UDC 547.918 : 582.951.6

29. **Atsuji Okano** : Studies on the Constituents of *Digitalis purpurea* L. VIII.[1] The Isolation of Neogitostin, a New Cardiotonic Glycoside.

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

It was reported in the preceding paper of this series that two kinds of new cardiotonic glycosides, gitostin[2] and glucodigifucoside,[3] had been isolated and characterized. In this paper will be described the isolation of another new glycoside, which was described in the previous paper as Substance A-IX, and it was proved to be a new cardiotonic glycoside.

The various fractions containing the substance A-IX were combined and purified by means of partition chromatography on Celite, developing with water-saturated methyl ethyl ketone in the same manner as reported previously.[3] Gitostin eluted more rapidly than the substance A-IX, but the latter eluted much slower and did not show any distribution on account of its very sparing solubility for water-saturated methyl ethyl ketone. The separation of the substance A-IX was carried out by partition chromatography on a cellulose column, developing with water-saturated isoamyl alcohol. The substance A-IX developed relatively fast and each fraction was submitted to paper partition chromotography. It was found that the substance A-IX had been isolated into fraction Nos. 8~13 by preceding paper chromatography as in Table I. Recrystallization of the residue from these fractions was attempted from various solvents, but crystallization was not effected.

Table I. Partition Chromatography of Neogitostin by Cellulose Column

Fract. No.	Weight (mg.)	Paper Partition Chromatography	
		H_2O-satd. MeCOEt	BuOH·toluene·H_2O (6:3:1)
1	450		
2	40	Gts	Gts, A-Ⅷ′
3	60	Gts	Gts, A-Ⅷ′
4	70	Gts	Gts, A-Ⅷ′
5	120	Gts, Neo	Gts, A-Ⅷ′, Neo
6	80	Gts, Neo	A-Ⅷ′, Neo
7	130	Gts, Neo	A-Ⅷ′, Neo
8	165	Neo	
9	240	Neo	
10	340	Neo	
11	340	Neo	
12	325	Neo	
13	320	Neo	
14	215	Neo, C-Ⅲ	
15	195	Neo, A-X, C-Ⅲ	
16	140	Neo, A-X	
17	200	Neo, A-X	
18	180	Neo, A-X	
19	160	Neo, A-X	
20	140	Neo, A-X	

Gts : Gitostin, Neo : Neogitostin.

The white crystalline powder that precipitated from methanol-ether mixture is hygroscopic, very soluble in water, methanol, hydrated ethanol, and insoluble in acetone,

* Hirakawabashi, Sumida-ku, Tokyo (岡野淳二).

1) Part Ⅶ : This Bulletin, **5**, 279(1957).
2) Part Ⅲ : *Ibid.*, **5**, 163(1957).
3) Part Ⅱ : *Ibid.*, **5**, 157(1957).

ethyl acetate, chloroform, and ether. It is extremely bitter to the taste and positive to both Legal and Raymond reactions. It does not give a positive reaction for a 2-desoxy-sugar with Keller-Kiliani reagent, but gives carmine red in sulfuric acid layer as gitoxigenin. It was acetylated in the usual manner and recrystallized from acetone-ether in colorless needles, m.p. 197~199°. When this glycoside acetate is deacetylated in accordance with the method of Reichstein,[4] deacetylated glycoside is easily crystallized from 80% ethanol-ether mixture in needles (Fig. 1), m.p. 249~252°. This crystalline

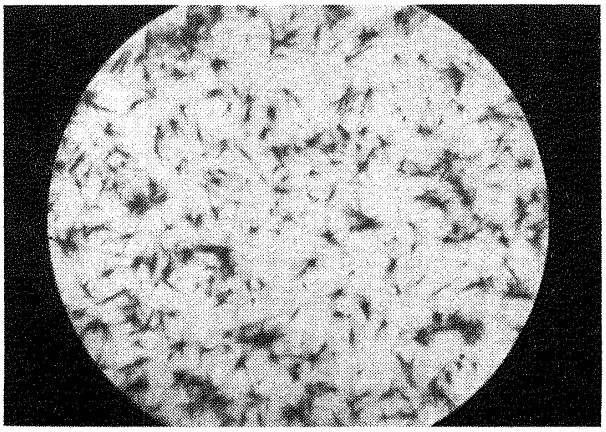

Fig. 1.
Neogitostin Monoacetate
(×30)

glycoside is not hygroscopic and gives the same color reactions as with amorphous substance A-IX. Its ultraviolet spectrum exhibits the maximum absorption at 218 mμ(EtOH), but Rf value of paper chromatogram is larger than that of amorphous substance A-IX (Fig. 3). It was positive to Frèrejacque's reaction,[4] the same as digitalinum verum monoacetate, and this supports the existence of an acyl group.

