Triterpenoids with α-glucosidase inhibitory activities from the roots of Codonopsis pilosula var. modesta

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Abstract
A new taraxerane-type triterpenoid, codopimodol A (1), together with two known triterpenoids (2 and 3), were isolated from the EtOH extract of the roots of Codonopsis pilosula var. modesta (Campanulaceae). The structure of the new compound was identified by high-resolution electrospray ionization mass spectrometry and extensive spectroscopic analyses, particularly one-dimensional and two-dimensional NMR and IR. All the compounds were first isolated from C. pilosula var. modesta. Compounds 1 and 3 exhibited potential α-glucosidase inhibitory activities.

Keywords
Codonopsis pilosula var. modesta, codopimodol A, α-glucosidase inhibitory activities, taraxerane, triterpenoid

Introduction
Codonopsis pilosula var. modesta, Codonopsis genus, which belongs to the family of Campanulaceae, is one of the medical plants of “Codonopsis Radix.”¹ As a traditional Chinese medicine (TCM), the roots of the plant have long been used for the therapy of body weakness, poor appetite, thirst, indigestion, chronic diarrhea, anemia, and leukemia.²,³ Modern pharmacological research shows that the “Codonopsis Radix” also has the function of regulating blood sugar.⁴ Previous phytochemical investigations revealed that Codonopsis genus has many chemical constituents such as sesquiterpenes, triterpenes, alkaloids, saponins, flavonoids, and phytosterols.⁵–¹⁰ In order to find out which of the active ingredients in “Codonopsis Radix” regulate blood sugar, C. pilosula var. modesta was chosen as a research subject. A study of “Codonopsis Radix” would also enrich studies on the chemical compositions of other plants in our search for bioactive compounds.¹¹–¹⁵ A new taraxerane-type triterpenoid, codopimodol A (1), together with two known triterpenoids 2 and 3, were identified in the current investigation (Figure 1). Herein, this study describes the isolation and structural elucidation of these compounds from C. pilosula var. modesta and their α-glucosidase inhibitory activities.

Results and discussion
Compound 1 was isolated as a white amorphous powder, with optical rotation [α]D20 = -1.1 (c 0.90, MeOH). The molecular formula of 1 was determined as C30H50O2 on the basis of high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) (m/z 443.3880 [M +H]⁺, calcd for 443.3884) and NMR data (Table 1), indicating six degrees of unsaturation. The IR spectrum of 1 showed absorption bands for OH groups (3443 cm⁻¹). The 1H NMR spectrum of 1 displayed resonances for a olefinic proton at δH 5.53 (1H, dd, J= 7.8, 3.0 Hz, H-15), two oxygenated methines at δH 3.14 (1H, dd, J= 10.8, 5.4 Hz, H-3) and 4.55 (1H, m, H-6), and eight methyl singlets at δH 0.83 (3H, s, H-28), 0.90 (3H, s, H-30), 0.91 (3H, s, H-27), 0.95 (3H, s, H-29), 1.07 (3H, s, H-23), 1.20 (3H, s, H-24), 1.32 (3H, s, H-25), and 1.40 (3H, s, H-26). Analysis of ¹³C NMR and distortionless enhancement by polarization transfer (DEPT) spectra of 1 showed 30 carbon signals including eight methylenes, nine methylenes, six methines (two oxygenated, one sp²), and seven quaternary carbons (one sp²). The double bond accounted for one degree of unsaturation, and the remaining five degrees of unsaturation were assumed for the presence of a pentacyclic system in compound 1.

