Andropanolide and Isoandrographolide, Minor Diterpenoids from *Andrographis paniculata*: Structure and X-ray Crystallographic Analysis[⊥]

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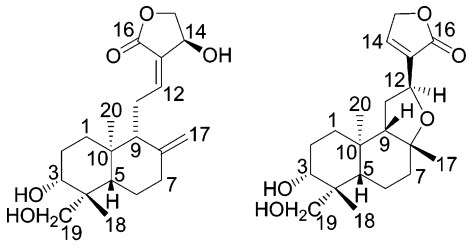
Phytochemical investigation of the leaves of *Andrographis paniculata* has led to the isolation of a new labdane type diterpenoid, andropanolide (**1**), along with seven known diterpenoids including isoandrographolide (**2**), previously reported as a rearrangement product of andrographolide. The structures and stereochemistry of compounds **1** and **2** were established by X-ray crystallographic analysis.

Andrographis paniculata Nees (Acanthaceae)[1] is a small herb found throughout southeast Asia and India. Extracts of this plant and its constituents are reported to exhibit a wide spectrum of biological activities including antibacterial,[2] antiinflammatory,[3] antimalarial,[4,5] antithrombotic,[6] hepatoprotective,[7] and immunostimulant[8] properties. An alcoholic extract of the plant and its major constituents have been shown to affect the cell cycle progression in prostrate and breast cancer cell lines[9] as well as to exhibit potent cell differentiation inducing activity in mouse myeloid leukemia (M1) cells.[10] Previous investigations on the chemical composition of *A. paniculata* showed that it is a rich source of labdane diterpenoids[6,10−18] and 2′-oxygenated flavonoids.[16,18−23] In the present study, we report the isolation of andropanolide (**1**), a new labdane diterpenoid, along with seven known diterpenoids, andrographolide,[6,10−18] andrograpanin,[10,14,18] isoandrographolide (**2**),[24,25] 14-deoxy-11,12-didehydroandrographolide,[10,13,17,18] 14-deoxyandrographolide,[10,12−14,18] isoandrographolide (**2**),[24,25] neoandrographolide,[10,12,14,26] and 14-deoxyandrographolide 19-glucoside.[10,14] The structures were determined mainly through the use of 1D and 2D NMR techniques. For **1** and **2** the deductions were confirmed by X-ray crystallographic analysis.



1

2

Andropanolide (**1**) displayed a quasimolecular ion peak at *m*/*z* 373 in the ESIMS (positive mode). This information, together with data from elemental analysis and the ^{13}C NMR DEPT spectrum, suggested the molecular formula to be $C_{20}H_{30}O_5$. The IR spectrum showed absorption bands at 3600 (hydroxy group), 1760 (α,β-unsaturated-γ-lactone), and 898 (exomethylene) cm^{-1}. The ^{1}H NMR spectrum of **1** indicated the presence of two methyl groups with three-proton singlets at δ 1.29 and 0.52 ppm. It also exhibited two singlets with fine splitting at δ 4.68 (1H) and 4.52 (1H) ascribed to the exomethylene group. A set of doublets (J = 11.0 Hz) at δ 4.24 and 3.41, each integrating for one proton, pointed to the presence of a hydroxymethylene group, with the doublet at δ 3.41 overlapping a signal assignable to H-3. The double triplet at δ 6.52 (J = 7.0, 1.0 Hz) and a broad singlet at δ 4.80 were attributable to H-12 and H-14. Appropriate ^{13}C NMR signals were observed at δ 17.0 (C-20), 25.42 (C-18), 65.9 (C-19), 70.8 (C-14), 81.6 (C-3), 109.8 (C-17), 131.5 (C-13), and 150.1 (C-12). The ^{13}C NMR data of **1** were comparable to those of andrographolide, except that the signals for C-12 and C-14 appeared downfield in **1** by 3.2 and 4.9 ppm, respectively. This was accompanied by an upfield shift of 0.65 ppm for H-12, which suggests that the geometry of the double bond may be reversed in **1**, so that the carbonyl group is oriented away from H-12. The downfield shift of C-14 in **1** can then be rationalized on the basis of the absence of a γ-effect from H-11. On the basis of the above data, andropanolide (**1**) was concluded to be a geometric isomer of andrographolide, although the stereochemistry at C-14 remained to be proved conclusively. Finally, the detailed structure and stereochemistry of **1** were established unambiguously from single-crystal X-ray analysis (ORTEP drawing in Figure 1). The absolute configuration has been assumed on the basis of that established for andrographolide from ORD studies[27] and X-ray crystallographic inferences.[28]

