Two New Phenylpropanoid Derivatives from *Codonopsis tangshen* OLIV.

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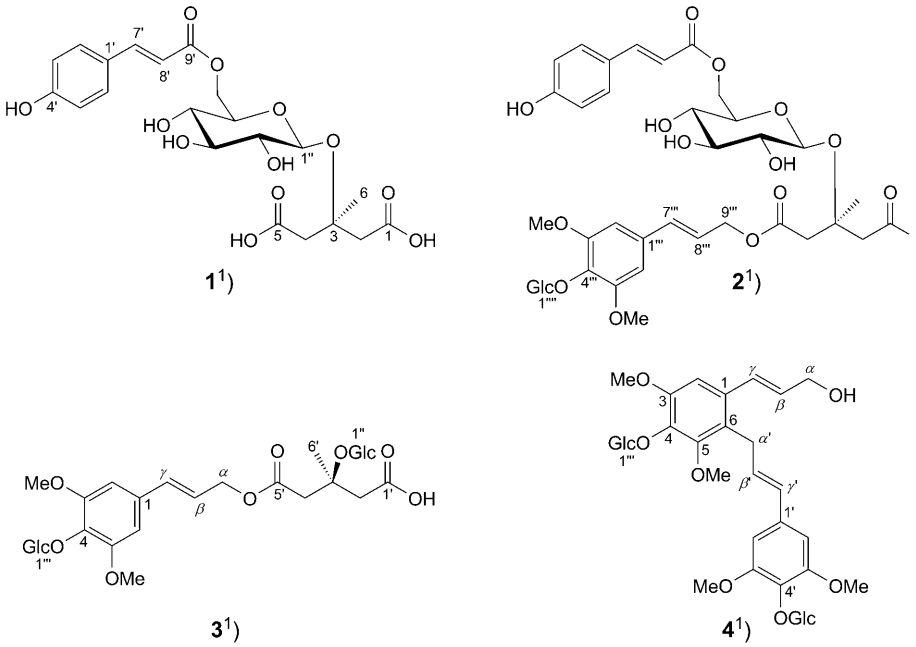
Two new 3-hydroxy-3-methylglutaric acid derived phenylpropanoid glucosides, tangshenoside V (**1**) and tangshenoside VI (**2**), were isolated from the roots of *Codonopsis tangshen* OLIV., along with the two known compounds tangshenoside I (**3**) and tangshenoside III (**4**). Their structures were elucidated by spectroscopic methods (IR and 1D- and 2D-NMR) and by mass spectrometry (HR-ESI-MS).

Introduction. – The roots of *Codonopsis tangshen* OLIV. (Dangshen in Chinese) is a very common traditional Chinese medicine, belonging to the family Campanulaceae. It is recorded in the Chinese Pharmacopoeia for the treatment of neurosis, hematopoietic diseases, gastric ulcer, and nephritis [1][2]. Dangshen was also administrated as a substitute for Asian ginseng, or as a tonic for immunoregulatory purposes [3][4]. Many compounds such as polyacetylenes, alkaloids, flavonoids, steroids, sesquiterpenes, triterpenoids, and polysaccharides have been isolated in the *Codonopsis* genus [5–9]. In previous works, four phenylpropanoid glucosides, tangshenoside I–IV, were isolated and identified from the roots of *C. tangshen* [6][7]. In the present paper, we describe the isolation and the structure elucidation of tangshenoside V (**1**) and tangshenoside VI (**2**), along with two derivatives previously isolated from this plant, tangshenosides I (**3**) and III (**4**).

Results and Discussion. – The air-dried and powdered roots were extracted with 70% EtOH to give the crude extract (10 kg). The total extract was suspended in H_2O and partitioned successively with petroleum ether, AcOEt, and BuOH. The BuOH fraction was separated by column chromatography over *D-101* macroporous resin, silica gel, and *Sephadex LH-20*, repeatedly, followed by *ODS* column chromatography; it afforded a series of phenylpropanoid derivatives, including two new compounds **1** and **2** and the two known ones **3** and **4**. The structures of the known compounds were confirmed as tangshenoside I (**3**) and tangshenoside III (**4**) by comparison of their physical and spectral data with the reported data [7][8].

Compound **1**, a colorless gum, showed a molecular formula $C_{21}H_{26}O_{12}$ as deduced from its positive-mode HR-ESI-MS (*m*/*z* 493.1334 ($C_{21}H_{26}NaO_{12}^{+}$)). The IR spectrum



revealed the absorption bands of OH (3432 cm^{-1}) and CO (1706 cm^{-1}) groups. From the ^{1}H- and ^{13}C-NMR (*Table*), HMQC, and HMBC (*Fig. 1*) data, the structure of **1** was elucidated as 3-[(6-*O*-(*p*-coumaroyl)-β-D-glucopyranosyl)oxy]-3-methylglutaric acid, named tangshenoside V. The configuration at the 3-hydroxy-3-methylglutaric acid derived moiety was not determined. However, it can be proposed as (3*S*), since all compounds containing such a residue reported until now in the family Campanulaceae possess this configuration [6–8].

