ORIGINAL ARTICLE

Acetylenes and fatty acids from *Codonopsis pilosula*

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**Abstract**

Four new acetylenes (1–4) and one new unsaturated *ω*-hydroxy fatty acid (5), together with 5 known analogues, were isolated from an aqueous extract of *Codonopsis pilosula* roots. Their structures were determined by spectroscopic and chemical methods. The new acetylenes are categorized as an unusual cyclotetradecatrienynone (1), tetradecenynetriol (2), and rare octenynoic acids (3 and 4), respectively, and 3 and 4 are possibly derived from oxidative metabolic degradation of 1 and/or 2. The absolute configuration of 1 was assigned by comparison of the experimental circular dichroism (CD) spectrum with the calculated electronic circular dichroism (ECD) spectra of stereoisomers based on the quantum-mechanical time-dependent density functional theory, while the configuration of 2 was assigned by using modified Mosher’s method based on the MPA determination rule of *ΔδRS* values for diols.

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1. Introduction

Codonopsis pilosula (Franch.) Nannf., a perennial plant of the Campanulaceae family, is widely cultivated in the northwest provinces of China to meet the demand of pharmaceutical and food industries. The root of this plant, known as “Dang shen” in Chinese, is one of the most common traditional Chinese medicines used for the treatment of body weakness, poor appetite, thirsty, indigestion, chronic diarrhea, anemia, and leukemia. It exhibits similar therapeutic effects of Panax ginseng as tonic agents and is used in many cases as a substitute of the more costly Panax ginseng. Previous studies showed that the extracts of the C. pilosula roots exhibited pharmacological effects in protecting against peptic ulceration and promoting its healing, enhancing immunity, and improving learning and memory behavior, as well as inhibiting inducible NO synthase and protein oxidation and attenuating the cardiac-impaired insulin-like growth factor II receptor pathway. Meanwhile, different types of chemical constituents were isolated from the extracts, such as phytoestrogens, sesquiterpenes, triterpenoids, alkali alcohol glycosides, and phenylpropanoid glycosides. Polyacetylene glycosides, phytosteroids, sesquiterpenes, triterpenes, alkaloids, alkyl alcohol chemical constituents were isolated from the extracts, such as cytokines.

2. Results and discussion

Compound 1 was obtained as a yellowish amorphous powder with [α]D +10.8 (c 0.07, MeOH). Its IR spectrum showed absorption bands for hydroxyl (3395 cm-1), double bond (3011 cm-1), triple bond (2191 and 2062 cm-1), and conjugated carbonyl (1720, 1685, and 1647 cm-1) functionalities. The molecular formula of 1, C_{14}H_{24}O_{3}, was indicated by HR-ESI-MS at 256.1527 [M+Na]+ (Calcd. for C_{14}H_{25}O_{3}Na, 256.1513) and NMR data (Table 1). The 1H NMR spectrum of 1 in CD_{3}OD showed signals attributed to a conjugated cis-disubstituted double bond at δH 7.13 (dd, J = 9.6, 6.0 and 2.4 Hz, H-4) and 6.07 (dd, J = 10.8 and 8.4 Hz, H-1b) and an aliphatic methylene at δH 2.73 (dd, J = 10.8, 6.0 and 1.0 Hz, H-3a) and 2.35 (dd, J = 10.8, 6.0, and 2.4 Hz, H-3b), as well as a multiplet due to a methine at δH 2.28 (m, H-2), which is vicinal to both the methylenes based on the splitting patterns and coupling constants. The 13C NMR and DEPT spectra of 1 exhibited 14 resonances corresponding to proton-bearing carbons of the above units and four quaternary carbons including a conjugated carbonyl (δC 199.6, C-6), a triple bond (δC 86.4 (C-10) and 91.2 (C-11)), and an oxygen-bearing (δC 80.2, C-7) carbon (Table 1). Together, the above spectroscopic data indicated that 1 is a monocyclic C_{14} hydroxytriene in an unusual structure feature, which was further elucidated by 2D NMR experimental data analysis. The assignments of proton and proton-bearing carbon signals in the NMR spectra were confirmed by cross-peaks in the 1H-1H COSY and HSQC spectra. The vicinal coupling cross-peaks H-2 ↔ H-3a ↔ H-2 ↔ H-3b ↔ H-5 in the 1H-1H COSY spectrum and three-bond hetero nuclear correlations (Fig. 2) from H-2 to C-3 and C-7; from H-2 to C-4 and C-7; from H-3 to C-1, C-5, and C-7; from H-4 to C-2 and C-6; and from H-5 to C-3 and C-7 in the HMBC spectrum, together with the chemical shifts of these protons and carbon resonances, revealed that the oxygen-bearing quaternary carbon (C-7) was connected with both the methine (CH-2) and the carbonyl (C-6) to form a cyclohexenone moiety in 1. The 1H-1H COSY cross-peaks H-8 ↔ H-9 and H-12 ↔ H-13 ↔ H-14, together with the HMBC correlations from H-8 to C-10, from H-9 to C-11, from H-12 to C-14; from H-13 to C-11, and from H-14 to C-12, in combination with their chemical shifts, confirmed the presence of a 8,12-dien-10-yn-8-yl chain moiety in 1. In addition, the HMBC correlations from H-2 to C-8, from H-8 to C-6 and C-7, and from H-9 to C-7 indicated a C-7-C-8 bond connection between the two moieties. To match requirement of the molecular composition, two hydroxyl groups must occur at the oxygen-bearing carbons (C-1 and C-7) in 1. Accordingly the planar structure of 1 is elucidated as shown in Fig. 2. In the NOE difference spectrum of 1, irradiation of H-3b enhanced H-2 and H-8 (Fig. 3), indicated that these protons were cofacial and that the chain moiety had a quasi-axial orientation on the cyclohexenone ring, which was supported by conformational analysis with the MMFP94 molecular mechanics force field using molecular operating environment (MOE) software package. The circular dichroism (CD) spectrum of 1 displayed a positive Cotton effect at 299 nm (Δε +1.41) for n→π* and a negative Cotton effect at 256 nm (Δε -4.71) for π→π*. Based on the octant rule for cyclohexenones, these Cotton effects suggest that 1 possesses the 2R,7S-configuration (Fig. 4). The suggestion was further supported by comparing the experimental CD spectrum with the electronic circular dichroism (ECD) spectrum predicted from the quantum-mechanical time-dependent density functional theory (TDDFT) calculations (Fig. 5). Therefore, compound 1 was determined as (+)-(2R,7S)-1,7-dihydroxy-2,7-cyclooctadeca-4,8,12-trien-10-yn-6-one.

