Studies on the Constituents of *Broussonetia* Species X. Six New Alkaloids from *Broussonetia kazinoki* Sieb.

Daisuke Tsukamoto, Makio Shibano, and Genjiro Kusano*

Osaka University of Pharmaceutical Sciences, 4–20–1 Nasahara, Takatsuki, Osaka 569–1094, Japan.
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Six new alkaloids, broussonetines W, X, M_1, U_1, J_1, and J_2 (1—6) were isolated from the branches of *Broussonetia kazinoki* Sieb. (Moraceae) as minor constituents. They were formulated as (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[7-(cyclohexy-2-on-1(6)-enyl)heptyl]pyrrolidine (1), (2*R*,3*S*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[7-(cyclohexy-2-on-1(6)-enyl)heptyl]pyrrolidine-4-*O*-β-D-glucopyranoside (2), (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(9*R*)-9,13-dihydroxytridecyl]pyrrolidine (3), (2*S*,3*S*,4*S*)-2-hydroxymethyl-3,4-dihydroxy-5-(10-oxo-13-hydroxytridecyl)-5-pyrroline (4), (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}piperidine (5), (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}piperidine (6).

Key words pyrrolidine alkaloid; pyrroline alkaloid; pyrrolidinyl piperidine alkaloid; broussonetine; *Broussonetia kazinoki*; Moraceae

Previously we reported the structures of broussonetines A—V and broussonetinines A and B from *Broussonetia kazinoki* Sieb. (Moraceae).[1—8] In our continuing studies, we obtained six new alkaloids, broussonetines W, X, M_1, U_1, J_1, and J_2 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] Six alkaloids, (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[7-(cyclohexy-2-on-1(6)-enyl)heptyl]pyrrolidine (**1**), (2*R*,3*S*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[7-(cyclohexy-2-on-1(6)-enyl)heptyl]pyrrolidine-4-*O*-β-D-glucopyranoside (**2**), (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(9*R*)-9,13-dihydroxytridecyl]pyrrolidine (**3**), (2*S*,3*S*,4*S*)-2-hydroxymethyl-3,4-dihydroxy-5-(10-oxo-13-hydroxytridecyl)-5-pyrroline (**4**), (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}piperidine (**5**), (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}piperidine (**6**) were isolated by preparative HPLC of the concentrated alkaloids. The structures were elucidated mainly on the basis of spectroscopic evidence, as shown in Fig. 1.

Compound **1**, broussonetine W, was obtained as a colorless oil, $[\alpha]_D$ +16.0° (c=0.07, MeOH), showing a brownish spot on TLC by ninhydrin reaction as previously.[1] The molecular formula was determined to be $C_{18}H_{31}NO_4$ by positive high-resolution secondary ion mass spectrometry (positive HR-SI-MS) (m/z: 326.2340 $[M+H]^+$, error, +1.1 mmu). The IR spectrum showed a strong OH and NH band at 3436 cm^{-1} and a carbonyl band at 1652 cm^{-1}. The UV spectrum showed an absorption maximum at 236 nm ($\log \varepsilon$ 3.59) due to an α,β-unsaturated ketone.

The ^{1}H-NMR spectrum of **1** was similar to those of broussonetines C and D,[8] and suggested the presence of 7 methylene groups [δ 1.15—2.10 (14H, m)], an oxymethylene group [δ 4.17 (1H, dd, J=11.0, 5.9 Hz), δ 4.23 (1H, dd, J=11.0, 4.1 Hz)], a methylene group attached to a carbonyl group [δ 2.38 (2H, t, J=6.7 Hz)], 2 methylene groups attached to a C=C bond [δ 2.15 (2H, m), δ 2.24 (2H, m)], 2 oxymethine groups [δ 4.38 (1H, t, J=6.4 Hz), δ 4.66 (1H, t, J=6.4 Hz)] and 2 methine groups attached to a nitrogen atom [δ 3.49 (1H, m), δ 3.79 (1H, m)].

