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44. Atsuji Okano, Kazuhiko Hoji, Tōsaku Miki, and Akio Sakashita : Studies on the Constituents of *Digitalis purpurea* L. XI.[1] Digifucocellobioside, a New Cardiotonic Glycoside from Digitalis Seeds.

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It was reported in the preceding paper[1] that several new cardiotonic glycosides had been isolated from the seeds of *Digitalis purpurea* L. The isolation of an unknown glycoside corresponding to substance C-II, which is more water-soluble than substance C-I, was described in Part X.[1] The present investigation was undertaken to determine the structure of substance C-II.

Repeated recrystallization of this substance from water-saturated butanol and hydrated ethanol-ether afforded colorless crystalline powder, m.p. 238~242° ; $[\alpha]_D^{20}$ −1.4° ±3°(c=1.23, MeOH), as indicated in Table I of Part X.[1] It is extremely bitter to the taste, easily soluble in methanol and hydrated ethanol, sparingly soluble in water, and insoluble in chloroform, ether, or benzene. It gives positive Legal and Raymond reactions and exhibits colorless glacial acetic acid layer and brown sulfuric acid layer in the Keller-Kiliani reaction, same as digitoxigenin. It also exhibits the maximum absorption, λ_{max}^{EtOH} 218 mμ, characteristic to cardiotonic glycoside in general. Its elemental analytical values agreed with the formula of $C_{41}H_{64}O_{18}\cdot 2H_2O$.

The sugar portion of substance C-II obtained by drastic hydrolysis of Kiliani method[2] was submitted to paper partition chromatography with three solvent systems.

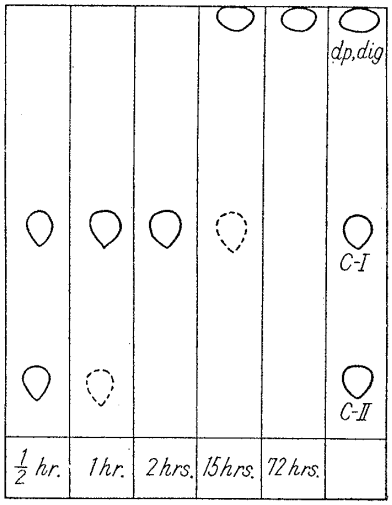

Fig. 1.
Moving phase : Water-satd. MeCOEt
Stationary phase : Water
Coloring agent : Raymond reagent

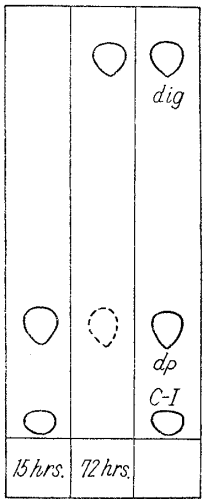

Fig. 2.
Formamide-satd. toluene-BuOH (3:1)
Formamide

C-I : Glucodigifucoside dp : Digiproside dig : Digitoxigenin
Toyo Roshi No. 50, ascending method, at 18~22°.

Figs. 1 and 2. Paper Partition Chromatography of Enzymatic Decomposition of Substance C-II

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1) Part X. A. Okano, *et al.* : This Bulletin, **7**, 212(1959).
2) H. Kiliani : Chem. Ber., **63**, 2866(1930).

Two spots appeared on the paper strip. These spots were found to agree with those of glucose and fucose, like the sugar portions of glucodigifucoside.[3]

In Part Ⅷ of this series,[4] enzymatic decomposition of glucodigifucoside (Ⅱ) with an enzyme obtained from a snail (*Euhadra quaesita* DESHAYES) was described and it had been indicated that application of an enzyme solution effected cleavage of glucose and fucose from (Ⅱ), but partial decomposition to digiproside (digitoxigenin β-D-fucoside) (Ⅲ)[5] was also possible by shortening the duration of this hydrolysis. Partial decomposition under the same conditions was attempted with substance C-Ⅱ. First, the glycosides formed by partial hydrolysis was examined at definite intervals of time by means of paper chromatography, as shown in Figs. 1 and 2. From these data, it was assumed that substance C-Ⅱ was transformed into glucodigifucoside (Ⅱ) in two hours and into digiproside (Ⅲ) in 15 hours, and this enzymatic hydrolysis proceeded in steps. Paper chromatographic analysis of the sugar portion of this hydrolysis product indicated only a clear spot of glucose.

