Steroidal Constituents of *Physalis minima* (Solanaceae)

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Four new steroidal lactones have been isolated from the leaves of *Physalis minima* (Solanaceae), along with the known physalin B (1) (major constituent). 5β,6β-Epoxyphysalin B (2b) is a 16α,24-cyclo-13,14-seco-steroid whose structure was established by comparison with the 5α,6α- and 5β,6β-epoxy-derivatives (2a and 6) and obtained by epoxidation of physalin B (1). Withaphysalins A (3) and B (7a) belong to the withanolide group, the first containing an 18,20-lactone and the latter an 18,20-lactol ring. The structure of withaphysalin C, $C_{28}H_{36}O_7$, is still under investigation.

In continuation of our studies [1] on steroidal constituents of plants of the Solanaceae family, *Physalis minima*, an annual herb growing wildly in India, Ceylon, and tropical regions of Africa, has been investigated. The plant has a bitter taste and Indian popular medicine recommends it, *inter alia*, as a diuretic and laxative and in the treatment of inflammations of the spleen. The work was done on plants collected around Pondicherry, India, and on plants grown in our uniform nursery at Bet Dagan, Israel, from seeds received from India.

Extraction of the leaves and chromatographic separation of the crude extract afforded the known physalin B (1) [2] as the major component, accompanied by small amounts of four new, related compounds: 5β,6β-epoxyphysalin B, $C_{28}H_{30}O_{10}$ (2b), withaphysalin A, $C_{28}H_{34}O_6$ (3), withaphysalin B, $C_{28}H_{36}O_6$ (7a), and withaphysalin C, $C_{28}H_{36}O_7$. The structures of compounds (2b), (3), and (7) have been assigned, but withaphysalin C, isolated only in minute quantities, is still under investigation; however, some features of its structure have been deduced.

The ' normal ' withanolides, as exemplified by withanolide G (9) ,[1] are natural C_{28} steroidal lactones built up on a relatively highly oxidized ergostane-type skeleton, characterized by a six-membered ring lactone in the side chain (in most of the compounds isolated so far, this lactone is αβ-unsaturated). The various members of this group differ in the substitution pattern of the carbocyclic system, although with few exceptions all possess a 2-en-1-one group in ring A.

The physalins,[2] as exemplified by physalin B (1), although biogenetically related to the withanolides, are characterized in particular by (*a*) oxidative 13,14-bond cleavage giving a nine-membered ring; (*b*) formation of a new six-membered carbocycle between C-16 and C-24; and (*c*) oxidation of the 13-methyl group to a carboxylic acid followed by 18,20-lactonization.

Our interest in compounds (3) and (7a) and withaphysalin C, although these were present in very small amounts in comparison with (1), is due to the fact that they seem to represent intermediate biogenetic stages between the ' normal ' withanolides and the physalins. This is the first instance of a typical physalin such as (1) coexisting with compounds in which the steroidal carbon skeleton is intact.

Before discussing the complete structures of the withaphysalins (3) and (7a), we note some common, characteristic features. Neither compound possesses a C-15 keto-group; consequently the conditions for an intramolecular Michael-type addition leading to 16,24-bond formation are not satisfied. Both compounds possess a masked tertiary 20-hydroxy-group: in (3) as an 18,20-lactone [C(18)H_3 oxidised to acid level], and in (7a) [and withaphysalin C] as an 18,20-lactol [C(18)H_3 oxidised to aldehyde level]. The 13,14 bond is intact.

5β,6β-Epoxyphysalin B (2b) exhibits three i.r. bands in the carbonyl region at 1775 (γ-lactone), 1740 (overlap of saturated δ-lactone and five-membered ring ketone), and 1650 cm^{-1} (αβ-unsaturated ketone). The single u.v. maximum at 222 nm (ε 9 500) indicates the presence of only one αβ-unsaturated carbonyl chromophore. The n.m.r. spectrum (Table 1) shows signals similar to those exhibited by physalin B (1), with the exception of the 6-H signal which is a doublet at δ 3.23 (J 2.5 Hz) instead of a narrow multiplet at δ 5.65. Comparison of the molecular weight of (2b) (M^+ 526) with that of (1) (M^+ 510), in conjunction with the foregoing data suggests the presence of a 5,6-epoxide ring in the former; according to the n.m.r. 6-H signal it should be β-oriented. C.d. measurements confirmed this in that a positive Cotton effect was observed at 340 and 352 nm (Δε +2.09 and +1.69), as expected for a 2-en-1-one in a *cis*-fused AB ring system.

