Tetrahedron. Vol. 29, pp. 1353 to 1364. Pergamon Press 1973. Printed in Great Britain.

CONSTITUENTS OF *WITHANIA SOMNIFERA* DUN—XIII[a]

THE WITHANOLIDES OF CHEMOTYPE III

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Abstract—Nine new steroidal lactones of the withanolide series (withanolides E–M) have been isolated from the leaves of *Withania somnifera* Dun (Solanaceae) growing in the southern coastal plane of Israel. This population of *W. somnifera* constitutes a new chemotype and is designated as chemotype III. The following structures have been assigned to seven of the above compounds:

Withanolide G, 20α-hydroxy-1-oxo-20R,22R-witha-2,5,8(14),24-tetraenolide (**1**); withanolide H, 20α,27-dihydroxy-1-oxo-20R,22R-witha-2,5,8(14),24-tetraenolide (**2**); withanolide I, 20α-hydroxy-1-oxo-20R,22R-witha-3,5,8(14),24-tetraenolide (**3**); withanolide J, 17α,20α-dihydroxy-1-oxo-20S,22R-witha-2,5,8(14),24-tetraenolide (**4**); withanolide K, 17α,20α-dihydroxy-1-oxo-20S,22R-witha-3,5,-8(14),24-tetraenolide (**5**); withanolide L, 17α,20α-dihydroxy-1-oxo-20S,22R-witha-2,5,14,24-tetraenolide (**6**); withanolide M, 17α,20α-dihydroxy-1-oxo-14α,15α-epoxy-20S,22R-witha-2,5,24-trienolide (**7**).

During our studies on *Withania somnifera* Dun (Solanaceae), three genetic types designated as chemotypes **1**, **2**, and **3** were found to occur in Israel.[2] The chemotypes have a definite geographic distribution area in their natural habitat and are identical from botanical and morphological point of view, but differ through their content in variously substituted steroidal lactones of the withanolide type.

The present work is concerned with the constituents of *chemotype III* growing in the southern coastal plane of Israel, and raised as well from seeds in a uniform nursery. Of the nine steroidal lactones isolated from the leaves of this chemotype, withanolides G–M (**1–7**) are hereby presented; the structure of the remaining two compounds, withanolide E (**8**) and F (**9**) will be discussed in detail in a forthcoming publication. An X-ray analysis performed on **8** has revealed its unusual 17α oriented side chain.[3]

Withanolide G, 20α-hydroxy-1-oxo-20R, 22R-witha-2,5,8(14),24-tetraenolide (**1**), $C_{28}H_{36}O_4$, exhibits two bands in the CO region of the IR at 1685 and 1692 cm^{-1} (αβ-unsaturated ketone and αβ-unsaturated lactone respectively) and maximum absorption in the UV at 223 nm (ε 19,400) followed by strong end absorption. Catalytic hydrogenation over Pd-$CaCO_3$ takes place with the rapid absorption of one mole of hydrogen to give the 2,3-dihydroderivative $C_{28}H_{38}O_4$ (**10**) characterized by the shift of the 1685 cm^{-1} band at 1704 cm^{-1} (saturated 6-membered ring ketone); the UV absorption of **10** has a lower intensity (224 nm, ε 10,500), due to the reduction of the unsaturated ketone chromophore. The hydrogenation is also characterised by the disappearance of two NMR signals from the low field region of the spectrum, the double quartets at δ 6·76 and 5·83 (Table 1), leaving only the signal of a third vinylic proton at δ 5·60. The NMR pattern displayed by **1** is in perfect agreement with the relevant signals observed for cholesta-2,5-diene-1-one.[4] In the Me region of the spectrum, **1** shows three signals for tertiary groups, one of them significantly deshielded (δ 1·30), and a broadened 6-proton signal (δ 1·91) accounting for two vinylic Me groups; the latter is characteristic for all the withanolides with a similarly substituted δ-lactone in the side chain. The second signal, characteristic for the lactone moiety, is related to the 22-H: in the withanolides with a 20-H it occurs as a double triplet, whereas now it is a double doublet (δ 4·21) indicating therefore the absence of the 20-H.

Compound **1** does not undergo acetylation with acetic anhydride in pyridine, however, it reacts easily, *in situ*, with trichloroacetyl isocyanate to afford a monocarbamate characterized in the NMR by a singlet at δ 8·70 for the trichloroacetyl carbamate proton. The tertiary OH responsible for this reaction is assigned the C-20 position in agreement with the position of the 21-Me (singlet δ 1·30) and

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Table 1. NMR signals of relevant protons in withanolides G–M and their derivatives

Compound	2H	3H	4H	6H	15H	22H	Methyl groups 19H	18H	21H	27 & 28H	Other signals
1	5·83dq (10; 3; 1)	6·76dq (10; 5; 2·5)		5·60 (W½ 8)		4·21dd (10; 6·5)	1·25s	1·05s	1·30s	1·91	
2	5·88dq (10; 3; 1)	6·83dq (10; 5; 2·5)		5·63 (W½ 8)		4·30dd (10; 6·5)	1·26s	1·07s	1·31s		27-methylene 4·90s; 28H and acetate 2·06; 2·08
3*		5·62dt (10)	6·08dt (10)	5·71t		4·25dd (11·5; 4·5)	1·40s	1·07s	1·30s	1·87 1·94	
4	5·84dq (10; 3; 1)	6·83dq (10; 5; 2·5)		5·59 (W½ 8)		4·62dd (10·5; 5·5)	1·24s	1·06s	1·31s	1·90	
5		5·64dt (10)	6·08dt (10)	5·70t		4·66dd (10; 5)	1·39s	1·07s	1·32s	1·88 1·95	
6	5·89dq (10; 3; 1)	6·81dq (10; 5; 2·5)		5·61 (W½ 8)	5·26 (W½ 5)	4·70dd (11; 5)	1·26s	1·21s	1·32s	1·93	
7	5·91dq (10; 3; 1)	6·81dq (10; 5; 2·5)		5·56 (W½ 8)	3·58s	4·70t (8)	1·28s	1·24s	1·26s	1·90	
10				5·60 (W½ 8)		4·21dd (10; 6·5)	1·30s	1·06s	1·30s	1·91	
11	5·86dq (10; 3; 1)	6·81dq (10; 5; 2·5)		3·63 (W½ 8)	3·23 (W½ 5)	4·28dd (11; 6)	1·23s	1·18s	1·33s	1·91	
12				3·33 (W½ 8)	3·23 (W½ 6)	4·18dd (11·5; 5)	1·31s	1·13s	1·33s	1·93	
13	6·01dq (10; 2·5; ~ 0·5)	6·88dq (10; 6; 2)		3·16 (W½ 5)	3·41s	4·18dd (11; 5·5)	1·30s	1·10s	1·26s	1·91	
14	6·60dq (10; 2; ~ 0·5)	6·76dq (10; 5; 2)		3·04t (W½ 6)	3·33s	4·20dd (11; 6·5)	1·39s	1·11s	1·27s	1·90	
15				3·15 (W½ 3·5)	3·42s	4·15dd (11·5; 5·5)	1·21s	1·06s	1·25s	1·87	
16				3·02t (W½ 6)	3·26s	4·13dd (11; 6·5)	1·44s	1·06s	1·23s	1·90	
17				3·30		4·23dd (11; 6)	1·03s	1·03s	1·28s	1·91	21H 3·73t (W½ 5)
18				5·50		4·23dd (11; 6)	1·05s	1·05s	1·28s	1·91	1H 3·41m (W½ 17)
19a				3·48 (W½ 8)		~ 3·50	1·03s	1·03s	1·23s	1·70 1·75	1H 3·75, 26H₂ (AB) 3·66d (11), 4·30d (11)
20a				3·46 (W½ 8)		~ 3·50	1·01	1·01s	1·21s	1·70 1·76	1H 3·40 26H₂ (AB) 3·67d (11), 4·30d (11)
20b				3·51 (W½ 8)		4·98dd (10·5; 3)	1·03s	1·13s	1·27s	1·69	26H₂ (AB) 4·61, 4·55

