The same product was also obtained by adding the solution of $HgCl_2$ and excess of AcONa to the solution of cycloheximide or by adding $HgCl_2$ to the solution of cycloheximide followed by adjusting to pH 6~7 with dil. NaOH solution.

N-Phenylmercuricycloheximide (III)——A solution of 5 g. of cycloheximide dissolved in 15 cc. of hot EtOH plus 250 cc. of H_2O was mixed with the solution of 6 g. of phenylmercuric acetate dissolved in 150 cc. of EtOH plus 250 cc. of H_2O, and a white precipitate deposited immediately. After standing in a refrigarator overnight, the precipitate was collected, washed with H_2O, and dried. Recrystallization of 10.5 g. of the crude product from 50% MeOH gave colorless scaly crystals, m.p. 161~162°. *Anal.* Calcd. for $C_{15}H_{22}O_4N \cdot HgC_6H_5 \cdot \frac{1}{2}H_2O$: C, 44.5; H, 4.95; N, 2.48; Hg, 35.4. Found: C, 44.4; H, 5.25; N, 2.55; Hg, 35.44, 35.22. IR (in Nujol) cm^{-1} : ν_{OH} 3448, $\nu_{C=O}$ 1712, 1681, 1613.

N-(2-Methyl-5-thienylmercuri)cycloheximide (IV)——To 1 g. of 2-methyl-5-thienylmercuric acetate dissolved in 30 cc. of EtOH and added with 15 cc. of H_2O, a solution of 770 mg. of cycloheximide dissolved in 60 cc. of H_2O was added. The deposited white precipitate was collected, washed with H_2O, and dried. The crude product (1.5 g.) was recrystallized repeatedly from 90% MeOH to colorless prisms, m.p. 149~150°. *Anal.* Calcd. for $C_{15}H_{22}O_4N \cdot HgC_5H_5S$: N, 2.43; Hg, 34.8. Found : N, 2.41; Hg, 34.73. IR (in Nujol) cm^{-1} : ν_{OH} 3472, $\nu_{C=O}$ 1715, 1689, 1617.

The authors express their deep gratitude to Dr. K. Abe, the Director of this Laboratory, for his encouragement. They are indebted to Mr. K. Kotera for infrared analysis and to Mrs. F. Hisamichi and Messers. T. Yoda and T. Kono for elementary analyses.

Summary

Among the water-insoluble compounds prepared by the addition of metal ions to a solution of cycloheximide, N-mercuricycloheximides were reported on their preparation, constitution, antifungal activity, and rodent reppellency.

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Kazuo Miyatake, Atsuji Okano, Kazuhiko Hoji, Tōsaku Miki, and Akio Sakashita : Studies on the Constituents of *Digitalis purpurea* L. XXII.[1] Alloneogitostin, a New Glycoside from Digitalis Seeds.

(*Research Laboratory, Daiichi Seiyaku Co., Ltd.*[*1])

It was previously reported that two cardiotonic glycosides, gitostin[2] and neogitostin,[3] had been isolated from the water-soluble fraction of digitalis seeds. In testing for other glycosides in the water-soluble fraction, a new glycosidal substance corresponding to substance A-X had already been observed on paper chromatogram.[4] This substance was isolated from residues obtained in the processing of gitostin and neogitostin, and its physical and chemical properties were determined.

The amount of the objective substance seemed to be too small for separation, and this substance was isolated and purified by partition chromatography using three systems of the developing solvent. These systems consisted of methyl ethyl ketone, a mixture of isoamyl alcohol and methyl ethyl ketone (3:1), and a mixture of butanol and chloroform (4:1).

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1) Part XXI : This Bulletin, **9**, 375 (1961).
2) Part III : *Ibid.*, **5**, 163 (1957).
3) Part VIII : *Ibid.*, **6**, 173 (1958).
4) Part II : *Ibid.*, **5**, 157 (1957).