The structure of (2b) was finally confirmed by comparison with the products of epoxidation of (1) with *m*-chloroperbenzoic acid; a 1 : 1 mixture of the 5α,6α- and 5β,6β-epoxides (2a and b) was obtained and the latter was identical with the naturally occurring compound.* The epoxidic 6-proton in (2a) resonates at δ 3.06 (J 6 Hz), and the 2-en-1-one system in this compound gives rise to a negative Cotton effect at 335 and 342 nm(Δε −1.89 and −1.82), characteristic of a *trans* AB ring junction.

Withaphysalin A (3) exhibits i.r. bands at 1753, 1690, and 1640 cm^{-1} (γ-lactone,αβ-unsaturated δ-lactone, and

* Compound (2b) has also been isolated from *Physalis alkekengi* raised in our nursery from seeds purchased on the market, in Teheran.

[1] E. Glotter, I. Kirson, A. Abraham, and D. Lavie, *Tetrahedron*, 1973, **29**, 1353 and references cited therein.
[2] T. Matsuura, M. Kawai, R. Nakashima, and Y. Butsugan, *J. Chem. Soc.* (*C*), 1970, 664.

αβ-unsaturated ketone, respectively), and maximum u.v. absorption at 224.5 nm (ε 18 500). Catalytic hydrogenation over Pd–$CaCO_3$ takes place with the rapid absorption of 1 mol. equiv. of hydrogen to give the 2,3-dihydro-derivative (4), characterized by a shift of the 1 640 i.r. band to 1 705 cm^{-1}, lowering of intensity of the u.v. absorption [226.5 nm (ε 9 600)], and the disappearance from the low-field region of the n.m.r. spectrum of two signals corresponding to the vinylic 2-H and 3-H; the only low-field signal remaining is a narrow multiplet at δ 5.58 (6-H).

The n.m.r. spectrum of compound (3) (Table 1) agrees with the signals observed for the vinylic protons in rings A and B of cholesta-2,5-dien-1-one and with the spectra of withanolides possessing a similar substitution pattern, such as (9).[1] The structural similarities between (3) and (9) include the side-chain δ-lactone system responsible for the 226.5 nm u.v. band and a broadened six-proton n.m.r. signal (δ 1.95, two vinylic Me) and a double doublet at δ 4.65 (22-H). The appearance of the 20-Me signal as a singlet implies the absence of the 20-H; its low-field position (δ 1.51) indicates the presence of an oxygen atom at C-20. The stronger deshielding of this group in (3) as compared to (9) (δ 1.30) is attributable to several factors, as will now become apparent.

The n.m.r. spectrum of (3) also discloses the presence of only two tertiary methyl groups, the 10-Me (δ 1.30) and the 20-Me discussed above. The lack of any 13-Me signal, in conjunction with the 1750 cm^{-1} i.r. band (γ-lactone) and the precedent furnished by physalin B (1), leads to the conclusion that withaphysalin A (3) contains an 18,20 γ-lactone. The unusual low-field position of the 20-Me n.m.r. signal is thus due to deshielding by the lactone carbonyl group. The presence of the 18,20-lactone group defines the stereochemistry at C-17: it rules out the possibility of a 17α-side chain. As to the configuration at C-20, although both possible orientations of the hydroxy-group would allow closure of the γ-lactone, the α-stereochemistry (Fieser's designation [3]) is supported by biogenetic arguments: in all the 20-OH withanolides, as well as in the physalins, the configuration at C-20 is α. The stereochemistry at C-22 is the same as in all the withanolides (22*R*) and has been determined by c.d. measurements on compound (6) [positive Cotton effect at 245 nm (Δε +4.55)].

