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Digitoxigenin Oleandroside and 5α-Adynerin in the Leaves of *Nerium odorum* (*Nerium* 9)[1]

FUMIKO ABE[2] and TATSUO YAMAUCHI[2a]

Faculty of Pharmaceutical Sciences, Fukuoka University[2]

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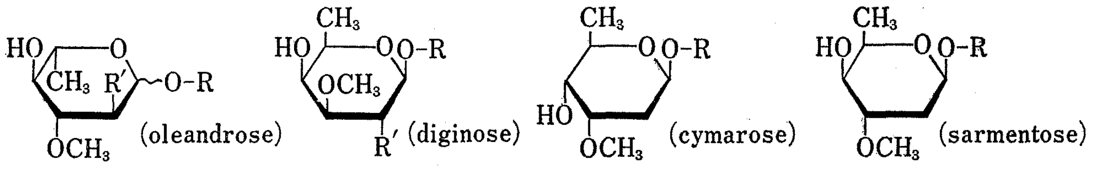
Digitoxigenin α-L-oleandroside and 5α-adynerin were isolated from the oven-dried leaves of *Nerium odorum*, and the structures were elucidated with the aid of ^{13}C nuclear magnetic resonance and finally by the conversion into the glycoside from oleandrin and by the synthesis of 5α-adynerigenin, respectively.

Keywords——CMR of 2,6-deoxy-3-O-methyl-sugars; CMR of 8,14β-epoxy-cardenolides; adynerin; 5α-adynerin; 16-anhydro-cardenolides; α-anhydro-uzarigenin; digitoxigenin oleandroside; 17βH-digitoxigenin oleandroside; *Nerium odorum*; Apocynaceae

Cardenolides of oleander leaves have been investigated by many workers.[3] Previously, we reported on the efficiential preparation of oleandrin from the native glycoside in the leaves, together with the isolation of adynerin, odoroside A,[4a] 16-dehydroadynerin,[4b] and on the isolation of the polar glycosides, including gentiobiosyl-oleandrin, -odoroside A, 16-O-acetyl digitalinum verum, glucosyl nerigoside and oleandrigenin glucoside from the air-dried leaves of *Nerium odorum*.[4c] In this paper, we describe the identification of two new cardenolides, digitoxigenin α-L-oleandroside (I) and 5α-adynerin (II), minor constituents in the leaves dried in the oven.



I: R = digitoxigenin
R′ = H
anomeric OH: α
III: R = oleandrigenin
R′ = H
anomeric OH: α
VIII: R = digitoxigenin
R′ = OH
anomeric OH: β

II: R = 5α-adynerigenin
R′ = H
IV: R = 5β-adynerigenin
R′ = H
VI: R = digitoxigenin
R′ = H
IX: R = digitoxigenin
R′ = OH
XIII: R = uzarigenin
R′ = H

V: R = strophanthidin

VII: R = sarmentogenin

Chart 1

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1) Part VIII: T. Yamauchi and F. Abe, *Tetrahedron Lett.*, **1978**, 1825; Part VII: F. Abe and T. Yamauchi, *Phytochemistry*, **15**, 1745 (1976).

2) Location: *Nanakuma 11, Nishi-ku, Fukuoka*; *a*) To whom all inquiries should be addressed.

3) *a*) W. Neumann, *Chem. Ber.*, **70**, 1547 (1939), R. Tschesche, *Chem. Ber. Ber.*, **70**, 1554 (1937); N.W. Cardwell and S. Smith, *J. Chem. Soc.*, **1954**, 2012; R. Tschesche and G. Snatzke, *Chem. Ber.*, **88**, 511 (1955); R. Tschesche, P.K. Chaudhri, and G. Snatzke, *Naturwissenschaften*, **51**, 139 (1964); M. Ishidate and Z. Tamura, *Yakugaku Zasshi*, **70**, 7 (1950); M. Okada, *Yakugaku Zasshi*, **73**, 86 (1953); T. Takemoto and K. Kometani, *Yakugaku Zasshi*, **74**, 1263 (1954); *b*) P.S. Janiak, E. Weiss, J.V. Euw, and T. Reichstein, *Helv. Chim. Acta*, **46**, 374 (1963); *c*) B. Görlich, *Planta Medica*, **9**, 442 (1961).

