Reactions of Nic-1 (Nicandrenone), a Naturally Occurring Ring-D-aromatic Steroid

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Some reactions of Nic-1 (nicandrenone) (2a), the main steroidal component of *Nicandra physaloides* (Solanaceae), and its relation to the steroidal lactones of the withanolide group are discussed.

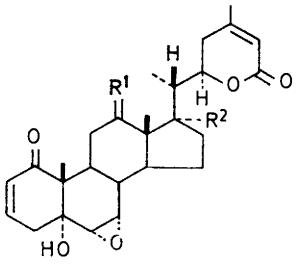
WE have previously [1] reported the isolation and characterization of withanicandrin (1) from a population of *Nicandra physaloides* (Solanaceae) growing in South India. While work towards the elucidation of the structure of nicandrenone,[2] the main constituent of this plant, was in progress, we learned that Crombie and his colleagues [3] had characterized a series of steroidal components from *N. physaloides*, including nicandrenone [renamed Nic-1 (2a)]. Similar conclusions were reached in an independent investigation by Bates.[4] The populations of *N. physaloides* studied by these authors contained neither withanicandrin (1) nor any other steroidal components with the unsaturated δ-lactone system in the side chain, as present in most of the withanolides investigated so far.

Our work on Nic-1 (2a) has been discontinued; we report here some chemical transformations originally directed towards the determination of its structure. Acetylation with acetic anhydride–pyridine afforded the lactol monoacetate (2b), displaying a significantly low-field n.m.r. singlet [δ 6.12 (26-H)]. Catalytic hydrogenation over Pd–$CaCO_3$ resulted in the 2,3-dihydro-derivative (3) (disappearance of the n.m.r. signals due to the vinylic 2- and 3-H). Jones reagent induced smooth oxidation of the epoxy-lactol system to give the corresponding epoxy-lactone (4), characterized by the disappearance of the 26-H signal and the significant shift of a broad multiplet from δ 3.87 [in (2a)] to δ 4.65 [in (4)]. The position and the pattern (double triplet) of this signal, in conjunction with the signals of the substituents of rings A and B, suggested the close relationship between Nic-1 and withanicandrin (1). The structure of (4) was substantiated by decoupling experiments (see Experimental section). The c.d. spectrum showed negative bands at 338 nm for the 2-en-1-one (*trans*-AB-system) and 244 nm for the side-chain epoxy-lactone, as well as a very weak triplet of bands at 275.5, 268, and 263 nm for the aromatic ring D absorption (optically active owing to its dissymmetric environment). Catalytic hydrogenation of (4) resulted in the 2,3-dihydro-derivative (5), alternatively obtained by oxidation (CrO_3) of compound (3). In order better to visualize the c.d. band of the epoxy-lactone system, compound (5) was reduced ($NaBH_4$) to the 1α-ol (7) (1β-H, narrow multiplet at δ 3.66), which showed a negative c.d. band at 233 nm. Reduction of the epoxy-lactone (5) ($CrCl_2$) afforded in good yield a compound (6) possessing the unsaturated δ-lactone unit normally present in the withanolides. This conversion was indicated in the n.m.r. spectrum by the shift of the 24- and 25-CH_3 signals from δ 1.43 and 1.55 [in (5)] to δ 1.89 [in (6)]. The c.d. behaviour of (6) supports the proposed structure [positive band at 245 nm for the αβ-unsaturated δ-lactone (22*R*)].

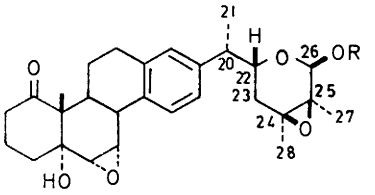
Oxidation of (6) by peroxy-acid proceeded in a stereoselective manner, albeit slowly, to give only the epoxy-lactone (5), with the epoxide possessing the same configuration as in the natural Nic-1. Owing to the influence of the 5α-OH, the 6α,7α-epoxide system in the withanolide (10) undergoes with hydrobromic acid a bidirectional opening to give mainly (70%) the corresponding *trans*-diequatorial bromohydrin.[5] To ascertain the influence of the aromatic ring on one side and of the hydroxy-group on the other side of the 6α,7α-epoxy-group in Nic-1, compound (6) was treated with hydrobromic acid, leading to a mixture of two compounds, separated by preparative layer chromatography. The upper band was due to the *trans*-diequatorial bromohydrin (8a), converted by acetylation into the bromoacetate (8b), $J_{6\beta,7\alpha}$ 10 Hz. The lower band did not contain the expected *trans*-diaxial bromohydrin (6β-bromo,7α-hydroxy) because the axial 5α-OH attacked the incipient C-6 carbocation to give a 5α,6α-epoxide (9a) isomeric with the starting compound (6). Acetylation of (9a) afforded the corresponding monoacetate (9b), $J_{6\beta,7\beta}$ 5 Hz. The axial orientation of the 7α-acetate in (9b) places its methyl group below the plane of the aromatic ring, thus resulting in a sizable upfield shift of its resonance (δ 1.80), as well as that of the aromatic 15-H (δ 7.02). The structures assigned to the bromoacetate (8b) and the epoxy-acetate (9b) were confirmed by spin decoupling and by high resolution mass spectrometry.

