



Two new alkaloids, pipericyclobutanamides A and B, from *Piper nigrum*

Yasuhiro Fujiwara,^{a,*} Kaname Naithou,^a Tomoko Miyazaki,^a Keiji Hashimoto,^a Kazuo Mori^b and Yasuo Yamamoto^c

^aKyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan

^bHyogo College, Hyogo University, Hiraoka, Kakogawa 675-0101, Japan

^cSonoda Women's College, Minamitsukaguchi, Amagasaki 661-0012, Japan

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Abstract—Two new alkaloids possessing a cyclobutane ring, named pipericyclobutanamides A (**1**) and B (**2**), have been isolated from the fruits of *Piper nigrum*. Their stereostructures were determined by extensive spectroscopic methods. © 2001 Elsevier Science Ltd. All rights reserved.

Black and white peppers *Piper nigrum* are well known world wide as a spice, and many studies on the constituents have been reported in the literature.^{1–5} In the course of our study on the chemistry of piperine in pepper,⁶ we found two new alkaloids, named pipericyclobutanamides A and B, in the fruits of black pepper. Thus, we report here the isolation and the structure elucidation of the two new alkaloids.

A chloroform extract of the dried and powdered fruits (50 g) of Indian black pepper *P. nigrum* was chromatographed on silica gel^{7a} and divided into six fractions. The ether-soluble constituents of the residual

solid (0.73 g) from fraction no. 5 were purified by prep. TLC on silica gel,^{7b} and HPLC on silica gel column^{7c} furnished pipericyclobutanamides A (**1**, 60 mg) and B (**2**, 3 mg).

Pipericyclobutanamide A (**1**) was obtained as a colorless amorphous powder. The IR spectrum of **1** in CHCl₃ showed carbonyl absorption at 1630 cm⁻¹. The UV spectrum of **1** in *n*-hexane showed absorption maxima at 265, 271, and 289 nm, suggesting the existence of a piperolein B chromophore.² No applicable Cotton effect was observed in the CD spectrum of **1** in methanol. The EIMS spectrum of **1** showed ion peaks^{8a}

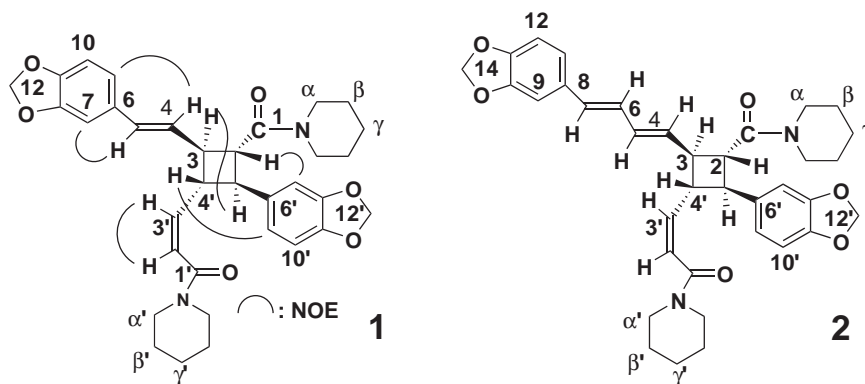


Figure 1. Structures (relative stereochemistry) of **1** and **2**, and NOE data summary of **1**.

Keywords: alkaloids; pipericyclobutanamides; *Piper nigrum*; structure elucidation; ¹H–¹H NOE.

* Corresponding author. Fax: +81-75-595-4766; e-mail: fujiwara@mb.kyoto-phu.ac.jp

at m/z 570 (M^+), 485, 458, 372, 345, 310 and 285. The peaks at 485 and 458 suggested the existence of a piperidinocarbo group and also an ion peak at 135 suggested the existence of a methylenedioxybenzyl and/or methylenedioxyethyl group(s).^{3,8a} The empirical formula of **1**, $C_{34}H_{38}O_6N_2$, was established by HREIMS of the molecular ion peak.^{9a} The ^{13}C NMR and DEPT spectra of **1** showed 34 carbon signals and 38 carbon-bonded protons: 12 methylene groups (including two methylenedioxy carbons), 14 methine groups (ten ethylenic and four tetrahedral carbons) and eight quaternary carbons (six ethylenic and two carbonyl carbons). Based on the data mentioned above and comparison of their data with those of piperoylpiperidine,^{6,10} the existence of two methylenedioxyphenyl and two piperidinocarbo groups in **1** was suggested as the partial structures. The ^{13}C and 1H NMR signals of **1** were assigned by DEPT, ^{13}C - 1H COSY, HMBC, 1H homodecoupling and 1H - 1H NOE experiments are listed in Table 1. On the basis of these spectral data, the elucidated structure of **1** is shown in Fig. 1. The ^{13}C - 1H COSY spectra of **1** established all the one-bond ^{13}C - 1H connectivities. The 1H homodecoupling NMR experiments of **1** revealed the 1H spin-coupling connectivities

