Studies on the Constituents of *Broussonetia* Species. IV. Two New Pyrrolidinyl Piperidine Alkaloids, Broussonetines I and J, from *Broussonetia kazinoki* SIEB.[1)]

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Two new pyrrolidinyl piperidine alkaloids called broussonetines I and J were isolated from the branches of *Broussonetia kazinoki* SIEB. (Moraceae). Broussonetines I and J were formulated as (2*R*)-2-{(1*S*,2*S*)-1,2-dihydroxy-8-[(2*R*,3*R*,4*R*,5*R*)-5-(2-hydroxymethyl-3,4-dihydroxypyrrolidinyl)]octyl}-1-acetylpiperidine (1) and (2*R*)-2-{(1*S*,2*S*)-1, 2-dihydroxy-8-[(2*R*,3*R*,4*R*,5*R*)-5-(2-hydroxymethyl-3,4-dihydroxy-1-acetylpyrrolidinyl)]octyl}-1-acetylpiperidine (2), respectively, by spectroscopic and chemical methods.

Key words pyrrolidinyl piperidine alkaloid; *Broussonetia kazinoki*; broussonetine I; broussonetine J; Moraceae

Recently we reported the structures of ten pyrrolidine alkaloids, broussonetines A—H and broussonetinines A and B as glycosidase inhibitors from *Broussonetia kazinoki* SIEB. (Moraceae).[1—3)] In our continuing studies, we obtained two new pyrrolidinyl piperidine alkaloids called broussonetines I (**1**) and J (**2**) (Fig. 1) from the same tree. The present study deals with isolation and structural elucidation.

The branches of this tree were extracted with hot water and the alkaloidal constituents were concentrated as described in the Experimental section. Compounds **1** and **2** were isolated by preparative HPLC.

Compound **1** was obtained as a colorless oil, $[\alpha]_D$+2.9° (c=0.26 MeOH), showing a brownish spot on TLC by spraying with ninhydrin reagent followed by heating on a hot plate (ninhydrin reaction). The molecular formula was determined to be $C_{20}H_{38}N_2O_6$ by positive high resolution secondary ion mass spectroscopy (pos. HR-SIMS) (m/z: 403.2811 [M+H]$^+$). The IR spectrum showed a strong hydroxyl band at 3400 cm^{-1} and an amide band at 1608 cm^{-1}.

Compound **2** was obtained as a colorless oil, $[\alpha]_D$ +2.1° (c=0.70 MeOH), showing a purplish spot on TLC by the ninhydrin reaction, and molecular formula was determined to

