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# TWO AMIDES FROM *PIPER BRACHYSTACHYUM*

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**Key Word Index**—*Piper brachystachyum*; Piperaceae; alkamides, brachystamides A and B; 2D NMR.

**Abstract**—Two new unsaturated amides, brachystamides-A and B, were isolated from the total above-ground parts of *Piper brachystachyum*. Brachystamide-A was shown to be *N*-isobutyl-15 (3′,4′-methylenedioxyphenyl) 2*E*,4*E*-pentadecadienamide from spectroscopic and chemical investigations. Brachystamide-B was *N*-isobutyl-15 (3′,4′-methylenedioxyphenyl) 2*E*,4*E*,14*E*-pentadecatrienamide.

## INTRODUCTION

We have investigated the constituents of several *Piper* species [1–5] as part of our research programme on the study of Indian medicinal plants, and the search for potent plant insecticides. Several new compounds have been isolated and characterized in the course of these studies. We now report the isolation and structure elucidation of two new amides from *Piper brachystachyum*. These amides were obtained in addition to the known compounds sesamin, β-sitosterol, crotepoxide, caryophyllene oxide and triacontanol, which were reported earlier from the same source [6–8].

## RESULTS AND DISCUSSION

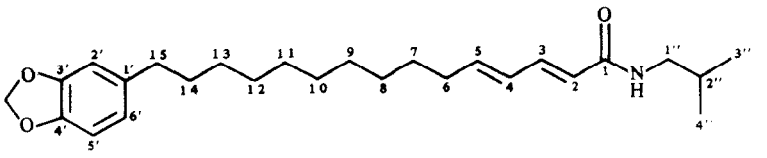
The total above-ground parts of *Piper brachystachyum* were extracted with petrol in a Soxhlet apparatus. The petrol extract on chromatography over silica gel yielded a crude mixture of amides in the chloroform eluates. On careful rechromatography and repeated recrystallization, this afforded the new compound brachystamide-A, whose structure and stereochemistry have been determined as **1**.

Brachystamide-A, $C_{26}H_{39}NO_3$ ([M]$^+$ *m/z* 413), mp 101°. $[\alpha]_D^{25}$ 0° ($CHCl_3$, EtOH) showed the UV characteristics ($\lambda_{max}^{EtOH}$ 260, 208 nm; log ε 4.41, 4.15; $\lambda_{min}^{EtOH}$ 220 nm; log ε 4.03) of a sorbamide chromophore [9, 10]. Its IR spectrum (KBr) exhibited a band for an –NH–function (3300 cm$^{-1}$) which was incorporated in a dienamide grouping (1654, 1626 and 1000 cm$^{-1}$). Further bands appeared for a methylenedioxy group (1040, 920 cm$^{-1}$) and for a 1,2,4-trisubstituted benzene (1614, 860 and 810 cm$^{-1}$). The 300 MHz $^1$H NMR spectrum confirmed the presence of a methylenedioxy group (2H, *s*, δ5.84) and an amide proton (1H, *br s*, δ5.31–5.55), and also showed signals for seven aromatic and/or olefinic, and 29 aliphatic protons.

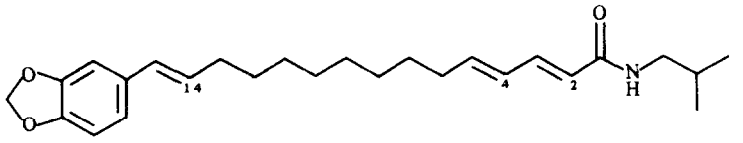
Catalytic hydrogenation of the amide over Adam's catalyst afforded a tetrahydro derivative, $C_{26}H_{43}NO_3$ ([M$^+$] *m/z* 417). The 300 MHz $^1$H NMR spectrum showed the presence of only three aromatic protons at δ6.5–6.8 and no olefinic protons. The position of this signal, as well as the appearance of IR bands at 1045, 930 cm$^{-1}$ (methylenedioxy), and 870, 800 cm$^{-1}$ (1,2,4-trisubstituted aromatic), indicated that a 3,4-methylenedioxyphenyl moiety was present. The spectral properties were closely similar to those reported for tetrahydroretrofactamide-C [12], except for the number of side-chain methylene protons in the $^1$H NMR.

