Anal. Required for $C_{28}H_{23}N_3O_6$: *m/e* 437.165. Found: *m/e* 437.163.

Attempted Cyclization of 15b.—A sample of **15b** (5 mg) was dissolved in 1 ml of acetic anhydride and the solution maintained at 100° for 18 hr. Removal of the solvent gave 4.5 mg of crude material which had spectral properties identical with those of **15b**.

Registry No.—**3**, 21902-11-4; **4**, 21902-12-5; **5**, 21902-13-6; **7**, 21902-14-7; **8**, 21902-15-8; **8**, free acid, 21902-25-0; **9**, 21902-16-9; **10**, 21902-17-0; **11**, 21902-18-1; **12**, 21902-19-2; **13**, 21902-20-5; **14**, 21902-21-6; **15a**, 21902-22-7; **15b**, 21902-23-8; **20**, 21902-24-9.

Acknowledgment.—The authors wish to thank Dr. David Rosenthal for obtaining and interpreting the mass spectra, and J. E. Mason and J. B. Thompson for technical assistance.

Tumor Inhibitors. XXXIX.[1a] Active Principles of *Acnistus arborescens*. Isolation and Structural and Spectral Studies of Withaferin A and Withacnistin

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Evidence is presented for assignment of structure for two cytotoxic compounds from *Acnistus arborescens* (L.), withaferin A (**4a**), and withacnistin (**14a**). Withaferin A (**4a**) was converted to the diacetate **4b**, the ene-dione **5**, and the methanol adduct **9**, and the functional groups of each were characterized. The complete structure and stereochemistry of **4a** were established by X-ray crystallographic analysis of the monoacetate *p*-bromobenzoate **4c**. A detailed discussion of the nmr and mass spectral data is presented for the cited compounds and their derivatives. The structure of withacnistin (**14a**) was deduced, largely on the basis of ORD, nmr, and mass spectral comparison with **4a** and its derivatives, as well as by nmr solvent shift data. Conversion to the acetate **14b**, the methanol adduct **15a**, and the deacetyl methanol adduct **15c** supported the structural assignment. A small quantity of loliolide (**11**) was also isolated.

The leaves of *Acnistus arborescens* (L.) Schlecht (Solanaceae) and related species have been used for many years to treat cancerous growths.[3] In the course of a continuing search for tumor inhibitors of plant origin, alcoholic extracts of dried *A. arborescens* leaves[4] were evaluated and found to show significant activity *in vitro* against cells derived from human carcinoma of the nasopharnyx (KB), and *in vivo* against sarcoma 180 (SA) in mice.[5] Consequently, a systematic study aimed at the isolation of the SA-inhibitory principles was undertaken. We report herein the systematic fractionation of an active extract of *A. arborescens* and the isolation and elucidation of withaferin A, a novel steroidal lactone which shows significant inhibitory activity against the SA tumor in mice and the Walker intramuscular carcinosarcoma 256 (WM) in rats (see Table I).[6] In addition, the isolation and characterization of a companion cytotoxic[7] withanolide, withacnistin, are described.

Fractionation of an ethanol extract guided by assay against sarcoma 180 revealed that the active principle was concentrated successively in the aqueous methanol layer of a 10% aqueous methanol–petroleum ether partition, the chloroform layer of a chloroform–water partition, the formamide layer of a formamide–benzene partition, and the chloroform layer of a chloroform–formamide partition. Chromatography of the chloroform-soluble material yielded withacnistin ($C_{30}H_{40}O_7$) and crystalline withaferin A ($C_{28}H_{38}O_6$). Rechromatography of mother liquors from withacnistin gave 3-ethoxy-2,3-dihydrowithacnistin and the known compound loliolide[8] (**11**).

The presence of an intense band at 214 mμ in the ultraviolet spectrum of withaferin A and a strong band at 5.92 μ in its infrared spectrum suggested the presence of α,β-unsaturated carbonyl groups. The nmr spectrum (see Table II) contained a low-field asymmetrical quartet at τ 3.05 (1 H, J_{AM} = 10.0 Hz and J_{AX} = 6.0 Hz) and doublets at τ 3.82 (1 H, J_{AM} = 10.0 Hz) and 6.25 (1 H, J_{AX} = 6.0 Hz) assignable to the AMX system **1**. Other signals were assigned to an allylic alcohol grouping, a proton on a carbon carrying an epoxide, a vinylic methyl group, and two tertiary and one secondary methyl groups.

Upon acetylation, a crystalline diacetate ($C_{32}H_{42}O_8$)

(1) (a) University of Wisconsin. Part XXXVIII: S. M. Kupchan and I. Ognyanov, *Tetrahedron Lett.*, 1709 (1969). The investigation at the University of Wisconsin was supported by grants from the National Cancer Institute (CA-04500) and the American Cancer Society (T-275). (b) Author to whom inquiries should be directed: Department of Chemistry, University of Virginia, Charlottesville, Va. 22901.

(2) University of California. This is part X in the series entitled "High Resolution Mass Spectrometry in Molecular Structure Studies." Part IX: H. K. Schnoes, D. H. Smith, A. L. Burlingame, P. W. Jeffs, and W. Döpke, *Tetrahedron*, **24**, 2825 (1968). The investigation at the University of California was supported in part by a grant from the National Aeronautics and Space Administration (NGL 05-003-003).

(3) R. de Grosourdy, "El Médico Botànico Criollo," F. Brachet, Paris, 1864; F. Häussler, *Schweiz. Apoth.-Ztg.*, **52**, 260, 275 (1914). We thank Dr. Jonathan L. Hartwell of the National Cancer Institute for calling these references to our attention.

(4) The plant material was collected in Costa Rica by Professor J. A. Saenz Renauld, Department of Biology, University of Costa Rica, San Jose, in Jan 1961.

(5) Cytotoxicity and *in vivo* inhibitory activity were assayed under the auspices of the Cancer Chemotherapy National Service Center (CCNSC), National Cancer Institute, by the procedures described in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

(6) *Cf.* B. Shohat, S. Gitter, A. Abraham, and D. Lavie, *ibid.*, **51**, 271 (1967).

(7) Withaferin A and withacnistin showed cytotoxicity (ED_{50}) against KB cell culture at 2.8×10^{-1} and 1.7×10^{-1} μg/ml, respectively.

(8) R. Hodges and A. L. Porte, *Tetrahedron*, **20**, 1463 (1964).

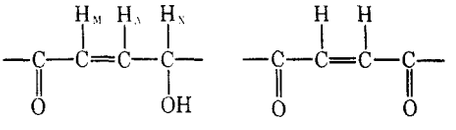
TABLE I

ACTIVITY OF WITHAFERIN A (4a) AGAINST *in Vivo* TUMOR SYSTEMS[a]

Tumor system	Dose, mg/kg	Survivors	Animal wt change difference, g T − C	Tumor wt, mg T/C	T/C × 100
SA[b]	30	0/4	...	...	...
	20	3/4	−2.4	407/1051	38
	10	4/4	+0.4	674/1051	64
WM[c]	60	3/6	−19	1400/11000	...
	40	6/6	−20	2700/11000	24
	20	6/6	−13	4200/11000	38

[a] T, treated animals; C, control animals. [b] Sarcoma 180. [c] Walker 256 intramuscular carcinosarcoma.

was formed. The changes in its nmr spectrum indicated the presence of a secondary and of a primary alcohol group in withaferin A. Manganese dioxide oxidation of withaferin A yielded a crystalline enedione,[9] showing that the secondary alcohol was allylic. The nmr spectrum lacked the AMX system attributed to partial structure **1** in withaferin A and instead contained a singlet at τ 3.20 (2 H) assignable to the two vinylic protons in partial structure **2**.



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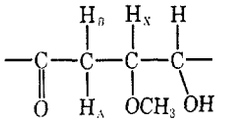

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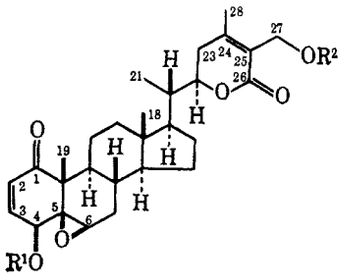
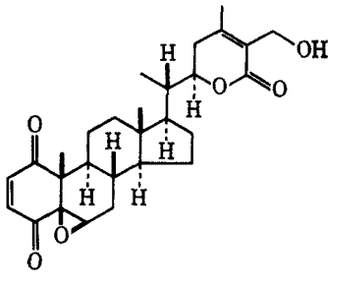
X-Ray crystallographic[10,11] analysis of the *p*-bromobenzoate monoacetate of withaferin A established its structure and stereochemistry as **4c**. Therefore, withaferin A, its diacetate, and the ene-dione could be respectively assigned the structures **4a**, **4b**, and **5**.

