It is easily soluble in MeOH and Me_2CO, and insoluble in H_2O. It gives positive Legal, Raymond, Gregg-Gisvold, and Frèrejacque reactions, and exhibits a carmine-red H_2SO_4 layer and a dark blue AcOH layer in the Keller-Kiliani reaction.

Acid Hydrolysis of Substance B-2——The same of hydrolysis of substance B-2 showed spots of gitoxigenin, acetyldigitoxose, and digitoxose on the paper chromatogram.

Substance B-3 (Gitoxigenin)——The crude substance B-3 obtained was recrystallized from MeOH and a mixture of Me_2CO-petr. ether, to colorless plates (40 mg.), m.p. 220～227°. Its mixed fusion with gitoxigenin, m.p. 220～225°, melted at 220～224°.

The author expresses his sincerest gratitude to Dr. J. Shinoda, President of this Company, to Dr. T. Ishiguro, Director of this Laboratory, and to Dr. M. Shimizu, Acting Director, for kind encouragement during the course of this work, and to Dr. K. Miyatake, Director of the Yanagishima Factory, and to Dr. A. Okano, for continued guidance. The author is much indebted to Messrs. T. Miki and A. Sakashita for technical help and to Messrs. B. Kurihara and K. Abe for elemental analyses.

Summary

It was already reported that purlanosides-A and -B were isomers of lanatosides-A and -B. By partial acid hydrolysis, purlanoside-A afforded three new products and one of them was found to be digitoxigenin acetyldigitoxoside, by subsequent deacetylation and acid hydrolysis. Therefore, it was assumed that the acetyl group of purlanoside-A is in the first digitoxose and its structure is formulated as digitoxigenin glucosido-bisdigitoxosido-acetyldigitoxoside.

Purlanoside-B also afforded gitoxigenin acetyldigitoxoside by the same hydrolysis. Therefore, the acetyl group is also in the first digitoxose, and its structure is formulated as gitoxigenin glucosido-bisdigitoxosido-acetyldigitoxoside.

Further, it was determined that the acetyl groups of acetyldigitoxin-γ and acetylgitoxin-γ are in the first digitoxose.

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92. Kazuhiko Hoji : Studies on the Constituents of *Digitalis purpurea* L. XXV.*1 A New Cardiotonic Glycoside, Acetylglucogitoroside and Digitalinum verum Monoacetate from Digitalis Seeds.

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

It was reported in Part XVII[1] of this series that several new cardiotonic glycosides had been isolated from the seeds of *Digitalis purpurea* L. and two of these glycosides, substances A-II′ and A-III′, were examined for their structural determination. Substance A-II′ corresponds to glucogitoroside monoacetate, and substance A-III′ to digitalinum verum monoacetate.

After isolating them from the seeds, substance A-II′ was repeatedly recrystallized from a mixture of acetone, ether, and petroleum ether to a colorless powder, m.p. 199～

*1 Part XXIV. K. Hoji : This Bulletin, **9**, 566 (1961).

*2 Hirakawabashi, Sumida-ku, Tokyo (傍士和彦).

1) Part XVII. K. Hoji, *et al.* : This Bulletin, **9**, 276 (1961).

205°, $[\alpha]_D^{21}$ +5.9° (methanol). It is easily soluble in methanol, acetone, and chloroform, soluble in water, slightly soluble in ether, and insoluble in petroleum ether. It has a bitter taste and gives positive Legal and Raymond reactions, the same as general cardiotonic glycosides. It also exhibits the maximum absorption at 219 mμ. Its positive Gregg-Gisvold reaction indicates the presence of 2,6-deoxysugar in its structure, and in the Keller-Kiliani reaction, it gives a carmine-red sulfuric acid layer and a brown glacial acetic acid layer, the same as glucogitoroside. The positive Frèrejacque reaction shows that it has some acyl group. The acyl group was converted to hydroxamic acid and determined as an acetyl group by paper chromatography. Its elemental analytical values and acetyl determination agree with the formula of $C_{37}H_{56}O_{14}\cdot 1\frac{1}{2}H_2O$, calculated as glucogitoroside plus one mole of acetyl group.

