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Unusually sulfated and oxygenated steroids from *Withania somnifera*

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Abstract

Four (**1**, **8**–**10**) and six known (**2**–**7**) withanolides were isolated from the leaves of *Withania somnifera*. Among the new compounds, **10** possessed the rare 3-*O*-sulfate group with the saturation in A ring and **9** contained unusual 1,4-dien-3-one group. Compound **8** did not have usual 2,3 unsaturation in A ring while **1** had the rare C-16 double bond. The structures of all the compounds were elucidated by spectroscopic methods and chemical transformation.

Keywords: *Withania somnifera* L.; Solanaceae; Ashwagandha; Withanolides; Withasteroids; Sulfated; Oxygenated withanolide

1. Introduction

Withania somnifera L. Dunal (Solanaceae), popularly known as "Ashwagandha", is one of the top rank medicinal plants of India and is highly valued for its medicinal and neutraceutical properties (Anonymous, 1962; Sangwan et al., 2004). It is an annual herb growing in dry and arid soil as a wild plant (Singh and Kumar, 1998). The plant is known to synthesize withasteroids (Ray and Gupta, 1994) which have shown antioxidant, anti-tumour (Jayaprakasam et al., 2003), adaptogenic, anti-stress, anti-convulsant, immuno-modulatory and neurological effects (Budhiraja et al., 2000; Furmanowa, 2001). The major source of withanolides in *W. somnifera* has been reported to be in its leaves possessing an excellent selective COX-2 inhibitory activity (Jayaprakasam and Nair, 2003; Glotter, 1991). In this study we report the isolation and characterization of four unusual withanolides along with six known withanolides from its leaves. The structures of new as well as known compounds were elucidated by spectroscopic techniques and chemical transformations.

2. Results and discussion

Although the leaves of *W. Somnifera* have been extensively investigated yielding large number of steroidal structures, our work has, now, afforded 10 steroidal compounds out of which four are new. Among the six known compounds (**2**–**7**), two (**2** and **3**) have been isolated for the first time from the leaves. The structure of the known compounds was compared with the spectral data available in the literature and were identified as: 24,25-dihydrowithanolide A (**2**); withanolide A (**3**); withanone (**4**); withaferin A (**5**); 27-hydroxy withanone (**6**) and 17-hydroxy withaferin A (**7**). The ^{13}C NMR data of **2** and **6** have also been included in Table 1 as they are not traceable in the literature. The structure elucidation of compounds **1**, **8**–**10** have been achieved by spectroscopic methods and are discussed hereunder.

Compound **1** in its IR spectrum showed bands at 3460, 1710, 1690 and 1120 cm^{-1} indicative of hydroxyl, α,β-unsaturated six membered δ-lactone, α,β-unsaturated six membered ketone and epoxide functionalities, respectively. HRMS showed the $[M]^+$ at m/z 452.5863 corresponding to the molecular formula $C_{28}H_{36}O_5$. The ^1H NMR spectrum of **1** showed signals for four tertiary methyls at δ 0.80,

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Table 1
^{13}C NMR spectral data for compound **1**, **2**, **8**–**10** (75 MHz)