Figure 1 The structures of compounds 1−5.
and Table 1) demonstrated that it was a C₁₄ enynetriol derivative with the same terminal unit trans-12-en-10-ynyl as that of 1. Comparison of the NMR spectroscopic data between 2 and 1 indicated replacement of the 2,7-cyclo-4,8-dien-6-one moiety in 1 by a linear diol moiety consisting of two hydroxyl-bearing methines δ₁₅(C-6) 3.34 (q, J = 4.0 Hz, H-6) and 3.46 (q, J = 4.0 Hz, H-7), δ₁₅(C-5) 75.3 (C-5) and 74.2 (C-7) and six aliphatic methylenes δ₁₅(C-13) 1.34-1.48 (8H, partially overlapped, H₂-2-H₂-5), 1.62 (m, H-8a), 1.57 (m, H-8b), and 2.34 (m, H-9); δ₁₅(C-9) 16.9 (C-9), 27.1 (C-4), 27.3 (C-3), 33.6 (C-8), 33.9 (C-2), and 34.2 (C-5) in 2. In addition, the two doublets of inequivalent H-1a and H-1b in 1 were replaced by a hydroxymethylene triplet at δ₁₅(C-1) 4.39 (J = 6.5 Hz, H-1) in 2, while the triple carbon resonances were shifted from δ₁₅(C-7) 86.4 (C-7) and 91.2 (C-11) in 1 to δ₁₅(C-7) 88.8 (C-10) and 80.6 (C-11) in 2. These data suggest that 2 differs from 1 in the 2,7-cyclo-4,8-dien-6-one moiety with cleavage of the C-2-C-7 bond and saturation of the 4,8-dien-6-one. This was further confirmed by 2D NMR data analysis, especially by the 13C-1H COSY cross-peaks H-12 ↔ H-13 ↔ H-14 and the HMBC correlations from H₂-8 to C-6 and C-10; from H₂-9 to C-7 and C-11; from H-13 to C-11 and C-14; and from H₂-14 to C-11, C-12, and C-13, combined with their chemical shifts. The absolute configuration of the 6,7-diol moiety in 2 was determined using the modified Mosher's method since the primary alcohol at C-1 is away from and has less shielding/deshielding effects on protons around the chiral centers. Treatment of 2 with (–)- and (+)-3,5-dimethoxyphenylacetic acid (R- and S-MPA) in anhydrous CH₂Cl₂ afforded the tri-(R)- and tri-(S)-MPA esters of 2. The δₑᵤₛ values of H-6 and H-7 were calculated by NMR data measurement of the esters. According to the Mosher's model of vicinal diols, the δₑᵤₛ values predict that 2 has the 6R,7R-configuration (Fig. 6). This was supported by Mos(OAc)₂-induced circular dichroism (ICD) spectrum of 2, which displayed a negative Cotton effect at 316 nm (Fig. S17, Supporting information). On the basis of the empirical rule proposed by Snatzke, the Cotton effect indicates that the O-C-C-O torsion angle of the diol moiety in 2 is negative and also predicts 6R,7R configuration. Thus, compound 2 was determined as (+)-(6R,7R,12E)-tetradeca-12-en-10-yn-1,6,7-triol.