Hence brachystamide-A could be formulated as a dienamide having a 3,4-methylenedioxyphenyl grouping. Detailed 300 MHz $^1$H NMR, 75.5 MHz $^{13}$C NMR and 2D-COSY studies were undertaken for complete structural elaboration of brachystamide-A as *N*-isobutyl-15 (3′,4′-methylenedioxyphenyl) 2*E*,4*E*-pentadecadienamide (**1**). The $^1$H NMR spectrum indicated signals characteristic of an *N*-isobutyl moiety. In addition it showed the signals for one allylic methylene at δ2.03–2.10 and one benzylic methylene at δ2.45. Of the four olefinic protons, H-2 and H-3 could be assigned on the following basis: H-2 being α- to the carbonyl group was somewhat upfield, and H-3 being β- to the carbonyl was very far downfield of the other olefinic protons. The assignments of H-2 and H-3 were confirmed by a 2D-COSY experiment. This also established the coupling pattern of all the protons in the molecule, and allowed complete assignments to be made (Table 1). The splitting pattern of the four olefinic protons were finally analysed by using the Bruker programme PANIC (Parameter Adjustment in NMR by Iteration Calculation); the values of the coupling constants and chemical shifts for the olefinic protons given in Table 1 were calculated by this procedure.

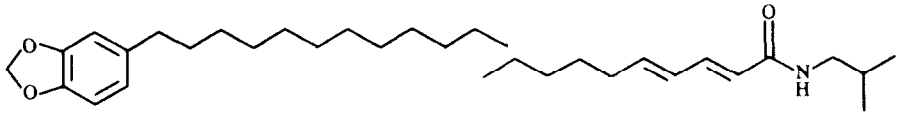
The assignments of H-2 and H-3 were confirmed by 2D-COSY and also by irradiation of the H-2 which resulted H-3 to collapse to a doublet. On the other hand irradiation of H-3 transformed the H-2 into a singlet. In order to simplify the splitting patterns of the olefinic protons and to gain information about the coupling between them, the allylic methylene protons were irradiated. This caused the signal at δ5.99 to collapse to a doublet indicating that the signal was due to the H-4 whereas the signal at δ6.07 remained unchanged as a double doublet indicating that this signal was due to the H-3. One of the coupling constants of all the olefinic



1



2



3 4

protons was 15–16 Hz. Hence both the double bonds had the *E*-configuration.

The 75.5 MHz $^{13}$C NMR spectrum (chemical shifts in $\delta$, ppm) of brachystamide-A, recorded in $CDCl_3$ solution, are also in accordance with the structure (**1**). The low-field region of the spectrum exhibited an amide carbonyl at $\delta$166.39, a methylenedioxy group at $\delta$100.66 and 10 olefinic and/or aromatic carbons. Signal assignments could be made from comparison of chemical shifts with those of dihydropipataline (**3**) and *N*-isobutyl-2*E*,4*E*-decadienamide (**4**) [11]. It was observed that the chemical shifts of the aromatic carbons of the unknown amide were similar to the corresponding values of the dihydropipataline. This indicated that a 3,4-methylenedioxyphenyl moiety was present in brachystamide-A. The close correspondence in chemical shifts of the four olefinic carbons of (**1**) to those of *N*-isobutyl 2*E*,4*E*-decadienamide further suggested that both the double bonds in (**1**) had *E*-configuration. All the $^{13}$C NMR assignments are listed in Table 1.

Brachystamide-B, $C_{26}H_{37}NO_3$ ([M$^+$] *m/z* 411), was obtained as an amorphous solid by PTLC. Its UV ($\lambda_{max}^{EtOH}$ 260, 208 nm; log $\varepsilon$ 4.40, 4.15) and IR ($\nu_{max}^{KBr}$ 1654, 1625 and 1000 cm$^{-1}$) showed the presence of a *E*,*E*-dienamide grouping as in brachystamide-A. In addition the IR band at 960 cm$^{-1}$ indicated the presence of another trans (*E*)-double bond. Its 300 MHz $^1$H NMR spectrum showed signals similar to those of brachystamide-A with the following differences: (i) two additional olefinic protons appeared in the region $\delta$6.0–6.2; (ii) two homoallylic methylenes appeared at $\delta$1.3–1.4, instead of one homoallylic and one homobenzylic methylene at $\delta$1.34 and 1.51 respectively; (iii) a proton count indicated two aliphatic methylene groups less.

