UDC 547.92.02

100. **Hayao Nawa and Masao Uchibayashi:** Studies on Steroids. XIII.[1] The Structure of Rhodexin-B and -C.*

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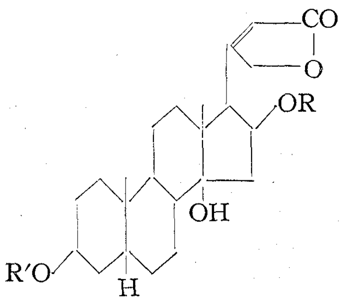
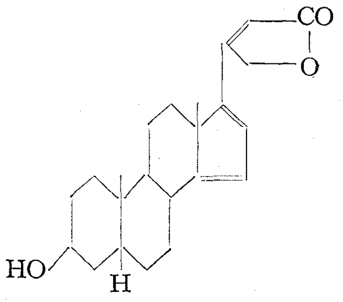
In the preceding paper of this series[1] which dealt with the Mannich hydrolysis of a cardiac glycoside possessing a 2-hydroxy sugar, gitorin, it was reported that two unidentified substances which were detected besides gitoxigenin and dianhydrogitoxigenin on paper chromatogram were supposed to be 14-anhydro- and 16-anhydrogitoxigenin, but could not be investigated further due to their meager amount. For the purpose of pursuing these substances, the Mannich hydrolysis was applied to rhodexin-B, a cardiac glycoside of *Rhodea japonica* ROTH., which seemed to have similarity with gitorin in constitution, and the unexpected findings obtained thereby required correction of the existing formulation of the structure of rhodexin-B and -C.

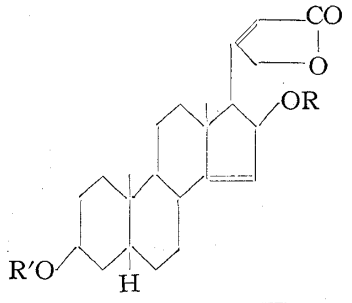
Mannich hydrolysis of rhodexin-B was effected in the following manner. Rhodexin-B dissolved in acetone containing hydrogen chloride was allowed to stand at room temperature as in the case of gitorin. The progress of the reaction was examined by paper partition



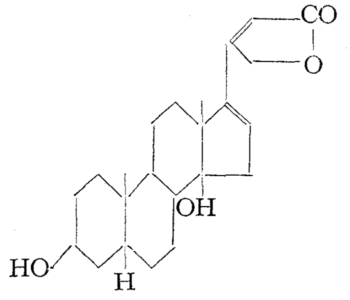
Gitorin (I) R=H, R'=glucose
Rhodexin-B (II) R=Ac, R'=rhamnose
Rhodexin-C (III) R=Ac, R'=rhamnose-glucose
Gitoxigenin (IV) R=R'=H
Oleandrigenin (16-Acetylgitoxigenin) (V) R=Ac, R'=H
Diacetylgitoxigenin (VI) R=R'=Ac



Dianhydrogitoxigenin (VII)



14-Anhydrogitoxigenin (VIII) R=R'=H
3, 16-Diacetyl-14-anhydrogitoxigenin (IX) R=R'=Ac

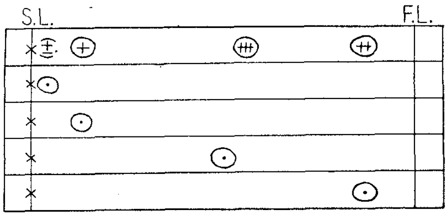


16-Anhydrogitoxigenin (X)

Fig. 1. Paper Chromatograms of the Hydrolysis Products of Rhodexin-B

Solvent system: Benzene-MeOH-H_2O=5:3:2

Ascending method; S.L.=starting line, S.F.=solvent front



Products
Rhodexin-B, Rf 0.04
Gitoxigenin, Rf 0.14
16-Anhydrogitoxigenin, Rf 0.52
Dianhyrogitoxigenin, Rf 0.89

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* A preliminary report of this investigation appeared in Chem. & Ind. (London), **1958**, 653.