Compound 3, a yellowish amorphous powder, has the molecular formula C₉H₆O₂ as indicated by HR-ESI-MS m/z 137.0595 [M+H]+ (Calcd. for C₉H₆O₂, 137.0597) and 159.0413 [M+Na]+ (Calcd. for C₉H₆O₂Na, 159.0417). Its IR spectrum showed the presence of OH (3395 cm⁻¹), C=O (3011 cm⁻¹), C=C (2185 cm⁻¹) and, carbonyl (1698 cm⁻¹) sh) functionalities. The 1H NMR data of 3 (Table 1) demonstrated occurrence of a terminal trans-propenyl (δ₁₅(C-3) 6.23 (dq, J = 16.0 and 7.0 Hz, H-7), 5.66 (d, J = 16.0 Hz, H-6), and 1.77 (d, J = 7.0 Hz, H-8)) and a trans-disubstituted double bond (δ₁₅(C-6) 6.08 (d, J = 16.0 Hz, H-2) and 6.74 (d, J = 16.0 Hz, H-3)). Besides the resonances corresponding to the proton-bearing carbons, the 13C NMR and DEPT spectral data confirmed the presence of the triple-bond (δ₁₅(C-4) 85.9 (C-4) and 98.3 (C-5)) and carbonyl (δ₁₅(C-9) 170.1 (C-1)) units. These spectroscopic data reveal that 3 is (2E,6E)-octa-2,6-dien-4-ynoic acid, which was further confirmed by 2D NMR data analysis, particularly by HMBC correlations from H-2 to C-1 and C-4 and from H-3 to C-1 and C-5 combined with their chemical shifts. Thus, compound 3 was determined as (2E,6E)-octa-2,6-dien-4-ynoic acid.

Compound 4 was isolated as a yellowish amorphous powder. The IR and HR-ESI-MS data (Section 4) indicated that 4 was a dihydro analogue of 3. Comparison of the NMR spectral data of 4 and 3 (Tables 1 and 2) demonstrated that the 2-en in 3 was saturated in 4. Therefore, compound 4 was determined as (E)-octa-6-en-4-ynoic acid, which was also verified by 2D NMR data analysis.

Compound 5 has the molecular formula C₁₂H₂₀O₃ as indicated by HR-ESI-MS at m/z 235.1296 [M+Na]+ (Calcd. for C₁₂H₁₇O₄Na, 235.1305). The IR spectrum showed the presence of OH (3200 cm⁻¹), C=O (3017 cm⁻¹), and C=O (1723 cm⁻¹) functionalities. The 1H NMR spectral data of 5 (Table 2) revealed the presence of two double bonds, an oxygen-bearing methylene, and six aliphatic methylenes. These spectroscopic data suggested that 5 is (8E,10E)-12-hydroxydodeca-8,10-dienoic acid though an expected carboxylic carbon resonance was extremely diminished and almost not recognized in the 13C NMR spectrum. The suggestion was confirmed by 2D NMR experiments. Especially, the HMBC spectrum exhibited correlations from H₂-2 and H₂-3 to a carbon resonated at δ₁₅(C-11) 178.9, leading to an ambiguous assignment of the carboxylic carbon in 5. In addition, the HMBC correlations from H-10 to C-8 and C-12 and from H₂-12 to C-10 and C-11 confirmed positions of the hydroxyl and double bonds. Therefore, compound 5 was determined as (8E,10E)-12-hydroxydodeca-8,10-dienoic acid.

The known compounds were identified by comparing their spectroscopic data with the reported data as (6R,7R,4E,8E,12E)-tetradeca-4,8,12-trien-10-yn-1,6,7-triol, (10E)-12-hydroxydocosa-10-enoic acid, hexadecanoic acid-2,3-dihydroxy propyl ester, fulgide acid, and pinolic acid.

Various biological activities of acetylene and unsaturated fatty acid derivatives were reported, such as adjuvant activity, cytotoxicity and anti-diabetic activity. This indicates that these types of compounds might play some biological roles in clinical effects of this herbal medicine. In the preliminary in vivo assays carried out in this study, the isolates were assessed for inhibitory activity against LPS-induced NO production in BV2-cells, protein tyrosine phosphatase 1B (PTP1B), HIV-1 replication and several human cancer cell lines, as well as antioxidant activity, but all were inactive at a concentration of 10 μmol/L. Therefore, their potential biological activity is still expected from a further in depth evaluation on other biological models.

3. Conclusions

From the aqueous extract of C. pilosula roots, four new acetylenes (1–4) and one new unsaturated α-hydroxy fatty acid (5), together with five known analogues, were isolated. The new acetylenes are categorized as an unusual cyclooctatetraene (1), tetradeceyne (2), and rare octenoic acids (3 and 4), respectively, of which 3 and 4 are possibly derived from oxidative metabolic degradation of 1 and/or 2. Although the new compounds were inactive in the assays carried out in this study, the results provide a clue for further studies of synthesis, chemical transformation, structural modification and biosynthesis, as well as biological evaluations on other pharmacological models, of the diverse ployacetylene derivatives from the C. pilosula.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on P-2000 polarimeter (JASCO, Tokyo, Japan). UV spectra were measured on a V-650 spectrometer (JASCO, Tokyo, Japan). IR spectra were recorded on a
Table 1  $^1$H and $^{13}$C NMR spectral data (δ) for compounds 1–5.  

