Structurally Diverse Terpenoids from the Rhizomes of *Cyperus rotundus* L.

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**Key words**

- *Cyperus rotundus*
- Cyperaceae
- terpenoids
- single-crystal X-ray diffraction analysis

**Abstract**

An extract of the rhizomes of *Cyperus rotundus* L. afforded two new sesquiterpenoids (2 and 3) with rearranged secoeudesmane and germacrane skeletons, and a new 9,10-seco-cycloartane triterpenoid (9), as well as seven previously reported terpenoids, including a monoterpenoid (1), five sesquiterpenoids (4–8) with guaiane, patchoulan, and eudesmane skeletons, and a 3,4-seco-dammarane nortriterpenoid (10). The structures of 1–10 were elucidated by extensive spectroscopic methods and comparison with the literature data. The structures of 2 and 6 were confirmed by single-crystal X-ray diffraction analysis. The carbon skeleton of 2 is the third example reported thus far of such a skeleton and the 9,10-seco-cycloartane framework of 9 has rarely been found from a natural source. Compound 7 was a new natural product and compounds 1–5 and 7–10 were discovered from the genus *Cyperus* for the first time. This study may provide some useful information for the chemotaxonomy for the plant *Cyperus rotundus*.

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**Introduction**

*Cyperus rotundus* L. (Cyperaceae), a perennial herb, is widespread in the tropical and subtropical regions all over the world [1,2]. The rhizomes of *C. rotundus* L., called Xiang-Fu in the traditional Chinese medicines (TCMs), have been used as hypotensive, estrogenic, anti-inflammatory, antipyretic, tonic, stomachic, sedative, and antiemetic agents for the treatment of dysentery and women’s disease for a long time [1–4]. Previous investigations showed that the phytochemical constituents of *C. rotundus* L. contained terpenoids [1,5–7], steroids [2], flavonoids [2,3], and cerebrosides [4]. Among them, the sesquiterpenoids are the characteristic secondary metabolites [1]. Up till now, a series of sesquiterpenoids possessing diverse skeletons, such as patchoulan, rotundane, eudesmane, guaiane, cadinane, caryophyllane, clavane, and copane, have been discovered from *C. rotundus* L. [1,5–7].

In recent years, we have put our efforts to the discovery of secondary metabolites with various frameworks, in particular terpenoids, from TCMs [8–13]. As part of this program, and considering the presence of terpenoids with diverse skeletons in this plant, a NMR-guided chemical investigation of the rhizomes of *C. rotundus* L. was carried out in our lab in order to discover new terpenoids with novel frameworks. As a result, a novel rearranged secoeudesmane sesquiterpenoid (2), a new germacrane sesquiterpenoid (3), and a new 9,10-seco-cycloartane triterpenoid (9), as well as seven known compounds, including a monoterpenoid (1), two guaiane sesquiterpenoids (4,5), a patchoulan sesquiterpenoid (6), two eudesmane sesquiterpenoids (7,8), and a 3,4-seco-dammarane nortriterpenoid (10), have been isolated (Fig. 1). Compound 7 was discovered from a natural source for the first time. The rearranged secoeudesmane skeleton of 2 is the third example reported thus far of such a skeleton and the 9,10-seco-cycloartane framework of 9 from a natural source has rarely been reported. Here, we report the isolation and structure elucidation of these terpenoid natural products.
Materials and Methods

Apparatus

Optical rotations were measured using a PerkinElmer model 341 polarimeter. UV spectra were measured on a New Century T6 spectrophotometer, supplied by PGeneral. CD spectra were recorded on an Olis DSM 1000 circular dichroism spectrometer. IR spectra were obtained on a Nicolet NEXUS 670 FT-IR spectrometer, supplied by GMI. NMR spectra were recorded on a Bruker AVANCE III-400 spectrometer. Chemical shifts are given as δ (ppm) using TMS as the internal standard. HR ESI-MS was performed on a Bruker APEX II mass spectrometer or a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer, supplied by Bruker. Single-crystal X-ray diffraction analysis was performed on a CAD4SDP-44 X-ray diffractometer, supplied by Enraf Nonius. Silica gel (200–300 mesh) used for column chromatography (CC) and silica GF254 (10–40 µm) for thin-layer chromatography (TLC) were both supplied by Qingdao Marine Chemical Factory. The RP-18 silica gel used for CC was supplied by YMC. Sephadex LH-20 was supplied by Amersham Biosciences. The analytical reagents, used in the extraction and isolation process, were supplied by Tianjin Chemical Reagent Company. The spots on TLC were detected at 254 nm UV light and visualized by spraying with 98% H2SO4-C2H5OH (5:95, v/v) followed by heating.

