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Chabamides F and G, two novel dimeric alkaloids from the roots of *piper chaba* hunter

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ABSTRACT

Two new dimeric alkaloids, chabamide F (**1**) and chabamide G (**2**), containing pyrrolidine rings, were isolated from the roots of *piper chaba* hunter. The structures of **1** and **2** were established on the basis of spectroscopic data, especially 2D NMR and mass spectral data.

The genus Piper (Piperaceae) comprises more than 700 species distributed in the tropical and subtropical regions of the world.[1] Piper is well reputed in the Indian Ayurvedic system of medicine.[2] Plants of the Piperaceae family are a well-known source of structurally diverse amides with the wide range of bioactivities such as cytotoxic, stomach aches, insect repellents, *anti*-inflammatory, insecticidal and *anti*-feedant activities.[3] The fascinating structural features and multiple biological activities of amides isolated from different species of piper from our laboratory[4] have encouraged us to continue the study of this family.

Piper chaba hunter (Piperaceae), a glabrous shrub, mainly grows in the subtropical areas of Asia, such as India, Malaysia and Bangladesh.[5] This plant has been traditionally used for many medicinal purposes in Ayurvedic formulations. The dried roots and fruits of this plant have been used to treat asthma, bronchitis, fever, pain in abdomen and as a stimulant in haemorrhoidal afflictions.[6]

Despite extensive biological and pharmacological studies, little is known about the chemical constituents of this medicinally important herb. In previous studies, a few chemical constituents were identified from this plant, including amides, one dimeric alkaloid and some miscellaneous substances.[7] As part of our ongoing efforts to search for novel metabolites from *piper* species, two novel dimeric alkaloids chabamide F (**1**) and chabamide G (**2**) were isolated together with known compounds such as piperine (**3**), guineensine (**4**) and brachystamide B (**5**) from the methanolic extract of roots of *piper chaba* (Fig. 1). The structures of the dimeric alkaloids were elucidated on the basis of extensive spectroscopic data (IR, MS and 1D and 2D NMR) analyses. In this Letter, we describe the isolation and structural elucidation of two novel dimeric alkaloids **1** and **2**.

Roots of *piper chaba* (1 kg) have been repeatedly extracted with MeOH in a soxhlet apparatus and combined extracts were concentrated to afford the crude extract (30 g). The crude extract (10 g) was subjected to column chromatography over silica gel (100–200 mesh) using an eluent system of increasing polarity from *n*-hexane to ethyl acetate (60:40) to yield six fractions. Homogeneous fractions were pooled together to give major three fractions (F1–F3). A portion of fraction F2, was subjected to open column chromatography over silica gel using EtOAc–hexane (35:65) as an eluent to give a mixture of two compounds. Subsequent purification by RP HPLC (C18, 60 × 16 mm, 15 μm) with acetonitrile–water (45:55) as an eluent system to afford the compounds **1** (17 mg) and **2** (10 mg). Similarly, fractions F1 and F3 were purified by silica gel column chromatography using EtOAc–hexane (40:60) eluent system to get compounds **3** (2 g), **4** (3 g) and **5** (1.6 g). Compounds **3**, **4** and **5** were identified by comparison with the reported literature data.[8]

Compound **1** was obtained as pale yellow liquid. The molecular formula of **1** was established as $C_{32}H_{34}N_2O_6$ by HRESIMS, which provided a molecular ion peak[9] at *m/z* 565.2316 $(M+Na)^+$, in conjunction with its ^{13}C NMR spectrum. The IR spectrum displayed absorption bands diagnostic of tertiary amide (1630 cm^{-1}) and aromatic (1637 and 1566 cm^{-1}) moiety. The 300 MHz ^1H NMR

