UDC 615.711.5 : 547.918

91. Kazuhiko Hoji : Studies on the Constituents of *Digitalis purpurea* L. XXIV.*1 On the Structures of Purlanosides-A and -B.

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

It was reported in Part XIX[1] that purlanosides-A and -B were isomers of lanatosides-A and -B, and that their acetyl group was situated at the first or second digitoxose. It was found in the present series of work, that the acetyl group is in the first digitoxose, by the result of partial acid hydrolysis.

Kaiser, *et al.*[2] reported that digitoxigenin monodigitoxoside and bisdigitoxoside were obtained from digitoxin by partial acid hydrolysis, and gitoxigenin monodigitoxoside and bisdigitoxoside, from gitoxin.

Therefore, similar partial acid hydrolysis was used on purlanoside-A and the product showed three new spots on paper chromatogram. The substances corresponding to these spots were provisionally named substances A-1, A-2, and A-3, according to their Rf values, as shown in Fig. 1. Substances A-1 and A-2 were not obtained from lanatoside -A by the same hydrolysis.

The hydrolyzed mixture was fractionated by a column partition chromatography using formamide-saturated benzene, and substance A-1 was separated and recrystallized from hydrous methanol to colorless needles, m.p. 115~120°, $[\alpha]_D^{25}$ +30.2° (methanol). Substance A-1 gives positive Legal and Raymond reactions and exhibits the maximum absorption (in ethanol) at 218 mμ, which is generally characteristic to cardiotonic glycoside. Its positive Gregg-Gisvold reaction shows the presence of 2,6-deoxysugar. In the Keller-Kiliani reaction, it exhibits a dark blue glacial acetic aicd layer and a brown sulfuric acid layer, the same as digitoxin. By the positive Frèrejacque reaction, it is assumed to possess an acetyl group. Its elemental analysis values agree with the formula $C_{31}H_{46}O_8$, calculated as monoacetyldigitoxigenin monodigitoxoside.

By deacetylation with potassium hydrogencarbonate, this glycoside changed into digitoxigenin monodigitoxoside*3 which was identified by paper chromatography. By mild acid hydrolysis, it afforded digitoxigenin as the aglycone and acetyldigitoxose as the sugar. In this experiment, it was found that most of the acetyldigitoxose was extracted by chloroform. Therefore, the chloroform extract had to be examined on the paper chromatogram, not only for the aglycone, but also for the sugar.

The foregoing results clearly show that substance A-1 is monoacetyldigitoxigenin monodigitoxoside. As this glycoside is obtained from purlanoside-A by hydrolysis, it is proved that the acetyl group is in the first digitoxose. If the acetyl group is in the second or third digitoxose, this glycoside will not be obtained. Therefore, the structure of purlanoside-A is formulated as digitoxigenin glucosido-bisdigitoxosido-acetyldigitoxoside.

Substance A-2 was formed in a very small amount and could not be isolated. This substance is considered to be a monoacetyldigitoxigenin bisdigitoxoside (digitoxigenin digitoxosido-acetyldigitoxoside). Substance A-3 was found to be identical with digitoxigenin by paper chromatography.

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*1 Part XXIII : This Bulletin, **9**, 524 (1961).

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*3 Grateful acknowledgement is expressed to Dr. F. Kaiser for the kind donation of digitoxigenin monodigitoxoside and bisdigitoxoside.

1) Part XIX. K. Hoji : This Bulletin, **9**, 291 (1961).

2) F. Kaiser, E. Haack, H. Spingler : Ann., **603**, 75 (1957).

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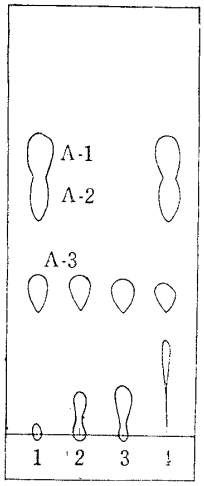

Fig. 1. Paper Partition Chromatography after Partial Acid Hydrolysis
Solvent I : Benzene saturated with $HCONH_2$
Paper : Toyo Roshi No. 50, impregnated with $HCONH_2$-Me_2CO (1:4)
Method : Ascending method at 20~25°
Coloring agent : Raymond reaction

1. Partial acid hydrolysis of purlanoside-A
2. Partial acid hydrolysis of purpurea glycoside-A
3. Partial acid hydrolysis of lanatoside-A
4. Partial acid hydrolysis of acetyldigitoxin-γ

