Cyclobutanoid Amides from *Piper arborescens*

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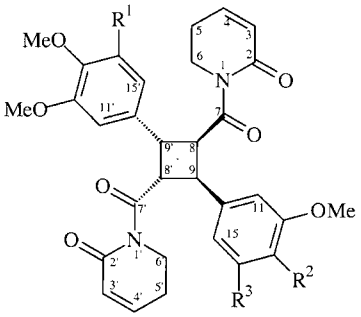
Two new cyclobutanoid amides, piperarborenine A (=1,1′-{[(1*α*,2*α*,3*β*,4*β*)-2,4-bis(3,4-dimethoxyphenyl)-cyclobutane-1,3-diyl]dicarbonyl}bis[5,6-dihydropyridin-2(1*H*)-one]; **1**) and piperarborenine B (=1,1′-{[(1*α*,2*α*,3*β*,4*β*)-2-(3,4-dimethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)cyclobutane-1,3-diyl]dicarbonyl}bis[5,6-dihydropyridin-2(1*H*)-one]; **2**) were isolated from the stem of *Piper arborescens*, besides two known cyclobutanoid amides, piperarboresine (=1,1′-{[(1*α*,2*α*,3*β*,4*β*)-2-(7-methoxy-1,3-benzodioxol-5-yl)-4-(3,4,5-trimethoxyphenyl)cyclobutane-1,3-diyl]dicarbonyl}bis[5,6-dihydropyridin-2(1*H*)-one]; **3**) and piplartine-dimer A (=1,1′-{[(1*α*,2*α*,3*β*,4*β*)-2,4-bis(3,4,5-trimethoxyphenyl)cyclobutane-1,3-diyl]dicarbonyl}bis[5,6-dihydropyridin-2(1*H*)-one]; **4**). The structures of the two new compounds were determined by spectral analyses.

Introduction. – *Piper arborescens* ROXB. (Piperaceae) is a creeping shrub distributed in Lanyu Island of Taiwan, Philippines, and the Malay peninsula and Archipelago [1]. Phytochemical [2] and cytotoxic [3][4] studies have been conducted on the stems and leaves of this plant. In a screening program of Formosan plants, the MeOH extract of the stem of *P. arborescens* showed significant antiplatelet aggregation activity and cytotoxicity activities. Investigation of the $CHCl_3$-soluble fraction of the stem of this plant led to the isolation of four cyclobutanoid alkaloids with *cis*,*trans*,*cis*-configuration, *i.e.*, two new cyclobutanoid amides piperarborenine A (**1**) and piperarborenine B (**2**), besides the two known (see below) cyclobutanoid amides piperarboresine (**3**) and piplartine-dimer A (**4**)[1]). This paper describes the structural elucidation of the two new alkaloids **1** and **2**.

Results and Discussion. – Piperarborenine A (**1**) was isolated as colorless needles. Its molecular formula $C_{32}H_{34}N_2O_8$ was determined by FAB-MS ($[M+H]^+$, *m/z* 575) and FAB-HR-MS ($[M+H]^+$, 575.2375). The IR spectrum exhibited the presence of a conjugated ketone at 1686 cm^{-1}. In the ^1H- and ^{13}C-NMR spectra, the number of signals observed was half that expected, suggesting that **1** had a symmetrical structure. According to the spectral evidences, the structure of piperarborenine A was elucidated as **1**, which was further supported by COSY, NOESY, HMQC, and HMBC experiments.

The ^1H-NMR data of **1** were very similar to that of piplartine-dimer A (**4**) [5], also isolated in this study, except for the presence of the *ABX* pattern at δ 6.77 (*d*, *J* = 8.4 Hz, 2 H, H–C(14,14′)), 6.82 (*d*, *J* = 2.0 Hz, 2 H, H–C(11,11′)), and 6.89 (*dd*, *J* = 8.4, 2.0 Hz, 2 H, H–C(15,15′)) for the substituted phenyl groups of **1** in place of the *s* at δ 6.51 (4 H) for the aromatic protons of **4**, and except for 2*s* at δ 3.84 and 3.86 (each 6 H) for

[1]) Arbitrary numbering; for systematic names, see *Exper. Part*.