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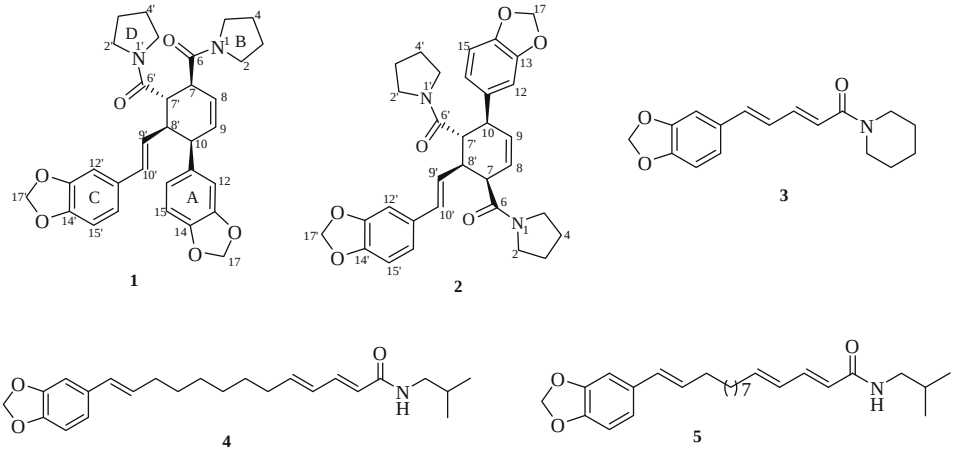


Figure 1. Compounds isolated from the roots of *p. chaba*.

Table 1
^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectral data for **1** and **2** (in $CDCl_3$)

No.	Chabamide F (**1**)		No.	Chabamide G (**2**)	
	^{13}C, δ	^{1}H, δ (mult, *J*, Hz)		^{13}C, δ	^{1}H, δ (mult, *J*, Hz)
1	—	—	1		
2	46.52	3.54–3.62 (m)	2	46.60	3.47–3.50 (m)
3	24.28	1.39–1.44 (m)	3	26.00	1.86–1.90 (m)
4	25.81	1.61–1.80 (m)	4	26.13	1.91–1.98 (m)
5	45.72	3.43–3.50 (m)	5	46.73	3.50–3.54 (m)
6	171.24	–C–	6	171.44	–C–
7	45.10	3.94–4.00 (dq, *J* = 10.0, 2.0)	7	38.81	3.05–3.15 (m)
8	124.81	5.76–5.82 (dt, *J* = 10.0, 1.5)	8	122.44	5.72–5.78 (dq, *J* = 10.0, 2.4)
9	130.50	5.85–5.91 (ddd, *J* = 10.0, 5.0, 2.6)	9	123.57	5.79–5.86 (ddd, J = 10.0, 5.0 2.4)
10	45.53	3.11–3.26 (m)	10	45.19	3.67–3.71 (dq, J = 10.0, 2.3)
11	133.50	–C–	11	133.15	–C–
12	107.93	6.81 (br s)	12	108.23	6.99–7.01 (d, J = 1.5)
13	147.50	–C–	13	146.37	–C–
14	146.78	–C–	14	146.36	–C–
15	110.80	6.85 (d, *J* = 1.5)	15	109.09	6.85–6.86 (d, *J* = 8.0)
16	123.53	6.82 (dd, *J* = 8.5, 1.5)	16	130.23	6.87–6.90 (dd, *J* = 8.0, 1.5)
17	100.94	5.91 (br s)	17	100.91	5.96–5.97 (d, *J* = 1.5)
1′	—	—	1′	—	—
2′	46.66	3.67–3.73 (m)	2′	46.73	3.33 (m)
3′	24.32	1.86–1.92 (m)	3′	24.42	1.25–1.31 (m)
4′	26.08	1.92–2.02 (m)	4′	24.45	2.02–1.92 (m)
5′	46.06	2.96–3.04 (m)	5′	46.81	3.65 (m)
6′	172.45	–C–	6′	172.36	–C–
7′	40.56	3.66–3.76 (dd, *J* = 10.1, 9.5)	7′	45.79	3.87–3.93 (dq, *J* = 10.0, 1.7)
8′	45.26	2.89–2.91 (ddd, *J* = 10.1, 9.5, 5.5)	8′	39.97	3.62–3.64 (td, *J* = 10.0, 5.2)
9′	127.33	5.24–5.34 (dd, *J* = 15.6, 10.1)	9′	130.44	4.65–4.75 (dd, *J* = 15.5, 10.0)
10′	131.35	6.30–6.40 (d, *J* = 15.6)	10′	130.90	6.28–6.33 (d, *J* = 15.5)
11′	131.97	–C–	11′	131.20	–C–
12′	105.25	6.67 (d, *J* = 1.5)	12′	107.80	6.91 (br s)
13′	147.80	–C–	13′	147.20	–C–
14′	146.45	–C–	14′	147.50	–C–
15′	108.19	6.64 (d, *J* = 8.5)	15′	111.12	6.78–6.80 (d, *J* = 7.9)
16′	120.66	6.61 (dd, *J* = 8.5, 1.5)	16′	125.12	6.81–6.83 (dd, *J* = 7.9, 1.6)
17′	100.91	5.97–5.98 (d, *J* = 1.32) and 5.99–6.00 (d, *J* = 1.32)	17′	100.86	5.94–5.95 (d, *J* = 1.3)

