Indian Journal of Chemistry
Vol.36B, May 1997, pp. 424 - 433

New withanolides from the roots of *Withania somnifera*

A S R Anjaneyulu* & D Satyanarayana Rao
School of Chemistry, Andhra University, Visakhapatnam 530 003, India

Received 16 September 1996; accepted 25 November 1996

The non-basic fraction of the benzene and ethyl acetate extracts of the roots of *Withania somnifera* furnish six withanolides, of which three are new, in addition to β-sitosterol, its 3-*O*-β-D(+)-glucoside and linear benzo[6:7]chroman. The structures of new withanolides, withasomniferols A to C have been established respectively as 5α, $20\alpha_F$*(R)*, 27-trihydroxy-6α,7α-epoxy-1-oxowitha-2,24-dienolide **5**, 5α,$20\alpha_F$*(R)*-dihydroxy-6α,7α-expoxy-1-oxowitha-2-enolide **6** and 5α,14α,$20\alpha_F$*(R)*-trihydroxy-1-oxowitha-2,7,24-trienolide **7** by a study of their physical and spectral (UV, IR, ^{1}H and ^{13}C NMR and Mass) characteristics.

Withania somnifera is well known for its medicinal properties[1]. Various parts of the plant have been extensively examined for their chemical constituents. A large number of withanolides, steroidal lactones have been reported besides a variety of other products[2-29]. The pharmacological activity of its roots is attributed to the presence of alkaloids. We present here our results on the chemical examination of the roots of *W. somnifera* roots collected from Tamilnadu region.

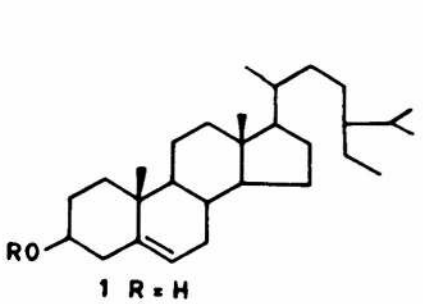
The roots were powdered and extracted with methanol. The total methanolic extract was concentrated under reduced pressure and the residue fractionated into benzene, ethyl acetate and methanolic extracts. The benzene extract was concentrated to a small volume and then washed with 1% aqueous HCl to remove basic substances. The non-basic portion after fractional crystallisation from $CHCl_3$-MeOH (1:1) furnished two solids which on chromatographic separation over silica gel columns furnished altogether nine compounds (**1-9**). Compounds **1** and **8** were characterised as β-sitosterol and its 3-*O*-β-D(+)-glucoside respectively by comparing their spectral data with those given in the literature and also by direct comparison. Compound **9** was identified as linear benzo[6:7]chroman. The remaining six compounds were found to be withanolides of which compounds **5, 6** and **7** were found to be new withanolides whose structures were established by a study of their spectral data.

Compound **2**, $C_{28}H_{38}O_5$, M^+ 452, m.p. 261-65°, $[\alpha]_D$+33.38° (*c* 0.99, $CHCl_3$) could be readily identified as the known withanolide, lycium substance-B by comparison of their physical and spectral data (Tables I and II). This was previously isolated from the leaves of *Lycium chinense*[20] and also very recently from *Datura* species[31].

Compound **3**, $C_{28}H_{38}O_6$, m.p. 313-14°, $[\alpha]_D$+80.0° (*c* 1.25, $CHCl_3$) was characterised as 5α,$20\alpha_F$ *(R)*-dihydroxy-6α, 7α-epoxywitha-2,24-dienolide by a comparison of its physical and spectral data (Table I) with those reported in literature. This was previously isolated from the roots of *W. coagulans*[32] and *W. somnifera*[29].

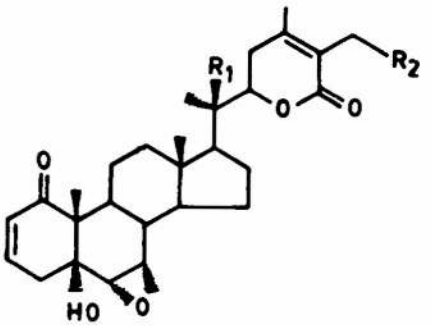
Compound **4**, $C_{28}H_{36}O_6$, M^+ 470, m.p. 277-79°, $[\alpha]_D$+120.22° (*c* 1.32, $CHCl_3$) was identified as another known withanolide, 5α,27-dihydroxy-6α,7α-epoxy-1-oxowitha-2,24-dienolide by comparing the physical and spectral characteristics (Tables I and II) of its acetate with those of the natural acetate isolated earlier from the leaves of *W. somnifera*[3] and from *W. coagulans*[33].

Compound **5** was obtained from chloroform-methanol eluates as colourless needles, m.p. 271-73°, $[\alpha]_D$+74.88° (*c* 0.44, $CDCl_3$). From elemental analysis and mass ion at m/z 468 (M^+–18) in its EIMS, its molecular formula was fixed as $C_{28}H_{38}O_7$. An examination of its physical and spectral characteristics revealed it to be a new withanolide which was named as withasomniferol A. It exhibited absorptions due to hydroxyl (broad band 3600-3400



1 R = H

8 R = B - D (+) glucose

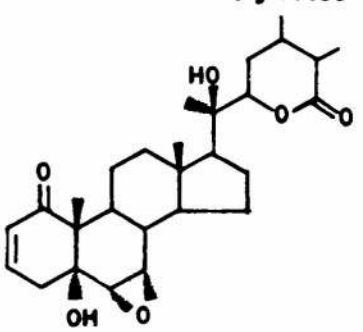


2 R_1 = H, R_2 = H

3 R_1 = OH, R_2 = H

4 R_1 = H, R_2 = OH

5 R_1 = R_2 = OH



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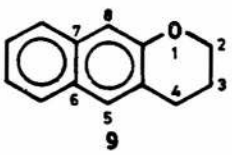

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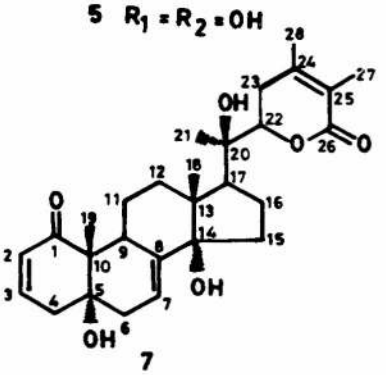

