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TWO SECURINEGA-TYPE ALKALOIDS FROM *PHYLLANTHUS AMARUS*

PETER J. HOUGHTON,* TIBEBE Z. WOLDEMARIAM, SIOBHAN O'SHEA† and S. P. THYAGARAJAN‡

Pharmacognosy Research Laboratories, Department of Pharmacy, King's College London, Manresa Road, London SW3 6LX; †Virology Department, St Thomas's Hospital Medical School, Lambeth Palace Road, London SE1 7AN, U.K.; Dr ALM Postgraduate Institute of Basic Medical Sciences, Taramani, Madras 600 113, India

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Key Word Index—*Phyllanthus amarus*; Euphorbiaceae; securinega-type alkaloids; isobubbialine; epibubbialine.

Abstract—Two new securinega-type alkaloids, isobubbialine and epibubbialine, were isolated from the leaves of *Phyllanthus amarus*, as well as the three known alkaloids, phyllanthine, securinine and norsecurinine. The structures of the unknown compounds were determined by means of UV, IR, mass and NMR spectroscopy.

INTRODUCTION

Phyllanthus amarus Schum. et Thonn. (syn. *P. niruri* L.) is widely used in Ayurvedic medicine for the treatment of liver ailments and has shown both *in vitro* and *in vivo* activity against hepatitis B virus [1, 2]. The plant has also been reported to possess antifungal, antibacterial and antiviral activities [3]. Earlier work on this plant showed the occurrence of lignans, flavonoids and alkaloids [4–6]. We report here the isolation and characterization of two novel alkaloids from the leaves.

RESULTS AND DISCUSSION

Three of the alkaloids isolated, PA1, PA2 and PA3, gave spectral features in accordance with the known compounds phyllanthine, securinine and norsecurinine, respectively, which have previously been isolated from this species [7–9]. The other two alkaloids isolated both showed a molecular ion peak of m/z 221 and fragments of m/z 134, 106 and 78. These fragments strongly suggested that the alkaloids were of the securinega type with a pyrrolidine A ring, as in norsecurinine [9, 10]. The observed M_r was 18 Da greater than that of norsecurinine, and the presence of only two signals ascribable to alkene C atoms in the ^{13}C NMR spectrum indicated that only one C–C double bond was present in the molecules. The peak at 3300 cm^{-1} in the IR spectrum, a carbinol signal at 72.1 ppm in the ^{13}C NMR spectrum and a proton signal at 4.05 and 4.25 ppm in the ^{1}H NMr spectra of PA4 and PA5, respectively, was evidence of a secondary alcohol.

The 2D COSY spectrum of PA4 showed weak coupling between the olefinic 12-H at 5.75 ppm and the two geminal protons at 2.98 and 2.68 ppm. These geminal protons in turn showed coupling with a poorly resolved multiplet at 3.32 ppm which was ascribed to 6-H by comparison with the literature values for norsecurinine [9, 10]. The signal at 3.32 ppm also coupled with the 1H signal at 4.25 ppm which coupled most strongly with the geminal protons giving signals at 2.38 and 1.88 ppm. These coupled with no other protons and this sequence of signals point to a structure similar to that observed in bubbialine and bubbiadialine [11] whereby this geminal pair would be due to the 8-CH_2. The ^{13}C NMR signals, supported by HETCOR spectra, also agree very closely with those reported for bubbialine and isobubbialine [11]. The geminal signals at 2.98 and 2.68 ppm and the signal at 3.32 ppm were therefore considered to be due to the 14-CH_2 and 6-CH, respectively, since the other signals observed are very similar to those reported for norsecurinine [9, 10]. The carbinol 7-H at 4.25 ppm showed nOe effects with protons giving signals at 3.32 ppm (6-H), 3.13 ppm (2-H) and 2.38 ppm (14-H_{ax}), which indicates that it is on the same side of the molecule as H-2. The configuration of the 2-H was suggested to be β since the chemical shift of 3.13 ppm was closer to that seen in norsecurinine (3.24 ppm), which also has 2-Hβ, than in its 2-Hα isomer [10]. The circulardichroic (CD) spectrum of PA4 resembled that of bubbialine, which has 2-Hβ rather than bubbialidine, where 2-H is α, since it gave a negative reading at 219 nm and a positive one at 272 nm [11]. The NOESY spectrum showed correlation with the 2-H and the 8-H signal at 2.38 ppm, which gave further evidence of its β configuration. TLC examination showed that PA4 was not identical to bubbialine, where the 7-H is β, and this agrees with PA4 being the 7-Hα isomer of bubbialine, as indicated

*Author to whom correspondence should be addressed.

