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# WITHANOLIDES FROM THE STEM BARK OF *WITHANIA SOMNIFERA*

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**Key Word Index**—*Withania somnifera*; Solanaceae; stem bark; withanolides; structural elucidation.

**Abstract**—Phytochemical studies on the stem bark of *Withania somnifera*, collected from the southern region of New Delhi, resulted in the isolation of five new withanolides, namely withasomnilide, withasomniferanolide, somniferanolide, somniferawithanolide and somniwithanolide. Their structures have been established as (20*R*, 22*R*)-1-oxo-5α, 8β-dihydroxywitha-6α, 7β-epoxide-2,24-dienolide, (20*R*, 22*R*)-1-oxo-8β, 11β, 16β-trihydroxywitha-2,5,24-trienolide, (20*R*, 22*R*)-1-oxo-8β, 11β-dihydroxywitha-16α, 17α-epoxy-2,5,24-trienolide, (20*R*, 22*R*)-1-oxo-8β,18,20β-trihydroxywitha-2,5,24-trienolide and (20*R*, 22*R*)-1-oxo-7β, 18,20β,27-tetrahydroxywitha-2,4,24-trienolide, respectively, on the basis of spectroscopic techniques and chemical means. 

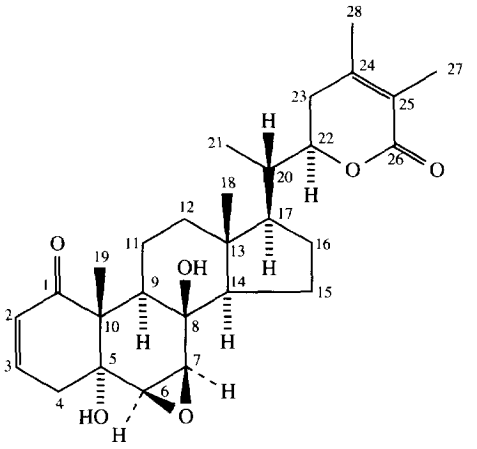
## INTRODUCTION

*Withania somnifera* Dunal is an erect, evergreen, perennial shrub which is reputed to have adaptogenic, tonic, analgesic, antipyretic, anti-inflammatory and abortifacient properties. It is used to treat various body disorders [1–3]. The plant is a source of various withanolides [4, 5]. The present paper describes the isolation and characterization of five new chemical constituents from the stem bark of the plant.

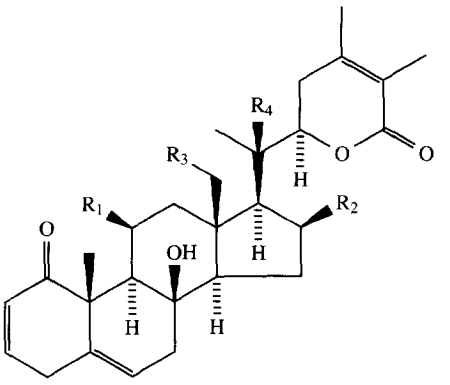
## RESULTS AND DISCUSSION

Compound **1**, named withasomnilide, was obtained as flakes from chloroform eluants. The compound had a UV maximum at 229 nm typical for withanolides with the 2-ene-1-one system [6, 7]. Its IR spectrum showed bands for hydroxyl (3460 $cm^{-1}$), α,β-unsaturated δ-lactone (1704 $cm^{-1}$) and conjugated carbonyl (1688 $cm^{-1}$) functions. The $^1H$ NMR spectrum of **1** displayed two distinct olefinic signals [6, 7] at δ 6.60 as a multiplet and δ 5.73 as a doublet ($J$ = 10.0 Hz) assigned correspondingly to H-3 and H-2. The C-22 carbinyl hydrogen appeared as a multiplet at δ 4.46 and other oxygen-substituted methine protons as doublets at δ 3.10 ($J$ = 10.0 Hz, H-7α) and 3.00 ($J$ = 10.0 Hz, H-6α). A six-proton signal at δ 1.86 was assigned to C-27 and C-28 methyl groups attached to olefinic carbons. The C-19 and C-18 tertiary methyls resonated at δ 1.16 and 0.83, respectively. A three proton doublet at δ 1.00 ($J$ = 7.0 Hz) was ascribed to a C-21 secondary methyl function. The $^{13}C$ NMR signals (Table 1) for olefinic carbons at δ 150.33 (C-24), 139.61 (C-3), 128.99 (C-2), and 121.44 (C-25) and oxygen substituted carbons at δ 84.57 (C-8), 78.75 (C-22), 73.22 (C-5), 57.11 (C-7) and 56.27 (C-6) were in good agreement with the proposed structure and compared with other similar withanolides [6, 8–10]. The multiplicity of each carbon was determined by DEPT spectra.

