Naturally Occurring Steroidal Lactones with a 17α-Oriented Side Chain. Structure of Withanolide E and Related Compounds

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Four related steroidal lactones of the withanolide group possessing a 17α-oriented side chain have been characterised as (17*S*.20*S*.22*R*)-5β.6β-epoxy-14α.17.20-trihydroxy-1-oxowitha-2.24-dienolide [withanolide E (1)]. (17*S*.20*S*.22*R*)-14α.17.20-trihydroxy-1-oxowitha-2.5.24-trienolide [withanolide F (2)]. (17*S*.20*S*.22*R*)-5α.6β.-14α.17.20-pentahydroxy-1-oxowitha-2.24-dienolide [withanolide S (3)]. and (17*R*.20*R*.22*R*)-14α.17-dihydroxy-1-oxowitha-2.5.24-trienolide [withanolide P (4)]. The structure of withanolide E deduced here has been confirmed by *X*-ray analysis (reported elsewhere): those of withanolide F and S are related to that of withanolide E on the basis of common degradation products. The identification of withanolide P is based on spectral analysis.

We have briefly reported the isolation of withanolides E (1) and F (2), two steroidal lactones of the withanolide group, characterised by the unusual α-orientation of the side chain.[1] The structure of withanolide E (1) was unequivocally determined by crystallographic analysis,[1] as (17*S*,20*S*,22*R*)-5β,6β-epoxy-14α,17,20-trihydroxy-1-oxowitha-2,24-dienolide, with a boat-like conformation for ring A, a distorted half chair for ring B, and a normal chair for ring C; ring D has an envelope form in which C(14) is out-of-plane. The compound crystallises with two molecules of water; the C(14) and C(20) hydroxy-groups are hydrogen bonded. As a result, the 17β-OH · · · C(13)Me and 17β-OH · · · 22-H distances are decreased, and the 20α-OH · · · C(13)Me distance is increased.

Withanolide F (2), a minor companion of withanolide E (1), possesses a double bond instead of the 5β,6β-epoxy-group present in the latter. Before the isolation of these two compounds, only a few natural steroids possessing an α-oriented side chain were known; to the best of our knowledge all are pregnane derivatives.[2] Since our preliminary communication,[1] the structures of several other 17α-alkyl steroids have been reported: Nic-2[3] and Nic-11[4] from *Nicandra physaloides*, and 4β-hydroxywithanolide E and dihydrowithanolide E[5] from *Physalis peruviana*. The present paper gives a detailed account of the chemical work on withanolide E (1) as well as of the identification of the related withanolides F (2), S (3), and P (4).[6]

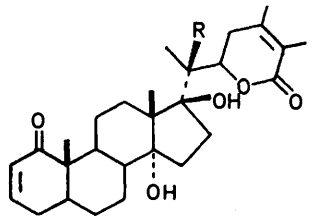
Hydrogenation of withanolide E over palladium–charcoal afforded the 2,3-dihydro-derivative (5), characterised by the lack of low-field vinylic proton n.m.r. signals (Table 1) and the lowering of the intensity of the u.v. absorption [$\lambda_{max.}$ 225 nm (ε 7 800)], now due only to the side-chain unsaturated δ-lactone chromophore. Treatment of this dihydro-derivative with acetone containing a trace of sulphuric acid, for 30 min at −10 °C, induced smooth elimination of a tertiary hydroxy-group, leaving intact the epoxide ring. Chromatography gave two isomeric products, a major deoxy-derivative with a trisubstituted double bond (6) (vinylic proton signal at δ 5.23), and a minor isomer with a tetrasubstituted double bond (7). Each deoxy-derivative still possessed two tertiary hydroxy-groups. This ready bidirectional elimination suggests that the 14α-OH was involved; the reaction would be facilitated by the release of strain imposed by the hydrogen bond keeping together the 14α-OH and the 20α-OH. The presence of two tertiary hydroxy-groups in compound (6) was confirmed by formation of a bis(trichloroacetylcarbamate) on treatment with trichloroacetyl isocyanate (δ 8.75 and 8.95 for NH). The same bis(trichloroacetylcarbamate) was obtained when dihydrowithanolide E (5) was treated with this reagent, thus pointing to the easy expulsion of the 14α-OH. Withanolide E (1) also afforded a bis(trichloroacetylcarbamate). It is possible that in the last two reactions a tricarbamate was formed initially, and that a molecule of trichloroacetylcarbamic acid was subsequently eliminated.

