

## Isolation of Triterpenoid and Phenylpropanoid from *Codonopsis ussuriensis*

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**Abstract** □ From the roots of *Codonopsis ussuriensis* (Rupr. et Maxim) Hemsley (Campanulaceae), taraxerol (mp. 280-282°), syringin (mp. 192°) and a new phenylpropanoid, ussurienoside I (syringin-3'-hydroxy-3'-methyl glutarate, mp. 102-104°) were isolated.

**Keywords** □ *Codonopsis ussuriensis*, Campanulaceae, taraxerol, syringin, ussurienoside I

*Codonopsis ussuriensis* (Rupr. et Maxim) Hemsley is a plant of the family Campanulaceae, which is distributed throughout Korea, Japan, and China. The pharmacological actions and components of this plant, including another *Codonopsis* spp. have been studied extensively by several researchers<sup>1-4</sup>. In the previous paper<sup>5</sup> we reported that the isolation and identification of a new compound, 4-(3-ethoxy-1-propenyl)-2,6-dimethoxyphenyl- $\beta$ -D-glucoside.

This paper deals with the isolation and structure elucidation of a new phenylpropanoid together with identification of the known phenylpropanoid and triterpenoid from the rhizome of the title plant.

### EXPERIMENTAL METHODS

#### Instrumental

Melting point was recorded on METTLER FP 62. UV spectra were measured on Shimadzu UV-visible recording spectrophotometer UV-240 Graphicord. <sup>13</sup>C-NMR and <sup>1</sup>H-NMR spectra were obtained on Bruker AMX-500 spectrometer. IR spectra were measured on PERKIN-ELMER 1420 Ratio recording spectrophotometer and Bruker Model IFS 66. Mass spectra were taken on high resolution Mass (Low EI<sup>+</sup>) VG 70 VSEQ.

#### Isolation

*Codonopsis ussuriensis* was collected in June (1990)

at Kwang-Neung, Kyungkido, Korea. Dried root (1.18 KG) was extracted with methanol (4h, 3 times). The methanol extract was evaporated in vacuum and fractionated with hexane, diethylether and then *n*-butanol.

After defating with hexane, TLC of the ether fraction revealed four major spots upon vanillin sulphuric acid spray (hexane:EtOAc=10:1). When it was subjected to column chromatography on silica gel (Merck, #7734) with a solvent system of hexane and ethylacetate (gradient), compound 1 was obtained at hexane/EtOAc (20:1).

TLC chromatogram of the *n*-butanol fraction on a silica gel plate (CHCl<sub>3</sub>:CH<sub>3</sub>OH:H<sub>2</sub>O=9:6:1.2) revealed five major spots upon vanillin sulphuric acid spray. Among them two kinds of phenylpropanoids were isolated in the pure state by the fractional procedure on the lipophillic Sephadex LH-20 column followed by repeated silica gel chromatography. Compounds 2 and 3 were obtained at CHCl<sub>3</sub>/MeOH (10:1).

#### Compound 1

Colorless crystal, mp. 280-282°; IR  $\nu_{max}$  cm<sup>-1</sup> 3480 (OH), 1642, 813 (trisubstituted double bond); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm) 0.81 (3H, s, CH<sub>3</sub>), 0.83 (3H, s, CH<sub>3</sub>), 0.93 (6H, s, 2CH<sub>3</sub>), 0.94 (3H, s, CH<sub>3</sub>), 0.95 (3H, s, CH<sub>3</sub>), 0.99 (3H, s, CH<sub>3</sub>), 1.10 (3H, s, CH<sub>3</sub>), 3.20 (1H, dd, *J*=5 & 11 Hz, 3 $\alpha$ -H), 5.54 (1H, dd, *J*=4 & 8 Hz); Mass (EI) 426 (M<sup>+</sup>, 31), 411 (M<sup>+</sup>-

CH<sub>3</sub>, 18), 302 (50.5), 287 (302-CH<sub>3</sub>, 30.5), 204 (D/E ring, 100), 189 (204-CH<sub>3</sub>, 24.5).