4) *a*) T. Yamauchi and Y. Ehara, *Yakugaku Zasshi*, **92**, 155 (1972); *b*) T. Yamauchi, T. Mōri, and Y. Ogata, *Phytochemistry*, **12**, 2737 (1973); *c*) T. Yamauchi, N. Takata, and T. Mimura, *Phytochemistry*, **14**, 1379 (1975).

During the fractionation of the mother liquor portion of oleandrin (III) and adynerin (=β-D-diginoside of 3β-hydroxy-8,14β-epoxy-5β-card-20(22)-enolide, IV), at least four unknown cardenolides were observed on thin-layer chromatography (TLC), and tentatively named Subst. X (I), -C(II), neriaside[1] and oleaside.[5]

TABLE I. ^{13}C Chemical Shifts of 2,6- and 6-Deoxy-3-O-methyl Sugars

Compounds	Carbon No.						
	1′	2′	3′	4′	5′	6′	-OCH_3
III	95.9	35.8	79.3	77.1	69.1	18.6	56.9
III-4′-O-Ac	95.6	35.7	76.2	77.0	66.6	17.9	56.7
V	97.6	36.4	78.7	74.0	71.1	18.9	58.1
VI	98.2	33.1	79.1	67.0	71.2	17.6	55.1
VI′-4-O-Ac	98.1	33.1	77.2	69.5	68.4	17.2	56.3
VII	97.3	33.6	80.3	67.9	69.9	17.5	56.7
VIII	98.9	73.8	84.8	76.6	68.9	18.5	60.6
VIII-2′-O-Ac	94.5	74.8	82.0	76.6	68.8	18.3	60.6
VIII-2′,4′-O-Ac	94.2	74.2	75.6	79.0	66.1	17.6	59.8
IX	103.6	70.9	85.1	68.7	71.0	17.4	57.2
IX-2′-O-Ac	99.9	71.9	82.3	68.1	71.3	17.2	56.5
IX-2′,4′-O-Ac	99.7	71.7	80.2	69.5	69.5	16.7	57.6
I	96.1	35.6	79.4	77.2	69.2	18.5	57.0
II	98.3	33.2	79.2	67.1	71.4	17.7	55.3

-$CO\underline{C}H_3$: 21.3±0.1, -$\underline{C}OCH_3$: 170.3±0.2

TABLE II. Comparison of ^{13}C Chemical Shifts of 14β- Hydroxy- and 8, 14β-Epoxy-cardenolides

Carbon	Compds.						
	IV	VI	$\delta_{(IV-VI)}$	II	XIII	$\delta_{(II-XIII)}$	I
1	31.0$^{a)}$	30.7		37.2	37.3		30.4
2	27.3	27.2		29.6	29.9		27.0$^{c)}$
3	72.9$^{b)}$	73.2		76.2	76.4		72.1
4	30.6$^{a)}$	30.7		34.6	34.8		31.1
5	37.1$^{c)}$	37.1		44.3	44.4		37.2
6	25.9	27.2	−1.3	27.2	29.1	−1.9	27.4$^{c)}$
7	25.1	22.0$^{a)}$	3.1	32.3	27.9	4.4	22.0$^{a)}$
8	65.2	41.9	23.3	64.7	41.6	23.1	42.2
9	36.7$^{c)}$	35.5$^{c)}$	1.2	50.2	49.9	0.3	36.1
10	36.7$^{c)}$	35.8$^{c)}$	0.9	37.2$^{a)}$	36.0	1.2	36.1
11	16.4	21.6$^{a)}$	−5.2	16.4	21.5	−5.1	21.7$^{a)}$
12	37.1$^{c)}$	39.9	−2.8	36.9$^{a)}$	39.7	−2.8	40.2
13	41.8	50.1	−8.3	41.7	49.9	−8.2	50.2
14	70.8	84.7	−13.9	70.6	84.5	−13.9	84.9
15	26.9	33.1	−6.2	25.8	33.1	−7.3	33.3
16	27.3	27.2		27.2	27.2		27.4$^{c)}$
17	51.4	51.5		51.4	51.4		51.6
18	16.4	16.2		16.4	16.1		16.2
19	25.1	24.0	1.1	12.7	12.2	0.5	24.1
20	170.6	175.9	−5.3	170.6	175.8	−5.2	175.9
21	73.5$^{b)}$	73.7		73.5	73.6		73.8
22	116.9	117.7		116.9	117.7		171.8
23	170.6	174.5	−3.9	170.6	174.4	−3.8	175.9