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The 1H–1H COSY spectrum showed the existence of correlations of H-2-1/H-2-2/H-3, H-5/H-6/H-7, H-9/H-11/H-12, H-15/H-16, H-18/H-19, and H-21/H-22. In the HMBC spectrum of 1, the presences of correlations of H-15/C-8, C-13, C-16, C-17, H-23/C-3, C-4, C-5, C-24, H-24/C-3, C-4, C-5, C-23, H-25/C-1, C-5, C-9, C-10, H-26/C-7, C-9, C-14, H-27/C-12, C-13, C-14, C-18, H-28/C-16, C-17, C-18, C-22, H-29/C-19, C-20, C-21, H-30/C-19, C-20, C-21, and C-29 were observed (Figure 2). It is worth noting that the correlations of δH 5.53 (1H, dd, J = 7.8, 3.0 Hz, H-15) with four carbons at δC 35.8 (C-17), 37.4 (C-13), 37.5 (C-8), and 37.6 (C-16) determine the position of double bond is δC 157.7 (C-14) and 117.2 (C-15), which is the typical characteristic of taraxerane-type triterpenoids. However, compared with taxaroxol, the 13C NMR spectrum of 1 showed an additional methine group at δC 69.0 (C-6) instead of a methylene group, indicating that there was another hydroxy group in compound 1. The above information indicated that compound 1 is a taraxerane-type triterpenoid with two hydroxy groups. Furthermore, the HMBC correlations of δH 3.14 (1H, dd, J = 10.8, 5.4 Hz, H-3) with carbon signals at δC 16.9 (C-24), 27.3 (C-2), 27.6 (C-23), 39.5 (C-4), 39.6 (C-1) and δH 4.55 (1H, m, H-6) with three carbons at δC 37.5 (C-8), 38.4 (C-10), and 56.2 (C-5) verified that the location of two hydroxy groups was attached at C-3 and C-6, respectively.

The relative configuration of 1 was confirmed by the NOESY spectrum, which showed correlations of H-3/H-5, H-3/H-23, and H-6/H-23, revealing the β-orientation of H-3 and H-6 (Figure 3).

The isomers of compound 1, such as daturadiol and 12-oleanene-3β-6α-diol which were oleanane-type triterpenoids with a double bond at C-12 and C-13, have been reported in previous literature. The NMR data of compound 1 were very different from the above two known triterpenoids daturadiol and 12-oleanene-3β-6α-diol. In addition, the application of two-dimensional (2D) NMR technology could well analyze the structure of compound 1. It is
could be potential α-glucosidase inhibitors with IC50 values and enzyme in vitro. 

Compounds pairing their NMR data with those reported in the literature. Of 72.5.

Cycloartane-type triterpenoids have been separated from 3. Taraxerane-type and cycloartane-type triterpenoid (2), and one known ursane-type triterpenoid (18)19 by comparing their NMR data with those reported in the literature. Compounds 2 and 3 were isolated from this plant for the first time.

The three isolated compounds were evaluated for their in vitro α-glucosidase inhibitory activities against the enzyme α-glucosidase, with acarbose as the positive control (Table 2). The results showed that compounds 1 and 3 could be potential α-glucosidase inhibitors with IC50 values of 72.5 ± 2.3 μM and 34.8 ± 1.4 μM, respectively.

Table 2. α-Glucosidase inhibitory activity of compounds 1–3.

| Compound | IC50 (μM) | Compound | IC50 (μM) |
|----------|----------|----------|----------|
| 1        | 72.5 ± 2.3 | 3        | 34.8 ± 1.4 |
| 2        | >200     | Acarbose | 34.8 ± 6.8 |

*IC50 values represent the mean values ± standard deviation (SD) of three parallel measurements.

reasonably to prove that compound 1 was a taraxerane-type triterpenoid and not an oleanane-type triterpenoid. Therefore, the structure of 1 was determined as taraxer-14-ene-3β,6β-diol, namely, codopimodol A.

The two known compounds were identified as α-amyrin caprylate (2)18 and 24-methylenecycloartanol (3)19 by comparing their NMR data with those reported in the literature. Compounds 2 and 3 were isolated from this plant for the first time.

In summary, a phytochemical investigation on C. pilosula var. C. pilosula led to the isolation of three triterpenoids including one new taraxerane-type triterpenoid (1), one known ursane-type triterpenoid (2), and one known cycloartane-type triterpenoid (3). Taraxerane-type and cycloartane-type triterpenoids have been separated from Codonopsis genus before.20 Their structures were determined by extensive spectroscopic methods. To the best of our knowledge, compounds 2 and 3 were isolated from C. pilosula var. modesta for the first time, and compound 2 was separated from the Codonopsis genus for the first time. Previous biological investigations of compounds 2 and 3 have demonstrated anti-inflammatory,21 anticonvulsant,21 and antifungal22 activities of 2, and anti-diabetes,23 anti-inflammatory,24 and antitubercular25 activities of 3.