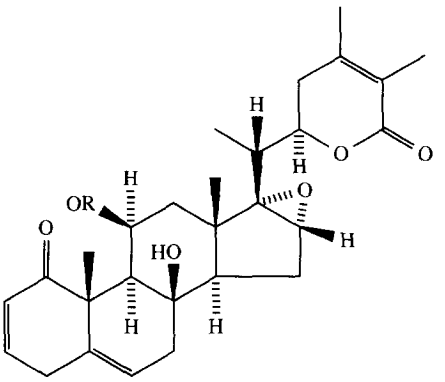
The mass spectrum of **1** showed a molecular ion peak at $m/z$ 470 ($C_{28}H_{38}O_6$) and the base peak at 125 due to a δ-lactone ring [11] without a hydroxyl group since hydroxylated lactones (C-27-OH) generated an intense ion peak at $m/z$ 141 corresponding to $C_7H_9O_3$. Successive removal of water and methyl groups formed ion peaks at $m/z$ 452 $[M-H_2O]^+$, 434 $[452-H_2O]^+$, 419 $[434-Me]^+$, 404 $[419-Me]^+$ and 389 $[404-Me]^+$. The ion fragments at $m/z$ 68, 402 ($C_{1,10}$-$C_{4,5}$ fission), 124, 346 ($C_{5,6}$-$C_{9,10}$ fission), 166 ($C_{7,8}$-$C_{9,10}$ fission), 208, 262 ($C_{8,14}$-$C_{9,10}$ fission), 384 $[402-H_2O]^+$, 366 $[384-H_2O]^+$, 106 $[124-H_2O]^+$, 148 $[166-H_2O]^+$, 190 $[208-H_2O]^+$ and 172 $[190-H_2O]^+$ suggested the presence of hydroxyl groups at C-5 and C-8 and an epoxy ring at C-6, C-7 of the steroidal skeleton. The saturated nature of rings C and D was inferred from the ion peaks appearing at $m/z$ 222, 248 ($C_{8,14}$-$C_{11,12}$ fission), 236, 234 ($C_{8,14}$-$C_{12,13}$ fission), 276, 194 ($C_{14,15}$-$C_{13,17}$ fission) and 290, 180 ($C_{15,16}$-$C_{13,17}$ fission). Compound **1** resisted acetylation with acetylating reagents supporting the tertiary nature of the hydroxyl groups. Based on the evidence the structure of **1** was elucidated as (20*R*, 22*R*)-1-oxo-5α,8β-dihydroxywitha-6β,7β-epoxide-2,24-dienolide.



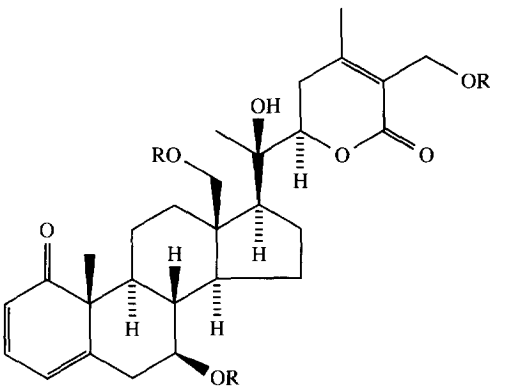
**1**



**2** $R_1=R_2=OH;R_3=R_4=H$
**2a** $R_1=R_2=OAc;R_3=R_4=H$
**4** $R_1=R_2=H;R_3=R_4=OH$
**4a** $R_1=R_2=H;R_3=OAc,R_4=OH$



**3** R=H
**3a** R=Ac



**5** R=H
**5a** R=Ac

Compound **2**, designated as withasomniferanolide, was obtained as an amorphous powder from chloroform eluants. Like a typical withanolide [12, 13], it exhibited a single absorption maximum at 225 nm indicating the presence of an enone and an $\alpha,\beta$-unsaturated $\delta$-lactone chromophore. Its IR spectrum showed absorption bands at 3400, 1710 and 1685 $cm^{-1}$ assignable, respectively, to hydroxyls, $\alpha,\beta$-unsaturated $\delta$-lactone and enone groupings. Three olefinic hydrogen signals discernible in its $^1H$ NMR spectrum at $\delta$ 6.50 (*ddd*, $J$ = 10.0, 2.0, 4.5 Hz, H-3), 5.70 (*dd*, $J$ = 10.0, 5.5 Hz, H-2) and 5.66 (*br s*, H-6) suggested the presence of a steroidal 2,5-diene-1-one system as is present in withametelin [6] and secowithametelin [13]. The oxygen-substituted methine protons appeared as one-proton each signals at $\delta$ 4.46 (*dd*, $J$ = 10.0, 4.5 Hz), 3.76 (*ddd*, $J$ = 9.5, 5.0, 4.0 Hz) and 3.53 (*ddd*, $J$ = 9.5, 4.5, 4.0, Hz) ascribable correspondingly to H-22, H-11$\alpha$ and H-16$\beta$. In addition to these the $^1H$ NMR spectrum showed signals for two angular C-methyls ($\delta$0.83; 1.20 for 18- and 19-methyls), two allylic methyls ($\delta$1.83; 1.80 for 28- and 27-methyls) and a secondary methyl ($\delta$1.13, *d*, $J$ = 6.5 Hz for 21-methyl). The $^{13}C$ NMR (Table 1) spectrum supported the proposed structure and the values were compared with withanolides possessing 2,5-diene-1-one system, such as withametelin [13], secowithametelin [13] and withaphysalin A [14]. It showed signals for two $sp^2$ carbons of a tetra-

