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Oleasides; Novel Cardenolides with an Unusual Framework in *Nerium* (*Nerium* 10)[1]

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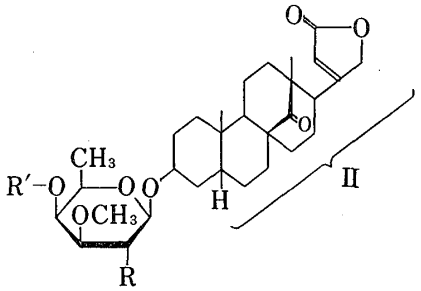
During the course of re-investigation of the cardiac glycosides of *Nerium*, new cardenolides with a novel aglycone moiety were isolated and named oleasides A—F. The structure of the aglycone, oleagenin, was established as 3β-hydroxy-15(14→8)abeo-5β-(8*R*)-14-oxo-card-20(22)-enolide, and the sugar moieties were identified as D-diginose (oleaside A), D-digitalose (-B), β-D-glucosyl-D-diginose (-C), 4-O-β-D-glucosyl-D-digitalose (-D), β-gentiobiosyl-D-diginose (-E) and 4-O-β-gentiobiosyl-D-digitalose (-F).

Keywords——*Nerium*; Apocynaceae; cardiac glycosides; 15(14→8)abeo-cardenolide; bicyclo[3,3,1]nonan-9-one system; Wagner-Meerwein rearrangement; 14-anhydro-Δ^8-cardenolides; 8,14-epoxy-cardenolides; 21-nor-Δ^8-pregnene; cardenolides

Cardenolides in the leaves of *Nerium oleander* L. have been investigated by many workers, and oleandrin, adynerin, neriantin, odoroside A, odoroside H,[3] and their glucosides have been identified.[3a,4] In the early studies of this series, we reported the isolation of oleandrin along with adynerin, odoroside A[5] and Δ^{16}-adynerin[6] (major monosides in the oven-dried leaves) as well as glucosyl-nerigoside and gentiobiosyl-oleandrin[7] from the air-dried leaves of *N. odorum* SOL. (=*N. indicum* MILL.). During the course of chemical identification studies of *N. oleander* and *N. odorum*, re-examination of the cardenolides of the oven-dried leaves of the latter species was conducted, and minor new cardenolides including digitoxigenin α-L-oleandroside, 5α-adynerin,[1] neriaside[8] and oleaside A were isolated. On the other hand, oleaside



I-a: R=H, R′=H I-b: R=OH, R′=H
I-c: R=H, R′=β-D-glucosyl I-d: R=OH, R′=β-D-glucosyl
I-e: R=H, R′=β-gentiobiosyl I-f: R=OH, R′=β-gentiobiosyl

Chart 1

1) *Nerium* 9: F. Abe and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **26**, 3023 (1978).
2) Location: *Nanakuma 11, Nishi-ku, Fukuoka*; *a*) To whom all inquiries should be addressed.
3) W. Neumann, *Chem. Ber.*, **70**, 1547 (1937); R. Tschesche, *Chem. Ber.*, **70**, 1554 (1937); N.W. Cardwell and S. Smith, *J. Chem. Soc.*, **1954**, 2012; T. Tschesche and G. Snatzke, *Chem. Ber.*, **88**, 511 (1955); L. Fauconnet and P.L. Pouly, *Pharm. Act. Helv.*, **37**, 301 (1962); *a*) R. Tschesche, P.K. Chaudhri, and G. Snatzke, *Naturwissenschaften*, **51**, 139 (1964); *b*) P.S. Janiak, E. Weiss, J.V. Euw, and T. Reichstein, *Helv. Chim. Acta*, **46**, 374 (1963).
4) B. Görlich, *Planta Medica*, **9**, 442 (1954).
5) T. Yamauchi and Y. Ehara, *Yakugaku Zasshi*, **92**, 155 (1972).
6) T. Yamauchi, T. Mōri, and Y. Ogata, *Phytochemistry*, **12**, 2737 (1973).
7) T. Yamauchi, N. Takata, and T. Mimura, *Phytochemistry*, **14**, 1379 (1975).
8) T. Yamauchi and F. Abe, *Tetrahedron Lett.*, **1977**, 175.

A was found as one of the major cardenolides in *N. oleander*, together with glycosides having the same aglycone as oleaside A; they were named oleasides B, -C, -D, -E and -F, in order of increasing polarity. This paper deals with the isolation and structure elucidation of oleasides A—F.

Oleaside A (mp 251—255°, $[\alpha]_D$ +25.4°)(I-a) was obtained as one of the major cardenolides along with a small amount of oleaside B (mp 267—277°, $[\alpha]_D$ +45.2°)(I-b), and many other known cardenolides in the monosides fraction of the leaves of *N. oleander* dried at 80°. Four more polar cardenolides, oleasides C (I-c), -D(I-d), -E(I-e) and -F(I-f), which gave the same characteristic blue staining with $SbCl_3$ or H_2SO_4 on thin-layer chromatography (TLC) as I-a or I-b, were detected in the air-dried leaves, I-e being the predominant component. The same compounds were also obtained from the trunk bark and root of *N. oleander*.