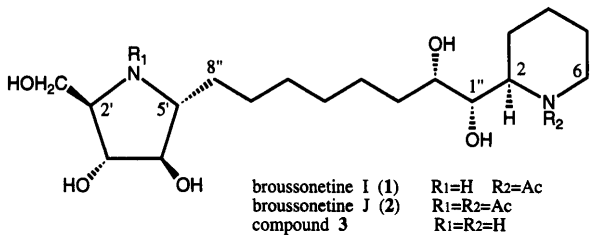

Fig. 1. Structures of Broussonetines I (**1**), J (**2**) and Compound **3**

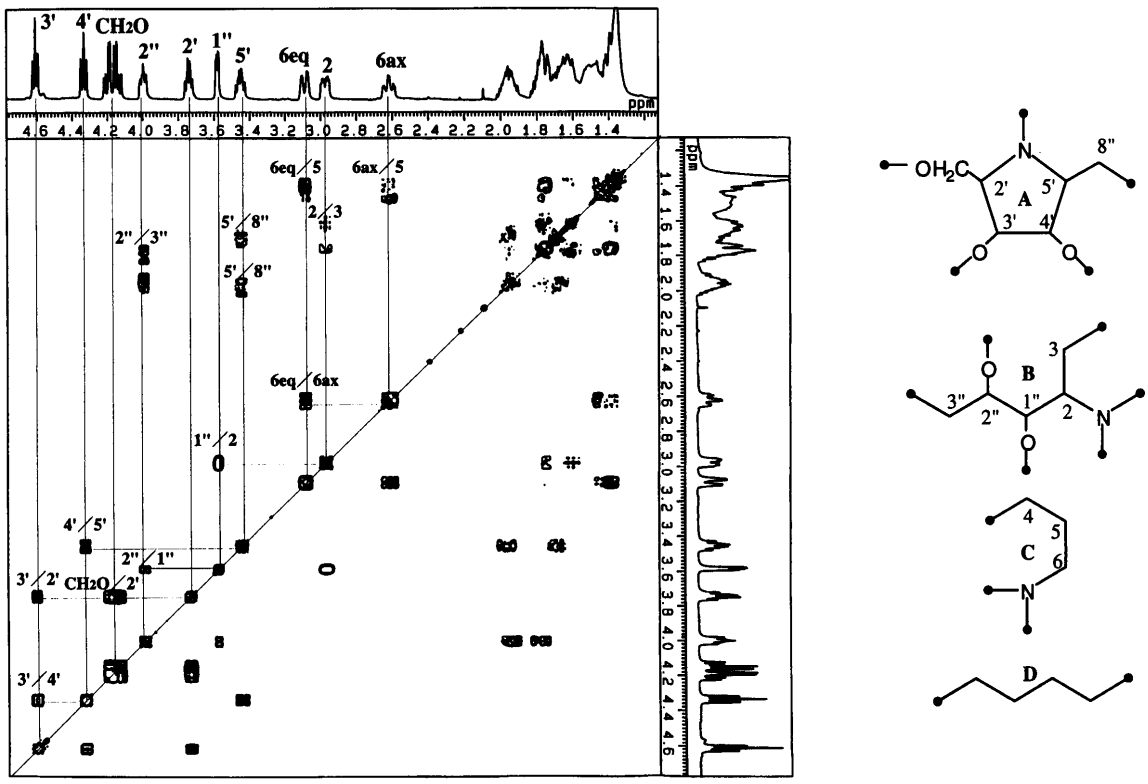

Fig. 2. ^{1}H–^{1}H COSY Spectrum and Partial Structures (**A**—**D**) of **3**

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be $C_{22}H_{40}N_2O_7$ by pos. HR-SIMS (*m/z*: 445.2904 $[M+H]^+$). The IR spectrum showed a strong hydroxyl band at 3400 cm^{-1} and an amide band at 1614 cm^{-1}.

The ^{1}H-NMR spectra of **1** and **2** were too complicated to analyze, suggesting the presence of conformers (rotational isomers of an amide[4] and/or isomers due to nitrogen inversion[5]). Therefore, compounds **1** and **2** were hydrolyzed with 1 N HCl to yield compound **3**, which was proven to be one conformer by NMR spectra.

Compound **3** was obtained as a colorless oil, $[\alpha]_D$ +2.7° (*c*=0.43 MeOH), showing a purplish yellow spot on TLC by the ninhydrin reaction, and the molecular formula was determined to be $C_{18}H_{36}N_2O_5$ by pos. HR-SIMS (*m/z*: 361.2699 $[M+H]^+$). The IR spectrum showed a strong hydroxyl band at 3400 cm^{-1}.

The ^{1}H-NMR spectrum of **3** was similar to those of brousonetines C and D[2] except for their side chains, and suggested the presence of nine methylene groups [δ 1.34—2.10, 18 H, one methylene group attached to a nitrogen atom [δ 2.61, ddd, *J*=11.8, 11.8, 2.5 Hz; δ 3.08, br d, *J*=11.8 Hz], one oxymethylene group [δ 4.15, dd, *J*=10.6, 6.2 Hz; δ 4.22, dd, *J*=10.6, 4.3 Hz], four oxymethine groups [δ 3.58, dd, *J*=5.2, 2.5 Hz; δ 4.00, ddd, *J*=7.5, 5.2, 1.9 Hz; δ 4.35, t, *J*=6.2 Hz; δ 4.63, t, *J*=6.2 Hz] and three methine groups at-

