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NEW WITHANOLIDES OF BIOGENETIC INTEREST FROM *WITHANIA SOMNIFERA*

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Key Word Index—*Withania somnifera*; Solanaceae; withanolides; steroidal lactones.

Abstract—The structure of 1α,3β,20α$_F$-trihydroxy-20*R*,22*R*-witha-5,24-dienolide, a new naturally occurring steroidal lactone of the withanolide group, isolated from *Withania somnifera*, has been elucidated. The presence of a 1-oxo derivative was detected and compared to a synthetic product. These two compounds are considered as key intermediates in the biosynthesis of the withanolides, and are at the origin of the 2-en-1-one system. Two other new withanolides are described: 1-oxo-14α,20α$_F$,27-trihydroxy-20*R*,22*R*-witha-3,5,24-trienolide and 5α-ethoxy-1-oxo-6β,14α,17β,20-tetrahydroxy-20*S*,22*R*-witha-2,24-dienolide.

INTRODUCTION

In the course of our studies on the withanolides, C-28 steroidal lactones isolated from *Withania somnifera* Dun. chemotype III (Solanaceae), large quantities of withanolide E [1,2] were required for biological testing. This compound exhibits antineoplastic activity and has immunosuppressive properties [3]. During this pilot scale preparation, three new withanolides (**1a**, **2**, **3a**) were isolated from the various fractions and fully identified. The biogenetic implications of one of these compounds are discussed.

RESULTS

The dried leaves of *W. somnifera* chemotype III, grown in our experimental plots, were collected and extracted with methanol as described [4, 5], and the mixture was resolved in this case first on a chromatographic column of alumina. Following elution of withanolide E, a fraction consisting of four products was obtained. These compounds were separated on a column of silica gel. Compounds **1a**, **2** and **3a** are described below and the fourth was found to be identical with withanolide H [4, 5].

Compound **1a**, $C_{28}H_{42}O_5$, in the UV spectrum showed absorption at λ_{max} 226 nm (ε 10 500). This value and intensity are characteristic for a single α,β-unsaturated carbonyl chromophore. The IR spectrum indicated an unsaturated six-membered lactone and a double bond (1670 and 1650 cm^{-1}).

The ^{1}H NMR spectrum (Table 1) was characteristic for the steroidal structure of the withanolide group. The signals of two vinylic methyl groups, 27- and 28-Me, and the characteristic 22-H signal confirmed the presence of the α,β-unsaturated lactone side chain of the withanolides. The pattern and the coupling constants of the 22-H signal and the chemical shift of the 21-Me were indicative of a 20-OH compound. Indeed, all withanolides isolated so far from chemotype III have this function [4, 5], and by analogy the configurations 20*R* (20α$_F$) and 22*R* are assigned. The position of the 18-Me signal was similar to that of the other withanolides characterized by unsubstituted C,D rings such as withanolide D [6]. The mass spectrum of compound **1a** confirmed the proposed structure. The fragments at *m/z* 333 ($C_{21}H_{33}O_3$) and 125 ($C_7H_9O_2$) are characteristic for the cleavage of the C(20)–C(22) bond. The cleavage of the C(17)–C(20) bond resulted in fragments *m/z* 289 ($C_{19}H_{29}O_2$) and 169 ($C_9H_{13}O_3$). Furthermore, in the fragmentation, there was the loss of 3 × H_2O from the molecular ion (M^+ at *m/z* 458) as well as from the ion at *m/z* 333 ($M^+ - 125$), and the loss of 2 × H_2O from the ion *m/z* 289 ($M^+ - 169$) was also observed. These fragmentations provide good evidence for the presence of one double bond and two OH groups on rings A–B, and a 20-OH group on the side chain.

In the low field ^{1}H NMR spectrum of compound **1a**, no signals were seen for the well known 2-en-1-one system of the withanolides. However, only one olefinic proton was present. The two other signals present in the spectrum were attributed to the protons adjacent to the OH groups (δ 3.99 broad octet, and 3.84 narrow multiplet).