Catalytic hydrogenation of withaferin A (**4a**) occurred in three stages. Rapid consumption of 1 mol equiv of hydrogen led to dihydrowithaferin A (**6**). Reduction of the ring-A double bond was rapidly followed by the uptake of a second mole equivalent of hydrogen, with hydrogenolysis of the allylic primary alcohol group, to yield deoxydihydrowithaferin A (**7a**). Further hydrogenation slowly reduced the tetrasubstituted double bond in the side chain to give deoxytetrahydrowithaferin A (**8a**). Both **7a** and **8a**, on acetylation, gave their respective monoacetates **7b** and **8b**.

As expected, the nmr spectrum of the dihydro derivative **6** lacked the signals associated with the vinylic protons in ring A. The C-4 α-proton signal had moved upfield to τ 6.50 to give a triplet (J = 3.0 Hz). The spectrum of the deoxydihydro derivative **7a** showed similar changes due to the reduction of ring A and also lacked the broad signal found in the spectra of **4a** and **6** for the C-27 methylene group. Instead, it contained a singlet at τ 8.06 (6 H), confirming



4a, $R^1 = R^2 = H$
b, $R^1 = R^2 = Ac$
c, $R^1 = Ac$; $R^2 = p\text{-}BrC_6H_4CO$



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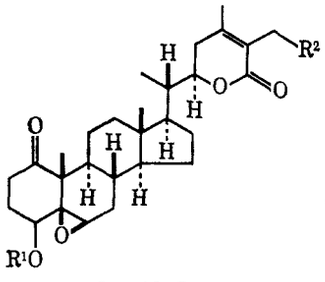
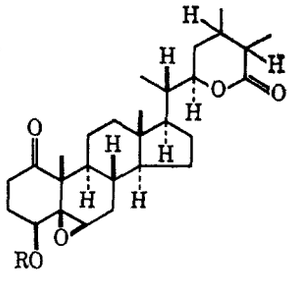


6, $R^1 = H$; $R^2 = OH$
7a, $R^1 = R^2 = H$
b, $R^1 = Ac$; $R^2 = H$



8a, R = H
b, R = Ac

that hydrogenolysis had occurred. In benzene–deuteriochloroform (1:1), the signal for the C-28 methyl group shifted by 0.32 ppm to τ 8.38 and the C-27 methyl group by 0.12 ppm to τ 8.18. In the spectrum of the acetate **7b**, the signal for the C-4 proton appeared as a triplet (τ 5.42, J = 3.0 Hz) and its infrared spectrum contained a new band at 5.76 μ. Saturation of the 24,25 bond to form **8b** resulted in changes in the nmr spectrum to give peaks at τ 8.87 (3 H, J = 7.0 Hz, C-27) and at τ 9.08 (6 H, J = 7.0 Hz, C-21 and C-28).

Treatment of withaferin A with methanolic sodium acetate afforded, in high yield, a methanol adduct (**9**, $C_{29}H_{42}O_7$), whose infrared spectrum contained a band at 5.86 μ assignable to a saturated cyclohexanone. Its ultraviolet absorption at 217 mμ was reduced in intensity in comparison with **4a** as expected following a Michael addition to the α,β-unsaturated cyclohexanone group. The nmr spectrum contained signals attributable to a methoxyl group (3 H, τ 6.67). The nonequivalent protons, adjacent to the carbonyl in partial structure **3**, appeared as an eight-line AB portion of an ABX system replacing the vinylic proton signals present in withaferin A.

Hydrogenation of the methanol adduct (**9**) caused smooth hydrogenolysis of the C-27 allylic hydroxyl group to give 27-deoxy-2,3-dihydro-3-methoxywithaferin A (**10a**), whose acetate (**10b**) could be readily

(9) *Cf.* the homocisoid ene-dione described by D. H. R. Barton and A. S. Lindsay, *J. Chem. Soc.*, 2988 (1951).

(10) S. M. Kupchan, R. W. Doskotch, P. Bollinger, A. T. McPhail, G. A. Sim, and J. A. Saenz Renauld, *J. Amer. Chem. Soc.*, **87**, 5805 (1965).

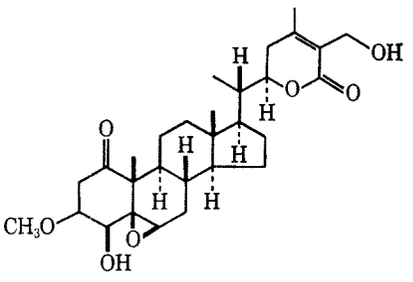
(11) A. T. McPhail and G. A. Sim, *J. Chem. Soc.*, *B*, 962 (1968).

TABLE II
NUCLEAR MAGNETIC RESONANCE DATA FOR WITHAFERIN A AND DERIVATIVES[a,b]

Compd	C-2	C-3	C-4	C-6	C-18	C-19	C-21	C-22[c]	C-27	C-28
4a	3.82 d (10.0)	3.05 dd (10.0, 6.0)	6.25 d (6.0)	6.78	9.28	8.58 [0]	9.02 d (6.5) [0.11]		5.64	7.95
4b	3.76 d (10.0)	2.98 dd (10.0, 6.0)	5.34 d (6.0)	6.78	9.30	8.60 [−0.07]	9.02 d (6.5)	5.60	5.13	7.97, 7.04 ($COCH_3$)
5	3.20	3.20		6.62 d (2.0)	9.28	8.63	8.98 d (6.5)		5.68	7.97
6			6.50 t (3.0)	6.88	9.33	8.70	9.02 d (6.5)		5.64	7.97
7a			6.50 t (3.0)	6.88	9.33	8.70 [−0.05]	9.02 d (6.5)	5.65	8.06 [0.12]	8.06 [0.32]
7b			5.42 t (3.0)	6.83	9.32	8.70	9.02 d (6.5)	5.63	8.07	8.07, 7.95 ($COCH_3$)
8a			6.50 t (3.5)	6.88	9.33	8.70	9.05[d] (7.0)	5.65	8.84 d (7.0)	9.05[d] (7.0)
8b			5.43 t (3.0)	6.87	9.33	8.71	9.08[d] (7.0)	5.66	8.87 d (7.0)	9.08[d] (7.0) 7.95 ($COCH_3$)
9	7.39 dd[e] 7.00 dd	6.3 m	6.51 d (3.0)	6.73 t (1.5)	9.32	8.71	9.02 d (6.5)		5.62	7.95 6.67 (OCH_3)
10a	7.40 dd[e] 7.01 dd	6.3 m	6.50 d (3.0)	6.80	9.33	8.69 [−0.04]	9.02 d (6.5)	5.63	8.07	8.07 6.63 (OCH_3)
10b	7.22 d[f] (6.0)	6.36[g]	5.37 d (2.5)	6.72	9.32	8.72 [−0.05]	9.02 d (6.5)	5.63	8.07	8.07 7.92 ($COCH_3$) 6.67 (OCH_3)

[a] Spectra were determined in deuteriochloroform solutions. Values are given in τ units relative to tetramethylsilane as internal standard. Multiplicity of signals is designated as follows: d, doublet; dd, doublet of doublets; t, triplet. Numbers in parentheses denote coupling constants in hertz. [b] Figures in square brackets are the solvent shifts $\Delta\tau = \tau$ [$CDCl_3$–benzene (1:1)] − τ ($CDCl_3$). [c] Doublet of triplets; J = 12.0 and 4.0 Hz. [d] The signals for C-21 and C-28 overlap. [e] AB portion of an ABX system: J_{AB} = 15.5 Hz; J_{AX} = 4.2 Hz; J_{BX} = 5.7 Hz. [f] Acetylation has apparently caused the ABX system to collapse to an A_2X system. [g] Doublet of triplets; J = 2.5 and 6.0 Hz.

prepared. The ultraviolet spectrum of **10a** showed the unusual but characteristic bathochromic shift, caused by loss of the hydroxyl group at C-27, from the abnormal value of 217 to 227 mμ. Its nmr spectrum contained the C-2 methylene proton signals as the AB proton of an ABX system (Table II). The C-3 proton (τ 6.3, H_X) was further coupled to the C-4 proton.



