UDC 547.918 : 582.951.6

49. Atsuji Okano : Studies on the Constituents of *Digitalis purpurea* L. VI.[1] Glucodigifucoside, a New Cardiotonic Glycoside.

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It was reported in Part II[2] of this series, that the presence of 17 kinds of unknown cardiotonic glycoside in the seed of *Digitalis purpurea* had been indicated by paper chromatography. Of these, three were considered not to belong to the gitoxigenin series from their reaction, not possessing 2,6-deoxy sugar as the sugar portion, and giving positive Raymond reaction. From these three, a substance corresponding to Substance C-I was isolated in crystalline form and it was proved to be a new cardiotonic glycoside.

The fraction Nos. 4~9[3] from partition chromatography for purification of digitalinum verum, reported in Part II, and fraction Nos. 1~6[4] obtained on purification and isolation of gitostin, reported in Part III, of this series, corresponding to the substance C-I, were purified through a column of Celite 535 (Johns-Manville product), with water-saturated methyl ethyl ketone as the developer, in exactly the same manner as reported previously.[2] The substance C-I developed rapidly and showed distribution as indicated in Fig. 1. It was found that the substance had been isolated and purified in fraction Nos. 8~12 in this paper partition chromatography. Recrystallization of the residue from these fractions from hydrous methanol and hydrous ethanol easily afforded lustrous, colorless needle crystals (cf. Fig. 2).

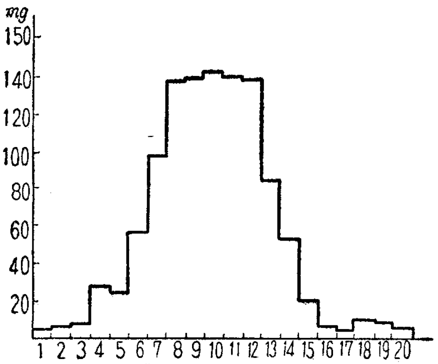

Fig. 1. Fractional Distribution of Substance C-I

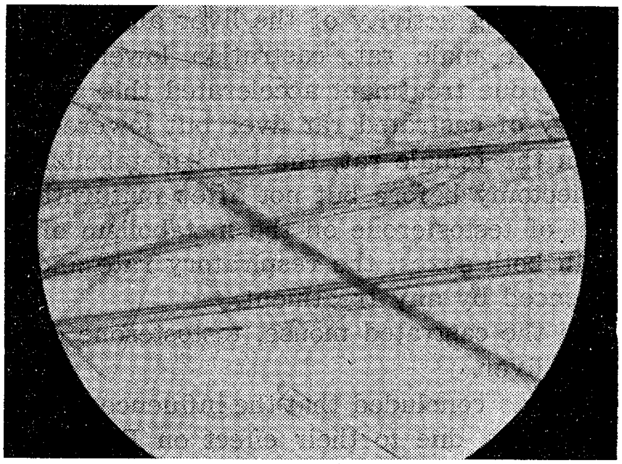

Fig. 2. Glucodigifucoside

The crystals had extremely bitter taste, melted at 194~197°(Kofler, uncorr.); easily soluble in ethanol, soluble in acetone and methanol, sparingly soluble in ethyl acetate and water, and almost insoluble in ether, chloroform, and benzene; $[\alpha]_D^{21}$ +1.9±2°(c=1.75, EtOH). The substance was clearly not of gitoxigenin series, since it exhibited colorless glacial acetic acid layer and brown sulfuric acid layer in the Keller-Kiliani reaction, and positive to both Legal and Raymond reactions. It also exhibited the absorption characteristic to cardiotonic glycoside in general, λ_{max}^{EtOH} 217 mμ. Its elemental analytical

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[1] Part V. A. Okano, *et al.* : This Bulletin, 5, 171(1957).

[2] Part II. K. Miyatake, *et al.* : *Ibid.*, **5**, 157(1957).

[3] cf. Fig. 4 in Part II.

[4] cf. Table I in Part III : This Bulletin, **5**, 164(1957).

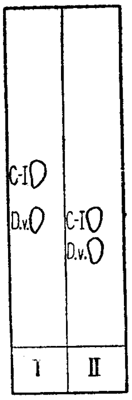

Fig. 3.

Paper Partition Chromatography of Substance C-I (Glucodigifucoside) and Digitalinum verum

Toyo Roshi No. 50, ascending method (20°C)
I : Water-saturated MeCOEt, water-impregnated paper.
II : Formamide satd.toluene-BuOH (1 : 1, v/v), paper impregnated with formamide

values agreed with the formula of $C_{35}H_{54}O_{13}\cdot 2H_2O$ and indicated Rf value close to that of digitalinum verum, as shown in Fig. 3, in paper partition chromatography.