| No.  | δH             | δC   | δH             | δC   | δH             | δC   |
|------|----------------|------|----------------|------|----------------|------|
|      | (δH, J H,H)    |      | (δH, J H,H)    |      | (δH, J H,H)    |      |
| 1a   | 3.86 dd (10.8, 4.8) | 62.8 | 3.49 t (6.5)   | 63.2 | 170.1          |
| 1b   | 3.45 dd (10.8, 8.4) |     | 1.48 m         | 33.9 | 6.08 d (16.0)  | 131.9 |
| 2    | 2.28 m          | 48.2 | 1.34 m         | 27.3 | 6.74 d (16.0)  | 126.2 |
| 3a   | 2.73 ddd (10.8, 6.0, 1.0) | 29.9 | 1.47 m         |      | 85.9           |
| 3b   | 2.35 ddd (10.8, 6.0, 2.4) |     | 1.71 m         |      | 98.3           |
| 4    | 7.13 ddd (9.6, 6.0, 2.4) | 152.4| 2.71 m         |      | 4.36 m         | 111.7 |
| 5    | 6.07 ddd (9.6, 3.0, 0.6) | 127.8| 3.97 m         | 34.2 | 1.77 d (7.0)   | 19.2  |
| 6    | 199.6           | 3.34 q (8.0) | 75.3 | 5.66 d (16.0) | 114.0 |
| 7    | 80.2            | 3.46 m | 74.2 | 6.23 dq (16.0, 7.0) | 144.0 |
| 8a   | 6.19 d (15.6)   | 138.2| 1.62 m         | 33.6 | 1.77 d (7.0)   | 19.2  |
| 8b   | 1.57 m          |      | 1.69 m         |      | 4.36 m         | 111.7 |
| 9    | 5.99 dd (15.6, 2.4) | 113.6| 2.34 m         | 16.9 |                |
| 10   | 86.4            |      | 88.8           |      |                |
| 11   | 91.2            |      | 80.6           |      |                |
| 12   | 111.9           |      | 112.6          |      |                |
| 13   | 140.8           |      | 138.9          |      |                |
| 14   | 18.7            |      | 18.7           |      |                |

aNMR data (δ) were measured in MeOH-d$_4$ for 2, 3, and 4 at 500 MHz for $^1$H NMR and at 125 MHz for $^{13}$C NMR and 1, 5 at 600 MHz for $^1$H NMR and at 150 MHz for $^{13}$C NMR. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, $^1$H-$^1$H COSY, HSQC, and HMBC experiments.

Table 2  $^1$H and $^{13}$C NMR spectral data (δ) for compounds 4 and 5.  

| No.  | δH             | δC   | δH             | δC   | δH             | δC   |
|------|----------------|------|----------------|------|----------------|------|
|      | (δH, J H,H)    |      | (δH, J H,H)    |      | (δH, J H,H)    |      |
| 1a   | 176.8          |      | 178.9          |      |                |
| 1b   | 2.40 t (7.5)   | 35.4 | 2.25 t (7.2)   | 35.7 |                |
| 2    | 2.47 t (7.5)   | 16.4 | 1.59 quin (7.2) | 26.3 |                |
| 3a   | 1.33 m         |      | 1.33 m         | 30.1 |                |
| 3b   | 87.6           |      | 80.7           | 30.3 |                |
| 4    | 1.37 dd (16.0, 1.5) | 112.4| 1.39 quin (7.2) | 30.3 |                |
| 5    | 5.95 dq (16.0, 6.5) | 139.4| 2.07 q (7.2)   | 33.5 |                |
| 6a   | 1.67 dd (6.5, 1.5) | 18.7 | 5.65 dt (15.0, 7.2) | 131.1 | 131.1 |
| 6b   | 6.04 dd (15.0, 4.2) | 131.0| 6.18 dd (15.0, 4.2) | 132.5| 132.5 |
| 7    | 5.68 dt (15.0, 6.0) | 135.6| 6.05 d (6.0)   | 63.4 |                |

aNMR data (δ) were measured in MeOH-d$_4$ for 4 at 500 MHz for $^1$H NMR and at 125 MHz for $^{13}$C NMR and 5 at 600 MHz for $^1$H NMR and at 150 MHz for $^{13}$C NMR. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, $^1$H-$^1$H COSY, HSQC and HMBC experiments.

Nicolet 5700 FT-IR microscope instrument (FT-IR microscope transmission) (Thermo Electron Corporation, Madison, USA). NMR spectra were obtained at 500 MHz or 600 MHz for $^1$H NMR, and 125 MHz or 150 MHz for $^{13}$C NMR, respectively, on Inova 500 or SYS 600 (Varian Associates Inc., Palo Alto, USA) or Bruker 600 NMR spectrometers (Bruker Corp. Switzerland) in MeOH-d$_4$, with solvent peak used as references. ESI-MS and HR-ESI-MS data were measured using an AccuToFCS JMS-T100CS spectrometer (Agilent Technologies, Ltd., Santa Clara, USA). Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), and CHP 20P (Mitsubishi Chemical Inc., Tokyo, Japan). HPLC separation was performed on an instrument consisting of an Agilent ChemStation for LC system, an Agilent 1200 pump, and an Agilent 1100 single-wavelength absorbance detector (Agilent Technologies, Ltd.) with a YMC-Pack Ph (250 mm × 10 mm, i.d.) column packed with Phenyl-silica gels (5 μm) (YMC Co., Ltd.,
Kyoto, Japan) or a Grace (250 mm / C2 10 mm, i.d.) semipreparative column packed with C18 reversed phase silica gel (5 μm) (W.R Grace & Co., USA). TLC was carried out with glass precoated silica gel GF254 plates (Qingdao Marine Chemical Inc.). Spots were visualized under UV light or by spraying with 7% H2SO4 in 95% EtOH followed by heating. Unless otherwise noted, all chemicals were obtained from commercially available sources and were used without further purification.