The mass spectral fragmentation of compound (3) (M^+ 466) supports the presence of the side chain δ-lactone (most important fragments at *m/e* 125 and 341, due to cleavage of the 20,22-bond) as well as of the second lactone group (*m/e* 422, due to loss of CO_2). A series of signals related to the loss of H_2O also point unequivocally to the presence of an OH group: *m/e* 448 ($M^+ - H_2O$), 404 [$M^+ - (CO_2 + H_2O)$], and 323 [$M^+ - (125 + H_2O)$]. In the absence of characteristic n.m.r. signals, this could be only a tertiary OH, the alternative locations being restricted to positions 14 and 17: for a 14-OH, the α-orientation would be biogenetically preferred since in the withanolide series a 14-OH, whenever present,[4] is α-oriented; for a 17-OH, the β-orientation is excluded by the presence of the 18,20-lactone. The following evidence supports the 14α-location for this tertiary OH. Treatment of compound (3) with thionyl chloride results in elimination of a molecule of water to give a deoxy-derivative (5), characterized by a new vinylic ^1H n.m.r.

TABLE 1

N.m.r. data*

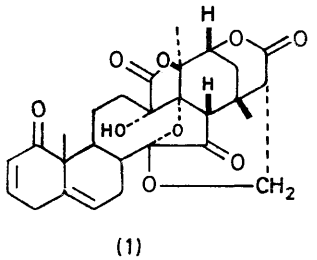
Compound	2-H	3-H	6-H	15-H	18-H	22-H	Me groups: 19-H	21-H	27- and 28-H
(1)	5.96dq	6.85dq	5.65m			4.60m	1.27s	2.00s	1.24 (28-H)
							[1.08]	[2.33]	[1.32]
(2a)	5.96dq	6.82dq	3.06d (6)			4.60m	1.32s	2.00s	1.25 (28-H)
(2b)	6.00dq	6.85dq	3.23d (2.5)			4.58m	1.31s	1.96s	1.26 (28-H)
(3)	5.90dq	6.83dq	5.62m			4.62dd (12, 4.5)	1.30s	1.51s	1.95
(4)			5.58m			4.65dd (12, 4)	1.36s	1.51s	1.95
			[5.52]			[4.85]	[1.31]	[1.48]	[1.74, 1.85]
(5)	5.93dq	6.85dq	5.65m	5.32 narrow m		4.58dd (12, 5)	1.32s	1.57s	1.89, 1.98
(6)			3.10d (3.5)			4.58dd (12, 5)	1.45s	1.45s	1.97
(7a)	6.03dq	6.9dq	3.15		5.17, 5.28 †	4.58dd	1.25s	1.28, 1.47	1.93
(7b)	6.1dq	6.9dq	3.17		6.17	4.50dd	1.13	1.25, 1.45	1.93
(8)	6.05dq (10, 3, *ca.* 1)	6.93dq (10, 5, 2.5)	3.17d (2.5)			4.57dd (11.5, 5)	1.30s	1.51s	1.92
	[6.20]	[6.87]	[3.22]			[4.62]	[1.48]	[1.48]	[1.80, 1.97]
Withaphysalin C	5.92dq	6.82dq	5.65m		5.10s †	4.32dd (12, 4)	1.27s	1.27s	1.90, 1.97

* Recorded at 60 MHz; solvent $CDCl_3$; δ values; data for C_5D_5N solutions in square brackets; coupling constants (Hz) in parentheses. † After addition of D_2O.

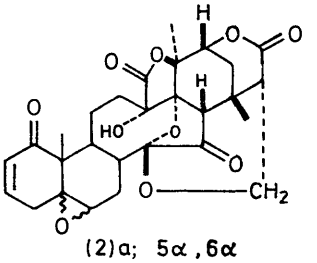
[1] L. Fieser and M. Fieser, 'Steroids,' Reinhold, New York, 1959, p. 344.

[4] E. Glotter, R. Waitman, and D. Lavie, *J. Chem. Soc.* (*C*), 1966, 1765.

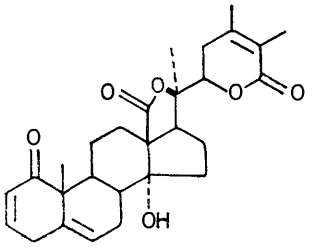
signal (narrow multiplet at δ 5.32). The transformation (3) ⟶ (5) has little influence on the chemical shift of the



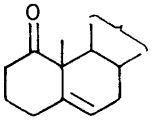
(1)