Then a large-scale enzymatic hydrolysis (substance C-Ⅱ ; 100 mg.) was performed under the same condition, the reaction mixture was extracted, and the extract was submitted to alumina column chromatography. (Ⅱ) was obtained as needles of m.p. 189~191°, and its acetate was crystallized from acetone-ether-petroleum ether mixture to needles, m.p. 227~230°. They were respectively identified with authentic glucodigifucoside and its acetate by mixed melting point and paper chromatographic analysis. (Ⅱ) was further treated for four days by the same enzymatic method and digitoxigenin (Ⅳ) was obtained as plates of m.p. 244~248°, as described in Part Ⅷ.[4] Paper chromatographic analysis of this sugar portion gave two spots of glucose and fucose. On the basis of these data, it seemed reasonable to assume that substance C-Ⅱ is a triglycoside and its terminal glucose is linked to the terminal glucose in (Ⅱ). The difference in molecular rotation[6] between substance C-Ⅱ and glucodigifucoside (Ⅱ) shows that the glucosidic linkage of the terminal glucose in substance C-Ⅱ is β-configuration, as shown in Table I.

It was reported in Part Ⅸ[7] of this series that for the glucosidic linkage of terminal disaccharide in cardiotonic triglycosides there were two types, cellobiose-type and gentiobiose-type, such as in gitostin and neogitostin. Therefore, as shown in Chart 1, substance C-Ⅱ is digitoxigenin β-cellobiosido-β-D-fucoside and it was named digifucocellobioside (Ⅰ), in accordance with its constitution.

TABLE I.

Substance	$[\alpha]_D$	$[M]_D$ Calcd.
Digifucocellobioside	− 1.4°	− 12.3°
Glucodigifucoside	+ 1.9°	+ 13.6°
$\Delta[M]_D$		− 25.9°
α-Methyl-D-glucopyranoside	+160.8°	+312°
β-Methyl-D-glucopyranoside	− 32.8°	− 64°

Triglycosides of digitoxigenin series which contained two moles of glucose have not been found in Digitalis and a new digifucocellobioside (Ⅰ) has now been added by the authors. It is very interesting that two triglycosides containing cellobiose, e.g., gitostin and digifucocellobioside (Ⅰ), were found in the seeds of *Digitalis purpurea*.

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3) Part Ⅵ. A. Okano : This Bulletin, **5**, 272(1957).
4) Part Ⅷ. A. Okano : *Ibid.*, **5**, 279(1957).
5) D. Satoh, *et al.* : *Ibid.*, **4**, 284(1956).
6) W. Klyne : Biochem. J. (London), **47**, xli(1950).
7) Part Ⅸ. A. Okano : This Bulletin, **6**, 178(1958).

