Tetrahedron. Vol. 26, pp. 2209 to 2219. Pergamon Press 1970. Printed in Great Britain

CONSTITUENTS OF *WITHANIA SOMNIFERA* DUN—XI*

THE STRUCTURE OF THREE NEW WITHANOLIDES†

I. KIRSON,[a]‡ E. GLOTTER,[a] A. ABRAHAM[b] and D. LAVIE[a]

[a] Department of Chemistry, The Weizmann Institute of Science, Rehovoth, Israel
[b] The National and University Institute of Agriculture, Rehovoth, Israel

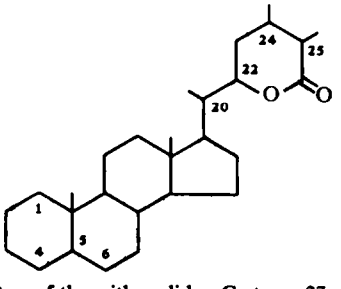
(*Received in the UK* 20 *December* 1969; *Accepted for publication* 2 *February* 1970)

Abstract—Three new withanolides (C_{28} steroids possessing a 6-membered ring lactone in the side chain) have been isolated from *Withania somnifera* (Solanaceae) growing in South Africa and characterized as 4β-hydroxy-1-oxo-5β,6β-epoxywitha-2,24-dienolide (IIIa), 4β,20α,(*R*)dihydroxy-1-oxo-5β,6β-epoxywith-2-enolide (IVa),§ and 4β-hydroxy-1-oxo-5β,6β-epoxywith-2-enolide (Va). The major steroidal components of the above plant are the known withaferin A (4β,27-dihydroxy-1-oxo-5β,6β-epoxywitha-2,24-dienolide) and withanolide D (4β,20α,(*R*)-dihydroxy-1-oxo-5β,6β-epoxywitha-2,24-dienolide). The structures assigned to compounds IIIa, IVa and Va are based on spectral evidence (NMR, IR and UV), analysis of their fragmentation under electron impact, as well as on chemical degradation to known compounds.

IN PREVIOUS publications[1–6] we dealt with the structure of the withanolides, a new series of C_{28} steroidal lactones occurring in plants of the Solanaceae family. In the present paper the results obtained following a chemical investigation of *Withania somnifera* raised on experimental plots in our nursery at Beth Dagan, Israel, from seeds obtained from South Africa‖ are described.

* Part X, see ref. 5.

† The name "withanolide" has been proposed by us[3] for this new type of steroidal lactones characterized by a 9 C atoms side chain with a 6-membered ring lactone.



In the description of the basic skeleton of the withanolides, C atoms 27 and 28 have not been included; since 1966 several members of this group of compounds, have been isolated from natural sources, and in all of them these two C atoms were present; it seemed therefore justified[5] to describe the withanolides with a C-28 basic skeleton; even more so, from a biogenetic point of view, the withanolides can be considered as possessing a highly oxygenated cholestane type side chain bearing an extra Me group at C-24.

‡ In partial fulfillment of the requirement for the Ph.D. degree at The Feinberg Graduate School, The Weizmann Institute of Science.

§ The stereochemistry at C-20 is according to Fieser's designation.[7a]

‖ A comparative study of *Withania somnifera* growing in different geographic areas will be published elsewhere.

Chromatography of the crude methanol extract obtained from the leaves resulted in the separation of five withanolides. The main component (about 90% of the total amount of crystalline product and ~0·9% of the dried leaves) was identified as withaferin A (Ia)[1–3] well known from our previous studies on *W. somnifera* chemotype I (Israeli origin). The second compound present in much smaller proportions (~0·05% of the dry leaves) was identified as withanolide D (IIa)[5] previously isolated as the main component of *W. somnifera* chemotype II (from Israel). The three compounds (IIIa, IVa and Va) which have been obtained in small quantities (~0·003, 0·05 and 0·003% respectively) have not yet been encountered.

The NMR spectrum of IIIa, m.p. 268–269°, mol wt 454, afforded all the structural information necessary for its characterization (see Table 1). The signals of the relevant protons of the carbocyclic system are found at the same position with similar patterns as the corresponding protons in compounds Ia and IIa. Analysis of the signals due to the protons of the side chain lactone leads to the conclusion that the C-22H exhibits the same multiplicity as in compound Ia whereas the position of the two olefinic Me groups at C-24 and C-25 parallels the chemical shift of the corresponding methyls in IIa.

