New Monoterpenoid and Hemiterpenoid Tetraol of the Crude Drug "She chuang zi"

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From the methanol extract of the crude drug "She chuang zi" [Japanese name "Jyashoshi", the fruit of *Cnidium monnieri* CUSSON (Umbelliferae)], two new substances were obtained as the main constituents of the water soluble portion. From the results of spectral investigations, they were characterized as 3,7-dimethyl-1,2,6,7-tetrahydroxyoct-3(10)-ene (obtained as a mixture of two epimers) and 3-methyl-1,2,3,4-tetrahydroxy-butane, respectively. It is interesting that such monoterpenoid and hemiterpenoid tetraols were obtained from the water soluble portion of an Umbelliferous crude drug which contained an abundance of essential oil and coumarin derivatives.

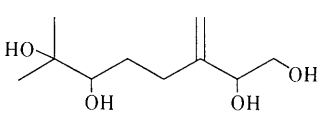
Keywords crude drug "She chuang zi"; monoterpenoid tetraol; hemiterpenoid tetraol; *Cnidium monnieri* fruit; Umbelliferae; water soluble portion

A crude drug "She chuang zi" (known in Japanese as "Jyashoshi") is a principal Chinese medicament used as a tonic, anti-uredo and as a drug for the treatment of eczema. She chuang zi is considered to be prepared from the fruits of *Cnidium monnieri* CUSSON (Umbelliferae) which is distributed in most regions of China.[1)]

Among the constituents of *C. monnieri* fruit, isolated are the essential oil components (−)-pinene, (−)-camphene, bornyl isovalerate and isoborneol[2)] and the coumarin derivatives osthol, archangelicin, bergapten, edultin, isopimpinellin, columbianetin, imperatorin and others.[1,3)] Recently, Yahara, *et al.* reported the finding of two new monoterpenoid derivatives 3,7-dimethyl-3β,8-dihydroxy-oct-1,6-diene 3-*O*-β-D-glucopyranoside (cnidinoside C, **3**) and 3,7-dimethyl-6β-hydroxy-oct-1-en-3(*S*),7-oxide (cnidiol C) with three benzofuran derivatives.[4)]

The present study was done in the hope of isolating the main constituents of the water soluble portion from the methanol extract of this crude drug.

The methanolic extract of commercial She chuang zi was partitioned between ethyl acetate and water. The aqueous layer was evaporated, the residue was heated under reflux with methanol, and the methanol insoluble portion (crystal of inorganic compounds) was removed. The methanol soluble fraction was chromatographed on Amberlite XAD-II and gave a water eluate fraction and a methanol eluate fraction. The methanol eluate fraction was subjected to a combination of Sephadex LH-20, silica gel and Lobar RP-8 column chromatographies to give monoterpenoid I (**1**) and **3**. From the water eluate fraction, a mixture of hemiterpenoid II (**2**) and glycerol was obtained by a combination of Sephadex LH-20 and silica gel chromatographies. This mixture was acetylated and the acetylated mixture was chromatographed on silica gel to isolate **2**-triacetate. Then, **2**-triacetate was alkaline hydrolyzed by 10% NH_4OH–methanol to **2**.



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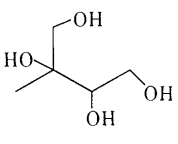

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Chart 1

Monoterpenoid I (**1**), a colorless oil, showed one peak by high-performance liquid chromatography (HPLC), but this was determined to be a mixture of two epimeric compounds [**1a** (main) and **1b** (minor), about 4:1] by nuclear magnetic resonance (NMR) speculation. Its chemical ionization mass spectrum (CI-MS) exhibited a M+H ion peak at *m/z* 205 with fragment peaks at *m/z* 187 $[M-H_2O+H]^+$, *m/z* 169 $[M-2H_2O+H]^+$ and *m/z* 151 $[M-3H_2O+H]^+$. The carbon-13 (^{13}C)-NMR (Table I) spectral data of **1** showed ten strong signals with eight weaker signals which were due to the minor epimeric compound. The proton (^1H)-NMR (Table II) signals showed the presence of two tertiary methyl, two methylene, two hydroxymethylene, one hydroxymethyl and one exo-methylene groups. Further, acetylation of **1** with Ac_2O and pyridine gave **1**-triacetate (**4**), and its ^1H- and ^{13}C-NMR (Tables I and II) supported these results. From the analysis of ^1H–^{13}C shift correlated spectroscopy (COSY), **1** was concluded to be a mixture of two acyclic monoterpenoid epimers which has two tertiary methyl, two methylene, two secondary alcohol, one primary alcohol, one tertiary alcohol and one *exo*-methylene groups.

