

## Note

### Phytoconstituents of *Phlogacanthus tubiflorus* Nees (Acanthaceae): Phlogacanthin, a new diterpene lactone (andrographolide) isolated from the root wood

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Phlogacanthin **2**, a naturally occurring andrographolide has been isolated from *Phlogacanthus tubiflorus* Nees (Acanthaceae) made available from humid rainforest of Arunachal Pradesh, India along with the known compounds  $\beta$ -sitosterol, lupeol and 3-epibetulin. The structure of the new compound has been elucidated on the basis of elemental analysis and spectral data.

Andrographolide **1**, the main crystalline bitter principle of *Andrographis paniculatus* Nees (Acanthaceae) has been the subject of a number of investigations<sup>1</sup> for its structural complexity and biological activity. In a systematic study<sup>2</sup> aimed at isolation of diterpene lactones from indigenously occurring medicinal plants of Arunachal Pradesh, we have isolated phlogacanthin **2** from *Phlogacanthus tubiflorus*<sup>3</sup> Nees (Acanthaceae) whose general skeleton resembles **1** with minor variations. The C-8/C-17 double bond of **1** is transformed into an epoxide followed by the introduction of -OH function at C-9 and acetylation of primary alcohol at C-19 in **2** accounting for a natural manipulation of **1** through probable epoxidation<sup>4,5</sup> of exocyclic double bond in the host domain.

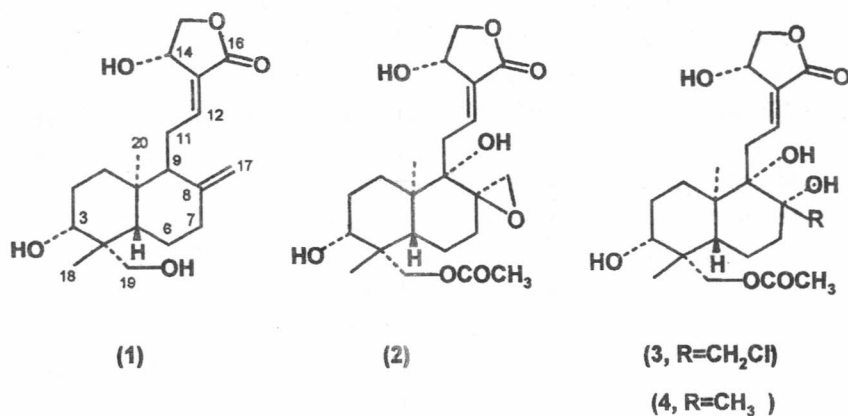
Ethyl acetate extract of the root material resulted in greenish solid which on repeated crystallisation from methanol yielded pure (TLC) compound **2** (yield 0.039 %) with a m.p. 230-31°C; UV (MeOH): 205 nm, IR (KBr): 3440, 3300, 1740 and 1720 cm<sup>-1</sup>. The compound was assigned the molecular formula C<sub>22</sub>H<sub>32</sub>O<sub>8</sub> on the basis of elemental analysis, EIMS, <sup>13</sup>C NMR and DEPT experiments.

In the <sup>13</sup>C NMR (Bruker WM-400, 100 MHz, Py-d<sub>5</sub>) **2** showed all the carbon (Table 1). The <sup>1</sup>H NMR (400 MHz, Py-d<sub>5</sub>) spectrum of **2** showed the presence of tertiary CH<sub>3</sub> signals at  $\delta$  1.02 and 1.06

Table I—<sup>13</sup>C NMR (100 MHz) data for compounds **1** and **2** (Py-d<sub>5</sub>)

Position	<b>1</b>		<b>2</b>	
	$\delta$ C	$\delta$ C	$\delta$ C	$\delta$ H (J=Hz)
1	37.6	36.1		1.80
2	29.1	29.6		1.85
3	80.1	78.3		3.05
4	43.4	36.0		
5	55.6	52.5		
6	24.5	18.8		1.90
7	38.3	38.4		1.95
8	148.1	59.5		
9	56.6	73.0		4.85
10	39.4	39.2		
11	25.1	27.7		
12	146.8	162.8		5.12
13	130.2	137.2		
14	66.2	64.3		4.85
15	75.2	75.0		
16	170.5	175.3		
17	108.7	50.7		2.32, 2.70
18	23.7	30.0		1.02
19	64.2	64.3		3.66
20	15.3	19.5		1.06