Partial structures A1, B1, and C1 were obtained by tracing ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) cross peaks and they were connected by the heteronuclear multiple-bond correlation (HMBC) spectrum to establish the planar structure (Fig. 2).

The ^{1}H- and ^{13}C-NMR signals were reasonably assigned to the structure by total correlation spectroscopy (TOCSY), het-

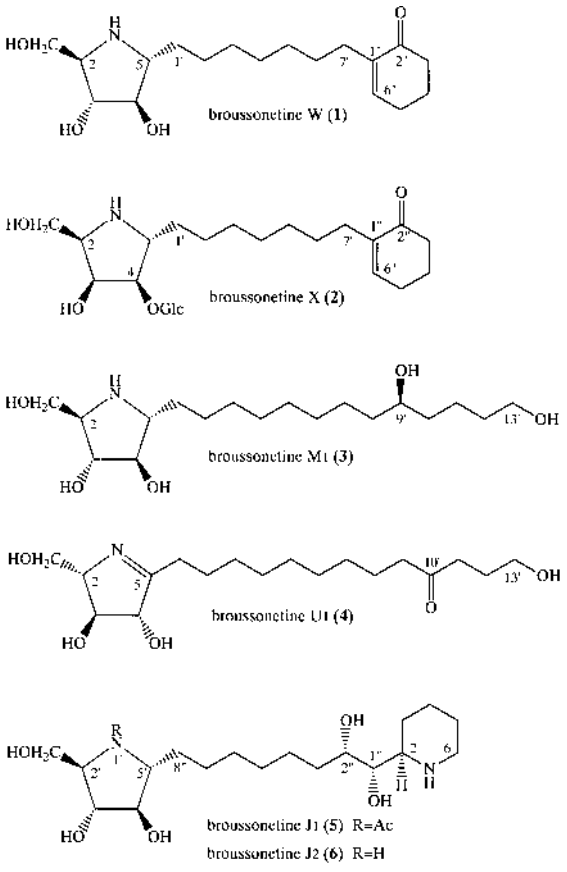

Fig. 1. Structures of **1**—**6**

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

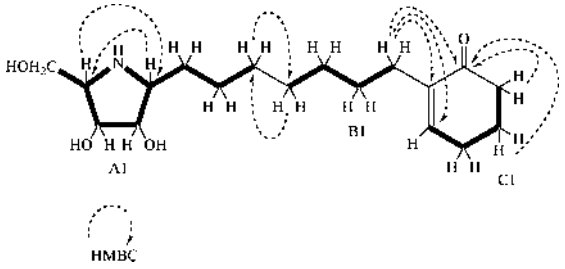

Fig. 2. Partial Structures and HMBC of **1**

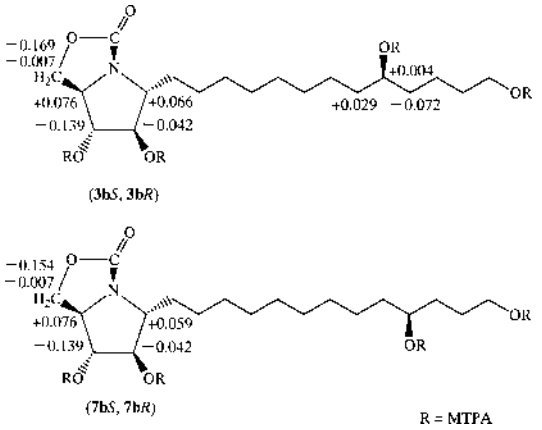

Fig. 3. $\Delta\delta$ Values Obtained for the MTPA Esters (**3b***S*, **3b***R*, **7b***S*, **7b***R*)

eronuclear single quantum coherence (HSQC), and distortionless enhancement by polarization transfer (DEPT), as shown in Tables 1 and 2.

