UDC 615.711.5-011

27. Hiroshi Ishii, Yoshio Nozaki, Tamotsu Okumura, and Daisuke Satoh : Studies on Digitalis Glycosides. XVII.[1] Transformation of Cardenolides by Microorganisms. (3). 1β,7β-Dihydroxydigitoxigenin and 5β,7β-Dihydroxydigitoxigenin.*1

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Recently, one of the authors, Nozaki,[2] has reported the isolation of two kinds of dihydroxydigitoxigenin (IIa and VIIIa) as well as that of acovenosigenin A, periplogenin and 7β-hydroxydigitoxigenin from culture broths of *Absidia orchidis* incubated with digitoxigenin I. In this paper the results of further investigations on the structures of these dihydroxyl derivatives are described.

One of the dihydroxydigitoxigenins, IIa, $C_{23}H_{34}O_6$, m.p. 244~245°, $[\alpha]_D$ +19.1°,[2] afforded a triacetate IIb when treated with a mixture of acetic anhydride and pyridine, so that two hydroxyl groups newly introduced were assumed to be either primary or secondary.

On oxidation over a platinum catalyst in an oxygen atmosphere,[3] IIa was converted into its 3-dehydro compound III, the Rf values of which were found to be smaller than those of the starting material IIa. It is generally recognized that 3-hydroxyl steroids have smaller Rf values than those of their corresponding 3-oxo-derivatives, but Fechtig, *et al.*[4] reported that 5β-hydroxyl cardiac aglycones, periplogenin and bipindogenin, possess the paper chromatographic mobilities greater than those of their 3-dehydro derivatives. They attributed these observations to the decreased polarity caused by a presence of the strong intramolecular hydrogen bond formed between 1,3-diaxial hydroxyl groups at 3β- and 5β-position. Authors have also observed such characteristic behaviors of the paper chromatography with periplogenin[1] and acovenosigenin A.[2] The fact mentioned above for IIa therefore suggests that one of the new acylable hydroxyl groups in IIa is at the position where it forms a diaxial 1,3-glycol with the 3β-hydroxyl group, that is, at 1β-position.

When III was refluxed with acetic acid, an anhydro compound IV was produced. This anhydro derivative IV shows an ultraviolet absorption maximum at 220 mμ and its spectrum agrees well with that of Δ^1-digitoxigenone.[5] Its infrared spectrum also shows absorptions for an α,β-unsaturated ketone so that IV is thought to be a Δ^1-3-ketone derivative of the cardiac aglycone. This fact shows that the hydroxyl group was liberated at 1-position. Catalytic hydrogenation of IV over palladium-charcoal yielded a dihydro-anhydro compound V, which was proved to be identical with 3-dehydro-7β-hydroxydigitoxigenin[6] by mixed melting point determination and by comparison of their infrared spectra. This result shows that the second hydroxyl group had been introduced into 7β-position of IIa.

The difference of −44° between M_D +91° of IIa and M_D +135° of 7β-hydroxydigitoxigenin[6] is comparable to the difference of −63° between M_D +9° of acovenosi-

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*1 A brief summarized report of this work was published in a Communication to the Editor in Yakugaku Zasshi, **81**, 1051 (1961).