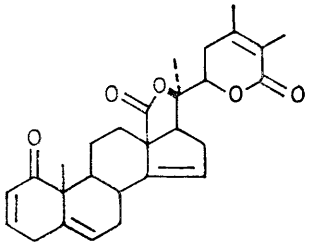
(2)a; 5α,6α
b; 5β,6β



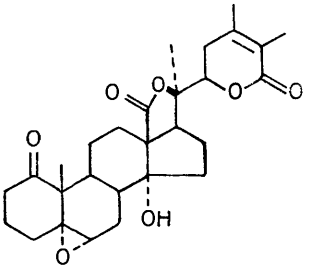
(3)



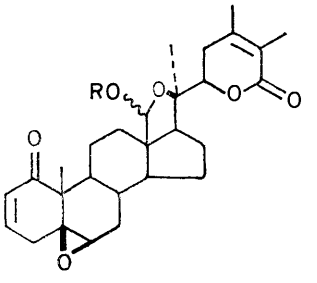
(4)



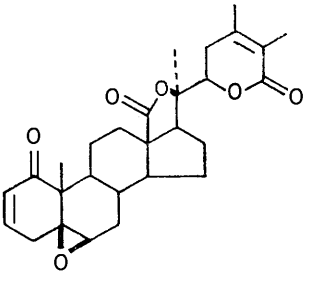
(5)



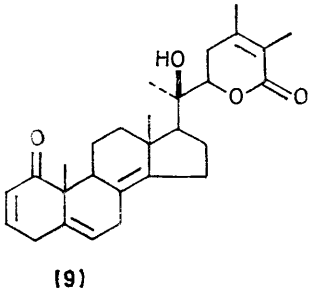
(6)



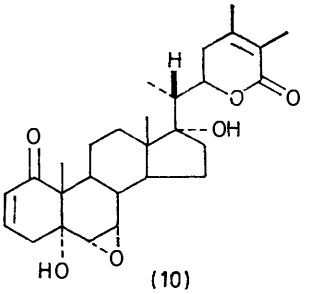
(7)a; R=H
b; R=Ac



(8)



(9)



(10)

10-Me and only a slight effect on the 20-Me signal (upfield shift of 0.06 p.p.m.). In Δ^{14}-withanolides [1] the vinylic 15-H resonates at δ 5.3, whereas in Δ^{16}-withanolides [5] the 16-H signal appears at δ 5.6.

Pyridine-induced n.m.r. shifts are very sensitive to the influence of nearby OH groups.[6] In structure (10) the 17α-OH deshields significantly both the 20-Me and the 22-H [Δ($CDCl_3$ — C_5D_5N) —0.16 and 0.22 p.p.m., respectively], whereas in (4) the 20-Me is practically unaffected, and the 22-H signal undergoes a significant shift (+0.03 and —0.20 p.p.m., respectively).

The 14α-position of the OH group provides an explanation for the stereoselectivity of the peroxy-acid epoxidation of compound (4). Treatment of the latter with *m*-chloroperbenzoic acid afforded almost quantitatively the 5α,6α-epoxide (6), characterized by the 6-H n.m.r. signal (doublet at δ 3.10, *J* 3.5 Hz) and the c.d. pattern (290 nm, Δε +1.34, inflection), confirming both the position and the orientation of the epoxide ring. The only plausible explanation for the steric course of this epoxidation is the directive influence of the 14α-OH. Peroxy-acid epoxidation of steroidal 5-en-1-ones usually affords mixtures of α- and β-epoxides, *e.g.* a 3 : 7 ratio for an isomer of (9) [Δ^{14} instead of $\Delta^{8(14)}$],[1] a 1 : 1 ratio for (1), and a 2 : 1 ratio for cholesta-2,5-dien-1-one.