The mass spectral fragmentation of Nic-1 and derivatives is dominated by the cleavage of the 20,22-bond, leading to the fragments *m/e* 323 in the Δ^2-1-one and *m/e* 325 in the 1-one derivatives, and the alternative fragments *m/e* 141 in compounds (4) and (5) and *m/e*

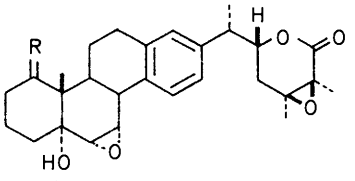
[1] I. Kirson, D. Lavie, S. S. Subramanian, P. D. Sethi, and E. Glotter, *J.C.S. Perkin I*, 1972, 1972.
[2] F. v. Gizycki and G. Kotitschke, *Arch. Pharm.*, 1951, **284**, 129; O. Nalbandov, R. T. Yamamoto, and G. S. Fraenkel, *J. Agric. Food Chem.*, 1964, **12**, 55.
[3] M. J. Begley, L. Crombie, P. J. Ham, and D. A. Whiting, *J.C.S. Chem. Comm.*, 1972, 1250.
[4] R. B. Bates and D. J. Eckert, *J. Amer. Chem. Soc.*, 1972, **94**, 8258.
[5] I. Kirson, E. Glotter, D. Lavie, and A. Abraham, *J. Chem. Soc.* (*C*), 1971, 2032.



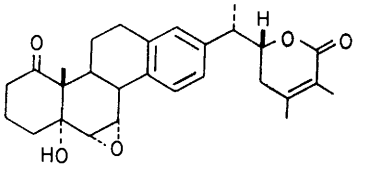
(1) R^1=O R^2=H
(10) R^1=H_2, R^2=OH



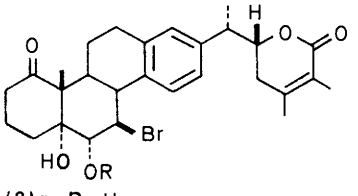
(2) a; Δ^2; R=H
b; Δ^2; R=Ac
(3) R=H



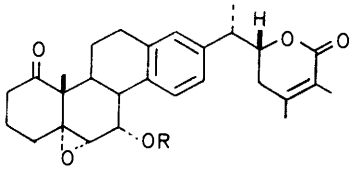
(4) Δ^2; R=O
(5) R=O
(7) R=α-OH, β-H



(6)



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



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

N.m.r. data *

Compound	2-H	3-H	6-H	7-H	Aromatic protons 15-H	16-H	17a-H	22-H	6-H	Methyl groups 19-H	21-H	27- and 28-H	Other
(2a)	5.88dt (10)	6.62m	3.22d (4)	4.0m narrow	7.38d (8)	7.05d (8)	7.0s	3.87m	4.98s [a]	1.22s	1.24d (7)	1.33;1.35	
(2b)	5.92dt	6.66m	3.28d [3.27]	4.06m [4.05]	7.38d	7.05d	6.97s	4.06m [4.47]	6.12s [6.47]	1.25s [1.20]	1.23d [1.26]	1.32;1.37 [1.25;1.32]	2.10 (OAc) [2.07]
(3)			3.26d	3.98m				3.83	5.00s [a]	1.27s	1.23d	1.35;1.37	
(4) †	5.88dt (10)	6.65m	3.25d (4)	4.04m narrow	7.39d (8)	7.05d (8)	7.01s	4.65dt (12;5)		1.24s	1.35d (7)	1.50;1.40	
(5)			3.23d (4)	4.02dd (4;3)	7.38d (8)	7.05d (8)	6.98s	4.68dt (10;6)		1.30s	1.35d (7)	1.43;1.55	
(6)			3.30d (4)	4.03dd (4;3)	7.43d (8)	7.02d (8)	7.08s	4.52dt (12;5)		1.31s	1.41d (8)	1.89	
(8a)			4.08d (10)	4.57dd (10;11)	7.50d (8)	7.12d (8)	7.05s	4.42		1.42s	1.40d (8)	1.85	
(8b)			5.58d (10)	4.55dd (10;11)	7.48d (8)	7.08d (8)	7.03s	4.47dt (12;5)		1.50s	1.38d (8)	1.85	2.21 (OAc)
(9a)			3.50d (5)	4.53	6.97; 7.13 (narrow multiplets)			*ca.* 4.5		1.42s	1.37d (8)	1.85	
(9b)			3.55d (5)	5.67t (5)	7.02 (narrow multiplet)			4.42dt (12;5)		1.42s	1.35d (8)	1.85	1.78 (OAc)

