TWO NEW WITHANOLIDES FROM *WITHANIA SOMNIFERA*

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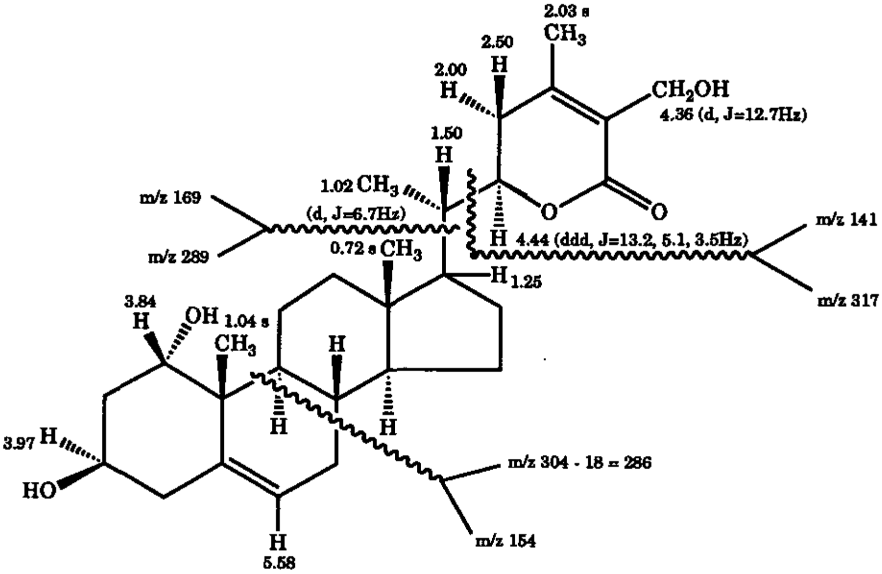
Abstract- Two new withanolides, sominone (1α, 3ß, 27-trihydroxy-22 (*R*)-witha-5, 24-dienolide) (1) and sominolide (4ß,27-dihydroxy- 14α,15α-epoxy-1-oxo-22- (*R*)-witha-2, 24-dienolide) (2) were isolated from the herbs of *Withania somnifera* Dun. (Solanaceae). The elucidation of their structures is based on extensive spectroscopic studies.

A number of highly oxygenated C-28 steroidal lactones have been isolated from various genera of the plant family Solanaceae including *Withania, Acnistus, Datura, Jaborosa, Lycium, Nicardra* and *Physalis*.[1] These compounds have considerable phytochemical, chemotaxonomic and medicinal interest.[2] Two species of the genera *Withania i.e. Withania somnifera* and *W. coagulans* are commonly found in Pakistan and are claimed to have uses in the indigenous system of medicine for the treatment of ulcers, tumors, tubercular glands, carbuncles, as diuretic and antipyretic.[1,2] In continuation of our interest on medicinal plants of Pakistan, we have investigated the chemical constituents of *Withania somnifera*. The present investigation on the ethanolic extracts of the herbs of *W. somnifera* revealed the presence of two new withanolides, sominone (1) and sominolide (2).

Sominone (1), $C_{28}H_{42}O_5$, showed a uv absorption maximum at 215 nm, characteristic of an α, ß-unsaturated lactone chromophore.[3] The ir spectrum displayed intense absorptions at 1710 (α,ß-unsaturated lactone) and 3500 (O-H) cm^{-1}. The high-resolution electron impact mass spectrum of 1 showed the molecular ion at m/z 458.3051 corresponding to the molecular formula $C_{28}H_{42}O_5$ (calcd 458.3032), indicating the presence of eight double bond equivalents in the molecule. Considerably strong peaks at m/z 440 and 422 were due to the loss of one and two water molecules respectively. The ion at m/z 169.0851 of composition $C_9H_{13}O_3$ resulted by the cleavage of the C-17/C-20 bond and indicated the presence of a six-membered lactone substituent at the C-20 side chain, while the fragment at m/z 289 ($C_{19}H_{29}O_2$) in-turn represented the remaining half of the molecule. The mass fragment at m/z 154 ($C_9H_{14}O_2$), formed by the cleavage of ring B, was indicative of the presence of two oxygen functions and one double bond in rings A and B of the molecule.[4] The prominent peak at m/z 141($C_7H_9O_3$) was originated by cleavage of the C-20/C-22 bond, whereas the base peak resulted by the further loss of carbonyl moiety from m/z 141, which were found in a number of withanolide, as shown in Figure 1.