spectrum (in $CDCl_3$) indicated the presence of two signals at δ 5.24–5.34 (dd, *J* = 15.6, 10.0 Hz) and 6.35–6.40 (d, *J* = 15.6 Hz), which were assigned to *trans*-olefinic protons by the coupling constant of 15.6 Hz. It also displayed two sets of aromatic protons due to two 1,3,4-trisubstituted aromatic rings at δ 6.85 (1H, d, *J* = 1.5 Hz), 6.82 (1H, dd, *J* = 8.5, 1.5 Hz), 6.81 (1H, br s) and 6.67 (1H, d, *J* = 1.5), 6.64 (1H, d, *J* = 8.5 Hz), 6.61 (1H, dd, *J* = 8.5, 1.5 Hz). In addition to the above-mentioned moieties, combined inspection of ^{1}H NMR and ^{1}H–^{1}H COSY revealed the presence of cyclohexene ring and two pyrrolidine rings. The ^{13}C NMR spectrum displayed the presence of 32 carbon atoms (Table 1), and were further classified by DEPT experiments into categories of 10 methylenes, 14 methines and 8 quaternary carbons including two carbonyls (δ 171.2 and 172.4). On the basis of these characteristic features, database and literature searches led the skeleton of **1** as a dimeric alkaloidal framework.

A comprehensive analysis of the 2D NMR data (Fig. 2) of **1** facilitated the proton and carbon assignments (Table 1). ^{1}H–^{1}H COSY spectrum suggested the sequential correlations of δ 3.94–4.00 (dq, *J* = 10.0, 2.0 Hz)/5.76–5.82 (dt, *J* = 10.0, 1.5 Hz)/5.85 (ddd, *J* = 10.0, 5.0, 2.6 Hz)/3.11–3.26 (m)/2.89–2.91 (ddd, *J* = 10.1, 9.5, 5.5 Hz)/3.66–3.76 (dd, *J* = 10.1, 9.5 Hz) assignable to H-7-H-8-H-9-H-10-H-8′-H-7′ of the cyclohexene ring. Concerning the connections of the two phenyl rings, HMBC spectrum showed correlations of H-16, H-12/C-10; H-10/C-11, H-9′/C-8′, which implies that these units (A and C) were bonded to the cyclohexene ring at C-10 and C-8′. Further, HMBC correlations of two methylene protons at δ 5.91 with 147.50 (C-13), 146.78 (C14), at δ 5.97 and 5.99 with δ 147.80 (C-13′) and δ 146.45 (C-14′) confirmed the location of the two methylenedioxy groups at C-13, and C-13′. Remaining units, B and D (pyrrolidine rings) were connected through carbonyl groups at C-7 and C-7′, which was confirmed by HMBC correlations of H-7 to C-6 (δ 171.24) and H-7′ to C-6′ (δ 172.45).

