Colorless needles (1 g.) of m.p. 111~112° were obtained. *Anal.* Calcd. for $C_{10}H_8O_2N_2$: C, 63.83; H, 4.25; N, 14.89. Found : C, 63.99; H, 4.44; N, 14.35.

2-Quinoxalinecarbonamide (V)—(IV) (1 g.) was dissolved in MeOH (30 cc.), saturated with ammonia, and kept standing for 12 hrs. Colorless needles deposited out from the solvent. m.p. 200° (from MeOH). *Anal.* Calcd. for $C_9H_7ON_3$: C, 62.42; H, 4.06; N, 24.27. Found : C, 62.26; H, 4.16; N, 23.95.

2-Quinoxalinecarbohydrazide (VI)—To the solution of (IV) (0.5 g.) in MeOH (10 cc.) was added hydrazine hydrate (60%, 4 cc.). The mixture was refluxed on a water bath for 2 hrs. On cooling, crystalline solid separated out and purified from 60% MeOH to colorless needless of m.p. 208°. *Anal.* Calcd. for $C_9H_8ON_4$: C, 57.44; H, 4.25; N, 29.78. Found : C, 57.56; H, 4.43; N, 29.83.

2-Quinoxalinehydroxamic Acid (VII)—Metallic Na (0.5 g.) was dissolved in MeOH (30 cc.), and to this a solution of hydroxylamine hydrochloride (0.8 g.) in MeOH (20 cc.) was added, and sodium chloride separated was filtered off. To this filtrate, (IV was added and boiled for 30 mins. After the reaction, MeOH was removed, and the remaining substance was washed with water and purified from MeOH to give colorless needles of m.p. 190°(decomp.). *Anal.* Calcd. for $C_9H_7O_2N_3$: C, 57.14; H, 3.70; N, 22.22. Found : C, 57.59; H, 4.43; N, 21.97.

Summary

Oxidation of phenazine gave 2,3-quinoxalinedicarboxylic acid in the yield of 70%, together with small amount of another acidic substance. The latter was obtained by the similar oxidation of 1- and 2-methoxyphenazine in the yields of 45% and 24%, respectively. From quinoxalinedicarboxylic acid, 2-quinoxaline-carbonamide, -carbohydrazide, and -hydroxamic acid were prepared and their antitubercular activities tested.

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Atsuji Okano : Studies on the Constituents of *Digitalis purpurea* L. VII.[1] Enzymatic Decomposition of Glucodigifucoside.

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The new cardiotonic glycoside, glucodigifucoside, isolated from the seeds of *Digitalis purpurea*, is a diglycoside with digitoxigenin as the aglycone and glucose and fucose as the sugars, as described in the preceding paper.[1] In Part IV[2] of this series, enzymatic decomposition of gitostin[3] with an enzyme obtained from a snail (*Euhadra quaesita* DESHAYSE) was described and the same enzymatic method was adopted in the examination of the structure of glucodigifucoside.

Glucodigifucoside was treated for 5 days by the usual method with the enzyme solution, prepared from the snail enzyme powder treated with acetate buffer (pH 5.4), and digitoxigenin was obtained, but not a monoglucoside formed from glucodigifucoside. Examination by paper chromatography of the sugar portion formed by this enzymatic hydrolysis indicated spots for glucose and fucose, giving identical Rf values as those of the sugar portion obtained by acid hydrolysis as reported in the preceding paper. This has shown that the enzyme had also hydrolysed fucose.

Reichstein and others used their Schneckenferment, obtained from Weinbergschnecke, to numerous kinds of glycoside and the sugars hydrolyzed reported in the literature are all glucose.[4] In the enzymatic decomposition of cheiroside-A (uzarigenin-

