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Studies on the Constituents of Leaves of *Citrus unshiu* MARCOV.

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Two new glycosides citroside A (**3**) and B (**4**), together with 2-phenylethyl β -D-glucopyranoside (**1**) and 2-phenylethyl D-rutinoside (**2**), were isolated from the methanol extract of leaves of *Citrus unshiu*, in addition to two known terpenoids, limonin (**5**) and friedelin (**6**). On the basis of spectral and chemical evidence the structures of the new glycosides were determined as (5-dehydroxy-grasshopper ketone-5-yl) 5- β -D-glucopyranoside (**3**) and a (5-dehydroxy-allenic ketodiol-5-yl) 5- β -D-glucopyranoside (**4**).

Keywords—*Citrus unshiu*; Rutaceae; citroside A; citroside B; 2-phenylethyl β -D-glucopyranoside; 2-phenylethyl D-rutinoside; limonin; friedelin

Citrus unshiu MARCOV (Rutaceae) is widely distributed in the southern part of Japan. Earlier investigations of the constituents of this plant dealt mainly with the fruit's pericarp (*Aurantii Nobilis Pericarpium*).¹⁾ This paper describes the isolation and structure elucidation of two new ionone-type glycosides named citrosides A (**3**) and B (**4**), as well as the phenylethyl glycosides 2-phenylethyl β -D-glucopyranoside (**1**) and 2-phenylethyl D-rutinoside (**2**), and the triterpenoids limonin (**5**) and friedelin (**6**) from the leaves of this plant.

2-Phenylethyl β -D-glucopyranoside (**1**), C₁₄H₂₀O₆ · 5/4H₂O, [α]_D –28.8° was isolated as a colorless amorphous powder. The infrared (IR) spectrum showed the presence of hydroxyl groups (3500 cm⁻¹). The proton nuclear magnetic resonance (¹H-NMR) spectrum exhibited a triplet signal due to benzylic methylene protons at δ 3.00 (2H, J = 7 Hz), a doublet signal due to an anomeric proton at δ 4.82 (1H, J = 7 Hz) and a singlet signal due to aromatic protons at δ 7.25 (5H, s). The carbon-13 nuclear magnetic resonance (¹³C-NMR) spectrum exhibited two methylene carbon signals at δ 36.6 and 71.6. The acid hydrolysis of **1** afforded glucose as the sugar moiety and phenylethanol as the aglycone. These data led us to conclude the structure of this compound to be **1**. This is the first report of the isolation of **1** from a natural origin.

2-Phenylethyl D-rutinoside (**2**), C₂₀H₃₀O₁₀ · H₂O, [α]_D –101.2°, was obtained as a colorless amorphous powder. The ¹H-NMR spectrum exhibited a triplet methylene proton at δ 3.00 (2H, J = 7 Hz), two doublet signals at δ 1.60 (3H, J = 5 Hz) due to C-6 protons of rhamnose, δ 4.81 (1H, J = 7 Hz) due to an anomeric proton and an aromatic proton signal at δ 7.25 (5H, s). On acid hydrolysis, **2** afforded D-glucose and L-rhamnose as sugar moieties and phenylethanol as the aglycone. In the ¹³C-NMR spectrum of **2**, the signal due to C-6 of glucose was shifted downfield by 5.4 ppm and that of C-5 of glucose was shifted upfield by 1.3 ppm in comparison with those of usual glucopyranosides. Thus, rhamnose was attached to C-6 of glucose. From these data, the structure of compound **2** was decided to be **2**.