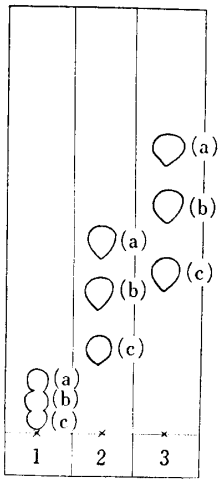


Fig. 1. Paper Partition Chromatography of Gitostin, Neogitostin, and Alloneogitostin

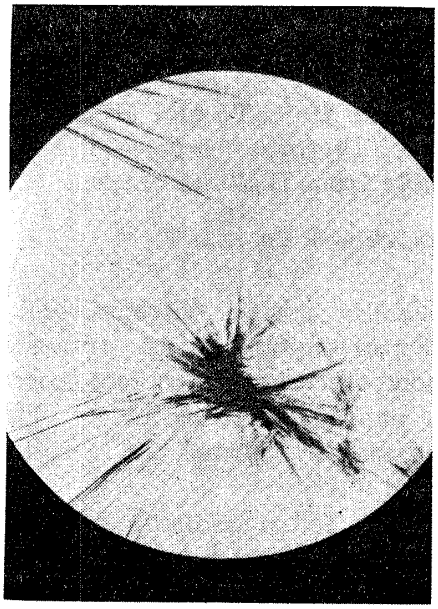
(a) Gitostin
(b) Neogitostin
(c) Alloneogitostin (Substance A-X)

Toyo Roshi No. 50; ascending method, at 18~22°
Moving phase: 1. MeCOEt saturated with H_2O
2. iso-AmOH-MeCOEt (3:1) saturated with H_2O
3. BuOH-$CHCl_3$ (4:1) saturated with H_2O
Stationary phase: Impregnated with Me_2CO-H_2O (4:1)
Coloring agent: 20% $SbCl_3$-$CHCl_3$ solution

These solvents were used by saturating with water and also applied to paper chromatography to effect separation (Fig. 1).

Substance A-X is a hygroscopic amorphous powder, easily soluble in water, methanol, and ethanol, and insoluble in chloroform and benzene. It is positive to both Legal and Raymond reactions, and negative to the Frèrejacque reaction as a test for acyl group, and exhibits a colorless glacial acetic acid layer and a carmine-red sulfuric acid layer in the Keller-Kiliani reaction. Its ultraviolet spectrum showed the absorption characteristic to cardiotonic glycoside, λ_{max}^{EtOH} 218 mμ.

Substance A-X was hydrolyzed with 3.5% hydrochloric acid to dianhydrogitoxigenin, digitalose, and glucose, which were identified by comparison of their Rf values with those of pure reference samples. Substance A-X was acetylated and the acetate was obtained



×30

Fig. 2. Alloneogitostin Nonaacetate

as needles, m.p. 170~175°, $[\alpha]_D^{28}$ −10.8°($CHCl_3$) (Fig. 2). It was shown earlier[3] that deacetylation of the acetates of gitostin and neogitostin with potassium hydrogencarbonate invariably left one acetyl group in the digitalose. Substance A-X acetate was also submitted to deacetylation with potassium hydrogencarbonate and converted to substance A-X monoacetate possessing one acetyl group in the digitalose portion. This monoacetate was

