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45. Kazuhiko Hoji : Studies on the Constituents of *Digitalis purpurea* L. XX.*1 New Cardiotonic Glycosides, Acetyl-digitoxin-γ and Acetyl-gitoxin-γ.

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In the preceding paper,*1 it was shown that the new cardiotonic glycoside, purlanoside-A, isolated from the seeds of *Digitalis purpurea* L., was a tetraglycoside with digitoxigenin as the aglycone, and one mole of glucose, two moles of digitoxose, and one mole of acetyldigitoxose as the sugars, and that purlanoside-B was a tetraglycoside with gitoxigenin as the aglycone and the same sugars as purlanoside-A. By hydrolysis with the snail enzyme these glycosides afforded digitoxin and gitoxin with loss of an acetyl group and a glucose. The present paper reports that by the action of an enzyme, Hemicellulase,*3 purlanoside-A was changed to acetyl-digitoxin and purlanoside-B to acetyl-gitoxin, with liberation of glucose alone in both cases.

It had been found that Hemicellulase has no faculty for elimination of an acetyl group, but only for glucose and this was found suitable for the present purpose. By the action of this enzyme, at pH 5.0 at 32° for five days, purlanoside-A was converted to a glycoside which was provisionally named substance E-B-0. It was purified through an alumina column and recrystallized from hydrous methanol to colorless needles, m.p. 134~140°, $[\alpha]_D^{21}$ +34.4°(MeOH). It gives positive Legal, Raymond, and Gregg-Gisvold reactions, and also positive Frèrejacque reaction, indicating the presence of an acyl group. This acyl group was converted to hydroxamic acid and was identified with acetyl group by paper chromatography. In Keller-Kiliani reaction, substance E-B-0 exhibits a dark blue glacial acetic acid layer and a brown sulfuric acid layer, same as digitoxin. Its ultraviolet absorption exhibits a maximum at 218 mμ (EtOH) and its analytical values agree well with $C_{43}H_{66}O_{14}$(monoacetyl-digitoxin). Determination of the acetyl group showed the presence of one mole in this molecule.

Substance E-B-0 was deacetylated by the snail enzyme and, after alumina chromatography, the reaction product was recrystallized from acetone-ether to colorless prisms, m.p. 243~247°, which were identified by mixed fusion and Rf values with digitoxin obtained from the leaves of *Digitalis purpurea*.

The foregoing results have shown that substance E-B-0 consists of one mole each of acetyl group and digitoxin. Two glycosides, acetyl-digitoxin-α and -β[1] are known to consist of the same components, but these glycosides are different from substance E-B-0 in their melting point, optical rotation, and solubility in various solvents. Therefore, substance E-B-0 is a new glycoside, an isomer of the known acetyl-digitoxins, and was named acetyl-digitoxin-γ.

Purlanoside-B was converted to a glycoside by enzymatic hydrolysis with Hemicellulase. This glycoside was provisionally named substance E-A-II. It was purified by an alumina column and recrystallized from hydrous methanol to colorless prisms, m.p. 164~167°, $[\alpha]_D^{21}$ +35.7°(MeOH). It gives positive Legal, Raymond, and Gregg-Gisvold reactions, and exhibits positive Frèrejacque reaction, indicating the presence of an acyl group which was found to be acetyl group through comparison of their hydroxamic acids. In the Keller-Kiliani reaction, it exhibits a dark blue glacial acetic acid layer and a carmine-red sulfuric acid

*1 Part XIX : This Bulletin, **9**, 291 (1961).
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*3 The product of Tokyo Kasei Kogyo Co., Ltd.
1) A. Stoll, W. Kreis : Helv. Chim. Acta, **35**, 1318 (1952).

layer, same as gitoxin. Its ultraviolet absorption spectrum exhibits a maximum at 219 mμ (EtOH) and the analytical values and quantitative acetyl group analysis agree with $C_{43}H_{66}O_{15}\cdot 2H_2O$ (monoacetyl-gitoxin).

Substance E-A-II was deacetylated by the snail enzyme and the deacetylated glycoside was recrystallized from pyridine-methanol to colorless prisms, m.p. 269~271°, which were identified as gitoxin by paper chromatography and mixed fusion.