In order to establish the relationships among I-a—I-f, the carbon nuclear magnetic resonance (CMR) spectra and enzymatic hydrolysis were investigated. In the CMR spectra of I-a, I-b, I-c, I-d and I-e, the peaks due to the aglycone are observed at the same chemical shifts, and the resonances of the sugar moieties are superimposed: the diginoside in I-a, the digitaloside in I-b, the neribioside (=β-D-glucosyl-D-diginoside) in I-c, the odorobioside (=4-O-β-D-glucosyl-D-digitaloside) in I-d and the neritrioside (=β-gentiobiosyl-D-diginoside) in I-e. I-f was hydrolyzed to I-d with the aid of a snail enzyme. Hence, I-a, I-c, and I-e can be classified in the neritrioside group, while I-b, I-d and probably I-f can be classified in the odorotrioside group, like other already known digitoxigenin or uzarigenin glycosides of this plant.[7,9,10]

The aglycone, oleagenin (mp 290—296°, $[\alpha]_D$ +58.7°) (II) was obtained by the acid hydrolysis of I-a. The CMR spectrum of II exhibits the same pattern as the aglycone moiety in I-a—I-e, except for C-2, C-3 and C-4, indicating that II retains the original structure as in the glycosides. II has only one hydroxyl group at C-3, to which the sugar moieties are attached, and one carbonyl carbon represented by the peak at 221.2 ppm, instead of the 14β-hydroxyl usually observed at 85 ppm.[11] Since II gave a negative circular dichroism (CD) curve at 309 nm, the carbonyl group appears to be located at C-1, C-6 or in the D-ring, if II is a normal steroid. On the other hand, the chemical shifts of C-1—C-6 are identical with those of digitoxigenin, the carbonyl carbon being assigned to the C- or D-ring. On the reaction of II-acetate (III) with SeO_2, however, no olefinic linkage conjugated to the carbonyl was formed, and C-15 was also ruled out by direct comparison with 3β-acetoxy-15-oxo-14β-card-20(22)-enolide.[12] Owing to the failure of the carbonyl group to react with $NaBH_4$, an attempt to reduce III to the corresponding hydroxyl derivative was unsuccessful. III, therefore, is assumed to be a cardenolide with an unusual framework.

Leaves of *N. oleander*, which contain a large amount of I-a (0.089%) have scarcely any adynerin (0.003%). However, adynerin is one of the major cardenolides in the form of *N. odorum* with double, pink-colored flowers usually planted in Japan (0.035%), while I-a is minor (0.005%). Based on this evidence, a close biogenetic relationship has been suggested between I-a and adynerin, and in fact, adynerigenin acetate (IV) was transformed chemically to III in a yield of 15% when IV was treated with BF_3-etherate; a similar reaction occurs in some epoxysteroids.[13] III, therefore, appeared to have a carbonyl group at C-8 or C-14,

9) S. Rangaswami and T. Reichstein, *Pharm. Act. Helv.*, **24**, 159 (1949); *Helv. Chim. Acta*, **32**, 939 (1949); A. Rheiner, A. Hunger, and T. Reichstein, *Helv. Chim. Acta*, **35**, 687 (1952); W. Rittel, A. Hunger, and T. Reichstein, *Helv. Chim. Acta*, **36**, 434 (1953); W. Rittel, and T. Reichstein, *Helv. Chim. Acta*, **36**, 554 (1953); *idem, ibid.*, **37**, 1361 (1954).

10) T. Yamauchi, M. Takahashi, and F. Abe, *Phytochemistry*, **15**, 1275 (1976).

11) K. Tori, S. Seo, Y. Yoshimura, H. Arita, and Y. Tomita, *Tetrahedron Lett.*, **1977**, 179.

12) H. Ishii, T. Tozyo, and D. Sato, *Chem. Pharm. Bull.* (Tokyo), **11**, 576 (1963).

13) B. Kirk and B. Petrow, *J. Chem. Soc.*, **1960**, 4657; J. P. Dusza, J.P. Joseph and S. Bernstein, *J. Org. Chem.*, **28**, 29 (1963); T. Masamune, "International Rev. of Science," Org. Chem. (Series 2), Vol. 8 (Steroids), ed. by W. F. Johns, Butter Worths & Co., Ltd. London, 1976, p. 237.