Hydrolysis of this substance by the Mannich method afforded β-anhydrodigitoxigenin and digitoxigenin, both of which showed no depression of the melting point on admixture respectively with authentic samples obtained from digitoxin. Their identity was also established by comparison of optical rotation and Rf values.[1]

The sugar portion obtained by the drastic hydrolysis by the Kiliani method[5] was submitted to paper partition chromatography with various developing solvents. Two spots appeared on the paper chromatogram, whose Rf values are indicated in Table I. These spots were found to agree respectively with those of glucose, run as a control, and of fucose given in literature. These two spots were extracted and the phenylosazones obtained therefrom agreed with those[6] of glucose and fucose. The sugars were thereby established as glucose and fucose.

Table I. Paper Partition Chromatography of Sugar Portion (Rf Values)

Solvent system[a]	Spots found		Glucose	Fucose
	1	2		
1	0.16	0.27	0.18	0.27[b]
2	0.23	0.40	0.23	0.41[9]
3	0.37	0.43	0.38	0.44[c]
Phenylosazone m.p. (°C	205～208	170～173	208	170～175[b]

Toyo Roshi No. 51, ascending method (18～23°)
a) Solvent system : (1) BuOH : AcOH : H_2O=4 : 1 : 5
(2) BuOH : AcOH : H_2O=4 : 1 : 2
(3) BuOH : pyridine : H_2O=3 : 2 : 1.5
b) S.M. Partridge : Biochem. J. (London), 42, 238(1948).
c) A. Jeanes, C.S. Weise, R.J. Dimler : Anal. Chem., 23, 415(1951).

It follows, therefore, that substance C-I is a new cardiotonic glycoside composed of digitoxigenin as the aglycone and glucose and fucose as the sugars.

The assay of glucose by the method of determination used in the case of gitostin,[7] as described in Part III of this series, gave value agreeing with that calculated for the presence of 1 mole of glucose in this substance. The number of fucose was also assumed to be 1 mole from the comparison of elemental analytical values, molecular absorbancy coefficient, and Rf values. Since the analytical value of the acetate agreed with that for hexaacetate, $C_{47}H_{54}O_{19}$ (Fig. 4), this substance was identified as diglycoside.

Tschesche and others[8] isolated a glycoside assumed to be gitoxigenin-glucosido-

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[5] H. Kiliani : Ber., **63**, 2866(1930).

[6] Fucose phenylosazone, m.p. 177° (E. Votocek : Ber., **37**, 3861(1904)), m.p. 170～175° (D. Satoh, *et al.* See Footnote (9)).

[7] Part III : This Bulletin, **5**, 165(1957).

[8] R. Tschesche, G. Grimmer : Chem. Ber., **88**, 1569(1955),

fucoside from the leaves of *Digitalis lanata* and named it glucogitofucoside. Although the structure of the new glycoside has not been established as yet, this substance C–I was named glucodigifucoside in accordance with the nomenclature adopted by Tschesche and others.

Recently, Satoh and others⁹⁾ isolated digiproside, a digitoxigenin-*d*-fucoside, from the leaves of *Digitalis purpurea*. It is assumed that the new glycoside obtained by the writer is a diglycoside formed by further bonding of 1 mole of glucose to digiproside. Further, the present glucodigifucoside is an isomer of cheiroside-A, an uzarigenin-β-*d*-glucosido-β-*d*-fucoside, obtained by Reichstein and others¹⁰⁾ from the seeds of *Cheiranthus cheiri* L., in steric configuration at 5-position in digitoxigenin.

Diglycosides of digitoxigenin series have not been found in Digitalis and a new glucodigifucoside has now been added by the writer. This is present in the seeds with glucogitofucoside, whose presence was indicated in Part II of this series.²⁾ It is interesting to note that a larger amount of glucodigifucoside is present. The order of the bonding of sugars and physiological action of this glucodigifucoside will be reported in a subsequent paper.