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Table I—^{1}H NMR (90 MHz) spectra of withanolides 2-7

Assignment	2†	3†	4†	5†	5*	6†	6*	7†	7*
18-CH_3	0.79 s(7)	0.95 s	0.78 s	0.99 s	0.93 s	1.15-1.25	1.15 s	0.75	0.83
19-CH_3	1.19 s	1.16 s	1.19 s	1.2 s	1.09 s	1.15-1.25	1.21 s	1.12 s	1.12 s
21-CH_3	0.93 d(7)	1.30 s	1.02 d(7)	1.3 s	1.27 s	0.92 s	0.92 s	1.30 s	1.36 s
27-CH_3	1.88 s	1.96 s	4.4 br s	4.4 br s	4.32 br s	1.15-1.25	1.9 d(6)	1.90 s	2.01 br s
			-CH_2OH	-CH_2OH	-CH_2OH				
28-CH_3	1.92 s	2.02 s	2.02 br s	2.1 s	1.91 s	1.15-1.25	1.15 d(6)	1.96 s	
H-22	4.4 dt(12,4)	4.19 dd(12,4)	4.5 m	4.3 m	4.21 m	4.14 m	4.1 m	4.21 m	4.3 m
H-6	3.02 d(4)	3.01 d(4)	3.02 d(4)	3.08 d(4)	2.9 d(4)	3.0 d(4)	2.91 d(4)	-	-
H-7	3.3 d/d(4,1)	3.29 dd(4,1)	3.3 dd(4,1)	3.3 dd(4,1)	3.17 m	3.29 dd(4,1)	3.15	5.09 dd(6,2)	5.14 dd(6,2)
H-2	5.83 d(10)	5.8 d(10)	5.85 d(10)	5.9 d(10)	5.8 m	5.8 d(10)	5.82 d(10)	5.97 d(10)	6.04 d(10)
H-3	6.6 m	6.5 m	6.6 m	6.65 m	‡	6.65 m	‡	6.73 m	‡
-OH		3.11 s				3.45 s	3.35 s	3.45 s	3.54 s

†in $CDCl_3$; * in $CDCl_3$+C_6D_6; ‡Merged with benzene peak

cm^{-1}), α,β-unsaturated six-membered ketone (1695 cm^{-1}) and α,β-unsaturated lactone (1710 cm^{-1}) functions in its IR spectrum. Its UV absorption at 225 nm supported the conjugated functionalities. On acetylation with pyridine and Ac_2O it yielded a monoacetate whose IR spectrum still showed hydroxylic absorption (3400 cm^{-1}) indicating the presence of either hindered secondary or tertiary hydroxyl group. Only one methyl on the double bond appeared at δ 2.1 in the ^{1}H NMR spectrum suggesting that the C_{27} methyl might be in the oxidised form as hydroxymethyl as in compound **4**. The hydroxymethyl protons appeared at δ 4.4 (br s). The 18-, 21- and 19-methyls appeared as singlets at δ 0.99, 1.3 and 1.2, respectively. The appearance of 21-methyl at δ 1.3 as a singlet suggested its attachment to an oxygenated carbon bearing a hydroxyl group as in compound **3**. The signals at δ

Table II—^{13}C NMR (25 MHz) spectra of withanolides **2**, **4** and **7**

Carbon No.	2[31]	4	7 (DMSO-d_6)
1	203.0	203.01	203.6
2	129.1	128.67	127.6
3	139.5	139.67	139.3
4	36.8	36.01	35.22
5	73.3	73.21	74.24
6	56.2	57.30	24.06
7	57.3	57.13	120.41
8	35.8	36.01	144.48
9	35.6	35.70	35.22
10	51.1	51.49	50.7
11	21.9	21.94	20.29
12	39.9	39.80	43.88
13	43.6	47.50	43.88
14	52.0	51.73	69.0
15	23.6	23.48	22.29
16	27.3	27.48	21.35
17	51.0	50.90	54.18
18	12.1*	12.09	12.29
19	14.7	14.78	16.29
20	39.2	38.8	76.06
21	13.3*	13.21	13.82
22	78.3	75.88	81.12
23	29.7	29.71	31.06
24	149.0	153.53	150.77
25	122.1	125.53	120.41
26	167.0	166.90	166.13
27	12.5	56.36	12.29
28	20.4	20.02	20.70

*Values interchangeable

3.08 (d, J=4 Hz) and 3.3 (d/d, J=4, 1 Hz) accounted for the geminal protons of 5α-OH-6α,7α-epoxy system as found in compound **4**. The solvent induced shift of 0.11 ppm $\Delta^{CDCl_3}_{C_6H_6}$ 1.2-1.09 ppm) of 19-CH_3 (cf. Table I) suggested A/B ring junction as *trans*. I ts CD spectrum was not good enough to derive any conclusion.

Availability of compound in very small quantity prevented obtaining its ^{13}C NMR data. However, from the foregoing information the structure of withasomniferol A could be tentatively established as 5α,20α$_F$*(R)*, 27-trihydroxy-6α,7α-epoxy-1-oxowitha-2,24-dienolide **5**.