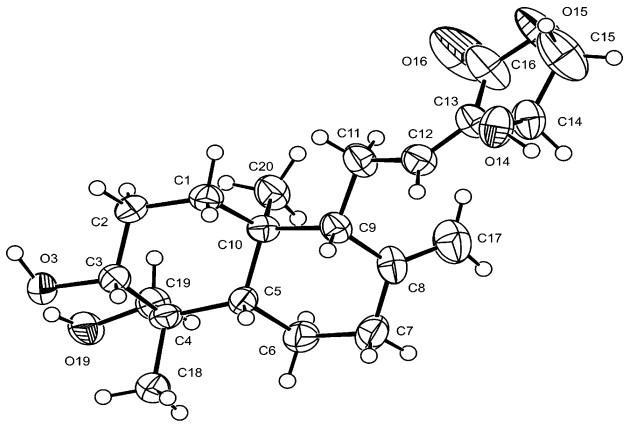


Figure 1. ORTEP drawing for compound **1**.

A compound with similar structure but with unknown C-14 stereochemistry has been reported earlier from *A. paniculata*[10] and as a metabolite of andrographolide in rats.[28] The spectroscopic data reported are in good agreement with those observed by us for andropanolide (**1**), which settles the structure of these products.

[⊥] Dedicated to Dr. Norman R. Farnsworth of the University of Illinois at Chicago for his pioneering work on bioactive natural products.

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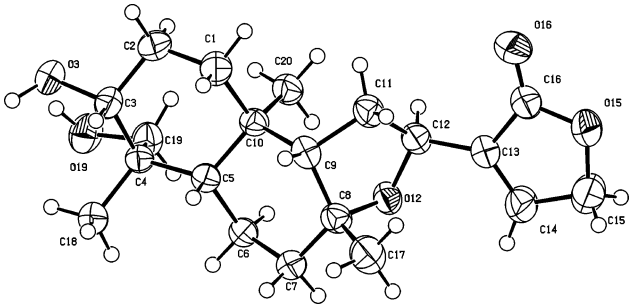

Figure 2. ORTEP drawing for compound **2**.

Isoandrographolide (**2**), obtained as a colorless crystalline solid, displayed 20 signals in the ^{13}C NMR spectrum (one carbonyl, four other nonprotonated carbons, five methines, seven methylenes, and three methyls) and gave a pseudomolecular ion peak at *m*/*z* 373 in the ESIMS (positive mode) attributed to the $[M + Na]^+$ ion, suggesting it to be an isomer of andrographolide ($C_{20}H_{30}O_5$). The ^1H NMR spectrum had several signals comparable to those of the latter, viz., a 1H downfield singlet (δ 7.29) assignable to the β-proton of an α,β-unsaturated carbonyl system, a signal at δ 3.44 for H-3, and a pair of AB doublets ($J = 10.8$ Hz) at δ 4.26 and 3.36 for the hydroxymethylene group (H_2-19). The major difference was in the presence of three methyl singlets (at δ 1.25, 1.10, and 0.95) coupled with the absence of signals for an exomethylene group, which pointed to the presence of a ring system involving C-8.

Detailed analysis of the 2D NMR spectra provided further structural information. Although correlations involving ring A and most of ring B were comparable to those of andrographolide, the chemical shifts of C-8 and C-12 (δ 83.1 and 73.5) identified them as being oxygenated, nonolefinic carbons. Evidence for a furanoid C-ring came from the observed HMBC correlation of the C-8 signal with that of H-12 (δ 4.68). The H-12 triplet showed, in addition, cross-peaks with signals for the olefinic carbons (δ 143.7 and 138.8), pointing to branching at C-12. The 2H singlet at δ 4.81 could then be due to the acyloxymethylene protons of the α,β-unsaturated-γ-lactone moiety constituting ring D. The location of the third methyl group at C-8 was evident from the HMBC correlation of C-17 with the H-9 signal.