Withaphysalin B (7a) shows two i.r. bands in the carbonyl region at 1 700 and 1 650 cm^{-1}, characteristic of an αβ-unsaturated ketone and an αβ-unsaturated δ-lactone, and maximum u.v. absorption at 226 nm (ε 17 000), characteristic of the overlap of these unsaturated chromophores. In the n.m.r. spectrum the side-chain signals are similar to those given by compound (3), and the signals corresponding to rings A and B indicate the presence of two vinylic protons only (2- and 3-H) and an epoxidic proton at C-6 (δ 3.15, narrow signal, $W_{\frac{1}{2}}$ 4 Hz). The position and nature of the latter signal are similar to those of the 6α-H in other withanolides possessing the same functionality in rings A and B, *e.g.* jaborosalactone A [7] and withanolide E.[8] In the methyl group region there are three singlets at δ 1.25, 1.28, and 1.47; however only the first signal is of three-proton intensity. This finding, in conjunction with the presence of two unequal C*H*·OH singlets at δ 5.17 and 5.28 (after addition of D_2O), led to the suspicion that (7a) is a mixture of two related compounds. Treatment of (7a) with acetic anhydride in pyridine afforded a mixture of monoacetates (7b) (M^+ 510) showing a C*H*·OAc signal at δ 6.17. The low-field position of this signal suggests a lactol acetate structure; indeed, mild oxidation of (7a) with CrO_3 in pyridine afforded quantitatively only one lactone (8) ($\nu_{max.}$ 1 760 cm^{-1}). The methyl region of the n.m.r. spectrum of (8) showed only two signals, at δ 1.30 and 1.51, attributable to the 10- and the 20-Me, respectively. The only plausible explanation is that (7a) is a mixture of 18,20-lactols epimeric at C-18, and that the δ 5.17 and 5.28

[5] I. Kirson, E. Glotter, D. Lavie, and A. Abraham, *J. Chem. Soc.* (*C*), 1971, 2032.

[6] D. V. Demarco, E. Farkas, D. Doddrell, B. L. Mylari, and E. Wenkert, *J. Amer. Chem. Soc.*, 1968, **90**, 5480.

[7] R. Tschesche, H. Schwang, H. W. Fehlhaber, and G. Snatzke, *Tetrahedron*, 1966, **22**, 1129.

[8] D. Lavie, I. Kirson, E. Glotter, D. Rabinovich, and Z. Shakked, *J.C.S. Chem. Comm.*, 1972, 877.

signals which disappear following the oxidation of (7a) to (8) belong to the 18-H.

C.d. measurements on compound (8) afforded information concerning the stereochemistry at C-5 and -22. The positive Cotton effect at 345 nm ($\Delta\varepsilon$ +1.30) is characteristic of a 2-en-1-one in a *cis* AB ring system and supports the conclusion drawn from the n.m.r. signal of the epoxidic proton. The positive Cotton effect at 256 nm ($\Delta\varepsilon$ +3.80) indicates the 22*R*-configuration.

Attempted separation of the lactol mixture (7a) by repeated fractional crystallization from ethyl acetate resulted in the isolation of one isomer in almost pure form, displaying in the n.m.r. only two signals for the tertiary methyl groups at δ 1.25 (10-Me) and 1.28 (20-Me, almost full intensity), and only two related doublets at δ 5.28 and 3.40 (*J* 5 Hz) for the mutually coupled 18-H and lactol proton. Upon addition of D_2O, the original equilibrium mixture was re-established in a few minutes, to give again the two singlets at δ 5.17 and 5.28. In the n.m.r. spectrum of the mother liquor from this crystallization, the most important signals in the methyl group region were those at δ 1.25 and 1.47.

Although the molecular ion was not observed in the mass spectrum of (7a), the highest mass fragment is at *m/e* 450 ($M^+ - 18$). The spectrum of the acetate (7b) shows signals at *m/e* 510 (M^+) and 450 ($M^+ - 60$). The identity of the side chain is confirmed by the *m/e* 125 signal (cleavage of the 20,22-bond) which is the base peak in the spectra of (7a and b) as well as (8).

EXPERIMENTAL

M.p.s were taken on a Fisher–Johns apparatus. Optical rotations were recorded with an automatic Perkin-Elmer 141 polarimeter and refer to solutions in chloroform. C.d. measurements were performed by Mrs. B. Romano with a Cary 60 instrument for solutions in ethanol. I.r. spectra were recorded on a Perkin-Elmer Infracord 137 spectrophotometer and refer to KBr pellets; u.v. spectra were recorded on a Cary 14 instrument for solutions in ethanol; n.m.r. spectra were determined on a Varian A-60 instrument for *ca.* 5% solutions in deuteriochloroform or deuteriopyridine containing tetramethylsilane as internal standard. T.l.c. was carried on chromatoplates of silica gel G (Merck) and spots were developed with iodine vapour. Preparative chromatoplates (1 mm thickness) were prepared from silica gel PF_{254} (Merck). Mass spectra were taken under the direction of Dr. Z. Zaretskii with an Atlas CH4 instrument. Analyses were performed in the microanalytical laboratory of the Weizmann Institute, under the direction of Mr. R. Heller.