4.2. Plant material

The roots of C. pilosula were collected in October 2012 from the culture field in Weiyuan, Gansu Province, China. Plant identity was verified by Mr. Lin Ma (Institute of Materia Medica, Beijing, China). A voucher specimen (No. ID-S-2503) was deposited at the herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Beijing, China.

4.3. Extraction and isolation

The dried and minced roots of C. pilosula (50 kg) were extracted with H2O (150 L, 3 × 1 h). The aqueous extracts were evaporated under reduced pressure to yield a dark brown residue (26 kg). The residue was dissolved in H2O (100 L), loaded on a macroporous adsorbent resin (HPD-110, 20 L) column (20 cm × 200 cm), and eluted successively with H2O (100 L), 50% EtOH (120 L), and 95% EtOH (80 L) to yield three corresponding fractions, A, B and C. Fraction C (31.0 g) was subjected to CC over silica gel, with elution by a gradient of increasing acetone concentration (0–100%) in petroleum ether, to yield fractions C1–C18 based on TLC analysis. Fraction C10 (1.0 g) was separated by CC over Sephadex LH-20, eluted with petroleum ether–CHCl3–MeOH (5:5:1, v/v/v), to give C10–1–C10–8. Hexadecanoicacid-2,3-dihydroxy propyl ester (7.0 mg) was precipitated from C10–3 (MeOH). Fraction C10–6 (80 mg) was separated by reversed-phase (Phenyl-silica gel) semipreparative HPLC, using MeOH–H2O (40:60, v/v) as the mobile phase (1.5 mL/min, UV 254 nm), to yield 1 (1.0 mg, tR = 36 min). Fraction C12 (0.8 g) was separated by CC over Sephadex LH-20, eluted with petroleum ether–CHCl3–MeOH (5:5:1, v/v/v), to give C12–1–C12–8, of which C12–6 (100 mg) was separated by reversed-phase (phenyl-silica gel) semipreparative HPLC, using MeOH–H2O (42:58, v/v) as the mobile phase (1.5 mL/min, UV 254 nm), to yield 4 (1.1 mg, tR = 23 min). Fraction C12–8 (60 mg) was isolated by preparative TLC, using petroleum ether–ethyl acetate (1:1, v/v) containing 0.1% HOAc as the mobile phase, to give (10E)-12-hydroxydodeca-10-enoic acid (6.9 mg). Fraction C14 (1.0 g) was separated by CC over Sephadex LH-20, eluted with petroleum ether–CHCl3–MeOH (5:5:1, v/v/v), to give C14–1–C14–8. Fraction C14–4 (80 mg) was separated by reversed-phase (C18) semipreparative HPLC, using MeOH–H2O (62:38, v/v) as the mobile phase (1.5 mL/min, UV 220 nm), to

Figure 2 The 1H–1H COSY (thick line) and key HMBC (arrows) correlations of compound 1.

Figure 3 The NOE enhancements induced by irradiation of H-3b (dashed arrows) for compound 1.

Figure 4 Absolute configuration of compound 1.

Figure 5 The experimental CD spectrum of 1 (black) and the calculated ECD spectra of (2R,7S)-1 (dashed blue) and (2S,7R)-1 (dashed red).

Figure 6 Diagnostic ΔδSRS values (δR−δS, blue data in ppm, left upper) and applied model for tri-MPA esters of compound 2.
yield 2 (1.0 mg, \( t_R = 26 \) min) and 3 (20 mg, \( t_R = 23 \) min). Fraction C_{14-6} (60 mg) was purified by reversed-phase (C18) semipreparative HPLC, using MeOH-H2O (63:37, v/v) containing 0.1% HOAc as the mobile phase (1.5 mL/min, UV 220 nm), to yield 5 (1.7 mg, \( t_R = 42 \) min). Fraction C_{17} (1.1 g) was separated by CC over Sephadex LH-20, eluted with CHCl3-1-MeOH (1:1, v/v), giving C_{17}-1→C_{17}-6. Fraction C_{17}-4 (300 mg) was separated by CC over silica gel, eluted by a gradient of increasing MeOH concentration (0–100%) in CHCl3, yielding C_{17}-4→C_{17}-8 based on TLC analysis. Full gelic acid (13.0 mg) was precipitated from C_{17}-4 (MeOH). Penicillic acid (15.0 mg) was precipitated from C_{17}-6 (MeOH). Purification of B3-2-1 (100 mg) by RP HPLC (C18) (49% CH3OH in H2O), afforded (8R,7R,12E)-tetradeca-4,12-trien-10-yn-1,6,7-triol (2.0 mg, 1.5 mL/min, UV 220 nm, \( t_R = 51 \) min).

