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Studies on Rhubarb (Rhei Rhizoma). XIV.[1] Isolation and Characterization of Stilbene Glucosides from Chinese Rhubarb

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Two major stilbene glucosides (**1** and **2**) have been newly isolated from Chinese rhubarb (commercial name: Gaoh, second grade), and characterized on the basis of chemical and spectroscopic evidence as piceatannol 4′-*O*-β-D-glucopyranoside (**1**) and its 6″-*O*-gallate (**2**). In addition, high-performance liquid chromatographic analysis has led to the characterization of minor components including sennoside, anthraquinones, and other stilbenes.

Keywords——rhubarb; Polygonaceae; stilbene glucoside; stilbene glucoside gallate; piceatannol; chemical evaluation; HPLC

In the course of chemical evaluation of various commercial rhubarbs by high-performance liquid chromatography (HPLC), we have encountered a different type of rhubarb which contains unidentified compounds as major components. As a result of a large-scale extraction, we have isolated the two major compounds and found them to be new stilbene glucosides (**1** and **2**). This paper describes the isolation and characterization of these compounds.

The rhubarb studied here is the one produced in Szechwan, China, and designated commercially as second-grade Gaoh. Examination of the ethyl acetate-soluble portion of the

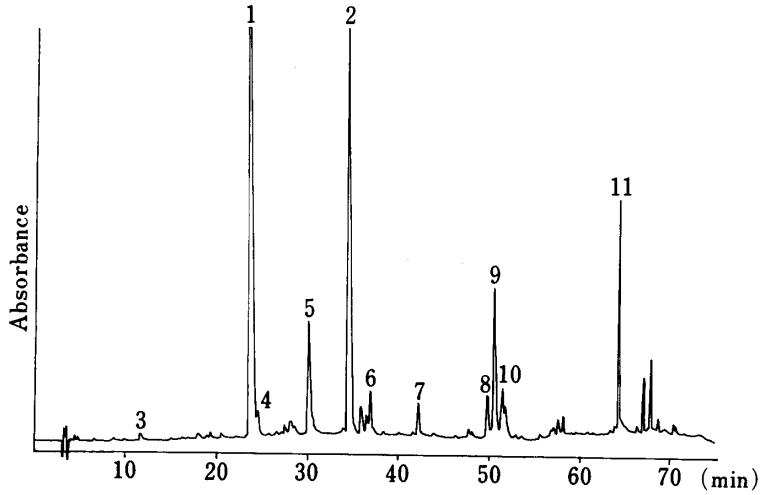


Fig. 1. High-Performance Liquid Chromatogram of Ethyl Acetate-Soluble Portion

Column: Nucleosil $5C_{18}$. Solvent: CH_3CN–0.05 M phosphoric acid buffer solution. Flow rate: 0.8 ml/min. Detection: 280 nm.

aqueous acetone extract by HPLC showed the presence of two major unknown peaks (peaks **1** and **2** in Fig. 1). Subsequent separation by Fuji-gel ODS G3 chromatography, followed by preparative-scale HPLC, yielded compounds **1** and **2**.

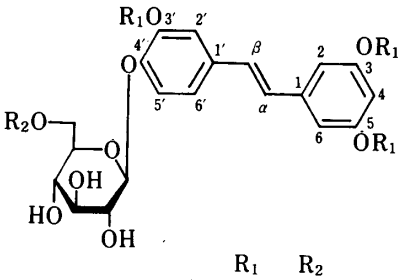
Compound **1** showed ultraviolet (UV) maxima at 305 and 319 nm, suggesting the presence of a highly conjugated system, while the infrared (IR) spectrum exhibited a strong hydroxyl absorption band at 3400 cm^{-1}. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed, together with a pair of *trans*-coupled olefinic signals (δ 6.94 and 7.02, each 1H, d, J=16 Hz), A_2X-type [δ 6.29 (1H, t, J=2 Hz) and 6.56 (2H, d, J=2 Hz)] and ABX-type [δ 6.97 (1H, dd, J=2, 8 Hz), 7.11 (1H, d, J=2 Hz) and 7.16 (1H, d, J=8 Hz)] aromatic signals suggestive of the presence of 1,3,5- and 1,3,4-trisubstituted aromatic rings. In addition, the existence of a sugar moiety was indicated by an anomeric proton resonance [δ 4.79 (1H, d, J=7 Hz)] and by carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy [δ 104.0, 77.8, 77.3, 74.5, 71.0 and 62.4]. From these spectral data, **1** was presumed to be a tetrahydroxystilbene glycoside. Acid hydrolysis of **1** yielded glucose and an aglycone, which was found to be identical with piceatannol, 3,3′,4′,5-tetrahydroxystilbene, by comparison with an authentic sample.[3] Since in the ^{1}H-NMR spectrum, the signal pattern of the resorcinol ring is symmetrical, the glucosyl moiety was presumed to be bound to either the C-3′ or C-4′ hydroxyl group in the piceatannol moiety.