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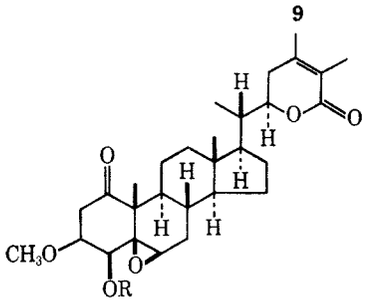
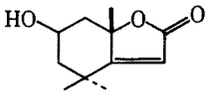


10a, R = H
b, R = Ac



11

While this work was in progress, Lavie and coworkers independently reported the isolation[12] and structure elucidation[13] of withaferin A and its 2,3-dihydro derivative, which they had isolated from *Withania somniferia* (L.) Dun. (Solanaceae). Similarities in the reported physical properties of withaferin A to those of our compound led to direct comparison,[14] by mixture melting point, mixture tlc, and infrared spectroscopy, which confirmed their identity. Consequently, we adopted the name withaferin A for our compound.

Further independent studies by Lavie and coworkers[15] have led to a proposal for the same stereochemistry for withaferin A. They have also isolated a number of closely related withanolides from the same species,[16,17] having the same skeleton but different substitution patterns. Studies on a related species, *Jaborosa integrifolia* Lam. (Solanaceae), by Tschesche, *et al.*,[18,19] have led to the isolation of 4-deoxywithaferin A and a related compound.

Withacnistin ($C_{30}H_{40}O_7$) was so named to reflect its botanical origin and its relationship to withaferin A. An intense ultraviolet absorption at 215 mμ and a strong infrared absorption band at 5.92 μ suggested the presence of α,β-unsaturated carbonyl groups similar to those of **4a**. An extra band at 5.78 μ indicated that an acetate group might also be present.

The nmr spectra of withacnistin (Table III) and of **4a** contain a number of common features. The spectrum of withacnistin also shows the ABX pattern, due to the C-2, -3, and -4 protons in partial structure **1**,

(12) A. Yarden and D. Lavie, *J. Chem. Soc.*, 2925 (1962).
(13) D. Lavie, E. Glotter, and Y. Shvo, *ibid.*, 7517 (1965), and earlier papers cited therein.
(14) We cordially thank Professor David Lavie for authentic samples of withaferin A and its methanol adduct.
(15) D. Lavie, S. Greenfield, and E. Glotter, *J. Chem. Soc., C*, 1753 (1966).
(16) E. Glotter, R. Waitman, and D. Lavie, *ibid.*, 1765 (1966).
(17) A. Abraham, I. Kirson, E. Glotter, and D. Lavie, *Phytochemistry*, **7**, 957 (1968).
(18) R. Tschesche, H. Schwang, and G. Legler, *Tetrahedron*, **22**, 1121 (1966).
(19) R. Tschesche, H. Schwang, H.-W. Fehlhaber, and G. Snatzke, *ibid.*, **22**, 1129 (1966).

TABLE III

NUCLEAR MAGNETIC RESONANCE DATA FOR WITHACNISTIN AND DERIVATIVES[a,b]

Compd	C-2	C-3	C-4	C-6	C-18[c]	C-19	C-21	C-27	C-28	-COCH$_3$	Other
14a	3.82 d	3.04 dd	6.24 d	6.78	6.17 d	8.60	8.90 d	8.08	8.08	7.93	
	(10.0)	(10.0, 6.0)	(6.0)		5.78 d	[−0.08]	(6.5) [0.04]	[0.07]	[0.25]		
14b	3.75 d	2.95 dd	5.30 d	6.78	6.17 d	8.62	8.90 d	8.08	8.08	7.95	5.64 (C-22)[d]
	(10.0)	(10.0, 6.0)	(3.0)		5.78 d	[−0.07]	(6.5)			7.93	
15a	7.38 dd[e]	6.3 m	6.50 d	6.77	6.16 d	8.89	8.89 d	8.07	8.07	7.94	6.62 (-OCH$_3$)
	6.97 dd		(3.0)		5.83 d	[−0.06]	(6.5)				
15b	7.19 d (2.5)	6.4 m	5.38 d	6.75	6.17 d	8.74	8.92 d	8.08	8.08	7.95	6.58 (-OCH$_3$)
	7.29 d (2.0)		(2.5)		5.85 d	[−0.10]	(6.5)			7.93	
15c	7.38 dd[e]	6.3 m	6.51 d	6.78	6.37 m	8.67	8.88 d	8.07	8.07		6.63 (-OCH$_3$)
	6.98 dd		(3.0)				(6.5)				5.58 (C-22)[d]
16a	7.40 dd[e]	6.2 m	6.50 d	6.80	6.17 d	8.71	8.90 d[f]	8.07	8.07	7.95	
	7.02 dd		(2.5)		5.84 d	[−0.13]	(7.0)				
16b	7.22 d	6.3 m	5.38 d	6.75	6.15 d	8.73	8.87 d	8.07	8.07	7.92	5.62 (C-22)[g]
	(6.0)		(2.5)		5.85 d	[−0.08]	(6.5)			7.92	

[a] See footnote *a*, Table II. [b] Figures in square brackets are $\Delta\tau = \tau$ [$CDCl_3$–benzene (1:1)] − τ ($CDCl_3$). [c] AB quartet; J_{AB} = 12.0 Hz (except for **15c**). [d] Doublet of triplets, J = 12.0 and 4.0 Hz. [e] AB portion of ABX system: J_{AB} = 15.5 Hz; J_{AX} = 4.2 Hz; J_{BX} = 5.7 Hz. [f] Overlap with ethoxyl signal. [g] Doublet of triplets; J = 12.5 and 3.5 Hz.

at τ 3.82, 3.04, and 6.24, respectively. The signals assignable to the C-6 epoxide proton (τ 6.78) and the C-19 methyl group (τ 8.60) were also present, as well as a singlet at τ 7.93 (3 H) assignable to an acetate group. Treatment with deuterium oxide exchanged one proton about τ 7.5.

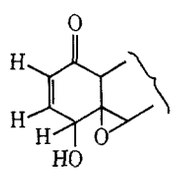
The mass spectrum of withacnistin also contained the characteristic ions from the fragmentation of ring B of withaferin A (see later) and together with the spectral data permit the partial structure **12** to be deduced.

Further study of the mass spectrum led to assignment of the intense peak at *m/e* 125 to a 27-deoxy side chain, by comparison with derivatives of withaferin A. The nmr spectrum contained a peak at τ 8.08 (6 H), which, as in **7a**, was shifted and split to τ 8.15 and 8.33 in benzene–deuteriochloroform (1:1) and can be assigned to the C-27 and C-28 methyl groups. Thus, the partial structure **13** can be deduced for the side chain.

A doublet at τ 8.90 (J = 6.5 Hz), which is due to the C-21 methyl group, showed a smaller solvent shift and was at a lower position than in withaferin A. Thus, the C-21 methyl group is probably in a different environment. A more significant difference between the spectra of the two compounds was the absence from the withacnistin spectrum of the signal (τ 9.28) attributed to the C-18 methyl group in withaferin A. Instead, the spectrum contained an AB quartet (τ 5.78 and 6.17; $\Delta\nu$ = 23 Hz; J = 12.0 Hz), which overlapped the signal for the C-22 proton. From its position it could be assigned to a C-18 primary acetate group.

Thus, by comparison with withaferin A, withacnistin can be assigned the structure and configuration **14a**. Acetylation gave a crystalline acetate (**14b**), whose infrared spectrum contained no absorption due to hydroxyl groups. Its nmr spectrum showed the expected acetylation shift of the secondary C-4 proton to τ 5.30.