Table 1. $^{13}C$ NMR chemical shift assignments of compounds **1–5** ($CDCl_3$)

| C | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 203.02 | 202.37 | 200.88 | 201.05 | 202.40 |
| 2 | 128.99 | 127.61 | 126.67 | 126.30 | 119.61 |
| 3 | 139.61 | 150.56 | 149.16 | 149.20 | 140.90 |
| 4 | 36.02 | 32.33 | 32.38 | 33.64 | 128.35 |
| 5 | 73.22 | 140.89 | 139.40 | 139.78 | 160.10 |
| 6 | 56.27 | 128.30 | 126.30 | 128.67 | 19.81 |
| 7 | 57.11 | 29.14 | 30.42 | 30.42 | 71.90 |
| 8 | 84.57 | 82.53 | 82.25 | 82.20 | 45.22 |
| 9 | 35.27 | 42.66 | 42.22 | 42.10 | 51.92 |
| 10 | 50.95 | 50.68 | 50.51 | 48.61 | 55.20 |
| 11 | 21.65 | 68.37 | 70.45 | 21.04 | 22.37 |
| 12 | 36.76 | 40.28 | 40.43 | 40.43 | 36.81 |
| 13 | 42.98 | 41.72 | 41.54 | 41.37 | 43.63 |
| 14 | 45.89 | 49.40 | 46.20 | 48.61 | 55.59 |
| 15 | 22.87 | 24.02 | 24.66 | 24.66 | 25.20 |
| 16 | 32.81 | 78.79 | 61.15 | 31.83 | 28.98 |
| 17 | 48.72 | 49.97 | 67.18 | 49.30 | 49.97 |
| 18 | 9.47 | 9.26 | 7.93 | 61.15 | 62.34 |
| 19 | 14.66 | 20.11 | 18.80 | 18.95 | 20.10 |
| 20 | 36.76 | 39.24 | 38.37 | 80.90 | 78.78 |
| 21 | 15.55 | 15.26 | 15.43 | 21.04 | 19.81 |
| 22 | 78.75 | 75.35 | 76.52 | 76.50 | 75.35 |
| 23 | 32.49 | 32.04 | 32.27 | 32.30 | 33.78 |
| 24 | 150.33 | 150.56 | 149.16 | 149.20 | 150.50 |
| 25 | 121.44 | 120.46 | 119.02 | 120.65 | 121.10 |
| 26 | 167.26 | 166.59 | 164.99 | 165.00 | 166.10 |
| 27 | 12.28 | 14.25 | 12.90 | 13.93 | 56.65 |
| 28 | 20.42 | 22.17 | 21.04 | 22.70 | 18.77 |

substituted olefin as a part of an unsaturated $\delta$-lactone at $\delta$150.56 (C-24) and 120.46 (C-25). It also showed signals for three oxymethine carbons at $\delta$68.37 (C-11), 78.79 (C-16) and 75.35 (C-22) and one tertiary oxycarbon signal at $\delta$ 82.53 (C-8). The nature of the hydroxyl groups was concluded from derivatization. Treatment of **2** with acetic anhydride and pyridine yielded a diacetyl product (**2a**) which still showed absorption bands for a tertiary hydroxyl group at 3400 $cm^{-1}$ and for two acetyl groups at 1740 and 1725 $cm^{-1}$. The C-11 and C-16 acetoxymethine proton signals shifted at $\delta$ 4.10 and 3.96 in the $^1H$ NMR spectrum of **2a**.