Sominone 1

The ^{1}H-nmr spectrum (400 MHz, $CDCl_3$) showed three three-proton singlets for the quaternary methyls at δ 0.72, 1.04 and 2.03 for the C-18, C-19 and C-28 protons respectively, while a three-proton doublet at δ 1.02 (J = 6.7 Hz) was due to the secondary methyl protons. A downfield methine signal at δ 4.44 was assigned to the C-22 proton of the lactone moiety. Two-proton AB double doublets at δ 4.34 and 4.39 (J = 12.7 Hz) were ascribed to the C-27 methylenic protons.[6] These observations convincingly support a tetracyclic steroidal skeleton with a lactone substituent, as found in a number of common withanolides.[7] Two interesting multiplets resonated at δ 3.84 and 3.97 due to two oxygenated methine protons ,[4] and showed vicinal coupling with the same set of methylenic protons, thereby indicating the presence of a fragment HOCH-CH_2- CHOH, possibly in ring A of the steroidal skeleton. The α and β stereochemistry of the C-1 and C-3 hydroxyl groups respectively, were assigned by comparing the ^{1}H-nmr chemical shifts with the earlier reported withanolide 1α, 3ß, 20α-trihydroxy (20*R*, 22*R*)-witha-5,24- dienolide (3)[4]. A downfield multiplet at δ 5.58 was assigned to the C-6 vinylic protons showing vicinal couplings with the C-7 methylenic protons[4,5]

The two-dimensional COSY 45° experiment was performed to determine the ^{1}H/^{1}H connectivities.[6,7] Strong cross peaks between the C-1 proton (δ 3.84) and the C-2 α and ß-protons (δ 2.10 and 1.78 respectively) appeared in the COSY 45° spectrum. The C-2 α and ß-protons also showed COSY coupling interactions with the C-3 proton which resonated at δ 3.97. The vicinal couplings between the C-22 proton (δ 4.44) and the C-23 ß and α-protons (δ 2.50 and 2.00) respectively, as well as with the C-20 methine proton (δ 1.50) were also apparent in the COSY 45° spectrum of compound (1). [8-10] The configuration at C-22 is assumed to be *R* on biogenetic grounds as in other withanolides[4,5,10,11] Other COSY 45° interaction are shown in Table 1.

