PERGAMON

Phytochemistry 59 (2002) 897–901

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Amides from *Piper brachystachyum* and *Piper retrofractum*

Avijit Banerji[a,*], Manjusha Sarkar[a], Ratna Datta[a,1], Piyali Sengupta[a], Koshy Abraham[b]

[a]*Centre of Advanced Studies on Natural Products, Department of Chemistry, University College of Science, 92, Acharya Prafulla Chandra Road, Calcutta 700 009, India*

[b]*Regional Research Centre, Government Central Pharmacy, Jayanagar, Bangalore 560 011, India*

Received 1 May 2001; received in revised form 8 August 2001

Abstract

Three unsaturated amides, designated brachystamides-C, D and E have been characterised from *Piper brachystachyum* Wall. Brachystamide-C, shown to be *N*-isobutyl-15-(3′,4′-methylenedioxyphenyl)-2*E*,4*E*,13*E*-pentadecatrienamide, was unusual in having a non-conjugated double bond. *Piper retrofractum* Vahl. yielded retrofractamide-D, which has been fully characterised.

Keywords: *Piper brachystachyum*; *Piper retrofractum*; Piperaceae; Alkamides; Brachystamides-C, D, E; Retrofractamide-D

1. Introduction

Previously we have reported the isolation and characterisation of several amide constituents from a number of *Piper* species (Banerji et al., 1985; Banerji and Das, 1989; Banerji, 1992). Two new unsaturated amides, viz. brachystamide-A (**1**) and brachystamide-B (**2**), were obtained earlier from *Piper brachystachyum* Wall. (Banerji and Das, 1989). Subsequently ridleyamide (**3**), a structural isomer of brachystamide-B was obtained from *Piper ridleyi* (Ahmad et al., 1995). We have now obtained three new amide components, designated brachystamides-C, D and E, and elucidated their structures as (**4**), (**5**) and (**6**), respectively.

Piper retrofractum Vahl. is a *Piper* species indigenous to India, which is used in the traditional systems of Indian medicine. Studies on its crude extracts also exhibited insecticidal properties. The following compounds were previously reported from this plant—retrofractamide-A (**10**), retrofractamide-B (pipericide) (**11**), retrofractamide-C (**12**), piperine, piperlonguminine, guineensine, piperlongumine, sylvatine, filfiline, sitosterol, fructose, glucose and methyl piperate (Krishnamoorthi, 1969; Banerji et al., 1985; Chatterjee, 1991). The present investigation reports the complete characterisation of another amide, viz. retrofractamide-D as (**13**).

2. Results and discussion

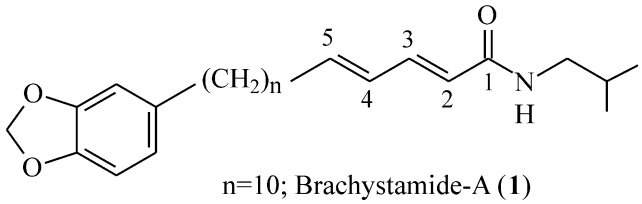
The aerial parts of *P. brachystachyum* were extracted with petrol in a Soxhlet apparatus. Successive column chromatographic resolution over silica gel, repeated crystallisations and PTLC afforded brachystamide-A (**1**), brachystamide-B (**2**), brachystamide-C (**4**), brachystamide-D (**5**) and brachystamide-E (**6**).

Brachystamide-C was an interesting exception to the normal series of amide derivatives, as it contained a non-conjugated double bond. It was obtained as an white amorphous solid, mp 90–95 °C. Its molecular formula of $C_{26}H_{37}NO_3$ ($[M]^+$ at m/z 411.2811) was determined by EI mass spectrometry. Its UV spectrum (λ_{max}^{EtOH} 260, 208 nm; log ε 4.41, 4.15; λ_{min}^{EtOH} 220 nm; log ε 4.03) and its IR spectrum (1652, 1625 and 1000 cm^{-1}) showed the presence of an *E,E*-dienamide (sorbamide) grouping (Crombie, 1955) as in (**1**–**3**). Further IR bands appeared for a methylenedioxy (1252, 1042 and 922 cm^{-1}), NH (3290–3295 cm^{-1}), 1,2,4-trisubstituted benzene (1612, 870 and 845 cm^{-1}) and a third *trans*-double bond (960 cm^{-1}). 300 MHz ^1H NMR and 75.5 MHz ^{13}C NMR investigations indicated the presence of a 3,4-methylenedioxyphenyl group, an N-isobutylamide moiety, six olefinic protons and sixteen other protons in the region δ 1.19–2.15. Catalytic hydrogenation of brachystamide-C furnished a hexahydro derivative, $C_{26}H_{43}NO_3$ ($[M^+]$ m/z 417), identical with tetrahydro-brachystamide-A, i.e. hexahydrobrachystamide-B (**7**), described

