

Withanicandrin, a Ring-c-substituted Withanolide from *Nicandra physaloides* (Solanaceae)

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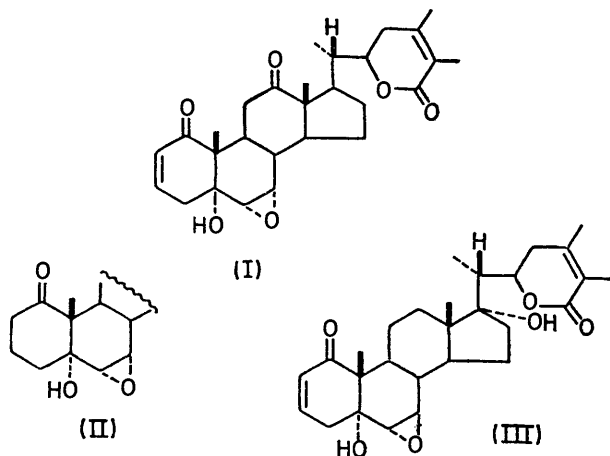
The title compound has been shown to be 6 α ,7 α -epoxy-5 α -hydroxy-1,12-dioxo-22*R*-witha-2,24-dienolide. This is the first natural 12-oxowithanolide isolated so far.

THE withanolides are a series of related steroidal lactones possessing an ergostane skeleton in which a six-membered-ring 22-hydroxy-26-oic acid lactone is present; more than 30 compounds of this type have been isolated so far^{1a-c} from different Solanaceae.

From *Nicandra physaloides*, nicandrenone, a probably impure compound, m.p. 102–105°, has been isolated and described without any assignment of structural formula;^{2a,b} the crude extract was reported^{2b} to contain a second compound, but no further information was given.

A reinvestigation of the constituents of *N. physaloides* was undertaken. The structural elucidation of nicandrenone is in progress, however, the second compound, for which the name withanicandrin is proposed, has now been identified as 6 α ,7 α -epoxy-5 α -hydroxy-1,12-dioxo-22*R*-witha-2,24-dienolide (I).

Withanicandrin, C₂₈H₃₆O₆ (I), which is the first natural occurring 12-oxowithanolide, was isolated by chromatographic fractionation of the extract of the leaves of *N. physaloides*. Following catalytic hydrogenation the dienolide (I) afforded the dihydro-derivative



(II); u.v. spectroscopy (see Experimental section) indicated the disappearance of an $\alpha\beta$ -unsaturated ketone chromophore.

¹ (a) I. Kirson, E. Glotter, D. Lavie, and A. Abraham, *J. Chem. Soc. (C)*, 1971, 2032, and references cited therein; (b) R. Tschesche, M. Baumgarth, and P. Welzel, *Tetrahedron*, 1968, **24**, 5169, and references cited therein; (c) S. M. Kupchan, W. K. Anderson, P. Bollinger, R. W. Doskotch, R. M. Smith, J. A. Saenz Renauld, H. K. Schnoes, A. L. Burlingame, and D. H. Smith, *J. Org. Chem.*, 1969, **34**, 3858.

The carbonyl region of the i.r. spectrum of (I) exhibits bands at 1677 (unsaturated ketone), 1700 (unsaturated six-membered-ring lactone) and 1711 cm⁻¹ (saturated six-membered-ring ketone), and the ketonic absorption being displaced to 1715 cm⁻¹ in the dihydro-compound (II).

Most of the n.m.r. signals of withanicandrin (I) (see Table 1), are similar to the corresponding signals of the

TABLE 1

N.m.r. signals for compounds (I) and (III) (CDCl ₃ solution)					
	2-H	3-H	6-H	7-H	22-H
(I) δ /p.p.m. (<i>J</i> /Hz)	5.81dq (10; 3; 1)	6.60dq (10; 4.5; 3)	3.08d (4)	3.40dd (4; 1)	4.40dt (12; 3.5)
(III) δ /p.p.m. (<i>J</i> /Hz)	5.81dq (10; 3; 1)	6.60dq (10; 4.5; 3)	3.06d (4)	3.34dd (4; 1)	4.63dt (8.5; 3)
Methyl groups					
	18-H ₃	19-H ₃	21-H ₃	27- and 28-H ₃	
(I) δ /p.p.m. (<i>J</i> /Hz)	1.11s	1.24s	0.98d (6)	1.92	
(III) δ /p.p.m. (<i>J</i> /Hz)	0.85s	1.18s	1.04d (7)	1.90	

withanolide (III),^{1a} pointing to the relationship between these compounds. However, a significant difference between the positions of the 22-H signal in the two compounds is observed. In all the withanolides devoid of the 17 α -OH, this signal appears at the same position as in withanicandrin (I). The downfield shift of ca. 0.2 p.p.m. experienced by the 22-H signal in compound (III), as well as the change in the *J* values, are characteristic for 17 α -hydroxywithanolides.^{1a}