Withacnistin (**14a**) underwent ready addition of methanol to give 2,3-dihydro-3-methoxywithacnistin (**15a**). Its nmr spectrum lacked the vinylic proton signals and instead contained two double doublets at τ 7.38 (J_{AB} = 15.5 Hz, J_{AX} = 4.2 Hz) and τ 6.97 (J_{AB} = 15.5 Hz, J_{BX} = 5.7 Hz) due to the C-2 methylenic protons, which constituted the AB portion of



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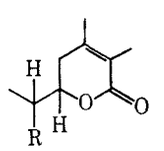
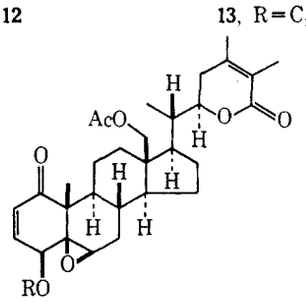


13, R = $C_{21}H_{27}O_5$



14a, R = H

b, R = Ac

an ABX system. The C-3 proton (τ 6.3, m) was further coupled to the C-4 proton. Acetylation of **15a** yielded crystalline **15b**, whose ultraviolet spectrum, λ_{max} 229 mμ (ϵ 7400), was consistent with an α,β-dimethyl-α,β-unsaturated δ-lactone.

This facile Michael addition to the ring-A α,β-unsaturated ketone apparently leads to the formation of artifacts during plant extraction, as 3-ethoxy-2,3-dihydrowithacnistin (**16a**) was also isolated.[20] Its spectra were in agreement with the proposed structure and it could be converted into its acetate (**16b**). Hydrolysis of **16a** with potassium hydroxide in methanol gave 18-deacetyl-2,3-dihydro-3-methoxywithacnistin (**15c**). Its formation must have proceeded *via* a retro Michael reaction and hydrolysis of the acetate, followed by Michael addition of methanol to ring A. Treatment of **15a** in the same way also led to the same diol **15c**, which on acetylation gave the diacetate **15b**. Partial acetylation of **15c** with acetic anhydride and a catalytic amount of pyridine in benzene gave the monoacetate **15a**. In the nmr spectrum of the diol **15c**, the AB quartet assigned to the C-18 methylene protons in the 18-acetoxyl derivatives was replaced

(20) *Cf.* the isolation of the methanol adduct of withaferin A by Lavie, *et al.*[13]

TABLE IV

MASS SPECTRAL DATA FOR WITHAFERIN A AND DERIVATIVES[a]

Compd	M+	a	b	c	d	e	f	g
4a	470 ($C_{28}H_{38}O_6$)	141 ($C_7H_9O_3$)	197 ($C_{11}H_{17}O_3$)	167 ($C_9H_{11}O_3$)[b]	154 ($C_8H_{10}O_3$)[b]	141 ($C_7H_9O_3$)	124 ($C_7H_8O_2$)	347 ($C_{21}H_{31}O_4$)
6	472 ($C_{28}H_{40}O_6$)	141 ($C_7H_9O_3$)	197 ($C_{11}H_{17}O_3$)	169 ($C_9H_{13}O_3$)	156 ($C_8H_{12}O_3$)	143 ($C_7H_{11}O_3$)	126 ($C_7H_{10}O_2$)	347 ($C_{21}H_{31}O_4$)
7a	456 ($C_{28}H_{40}O_5$)	125 ($C_7H_9O_2$)	181 ($C_{11}H_{17}O_2$)	169 ($C_9H_{13}O_3$)	156 ($C_8H_{12}O_3$)	143 ($C_7H_{11}O_3$)	126 ($C_7H_{10}O_2$)	331 ($C_{21}H_{31}O_3$)
7b	498 ($C_{30}H_{42}O_6$)	125 ($C_7H_9O_2$)	181 ($C_{11}H_{17}O_2$)	211 ($C_{11}H_{15}O_4$)	138 ($C_8H_{10}O_2$) (d − C_2H_4O)	185 ($C_9H_{13}O_4$)	168 ($C_9H_{12}O_3$)	331 ($C_{21}H_{31}O_3$)
9	502 [$C_{29}H_{42}O_7$][c]	141 ($C_7H_9O_3$)	197 ($C_{11}H_{17}O_3$)	199 ($C_{10}H_{15}O_4$)	186 ($C_9H_{14}O_4$)	155 ($C_8H_{11}O_3$) (e − H_2O)	156 ($C_8H_{12}O_3$)	347 ($C_{21}H_{31}O_4$)
10a	486 ($C_{29}H_{42}O_6$)	125 ($C_7H_9O_2$)	181 ($C_{11}H_{17}O_2$)	199 ($C_{10}H_{15}O_4$)	186 ($C_9H_{14}O_4$)[b]	173 ($C_8H_{13}O_4$)	156 ($C_8H_{12}O_3$)	331 ($C_{21}H_{31}O_3$)
10b	528 ($C_{31}H_{44}O_7$)	125 ($C_7H_9O_2$)	181 ($C_{11}H_{17}O_2$)	241 ($C_{12}H_{17}O_5$)	228 ($C_{11}H_{16}O_5$)	215 ($C_{10}H_{15}O_5$)	198 ($C_{10}H_{14}O_4$)	331 ($C_{21}H_{31}O_3$)

[a] In all cases, the appropriate fragment ions formed by losses of water, methanol, or acetic acid from the quoted ions were also observed. [b] While these ions were present, the major ion from these fragmentations appeared to be one hydrogen less. [c] In this case, the molecular ion was observed only in the low-resolution spectrum but not in the high-resolution spectrum.

TABLE V

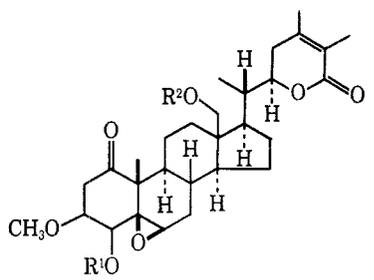
MASS SPECTRAL DATA FOR WITHACNISTIN AND DERIVATIVES[a]

[in every case, ions were present at *m/e* 125 ($C_7H_9O_2$, a) and 181 ($C_{11}H_{17}O_2$, b)]

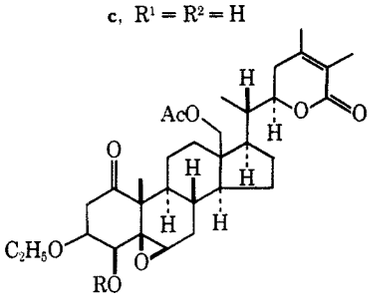
Compd	M+[b]	c	d	e	f	g
14a	512 ($C_{30}H_{40}O_7$)	167 ($C_9H_{11}O_3$)	154 ($C_8H_{10}O_3$)	141 ($C_7H_9O_3$)	124 ($C_7H_8O_2$)	389 ($C_{23}H_{33}O_5$)
15b	586 [$C_{33}H_{46}O_9$]	241 ($C_{12}H_{17}O_5$)	168 ($C_9H_{12}O_3$) (d − $C_2H_4O_2$)	215 ($C_{10}H_{15}O_5$)	198 ($C_{10}H_{14}O_4$)	389 ($C_{23}H_{33}O_5$)
15c	502 [$C_{29}H_{42}O_7$]	199 ($C_{10}H_{15}O_4$)	186 ($C_9H_{14}O_4$)	173 ($C_8H_{13}O_4$)	156 ($C_8H_{12}O_3$)	347 ($C_{21}H_{31}O_4$)
16a	558 [$C_{32}H_{46}O_8$]	213 ($C_{11}H_{17}O_4$)	200 ($C_{10}H_{16}O_4$)	187 ($C_9H_{15}O_4$)	170 ($C_9H_{14}O_3$)	389 ($C_{23}H_{33}O_5$)
16b	600 [$C_{34}H_{48}O_9$]	255 ($C_{13}H_{19}O_5$)	182 ($C_{10}H_{14}O_3$) (d − $C_2H_4O_2$)	229 ($C_{11}H_{17}O_5$)	212 ($C_{11}H_{16}O_4$)	389 ($C_{23}H_{33}O_5$)

[a] In all cases, the appropriate fragment ions formed by losses of water, methanol, ethanol, or acetic acid from the quoted ions were also observed. [b] Ions whose formulae are given in square brackets were observed in low-resolution spectra but not in high-resolution spectra.

by a broad singlet (τ 6.37), the upfield shift corresponding to the deacetylation of a primary acetate.



