UDC 547.918:615.711.5

43. Kazuhiko Hoji: Studies on the Constituents of *Digitalis purpurea* L. XVIII.[*1] Odorobioside-G from Digitalis Seeds.

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

It was reported in the preceding paper[*1] that several cardiotonic glycosides had been isolated from the seeds of *Digitalis purpurea* L. One of them, substance C-0, was identified as odorobioside-G which had been isolated from *Nerium odorum* by Reichstein, *et al.*1)

This glycoside was repeatedly recrystallized from hydrous methanol-acetone to colorless prisms, m.p. 240～242°, $[\alpha]_D^{16}$ −8.1°(MeOH). It has extremely bitter taste, is easily soluble in methanol, soluble in acetone, and insoluble in ether and water. It gives positive Legal and Raymond reactions, and negative Gregg-Gisvold and Frèrejacque reactions. It exhibits a colorless glacial acetic acid layer and a brown sulfuric acid layer in the Keller-Kiliani reaction. It also exhibits the maximum absorption, λ_{max}^{EtOH} 218 mμ, which is generally characteristic to cardiotonic glycosides. Its analytical values agree with the formula of $C_{36}H_{56}O_{13}$.

The sugar portion of the substance C-0, obtained by its drastic hydrolysis, was submitted to paper chromatography and two spots were revealed on the paper chromatogram. These spots were found to agree with those of glucose and digitalose, and they are the same as the sugar portion of digitalinum verum. The aglycone was obtained as plate crystals, m.p. 247～252°, by the Mannich hydrolysis in an ice storage and it was identical with digitoxigenin.

The foregoing results have shown that the substance C-0 is composed of one mole each of digitoxigenin, glucose, and digitalose. It was shown by Reichstein, *et al.*2) that odorobioside-G and graciloside (odoroside-F) had the same component. Substance C-0 has a similar melting point and optical rotation as odorobioside-G (Table I). It was then identified with odorobioside-G[*3] by the mixed melting point and paper chromatographic methods.

Table I. Comparison of Odorobioside-G and Substance C-0

Substance	m.p. (°C)	Recrystn. solvent	$[\alpha]_D$
Odorobioside-G1)	243～245	MeOH-$CHCl_3$-Et_2O	−7.4° (MeOH)
Substance C-0	240～242	MeOH-Me_2CO-H_2O	−8.1° (MeOH)
Odorobioside-G monoacetate1)	288～294	MeOH-Et_2O	−7.7° (MeOH-$CHCl_3$)
	276～286	MeOH-H_2O	
Substance C-0 monoacetate	274～277	〃	−8.3° (MeOH-$CHCl_3$)
Odorobioside-G pentaacetate1)	236～239	〃	−8.9° ($CHCl_3$)
	169/218/238～241	Me_2CO-Et_2O	
Substance C-0 acetate	239～243	MeOH-H_2O	−12.6° ($CHCl_3$)
	167/220/238～242	Me_2CO-Et_2O	

Acetylation of substance C-0 afforded an acetate, crystallizing from hydrous methanol to colorless needles, m.p. 239～243°, and from acetone-ether to colorless needles, m.p. 167～169°/220～223°/238～242°, $[\alpha]_D^{18}$ −12.6°($CHCl_3$). The physical properties, elemental analysis, and quantitative analysis of acetyl group agree with those of odorobioside-G acetate, $C_{46}H_{66}O_{18}$.

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

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

[*3] Grateful acknowledgement is expressed to Prof. Dr. T. Reichstein for kind donation of odorobioside-G and odorobioside-G monoacetate.

1) A. Rheiner, A. Hunger, T. Reichstein: Helv. Chim. Acta, **35**, 687 (1952).

2) W. Rittel, T. Reichstein: *Ibid.*, **36**, 554 (1953).

When this acetate was deacetylated by the usual method, a deacetylated glycoside crystallized from hydrous methanol to colorless needles, m.p. 274~277°, $[\alpha]_D^{18}$ $-8.3°$($CHCl_3$-MeOH=1:1). It is positive to Frèrejacque reaction, supporting the presence of an acetyl group. The physical properties, elemental analysis, and quantitative analysis of the acetyl group agree with those of odorobioside G monoacetate, $C_{38}H_{58}O_{14}$. It was identified with odorobioside G monoacetate[*3] by the mixed melting point and paper chromatography.

Enzymatic hydrolysis of substance C-0 afforded a substance which was identical with odoroside-H,[*4] a digitoxigenin digitaloside, which had been isolated from the leaves of *Digitalis purpurea* by Satoh, *et al.*[3)]

This is the first time that odorobioside G, a digitoxigenin glucosido-digitaloside, was isolated from the seeds of *Digitalis purpurea* in this labolatory, but Haack, *et al.*[4)] assumed from his studies with paper chromatography that the glycoside might be present in the seeds.

