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Two new polyacetylene glycosides from the roots of *Codonopsis tangshen* Oliv.

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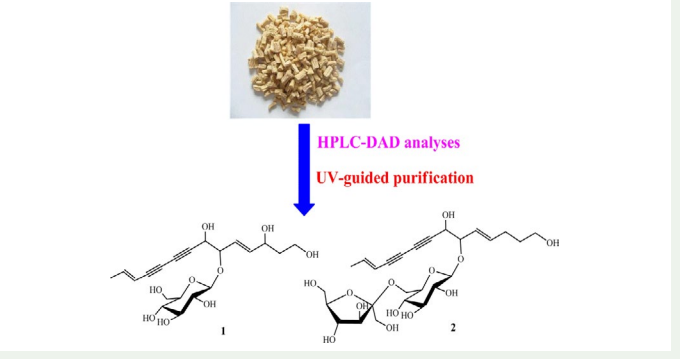
ABSTRACT

Two new polyacetylene glycosides, tangshenyne A (**1**) and tangshenyne B (**2**), along with six known polyacetylenes were isolated from an 85% MeOH extract of the roots of *Codonopsis tangshen* Oliv. The chemical structures of the new compounds were determined on the basis of extensive spectroscopic analyses, including UV, IR, 1D and 2D NMR (^{1}H–^{1}H COSY, HSQC and HMBC) and HR-ESI-MS.



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1. Introduction

Codonopsis tangshen Oliv. belongs to the genus of *Codonopsis* (Campanulaceae), containing approximately 40 species mainly distributed in Central and East Asia and 39 species exist in China (Bi et al. 2008). It is usually used as a Traditional Chinese Medicine for the treatment of neurosis, haematopoietic diseases, gastric ulcer and nephritis (Feng et al. 2012). Previous phytochemical investigations on *C. tangshen* Oliv. revealed the presence of alkaloids, phenylpropanoids, triterpenoids, flavonoids, organic acids and polyacetylenes (Lin et al. 2013; He et al. 2015). In the last decade, much more attention had been paid to polyacetylenes due to their exhibiting various biological activities (Dumlu et al. 2008; Song et al. 2008a; Song et al. 2008b). However, just a limited number of polyacetylenes and their glycosides from *C. tangshen* Oliv. had been reported (Negri 2015). Therefore, the present study focused

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on the isolation and structural elucidation of the minor polyacetylene analogues from the roots of *C. tangshen* Oliv. Herein, two new polyacetylene glycosides including six polyacetylenes are described (Figure 1).

2. Results and discussion

Compound **1** was obtained as a colourless syrup. The molecular formula of compound **1** was determined as $C_{20}H_{28}O_9$ according to the pseudo-molecular ion at *m/z* $[M + Na]^+$ 435.1616 (Calcd for $C_{20}H_{28}O_9Na$, 435.1626) in HR-ESI-MS, requiring 7 degrees of unsaturation. The specific absorption in the UV (284, 268, 254 and 246 nm) spectrum indicated the presence of one C=C bond and two C≡C bonds in conjugation (Mei et al. 2008). The ^{1}H NMR spectrum suggested the existence of two pairs of olefinic protons at δ_H 6.24 (1H, dq, *J* = 15.8, 6.8 Hz), 5.95 (1H, dd, *J* = 15.6, 8.0 Hz), 5.83 (1H, dd, *J* = 15.6, 6.3 Hz), 5.48 (1H, dd, *J* = 15.8, 1.2 Hz) and a sugar moiety with an anomeric proton at δ_H 4.47 (1H, d, *J* = 6.3 Hz). The large coupling constants (nearly 16.0 Hz) of