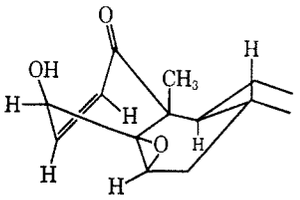
15a, $R^1 = H$; $R^2 = Ac$
b, $R^1 = R^2 = Ac$
c, $R^1 = R^2 = H$



16a, R = H
b, R = Ac

Comparison of the ORD spectra of **4a** and **14a** showed them to be very similar and supported the assignment of an A/B-*cis* ring fusion to withacnistin **14a**. This argument was supported by the similarity between the chemical shifts and coupling constants of the C-4 and C-6 proton signals. Thus, **14a** was assigned a structure with a 4β-hydroxyl group and 5,6β-epoxide, as was found for **4a**.

The solvent-induced shifts of the C-19 methyl group between deuteriochloroform and benzene–deuteriochloroform (1:1) were studied in derivatives of withaferin A (Table II) and withacnistin (Table III). In all cases, the small downfield shift led to the conclusion[21] that ring A was in a boat-type orientation (**17**)



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in which the hydroxyl group at C-4 was in an axial β position and the C-1 carbonyl group pointed upwards toward the C-19 methyl group.

These conclusions were independently confirmed by the X-ray crystallographic analysis[11] of **4c**, which showed that ring A was in a boatlike configuration.

The fragmentations of derivatives of withaferin A (Table IV, Figures 1 and 2) and withacnistin (Table V, Figures 3 and 4) in the mass spectrometer follow basically the same pathways. The variations in substitution appear to have little effect on the breakdown of the carbon skeleton. Both the molecular ions and the major fragmentation ions showed a series of further fissions corresponding to the losses of their functional groups. The presence of these ions was used to confirm the assignments of the proposed fragments, losses of water (−18), methanol (−32), ethanol (−46), or acetic acid (−60) suggesting the presence of hydroxyl, methoxyl, ethoxyl, or acetoxyl groups, respectively. The assignment of the proposed fragments was also aided by comparison of the high-resolution spectra[22] of related compounds, although because of the complexity of these molecules there may be alternative sequences leading to a particular fragment.

(21) *Cf.* E. Glotter and D. Lavie, *J. Chem. Soc., C*, 2298 (1967).
(22) A. L. Burlingame and D. H. Smith, *Tetrahedron*, **24**, 5749 (1968).

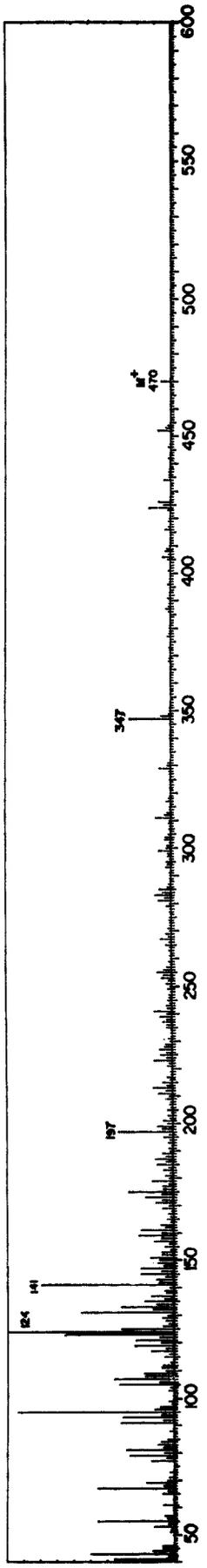


Figure 1.—Mass spectrum of withaferin A (4a).

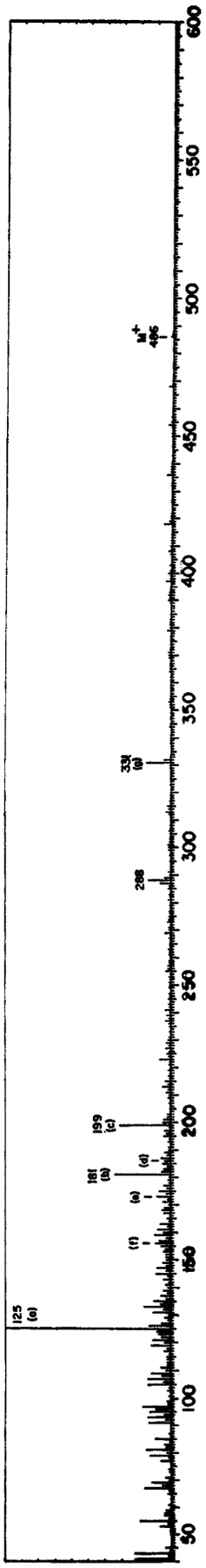


Figure 2.—Mass spectrum of 27-deoxy-2,3-dihydro-3-methoxywithaferin A (10a).

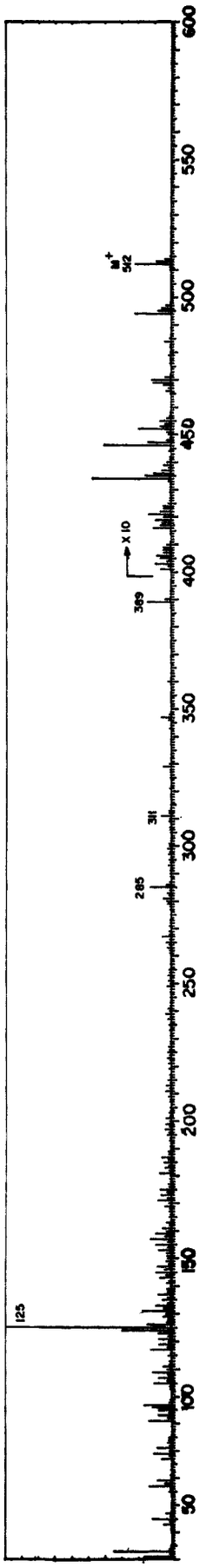


Figure 3.—Mass spectrum of withacnistin (14a).

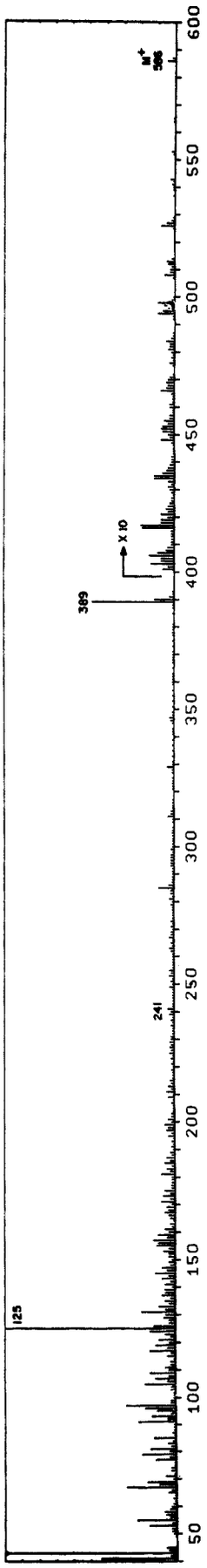
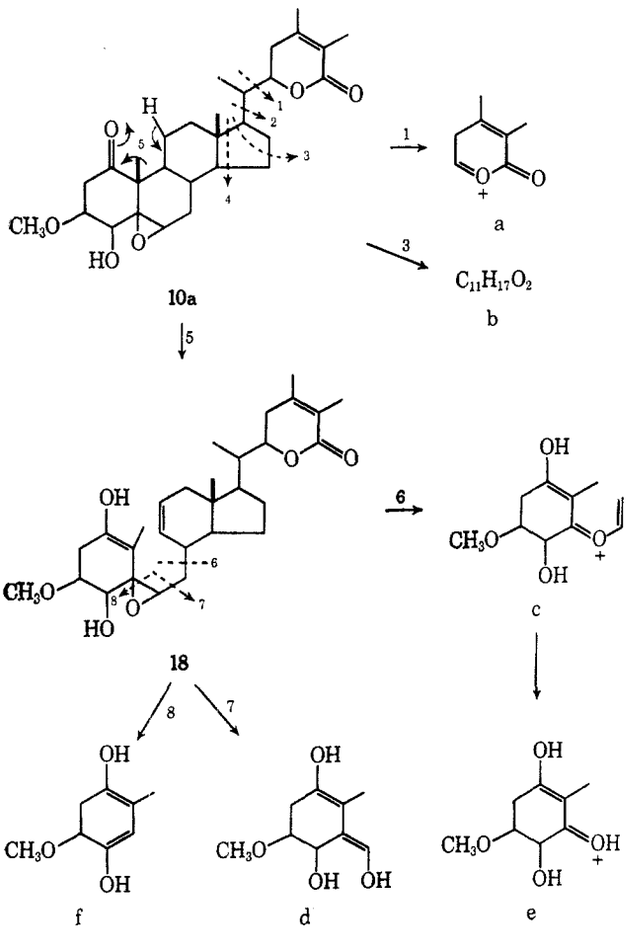


Figure 4.—Mass spectrum of 2,3-dihydro-3-methoxywithacnistin acetate (15b).

