

## Structures of Three Oxygenated 24-Methyl-steroids (Nic-3, -7, and -11) from the Insect Repellent Plant *Nicandra physaloides* (Solanaceae): X-Ray Analysis of Nic-3 Acetate and Nic-11 Ethyl Ether

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Seventeen compounds (Nic 1—17) have been isolated from aqueous extracts of the leaves of *Nicandra physaloides* which inhibit the feeding of certain insect larvae. X-Ray analysis of its acetate (direct methods) showed Nic-3 to be (20*S*,22*R*,24*S*,25*S*,26*R*)-6 $\alpha$ ,7 $\alpha$ :22,26:24,25-triepoxy-5,26-dihydroxy-5 $\alpha$ -ergost-2-en-1-one (3). Similar examination of Nic-11 as its ethyl ether showed it to be the 6 $\alpha$ ,7 $\alpha$ :17,24:22,26-triepoxy-5,25,26-trihydroxy-5 $\alpha$ -17 $\alpha$ -ergost-2-en-1-one (6). Spectroscopic correlation of Nic-7 with Nic-3 shows the former to be the corresponding 1,12-dione (4). Sneath's chiroptical correlation for cyclohexenones supports the assigned absolute configuration.

Biogenetic relationships between these compounds, withanolides, physalins, and the aromatic ring D steroids of the following paper are outlined.

THE tobacco hornworm (*Protoparce sexta*) feeds preferentially on plants of the Solanaceae family, although it avoids the leaves of *Nicandra physaloides* (Solanaceae),<sup>1</sup> a reputed fly-repellant of Peruvian origin. Aqueous extracts of the leaves of this plant have been shown to inhibit feeding of larvae of various insect species. Two crystalline substances were isolated from such extracts by Fraenkel and co-workers,<sup>2</sup> the major component ('nicandrenone,' C<sub>34</sub>H<sub>42</sub>O<sub>7</sub>, m.p. 102—105 °C) having a bitter taste and insecticidal properties, but the structures of these compounds were not elucidated.

In the present work, the annual *N. physaloides* was grown from seed during several years, and the constituents of the aqueous extract of leaf were examined. It was found that the composition of the natural product mixture varied with each succeeding crop and it seems likely that there was genotypic variation in the commercial seed used. On the other hand, there may be relationships with climate, time of harvesting, and habitat, and these factors have not been unravelled.

were non-crystalline, probably saturated and acyclic substances (mass-spectrometry) which were not further investigated. Structures, based on crystallographic and spectroscopic investigation, have been defined for seven of the remaining compounds. This paper deals

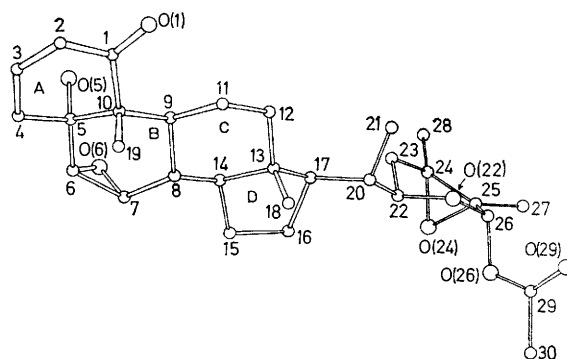
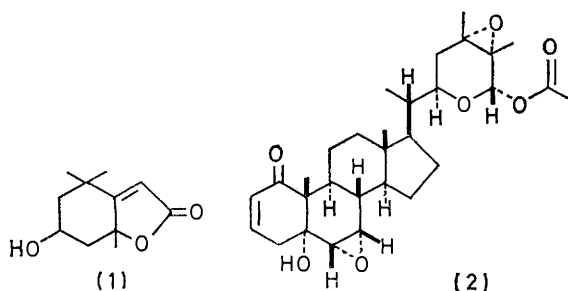


FIGURE 1 Nic-3 acetate (2): a general view projected down the *b* axis showing the crystallographic numbering system



In all, 17 distinct products were characterised, and for convenience were labelled Nic-1 to Nic-17. One compound (Nic-15) was identified as loliolide (1),<sup>3</sup> and six

with the constitution of one structurally related group (Nic-3, -7, and -11), while the nature of a second family (Nic-1, -10, -12, and -17) is examined in the following paper.

Since the quantities of the compounds available were limited (mean total content 0.0005% based on dry leaf), crystallographic analysis appeared most likely to be fruitful. Attempts to prepare heavy-atom derivatives were frustrated by the (subsequently revealed) complex functionalities of the molecules, and direct methods were employed. Adequately large and well formed crystals were first obtained from the acetate of Nic-3, C<sub>30</sub>H<sub>42</sub>O<sub>7</sub>, m.p. 262 °C.

The structure was solved from diffractometer data (1 630 reflections) by direct methods and refined by least-squares and difference-Fourier methods to *R*

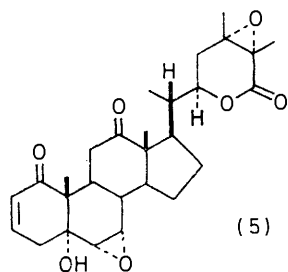
<sup>1</sup> R. T. Yamamoto and G. S. Fraenkel, *Ann. Entomol. Soc. Amer.*, 1960, **53**, 503; G. Fraenkel, J. Nayar, O. Nalbandov, and R. T. Yamamoto, Proc. 11th Internat. Congr. Entomol., Vienna, 1960, **3**, 122.

<sup>2</sup> O. Nalbandov, R. T. Yamamoto, and G. Fraenkel, *J. Agric. Food Chem.*, 1964, **12**, 55; cf. F. v. Gizycki and G. Kotitschke, *Arch. Pharm.*, 1951, **284**, 129.

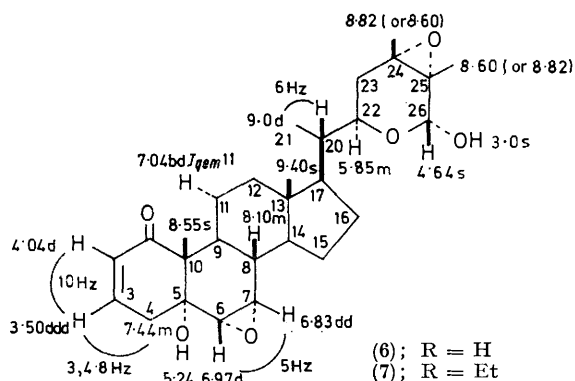
<sup>3</sup> R. Hodges and A. L. Porte, *Tetrahedron*, 1964, **20**, 1463; T. Wada, *Chem. and Pharm. Bull. (Japan)*, 1965, **13**, 43; S. M. Kupchan, W. K. Anderson, P. Bollinger, R. W. Doskotch, R. M. Smith, J. A. S. Renauld, H. K. Schnoes, A. L. Burlingame, and D. H. Smith, *J. Org. Chem.*, 1969, **34**, 3858.



