Disesquiterpenoid and Sesquiterpenes from the Flos of *Chrysanthemum indicum*

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One new disesquiterpenoid (1), two new sesquiterpenoids (2, 3), were isolated from the dry flos of *Chrysanthemum indicum*. The structures were elucidated by spectroscopic analysis, the absolute configurations of 1 and 3, were determined by single X-ray diffraction study.

Key words  *Chrysanthemum indicum*; disesquiterpenoid; sesquiterpene; X-ray crystallography

*Chrysanthemum indicum* Linné, a perennial herb belonging to the family Compositae. It is a cross-pollination species, which can reproduce both sexually by out-crossed seeds and asexually through rhizomes.\(^1\)\(^2\) Pollens and seeds of this species are dispersed by wind or animals. It is one of the most widespread species in the genus Dendranthema ranging from north of Russia to the Southeastern part of Asia and Europe. In China, this species covers a broad geological range, distributing in nearly the entire mainland except for the western desert area. It is confined to mountain slope and grass land, shrubs, wetlands, coast saline land and roadside.\(^3\) It has been used for the treatment of inflammation, headache, ulcerative diseases.\(^4\)\(^9\) Modern pharmacological research demonstrated that the extracts of *C. indicum* also possess certain anti-tumor properties associated with the presence of flavonoids, terpenoids, phenolic compounds and several other chemical constituents.\(^10\)\(^13\)

The aim of the present study was to establish a further insight into the phytochemical constituents of the title plant.

Results and Discussion

The ethanolic extract of *C. indicum* was partitioned with CH\(_2\)Cl\(_2\), then chromatographed on a combination of silica gel, octadecyl silica (ODS), and finally purified by preparative HPLC to yield compounds 1–3 (Fig. 1).

Compound 1 was obtained as colorless crystals (MeOH–H\(_2\)O). Its molecular formula was determined to be C\(_{32}\)H\(_{40}\)O\(_9\) by high resolution electrospray ionization mass spectrometry (HR-ESI-MS), as indicated by pseudo-molecular ions that the extracts of *Chrysanthemum* were used for the treatment of inflammation, headache, ulcerative diseases, and respiratory diseases.\(^4\)\(^9\) Modern pharmacological research demonstrated that the extracts of *C. indicum* also possess certain anti-tumor properties associated with the presence of flavonoids, terpenoids, phenolic compounds and several other chemical constituents.\(^10\)\(^13\)

The aim of the present study was to establish a further insight into the phytochemical constituents of the title plant.

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of H-6’/Me-15’, H-6’/H-9’α, H-9’α/Me-14’, Me-14’/Me-15’, indicated that they were co-facial, and were arbitrarily assigned to be in α-orientation, and a β configuration of 10-OH. Likewise, the ROESY correlations of H-7/H-5, H-7/H-8β, and H-9β/H-8β, suggested that they were β-oriented.

A single crystal of 1 (Fig. 3) was obtained and analyzed on an X-ray diffractometer with a mirror CuKα (λ=1.54184 Å) radiation (ω scans, 2θ max=71.94). By anomalous dispersion methods with Flack=0.02 (14),15) the absolute configuration of 1 was determined to be 1R, 3R, 5R, 6R, 7R, 8S, 10R, 11R, 1’R, 4’R, 5’S, 6’R, 7’S, and 10’R.

Compound 2 was isolated as a white, amorphous powder. Its molecular formula, C15H26O3, was established by HR-ESI-MS (m/z 277.1772 [M+Na]+), indicating 3 degrees of unsaturation. The 1H-NMR spectrum (Table 2, in DMSO-d6) showed three singlet tertiary methyls (δH 0.92, 1.08, 1.11), two exocyclic

\[ \text{Table 1. 1H-NMR (500 MHz) and 13C-NMR (125 MHz) Data of 1 in Methanol-d}_4 \]

<table>
<thead>
<tr>
<th>Position</th>
<th>( \delta_c )</th>
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<th>( \delta_c )</th>
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\( a \) Signal pattern unclear due to overlapping.

Fig. 1. Structures of Isolated Compounds

Fig. 2. Selected HMBC and ROESY Correlations of 1
olefinic protons (δ_H 4.69, 4.61), two oxygenated protons (δ_H 4.12, 3.23), two exchangeable hydroxy signals [δ_H 4.62 (OH-5) and 4.50 (OH-10)]. The 13C-NMR spectrum (Table 3), aided with HSQC technique, showed the presence of three methyls, six methylenes (one olefinic), three methines (two oxygenated), three quaternary carbons (one olefinic and two oxygenated). From the 1H–1H correlation spectroscopy (COSY) spectrum (Fig. 4), the partial structures of CH_3–CH_2–CH (1), CH (1)–CH_2 (6), CH (1)–CH (5), and CH_2 (8)–CH_2 (9)–CH (10) were established. HMBC correlations from Me-14 (δ_C 23.4) to C-6 (δ_C 70.6 (C-10)) allowed the location of the remaining hydroxyl group (δ_C 74.2 (C-11)). The HMBC cross peaks from the oxygenated tertiary carbon δ_C 74.1 and C-7 δ_C 74.5 demonstrated the connection of a gem-dimethyl group at C-11. The HMBC correlations from H_2-8 and H_1-9 to the oxygenated methine carbon δ_C 151.3 established the planar structure of 2 (Fig. 4).

