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β-PHENYLETHYLAMINE-DERIVED AMIDES FROM *PIPER GUAYRANUM*

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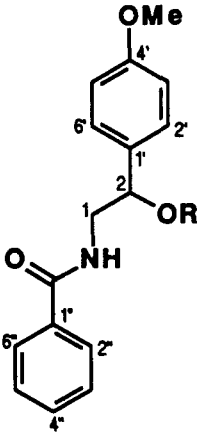
ABSTRACT.—The acetone extract of the aerial parts of *Piper guayranum* yielded tembamide acetate [**1**] and alatamide [**2**] but not tembamide [**3**]. Compound **1** has not been previously reported as a natural product while both **2** and **3** has only been encountered so far in species of Rutaceae.

Piper guayranum C.DC. (Piperaceae) is a tall, slender shrub that is found in shady, elevated regions of Trinidad, Tobago, and Venezuela (1). There is no record of folkloric medicinal use of this plant, and there appear to be no reported chemical studies either. We wish to report the results of our studies on the aerial parts of *P. guayranum* occurring in Trinidad.

Repeated vacuum liquid chromatography (vlc) of the Me_2CO extract resulted in the isolation of three crystalline compounds. The most polar compound **1** was obtained as white needles, mp 159° (petroleum ether/Me_2CO). High resolution eims gave an $[M]^+$ (at *m/z* 313.1308) which indicated a molecular formula of $C_{18}H_{19}NO_4$ ($[M]^+$ calculated 313.1314). Four bands, at 208, 225, 270, and 278 nm, were observed in the uv spectrum. The ir spectrum exhibited absorptions at 3315, 1727, 1645, 1532, and 1510 cm^{-1} indicating the presence of both amide and ester groups. ^1H nmr (Table 1) suggested a 1,4-disubstituted aromatic ring (AB type four-proton doublet of doublets at δ 6.92 and 7.35) as well as a monosubstituted one (five-proton multiplet at δ 7.45–7.83). Acetate (three-proton singlet at δ 2.12) and aromatic methoxy (three-proton singlet at δ 3.82) groups were also indicated. On complete exchange after the addition of D_2O, the broad signal at δ 6.40 disappeared, and, significantly, the two-proton signal at δ 3.85, originally a triplet, became a doublet ($J = 6$ Hz). This suggested that these two protons are adjacent to the nitrogen atom in compound **1** and are probably coupled to the methine proton at δ 5.97. Decoupling studies confirmed this, because irradiation of the triplet at δ 5.97 caused



1 R=Ac
3 R=H

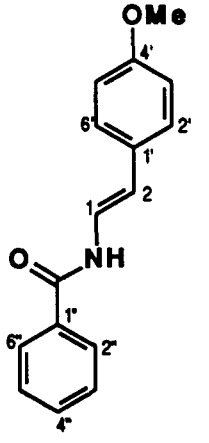


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TABLE 1. ^{1}H nmr (80 MHz) of Compounds **1–3** in $CDCl_3$.[a]

Proton	Compound		
	1	**2**	**3**
H-1	3.85 (t, J = 6)	7.65 (d, J = 14)	3.70 (m)
H-2	5.97 (t, J = 6)	6.22 (d, J = 14)	4.93 (dd, J = 3.5, 7.5)
H-2′, 6′	7.35 (d, J = 9)	7.30 (d, J = 9)	7.35 (d, J = 9)
H-3′, 5′	6.92 (d, J = 9)	6.85 (d, J = 9)	6.93 (d, J = 9)
H-2″, 6″	7.75 (m)	7.80 (m)	7.80 (m)
H-3″, 4″, 5″	7.50 (m)	7.50 (m)	7.47 (m)
Ac	2.12 (s)		
4′-OMe	3.83 (s)	3.81 (s)	3.84 (s)
N-H	6.40 (br s)	—[b]	6.60 (br s)

[a]All chemical shifts (relative to TMS) are given in δ (ppm) and coupling constants in Hz.
[b]Signal was not observed.

the triplet at δ 3.85 to collapse to a doublet while irradiation at δ 3.85 resulted in a singlet at δ 5.97.