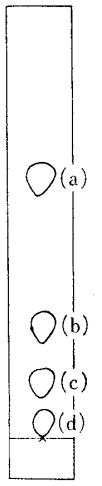
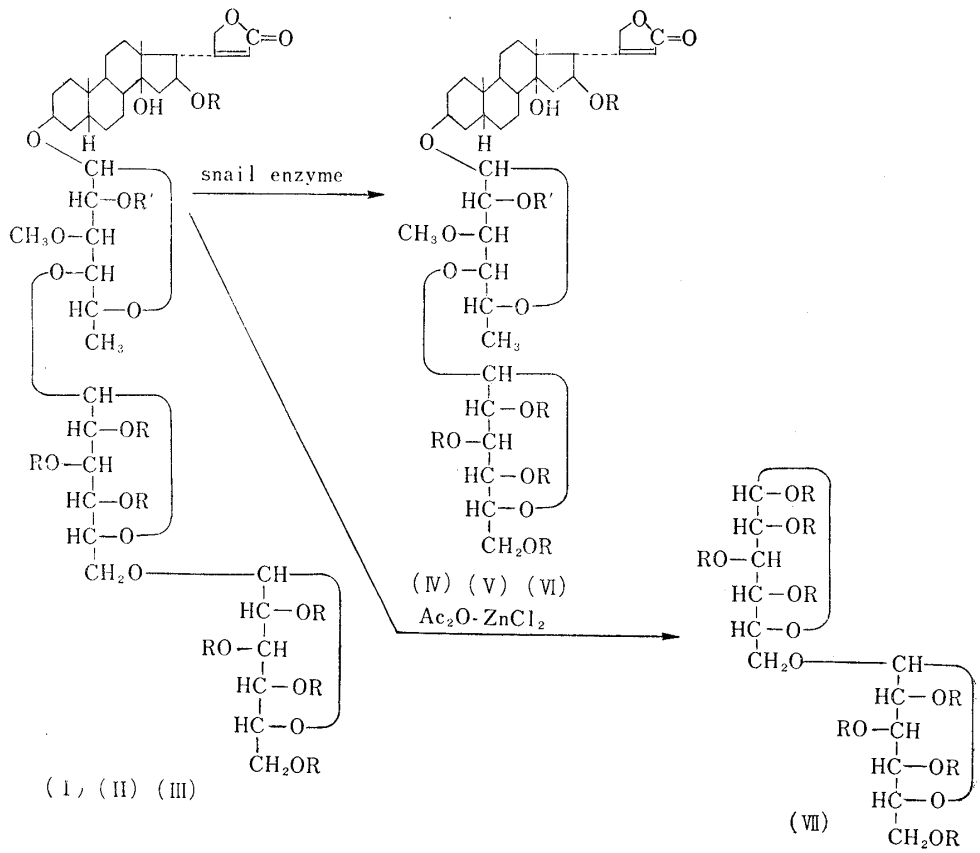


Fig. 3. Paper Partition Chromatography of Allodigitalinum verum, Alloneogitostin and their Monoacetyl Derivatives

(a) Allodigitalinum verum monoacetate
(b) Allodigitalinum verum
(c) Alloneogitostin monoacetate (Substance A-X monoacetate)
(d) Alloneogitostin (Substance A-X)

Toyo Roshi, No. 50; ascending method, at 18~22°
Moving phase : MeCOEt saturated with H_2O
Stationary phase : Impregnated with Me_2CO-H_2O (4:1)
Coloring agent : 20% $SbCl_3$-$CHCl_3$ solution



(I)	(R=R′=H)	Alloneogitostin
(II)	(R=R′=CH_3CO)	Alloneogitostin nonaacetate
(III)	(R=H, R′=CH_3CO)	Alloneogitostin monoacetate
(IV)	(R=R′=H)	Allodigitalinum verum
(V)	(R=R′=CH_3CO)	Allodigitalinum verum hexaacetate
(VI)	(R=H, R′=CH_3CO)	Allodigitalinum verum monoacetate
(VII)	(R=CH_3CO)	Octaacetyl α-gentiobiose

Chart 1.

recrystallized from a mixture of hydrous isopropanol and ether to give needle crystals, m.p. 194~198°, $[\alpha]_D^{17}$ +4.5°(MeOH). It is positive to the Frèrejacque reaction.

Various degrees of hydrolysis effected by snail enzyme have been described in previous papers of this series.[5,6] Decomposition with the snail enzyme was carried out on substance A-X monoacetate and there was formation of allodigitalinum verum monoacetate (VI)[1] by liberation of one mole of glucose from substance A-X monoacetate (Fig. 3) (Chart 1). It was shown in Part XXI[1] of this series that allodigitalinum verum (IV) isolated from digitalis seeds was found to be a 17α-glycoside.

The structure of substance A-X in the sugar portion was further confirmed by the use of acetolysis[6] of substance A-X acetate to octaacetyl-α-gentiobiose (VII)[7] (Chart 1) and it was presumed that the sugar portion is composed of digitalose and gentiobiose.

