Constituents of *Withania somnifera* Dun. III. The Side Chain of Withaferin A*,[1]

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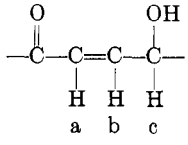
Withaferin A, $C_{28}H_{38}O_6$, a compound isolated from the leaves of *Withania somnifera*, was studied. All functional groups have been determined and interrelated. The side chain is an α,β-unsaturated lactone of structure Ia.

In our investigations dealing with the constituents of the leaves of the perennial herb, *Withania somnifera* Dun (*Solanaceae*), growing in Israel, several crystalline compounds have been isolated, following a careful chromatography of the crude extraction mixture.[2] These compounds have been described as A_1, A_2, A_3, etc., the major constituent being A_2. The present paper deals with studies directed toward the elucidation of the structure of A_2 for which the name of withaferin A is proposed.

Withaferin A is a white crystalline substance, m.p. 243–245°, $[\alpha]_D$ + 114°, λ_{max} 214 mμ (ϵ 17,500), and ν_{max} 1692 cm.$^{-1}$ (carbonyl region), analyzing for an empirical formula, $C_{28}H_{38}O_6$. Upon acetylation a diacetate is formed accounting for two acylatable hydroxyl groups in the molecule, one being primary as described subsequently. Stepwise hydrogenation of withaferin A over palladium on charcoal as catalyst indicated the absorption of 3 moles of hydrogen, all three products being isolated and characterized. The spectroscopic characteristics of dihydrowithaferin A, λ_{max} 210 mμ (ϵ 10,000) and ν_{max} 1700 cm.$^{-1}$, revealed that in the original compound the maximum of absorption in the ultraviolet is due to the overlapping of two different chromophores; it also yielded a diacetate. Dihydrowithaferin A was found to be identical with A_3, one of the compounds isolated from the original crude mixture,[2] setting thereby the relationship between these two naturally occurring substances. Absorption of the second mole of hydrogen resulted in hydrogenolysis of the primary hydroxyl, a behavior characteristic for such a group in an allylic position. The loss of the hydroxyl was indicated by the formation of a monoacetate as well as by other evidence given below. Dihydrowithaferin A diacetate underwent hydrogenolysis even faster leading to the same product, dihydrodesoxywithaferin A monoacetate, λ_{max} 226 mμ, ν_{max} 1730 (acetate) and 1698 cm.$^{-1}$ (overlapping of two carbonyl groups). The third mole of hydrogen, when introduced into the above monoacetate, leads to tetrahydrodesoxywithaferin A monoacetate, a compound lacking strong absorption in the ultraviolet and displaying two bands in the carbonyl region of the infrared, ν_{max} 1739 and 1715 cm.$^{-1}$. Withaferin A has, therefore, two double bonds, both conjugated to carbonyl groups.

With these data in hand, the n.m.r. spectra of the various derivatives were most instructive and contributed to the determination of all the groupings. The methyl region of the spectrum of withaferin A revealed the presence of four methyl groups as follows: two singlets at δ 0.68 and 1.38 for two tertiary methyl groups, a doublet centered at 0.97 (J = 6.5 c.p.s.) for one secondary methyl, and a singlet at 2.03 related to a vinylic methyl. A sharp signal at δ 4.35 accounting for two equivalent protons was ascribed to the primary alcohol group mentioned above; in the acetylated product (Figure 1), this signal is found shifted to δ 4.87. The downfield part of the spectrum is due to the interaction of two vinylic protons which are correlated in the following system.



Signals of these two vinylic protons appear for H_a as a doublet at δ 6.18 (J_{ab} = 10 c.p.s.) and for H_b as a quartet centered at 6.97 (J_{ab} = 10 c.p.s. and J_{bc} = 6 c.p.s.) while the doublet centered at δ 3.75 (J_{cb} = 6 c.p.s.) is related to H_c; upon acetylation this signal is found shifted to δ 4.66. The doublet displayed by this proton indicates that it has no other neighboring protons. The double bond carrying these two vinylic protons was hydrogenated in dihydrowithaferin A as demonstrated by the disappearance of the signals in the low-field region of its spectrum. However, as expected, the pattern of H_c was now converted to a triplet centered at δ 3.55 due to coupling with the two adjacent protons of the newly formed methylene group. In the corresponding dihydrowithaferin A acetate, this proton is at δ 4.62 (triplet).