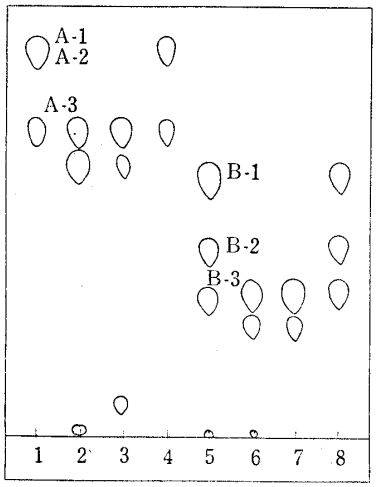

Fig. 2. Paper Partition Chromatography after Partial Acid Hydrolysis
Solvent II : MeCOEt-xylene (1:1) saturated with $HCONH_2$
Paper : Toyo Roshi No. 50, impregnated with $HCONH_2$-Me_2CO (1:4)
Method : Ascending method at 20~25°
Coloring agent : Raymond reaction or 20% $SbCl_3$-$CHCl_3$ solution

5. Partial acid hydrolysis of purlanoside-B
6. Partial acid hydrolysis of purpurea glycoside-B
7. Partial acid hydrolysis of lanatoside-B
8. Partial acid hydrolysis of acetyldigitoxin-γ

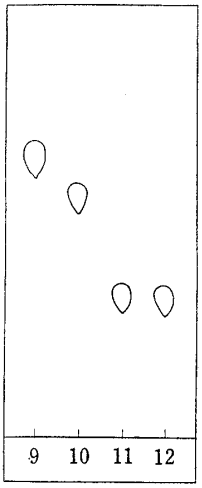

Fig. 3. Paper Partition Chromatography of Substances obtained from Purlanoside-A
Solvent I.

9. Substance A-1 (monoacetyldigitoxigenin-monodigitoxoside)
10. Substance A-2 (monoacetyldigitoxigenin-bisdigitoxoside ?)
11. Substance A-3 (digitoxigenin)
12. Digitoxigenin

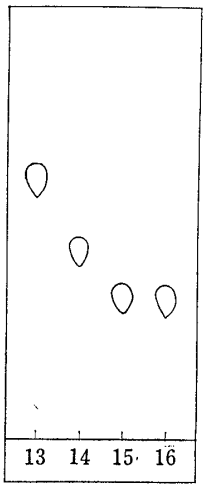

Fig. 4. Paper Partition Chromatography of Substances obtained from Purlanoside-B
Solvent II.

13. Substance B-1 (monoacetylgitoroside)
14. Substance B-2 (monoacetylgitoxigenin-bisdigitoxoside)
15. Substance B-3 (gitoxigenin)
16. Gitoxigenin

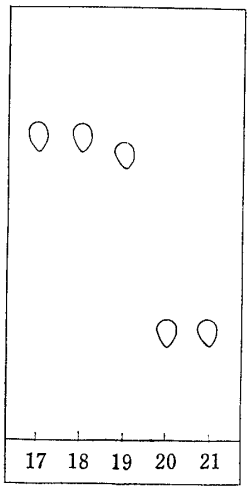

Fig. 5. Paper Partition Chromatography after Deacetylation with Potassium Hydrogencarbonate
Solvent II.

17. Deacetylation of substance A-1
18. Digitoxigenin-monodigitoxoside*3
19. Digitoxigenin-bisdigitoxoside*3
20. Deacetylation of substance B-1
21. Gitoroside

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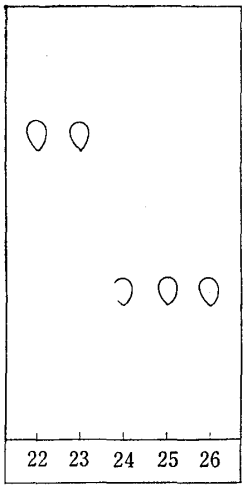

Fig. 6. Paper Partition Chromatography of Aglycones
Solvent II.