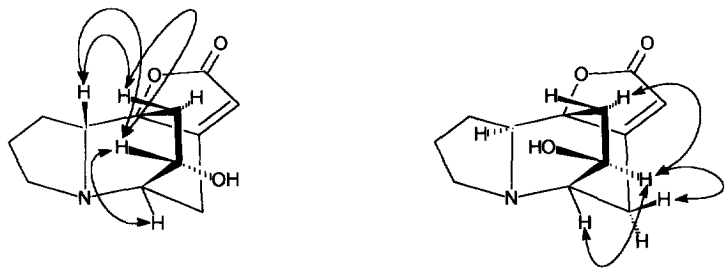

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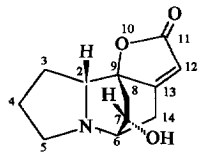
Fig. 1. NOE relationships observed for 7-H in isobubbialine (**1**) and epibubbialine (**2**).



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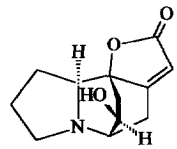

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by the NOE data (Fig. 1). This molecule is consequently considered to have structure **1** and is named isobubbialine.

Compound PA5 showed a similar pattern in its ^{1}H and ^{13}C NMR spectra to PA4 and was considered to be a stereoisomer. Three asymmetric centres are present in the molecule at C-2, C-6 and C-7. The NOESY spectrum showed a close spatial relationship between the 7-H signal at 4.05 ppm and the 14-CH_{ax} signal at 2.70 ppm, and the 8-CH at 2.05 ppm and the 6-H signal at 3.18 ppm; this indicates that the 7-H is α (Fig. 1). The 2-CH signal at 3.64 ppm is considerably higher than the equivalent signal in PA4 and this indicates that it is in the α configuration since the 2-H isomers 4-epi-phyllanthine (2-Hβ) and securinitine (2-Hα) give

Table 1. ^{1}H NMR signals and COSY correlations for PA4 (**1**) and PA5 (**2**)

Proton at C	PA4	COSY correlations	PA5	COSY correlations
2	3.13 *m*	2.22, 1.92	3.64 *d* (J = 4.2 Hz)	1.84
3	2.22 *m*	3.13, 2.12 1.92, 1.70	1.84 *m*	1.71, 1.11
3	1.92 *m*	3.13, 2.22, 2.12, 1.70	1.84 *m*	1.71, 1.11
4	1.70 *m*	3.74, 3.08 2.22, 3.08, 2.12, 1.92	1.11 *m*	3.02, 2.70, 1.84, 1.71
4	2.12 *m*	3.74, 3.08, 2.22, 1.92, 1.70	1.71 *m*	3.02, 2.70, 1.84, 1.11
5	3.08 *m*	3.74, 2.12, 1.70	2.70 *m*	3.02, 1.71, 1.11
5	3.74 *m*	3.08, 2.12, 1.70	3.02 *m*	2.70, 1.71, 1.11
6	3.32 *prt*	4.25, 2.68, 2.98	3.18 *prt*	2.99
7-H	4.25 *dd* (J = 4.2, 10.1 Hz)	3.32, 2.38 1.88	4.05 *dd* (J = 4.1, 8.6 Hz)	3.18, 2.07, 2.05
7-OH	5.40 *brs*		5.34 *brs*	
8	1.88 *dd*	4.25	2.05 *dd*	4.05, 2.07
8	2.38 *dd*	4.25	2.07 *dd*	4.05, 2.05
12	5.75 *t* (J = 1.8 Hz)	2.68, 2.98	5.80 *t* (J = 1.7 Hz)	2.99
14	2.68 *dt* (J = 18 Hz, 2.2 Hz)	3.32, 5.75	2.70 *dt* (J = 12, 1.8 Hz)	5.80, 3.18
14	2.98 *dt* (J = 18, 2.2 Hz)	3.32, 5.75	2.97 *dt* (J = 12, 1.8 Hz)	5.80, 3.18