SCHEME I



The fragmentation of the carbon skeleton can be considered in two parts, that of ring D and the side chain and that of ring B. As 2,3-dihydro-3-methoxywithaferin A (**10a**) is typical of this series of compounds, it will be used as a model (Figure 2 and Scheme I).

The base peak (*m/e* 125) of the spectrum can be assigned to the ion a, formed by fission 1, which is prominent in the other derivatives. Fission 3 of ring D leads to the ion b at *m/e* 181. There are also smaller ions at *m/e* 331, 306, and 291, corresponding to the losses of the side chain (2) and the side chain with two (3) or three carbons (4) from ring D, respectively.[2,3] These peaks, whose formation may involve hydrogen transfers or rearrangement, were usually small and were therefore not included in the tables.

The fission of ring B can be considered to be promoted by a McLafferty rearrangement[19] (5) to give the ion **18**. Fission 6 with rearrangement of the epoxide can lead to the ion c at *m/e* 199, which can further lose C_2H_2 to give the ion e at *m/e* 173, whereas fission 7 can give the ion d at *m/e* 186.

The loss of fragment c with a hydrogen transfer can be assigned to the ion *m/e* 285. The double fission 8 is responsible for the ions f (*m/e* 156) and g (*m/e* 331 after hydrogen transfer).

These fragmentations, with the expected changes on differing substitution, are also found in the high-resolution spectra of the other withaferin A and withacnistin derivatives, as shown in Tables IV and V.

(23) L. Tökés, G. Jones, and C. Djerassi, *J. Amer. Chem. Soc.*, **90**, 5465 (1968).

Experimental Section

Melting points were determined on a Hoover Uni-Melt apparatus in a capillary tube and have been corrected. Ultraviolet absorption spectra were determined in 95% ethanol on a Beckman DK-2A ratio-recording spectrophotometer. Infrared spectra were determined on Beckman IR-5A and IR-9 recording spectrophotometers in chloroform unless otherwise stated. Optical rotations were determined on a Zeiss–Winkel polarimeter in chloroform solution and values of $[\alpha]_D$ have been approximated to the nearest degree. Optical rotatory dispersion spectra were determined on a Cary model 60 spectropolarimeter in dioxane solution. Nuclear magnetic resonance spectra were determined on Varian Associates A-60A and HA-100 recording spectrometers using tetramethylsilane as an internal standard. A C.E.C.-21-1103 mass spectrograph was used for both low- and high-resolution mass spectra; ionizing current was 70 eV. Solid samples were introduced directly into the ion source and complete high-resolution mass spectra were recorded on a photoplate. Spectra were usually determined at an ion-source temperature of 200–220°; however, some samples (particularly in the withacnistin series) required temperatures up to 270°. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. Petroleum ether refers to the fraction with bp 60–68°. Alumina refers to acid-washed aluminum oxide. Hydrogenations were carried out at 25° and atmospheric pressure. Solutions were evaporated at room temperature under reduced pressure.

Isolation of Withaferin A (4a), Withacnistin (14a), Ethanol Adduct 16a, and Loliolide (18).—An ethanolic extract (260 g) of the leaves (air dried, 2.2 kg) of *Acnistus arborescens* (L.) was dissolved in methanol–water (9:1, 2.2 l.) and extracted with petroleum ether (five 1-l. portions). The methanol solution was evaporated and the residue (227 g) was dissolved in chloroform (1.5 l.), which was washed with water (four 1-l. portions) and evaporated. The residue was dissolved in formamide (1 l.) and washed with benzene (five 1-l. portions). It was then extracted with chloroform (seven 1-l. portions), which was concentrated slightly, washed with water (two 1-l. portions), dried (Na_2SO_4), and evaporated to give a residue (18.1 g).

This material (18.1 g) was chromatographed on alumina (550 g) to give fraction A (5.5 g) on elution with chloroform, fraction B (2.8 g) with 2% methanol–chloroform (4 l.), fraction C (1.1 g) with 5% methanol–chloroform (4 l.), fraction D (0.8 g) with methanol (6 l.), and fraction E with acetic acid–methanol (1:9).

Fraction A (5.5 g) was chromatographed on alumina to give a mixture (1.1 g), which on rechromatography on silica gel yielded A_1 (0.2 g) and A_2 (0.1 g) on elution respectively with benzene–ether (1:1) and benzene–ether (1:4). Fraction A_1, on further chromatography on silica gel, gave, on elution with chloroform, 3-ethoxy-2,3-dihydrowithacnistin (**16a**, 22 mg): mp 134–136° (sintered *ca.* 112°); $[\alpha]^{24}D$ +29° (*c* 0.46); uv λ_{max} 227 mμ (ε 8600); ir λ_{max} 2.78, 2.90, 3.31, 5.78, 5.88, 8.95, and 9.62 μ.

Anal. Calcd for $C_{32}H_{46}O_8$: C, 68.79; H, 8.30. Found: C, 69.01; H, 8.42.

Precipitation of fraction A_2 from benzene gave crude withacnistin (**14a**). Further treatment with benzene yielded pure **14a** (25 mg) as an amorphous solid: mp 130–135°; $[\alpha]^{27}D$ +123° (*c* 1.21); uv λ_{max} 215 mμ (ε 16,100); ir λ_{max} 2.81, 2.93, 3.31, 5.78, 5.92, 8.05, and 9.63 μ; ORD (*c* 0.033) $[\phi]_{600}$ +461°, $[\phi]_{589}$ +461°, $[\phi]_{500}$ +778°, $[\phi]_{400}$ +2305°, $[\phi]_{368}$ +4148°, $[\phi]_{343}$ +1440°, λ_0 334 mμ, $[\phi]_{317.5}$ −1095°, λ_0 307 mμ, $[\phi]_{272}$ +7202°, $[\phi]_{261}$ +5992°, $[\phi]_{250}$ +4206°, $[\phi]_{243}$ +6741°.

Anal. Calcd for $C_{30}H_{40}O_7$: C, 70.29; H, 7.87. Found: C, 69.96; H, 7.76.

Rechromatography on silica gel of fractions (1.3 g) from the purification of **14** and **16a**, on elution with benzene–chloroform (3:2), gave a solid which, on repeated crystallization from chloroform–cyclohexane, yielded loliolide (**11**, 7 mg): mp 152–152.5°; $[\alpha]^{28}D$ −111° (*c* 1.04); uv λ_{max} 212.5 mμ (ε 11,500); ir λ_{max} 2.77, 2.89, 5.75, and 6.16 μ. The compound was characterized by comparison with its reported physical and spectral properties.[8]

Anal. Calcd for $C_{11}H_{16}O_3$: mol wt, 196.1099. Found: mol wt, 196.1102 (mass spectrum).

Fraction B was crystallized from ethyl acetate to give a colorless solid (0.76 g), mp 230–237°. Two recrystallizations from acetone–petroleum ether yielded crude withaferin A (0.45 g) as small colorless prisms, mp 243–244°. Repeated recrystallization from acetone–petroleum ether gave white prisms of with-

aferin A (**4a**): mp 252–253°; $[\alpha]^{28}D$ +125° (*c* 1.30); uv λ_{max} 214 (ϵ 17,300) and 335 mμ (ϵ 165); ir λ_{max} 2.81, 2.94 (hydroxyl), 3.33, and 5.92 μ (α,β-unsaturated δ-lactone and α,β-unsaturated cyclohexanone); ORD (*c* 0.0944) $[\phi]_{600}$ +522°, $[\phi]_{589}$ +522°, $[\phi]_{500}$ +820°, $[\phi]_{400}$ +2609°, $[\phi]_{367}$ +4510°, $[\phi]_{343}$ +1603°, λ_0 333.5 mμ, $[\phi]_{319}$ −895°, λ_0 309 mμ, $[\phi]_{278}$ +7679°, $[\phi]_{262.5}$ +5628°, $[\phi]_{252}$ +3504°, $[\phi]_{247}$ +5591°.