The assignment of the relative configuration of chabamide F (**1**), and confirmation of overall structure were achieved by the interpretation of the NOESY spectral data (Fig. 2) and by analysis of ^{1}H NMR coupling constants. The large vicinal coupling constants of H-7′/H-7 (10.0 Hz) and H-7′/H-8′ (10.0 Hz) indicated *anti*-relations of H-7′/H-7 and H-7′/H-8′ and the axial orientations for these protons. In the NOESY spectrum, the occurrence of the correlations between H-7/H-8′ and the absence of NOE effects between H-7/H-7′ and H-7′/H-8 supported the above result. These data indicated β-orientation for H-7′ and α-orientation for H-7 and H-8′. The α-orientation of H-10 was suggested by the coupling constant of H-10/H-8′ (5.5 Hz) and the absence of the NOESY correlations between H-8′

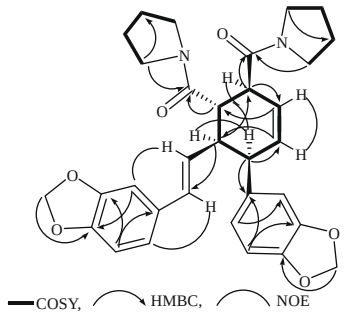


Figure 2. Key COSY, HMBC and NOE correlations of compound **1**.

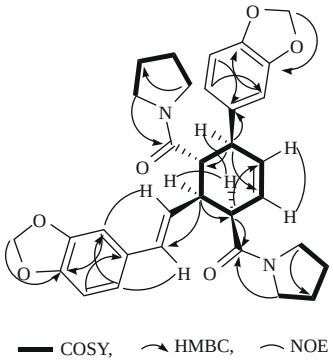


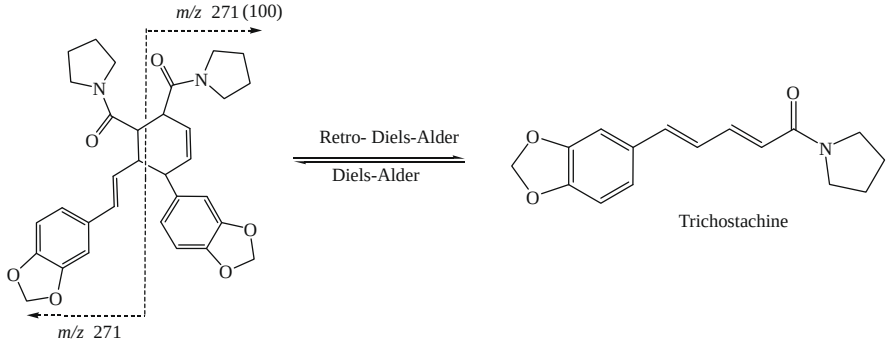
Figure 3. Key COSY, HMBC and NOE correlations of compound **2**.

and H-7′. Further, zero optical rotation[9] of **1** indicated to be a racemate. On the basis of these spectral data, the structure of **1** was unambiguously established and trivially named as chabamide F.

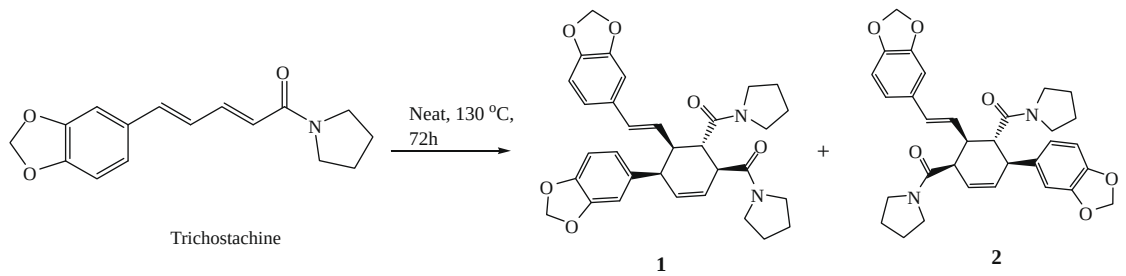
Compound **2**, obtained as yellow oil, had the same molecular formula $C_{32}H_{34}N_2O_6$ as that of the **1** on the basis of the HRESIMS analysis[9] (*m/z* 543.2489 $[M+1]^+$). Initial analysis of the ^1H NMR and ^{13}C NMR data for **2** (Table 1), illustrated similar features to that of **1** except for the *meta*-orientation of the two carbonyl groups in **2** instead of *ortho*-orientation in **1**. From this observation, it was deduced **2** to be a stereoisomer similar to that of **1.** As in **1**, major portion of **2** was assembled by the interpretation of COSY, HSQC and HMBC spectral data (Fig. 3).