Table 1. (*Cont.*)

Compound	2H	3H	4H	6H	15H	22H	Methyl groups 19H	18H	21H	27 & 28H	Other signals
21				5·52 ($W_{\frac{1}{2}}$ 8)			1·00s	0·76s	2·12s		1H 3·80t ($W_{\frac{1}{2}}$ 5)
22				5·54 ($W_{\frac{1}{2}}$ 8)			1·03s	0·76s	2·12s		1H 3·40m ($W_{\frac{1}{2}}$ 17)
23				5·58 ($W_{\frac{1}{2}}$ 8)			1·30s	0·78s	2·13s		
25		5·62dt (10)	6·19dt (10)	5·71t	5·26	4·28dd	1·38s	1·17s	1·33s	1·91	
26				5·54 ($W_{\frac{1}{2}}$ 8)		4·63dd (11; 5·5)	1·28s	1·05s	1·31s	1·91	
27				5·56 ($W_{\frac{1}{2}}$ 8)	5·30 ($W_{\frac{1}{2}}$ 5)	4·70dd (11·5; 5)	1·31s	1·20s	1·31s	1·91	
28	6·04dq (10; 2·5; ~ 0·5)	6·88dq (10; 6; 2)		3·18 ($W_{\frac{1}{2}}$ 5)	3·64s	4·68t (8)	1·31s	1·20s	1·23s	1·91	
29	5·96dq (10; 2·5; ~ 0·5)	6·72dq (10; 6; 2)		3·11 ($W_{\frac{1}{2}}$ 5)	3·52s	4·68t (8)	1·38s	1·18s	1·25s	1·90	
30				3·18 ($W_{\frac{1}{2}}$ 3·5)	3·64s	4·68t (8)	1·23s	1·16s	1·23s	1·90	
31				3·01t ($W_{\frac{1}{2}}$ 5)	3·50s	4·67t (8)	1·43s	1·22s	1·30s	1·90	
33				5·50 ($W_{\frac{1}{2}}$ 8)			1·04s	0·73s	2·24s		1H 4·74t ($W_{\frac{1}{2}}$ 5)
34				5·54 ($W_{\frac{1}{2}}$ 8)			1·03s	0·72s	2·23s		1H 3·42m ($W_{\frac{1}{2}}$ 18)
35				5·54 ($W_{\frac{1}{2}}$ 8)			1·30s	0·73s	2·23s		

Spectra are recorded at 60 MHz in $CDCl_3$ solution; chemical shifts are in δ units; coupling constants (in Hz) are in parentheses.

*Spectrum recorded at 100 MHz.

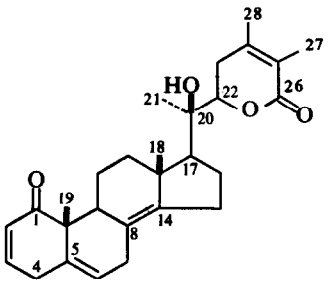
Abbreviations: s = singlet; d = doublet; t = triplet; dd = double doublet; dt = double triplet; dq = double quartet; m = multiplet.

the pattern of the 22-H referred above. This assignment is corroborated by the impressive shifts of these signals in the monocarbamate, the 21-Me at δ 1·90 and the 22-H at δ 4·86 (similar down-field shifts have been encountered in withanolide D).[5]

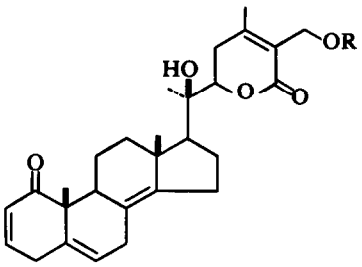
Under electron impact, the usual cleavage of the C(20)-C(22) bond is accompanied by the fragmentation of the C(17)-C(20) bond, leading to the most significant ions *m/e* 169 and 267 (for the side chain and the rest of the molecule, respectively).

In view of all these data and the empirical formula of the compound an additional tetrasubstituted double bond has to be present in the molecule, at 8-9 or 8-14; such double bonds are known[6] to migrate in acidic conditions to position 14-15. Indeed, both **1** and **10** undergo this reaction in the presence of dry HCl in cold chloroform solution to give the isomeric compounds **11** and **12** respectively, characterised by the appearance of a new vinylic proton signal (δ 5·25 and 5·23, respectively). The same reaction accompanies hydrogenation of **1** over Pd-C as catalyst.

Epoxidation of **11** with two equivalents of perbenzoic acid afforded a mixture of diepoxides, the 5β,6β-14α,15α-(**13**) and the 5α,6α-14α,15α diepoxide (**14**), the former being predominant. Similarly, the 2,3-dihydro-derivative **12** afforded the diepoxides **15** and **16**, again the compound possessing the



1
withanolide G



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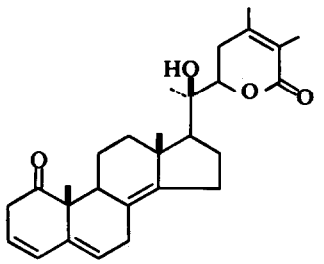
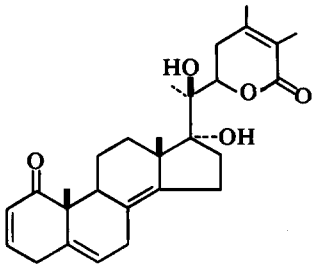
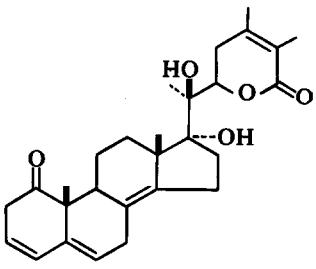
withanolide H



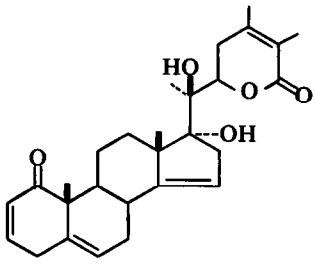
3
withanolide I



4
withanolide J



5
withanolide K



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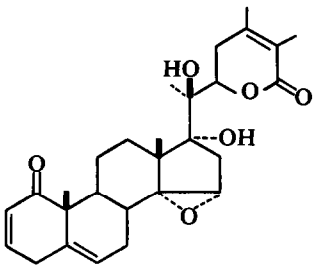
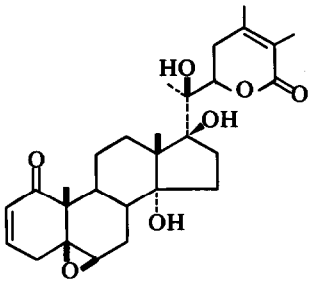
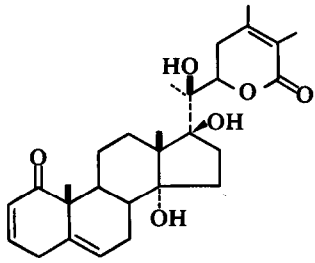
withanolide L



7
withanolide M



8
withanolide E



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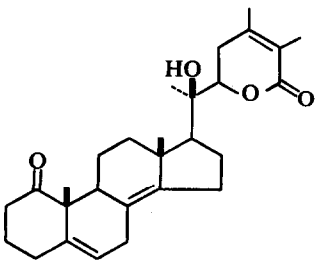
withanolide F



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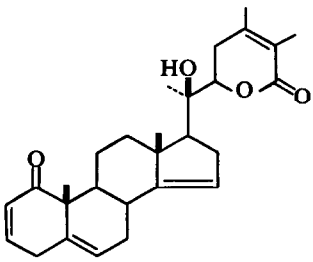

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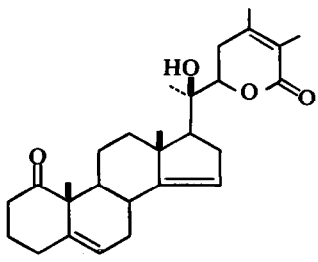