Anal. Calcd for $C_{28}H_{38}O_6$: C, 71.46; H, 8.14. Found: C, 71.34; H, 8.06.

Withaferin A Diacetate (4b).—A solution of withaferin A (**4a**, 46 mg) in anhydrous pyridine (1 ml) and acetic anhydride (1 ml) was allowed to stand overnight at room temperature. Normal work-up followed by two crystallizations from acetone–petroleum ether yielded **4b** (33 mg): mp 201–202°; $[\alpha]^{30}D$ +192° (*c* 1.09); uv λ_{max} 214 mμ (ϵ 18,000); ir λ_{max} 3.33, 5.76, 5.86, 5.94, and 8.08 μ.

Anal. Calcd for $C_{32}H_{42}O_8$: C, 69.29; H, 7.63. Found: C, 69.57; H, 7.58.

4-Dehydrowithaferin A (5).—Withaferin A (**4a**, 220 mg) was added to a stirred suspension of activated manganese dioxide (1 g) in chloroform (70 ml) at 25°. After 2 days, the reaction mixture was filtered and evaporated to yield a yellow oil (203 mg). Chromatography of a small sample (32 mg) on alumina gave a solid, pale yellow foam (24 mg) on elution with chloroform. Crystallization from acetone–petroleum ether yielded 4-dehydrowithaferin A (**5**, 18 mg) as pale yellow prisms: mp 276–277°; $[\alpha]^{27}D$ +106° (*c* 0.786); uv λ_{max} 223 mμ (ϵ 14,700); ir λ_{max} 2.80, 5.85–5.91 (α,β-unsaturated δ-lactone and ene-dione), and 6.17 μ (C=C).

Anal. Calcd for $C_{28}H_{36}O_6$: C, 71.77; H, 7.74. Found: C, 71.66; H, 7.70.

2,3-Dihydrowithaferin A (6).—A solution of withaferin A (**4a**, 500 mg) in dioxane (50 ml) was added to a prereduced suspension of platinum oxide (300 mg) in dioxane (20 ml). The stirred mixture was hydrogenated for 3 hr, filtered, and evaporated. Repeated chromatography of the residue on silica gel and elution with 3% methanol–chloroform gave crude **6** (175 mg). Several crystallizations from acetone–petroleum ether afforded **6** as colorless platelets: mp 229–230°; uv λ_{max} 217 mμ (ϵ 8800); ir λ_{max} 2.78, 2.89, and 5.88 μ.

Anal. Calcd for $C_{28}H_{40}O_6$: mol wt, 472.2825. Found: mol wt, 472.2831 (mass spectrum).

27-Deoxy-2,3-dihydrowithaferin A (7a).—A solution of withaferin A (**4a**, 115 mg) in 95% ethanol (50 ml) was hydrogenated over 10% Pd–C (50 mg). After 2 mol equiv of hydrogen had been consumed (*ca.* 45 min), the reaction mixture was filtered and evaporated to give a solid white foam. Crystallization from acetone–petroleum ether yielded **7a**: mp 246–247° *in vacuo*; $[\alpha]^{28}D$ −8° (*c* 0.61); uv λ_{max} 227 mμ (ϵ 7100); ir λ_{max} 2.79, 2.91, 3.33, 5.86, and 5.90 μ.

Anal. Calcd for $C_{28}H_{40}O_5$: C, 73.65; H, 8.83; mol wt, 456.2875. Found: C, 73.69; H, 9.06; mol wt, 456.2873 (mass spectrum).

27-Deoxy-2,3-dihydrowithaferin A Acetate (7b).—Acetic anhydride (2 ml) was added to a solution of **7a** (75 mg) in anhydrous pyridine. After 20 hr at 25°, the reaction mixture was worked up to give, after three crystallizations from acetone–petroleum ether, **7b** (25 mg) as fine colorless needles: mp 260–262° *in vacuo*; uv λ_{max} 226 mμ (ϵ 9100); ir λ_{max} 3.32, 5.76, 5.87, and 8.08 μ.

Anal. Calcd for $C_{30}H_{42}O_6$: mol wt 498.2981. Found: mol wt, 498.2975 (mass spectrum).

27-Deoxy-2,3:24,25-tetrahydrowithaferin A (8a).—A mixture of withaferin A (**4a**, 204 mg) and 10% Pd/C (100 mg) in 95% ethanol (50 ml) was hydrogenated. After 3 mol equiv of hydrogen had been consumed (*ca.* 16 hr), the reaction mixture was filtered and evaporated to give a solid white foam. Silica gel chromatography (elution with chloroform) followed by crystallization from acetone–petroleum ether yielded crude **8a** (124 mg). Three recrystallizations from acetone–petroleum ether gave pure **8a** (109 mg): mp 228–229° (Fisher-Johns melting point block); ir λ_{max} 2.79, 5.78, and 5.86 μ.

Anal. Calcd for $C_{28}H_{42}O_5$: C, 73.32; H, 9.23. Found: C, 73.51; H, 9.12.

27-Deoxy-2,3:24,25-tetrahydrowithaferin A Acetate (8b). A. From 8a.—A solution of **8a** (59 mg) in anhydrous pyridine (4 ml) was treated with acetic anhydride (4 ml). After 16 hr at 25°, the reaction mixture was worked up in the normal way to give a solid white foam. Silica gel chromatography (elution with chloroform) followed by three crystallizations from acetone–petroleum ether gave **8b** (32 mg): mp 188–189°; ir λ_{max} 3.31, 5.76, 5.82, and 8.04 μ.

Anal. Calcd for $C_{30}H_{44}O_6$: mol wt, 500.3137. Found: mol wt, 500.3159 (mass spectrum).

B. From 7b.—**8b** was also obtained on hydrogenation of 27-deoxy-2,3-dihydrowithaferin A acetate (**7b**) for 23 hr.

2,3-Dihydro-3-methoxywithaferin A (9).—A solution of withaferin A (**4a**, 400 mg) in 0.05 *N* methanolic sodium acetate (100 ml) was allowed to stand at room temperature for 17 hr and was then heated under reflux for 2 hr. The methanol was evaporated, and the residue was dissolved in chloroform, filtered, and evaporated to give 433 mg of solid white foam. Three crystallizations from acetone–petroleum ether afforded **9** as colorless needles: mp 242–243°; $[\alpha]^{35}D$ +19° (*c* 0.99); uv λ_{max} 217 mμ (ϵ 9500); ir λ_{max} 2.79, 2.94, 5.86, and 5.91 μ.

Anal. Calcd for $C_{29}H_{42}O_7$: C, 69.29; H, 8.42. Found: C, 68.79; H, 8.33.

27-Deoxy-2,3-dihydro-3-methoxywithaferin A (10a).—A mixture of 2,3-dihydro-3-methoxywithaferin A (**9**, 105 mg) and 10% Pd–C (60 mg) in 95% ethanol (50 ml) was hydrogenated. After 1 mol equiv of hydrogen had been consumed (*ca.* 1.5 hr), the reaction mixture was filtered and evaporated to give a solid white foam. Two crystallizations from acetone–petroleum ether yielded **10a** as large colorless needles: mp 243–244°; $[\alpha]^{28}D$ +6° (*c* 0.33); uv λ_{max} 227 mμ (ϵ 8200); ir λ_{max} 2.78, 2.91, 3.32, and 5.88 μ.

Anal. Calcd for $C_{29}H_{42}O_6$: C, 71.57; H, 8.76; mol wt, 486.2981. Found: C, 71.42; H, 8.95; mol wt, 486.2988 (mass spectrum).

27-Deoxy-2,3-dihydro-3-methoxywithaferin A Acetate (10b).—Acetic anhydride (2 ml) was added to a solution of **10a** (37 mg) in anhydrous pyridine (2 ml). After 18 hr at 25°, the reaction mixture was worked up in the normal way. Silica gel chromatography yielded crude **10b** (30 mg) on elution with benzene–chloroform (1:4). Two crystallizations from acetone–cyclohexane afforded **10b** as fine colorless needles, mp 196–199°.

Anal. Calcd for $C_{31}H_{44}O_7$: mol wt, 528.3086. Found: mol wt, 528.3104 (mass spectrum).