Earlier in our presentation it was said that absorption of the second mole of hydrogen induced hydrogenolysis, and indeed in the n.m.r. spectrum of dihydrodesoxywithaferin A, instead of the signal related to the $-CH_2$-OH, a new vinylic methyl group is found at δ 1.93 overlapping the signal of the previously existing similar group. In tetrahydrodesoxywithaferin A acetate these two methyl groups are shifted upfield and appear now as two new sets of doublets centered at δ 0.92 and 1.13 indicating unequivocally that the two vinylic methyl groups in dihydrodesoxywithaferin A are both located on the same double bond, and therefore, in withaferin A the primary alcohol and the vinylic methyl group are placed as well on that same double bond.

Throughout the n.m.r. spectra of all these derivatives, there are two signals which remained unchanged in location and pattern, one being a rather broad peak at δ 3.20 (half-height width 4 c.p.s.), while the other forms a double triplet centered at δ 4.40, accounting each for one proton. The location of the former signal (δ 3.20) could well be that of a proton of an epoxide, a fact which was indeed disclosed by various chemical

* To Professor Louis F. Fieser.

(1) Paper II: D. Lavie, E. Glotter, and Y. Shvo, *Israel J. Chem.*, **2**, 247 (1964).

(2) A. Yarden and D. Lavie, *J. Chem. Soc.*, 2925 (1962).

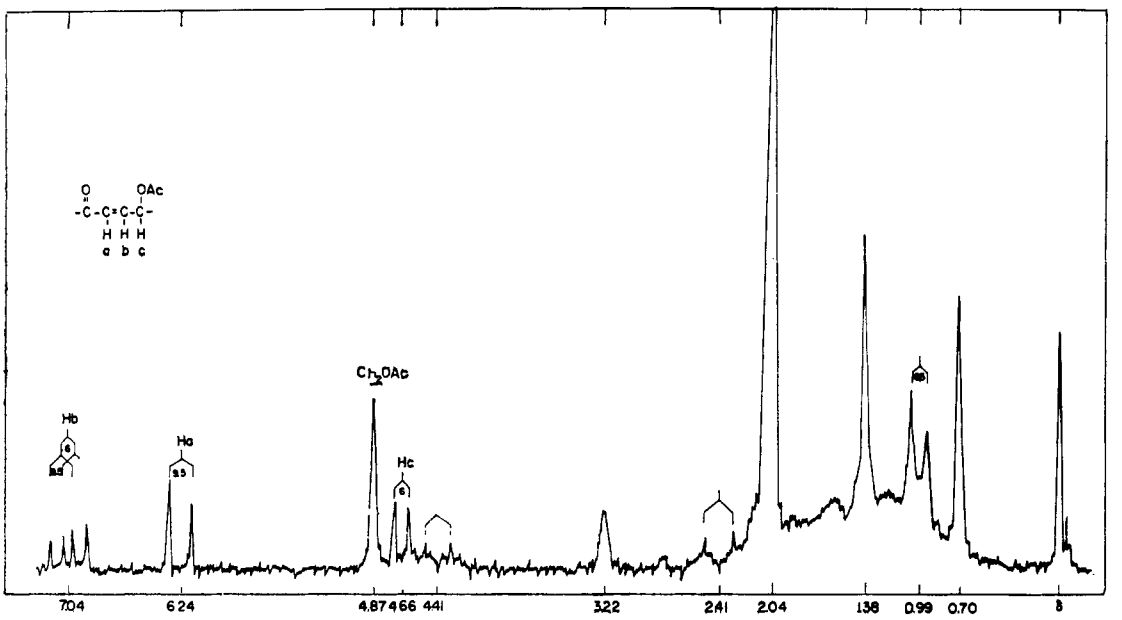
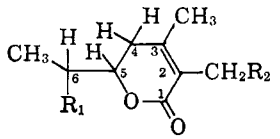


Figure 1.—N.m.r. spectrum in chloroform-*d* of withaferin A diacetate.

means and will be described in details in a subsequent publication. Summing up, of the six oxygen atoms present in the molecule four are accounted at this stage of our presentation; the remaining two are, as will be demonstrated, part of a lactone ring (I).[3] In such a