The foregoing results have shown that substance E-A-II consists of an acetyl group and gitoxin. Two glycosides, acetyl-gitoxin-α and -β[2] are known to consist of the same components, but these glycosides are different from substance E-A-II in melting point, optical rotation, and solubility in various solvents. Therefore, substance E-A-II was found to be a new glycoside, an isomer of the known acetyl-gitoxins, and was named acetyl-gitoxin-γ.

It has been found that purlanosides-A and -B are changed into secondary glycosides, acetyl-digitoxin-γ and acetyl-gitoxin-γ. Therefore, it is further confirmed that the original glycosides are the isomers of lanatosides-A and -B. The position of the acetyl group will be examined and reported later.

Experimental*4

Formation of Acetyl-digitoxin-γ (Substance E-B-0) from Purlanoside-A——To a solution of 2 g. of purlanoside-A dissolved in 100 cc. of MeOH, 1,500 cc. of distilled water was added, MeOH was evaporated in a reduced pressure, and distilled water was added to make the whole volume 1,600 cc. Buffer solution (pH 5.0) consisting of 100 cc. of 0.2*N* AcOH and 260 cc. of 0.2*N* AcONa was added to this solution, 1.2 g. of Hemicellulase was then added, together with 10 cc. of toluene, and the mixture was allowed to stand in a thermostat at 32° for 5 days. The mixture was evaporated in a reduced pressure, the residue was extracted with 50 cc. of MeOH-$CHCl_3$ (1:1), and the extract was filtered. The filtrate was evaporated and the residue (1.46 g.) was chromatographed through a column containing a mixture of 140 g. of Celite 535 and 140 cc. of $HCONH_2$-H_2O (2:1). The column was eluted with benzene, 100-cc. fractions were collected, and fraction Nos. 5~14 contained degluco-purlanoside-A (acetyl-digitoxin-γ).

The combined fraction Nos. 5~14 (1.1 g.) was chromatographed through a column of alumina (40 g.), which was eluted successively with $CHCl_3$ (400 cc.), 1% MeOH-$CHCl_3$ (900 cc.), and 20% MeOH-$CHCl_3$ (1,000 cc.). 1% MeOH-$CHCl_3$ portion was found to be crude acetyl-digitoxin-γ (Rf 0.75) by paper chromatography (Fig. 1).

Acetyl-digitoxin-γ——The crude acetyl-digitoxin-γ was recrystallized from hydr. MeOH to colorless needles (330 mg.), m.p. 135~142°, $[\alpha]_D^{21}$ +32.4° (c=1.37, MeOH). UV : λ_{max}^{EtOH} 218 mμ (log ε 4.22). *Anal.* Calcd. for $C_{43}H_{66}O_{14}$: C, 64.00; H, 8.24; CH_3CO, 5.33. Found : C, 64.47; H, 8.57; CH_3CO, 5.93.

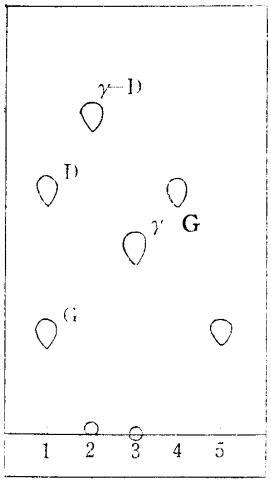

Fig. 1. Paper Partition Chromatography

Moving phase : Xylene-MeCOEt (2:1) saturated with $HCONH_2$
Paper : Toyo Roshi No. 50 filter paper impregnated with $HCONH_2$-Me_2CO (1:4) and Me_2CO evaporated.
Method : Ascending method
Coloring agent : Raymond reaction agent or 20% $SbCl_3$ solution

1. Digitoxin (D) and gitoxin (G)
2. Acetyl-digitoxin-γ (γ-D) from purlanoside-A by Hemicellulase
3. Acetyl-gitoxin-γ (γ-G) from purlanoside-B by Hemicellulase
4. Digitoxin from acetyl-digitoxin-γ by the snail enzyme
5. Gitoxin from acetyl-gitoxin-γ by the snail enzyme

*4 All melting points were measured on a Kofler block and are uncorrected.
2) A. Stoll, A. von Wartburg, W. Kreis : Helv. Chim. Acta, 35, 1324 (1952).