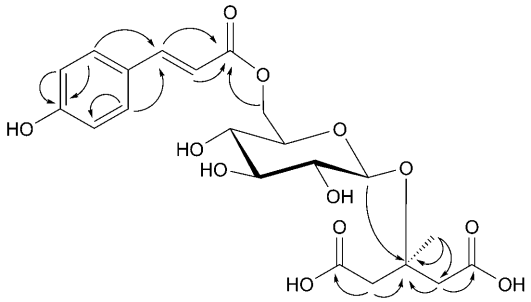


Fig. 1. *Selected HMBC* (H → C) *of* **1**

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; CD_3OD) *of* **1** *and* **2**[1]). δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)		174.6		174.6
CH_2(2)	2.80–2.92 (*m*)	44.5	2.80–2.92 (*m*)	44.5
C(3)		77.8		77.9
CH_2(4)	2.80–2.92 (*m*)	44.5	2.80–2.92 (*m*)	44.7
C(5)		174.7		172.6
Me(6)	1.49 (*s*)	25.0	1.50 (*s*)	25.4
C(1′)		127.5		127.5
H–C(2′)	7.42 (*d*, *J* = 8.6)	131.4	7.39 (*d*, *J* = 8.2)	131.5
H–C(3′)	6.78 (*d*, *J* = 8.6)	117.1	6.78 (*d*, *J* = 8.2)	117.1
C(4′)		161.5		161.5
H–C(5′)	6.78 (*d*, *J* = 8.6)	117.1	6.78 (*d*, *J* = 8.2)	117.1
H–C(6′)	7.42 (*d*, *J* = 8.6)	131.4	7.39 (*d*, *J* = 8.2)	131.5
H–C(7′)	7.62 (*d*, *J* = 15.9)	147.0	7.60 (*d*, *J* = 15.9)	147.0
H–C(8′)	6.33 (*d*, *J* = 15.9)	115.3	6.29 (*d*, *J* = 15.9)	115.4
C(9′)		169.3		169.3
H–C(1″)	4.65 (*d*, *J* = 7.7)	98.6	4.65 (*d*, *J* = 7.7)	98.7
H–C(2″)	3.22 (*t*, *J* = 8.9)	75.3	3.22–3.49 (*m*)	75.4
H–C(3″)	3.42 (*t*, *J* = 8.9)	78.2	3.22–3.49 (*m*)	78.1
H–C(4″)	3.30–3.34 (*m*)	72.1	3.22–3.49 (*m*)	72.2
H–C(5″)	3.53–3.56 (*m*)	75.5	3.22–3.49 (*m*)	75.5
CH_2(6″)	4.48 (*dd*, *J* = 11.8, 2.0), 4.28 (*dd*, *J* = 11.8, 6.8)	65.1	4.48 (*dd*, *J* = 11.4, 2.0), 4.28 (*dd*, *J* = 11.4, 6.8)	65.1
C(1‴)				134.9
H–C(2‴)			6.71 (*s*)	106.3
C(3‴)				154.6
C(4‴)				136.6
C(5‴)				154.6
H–C(6‴)			6.71 (*s*)	106.3
MeO–C(3‴)			3.83 (*s*)	57.4
MeO–C(5‴)			3.83 (*s*)	57.4
H–C(7‴)			6.56 (*d*, *J* = 16.0)	135.2
H–C(8‴)			6.23 (*ddd*, *J*= 16.0, 6.5, 6.5)	124.6
CH_2(9‴)			4.65 (*d*, *J* = 6.5)	66.3
H–C(1⁗)			4.86 (*d*, *J* = 7.4)	105.7
H–C(2⁗)			3.22–3.49 (*m*)	76.0
H–C(3⁗)			3.22–3.49 (*m*)	78.6
H–C(4⁗)			3.22–3.49 (*m*)	71.6
H–C(5⁗)			3.22–3.49 (*m*)	78.2
CH_2(6⁗)			3.79 (*dd*, *J* = 12.0, 2.0), 3.67 (*dd*, *J* = 12.0, 4.9)	62.9