* At 60 MHz; solvent $CDCl_3$; δ values; data for C_5D_5N solutions in square brackets; coupling constants (Hz) in parentheses.
† Measured at 90 MHz on a Brucker instrument.

[a] After addition of D_2O.

125 in compounds (6), (8), and (9). Further fragmentation of the *m/e* 323 and 325 ions involves cleavage of the 7,8- and 9,10-bonds, resulting in two strong signals at *m/e* 155 and 157, containing rings C and D. The fragmentation of the epoxy-acetate (9b) follows a different path owing to the ready elimination of acetic acid [*m/e* 432 ($M^+ - 60$)], thus precluding cleavage of the 7,8-bond; the *m/e* 155 and 157 signals are therefore of low intensity. Alternatively, this spectrum is characterized by the intense formation of two fragments: $C_{21}H_{24}O_2$ [*m/e* 308 ($M^+ - 125 - 59$)] which results from rings A—D and a C_2 side chain, and $C_{17}H_{17}$ (*m/e* 221) which results from rings B—D and the same C_2 side chain.

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 on a Perkin-Elmer Infracord 137 spectrophotometer and refer to solutions in chloroform; u.v. spectra were recorded on a Cary 14 instrument for solutions in ethanol; n.m.r. spectra were determined on a Varian NV-14 instrument for *ca.* 5% solutions in deuteriochloroform containing tetramethylsilane as internal standard. T.l.c. was carried on plates of silica gel G (Merck) and spots were developed with iodine vapour. Preparative chromatoplates (1 mm thick) were prepared from silica gel PF_{254} (Merck). Mass spectra were taken under the direction of Dr. Z. Zaretskii with a Varian MAT 731 HR instrument. Analyses were performed in the microanalytical laboratory of the Weizmann Institute, under the direction of Mr. R. Heller.

Plant Material.—*Nicandra physaloides* was raised by Mr. A. Abraham in the nursery of the Agricultural Research Organization, Volcani Center, Israel, from seeds received from India (courtesy of Dr. P. D. Sethi and Dr. S. Sankara Subramanian, Pondicherry). The isolation procedure was as described.[1] The amount of Nic-1 (nicandrenone) (2a) isolated following the processing of 1 kg of dry leaves was 800 mg. The product was further purified by preparative layer chromatography (p.l.c.) in benzene–ethyl acetate (1 : 4) and recrystallization from benzene; m.p. 138—139° (lit.,[3] 137°); c.d. $\Delta\varepsilon_{338}$ −1.7, $\Delta\varepsilon_{224}$ +6.22.

Nic-1 Acetate (2b) [26-*Acetoxy*-6α,7α:22,26:24,25-*triepoxy*-5-*hydroxy*-17(13 ⟶ 18)abeo-5α-*ergosta*-2,13,15,17-*tetraen*-1-*one*].—Nic-1 (50 mg) was acetylated with acetic anhydride (0.5 ml) and pyridine (0.5 ml), overnight at room temperature. The *product* was precipitated with ice–water, isolated by filtration, and crystallized from ethanol; m.p. 180—181°, $[\alpha]_D$ +22.5° (*c* 0.4), $\nu_{max.}$ 1 690 and 1 740 cm^{-1}; $\lambda_{max.}$ 275.5 (ε 1 000), 265 (1 150), 217.5sh (15 500), and 222 nm (16 000) (Found: C, 70.9; H, 7.1. $C_{30}H_{36}O_7$ requires C, 70.8; H, 7.1%).