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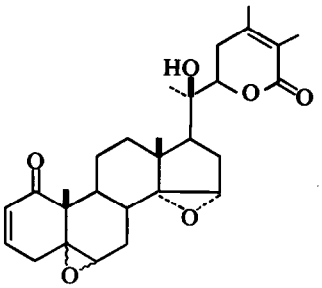
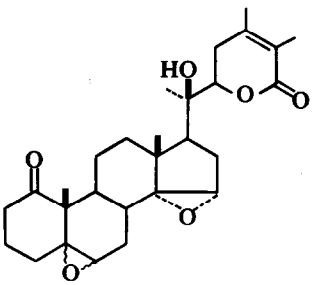
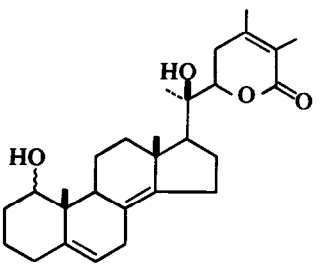


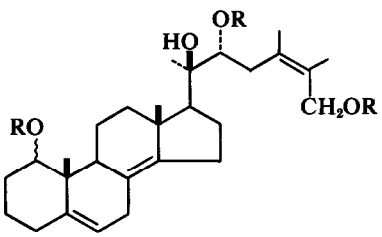
13: 5β6β, 14α15α
14: 5α6α, 14α15α



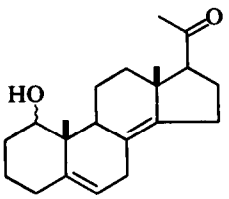
15: 5β6β, 14α15α
16: 5α6α, 14α15α



17: 1α-OH
18: 1β-OH



19: 1α-OH
20: 1β-OH



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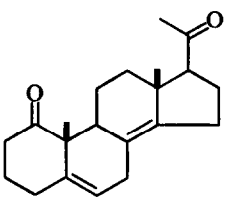
22: 1β-OH



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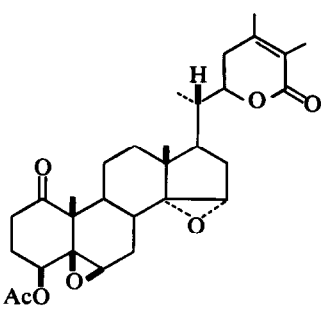

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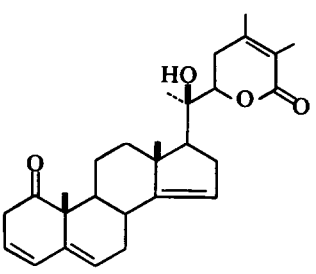

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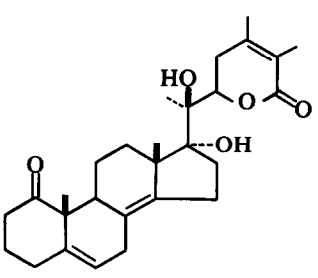

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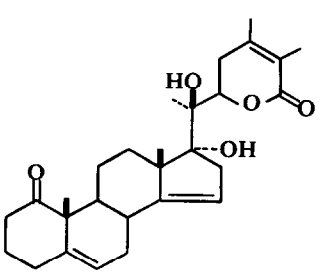

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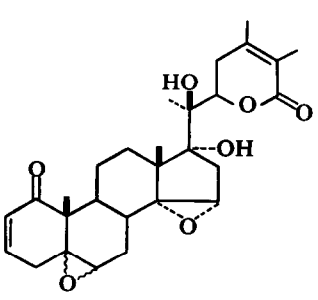
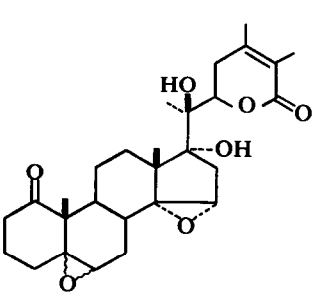
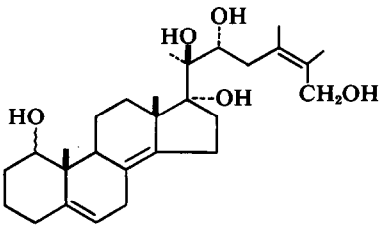


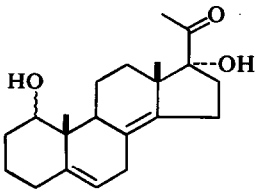
28: 5β6β, 14α15α
29: 5α6α, 14α15α



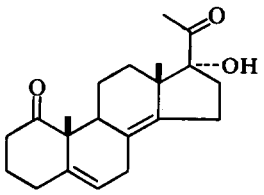
30: 5β6β, 14α15α
31: 5α6α, 14α15α



32: 1α-OH + 1β-OH



33: 1α-OH
34: 1β-OH



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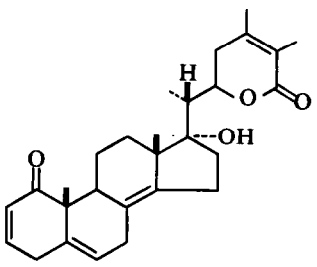


36

a: R = H
b: R = Ac

5β,6β-epoxide being the major component. The selective formation of the 14α,15α epoxide is in line with the course of this reaction in 17β-substituted steroids, the β configuration of the 15-H in all these compounds (**13–16**) being confirmed by its NMR signal, which is practically a singlet.[7]

The configurational assignments of the 5,6-epoxides are based on the following data: the signals of the 6-H in the 5β,6β-epoxides (**13** and **15**) are by ~ 0·12 ppm at lower field than in the corresponding 5α,6α-epoxides (**14** and **16**); the solvent shift $\Delta^{CDCl_3}_{C_6H_6}$ of the 19-Me is negative (−5 Hz) in **15** and positive (+11 Hz) in **16**. Ultimately, the Cotton effects of the Δ²-1-one in **13** and **14** and of the 1-one in **15** and **16** are consistent with the assigned structures.