Carbon	**1**($CDCl_3$)	**2**($CDCl_3$)	**6**($CDCl_3$)	**8**($CDCl_3$)	**9**($CDCl_3$)	**10**(CD_3OD)
1	202.4 *s*	202.0 *s*	203.4 *s*	212.0 *s*	154.9 *d*	210.6 *s*
2	131.9 *d*	128.3 *d*	129.4 *d*	41.3[a] *t*	129.2 *d*	39.7 *t*
3	143.0 *d*	138.5 *d*	140.4 *d*	66.0 *d*	186.7 *s*	73.0 *d*
4	69.4 *d*	37.0 *t*	37.7 *t*	47.0[a] *t*	123.5 *d*	45.7 *t*
5	63.0 *s*	72.5 *s*	73.8 *s*	73.2 *s*	147.8 *s*	73.5 *s*
6	60.0 *d*	56.5 *d*	56.7 *d*	56.6 *d*	29.4[a] *t*	57.3 *d*
7	30.6 *t*	55.5 *d*	56.7 *d*	56.8 *d*	29.3[a] *t*	57.0 *d*
8	28.0 *d*	35.3 *d*	36.0[a] *d*	35.3 *d*	30.1 *d*	36.9 *d*
9	44.2 *d*	35.8 *d*	36.9[a] *d*	44.9 *d*	42.6 *d*	44.6 *d*
10	46.3 *s*	50.4 *s*	51.8 *s*	51.6 *s*	47.0 *s*	53.0 *s*
11	20.5 *t*	21.0 *t*	22.4 *t*	21.2 *t*	21.7 *t*	22.2 *t*
12	33.9 *t*	40.9 *t*	37.7 *t*	36.6 *t*	39.3 *t*	37.9 *t*
13	48.0 *s*	43.4[b] *s*	49.3[b] *s*	48.3[b] *s*	43.0 *s*	50.0[b] *s*
14	56.6 *d*	54.9[b] *d*	46.5[b] *d*	45.6[b] *d*	56.6 *d*	46.7 *d*
15	32.2 *t*	23.5 *t*	23.3 *t*	22.6 *t*	24.7 *t*	23.8 *t*
16	124.0 *d*	21.0 *t*	34.0 *t*	32.6 *t*	27.6 *t*	33.9 *t*
17	154.8 *s*	52.3 *d*	84.6 *s*	84.9 *s*	51.9 *d*	85.0 *s*
18	15.5 *q*	13.5 *q*	10.0 *q*	9.3 *q*	11.9 *q*	10.2 *q*
19	16.2 *q*	13.9 *q*	15.3 *q*	15.9 *q*	19.6 *q*	16.6 *q*
20	35.5 *d*	75.8 *s*	44.0 *d*	35.6 *d*	39.2 *d*	36.6 *d*
21	15.7 *q*	19.9 *q*	14.9 *q*	15.0 *q*	13.3 *q*	15.6 *q*
22	78.6 *d*	79.7 *d*	79.6 *d*	78.9 *d*	78.2 *d*	80.5 *q*
23	30.6 *t*	31.6 *t*	33.3 *t*	32.2 *t*	31.7 *t*	33.6 *t*
24	149.9 *s*	31.4[b] *d*	154.8 *s*	151.6 *s*	156.9 *s*	153.2 *s*
25	121.0 *s*	40.6[b] *d*	126.9 *s*	120.0 *s*	122.9 *s*	121.7 *s*
26	167.3 *s*	176.0[b] *s*	166.6 *s*	168.0 *s*	166.8 *s*	168.8 *s*
27	11.6 *q*	12.9 *q*	56.7 *t*	12.1 *q*	58.0 *t*	12.6 *q*
28	19.7 *q*	20.5 *q*	19.9 *q*	20.7 *q*	20.5[a] *q*	20.7 *q*
OAC	–	–	–	–	171.7 *s*	–
					20.8[a] *q*	

[a] Values interchangeable for substance.
[b] Assignment by comparison to the analogous steroids (Su et al., 2002; Choudhary et al., 2004).

1.44, 1.86, 1.95. The doublet at δ 1.09 ($J = 7.0$ Hz) suggested that C-21 is a secondary methyl having the usual α-orientation. The down field chemical shifts of the C-27 and C-28 methyl singlets at δ 1.86 and 1.95 supported that both of them are located on a double bond. The relatively more downfield signals and their typical coupling constants at δ 6.21 (*d*, $J = 10.0$ Hz, H-2) and 7.01 (*dd*, $J = 10.0$, 6.0 Hz, H-3), clearly indicated that **1** belongs to the withaferin A type of carbon skeleton with C-4, 5 and 6 functionalities as β-oriented owing to the *cis*-fusion of A/B ring as evidenced by the downfield chemical shift (δ 1.44) of H-19 methyl in the ^1H NMR spectrum (Kirson et al., 1971; Lavie et al., 1966; Anjaneyulu and Rao, 1997a,b; Fuska et al., 1982). A broad singlet at δ 5.52 suggested that the compound contains another double bond with a proton on only one of the carbons (CH=C). ^1H ^1H COSY showed that this singlet has correlation with H-15 which clearly indicated that the double bond is present at C-16. The doublet at δ 3.70 ($J = 6.0$ Hz) and a broad singlet at δ 3.20 were assigned to 4β-hydroxy-5β,6β-epoxy system. The typical downfield methine double triplet at δ 4.39 ($J = 12.0$, 5.5, 3.5 Hz) was assigned to the H-22 of the lactone moiety. In $^1H^1H$ COSY spectrum of **1**, the H-3 correlated with the H-2 and H-4, whereas the H-6 interacted with H-7. The H-22 also showed the vicinal coupling with H-23. These data supported that a double bond is unusually present at C-16 along with the usual unsaturation at C-2 and C-24 (Jayaprakasam and Nair, 2003).

