treated with three 2-cc. portions of acetate buffer solution (pH 5.4), was added to the solution of substance C-0, together with 1 cc. of toluene, and the mixture was allowed to stand at 32° for 5 days. The solution was evaporated to dryness and the residue was submitted to paper chromatography using a mixture of xylene-MeCOEt (1:1) saturated with formamide on a filter paper impregnated with formamide-$Me_2CO$ (1:4). The spot (Rf 0.28) corresponding to odoroside-H[*4] (Rf 0.275) was found.

The writer expresses deep 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 writer 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 substance C-0, newly isolated from the digitalis seeds, was examined. From the experimental results, this substance was identified as odorobioside-G, a digitoxigenin glucosido-digitaloside.

(Received July 5, 1960)

---

*UDC 547.918:615.711.5*

# 44. **Kazuhiko Hoji** : Studies on the Constituents of *Digitalis purpurea* L. XIX.[*1] New Cardiotonic Glycosides, Purlanoside-A and Purlanoside-B from Digitalis Seeds.

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

It was reported in the preceding paper[1] that several new cardiotonic glycosides had been isolated from the seeds of *Digitalis purpurea* L. The present investigation was undertaken to determine the structures of substances B-0 and A-II.

Substance B-0 did not crystallize and was obtained in a syrupy form, but the result of paper chromatography showed it to be homogeneous. It gives positive Legal and Raymond reactions, and exhibits a dark blue glacial acetic acid layer and a brown sulfuric acid layer in the Keller-Kiliani reaction, the same as digitoxin. Its positive Gregg-Gisvold reaction shows that it contains some 2,6-deoxysugar. Its ultraviolet spectrum exhibits a maximum absorption at 218 mμ (ethanol), indicating the presence of an $\alpha,\beta$-unsaturated lactone, characteristic to cardiotonic glycosides. Its optical rotation is $[\alpha]_D^{21}$ $+22.0°$ (75% EtOH) and its analytical values agree with $C_{49}H_{76}O_{19}$.

It gives positive Frèrejacque reaction, indicating the presence of an acyl group. The acyl group was changed into hydroxamic acid and identified as the acetyl group on paper chromatogram. The quantitative determination of acetyl group agreed with one mole.

Acetylation of substance B-0 by the usual method afforded colorless needles, m.p. 146~152°, from hydrous methanol. This acetate was identified with purpurea glycoside-A acetate[2] by mixed fusion.

---

[*1] Part XVIII: This Bulletin, **9**, 289 (1961).

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

1) Part XVII: This Bulletin, **9**, 276 (1961).

2) Part V: *Ibid.*, **5**, 171 (1957).

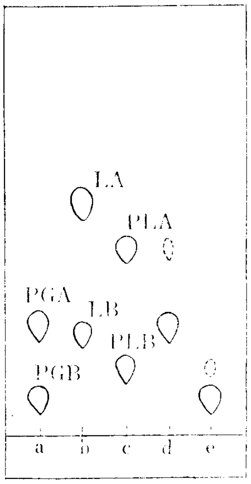


Fig. 1. Paper Partition Chromatography

Solvent : BuOH-toluene (1:2) saturated with $HCONH_2$
Paper : Toyo Roshi No. 50 filter parer, impregnated with $HCONH_2$-$Me_2CO$ (1:4) and $Me_2CO$ evaporated.
Method : Ascending method
Coloring agent : 20% $SbCl_3$ and Raymond reaction

a : Purpurea glycoside-A (PGA) and -B (PGB)
b : Lanatoside-A (LA) and -B (LB)[*3]
c : Purlanoside-A (PLA) and -B (PLB)
d : Purpurea glycoside-A from purlanoside-A by $KHCO_3$ deacetylation
e : Purpurea glycoside-B from purlanoside-B by $KHCO_3$ deacetylation

