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A new withanolide from the leaves of *Withania somnifera*

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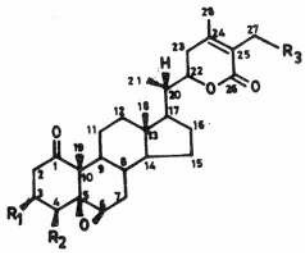
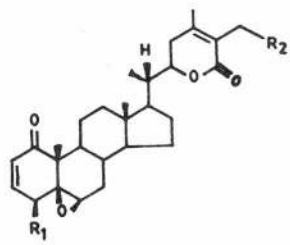
From the leaves of *Withania somnifera* (Solanaceac), collected from Tamil Nadu, a new withanolide, 3α-methoxy-2,3-dihydro-27-deoxywithaferin A (**1**) in addition to the known derivatives withaferin A (**4**), 2,3-dihydrowithaferin A (**5**) 3β-methoxy-2,3-dihydrowithaferin A (**2**) and 27-deoxywithaferin A (**3**) have been isolated, and their structures elucidated on the basis of physical , chemical and spectral methods.

Withania somnifera[1] (fam: *Solanaceac* , Vern: Sanskrit, Aswagandha; Telugu : Panneru; Trade name :Aswagandha) is an important drug in the ancient system of Ayurveda used for curing of a variety of ailments. The crude extracts of the roots and leaves are reported to exhibit sedative, hypnotic and antiseptic activities[2a]. Recent studies[2b,c] on the glyowithanolides from *W.somnifera* lent support to validate the potential of the plant in alleviating the adverse effects of morphine and the attendant immunosuppression, and in promoting learning and memory (medharsayan) as recorded in Ayurveda system of medicine. The different species distributed all over the world have been extensively examined and found to contain withanolides, steroidal ·lactones, alkaloids and other chemical constituents[3-31]. We have reported recently the chemical examination of the roots of *W. somnifera* collected from Tamil Nadu, which resulted in the isolation of three new withanolides, withasomniferols A to C ,their structure elucidation[32]. This paper deals with the chemical examination of its leaves collected from the same place.

The methanolic extract of the leaves after concentration was fractionated into *n*-hexane, ether, ethyl acetate and methanol successively. The hexane extract contained only chlorophyll and waxes.The ether fraction on column chromatography over silica gel furnished two pure compounds, compounds **1** and **2** and an inseparable mixture of compounds **1** and **3**. The dark brown ethyl acetate extract was concentrated and dissolved in ether. The ether insoluble portion contained mainly tannins. The ether soluble portion on column chromatography over silica gel furnished two pure compounds **4** and **5** besides compound **2**.





1 , R_1 = OMe , R_2 = OH , R_3 = H
1a , R_1 = OMe , R_2 = OAc, R_3 = H
2 , R_1 = OMe , R_2 = R_3 = OH
2a, R_1 = OMe , R_2 = R_3 = OAc
5 , R_1 = H , R_2 = R_3 = OH
5a, R_1 = H , R_2 = R_3 = OAc

3, R_1 = OH, R_2 = H
4, R_1 = R_2 = OH
4a, R_1 = R_2 = OAc

Compound **1** crystallised from methanol as colourless needles m.p. 256-57°, $[\alpha]_D$ – 40.47°. Its molecular formula was assigned as $C_{29}H_{42}O_6$ on the basis of elemental analysis and the molecular ion in EIMS at m/z, 486. A study of its physical and spectral characteristics revealed it to be a new withanolide. Its IR spectrum showed the presence of a chelated hydroxyl (3395 cm^{-1}), a saturated six-membered ketone (1702 cm^{-1}) and an α,β-unsaturated δ-lactone (1670 cm^{-1}). The UV absorption maxima at 224 and 280-90nm(sh) supported the conjugation and n-π* absorption of a ketone function. The presence of a saturated six-membered ketone moiety suggested the compound to be a 2,3-dihydrowithanolide[6,7]. On acetylation it yielded a monoacetate **1a** $C_{31}H_{44}O_7$, M^+ 528 whose IR spectrum exhibited absorptions characteristic of an acetate (1735 cm^{-1}), a conjugated δ-lactone (1690 cm^{-1}) and a saturated six-membered ketone (1710 cm^{-1}) groups but not the hydroxylic absorption suggesting that the compound has an acylable hydroxyl group.