6α,7α:22,26:24,25-*Triepoxy*-5,26-*dihydroxy*-17(13 ⟶ 18)abeo-5α-*ergosta*-13,15,17-*trien*-1-*one* (3).—Nic-1 (100 mg) in absolute ethanol was hydrogenated (10% Pd–$CaCO_3$) at room temperature and atmospheric pressure. After absorption of 0.9 mol. equiv. the *product* was purified by p.l.c. [benzene–ethyl acetate (1 : 4)] and crystallized from acetone–hexane; m.p. 125—127°; $[\alpha]_D$ +51.8 (*c* 0.45); $\nu_{max.}$ 1 705 cm^{-1}; $\lambda_{max.}$ 220sh (ε 10 500) and 215sh nm (12 000); c.d. $\Delta\varepsilon_{295}$ +0.73 and positive at wavelengths shorter than 220 nm (Found: C, 71.7; H, 7.8. $C_{28}H_{36}O_6$ requires C, 71.8; H, 7.7%).

6α,7α:24,25-*Diepoxy*-5-*hydroxy*-17(13 ⟶ 18)abeo-5α-*ergosta*-13,15,17-*trien*-1-*one* 26,22-*Lactone* (5).—To a solution of compound (3) (50 mg) in acetone (25 ml), a solution of Jones reagent was added dropwise at 16—18 °C. After 30 min the excess of reagent was destroyed with a few drops of methanol, the solvent was removed under vacuum, and the *residue* was crystallized (yield 40 mg) from chloroform–ethyl acetate; m.p. 243—245°; $[\alpha]_D$ +62.6° (*c* 0.4); $\nu_{max.}$ 1 708 and 1 715 cm^{-1}; $\lambda_{max.}$ 275.5 (ε 1 050), 267 (1 100), 261 (950), 220sh (8 700), and 214 nm (10 000); c.d. $\Delta\varepsilon_{293}$ +0.70, $\Delta\varepsilon_{234}$ −2.3 (Found: C, 72.0; H, 7.25%; M^+, 466. $C_{28}H_{34}O_6$ requires C, 72.1; H, 7.35%; *M*, 466).

6α,7α:24,25-*Diepoxy*-5-*hydroxy*-17(13 ⟶ 18)abeo-5α-*ergosta*-2,13,15,17-*tetraen*-1-*one* 26,22-*Lactone* (4).—The oxidation of Nic-1 (1 g) was performed as described for compound (3) and the *product* was crystallized (yield 700 mg) from acetone–hexane; m.p. 230—232°, $[\alpha]_D$ +34.6° (*c* 0.5); $\lambda_{max.}$ 1 698 and 1 715 cm^{-1}; $\lambda_{max.}$ 216 nm (ε 16 600); c.d. $\Delta\varepsilon_{338}$ −2.25, $\Delta\varepsilon_{275.5}$ +0.11, $\Delta\varepsilon_{268}$ +0.10, $\Delta\varepsilon_{263}$ 0.07sh, $\Delta\varepsilon_{244}$ −0.39, $\Delta\varepsilon_{224}$ +5.29; n.m.r. decoupling: irradiation of 20-H ⟶ 20-CH_3 singlet and 22-H double doublet; irradiation of 7β-H ⟶ 6β-H singlet; irradiation of 6β-H ⟶ 7β-H narrow band $W_{\frac{1}{2}}$ 3 Hz; irradiation of 8β-H ⟶ 7β-H doublet (*J* 4 Hz) (Found: C, 72.5; H, 7.0%; M^+, 464. $C_{28}H_{32}O_6$ requires C, 72.4; H, 6.9%; *M*, 464).

Hydrogenation of the Lactone (4).—Compound (4) (330 mg) in absolute ethanol (140 ml) was hydrogenated (10% Pd–$CaCO_3$) at room temperature and atmospheric pressure. After absorption of 1 mol. equiv. the product was crystallized from chloroform–ethanol. It was identical with compound (5).

Reduction of the Lactone (5) *with Chromium*(II) *Chloride.*—A solution of chromium(II) chloride [from $CrCl_3,6H_2O$ [6] (3.2 g)] was added at room temperature under CO_2 to a solution of compound (5) (220 mg) in acetone (15 ml) and acetic acid (10 ml). The mixture was heated during 1 h at 50 °C, then ice–water was added and the product was isolated by filtration. 6α,7α-*Epoxy*-5-*hydroxy*-17(13 ⟶ 18)abeo-5α-*ergosta*-13,15,17,24-*tetraen*-1-*one* 26,22-*Lactone* (6) crystallized from acetone–hexane (yield 100 mg); m.p. 186—187°, $[\alpha]_D$ +136.4° (*c* 0.4); $\nu_{max.}$ 1 700 cm^{-1}; $\lambda_{max.}$ 275 (ε 730), 266 (1 140), 261 (1 500), and 213 nm (19 000); c.d. $\Delta\varepsilon_{290}$ +0.63sh, $\Delta\varepsilon_{245}$ +7.00sh, $\Delta\varepsilon_{229}$ +8.17 (positive at shorter wavelengths) (Found: C, 74.5; H, 7.7%; M^+, 450. $C_{28}H_{34}O_5$ requires C, 74.6; H, 7.6%; *M*, 450).