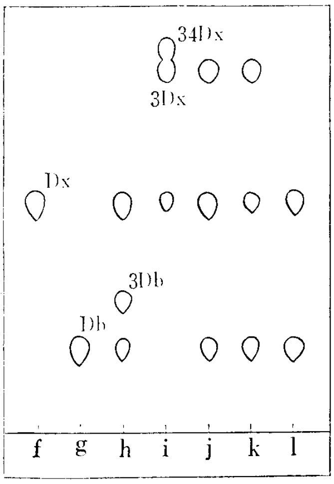


Fig. 2. Paper Partition Chromatography of Sugars

Solvent : BuOH-AcOH-$H_2O$ (4:1:5), upper layer
Paper : Toyo Roshi No. 50 filter paper
Method : Ascending method
Coloring agent : 1% HCl-MeOH solution (Gregg-Gisvold reaction)

f : Digitoxose (Dx)
g : Digilanidobiose (Db)
h : Sugars from lanatoside-B[*3] (3Db : acetyldigilanidobiose)
i : Sugars from gitoxin acetate[2] (3Dx : 3-acetyldigitoxose ?, 34Dx : 3,4-diacetyldigitoxose ?)
j : Sugars from purlanoside-A
k : Sugars from purlanoside-B
l : Sugars from purpurea glycoside-B

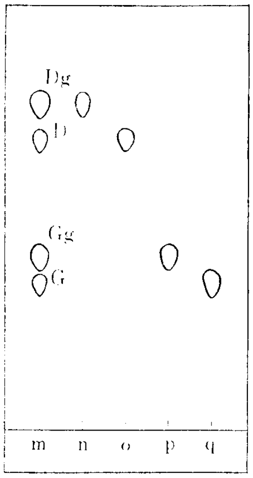


Fig. 3. Paper Partition Chromatography of Secondary Glycosides and Aglycones

Solvent : MeCOEt-xylene (1:1) 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 : 20% $SbCl_3$ and Raymond reaction

m : Digitoxigenin (Dg), digitoxin (D), gitoxigenin (Gg), and gitoxin (G)
n : Aglycone from purlanoside-A
o : Secondary glycoside from purlanoside-A, by the snail enzyme hydrolysis
p : Aglycone from purlanoside-B
q : Secondary glycoside from purlanoside-B, by the snail enzyme hydrolysis

Deacetylation of substance B-0 was effected with potassium hydrogencarbonate and the deacetylated glycoside was chromatographically identified with purpurea glycoside-A (Fig. 1).

By the hydrolysis of substance B-0 with snail enzyme, digitoxin was obtained by elimination of an acetyl group and a glucose (Fig. 3).

The foregoing results have shown that substance B-0 is composed of purpurea glycoside-A and one mole of acetyl group. Lanatoside-A (digilanide-A) had been isolated from the leaves of *Digitalis lanata* by Stoll, *et al.*[3] and this glycoside is composed of the same components, but these two glycosides, lanatoside-A and substance B-0 are different in their solubility in various solvents and their paper chromatography differed widely in the Rf values (Fig. 1).

A mild acid hydrolysis of substance B-0 afforded digitoxigenin as the aglycone and three sugars, digilanidobiose, digitoxose, and an unknown sugar which runs faster than digitoxose on paper chromatogram. On comparison with a sugar obtained from gitoxin acetate,[2] it seemed that the unknown sugar may be 3-acetyl-digitoxose. In this case, acetyldigilanidobiose[4] was not formed, but its spot was found from the hydrolysis product of lanatoside-A[*3] (Fig. 2). It had already been found that the acetyl group of lanatoside-A is situated at the third digitoxose, but the present results have shown that the acetyl group of substance B-0 is situated at the first or the second digitoxose. Therefore, substance B-0 is a new glycoside and has been named purlanoside-A.

Substance A-II was obtained in a syrupy form, but it was found to be homogeneous by the result of paper chromatography. It gives positive Legal, Raymond, and Gregg-Gisvold reactions and exhibits a dark blue glacial acetic acid layer and a carmine-red sulfuric acid layer in the Keller-Kiliani reaction, the same as gitoxin. Its ultraviolet spectrum exhibits a maximum absorption at 218 mμ (ethanol), indicating the presence of an $\alpha,\beta$-unsaturated lactone. Its optical rotation shows $[\alpha]_D^{21}$ +27.8° (EtOH) and its analytical values agreed with $C_{49}H_{76}O_{20}$. It gives positive Frèrejacque reaction. Its acyl group was changed into hydroxamic acid and identified with acetyl group by paper chromatography. Quantitative determination of the acetyl showed one mole.

Substance A-II was acetylated by the usual method and the acetate obtained was recrystallized from ethanol to colorless needles, m.p. 149~153°/222~232°, which were identified as purpurea glycoside-B acetate.[2]

Deacetylation with potassium hydrogencarbonate was carried out and the deacetylated glycoside was identified with purpurea glycoside-B by paper chromatography (Fig. 1).

