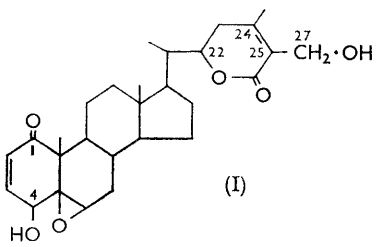
1371. *Constituents of* Withania somnifera *Dun. Part IV.*[1] *The Structure of Withaferin A*

By D. Lavie, E. Glotter, and Y. Shvo

Withaferin A, $C_{28}H_{38}O_6$, isolated along with its dihydro-derivative from the leaves of *Withania somnifera* Dun. (Solanaceae), has been shown to possess structure (I). The compound previously called withaferin has now been identified as 2,3-dihydro-3-methoxywithaferin A.

Withaferin A, $C_{28}H_{38}O_6$, is a crystalline compound which has been isolated [2] from the leaves of *Withania somnifera* Dun. (Solanaceae). In a previous Paper of this Series the functional groups of this compound were discussed and a partial structure was proposed.[1] From the data described there it could be inferred that withaferin A was identical with the " unsaturated lactone " isolated by Kurup,[3] and was given the empirical formula $C_{24}H_{36}O_6$.*

In this Paper we propose that withaferin A possesses the structure (I). The nature of the skeleton was arrived at after several selenium dehydrogenation experiments which led to the isolation of a derivative of cyclopentenophenanthrene and of a trimethylnaphthalene.[4] The dehydrogenation experiments were carried out on a product obtained by lithium aluminium hydride reduction of dihydrowithaferin A (IIa) and subsequent acetylation. Since only limited amounts of substance were available for these degradation experiments, the rather small quantities of the aromatic hydrocarbons, which were obtained from the chromatography of the reaction mixture, were identified spectroscopically.



(I)

The ultraviolet absorption spectrum of the phenanthrene derivative was found to be superimposable on that of 1,2-cyclopentenophenanthrene.[4] The n.m.r. spectrum of the above derivative was readily identified inasmuch as all the low-field signals could be related to the appropriate aromatic protons: one multiplet for two protons at δ 8·50, a second accounting for 4 protons between δ 7·20—7·80, and a sharp two-proton singlet at δ 7·60. The detection of only eight aromatic protons confirms that the phenanthrene derivative is disubstituted. The assignment of these various signals was done on the basis of the detailed study by Martin and co-workers on the n.m.r. spectra of aromatic polycyclic hydrocarbons.[5] Accordingly, the 8·50 multiplet is due to the two protons located at positions 4 and 5 (phenanthrene numbering), while the 7·60 singlet is related to the protons at positions 9 and 10; the phenanthrene nucleus should be substituted, therefore, at two of the remaining positions. In the high-field region of the spectrum, the signals of several methylenic protons were present, as well as a triplet due to a primary methyl group; these data, in conjunction with the ultraviolet absorption spectrum, identify the compound as an ethyl-1,2-cyclopentenophenanthrene. The isolation of this aromatic hydrocarbon indicates a perhydrocyclopentanophenanthrene skeleton in withaferin A, a fact well

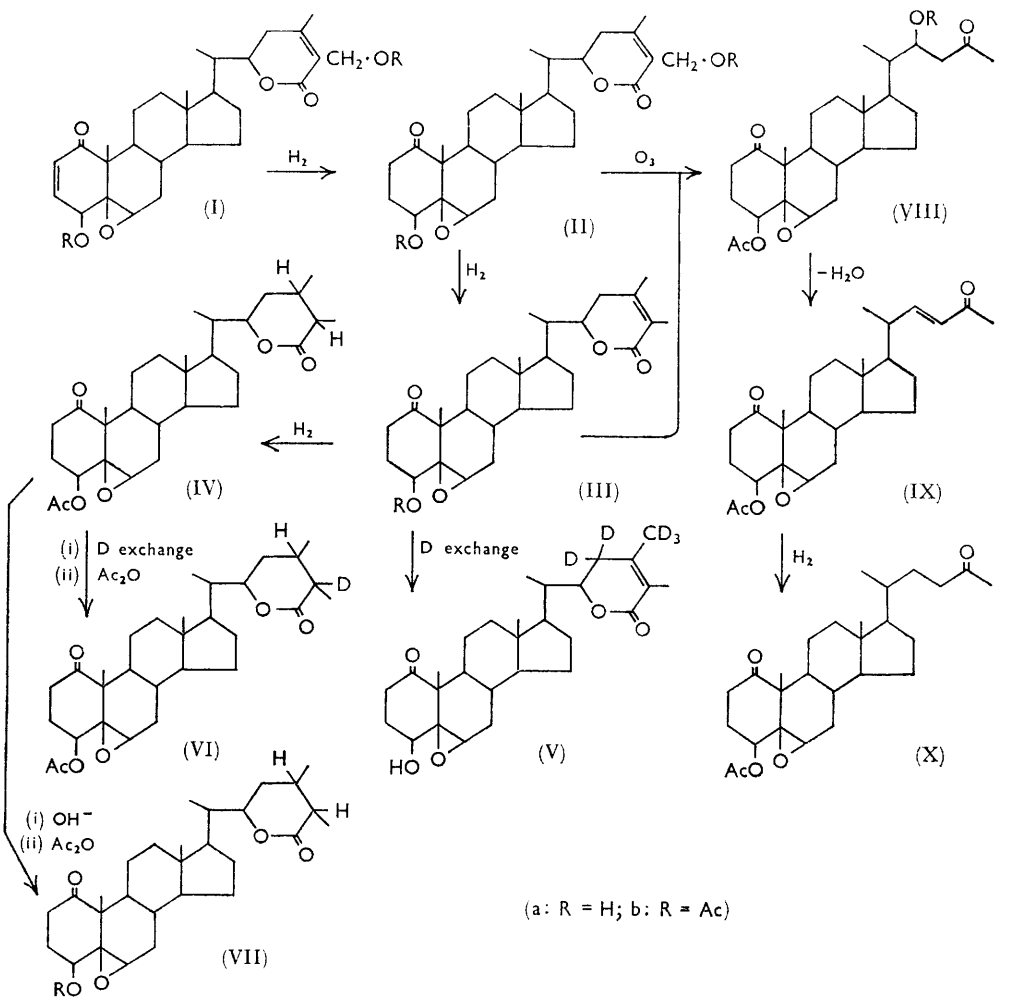
* While the substance described by Kurup had m. p. 157—159° (from ethanol–water), $[\alpha]_D$ +101·7°, $\lambda_{max.}$ 216 mμ and $\nu_{max.}$ 1690 cm.$^{-1}$, our substance melted at 243—245° from ethyl acetate, and possessed $[\alpha]_D$ +114°, $\lambda_{max.}$ 214 mμ and $\nu_{max.}$ 1692 cm.$^{-1}$. However if crystallised from ethanol–water the product melted as described by Kurup.[3]

[1] Part III, D. Lavie, E. Glotter, and Y. Shvo, *J. Org. Chem.*, 1965, **30**, 1774.
[2] D. Lavie and A. Yarden, *J.*, 1962, 2925.
[3] P. A. Kurup, *Current. Sci.*, 1956, **25**, 57; *Antibiotics and Chemotherapy*, 1958, **8**, 511; a similar compound has also been isolated by S. Kohlmuenzer and J. Krupinska, *Diss. Pharm.*, 1963, **14**, 501 (*Chem. Abs.*, 1963, **59**, 6866).
[4] J. W. Cook and C. L. Hewett, *J.*, 1933, 1098; E. Heilbronner, H. U. Däniker, and Pl. A. Plattner, *Helv. Chim. Acta*, 1949, **32**, 1723; E. Heilbronner, U. Fröhlicher, and Pl. A. Plattner, *ibid.*, p. 2479.
[5] R. H. Martin, *Tetrahedron*, 1964, **20**, 897; R. H. Martin, N. Defay, F. Geerts-Evrard, and S. Delavarenne, *ibid.*, p. 1073; R. H. Martin, N. Defay, F. Geerts-Evrard, and H. Figeys, *Bull. Soc. chim. belges*, 1964, **73**, 199.

supported by the isolation of a trisubstituted derivative of naphthalene formed by the cleavage of the highly oxygenated ring A.

A similar observation was made in the cucurbitacin series,[6] a group of tetracyclic triterpenes possessing two oxygen atoms substituted on ring A, the dehydrogenation of which yielded small amounts of a naphthalene derivative along with the main phenanthrenic hydrocarbon.

It will be useful to recapitulate briefly the work previously described [1] for withaferin A so as to provide the background for the present work. Stepwise hydrogenation of withaferin A led to the formation of dihydrowithaferin A (IIa),* and through hydrogenolysis of the primary hydroxyl group to deoxydihydrowithaferin A (IIIa); the hydrogenation of deoxydihydrowithaferin A acetate (IIIb) yielded the tetrahydro-derivative (IV).



