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# PHENYLALKANOIDS FROM *PIPER MARGINATUM*‡

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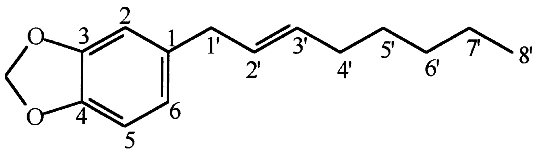
**Key Word Index**—*Piper marginatum*; Piperaceae; phenylpropanoids; NMR.

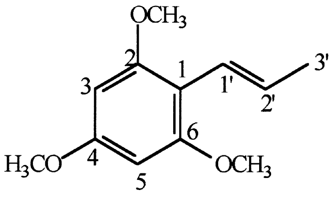
**Abstract**—Phenylpropanoids, and a new phenyloctanoid were isolated from the roots of *Piper marginatum* and their structures were elucidated by spectroscopic methods. The compounds were: 3,4-methylenedioxy-1-(2E-octenyl)-benzene, 2,6-dimethoxy-3,4-methylenedioxy-1-(2-propenyl)-benzene, 1-(1E-propenyl)-2,4,6-trimethoxybenzene; apiole; isoasarone. © 1998 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

Many plants of the genus *Piper* are used as food flavoring agents and for the treatment for numerous diseases [1–3]. *P. marginatum* has previously only been shown to produce the phenylpropanoid, croweacin (2-methoxy-3,4-methylenedioxy-1-(2-propenyl)-benzene [4]. Herein we describe the isolation and structure elucidation of 3,4-methylenedioxy-1-(2E-octenyl)-benzene(**1**) and 1-(1E-propenyl)-2,4,6-trimethoxybenzene (**2**) from the dried ground root of *P. marginatum*. The plant popularly known as "mavaisco", is used in the Brazilian state of Paraíba as a food flavouring agent (seeds) and as an antidote for snakebite (root) [5]. We named the new compounds marginatine (**1**) and pipermargine (**2**), respectively. Complete $^1$H and $^{13}$C NMR assignments for apiole (**4**) and isoasarone (**5**) are given here for the first time [6].



**1**



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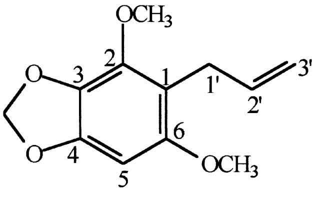

**3**

## RESULTS AND DISCUSSION

The $^1$H NMR spectrum of compound **1** showed the presence of an ABX system, with signals centred at $\delta$ 6.69 (1H, *brd*, $J = 1.6$ Hz), 6.64 (1H, *brdd*, $J = 7.9$, 1.6 Hz) and 6.74 (1H, *d*, $J = 7.9$ Hz), of an aromatic ring and a singlet at $\delta$ 5.92 (2H) for a methylenedioxy group indicating a piperonyl moiety; a series of signals indicating a long chain with integrations totalling 15 H. Analysis of the $^{13}$C NMR spectrum confirmed the presence of a methylenedioxy group ($\delta$ 101.0) and a piperonyl substituent. It also showed evidence of a $C_8$ side-chain with one double bond ($\delta$ 14.3, 22.7, 29.4, 31.7, 32.7, 39.0, 129.0 and 132.3). EI mass spectrometry gave a $[M]^+$ at $m/z$ at 232 which is com-

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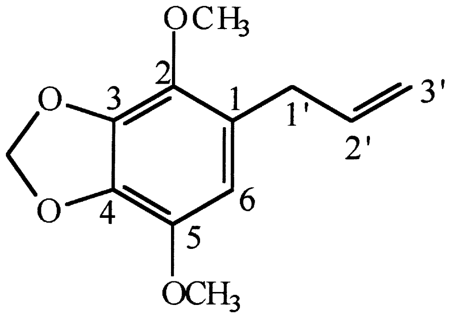

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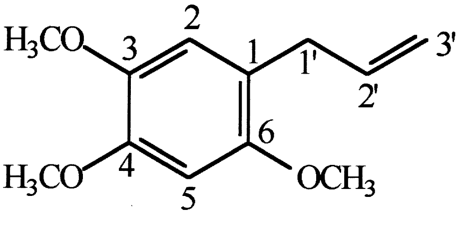

**5**

patible with the molecular formula $C_{15}H_{20}O_2$. The NMR HMBC spectrum gave evidence for one methylene group between the double bond of the side chain and the piperonyl moiety. The large *J* value obtained for the olefinic protons, H-2′ and H-3′, pointed to an *E* configuration for the double bond in the 2-octenyl side-chain.

