Studies on the Constituents of *Broussonetia* Species VIII. Four New Pyrrolidine Alkaloids, Broussonetines R, S, T, and V and a New Pyrroline Alkaloid, Broussonetine U, from *Broussonetia kazinoki* SIEB.[1)]

Daisuke TSUKAMOTO, Makio SHIBANO, Rieko OKAMOTO, and Genjiro KUSANO*

Osaka University of Pharmaceutical Sciences, 4–20–1 Nasahara, Takatsuki, Osaka 569–1094, Japan.
Received November 6, 2000; accepted December 26, 2000

Four new pyrrolidine alkaloids, broussonetines R, S, T, and V and a new pyrroline alkaloid, broussonetine U were isolated from the branches of *Broussonetia kazinoki* SIEB. (Moraceae) in low yield. Broussonetines R, S and T were formulated as (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-{(1*R*)-1-hydroxy-3-[6-(4-hydroxybutyl)-cyclohexy-2-on-1(6)-enyl]propyl} pyrrolidine (1), (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(1*R*,10*S*)-1,10,13-trihydroxytridecyl] pyrrolidine (2), (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(1*R*,5*S*)-1,5, 13-trihydroxy-10-oxo-tridecyl] pyrrolidine (3). And broussonetines U and V were proposed to be (2*S*,3*S*,4*S*)-2-hydroxymethyl-3, 4-dihydroxy-5-(9-oxo-13-hydroxytridecyl)-5-pyrroline (4), (2*R*,3*S*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(*E*)-9-oxo-13-hydroxy-3-tridecenyl] pyrrolidine (5), respectively, by spectroscopic and chemical methods.

Key words pyrrolidine alkaloid; pyrroline aklkaloid; *Broussonetia kazinoki*; Moraceae

Recently we reported the structures of seventeen pyrrolidine or pyrrolizidine alkaloids, broussonetines A—H, K, L, M—Q, and broussonetinines A and B as glycosidase inhibitors and two pyrrolidinyl piperidine alkaloids, broussonetines I and J from *Broussonetia kazinoki* SIEB. (Moraceae)[1—7)]. In our continuing studies, we obtained four new pyrrolidine alkaloids, broussonetines R, S, T, and V and a new pyrroline alkaloid, broussonetine U, from the same tree in low yield. The present study deals with the isolation and structural elucidation of these minor constituents.

The branches of this tree were extracted with hot water and the alkaloidal fractions were concentrated as previously reported[1)]. Compounds **1**—**5** were isolated by preparative HPLC of the concentrated alkaloids.

Compound **1** was obtained as a colorless oil, $[\alpha]_D$ +21.8° (MeOH, c=0.27), and was named broussonetine R, showing a brownish spot on TLC when sprayed with ninhydrin reagent followed by heating on a hot plate (ninhydrin reaction). The molecular formula was determined as $C_{18}H_{31}NO_6$ on the basis of positive high resolution secondary ion mass spectroscopy (pos. HR-SI-MS) (*m/z*: 358.2224 $[M+H]^+$, error, −0.4 mmu). The IR spectrum showed a strong OH and NH band at 3392 cm^{-1} and a carbonyl band at 1646 cm^{-1}. The UV spectrum showed an absorption maximum at 247 nm (log ε 3.98) derived from an α,β-unsaturated ketone.

The ^{1}H-NMR spectrum of **1** was similar to those of broussonetines E and F[6)] in the pyrrolidine moiety, and suggested the presence of four methylene groups [δ 1.62—2.20 (8H, m)], two oxymethylene groups [δ 4.23 (1H, dd, J=11.0, 4.1 Hz), δ 4.19 (1H, dd, J=11.0, 5.7 Hz), δ 3.86 (2H, t, J=6.2 Hz)], three oxymethine groups [δ 4.95 (1H, t, J=6.4 Hz), δ 4.70 (1H, t, J=6.4 Hz), δ 4.20 (1H, m)], a methylene group attached to a carbonyl group [δ 2.34 (2H, t, J=6.5 Hz)], three methylene groups attached to a C–C double bond [δ 2.94 (1H, m), δ 2.73 (1H, m), δ 2.35 (2H, m), δ 2.16 (2H, m)] and two methine groups attached to a nitrogen atom [δ 3.79 (1H, m), δ 3.67 (1H, t, J=6.4 Hz)].