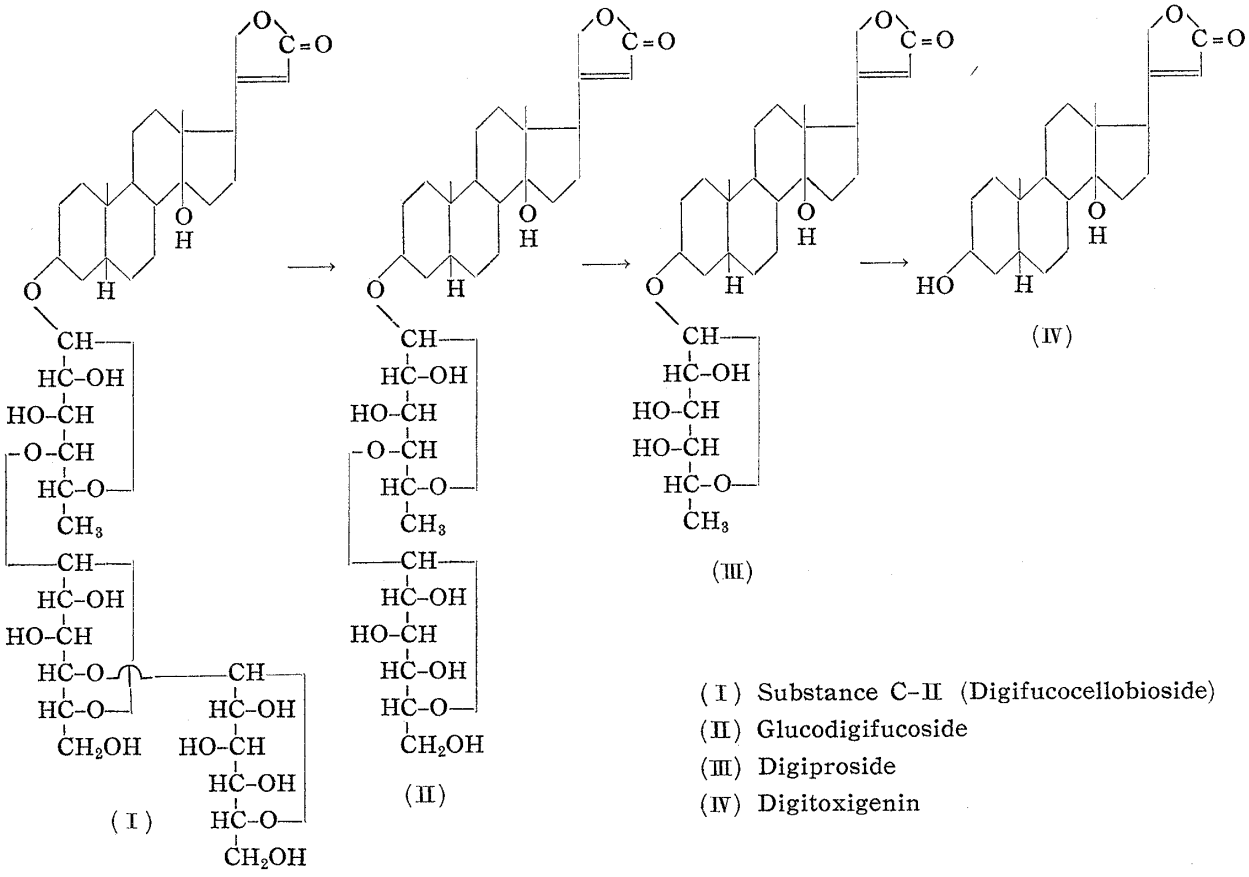

Chart 1. Enzymatic Decomposition of Substance C-II

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Experimental[8]

Substance C-II (Digifucocellobioside) (I)—Recrystallization from water-satd. BuOH or hydrated EtOH-Et_2O gives white crystalline powder, m.p. 238~242°; $[\alpha]_D^{20}$ $-1.4° \pm 3°$(c=1.23, MeOH); U.V. λ_{max}^{EtOH} 218 mμ(log ε 4.22). Other properties are given in the main text. *Anal.* Calcd. for $C_{41}H_{64}O_{18}$: C, 58.28; H, 7.64. Calcd. for $C_{41}H_{64}O_{18} \cdot 2H_2O$: C, 55.90; H, 7.78. Found (dried over P_2O_5 for 3 hrs. at 100°, under 0.1 mm. Hg): C, 56.13; H, 7.77.

Sugar Portion of Substance C-II—A solution of 10 mg. of C-II dissolved in 0.6 cc. of Kiliani mixture (AcOH : H_2O : conc. HCl = 3.5 : 5.5 : 1) was refluxed for 1 hr. on a boiling water bath and the mixture was extracted with $CHCl_3$. The aqueous layer containing the sugar portion of C-II, was treated with ion-exchanger, Amberlite IR-4B, and the residue obtained on evaporation of solvent was submitted to paper partition chromatography with 3 kinds of solvent systems,[8,9] giving results as shown in Table II. The Rf values of spot No. 1 and spot No. 2 agreed well with those of glucose and fucose.

Table II. Paper Partition Chromatography of Sugar Portion

Solvent system	Rf Value	
	Spot No. 1	Spot No. 2
BuOH:AcOH:H_2O (4:1:5)	0.15	0.26
BuOH:AcOH:H_2O (4:1:2)	0.23	0.36
BuOH:Pyridine:H_2O (3:2:1.5)	0.38	0.47

8) All m.p.s were measured on a Kofler block and are uncorrected.
9) Toyo Roshi No. 50; developed by the ascending method at 18~22°.

Enzymatic Decomposition of Substance C-II—To a solution of 40 mg. of C-II dissolved in 80 cc. of distilled water, enzyme solution prepared from 10 mg. of enzyme powder and 10 cc. of distilled water was mixed, 3 cc. of toluene added, and the mixture was allowed to stand in a thermostat of 32°. A small quantity of the reaction mixture was taken out at intervals of 0.5, 1, 2, 15, and 72 hrs., and examined by paper partition chromatography, as shown in Fig. 1. It was found that C-II was completely transformed into (II) in only 2 hrs., (II) almost completely into (III) in 15 hrs., and completely into (IV) in 72 hrs.