The structure of the side-chain was conclusively demonstrated by a series of reactions: deuterium exchange undertaken on compound (IIIa) yielded a product (V) in which five

* Dihydrowithaferin A is identical with the naturally occurring compound previously described [2] as A_3.

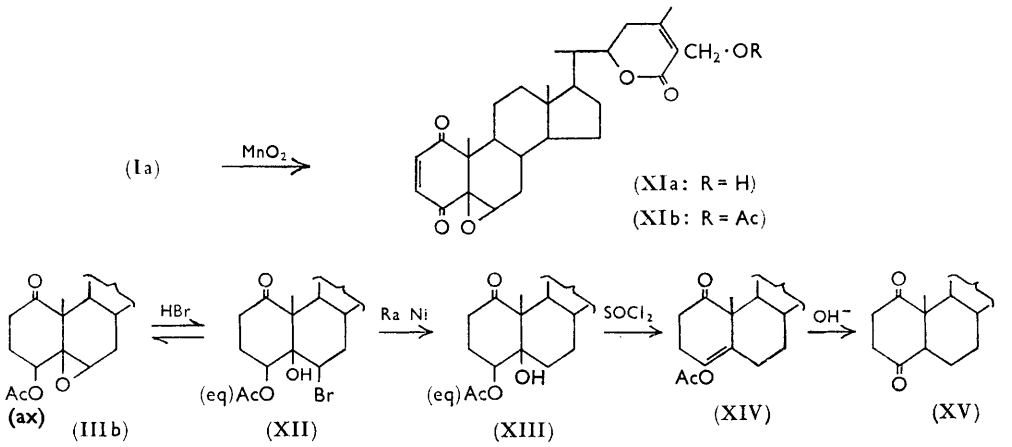
[6] D. Lavie, Y. Shvo, O. R. Gottlieb, and E. Glotter, *J. Org. Chem.*, 1962, **27**, 4546.

deuterium atoms were introduced, while when the exchange was performed on the tetrahydro-derivative (IV), only one hydrogen was exchanged [at C-25, compound (VI)]. During alkaline treatment of compound (IV) epimerisation occurred at C-25 as was shown by the formation of compound (VIIa).

The structure of the αβ-unsaturated lactone ring was confirmed by the ozonolysis of (IIIb) resulting in cleavage of the ring and production of the β-hydroxy-ketone (VIIIa), which, under extremely mild acidic conditions, afforded the unsaturated ketone (IX). The same hydroxy-ketone (VIIIa) was also obtained by ozonolysis of (IIb), thus establishing the position of the primary alcohol group at C-27 on the lactone ring of withaferin A.

The location of the secondary methyl group at C-20 was deduced from considerations concerning the fragmentation pattern in the mass spectra of a series of derivatives,* as well as by studying the position and the pattern of its n.m.r. signal in compounds (VIIIa), (VIIIb), (IX), and (X). Specifically, this signal appears in the unsaturated ketone (IX) as a doublet centred at δ 1·08, while reduction of the double bond to yield compound (X) induces a shift of the signal to δ 0·97, which appears now as a peak with a shoulder towards high-field. Compound (X) has now been fully characterised.

From the n.m.r. data, the presence of a Δ^2-1,4-oxo-hydroxy-system was inferred in withaferin A: the 2- and 3-protons gave rise to a doublet ($\delta = 6{\cdot}18$, $J_{2,3} = 10$ c./sec.) and a double doublet ($\delta = 6{\cdot}97$, $J_{3,2} = 10$ c./sec.; $J_{3,4} = 6$ c./sec.) respectively, while the 4-proton appeared as a doublet ($\delta = 3{\cdot}75$; $J_{4,3} = 6$ c./sec.) suggesting thereby the presence of one neighbouring proton only. Upon acetylation, the latter signal shifted to δ 4·66. The allylic position of the 4-hydroxyl group was ascertained by the oxidation of withaferin A with manganese dioxide [7] in excellent yields, to the enedione (XIa); this product was characterised as such and as its monoacetate (XIb): the ultraviolet absorption



band at 222 mμ is due to the overlapping of the 210-mμ band of the unsaturated lactone in the side-chain, and the 227-mμ band of the newly created 1,4-enedione system,[8] while in the n.m.r. spectrum, the 8-line pattern characteristic of the three protons at C-2, C-3, and C-4 in withaferin A disappears and a sharp singlet at δ 6·89, due to the two now equivalent protons at C-2 and C-3 is present. In a steroidal molecule there is only one possibility of rationalising the Δ^2-1,4-oxo-hydroxy-system in compound (I) as well as the homocisoid enedione in compound (XI), *i.e.*, by placing them in ring A. It is noteworthy that the rate

* A detailed study of the mass-spectral analysis of withaferin A and of several derivatives will be given in a forthcoming publication.

[7] J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen, and T. Walker, *J.*, 1952, 1094.

[8] A. I. Scott, "Interpretation of the Ultra-violet Spectra of Natural Products," Pergamon Press, London, 1964, p. 61.

Nuclear magnetic resonance line positions

Cpd.	2-H	3-H	4-H	6-H	22-H	23-H	10-Me	13-Me	20-Me	24-Me	25-Me	Other
(Ia)	6·18d (10)	6·97dd (10; 6)	3·75d (6)	3·20b	4·40dt		1·38	0·68	0·97d (6·5)	2·03		4·35A
(Ib)	6·24d (9·5)	7·04dd (9·5; 6)	4·66d (6)	3·22b	4·41dt (12·5)		1·38	0·70	0·99d (6·5)	2·04		4·87A 2·04B
(IIa)			3·55t (3·5)	3·18b	4·40dt		1·32	0·70	1·01d (6·8)	2·09		4·41A
(IIb)			4·62t (3·5)	3·18b	4·41dt (12)		1·32	0·70	1·01d (6·7)	2·04		4·88A 2·04B
(IIIa)			3·50t (3·5)	3·13b	4·37dt		1·30	0·67	0·99d (6·5)	1·93	1·93	
(IIIb)			4·62t (3·3)	3·18b	4·41dt (12)		1·29	0·69	1·00d (6·7)	1·95	1·95	2·06B
(IV)			4·57t	3·17b	4·22dt		1·27	0·66	0·92d (6·5)	1·13d (6·6)	0·92d (6·5)	2·03B
(V)			3·50t (3·5)	3·12b	4·37d (4)		1·30	0·67	0·98d (6·5)		1·87	
(VI)			4·58t	3·18b	4·37dt		1·28	0·67	0·90d (6·5)	1·07d (5·8)	1·30	2·05B
(VIIb)			4·58t	3·17b	4·37dt		1·28	0·67	0·91d (6·5)	1·08d (5·6)	1·32d (5·6)	2·05B
(VIIIa)			4·59t (3·8)	3·17b	4·11dt	2·45d (6)	1·28	0·66	0·91d (6·5)	2·20		2·05B
(VIIIb)			4·59t (3·8)	3·17b	5·36dt	2·57d (7)	1·28	0·66	0·91d (6·5)	2·17		2·00B 2·05B
(IX)			4·57t (3·8)	3·15b	6·67dd (16; 8)	5·98d (16)	1·28	0·67	1·08d (6·3)	2·21		2·04B
(X)			4·55t	3·16b			1·27	0·62	0·96b	2·12		2·04B
(XIa)	6·89			3·42d (3)			1·38	0·72	1·02d (6·5)	2·06		4·32A
(XIb)	6·90			3·43d (3)	4·41dt		1·38	0·72	1·02d (6·5)	2·05 or 2·08		2·08 or 2·05B 4·90A
(XII)			6·08dd (6·5; 8·5)	4·50	4·38dt		1·19	0·68	0·97d (6·5)	1·96	1·96	2·12B
(XIII)			5·76dd (6·5; 8·5)		4·34dt		1·14	0·67	0·97d (7)	1·94	1·94	2·11B
(XV)					4·37dt		1·26	0·69	0·95d (6)	1·96	1·96	
(XVI)				3·3d (2)	4·38dt		1·18	0·70	0·99d (6·5)	1·92	1·92	
(XVII)	2·73 (4H)			6·80dd (5; 2)	4·40dt 12·5		1·28	0·72	1·00d (6·5)	1·95	1·95	
(XVIII)				3·30d (2)	4·39dt		1·17	0·69	0·92d (6·5)	1·08d (5·2)	1·30d (6·0)	
XIX)	2·72 (4H)			6·77dd	4·38dt		1·29	0·72	0·92d	1·12d	1·32d	
(XXa)			5·77t (3)	4·40			1·42	0·74	0·99d (6)	1·93	1·93	
(XXb)			5·96t	5·41t (3)	4·37dt		1·32	0·77	1·00d (6)	1·93	1·93	2·04B
(XXI)				4·6c	6·68dd (16; 8)	5·99d (16)	1·12	0·66	1·07d (7)	2·23		1·99B 2·46C 7·39d (8) D 7·82d (8) D
(XXIII)			Overlapping of signals between 4·20—4·80				1·11	0·65	0·97d (6·5)	1·95	1·95	2·00B 2·47C 7·40d (8) 7·82d (8) D
(XXIVa)	2·81c 2H	3·67c	3·48d (2·7)	3·21b			1·30	0·68	0·99d (6·5)	2·05		4·37A 3·37E
(XXIVb)	2·78c 2H	3·67c	4·62d (2·3)	3·28b	4·42dt		1·26	0·68	0·99d (6·7)	2·04		4·90A 2·04B 3·41E
(XXVa)	2·80c 2H	3·69c	3·50d (3)	3·21b	4·39dt		1·30	0·68	0·99d (6·3)	1·94	1·94	3·37E
(XXVb)	2·80c 2H	3·68c		3·28b	4·40dt		1·25	0·68	0·99d (6·8)	1·93	1·93	2·05B 3·42E
(XXVII)	2·75d (7)	3·60c	4·60d (2·8)	3·26b	4·10dt	2·43d (5·8) 2H	1·23	0·65	0·90d (6·5)	2·19		2·03B 3·39E

