Note

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Kazuhiko Hoji : Studies on the Constituents of *Digitalis purpurea* L. XXVI.*1 Purpurea Glycoside-A and Purpurea Glycoside-B from Digitalis Seeds.

(*Research Laboratory, Daiichi Seiyaku Co., Ltd.**2)

Substances PGA and PGB were isolated from digitalis seeds and their properties were described in Part XVII[1] of this series. These glycosides were examined and were identified as purpurea glycosides-A and -B which were first isolated from the leaves of *Digitalis purpurea* by Stoll, *et al.*[2]

Substance PGA was repeatedly recrystallized from ethanol-ether to a colorless powder, m.p. 248~254°, $[\alpha]_D^{21}$ −5.2° (pyridine), $[\alpha]_D^{21}$ +7.7° (75% ethanol). It has an extremely bitter taste and is easily soluble in methanol-chloroform (1:1), soluble in methanol, and insoluble in acetone, ether, and water. It gives positive Legal, Raymond, and Gregg-Gisvold reactions, negative Frèrejacque reaction, and exhibits a dark blue glacial acetic acid layer and a brown sulfuric acid layer in the Keller-Kiliani reaction. Therefore, it is reasonable to assume that the glycoside contains some 2,6-deoxysugars. Its ultraviolet spectrum exhibits the maximum absorption at 217 mμ (ethanol), indicating the presence of an α,β-unsaturated lactone, the characteristics of cardiotonic glycosides. By the calculation of absorption intensity, the glycoside was found to be a tetraglycoside. The elemental analysis agrees with the formula $C_{47}H_{74}O_{18}$, calculated as purpurea glycoside-A. The Rf values in various solvent systems were compared with those of purpurea glycoside-A from digitalis leaves, and these glycosides were found to be identical.

Mild acid hydrolysis of substance PGA with 0.05*N* methanolic sulfuric acid afforded an aglycone, digitoxigenin, and two sugars, digitoxose and digilanidobiose. Enzymatic hydrolysis of substance PGA for five days afforded colorless needles, m.p. 247~249°, from methanol-ether, after purification by an alumina chromatography. This substance was identified with digitoxin from digitalis leaves, by paper chromatography and mixed fusion. Acetylation of substance PGA by the usual method afforded colorless needles, m.p. 141~148°, $[\alpha]_D^{21}$ +44.0° (chloroform). This product was identified as purpurea glycoside-A acetate[3] by mixed fusion. From the foregoing results, it is confirmed that substance PGA is purpurea glycoside-A.

Substance PGB was isolated as shown in Part XVII.[1] It was recrystallized from pyridine-ethanol to a colorless powder, m.p. 228~233°, $[\alpha]_D^{21}$ +2.3° (pyridine), $[\alpha]_D^{21}$ +13.2° (75% ethanol). It has a bitter taste and is easily soluble in pyridine and methanol-chloroform (1:1), soluble in methanol, sparingly soluble in ethanol, and insoluble in acetone, ether, and water. It gives positive Legal, Raymond, and Gregg-Gisvold reactions and negative Frèrejacque reaction. It exhibits a dark blue glacial acetic acid layer and a carmine-red sulfuric acid layer in the Keller-Kiliani reaction. Therefore, it is reasonable to assume that the glycoside contains some 2,6-deoxysugars and gitoxigenin. Its ultraviolet spectrum shows the maximum absorption at 219 mμ (ethanol), indicating the presence of an α,β-unsaturated lactone. The elemental analysis agrees with the formula $C_{47}H_{74}O_{19}$, calculated

*1 Part XXV. K. Hoji : This Bulletin, **9**, 571 (1961).
*2 Hirakawabashi, Sumida-ku, Tokyo (傍士和彦).
1) Part XVII. K. Hoji, *et al.* : This Bulletin, **9**, 276 (1961).
2) A. Stoll, W. Kreis : Helv. Chim. Acta, **18**, 120 (1935).
3) Part V. A. Okano, *et al.* : This Bulletin, **5**, 171 (1957).

as purpurea glycoside-B. The Rf values in various solvent systems were compared with that of purpurea glycoside-B from digitalis leaves and these glycosides were found to be identical.

