Monoterpenoid Polyols in Fruit of *Cnidium monnieri*

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Five new monoterpenoid polyols were obtained from the fruits of *Cnidium monnieri* CUSSON (Umbelliferae). They were characterized as diastereomers of 3,7-dimethyloctane-1,2,6,7-tetrols (3 and 4), (6,7-*threo*)- and (6,7-*erythro*)-3,7-dimethyloct-3(10)-ene-1,2,6,7,8-pentols (5 and 6) and *trans-p*-menthane-1β,2α,8,9-tetrol (7), respectively.

Key words monoterpenoid tetrol; monoterpenoid pentol; *Cnidium monnieri* fruit; acyclic monoterpenoid; menthane-type monoterpenoid; Umbelliferae

We earlier reported,[1] the separation and characterization of 3,7-dimethyloct-3(10)-ene-1,2,6,7-tetrol (**1**; a mixture of two stereoisomers) and (2*S*,3*R*)-2-methylbutane-1,2,3,4-tetrol (**8**; 2-*C*-methyl-D-erythritol) from the water-soluble portion of an herbal medicine, She chuang zi [known in Japanese as "Jyashōshi"], the fruit of *Cnidium monnieri* CUSSON (Umbelliferae). Recently, anti-HIV activity was reported for this crude drug.[2] The present study was done in the hope of isolating monoterpenoid polyols along with **1** from this herbal medicine.

The methanolic extract of commercial She chuang zi was partitioned between ethyl acetate and water. The aqueous layer was treated as described in Experimental, with Amberlite XAD-II, Sephadex LH-20, silica gel, Lobar RP-8, octadecyl silica (ODS) and carbohydrate analysis column chromatographies to give monoterpenoids **2** to **7**.

Monoterpenoid **2** ($C_{10}H_{20}O_3$, a colorless syrup, $[\alpha]_D^{23}$ −22.0°) showed an $[M+H]^+$ ion peak at *m/z* 189 in the positive FAB-MS. The ^{1}H- and ^{13}C-NMR spectral data (*vide* Experimental) for **2** revealed the presence of three *tert*-methyls, two methylenes, one hydroxylated methine, two hydroxylated quaternary carbons and a monosubstituted double bond. So, **2** was suggested to be an acyclic monoterpenoid triol having hydroxyl groups at C-3, C-7 and a double bond at C-1(2). From comparison of NMR data with those published, **2** was identified as 3,7-dimethyloct-1-ene-3,6,7-triol, which was obtained from the fruit of *Vitis vinifera*[3] and leaves of *Cunila spicata*.[4]

Monoterpenoid **3** ($C_{10}H_{22}O_4$, a colorless syrup, $[\alpha]_D^{23}$ +17.6°) showed an $[M+H]^+$ ion peak at *m/z* 207 in the positive FAB-MS. The ^{1}H-, ^{13}C- and ^{13}C–^{1}H correlation spectroscopy (COSY) NMR spectral data (Tables 1 and 2) revealed that **3** was an acyclic monoterpenoid tetrol having one *prim*-hydroxyl, two *sec*-hydroxyl and one *tert*-hydroxyl group. From the analysis of heteronuclear multiple-bond correlation (HMBC) spectral data of **3**, the position of the hydroxyl groups was indicated at C-1, C-2, C-6 and C-7, respectively. So, **3** was characterized as 3,7-dimethyloctane-1,2,6,7-tetrol.

Monoterpenoid **4** ($C_{10}H_{22}O_4$, a colorless syrup, $[\alpha]_D^{22}$ −18.1°) showed an $[M+H]^+$ ion peak at *m/z* 207 in the positive FAB-MS. Though **4** showed one peak in the HPLC, this was determined to be a mixture of two isomeric compounds (**4a** and **4b**, about 3 : 1) by the doubling of the signals of NMR. From the ^{1}H-, ^{13}C- and ^{13}C–^{1}H COSY NMR spectral data (Tables 1 and 2) and the result of HMBC experiment, **4** was concluded to be 3,7-dimethyloctane-1,2,6,7-tetrol as **3**. If optical isomers are not counted, 3,7-dimethyloctane-1,2,3,7-tetrol has four stereoisomers, and **3**, **4a** and **4b** must be three of them.

