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Studies on Digitalis Glycosides. XXIX.[1] The Structure of Digiprogenin. (4). Partial Synthesis of Dihydro-α-digiprogenin Acetate[2]

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14-Position of the tertiary hydroxyl group of γ- and α-digiprogenin (I and II) was established by partial synthesis of dihydro-α-digiprogenin 3-acetate (IIIb) from 11-oxotigogenin 3-acetate. Dehydration mechanism of α-digiprogenin (14-hydroxy-15,20-dione type) to β-digiprogenin (16-ene-15,20-dione type) was presented.

In the preceding paper,[1] the authors reported that the tertiary hydroxyl groups of γ-digiprogenin (Ia) and α-digiprogenin (IIa, 17-epimer of Ia) were considered to be at C-14 from the results of oxidative cleavages of D-ring of γ-digiprogenin 3-acetate (Ib). This position was confirmed by subsequent partial synthesis of dihydro-α-digiprogenin 3-acetate (IIIb) from 11-oxotigogenin 3-acetate. This paper concerns with this studies.

Catalytic hydrogenation of IIa over palladium–on–charcoal in ethanol gave dihydro-α-digiprogenin (IIIa), together with an unidentified by–product. In the infrared (IR) spectrum of IIIa, the absorption at 1710 cm^{-1} appeared with twofold intensity of that at 1746 cm^{-1}, showing that the three carbonyl groups were retained intact in IIIa. The fact that IR spectrum of the dioxime of IIIa exhibited an absorption of a six-membered ring ketone at 1705 cm^{-1} supported this consideration. In the nuclear magnetic resonance (NMR) spectrum of IIIa, the signal of 6-vinyl proton was not observed, while the signal of C-3 proton (axial) appeared as a broad multiplet at 6.42 τ. These data indicated that the 5,6-double bond of IIa was hydrogenated from rear side to give IIIa. Acetylation of IIIa with acetic anhydride in pyridine gave dihydro-α-digiprogenin 3-acetate (IIIb), mp 200—202°, which was synthesized from 11-oxotigogenin as follows.

Thus, 11-oxotigogenin 3-acetate was converted to 3β-acetoxy-5α-pregn-16-ene-11,20-dione (IV) by the known method,[4] and IV was treated with N-bromosuccinimide (NBS) and subsequently with sodium iodide[5] to give 3β-acetoxy-5α-pregn-14,16-diene-11,20-dione (V). Ultraviolet (UV) absorption of V at 303.5 mμ (ε=10480) indicated the formation of a dienone system in V, which was supported by NMR spectrum whose signals were assigned as follows: 8.84 τ (19-CH_3), 8.81 τ (18-CH_3), 7.67 τ (21-CH_3), 3.79 τ (1H, t, J=2.0 cps, 15-vinyl proton), 2.76 τ (1H, d, J=2.0 cps, 16-vinyl proton).

Oxidation of V with *m*-chloroperbenzoic acid in chloroform afforded an epoxide (VI), whose UV absorption at 240 mμ (ε=7595) showed that the dienone system in V was converted into a monoenone system in VI, and NMR spectrum assigned as 8.89 τ (19-CH_3), 8.65 τ (18-CH_3), 7.75 τ (21-CH_3), 6.03 τ (1H, d, J=1.5 cps, 15-proton connects with an epoxide), 3.04 τ (1H, d, J=1.5 cps, 16-vinyl proton) proved VI to be a 14,15-epoxide. As it is well known[6,7] that epoxidation of pregn-14,16-dien-20-one type steroids gave predominantly 14β,15β-epoxide,

1) Part XXVIII: D. Satoh, M. Miyamura, and S. Nishii, *Chem. Pharm. Bull.* (Tokyo), **17**, 1395 (1969).
2) Preliminary communication was reported in *Chem. Pharm. Bull.* (Tokyo), **15**, 248 (1967).
3) Location: *Sagisu, Fukushima-ku, Osaka.*
4) G. P. Mueller, *Nature*, **76**, 771 (1958).
5) A. J. Sole and B. Singh, *J. Org. Chem.*, **30**, 1658 (1965).
6) Pl. A. Plattner, L. Ruzicka, H, Heusser, and E. Angliker, *Helv. Chim. Acta*, **30**, 385 (1947).
7) H. Mitsuhashi and T. Nomura, *Steroids*, **3**, 271 (1964).

the structure of VI was considered to be 3β-acetoxy-14β,15β-epoxy-5α-pregn-16-ene-11,20-dione.