In its mass spectrum, **2** displayed a molecular ion peak at *m/z* 470 ($C_{28}H_{38}O_6$) and the base peak at *m/z* 125 due to $\delta$-lactone moiety. The fragments at *m/z* 68, 402 $[M-68]^+$ and 277 $[402-125]^+$ generated due to cleavage of ring A across $C_{1,10}$-$C_{4,5}$. The ion fragments appearing at *m/z* 120, 134, 225 $[M-120-\delta$-lactone$]^+$, 336 $[M-134]^+$ and 211 $[336-\delta$-lactone$]^+$ formed due to cleavage of ring **B** and at *m/z* 176, 206, 220, 294 $[M-176]^+$, 264 $[M-206]^+$, 250 $[M-220]^+$, 158 $[176-H_2O]^+$, 188 $[206-H_2O]^+$, 170 $[188-H_2O]^+$, 202 $[220-H_2O]^+$, 184 $[202-H_2O]^+$, 169 $[294-\delta$-lactone$]^+$ and 139 $[264-\delta$-lactone$]^+$ due to cleavage of ring C indicated the presence of hydroxyl groups at C-8 and C-11. The location of the third hydroxyl group in ring D at C-16 was deduced from the ion peaks appearing at *m/z* 260, 210 $[C_{14,15}$-$C_{13,17}$ fission$]^+$ 274, 196 $[C_{15,16}$-$C_{13,17}$ fission$]^+$ and 304, 166 $[C_{16,17}$-$C_{13,17}$ fission$]^+$. On the basis of the spectral data and chemical reaction the structure of **2** was established as (20*R*, 22*R*)-1-oxo-8$\beta$, 11$\beta$, 16$\beta$-trihydroxywitha-2,5,24-trienolide.

Compound **3**, somniferanolide, was obtained as flakes from chloroform–methanol (19:5) eluants. A single UV absorption maximum at 225 nm, **IR** absorption bands at 3420 (OH), 1710 ($\alpha,\beta$-unsaturated $\delta$-lactone) and 1676 $cm^{-1}$ (enone group), $^1H$ NMR signals at $\delta$ 6.60 (*ddd*, $J$ = 10.1, 4.5, 2.5, Hz, H-3), 5.66 (*dd*, $J$ = 10.1, 5.0 Hz, H-2) and 5.50 (*d*, $J$ = 6.5 Hz, H-6) and the ion peaks at *m/z* 120, 134, 176, 206, 220, 234 $[M-134]^+$, 292 $[M-176]^+$, 262 $[M-206]^+$ and 248 $[M-220]^+$ in its mass spectrum ($M^+$ at 468, $C_{28}H_{36}O_6$) suggested the identical substitution pattern of rings A, B and C of the steroidal carbon framework to that present in compound **2**. The presence of a *cis*-epoxide ring at C-16(17) was deduced from the ion fragments appearing at *m/z* 260, 208 ($C_{14,15}$-$C_{13,14}$ fission), 274, 194 ($C_{15,16}$-$C_{13,14}$ fission), and 315, 153 ($C_{17}$-$C_{20}$ fission), $^1H$ NMR signals at $\delta$ 3.13 (1H, *br s*, H-16) and the signals in the $^{13}C$ NMR spectrum at $\delta$ 61.15 (C-16) 67.18 (C-17). The identification of the carbocyclic skeleton was supported by the $^{13}C$ NMR signals (Table 1) which were compared with the withanolides of identical carbon skeleton [13, 14].

Acetylation of **3** with acetic anhydride–pyridine yielded a monoacetyl derivative **3a**. Its IR spectrum still showed the presence of a hydroxyl group ($\nu$ max 3450 $cm^{-1}$) supporting the presence of one tertiary hydroxyl group in the molecule. The C-11 carbinol proton shifted at $\delta$ 4.13 in the $^1H$ NMR spectrum of **3a**. Based on the evidence the structure of **3** was characterized as (20*R*, 22*R*)-1-oxo-8$\beta$, 11$\beta$-dihydroxywitha-16$\alpha$, 17$\alpha$-epoxy-2,5,24-trienolide.

