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Flower Fragrance Precursors from Flower Buds of *Citrus unshiu* MARCOV.

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A new aroma glycoside, unshuoside A, and four known compounds including prenyl glucoside, 2-endo-cineolylol glucoside, phenethyl glucoside and solidroside, were isolated from fresh flower buds of *Citrus unshiu* MARCOV.

Keywords——*Citrus unshiu*; Rutaceae; fragrance precursor; aroma glucoside; hemiterpenoid glucoside; terpenyl glucoside

It has been reported that monoterpene glycosides are aroma precursors in tea fruits,[1-5] and leaves[6,7] contributing to their floral fragrance when hydrolysed. Generally, flower buds do not smell and floral note is released from flowers during anthesis. Watanabe *et al.*[8] suggested that β-glucosides of linalool, eugenol, borneol, and isoeugenol were precursors of the fragrance compounds in *Gardenia jasminoides* ELLIS.

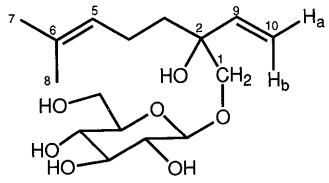
During our studies[9] on glycosidically bound volatiles in plants, we isolated a new aroma component together with four known aroma glycosides from fresh flower buds of *Citrus unshiu* MARCOV.

By repeated ordinary-phase (SiO_2) and reversed-phase (ODS) column chromatography of an EtOH extract of fresh flower buds of *C. unshiu* (8.5 kg), a new aroma glycoside named unshuoside A (**1**) was obtained along with known aroma glycosides, prenyl (3,3-dimethylallyl alcohol) glucoside (**2**), 2-endo-cineolylol glucoside (**3**), solidroside (**4**) and phenethyl glucoside (**5**).

Unshuoside A (**1**), isolated as an amorphous powder, exhibited an $[M+H]^+$ peak at m/z 333.1950 in the high-resolution chemical ionization (HRCI) MS, consistent with the molecular formula $C_{16}H_{28}O_7$. The ^{1}H-NMR and ^{1}H-^{1}H correlation spectroscopy (COSY) spectra revealed the existence of a vinyl group [δ 5.14 (1H, dd, J=11.0, 1.7 Hz), 5.30 (1H, dd, J=17.3, 1.7 Hz), 5.88 (1H, dd, J=17.3, 11.0 Hz)], a 5-methyl-3-pentenyl group [δ 1.59 and 1.65 (each 3H, s), δ 1.50 (1H, ddd, J=12.0, 12.0, 4.0 Hz), 1.58 (1H, ddd, J=12.0, 12.0, 4.0 Hz), δ 1.95-2.05 (2H, m), δ 5.09 (1H, br t, J=7.1 Hz)], a hydroxymethyl group [δ 3.43 and 3.88 (each 1H, d, J=10.0 Hz)] and one anomeric proton [δ 4.25 (1H, d, J=7.8 Hz)]. The distorsionless enhancement by polarization transfer (DEPT) spectrum indicated the presence of two methyl carbons, two methylene carbons, one oxygen-bearing methylene carbon, one oxygen-bearing tertiary carbon and one secondary, two tertiary and one quaternary carbons due to two olefinic bonds and a hexosyl moiety. Acid hydrolysis of **1** gave D-glucose, which was confirmed by the specific rotation measurement using HPLC with chiral detection. The β-configuration of the anomeric center of D-glucopyranosyl group was suggested by the coupling constant (J=7.8 Hz). Comparison of the ^{13}C-NMR spectra of **1** with those of the genin[10] showed glycosylation shifts[11,12] by +9.1 ppm at the C-1 signal and −1.1 ppm at the C-4 signal, demonstrating that the sugar linkage was at C-1-OH. This was further confirmed by the heteronuclear multiple-bond correlation (HMBC) and rotating frame Overhauser enhancement spectroscopy (ROESY) experiments. Long-range correlations were seen between H-1′ (δ 4.25) (Glc) and C-1 (δ 77.4) in the HMBC spectrum, and NOEs were detected between H-1′ (δ 4.25) (Glc) and H-1 (δ 3.88) and between H-1′ (δ 4.25) (Glc) and H-1 (δ 3.43). Thus, by analysis of the NMR data including ^{1}H-^{1}H COSY, ^{1}H detected multiple quantum cohence (HMQC), HMBC, and ROESY experiments (TABLE I), the structure of unshuoside A was established to be 6-methyl-2-vinyl-5-heptene-1,2-diol-1-*O*-β-D-glucopyranoside. The absolute configuration of the remaining chiral center C-2 is not determined yet.