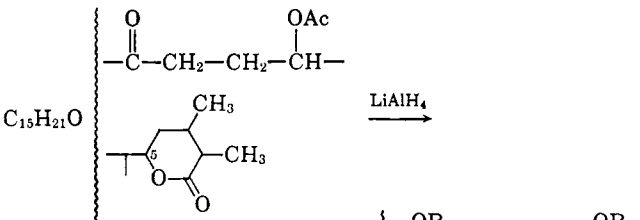


Ia, withaferin A, $R_1 = C_{19}H_{24}O_3$; $R_2 = OH$
b, dihydrowithaferin A, $R_1 = C_{19}H_{26}O_3$; $R_2 = OH$
c, dihydrodesoxywithaferin A, $R_1 = C_{19}H_{26}O_3$; $R_2 = H$

system, the δ 4.40 signal referred to above fits well to the C-5 proton, both in location and in splitting pattern. Notwithstanding the fact that λ_{max} 210 mμ in dihydrowithaferin A (Ib) is incompatible with such a structure, the λ_{max} 226 mμ in Ic is in a rather usual position for α,β-unsaturated lactones carrying two alkyl substituents on the double bond; the obvious deduction is therefore that the hypsochromic shift of 16 mμ in the former is due to the primary alcohol group. To the best of our knowledge no analogy has been recorded yet.

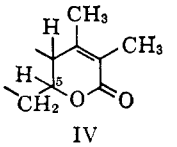
Repeated attempts of hydrolytic opening of the lactone to yield the corresponding hydroxy acid were unsuccessful. Alkaline treatment of Ic followed by acidification yielded quantitatively the original unchanged lactone. However, in order to ascertain that the lactone did open in alkaline medium, the aqueous basic solution, having been thoroughly extracted with ether in order to remove any neutral substances, was acidified and re-extracted with ether, thus resulting in recovery of the reclosed lactone.

Upon reduction of tetrahydrodesoxywithaferin A acetate II with $LiAlH_4$ or $NaBH_4$, a tetrol (IIIa) was obtained in which two secondary hydroxyl groups originated from the acetate and the ketone groups present in the carbocyclic system, while the two other, a primary and a secondary hydroxyl group, were obtained from the reductive opening of the lactone. It was expected that the epoxide should have also been reduced during the process; however, evidence indicated that it remained unchanged. The n.m.r. spectrum of IIIb displayed the presence of three separate signals corresponding to one hydrogen each, centered at δ 4.48, 4.88, and 5.00. The last signal appeared as a double triplet and is attributed to the C-5 proton; the corresponding signal in II is at δ 4.22. As to the two protons of the primary acetate group (IIIb) they are the AB part of an ABX system (X being the C-2 proton) and display two signals centered st δ 4.06 and 4.09. Obviously, the six-membered ring lactone could



IIIa, R = H
b, R = Ac

as well be fused to the carbocyclic skeleton as shown in IV. In such a case, the C-5 proton should also display



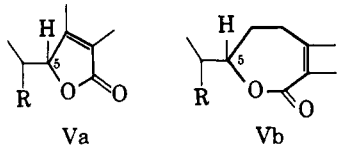
IV

a double triplet pattern as referred to above, involving however only one vicinal allylic proton instead of two. In order to settle this point deuterium-exchange experiments were undertaken. Dihydrodesoxywithaferin A

(3) The numbering in the lactone ring is intended for the present discussion only and has no relationship to other parts of the molecule.

(Ic) was treated with deuterated methanol in the presence of sodium methoxide resulting in a product in which at least five hydrogen atoms were exchanged by deuterium as shown in the mass spectrum. The deuterated compound indicates unequivocally in its n.m.r. spectrum that the C-5 proton displays now a doublet at δ 4.37 (J = 4 c.p.s.) and has therefore only one neighboring proton; two allylic hydrogens which were previously present in the molecule have then been exchanged with deuterium and the part structure IV could be eliminated. It is noteworthy that, of the two vinylic methyl groups, only one has undergone total exchange, shown in the n.m.r. spectrum by the sharpness of the signal of the remaining CH_3 and its integral. To ascertain that the two hydrogens which underwent exchange are indeed in an allylic position, tetrahydrodesoxywithaferin A acetate (II) in which no double bonds are present was submitted to the same deuteration procedure. Following reacetylation, the C-5 proton exhibited the same double triplet pattern as in II. Actually, earlier decoupling experiments[4] performed on withaferin A diacetate disclosed the relationship existing between the double triplet at δ 4.41 (J = 12.5 c.p.s.) and the two allylic protons (two multiplets apart by 12.5 c.p.s., centered at δ 2.37, with no determinable patterns); indeed irradiation of this part of the field induced the collapse of the δ 4.41 double triplet to a doublet. Preceding the latter deuteration experiment, the stability of tetrahydrodesoxywithaferin A acetate (II) to alkaline conditions was tested, and following the reacetylation procedure it was found that a new substance was obtained, isomeric with the starting compound. It has been reported in several instances[5] that the alkaline treatment of lactones may result in an epimerization at the carbon atom neighboring the carbonyl function, in the present case at C-2, induced by the formation of an intermediate enolate which rearranges to the most stable epimer. The two hydrogen atoms at C-2–C-3 in II should be, following the catalytic hydrogenation, in a *cis* relationship, and in the epimer therefore this relationship will be *trans*. The configurational change undergone by one of the secondary methyl groups could be well observed by the shift of the doublet centered at δ 0.92 (J = 6.5 c.p.s.) to δ 1.32 (J = 5.7 c.p.s.) in the epimerized compound. During the deuteration process the formation of an enolate involves, in addition to the epimerization, the exchange of the C-2 hydrogen atom. Indeed, at least one deuterium enters the molecule and this fact was also observed indirectly in the n.m.r. spectrum of the deuterated epimer, the C-2 methyl appearing now as a singlet at δ 1.30.

