Studies on the Constituents of *Broussonetia* Species. VII. Four New Pyrrolidine Alkaloids, Broussonetines M, O, P, and Q, as Inhibitors of Glycosidase, from *Broussonetia kazinoki* Sieb.

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Four new pyrrolidine alkaloids, broussonetines M, O, P, and Q, were isolated from the branches of *Broussonetia kazinoki* Sieb. (Moraceae). Broussonetines M, O, P, and Q were formulated as (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(10*S*)-10,13-dihydroxy-tridecyl]pyrrolidine (1), (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(*E*)9-oxo-13-hydroxy-3-tridecenyl]pyrrolidine (2), (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(*E*)10-oxo-13-hydroxy-3-tridecenyl)pyrrolidine (3), and (2*R*,3*S*,4*R*,5*R*)-2-hydroxymethyl-3-hydroxy-4-(β-D-glucopyranosyloxy)-5-[10-oxo-13-(β-D-glucopyranosyloxy)tridecyl]pyrrolidine (4) respectively, by spectroscopic and chemical methods. 1—4 inhibited β-glucosidase, β-galactosidase and β-mannosidase.

Key words pyrrolidine alkaloid; glycosidase inhibitor; *Broussonetia kazinoki*; Moraceae

Recently, we reported the structures of thirteen pyrrolidine or pyrrolizidine alkaloids, broussonetines A—H, K, L, N and broussonetinines A and B, as glycosidase inhibitors and two pyrrolidinyl piperidine alkaloids, broussonetines I and J, from *Broussonetia kazinoki* Sieb. (Moraceae).[1—6] In our latest work, we obtained four new pyrrolidine alkaloids, broussonetines M (**1**), O (**2**), P (**3**) and Q (**4**) (Fig. 1), from the same tree. The present report deals with the isolation, structural elucidation including absolute stereostructures and inhibitory activity on some glycosidases.

The branches of this tree were extracted with hot water and the alkaloid constituents were concentrated as described in the Experimental section. Compounds **1**—**4** were isolated by preparative HPLC of the concentrated alkaloids.

Compound **1** was obtained as a colorless powder, $[\alpha]_D$ +5.9° (c=0.30, MeOH), showing a yellowish spot on TLC when sprayed with ninhydrin reagent followed by heating on a hot plate (ninhydrin reaction). The molecular formula was determined as $C_{18}H_{37}NO_5$ on the basis of positive high resolution secondary ion mass spectroscopy (pos. HR-SI-MS) (m/z: 348.2741, $[M+H]^+$, error, −0.7 mmu). The IR spectrum showed a strong OH and NH band at 3275 cm^{-1}.

The ^{1}H-NMR spectrum of **1** suggested the presence of seven methylene groups [δ: 1.16—1.66 (14H, m)], two oxymethylene groups [δ: 4.22 (1H, dd, J=11.0, 4.1 Hz), 4.15 (1H, dd, J=11.0, 5.9 Hz), 3.95 (2H, t, J=6.4 Hz)], three oxymethine groups [δ: 4.62 (1H, t, J=6.4 Hz), 4.36 (1H, t, J=6.4 Hz), 3.90 (1H, m)], and two methine groups attached to a nitrogen atom [δ: 3.75 (1H, m), 3.45 (1H, m)]. These data were strikingly similar to those of broussonetine C,[1] except for an additional oxymethine signal and disappearance of two signals due to methylene groups attached to a carbonyl group. Thus, **1** was concluded to be the compound following reduction of the carbonyl group of broussonetine C. The ^{1}H- and ^{13}C-NMR signals were assigned by ^{1}H–^{1}H correlated spectroscopy (^{1}H–^{1}H COSY), total correlation spectroscopy (TOCSY), heteronuclear signal quantum coherence (HSQC), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple bond correlation (HMBC), as shown in Tables 1 and 2.

The relative stereochemistry 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) in the nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum: 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 stereochemistry of the pyrrolidine moiety and C-10′ in **1** was determined by a new modification of