a) or *b*), *c*) in vertical column may be reversed.

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5) F. Abe and T. Yamauchi, "21th Symposium on the Chemistry of Natural Products," at Sapporo, 1978.

I, mp 205—208°, $[\alpha]_D$ −27.7°, was presumed to be a glycoside composed of 2,6-dideoxy-3-O-methyl sugar, according to its polarity on TLC and proton magnetic resonance (PMR) at δ 1.25 (3H, d, $J=6$ Hz) for 6′-Me, and 3.40 (3H, s, -OMe). In order to identify the sugars moiety, ^{13}C nuclear magnetic resonance (CMR) of known 2,6-dideoxy-3-O-methyl sugars, L-oleandrose, D-cymarose, D-diginose and D-sarmentose was investigated as their glycoside; III, cymarin (V), odoroside A (VI) and sarmentocymarin (VII),⁶⁾ respectively (Table I). The signal assignment was based on the comparison of the resonances of each glycoside, its 2′-hydroxyl homolog such as L-thevetoside (as neriifolin, VIII), or D-digitaloside (odoroside H, IX), and its acetates. On the CMR of I, the resonances are superimposable with those of L-oleandrose in the sugar moiety and of digitoxigenin in the aglycone (Table I and II). I, therefore, has been found to be digitoxigenin oleandroside.

The final determination of the structure was conducted by the conversion of III into I. Whereas 16-O-acetyl cardenolides have been known to be derived to Δ^{16}-derivatives by passing through alumina column,⁷⁾ III was transformed into 16-anhydro-oleandrin (X), without any by-products, by heating in dimethylformamide with NaOTs and NaOAc for 30 min. X was subjected to a catalytic hydrogenation to afford I as a minor product, along with a large amount of 17βH-isomer.

II, mp 222—224°, $[\alpha]_D$ −2.0°, was also obtained in a small amount. Its acetate shows no free hydroxyl on infrared (IR). II indicates, on the CMR, the presence of D-diginose in the sugar moiety. 5α-Structure and the presence of the butenolide ring are suggested according to the resonances of C-19 methyl at δ 12.7 ppm⁸⁾ and from the resonances at 170.6