TABLE (*Continued*)

Cpd.	2-H	3-H	4-H	6-H	22-H	23-H	10-Me	13-Me	20-Me	24-Me	25-Me	Other
(XXVIII)	2·77d (6·5)	3·61c	4·61d (2·1)	3·27b	6·65dd (16; 8)	5·97d (6)	1·25	0·69	1·08d (6·7)	2·20		2·05B 3·40E
(XXIXa)				3·15b			1·30	0·68	1·00d (6·8)	2·08		4·26A 3·39E
(XXIXb)			4·58t (3·5)	3·19b			1·29	0·69	0·99d (6·5)	2·06		4·22A 2·06B 3·39E
(XXXa)	2·79c	3·71c	3·41d (2·5)	3·21b			1·29	0·67	0·99d (6·5)	2·08		4·22A 3·38E
(XXXb)	2·74d (6·5)	3·62c	4·55d (2·5)	3·24b			1·26	0·71	0·99d (6)	2·04		4·21A 2·04B 3·35; 3·39E

Positions are given in δ units. Numbers in parentheses present the coupling constants in c./sec. Multiplicity of signals are designated as follows: d, doublet; t, triplet; dd, double doublet; dt, double triplet; b, broad; c, complex pattern. Other signals: A, C_{27}-protons; B, O·CO·CH_3; C, CH_3 on benzene ring; D, aromatic protons; E, OCH_3.

of oxidation of the two allylic alcohol groups present in (I) is quite different, and under the conditions of the reaction only minor quantities (3—5%) of a compound in which both hydroxyl groups underwent oxidation were obtained.

A rather broad signal (half-height width 4 c./sec.) at δ 3·20, accounting for one proton, in the n.m.r. spectra of withaferin A and of a series of derivatives suggested an epoxide ring in the molecule; protons attached to carbon atoms of such a ring are known to resonate in this region of the spectrum. Furthermore, the presence of one proton only of this type indicated that the second carbon atom in this system is tetrasubstituted.

Treatment of deoxydihydrowithaferin A acetate (IIIb) with hydrobromic acid in glacial acetic acid solution at 15° induced the opening of the epoxide yielding a bromohydrin (XII); the hydroxyl group in this compound is tertiary and possesses therefore no neighbouring protons observable in the n.m.r. spectrum. However, a signal at δ 4·50, accounting for the hydrogen α to the bromine atom, is now to be seen. When this compound was exposed to alkaline conditions the expected reaction, inducing epoxide formation, took place yielding thereby deoxydihydrowithaferin A (IIIa); upon acetylation it afforded the original compound (IIIb).

The assignment of the 4·50 signal to the hydrogen attached to the same carbon atom as the bromine is unambiguously demonstrated by the debromination of (XII) with Raney nickel to compound (XIII), in which the signals of the C-4 and C-22 protons remained almost unaffected, while the one relating to the C-6 proton disappeared from this part of the spectrum.

Careful examination of the 4-hydrogen signal in the n.m.r. spectra of all these compounds led us to interesting observations concerning its orientation; whereas in the epoxy-acetate (IIIb) this proton is equatorial and displays a triplet at δ 4·62, in the bromohydrin the signal is shifted to δ 6·08 appearing as a double doublet and implying an axial orientation of the same proton. In the epoxy-acetate (IIIb) which is reformed following the alkaline treatment,[9] this proton assumes its original equatorial orientation. However, following the debromination procedure, the C-4-hydrogen in compound (XIII) retains the same orientation as in the bromohydrin which is disclosed by the double doublet pattern at 5·76.

This sequence which is intimately related to the relative stereochemistry of rings A and B and thereby to the orientation of the epoxide in the molecule will be discussed in detail in a forthcoming publication dealing with the stereochemistry of withaferin A.

The formation of a C-4 enol acetate (XIV), following treatment of compound (XIII) with thionyl chloride at room temperature, ascertains the location of the tertiary hydroxyl group at position C-5 of the hydroxy-acetate (XIII), as well as of the bromohydrin (XII)

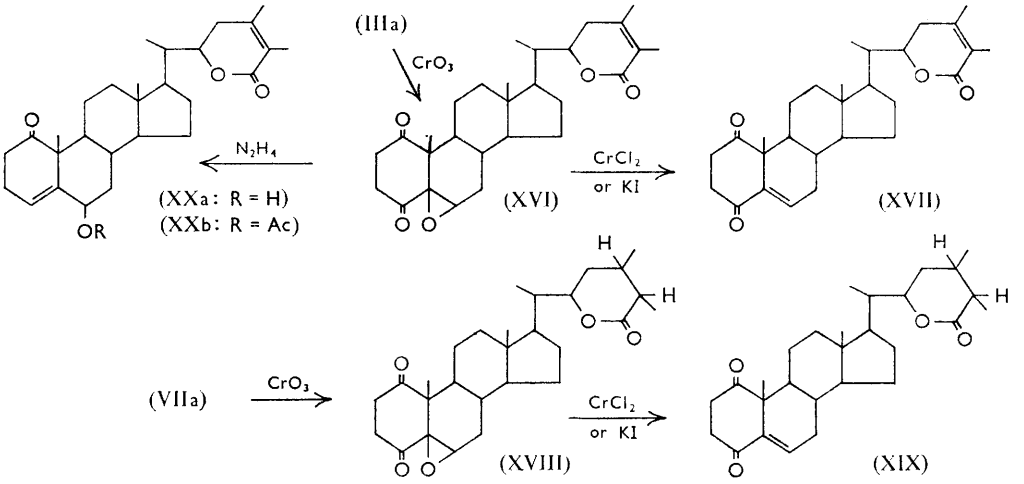
[9] R. E. Parker and N. S. Isaacs, *Chem. Rev.*, 1959, **59**, 737, and references cited therein.

from which it was prepared; it provides therefore an unequivocal indication that the epoxide occupies the 5,6-position.

The enol acetate (XIV) was smoothly hydrolysed to the crystalline 1,4-diketone (XV), which gave good analytical figures and showed one spot only on a chromatoplate; however, in the n.m.r. spectrum, in addition to a strong signal at δ 1·26 attributed to the 10-methyl group, there is also a relatively smaller singlet at δ 1·10, which may well be due to the fact that the hydrolysis of the enol acetate (XIV) actually yielded a mixture of two epimers at C-5, the minor component being responsible for the δ 1·10 signal. Attempts to separate the two components were so far unsuccessful.

The presence of the 5,6-epoxide was further substantiated when deoxydihydrowithaferin A was oxidised to the corresponding α-epoxy-ketone (XVI) and subjected subsequently to several characteristic reactions for such a system.

(*a*) Heating the epoxy-ketone (XVI) in glacial acetic acid with potassium iodide [10] resulted in the elimination of the epoxidic oxygen, yielding thereby the αβ-unsaturated ketone (XVII), $\lambda_{max.}$ 232 mμ (ε 12,000), which is the overlapping of the newly introduced chromophore with the existing one of the unsaturated lactone of the side-chain. In the n.m.r. spectrum of this compound a signal for one vinylic proton appeared at δ 6·80 as a double doublet. However, the outstanding feature of this spectrum is the appearance of a sharp peak at δ 2·73 accounting for the 4 protons at C-2 and C-3 between the two carbonyl groups; this observation can be rationalised by assuming a flattening of ring A.



In order to disclose the actual position of the ultraviolet absorption band of the Δ^5-4-oxo-system, the same reaction was performed on the epoxy-ketone (XVIII), obtained by chromium trioxide oxidation of deoxytetrahydrowithaferin A (VIIa). Effectively, the resulting compound (XIX) displayed an ultraviolet absorption band at the expected location for this chromophore, 239 mμ.

(*b*) While in the previous procedure, tedious and careful purification of the reaction products was necessary, the use of chromous chloride [11] on the epoxy-ketone (XVI) effected its conversion smoothly and in almost quantitative yield to the same compound, (XVII).