The structure of **1** was determined by the ^1H-detected heteronuclear multiple bond connectivity (HMBC) spec-

TABLE I. ^{13}C-NMR Assignments of **1**, **2**, **4** and **5**

	1	4	2	5
C-1	66.71 [66.74]	64.39 [64.36]	63.60	62.84
C-2	76.51 [76.27]	73.71 [73.50]	75.73	72.66
C-3	151.80 [151.70]	143.83 [143.51]	74.54	72.17
C-4	30.50 [30.45]	27.69 [27.25]	68.42	68.16
C-5	30.93 [30.78]	29.46 [29.40]	20.24	19.98
C-6	78.62 [78.36]	79.28 [78.92]		
C-7	72.71 [72.63]	72.35 [72.32]		
C-8	25.89[a)]	25.13 [25.28][a)]		
C-9	25.93[a)]	26.48 [26.25][a)]		
C-10	109.86 [110.07]	113.22		
OAc		20.79, 170.05		20.78, 170.14
		21.03, 170.71		20.81, 170.96
		21.06, 171.18		20.89, 171.04

Chemical shifts written in [ ] were alloted to the minor epimeric component. Solvent: C_5D_5N for **1** and **2**, $CDCl_3$ for **4** and **5**. *a*) Assignment may be reversed.

TABLE II. ^{1}H-NMR Assignments of **1** and **4**

	1	**4**
1-H_2	4.064 (dd, J=8.5, 10.0 Hz), 4.163 (br d, J=10.0 Hz)	4.099 (dd, J=8.0, 11.9 Hz) [4.083 (dd, J=8.0, 11.9 Hz)], 4.245 (dd, J=3.3, 11.9 Hz)
2-H	4.742 (m)	5.363 (dd, J=3.3, 8.0 Hz) [5.333 (dd, J=3.3, 8.0 Hz)]
4-H_2	2.497 (ddd, J=6.1, 10.4, 15.9 Hz) [2.642 (ddd, J=7.0, 9.8, 15.9 Hz)], 3.036 (ddd, J=4.2, 10.4, 15.2 Hz) [2.874 (ddd, J=4.6, 9.8, 15.2 Hz)]	1.668—1.940 (4H, m)
5-H_2	1.948 (m), 1.497 (m)	
6-H	3.801 (m)	4.834 (dd, J=2.6, 10.2 Hz)
8-H_3	1.416 [1.453],[a]	1.206,[a]
9-H_3	1.497 [1.489][a]	1.212[a]
10-H_2	5.188 (br s), 5.530 (br s)	5.006 (br s), 5.127 (br s)
OAc		2.058, 2.094 [2.099], 2.119 [2.123]

Chemical shifts written in [ ] were alloted to the minor epimeric component. *a*) Assignment may be reversed.

TABLE III. ^{1}H-NMR Assignments of **2** and **5**

	2	**5**
1-H_2	4.277 (dd, J=7.0, 11.0 Hz), 4.504 (dd, J=3.8, 11.0 Hz)	4.143 (dd, J=8.2, 12.2 Hz), 4.564 (dd, J=2.7, 12.2 Hz)
2-H	4.457 (dd, J=3.8, 7.0 Hz)	5.198 (dd, J=2.7, 8.2 Hz)
4-H_2	4.087 (d, J=10.8 Hz), 4.200 (d, J=10.8 Hz)	3.908 (d, J=11.5 Hz), 4.140 (d, J=11.5 Hz)
5-H_3	1.611	1.244
OAc		2.043, 2.090, 2.105

trum. Analysis of this spectrum revealed the sequence of ten carbons, C1–C2–C3(C10)–C4–C5–C6–C7–C8(C9), and the positions of four hydroxyl groups were located as C-1, C-2, C-6 and C-7. So, **1** was characterized as 3,7-dimethyl-3(10)-octene-1,2,6,7-tetraol. Previous attempts to separate these two epimers had not been successful.