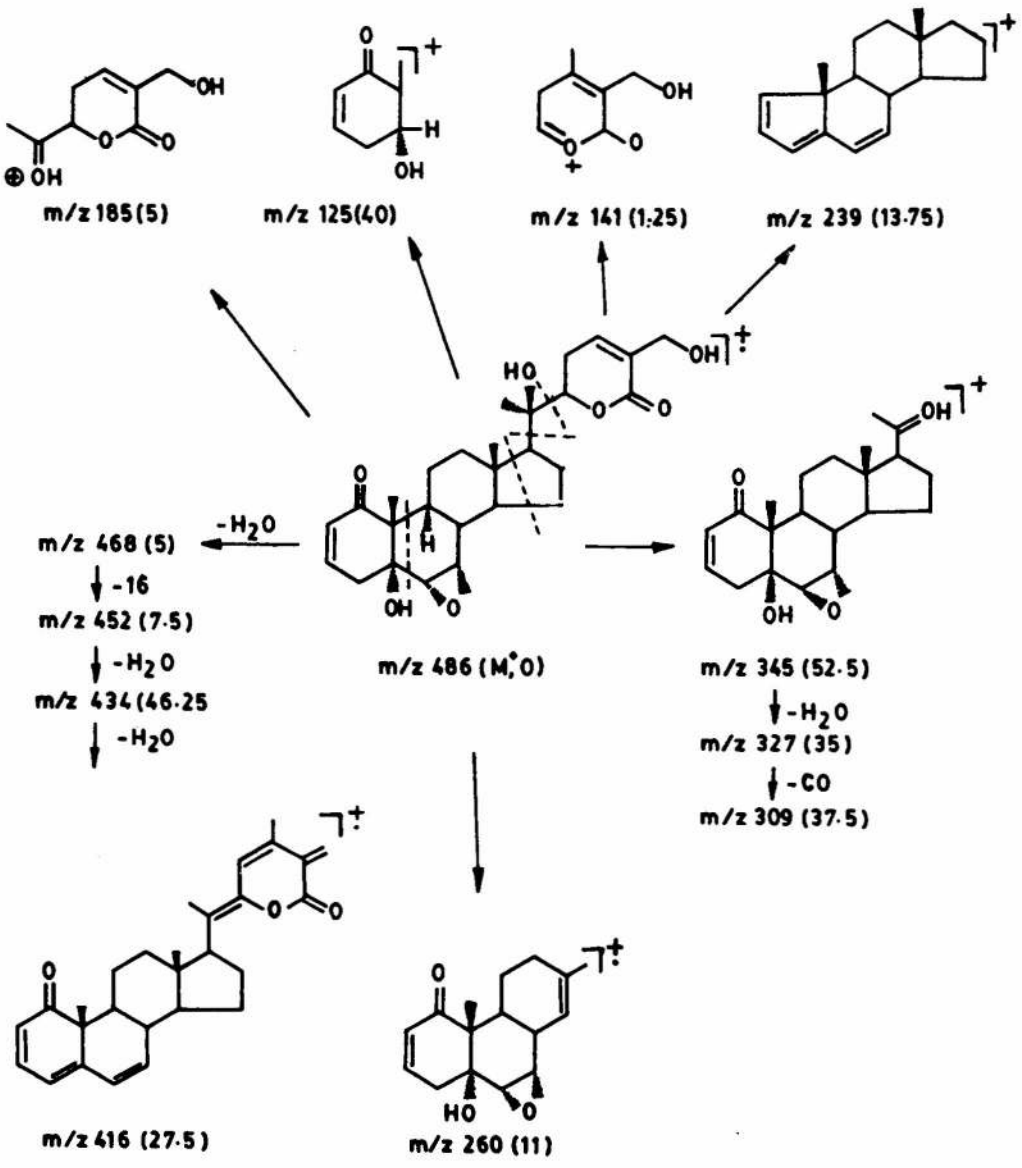
The mass spectral fragmentation given in detail (cf. Scheme I) supported its structure. The molecular ion (m/z 486) could not be noticed in its mass spectrum, and only the ion found by the loss of water appeared at m/z 468 (M^+ –18). The presence of a characteristic peak at m/z 125 (40%) confirmed that the compound contains 5α-hydroxy-6α,7α-epoxy system whereas the ions m/z 345 (52.5%) and 186 (5%) supported the presence of 20-hydroxyl. Further, the ions m/z 171 (28.75%) and 169 (15%) supported the presence of hydroxymethyl in the side chain.

Compound **6** was obtained from chloroform-methanol eluates as colourless needles, m.p., 281-83°; $[\alpha]_D$ –123.39° (*c* 0.53, $CHCl_3$). Its molecular formula was assigned as $C_{28}H_{40}O_6$ from elemental analysis and the mass ion m/z 454 (M^+ –H_2O) in EIMS. From its physical and spectral characteristics it was also recognised as a new withanolide, and named as withasomniferol B. Its IR spectrum showed peaks at 3400 cm^{-1} (hydroxyls), 1735 cm^{-1} (saturated δ-lactone) and 1680 cm^{-1} (α,β-unsaturated six-membered ketone). The latter functionality was supported by the strong UV absorption at 228 nm. The compound resisted acetylation with Ac_2O and pyridine indicating the presence of either tertiary or hindered secondary hydroxyl.

Studies on withasomniferol B indicated it to be a 2-en-1-one derivative with saturated δ-lactone moiety in the side chain. Its ^{1}H NMR spectrum (Table I) showed signals for the olefinic protons of C-2 and C-3 at δ 5.9 (d) and 6.65 (m) and for the geminal protons of 6,7-epoxy system at 3.0 (d, J=4 Hz) and δ 2.29 (d/d, J=4, 1 Hz) as in withasomniferol A **5**. The proton at C-22 appeared as a multiplet centred at δ 4.14 suggesting the absence of hydroxyl substituent at C-23. The spectrum further showed a peak at δ 0.92 (s) for 21-methyl, and peaks at 1.15 and 1.25 together accounting for 4 methyls. The solvent induced shift with benzene, however, resolved the peaks at δ 0.92 (s, 21-CH_3), 1.19 (d, J=27-CH_3), 1.15 (d, J=6 Hz, 28-CH_3), 1.15 (d, J=6 Hz, 18-CH_3) and 1.21 (s, 19-CH_3. A singlet at δ 3.35 (exchangeable with D_2O) acounted for 5α-hydroxyl.

The 24,25-dihydro system was further supported by comparing its ^{1}H NMR data with those of 24,25-dihydrowithanolide D and 24,25-dihydro-27-deoxywithaferin-A-4-monoacetate. The hydroxylic proton was observed as a singlet at δ 3.45 in its ^{1}H NMR spectrum in $CDCl_3$ and the same signal appeared at 3.35 as singlet in benzene showing a solvent induced shift of 0.1 ppm as noticed in related compounds having 5α-OH-6α,7α-epoxy system.