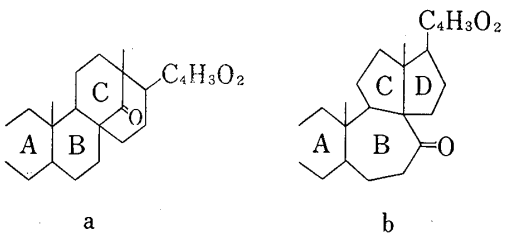


Chart 2

and structure a or b seems most likely on the basis of reaction mechanism and a consideration of structure models, since the epoxide ring cleavage is accompanied by a 1,2-shift of the carbon-carbon bond into the carbonium center.

Prior to reduction of the carbonyl group, the butenolide ring in III was cleaved with O_3 to afford the corresponding 3β-acetoxy-etianic acid (V), which was then treated with CH_2N_2 to yield the methyl ester (VI). $LiAlH_4$ reduction of VI afforded two triols (VII: minor, solid, VII-acetate (IX): mp 174—181°; VIII: major, mp 198—201°). In VIII, a newly formed methine bearing a hydroxyl function is observed at δ 3.53 as a singlet, which is transformed to a doublet (δ 3.18, J=4 Hz) coupled with the hydroxyl proton in the proton magnetic resonance (PMR) spectrum of VIII-diacetate (XI). These PMR data strongly support structure a, in which both carbons adjacent to the carbonyl group are quaternary.

$LiBH_4$ reduction of V furnished an amorphous dihydroxy-carboxylic acid (XII), which, without further purification, was converted into a less polar compound (XIII) on reflux with

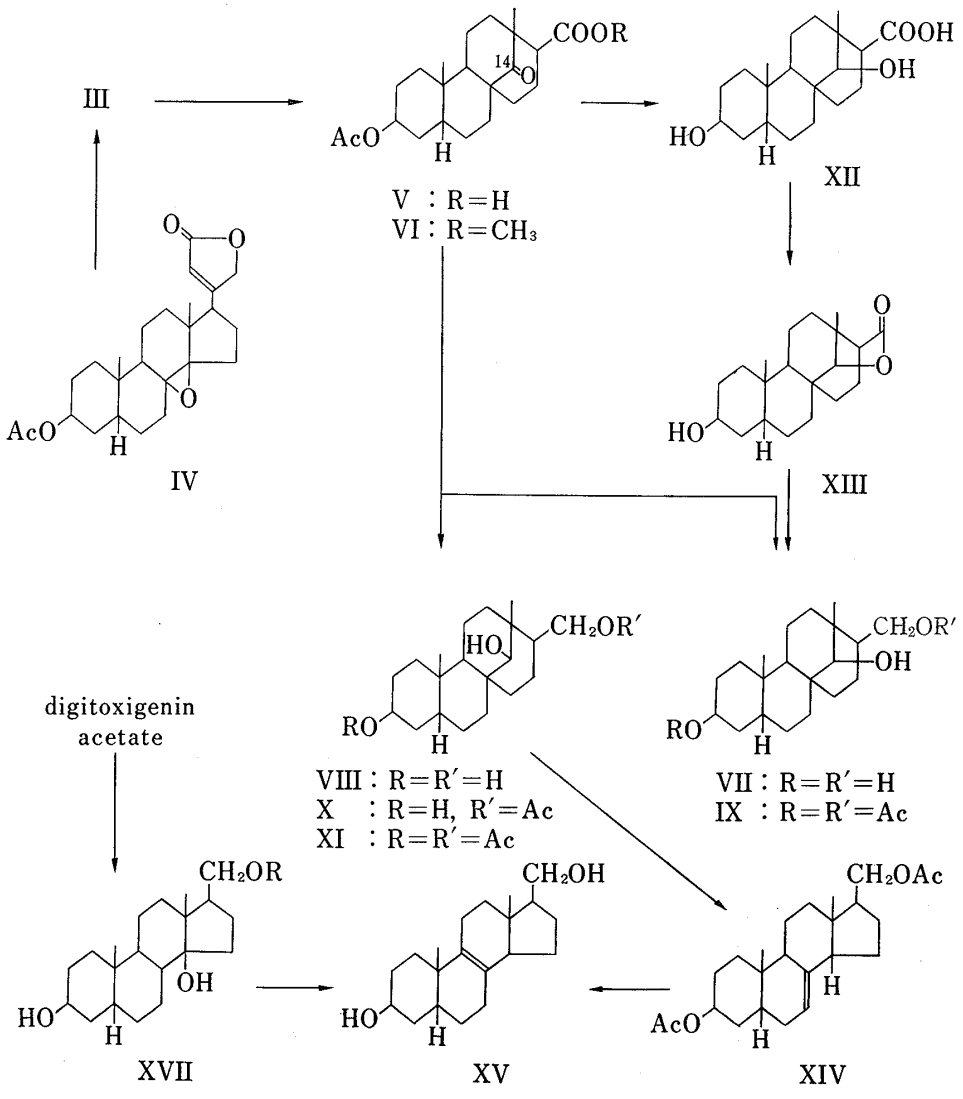


Chart 3

5% hydrochloric acid in 50% MeOH. Five-membered lactone ring formation in XIII was confirmed by the absorption band at 1765 cm^{-1} in the infrared (IR) spectrum, the molecular peak in the mass spectrum (MS) (*m/e*: 318.2187, M^+: $C_{20}H_{30}O_3$), and deshielding of the C-14 methine proton from δ 3.76 in IX to 4.70. Since XIII was converted into VII by $LiAlH_4$ reduction, the configuration of the hydroxyl group formed from the carbonyl in VII or XII is considered to be *cis* with respect to the C-17 side chain, and *trans* in VIII.