The presence of only one secondary OH group (at C-4) was confirmed by preparing a monoacetate IIIb characterized by the expected shift of the C-4H signal, from δ 3·76 (IIIa) to δ 4·60 (IIIb). The UV absorption of IIIa (λ_{max} 215 nm ε 17,500) indicates two overlapping bands, one due to the $\alpha\beta$-unsaturated ketone in ring A and the second to the $\alpha\beta$-unsaturated lactone. Catalytic hydrogenation of IIIb proceeded with the rapid absorption of one mole of hydrogen to yield a dihydroderivative (VI) identical with that obtained by reduction of Ib (following absorption of two moles of hydrogen).

The structure of IIIa is therefore unequivocally established as 4β-hydroxy-1-oxo-5β,6β-epoxywitha-2,24-dienolide.

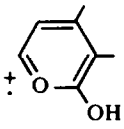
The characterization of compound IVa is based on the analysis of the spectral data as well as by degradation to the pregnane derivative VII which has been independently obtained by a corresponding sequence performed on IIa.[5]

Comparison of the NMR signals of IVa and IIa shows a close similarity as far as the characteristic protons of the carbocyclic system are involved. The high field region of the spectrum of IVa was clearly different from that of IIa. Instead of the signals of the two vinylic Me groups in IIa, two doublets [at δ 1·21 (d, J = 6 Hz) and δ 1·16 (d, J = 6 Hz)] were present implying two secondary Me groups. Since the UV absorption band of IVa (λ_{max} 214 nm, ε 9500) pointed towards the presence of only one $\alpha\beta$-unsaturated carbonyl system in this molecule, it can be concluded that the 6-membered ring lactone is saturated. Supporting evidence was found in the IR spectrum which showed in the carbonyl region two bands at 1681 and 1730 cm^{-1} (unsaturated 6-membered ring ketone and saturated 6-membered ring lactone, respectively).

Upon acetylation of IVa the monocetate IVb was obtained, characterized by the expected shift of the C-4H from δ 3·75 to δ 4·60, together with the new signal of the acetoxy Me group. The catalytic hydrogenation proceeded with the absorption of only one mole of hydrogen, yielding the dihydroderivative VIII devoid of any absorption in the UV region. The reduction of the double bond (Δ^2) in IV could be also followed in the NMR spectrum of VIII by the disappearance of the signals of

the vinylic C-2H and C-3H and the change in the pattern of the C-4H (from a doublet at δ 4·60 to a narrow triplet at δ 4·58) pointing unequivocally to the equatorial orientation of the C-4H.

These structural assignments are confirmed through the analysis of the mass spectra of compounds IVa and VIII (molecular ions M^+ 472 and 516, respectively). These spectra do not have the *m/e* 125 peak which is present in all the withanolides with a double bond (Δ^{24}) in the lactone ring. This peak is due to cleavage of the C_{20}—C_{22} bond, resulting in the ion:



In these two compounds the 100% peak (*m/e* 345 and 389, respectively) is due to the fission of the same bond and loss of 127 m.u. (the saturated lactone). The presence of the C-20 OH facilitates the cleavage of the C_{17}—C_{20} bond in IVa as well as in VIII yielding a fragment *m/e* 171 (the whole side chain); the same cleavage in IIa leads to the formation of the ion *m/e* 169. Other important peaks in the spectrum of IVa, related to these fragmentations, are: *m/e* 327 (M^+ −127–18), 309 (M^+ −127–2 × 18), and 283 (M^+ −171–18); the corresponding peaks in the dihydroacetate VIII are at *m/e* 371 (M^+ −127–18), 329 (M^+ −127–60), 311 (M^+ −127–60–18) and 285 (M^+ −171–60).