Table 1. ^{1}H-NMR Spectral Data for **1**, **2** and **3**

		3[a]	**1a**[a]	**1b**[a]	**2a**[b]	**2b**[b]
Piperidine moiety	2	2.98 ddd (11.3, 4.4, 2.5)	4.57 br dd (10.0, 4.4)	5.45 m	4.54[c]	5.44m
	3	1.59[c], 1.75[c]	1.68[c], 2.08[c]	1.56[c], 1.86[c]	1.64[c], 2.05[c]	1.56[c], 1.90[c]
	4	1.39[c], 1.48[c]	1.51[c]	1.62[c]	1.50[c]	1.62[c]
	5	1.40[c], 1.78[c]	1.39[c], 1.53[c]	1.39[c], 1.53[c]	1.36[c], 1.55[c]	1.34[c], 1.55[c]
	6-ax	2.61 ddd (11.8, 11.8, 2.5)	2.82 br t (13.1)	3.39[c]	2.87 br t (13.0)	3.42m
	6-eq	3.08 br d (11.8)	4.95 br d (13.1)	3.58 br d (13.1)	4.85 br d (13.0)	3.64 br d (13.0)
	Ac		2.39 s	2.08 s	2.39 s	2.13 s
Pyrrolidine moiety	2′	3.76 ddd (6.2, 6.2, 4.3)	3.73 ddd (6.2, 6.2, 5.0)	3.73 ddd (6.2, 6.2, 5.0)	4.77 br t (5.3)	4.51[c]
	3′	4.63 t (6.2)	4.63 t (6.2)	4.63 t (6.2)	4.91 br s	5.06 br s
	4′	4.35 t (6.2)	4.35 t (6.2)	4.35 t (6.2)	4.61 br s	4.66 br s
	5′	3.46 ddd (6.2, 6.2, 4.3)	3.46 ddd (6.2, 6.2, 5.6)	3.46 ddd (6.2, 6.2, 5.6)	4.07 dd (11.4, 2.1)	4.49[c]
	CH_2OH	4.15 dd (10.6, 6.2)	4.13 dd (10.6, 6.2)	4.13 dd (10.6, 6.2)	4.36 dd (10.7, 3.4)	4.13 dd (10.9, 3.6)
		4.22 dd (10.6, 4.3)	4.19 dd (10.6, 5.0)	4.19 dd (10.6, 5.0)	4.64 t (10.7)	4.44 dd (10.9, 8.2)
	Ac				2.19 s	2.27 s
Octyl moiety	1″	3.58 dd (5.2, 2.5)	4.22 br d (8.8)	4.05 br d (8.8)	4.22 dd (9.8, 1.0)	4.07[c]
	2″	4.00 ddd (7.5, 5.2, 1.9)	3.92 m	3.92[c]	3.92 ddd (5.7, 5.7, 1.0)	3.92
	3″	1.78[c], 1.95[c]	1.89[c], 2.08[c]	1.89[c], 2.08[c]	1.89[c], 2.08[c]	1.89[c], 2.08[c]
	4″	1.65[c]	1.66[c]	1.66[c]	1.56[c]	1.56[c]
	5″	1.36[c]	1.38[c]	1.38[c]	1.22—1.40[c]	1.22—1.40[c]
	6″	1.36[c]	1.38[c]	1.38[c]	1.22—1.40[c]	1.22—1.40[c]
	7″	1.63[c]	1.65[c]	1.65[c]	1.52[c]	1.52[c]
	8″	1.68[c], 1.97[c]	1.68[c], 2.00[c]	1.68[c], 2.00[c]	2.10[c], 2.56[c]	2.10[c], 2.56[c]

a) δ in pyridine-d_5 at 400 MHz. *b*) δ in pyridine-d_5 at 500 MHz. *c*) Overlapped signals.

Table 2. ^{13}C-NMR Spectral Data for **1**, **2** and **3**

		3[a]	**1a**[a]	**1b**[a]	**2a**[b]	**2b**[b]
Piperidine moiety	2	60.58	56.18	50.72	56.14	50.75
	3	29.94	26.53	26.23	26.33	26.05[c]
	4	27.00	20.23	19.92	19.90	19.77
	5	25.08	25.90	26.04	25.67	26.00[c]
	6	46.75	37.13	42.90	37.21	42.84
	Ac		22.58 (CH_3)	22.03 (CH_3)	22.40 (CH_3)	21.96 (CH_3)
			170.79 (CO)	170.40 (CO)	171.75 (CO)	171.25 (CO)
Pyrrolidine moiety						
	2′	65.18	65.26	65.26	69.84	69.98
	3′	80.44	80.61	80.61	79.79	80.12
	4′	84.39	84.55	84.55	79.00	79.14
	5′	62.93	63.02	63.02	69.98	69.12
	CH_2OH	63.84	63.90	63.90	60.75	62.51
	Ac				22.81 (CH_3)	23.05 (CH_3)
					170.70 (CO)	171.23 (CO)
Octyl moiety	1″	75.91	69.29	72.24	69.02	72.22
	2″	73.48	71.22	71.02	70.96	70.92
	3″	35.01	35.55	35.55	35.03	35.03
	4″	26.53	26.75	26.75	26.52[c]	26.52[d]
	5″	30.33[c]	30.27[c]	30.27[c]	29.92[d]	29.92[e]
	6″	30.26[c]	30.25[c]	30.25[c]	29.70[d]	29.70[e]
	7″	27.28	27.27	27.27	26.52[c]	26.52[d]
	8″	35.70	35.78	35.78	33.65	33.65

a) δ in pyridine-d_5 at 100 MHz. *b*) δ in pyridine-d_5 at 125 MHz. *c*—*e*) Assignments may be interchangeable within the same column.

tached to a nitrogen atom [δ 2.98, ddd, J=11.3, 4.4, 2.5 Hz; δ 3.46, ddd, J=6.2, 6.2, 4.3 Hz; δ 3.76, ddd, J=6.2, 6.2, 4.3 Hz].