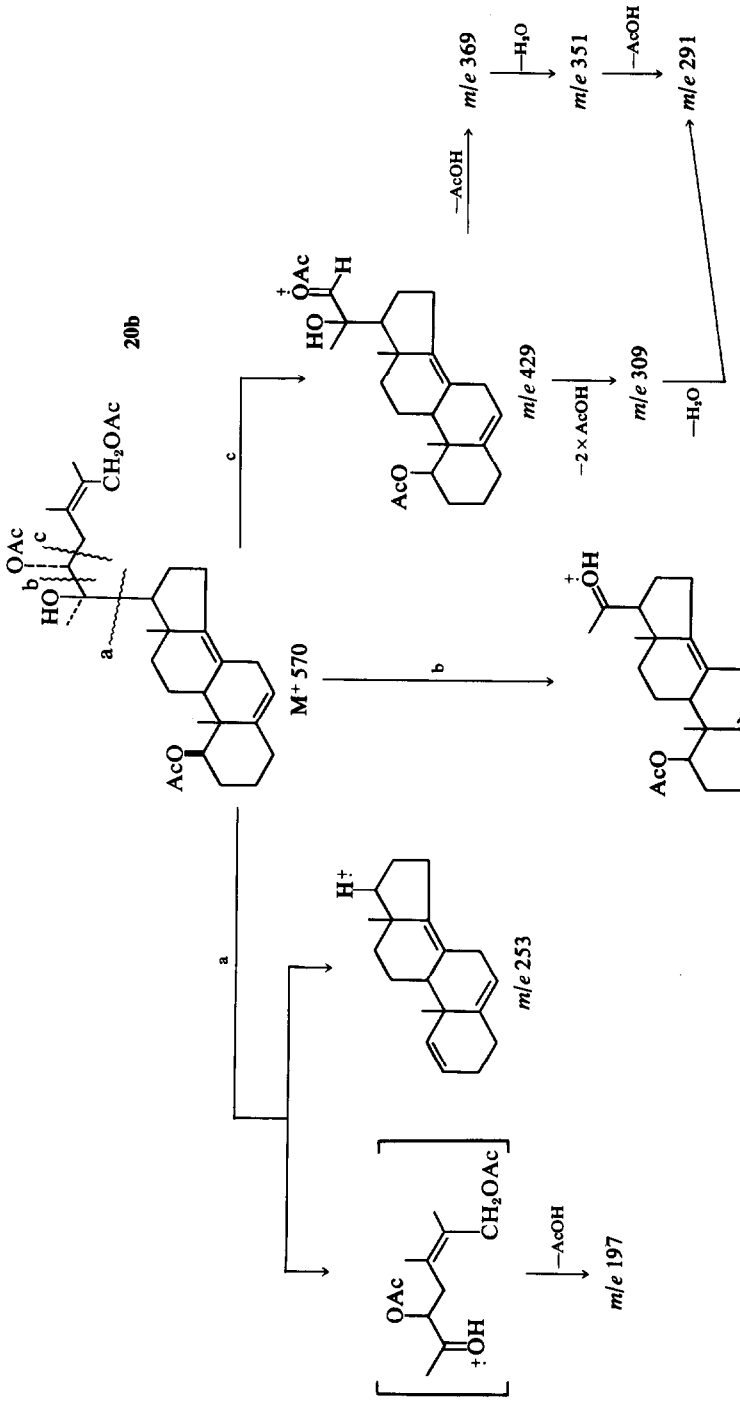
In order to confirm in a chemical way the presence of a C-20 OH in **1**, its degradation to a pregnan-20-one derivative was undertaken. Reduction of **10** with $NaBH_4$ resulted in a 7:3 mixture of the 1α-OH and 1β-OH derivatives, **17** and **18**, respectively. The configurational assignments at C-1 are based on the position and pattern of the NMR signal of 1-H, narrow triplet δ 3·75 ($W_{\frac{1}{2}}$ 5 Hz) for **17** and broad multiplet δ 3·41 ($W_{\frac{1}{2}}$ 17 Hz) for **18**, in agreement with an equatorial and axial orientation respectively of this proton. The pyridine induced shifts $\Delta^{CDCl_3}_{C_5D_5N}$ of the 19-Me in these compounds support very well the above assignments: in **17** the downfield shift is negligible (−0·05 ppm) as expected for a *trans* diaxial relationship between the 1α-OH and the 19-Me, whereas the *cis* relation between the 1β-OH and the same Me group in **18** leads to a very significant shift (−0·30 ppm). This reaction contrasts markedly by its nonstereospecificity with the $NaBH_4$ reduction of a 1-one in a 5α-steroid, leading exclusively to the 1α-OH derivative.

Table 2. Cotton effects of the Δ²-1-one and of the 1-one chromophores

Compound	λ_{max} (nm)	Δε
13	345	+0·96
14	335	−0·74
15	294	−3·32
16	291	+0·67 (shoulder)

Following this preliminary study of the course of the hydride reduction of the 1-one in the present series, compound **10** was treated with LAH; the reaction took place with the concomitant reduction of the 1-one and the reductive opening of the side chain lactone to give the isomeric tetrols **19** and **20** (1:1 ratio). Their identification was based on the NMR spectra, as well as on the fragmentation under electron impact of the triacetate **20b**, as shown in scheme (a) (the very abundant fragments obtained by simple elimination of AcOH and/or H_2O are omitted).

Oxidation of each of the tetrols with $NaIO_4$ in water-dioxane solution afforded the isomeric 1-hydroxypregna-5,8(14)-dien-20-ones (**21** and **22**), characterized by the appearance of the $COCH_3$ signal at δ 2·12. Mild oxidation of both products afforded the same diketone **23**.



SCHEME (a). Significant fragments obtained from **20b** under electron impact.

Of all the functional groups present in the original compound 1, only the tetrasubstituted double bond was assigned in an indirect way, the choice remaining between positions 8-9 and 8-14. Assignment at the latter position is based on the chemical shifts of the angular Me groups. Although there is no perfect agreement with the values calculated according to the increments given by Zürcher,[8] the same calculations performed for the 8-9 assumption (in both the 14α- and 14β-H alternative) give quite different results; one has also to consider that Zürcher's calculations are based on experimental data obtained mainly with 5β-steroids. A few examples of such comparisons are supplied.

		19-H	18-H
21	Found	1·00	0·76
	Calcd for $\Delta^{8(14)}$	0·92	0·81
	Calcd for Δ^{8} (14αH)	1·16	0·58
23	Found	1·30	0·78
	Calcd for $\Delta^{8(14)}$	1·28	0·84
	Calcd for Δ^{8} (14αH)	1·51	0·58
	Calcd for Δ^{8} (14βH)	1·49	0·88

The last problem which remained to be solved was the stereochemistry at the asymmetric centers C-17, C-20 and C-22. The configuration at C-17 was determined by comparing the CD and ORD curves of **22** with those of 3β-hydroxypregn-5-en-20-one (Table 3). For the orientation of the 20-OH the NMR criteria successfully applied for the same purpose in 20α- and 20β-hydroxycholesterol[9] and subsequently for the configurational assignment at C-20 in withanolide D[5] were used. To this end, compound **15** was compared with a derivative (**24**) of 27-desoxy-14α-hydroxywithaferin[7b] (20S, no hydroxyl at this carbon). The difference between the chemical shifts (chloroform solution) of the 21-Me groups is −0·26 ppm (δ 0·99 in **24**; δ 1·25 in **25**); this value is in good agreement with a 20α-OH configuration in **15** and implicitly at **1**. CD was used to determine the stereochemistry at C-22[10]; compound **1** shows a positive Cotton effect at 250 nm (Δε + 3·7), indicative for the 22R configuration. These data complete the structure of withanolide G (**1**).

Withanolide H, 20α,27-dihydroxy-1-oxo-20R, 22R-witha-2,5,8(14),24-tetraenolide (**2**), $C_{28}H_{36}O_5$, is one of the minor constituents of the steroidal lactones mixture. The NMR spectrum of its acetate is similar to that of **1**, with the exception of the signals of the lactone substituents, resembling those of withaferin A diacetate: only one vinylic Me group at δ 2·06 (or 2·08) and a CH_2OAc signal at δ 4·90. The catalytic hydrogenation (Pd-$CaCO_3$) of **2b** takes place with reduction of the Δ^2 and hydrogenolysis of the 27-allylic acetate to yield a dihydro-deoxyderivative (**10**) identical with the dihydro-derivative of **1**.

Withanolide I, 20α-hydroxy-1-oxo-20R,22R-witha-3,5,8(14),24-tetraenolide (**3**), is isomeric with **1**, differing only in the position of the ring A double bond; the CO band at 1694 cm^{-1} accounting for both the ketone and the unsaturated lactone, and more specifically the Cotton effect (308·5 nm, Δε −1·00) suggest that the ring A enone in this compound is not αβ-conjugated. The compound possesses an intense UV band at 232 nm (ε 24,000), due to the overlap of the unsaturated lactone chromophore with that of the $\Delta^{3,5}$ heteroannular diene.

The NMR of **3** exhibits three vinylic protons; in acidic conditions the compound is easily isomerized to **25** characterized by the appearance of a fourth signal for a vinylic proton. This change is explained by a double bond migration ($\Delta^{8(14)} \rightarrow \Delta^{14}$) similar to that occurring in the conversion of **1** into **11**. Compounds **1** and **3** could be interrelated by prolonged treatment with HCl in dry chloroform of the former, leading to the out of conjugation migration of the ring A double bond ($\Delta^2 \rightarrow \Delta^3$):

$$\mathbf{1} \xrightarrow{\text{HCl, 1 hr}} \mathbf{11}$$

$$\downarrow \text{HCl, 24 hr}$$

$$\mathbf{3} \xrightarrow{\text{HCl, 1 hr}} \mathbf{25}$$

A similar reaction has been recently reported for physalin B.[11] To discard an eventual $\Delta^{4,6}$ arrangement of the heteroannular diene, the vinylic region of the NMR of **3** was compared with that of a known $\Delta^{4,6}$-diene obtained in the course of the degradation of another withanolide.[1] The differences between their spectra were significant enough to support the alternative $\Delta^{3,5}$ assignment for **3**.