Partial structures A, B, and C were obtained by tracing ^{1}H–^{1}H correlated spectroscopy (^{1}H–^{1}H COSY) cross peaks and they were connected on the basis of the heteronuclear multiple band correlation (HMBC) spectrum to establish the planar structure (Fig. 2).

The ^{1}H-NMR and ^{13}C-NMR signals were reasonably assigned to 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.

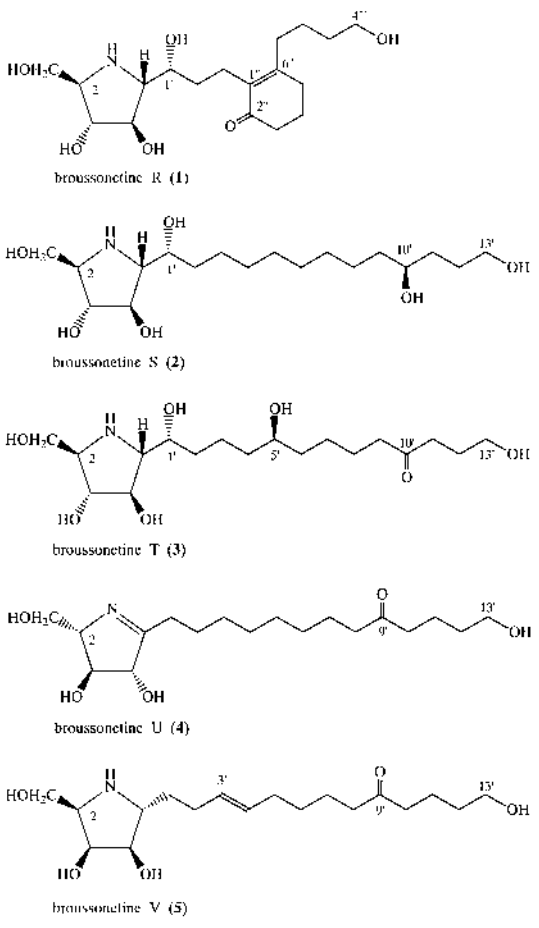

Fig. 1. Structures **1**—**5**

* To whom correspondence should be addressed. e-mail: kusano@oups.ac.jp

The relative stereostructure of the pyrrolidine moiety in **1** was disclosed by the the vicinal coupling constants ($J_{2,3}$= $J_{3,4}$=$J_{4,5}$=6.4 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, and H-3 and H-5 to establish the 2β-hydroxymethyl-3α,4β-dihydroxy-5α-alkylpyrrolidine structure.

The absolute stereostructure of the pyrrolidine moiety and C-1′ in **1** was determined as follows.[8] A cyclic carbamate (**1a**) was prepared from **1** by reaction with phenyl chlorocarbonate in tetrahydrofuran (THF) : H_2O (7 : 3). The ^{1}H-NMR signals of the tetra (*S*)- and (*R*)-2-methoxy-2-phenyl-2-(trifluoromethyl) acetic acid (MTPA) esters (**1b***S*, **1b***R*) prepared from **1a** were assigned by analyzing the ^{1}H–^{1}H COSY (500 MHz) spectra, and the $\Delta\delta$ ($=\delta_S-\delta_R$) values were similar to those in tetra-MTPA esters (**6b***S*, **6b***R*) prepared from broussonetine F[3] (Fig. 3).

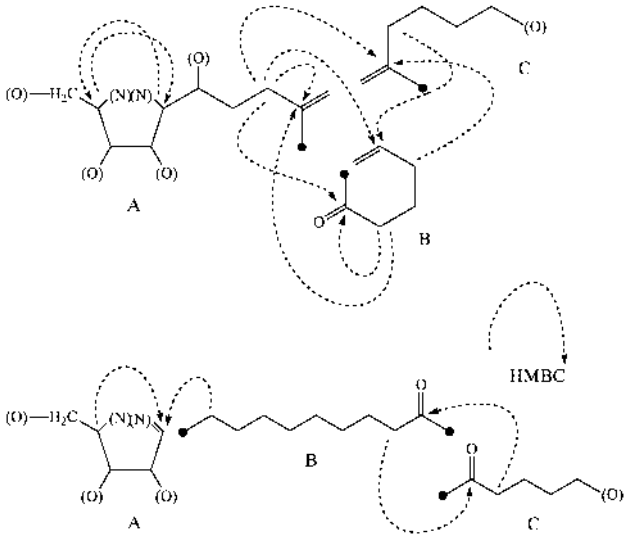

Fig. 2. Partial Structures and HMBC of **2** and **4**

Thus, the absolute stereostructure of **1** was formulated as (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-{(1*R*)-1-hydroxy-3-[6-(4-hydroxybutyl)-cyclohexy-2-on-1(6)-enyl] propyl} pyrrolidine (=2-[(3*R*)-3-{(2*R*,3*R*,4*R*,5*R*)-3,4-dihydroxy-5-hydroxymethyl-pyrrolidin-2-yl}-3-hydroxypropyl]-3-(4-hydroxybutyl)-cyclohex-2-enone).