Two structures can account for the above data [7]: a 1α,3β-dihydroxy-Δ^5 or a 2α,7α-dihydroxy-Δ^4 system. Comparison with the signals observed for 1α,3β,5α-trihydroxy-6α,7α-epoxy-22*R*-withanolide, isolated earlier from an Indian chemotype [8], as well as with 1α,3β-dihydroxycholesterol [9], 1α,3α,5α-trihydroxycholestane [10] and 1β,3β,3β-trihydroxycholestane [10], provided support for the proposed 1α,3β-dihydroxy-Δ^5 substitution. The study of the pyridine-induced shift in the ^{1}H NMR spectrum confirmed the respective 1α- and 3β-orientations of the OH groups. The sizable shift ($\Delta^{CDCl_3}_{C_5D_5N}$ 0.696 ppm) observed for the octet signal at δ 3.99 was due to a 1–3-diaxial interaction between the 1α-OH and the 3α-H. In addition, the very low pyridine solvent shift effect observed for the 19-Me group (0.006 ppm) supports the axial α-orientation of the OH at C-1, and the equatorial configuration of the 3β-OH. The other values of the pyridine shift confirm structure **1a** and eliminate the alternative possibility, 2α,7α-dihydroxy-Δ^4. The observed signal location for the 19-Me (δ 1.03) is in good agreement with the calculated value, δ 1.05 [7].

The ^{13}C NMR spectral data of compound **1a** (Table 2) can be divided into two parts: values related to rings C, D

Table 1. ^{1}H NMR signals of relevant protons in withanolides

Compound	2-H	3-H	4-H	6-H	22-H	Methyl groups: 18	19	21	27 and 28	Other signals
1a		3.99 (*br.octet*) [4.68]		5.58 ($W_{\frac{1}{2}}$ 9.9) [5.64]	4.23 (13-3.6) [4.38]	0.87 [1.11]	1.03 [1.09]	1.25 [1.41]	1.88 1.96 [1.82–1.91]	1β-H 3.84 ($W_{\frac{1}{2}}$ 7.2) [4.11]
1b		5.04 ($W_{\frac{1}{2}}$ 21.6)		5.60	4.22 (12-4)	0.88	1.04	1.20	1.88–1.99	1β-H 3.85 ($W_{\frac{1}{2}}$ 8.6) 3β-OAc 2.03
1c		4.92 ($W_{\frac{1}{2}}$ 15)		5.54	4.22 (12-4)	0.87	1.09	1.25	1.89–1.99	1β-H 5.06 ($W_{\frac{1}{2}}$ 7) AcO 2.02 and 2.05
2	2.76 (*dd*20-6) 3.29 (*bd*,20)	5.62 (10-4)	6.06 (*br.d*,10)	5.70 ($W_{\frac{1}{2}}$ 10)	4.22 (13-4)	1.07	1.37	1.28	2.03	27 C$\underline{H}_2$OH 4.37
3a	5.83 (*dd*,10-4) [6.05]	6.52 (*dq*,10-4) [6.53]	2.34 (*dd*,20-6) [2.45] 3.02 (*br.d*,20) [3.40]	3.99 ($W_{\frac{1}{2}}$ 6) [4.28]	4.93 (*br.t*,$W_{\frac{1}{2}}$20) [5.32]	1.15 [1.48]	1.30 [1.68]	1.41 [1.77]	1.88–1.94 [1.71–1.90]	5α-O-C$\underline{H}_2$-Me 3.06 and 3.20 (*dq*, 6.8–8.3) (*dq*, 6.8–8.3) [3.10] and [3.27] 5α-O-CH$_2$-C$\underline{H}_3$ 1.02 (*t*, 6.8) [0.92]
3b	5.82 (*dd*,10-3)	6.44 (*dq*,10-3)	2.34 (*dd*,22-6) 2.55 (*br.d*,22-4)	5.15 ($W_{\frac{1}{2}}$8.2)	4.94 (*br.t*.$W_{\frac{1}{2}}$20)	1.15	1.27	1.41	1.88–1.94	5α-O-C$\underline{H}_2$-Me 3.14 and 3.34 (*dq*, 7-8) (*dq*, 7-8) 5α-O-CH$_2$-C$\underline{H}_3$ 1.05 (*t*, 7) 6β-OAc 2.11
4a		3.87 ($W_{\frac{1}{2}}$ 19.5)		5.62	4.22 (*dd*,12-14)	0.89	1.29	1.25	1.89–1.95	
4b		4.89 ($W_{\frac{1}{2}}$ 19.5)		5.65	4.21 (*dd*,12-4)	0.88	1.28	1.27	1.88–1.94	3β-OAc 2.03
5	5.89 (*dd*,10-3)	6.80 (*dq*,10-5-2.5)	2.82 (*dd*,22-4) 3.30 (*br.dd*,22-3)	5.52	4.23 (*dd*,13-4)	0.91	1.30	1.22	1.88–1.94	
7	3.08 (*d*,17.3) 3.17 (*d*,17.3)		3.24 (*d*,15.2) 3.67 (*d*,15.2)	5.76	4.24 (*dd*,12-4)	0.91	1.29	1.28	1.89–1.95	