( $\tau$  9.03, s) and 11-H<sub>eq</sub> (6.15bd,  $J_{\text{gem}}$  11.5 Hz) resonate at significantly lower fields than their counterparts in (3) {13-Me (9.40s), 11-H<sub>eq</sub> (7.04bd)}. Nic-7 could be oxidised with acetyl hypobromite or *N*-bromoacetamide to the  $\delta$ -lactone (5), (an epoxywithanolide),  $\nu_{\text{max}}$ . 1730 cm<sup>-1</sup>.



A third methyl steroid was discovered in Nic-11, C<sub>28</sub>H<sub>40</sub>O<sub>7</sub>, m.p. 277 °C,  $\lambda_{\text{max}}$ . (EtOH) 224 (6000) nm (enone). Repeated crystallisation from ethanol gave crystals which eventually proved to be the 26-*O*-ethyl derivative (7) of the original natural product. The structure was solved by direct methods as for Nic-3 and refined to *R* 0.059 (2140 reflections). A general view of the resulting structure is shown in Figure 5. The parent Nic-11 is thus the oxidised hexacyclic methylsteroid (6), carrying a dioxaspirobicyclic system at C-17, with a 17- $\alpha$  carbon chain. Proton n.m.r. correlations are also displayed on (6). For crystallographic purposes only, the numbering of Figure 5 was



<sup>1</sup>H n.m.r. data refer to (6); Me signals: 8.46s (3 H), 8.63s (3 H), 8.79s (3 H), and 8.98s (6 H).

employed; bond lengths and angles are given in Figures 6 and 7, and the arrangement of molecules in the unit cell in Figure 8. The conformation of the all-*trans*-ABCD system is very similar to that in Nic-3, but the orientation of the C-17 substituents was such that C(20) was axial ( $\alpha$ ) and O(17) equatorial whereas in Nic-3 it is C(20) which is equatorial ( $\beta$ ). In the dioxaspirobicyclic system, ring E was found to show a slightly distorted boat conformation with C(17) and C(23) below the mean plane of the ring. Substituents C(16) and C(28) were equatorial to the ring while C(13), C(21), O(22), and

C(25) were axial. Ring F adopts the chair conformation with substituents O(26), C(27), and C(28) equatorial and

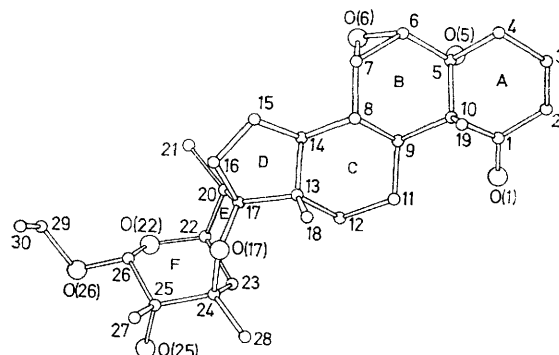


FIGURE 5 Nic-11 ethyl ether (7): a general view of the structure showing the crystallographic numbering system

O(17), C(20), and O(25) axial. Weak intermolecular hydrogen bonds were discovered in the crystal structure: these were OH(25)  $\cdots$  O(6') 3.12, OH(5)  $\cdots$  O(22')

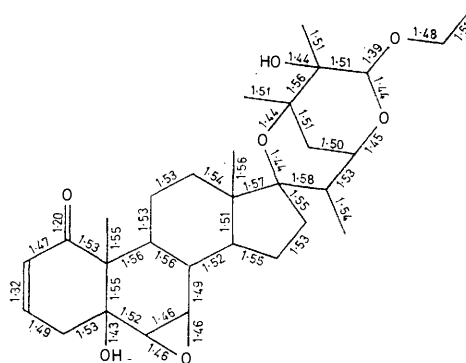


FIGURE 6 Bond lengths (Å) in (7); mean  $\sigma$  < 0.01 Å

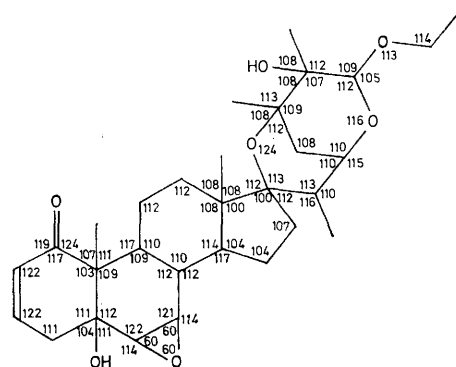


FIGURE 7 Bond angles (°); mean  $\sigma$  < 0.7°. Additional angles: C(4)-C(5)-C(6) 110, O(5)-C(5)-C(10) 109, C(1)-C(10)-C(9) 116, C(5)-C(10)-C(19) 111, C(12)-C(13)-C(17), 121, C(14)-C(13)-C(18) 112, C(13)-C(17)-C(20) 113, C(16)-C(17)-O(17) 105, O(17)-C(24)-C(25) 103, C(23)-C(24)-C(28) 111, C(24)-C(25)-C(27) 112, and O(25)-C(25)-C(26) 110°

3.12, OH(5)  $\cdots$  O(26') 3.15 Å, with the hydrogen in the latter case midway between the two possible oxygens O(22') and O(26').

The (5*R*,10*R*) absolute configuration shown for Nic-3, -7, and -11 is that expected on biogenetic grounds. Confirmation is provided by o.r.d. data, as all three

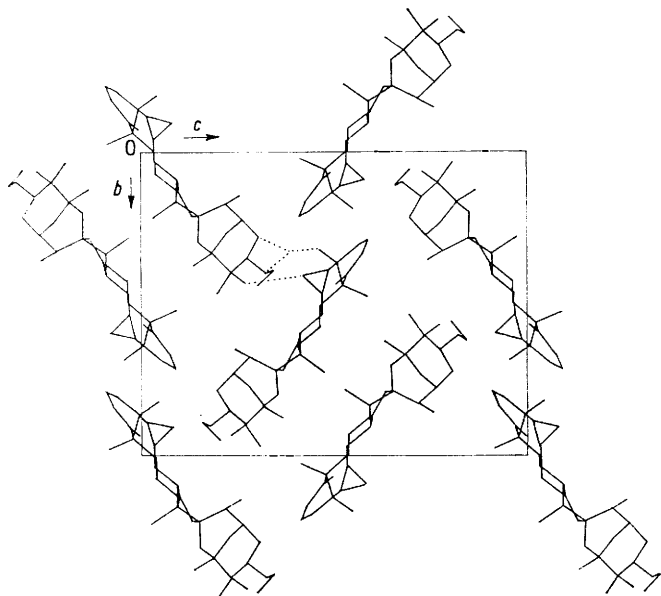


FIGURE 8 Arrangement of molecules of (7) in the unit cell

compounds display strong negative Cotton effects centred around 330 nm (the enone *R*-band,  $n \rightarrow \pi^*$ ). Snatzke<sup>4</sup> has co-ordinated data for chiral cyclohexenones and as a result the quadrant rule shown in Figure 9 has been suggested. Reference to the *X*-ray results for

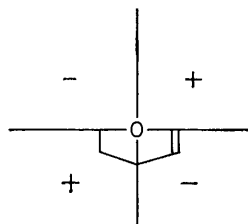


FIGURE 9 Quadrant rule for enones (*R* band)

Nic-3 acetate and Nic-11 ethyl ether indicate the conformation shown in Figure 10 for ring A of the compound in the (5*R*,10*R*)-configuration. Torsion angles O(1)-C(1)-C(2)-C(3) are 165° and 164° for Nic-3 acetate and Nic-11 ethyl ether, and such stereochemistry would give rise to a negative Cotton effect when Snatzke's rule is applied. The closest model compounds we know are the ring B- and ring C-enones<sup>5</sup> (8) and (9), which show positive and negative *R*-band Cotton effects respectively, in concord with the present case. The total stereo-

<sup>4</sup> 'Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry,' ed. G. Snatzke, Heyden, London, 1967.