22. Aglycone of substance A-1
23. Digitoxigenin
24. Aglycone of substance B-1
25. Aglycone of substance B-2
26. Gitoxigenin

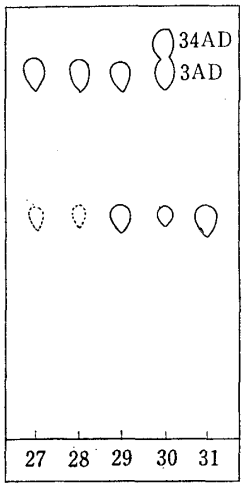

Fig. 7. Paper Partition Chromatography of Sugars
Solvent : BuOH-AcOH-H_2O (4:1:5)
Paper : Toyo Roshi No. 50
Method : Ascending method at 20~25°
Coloring agent : Gregg-Gisvold reaction
27. Sugar of substance A-1
28. Sugar of substance B-1
29. Sugars of substance B-2
30. Sugars of gitoxin acetate
31. Digitoxose
34AD=3,4-diacetyldigitoxose ?
3AD=3-acetyldigitoxose ?

Purlanoside-B was decomposed by the same partial acid hydrolysis and the reaction material showed three new spots on the paper chromatogram. These substances were provisionally named B-1, B-2, and B-3, according to their Rf values, as shown in Fig. 2. Substances B-1 and B-2 were not formed from lanatoside-B by the same hydrolysis.

The hydrolyzed mixture was submitted to partition column chromatography using formamide-saturated benzene-methyl ethyl ketone, and the separated substance B-1 was recrystallized from hydrous methanol to colorless needles, m.p. 176~181°, $[\alpha]_D^{25}$ +14.8° (methanol). Substance B-1 gives the color reaction of monoacetyldigitoxigenin monodigitoxoside (substance A-1), except a carmine red sulfuric acid layer in the Keller-Kiliani reaction. Its elemental analytical values agree with the formula $C_{31}H_{46}O_9$, calculated as monoacetylgitoroside. The quantitative analysis of digitoxose shows that it possesses one mole of digitoxose.

This glycoside was deacetylated by potassium hydrogencarbonate and the spot corresponding to gitoroside[3,4] was found on the paper chromatogram. By mild acid hydrolysis, it afforded gitoxigenin and acetyldigitoxose.

From the foregoing results, substance B-1 is monoacetylgitoroside. From the fact that purlanoside-B afforded this glycoside by hydrolysis, it is assumed that the acetyl group is in the first digitoxose. Therefore, purlanoside-B is formulated as gitoxigenin glucosido-bisdigitoxosido-acetyldigitoxoside.

Substance B-2 gives the same color reactions as monoacetylgitoroside (substance B-1) and it was obtained as a colorless powder, m.p. 171~173°, from methyl ethyl ketone-petroleum ether. Its elemental analysis and quantitative analysis of digitoxose agree with the formula $C_{37}H_{56}O_{12}$, calculated as monoacetylgitoxigenin bisdigitoxoside. By acid hydrolysis, it afforded gitoxigenin as the aglycone, and digitoxose and acetyldigitoxose as the sugar. According to these results its structure is formulated as gitoxigenin digitoxosido-acetyldigitoxoside.

Substance B-3 was recrystallized from acetone-petroleum ether to colorless plates, m.p. 220~227°. It was found to be identical with gitoxigenin by paper chromatography and mixed fusion.

Acetyldigitoxin-γ,[5] which was obtained from purlanoside-A, also showed a spot of

3) D. Satoh, T. Wada, H. Ishii, Y. Oyama, T. Okumura : This Bulletin, **5**, 253 (1957) (gitoroside is the same substance as gitoxigenin monodigitoxoside). J.E. Murphy : J. Am. Pharm. Assoc., Sci. Ed., **46**, 170 (1957) (the same substance was named gitoside).
4) Part XII. A. Okano, *et al.* : This Bulletin, **7**, 226 (1959) (the same substance was obtained from gitorocellobioside).
5) Part XX. K. Hoji : This Bulletin, **9**, 296 (1961).

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monoacetyldigitoxigenin monodigitoxoside by the same partial acid hydrolysis. In the case of acetylgitoxin-γ[5] obtained from purlanoside-B, monoacetylgitoroside was found on the paper chromatogram. Therefore, it is also assumed that the acetyl group of these glycosides is in their first digitoxose.