Substance A-X was identified as 16ξ,17α-gitoxigenin-β-gentiobiosido-β-D-digitaloside. It is very similar in structure to neogitostin, except for the difference of configuration at 17-position, and has been named alloneogitostin*[2] (I). It is very interesting that two 17α-glycosides, allodigitalinum verum (IV) and alloneogitostin (I), are present in the seeds of *Digitalis purpurea* L.

Experimental*[3]

Isolation of Substance A-X——As reported in Part X,[8] the easily water-soluble fraction was obtained from 100 kg. of digitalis seeds and submitted to column chromatography by the several methods described in Part II.[4] The eluted fractions containing a large amount of gitostin and neogitostin were collected, and after separation of gitostin and neogitostin from these fractions, substance A-X was isolated from 70 g. of the residue.

This residue was submitted to partition chromatography using 1 kg. of a mixture (1:1) of Celite and water, water-saturated mixture (3:1) of AmOH and MeCOEt as the developing solvent, and 500-cc. fractions were collected. The fraction Nos. 1~16 gave gitostin and neogitostin, the fraction Nos. 17~34 (34 g.) contained substance A-X and neogitostin, and the fractions later than No. 35 gave sugars and the Legal reaction-negative materials.

The residue from the fraction Nos. 17~34 was submitted to partition chromatography using 1 kg. of a mixture (3:2) of Celite and water, water-saturated MeCOEt as the solvent, and 1000-cc. fractions were collected. The fraction Nos. 1~5 gave neogitostin, the fraction Nos. 6~18 (10.2 g.) contained substance A-X, and the fractions later than No. 19 gave the Legal reaction-negative materials.

The fraction (10.2 g.) containing substance A-X was submitted to partition chromatography using 1 kg. of a mixture (1:1) of Celite and water, water-saturated mixture (4:1) of BuOH and $CHCl_3$, and 500-cc. fractions were collected. The fraction Nos. 6~11 afforded 2.0 g. of pure substance A-X.

Allo-neogitostin (I) (Substance A-X)——Hygroscopic colorless powder, soluble in H_2O, MeOH, and EtOH, and insoluble in $CHCl_3$ and benzene; UV λ_{max}^{EtOH} 218 mμ. Other properties are given in the main text.

A solution of 10 mg. of (I) dissolved in 5 cc. of 3.5% HCl-MeOH was refluxed on a water bath for 6 hr. The reaction product was deacidified by Amberlite IR-4B, extracted with $CHCl_3$ and H_2O, and submitted to paper chromatography. In the $CHCl_3$ extract, a spot was observed by the use of a mixture (100:30:20:1) of cyclohexane-AcOH-$CHCl_3$-H_2O and identified as dianhydrogitoxigenin. In the aqueous extract, two spots were detected by the use of a mixture (4:1:5) of BuOH-AcOH-H_2O and identified as digitalose and glucose.

Allo-neogitostin Nonaacetate (II)——340 mg. of (I) dissolved in 5.1 cc. of pyridine was acetylated with 3.4 cc. of Ac_2O and the crude acetate was recrystallized from a mixture of MeOH-Et_2O-petr. ether, affording 370 mg. of (II) as needles, m.p. 170~175°; $[\alpha]_D^{28}$ −10.8°(c=0.93, $CHCl_3$), UV : λ_{max}^{EtOH} 218 mμ (log ε 4.17). *Anal.* Calcd. for $C_{60}H_{84}O_{28}$: C, 57.50; H, 6.76; CH_3CO, 30.91. Found : C, 57.39; H, 6.95; CH_3CO, 30.80.

*[2] The configuration of 16-hydroxyl group in alloneogitostin is not clear. If it is found that the configuration of its 16-hydroxyl group is in β-position, the same as that of gitoxigenin, its name will be changed to 17α-neogitostin, in accordance with Reichstein's proposal.

*[3] All melting points were measured on a Kofler block and are uncorrected.

5) Part IV : This Bulletin, **5**, 167 (1957).
6) Part IX : *Ibid.*, **6**, 178 (1958).
7) C.S. Hudson, J.M. Johnson : J. Am. Chem. Soc., **39**, 1272 (1917).
8) Part X : This Bulletin, **7**, 212 (1959).