Prenyl glucoside (**2**), an amorphous powder, showed an $[M+H]^+$ peak at m/z 249 in the CIMS, corresponding to the molecular formula of $C_{11}H_{20}O_6$. The ^{1}H-NMR spectrum revealed the existence of a 3-methyl-2-butenyl group [δ 5.37 (1H, t, J=7.7 Hz), 4.27 (2H, d, J=7.7 Hz), 1.70 and 1.76 (each, 3H, s) and one anomeric proton [δ 4.80 (1H, d, J=8.0 Hz)]. The DEPT spectrum indicated the presence of two methyl carbons, one oxygen-bearing methylene carbon, and one tertiary and one quaternary carbons due to olefinic bond and a glucosyl moiety. Those data concluded that the structure of this compound was prenyl glucoside, which had been synthesized by Ackermann *et al.*[13] This is the first report which describes isolation of glucoside from natural sources.

2-Endo-cineolylol glucoside (**3**), isolated as an amor-



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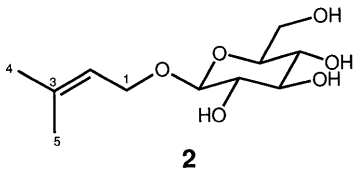

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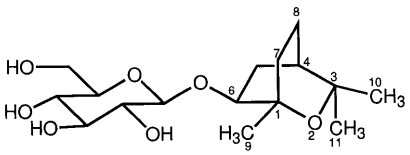

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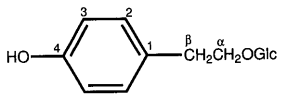

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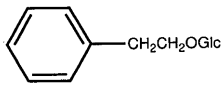

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TABLE I. NMR Spectral Data for Unshuoside A (**1**) in CD_3OD

Carbon	δ_C	DEPT	δ_H	HMBC	ROESY
1	77.4	$-CH_2$	3.88 (H_a, d, 10.0)	H-1′	H-1′
			3.43 (H_b, d, 10.0)	H-1′	H-1′
2	76.1	>C<		H_a10, H_b10, H-9, H-3	
3	38.5	$-CH_2$	1.58 (H_a, ddd, 12.0, 12.0, 4.0)	H_2-4, H-1	H-4
			1.50 (H_b, ddd, 12.0, 12.0, 4.0)		H-4
4	23.0	$-CH_2$	2.05–1.95 (2 H, m)	H_2-3	H-3
5	125.6	-CH=	5.09 (br t, 7.1)	H-4, H-7, H-8	H-8
6	132.2	>C=		H-7, H-8	
7	25.9	$-CH_3$	1.65 (3 H, s)	H-5, H-8	
8	17.7	$-CH_3$	1.59 (3 H, s)	H-5, H-7	
9	142.4	-CH=	5.88 (dd, 17.3, 11.0)	H_a-10	H_b-10
10	114.4	$=CH_2$	5.30 (H_a, dd, 17.3, 1.7)		H_b-10
			5.14 (H_b, dd, 11.0, 1.7)		H_a-10
1′	105.1	-CH-	4.25 (d, 7.8)	H-2′, H-3′, H-5′, H-1	H-3′, H-5′, H-1
2′	75.2	-CH-	3.20 (dd, 9.0, 7.8)	H-3′	
3′	77.9	-CH-	3.34 (dd, 9.0, 9.0)	H-2′	
4′	71.6	-CH-	3.27 (dd, 9.0, 9.0)	H-5′, H-6′	
5′	78.0	-CH-	ca. 3.26 (m)	H-1′, H-6′	
6′	62.7	$-CH_3$	3.86 (H_a, dd, 12.0, 2.5)	H-4′, H_b-6′	
			3.65 (H_b, dd, 12.0, 5.4)	H-4′, H_a-6′	