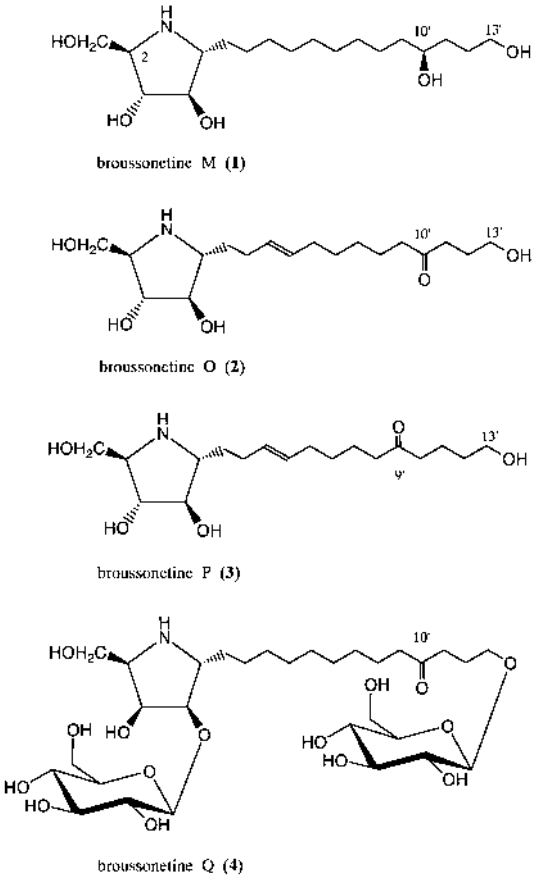

Fig. 1. Structures of **1**—**4**

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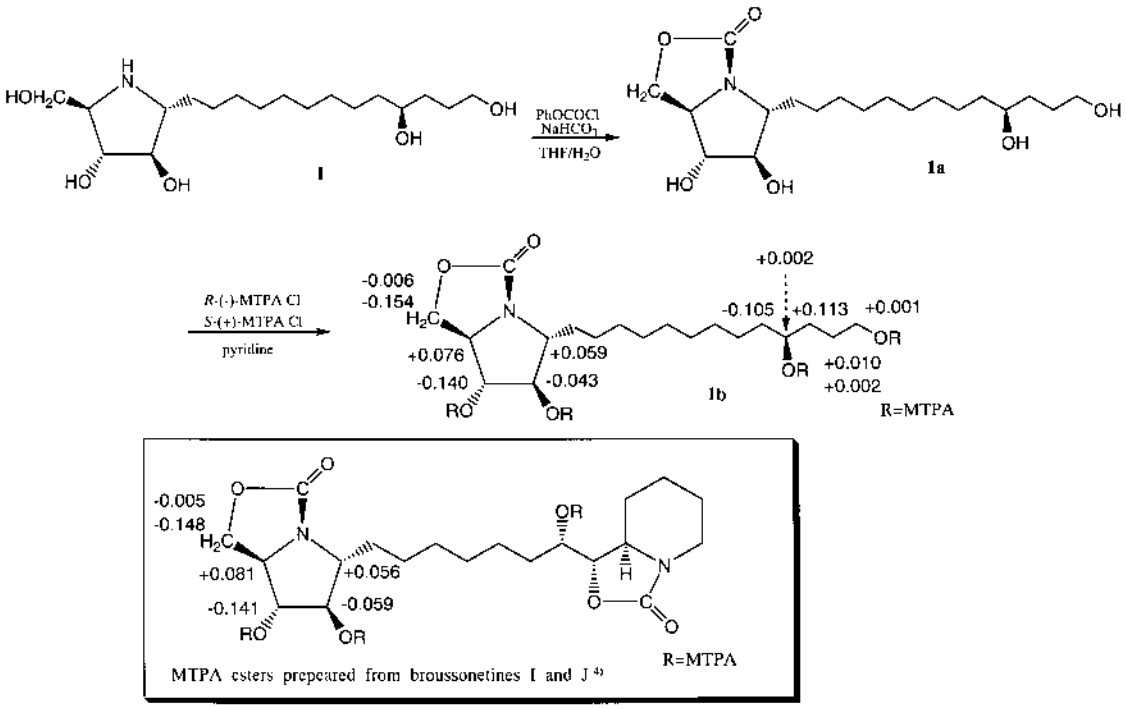

Fig. 2. $\Delta\delta$ Values Obtained for the MTPA Esters of **1**

Mosher's method.[7] A cyclic carbamate (**1a**) was prepared from **1** by reaction with phenyl chlorocarbonate in teterahydrofuran (THF) : H_2O (7 : 3). ^{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 measured respectively: these values established the (*S*) configuration at C-10′ (Fig. 2). In addition, the $\Delta\delta$ values of the pyrrolidine moiety in **1b** coincided with those in the tri-MTPA esters prepared from broussonetines I and J.[4]

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

Compound **2** was obtained as a colorless powder, $[\alpha]_D$ +22.7° (c=0.37, MeOH), showing a brownish spot on TLC with the ninhydrin reaction, and the molecular formula was determined as $C_{18}H_{33}NO_5$ on the basis of pos. HR-SI-MS (*m*/*z*: 344.2435, $[M+H]^+$, error, 0.0 mmu). The IR spectrum showed a strong OH and NH band at 3406 cm^{-1} and a carbonyl band at 1706 cm^{-1}. The ^{1}H-NMR spectrum was strikingly similar to that of broussonetine C,[1] except for two additional olefin proton signals [δ: 5.53 (1H, m), 5.45 (1H, m)]. These signals were assigned as in **1** and summarized in Tables 1 and 2. The vicinal coupling constant (J=15.3 Hz), which was shown by decoupling a methylene signal [δ: 1.93 (2H, dd)], of C′-3 and C′-4 in **2** established the (*E*) conformation of this olefin.

Compound **3** was obtained as a colorless powder, $[\alpha]_D$ +28.8° (c=0.96, MeOH), showing a brownish spot on TLC with the ninhydrin reaction, and the molecular formula was determined as $C_{18}H_{33}NO_5$ on the basis of pos. HR-SI-MS (*m*/*z*: 344.2442, $[M+H]^+$, error, +0.7 mmu). The IR spectrum showed a strong OH and NH band at 3308 cm^{-1} and a carbonyl band at 1705 cm^{-1}.