ppm. Signals for two acetoxy methylene protons (-CH<sub>2</sub>OAc) appeared as a pair of doublets centered at  $\delta$  3.66 (1H,  $J=11$ Hz) and  $\delta$  3.96 (1H,  $J=11$ Hz). Carbinol proton of C-3 appeared as a multiplet at  $\delta$  3.05. The C-12 and C-14 protons appeared as singlet and triplet at  $\delta$  5.12 and 4.85 ( $J=8$  Hz) respectively. The epoxymethylene protons appeared as doublets at  $\delta$  2.32 (1H, d,  $J=8$ Hz) and 2.70 (1H, d,  $J=8$ Hz). The presence of the terminal epoxide was also confirmed by performing two chemical transformations<sup>6</sup>. First, the reaction of POCl<sub>3</sub> on **2** gave a product **3** containing a chloro methyl group (AB, q, at  $\delta$  3.85, 3.98 ppm) through the addition of HCl to the terminal epoxide in the anti-Markonikov sense. Another transformation was conducted by reducing the compound **2** with LAH in THF which gave the product **4** with a new methyl group ( $\delta$  1.24 ppm) indicative of its placement on a carbon bearing an oxygen function. On the basis of the above observations the structure of the new compound is presented as **2** (cf Scheme 1). The fragmentation pattern in the mass spectrum was consistent with the proposed above structure. In the



Scheme I

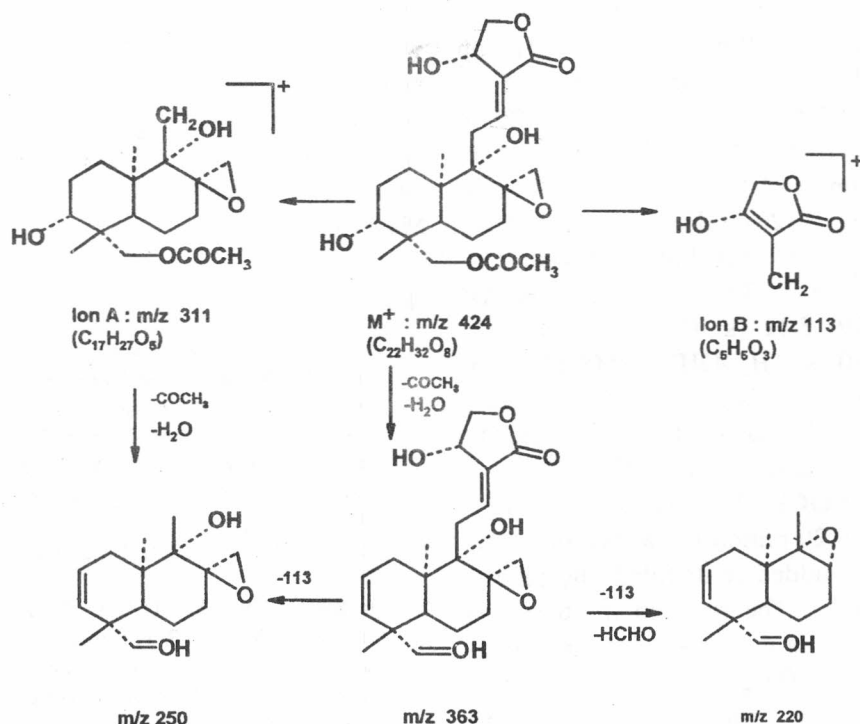


Chart 1

mass spectrum the molecular ion peak at  $m/z$  311 (fragment ion A) supports the proposed structure of ring A and an ion at  $m/z$  113 (fragment B) gives evidence for the position of the furanone ring. The probable fragments in the mass spectrum are represented in Chart I.

### Experimental Section

Melting points were determined in open capillaries on a Buchi oil heated (Model 510) apparatus and are uncorrected. UV spectra were recorded in MeOH on a Hitachi 320 and Jasco UV-Vis 7800 spectrophotometers; IR on a Perkin-Elmer 983

spectrometer ( $\nu_{\max}$  in  $\text{cm}^{-1}$ ), and NMR spectra on a Varian EM-390, Bruker WM-400 spectrometer using TMS as internal reference (chemical shifts in  $\delta$  ppm). Mass spectra were recorded on Jeol-300 or INCOS-50 GC/MS/DS mass spectrometer. TLC and Preparative TLC were performed on Si gel-G (E. Merck) and Column Chromatography on Si gel (BDH) of 60-120 mesh size.