Compound **2** was obtained as a colorless powder, $[\alpha]_D$ +25.1° (MeOH, *c*=0.18), and was named broussonetine S, showing a brownish spot on TLC by ninhydrin reaction, and the molecular formula was determined as $C_{18}H_{37}NO_6$ on the basis of pos. HR-SI-MS (*m/z*: 364.2692 $[M+H]^+$, error, −0.5 mmu). The IR spectrum showed a strong OH and NH band at 3392 cm^{-1}. The ^{1}H-NMR spectrum was strikingly similar to that of broussonetine E,[6] except for an additional oxymethine signal and the disappearance of 2 signals due to 2 methylene groups flanked a carbonyl group. These signals were assigned as in **1** and summarized in Tables 1 and 2. The $\Delta\delta$ values in the penta-MTPA esters (**2b***S*, **2b***R*) prepared from a cyclic carbamate (**2a**) established the (*S*)- configuration at C-10′. And the $\Delta\delta$ values of the pyrrolidine moiety, and H-1′ and H-2′ in **2b***S* and **2b***R* coincided with those in **6b***S* and **6b***R* (Fig. 3).

Thus, the absolute stereostructure of **2** was formulated as (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(1*R*,10*S*)-1,10,13-trihydroxytridecyl] pyrrolidine.

Compound **3** was obtained as a colorless oil, $[\alpha]_D$ +11.0° (MeOH, *c*=0.49), and was named broussnetine T, showing a yellowish spot on TLC with ninhydrin reaction, and the molecular formula was determined as $C_{18}H_{35}NO_7$ on the basis of pos. HR-SI-MS (*m/z*: 378.2485 $[M+H]^+$, error, +0.3 mmu). The IR spectrum showed a strong OH and NH band at 3316

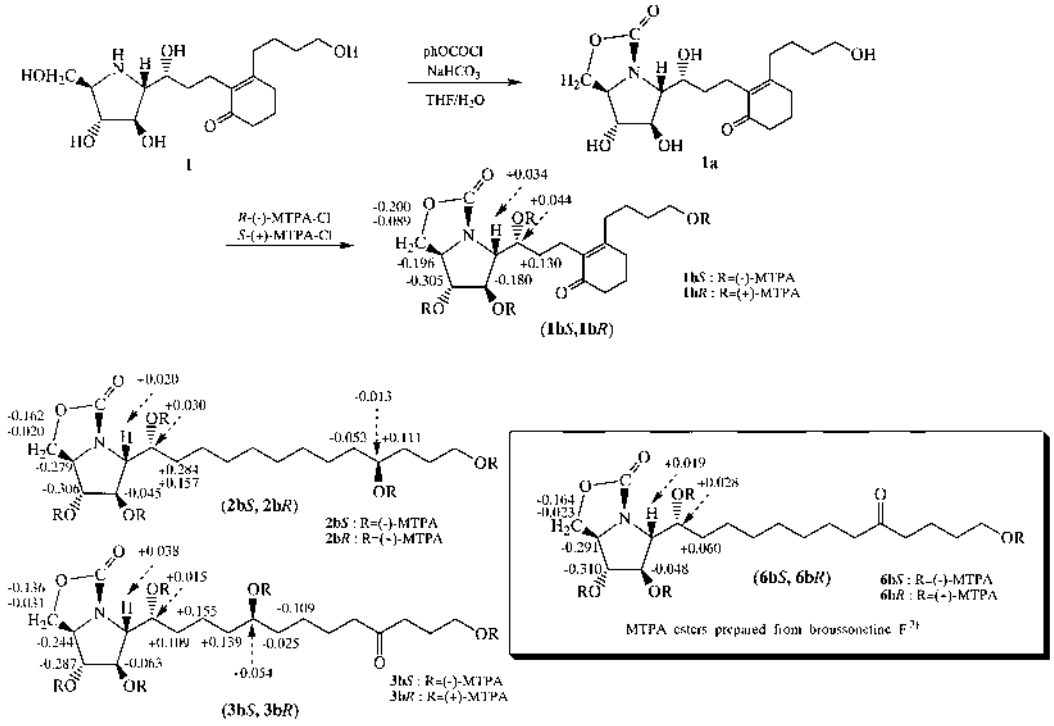

Fig. 3. $\Delta\delta$ Values Obtained for the MTPA Esters of **1**—**3**, and **6**