Hemiterpenoid II (**2**), a colorless oil, $[\alpha]_D$ +13.5°, showed a M+H ion peak at 137 in the CI-MS. Its EI-MS exhibited a weak M+H ion peak with fragment peaks at m/z 119 $[M+H-H_2O]^+$, m/z 105 $[M-CH_2OH]^+$ and m/z 101 $[M+H-2H_2O]^+$. The ^{13}C-NMR spectrum of **2** showed five signals (Table I) and ^{1}H-NMR spectral data of **2** (Table III) showed the presence of one tertiary methyl, two hydroxymethyl and one hydroxymethylene groups. Acetylation of **2** gave **2**-triacetate (**5**) and its NMR spectral data (Tables I and III) supported those results. From these data, **2** was concluded to be a hemiterpenoid which has two primary alcohol, one secondary alcohol, one tertiary alcohol and one tertiary methyl groups, and it was therefore characterized as 3-methyl-1,2,3,4-tetrahydroxy-butane.

Though the configuration of C-2, C-6 of **1** and C-2, C-3 of **2** were not defined, this is the first report of isolation of acyclic monoterpenoid tetraol and hemiterpenoid tetraol, and **2** is believed to be the most polar free terpenoid isolated from the plant.

She chuang zi contains essential oil equalling about 1.3% of its total weight,[2] the main constituents of which are the monoterpenoids (−)-pinene, (−)-camphene, isovalerate and isoborneol. This crude drug also contains coumarin derivatives which have an angeloyl unit (osthol, archangelicin). To date, however, polyhydroxy terpenoid has not been detected.

It is worthy of note that monoterpenoid and hemiterpenoid tetraol such as **1** and **2** were found to be the main constituents from the water soluble portion of this Umbelliferous crude drug which contains an abundance of essential oil and coumarin derivatives.

Experimental

Optical rotations were measured on a JASCO DIP-140 automatic polarimeter at 22—25 °C. MS were recorded with a JEOL JMS D-300 and HX-110 spectrometer. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL GSX-500 spectrometer in C_5D_5N or $CDCl_3$ with tetramethylsilane as an internal standard, and chemical shifts were recorded in δ value. ^{1}H–^{13}C COSY and HMBC were obtained with the usual pulse sequence and data processing was performed with the standard JEOL software. Column chromatography was carried out under TLC monitoring using Kieselgel 60 (70—230 mesh, Merck), Silica Woelm TSC (silica gel for dry column, Woelm), Sephadex LH-20 (25—100 μm, Pharmacia) and Amberlite XAD-II (Organo). TLC was performed on Kieselgel 60 (Merck 5721) and spots were located by using an anisaldehyde reagent. HPLC separation was carried out on a JASCO liquid chromatograph (880-system) with a JASCO 830 RI detector and ODS-3251-D [Senshu pack; column size, 8 × 250 mm].

Extraction and Separation of the Constituents of the Crude Drug She chuang zi She chuang zi (Japanese name Jyashyoshi) was purchased from Kinokuniya Chinese Medicine Pharmacy, Ltd. (lot. No. MU961715Y).

The crude drug (970 g) were extracted with methanol (5 l) at room temperature. After evaporation of solvent, the residue (88.0 g) was partitioned into ethyl acetate and water. Removal of solvent from both phases gave an ethyl acetate (51.1 g) and an aqueous (26.9 g) residue. The aqueous residue was then extracted with hot methanol (200 ml) and the methanol insoluble portion (1.8 g) removed. The hot methanol soluble fraction (25.1 g) was subjected to column chromatography on Amberlite XAD-II (H_2O→MeOH). The methanol eluate (8.0 g) was repeatedly purified by column chromatography on Sephadex LH-20 (MeOH), silica gel ($CHCl_3$: MeOH : H_2O = 8.5 : 1.5 : 0.1→7 : 3 : 0.5, $CHCl_3$: acetone = 1 : 1) and a Lobar RP-8 column (50% MeOH) and finally **1** (0.40 g) and **3** (0.19 g) were obtained. Meanwhile, the water eluate (17.1 g) was subjected to column chromatography on Sephadex (MeOH) and silica gel ($CHCl_3$: MeOH : H_2O = 8 : 2 : 0.2→7 : 3 : 0.5). The fraction containing **2** and glycerol (2.47 g) was acetylated with Ac_2O and pyridine, and the acetylated fraction was purified by repeated silica gel column chromatography (*n*-hexane : EtOAc = 3 : 2) to afford **2**-triacetate (**5**, 1.72 g) and glycerol triacetate (0.85 g). These were then hydrolyzed to **2** (0.83 g) and glycerol (0.35 g) by heating in a water bath with 10% NH_4OH–MeOH for 2 h, respectively.

