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Andrographolactone, a unique diterpene from *Andrographis paniculata*

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ABSTRACT

Andrographolactone (**1**), possessing an unprecedented diterpene skeleton, was isolated from the EtOAc extract of the aerial parts of *Andrographis paniculata*. Its structure was established by NMR, IR, UV, and HRESIMS data and subsequently confirmed by X-ray diffraction analysis. A possible biogenetic pathway of **1** was also proposed. Bioassay showed that **1** exhibited cytotoxic activity.

The plant *Andrographis paniculata* Nees (Acanthaceae) is widely used as a traditional medicine in India, Southeast Asia, and China.[1] The aerial part of this plant was recorded for the therapy of some diseases such as wounds, ulcers, chronic fever, cough, and inflammations. The labdan-type diterpenes of the plant were considered as important bioactive components and had been extensively investigated in the past decades. So far, phytochemical investigation on this plant has led to the isolation of more than 30 labdan-type diterpenoids.[2–5] As a part of our study on diterpene constituents of medicinal plants, we had reported some new labdan-type diterpenoids from *A. paniculata*.[6] Recently, a novel diterpene, andrographolactone (**1**), with an unprecedented skeleton was isolated from the aerial part of this plant. In this Letter, we describe the isolation and structural elucidation of **1**.

The dried and powdered aerial parts of *Andrographis paniculata* (20 kg)[7] were extracted with 95% EtOH (60 l × 3), and the solution was evaporated in vacuo. The extract was subsequently suspended in distilled water and was successively partitioned with cyclohexane, ethyl acetate, and *n*-butanol to afford 115 g, 1520 g, and 245 g residues, respectively, as well as the remaining-soluble residue (620 g). The EtOAc extract was chromatographed over silica gel, Sephadex LH-20, and ODS columns. Andrographolactone (**1**, 9 mg) was finally obtained by preparative reverse phase HPLC (Lichrosorb RP-18, 5 μm, 10 × 250 mm) using 65% aqueous methanol as eluent.

1[8] was obtained as colorless needles, mp 96–98 °C. The molecular formula $C_{20}H_{24}O_2$ was established by HRESIMS (*m/z* 319.1687 $[M+Na]^+$, calcd for $C_{20}H_{24}O_2Na$ *m/z* 319.1674). The positive reactions with Legal and Kedde reagents indicated the presence of α,β-unsaturated γ-lactone ring. The absorption bands in its IR spectrum suggested the presence of α,β-unsaturated γ-lactone ring (1755, 1651 cm^{-1}) and aromatic ring (1590, 1516 cm^{-1}). The presence of α,β-unsaturated γ-lactone and aromatic rings was also supported by the UV spectrum [λ_{max} 225 nm (ε 13,200), 259 nm (ε 12,100)].

The ^1H and ^{13}C NMR spectra displayed signals for a pentasubstituted benzene ring [δ_C 138.1 (s, C-1), 136.5 (s, C-7), 129.9 (d, C-8), 133.2 (s, C-9), 135.9 (s, C-10), 133.3 (s, C-11); δ_H 6.81 (1H, br s, H-8)] and an α,β-unsaturated γ-lactone ring [δ_C 133.2 (s, C-14), 146.1 (d, C-15), 70.8 (t, C-16), 174.6 (s, C-17); δ_H 4.68 (2H, m, H-16), 7.15 (1H, t, *J* = 1.6 Hz, H-15)]. In addition, an olefinic bond [δ_C 139.7 (s, C-5), 127.0 (d, C-6); δ_H 6.31 (1H, br s, H-6)], five methylenes [δ_C 25.7 (t), 28.7 (t), 29.5 (t), 29.9 (t), 34.6 (t); δ_H 1.87 (2H, m), 2.00 (2H, m), 2.37 (2H, m), 2.63 (2H, m), 2.82 (2H, t, *J* = 8.3 Hz)] and three methyls [δ_C 15.7 (q), 20.0 (q), 26.3 (q); δ_H 1.82 (3H, br s), 2.23 (3H, br s), 2.24 (3H, br s)] were observed in the 1D NMR spectra of **1**. With the aid of 1D and 2D NMR experiments, all the ^1H and ^{13}C NMR signals of **1** were assigned and are shown in Table 1.

