Monoterpenoid Glucosides of *Cnidium monnieri* Fruit

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Four new monoterpenoid glucosides were isolated from the methanolic extract of the fruit of *Cnidium monnieri* CUSSON (Umbelliferae) together with four known glucosides of aromatic compounds. Their structures were clarified by spectral investigation.

Key words *Cnidium monnieri* fruit; acyclic monoterpenoid glucoside; menthane-type monoterpenoid glucoside; ^{13}C-NMR

In previous papers,[1)] we reported the separation and characterization of monoterpenoid polyols and hemiterpenoid tetrol from the methanolic extract of *Cnidium monieri* CUSSON fruit (Umbelliferae; known in Japanese as "Jyashōshi"). The present study was performed with the aim of isolating glycosidic constituents other than cnidiosides A, B and C, which were reported as the constituents of this fruit by Yahara *et al*.[2)]

The methanolic extract of commercial fruit of *C. monieri* was worked up as described in the previous paper,[1b)] and from the same aqueous portion, four new monoterpenoid glycosides (**1** to **4**), cnidioside C, two furocoumarin glycosides (**5** and **6**), two chromone glycosides (**7** and **8**) and (*S*)-peucedanol[3)] were isolated. All glycosides were found to be β-D-glucopyranoside as evidenced from their ^{13}C-NMR data (Table 2), and this was confirmed by enzymatic hydrolysis to yield D-glucose. Their molecular formulae were suggested from the accurate mass number of $[M+H]^+$ or $[M+Na]^+$ ion peaks in the high-resolution positive FAB-MS.

Glycoside **1** ($C_{16}H_{30}O_8$, an amorphous powder, $[\alpha]_D^{23}$ $-21.3°$) showed, in addition to the β-glucopyranosyl moiety, three *tert*-methyls, two methylenes, one hydroxylated methine and two hydroxylated quaternary carbons, and one monosubstituted double bond, in the ^{1}H- and ^{13}C-NMR data (Tables 1 and 2). Enzymatic hydrolysis of **1** gave an aglycone which was identified as 3,7-dimethyloct-1-ene-3,6,7-triol (**9**).[1b)] The position of the glucosyl unit was ascribed to C-3 not only from the downfield shift of the C-3 signal when compared with that of **9** (by 7.60 ppm), but also from the observed correlation between the glucosyl anomeric proton signal and the C-3 carbon in the heteronuclear multiple-bond correlation (HMBC) spectrum. So, **1** was characterized as 3,7-dimethyloct-1-ene-3,6,7-triol 3-*O*-β-D-glucopyranoside.

Enzymatic hydrolysis of glycoside **2** ($C_{16}H_{30}O_9$, mp 69—70 °C, $[\alpha]_D^{25}$ $-8.5°$) gave an aglycone 3,7-dimethyloct-3(10)-ene-1,2,6,7-tetrol (**10a**; $C_{10}H_{20}O_4$, a colorless syrup, $[\alpha]_D^{23}$ $-25.3°$), which was the main monoterpenoid constituent of this fruit existing with its stereoisomer (**10b**).[1a)] The position of attachment of the glucosyl unit was revealed to be C-2 from the H–C long-range correlation between the glucosyl anomeric proton signal and the C-2 carbon in the HMBC spectrum, and the downfield shift of C-2 (by 8.62 ppm). So, **2** was characterized as 3,7-dimethyloct-3(10)-ene-1,2,6,7-tetrol 2-*O*-β-D-glucopyranoside. The absolute configuration at C-2 of **2** was confirmed to be *S* by comparison of its ^{13}C-NMR spectrum with those of **10a** and **10b**, and the 2*R* isomer of **2** (**11**) which was isolated from fennel [fruit of *Foeniculum vulgare* MILLER (Umbelliferae)] by us (Table 2).[4,5)] Therefore, **2**

Table 1. ^{1}H-NMR Chemical Shifts of **1**—**4** and **10a** (in Pyridine-d_5, 500 MHz)