<sup>5</sup> L. Velluz and M. Legrand, *Angew. Chem.*, 1961, **73**, 603; H. Vorbrüggen, S. C. Pakrashi, and C. Djerassi, *Annalen*, 1963, **668**, 57.

<sup>6</sup> P. L. C. Yu, M. M. El-Olemy, and S. J. Stohs, *Lloydia*, 1974, **37**, 593.

chemistry of the fourteen chiral centres of Nic-3 are thus completely defined as (5*R*,6*S*,7*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*,20*S*,22*R*,24*S*,25*S*,26*R*).

Likely biosynthetic relationships between these three methylsteroids of *N. physaloides* are shown in the Scheme. Possible progenitors of the group include

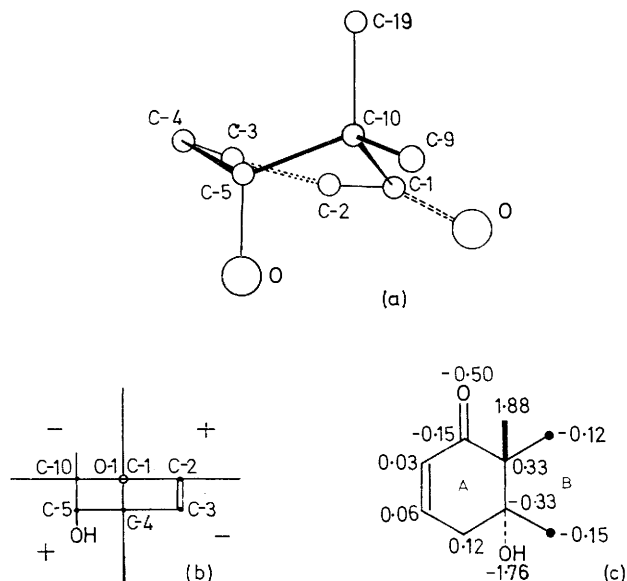
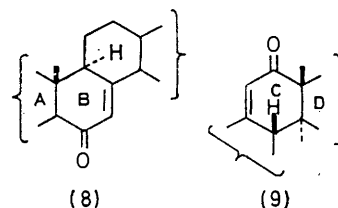


FIGURE 10 Ring A conformation for Nic-3 and -11 derivatives: (a) general view, (b) quadrant projection O-1  $\rightarrow$  C-1, (c) deviations from the mean plane of ring A carbons (Nic-3 acetate)

campesterol, 24-methylidenecholesterol (both found in tissue cultures of the related *Withania somnifera*<sup>6</sup>), a dihydroergosterol, or 24,25-didehydro-24-methylcholesterol (13) (also a constituent of *W. somnifera*<sup>7</sup>). An oxidation sequence leading ultimately to Nic-3 is envisaged. Further oxidation at C-12 would form Nic-7, whilst alternative 17 $\beta$ -hydroxylation could give Nic-2, a compound reported from *N. physaloides* by Bates and Morehead<sup>8</sup> soon after the appearance of our preliminary publications.<sup>9</sup> Nic-11 can be formed from



Nic-2 by intramolecular opening of the 24,25-epoxide by the 17 $\beta$ -hydroxy-group, as shown. The stereochemistry of Nic-11 accords with a *trans*-diaxial ring opening.

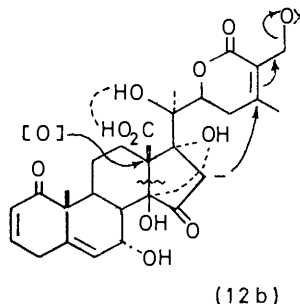
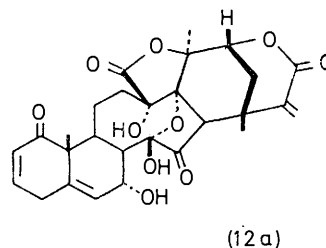
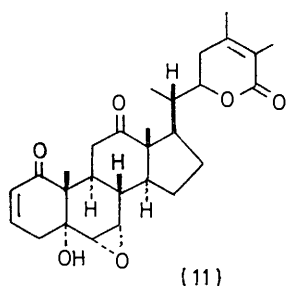
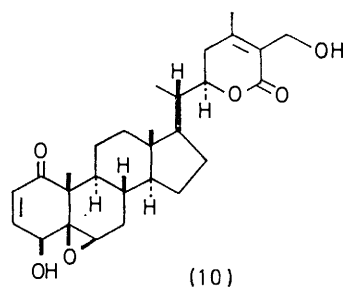
<sup>7</sup> W. J. S. Lockley, D. P. Roberts, H. H. Rees, and T. W. Goodwin, *Tetrahedron Letters*, 1974, 3773.

<sup>8</sup> R. B. Bates and S. R. Morehead, *J.C.S. Chem. Comm.*, 1974, 125.

<sup>9</sup> M. J. Begley, L. Crombie, P. Ham, and D. A. Whiting, *J.C.S. Chem. Comm.*, 1972, 1108; 1973, 821.

The closest relatives to these *Nicandra* extractives are the lactonic withanolides characteristic of, *inter alia*, the *Acnistus*, *Datura*, *Dunalia*, *Jaborosa*, and *Withania* genera of the Solanaceae.<sup>10</sup> Withaferin A (10)<sup>10</sup> is a well-known example and withanicandrin (11)<sup>11</sup> has been found in *N. physaloides*. 17-Hydroxylation is featured in various withanolides both with  $\alpha$ -orientation<sup>8,12</sup> and also with the rarer  $\beta$ -disposition:<sup>13</sup> 17 $\beta$ -hydroxylation is otherwise known in relatively few pregnane relatives.<sup>14</sup>

The physalins<sup>15</sup> represent more oxidatively elaborated relatives of these *Nicandra* compounds, and hypothetical



connection<sup>15</sup> between physalin A (12a), found in *Physalis alkengi* var. Franchetti (Solanaceae), and a highly oxidized withanolide such as (12b) is indicated. Our *Nicandra* compounds and those having an aromatic ring D reported in the following paper, together with the withanolides and the physalins, form a biogenetic group within the Solanaceae, the apparent outcome of characteristic oxidation processes operating on a fundamental ergostane-like framework.

#### EXPERIMENTAL

*Extraction of Nicandra physaloides* Leaves.—Seeds were supplied by Thompson and Morgan, Ipswich, and Sharpes,

\* For details of Nic-1, -10, -12, and -17 see following paper.