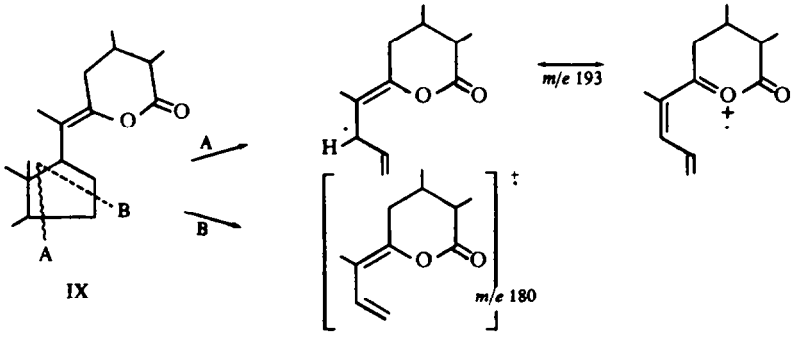
In order to confirm the assignments of the peaks obtained in the mass spectrum of VIII, this compound was treated with MeOD/MeONa in order to exchange the two hydrogens at C_2 and the one at C_{25} with deuterium; indeed the deuteriated product VIII showed M^+ 519. In this compound the peaks due to fragments derived from the carbocyclic skeleton (with the two deuterium at C_2) had two m.u. more than the corresponding signals in the nondeuteriated VIII (*m/e* 391, 373, 331, 313, 287); the peak due to the whole side chain was now at *m/e* 172, one m.u. more than before deuteriation.

The NMR indication for the replacement of the C-25 H by a D atom was obtained from the signal of the C-25 Me group; in the original compound it was split into a doublet δ 1·21 (J = 6 Hz), whereas after deuteriation it appeared as a singlet at the same position (δ 1·21).

Elimination of the tertiary C-20 OH in VIII was smoothly performed with thionyl chloride in excess pyridine to give the corresponding crystalline enol-lactone IX. A similar elimination has been previously performed on a derivative of IIa but the product (enol-lactone) could not be purified, since already during chromatography on silica gel the exocyclic Δ^{20} entered into conjugation with the endocyclic Δ^{24} to give a mixture of two stereoisomeric pyrone derivatives.[5]

The structure of the enol-lactone IX was determined by inspection of its NMR spectrum and comparison with that of VIII; one could observe the disappearance of the signal of C-22 as well as the expected changes in the pattern of the C-20Me (downfield shift from δ 1·26 in VIII to δ 1·51 in IX) in agreement with its olefinic character.

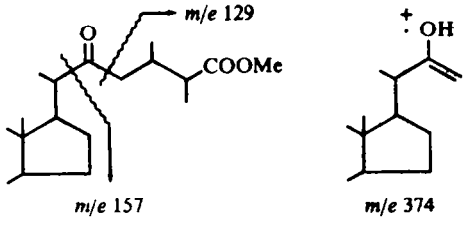
The enol-lactone was further characterized by three important peaks in its mass spectrum: 498 (M^+; 100% peak), 193 and 180. The *m/e* 193 signal arises presumably through the fragmentation path A while the fragment *m/e* 180 is due to cleavage of ring D according to path B.



To ascertain these fragmentations, the $SOCl_2$ induced elimination of the C-20 OH was repeated with the deuteriated compound VIII, yielding the enol-lactone IX in which the two C-2 hydrogens and the C-25 H are exchanged by deuterium (M^+ 501). The mass spectrum of deuteriated IX shows peaks at *m/e* 194 and 181 instead of the peaks at *m/e* 193 and 180 in the non deuteriated compound, i.e. with one m.u. more, respectively. The assumption that these ions contain the lactone ring is thereby confirmed.

The chemical confirmation for structure IX was adduced by conversion into the δ-ketoester X upon treatment with methanolic sodium methoxide. The structural changes in X as compared to IX were nicely illustrated by a new signal for the Me group of the carbomethoxy moiety at δ 3·68, and by the upfield shift of the C-20 Me signal, now exhibiting a doublet pattern at δ 0·98 (J = 6 Hz). The IR spectrum of X (CO region) showed two bands, one at 1739 cm^{-1} for the ester carbonyl, and a second more intense band at 1709 cm^{-1} for the two ketones at C-1 and C-22.

The mass spectrum of the δ-ketoester showed in addition to the molecular ion (M^+ 488) a series of significant peaks related to different cleavage processes of the side chain. Cleavage of the bonds on the two sides of the C-22 ketone gives the very important peaks *m/e* 157 (100% peak) and 129. The peaks at *m/e* 470, 457 and 456 are due to the loss of H_2O, MeO and MeOH respectively. The peak corresponding to the McLafferty rearrangement involving the C-22 ketone and the γ H(C-25 H) (*m/e* 374) is present, however weaker than the previous signals.