### Compound 2

Colorless crystal. mp. 192°; UV  $\lambda_{max}$  (MeOH) nm 265; IR  $\nu_{max}$  cm<sup>-1</sup> 3560, 3391 (OH), 3030, 1650, 1589 (olefin, aromatic); <sup>1</sup>H-NMR (CD<sub>3</sub>OD)  $\delta$  (ppm) 3.86 (6H, s, 2×OCH<sub>3</sub>), 4.21 (2H, dd,  $J=5.5$  & 1.2 Hz, -CH=CH-CH<sub>2</sub>), 6.33 (1H, dt,  $J=15.8$  & 5.5 Hz, -CH=CH-CH<sub>2</sub>), 6.55 (1H, dt,  $J=15.8$  & 1.2 Hz, -CH=CH-CH<sub>2</sub>), 6.75 (2H, s, aromatic 2H); <sup>13</sup>C-NMR (CD<sub>3</sub>OD)  $\delta$  (ppm) 57.2, 62.6, 63.7, 71.4, 75.8, 77.9, 78.4, 105.5, 105.6, 130.2, 131.3, 135.4, 136.0, 154.4; Mass(EI) 285 (6.18), 254 (45), 211 (100), 192 (14), 183 (23).

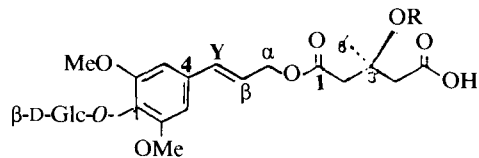
### Compound 3

Colorless crystal. mp. 102-104°; UV  $\lambda_{max}$  (MeOH) nm 267; IR  $\nu_{max}$  cm<sup>-1</sup> 3400-2400 (br, COOH), 1732 (C=O, 1653, 1587 (olefin, aromatic), 1244, 1123 (C-O); <sup>1</sup>H-NMR (CD<sub>3</sub>OD)  $\delta$  (ppm) 1.33 (3H, s), 2.34 (1H, d,  $J=15.3$  Hz), 2.52 (1H, d,  $J=15.3$  Hz), 2.65 (2H, s), 3.90 (6H, s, 2×OCH<sub>3</sub>), 4.73 (2H, dd,  $J=6.1$  & 1.2 Hz, -CH=CH-CH<sub>2</sub>), 6.17 (1H, dt,  $J=6.1$  & 15.9 Hz, -CH=CH-CH<sub>2</sub>), 6.52 (1H, dt,  $J=15.9$  & 1.2 Hz, -CH=CH-CH<sub>2</sub>), 6.66 (2H, s, aromatic 2H); <sup>13</sup>C-NMR (CD<sub>3</sub>OD)  $\delta$  (ppm) Table I; Mass (EI) 279 (8), 253 (52), 211 (47), 192 (14), 168 (12).

## RESULTS AND DISCUSSION

Compound **1** was a colorless crystal and its melting point was 280-282°. In Liebermann-Burchard test, it indicated positive reaction. IR spectrum revealed the presence of hydroxyl group (3480 cm<sup>-1</sup>) and trisubstituted double bond (1642, 813 cm<sup>-1</sup>). The <sup>1</sup>H-NMR spectrum showed eight angular methyl groups at  $\delta$  0.81-1.10, olefinic proton of trisubstituted double bond at  $\delta$  5.54 (1H, dd,  $J=4$  & 8 Hz), and the presence of hydroxyl group at  $\delta$  3.20 (1H, dd,  $J=5$  & 11 Hz). Thus, compound **1** is supposed to be pentacyclic triterpenoid which has a hydroxyl group bonded by  $\beta$ -position at C-3. The splitting pattern of olefinic proton indicated that compound **1** is not olean-12-ene but taraxer-14-ene. In Mass spectrum the structure of compound **1** was obvious. Molecular ion peak ( $m/z$  426) and several characteristic fragment ion peak ( $m/z$  302, 287, 269, 204, 189) of its retro-Diels Alder reaction indicated

**Table I.** <sup>13</sup>C-NMR data of compound **3** comparing with that of tangshenoside **1**



compound **3**; R=H

tangshenoside **1**; R=- $\beta$ -D-Glc' (G'-1)

Carbon number	Compound 3	Tangshenoside 1	HMG*
C-1	134.9	134.4	
2, 6	154.4	153.3	
3, 5	105.8	105.1	
4	134.8	134.2	
-OCH <sub>3</sub>	57.3	57.0	
$\alpha$	66.1	66.3	
$\beta$	124.8	124.3	
$\gamma$	136.2	134.4	
1'	180.2	176.7	175.8
2'	48.0	47.4	46.0
3'	71.1	78.2	70.7
4'	47.3	44.3	46.0
5'	172.9	173.3	175.8
6'	28.0	24.8	27.2
G-1	105.6	103.8	
2	75.8	74.5	
3	78.4	77.0	
4	71.4	70.3	
5	77.8	76.6	
6	62.3	61.5	
G'-1		97.2	
2		74.0	
3		76.6	
4		70.0	
5		76.5	
6		61.2	

the form of taraxer-14-ene. Based on these results of above mentioned, and the comparison of the data<sup>1,2,6)</sup> which were reported previously, compound **1** was identified as the taraxerol.