Compound **4**, named somniferawithanolide, was obtained as an amorphous powder from chloroform–methanol (19:5) eluants. Its UV absorption maximum at 228 nm, IR absorption bands at 3445 (OH), 1710 ($\alpha,\beta$-unsaturated $\delta$-lactone) and 1695 $cm^{-1}$ (conjugated CO), three one-proton each downfield $^1H$ NMR signals at $\delta$ 6.61 (*ddd*, $J$ = 10.5, 2.0, 5.0 Hz, H-3), 5.70 (*dd*, $J$ = 10.5, 5.0 Hz, H-2), 5.50 (*br s*, H-6) and the identical fragmentation pattern of rings A and B in the mass spectrum ($M^+$ at 470, $C_{28}H_{38}O_6$) to that of compounds **2** and **3** were found to be in perfect agreement with a withanolide 2,5-diene-1-one system and it was thus inferred that the same steroidal carbocyclic moiety was present in **4**. This assumption was further substantiated from a comparison of their $^{13}C$ NMR spectra, which revealed that most of the resonance signals from C-1 to C-19 of **4** were also discernible in the spectra of **2** and **3** at the same positions. In addition to a six-proton signal for two angular C-methyls ($\delta$ 1.20 for C-19 and C-21 methyls) and two allylic methyls ($\delta$ 2.00, 1.96; C-28 and C-27 methyls) the $^1H$ NMR spectrum of **4** showed two one-proton doublets at $\delta$ 4.10 ($J$ = 10.0 Hz) and 3.90 ($J$ = 10.5 Hz) characteristic of a hydroxymethylene grouping which was readily recognized as a C-18 hydroxylmethylene from their splitting patterns, as well as from their downfield shifts to $\delta$ 4.36 (*d*, $J$ = 10.5 Hz) and 4.27 (*d*, $J$ = 10.5 Hz) in the spectrum of its acetate derivative (**4a**). On the basis of the information compound **4** was formulated as (20*R*, 22*R*)-1-oxo-8$\beta$, 18, 20$\beta$-trihydroxywitha-2,5,24-trienolide.

Compound **5**, named somniwithanolide, was obtained as an amorphous powder from chloroform–methanol (9:1) eluants. It had a molecular ion peak at $m/z$ 486 ($C_{28}H_{38}O_7$). Its IR spectrum showed bands for hydroxyl (3428 $cm^{-1}$), $\alpha,\beta$-unsaturated $\delta$-lactone (1700 $cm^{-1}$) and conjugated carbonyl (1655 $cm^{-1}$) functions. Its UV absorption maxima at 230, 286 and 317 nm indicated the presence of a steroidal 2,4-diene-1-one chromophore [15] which was corroborated by one-proton signals of three contiguous olefinic hydrogens in its $^1H$ NMR spectrum as double doublets at $\delta$ 6.90 ($J$ = 9.0, 5.5 Hz), 6.60 ($J$ = 9.0, 6.0 Hz), and 6.46 ($J$ = 6.0, 2.5 Hz) assigned to H-3, H-1 and H-4, respectively. The appearance of an intensified ion peak at $m/z$ 141 and a two-proton signal at $\delta$ 4.20 ($HOCH_2$-27) supported the presence of a hydroxyl group at C-27 as well as that of 12-deoxywithastramonolide [6]. It also showed, like typical 20-H withanolides, a diagnostic one-proton multiplet at $\delta$ 4.30 for carbonylic H-22, two one-proton doublets at $\delta$ 3.70 and 3.50 with coupling interactions of 10.5 Hz for C-18 hydroxymethylene protons, and a one-proton signal at $\delta$ 3.30 (*ddd*, $J$ = 4.5, 9.5, 5.0 Hz) due to H-7$\alpha$. The methyl signals resonated as broad singlets at $\delta$ 2.06 (Me-28) and 1.23 (6H, Me-19, Me-21). The structure of **5** was supported by its $^{13}C$ NMR data [6, 14]. Treatment of **5** with acetic anhydride–pyridine yielded a triacetyl product (**5a**) which still demonstrated an IR absorption band for a hydroxyl group (3430 $cm^{-1}$) indicating the existence of one tertiary hydroxyl group in the molecule. The carbinol proton signals shifted to $\delta$4.50 (2H, $H_2$-27), 4.13 (1H, *d*, $J$ = 10.5 Hz, H-18a), 3.90 (1H, *d*, $J$ = 10.5 Hz, H-18b) and 3.77 (1H, *m*, H-7) in the $^1H$ NMR spectrum of **5a**. The data indicated that the structure of **5** must be (20*R*, 22*R*)-1-oxo-7$\beta$, 18, 20$\beta$, 27-tetrahydroxywitha-2,4.24-trienolide.

## EXPERIMENTAL

*General experimental procedures.* Mps were determined in capillaries on a Perfit melting point apparatus and are uncorr. IR spectra ($\nu_{max}$) were measured by Perkin Elmer 577 spectrophotometer in KBr pellets. UV spectra ($\lambda_{max}$) were obtained on a Hitachi 320 spectrophotometer in MeOH. $^1H$ NMR spectra were recorded on a Perkin Elmer 100 MHz in $CDCl_3$. $^{13}C$ NMR spectra were scanned on a Bruker VM-40 100 MHz instrument in $CDCl_3$. The EI-MS were screened on a Jeol D-300 instrument. CC was carried out using silica gel (60–120 mesh). TLC was performed on silica gel G. Iodine vapours, caric ammonium sulphate and UV light were used for visualization of TLC spots.