The ^{13}C NMR spectrum indicated the presence of 28 carbon resonances including five methyl, five methylene, 10 methine and eight quaternary carbons. The characteristic down field signals at δ 202.4 and 167.3 were due to α,β-unsaturated ketone and lactone carbonyls, respectively, along with the characteristic doublets at δ 143.0 and 131.9 for the vinylic carbons at C-3 and C-2, respectively, in ring A. The singlets at δ 149.9 and 121.0 were attributed to the quaternary vinylic carbons at C-24 and C-25, respectively (Fuska et al., 1982), while a doublet at δ 124.0 and a singlet at δ 154.8 suggested that an additional CH=C is present in the molecule which has been placed at C-16, 17 as H-16 correlated with the H-15 in $^1H^1H$ COSY (Chen et al., 1990). The typical signals at δ 78.6, 69.4, 63.0 and 60.0 were assigned to the oxygenated carbons at C-22, C-4, C-5 and C-6, respectively. The signals appearing at δ 19.7, 16.2, 15.7, 15.5 and 11.6 were assigned to the C-28, C-19, C-21, C-18 and C-27 methyls, respectively (Fuska et al., 1982). On acetylation it gave an acetate which in its ^1H NMR spectrum

showed shifting of the doublet to δ 4.65 for H-4 along with the additional singlet at δ 2.01 for the acetate. These data clearly established the structure of compound **1** as 27-deoxy-16-en-withaferin A.

Compound **8** in its IR spectrum showed bands at 3420, 1720, 1700 and 1120 cm^{-1} indicative of hydroxyl, α,β-unsaturated six membered δ-lactone, α,β-unsaturated six membered ketone and epoxide functionalities, respectively. HRMS showed the $[M]^+$ at *m/z* 488.6090 corresponding to the molecular formula $C_{28}H_{40}O_7$. The ^1H NMR spectrum of **8** showed four quartets at δ 0.83, 1.23, 1.85, 1.92 for methyls at H-19, H-18, H-28 and H-27, respectively. The doublet at δ 1.02 ($J = 7.0$ Hz) suggested that C-21 is a secondary methyl having the usual α-orientation. In the ^1H NMR, the typical vinylic signals for the H-2 and H-3, were absent while an additional signal resonated at δ 4.32 which showed correlation with H-2 and H-4 at δ 2.68, 2.92 and 2.28 in the $^1H^1H$ COSY. These observations clearly indicated that C-$_{2,3}$ double bond is saturated and C-3 is oxygenated to a hydroxy ($W_{1/2} = 17.5$ Hz). The doublet ($J = 3.2$ Hz) at δ 3.03 for H-6 and a double doublet at δ 3.30 ($J = 3.2$, 2.1 Hz) for H-7 along with a broad singlet overlapped with H-7 at δ 3.27 for 5-OH were also present. The oxygenation at 5,6,7 carbons were comparable to the well established withanone type of carbon skeleton having α-orientation owing to the *trans*-junction of A/B ring as evidenced by the 0.26 ppm upfield chemical shift of H-19 protons in the ^1H NMR spectrum (Kirson et al., 1971). The typical down field double triplet at δ 4.63 ($J = 11.0$, 5.5, 3.0 Hz) was assigned to the H-22 of a withasteroidal lactone (Jayaprakasam and Nair, 2003; Abou-Douh, 2002; Anjaneyulu and Rao, 1997a,b).

The ^{13}C NMR spectrum indicated the presence of 28 carbon resonances including five methyl, seven methylene, eight methine and eight quaternary carbons. The down field signals at δ 212.0 and 168.0 supported the presence of a saturated ketone in A ring and an unsaturated δ-lactone carbonyl of E ring, respectively. The singlets at δ 151.7 and 120.0 were attributed to the quaternary vinylic carbons at C-24 and C-25, respectively. Further characteristic signals at δ 84.9, 78.9, 73.9, 66.0, 56.8 and 56.6 supported the presence of the oxygenation at C-17, C-22, C-5, C-3, C-7 and C-6, respectively. Rest of the signals appearing at δ 20.7, 15.9, 15.0, 12.1 and 9.3 were assigned to the C-28, C-19, C-21, C-27 and C-18 methyls, respectively (Anjaneyulu and Rao, 1997a,b; Shingu et al., 1989).