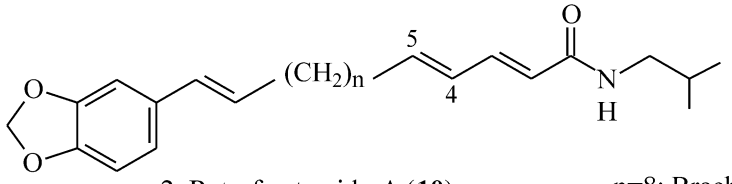
* Corresponding author. Fax: +91-033-351-9755.
E-mail address: ab@cucc.ernet.in (A. Banerji).

[1] (Née Sengupta)

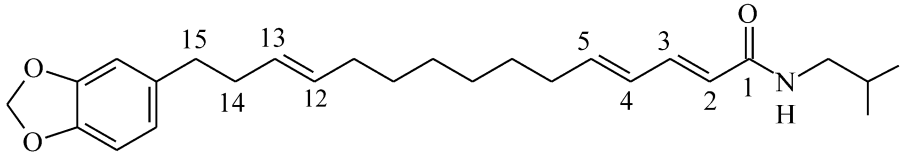
0031-9422/02/$ - see front matter
PII: S0031-9422(01)00364-8



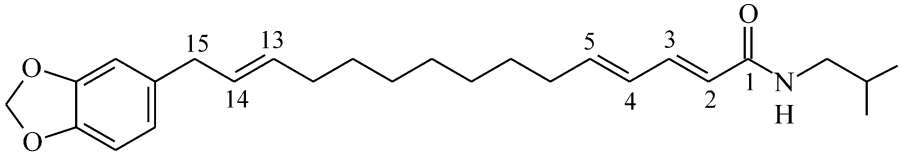
n=10; Brachystamide-A (**1**)
n=11; Brachystamide-E (**6**)



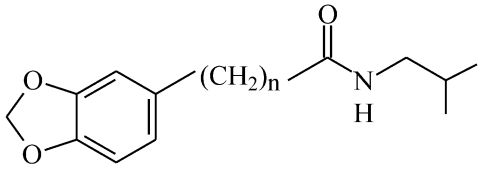
n=2; Retrofractamide-A (**10**)
n=3; Retrofractamide-D (**13**)
n=4; Retrofractamide-B (Pipericide) (**11**)
n=8; Brachystamide-B (**2**)
n=9; Brachystamide-D (**5**)



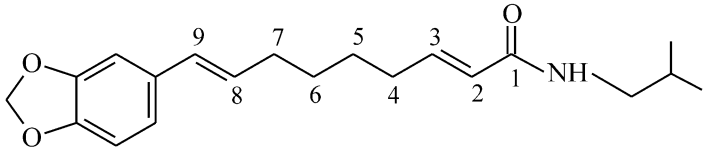
Ridleyamide (**3**)



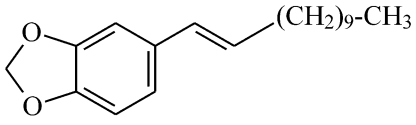
Brachystamide-C (**4**)



n=14; Hexahydrobrachystamide-C (**7**)
n=15; Hexahydrobrachystamide-D≡ Tetrahydrobrachystamide-E (**8**)



Retrofractamide-C (**12**)



Pipataline (**9**)

earlier by us (Banerji and Das, 1989). Thus brachystamide-C had the same carbon skeleton as the isomeric (**2**) and (**3**), differing from these only with respect to the double bond pattern. The ^{1}H and ^{13}C NMR spectra of brachystamide-C were similar to those of (**1**), (**2**) and (**3**) with small but significant variations. Near identity in signal positions and coupling patterns of four of the ^{1}H and ^{13}C NMR olefinic signals confirmed the presence of