	1	2	10a	3	4
H_2-1	5.15 dd (1.5, 11.0)	4.11 dd (4.5, 14.0)	4.08 dd (7.5, 11.0)	4.13 (2H) dd (5.0, 10.0)	—
	5.34 dd (1.5, 17.5)	4.17 dd (7.0, 14.0)	4.18 dd (4.0, 11.0)		—
H-2	6.42 dd (11.0, 17.5)	4.78 dd (4.5, 7.0)	4.76 dd (4.0, 7.5)	4.75 dd (5.0, 6.5)	5.90 br d (3.0)
H_2-3	—	—	—	—	2.22 m
H-4	—	—	—	—	1.89 br dd (13.0, 13.0))
H_2-4	2.07 ddd (4.0, 11.5, 12.5)	2.39 ddd (6.0, 10.0, 15.0)	2.51 ddd (6.0, 10.5, 15.5)	2.24 m	—
	2.55 ddd (4.0, 12.5, 12.5)	3.13 ddd (4.0, 11.0, 15.0)	3.05 ddd (4.0, 10.5, 15.5)	2.42 m	—
H_2-5	1.92 br dd (12.5, 12.5)	1.95 m	1.96 m	2.25 (2H) m	1.30 br ddd (5.0, 13.0, 13.0)
	2.47 br ddd (4.0, 11.5, 12.5)	2.21 m	2.24 m		2.23 br d (13.0)
H-6	3.86 br d (11.5)	3.81 br d (10.5)	3.82 br d (9.0)	5.18 dd (5.0, 6.0)	—
H_2-6	—	—	—	—	1.95 br dd (13.0, 13.0)
	—	—	—	—	2.32 br d (13.0)
H_2-7	—	—	—	—	4.26 d (9.0)
	—	—	—	—	4.27 d (9.0)
H_3-8	1.50[a)] s	1.49[a)] s	1.47[a)] s	1.55[a)] s	—
H_3-9	1.51[a)] s	1.53[a)] s	1.50[a)] s	1.62[a)] s	1.40[b)] s
H_3-10	1.56 s	—	—	—	1.35[a)] s
H_2-10	—	5.14 br s	5.20 br s	5.07 br s	—
	—	5.39 br s	5.55 br s	5.43 br s	—
Glc-1	5.00 d (8.0)	5.17 d (8.0)		5.27 d (7.5)	5.02 d (7.5)

δ in ppm from tetramethylsilane (TMS) [coupling constants (J) in Hz are given in parentheses]. *a*, *b*) Assignments may be interchanged in each column.

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Table 2. ^{13}C-NMR Chemical Shifts of **1**—**4**, **9**, **10a**, **10b**, **11** and **12** (in Pyridine-d_5, 125 MHz)

	1	**9**	**2**	**10a**	**11**	**10b**	**3**	**4**	**12**
C-1	113.79	111.19	64.87	66.75	65.25	66.79	65.44	139.01	139.26
C-2	145.17	147.49	85.18	76.56	82.72	76.30	85.38	121.31	121.33
C-3	80.18	72.58	148.96	151.88	147.49	151.72	148.32	26.99	27.14
C-4	37.42	41.14	30.19	30.54	29.56	30.49	33.01	44.50	44.59
C-5	26.10	26.91	30.19	30.97	30.16	30.83	26.76	24.00	23.96
C-6	79.53	79.78	78.42	78.66	78.04	78.38	124.91	26.95	27.12
C-7	72.89	72.78	72.66	72.71	72.63	72.68	131.40	66.43	66.55
C-8	25.12[a)]	26.07[a)]	25.51[a)]	25.94	25.96[a)]	25.94	17.73[a)]	79.33	79.30
C-9	24.98[a)]	25.92[a)]	25.85[a)]	25.94	26.08[a)]	25.94	25.73[a)]	24.96[b)]	24.34[b)]
C-10	26.78	28.67	111.22	109.85	113.40	110.07	111.37	23.08[b)]	23.71[b)]
Glc-1	99.37		104.71		101.69		105.25	98.54	98.63
Glc-2	75.22		75.54		75.26		75.95	75.33	75.43
Glc-3	78.63		78.24		78.68		78.63	78.72	78.96
Glc-4	71.58		71.33		71.76		71.64	71.74	71.94
Glc-5	78.05		78.24		78.53		78.60	78.86	78.15
Glc-6	62.64		62.33		62.64		62.67	62.89	63.05

δ in ppm from TMS. *a*, *b*) Assignments may be interchanged in each column.