Brachystamide-B on hydrogenation gave the same product ($C_{26}H_{43}NO_3$), as obtained from brachystamide-A. Thus the former was a trienamide having the same carbon skeleton as the latter. A comparison of its $^1$H and $^{13}$C NMR data with those of retrofractamide-A, pipericide [12], and guineensine (PS-A) [5] showed a close correspondence of the chemical shifts and splitting patterns ($^1$H NMR) with these compounds. Hence brachystamide-B has the structure (**2**).

## EXPERIMENTAL

Mps: uncorr. $^1$H NMR spectra were recorded at 300 MHz while the $^{13}$C NMR spectra were taken at 75.5 MHz. Analytical samples were routinely dried at 30° *in vacuo*. Dry $Na_2SO_4$ was used for drying extracts. CC and TLC were carried out using silica gel (60–120 mesh) and silica gel G, respectively.

*Plant material.* Piper brachystachyum Wall. was collected in Kerala. A voucher specimen (PR-WP) has been preserved in our laboratory.

*Isolation.* Crushed and dried aerial parts of *P. brachystachyum* (2 kg) were extracted with petrol (bp 60–80°) (8 l) for 72 hr in a Soxhlet apparatus. The petrol extract was concd and chromatographed over silica gel. The $CHCl_3$ eluate furnished a mixture of closely related amide derivatives. The mixture was subjected to repeated chromatography and prep. TLC to obtain brachystamide-A, mp 101°, $[\alpha]_D^{25}$ 0° ($CHCl_3$, EtOH) and brachystamide-B, isolated as an amorphous solid, $[\alpha]_D^{25}$ 0° ($CHCl_3$, EtOH).

*Brachystamide-A.* Found: C 75.2 H 9.1 N. 3.1% $C_{26}H_{39}NO_3$ requires C 75.5 H 9.4 N 3.4%. MS (70 eV); *m/z*: [M$^+$] 413, 398 [M−Me]$^+$, 385 [M−CO]$^+$, 357 [M−$Me_2C$=$CH_2$]$^+$, 314 [M−NHBu*i*+H]$^+$, 313 [M−NHBu*i*]$^+$, 312, 278, 180, 152, 149, 136, 135, 115, 107, 105, 72.

*Brachystamide-B.* Found: C 75.2% H 8.7% N 3.2% $C_{26}H_{37}NO_3$ requires C 75.9% H 9.0% N 3.4%. UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$): 260 (4.4), 208 (4.15). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3300 (–NH–), 1654,

Table 1. $^1$H NMR (300 MHz, $CDCl_3$) and $^{13}$C NMR (75.5 MHz, $CDCl_3$) of brachystamide-A; $^1$H NMR (300 MHz, $CDCl_3$) and $^{13}$C NMR (75.5 MHz, $CDCl_3$) of brachystamide-B