Hydrolysis of amorphous substance A-IX by heating with 3.5% hydrochloric acid affords needles, m.p. 214~216°, as the aglycone, and this was identified as dianhydrogitoxigenin. The sugar portion gave two spots respectively identical with digitalose and glucose spots, and coloration of the glucose spot was more intense in paper chromatography like gitostin.

Odoroside G[4] and digitalinum verum,[6] which are composed of digitalose and glucose, retained one acetyl group in digitalose part when acetates of their glycosides were deacetylated. Therefore, the above-mentioned crystalline glycoside is assumed as the monoacetylglycoside.

The assay of glucose by the method of determination used in the case of gitostin and glucodigifucoside, as described in Part III[2] and VI of this series, gave a value agreeing with that calculated for the presence of 2 moles of glucose in this substance. Therefore, the component of this glycoside is one and the same with gitostin.

To compare with this monoacetylglycoside, gitostin monoacetate was prepared in the same manner. It was easily crystallized (Fig. 2) and its character is distinguished from the new glycoside monoacetate, but their elemental analytical values and molecular extinction at 218 mμ agree with each other. When substance A-IX acetate was chromatographed through an alumina column, it was found to give the 16-anhydro compound, as in the case of gitostin, from the ultraviolet absorption spectrum.[7]

4) A. Rheiner, A. Hunger, T. Reichstein: Helv. Chim. Acta, **35**, 687(1952).
5) M. Frèrejacque: Compt. rend., **240**, 1804(1955)(C. A., **49**, 12774(1955)).
6) W. Rittel, A. Hunger, T. Reichsten: Helv. Chim. Acta, **35**, 434(1952).
7) Alumina chromatography of the acetate gave a fraction whose ultraviolet spectrum exhibited a new absorption maximum at 270 mμ and it showed a bluish white fluorescence on the filter paper under ultraviolet without spraying any coloring agent.

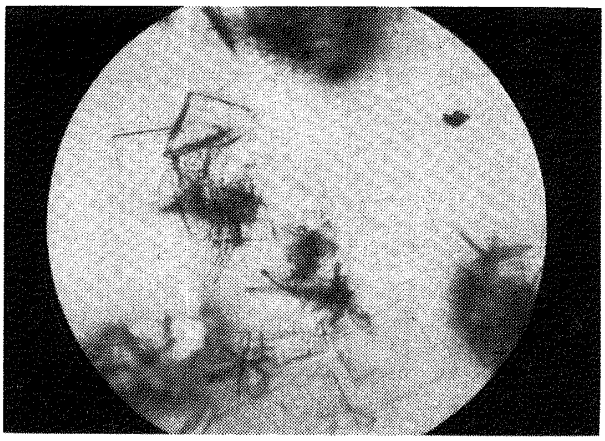

Fig. 2.
Gitostin Monoacetate

(×75)

It was thereby assumed that substance A-IX is an isomer of gitostin in sugar linkage and this glycoside was named neogitostin.

In fraction Nos. 2~7 of Table I, only gitostin spot was found by paper chromatography, but crystalline gitostin was obtained in only a small amount by recrystallization. To make clear this strange phenomenon, paper chromatography of various kinds of solvents was examined, and it was found that these fractions contained not only gitostin but also unknown glycoside which was neither gitostin nor neogitostin, when developed on the water-acetone (1:1) impregnated paper with butanol-toluene-water (6:3:1) as a developing solvent. This unknown glycoside is described in Fig. 4, as the substance A-Ⅷ′. It had been observed that gitostin fractions which contained a large amount of substance A-Ⅷ′ were not easily crystallized. The content of substance A-Ⅷ′ in the digitalis seed seems to be far smaller than that of gitostin. The spots of digitalinum verum, gitostin, gitostin monoacetate, neogitostin, and neogitostin monoacetate, by the use of above-mentioned developing mixture, are also shown in Fig. 4.

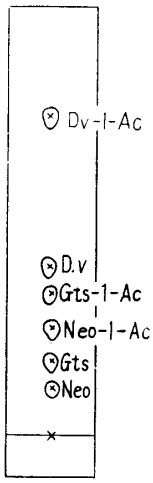

Fig. 3.

Figs. 3 and 4.
Paper Partition Chromatography of
Digitalinum verum, Gitostin,
Neogitostin, and their
Monoacetyl Derivatives

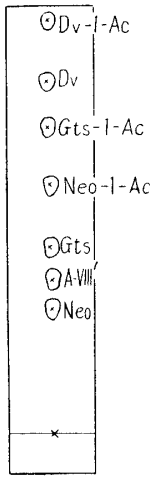

Fig. 4.