Monoterpenoid **5** ($C_{10}H_{20}O_5$, a colorless syrup, $[\alpha]_D^{22}$ +2.5°) showed an $[M+Na]^+$ ion peak at *m/z* 243 in the positive FAB-MS and an $[M-H]^-$ ion peak at *m/z* 219 in the negative FAB-MS. Though **5** showed one peak in the HPLC, this was also determined to be a mixture of two isomeric compounds (**5a** and **5b**, about 2 : 1) by the doubling of the signals of NMR. The ^{1}H-, ^{13}C- and ^{13}C–^{1}H COSY NMR spectral data (Table 2) showed that **5** was an acyclic monoterpenoid pentol having two *prim*-hydroxyl, two *sec*-hydroxyl, one *tert*-hydroxyl group and one terminal-methylene. The result of heteronuclear multiple bond correlation (HMBC) experiment revealed that the hydroxyl groups and the double bond were located at C-1, C-2, C-6, C-7, C-8 and C-3(10), respectively, and **5** was characterized as 3,7-dimethyloct-3(10)-ene-1,2,6,7,8-pentol.

Monoterpenoid **6** ($C_{10}H_{20}O_5$, a colorless syrup, $[\alpha]_D^{22}$ −25.4°) showed an $[M+H]^+$ ion peak at *m/z* 221 in the positive FAB-MS. The doubling of the signals of NMR showed that **6** was also a mixture of two epimeric compounds (**6a** and **6b**, about 1 : 1). The ^{1}H-, ^{13}C- and ^{13}C–^{1}H COSY NMR spectral data (Tables 1 and 2) showed that **6** has the same plane structure as **5**, and the results of HMBC experiment also confirmed this conclusion. If optical isomers are not counted, 3,7-dimethyloct-3(10)-ene-1,2,6,7,8-pentol has four stereoisomers, corresponding to **5a**, **5b**, **6a**, **6b**. The stereochemical relations among these compounds were deduced by comparison of their ^{13}C-NMR spectra with those of 2-methylbutanetetrols **8** (*erythro*) and **9** (*threo*), where C-5 in **9** appeared significantly downfield from that in **8**.[5] The ^{13}C chemical shift at C-9 of **5** was significantly downfield to that of **6**. Thus, the stereochemical relationship between C-6 and C-7 in **5** and **6** were considered to be *threo* for **5** and *erythro* for **6** as true in **9** and **8**. Then, **5a** and **5b**, **6a** and **6b** should be epimers at C-2, respectively. However, the absolute configuration of these pentols could not be determined from available data.

Monoterpenoid **7** ($C_{10}H_{20}O_4$, an amorphous powder, $[\alpha]_D^{23}$ +17.6°) showed an $[M+H]^+$ ion peak at *m/z* 205 in the positive FAB-MS and an $[M-H]^-$ ion peak at *m/z* 203 in the negative FAB-MS. The ^{1}H-, ^{13}C- and ^{13}C–^{1}H COSY NMR spectral data for **7** (*vide* Experimental) revealed the presence of two *tert*-methyls, four methylenes (one of them was hy-

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Table 1. ^{1}H-NMR Chemical Shifts of **3**—**6** (in Pyridine-d_5, 500 MHz)