Chemical shifts are in δ units; coupling constants (in Hz) are in parentheses. Data for spectra in C_5D_5N are in square brackets.

Table 2. ^{13}C NMR spectral data of compounds **1a** and **3a**

	1a	3a		1a	3a
C-1	72.9	204.9	C-16	22.0	34.5
C-2	38.3	129.4	C-17	54.7	88.2
C-3	66.3	139.6	C-18	13.6	21.2*
C-4	41.4	31.0	C-19	19.4	15.6‡
C-5	137.6	81.5	C-20	75.2	79.2
C-6	125.2	69.9	C-21	20.8	20.1*
C-7	31.7	28.5	C-22	81.0	80.45
C-8	31.3	33.9	C-23	31.5	32.8
C-9	41.5	34.1	C-24	149.1	151.3
C-10	41.7	53.2†	C-25	122.0	121.5
C-11	20.2	22.9	C-26	166.2	166.6
C-12	39.8	37.7	C-27	12.5	12.5
C-13	43.0	55.1†	C-28	20.5	20.9*
C-14	56.8	83.0	5α-O-$\underline{C}H_2$-Me		57.5
C-15	23.9	30.1	5α-O-CH_2-$\underline{C}H_3$		16.0‡

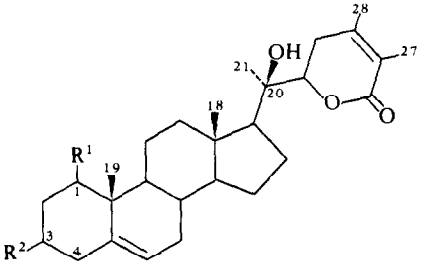
*,†,‡ refer to interchangeable data.

and the lactone side chain which are similar to those obtained for withanolide D [11, 12], and those of rings A and B which are comparable to cholesterol [13], except for the 1α-OH group responsible for the two γ-effects observed at C-3 and C-9 (C-3: 71.6 and 66.3; C-9 50.2 and 41.5 for cholesterol and **1a** respectively). Chemical confirmation for the structure of compound **1a** was provided by oxidation of the two secondary hydroxy groups at C-1 and C-3 with Jones reagent to give the 1,3-dioxo compound **7**.

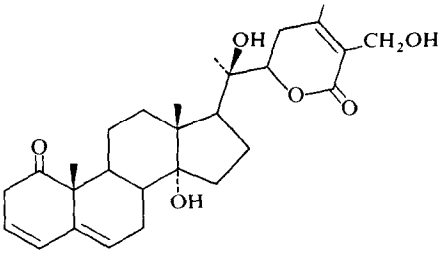
Since compound **1a** may be a key intermediate in the biogenesis of the withanolides, it was further subjected to biomimetic reactions, namely, the formation of a 1-oxo derivative **4**, and then elimination of the 3β-OH, thereby producing the characteristic 2-en-1-one system of the withanolides (**5**). The first step for this sequence was the selective acetylation of the 3β-OH in **1a** [14]. Oxidation of the 1α-OH, in **1b** was again performed with Jones reagent, followed by elimination of the 3-acetoxy group with alkali in dioxane. A mixture was obtained, which was resolved by chromatography. One compound was found to be unreacted material (**4b**), while the other two were identified as the required 2-en-1-one (**5**), and the 3β-hydroxy-1-one derivative (**4a**). This synthetic sequence was identical with that previously used with 1α-hydroxycholesterol [9, 15, 16].