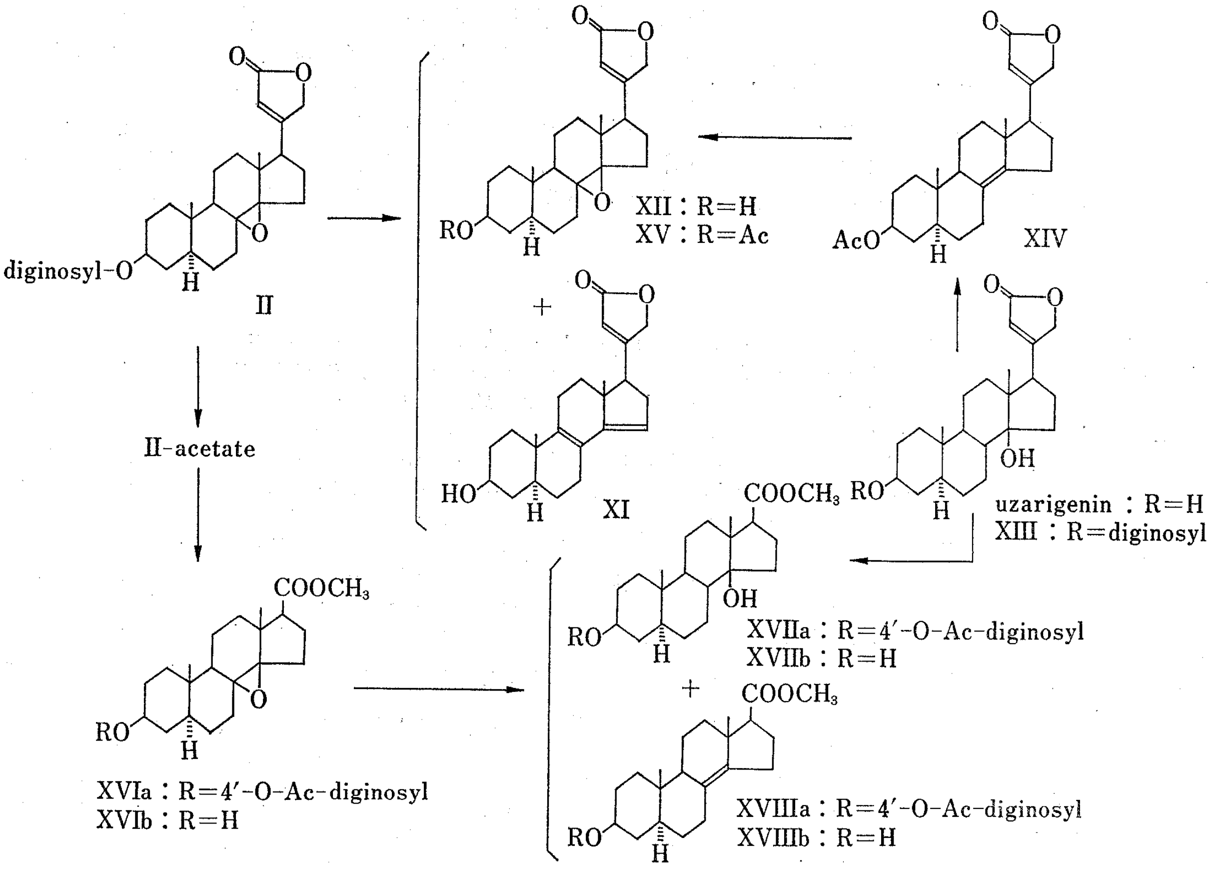

Chart 2

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6) Obtained through the courtesy of Prof. T. Reichstein.
7) K. Meyer, *Helv. Chim. Acta*, **29**, 718 (1946).
8) T. Yamauchi, F. Abe, and M. Nishi, *Chem. Pharm. Bull.* (Tokyo), **26**, 2894 (1978).

(C-20 and C-23), 73.5 (C-21), and 116.9 ppm (C-22), as well as PMR at δ 4.77 (C-21) and 5.89 ppm (C-22). While there are four resonances (64.7, 70.6, 73.5 and 76.2 ppm) arised from the aglycone moiety over the range of 60—90 ppm, no line is found at 85 ppm due to C-14 hydroxyl.

When II was refluxed for 5 min with 0.05 N H_2SO_4–50% EtOH, two aglycones (XI and XII) were obtained and the less polar one (XI) exhibited absorption band at 244 nm, representing to be $\Delta^{8,14}$-diene compound. Since IV, one of the major cardenolide in the oven-dried leaves, also have been known to form this diene on acid hydrolysis, II was considered to be 5α-adynerin. On the comparison of the chemical shift differences [$\delta_{(\text{IV}-\text{VI})}$] between IV and a corresponding normal cardenolide glycoside, VI, with those [$\delta_{(\text{II}-\text{XIII})}$] between II and odoroside B (=uzarigenin β-D-diginoside, XIII), shielding of C-6, -11, -12, -13, -14 and C-15, deshielding of C-7, -8, -9 and C-10 are coincided with those of XIII to II (Table II).