Table 1. ^{1}H-NMR Spectral Data for **1**—**5** (500 MHz, Pyridine-d_5)

	1		**2**	**3**	**4**	**5**
2	3.79 m	2	3.80 m	3.79[a)]	4.47 m	3.72 m
3	4.70 t (6.4)	3	4.70 t (6.4)	4.71 t (6.2)	5.01 t (3.4)	4.58 t (4.8)
4	4.95 t (6.4)	4	4.94 t (6.4)	4.95 t (6.2)	5.21 d (3.4)	4.09 m
5	3.67 t (6.4)	5	3.65 t (6.4)	3.66 t (6.2)		3.55 m
1′	4.20[a)]	1′	4.14 m	4.18[a)]	2.71 m, 2.91 m	1.69[a)] m, 1.98[a)] m
2′	2.07 m, 2.16[a)] m	2′	1.95[a)] m	2.05[a)]	1.74[a)]	2.29[a)] m, 2.37[a)] m
3′	2.73 m, 2.94 m	3′	1.55[a)] m, 1.76[a)] m	1.98[a)]	1.30 m	5.54 m
1″		4′		1.68[a)]		5.46 m
2″		5′	1.19[a)]—1.36[a)]	3.80[a)]	1.08[a)]—1.19[a)]	1.95[a)] dd (14.0, 7.3)
3″	2.34[a)] t (6.5)	6′		1.57[a)]		1.30 q (7.3)
4″	1.70[a)]	7′		1.48[a)], 1.63[a)]	1.51 m	1.59 q (7.3)
5″	2.16[a)] m	8′	1.47[a)] m, 1.60[a)] m	1.65[a)]	2.34 t (7.3)	2.35 t (7.3)
6″		9′	1.60[a)] m, 1.69[a)] m	2.42 t (7.1)		
1‴	2.35[a)] m	10′	3.89 m		2.47 t (7.3)	2.46 t (7.3)
2‴	1.70[a)]	11′	1.85 m	2.64 t (7.3)	1.86 m	1.86 m
3‴	1.70[a)]	12′	2.01[a)] m, 2.12 m	2.05[a)]	1.74[a)]	1.73[a)] m
4‴	3.86 t (6.2)	13′	3.95 t (6.5)	3.85 t (6.4)	3.85 t (6.4)	3.84 t (6.4)
CH_2OH	4.19 [a)] (11.0, 5.7)	CH_2OH	4.20 dd (11.0, 5.7)	4.18[a)] dd (11.0, 5.5)	4.22 dd (11.8, 3.2)	4.26 dd (10.8, 5.9)
	4.23[a)] (11.0, 4.1)		4.24 dd (11.0, 4.1)	4.23 dd (11.0, 4.1)	4.83 dd (11.8, 3.2)	4.32 dd (10.8, 5.9)

a) Overlapped signals. ppm (Hz)

cm^{-1} and a carbonyl band at 1704 cm^{-1}. The ^{1}H-NMR spectrum was strikingly similar to that of broussonetine E[6], except for an additional oxymethine signal. These signals were assigned as in **1** and summarized in Tables 1 and 2. The $\Delta\delta$ values in the penta-MTPA esters (**3b***S*, **3b***R*) prepared from **3** established (*S*)-configuration at C-5′ (Fig. 2). And the $\Delta\delta$ values of the pyrrolidine moiety, and H-1′ and H-2′ in **3b***S* and **3b***R* coincided with those in **6b***S* and **6b***R*.

Thus, the absolute stereostructure of **3** was formulated as (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(1*R*,5*S*)-1,5,13-trihydroxy-10-oxo-tridecyl] pyrrolidine.

Compound **4** was obtained as a colorless powder, $[\alpha]_D$ −33.3° (MeOH, c=0.20), and was named broussonetine U. The molecular formula was determined as $C_{18}H_{33}NO_5$ on the basis of pos. HR-SI-MS (*m/z*: 344.2438 $[M+H]^+$, error, +0.4 mmu). The IR spectrum showed a strong OH and NH band at 3338 cm^{-1} and a carbonyl band at 1704 cm^{-1}. The ^{1}H-NMR spectrum of **4** was similar to those of broussonetine D[7], except for the pyrrolidine moiety. Partial structures A, B and C were obtained by ^{1}H–^{1}H COSY cross peaks and they were connected on the basis of HMBC spectrum (Fig. 2). The ^{1}H-NMR and ^{13}C-NMR signals were assigned as in **1** and summarized in Tables 1 and 2.