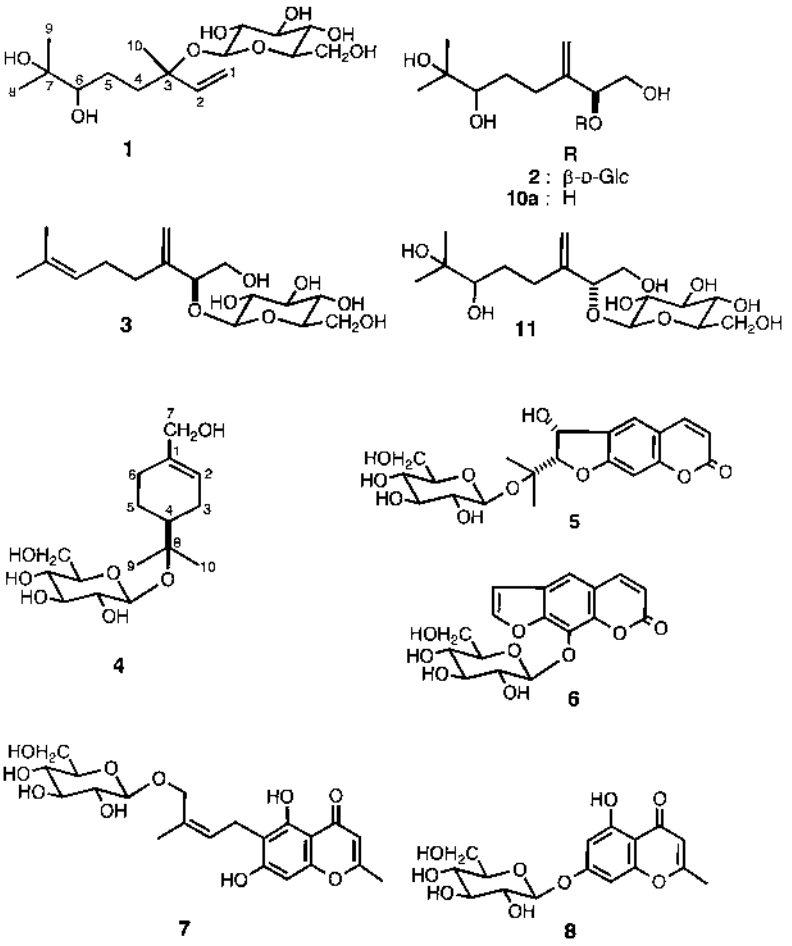

Fig. 1. Structures of **1**—**8**, **10a** and **11**

was determined to be (2*S*,6ζ)-3,7-dimethyloct-3(10)-ene-1,2,6,7-tetrol 2-*O*-β-D-glucopyranoside.

Glycoside **3** ($C_{16}H_{28}O_7$, an amorphous powder, $[\alpha]_D^{23}$ −27.2°) showed the presence of two *tert*-methyls, three methylenes (one of them was hydroxylated), one hydroxylated methine and one trisubstituted double bond, and one terminal-methylene group in its aglycone moiety, from the inspection of the ^{1}H-, ^{13}C-NMR and ^{13}C–^{1}H correlation spectroscopy (COSY) NMR spectral data (Tables 1 and 2). The planar structure of **3** was obtained from the HMBC spectrum which showed H–C long-range correlations between the two methyl protons (H_3-8 and H_3-9) and the C-6, C-7 carbons, between the hydroxylated methylene protons (H_2-1) and the C-2, C-3 carbons, between the terminal-methylene protons (H_2-10) and the C-2, C-4 carbons, between the hydroxylated methine proton (H-2) and the C-1, C-3, C-4, C-10 carbons,

and between the olefinic proton (H-6) and the C-4 carbon. The position of attachment of the glucosyl unit was ascribed to C-2 from the correlation between the glucosyl anomeric proton signal and the C-2 carbon in the HMBC spectrum, and **3** was characterized as 3,7-dimethyloct-3(10),6-diene-1,2-diol 2-*O*-*β*-D-glucopyranoside. Comparison of the chemical shifts of C-2 (δ 85.38) and glucosyl C-1 (δ 105.25) with those of **2** (C-2; δ 85.18, glucosyl C-1; δ 104.71) and **11** (C-2; δ 82.72, glucosyl C-1; δ 101.69) suggested that the absolute configuration at C-2 of **3** was *S*, the same as **2**. From these facts, **3** was determined to be (2*S*)-3,7-dimethyloct-3(10),6-diene-1,2-diol 2-*O*-*β*-D-glucopyranoside.

Glycoside **4** ($C_{16}H_{28}O_7$, an amorphous powder, $[\alpha]_D^{25}$ −50.0°) was indicated to contain two *tert*-methyls, four methylenes (one of them was oxygenated), one methine and one oxygenated quaternary carbon, and one trisubstituted double bond by the ^{1}H-, ^{13}C-NMR and ^{13}C–^{1}H COSY NMR spectral data (Tables 1 and 2). From the results of HMBC experiment, the planar structure of **4** was obtained, and **4** was concluded to be a glucoside of *p*-menth-1-ene-7,8-diol 8-*O*-*β*-D-glucopyranoside. The $[M]_D$ value (−132°) calculated with the value of methyl *β*-D-glucopyranoside (−62°, $[M]_D$ of **4** −$[M]_D$ of methyl *β*-D-glucopyranoside; Δ −70°) suggested the aglycone of **4** should be (−) form.[6] As the (−)-*p*-menth-1-ene-7,8-diol was known to have 4*S* configuration,[7] **4** was determined to be 4(*S*)-*p*-menth-1-ene-7,8-diol 8-*O*-*β*-D-glucopyranoside as shown in Fig. 1. It was also supported by comparison of the $[M]_D$ value with that of the 4*R* epimer of **4** (**12**: +25°; Δ+87°), which was isolated from fennel by us.[8]