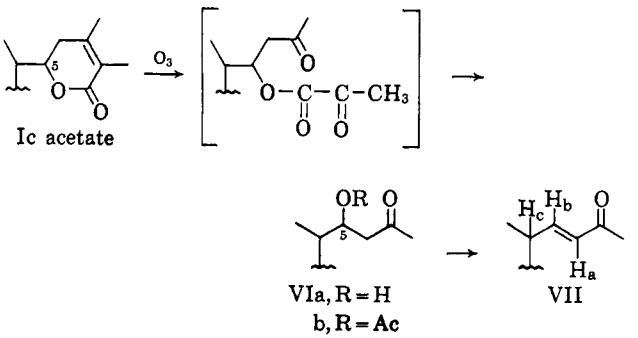
With these results in hand one can rule out definitely the possibilities of five- and seven-membered ring α,β-unsaturated lactones (Va and b) inasmuch as in the former the corresponding C-5 proton could not possibly display a double triplet, while in the latter, the corresponding double triplet should have remained unchanged following the deuteration experiments.



Va Vb

The carbonyl region of the infrared spectra of these compounds is interesting to note. While in tetrahydrodesoxywithaferin A acetate (II) bands are recorded at 1739 (overlapping of acetate and lactone) and 1715 cm.$^{-1}$, in the epimerized compound, the carbonyl absorption bands are at 1712 cm.$^{-1}$ (overlapping of ketone and lactone, resolved in a KBr pellet to 1718 and 1706 cm.$^{-1}$), while after reacetylation the appropriate bands are at 1730 (acetate) and 1712 cm.$^{-1}$ (overlapping of ketone and lactone).[6]

In order to ascertain by chemical means the structure of the α,β-unsaturated lactone ring, dihydrodesoxywithaferin A acetate was subjected to ozonolysis Reductive decomposition of the ozonide yielded directly[7] the β-hydroxy ketone VIa which was characterized as its crystalline acetate (VIb). Both VIa and VIb



Ic acetate

VIa, R = H
b, R = Ac

VII

have no strong absorption in the ultraviolet, while in the n.m.r. spectra both vinylic methyl groups disappeared and a new signal related to the methyl ketone group was observed. Considering the signal related to the C-5 proton in the original acetate of Ic (δ 4.41), in the hydroxy ketone VIa it is now found at δ 4.11, and in its acetate VIb at an expected location, δ 5.36, all keeping the same pattern of a double triplet. Due to the sensitivity of the epoxide group present in the molecule (*vide supra*) even to mild acidic conditions, elimination of the hydroxyl group in VIa was performed by storage on acid-washed alumina forming thereby the α,β-unsaturated methyl ketone VII, λ_{max} 225 mμ (ϵ 18,300), ν_{max} 1672 and 1642 cm.$^{-1}$. The n.m.r. spectrum contains a pattern of lines for the two vinylic protons, a doublet for H_a at δ 5.98 (J_{ab} = 16 c.p.s.) and a quartet for H_b centered at δ 6.67 (J_{ba} = 16 c.p.s.) J_{bc} = 8 c.p.s.). The large coupling constant, J_{ab} = 16 c.p.s., confirms that the two vinylic hydrogens are in a *trans* position.

