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58. **Kazuo Miyatake, Atsuji Okano, Kazuhiko Hoji, Tōsaku Miki, and Akio Sakashita** : Studies on the Constituents of *Digitalis purpurea* L. XXI.[*1] Allo-digitalinum verum, a New Glycoside from Digitalis Seeds.

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It was reported in Part X of this series[1] that several new cardiotonic glycosides had been isolated from the seeds of *Digitalis purpurea* L. Three of these glycosides have already been studied and they were named digifucocellobioside,[2] gitorocellobioside,[3] and gitoxin cellobioside.[3] The present investigation was undertaken to determine the structure of substance A-VI.

Substance A-VI is in a syrupy form, but it was found to be homogeneous by the results of paper chromatography. It is easily soluble in methanol and ethanol, soluble in water and acetone, and insoluble in chloroform and ether. It has only a very slightly bitter taste, differing from other cardiotonic glycosides. It gives positive Legal and Raymond reactions, and negative Gregg-Gisvold reaction. In the Keller-Kiliani reaction, it exhibits a colorless glacial acetic acid layer and a carmine-red sulfuric acid layer, the same as digitalinum verum does.

By hydrolysis with 3.5% hydrochloric acid-methanol, it afforded dianhydrogitoxigenin as the aglycone, and digitalose and glucose as the sugars, the same as digitalinum verum.

It was described in Part X[1] that the acetate of this glycoside was obtained by the usual acetylation and the acetate obtained was recrystallized from ethanol to colorless needles, m.p. 237~239°, $[\alpha]_D^{18}$ +14.5°(chloroform). Its elemental and quantitative acetyl analyses agree with the formula $C_{48}H_{68}O_{20}$, calculated as digitalinum verum hexaacetate.

By deacetylation with potassium hydrogencarbonate, this acetate changed into a substance different from substance A-VI. After a column partition chromatography, it was recrystallized from ethanol-water-ether to colorless needles, m.p. 197~200°, $[\alpha]_D^{18}$ +17.4° (methanol). It gives the same reactions as substance A-VI, except a positive Frèrejacque reaction which shows that an acetyl group remains. Its elemental and quantitative acetyl analyses agree with the formula $C_{38}H_{58}O_{15}\cdot 2H_2O$, calculated as digitalinum verum monoacetate plus two moles of water.

The foregoing results show that substance A-VI has an aglycone which changes to dianhydrogitoxigenin by losing two moles of water during acid hydrolysis, and two sugars, digitalose and glucose. Therefore, substance A-VI is considered to be an isomer of digitalinum verum.

Substance A-VI acetate was treated by the same method as that used to obtain 16-anhydro derivative from digitalinum verum hexaacetate, i.e. an active alumina. The reaction material was deacetylated with potassium hydrogencarbonate and after purification through an alumina column, was recrystallized from hydrous methanol and methanol-chloroform-ether to colorless needles, m.p. 276~284°. Its ultraviolet spectrum exhibits maximum at 270 mμ (log ε 4.22) and the substance was found to be identical with 16-anhydrodigitalinum verum monoacetate[4] by paper chromatography and mixed fusion.

[*1] Part XX : This Bulletin, **9**, 296 (1961).

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1) Part X. A. Okano, *et al.* : This Bulletin, **7**, 212 (1959).
2) Part XI. A. Okano, *et al.* : *Ibid.*, **7**, 222 (1959).
3) Part XII. A. Okano, *et al.* : *Ibid.*, **7**, 226 (1959).
4) Part XIII. A. Okano, *et al.* : *Ibid.*, **7**, 627 (1959).

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Therefore, the aglycone of substance A-Ⅵ is an isomer of gitoxigenin, as far as 16- and 17-positions are concerned, and it is assumed that substance A-Ⅵ is an isomer of digitalinum verum.

Substance A-Ⅵ monoacetate has no bitter taste and was found to have inactive pharmaceutical effect (LD_{100} 32 mg./kg.) by the pigeon method.*[3] It is known that 17α-glycosides, allo-emicymarin,[5,6] allo-cymarin[5,7] and allo-periplocymarin,[5] also have no bitter taste and have inactive effect. Further, it was found that 17α-glycosides gave smaller Rf values[8] than the corresponding normal glycosides. In fact, the Rf value of substance A-Ⅵ is smaller than that of digitalinum verum (Fig. 1).

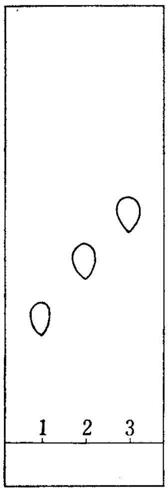
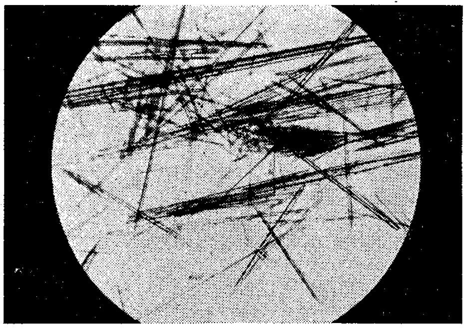


Fig. 1.