† The term 'Nic-2' has been used elsewhere (ref. 8).

<sup>10</sup> See *inter alia*: D. Lavie, E. Glotter, and Y. Schvo, *J. Chem. Soc. (C)*, 1965, 7517; S. M. Kupchan, R. W. Doskotch, P. Bollinger, A. T. McPhail, G. A. Sim, and J. A. S. Renault, *J. Amer. Chem. Soc.*, 1965, **87**, 5805; A. T. McPhail and G. A. Sim, *J. Chem. Soc. (B)*, 1968, 962; R. Techsche, M. Baumgarth, and P. Welzel, *Tetrahedron*, 1968, **24**, 5169; S. M. Kupchan, W. K. Anderson, P. Bollinger, R. W. Doskotch, R. M. Smith, J. A. S. Renault, H. K. Schnoes, A. L. Burlingame, and D. H. Smith, *J. Org. Chem.*, 1969, **34**, 3858; S. M. Kupchan, R. W. Doskotch, P. Bollinger, A. T. McPhail, G. A. Sim, and J. A. S. Renault, *Tetrahedron*, 1970, **26**, 2209, 5062; 1973, **29**, 1353; A. Abraham, I. Kirson, D. Lavie, and E. Glotter, *Phytochemistry*, 1975, **14**, 189.

Sleaford, and plants were raised as half-hardy annuals. After harvesting whole plants, the leaves were removed as quickly as possible and oven-dried over 10 days, initially at 30–40 and finally at 50–60 °C. Powdered dry leaf was suspended with stirring in water (1 dm<sup>3</sup> per 100 g leaf) and heated under reflux overnight. The extract was decanted and the residue again treated with boiling water (500 cm<sup>3</sup> per 100 g leaf) and set aside overnight before filtration. The combined dark brown aqueous extracts were extracted with chloroform (1 dm<sup>3</sup> per 2 dm<sup>3</sup> extract); extraction was made difficult by the formation of emulsions which resolved slowly when set aside. The chloroform extracts were

concentrated, dried, and evaporated to yield a light brown glass. Separation of the terpenoids was achieved by preparative t.l.c. (40 × 40 × 0.03 cm layers of Kieselgel HF<sub>254</sub>); ca. 50 mg of crude terpenoids were separated on each plate by use of multiple development (3–5 runs) with chloroform–methanol eluant. Results in various years are as follows: (a) 1967: leaves (1 kg) afforded Nic-1\* (37 mg); (b) 1968: leaves (46 kg) afforded 2.2 g extractives; p.l.c. with chloroform–methanol (199:1) gave Nic-2A† (28 mg), Nic-3 (562 mg), Nic-4 (23 mg), Nic-5 (6 mg), Nic-6 (61 mg), Nic-7 (1 450 mg), and Nic-8 (14 mg); (c) 1969: similar pattern to 1968 [crops (a), (b), and (c) were grown at Leckwith, Glamorgan]; (d) 1970: leaves (9 kg) gave 566 mg extractives Nic-10 (55 mg), Nic-11 (127 mg), Nic-12 (100 mg), and Nic-13 (283 mg); (e) 1971:

<sup>11</sup> K. Kirson, D. Lavie, S. Sankara Subramanian, P. D. Sethi, and E. Glotter, *J.C.S. Perkin I*, 1972, 2109.

<sup>12</sup> K. L. Dhar and M. L. Rama, *Phytochemistry*, 1973, **12**, 476.

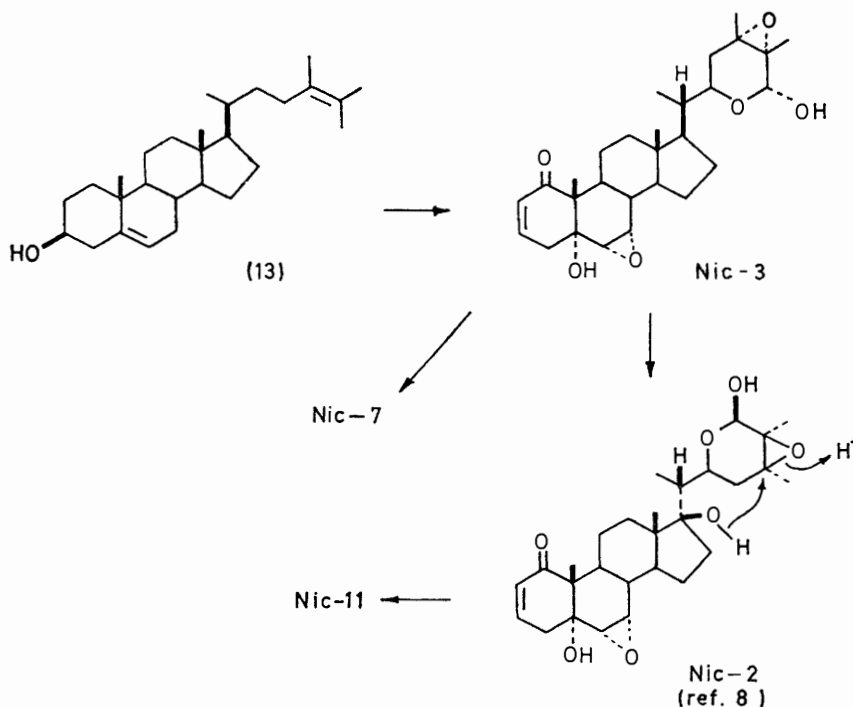
<sup>13</sup> D. Lavie, I. Kirson, E. Glotter, D. Rabinovich, and Z. Shakked, *J.C.S. Chem. Comm.*, 1972, 877.

<sup>14</sup> K. A. Jaeggi, E. Weiss, and T. Reichstein, *Helv. Chim. Acta*, 1963, **46**, 694; C. W. Shoppee and R. E. Lack, *J. Chem. Soc.*, 1964, 3611; H. Mitsuhashi, T. Sato, T. Nomura, and I. Takemori, *Chem. and Pharm. Bull. (Japan)*, 1964, **12**, 981; M. Fukuoka and H. Mitsuhashi, *ibid.*, 1968, **16**, 553.

<sup>15</sup> T. Matsuura, M. Kawai, R. Nakashima, and Y. Butsugan, *J. Chem. Soc. (B)*, 1970, 664; M. Kawai, T. Matsuura, T. Taga, and K. Osaki, *J. Chem. Soc. (B)*, 1970, 812; M. Kawai and T. Matsuura, *Tetrahedron*, 1970, **26**, 1743.

leaves (75 kg) gave 3 g extractives. Elution with chloroform-methanol (96:4) gave Nic-1 (1 000 mg), Nic-9 (306 mg), Nic-10 (139 mg), Nic-14 (54 mg), Nic-15 (30 mg), Nic-16 (32 mg), and Nic-17 (539 mg) [crops (d) and (e)

$C_{28}H_{40}O_8$  requires  $M$ , 472.2825),  $\nu_{max}$  (KBr) 3 448 (OH), 1 693 (C=O), 1 466, 1 395, 1 295, 1 091, 1 038, 905, and 786  $cm^{-1}$ ;  $\tau^*$  (100 MHz,  $CD_3 \cdot SO \cdot CD_3$ ) 3.39 (1 H, ddd,  $J$  10 4.8, 3 Hz, 3-H), 4.02 (1 H, d,  $J$  7 Hz, 26-OH), 4.33



SCHEME Possible biogenetic relationships among extractives of Solanaceae

cultivated at Sutton Bonington, Nottingham].  $R_F$  values ( $\times 10^2$ ) are listed in Table 1.