The relative stereostructure of the pyrrolidine moiety in **1** was disclosed by the vicinal coupling constants ($J_{2,3}$=$J_{3,4}$=$J_{4,5}$=6.4 Hz) and nuclear Overhauser effects (NOEs) between H-2 and H-4, and H-3 and H-5 in the nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum to establish the 2β-hydroxymethyl-3α,4β-dihydroxy-5α-alkylpyrrolidine structure.

The absolute stereostructure of the pyrrolidine moiety was concluded to be (2*R*,3*R*,4*R*,5*R*), by comparison of the $[\alpha]_D$ value (+16.0°) of **1** with those of broussonetines C (+25.0°) and D (+22.9°).[8] Thus, **1** was formulated as (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[7-(cyclohexy-2-on-1(6)-enyl)heptyl] pyrrolidine.

Compound **2**, broussonetine X, was obtained as a colorless oil, $[\alpha]_D$ +13.7° (c=0.51, MeOH), showing a brownish spot on TLC by ninhydrin reaction. The molecular formula was determined to be $C_{24}H_{41}NO_9$ by positive HR-SI-MS (m/z: 488.2856 $[M+H]^+$, error, −0.1 mmu). The IR spectrum showed a strong OH and NH band at 3415 cm^{-1} and a carbonyl band at 1670 cm^{-1}. The UV spectrum showed an absorption maximum at 236 nm (log ε 3.96) due to an α,β-unsaturated ketone.

The ^{1}H-NMR spectrum of **2** showed an anomeric proton [δ 4.98 (1H, d, J=7.8 Hz)]. Hydrolysis of **2** with 1 N HCl provided a genuine aglycone (**2a**) and D-glucose ($[\alpha]_D$ +58.6°). The ^{1}H- and ^{13}C-NMR spectra of **2** were strikingly similar to those of broussonetines A and B,[7] especially those of pyrrolidine moiety (2*R*-hydroxymethyl-3*S*,4*R*-dihydroxy-5*R*-alkylpyrrolidine structure) and 4-*O*-β-D-glucopyranosyl group, and those of the side chain in **1** as summarized in Tables 1 and 2. The glucosyl moiety (4-*O*-β-D-glucopyranosyl group) was established by the vicinal coupling constants ($J_{1'',2''}$= 7.8 Hz) and HMBC (between H-4 and the anomeric carbon, and the anomeric proton and C-4).

The absolute stereostructure of the pyrrolidine moiety was concluded to be (2*R*,3*S*,4*R*,5*R*) by comparison of the $[\alpha]_D$ value (+30.7°) with those of broussonetines A (+32.1°) and B (+29.8°).[7] Thus, **2** was formulated as (2*R*,3*S*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[7-(cyclohexy-2-on-1(6)-enyl)heptyl]pyrrolidine-4-*O*-β-D-glucopyranoside.

Compound **3**, broussonetine M_1, was obtained as a colorless powder, $[\alpha]_D$ +18.3° (c=0.56, MeOH), showing a yellowish spot on TLC by ninhydrin reaction. The molecular formula was determined to be $C_{18}H_{37}NO_5$ by positive HR-SI-MS (m/z: 348.2749 $[M+H]^+$, error, +0.1 mmu). The IR spectrum showed a strong OH and NH band at 3370 cm^{-1}.