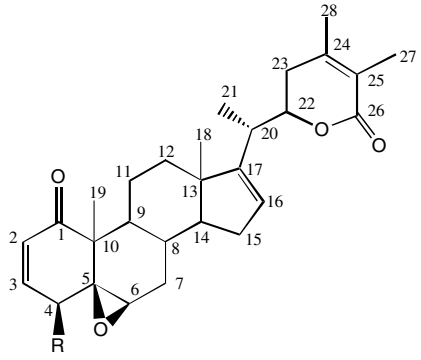
The HSQC spectrum of **8** showed that the carbon signal resonating at δ 66.6 had a correlation with H-3 at δ 4.32 while in the HMBC, this signal showed correlations with H-2 at δ 2.68 and 2.92 as well as H-4 at δ 2.28 supporting that a hydroxyl is present at C-3 without the typical unsaturation at C-2,3. The stereochemistry was assigned as β since the multiplet at δ 4.32 showed $W_{1/2} = 17.0$ Hz (Choudhary et al., 2004). However, the stereochemistry at C-5, C-6 and C-7 was assigned as α by comparing the ^1H NMR chemical shift for H-19 at δ 1.23 and the typical coupling constants in the known compounds like withanone (**4**) and withanolide-A (**3**) (Kirson et al., 1971; Anjaneyulu and Rao, 1997a,b). On acetylation **8** gave an acetate with its multiplet for H-3 shifting to δ 5.02 along with an additional singlet at δ 2.06 for the acetate. These data clearly established the structure of compound **8** as 2,3-dihydro-3β-hydroxy withanone.

Compound **9a** was obtained on separation from the acetylated complex fraction 8. The IR spectrum of **9a** showed bands at 1765, 1710 and 1665 cm^{-1} indicative of ester, α,β-unsaturated six membered δ-lactone and cross conjugated cyclohexadienone functionalities, respectively. HRMS showed the $[M]^+$ at *m/z* 480.3856 corresponding to the molecular formula $C_{30}H_{40}O_5$. The ^1H NMR spectrum of **9a** showed quartet for three tertiary methyls of a withasteroid at δ 0.78, 1.25, 2.06. The doublet at δ 1.03 ($J = 7.0$ Hz) suggested that C-21 is a secondary methyl with the usual α-orientation. The singlets for H-28 appeared at δ 2.06 and for H-27 (2H) at δ 4.89 along with a singlet at δ 2.07 for $OCOCH_3$ suggesting it to possess an acetate at C-27 (Furmanowa et al., 2001). The unusual downfield signals at δ 7.60 (*d*, $J = 5.5$ Hz), 5.96 (*d*, $J = 5.5$ Hz) and 5.80 (*br s*) were attributed to vinylic protons at H-1, H-2 and H-4, respectively, typical to the 1,4-dien-3-one minabeolide steroids earlier isolated from a soft coral *Minabea* sp. (Ksebati and Schmitz, 1988). The characteristic down field double triplet at δ 4.43 ($J = 12.0$, 5.5, 3.0 Hz) was assigned to H-22 of the lactone moiety. In $^1H^1H$ COSY spectrum, the H-1 at δ 7.60 showed correlation with the H-2 at δ 5.96 while H-4 did not show any correlation supporting the placement of double bonds at C-1 and C-4. The possibility of linear dienone systems, like 2,4-dien-1-one or 4,6-dien-1-one, is ruled out by the presence of a band at 240 nm in the UV spectrum of **9a**. This fact was further supported by the typical signals for the protons at C-1 and C-2 for the α,β-unsaturated ketone at δ 7.60 and 5.96 and a single singlet at δ 5.80 for second conjugated double bond which clearly supported the placement of the second conjugated double bond at C-4,5.

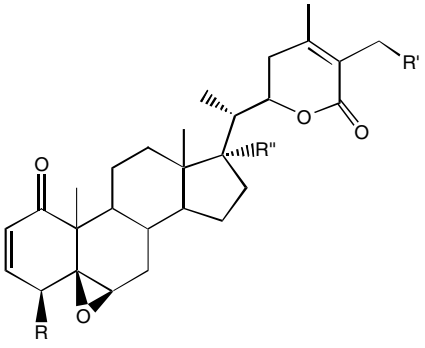
The ^{13}C NMR spectrum also supported above structure by indicating the presence of 28 carbon resonances including four methyl, eight methylene, nine methine and seven quaternary carbons. The down field signals at δ 186.7 and 166.8 were due to the ketone and lactone carbonyls, respectively. The signals at δ 154.9, 123.5, 129.2 and 147.8 were assigned to the vinylic carbons at C-1, C-2, C-4 and C-5, respectively, in ring A while the typical singlet at δ 156.9 and 122.9 were attributed to the quaternary vinylic carbons at C-24 and C-25, respectively. The characteristic signals at δ 78.2 and 58.0 were assigned to oxygenated carbons at C-22 and C-27, respectively. The signals for acetate at C-27 appeared at δ 20.8 and 171.7. The quartets at δ 20.5, 19.6, 13.3 and 11.9 were assigned to the C-28, C-19, C-21 and C-18 methyls, respectively (Ksebati and Schmitz, 1988; Ray and Gupta, 1994). These data clearly suggested that compound **9a** is 27-acetoxy-3-oxo-witha-1,4,24-trienolide, which was isolated after the acetylation of the original hydroxy compound (**9**).