The acetate XI afforded, on reaction with MsCl/pyridine, an anhydrocompound (XIV) which showed one olefinic proton as a doublet (J=5 Hz) at 5.32. XIV was then isomerized by heating with HCOOH at 70° according to the procedure of Janiak *et al.*,[3b] followed by hydrolysis to give a compound having a tetrasubstituted olefinic linkage (XV). By direct comparison with an authentic sample,[14] prepared from digitoxigenin acetate *via* 21-nor-5β,14β-pregnane-3β,14,20-triol, XV was identified as 21-nor-5β,14β-pregn-8-ene-3β,20-diol, and therefore, XIV was considered to be a compound with an olefinic linkage at either C-7 or C-9 (11). Taking into account the Wagner-Meerwein rearrangement in the conversion of XI to XIV, a 15 (14→8) abeo type structure for XI appears likely; this can furnish the 14β-Δ^7-compound. XIV can then be assigned as 21-nor-5β,14β-pregn-7-ene-3β,20-diol diacetate. Chemical shifts of the C-18 and C-19 methyl protons in XV and XIV, respectively, are also in good agreement with those of the corresponding Δ^8- and Δ^7-compounds, calculated from the "substitution effect" of Bhacca *et al.*[15,16]

The sugar attached to I-a can be tentatively regarded as D-diginose, since no enantiomer of diginose has been found in the genus *Nerium*. The stereochemistry at C-8 can be assigned as *R*, based on the reaction mechanism from IV to III. The linkage of D-diginose to C-3 hydroxyl was determined to be β from the dd-line of the anomeric proton in diginose. Consequently, the structure of I-a is established as the β-D-diginoside of 3β-hydroxy-15(14→8) abeo-5β-(8*R*)-14-oxo-card-20(22)-enolide.

It is noteworthy that oleasides are the first naturally occurring cardenolides having a bicyclo[3,3,1]nonan-9-one C/D ring system. As regards the distribution of oleasides in the genus *Nerium*, the presence of two types, that is, adynerin and oleaside producers, seems likely.

Experimental

Extraction and Isolation of Oleasides A (I-a) and B (I-b)——Fresh leaves of *N. oleander* (cultivation No.: N-16) were harvested in July, 1977, and dried in an oven at 80°. The dried leaves (600 g) were powdered and percolated with MeOH. The MeOH percolate was concentrated *in vacuo* and diluted with water to give a 50% aqueous solution. The deposit was removed by filtration and the filtrate was extracted with benzene. The benzene extracts were chromatographed on silica gel (s.g.) and eluted with a benzene–acetone system (10:1—3:1). The fraction between Δ^{16}-adynerin and oleandrin was crystallized from MeOH or EtOAc/hexane to give prisms (I-a, 0.089%). The fraction following nerigoside was crystallized from EtOAc to give prisms (I-b, 0.003%). Oleandrin, adynerin, Δ^{16}-adynerin and nerigoside were isolated in yields of 0.067%, 0.005%, 0.020% and 0.01%, respectively, and odoroside A and Δ^{16}-nerigoside were also detected on TLC.

14) The configuration at C-14 was assigned on the basis of the following evidence; digitoxigenin was converted into the 14-anhydro-Δ^8 derivative (mp 145—147°, $[\alpha]_D$ +108.0°) and a minor amount of α-anhydrodigitoxigenin ($\Delta^{8(14)}$) by heating with HCOOH at 70°.[3b] On the other hand, another Δ^8-isomer (mp 205—208°, $[\alpha]_D$ −2.1°), along with α-anhydrodigitoxigenin, was obtained by catalytic hydrogenation over PdO in EtOH of the $\Delta^{8,14}$-diene compound, prepared from adynerigenin by treatment with acid. The chemical shifts of the angular methyl protons in the former Δ^8-compound (δ 0.79 and 1.05) coincided with the calculated values[15] for 3β-hydroxy-5β,14β-carda-8,20(22)-dienolide (C-18: 0.82, C-19: 1.07) and those in the latter (0.60 and 1.08) with the values for the 14α-isomer (C-18: 0.55, C-19: 1.10).

15) N.S. Bhacca and D.H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, 1964.