3,7-Dimethyl-1,2,6,7-tetrahydroxy-oct-3(10)-ene (1) Colorless oil. HPLC (t_R): 5.40 min (solvent, CH_3CN : H_2O = 1 : 9, flow rate, 3 ml/min). CI-MS m/z: 205 $[M(C_{10}H_{20}O_4)+H]^+$, 187 $[M-H_2O+H]^+$, 169 ($[M-2H_2O+H]^+$, base), 151 $[M-3H_2O+H]^+$. ^{1}H-NMR (C_5D_5N) δ: Table II. ^{13}C-NMR (C_5D_5N) δ: Table I.

1-Triacetate (4) Colorless oil. HPLC (t_R): 13.79 min (solvent, CH_3CN : H_2O = 1 : 2; flow rate, 5 ml/min). ^{1}H-NMR ($CDCl_3$) δ: Table II. ^{13}C-NMR ($CDCl_3$) δ: Table I.

3-Methyl-1,2,3,4-tetrahydroxy-butane (2) Colorless oil, $[\alpha]_D^{25}$ +13.5° (c = 1.6, methanol). CI-MS m/z: 137 $[M(C_5H_{12}O_4)+H]^+$, 119 $[M-H_2O+H]^+$, 101 ($[M-2H_2O+H]^+$, base). EI-MS m/z: 119 $[M-H_2O+H]^+$, 105.0552 ($[M-CH_2OH]^+$, base), 101.0594 $[M-2H_2O+H]^+$. ^{1}H-NMR (C_5D_5N) δ: Table III. ^{13}C-NMR (C_5D_5N) δ: Table I.

2-Triacetate (5) Colorless oil, $[\alpha]_D^{22}$ +19.1° (c = 1.5, methanol). ^{1}H-NMR ($CDCl_3$) δ: Table III. ^{13}C-NMR ($CDCl_3$) δ: Table I.

3,7-Dimethyl-3β,8-dihydroxy-oct-1,6-diene 3-*O*-β-D-Glucopyranoside (3) Amorphous. CI-MS m/z: 333 $[M(C_{16}H_{28}O_7)+H]^+$, 315 $[M-H_2O+H]^+$, 297 $[M-2H_2O+H]^+$, 163 $[C_6H_{10}O_5+H]^+$, 153 ($[C_{10}H_{18}O_2-H_2O+H]^+$, base). ^{1}H-NMR ($C_5D_5N$) δ: 1.542 (3H, s, 10-H_3), 1.850 (3H, s, 9-H_3), 4.263, 4.276 (each 1H, d, J=9.3 Hz, 8-H_2), 4.972 (1H, d, J=7.6 Hz, anomeric proton of glucose), 5.195 (1H, dd, J=1.5, 11.0 Hz, 1-H_a), 5.323

(1H, dd, $J=1.5$, 6.7 Hz, 1-H_b), 5.794 (1H, t, $J=7.3$ Hz, 6-H), 6.397 (1H, dd, $J=6.7$, 11.0 Hz, 2-H). ^{13}C-NMR (C_5D_5N) δ: 13.99 (C-10), 22.63 (C-5), 24.20 (C-9), 40.69 (C-4), 68.12 (C-8), 79.98 (C-3), 113.97 (C-1), 125.02 (C-6), 136.34 (C-7), 114.47 (C-2), glucosyl [62.94, 71.79, 75.28, 78.15, 78.83, 99.46].

Glycerol Colorless oil. ^{13}C-NMR (C_5D_5N) δ: 64.58 (t × 2), 73.86 (d).

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References and Notes

1) Based on the results of analysis of coumarin constituents (in which osthol and imperatorin were isolated as the main substances), the original plant of this commercial sample was identified as the fruit of *C. monnieri*; *a*) G. Honda, M. Tabata, K. Baba, M. Kozawa, *Shoyakugaku Zasshi*, **38**, 221 (1984); *b*) K. Baba, F. Hamasaki, Y. Tabata, M. Kozawa, G. Honda, M. Tabata, *ibid.*, **39**, 282 (1985).
2) T. Namba, "Gensyoku Wakanyaku Zukan I," Hoikusya, Osaka, 1980, pp. 233—235.
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