The ^{1}H-NMR spectrum of **3** was similar to that of broussonetine M.[2] The ^{1}H–^{1}H COSY spectrum of **3** suggested the presence of 9-hydroxytridecyl in the side chain, because $(CH_2)_3$ signals were confirmed between CH(OH) and CH_2OH signals in **3**, while $(CH_2)_2$ signals were confirmed between them in compared broussonetine M (10-hydroxytridecyl moiety) as shown in Tables 1 and 2. The absolute stereostructure of the pyrrolidine moiety and C-9′ of **3** was determined by a modification of Mosher's method.[9] A cyclic carbamate (**3a**) was prepared from **3** 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 (**3b***S*, **3b***R*) prepared from **3a** were assigned by analyzing the ^{1}H–^{1}H COSY (500 MHz) spectrum, and the $\Delta\delta$ ($=\delta_S-\delta_R$) values established the (*R*) configuration at C-9′ (Fig. 3). In addition, the $\Delta\delta$ values of the pyrrolidine moiety in MTPA esters (**3b***S*, **3b***R*) coincided with those in the tetra-MTPA esters (**7b***S*, **7b***R*) prepared from broussonetine M (**7**)[2] (Fig. 3), establishing the same absolute stereostructure of **3** as that of **7**. Thus, the absolute stereostructure of **3** was determined to be (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(9*R*)-9,13-dihydroxytridecyl]pyrrolidine.

Compound **4**, broussonetine U_1, was obtained as a colorless powder, $[\alpha]_D$ −30.2° (c=0.09, MeOH). The molecular formula was determined to be $C_{18}H_{33}NO_5$ by positive HR-SI-MS (m/z: 344.2426 $[M+H]^+$, error, −0.9 mmu). The IR spectrum showed a strong OH and NH band at 3431 cm^{-1} and a carbonyl band at 1706 cm^{-1}.

The ^{1}H-NMR spectrum of **4** was strikingly similar to that of broussonetine U,[1] except for 2 methylene signals attached to a carbonyl group. The ^{1}H–^{1}H COSY spectrum of **4** showed the presence of 10-oxo in the side chain, because $(CH_2)_2$ signals flanked with CO and CH_2OH groups were confirmed in **4**, while $(CH_2)_3$ signals flanked with them were confirmed in broussonetine U (9-oxo moiety). These signals were assigned as summarized in Tables 1 and 2.

The configuration of the asymmetric carbons of **4** was proposed as (2*S*, 3*S*, 4*S*) by comparison of the $[\alpha]_D$ value (−30.2°) with those of broussonetine U (−33.3°),[1] 2*R*-hydroxymethyl-3*R*,4*R*-dihydroxy-2H-pyrrole (+21.8°),[10] 2*R*-hydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (+7.8°),[11] and (2*S*,3*S*,4*S*)-1,4-dideoxy-1,4-imino-L-arabinitol (−34.6°).[12] Thus, **4** was formulated as (2*S*,3*S*,4*S*)-2-hydroxymethyl-3,4-

Table 1. ^{1}H-NMR Spectral Data for **1**—**6** (500 MHz, Pyridine-d_5)