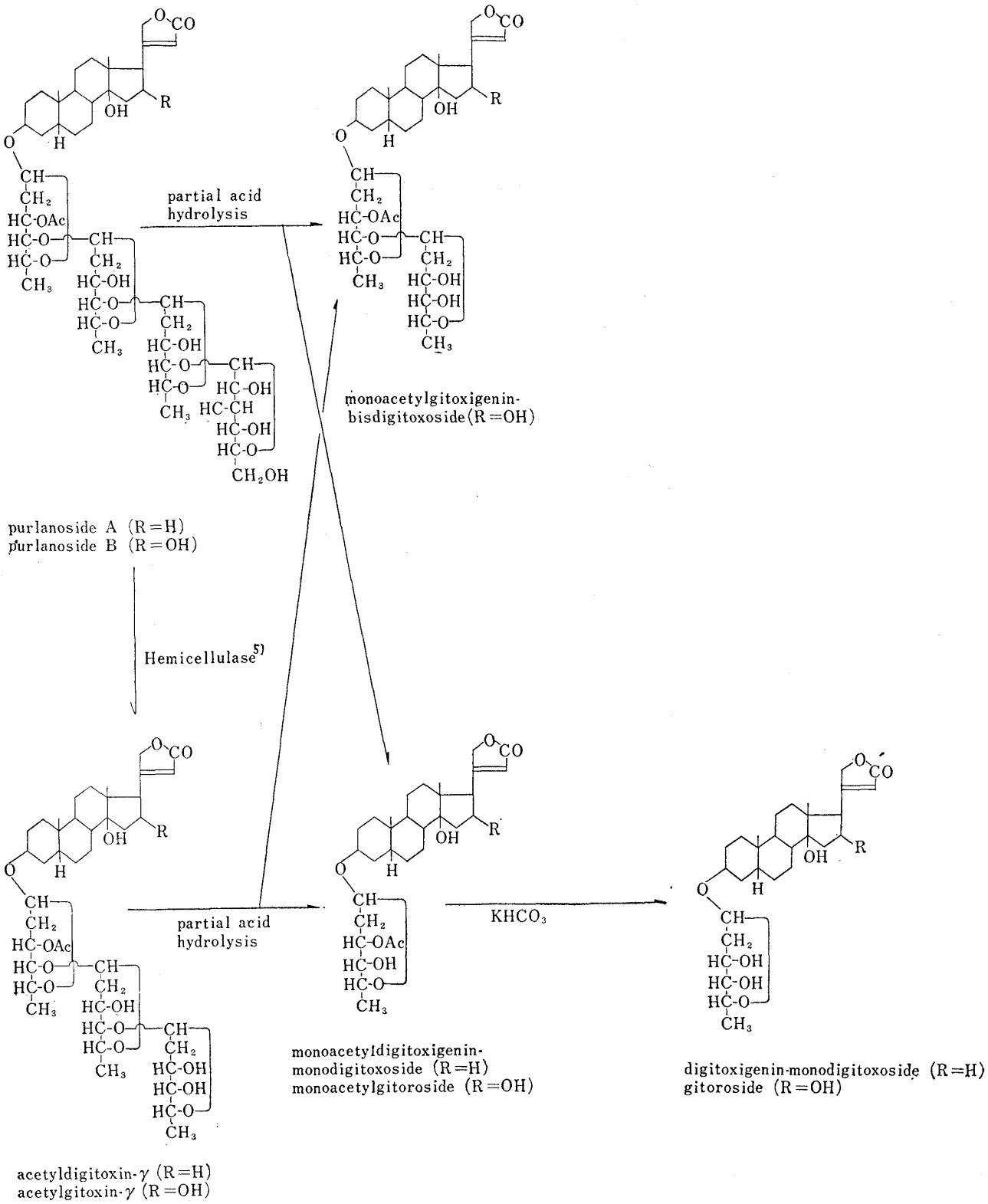

Chart 1. Partial Acid Hydrolysis of Purlanosides

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The monoacetyldigitoxigenin monodigitoxoside, monoacetylgitoroside, and monoacetylgitoxigenin bisdigitoxoside obtained in the present experiment are new glycosides.

Experimental*4

Partial Acid Hydrolysis of Purlanoside-A——To a solution of 500 mg. of purlanoside-A dissolved in 180 cc. of MeOH, 30 cc. of 0.05*N* H_2SO_4 was added and the mixture was refluxed for 2.5 hr. To the reaction mixture, 180 cc. of H_2O was added and extracted with five 50-cc. portions of $CHCl_3$. The combined extract was washed with a small quantity of H_2O, evaporated to dryness (280 mg.), and submitted to paper chromatography. Three spots, substances A-1, A-2, and A-3, were revealed. The combined extract was chromatographed over a Celite column (30 g. of Celite and 30 cc. of formamide-H_2O (4:1)), and 30-cc. fractions were collected. Fraction Nos. 2～4 contained substance A-1 alone. Fraction No. 6 contained substance A-2 and A-3, and fraction Nos. 7～12 contained substance A-3.