| C | $^{13}$C NMR chemical shifts (ppm) | $^1$H NMR chemical shifts (ppm) | Multiplicity and $J$ in Hz | COSY-correlations with | C | $^{13}$C NMR chemical shifts (ppm) | $^1$H NMR chemical shifts (ppm) | Multiplicity and $J$ in Hz |
|---|---|---|---|---|---|---|---|---|
| 1 | 166.39 | — | | | 1 | 166.39 | — | — |
| 2 | 121.85 | 5.68 | *d* (15.1)* | H-3 | 2 | 121.85 | 5.68 | *d* (15.1)* |
| 3 | 143.07 | 7.12 | *dd* (15.1, 11.3)* | H-2, H-4 | 3 | 142.92 | 7.12 | *dd* (15.1, 11.3)* |
| 4 | 128.26 | 6.05 | *dd* (16.0, 11.3)* | H-3, H-5 | 4 | 128.33 | 6.05 | *dd* (16.0, 11.3)* |
| 5 | 141.23 | 5.99 | *td* (16.0, 7)* | H-4, H-6 | 5 | 141.23 | 5.99 | *td* (16.0, 7)* |
| 6 | 32.91 | 2.03–2.10 | *br quartet* | H-5, H-7 | 6 | 32.86 | 2.00–2.25 | *m* |
| 7 | 28.64–29.77 | 1.34 | *br quintet* | H-6† | 7 | 28.64–29.51 | 1.3–1.4 | *m* |
| 8–13 | 28.64–29.77 | 1.20 | *br* | | 8–11 | 28.64–29.51 | 1.20 | *br s* |
| | | | | | 12 | 28.64–29.51 | 1.3–1.4 | *m* |
| | | | | | 13 | 32.81 | 2.00–2.25 | *m* |
| 14 | 31.68 | 1.51 | *br quintet* | H-15† | 14 | 129.36 | 6.04 | *m* |
| 15 | 35.69 | 2.45 | *t* (7.7) | H-14 | 15 | 129.42 | 6.21 | *d* (16.0) |
| 1′ | 136.85 | — | — | — | 1′ | 132.58 | — | — |
| 2′ | 108.02 | 6.60 | *d* (1.6) | H-6′ | 2′ | 105.48 | 6.68 | *br s* with *f.s.* |
| 3′ | 147.50 | — | — | — | 3′ | 147.96 | — | — |
| 4′ | 145.43 | — | — | — | 4′ | 146.59 | — | — |
| 5′ | 108.86 | 6.65 | *d* (7.9) | H-6 | 5′ | 108.21 | 6.68 | *br s* with *f.s.* |
| 6′ | 121.02 | 6.55 | *dd* (7.9, 1.6) | H-2′, H-5′ | 6′ | 120.18 | 6.83 | *br s* with *f.s.* |
| 1″ | 46.97 | 3.10 | *t* (6.5) | NH, H-2″ | 1″ | 46.97 | 3.10 | *t* (6.5) |
| 2″ | 28.64 | 1.73 | 9 *line multiplet* (6.6) | H-4″, H-3″, H-1″ | 2″ | 28.66 | 1.73 | 9 *line multiplet* (6.6) |
| 3″,4″ | 20.09 | 0.86 | *d* (6.5) | H-2″ | 3″,4″ | 20.69 | 0.86 | *d* (6.5) |
| O–$CH_2$–O | 100.66 | 5.84 | *s* | — | O–$CH_2$–O | 100.88 | 5.84 | *s* |
| NH | — | 5.43 | *br* | H-1″ | NH | — | 5.63 | *br* |

*Chemical shifts and coupling constants determined accurately using Bruker-PANIC programme and spectrum simulation.

†Both these signals also correlate with the broad signal at $\delta$1.19.

1626, 1000 (dienamide) 1250, 1040, 920 (–O–$CH_2$–O–), 960 (–CH $\overset{E}{=}$CH–), 1614, 860, 810 (1,2,4-trisubstituted aromatic ring). MS (70 eV); *m/z*: $[M^+]$ 411, 396 $[M-Me]^+$, 383 $[M-CO]^+$, 312 $[M-NHBui+H]^+$, 278, 180, 152, 147, 136, 135, 115, 107, 105, 72.

*Hydrogenation of brachystamide-A.* Brachystamide-A (30 mg) in EtOAc soln (20 ml) was hydrogenated under atmos. pres. over Adam's catalyst. After the uptake of $H_2$ was complete, the reaction mixture was filtered and evapd to dryness. The residue was crystallized from MeOH to yield tetrahydrobrachystamide-A, mp 62°, Found: C 74.2%; H 9.9%; N 3.2%, $C_{26}H_{43}NO_3$ requires C 74.8%; H 10.3%; N 3.4%.

*Hydrogenation of brachystamide-B.* Brachystamide-B was hydrogenated under conditions similar to those described above. Hexahydrobrachystamide-B, mp 62° was found to be identical to tetrahydrobrachystamide-A by direct comparison (IR, $^1$H NMR, MS, mixed mp and Co-TLC).

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