TABLE 1  
 $R_F$  ( $\times 10^2$ )<sup>a</sup> for *Nicandra* extractives

Compound	Solvent			
	(1) <sup>b</sup>	(2)	(3)	(4) <sup>c</sup>
Nic-1	80	70		
2A	70			
3	55		55	
4	50			
5	40			
6	35			
7	30		35	
8	87			
9		90		
10		90	55	
11			15	70
12			65	
13			5	40
14		85		
15		60		
16		95		
17		70		

Solvent: chloroform-methanol in ratios (1) 199:1, (2) 96:4, (3) 25:1, and (4) 92:8. <sup>a</sup> Kieselgel G. <sup>b</sup> 4 Runs. <sup>c</sup> 3 Runs.

Nic-3 [(20S,22R,24S,25S,26R)-6 $\alpha$ ,7 $\alpha$ :22,26:24,25-triepoxy-5,26-dihydroxy-5 $\alpha$ -ergost-2-en-1-one] (3) crystallised from benzene-chloroform, m.p. 248 °C (Found:  $M$ , 472.2818.

(1 H, d,  $J$  10 Hz, 2-H), 5.05 (1 H, d,  $J$  7 Hz, 26-H), 6.18 (1 H, m, 22-H), 6.34 (1 H, bd,  $J_{gem}$  11.5 Hz, 11-H<sub>eq</sub>), 6.86 (1 H, dd,  $J_{6,7}$  5 Hz, 7-H), 7.08 (1 H, d,  $J$  5 Hz, 6-H), 8.68 (3 H, s, 19-H<sub>3</sub>), 8.73, 8.93 (both 3 H, s, 27-H<sub>3</sub> and 28-H<sub>3</sub>), 9.15 (3 H, d,  $J$  6 Hz, 21-H<sub>3</sub>), and 9.30 (3 H, s, 18-H<sub>3</sub>);  $[M]_{281}^{22} nm + 6 940^\circ$  (min.),  $[M]_{305}^{22} nm + 9 480^\circ$  (max.),  $[M]_{364}^{22} nm - 3 780^\circ$  (min.) (dioxan).

Nic-3 26-acetate (2) was obtained by treatment of Nic-3 with pyridine-acetic anhydride (2:1) at 0 °C for 36 h; m.p. 262 °C (Found:  $M$ , 514.292.  $C_{30}H_{42}O_7$  requires  $M$ , 514.293);  $\nu_{max}$  ( $CHCl_3$ ) 1 744 (Ac) and 1 693  $cm^{-1}$  (C=O).

Nic-7 [(20S,22R,24S,25S,26R)-6 $\alpha$ ,7 $\alpha$ :22,26:24,25-triepoxy-5,26-dihydroxy-5 $\alpha$ -ergost-2-ene-1,12-dione] (4) had m.p. 263 °C from benzene-chloroform (Found: C, 69.55; H, 8.0%;  $M$ , 486.264;  $M - 18$ , 468.250.  $C_{28}H_{38}O_7$  requires C, 69.1; H, 7.9%;  $M$ , 486.262;  $M - 18$ , 468.252);  $\nu_{max}$  (KBr) 3 533 (OH), 3 444 (OH), 1 700 (C=O), 1 688 (C=O), 1 464, 1 396, 1 297, 1 262, 1 091, 1 037, and 906  $cm^{-1}$ ;  $\tau^*$  (100 MHz,  $CD_3 \cdot SO \cdot CD_3$ ) 3.48 (1 H, ddd,  $J$  10, 5, 3 Hz, 3-H), 4.18 (1 H, d,  $J$  7 Hz, 26-OH), 4.44 (1 H, d,  $J$  10 Hz, 2-H), 5.18 (1 H, d,  $J$  7 Hz, 26-H), 5.30 (1 H, s, 5-OH), 6.95 (1 H, bd,  $J$  4 Hz, 7-H), 8.68 (3 H, s, 19-H<sub>3</sub>), 8.96 (3 H, s, 18-H<sub>3</sub>), 8.84 and 8.73 (both 3 H, s, 28-H<sub>3</sub> and 27-H<sub>3</sub>), and 9.28 (3 H, d, 21-H<sub>3</sub>);  $[M]_{274}^{22} nm + 4 450^\circ$  (min.),  $[M]_{306}^{22} nm + 6 940^\circ$  (max.),  $[M]_{364}^{22} nm - 1 470^\circ$  (min.).

Nic-7 26-acetate, prepared by keeping a solution of Nic-7 (23 mg) in pyridine (0.4 cm<sup>3</sup>) and acetic anhydride (0.2 cm<sup>3</sup>) at 0° for 36 h (yield 15 mg), crystallised from methanol,

\* Data for [<sup>2</sup>H<sub>6</sub>]pyridine solutions given in Discussion section.

m.p. 274—275 °C (Found: C, 68.0; H, 7.7%;  $M$ , 528.274.  $C_{30}H_{40}O_8$  requires C, 68.15; H, 7.65%;  $M$ , 528.273);  $\lambda_{\max}$  (EtOH) 224 nm (5 860);  $\nu_{\max}$  (KBr) 3 540 (OH), 1 735 (Ac), 1 700 (C=O), and 1 695 (C=O)  $cm^{-1}$ ;  $\tau$  ( $CD_3SO-CD_3$ ) 3.41 (1 H, ddd,  $J$  10, 5, 2 Hz, 3-H), 4.02 (1 H, s, 26-H), 4.37 (1 H, d,  $J$  10 Hz, 2-H), 5.32 (1 H, s, OH), 6.22 (1 H, m, 22-H), 6.93 (1 H, d,  $J$  5 Hz, 7-H), 7.07 (1 H, d, 6-H), 7.97 (3 H, s,  $CO-CH_3$ ), 8.66, 8.76, and 8.87 (all 3 H, s, 19- $H_3$ , 27- $H_3$ , and 28- $H_3$ ), 9.00 (3 H, s, 18- $H_3$ ), and 9.28 (3 H, d,  $J$  7 Hz, 21- $H_3$ ).