The mild acid hydrolysis of substance A-II′ afforded gitoxigenin as the aglycone and acetyldigilanidobiose[2] as the sugar. The sugar was compared by paper chromatography, run together with the sugars obtained from lanatoside-B*[3] by the same method of hydrolysis.

Deacetylation with potassium hydrogencarbonate converted substance A-II′ into another glycoside which was found to be identical with glucogitoroside,[3] by paper chromatography.

The foregoing results have shown that substance A-II′ is obviously a hitherto unknown glycoside, consisting of one mole of acetyl group and one mole of glucogitoroside. Therefore, substance A-II′, a new glycoside, was named acetylglucogitoroside.

As in the formation of acetyldigitoxin-γ from purlanoside-A in Part XX,[4] Hemicellulase*[4] was used for the liberation of glucose from acetylglucogitoroside. The experiment failed as this glycoside did not convert into any degluco-glycoside. Lanatoside-B*[3] was then used for the starting material and it did not convert into degluco-glycoside, acetylgitoxin-α, either. From these results, it is assumed that the enzyme has no capacity for degradation of linkage between 3-acetyldigitoxose and glucose.

Further, when the glycoside was treated with the snail enzyme, it lost both glucose and acetyl groups, and changed into gitoroside, which was determined by paper chromatography. It was then treated with strophanthobiase*[5] and by losing glucose only, it converted to a second glycoside which was found to be identical with monoacetylgitoroside*[1] on the paper chromatogram.

The isolated substance A-III′ was repeatedly recrystallized from hydrous methanol to colorless needles, m.p. 248~250°, $[\alpha]_D^{21}$ −2.6° (methanol). It has a bitter taste and is easily soluble in methanol-chloroform (1:1), soluble in methanol, and insoluble in ether and water. It gives positive Legal and Raymond reactions, and exhibits a colorless glacial acetic acid layer and a carmine-red sulfuric acid layer in the Keller-Kiliani reaction. Its negative Gregg-Gisvold reaction shows a complete absence of 2,6-deoxysugar. It gives positive Frèrejacque reaction, so it possesses an acyl group which was changed into hydroxamic acid and identified as an acetyl group on the paper chromatogram. Its ultraviolet spectrum exhibits the maximum absorption at 219 mμ (ethanol), indicating the presence of an α,β-unsaturated lactone. Its elemental analytical values and the acetyl determination agree with the formula of $C_{38}H_{58}O_{15}$, calculated as digitalinum verum monoacetate.

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*3 Grateful acknowledgement is expressed to Prof. Dr. A. Stoll and Dr. M. Okada for the kind donation of lanatoside-B.

*4 Product of Tokyo Kasei Co., Ltd.

*5 Grateful acknowledgement is expressed to Prof. Dr. T. Reichstein for the kind donation of strophanthobiase.

2) R. Tschesche, B. Niyomporn, H. Machleidt: Chem. Ber., **92**, 2258 (1959).
3) Part XII. A. Okano, *et al.*: This Bulletin, **7**, 226 (1959).
4) Part XX. K. Hoji: *Ibid.*, **9**, 296 (1961).

By drastic acid hydrolysis, substance A-III′ afforded dianhydrogitoxigenin as the aglycone, and digitalose and glucose as the sugars which were found to be identical with the known substances by paper chromatography. By the Mannich hydrolysis, substance A-III′ afforded gitoxigenin as the aglycone.

Substance A-III′ was acetylated by the usual method to an acetate, which was then recrystallized from acetone-ether to needles, m.p. 179~184°/225~229°. This acetate was found to be identical with digitalinum verum hexaacetate by the mixed fusion method.

From the foregoing results, substance A-III′ was thought to be digitalinum verum monoacetate and the two were compared by their melting points, optical rotation, and paper chromatographic Rf values, by which they were found to be identical. The mixed fusion result showed no depression and therefore, A-III′ is digitalinum verum monoacetate.

Digitalinum verum monoacetate is easily obtained from digitalinum verum hexaacetate by deacetylation with potassium hydrogencarbonate, but it is not found in natural products of the digitalis species. It is interesting that this glycoside was isolated from the seeds of *Digitalis purpurea*, though in a very small quantity.

