H_b-12 (δ_H 2.07; δ_c 31.8). The proton 12 did not show further COSY cross peaks and was therefore adjacent to quaternary C-13 (δ_c 47.4). COLOC in conjunction with COSY experiments were also instrumental in establishing the positions of two methylenes at C-15, C-16 and methine at C-17 as cross interactions were observed between C-8 (δ_c 164.5) ↔H-15 (δ_H 1.85; δ_c 31.0), H-15 (δ_H 1.85) ↔ H-16 (δH 1.82; δ_c 20.4) ↔ H-17 (δ_H 2.32; δ_c 49.4). Proton H-15 and H-17 did not show further COSY cross peaks and were therefore adjacent to quaternary C-14 (δ_c 84.4) and C-13 (δ_c 47.4), C-20 (δ_c 77.0) respectively. The unusual downfield shift of C-14 and C-20 signified that these could be attached to oxygen bonded functionalities and thus supporting the possibility of tertiary hydroxyl groups at these positions. The positions of two angular methyl groups at C-18 and C-21 were assigned on the basis of COLOC interactions between C-14 (δ_c 84.4) ↔ H-18 methyl (δ_H 0.83;δ_c 17.3), C-17 (δ_c 49.4) ↔ H-21 methyl (δ_H 1.22; δ_c 21.0) and C-20 (δ_c 77.0) ↔ H-21 COSY experiment established the positions of two methylenes at C-23, C-24 and methine at C-22 on the basis of scalar coupling observed between H-22 (δ_H 4.82; δ_c 79.7) ↔ $H_{a,b}$-23 (H_a 1.56, H_b 1.78; δ_c 24.6) ↔ H-24 (δ_H 1.47; δ_c 40.3). The deshielding of C-22 suggested that it could be attached to oxygen bonded functionality and thus supporting the presence of the third secondary hydroxyl group at this position. The proton H-24 did not show further COSY cross peaks and was therefore adjacent to quaternary C-25 (δ_c 70.5). The two tertiary methyl groups were assigned the positions C-26 and C-27 as COLOC interactions were observed between quaternary C-25 (δ_c 70.5) ↔ H-26 methyl (δ_H 1.20; δ_c 28.4) and C-25 ↔ H-27 methyl (δ_H 1.21; δ_c 30.3). The downfield shift of C-25 signified that it could also be attached to oxygen bonded functionality and thus supporting the presence of tertiary hydroxyl group at C-25. Other COSY and COLOC interactions are as given in the Table I.

The relative stereochemistry of the compound was fixed on the basis of ^1H-^1H NOESY spectrum. The important nOe interactions observed were between H-2 (δ_H 5.09) ↔ H-9 (δ_H 3.12), H-3 (δ_H 5.38) ↔ H-4 (δ_H 1.82), H-5 (δ_H 2.42) ↔ H-19 methyl (δ_H 1.01),