The COSY spectrum of **2** revealed the presence of a cyclohexene ring (-C-7-C-8-C-9-C-10-C-8′-C-7′-) and *trans*-olefinic double bond (C-9′-C-10′), which was bonded to C-8′, respectively. The presence of the carbonyl groups at C-6 and C-6′ was implied by HMBC cross peaks at δ 3.05 (H-7) and 3.62 (H-8′)/δ 171.44 (C-6) and δ 3.67-3.71 (H-10) and 3.89 (H-7′)/δ 172.36 (C-6′). The analysis of ^1H–^1H coupling and NOESY data allowed us to determine the stereochemistry of **2**. The large coupling constants for H-7′/H-10 and H-7′/H-8′, small coupling constants for H-7/H-8′ and NOESY correlations for H-10, H-7 and H-8′ suggested that **2** had the same relative stereochemistry as that of **1.** Thus, the stereostructure of **2** was established.

The dipiperamides are a family of structurally diverse class of metabolites and usually isolated from the piper species. These compounds are presumably generated by intermolecular Diels–Alder reaction of the same or different two monomeric alkaloids to afford a fused cyclic (cyclohexene) system. Recently, Wei et al. reported the biomimetic synthesis of the dimeric alkaloids via exo-selective [4+2] cycloaddition reaction to prove above hypothesis.[10] A comparison of the NMR spectroscopic data revealed that the structures of **1** and **2** closely related to chabamide[6b] and nigramide B[10] except for the presence of pyrrolide ring instead of the piperidine ring. This was also supported by the molecular weights of **1** and **2**, which were 14 amu lower than those of chabamide and nigramide B, as indicated by the HRESIMS data. However, Compounds **1** and **2** are novel natural products and, to the best of our



Scheme 1. Retro Diels–Alder fragmentation of **1**.



Scheme 2. Diels–Alder reaction of trichostachine.

knowledge these compounds represent the first bisalkaloids possessing two pyrrolidine rings. From the biosynthetic view point, chabamide F and G (**1** and **2**) may be presumably generated by the intermolecular Diels–Alder reaction of the trichostachine (Scheme 1).

This hypothesis was further confirmed by the mass spectrum, which showed a significant peak at *m*/*z* 294.113 [M^++Na], assigned to the trichostachine ion arising by the retro-Diels–Alder cleavage of molecular ion into two halves. Finally, to confirm the existence of the compounds **1** and **2**, we extracted the roots of *p. chaba* with MeOH at room temperature followed HPLC/electron spray ionization (ESI) MS experiments. In HPLC/ESIMS[11] of the MeOH extract showed the presence of peaks at *m*/*z* 563 [M^++Na] and 543 [M^++1] at about 8.8 min and 10.6 min of LC retention time, respectively.

Cycloaddition reactions of trichostachine: To support the above-described biosynthetic hypothesis, we have carried out the intermolecular [4+2] cycloaddition reaction with the trichostachine[12] under solvent-free conditions (Scheme 2).

Reaction mixture was analysed by the LC–MS, which clearly indicted the presence of the compounds **1** and **2** (retention time and mass). In HPLC analysis,[11] retention times of the synthetic **1** and **2** were identical to those of chabamide F and G, confirming that the structure and stereochemistry are the same as those of isolated alkaloids. Detailed synthetic experiments are in progress in our laboratory and will be published in due course.