Plant material

The dried rhizomes of *C. rotundus* L. were purchased from Lanzhou Traditional Chinese Medicine Market in 2009 and identified by Associate Professor Huan-Yang Qi, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, China. Voucher specimens (No. ZY2009C002) were deposited in the Key Laboratory of Chemistry of Northwestern Plant Resources, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, China.

Extraction and isolation

The dried and powdered rhizomes of *C. rotundus* L. (9.0 kg) were extracted with EtOH (95%) three times (3 hours for each time) at ca. 50 °C and concentrated under reduced pressure to afford a crude extract (98.6 g). This crude extract was suspended in H2O (40 °C, 1.5 L) and then partitioned against petroleum ether (PE; 60–90 °C). The PE-soluble part (68.2 g) was subjected to Si gel CC (1000.9 g) eluting with a PE-EtOAc gradient system (v:v = 60:1, 40:1, 15:1, 8:1, 5:1, 2:1, 1:1, and CH3OH) to give fractions 1–8 according to TLC analysis. Fraction 3 (18.3 g) was isolated over Si gel (80.2 g) CC (3.5 cm i.d. × 56 cm) eluting with gradient PE-EtOAc (v:v = 30:1, 15:1, 5:1, each for 1000 mL) to give a crystalline product, which was further purified using preparative TLC developing with PE-EtOAc (v:v = 10 :1, 40 mL) to yield compound 6 (Rf = 0.65, 14.9 mg). Fraction 4 (8.1 g) was fractionated by Si gel (70.2 g) CC (4.5 cm i.d. × 63.0 cm) with a gradient PE-EtOAc system (v:v = 15:1, 10:1, 5:1, each for 800 mL) as the eluant, providing subfractions 4a, 4b, and 4c (NMR and TLC guided). Subfraction 4a (1.1 g) was further purified using Si gel CC (2.5 cm i.d. × 40.0 cm) eluting with gradient PE-EtOAc (v:v = 20:1, 8:1, 3:1, each for 500 mL), to give compounds 3 (3.4 mg) and 9 (10.3 mg). Subfraction 4c (0.8 g) was purified by Sephadex LH-20 CC eluting with EtOH to yield compound 6 (Rf = 0.62, 7.1 mg). Fraction 5 (3.4 g) was subjected to Sephadex LH-20 CC eluting with EtOH to afford subfractions 5a and 5b (NMR and TLC guided). Subfraction 5b (0.8 g) was further fractionated by Si gel (10.3 g) CC (1.5 cm i.d. × 30.0 cm) eluting with a gradient CHCl3-EtOAc system (v:v = 60:1, 20:1, 5:1, each for 1000 mL) to give compounds 1 (7.6 mg) and 4 (2.2 mg). Fraction
Rotundusolide C (9): colorless gum; Rf ca. 0.65 (CH3Cl:EtOAc, 10:1, v/v); [α]D30 +30 (c 0.1, CHCl3); UV (CH3Cl) λmax 241 (log ε) 4.21 nm; IR (film) νmax 2924, 1761, 1675, 1452, 1099, 985 cm−1; 1H (CDCl3, 400 MHz) and 13C (CDCl3, 100 MHz) NMR data, see Table 2; HR-APCI-MS (positive) m/z 336.033 (M + Na+) (calcd. for C18H24O6Na, 336.0345).

Crystallographic data of 2 and 6

Crystallographic data of 2 and 6 have been deposited in the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC 828809 and 828810, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, [fax: +44(0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

Results and Discussion

Rotundusolide A (2) was obtained as colorless needles. Its positive HRESIMS afforded a pseudomolecular ion peak at m/z 359.1469 ([M + Na]+, calcd. 359.1465), which indicated a molecular formula of C18H24O6Na corresponding to seven unsaturation degrees. The IR absorptions indicated the presence of the carbonyl (ν = 1694 cm−1) and hydroxyl (ν = 3394 cm−1) functionalities. The 1H and 13C NMR data (400 MHz and 100 MHz, respectively) of compound 9, see Table 2; HR-APCI-MS (positive) m/z 245.3779 (M + NH4)+ (calcd. for C18H24O5N, 245.3785).