The $^1$H NMR spectrum of **2** indicated the symmetrical nature of the molecule. It showed one *s* at $\delta$ 6.15 (2H) for H-3 and H-5; one *s* at $\delta$ 3.83 (6H) attributable to two methoxy groups (2-C-OMe and 6-C-OMe); a further methoxy at $\delta$ 3.82 (3H) was assigned to the 4-C-OMe; one allylic methyl *dd* centred at $\delta$ 1.90 (C-3′) was coupled to two *trans* olefinic protons with signals centred at $\delta$ 6.57 (H-1′) and $\delta$ 6.47 (H-2′). $^{13}$C NMR chemical shifts of the three methoxy groups ($\delta$ 55.9 for 2-C-Ome, 6-C-Ome and 55.5 for 4-C-Ome) indicated that all these moieties should have at least one of the *ortho* carbons unsubstituted. This fact, together with protons H-3 and H-5 being equivalent, confirms the substitution pattern for the aromatic ring proposed for compound **2**. EI mass spectrometry gave the $[M]^+$ at *m/z* 206 which is consistent with the molecular formula $C_{12}H_{14}O_3$. All the NMR assignments presented for all the compounds were based on 2D experiments such as HMBC (with *J* optimised for 7 Hz), $^1$H–$^1$H COSY, NOESY and H–C COBI. Complete $^1$H and $^{13}$C NMR data for compounds **1** and **2** are given in Tables 1 and 2, respectively. The complete spectroscopy analysis of **3** is in accordance with the literature [7] while complete and unambiguous $^1$H and $^{13}$C NMR data for the compounds **4** and **5** are presented here for the first time (Table 3).

## EXPERIMENTAL

### *General methods*

CC: silica gel; Prep. and analyt. TLC: silica gel 60 $PF_{254}$. Compounds were detected using UV light and/or spraying with $H_2SO_4$-vanillin reagent. IR: liquid film; EIMS: GC-MS at 70 eV (Column HP-1, Init. temp. 70°, rate 20°, final temp. 220°); NMR: 400.14 MHz for $^1$H and 100.62 MHz for $^{13}$C. Chemical shifts are reported in ppm relative to the solvent ($CDCl_3$) at 27°.

Table 1. $^{13}$C and $^1$H NMR ($CDCl_3$) data for marginatine (**1**) with correlations based on H-C COBIdec and HMBC

| | H | $^1J$ | $^2J$ | $^3J$ |
|---|---|---|---|---|
| 1 | — | 135.3 | — | — |
| 2 | 6.69 (1H, *brd*, $J = 1.6$ Hz) | 109.2 | — | 121.3, 145.9 |
| 3 | — | 147.8 | — | — |
| 4 | — | 145.9 | — | — |
| 5 | 6.74 (1H, *d*, $J = 7.9$ Hz) | 108.3 | 145.9 | 135.3, 147.8 |
| 6 | 6.64 (1H, *brdd*, $J = 1.6, 7.9$ Hz) | 121.3 | — | 39.0, 109.2, 145.9 |
| 1′ | 3.25 (2H, *brd*, $J = 4.8$ Hz) | 39.0 | 129.0, 135.3 | 109.2, 121.3, 132.3 |
| 2′ | 5.52 (1H, *dm*, $J = 15.4$ Hz) | 129.0 | — | 32.7 |
| 3′ | 5.50 (1H, *dm*, $J = 15.4$ Hz) | 132.3 | — | 39.0 |
| 4′ | 2.02 (2H, *m*) | 32.7 | 132.3 | 129.0 |
| 5′ | 1.38 (2H, *m*) | 29.4 | 31.7 | — |
| 6′ | 1.30 (2H, *m*) | 31.7 | — | — |
| 7′ | 1.30 (2H, *m*) | 22.7 | — | — |
| 8′ | 0.90 (3H, *m*) | 14.3 | 22.7 | 31.7 |
| $OCH_2O$ | 5.92 (2H *s*) | 101.0 | | 145.9, 147.8 |

Table 2. $^{13}C$ and $^{1}H$ NMR ($CDCl_3$) data for pipermargine (**2**) with correlations based on H-C COBIdec and HMBC

| | H | $^{1}J$ | $^{2}J$ | $^{3}J$ |
|---|---|---|---|---|
| 1 | — | 108.7 | — | — |
| 2 | — | 159.0 | — | — |
| 3/5 | 6.15 (2H, *s*) | 91.0 | 159.0, 159.6 | 91.0, 108.7 |
| 4 | — | 159.6 | — | — |
| 6 | — | 159.0 | — | — |
| 1′ | 6.57 (1H, *dq*, $J$ = 1.3, 16.0 Hz) | 121.2 | 121.2 | 20.2, 159.0 |
| 2′ | 6.47 (1H, *dq*, $J$ = 6.0, 16.0 Hz) | 128.2 | 20.2, 128.2 | 108.7 |
| 3′ | 1.90 (3H, *dd*, $J$ = 1.3, 6.0 Hz) | 20.2 | 121.2 | 128.2 |
| C2/6–OMe | 3.38 (6H, *s*) | 55.9 | — | 159.0 |
| C4–OMe | 3.82 (3H, *s*) | 55.5 | — | 159.6 |