Plant Material.—*Physalis minima* was collected (S. S. S. and P. D. S.) in India, near Pondicherry, during the summer of 1971, and then raised (A. A.) in our nursery at Bet Dagan from seeds of the above specimens.

Isolation Procedure.—Crushed air-dried leaves (1 kg) were exhaustively extracted with methanol; the extract was concentrated to *ca.* 2.5 l, a similar volume of water was added, and the mixture was extracted with hexane to remove chlorophyll and other pigments. The residual solution was re-extracted with ether; the extract was washed with water, dried (Na_2SO_4), and evaporated to leave a green residue (*ca.* 18 g) which was then chromatographed on silica gel H (Merck) (800 g); the column was eluted with benzene–ethyl acetate mixtures (35 ml fractions) (Table 2).

TABLE 2

PhH–EtOAc	Compound	Amount
8 : 2	(1)	4 g
8 : 2	(2b)	185 mg
7 : 3	(3)	180 mg
7 : 3	Withaphysalin C	92 mg
6 : 4	(7a)	460 mg

Physalin B (1), m.p. 268—270° (from methanol), was identified by comparison with an authentic sample.

5β,6β-*Epoxyphysalin B* (2b) had m.p. 262—264° (from ethyl acetate); $[\alpha]_D$ −60.7° (*c* 0.17); c.d. $\lambda_{max.}$ 352 ($\Delta\varepsilon$ +1.69), 340 (+2.09), 290 (−0.52), 270 (−0.17), 240 (−3.14), and 211 nm (+2.13) (strongly negative at shorter wavelengths); $\nu_{max.}$ 1775, 1740, and 1650 cm^{-1}; $\lambda_{max.}$ 222 nm (ε 9 500) (Found: C, 63.7; H, 5.8%; M^+, 526. $C_{28}H_{30}O_{10}$ requires C, 63.85; H, 5.75%; *M*, 526).

Withaphysalin A (3) had m.p. 222—223° (from ethyl acetate), $[\alpha]_D$ +43.6° (*c.* 0.18); $\nu_{max.}$ 1753, 1690, and 1640 cm^{-1}; $\lambda_{max.}$ 224.5 nm (ε 18 500) (Found: C, 72.0; H, 7.4%; M^+, 466. $C_{28}H_{34}O_6$ requires C, 72.1; H, 7.35%; *M*, 466).

Withaphysalin B (7a) had m.p. 161—162° (from ethyl acetate) (mixture of C-18 epimers); $\nu_{max.}$ 1700 and 1650 cm^{-1}; $\lambda_{max.}$ 226 nm (ε 17 000) [Found: C, 71.9; H, 7.8%; *m/e* 450 ($M^+ - 18$). $C_{28}H_{36}O_6$ requires C, 71.75; H, 7.75%; *M*, 468].

Withaphysalin C had m.p. 202—203° (from ethyl acetate), $[\alpha]_D$ + 33.4° (*c* 0.15); $\nu_{max.}$ 1712 and 1685 cm^{-1}; $\lambda_{max.}$ 225 nm (ε 17 000) (Found: C, 69.25; H, 7.4%; M^+, 484. Calc. for $C_{28}H_{36}O_7$: C, 69.4; H, 7.5; *M*, 484).

Epoxidation of Physalin B (1).—*m*-Chloroperbenzoic acid (50 mg) was added to a solution of physalin B (1) (100 mg) in chloroform (15 ml); the mixture was kept for *ca.* 24 h at room temperature, and was then washed with dilute sodium hydrogen carbonate solution and water, dried, and evaporated. The residue (showing two spots on a chromatoplate) was separated by thick-layer chromatography in ethyl acetate–benzene (4 : 1), yielding the epoxides (2a) (45 mg, lower spot) and (2b) (47 mg), the latter being identical with the natural compound described above. 5α,6α-Epoxyphysalin B (2a) had m.p. 243—245° (from ethyl acetate), $[\alpha]_D$ −67.2° (*c* 0.16); c.d. $\lambda_{max.}$ 342 ($\Delta\varepsilon$ −1.82), 335 (−1.89), 275 (−0.14), 238 (−2.12), and 208sh nm (+6.95) (strongly positive at shorter wavelengths); $\nu_{max.}$ 1775, 1755, 1740, and 1660 cm^{-1}; $\lambda_{max.}$ 222 nm (ε 10 000) (Found: C, 64.0; H, 5.7%; M^+, 526. Calc. for $C_{28}H_{30}O_{10}$: C, 63.85; H, 5.7%; *M*, 526).