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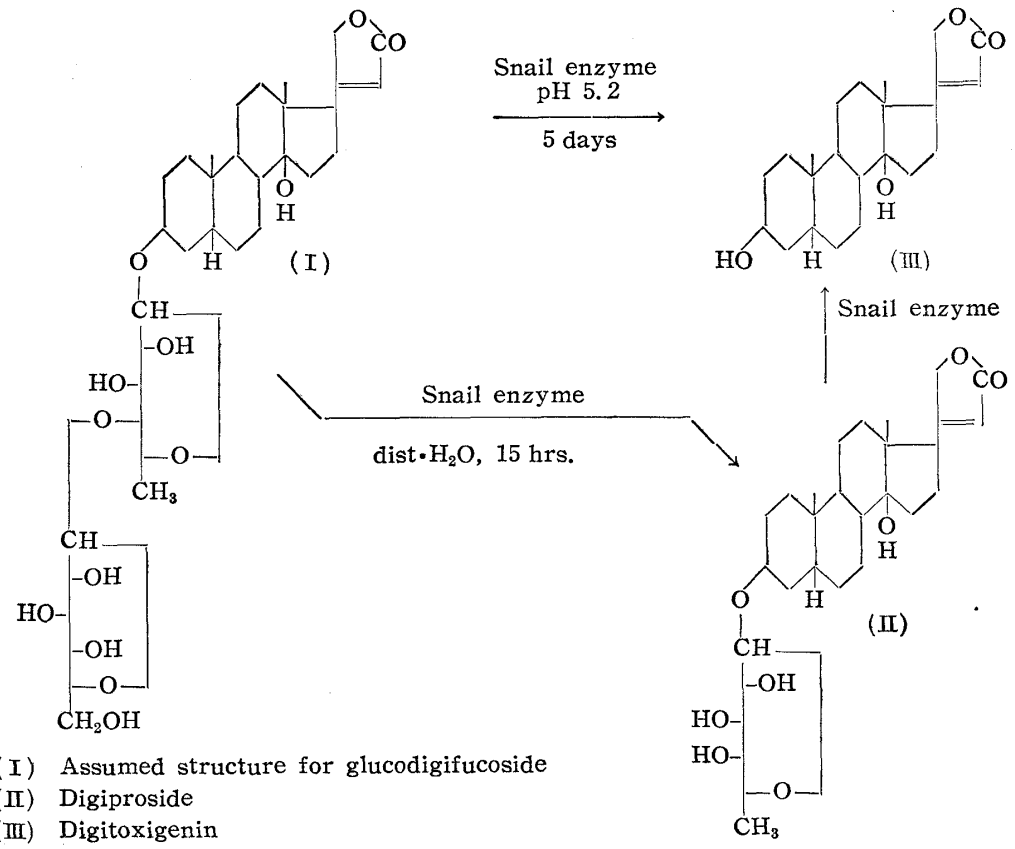
1) Part VI : This Bulletin, **5**, 272(1957).
2) Part III : *Ibid.*, **5**, 163(1957).
3) Part IV : *Ibid.*, **5**, 167(1957).
4) O. Schindler, T. Reichstein : Helv. Chim. Acta, **34**, 68(1951).

β-*d*-glucosido-β-*d*-fucoside),[5] an enzyme obtained from the seeds of *Adenium multiflorum* was used to hydrolyze glucose alone and a monofucoside, degluco-cheiroside-A, was obtained.

It had been shown in a previous paper[3] that application of an enzyme solution obtained from a snail and treated with distilled water, effected cleavage of only one mole of glucose from gitostin and digitalinum verum was obtained in a good yield. Decomposition under the same conditions was attempted with glucodigifucoside. The glycoside formed by hydrolysis was examined at definite intervals of time by paper partition chromatography and it was found that glucodigifucoside did not form digitoxigenin directly but that only one mole of glucose is first hydrolyzed and then fucose is hydrolyzed to form digitoxigenin. It was also established through paper partition chromatography of the sugar portion that fucose is not formed by hydrolysis for 20 hours by this reaction.

Therefore, glucodigifucoside was treated for 15 hours under the foregoing hydrolytic conditions, the reaction mixture was extracted with chloroform-ethanol mixture (2 : 1), and the chloroform-soluble portion was purified by adsorption chromatography through alumina, from which a monoglycoside was obtained as plate crystals of m.p. 190~192° (Kofler, uncorr.) and a small amount of glucodigifucoside was recovered. Examination of the aqueous layer by paper partition chromatography revealed only a clear spot of glucose.

The substance obtained by this hydrolysis, therefore, is a digitoxigenin-fucoside and it showed no depression of the melting point on admixture with digiproside,[6] isolated



(I) Assumed structure for glucodigifucoside
(II) Digiproside
(III) Digitoxigenin

Chart 1. Enzymatic Decomposition of Glucodigifucoside

5) J. A. Moore, Ch. Tamm, T. Reichstein : Helv. Chim. Acta, **37**, 755(1954).
6) Grateful acknowledgement is expressed to Mr. D. Satoh of Shionogi Research Laboratory for the kind donation of digiproside crystals.
7) D. Satoh, *et al.* : This Bulletin, **4**, 284(1956).

by Satoh and others,[7] and the Rf values also agreed well. The melting point of the acetate, obtained by acetylation by the usual method, also agreed with that given in the literature. This has established the identity of this digitoxigenin-fucoside as digiproside.

It was identified through paper partition chromatogrphy that this digiproside also is hydrolyzed by the enzyme to digitoxigenin and fucose.

The foregoing results have shown that the glucodigifucoside isolated by the writer is digitoxigenin-glucosido-fucoside and its structure would be that formed by the bonding of glucose to the fucose portion in digiproside. If the bonding of the sugar were taken to be the same as that presumed for cheiroside-A and glucogitofucoside, the structural formula would be the one indicated in Chart 1.