Table 3. $^{13}C$ and $^{1}H$ NMR ($CDCl_3$) data for apiole (**4**) and isoasarone (**5**)

| | Apiole | | Isoasarone | |
|---|---|---|---|---|
| | H | C | H | C |
| 1 | — | 110.8 | — | 120.3 |
| 2 | — | 136.5 | 6.70 (1H, *s*) | 114.3 |
| 3 | — | 139.0 | — | 143.3 |
| 4 | — | 135.2 | — | 148.2 |
| 5 | — | 139.2 | 6.54 (1H, *s*) | 98.4 |
| 6 | 6.31 (1H, *s*) | 108.5 | — | 151.6 |
| 1′ | 3.31 (1H, *dm*, $J$ = 6.4 Hz) | 34.3 | 3.30 (2H, *dm*, $J$ = 6.4 Hz) | 33.8 |
| 2′ | 5.94 (1H, *m*) | 137.6 | 5.97 (1H, *m*) | 137.5 |
| 3′ | 5.05 (2H, *m*) | 115.6 | 5.04 (2H, *m*) | 115.4 |
| C2-OMe | 3.88 (3H, *s*) | 60.4 | — | — |
| C3-OMe | — | — | 3.83 (3H, *s*) | 56.8 |
| C4-OMe | — | — | 3.88 (3H, *s*) | 56.5 |
| C5-OMe | 3.86 (3H, *s*) | 57.1 | — | — |
| C6-OMe | — | — | 3.81 (3H, *s*) | 56.8 |
| $OCH_2O$ | 5.96 (2H, *s*) | 101.7 | — | — |

### *Plant material*

The root of *P. marginatum* Jacq. was collected in September 1993 near the city of João Pessoa, PB, Brazil. A voucher specimen (Agra 1500-JPB) is deposited at the Herbarium Lauro Pires Xavier of the Universidade Federal da Paraíba.

### *Extraction and isolation*

The powdered root of *P. marginatum* (4.2 kg) was moistened with a soln. of 40% conc. $NH_4OH$ in $H_2O$ and extracted with EtOH at room temp. The resultant extract (170 g) was treated with a soln. of 2% HCl in $H_2O$ and extracted with $CHCl_3$. This extract (22 g) was then subjected to cc (column 1) over silica gel, elution with hexane, hexane–$CHCl_3$ mixtures, $CHCl_3$, $CHCl_3$–MeOH mixtures and finally MeOH (100 fractions). The fractions 10–15 (1.0 g) eluted with hexane–$CHCl_3$ (1 :4) were subjected to a second column (column 2) over silica gel using hexane and $CHCl_3$, (15 fractions). Fractions 2–3 (70 mg) eluted with pure hexane, were subjected to prep. TLC developed with pure hexane. Fraction 1 (48 mg) was marginatine (**1**). The fractions 18–19 of column 1 (80 mg) when subjected to prep. TLC developed with hexane–$CHCl_3$ (9:1) gave pipermargine (**2**) (20 mg). A column of the fractions 23–26 from column 1 (1.02 g) (column 3) eluted with hexane and $CHCl_3$ mixtures of increasing polarity gave 14 fractions. Fractions 3–4 (27 mg) contained 2,6-dimethoxy-3,4-methylenedioxy-1-(2-propenyl)-benzene (**3**), which was previously isolated from *P. sarmentosum* [7]. The fraction 8 of column 3 when purified by prep. TLC developed with hexane–EtOAc (9:1) gave apiole (26 mg) (**4**). Fractions 11–14 of column 3 purified in the same way gave isoasarone (6.6 mg) (**5**).

*Marginatine* (**1**). Pale yellow oil. IR $\nu_{max}$ $cm^{-1}$: 2933, 2854, 2360, 1488, 1442, 1241, 1041; UV $\lambda_{max}$ nm: 288, 233; EIMS $m/z$ (rel. int.): 232 [$M^+$] (75), 161 (68), 131 (100); NMR: Table 1.

*Pipermargine* (**2**). Pale yellow oil. IR $\nu_{\max}$ cm$^{-1}$: 2923, 2846, 1604, 1457, 1203, 1126; UV $\lambda_{\max}$ nm: 268 nm; IEMS *m*/*z* (rel. int.): 208 [M$^{+}$] (100), 180 (51); NMR: Table 2.

2,6-Dimethoxy-3,4-methylenedioxy-1-(2-propenyl)-benzene (**3**). Pale yellow oil. Spectroscopy data identical to literature [7].

*Apiole* (**4**). Pale yellow oil. IR $\nu_{\max}$ cm$^{-1}$: 2923, 2846, 1511, 1457, 1203, 1033; UV $\lambda_{\max}$ nm: 280 nm; EIMS *m*/*z* (rel. int.): 222 [M$^{+}$] (100), 207 (47), 195 (20), 191 (17), 178 (33); NMR: see Table 3.

*Isoasarone* (**5**). Pale yellow oil. IR $\nu_{\max}$ cm$^{-1}$: 2923, 2846, 1511, 1458, 1203, 1033; UV $\lambda_{\max}$ nm): 291, 350; EIMS *m*/*z* (rel. int.): 208 [M$^{+}$] (100), 193 (52), 177 (16), 164 (19); NMR: see Table 3.

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