All the evidence clearly pointed to the structure indicated for **1**. ^{13}C nmr (Table 2) fully corroborated this structure which was also supported by eims. The latter showed important fragments at *m/z* $[M-HOAc]^+$ 253.1121, $[M-C_6H_5CONH_2]^+$ 192.0805, $[M-C_6H_5CO-HOAc-OMe]^+$ 117.0582, and $[C_6H_5CO]^+$ 105.0341.

TABLE 2. ^{13}C-nmr Data of Compound **1** in $CDCl_3$.[a]

Carbon	Chemical Shift
1	45.0
2	75.0
1′	130.8
2′ and 6′	128.0[b]
3′ and 5′	115.3
4′	161.0
1″	135.5
2″ and 6″	129.7
3″ and 5″	129.2[b]
4″	132.6
4′-OMe	55.6
-CO*Me*	21.5
-OC=O	172.0
-CONH	168.5

[a]Chemical shifts relative to TMS are given in δ (ppm). Assignment of the signals was based on the *J*-Modulated Spin Echo ^{13}C-nmr spectra and on calculations of chemical shifts from empirical rules.
[b]Assignments may be interchanged.

Mild hydrolysis of compound **1** gave the crystalline alcohol **3**, mp 147–148°. All the spectral data indicated its identity with the known tembamide [**3**], which has been prepared synthetically (2–4) and has also been also isolated from several species of Rutaceae (4–10). For example, the uv spectrum gave bands at 209, 224, 270, and 279 nm, and the ir spectrum showed diagnostic absorptions at 3440 (br) and 1655 cm^{-1}. The ^{1}H-nmr spectrum of **3** (Table 1), while virtually identical in the aromatic region to that of **1**, differed from it in three significant respects: (a) the singlet at δ 2.12 in the spectrum of **1** was absent in the spectrum of **3**; (b) the signal assignable to the benzylic methine proton appeared at δ 4.93 (doublet of doublets) in the spectrum of **3**, whereas it occurred at δ 5.97 (triplet) in the spectrum of **1**; and (c) the signal due to the methylene protons α to nitrogen, which was a simple triplet at δ 3.85 in the spectrum of **1**, appeared as a complex multiplet (δ 3.35–4.08) in the spectrum of **3**. Interestingly, after D_2O exchange this multiplet simplified to a double AB doublet of doublets. As expected, the eims closely resembled that of **1**. Significantly, the fragment ion corresponding to loss of $C_6H_5CONH_2$ was found at *m/z* 150, replacing the fragment at *m/z* 192 observed in the case of **1**.

Compound **2** was isolated as white

needles, mp 188–189° (petroleum ether/Me_2CO). It gave bands at 204, 222, and 308 in the uv spectrum and showed ir absorptions at 3320, 1645, and 945 cm^{-1}. Consideration of these data and comparison of the ^{1}H-nmr spectrum (Table 1) with that of **1** strongly suggested that **2** was related to **1** via loss of HOAc. Thus, the methyl singlet at δ 2.12 and the triplets at δ 3.85 and 5.97 in the spectrum of **1** were absent in that of **2**, and in their place two doublets (J = 14 Hz) at δ 6.22 and 7.65 appeared. The aromatic regions of both spectra were identical. These data indicated that **2** is identical with alatamide, which was previously isolated (11) from the South Indian tree *Pleiospermium alatum* (Wight and Arn.) Swingle (Rutaceae). Full ^{1}H-nmr data were, however, not published.

A third crystalline compound isolated was readily shown to be stigmasterol by comparison of mp, ir, and ^{1}H-nmr spectra with those of an authentic sample.

Compound **1** has been known for a long time (2,4), but this is the first report of its occurrence as a natural product and of its full spectral data.