The methyl group at C-10 (supposed to be α as in case of andrographolide) showed a strong NOE interaction with H-12; the latter must therefore be α-oriented. Absence of any NOE correlations further indicated that H_3-17 is *trans* to both H_3-20 and H-12. The evidence suggested that the compound could be identical with isoandrographolide, reported earlier[24,25] as the acid-catalyzed rearrangement product of andrographolide but not so far isolated from a natural source.[29] However the stereochemistry, particularly at C-8 and C-12, remained to be settled. Therefore, we undertook an X-ray crystallographic study. The results prove that the stereochemistry at C-8 and C-12 should be as shown (8*R*,12*S*) in the ORTEP diagram (Figure 2).

Experimental Section

General Experimental Procedures. TLC was carried out on silica gel 60 F_{254} (Merck) plates, and spots were visualized by spraying with Liebermann-Burchard reagent followed by heating at 120 °C. Column chromatography was performed on silica gel mesh 60−120 (Merck). Melting points were measured on a Yanagimoto micro-melting point apparatus and were uncorrected. Specific rotations were measured on a JASCO DIP-370 polarimeter. IR spectra were recorded as KBr pellets using a JASCO 7300 FTIR spectrometer. ^1H and ^{13}C NMR spectra were recorded using a Bruker DRX spectrometer (500 MHz). The mass spectra were recorded on a Q-TOF-Micromass spectrometer.

Plant Material. The leaves of *Andrographis paniculata* were collected from suburbs of Kolkata in January 2005 and identified at the Indian Botanical Garden, Howrah, India. A voucher specimen (No. 338) has been deposited at the Medicinal Chemistry Department of this institute.

Table 1. X-ray Crystallographic Data Processing and Refinement Statistics for Andropanolide (**1**) and Isoandrographolide (**2**)

	andropanolide (**1**)	isoandrographolide (**2**)
formula (asymmetric unit)	$C_{20}H_{30}O_5$	
molecular weight	350.44	
space group	$P2_12_12_1$	$P2_12_12_1$
Z	4	4
cell params (Å, deg)		
a	7.435(1)	7.004(1)
b	10.636(1)	11.484(1)
c	23.951(2)	22.831(1)
volume (Å^3)	1894.1(7)	1836.3(3)
no. of reflns measd	9221	8323
completeness (%)	98.5	99.0
R sym (%) overall	5.1	4.4
no. of indep reflns	2796	2729
no. of obsd reflns[a]	2153	2339
R factor (observed *F* data)	0.043	0.039
R factor (all *F* data)	0.062	0.052
R factor (observed F^2 data)	0.101	0.100
no.of params	271	232
min./max. (e^-) in last electron density	−0.16/+0.14	−0.14/+0.11
CCDC deposit number	259885	274843

[a] Criteria for observation: $F > 2\sigma(F)$.

Extraction and Isolation. The air-dried powdered leaves of *A. paniculata* (1 kg) were subjected to extraction in a Soxhlet apparatus with light petroleum ether (bp 60−80 °C), $CHCl_3$, and MeOH, respectively. The MeOH extract was partitioned between *n*-BuOH and water. The *n*-butanol extract (15 g), obtained after evaporation of solvent in vacuo, was chromatographed on a column of silica gel (mesh 60−120). Gradient elution was carried out with chloroform followed by various mixtures of $CHCl_3$ and MeOH (19:1, 9:1, 17:3, and 4:1). A total of 50 fractions (each 250 mL) were collected, and fractions giving similar spots on TLC were combined. Repeated chromatography of the fractions followed by preparative TLC resulted in the isolation of andrographolide (2.5 g), andropanolide (14 mg) (**1**), andrograpanin (40 mg), 14-deoxy-11,12-didehydroandrographolide (80 mg), 14-deoxyandrographolide (500 mg), isoandrographolide (25 mg) (**2**), neoandrographolide (1.5 g), and 14-deoxyandrographolide 19-glucoside (40 mg).