4.3.1. (a)-2(7R,5R)-1,7-dihydroxytetradeca-4,8,12-trien-10-yn-6-one (1)

Yellowish amorphous powder; \([\alpha]_D^{19} +10.8 (c 0.07, MeOH); UV (MeOH) \( \lambda_{max} (log e) = 270 (3.22) \) nm; CD (MeOH) 256 (\( \Delta \epsilon = -4.71 \)), 299 (\( \Delta \epsilon = 1.41 \)) nm; IR \( \nu max = 3395, 3017, 2920, 2150, 2061, 1721, 1684, 1647, 1420, 1134, 1120, 1092, 957, 648 \) cm\(^{-1}\); \( ^1\)H NMR (CD3OD, 250 MHz) data, see Table 1; \( ^{13}\)C NMR (CD3OD, 150 MHz) data, see Table 1; (+)-ESI-MS \( m/z = 255 [M+Na]^+ \), 271 [M+K]*; HRESI-MS \( m/z = 255.0999 [M+Na]^+ \) (Calcd. for C17H16O4Na, 255.0992).

4.3.2. (a)-6(8R,7R,12E)-tetradeca-12-en-10-yn-1,6-triol (2)

Yellowish amorphous powder; \([\alpha]_D^{19} +41.5 (c 0.06, MeOH); UV (MeOH) \( \lambda_{max} (log e) = 227 (3.60), 255 (3.77), 229 (4.30) \) nm, \( \nu max 3200, 3017, 2922, 2847, 2173, 1571, 1462, 1439, 986, 725 \) cm\(^{-1}\); \( ^1\)H NMR (CD3OD, 250 MHz) data, see Table 1; \( ^{13}\)C NMR (CD3OD, 150 MHz) data, see Table 1; (+)-ESI-MS \( m/z = 235 [M+Na]^+ \), 251 [M+K]*; (–)-ESI-MS \( m/z = 211 [M–H]^- \); HRESI-MS \( m/z = 235.1296 [M+Na]^+ \) (Calcd. for C_{16}H_{16}O_{12}Na, 235.1305).

4.4. ECD calculation of 1

Conformational analysis of 1 was performed with the MMFF94 molecular mechanics force field using Molecular Operating Environment (MOE) software package\(^{26}\). The lowest-energy conformers having relative energies within 2 kcal/mol were optimized with the Gaussian09 program\(^{11}\) at the B3LYP/6-31+G(d) level in MeOH (Fig. S1, Supporting information). The stabilities of these conformers were confirmed by harmonic vibrational frequency calculations at the B3LYP/6-31+G(d) level. The energies, oscillator strengths, and rotational strengths of the electronic transitions of the lowest-energy conformers were calculated using the TDDFT method at the B3LYP/6-311++G(2d,2p) level in MeOH, and ECD spectra were then simulated by the Gaussian function. The final ECD spectrum of 1 was obtained according to Boltzmann weighting of each conformer.

4.5. Synthesis of tri-(R)- and tri-(S)-MPA esters of 2

Compound 2 (0.3 mg) was stored in 10 mL round-bottomed flask and dried under vacuum. Anhydrous CH2Cl2 (3.0 mL) and R-MPA or S-MPA (5.3 mg), 1-ethyl-3-(3-dimethyloxallyl) carbodiimide hydrochloride (EDCI) (6.1 mg), and 4-dimethylaminopyridine (DMAP) (3.9 mg) were added to the round-bottomed flask. The reaction round-bottomed flask was permitted to stand at room temperature for 15 min. The residue obtained after evaporation of the solvent was applied to preparative TLC (petroleum ether-acetone (3:1, v/v)) to give 2-tri-(R)-MPA and 2-tri-(S)-MPA. 2-tri-(R)-MPA: \( ^1\)H NMR (600 MHz, CD3OD) \( \delta_H = 7.28–7.37 (15H, m, aromatic H), 5.96 (1H, dq, \( J = 15.6, 6.6 \) Hz, H-13), 5.39 (1H, d, \( J = 15.6, 6.6 \) Hz, H-14), 4.95 (1H, m, H-7), 4.80 (1H, overlapped, H-6), 4.79 (1H, s, H-α), 4.77 (1H, s, H-α), 4.74 (1H, s, H-α), 3.80 (1H, dt, \( J = 4.2, 12.6 \) Hz, H-1a), 3.90 (1H, dt, \( J = 12.6, 6.6 \) Hz, H-1b), 3.33 (3H, s, H-αOCH2), 3.31 (3H, s, H-βOCH2), 1.95 (2H, m, H-9), 1.69 (3H, d, \( J = 6.6 \) Hz, H-14), 1.23 (10H, m, H-2, 3, 4, 5, 8); 2-tri-(S)-MPA: \( ^1\)H NMR (600 MHz, CD3OD) \( \delta_H = 7.24–7.40 (15H, m, aromatic H), 5.95 (1H, dq, \( J = 15.6, 6.6 \) Hz, H-13), 5.35 (1H, d, \( J = 15.6, 6.6 \) Hz, H-12), 4.95 (1H, m, H-7), 4.80 (1H, overlapped, H-6), 4.79 (1H, s, H-α), 4.77 (1H, s, H-α), 4.74 (1H, s, H-α), 3.90 (1H, dt, \( J = 12.6, 6.6 \) Hz, H-1a), 3.84 (1H, dt, \( J = 12.6, 6.0 \) Hz, H-1b), 3.33 (6H, s, H-αOCH2), 3.31 (3H, s, H-βOCH2), 1.67 (3H, d, \( J = 6.6 \) Hz, H-14), 1.50 (2H, m, H-9), 1.15 (10H, m, H-2, 3, 4, 5, 8).