The relative configuration of 2 was mainly deduced from the analysis of its ROESY correlations. As shown in Fig. 4, the observed nuclear Overhauser effect (NOE) correlation from H-1 to Me-14, together with the cross-peaks of Me-12 with OH-5, OH-10, and Me-14, indicated that H-1, OH-5,
OH-10, Me-12, and Me-14 were co-facial and arbitrarily assigned as in β-orientation, and accordingly, the α-orientation of H-5 and H-10. In consequence, the ROESY correlation between H-10 and Me-13 determined Me-13 to be α-directed.

Compound 3 was obtained as colorless crystals (MeOH/H2O). Its HR-ESI-MS exhibited a pseudo-molecular ion at m/z 335.1832 [M+Na]+ (Calcd for C17H28O5Na, 335.1829), consistent with a molecular formula of C17H28O5, indicating 4 degrees of unsaturation. The IR spectrum showed absorptions at 3455, 3379, 1728, and 1695 cm⁻¹, suggesting the presence of hydroxyl and carbonyl groups. The 1H-NMR spectrum of 3 exhibited 28 protons: including three methine protons (δH 4.74, 3.46, 1.64), five methyl protons (δH 2.17, 2.01, 1.18, 1.17, 0.95), two exchangeable hydroxy protons (δH 3.28, 3.13), and four methylene protons. The 13C-NMR spectrum of 3 gave signals for five quaternary carbons (δC 209.0, 170.9, 84.5, 72.0, 50.5), three methine carbons (δC 83.0, 56.6, 46.9), four methylene carbons (δC 36.2, 34.0, 32.7, 23.3), five methyl carbons (δC 31.8, 28.2, 27.8, 21.5, 14.3). These groups accounted for two out of four degrees of unsaturation, the remaining two degrees of unsaturation suggest that 3 contains two rings. The 1H–1H COSY spectrum (Fig. 5) indicated the partial structures of CH (1)–CH2 (2)–CH (3), CH2 (6)–CH (7)–CH2 (8), and CH2 (8)–CH2 (9). Deducting the acetyl group, the remaining 15 carbons and the NMR data (Tables 2, 3) suggested compound 3 to be a sesquiterpene, similar to lucinone, which was an eudesmanoid-type terpene, isolated from Jasione glutinosa. In the HMBC experiment (Fig. 5), correlations from H-15 (δH 2.17) to C-3 and C-4, and from H-1 (δH 4.74) to the ester carbonyl carbon located the ketone carbonyl and the acetyl at C-4 and C-1, respectively. The presence of gem-dimethyl (Me-12, -13) groups linked to a quaternary carbon at δC 72.0 (C-11) was determined by HMBC cross-peaks from both Me-12 (δH 1.18) and Me-13 (δH 1.17) to C-7 and C-11. HMBC correlations from Me-14 (δH 0.95) to C-1, C-5, C-9, and C-10, assigned the remaining methyl to be at C-10. The hydroxyl proton at δH 3.13 correlated with C-3 and C-5 in the HMBC spectrum, assigned itself to C-5. On the basis of the above spectroscopic analysis, the gross structure of 3 was established as shown in Fig. 5.

The relative configuration of compound 3 was mainly determined by a ROESY experiment (Fig. 5), in which the correlation of H-1/H-9α, indicated that they were co-facial, and were arbitrarily assigned as an α-orientation. The cross-peaks of Me-14 with H-9β, H-6β, OH-5, indicated that they were assigned to be β-orientation. The ROESY correlations of H-αα/H-7, H-3/H-7, suggested that they were α-oriented.

Fortunately, a single crystal of 3 (Fig. 6) was analyzed on an X-ray diffractometer with a mirror CuKα (λ=1.54184 Å) radiation (ω scans, 2θmax=72.26). By anomalous dispersion methods with Flack=0.02 (13), the absolute configuration of 3 was determined to be 1R, 3S, 5S, 7R, and 10S. 