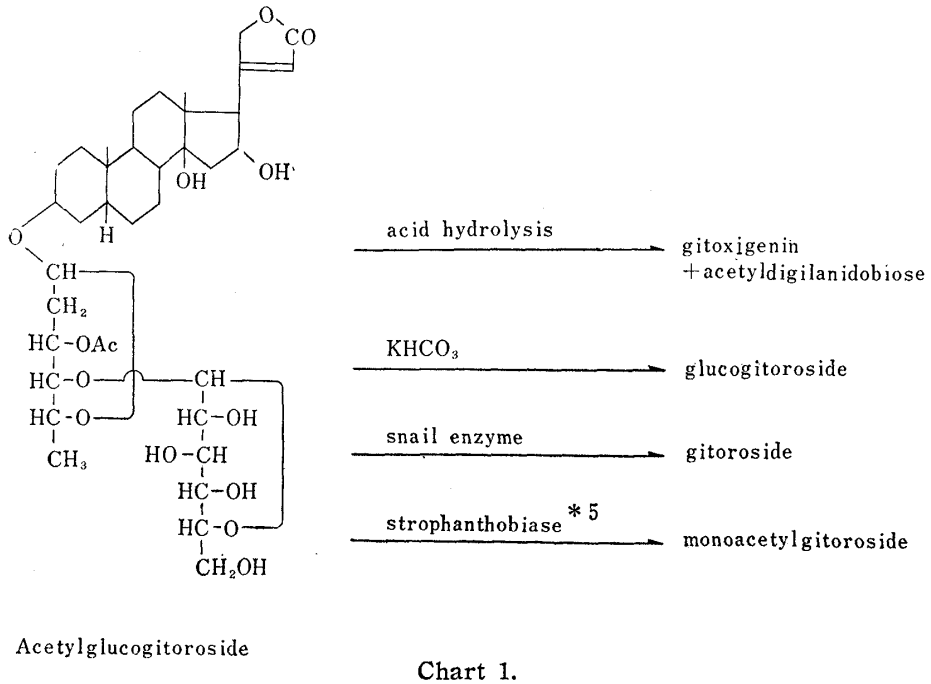

Chart 1.

Experimental*6

Acetylglucogitoroside (Substance A-II′)——The method of isolation was reported in Part XVII.[1] This substance was recrystallized from Me_2CO-Et_2O-petr. ether to a colorless powder, m.p. 199~205°, $[\alpha]_D^{21}$ +5.9° (c=1.32, MeOH), UV : λ_{max}^{EtOH} 219 mμ (log ε 4.13). *Anal.* Calcd. for $C_{37}H_{56}O_{14}\cdot 1\frac{1}{2}H_2O$: C, 59.11; H, 7.91; CH_3CO, 5.73. Found : C, 59.20; H, 8.03; CH_3CO, 5.83.

Estimation of the Acetyl Group in Acetylglucogitoroside——To a solution of 5 mg. of acetylglucogitoroside dissolved in 0.4 cc. of MeOH, 0.2 cc. of 5% EtOH solution of $NH_2OH\cdot HCl$ and 0.2 cc. of 12.5% NaOH in EtOH-H_2O (1:1) solution 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 thereby 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% aq. solution of $FeCl_3\cdot 6H_2O$ as the coloring agent. A spot (Rf 0.46) of hydroxamic acid of the acetyl group was detected and was respectively run with hydroxamic acid from digitalinum verum monoacetate as the standard substance (Rf 0.46).

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

Acid Hydrolysis of Acetylglucogitoroside——A solution of 70 mg. of acetylglucogitoroside dissolved in a mixture of 10 cc. of MeOH and 10 cc. of 0.1*N* H_2SO_4 was refluxed for 45 min. MeOH was distilled off in a reduced pressure, the residual mixture was kept warm at 80° for 10 min., and extracted 5 times with 10-cc. each of $CHCl_3$.

i) Aglycone : The combined $CHCl_3$ extract was washed with two 10-cc. portions of water and evaporated to dryness (20 mg.). The residue was recrystallized from hydr. MeOH to plates, m.p. 216~221°, which did not show any depression on admixture with gitoxigenin, m.p. 218~222°. The aglycone was found to be identical with gitoxigenin by paper chromatography. *Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found : C, 70.28; H, 9.18.

ii) Sugar moiety : The aqueous solution was deionized by Amberlite IR-4B (2 cc.) and evaporated to dryness. The residue (40 mg.) failed to crystallize, but it was identified chromatographically as acetyldigilanidobiose,[2] which had been obtained by acid hydrolysis of lanatoside-B*[3] revealed on the paper chromatogram (Fig. 1).