Toyo Roshi, No. 50, ascending method, at 18~25°

Moving phase	: Water-saturated MeCOEt	BuOH•toluene•H_2O (6:3:1)
Stationary phase	: Impregnated with H_2O•acetone (1:4)	Impregnated with H_2O•acetone (1:1)
Coloring agent	: 20% $SbCl_3$-$CHCl_3$ solution	

Dv : Digitalinum verum
Dv-1-Ac : Digitalinum verum monoacetate
Gts : Gitostin
Gts-1-Ac : Gitostin monoacetate
Neo : Neogitostin
Neo-1-Ac : Neogitostin monoacetate

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Experimental[8]

Separation of Substance A-IX—The fractions containing substance A-IX, confirmed by paper chromatography, indicated as fraction Nos. 17~20 in Table I, Part II,[3] the fraction Nos. 17~23 in Part III,[2] and mother liquor of crystallization of gitostin in Part III, were combined and submitted to partition chromatography through a column with 300 g. of Celite 535 as a carrier and water-saturated MeCOEt as the developer. Effluent was collected in 200-cc. fractions and each fraction was examined by paper partition chromatography, Gitostin collected in the fraction Nos. 10~19 (ca. 500 mg.) and substance A-IX was found in the fraction Nos. 20~35 containing substance A-X and a small quantity of gitostin. The residue from this fraction was crystallized, but crystals were not obtained.

Isolation of Substance A-IX—a) Preparation of Cellulose Column: Cellulose powder (Schleicher & Schüll, Nr. 123) was soaked in H_2O-Me_2CO(2:3) mixture and the powder was spread on a tray for 5 hrs. in ventilator until the odor of acetone disappeared. Then it was filled in a glass tube (internal diam., 5 cm.) by pressing with a glass rod to the height of 40 cm. The glycoside mixture was dissolved in $CHCl_3$-MeOH mixture and added to 20 g. of the cellulose powder, stirred thoroughly, and the organic solvent was evaporated in a ventilater. Further 20 g. of water-impregnated powder as described above was added to this mixture, stirred thoroughly, and placed on the column. Further 30 g. of the powder was piled and pressed on top of the column.

b) Partition Chromatography of Substance A-IX by Cellulose Column: The foregoing cellulose column was developed with water-saturated isoamyl alcohol and fractions of 200 cc. each were collected. The weight of residue and composition of each fraction are indicated in Table I. Fraction Nos. 1~7 was not distinguished from gitostin by paper chromatography using a solvent of water-saturated MeCOEt, but it did not crystallize from any solvents. By paper chromatograpny using a system of BuOH-toluene-H_2O (6:3:1) as a developing solvent, and impregnated with 1:1 mixture of water and acetone, this fraction was completely separated from gitostin, as shown in Fig. 4. This unknown spot showed the same coloration as gitostin and was numbered A-VIII'. These fractions were found to consist of a small quantities of gitostin besides A-VIII'.

Neogitostin was found to be eluted in fraction Nos. 8~13 and the portion was recrystallized from hydrated MeOH-Et_2O mixture and was obtained as a light yellow crystalline powder, whose properties were described in the text.

Fraction Nos. 14~20 contained a small amount of neogitostin and substance A-X.

Acetylation of Amorphous Neogitostin—Acetylation of 460 mg. of amorphous substance A-IX (neogitostin) by the usual method by standing with 7.5 cc. of pyridine and 5.5 cc. of Ac_2O for 72 hrs. and recrystallization from acetone-Et_2O afforded colorless needles (370 mg.), m.p. 197~199°; $[\alpha]_D^{21}$ −32.5° ±2°(c=1.58 in $CHCl_3$). *Anal.* Calcd. for $C_{60}H_{84}O_{28}$ (Neogitostin nonaacetate): C, 57.50: H, 6.76. Found: C, 57.00; H, 6.61.