phous powder, showed an $[M+H]^+$ peak at m/z 333.1930 in the HRCIMS, consistent with molecular formula $C_{16}H_{28}O_7$. The DEPT spectrum indicated the presence of three methyl carbons, three methylene carbons, one methine carbon, one oxygen-bearing methine carbon, and two oxygen-bearing tertiary carbons in addition to six carbons due to glucosyl moiety. By analysis of the NMR data including ^1H-^1H COSY, HMQC, HMBC and ROESY experiments, the structure of **3** was determined to be (1*S*, 4*R*, 6*S*)-1,3,3-trimethyl-2-oxabicyclo[2,2,1]-octane-6-*O*-*β*-D-glucopyranoside, which had been isolated from peels of young *C. unshiu* by SAWABE *et al.*[14]

Compounds **4** and **5** were identified as solidroside and phenethyl glucoside, respectively, by comparing the physical and spectral data with those in the literature.[15,16] These compounds, isolated from this plant for the first time are considered to be constituents of fragrance precursors in the flower.

EXPERIMENTAL

Optical rotations were taken on a JASCO DIP-140 digital polarimeter. NMR spectra were recorded on a Varian UNITY 200 and 600 spectrometers in CD_3OD solution using TMS as an internal standard. NMR experiments included ^1H-^1H-COSY, ^{13}C-^1H-COSY, HMBC and ROESY. Coupling constants (J values) are given in Hz. The CIMS were measured on a JEOL JMS-PX303 mass spectrometer. HPLC was carried out with a Waters ALC/GPC 244 instrument by using Octadecyl-functionalized Si gel (Daiso). Flash chromatography was carried out by using Kiesel gel 60 (230–400 mesh, Merck) and TLC Merck DC-Platten Kiesel

gel 60 F_{254}.

Plant materials Flower buds of *C. unshiu* were collected in Tokushima prefecture, in May, 1994. A voucher specimen has been deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and isolation Fresh flower buds of *C. unshiu* (8.5 kg) were extracted with EtOH at room temperature for two weeks. After removal of the solvent, the MeOH-soluble portion was subjected to column chromatography {SiO_2 : MeOH : H_2O (25 : 4 : 0.1→25 : 6 : 0.1)} and subsequently to HPLC on reverse-phase DAISOPAK $^5C_{18}$ {CH_3CN-H_2O (5→25%)} to give unshuoside A (**1**, 5 mg), prenyl (3,3-dimethylallyl alcohol) glucoside (**2**, 3 mg), 2-endo-cineolylol glucoside (**3**, 16 mg), solidroside (**4**, 2 mg) and phenethyl glucoside (**5**, 4 mg).

Unshuoside A (**1**): Amorphous powder; $[\alpha]_D$ −5.0° (c =0.4 in MeOH); HRCIMS (isobutane): Calcd. for $[M+H]^+$, $C_{16}H_{29}O_7$: 333.1913. Found: 333.1950. NMR data (600 MHz), see TABLE I.

Prenyl glucoside (**2**): Amorphous powder, $[\alpha]_D$ −20.0° (c=0.2 in MeOH). CIMS (isobutane): m/z 249 $[M(C_{11}H_{20}O_6)+H]^+$. ^{1}H-NMR (200 MHz) δ: 1.70 and 1.76 (each 3H, s), 4.27 (2H, t, J=7.7 Hz, H-1), 4.80 (1H, d, J=8.0 Hz, H-1 of Glc), 5.37 (1H, t, J=7.7 Hz, H-2). ^{13}C-NMR (50 MHz) δ: 18.1 (C-5), 26.0 (C-4), 62.9 (C-6′), 66.4 (C-1), 71.9 (C-4′), 75.2 (C-2′), 78.1 (C-3′), 78.2 (C-5′), 102.2 (C-1′), 121.8 (C-2), 138.3 (C-3).