Ozonolysis of IX produced the methyl ketone VII, identical with that obtained from the degradation of IIa.[5] The stereochemistry at C-20 in IVa is 20α(*R*) using the same criteria which served for this stereochemical assignment in compound II, i.e. comparison with the positions of the C-13 Me and C-20 Me in the NMR spectra of cholesterol, 20α- and 20β-hydroxy-cholesterol.[5,7] The data presented herewith, firmly establish the structure of IVa as 4β,20α,(*R*)-dihydroxy-1-oxo-5β,6β-epoxywith-2-enolide; the only points which remain for further study are the asymmetric centers of the lactone ring (C_{22}, C_{24} and C_{25}).

A final interrelation between IVa and IIa was attempted by preparing a tetrahydroderivative of the latter, assuming that such a compound would be identical with VIII. Whereas the reduction of the Δ^2 in IIb proceeded smoothly it was however, impossible to hydrogenate the Δ^{24} double bond in this compound (only different palladium catalysts were used).

The last compound which was isolated from the crude extract only in minute amounts was Va; it is related to IIIa in the same way as compound IVa is related to IIa, i.e. Va is the 24-dihydroderivative of IIIa. The relevant signals of the protons on the carbocyclic skeleton of Va are at the same positions and have the same multiplicities as the corresponding signals in compounds I–IV (see Table 1). In contrast to compound IIIa, Va features two doublets at δ 1·21 and 1·11 indicating two secondary Me groups on saturated C atoms. These data, in conjunction with the ultraviolet spectrum (λ_{max} 214 nm, ε 9550) similar to that of IVa, and infrared absorption bands at 1730 and 1681 cm^{-1}, point unambiguously to the presence of a saturated lactone ring in Va.

Further insight into the structure of Va was obtained by acetylation (Vb) and catalytic hydrogenation to a dihydroderivative XI devoid of major UV absorption.

At this stage it is worthwhile to refer to the catalytic hydrogenation of withaferin A diacetate (Ib).[1] The hydrogenation of Ib could be performed stepwise: reduction of Δ^2, hydrogenolysis of the allylic C-27 OH, and saturation of the tetrasubstituted double bond in the lactone ring. The product tetrahydrodesoxywithaferin A acetate (XII) was however different from compound XI.

The close similarity of these two isomeric compounds was revealed by a quasi identical fragmentation pattern under electron impact.

Comparison of their NMR spectra suggested however, that the difference should be in the stereochemistry of the lactone ring.

Since compound XII was obtained by catalytic reduction, a *cis* orientation was assigned to the C-24 and C-25 Me groups; when exposed to sodium methoxide in methanol[1] the product underwent epimerization to XIII, the Me groups becoming *trans*.

Under similar alkaline conditions followed by reacetylation of the C-4 OH, XI was left unchanged; it was however, different from compound XIII. When the reaction was performed in methanol-d_1, only exchange of the two C-2H and of the C-25 H with deuterium took place. Compound VIII behaved in the same manner, suggesting a similarity in the stereochemistry of the lactone ring in both VIII and XI (and correspondingly in IVa and Va).

Additional proof for the stereochemical similarity of the lactone ring in compounds VIII and XI was found using molecular rotation differences (ΔM_D) between the saturated lactones and the corresponding Δ^{24} derivatives (Table 2).

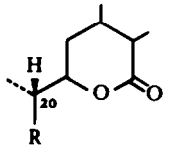
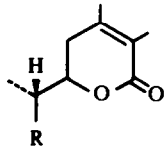
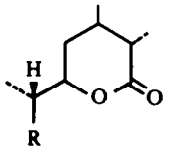
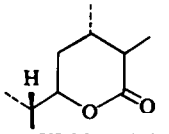
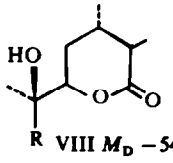
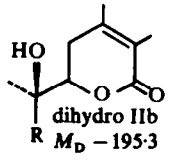
TABLE 1. CHEMICAL SHIFTS OF SIGNIFICANT PROTONS IN CERTAIN WITHANOLIDES (IN δ UNITS)