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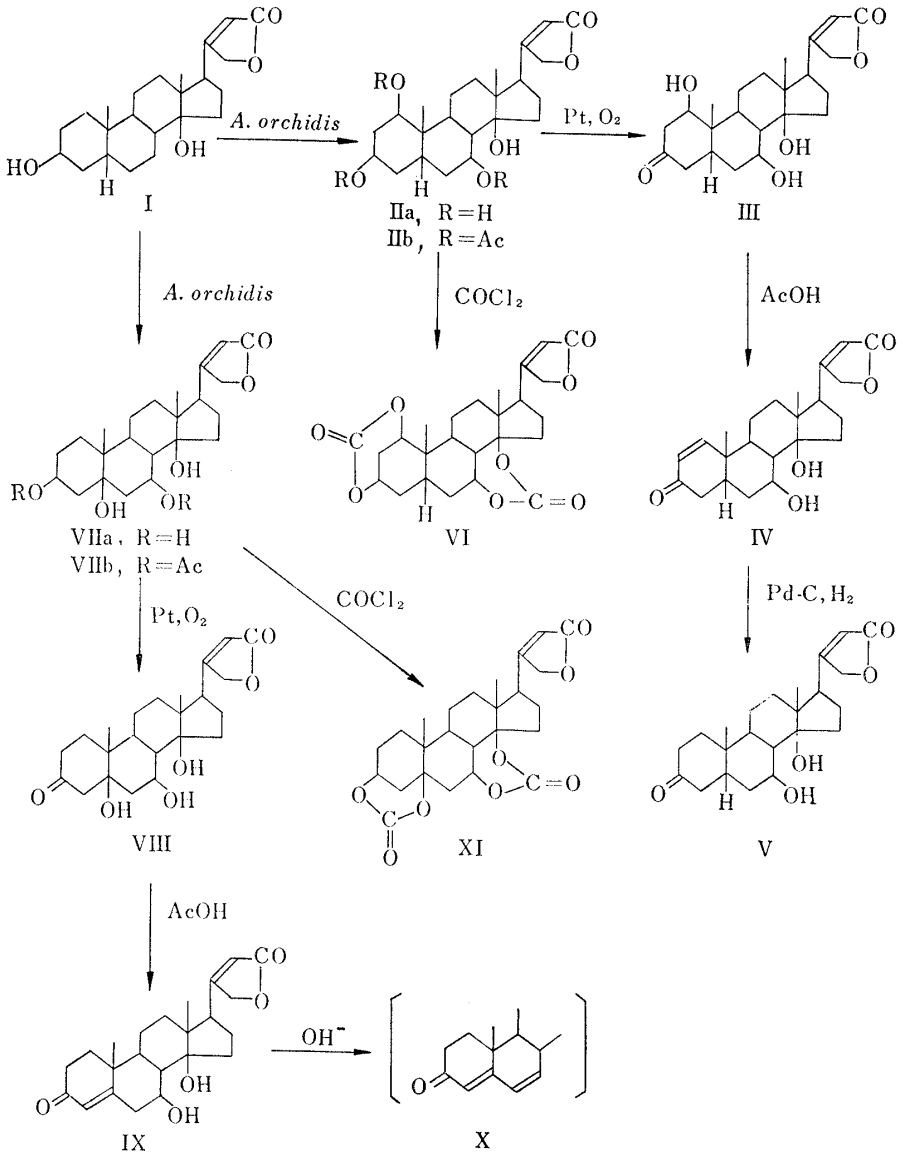
2) Y. Nozaki : Agr. Biol. Chem., **25**, 879 (1961).
3) R. P. A. Sneeden, R. B. Turner : J. Am. Chem. Soc., **77**, 190 (1955).
4) B. Fechtig, J. v. Euw, O. Schindler, T. Reichstein : Helv. Chim. Acta, **43**, 1570 (1960).
5) D. Satoh, T. Wada : Yakugaku Zasshi, **80**, 1314 (1960).
6) H. Ishii, Y. Nozaki, T. Okumura, D. Satoh : *Ibid.*, **81**, 805 (1961).



genin A and M_D +72° of I. These molecular rotational data, together with the result on paper chromatography mentioned above, indicated that the hydroxyl group at 1-position of IIa is β-oriented. Treatment of IIa with phosgene afforded a dicarbonate VI, $C_{25}H_{30}O_8$, m.p. 321~331°(decomp.) in good yield. This result shows that VI is 1β,3β; 7β,14β-dicarbonate of IIa and the hydroxyl at 1-position is in the *cis*-position to the 3-hydroxyl, taking β-configuration. Consequently, it is confirmed that the structure of IIa is 1β,7β-dihydroxydigitoxigenin.

The other dihydroxyl derivative VIIa, $C_{23}H_{34}O_6$, m.p. 245~250°, $[\alpha]_D$ +49.1°,[2] gave a diacetate VIIb on acetylation with acetic anhydride and pyridine. Hence one of the newly introduced hydroxyls was assumed to be secondary and the other tertiary.

The 3-dehydro compound VIII, obtained by dehydrogenation of VIIa over platinum in an oxygen,[3] gave smaller Rf values on paper chromatograms than those of VIIa which was observed similarly to the case of 3-dehydro derivatives of 1β-[2] and 5β-hydroxyl cardiac aglycones.[1,4,7] Based upon the same reason as described above with IIa, it is considered that one of the hydroxyl groups in VIIa is at either 1β- or 5β-position.

7) J. Polonia, A. Kuritzkes, H. Jäger, T. Reichstein : Helv. Chim. Acta, **42**, 1437 (1959).

When VIIIa was heated in acetic acid, an anhydro compound IX was formed. The absorption maximum of its ultraviolet spectrum at 224 mμ is in good agreement with the spectrum of 4,5-anhydroperiplogenone,[1] and the infrared spectrum of IX shows absorptions for an α,β-unsaturated ketone. So it may be considered reasonable that IX is a Δ^4-3-ketone derivative of the cardiac aglycone and that the tertiary hydroxyl group introduced microbiologically into VIIIa is located at 5β.