The double bonds (14,15- and 24,25-) in the deoxy-derivative (6) were further characterised by treatment with perbenzoic acid, leading to two epoxy-derivatives, a 5,6:14,15-diepoxide (8) (very narrow signal at δ 3.58 for 15-H) and a 5,6:14,15:24,25-triepoxide (9), characterised by a similar signal for the 15-H and a significant upfield shift of the signals due to the 24- and 25-methyl groups [from δ 1.92 in compound (8) to δ 1.48 and 1.57 in compound (9)]. Although the pattern of the 15-H signal in compounds (8) and (9) suggests[7] an α-orientation of the 14,15-epoxide, there is not enough evidence for an unequivocal configurational assignment. By analogy with the epoxidation with peroxy-acid of the 24,25-double bond in a similar unsaturated δ-lactone [compound (6) in ref. 8, a derivative of Nic-1], the 24,25-epoxy-group in compound (9) must be *syn* with respect to the 22-H.

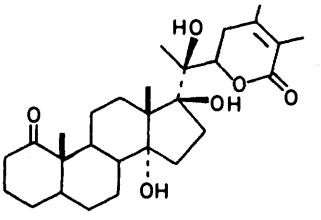
The following reactions were performed in order to confirm the position and the orientation of the epoxide ring in withanolide E (1); the previous indications were

[1] D. Lavie, I. Kirson, E. Glotter, D. Rabinovich, and Z. Shakked, *J.C.S. Chem. Comm.*, 1972, 877.
[2] See for instance K. A. Jaeggi, E. Weiss, and T. Reichstein, *Helv. Chim. Acta*, 1963, **46**, 694; C. W. Shoppee and R. E. Lack, *J. Chem. Soc.*, 1964, 3611.
[3] R. B. Bates and S. R. Morehead, *J.C.S. Chem. Comm.*, 1974, 125.
[4] M. J. Begley, L. Crombie, P. J. Ham, and D. A. Whiting, *J.C.S. Chem. Comm.*, 1973, 821; *J.C.S. Perkin I*, 1976, 296.
[5] I. Kirson, A. Abraham, P. D. Sethi, S. S. Subramanian, and E. Glotter, *Phytochemistry*, 1976 **15**, 340.
[6] A. Abraham, I. Kirson, D. Lavie, and E. Glotter, *Phytochemistry*, 1975, **14**, 189.
[7] K. Tori, T. Komeno, and T. Nagakawa, *J. Org. Chem.*, 1964, **29**, 1136.
[8] E. Glotter, P. Krinsky, and I. Kirson, *J.C.S. Perkin I*, 1976, 669.

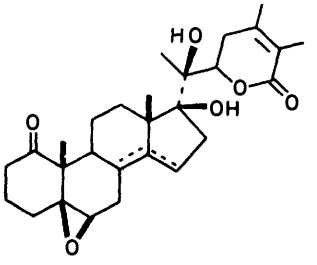
based on n.m.r. (narrow signal, $W_{\frac{1}{2}}$ 5 Hz at δ 3.20 for the 6-H) and c.d. data [$\Delta\varepsilon_{340}$ +1.69 for compound (1) and $\Delta\varepsilon_{295}$ −4.18 for compound (5)]. Treatment of dihydrowithanolide E (5) with hydrobromic acid in acetone at −12 °C afforded a mixture of the bromohydrins (10a) and (11a), resulting from diaxial opening of the epoxide ring; whereas in the former (10a) the reagent attacked only the epoxide ring, in the latter



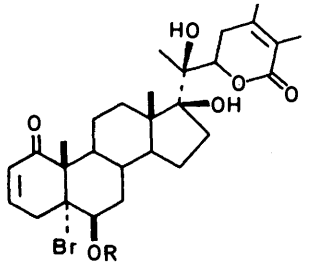
(1) 5β,6β-epoxy, R = OH
(2) 5,6-didehydro, R = OH
(3) 5α-OH, 6β-OH, R = OH
(4) 5,6-didehydro, R = H



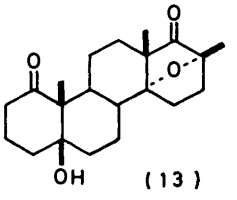
(5) 5β,6β-epoxy
(12) 5,6-didehydro



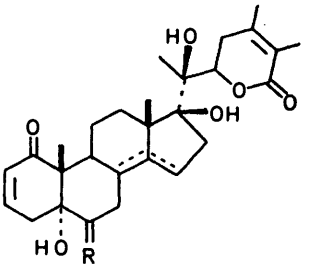
(6) Δ^{14}
(7) $\Delta^{8(14)}$
(8) 14,15-epoxy-14,15-dihydro
(9) 14,15:24,25-diepoxy-14,15,24,25-tetrahydro



(10) a; 14α-OH, R = H
b; 14α-OH, R = Ac
(11) a; 14,15-didehydro, R = H
b; 14,15-didehydro, R = Ac



(13)