Neogitostin Monoacetate—1.14 g. of the foregoing nonaacetate (m.p. 190~197°) was dissolved in 131 cc. of MeOH, a solution of 1.31 g. of $KHCO_3$ dissolved in 26.2 cc. of water was added, the vessel was stoppered closely, and allowed to stand for 15 days at room temperature. To this mixture, 20 cc. of water was added, evaporated to about 40 cc. under a reduced pressure at 20°, and the residual solution was extracted 5 times with 45 cc. each of a mixture of $CHCl_3$-EtOH (2:1). The combined extract solution was washed with a small amount of water, dried over anhyd. Na_2SO_4, and concentrated under a reduced pressure. The residue was submitted to chromatography through a column with 95 g. of Celite 535 as a carrier and water-saturated MeCOEt as the developer. Effluent (600~1300 cc.) was combined and evaporated under a reduced pressure. The residue was recrystallized from hydrated EtOH-Et_2O to needles, m.p. 249~252°; $[\alpha]_D^{24}$ −14.2°±3°(c=1.243 in MeOH). U. V. λ_{max}^{EtOH} 218 mμ(log ε 4.18. Calcd. as $C_{44}H_{68}O_{20}\cdot H_2O$: mol. wt., 935.00). Paper partition chromatography of this crystalline product with water-saturated MeCOEt showed larger Rf value than gitostin (Figs. 3 and 4). Qualitative examination of acyl group on a small strip of filter paper by Frèrejacque's coloration showed reddish violet same as digitalinum verum monoacetate. *Anal.* Calcd. for $C_{44}H_{68}O_{20}$ (monoacetate): C, 57.63; H, 7.47. Calcd. for $C_{44}H_{68}O_{20}\cdot H_2O$: C, 56.52; H, 7.55. Found: C, 56.18; H, 7.44.

Reacetylation of the above monoacetate by the usual manner gave the same nonaacetate, as needles, m.p. 193~195°

8) All m.p.s. were measured on a Kofler block and uncorrected.

Sugar Portion of Amorphous Neogitostin—A mixture of 50 mg. of amorphous neogitostin and 1.5 cc. of Kiliani mixture (AcOH:H_2O:conc. HCl=3.5:5.5:1) was refluxed for 1 hr. on a boiling water bath, 1.5 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-4B, and the extract obtained on evaporation of deionized solution was submitted to paper partition chromatography with sugar portions of gitostin and digitalinum verum using a mixture of BuOH-AcOH-H_2O(4:1:5), and the Rf values of every substance obtained were 0.11 and 0.40. Then it was found that the sugar portion of neogitostin also consisted of glucose and digitalose.

Gitostin Monoacetate—620 mg. of gitostin-nonaacetate, m.p. 163~166°, was dissolved in 62 cc. of MeOH, a solution of 620 mg. of $KHCO_3$ dissolved in 11 cc. of water was added, stoppered closely, and allowed to stand for 14 days at room temperature. This was treated as in the case of neogitostin monoacetate, and chromatographed on 32 g. of Celite 535, developing with water-saturated MeCOEt. Effluent (230~460 cc.) was combined and evaporated under a reduced pressure. The residue was recrystallized from MeOH-Et_2O and 80% EtOH-Et_2O to plates (Fig. 2), m.p. 257~260°, $[\alpha]_D^{24}$ +1.6°±1.5° (c=1.24 in 70% MeOH). Paper chromatogram of this crystalline product is shown in Figs. 3 and 4. The same color as neogitostin monoacetate was shown by Frèrejacque's coloration. *Anal.* Calcd. for $C_{44}H_{68}O_{20}$(Monoacetate): C, 57.63; H, 7.47. Calcd. for $C_{44}H_{68}O_{20}\cdot H_2O$: C, 56.52; H, 7.55. Found: C, 56.16; H, 7.79.

Determination of Glucose in Neogitostin Monoacetate—Glucose was determined by the method described in Part III,[2] using 29.85 mg. of crystalline neogitostin monoacetate, m.p. 245~249°, and resultant values were 10.45 and 11.73 mg., or 35.0% and 39.3%, agreeing well with 38.5% calculated as triglycoside monoacetate, $C_{44}H_{68}O_{20}\cdot H_2O$.

Summary

Of the 17 kinds of unknown cardiotonic glycoside-like substances contained in the seeds of *Digitalis purpurea*, described in Part II of this series,[3] a glycoside corresponding to substance A-IX was isolated. This substance was not obtained in crystalline form, but by the usual deacetylation of its acetate, monoacetate came as needle crytals of m.p. 249~252°(Kofler, uncorr.); $[\alpha]_D^{24}$ −14.2°±3°; $C_{44}H_{68}O_{20}\cdot H_2O$; negative to Keller-Kiliani reaction, giving carmine red sulfuric acid layer, and positive to Legal and Raymond reactions; U. V. λ_{max}^{EtOH} 218 mμ (log ε 4.18). These results indicated the substance to be a cardiotonic glycoside. Its hydrolysis with 3.5% hydrochloric acid afforded dianhydrogitoxigenin and the sugar portion was found to be digitalose and glucose by paper partition chromatography. Determination of glucose showed the presence of two moles. Therefore this substance was found to be a new glycoside, an isomer of gitostin which was reported in Part III, and was named neogitostin. Gitostin monoacetate was prepared and compared with neogitostin monoacetate.

By paper chromatography for strong polar glycoside portion, a new spot was confirmed between the spots of gitostin and neogitostin, and numbered A-VIII′.

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