For compound **2**, the ^1H NMR spectrum showed the three methyl groups characteristic of a steroidal structure (Table 1). The interpretation of the spectra was facilitated by comparison with several well-known withanolides. The singlet at δ 1.28 for the 21-Me and the signal observed for 22-H (4.28) indicate that this compound had a 20-OH group. The low value of the signal observed for the 19-Me (1.37), together with the coupling pattern of the three olefinic protons, were consistent with a 3,5-dien-1-one system, since the usual 2,5-dien-1-one would display quite a different pattern. Confirmation was given by decoupling all the protons in turn and by comparing with withanolide I and K [4, 5]. The fact that only one three-proton signal appeared for the 28-Me, and that a two-proton signal was present at δ 4.37 was good evidence for a 27-OH group.

The ^1H NMR spectrum of compound **3a** (Table 1) showed the characteristic signals of the withanolides. In particular, the 18- 21- 27- and 28-Me groups were similar to those observed for withanolide E and the other steroidal lactones having a 17α-oriented side chain [1]. By comparison with withanolide E, the signal due to the 3-H appeared at higher field (δ 6.52), which is usually the case when an oxygenated substituent is in the 5α-position [2]. The multiplicity of the 2-H and 3-H signals indicated that position C-4 was unsubstituted, and through double irradiation the two geminal C-4 protons could be identified: the equatorial 4α-H at δ 2.34 (J = 20 and 6 Hz, *dd*) and the axial 4β-H at 3.02 (J = 20 Hz, *br. d*). After acetylation of **3a**, the narrow multiplet, initially present at 3.99, was shifted to 5.15, suggesting a secondary alcohol at C-6. Only an axial 6β-OH can influence directly the



1a R^1 = α-OH, β-H; R^2 = α-H, β-OH
1b R^1 = α-OH, β-H; R^2 = α-H; β-OAc
1c R^1 = α-OAc, β-H; R^2 = α-H, β-OAc
4a R^1 = O; R^2 = α-H, β-OH
4b R^1 = O; R^2 = α-H, β-OAc
5 R^1 = O; R^2 = H; Δ^2
6 R^1 = O; R^2 = H; Δ^3
7 $R^1 = R^2$ = O



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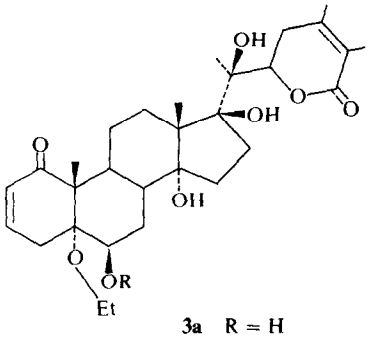


3a R = H
3b R = Ac

position of the 19-Me signal (1.30), an influence well seen following the acetylation reaction (1.27 in **3b**), and also observed for the 6β-OAc-withanolide S [2]. In this acetoxy derivative (**3b**), the change observed for the 4β-H, now at δ 2.55, confirmed the 1–3-diaxial configuration with the 6β-OAc group.

Two additional signals were present in the spectrum of **3a**. One was a triplet at δ 1.02, whereas the second appeared as two double quartets at δ 3.06 and 3.20. Selective decoupling of each proton signal in turn enabled the identification of an ethoxy group. In the acetate **3b**, the change observed in the chemical shift of the signal of the ethoxy group suggested its proximity to the 6-OAc. It can be concluded, therefore, that the ethoxy group is at C-5 and α-oriented.