Withaferin A *p*-Bromobenzoate Monoacetate (4c).—*p*-Bromobenzoyl chloride (180 mg) was added to a solution of withaferin A (**4a**, 172 mg) in benzene (15 ml) and anhydrous pyridine (1 ml) and heated under reflux for 1 hr. Working up in the normal way followed by chromatography on silica gel gave, on elution with chloroform and crystallization from acetone–petroleum ether, withaferin A *p*-bromobenzoate: mp 198–199°; $[\alpha]^{28}D$ +63° (*c* 0.75); ir λ_{max}^{Nujol} 5.82, 5.90, 5.98, 6.29, 6.81, and 7.22 μ.

Anal. Calcd for $C_{35}H_{41}BrO_7$: C, 64.30; H, 6.43; Br, 12.22. Found: C, 64.20; H, 6.33; Br, 12.19.

A solution of the *p*-bromobenzoate (60 mg) in acetic anhydride (3 ml) and anhydrous pyridine (3 ml) was kept at 25° for 16 hr. Normal work-up followed by three crystallizations from acetone–petroleum ether gave crude **4c**. Recrystallization from ethyl acetate–petroleum ether yielded **4c** (30 mg): mp 179–180°; $[\alpha]^{28}D$ +101° (*c* 0.73); ir λ_{max}^{Nujol} 5.72, 5.82, 5.89, 5.93, 6.29, 6.82, 7.24, and 7.83 μ.

Anal. Calcd for $C_{37}H_{43}BrO_8$: C, 63.88; H, 6.23; Br, 11.49. Found: C, 64.47; H, 6.62; Br, 11.90.

Withacnistin Acetate (14b).—A solution of withacnistin (**14a**, 70 mg) in anhydrous pyridine (2 ml) was treated with acetic anhydride (2 ml). After 16 hr at 25°, the reaction mixture was worked up in the normal way to give a solid white foam. Chromatography on silica gel yielded, on elution with benzene–chloroform (1:9), crude **14b** (62 mg). Several crystallizations from acetone–cyclohexane afforded withacnistin acetate (**14b**) as fine white needles: mp 131–132°; $[\alpha]^{25}D$ +180° (*c* 1.90); uv λ_{max} 217 mμ (ϵ 15,500); ir λ_{max} 3.31, 5.76, 5.90, 8.06, and 9.62 μ.

Anal. Calcd for $C_{32}H_{42}O_8$: C, 69.29; H, 7.63. Found: C, 69.66; H, 7.95.

2,3-Dihydro-3-methoxywithacnistin (15a). A. From Withacnistin (14a).—A solution of **14a** (51 mg) in 0.05 *N* methanolic sodium acetate (10 ml), under nitrogen, was allowed to stand for 16 hr at 25° and was then heated under reflux for 3 hr. The reaction mixture was cooled and evaporated. The residue was dissolved in chloroform and filtered. Silica gel chromatography gave 2,3-dihydro-3-methoxywithacnistin (**15a**, 49 mg), on elution with chloroform, as a solid white foam which could not be induced to crystallize: ir λ_{max} 2.79, 2.90, 3.32, 3.36, 5.78, 5.89, 7.17, 8.08, 8.87, and 9.62 μ.

B. From 18-Deacetyl-2,3-dihydro-3-methoxywithacnistin (15c).—A solution of 18-deacetyl-2,3-dihydro-3-methoxywith-

acnistin (**15c**, 20 mg) in benzene (5 ml) and anhydrous pyridine (0.4 ml) was treated with acetic anhydride (0.2 ml). After 24 hr at 7°, normal work-up followed by elution from a silica gel column with chloroform gave pure **15a** with tlc behavior and ir and nmr spectral properties identical with those of 2,3-dihydro-3-methoxywithacnistin (**15a**) prepared in part A.

2,3-Dihydro-3-methoxywithacnistin Acetate (15b). A. From 2,3-Dihydro-3-methoxywithacnistin (15a).—A solution of **15a** (54 mg) in anhydrous pyridine (2 ml) was treated with acetic anhydride (2 ml) for 15 hr at 26°. Normal work-up followed by silica gel chromatography gave crude **15b** on elution with benzene–chloroform (1:4). Three crystallizations from acetone–cyclohexane afforded 2,3-dihydro-3-methoxywithacnistin acetate (**15b**, 11 mg) as white needles: mp 242–244° dec; $[\alpha]^{23}D$ +18° (*c* 1.43); uv λ_{max} 229 mμ (ε 7400); ir λ_{max} 3.31, 3.52, 5.75, 5.88, 8.06, and 9.63 μ.

Anal. Calcd for $C_{33}H_{46}O_9$: C, 67.55; H, 7.90. Found: C, 67.58; H, 8.01.

B. From 18-Deacetyl-2,3-dihydro-2-methoxywithacnistin (15c).—A solution of **15c** (7 mg) in anhydrous pyridine (1 ml) was treated with acetic anhydride (1 ml) for 17 hr at 25°. Normal work-up followed by silica gel chromatography yielded crude **15b** (4 mg) on elution with benzene–chloroform (1:4). Crystallization of the diacetate from acetone–cyclohexane gave colorless needles (**15b**, 2 mg): mp 242–244° dec.

18-Deacetyl-2,3-dihydro-3-methoxywithacnistin (15c). A. From 3-Ethoxy-2,3-dihydrowithacnistin (16a).—A solution of **16a** (129 mg) in 0.16 *N* methanolic potassium hydroxide (15 ml) was heated at 55° for 5 hr under nitrogen. The orange reaction mixture was cooled, poured into water (100 ml), acidified with 2 *N* sulfuric acid, and extracted with chloroform. The chloroform solution was washed with water, dried (Na_2SO_4), and evaporated to give 109 mg of a pale yellow, solid foam. Chromatography on silica gel yielded crude **15c** (43 mg) on elution with 1% methanol–chloroform. Three crystallizations from acetone–cyclohexane afforded 18-deacetyl-2,3-dihydro-3-methoxywithacnistin (**15c**, 16 mg) as colorless needles: mp 227–229° dec; $[\alpha]^{24}D$ +34° (*c* 1.12); uv λ_{max} 228 mμ (ε 7700); ir λ_{max} 2.75, 2.86, 3.31, 3.53, 5.89, and 9.65 μ.

Anal. Calcd for $C_{29}H_{42}O_7$: C, 69.29; H, 8.42. Found: C, 69.56; H, 8.51.

B. From 2,3-Dihydro-3-methoxywithacnistin (15a).—A solution of **15a** (10 mg) in 0.16 *N* methanolic potassium hydroxide (5 ml) was heated, under nitrogen, at 55° for 5 hr. Work-up as in part A yielded 6 mg of material. Silica gel chromatography gave 3 mg of **15c**.

3-Ethoxy-2,3-dihydrowithacnistin Acetate (16b).—A solution of **16a** (128 mg) in anhydrous pyridine (5 ml) was treated with acetic anhydride (5 ml). After 14 hr at 25°, the reaction mixture was worked up in the normal way followed by silica gel chromatography, which gave a solid white foam on elution with chloroform. Two crystallizations from acetone–cyclohexane afforded pure **16b** (66 mg) as colorless needles: mp 216–217° dec, sintered at 213°; $[\alpha]^{23}D$ +16° (*c* 1.16); uv λ_{max} 227 mμ (ε 8900); ir λ_{max} 3.30, 5.75, 5.88, 8.06, and 9.65 μ.

Anal. Calcd for $C_{34}H_{48}O_9$: C, 67.98; H, 8.05. Found: C, 68.06; H, 7.94.

Registry No.—**4a**, 5119-48-2; **4b**, 5234-92-4; **4c**, 6850-31-3; **5**, 6850-30-2; **6**, 5589-41-3; **7a**, 19317-85-2; **7b**, 21902-93-2; **8a**, 21902-94-3; **8b**, 21902-95-4; **9**, 21902-96-5; **10a**, 21902-97-6; **10b**, 21902-98-7; **14a**, 21902-99-8; **14b**, 21903-00-4; **15a**, 21903-01-5; **15b**, 21903-02-6; **15c**, 21903-03-7; **16a**, 21903-04-8; **16b**, 21903-05-9; withaferin A *p*-bromobenzoate, 21902-90-9.