2-Endo-cineolylol glucoside (**3**): Amorphous powder, $[\alpha]_D$ +1.5° (c=0.8 in MeOH). HRCIMS: Calcd. for $[M+H]^+$, $C_{16}H_{29}O_7$: 333.1913. Found: 333.1930. ^{1}H-NMR (600 MHz, C_5D_5N) δ: 1.15 (3H, s, H-11), 1.23 (3H, s, H-10), 1.32 (1H, m, H-4), 1.46 (3H, s, H-9), 1.50, 2.12 (each, 1H, m, H_2-7), 1.52, 1.82 (each, 1H, m, H_2-8), 1.91 (1H, ddd, J=14.5, 4.0, 3.2 Hz, H-5_{exo}), 2.59 (1H, dddd, J=14.5, 9.6, 3.6, 3.2 Hz, H-5_{endo}), 3.96 (1H, ddd, J=9.3, 5.1, 2.6 Hz, H-5′), 4.00 (1H, ddd, J=9.6, 4.0, 2.0 Hz, H-6), 4.03 (1H, dd, J=8.8, 7.8 Hz, H-2′), 4.22 (1H, dd, J=8.8, 8.8 Hz, H-3′), 4.29 (1H, dd, J=9.3, 8.8 Hz, H-4′), 4.44 (1H, dd, J=11.6, 5.1 Hz, H-6′), 4.56 (1H, dd, J=11.6, 2.6 Hz, H-6′), 4.93 (1H, d, J=7.8 Hz, H-1′). ^{13}C-NMR (150 MHz, C_5D_5N) δ: 22.5 (C-8), 24.9 (C-9), 26.3 (C-7), 28.9 (C-10), 29.1 (C-11), 34.3 (C-4 and C-5), 62.9 (C-6′), 71.7 (C-4′), 72.3 (C-1), 73.2 (C-3), 75.6 (C-2′), 78.4 (C-5′), 78.6 (C-3′), 80.1 (C-6), 106.5 (C-1′).

Solidroside (**4**): Amorphous powder, $[\alpha]_D$ −26.9° (c=0.2 in MeOH). CIMS (isobutane): m/z 301 $[M(C_{14}H_{20}O_7)+H]^+$. ^{13}C-NMR (50 MHz) δ: 36.4 (C-β), 62.8 (C-6′), 71.7 (C-4′), 72.2 (C-α), 75.2 (C-2′), 78.0 (C-3′, C-5′), 104.4 (C-1′), 111.6 (C-3, C-5), 130.9 (C-1, C-2, C-6), 157.0 (C-4).

Phenethyl glucoside (**5**): Amorphous powder, $[\alpha]_D$ −25.0° (c=0.4 in MeOH). CIMS (isobutane): m/z 285 $[M(C_{14}H_{20}O_6)+H]^+$. ^{13}C-NMR (50 MHz) δ: 37.3 (C-β), 62.8 (C-6′), 71.7 (C-4′), 71.8 (C-α), 75.2 (C-2′), 78.0 (C-3′), 78.5 (C-5′), 104.4 (C-1′), 127.3 (C-4), 129.4 (C-2, C-6), 130.3 (C-3, C-5), 140.1 (C-1).

Acid hydrolysis of compound 1 Compound **1** (1 mg) was heated in 5% H_2SO_4 : dioxane (1 : 1) at 100℃ for 1 h. The reaction mixture was diluted with H_2O, neutralized with Amberlite IR-45 and concentrated *in vacuo* to dryness. The sugar was identified and the configuration was determined as D by the RI detection (Waters 410) and chiral detection (Shodex OR-1) in HPLC (Shodex RSpak DC-613, 80% CH_3CN, 1 ml/min, 70℃). In HPLC, the sugar gave a peak at the same location as that of authentic D-(+)-Glc (10.30 min).

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