Experimental[*5]

Odorobioside-G (Substance C-0)——The methods of isolation were as reported in Part XVII.[*1] The substance C-0 was recrystallized from MeOH-Me_2CO-H_2O to colorless prisms, m.p. 240~242°, $[\alpha]_D^{18}$ $-8.1°$(c=1.04, MeOH), UV : λ_{max}^{EtOH} 218 mμ (log ε 4.19). *Anal.* Calcd. for $C_{36}H_{56}O_{13}$: C, 62.05; H, 8.10. Found : C, 62.30; H, 7.97. When it was mixed with Reichstein's odorobioside-G[*3] (m.p. 241~244°), the m.p. was 240~243°.

Acid Hydrolysis of Substance C-0——CO_2 was bubbled through the solution of 10 mg. of the substance C-0 dissolved in a mixture of 1 cc. of MeOH and 1 cc. of 7% HCl while refluxing for 6 hr. MeOH was distilled off in a reduced pressure and the residual solution was extracted with $CHCl_3$. The aqueous layer was deacidified by Amberlite IR-4B (5 cc.), evaporated until dry, and a syrupy sugar was obtained. The sugar obtained was submitted to paper chromatography with BuOH-AcOH-H_2O (4:1:5) and two spots corresponding to glucose and digitalose were found.

The Mannich Hydrolysis of Substance C-0——A solution of 50 mg. of the substance C-0 dissolved in a mixture of 10 cc. of Me_2CO and 0.1 cc. of 36% HCl was allowed to stand at 5° for 30 days. To the reaction mixture, 20 cc. of H_2O was added, Me_2CO was evaporated in a reduced pressure, and the residue was extracted with five 10-cc. portions of $CHCl_3$. The combined $CHCl_3$ extract was washed with two 10-cc. portions of water and evaporated until dry. The residue was recrystallized from Me_2CO-Et_2O to prisms, m.p. 247~252°, which were identified as digitoxigenin by paper chromatography and mixed m.p. determination, and its analytical values agreed with those of digitoxigenin. *Anal.* Calcd. for $C_{23}H_{34}O_4$ (Digitoxigenin) : C, 73.76; H, 9.15. Found : C, 74.03; H, 9.08.

Acetylation of Substance C-0——Substance C-0 (100 mg.) was acetylated by the usual method, using 1.5 cc. of pyridine and 1 cc. of Ac_2O, and leaving it at room temperature for 48 hr. Pyridine and Ac_2O were evaporated in a reduced pressure and the residue was recrystallized from hydr. MeOH to colorless needles, m.p. 239~243° (125 mg.). The crystals of acetate were repeatedly recrystallized from Me_2CO-Et_2O to needles, m.p. 167~169°/220~223°/238~242°; $[\alpha]_D^{18}$ $-12.6°$(c=1.11, $CHCl_3$); UV: λ_{max}^{EtOH} 218 mμ (log ε 4.19). *Anal.* Calcd. for $C_{46}H_{66}O_{18}$ (Pentaacetylodorobioside-G) : C, 60.91; H, 7.34; CH_3CO, 23.76. Found : C, 60.65; H, 7.10; CH_3CO, 24.24.

Deacetylation of Substance C-0 Acetate——The substance C-0 acetate (400 mg.) was dissolved in 50 cc. of MeOH, a solution of $KHCO_3$ (400 mg.) dissolved in 5 cc. of H_2O was added, and the mixture was allowed to stand for 12 days at room temperature. To this mixture, 15 cc. of H_2O was added, evaporated to about 15 cc. in a reduced pressure, and the residual solution was extracted 5 times with 15-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 in a reduced pressure. The residue was recrystallized from hydr. MeOH to colorless needles (180 mg.), m.p. 274~277°; $[\alpha]_D^{18}$ -8.3 (c=1.48, $CHCl_3$-MeOH (1:1)); UV: λ_{max}^{EtOH} 218 mμ (log ε 4.19). *Anal.* Calcd. for $C_{38}H_{58}O_{14}$ (Odorobioside-G monoacetate) : C, 61.63; H, 7.91; CH_3CO, 7.46. Found : C, 61.28; H, 7.99; CH_3CO, 7.87.

Formation of Odoroside-H from Substance C-0——To a solution of 5 mg. of the substance C-0 dissolved in 1 cc. of MeOH, 20 cc. of distilled water was added, and MeOH was evaporated from this solution in a reduced pressure. The filtrate, obtained from 10 mg. of the snail enzyme powder

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[*4] Grateful acknowledgement is expressed to Dr. Satoh for kind donation of odoroside-H.

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

3) D. Satoh, H. Ishii, Y. Oyama, T. Wada, T. Okumura : This Bulletin, **4**, 284 (1956).

4) E. Haack, F. Kaiser, M. Gube, H. Spingler : Naturwiss., **43**, 301 (1956).

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 α,β-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).