the 2*E*,4*E*-dienamide moiety in the amide. The ^{13}C NMR chemical shifts of the aromatic nucleus were very close to those of (**1**) and dihydropipataline, and significantly different from those of (**2**) and pipataline (**9**) (Banerji et al., 1984; Banerji and Das, 1989). This indicated that the 3,4-methylenedioxyphenyl moiety was not conjugated with a double bond. The position of the third double bond was determined to be at $\Delta^{13,14}$ essentially on the basis of NMR data. The ^1H NMR signals for the third olefinic bond appeared as a broad overlapped signal in the region δ 5.4–5.6, though the olefinic ^{13}C NMR signals were well-differentiated (δ 132.0, δ 129.5). Brachystamide-C showed ^{13}C and ^1H NMR signals for three allylic/benzylic methylenes compared to two in (**2**) and four in (**3**). The comparatively low-field chemical shifts of the ^1H (δ 3.18) and ^{13}C (δ 38.73) signals of one of the methylene groups indicated its doubly allylic-benzylic position, viz. C-15. The methylene protons at δ 3.18 were coupled only to the olefinic signal at δ 5.4–5.6—this confirmed its positional assignment. All the proton assignments were in agreement with the ^1H–^1H COSY 2D-spectrum of brachystamide-C. Brachystamide-C showed a MS fragment at *m*/*z* 175.0661 [$C_{11}H_{11}O_2$] corresponding to cleavage at C_{12}. Hence the structure and stereochemistry of brachystamide-C can be represented as *N*-isobutyl-15-(3′,4′-methylenedioxyphenyl)2*E*,4*E*,13*E*-pentadecatrienamide (**4**).

Extensive fractionation yielded two minor components, designated brachystamide-D (**5**), and brachystamide-E (**6**). While brachystamide-D could be obtained in a pure state, brachystamide-E (**6**) could be detected in a ~1:2 mixture with (**5**). Brachystamide-D showed a molecular-ion peak at M^+ 425.2968, corresponding to the molecular formula $C_{27}H_{39}NO_3$. Its 300 MHz ^1H NMR and 75.5 MHz ^{13}C NMR spectra were closely similar to those of brachystamide-B (**2**), the only difference being the presence of an extra methylene group. Thus brachystamide-D was *N*-isobutyl-16-(3′,4′-methylenedioxyphenyl)2*E*,4*E*,15*E*-hexadecatrienamide (**5**). The signal assignments, given in the Experimental were confirmed by ^1H–^1H decoupling, COSY-90° two-dimensional experiments, and DEPT-spectra.

A ~2:1 mixture of (**5**) and (**6**), which could not be resolved further, showed molecular ion-peaks at M^+ 425 and M^+ 427.3124, corresponding to (**5**) and its dihydro derivative (**6**) respectively. Catalytic hydrogenation of this mixture furnished a single reduced derivative (**8**), M^+ 431, the spectroscopical properties of which were in accord with its structure. Additional weaker signals for brachystamide-E appeared in the 75.5 MHz ^{13}C NMR spectrum of the mixture at 136.78 (C-1′), 107.98 (C-2′), 147.40 (C-3′), 145.38 (C-4′), 108.76 (C-5′), 120.97 (C-6′), 31.60 (C-15), 35.63 (C-16); the other signals were overlapped with those of brachystamide-D. The similarity in chemical shifts of these signals (C-1′ to 6′, benzylic and homobenzylic carbons) to the corresponding signals in its lower homologue brachystamide-A (**1**) seemed to indicate their structural similarity. Thus, brachystamide-E differed from (**5**) in having the styryl double-bond saturated, and hence could be assigned structure (**6**), viz. *N*-isobutyl-16-(3′,4′-methylenedioxyphenyl)-2*E*,4*E*-hexadecadienamide (**6**).