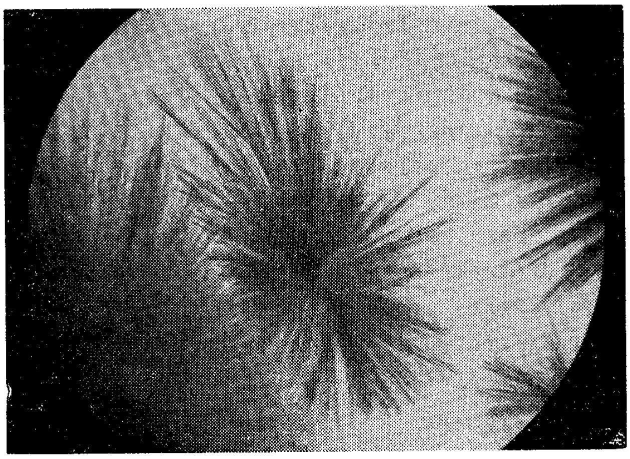
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Fig. 4.

Glucodigifucoside Hexaacetate

The writer expresses his deep gratitude to Prof. Shoji Shibata of the University of Tokyo for reviewing the manuscript, to Dr. Junzo Shinoda, President of this Company, and to Mr. Isamu Nakano, Director of the Yanagishima Factory, for encouragement during the course of this work and for permission to publish the work, and to Dr. Kazuo Miyatake for continued guidance. The writer is also indebted to Messrs. Hoji and Miki for technical help and to Messrs. Negishi and Abe for microanalytical data.

Experimental¹¹⁾

Isolation of Substance C-I—The fractions containing substance C-I obtained by paper chromatography, indicated as fraction Nos. 4~9 in Fig. 4, Part II,²⁾ and fraction Nos. 1~6 in Table I, Part III,⁴⁾ were combined (total, ca. 2.5 g.) and submitted to partition chromatography through a column with 700 g. of Celite 535 as a carrier and water-saturated MeCOEt as the developer. Effluent was collected in 100-cc. fractions and indicated distribution as shown in Fig. 1. Substance C-I was found to be isolated and collected in the fraction Nos. 8~12 (ca. 720 mg.). Therefore, the residue from this fraction was recrystallized from hydr. MeOH and lustrous, colorless needles were obtained. Other fractions containing substances other than C-I were treated with a small amount of EtOH and soluble portion was repeatedly recrystallized from MeOH and hydr. MeOH. A total of 760 mg. of substance C-I was obtained as crystals, yield being 0.038% from the seeds.

Crystalline C-I (Glucodigifucoside)—Crystallizes from hydr. MeOH or hydr. EtOH as lustrous, colorless needles, m.p. 194~197°; $[\alpha]^{21}_{D}$ $+1.9\pm2°$ (c=1.75, EtOH), $[\alpha]^{21}_{D}$ $-3°\pm2°$ (c=1.45, pyridine);

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9) D. Satoh, *et al.*: This Bulletin, **4**, 284(1956).
10) N. M. Shah, K. Meyer, T. Reichstein: Pharm. Acta Helv., **24**, 113(1949).
11) All m.p.s were measured on a Kofler block and are uncorrected.

λ_{max}^{EtOH} 217 mμ (log ε 4.23). Other properties are given in the main text. There was no loss in weight on drying (dried over P_2O_5 for 10 hrs. at 100°, under 0.1 mm. Hg). *Anal.* Calcd. for $C_{25}H_{54}O_{13}$: C, 61.57; H, 7.97. Calcd. for $C_{35}H_{54}O_{13} \cdot 2H_2O$: C, 58.48; H, 8.13. Found : C, 56.95; H, 8.15.

Acetylation of Crystalline Substance C-I—Crystalline substance C-I (50 mg.) was acetylated by the usual method with 1 cc. of pyridine and 0.8 cc. of Ac_2O by leaving at room temperature for 48 hrs. and recrystallized from acetone-ether-petr. ether mixture to clustered needles, m.p. 232~235°; yield, 48 mg.; $[\alpha]_D^{21}$ −2.8°±1.5° (c=1.41, $CHCl_3$); λ_{max}^{EtOH} 216 mμ (log ε 4.21, calcd. as hexaacetate, $C_{47}H_{54}O_{19}$). Paper partition chromatography was carried out by the method reported in Part V,[1] using solvents (1), (2), and (3), and respective Rf values of 0.00, 0.06, and 0.42 were obtained. Its relation to digitalinum verum hexaacetate, used as the control, is indicated in Fig. 5. *Anal.* Calcd. for $C_{47}H_{54}O_{19}$ (Hexaacetate): C, 60.37; H 7.12. Found : C, 59.59; H, 7.39.

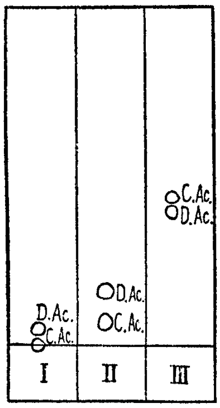

Fig. 3.