of H5 to H2' via cyclobutane ring protons. Concerning the connections of the two phenyl groups with vinyl and cyclobutane groups in **1**, the bond of C5-C6 was determined on the basis of the large NOEs between the phenyl protons at $\delta \sim 6.65$ (H7 and H11) and the two vinyl protons (H5 and H4). Also, the bond of C5'-C6' was assigned by the large NOEs between the phenyl protons at δ 6.83 and 6.81 (H7' and H11') and the two aliphatic protons (H2 and H4'). The connections of C5-C6 and C5'-C6' in **1** were also supported by HMBC.¹¹ Thus, the two remaining piperidinocarbo groups should be connected to C2 and C2'. The *trans* and *cis* geometries between H4 and H5, and between H2' and H3' on the double bonds in **1** were determined on the basis of the magnitude [15.6 (J_{4-5}) and 10.0 Hz ($J_{2'-3}$)] of their 1H spin-coupling constants, respectively.¹² The *cis* geometry between H2' and H3' was also supported by the NOE between them. As for the stereochemistry of the cyclobutane moiety in **1**, the H3 and H5', each of which is located in a diagonal position on the cyclobutane ring, had *cis* relationship with each other, since NOE between the protons was observed. The *trans* configurations between H2, H4'-H5' were characterized by the large NOEs between H7', H11'-

Table 1. ^{13}C (75.4 MHz) and 1H (300 MHz) NMR data for **1** and **2** in $CDCl_3$

Carbon no.	1		Carbon No.	2	
	^{13}C , δ mult	1H , δ (mult, J , Hz)		^{13}C , δ	1H , δ (mult, J , Hz)
α, α'	42.9, t, 46.9, t 43.3, t, 47.1, t	3.31, 3.56 (m)	α, α'	42.9, 46.9 43.3, 47.1	3.29–3.58 (m)
β, β'	25.6, t, 26.4, t 25.8, t, 26.7, t	1.3–1.6 (m)	β, β'	25.7, 26.4 25.8, 26.7	1.3–1.6 (m)
γ, γ'	24.5, t, 24.7, t	1.62 (m)	γ, γ'	24.6, 24.6	1.6 (m)
1	172.2, s	–	1	172.2	–
2	37.7, d	3.66 (dd, 11.7, 9.6)	2	37.7	3.63 (dd, 11.9, 9.6)
3	45.4, d	2.93 (ddd, 11.7, 10.2, 5.6)	3	45.3	2.89 (ddd, 11.9, 10.0, 5.6)
4	128.0, d	5.21 (dd, 15.6, 10.2)	4	133.4	4.95 (dd, 15.0, 10.0)
5	131.0, d	6.32 (d, 15.6)	5	132.4	6.19 (dd, 15.0, 10.3) ^a
–	–	–	6	127.1	6.38 (dd, 15.5, 10.3) ^a
–	–	–	7	131.2	6.36 (d, 15.5) ^a
6	131.9, s	–	8	131.9	–
7	105.3, d	6.65 (d, 2.0)	9	105.3	6.89 (d, 1.6)
8	147.8, s	–	10	148.0	–
9	146.7, s	–	11	147.0	–
10	108.2, d	6.69 (d, 7.9)	12	108.3	6.73 (d, 8.0)
11	120.8, d	6.64 (dd, 7.9, 2.0)	13	121.1	6.77 (dd, 8.0, 1.6)
12	100.9, t	5.92 (s)	14	101.0	5.93 (s)
1'	171.1, s	–	1'	171.1	–
2'	125.5, d	5.75 (dt, 10.0, 1.7)	2'	125.3	5.73 (dt, 9.9, 1.7)
3'	130.3, d	5.86 (ddd, 10.0, 4.9, 2.7)	3'	130.3	5.85 (ddd, 9.9, 5.0, 2.7)
4'	45.9, d	3.48 (dddd, 5.6, 4.9, 2.7, 1.7)	4'	45.7	3.45 (dddd, 5.6, 5.0, 2.7, 1.7)
5'	42.8, d	4.14 (dddd, 9.6, 2.7, 2.7, 1.7)	5'	42.9	4.12 (dddd, 9.6, 2.7, 2.7, 1.7)
6'	133.6, s	–	6'	133.5	–
7'	110.8, d	6.83 (d, 1.5)	7'	110.9	6.82 (s)
8'	147.4, s	–	8'	147.5	–
9'	146.4, s	–	9'	146.4	–
10'	107.9, d	6.81 (s)	10'	108.0	6.82 (s)
11'	123.4, d	6.81 (d, 1.5)	11'	123.5	6.82 (s)
12'	100.9, t	5.96 (d, 1.4), 5.99 (d, 1.4)	12'	100.9	5.95 (d, 1.4), 5.99 (d, 1.4)

1H and ^{13}C chemical shifts are reported downfield from internal TMS. Assignments are based on 1H homodecoupling, 1H - 1H NOE, DEPT, ^{13}C - 1H COSY and HMBC experiments in $CHCl_3$ and/or acetone- d_6 , and on comparison with 1-piperoylpiperidines (cf. Refs. 6 and 10).

^a In acetone- d_6 .