Extraction of petrol extract of the roots of *Piper retrofractum* followed by fractionation gave a mixture of amides (Banerji et al., 1985). Our previous studies on this mixture of alkamides obtained from *Piper retrofractum* showed molecular-ion peaks at *m*/*z* 357, 355 (retrofractamide-B), 353, 345, 343, 341, 329 (retrofractamide-C) and 327 (retrofractamide-A) (Banerji et al., 1985). The component with M^+ 341 was designated retrofractamide-D; however, this could not be isolated earlier. By repeated chromatographic resolution it has been now possible to obtain retrofractamide-D which has been fully characterised as (**13**). Retrofractamide-D, obtained as a white solid, mp 118–120 °C: showed UV absorption (λ_{max}^{EtOH} 262, 211 nm, log ε 4.50, 4.42; λ_{min}^{EtOH} 230 nm, log ε 4.26) characteristic of a modified sorbamide chromophore. Its IR spectrum corroborated this by showing bands at 1625 and 1000 cm^{-1}. In addition IR bands were observed for 3,4-methylenedioxyphenyl nucleus (1615, 870 and 815 cm^{-1}; 1043, 922 cm^{-1}). The ^1H and ^{13}C NMR data indicated the presence of six olefinic protons, a 3,4-methylenedioxyphenyl nucleus, an NH–Bu^i group, and six other protons attached to sp^3 carbons. The positions and multiplicites of the olefinic protons indicated the presence of the *E,E*-dienamide moiety and a styryl double bond with the *E*-configuration. MS, ^1H and ^{13}C NMR data indicated that a three methylene chain linked the dienamide grouping with the third double bond which was conjugated to the 3,4-methylenedioxyphenyl unit. Taking into account all the spectroscopical data retrofractamide-D was assigned structure (**13**).

3. Experimental

3.1. General

Mps: uncorr. IR: KBr pellets. UV: EtOH. 300 MHz ^1H and 75.5 MHz ^{13}C NMR: $CDCl_3$. MS: 70 eV. CC and PTLC: silica gel.

3.2. Plant material

Piper brachystachyum Wall. and *Piper retrofractum* Vahl. were collected in Kerela, and identified by Koshy Abraham. Voucher specimens (PBr-WP) and (PR-WP) have been preserved at the Centre of Advanced Studies on Natural Products, Chemistry Department, University of Calcutta.

3.3. Extraction and isolation

Air-dried and crushed aerial parts (2 kg) were extracted with petrol (bp 60–80 °C) (8 l) for 72 h in a Soxhlet apparatus. The conc. petrol extract on chromatography furnished a mixture of amides (0.8 g) in the $CHCl_3$ eluates. The mixture could be resolved on careful rechromatography, repeated recrystallisation and PTLC to yield **1** (250 mg), **2** (86 mg), **4** (54 mg), **5** (16 mg) and a ~ 2:1 mixture of 5:6 (56 mg).

3.3.1. Brachystamide-C (*4*)

White amorphous solid, mp 90–95 °C. UV and IR data in Results and Discussion. ^{1}H NMR: δ 5.68 (1H, *d*, J = 15.1 Hz, H-2), 7.12 (1H, *dd*, 15.1, 11.3, H-3), 6.05 (1H, *dd*, 16.0, 11.3, H-4), 5.99 (1H, *dt*, 16.0, 7.0, H-5), 2.10–2.15 (2H, *br*. quartet, H-6), 1.34–1.40 (2H, *br*. quintet, H-7), 1.19–1.34 (8H, *br.m*, H-8-11), 1.96 (2H, *br*. quartet, H-12), 5.40–5.60 (2H, *br.m*, H-13,14; overlapped by –NH– signal; apparent after D_2O-exchange), 3.18 (2H, *br.d*, 6.4, H-15), 6.60 (1H, *d*, 1.6, H-2′), 6.65 (1H, *d*, 7.9, H-5′), 6.55 (1H, *dd*, 7.9, 1.6, H-6′), 3.10 (2H, *t*, 6.6, –NH–CH_2–CH <), 1.73 (1H, 9-line multiplet, 6.6, –CH_2–*CH*$(CH_3)_2$), 0.86 (6H, *d*, 6.6, CH(*CH*$_3$)$_2$), 5.91 (2H, *s*, –O–CH_2–O–), 5.3–5.5 (*br*., D_2O-exchangeable, –NH–). Chemical shifts and coupling constants for 2*E*,4*E*-diene protons and aromatic protons were determined by using Brüker–parameter adjustment in NMR by iterative calculations (PANIC) programme and spectrum-simulation; ^{1}H–^{1}H coupling relationships were confirmed from COSY-90° 2D-spectrum. ^{13}C NMR: 166.39 (C-1), 121.85 (C-2), 143.07 (C-3), 128.26 (C-4), 141.23 (C-5), 32.91 (C-6), 29.51, 29.40, 29.24, 29.15, 28.98 (C-7-11), 33.85 (C-12), 129.50 (C-13), 132.00 (C-14), 38.73 (C-15), 136.85 (C-1′), 108.09 (C-2′), 147.50 (C-3′), 145.43 (C-4′), 109.00 (C-5′), 121.12 (C-6′), 46.97 (–NH–*CH*$_2$–CH <), 28.64 (–CH_2–*CH*$(CH_3)_2$), 20.09 (–CH(*CH*$_3$)$_2$), 100.70 (–O–CH_2–O–). MS *m*/*z*: 411.2811 (M^+), 396 (M^+–Me), 383 (M^+–CO), 355 (M^+–$Me_2C=CH_2$), 312 (M^+–CONHBu^i + H), 175, 152, 136, 135, 115.