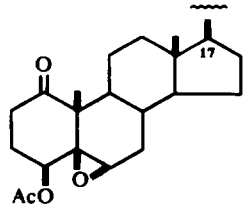
Compound	C-2 H	C-3 H	C-4 H	C-6 H	C-22 H	C-10 CH_3	C-13 CH_3	C-20 CH_3	C-24 CH_3	C-25 CH_3
Ia	6·18(d) J 10 Hz	6·97(dd) J 10; 6 Hz	3·75(d) J 6 Hz	3·20 $W_{\frac{1}{2}} \sim 4$ Hz	4·40(dt) J 12; 3·5 Hz	1·38(s)	0·68(s)	0·97(d) J 6 Hz	2·03(s)	4·35(s) C-25 $C\underline{H}_2OH$
IIa	6·21(d) J 10 Hz	6·97(dd) J 10; 6 Hz	3·77(d) J 6 Hz	3·25 $W_{\frac{1}{2}} \sim 4$ Hz	4·25(dd) J 12; 5 Hz	1·41(s)	0·85(s)	1·27(s)	1·91 two methyl signal	
IIIa	6·20(d) J 10 Hz	7·08(dd) J 10; 6 Hz	3·76(d) J 6 Hz	3·23 $W_{\frac{1}{2}} \sim 4$ Hz	4·37(dt) J 12; 3·5 Hz	1·39(s)	0·68(s)	0·98(d) J 6 Hz	1·91 two methyl signal	
IVa	6·20(d) J 10 Hz	6·96(dd) J 10; 6 Hz	3·75(d) J 6 Hz	3·25 $W_{\frac{1}{2}} \sim 4$ Hz	4·16(dd) J 10·5; 4 Hz	1·40(s)	0·86(s)	1·26(s)	1·16(d) J 6 Hz	1·21(d) J 6 Hz
Va	6·21(d) J 10 Hz	7·07 J 10; 6 Hz	3·76(d) J 6 Hz	3·21 $W_{\frac{1}{2}} \sim 4$ Hz	4·31(dt) J 11; 3 Hz	1·41(s)	0·69(s)	0·95(d) J 6 Hz	1·11(d) J 6 Hz	1·21 J 6 Hz

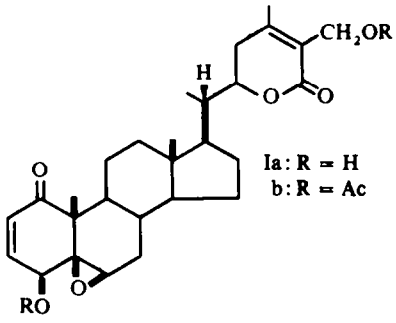
It can be concluded therefore that there are subtle differences between the lactone rings in the natural occurring withanolides IVa and Va and the saturated compounds (XII, XIII) prepared in the laboratory through hydrogenation and epimerization procedures.

The conformation of the lactone ring as well as the configuration of the substituents at this ring (C-22 H, C-24 Me, C-25 Me) were elucidated by analysis of the solvent shifts of the corresponding NMR signals together with circular dichroism measurements. The following paper deals with this analysis.

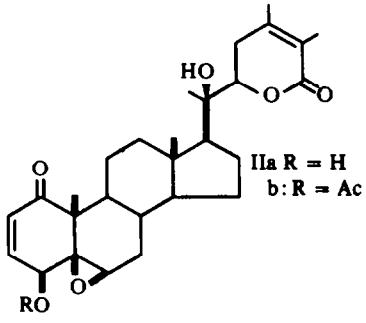
TABLE 2. MOLECULAR ROTATION DIFFERENCES (ΔM_D) IN CERTAIN WITHANOLIDES

Saturated lactones	Unsaturated lactones	ΔM_D
 XII M_D −495	 VI M_D −69·7	−425·3
 XIII M_D −225	ibid	−155·3
 XI M_D −412·5	ibid	−342·8
 VIII M_D −546·9	 dihydro IIb M_D −195·3	−351·6