Compared to acarbose, compounds 1 and 3 showed inhibitory activity against α-glucosidase with the IC50 values of 72.5 ± 2.3 μM and 34.8 ± 1.4 μM, respectively, indicating that these two compounds have potential α-glucosidase inhibitory activities. However, compound 2 did not show inhibitory activity against α-glucosidase even at the concentration of 200 μM.

Experimental

General experimental procedures

Optical rotation was measured on a Perkin Elmer 341 polarimeter. IR spectra were obtained on a Nicolet NEXUS 670 FTIR spectrometer in KBr. 1H, 13C NMR (DEPT), and 2D NMR spectra were recorded on the Bruker Avance NEO-600 (Bruker, Germany) and Varian Mercury-300 BB (300 MHz). HR-ESI-MS spectra were obtained with a Thermo LTQ-Orbitrap Elite mass spectrometer (Thermo, USA). Silica gel (200-300 mesh) used for thin layer chromatography (CC) and silica gel GF254 (10–40 μm) used for thin layer chromatography (TLC) were purchased from Qingdao Marine Chemical Factory in China. MCI GEL CHP 20P (75–150 μm) was purchased from the Mitsubishi Chemical Holdings in Japan. Sephadex LH-20 was supplied by the GE Healthcare Biosciences AB, Uppsala, Sweden. The purity of all compounds was inspected by TLC under UV light at 254 nm and heating after spraying with 5% H2SO4 in C6H5OH (V/V).

Plant material

The roots of C. pilosula var. modesta were collected in July 2018 from Wenxian district, Gansu province, P.R. China, and were identified by Professor Fang-Di Hu (from the School of Pharmacy, Lanzhou University). A voucher specimen (accession no. CPM201807) has been deposited in the Laboratory of Phytochemistry, School of Pharmacy, Lanzhou University.

Extraction and isolation

The air-dried and powdered roots of C. pilosula var. modesta (21 kg) were extracted with 95% EtOH (3 x 70 L, 7 days, each) at room temperature. The extract was concentrated under reduced pressure to obtain a crude brown residue (7.5 kg), which was suspended in H2O and extracted successively with EtOAc and n-butanol, respectively. The EtOAc extract (265 g) was subjected to silica gel CC, eluted with a gradient of petroleum ether-acetone (from 40:1 to
1:1, V/V) to acquire six fractions (Fr.1–Fr.6). Fr.3 was divided into two parts (Fr.3.1 and Fr.3.2). Fr.3.1 (16.5 g) was subjected to silica gel CC (petroleum ether-acetone, from 40:1 to 1:1) to obtain five fractions (Fr.3.1.1–Fr.3.1.5). Fr.3.1.5 (3 g) was subjected to Sephadex LH-20 column (CHCl₃-MeOH, 2:3, V/V) to give 3 (40 mg). Fr.4 (35 g) was separated over MCI gel CC using MeOH-H₂O (from 3:7 to 10:0, V/V) to obtain four fractions (Fr.4.1–Fr.4.4). Fr.4.2 (7.4 g) was purified by silica gel CC (petroleum ether-acetone, from 100:1 to 1:1, V/V) and different concentrations of the drugs using as the therapy for type-II diabetes, was chosen as the positive control. The positive control acarbose was incubated for 30 min at 37 °C. Finally, 80 μL of PBS, 20 μL of test sample, the difference between test groups and negative control and the absorbance of the released product (α-nitrophenyl-β-D-glucopyranoside (α-D-glucopyranoside (α-nitrophenyl-NPG) (15 mM, 0.2 M, dissolved in PBS) was added to terminate the reaction and the absorbance of the released product (α-nitrophenol) was recorded at 405 nm by the microplate reader. The α-glucosidase inhibition ratio (%) is calculated as follows

\[
\frac{(OD_{\text{negative}} - OD_{\text{negative blank}})}{(OD_{\text{test}} - OD_{\text{test blank}})} \times 100\%
\]

All tests were accomplished in triplicate, and IC₅₀ values were calculated through the probit regression using IBM SPSS Statistics 21.0.

**Declaration of conflicting interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**Supplemental material**

Supplemental material for this article is available online.

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