

Article

Norcyperone, a Novel Skeleton Norsesquiterpene from *Cyperus rotundus* L.

Yan Xu ¹, Hong-Wu Zhang ¹, Chang-Yuan Yu ^{2,*}, Yang Lu ³, Ying Chang ³, and Zhong-Mei Zou ^{1,*}

¹ Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, P. R. China; E-mail: xuyantea@163.com (Y. X.), hongwuzhang77@hotmail.com (H-W. Z.)

² College of Life Science and Technology, Beijing University of Chemical Technology, Beijing 100029, P. R. China; E-mail: yucy@mail.buct.edu.cn (C-Y. Y.)

³ Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, P. R. China; E-mail: luy@imm.ac.cn (Y. L.),

* Authors to whom correspondence should be addressed: E-mail: zmozou@implad.ac.cn (Z-M. Zou), yucy@mail.buct.edu.cn (C-Y. Y.); Tel: +86 -10-62899756; Fax: +86 -10-62899756.

Received: 21 August 2008; in revised form: 25 September 2008 / Accepted: 26 September 2008 / Published: 10 October 2008

Abstract: A novel norsesquiterpene, named norcyperone (**1**), and three known compounds: (-)-clovane-2,9-diol (**2**), rosenonolactone (**3**), and 5 α ,8 α -epidioxy-(20*S*,22*E*,24*R*)-ergosta-6,22-dien-3 β -ol (**4**) were isolated from the rhizomes of *Cyperus rotundus* L. The structure of **1** was elucidated as 8,11,11-trimethylbicyclo[5.3.1]undecane-5 α , 8 α -epoxy-3-one on the basis of extensive spectroscopic analyses, including 1D- and 2D-NMR, MS experiments, and single-crystal X-ray diffraction. This is the first report of a 8,11,11-trimethyl- bicyclo[5.3.1]undecane-3-one type norsesquiterpene with a tetrahydrofuran ring at C-5 and C-8.

Keywords: *Cyperus rotundus* L.; norsesquiterpene; norcyperone; bicyclo[5.3.1]undecane-3-one

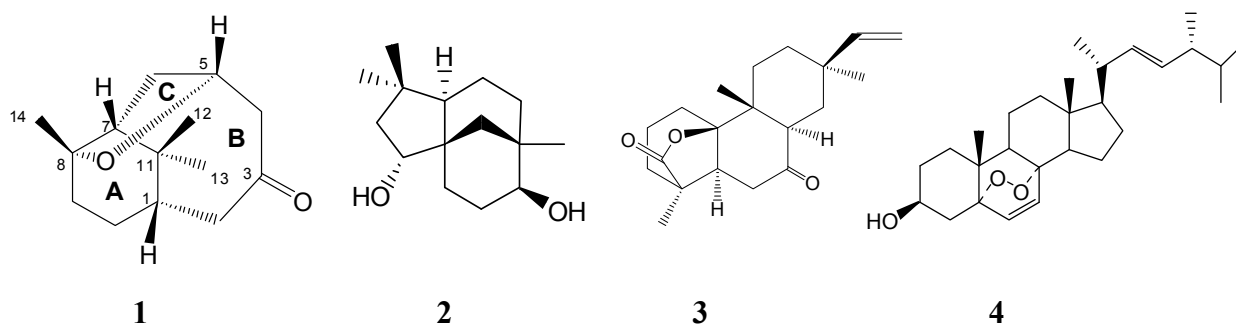
Introduction

The rhizomes of *Cyperus rotundus* L. have been used in traditional Chinese medicine as an estrogenic and anti-inflammatory agent for the treatment of women's diseases and also used for treatment of stomach ache and bowel disorders [1]. The extract of the rhizomes of *C. rotundus* L. showed anti-diabetic activity [2], acetylcholinesterase inhibitory activity [3] and antidiarrhoeal activity [4], as well as inhibition of nitric oxide and superoxide production [5]. Previous phytochemical studies on *C. rotundus* L. have led to the identification of more than 60 sesquiterpenes with a diversity of skeletons [6, 7] besides flavonoids, furochromones, triterpenes and sterols [8, 9]. In the course of our phytochemical investigation of this species, a novel norsesquiterpene **1**, named norcyperone, together with three known compounds: (-)-clovane-2,9-diol (**2**), rosenonolactone (**3**), and 5 α ,8 α -epidioxy-(20*S*, 22*E*, 24*R*)-ergosta-6,22-dien-3 β -ol (**4**) were isolated from the rhizomes of *C. rotundus* L. Herein we report the isolation and structural elucidation of these compounds.