### Extraction and Isolation

Dried root wood (700 g) of *Phlogacanthus tubiflorus*<sup>3</sup> Nees (collected from Namsai and Chessa reserve forest of Itanagar, Arunachal Pradesh, In-

dia during November 1990, identified by Forest Research institute, Chessa, Itanagar, Government of India from the Herbarium species maintained in the Institute) were extracted with pet. ether (60-80°) and ethyl acetate successively at room temperature. The gum obtained after concentration of pet. ether extract resulted in the isolation of known compounds  $\beta$ -sitosterol, lupeol and 3-epibetulin. The greenish ethyl acetate extract obtained after keeping the plant material over 3 litre of ethyl acetate for 30 days, resulted in 1.3 g of a solid after concentrating to 200 mL. This isolation was repeated 4 times to get 5.5 g of the solid product. The solid on repeated crystallization from methanol yielded 1.08 g of compound 2, phlogacanthin.

**Phlogacanthin.** Crystalline solid (MeOH); mp 230-31°C; UV (MeOH)  $\lambda_{\max}$ : 205 nm;  $[\alpha]_D -85^\circ$  (c 1.05, CHCl<sub>3</sub>); IR (KBr): 3400-3300, 2970, 2930, 2860, 1740, 1720, 1680, 1460, 1385, 1230, 1020, 995, 900 and 768 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 Mhz, Py-d<sub>5</sub>):  $\delta$  1.02, 1.06 (s, 6H), 2.32 (d, 1H,  $J=4$ Hz), 2.70 (d, 1H,  $J=13$  Hz), 1.80, 1.85 (6H, both methyls), 2.12 (H-5), 3.05 (m, 1H, H-3), 3.66, 3.96 (each 1H, AB q,  $J=11$  Hz, H-19) 5.12 (s, 1H, H-12), 4.85 (t,  $J=8$ Hz, H-14), 4.80 (s, 1H, -OH): EIMS (70 eV): m/z 424 [M]<sup>+</sup> 406, 381, 363, 311, 268, 250, 220, 180, 123, 113, 95, 85. Anal. Calcd. for C<sub>22</sub>H<sub>32</sub>O<sub>8</sub>: (C, 62.24, H, 7.59. Found: C, 62.30; H 7.62%).

**Reaction with POCl<sub>3</sub>.** To a solution of compound 2 (84.8 mg, 0.20 mmole) in anhydrous pyridine (10 mL), was added re-distilled phosphorus oxychloride (1.0 mL). The reaction mixture was then stirred at 100 °C for 4 hr, cooled to room temperature, poured into 100 g crushed ice and then extracted with 150 mL ether. The extract was

washed with 125 mL (2X) portions of water, dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated. The residue was crystallized from chloroform: hexane to give the compound 3, mp 112-13°C, <sup>1</sup>H NMR:  $\delta$  3.85, 3.98 (AB q, 2H, CH<sub>2</sub>Cl), Mass: (M<sup>+</sup>) m/z 461 (typical of chloro compound).

**Reduction with LAH.** To a solution of 2 (84.8 mg, 0.20 mmole) in anhydrous tetrahydrofuran (5 mL), was added with stirring a suspension of lithium aluminium hydride (400 mg) in anhydrous tetrahydrofuran (10 mL). The mixture was then heated under reflux with stirring for 3 hr, cooled in an ice-bath and treated with ethyl acetate to destroy the excess LAH. The cold mixture was added to an ice cold H<sub>2</sub>SO<sub>4</sub> (10 mL, 5%) and extracted with ethyl acetate (2×50 ml). The extract was washed with saturated brine, dried (MgSO<sub>4</sub>) and evaporated to give a gum which on addition of hexane gave 72 mg of compound 4, as a solid mp 140-41°C, <sup>1</sup>H NMR  $\delta$ , 1.24 (s, 3H, extra CH<sub>3</sub>), Mass: M<sup>+</sup> m/z 426.

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