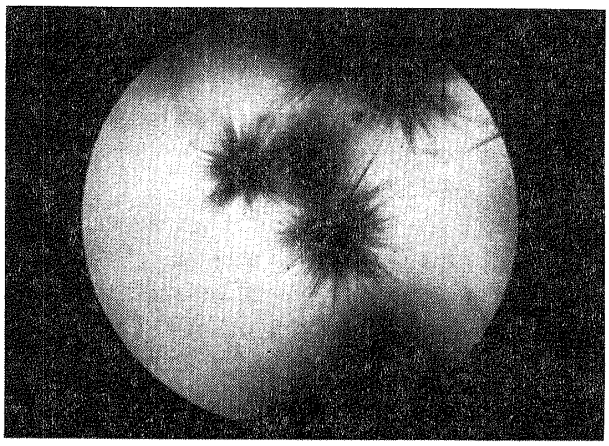

Fig. 2. Acetyl-digitoxin-γ

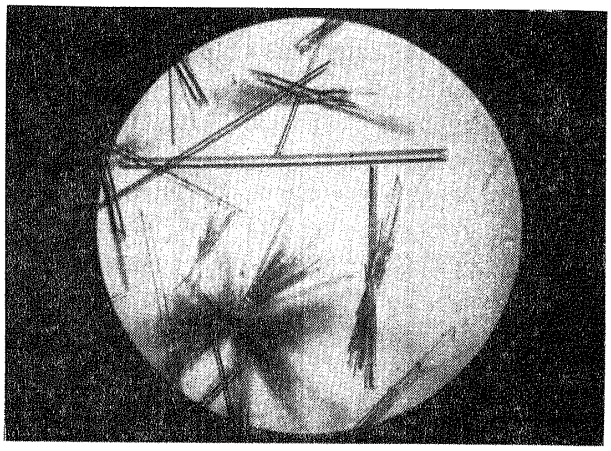

Fig. 3. Acetyl-gitoxin-γ

This substance gives positive Legal, Raymond, Gregg-Gisvold, and Frèrejacque reactions, and exhibits a dark blue glacial AcOH layer and a brown H_2SO_4 layer in Keller-Kiliani reaction. It is easily soluble in MeOH and EtOH, soluble in $CHCl_3$ and Me_2CO, and sparingly soluble in Et_2O and H_2O.

Estimation of the Acetyl Group in Acetyl-digitoxin-γ——To a solution of 5 mg. of acetyl-digitoxin-γ dissolved in 0.4 cc. of MeOH, 0.2 cc. of 5% $NH_2OH \cdot HCl$-EtOH solution and 0.2 cc. of 12.5% EtOH-H_2O (1:1) solution of NaOH were added and the mixture was allowed to stand at room temperature for 20 min. This reaction mixture was neutralized with 7% HCl-MeOH solution, the precipitate formed was removed, and the solution was evaporated to a small volume. The residual solution was submitted to paper chromatography, using Toyo Roshi No. 50 filter paper and BuOH-AcOH-H_2O (4:1:5) as the developing solvent, at 18~22°, with 16% aqueous solution of $FeCl_3 \cdot 6H_2O$ as the coloring agent. A spot (Rf 0.46) of hydroxamic acid derived from acetyl group was detected and was identified with hydroxamic acid from digitalinum verum monoacetate (Rf 0.46).

Formation of Digitoxin from Acetyl-digitoxin-γ——To a solution of 230 mg. of acetyl-digitoxin-γ dissolved in 150 cc. of 2% EtOH-H_2O, a filtrate obtained from 110 mg. of the snail enzyme powder treated 3 times with 10 cc. each of H_2O was added, together with 1 cc. of toluene, the mixture was allowed to stand at 32° for 3 days, and evaporated to dryness in a reduced pressure. The residue was treated with 50 cc. of MeOH-$CHCl_3$ (1:1) and evaporated. The reaction product was submitted to paper chromatography and a spot was found, whose Rf value was the same as that of digitoxin (Fig. 1).