δ ppm from TMS in $CDCl_3$.
prt = poorly resolved triplet.

signals at 2.09 and 3.90 ppm, respectively [12]. The 2-H signal in PA5 also showed no NOE correlations, and the molecule gave a CD spectrum opposite to that given by PA4. In the light of this evidence it is proposed that PA5 is the 7-Hα and 2-Hα isomer of bubbialine (**2**) and has been named epibubbialine.

EXPERIMENTAL

Plant material. The aerial parts of *P. amarus* were obtained from Madras, India. The plants were collected and authenticated by Prof. A. Mahaddevan of the centre for Advanced Studies in Botany, University of Madras, Guinde Campus, and a voucher specimen (Mah 2331) is deposited in the herbarium of the same institute. The leaves were air-dried prior to extraction.

General. ^{1}H and ^{13}C NMR and ^{1}H–^{13}C short range correlation HETCOR and HMBC spectra were obtained at 400/100 MHz on an AMX 400 NMR spectrometer using $CDCl_3$ as solvent with TMS as int. standard.

TLC and prep. TLC. Merck silica gel GF_{254} plate (0.25 mm) and silica gel PF_{254} (Merck) (1 mm). Spots and bands were detected by UV irradiation (254 and 365 nm). The following solvents were used: (*a*) $CHCl_3$–MeOH (6:1) and (*b*) Me_2CO–MeOH (9:1). Spray reagent for TLC: Dragendorff reagent.

Extraction and isolation of alkaloids. Air-dried leaves of *P. amarus* (100 g) were extracted with 0.5 M HCl and filtered. The filtrate was basified with 10% aq. Na_2CO_3 and extracted with $CHCl_3$ to yield an oily residue (165 mg). Examination by TLC showed the presence of 5 Dragendorff-positive zones. The residue was then subjected to prep. TLC on silica gel (solvents *a* and *b*) to yield 5 compounds (PA1–PA5).

PA1 (26 mg, 0.026% yield), PA2 (21 mg, 0.021% yield) and PA3 (18 mg, 0.018% yield) were characterized as phyllanthine, securinine and norsecurinine, respectively, by comparison of their spectral data with values [7–9]. PA4 (12 mg, 0.012% yield), crystalline powder mp 112–113° $[\alpha]_D$ +11.7°, $\Delta\varepsilon$ 219 nm −3.87, 272 nm +2.10; IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1766 (lactone CO), 1696 (C=C), 1412, 1110, 1080, 981. EIMS (probe) *m/z* (rel. int.): 221.0504 (100) $[M]^+$ ($C_{12}H_{15}O_3N$ calc. as 221.0505). 177 (95), 162 (30), 120 (47), 96 (60). ^{1}H and ^{13}C NMR: Tables 1 and 2.

PA5 (10 mg, 0.010% yield), crystalline powder mp 107–108°; $[\alpha]_D$ − 14.5°, $\Delta\varepsilon$ 219 nm + 3.21, 272 nm − 2.02; IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1766 (lactone CO), 1696 (C=C), 1412, 1110, 1080, 981. EIMS *m/z* (rel. int.): 221.0504 (100) $[M]^+$ ($C_{12}H_{15}O_3N$ calc. as 221.0505) 177 (93), 162 (33), 120 (45), 96 (63). ^{1}H and ^{13}C NMR: Tables 1 and 2.

Table 2. ^{13}C NMR signals for PA4 (**1**) and PA5 (**2**) in $CDCl_3$

C	1	2
2	63.7	62.9
3	25.8	24.7
4	27.2	27.1
5	52.8	50.6
6	56.5	55.5
7	72.1	67.4
8	33.3	40.7
9	85.2	84.5
11	175.7	173.2
12	111.3	113.4
13	174.0	169.5
14	31.7	24.1

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