*Oxidation of Nic-7.*—Bromine (0.25  $cm^3$ ) in carbon tetrachloride (5  $cm^3$ ) was added, with stirring, to silver acetate (1 g) in carbon tetrachloride (40  $cm^3$ ) at 0°. After 90 min at 0 °C (stirring maintained) the yellow solution (ca. 0.1M) of acetyl hypobromite was decanted. This solution (0.5  $cm^3$ ) was added at 0° to Nic-7 (22 mg) in chloroform (5  $cm^3$ ) and the mixture kept overnight. The product, after washing with aqueous sodium sulphite and water, was evaporated. The residue crystallised from methanol to give the lactone (i.e. the 1,12,26-trione) (5), (16 mg), m.p. 307—309 °C (Found:  $M$ , 484;  $M - 15$ , 469.225;  $M - 18$ , 446.237.  $C_{28}H_{36}O_7$  requires  $M$ , 484;  $M - 15$ , 469.223;  $M - 18$ , 466.236);  $\lambda_{\max}$  (EtOH) 224 nm (6 880),  $\nu_{\max}$  (KBr) 1 730 ( $\delta$ -lactone), 1 698 (C=O), and 1 683 (C=O);  $\tau$  ( $CD_3SO-CD_3$ ) 3.39 (1 H, ddd,  $J$  10, 5, 2 Hz, 3-H), 4.35 (1 H, d,  $J$  10 Hz, 2-H), 5.32 (1 H, s, OH), 5.59 (1 H, m, 22-H), 6.86 (1 H), 7.04 (1 H, d,  $J$  5 Hz, 6-H), 7.20 (1 H), 8.52, 8.57, and 8.84 (all 3 H, s, 19- $H_3$ , 27- $H_3$ , and 28- $H_3$ ), 8.95 (3 H, s, 18- $H_3$ ), and 9.22 (3 H, d,  $J$  7 Hz, 21- $H_3$ ).

*Nic-11* (6 $\alpha$ ,7 $\alpha$ :17,24:22,26-triepoxy-5,25,26-trihydroxy-5 $\alpha$ ,17 $\alpha$ -ergost-2-en-1-one) (6).—This was crystallised from ethanol, m.p. 277 °C (Found:  $M$ , 488.275;  $M - CH_2O_2$ , 442.272.  $C_{28}H_{40}O_7$  requires  $M$ , 488.277;  $M - CH_2O_2$ , 442.272),  $\lambda_{\max}$  (EtOH) 224 nm (6 210),  $\nu_{\max}$  (KBr) 3 525 (OH), 1 683 (C=O), 1 625, 1 350, 1 252, 1 102, 1 061, 1 009, and 902  $cm^{-1}$ ;  $[M]_{271}^{22} nm + 3 000^\circ$  (min.),  $[M]_{305}^{22} nm + 3 860^\circ$  (max.),  $[M]_{360}^{22} nm - 4 250$  (min.).

On repeated crystallisation from ethanol, Nic-11 gave a 26-O-ethyl derivative (Found:  $M$ , 516.309.  $C_{30}H_{44}O_7$  requires  $M$ , 516.309).

*Nic-15* was identified as loliolide<sup>3</sup> and crystallised from chloroform—light petroleum as plates, m.p. 149 °C (lit.,<sup>3</sup> m.p. 145—149, 152—152.5 °C) (Found:  $M$ , 196. Calc. for  $C_{11}H_{16}O_3$ :  $M$ , 196);  $\lambda_{\max}$  (EtOH) 214 nm (10 830) [lit.,<sup>3</sup>  $\lambda_{\max}$  213 nm (11 500), 215 (14 800)];  $\nu_{\max}$  ( $CHCl_3$ ) 3 635, 3 455, 1 850w, 1 738, and 1 630  $cm^{-1}$  (lit.,<sup>3</sup> 3 580, 3 440, 1840, 1 741, and 1 632  $cm^{-1}$ );  $\tau$  ( $CDCl_3$ ) 4.32 (1 H, 3-H), 5.68 (1 H, 6-H), 7.51 and 7.97 (2 H,  $J_{gem}$  14.5 Hz, 7- $H_2$ ), 7.36 (1 H, s, OH), 8.24 and 8.49 (2 H,  $J_{gem}$  14 Hz, 5- $H_2$ ), 8.22 (3 H, s, 7 $\alpha$ - $CH_3$ ), 8.53 and 8.74 (both 3 H, s, 5-Me<sub>2</sub>). These data are closely similar to those reported.<sup>3</sup>

*Crystallographic Analysis of Nic-3 Acetate* (2).—Oscillation and Weissenberg photographs were taken about the axis to establish unit-cell dimensions and space group. For intensity measurement a crystal of dimensions ca. 0.6  $\times$  0.4  $\times$  0.4  $mm^3$  was mounted about the  $a$  axis on a Hilger and Watts four-circle diffractometer. Unit-cell dimensions were refined by a least-squares fit on the positions of 12 peaks found on the diffractometer. Intensity data were collected with Cu- $K_\alpha$  radiation for  $2\theta \leq 154^\circ$  by use of an  $\omega$ -2 $\theta$  scan. Of 3 300 independent reflections measured, 1 630 were considered observed, having  $I > 3\sigma(I)$  and were used in the structure refinement. No absorption corrections were made. Data reduction

TABLE 2

Fractional atomic co-ordinates for Nic-3 acetate, with standard deviations in parentheses

Atom	$x$	$y$	$z$
C(1)	1.1841(14)	0.5085(5)	-0.0753(4)
C(2)	1.2247(15)	0.5347(5)	-0.1364(4)
C(3)	1.1165(16)	0.5188(6)	-0.1855(5)
C(4)	0.9291(17)	0.4736(5)	-0.1828(4)
C(5)	0.9226(14)	0.4351(5)	-0.1218(4)
C(6)	0.7226(15)	0.3943(5)	-0.1171(4)
C(7)	0.6233(14)	0.3836(4)	-0.0566(4)
C(8)	0.7085(12)	0.4129(4)	0.0009(4)
C(9)	0.9371(11)	0.4371(4)	-0.0057(4)
C(10)	0.9595(12)	0.4797(5)	-0.0654(4)
C(11)	1.0009(13)	0.4705(5)	0.0548(4)
C(12)	0.9751(12)	0.4221(5)	0.1100(4)
C(13)	0.7528(12)	0.3958(4)	0.1167(4)
C(14)	0.6990(13)	0.3635(4)	0.0545(4)
C(15)	0.4948(14)	0.3261(5)	0.0672(4)
C(16)	0.5098(15)	0.3036(5)	0.1358(4)
C(17)	0.7212(13)	0.3326(5)	0.1597(4)
C(18)	0.6169(15)	0.4556(4)	0.1352(5)
C(19)	0.8225(13)	0.5451(4)	-0.0647(4)
C(20)	0.7197(15)	0.3441(5)	0.2291(4)
C(21)	0.9272(17)	0.3731(6)	0.2525(5)
C(22)	0.6599(16)	0.2776(5)	0.2625(4)
C(23)	0.8065(17)	0.2187(5)	0.2537(4)
C(24)	0.7559(18)	0.1591(5)	0.2959(5)
C(25)	0.6370(16)	0.1728(5)	0.3526(4)
C(26)	0.5914(14)	0.2450(5)	0.3681(4)
C(27)	0.6336(19)	0.1265(6)	0.4079(5)
C(28)	0.9034(22)	0.0970(6)	0.2895(6)
C(29)	0.3008(16)	0.2835(6)	0.4200(4)
C(30)	0.0601(16)	0.2861(8)	0.4212(6)
O(1)	1.3060(9)	0.5120(4)	-0.0334(3)
O(5)	1.0845(10)	0.3841(3)	-0.1251(3)
O(6)	0.7396(10)	0.3298(3)	-0.0864(3)
O(22)	0.6556(10)	0.2956(3)	0.3271(3)
O(24)	0.5401(12)	0.1442(3)	0.2986(3)
O(26)	0.3676(10)	0.2506(4)	0.3725(3)
O(29)	0.3989(12)	0.3098(4)	0.4599(4)
H(2)	1.353	0.570	-0.143
H(3)	1.168	0.539	-0.230
H(4a)	0.929	0.437	-0.221
H(4b)	0.821	0.516	-0.173
H(6)	0.679	0.384	-0.166
H(8)	0.606	0.455	0.010
H(9)	1.045	0.395	-0.012
H(11a)	1.159	0.487	0.052
H(11b)	0.911	0.517	0.062
H(12a)	1.076	0.378	0.105
H(12b)	1.017	0.448	0.152
H(14)	0.814	0.327	0.040
H(15a)	0.475	0.283	0.036
H(15b)	0.366	0.360	0.059
H(16a)	0.501	0.274	0.141
H(16b)	0.384	0.324	0.164
H(17)	0.851	0.297	0.157
H(18a)	0.471	0.439	0.142
H(18b)	0.654	0.477	0.178
H(18c)	0.642	0.499	0.108
H(19a)	0.837	0.576	-0.105
H(19b)	0.671	0.527	-0.057
H(19c)	0.846	0.576	-0.026
H(20)	0.609	0.384	0.241
H(21a)	0.975	0.420	0.230
H(21b)	0.929	0.384	0.299
H(21c)	1.042	0.334	0.241
H(22)	0.487	0.270	0.257
H(23a)	0.946	0.230	0.277
H(23b)	0.779	0.211	0.206
H(26)	0.671	0.267	0.409
H(27a)	0.633	0.074	0.397
H(27b)	0.787	0.139	0.431
H(27c)	0.479	0.139	0.431
H(28a)	0.904	0.077	0.245
H(28b)	1.071	0.096	0.307
H(28c)	0.808	0.075	0.326