3.3.2. Hydrogenation of brachystamide-C (*4*)

This sample (15 mg) in EtOAc soln. (15 ml) was hydrogenated under atmos. pres. over Adam's catalyst (PtO_2). Usual work-up gave hexahydrobrachystamide-C (**5**), mp 63 °C, identical in all respects with tetrahydro brachystamide-A (Banerji and Das, 1989). MS *m*/*z* 417 (M^+), 402 (M^+–Me), 318 (M-CONHBu^i + H), 136, 135 ($CH_2O_2C_6H_4CH_2^+$), 107, 105.

3.3.3. Brachystamide-D (*5*)

White amorphous solid, mp 88–90 °C. ^{1}H NMR: δ 5.69 (1H, *d*, *J* = 15 Hz, H-2), 7.11 (1H, *dd*, 15, 11 Hz, H-3), 6.06 (1H, *dd*, 16, 11, H-4), 6.00 (1H, *dt*, 16, 6.8, H-5), 2.00–2.25 (4H, *m*, H-6, H-14), 1.38–1.42 (4H, *m*, H-7, H-12), 1.20–1.38 (14H, *br.m*, H-7-13), 6.04 (*m*, H-15), 6.20 (*d*, 7, H-16), 6.60 (1H, *d*, 1.5, H-2′), 6.66 (2H, *d*, 8, H-5′), 6.55 (1H, *d*, 8, 1.5, H-6′), 3.10 (2H, *t*, 6.6, –NH–*CH*$_2$–CH <), 1.73 (1H, 9-line multiplet, 6.6, –CH_2–*CH*$(CH_3)_2$), 0.86 (6H, *d*, 6.6, CH(*CH*$_3$)$_2$), 5.91 (2H, *s*, –O–CH_2–O–), 5.4–5.5 (*br*., D_2O-exchangeable, –NH–). ^{1}H–^{1}H coupling relationships were confirmed from decoupling and COSY-90 °C 2D-spectrum. ^{13}C NMR: 166.42 (C-1), 121.88 (C-2), 142.96 (C-3), 128.36 (C-4), 141.23 (C-5), 32.47 (C-6), 28.8-29.4 (C-7-12), 32.90 (C-14), 129.30 and 129.46 (C-15,16), 132.62 (C-1′), 108.10 (C-2′), 147.86 (C-3′), 145.48 (C-4′), 108.40 (C-5′), 120.12 (C-6′), 46.97 (–NH–*CH*$_2$–CH <), 28.64 (–CH_2–*CH*$(CH_3)_2$), 20.10 (–CH(*CH*$_3$)$_2$), 100.78 (–O–CH_2–O). MS *m*/*z*: 425.2968 (M^+), 410 (M^+–Me), 397 (M^+–CO), 369 (M^+–$Me_2C=CH_2$), 326 (M^+–CONHBu^i + H), 189, 166, 150, 149, 129.

3.3.4. Hydrogenation of a mixture of brachystamides-D and E

This sample (8 mg) in EtOAc solution (6 ml) was hydrogenated under atmospheric pressure over Adam's catalyst. After 6 h, the mixture was filtered and evaporated to dryness to yield hexahydrobrachystamide-D (8 mg), mp 61 °C; MS *m*/*z* 431 (M^+), 416 (M^+–Me), 332 (M–CONHBu^i + H), 136, 135 ($CH_2O_2C_6H_4CH_2^+$), 107,105.