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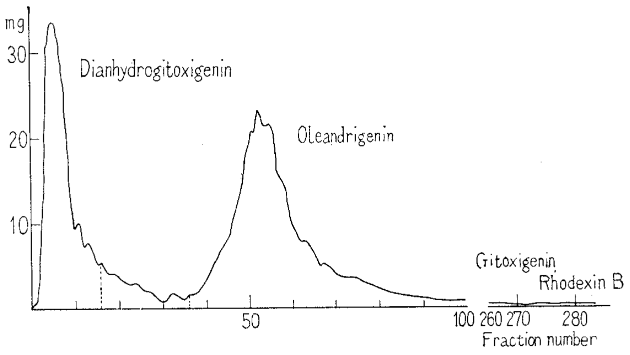
[1] Part XII. M. Uchibayashi: This Bulletin, **6**, 504 (1958).

chromatography and on the 13th day disappearance of almost all of rhodexin-B and formation of gitoxigenin (IV), dianhydrogitoxigenin (VII), and an appreciable amount of an unidentified substance were observed (Fig. 1). Treatment of the reaction mixture gave an aglycone and sugar fractions, and in the latter the presence of rhamnose was confirmed by paper partition chromatography.

The aglycone fraction was chromatographed on a powdered cellulose column to separate into its components. Fig. 2 shows the weight of substances present in each fraction (10 cc.). The fractions 1∼16 and 267∼276 respectively gave dianhydrogitoxigenin (VII) and gitoxigenin (IV), and the fractions 277∼284 yielded a small amount of the recovered rhodexin-B. From the fractions 36∼100 an unknown substance was successfully isolated as colorless thin plates which melted at 223∼224°. This substance was evidently the main product in the hydrolysis since its yield was the highest among the three products.

Fig. 2. Separation of the Hydrolysis Product of Rhodexin-B



Considering the proposed structure of rhodexin-B, it was tentatively assumed that the compound obtained might be a monoanhydrogitoxigenin (VIII or X) derivable from gitoxigenin (IV) by removal of either of its hydroxyl groups at 14- and 16-positions. The product shows similarity in its analytical values of carbon and hydrogen to a hydrated monoanhydro compound, and has an absorption maximum at 215 mμ (log ε 4.24) in its ultraviolet spectrum, and the specific rotation, $[\alpha]_D$ −4° ($CHCl_3$), −5° (MeOH). Since these data clearly exclude the possibility of 16-anhydrogitoxigenin (X), m.p. 227°,[2] 245∼247°,[3] λ_{max} 271 mμ (log ε 4.23),[3] $[\alpha]_D$ +93.1° ($CHCl_3$), [3] +92.7°±3° (MeOH),[2] the product was thought likely to be 14-anhydrogitoxigenin (VIII) which has been so far unknown in the literature. Therefore acetylation was performed on the product to examine this assumption. The diacetate, m.p. 241∼242°, thus obtained was apparently different from 3,16-diacetyl-14-anhydrogitoxigenin (IX),[4] m.p. 168∼170°, and moreover it was unexpectedly found to be 3,16-diacetylgitoxigenin (VI) judging from its analytical values, melting point, and optical rotation. Thus, these findings led to the examination of the presence of an acetyl group in the substance which had been tentatively considered as a monoanhydro compound. As a result, the substance was not only revealed to be certainly a monoacetyl compound, but also identified, from analytical values, melting point, optical rotation, and ultraviolet spectrum, to be oleandrigenin or 16-acetylgitoxigenin (V), which was first obtained from *Nerium oleander* in 1937[5] and has been known as the aglycone of various cardiac glycosides.[6]

One of the authors (Nawa) had presented[7] a supposed structure of rhodexin-B as gitoxigenin rhamnoside on the ground that it gives dianhydrogitoxigenin (VII) and rhamnose on acid hydrolysis under drastic conditions. As compounds giving the dianhydroaglycone under such conditions, however, glycosides containing 16-acylgitoxigenin, such as oleandrigenin and gitaloxigenin, should also be taken into consideration, and in the present work oleandrigenin (V) together with rhamnose was actually obtained on mild hydrolysis. Thus, reëxamination of the previous analytical values of rhodexin-B gave a good support to the for-