The CD spectrum of withasomniferol B showed a negative Cotton effect at 326 nm indicative of *trans* A/B junction as in other withanolides[34] and a strong positive band at lower wavelength[35] (between 220-240nm) in conformation of the side chain in ring-E



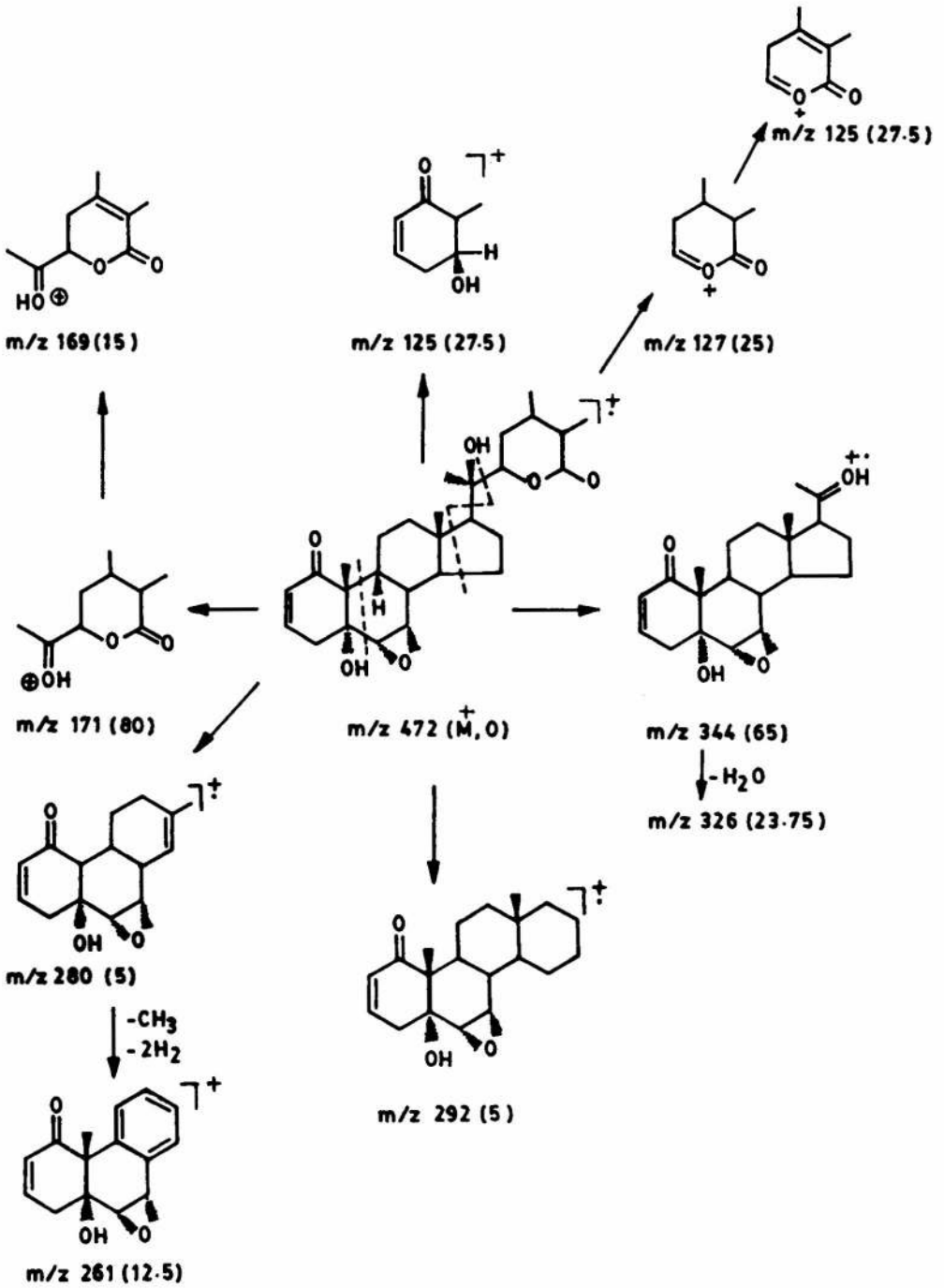
Scheme I—Mass fragmentation of 5α,20α$_F$*(R)*,27-trihydroxy- 6α,7α-epoxy-1-oxowitha-2,24-dienolide **5**.

as half chair. From the foregoing data withasomniferol B could be characterised as 5α,20α$_F$*(R)*-dihydroxy-6α, 7α-epoxy-1-oxowitha-2-enolide **6**.

The structure was further supported by its mass fragmentation (Scheme II). The ion m/z 127 (25%) accounted for the scission of C_{20}-C_{22} bond as in other dihydro derivatives. The ion at m/z 344 (65%) confirmed the presence of 20-hydroxyl as in 5α,20α$_F$*(R)*-dihydroxy-1-oxo-6α,7α-epoxy-witha-2,24-enolide **3**.