There remained to be determined the position of the primary alcohol group on the lactone ring of withaferin A, either at C-2 or at C-3 (*cf.* Ia). Ozonolysis of dihydrowithaferin A diacetate (Ib diacetate) yielded, following the procedure described above, a product identical with the α,β-unsaturated methyl ketone VII; the alcohol group is therefore placed at C-2. Should the alcohol group have been placed in the alterna-

(4) Decoupling was performed using the Varian n.m.r. integrator, Model V 3521, according to the procedure of L. F. Johnson, Varian Associates Publication No. 87-100-082, Palo Alto, Calif., 1962.

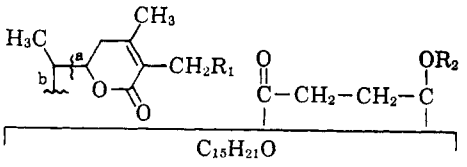
(5) C. Asselineau, S. Bory, and E. Lederer, *Bull. soc. chim. France*, 1524 (1955), and references cited therein.

(6) This frequency value for the δ-lactone is rather low. However such values or even lower have been recorded: see R. N. Jones and B. S. Gallagher, *J. Am. Chem. Soc.*, **81**, 5242 (1959); T. Kubota, T. Matsuura, T. Tsutsui, and K. Naya, *Bull. Chem. Soc. Japan*, **34**, 1737 (1961).

(7) The expected pyruvic ester could not be isolated from the reaction mixture and is probably hydrolyzed during the work-up.

tive C-3 position, ozonolytic cleavage would result in an α-acetoxy ketone, $RCH(CH_3)CH(OH)CH_2COCH_2OAc$. Cleavage of the double bond by an alternative route, the osmium tetroxide–sodium periodate procedure,[8] afforded the same compound VII, pointing unequivocally to the C-2 position of the primary alcohol group.

Mass spectral measurements performed on withaferin A as well as on several of its derivatives supplied useful information which supported well and completed the structural data presented herewith. The principal



VIIIa, R_1 = OH; R_2 = OH
b, R_1 = H; R_2 = OH
c, R_1 = H; R_2 = OAc

peaks in the high-mass range of dihydrodesoxywithaferin A (VIIIb) which was first selected for our measurements occurred at *m/e* 456, 438, 331, 313, 303, and 285. The first peak is due to the molecular ion and confirms the empirical formula, $C_{28}H_{40}O_5$; elimination of the elements of water (secondary alcohol) gave rise to the second peak, *m/e* 438. Cleavage a gave the fragments *m/e* 331 and 313 due to elimination of the lactone ring alone or in conjunction with water, respectively. Cleavage b resulted in the fragments *m/e* 303 and 285, loss of the whole side chain alone or again with water; however, stronger peaks were present at *m/e* 301 and 283, *i.e.*, less two hydrogens. In the deuterated dihydrodesoxywithaferin A loss of elements of water gave rise to the peak *m/e* 443 indicating an exchange introducing five deuterium atoms. All other peaks were identical with the corresponding ones in the nondeuterated compound, indicating thereby unequivocally that deuterium exchange involved the lactone ring only. The high mass range peaks of VIIIa and VIIIc agreed well with the fragmentation patterns described above.

The fragmentation pattern b assumes the secondary methyl group as part of the side chain. This fact was well supported by the chemical shift of this methyl group in the n.m.r. spectra of several compounds. In the β-hydroxy ketone VIa or its acetate VIb this signal is centered at δ 0.91; in the α,β-unsaturated ketone VII, as expected, it is shifted downfield to δ 1.08, while following the reduction of the double bond the position of the signal was reverted to δ 0.96.

Experimental

Melting points were taken on a Kofler hot-stage microscope and are corrected. All optical rotation measurements were carried out in chloroform solution. Ultraviolet absorption spectra were done on a Cary 14 spectrophotometer in ethanol solution. Infrared spectra were recorded on a Perkin-Elmer Infracord Model 137 spectrometer 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. The line positions given are δ values. The spectra were determined in deuterated chloroform solutions of about 5–10% concentration and containing tetramethylsilane as internal standard. Thin layer chromatography (t.l.c.) was done on chromatoplates of silica gel G (Merck) and spots were developed with 0.5% potassium permanganate solution in a saturated cupric acetate solution or iodine vapors. In the chromatographies, alumina refers to acid-washed alumina (Merck).