By hydrolysis with snail enzyme, an acetyl group and a glucose were eliminated from substance A-II and gitoxin was found on the paper chromatogram (Fig. 3).

The foregoing results have shown that substance A-II is composed of purpurea glycoside-B and one mole of acetyl group. Lanatoside-B had been isolated from the leaves of *Digitalis lanata* by Stoll, *et al.*[3] and it is composed of the same components, but these two glycosides are different in their solubility in various solvents and their paper chromatography differed widely in the Rf values (Fig. 1).

A mild acid hydrolysis of substance A-II afforded gitoxigenin as the aglycone and three sugars, digilanidobiose, digitoxose, and 3-acetyl-digitoxose, the same as substance B-0. Therefore, the acetyl group of substance A-II is in the first or the second digitoxose, differing from lanatoside-B. The substance A-II was therefore named purlanoside-B.

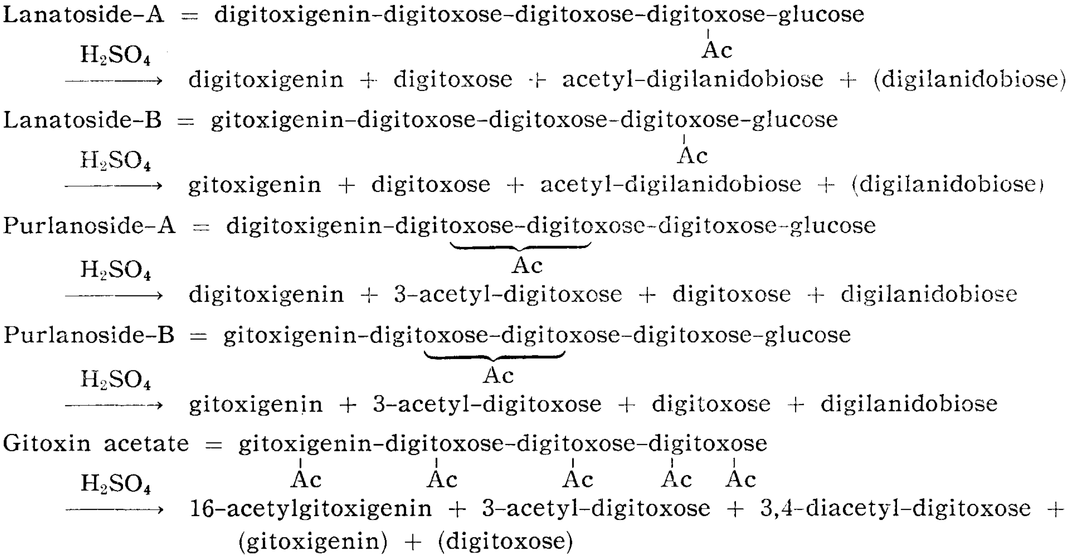
Acetyl-glycosides, lanatosides, had been isolated from the leaves of *Digitalis lanata*,

---

[*3] Grateful acknowledgement is expressed to Prof. Dr. A. Stoll and to Dr. M. Okada for kind donation of valuable samples of lanatosides-A and -B.

3) A. Stoll, W. Kreis : Helv. Chim. Acta, **16**, 1049 (1933).

4) R. Tschesche, B. Niyomporn, H. Machleidt : Chem. Ber., **92**, 2258 (1959).



$$\text{Lanatoside-A} = \text{digitoxigenin-digitoxose-digitoxose-}\underset{\mathrm{Ac}}{\underset{|}{\text{digitoxose}}}\text{-glucose}$$

$$\xrightarrow{H_2SO_4} \text{digitoxigenin} + \text{digitoxose} + \text{acetyl-digilanidobiose} + \text{(digilanidobiose)}$$

$$\text{Lanatoside-B} = \text{gitoxigenin-digitoxose-digitoxose-}\underset{\mathrm{Ac}}{\underset{|}{\text{digitoxose}}}\text{-glucose}$$

$$\xrightarrow{H_2SO_4} \text{gitoxigenin} + \text{digitoxose} + \text{acetyl-digilanidobiose} + \text{(digilanidobiose)}$$

$$\text{Purlanoside-A} = \text{digitoxigenin-}\underbrace{\text{digitoxose-digitoxose}}_{\mathrm{Ac}}\text{-digitoxose-glucose}$$

$$\xrightarrow{H_2SO_4} \text{digitoxigenin} + \text{3-acetyl-digitoxose} + \text{digitoxose} + \text{digilanidobiose}$$