1 $R^1 = R^3 = H$, $R^2 = MeO$
2 $R^1 = R^2 = MeO$, $R^3 = H$
3 $R^1 = MeO$, $R^2, R^3 = OCH_2O$
4 $R^1 = R^2 = R^3 = MeO$

MeO–C(12,12′) and MeO–C(13,13′)[1]), respectively, in **1**. Four cyclobutane protons were determined by HMBC; the signal at δ 4.75 (*dd*, $J = 11.2$, 8.0 Hz, 2 H) was assigned to H–C(9,9′) due to its ^3J correlation with δ 112.1 (C(11,11′)) and 120.0 (C(15,15′)) and the signals at δ 6.82 (H–C(11,11′)) and 6.89 (H–C(15,15′)) showing ^3J correlation with δ 41.6 (C(9,9′)). Thus, δ 4.89 (2*dd*, $J = 11.2$, 8.0 Hz, 2 H) was assigned to H–C(8,8′). The EI-MS spectrum of **1** showed fragments at *m*/*z* 287 but not at *m*/*z* 274 or 300, indicating a head-to-tail structure [6]. Furthermore, the coupling constants ($J = 11.2$, 8.0 Hz) of the cyclobutane protons of **1** were very similar to those of **3** ($J = 11.3$, 6.8 Hz) or α-truxillic acid (=(1α,2α,3β,4β)-2,4-diphenylcyclobutane-1,3-dicarboxylic acid; $J = 11.7$, 6.3 Hz) [6], and suggested a relative *cis*,*trans*,*cis*-configuration of the cyclobutane ring of **1**.

Piperarborenine B (**2**) was obtained as colorless needles. The molecular formula $C_{33}H_{36}N_2O_9$ was established by FAB-MS ($[M+H]^+$, *m*/*z* 605) and HR-FAB-MS spectrometry. The IR spectrum showed the presence of conjugated-ketone moieties at 1671 and 1690 cm^{-1}. According to spectral data, the structure of **2** was elucidated as 1,1′-{[(1α,2α,3β,4β)-2-(3,4-dimethoxy)-4-(3,4,5-trimethoxyphenyl)cyclobutane-1,3-diyl]-dicarbonyl}bis[5,6-dihydropyridin-2(1*H*)-one], which was further confirmed by COSY, NOESY, DEPT, ^{13}C-NMR, HMQC, and HMBC experiments.

The ^{1}H-NMR spectrum of **2** was similar to that of piperarboresine (**3**) [2], which was also isolated in this study, except for a total of five aromatic protons at δ 6.52 (*s*, H–C(11′), H–C(15′)), 6.82 (*d*, $J = 2.0$ Hz, H–C(11)), 6.77 (*d*, $J = 8.4$ Hz, H–C(14)), and 6.88 (*dd*, $J = 8.4$, 2.0 Hz, H–C(15)) and five MeO groups at δ 3.79 (*s*, MeO–C(13′)), 3.85 (*s*, MeO–C(12′), MeO–C(14′)), 3.84 (*s*, MeO–C(12)), and 3.86 (*s*, MeO–C(13))[1]) in **2**, which arose from 3,4,5-trimethoxyphenyl and 3,4-dimethoxyphenyl groups. The four cyclobutane protons were also determined by HMBC and identified as H–C(8) at δ 4.89 (*dd*, $J = 11.4$, 7.4 Hz), H–C(8′) at δ 4.92 (*dd*, $J = 11.4$, 7.4 Hz), H–C(9) at δ 4.73 (*dd*, $J = 11.4$, 7.4 Hz), and H–C(9′) at δ 4.76 (*dd*, $J =$ 11.4, 7.4 Hz). The mass spectrum of **2** showed fragments at *m*/*z* 317 and 287 but not at *m*/*z* 274 and 330, and also indicated a head-to-tail structure [6]. The coupling constants ($J = 11.4$, 7.4 Hz) of the cyclobutane protons were very similar to those of **1**, **3**, and α-truxillic acid and also suggested a *cis*,*trans*,*cis*-configuration of the cyclobutane moiety of **2**.

Compound **3** was obtained as colorless needles. The IR spectrum showed the presence of conjugated-ketone moieties at 1670 and 1691 cm^{-1}. The ^{1}H-NMR spectrum was very similar to that of piperarboresine [2]. According to the spectral data, the structure of **3** was elucidated as 1,1′-{[(1α,2α,3β,4β)-4-(7-methoxy-1,3-benzodioxol-5-yl)-4-(3,4,5-trimethoxyphenyl)cyclobutane-1,3-diyl]dicarbonyl}bis[5,6-dihydropyridin-2(1*H*)-one], which was further confirmed by comparison of its UV, IR, ^{1}H-NMR, $[\alpha]_D$, and MS data with those of piperarboresine (**3**) which had already been isolated previously from the leaves of *Piper arborescens* [2].