Paper Partition Chromatography

Moving phase : Water-saturated MeCOEt
Paper : Impregnated with $Me_2CO-H_2O(4:1)$ and Me_2CO evaporated
Coloring agent : 20% $SbCl_3-CHCl_3$ solution

1. Allo-digitalinum verum
2. Digitalinum verum
3. Allo-digitalinum verum monoacetate



(×30)

Fig. 2.

Allo-digitalinum verum Hexaacetate

From the foregoing results, it is confirmed that substance A-Ⅵ is formulated as 16ξ, 17α-gitoxigenin glucosidodigitaloside and it was named allo-digitalinum verum. If the configuration of 16-hydroxyl group is found to be β-, the same as gitoxigenin, this name will be changed to 17α-digitalinum verum, in accordance with Reichstein's proposition.[9]

It has been reported[6,7] that some glycosides are converted to the corresponding 17α-glycosides, by the action of enzyme in seeds. Therefore, it could be considered that allo-digitalinum verum will also be derived from digitalinum verum.

Recently, Reichstein, *et al.*[10] reported that gitoxigenin converted to 17α-gitoxigenin by chemical reaction. Therefore, the aglycone of allo-digitalinum verum must be compared

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*[3] Japanese Pharmacopoeia, Ed. Ⅵ (Supplement).

[5] Ch. Tamm : Fortschr. Chem. org. Naturstoffe, **13**, 137 (1956).

[6] I. D. Lamb, S. Smith : J. Chem. Soc., **1936**, 442.

[7] W. A. Jacobs : J. Biol. Chem., **88**, 519 (1930).

[8] R. Zelnik, O. Schindler : Helv. Chim. Acta, **40**, 2110 (1957).

[9] A. Kuritzkes, J. v. Euw, T. Reichstein : *Ibid.*, **42**, 1502 (1959).

[10] J. H. Russel, O. Schindler, T. Reichstein : *Ibid.*, **43**, 167 (1960).

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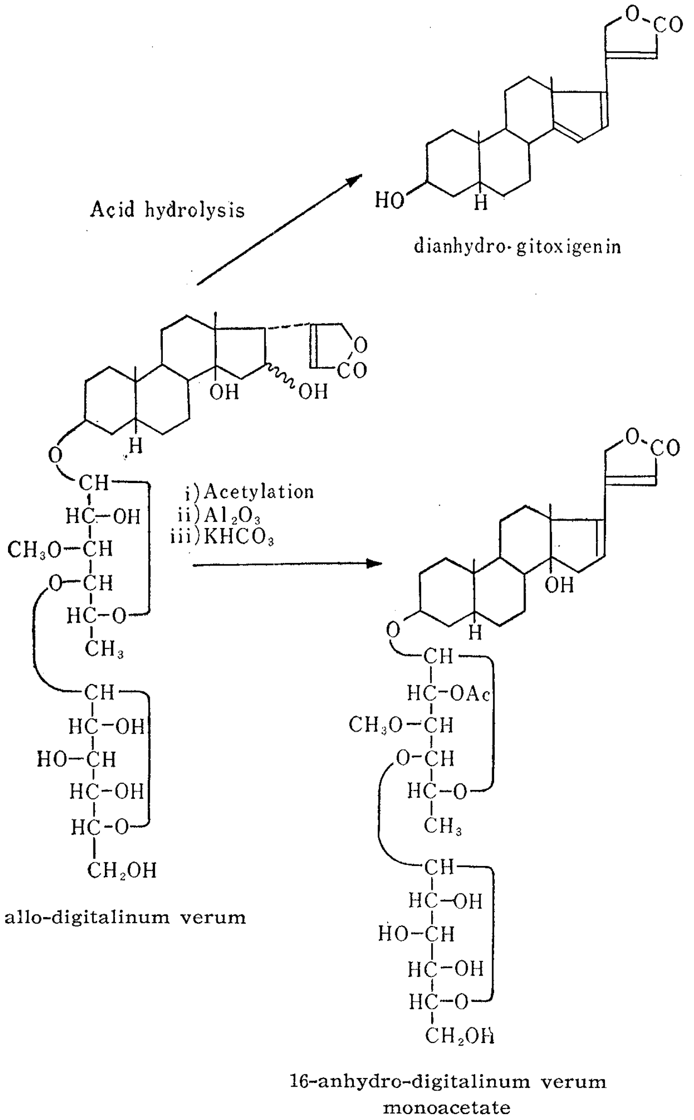

Chart 1.

with 17α-gitoxigenin, but the aglycone has not completely been examined. This point will be reported later.