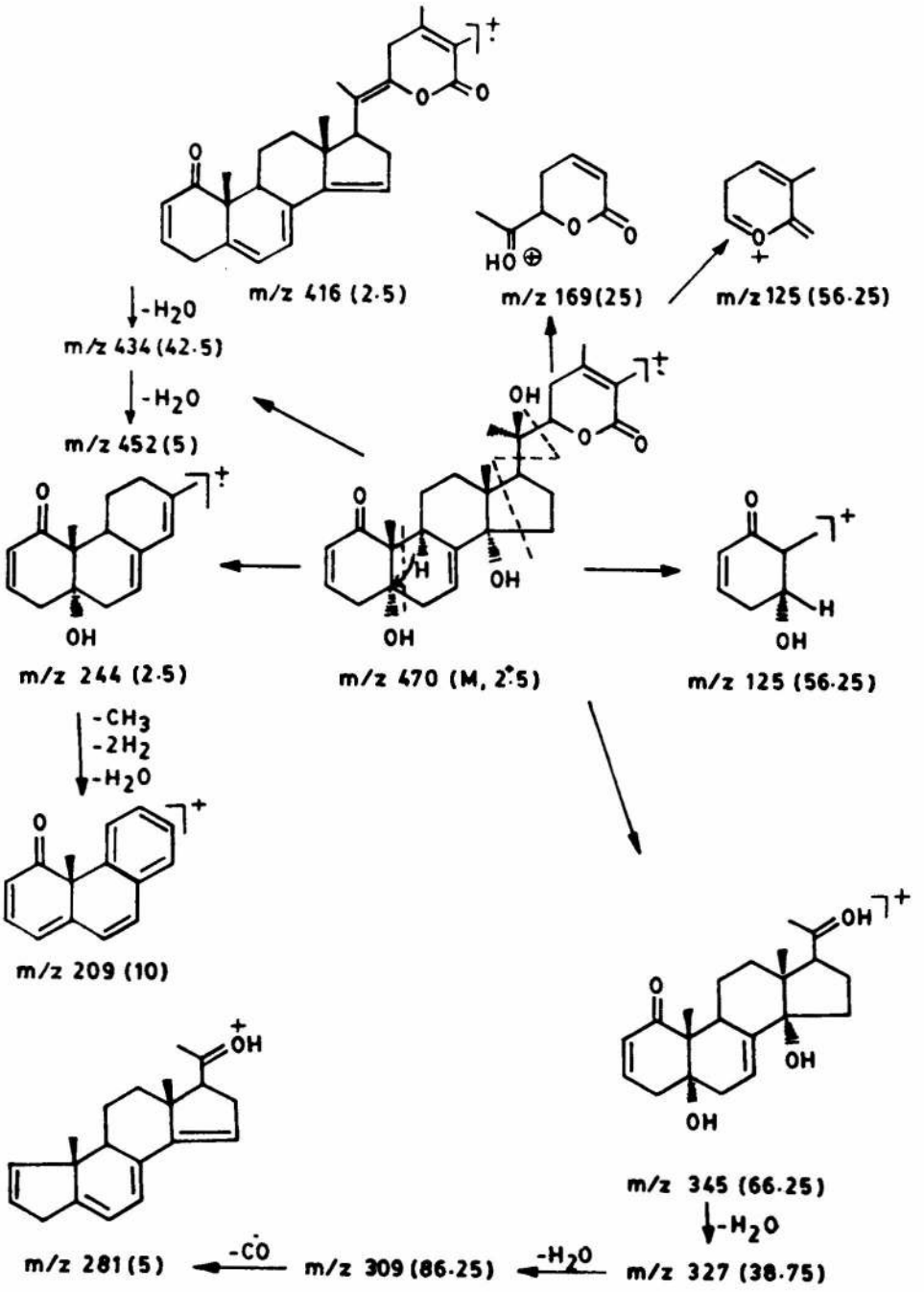
Compound **7** crystallised from $CHCl_3$-MeOH as colourless buttons (150 mg), m.p. 294-95°; R_f 0.34 (benzene-ethyl acetate, 2:8); $[\alpha]_D$+67.78 (*c* 0.09, $CHCl_3$). Its molecular formula was assigned as $C_{28}H_{38}O_6$ from elemental analysis and the molecular ion (M^+ 470) in its EIMS. From its physical and spectral characteristics it was recognised as a new withanolide, and thus named as withasomniferol C.

Its UV spectrum exhibited a peak at 219 nm indicating the presence of conjugation. Its IR spectrum showed a broad peak between 3500 and 3350 for hydroxyl groups, at 1710 for an α,β-unsaturated lactone and at 1675 cm^{-1} for an α,β-unsaturated ketone moiety. The compound remained unchanged on acetylation with Ac_2O and pyridine indicating that the hydroxyls might be tertiary or hindered secondary in nature.



Scheme II—Mass fragmentation of 5α,20α$_F$*(R)*-dihydroxy-6α,7α-epoxy-1-oxowitha-2-enolide **6**.

The ^{1}H NMR spectrum of withasomniferol C **7** (Table I), reminiscent of withanolides, showed close resemblance to that of compound **4**. It showed three singlets at δ 0.75, 1.12 and 1.3 for 18-, 19- and 21-methyls. The deshielded C_{21}-methyl singlet appearing at δ 1.3 indicated its attachment to an oxygenated carbon thus establishing the presence of C_{20}-hydroxyl as in withasomniferols A (**5**) and B(**6**). The two methyls appearing at δ 1.90 and 1.96 accounted for the olefinic 27- and 28-methyls on the α,β-unsaturated-δ-lactone in ring-E. A multiplet at δ 4.21 assignable for C_{22}-H indicated C-23 to be unsubstituted. The olefinic protons at C-2 and C-3 were observed as a doublet at δ 5.97 d(*J*=10 Hz) and



Scheme III—Mass fragmentation of 5α,14α,20α$_F$*(R)*-trihydroxy-1-oxowitha-2,7,24-trienolide 7.

a multiplet at 6.73 respectively indicating the presence of an α,β-unsaturated ketonic system in ring-A as in other withanolides.

The singlet at δ 3.4 disappeared on the addition of the shift reagent trichloroacetyl isocyanide (TAI) with the appearance of carbamate protons at δ 8.39 and 8.72, and accounted for three tertiary hydroxyls. Additionally, a triplet was observed at δ 5.09 accounting for a proton on a trisubstituted double bond. The spectrum further revealed that the compound did not belong to the 5α-hydroxy-6α,7α-epoxy series or 5β,6β-epoxy series or to the 5-enes.

Appearance of the ion m/z 125 in its mass spectral fragmentation (Scheme I) showed the presence of a hydroxyl at C-5 and also the loss of ring-E. The ion m/z 169 (25%) confirmed the

Table III—Comparison of the chemical shifts of olefinic protons in a few steroid enes with that of withasomniferol C(7)

Compound	Position of the double bond	Chemical shift (δ, ppm)
24(*R*+*S*)-isopropenylchloset-7-ene-3-01[37]	7	5.16
24_F (*R*)-hydroxy-1-oxowitha-2,5,14,24-tetraenolide[21]	14	5.25
Δ^{16}-Withanolide[22]	16	5.57
Holothurinogenin[38]	9(11)	5.30 m
Withasomniferol C(**7**)	7	5.09 d

presence of a hydroxyl at C-20 which was further confirmed by a singlet at δ 1.30 for the C-21 methyl. and solvent induced shift ($\Delta^{CDCl_3}_{Benzene}$ 1.36-1.30=0.06 ppm) on 21-CH_3 (Table I).

Of the three tertiary hydroxyls in the molecule two could be located at C-20 and C-5. The third tertiary hydroxyl might be present at C-14 as found commonly in withanolides. The presence of a hydroxyl group at C-14 was supported by the prominent mass ion m/z 345 (66.25%) in its mass spectrum. The solvent induced shift of 18-methyl ($\Delta^{CDCl_3}_{Benzene}$ 0.83-0.75=0.08 ppm) indicated its 14-axial configuration.