Hydrogenation of Withaphysalin A (3).—Compound (3) (50 mg) in absolute ethanol was hydrogenated over 10% Pd–$CaCO_3$ at room temperature and atmospheric pressure. The reaction was discontinued after absorption of 1 mol. equiv. and the product 2,3-*dihydrowithaphysalin A* (4), was crystallized from ethyl acetate; m.p. 236—238°, $[\alpha]_D$ +86° (*c* 1.01); $\nu_{max.}$ 1750 and 1705 cm^{-1}; $\lambda_{max.}$ 226.5 nm (ε 9 600) (Found: C, 71.9; H, 7.8%; M^+, 468. $C_{28}H_{36}O_6$ requires C, 71.75; H, 7.75%; *M*, 468).

Dehydration of Withaphysalin A (3).—An ice–salt cooled solution of (3) (50 mg) in dry pyridine (5 ml) was treated with freshly distilled thionyl chloride (1 ml) in pyridine (4 ml). After 30 min the mixture was poured on ice and the crude

product was purified by thick-layer chromatography, yielding 14-deoxy-**14**,15-didehydrowithaphysalin A (5) (30 mg), m.p. 245° (from acetone); $[\alpha]_D$ +110.1° (*c* 0.11); c.d. $\lambda_{max.}$ 340 ($\Delta\varepsilon$ −2.54) and 254 nm (+4.93); $\nu_{max.}$ 1750, 1700, and 1650 cm^{-1}; $\lambda_{max.}$ 224 nm (ε 18 100) (with strong end absorption); M^+ 448 ($C_{28}H_{32}O_5$).

Epoxidation of 2,3-*Dihydrowithaphysalin A* (4).—Compound (4) (50 mg) was epoxidised as above, and the crude product was purified on a preparative chromatoplate in benzene–ethyl acetate (3 : 7) to give 5α,6α-*epoxy*-2,3-*dihydrowithaphysalin A* (6), m.p. 226—228° (from acetone), $[\alpha]_D$ +104.5° (*c* 1.015); c.d. $\lambda_{max.}$ 290 ($\Delta\varepsilon$ +1.34) and 247 nm (+4.55); $\nu_{max.}$ 1750 and 1700 cm^{-1}; $\lambda_{max.}$ 228 nm (ε 9 000) (Found: C, 69.55; H, 7.4%; M^+, 484. $C_{28}H_{36}O_7$ requires C, 69.4; H, 7.5%; *M*, 484).

Acetylation of Withaphysalin B (7a).—Compound (7a) (50 mg) was treated with acetic anhydride (1 ml) and pyridine (1 ml) overnight at room temperature. The crude product showed two spots on a chromatoplate. Attempted separation by thick-layer chromatography in benzene–ethyl acetate (1 : 1) gave one of the isomeric acetates (7b) [δ 1.45 (10-Me)], m.p. 177—178° (from acetone–hexane); $[\alpha]_D$ +62.2° (*c* 0.17); $\nu_{max.}$ 1745, 1712, and 1680 cm^{-1}; $\lambda_{max.}$ 226.5 nm (ε 18 600); M^+ 510 ($C_{30}H_{38}O_7$).

Oxidation of Withaphysalin B (7a).—Freshly prepared trioxodipyridinechromium [9] (200 mg) was added to a stirred solution of compound (7a) (100 mg) in acetone (25 ml). After 10 h stirring at room temperature, the excess of reagent was destroyed with a few drops of methanol, the solvent was removed under vacuum, and the residue was purified by thick-layer chromatography to give the *lactone* (8), m.p. 224—225° (from ethanol), $[\alpha]_D$ +83.0° (*c* 0.15); c.d. $\lambda_{max.}$ 345 ($\Delta\varepsilon$ +1.30) and 256 nm (+3.80); $\nu_{max.}$ 1760, 1716, and 1685 cm^{-1}; $\lambda_{max.}$ 226 nm (ε 17 800) (Found: C, 72.0; H, 7.5%; M^+, 466. $C_{28}H_{34}O_6$ requires C, 72.1; H, 7.35%; *M*, 466).

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