A New Glycoside, Kusaginin Isolated from *Clerodendron trichotomum*

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Synopsis. A new glycoside, kusaginin was isolated from *Clerodendron trichotomum* Thunb. and 2-(3,4-dihydroxyphenyl)ethyl 3-*O*-α-L-rhamnopyranosyl-4-*O*-(3,4-dihydroxycinnamoyl)-β-D-glucopyranoside was assigned to this substance from studies on the hydrolysis products and from analyses of the ^{1}H NMR and ^{13}C NMR spectra.

From the leaves of *Clerodendron trichotomum* Thunb. (Kusagi in Japanese) a flavonoid glycoside, acacetin 7-*O*-2-β-D-glucopyranuronosyl-β-D-glucopyranuronoside[1] and a bitter principle, clerodendrin A,[2] and from the fruits of the plant two blue pigments, trichotomin and trichotomin G_1,[3] have been isolated.

During our studies on the constituents of this plant we isolated a new glycoside and named it kusaginin (**1**). In this paper we wish to report the isolation and structure of **1**.

The methanol extract of the fresh leaves of the plant was triturated with water and ethyl acetate and the aqueous layer was subjected to chromatography on a column of Sephadex LH-20 eluting with methanol. The last effluent gave **1** as colorless crystals, mp 154—156 °C in 0.3% yield from wet plant. Compound **1** is optically active, $[\alpha]_D$ −104° and from its elemental analyses was suggested the formula $C_{29}H_{36}O_{15}$. The IR spectrum of **1** shows absorption bands indicative of the existence of a conjugated ester group at 1716 and 1278 cm^{-1} and the UV spectrum of **1** in ethanol shows absorption maxima characteristic of an ester of 3-(3,4-dihydroxyphenyl)-2-propenoic acid[4] at 202, 219, 245, 290, and 332 nm. From the IR spectrum coupled with ^{1}H NMR spectrum of **1** which has complex signals corresponding to the protons on oxygenated carbon atoms at δ 3.0—4.0, it is considered that **1** has sugar portions.

The ^{1}H NMR spectrum of **1** in DMSO-d_6 shows the signals attributed to the protons attached to two 1,3,4-trisubstituted benzene rings at δ 6.58 (1H, dd, J=1.5 and 8 Hz), 6.69 (1H, d, J=8 Hz), 6.71 (1H, d, J=1.5 Hz), 6.80 (1H, d, J=7.5 Hz), 6.95 (1H, dd, J=2 and 7.5 Hz), and 7.07 (1H, d, J=2 Hz), two *trans* olefinic protons at δ 6.28 (1H, d, J=16 Hz) and 7.58 (1H, d, J=16 Hz), two anomeric protons of sugar portions at δ 4.35 (1H, d, J=8 Hz) and 5.00 (1H, d, J=2 Hz), a proton attached to a carbon atom bearing an ester group at δ 4.67 (1H, t, J=9 Hz), methylene protons of a benzyl group at δ 2.67 (2H, t, J=7.5 Hz), and methyl protons at δ 0.98 (3H, d, J=6 Hz), besides them.

Acetylation of **1** with acetic anhydride and pyridine gave a nonaacetate of **1** as colorless crystals, mp 92—96 °C and its ^{1}H NMR spectrum shows the signals ascribed to the methyl protons of acetoxyl groups at δ 1.89 (3H, s), 1.97 (3H, s), 2.02 (3H, s), 2.08 (3H, s), 2.10 (3H, s), 2.26 (3H, s, aromatic acetoxyl), 2.28 (3H, s, aromatic acetoxyl), and 2.29 (6H, s, aromatic acetoxyl).

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Treatment of **1** with 10% hydrogen chloride in methanol gave 2-(3,4-dihydroxyphenyl)ethanol (**2**),[5] methyl caffeate (**3**),[4] a substance **4**, methyl α-L-rhamnoside (**5**), methyl α-D-glucoside (**6**), and small amounts of methyl β-L-rhamnoside (**7**) and methyl β-D-glucoside (**8**). Since **4** gives **2**, **6**, and **8** by further treatment with hydrogen chloride in methanol and the ^{1}H NMR spectrum of **4** in CD_3OD shows a sharp doublet of 1′-proton of the D-glucose at δ 4.27 (J=7.5 Hz, axial/axial coupling indicative of β-D-glucoside), D-glucose and **2** should be linked by a β-glycoside bond. In the ^{13}C NMR spectra of **2**, **4**, methyl α-D-glucopyranoside (**6**), and methyl β-D-glucopyranoside (**8**) as shown in Table 1, the 8-carbon in **4** appears at a field lower by 7.5 ppm than that of **2** and the 7-carbon in **4** also appears at a field higher by 3.1 ppm than that of **2**. These results[6] indicate that the D-glucose in **4** might be linked by a β-glycoside bond to the C-8 of **2**.