Partial structures **A**—**D** were obtained by tracing ^{1}H–^{1}H correlated spectroscopy (^{1}H–^{1}H COSY) cross peaks, as shown in Fig. 2, and they were connected on the basis of the heteronuclear multiple bond correlation (HMBC) spectrum to establish the planar structure (Fig. 3).

The ^{1}H- and ^{13}C-NMR signals were reasonably assigned on the structure by total correlation spectroscopy (TOCSY), heteronuclear signal quantum coherence (HSQC), and distortionless enhancement by polarization transfer (DEPT), as shown in Tables 1 and 2.

The relative stereochemistry of the pyrrolidine moiety in **3** was disclosed by the vicinal coupling constants ($J_{2'\text{-}3'}$= $J_{3'\text{-}4'}$=$J_{4'\text{-}5'}$=6.2 Hz) and nuclear Overhauser effects (NOEs) in the nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum, that is, NOEs were observed between H-2′ and H-4′, H-3′ and H-5′ to establish 2β-hydroxymethyl-3α,4β-dihydroxy-5α-alkylpyrrolidine structure. The 1″,2″-diol moiety attached to the piperidine moiety was proven to have a *threo* configuration by the formation of an acetonide derivative (**3a**) which showed an unsplit singlet [δ 1.43, s, 6H] of the geminal dimethyl groups, and a visinal coupling constant ($J_{1''\text{-}2''}$=7.5 Hz) in the ^{1}H-NMR spectrum[6] and NOEs between H-2″ and H-2, and H-3″ and H-1″ in the NOESY spectrum (Chart 1, Fig. 4). Furthermore, the 2, 1″ anti-configuration (Fig. 5) was proven by the cyclic carbamate derivative (**3b**) (Chart 1), which showed visinal coupling constants ($J_{2\text{-}1''}$=6.4 Hz) of H-2 and H-1″ in the ^{1}H-NMR spectrum; NOEs were observed between H-2 and H-6ax, H-2 and H-1″, and H-1″ and H-3 in the NOESY spectrum.

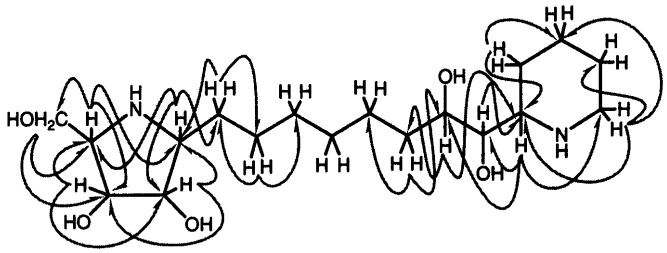
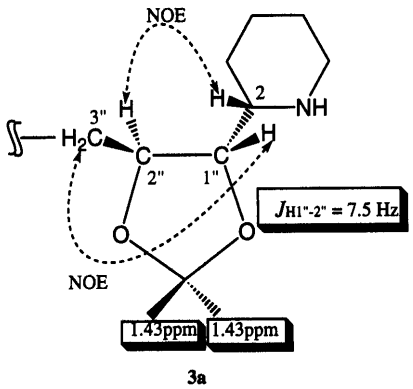


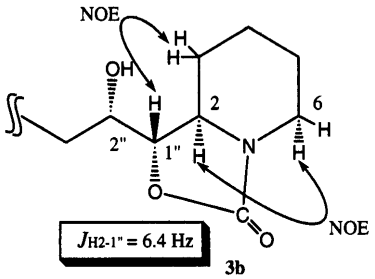
Fig. 3. HMBC of **3**

The absolute stereostructure of the pyrrolidine moiety was determined to be (2′*R*, 3′*R*, 4′*R*, 5′*R*) using a benzoate chirality method as follows. An acetylacetoamide (**3c**) was selectively prepared from **3a** (12 mg) by treatment with acetic anhydride (20 mg) in pyridine at room temperature, and then a dibenzoate (**3d**) was obtained by benzoylation of **3c** and purification of the products in preparative HPLC. The circular dichroism (CD) curve of **3d** showed a negative Cotton effect ($\Delta\varepsilon_{237}$−10.9) and a positive effect ($\Delta\varepsilon_{223}$+12.1) to establish the chiral arrangement in a counter-clockwise manner.[7,8] Moreover, a new version of Mosher's method[9] was applied to determine the absolute configuration of C-2″ in **3**. The ^{1}H-NMR spectra of the (*S*)- and (*R*)-2-methoxy-2-phenyl-2-(trifluoromethyl) acetic acid (MTPA) esters (**3e**, **3f**) prepared from **3b** were assigned and recorded by double quantum filtered correlated spectroscopy (DQF-COSY) (500 MHz) and the $\Delta\delta$ (=δ_S−δ_R) values were measured: these values (Fig. 6) established the (*S*) configuration at C-2″ of **3b**. Thus, compound **3** was formulated as (2*R*)-2-{(1*S*,2*S*)-1,2-dihydroxy-8-[(2*R*,3*R*,4*R*,5*R*)-5-(2-hydroxymethyl-3,4-dihydroxy-pyrrolidinyl)]octyl}piperidine. Compounds **1** and **2** were a mo-