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[2] A. Hunger, T. Reichstein: Helv. Chim. Acta, **33**, 76(1950).
[3] M. Okada: Yakugaku Zasshi, **73**, 86(1953).
[4] R. Tschesche: Ber., **71**, 1927(1938).
[5] W. Neumann: *Ibid.*, **70**, 1547(1937).
[6] e.g. Cryptograndoside-A and -B. A. Abei, T. Reichstein: Helv. Chim. Acta, **33**, 1013(1950); Hongheloside-A and -C. cf. Footnote 2); Urechitoxin-C. H. Hassall: J. Chem. Soc., **1951**, 3193.
[7] H. Nawa: Proc. Japan Acad., **27**, 436(1951); Yakugaku Zasshi, **72**, 404(1952).

mula of oleandrigenin rhamnoside as was expected, and accordingly the structure of rhodexin-B is now best represented by 16-acetylgitoxigenin rhamnoside (II). Another cardiac glycoside, rhodexin-C,[8] had been isolated from *Rhodea japonica* Roth. and formulated as gitoxigenin rhamnosido-glucoside from the fact that it splits into rhodexin-B and glucose by the action of enzymes. This glycoside has now to be denoted as 16-acetylgitoxigenin rhamnosido-glucoside (III) according to the findings obtained above and the analytical data also agreed well with this formulation.

Although investigation of the unidentified substances which were detected as the Mannich hydrolysis product of gitorin could not be accomplished, the isolation of oleandrigenin as the hydrolysis product of rhodexin-B was achieved, which enabled the authors to correct the proposed structures for rhodexin-B and -C to a much more probable ones. Application of the Mannich hydrolysis to the oleandrigenin glycosides of 2-hydroxy sugar has not been reported yet. It is now clarified that while dianhydrogitoxigenin and gitoxigenin are the major product and the substances supposed to be 14-anhydro- and 16-anhydrogitoxigenin are only minor ones in the case of the Mannich hydrolysis of the gitoxigenin glycoside, gitorin, the predominant product is 16-acetylgitoxigenin in the case of the hydrolysis of the oleandrigenin glycoside, rhodexin-B, and no by-products other than a small amount of dianhydrogitoxigenin and gitoxigenin can be detected. These facts may indicate that the acetoxyl group at 16-position of cardiac aglycones is more stable and less likely to be removed by the action of acids as compared with the hydroxyl in the same position, and may be particularly interesting when considered in connection with a possible pathway from gitoxigenin to its dianhydro compound.

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Experimental

(All melting points are uncorrected.)

Mannich Hydrolysis of Rhodexin-B (II)—Rhodexin-B (II) weighing 819.7 mg. was dissolved in 130 cc. of acetone containing 0.4% of HCl and the mixture was allowed to stand in a closed flask at room temperature. The progress of the reaction was examined by paper partition chromatography (solvent system, benzene : MeOH : $H_2O=5:3:2$) and on the 13th day the hydrolysis was found nearly complete with disappearance of almost all of rhodexin-B. After being neutralized with $KHCO_3$ solution, the reaction mixture was concentrated *in vacuo* at low temperature and the residue was extracted with $CHCl_3$.

(a) Identification of the sugar fraction: The aqueous layer of the above extraction was concentrated *in vacuo* and the residue was extracted with MeOH. The residual substance obtained by concentration of the MeOH extract, showed on the paper chromatogram (solvent system, AcOH : *n*-BuOH : $H_2O=1:4:5$, ascending method; reagent, benzidine) Rf value of 0.31 which was undistinguishable from the Rf value of authentic L-rhamnose.

(b) Identification of the aglycone fraction: The $CHCl_3$ layer, obtained by the extraction of the hydrolysis products, was washed with dil. $KHCO_3$ solution and water, dried over $MgSO_4$, and the solvent was removed. The residue was chromatographed on a powdered cellulose column. Through a column of 280 g. of powdered cellulose was passed the aqueous layer of a mixture of benzene : MeOH : $H_2O=5:3:2$ and then the organic layer of the mixture until the eluate became homogeneous. MeOH solution of the residue obtained above was added to this column and developed with the same organic solvent mixture. The eluate collected in 10-cc. portions was examined by paper partition chromatography (benzene : MeOH : $H_2O=5:3:2$). The fractions 1～16, 267～276, and 277～284 gave dianhydrogitoxigenin (VII) (97.6 mg.), gitoxigenin (IV) (44.3 mg.), and rhodexin-B (II) (32.6 mg.), respectively, and they were identified by comparison with respective authentic samples. The fractions 36～100 were combined and concentrated, and the residue was recrystallized from benzene-MeOH to yield 285.6 mg. of colorless plates (V), m. p. 223～224°. *Anal.* Calcd. for $C_{25}H_{36}O_6$ (Oleandrigenin): C, 69.42; H, 8.39; $COCH_3$, 9.94. Found: C, 69.40, H, 8.32; $COCH_3$, 10.58. $[\alpha]_D^{20}$ $-4°$ (c=0.5%, $CHCl_3$), $-5°$ (c=0.5%, MeOH), λ_{max}^{EtOH} 215 mμ (log ε 4.24).

