Withaphysalin C, a Naturally Occurring 13,14-Seco-steroid

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The structure of withaphysalin C, a new naturally occurring steroidal lactone of the withanolide group, isolated from *Physalis minima* (Solanaceae), has been elucidated. This is a 13,14-seco-steroid closely related to withaphysalin A. The lack of a 17α-OH precluded the formation of a 17 ⟶ 14 hemiacetal as in the physalins, leading to the alternative formation of a 13 ⟶ 14 hemiacetal.

We reported recently [1] the isolation and characterisation of several steroidal lactones from *Physalis minima* (Solanaceae), representing intermediate stages in the biogenetic pathway from the withanolides [2] to the physalins.[3] We now present the work leading to the identification of withaphysalin C(1a), isolated from the same plant.

Withaphysalin C (1a) shows n.m.r. signals (Table 1) similar to those exhibited by withaphysalin A (6),[1] consistent with a 2,5-dien-1-one system in rings A and B, an αβ-unsaturated δ-lactone in the side chain, and the absence of the 13-Me group. The spectrum also displays a singlet at δ 4.48 and two inter-related doublets at δ 4.42 and 5.10 (J 11 Hz). In the presence of D_2O, only the last signal persists, as a singlet at δ 5.10, attributed to the lactolic 18-H. Treatment of withaphysalin C (1a) with trichloroacetyl isocyanate (in the n.m.r. tube), afforded the bis(trichloroacetylcarbamate) (1c), showing the presence of only two hydroxy-groups in the molecule, one involved in the γ-lactol (downfield shift of the 18-H signal to δ 6.12) and the other without a proton α to OH. The n.m.r. spectrum of compound (1a) in pyridine [Δ($CDCl_3$ — C_5D_5N) +0.12 (10-Me) and −0.01 p.p.m. (20-Me)] excludes [4] the C(17) location for the tertiary OH.

The natural product (1a), which is a mixture of

[1] E. Glotter, I. Kirson, A. Abraham, S. Sankara Subramanian, and P. D. Sethi, *J.C.S. Perkin I*, 1975, 1370.
[2] I. Kirson, E. Glotter, A. Abraham, and D. Lavie, *Tetrahedron*, 1970, **26**, 2209.
[3] T. Matsuura, M. Kawai, R. Nakashima, and Y. Butsugan, *J. Chem. Soc.* (*C*), 1970, 664.
[4] I. Kirson, E. Glotter, D. Lavie, and A. Abraham, *J. Chem. Soc.* (*C*), 1971, 2032.

C-18 epimers [($1a_1$) and ($1a_2$)], is accompanied by *ca.* 5% of the 2,3-dihydro-derivatives ($2a_1$) and ($2a_2$). The mass spectra (Table 2 *) of ($1a_1$) and ($1a_2$) [contaminated by ($2a_1$) and ($2a_2$)] differ only in the relative intensities of the fragments due to elimination of H_2O. Almost complete separation could be achieved, following acetylation, by thick-layer chromatography, resulting in the monoacetates ($1b_1$) and ($1b_2$). A remarkable feature in their mass spectra is the sharp difference between the $(M - AcOH)/M^+$ intensity values [1.5 ($1b_1$) and 14 ($1b_2$)].

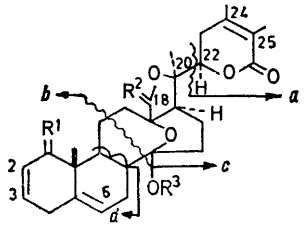
Catalytic hydrogenation of compound (1a) over Pd–$CaCO_3$, followed by thick-layer chromatography, afforded the pure C-18 epimeric dihydro-derivatives ($2a_1$) and ($2a_2$). Oxidation of each stereoisomer with trioxodipyridinechromium in acetone [5] yielded the same dihydrowithaphysalin C lactone (4); the significant features of its n.m.r. spectrum are the shift of the 20-Me signal to δ 1.45 and the disappearance of the 2-, 3-, and 18-H signals. The same compound (4) was obtained by a similar oxidation of (1a) to give withaphysalin C lactone (3), which was then catalytically reduced to (4).