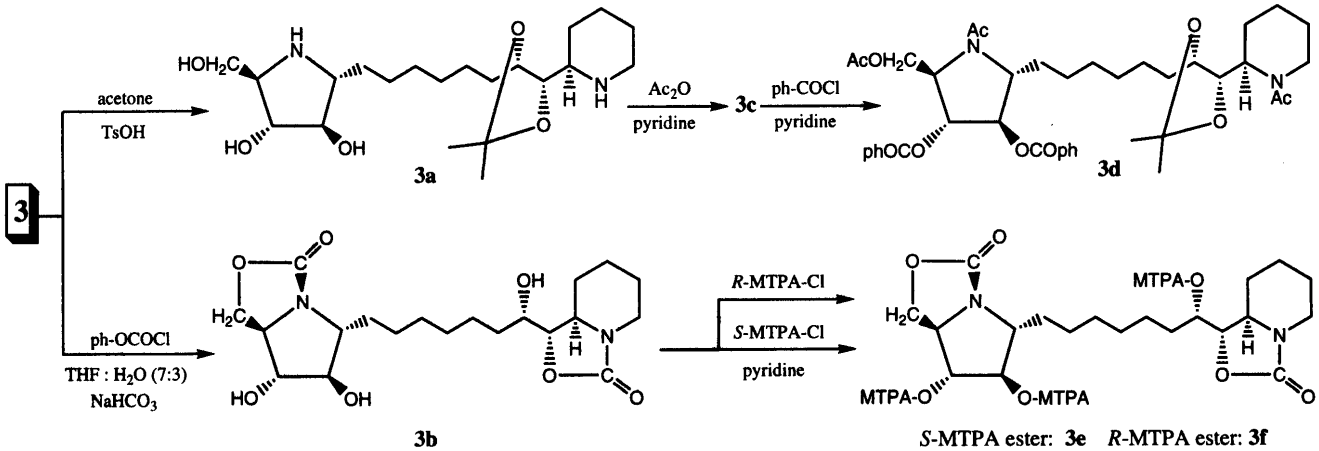
3a

Fig. 4



3b

Fig. 5



3b *S*-MTPA ester: **3e** *R*-MTPA ester: **3f**

Chart 1

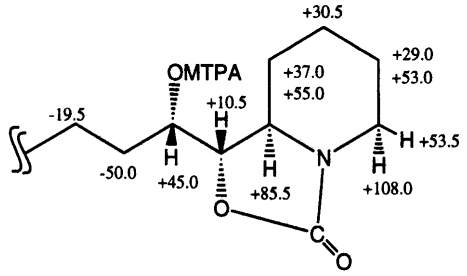


Fig. 6

noacetoamide and a diacetoamide of **3** on the basis of the spectroscopic data, and were formulated as (2*R*)-2-{(1*S*,2*S*)-1,2-dihydroxy-8-[(2*R*,3*R*,4*R*,5*R*)-5-(2-hydroxymethyl-3,4-dihydroxypyrrolidinyl)]octyl}-1-acetylpiperidine and (2*R*)-2-{(1*S*,2*S*-)-1,2-dihydroxy-8-[(2*R*,3*R*,4*R*,5*R*)-5-(2-hydroxymethyl-3,4-dihydroxy-1-acetylpyrrolidinyl)]octyl}-1-acetylpiperidine, respectively. ^{1}H- and ^{13}C- NMR spectra of **1** and **2** showed the presence of conformational isomers (**1a** and **1b**, **2a** and **2b**) in the solution. Moreover, the NOESY spectra of **1** and **2** showed conformational exchange cross peaks.[10,11] The isomerism of the *N*-acetylpiperidine moieties showed a ratio of signals (4 : 1) due to **1a** and **1b** or **2a** and **2b**, while that of the *N*-acetylpyrrolidine moieties showed the ratio (3 : 2) due to **2a** and **2b**.