Formation of Diacetylgitoxigenin (VI) from Oleandrigenin (V)—A portion of 225 mg. of oleandrigenin (V) obtained above was dissolved in 2.6 cc. of pyridine, mixed with 2.6 cc. of Ac_2O, and the mixture was allowed to stand at room temperature for 52 hrs. The red-colored reaction mixture was

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8) H. Nawa: Yakugaku Zasshi **72,** 507(1952).

poured into ice water containing 4.0 cc. of conc. HCl. The separated crystals were collected, washed with water, and dissolved in 50 cc. of $CHCl_3$. The $CHCl_3$ solution was washed with dil. $NaHCO_3$ solution and water, dried over $MgSO_4$, and the solvent was removed. The residue was recrystallized from AcOEt-$CHCl_3$ to 221 mg. of colorless plates, m.p. 241~242°. *Anal.* Calcd. for $C_{27}H_{38}O_7$ (Diacetylgitoxigenin): C, 68.33; H, 8.07; $COCH_3$, 18.14. Found: C, 68.30; H, 8.02; $COCH_3$, 17.76. $[\alpha]_D^{17}$ −6° (c=1.0%, $CHCl_3$), λ_{max}^{EtOH} 216 mμ (log ε 4.14).

Summary

Rhodexin-B, a cardiac glycoside of *Rhodea japonica* ROTH., was subjected to the Mannich hydrolysis and oleandrigenin (16-acetylgitoxigenin) was obtained as the main product, together with gitoxigenin, dianhydrogitoxigenin, and rhamnose. From these results the existing formulation for the structure of rhodexin-B and -C were corrected to 16-acetylgitoxigenin rhamnoside (II) and 16-acetylgitoxigenin rhamnosido-glucoside (III), respectively. This report constitutes the first example of the Mannich hydrolysis of the glycosides of oleandrigenin linking with a 2-hydroxy sugar.

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101 **Mitiiti Fujita and Tsutomu Furuya**: Studies on the Metabolism of Naturally Occurring Coumarins. I. Separation and Identification of Coumarin Derivatives by Paper Chromatography.

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Studies on the metabolism of drugs are usefully applied for finding a drug which is stable in human body keeping its direct effectivity. As a general method for such studies, several workers have carried out chemical investigation on metabolic products excreted in urine after administration of a certain drug in animals. The knowledge induced from the studies concerning the fate of a drug in body and the maintenance period of effectiveness would play an important part in improvement of medicaments.

A number of metabolic studies on chemicals have been reported, whereas constituents of crude drugs has not been sufficiently studied.

The present authors commenced studies on the metabolism of coumarin derivatives which frequently occur in plants to examine their mode of action and the mechanism of detoxication. Difficulties of this study arise from the coëxistence of similar constituents in plants and the poor recovery of excreted product. In such a case paper chromatography was usefully applied to analyse the excreted substances.

As a first step in this study, separation and identification of more than 30 kinds of natural and synthetic coumarins were studied by paper chromatography.

The following works have already been published to deal with the paper chromatography of coumarins:

Swendsen[1] separated and identified coumarins from *Pimpinella saxifraga* L., *P. magna* L., and *Angelica* spp. using petroleum ether-benzene-methanol (25 : 20 : 10) as a developing solvent system, but the result was not so satisfactory with variable Rf values.

Riedl and Neugebauer[2] employed the filter paper impregnated with ethylene glycol or propylene glycol, and benzine as a mobile phase. Although this system gives fairly good result for the separation of furocoumarins, it is not so easy to get a uniformly saturated

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* Hongo, Tokyo (藤田路一, 古谷 力).

[1] A. B. Swendsen: Pharm. Acta Helv., **27**, 44(1952).

[2] K. Riedl, L. Neugebauer: Monatsh., **83**, 1083(1952).