16) The shift values of the C-19 and C-18 protons with 17β-CH_2OH were given as −0.028 and −0.022 in the 14β-series, and +0.006 and −0.17 in the 14α-series, based on the differences between 21-nor-5β,14β- and 14α-pregnane-3β,20-diols, and the corresponding 5β,14β- and 14α-androstan-3β-ols, respectively.

I-a was obtained in a yield of less than 0.005% from the oven-dried leaves of *N. odorum* (double, pink flower).

Extraction and Isolation of Oleasides C (I-c), D (I-d), E (I-e) and F (I-f)——Air-dried leaves of *N. oleander* (leaves of several plants were combined; 7 kg) were percolated with MeOH (60 l) then the concentrated MeOH solution (7 l) was diluted with the same volume of water and filtered. The filtrate was extracted with benzene (fraction 1, ext. 11.2 g), $CHCl_3$–EtOH (2: 1) (fr. 2, 89.1 g) and then with 3: 2 (fr. 3, 51 g). Fr. 2 (45 g) was chromatographed on s.g., eluting with $CHCl_3$–MeOH (20: 1—5: 1) to fractionate biosides (*ca.* 10 g) and triosides. The biosides fraction was subjected to droplet counter current chromatography (DCCC) using $CHCl_3$–MeOH–H_2O (5: 10: 6) as a solvent system, by the ascending method. The fractions in which new compounds were detected were successively fractionated by DCCC and finally crystallized from MeOH to give prisms (I-c, 0.0043%; I-d, 0.0006%).

A half of fr. 3 was passed through a polyamide column and the effluent with water was chromatographed on s.g. with $CHCl_3$–MeOH as an eluant. The fractions containing triosides (21.7 g) were combined with those from fr. 2 (total 43 g) and subjected to DCCC with $CHCl_3$–MeOH–H_2O (5: 9: 7, ascending). A major fraction, giving a light blue spot with $SbCl_3$ reagent, was obtained following gentiobiosyloleandrin and was crystallized from MeOH to give needles (I-e, 0.098%). A compound, showing the same color with $SbCl_3$ reagent was also obtained as a solid from a more polar fraction (I-f, 0.0004%).

I-f (10 mg) was dissolved in 30% EtOH and shaken for 10 hr with β-glucosidase prepared from snail digestive juice. The hydrolysate was compared with I-d by TLC (solv. $CHCl_3$–MeOH–H_2O 7: 3: 1, lower layer) and the two spots were found to coincide.

Small amounts of the new glycosides, gentiobiosyl-nerigoside (=oleandrigenin neritrioside) (mp 175—180°, $[\alpha]_D^{20}$ −32.3° (c=0.167, MeOH)) and digitoxigenin neritrioside (solid) were isolated together with known glycosies such as odoroside G, 16-O-acetyl neogitostin, gentiobiosyl-odoroside A, gentiobiosyl-oleandrin and gentiobiosyl-Δ^{16}-adynerin[7,10] from the same source.

Oleasides were also obtained from the air-dried bark (600 g) and the root of the same plant: I-c (bark: 60 mg, root: trace), I-d (50 mg, +), I-e (108 mg, 21 mg) and I-f (20 mg, +).

Physical and Chemical Properties of Oleasides——Oleaside A (I-a): mp 251—255°, $[\alpha]_D^{25}$ +25.4° (c= 0.134, MeOH), λ_{max}^{MeOH} nm (ε): 211 (19000). *Anal.* Calcd. for $C_{30}H_{44}O_7$: C, 69.74; H, 8.58. Found: C, 69.69; H, 8.46. PMR ($CDCl_3$) δ: 0.78 (3H, s), 0.94 (3H, s), 1.24 (3H, d, J=6 Hz, 6′-Me), 2.51 (1H, bs), 2.88 (1H, m), 3.13 (1H, m, C-17), 3.47 (3H, s, 3′-OMe), 3.75 (1H, d, J=3 Hz, C-4′), 4.12 (1H, m, C-3), 4.52 (1H, dd, J=3, 9 Hz, C-1′), 4.67 (2H, bs, C-21), 5.73 (1H, bs, C-22). CMR[17] (pyridine-d_5): 29.4 (C-1), 26.9 (C-2), 73.0 (C-3), 30.6 (C-4), 37.5 (C-5), 26.9 (C-6), 47.5 (C-8), 46.1 (C-9), 37.6 (C-10), 48.9 (C-13), 221.2 (C-14), 26.4 (C-16), 53.1 (C-17), 171.7 (C-20), 73.2 (C-21), 116.4 (C-22), 173.9 (C-23), 44.1, 42.6, 31.5, 24.7, 23.4, 21.4 (C-7, -11, -12, -15, -18, -19), 99.0 (C-1′), 33.1 (C-2′), 79.1 (C-3′), 67.1 (C-4′), 71.4 (C-5′), 17.6 (C-6′), 55.3 (3′-OMe). On acid hydrolysis with 0.05 N H_2SO_4–50%EtOH for 5 min, the aglycone, oleagenin (II), and D-diginose (TLC 7: 3: 1, and BuOH–AcOH–H_2O 4: 1: 5) were obtained.