When VI was oxidized with chromium trioxide in acetic acid, a hydroxyketone (VII) was obtained. UV absorption at 241 mμ (ε=11000) was ascribable to a monoenone system, and the IR absorption at 3540 cm^{-1} showed a formation of hydroxyl group in the oxidation, which was thought to be tertiary because it resisted oxidation. NMR spectrum assigned as 9.15 τ (19-CH_3), 8.60 τ (18-CH_3), 7.61 τ (21-CH_3), 3.40 τ (1H, s, 16-vinyl proton) was comparable to that of β-digiprogenin (IX) having a 16-ene-15,20-dione system as follows: 8.96 τ (19-CH_3), 8.43 τ (18-CH_3), 7.58 τ (21-CH_3), 3.48 τ (1H, s, 16-vinyl proton). The signal of 15-proton of VI at 6.03 τ disappeared in VII and the signal of 16-vinyl proton changed from doublet into singlet. These data indicated that the oxidative cleavage of the 14β,15β-epoxide of VI afforded a 14-hydroxy-15-ketone grouping, and hence VII has a partial structure of 14-hydroxy-16-ene-15,20-dione. Since 16-ene-14,15-epoxide[8] as well as 17α-H-14,15-epoxides[9,10] were reported to give 14β-hydroxy-15-ketone on chromium trioxide oxidation, VII was considered to be 3β-acetoxy-14-hydroxy-5α,14β-pregn-16-ene-11,15,20-trione. This compound (VII) was also prepared by the following route. Thus, treatment of V with osmium tetraoxide gave a 14,15-glycol (VIII), whose structure was proved by UV absorption at 223 mμ (ε=6986) and NMR signals at 5.11 τ (1H, d, J=2.5 cps, 15-proton) and 3.52 τ (1H, d, J=2.5 cps, 16-vinyl proton). Oxidation of VIII with chromium trioxide gave VII. This fact indicated that VIII is a 14β, 15β-glycol providing a further proof for the 14β-hydroxy-15-ketone grouping of VII.