Reduction of α-epoxy-ketones with chromous chloride proceeded as reported,[11] by formation of the corresponding β-hydroxy-ketone, which under the acidic conditions of the reaction was dehydrated to a certain extent to the unsaturated ketone. The reaction was usually taken to completion by acid treatment of the crude mixture, yielding thereby

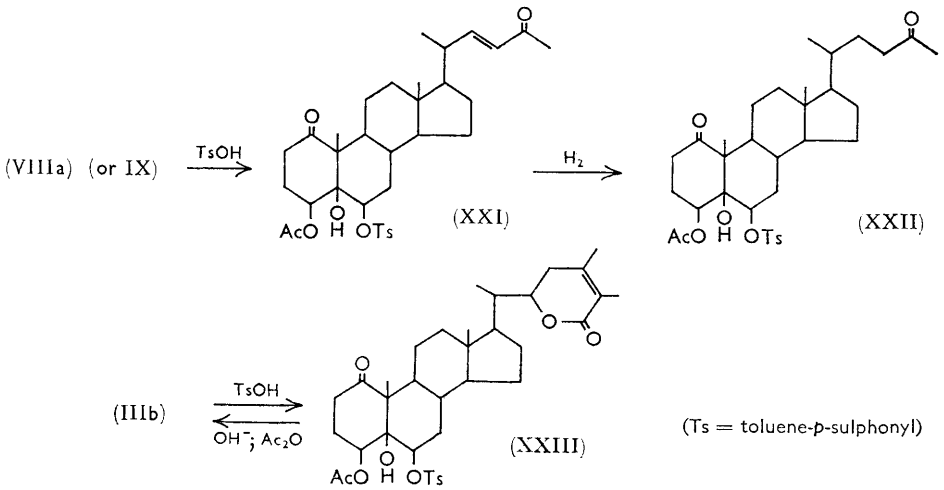
[10] S. Bodforss, *Ber.*, 1916, **49**, 2795; W. Bergmann and M. B. Meyers, *Chem. and Ind.*, 1958, 655.

[11] (*a*) W. Cole and P. L. Julian, *J. Org. Chem.*, 1954, **19**, 131; (*b*) P. Crabbé, M. Perez, and G. Vera, *Canad. J. Chem.*, 1963, **41**, 156.

preponderantly the unsaturated ketone. Interestingly enough, in the present case elimination of the hydroxyl in the β-hydroxy-ketone took place quantitatively during the reaction itself and no additional treatment with acid was necessary.

(*c*) Hydrazine hydrate has been shown to reduce α-epoxy-ketones to allylic alcohols in which the hydroxyl group occupies the β-position with respect to the previously exisiting carbonyl function.[12] Upon treating the epoxy-ketone (XVI) with the reagent at room temperature, it was smoothly converted into the Δ^4-6-hydroxy-derivative (XXa) which was then acetylated to (XXb) for further identification. Despite the fact that neither of these compounds could be crystallised, they were fully characterised spectroscopically. The n.m.r. spectrum of the alcohol (XXa) reveals the signal of the vinylic 4-hydrogen as a triplet at δ 5·77, while the 6-proton appears at δ 4·40 overlapping the signal of the 22-hydrogen; in the corresponding acetate (XXb) the signals are at δ 5·96 for the C-4 vinylic hydrogen and at δ 5·41 (triplet) for the 6-proton. In both signals a mutual allylic splitting of about 1·5 c./sec. was observable. In the infrared spectrum of (XXa) the band at 1727 cm.$^{-1}$ due to the 4-carbonyl in the original epoxy-ketone (XVI) was not present.

Among several reactions leading to the opening of the epoxide ring, an outstanding observation was made when toluene-*p*-sulphonic acid was used for the elimination of the 22-hydroxyl group in compound (VIIIa). The reaction was first performed in benzene solution with catalytic amounts of acid, and in addition to the expected αβ-unsaturated ketone previously described (IX), a second unsaturated ketone (XXI) was obtained, in which the epoxide ring had been opened to the corresponding 5-hydroxy-6-toluene-*p*-sulphonyloxy-derivative. However, when the reaction was repeated at room temperature in acetic acid solution with a slight excess of toluene-*p*-sulphonic acid, it proceeded smoothly and almost quantitatively to the hydroxy-ester (XXI).



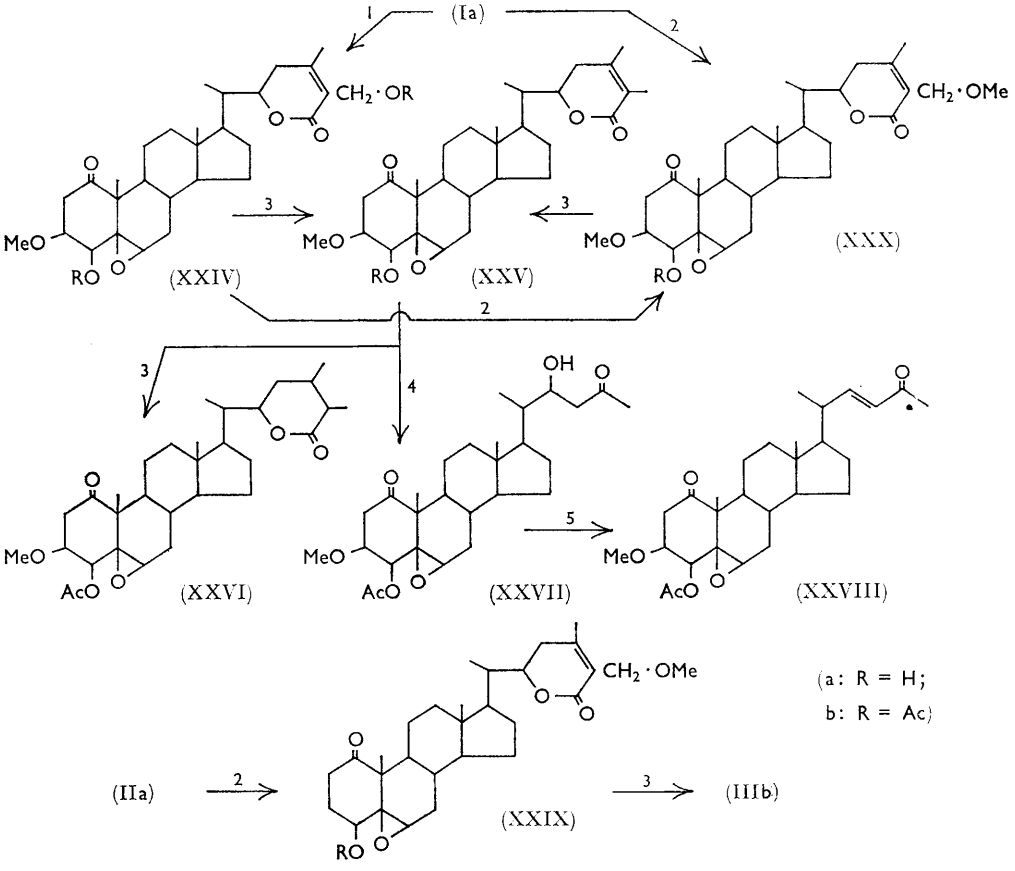
The structure of this compound was disclosed by its n.m.r. spectrum. The signal of the epoxidic proton present in the original compound (IX) disappeared, while two sets of doublets for two protons each, centred at δ 7·39 and 7·82 and a three-proton singlet at δ 2·46, accounted, respectively, for the four hydrogen atoms and the methyl group on the aromatic ring. Following the reduction of the C-22 double bond, the ultraviolet absorption spectrum of the compound (XXII) disclosed the characteristic bands of the assigned structure: * $\lambda_{max.}$ 272, 265, 261, 256, 223 mμ (ε 400, 440, 470, 440, 7600).

* For reference, 3β-acetoxy-17β-toluene-*p*-sulphonyloxy-5α-androstane, kindly supplied by Professor Y. Mazur of our Institute was used. The ultraviolet absorption spectrum of the aromatic system was superimposable on that of compound (XXII), while in the n.m.r. spectrum all the signals related to the ester group were identical as well.

[12] P. S. Wharton and D. H. Bohlen, *J. Org. Chem.*, 1961, **26**, 3615.

The characterisation of the hydroxy-ester system was completed by taking advantage of the known displacement in alkaline conditions of the ester group in vicinal *trans*-hydroxy-toluene-*p*-sulphonates leading to an epoxide ring.[13] In order to avoid secondary reactions involving the unsaturated ketone in the side-chain (XXI), the hydroxy-ester (XXIII) was prepared by the action of toluene-*p*-sulphonic acid on deoxydihydrowithaferin A acetate (IIIb). Indeed, alkaline treatment of (XXIII) resulted in the formation of an epoxide, yielding the original deacetylated compound (IIIa). This reaction parallels the alkaline treatment of the bromohydrin (XII) referred to above.

Withaferin A in methanol solution when treated with a catalytic amount of toluene-*p*-sulphonic acid, yields a compound which is assigned structure (XXIVa), 3-methoxy-2,3-dihyrowithaferin A: $C_{29}H_{42}O_7$, m. p. 243—245°, $[\alpha]_D$ +17·5°, $\lambda_{max.}$ 212 mμ (ε 9500). [In a previous publication,[2] in addition to several crystalline products described as A_2,A_3, etc., the isolation of a compound A_1 (denominated withaferin), was described. The structures of A_2 and A_3 have now been elucidated, they are withaferin A and its dihydro-deriva-



a: R = H; b: R = Ac.