Oleaside B (I-b): mp 267—277°, $[\alpha]_D^{14}$ +45.2° (c=0.42, MeOH), λ_{max}^{MeOH} 207 (19000). *Anal.* Calcd. for $C_{30}H_{44}O_8$: C, 67.64; H, 8.33. Found: C, 67.26; H, 8.03. CMR- 103.7 (C-1′), 71.0 (C-2′), 85.1 (C-3′), 68.7 (C-4′), 71.6 (C-5′), 17.0 (C-6′), 57.3 (3′-OMe); the peaks due to the aglycone moiety in I-b, I-c, I-d and I-e gave the same principal chemical shifts as those of I-a.

Oleaside C (I-c): mp 198—205°, $[\alpha]_D^{15}$ +13.9° (c=0.50, MeOH), λ_{max}^{MeOH} 208 (18300). *Anal.* Calcd. for $C_{36}H_{54}O_{12}\cdot H_2O$: C, 62.05; H, 8.10. Found: C, 62.69; H, 8.13. CMR: 98.7 (C-1′), 33.2 (C-2′), 80.1 (C-3′), 74.3 (C-4′), 70.8 (C-5′), 17.9 (C-6′), 56.2 (3′-OMe), 105.0 (C-1″), 76.0 (C-2″), 78.4 (C-3″), 71.9 (C-4″), 78.4 (C-5″), 63.2 (C-6″).

Oleaside D (I-d): mp 232—235°, $[\alpha]_D^{15}$ +37.0° (c=0.24, MeOH), λ_{max}^{MeOH} 207 (14300). *Anal.* Calcd. for $C_{36}H_{54}O_{13}\cdot H_2O$: C, 60.66; H, 7.91. Found: C, 60.85; H, 7.80. CMR: 103.1 (C-1′), 70.3 (C-2′), 85.4 (C-3′), 75.5 (C-4′), 71.3 (C-5′), 17.9 (C-6′), 58.7 (3′-OMe), 105.0 (C-1″), 75.5 (C-2″), 78.2 (C-3″), 71.5 (C-4″), 78.2 (C-5″), 62.6 (C-6″).

Oleaside E (I-e): mp 182—184°, $[\alpha]_D^{25}$ +16.0° (c=0.10, MeOH), λ_{max}^{MeOH} 208 (21600). *Anal.* Calcd. for $C_{42}H_{64}O_{17}\cdot 2H_2O$: C, 57.51; H, 7.82. Found: C, 57.27; H, 8.05. CMR: 98.6 (C-1′), 33.2 (C-2′), 79.9 (C-3′), 73.4 (C-4′), 70.7 (C-5′), 18.0 (C-6′), 56.2 (3′-OMe), 105.3 (C-1″), 74.9 (C-2″), 78.1 (C-3″), 71.5 (C-4″), 77.4 (C-5″), 70.5 (C-6″), 104.6 (C-1‴), 75.5 (C-2‴), 78.1 (C-3‴), 71.5 (C-4‴), 78.1 (C-5‴), 62.6 (C-6‴). On acid hydrolysis with 1% HOAc for 1 hr at 100°, oleagenin (II) and neritriose were detected. Sugar: R_{Glc}, 0.63 (authentic oleandrotriose (=β-gentiobiosyl-L-oleandrose) 0.80, neritriose 0.64, Solv. 4: 1: 5), GLC: t_R of the triose acetate (acetylated with Ac_2O/pyridine): 27.5 min (authentic neritriose acetate 27.5 min, oleandrotriose acetate 25.6 min, column 2.1 m, SE-52, 248°, N_2: 1.4 kg/cm²).

Oleagenin (II)——The aglycone of oleasides was crystallized from hexane/EtOAc to give prisms; mp 290—296°, $[\alpha]_D^{20}$ +58.7° (c=0.13, MeOH), λ_{max}^{MeOH} 207 (20900). MS m/e: Calcd. for $C_{23}H_{32}O_4$: 372.2300. Found: 372.2269. CD (c=0.060, dioxane) $[\theta]^{20}$ (nm) −3300 (309).

17) CMR was run on a Hitachi R-22 Ft unit operating at 22.63 MHz using the procedure described in the preceding papers.[1,18]

18) T. Yamauchi, F. Abe, and M. Nishi, *Chem. Pharm. Bull.* (Tokyo), **26**, 2894 (1978).