The 8,14-epoxide structure was confirmed by the synthesis of the genuine aglycone (XII). $\Delta^{8,14}$-Anhydrouzarigenin acetate (XIV) was prepared according to the procedure of the synthesis of adynerigenin by Janiak, *et al.*[1b,8] On the reaction of XIV with *m*-chloroperbenzoic acid, a 8,14-epoxide was yielded and identified as the acetate of XII (XV), although the isolation of an isomeric epoxide from the reaction mixture was unsuccessful. The configuration of the oxide in II was determined by the reductive cleavage of the epoxide ring. Acetate of II was converted into the corresponding methyl 8,14-epoxy-etianoate (XVIa) by means of $KMnO_4$ or O_3 oxidation, in the latter case, diginose was splitted by use of HIO_4 to give XVIb. The catalytic reduction over PtO_2 in acetic acid furnished methyl 3β,14β-dihydroxy-5α-etianoate (XVIIb) or its 4′-O-acetyl-diginoside (XVIIa), and their 14-anhydrides (XVIIIa and XVIIIb).[9] As a result of the isolation of XVIIa, β-configuration of the epoxide ring, as well as the β-linkage of D-diginose moiety, was established. Consequently, II has been elucidated as β-D-diginoside of 3β-hydroxy-8,14β-epoxy-5α-card-20(22)-enolide.

In oleander, I is a rare glycoside since L-oleandrose has been found only as the glycosides of oleandrigenin, its desacetyl or anhydro-derivative.[3a,c,4,10] Although the root bark contains a large amount of uzarigenin glycosides,[11] II is the first 5α-cardenolide isolated from the leaves.

Experimental

CMR of 2,6- and 6-Deoxy-3-O-methyl Sugar Glycosides and the Epoxycardenolides——CMR was run using Hitachi R-22 Ft operating at 22.63 MHz in the same procedure described in the preceding paper.[8] The spectra were obtained in pyridine-d_5 at 35° with internal TMS as standard and 8 mm tube was used.

Extraction and Isolation of Subst.X (I) and -C (II)——Fresh leaves of *Nerium odorum* were dried in the oven at 80° within at most 2 days after harvest. The dried leaves (10 kg) were powdered and percolated with MeOH (70 l). The whole MeOH solution was concentrated *in vacuo* to 5 l, and same volume of water was added. The ppt. was removed by filtration and the filtrate was partitioned with benzene. The benzene extractives were crystallized from MeOH to give adynerin (IV) and the mother liquor fraction was again crystallized from hexane–EtOAc to give oleandrin (III). The mother liquor fraction of III was subjected to silica gel column chromatography and eluted with benzene–acetone (10:1—3:1). The fraction containing 16-dehydro-adynerin, IV, and III, obtained prior to the major fraction of III by the elution with 8:1 mixture, was rechromatographed over silica gel with hexane–EtOAc (2:1—1.5:1). I shows overlapping on TLC with III but slightly less polar, and was obtained by crystallization from hexane–EtOAc as needles (35 mg), mp 205—208°, $[\alpha]^{25}_{D}$ −27.7° (*c*=0.09, MeOH), PMR (in $CDCl_3$) δ (ppm): 0.87 and 0.94 (3H of each, s, C-18 and C-19), 1.25 (3H, d, *J*=6 Hz, C-6′), 2.78 (1H, m, C-17), 3.40 (3H, s, 3′-OMe), 3.90 (1H, m, C-3), 4.77 (1H, dd, *J*=17, 2 Hz, C-21), 4.98 (1H, m, C-1′), 5.04 (1H, dd, *J*=17, 2 Hz, C-21), 5.86 (1H, d, *J*=2 Hz, C-22).