Hydrolysis of **1** with sodium hydroxide in aqueous methanol gave caffeic acid (**9**)[5b,7] and a substance **10**. Since **10** gives **2**, **4**, **5**, and **6** by further treatment with hydrogen chloride in methanol and the ^{1}H NMR spectrum of **10** in CD_3OD shows a sharp doublet of 1″-proton of the L-rhamnose at δ 5.13 (J=1.8 Hz, equatorial/equatorial coupling indicative of α-L-rhamnoside), L-rhamnose and **4** should be linked by an α-glycoside bond. In the ^{13}C NMR spectra of **4**, methyl α-L-rhamnopyranoside (**5**), methyl β-L-rhamnopyranoside (**7**), and **10** as shown in Table 1, the 3′-carbon of the glucose in **10** appears at a field lower by 6.3 ppm than that of **4** and the 4′-carbon of the glucose in **10** also appears at a field higher by 1.5 ppm than that of **4**. These results indicate that the L-rhamnose in **10** might be linked by an α-glycoside bond to the C-3′ of **4**.

TABLE 1. CARBON-13 NMR CHEMICAL SHIFTS OF **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, AND **10** IN CD_3OD

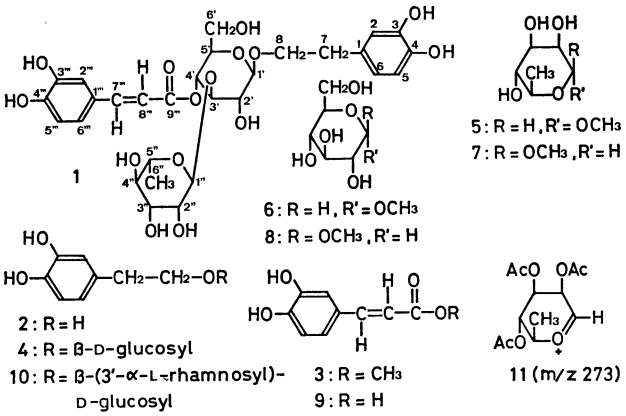
Compound		1	10	4	3	2	6	8	5	7
2-(3,4-Dihydroxy-phenyl)ethanol	1	131.4	131.4	131.4		131.7	—	—	—	—
	2	116.3*	116.3*	116.3*	—	116.2*	—	—	—	—
	3	145.8**	145.9**	145.9**	—	145.9**	—	—	—	—
	4	144.4**	144.4**	144.5**	—	144.4**	—	—	—	—
	5	117.0*	117.0*	117.0*	—	117.0*	—	—	—	—
	6	121.3	121.2	121.3	—	121.2	—	—	—	—
	7	36.4	36.3	36.4	—	39.5	—	—	—	—
	8	72.1	72.0	72.0	—	64.5	—	—	—	—
D-Glucose	1	103.9	103.9	104.2	—	—	101.1	105.1	—	—
	2	75.9	75.4	75.0	—	—	73.5	74.8	—	—
	3	81.6	84.2	77.9	—	—	75.1	77.7	—	—
	4	70.4	70.0	71.5	—	—	71.7	71.3	—	—
	5	75.9	77.5	77.9	—	—	73.5	77.7	—	—
	6	62.2	62.3	62.6	—	—	62.6	62.5	—	—
L-Rhamnose	1	102.8	102.5	—	—	—	—	—	102.6	102.7
	2	72.1	72.0	—	—	—	—	—	72.3	72.3
	3	72.1	72.0	—	—	—	—	—	72.0	74.9
	4	73.7	73.8	—	—	—	—	—	73.8	73.9*
	5	70.4	70.0	—	—	—	—	—	69.5	73.5*
	6	18.4	17.9	—	—	—	—	—	18.0	17.9
Caffeic acid	1	127.5	—	—	127.5	—	—	—	—	—
	2	114.5***	—	—	114.7*	—	—	—	—	—
	3	149.5	—	—	149.2	—	—	—	—	—
	4	146.5	—	—	146.5	—	—	—	—	—
	5	115.2***	—	—	115.1*	—	—	—	—	—
	6	123.2	—	—	122.9	—	—	—	—	—
	7	148.0	—	—	148.0	—	—	—	—	—
	8	116.3***	—	—	116.3*	—	—	—	—	—
	9	168.2	—	—	169.6	—	—	—	—	—
	CH_3	—	—	—	51.9	—	55.5	57.4	55.1	57.0

In parts per million downfield from tetramethylsilane. Asterisks indicate that assignments are unambigous.

Methanolysis and base catalyzed hydrolysis of **1** gave methyl caffeate (**3**) and caffeic acid (**9**) respectively as described above, which indicates that caffeic acid and **10** might be linked by an ester bond. In the in-beam electron impact mass spectrum[8,9] of an acetate of **1** could not be observed the molecular ion peak, however the spectrum exhibits a fragment ion peak corresponding to the ion structure **11** shown in Figure at *m/z* 273 as base peak, which indicates that caffeic acid should not be linked to the L-rhamnose in **1**. In the ^{1}H NMR spectrum of **1** irradiation of the proton on a carbon atom bearing the ester group at δ 4.67 shows no change in the spectral pattern of the anomeric proton of glucose at δ 4.35 and *vice versa*, which indicates that caffeic acid should not be linked to the 2-position of glucose in **1**.