Withanolide J, 17α,20α-dihydroxy-1-oxo-20S, 22R-witha-2,5,8(14),24-tetraenolide (**4**), $C_{28}H_{36}O_5$, possesses one tertiary OH group more than **1**; actually, the presence of two OH groups was disclosed by esterification with trichloroacetyl isocyanate[12] resulting in the fast appearance of a two proton signal at δ 6·56 for the trichloroacetylcarbamate protons. Comparison of the UV, IR and NMR spectra of **1** and **4** revealed their close similarity, the only significant difference refers to the 22-H NMR signal which shows the same double doublet pattern as in **1**, however, it is appreciably shifted downfield (δ 4·62), suggesting that the second OH should be located in the proximity of the 22-H.

The mass spectrum of **4** exhibits the well known[13] fragmentation pattern of the side chain characterized by the peaks *m/e* 125 and 289 (cleavage of the C(20)–C(22) and C(17)–C(20) bonds). Among the peaks corresponding to the carbocyclic part of the molecule, the *m/e* 327 and 283 signals are by 16 m.u. higher than the corresponding peaks in **1**. It can be concluded therefore that the additional tertiary OH is located on the steroidal framework, in the proximity of the side chain.

To assess by chemical means the above struc-

tural indications a series of reactions paralleling those performed on **1** was undertaken. Catalytic hydrogenation in the presence of Pd-$CaCO_3$ afforded the 2,3-dihydro derivative **26** whereas the use of Pd-C as catalyst was accompanied by the concomitant migration of the $\Delta^{8(14)}$ to Δ^{14} (**27**). The isomerization alone could be induced by treatment of either **4** or **26** with HCl in chloroform over a short period of time, leading to **6** and **27**, respectively. The isomerization product of **6** was identical with one of the minor components of the crude extract.

Epoxidation with peracid of **6** and **27** led to the pairs of epoxides **28**, **29** and **30**, **31**, respectively, the epoxidation of Δ^{14} being again stereospecific, whereas the Δ^{5} afforded a mixture of the two possible derivatives.

The parallelism of the reactions as well as the close values recorded for the angular Me group signals in the corresponding derivatives of **1** and **4** imply a similar distribution of the double bonds. There are accordingly only two possible assignments for the second tertiary OH group, C-9 or C-17. The latter is favoured since there is no reason for a 9-OH group to exert such a strong deshielding on the 22-H signal without affecting the signals of other protons in the molecule.

The demonstration of the 17α assignment of this group is based on the following reactions. LAH reduction of **26** afforded a mixture of two pentols (**32**) isomeric at C-1, which was directly submitted to cleavage with $NaIO_4$, to yield the two corresponding pregnan-20-one derivatives **33** and **34** which were separated by chromatography. Comparison of the CD and ORD curves of **34** with those of 3β,17α-dihydroxypregn-5-en-20-one was conclusive for the location and orientation of the OH group (17α-OH) and thereby for the 17β orientation of the side chain. Chromium trioxide oxidation of the mixture of 1-ols (**33** and **34**) afforded the 17α-hydroxypregna-5,8(14)-diene-1,20-dione (**35**).

In a previous publication in this series[1] dealing with the structure of another withanolide possessing a 17α-OH group (5α,17α-dihydroxy-1-oxo-6α,7α-epoxy-22R-witha-2,24-dienolide), a conformational analysis was done by NMR to ascertain the orientation of this OH.

The same analysis, taking advantage of the shifts induced by pyridine on the 18-H, 21-H and 22-H signals, was now applied for compound **4**. The Newman projection along the C(17)–C(20) bond of this compound illustrates the existing relationship between the 22-H and the 17α-OH, responsible for the 0·41 ppm downfield shift of the former, as compared to its position in **1**. Even more so, the pyridine induced shift $\Delta^{CDCl_3}_{C_5D_5N}$ of the 22-H in **4** is −0·33 ppm, as compared to −0·16 ppm in **1**, the difference being due to the presence of the 17α-OH. In this connection it can be added that the pyridine molecule, which acts by H-bonding with OH groups, may induce a distortion of adjacent bonds of the solute molecule; this manifests itself by a change in pattern of the 22-H signal, from a double doublet (δ 4·62, J 10·5; 5·5 Hz) in $CDCl_3$ solution, to a triplet (δ 4·95, J 8 Hz) in C_5D_5N solution. No such changes of pattern are observed with compound **1**.

The stereochemistry at the asymmetric carbons 20 and 22 rests on arguments similar to those used for **1**. The difference between the chemical shifts of the 21-Me in **4** (δ 1·31) and the corresponding signal

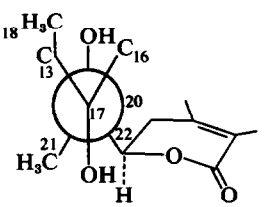


Table 3. CD and optical rotatory dispersion curves of the 20-one chromophore in several compounds (methanol solution)

Compound	CD		ORD		
	λ_{max}nm	$\Delta\epsilon$	λ_{max}nm	[Φ]	*a*
3β-Hydroxypregn-5-en-20-one	287·5	+3·00	307·5	+2356	+5657[b]
	294*[a]	+3·59	262·5	−3301	
22	285·8	+4·03	305·5	+2610	+5550
			263·0	−2940	
3β,17α-Dihydroxy-pregn-5-en-20-one	298·2	+2·83	320·0	+1374	+3819[b]
	303*[a]	+2·29	275·0	−2445	
34	296·6	+3·85	316·0	+2030	+4910
			270·0	−2880	

*Dioxane solution.

[a]L. Velluz, M. Legrand, M. Grosjean *Optical circular dichroism principles, measurement and applications*. Academic Press (1965).

[b]C. Djerassi, O. Halpern, W. Halpern, O. Schindler and Ch. Tamm, *Helv. Chim. Acta* **41**, 250 (1958).

(δ 1·03) in a withanolide without the 20-OH, however possessing a 17α-OH [17α-hydroxy-1-oxo-20R,22R-witha-2,5,8(14),24-tetraenolide (**36**)[14]] is characteristic for the α-orientation of the 20-OH (20S). The positive Cotton effect at 253 nm (Δε + 3·64) is indicative for the 22R configuration.

Three other withanolides related to **4** were isolated in minute quantities from the crude extract. Withanolide K (**5**) is the 17α-OH analog of **3** and its structure is supported by the similarity of their spectral data (Experimental).

Withanolide L (**6**), which is assigned the 17α-20α-dihydroxy - 1 - oxo - witha - 2,5,14,24 - tetraenolide structure has been already interrelated with **4** by isomerization of the latter.

As for the last compound of this group, withanolide M (**7**) $C_{28}H_{36}O_6$, it exhibits a singlet at δ 3·58 characteristic for a C-15 epoxidic proton. Epoxidation with one mole of perbenzoic acid afforded a mixture of 5,6 epoxides identical with the mixture **28** and **29**. Compound **7** is therefore 17α,20α-dihydroxy-1-oxo-14α,15α-epoxy-20S,22R-witha-2,5,24-trienolide.