4.6. Measurement of MoO\( (OAc)_2\)-induced circular dichroism (ICD) spectrum of 2

According to the published approach, a solution of 2 (0.5 mg) in dry DMSO (1 mL) was mixed with dimolybdenumtetraacetate (1.0 mg). The first CD of the mixture (ca. 1:1 diol/dimolybdenumtetraacetate) was recorded immediately after mixing, and its time evolution was monitored until stationary. The observed sign of the diagnostically band at 316 nm in the ICD was correlated to the 6R,7R-configuration of the 6,7-diol moiety in 2.
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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.apsb.2015.03.005.

References

1. Jiangsu New Medical College. Dictionary of traditional Chinese medicine. Shanghai: Shanghai Sci Technol Publ House 1986:1 p.1837–9.
2. Wang ZT, Du Q, Xu GJ, Wang RJ, Fu DZ, Ng TB. Investigations on the protective action of Codonopsis pilosula (Dangshen) extract on experimentally-induced gastric ulcer in rats. Gen Pharmacol 1997;28:469–73.
3. Shan BE, Yaoshida Y, Sugita T, Yamashita U. Stimulating activity of Chinese medicinal herbs on human lymphocytes in vitro. Int J Immunopharm 1999;21:149–59.
4. Singh B, Song H, Liu XD, Hardy M, Liu GZ, Vinjamury SP, et al. Dangshen (Codonopsis pilosula) and baiguo (Gingko biloba) enhancing learning and memory. Altern Ther 2004;10:52–6.
5. Yoo CS, Kim SJ. Methanol extract of Codonopsis pilosula inhibits inducible nitric oxide synthase and protein oxidation in lipopolysaccharide-stimulated raw cells. Trop J Pharm Res 2013;12:705–10.
6. Tsai KH, Lee NH, Chen GY, Hu WS, Tsai CY, Chang MH, et al. Dung-shen (Codonopsis pilosula) attenuated the cardiac-impaired insulin-like growth factor II receptor pathway on myocardial cells. Food Chem 2013;138:1856–67.
7. Wang ZT, Xu GJ, Hattori M, Namba T. Constituents of the roots of Codonopsis pilosula. Shoyakagaku Zasshi 1988;42:339–42.
8. Thuy TT, Sung TV, Wessjohann L. Chemical constituents of the roots of Codonopsis pilosula. J Chem 2003;41:119–23.
9. He Q, Zhu EY, Wang ZT, Yu GX, Xu LS, Hu ZB. Study on chemical constituents of Codonopsis pilosula. Chin Pharm J 2006;41:10–6.
10. Qi HY, Wang R, Liu Y, Shi YP. Studies on the chemical constituents of Codonopsis pilosula. J Chin Med Mater 2011:34:546–8.
11. Wakan D, Kawahara N, Goda Y. Two new pyrrolidinealkaloids, cocnophosin C and cocnopholides A, isolated from Codonopsis pilosula. Chem Pharm Bull 2013;61:1315–7.
12. Yang CX, Guo YQ, Chen JY, An J, Chen WX, Hu FD. Structural characterization and antitumor activity of a pectic polysaccharide from Codonopsis pilosula. Carbohydr Poly 2013;98:886–95.
13. Sun YX, Liu JC. Structural characterization of a water-soluble polysaccharide from the roots of Codonopsis pilosula and its immunity activity. Int J Biol Macromol 2008;43:279–82.
14. Li ZT, Zhu LB, Zhang H, Yang J, Zhao J, Du DW, et al. Protective effect of a polysaccharide from stem of Codonopsis pilosula against renal ischemia/reperfusion injury in rats. Carbohydr Polym 2012;90:1739–43.
15. Xu C, Liu Y, Yuan GX, Guan M. The contribution of side chains to antitumor activity of a polysaccharide from Codonopsis pilosula. Int J Biol Macromol 2012;50:891–4.
16. Xin T, Zhang FB, Jiang QY, Chen CH, Huang DY, Li YJ, et al. The inhibitory effect of a polysaccharide from Codonopsis pilosula on tumor growth and metastasis in vitro. Int J Biol Macromol 2012;51:788–91.
17. Zhao XN, Hu YL, Wang DY, Liu JZ, Guo LW. The comparison of immune-enhancing activity of sulfated polysaccharides from Tremella and Codonopsis pilosula. Carbohydr Poly 2013;98:438–43.
18. Chen MH, Lin S, Li L, Zhu CG, Wang XL, Wang YN, et al. Enantiomers of an indole alkaloid containing unusual dihydrotropane and 1,2,4-thiadiazole rings from the root of Iasitis indigotica. Org Lett 2012;14:5668–71.
19. Zhao F, Wang SJ, Lin S, Zhu CG, Yue ZG, Yu Y, et al. Natural and unnatural arachnoquiones isolated from the ethanol extract of the roots of Knoxia valerianoides. Acta Pharm Sin B 2012;2:260–6.