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

Experimental

Formation of Digitoxigenin from Glucodigifucoside—To a solution of 100 mg. of glucodigifucoside, m.p. 193~197°, dissolved in 5 cc. of EtOH, 350 cc. of distilled water of 30° was added and EtOH was distilled off under a reduced pressure. A supernatant solution from 300 mg. of enzyme powder treated with 10 cc. of acetate buffer (pH 5.4) and centrifuged, was added to this solution, together with 5 cc. of toluene, and the mixture was allowed to stand in a thermostat of 32° for 5 days. The reaction mixture was concentrated to 50 cc. under a reduced pressure, at a bath temp. of 45°, 250 cc. of EtOH was added to the concentrated solution, and the enzyme that precipitated was filtered off, using Hyflo Super Cel. The filtrate was again concentrated to 40 cc. under a reduced pressure, the concentrated solution was shaken with a mixture (2:1) of $CHCl_3$ and EtOH, and $CHCl_3$ layer was evaporated under a reduced pressure. Two g. of the $CHCl_3$ extract was chromatographed on 2 g. of alumina (Merck) and the portion eluted with $CHCl_3$ was recrystallized from MeOH-ether mixture to 40 mg. of prisms, m.p. 250~252°; $[\alpha]_D^{26}$ +14°±1.5°(c=1.65, MeOH). These crystals showed no depression of m.p. on admixture with digitoxigenin, m.p. 246~248°, and paper partition chromatography gave identical results of Rf 0.80(22°) on formamide-impregnated Toyo Roshi No. 50, developed with formamide-saturated xylene-MeCOEt (1:1) mixture, by the ascending method.

Further elution of the foregoing alumina column failed to afford any further substance.

Formation of Digiproside from Glucodigifucoside—To a solution of 190 mg. of glucodigifucoside, m.p. 191~195°, dissolved in 15 cc. of EtOH, 800 cc. of distilled water was added, EtOH was evaporated from this solution under a reduced pressure, and 7 cc. of the supernatant, obtained from 60 mg. of enzyme powder treated with 5 cc. of distilled water and centrifuged, was added, together with 7 cc. of toluene. The mixture was allowed to stand in a thermostat of 32° for 15 hrs., the solution was concentrated under a reduced pressure to 70 cc., and 350 cc. of EtOH was added to the concentrated solution. The enzyme that precipitated out was filtered off with Hyflo Super Cel, filtrate was concentrated to 50 cc. under a reduced pressure, and the concentrated solution was shaken with a mixture (2:1) of $CHCl_3$-EtOH. $CHCl_3$ layer was evaporated under a reduced pressure and the extract thereby obtained was chromatographed on 40 g. of alumina . The portion eluted with $CHCl_3$ contained a minute amount of substance that gave a weak positive result to the Legal reaction. A portion that eluted with $CHCl_3$ containing 5% MeOH was recrystallized from acetone-ether to 85 mg. of plates, m.p. 190~192°, undepressed on admixture with digiproside (from MeOH), m.p. 147~150°/190~194°. Paper partition chromatography with the following two kinds of solvent also gave identical Rf values.

i) Toyo Roshi No. 51, impregnated with 1:4 mixture of formamide and acetone, developed by the ascending method with formamide-saturated toluene-BuOH (3:1). Rf 0.26(25°).

ii) Toyo Roshi No. 51, impregnated with formamide as above and developed for 2.5 hrs. by the ascending method using $CHCl_3$ saturated with formamide. Rf 0.36. Strospeside, Rf 0.44; gitoxigenin, Rf 0.53(21°).

The aqueous layer separated from the $CHCl_3$ extract was concentrated under a reduced pressure and the extract thereby obtained was submitted to paper partition chromatography as described in the preceding paper,[1] from which a spot corresponding to glucose alone was obtained.

Enzymatic Decomposition of Digiproside—To a solution of 9 mg. of digiproside, m.p. 190~192°, formed by enzymatic decomposition of glucodigifucoside, dissolved in 5 cc. of EtOH, 40 cc. of distilled water was added, and EtOH was distilled off under a reduced pressure. A supernatant obtained from 10 mg. of the enzyme solution treated with 5 cc. of distilled water and centrifuged, was added

to this solution, together with 2 cc. of toluene, and the mixture was allowed to stand in a thermostat of 32° for 5 days. This reaction mixture was treated as usual and the $CHCl_3$ extract was examined by paper partition chromatography. Only a spot giving the same Rf value as that of digitoxigenin was detected and not the spot of digiproside.

The sugar portion in the aqueous layer was examined by paper partition chromatography as described in the preceding paper[1] and only one spot whose Rf value agreed with that reported for fucose was obtained.

Summary

The structure of glucodigifucoside, newly isolated by the writer and described in the preceding paper,[1] was examined by enzymatic decomposition. The enzyme used was the same enzyme isolated from a snail (*Euhadra quaesita* DESHAYES), described in Part IV of this series.[3] It was found that hydrolysis under a general condition afforded digitoxigenin, with both glucose and fucose hydrolyzed, but under the same conditions as those used in the partial decomposition of gitostin to form digitalinum verum, by shortening the duration of hydrolysis, glucose alone was found to be hydrolyzed, affording a digiproside. From these results, it was established that glucodigifucoside is a diglycoside formed by the bonding of one mole of glucose to digiproside. It was also found that this digiproside is also hydrolyzed by this enzyme and that this enzyme also hydrolyzed fucose.

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