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References and notes

1. Parmar, V. S.; Jain, S. C.; Bisht, K. S.; Jain, R.; Taneja, P.; Jha, A.; Tyagi, O. D.; Prasad, A. K.; Wengel, J.; Olsen, C. E.; Boll, P. M. *Phytochemistry* **1997**, *46*, 597–673.
2. (a) Kirtikar, K. R.; Basu, B. D. In *Indian Medicinal Plants*; Periodical Experts Book Agency: New Delhi, India, 1933; Vol. III, p 2128.; (b) *The Wealth of India*, Raw Materials; CSIR: New Delhi, 1969; Vol. VIII, p 83.
3. (a) Geran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3*, 23825; (b) Asprey, G. F.; Thornton, P.. *Medicinal Plants of Jamaica 17*. In *West Indies Medicinal Journal*; Kingston: Jamaica, 1954; (c) Kirtikar, K. R.; Basu, B. D. In *Indian Medicinal Plants*; Periodical Experts Book Agency: New Delhi, India, 1933; Vol. III, p 2130.; (d) Jacobson, M.; Crosby, D. G. *Naturally Occurring Insecticides* **1971**, *144*, 226. Marcel Dekker; (e) Matsui, K.; Manakata, K. *Tetrahedron Lett.* **1975**, *16*, 1905–1908.
4. (a) Sumathykutty, M. A.; Rao, J. M. *Phytochemistry* **1991**, *30*, 2075–2076; (b) Reddy, S. V.; Srinivas, P. V.; Praveen, B.; Hara, K. K.; Raju, B. C.; Murthy, U. S. N.; Rao, J. M. *Phytomedicine* **2004**, *11*, 697–700; (c) Srinivas, P. V.; Rao, J. M. *Phytochemistry* **1999**, *52*, 957–958; (d) Srinivas, P. V.; Anuradha, V.; Tiwari, A. K.; Rao, J. M. *J. Piper Ind.: Focus Pepper* **2005**, *2*, 21; (e) Srinivas, P. V.; Tiwari, A. K.; Sarma, V. U. M.; Anuradha, V.; Haribabu, T.; Rao, D. K.; Khan, I. A.; Rao, J. M. *J. Ethnopharmacol.* **2006**, *108*, 445–449.
5. Tewtrakul, S.; Hase, K.; Kadota, S.; Namba, T.; Komatsu, K.; Tanaka, K. *J. Essent. Oil Res.* **2000**, *12*, 603–608.
6. (a) Dominguez, X. A.; Alcorn, J. B. *J. Ethanopharmacol.* **1985**, *13*, 139–156; (b) Toshio, M.; Hisashi, M.; Itadaki, Y.; Yutana, P.; Masayuki, Y. *Planta Med.* **2004**, *70*, 152–159.
7. Rukachaisirikul, T.; Prabpai, S.; Champung, P.; Suksamrarn, A. *Planta Med.* **2002**, *68*, 850–853.
8. Matsuda, H.; Ninomiya, K.; Morikawa, T.; Yasuda, D.; Yamaguchi, I.; Yoshikawa, M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2038–2042.
9. (a) Compound **1**: Yellow liquid; $[\alpha]_D^{25}$ ±0 (*c* 0.755, $CHCl_3$); IR (KBr) ν_{max} 3025, 2940, 2920, 2850, 1630, 1637, 1566, 1490, 1230 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); HRESIMS *m*/*z*, 565.2316 (M^++Na), calcd for $C_{32}H_{34}N_2O_6$ 565.2309; (b) **2**: Yellow liquid; $[\alpha]_D^{25}$ ±0 (*c* 0.815, $CHCl_3$); IR (KBr) ν_{max} 2935, 2855, 1627, 1500, 1490, 1245, 1120 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); HRESIMS *m*/*z*, 543.2489 (M^++H), calcd for $C_{32}H_{34}N_2O_6$ 543.2490.
10. Wei, K.; Li, W.; Koike, K.; Chen, Y.-J.; Nikaido, T. *J. Org. Chem.* **2005**, *70*, 1164–1176.
11. *Operating conditions*: Column Phenomenex Luna (C18, 150 × 4.6 mm, 5 μm); Solvent system: gradient elution, 0–10 min, acetonitrile:water (1:1); 10–35 min, acetonitrile:water (70:30); 36–40 min, acetonitrile:water (1:1).
12. Chandrasekhar, S.; Reddy, M. V.; Reddy, K. S.; Rama Rao, C. *Tetrahedron Lett.* **2000**, *41*, 2667–2670.