(14) a; Δ^{14}, R = α-H, β-OH
b; Δ^{14}, R = α-H, β-OAc
(15) a; $\Delta^{8(14)}$, R = α-H, β-OH
b; $\Delta^{8(14)}$, R = α-H, β-OAc
(16) Δ^{14}, R = O
(17) $\Delta^{8(14)}$, R = O

(11a) elimination of the 14α-OH took place as well. The compounds were characterised as the corresponding bromo-acetates (10b) and (11b). The benzene-induced shift [$\Delta(CDCl_3 - C_6H_6)$] of the n.m.r. signal due to C(10)Me in compound (10b) is +0.25 p.p.m., which is conclusive evidence for the *trans*-AB ring junction. On treatment with Raney nickel, the bromo-acetate (10b) underwent reductive elimination to give the 5-ene (12), identical with 2,3-dihydrowithanolide F (2). The final proof for the structure assigned to the latter was obtained by epoxidation of compound (12), leading to dihydrowithanolide E (5).

In order to confirm the location and orientation of the unusual D-homo rearrangement triggered by the 14α-OH group. This failure led us to ask Dr. D. Rabinovich [1] to perform an *X*-ray analysis of withanolide E. The structure of the main rearrangement product (13) obtained in this reaction was also determined by *X*-ray analysis, and made the object of a separate communication [9] in which the mechanistic aspects of the rearrangement are outlined.

Withanolide S (3) was obtained during a study of the biogenesis of the withanolides in *Withania somnifera*, involving combination of various types through cross

[9] D. Rabinovich, Z. Shakked, I. Kirson, G. Günzberg, and E. Glotter, *J.C.S. Chem. Comm.*, 1976, 461.

pollination.* This is a means of obtaining offspring of the naturally occurring populations of the plant, in which the biosynthetic processes can be directed towards the production of new related compounds, or at least to changes in the relative concentrations of the various constituents. Cross pollination of *W. somnifera* chemotypes I [6] by III [10] resulted in F_1 offspring which led, following self-pollination, to several types of F_2 offspring (due to rearrangements of the genes). One of these types was again self-pollinated and afforded F_3 offspring identical with the parent F_2 plants, thus proving that they represent a true breeding new type of *W. somnifera*. Cross pollination of this type with the natural chemotype II [6] resulted in a new artificial chemotype. Extraction of the leaves in the usual manner afforded two major components, withanolide D [11] and withanolide S (3), in the ratio 4 : 1.

Spectral analysis of withanolide S revealed its close similarity to withanolide E. In the n.m.r. spectrum (pyridine solution) the only significant differences are the lack of the epoxidic 6-H signal at δ 3.20 and the appearance of a new signal at δ 4.10, attributed to a secondary, axial hydroxy-group; the low-field position of the C(10)Me resonance suggests its 6β-location. The compound afforded a monoacetate. Whereas the c.d. spectrum of withanolide E indicates a *cis*-fusion of rings A and B, that of withanolide S points towards a *trans*-