The relative stereostructure of the pyrroline moiety in **4** was disclosed by the vicinal coupling constants ($J_{2,3}$=$J_{3,4}$= 3.4 Hz) and NOEs in the NOESY spectrum, that is NOEs were observed between H-2 and H-4 to establish 2α-hydroxymethyl-3β,4α-dihydroxy-5-alkyl 5-pyrroline.

The absolute stereostructure of the pyrroline moiety was deduced to be the (2*S*,3*S*,4*S*) configuration, by comparison of the value of $[\alpha]_D$ −33.3° with those of (2*R*,3*R*,4*R*)-3,4-dihydroxy-2-(hydroxymethyl)-2H-pyrrole (+21.8°),[9] (2*R*,3*R*,4*R*)-3,4-dihydroxy-2-(hydroxymethyl)-pyrrolidine (+7.8°)[10] and (2*S*,3*S*,4*S*)-1,4-dideoxy-1,4-imino-L-arabinitol (−34.6°).[11]

Thus, **4** was proposed to be (2*S*,3*S*,4*S*)-2-hydroxymethyl-3,4-dihydroxy-5-(9-oxo-13-hydroxytridecyl)-5-pyrroline.

Compound **5** was obtained as a colorless powder, $[\alpha]_D$ +10.9° (MeOH, c=0.09), and was named broussonetine V, showing a brownish spot on TLC by ninhydrin reaction, and

Table 2. ^{13}C-NMR Spectral Data for **1**—**5** (125 MHz, Pyridine-d_5)

	1		**2**	**3**	**4**	**5**
2	63.88	2	65.05	65.54	80.85	61.75
3	78.67	3	79.85	80.21	74.83	73.84
4	78.37	4	79.48	79.95	79.72	79.55
5	65.47	5	66.78	67.23	149.89	61.75
1′	72.28	1′	73.24	73.18	25.24	35.70
2′	33.28	2′	34.47	34.99	25.17	30.65
3′	21.01	3′	26.39	23.11	29.94	131.01
1″	158.69	4′		38.50		130.22
2″	197.80	5′	29.85, 29.85	70.69	29.47, 29.39	32.68
3″	36.84	6′	29.69, 29.69	38.07	29.34	29.37
4″	21.34	7′		25.96	24.04	23.64
5″	29.07	8′	26.03	24.43	42.66	42.53
6″	134.57	9′	38.00	42.94	211.01	217.54
1‴	33.31	10′	70.70	210.83	42.56	42.58
2‴	23.34	11′	34.65	39.48	20.89	20.96
3‴	31.88	12′	29.80	27.77	32.91	33.05
4‴	60.08	13′	62.10	61.22	61.62	61.64
CH_2OH	61.57	CH_2OH	62.78	63.10	58.50	62.47

ppm

the molecular formula was determined as $C_{18}H_{33}NO_5$ on the basis of pos. HR-SI-MS (*m/z*: 344.2737 $[M+H]^+$, error, +0.2 mmu). The IR spectrum showed a strong OH and NH band at 3379 cm^{-1} and a carbonyl band at 1708 cm^{-1}. The ^{1}H-NMR spectrum was strikingly similar to that of broussonetinine B[6], except for two additional olefin proton signals [δ 5.54 (1H, m), δ 5.46 (1H, m)]. These signals were assinged as in **1** and summarized in Tables 1 and 2. The vicinal coupling constant ($J_{3',4'}$=15.0 Hz), which was confirmed by decoupling experiment on the methylene signal [δ 1.95, (2H, dd) on H-5′] in **5**, established the (*E*) conformation of this olefin. The $[\alpha]_D$ of **5** (+10.9°) was similar to that of broussonetinine B (+15.3°)[6] and the relative stereostructures of the pyrrolidine moiety were the same.

Thus, the absolute stereostructure of **5** was proposed to be (2*R*,3*S*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(*E*) 9-oxo-13-hydroxy-3-tridecenyl] pyrrolidine.