The four aromatic protons at δ 6.50 (*s*, H–C(11′), H–C(15′)), 6.54 (*d*, $J = 1.4$ Hz, H–C(11)), and 6.48 (*d*, $J = 1.4$ Hz, H–C(15)), the four MeO groups at δ 3.78 (*s*, 3 H) and 3.85 (*s*, 9 H), and a OCH_2O moiety at δ 5.91 (*m*) in the ^{1}H-NMR spectrum of **3** suggested the presence of a 3,4,5-trimethoxyphenyl group and a 7-methoxy-1,3-benzodioxol-5-yl substituent. The four cyclobutane protons appeared at δ 4.87 and 4.71 (each *m*, each 2 H, H–C(8,8′) and H–C(9,9′), resp.), very similarly to those of **1** and **2**, thus suggesting a *cis*,*trans*,*cis* configuration of the cyclobutane moiety of **3**. The EI-MS of **3** showed fragments at *m*/*z* 317 and 301 but not at *m*/*z* 344, and also indicated a head-to-tail structure [6].

The known compound, piplartine-dimer A (**4**) [7] was readily identified by comparison of its UV, IR, ^{1}H-NMR, $[\alpha]_D$, and MS data.

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Experimental Part

General. TLC: silica gel *60* F_{254} precoated plates (*Merck*). Column chromatography (CC): silica gel *60* (*Merck* 70–230 mesh, 230–400 mesh, ASTM). M.p.: *Yanaco* micro-melting-point apparatus; uncorrected. Optical rotation: *Jasco DIP-370* polarimeter; in $CHCl_3$. UV Spectra: *Jasco UV-240* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Perkin-Elmer 2000* FT-IR spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H-, ^{13}C-, and 2D-NMR Spectra: *Varian Unity-Plus-400* and *Varian Gemini-200* spectrometers; δ in ppm rel. to $SiMe_4$, *J* in Hz. EI-MS: *VG-Biotech Quatro-5022* spectrometer; *m*/*z* (rel. %). FAB-MS and HR-FAB-MS: *Jeol JMX-HX-110* mass spectrometer.

Plant Material. The stem of *Piper arborescens* was collected from Lanyu Island, Taitung County, Taiwan, in July, 2000. A voucher specimen (Chen 6101) was deposited in the Herbarium of the School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.

Extraction and Isolation. Air-dried stem (7.2 kg) of *P. arborescens* was extracted repeatedly with cold MeOH. After evaporation, the extract (320 g) was partitioned between hexane (*Fraction B*, 40 g), $CHCl_3$ (*Fraction A*, 60 g), BuOH (*Fraction C*, 30 g), and H_2O (*Fraction D*, 170 g). The $CHCl_3$ fraction (60 g) was submitted to CC (silica gel, $CHCl_3$ and $CHCl_3$/acetone of increasing polarity): *Fr. A.1–A.18*. *Fr. A.9* (1.89 g) was separated by CC (silica gel, $CHCl_3$ and $CHCl_3$/acetone of increasing polarity): *Fr. A.9.1–A.9.8. Fr. A. 9.5* (115 mg) was submitted to CC (silica gel, CH_2Cl_2 and CH_2Cl_2/acetone of increasing polarity): *Fr. A. 9.5.1–A9.5.6*. *Fr. A.9.5.2* (14 mg) was purified by prep. TLC ($CHCl_3$/acetone 15:1): **1** (2.4 mg) and **2** (2.7 mg). *Fr. A.9.5.5* (75.2 mg) was washed with MeOH to yield **3** (8.8 mg). *Fr. A.10* was submitted to CC (silica gel, CH_2Cl_2/acetone step gradients): *Fr. A.10.1–A.10.7. Fr. A.10.6* (740 mg) was washed with MeOH to afford **4** (129.8 mg).

*1,1′-{[(1α,2α,3β,4β)-2,4-Bis(3,4-dimethoxyphenyl)cyclobutane-1,3-diyl]dicarbonyl}bis[5,6-dihydropyridin-2(1*H*)-one]* (= *Piperarborenine A*; **1**). Colorless needles (MeOH). M.p. 220–222°. $[\alpha]_D^{25} = +5.5$ ($c = 0.055$, $CHCl_3$). UV (MeOH): 280 (sh, 3.92), 240 (sh, 4.34), 212 (4.53). IR (KBr): 1686 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 100 MHz): *Table 2*. EI-MS: 574 (0.04, M^+), 287 (21), 259 (7), 191 (100), 163 (16), 147 (10), 148 (10). FAB-MS: 575 (3, $[M+H]^+$), 287 (33), 191 (49), 107 (18), 73 (14). HR-FAB-MS: 575.2375 ($C_{32}H_{35}N_2O_8^+$; calc. 575.2393).