TABLE 1

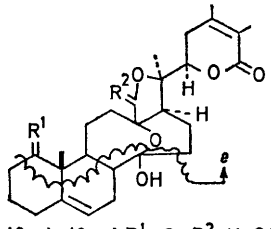
N.m.r. data (60 MHz; solvent chloroform; δ values)

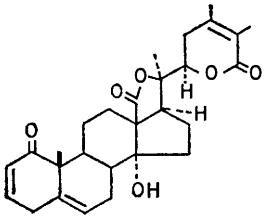
Cpd.	2-H (dq)	3-H (dq)	6-H (m)	18-H	22-H (dd)	10-CH_3 (s)	20-CH_3 (s)	24- and 25-CH_3	Other
(1a)	5.92	6.82	5.65	5.10 (d) *	4.32	1.27	1.27	1.90, 1.97	
($1b_1$)	5.85	6.72	5.62	6.07 (s)	4.27	1.25	1.27	1.95, 1.88	OAc 2.07
($1b_2$)	5.85	6.72	5.58	5.95 (s)	4.35	1.25	1.30	1.95, 1.87	OAc 1.95
(1c)	5.93	6.83	5.58	6.12 (s)	4.53	1.30	1.33	1.93	NH 8.98, 8.27
($2a_1$)			5.53	5.08 (d) *	4.25	1.30	1.23	1.88	
($2a_2$)			5.53	5.10 (d) *	4.25	1.30	1.25	1.89	
(3)	5.88	6.75	5.70		4.40	1.29	1.47	1.95, 1.87	
(4)			5.52		4.38	1.37	1.45	1.97, 1.87	
(5a)			5.60		4.38	1.03	1.43	1.93, 1.83	1β-H 3.83 †
(5b)			5.50		4.38	1.07	1.43	1.93, 1.83	1α-H 3.42 ‡

* Singlet following exchange with D_2O (this finding excludes the presence of a 13-H). † Broad m. ‡ Narrow m.



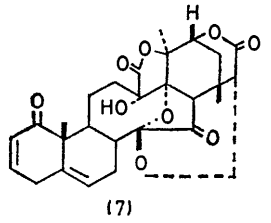
($1a_1$), ($1a_2$) R^1 = O, R^2 = H,OH, R^3 = H
($1b_1$), ($1b_2$) R^1 = O, R^2 = H,OAc, R^3 = H
(1c) R^1 = O, R^2 = H, $O_2C \cdot NH \cdot CO \cdot CCl_3$, R^3 = $O_2C \cdot NH \cdot CO \cdot CCl_3$
(3) R^1 = R^2 = O, R^3 = H





(6)

($2a_1$), ($2a_2$) R^1 = O, R^2 = H,OH
($2b_1$), ($2b_2$) R^1 = O, R^2 = H,OAc
(4) R^1 = R^2 = O
(5a) R^1 = α-OH,H, R^2 = O
(5b) R^1 = β-OH,H, R^2 = O



(7)

* The mass spectrometric investigation of the withanolides in general and of withaphysalin C in particular, will be the subject of a separate report.

The mass spectral fragmentation pattern of the lactone (3) reveals the elimination of only one molecule of H_2O from M^+. The *m/e* values of all ions containing the γ-lactone are two mass units less than those of the lactols ($1a_1$) and ($1a_2$). The mass spectrum of the dihydro-lactone (4) is dominated by the abundant ion *b*.

Its elemental composition shows that withaphysalin C possesses an additional oxygen atom, which may be either the bridge of an internal hemiacetal or an oxo-group (if its i.r. absorption is obscured by that of the 1-one). Biogenetically, the structure (1a) proposed for withaphysalin C is based on its coexistence with withaphysalin A (6), possessing the intact steroidal skeleton, and with physalin B (7) in which the 13,14-bond is cleaved. The reasonable steps leading in nature to the modified ring CD pattern of the physalins require a 14,17-dihydroxy-steroid precursor (i), oxidatively cleaved to a 13,14-seco-13α-hydroxy-14-one intermediate (ii), in which the 17α-ol attacks the 14-one to give the hemiacetal (iii) (abstraction is made of other structural features of the physalins).