Experimental

General The instruments used in this work were: a JASCO digital polarimeter (for specific rotation, measured at 25°C); a Perkin-Elmer 1720X-FTIR spectrometer (for IR spectra); a Hitachi M-80 spectrometer (for MS spectra); a Varian Mercury 300, unity Inova-500 (for NMR spectra, measured in pyridine-d_5, on the δ scale using tetramethylsilane as an internal standard); a Shimadzu spectrophotometer UV 1200 (for UV spectra).

Column chromatography was carried out on ion exchange resin (Amberlite CG-50, Amberlite IRA-67/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 UV-970 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—5 Dried branches of *Broussonetia kazinoki* (7.5 kg, collected in Takatsuki City (Osaka) in 1998) were treated as described in the experimental section of the previous paper.[1] The fraction containing **1**—**5** was rechromatographed on silica gel (Chromatorex DM1020) using $CHCl_3$ and MeOH, followed by preparative HPLC [column : Asahipak ODP 10E (i.d. 10×250 nm); solvent: CH_3CN–H_2O (7 : 93) in **1** and **3**, and (15 : 85) in **2**, **4** and **5**, adjusted to pH 12.0 with ammonia solution; flow rate : 1.5 ml/min; detection, RI; column temperature: ambient]. **1** (5 mg), **2** (6 mg), **3** (6 mg), **4** (2 mg), and **5** (2 mg) were finally obtained.

Broussonetine R (**1**): Colorless oil, ninhydrin reaction: positive (a brown spot on TLC), t_R 42 min, $[\alpha]_D$ +21.8° (MeOH c=0.27), $C_{18}H_{31}NO_6$, pos. HR-SI-MS *m/z*: 358.2224 ([M+H]+) error: −0.4 mmu, IR ν (KBr) cm^{-1}: 3392 (OH, NH), 1646 (CO), UV (MeOH) λ_{max} nm (log ε): 247 (3.98), ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1, 2.

Broussonetine S (**2**): Colorless powder, ninhydrin reaction: positive (a brown spot on TLC), t_R 20 min, $[\alpha]_D$ +25.1° (MeOH c=0.18), $C_{18}H_{37}NO_6$, pos. HR-SI-MS *m/z*: 364.2692 ($[M+H]^+$) error: −0.5 mmu, IR ν (KBr) cm^{-1}: 3392 (OH, NH) ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1, 2.

Broussonetine T (**3**): Colorless oil, ninhydrin reaction: positive (a yellow spot on TLC), t_R 20 min, $[\alpha]_D$ +11.0° (MeOH c=0.49), $C_{18}H_{35}NO_7$, pos. HR-SI-MS *m/z*: 378.2485 ($[M+H]^+$) error: −0.5 mmu, IR ν (KBr) cm^{-1}: 3316 (OH, NH), 1704 (CO), ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1, 2.

Broussonetine U (**4**): Colorless oil, t_R 27 min, $[\alpha]_D$ −33.3° (MeOH c=0.20), $C_{18}H_{33}NO_5$, pos. HR-SI-MS *m/z*: 344.2438 ($[M+H]^+$) error: +0.3 mmu, IR ν (KBr) cm^{-1}: 3343 (OH, NH), ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1, 2.

Broussonetine V (**5**): Colorless powder, ninhydrin reaction: positive (a brown spot on TLC), t_R 27 min, $[\alpha]_D$ +10.9° (MeOH c=0.09), $C_{18}H_{33}NO_5$, pos. HR-SI-MS *m/z*: 344.2437 ($[M+H]^+$) error: +0.2 mmu, IR ν (KBr) cm^{-1}: 3379 (OH, NH), 1708 (CO), ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1, 2.

Carbamate (1a) **1** (5 mg) was treated with phenyl chloroformate (1.5 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 (5 : 95→30 : 70, 40 min), adjusted to pH 12.0 with ammonia solution; flow rate, 1.0 ml/min; detection, UV (247 nm); column temperature, ambient]. Carbamate (**1a**) was obtained as a colorless oil (3 mg).

(*S*)-(−)-MTPA Ester (1b*S*) **1a** (1.5 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]. **1b***S* was obtained as a colorless oil (1.5 mg), $C_{59}H_{57}NO_{15}F_{12}$, pos. SIMS *m/z*: 1247 $(M+H)^+$, 189 (base peak); ^{1}H-NMR ($CDCl_3$) δ: 1.400*—2.407* (CH_2), 1.547* (2′-H), 2.376* (3′-H), 3.109 (1H, m, 2-H), 3.320, 3.490, 3.534, 3.714 (each 3H, s, OCH_3), 4.088* (1H, 5-H), 4.116* (1H, C$\underline{H}_2$O), 4.202 (1H, dd, J=9.8, 4.6 Hz, C$\underline{H}_2$O), 4.721 (1H, m, 3-H), 5.218 (1H, m, 1′-H), 5.548 (1H, t, J=4.8 Hz, 4-H), 7.250*—7.540* (20H, m, MTPA-ArH). *: Overlapped signals.