It is noteworthy that five of the seven naturally occurring steroids studied in the present work possess the $\Delta^{8(14)}$ double bond. This finding is in contrast with the scarceness of natural steroids, of plant or animal source, possessing such a double bond.[15]

EXPERIMENTAL

M.ps were taken on a Fisher-Johns apparatus. Optical rotations were recorded with an automatic Perkin-Elmer 141 polarimeter and refer to $CHCl_3$ solns. IR spectra were recorded on a Perkin-Elmer infracord model 137 spectrophotometer equipped with a NaCl prism and refer to $CHCl_3$ solns. UV spectra were recorded on a Cary 14 instrument (EtOH as solvent); NMR spectra were determined on Varian A-60 and HA-100 spectrometers for 5–10% solns in $CDCl_3$, containing TMS as internal standard. CD and ORD spectra were recorded by Mrs. Batia Romano with a Cary 60 spectropolarimeter. TLC were carried on chromatoplates of silica gel G (Merck) and spots were developed with iodine vapour. Preparative chromatoplates were prepared from silica gel PF_{254}-7747 (Merck). MS were taken by Mr. S. Gattegno and Mr. M. Greenberg with an Atlas CH4 instrument. Analyses were performed in the microanalytical laboratory of our Institute, under the direction of Mr. R. Heller.

Plant material. Withania somnifera, chemotype III, was collected around the village of Yavneh (southern coastal plane of Israel), and raised as well from seeds in a uniform nursery.

Isolation procedure. Crushed air-dried leaves (2 kg) were exhaustively extracted with MeOH; the extract was concentrated to a volume of *ca* 31, a similar volume of water was added and the mixture was extracted with hexane to remove chlorophyll and other pigments. The residual soln was then re-extracted with ether; the ethereal extracts were washed with water, dried (Na_2SO_4), and the solvent removed to leave a green residue (*ca* 20 g). This crude product was introduced at the top of a chromatographic column made up with silica gel H (Merck) (800 g); the column was eluted with mixtures of C_6H_6: EtOAc, 35 ml fractions being collected.

Fraction No.	Benzene-ethyl acetate	Compound	Amount
41–45	9:1	withanolide L (**6**)	29 mg
46–48	9:1	withanolide M (**7**)	32 mg
50–54	9:1	withanolide K (**5**)	27 mg
97–120	9:1	withanolide J (**4**)	300 mg
124–130	8·5:1·5	withanolide I (**3**)	40 mg
136–162	8·5:1·5	withanolide G (**1**)	450 mg
285–290	1:1	withanolide F (**9**)	40 mg
292–356	1:1	withanolide E (**8**)	10·5 g
360–407	1:1	withanolide H (**2**)	200 mg

Withanolide G (**1**), *20α-hydroxy-1-oxo-20R,22R-witha-2,5,8(14),24-tetraenolide*. M.p. 194–195° (EtOAc), $[\alpha]_D$ +52·5° (*c* 0·50); ν_{max} 1685 and 1692 cm^{-1}; λ_{max} 223 nm (ε 19,400); CD (*c* 0·49); 390 (0); 339 (−0·98); 250 (+3·76). (Found: C, 76·9; H, 8·4; M^+, 436. $C_{28}H_{36}O_4$ requires: C, 77·03; H, 8·31%; M. wt. 436·57.)

Withanolide H (**2**), *20α,27-dihydroxy-1-oxo-20R,22R-witha-2,5,8(14),24-tetraenolide*. The compound could not be induced to crystallize and was characterized as its monoacetate (**2b**), m.p. 141–142° (EtOAc), $[\alpha]_D$ +35·5° (*c* 0·5); ν_{max} 1689 and 1730 cm^{-1}; λ_{max} 220 nm (ε 17,200). (Found: M^+ 494 . $C_{30}H_{38}O_6$ requires M. wt. 494·60.)

Withanolide I (**3**), *20α-hydroxy-1-oxo-20R,22R-witha-3,5,8(14),24-tetraenolide*. M.p. 184° (EtOAc), $[\alpha]_D$ +118° (*c* 0·3); ν_{max} 1706 cm^{-1}; λ_{max} 232 nm; CD (*c* 0·92); 362 (0), 308·5 (−0·92); 285i (0); 247 (+11·47); 231·7i (+6·00); 221 (0), 219·4i (−1·09); 209·4 (−6·96); 196·7 (0) positive at shorter wavelengths. (Found: M^+, 436. $C_{28}H_{36}O_4$ requires: M. wt. 436·57.)

Withanolide J (**4**), *20α,17α-dihydroxy-1-oxo-20S,22R-witha-2,5,8(14),24-tetraenolide*. M.p. 215–216° ($CHCl_3$-EtOAc), $[\alpha]_D$ + 32·7° (*c* 0·65); ν_{max} 1685 and 1692 cm^{-1}; λ_{max} 224 nm (ε 18,000); CD (*c* 0·45): 391 (0), 338·5 (−0·95), 252 (+3·82). (Found: C, 74·27; H, 8·12; M^+, 452. $C_{28}H_{36}O_5$ requires: C, 74·30; H, 8·02%; M. wt. 452·57.)

Withanolide K (**5**), *20α,17α-dihydroxy-1-oxo-20S,22R-witha-3,5,8(14)-tetraenolide*. M.p. 218–219° ($CHCl_3$–EtOAc), $[\alpha]_D$ +92° (*c* 0·30); ν_{max} 1706 cm^{-1}; λ_{max} 231 nm (ε 25,000) CD (*c* 0·36): 360 (0); 308·8 (−1·00); 286i (0); 248 (+11·40), 232·5i (+6·02), 220 (0), 211 (−6·26), 197 (0), positive at shorter wavelengths (Found: M^+ 452. $C_{28}H_{36}O_5$ requires: M. wt. 452·60).

Withanolide L (**6**), *20α,17α-dihydroxy-1-oxo-20S,22R-witha-2,5,14,24-tetraenolide*. M.p. 213° (EtOAc), $[\alpha]_D$ +9·6°; ν_{max} 1698 cm^{-1}; λ_{max} 220 nm (sh) (ε 18,600) and strong end absorption. (Found: M^- 452. $C_{28}H_{36}O_5$ requires M. wt. 452·57.)

Withanolide M (**7**), *20α,17α-dihydroxy-14α,15α-epoxy-20S,22R-witha-2,5,24-trienolide*. M.p. 240° (EtOAc); $[\alpha]_D$ +44·6°; ν_{max} 1692 cm^{-1}; λ_{max} 225 nm (ε 19,200). (Found: M^+ 468. $C_{28}H_{36}O_6$ requires: M. wt. 468·57.)

The physical constants of withanolide E (**8**) and F (**9**) will be given in a detailed paper dealing with their structures.

Hydrogenation of compound **1** *to* **10**. Compound **1** (150 mg) in abs EtOH (200 ml) was hydrogenated over 10% Pd-$CaCO_3$ at room temp and atm pressure. The reaction was discontinued after the absorption of one molar equivalent of H_2 and the product was crystallised

from EtOAc (150 mg), m.p. 202°, $[\alpha]_D$ +42·5° (*c* 0·5); ν_{max} 1704 cm^{-1}; λ_{max} 224 nm; CD (*c* 0·42): 340 (0); 302 (−2·56), 250 (+3·76); ~200 (+14·0). (Found: M^+ 438. $C_{28}H_{38}O_4$ requires: M. wt. 438·58.)

Isomerisation of **1** *to give* **11**. Dry HCl was bubbled for 1 hr at 0° through a soln of **1** (100 mg) in $CHCl_3$ (20 ml). The solvent was removed in vacuum, the residue dissolved in ether, shaken with 2% $NaHCO_3$aq, washed with water and dried over Na_2SO_4. The ether was evaporated and the residue chromatographed on silica gel; elution with $CHCl_3$ yielded **11** (92 mg), m.p. 184–185°; $[\alpha]_D$ +7·3° (*c* 0·2); ν_{max} 1695 and 1681 cm^{-1}; λ_{max} 220 nm (sh) (ϵ 18,500) and strong end absorption (Found: M^+ 436. $C_{28}H_{36}O_4$ requires: M. wt. 436·57).