N.m.r. data *

						Me groups				
Compd.	2-H	3-H	6-H	15-H	22-H	19-H	18-H	21-H	27- and 28-H	Other
(1)	6.03dq (10, 2.5, 1)	6.87dq (10, 5.5, 2.5)	3.20nm $w_{\frac{1}{2}}$ 5 [3.18]		4.88t (8) [5.16]dd (11.5, 5.5)	1.25s	1.10s	1.42s	1.93; 1.88 [1.92; 1.68]	
(2)	5.84dq	6.88dq	5.60		4.92t	1.24	1.13	1.42	1.93	
(3a)	[6.98]	[6.56]	[4.10] $W_{\frac{1}{2}}$ 6			[1.63]	[1.45]	[1.71]	[1.92; 1.71]	
(3b)	5.82	6.53	4.87 $W_{\frac{1}{2}}$ 6		Obscured by 6-H	1.27	1.15	1.43	1.89; 1.85	2.11 (OAc)
	[6.07]	[6.52]	[5.42]			[1.52]	[1.52]	[1.77]	[1.97; 1.76]	[2.18]
(4)	5.87dq (10, 3, 1)	6.79dq (10, 5, 3)	5.61d (6)		4.70dt (12, 4.5)	1.23	1.12	1.06d (7)	1.93	
						[1.21]	[1.45]	[1.41]d	[1.92; 1.70]	
(5)			3.23nm		4.85	1.15	1.05	1.43	1.97; 1.93	
(6)			3.25 $W_{\frac{1}{2}}$ 5	5.23 $W_{\frac{1}{2}}$ 5	4.65t (8)	1.23	1.14	1.30	1.95; 1.88	
(7)			3.16 $W_{\frac{1}{2}}$ 5		4.60	1.12	1.02	1.28	1.95; 1.87	
(8)			3.18nm $W_{\frac{1}{2}}$ 5	3.58 very nm $W_{\frac{1}{2}}$ 3	4.55dd (12, 4.5)	1.25	1.12	1.25	1.92	
(9)			3.18nm $W_{\frac{1}{2}}$ 5	3.57 very nm $W_{\frac{1}{2}}$ 3	4.78dd (11.5, 3)	1.17	1.17	1.26	1.57; 1.48	
(10a)			4.26 $W_{\frac{1}{2}}$ 6			1.53	1.13	1.42	1.95; 1.88	
(10b)			5.43 $W_{\frac{1}{2}}$ 5		4.92t	1.48 {1.23}	1.13 {1.12}	1.43 {1.50}	1.93 {1.82; 1.48}	2.12 (OAc)
(11b)			5.45 $W_{\frac{1}{2}}$ 5	5.22	4.70dd (10, 6)	1.50	1.22	1.32	1.96; 1.89	2.13 (OAc)
(12)			5.53		4.90t	1.24	1.12	1.43	1.92	
(14a)	5.90m	6.67m	3.76m $W_{\frac{1}{2}}$ 6 [4.21]	5.21m $W_{\frac{1}{2}}$ 5	4.70t (8)	1.37 [1.75]	1.23 [1.56]	1.38 [1.64]	1.95; 1.88 [1.93; 1.75]	
(14b)	5.90	6.68	4.90m $W_{\frac{1}{2}}$ 6	5.17m $W_{\frac{1}{2}}$ 5	4.68dd (9.5, 6)	1.30	1.23	1.33	1.93	
(15a)	[6.05]	[6.57]	[4.18]			[1.66]	[1.23]	[1.47]	[1.79; 1.52]	
(15b)	5.83 [6.08]	6.55 [6.59]	4.75 [5.37]		Obscured by 6-H	1.23 [1.42]	1.07 [1.23]	1.23 [1.44]	1.97 [1.69; 1.53]	
(16)	5.88m	6.62m		5.23 $W_{\frac{1}{2}}$ 5	4.66dd (9, 7.5)	1.11	1.17	1.32	1.93	
(17)	5.88	6.69			4.75dd (13, 4)	1.04	1.04	1.31	1.93	

* Recorded at 60 MHz; solvent $CDCl_3$; δ values; data for solutions in C_5D_5N in square brackets; data for solutions in C_6D_6 in braces; coupling constants (Hz) in parentheses; nm = narrow multiplet.

* The genetic aspects of this work will be published elsewhere.

[10] E. Glotter, I. Kirson, A. Abraham, and D. Lavie, *Tetrahedron*, 1973, **29**, 1353.

[11] D. Lavie, I. Kirson, and E. Glotter, *Israel J. Chem.*, 1968, **6**, 671.

fusion ($\Delta\varepsilon_{336}$ −1.32); the positive Cotton effect at *ca.* 250 nm in both compounds is characteristic of the 22*R*-configuration.

The structure (3) assigned to withanolide S is based on the identity of degradation products with those obtained from withanolide E. Thus, treatment of compound (1) with sulphuric acid in acetone for 4 h at room temperature led to opening of the epoxide ring to the corresponding diaxial glycol, as well as to the elimination of the 14α-hydroxy-group. The elimination proceeded in both possible directions, leading to the isomeric compounds (14a) (Δ^{14}; major component) and (15a) [$\Delta^{8(14)}$; minor component]. The same compounds were obtained from withanolide S (3). The compounds were also characterised as the corresponding monoacetates, as well as by smooth oxidation to the corresponding ketols (16) and (17).

Withanolide P (4) is one of the minor constituents of *W. somnifera*, chemotype I.[6] The common feature of all the withanolides isolated from this chemotype is the lack of a 20-OH group (in contrast to the withanolides of chemotypes II [6] and III [10]). The assignment of structure (4) to withanolide P is based on analysis of its spectral behaviour. The n.m.r. spectrum shows signals for two tertiary [C(10)Me δ 1.23; C(13)Me δ 1.12] and one secondary methyl group [C(20)Me, doublet, δ 1.06]. The three vinylic protons possess the same chemical shift and splitting pattern as in withanolide G,[10] thus leading to the assignment of a 2,5-dien-1-one partial structure. The δ-lactone system in the side chain is characterised by two vinylic methyl signals (δ 1.93) and a double triplet for the 22-H with the same splitting pattern as that in withaferin A,[12] though at significantly lower field (δ 4.7 as compared with 4.4). The position of this signal suggests the proximity of a hydroxy-group, the only possible location of this being C(17). Its β-orientation is assigned on the basis of pyridine-induced shifts [$\Delta(CDCl_3 - C_5D_5N)$]: these are significant only for the C(13)Me (−0.33 p.p.m.) and the C(20)Me (−0.35 p.p.m.). Models show that a 17β-OH should strongly influence both signals, whereas a 17α-OH should induce a sizeable downfield shift in only the C(20)Me signal. These conclusions are supported by the pyridine-induced shifts of the signals for the methyl groups in withanone, a related compound possessing a 17α-hydroxy-group [compound (4) in ref. 13].