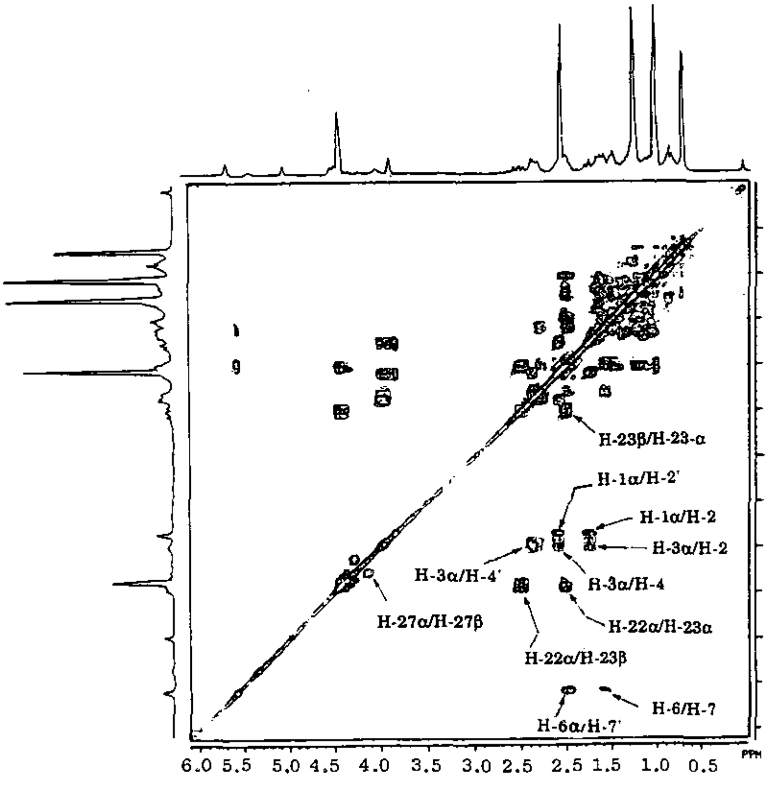


Figure 1: COSY-45° spectrum of sominone.

Table 1

COSY-45° connectivity of compound (1)

H-1α	3.84	H-2’ (2.10), H-2 (1.78)
H’-2	2.10	Hα-1 (3.84), H-2 (1.78), Hα-3 (3.97)
H-2	1.78	Hα-1 (3.84), H’-2 (2.10), Hα-3 (3.97)
H-3α	3.97	H’-2 (2.10), H-2 (1.78), H’-4 (2.38), H-4 (2.10)
H’-4	2.38	H–3α (3.97), H-4 (2.10)
H-4	2.10	H-3α (3.97), H’-4 (2.38)
H-6	5.58	H’-7 (2.30), H-7 (1.60)
H’-7	2.30	H-6 (5.58), H-7 (1.60)
H-7	1.60	H-6 (5.58), H’-7(2.30)
H-17	1.25	H-20 (1.5)
H-20	1.5	H-17 (1.25), CH_3-21 (1.04), H-22α (4.44)
CH_3-21	1.04	1.5 (H-20)
H-22α	4.44	1.5 (H-20), H-23ß (2.50), H-23α (2.00)
H-23β	2.50	H-23α (2.00), H-22α (4.44) CH_3-28 (2.03)
H-23α	2.00	H-23ß (2.50), H-22α (4.44), CH_3-28 (2.03)
CH_3-28	2.03	H-23ß (2.50)

COSY-45° connectivity of compound (2)

H-2	6.19	H-3 (6.92)
H-3	6.92	H-2 (6.19), 3.96 (Hα-4)
H-4α	3.96	H-3 (6.92), 2.40 (Hα-5)
H-5α	2.40	H-4α (3.96)
H-15ß	3.22	H’-16 (2.30), H-16 (1.32)
H’-16	2.30	H-15ß (3.22), H-16 (1.32), H-17 (1.53)
H-16	1.32	H-15ß (3.22), H’-16 (2.30), H-17 (1.53)
H-17	1.53	H’-16 (2.30), H-16 (1.32), H-20 (1.95)
H-20	1.95	H-17 (1.53), CH_3-21 (1.00), 4.44 (H-22α)
CH_3-21	1.00	H-20 (1.95)
H-22α	4.44	H-20 (195), 2.50 (H-23ß), 2.00 (H-23α)
H-23ß	2.50	4.44 (H-22α), 2.00 (H-23α)
H-23α	2.00	4.44 (H-22α), 2.50 (H-23ß)

The ^{13}C-nmr spectrum (100 MHz, $CDCl_3$) (DEPT and Broad Band)[6] showed signals for all twenty eight carbons. The signals at δ 152.8, 125.4 and 167.0 were assigned to C-24, C-25 vinylic and C-26 carbonyl carbons respectively. The oxygen bearing C-1 and C-3 carbons resonated at δ 72.9 and 66.4 respectively. The chemical shift assignments for the various carbons of compound (**1**) are presented in Table **2** and compared with the previously known withanolide (**3**)[4]. The above mentioned spectroscopic studies led to structure (1) (1ß,3ß,27-trihydroxy-22(*R*)-witha-5,24-dienolide) for sominone.