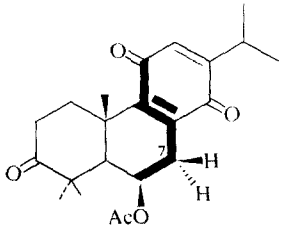
The deuterated pyridine shifts observed for **3a** were in good agreement with those observed for withanolide S. Furthermore, the shifts for the two geminal 4-H were quite different, Δ 0.1 and 0.38 ppm for the 4α-H and 4β-H, respectively and corroborated the presence of the 6β-OH.

It is interesting to note that the axial orientation of the 5α-ethoxy group and the 2-en-1-one function of **3a** confer upon ring A a configuration which is reflected by the unusually large value of the two 4-H geminal coupling constants (J = 20 Hz). A similar value has been observed for the geminal 7-H present in compound **8**, which was previously analysed and for the NMR spectrum of which a detailed explanation was provided, together with a correlation with the X-ray data [17]. In that case, it was shown that the plane which bisects the Hα–C–Hβ group is practically perpendicular to the direction of both the acetoxy group and the π-orbitals of the α,β-unsaturated ketone.

In compound **3a** the overall system of substituents is characteristically the same, and therefore the contributing factors discussed in the previous publication [17] also apply in this case, namely, there is a tendency of the plane bisecting the angle formed by the C–H geminal hydrogens to be almost perpendicular to the directions of both the 5α-ethoxy group and the π-orbital of the 2-en-1-one system. The ^{13}C NMR spectral data of compound **3a** (Table 2) are similar to those obtained for withanolide S [12].

Epoxides are known to undergo solvolytic reactions in the presence of acid. When withanolide E was treated in ethanol solution with traces of *p*-toluenesulfonic acid, two products were produced, one identical with **3a** and the second with the known withanolide S (5α,6β-dihydroxy-).

The presence in the plant of compound **3a**, having a 5α-OEt-6β-OH, has to be interpreted with caution. The stereochemistry is identical with that of withanolide S as well as that of jaborosalactone D [18]. Such a stereochemistry indicates a possible derivation from a 5β,6β-epoxide obtained by chemical reactions. The possibility of artefact formation was considered, but since the extraction was carried out in MeOH and no 5α-OMe-6β-OH has ever been encountered, this possibility was eliminated. Although EtOAc was, in fact, used for chromatography, a sample of withanolide E dissolved in EtOH, and left in contact with silica gel and alumina for several months did not produce even traces of a compound similar to **3a**. Therefore, it seems that this compound must be considered as of natural occurrence and as an unusual observation in this context. Recently, the isolation of a 5α-MeO, 6β-OH compound was reported and considered as a natural derivative [19].



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DISCUSSION

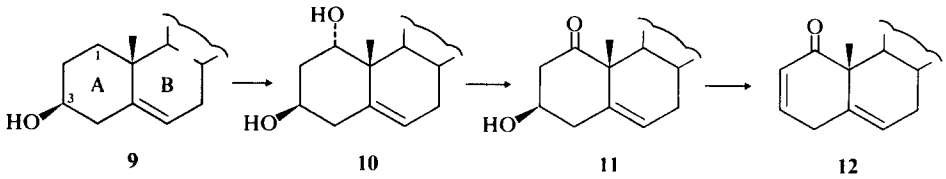
A number of years ago, two biogenetic schemes were suggested in order to explain the formation of the withanolides: one relates to the substitution patterns of rings A and B, and the second to the side chain [20, 21]. These schemes were worked out following several cross-breedings done with different chemotypes of *W. somnifera*. They were based on the different combinations of substituents found in the various compounds. With regard to rings A and B, a 1-OH, 3β-OH structure (**10**), as shown in Scheme 1, was postulated to be formed from a 3β-OH precursor (**9**) which, following oxidation at position C-1 (**11**) and subsequent elimination of the 3β-OH, would result in the basic 2-en-1-one structure (**12**) of the withanolide group. The present isolation from the leaves of the plant of compound **1a**, having a 1α-OH, 3β-OH grouping, indicates the *in vivo* operation of the sequence in the proposed scheme, and this compound can be regarded as a key intermediate in the formation of the basic substitution pattern of ring A. Furthermore, the fact that compound **1a** has the unsaturated lactone side chain clearly indicates that, at the stage of the C-1 oxidation *in vivo*, the side chain is already fully formed and in this case the introduction of the 20-OH has taken place, both preceding the oxidation reaction of ring A.