Compound **10** in its IR spectrum showed bands at 3420, 1720, 1710, 1230 and 1120 cm^{-1} indicative of hydroxyl, saturated ketone, α,β-unsaturated six membered δ-lactone, sulfate and epoxide functionalities, respectively. HRMS showed the $[M]^+$ at *m/z* 568.6905 corresponding to the molecular formula $C_{28}H_{40}O_{10}S$. The ^1H NMR spectrum of **10** showed almost similar signals as in case of **8** whose structure elucidation has already been described above. However, when subjected to acetylation by acetic anhydride in the presence of pyridine, it yielded withanone (**4**) which is a typical conversion for 3-*O*-sulfates to a 2-ene steroids (Shingu et al., 1989). Compound **10** when refluxed with dioxane and pyridine, it got converted to **8**. This reaction is again typical for conversion of sulfate group into the corresponding hydroxy group (Shingu et al., 1989). The ^1H NMR and ^{13}C NMR data of the product (**8**) matched well with the authentic sample of 3β-hydroxy-2,3-dihydrowithanone available with us. The compound **8** thus formed, was acetylated to match with **8a** confirming the proposed structure. The presence of sulfate group in the molecule was further supported by the fact that **10** showed high polarity (R_f 0.25, $CHCl_3$:EtOAc:MeOH:C_6H_6 70:2:8:20) as compared to its corresponding hydroxy compound with lower polarity (R_f 0.38, $CHCl_3$:EtOAc:MeOH:C_6H_6, 70:2:4:24) on the TLC (Shingu et al., 1989). In the ^1H NMR spectrum of **10**, the multiplet at δ 4.93 for H-3 got also shifted to upfield at δ 4.32 when converted to **8**. In the $^1H^1H$ COSY spectrum of **10**, the H-3 showed correlation with H-2 and H-4 supporting the placement of sulfate group at C-3. The rest of the ^1H NMR and ^{13}C NMR signals of **10** (see Section 3) also supported its structure as 2,3-dihydro withanone-3β-O-sulfate.

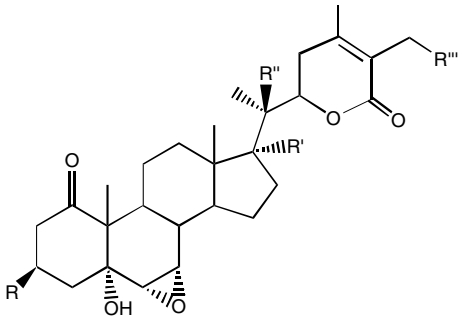
The configuration at C-22 in all the isolated steroids as been assigned as *R*, since in their ^1H NMR spectra, the H-22 has appeared as double triplet by having couplings with the protons of C-23 whereas in the case of *S* configuration, it shows negligible coupling with H-23 (Rahman et al., 1998) which is a normal configuration for the steroids isolated from *W. somnifera*, so far (Ray and Gupta, 1994) and has got the biogenetic support also (Glotter, 1991). However, the presence of sulfate group in steroids is quite rare and has once been reported from *Datura metel* (Shingu et al., 1989). Further isolation of such molecules and the studies on their biogenetic aspects, will certainly help reveal the role of sulfated steroids in *W. somnifera*.