The fraction containing III, odoroside A (VI), and Subst. C (II), was eluted with benzene–acetone (5:1) mixture, following the elution of III, and rechromatographed with hexane–EtOAc (1:1—4:5). II was obtained as needles, on crystallization from hexane–EtOAc (60 mg), mp 222—224°, $[\alpha]^{25}_{D}$ −2.0° (*c*=0.20,

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9) Olefinic linkage is tentatively assigned at $\Delta^{8,14)}$ according to the result on adynerigenin by Janiak, *et al.*[3b]

10) H. Jäger, O. Schindler, and T. Reichstein, *Helv. Chim. Acta*, **42**, 977 (1959).

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

MeOH), *Anal.* Calcd. for $C_{30}H_{44}O_7$: C, 69.74; H, 8.58. Found: C, 69.71; H, 8.53. PMR: 0.86 and 0.89 (3H of each, s, C-18 and C-19), 1.34 (3H, d, $J=6$ Hz, C-6′), 2.60 (1H, m, C-17), 3.40 (3H, s, 3′-OMe), 4.57 (1H, dd, $J=2$, 9 Hz, C-1′), 4.77 (2H, s, C-21), 5.89 (1H, bs, C-22).

Conversion of Oleandrin (III) into Subst.X (I)——III (900 mg) was heated at 125° for 30 min in DMF with NaOTs (2.5 g) and NaOAc (1.1 g) and the reaction mixture was poured into water, followed by extraction with $CHCl_3$. The $CHCl_3$ ext. was chromatographed over silica gel to afford 16-anhydro-oleandrin (X) (470 mg), mp 230—235°, $[\alpha]_D^{15}$ +173° ($c=0.60$, MeOH), after crystallization from hexane–EtOAc. X (320 mg) was hydrogenated over Pd–$BaSO_4$ as catalyst in atmosphere of hydrogen, and subjected to column chromatography. On benzene–acetone elution, 10 mg of digitoxigenin α-L-oleandroside [mp 209—212°, $[\alpha]_D^{25}$ −26.0° ($c=0.10$, MeOH)] and 250 mg of 17βH-digitoxigenin α-L-oleandroside [mp 216—220°, $[\alpha]_D^{25}$ −29.5° ($c=0.15$, MeOH)] were obtained. The former was in good agreement with I on mixed melting point and on IR.

Hydrolysis of II——II (50 mg) was refluxed with 5 ml of 0.05 N H_2SO_4–50%EtOH for 5 min, EtOH was evaporated *in vacuo*, water was added and the hydrolyzate was extracted with $CHCl_3$. The $CHCl_3$ ext. was chromatographed over silica gel with benzene–acetone. Aglycone-1 (less polar, XI) (10 mg) was crystallized from hexane–EtOAc to give prisms, mp 232—235°, λ_{max}^{EtOH} nm (ε): 217 (32500), 244 (27000), MS *m/e*: 354 (M^+: $C_{23}H_{30}O_3$). Aglycone-2 (genuine, polar, XII) (20 mg) was crystallized from hexane–EtOAc to give prisms, mp 228—230°, λ_{max}^{EtOH} 215 (19700), MS *m/e*: 372.2318 (Calcd. for $C_{23}H_{32}O_4$: 372.2300). PMR: 0.86 and 0.89 (3H of each, s, C-18 and C-19), 2.60 (1H, m, C-17), 3.60 (1H, m, C-3), 4.77 (2H, d, $J=1$ Hz, C-21), 5.89 (1H, d, $J=1$ Hz, C-22). Acetate of XII: On usual acetylation, followed by crystallization from MeOH, prisms were obtained, mp 260—267°.