The last problem was to fix the trisubstituted double bond, the proton of which was observed at δ 5.09 as a doublet of a doublet (*J*=6, 2 Hz). A comparison of the chemical shift of this proton with those of the olefinic protons in other ocmpounds, e.g. C-7, C-14, C-16 and C-9(11) (Table III), suggested that it could be placed at C-7. This was further supported by the mass spectral fragmentation (Scheme III).

The stereochemistry of **7** was established from its CD spectrum as it showed a negative Cotton effect at 330 nm indicative of *trans* A/B ring junction and a positive Cotton effect at 255 nm indicative of 22*R* configuration in ring-E (α,β-unsaturated-δ-lactone) as in other withanolides[3].

The ^{13}C NMR spectrum of **7** was taken in DMSO-d_6 and the chemical shifts of various carbons (Table II) were assigned by a comparison of the data with those of the closely related 5α, $20\alpha_F$(*R*)-dihydroxy-6α, 7α-epoxy-1-oxowitha-2, 24-dienolide (compound **3**) except for the carbons in ring-B.

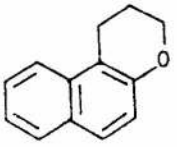
The carbonyl carbon of the α,β-unsaturated ketone at C-1 and the lactone carbon at C-26 were observed at δ 203.6 and 166.13 ppm respectively. The signals at δ 127.6 and 139.3 were assigned to C-2 and C-3 carbons respectively of ring-A as in other withanolides. The signals at δ 150.77 and 120.41 were assigned to the olefinic carbons C-26 and C-27 of the unsaturated-δ-lactone. The chemical shift at δ 74.24 was assigned to the C-5 carbon as in lycium substance-B (**2**) and the signal at δ 76.06 to the C-20 as in withanolide D. Additionally, a signal at δ 69.0 was noticed accounting for the presence of a hydroxyl at C-14 as observed in other 14α-hydroxy compounds[12].

The presence of two more olefinic carbons at δ 120.41 and 144.48 could be assigned to C-7 and C-8 carbons in support of the trisubstituted double bond. Location of this double bond at C-7 is consistent with the absence of 6α,7α-epoxide system unlike in other compounds in the same series. This is the first compound to be isolated in withanolides with a Δ^7 ene system and also lacking any epoxide system in ring-B.

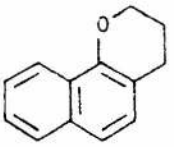
The structure of withasomniferol C (Compound **7**) was thus established as 5α,14α,$20\alpha_F$(*R*)-trihydroxy-1-oxowitha-2,7,24-trienolide which was further supported by its mass fragmentation pattern (Scheme III).

Compound **9** was obtained as colourless crystals from ethyl acetate-hexane, m.p. 127-09°. Its molecular formula was established as $C_{13}H_{12}O$ by elemental analysis and molecular ion m/z 184 in its EIMS. The IR spectrum showed strong peaks between 1610 and 1570 cm^{-1} suggesting its aromatic nature, which was further supported by its UV absorption at 248 nm. No carbonyl or hydroxylic absorption was observed in its IR spectrum indicating the involvement of oxygen of the molecule as ether.

Its ^1H NMR spectrum exhibited a pentet at δ 2.55 (*J*=8 Hz) for two protons, a triplet at 2.94 (*J*=8 Hz) integrating for two benzylic protons and a triplet at 4.0 (*J*=8 Hz) integrating for two oxymethylene protons. This pattern suggested a benzopyran system. The aromatic region showed six protons of which one appeared distinctly as a singlet at δ 7.6 and the remaining five as a multiplet centred at 7.25. The appearance of this deshielded proton as a singlet favoured a linear benzo[6:7]chroman **9** over the angular ones **9a** and **9b** where, in either case this proton would have appeared as a multiplet coupled to the other protons The benzo[6:7]chroman



9a



9b

structure for compound **9** was further supported by its mass fragmentation on the expected lines.

Experimental Section

General. Melting points were determined on VEB Analytik Dresdon HMK hot plate and are uncorrected. Compounds were dried at room temperature or at 100°C/0.2 mm for 6 hr. Acme's silica gel G was used for column chromatography and TLC. IR spectra were recorded on a Shimadzu IR-408 spectrometer and UV spectra on a Shimadzu UV-140 or Shimadzu UV-260 UV-Vis spectrometer. ^{1}H NMR spectra were recorded on a Perkin-Elmer R-32 instrument at 90 MHz (chemical shifts are given in δ-scale with TMS as internal standard). ^{13}C NMR spectra were recorded on a Brucker instrument at 25 MHz. Optical rotations were measured on an OC Rudolph instrument model 60, and CD spectra on a J-20 spectrophotometer of Japan Spectroscopic Company Limited.

Collection, extraction and isolation. The roots of *Withania somnifera* (25 kg), collected from Tamil Nadu region and supplied by Unichem Laboratories, were powdered and percolated with methanol (50 L). The process was repeated four times and the combined methanolic extract concentrated under reduced pressure to a small volume (4 L) which was fractionated into benzene, ethyl acetate and methanol. The benzene extract (10 L) after concentration to a small volume (1 L) was washed with 1% aq. HCl (500 mL) and evporated to give a solid (11.5 g) which on fractional crystallisation from methanol furnished two solids B_1 (9.3 g) and B_2 (2.18 g). Solid B_1 was found to be a mixture and showed several spots on TLC. It was adsorbed on a column of silica gel and eluted with benzene, benzene-EtOAc and EtOAc. Several fractions of 250 mL each were collected and the individual fractions monitored by TLC. Fractions of similar nature were collected and further purified by either column chromatography or crystallisation from appropriate solvents to furnish compounds **1-8**. Solid B_2 on similar treatment furnished compounds **1-6** but not **7** and **8**. The dark brown gum (14.5 g) from the benzene extract after separation of B_1 and B_2 on chromatographic separation over silica gel column furnished waxes and compound **9** in addition to compounds **4** and **5**. The dark brown ethyl acetate extract (0.75 L) was treated with basic lead acetate to remove tannins. The tannin-free extract on concentration left a residue (2.5 g) which was adsorbed over silica gel (100-200 mesh) column and eluted with benzene, benzene ethyl acetate and ethyl-acetate. The eluates gave compounds **2, 3** and **9** in pure state.