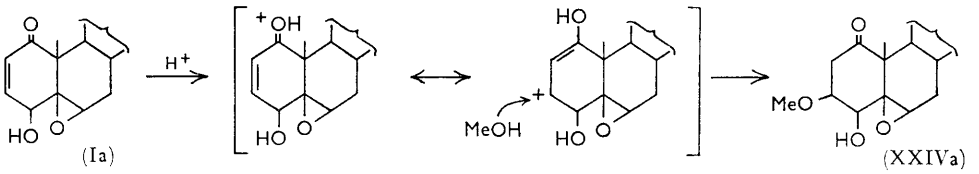
Reagents: 1, MeOH–H^+; 2, MeOH–OH^-; 3, H_2; 4, O_3; 5, $-H_2O$.

tive, respectively, while compound A_1 turned out to be an artefact which was formed during the processing of the plant material. This compound, which is identical with (XXIVa), must have been formed from withaferin A during the prolonged extraction of the leaves of *W. somnifera* with industrial methanol. When the extraction procedure was shortened,

[13] R. P. Linstead, L. N. Owen, and R. F. Webb, *J.*, 1953, 1218; F. H. Newth, *Quart. Rev.*, 1959, **13**, 30.

or performed with ethanol, this compound could not be obtained again. Compound A_1 had been assigned the wrong formula, $C_{21}H_{30}O_5$, instead of $C_{29}H_{42}O_7$; at the time, the presence of an epoxide ring in the molecule had not been detected.]

The assignment of structure (XXIVa) to the methoxy-compound is well supported by its n.m.r. spectrum: the differences encountered between the spectra of this compound and of the original withaferin A (Ia) refer to changes involving exclusively signals related to protons in ring A. While the low-field signals of the vinylic 2- and 3-protons (*vide supra*) disappeared, a new sharp signal with the intensity of three protons appeared at δ 3·37 due to the methoxyl group. The 3-proton now gave rise to a multiplet centred at δ 3·67 formed by its interaction with the two hydrogens at C-2 and the one at C-4. The signal of the 4-proton appeared as a doublet at δ 3·48 which on acetylation was shifted to δ 4·62. The two C-2 protons gave a multiplet centred at δ 2·81. The formation of such a structure could well be explained through nucleophilic addition of the solvent at C-3.

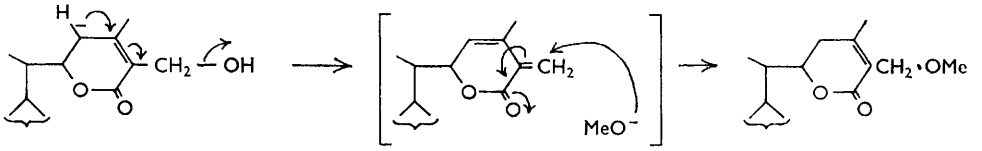


In order to substantiate the structure of this compound, a sequence of reactions previously used for the characterisation of the side-chain in withaferin A was performed. Catalytic reduction of compound (XXIVa) induced the hydrogenolysis of the allylic 27-hydroxyl group, yielding the deoxy-derivative (XXVa), $\lambda_{max.}$ 226 mμ. Upon acetylation a monoacetate (XXVb) was obtained; this compound was also formed by hydrogenolysis of the diacetate (XXIVb). The reduction of the tetra-substituted double bond (Δ^{24}) proceeded at a slow rate, giving the 27-deoxytetrahydro-3-methoxy-derivative (XXVI). As expected, this compound had no strong absorption in the ultraviolet, while in its n.m.r. spectrum, two doublets, centred at δ 0·94 and 1·07, accounted for the two new secondary methyl groups which, following the hydrogenation, replaced the two vinylic methyls of compound (XXVb).

Cleavage of the double bond in (XXVb) was done by ozonolysis followed by reductive decomposition of the ozonide, yielding the β-hydroxy-ketone (XXVII), devoid of strong absorption in the ultraviolet, while in the n.m.r. spectrum a methyl ketone group signal appeared at δ 2·19. Elimination of the 22-hydroxyl group in (XXVII) was effected by contact with acid-washed alumina, leading to the α,β-unsaturated ketone (XXVIII), $\lambda_{max.}$ 225 mμ, which in the n.m.r. spectrum presented the characteristic pattern for the two vinylic protons: doublet for H-23 at δ 5·97 and double doublet for H-22 at δ 6·65 ($J_{22,23} = 16$ c./sec.; $J_{22,20} = 8$ c./sec.). The large coupling constant between the two vinylic protons is indicative of their *trans* orientation.

A rather unusual and interesting reaction was encountered when dihydrowithaferin A (IIa) was exposed in methanol solution to alkaline conditions at room temperature. Within a few hours, a new product was isolated in almost quantitative yield, and characterised as the methyl ether (XXIXa). While in the ultraviolet spectrum no difference from the original compound was observed, in the n.m.r. spectrum, the only outstanding change consisted in the appearance of a three-proton singlet at δ 3·39 accounting for a methoxyl group. This compound gave a monoacetate (XXIXb) which was also fully characterised. Unequivocal proof of the location of the methoxyl group at C-27 was obtained by the hydrogenolysis of this group in (XXIXb) to yield the deoxydihydrowithaferin A acetate (IIIb) described earlier.

This reaction can be rationalised by assuming a process of elimination of the hydroxyl group followed by a Michael-type addition of the solvent molecule.



Using the same conditions of reaction, withaferin A (Ia) was converted into a new derivative (XXX), in which the nucleophilic reagent attacked the molecule at two centres: in the side-chain the reaction proceeded as described above for dihydrowithaferin A, leading to the formation of the 27-methyl ether, while the β-position of the unsaturated ketone in ring A afforded a second site of attack, through a Michael-type addition, leading to the introduction of a methoxyl group at C-3.

As expected, compound (XXXa) yielded a monoacetate (at C-4 in XXXb). All the spectroscopic evidence for both compounds is in full agreement with the proposed structures. Furthermore, when subjected to hydrogenolysis, compound (XXXa) was converted into deoxydihydro-3-methoxywithaferin A (XXVa), described in the above sequence. The identity of the products obtained by both routes was unequivocal, indicating that the same 3-methoxy-derivative is obtainable either in alkaline or in acidic conditions.

In order to complete the interconversion of the various derivatives in this group of compounds, 3-methoxydihydrowithaferin A (XXIVa), in alkaline methanolic solution was converted into the dimethoxy-derivative (XXXa).

The assignment of the two angular methyl groups to positions 10 and 13 of the proposed skeleton of withaferin A rests upon considerations of its steroid structure. Some evidence could be adduced from the positions of their signals in the n.m.r. spectra of the whole series of derivatives discussed above (see Table). As expected, the chemical shift of the 13-methyl group is almost unaffected by the changes involving rings A and B of the molecule, and its position is in outstanding agreement with data reported [14] for the signal of this group in known steroid compounds. The only case in which the position of this signal appears at higher field (δ 0·62) is in compound (X) in which the nearest deshielding factors, namely the substituent at C-22, are eliminated.

In contrast, the position of the 10-methyl signal is highly influenced by the various changes involving rings A and B. Should this methyl group have been located at C-9, variations of the functional groups would exert a much smaller influence on its chemical shift, while the alternative C-5 location could be eliminated by the intrinsic relative distribution of the substituents in this part of the molecule.

The six-membered-ring lactone in the side-chain of withaferin A can be conceived as arising from the cyclisation of a highly oxygenated steroidal side-chain, carrying an extra methyl group at C-24. To our knowledge, no such lactone side-chain has been encountered so far in naturally occurring steroids or tetracyclic triterpenoids. As to the substitution pattern of rings A and B, although it seems to be rather unusual, it should be mentioned that kitigenin,[15] a sapogenin isolated from the saponin of *Reineckia carnea* contains four hydroxyl groups in ring A, at positions 1, 3, 4, and 5.

Experimental

Melting points were taken on a Kofler hot-stage apparatus and are corrected. Optical rotations refer to chloroform solutions. Ultraviolet absorption spectra were determined in a Cary 14 spectrophotometer in ethanol solution. Infrared spectra were recorded on a Perkin-Elmer Infracord model 137 spectrophotometer equipped with a sodium chloride prism and, unless otherwise stated, were determined in chloroform solution of 5—10% concentration. N.m.r. spectra were recorded on a Varian A-60 spectrometer for 5—10% solutions in $CDCl_3$ containing tetramethylsilane as internal standard, and the data are collected in the Table. Thin-layer

[14] R. F. Zürcher, *Helv. Chim. Acta*, 1963, **46**, 2054.
[15] K. Sasaki, *Chem. Pharm. Bull.* (*Tokyo*), 1961, **9**, 684 (*Chem. Abs.*, 1962, **57**, 8637).

chromatography (t.l.c.) was done on chromatoplates of silica gel G (Merck) and spots were developed with iodine vapour. In chromatography, alumina refers to acid-washed alumina Merck and neutral alumina refers to alumina Woelm grade III. The molecular weights, whenever given, were determined by mass spectrometry on an Atlas CH4 instrument.