The ^1H NMR spectra of **1** and its acetate **1a**

(Table I) showed a close resemblance to 27-deoxywithaferin A(3)[8,9] except for ring A pattern. Compound **1** showed methyl signals at δ 0.67 (s, 18-CH_3), 1.30 (s, 19-CH_3), 1.90 (br s, 27-CH_3 and 28-CH_3) and 0.97 (d, J=7 Hz, 21-CH_3). The appearance of 21-CH_3 at δ 0.97 as a doublet and H-22 at δ 4.29 (dt) indicated secondary nature of the C-21 methyl group and the absence of hydroxyl at C-20. A one proton broad singlet at δ 3.19 ($W_{1/2}$ ~ 4 Hz) was reminiscent of the H-6 of 5β-6β-epoxywithanolides[10]. A three-proton signal at δ3.34 in the compound **1**(δ 3.37 in its acetate) was assigned to a methoxy group which, in the absence of 2,3-double bond could be located at C_3 as in 1-oxo-3-methoxy compounds such as 3-methoxy-2,3-dihydrowithaferin[8,9]. The acylable hydroxyl was deduced to be secondary as the carbinolic proton appearing as a doubet at δ 3.47 (d, J=2 Hz) in **1** shifted to δ 4.4 (J=2 Hz) in its acetate **1a**. In view of its co-occurrence with 4-hydroxy compounds the secondary hydroxyl could be located at C-4 as in withaferin A[8,9]. The foregoing evidences suggested that the structure of compound **1** is 3β-methoxy-2,3-dihydro-27-deoxywithaferin A. The ^{13}C NMR spectra of **1** and **1a** could not be obtained for want of enough samples.

The structure of compound **1** was supported by the mass fragmentation of **1** and **1a**. In both the spectra the peak at m/z 125 was prominent (100% and 87.5%) supporting the presence of an α,β-unsaturated δ-lactone with C_{24} and C_{25}-methyls in the side chain. Loss of methanol from the original compound supported the presence of a methoxyl group at C-3. Other fragmentations of compound **1** (Chart 1) led to its gross structure while the stereochemical aspects of the various chiral centres including the configuration of methoxyl at C-3 and the hydroxyl at C-4 were taken as in related withanolides. Compound **1** could be an artifact formed by the addition of a methanol molecule to the unsaturated 1-keto-2-en moiety.This assumption was based on the earlier experience of Lavie *et al.*[8,9] with compound **2**, but could not be verified with **1**.

Compound **2** , $C_{29}H_{42}O_7$, M^+ 502 had a hydroxymethyl group at 27-position in place of a methyl in compound **1** and thus formed a diacetate, $C_{33}H_{46}O_9$,M^+ 586 on acetylation. The physical and spectral characteristics of **2** and its acetate **2a** (Table I) were identical with those of 3β-methoxy-2,3-dihydrowithaferin A and its acetate respectively. This compound was considered as an artifact, by Lavie *et al* [8,9].

Compound **3** could not be separated as such. But it was identified as 27-deoxywithaferin found as an inseparable mixture along with compound **1** by analysing the ^1H NMR spectrum of their mixture obtained from the column fractions. The mixture of acetates was also found to be inseparable.