Compound 1: β-sitosterol. The benzene-EtOAc (9:1) fractions (9-27) of solid B_2 and the benzene-EtOAc (9:1) fraction (20-27) of solid B_1 furnished compound **1** on crystallisation from chloroform-methanol as colourless wooly crystals (100 mg), m.p. 135-37°; $[\alpha]_D$ -370° (*c* 1.2, $CHCl_3$); R_f 0.79 (benzene-EtOAc, 6:4). It gave +ve LB test for steroids, and was identified as β-sitosterol by physical and spectral characteristics and by comparison with an authentic sample.

Compound 2: Lycium substance-B. The benzene-EtOAc (8:2) fractions (45-48) of solid B_1 and the benzene EtOAc (9:1) fractions (37-53) of solid B_2 on mixing together and crystallisation from chloroform-methanol furnished compound **2** as colourless needles (750 mg), m.p. 261-65°; $[\alpha]_D$ +33.78 (*c* 0.99, $CHCl_3$); R_f 0.58 (benzene-EtOAc, 6:4); UV (EtOH) 224 nm (log ε, 4.18); IR (Nujol): 3430 (-OH), 2920, 2870, 1700 (δ-lactone), 1685 (α,β-unsaturated six-membered ketone), 1660, 1375, 1295, 1130, 1020 (epoxy), 900 cm^{-1}; ^{1}H NMR data (cf. Table I); MS (70 eV) (rel. int.): m/z 454 M^+ (11.11), 436 (8.33), 329 (52.77), 312 (18.05), 293 (5.55), 259 (2.77), 241 (19.44), 223 (22.22), 153 (6.8), 125 (100). It was recovered unchanged after acetylation with Py+Ac_2O. Compound **2** was identified as lycium substance-B[31] by comparing its physical and spectral characteristics.

Compound 3: 5α,20α$_F$*(R)*-dihydroxy-6α,7α-epoxy-1-oxowitha-2,24-dienolide. The benzene-EtOAc (8:2) column fractions (71-77) of B_1 and benzene-EtOAc (9:1) column fractions (69-79) of B_2 furnished compound **3** which crystallized chloroform-methanol as colourless wooly crystals, m.p. 313-14° (the compound crystallizing from EtOAc melted at 283/84°), yield 4.5 g; $[\alpha]_D$ +80° (*c* 1.25, $CHCl_3$); R_f 0.36 (benzene-EtOAc, 1:1); UV (EtOH): 223 nm (log ε, 4.15); IR (Nujol): 3450

(OH), 2920, 2880, 1715 (αβ,-unsaturated-δ-lactone), 1685 (α,β-unsaturated six-membered ketone), 1460, 1375, 1290, 1134, 1020 (epoxy), 910 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) Table I; addition of the shift reagent TAI showed a new peak at δ 8.7 for the carbamate protons in its ^1H NMR spectrum indicating the presence of hydroxyls.

Compound **3** on acetylation with Py and Ac_2O was recovered unchanged. It was identified as 5α, $20\alpha_F(R)$-dihydroxy-6α, 7α-epoxy-1-oxowitha-2,24-dienolide **3** by comparing its physical and spectral characteristics with the literature values of the compound[29,32].

Compound 4: 5α,27-dihydroxy-6α,7α-epoxy-1-oxowitha-2-,24-dienolide. The benzene-EtOAc (8:2) column fractions (78-80) of B_1 and benzene-EtOAc (8:27) column fractions of B_2 furnished compound **4** which crystallised from chloroform-methanol as colourless needles (130 mg), m.p. 277-79°; $[\alpha]_D$ +120.22° (*c* 1.32, $CHCl_3$); R_f 0.26 (benzene-EtOAc, 1:1); UV (EtOH): 225 nm (log ε, 4.20); IR (Nujol) 3420 (OH), 2920, 2880, 1690 (α,β-unsaturated δ-lactone), 1675 (α,β-unsaturated six-membered ketone), 1460, 1395, 1375, 1240, 1020 cm^{-1} (epoxy); ^1H NMR (δ, 90 MHz, $CDCl_3$) Table I. Addition of shift reagent TAI showed a singlet at δ 8.82 (carbamate protons)

Compound 4 acetate: Compound **4** (30 mg) was acetylated with Py (1 mL) and Ac_2O (1 mL) under reflux for 4 hr, and the aetate obtained after usual work-up was crystallised from chloroform-methanol as colourless needles (25 mg), m.p. 231-33°; $[\alpha]_D$ +83.44° (*c* 1.2, $CHCl_3$); R_f 0.75 (benzene-EtOAc, 4:6); UV (EtOH): 221 nm (log ε, 4.22); IR (KBr): 1730-(OAc), 1710 (α,β-unsaturated δ lactone), 1680 (α,β-unsaturated six-membered ketone), 1385, 1230, 1132, 1020 (epoxy), 900, 780 cm^{-1}; ^1H NMR (δ, 90 MHz, $CDCl_3$): δ 0.79 (s, 18-CH_3), 1.18 (s, 19-CH_3), 1.01 (d, *J*=7 Hz, 21-CH_3), 4.9 (br s, 27-CH_2OAc), 2.05 (s, 28-H_3), 2.08 (s, -OCOCH_3), 4.4 (m, 22-H), 5.85 (d, *J*=10 Hz, 2-H), 6.6 (m, 3-H), 3.02 (d, *J*=4 Hz, 6-H), 3.3 (m, 7-H). Addition of shift reagent TAI showed a peak at δ 9.4 (carbamate protons). Compound **4** and its acetate were identical in every respect with 5α,27-dihydroxy-6α ,7α-epoxy-1-oxowitha-2,24-dienolide **4**[3,33] and its acetate, respectively.