20. Yu Y, Zhu CG, Wang SJ, Song WX, Yang YC, Shi JG. Homosecoiridoid alkaloids with amino acid units from the flower buds of Lonicerajaponica. J Nat Prod 2013;76:2226–33.
21. Wang F, Jiang YP, Wang XL, Wang SJ, Bu PB, Lin S, et al. Aromatic glycosides from the flower buds of Lonicerajaponica. J Asian Nat Prod Res 2013;15:492–501.
22. Tian Y, Guo QL, Xu WD, Zhu CG, Yang YC, Shi JG. A minor diterpenoid with a new 6/9/7 fused-ring skeleton from Euphorbia microactina. Org Lett 2014;16:3950–3.
23. Xu WD, Tian Y, Guo QL, Yang YC, Shi JG. Secoephoraritin a minor diterpenoid with a new skeleton from Euphorbia microactina. Chin Chem Lett 2014;25:1531–4.
24. Song WX, Yang YC, Shi JG. Two new β-hydroxy amino acid-coupled secoiridoids from the flower buds of Lonicerajaponica: isolation, structure elucidation, semisynthesis, and biological activities. Chin Chem Lett 2014;25:1215–9.
25. Yu Y, Jiang ZB, Song WX, Yang YC, Li Y, Jiang JD, et al. Glucosylated caffeoylquinic acid derivatives from the flower buds of Lonicerajaponica. Acta Pharm Sin B 2015; in press, 10.1016/j.apsb.2015.01.012.
26. Molecular Operating Environment software package; 2008.10; Chemical Computing Group Inc., www.chemcomp.com.
27. Ye XL. Stereochimistry. Beijing: Peking University Express; 1999 p. 257–9.
28. Li XC, Ferreira D, Ding YQ. Determination of absolute configuration of natural products: theoretical calculation of electronic circular dichroism as a tool. Curr Org Chem 2010;14:1678–97.
29. Freire F, Seco JM, Quinoa E, Rigueria R. Determining the absolute stereochemistry of secondary/diaryls by 1HNMR; basis and applications. J Org Chem 2005;70:3778–90.
30. Frelek J, Ikekawa N, Takatsuto S, Sznazke G. Application of [Mo2(OAc)6] for determination of absolute configuration of brassinosteroiddicbicylic circular dichroism. Chirality 1997;9:578–82.
31. Gorecki M, Jablonska E, Kruszewska A, Suszynska A, Urbanczyk-Lipkowska Z, Gerards M, et al. Practical method for the absolute configuration assignment oftet/tet 1,2-diols using their complexes with Mo2(OAc)6. J Org Chem 2007;72:2906–16.
32. Sungwon H, Yon G, Kang K, Shin SY, Lee YH, Lim Y. NF-κB activation by compounds found in Platycodon grandiflorum extract. J Microbiol Biotechnol 2009;19:556–9.
33. Cameron AG, Knight DY. Model studies on the synthesis of medium-sized and large carboxylic acids by their elandenolate claisen rearrangement. J Chem Soc Perkin Trans 1986;1:161–7.
34. Rahman AU, Sultana N, Shahwar D, Choudhary ML. Two new fatty acesters from the roots (Apocynanaceae). J Org Chem 1999;64:1215–20.
35. Kurashina Y, Miura A, Enomoto M, Kuwahara S. Stereoselective activation by compounds found in Bupleurumlongiradiatum. J Nat Prod 2011;74:1215–20.
36. Shirahata T, Sunazuka T, Yoshida K, Yamamoto D, Harigaya Y, Knoxia valerianoides. J Nat Prod Res 2012;4:52–6.
37. Gaussian 09, Gaussian, Inc., www.gaussian.com.
38. Huang HQ, Zhang X, Shen YH, Sun J, Liu XH, Tian JM, et al. Polyacetylenes from Bupleurum longiradiatum. J Nat Prod 2009;72:2153–7.
39. Lai WC, Wu YC, Danko B, Cheng YB, Hsieh TJ, Hsieh CT, et al. Bioactive constituents of Cirsium japonicum var. austral. *J Nat Prod* 2014;77:1624–31.

40. Qu J, Fang L, Ren XD, Liu YB, Yu SS, Li L, et al. Bisindole alkaloids with neural anti-inflammatory activity from Gelsemium elegans. *J Nat Prod* 2013;76:2203–9.

41. Xiong L, Zhu M, Zhu CG, Lin S, Yang YC, Shi JG. Structure and bioassay of triterpenoids and steroids isolated from Sinocalamus affinis. *J Nat Prod* 2012;75:1160–6.

42. Tian Y, Xu WD, Zhu CG, Lin S, Guo Y, Shi JG. Diterpenoids with diverse skeletons from the roots of Euphorbia microactina. *J Nat Prod* 2013;76:1039–46.

43. Sun J, Shi DY, Li S, Wang SJ, Han LJ, Fan X, et al. Chemical constituents of the red alga Laurencia tristicha. *J Asian Nat Prod Res* 2007;9:725–34.

44. Wang HF, Xie WD, Zhang Z, Xing DM, Ding Y, Wang W, et al. Bioactive compounds from the seeds of Punica granatum (Pomegranate). *J Nat Prod* 2004;67:2096–8.