(*R*)-(+)-MTPA Ester (1b*S*) **1a** (1.5 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]. **1b***R* was obtained as a colorless oil (1.5 mg), $C_{59}H_{57}F_{12}NO_{15}$, pos. SIMS *m/z*: 1247 $(M+H)^+$, 189 (base peak); ^{1}H-NMR ($CDCl_3$) δ: 1.368*—2.371* (CH_2), 1.420* (2′-H), 2.148* (3′-H), 3.300 (1H, m, 2-H), 3.359, 3.408, 3.474, 3.548 (each 3H, s, OCH_3), 4.060* (1H, t, J=4.3 Hz, 5-H), 4.225 (1H, m, C$\underline{H}_2$O), 4.328* (1H, C$\underline{H}_2$O), 5.027 (1H, m, 3-H), 5.173 (1H, m, 1′-H), 5.729 (1H, t, J=4.3 Hz, 4-H), 7.250*—7.540* (20H, m, MTPA-ArH). *: Overlapped signals.

Carbamate (2a) **2** (5 mg) was treated with phenyl chloroformate(1.5 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 (15 : 85), adjusted to pH 12.0 with ammonia solution; flow rate, 1.0 ml/min; detection, RI; column temperature, ambient]. Carbamate (**1a**) was obtained as a colorless oil (3 mg).

(*S*)-(−)-MTPA Ester (2b*S*) **2a** (1.5 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]. **2b***S* was obtained as a colorless oil (1.5 mg), $C_{69}H_{70}F_{15}NO_{17}$, pos. SIMS *m/z*: 1470 $(M+H)^+$, 189 (base peak); ^{1}H-NMR ($CDCl_3$) δ: 1.12*—1.65* (CH_2), 1.524* (1H, 2′-H), 1.549* (1H, 12′-H), 1.599* (2H, 9′-H), 1.600* (1H, 12′-H), 1.647* (2H, 11′-H), 1.647* (1H, 2′-H), 2.873 (1H, m, 2-H), 3.346 (3H, s, OCH_3), 3.482*—3.538* (9H, OCH_3), 3.616 (3H, s, OCH_3), 3.980 (1H, m, C$\underline{H}_2$O), 4.030 (1H, t, J=4.3 Hz, 5-H), 4.216 (2H, m, 13′-H), 4.259 (1H, dd, J=9.8, 4.3 Hz, C$\underline{H}_2$O), 4.764 (1H, m, 3-H), 5.057 (1H, m, 10′-H), 5.221 (1H, m, 1′-H), 5.757 (1H, t, J=5.3 Hz, 4-H), 7.310*—7.530* (25H, m, MTPA-ArH). *: Overlapped signals.

(*R*)-(+)-MTPA Ester (2b*S*) **2a** (1.5 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]. **2b***R* was obtained as a colorless oil (1.5 mg), $C_{69}H_{70}F_{15}NO_{17}$, pos. SIMS *m/z*: 1470 $(M+H)^+$, 189 (base peak); ^{1}H-NMR ($CDCl_3$) δ: 1.08*—1.79* (CH_2), 1.240* (1H, 2′-H), 1.490* (1H, 2′-H), 1.536* (2H, 11′-H), 1.549* (1H, 12′-H), 1.652* (2H, 9′-H), 1.681* (1H, 12′-H), 3.152 (1H, m, 2-H), 3.325 (3H, s, OCH_3), 3.46*—3.55* (12H, OCH_3), 4.010 (1H, t, J=4.8 Hz, 5-H), 4.142* (2H, 13′-H), 4.142* (1H, C$\underline{H}_2$O), 4.279* (1H, C$\underline{H}_2$O), 5.070* (1H, 3-H), 5.070* (1H, 10′-H), 5.191 (1H, m, 1′-H), 5.802 (1H, t, J=4.8 Hz, 4-H), 7.310*—7.530* (25H, m, MTPA-ArH). *: Overlapped signals.