It is interesting to note that, *in vitro*, compound **4b** gives a regiospecific elimination to form the α,β-unsaturated system present in **5**. Only under acidic conditions does the migration of Δ^2 to Δ^3 (**6**) take place. Similarly, elimination *in vivo* of the same 3β-OH groups should take place to form the predominant system observed in the 2-en-1-one ring A substitution pattern. Only in a few cases, and usually in very small quantities, is the 3,5-dien-1-one observed, as in withanolide I and K [4, 5]. This is true for the diene **2** now isolated and which co-exists with the usual 2-en-1-one products extensively found in the plant but already described.

Dr. I. Kirson kindly informed us that he has now isolated a new withanolide having the A,B-ring system shown in **11** from a *W. somnifera* growing in the Faluga region (Israel). This compound (which after acetylation gives the same ^{1}H NMR spectrum as our derivative **4b**) thus completes the biogenetic sequence **9** → **12**.

EXPERIMENTAL

Mps were measured on a Fischer–Johns apparatus and are uncorr. Optical rotations were determined with $CHCl_3$ solns. IR spectra were recorded with KBr pellets; UV spectra were recorded for EtOH solns; ^{1}H NMR spectra were determined for 5–10% solns in $CDCl_3$, containing TMS as internal standard. Analytical TLC was carried out on chromatoplates of 50 × 75 mm, Kieselgel F_{254}, R_f value in EtOAc–C_6H_6–EtOH



9 10 11 12

Scheme 1.

(180:20:3) and prep. TLC on 200 × 200 × 2 mm, Kieselgel 60 F_{254}. Mass spectra were determined under the direction of Dr. Z. Zaretskii. Analyses were performed in the microanalytical laboratory of our Institute by Mr. R. Heller.

1α,3β,20α$_F$-*Trihydroxy*-20R,22R-*witha*-5-,24-*dienolide* (**1a**). Mp 273° from EtOAc; $[\alpha]_D$ + 19.8° (*c* 0.11); R_f 0.41. MS *m/z* (rel. int.): 458.300 (M^+ 4.01) ($C_{28}H_{42}O_5$), 440.289 (11.37) ($C_{28}H_{40}O_4$), 422.282 (4.91) ($C_{28}H_{38}O_3$), 404.272 (2.38) ($C_{28}H_{36}O_2$), 333.243 (13.89) ($C_{21}H_{33}O_3$), 315.231 (47.34) ($C_{21}H_{31}O_2$), 297.223 (41.84) ($C_{21}H_{29}O$), 289.219 (3.40) ($C_{19}H_{29}O_2$), 279.210 (14.16) ($C_{21}H_{27}$), 271.208 (3.68) ($C_{19}H_{27}O$), 253.197 (8.06) ($C_{19}H_{25}$). (Found: C, 73.29; H, 9.15%. $C_{28}H_{42}O_5$ requires C, 73.32; H, 9.23%. MW, 458.30).

3β-*Acetoxy*-1α,20α$_F$-*dihydroxy*-20R,22R-*witha*-5,24-*dienolide* (**1b**). Mp 144–146° from EtOAc; $[\alpha]_D$ + 8.8° (*c* 0.11); UV λ_{max}^{EtOH} nm: 227 (ε 7164); IR ν_{max}^{KBr} cm^{-1}: 3500, 1725 and 1700; R_f 0.75. MS *m/z* (rel. int.): 500 (M^+, <0.1), 440 (0.6) (M^+ −60 HOAc), 422 (0.3) (M^+ −60 − H_2O) (M^+ −125 cleavage of 20C–22C), 315 (0.78 (M^+ −125 − 60), 297 (2) (M^+ −125 − 60 − H_2O), 279 (0.8) (M^+ −125 − 60 − 2 × H_2O), 253 (0.4) (M^+ −169 − 60 − H_2O cleavage of 17C–20C), 126 (8), 125 (5).