Further support for the structure of withanolide P is obtained from its fragmentation under electron impact. The most prominent signals are due to the gradual loss of up to two molecules of water (*m/e* 436 and 418), as well as to cleavage of the C(17)–C(20) bond (*m/e* 301), also accompanied by loss of water (*m/e* 283 and 265). The presence of a 17-OH is indicated by formation of an ion *m/e* 209 due to cleavage of ring D along the C(13)–C(17) and C(14)–C(15) bonds.[13] The base peak of withanolide P (*m/e* 125) is due to cleavage of the C(20)–C(22) bond and is a common feature of all the withanolides possessing the unsaturated δ-lactone system.

[12] D. Lavie, E. Glotter, and Y. Shvo, *J. Chem. Soc.*, 1965, 7517.
[13] I. Kirson, E. Glotter, D. Lavie, and A. Abraham, *J. Chem. Soc.* (*C*), 1971, 2032.

EXPERIMENTAL

M.p.s were taken with a Fisher-Johns apparatus. Optical rotations were recorded with an automatic Perkin-Elmer 141 polarimeter and refer to solutions in chloroform, unless otherwise stated. C.d. measurements were performed by Mrs. B. Romano with a Cary 60 instrument for solutions in ethanol. I.r. spectra were recorded on a Perkin-Elmer Infracord 137 spectrophotometer and refer to chloroform solutions; u.v. spectra were recorded on a Cary 14 instrument for solutions in ethanol; n.m.r. spectra were determined on Varian A-60 and NV-14 instruments for *ca.* 5% solutions in deuteriochloroform, pyridine, or benzene (as stated), containing tetramethylsilane as internal standard. T.l.c. was carried out on chromatoplates of silica gel G (Merck), and spots were developed with iodine vapour. Preparative chromatoplates (1 mm thickness) were prepared from silica gel PF_{254} (Merck). Column chromatography was carried out on silica gel H (Merck). Mass spectra were taken under the direction of Dr. Z. Zaretskii with a Varian MAT 731 HR instrument. Analyses were performed in the microanalytical laboratory of the Weizmann Institute, under the direction of Mr. R. Heller.

Isolation Procedure.—Withanolides E (1) and F (2) were isolated from the crude extract of the leaves of *Withania somnifera* chemotype III, as previously described.[10] Similarly, a crude extract was obtained by processing the leaves (1 kg) of the artificial chemotyoe of *W. somnifera* described in the Discussion section. Chromatography afforded two main components: withanolide D (by elution with benzene–ethyl acetate, 7 : 3) (10.5 g), identified by comparison with an authentic sample; and withanolide S (3) (by elution with ethyl acetate–methanol, 9 : 1) (3.0 g). Several other steroidal components isolated in minute quantities are still being investigated. Withanolide P (4) was obtained during the processing of the leaves of *W. somnifera* chemotype I, as already described.[6]

Withanolide E (1) had m.p. 167—168° (from acetone); $[\alpha]_D$ +103.5° (*c* 0.7); $\lambda_{max.}$ 226 nm (ε 18 000); $\nu_{max.}$ 1 675 cm^{-1}; c.d. (*c* 0.48 in EtOH) $\Delta\varepsilon_{390}$ 0, $\Delta\varepsilon_{340}$ +1.69, $\Delta\varepsilon_{298}$ 0, $\Delta\varepsilon_{295}$ −0.05, $\Delta\varepsilon_{282}$ 0, $\Delta\varepsilon_{255}$ +4.05 [Found: C, 64.4; H, 8.15%; M^+, 486. $C_{28}H_{38}O_7,2H_2O$ requires C, 64.35; H, 8.1%; *M* (anhyd.), 486.6]. *Withanolide F* (2) had m.p. 192—193° (from acetone–hexane); $\lambda_{max.}$ 226 nm (ε 17 500); $\nu_{max.}$ 1 678 cm^{-1} (Found: C, 71.2; H, 8.2%; M^+, 470. $C_{28}H_{38}O_6$ requires C, 71.45; H, 8.15%; *M*, 470.6). *Withanolide S* (3) had m.p. 272° (decomp.) (from ethyl acetate); $[\alpha]_D$ +95.5° (*c* 0.2 in MeOH); $\lambda_{max.}$ 225 nm (ε 18 100); $\nu_{max.}$ 1 685 cm^{-1}; c.d. (*c* 0.46 in EtOH), $\Delta\varepsilon_{380}$ 0, $\Delta\varepsilon_{336}$ −1.32, $\Delta\varepsilon_{290}$ 0, $\Delta\varepsilon_{256}$ +4.31, $\Delta\varepsilon_{236}$ −1.96 (positive at shorter wavelengths) (Found: C, 66.1; H, 8.3%; M^+, 504. $C_{28}H_{40}O_8$ requires C, 66.6; H, 7.9%; *M*, 504.6). *Withanolide P* (4) had m.p. 216—217° (from ethyl acetate); $[\alpha]_D$ +51° (*c* 0.2), $\lambda_{max.}$ 225 nm (ε 17 900); $\nu_{max.}$ 1 680 cm^{-1} (Found: C, 73.85; H, 8.5%; M^+, 454. $C_{28}H_{38}O_5$ requires C, 74.0; H, 8.45%; *M*, 454.6).