Table 1 1H and 13C NMR data (400 MHz and 100 MHz, respectively) of compounds 2 and 3.

<table>
<thead>
<tr>
<th>Position</th>
<th>δH (J in Hz)</th>
<th>δC</th>
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<tbody>
<tr>
<td>1</td>
<td>1.27 m</td>
<td>31.7 d</td>
</tr>
<tr>
<td>2a</td>
<td>1.30 m</td>
<td>6.2 t</td>
</tr>
<tr>
<td>2b</td>
<td>0.56 m</td>
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<tr>
<td>3</td>
<td>1.59 m</td>
<td>27.6 d</td>
</tr>
<tr>
<td>4</td>
<td>91.8 s</td>
<td>2.61 m</td>
</tr>
<tr>
<td>5a</td>
<td>1.97 d (4.8)</td>
<td>57.8 d</td>
</tr>
<tr>
<td>5b</td>
<td>2.28 d (12.8)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5.66 d (4.8)</td>
<td>66.4 d</td>
</tr>
<tr>
<td>7</td>
<td>139.6 s</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>169.5 s</td>
<td>5.44 br s</td>
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<td>9a</td>
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<td>34.9 q</td>
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<tr>
<td>9b</td>
<td>2.76 dd (14.6, 2.4)</td>
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<tr>
<td>10</td>
<td>43.6 s</td>
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<tr>
<td>11</td>
<td>128.3 s</td>
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<td>13</td>
<td>2.16 s</td>
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<td>14</td>
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<td>C=O(OAc)</td>
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</table>

Crystallographic data of 2 and 6

6 (5.1 g) was chromatographed on Si gel (60.2 g) CC (2.0 cm i.d. × 30.0 cm) eluting with CHCl3-EtOAc (v:v = 10:1, 1500 mL) to give subfractions 6a, 6b, and 6c (NMR and TLC guided). Sub-fraction 6b (0.2 g) was purified using RP-18 silica gel CC with CHCl3-H2O (1:1) as the eluant, yielding compounds 2 (6.3 mg) and 5 (3.1 mg). Sub-fraction 6c (1.4 g) was subjected to Sephadex LH-20 CC eluting with CHCl3-MeOH (2:1, v:v); [α]D20 −2957, 1737, 1663, 1308, 1227, 1082, 956 cm−1; m.p. 168°C; [α]D20 +1 5 ([15]D20 +1 5) revealed the existence of an OAc group (δH 3.76 s; δC 52.8). Be-
to an olefinic bond), one methylene, four methines (including one oxygenated methine), four sp² (including two carbonyl carbons and two olefinic carbons) and two sp³ (one of them being oxygenated) quaternary carbons. The ¹H NMR spectrum demonstrated an oxygen-bearing methine proton at δ_H 5.66 (d, J = 4.8 Hz) and four methyl singlets at δ_H 2.16, 1.69, 1.28, and 1.08. A pair of methylene protons at δ_H 1.30 and 0.56 in the ¹H NMR spectrum, together with the ¹³C NMR signal at δ_C 62.2 (CH₂) and the HSQC spectrum, indicated the existence of a cyclopropane ring [14–16]. The ¹H-¹H COSY experiment further supported the presence of the cyclopropane ring and another spin system H-5 and H-6 (Fig. 2).

The moiety A in the structure of 2 was further constructed using the following HMBC correlations: the correlations from H3-9/14 to C-1, C-5, C-10, and C-14/9, and the correlations from H3-15 to C-3, C-4, and C-5, and the correlation from H-6 to the carbonyl carbon of the OAc group (Fig. 2). The moiety B was elucidated by the HMBC correlations from H3-13 to C-7, C-8, C-11, and C-12, and from the OMe proton to C-12 (Fig. 2). Furthermore, the HMBC correlations from H-6 to C-7, C-8, and C-11 confirmed the linkage of C-6 and C-7. Finally, considering the diagnostic chemical shift of C-4 (δ_C 91.8) and the established molecular formula, the structure of 2 should contain a 4,8-lactone moiety. The relative configuration was established by the NOE difference spectrum. The NOE correlation between H-6 and H3-13 indicated the Z-type Δ⁷, ¹¹ olefinic bond. The NOE correlations between H-5 and H-6, H-5 and H3-15, and H3-15 and H-3, together with a molecular modeling analysis, determined the relative configuration as shown in Fig. 1. Furthermore, the structure of 2 was confirmed by the single-crystal X-ray diffraction method (Fig. 3). Rotundusolide A (2) possessed a novel rearranged secoeudesmane sesquiterpenoid skeleton. To the best of our knowledge, this is the third example of such a type of sesquiterpenoid up till now [14–16].