Table **2**

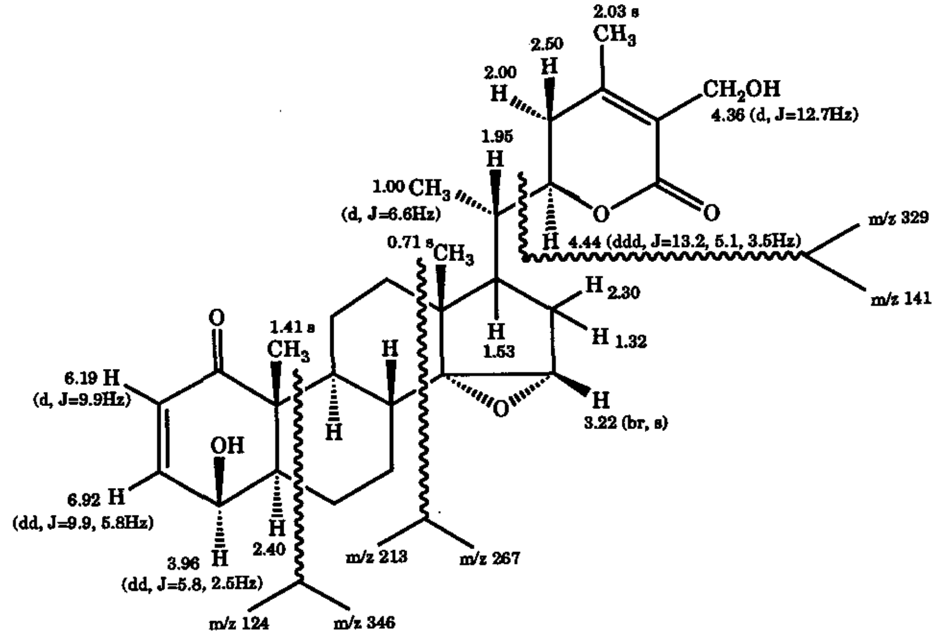
^{13}C-Nmr spectral data of compounds (**1**)[+] and (**3**)

	1	3		1	3
C-1	72.9 (CH)	72.9 (CH)	C-15	27.4 (CH_2)	23.9 (CH_2)
C-2	38.4 (CH_2)	38.3 (CH_2)	C-16	24.4 (CH_2)	22.0 (CH_2)
C-3	66.4 (CH)	66.3 (CH)	C-17	52.1 (CH)	54.7 (CH)
C-4	41.4 (CH_2)	41.4 (CH_2)	C-18	11.7 (CH_3)	13.6 (CH_3)
C-5	137.4 (C)	137.6 (C)	C-19	14.0 (CH_3)	19.4 (CH_3)
C-6	125.4 (CH)	125.2 (CH)	C-20	38.4 (CH)	75.2 (C)
C-7	31.80 (CH_2)	31.7 (CH_2)	C-21	19.4 (CH_3)	20.8 (CH_3)
C-8	31.9 (CH)	31.3 (CH2)	C-22	79.9 (CH)	81.0 (CH)
C-9	41.6 (CH)	41.5 (CH)	C-23	29.9 (CH_2)	31.5 (CH_2)
C-10	52.1*(C)	47.7* (C)	C-24	152.8 (C)	149.1 (C)
C-11	20.2 (CH_2)	20.2 (CH_2)	C-25	125.4 (C)	122.0 (C)
C-12	39.5 (CH_2)	39.8 (CH_2)	C-26	167.0 (C)	166.2 (C)
C-13	42.8*(C)	43.0* (C)	C-27	57.5 (CH_2)	12.5 (CH_3)
C-14	56.3 (CH)	56.0 (CH)	C-28	19.9 (CH_3)	20.5 (CH_3)

Note: * refer to interchangeable data.