Substance A-1 (Monoacetyldigitoxigenin Monodigitoxoside)——The above-mentioned Fraction Nos. 2～4 was recrystallized from hydr. MeOH to colorless needles (75 mg.), m.p. 115～120°, $[\alpha]_D^{25}$ +30.2°(c=0.83, MeOH); UV : λ_{max}^{EtOH} 218 mμ (log ε 4.20). *Anal.* Calcd. for $C_{31}H_{46}O_8$: C, 68.10; H, 8.48; digitoxose, 27.10. Found : C, 68.20; H, 8.13; digitoxose,*5 28.28.

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

Deacetylation of Substance A-1——To a solution of 5 mg. of substance A-1 dissolved in 2 cc. of MeOH, a solution of 5 mg. of $KHCO_3$ in 0.5 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 solution was evaporated to about 5 cc. in a reduced pressure, and the residual mixture was extracted 3 times with 5 cc. each of $CHCl_3$. The combined extract was washed with a small amount of H_2O and concentrated in a reduced pressure. The residue was submitted to paper chromatography and a spot identical with that of digitoxigenin monodigitoxoside*3 was found.

Acid Hydrolysis of Substance A-1——Substance A-1 (5 mg.) was dissolved in 2 cc. of MeOH and 1 cc. of 0.1*N* H_2SO_4 was added. This mixture was refluxed on a water bath for 45 min., 2 cc. of H_2O added, and MeOH was evaporated. The residual mixture was extracted with three 2-cc. portions of $CHCl_3$.

The $CHCl_3$ extract was submitted to paper chromatography. On the paper chromatogram, a spot corresponding to digitoxigenin and another considered to be of acetyldigitoxose were found.

The aqueous layer was deionized by Amberlite IR-4B and submitted to paper chromatography. Spots corresponding to digitoxose and acetyldigitoxose were found.

Partial Acid Hydrolysis of Purlanoside-B——The same reaction as for purlanoside-A was used on 800 mg. of purlanoside-B. The reaction product (410 mg.) was obtained by extraction with $CHCl_3$. It was submitted to paper chromatography and three spots for substances B-1, B-2, and B-3, were found. This was chromatographed over a Celite column (100 g. of Celite and 100 cc. of formamide-H_2O (4:1)), eluted with MeCOEt-benzene (1:4), and 50-cc. fractions were collected. Fraction Nos. 15～24 contained substance B-1 alone. Fraction Nos. 30～36 contained substance B-2 and fraction Nos. 42～48, substance B-3.

Substance B-1 (Monoacetylgitoroside)——The crude substance B-1 obtained was recrystallized from MeCOEt-petr. ether to a colorless powder (140 mg.), m.p. 184～189°, $[\alpha]_D^{25}$ +14.8°(c=1.19, MeOH), UV : λ_{max}^{EtOH} 219 mμ (log ε 4.17). It was further recrystallized from hydr. MeOH to colorless needles, m.p. 176～181°. *Anal.* Calcd. for $C_{31}H_{46}O_9$: C, 66.17; H, 8.24; digitoxose, 26.33. Found : C, 65.60; H, 8.03; digitoxose,*5 25.45.

It is easily soluble in MeOH, $CHCl_3$, and Me_2CO, soluble in Et_2O, and insoluble in H_2O and petr. ether. 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.

Deacetylation of Substance B-1——Substance B-1 (5 mg.) was treated by the same method as for substance A-1 and the spot corresponding to gitoroside[4] was found on the paper chromatogram.

Acid Hydrolysis of Substance B-1——The same hydrolysis of substance B-1 showed spots of gitoxigenin, acetyl digitoxose, and digitoxose on the paper chromatogram.

Substance B-2 (Monoacetylgitoxigenin bisdigitoxoside)——The crude substance B-2 obtained was recrystallized from hydr. MeOH to an amorphous product (35 mg.), m.p. 171～173°, UV : λ_{max}^{EtOH} 219 mμ (log ε 4.20). *Anal.* Calcd. for $C_{37}H_{56}O_{12}$: C, 64.14; H, 8.15; digitoxose, 42.77. Found : C, 64.51; H, 8.14; digitoxose,*5 40.30.

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

*5 The analysis of digitoxose was made by the method of Kaiser, *et al.*[2]

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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).

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