*Preparation of plant material.* The stem bark of *W. somnifera* was collected from the South Delhi region and air-dried. The plant was identified by Dr M. P. Sharma, taxonomist, Department of Botany, Jamia Hamdard, New Delhi, where a voucher specimen is preserved.

*Isolation of compounds.* The pulverized sample (2.80 kg) was extracted exhaustively in a Soxhlet apparatus with EtOH (95%). The extract was concd *in vacuo* to yield a thick, viscous dark greenish mass (255 g). This material was adsorbed on silica gel (100 g) with constant stirring until completely dried and subjected to a silica gel column prepared in petrol (bp 60–80°). The column was eluted successively with petrol, $CHCl_3$ and MeOH in order of increment of polarity to isolate the following compounds.

*Withasomnilide* (**1**). Elution of the column with $CHCl_3$ afforded flakes of **1**, crystallized from $CHCl_3$–$C_6H_6$ (1:1), 0.76 g (0.027% yield), mp 251–252°. UV $\lambda_{max}^{MeOH}$ 229 nm (log $\varepsilon$ 6.1). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3460, 2932, 1704, 1688, 1426, 1380, 1300, 1268, 1130, 1030, 910, 802, 784, 664. $^1H$ NMR: $\delta$ 6.60 (1H, *m*, H-3), 5.73 (1H, *d*, $J$ = 10.0 Hz, H-2), 4.46 (1H, *m*, H-22), 3.20 (1H, *m*, H-4), 3.10 (1H, *d*, $J$ = 10.0 Hz, H-7$\alpha$), 3.00 (1H, *d*, $J$ = 10.0 Hz, H-6$\alpha$), 1.86 (6H, *br s*, Me-27, Me-28), 1.55 (1H, *br s*, H-20), 1.16 (3H, *br s*, Me-19),

1.00 (3H, *d*, $J = 7.0$ Hz, Me-21), 0.83 (3H, *br s*, Me-18). EI-MS *m/z* (rel. int.): 470 $[M]^+$ ($C_{28}H_{38}O_6$) (1.3), 452 (2.1), 437 (1.9), 434 (12.7), 422 (2.3), 419 (2.1), 404 (2.1), 402 (1.9), 389 (2.3), 384 (3.4), 366 (4.1), 346 (3.1), 328 (3.6), 290 (2.1), 276 (3.9), 262 (5.5), 248 (2.6), 234 (4.8), 240 (4.7), 236 (35.3), 222 (7.5), 218 (2.8), 208 (17.8), 204 (3.0), 202 (17.0), 200 (3.4), 194 (7.4), 196 (14.5), 190 (4.3), 186 (6.0), 180 (6.6), 172 (10.9), 170 (22.3), 166 (2.9), 148 (6.1), 125 (100), 124 (20.2), 109 (44.9), 106 (18.2), 96 (22.1), 80 (20.6), 68 (24.3). $^{13}C$ NMR (Table 1).

*Withasomniferanolide* (**2**). Further elution of the column with $CHCl_3$ furnished amorphous powder of **2**, re-crystallized from $CHCl_3$–MeOH (9:1), 0.62 g (0.022% yield), mp 225–226°. UV $\lambda_{max}^{MeOH}$ 225 nm (log ε 7.4), IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 2928, 1710, 1685, 1480, 1390, 1196, 1134, 984, 805, 762, 716. $^1H$ NMR: δ 6.50 (1H, *ddd*, $J = 10.0$, 2.0, 4.5 Hz, H-3), 5.70 (1H, *dd*, $J = 10.0$, 5.5 Hz, H-2), 5.56 (1H, *br s*, H-6), 4.46 (1H, *dd*, $J = 10.0$, 4.5 Hz, H-22), 3.76 (1H, *ddd*, $J = 9.5$, 4.0 Hz, H-11α), 3.53 (1H, *ddd*, $J = 9.5$, 4.5, 4.0 Hz, H-16β), 1.83 (3H, *br s*, Me-28), 1.80 (3H, *br s*, Me-27), 1.20 (3H, *br s*, Me-19), 1.13 (3H, *d*, $J = 6.5$ Hz, Me-21), 0.83 (3H, *br s*, Me-18). EI-MS *m/z* (rel. int.): 470 $[M]^+$ ($C_{28}H_{38}O_6$) (1.2), 402 (1.3), 336 (5.3), 332 (13.5), 304 (1.3), 297 (18.4), 294 (2.7), 279 (22.5), 277 (3.9), 274 (4.3), 264 (26.1), 260 (7.3), 250 (9.4), 242 (3.4), 238 (5.6), 236 (27.4), 225 (18.8), 220 (14.1), 211 (12.6), 210 (9.4), 208 (21.1), 206 (6.0), 202 (4.6), 196 (16.1), 188 (8.3), 184 (10.1), 176 (5.5), 174 (10.1), 170 (20.3), 169 (11.0), 166 (5.5), 158 (20.3), 139 (21.3), 134 (23.2), 125 (100), 120 (27.7), 108 (55.6), 96 (48.3), 68 (36.3). $^{13}C$ NMR (Table 1). Acetylation of **2** with $Ac_2O$–pyridine and usual work-up yielded a diacetyl product (**2a**), mp 189–191. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH), 1740 (CO), 1725 (CO). $^1H$ NMR ($CDCl_3$) δ 6.53 (1H, *m*, H-3), 5.66 (1H, *d*, $J = 10.0$ Hz, H-2), 5.23 (1H, *m*, H-6), 4.10 (1H, *m*, H-11α), 3.96 (1H, *m*, H-16), 1.86 (3H, *br s*, Me-27), 1.83 (3H, *br s*, Me-28), 1.23 (3H, *br s*, Me-21), 0.96 (3H, *br s*, Me-19).