Synthesis of 3β-Acetoxy-8,14β-epoxy-5α-card-20(22)-enolide (XV)——To the solution of $\Delta^{8(14)}$-anhydro-uzarigenin acetate[8] (70 mg) in 10 ml of $CHCl_3$, was added 80 mg of *m*-chloroperbenzoic acid. The solution was allowed to stand overnight in refrigirator, and then washed with dil.alkali, and with water. The $CHCl_3$ ext. was subjected to column chromatography with benzene–acetone (20:1). The first fraction was crystallized from MeOH to give prisms (30 mg), mp 260—268°, MS *m/e*: 415.2539 (Calcd. for $C_{25}H_{34}O_5+1H$: 415.2484). On admixture with aglycone-2 acetate, no melting point depression was observed and IR spectra of the both samples were superimposable.

4′-O-Acetyl-β-D-diginoside of Methyl 3β,14β-dihydroxy-5α-etianoate (XVIIa)——1) From XIII-Acetate: To the solution of 200 mg of XIII-acetate in 12 ml of acetone, 360 mg of $KMnO_4$ was added portionwise and stirred for 5 hr at room temp. The acetone was evaporated *in vacuo*, and the residue was diluted with water and filtered. The filtrate was extracted with $CHCl_3$. The $CHCl_3$ ext. was dissolved in ether and treated with CH_2N_2, followed by silica gel column chromatography. The fraction containing the product (52 mg) was crystallized from ether–hexane to give prisms (XVIIa), mp 185—191°, $[\alpha]_D^{15}$ −7.3° ($c=0.52$, $CHCl_3$); PMR: 0.80 and 0.97 (3H of each, s, C-18 and C-19), 1.20 (3H, d, $J=6$ Hz, C-6′), 2.13 (3H, s, –OAc), 2.58 (1H, m, C-17), 3.35 (3H, s, C-3′-OCH_3), 3.72 (3H, s, –$COOCH_3$), 4.02 (1H, s, –OH), 4.60 (1H, dd, $J=4$, 8 Hz, C-1′), 5.18 (1H, d, $J=4$ Hz, C-4′).

2) From II-Acetate: To the solution of II-acetate (50 mg) in 6 ml of acetone, was added 150 mg of $KMnO_4$ and the reaction was continued for 5 hr. The mixture was worked up in the same manner as in 1). The $CHCl_3$ ext. (15 mg) was hydrogenated over PtO_2 in AcOH and the products were fractionated. From the first fraction, anhydride (XVIIIa) was obtained, mp 124—131°. From the second fraction, after crystallization from ether–hexane, prisms were obtained, mp 183—186°. On the comparison with the sample of XVIIa prepared from XIII, no melting point depression was observed and IR spectra of the both samples were in good agreement.

Methyl 3β,14β-dihydroxy-5α-etianoate (XVIIb)——1) From XIII-Acetate: The solution of XIII-acetate (200 mg) in EtOAc was treated with O_3 for 2 hr, and the conjugated ozone was removed with Zn–AcOH. The resultant ester was saponified in $KHCO_3$ solution. Finally, the α-ketol compound was dissolved in 18 ml of EtOH and oxidized with HIO_4 (220 mg in 6 ml of water) for 3 hr at room temp. to give 26 mg of prisms, on crystallization from hexane–EtOAc, mp 146—152°. PMR: 0.80 and 0.95 (3H of each, s, C-18 and C-19), 2.60 (1H, t, $J=6$ Hz, C-17), 3.72 (3H, s, –$COOCH_3$), 3.40—3.75 (1H, m, C-3), 4.02 (1H, s, –OH).

2) From II-Acetate: II-Acetate (50 mg) was treated as same manner as in 1). The corresponding methyl 8,14β-epoxy-etianoate was hydrogenated over PtO_2 in AcOH and purified through silica gel column. The first fraction was crystallized from hexane–EtOAc to give 2 mg of anhydro-compound; mp 168—171°. The second fraction was compared by use of GLC with XVIIb prepared from XIII; relative t_R: 1.00 (t_R of specimen XVIIb: 18.8 min; 1.5% QF-1, 2.1 m, temp. 200°, N_2: 1.0 kg/cm^2).

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