Experimental

General The instruments used in this work were: Yanagimoto micromelting point apparatus (for melting points, uncorrected); JASCO digital polarimeter (for specific rotation, measured at 25 °C) and JASCO J-20A spectrometer (for CD, measured at 25 °C); Perkin-Elmer 1720X-FTIR spectrometer (for IR spectra); Hitachi M-80 spectrometer (for MS spectra); Varian Mercury-300, unity INOVA-500, JEOL α-400 (for NMR spectra, measured in pyridine-d_5 or $CDCl_3$, on the δ scale using tetramethylsilane as an internal standard).

Column chromatography was carried out on ion exchange resin (Amberlite CG-50/Orugano Company and Dowex 50W-X4/the Dow Chemical Company) and silica gel (Chromatorex DM1020/Fuji Silysia Chemical, Ltd.). HPLC was conducted on a Gilson 305 pump or a JASCO PU 980 equipped with a JASCO 830-RI or 875-UV/VIS as a detector. Silica gel 60 F_{254} (Merck) precoated TLC plates were used, developed with a $CHCl_3$–MeOH–AcOH–H_2O (20 : 10 : 7 : 5) solvent system, and detection was carried out by ninhydrin reagent followed by heating.

Isolation of 1 and 2 Dried branches of *Broussonetia kazinoki* (9.5 kg, collected in Takatsuki City (Osaka) in 1995) were cut finely and then extracted with hot water (40 l×3) for 2 h each. The extracted solution was chromatographed on an Amberlite CG-50 (H^+-form) column (8 l, i.d. 6.5×30 cm, repeated 8 times). After washing the column with water and then 50% MeOH, the adsorbed material was eluted with 50% MeOH–28% ammonia solution (9 : 1). The eluted fraction was concentrated *in vacuo* to give a basic fraction (46.0 g). This fraction was chromatographed on a Dowex 50W-X4 column (200—400 mesh, 500 ml, i.d. 5.0×30 cm) pretreated with formic acid–ammonium formate buffer (0.2 M ammonia formate, adjusted to pH 5.7 with 1 N formic acid), and eluted with gradient elution {H_2O (2.0 l)→H_2O–28% ammonia solution (9 : 1, 2.0 l)}. The fraction containing **1** and **2** was rechromatographed on silica gel (Chromatorex DM1020) using $CHCl_3$ and MeOH, followed by preparative HPLC [column, Asahipak ODP 5E (i.d. 10×250 mm); solvent, CH_3CN–H_2O (12 : 88), adjusted to pH 12.0 with ammonia solution; flow rate, 1.5 ml/min; column temperature, ambient]. **1** (105 mg) and **2** (120 mg) were finally obtained.

Broussonetine I (**1**): A colorless oil; ninhydrin reaction, positive (a brownish spot with *Rf*=0.65 on TLC), $[\alpha]_D$ +2.9° (*c*=0.26, MeOH). $C_{20}H_{38}N_2O_6$. pos. HR-SIMS *m/z*: 403.2811 ($[M+H]^+$): error, 0.6 mmu. IR ν (KBr) cm^{-1}: 3400 (OH, NH), 1608 (NH-CO). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1, 2.

Broussonetine J (**2**): A colorless oil; ninhydrin reaction, positive (a purplish spot with *Rf*=0.83 on TLC), $[\alpha]_D$ +2.1° (*c*=0.70, MeOH). $C_{22}H_{40}N_2O_7$. pos. HR-SIMS *m/z*: 445.2904 ($[M+H]^+$): error: −0.7 mmu. IR ν (KBr) cm^{-1}: 3400 (OH, NH), 1614 (NH-CO). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1, 2.

Hydrolysis of **1** and **2** with 1 N HCl: **1** (20 mg) was dissolved in 1 N HCl (10 ml) and the solution was refluxed on a water bath for 1 h. After cooling, the reaction mixture was passed through an Amberlite IRA-67 (OH^- form) column (i.d. 2.0×10.0 cm) to neutralize it. The hydrolysate was purified by HPLC [column, Asahipak ODP-5E (i.d. 6.0×250 mm); solvent, CH_3CN–H_2O (10 : 90), adjusted to pH 12.0 with ammonia solution; flow rate: 1.0 ml/min; detection, refractive index (RI); column temperature, ambient] to give 15 mg of pure **3**. **3** (23 mg) was also obtained from **2** (30 mg) by the same method. **3**: a colorless oil, ninhydrin reaction: positive (a purplish yellow spot with *Rf*=0.43 on TLC), $[\alpha]_D$ +2.7° (*c*=0.43, MeOH). $C_{18}H_{36}N_2O_5$. pos. HR-SIMS *m/z*: 361.2699 ($[M+H]^+$), error, −0.1 mmu. IR ν (KBr) cm^{-1}: 3400 (OH, NH). ^{1}H- and ^{13}C-NM R(pyridine-d_5): Tables 1, 2.