By mild acid hydrolysis of substane PGB, gitoxigenin was obtained as the aglycone, and digitoxose and digilanidobiose as the sugar moiety. After enzymatic hydrolysis for five days and alumina chromatography, substance PGB afforded colorless prisms, m.p. 271~275°, by recrystallization from pyridine-methanol. They were identified with gitoxin from digitalis leaves by paper chromatography and mixed fusion. Acetylation of substance PGB by the usual method afforded colorless needles, m.p. 151~154°/223~227°, $[\alpha]_D^{22}$ +34.5° (chloroform). They were identified as purpurea glycoside-B acetate[3] by mixed fusion. It is confirmed from the foregoing results that substance PGB is purpurea glycoside-B.

Satoh, *et al.*[4] had reported that gitoxin was isolated from seeds of *Digitalis purpurea*. Haack *et al.*[5] had assumed by chromatography that purpurea glycosides-A and -B were present in the seeds. It was reported in Part II[6] of this series that substances PGA and PGB might correspond to purpurea glycosides-A and -B, and their isolation and identification were accomplished for the first time, although they were not obtained in crystalline form. It is interesting that there is larger amount of purpurea glycosides-A and -B than digitalinum verum in the leaves, but the latter was more predominant than the former in the seeds. This is the reason why the latter was more easily obtained than the former from the seeds.

Experimental*3

Substance PGA (Purpurea Glycoside-A)——The method of isolation was reported in Part XVII.[1] This substance was recrystallized from EtOH-Et_2O to a colorless powder, m.p. 248~254°; $[\alpha]_D^{21}$ −5.2° (c=1.88, pyridine), $[\alpha]_D^{21}$ +7.7° (c=0.41, 75% EtOH); UV: λ_{max}^{EtOH} 217 mμ (log ε 4.23). *Anal.* Calcd. for $C_{47}H_{74}O_{18}$: C, 60.87; H, 8.05. Found: C, 61.06; H, 8.30.

Acid Hydrolysis of Substance PGA——A solution of 10 mg. of substance PGA dissolved in a mixture of 2 cc. of MeOH and 2 cc. of 0.1*N* H_2SO_4 was refluxed for 30 min. MeOH was distilled off in a reduced pressure and the residue was extracted 5 times with 5 cc. each of $CHCl_3$.

i) Aglycone: The combined $CHCl_3$ extract was washed with 10 cc. of water and $CHCl_3$ was evaporated. The residue was submitted to paper chromatography, using a solvent system of xylene-MeCOEt (1:1) saturated with formamide, on a paper (Toyo Roshi No. 50) impregnated with formamide-Me_2CO (1:4), and the aglycone (Rf 0.78) was found to be identical with digitoxigenin (Rf 0.78).

ii) Sugar moiety: The aqueous solution was deacidified by Amberlite IR-4B (2 cc.), evaporated to dryness, and the residue was submitted to paper chromatography, using the solvent system of BuOH-AcOH-H_2O (4:1:5), and two sugar spots were found. One (Rf 0.54) was identified as that of digitoxose (Rf 0.54) and the other (Rf 0.19) as that of digilanidobiose (Rf 0.19).

Formation of Digitoxin from Substance PGA——To a solution of 200 mg. of substance PGA dissolved in 30 cc. of MeOH, 400 cc. of distilled water and 200 mg. of the snail enzyme powder were added, and the mixture was allowed to stand at 32° for 5 days. The reaction mixture was evaporated to dryness in a reduced pressure, and the residue was extracted with MeOH-$CHCl_3$ (1:1). MeOH-$CHCl_3$ solution was evaporated to dryness (130 mg.) and the residue was chromatographed on 6 g. of Al_2O_3 with 10% MeOH-$CHCl_3$. The colorless material obtained (90 mg.) was recrystallized from MeOH-Et_2O to needles, m.p. 242~244°. When this product was mixed with digitoxin, m.p. 242~245°, the mixture melted at 241~244°. Paper chromatography was carried out on these glycosides, using a mixture of xylene-MeCOEt (1:1) saturated with formamide on a formamide-Me_2CO (1:4) impregnated paper. Rf values were 0.72 for this product and 0.725 for digitoxin.