Rotundusolide B (3) was isolated as colorless oil. Its molecular formula was determined as C₁₆H₂₀O₄ based on its HRESIMS ([M + NH₄]⁺, obs.d. 294.1702, calcd. 294.1700), requiring seven unsaturation degrees. The IR absorptions indicated the presence of the carbonyl (1761 cm⁻¹) and olefin (1675 cm⁻¹) functionalities. The ¹H and ¹³C NMR data (Table 1) revealed the existence of an OMe group (δ_H 3.23 s; δ_C 57.1). In addition, the ¹³C NMR spectrum showed 15 carbon signals, including two methyls (one of them bonded to an olefinic bond), three methylenes (including one olefinic terminal methylene), five methines (including two oxygenated methines and two olefinic quaternary carbons), and five sp³ quaternary carbons (including two carbonyl and three olefinic quaternary carbons). The ¹H-¹H COSY experiment, together with the HMBC spectrum, provided the correlations from H2-14 to C-1, C-2, C-8, and C-9, and from H-1/9 to C-10 strongly supported the presence of the Δ¹, ² and Δ¹⁰, ¹⁴ olefinic systems (Fig. 4). Furthermore, the HMBC correlations from H3-13 to C-6, C-7, C-8, C-11, and C-12, and from H₂-5 to C-6 determined the existence of the α,β-unsaturated lactone moiety and the carbonyl function at C-6 (Fig. 4). The relative configuration of 3 was determined as follows: The large coupling constant J = 16.0 Hz indicated the E-type Δ¹, ³ dou-
ble bond. The NOE difference spectrum provided the NOE correlation between H-3 and H-15, revealing the β-orientation of the OMe group at C-3 and the α-orientation of Me-15. Furthermore, the NOE difference experiment provided the NOE correlations between H-3 and H-1, H-1 and H2-14, H2-14 and H-9b, and H-2 and H-9a. A detailed molecular modeling analysis, combined with the H-3 coupling pattern (t, J = 9.6 Hz), suggested H-3 was axially α-oriented and H-9a was axially β-oriented. The H-8 proton showed a broad singlet in the 1H NMR spectrum, which required that the dihedral angle between H-8 and H-9a be ca. 90°, and H-8 should be equatorially β-oriented. Hence, the structure of rotundusolide B (3) was determined as depicted.