The crude digitoxin obtained (200 mg.) was chromatographed through a column of alumina (5 g.). This column was eluted successively with 50 cc. of $CHCl_3$, and 50 cc. each of 1%, 2%, and 5% MeOH-$CHCl_3$. The residue from 2% MeOH-$CHCl_3$ portion was recrystallized from Me_2CO-Et_2O to colorless prisms, m.p. 243~247°, which, mixed with digitoxin, m.p. 242~245°, melted at 240~244°.

Formation of Acetyl-gitoxin-γ (Substance E-A-II) from Purlanoside-B——To a solution of 2 g. of purlanoside-B dissolved in 100 cc. of MeOH, 7 L. of distilled water was added, MeOH was evaporated in a reduced pressure, and distilled water was added to bring the whole volume to 7.5 L. The buffer solution (pH 5.0) consisting of 200 cc. of 0.2*N* AcOH and 520 cc. of 0.2*N* AcONa, and 1 g. of Hemicellulase were added to this solution, together with 10 cc. of toluene, and the mixture was allowed to stand in a thermostat at 32° for 5 days. The mixture was evaporated in a reduced pressure to 2 L., extracted with BuOH-$CHCl_3$ (1:2), and BuOH-$CHCl_3$ solution was evaporated. The residue (1.7 g.) was chromatographed through an alumina column (40 g.) and this was eluted with $CHCl_3$ (150 cc.), and 2% (600 cc.), 5% (300 cc.), and 10% MeOH-$CHCl_3$ (500 cc.). The residue from 2% MeOH-$CHCl_3$ portion was found to contain crude acetyl-gitoxin-γ (Rf 0.44) by paper chromatography (Fig. 1).

Acetyl-gitoxin-γ——The crude acetyl-gitoxin-γ was recrystallized from hydr. MeOH to colorless prisms (740 mg.), m.p. 164~167°, $[\alpha]_D^{21}$ +35.7° (c=1.19, MeOH). UV : λ_{max}^{EtOH} 219 mμ (log ε 4.20). *Anal.* Calcd. for $C_{42}H_{66}O_{15} \cdot 2H_2O$: C, 60.12; H, 8.21; CH_3CO, 5.01. Found : C, 60.46; H, 8.53; CH_3CO, 5.17.

This substance gives positive Legal, Raymond, Gregg-Gisvold, and Frèrejacque reactions, and exhibits a dark blue glacial AcOH layer and a carmine-red H_2SO_4 layer in Keller-Kiliani reaction. They are easily soluble in MeOH and EtOH, soluble in Me_2CO, and sparingly soluble in $CHCl_3$, Et_2O and H_2O.

Estimation of the Acetyl Group in Acetyl-gitoxin-γ——Acetyl-gitoxin-γ was treated by the same method as acetyl-digitoxin-γ and the hydroxamic acid obtained was submitted to paper chromatography. A spot (Rf 0.46) of the hydroxamic acid derived from acetyl group was detected.

Formation of Gitoxin from Acetyl-gitoxin-γ——To a solution of 130 mg. of acetyl-gitoxin-γ dissolved in 150 cc. of 2% EtOH-H_2O, a filtrate obtained from 60 mg. of the snail enzyme powder treated 3 times with 10 cc. each of H_2O was added, together with 1 cc. of toluene, the mixture was allowed to stand at 32° for 3 days, and evaporated to dryness in a reduced pressure. The residue was treated with 50 cc. of MeOH-$CHCl_3$ (1:1) and evaporated to leave 110 mg. of a solid. The reaction product was submitted to paper chromatography and a spot was found, whose Rf value was the same as that of gitoxin (Fig. 1).

The crude gitoxin was recrystallized from pyridine-MeOH to colorless prisms, m.p. 269~271°, which on admixture with gitoxin, m.p. 267~272°, melted at 266~270°.

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Summary

The structure of purlanosides-A and -B, newly isolated from the digitalis seeds, was examined by enzymatic decomposition. By the action of Hemicellulase, purlanoside-A was changed to acetyl-digitoxin-γ, an isomer of the known acetyl-digitoxins. Acetyl-digitoxin-γ was obtained as crystals of m.p. 135~142°. Purlanoside-B was changed to acetyl-gitoxin-γ by Hemicellulase. Acetyl-gitoxin-γ is an isomer of the known acetyl-gitoxins and was obtained as crystals of m.p. 164~167°.

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