Table I — 1D and 2D NMR data of ecdysterone 2,3,22-triacetate **1a** in $CDCl_3$

Pos.	δc	DEPT	δ_H*	COSY	NOESY	COLOC
1	33.9	$-CH_2-$	1.52(m,Ha)	H-2,Hb-1		H-19
			1.82(m,Hb)	H-2,Ha-1		
2	68.5	>CH-	5.09(m,1H)	H-3,Ha,b-1	H-9	Hb-1
3	67.0	>CH-	5.38(br s,1H)	H-2,H-4	H-4	
4	29.1	$-CH_2-$	1.82(m,2H)	H-3,H-5	H-3	
5	50.9	>CH-	2.42(m,1H)	H-4,H-7(1r)	H-19	H-19
6	202.0	>C=O				H-19
7	121.5	-CH=	5.89(br s,1H)	H-9 (1r),H-5 (1r)		
8	164.5	=C<				H-9,H-15
9	33.5	>CH-	3.12(m,1H)	Ha,b-11,H-7(1r)	H-2	.
10	38.2	>C<				H-19,Hb-1
11	20.4	$-CH_2-$	1.48(m,Ha)	H-9		.
			1.8(m,Hb)	H-9,H_b-12		.
12	31.8	$-CH_2-$	1.59(m,Ha)	H_b-12		
			2.07(m,Hb)†	Ha-12,Hb-11	H-18	.
13	47.4	>C<				.
14	84.4	>C<				H-7,H-18
15	31.0	$-CH_2-$	1.85(m,2H)	H-16		
16	20.4	$-CH_2-$	1.82(m,2H)	H-15,H-17		
17	49.4	>CH-	2.32(m,1H)	H-16	H-22	H-21,Hb-12
18	17.3	$-CH_3$	0.83(s,3H)		H_b-12	
19	23.8	$-CH_3$	1.01(s,3H)		H-5	
20	77.0	>C<				H-21
21	21.0	$-CH_3$	1.22(s,3H)			
22	79.7	>CH-	4.82(dd,8.6,1,1H)	Ha,b-23	H-17,H-24	H-21
23	24.6	$-CH_2-$	1.56(m,Ha)	H-22,H-24		
			1.78(m,Hb)	H-22,H-24		
24	40.3	$-CH_2-$	1.47(m,2H)	Ha,b-23	H-22	
25	70.5	>C<				H-26,H-27
26	28.4	$-CH_3$	1.20(s,3H)			
27	30.3	$-CH_3$	1.21(s,3H)			
OCOMe	170.2	>C=O				
	170.4	>C=O				
	172.4	>C=O				
OCOMe	21.0×3	$3\times CH_3$	2.1(s,9H)			

*Proton assignments are based on ^{13}C - ^{1}H HETCOR and ^{1}H - ^{1}H COSY spectra.

† Signal overlapping with acetyl methyl protons.

H_b-12 (δ_H 2.07) ↔ H-18 methyl (δ_H 0.83), and H-17 (δ_H 2.32) ↔ H-22 (δ_H 4.82) ↔ H-24 (δ_H 1.47). No nOe interactions were observed between (H-2, H-9), (H-3, H-4) pairs with (H-5, H-19), (H_b-12, H-18), (H-17, H-22), (H-22, H-24) pairs, indicating that formers were on one side of the molecule whereas latters on other side of the molecule. The important nOe interactions are as given in Table I.

Ecdysterone 2,3,22,25-tetraacetate 1b The assignments of methyls, methylenes and methines were made on the basis of 2D experiments. Compound **1b** showed similar types of cross peak patterns as indicated for **1a**.

On comparing the ^{13}C chemical shift values of **1a** with those of **1b** at position C-25 indicated that comparative downfield shift was observed in **1b** (δc

Table I — 1D and 2D NMR data of ecdysterone 2,3,22-triacetate **1a** in $CDCl_3$

Pos.	δc	DEPT	δ_{H}*	COSY	NOESY	COLOC
1	33.9	$-CH_2-$	1.52(m,Ha)	H-2	Hb-1	
			1.82(m,Hb)	H-2,Ha-1		
2	68.5	>CH-	5.09(m,1H)	H-3,$H_{a,b}$-1	H-9	H_b-1
3	67.0	>CH-	5.38(br s,1H)	H-2,H-4	H-4	
4	29.1	$-CH_2-$	1.82(m,2H)	H-3,H-5	H-3	
5	50.9	>CH-	2.42(m,1H)	H-4,H-7(1r)	H-19	H-19
6	202.0	>C=O				H-19
7	121.5	-CH=	5.89(br s,1H)	H-9 (1r),H-5 (1r)		
8	164.5	=C<				H-9,H-15
9	33.5	>CH-	3.12(m,1H)	Ha,b-11,H-7(1r)	H-2	
10	38.2	>C<				H-19,Hb-1
11	20.4	$-CH_2-$	1.48(m,Ha)	H-9		
			1.8(m,Hb)	H-9,H_b-12		
12	31.8	$-CH_2-$	1.59(m,Ha)	H_b-12		
			2.07(m,Hb)†	Ha-12,Hb-11	H-18	
13	47.4	>C<				
14	84.4	>C<				H-7,H-18
15	31.0	$-CH_2-$	1.85(m,2H)	H-16		
16	20.4	$-CH_2-$	1.82(m,2H)	H-15,H-17		
17	49.4	>CH-	2.32(m,1H)	H-16	H-22	H-21,Hb-12
18	17.3	$-CH_3$	0.83(s,3H)		H_b-12	
19	23.8	$-CH_3$	1.01(s,3H)		H-5	
20	77.0	>C<				H-21
21	21.0	$-CH_3$	1.22(s,3H)			
22	79.7	>CH-	4.82(dd,8.6,1,1H)	Ha,b-23	H-17,H-24	H-21
23	24.6	$-CH_2-$	1.56(m,Ha)	H-22,H-24		
			1.78(m,Hb)	H-22,H-24		
24	40.3	$-CH_2-$	1.47(m,2H)	Ha,b-23	H-22	
25	70.5	>C<				H-26,H-27
26	28.4	$-CH_3$	1.20(s,3H)			
27	30.3	$-CH_3$	1.21(s,3H)			
OCOMe	170.2	>C=O				
	170.4	>C=O				
	172.4	>C=O				
OCOMe	21.0×3	3×CH_3	2.1(s,9H)			