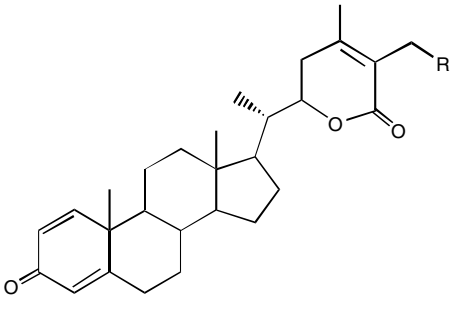
1, R= OH
1a, R= OAc



5, R= R'= OH, R''= H
5a, R= R'= OAc, R''= H
7, R= R'= R''= OH
7a, R= R'= OAc, R''= OH



2, 2-ene, R= R'= R'''= H, R''= OH, 24,25-dihydro
3, 2-ene, R= R'= R'''= H, R''= OH
4, 2-ene, R= R''= R'''= H, R'= OH
6, 2-ene, R= R''= H, R'= R'''= OH
6a, 2-ene, R= R''= H, R'= OH, R'''= OAc
8, R''= R'''= H, R= R'= OH
8a, R''= R'''= H, R= OAc, R'= OH
10, R''= R'''= H, R= OSO_3H, R'= OH



9, R= OH
9a, R= OAc

3. Experimental

3.1. General

Melting point of compounds was recorded on Fisher Johns melting point apparatus. NMR spectra were recorded on a 300 MHz Bruker AV-300 FTNMR spectrometer using TMS as internal standard and FT-IR on a Perkin–Elmer 1710B instruments. FAB HRMS spectra were recorded on JEOL SX 102/DA-6000 mass spectrometer using Argon/Xenon (6 kV, 100 mA) as the FAB gas.

3.2. Plant material

The *W. somnifera* (Ashwagandha) leaves were collected from Lucknow, India in July 2001 and identification of the plant material was done by the taxonomists of CIMAP and the accession is being maintained by us in the institute farm. The plant genotype is deposited (No. RS-NMITLI-II.A) in the National Gene Bank at CIMAP, Lucknow for the field conservation and maintenance.

3.3. Extraction and isolation of compounds

The shade dried leaves (850 g) were ground and defatted three times with *n*-hexane by keeping at RT overnight. The spent material was further extracted with MeOH (3× 1 l) at RT overnight. The methanol extract (108 g) was chromatographed over a column of silica gel (720 g) with *n*-hexane as mobile phase and then elution was carried out in *n*-hexane and EtOAc with solvent gradient. The polarity was increased by sequentially adding 5%, 25%, 50%, 75% ethyl acetate, pure ethyl acetate and finally 5%, 10%, 15% and 20% methanol was added in the ethyl acetate. The fractions (150 ml each) of CC were collected and pooled into nine major fractions based on their TLC pattern. Fr. 1 yielded oleic, linoleic and palmitic acids while fr. 2 and 3 were discarded as they contained chlorophyll and other pigments. Fr. 4 yielded compound **1** (320 mg, R_f 0.72, $CHCl_3$:EtOAc:MeOH:C_6H_6 70:2:4:24), **2** (70 mg) and **3** (52 mg). Fr. 5 on further CC and crystallization yielded mainly compound **4** (1.95 g) and **5** (450 mg). Fr. 6 after further CC gave **4** (675 mg), **5** (2.5 g), **6** (155.0 mg) and **7** (263 mg). Fr. 7 after CC afforded **8** (135 mg, R_f 0.38, $CHCl_3$:EtOAc:-MeOH:C_6H_6 70:2:4:24) while fr. 8 after further CC yielded sucrose (315 mg), and **10** (335 mg, R_f 0.25, $CHCl_3$:EtOAc:-MeOH:C_6H_6 70:2:8:20). Fr. 9 after acetylation and CC yielded **9a** (25 mg, R_f 0.30, $CHCl_3$:EtOAc:MeOH:C_6H_6 70:2:4:24) glucose penta acetate (310 mg) and sucrose octa acetate (265 mg).

3.3.1. 5β,6β-epoxy-4β-hydroxy-1-oxo-witha-2,16,24-trienolide (*1*)

M.p.: 268 °C; $[\alpha]_D^{30}$: +92.60° $CHCl_3$, $c = 0.25$); IR (KBr) cm^{-1}: 3460 (OH), 1710, 1690, 1120; HRMS: 452.5863 (Calc. for $C_{28}H_{36}O_5$ 452.5874); FABMS *m/z* (rel. int.): 452 (10) $[M]^+$, 434 (5) $[M - H_2O]^+$, 416 (5) $[M - 2H_2O]^+$, 327 (24), 285 (22), 125 (100); ^{1}H NMR spectral data (300 MHz, $CDCl_3$) δ H: 6.21 (1H, *d* ($J = 10.0$ Hz), H-2), 7.01 (1H, *dd* ($J = 10.0$, 6.0 Hz), H-3), 3.70 (1H, *d* ($J = 6.0$ Hz), H-4), 3.20 (1H, *br s*, H-6), 2.20 (2H, *dd* ($J = 6.0$, 4.0 Hz), H-15), 5.52 (1H, *br s*, H-16), 0.80 (3H, *s*, H-18), 1.44 (3H, *s*, H-19), 1.09 (3H, *d* ($J = 7.0$ Hz), H-21), 4.39 (1H, *dt* ($J = 12.0$, 5.5, 3.5 Hz), H-22), 2.49 (2H, *br s*, H-23), 1.86 (3H, *s*, H-27), 1.95 (3H, *s*, H-28); ^{13}C NMR spectral data (75 MHz, $CDCl_3$) Table 1.