Experimental[*4]

Drastic Acid Hydrolysis of Substance A-VI (Allo-digitalinum verum)——A solution of 10 mg. of substance A-VI dissolved in 1 cc. of MeOH and 1 cc. of 7% HCl was refluxed on a water bath for 6 hr. while bubbling CO_2. MeOH was distilled off in a reduced pressure and the residue was extracted with $CHCl_3$. The extract was chromatographically identified as dianhydrogitoxigenin. The residual aqueous layer was deacidified by Amberlite IR-4B, evaporated, and submitted to paper chromatography using BuOH-AcOH-H_2O (4:1:5). Two spots (Rf 0.43 and 0.14) of sugar were revealed and identified as those of digitalose (Rf 0.43) and glucose (Rf 0.14) from digitalinum verum.

Substance A-VI Acetate (Allo-digitalinum verum Hexaacetate)——This acetate was obtained by the usual method as reported in Part X.1) It was repeatedly recrystallized from EtOH to colorless needles, m.p. 237~239°, $[\alpha]_D^{18}$ +14.5° (c=1.10, $CHCl_3$). UV : λ_{max}^{EtOH} 217 mμ (log ε 4.22). *Anal.* Calcd. for $C_{48}H_{68}O_{20}$: C, 59.73; H, 7.12; CH_3CO, 26.76. Found : C, 59.19; H, 6.90; CH_3CO, 26.25.

It gives positive Legal and Raymond reactions, negative Gregg-Gisvold reaction, and exhibits a

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

colorless glacial AcOH layer and a carmine-red H_2SO_4 layer in Keller-Kiliani reaction. It is easily soluble in $CHCl_3$, soluble in Et_2O and Me_2CO, sparingly soluble in EtOH, and insoluble in H_2O.

Substance A-VI Monoacetate from Substance A-VI Acetate——To a solution of 500 mg. of substance A-VI acetate dissolved in 80 cc. of MeOH, 20 cc. of 2.5% $KHCO_3$ solution was added and this mixture was allowed to stand for 14 days at room temperature. MeOH was evaporated in a reduced pressure from the reaction mixture and the residual material was extracted with five 10-cc. portions of $BuOH-CHCl_3$ (1:2). The combined extract was washed twice with a small quantity of H_2O and evaporated to dryness (315 mg.) in a reduced pressure. It was treated by a Celite column (60 g. of Celite 535 and 60 cc. of H_2O) with MeCOEt, and 50-cc. fractions were collected. Fraction Nos. 5~7 contained substance A-VI monoacetate.

Substance A-VI Monoacetate (Allo-digitalinum verum Monoacetate)——The material was recrystallized from hydr. $EtOH-Et_2O$ to colorless needles, m.p. 197~200°, $[\alpha]_D^{18}$ +17.4°(c=1.51 MeOH). UV: λ_{max}^{EtOH} 219 mμ (log ε 4.22). *Anal.* Calcd. for $C_{38}H_{58}O_{15} \cdot 2H_2O$: C, 57.71; H, 8.09; CH_3CO, 5.56. Found: C, 56.32; H, 7.90; CH_3CO, 6.24.

It gives positive Legal and Raymond reactions, positive Frèrejacque reaction, and negative Gregg-Gisvold reaction, and exhibits a colorless glacial AcOH layer and a carmine-red H_2SO_4 layer in Keller-Kiliani reaction. It is easily soluble in MeOH, sparingly soluble in Me_2CO and H_2O, and insoluble in Et_2O.

Formation of 16-Anhydro-digitalinum verum Monoacetate from Allo-digitalinum verum Hexaacetate——A solution of 450 mg. of allo-digitalinum verum hexaacetate dissolved in 20 cc. of benzene was adsorbed on 25 g. of activated alumina and this was covered with benzene. After allowing this mixture to stand at room temperature for 5 days, the reaction mixture was eluted with hydr. BuOH and evaporated to dryness (380 mg.) in a reduced pressure. The residue was dissolved in 100 cc. of MeOH, 20 cc. of 2.5% $KHCO_3$ solution was added, and the mixture was allowed to stand at room temperature for 7 days. MeOH was evaporated and the residual solution was extracted with five 10-cc. portions of $BuOH-CHCl_3$ (1:1). The combined extract was washed with 20 cc. of H_2O and evaporated to dryness (270 mg.). The crude material was chromatographed through a Celite column (100 g. of Celite 535 and 100 cc. of H_2O) eluted with H_2O-saturated iso-BuCOMe-MeCOEt (1:1), and 100-cc. fractions were collected. The crude crystals were obtained from fraction Nos. 3~5, which were recrystallized from $MeOH-CHCl_3-Et_2O$ to colorless needles, m.p. 276~284°, showing no depression of melting point on admixture with 16-anhydro-digitalinum verum monoacetate from digitalinum verum hexaacetate. They were further identified by paper chromatography.

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Summary

The structure of substance A-VI, isolated by the writers and described in Part X,[1] was examined. This substance did not crystallize, but its acetate was obtained as needles, m.p. 237~239°. By acid hydrolysis, it afforded dianhydrogitoxigenin, digitalose, and glucose. Its treatment with activated alumina and deacetylation with potassium hydrogencarbonate changed its acetate into 16-anhydro-digitalinum verum monoacetate. Therefore substance A-VI is formulated as $16\xi,17\alpha$-gitoxigenin glucosidodigitaloside and it was named allo-digitalinum verum.

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