1α,3β-*Diacetoxy* 20α$_F$-*hydroxy*-20R,22R-*witha*-5,24-*dienolide* (**1c**). Mp 85–86° from EtOAc; $[\alpha]_D$ +17.6° (*c* 0.11); UV λ_{max}^{EtOH} nm: 226 (ε 7480); IR ν_{max}^{KBr} m^{-1}: 3500, 1735 and 1710; R_f 0.79; MS *m/z* (rel. int.): M^+ 542 (<0.1), 422 (46.8) (M^+ −2 × HOAc), 417 (1.25) (M^+ −125, cleavage of 20C–22C), 357 (8.8) (M^+ −125 − 60), 297 (39.6) (M^+ −125 − 2 × 60), 279 (10) (M^+ − 125 − 2 × 60 − H_2O), 253 (6.6) (M^+ −169 − 2 × 60 cleavage of 17C–20C), 169 (63), 126 (100), 125 (38.1).

1-*Oxo*-14,20α$_F$, 27-*trihydroxy*-20R,22R-*witha*-3,5,24-*trienolide* (**2**). Mp 184° from EtOAc; $[\alpha]_D$ +98.7° (*c* 0.12); IR ν_{max}^{KBr} cm^{-1}: 1650, 1680, 1700, 3400 and 3550. MS *m/z* (rel. int.): 452 (3.2) (M^+ −H_2O), 438 (1.2) (M^+ −MeOH), 437 (3.5) (M^+ −H_2O − Me), 434 (2) (M^+ −2 × H_2O), 424 (1.6) (M^+ −CO − H_2O), 419 (1.8) (M^+ −2 × H_2O − Me), 416 (1.2) (M^+ −3 × H_2O), 406 (1.8) (M^+ −CO − 2 × H_2O), 401 (3.7) (M^+ −3 × H_2O − Me), 398 (0.9) (M^+ −CO − 3 × H_2O).

5α-*Ethoxy*-1-*oxo*-6β-14α,17β,20α$_F$-*tetrahydroxy*-20S,22R-*witha*-2,24-*dienolide* (**3a**). Mp 167° from EtOAc; $[\alpha]_D$ +62.7° (*c* 0.11); UV λ_{max}^{EtOH} nm: 224 (ε 17 739), IR ν_{max}^{KBr} cm^{-1}: 3400, 1680, and 1670. MS *m/z* (rel. int.): 514.290 (0.7) $C_{30}H_{42}O_7$ (M^+ −H_2O), 468.248 (1.46) $C_{28}H_{36}O_6$ (M^+ −H_2O − EtOH), 389.232 (2.52) $C_{23}H_{33}O_5$ (M^+ −H_2O − 125), 371.222 (5.59) $C_{23}H_{31}O_4$ (M^+ −2 × H_2O − 125), 345.209 (4.93) $C_{21}H_{29}O_4$ (M^+ −H_2O − 169), 343.188 (3.32) $C_{21}H_{27}O_4$ (M^+ −H_2O − EtOH − 125), 325.180 (4.08) $C_{21}H_{25}O_3$ (M^+ −2 × H_2O − EtOH − 125), 307.17 (1.70) $C_{21}H_{23}O_2$ (M^+ −3 × H_2O − EtOH − 125), 299.164 (9.37) $C_{19}H_{23}O_3$ (M^+ −H_2O − EtOH − 169), 281.153 (4.36) $C_{19}H_{21}O_2$ (M^+ −2 × H_2O − EtOH − 169), 263.142 (1.68) $C_{19}H_{19}O$ (M^+ −3 × H_2O − EtOH − 169), 170.094 (12.98) $C_9H_{14}O_3$, 169.090 (9.93) $C_9H_{13}O_3$, 153.089 (8.86) $C_9H_{13}O_2$ and 152.082 (33.31) $C_9H_{12}O_2$ (cleavage of 6C–7C, 9C–10C). (Found: C, 65.24; H, 8.45. $C_{30}H_{44}O_8 \cdot H_2O$ requires C, 65.45; H, 8.35%; MW 550).