	1	**2**		**3**	**4**		**5a**	**5b**
2	3.79 m	3.70 m	2	3.77 m	4.44 m	2	2.94 ddd (11.0, 4.9, 2.4)	2.94 ddd (11.0, 4.9, 2.4)
3	4.66 t (6.4)	4.74 t (4.1)	3	4.65 t (6.4)	5.00 t (3.4)	3	1.59[a], 1.73[a]	1.59[a], 1.73[a]
4	4.38 t (6.4)	4.05 dd (8.0, 4.1)	4	4.38 t (6.4)	5.20 m	4	1.37[a], 1.76[a]	1.37[a], 1.76[a]
5	3.49 m	3.64 m	5	3.48 m		5	1.42[a]	1.42[a]
1′	1.72[a] m, 2.00	1.52[a], 1.90 m	1′	1.71[a], 2.01[a]	2.71[a] m, 2.91 m	6ax	2.61 ddd (11.9, 11.9, 2.8)	2.61 ddd (11.9, 11.9, 2.8)
2′	1.50 m, 1.60 m	1.36[a], 1.56[a]	2′	1.50[a], 1.62[a]	1.76 m	6eq	3.03 br d (12.3)	3.03 br d (12.3)
3′	1.28[a]		3′		1.32 m	2′	4.78 br t (4.3)	4.50[a]
4′		1.10[a]—1.23[a]	4′			3′	4.90 br s	5.08 br s
5′	1.20[a]—1.25[a]		5′	1.14[a]—1.70[a]	1.08[a]—1.21[a]	4′	4.59 br s	4.65 br s
6′	1.39 m	1.38[a]	6′			5′	4.08 dd (11.4, 2.5)	4.52[a]
7′	2.24 m	2.24 m	7′			CH_2OH	4.38 dd (10.7, 3.3)	4.17 dd (9.0, 1.6)
1″			8′	1.64[a]	1.56 quintet (7.3)		4.72 dd (10.7, 5.4)	4.48[a] t (8.9)
2″			9′	3.86 m	2.39 t (7.3)	Ac	2.16 s	2.24 s
3″	2.38 t (6.7)	2.38 t (6.6)	10′	1.73[a]		1″	3.56 dd (4.9, 2.0)	3.56 dd (4.9, 2.0)
4″	1.77[a]	1.77 quintet (6.2)	11′	1.77[a], 1.93[a]	2.67 t (7.3)	2″	3.99 ddd (8.1, 5.2, 2.2)	3.99 ddd (8.1, 5.2, 2.2)
5″	2.15 m	2.15 m	12′	1.85[a]	2.07 quintet (7.3)	3″	1.75[a], 1.94 m	1.75[a], 1.94 m
6″	6.58 t (4.1)	6.58 t (4.2)	13′	3.92 t (6.4)	3.87 t (6.3)	4″	1.48[a], 1.62[a]	1.48[a], 1.62[a]
CH_2OH	4.17 dd (11.0, 5.9)	4.21[a]	CH_2OH	4.17 dd (11.0, 5.9)	4.21 dd (11.7, 3.4)	5″		
	4.23 dd (11.0, 4.1)	4.31[a]		4.24 dd (11.0, 5.9)	4.82 dd (11.7 3.0)	6″	1.24[a]—1.41[a]	1.24[a]—1.41[a]
1‴		4.98 d (7.8)				7″	1.42[a]	1.42[a]
2‴		4.01[a]				8″	1.70[a], 2.46[a]	1.70[a], 2.46[a]
3‴		4.24[a]						
4‴		4.20[a]						
5‴		3.90[a]						
6‴		4.33[a]						
		4.51 dd (11.7, 2.5)						

a) Overlapped signals. ppm (Hz)

Table 2. ^{13}C-NMR Spectral Data for **1**—**6**

	1[a]	**2**[a]		**3**[a]	**4**[a]		**5a**[a]	**5b**[a]	**6**[b]
2	64.97	61.60	2	65.00	80.99	2	60.39	60.39	60.61
3	80.07	73.44	3	80.22	74.96	3	29.75	29.75	30.15
4	84.06	89.24	4	84.22	79.80	4	25.05	25.05	27.27
5	62.74	59.95	5	62.77	150.00	5	26.95	26.95	25.34
1′	35.43	35.57	1′	35.61	25.30	6	46.62	46.62	46.85
2′	27.23	27.62	2′	27.29	25.24	2′	70.13	70.49	65.10
3′	30.12		3′		30.01	3′	80.08	80.48	80.35
4′		30.12, 29.80,	4′	30.23, 30.19,		4′	79.23	79.23	84.35
5′	29.74, 29.70	29.73	5′	30.00, 29.95	29.62, 29.55	5′	70.13	69.34	62.85
6′	28.98	28.99	6′	26.36	29.53, 29.41	CH_2OH	61.10	62.72	63.81
7′	29.96	29.98	7′			Ac	170.63	169.94	
1″	139.79	139.81	8′	38.40	24.15	1″	75.79	75.79	75.94
2″	198.81	198.79	9′	70.89	42.82	2″	73.30	73.30	73.55
3″	38.81	38.82	10′	38.28	210.77	3″	34.80	34.80	35.07
4″	23.43	23.44	11′	23.03	39.52	4″	26.34	26.34	26.67
5″	26.09	26.10	12′	33.91	27.83	5″			
6″	145.09	145.07	13′	62.11	61.30	6″	30.04, 29.83	30.04, 29.83	30.47, 30.40
CH_2OH	63.45	62.40	CH_2OH	63.63	58.70	7″	27.01	27.01	27.46
1‴		105.54				8″	33.79	33.79	35.84
2‴		74.76							
3‴		78.16							
4‴		71.34							
5‴		78.46							
6‴		62.40							