*Somniferanolide* (**3**). Elution of the column with $CHCl_3$–MeOH (19:5) afforded flakes of **3**, re-crystallized from $CHCl_3$–MeOH (9:1), 0.39 (0.14% yield), mp 230–231°. UV $\lambda_{max}^{MeOH}$ 225 nm (log ε 7.6). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420, 2944, 1710, 1676, 1400, 1384, 1212, 1126, 1068, 978, 892, 762, 720. $^1H$ NMR: δ 6.60 (1H, *ddd*, $J = 10.1$, 4.5, 2.5 Hz, H-3), 5.66 (1H, *dd*, $J = 10.1$, 5.0 Hz, H-2), 5.50 (1H, *d*, $J = 6.50$ Hz, H-6), 4.40 (1H, *dd*, $J = 10.5$, 4.5 Hz, H-22), 3.76 (1H, *ddd*, $J = 9.5$, 5.0, 4.5 Hz, H-11α), 3.13 (1H, *br s*, H-16 β), 1.86 (3H, *br s*, Me-28), 1.73 (1H, *br s*, Me-27), 1.20 (3H, *br s*, Me-19), 1.00 (3H, *d*, $J = 6.5$ Hz, Me-21), 0.80 (3H, *br s*, Me-18). EI-MS *m/z* (rel. int.): 468 $[M]^+$ ($C_{28}H_{36}O_6$) (1.0), 432 (3.9), 417 (3.4), 400 (2.2), 334 (4.0), 316 (2.5), 315 (2.5), 292 (7.3), 274 (5.0), 262 (36.4), 260 (12.7), 256 (3.6), 248 (11.6), 242 (6.4), 238 (2.7), 234 (7.7), 220 (8.1), 208 (8.7), 206 (5.1), 202 (3.3), 194 (7.2), 184 (16.5), 176 (3.2), 170 (18.6), 158 (16.2), 153 (9.6), 134 (18.7), 125 (100), 120 (27.3) 108 (24.8), 98 (36.5), 68 (24.3). $^{13}C$ NMR (Table 1). Acetylation of **3** with $Ac_2O$–pyridine gave monoacetyl derivative (**3a**), mp 207–209°. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3450, 1725, 1710, 1680. $^1H$ NMR: δ6.26 (1H, *m*, H-3), 5.70 (1H, *m*, H-2), 5.53 (1H, *d*, $J = 6.5$ Hz, H-16), 4.30 (1H, *br s*, H-22), 4.13 (1H, *m*, H-11), 2.10 (3H *br s*, COMe), 1.88 (3H, *br s*, Me-27), 1.76 (3H, *br s*, Me-28), 1.25 (3H, *br s*, Me-19), 1.00 (3H, *d*, $J = 6.0$ Hz, Me-21), 0.83 (3H, *br s*, Me-18).