	3	**4a**	**4b**
H_2-1	4.07 br d (6.0)	4.06 br d (6.0)	3.98 br d (6.5)
	—	4.07 br d (6.0)	4.02 br d (6.5)
H-2	4.14 ddd (3.5, 6.0, 6.0)	4.17 ddd (4.0, 6.0, 6.0)	3.98 ddd (3.5, 6.5, 6.5)
H-3	2.09 m	2.06 m	2.07 m
H_2-4	2.02 m	1.63 dddd (3.0, 6.0, 6.0, 13.0)	*ca.* 1.87 m
	2.07 m	2.48 dddd (4.5, 6.0, 10.0, 13.0)	2.20 m
H_2-5	1.82 m	1.77 dddd (3.0, 4.5, 10.0, 13.0)	1.92 m
	1.99 m	2.10 m	*ca.* 2.03 m
H-6	3.78 br d (9.5)	3.79 br d (10.0)	3.79 br d (10.0)
H_3-8	1.47[a] s	1.48[a] s	1.48[a] s
H_3-9	1.51[a] s	1.51[a] s	1.51[a] s
H_3-10	1.22 d (6.0)	1.23 d (6.5)	1.14 d (6.5)

	5a	**5b**	**6a**	**6b**
H_2-1	4.07 dd (7.5, 11.0)	4.07 dd (7.5, 11.0)	4.06 dd (7.5, 11.0)	4.06 dd (7.5, 11.0)
	4.17 dd (4.0, 11.0)	4.15 dd (4.0, 11.0)	4.16 dd (4.0, 11.0)	4.17 dd (4.0, 11.0)
H_2-2	4.76 dd (4.0, 7.5)	4.75 dd (4.0, 7.5)	4.77 dd (4.0, 7.5)	4.75 dd (4.0, 7.5)
H_2-4	2.67 ddd (6.0, 10.0, 15.0)	2.51 ddd (5.5, 10.0, 15.0)	2.65 ddd (6.0, 10.0, 15.0)	2.53 m
	2.91 ddd (4.5, 10.0, 15.0)	3.06 ddd (4.5, 10.5, 15.0)	2.91 ddd (4.5, 10.0, 15.0)	3.07 ddd (4.5, 10.5, 15.0)
H_2-5	2.12 dddd (2.0, 4.5, 10.0, 15.0)	2.09 dddd (2.0, 4.5, 10.5, 15.0)	2.11 m	2.11 m
	2.27 m	2.31 m	2.50 m	2.46 m
H-6	4.20 dd (2.0, 10.5)	4.19 dd (2.0, 10.5)	4.23 dd (1.5, 10.5)	4.21 dd (1.5, 10.5)
H_2-8	4.06 d (11.0)	4.07 d (11.0)	4.08 d (10.5)	4.08 d (10.5)
	4.15 d (11.0)	4.18 d (11.0)	4.26 d (10.5)	4.28 d (10.5)
H_3-9	1.55 s	1.56 s	1.58 s	1.58 s
H_2-10	5.20 br s	5.20 br s	5.21 br s	5.21 br s
	5.55 br s	5.53 br s	5.56 br s	5.53 br s

δ in ppm from TMS [coupling constants (J) in Hz are given in parentheses]. *a*) Assignments are interchangeable in each column.

Table 2. ^{13}C-NMR Chemical Shifts of **3**—**6**, **8** and **9**

	3	**4a**	**4b**	**5a**	**5b**	**6a**	**6b**	**9**[5]	**8**[5]
C-1	65.53	65.60	65.17	66.79 (65.0)	66.75 (65.0)	66.83 (65.0)	66.79 (64.9)	(67.2)	(67.3)
C-2	75.88	75.31	76.89	76.31 (76.3)	76.61 (76.3)	76.35 (75.9)	76.57 (75.9)	(74.9)	(75.0)
C-3	36.30	36.53	36.79	151.72 (148.7)	151.84 (148.9)	151.83 (148.8)	151.98 (148.9)	(76.0)	(75.9)
C-4	31.78	32.06	32.06	30.30 (29.7)	30.30 (29.7)	30.56 (29.4)	30.67 (29.4)	(62.9)	(63.0)
C-5	30.01	30.20	29.85	30.68 (29.8)	30.81 (30.1)	30.56 (29.7)	30.62 (29.8)	(20.4)	(19.4)
C-6	79.06	79.53	79.07	75.43 (75.5)	75.65 (75.9)	75.88 (75.5)	75.66 (75.2)		
C-7	72.73	72.76	72.71	75.02 (75.1)	75.02 (75.3)	74.74 (75.0)	74.77 (75.0)		
C-8	26.12[a]	26.03[a]	26.13[a]	68.59 (67.3)	68.59 (67.3)	69.08 (67.5)	69.10 (67.5)		
C-9	25.77[a]	25.82[a]	25.80[a]	21.35 (19.5)	21.35 (19.5)	20.09 (18.9)	20.11 (18.9)		
C-10	14.73	15.03	16.28	110.07 (112.6)	109.89 (112.5)	110.03 (112.6)	109.83 (112.6)		