Oleagenin Acetate (III): mp 238—241°, $[\alpha]_D^{20}$ +60.3° (c=0.13, $CHCl_3$). PMR: 0.79 (3H, s), 0.92 (3H, s), 2.02 (3H, s, –OAc), 2.30—2.60 (2H), 2.83 (1H, m), 2.60—3.00 (1H), 3.12 (1H, m), 4.63 (2H, C-21), 5.09 (1H, bs, C-3), 5.70 (1H, C-22). On heating II in EtOH or iso-PrOH with excess SeO_2 for 9 hr, only II was recovered.

Oleagenin Acetate (III) from Adynerigenin Acetate (IV)——IV (60 mg) was dissolved in ether (20 ml) and the solution was stirred with BF_3–etherate. After stirring for 2 hr, the solution was washed with water, dried over Na_2SO_4 and the ether was evaporated off. The residue was subjected to s.g. chromatography to give a homogeneous fraction. On crystallization from hexane/EtOAc, prisms were obtained (10 mg), mp 237—240°. On direct comparison with authentic III, the properties of the two samples were in good agreement.

Degradation of III with O_3——III (200 mg) was dissolved in EtOAc (20 ml) and O_3 was introduced into the solution at −40° for 12 hr. After concentration to half volume, Zn and AcOH were added and the mixture was stirred overnight. After filtration, the filtrate was washed with water and EtOAc was evaporated off *in vacuo*. The residue was dissolved in MeOH and saponified with $KHCO_3$. The product was treated with 150 mg of HIO_4 in 16 ml of dil.EtOH for 24 hr, then the mixture was acidified with H_2SO_4, diluted with water and extracted with $CHCl_3$. The $CHCl_3$ ext. was crystallized from MeOH to give needles (V), mp 227—237°. V was dissolved in ether and methylated with CH_2N_2 in ether. After purification on an s.g. column, followed by crystallization from Et_2O/hexane, prisms (VI, 63 mg) was obtained, mp 141—144°, MS *m/e*: Calcd. for $C_{23}H_{34}O_5$: 390.2406. Found: 390.2379. PMR: 0.83 (3H, s), 0.94 (3H, s), 2.03 (3H, s, –OAc), 2.72 (1H, m), 3.00 (1H, dd, J=2, 6 Hz), 3.62 (3H, s, –COOMe), 5.07 (1H, C-3).

$LiAlH_4$ Reduction of VI——VI (200 mg) was dissolved in ether and an excess of $LiAlH_4$ was added portionwise. The solution was stirred for 2 hr, then the product was subjected to s.g. column chromatography to give two triols, VII (solid, 11 mg) and VIII (105 mg), of which the latter was crystallized from Et_2O/hexane to give prisms, mp 198—201°. MS *m/e*: 304 (M^+-18: $C_{20}H_{34}O_3$). PMR (pyridine-d_5): 1.25 (3H, s), 1.50 (3H, s), 3.53 (1H, bs, C-14), 3.90 (1H, dd, J=9, 10 Hz), 4.13 (1H, dd, J=4, 10 Hz), 4.36 (1H, m, C-3), 5.80 (1H, bs, –OH).

VIII-Monoacetate (X): VIII was acetylated with Ac_2O/pyridine for 1 hr at room temp. and the monoacetate (X) obtained was crystallized from hexane/EtOAc to give prisms, mp 186—190°, MS *m/e*: 362 (M^+, $C_{22}H_{36}O_4$). PMR: 1.00 (3H, s), 1.10 (3H, s), 2.03 (3H, s, –OAc), 3.17 (1H, s, C-14), 4.05 (1H, m, C-3), 4.06 (1H, dd, J=9, 11 Hz), 4.24 (1H, dd, J=4, 11 Hz).

VIII-Diacetate (XI): VIII was acetylated with Ac_2O/pyridine for 24 hr at room temp. and the diacetate (XI) was crystallized from Et_2O/hexane to give needles, mp 130—132°, MS *m/e*: 406 (M^+, $C_{24}H_{38}O_5$). PMR: 1.00 (3H, s), 1.12 (3H, s), 2.04 (6H, s, 2×–OAc), 3.18 (1H, d, J=4 Hz, C-14), 4.04 (1H, dd, J=9, 11 Hz), 4.24 (1H, dd, J=4, 11 Hz), 5.00 (1H, m, C-3).

VII-Diacetate (IX): IX was obtained by the usual acetylation procedure with Ac_2O/pyridine, mp 174—181° (hexane/EtOAc). PMR: 1.05 (6H, s), 2.03 (6H, s, 2×–OAc), 3.76 (1H, d, J=4 Hz, C-14), 4.24 (2H, d, J=6 Hz, C-20), 5.05 (1H, m, C-3).