Andropanolide (1): colorless needles (MeOH); mp 210−211 °C; $[\alpha]^{25}_D$ − 31.5° (*c* 0.5, C_5H_5N); IR (KBr) ν_{max} 3600, 1760, 898 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) δ 6.52 (1H, dt, $J = 7.0$, 1.0 Hz, H-12), 4.68 (1H, s, H-17a), 4.52 (1H, br s, H-17b), 4.33 (1H, dd, $J = 9.5$, 6.0 Hz, H-15a), 4.24 (1H, d, $J = 11.0$ Hz, H-19a), 4.19 (1H, dd, $J = 9.5$, 3.5 Hz, H-15b), 3.44 (1H, m, overlapped signal, H-3), 3.41 (1H, d, $J = 11.0$ Hz, H-19b), 2.92 (1H, brdd, $J = 16.0$, 6.0 Hz, H-11a), 2.79 (1H, ddd, $J = 16.0$, 11.0, 7.5 Hz, H-11b), 2.11 (1H, brd, $J = 12.5$ Hz), 1.85−1.50 (several protons), 1.29 (3H, s, H-18), 1.2−0.9 (several protons), 0.52 (3H, s, H-20), signal for H-14 apparently merged in solvent signal at 4.80 ppm; ^{13}C NMR (C_5D_5N, 125 MHz) δ 171.6 (C, C-16), 150.1 (CH, C-12), 149.9 (C, C-8), 131.5 (C, C-13), 109.8 (CH_2, C-17), 81.6 (CH, C-3), 76.0 (CH_2, C-15), 70.8 (CH, C-14), 65.9 (CH_2, C-19), 58.4 (CH, C-5), 57.1 (CH, C-9), 44.9 (C, C-4), 41.1 (C, C-10), 40.0 (CH_2, C-7), 39.0 (CH_2, C-1), 30.7 (CH_2, C-2), 26.2 (CH_2, C-11), 25.42 (CH_3, C-18), 25.36 (CH_2, C-6), 17.0 (CH_3, C-20); ESIMS (positive mode) *m*/*z* 373 $[M + Na]^+$; *anal*. C 68.44%, H 8.62%, calcd for $C_{20}H_{30}O_5$, C 68.54%, H 8.63%.

Isoandrographolide (2): colorless prisms (ethyl acetate−hexane); mp 198−200 °C; $[\alpha]^{25}_D$ −40.4° (*c* 1.09, $CHCl_3$); IR (KBr) ν_{max} 3268, 1754, 1021 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.29 (1H, s, H-14), 4.81 (2H, s, H-15), 4.68 (1H, t, $J = 4.6$ Hz, H-12), 4.26 (1H, d, $J = 10.8$ Hz, H-19a), 3.44 (1H, d, $J = 9.3$ Hz, H-3), 3.36 (1H, d, $J = 10.8$ Hz, H-19b), 2.43 (1H, dd, $J = 8.3$, 4.6 Hz, H-11a), 2.20 (1H, m, H-1a), 2.01 (1H, dd, $J = 8.3$, 4.6 Hz, H-11b), 1.77 (3H, m, H-7a, H-2), 1.53 (3H, m, H-1b, H-6a, H-9), 1.44 (1H, m, H-6b), 1.25 (3H, s, H-18), 1.10 (3H, s, H-17), 1.04 (1H, m, H-7b), 0.98 (1H, m, H-5), 0.95 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.1 (C, C-16), 143.7 (CH, C-14), 138.8 (C, C-13), 83.1 (C, C-8), 81.2 (CH, C-3), 73.5 (CH, C-12),

71.0 (CH_2, C-15), 64.6 (CH_2, C-19), 58.4 (CH, C-9), 53.1 (CH, C-5), 42.9 (C, C-4), 39.4 (CH_2, C-7), 36.6 (CH_2, C-1), 36.0 (C, C-10), 33.3 (CH_2, C-11), 32.0 (CH_3, C-17), 27.8 (CH_2, C-2), 23.2 (CH_3, C-18), 18.6 (CH_2, C-6), 16.8 (CH_3, C-20); ESIMS (positive mode) *m*/*z* 373 $[M + Na]^+$; *anal*. C 68.46%, H 8.61%, calcd for $C_{20}H_{30}O_5$, C 68.54%, H 8.63%.

X-ray Crystallographic Analysis of Andropanolide (1) and Isoandrographolide (2). The two compounds were crystallized from a methanol−anisole mixture. In both cases, a monocrystal was selected, glued on a glass fiber, and mounted on a Nonius CCD Kappa diffractometer using Mo Kα radiation ($\lambda = 0.7107$ Å). The structures were solved using the SHELXS[30] program and refined with SHELXL. Diffraction data and refinement statistics are given in Table 1.

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