a) δ in pyridine-d_5. ^{13}C-NMR at 125 MHz. *b*) δ in pyridine-d_5. ^{13}C-NMR at 75 MHz. ppm

dihydroxy-5-(10-oxo-13-hydroxytridecyl)-5-pyrroline.

Compound **5**, broussonetine J_1, was obtained as a colorless oil, $[\alpha]_D$ −17.5°(c=0.30, MeOH), showing a purplish spot on TLC by ninhydrin reaction. The molecular formula was determined to be $C_{20}H_{38}N_2O_6$ by positive HR-SI-MS (m/z: 403.2802 $[M+H]^+$, error, −0.3 mmu). The IR spectrum showed a strong OH and NH band at 3387 cm^{-1} and an amide band at 1615 cm^{-1}.

Compound **6**, broussonetine J_2, was obtained as a colorless oil, $[\alpha]_D$ +13.8° (c=0.42, MeOH), showing a purplish-yellow spot on TLC by ninhydrin reaction. The molecular formula was determined to be $C_{18}H_{36}N_2O_5$ by positive SI-MS (m/z: 361 $[M+H]^+$). The IR spectrum showed a strong OH and NH band at 3392 cm^{-1}.

The ^{1}H-NMR spectrum of **5** was complicated for the rotational isomers (**5a**, **5b**) due to an amide bond. Then, **5** was hydrolyzed with 1 N HCl to yield **6**. The ^{1}H- and ^{13}C-NMR spectra of **6** were identical to those of the hydrolyzed compound of broussonetines I and J.[5] Spectroscopic data including NOEs showed that **5** should be the *N*-acetylpyrrolidine of **6**, mainly because of the complicated signals of the pyrrolidine moiety. These signals of **5** and **6** were assigned as summarized in Tables 1 and 2.

Thus, **5** and **6** 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-dihydroxy-1-acetylpyrrolidinyl)]octyl}piperidine 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-dihydroxypyrrolidinyl)]octyl}piperidine, respectively. This was, to our knowledge, the first isolation of **6** from the plant, although its acetamide (broussonetine I) and acetodiamide (broussonetine J) were isolated previously and the free base (**6**) was obtained as a hydrolyzed compound.

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); and a Shimadzu spectrophotometer UV 1200 (for UV spectra).

Column chromatography was carried out on ion-exchange resin (Amberlite CG-50, Amberlite IRA-67, Organo Company, and Dowex 50W-X4, 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 spraying with ninhydrin reagent followed by heating.

Isolation of 1—6 Dried branches of *B. kazinoki* (7.5 kg, collected in Takatsuki ward, Osaka, in 1998) were treated as described in the experimental section of a previous paper.[1] Compounds **5** and **6** were obtained from the fraction, which was eluted with MeOH–28% ammonia solution (1 : 1) on a Dowex 50W-X4 column pretreated with formic acid–ammonium formate buffer (0.2 M ammonia formate, adjusted to pH 5.7 with 1 N formic acid) and had not been examined previously. The fraction containing **1**—**6** was rechromatographed on silica gel (Chromatorex DM1020) using $CHCl_3$ and MeOH, followed by preparative HPLC [column: Asahipak ODP 50 10E (i.d. 10×250 mm); solvent: CH_3CN–H_2O (15 : 85) in **4** and **6**, (18 : 82) in **3**, (20 : 80) in **5**, and (25 : 75) in **1** and **2**, adjusted to pH 12.0 with ammonia solution; flow rate: 1.5 ml/min; detection: refractive index (RI); column temperature: ambient]. **1** (5 mg), **2** (5 mg), **3** (10 mg), **4** (1 mg), **5** (15 mg), and **6** (10 mg) were finally obtained.