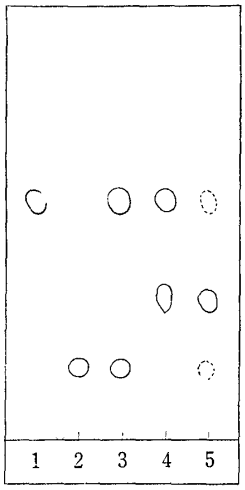

Fig. 1. Paper Partition Chromatography of Sugars
Moving Phase : Upper layer of BuOH-AcOH-H_2O (4:1:5)
Paper : Toyo Roshi No. 50.
Coloring agent : 5% HCl-MeOH (Gregg-Gisvold reaction)
1. digitoxose
2. digilanidobiose
3. sugars from purpurea glycoside-B
4. sugars from lanatoside-B
5. sugars from acetylglucogitoroside (substance A-II′)

Formation of Glucogitoroside from Acetylglucogitoroside——To a solution of 5 mg. of acetylglucogitoroside dissolved in 2 cc. of MeOH, a solution of 5 mg. of $KHCO_3$ dissolved in 0.3 cc. of H_2O was added and the mixture was allowed to stand for 15 days at room temperature. To this mixture, 2 cc. of H_2O was added, evaporated to about 1.5 cc. in a reduced pressure, and the residual mixture was extracted 3 times with 1 cc. each of a mixture of $CHCl_3$-BuOH (1:1). The combined extract solution was washed with a small amount of H_2O and evaporated in a reduced pressure. The residue was submitted to paper chromatography, using a mixture of BuOH-benzene (1:2) saturated with $HCONH_2$, and the spot (Rf 0.46) was found to be identical with that of glucogitoroside[3] (Rf 0.46).

Formation of Gitoroside from Acetylglucogitoroside——To a solution of 5 mg. of acetylglucogitoroside dissolved in 1 cc. of MeOH, 10 cc. of distilled H_2O was added and MeOH was evaporated from this solution in a reduced pressure. A filtrate obtained from 5 mg. of the snail enzyme powder, treated twice with 1 cc. each of distilled H_2O and filtered, was added to this solution, together with 0.5 cc. of toluene, and the mixture was allowed to stand at 32° for 1 day. The mixture was evaporated, the residue was submitted to paper chromatography, using xylene-MeCOEt (1:1) saturated with $HCONH_2$, and the spot (Rf 0.35) was identified as that of gitoroside (Rf 0.36).

Formation of Monoacetylgitoroside from Acetylglucogitoroside——To a solution of 1 mg. of acetylglucogitoroside dissolved in 1 cc. of H_2O, 2 mg. of strophanthobiase*[5] was added, together with one drop of toluene, and the mixture was allowed to stand at 32° for 2 days. The mixture was evaporated, submitted to paper chromatography, using xylene-MeCOEt (1:1) saturated with $HCONH_2$, and the spot (Rf 0.60) was identified as that of monoacetylgitoroside*[1] (Rf 0.60) obtained from purlanoside-B.

Substance A-III′ (Digitalinum verum Monoacetate)——The method of isolation was reported in Part XVII.[1] This substance was recrystallized from hydr. MeOH to colorless needles, m.p. 248~250°, $[\alpha]_D^{21}$ −2.6° (c=1.11, MeOH), UV : λ_{max}^{EtOH} 219 mμ (log ε 4.20). *Anal.* Calcd. for $C_{38}H_{58}O_{15}$: C, 60.46; H, 7.73; CH_3CO, 5.70. Found : C, 59.09; H, 7.73; CH_3CO, 5.98.

It showed the same Rf value as digitalinum verum monoacetate on the paper chromatogram. This substance showed no depression of melting point on admixture with digitalinum verum monoacetate, m.p. 247~251°, $[\alpha]_D^{21}$ −2.7° (c=1.28, MeOH).