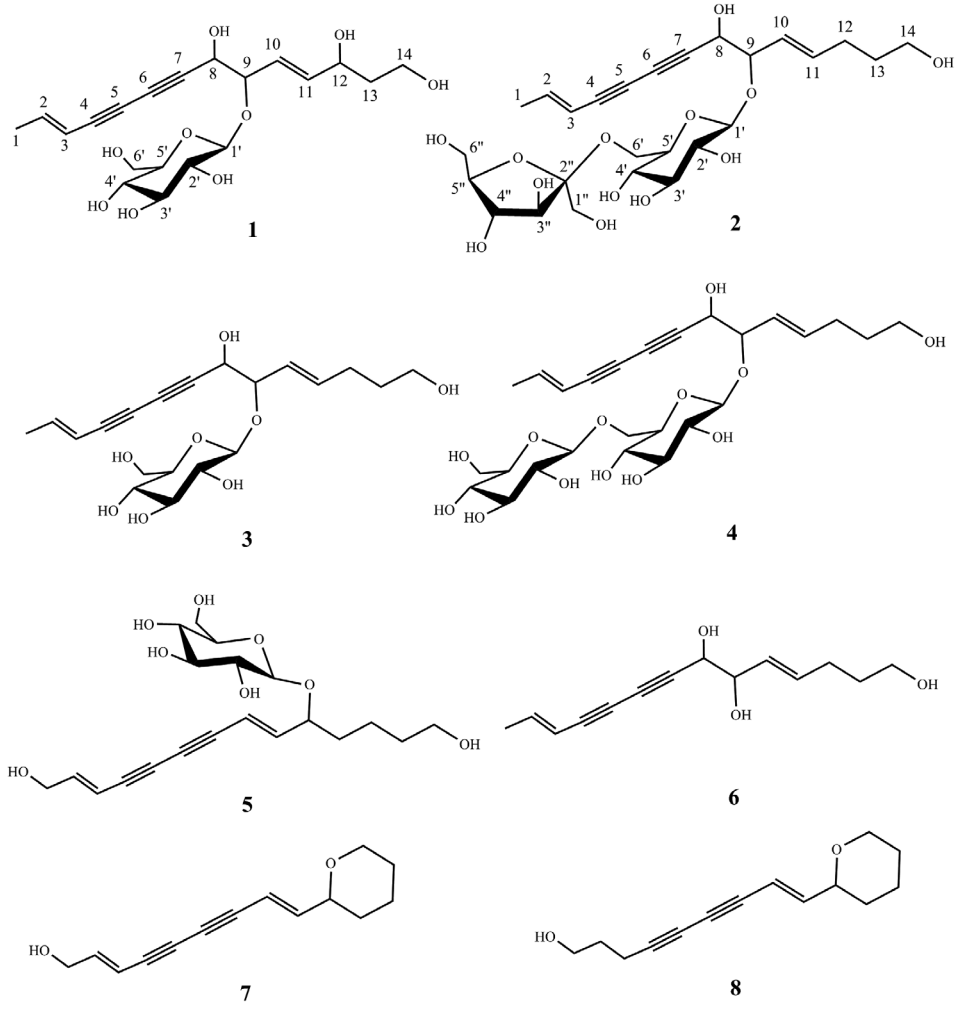

Figure 1. Chemical structures of compounds **1**–**8**.

the two pairs of olefinic protons suggested the *trans*-forms of the double bonds. The ^{13}C NMR and DEPT spectra presented four signals for alkyne carbon atoms, four signals ascribable to olefinic carbon atoms, six signals belonging to a hexose residue and other three O-bearing carbon atoms. The hexose was determined as D-glucose by TLC analysis and measuring the optical rotation of the acid hydrolysis solution of **1** (He et al. 2014). The coupling constant of the anomeric proton suggested a *β*-configuration (Li et al. 2012). The NMR data of **1** were similar to those of the known compound **3**, the main component in *C. tangshen* Oliv., except that a hydrogen at C-12 in **3** was replaced by a hydroxyl group in **1**. This deduction was confirmed by the dd peak of H-11 at δ_H 5.83 (1H, dd, $J = 15.6$, 6.3 Hz) and the chemical shift of C-12 at δ_C 71.1 in **1**. The inversion of a hydroxyl group at C-12 was further verified by the fact that the molecular weight of **1** was 16 Da higher than that of **3**. Unfortunately, the data were not conclusive enough to determine the absolute configuration of C-12. The two spin systems of CH_3–CH–CH– and –CH–CH–CH–CH–CH–CH_2–CH_2– due to C-1, C-2, C-3 and C-8, C-9, C-10, C-11, C-12, C-13, C-14, respectively, were also deduced from the ^1H–^1H COSY correlations (Figure S24). The HMBC correlation (Figure S24) between H-1′ (δ_H 4.47) and C-9 (δ_C 82.3) revealed that the glucose residue was connected to C-9. Based on the above evidences, the structure of compound **1** was established as 9-(*β*-D-glucopyranosyloxy) tetradeca-2,10-diene-4,6-diyne-8,12,14-triol and named tangshenyne A.