$$\text{Purlanoside-B} = \text{gitoxigenin-}\underbrace{\text{digitoxose-digitoxose}}_{\mathrm{Ac}}\text{-digitoxose-glucose}$$

$$\xrightarrow{H_2SO_4} \text{gitoxigenin} + \text{3-acetyl-digitoxose} + \text{digitoxose} + \text{digilanidobiose}$$

$$\text{Gitoxin acetate} = \underset{\mathrm{Ac}}{\underset{|}{\text{gitoxigenin}}}\text{-}\underset{\mathrm{Ac}}{\underset{|}{\text{digitoxose}}}\text{-}\underset{\mathrm{Ac}}{\underset{|}{\text{digitoxose}}}\text{-}\underset{\mathrm{Ac}\ \ \mathrm{Ac}}{\underset{|\ \ \ \ |}{\text{digitoxose}}}$$

$$\xrightarrow{H_2SO_4} \text{16-acetylgitoxigenin} + \text{3-acetyl-digitoxose} + \text{3,4-diacetyl-digitoxose} + \text{(gitoxigenin)} + \text{(digitoxose)}$$

Chart 1. Acid Hydrolysis of Acetylated Glycosides

but were not isolated from the leaves of *D. purpurea*, except digacetinin.[5] Formyl-glycosides belonging to the gitaloxigenin series had also been found. It is interesting that acetylated glycosides, purlanosides-A and -B, were isolated from the seeds of *D. purpurea* and that they are isomers of lanatosides-A and -B.

## Experimental[*4]

**Purlanoside-A (Substance B-0)**——The method of isolation was reported in Part XVII[1] of this series. This is a colorless syrupy substance, $[\alpha]_D^{21}$ +22.0°(c=1.55, 75% EtOH), $[\alpha]_D^{21}$ +24.6°(c=1.37, MeOH). UV: $\lambda_{max}^{EtOH}$ 217 mμ (log ε 4.23). *Anal.* Calcd. for $C_{49}H_{76}O_{19}$: C, 60.71; H, 7.91; $CH_3CO$, 4.44. Found: C, 61.23; H, 7.76; $CH_3CO$, 4.95.

**Estimation of the Acetyl Group in Purlanoside-A**——To a solution of 5 mg. of purlanoside-A 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% EtOH-$H_2O$ (1:1) solution of NaOH were added, and the mixture was allowed to stand at room temperature for 20 min. The reaction mixture was neutralized with 7% HCl-MeOH solution, a 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% solution of $FeCl_3 \cdot 6H_2O$ as the coloring agent. The spot (Rf 0.46) of hydroxamic acid derived from the acetyl group was detected and was identified with that of hydroxamic acid from digitalinum verum monoacetate, as the standard substance (Rf 0.46).

**Acetylation of Purlanoside-A**——Purlanoside-A (100 mg.) was acetylated by the usual method, using 1.5 cc. of pyridine and 1 cc. of $Ac_2O$ and leaving at room temperature for 5 days. The reaction mixture was evaporated until dry and the residue was recrystallized from hydr. MeOH to needles (55 mg.), m.p. 146～152°. When these needles were mixed with purpurea glycoside-A acetate,[2] m.p. 148～153°, the melting point was 145～152°.

**Formation of Purpurea Glycoside-A from Purlanoside-A**——Purlanoside-A (10 mg.) was dissolved in 2 cc. of MeOH, a solution of $KHCO_3$ (10 mg.) dissolved in 0.2 cc. of $H_2O$ was added, and allowed to stand for 14 days at room temperature. To this mixture, 5 cc. of $H_2O$ was added, the mixture was evaporated to about 5 cc. in a reduced pressure, and the residual solution was extracted 3 times with 3 cc. each of a mixture of BuOH-$CHCl_3$ (1:1). The combined extract was washed with a small amount of $H_2O$ and concentrated under a reduced pressure. The residue was submitted to paper chromatography and revealed a spot corresponding to purpurea glycoside-A as shown in Fig. 1.

**Acid Hydrolysis of Purlanoside-A**——A solution of 10 mg. of purlanoside-A 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, the residue was warmed at 80° for 10 min. for decomposition of methyldigitoxoside formed, and extracted 5 times with 2 cc. each of $CHCl_3$.

---

[*4] All m.p.s were measured on a Kofler block and are uncorrected.

