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Constituents of *Rhodea japonica* Roth

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The upper ground portions of *Rhodea japonica* Roth were proved to contain a glycoside mixture. A new glycoside, Rhodexin D (oleandrigenin–glucose–glucose), was isolated and its structure was determined.

With respect to the components of the leaves of *Rhodea japonica* Roth (Liliaceae) (Japanese name "Omoto"), isolation of Rhodexin A, B, and C has been reported by Nawa and Uchibayashi.[2] With the intention of isolating C_{21}-type steroids from this plant in connection with the hypothesis on the biogenesis of cardiac glycosides,[3] we reinvestigated the chemical components of the leaves of *Rhodea* plants. With the chloroform extract of the leaves, after usual C_{21}-glycoside isolation procedure,[4] mild acid hydrolysis of the glycoside mixture was attempted, but we could not isolate the aglycone of C_{21}-type.

The crude glycoside mixture was submitted to column chromatography over silica gel. Elution with acetone–benzene mixture gave four kinds of crystalline substances as shown in Table I.

Table I

	Fract. No.	mg	Note	K.K. react[a]	L.B. react[b]	Kedde react[c]
Crystal I	282—324	236	white powder	−	+	+
Crystal II	199—221	77	yellow green	−	++	−
Crystal III	87— 94	3.3	yellow green	+	pink	−
Crystal IV	99—101	4.1	brown	+	−	−

a) Keller-Kiliani reaction *b*) Liebermann-Burchard reaction
c) One g of 3,5-dinitrobenzoic acid in methanol (50 ml) was mixed with 2N KOH (50 ml).

Crystal-I forms white crystalline powder (from EtOH–AcOEt), mp 181—184°, $[\alpha]_D$ −42.4°, for which the molecular formula $C_{37}H_{56}O_{16}$ was proposed from its elemental analysis. It gave positive reaction with the Kedde reagent, its ultraviolet (UV) and infrared (IR) spectra showed the presence of a cardenolide, but a negative test with the Keller–Kiliani reagent.[5,6] The nuclear magnetic resonance (NMR) peaks δ 3.1—5.5 suggested the presence of sugar groups. Acetylation of I with acetic anhydride–pyridine afforded an acetate, mp 157—160.5°, $C_{51}H_{70}O_{23}$, which showed the peaks of acetyl group at δ 1.96—2.11.

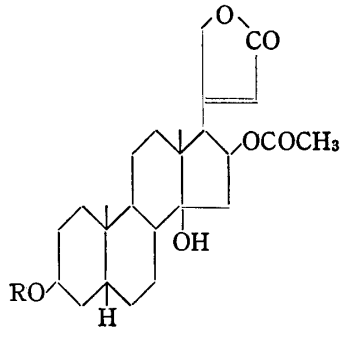
Crystal-I was hydrolyzed with emulsin[7] and gave genin-I and D-glucose. Genin-I, mp 234—238°, was identical with oleandrigenin (gitoxigenin 16-O-acetate). From the re-

1) Location: *Kita-12-jo, Nishi-6-chome, Sapporo, Hokkaido.*
2) H. Nawa, *Yakugaku Zasshi*, **72**, 404 (1952); H. Nawa and M. Uchibayashi, *Chem. Ind.* (London), **1958**, 653.
3) J.v. Euw and T. Reichstein, *Helv. Chim. Acta*, **47**, 711 (1964).
4) H. Mitsuhashi and Y. Shimizu, *Steroids*, **2**, 1373 (1963); *idem*, *Tetrahedron*, **24**, 4143 (1968).
5) W. Schmid, H.P. Uehlinger, Ch. Tamm and T. Reichstein, *Helv. Chim. Acta*, **42**, 72 (1959).
6) S. Bauer, L. Masler, O. Bauerova and D. Sikl, *Experientia*, **17**, 15 (1961).
7) S. Akabori, "Kôso Kenkyuhô 2," 7th Ed. Asakura Shoten, Tokyo, 1966.

sults of paper chromatography, the sugar portion was identified as D-glucose [solvent system (a) BuOH:AcOH:H_2O=4:2:5, (b) phenol saturated with water]. Crystal-I was also submitted to the Mannich hydrolysis.[8] A thin-layer chromatography (TLC) of the reaction mixture gave oleandrigenin, gitoxigenin, and two other spots.

These facts suggest that crystal-I seems to be oleandrigenin–glucose–glucose, and it is named as Rhodexin D (Fig. 1).

Crystal-II forms white crystalline powder from chloroform–MeOH, mp 280—285°. From its IR spectrum (1200—1100 and 3400 cm^{-1}) and NMR spectrum the presence of a sugar group was anticipated. By thin-layer chromatographic comparison (20% MeOH in chloroform) of crystal-II with authentic β-sitosterol β-D-glucoside, the identity of these compounds was established. In the mass spectral data of crystal-II, the prominent peaks can be accounted for by the scheme (Fig. 2).[9] A minute amount of crystal-III (mp 58—68°) and crystal-IV (mp 149.5—159°) was obtained but they were not examined further due to lack of material.