Although the position and the stereochemistry of the tertiary hydroxy-group and the disubstituted epoxide in withanicandrin (I) are assigned only by interpretation of the n.m.r. signals, they are fully supported by the chemical evidence, on which are based the corresponding assignments in compound (III).^{1a}

Two of the three carbonyl bands in the i.r. spectrum of withanicandrin (I) are accounted for and corroborated by the u.v. and n.m.r. data ($\alpha\beta$ -unsaturated ketone and lactone). The only available positions accounting for the third carbonyl group (ν_{\max} 1711 cm⁻¹) are in ring c, either at C-11 or C-12. The differences between the chemical shifts of the angular methyl groups in (I) and (III) reflect the contribution of a 12-ketone (according to

² (a) F. v. Gizycki and G. Kotitschke, *Arch. Pharm.*, 1951, **284**, 129; (b) O. Nalbandov, R. T. Yamamoto, and G. S. Fraenkel, *Agric. Food Chem.*, 1964, **12**, 55.

Zürcher,³ downfield shifts of 0.10 and 0.37 p.p.m. for the 19-H and 18-H, respectively, are expected).

The C-12 assignment is further supported by n.m.r. solvent shifts Δ and c.d. measurements. The solvent shifts [$\delta(\text{CDCl}_3) - \delta(\text{C}_6\text{H}_6)$] experienced by the angular methyl protons in steroidal ketones are well documented: ^{4a-e} there are only two ketones [a 1-one (in a 5β -steroid),^{4c,d} and an 11-one^{4b}], which show a negative solvent shift. The data for compounds (I) and (III) are summarised in Table 2. Subtraction of the solvent shift [$\Delta(\text{I}) - \Delta(\text{III})$] gives the contribution of the 12-one, +0.38 p.p.m. for the 18-H and +0.15 p.p.m. for the 19-H. The increment of the 19-H signal allows an unequivocal differentiation between the C-11 and the C-12 position of the ring c ketone. The above contributions are in agreement with the trend of the solvent shifts determined in hecogenin acetate:^{4a} a strong shift for the 18-H (+0.51 p.p.m.) and a small one for the 19-H (+0.02 p.p.m.). The solvent shifts measured for 5α -androstan-12-one follow the same tendency toward upfield values; the agreement with our data is excellent if we reverse the assignments for the benzene solution of this compound (ref. 4b, see Table 2), thus leading to a value of Δ of +0.38 and +0.12 p.p.m. for 18-H and 19-H, respectively.

TABLE 2

Solvent shifts Δ [$\delta(\text{CDCl}_3) - \delta(\text{C}_6\text{H}_6)$] of 18-H and 19-H in compounds (I) and (III) (in p.p.m.)

		$\delta(\text{CDCl}_3)$	$\delta(\text{C}_6\text{H}_6)$	Δ
(I)	18-H	1.11	0.56	+0.55
	19-H	1.24	0.88	+0.36
(III)	18-H	0.85	0.68	+0.17
	19-H	1.18	0.97	+0.21
5α -Androstan-12-one ^{4b}	18-H	1.01	0.75	
	19-H	0.87	0.63	

Comparison of the circular dichroism spectra of compounds (I) and (II), with those of the withanolide (III) and its 2,3-dihydro-derivative, respectively, afforded clear evidence for the presence of a saturated ketone-chromophore in ring c of withanicandrin (I) (see Table

TABLE 3

Circular dichroism spectra of compounds (I), (II), (III), and dihydro-(III) (for ethanolic solutions)

Compound	$\lambda_{\text{max}}/\text{nm}$ ($\Delta\epsilon$)
(I)	336.5 (-1.96); ca. 287i (+0.80); 252 (+5.01); ca. 195 (+31.0)
(III)	338.5 (-2.16); 252.3 (+5.02); ca. 195 (+29.5)
(II)	289.4 (+1.93); 252 (+4.65); ca. 200 (+14.9)
Dihydro-(III)	301i (+0.36); 252 (+4.51); ca. 200 (+12.9)

3). The Cotton effect at ca. 285 nm in (I) and (II) compares well with that reported for 5α -pregnan-12-

one^{5a} and other 12-ketosteroids.^{5b} The stereochemistry at C-22 (22R) is confirmed by the positive effect in the range of the R band (ca. 250 nm) of the conjugated lactone.⁶

The assignment of structure (I) to withanicandrin is supported by mass spectrometry (M^+ , 468). Besides the trivial fragments due to elimination of H_2O and CH_3 (m/e 450 and 435), the spectrum is dominated by the cleavage of the C(20)-C(22) bond, common to all the withanolides, giving rise to the base peak m/e 125, as well as to peaks at 344, 329, and 326; the last three signals are higher than expected by one mass unit due to the transfer of a proton, and are assigned to ($M^+ - 125$), ($M^+ - 125 - 15$), and ($M^+ - 125 - 18$), respectively. Cleavage of the C(17)-C(20) bond affords the fragment m/e 315 ($M^+ - 153$). Compound (III), as well as other 17-hydroxywithanolides, show significant peaks due to a fragmentation induced by this hydroxy-group, taking place with the cleavage of the C(13)-C(17) and C(14)-C(15) bonds^{1a} [m/e 209 in (III)]. Such signals do not appear in the spectrum of (I), substantiating thereby the conclusion derived from the n.m.r. data about the absence of a 17-hydroxy-group. Studies on pregnan-12-one⁷ have shown that the carbonyl group triggers a fragmentation involving the cleavage of the C(14)-C(15) bond, and a McLafferty rearrangement with the participation of the 20-proton and the 12-ketone leading to a peak m/e 233. Such a fragmentation seems to take place also in (I), yielding a fragment m/e 275.