*Proton assignments are based on ^{13}C-^{1}H HETCOR and ^{1}H-^{1}H COSY spectra.

† Signal overlapping with acetyl methyl protons.

81.7) as compared to **1a** (δc 70.5). This argument could be explained by considering that under normal acetylating condition, the tertiary OH at C-25 also gets acetylated and responsible for causing such deshielding which was in agreement with literature values[10].

Makisterone A 2,3,22-triacetate 2a. The assignment of the structure **2a** was also based on 1D and 2D NMR data. Compound **2a** also displayed similar types of cross peak patterns as indicated for **1a** and **1b** and shown in Table II.

The only difference between **1a** and **2a** was that the latter contained an additional carbon as compared to the former and was assigned C-24 in the form of secondary methyl group (δ_H 0.91, d,J = 6.2 Hz, 3H).

Experimental Section

General. Mps are uncorrected. Optical rotations were recorded on a JASCO polarimeter, (DIP-370). IR spectra were scanned as KBr pellets on a Shimadzu FTIR spectrometer (model-8101). UV spectra were recorded on a Shimadzu UV-visible spectrophotometer (model Graphicord UV-240) using spectroscopic grade MeOH as the solvent. ^1H and ^{13}C NMR spectra were recorded at 200 and 50 MHz respectively on a Bruker AC 200 spectrometer. All the 2D experiments were carried out with 0.001 *M* solutions in $CDCl_3$. For DEPT, signal editings were done by varying the pulse width of the last polarisation pulse as 14.5, 29 and 43.5 μs. In COLOC, 512 transients were recorded for each t_1 experiment of 80 increments. It was then zero-filling to 256W. In the W_2 dimension (^{13}C) 4K data points were recorded without zero-filled. The delays used were: D_1 = 1s, D_2 = 0.030s and D_3 = 0.015s. The NOESY and COSY spectra were recorded with following parameters; TD_1 = 256W, SI_1 = 512W and SI = TD = 1K with square sine bell multiplications in both dimensions. For NOESY a mixing time of 1 s was used.

Isolation of ecdysterone 2,3,22-triacetate 1a, ecdysterone 2,3,22,25-tetraacetate 1b and makisterone A 2,3,22-triacetate 2a. The plant material (fresh wt., 5.8 kg was collected from Trombay campus, Bhabha Atomic Research Centre, Bombay and identified by Dr V Abraham of the Nuclear Agriculture Division, BARC. Fresh stems were subjected to cold extraction with MeOH through percolation. The non-polar compounds were removed by extraction with petrol and EtOAc. It was then extracted with n-BuOH and subjected to column chromatography over silica gel, using increasing polarities of MeOH in $CHCl_3$. The crude fractions 2 and 3 were acetylated using Ac_2O and pyridine at room temperature for 48 hr. Further fractionation was achieved by repeated radial chromatography of the acylated crude fractions as well as by preparative TLC. This resulted in the isolation of two more compounds, in addition to previously reported cordifolisides A,B,C[7], cordifolisides D,E[8] and palmatosides C,F[9]. These were ecdysterone **1** and makisterone A **2** and were characterized as ecdysterone 2,3,22 triacetate **1a**, ecdysterone 2,3,22,25 – tetraacetate **1b** and makisterone A 2,3,22-triacetate **2a**, respectively.