Glycoside **5** ($C_{20}H_{24}O_{10}$), **6** ($C_{17}H_{16}O_9$), **7** ($C_{21}H_{26}O_{10}$) and **8** ($C_{16}H_{18}O_9$) were identified as (3′*R*)-hydroxymarmesin 4′-*O*-*β*-D-glucopyranoside (isolated from *Angelica archangelica* LINN. subsp. *littoralis* and *Glehnia littoralis* FR. SCHMIDT *ex* MIQ.),[9] xanthotoxol 8-*O*-*β*-D-glucopyranoside (isolated from *Angle marmelos* CORR.),[10] cnidimoside (isolated from *Cnidium japonicum* MIQ.)[11] and 2-methyl-5,7-dihydroxychromone 7-*O*-*β*-D-glucopyranoside (isolated from *Tecomella undulata*)[12] by comparison of the NMR data with those published and the results of HMBC experiments.

Experimental

The instruments and experimental conditions for obtaining spectral data for chromatography were the same as in the preceding papers.[1b]

Extraction and Separation of Glycosides As reported in the previous paper,[1b] commercial fruit of *C. monnieri* CUSSON (1 kg) was extracted with methanol (5 l). The aqueous portion of the methanol extract was extracted with hot methanol and the hot methanol soluble portion was subjected to column chromatography on Amberlite XAD-II (H_2O→MeOH) to afford water and methanol eluates (21.2 g and 10.6 g, respectively). The methanol eluate fraction was chromatographed on Sephadex LH-20 (MeOH) which furnished six fractions (frs. 1 to 6). Fraction 2 (8.4 g) was purified by silica gel [$CHCl_3$–MeOH–H_2O (9 : 1 : 0.1→17 : 3 : 0.3→4 : 1 : 0.1→7 : 3 : 0.5)→MeOH] column chromatography to afford fourteen fractions (frs. 2-1 to 2-14). From fr. 2-2 (165 mg), (*S*)-peucedanol (16 mg) was isolated by silica gel [$CHCl_3$–MeOH (19 : 1)] and Sephadex LH-20 (MeOH) column chromatographies. From fr. 2-6 (1.02 g), **3** (7 mg), **5** (23 mg), **6** (12 mg), **7** (56 mg) and **8** (8 mg) were isolated by a Lobar RP-8 [CH_3CN–H_2O (3 : 17)], Sephadex LH-20 (MeOH) and silica gel [$CHCl_3$–MeOH–H_2O (4 : 1 : 0.1)] column chromatograpies, and HPLC [carbohydrate analysis, CH_3CN–H_2O (97 : 3) for **3**; CH_3CN–H_2O (19 : 1) for **6** and **7**; symmetryprep C_{18} (Waters, column size, 7.8×300 mm), CH_3CN–H_2O (1 : 4) for **8**]. From fr. 2-7 (1.70 g), **1** (28 mg) was isolated by a Lobar RP-8 [CH_3CN–H_2O (1 : 9)] column chromatography and HPLC [carbohydrate analysis, CH_3CN–H_2O (19 : 1)]. From fr. 2-8 (396 mg), cnidioside C (160 mg) and **4** (14 mg) were isolated by a Lobar RP-8 [CH_3CN–H_2O (3 : 17)] column chromatography and HPLC [octadecyl silica (ODS), CH_3CN–H_2O (3 : 17) for **4**], and from fr. 2-10 (308 mg), **2** (61 mg) was isolated by a Lobar RP-8 [CH_3CN–H_2O (1 : 9)] column chromatography and HPLC [ODS, CH_3CN–H_2O (1 : 19)].

3,7-Dimethyloct-1-ene-3,6,7-triol 3-*O*-*β*-D-Glucopyranoside (1) An amorphous powder, $[\alpha]_D^{23}$ −21.3° (*c*=1.0, MeOH). Positive FAB-MS *m/z*: 701 $[2M+H]^+$, 351.2026 $[M+H]^+$ (base, Calcd for $C_{16}H_{31}O_8$: 351.2019).