Hydrolysis of Alloneogitostin Nonaacetate (II) with $KHCO_3$——A solution of 250 mg. of $KHCO_3$ dissolved in 5 cc. of water was added to a solution of 220 mg. of (II) dissolved in 25 cc. of MeOH and the mixture was allowed to stand for 2 weeks at a room temperature. The reaction mixture was neutralized with Amberlite IRC-50 and evaporated in a reduced pressure at below 50°. This residue was submitted to partition chromatography using 60 g. of a mixture (1:1) of Celite and water, water-saturated MeCOEt as the developing solvent, and 50-cc. fractions were collected. The fraction Nos. 13～24 (135 mg.) gave alloneogitostin monoacetate (III).

Alloneogitostin Monoacetate (III)——The residue from the fraction Nos. 13～24 was recrystallized from hydr. iso-PrOH-Et_2O, and 100 mg. of (III) was obtained as needles, m.p. 194～198°; $[\alpha]_D^{17}$ +4.5° (c=1.33, MeOH). UV : λ_{max}^{EtOH} 219 mμ (log ε 4.10). This substance is soluble in MeOH, EtOH and H_2O, and insoluble in $CHCl_3$ and Et_2O. *Anal.* Calcd. for $C_{44}H_{68}O_{20}$: C, 57.63; H, 7.47; CH_3CO, 4.69. Calcd. for $C_{44}H_{68}O_{20}\cdot H_2O$: C, 56.52; H, 7.55; CH_3CO, 4.60. Found : C, 56.25; H, 7.92; CH_3CO, 5.68.

Enzymatic Decomposition of Alloneogitostin Monoacetate (III)——An enzyme solution obtained by extracting 40 mg. of snail enzyme with two 10-cc. portions of H_2O was added to a solution of 120 mg. of (III) dissolved in 240 cc. of H_2O, the mixture was covered with 5 cc. of toluene, and allowed to stand at 32°. After 2 hr., (III) was almost completely hydrolyzed by the enzyme and formed allodigitalinum verum monoacetate (VI), as evidenced by paper chromatography (Fig. 3). The solution was concentrated to 20 cc. in a reduced pressure, 100 cc. of EtOH was added to the residue, the precipitate thereby formed was removed, and the solution was further concentrated. The residue was recrystallized from MeOH-H_2O and hydr. EtOH-Et_2O to 55 mg. of needles, m.p. 192～197°; $[\alpha]_D^{17}$ +19.5° (c=1.23, MeOH). These crystals showed no depression of melting point on admixture with allodigitalinum verum monoacetate (VI).

This substance was acetylated with pyridine and Ac_2O, and the crude acetate was recrystallized from hydr. EtOH to needles, m.p. 144～149°/230～233°; $[\alpha]_D^{14}$ +18.9° (c=1.32, $CHCl_3$). The acetate gave no depression of melting point on admixture with allodigitalinum verum hexaacetate (V).

Acetolysis of Alloneogitostin Nonaacetate (II)——To a solution of 90 mg. of (II) dissolved in 1.65 cc. of Ac_2O, 23 mg. of $ZnCl_2$ was added and the mixture was heated for 30 min. at 100°. The reaction mixture was poured into ice water, allowed to stand for 15 hr., and extracted with two 25-cc. portions of $CHCl_3$. The extract was concentrated and submitted to adsorption chromatography using 2.5 g. of a mixture (5:1) of Florisil (Floridin Co., U.S.A.) and Celite. The fraction eluted with a mixture (99:1) of benzene and EtOH was recrystallized from hydr. EtOH to 25 mg. of needles, m.p. 186～189°; $[\alpha]_D^{14}$ +48.7° (c=1.40, $CHCl_3$). This substance showed no depression of melting point on admixture with octaacetyl-α-gentiobiose (VII), m.p. 185～188°; $[\alpha]_D^{24}$ +52.7°.

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Summary

An unknown 17α-glycoside was isolated from the seeds of *Digitalis purpurea* L. and found to be closely related to allodigitalinum verum, another 17α-glycoside, reported in Part XXI.[1] This glycoside was identified as 16ξ,17α-gitoxigenin-β-gentiobiosido-β-D-digitaloside and was named alloneogitostin.

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