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A New Rosane Diterpene from *Lycoris aurea*

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ABSTRACT

Plants of the Amaryllidaceae are known to produce structurally unique alkaloids with a wide range of interesting physiological effects. *Lycoris aurea* (Amaryllidaceae), a popular ornamental plant in China, has a wide distribution in the tropics and warm temperature regions. In order to search new bioactive alkaloids from this plant, we investigated the chemical constituents of the bulbs of *L. aurea* collected in Kunming of Yunnan province. A new rosane diterpene, lyrosenonolactone (**1**), has been isolated from the whole plant of *L. aurea*. Its structure was elucidated on the basis of extensive spectroscopic analysis. Additionally, rosololactone (**2**) and seven known alkaloids were also isolated from this plant. To the best of our knowledge, this is the first report of a diterpene from *L. aurea*. Moreover, on the basis of detailed 2D NMR data analysis, the chemical shifts of rosolactone (**2**) reported previously were revised.

Keywords: Amaryllidaceae, diterpenoid, lactone, lyrosenonolactone, rosololactone
Abbreviations: **COSY**, Correlation spectroscopy; **DEPT**, Distortion enhancement by polarization transfer; **HMBC**, Heteronuclear multiple bond coherence; **HMQC**, Heteronuclear multiple quantum coherence; **HRESIMS**, High resolution Electrospray ionization mass spectrum; **IR**, Infrared spectroscopy; **NMR**, Nuclear magnetic resonance; **ROESY**, Rotating frame Overhauser effect spectroscopy; **TLC**, Thin layer chromatography

INTRODUCTION

The genus *Lycoris* (Amaryllidaceae) is one of the 10 genera of the tribe Amaryllidaceae that has a wide distribution in the tropics and warm temperature regions (We *et al*. 1982). The family Amaryllidaceae has been a rich source of structurally diverse alkaloids with a wide range of interesting physiological effects, including antiviral, acetylcholinesterase inhibitory, immunostimulatory and antimalarial activities (Jin 2003). *Lycoris aurea*, a plant distributed in Yunnan province of China, was traditionally used for the treatment of sores and tumors (Deng *et al*. 1963). In order to search new bioactive compounds from this plant, we investigated the chemical constituents of *L. aurea* collected in Kunming of Yunnan province, which has not been reported previously. During our systematic chemical investigation, a new rosane diterpenoids, lyrosenonolactone (**1**), together with rosololactone (**2**) and seven known alkaloids, galanthamine (Kobayashi *et al*. 1985; Kihara *et al*. 1991), hippeastrine (Kihara *et al*. 1991), 9-demethylhomolycorine (Kobayashi *et al*. 1985), *O*-demethylgalanthamine (Kobayashi *et al*. 1985), *O*-demethyllycoramine (Kobayashi *et al*. 1980), lycoramine (Kobayashi *et al*. 1980) and pluviine (Kreh *et al*. 1995) were isolated from the whole plant of *L. aurea*. The known compounds were identified by comparisons of spectroscopic data with those of reported ones. This is the first report of a rosane diterpene isolated from the Amaryllidaceae family. This type of diterpene has demonstrated specific activity as CETP inhibitors in the treatment of atherosclerotic cardiovascular diseases (Kwon *et al*. 1995).

MATERIALS AND METHODS

General experimental procedures

Melting point was obtained on an XRC-1 apparatus and is uncorrected. Optical rotations were carried out on a HORIBA SEPA-300 high sensitive polarimeter. IR spectrometer was measured in a Bio-Rad FTS-135 spectrometer with KBr pellets. MS were recorded on a VG Auto spec-3000 spectrometer or on a Finnigan MAT 90 instrument. 1D and 2D NMR spectra were taken on a Bruker AM-400 and DRX-500 instrument with TMS as internal standard, respectively. Column chromatography were performed either on silica gel (200-300 mesh; Qingdao Marine Chemical Inc., China), silica gel H (10-40 μm, Qingdao Marine Chemical Inc., China), or Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany). Spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

Plant material

Fresh whole plants of *L. aurea* were collected in Kunming, Yunnan Province of China, in March 2004, and were identified by Prof. Xiao Chen of Kunming Institute of Botany, Chinese Academy of Sciences, where a voucher specimen (KIB L00401) is deposited.