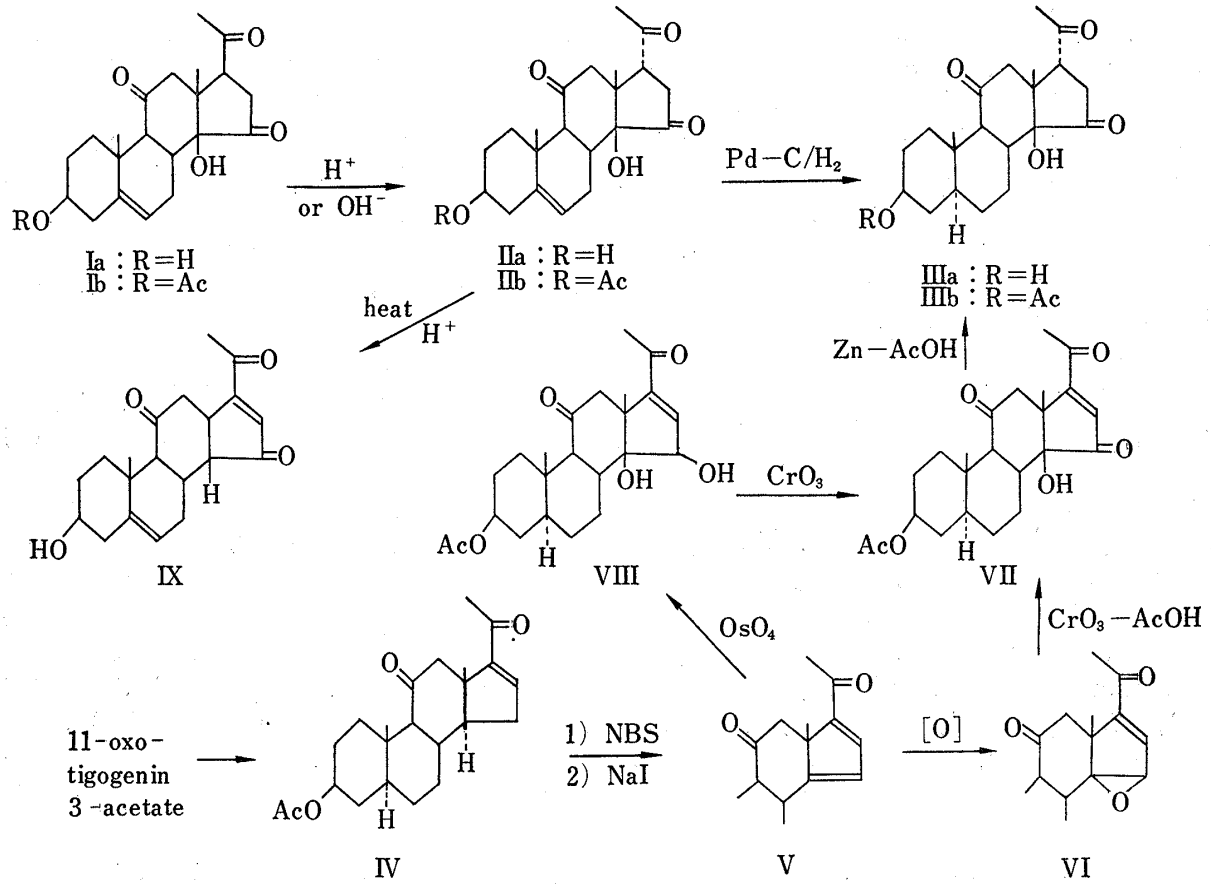
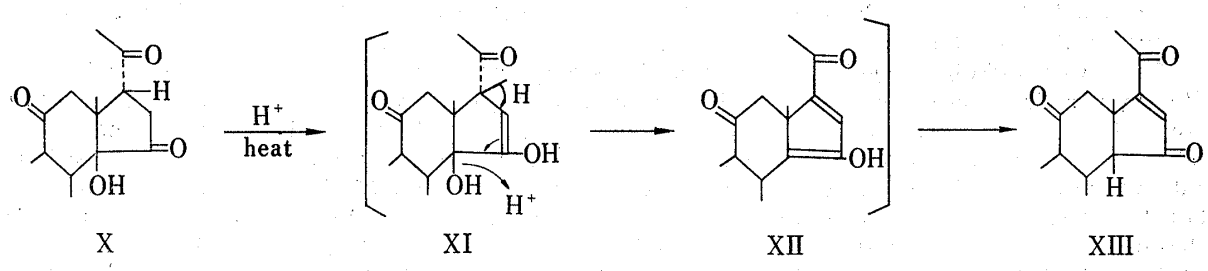


Chart 1

8) H. Mitsuhashi and M. Fukuoka, *Chem. Pharm. Bull.* (Tokyo), **14**, 809 (1966).
9) A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **45**, 943 (1962).
10) M. Okada and M. Hasunuma, *Yakugaku Zasshi*, **85**, 822 (1965).



X XI XII XIII

Chart 2

Reduction of VII with zinc powder in acetic acid at room temperature gave a dihydro derivative, mp 199—201°. The UV and IR spectra of this product showed that 16,17-double bond in VII has been saturated. As it is well known that 16,17-double bond of the C/D-*cis* steroid is hydrogenated predominantly from the front side to give 17α-steroid, the structure of dihydro derivative should be 3β-acetoxy-14-hydroxy-5α,14β,17α-pregnane-11,15,20-trione.

This dihydro derivative proved to be identical with IIIb derived from α-digiprogenin by mixed melting point and comparisons of thin-layer chromatograms (TLC) and IR spectra, thus establishing definitely the 14-position of the tertiary hydroxyl group as well as the positions of the other functional groups of digiprogenin.

Formation of β-digiprogenin (IX) from α-digiprogenin (II) with acid can be explained by 1,4-elimination of water in the sequence from X to XIII as indicated in Chart 2. An analogous elimination was observed in digacetigenin.[11,12]

Experimental[13]

Dihydro-α-digiprogenin (IIIa)——A solution of IIa (200 mg) in EtOH (40 ml) was hydrogenated over 5% Pd–on–charcoal (100 mg). After the absorption of H_2 ceased (H_2=16.3 ml), the catalyzer was filtered off and the filtrate was evaporated *in vacuo* to dryness to give a residue (198 mg) which was separated into following two fractions by preparative thin-layer chromatography (TLC) (SiO_2, AcOEt: benzene=5:1).

i) The first fraction (less polar, 60 mg) was recrystallized from acetone to afford an unidentified by-product (26 mg), mp 187—191°. *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.95; H, 8.73.

ii) The second fraction (more polar, 113 mg) was recrystallized from acetone to afford IIIa (74 mg) as colorless crystals, mp 215—218°. *Anal.* Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.51; H, 8.18. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3583 (OH), 1746 (15-CO), 1710 (11-CO, 20-CO).