Hydrogenation of Compound (1) *to* (17S,20S,22R)-5β,6β-*Epoxy*-14α,17,20-*trihydroxy*-1-*oxowith*-24-*enolide* (5).—Withanolide E (1) (500 mg) in absolute ethanol (200 ml) was hydrogenated over 10% palladium–charcoal at room temperature and atmospheric pressure. After absorption of 1 mol. equiv. of hydrogen the *product* was crystallised from ethanol; m.p. 264—265° (from acetone–hexane, m.p.

247—248°); $[\alpha]_D$ −42° (*c* 0.3), $\lambda_{max.}$ 225 nm (ε 7 800); $\nu_{max.}$ 1 711 cm^{-1}; c.d. $\Delta\varepsilon_{295}$ −4.18, $\Delta\varepsilon_{268}$ 0, $\Delta\varepsilon_{251}$ +3.84 (Found: M^+, 488.2757. $C_{28}H_{40}O_7$ requires *M*, 488.2774).

Dehydration of Compound (5) *to* (17S,20S,22R)-5β,6β-*Epoxy*-17,20-*dihydroxy*-1-*oxowitha*-14,24-*dienolide* (6) *and* 5β,6β-*Epoxy*-17,20-*dihydroxy*-1-*oxowitha*-8(14),24-*dienolide* (7).—Dihydrowithanolide E (500 mg) in acetone (400 ml) at −10 °C was treated with 8N-H_2SO_4 (5 capillary drops). After 1 h the solution was neutralised with aqueous 10% sodium hydrogen carbonate, most of the solvent was evaporated off under vacuum and the residue was isolated with ether. The crude product (450 mg) was chromatographed on silica gel H (200 g); elution with benzene–ethyl acetate (7 : 3) yielded *compound* (6) (400 mg), m.p. 188—190° (from acetone–hexane); $[\alpha]_D$ +80.5 (*c* 0.2); $\lambda_{max.}$ 226 nm (ε 7 750) (with strong end absorption); $\nu_{max.}$ 1 705 cm^{-1} (Found: C, 71.7; H, 8.2%; M^+, 470. $C_{28}H_{38}O_6$ requires C, 71.45; H, 8.15%; *M*, 470.6). Further elution with the same solvent yielded *compound* (7) (15 mg), m.p. 204—207° (from acetone–hexane); $\lambda_{max.}$ 226 nm (16 900); $\nu_{max.}$ 1 705 cm^{-1} (Found: M^+, 470.2665. $C_{28}H_{38}O_6$ requires *M*, 470.2668).

Epoxidation of Compound (6) *to* (17S,20S,22R)-5β,6β:14,15-*Diepoxy*-17,20-*dihydroxy*-1-*oxowith*-24-*enolide* (8) *and* 5β,6β:14,15:24,25-*Triepoxy*-17,20-*dihydroxy*-1-*oxowithanolide* (9).—*m*-Chloroperbenzoic acid (55 mg) was added to a solution of compound (6) (100 mg) in benzene (10 ml). After 24 h at room temperature, the solution was washed with aqueous sodium hydrogen carbonate, then with water, dried (Na_2SO_4), and evaporated. The crude product (two close spots on a chromatoplate) was separated by preparative layer chromatography (p.l.c.) on plates 40 cm in length [benzene–ethyl acetate (2 : 8)]. The upper band (R_F 0.42) yielded *compound* (8) (60 mg), m.p. 224° (from ethyl acetate); $\lambda_{max.}$ 226 nm (ε 8 000); $\nu_{max.}$ 1 704 cm^{-1} (Found: C, 69.15; H, 7.95%; M^+, 486. $C_{28}H_{38}O_7$ requires C, 69.1; H, 7.85%; *M*, 486.6). From the lower band (R_F 0.38), *compound* (9) (24 mg) was extracted, m.p. 241° (from ethyl acetate); $\nu_{max.}$ 1 712 cm^{-1} (Found: C, 67.0; H, 7.7%; M^+, 502. $C_{28}H_{38}O_8$ requires C, 66.9; H, 7.6%; *M*, 502.6).