It is interesting that both tembamide acetate [**1**] and alatamide [**2**] were isolated from the plant *P. guayranum*, while there was no evidence of tembamide [**3**], their putative biogenetic precursor. Further, it is noteworthy that β-phenylethylamine-derived amides, which are of taxonomic significance in the Rutaceae family, have now been isolated from a member of the Piperaceae.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Reichert micro melting point apparatus and are uncorrected. Uv spectra were recorded on a Perkin-Elmer 552A uv-vis spectrophotometer, and ir spectra were run as Nujol mulls using a Pye Unicam SP3-200 instrument. ^{1}H- (80 MHz) and ^{13}C-(20 MHz) nmr spectra were run on a Bruker WP 80 SY FT nmr spectrometer with TMS as internal standard. Lreims were obtained at 70 eV on a Kratos/AEI MS902 or a Finnegan 4000 mass spectrometer while hreims were obtained with a Kratos/AEI MS50 instrument. Si gel 60 PF-254 and 366 (Merck) was used for analytical (0.25 mm) and preparative (1 mm) tlc and for vlc (12).

PLANT MATERIAL.—Aerial parts of the plant *P. guayranum* were collected in April 1985 near the 7¾ mile post along the Blanchisseuse Road, Arima, Trinidad. A voucher specimen is on deposit at the National Herbarium of Trinidad and Tobago. The plant material was air-dried (ca. 30°) for 1 week.

EXTRACTION, SEPARATION, AND ISOLATION.—The dried, ground plant material (0.7 kg) was exhaustively extracted with Me_2CO (10 liters) over 2 days. Evaporation of the Me_2CO gave the crude extract (32.8 g). A portion (15 g) of the extract was subjected to vlc eluting first with petroleum ether and then with increasing proportions of Me_2CO. Fractions 3–24 were combined to give a mixture (4 g) which was rechromatographed as described above, to yield three crystalline compounds.

The major, most polar compound, amide **1**, was obtained as white crystalline needles (36 mg), mp 159° (petroleum ether/Me_2CO) [lit. (4) 140–141°]; uv (MeOH) λ max 208, 225, 270, 278 nm (ε 14200, 28200, 3100, 2300); ir ν max 3315, 1727, 1637, 1532, 1510, 1250, 1232, 1026, 817, 798, 715, 687 cm^{-1}; eims *m/z* (%) $[M]^+$ 313.1308 (0.7), 253.1121 (12), 192.0805 (14.5), 137.0607 (27), 134.0627 (11), 117.0582 (100), 105.0341 (24), 105.0702 (12), 77.0388 (31); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

Compound **2** was also obtained as white needles (6 mg), mp 188–189° (petroleum ether/Me_2CO) [lit. (11) 178–180°]; uv (MeOH) λ max 204, 222, 308 nm (ε 10700, 13400, 17200); ir ν max 3320, 1640, 1290, 1250, 1170, 1030, 945 cm^{-1}; eims *m/z* (%) $[M]^+$ 253 (40), 150 (6), 149 (6), 148 (6), 135 (13), 134 (21), 121 (11), 105 (100), 77 (30); ^{1}H nmr see Table 1. The least polar compound, obtained as white crystals (10 mg), was identical in its mp and in its ir and ^{1}H-nmr spectra with stigmasterol.

MILD ALKALINE HYDROLYSIS OF **1**.—Compound **1** (12 mg) was stirred overnight at room temperature with 10 ml of 10% Na_2CO_3 in MeOH/H_2O. After the usual workup white crystals (10 mg) of compound **3** were obtained, mp 147–148° [lit. (7) 149–151°]; uv (MeOH) λ max 209, 224, 270, 279 nm (ε 11400, 20500, 2190, 1490); ir ν max 3440, 1655, 1595, 1520, 1225 cm^{-1}; eims *m/z* (%) $[M-H_2O]^+$ 253 (6), 150 (37), 138 (11), 137 (32), 136 (38), 135 (100), 134 (58), 117 (48), 109 (11), 105 (71), 94 (11), 78 (10), 77 (60); ^{1}H nmr see Table 1.

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