Compound **2** was obtained as a colourless crystal. Its molecular formula was assigned as $C_{26}H_{38}O_{13}$ by positive ion HR-ESI-MS, which exhibited a pseudo-molecular ion at *m/z* $[M + Na]^+$ 581.2198 (Calcd for $C_{26}H_{38}O_{13}Na$, 581.2205). Comparison of the UV and 1D NMR data of compound **2** with those of compound **3** indicated that these two compounds shared similar chemical structures except the presence of one more sugar residue in **2**. In contrast to **3**, the DEPT and HSQC spectra of **2** displayed one additional quaternary carbon at δ_C 106.1 (C-2″), two O-bearing methylenes at δ_C 63.0 (C-1‴) and 64.8 (C-6″) and three O-bearing methines at δ_C 79.9 (C-3″), 77.2 (C-4″) and 84.3 (C-5″), suggesting the presence of a non-reducing sugar moiety which was further determined to be D-fructose by the method as described above. The chemical shift of the anomeric carbon at δ_C 106.1 indicated a *β*-configuration for the fructose residue, while the IR absorption bands at 931 and 820 cm^{-1} suggested its furanose form (Qiu et al. 2015). The key HMBC correlation (Figure S24) between H-6′ (δ_H 3.63 and 3.71) and C-2″ (δ_C 106.1) implied that the fructose moiety was linked to the C-6 of the glucose residue. Accordingly, compound **2** was unequivocally identified as 9-(*β*-D-fructofuranosyl (2 → 6)-*β*-D-glucopyranosyloxy) tetradeca-2, 10-diene-4,6-diyne-8,12-diol and named tangshenyne B.

The known compounds were identified as lobetyolin (**3**) (Ishimaru et al. 2003), lobetyolinin (**4**) (Ishimaru et al. 1992), cordifolioidyne B (**5**) (Mei et al. 2008), lobetyol (**6**) (Ishimaru et al. 2003), 9-(tetrahydropyran-2-yl)-non-*trans*-2,8-diene-4,6-diyn-l-ol (**7**) (Bentley et al. 1969) and 9-(tetrahydropyran-2-yl)-non-*trans*-8-ene-4,6-yn-l-ol (**8**) (Bentley et al. 1969) by comparison with their spectroscopic data with those reported in the literature.

3. Experimental

3.1. General experimental procedures

Optical rotations were determined in methanol on a PerkinElmer 341 polarimeter (PerkinElmer Corporation, Wellesley, MA, USA). IR spectra were carried out on a PerkinElmer Spectrum One FTIR spectrometer (PerkinElmer Corporation, Wellesley, MA, USA). High-resolution mass

spectrometry was measured on a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) with electrospray ionisation sources operated in positive ion mode. NMR spectra, including ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC and HMBC experiments, were recorded on an Avance III NMR spectrometer (Bruker Group, Fallanden, Switzerland) operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C with TMS as an internal standard. Semi-preparative HPLC purifications were performed on a CXTH system, equipped with a UV3000 detector at 254 nm (Beijing Chuangxintongheng Instruments Co Ltd, Beijing, People's Republic of China). The preparative HPLC column used was a 20 × 250 mm i.d., 10 μm, YMC-pack ODS-AM (YMC Co Ltd, Kyoto, Japan) with a flow rate of 12 mL/min. Silica gel (100–200 mesh, Qingdao Marine Chemical Factory, Qingdao, China) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden) were used for open column chromatography. TLC was performed on pre-coated silica gel plates (GF254, 0.25 mm, Kang-Bi-Nuo Silysia Chemical Ltd, Yantai, China). Chemical reagents for isolation were of analytical grade and purchased from Chengdu Kelong Chemical Reagent Co Ltd (Chengdu, China).