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 chloroform solutions; c.d. spectra were measured with a Cary 60 spectropolarimeter. I.r. spectra were recorded on a Perkin-Elmer Infracord model 137 spectrophotometer and refer to KBr pellets; u.v. spectra were determined on a Cary 14 instrument (ethanol as solvent); n.m.r. spectra were recorded with a Varian A-60 spectrometer, with tetramethylsilane as internal standard. T.l.c. were carried on chromatoplates of silica gel G (Merck) and spots were developed with iodine vapour. Mass spectra were taken by Mr. M. Greenberg with an Atlas CH4 instrument. Analyses were performed in the micro-analytical laboratory of the Weizmann Institute, under the direction of Mr. R. Heller.

Plant Material.—*Nicandra physaloides* was collected in South India (the Ooty Hills) in July–August 1970 and 1971. The material collected at this period yielded mainly withanicandrin and only trace amounts of the lower m.p. compound, in contradistinction to the material collected in November, where withanicandrin was only a minor component.

Isolation.—Crushed air dried leaves (1 kg) were exhaus-

³ R. F. Zürcher, *Helv. Chim. Acta*, 1963, **46**, 2054.

⁴ (a) D. H. Williams and N. S. Bhacca, *Tetrahedron*, 1965, **21**, 1461; (b) *ibid.*, p. 2021; (c) J. E. Bridgeman, P. C. Cherry, E. R. H. Jones, and C. D. Meakins, *Chem. Comm.*, 1967, 483; (d) E. Glotter and D. Lavie, *J. Chem. Soc. (C)*, 1967, 2298; (e) P. C. Cherry, W. R. T. Cottrell, G. D. Meakins, and E. E. Richards, *ibid.*, p. 181.

⁵ (a) D. N. Kirk, W. Klyne, and S. R. Wallis, *J. Chem. Soc. (C)*, 1970, 350; (b) L. Velluz, M. Legrand, and M. Grosjean, 'Optical Circular Dichroism, Principles, Measurements and Applications,' Academic Press, 1965, p. 214.

⁶ G. Snatzke, *Angew. Chem. Internat. Edn.*, 1968, **7**, 14.

⁷ M. Spitteller-Friedman and G. Spitteller, *Fortschr. chem. Forsch.*, 1969, **12** (3), 440.

tively extracted with methanol; the extract was concentrated to ca. 2.5 l, water was added, and the mixture was extracted with hexane to remove chlorophyll and other pigments. The residual solution was then re-extracted with ether; the ethereal extract was washed with water, dried (Na_2SO_4), and the solvent was removed. The residue was chromatographed [silica gel H (Merck) column (500 g); benzene-ethyl acetate mixtures]. Elution with a 4:1 mixture afforded nicandrenone (100 mg), m.p. 106–107° (from benzene).

Further elution with a 1:1 mixture afforded withanicandrin (I), fine needles, m.p. 267–269° (from ethyl acetate), $[\alpha]_D^{25} +105^\circ$ (c 0.25), ν_{max} 1677, 1700, and 1711 cm^{-1} , λ_{max} 226 nm (ϵ 18,100) (Found: M^+ , 468. $\text{C}_{28}\text{H}_{36}\text{O}_6$ requires M , 468.57).

Hydrogenation of Withanicandrin (I).—Compound (I) (100 mg) in absolute ethanol (100 ml) was hydrogenated over 10% Pd- CaCO_3 at room temperature and atmospheric pressure. The reaction was discontinued after the absorption of 1 mol. equiv. of hydrogen, and 6 α ,7 α -epoxy-5 α -hydroxy-1,12-dioxo-22R-with-24-enolide (II) was crystallised from ethyl acetate-chloroform, m.p. 274–275°, $[\alpha]_D^{25} +166.6^\circ$ (c 0.1), ν_{max} 1700, 1711, and 1715 cm^{-1} , λ_{max} 226.5 nm (ϵ 8900), δ 0.98 (d, J 6 Hz, 21- H_3), 1.09 (s, 18- H_3), 1.19 (s, 19- H_3), 1.92 (27- and 28- H_3), 3.10 (d, J 4 Hz, 6-H), 3.40 (dd, J 4, Hz, 7-H), and 4.43 p.p.m. (dt, J 12, 3.5 Hz, 22-H) (Found: C, 71.6; H, 8.1. $\text{C}_{28}\text{H}_{36}\text{O}_6$ requires C, 71.5; H, 8.1%).

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