Rotundusolide C (9) was isolated as colorless gum. The positive ESI MS gave a protonated pseudomolecular ion peak at m/z 425.4, together with the 13C NMR data, corresponding to a molecular formula of C30H48O. The proposed molecular formula C30H48O was supported by the positive HRAPCI MS ([M + H]+, obsd. 425.3797; calcld. 425.3778). The IR spectrum showed the absorptions for the hydroxyl (3394 cm⁻¹) and olefin (1662 cm⁻¹) functionalities. The 13C NMR data (Table 2) provided thirty carbon signals, including seven methyls (two of them bonded to an olefinic bond), nine methylenes, eight methines (including three olefinic methines and one oxygenated methine), and three sp² olefinic and three sp³ quaternary carbons. The 1H NMR spectrum (Table 2) showed six methyl singlets at δH 1.68, 1.60, 1.00, 0.86, 0.69, 0.68, one methyl doublet at δH 0.90 (J = 6.4 Hz), one oxygenated methine proton at δH 3.74 (dd, J = 9.6, 6.0 Hz), and three olefinic protons at δH 5.25 (dd, J = 4.4, 2.4 Hz), 5.22 (t, J = 3.6 Hz), 5.10 (br t, J = 7.2 Hz). Furthermore, the 1H NMR spectrum gave a pair of diagnostic methylene protons at δH 2.90 (br d, J = 14.4 Hz) and 2.70 (br d, J = 14.4 Hz). The above information indicated that compound 3 might possess a 9,10-seco-cycloartane triterpenoid skeleton [17–21]. This was confirmed as follows: The 1H-1H COSY spectrum provided five spin systems as shown in Fig. 5. In the HMBC spectrum, H2-19 showed the correlations to C-1, C-5, C-8, C-9, C-10, and C-11, and H3-29/30 showed the correlations to C-3, C-4, and C-5, which determined the construction of rings A and B, as well as the presence of a hydroxyl group at C-3 and an Δ1,10 olefinic bond (Fig. 5). The establishment of rings C and D was confirmed by the HMBC correlations from H2-18 to C-12, C-13, C-14, and C-17, from H2-19 to C-9 and C-11, and from H3-28 to C-8, C-14, and C-15 (Fig. 5). The construction of the side chain was established by the HMBC correlations from H2-21 to C-20 and C-22, and from H3-26/27 to C-24 and C-25, which also determined the presence of the Δ14,25 olefinic system (Fig. 5). The relative configuration at C-3 was determined by the NOE difference experiment, which provided the NOE correlations between H-3 and the equatorially α-oriented Me-29 signal (stronger) and the axially β-oriented Me-30 signal (weaker), and no NOE correlation between H-3 and the axially α-oriented H-5 signal. A detailed molecular modeling analysis showed that only the equatorially β-oriented H-3 satisfied such stereochemistry. The stereochemistry at C-17 and C-20 of 9 was determined by comparing the chemical shifts (δC 50.9, δC 50.8, δC 35.9; in CDCl3) with those of its analogues 9a (δC 50.4, δC 36.6; in CDCl3) and 9b (δC 50.8, δC 36.3; in CDCl3) [17, 19]. Finally, the structure of rotundusolide C (9) was elucidated as depicted. Rotundusolide C (9) was a 9,10-seco-cycloartane triterpenoid. A literature search showed that, so far, there have been only eleven such triterpenoid types discovered from a natural source [17–21].

A literature search showed that secomacrogenin B (9b), an analogue of rotundusolide C (9), shared the same coupling pattern for H-3 (dd, J = 9.6, 6.0 Hz) as that of rotundusolide C (9) [19]. However, the OH-3 was determined as equatorially β-oriented based on the coupling patterns, but without the NOE difference correlation or NOESY experiment, which was dubitable according to our study.

Besides the above chemical constituents, based on the ESI MS, NMR, optical rotation data, and comparison with data from the literature, the other compounds were elucidated as 3,4-O-isopropylidenedeshikimic acid (1) [22], dehydrocostuslactone (4) [23], (+)-alismoxide (5) [24], sugretriol triacetate (6) [1], 2β-hydroxy-α-cyperone (7) [25, 26], eudesma-4(14),11(13)-diene-7α,8a,12-triol (8) [27], and 3,4-seco-mansambinonic acid (10) [28]. The sesquiterpenoid 6 possessed the patchoulane-type skeleton. A literature search showed that such types of sesquiterpenoids are the characteristic chemical constituents of the genus Cyperus, and they are a class of promising plant growth inhibitors [5, 6]. Here, its structure was further confirmed by the single-crystal X-ray diffraction method (Fig. 3).

2β-Hydroxy-α-cyperone (7) was discovered from natural sources for the first time. Its structure was closely similar to that of α-cyperone, which is abundant in the rhizomes of C. rotundus L. From a biogenetic viewpoint, the absolute configuration of 7 might be the same as that of α-cyperone with 7R and 10S [25, 26]. We tried to prove this deduction using the CD exciton chirality method [10], but the CD spectrum of 7 did not expressly give the Cotton effect resulting from the exciton coupling between the α,β-unsaturated ketone and the Δ11,12 olefinic system (Fig. 6). The absolute configuration of 7 could not yet be confirmed by the CD exciton chirality method.

This study, to the best of our knowledge, is the first report on the discovery of the carbon skeletons of 2 and 9 from the genus Cyperus, and the first report on the skeletons of 3 and 10 from the title plant. Furthermore, compounds 1–5 and 7–10 were isolated from the genus Cyperus for the first time. These findings may provide
some useful information for the chemotaxonomic study for the plant *Cyperus rotundus*.

### Acknowledgements

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### Conflict of Interest

None of the authors have conflicts of interest in this study.

### References