Methylation of **1** with dimethyl sulfate and potassium carbonate in dry acetone, followed by hydrolysis with sulfuric acid, gave the aglycone methyl ether (**1b**) and glucose. The ^{1}H-NMR spectrum of **1b** showed three methoxyl signals, among which irradiation at the frequency of δ 3.88 caused a 19% increase in the integral intensity of the H-2′ signal. This

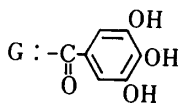
TABLE I. ^{13}C-NMR Chemical Shifts for Compounds **1**, **1a** and **2**[a]

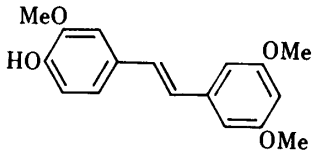
		1[b]	**1a**[c]	**2**[b]
Aglycone moiety	C-1	140.4	139.4	140.4
	C-2	105.9 (2C)	104.2 (2C)	105.9 (2C)
	C-3	159.5 (2C)	160.6 (2C)	159.5 (2C)
	C-4	103.0	99.6	103.0
	C-1′	134.4	131.1	134.1
	C-2′	114.3	110.0	114.4
	C-3′	145.9	146.3	145.8
	C-4′	148.6	149.0	148.2
	C-5′	119.4	115.3	119.3
	C-6′	119.1	119.8	118.6
	$C_{\alpha,\beta}$	128.7 (2C)	126.8 128.8	128.6 (2C)
Glucosyl moiety	C-1	104.0	99.9	103.8
	C-2	74.5	73.1	74.5
	C-3	77.8	76.8	77.3
	C-4	71.0	69.6	71.4
	C-5	77.3	76.5	75.3
	C-6	62.4	60.5	64.5
Galloyl moiety	C-1			121.6
	C-2			110.1 (2C)
	C-3			146.1 (2C)
	C-4			139.0
	–COO–			166.8
	Me		55.2 (2C) 55.7	

a) Spectra were measured at 25.05 MHz. *b*) Measured in acetone-d_6 + D_2O. *c*) Measured in DMSO-d_6 + D_2O.



	R_1	R_2
1	H	H
1a	Me	H
2	H	G





1b

TABLE II. Constituents of Ethyl Acetate-Soluble Portion

No.	Compound
1	Piceatannol 4′-*O*-*β*-D-glucopyranoside
2	Piceatannol 4′-*O*-(6″-*O*-galloyl)-*β*-D-glucopyranoside
3	(+)-Catechin[5]
4	Resveratrol 4′-*O*-*β*-D-glucopyranoside[6]
5	Aloe-emodin 8-*O*-*β*-D-glucopyranoside[7]
6	Resveratrol 4′-*O*-(6″-*O*-galloyl)-*β*-D-glucopyranoside[6]
7	Sennoside A
8	Chrysophanol 1-*O*-*β*-D-glucopyranoside[7]
9	Emodin 8-*O*-*β*-D-glucopyranoside[7]
10	Chrysophanol 8-*O*-*β*-D-glucopyranoside[7]
11	Piceatannol[3b]

result indicated that one of the methoxyl groups is present at the C-3′ position, and that glucose is therefore located at the C-4′ hydroxyl group. The mode of the glucosyl linkage was determined to be *β* from the coupling constant value (d, $J=7$ Hz) of the anomeric proton signal. On the basis of these chemical and spectral results, **1** was established as piceatannol 4′-*O*-*β*-D-glucopyranoside.