δ in ppm from TMS. Solvent: pyridine-d_5, 125 MHz. ( ): D_2O, 25 MHz. *a*) Assignments are interchangeable in each column.

droxylated), two methines (one of them also hydroxylated) and two hydroxylated quaternary carbons. **7** was thus considered to be a menthane tetrol. From analysis of the HMBC and ^{1}H–^{1}H COSY NMR spectral data, the position of the hydroxyl groups was indicated at C-1, C-2, C-8 and C-9, respectively, and **7** was characterized as *p*-menthane-1,2,8,9-tetrol. The configuration of C-2 hydroxyl was suggested to be *axial* by a small coupling constant (br t, J=3.0 Hz) of H-2 signal. The configuration of H-4 was suggested to be *axial* by a large coupling constant between H-4 and H-3*ax*, H-5*ax* (13.0 Hz) and a small coupling constant between H-4 and H-3*eq*, H-5*eq* (5.0 Hz) in its ^{1}H-NMR spectrum. The observed cross peaks between H-3*eq* and H-5*eq*, and between H-2*eq* and H-6*eq* in its ^{1}H–^{1}H COSY NMR by long-range coupling (W type) also supported this conclusion (Fig. 1). The stereochemistry of **7** was confirmed to be 7—8 *trans* form by the observed cross peaks described in Fig. 1 in its nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum. From these facts, **7** was characterized as *trans-p*-menthane-1β,2α,8,9-tetrol, but its absolute configuration could not be decided from available data.

It is noteworthy that the tetra and penta hydroxy-monoterpenoids reported in this paper are the first examples from the natural source following our previous isolation of 3,7-dimethyloct-3(10)-ene-1,2,6,7-tetrol.[1]

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. MS were recorded with a JEOL HX-110 spectrometer, and in the case of FAB-MS, glycerol was used as matrix. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM A-500 spectrometer with tetramethylsilane as an internal standard, and chemical shifts were recorded

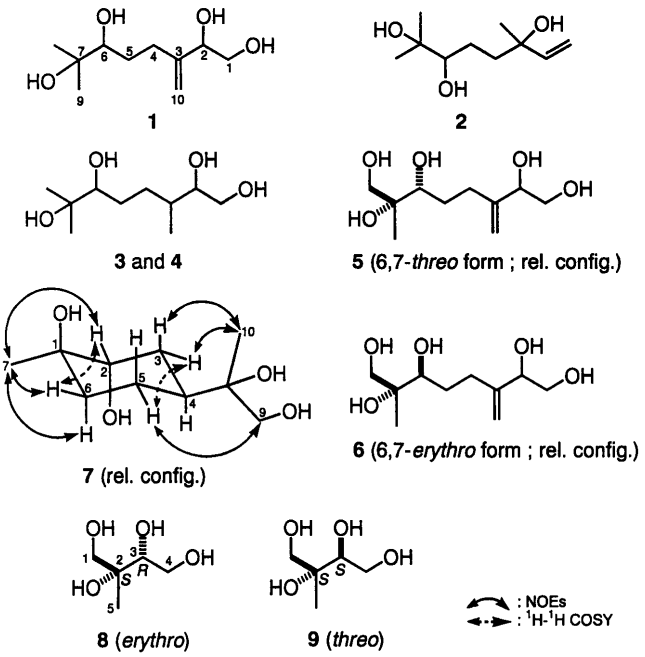

Fig. 1. Structures of **1** to **9**, and NOE and ^{1}H–^{1}H COSY Correlations of **7**