In the ^{1}H- and ^{13}C-NMR spectra data of **1** (*Table*), three structural moieties were clearly observed. Two *d* at δ(H) 6.78 (*d*, *J* = 8.6, 2 H) and 7.42 (*d*, *J* = 8.6, 2 H), two olefinic H-atoms at δ(H) 6.33 (*d*, *J* = 15.9, 1 H) and 7.62 (*d*, *J* = 15.9, 1 H), together with four aromatic C-atoms, two olefinic C-atoms as well as one carboxy C-atom indicated the presence of a *p*-coumaroyl (= 3-(4-hydroxyphenyl)-1-oxoprop-2-en-1-yl) group. A Me *s* at δ(H) 1.49 (*s*, Me(6)), and the four H-atoms at δ(H) 2.80–2.92 (*m*, CH_2(2), CH_2(4)) corresponding to two CH_2 groups, together with two CO signals at δ(C) 174.6 (C(1)) and 174.7

(C(5)) demonstrated the presence of a 3-hydroxy-3-methylglutaric acid moiety [5]. The anomeric signal at δ(H) 4.65 (*d*, *J* = 7.7, 1 H), the corresponding ^{13}C-NMR signal at δ(C) 98.6, together with the signals in the region δ(H) 3.22–4.48 and relevant ^{13}C-NMR resonances, indicated the presence of a β-glucopyranosyl (Glc) unit. Interpretation of the HMQC and HMBC data of **1** (*Fig. 1*) revealed the substitution pattern and allowed us to assign all the ^{1}H- and ^{13}C-NMR signals. The *p*-coumaroyloxy group was located at C(6″) of the Glc moiety because of the downfield shift observed for this H-atom in the ^{1}H-NMR spectrum, as corroborated by the HMBC cross-peaks CH_2(6″)/C(9′). The attachment of the β-glucopyranose moiety at C(3) was established by the HMBC cross-peak H–C(1″)/C(3).

Compound **2**, a colorless gum, had a molecular formula $C_{38}H_{48}O_{20}$ as shown by its positive-mode HR-ESI-MS (*m*/*z* 847.26298 ($C_{38}H_{48}NaO_{20}^{+}$)). The IR spectrum revealed the absorption bands of OH (3432 cm^{-1}) and CO (1706 cm^{-1}) groups. The UV spectrum of **2** showed absorptions at λ_{max} (MeOH) 227 and 277 nm, which suggested that there was an aromatic system conjugated with an unsaturated side chain. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** (*Table*) with those of **1** indicated that **2** is a monoesterified derivative of **1**. From these and the HMBC data (*Fig. 2*), the structure of **2** was elucidated as 3′-*O*-[6-*O*-(*p*-coumaroyl)-β-D-glucopyranosyl]ussurienoside, named tangshenoside VI.

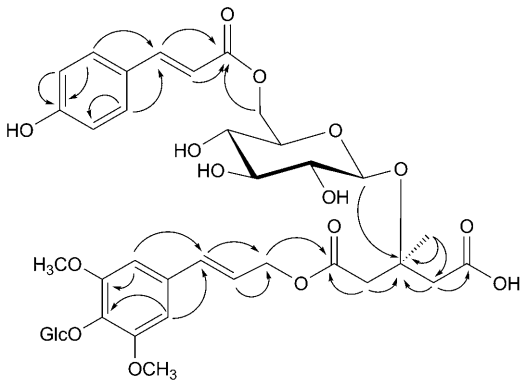


Fig. 2. *Selected HMBC* (H → C) *of* **2**

The presence of a (2*E*)-prop-2-en-1-ol derived moiety was indicated by a pair of olefinic signals at δ(H) 6.56 (*d*, *J* = 16.0, 1 H) and 6.23 (*ddd*, *J* = 16.0, 6.5, 6.5, 1 H), and an oxygenated-CH_2 *d* at δ(H) 4.65 (*d*, *J* = 6.5, 2 H). Furthermore, two aromatic H-atoms at δ(H) 6.71 (*s*, 2 H) were attributed to a 1,3,4,5-tetrasubstituted phenyl ring. A six H-atoms *s* at δ(H) 3.83 (*s*, 6 H) was characteristic of the two MeO groups symmetrically situated at a benzene ring. One *d* with a large coupling constant due to an anomeric H-atom at δ(H) 4.86 (*d*, *J* = 7.4, 1 H) in the ^{1}H-NMR spectrum and one anomeric C-atom at δ(C) 105.7 in the ^{13}C-NMR spectrum revealed that **2** possesses an additional β-linked sugar unit. The spectral data of the remaining resonances were almost the same as those of **1** [10][11]. The above data suggested that **2** is a 'tangshenoside-type' phenylpropanoid derivative [6]. The esterification of the 3-hydroxy-3-methylglutaric acid moiety was confirmed by the downfield shifted CH_2O *d* at δ(H) 4.65 (CH_2(9‴)), and further supported by a HMBC cross-peak CH_2(9‴)/C(5) (*Fig. 2*). The attachment of the β-glucopyranosyl moiety at C(4‴) was established by an HMBC cross-peak H–C(1⁗)/C(4‴).