Results and Discussion

The 95% EtOH extract of dried and powdered rhizomes of *C. rotundus* L. was suspended in H₂O and then extracted successively with petroleum ether, CH₂Cl₂, EtOAc and n-BuOH. The CH₂Cl₂-soluble fraction was separated by repeated column chromatography to afford a novel norsesquiterpene, norcyperone (**1**) and three known compounds **2-4** (Figure 1). Compounds **2**, **3** and **4** were identified as (-)-clovane-2,9-diol (**2**) [10], rosenonolactone (**3**) [11], and 5 α ,8 α -epidioxy-(20*S*, 22*E*, 24*R*)-ergosta-6,22-dien-3 β -ol (**4**) [12-13] by comparing their physical and spectroscopic data with those reported in the literatures.

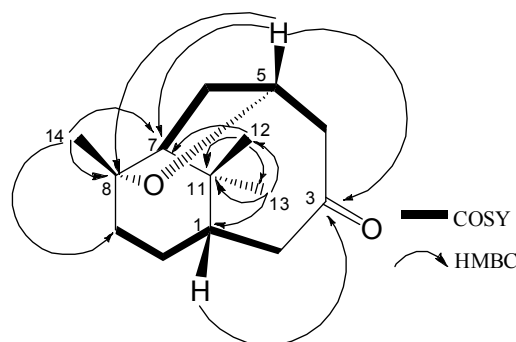
Figure 1. Structures of compounds **1-4**.



Compound **1** was obtained as colourless needles in CH₂Cl₂, mp 146-148°C, $[\alpha]_D^{20}$ -54.3° (*c*=0.09, CH₂Cl₂). Its IR spectrum showed a strong carbonyl group absorption at 1693 cm⁻¹. The molecular formula of **1** was assigned as C₁₄H₂₂O₂ by HR-ESI-MS (*m/z* 245.1514 [M+Na]⁺, calcd. 245.1512 [M+Na]⁺), with four degrees of unsaturation. The complete assignment of all protons and carbons for **1** was made by ¹H-NMR, ¹³C-NMR, ¹H-¹H COSY, HMQC and HMBC (Table 1). Its ¹³C-NMR spectrum gave 14 carbon signals including two oxygenated ones at δ_C 83.8 and 72.2, respectively, and a ketone carbon signal at δ_C 211.3. The remaining carbon signals were three methyl groups, five methylene units, two methine units and one quaternary carbon. The ¹H-NMR spectrum of **1** exhibited

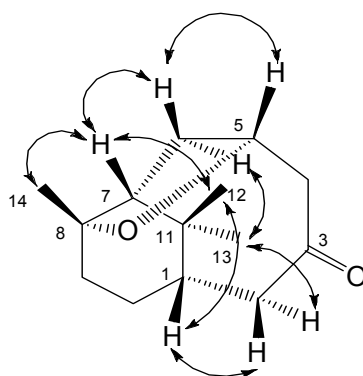
three singlet methyls at δ_H 1.11, 1.14 and 1.17, as well as a proton signal of oxygen-bearing carbon at δ_H 4.39. The 1H - 1H COSY correlations between H-1/H-2a, 2b, H-1/ H-10a, 10b, H-9b/H-10b, H-5/H-4a, 4b, H-6a/H-5, 7, and H-6b/H-5, 7 revealed partial structures shown by bold lines in Figure 2.

Figure 2. Key HMBC and 1H - 1H COSY correlations of **1**.



The location of the ketone carbonyl group was established at C-3 by the HMBC correlations of the carbonyl carbon signal (δ_C 211.3) with the resonances of H-1, H-2, H-4 and H-5, while the protons of the methyl singlet at δ_H 1.11 and 1.14 gave HMBC correlations to δ_C 38.6 (C-1), δ_C 50.6 (C-7), and δ_C 35.1 (C-11) suggesting the two geminal methyls linked at C-11. In turn, the HMBC spectrum exhibited correlations of the methyl protons (δ_H 1.17) with δ_C 50.6 (C-7), δ_C 83.8 (C-8), and δ_C 30.3 (C-9), in good agreement with a methyl group linked at C-8. The presence of a tetrahydrofuran ring was deduced by the molecular formula as well as the HMBC correlation between the proton of oxygen-bearing carbon at δ_H 4.39 (H-5) and C-8 (Figure 2). All above data indicated that compound **1** was a 8, 11, 11-trimethyl- bicyclo[5.3.1]undecane-3-one type norsesquiterpene with a tetrahydrofuran ring at C-5 and C-8.