Extraction and isolation

Fresh *L. aurea* Herb. plants (3 kg) were extracted with 95% EtOH under reflux for 5 × 24 h at room temperature. The extract was concentrated *in vacuo* and fractionated by Si gel column chromatography (1 kg, 200-300 mesh) eluting with petroleum ether (60~90°C)-EtOAc-Et2NH (9.5:0:0.5~0:9.5:0.5). Based on TLC analysis; 9 fractions were obtained. Repeated chromatography of fractions 1-9 on either a silica gel column or an RP-18 gel column yielded 7 alkaloid compounds. The residual (48.5 g) was combined and fractionated on a silica gel column; elution with cyclohexane-$CHCl_3$-Et_2NH from 8:1.5:0.5 to 3:6.5:0.5 afforded 12 fractions. Of the fractions, the 1st was chromatographed over RP-18 silica gel by eluting with MeOH-H_2O (20:80~80:20), then was recrystallized from MeOH to afford compounds **1** (15 mg) and **2** (16 mg).

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Short Communication

RESULTS AND DISCUSSION

Compound **1** was obtained as colorless needles (MeOH), giving the molecular formula $C_{20}H_{28}O_4$ by HRESIMS $[M+Na]^+$ (obsd 355.1892, calcd 355.1885), indicating 7 sites of unsaturation. Its IR spectrum showed strong absorptions at 3408, 1796, 1701 and 1635 cm^{-1}, indicating the presence of a hydroxyl group, a γ-lactone group, a ketone group and an olefinic group. Observed in ^1H NMR and ^{13}C NMR (including DEPT) spectra (see **Table 1**) there were three tertiary methyl groups [δ_H 0.9 (3H, s), 1.17 (3H, s) and 1.31 (3H, s); δ_C 25.6 (q), 17.1 (q) and 14.6 (q)], an olefinic group [δ_H 5.81 (1H, dd, J = 10.7, 17.4 Hz), 4.96 (1H, d, J = 17.4 Hz), 4.88 (1H, d, J = 10.7 Hz); δ_C 150.1 (d), 109.4 (t)], a hydroxyl group [δ_H 3.27 (1H, s)], two oxygenated quaternary carbons [δ_C 77.3 (s), 88.3 (s)], a lactone carbon [δ_C 178.4 (s)], and a ketone carbon [δ_C 206.1 (s)]. In addition, the upfield region of ^{13}C NMR and DEPT spectra exhibited seven methylenes, one methenyl and three quaternary carbons. All these spectral data show that it may be a rosane diterpenoid (Achilladelis *et al*. 1969; Cane *et al*. 1977; Dockerill *et al*. 1978). Comparison data of **1** with those of rosenonolactone indicated that **1** was very similar to rosenonolactone except for a hydroxyl group at C-8.

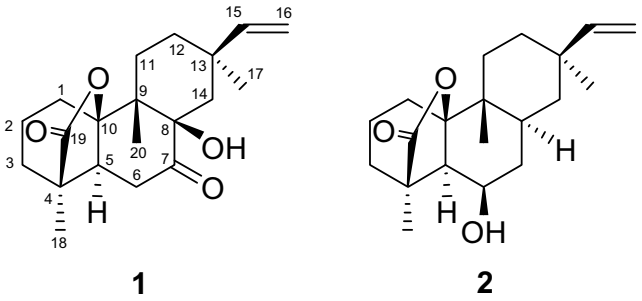

Fig. 1 The structure of compounds **1** and **2**.

Table 1 ^1H and ^{13}C NMR spectral data of compound **1** ($CDCl_3$ ∗ values may be interchanged).