Selenium Dehydrogenation of Dihydrowithaferin A.—Dihydrowithaferin A[1] (IIa) was treated with lithium aluminium hydride until all the carbonyl groups had been reduced, as indicated by the infrared spectrum of the reaction product. This crude product was then acetylated in the usual manner at room temperature. The crude acetate (2 g.), after being thoroughly mixed with selenium powder (2 g.) was heated at 270—280° in a stream of dry nitrogen during 24 hr. The mixture was exhaustively extracted with ether (Soxhlet). The ethereal extract was washed with aqueous sodium hydroxide (4%) and water, then dried (Na_2SO_4). Removal of the solvent left a brown oily residue (250 mg.)which was percolated in pentane solution through a column of alumina (Alcoa F_{20}; 50 g.). The resulting yellowish oily material (120 mg.) was rechromatographed three times through alumina (100 g. each) and fractions were combined according to their ultraviolet absorption. The naphthalenic fraction (10 mg.) had $\lambda_{max.}$ 230 (ε 62,000), 275sh (4100), 281 (4400), 290sh (3700), 315 (850), 322 (700), and 330 mμ (850). The phenanthrenic fraction (40 mg.) had $\lambda_{max.}$ 214 (ε 39,600), 258 (54,000), 279 (13,700), 300 (10,000), 318 (930), 333 (690), and 351 mμ (500).

Manganese Dioxide Oxidation of Withaferin A[1] (Ia) *to* (XIa).—Withaferin A (200 mg.) in a mixture of chloroform (10 ml.) and ethyl acetate (15 ml.) was stirred overnight with freshly precipitated manganese dioxide[7] (1 g.). The mixture was filtered, the solvent was removed *in vacuo* and the residue (190 mg.) was chromatographed through alumina (20 g.). Elution with hexane–chloroform (4 : 1) yielded in the first fractions minute quantities (8 mg.) of the aldehyde, while the latter fractions contained the main *product* (XIa). Two crystallisations from acetone–hexane yielded yellowish crystals (150 mg.), m. p. 272—275°; $[\alpha]_D$ +147° (*c* 0·83); $\nu_{max.}$ 1692 (overlapping of three carbonyl groups) and 1618 cm.$^{-1}$; $\lambda_{max.}$ 222 mμ (ε 19,300) (Found: C, 71·6; H, 7·8. $C_{28}H_{36}O_6$ requires C, 71·75; H, 7·75%).

The *acetate* (XIb) was obtained by treating compound (XIa) (100 mg.) in pyridine solution (1 ml.) with acetic anhydride (1 ml.) overnight, at room temperature. The mixture was poured on to ice and the *product* was collected, washed with water, and crystallised from acetone–hexane, yielding yellowish crystals (90 mg.), m. p. 256—257°; $[\alpha]_D$ +125° (*c* 0·80); $\nu_{max.}$ 1736 (acetate), 1695 (three carbonyls), and 1613 cm.$^{-1}$; $\lambda_{max.}$ 222 mμ (ε 22,600) (Found: C, 70·65; H, 7·6. $C_{30}H_{38}O_7$ requires C, 70·55; H, 7·5%).

Preparation of the Bromohydrin (XII).—To a solution of deoxydihydrowithaferin A acetate[1] (IIIb; 200 mg.) in glacial acetic acid (10 ml.), an acetic acid solution of hydrobromic acid (45%; 0·5 ml.) was added at 15°, and the mixture was stirred for 2 hr. at this temperature. Ice-water was added and the solid which separated was filtered and washed with water. The product, which was almost pure (t.l.c. evidence), was nevertheless chromatographed through neutral alumina. Elution with hexane–chloroform (4 : 1) yielded the pure *bromohydrin* (XII) which crystallised from methanol–water, m. p. 152—155°; $[\alpha]_D$ +38° (*c* 0·57); $\nu_{max.}$ 1727 and 1701 cm.$^{-1}$; $\lambda_{max.}$ 226 mμ (ε 8300) (Found: C, 61·7; H, 7·4; Br, 13·5. $C_{30}H_{43}BrO_6$ requires C, 62·15; H, 7·5; Br, 13·8%).

Alkaline Treatment of the Bromohydrin (XII).—The bromohydrin (100 mg.) in methanol solution (20 ml.) was heated during 1 hr. under reflux with 2% methanolic solution of sodium methoxide (10 ml.). The solution was acidified to pH ~ 3 (HCl), filtered, then most of the solvent was removed *in vacuo*; following addition of water the solid was filtered and washed. The product (70 mg.) crystallised from ethyl acetate and was identical with deoxydihydrowithaferin A (IIIa). Acetylation with acetic anhydride in pyridine gave the corresponding acetate (IIIb), identical (mixed m. p., R_F on t.l.c., and infrared spectrum) with an authentic sample.

Debromination of the Bromohydrin (XII) *to the α-Hydroxy-acetate* (XIII).—The bromohydrin (110 mg.) in absolute ethanol (50 ml.) was heated under reflux with Raney nickel (*ca.* 1 g.) with stirring for 24 hr. After filtration, the solvent was removed *in vacuo* and the residue was dissolved in chloroform and percolated through neutral alumina, thus yielding 77 mg. of pure compound (XIII) (one spot on t.l.c.) which crystallised from methanol, m. p. 263—266°; $[\alpha]_D$ +28° (*c* 0·50); $\nu_{max.}$ 1730 and 1695 cm.$^{-1}$; $\lambda_{max.}$ 226 mμ (ε 8900) (Found: C, 71·9; H, 8·75. $C_{30}H_{44}O_6$ requires C, 71·95; H, 8·85%).

Dehydration of Compound (XIII) *to the Enol Acetate* (XIV).—To the α-hydroxy-acetate (XII) (100 mg.) in dry pyridine (3 ml.), freshly distilled thionyl chloride (0·4 ml.) was added at room

temperature. After 1 hr., the mixture was carefully poured on to ice and the gummy material was extracted with chloroform and washed with dilute hydrochloric acid and water. After removal of the solvent, a residue (90 mg.) was obtained which could not be induced to crystallise: on a chromatoplate it exhibited only one spot, $\nu_{max.}$ 1750 (enol acetate) and 1698 cm.$^{-1}$ (two carbonyls).

Hydrolysis of the Enol Acetate to the Diketone (XV).—The above product (80 mg.) in methanol (5 ml.) was left overnight with a few drops of 2% methanolic potassium hydroxide solution. The solution was then acidified to pH ~ 3 (HCl), most of the solvent removed, water added, and the solid filtered, washed, and dried. The crude product (65 mg.) was chromatographed through neutral alumina. Elution with hexane–chloroform (4 : 1) yielded fractions which were combined and evaporated, and the resulting *product* was crystallised from ethanol, m. p. 211—216°; $[\alpha]_D$ +80° (*c* 0·67); $\nu_{max.}$ 1715 and 1701 cm.$^{-1}$; $\lambda_{max.}$ 226 mμ (ε 8800) (Found: C, 76·45; H, 9·2. $C_{28}H_{40}O_4$ requires C, 76·3; H, 9·15%).

Chromium Trioxide Oxidation of Deoxydihydrowithaferin A (IIIa) *to the Epoxy-ketone* (XVI).—Deoxydihydrowithaferin A[1] (100 mg.) in pure acetone solution (50 ml.) was oxidised at 0—5° with a solution of chromium trioxide in dilute sulphuric acid (Jones reagent). The excess of oxidant was destroyed with methanol, water was added, and the product extracted with chloroform. The solution was washed and dried. Evaporation of the solvent left a residue which was chromatographed through neutral alumina. Elution with hexane–chloroform (7 : 3) yielded fractions which were combined and evaporated, and the crude *product* (60 mg.) was crystallised from acetone–hexane, m. p. 251—253°; $[\alpha]_D$ −79° (*c* 2·2); $\nu_{max.}^{KBr}$ 1727, 1718, and 1698 cm.$^{-1}$; $\lambda_{max.}$ 226 mμ (ε 9200) (Found: C, 73·95; H, 8·4. $C_{28}H_{38}O_5$ requires C, 74·0; H, 8·45%).

Chromium Trioxide Oxidation of Deoxytetrahydrowithaferin A (VIIa) *to the Epoxy-ketone* (XVIII).—Deoxytetrahydrowithaferin A[1] (100 mg.) was oxidised with chromium trioxide as described above. Following a similar work-up, the epoxy-ketone (XVIII) was obtained, which crystallised from acetone–hexane, m. p. 223°; $[\alpha]_D$ −136° (*c* 0·68); $\nu_{max.}$ 1721 cm.$^{-1}$ (overlapping of three carbonyls); no strong ultraviolet absorption (Found: C, 73·65; H, 8·85. $C_{28}H_{40}O_5$ requires C, 73·65; H, 8·85%).