Provided that withaphysalin C is formed from a 14α-hydroxy-compound like (6), but with C-18 at a lower oxidation level (iv), one may conceive an oxidative cleavage of the 13,14-bond, resulting in a nine-membered-ring intermediate (v) in which the 13-ol attacks the 14-one to give the hemiacetal (vi). To distinguish between possibilities (v) and (vi), dihydrowithaphysalin C lactone (4) was reduced with borohydride, resulting in a mixture containing only the corresponding 1α-ol (5a) and 1β-ol (5b). The absence of any saturated ketone group in compounds (5a and b) was confirmed by c.d. measurements. Whereas compound (4) is characterised

[5] J. R. Holum, *J. Org. Chem.*, 1961, **26**, 4814.

by a negative band at 307 nm ($\Delta\varepsilon$ −0.65) for the 5-en-1-one and a positive band at 248 nm ($\Delta\varepsilon$ +3.12) for the side chain lactone (22*R*), compound (5a) shows only the band at 248 nm ($\Delta\varepsilon$ +4.05). Oxidation of the stereoisomeric alcohols (5) afforded the original 1-one (4).

The oxygen bridge between C-13 and C-14 may be either α- or β-oriented, requiring these atoms in the *SS* or in the *RR*-configuration, respectively. Although the α-orientation of the oxygen bridge seems plausible since it corresponds to cleavage of the 13,14-bond as in the

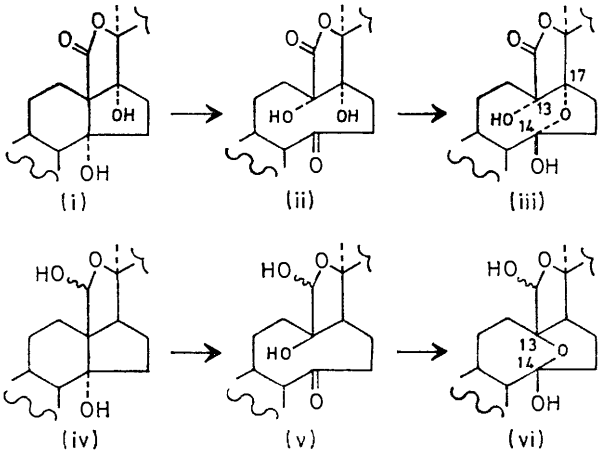
TABLE 2

Characteristic peaks in the mass spectra of compounds (1)—(5) [*m/e* (%)]

Cpd.	M^+	$M - H_2O$	$M - 2H_2O$	$M - 125$	$M - 125 - H_2O$	$M - 125 - 2H_2O$	*a*	*b*	*b* − H_2O	*c*	*d*	*e*
($1a_1$)	484	466	448	359	341	323	125	174		293	159	363
	(5)	(12)	(6)	(6)	(65)	(9)	(100)	(16)		(12)	(32)	(2.5)
($1a_2$)	484	466	448	359	341	323	125	174		293	159	363
	(15)	(25)	(50)	(12)	(60)	(50)	(100)	(20)		(20)	(35)	(2.5)
($1b_1$)	526	508 †	‡	401	§	¶	125	174		293	159	
	(9)	(7)		(11)			(100)	(21)		(14)	(28)	
($1b_2$)	526	508 †	‡	401	§	¶	125	174		293	159	
	(1.6)	(0.8)		(13)			(100)	(16)		(23)	(32)	
($2a_1$)	486	468	450	361	343	325	125	176		293	161	363
	(96)	(55)	(20)	(11)	(70)	(20)	(100)	(50)		(17)	(23)	(5)
($2a_2$)	486	468	450	361	347	325	125	176		293	161	363
	(72)	(38)	(10)	(10)	(50)	(12)	(100)	(50)		(14)	(26)	(4)
(3)	482	464		357	339		125	174		291	159	361
	(16)	(11)		(2)	(2)		(100)	(44)		(10)	(42)	(8)
(4)	484	466		359			125	176		291	161	361
	(62)	(12)		(4)			(50)	(100)		(6)	(28)	(18)
(5a)	486	468	450				125		160	291		
	(0.5)	(40)	(10)				(100)		(96)	(7)		
(5b)	486	468	450				125		160	291		
	(1.5)	(17)	(6)				(100)		(80)	(15)		

† M − AcOH, 466 [14($1b_1$), 23($1b_2$)]. ‡ M − AcOH − H_2O, 448 [7($1b_1$), 11($1b_2$)]. § M − AcOH − 125 341 [35($1b_1$), 45($1b_2$)]. ¶ M − AcOH − 125 − H_2O 323 [16($1b_1$), 19($1b_2$)].