Treatment of IX with tetramethylammonium hydroxide[8] resulted in a disappearance of the ultraviolet absorption at 224 mμ and a new absorption maximum appeared at 285 mμ characteristic for $\Delta^{4,6}$-3-ketone (X). This fact indicates that the newly introduced secondary hydroxyl into VIIIa was at 7-position since it is well known that Δ^4-3-oxo-7-hydroxyl steroid easily undergoes dehydration at C-7 to give $\Delta^{4,6}$-3-oxo-steroid.[8]

When VII was subjected to the reaction with phosgene, it gave a dicarbonate XI, $C_{25}H_{30}O_8$, m.p. 300~308° (decomp.). This fact reveals that XI is 3β,5β; 7β,14β-dicarbonate of VIIIa and the hydroxyl at 7-position is at the *cis*-position to the 14-hydroxyl, taking β-configuration. This is also confirmed by the fact that the difference of +86° between M_D +203° of VIIIa and M_D +117° of periplogenin corresponds to the difference +63° between M_D +135° of 7β-hydroxydigitoxigenin[6] and M_D +72° of I. It follows, therefore, that the structure of the compound VIIIa is 5β,7β-dihydroxydigitoxigenin.

The formation of these two compounds IIa and VIIIa is considered to be the first example of the microbiological dihydroxylation of digitoxigenin.

Experimental*3

1β,7β-Dihydroxydigitoxigenin Triacetate (IIb)——1β,7β-Dihydroxydigitoxigenin (IIa) (29 mg.) was acetylated in the usual way with Ac_2O and pyridine to give the triacetate IIb, m.p. 134~143°, as prisms (from aq. EtOH). *Anal.* Calcd. for $C_{29}H_{40}O_9 \cdot H_2O$: C, 63.25; H, 7.69; CH_3CO, 23.45. Found: C, 63.45; H, 7.85; CH_3CO, 24.97.

3-Dehydro-1β,7β-dihydroxydigitoxigenin (III)——A suspension of 50 mg. of $PtO_2 \cdot H_2O$ in 1 cc. of H_2O was shaken in H_2 until the reduction was complete to Pt. A solution of 50 mg. of IIa in 12 cc. of Me_2CO was added and the mixture was shaken in O_2 for 25 hr. The catalyst was filtered off and the filtrate gave, after removal of the solvent *in vacuo*, 48 mg. of residue. Recrystallization from MeOH afforded 36 mg. of plates of III, m.p. 302~314° (decomp.); $[\alpha]_D^{24}$ +52.4° (c=0.719, $CHCl_3$-MeOH (1:1)). *Anal.* Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 67.96; H, 8.12. IR λ_{max}^{Nujol} μ: 2.93, 5.79, 5.82, 6.16.

Rf values of 0.40 for IIa and of 0.25 for III were given by paper chromatography, on which a mixture of toluene and BuOH (3:1) was used as the developing solvent and a mixture of formamide and Me_2CO (1:4) as the stationary phase.

Δ^1-3-Dehydro-7β-hydroxydigitoxigenin (IV)——III (25 mg.) was refluxed for 10 min. with 2 cc. of AcOH. After distillation of AcOH *in vacuo*, the residue was crystallized from MeOH to 19 mg. of IV as needles, m.p. 304~309° (decomp.). UV λ_{max}^{EtOH}: 220 mμ (log ε 4.38). IR λ_{max}^{Nujol} μ: 2.96, 5.78, 5.96, 6.20. *Anal.* Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.72; H, 7.94.

3-Dehydro-7β-hydroxydigitoxigenin (V)——A solution of 44 mg. of IV in 65 cc. of MeOH was shaken with 40 mg. of 5% Pd-C in H_2 until the uptake of H_2 ceased. After the removal of the catalyst, the product was crystallized from MeOH-Et_2O to give 37 mg. of prisms of V, m.p. 267~273°, which were shown to be identical with the authentic sample of 3-dehydro-7β-hydroxydigitoxigenin[6] by mixed melting point determination and by direct IR comparison. *Anal.* Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.00; H, 8.41. IR λ_{max}^{Nujol} μ: 2.93, 5.75, 5.83, 6.20.