Acetonide (**3a**): **3** (16 mg) was treated with 1% *p*-toluenesulfonic acid in acetone to provide an acetonide (**3a**). The reaction mixture was purified by HPLC [column, Asahipak ODP-5E (i.d. 6.0×250 mm); solvent, CH_3CN–H_2O (25 : 75), adjusted to pH 12.0 with ammonia solution; flow rate, 1.0 ml/min; detection, RI; column temperature, ambient] to yield 13 mg of pure **3a**. **3a**: a colorless oil. pos. SIMS *m/z*: 401 ($[M+H]^+$, 88%), 84 (base peak). ^{1}H-NMR (pyridine-d_5) δ:1.43 (6H, s, acetonyl dimethyl), 2.56 (1H, ddd, *J*=12.0, 12.0, 2.5 Hz, 6-Hax), 2.60 (1H, ddd, *J*=10.7, 6.6, 2.5 Hz, 2-H), 3.05 (1H, br d, *J*=12.0 Hz, 6-Heq), 3.38 (1H, m, 5′-H), 3.63 (1H, dd, *J*=7.5, 6.6 Hz, 1″-H), 3.66 (1H, m, 2′-H), 4.06 (1H, ddd, *J*=7.5, 7.0, 2.2 Hz, 2″-H), 4.08 (1H, dd, *J*=10.5, 6.2 Hz, CH_2OH), 4.14 (1H, dd, *J*=10.5, 4.1 Hz, CH_2OH), 4.26 (1H, t, *J*=6.2 Hz, 4′-H), 4.53 (1H, t, *J*=6.2 Hz, 3′-H).

Dibenzoate (**3d**): **3a** (12 mg) was treated with acetic anhydride (20 mg) in pyridine at room temperature, and after the usual treatment and HPLC purification [column, Cosmosil C18-AR-II (i.d. 10×250 mm); solvent, CH_3CN–H_2O (20 : 80); flow rate, 1.5 ml/min; detection, UV 220 nm; column temperature, 40 °C], acetylacetoamide (**3c**) was obtained as a colorless oil (10 mg). Acetoamide (**3c**) (10 mg) was dissolved to pyridine (500 μl), benzoylchloride (100 μl) was added, and the solution was stirred at room temperature overnight. The reaction products were subjected to HPLC [column, Cosmosil C18-AR-II (i.d. 10×250 mm); solvent, CH_3CN–H_2O (20 : 80→100 : 0 60 min); flow rate, 1.5 ml/min; detection, UV 254 nm; column temperature, 40 °C]. Dibenzoate (**3d**) was obtained as a colorless oil (12 mg). **3d**: pos. SIMS *m/z*: 757 ($[M+Na]^+$, 11%), 735 ($[M+H]^+$, 27%), 105 (base peak). ^{1}H-NMR ($CDCl_3$) δ: 2.15—2.25 (3H×3, each s, $COCH_3$), 2.75 (1H, ddd, *J*=12.1, 12.1, 2.3 Hz, 6-Hax), 3.98 (1H, br d, *J*=12.1 Hz, 6-Heq), 4.44 (1H, t, *J*=11.0 Hz, CH_2OAc), 4.68 (1H, dd, *J*=11.0, 4.1 Hz, CH_2OAc), 7.40—7.60 (6H, m, phenyl H), 8.00—8.10 (4H, m, phenyl H). CD (*c*=3.0×10^{-5}, MeOH) $\Delta\varepsilon^{25}$: +12.1 (223), −10.9 (237).