Compound **3** (citroside A), [α]_D –95.7°, C₁₉H₃₀O₈ · 1/2H₂O, was obtained as an amorphous powder. The ultraviolet (UV) spectrum showed an absorption maximum at 232 nm (log ϵ 4.12) and the IR spectrum showed the presence of hydroxyl groups (3400 cm⁻¹),

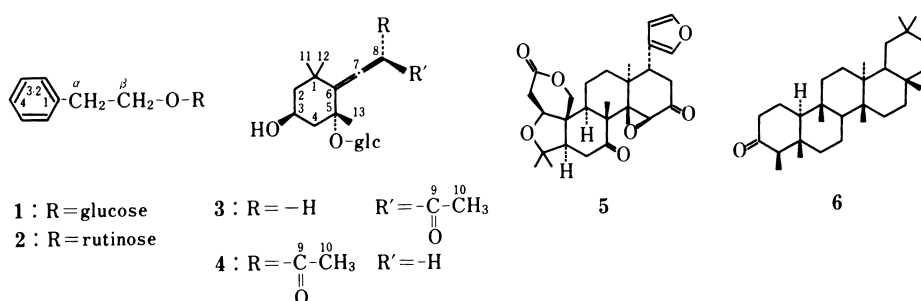


Chart 1

TABLE I. ^{13}C -NMR Chemical Shifts

1		2		3		4	
Aglycone moiety				Aglycone moiety			
1	139.3	139.4	1	36.4	36.2		
2	128.6	128.6	2	50.1	50.1		
3	129.3	129.4	3	62.5	62.4		
4	126.4	126.4	4	47.5	46.8		
			5	78.1	78.6		
α	36.6	36.6	6	118.7	118.8		
β	71.6	71.7	7	197.6	199.0		
			8	100.8	100.9		
Sugar moiety				9	211.4	211.2	
1'	104.6	104.5	10	26.5 ^{a)}	27.1 ^{a)}		
2'	75.0	74.9	11	27.0 ^{a)}	27.6 ^{a)}		
3'	78.3	78.4	12	29.7 ^{a)}	29.6 ^{a)}		
4'	70.5	70.5	13	32.3 ^{a)}	32.5 ^{a)}		
5'	78.3	77.0	Sugar moiety				
6'	62.8	68.2	1'	98.4	98.4		
1''		102.3	2'	75.1	75.2		
2''		72.1 ^{a)}	3'	79.0	79.1		
3''		72.7 ^{a)}	4'	71.7	71.7		
4''		74.0	5'	78.0	78.0		
5''		69.7	6'	62.8	62.8		
6''		18.6					

Run at 22.5 MHz in pyridine-*d*₅ solution. a) Interchangeable in each column.

an allenic structure (1945 cm⁻¹) and a conjugated ketone group (1670 cm⁻¹). The ¹H-NMR spectrum exhibited four singlet methyl signals at δ 1.17 (3H), 1.62 (6H), 2.20 (3H), the last one being due to a methyl ketone, an anomeric proton signal at δ 5.04 (1H, d, *J* = 7 Hz) and an olefinic proton signal at δ 6.00 (1H, s). The acid hydrolysis of **3** afforded glucose as the sugar moiety and the aglycone **3a**. In the ¹H-NMR spectrum of **3a**, a carbinol proton signal was observed at δ 4.36 (m, *W*_{1/2} = 23 Hz). From these data, **3a** was assumed to be grasshopper ketone, previously isolated from *Romalea microptera*.²⁾ The identity of **3a** was established by direct comparison with an authentic sample [high-performance liquid chromatography (HPLC), mp, ¹H-NMR].³⁾ In the ¹³C-NMR spectrum of **3a**, two carbinol carbon signals were observed at δ 63.8 (d) and 72.3 (s). The former was shifted upfield by only 1.3 ppm in the ¹³C-NMR spectrum of **3**, but the latter was shifted downfield by 5.8 ppm. Therefore, the glucosidation position was decided to be at C-5. These results led us to conclude the structure of citroside A to be **3**.