The ^1H–^1H COSY experiment of **1** indicated the presence of three structural fragments [**a** (C-2 to C-4), **b** (C-19 to C-6), and **c** (C-12 to C-16)] (Fig. 1). The linkages of these fragments could be established by a HMBC experiment. Thus, in the HMBC spectrum, the correlations between H_2-2 (δ_H 2.63) and C-7 (δ_C 136.5), C-11 (δ_C 133.3), and C-1 (δ_C 138.1), as well as between H-6 (δ_H 6.31) and C-1 (δ_C 138.1) and C-8 (δ_C 129.9) could be observed, suggesting that fragments **a** and **b** were connected to the benzene ring via

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Table 1
NMR data of **1** (pyridine-d_5, J in Hz)

	δ_H	δ_C	$^1H-^1H$ COSY	HMBC
1	—	138.1 (s)		
2	2.63 (2H, m)[a]	29.5 (t)	H_2-3	C-1, -3, -4, -7, -11
3	1.87 (2H, m)[a]	29.9 (t)	H_2-2, H_2-4	C-1, -2, -4, -5
4	2.00 (2H, m)[a]	34.6 (t)	H_2-3	C-2, -5, -6, -19
5	—	139.7 (s)		
6	6.31 (1H, br s)	127.0 (d)	H_3-19	C-1, -4, -8, -19
7	—	136.5 (s)		
8	6.81 (1H, br s)	129.9 (d)	H_3-18	C-1, -6, -10, -18
9	—	133.2 (s)		
10	—	135.9 (s)		
11	—	133.3 (s)		
12	2.82 (2H, t, 8.3)	28.7 (t)	H_2-13	C-9, -10, -13, -14
13	2.37 (2H, m)	25.7 (t)	H_2-12, H-15, H_2-16	C-12, -14, -15, -17
14	—	133.2 (s)		
15	7.15 (1H, t, 1.6)	146.1 (d)	H_2-13, H_2-16	C-13, -14, -16, -17
16	4.68 (2H, m)	70.8 (t)	H_2-13, H-15	C-14, -15
17	—	174.6 (s)		
18	2.24 (3H, br s)	20.0 (q)	H-8	C-8, -9, -10
19	1.82 (3H, br s)	26.3 (q)	H-6	C-4, -5, -6
20	2.23 (3H, br s)	15.7 (q)		C-1, -10, -11

[a] The geminal protons attached to C-2, -3, and -4 positions showed the same chemical shift, respectively, which could be attributed to the chemical shift equivalence environment due to the thermal motion of the single bond fragment from C-2 to C-4 in the seven-membered ring.

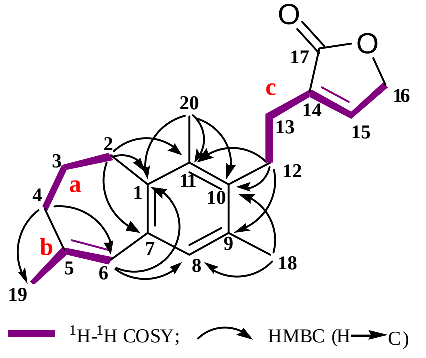


Figure 1. Key $^1H-^1H$ COSY and HMBC correlations of **1**.

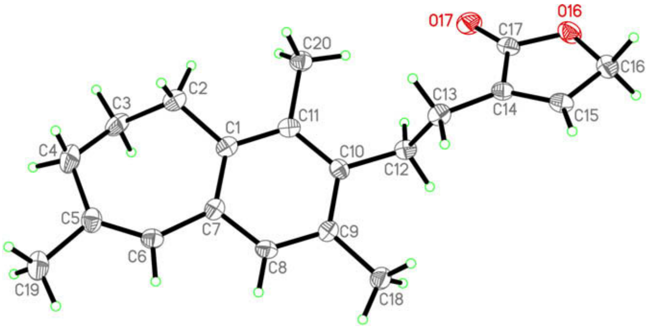
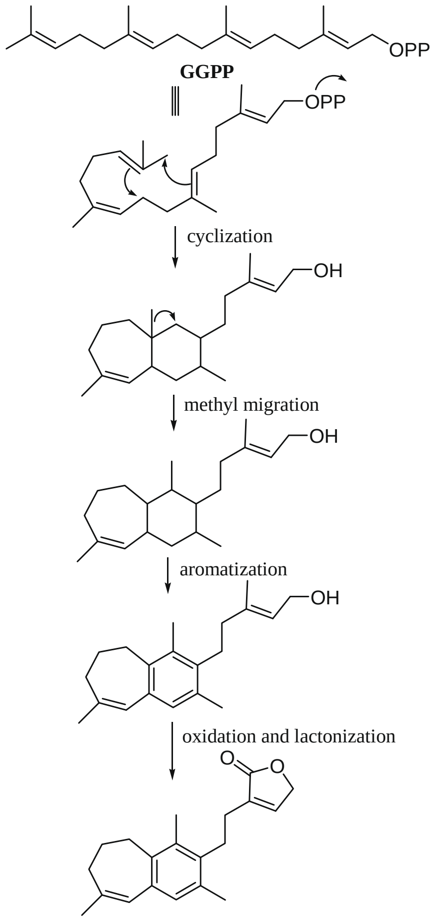


Figure 2. Perspective drawing of X-ray structure of **1**.