Carbamate (**3b**): **3** (20 mg) was treated with phenyl chloroformate (1.0 ml) in THF–H_2O (7 : 3) (10 ml) and $NaHCO_3$ (0.5 g) at 2 °C for 3 h followed by warming to room temperature for 36 h. The reaction products were subjected to HPLC [column, Asahipak ODP-5E (i.d. 6.0×250 mm); solvent, CH_3CN–H_2O (19 : 81), adjusted to pH 12.0 with ammonia solution; flow rate, 1.0 ml/min; detection, RI; column temperature, ambient]. Carbamate (**3b**) was obtained as a colorless oil (13 mg). **3b**: pos. SIMS *m/z*: 435 ($[M+Na]^+$, 19%), 413 ($[M+H]^+$, 66%), 73 (base peak). ^{1}H-NMR (pyridine-d_5) δ: 2.70 (1H, ddd, *J*=12.0, 12.0, 3.0 Hz, 6-Hax), 3.76 (1H, ddd, *J*=10.5, 6.4, 3.7 Hz, 2-H), 3.85 (1H, ddd, *J*=9.2, 3.9, 3.9 Hz, 2″-H), 3.94 (1H, br d, *J*=12.0 Hz, 6-Heq), 4.17 (1H, dd, *J*=6.4, 3.9 Hz, 1″-H), 4.22 (2H, m, 2′, 5′-H), 4.43 (1H, dd, *J*=7.5, 5.5 Hz, 4′-H), 4.50 (2H, m, CH_2O, 3′-H), 4.57 (1H, t, *J*=9.5 Hz, CH_2O).

(*S*)-(−)-MTPA Ester (**3e**): **3b** (6 mg) was treated with (*R*)-(−)-MTPA-Cl (20 μl) in pyridine (300 μl) at room temperature overnight, and then *N*, *N*-dimethyl-1,3-propanediamine was added. The reaction products were subjected to HPLC [column, Cosmosil C18-AR-300 (i.d. 4.6×150 mm); solvent, CH_3CN–H_2O (20 : 80→100 : 0 40 min); flow rate, 1.0 ml/min; detection, UV 230 nm; column temperature, 40 °C]. **3e** was obtained as a colorless oil (2.5 mg). **3e**: pos. SIMS *m/z*: 1061 ($[M+H]^+$, 5.9%), 84 (base peak). ^{1}H-NMR ($CDCl_3$) δ: 1.292*, 1.733* (3-H), 1.330*, 1.895 (4-H), 1.391*, 1.660* (5-H), 1.213* (4″-H), 1.648* (3″-H), 2.684 (1H, ddd, *J*=13.1, 13.1, 3.2 Hz, 6-Hax), 3.247 (1H, m, 2-H), 3.487, 3.521, 3.530 (each 3H, s, O-Me), 3.827 (1H, br d, *J*=13.1 Hz, 6-Heq), 3.978 (1H, m, 2′-H), 4.011 (1H, m, 5′-H), 4.122 (1H, dd, *J*=6.0, 4.7 Hz, 1″-H), 4.240 (1H, dd, *J*=9.5, 4.8 Hz, CH_2O), 4.607 (1H, dd, *J*=9.5, 8.0 Hz, CH_2O), 4.871 (1H, m, 3′-H), 5.190 (1H, t, *J*=3.0 Hz, 4′-H), 5.212 (1H, m, 2″-H), 7.400—7.575 (15H, m, phenyl H). *: overlapped signals.

(*R*)-(+)-MTPA Ester (**3f**): **3b** (6 mg) was treated with (*S*)-(+)-MTPA-Cl (20 μl) in pyridine (300 μl) at room temperature overnight, then *N*,*N*-dimethyl-1,3-propanediamine was added. The reaction products were sub-

jected to HPLC [column, Cosmosil C18-AR-300 (i.d. 4.6×150 mm); solvent, CH_3CN–H_2O (20 : 80→100 : 0 40 min); flow rate, 1.0 ml/min; detection, UV 230 nm; column temperature, 40 °C]. **3f** was obtained as a colorless oil (4.3 mg). **3f**: pos. SIMS *m/z*: 1061 ($[M+H]^+$, 6.0%), 58 (base peak). ^{1}H-NMR ($CDCl_3$) δ: 1.218*, 1.663* (3-H), 1.240*, 1.834 (4-H), 1.333*, 1.554* (5-H), 1.252* (4″-H), 1.748* (3″-H), 2.468 (1H, ddd, J=13.5, 13.5, 3.0 Hz, 6-Hax), 3.076 (1H, m, 2-H), 3.468, 3.515, 3.536 (each 3H, s, O-Me), 3.720 (1H, br d, J=13.5 Hz, 6-Heq), 3.897 (1H, m, 2′-H), 3.955 (1H, m, 5′-H), 4.101 (1H, dd, J=5.3, 3.4 Hz, 1″-H), 4.388 (1H, dd, J=9.6, 4.5 Hz, CH_2O), 4.612 (1H, dd, J=9.6, 8.0 Hz, CH_2O), 5.012 (1H, dd, J=5.0, 3.0 Hz, 3′-H), 5.122 (1H, ddd, J=6.4, 6.4, 3.4 Hz, 2″-H), 5.249 (1H, t, J=3.0 Hz, 4′-H), 7.400—7.575 (15H, m, phenyl H). *: overlapped signals.

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