The isomerisation was also accomplished with a few drops of 8N H_2SO_4 added to an acetone soln of **1**

Isomerisation of **10** *to* **12**. The isomerization was performed with HCl as described above. The product **12** (95 mg) could not crystallize but showed one spot on a chromatoplate, $[\alpha]_D$ +50·5° (*c* 0·18); ν_{max} 1695 cm^{-1}; λ_{max} 224 nm (sh) (ϵ 10,000) and strong end absorption. (Found: M^+ 438. $C_{28}H_{38}O_4$ requires: M. wt. 438·58.)

Compound **12** was also obtained by catalytic hydrogenation of **1** over 10% Pd-C.

Epoxidation of **11** *to* **13** *and* **14**. *m*-Chloroperbenzoic acid (0·30 mmole) was added to a soln of **11** (60 mg, 0·137 mmole) in C_6H_6–$CHCl_3$ soln (5:1) (20 ml); the mixture was kept for *ca* 24 hr at room temp, then washed with dil $NaHCO_3$aq and water, dried (Na_2SO_4) and the solvent evaporated. The residue (60 mg) which showed two spots on a chromatoplate (EtOAc–C_6H_6, 7:3), was resolved by thick-layer chromatography on plates of 40 cm length, using the same solvent system. The upper band afforded, upon extraction with $CHCl_3$–MeOH (8:2) 44 mg of **13** m.p. 239–240° (EtOAc); $[\alpha]_D$ +88·5° (*c* 0·25); ν_{max} 1695 cm^{-1}; λ_{max} 226 nm (ϵ 18,500); CD (*c* 0·61): 380 (0), 356i (+0·86), 345 (+0·96), 315 (0), 300 (−0·56), 278 (0), 251 (+3·86). (Found: M^+ 468. $C_{28}H_{36}O_6$ requires: M. wt. 468·57). The lower band yielded 13 mg of **14** m.p. 217° (acetone–hexane); $[\alpha]_D$ +100° (*c* 0·10); ν_{max} 1689 cm^{-1} λ_{max} 226 nm (ϵ 18,000); CD (*c* 0·91): 368 (0), 335 (−0·74), 303i (−0·16), 290 (0), 250 (+3·77). (Found: M^- 468. $C_{28}H_{36}O_6$ requires: M. wt. 468·57.)

Epoxidation of **12** *to* **15** *and* **16**. The reaction was performed with compound **12** (60 mg) as described for **11**. Thick layer chromatography afforded **15** (42 mg) and **16** (13 mg). Compound **15**, m.p. 245–246° (EtOAc), $[\alpha]_D$ −7·3° (*c* 0·14); ν_{max} 1709, 1695 cm^{-1}; λ_{max} 225 nm (ϵ 9000). CD (*c* 0·42): 330 (0), 294 (−3·37), 250 (+4·94), ~200 (+12·6). (Found: C, 71·3; H, 8·1%; M^+ 470. $C_{28}H_{38}O_6$ requires: C, 71·46; H, 8·14%; M, 470·58). Compound **16**, m.p. 221–222° (acetone-hexane), $[\alpha]_D$ +14° (*c* 0·10), ν_{max} 1707, 1698 cm^{-1}, λ_{max} 225 nm (ϵ 8,900). CD (*c* 0·98) 329 (0), 291i (+0·67), 251 (+4·39), ~200 (+10·1). (Found: M^+ 470. $C_{28}H_{38}O_6$ requires: M. wt. 470·58.)

$NaBH_4$ *Reduction of* **10** *to give* **17** *and* **18**. $NaBH_4$ (60 mg) was added to a soln of **10** (50 mg) in MeOH (100 ml) and the mixture was stirred for 2 hr at room temp. The solvent was then evaporated, the product (50 mg) isolated with $CHCl_3$ and separated by thick layer chromatography (C_6H_6–EtOAc, 3:7). Compound **17** (32 mg), m.p. 219° ($CHCl_3$–EtOAc); $[\alpha]_D$ +44·5° (*c* 0·5); ν_{max} 1695 cm^{-1}, λ_{max} 225 nm (ϵ 9500). (Found: M^+ 440 $C_{28}H_{40}O_4$ requires: M. wt. 440·65). Compound **18** (14 mg), m.p. 229° (EtOAc); $[\alpha]_D$ +69·5°; ν_{max} 1695 cm^{-1}; λ_{max} 225 nm (ϵ 9700). CD (*c* 0·47): 303 (0), 251 (+4·03). (Found: M^+ 440. $C_{28}H_{40}O_4$ requires: M. wt. 440·65.)

LAH *reduction of* **10** *to give* **19a** *and* **20a**. To a slurry of LAH (2·0 g) in dry THF (250 ml), a soln of **10** (500 mg) in 50 ml of the same solvent was added dropwise. The stirred soln was heated to reflux for 16 hr. The excess reducing agent was destroyed by the dropwise addition of EtOAc followed by sat Na_2SO_4aq to the ice-cooled mixture. The soln was then thoroughly extracted with ether, the combined extracts washed with water, dried over Na_2SO_4 and distilled under vacuum. The residue (495 mg) (no IR absorption in the CO region) was chromatographed on silica gel H (Merck) (150 g). Elution with C_6H_6–EtOAc (1:1) yielded first **19a** and then **20a**. Compound **19a** (130 mg) m.p. 162–163° (EtOAc); $[\alpha]_D$ (MeOH) 0° (*c* 0·5); ν_{max} 3300 cm^{-1}; UV end absorption. (Found: M^+ 444. $C_{28}H_{44}O_4$ requires: M. wt. 444·63). Compound **20a** (115 mg), m.p. 171–172° (EtOAc); $[\alpha]_D$ (MeOH) +2° (*c* 0·5); ν_{max} 3300 cm^{-1}; UV end absorption (Found: M^+ 444. $C_{28}H_{44}O_4$ requires: M. wt. 444·63.)

Acetylation of **20a**. The acetate (**20b**) (14 mg) was obtained by treatment of **20a** (15 mg) with Ac_2O (0·2 ml) and pyridine (0·2 ml) overnight at room temp, crystallised from acetone-hexane, m.p. 132–133° $[\alpha]_D$ −7·7° (*c* 0·92); ν_{max} 1738 and 1733 cm^{-1}. (Found: M^+ 570. $C_{34}H_{50}O_7$ requires: M. wt. 570·74.)

Periodate oxidation of **19** *and* **20** *to give* **21** *and* **22**. To a soln of **19** (100 mg) in dioxane (50 ml) an aq soln of sodium *meta*-periodate (150 mg in 10 ml water) was added, and left overnight at room temp. The excess oxidizing reagent was destroyed with ethylene glycol (1 ml); most of the dioxane was distilled under vacuum, water (50 ml) was then added, the product extracted with ether and chromatographed on silica gel; elution with $CHCl_3$ yielded **21** (56 mg), m.p. 192° (EtOAc); $[\alpha]_D$ +48·5° (*c* 0·32); ν_{max} 1700 cm^{-1} (Found: M^+ 314. $C_{21}H_{30}O_2$ requires: M. wt. 314·45. A similar treatment of **20** (100 mg) afforded **22** (62 mg), m.p. 198° (EtOAc); $[\alpha]_D$ +63·5° (*c* 0·32). CD (CH_3OH, *c*, 0·85): 340 (0), 285·8 (+4·03), 235 (0), ~200 (+15·7). ORD (MeOH, *c* 0·85): $[\phi]_{305·5}$ (+2610°), $[\phi]_{263}$ (−2940°). (Found: M^+ 314. $C_{21}H_{30}O_2$ requires: M. wt. 314·45.)

CrO_3-*Pyridine complex oxidation of* **21** *and* **22** *to give* **23**. Freshly prepared CrO_3 (pyridine)$_2$ complex[16] (49 mg) (0·2 mmol) was added to a magnetically stirred soln of **21** (20 mg; 0·06 mmol) in acetone (10 ml). After 10 hr of stirring at room temp the solvent was removed in vacuum, the residue worked up to give **23** (17 mg), purified by chromatography on silica gel, m.p. 168–169° (abs EtOH); $[\alpha]_D$ +7·42 (*c* 0·30), ν_{max} 1706 and 1701 cm^{-1}. (Found: M^+, 312 $C_{21}H_{28}O_2$ requires: M. wt. 312·44). Oxidation of **22** (20 mg) under the same conditions gave again **23**.