*Somniferawithanolide* (**4**). Further elution of the column with $CHCl_3$–MeOH (19:5) yielded an amorphous powder of **4**, re-crystallized from $CHCl_3$–MeOH (1:1), 0.43 g (0.015% yield), mp 127–128°, UV $\lambda_{max}^{MeOH}$ 228 nm (log ε7.1), IR $\nu_{max}^{KBr}$: 3445, 2940, 1710, 1695, 1518, 1456, 1380, 1130, 1032, 732. $^1H$ NMR: δ 6.61 (1H, *ddd*, $J = 10.5$, 2.0, 5.0 Hz, H-3), 5.70 (1H, *dd* $J = 10.5$, 5.0 Hz, H-2), 5.50 (1H, *br s*, H-6), 4.46 (1H, *m*, H-22), 4.10 (1H, *d*, $J = 10.5$ Hz, H-18a), 3.90 (1H, *d*, $J = 10.5$ Hz, H-18b), 2.00 (3H, *br s*, Me-27), 1.96 (3H, *br s*, Me-28), 1.20 (6H, *br s*, Me-19, Me-21). EI-MS *m/z* (rel. int.): 470 $[M]^+$ ($C_{28}H_{38}O_6$) (1.2), 454 (1.3), 402 (1.7), 350 (2.4), 336 (1.2), 332 (1.6), 314 (20.6), 301 (1.3), 294 (5.0), 266 (8.6), 262 (25.1), 260 (8.8), 210 (10.7), 204 (9.8), 192 (22.2), 190 (18.1), 176 (35.2), 172 (13.8), 169 (20.2), 158 (20.1), 151 (11.8), 134 (22.3), 125 (82.0), 120 (34.5), 68 (36.5). $^{13}C$ NMR (Table 1). Acetylation of **4** with $Ac_2O$–pyridine formed a monoacetyl product **4a**, mp 118–119°. IR $\nu_{max}^{KBr}$ $cm^{-1}$ 1735, 1710, 1785. $^1H$ NMR ($CDCl_3$): δ6.67 (1H, *m*, H-3), 5.73 (1H, *d*, $J = 10.0$ Hz, H-2), 5.53 (1H, *br s*, H-6), 4.46 (1H, *br s*, H-22), 4.36 (1H, *d*, $J = 10.5$ Hz, $H_2$-18a), 4.27 (1H, *d*, $J = 10.5$ Hz, H-18b), 2.13 (3H, *br s*, OAc), 2.00 (3H, *br s*, Me-27), 1.96 (3H, *br s*, Me-28), 1.20 (6H, *br s*, Me-19, Me-21).

*Somniwithanolide* (**5**). Elution of the column with $CHCl_3$–MeOH (9:1) gave an amorphous powder of **5**, re-crystallized from MeOH, 0.41 g (0.015% yield), mp 144–146°, UV $\lambda_{max}^{MeOH}$ nm: 230, 286, 317 (log ε 7.5, 3.4, 13.3). IR $\lambda_{max}^{KBr}$ $cm^{-1}$: 3428, 2936, 1700, 1655, 1605, 1518, 1455, 1395, 1315, 1032. $^1H$ NMR: δ6.90 (1H, *dd*, $J = 9.0$, 5.5 Hz, H-3), 6.60 (1H, *dd*, $J = 9.0$, 6.0 Hz, H-1), 6.46 (1H, *dd*, $J = 6.0$, 2.5 Hz, H-4), 4.30 (1H, *m*, H-22), 4.20 (2H, *br m*, $H_2$-27), 3.70 (1H, *d*, $J = 10.5$ Hz, H-18a), 3.50(1H, *d*, $J = 10.5$ Hz, H-18b), 3.30 (1H, *ddd*, $J = 4.5$, 9.5, 5.0 Hz, H-7a), 2.06 (3H, *br s*, Me-28), 1.23 (6H, *br s*, Me-19), Me-21), EI-MS *m/z*: 486 $[M]^+$ ($C_{28}H_{38}O_7$) (1.3), 450 (2.2), 380 (15.0), 366 (5.6), 336 (4.0), 310 (20.2), 296 (2.4), 282 (18.3), 260 (26.2), 242 (11.1), 229 (12.0), 226 (14.5), 204 (4.9), 190 (26.6), 186 (9.4), 185 (8.5), 176 (54.4), 172 (12.4), 158 (16.8), 150 (13.3), 141 (63.7), 132 (25.7), 120 (34.7), 106 (87.6). $^{13}C$ NMR (Table 1). Acetylation of **5** with acetic anhydride–pyridine produced a triacetyl product **5a**, mp 121–122°. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3430, 1735, 1725, 1710, 1660. $^1H$ NMR: δ 7.00 (1H *m*, H-3), 6.77 (1H, *d*, $J = 10.5$ Hz, H-2), 6.36 (1H, *d*, $J = 10.5$ Hz, H-4), 4.50 (2H, *br s*, $H_2$-27), 4.23 (1H, *br s*, H-22), 4.13 (1H, *d*, $J = 10.5$ Hz, H-18a), 3.90 (1H, *d*, $J = 10.5$, Hz, $H_2$-18b), 3.77 (1H, *br m*, $w_{1/2} = 18.50$ Hz, H-7 α), 2.16 (6H, *br s*, 2 × COMe), 2.00 (6H, *br s*, COMe, Me-28), 1.27 (6H, *br s*, Me-19, Me-21).

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