Withaferin A.—Withaferin A was isolated from *Withania somnifera* leaves according to a simplified procedure.[2] Crushed air-dried leaves (1 kg.) were extracted continuously for 24 hr. with methanol. The solution was concentrated to a small volume, the residue was dissolved in acetic acid (200 ml.), and water (2 l.) was added while stirring. After 24 hr. the solution was filtered and extracted exhaustively with ether. The ethereal layer was shaken with 10% aqueous ammonia and washed with water. Upon concentration a residue was obtained (8–10 g.) which was rapidly passed in chloroform solution through alumina (300 g.). Elution with chloroform and recrystallization from ethyl acetate yielded a mixture (2–3 g.) of withaferin A and dihydrowithaferin A previously described[2] as A_2 and A_3, respectively.

This mixture (5 g.) in purified chloroform was carefully chromatographed on alumina (300 g.); elution with chloroform–methanol, 99:1, yielded fractions containing first withaferin A and then the dihydro derivative. The various fractions were combined according to t.l.c. indications and resulted in 3 g. of the former and 1.5 g. of the latter. Both were recrystallized several times from ethyl acetate. Withaferin A had m.p. 243–245°, $[\alpha]_D$ +114° (*c* 0.56), λ_{max} 214 mμ (ϵ 17,500), and ν_{max} 1692 cm.$^{-1}$.

Anal. Calcd. for $C_{28}H_{38}O_6$: C, 71.46; H, 8.14. Found: C, 71.50; H, 8.28.

Dihydrowithaferin A had m.p. 229–230°, $[\alpha]_D$ +8° (*c* 0.91), λ_{max} 210 mμ (ϵ 10,000), and ν_{max} 1700 cm.$^{-1}$.

Anal. Calcd. for $C_{28}H_{40}O_6$: C, 71.16; H, 8.53. Found: C, 71.06; H, 8.30.

Withaferin A diacetate was obtained from withaferin A by the usual acetic anhydride–pyridine procedure at room temperature: crystallized from methanol, m.p. 201°; $[\alpha]_D$ +193° (*c* 1.22); λ_{max} 214 mμ (ϵ 17,800); ν_{max} 1736 (2 acetates), 1706 (unsaturated lactone), and 1692 (unsaturated ketone) cm.$^{-1}$.

Anal. Calcd. for $C_{32}H_{42}O_8$: C, 69.29; H, 7.63. Found: C, 69.24; H, 7.45.

Hydrogenation of Withaferin A to Dihydrowithaferin A.—Withaferin A (500 mg.) was hydrogenated over 10% palladium on charcoal at room temperature and atmospheric pressure. The reaction was discontinued when 1 mole of hydrogen had been absorbed. The crude reaction product was chromatographed on alumina. Elution with hexane–chloroform, 1:1, afforded small quantities of dihydrodesoxywithaferin A while with chloroform the product which emerged and crystallized from ethyl acetate was found identical in all respects with the natural dihydrowithaferin A.

Dihydrowithaferin A diacetate was obtained from dihydrowithaferin A by the acetic anhydride–pyridine procedure: crystallized from methanol, m.p. 205–208°; $[\alpha]_D$ +10° (*c* 1.13); λ_{max} 214 mμ (ϵ 9700); ν_{max} 1730 (2 acetates) and 1706 cm.$^{-1}$.

Anal. Calcd. for $C_{32}H_{44}O_8$: C, 69.04; H, 7.97. Found: C, 69.03; H, 7.80.

Dihydrodesoxywithaferin A.—Dihydrowithaferin A (500 mg.) in ethanol (100 ml.) was hydrogenated over 10% palladium on charcoal at room temperature and atmospheric pressure. The reaction was discontinued when 1 mole of hydrogen had been absorbed. The crude hydrogenated product was chromatographed on alumina. Elution with hexane–chloroform, 1:1, yielded a pure crystalline substance which was recrystallized from acetone: m.p. 195–198° (opaque at 150°), $[\alpha]_D$ +9° (*c* 0.92), λ_{max} 226 mμ (ϵ 7900), ν_{max} 1695 cm.$^{-1}$ (overlapping of ketone and unsaturated lactone). The molecular weight was determined by mass spectrometry to be 456 ($C_{28}H_{40}O_5$); however it consistently crystallized with water.

Anal. Calcd. for $C_{28}H_{40}O_5 \cdot 0.5H_2O$: C, 72.22; H, 8.87. Found: C, 72.49; H, 8.78.

The same product was obtained when withaferin A was allowed to absorb 2 moles of hydrogen.

Dihydrodesoxywithaferin A monoacetate was prepared by the acetic anhydride–pyridine procedure at room temperature: crystallized from acetone–hexane mixture, m.p. 255–258° dec.; $[\alpha]_D$ −14° (*c* 0.91); λ_{max} 226 mμ (ϵ 8200); ν_{max} 1730 (acetate) and 1698 (overlapping of ketone and unsaturated lactone) cm.$^{-1}$.