5) R. Tschesche, W. Hammerschmidt, G. Grimmer: Ann., **614**, 136 (1958).

i) Aglycone : The combined $CHCl_3$ extract was washed with a small amount of $H_2O$ and evaporated until dry. The residue was submitted to paper chromatography and revealed a spot identical with that of digitoxigenin (Fig. 3).

ii) Sugar Moiety : The aqueous solution was deionized by Amberlite IR-4B (1 cc.), evaporated until dry, and the syrupy sugar was submitted to paper chromatography, by which three spots were found. The one was identified with digilanidobiose, the second as digitoxose, and the third as 3-acetyl-digitoxose (Fig. 2).

**Formation of Digitoxin from Purlanoside-A**——To a solution of 10 mg. of purlanoside-A dissolved in 20 cc. of distilled water, a filtrate obtained from 10 mg. of a snail enzyme powder and 2 drops of toluene were added. This mixture was allowed to stand at 32° for 5 days and evaporated until dry. The residue was extracted with MeOH-$CHCl_3$ (1:1) and MeOH-$CHCl_3$ solution was evaporated. The residue was submitted to paper chromatography and the spot found was identified with digitoxin (Fig. 3).

**Purlanoside-B (Substance A-II)**——The method of isolation was reported in Part XVII.[1] This comes as a colorless syrupy substance, $[\alpha]_D^{21}$ +27.8° (c=1.17, EtOH). UV : $\lambda_{max}^{EtOH}$ 218 mμ (log $\varepsilon$ 4.18). *Anal.* Calcd. for $C_{49}H_{76}O_{20}$ : C, 59.72; H, 7.78; $CH_3CO$, 4.37. Found : C, 60.34; H, 8.00; $CH_3CO$, 4.89.

**Estimation of the Acetyl Group in Purlanoside-B**——The same method as used for purlanoside-A was used for purlanoside-B. Hydroxamic acid from purlanoside-B (Rf 0.46) was found on the paper chromatogram and was identified with that from digitalinum verum monoacetate (Rf 0.46).

**Acetylation of Purlanoside-B**——Purlanoside-B (150 mg.) was acetylated by the usual method of leaving at room temperature with 2 cc. of pyridine and 2 cc. of $Ac_2O$ for 5 days. The reaction mixture was evaporated until dry and the residue was recrystallized from EtOH to needles (80 mg.), m.p. 149~153°/222~232°; $[\alpha]_D^{20}$ +35.8° (c=1.23, $CHCl_3$). This substance was mixed with purpurea glycoside-B acetate,[2] m.p. 149~153°/228~235°, and melted at 150~156°/222~233°. *Anal.* Calcd. for $C_{63}H_{90}O_{27}$: C, 59.16; H, 7.05. Found : C, 58.85; H, 7.24.

**Formation of Purpurea Glycoside-B from Purlanoside-B**——Purlanoside-B (10 mg.) was deacetylated by the same method as for purlanoside-A and the reaction mixture was submitted to paper chromatography. The spot revealed was identified with purpurea glycoside-B as shown in Fig. 1.

**Acid Hydrolysis of Purlanoside-B**——Purlanoside-B (10 mg.) was hydrolized by 0.05*N* $H_2SO_4$ and MeOH mixture, the same as for purlanoside-A. Gitoxigenin was found as the aglycone (Fig. 3) and three sugars, digilanidobiose, digitoxose, and 3-acetyl-digitoxose were found on paper chromatogram (Fig. 2).

**Formation of Gitoxin from Purlanoside-B**——Purlanoside-B (10 mg.) was treated with the snail enzyme, the same as for purlanoside-A, and the reaction mixture was submitted to paper chromatography. The spot revealed was identified with gitoxin.

The writer expresses deep 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 writer 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 structures of substances B-0 and A-II, newly isolated from the digitalis seeds, were examined. The substance B-0 has an acyl group, because its Frèrejacque reaction was positive and this acyl was determined as an acetyl group by hydroxamic acid method. It afforded purpurea glycoside-A by deacetylation with potassium hydrogencarbonate and the substance B-0 was found to be composed of the same components as lanatoside-A, but they were different. Its mild acid hydrolysis afforded digitoxigenin, and digilanidobiose, digitoxose, and 3-acetyl-digitoxose. Therefore, substance B-0 is formulated as monoacetyl-purpurea glycoside-A, an isomer of lanatoside-A, and was designated as purlanoside-A.

Substance A-II was examined by the same method. It was found to be an isomer of lanatoside-B and was named purlanoside-B.

(Received July 5, 1960)