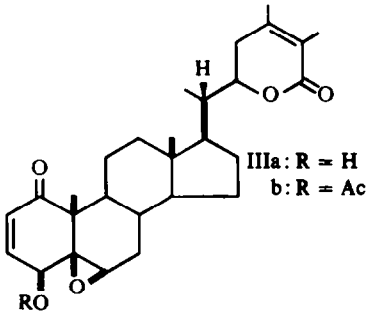
R = 



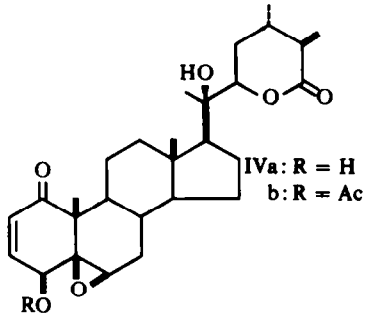
Ia: R = H
b: R = Ac



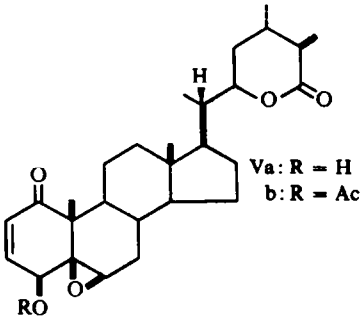
IIa R = H
b: R = Ac



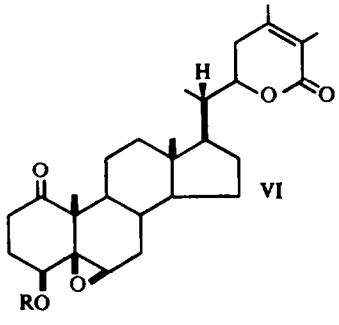
IIIa: R = H
b: R = Ac



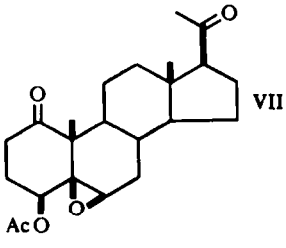
IVa: R = H
b: R = Ac



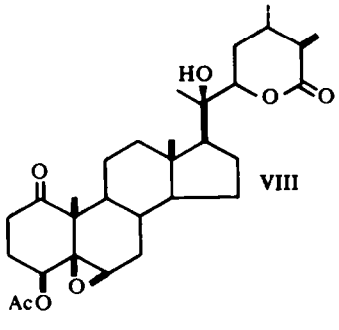
Va: R = H
b: R = Ac



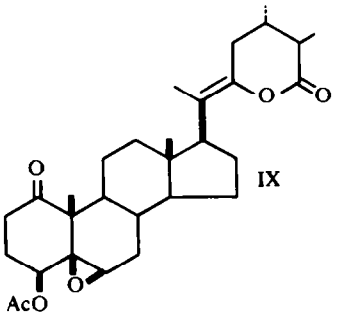
VI



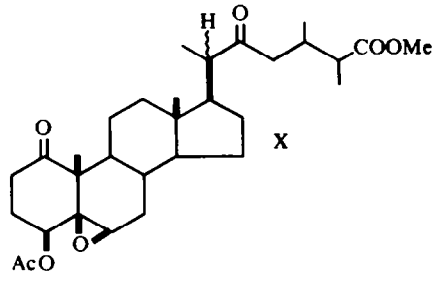
VII



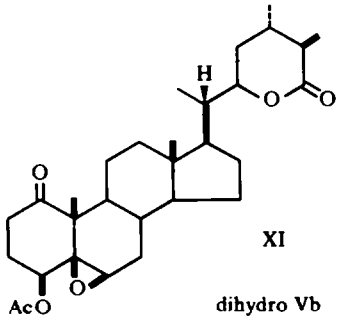
VIII



IX

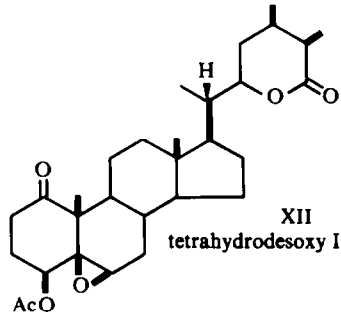


X



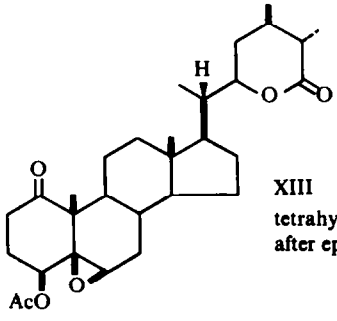
XI

dihydro Vb



XII

tetrahydrodesoxy Ib



XIII

tetrahydrodesoxy Ib
after epimerization

EXPERIMENTAL

M.ps were taken on a Fisher–Johns apparatus and are uncorrected. Optical rotations were recorded with an automatic Perkin–Elmer 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 KBr pellets; UV spectra were recorded on a Cary 14 instrument; NMR spectra were determined on a Varian A-60 spectrometer, for 5–10% solns in $CDCl_3$, containing TMS as internal standard. TLC were carried on chromatoplates of silica gel G (Merck) and spots were developed with iodine vapors. Mass spectra were taken with an Atlas CH4 instrument.