Reduction of Compound (5) *with Borohydride.*—Sodium borohydride (50 mg) was added to a solution of compound (5) (50 mg) in methanol (20 ml). After 1 h at room temperature the solution was neutralized with dilute hydrochloric acid (1 : 4), the solvent was removed, water was added, and the product was filtered off. 6α,7α:24,25-*Diepoxy*-1α,5-*dihydroxy*-17(13 ⟶ 18)abeo-5α-*ergosta*-13,15,17-*triene* 26,22-*lactone* (7) was purified by p.l.c. [benzene–ethyl acetate (1 : 4)] and crystallized from ethanol; m.p. 185—187°, $[\alpha]_D$ −23.5° (*c* 0.3); $\nu_{max.}$ 1 715 cm^{-1}; c.d. $\Delta\varepsilon_{233}$ −2.96 (Found: C, 71.6; H, 7.9. $C_{28}H_{36}O_6$ requires C, 71.8; H, 7.7%).

[6] L. Fieser and M. Fieser, ' Reagents for Organic Synthesis,' vol. I, Wiley, 1967, p. 149.

Epoxidation of Compound (6).—To a solution of (6) (35 mg) in benzene (2 ml), a solution of perbenzoic acid (30 mg) in benzene was added and the mixture was kept for 1 week at room temperature. The solution was then washed with dilute aqueous sodium carbonate and water, and evaporated. The residue was purified by p.l.c. [benzene–ethyl acetate (1 : 4)]. The main band gave the lactone (5) (30 mg), identical with the compound described above.

Treatment of Compound (6) *with Hydrobromic Acid.*—To a solution of (6) (100 mg) in acetone (90 ml), aqueous 45% hydrobromic acid (3 ml) was added. After 7 h at room temperature the solution was neutralized with sodium carbonate, the solvent was removed, and the residue was extracted with methylene chloride. The crude product (105 mg; two spots on a chromatoplate) was separated by p.l.c. [benzene–ethyl acetate (1 : 4)]. The upper band yielded 7β-*bromo*-5,6α-*dihydroxy*-17(13 ⟶ 18)abeo-5α-*ergosta*-13,15,17,24-*tetraen*-1-*one* 26,22-*lactone* (8a) (20 mg), m.p. 218—220° (from acetone); $[\alpha]_D$ +311.5° (*c* 0.15); $\nu_{max.}$ 1 690 and 1 710 cm^{-1}; $\lambda_{max.}$ 213 nm (ε 20 000) (Found: C, 63.3; H, 6.7%; M^+, 530/532. $C_{28}H_{35}BrO_5$ requires C, 63.3; H, 6.6; *M*, 530/532). Acetylation afforded the non-crystalline 6-*monoacetate* (8b); n.m.r. decoupling: irradiation of 6β-H ⟶ 7α-H doublet (*J* 11 Hz); irradiation of 7α-H ⟶ 6β-H slightly broadened singlet (Found: M^+, 572.177 2/574.178 0. $C_{30}H_{37}BrO_6$ requires *M*, 572.177 3/574.175 4).

The lower band yielded 5, 6α-*epoxy*-7α-*hydroxy*-17(13 ⟶ 18)abeo-5α-*ergosta*-13,15,17,24-*tetraen*-1-*one* 26,22-*lactone* (9a) (40 mg), m.p. 195—197° (from aqueous methanol); $[\alpha]_D$ −9° (*c* 0.1); $\nu_{max.}$ 1 710 cm^{-1}; $\lambda_{max.}$ 216 nm (ε 16 200) (Found: C, 74.5; H, 7.6. $C_{28}H_{34}O_5$ requires C, 74.6; H, 7.6%). Upon acetylation with acetic anhydride and pyridine overnight at room temperature, the non-crystalline 7-*monoacetate* (9b) was obtained; n.m.r. decoupling: irradiation of 6β-H ⟶ 7β-H doublet (*J* 5 Hz); irradiation of 7β-H ⟶ 6β-H singlet (Found: M^+, 492.253 9. $C_{30}H_{36}O_6$ requires *M*, 492.251 2).

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