Treatment of Dihydrowithanolide E (5) *with Hydrobromic Acid.*—To a cooled solution (*ca.* −12 °C) of compound (5) (100 mg) in acetone (100 ml), 45% hydrobromic acid in acetic acid (3 ml) was added dropwise. After 1 h at the same temperature, the solution was neutralised with aqueous 5% sodium carbonate, part of the acetone was removed, water was added, and the product was extracted with chloroform; it showed two close spots on a chromatoplate. Separation was achieved after acetylation with acetic anhydride (0.5 ml) and pyridine (1 ml) overnight at room temperature, by p.l.c. on plates 40 cm in length [ethyl acetate–benzene (4 : 1)]. The lower band yielded (17S,20S,22R)-6β-*acetoxy*-5α-*bromo*-14α,17,20-*trihydroxy*-1-*oxowith*-24-*enolide* (10b) (75 mg), m.p. 239° (decomp.) (from methanol), $[\alpha]_D$ −32° (*c* 0.2), $\lambda_{max.}$ 225 nm (ε 8 100), $\nu_{max.}$ 1 742 and 1 700 cm^{-1} (Found: C, 57.6; H, 7.5; Br, 12.3. $C_{30}H_{43}BrO_8,CH_3OH$ requires C, 57.85; H, 7.35; Br, 12.4%). The upper band yielded (17S,20S,22R)-6β-*acetoxy*-5α-*bromo*-17,20-*dihydroxy*-1-*oxowitha*-14,24-*dienolide* (11b) (21 mg), m.p. 251° (decomp.) (from ethanol) (Found: C, 61.0; H, 7.1; Br, 13.9%; $M^+ - 2H_2O$, 556 and 558. $C_{30}H_{41}BrO_7$ requires C, 60.7; H, 6.95; Br, 13.45%).

Treatment of the Bromo-acetate (10b) *with Raney Nickel to give* (17S,20S,22R)-14α,17,20-*Trihydroxy*-1-*oxowitha*-5,24-*dienolide* (*Dihydrowithanolide F*) (12).—A solution of compound (10b) (60 mg) in ethanol was heated to reflux with Raney nickel (1 g) for 4 h. The mixture was filtered and evaporated, and the residue was filtered through a short silica gel column. The product (55 mg) was purified by p.l.c. [benzene–ethyl acetate (2 : 8)]. The main band was extracted and the product crystallised from acetone–hexane, m.p. 211°. The same compound was obtained by catalytic hydrogenation of withanolide F (2), as described for withanolide E (1).

Epoxidation of Compound (12) *to give Dihydrowithanolide E* (5).—Compound (12) (40 mg) was treated with *m*-chloroperbenzoic acid (1.1 equiv), as described above. The product was purified by p.l.c. [benzene–ethyl acetate (2 : 8)] and crystallised from ethanol. It was identical with dihydrowithanolide E (5).

Treatment of Compound (1) *with Sulphuric Acid to give* (17S,20S,22R)-5α,6β,17,20-*Tetrahydroxy*-1-*oxowitha*-2,14,24-*trienolide* (14a) *and* 5α,6β,17,20-*Tetrahydroxy*-1-*oxowitha*-2,8(14),24-*trienolide* (15a).—8N-Sulphuric acid (3 ml) was added to a solution of compound (1) (600 mg) in acetone (400 ml). After 4 h at room temperature, the solution was neutralised with aqueous sodium hydrogen carbonate, water was added, and the product was extracted with ether (3 × 300 ml). The extract was washed with aqueous 10% sodium chloride, dried (Na_2SO_4), and evaporated, and the residue was separated by p.l.c. [benzene–ethyl acetate–ethanol (2 : 18 : 1)]. The upper band was extracted with chloroform–methanol (6 : 4) to give *compound* (14a) (450 mg), which could not be induced to crystallise; $[\alpha]_D$ +121° (*c* 0.4 in MeOH); $\lambda_{max.}$ 226 nm (ε 17 600); $\nu_{max.}$ 1 680 cm^{-1}; c.d. (*c* 0.52 in EtOH) $\Delta\varepsilon_{380}$ 0, $\Delta\varepsilon_{336}$ −1.29, $\Delta\varepsilon_{290}$ 0, $\Delta\varepsilon_{256}$ +4.52, and $\Delta\varepsilon_{239}$ 0 (positive at shorter wavelengths) (Found: M^+, 486.2745. $C_{28}H_{38}O_7$ requires *M*, 486.2617). Similarly, the lower band gave *compound* (15a) (120 mg), which could not be induced to crystallise; $[\alpha]_D$ +12.5° (*c* 0.2 in MeOH); $\lambda_{max.}$ 226 nm (ε 17 800); $\nu_{max.}$ 1 680 cm^{-1}; c.d. (*c* 0.5 in EtOH) $\Delta\varepsilon_{380}$ 0, $\Delta\varepsilon_{337}$ −1.31, $\Delta\varepsilon_{290}$ 0, $\Delta\varepsilon_{254}$ +4.34, and $\Delta\varepsilon_{239}$ −2.80 (positive at shorter wavelengths) (Found: M^+, 486.2739. $C_{28}H_{38}O_7$ requires *M*, 486.2617).