The main differences between the fragmentations of the 1-hydroxy-derivatives (5a and b) are due to the ease



of elimination of H_2O from M^+. The abundance of the ion $b - H_2O$ (*m/e* 160) instead of the ion *b* (*m/e* 178) suggests that the first molecule of H_2O lost from M^+ is that given by the 1-OH. The easy elimination of H_2O from ring A suppresses the formation of ion *e*. The $(M - H_2O)/M^+$ intensity values for (5a) (80) and for (5b) (*ca.* 10) are in agreement [6] with the axial and equatorial orientation, respectively, of the 1-OH.

* *Note added in proof:* The structure assigned to withaphysalin C has been confirmed (by D. Rabinovich and F. Frolow) by *X*-ray analysis of dihydrowithaphysalin C lactone (4). The stereochemistry at C-13 and C-14 is indeed 13*R*,14*R*.

physalins, there are indications favouring the alternative β-orientation. The chemical shift of the acetoxy-protons in the monoacetate ($1b_2$) (δ 1.95) can be rationalised by assuming that the acetoxy-group enters the shielding region of the side-chain lactone carbonyl. Models indicate that such a possibility exists only in a compound with the oxygen bridge β-oriented.*

EXPERIMENTAL

M.p.s were taken with 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 with a Perkin-Elmer Infracord 137 spectrophotometer and refer to KBr pellets; u.v. spectra were recorded with a Cary 14 instrument for solutions in ethanol; n.m.r. spectra were determined with a Varian NV-14 instrument (60 MHz) for *ca.* 5% solutions in deuteriochloroform or pentadeuteriopyridine containing tetramethylsilane as internal standard. T.l.c. was performed 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). High resolution mass spectra were recorded on a Varian MAT 731 spectrometer in conjunction with a Spectro-System 100MS, at resolving power 10 000 and 70 eV. The elemental compositions of all ions referred to were determined. Elemental analyses were obtained by high resolution mass spectrometry, or by microanalysis and

[6] V. I. Zaretskii and V. G. Zaikin, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1969, 1722.

low resolution mass spectrometry. Microanalyses were performed in the microanalytical laboratory of the Weizmann Institute, under the direction of Mr. R. Heller.

Plant Material.—Physalis minima was raised by Mr. A. Abraham in the nursery of the Agricultural Research Organisation, Volcani Center, Bet Dagan, Israel. The isolation procedure was as previously described.[1]

Withaphysalin C (1a) had m.p. 202—203° (from ethyl acetate); $[\alpha]_D$ +33.4° (*c* 0.15); $\nu_{max.}$ 1 712 and 1 685 cm^{-1}; $\lambda_{max.}$ 225 nm (ε 17 000); c.d. $\Delta\varepsilon_{340}$ −3.64, $\Delta\varepsilon_{251}$ +5.80 {Found: C, 69.25; H, 7.4%; M^+, 484 and 486 [impurity of the dihydro-derivative (2a)]. $C_{28}H_{36}O_7$ requires C, 69.4; H, 7.5%; *M*, 484}; high resolution M^+ 484.2472 (calc. 484.2453).

Acetylation of Withaphysalin C (1a).—Compound (1a) (20 mg) was treated with acetic anhydride (0.5 ml) and pyridine (0.5 ml) overnight at room temperature. The crude product was separated by thick-layer chromatography in benzene–ethyl acetate (3 : 7) to give two stereoisomeric monoacetates. The upper band contained *withaphysalin C* 18-*monoacetate* ($1b_1$), m.p. 162—163° (from acetone–hexane); $\nu_{max.}$ 1 730 and 1 700 cm^{-1} [Found: M^+, 526 and 528 (impurity of the dihydro-acetate). $C_{30}H_{38}O_8$ requires *M*, 526]. The lower band contained the stereoisomeric *acetate* ($1b_2$), m.p. 167—168° (from acetone–hexane); $\nu_{max.}$ 1 730 and 1 700 cm^{-1} (Found: M^+, 526 and 528).