and subsequent crystallographic calculations were performed using the National Research Council (Ottawa) programs of Ahmed, Hall, Pippy, and Saunders. Atomic scattering factors were taken from ref. 16.

*Crystal data.*  $C_{30}H_{42}O_7$ ,  $M = 514.666$ . Orthorhombic  $a = 6.580 \pm 0.003$ ,  $b = 19.493 \pm 0.005$ ,  $c = 21.763 \pm 0.004$  Å,  $U = 2791.4$  Å<sup>3</sup>,  $Z = 4$ ,  $D_c = 1.23$  g cm<sup>-3</sup>,  $F(000) = 1112$ . Space group  $P2_12_12_1$  uniquely from systematic absences, Cu- $K_\alpha$  radiation,  $\lambda = 1.54178$  Å;  $\mu(\text{Cu-}K_\alpha) = 7.05$  cm<sup>-1</sup>.

The structure was determined by direct methods by use of the MULTAN program.<sup>17</sup> This program had to be run a few times, during which the choice of starting reflections

TABLE 3

Fractional atomic co-ordinates for Nic-11 26-ethyl ether, with standard deviations in parentheses

Atom	$x$	$y$	$z$
C(1)	1.2615(6)	-0.0950(3)	-0.0306(2)
C(2)	1.2776(6)	-0.1666(3)	-0.0733(2)
C(3)	1.1484(7)	-0.2120(3)	-0.0899(2)
C(4)	0.9774(6)	-0.1959(3)	-0.0646(2)
C(5)	0.9308(5)	-0.1367(3)	-0.0066(2)
C(6)	0.8040(6)	-0.1146(3)	-0.0127(2)
C(7)	0.7581(5)	-0.0315(3)	0.0320(2)
C(8)	0.8830(5)	0.0357(3)	0.0333(2)
C(9)	1.0651(5)	0.0031(2)	0.0343(2)
C(10)	1.0865(5)	-0.0600(3)	-0.0221(2)
C(11)	1.1879(5)	0.0743(3)	0.0366(2)
C(12)	1.1519(6)	0.1327(3)	0.0933(2)
C(13)	0.9726(5)	0.1660(3)	0.0912(2)
C(14)	0.8544(5)	0.0941(2)	0.0904(2)
C(15)	0.6800(6)	0.1342(3)	0.0961(2)
C(16)	0.7115(6)	0.2146(3)	0.1328(2)
C(17)	0.8993(6)	0.2157(3)	0.1506(2)
C(18)	0.9652(7)	0.2203(3)	0.0280(2)
C(19)	1.0348(6)	-0.0221(3)	-0.0884(2)
C(20)	0.9350(6)	0.1740(3)	0.2188(2)
C(21)	0.7823(7)	0.1446(3)	0.2575(2)
C(22)	1.0471(7)	0.2256(3)	0.2633(2)
C(23)	1.1741(7)	0.2708(3)	0.2234(2)
C(24)	1.0840(7)	0.3349(3)	0.3347(2)
C(25)	1.0001(8)	0.3974(3)	0.2312(2)
C(26)	0.8799(7)	0.3499(3)	0.2736(2)
C(27)	0.9135(9)	0.4658(3)	0.1946(3)
C(28)	1.1986(9)	0.3750(4)	0.1347(3)
C(29)	0.6486(10)	0.3876(5)	0.3413(4)
C(30)	0.5290(11)	0.4408(7)	0.3034(6)
O(1)	1.3841(5)	-0.0669(3)	-0.0057(2)
O(5)	1.0601(4)	-0.1829(2)	0.0443(1)
O(6)	0.7842(4)	-0.0942(2)	0.0820(1)
O(17)	0.9448(5)	0.3005(2)	0.1487(1)
O(22)	0.9604(5)	0.2824(2)	0.3056(1)
O(25)	1.1284(5)	0.4339(2)	0.2711(1)
O(26)	0.8256(5)	0.4005(2)	0.3241(1)
H(2)	1.388(8)	-0.196(4)	-0.089(3)
H(3)	1.164(6)	-0.264(3)	-0.113(2)
H(4a)	0.936(6)	-0.245(3)	-0.046(2)
H(4b)	0.906(6)	-0.164(3)	-0.108(2)
H(6)	0.712(6)	-0.154(3)	-0.007(2)
H(7)	0.644(7)	-0.010(3)	0.026(2)
H(8)	0.865(6)	0.068(3)	-0.007(2)
H(9)	1.074(4)	-0.028(2)	0.080(2)
H(11a)	1.193(6)	0.106(3)	-0.003(2)
H(11b)	1.305(6)	0.045(3)	0.043(2)
H(12a)	1.172(5)	0.099(2)	0.141(2)
H(12b)	1.221(6)	0.190(3)	0.098(3)
H(14)	0.879(5)	0.061(2)	0.127(2)
H(15a)	0.607(8)	0.099(4)	0.114(3)
H(15b)	0.635(7)	0.144(3)	0.048(2)
H(16a)	0.707(6)	0.269(3)	0.107(2)
H(16b)	0.645(7)	0.219(3)	0.171(3)
H(18a)	1.002(6)	0.274(3)	0.038(2)
H(18b)	0.979(6)	0.200(3)	-0.010(2)