3.2. Plant material

The roots of *C. tangshen* Oliv. were purchased on April 2015 from Lotus Pond Chinese Herbal Medicine Market, Sichuan province, People's Republic of China. The plant material was identified by Professor Weikai Bao, Chengdu Institute of Biology, Chinese Academy of Sciences. A voucher specimen (NO. DS20150410) was deposited in the herbarium of Chengdu Institute of Biology, Chinese Academy of Sciences.

3.3. Extraction and isolation

The air-dried roots of *C. tangshen* Oliv. (30.0 kg) were extracted with 85% MeOH (30.0 L × 3, v/v) three times for 24 h each time at room temperature. The extracted solution was combined and concentrated to give a MeOH extract (4.3 kg), which was suspended in water and partitioned with EtOAc and *n*-BuOH, successively.

The EtOAc fraction (202.3 g) was subjected to silica gel column chromatography, eluting with increasing polarity CH_2Cl_2–MeOH (50:1 → 50:25) to obtain five fractions (Fr.A–Fr.E) combined by TLC. Fr. B (11.8 g) was subjected to a silica gel column eluted with CH_2Cl_2–MeOH (20:1 → 20:10) and further purified with semi-preparative HPLC (15% CH_3CN in H_2O) yield compounds **7** (40.2 mg) and **8** (12.4 mg). Fr. C (20.7 g) was separated by silica gel column chromatography to obtain compound **6** (37.5 mg). Fr. E (40.6 g) was purified on Sephadex LH-20 column chromatography with a step gradient of MeOH–H_2O (20, 40, 60 and 80%), followed by semi-preparative HPLC (45% MeOH) to afford compounds **1** (58.6 mg), **3** (4.2 g) and **5** (5.0 mg). The *n*-BuOH fraction (110.0 g) was subjected to silica gel column chromatography eluted with CH_2Cl_2–MeOH (10:1 → 10:10) to obtain four subfractions (Fr.F–Fr.I). Fraction G (18.9 g) was separated using Sephadex LH-20 column chromatography with a step gradient of MeOH–H_2O (10, 30, 50 and 70%), followed by semi-preparative HPLC (45% MeOH) to yield compounds **2** (48.2 mg) and **4** (18.7 mg).

3.3.1. Tangshenyne A (1)

Colourless syrup (MeOH). $[\alpha]_D^{20}$–41.0° (*c* = 0.07, MeOH). IR (KBr) ν_{max} 3393, 2908, 1653, 1567, 1411, 1052. HR-ESI-MS *m/z*: $[M + Na]^+$ 435.1616 (Calcd for $C_{20}H_{28}O_9Na$, 435.1626). ^{1}H NMR (CD_3OD, 400 MHz): δ_H 1.83 (3H, dd, *J* = 6.9, 1.7 Hz, H-1), 6.24(1H, dq, *J* = 13.8, 6.8 Hz, H-2), 5.48

(1H, dd, *J* = 15.8, 1.2 Hz, H-3), 4.38 (1H, d, *J* = 7.5 Hz, H-8), 5.95 (1H, dd, *J* = 15.6, 8.0 Hz, H-10), 5.83(1H, dd, *J* = 15.5, 6.3 Hz, H-11), 4.3 (1H, m, H-12), 1.78 (2H, dt, *J* = 5.7, 3.9 Hz, H-13), 3.37 (2H, t, *J* = 6.5 Hz, H-14), 4.47 (1H, d, *J* = 6.3 Hz, H-1′); ^{13}C NMR (CD_3OD, 100 MHz): δ_C 19.7 (C-1), 146.2 (C-2), 111.3 (C-3), 79.1 (C-4), 75.7 (C-5), 72.4 (C-6), 81.6 (C-7), 67.2 (C-8), 82.3 (C-9), 127.1 (C-10), 142.3 (C-11), 71.1 (C-12), 41.6 (C-13), 60.6 (C-14), 101.7 (C-1′), 73.2 (C-2′), 78.8 (C-3′), 72.3 (C-4′), 78.8 (C-5′), 63.5 (C-6′).