Broussonetine W (**1**): Colorless oil, ninhydrin reaction: positive (a brown spot on TLC), t_R 22 min, $[\alpha]_D$ +16.0° (c=0.07, MeOH), $C_{18}H_{31}NO_4$, positive HR-SI-MS m/z: 326.2340 ($[M+H]^+$), error: +1.1 mmu, IR ν (KBr) cm^{-1}: 3436 (OH, NH), 1652 (CO), UV (MeOH) λ_{max} nm (log ε): 236 (3.59), ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

Broussonetine X (**2**): Colorless oil, ninhydrin reaction: positive (a brown spot on TLC), t_R 13 min, $[\alpha]_D$ +13.7° (c=0.51, MeOH), $C_{24}H_{41}NO_9$, positive HR-SI-MS m/z: 488.2856 ($[M+H]^+$), error: −0.1 mmu, IR ν (KBr) cm^{-1}: 3415 (OH, NH), 1670 (CO), UV (MeOH) λ_{max} nm (log ε): 236 (3.96), ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

Broussonetine M_1 (**3**): Colorless powder, ninhydrin reaction: positive (a yellow spot on TLC), t_R 20 min, $[\alpha]_D$ +18.3° (c=0.56, MeOH), $C_{18}H_{37}NO_5$, positive HR-SI-MS m/z: 348.2749 ($[M+H]^+$), error: +0.1 mmu, IR ν (KBr) cm^{-1}: 3370 (OH, NH), ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

Broussonetine U_1 (**4**): Colorless powder, t_R 30 min, $[\alpha]_D$ −30.2° (c=0.09, MeOH), $C_{18}H_{33}NO_5$, positive HR-SI-MS m/z: 344.2426 ($[M+H]^+$), error: −0.9 mmu, IR ν (KBr) cm^{-1}: 3431 (OH, NH), 1706 (CO), ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

Broussonetine J_1 (**5**): Colorless oil, ninhydrin reaction: positive (a purple spot on TLC), t_R 12 min, $[\alpha]_D$ −17.5° (c=0.30, MeOH), $C_{20}H_{38}N_2O_6$, positive HR-SI-MS m/z: 403.2802 ($[M+H]^+$), error: −0.3 mmu, IR ν (KBr) cm^{-1}: 3387 (OH, NH), 1615 (CONH), ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

Broussonetine J_2 (**6**): Colorless oil, ninhydrin reaction: positive (a purplish-yellow spot on TLC), t_R 12 min, $[\alpha]_D$ +13.8° (c=0.42, MeOH), $C_{18}H_{36}N_2O_5$, positive SI-MS m/z: 361($[M+H]^+$), IR ν (KBr) cm^{-1}: 3392 (OH, NH), ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1 and 2.

Hydrolysis of 2 with 1 N HCl Compound **2** (3.5 mg) was dissolved in 1 N HCl (4 ml) and the solution was refluxed on a water bath for 2 h. After cooling, the reaction mixture was passed through an Amberlite IRA-67 (OH^- form) column (i.d. 2.0×5.0 cm) to neutralize it. The resulting solution was chromatogaphed on a Sep-Pak C-18 column (Waters), and elution with water afforded D-glucose (0.8 mg), $[\alpha]_D$ +58.6° (c=0.08, H_2O), which was identified by HPLC (t_R=14.5 min) [column, CAPCELL PAK NH_2 (i.d. 4.6×250 mm); solvent, CH_3CN–H_2O (80 : 20); flow rate, 1.0 ml/min; detection, RI; column temperature, 30 °C], TLC (Rf=0.34, AcOEt : AcOH : MeOH : H_2O=6 : 1.5 : 1.5 : 1), ^{1}H- and ^{13}C-NMR. Elution with MeOH afforded an aglycone (1.7 mg) as a colorless powder.