Isolation procedure. Crushed air dried leaves (960 g) of *Withania somnifera* raised from seeds obtained from South Africa were exhaustively extracted with MeOH (Soxhlet). The methanolic extract was then concentrated to a volume of ~3 l., the same quantity of water was added and the mixture extracted with hexane to remove chlorophyl and other pigments. The residual aqueous methanolic soln was reextracted with ether, the ethereal extract washed with water, dried (Na_2SO_4) and the solvent removed to leave a

green residue (~12 g). This crude product was introduced at the top of a chromatographic column made up with silica gel H (Merck) (300 g), and elution was performed with mixtures of benzene: EtOAc, fractions of 35 ml each being collected.

Compound	Fractions No.	Eluted with benzene: ethyl acetate
Va	141–146	8:2
IIIa	151–158	8:2
IVa	185–217	8:2
IIa	229–283	7:3
Ia	327–496	6:4

Compounds Ia and IIa crystallized from $CHCl_3$–EtOAc to yield 8·25 g and 0·47 g of withaferin A and withanolide D respectively, identified by direct comparison with authentic samples.[1,5].

Compound IIIa (28 mg) crystallized from EtOAc, m.p. 268–269°; $[\alpha]_D$ +101·5° (c, 0·5); ν_{max} 1690 cm^{-1}; λ_{max} 215 nm (ε 17,500). (Calcd for $C_{28}H_{38}O_5$; M wt 454·6, Found: M^+ 454).

Compound IVa (500 mg), m.p. 275° (from EtOAc), $[\alpha]_D$ +14° (c, 0·3); ν_{max} 1730, 1681 cm^{-1}; λ_{max} 214 nm (ε 9500) (Calcd for $C_{28}H_{40}O_6$, M wt 472·6. Found: M^+ 472).

Compound Va (37 mg), m.p. 252° (from $CHCl_3$–EtOAc), $[\alpha]_D$ +32·5° (c, 0·52); ν_{max} 1730, 1681 cm^{-1}; λ_{max} 214 nm (ε 9550). (Calcd for $C_{28}H_{40}O_5$, M wt 456·6. Found: M^+ 456).

Acetylation of IIIa. Compound IIIa (20 mg) was treated with Ac_2O (0·5 ml) in pyridine (0·5 ml) at room temp to yield IIIb (20 mg), m.p. 227–228° (from EtOAc), $[\alpha]_D$ +191° (c, 0·45); ν_{max} 1730, 1690 and 1250 cm^{-1}; λ_{max} 217 nm (ε 16,900). (Calcd for $C_{30}H_{40}O_6$, M wt 496·6. Found: M^+ 496).

Hydrogenation of IIIb to VI. Compound IIIb (16 mg) in abs EtOH (10 ml) was hydrogenated over Pd–C 10% at room temp and atm press. The reaction was discontinued after 1/2 hr and the product crystallized from acetone–hexane (14 mg), m.p. 258–9°. It was identified as VI by direct comparison with an authentic sample.[1]

Acetylation of IVa. Compound IVa (450 mg) was treated as above with Ac_2O (4 ml) in pyridine (4 ml) to yield IVb (445 mg), m.p. 237–238° (from acetone–hexane), $[\alpha]_D$ +112·6° (c, 0·27); ν_{max} 1735, 1681, 1250 cm^{-1}; λ_{max} 216 nm (ε 9400). (Calcd for $C_{30}H_{42}O_7$; M wt 514·6. (Found: M^+ 514).

Hydrogenation of IVb to VIII. Compound IVb (400 mg) was hydrogenated over 10% Pd–C in EtOH soln, at room temp and atm press. The absorption ceased after ~1 hr. The product crystallized from acetone–hexane, m.p. 274–276°; $[\alpha]_D$ −106° (c, 0·26); ν_{max} 1739, 1715 and 1250 cm^{-1}; no major maximum absorption in the UV. (Calcd for $C_{30}H_{44}O_7$; M wt 516·6. Found: M^+ 516).

Conversion of VIII into the enol-lactone IX. To a soln of VIII (200 mg) in dry pyridine (5 ml) at −13° (ice salt bath) a soln of freshly distilled $SOCl_2$ (1 ml) in pyridine (1 ml) was slowly added. After 10 min at the same temp the mixture was poured onto ice, filtered and the solid washed with water, and isolated with $CHCl_3$. The solvent was then removed and the residue (180 mg) chromatographed on silica gel (0·05–0·2 mm). Elution with benzene–EtOAc (9:1) yielded crystalline product (160 mg) m.p. 163–165° (from MeOH), $[\alpha]_D$ −122·5° (c, 0·46); ν_{max} 1739, 1715 and 1250 cm^{-1}; strong end absorption in the UV. (Calcd for $C_{30}H_{42}O_6$; M wt 498·6. Found: M^+ 498).