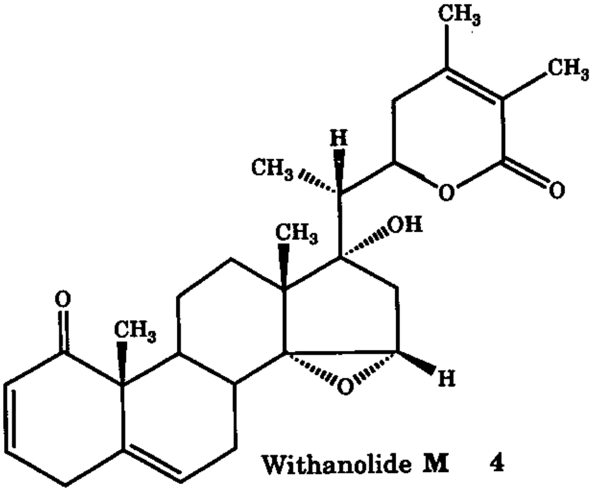
+ assignment of multiplicity by DEPT.

Our second compound, sominolide (**2**), $C_{28}H_{38}O_6$, showed an intense uv absorption at 215 nm.[3] The ir spectrum exhibited bands at 3430 (O H), 1705 (α,ß-unsaturated lactone) and 1690 (α,ß-unsaturated ketone) cm^{-1}. The high-resolution electron impact mass spectrum displayed the molecular ion at m/z 470.2670 in agreement with the molecular composition $C_{28}H_{38}O_6$ (calcd 470.2668) indicating ten degrees of unsaturation. The peaks at m/z 452 and 434 were representative of the loss of one and two water molecules respectively. The peak at m/z 346 arose by the loss of a $C_7H_8O_2$ fragment from the molecular ion. The ions at m/z 264 and 206 were due to the fragments resulting from the cleavages of ring C. The chemical composition of the ion at m/z 264 i.e $C_{14}H_{20}O_4$ indicated the presence of four oxygens as lactone, hydroxy and epoxide functions. The fragment at m/z 196 ($C_{11}H_{16}O_3$) resulting from the cleavage of ring D unambiguously determined the position of the epoxide functionality at C-14/C-15. The base peak at m/z 95 again resulted from the cleavage of the six-membered lactone moiety.

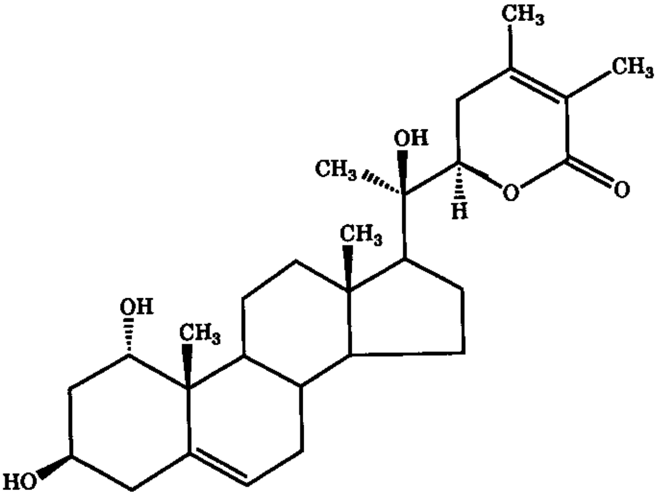


Sominolide **2**

The ^{1}H-nmr spectrum of **2** exhibited three three-proton singlets at δ 0.71, 1.41 and 2.03 as well as a three-proton doublet at δ 1.00 (J = 6.7 Hz). These were assigned to the protons of the C-18, C-19 and C-28 quaternary methyls,[2] and the C-21 secondary methyl respectively. A doublet resonating at δ 6.19 ($J_{2,3}$ = 9.9 Hz) was assigned to the C-2 vinylic proton which was coupled to the C-3 vinylic proton. The latter appeared as a doublet of doublet at δ 6.92 ($J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 5.8 Hz). The C-3 proton was also coupled with the C-4 methine proton, which appeared as a doublet of doublet at δ 3.96 ($J_{4,3}$ = 5.8 Hz, $J_{4,5}$ = 2.5 Hz). The downfield chemical shift of the C-4 methine proton indicated the presence of a geminal hydroxyl substituent. The C-5 methine proton resonated at δ 2.40 as multiplet. The α-stereochemistry of the C-5 proton is based on the coupling constant ($J_{4,5}$ = 2.4 Hz) representing a equatorial-axial coupling. Since the C-4 proton was assumed to be equatorially oriented (based on the chemical shift comparision with reported withanolides), the C-5 proton should therefore be axial. Regarding the stereochemistry at C-20, the β-configuration is supported by biogenetic arguments that all the reported withanolides unsubstituted at C-20 have the C-20 β-configurations.[12] The C-22 methine proton appeared at δ 4.44 ($J_{22\alpha,23\alpha}$ = 13.2 Hz, $J_{22\alpha,23\beta}$ = 3.5 Hz, $J_{22\alpha,20}$ = 5.1 Hz)[10] whereas the vicinal C-20 methine proton resonated as a doublet of double quintet at δ 1.95 ($J_{20,21}$ = 6.6Hz, $J_{20,22a}$ = 5.1 Hz, $J_{20,17}$ = 2.0 Hz). A broad singlet at δ 3.22 was assigned to the C-15 proton of the oxirane ring. The stereochemical assignment of oxirane is based on chemical shift comparisons with those of the earlier reported withanolide, withanolide M (4).[10]