Experimental
General Experimental Procedures
The melting point was recorded on an XT-4 micromelting point apparatus, uncorrected. Optical rotations were determined with a JASCO P-1020 polarimeter (Na filter, λ=589 nm). IR spectra were recorded on a Bruker Tensor 27 spectrometer with KBr-disks. Mass spectra were obtained on a MS Agilent 1100 Series LC/MSD Trap mass spectrometer (ESI-MS) and an Agilent 6520B Q-TOF spectrometer (HR-ESI-MS), respectively. NMR spectra were recorded on a Bruker AVANCE-III NMR instrument (1H: 500 MHz, 13C: 125 MHz) with tetramethylsilane (TMS) as internal standard. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd., China), and RP-C18 (40–63 μm, FUji, Japan) were used for column chromatography (CC). Preparative HPLC was carried out using Agilent 1100 Series with a Shimpack RP-C18 column (20×200 mm, i.d.) and 1100 Series Multiple Wavelength detector. All solvents used were of analytical grade (Jiangsu Hanbang Sci. and Tech. Co., Ltd., China). Silica gel GF254 plates (Qingdao Marine Chemical Co., Ltd., China) were used for thin-layer chromatography (TLC), and spots were visualized by heating silica gel plates immersed in vanillin–H2SO4 in ethanol.

Plant Material
The air-dried flos of C. indicum (7.0 kg) were purchased from Bozhou, Anhui Province, China, in May 2010, and were identified by Prof. Min-jian Qin, Department of Pharmacognosy, China Pharmaceutical University. Specimen (No. CI-Flos-2010-ZJ) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.
Extraction and Isolation The dry flos of *C. indicum* (7.0 kg) were extracted with 95% EtOH under reflux three times (14 L × 3, 3 h, 3 h, 2 h). After filtration, the extraction solution were combined and dried at reduced pressure to yield 1.0 kg crude extract. The product was suspended in H2O, and was sequentially extracted with petroleum ether, CH2Cl2, and EtOAc. The CH2Cl2 extract (193 g) was chromatographed over a silica gel column, by gradient elution with MeOH–CH2Cl2 (0 : 100, 1 : 99, 2 : 98, 5 : 95, 10 : 90, 20 : 80, 100 : 0) to yield 7 fractions [A (17.4 g), B (13.0 g), C (26.1 g), D (18.4 g), E (8.2 g), F (27.0 g), G (30.9 g)], combined according to TLC analysis. They were fractionated by CC over D101 porous resin with a gradient of H2O–EtOH to give different fractions (A1, A2, B10, B90, C100, D90, D90, E90, F90, F70, and F60). A2 was separated further on a reversed-phase (RP) C18 open column eluted with MeOH–H2O, resulting in a fraction containing mainly 3, which was rechromatographed on a RP-C18 silica gel CC followed by crystallization from MeOH to give compound 3 (92.3 mg). Compound 2 (2.0 mg) was isolated from fraction B90 by a RP-C18 silica gel CC with MeOH–H2O. Fraction D70 was also subjected to RP-C18 silica gel CC to get five subfractions (D70, D60, D50, D40, D30). D70 was purified by HPLC eluted with MeOH–H2O (60 : 40) and by crystallization from MeOH to give 1 (7.8 mg).

Compound 1: Colorless crystals (MeOH–H2O). mp 121.5–122.5 °C. [α]D23 = −26.5 (c = 0.15, MeOH). IR (KBr) cm⁻¹: 3418, 2991, 2973, 2953, 2935, 1641, 1460, 1400, 1377, 1226, 1105, 1030, 982, 890. 1H- and 13C-NMR spectroscopic data, see Table 1. HR-ESI-MS [M + Cl]⁻ (Calcd for C17H28O5NaCl, 335.1829), 337.0 (3) Å; the crystal dimensions 0.36×0.33×0.31 mm; the final indices were R₁ = 0.0386, wR₂ = 0.1091. Colorless crystals of I were obtained in a mixed solvent of methanol and water.

Crystal data for Compound 3: formula C32H40O9Cl; M₁ = 560.74; Monoclinic crystal system; space group P2₁/n; a = 8.1136 (1) Å, b = 23.6618 (3) Å, c = 9.1913 (1) Å; V = 1687.15 (4) Å³; Z = 2; Dcalcd = 1.281 mg/m³; crystal dimensions 0.36×0.33×0.31 mm; the final indices were R₁ = 0.0386, wR₂ = 0.1091. Colorless crystals of 3 were obtained in a mixed solvent of methanol and water.

Crystal data were obtained on a Bruker Smart-1000 CCD with a graphite monochromator with CuKα radiation at (λ = 1.54184 Å) 291(2)K. The structures were determined by direct methods using SHELX-97 and expanded using difference Fourier techniques, refined by SHELX-97 (CCDC-864120 (I), CCDC-864119 (3), contain the supplementary crystallographic data for this paper. Copies of the data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K. [fax: (+44) 1223–336–033; or e-mail: deposit@ccdc.cam.ac.uk].

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