1β,7β-Dihydroxydigitoxigenin 1β,3β;7β,14β-Dicarbonate (VI)——IIa (50 mg.) was suspended in alcohol-free $CHCl_3$ (10 cc.) and about 2 cc. of the $CHCl_3$ was removed by distillation. To this suspension 4 cc. of pyridine was added to dissolve IIa completely by warming. The mixture was cooled to −15° and a 10% $COCl_2$-toluene solution (10 cc.) was added dropwise. The reaction mixture was allowed to stand at room temperature overnight. After decomposing an excess of $COCl_2$ with ice, H_2O and $CHCl_3$ were added. The solvent layer was washed successively with dil. HCl, $NaHCO_3$ solution and H_2O, dried over anhyd. Na_2SO_4, and concentrated to dryness *in vacuo*. The residue (54 mg.) was crystallized

*3 All melting points are uncorrected.

8) A. S. Meyer: J. Org. Chem., **20**, 1240 (1955).

from Me_2CO to give needles of VI, m.p. 321~331°(decomp.). *Anal.* Calcd. for $C_{25}H_{30}O_8$: C, 65.49; H, 6.60. Found : C, 65.42; H, 6.89.

5β,7β-Dihydroxydigitoxigenin Diacetate (VIIb)——Acetylation of 30 mg. of VIIa in the usual manner with Ac_2O and pyridine yielded the diacetate VIIb as prisms (from MeOH-Et_2O), m.p. 219~222°. *Anal.* Calcd. for $C_{27}H_{38}O_8$: C, 66.10; H, 7.81; CH_3CO, 17.55. Found : C, 66.23; H, 7.88; CH_3CO, 17.32.

3-Dehydro-5β,7β-dihydroxydigitoxigenin (VIII)——VIIa (60 mg.) was dehydrogenated to VIII, m.p. 247~255°; $[\alpha]_D^{24}$ +67.1°(c=0.760, $CHCl_3$-MeOH (1:1)), according to the procedure described above for that of IIa. *Anal.* Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found : C, 68.27; H, 8.16. IR λ_{max}^{Nujol} μ : 2.94, 5.77, 5.84, 6.19.

Rf values of 0.50 for VIIa and of 0.35 for VIII were shown on the paper chromatogram carried out with the same solvent as in the preceding experiment (*vide supra*).

Δ^4-3-Dehydro-7β-hydroxydigitoxigenin (IX)——VIII (45 mg.) was refluxed for 5 min. with 2 cc. of AcOH. The solution was evaporated to dryness under reduced pressure and the residue (44 mg.) was recrystallized from MeOH to give needles of IX, m.p. 272~276°(decomp.); $[\alpha]_D^{26}$ +85.2° (c=1.059, $CHCl_3$MeOH (1:1)). UV λ_{max}^{EtOH} : 224 mμ (log ε 4.35). IR λ_{max}^{Nujol} μ : 2.96, 5.77, 5.99, 6.19. *Anal.* Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found : C, 71.39; H, 7.80.

Treatment of Δ^4-3-Dehydro-7β-hydroxydigitoxigenin (IX) with Tetramethylammonium Hydroxide——One milligram of IX was dissolved in the reagent[8] prepared by mixing 94 cc. of 95% EtOH with 6 cc. of 10% aq. tetramethylammonium hydroxide. The solution was allowed to stand at room temperature for 19 hr. and its UV spectrum was measured. The absorption maximum was obtained at 285 mμ (log ε 4.38).

5β,7β-Dihydroxydigitoxigenin 3β,5β;7β,14β-Dicarbonate (XI)——The reaction of VIIa (30 mg.) with $COCl_2$ was carried out in the similar manner described above to that of IIa. Recrystallization of the product (27 mg.) from MeOH afforded XI as plates, m.p. 300~308°(decomp.). *Anal.* Calcd. for $C_{25}H_{30}O_8$: C, 65.49; H, 6.60. Found : C, 65.06; H, 6.98.

The authors express their deep gratitude to Dr. K. Takeda, Director of this Laboratory, and Dr. E. Masuo of this Laboratory, for their kind encouragements. Thanks are also due to the members of the Analysis Room of this Laboratory for microanalyses and to the members of the Section of Physical Chemistry for the measurement of infrared, ultraviolet spectra and optical rotations.

Summary

The structure and configuration of two kinds of dihydroxyl derivatives obtained by microbiological transformation of digitoxigenin with *Absidia orchidis* were discussed. One of them was shown to be 1β,7β-dihydroxydigitoxigenin and the other 5β,7β-dihydroxydigitoxigenin. This represents the first instance of the introduction of two hydroxyl groups into a cardiac aglycone by microbial oxidation.

(Received February 24, 1962)