R: H Oleandrigenin
R: glucose–glucose, Rhodexin D

Fig. 1

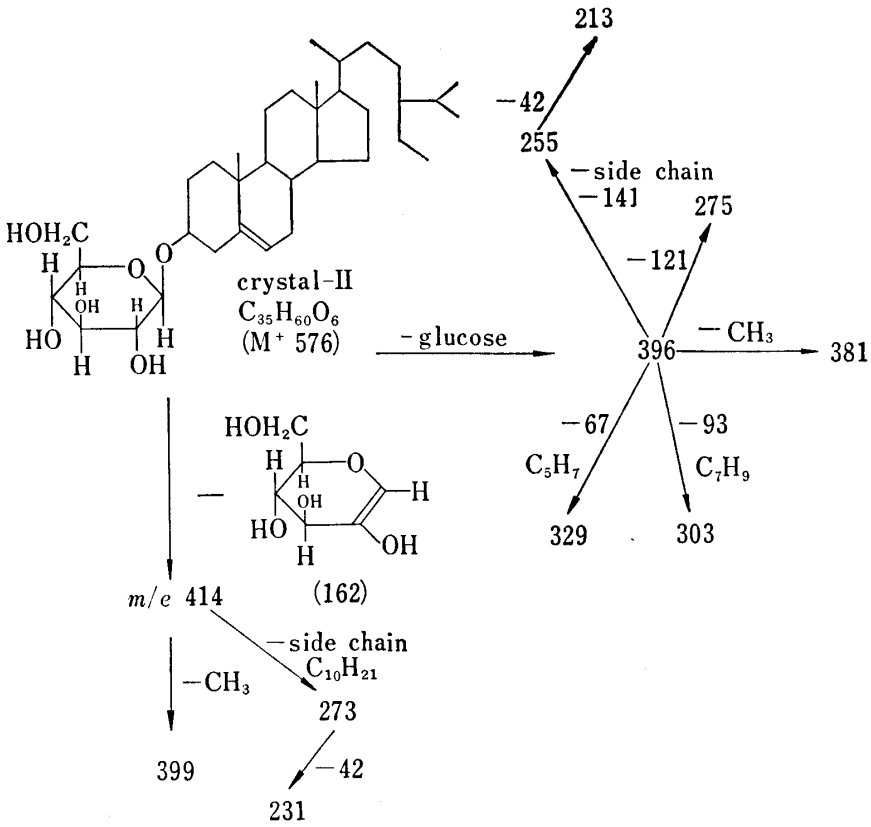


Fig. 2

Experimental

Extraction——The leaves of *Rhodea japonica* Roth, collected in the Awaji Island on December 1968, were dried and powdered, and 6.5 kg of the powdered material was extracted with $CHCl_3$. The deep greenish extract was concentrated at a temperature below 60° *in vacuo*. Evaporation of $CHCl_3$ gave 127.5 g of deep green residue (Keller–Kiliani (+), Liebermann–Burchard (+), Kedde (+)). The residue was digested with hexane and the insoluble part (84.7 g) was then extracted with methanol.[4] The methanolic solution was evaporated below 60° *in vacuo*, giving 28 g of crude glycoside, which showed positive Keller–Kiliani reaction and Liebermann–Burchard reaction. The crude glycoside mixture was examined by thin-layer chromatography (5% MeOH/$CHCl_3$, $SbCl_3$), and separated into 5—6 spots.

8) C. Mannich and G. Siewert, *Chem. Ber.*, **75**, 737 (1947).
9) B.A. Knight, *J. Gas. Chromatogr.*, **5**, 273 (1967).

Hydrolysis of the Crude Glycoside——The crude glycoside (1 g) was dissolved in a mixture of 30 ml each of MeOH and 0.1N H_2SO_4, and the mixture was refluxed for 1.5 hr, but hydrolysis did not progress. Several attempts were made but in vain.

Chromatography of the Crude Glycoside——The crude glycoside was submitted to chromatography over silica gel, giving a result shown in Table II.

Table II

Fract. No.	Solvent system	Eluted product (mg)	Note
1— 8	benzene	559.6	
9— 14	1% acetone–benzene	30.6	
15— 22	2% acetone–benzene	97.3	
23— 68	3% acetone–benzene	1748.2	
69— 83	4% acetone–benzene	383.9	
84— 94	5% acetone–benzene	321.0	Crystal III
95—113	8% acetone–benzene	631.6	Crystal IV
114—146	10% acetone–benzene	839.2	
147—172	20% acetone–benzene	1225.2	
173—188	30% acetone–benzene	574.1	
189—225	40% acetone–benzene	1414.0	Crystal II
226—253	50% acetone–benzene	926.3	
254—279	60% acetone–benzene	782.4	
280—281	70% acetone–benzene	43.5	
282—329	acetone	3477.7	Crystal I
330—350	10% methanol–acetone	2320.6	
351—370	20% methanol–acetone	2343.6	
371—378	50% methanol–acetone	1086.7	
379—399	methanol	2355.6	