Table I– ^1H NMR (90 MHz) spectra of withanolides **1, 1a, 2, 2a, 3, 4, 4a, 5** and **5a** (taken in $CDCl_3$, δ-scale, J values in parenthesis)

Assignment	1	1a	2	2a	3[10]	4	4a	5	5a
C_2 - H	--	--	--	--	6.20 d(10)	6.15 d(10)	6.05 d(10)	--	--
C_3 - H	3.62m ($W_{1/2}$≈11Hz)	3.5m	3.69m	4.48 d	7.08 dd(10,6)	6.86 dd(10,6)	6.85 dd(10,6)	--	--
C_4 - H	3.47 d(2)	4.4 d(2)	3.46 br s	3.50 m	3.76 d(6)	3.7 d(6)	4.51 d(7.5)	3.58 d(2)	4.52 d(2)
C_6 - H	3.19 s ($W_{1/2}$ ≈ 4Hz)	3.1 s	3.19 s	3.08 s	3.23 ($W_{1/2}$ ≈ 4Hz)	3.18 br s	3.02 br s	3.15 s	3.11
C_{22} - H	4.29 dt (12,3.5)	4.21 dt(12,3.5)	4.46 dt(12,3.5)	4.23 dt(12,3.5)	4.37 dt(12,3.5)	4.40 dt(12,3.5)	4.24 dt(12,3.5)	4.42 dt(12,3.5)	4.34 dt(12,3.5)
18 -CH_3	0.67 s	0.69 s	0.68 s	0.68 s	0.68 s	0.69 s	0.73 s	0.67 s	0.67 s
19 -CH_3	1.30 s	1.16 s	1.3 s	1.15 s	1.39 s	1.29 s	1.29 s	1.29 s	1.25 s
21 -CH_3	0.97 d(7)	0.96 d(7)	0.99 d(6.5)	0.96 d(6.5)	0.98 d(6)	0.97 d(6.5)	0.97 d(6.5)	0.98 d(7)	0.98 d(7)
27 -CH_3	1.90 s	1.80 s	4.3 br d (-CH_2OH)	4.68 br s (CH_2-OAc)	1.91 s	4.31 br s (-CH_2OH)	4.69 br s (-CH_2OAc)	4.82 br s (-CH_2OH)	4.82 (-CH_2OAc)
28 -CH_3	1.90 s	1.88 s	2.02 s	1.95 s	1.91 s	2.0 s	1.96 s	2.05 s	2.01 s
O-*Me*	3.34 s	3.37 s	3.34 s	3.35 s	--	--	--	--	--
>CHO*Ac*	--	2.03 s	--	2.04 s	--	--	2.00 s	--	2.01 s