Dioxime of IIIa——To a solution of IIIa (30 mg) in MeOH (1.5 ml) was added $NH_2OH \cdot HCl$ (72 mg) and $AcONa \cdot 3H_2O$ (138 mg), and the mixed solution was refluxed for 3 hr. After dilution with H_2O, the precipitate was collected by filtration and recrystallized from acetone-*n*-hexane to give dioxime of IIIa (16 mg), mp 236—240° (decomp.). *Anal.* Calcd. for $C_{21}H_{32}O_5N_2 \cdot H_2O$: C, 61.44; H, 8.35; N, 6.82. Found: C, 61.30; H, 8.32; N, 7.14.

Dihydro-α-digiprogenin 3-Acetate (IIIb) from IIIa——A mixed solution of IIIa (34 mg), Ac_2O (0.4 ml) and pyridine (0.4 ml) was allowed to stand at 0—5° overnight and diluted with H_2O. The crude acetate there deposited was collected by filtration and recrystallized from acetone to give IIIb (26 mg) as colorless crystals, mp 200—202°, $[\alpha]_D^{24}$ −40.2° (c=0.910, MeOH). *Anal.* Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.05; H, 8.13. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3575 (OH), 1745 (15-CO), 1720 (Ac), 1713 (11-CO, 20-CO).

3β-Acetoxy-5α-pregn-14,16-diene-11,20-dione (V)——To a solution of IV (2 g) in CCl_4 (37 ml) was added NBS (2 g, 2 moles) and 2,2′-azobisisobutyronitrile (8 mg), and the mixture was refluxed in an oil bath (90—100°) under N_2 for 1 hr. Succinimide there deposited was filtered off and the filtrate was evaporated *in vacuo* to dryness. The residue was dissolved in acetone (37 ml) and NaI (3.7 g) was added, and the mixed solution was refluxed in an oil bath (80—85°) under N_2 for 3 hr. The resulted solution was evaporated *in vacuo* to dryness. The residue was dissolved in $CHCl_3$ and the $CHCl_3$ solution was washed with 5% $Na_2S_2O_3$ to remove I_2, washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to dryness. The crude product (2.04 g) was recrystallized from acetone to afford V (950 mg) as pale yellow crystals, mp 211—212°, $[\alpha]_D^{23}$ +306.3° (c=1.044, MeOH). *Anal.* Calcd. for $C_{23}H_{30}O_4$: C, 74.56; H, 8.16. Found: C, 74.46; H, 8.18.

11) C. W. Shoppee, N. W. Hughes, R. E. Lack, and B. C. Newman, *Tetrahedron Letters*, **1967**, 3171.
12) R. Tschesche and H. G. Berscheid, *Chem. Ber.*, **100**, 3289 (1967).
13) All melting points are uncorrected, and all NMR spectra were measured at 60 Mc in $CDCl_3$.

IR ν_{max}^{Nujol} cm^{-1}: 1722 (Ac), 1706 (11-CO), 1642 and 1532 (14,16-dien-20-one). UV and NMR spectra were described in the main text.

3β-Acetoxy-14β,15β-epoxy-5α-pregn-16-ene-11,20-dione (VI)——To a solution of V (1877 mg) in $CHCl_3$ (22 ml) was added *m*-chloroperbenzoic acid (2058 mg, 2 moles) portionwise under stirring at room temperature, and the mixture was allowed to stand at the same temperature for 2 hr and then in a refrigerator overnight. After filtration of *m*-chlorobenzoic acid, NaI was added to decompose the excess of *m*-chloroperbenzoic acid and the $CHCl_3$ solution was washed successively with 5% $Na_2S_2O_3$, 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to dryness. The crude product (1950 mg) was recrystallized from AcOEt–*n*-hexane to afford VI (680 mg) as colorless crystals, mp 170—173°, $[\alpha]_D^{23}$ +144.4° (c=0.943, MeOH). *Anal.* Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.57; H, 7.79. IR ν_{max}^{Nujol} cm^{-1}: 1729 (Ac), 1715 (11-CO), 1665 and 1595 (16-en-20-one). UV and NMR spectra were described in the main text.