Estimation of the Acetyl Group in Substance A-III′——Substance A-III′ was treated by the same method as for acetylglucogitoroside, a hydroxamic acid obtained was submitted to paper chromato-

graphy, and the spot (Rf 0.46) was identified with that from digitalinum verum monoacetate (Rf 0.46).

Drastic Acid Hydrolysis of Substance A-III′——To a solution of 1 mg. of substance A-Ⅲ′ dissolved in 0.5 cc. of MeOH, 0.5 cc. of 7% HCl was added and the mixture was refluxed for 6 hr. in CO_2 atmosphere, in a water bath. To the reaction mixture, 2 cc. of H_2O was added and MeOH was distilled off in a reduced pressure. The residue was extracted with three 1-cc. portions of $CHCl_3$.

Aglycone : The combined $CHCl_3$ extract was washed with a small quantity of H_2O and evaporated to dryness. It was submitted to paper chromatography, using a mixture of cyclohexane-AcOH-$CHCl_3$ (10:3:3), and the spot (Rf 0.58) was identical with that of dianhydrogitoxigenin (Rf 0.58).

Sugar moiety : The aqueous solution was treated with Amberlite IR-4B and the residue from its effluent was submitted to paper chromatography, using an upper layer of BuOH-AcOH-H_2O (4:1:5). Two spots revealed by coloring agent, at Rf 0.12 and 0.39, agreed respectively with the Rf values of glucose (Rf 0.12) and digitalose (Rf 0.39).

Mannich Hydrolysis of Substance A-III′——To a solution of 1 mg. of substance A-Ⅲ′ dissolved in 1 cc. of Me_2CO, 0.01 cc. of conc. HCl was added and the mixture was allowed to stand for 15 days at room temperature. To the reaction mixture, 1 cc. of H_2O was added and Me_2CO was distilled off in a reduced pressure. The residual mixture was extracted with two 1-cc. portions of $CHCl_3$. The combined $CHCl_3$ extract was washed with a small quantity of H_2O and $CHCl_3$ was distilled off in a reduced pressure. The residue was submitted to paper chromatography and the spot obtained was identical with that of gitoxigenin.

Acetylation of Substance A-III′——Substance A-Ⅲ′ (10 mg.) was acetylated by the usual method using 0.2 cc. of pyridine and 0.2 cc. of Ac_2O, and leaving the mixture at room temperature for 2 days. The pyridine-Ac_2O was distilled off in a reduced pressure. The residue was recrystallized from Me_2CO-Et_2O to colorless needles (10 mg.), m.p. 179～184°/225～229°. When it was mixed with digitalinum verum hexaacetate, m.p. 179～184°/228～234°, it melted at 179～182°/228～234°. *Anal.* Calcd. for $C_{48}H_{68}O_{20}$: C, 59.73; H, 7.12. Found : C, 60.01; H, 7.53.

The author expresses his sincerest gratitude to Dr. J. Shinoda, President of this Company, to Dr. T. Ishiguro, Director of this Laboratory, and to Dr. M. Shimizu, Acting Director, for kind encouragement during the course of this work, and to Dr. K. Miyatake, Director of the Yanagishima Factory, and Dr. A. Okano, for continued guidance. The author is much indebted to Messrs. T. Miki and A. Sakashita for technical help and to Messrs. B. Kurihara and K. Abe for elemental analyses.

Summary

The structure of substances A-Ⅱ′ and A-Ⅲ′, newly isolated from digitalis seeds, was examined. It was found that the mild acid hydrolysis of substance A-Ⅱ′ afforded gitoxigenin and acetyldigilanidobiose, and by strophanthobiase, this glycoside changid to monoacetylgitoroside. Therefore, substance A-Ⅱ′ is formulated as gitoxigenin glucosido-acetyldigitoxoside and it was named acetylglucogitoroside.

Substance A-Ⅲ′ was examined by drastic acid hydrolysis and Mannich hydrolysis. Its acetylation gave digitalinum verum hexaacetate. Further, this glycoside was found to be identical with digitalinum verum monoacetate.

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