C-2–C-1 and C-6–C-7 bonds, respectively. In addition, the linkage of **a** and **b** (via C-4–C-5 bond) was supported by the correlations between H_2-4 (δ_H 2.00) and C-6 (δ_C 127.0) and C-19 (δ_C 26.3). The above HMBC correlations led to the assignment of a seven-membered ring and a benzene ring in **1**. Moreover, the HMBC correlations between H_2-12 (δ_H 2.82) and C-9 (δ_C 133.2), C-10 (δ_C 135.9), and C-11 (δ_C 133.3) indicated that **c** (including an α,β-unsaturated γ-lactone ring) was connected to the benzene ring via C-12–C-10 bond. The positions of the two methyl groups were also confirmed by the HMBC experiment. The cross-peaks between H_3-18 (δ_H 2.24) and C-8 (δ_C 129.9), C-9 (δ_C 133.2), and C-10 (δ_C 135.9), as well as between H_3-20 (δ_H 2.23) and C-1 (δ_C 138.1), C-10 (δ_C 135.9), and C-11 (δ_C 133.3) suggested that the two methyl groups were located at C-9 and C-11 positions of the benzene ring, respectively.

Finally, the structure of **1** was confirmed by the single crystal X-ray diffraction analysis (Fig. 2),[9] which revealed a novel diterpene with an unprecedented carbon skeleton constructed by an unsaturated seven-membered ring, fused benzene ring moiety, and an α,β-unsaturated γ-lactone ring connected via two methylenes. **1** was designated with the trivial name andrographolactone. In the solid state, the average displacement parameter of the fragment from C-2 to C-4 is 47.8% larger than that of the remaining four atoms of the seven-membered ring. In addition, the α,β-unsaturated γ-lactone ring was extended on the opposite side of the seven-membered ring and offered no electronic influence on the fragment from C-2 to C-4. Accordingly, the geminal protons on this fragment existed in a chemical shift equivalence environment and showed the same chemical shift as shown in Table 1.

Biogenetically, andrographolactone (**1**) was also derived from geranylgeranyl diphosphate (GGPP) as other diterpenes. A plausible biogenetic pathway of **1** was proposed (Scheme 1). **1** was obtained by the cyclization of GGPP, methyl migration, aromatization, oxidation, and lactonization processes.



1

Scheme 1. Proposed biogenesis of **1**.

The cytotoxicity of **1** was measured by MTT assay.[10] **1** exhibited cytotoxic effects on two human cancer cell lines, with IC_{50} values of 0.05 mM (LoVo, colon cancer) and 0.06 mM (NCI-H460, non-small cell lung cancer).

Acknowledgments

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Supplementary data

NMR and X-ray data for **1** are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.05.097.

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7. Plant Material: The aerial parts of *A. paniculata* were collected in Ruijin county, Jianxi province of China, in July of 2005, and were authenticated by Professor Min-Jian Qin of China Pharmaceutical University. A voucher specimen (No. 050711) was deposited in the herbarium of China Pharmaceutical University, Nanjing.
8. Compound **1**: Colorless needles, mp 96–98 °C; IR (KBr) ν_{max} 3477, 1755, 1651, 1590, 1516, 1288, 1158, 1080, 916, 829 cm^{-1}; UV (MeOH) λ_{max} 225, 259 nm; HRESIMS *m/z* 319.1687 [M+Na]$^+$ (calcd for $C_{20}H_{24}O_2Na$, 319.1674). ^1H (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) data, see Table 1.
9. X-ray diffraction studies on compound **1**: $C_{20}H_{24}O_2$, $M = 296.39$, crystal size $0.40 \times 0.20 \times 0.15$ mm^3, space group: triclinic, $P\bar{1}$, $T = 100(2)$K, $a = 6.3213$ (7) Å, $b = 10.2760$ (12) Å, $c = 12.5886$ (15) Å, $\alpha = 77.654$ (2)°, $\beta = 85.326$ (2)°, $\gamma = 81.066$ (2)°, $V = 788.14$ (16) Å^3, $F_{(000)} = 320$, $Z = 2$, $d = 1.249$ mg/m^3. Data were collected on a Bruker APEX CCD diffractometer with graphite-monochromated Mo–Kα radiation ($\lambda = 0.71073$ Å). Lattice determination and data collection using SMART v.5.625, data reduction and absorption correction by empirical methods were performed by SAINT v. 6.26 and SADABS v.2.03. The crystal structure was solved by direct methods, expanded by difference Fourier syntheses, and refined by full-matrix least-squares on F^2 using the SHELXTL v.6.10 software package. 4005 reflections collected with 2721 independent [$R_{(int)} = 0.0213$], data/restraints/parameters 2721/0/201, goodness-of-fit on $F^2 = 1.033$, final indices $R_1 = 0.0526$, w$R_2 = 0.1211$, largest difference peak and hole, 0.249 and -0.177 e Å^{-3} (CCDC deposition number: CCDC 679530).
10. MTT assay: Cells (2×10^4 cells/well) were treated with various concentrations of compound **1** in 96-well culture plates (Costar, USA) for 48 h. After that, cells were cultured with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) for 4 h at 37 °C. The formazan crystal formed was dissolved with DMSO, and the absorbance was colorimetrically measured at 570 nm. Data represent the mean value of three independent experiments.