Compound **2** was colorless crystal and its melting point was 192°. The presence of aromatic group was shown in UV ( $\lambda_{max}^{MeOH}$  265 nm) and IR spectrum (3030, 1650, 1589 cm<sup>-1</sup>). In <sup>1</sup>H-NMR the signals of 3.86 ppm (s, 6H) indicated the two symmetrical methoxyl groups which were bonded to benzene

ring directly. Thus, compound **2** has an aromatic group to which two methoxyl radicals are bonded symmetrically. In  $^1\text{H-NMR}$  spectrum the multiple peak of  $\delta$  3.22-3.80 ppm and the anomeric proton peak ( $\delta$  4.86, d,  $J=7.5$  Hz) revealed the presence of sugar. The hydroxyl groups were shown in IR spectrum ( $3560, 3391\text{ cm}^{-1}$ ).  $^1\text{H-NMR}$  spectrum showed the signal of two symmetrical protons at 6.75 (s, 2H). The signals of  $\delta$  4.21 (dd,  $J=5.5$  & 1.2 Hz, 2H), 6.33 (dt,  $J=15.8$  & 5.5 Hz, 1H) and 6.55 (dt,  $J=15.8$  & 1.2 Hz, 1H) indicate the presence of  $-\text{CH}=\text{CH}-\text{CH}_2\text{O}-$  group. From the  $J$  value, we can predict that the proton 1 and 2 have trans type. Thus, benzene ring of compound **2** has sugar linked by  $\beta$ -position at C-1, propenyl group at C-4, two symmetrical methoxyl radicals and protons at C-2, 6 or C-3, 5. From the above findings, we expected that the structure of compound **2** should be similar to that of syringin. And the spectral data of compound **2** was compared with those of syringin<sup>7)</sup>. Therefore compound **2** was identified as syringin, consequently.

Compound **3** was obtained in the form of colorless crystal and its melting point was  $102-104^\circ$ . Its UV ( $\lambda_{\text{max}}^{\text{MeOH}}$  267 nm) and  $^1\text{H-NMR}$  spectrum ( $\delta$  6.66) showed the presence of aromatic group. In  $^1\text{H-NMR}$  the multiple peak of 3.2-3.9 ppm and the anomeric proton peak ( $\delta$  4.86, d,  $J=7.5$  Hz) revealed the presence of sugar. The signals at 105.6, 75.8, 78.4, 71.3, 77.8, 62.3 ppm in the  $^{13}\text{C-NMR}$  spectrum showed the carbons of glucose.  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  data showed that compound **3** has an  $-\text{CH}=\text{CH}-\text{CH}_2\text{O}-$  group as compound **2** has. Different from compound **2**, the  $\alpha\text{-H}$  peak of compound **3** revealed down field shift. So we can predict that the group which decreases the electron density is bonded to the proton **3**. In IR spectrum, carbonyl group was shown at  $1732\text{ cm}^{-1}$  and carboxyl group at  $3400-2400$  (br),  $1123\text{ cm}^{-1}$ . The comparison of  $^{13}\text{C-NMR}$  of compound **3** with those of known compound, tangshenoside I<sup>8)</sup> showed the presence of 3'-hydroxy-3'-methyl glutarate. Based on the above observation, the structure of compound **3** can be formulated as syringin linked 3'-hydroxy-3'-methyl glutarate. Furthermore, TLC of MeOH extract of the

sample at room temperature verified that compound **3** is not artificial compound but natural one. Due to the small amount, it was unable to hydrolyze or to make methyl ether of compound **3**. However, comparisons of  $^{13}\text{C-NMR}$  data of compound **3** ( $\delta$  71.1 and 28.0) with the chemical shifts of C-3' and C-6' of HMG ( $\delta$  70.7 and 27.2) as the above Table I indicated that the stereochemistry of C-3' was the same as that of HMG. Therefore, we proposed compound **3**, which has not been reported so far, as syringin-3'-hydroxy-3'-methyl glutarate and named as ussurienoside I.

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