*Acetylation of **1***. Compound **1** (5 mg) was taken in a flask and to it acetic anhydride (1 ml) was added in presence of 1–2 drops of pyridine which after usual work up gave **1a** (5 mg). ^{1}H NMR spectral data (300 MHz, $CDCl_3$) δ H: 6.25 (1H, *d* ($J = 10.0$ Hz), H-2), 7.05 (1H, *dd* ($J = 10.0$, 6.0 Hz), H-3), 4.65 (1H, *d* ($J = 6.0$ Hz), H-4), 3.23 (1H, *br s*, H-6), 2.20 (2H, *dd* ($J = 6.0$, 4.0 Hz), H-15), 5.53 (1H, *br s*, H-16), 0.81 (3H, *s*, H-18), 1.42 (3H, *s*, H-19), 1.09 (3H, *d* ($J = 7.0$ Hz), H-21), 4.37 (1H, *dt* ($J = 12.0$, 5.5, 3.5 Hz), H-22), 2.50 (2H, *br s*, H-23), 1.87 (3H, *s*, H-27), 1.93 (3H, *s*, H-28), 2.05 (3H, *s*, OCOCH_3).

3.3.2. 6α,7α-epoxy-3β,5α,17α-trihydroxy-1-oxo-witha-24-enolide (*8*)

M.p.: 258 °C; $[\alpha]_D^{30}$: +66.00° MeOH, $c = 0.25$); IR (KBr) cm^{-1}: 3420 (OH), 1710, 1690, 1120; HRMS: 488.6080 (Calc. for $C_{28}H_{40}O_7$ 488.6170); FABMS *m/z* (rel. int.): 488 (2.5) $[M]^+$, 470 (2.5) $[M - H_2O]^+$, 452 (5) $[M-2H_2O]^+$, 345 (70), 125 (100); ^{1}H NMR spectral data (300 MHz, $CDCl_3$) δ H: 2.68 and 2.92 (2H, *d* ($J = 8.5$ Hz), H-2), 4.32 (1H, *m* ($W_{1/2} = 17$ Hz), H-3), 2.28 (2H, *d* ($J = 8.0$ Hz), H-4), 3.27 (1H, *br s*, OH at C-5), 3.03 (1H, *d* ($J = 3.2$ Hz), H-6), 3.30 (1H, *dd* ($J = 3.2$, 2.1 Hz), H-7), 0.83 (3H, *s*, H-18), 1.23 (3H, *s*, H-19), 1.02 (3H, *d* ($J = 7.0$ Hz), H-21), 4.63 (1H, *dt* ($J = 11.0$, 5.5, 3.0 Hz), H-22), 2.52 (2H, *d* ($J = 4.5$ Hz), H-23), 1.85 (3H, *s*, H-27), 1.92 (3H, *s*, H-28); ^{13}C NMR spectral data (75 MHz, $CDCl_3$) Table 1.

*Acetylation of **8***. Compound **8** (5 mg) was taken in a flask and to it acetic anhydride (1 ml) was added in the presence of pyridine and after usual work up **8a** (5 mg) was obtained.^{1}H NMR spectral data (300 MHz, $CDCl_3$) δ H: 2.65 and 2.90 (2H, *d* ($J = 8.5$ Hz), H-2), 5.02 (1H, *m* ($W_{1/2} = 17$ Hz), H-3), 2.24 (2H, *d* ($J = 8.0$ Hz), H-4), 3.27 (1H, *br s*, OH at C-5), 3.03 (1H, *d* ($J = 3.2$, Hz), H-6), 3.30 (1H, *dd* ($J = 3.2$, 2.1 Hz), H-7), 0.83 (3H, *s*, H-18), 1.23 (3H, *s*, H-19), 1.02 (3H, *d* ($J = 7.0$ Hz), H-21), 4.63 (1H, *dt* ($J = 11.0$, 5.5, 3.0 Hz), H-22), 2.52 (2H, *d* ($J = 4.5$ Hz), H-23), 1.85 (3H, *s*, H-27), 1.94 (3H, *s*, H-28), 2.06 (3H, *s*, $OCOCH_3$).