Opening of the Epoxide Ring in (XVI) *to the Unsaturated Ketone* (XVII).—(*a*) *Potassium iodide procedure.* The epoxy-ketone (XVI) (150 mg.) in glacial acetic acid (15 ml.) was heated on a steam-bath with potassium iodide (150 mg.) for 1 hr. After cooling, water was added, the product extracted with chloroform, and the solution washed with water, dilute aqueous sodium hydrogen sulphite, and sodium hydrogen carbonate. The crude product (130 mg.), obtained following removal of the solvent, was chromatographed three times through neutral alumina. The fractions which showed one spot on a chromatoplate were combined (40 mg.) and crystallised from acetone–hexane, yielding slightly yellowish crystals, m. p. 135—140°; repeated crystallisations did not improve the m. p.

(*b*) *Chromous chloride procedure.* To the epoxy-ketone (XVI) (125 mg.), in a mixture of acetone (8 ml.) and acetic acid (5 ml.), a solution of chromous chloride was added under carbon dioxide. The reagent was prepared from chromic chloride (1·6 g.).[10b] After 30 min. water was added and the product was extracted with chloroform, washed with water, and dried. Removal of the solvent left a residue (120 mg.) which exhibited on a chromatoplate only one spot having the same R_F value as the crystalline product of the preceding experiment. Recrystallisation from acetone–hexane yielded white *crystals*, m. p. 198°; $[\alpha]_D$ −10° (*c* 0·62); $\nu_{max.}$ 1712, 1695, and 1618 cm.$^{-1}$; $\lambda_{max.}$ 232 mμ (ε 12,000) (Found: C, 76·65; H, 8·65. $C_{28}H_{38}O_4$ requires C, 76·65; H, 8·75%).

Opening of the Epoxide Ring in (XVIII) *to the Unsaturated Ketone* (XIX).—The epoxy-ketone (XVIII) (100 mg.) was treated with a chromous chloride solution as described above. The crude product, which displayed one major spot on a chromatoplate, was chromatographed through neutral alumina. Elution with hexane–chloroform (9 : 1) yielded fractions which were combined (70 mg.). Notwithstanding the fact that the product showed only one spot on t.l.c., it could not be induced to crystallise, $\nu_{max.}$ 1712, 1695, and 1623 cm.$^{-1}$; $\lambda_{max.}$ 239 mμ (ε 4400).

Preparation of the Allylic Alcohol (XXa).—To a methanolic solution (20 ml.) of the epoxy-ketone (XVI) (100 mg.) under nitrogen, hydrazine hydrate (98%; 0·1 ml. in 1 ml. methanol) and acetic acid (0·1 ml.) were added. The solution, which turned immediately yellow, was left aside overnight at room temperature. The yellow colour slowly disappeared. Most of the solvent was then removed *in vacuo* (avoiding heating), water added, and the product collected

by filtration and chromatographed through neutral alumina. Elution with hexane–chloroform (8 : 2) yielded in the first fractions minute quantities of impurities followed by the main product (60 mg.) which was homogeneous on t.l.c. but could not be induced to crystallise; [molecular ion, *m/e* 440 ($C_{28}H_{40}O_4$)], $\nu_{max.}$ 1695 cm.$^{-1}$ (two carbonyls); $\lambda_{max.}$ 226 mμ (ε 9000), and strong end-absorption.

The *acetate* (XXb) was prepared from the alcohol (XXa) by the usual acetic anhydride–pyridine procedure, overnight at room temperature. It could not be induced to crystallise notwithstanding the fact that it exhibited only one spot on a chromatoplate [molecular ion, *m/e* 482 ($C_{30}H_{42}O_5$)]; $\nu_{max.}$ 1727 and 1701 cm.$^{-1}$.

Formation of the Hydroxy-ester (XXI).—(*a*) *From the* β*-hydroxy-ketone* (VIII). To a solution of the β-hydroxy-ketone (VIII) [1] (100 mg.) in benzene (50 ml.); toluene-*p*-sulphonic acid dihydrate (30 mg.) was added and the mixture boiled during 2 hr. with azeotropic removal of the water. The solution was washed with water; evaporation of the solvent left a residue (80 mg.) shown by t.l.c. to be a complex mixture. Two repeated chromatographies on alumina resulted on the separation of the two main products: one (15 mg.) was identified as the *unsaturated ketone* (IX) (R_F value on t.l.c., spectral behaviour, and mixed m. p.), while the second (18 mg.), which crystallised from acetone–hexane, was the required *product* (XXI), m. p. 157—159°; $[\alpha]_D$ −67° (*c* 0·42); $\nu_{max.}^{KBr}$ 1739 (acetate), 1715 (ketone), 1678, 1626 (unsaturated ketone), 1603, 1193, and 1176 cm.$^{-1}$; $\lambda_{max.}$ 224 (ε 23,000), 260sh, 265sh, and 272 mμ (400); (Found: C, 64·6; H, 7·65; S, 5·2. $C_{34}H_{46}O_8S,H_2O$ requires C, 64·6; H, 7·65; S, 5·05%) [molecular ion, *m/e* 614 ($C_{34}H_{46}O_8S$)].

(*b*) *From the unsaturated ketone* (IX). Compound (IX) [1] (100 mg.) treated with toluene-*p*-sulphonic acid as described above, yielded a mixture from which the same hydroxy-toluene-*p*-sulphonate (XXI) was isolated along with unreacted material. The yield of compound (XXI) could be appreciably increased (to *ca.* 70%) by modifying the experimental procedure.

To the unsaturated ketone (IX) (100 mg.) in glacial acetic acid (5 ml.), toluene -*p*-sulphonic acid (100 mg.) was added and the mixture left aside at room temperature during 3 days. Water was then added, the product was extracted with chloroform, and the solution was washed with water and aqueous sodium hydrogen carbonate. Evaporation of the solvent left a residue which consisted predominantly of compound (XXI). After chromatography on alumina, the fractions which were homogeneous on t.l.c. were combined; the product crystallised from acetone–hexane, m. p. 157—159°.

Hydrogenation of the Ester of (XXI) *to* (XXII).—Compound (XXI) (5 mg.) in ethanol was hydrogenated in a microhydrogenation apparatus over palladium–charcoal. One equivalent of hydrogen was rapidly absorbed. Filtration of the catalyst and removal of the solvent left a residue which showed one spot on t.l.c. and had $\lambda_{max.}$ 223 (ε 7600), 256 (440), 261 (470), 265 (440), and 272 mμ (400).

Hydrogenation of the Enedione (IX) *to* (X).—The unsaturated ketone (IX) (50 mg.) in ethanol was hydrogenated over palladium–charcoal. One equivalent of hydrogen was absorbed. The *product* crystallised from acetone–hexane, m. p. 161—163°; $[\alpha]_D$ −104° (*c* 0·45); $\nu_{max.}$ 1739 (acetate) and 1712 cm.$^{-1}$ (two carbonyls); no strong ultraviolet absorption (Found: C, 72·8; H, 9·0. $C_{27}H_{40}O_5$ requires C, 72·95; H, 9·05%).

Preparation of the Hydroxy-ester (XXIII).—A solution of deoxydihydrowithaferin A acetate (IIIb; 200 mg.) in glacial acetic acid (5 ml.) containing toluene-*p*-sulphonic acid (200 mg.) was left aside for 3 days. Following a work-up similar to the one described for compound (XXI), the crude product (225 mg.) was chromatographed on alumina, and the fractions eluted with hexane–chloroform (7 : 3) were combined (190 mg.). Notwithstanding the fact that the *product* was pure (one spot on t.l.c.) it could not be induced to crystallise; $\nu_{max.}$ 1736 (acetate), 1709 (two carbonyls), 1603, 1193, and 1176 cm.$^{-1}$; $\lambda_{max.}$ 226 and 272 mμ (ε 17,800 and 500) (Found: S, 4·9. $C_{37}H_{50}O_9S$ requires S, 4·77%).

*Alkaline Treatment of the Toluene-*p*-sulphonate* (XXIII).—A solution of the hydroxy-ester (XXIII) in methanol was treated with 2% sodium methoxide in methanol, as described above for the bromohydrin (XII). Following a similar work-up, the product (60 mg.) was identified as deoxydihydrowithaferin A (IIIa). Acetylation with acetic anhydride in pyridine yielded the corresponding acetate (IIIb).

Preparation of 2,3*-Dihydro-*3*-Methoxywithaferin A* (XXIVa).—Withaferin A (Ia; 500 mg.) in methanol (100 ml.) containing toluene-*p*-sulphonic acid (50 mg.) was heated under reflux for 4 hr. After neutralisation of the solution with aqueous soldium hydrogen carbonate, most

of the solvent was removed *in vacuo*, water added, and the solid collected and washed. The crude product was chromatographed on alumina. Elution with chloroform–methanol (99 : 1) yielded fractions which were combined according to t.l.c. indications. The first fractions contained some unreacted substance while the later fractions yielded the *product* (300 mg.); after repeated crystallisations from ethyl acetate, m. p. 243—245°; $[\alpha]_D$ +17·5° (*c* 0·89); $\nu_{max.}$ 1709sh and 1704 cm.$^{-1}$; $\lambda_{max.}$ 212 mμ (ε 9500) (Found: C, 69·4; H, 8·35. $C_{29}H_{42}O_7$ requires C, 69·3; H, 8·4%).