Anal. Calcd. for $C_{30}H_{42}O_6$: C, 72.26; H, 8.49. Found: C, 71.98; H, 8.45.

The same product was obtained as well by hydrogenation of dihydrowithaferin A diacetate under the same conditions.

(8) R. Pappo, D. S. Allen, Jr., R. U. Lemieux, W. S. Johnson, *J. Org. Chem.*, **21**, 478 (1956).

Titration of an aliquot of the ethanolic solution revealed the presence of 1 equiv. of acetic acid.

Tetrahydrodesoxywithaferin A Acetate.—The above dihydrodesoxywithaferin A acetate (300 mg.) was further hydrogenated as above; absorption was now very slow. The product was collected and chromatographed over alumina and elution was performed with hexane–chloroform, 4:1. The main homogeneous fractions (t.l.c. evidence) were combined and recrystallized from acetone–hexane: m.p. 220°, $[\alpha]D$ −99° (*c* 1.03), no major absorption in the ultraviolet, ν_{max} 1739 (overlapping of acetate and lactone) and 1715 (ketone) cm.$^{-1}$.

Anal. Calcd. for $C_{30}H_{44}O_6$: C, 71.97; H, 8.86. Found: C, 71.87; H, 8.61.

Epimerization of Tetrahydrodesoxywithaferin A Acetate.—The compound (100 mg.) was heated to reflux during 4 hr. in a methanolic solution of sodium methoxide (50 mg. of sodium in 20 ml. of methanol). After cooling the solution was acidified (HCl), diluted with water, and extracted with chloroform. The residue was passed through alumina using hexane–chloroform, 1:1; the major fractions were collected and recrystallized from acetone–hexane: m.p. 225–227°, $[\alpha]D$ −73° (*c* 0.75), ν_{max} 1712 (overlapping of lactone and ketone) cm.$^{-1}$, ν_{max}^{KBr} 1718 and 1706 cm.$^{-1}$.

The product was acetylated using the usual procedure and recrystallized from acetone–hexane: m.p. 238–240°, $[\alpha]D$ −45° (*c* 1.05), ν_{max} 1730 (acetate) and 1712 (overlapping of lactone and ketone) cm.$^{-1}$.

Anal. Calcd. for $C_{30}H_{44}O_6$: C, 71.97; H, 8.86. Found: C, 71.69; H, 8.72.

Hydrolysis of the Lactone Ring.—Dihydrodesoxywithaferin A (100 mg.) in 4% methanolic solution of KOH (15 ml.) was heated to reflux during 4 hr. Following dilution with water the solution was extracted exhaustively with ether in order to remove any neutral substance. The ethereal solution was dried over sodium sulfate and evaporated, leaving a residue (30 mg.) consisting mainly of unreacted starting material. The aqueous alkaline solution was acidified to pH 3 and re-extracted with ether the residue of which (60 mg.) after crystallization was identical with dihydrodesoxywithaferin A.

Hydride Reduction of Tetrahydrodesoxywithaferin A Acetate. A.—This compound (100 mg.) in dry tetrahydrofuran (30 ml.) was added slowly to a stirred suspension of lithium aluminum hydride (100 mg.) in the same solvent (20 ml.). The mixture was heated to reflux while stirring during 4 hr., thereafter the excess reagent was destroyed with ethyl acetate and a saturated solution of sodium sulfate was added. Removal of the solvent yielded a residue, exhibiting no more absorption in the carbonyl region. The product was acetylated in the usual way, yielding a crude tetraacetate which was chromatographed through alumina. Elution with hexane–chloroform, 4:1, yielded the main homogeneous (t.l.c. evidence) fraction (90 mg.) which could not be induced to crystallize, ν_{max} 1730 cm.$^{-1}$.

B.—The compound (100 mg.) in dioxane (15 ml.) was added to sodium borohydride (150 mg.) and heated to reflux while stirring during 1 hr. The solution was poured on ice, acidified to pH 3, and extracted with chloroform; evaporation of the solvent left a residue (85 mg.) which was acetylated by the usual procedure. The tetraacetate which was obtained proved to be identical with the above compound having identical infrared as well as n.m.r. spectra. On chromatoplate, both displayed the same R_f value.