A sample of compound **3a** prepared from withanolide E by the addition of a few crystals of *p*-toluenesulphonic acid to an EtOH soln was found identical with the natural compound: mp and mmp 167°; $[\alpha]_D$ + 61° (*c* 0.2). All spectroscopic data were also found identical.

The acetate **3b** was prepared using the usual method; mp 132° from EtOAc; $[\alpha]_D$ +23.8° (*c* 0.07); UV λ_{max}^{EtOH} nm: 224 (ε 16 359); IR ν_{max}^{KBr} cm^{-1}: 3400, 1730 and 1680. MS *m/z* (rel. int.): 574 (M^+, 0.3), 528 (0.3) (M^+ −EtOH), 450 (2.4) (M^+ −EtOH − H_2O − HOAc), 449 (1.1) (M^+ −125 cleavage of 20C–22C).

3β,20α$_F$-*Dihydroxy*-1-*oxo*-20R,22R-*witha*-5,24-*dienolide* (**4a**). Mp 91–92° from EtOAc; $[\alpha]_D$ +30° (*c* 0.13); UV λ_{max}^{EtOH} nm: 224 (ε 9747); IR ν_{max}^{KBr} cm^{-1}: 3500, 1700; R_f 0.69. MS *m/z* (rel. int.): 456 (M^+, 1), 438 (0.1) (M^+ −H_2O), 420 (0.1) (M^+ −2 × H_2O), 410 (0.1) (M^+ −H_2O − CO), 392 (0.2) (M^+ − 28 − 2 × H_2O), 331 (1.2) (M^+ −125 cleavage 20C–22C), 313 (1.3) (M^+ −125 − H_2O), 295 (1) (M^+ −125 − 2 × H_2O), 285 (1.6) (M^+ −125 − 28 − H_2O), 284 (2.9), 266 (1.6) (M^+ −125 − 28 − 2 × H_2O), 256 (3.7).

3β-*Acetoxy*-20α$_F$-*hydroxy*-1-*oxo*-20R,22R-*witha*-5,24-*dienolide* (**4b**). Mp 169–171° from EtOAc; $[\alpha]_D$ +29.8° (*c* 0.11); UV λ_{max}^{EtOH} nm: 224 (ε 9357); IR ν_{max}^{KBr} cm^{-1}: 3500, 1735 and 1710; R_f 0.79. *m/z* (rel. int.): MS 498 (M^+, 0.33), 438 (36.9) (M^+ −HOAc), 420 (4.7) (M^+ −60 − H_2O), 410 (7.5) (M^+ −60 − 28), 373 (6) (M^+ −125 cleavage of 20C–22C), 355 (2.2) (M^+ −125 − H_2O), 329 (2.6) (M^+ −169 cleavage of 17C–20C), 313 (70.9) (M^+ −125 − 60), 295 (19.7) (M^+ −125 − 60 − H_2O), 285 (45.2) (M^+ −125 − 60 − 28), 284 (3), 269 (11) (M^+ −169 − 60), 267 (12.3) (M^+ −125 − 60 − 28 − H_2O), 256 (99.7), 241 (4.1) (M^+ −169 − 60 − 28), 169 (100), 126 (100), 125 (73).

20α$_F$-*Hydroxy*-1-*oxo*-20R,22R-*witha*-2,5,24-*trienolide* (**5**). $[\alpha]_D$ +4.1° (*c* 0.38); UV λ_{max}^{EtOH} nm: 225; IR ν_{max}^{KBr} cm^{-1}: 3500, 1710 and 1680; R_f 0.78. MS *m/z* (rel. int.): 438 (M^+, 2), 420 (1.5) (M^+ −H_2O), 313 (29.3) (M^+ −125 cleavage 20C–22C), 295 (6.8) (M^+ −125 − H_2O), 284 (21), 126 (49.7), 125 (29.7).

1,3-*Dioxo*-20α$_F$-*hydroxy*-20R,22R-*witha*-5,24-*dienolide* (**7**). Mp 154–155° from EtOAc, UV λ_{max}^{EtOH} nm: 224 (ε 12 746); IR ν_{max}^{KBr} cm^{-1}: 3500 and 1690.

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