in δ value. The ^{13}C–^{1}H COSY, HMBC and NOESY spectra were obtained with the usual pulse sequence and data processing was performed with standard JEOL software. Column chromatography was carried out under TLC monitoring using Kieselgel 60 (70—230 mesh, Merck), Sephadex LH-20 (25—100 μm, Pharmacia), Lobar RP-8 column (Merck) and Amberlite XAD-II (Organo). TLC was performed on silica gel (Merck 5721) and spots were detected with *p*-anisaldehyde–H_2SO_4 reagent. HPLC separation was carried out on a JASCO chromatograph (980-system) with a JASCO RI-930 detector and ODS-3251-D (Senshu pak; column size, 8×250 mm) or carbohydrate analysis (Waters; column size, 3.9×300 mm).

Extraction and Separation of Monoterpenoid Polyols Fruit of *Cnidium monnieri* Cusson (She chuang zi) (1000 g) were purchased from Kinokuniya Chinese Medicine Pharmacy, Ltd. (lot. No. MU961715Y).[6]

They were extracted with methanol (5 l) at room temperature. After evaporation of the solvent, the residue (80.7 g) was suspended in water and extracted with ether. Removal of the solvent from both phases gave an ether-soluble (45.2 g) and an aqueous (35.5 g) residue. The aqueous residue was then extracted with hot methanol (300 ml) and the methanol insoluble portion (inorganic substance, 1.7 g) was removed. The hot methanol soluble fraction (33.8 g) was subjected to column chromatography on Amberlite XAD-II (H_2O→MeOH) to afford water eluate (21.2 g) and methanol eluate (10.6 g). 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. 2-3 (384 mg), **2** (16 mg) was isolated by silica gel [$CHCl_3$–MeOH (19 : 1)] column chromatography and HPLC [ODS, MeOH–H_2O (1 : 9)]. From fr. 2-6 (1.02 g), **1** (400 mg), **3** (21 mg) and **4** (23 mg) were obtained by a Lobar RP-8 column [CH_3CN–H_2O (3 : 17)] chromatography and HPLC [ODS, CH_3CN–H_2O (1 : 19)]. From fr. 2-7 (1.70 g), **5** (26 mg) and **6** (38 mg) were obtained by a Lobar RP-8 column [CH_3CN–H_2O (1 : 9)] chromatography and HPLC [ODS, CH_3CN–H_2O (3 : 197)]. The water eluate (21.2 g) was subjected to Sephadex LH-20 (MeOH) column chromatography to afford five fractions (frs. 1′ to 5′). Fraction 2′ (15.3 g) was purified by silica gel [$CHCl_3$–MeOH–H_2O (17 : 3 : 0.3→4 : 1 : 0.1→7 : 3 : 0.5)→MeOH] column chromatography to afford nine fractions. Fraction 2′-5 (1.23 g) was subjected to a Lobar RP-8 column [CH_3CN–H_2O (1 : 99)] and HPLC [carbohydrate analysis, CH_3CN–H_2O (19 : 1)] to give a fraction containing **7** (18 mg). This fraction was acetylated with Ac_2O and pyridine, and the acetylated fraction was purified by HPLC [ODS, MeOH–H_2O (3 : 7)] to afford acetylated **7**, then it was hydrolyzed to **7** (7 mg) by heating in a water bath with 5% NH_4OH–MeOH for 3 h.

3,7-Dimethyloct-1-ene-3,6,7-triol (2) A colorless syrup, $[\alpha]_D^{23}$ −22.0° (*c*=1.2, MeOH).