each fraction, 200 ml recovery, 21.26 g

Crystal-I——Combined fraction 282—324 was recrystallized four times from EtOH–AcOEt to give a white crystalline powder (236 mg), 181—184°, Keller–Kiliani reaction (−), Liebermann–Burchard reaction (+), Kedde (+). IR ν_{max}^{Nujol} cm^{-1}: 3400—3300, 1760, 1740, 1640, 1200—1100. NMR (in DMSO-d_6) δ: 0.83, 0.86 (angular Me), 1.88 (–OAc), 3.1—5.5, 5.94 (1H, m, vinylic). UV λ_{max}^{EtOH}: 217 mμ, $[\alpha]_D$ −42.4° (c=0.66, pyridine). *Anal.* Calcd. for $C_{37}H_{56}O_{16}$: C, 58.72; H, 7.46. Found: C, 58.44; H, 8.05.

Acetylation of Crystal-I——Crystal-I (50 mg) was dissolved in 1.5 ml of pyridine and 1 ml of Ac_2O was added. The mixture was allowed to stand for 24 hr at room temperature, poured into ice–water (100 ml), and the white powder which appeared was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with 2N HCl (50 ml), 5% $NaHCO_3$, and H_2O saturated with NaCl, and dried over Na_2SO_4. Evaporation of $CHCl_3$ gave a crystalline mass. Recrystallization of this product from PrOH afforded 49.6 mg of crystal-I acetate, mp 157—160.5°, $[\alpha]_D$ −55.1° (c=0.452, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3500, 1760, 1740, 1620, 1240, 1060. NMR δ (in $CDCl_3$): 0.95 (6H, s), 1.96—2.11 (–O–Ac), 5.97 (1H, m, vinylic). *Anal.* Calcd. for $C_{51}H_{70}O_{23}$: C, 58.27; H, 6.71. Found: C, 58.81; H, 7.37.

Hydrolysis of Crystal-I——i) Hydrolysis with Emulsin: Crystal-I (40 mg) was dissolved in 20 ml of acetate buffer (148 ml of 0.2N AcOH and 35.2 ml of 0.2N AcONa) and 20 mg of emulsin and 1 drop of toluene were added. The mixture was kept at 32°. The reaction mixture was checked by TLC and after 15 days, 20 mg of emulsin was added. After 23 days, white powder precipitated from the mixture. The precipitate was recrystallized from $CHCl_3$ and gave genin-I, mp 234—238°. $[\alpha]_D$ −62.3° (c=0.513, pyridine). UV λ_{max}^{EtOH} 215 mμ. IR ν_{max}^{Nujol} cm^{-1}: 3400, 1780 (sh), 1750 (sh), 1735, 1620, 1255, 1140. NMR (in $CDCl_3$) δ: 0.94 (6H, s, C-18, C-19 Me), 1.98 (3H, s, –OAc), 3.9 (2H, broad), 4.92 (2H, broad m, C-21–CH_2–O–), 6.00 (1H, broad m, C-22 vinylic H)

ii) Mannich Hydrolysis[8]: A mixture of crystal-I (30 mg), $(CH_3)_2CO$ (12.5 ml), and conc. HCl (0.075 ml) was maintained for 5 days at room temperature and the reaction mixture was examined by TLC (SiO_2 HF_{254}, 20% MeOH/$CHCl_3$, $SbCl_3$) and formation of oleandrigenin, gitoxigenin and two unidentified products were observed.

iii) Sugar Fraction: The aqueous layer from (i) was treated with a column containing 43 ml of Amberlite IR-120 (activated with 280 ml of 2N HCl and washed with H_2O), and eluted with H_2O. The eluate was concentrated to a syrup under a reduced pressure.

The syrupy sugar fraction thus obtained was submitted to paper chromatography for comparison with authentic samples and detected with $AgNO_3$–NH_3.[10,11]

	BuOH:AcOH:H_2O (4:2:5) (room temp., 24 hr)	Phenol satd. with H_2O (room temp., 24 hr)
D-Glucose	0.48	0.39
Syrup	0.48	0.39

Crystal-II (β-Sitosterol β-D-glucoside)——After repeated recrystallization from $CHCl_3$–MeOH, 77 mg of white powder was obtained, mp 280—285°, Keller–Kiliani reaction (−), Liebermann–Burchard reaction ++), Kedde (−). IR ν_{max}^{Nujol} cm^{-1}: 3400, 1200, 1000. $[\alpha]_D^{21}$ −43.9° (c=0.635, pyridine). NMR (in DMSO-d_6): δ 4.3—5.3. Mass spectrum m/e: 414 (M^+−162), 399 (M^+−162−15), 396 (M^+−180), 381 (M^+−180−15), 329 (M^+−180−67), 303 (M^+−180−93), 275 (M^+−180−121), 273 (M^+−162−141), 255 (M^+−180−141), 213 (M^+−180−141−42).

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10) S.M. Partridge, *Biochem. J.*, **42**, 238 (1948).
11) V.V. Rachinskii and E.I. Knyazyatova, *Doklady Akad Nauk, S.S.S.R.*, **85**, 1119 (1952) [*Chem. Abstr.*, **47**, 448 (1953)].