Compound **2** showed UV absorption bands similar to those of **1**, and gave a blue coloration with the ferric chloride reagent. The ^{1}H-NMR spectrum of **2** was similar to that of **1**, except for the appearance of a two-proton aromatic singlet at δ 7.23 due to a galloyl group. Tannase hydrolysis of **2** afforded gallic acid and a hydrolysate, which was found to be identical with **1** by comparison of the physical and spectral data. The location of the galloyl group was determined to be at the C-6 position in the glucosyl moiety from the downfield shift of the glucose C-6 methylene signals in the ^{1}H- and ^{13}C-NMR spectra [δ 4.40 (1H, dd, $J=$ 7, 12 Hz) and 4.69 (1H, dd, $J=2$, 12 Hz); δ 64.5]. From these observations, **2** was characterized as piceatannol 4′-*O*-(6″-*O*-galloly)-*β*-D-glucopyranoside.

Other minor compounds existing in the ethyl acetate-soluble portion were characterized by HPLC (Fig. 1). Although the chromatogram showed many peaks, nine compounds with relatively prominent peaks could be identified, as listed in Table II, by comparison of their t_R-values with those of authentic samples or by co-chromatography. It is clear that this rhubarb is quite unique in that it contains the stilbene glucosides **1** and **2** as major constituents, together with sennoside and anthraquinone glucosides.

Experimental

The instruments and chromatographic conditions used in this study were essentially the same as described in the

previous paper,[4] except for the following. Preparative-scale HPLC was carried out on a Hitachi model 655 liquid chromatograph equipped with a Hitachi variable-wavelength spectrometer and a Nucleosil $5C_{18}$ (M. Nagel) column (8 mm i.d. × 250 mm). Analytical HPLC was performed on a Toyo Soda apparatus equipped with a CCPM solvent delivery system, a UV-8000 spectrometer and a Nucleosil $5C_{18}$ (M. Nagel) column (4.0 mm i.d. × 250 mm). Elution was executed at a flow rate of 0.8 ml/min with increasing amounts of CH_3CN in 0.05 M phosphoric acid buffer solution.

Isolation——The powdered rhubarb [30 g, Gaoh (grade II)], kindly donated by Tochimoto Tenkaido Co., Ltd., was extracted three times with 300 ml each of 50% aqueous acetone. The combined extracts were concentrated under reduced pressure to give an aqueous solution, which was extracted three times with 200 ml each of ethyl acetate. The ethyl acetate-soluble portion, after removal of the solvent by evaporation, was subjected to chromatography over Fuji-gel ODS G3. Elution first with 300 ml of H_2O, followed by CH_3CN–H_2O–formic acid (10 : 89 : 1), afforded a fraction consisting mainly of stilbenes, which was subsequently applied to a column of Nucleosil $5C_{18}$ (8 mm i.d. × 250 mm). Elution with CH_3CN–H_2O–formic acid (20 : : 80 : 1) afforded compounds **1** (*ca.* 1 g) and **2** (250 mg).

Compound 1——A white amorphous powder, $[\alpha]_D^{22}$ −35.8° (c=0.84, acetone). *Anal.* Calcd for $C_{20}H_{22}O_9 \cdot 3/2\ H_2O$: C, 55.42; H, 5.81. Found: C, 55.12; H, 5.65. FD-MS m/z: 406 $(M)^+$, 244 $(M+H-glucosyl)^+$, 163. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1580, 1500. UV λ_{max}^{MeOH} nm (log ε): 305 (4.39), 319 (4.42). ^{1}H-NMR (acetone-d_6): 3.4—4.0 (6H, m, sugar-H), 4.79 (1H, d, J=7 Hz, anomeric-H), 6.29 (1H, t, J=2 Hz, 4-H), 6.56 (2H, d, J=2 Hz, 2, 6-H), 6.94, 7.02 (each 1H, d, J=16 Hz, olefinic-H), 6.97 (1H, dd, J=2, 8 Hz, 6′-H), 7.11 (1H, d, J=2 Hz, 2′-H), 7.16 (1H, d, J=8 Hz, 5′-H), ^{13}C-NMR: Table I.

Acid Hydrolysis of 1——A solution of **1** (5 mg) in 5% aqueous sulfuric acid (2 ml) was heated in a water bath for 4 h. The reaction mixture was neutralized with barium carbonate, filtered and concentrated to dryness under reduced pressure. The residue was subjected to TLC examination to detect piceatannol [silica gel; benzene–ethyl formate–formic acid (6 : 4 : 3); *Rf* 0.51] and glucose [cellulose; *n*-BuOH–pyridine–H_2O (6 : 4 : 3); *Rf* 0.42].