Figure 3. Key NOESY correlations of **1**.



The relative stereochemistry of **1** was determined on the basis of a NOESY spectrum. The NOESY correlations between H-1/CH₃-12, CH₃-12/ H-7, H-7/CH₃-14, H-5/H-6a, H-6a/H-7 suggested that all these atoms were in β -orientation (Figure 3), which further confirmed by single-crystal X-ray diffraction (Figure 4). The crystal structure showed a six-numbered ring *A* in 'twist boat' conformation, an eight-numbered ring *B* in 'boat' conformation, and a five-numbered tetrahydrofuran ring *C* in an 'envelope' conformation, in which rings *A* and *C* are cis-fused. Thus, the structure of **1**

was elucidated as 8,11,11-trimethylbicyclo[5.3.1]undecane-5 α ,8 α -epoxy-3-one (1 β -H,7 β -H), named norcyperone.

Figure 4. X-ray structure of compound **1**.

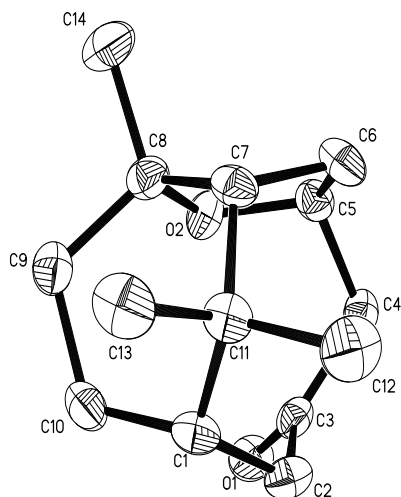


Table 1. The NMR data of compound **1**^a.

Position	¹ H-NMR	¹³ C-NMR	HMBC (H→C)
1	1.88 (1H, m)	38.6	C-3, 7, 9, 12, 13
2a	2.86 (1H, dd, 12.6, 1.8)	47.9	C-1, 3, 10
2b	2.34 (1H, ddd, 12.6, 7.8, 1.2)		C-1, 3, 4, 10, 11
3	-	211.3	
4a	2.88 (1H, ddd, 13.2, 7.8, 1.8)	52.6	C-2, 3, 5, 6
4b	2.62 (1H, dd, 13.2, 3.0)		C-3, 5, 6
5	4.39 (1H, ddt, 10.2, 7.2, 1.8)	72.2	C-3, 7, 8
6a	2.64 (1H, dd, 14.4, 10.2)	35.4	C-4, 5, 7, 11
6b	2.18 (1H, br d, 14.4)		C-4, 7, 8, 11
7	1.77 (1H, d, 10.2)	50.6	C-1, 5, 12, 14
8	-	83.8	
9a	1.85 (1H, m)	30.3	C-1, 7, 8, 10
9b	1.79 (1H, m)		C-8, 10
10a	1.94 (1H, m)	19.6	C-1, 2, 8, 9
10b	1.90 (1H, m)		C-1, 2, 8, 9
11	-	35.1	
12	1.14 (3H, s)	34.3	C-1, 7, 11, 13
13	1.11 (3H, s)	28.4	C-1, 7, 11, 12
14	1.17 (3H, s)	30.1	C-7, 8, 9

^a ¹H-NMR (600 MHz), ¹³C-NMR (150 MHz) spectra of compound **1** were measured in CDCl₃ on a Varian Inova-600 MHz spectrometer, δ in ppm, *J* in Hz.

Conclusions

A novel skeleton norsesquiterpene, norcyperone, together with three known compounds **2-4** were isolated from the rhizomes of *C. rotundus* L. Although a 8,11,11-trimethylbicyclo[5.3.1]undecane has been reported as a synthetic intermediate derived in the course of synthesis of the taxane-AB-fragment with a spiro-cyclopropyl group [14], this is the first report of a 8,11,11-trimethylbicyclo[5.3.1]undecane-3-one type norsesquiterpene with a tetrahydrofuran ring at C-5 and C-8. Compounds **2-4** were isolated from the genus *Cyperus* for the first time. These findings enrich our knowledge of the chemical constituents of *C. rotundus* L.