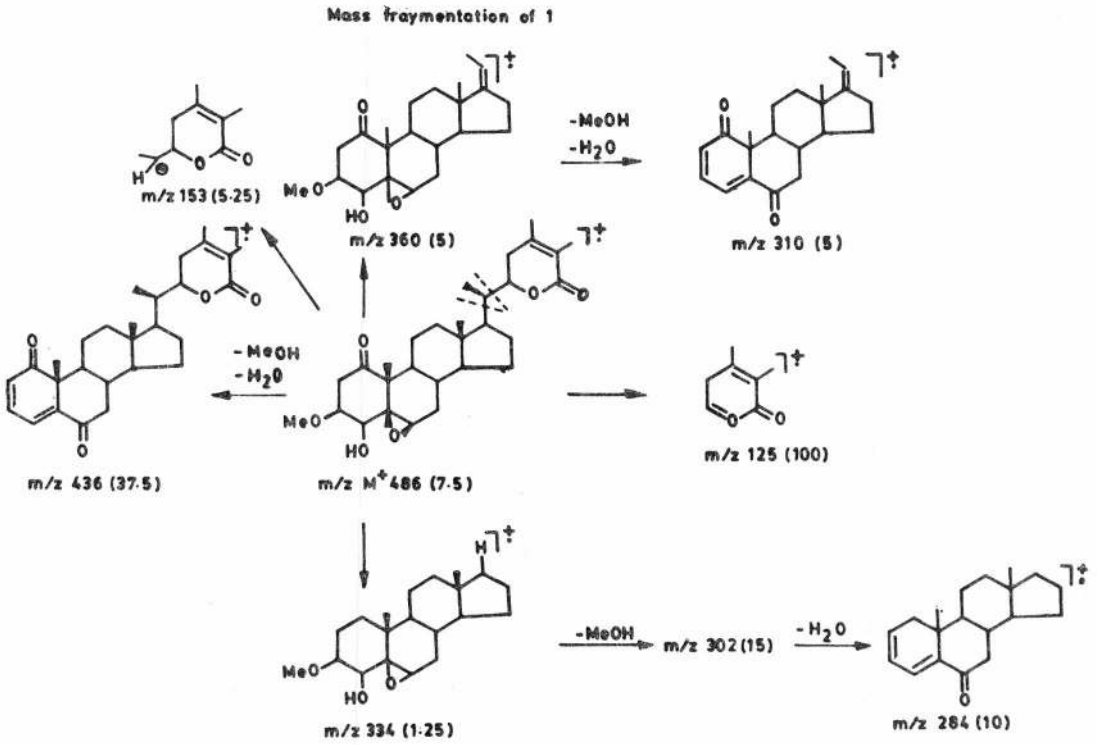


Chart 1

Compound **4**, $C_{28}H_{38}O_6$ M^+ 486,and compound **5**, $C_{28}H_{40}O_6$, M^+ 470 were identified as, withaferin A (**4**)[8,9] and 2,3-dihydrowithaferin A (**5**)[10] in every respect by comparing their physical and spectral characteristics (^{1}H NMR spectra Table I) and the mass fragmentation patterns with those reported in literature.

Experimental Section

Extraction and isolation of compounds. The leaves of *W. somnifera* (1.5 kg), collected from Tamil Nadu and supplied by Unichem Laboratories, were shade dried, powdered and exhaustively extracted with methanol. The combined methanolic extract (15 L) was concentrated to a small volume (1 L), left overnight in refrigerator and filtered to remove the waxy material.The filtrate was dried and the residue chromatographed over a silica gel column eluting successively with hexane, ether, ethyl acetate and methanol. The *n*-hexane eluates yielded only waxy material. The combined ether eluates on evaporation left a dark green solid (28 g) which on further chromatography over silica gel column furnished compounds **1**, **2** and an inseparable mixture of **1** and **3**. The solid (25 g) from the combined ethyl acetate eluates of the initial column was rechromatographed on a column of silica gel eluting with benzene and benzene-ethyl acetate mixtures to furnish compounds **2**, **4** and **5** along with a mixture of **4** and **5**.

3β-methoxy-2,3-dihydro-27-deoxywithaferin A (1). It was crystallised from methanol as colourless needles, 100 mg, m.p. 256-57°, $[\alpha]_D$– 40.47° (*c*, 0.43 in $CHCl_3$) R_f 0.36 (benzene-EtOAc, 7 : 3) (Found : C, 70.26; H, 8.97 $C_{29}H_{42}O_6$ requires C, 71.57, H 8.70%); UV (EtOH) 224 , 280-90 (sh) nm. IR (KBr): 3395 , 1702,1670 cm^{-1}; ^{1}H NMR (seeTable I); Ms (70 eV) (rel in %): m/z 486 (M^+; 7.5), 468 (2.5), 454 (12.5), 436 (37.5), 408 (37.5), 360 (5), 342(2.5), 328 (5), 310 (5), 334 (1.25), 302 (15), 284 (10), 153(5.25), 125(100).

Compound 1 acetate : 3β-methoxy-2,3-dihydro-27-deoxywithaferin-A monoacetate 1a. Compound **1** (30 mg) was refluxed with pyridine / Ac_2O (2 mL each) on a steam bath for 4 hr. After usual work-up and crystallisation from methanol the acetate **1a** was obtained as colourless needles. (25 mg), m.p. 210°, $[\alpha]_D$+ 18.50° (*c*, 1.47, $CHCl_3$) R_f 0.60 (benzene-EtOAc, 1 : 1) (Found : C, 70.98; H, 8.01. $C_{31}H_{44}O_7$ requires C, 70.45; H, 8.4%); UV ($CHCl_3$): 225, 238 nm; IR(KBr): 1735,1710 and 1690 cm^{-1} ^{1}H NMR (see Table I); Ms (70 eV) (rel int %):m/z M^+; 528 (2.5), 468 (1.25), 436

(2.5), 408 (5), 418 (12.5), 400 (7.5), 402 (2.5), 370 (1.25), 310 (10), 317 (1.25), 268 (13.5), 208 (7.5), 125 (87.5), 108 (30).