Position	δ_H (mult. J in Hz)	δ_C (mult.)	HMBC
1α	1.62 (m)	29.5 (t)	H-2αβ, H-3β
2			
3			
1β	2.14 (m)		
2α	1.88 (m)∗	19.7 (t)	H-1αβ, H-3αβ, Me-18
2β	1.68 (m)∗		
3α	1.56 (m)	34.4 (t)	H-1αβ, H-2β, H-5, Me-18
3β	1.76 (m)		
4		50.8 (s)	H-2α, H-3αβ, H-5, H-6β, Me-18
5	2.38 (dd, 11.0, 7.0)	48.6 (d)	H-1αβ, H-3αβ, H-6αβ, Me-18
6α	2.49 (dd, 16.4, 7.0)	34.6 (t)	H-5, Me-18
6β	2.65 (dd, 16.4, 11.0)		
7		206.1 (s)	H-5, H-6αβ, H-14αβ
8		77.4 (s)	OH-8, H-6α, H-11αβ, H-14αβ, Me-20
9		44.8 (s)	OH-8, H-1β, H-11αβ, H-12αβ, H-14αβ, Me-20
10		88.3 (s)	H-1β, H-2α, H-6αβ, H-11β, Me-20
11α	1.26 (m)	27.7 (t)	H-1β, H-12β, Me-20
11β	1.53 (m)		
12α	1.23 (m)	30.7 (t)	H-11αβ, H-14α, H-15, Me-17
12β	1.66 (m)		
13		35.9 (s)	OH-8, H-11αβ, H-12αβ, H-14αβ, H-15, H-16, Me-17
14α	2.16 (br.d, 14.1)	38.0 (t)	OH-8, H-12αβ, H-15, Me-17
14β	1.49 (d, 14.1)		
15	5.81 (dd, 17.4, 10.7)	150.1 (d)	H-12β, H-14β, H-16, Me-17
16a	4.88 (d, 10.7)	109.4 (t)	Me-17
16b	4.96 (d, 17.4)		
17	0.90 (s)	25.6 (q)	H-12αβ, H-14αβ, H-15
18	1.17 (s)	17.1 (q)	
19		178.4 (s)	H-3α, H-5, Me-18
20	1.31 (s)	14.6 (q)	H-11αβ
OH	3.27 (s)		

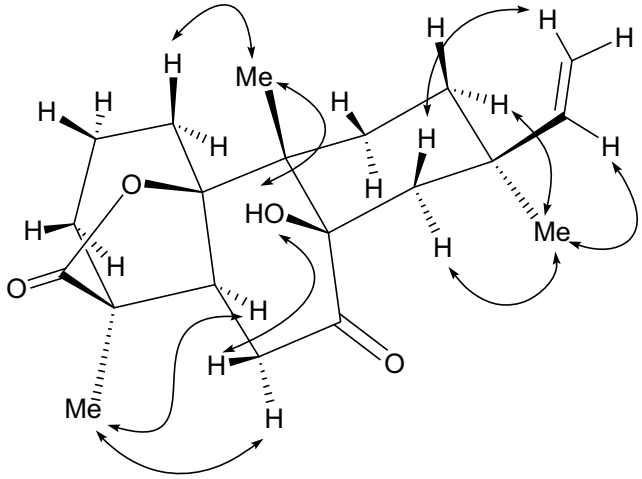

Fig. 2 The key ROESY correlations of **1**.

From the HMBC correlations (see **Table 1**) between the oxygenated quaternary carbon [δ_C 88.3 (s)] and H-1β, H-2α, H-6α, H-11β, Me-20, it could be concluded that the oxygenated quaternary carbon was ascribable to C-10. The signal at δ 77.3 (s) was assigned to C-8 by the observation of HMBC correlations of Me-20/ C-8, H_2-14/C-8, H-6α/C-8 and OH-8/C-8. Correlations between the hydroxyl proton [δ_H 3.27 (1H, s)] and C-8, C-9, C-13, C-14 further confirmed that the position of the hydroxyl group was designated at the C-8 position. HMBC correlations of Me-20 [δ_H 1.31 (3H, s)] with C-8, C-9, C-10 and C-11 revealed the presence of a methyl group at C-9. The relative downfield chemical shift of C-10 and C-19 was corresponding to the lactones at C-10 and C-19 of rosane diterpenoid lactones. In addition, HMBC correlations between the carbonyl carbon [δ_C 206.1 (s)] and H-5, H_2-6, H_2-14 were apparent, which led to the conclusion that the carbonyl was at C-7. Through careful analysis of the ^1H-^1H COSY spectrum of **1**, we concluded the presence of a -CH=CH_2 fragment due to coupling between H-15 and H_2-16. Furthermore, the HMBC correlations of H-15, H_2-16 and Me-17/C-13 confirmed a methyl group and a vinyl group at C-13. The relative stereochemistry of **1** was established by experimental ROESY (see **Fig. 2**). The ROESY correlations of Me-20/OH-8 and H-6β/OH-8 indicated that the conformation of OH-8 was β orientated, while the correlations of H-6α and H-5/Me-18 confirmed that the conformation of Me-18 was α orientated. Furthermore, the α-conformation of Me-17 was supported by the correlations of Me-17/H-14α, H-12α. Therefore, compound **1** was elucidated as 8β-hydroxyrosenonolactone, and named lyrosenonolactone.