Withanolide M 4



1α,3β,20α-Trihydroxy-20*R*,22*R*-withan-5,24-dienolide **3**

The COSY 45° spectrum of **2** served to establish proton-proton connectivities. Strong cross- peaks appeared between C-2/C-3, C-3/C-4 and C-4/C-5 protons and thus indicated the presence of the fragment O = C-CH = CH-CHOH-CH- in ring A of the steroidal skeleton. The proton of the oxirane (C-15ß H) on ring D of the steroidal skeleton showed COSY couplings with the C-16α and ß- protons (δ 2.30 and 1.32 respectively). The important COSY interactions are shown in Figure 2 and Table 1.

The above mentioned spectral data led to the assignment of structure (**2**) (4ß,27- dihydroxy-14,15 - epoxy-1-oxo-22-(*R*)-withanolide -2,24-dienolide) to sominolide.

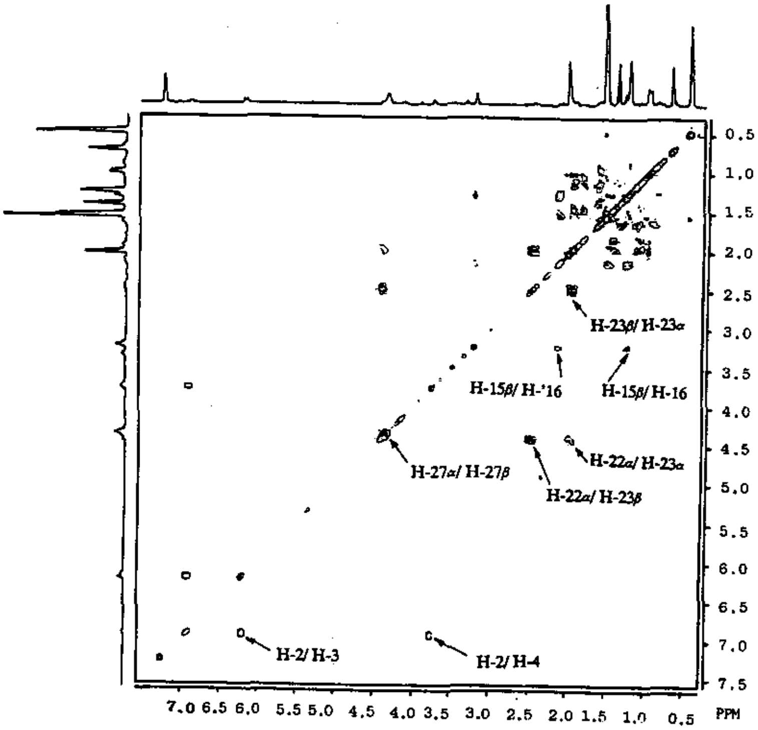


Figure 2: COSY-45° spectrum of sominolide.