Compound (2) : 3β-methoxy-2,3-dihydroxy–withaferin A[8,9] (2). It was crystallised from methanol as colourless needles (2g), m.p. 245-47°, $[\alpha]_D$ + 11.5 (*c*, 1.58,$CHCl_3$);R_f 0.41 (benzene-EtOAc, 2:3);UV (EtOH):212, 290-95 nm;IR (Nujol):3443, 3340, 1682 and 1710 cm^{-1}; ^1H NMR (see Table I).

Compound 2 acetate : 3β-methoxy-2,3-di–hydro–withaferin-A diacetate[8,9] 2a: Compound **2** (20 mg) on refluxing with pyridine / Ac_2O (1 mL each) for 4hr on a steam-bath and usual work-up gave a diacetate, which crystallised from methanol as colourlees needles (25 mg), m.p. 168-70°, $[\alpha]_D$–17.03 (*c*, 0.63, $CHCl_3$) R_f 0.84 (benzene-EtOAc, 6: 4); ;UV ($CHCl_3$):216, 238 nm; IR 1740, 1710 and 1682 cm^{-1}; ^1H NMR. (see Table I).

Compound 3. It could not be separated from compound **1** in the mixture as such.The mixture was acetylated using pyridine and Ac_2O. The acetates were again very close on TLC R_f 0.63 and 0.60 (benzene-EtOAc, 1:1) respectively and could not be separated any further. The ^1H NMR spectrum of the mixed acetates indicated the presence of the acetate **3a**[10] of 27-deoxywithaferin A and the acetate **1a**[8,9] of 3β-methoxy-2,3-dihydro-27 deoxywithaferin A.

Compound 4 : Withaferin A (4)[8,9]. It crystallised from methanol as colourless needles,(500 mg), m.p. 248-49°; R_f 0.79 (benzene-EtOAc, 2:8), $[\alpha]_D$+ 128.17° (*c*, 1.26, $CHCl_3$);UV ($CHCl_3$): 213, 238 nm; IR (Nujol): 3240 (OH), 1704 and 1680 cm^{-1}; ^1H NMR (see Table I).

Compound 4 acetate : Withaferin A diacetate 4a[8,9]. Compound **4** (30 mg) on acetylation by refluxing with pyridine/Ac_2O (1 mL each) on a steam-bath for 4hr gave withaferin-A diacetate which crystallised from methanol as colourless needles (20 mg), m.p. 203-4°, R_f 0.79 (benzene-ethyl acetate , 4:6) ; $[\alpha]_D$+176.93° (*c*, 1.37 $CHCl_3$); UV ($CHCl_3$): 214, 238 nm; IR ($CHCl_3$): 1738, 702 and 1670 cm^{-1}; ^1H NMR (see Table I).

Compound 5 : 2:3-dihydrowithaferin A 5[10]. It crystallised from methanol as an amorphous powder (200 mg), m.p. 228-30°, R_f 0.38 (benzene-ethyl acetate, 2:8) $[\alpha]_D^{23}$ + 14.53 (*c*, 1.08, $CHCl_3$); UV ($CHCl_3$):212, 238 nm; IR (KBr):3500-3250 br (OH), 1710 (six membered ketone), 1660 cm^{-1} (α,β-unsaturated δ-lactone); ^1H NMR (see Table I).

Compound 5 acetate : 2:3-dihydrowithaferin A diacetate 5a[10]. Compound **5** (30 mg) on acetylation with (pyridine+Ac_2O) gave a diacetate which crystallised from methanol as colourless needles (25 mg), m.p.206-7°, R_f 0.75 (benzene-EtOAc, 4:6); $[\alpha]_D^{23}$ + 41.73° (*c* 0.52, $CDCl_3$); UV ($CHCl_3$): 214, 237 nm; IR (KBr): 1730 br, 1710 and 1690 cm^{-1}; ^1H NMR (see Table I).

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