Chromatographic system. Thin layer chromatography: TLC plates were prepared using silica gel G (Acme, Bombay). $CHCl_3$- MeOH (97:3) was used as the solvent system. Detection was done by spraying with 20% $SbCl_3$ ($CHCl_3$) and heating (100°C, 10 min., yellowish spot under long UV).

Radial chromatography: The circular glass plates of thickness 1 mm, were prepared by using silica gel (PF_{254}, E. Merck, 50 g) in cold distilled water (105 mL). For elution, gradually increasing concentrations of MeOH in $CHCl_3$ were employed.

Ecdysterone 2,3,22 — triacetate 1a. Colourless solid (10 mg); R_f 0.36 ($CHCl_3$ - MeOH, 97:3); $C_{33}H_{50}O_{10}$; m.p. 145°; $[\alpha]_{D26}$ +63.8° (MeOH, *c*0.170); IR (KBr): 3525, 3496, 2967, 2876, 1750, 1730, 1659, 1626, 1383, 1368, 1318, 1267, 1250, 1146, 1125, 1090, 1048, 1030, 974, 947, 907, 874, 841, 804, 668, 552, 521cm^{-1}; UV (MeOH): 229.6 sh (ε 3790), 241.2 sh (ε 3228) nm; ^1H NMR (200 MHz, $CDCl_3$) and ^{13}C NMR (50 MHz, $CDCl_3$): see Table I, FAB MS: m/z 629 $[M + Na]^+$, 607 $[M + H]^+$, 606 $[M]^+$, 590, 589, 571, 511, 493, 460, 437, 409, 387, 327, 307, 289, 283, 213, 155, 154, 136, 120, 107, 89, 77, 69, 56.

Ecdysterone 2,3,22,25 — tetraacetate 1b. Colourless solid (10 mg); R_f 0.77 ($CHCl_3$ - MeOH, 97:3); $C_{35}H_{52}O_{11}$; m.p. 199°; $[\alpha]_D^{26}$ + 74.1É (MeOH, c 0.130); IR (KBr): 3450, 2934, 2855, 1740, 1661, 1507, 1449, 1372, 1320, 1244, 1217, 1146, 1048, 992, 949, 882 cm^{-1}; UV(MeOH): 226 sh (ε 5533), 246 sh (ε 3494)nm; ^1H NMR (200 MHz, $CDCl_3$): 0.83 (s, 3H, CH_3, H-18), 1.01(s, 3H, CH_3, H-19), 1.21 (s, 3H, CH_3, H- 21), 1.38 (s, 3H, CH_3, H-26), 1.41(s, 3H, CH_3, H-27), 1.45 (m, H_a-23), 1.54 (m, H_a-1), 1.59 (m, H_b-15), 1.60 (m, H_b-23), 1.61(m, H_b-12), 1.65 (m, H_a-11), 1.71(m, H_b-4), 1.76 (m, 2H, H-24), 1.8