In the ^{13}C NMR spectra of **1**, **3**, and **10**, the 9'''-carbonyl carbon in **1** appears at a field higher by 1.4 ppm than that of **3** and both 3'- and 5'-carbons in **1** also appear at fields higher by 2.6 and 1.6 ppm respectively than those of **10**. This fact is the consequence of steric compression[10] between the C-9 of caffeic acid and the C-3 or C-5 of D-glucose. These results indicate that caffeic acid might be linked by an ester bond to the C-4' of **10**.

These ^{1}H NMR and ^{13}C NMR spectroscopic data and all experimental results described above support the structure of kusaginin (**1**) to be 2-(3,4-dihydroxyphenyl)ethyl 3-*O*-α-L-rhamnopyranosyl-4-*O*-(3,4-dihydroxycinnamoyl)-β-D-glucopyranoside.



Experimental

All melting points are uncorrected. The ^{1}H NMR spectra were measured with a Varian EM-390 90 MHz NMR spectrometer. The ^{13}C NMR spectra were measured with a JEOL JNM-PFT-60 NMR spectrometer at 15.04 MHz. Chemical shifts were obtained by δ value (ppm) from tetramethylsilane as internal standard.

Isolation of Kusaginin (1). The fresh leaves of the plant (1.5 kg)[11] were extracted with methanol. The extract was condensed to a syrup under reduced pressure and the syrup was triturated with water (300 cm^3) and ethyl acetate (500 cm^3). The aqueous layer was condensed to a syrup under reduced pressure and the syrup was dissolved in methanol (150 cm^3). Each 50 cm^3 of the methanol solution was subjected to chromatography on a column of Sephadex LH-20 (3.5×120 cm) eluting with methanol. The last effluents gave **1** as colorless crystals after recrystallization from a mixture of methanol and chloroform: mp 154—156 °C; yield, 4.5 g (0.3% from wet plant); $[\alpha]_D^{20}$ −104° (*c* 1.0, MeOH); UV (EtOH) 202 (ε 41000), 219 (19300), 237 (sh 12500), 245 (11200), 290 (13200), 300 (sh 13600), and 332 nm (13600); IR (KBr) 3535, 3400—3100, 1716, 1640, 1606, 1598, 1523, 1445, 1390, 1310, and 1278 cm^{-1}. Found: C, 55.38; H, 5.79%. Calcd for $C_{29}H_{36}O_{15}$: C, 55.77; H, 5.81%.

Acetylation of **1** with acetic anhydride and pyridine at room temperature gave a nonaacetate of **1** as colorless crystals: mp 92—96 °C. Found: C, 56.23; H, 5.40%. Calcd for $C_{47}H_{54}O_{24}$: C, 56.29; H, 5.43%.

Methanolysis of 1. Compound **1** (500 mg) was dissolved in dry methanol containing 10 wt% of hydrogen chloride (30 cm^3), which was allowed to stand at room temperature for 20 h under nitrogen atmosphere. The mixture was concentrated to dryness under reduced pressure. The residue was subjected to chromatography on a column of Sephadex LH-20 in the same manner as described above and gave **2** (colorless crystals, mp 80—82 °C; yield, 85 mg), **3** (colorless crystals, mp 151—152 °C; yield, 135 mg), **4** (colorless oil; yield, 120 mg), and a mixture of sugars.

Acetylation of **4** with acetic anhydride and pyridine gave a hexaacetate of **4** as colorless crystals: mp 40.5—42 °C. Found: C, 54.82; H, 5.71%. Calcd for $C_{26}H_{32}O_{14}$: C, 54.93; H, 5.67%.

The sugar fraction was subjected to thin layer chromatography and the sugars were proved to be identical with authentic **5**, **6**, **7**, and **8**.

Hydrolysis of 1 with Sodium Hydroxide. Compound **1** (325 mg) was dissolved in a mixture of methanol (4 cm^3) and 1 M (1 M=1 mol dm^{-3}) aqueous sodium hydroxide (4 cm^3), which was allowed to stand at room temperature for 4 h under nitrogen atmosphere. After being acidified with hydrochloric acid, the mixture was concentrated to dryness under reduced pressure. The residue was subjected to chromatography on a column of Sephadex LH-20 and gave **9** (colorless crystals, mp 207—208 °C; yield, 51 mg) and **10** (colorless oil; yield, 195 mg).

Acetylation of **10** with acetic anhydride and pyridine gave an octaacetate of **10** as colorless crystals: mp 71—73 °C. Found: C, 54.04; H, 5.64%. Calcd for $C_{36}H_{46}O_{20}$: C, 54.13; H, 5.80%.

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11) The plant was collected at Mt. Udo of Shizuoka City in the autumn of 1981.