3.3.3. 27-acetoxy-3-oxo-witha-1,4,24-trienolide (*9a*)

M.p.: 213–15 ° C; $[\alpha]_D^{30}$: +24.60° MeOH, $c = 0.25$); UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 240 (2.72); IR (KBr) cm^{-1}: 1765, 1710, 1665; HRMS: 480.3856 (Calc. for $C_{30}H_{40}O_5$ 480.3862); FABMS *m/z* (rel. int.): 480 (15) $[M]^+$, 438 (10) $[M - COCH_2]^+$, 420 (15), 313 (25), 141 (100), 123 (55);

^{1}H NMR spectral data (300 MHz, $CDCl_3$) δ H: 7.60 (1H, *d* ($J = 5.5$ Hz), H-1), 5.96 (1H, *d* ($J = 5.5$ Hz), H-2), 5.80 (1H, *s*, H-4), 0.78 (3H, *s*, H-18), 1.25 (3H, *s*, H-19), 1.03 (3H, *d* ($J = 7.0$ Hz), H-21), 4.43 (1H, *dt* ($J = 12.0$, 5.5, 3.0 Hz), H-22), 2.50 (2H, *d* ($J = 4.5$ Hz), H-23), 4.89 (2H, *br s*, H-27), 2.06 (3H, *s*, H-28), 2.07 (3H, *s*, OCOCH_3); ^{13}C NMR spectral data (75 MHz, $CDCl_3$) Table 1.

3.3.4. 5α,17α-dihydroxy-6α,7α-epoxy-1-oxo-3β-O-sulfate-witha-24-enolide (*10*)

M.p.: 158 °C; $[\alpha]_D^{30}$: +59.40° MeOH, $c = 0.25$); IR (KBr) cm^{-1}: 3420 (OH), 1720, 1710, 1230, 1120; HRMS: 568.6905 (Calc. for $C_{28}H_{40}O_{10}S$ 568.6900); FABMS *m/z* (rel. int.): 568 (2) $[M]^+$, 488 (5) $[M - SO_3]^+$, 470 (5) $[M - H_2SO_4]^+$, 452 (5), 345 (70), 325 (25), 125 (100), 123 (40); ^{1}H NMR spectral data (300 MHz, CD_3OD) δ H: 2.74 and 2.99 (2H, *dd* ($J = 12.5$, 5.5 Hz), H-2), 4.93 (1H, *m* ($W_{1/2} = 17$ Hz), H-3), 2.54 and 2.38 (2H, *d* ($J = 7.5$ Hz), H-4), 3.30 (1H, *br s*, OH at C-5), 3.06 (1H, *d* ($J = 3.2$, Hz), H-6), 3.24 (1H, *dd* ($J = 3.2$, 2.1 Hz), H-7), 0.88 (3H, *s*, H-18), 1.25 (3H, *s*, H-19), 1.05 (3H, *d* ($J = 7.0$ Hz), H-21), 4.63 (1H, *br dt* ($J = 11.0$, 5.5, 3.0 Hz), H-22), 2.52 (2H, *d* ($J = 4.5$ Hz), H-23), 1.84 (3H, *s*, H-27), 2.06 (3H, *s*, H-28); ^{13}C NMR spectral data (75 MHz, CD_3OD) Table 1.

Solvolysis of ***10***. A solution of **10** (10 mg) in pyridine–dioxane (4:1, v/v, 3.0 ml) was heated on a water bath at 80 °C for 5 h. The reaction mixture was dried completely under reduced pressure and the residue was purified by preparative TLC on silica gel ($CHCl_3$:EtOAc:MeOH:C_6H_6 70:2:10:18) to afford the hydrolysate **8** (5 mg). ^{1}H NMR spectral data (300 MHz, $CDCl_3$) were similar to that of **8**. The product **8**, thus, obtained was further acetylated by the usual method affording its acetate which was similar to **8a**.

Elimination of sulfate from **10**. A solution of **10** (5 mg) in pyridine–acetic anhydride (1:3 v/v, 3.0 ml) was heated on a water bath at 80 °C for 5 h and reaction mixture was dried completely under reduced pressure. The residue was purified by crystallization (EtOAc–$CHCl_3$) to afford a compound identical with withanone (**4**).

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