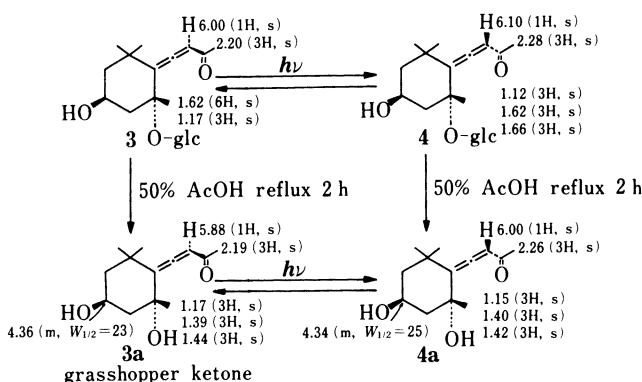


Chart 2

TABLE II. ^1H -NMR Chemical Shifts and Coupling Constants

Proton No.	3	3a	4	4a
3	a)	4.36 (1H, m, $W_{1/2} = 23$ Hz)	a)	4.34 (1H, m, $W_{1/2} = 25$ Hz)
8	6.00 (1H, s)	5.88 (1H, s)	6.10 (1H, s)	6.00 (1H, s)
10	2.20 (3H, s)	2.19 (3H, s)	2.28 (3H, s)	2.26 (3H, s)
11	1.17 (3H, s)	1.17 (3H, s)	1.12 (3H, s)	1.15 (3H, s)
12	1.17 (3H, s)	1.39 (3H, s)	1.62 (3H, s)	1.40 (3H, s)
13	1.62 (6H, s)	1.44 (3H, s)	1.66 (3H, s)	1.42 (3H, s)
Anomeric	5.04 (1H, d, $J = 7$ Hz)		5.16 (1H, d, $J = 7$ Hz)	

Run at 89.55 MHz in pyridine- d_5 solution. a) Overlapped with H_2O .

Compound **4** (citroside B), $[\alpha]_D -48.2^\circ$, $\text{C}_{19}\text{H}_{30}\text{O}_8$ was obtained as a colorless amorphous powder. The IR spectrum showed allenic structure (1945 cm^{-1}) and a conjugated ketone group (1675 cm^{-1}). The ^1H -NMR spectrum exhibited four singlet methyl signals at δ 1.12 (3H), 1.62 (3H), 1.66 (3H), 2.28 (3H), the last one being due to a methyl ketone, an anomeric proton signal at δ 5.16 (1H, d, $J = 7$ Hz) and an olefinic proton signal at δ 6.10 (1H, s). On comparison of the ^1H -NMR spectra of **3** and **4**, the signal of an olefinic proton signal at C-8 was shifted downfield by 0.1 ppm and acetyl methyl signal at C-10 was shifted downfield by *ca.* 0.1 ppm. Acid hydrolysis of **4** with 50% AcOH gave **4a** as the aglycone moiety and glucose as the sugar moiety.⁴⁾ Citrosides A and B were indicated to be epimeric at the C-8 position. It was reported by Ise *et al.* that grasshopper ketone in EtOH could be inverted at C-8 by irradiation with a high-pressure mercury lamp.⁵⁾ Compound **3a** gave a product identical with **4a** on irradiation with a high-pressure mercury lamp. Compound **3**, the glucoside of **3a**, gave a product identical with **4** (^1H -NMR, HPLC) on irradiation with a high-pressure mercury lamp. These results confirmed that **3** and **4** are C-8 epimers.

Limonin (**5**) was identified by direct comparison (thin layer chromatography (TLC), mp, ^1H -NMR and ^{13}C -NMR spectra) with an authentic sample.

Friedelin (**6**) was identified by comparison of various data (mp, ^1H -NMR) with reported values.⁶⁾

Experimental

Melting points were taken on a Yanaco MP-500 micromelting point apparatus and are uncorrected. IR spectra

were run on a JASCO A-201 IR spectrometer and UV spectra on a Shimadzu UV-360 recording spectrometer. Mass spectra (MS) were measured on a JEOL LMS-D 100 mass spectrometer. ^1H - and ^{13}C -NMR spectra were recorded on a JEOL FX-90Q NMR spectrometer (89.55 and 22.5 MHz, respectively). Chemical shifts are given on the δ scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). Gas chromatography (GC) was done on a Hitachi K53 gas chromatograph. HPLC was conducted on a JASCO model 880 PU with UVDEC 100V systems.

