

## PHYLLANTHIMIDE, A NEW ALKALOID FROM *PHYLLANTHUS SELLOWIANUS*

MICHAEL S. TEMPESTA,\* DAVID G. CORLEY,

Department of Chemistry, University of Missouri-Columbia, Columbia, Missouri 65211

JOHN A. BEUTLER,\* CLIMACO J. METRAL,

Chemical Synthesis and Analysis Laboratory, Program Resources, Inc.,  
National Cancer Institute—Frederick Cancer Research Facility, Frederick, Maryland 21701

ROSENDO A. YUNES,\* CESAR A. GIACOMOZZI, and JOÃO B. CALIXTO

Universidade Federal de Santa Catarina, 88.049 Florianopolis SC, Brazil

An alkaloidal fraction of *Phyllanthus sellowianus* Muell. Arg. (Euphorbiaceae) has previously been reported to have antispasmodic activity in several pharmacologic models (1). We report here the structure of the major component of that fraction. Recent publications have shown the presence of a sterol, phyllanthol (2), from this species, as well as a biflavonoid (3). No bioactive compounds or alkaloids have been reported.

High resolution eims of the fraction indicated a mol wt of 292 and a formula of  $C_{16}H_{24}N_2O_3$ . This proved to be a misleading result. We believe on the basis of mass spectroscopy that phyllanthimide (**1**) was esterified by the methanolic HCl in which it was shipped, to give the open chain methyl ester **2**. Preparative reversed-phased tlc, however, provided pure material of **1** which gave consistent uv, ir, ms, and nmr spectra.

High resolution eims of the pure material then showed that the correct formula was  $C_{15}H_{20}N_2O_2$ . Analysis of the  $^1H$ -nmr and COSY (4) spectra (Table 1) indicated that a monosubstituted benzene was present along with an amino acid moiety. We attribute the fine splitting of the resonance at 4.05 ppm to

slight nonequivalence of the methylene protons. The ms fragmentation and  $^{13}C$ -nmr spectra substantiated the structure as **1**. The observed lack of optical activity is probably due to racemization during the facile opening of the imide ring.

Preliminary pharmacological testing on the isolated rat uterus has shown that this major component does not possess antispasmodic activity at the maximal dosage tested.

### EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were obtained on a Nicolet NT-300 WB spectrometer operating at 300 MHz ( $^1H$ ), and 75 MHz ( $^{13}C$ ).  $^{13}C$ -nmr multiplicities were determined by DEPT (5). Mass spectra were obtained on either a VG ZAB-2F or 70-250. Fab spectra were obtained in positive ion mode from "magic bullet" matrix. Optical rotation was determined on a Rudolph Autopol III polarimeter. Ft-ir spectra were obtained on a Nicolet 20-DXB, as a film on an NaCl plate.

ISOLATION.—The initial purification of the alkaloid fraction from leaf and stem material was described by Calixto *et al.* (1). Vouchers were deposited in FLOR (#2757, 3392, 3884). Eims of the basified material gave a weak molecular ion at  $m/z$  292. Linked scans established the base peak at  $m/z$  144 as a daughter, as well as  $m/z$  84 (60%). Losses of MeOH ( $m/z$  260) and then CO ( $m/z$  233) were also established by linked scans. Positive ion

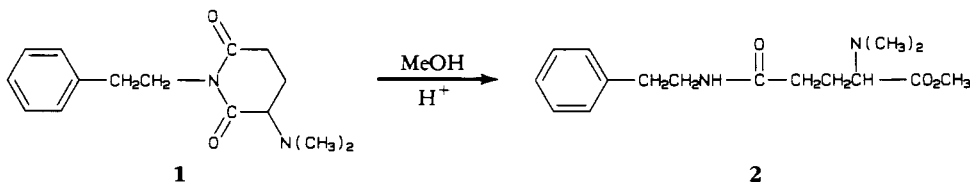


TABLE 1.  $^1\text{H}$ -nmr Data for **1** (300 MHz,  $\text{C}_6\text{D}_6$ ).