3,7-Dimethyloctane-1,2,6,7-tetrol (3) A colorless syrup, $[\alpha]_D^{23}$ +17.6° (*c*=0.7, MeOH). Positive FAB-MS *m/z*: 413 $[2M+H]^+$, 245 $[M+K]^+$, 229 $[M+Na]^+$, 207.1629 $[M+H]^+$ (base, Calcd for $C_{10}H_{23}O_4$: 207.1596).

3,7-Dimethyloctane-1,2,6,7-tetrol (4a and 4b) A colorless syrup, $[\alpha]_D^{22}$ −18.1° (*c*=0.4, MeOH). Positive FAB-MS *m/z*: 413 $[2M+H]^+$, 245 $[M+K]^+$, 229 $[M+Na]^+$, 207.1616 $[M+H]^+$ (base, Calcd for $C_{10}H_{23}O_4$: 207.1596).

(6,7-*threo*)-3,7-Dimethyloct-3(10)-ene-1,2,6,7,8-pentol (5a and 5b) A colorless syrup, $[\alpha]_D^{22}$ +2.5° (*c*=1.0, MeOH). Positive FAB-MS *m/z*: 243.1234 $[M+Na]^+$ (Calcd for $C_{10}H_{20}O_5Na$: 243.1208), 185 $[M-2H_2O+H]^+$ (base), 167 $[M-3H_2O+H]^+$, 131 $[M-5H_2O+H]^+$. Negative FAB-MS *m/z*: 219 $[M-H]^-$ (base).

(6,7-*erythro*)-3,7-Dimethyloct-3(10)-ene-1,2,6,7,8-pentol (6a and 6b) A colorless syrup, $[\alpha]_D$ −25.4° (*c*=0.7, MeOH). Positive FAB-MS *m/z*: 259 $[M+K]^+$, 243. $[M+Na]^+$ (base), 221.1414 $[M+H]^+$ (Calcd for $C_{10}H_{21}O_5$: 221.1389), 167 $[M-3H_2O+H]^+$, 131 $[M-5H_2O+H]^+$. Negative FAB-MS *m/z*: 219 $[M-H]^-$ (base).

***trans-p*-Menthane-1β,2α,8,9-tetrol (7)** An amorphous powder, $[\alpha]_D^{23}$ −12.0° (*c*=0.3, MeOH). Positive FAB-MS *m/z*: 205.1458 $[M+H]^+$ (Calcd for $C_{10}H_{21}O_4$: 205.1440), 169 $[M-2H_2O+H]^+$ (base). Negative FAB-MS *m/z*: 203 $[M-H]^-$ (base). ^{1}H-NMR (pyridine-d_5) δ: 1.49 (3H, s, H_3-10), 1.71 (3H, s, H_3-7), 1.90 (1H, br dddd, *J*=3.0, 4.5, 5.0, 13.0, H-5*eq*), 1.91 (1H, br ddd, *J*=3.0, 3.0, 13.0 Hz, H-6*eq*), 2.15 (1H, ddd, *J*=3.0, 13.0, 13.0 Hz, H-5*ax*), 2.26 (1H, ddd, *J*=4.5, 13.0, 13.0 Hz, H-6*ax*), 2.53 (1H, br ddd, *J*=3.0, 5.0, 13.0, H-3*eq*), 2.62 (1H, ddd, *J*=3.0, 13.0, 13.0 Hz, H-3*ax*), 2.76 (1H, dddd, *J*=5.0, 5.0, 13.0, 13.0 Hz, H-4), 3.94 (1H, d, *J*=10.5 Hz, H-9a), 4.02 (1H, d, *J*=10.5 Hz, H-9b), 4.27 (1H, br t, *J*=3.0 Hz, H-2). ^{13}C-NMR (pyridine-d_5) δ: 21.57 (C-10), 23.30 (C-5), 28.86 (C-7), 30.47 (C-3), 34.88 (C-6), 37.87 (C-4), 69.21 (C-9), 70.87 (C-1), 74.34 (C-2), 74.44 (C-8).

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References and Notes

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