*1,1′-{[(1α,2α,3β,4β)-2-(3,4-Dimethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)cyclobutane-1,3-diyl]dicarbonyl}bis[5,6-dihydropyridin-2(1*H*)-one]* (= *Piperarborenine B*; **2**). Colorless needles (MeOH). M.p. 158–162°. $[\alpha]_D^{25} = 0$ ($c = 0.11$, $CHCl_3$). UV (MeOH): 280 (sh, 3.67), 240 (sh, 4.31), 214 (4.52). IR (KBr): 1690, 1671 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 100 MHz): *Table 2*. FAB-MS: 604 (6, M^+), 317 (52), 288 (29), 221 (44), 191 (53), 107 (20), 89 (18), 69 (15), 57 (12), 55 (11), 43 (10). HR-FAB-MS: 604.2386 ($C_{33}H_{36}N_2O_8^+$; calc. 604.2421).

*1,1′-{[(1α,2α,3β,4β)-2-(7-Methoxy-1,3-benzodioxol-5-yl)-4-(3,4,5-trimethoxyphenyl)cyclobutane-1,3-diyl]dicarbonyl}bis[5,6-dihydropyridin-2(1*H*)-one]* (= *Piperarboresine*; **3**). Colorless needles (MeOH). M.p. 182–184°. $[\alpha]_D^{25} = 0$ ($c = 0.4$, $CHCl_3$). UV (MeOH): 240 (sh, 4.11), 213 (4.55). IR (KBr): 1691, 1670 (C=O). ^{1}H-NMR

Table 1. *^{1}H-NMR Data* ($CDCl_3$) *of Compounds* **1**–**4**. Chemical shifts δ in ppm rel. to $SiMe_4$, J in Hz. Arbitrary numbering[1]).

	1[a])	**2**[a])	**3**[b])	**4**[b])
H–C(3,3′)	5.77 (br. *d*, *J* = 9.6, 2 H)	5.76 (*m*, 2 H)	5.77 (each *d*, *J* = 9.8, each 1 H)	5.75 (br. *d*, *J* = 9.6, 2 H)
H–C(4,4′)	6.65 (*m*, 2 H)	6.65 (*m*, 2 H)	6.71 (*m*, 2H)	6.66 (*m*, 2 H)
H–C(5,5′)	1.64 (*m*, 2 H) 2.05 (*m*, 2 H)	1.65 (*m*, 2 H) 2.06 (*m*, 2 H)	1.74 (*m*, 2 H) 2.11 (*m*, 2 H)	1.66 (*m*, 2 H) 2.07 (*m*, 2 H)
H–C(6,6′)	3.45 (*m*, 2 H) 3.76 (*m*, 2 H)	3.41 (*m*, 2 H) 3.82 (*m*, 2 H)	3.42 (*m*, 2 H) 3.84 (*m*, 2 H)	3.39 (*m*, 2 H) 3.83 (*m*, 2 H)
H–C(8,8′)	4.89 (*dd*, *J* = 11.2, 8.0, 2 H)	4.89, 4.92 (2*dd*, each *J* = 11.4, 7.4, each 1 H)	4.87 (*m*, 2 H)	4.92 (*dd*, *J* = 11.3, 6.8, 2 H)
H–C(9,9′)	4.75 (*dd*, *J* = 11.2, 8.0, 2 H)	4.76, 4.73 (2*dd*, each *J* = 11.4, 7.4, each 1 H)	4.71 (*m*, 2 H)	4.74 (*dd*, *J* = 11.3, 6.8, 2 H)
H–C(11,11′)	6.82 (*d*, *J* = 2.0, 2 H)	6.82 (*d*, *J* = 2.0, H–C(11)), 6.52 (*s*, H–C(11′))	6.54 (*d*, *J* = 1.4, H–C(11)), 6.50 (*s*, H–C(11′))	6.51 (*s*, 2 H)
H–C(14,14′)	6.77 (*d*, *J* = 8.4, 2 H)	6.77 (*d*, *J* = 8.4, H–C(14))		
H–C(15,15′)	6.89 (*dd*, *J* = 8.4, 2.0, 2 H)	6.88 (*dd*, *J* = 8.4, 2.0, H–C(15)), 6.52 (*s*, H–C(15′))	6.48 (*d*, *J* = 1.4, H–C(15)), 6.50 (*s*, H–C(15′))	6.51 (*s*, 2 H)
MeO	3.84 (*s*, 6 H, MeO–C(12,12′)), 3.86 (*s*, 6 H, MeO–C(13,13′))	3.79 (*s*, MeO–C(13′)), 3.84 (*s*, MeO–C(12)), 3.85 (*s*, MeO–C(12′), MeO–C(14′)), 3.86 (*s*, MeO–C(13))	3.78 (*s*, 3 H), 3.85 (*s*, 9 H)	3.79 (*s*, 6 H, MeO–C(13,13′)), 3.85 (*s*, MeO–C(12,12′), MeO–C(14,14′))
OCH_2O			5.91 (*m*, 2 H)	