Hydrogenation of Withaphysalin C (1a).—Compound (1a) (50 mg) in absolute ethanol (25 ml) was hydrogenated over Pd–$CaCO_3$ at room temperature and atmospheric pressure. The reaction was discontinued after the absorption of 1 mol. equiv. The crude product was separated by thick-layer chromatography in benzene–ethyl acetate (3 : 7), yielding two stereoisomeric 2,3-dihydro-derivatives. The upper band contained *compound* ($2a_1$), m.p. 223—225° (from ethyl acetate); $\nu_{max.}$ 1 705 cm^{-1}; $\lambda_{max.}$ 227 nm (ε 9 500) (Found: M^+, 486.2613. $C_{28}H_{38}O_7$ requires *M*, 486.2617). The lower band contained *compound* ($2a_2$), m.p. 219—220° (from ethyl acetate), $\nu_{max.}$ 1 705 cm^{-1}; $\lambda_{max.}$ 227 (ε 9 700) (Found: M^+, 486.2603).

Oxidation of Dihydrowithaphysalin C [($2a_1$) *and* ($2a_2$)].—Freshly prepared trioxodipyridinechromium (20 mg) was added to a stirred solution of compound ($2a_1$) (10 mg) in acetone (5 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 in benzene–ethyl acetate (3 : 7) to give *dihydrowithaphysalin C lactone* (4), m.p. 249—251° (from ethanol); $[\alpha]_D$ +95.9 (*c* 0.2); c.d. $\Delta\varepsilon_{307}$ −0.65, $\Delta\varepsilon_{248}$ +3.12; $\nu_{max.}$ 1 709 (double intensity) and 1 790 cm^{-1}; $\lambda_{max.}$ 227 nm (ε 9 650) (Found: M^+, 484.2453. $C_{28}H_{36}O_7$ requires 484.2460).

Similarly, oxidation of compound ($2a_2$) afforded the same lactone (4), m.p. and mixed m.p. 249—251°.

Oxidation of Withaphysalin C (1a).—Compound (1a) (50 mg) was oxidised as described above, yielding *withaphysalin C lactone* (3), which was purified by thick layer chromatography in benzene–ethyl acetate; m.p. 231—232° (from ethanol); $[\alpha]_D$ +30.2° (*c* 0.17); $\nu_{max.}$ 1 680, 1 710, and 1 783 cm^{-1}; $\lambda_{max.}$ 227 nm (ε 17 200) (Found: C, 69.6; H, 7.2%; M^+, 482. $C_{28}H_{34}O_7$ requires C, 69.7; H, 7.1%; *M*, 482).

Hydrogenation of Withaphysalin C Lactone (3).—Compound (3) (20 mg) was hydrogenated as described for compound (1a). The product crystallised from ethanol; m.p. and mixed m.p. 249—251° [with dihydrowithaphysalin C lactone (4)].

Reduction of Dihydrowithaphysalin C Lactone (4) *with Sodium Borohydride.*—Compound (4) (30 mg) in methanol (10 ml) was treated with sodium borohydride (30 mg) for 2 h at room temperature. The mixture was neutralised with dilute hydrochloric acid and concentrated to a small volume; water was then added and the product extracted with ether and separated by thick-layer chromatography into two fractions. The upper band gave the 1α-*alcohol* (5a) (18 mg), m.p. 173° (from ethyl acetate), $\nu_{max.}$ 1 700 and 1 775 cm^{-1}; $\Delta\varepsilon_{248}$ +4.05 (Found: M^+, 486. $C_{28}H_{38}O_7$ requires *M*, 486. Found: $M^+ - H_2O$, 468.2532. $C_{28}H_{36}O_6$ requires 468.2511). The lower band gave the 1β-*alcohol* (5b) (8 mg), m.p. 168—170° (from ethyl acetate); $\nu_{max.}$ 1 700 and 1 775 cm^{-1} (Found: M^+, 486; $M^+ - H_2O$, 468.2515).

Re-oxidation of the Alcohols (5a *and* b).—Compound (5a) (10 mg) in acetone solution (5 ml) was oxidised with trioxodipyridinechromium (20 mg), as described for compound (2a). The crude product was filtered through silica gel and crystallised from ethanol. It was identical with dihydrowithaphysalin C lactone (4). The same compound was obtained following oxidation of the alcohol (5b).

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