Ozonolysis of IX to compound VII. A soln of IX (100 mg) in EtOAc (20 ml) was ozonized at −10°. The reaction was discontinued after 10 min, when the excess O_3 was removed under reduced press and the residue dissolved in AcOH (5 ml) and stirred with Zn powder (100 mg) during 4 hr. Following filtration, ether (200 ml) was added, the acid removed by shaking with $NaHCO_3$ aq. the ethereal soln dried (Na_2SO_4), the solvent removed and the residue (60 mg) chromatographed over silica gel H. Elution with benzene–EtOAc (7:3) yielded a crystalline product (32 mg), m.p. 204° (from EtOAc). The compound was identified as VII by direct comparison with an authentic sample.[5]

Alkaline treatment of IX. To a soln of IX (40 mg) in MeOH (1 ml) a methanolic soln of MeONa (from 10 mg Na in 2 ml MeOH), was added. After 3 hr at room temp the pH was adjusted to ~5 (dilute HCl); most of the solvent was then removed, water was added and the solid which separated was isolated with $CHCl_3$. Following evaporation of the solvent the residue (30 mg) was filtered through silica gel 0·05–0·2 mm, yielding an amorphous white product which could not be induced to crystallize (Calcd for $C_{29}H_{44}O_6$ M wt 488·6. Found: M^+ 488).

Attempted equilibration of VIII. The dihydroacetate VIII (40 mg) in methanolic MeONa (10 mg Na in 2 ml MeOH) was heated to reflux under N_2 during 4 hr, then acidified to pH ~5. The product was isolated with $CHCl_3$, the solvent was removed and the residue reacetylated with Ac_2O in pyridine. The product which was obtained following this treatment was found identical in all respects with the original compound VIII.

Deuteriation of VIII. A similar treatment as above but performed in presence of MeOD yielded the trideuterio VII. Following reacetylation, the M wt of the product was found to be 519 (mass spectrometry).

Acetylation of Va. Compound Va was acetylated in the usual conditions and afforded the monoacetate Vb, m.p. 213° (from EtOAc), $[\alpha]_D$ +124° (*c*, 0·50); ν_{max} 1739, 1681 and 1250 cm^{-1}; λ_{max} 216 nm (ε 9480). (Calcd for $C_{30}H_{42}O_6$, M wt, 498·6. Found: M^+ 498).

Hydrogenation of Vb to the dihydroderivative XI. The reaction was performed with 28 mg of product as described for IVb. Compound XI (27 mg), crystallized from MeOH, m.p. 241–242°, $[\alpha]_d$ −85·5° (*c*, 0·22); ν_{max} 1739, 1715 and 1250 cm^{-1}; no major maximum absorption in the UV. (Calcd for $C_{30}H_{44}O_6$, M wt 500·6. Found: M^+ 500).

Attempted equilibration of XI. The reaction was performed on 16 mg of product as described for VIII. The unreacted starting material was recovered.

Deuteriation of XI. The deuteriation was done as for VIII. In the NMR spectrum, the doublet of the C-25 Me (δ 1·22 J = 6 Hz) present in XI before deuteriation appeared now as a singlet with the same chemical shift. M wt (following reacetylation) 503.

REFERENCES

[1] D. Lavie, E. Glotter and Y. Shvo, *J. Org. Chem.* **30**, 1774 (1965)
[2] D. Lavie, E. Glotter and Y. Shvo, *J. Chem. Soc.* 7517 (1965)
[3] D. Lavie, S. Greenfield and E. Glotter, *Ibid.* (C), 1753 (1966)
[4] E. Glotter, D. Lavie and R. Weitman, *Ibid.* (C), 1765 (1966)
[5] D. Lavie, I. Kirson and E. Glotter, *Israel J. Chem.* **6**, 671 (1968)
[6] A. Abraham, I. Kirson, E. Glotter and D. Lavie, *Phytochemistry* **7**, 957 (1968)
[7] [a] L. F. and M. Fieser, *Steroids*, p. 344. Reinhold, New York (1959)
[b] A. Mijares, D. I. Cargill, J. A. Glasel and S. Lieberman, *J. Org. Chem.* **32**, 810 (1967)