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 100–200 or 200–300 mesh; *Qingdao Ocean Chemical Co.*, *Ltd*, Qingdao, China), *ODS* (50 μm; *YMC*, Japan), and *Sephadex LH-20* (*Amersham Biosciences, GE Health Care*). Thin-layer chromatography (TLC): silica gel GF_{254} plates (*Qingdao Ocean Chemical Co.*, *Ltd*, Qingdao, China). Optical rotation: *Perkin-Elmer 341* polarimeter; in MeOH at 22°. UV Spectra: *Shimadzu UV-240A* apparatus; λ_{max} (log ε) in nm. IR Spectra: *Nicolet FT-IR-380* spectrometer; KBr pellets; in cm^{-1}. ^1H- (500 MHz), ^{13}C- (125 MHz), and 2D-NMR (HMBC, HSQC) Spectra: *Bruker AV-500* spectrometer; in CD_3OD; chemical shifts δ in ppm and coupling constants *J* in Hz. ESI-MS and HR-ESI-MS: *LCQ-Deca-XP*^{plus} (*Thermo Finnigan*) and *Finnigan MAT-95* spectrometer, resp.; in *m*/*z*.

Plant Material. The roots of *C. tangshen* were collected in Chongqing, China, in January 2006, and identified by Prof. *Rui Peng* (Chongqing Academy of Chinese Materia Medica). A voucher (No. 06-01-28) specimen was deposited with the Herbarium of Chongqing Academy of Chinese Materia Medica.

Extraction and Separation. The air-dried, finely sliced roots of *C. tangshen* (25 kg) were extracted with 70% EtOH (3 × 120 l) under reflux for 2.0 h. After removal of the EtOH by evaporation, the resulting residue (7.5 kg) was suspended in H_2O (8 l) and then partitioned successively with petroleum ether (1 × 4 l, 2 × 3 l), AcOEt (1 × 4 l, 2 × 3 l), and BuOH (1 × 4 l, 2 × 3 l). The BuOH fraction (1.5 kg) was dissolved in H_2O, the soln. filtered, and the filtrate purified with the aid of a *D-101* macroporous resin column (20 × 80 cm), eluting successively with H_2O, 30%, 50%, and 95% (*v*/*v*) EtOH: *Fractions 1–4*. *Fr. 2* (170 g) was firstly separated by CC (*ODS*, MeOH/H_2O 1:9 → 4:6, then 100:0): *Fr. 2a–Fr. 2e*. *Fr. 2a* was subjected to CC (*ODS*, MeOH/H_2O 2:8): **3** (30 mg). *Fr. 2b* was submitted to repeated CC (*Sephadex LH-20*, MeOH/H_2O 1:1; then *RP-18* gel, MeOH/H_2O 3:7): **1** (60 mg) and **4** (15 mg). *Fr. 3* (50 g) was subjected to CC (SiO_2, gradient $CHCl_3$/MeOH 80:1 → 1:1): *Fr. 3a–Fr. 3j*. *Fr. 3h* was subjected to CC (silica gel, $CHCl_3$/MeOH 10:1 → 0:1); then *RP-18* gel (MeOH/H_2O 4:6): **2** (22 mg).

*3-{{6-*O*-[(2*E*)-3-(4-Hydroxyphenyl)-1-oxoprop-2-en-1-yl]-β-*D*-glucopyranosyl}oxy}-3-methylglutaric Acid* (= *Tangshenoside V*; **1**): Colorless gum. $[\alpha]_D^{22} = -3.40$ ($c = 0.65$, MeOH). UV (MeOH): 311 (4.32). IR (KBr): 3432, 2924, 1706, 1634, 1604, 1174, 835. ^1H- and ^{13}C-NMR: *Table*. HR-ESI-MS (pos.): 493.1334 ($[M + Na]^+$; calc. 493.1316).

*3-{{6-*O*-[(2*E*)-3-(4-Hydroxyphenyl)-1-oxoprop-2-en-1-yl]-β-*D*-glucopyranosyl}oxy}-3-methylglutaric Acid (2*E*)-3-[4-(β-*D*-Glucopyranosyloxy)-3,5-dimethoxyphenyl]prop-2-en-1-yl Ester* (= *Tangshenoside VI*; **2**): Colorless gum. $[\alpha]_D^{22} = -10.2$ ($c = 0.10$, MeOH). UV (MeOH): 227 (4.68), 277 (4.44), 309 (4.32). IR (KBr): 3432, 2927, 1706, 1633, 1604, 1172, 1126, 1072, 833. ^1H- and ^{13}C-NMR: *Table*. HR-ESI-MS (pos.): 847.2629 ($[M + Na]^+$; calc. 847.2631).

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