Isolation—Fresh leaves of *C. unshiu* (10 kg) collected in May 1986, in Shizuoka, Japan, were extracted twice with hot methanol. The water-soluble fraction of the extract was passed through a DIAION HP-20 column and the absorbed material was eluted with 50% MeOH, 75% MeOH and MeOH successively. The 50% MeOH eluate (70 g) was chromatographed on a TOYOPEARL HW-40 column with water. After repeated chromatography of the eluate on silica gel with a chloroform-methanol system, four glycosides were isolated. The ethyl acetate-soluble fraction of the methanol extract was repeatedly chromatographed on a silica gel column with a hexane-ethyl acetate and benzene-ethyl acetate to give two terpenoids.

Phenylethyl β -D-Glucoside (1)—Colorless amorphous powder (5 mg), $[\alpha]_D^{23}$ -28.8° ($c=0.40$, methanol). *Anal.* Calcd for $\text{C}_{14}\text{H}_{20}\text{O}_6$: C, 54.89; H, 6.86. Found: C, 54.80; H, 6.57. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 2930, 1500, 1450, 1375, 1050. ^1H -NMR (CDCl_3) δ : 3.00 (2H, t, $J=7$, H- α), 4.82 (1H, d, $J=7$, H-1'), 7.25 (5H, s, H-2, H-3, H-4, H-5, H-6), ^{13}C -NMR: Table I.

Phenylethyl D-Rutinoside (2)—Colorless amorphous powder (20 mg), $[\alpha]_D^{23}$ -101.2° ($c=0.10$, methanol). *Anal.* Calcd for $\text{C}_{20}\text{H}_{30}\text{O}_{10} \cdot \text{H}_2\text{O}$: C, 53.51; H, 6.92. Found: C, 53.56; H, 6.74. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 2930, 1500, 1450, 1375, 1050. ^1H -NMR (CDCl_3) δ : 1.60 (3H, d, $J=5$, H-6'), 3.00 (2H, t, $J=7$, H- α), 4.81 (1H, d, $J=7$, H-1'), 7.25 (5H, s, H-2, H-3, H-4, H-5, H-6), ^{13}C -NMR: Table I.

Citroside A (3)—Colorless amorphous powder (200 mg), $[\alpha]_D^{23}$ -95.7° ($c=0.44$, methanol). *Anal.* Calcd for $\text{C}_{19}\text{H}_{30}\text{O}_8 \cdot 1/2\text{H}_2\text{O}$: C, 57.71; H, 7.90. Found: C, 57.58; H, 7.73. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 1945, 1670, 1360, 1240. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 230 (4.12). ^1H -NMR: Table II and ^{13}C -NMR: Table I.

Citroside B (4)—Colorless amorphous powder (10 mg), $[\alpha]_D^{23}$ -48.2° ($c=0.28$, methanol). *Anal.* Calcd for $\text{C}_{19}\text{H}_{30}\text{O}_8 \cdot 1/2\text{H}_2\text{O}$: C, 57.71; H, 7.90. Found: C, 57.85; H, 7.96. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 2950, 1945, 1675, 1380, 1070. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 230 (4.17). ^1H -NMR: Table II and ^{13}C -NMR: Table I.

Limonin (5)—Colorless needles (85 mg), mp $271-275^\circ\text{C}$ (AcOEt). ^1H -NMR (CDCl_3) δ : 1.07 (3H, s, H-30), 1.18 (6H, s, H-18, 29), 1.29 (3H, s, H-28), 4.04 (2H, brs, H-1, 15), 4.45 (1H, d, $J=13$, H-19), 4.79 (1H, d, $J=13$, H-19), 5.46 (1H, s, H-17), 6.36 (1H, m, H-20), 7.40 (1H, s, H-23), 7.43 (1H, s, H-21). ^{13}C -NMR (pyridine- d_5) δ : 17.7 (C-30), 18.7 (C-11), 20.3 (C-18), 21.7 (C-29), 30.0 (C-28), 30.2 (C-12), 36.4 (C-2), 36.8 (C-6), 38.6 (C-13), 46.4 (C-10), 48.1 (C-9), 51.5 (C-8), 54.8 (C-15), 60.0 (C-5), 65.7 (C-19), 67.0 (C-14), 78.2 (C-17), 79.8 (C-1), 80.3 (C-4), 110.5 (C-22), 121.2 (C-20), 141.8 (C-21), 143.6 (C-23), 167.5 (C-16), 170.1 (C-3), 207.7 (C-7).