The *diacetate* (XXIVb) was obtained by acetylation of (XXIVa) with acetic anhydride in pyridine overnight at room temperature. Following the usual work-up, the crude acetate crystallised from methanol, m. p. 166—167°; $[\alpha]_D$ −12° (*c* 0·79); $\nu_{max.}$ 1739 and 1712 cm.$^{-1}$; $\lambda_{max.}$ 215 mμ (ε 8800) (Found: C, 67·35; H, 7·85. $C_{33}H_{46}O_9$ requires C, 67·55; H, 7·9%).

Hydrogenolysis of Compound (XXIVa) *to the Deoxy-derivative* (XXVa).—Compound (XXIVa) (200 mg.) in ethanol (50 ml.) was hydrogenated at room temperature and atmospheric pressure over palladium–charcoal. The reaction was discontinued after absorption of one equivalent of hydrogen and the crude product obtained on removal of the solvent was chromatographed through alumina. Elution with hexane–chloroform (3 : 2) and crystallisation from acetone gave the product (150 mg.), m. p. 259—261° (decomp.); $[\alpha]_D$ +6° (*c* 0·86); $\nu_{max.}$ 1701 cm.$^{-1}$; $\lambda_{max.}$ 226 mμ (ε 8000) (Found: C, 71·7; H, 9·0. $C_{29}H_{42}O_6$ requires C, 71·57; H, 8·70%).

The *acetate* (XXVb) was obtained by acetylation of (XXVa) with acetic anhydride in pyridine overnight at room temperature. It crystallised from acetone–hexane, m. p. 200—202°; $[\alpha]_D$ −18° (*c* 0·79); $\nu_{max.}$ 1736 and 1695 cm.$^{-1}$; $\lambda_{max.}$ 226 mμ (ε 8500) (Found: C, 70·6; H, 8·3. $C_{31}H_{44}O_7$ requires C, 70·45; H, 8·4%). The same acetate (XXVb) was obtained by the hydrogenation of the diacetate (XXIVb) in ethanol over palladium–charcoal. After the absorption of one equivalent of hydrogen, the reaction was discontinued and the crude product obtained on the removal of the solvent was chromatographed through alumina. The product was eluted with hexane–chloroform (7 : 3) and crystallised from acetone–hexane, m. p. and mixed m. p. 200—202°.

Hydrogenation of the Acetate (XXVb) *to Tetrahydro-3-methoxywithaferin A Acetate* (XXVI).—The acetate (XXVb) (100 mg.) in ethanol, was hydrogenated over palladium–charcoal; the absorption was very slow. After *ca.* 24 hr. the catalyst was filtered off, the solvent removed, and the crude product chromatographed through alumina. Elution with hexane–chloroform (8 : 2) yielded fractions which were combined and evaporated, and the *product* was crystallised several times from methanol, m. p. 177—178°; $\nu_{max.}$ 1735 and 1725 cm.$^{-1}$; no strong ultraviolet absorption (Found: C, 70·35; H, 8·65. $C_{31}H_{46}O_7$ requires C, 70·15; H, 8·75%).

Ozonolysis of the Acetate (XXVb) *to the* β*-Hydroxy-ketone* (XXVII).—The ozonolysis of compound (XXVb) (140 mg.) was performed at 0° in a mixture of chloroform (5 ml.) and ethyl acetate (12 ml.). The reaction was discontinued when *ca.* 1·3 equivalents of ozone had been introduced, thereafter the solvent was removed and the crude ozonide was dissolved in acetic acid (5 ml.) and stirred for several hours with zinc powder (100 mg.). After filtration, water was added, the product extracted with chloroform, and the solution washed with water and aqueous sodium hydrogen carbonate. Evaporation of the solvent left a residue which was rapidly chromatographed through alumina. Elution with hexane–chloroform (1 : 1) yielded fractions which were combined according to t.l.c. indications (105 mg.), and crystallised twice from acetone–hexane, m. p. 213—215°; $[\alpha]_D$ −81° (*c* 0·48); $\nu_{max.}$ 1736 and 1706 cm.$^{-1}$; no strong ultraviolet absorption (Found: C, 68·6; H, 8·6. $C_{28}H_{42}O_7$ requires C, 68·55; H, 8·65%).

Preparation of the Unsaturated Ketone (XXVIII).—A solution of the β-hydroxy-ketone (XXVII) (80 mg.) in chloroform (2 ml.) was introduced into a column of alumina (10 g.) made up with benzene–chloroform (1 : 1) and left aside for 3 days. The product was then eluted with chloroform; the residue which was obtained following the removal of the solvent showed two spots on t.l.c. Careful chromatography on alumina resulted in the separation of two compounds. Elution with hexane–chloroform (4 : 1) yielded the product showing the upper spot (45 mg.) while hexane–chloroform (1 : 1) eluted the second product, identical with the unreacted material. The former *compound* (XXVIII) crystallised from acetone–hexane, m. p. 185—187°; $[\alpha]_D$ −105° (*c* 0·57); $\nu_{max.}$ 1736, 1709, 1664, and 1626 cm.$^{-1}$; $\lambda_{max.}$ 225 mμ (ε 13,800) (Found: C, 71·0; H, 8·65. $C_{28}H_{40}O_6$ requires C, 71·15; H, 8·55%).

Preparation of Dihydrowithaferin 27*-Methyl Ether* (XXIXa).—To a solution of dihydrowithaferin A (IIa; 200 mg.) in methanol (20 ml.) a methanolic solution of potassium hydroxide (2%, 30 ml.) was added and the mixture was left aside overnight at room temperature. Water (60 ml.) was then added, the solution was acidified to pH ~ 3 and most of the methanol was distilled off *in vacuo*. The precipitated *solid* was filtered and washed; it crystallised from ethyl

acetate and then from acetone–hexane, m. p. 183—185°; $[\alpha]_D$ +7° (*c* 0·69); $\nu_{max.}$ 1706 cm.$^{-1}$; $\lambda_{max.}$ 214 mμ (ε 9200) (Found: C, 71·35; H, 8·55. $C_{29}H_{42}O_6$ requires C, 71·55; H, 8·7%).

The *acetate* (XXIXb) was prepared by the acetylation of the crude ether (XXIXa) with acetic anhydride in pyridine overnight at room temperature. The product was chromatographed on alumina and the fractions eluted with hexane–chloroform (7 : 3) were combined according to their homogeneity on t.l.c. The product was used for the following reaction without any further purification.

Hydrogenolysis of (XXIXb) *to* (IIIb).—Compound (XXIXb) (65 mg.) was hydrogenated in ethanol solution (25 ml.) over palladium–charcoal. The reaction was discontinued after the absorption of one equivalent of hydrogen. Removal of the solvent left a residue which crystallised from acetone–hexane and was identified as deoxydihydrowithaferin A acetate (IIIb) (spectral data, R_F values on t.l.c., and mixed m. p.).

Preparation of Dihydro-3-methoxywithaferin A 27-Methyl Ether (XXXa).—(*a*) *From withaferin A*. Withaferin A (200 mg.) was treated with methanolic potassium hydroxide as described above for compound (XXIXa). After the work-up, the crude product was chromatographed on alumina and the fractions which were eluted with hexane–chloroform (1 : 1) and exhibited only one spot on t.l.c. were combined (140 mg.). The *product* (XXXa) crystallised from methanol, m. p. 217—218°; $[\alpha]_D$ +22° (*c* 0·68); $\nu_{max.}$ 1695 cm.$^{-1}$; $\lambda_{max.}$ 215 mμ (ε 9100) (Found: C, 69·85; H, 8·35. $C_{30}H_{44}O_7$ requires C, 69·75; H, 8·6%).

(*b*) *From dihydro-3-methoxywithaferin A* (XXIVa). Compound (XXIVa) (100 mg. when subjected to the same reaction, yielded a compound (40 mg.) identical with the compound (XXXa) described above.

The *acetate* (XXXb), obtained by acetylation of (XXXa) under the usual conditions, crystallised from acetone–hexane, m. p. 173°; $[\alpha]_D$ −8° (*c* 0·53); $\nu_{max.}$ 1739 and 1706 cm.$^{-1}$; $\lambda_{max.}$ 214 mμ (ε 9000) (Found: C, 68·75; H, 8·5. $C_{32}H_{46}O_8$ requires C, 68·8; H, 8·3%).

Hydrogenolysis of (XXXa) *to* (XXVa).—Compound (XXXa) (80 mg.) in ethanol was hydrogenated over palladium–charcoal, and the reaction was discontinued after the absorption of one equivalent of hydrogen. After the work-up, the product crystallised from acetone (50 mg.) and was identified as (XXVa). Acetylation of this product yielded the corresponding acetate (XXVb).

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