Acetylation of Substance PGA——Substance PGA (70 mg.) was acetylated by the usual method of leaving at room temperature with 1 cc. of pyridine and 1 cc. of Ac_2O for 5 days. It was evaporated to dryness and recrystallized from hydr. MeOH to needles (50 mg.), m.p. 141~148°, $[\alpha]_D^{21}$ +44.0° (c=1.19, $CHCl_3$). When this product was mixed with purpurea glycoside-A acetate,[3] m.p. 143~148°,

*3 All melting points were measured on a Kofler block and are uncorrected.

4) D. Satoh, H. Ishii, Y. Oyama: Ann. Reports Shionogi Lab. (Japan) **5**, 113 (1955).
5) E. Haack, F. Kaiser, M. Gube, H. Spingler: Naturwiss., **43**, 301 (1956).
6) Part II. K. Miyatake, *et al.*: This Bulletin, **5**, 157 (1957).

$[\alpha]_D^{21}$ +43.2° (c=0.96, $CHCl_3$), the melting point was 140~148°.

Substance PGB (Purpurea Glycoside-B)——The method of isolation was reported in Part XVII.[1] This substance was recrystallized from pyridine-EtOH to a colorless powder, m.p. 224~230°; $[\alpha]_D^{21}$ +2.3° (c=1.40, pyridine), $[\alpha]_D^{21}$ +13.2° (c=0.38, 75%-EtOH); UV : λ_{max}^{EtOH} 219 mμ (log ε 4.27). *Anal.* Calcd. for $C_{47}H_{74}O_{19}$: C, 59.83; H, 7.91. Found : C, 59.71; H, 8.03.

Acid Hydrolysis of Substance PGB——Substance PGB (10 mg.) was hydrolyzed by the same method as for substance PGA.

i) Aglycone : The aglycone (Rf 0.45) was identified as gitoxigenin (Rf 0.46).

ii) Sugar moiety : The sugars (Rf 0.55 and 0.19) were identified as digitoxose (Rf 0.55) and digilanidobiose (Rf 0.19).

Formation of Gitoxin from Substance PGB——Substance PGB was hydrolyzed with the snail enzyme by the same method as for substance PGA. The crude reaction mixture was chromatographed on 6 g. of Al_2O_3 with 10% MeOH-$CHCl_3$. The colorless material so obtained (100 mg.) was recrystallized from pyridine-MeOH to prisms, m.p. 269~272°. When this product was mixed with gitoxin, m.p. 269~272°, the mixture melted at 266~270°, and its Rf was 0.41 and Rf of gitoxin, 0.41.

Acetylation of Substance PGB——Substance PGB (120 mg.) was acetylated by the same method as for substance PGA. The crude acetate obtained was recrystallized from EtOH to needles (100 mg.), m.p. 151~154°/223~227°, $[\alpha]_D^{22}$ +34.5° (c=1.02, $CHCl_3$), melting at 152~156°/219~227° on admixture with purpurea glycoside-B acetate,[3] m.p. 152~155°/228~232°.

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Summary

The structure of substances PGA and PGB, newly isolated from digitalis seeds, was examined. It was found that the enzymatic hydrolysis of substance PGA afforded digitoxin, and mild acid hydrolysis afforded digitoxigenin, digitoxose, and digilanidobiose. Further, purpurea glycoside-A acetate was formed from substance PGA by acetylation. Therefore, substance PGA was found to be identical with purpurea glycoside-A.

Substance PGB was examined by the same methods and was assumed to be purpurea glycoside-B.

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