Paper Partition Chromatography of Substance C-I Acetate and Digitalinum verum Hexaacetate

D.Ac. : Digitalinum verum hexaacetate
C.Ac. : Subst. C-I acetate
Toyo Roshi No. 51, ascending method (22°)

	Cyclohexane	AcOH	$CHCl_3$	H_2O
(1)	100	30	20	1
(2)	100	30	30	1
(3)	100	30	40	1

Mannich Hydrolysis of Crystalline Substance C-I—To a solution of 60 mg. of C-I dissolved in 15 cc. of acetone, 0.15 cc. of conc. HCl was added and the mixture was allowed to stand for 13 days at room temp. Acetone was distilled off under a reduced pressure, 10 cc. of MeOH added, and the mixture was refluxed for 30 mins. MeOH was distilled off at 20° under a reduced pressure, the residual suspension was extracted with $CHCl_3$, and $CHCl_3$ layer was washed consecutively with water, dil. alkali, and water, dried over anhyd. Na_2SO_4, and the solvent evaporated under a reduced pressure. The residual extract was submitted to alumina chromatography and a portion eluted with benzene-$CHCl_3$ (9 : 1) was recrystallized from acetone-ether-petr. ether mixture to prisms, m.p. 190~194°, $[\alpha]_D^{22}$ −16.4°±3° (c=0.381, $CHCl_3$), positive to tetranitromethane reaction.

This substance was acetylated with pyridine-Ac_2O and the product was recrystallized from benzene-ether-petr. ether mixture to needles, m.p. 180~184°.

These substances showed the same crystalline form and no depression of m.p. on admixture with β-anhydrodigitoxigenin, m.p. 188~192°, formed by the usual method[12] from digitoxigenin, and its acetate, m.p. 182~184°. They were also identified by the same Rf values obtained by paper chromatography, using the solvents (1), (2), and (3) reported in the preceding paper.[1]

The portion developed and eluted with benzene-$CHCl_3$ (4 : 1) was recrystallized from MeOH-ether to a small amount of prisms, m.p. 250~254°, which were identified with digitoxigenin by m.p. and paper partition chromatography.[1,8]

Sugar Portion of Crystalline Substance C-I—A mixture of 50 mg. of C-I and 3 cc. of Kiliani mixture (AcOH : H_2O : conc. HCl=3.5 : 5.5 : 1) was refluxed for 1 hr. on a boiling water bath, 3 cc. of distilled water was added, and extracted with $CHCl_3$. The aqueous layer and aqueous washing from $CHCl_3$ extract were combined, treated with ion exchanger, Amberlite IR-4 B, and the extract obtained on evaporation of the solvent was submitted to paper partition chromatography with 3 kinds of solvents, giving results shown in Table I. Each spot zone was extracted, treated with phenylhydrazine hydrochloride, and phenylosazones were obtained. As shown in Table I, the Rf values and m.p. of the osazone of glucose agreed well with those of glucose run as a control. Rf value of fucose and m.p. of its osazone agreed well with these values in literature.

Determination of Glucose in Substance C-I—Glucose was determined by the method described in Part III, using 40 mg. of crystalline substance C-I, m.p. 193~196°, and resultant values were 11.1 and 11.8 mg., or 27.7% and 29.5%, agreeing well with 27.0% calculated as diglycoside, $C_{35}H_{54}O_{13}$.

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[12] S. Smith : J. Chem. Soc., **1935**, 1050.

Summary

Of the 17 kinds of unknown cardiotonic glycoside-like substance contained in the seeds of *Digitalis purpurea*, described in Part II of this series, a glycoside corresponding to substance C-I was isolated. This substance had a bitter taste and came as needle crystals of m.p. 194~197°(Kofler, uncorr.); $[\alpha]_D^{21}$ $+1.9\pm2°$; $C_{35}H_{54}O_{13}\cdot2H_2O$; negative to Keller-Kiliani reaction, giving brown sulfuric acid layer, and positive to Legal and Raymond reactions; U.V. λ_{max}^{EtOH} 217 mμ (log ε 4.23). These results indicated the substance to be a cardiotonic glycoside. Hydrolysis by the Mannich method afforded β-anhydrodigitoxigenin and digitoxigenin, and the sugar portion was identified as fucose and glucose by paper chromatography. Determination of glucose by the Borel-Hostettler-Deuel method, as in the case of gitostin, indicated the presence of 1 mole. Confirming this substance to be diglycoside, a new cardiotonic glycoside of digitoxigenin series, it was named **glucodigifucoside.**

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