[a]) At 400 MHz. [b]) At 200 MHz.

Table 2. *^{13}C-NMR Data* ($CDCl_3$) *of Compounds* **1**–**4**. Chemical shifts δ in ppm rel. to $SiMe_4$. Arbitrary numbering[1]).

	1[a])	**2**[a])	**3**[b])	**4**[b])
C(2,2′)	164.4	164.4	164.4, 164.5	164.4
C(3,3′)	125.6	125.4, 125.5	125.4, 125.6	125.3
C(4,4′)	144.8	145.0	144.9, 145.0	145.1
C(5,5′)	24.1	24.1	24.1, 24.2	24.1
C(6,6′)	40.8	40.7, 40.8	40.8	40.8
C(7,7′)	174.2	174.1	173.9, 174.0	173.9
C(8,8′)	51.6	51.4	51.2, 51.6	51.3
C(9,9′)	41.6	41.6, 42.2	42.1, 42.3	42.2
C(10,10′)	133.3	133.1 (C(10)), 136.6 (C(10′))	133.9 C(10)), 136.7 (C(10′))	136.6
C(11,11′)	112.1	112.0 (C(11)), 105.2 (C(11′))	102.1 (C(11)), 105.2 (C(11′))	105.1
C(12,12′)	147.8	147.8 (C(12)), 152.7 (C(12′))	148.5 (C(12)), 152.8 (C(12′))	152.7
C(13,13′)	148.3	148.3 (C(13)), 136.4 (C(13′))	135.1 (C(13)), 136.2 (C(13′))	136.2
C(14,14′)	111.1	111.0 (C(14)), 152.7 (C(14′))	143.1 (C(14)), 152.8 (C(14′))	152.7
C(15,15′)	120.0	120.0 (C(15)), 105.2 (C(15′))	107.9 (C(15)), 105.1 (C(15′))	105.1
MeO	55.8, 56.0 (*Me*O–C(12,12′), *Me*O–C(13,13′))	55.8, 56.0 (*Me*O–C(12), *Me*O–C(13)), 56.1 (*Me*O–C(12′), *Me*O–C(14′)), 60.7 (*Me*O–C(13′))	56.2 (*Me*O–C(12′), *Me*O–C(14′)), 56.4 (*Me*O–C(14)), 60.7 (*Me*O–C(13′))	56.1 (*Me*O–C(12,12′), *Me*O–C(14,14′)), 60.7 (MeO–C(13,13′))
OCH_2O			101.3	

[a]) At 100 MHz. [b]) At 50 MHz.

($CDCl_3$, 200 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 50 MHz): *Table 2*. EI-MS: 618 (0.7, M^+), 318 (14), 317 (100), 301 (21), 221 (25), 205 (28), 175 (14), 147 (14), 119 (12), 98 (35), 81 (10), 68 (14).

*1,1′-{[(1α,2α,3β,4β)-2,4-Bis(3,4,5-trimethoxyphenyl)cyclobutane-1,3-diyl]dicarbonyl}bis[5,6-dihydropyridin-2(1*H*)-one]* (= *Piplartine-Dimer A*; **4**). Colorless needles (MeOH). M.p. 262–264°. $[\alpha]_D^{25} = 0$ ($c = 1.0$, $CHCl_3$). UV (MeOH): 240 (sh, 4.25), 214 (4.53). IR (KBr): 1693, 1673 (C=O). ^{1}H-NMR ($CDCl_3$, 200 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 50 MHz): *Table 2*. EI-MS: 634 (3, M^+), 318 (24), 317 (100), 221 (48), 190 (11), 177 (10), 98 (23), 97 (13), 95 (12), 85 (10), 84 (13), 83 (16), 71 (16), 69 (21), 67 (11), 57 (32), 56 (13), 55 (30).

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