Hydrogenation of **2b** *to give* **10**. The reaction was done as described for **1**, over Pd–$CaCO_3$; following the absorption of two molar equivalents of H_2 the product was chromatographed on a thick-layer chromatoplate and isolated with $CHCl_3$. It was identified as **10** by direct comparison with a sample.

Isomerisation of **1** *to give* **25**. Dry HCl was bubbled for 24 hr at 0° through a soln of **1** (100 mg) in $CHCl_3$ (20 ml). Chromatography on silica gel, elution with $CHCl_3$–C_6H_6 (1:1), yielded **25** (70 mg), m.p. 181° (EtOAc); $[\alpha]_D$ +53·7° (*c* 0·36); ν_{max} 1701 and 1689 cm^{-1}; λ_{max} 228 nm (sh) (ϵ 24,500). (Found: M^+, 436. $C_{28}H_{36}O_4$ requires: M. wt. 436·57.)

Isomerisation of **3** *to give* **25**. The isomerisation was performed as above, for 1 hr. The product was identified as **25** by direct comparison.

Hydrogenation of **4** *to give* **26**. Compound **4** (100 mg) in abs EtOH was hydrogenated over 10% Pd–$CaCO_3$. The reaction was discontinued after the absorption of one molar equivalent of H_2. The product **26** crystallised from acetone, m.p. 240–242° (98 mg), $[\alpha]_D$ +32° (*c* 0·97); ν_{max} 1704 cm^{-1}; λ_{max} 224 nm (ϵ 10,500) and end absorption. CD (*c* 0·50): 340 (0), 303 (− 2·56), 253 (+3·64), ~200 (+11·5). (Found: C, 73·90; H, 8·35; M^+ 454. $C_{28}H_{38}O_5$ requires: C, 73·98; H, 8·43%; M. wt. 454·58). When 10% Pd–C was used the isomeric compound **27** was obtained.

Isomerisation of **4** *to give* **6**. A few drops of 8N H_2SO_4 were added to an acetone soln (100 ml) of **4** (100 mg), the soln stirred for 15 hr at room temp, then neutralised with dil $NaHCO_3$, the solvent removed and the product isolated with $CHCl_3$. Chromatography on silica gel H afforded **6** identified by direct comparison with the natural product.

Isomerisation of **26** *to give* **27**. Compound **26** was submitted to the same reaction as described for the conversion **4** → **6**, to yield **27**, m.p. 257°; $[\alpha]_D$ −34·6° (*c* 0·41); ν_{max} 1706 and 1695 cm^{-1}; λ_{max} 220 nm (sh) (ϵ 10,000) and strong end absorption. (Found: M^+, 454. $C_{28}H_{38}O_5$ requires: M. wt. 454·58.)

Epoxidation of **6** *to give* **28** *and* **29**. A $CHCl_3$–C_6H_6 (3:1) soln (6 ml) of **6** (70 mg, 0·15 mmol) was treated with *meta* chloroperbenzoic acid (0·34 mmol). After stirring for 14 hr, the product was isolated in the usual manner. The crude epoxide (70 mg) showed two spots on a thin layer chromatoplate. Compounds **28** and **29** were separated by thick layer chromatography (C_6H_6–EtOAc 3:7). Compound **28** (49 mg) m.p. 267° (EtOAc); $[\alpha]_D$ +96° (*c* 0·25); ν_{max} 1695 cm^{-1}; λ_{max} 226 nm (ϵ 19,400). CD (*c* 0·56): 381 (0); 356i (+0·87); 346·5 (+1·01); 315 (0); 300 (−0·52); 276 (0); 255 (+3·91). (Found: M^+ 484. $C_{28}H_{36}O_7$ requires: M. wt. 484·57.) Compound **29**, (17 mg) m.p. 251–252° (acetone–hexane); $[\alpha]_D$ +109·5° (*c* 0·21); ν_{max} 1695 cm^{-1}; λ_{max} 225·5 nm (18,500). CD (*c* 0·57): 370 (0), 337 (−0·76), 302i (−0·19), 291 (0), 255 (+4·00). (Found: M^+ 484, $C_{28}H_{36}O_7$ requires: M. wt. 484·57.)

Epoxidation of **27** *to give* **30** *and* **31**. Compound **27** (50 mg; 0·11 mmol) was treated with *meta* chloroperbenzoic acid (0·25 mmol) overnight at room temp. The crude product was isolated as above to yield a mixture of **30** and **31**, separated by thick layer chromatography. Compound **30** (38 mg) m.p. 283–284° (EtOAc); $[\alpha]_D$ −24° (*c* 0·40); ν_{max} 1689 cm^{-1}; λ_{max} 224 nm (ϵ 8500). CD (*c* 0·45): 331 (0), 294 (−3·41), 255 (+5·01). (Found: C, 68·9; H, 8·0; M^+, 486). $C_{28}H_{38}O_7$ requires: C, 69·11; H, 7·87%; M. wt. 486·58). Compound **31** (11 mg) m.p. 267–268° (acetone); $[\alpha]_D$ −11°; ν_{max} 1692 cm^{-1}, λ_{max} 225 nm (ϵ 9000). CD (*c* 0·99): 331 (0), 289i (+0·65), 254 (+4·95). (Found: M^+ 486. $C_{28}H_{38}O_7$ requires: M. wt. 486·58.)

LAH *reduction of* **26** *to give the mixture* **32**. Compound **26** (500 mg) was reduced with LAH (2 g) in THF for 16 hr, as described above. The crude product was chromatographed on silica gel (elution with $CHCl_3$–EtOAc 7:3) but no separation could be obtained. The mixture (350 mg) did not show any carbonyl absorption in the IR).

Periodate oxidation of **32** *to give* **33** *and* **34**. The mixture (340 mg) was oxidized with sodium *meta* periodate (500 mg) as described above, and the crude product (200 mg) chromatographed on silica gel H (Merck). Elution with C_6H_6–EtOAc (7:3) yielded first **33** (88 mg) followed by **34** (93 mg). Compound **33** m.p. 204° ($CHCl_3$–EtOAc), $[\alpha]_D$ +36° (*c* 0·13); ν_{max} 1709 cm^{-1}. (Found: C, 76·2; H, 9·1, M^+ 330. $C_{21}H_{30}O_3$ requires: C, 76·32; H, 9·15%; M. wt. 330·45). Compound **34** m.p. 212° (EtOAc); $[\alpha]_D$ +22·6° (*c* 0·36); ν_{max} 1709 cm^{-1}. CD (MeOH) (*c* 0·70) 296·6 (+3·85), 235 (0), 221 (−0·31), ~200 (+10); ORD (MeOH) (*c*, 0·70), $[\phi]_{316}$ +2030°, $[\phi]_{270}$ −2880°. (Found: M^+ 330. $C_{21}H_{30}O_3$ requires: M. wt. 330·45).

CrO_3 *Pyridine complex oxidation of* **33** *and* **34** *to give* **35**. The reaction was done as described above. Oxidation of either **33** or **34** yielded the same product (**35**), m.p. 174–175° (acetone–hexane); $[\alpha]_D$ +59·2° (*c* 0·24) ν_{max} 1709 and 1701 cm^{-1}. (Found: M^+ 328. $C_{21}H_{28}O_3$ requires: M. wt. 328·44.)

Epoxidation of **2** *to give* **28** *and* **29**. A $CHCl_3$–C_6H_6 (1:1) solution (10 ml) of **7** (25 mg) was treated overnight at room temp with a slight excess of *meta* chloroperbenzoic acid. After the usual isolation procedure and chromatography two crystalline products were obtained (15 and 5 mg), identified by direct comparison as **28** and **29** respectively.

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