Acetylation of Compounds (14a) *and* (15a).—Each compound (50 mg) was acetylated with acetic anhydride (0.5 ml) and pyridine (1 ml) to give the corresponding 6-monoacetate. *Compound* (14b) had m.p. 162—164° (from acetone); $[\alpha]_D$ +147.5° (*c* 0.2); $\lambda_{max.}$ 226 nm (ε 18 000); $\nu_{max.}$ 1 735 and 1 680 cm^{-1} (Found: C, 68.25; H, 7.6%; M^+, 528. $C_{30}H_{40}O_8$ requires C, 68.15; H, 7.65%; *M*, 528.6). *Compound* (15b) had m.p. 203—204° (from acetone–hexane); $[\alpha]_D$ +26° (*c* 0.2); $\lambda_{max.}$ 226 nm (ε 17 900); $\nu_{max.}$ 1 735 and 1 680 cm^{-1} (Found: C, 68.3; H, 7.5%; M^+, 528).

Oxidation of Compounds (14a) *and* (15a) *to the Ketols* (16) *and* (17), *respectively.*—The oxidation was carried out with trioxodipyridinechromium in methylene chloride according to the described procedure.[14] The reaction with (14a) (200 mg) yielded 5α,17β,20α-*trihydroxy*-1,6-*dioxowitha*-2,14,24-*trienolide* (16) (160 mg), m.p. 248—250°; $[\alpha]_D$ +150° (*c* 0.2); $\lambda_{max.}$ 224 nm (ε 16 800) (followed by end absorption); $\nu_{max.}$ 1 715, 1 680, and 1 675 cm^{-1}; c.d. (*c* 0.23 in EtOH) $\Delta\varepsilon_{390}$ 0, $\Delta\varepsilon_{345}$ −1.58, $\Delta\varepsilon_{332}$ −1.26, $\Delta\varepsilon_{306}$ −5.05, and $\Delta\varepsilon_{254}$ +4.11 (negative at shorter wavelengths) (Found: M^+, 484.2515. $C_{28}H_{36}O_7$ requires *M*, 484.2460). Oxidation of (15a) (100 mg) gave 5α,17β,20α-*trihydroxy*-1,6-*dioxowitha*-2,8(14),24-*trienolide* (17) (89 mg), m.p. 277—278° (from

[14] W. G. Dauben, M. Lorber, and D. S. Fullerton, *J. Org. Chem.*, 1969, **34**, 3587.

ethyl acetate), $[\alpha]_D$ +32.5° (*c* 0.2); $\lambda_{max.}$ 224 nm (ε 16 900); $\nu_{max.}$ 1 715, 1 680, and 1 675 cm^{-1}; c.d. (*c* 0.2 in EtOH) $\Delta\varepsilon_{390}$ 0, $\Delta\varepsilon_{345}$ −1.79, $\Delta\varepsilon_{332}$ −1.45, $\Delta\varepsilon_{306}$ −5.6, and $\Delta\varepsilon_{256}$ +4.2 (negative at shorter wavelengths) (Found: M^+, 484.2396. $C_{28}H_{36}O_7$ requires *M*, 484.2460).

Acetylation of Withanolide S (3).—The 6-*monoacetate* was obtained as described above; m.p. 245—250° (decomp.) (from ethyl acetate); $[\alpha]_D$ +45° (*c* 0.21); $\lambda_{max.}$ 225 nm (ε 17 900); $\nu_{max.}$ 1 737 and 1 685 cm^{-1} (Found: C, 65.5; H, 7.9%; M^+, 546. $C_{30}H_{42}O_9$ requires C, 65.9; H, 7.8; ·*M*, 546.6).

Dehydration of Withanolide S (3) *to Compounds* (14a) *and* (15a).—The reaction was performed as described for withanolide E (1). Separation by p.l.c. gave two products, identical with compounds (14a) and (15a). (The comparison was also made with the corresponding monoacetates.)

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