Methylation of 1——A mixture of **1** (75 mg), dimethyl sulfate (0.5 ml) and potassium carbonate (750 mg) in dry acetone (15 ml) was refluxed for 1.5 h with stirring. After removal of inorganic salts by filtration, the filtrate was concentrated, and the residue was chromatographed over silica gel. Elution with $CHCl_3$–MeOH–H_2O (10 : 1 : 0.1) furnished the trimethyl ether (**1a**) (38 mg) as colorless needles ($CHCl_3$–MeOH), mp 108—110 °C, $[\alpha]_D^{22}$ −59.9° [c=0.81, acetone–H_2O (1 : 1)]. *Anal.* Calcd for $C_{23}H_{28}O_9 \cdot 3/2H_2O$: C, 58.10; H, 6.57. Found: C, 58.28; H, 6.36. FD-MS m/z: 448 (M^+), 286. ^{1}H-NMR (DMSO-d_6+D_2O): 3.2—3.9 (6H, m, sugar-H), 3.76, 3.82 (9H in total, each s, –OMe), 4.95 (1H, d, J=7 Hz, anomeric-H), 6.39 (1H, t, J=2 Hz, 4-H), 6.76 (2H, d, J=2 Hz, 2,6-H), 7.04, 7.20 (each 1H, d, J=16 Hz, olefinic-H), 7.10 (2H, br s, 5′,6′-H), 7.25 (1H, d, J=2 Hz, 2′-H). ^{13}C-NMR: Table I.

Acid Hydrolysis of 1a——A solution of **1a** (30 mg) in 5% sulfuric acid [H_2O–acetone (1 : 1)] (15 ml) was refluxed for 4 h. The reaction mixture was concentrated to give an aqueous solution, which was extracted with ethyl acetate. The ethyl acetate-soluble portion, after removal of the solvent by evaporation, was chromatographed over silica gel. Elution with *n*-hexane gave an aglycone (**1b**) as a colorless syrup. EI-MS m/z: 286 $(M)^+$. ^{1}H-NMR (acetone-d_6): 3.80, 3.88 (9H in total, each s, –OMe), 6.38 (1H, t, J=2 Hz, 4-H), 6.72 (2H, d, J=2 Hz, 2,6-H), 6.82 (1H, d, J=8 Hz, 5′-H), 6.97, 7.19 (each 1H, d, J=16 Hz, olefinic-H), 7.03 (1H, dd, J=2, 8 Hz, 6′-H), 7.22 (1H, d, J=2 Hz, 2′-H).

Compound 2——An off-white amorphous powder, $[\alpha]_D^{22}$ −73.2° (c=0.70, acetone). *Anal.* Calcd for $C_{27}H_{28}O_{14} \cdot 3/2H_2O$: C, 53.73; H, 5.18. Found: C, 53.74; H, 4.96. FD-MS m/z: 581 $(M+Na)^+$, 558 $(M)^+$, 406 $(M+H-galloyl)^+$, 243, 170. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1690 (–COO–), 1590, 1500. UV λ_{max}^{MeOH} nm (log ε): 301 (4.33), 322 (4.31). ^{1}H-NMR (acetone-d_6): 3.5—3.9 (4H, m, sugar-H), 4.40 (1H, dd, J=7, 12 Hz, 6″-H), 4.69 (1H, dd, J=2, 12 Hz, 6″-H), 4.86 (1H, d, J=7 Hz, anomeric-H), 6.29 (1H, t, J=2 Hz, 4-H), 6.57 (2H, d, J=2 Hz, 2, 6-H), 6.91, 6.98 (each 1H, d, J=16 Hz, olefinic-H), 6.96 (1H, dd, J=2, 8 Hz, 6′-H), 7.07 (1H, d, J=2 Hz, 2′-H), 7.21 (1H, d, J=8 Hz, 5′-H), 7.23 (2H, s, galloyl-H). ^{13}C-NMR: Table I.

Tannase Hydrolysis of 2——A solution of **2** (25 mg) in H_2O (10 ml) was treated with tannase at room temperature for 1 h. The reaction mixture was concentrated to dryness under reduced pressure, and the residue was treated with MeOH. The MeOH-soluble portion was subjected to chromatography over MCI-gel CHP 20P. Elution with H_2O afforded gallic acid. Subsequent elution with H_2O–MeOH (1 : 1) furnished the hydrolysate (15 mg), which was identified as piceatannol 4′-*O*-β-D-glucopyranoside (**1**) by comparison of the physical and spectral data with those of an authentic sample.

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