3.3.2. Tangshenyne B (2)

Colourless crystals (MeOH). $[\alpha]_D^{20}$–18.6° (*c* = 0.04, MeOH). IR (KBr) ν_{max} 3413, 2922. 2880, 2201, 1636, 1160, 931, 820. HR-ESI-MS *m/z*: $[M + Na]^+$ 581.2198 (Calcd for $C_{26}H_{38}O_{13}Na$, 581.2205). ^{1}H NMR (CD_3OD, 400 MHz): δ_H 1.83 (3H, dd, *J* = 6.9, 1.8 Hz, H-1), 6.36 (1H, dq, *J* = 15.8, 6.9 Hz, H-2), 5.61 (1H, d, *J* = 15.8 Hz, H-3), 4.50 (1H, d, *J* = 6.2 Hz, H-8), 4.26 (1H, dd, *J* = 8.0, 6.4 Hz, H-9), 5.47 (1H, dd, *J* = 15.4, 7.5 Hz, H-10), 5.93 (1H, dt, *J* = 15.4, 6.9 Hz, H-11), 2.11 (2H, m, H-12), 1.68 (2H, m, H-13), 3.60 (2H, m, H-14), 4.38 (1H, d, *J* = 7.7 Hz, H-1′), 3.63 (1H, m, H-6a′), 3.71 (1H, m, H-6b′), 4.16(1H, d, *J* = 8.2 Hz, H-3″); ^{13}C NMR (CD_3OD, 100 MHz): δ_C 19.8 (C-1), 146.3 (C-2), 111.3 (C-3), 79.1 (C-4), 73.4 (C-5), 72.2 (C-6), 82.0 (C-7), 67.4 (C-8), 83.2 (C-9), 127.2 (C-10), 140.0 (C-11), 30.6 (C-12), 33.7 (C-13), 63.0 (C-14), 101.8 (C-1′), 75.6 (C-2′), 77.4 (C-3′), 71.9 (C-4′), 78.5 (C-5′), 63.0 (C-6′). 63.0 (C-1″), 106.1 (C-2″), 79.9 (C-3″), 77.2 (C-4″), 84.3 (C-5″), 64.8 (C-6″).

3.4. Acid hydrolysis of compounds 1 *and* 2 *and measurement of optical rotations of the solution*

Each compound (5 mg) was heated with 5% H_2SO_4 (2 mL) under reflux for 10 h. The reaction solution was extracted with EtOAc for three times. The H_2O layer was neutralised with $Ba(OH)_2$, filtrated and concentrated to afford a sugar solution. The sugars were analysed by TLC over silica gel with authentic sugars. The optical rotations of the water solutions of **1** and **2** were determined to be $[\alpha]_D^{20}$ + 50.2° (*c* = 0.05, H_2O) and $[\alpha]_D^{20}$–23.5° (*c* = 0.03, H_2O), respectively, suggesting the D configuration for both glucose and fructose moieties.

4. Conclusion

Polyacetylenes from the Campanulaceae family possessed unique chemical structures and shared similar characteristic UV spectra. The UV-guided purification of the polyacetylenes from the roots of *C. tangshen* Oliv. yielded eight compounds including two new ones, tangshenyne A (**1**) and tangshenyne B (**2**). It was interesting that compound **2** contained a terminal, non-reducing fructose residue, which was sometimes described in natural polysaccharides but rarely reported in natural glycosides (Xu et al. 2005; Lyantagaye 2013). Besides the compounds reported in this paper, some other polyacetylene analogues were also detected but not obtained due to their low content in the plant source and the serious overlap in normal and reversed phase silica gel chromatography. Thus, further phytochemical investigation of this medicinal plant is practically needed. Moreover, the biological activities of the isolated individuals should be evaluated in the next work.

Disclosure statement

No potential conflict of interest was reported by the authors.

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