3.3.5. Isolation of the compounds from Piper retrofractum

Air-dried above-ground parts of *P. retrofractum* Vahl. (2 kg) were powered and extracted in two batches with petroleum ether (bp 60–80 °C) (4 l per batch) for 60 h in a Soxhlet apparatus. The combined petrol extract was concentrated (100 ml) and chromatographed over silica gel, when the following compounds were obtained: the petrol–benzene (2:1) eluates furnished white crystals of sesamin, mp 120 °C, $[\alpha]_D^{20°}$ + 68.2 °C ($CHCl_3$). The benzene-ethyl acetate (4:1) eluates furnished 3,4,5-trimethoxyphenyl propionic acid, mp 99 °C. A complex mixture of closely related amide derivatives was obtained in the benzene eluate which on mass spectroscopical analysis showed the presence of the following compounds: retrofractamide-A (M^+ 327), retrofractamide-C (M^+ 329), retrofractamide-B (M^+ 355), retrofractamide-D (M^+ 341) and compounds with molecular ion peaks at *m*/*z* 357, 353, 345 and 343. Rechromatography and repeated preparative TLC and repeated crystallisations furnished retrofractamide-A, mp 129 °C, retrofractamide-C, mp 120 °C, and retrofractamide-D, mp 118–120 °C.

3.3.6. Retrofractamide D (*13*)

White amorphous solid, mp 118–120 °C. UV and IR data in Section 2. 200 MHz ^{1}H NMR: δ 5.78 (1H, *d*, *J* = 15 Hz, H-2), 7.20 (1H, *dd*, 15, 10, H-3), 6.19 (1H, *dd*, 15, 10, H-4), 6.30 (1H, *d*, 16, H-10), 6.00–6.15 (2H, *m*, H-5, H-9; decoupling of allyic protons at δ 2.0–2.2 gave 6.11, *d*, 15, H-5 and 6.94, *d*, 16, H-9); 2.0–2.2 (4H, *br*., H-6, H-8);

1.5 (2H, *m*, H-7), 6.75–6.92 (3H, *m*, H-2′, H-5′, H-6′), 3.15 (2H, *t*, 7, –NH–CH_2–CH<), 1.75 (1H, *m*, –CH_2–$CH(CH_3)_2$), 0.90 (6H, *d*, 7, $CH(CH_3)_2$), 5.95 (2H, *s*, –O–CH_2–O–), 5.3–5.5 (*br*., D_2O-exchangeable, –NH–). 50 MHz ^{13}C NMR: 166.3 (C-1), 122.3 (C-2), 141.0 (C-3), 127.4 (C-4), 143.6 (C-5), 31.4 (C-6), 28.6 (C-7), 32.2 (C-8), 129.6 (C-9), 128.9 (C-10), 131.9 (C-1′), 105.2 (C-2′), 147.7 (C-3′), 146.4 (C-4′), 108.0 (C-5′), 120.1 (C-6′), 46.8 (–NH–CH_2–CH<), 28.4 (–CH_2–$CH(CH_3)_2$), 19.9 (–$CH(CH_3)_2$), 100.7 (–O–CH_2–O–). MS *m*/*z*: 341 (M^+), 326 (M^+–Me), 313 (M^+–CO), 285 (M^+–$Me_2C=CH_2$), 242 (M^+–$CONHBu^i$ + H), 136, 135, 115.

Acknowledgements

The authors thank University Grants Commission (India) (Ratna Dutta) and Council for Scientific and Industrial Research (India) (Manjusha Sarkar and Piyali Sengupta) for providing necessary financial assistance.

References

Ahmad, F., Shajarahtunnur, J., Ward, R.W., 1995. Ridleyamide—a new amide from *Piper ridelyi*. Phytochemistry 40 (4), 1163.

Banerji, A., 1992. Studies on natural products. J. Ind. Chem. Soc. 69 (2), 53.

Banerji, A., Sarkar, M., Ghosal, T., Pal, S.C., 1984. Carbon-13 NMR spectra of *Piper* alkamides and related compounds. Organic Magnetic Resonance 22 (11), 734.

Banerji, A., Bandyopadhyay, D., Sarkar, M., Siddhanta, A.K., Pal, S.C., Ghosh, S., Abraham, K., Shoolery, J.N., 1985. Structural and synthetic studies on the retrofractamides—new amide constituents of *Piper retrofractum*. Phytochemistry 24 (2), 279.

Banerji, A., Das, C., 1989. Two amides from *Piper brachystachyum*. Phytochemistry 28 (11), 3039.

Chatterjee, A., 1991. The Treatise on Indian Medicinal Plants, Vol. 1. Council of Scientific and Industrial Research (India), New Delhi.

Crombie, L., 1955. Amides from vegetable origin Part IV and V. J. Chem. Soc. 995, 999, 1007.

Krishnamurthi, A., 1969. Wealth of India. Council of Scientific and Industrial Research (India), New Delhi.