Compound 5: Withasomniferol A: $5\alpha,20\alpha_F(R)$, 27 - trihydroxy - 6α, 7α- epoxy-1-oxowitha -2, 24-dienolide. The benzene-EtOAc (8:2) column fractions (155-98) of B_1 and benzene-EtOAc (7:3) fraction (105-98) of B_2 furnished compound **5** which crystallised from chloroform-methanol as colourless needles (35 mg), m.p. 271-73°; $[\alpha]_D$ +74.88° (*c* 0.44, methanol); R_f 0.1 (benzene-EtOAc, 1:1) (Found: C, 69.9; H, 7.92. $C_{28}H_{38}O_7$ requires C, 69.13; H, 7.81%); UV (EtOH): 225 nm (log ε, 4.19); IR (Kbr): 3600-3400 br (OH),: 2900, 1710 (α,β-unsaturated δ-lactone), 1680 (α,β-unsaturated six-membered ketone), 1380, 1020 cm^{-1} (epoxy); ^1H NMR (δ, 90 MHz, $CDCl_3$) Table I; MS (70 eV): m/z 486 (M^+, 0), 468 (5), 452 (7.5), 434 (46.25), 416 (27.5), 345 (52.5), 327 (35), 309 (37.5), 301 (15), 291 (5), 260 (11), 239 (13.75), 185 (5), 169 (15), 141 (1.25), 125 (40%).

Compound 5 acetate: Compound **5** (5 mg) was refluxed with pyridine (0.5 mL) and Ac_2O (0.5 mL) for 4 hr and kept overnight at room temperature. The product after usual work-up yielded the acetate as a gummy substance (5 mg); $[\alpha]_D$ +192.85° (*c* 0.21, $CHCl_3$); UV (EtOH): 226 nm (log ε, 4.19); IR ($CHCl_3$): 3400 (-OH), 1730 and 1230 (-OCOCH_3), 1710 (α,β-unsaturated δ-lactone), 1680 (α,β-unsaturated six-membered ketone), 1385, 1130, 1020 (epoxy), 900, 870 cm^{-1}.

Compound 6: Withasomniferol B: $5\alpha,20\alpha_F(R)$-dihydroy-6α,7α-epoxy-1-oxowitha-2-enolide. The benzene EtOAc (6:4) column fractions (199-240) of B_1 furnished compound **6** which crystallised from chloroform-methanol as colourless needles (100 mg), m.p. 281-83°; $[\alpha]_D$ −123.99° (*c* 0.53, $CHCl_3$); R_f 0.6 (benzene-EtOAc, 2:8) (Found: C, 71.32; H, 8.40. $C_{28}H_{40}O_6$ requires C, 71.18; H, 8.47%); UV (EtOH): 228 nm (log ε, 4.18); IR (KBr): 3400 (-OH), 2900, 1730 (saturated δ-lactone), 1680 (α,β-unsaturated six-membered ketone), 1385, 1090 (epoxy), 900 cm^{-1}; ^1H NMR (δ, 90 MHz, $CDCl_3$) Table I; MS (70 eV) (rel. int.): m/z 472 (M^+, 0) 344 (65), 326 (23.75), 292 (5), 280 (5), 261 (12.5), 171 (80), 169 (15), 127 (2.5), 125 (27.5). It did not undergo acetylation with Ac_2O and pyridine.

Compound 7: Withasomniferol C: $5\alpha,20\alpha_F(R)$-trihydroxy - 6α, 7α-epoxy -1 -oxowitha - 2, 7, 24-trienolide. The benzene-EtOAc (1:1) column fractions (112-119) of solid B_1 furnished compound **7** which crystallised from chloroform-methanol as colourless buttons (150 mg), m.p. 294-95°; $[\alpha]_D$ +67.77° (*c* 0.09, $CHCl_3$); R_f 0.34 (EtOAc-benzene, 8:2) (Found: C, 71.56; H, 7.96. $C_{28}H_{38}O_6$ requires C, 71.4; H, 8.08%); UV (EtOH): 229 nm (log ε, 4.2);

IR (KBr): 3500-3350 br (-OH), 2920, 2880, 1710 (α,β-unsaturated δ-lactone), 1675 (α,β-unsaturated six-membered ketone), 1630, 1375, 1120, 1100, 980 cm^{-1}; ^1H NMR (δ, 90 MHz, $CDCl_3$) Table I; ^{13}C NMR (δ, 22.5 MHz, $CDCl_3$) Table II; MS (70 eV) (rel. int.): m/z 470 (M^+, 2.5), 452 (5), 434 (42.5), 416 (2.5), 345 (66.25), 327 (38.75), 309 (86.25), 281 (5), 244 (2.5), 209 (10), 169 (25), 125 (10). It did not undergo acetylation with Py+Ac_2O.

Compound 8: β-Sitosterol-3-O-β-D(+) glucoside. The ethyl acetate column fractions of B_1 furnished compound **8** which crystallised from chloroform-methanol as colourless fluffy solid (50mg), m.p. 278-80°; $[\alpha]_D$ –38°. (*c* 1.0, pyridine); R_f 0.36 (chloroform-methanol, 9:1). It gave +ve Molisch test for carbohydrates and LB test for steroids. Compound **8** was identified as β-sitosterol-3-β-O-D-(+)-glucoside by comparing its physical and spectral characteristics and by direct comparison with an authentic sample.

Compound 9: Benzo[6:7]chroman. The benzene-EtOAc (4:6) column fractions (81-103) of the ethyl acetate extract furnished compound **9** which crystallised from ethyl acetate and benzene as salt like crystals, m.p. 127-29°; R_f 0.46 (benzene-EtOAc, 7:3) (Found: C, 84.78; H, 6.52. $C_{13}H_{12}O$ requires C, 84.78; H, 6.52%); UV (EtOH): 248 nm; IR ($CHCl_3$): 2995, 1610, 1580, 1570, 1492, 1413, 1355, 1320, 1150, 1070, 975 cm^{-1}; ^1H NMR (δ, 90 MHz, $CDCl_3$): 2.55 (pentet, *J*=8 Hz, 2H), 2.94 (t, *J*=8 Hz, 2H), 4.0 (t, *J*=8 Hz, 2H), 7.25 (br, 5H), 7.6 (s, 1H); MS (70 eV) (rel. int.): m/z 184 (M^+, 100), 156 (9.67), 128 (12.37), 127 (4.3), 101 (6.45), 79 (7), 53 (5.4).

Acknowledgement

We thank Unichem Laboratories for the supply of plant material and the CSIR for the award of JRF/SRF to D S R. We are also indebted to Late Prof. L R Row for his encouragement during the course of this work.

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