3β-Acetoxy-14,15β-dihydroxy-5α,14β-pregn-16-ene-11,20-dione (VIII)——To a mixture of solution of V (500 mg) in dioxane (5 ml) and solution of OsO_4 (378 mg, 1.1 moles) in dioxane (2 ml) was added pyridine (0.5 ml) and the mixture was allowed to stand at room temperature in the dark for 2 days After removing osmium as OsS_2 with H_2S, the filtrate was evaporated *in vacuo* to dryness and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and $CHCl_3$ was distilled off to give a dark-brown residue (515 mg) which was shown to consist of four compounds by TLC (SiO_2, AcOEt:benzene=2:1). The crude product was separated into four fractions by preparative TLC using the same system, and the main fraction (the third fraction, 241 mg) was recrystallized from acetone–*n*-hexane to afford VIII (178 mg) as colorless crystals, mp 208—212°, $[\alpha]_D^{23}$ −24.7° (c=0.955, MeOH). *Anal.* Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 67.92; H, 7.95. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540 (OH), 3420 (OH), 1732 (Ac), 1715 (11-CO), 1683 and 1630 (16-en-20-one). UV and NMR spectra were described in the main text.

3β-Acetoxy-14-hydroxy-5α,14β-pregn-16-ene-11,15,20-trione (VII)——i) From VI: To a solution of VI (300 mg) in AcOH (6 ml) was added 2% CrO_3 solution in 90% AcOH (4 ml) dropwise under stirring at room temperature for 2 hr and the mixed solution was allowed to stand at the same temperature overnight. MeOH (5 ml) was added to reduce the excess of CrO_3 and the resulting solution was concentrated *in vacuo* and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with 2% $NaHCO_3$ and H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to dryness. The crude product (310 mg) was separated into three fractions by preparative TLC (Al_2O_3, AcOEt:benzene=5:1).

i) The least polar fraction (30 mg) was not clarified.

ii) The less polar fraction (102 mg) was recrystallized from AcOEt–*n*-hexane to afford VI recovered intact (33 mg), mp 170—172°.

iii) The more polar fraction (123 mg) was recrystallized from acetone–*n*-hexane to afford VII (73 mg) as colorless crystals, mp 165—168°, $[\alpha]_D^{23}$ −46.0° (c=0.522, MeOH). *Anal.* Calcd. for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.89; H, 7.42. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540 (14-OH), 1716 (broad, Ac, 11-CO), 1716, 1693 and 1596 (16-ene-15,20-dione). UV and NMR spectra were described in the main text.

ii) From VIII: To a solution of VIII (50 mg) in acetone (10 ml) was added Kiliani reagent[14] (0.26 ml) under stirring at 0° and the mixed solution was allowed to stand at the same temperature for 30 min. After reducing the excess of CrO_3 with MeOH (0.5 ml), the resulting solution was diluted with H_2O (14 ml), neutralized with 5% $NaHCO_3$, concentrated *in vacuo* and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with 3% $NaHCO_3$ and H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to dryness. The crude product (27 mg) was recrystallized from acetone–*n*-hexane to give VII (15 mg), mp 165—168°.

3β-Acetoxy-14-hydroxy-5α,14β,17α-pregnane-11,15,20-trione (Dihydro-α-digiprogenin 3-Acetate, IIIb) from VII——A mixture of a solution of VII (56 mg) in AcOH (5.6 ml) and zinc powder (280 mg) was stirred at room temperature for 2 hr, and then the excess of zinc powder was removed by filtration. The filtrate was concentrated *in vacuo* and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with 3% $NaHCO_3$ and H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to dryness. The crude product (56 mg) was separated into the two fractions by preparative TLC (SiO_2, AcOEt:benzene=1:3).

i) The less polar fraction (15 mg) was recrystallized from MeOH to give an unidentified by-product (7 mg) as colorless crystals, mp 184—186°. *Anal.* Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.26; H, 8.22. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1741, 1732, 1720, 1712.

ii) The more polar fraction (34 mg) was recrystallized from MeOH to give IIIb (20 mg) as colorless crystals, mp 199—201°, $[\alpha]_D^{25}$ −36.8° (c=1.098, MeOH). *Anal.* Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.11; H, 7.70. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3568 (14-OH), 1745 (15-CO), 1721 (Ac), 1712 (11-CO, 20-CO).

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14) A mixed solution of CrO_3 (2.6 g), conc. H_2SO_4 (2.3 ml) and H_2O (7 ml).