Carbamate (3a) Compound **3** (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 ODP50 6E (i.d. 6.0×250 mm); solvent: CH_3CN–H_2O (25 : 85), adjusted to pH 12.0 with ammonia solution; flow rate, 0.8 ml/min; detection: RI; column temperature: ambient]. Carbamate (**3a**) was obtained as a colorless oil (3 mg).

(*S*)-(−)-MTPA Ester (3b*S*) Compound **3a** (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]. **3b***S* was obtained as a colorless oil (1.0 mg), $C_{59}H_{63}F_{12}NO_{14}$, positive SI-MS m/z: 1260 $(M+Na)^+$, 319 (base peak); ^{1}H-NMR ($CDCl_3$) δ: 1.17*—1.69* (CH_2), 1.193* (2H, 11′-H), 1.381* (1H, 1′-H), 1.520* (1H, 1′-H), 1.546* (2H, 8′-H), 1.546* (2H, 10′-H), 1.608* (2H, 12′-H), 3.410*—3.550* (12H, OCH_3), 3.961 (1H, quintet, J=4.3 Hz, 2-H), 4.026 (1H, m, 5-H), 4.191* (1H, 13′-H), 4.215* (1H, C<u>H</u>$_2$O), 4.235* (1H, 13′-H), 4.584 (1H, t, J=9.2 Hz, C<u>H</u>$_2$O), 4.869 (1H, m, 3-H), 5.035 (1H, m, 9′-H), 5,203 (1H, t, J=1.6 Hz, 4-H), 7.360*—7.573* (20H, m, MTPA-ArH). *Overlapped signals.

(*R*)-(+)-MTPA Ester (3b*S*) Compound **3a** (1.5 mg) was treated with (*S*)-(+)-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 (1.0 mg), $C_{59}H_{63}F_{12}NO_{14}$, positive SI-MS m/z: 1260 $(M+Na)^+$, 319 (base peak); ^{1}H-NMR ($CDCl_3$) δ: 1.130*—1.750* (CH_2), 1.333* (2H, 11′-H), 1.333* (1H, 1′-H), 1.517* (1H, 1′-H), 1.517* (2H, 8′-H), 1.618* (2H, 10′-H), 1.683* (2H, 12′-H), 3.410*—3.546* (12H, OCH_3), 3.885 (1H, quintet, J=4.3 Hz, 2-H), 3.960 (1H, m, 5-H), 4.278 (2H, m, 13′-H), 4.384 (1H, dd, J=9.8, 4.1 Hz, C<u>H</u>$_2$O), 4.591 (1H, t, J=9.2 Hz, C<u>H</u>$_2$O), 5.008* (1H, m, 3-H), 5.031* (1H, m, 9′-H), 5.245 (1H, t, J=2.3 Hz, 4-H), 7.360*—7.544* (20H, m, MTPA-ArH). *Overlapped signals.

Hydrolysis of 5 with 1 N HCl Compound **5** (4.2 mg) was dissolved in 1 N HCl (4.0 ml) and the solution was refluxed in a water bath for 2 h. After cooling, the reaction mixture was passed though an Amberlite IRA-67 (OH^- form) column (i.d. 2.0×5.0 cm) to neutralize it. The resulting solution was purified by HPLC [column: Asahipak ODP 50 6E (i.d. 6.0×250 mm); solvent: CH_3CN–H_2O (15 : 85), adjusted to pH 12.0 with ammonia solution; flow rate: 0.8 ml/min; detection: RI; column temperature: ambient] to give 3.0 mg of **6**.

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