Deuterium-Exchange Experiments. A.—Dihydrodesoxywithaferin A (150 mg.) in CH_3OD (15 ml., 99% D) to which sodium metal (75 mg.) was added, was heated to reflux during 4 hr. under dry nitrogen. The cooled solution was acidified (HCl) and filtered, and the residue, after evaporation of the solvent, was crystallized twice from ethyl acetate and acetone; pure deuterated product (105 mg.) was collected. The exchange of five deuterium atoms was observed in the n.m.r. as well as in the mass spectra, *m/e* 443 for M − 18, compared with the corresponding *m/e* 438 for M − 18 in dihydrodesoxywithaferin A itself.

Prior to the deuteration experiment, the stability of the compound to the conditions of the reaction was tested, and the product was recovered unchanged.

B.—Tetrahydrodesoxywithaferin A acetate (150 mg.) was exposed to the same deuterium-exchange procedure. The crude product however was now reacetylated by the usual procedure and crystallized from an acetone–hexane mixture yielding the pure deuterated compound (90 mg.). The changes induced in the molecule were observed in the n.m.r. spectrum as described.

Ozonolysis of Dihydrodesoxywithaferin A Acetate.—The compound (500 mg.) was dissolved in a mixture of chloroform (10 ml.) and ethyl acetate (10 ml.) and ozonized oxygen (1.5 equiv.) was passed into the solution at 0°. The solvent was removed *in vacuo* and the ozonide was decomposed with powdered zinc (500 mg.) in acetic acid (10 ml.). The solution was filtered, diluted with water, and extracted with chloroform; the organic layer was washed with water and sodium bicarbonate solution to neutral reaction. The residue left after evaporation of the solvent could not be induced to crystallize; however, it showed only one clear spot on a chromatoplate. The product, which is the β-hydroxy ketone VIa, ν_{max} 1730 (overlapping of acetate and methyl ketone) and 1709 (ketone) cm.$^{-1}$, was characterized as its acetate VIb, which was prepared by the usual procedure: crystallized from an acetone–hexane mixture, m.p. 158–159°; no major absorption in the ultraviolet; ν_{max} 1730 and 1709 cm.$^{-1}$.

Anal. Calcd. for $C_{29}H_{42}O_7$: C, 69.29; H, 8.42. Found: C, 69.06; H, 8.23.

Preparation of the α,β-Unsaturated Ketone VII.—The above crude hydroxy ketone VIa (300 mg.) was introduced on an alumina column (20 g.) in a mixture of chloroform–benzene, 1:1, and allowed to stand for a period of 3 days. Then the product was washed out with chloroform and the mixture was carefully rechromatographed on alumina (30 g.). Elution with hexane–chloroform, 4:1, yielded the new compound VII (200 mg.), while with hexane–chloroform, 1:1, unreacted material (50 mg.) was collected. The first compound crystallized twice from methanol: m.p. 195–197°; $[\alpha]D$ −108° (*c* 0.97); λ_{max} 225 mμ (ε 18,300); ν_{max}^{KBr} 1733 (acetate), 1718 (ketone), 1672, and 1642 (unsaturated methyl ketone) cm.$^{-1}$. The unreacted material could be subjected to the same procedure yielding additional crops of compound VII.

Anal. Calcd. for $C_{27}H_{38}O_5$: C, 73.27; H, 8.65. Found: C, 73.13; H, 8.46.

Ozonolysis of Dihydrowithaferin A Diacetate.—This compound (200 mg.) was submitted to the same ozonolysis procedure described above. The crude product was then stored on alumina as well, yielding an α,β-unsaturated ketone which proved to be identical in all respects with the above compound VII, showing no depression on mixture melting point and having all spectroscopic data superimposable.

Cleavage of the Double Bond by the Osmium Tetroxide–Sodium Periodate Procedure. A.—Dihydrodesoxywithaferin A acetate (200 mg.) was dissolved in dioxane (15 ml.), osmium tetroxide (15 mg.) was added, and the mixture was stirred for 15 min.; then sodium periodate (200 mg.) in water (2 ml.) was added and stirring was continued for the following 4 days. Thereafter the mixture was poured on ice–water and extracted with chloroform, and the organic layer was washed with sodium bisulfite. Evaporation of the solvent left a residue (150 mg.) having no major ultraviolet absorption. The crude reaction product was introduced onto an alumina column as described above for the formation of compound VII. Elution with hexane–chloroform, 4:1, yielded a product (70 mg.) which proved to be identical in all respects with the above compound VII.

B.—Dihydrowithaferin A diacetate (200 mg.) was submitted to the same cleavage procedure yielding again the same compound VII (50 mg.)

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