XI was dissolved in acetone and stirred with Jones' reagent for 30 min, then the product was crystallized from Et_2O/hexane to give prisms, mp 123—125°, MS *m/e*: 404 (M^+, $C_{24}H_{36}O_5$). PMR: 0.78 (3H, s), 1.00 (3H, s), 1.98 (3H, s, –OAc), 2.02 (3H, s, –OAc), 3.80 (1H, dd, J=8, 12 Hz), 4.04 (1H, dd, J=4, 12 Hz), 5.06 (1H, m, C-3). CD (c=0.048, dioxane) $[\theta]^{20}$ −3500 (305).

$LiBH_4$ Reduction of V——V (100 mg) was dissolved in ether and stirred with an excess of $LiBH_4$ for 24 hr at room temp. The mixture was diluted with ether and washed with water. The ether layer was concentrated to dryness. The residue (XII) was refluxed, without further purification, with 5%HCl–50%MeOH for 30 min. The product was purified on an s.g. column to yield a lactone (XIII, 21 mg), which was crystallized from Et_2O/hexane to give prisms, mp 188—189°. PMR: 1.02 (3H, s), 1.08 (3H, s), 2.24 (1H, bs, C-17), 4.11 (1H, m, C-3), 4.70 (1H, s, C-14). MS: Calcd. for $C_{20}H_{30}O_3$: 318.2195. Found: 318.2187.

$LiAlH_4$ Reduction of XIII——XIII was treated with $LiAlH_4$ in the usual manner and the product was acetylated, followed by crystallization from Et_2O/hexane to give prisms, mp 175—181°. On comparison with a sample (IX) prepared by $LiAlH_4$ reduction of VI, the properties of the two samples were in good agreement.

Reaction of XI with MsCl——XI (40 mg) was dissolved in pyridine and allowed to stand with 0.5 ml of MsCl for 5 hr. The solution was diluted with water, and extracted with $CHCl_3$. The $CHCl_3$ ext. was purified through an s.g. column and the product was crystallized from hexane to give prisms (XIV, 24 mg), mp 122—125°, $[\alpha]_D^{15}$ +39.6° (c=0.943, $CHCl_3$), MS: Calcd. for $C_{24}H_{36}O_4$: 388.2613. Found: 388.2577. PMR: 0.88 (3H, s), 0.92 (3H, s), 2.04 (6H, s, 2×–OAc), 3.90 (1H, dd, J=7, 11 Hz), 4.14 (1H, dd, J=5, 11 Hz), 5.04 (1H, m, C-3), 5.32 (1H, d, J=5 Hz). XIV was also obtained (mp 124—126°) from the reaction of XI with $SOCl_2$ in pyridine.

Isomerization of XIV with HCOOH——XIV (90 mg) was heated at 70° for 1 hr with 4 ml of HCOOH. The solution was diluted with $CHCl_3$ and washed with water. The $CHCl_3$ ext. was then treated overnight with 1% KOH. The product was passed through an s.g. column and crystallized from EtOAc/hexane to give prisms (XV, 17 mg), mp 145—150°, MS: Calcd. for $C_{20}H_{32}O_2$: 304.2402. Found: 304.2407. On comparison with authentic 21-nor-5β,14β-pregn-8-ene-3β,20-diol, prepared from digitoxigenin acetate, the physical properties of samples were in good agreement (GLC: t_R 6.2 min, authentic 8-ene compound 6.2 min; 14-ene

compound 7.1 min; 8(14)-ene compound 7.95 min, 2.1 m QF-1, 200°, N_2: 1 kg/cm^2). The 8(14)-ene derivative (2 mg, mp 200—202°) was obtained from the fraction prior to XV.

Preparation of 21-Nor-5β,14β-pregn-8-ene-3β,20-diol (XV) from Digitoxigenin Acetate——The methyl-ester of 21-nor-5β,14β-pregn-8-ene-3β,14-diol-20-carboxylic acid (XVI, mp 155—158°) was obtained from digitoxigenin acetate by the procedure described for the preparation of V. XVI (500 mg) was subjected to $LiAlH_4$ reduction in ether and 21-nor-5β-pregnane-3β,14,20-triol (XVII, mp 189—197°, 300 mg) was obtained. XVII (200 mg) was heated at 70—75° with HCOOH for 1.5 hr. The mixture was diluted with ice-water and extracted with $CHCl_3$. The $CHCl_3$ ext. was saponified overnight with 1%KOH–MeOH. The product, showing two spots on TLC, was purified on an s.g. column. The less polar spot on TLC was found to be a mixture of the $\Delta^{8(14)}$- and Δ^{14}-derivatives. The fraction giving a polar spot was subjected to crystallization from EtOAc/hexane to give XV as prisms (26 mg), mp 147—149°, $[\alpha]_D^{23}$ +111.6° (c=0.381, $CHCl_3$). PMR: 0.87 (3H, s), 1.04 (3H, s), 3.51 (1H, dd, J=8, 12 Hz, C-20), 3.76 (1H, dd, J=6, 12 Hz, C-20), 3.95 (1H, m, C-3).

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