Compound **2** was identified as rosololactone by comparison of its spectral data with those in the literature (Dockerill *et al*. 1978; Loukaci *et al*. 2000). However, the NMR data reported in the literature were inexact. For instance, the isolation of **2** from *Trichothecium roseum* has been reported along with its ^{13}C NMR data which mixed the C-18 and C-20 methyl carbon (Dockerill *et al*. 1978). Another report on the purification of **2** from *Holarrhena floribunda* incurrectly reported the ^{13}H NMR data for this compound, and the H-7 and H-1 data is inexact (Loukaci *et al*. 2000). On the basis of ^1H-^1H COSY, HMQC, HMBC and ROESY spectra, we assigned all proton signals and carbon signals (see experimental section).

The origin of **1** and **2** in *L. aurea* Herb. remains to be explained. It is well known that rosane diterpenoids are metabolites produced by fungi. We cannot rule out those compounds isolated from the whole plant of *L. aurea* result

from molds empoison, although careful examination of the plant material did not reveal any contamination. Another tentative explanation is that these metabolites were produced by fungi in the soil and then absorbed and translocated in the plant. The same circumstances were observed by Loukaci *et al*. (2000), in which a new trichothecene isolated from *Holarrhena floribunda*.

Identification

Lyrosenonolactone (**1**): Colorless needles (MeOH); mp 194-195°C; $[\alpha]_D^{22}$ –13.2° (*c* 0.21, $CHCl_3$); IR ν_{max} (cm^{-1}) (KBr): 3408, 2959, 1769, 1701, 1635, 1121, 946 cm^{-1}; HRESIMS *m/z* $[M+Na]^+$ (obsd 355.1892, calcd for $C_{20}H_{28}NaO_4$); ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100MHz) spectral data see **Table 1**.

Rosololactone (**2**): Colorless needles; $[\alpha]_D^{22}$ –8.0° (*c* 0.13, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz * values may be interchanged) δ: 5.77 (1H, d, *J* = 10.8, 17.6 Hz, H-15), 4.90 (1H, dd, *J* = 17.2, 1.1 Hz, H-16a), 4.85 (1H, dd, *J* = 10.7, 1.1 Hz, H-16b), 4.18 (1H, m, H-6), 2.13 (1H, m, H-1β), 1.99 (1H, m, H-7α), 1.83 (1H, m, H-2α*), 1.76 (1H, overlap, H-11β), 1.74 (1H, overlap, H-5), 1.72 (2H, overlap, H-2β*, H-3β), 1.60 (1H, m, H-12β), 1.54 (3H, overlap, H-1α, H-3α, H-8), 1.48 (1H, m, OH), 1.44 (1H, ddd, *J* = 13.7, 3.7, 2.5 Hz, H-11α), 1.38 (1H, m, H-14α), 1.33 (1H, m, H-7β), 1.30 (3H, s, H-18), 1.27 (1H, m, H-12α), 1.24 (3H, s, H-20), 1.19 (1H, m, H-14β), 0.99 (3H, s, H-17); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 31.08 (t, C-1), 19.88 (t, C-2), 36.42 (t, C-3), 45.64 (s, C-4), 55.56 (d, C-5), 63.81 (d, C-6), 37.47 (t, C-7), 32.04 (d, C-8), 37.89 (s, C-9), 87.65 (s, C-10), 31.75 (t, C-11), 32.26 (t, C-12), 36.05 (s, C-13), 39.95 (t, C-14), 150.47 (d, C-15), 109.20 (t, C-16), 22.38 (q, C-17), 16.91 (q, C-18), 181.03 (s, C-19), 13.41 (q, C-20).

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