Experimental

General

Melting points were determined on Fisher-Johns Melting Point Apparatus and are uncorrected. IR spectra were measured with a Bruker IFS-55 infrared spectrometer (KBr). Optical rotations were recorded on a Perkin-Elmer 241 automatic recording spectropolarimeter. EI mass spectra were recorded on a Zabspec E mass spectrometer. HR-ESI-MS was taken on a LTQ OrbitrapTM Mass spectrometer. Column chromatography was performed over Silica gel (200-300 mesh) (Qingdao Marine Chemical Factory) and Sephadex LH-20 (Pharmacia). Nuclear magnetic resonance (NMR) spectra were measured on a Varian Inova-600 spectrometer operating at 600 MHz (¹H) and 150 MHz (¹³C), respectively.

Plant material

The dried rhizomes of *C. rotundus* L. were collected from Dabieshan Mountains of Anhui Province, P.R. China in Sep. 2006. A voucher specimen (No: 20060825) was authenticated by Prof. Yulin Lin and deposited at the Herbarium of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

Extraction and isolation

The dried and powdered rhizomes of *C. rotundus* L. (15 kg) were extracted with 95% EtOH three times under reflux, and the solvent was evaporated *in vacuo*. The residues were partitioned in H₂O and extracted successively with petroleum ether, CH₂Cl₂, EtOAc and n-BuOH. The CH₂Cl₂ extract (158 g) was submitted to column chromatography on silica gel eluting with petroleum ester/EtOAc (50:1 to 0:1) to afford eight fractions (F₁-F₈). Fraction F₂ (22 g) was further separated by silica gel column chromatography eluting with petroleum ether/acetone (20:1 to 2:1) to give 8 fractions (F_{2a}-F_{2h}). Fraction F_{2d} was purified by silica gel column chromatography eluting with petroleum ether/acetone (10:1) and Sephadex LH-20 eluted with CH₂Cl₂/MeOH (1:1) to obtain **3** (14 mg). Fractions F_{2f} was purified by silica gel column chromatography eluting with petroleum ester/acetone (4:1) to obtain **4** (24 mg). F₅ (18 g) was further chromatographed on silica gel eluted with petroleum ester/acetone (6:1

to 1:1) to give 10 fractions F_{5a}-F_{5j}. Fraction F_{5b} was purified by silica gel eluted with petroleum ether/acetone (5:1) and Sephadex LH-20 eluted with CH₂Cl₂/MeOH (1:1) to obtain **1** (16 mg). Fraction F_{5e} was purified by Sephadex LH-20 eluting with CH₂Cl₂/MeOH (1:1) to obtain **2** (10 mg).

Norcyperone (1): colorless needles; mp 146-148°C; $[\alpha]^{20}_D -54.3^\circ$ (c=0.09, CH₂Cl₂); HR-ESI-MS m/z 245.1514 [M+Na]⁺, calcd. 245.1512 [M+Na]⁺. IR $\nu_{\max}$ cm⁻¹: 2968, 2935, 2881, 1693, 1371, 1066; ¹H-NMR and ¹³C-NMR data: see Table 1.

(-)-Clovane-2, 9-diol (2): colourless needles; mp 162-164°C; EI-MS m/z : 238(M), 220, 182, 164, 135, 107, 93, 41. ¹H-NMR (CD₃COCD₃) δ_H : 0.85 (3H, s, CH₃-13), 0.91 (3H, s, CH₃-15), 1.01 (3H, s, CH₃-14), 3.22 (1H, m, H-9), 3.72 (1H, m, H-2), 1.43 (1H, m, H-5); ¹³C-NMR (CD₃COCD₃) δ_C : 45.4 (C-1), 80.8 (C-2), 48.5 (C-3), 37.6 (C-4), 51.5 (C-5), 34.1 (C-6), 21.5 (C-7), 35.4 (C-8), 74.9 (C-9), 27.2 (C-10), 27.5 (C-11), 36.4 (C-12), 25.8 (C-13), 31.9 (C-14), 29.2 (C-15).