TABLE 3 (Continued)

Atom	$x$	$y$	$z$
H(18c)	0.828(11)	0.233(5)	0.024(4)
H(19a)	1.067(6)	-0.056(3)	-0.130(2)
H(19b)	1.096(7)	0.042(3)	-0.094(3)
H(19c)	0.929(10)	-0.009(4)	-0.091(3)
H(20)	1.010(6)	0.120(3)	0.215(2)
H(21a)	0.723(7)	0.194(3)	0.260(3)
H(21b)	0.819(6)	0.115(3)	0.296(2)
H(21c)	0.721(6)	0.107(3)	0.227(3)
H(22)	1.088(5)	0.186(2)	0.292(2)
H(23a)	1.259(7)	0.301(3)	0.247(2)
H(23b)	1.229(8)	0.236(4)	0.201(3)
H(26)	0.775(7)	0.333(3)	0.249(3)
H(27a)	0.845(7)	0.443(4)	0.159(3)
H(27b)	0.891(10)	0.505(4)	0.233(3)
H(27c)	1.011(7)	0.501(3)	0.173(3)
H(28a)	1.246(10)	0.334(5)	0.103(4)
H(28b)	1.114(7)	0.403(3)	0.094(3)
H(28c)	1.255(8)	0.417(4)	0.160(3)
H(29a)	0.623(9)	0.325(4)	0.309(3)
H(29b)	0.634(14)	0.418(6)	0.405(5)
H(30a)	0.465(6)	0.400(3)	0.332(3)
H(30b)	0.542(18)	0.430(8)	0.242(8)
H(30c)	0.536(11)	0.498(5)	0.316(4)
H(05)	1.024(6)	-0.161(3)	0.083(2)
H(025)	1.143(16)	0.481(7)	0.303(5)

was altered manually, before it yielded a set of phases with a figure-of-merit 1.1187. A subsequent  $E$  map based on these phases showed 37 large peaks, 34 of which corresponded to chemically reasonable atomic positions.

A structure-factor calculation following one cycle of block-diagonal least-squares refinement gave  $R$  0.26. A subsequent difference-Fourier synthesis revealed the positions of the remaining 3 atoms. Two further cycles of least-squares refinement of atomic positions and isotropic temperature factors, using all data and unit weights, reduced  $R$  to 0.16. Analysis of the agreement of  $F_o$  and  $F_c$  suggested the adoption of a weighting scheme of the form  $w = 1$  for  $|F_o| < 45.0$  and  $w = (45.0/|F_o|)^2$  for  $|F_o| > 45.0$ . Three further cycles of refinement in which the atomic temperature factors were allowed to vary anisotropically reduced  $R$  to 0.106. A difference-Fourier synthesis was next calculated which revealed the approximate positions of all 42 hydrogen atoms. The hydrogen atoms were then included in the structure-factor calculations but their positions were not refined. Two further cycles of refinement lowered  $R$  to 0.073 when the largest parameter shifts were  $< 1.0\sigma$ , indicating that refinement had converged after a total of 8 cycles. A final difference map showed no fluctuations  $> 0.4$  eÅ<sup>-3</sup>. Final atomic coordinates are listed in Table 2; temperature factors and observed and calculated structure factors for (2) and (7) are listed in Supplementary Publication No. SUP 21580 (38 pp., 1 microfiche).\*

*Crystallographic Analysis of Nic-11 26-Ethyl Ether (7).*—For intensity measurement a crystal  $ca. 0.4 \times 0.55 \times 0.65$  mm<sup>3</sup> was mounted about the  $a$  axis on a Hilger and Watts four-circle diffractometer. The experimental procedures described for Nic-3 acetate were adopted. Intensity data were collected as before for  $2\theta \leq 156^\circ$  and 2140 independent observed reflections, having  $I > 3\sigma(I)$  were used

\* See Notice to Authors No. 7, in *J.C.S. Perkin I*, 1974, Index issue.

<sup>16</sup> 'International Tables for X-Ray Crystallography,' vol. III, Kynoch Press, Birmingham, 1962.

<sup>17</sup> G. Germain, P. Main, and M. M. Woolfson, *Acta Cryst.*, 1971, **A27**, 360.



in the structure refinement. No absorption corrections were made. Data reduction and subsequent crystallographic calculations were performed using the 'X-ray 70' system of programs. Normalised structure factors were calculated using the SAP programs of National Research Council (Ottawa) with a manually plotted  $k(s)$  curve. Atomic scattering factors were taken from ref. 16.

*Crystal data.*  $C_{30}H_{44}O_7$ ,  $M = 516.682$ . Orthorhombic,  $a = 8.043 \pm 0.003$ ,  $b = 16.339 \pm 0.007$ ,  $c = 20.435 \pm 0.005$  Å,  $U = 2685.5$  Å<sup>3</sup>,  $Z = 4$ ,  $D_c = 1.28$  g cm<sup>-3</sup>,  $F(000) = 1120$ . Space group  $P2_12_12_1$  uniquely from systematic absences. Cu- $K_\alpha$  radiation,  $\mu(\text{Cu-}K_\alpha) = 7.32$  cm<sup>-1</sup>.

The structure was again determined by the MULTAN program,<sup>17</sup> which was used as before until a set of phases with a figure-of-merit 0.9677 was obtained. A subsequent  $E$  map based on these phases revealed the positions of 32 chemically reasonable atomic positions within the top 38 peaks on the map.

A structure-factor calculation following two cycles of least-squares refinement gave  $R$  0.30 and a subsequent Fourier synthesis revealed five additional atom positions and that two of the originally chosen atom positions were incorrect. Four cycles of block-diagonal least-squares refinement of these 35 atomic positions and isotropic temperature factors were carried out with all the data and unit weights. After the fourth cycle  $R$  was 0.176, and in

subsequent refinement the atomic temperature factors were allowed to vary anisotropically. Two further cycles reduced  $R$  to 0.143. A weighting scheme of the form  $w = 1$  for  $|F_o| \leq 12.0$  and  $w = (12.0/|F_o|)^2$  for  $|F_o| > 12.0$  was then adopted. Two more rounds then reduced  $R$  to 0.129. A difference-Fourier synthesis was then calculated which revealed the positions of the two carbon atoms of the  $O$ -ethyl derivative which had been formed during crystallisation from ethanol. With these extra atoms included, four further cycles of least-squares refinement lowered  $R$  to 0.095. A second difference-Fourier synthesis was then calculated which revealed the approximate positions of all 44 hydrogen atoms. The hydrogen atoms were then included in the subsequent refinement with isotropic temperature factors and four further cycles reduced  $R$  to 0.064. Finally, four more cycles of full-matrix least-squares refinement lowered  $R$  to 0.059 after a total of 22 cycles, the largest parameter shifts being then of the order  $0.3\sigma$ , indicating that the refinement had converged. A final difference map showed no peaks or depressions  $> 0.2$  eÅ<sup>-3</sup>. Final atomic co-ordinates are listed in Table 3.

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