Enzymatic Hydrolysis of 1 A mixture of **1** (13 mg) and hesperidinase (5 mg) in water (5 ml) was shaken in a water bath at 37 °C for 5 d. The mixture was evaporated *in vacuo* to dryness and the residue was chromatographed on silica gel [$CHCl_3$–MeOH–H_2O (9 : 1 : 0.1 and 7 : 3 : 0.5)] to give 3,7-dimethyloct-1-ene-3,6,7-triol (**9**; 4 mg) and the sugar fraction. The sugar fraction was passed through Sephadex LH-20 (MeOH) to give a syrup. This was analyzed by HPLC [column; carbohydrate analysis (Waters: size, 3.9×300 mm), detector; JASCO RI-930 and OR-990 chiral detector: CH_3CN–H_2O (17 : 3), 2 ml/min; t_R 4.7 min] which revealed the presence of D-glucose.

(2*S*,6*ζ*)-3,7-Dimethyloct-3(10)-ene-1,2,6,7-tetrol 2-*O*-*β*-D-Glucopyranoside (2) Colorless needles (MeOH), mp 69—70 °C, $[\alpha]_D^{25}$ −8.5° (*c*=2.0, MeOH). Positive FAB-MS *m/z*: 405 $[M+K]^+$, 389 $[M+Na]^+$ (base), 367.1963 $[M+H]^+$ (Calcd for $C_{16}H_{31}O_9$: 367.1968), 187 $[M-C_6H_{12}O_6+H]^+$.

Enzymatic Hydrolysis of 2 A mixture of **2** (12 mg) and hesperidinase (5 mg) in water (5 ml) was shaken in a water bath at 37 °C for 5 d. The mixture was evaporated *in vacuo* to dryness and the residue was chromatographed on silica gel [$CHCl_3$–MeOH–H_2O (9 : 1 : 0.1 and 7 : 3 : 0.5)] to give **10a** (3.6 mg) and the sugar fraction. From the sugar fraction, the presence of D-glucose was revealed as **1**.

(2*S*,6*ζ*)-3,7-Dimethyloct-3(10)-ene-1,2,6,7-tetrol (10a) A colorless syrup, $[\alpha]_D^{23}$ −25.3° (*c*=0.3, MeOH). Positive FAB-MS *m/z*: 205 $[M+H]^+$, 187 $[M-H_2O+H]^+$, 169 $[M-2H_2O+H]^+$ (base), 151 $[M-3H_2O+H]^+$.

(2*S*)-3,7-Dimethyloct-3(10),6-diene-1,2-diol 2-*O*-*β*-D-Glucopyranoside (3) An amorphous powder, $[\alpha]_D^{23}$ −27.2° (*c*=0.5, MeOH). Positive FAB-MS *m/z*: 355 $[M+Na]^+$ (base), 333.1898 $[M+H]^+$ (Calcd for $C_{16}H_{29}O_7$: 333.1914).

(4*S*)-*p*-Menth-1-ene-7,8-diol 8-*O*-*β*-D-Glucopyranoside (4) An amorphous powder, $[\alpha]_D^{25}$ −50.0° (*c*=1.9, MeOH). Positive FAB-MS *m/z*: 371 $[M+K]^+$, 355.1738 $[M+Na]^+$ (base, Calcd for $C_{16}H_{28}O_7Na$: 355.1733), 333 $[M+H]^+$, 153 $[M-C_6H_{12}O_6+H]^+$. Negative FAB-MS *m/z*: 331 $[M-H]^-$ (base).

(3′*R*)-Hydroxymarmesin 4′-*O*-*β*-D-Glucopyranoside (5) Colorless needles (MeOH), mp 267—269 °C, $[\alpha]_D^{22}$ −18.0° (*c*=0.8, pyridine) [lit.,[9a] $[\alpha]_D$ −14° (pyridine)].

Xanthotoxol 8-*O*-*β*-D-Glucopyranoside (6) An amorphous powder, $[\alpha]_D^{23}$ −43.8° (*c*=0.5, MeOH).

Cnidimoside A (7) Colorless needles (MeOH), mp 137—139 °C, $[\alpha]_D^{23}$ −14.5° (*c*=1.2, MeOH) [lit.,[11] mp 137—139 °C, $[\alpha]_D$ −13.79° (*c*=0.58, MeOH)].

2-Methyl-5,7-dihydroxychromone 7-*O*-*β*-D-Glucopyranoside (8) An amorphous powder, $[\alpha]_D^{23}$ −64.6° (*c*=0.6, MeOH).

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