Friedelin (6)—Colorless needles (40 mg), mp $261-262^\circ\text{C}$ (AcOEt). ^1H -NMR (CDCl_3) δ : 0.76 (3H, s), 0.88 (3H, d, $J=7.2$), 0.87 (3H, s), 0.96 (3H, s), 1.02 (6H, s), 1.06 (3H, s), 1.20 (3H, s).

Acid Hydrolysis of Phenylethyl β -D-Glucoside (1) and Phenylethyl D-Rutinoside (2)—A solution of glycoside (5 mg) in 10% H_2SO_4 (1–2 drops) was well stirred in a boiling water bath for an hour. The reaction mixture was diluted with water and extracted with ethyl acetate 3 times. Ethyl acetate was evaporated off and the residue was purified by HPLC (YMC-ODS, 4.6×250 mm; $\text{H}_2\text{O}-\text{CH}_3\text{CN}$ (85:15)) to give the aglycone as a colorless oil (2 mg). ^{13}C -NMR ($\text{C}_5\text{D}_5\text{N}$) δ : 40.5 (C- α), 63.6 (C- β), 126.6 (C-4), 128.8 (C-2), 129.8 (C-3), 140.4 (C-1). The water-soluble fraction was passed through an Amberlite IRA-45 column and the eluate was concentrated to give a residue, which was reduced with sodium borohydride (*ca.* 2 mg) for 1 h at room temperature. The reaction mixture was passed through an Amberlite IR-120 column and the eluate was concentrated to dryness. Boric acid was removed by co-distillation with methanol and the residue was acetylated with acetic anhydride and pyridine (1 drop each) at room temperature overnight. The reagents were evaporated off *in vacuo*. From **1**, glucityl acetate was detected by GC. From **2**, glucityl acetate and rhamnityl acetate were detected by GC. Conditions: column, SUPELCO capillary column SPB-35; carrier gas, N_2 10 ml/min; column temperature, 170°C ; t_R 10.4 min (rhamnityl acetate); column temperature, 200°C ; t_R 10.8 min (glucityl acetate).

Acid Hydrolysis of Citrosides A (3) and B (4)—A solution of citroside A (5 mg) in 50% AcOH (2–3 drops) was heated at 70°C for 2 h, then the solvent was evaporated off, and the residue was partitioned in water-ethyl acetate. From the water-soluble fraction, glucose was detected as the glucityl acetate by GC. The ethyl acetate fraction was worked up in the same way as described for **1** to give the aglycone (**3a**) (2 mg) as colorless needles (acetone-benzene), mp $134-136^\circ\text{C}$. ^1H -NMR: Table II. From **4** (5 mg), **4a** (1 mg) was obtained in the same manner as colorless needles (ethyl acetate-hexane), mp $150-152^\circ\text{C}$. ^1H -NMR: Table II. GC conditions: column, SUPELCO capillary column SPB-35; carrier gas, N_2 ; column temperature, 200°C ; t_R 10.8 min (glucityl acetate).

Photo-epimerization of Citrosides A (3) and B (4)—An ethanolic solution (2 ml) of citroside B (**4**) (5 mg) was irradiated with a high-pressure mercury lamp (400 W) at room temperature through a Pyrex glass filter under a 1:1 nitrogen-air mixture for 30 min. The reaction mixture was purified by HPLC to give citroside A (2.5 mg) and B (1 mg). ^1H -NMR: Table II. From citroside A (**3**), citroside B (**4**) was obtained in the same manner. ^1H -NMR: Table II. HPLC conditions: column, YMC-ODS ($4.6 \text{ mm} \times 25 \text{ cm}$); $\text{H}_2\text{O}-\text{CH}_3\text{CN}$ (85:15); flow rate, 1 ml/min; t_R 11 min

(citroside A), 16 min (citroside B).

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