H4' and between H7', H11'–H2, as shown in Fig. 1; the phenyl group (including H7' and H11'), H4', and H2 had a *cis* relationship with each other. Therefore, the relative stereostructure of **1** was confirmed as shown in Fig. 1. The structure of **1** is distinct from that of the synthetic piperine dimers.¹³

Pipercyclobutanamide B (**2**) was obtained as a colorless amorphous powder. The IR spectrum of **2** in CHCl₃ showed carbonyl absorption at 1630 cm⁻¹. The UV spectrum of **2** in *n*-hexane showed absorption maxima at 285, 296, and 309 nm. The UV spectral pattern of **2** was similar to that of **1**, but each wavelength of the corresponding absorption maxima in **2** was shifted by ca. 20 nm to a longer wavelength than that in **1**. The EIMS spectrum of **2** showed ion peaks^{8b} at *m/z* 596 (M⁺), 511, 484, 398, 371, 336, and 311. The MS spectral pattern of **2** was similar to that of **1**, but each mass number of the corresponding ion peaks in **2** was 26 units larger than that in **1**. The empirical formula of **2**, C₃₆H₄₀O₆N₂, was established by HREIMS.^{9b} The ¹H and ¹³C NMR signals of **2** in CDCl₃ were almost identical with those of **1** except the following signals: three olefinic protons including two additional protons resonated at δ 6.38 (2H, m) and 6.19 (1H, m), and three olefinic carbons including two additional carbons resonated at δ 127.1, 132.4, and 133.4. As to the connections of these three protons and three carbons, the ¹H-homodecoupling experiments of **2** in acetone-*d*₆ revealed the ¹H spin-coupling connectivities of H5 (δ 6.14, *J*=15.0 and 10.3 Hz) to H6 (δ 6.57, *J*=10.3 and 15.5 Hz) and H7 (δ 6.43, *J*=15.5 Hz). The ¹³C-¹H COSY of **2** in acetone-*d*₆ clarified the assignments of C4, C5, and C6, as shown in Table 1. Each of the ¹H spin-coupling constants between the phenyl protons and between the protons on the cyclobutane ring in **2** agreed with their respective ones in **1** within experimental error. These results indicated that **2** was an analog of **1** adding one ethylene unit to the chromophore of **1**. The *trans* geometries between H4 and H5 and between H6 and H7 on the double bonds in **2** were determined by the magnitude (15.0 and 15.5 Hz) of their ¹H spin-coupling constants.¹² Thus, the stereostructure of **2** was established as shown in Fig. 1.

Finally, in order to examine the existence of the alkaloids **1** and **2** in other peppers, HPLC/atmospheric pressure chemical ionization (APCI) MS experiments were undertaken. The APCIMS spectra of **1** and **2** by using MeOH as an ionization agent showed the *quasi*-molecular ions (MH⁺) at *m/z* 571 and 597, respectively. In HPLC/APCIMS¹⁴ of the CHCl₃ extract of the presently used Indian black pepper, the MH⁺ ions at *m/z* 571 and 597 were recognized in the peaks at about 26 and 28 minutes of LC retention time, respectively.

Each HPLC/APCIMS of the CHCl₃ extracts of Indian white pepper, and Malaysian black and white peppers also showed the *quasi*-molecular ions (MH⁺) at *m/z* 571 and 597 in a similar manner as above.

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- (a) Elution solvent: CH₂Cl₂. (b) Developing solvent: ether. (c) Mobile phase: *n*-hexane/CH₂Cl₂/ethanol=40/4/1 and/or *n*-hexane/2-propanol=9/1.
- (a) **1**: EIMS *m/z* (%) 570 (M⁺, 43), 485 (M⁺-C₅H₁₀N, 93), 458 (M⁺-C₆H₁₀NO, 21), 373 (48), 372 (53), 345 (14), 310 (61), 285 (M⁺-C₁₇H₁₉NO=M⁺/2, 100), 251 (10), 223 (8), 201 (56), 173 (28), 141 (9), 135 (35), 115 (27), 112 (49), 84 (27), 69 (46). (b) **2**: EIMS *m/z* (%) 596 (M⁺, 49), 511 (M⁺-C₅H₁₀N, 81), 484 (M⁺-C₆H₁₀NO, 19), 398 (22), 399 (20), 371 (14), 336 (32), 311 (M⁺-C₁₇H₁₉NO, 100), 310 (67), 285 (44), 251 (13), 226 (38), 201 (48), 173 (20), 169 (29), 141 (34), 135 (72), 115 (38), 112 (96), 84 (41), 69 (85).
- (a) **1**: HREIMS *m/z*, 570.2735 (M⁺), calcd for C₃₄H₃₈O₆N₂ 570.2730; (b) **2**: HREIMS *m/z*, 598.2864 (M⁺), calcd for C₃₆H₄₀O₆N₂, 598.2886.
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- Operating conditions: column, silica gel (250×4.6 mm i.d., 5 μm); mobile phase, *n*-hexane/dichloromethane/ethanol=40/4/1; flow rate, 1 ml/min; nebulaizer temp., 250°C; drift voltage, 25 V (HITACHI M-1000).