Rosenonolactone (3): white needles, mp 224-226°C; EI-MS m/z : 316(M), 301, 220, 163, 108, 93, 41. ¹H-NMR (CDCl₃) δ_H : 0.95 (3H, s, CH₃-20), 0.97 (3H, s, CH₃-17), 1.13 (3H, s, CH₃-18), 4.92 (1H, dd, $J=10.8, 0.6$ Hz, H-16a), 4.99 (1H, dd, $J=17.4, 1.2$ Hz, H-16b), 5.83 (1H, dd, $J=17.4, 10.8$ Hz, H-15); ¹³C-NMR (CDCl₃) δ_C : 30.3 (C-1), 19.8 (C-2), 35.5 (C-3), 47.3 (C-4), 50.9 (C-5), 35.8 (C-6), 210.2 (C-7), 47.4 (C-8), 38.9 (C-9), 86.9 (C-10), 30.8 (C-11), 31.4 (C-12), 35.1 (C-13), 31.7 (C-14), 149.6 (C-15), 109.9 (C-16), 21.9 (C-17), 16.9 (C-18), 179.2 (C-19), 16.8 (C-20).

5 α ,8 α -Epidioxy-(20S,22E,24R)-ergosta-6,22-dien-3 β -ol (4): colourless needles; mp 155-157°C; EI-MS m/z : 428 (M), 396, 251, 152, 107, 93, 81, 69; ¹H-NMR (CDCl₃) δ_H : 6.50 (1H, d, $J=8.5$ Hz, H-6), 6.24 (1H, d, $J=8.5$ Hz, H-7), 5.21 (1H, dd, $J=15.5, 8.0$ Hz, H-23), 5.14 (1H, dd, $J=15.5, 8.0$ Hz, H-22), 3.97 (1H, m, H-3), 1.00 (3H, d, $J=7.0$ Hz, H-21), 0.91 (3H, d, $J=7.0$ Hz, H-28), 0.88 (3H, s, H-19), 0.84 (3H, s, H-18), 0.82 (3H, d, $J=5.0$ Hz, H-26), 0.81 (3H, d, $J=3.0$ Hz, H-27); ¹³C-NMR (CDCl₃) δ_C : 34.7 (C-1), 30.1 (C-2), 66.5 (C-3), 36.9 (C-4), 82.1 (C-5), 135.4 (C-6), 130.8 (C-7), 79.4 (C-8), 51.1 (C-9), 36.8 (C-10), 23.4 (C-11), 39.4 (C-12), 44.6 (C-13), 51.7 (C-14), 20.6 (C-15), 28.6 (C-16), 56.2 (C-17), 13.0 (C-18), 18.2 (C-19), 39.9 (C-20), 20.9 (C-21), 135.2 (C-22), 132.3 (C-23), 42.8 (C-24), 33.1 (C-25), 19.9 (C-26), 19.6 (C-27), 17.6 (C-28).

Crystallographic Data

Crystallographic data for **1**: formula: C₁₄H₂₂O₂; molecular weight: 222.32; monoclinic, space group $P2_12_12_1$, $a = 7.450$ (15), $b = 8.691$ (17), $c = 18.981$ (4) Å; $V = 1228.8$ (4) Å³, $Z = 4$, $d = 1.202$ g/cm³; crystal dimensions, 0.15×0.15×0.30 mm. The measurements were performed on a MAC DIP-2030K diffractometer with a graphite monochromator (ω -2 θ scans, 2 θ max = 50.0°), MoK α radiation. The total number of independent and observed reflections was 2423 ($|F|^2 \geq 2\sigma|F|^2$). The crystal structure was solved by direct methods using SHELXS-97, expanded using difference Fourier techniques, and refined with NOMCSDP using full-matrix least-squares calculations. Final indices: $R1 = 0.0395$, $wR2 = 0.0991$, $S = 1.038$. Crystallographic data for **1** has been deposited at the Cambridge Crystallographic Data Center. CCDC 694682 contains the supplementary crystallographic data for this paper. These

data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

Acknowledgements

This work was supported by the National Natural Science Foundation of China (30772711) and the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry of China. We are grateful to Professor Yulin Lin, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College for the identification of *C. rotundus* L. We are also thankful to Mr. He-bin Chen, the Academy of Military Medical Sciences for NMR measurements.

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Sample Availability: Samples are available from the authors.

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