Enzymatic Decomposition—a) Formation of Glucodigifucoside (II) from Substance C-II: The enzyme solution prepared from 20 mg. of enzyme powder and 15 cc. of distilled water was added to a solution of 100 mg. of C-II dissolved in 200 cc. of distilled water, 5 cc. of toluene added, and the mixture was allowed to stand at 32° for 4 hrs. The white turbid liquid was concentrated to 20 cc. under a reduced pressure at a bath temperature of 50° and 100 cc. of EtOH was added to the concentrated solution. The enzyme that precipitated out on standing was removed by the use of Hyflo Super Cel (Johns-Manville product), the filtrate was concentrated to 20 cc. under a reduced pressure, and the concentrated solution was shaken 3 times with a mixture of $CHCl_3$-BuOH (1:2). $CHCl_3$-BuOH layer was evaporated under a reduced pressure and the residue was chromatographed on 1 g. of alumina. The portion eluted with $CHCl_3$-MeOH(9:1) was recrystallized from hydrated EtOH to 40 mg. of needles, m.p. 189~191°, undepressed on admixture with glucodigifucoside, m.p. 191~193°. This substance was acetylated with pyridine-Ac_2O and the acetate was crystallized from Me_2CO-Et_2O-petr. ether to needles, m.p. 227~230°, undepressed on admixture with glucodigifucoside acetate, m.p. 228~230°.

The residue obtained from the aqueous layer gave only one spot of glucose by paper chromatographic analysis.

b) Formation of Digitoxigenin (IV) from Glucodigifucoside (II): To a solution of 17 mg. of above-mentioned (II) dissolved in 2 cc. of EtOH, 70 cc. of distilled water was added and EtOH was distilled off under a reduced pressure. A supernatant solution prepared from 35 mg. of enzyme powder and 10 cc. of distilled water was added to this solution, together with 3 cc. of toluene, and the mixture was allowed to stand at 32° for 4 days. This was treated as in the case of (a), chromatographed on alumina, and the portion eluted with benzene-$CHCl_3$(1:3) was recrystallized from MeOH-H_2O and Me_2CO-petr. ether to plates, m.p. 244~248°, undepressed on admixture with digitoxigenin, m.p. 246~248°. Paper chromatography with xylene-MeCOEt(1:1) saturated with formamide[10] also gave identical Rf value with that of digitoxigenin.

α-Octaacetylcellobiose from Substance C-II—Substance C-II (80 mg.) was acetylated by the usual method with pyridine and Ac_2O. The crude acetate was dissolved in 1.85 cc. of Ac_2O, together with 26 mg. of $ZnCl_2$, and the mixture was heated for 30 mins. in a boiling water bath. The acetolysis mixture was poured into ice water and allowed to stand for 16 hrs. at room temperature. The precipitate was collected by filtration, dissolved in $CHCl_3$, and this solution was washed successively with water, 5% NaOH, and water. After drying over anhyd. Na_2SO_4, the solvent was evaporated under a reduced pressure and the residue was submitted to chromatography on 2.5 g. of a mixture of Florisil-Celite (5:1). The portion eluted with EtOH-benzene(1:99) was recrystallized from $CHCl_3$-MeOH(1:1) to 25 mg. of needles, m.p. 224~227°; $[\alpha]_D^{20}$ +37.8°±3°(c=1.39, $CHCl_3$). This substance gave no depression of m.p. on admixture with α-octaacetylcellobiose, m.p. 225~228°; $[\alpha]_D^{24}$ +41.4°±2° (c=0.942, $CHCl_3$).

Summary

The structure of substance C-II, newly isolated by the writers and described in the preceding paper,[1] was examined. This substance occurs as a crystalline powder, m.p. 238~242°(Kofler, uncorr.), $C_{41}H_{64}O_{18}\cdot 2H_2O$; $[\alpha]_D^{20}$ −1.4°; U.V. λ_{max}^{EtOH} 218 mμ(log ε 4.22). The sugar portion obtained by hydrolysis of Kiliani method was identified as fucose and glucose on paper chromatography. The partial decomposition of substance C-II afforded glucodigifucoside (digitoxigenin β-D-glucosido-β-D-fucoside), by elimination of one mole of glucose. The acetolysis of the acetate of substance C-II gave α-octaacetylcellobiose and it was established that substance C-II is digitoxigenin β-cellobiosido-β-D-fucoside. This is a new cardioglycoside and was named digifucocellobioside.

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10) F. Kaiser: Chem. Ber., 88, 556(1955).