Carbamate (3a) **3** (9 mg) was treated with phenyl chloroformate(1.5 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 (15 : 85), adjusted to pH 12.0 with ammonia solution; flow rate, 1.0 ml/min; detection, RI; column temperature, ambient]. Carbamate (**3a**) was obtained as a colorless oil (4 mg).

(*S*)-(−)-MTPA Ester (3b*S*) **3a** (2 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]. **3b***S* was obtained as a colorless oil (1.5 mg), $C_{69}H_{68}F_{15}NO_{18}$, pos. SIMS *m/z*: 1484 $(M+H)^+$, 189 (base peak); ^{1}H-NMR ($CDCl_3$) δ: 1.09*—1.75* (CH_2), 1.134* (2H, 7′-H), 1.377* (2H, 3′-H), 1.516* (2H, 6′-H), 1.536* (2H, 2′-H), 1.602* (2H, 4′-H), 1.952* (2H, q, J=6.7 Hz, 12′-H), 2.276 (2H, m, 9′-H), 2.392 (2H, m, 11′-H), 2.948 (1H, m, 2-H), 3.330*—3.600* (15H, OCH_3), 3.999* (1H, 5-H), 4.040* (1H, C$\underline{H}_2$O), 4.264* (1H, C$\underline{H}_2$O), 4.320* (2H, 13′-H), 4.774 (1H, t, J=5.3 Hz, 3-H), 4.986 (1H, m, 5′-H), 5.146 (1H, m, 1′-H), 5.678 (1H, t, J=5.3 Hz, 4-H), 7.300*—7.580* (25H, m, MTPA-ArH). *: Overlapped signals.

(*R*)-(+)-MTPA Ester (3b*S*) **3a** (2 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]. **3b***R* was obtained as a colorless oil (1.5 mg), $C_{69}H_{68}F_{15}NO_{18}$, pos. SIMS *m/z*: 1484 $(M+1H)^+$, 189 (base peak); ^{1}H-NMR ($CDCl_3$) δ: 1.07*—1.60* (CH_2), 1.222* (2H, 3′-H), 1.243* (2H, 7′-H), 1.427* (2H, 2′-H), 1.463* (2H, 4′-H), 1.541* (2H, 6′-H), 1.962 (2H, m, 12′-H), 2.280 (2H, m, 9′-H), 2.398 (2H, m, 11′-H), 3.192 (1H, m, 2-H), 3.300*—3.550* (15H, OCH_3), 3.961 (1H, m, 5-H), 4.176 (1H, m, C$\underline{H}_2$O), 4.295* (1H, C$\underline{H}_2$O), 4.325* (2H, 13′-H), 4.932* (1H, m, 5′-H), 5.061 (1H, dd, J=5.7, 8.0 Hz, 3-H), 5.131 (1H, m, 1′-H), 5.741 (1H, m, 4-

H), 7.300*—7.540* (25H, m, MTPA-ArH). *: Overlapped signals.

References

1) Shibano M., Tsukamoto D., Fujimoto R., Masui Y., Sugimoto H., Kusano G., *Chem. Pharm. Bull*., **48**, 1281—1285 (2000).
2) Shibano M., Tsukamoto D., Kusano G., *Chem. Pharm. Bull*., **47**, 907—908 (1999).
3) Shibano M., Nakamura S., Motoya N., Kusano G., *Chem. Pharm. Bull*., **47**, 472—476 (1999).
4) Shibano M., Nakamura S., Kubori M., Minoura K., Kusano G., *Chem. Pharm. Bull*., **46**, 1416—1420 (1998).
5) Shibano M., Nakamura S., Akazawa N., Kusano G., *Chem. Pharm. Bull*., **46**, 1048—1050 (1998).
6) Shibano M., Kitagawa S., Nakamura S., Akazawa N., Kusano G., *Chem. Pharm. Bull*., **45**, 700—705 (1997).
7) Shibano M., Kitagawa S., Nakamura S., Kusano G., *Chem. Pharm. Bull*., **45**, 505—508 (1997).
8) Ohtani I., Kusumi T., Kashman Y., Kakisawa H., *J. Am. Chem. Soc*., **113**, 4092—4096 (1991).
9) Kim J. Y., Takatsuki A., Kogoshi N., Kitahara T., *Tetrahedron*, **55**, 8353—8364 (1999).
10) George W. J. F., Paul W. S., *Tetrahedron*, **42**, 5685—5692 (1986).
11) Jone D. W. C., Nashi R. J., Bell E. A., Williams J. M., *Tetrahedron Lett*., **26**, 3125—3126 (1985).