Shift	Mult	$J$ (Hz)	Integration	COSY
1.25	m		1H	a
1.35	m		1H	a
1.82	ddd	(6.2, 9.4, 17.5)	1H	ab
2.20	s		6H	—
2.33	dt	(5.3, 17.6)	1H	ab
2.65	dd	(5.2, 9.1)	1H	a
2.85	t	(7.8)	2H	c
4.05	m		2H	c
7.2	m		5H	—

fabms of the same material gave a molecular ion  $[\text{M} + \text{H}]$  at 293 (100%), with the same fragmentation as seen in the eims. Nmr data of this fraction could not be correlated with the mass spectral information, however. Further purification of the alkaloidal fraction was effected by reversed-phase tlc on Whatman KC18F plates 200  $\mu\text{m}$  thick. Two  $10 \times 20$ -cm plates, each with 10 mg of the fraction applied, were developed using  $\text{MeOH-H}_2\text{O}$  (70:30 v/v). A thin slice from the middle of the plate was removed and sprayed with Dragendorff's spray reagent to locate the alkaloidal zone. The Dragendorff's-positive band was scraped and eluted with  $\text{Me}_2\text{CO}$  for the nmr and ms analysis and pharmacologic testing.

**PHYLLANTHIMIDE [1].**—Uv  $\lambda$  max (EtOH) 259 nm (3.14), 253 nm (3.21), 248 nm (3.23), 243 nm (3.23), 238 nm (3.18);  $[\alpha]_D$  (EtOH,  $c = 2$  mg/ml)  $0 \pm 3^\circ$ ; Ft-ir ( $\text{cm}^{-1}$ ) 3023, 2956, 2921, 2859, 1722, 1677, 1672, 1345, 1145; eims 260 (28%), 217 (13%), 188 (40%), 141 (50%), 111 (75%), 84 (100%), 44 (76%); hr fabms measured mass  $[\text{M} + \text{H}]$  261.1603, calcd 261.1602;  $^1\text{H}$  nmr see Table 1;  $^{13}\text{C}$  nmr (75 MHz,  $\text{C}_6\text{D}_6$ ) 21.98 (t), 31.03 (t), 34.58 (t), 41.06 (t), 41.87 (q,  $2 \times \text{C}$ ), 64.76 (d), 126.64 (d), 128.68 (d,  $2 \times \text{C}$ ), 129.38 (d,  $2 \times \text{C}$ ), 139.00 (s), 171.11 (s), 171.92 (s).

#### ACKNOWLEDGMENTS

We wish to acknowledge the initial nmr ef-

forts of Peter Livant, Department of Chemistry, Auburn University. This research was supported, at least in part, by the National Cancer Institute, DHHS, under contract NO1-CO-74102 with Program Resources, Incorporated. The Brazilian work was supported by FINEP and CNPQ. Support from the National Science Foundation for the nmr facility (PCM-8115599) and the University of Missouri Institutional Biomedical Research Support Grant (RR07053) from the NIH to M.S.T. is gratefully acknowledged. The contents of this publication do not necessarily reflect the views or policies of the DHHS nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

#### LITERATURE CITED

1. J.B. Calixto, R.A. Yunes, A.S.O. Neto, R.M.R. Valle, and G.A. Rae, *Braz. J. Med. Biol. Res.* **17**, 313 (1984).
2. O. Hnatyszyn and G. Ferraro, *Planta Med.* **5**, 467 (1985).
3. O. Hnatyszyn, G. Ferraro, and J.D. Cousio, *J. Nat. Prod.* **50**, 1156 (1987).
4. A. Bax and R. Freeman, *J. Magn. Reson.* **44**, 542 (1981), and references therein.
5. D.M. Doddrell, D.T. Pegg, and M.R. Bendall, *J. Magn. Reson.* **48**, 323 (1982).

Received 14 December 1987