(m, H_b-15), 1.60 (m, H_b-23), 1.61(m, H_b-12), 1.65 (m, H_a-11), 1.71(m, H_b-4), 1.76 (m, 2H, H-24), 1.8 (m, H_b-11), 1.81 (m, H_a-4), 1.84 (m, H_b-1), 1.87 (m, H_a-15), 1.96 (s, 3H), 1.98 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H) (4 ×-$OCOCH_3$), 1.98 (m, H_a-12), 2.04 (m, 2H, H-16), 2.33 (m, 1H), 2.38 (m, 1H), 3.12 (m, 1H), 4.82 (dd, J = 9.8, 1.5 Hz, 1H), 5.07 (m, 1H), 5.33 (br s, 1H), 5.84 (br s, 1H). Proton assignments are based on ^{13}C-^1H HETCOR and ^1H-^1H COSY spectra. ^{13}C NMR (50 MHz, $CDCl_3$): 17.4 (C-18), 20.3 (C-11), 20.5 (C-16), 21.0 (3 ×-OCO*C*H_3), 21.1 (C-21), 22.3 (1 × -OCO*C*H_3), 23.7 (C-19), 24.5 (C-23), 25.9 (C-26), 26.2 (C-27), 29.1 (C-4), 31.0 (C-15), 31.6 (C-12), 33.5 (C-9), 33.9 (C-1), 37.4 (C-24), 38.2 (C-10), 47.4(C-13), 49.5 (C-17), 50.8 (C-5), 66.9 (C-3), 68.5 (C-2), 76.8(C-20), 79.3(C-22), 81.7 (C-25), 84.4(C-14), 121.5(C-7), 164.5(C-8), 170.2, 170.5 (2 x >*C*= O), 172.3 (four acetate carbonyls -O*C*OCH_3), 202.0 (C-6); FAB MS: m/z 671 $[M + Na]^+$, 649 $[M + H]^+$, 631, 589, 571, 553, 529, 511, 469, 493, 469, 447, 429, 409, 387, 363, 334, 154, 137, 107, 77, 52.

Makisterone A 2,3,22-triacetate 2a. Colourless solid (12 mg); R_f 0.33 ($CHCl_3$ - MeOH, 97:3); $C_{34}H_{52}O_{10}$; m.p. 208°; $[\alpha]_D^{26}$ + 60.0° (MeOH, *c* 0.160); IR (KBr): 3525, 2971, 2944, 2878, 1736, 1655, 1559, 1541, 1507, 1447, 1372, 1320, 1242, 1219, 1144, 1121, 1048, 1030, 992, 953, 922, 874, 606 cm^{-1}; UV (MeOH): 226 sh (ε 3067), 246 sh (ε 1631)nm; ^1H NMR (200 MHz, $CDCl_3$) and ^{13}C NMR (50 MHz, $CDCl_3$): see Table II; FAB MS m/z: 643 $[M + Na]^+$, 621 $[M + H]^+$, 620 $[M]^+$, 585, 567, 543, 525, 507, 483, 465, 441, 437, 408, 388, 327, 285, 237, 191, 165, 154, 136, 107, 89, 77, 69, 56.

Acknowledgement

We thank Dr O Seligmann and Prof Dr H Wagner of the Institute of Pharmaceutical Biology, University of Munchen, Germany for recording the FAB MS. One of the authors (V D G) thanks the Department of Atomic Energy for the award of senior research fellowship.

References

1 Kirtikar K R & Basu B D, In: *Indian medicinal plants*, edited by E Blatter, J R Cauis and K S Mhaskar Vol. I (Lalit Mohan Basu, Allahabad, India) **1933**, p.77.

2 Nayampalli S, Ainapore S S & Nadkarni P M, *Indian J Pharmacol*, 14, **1982**, 64.

3 Thatte U M & Dahanukar S A, *Phytotherapy Res*, 3, **1989**, 43.

4 Bhatt R K & Sabata B K, *Phytochemistry*, 28, **1989**, 2419.

5 Hanuman J B, Mishra A K & Sabata B K, *J Chem Soc, Perkin Trans-I*, **1986**, 1181.

6 Sipahimalani A T, Norr H & Wagner H, *Planta Medica*, 60,**1984**, 596.

7 Gangan V D, Pradhan P, Sipahimalani A T & Banerji A, *Phytochemistry*, 37, **1994**, 781.

8 Gangan V D, Pradhan P, Sipahimalani A T & Banerji A, *Phytochemistry*, 39, **1995**, 1139.

9 Gangan V D, Pradhan P, Sipahimalani A T & Banerji A, *Indian J Chem*, 35B, **1996**, 630.

10 Bandra B M R, Jayasinghe L, Karunaratne V, Wannigama G P, Bokel M, Kraus W & Sotheeswaran, *Phytochemistry*, 28, **1989**, 1073 and the references cited therein.

11 Miller R W, Clardy J, Kozlowski J, Mikolajczak K L, Platter R D, Powell R G, Smith R, Weisleder D & Zheng Qi-Tai, *Planta Medica*, **1984**, 40.