EXPERIMENTAL

Optical rotations were meassured on JASCO DIP-360. Ir spectra were recorded on JASCO 302 A spectrophotometer. Uv spectra were recorded on a Hitachi U 3200 spectrophotometer. Ei and hrei mass spectra were recorded on JMS-HX 110 mass spectrometer and data system JMA-DA 5000. The ^{1}H-nmr spectra were recorded on Bruker AM-400 NMR spectrometer. The ^{13}C-nmr spectrum was recorded on Bruker AC-300 spectrometer.

Plant Material: The fresh plant material (100 kg) was collected in Nov. 1989 from suburban areas of Karachi (Pakistan) and identified by the plant taxonomist of Botany Department at University of Karachi.

Isolation of sominone (1) and sominolide (2). The methanolic extracts of the air-dried whole plant (100 kg) were evaporated to a gum (2.70 kg). This gum was dissolved in MeOH (10 l) and defatted with hexane 100 l). The defatted methanolic extract was again evaporated and redissolved in water (10 l). The aqueous extract was extracted with $CHCl_3$ at different pH values. The fraction obtained at pH 7.0 (500 gm) was loaded on a silica gel column (3 kg) and eluted first with hexane and then with hexane-chloroform mixtures. Several fractions were thus obtained. The fraction collected with hexane-chloroform (3.3 : 6.7) was subjected to preparative tlc in methonol-chloroform (1:49) to afford **1** (8 mg) (Rf =0.34) and **2** (5 mg) (Rf =0.64) respectively.

Sominone (1):

Colorless amorphous powder, $[\alpha]^{18}_D$ = - 4.72° (c = 0.21 $CHCl_3$), hreims m/z 458.3051 (M^+, calcd for $C_{28}H_{42}O_5$, 458.3032); ir ν_{max} 3500 and 1710 cm^{-1}, uv λ_{max} 215.0 nm; ^{1}H-nmr ($CDCl_3$, 400 MHz); δ 0.72 (3H, s, 18-Me), 1.04(3H, s,19-Me), 1.02 (3H, d, J = 6.7, 21-Me), 1.50 (1H, m, H-20), 2.00 (1H, m, Hα-23), 2.03 (3H, s, 28-Me), 2.50 (1H, m, Hβ-23), 3.84(1H, t, J = 2.8 Hz, Hα-1), 3.97 (1H, m, Hα-3), 4.36 (2H, d, J = 12.7 Hz, H-27), 4.44 (1H, ddd, J = 13.3, 5.1, 3.5 Hz, Hα-22) and 5.58 (1H, d, J = 5.5 Hz, H-6); eims m/z (80 ev, rel. int., %), 458 (5), 440 (22), 422 (10), 384 (4), 317 (8), 287 (14), 123 (36), 55 (100); ^{13}C-nmr; see Table 2.

Sominolide (2):

Yellowish amorphous powder, $[\alpha]^{18}_{D}$ = - 2.66° (c = 0.6, $CHCl_3$), hreims m/z 470.2670 (M^+, calcd for $C_{28}H_{38}O_6$, 470.2668), ir ν_{max} ($CHCl_3$) 3430, 1705, and 1690 cm^{-1}; uv λ_{max} (MeOH) 214.8 nm; ^{1}H-nmr ($CDCl_3$, 400 MHz); δ 0.71 (3H, s, 18-Me), 1.00 (3H, d, J = 6.7 Hz, 21-Me), 1.41 (3H, s, 19-Me), 1.95 (1H, dd, J = 8.7, 5.1 Hz, H-20), 2.03 (3H, s, 28 Me), 3.96 (1H, dd, J = 5.8, 2.45 Hz, Hα-4), 4.36 (2H, d, J = 12.7 Hz, H-27), 4.44 (1H, ddd, J = 13.2, 5.1, 3.5 Hz, Hα-22), 6.19 (1H, d, $J_{2,3}$ = 9.9 Hz, H-2) and 6.92 (1H, dd, J = 9.9, 5.8 Hz, H-3); eims m/z (80 ev, rel. int.,%) 470 (38), 452 (30), 434 (29), 346 (10), 329 (5), 267 (18), 125 (90), 95 (100).

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