The ^{1}H-NMR spectrum was strikingly similar to **2** except for the signals due to a partial structure, $CO(CH_2)_3CH_2OH$ instead of $CO(CH_2)_2CH_2OH$. The ^{1}H- and ^{13}C-NMR signals were assigned as above and summarized in Tables 1 and 2.

The absolute stereochemistry of the pyrrolidine ring moieties in **2** and **3** was established by comparison of the values of $[\alpha]_D$ +22.7° (**2**) and +28.8° (**3**), with that of broussonetine C (+25.0°).

Thus, **2** and **3** were formulated as (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(*E*) 9-oxo-13-hydroxy-3-tridecenyl]pyrrolidine and (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(*E*)10-oxo-13-hydroxy-3-tridecenyl)pyrrolidine.

Compound **4** was obtained as a colorless powder, $[\alpha]_D$ +8.8° (c=0.25, MeOH), showing a brownish spot on TLC with the ninhydrin reaction, and the molecular formula was determined as $C_{30}H_{55}NO_{15}$ on the basis of pos. HR-SI-MS (*m*/*z*: 670.3664, $[M+H]^+$, error, +1.7 mmu). The IR spectrum showed a strong OH and NH band at 3402 cm^{-1} and a carbonyl band at 1705 cm^{-1}.

The ^{1}H-NMR spectrum of **4** showed two anomeric protons [δ: 4.79 (1H, d, J=7.8 Hz), 4.94 (1H, d, J=7.8 Hz)]. Hydrolysis of **4** with 1 N HCl provided a genuine aglycone (**4a**) and D-glucose ($[\alpha]_D$ +40.5°). The ^{1}H- and ^{13}C-NMR spectra of **4a** were identical with those of broussonetinine A.[2,6]

The structure of **4** was concluded to be 4-*O*-β-D-glucopyranosyl 13′-*O*-β-D-glucopyranosyl broussonetinine A: the glucosylation shift was 9.02 and 7.92 ppm between the C-4, 13′ of **4** and that of **4a** (Table 1) and the HMBC spectrum of **4** showed long-range correlations between H-4, 13′ and two anomeric carbons (δ: 105.35, 104.41), and two anomeric protons and C-4, 13′, respectively.

Thus, the structure of **4** was formulated as (2*R*,3*S*,4*R*,5*R*)-2-hydroxymethyl-3-hydroxy-4-(β-D-glucopyranosyloxy)-5-[10-oxo-13-(β-D-glucopyranosyloxy)tridecyl]pyrrolidine.

Table 1. ^{1}H-NMR Spectral Data for **1**—**4** (500 MHz, Pyridine-d_5)

	1	**2**	**3**	**4**
2	3.75 m	3.72 m	3.74 m	3.75[a]
3	4.62 t (6.4)	4.62 t (6.4)	4.61 t (6.4)	4.75 t (4.0)
4	4.36 t (6.4)	4.36 t (6.4)	4.36 t (6.4)	4.07 dd (3.5, 4.5)
5	3.45 m	3.49 m	3.48 m	3.66[a]
1′	1.70[a], 2.00[a]	1.82 m, 2.09[a]	1.83[a], 2.09 m	1.59[a], 1.93 m
2′	1.16—1.66	2.29 m, 2.39[a]	2.26 m, 2.36[a]	1.39 m, 1.57[a]
3′	1.16—1.66	5.53 m	5.50 m	1.16—1.66
4′	1.16—1.66	5.45 m	5.43 m	1.16—1.66
5′	1.16—1.66	1.93 dd (13.5, 7.3)	1.93 dd (14.0, 7.3)	1.16—1.66
6′	1.16—1.66	1.26[a]	1.29 quin (7.3)	1.16—1.66
7′	1.16—1.66	1.22[a]	1.57 quin (7.3)	1.16—1.66
8′	1.16—1.66	1.57 quin (7.3)	2.36 t (7.3)	1.53[a]
9′	1.63[a], 1.70[a]	2.39 t (7.3)	—	2.35 t (7.3)
10′	3.90 m	—	2.46 t (7.3)	—
11′	1.86 m	2.65 t (7.3)	1.83	2.62 t (7.3)
12′	2.00[a], 2.12 m	2.06	1.72 m	2.02 quin (7.3)
13′	3.95 t (6.4)	3.85 t (6.4)	3.83 t (6.4)	3.66[a], 4.15[a]
CH_2OH	4.15 dd (11.0, 5.9)	4.14 dd (11.0, 5.8)	4.14 dd (11.0, 5.9)	4.23[a]
	4.22 dd (11.0, 4.1)	4.19 dd (11.0, 4.2)	4.21 dd (11.0, 4.0)	4.31[a]
Glc				
1″				4.94 d (7.8)
2″				4.01[a]
3″				4.24[a]
4″				4.17[a]
5″				3.98[a]
6″				4.31[a], 4.52[a]
Glc				
1‴				4.79 d (7.8)
2‴				4.01[a]
3‴				4.24[a]
4‴				4.17[a]
5‴				3.94[a]
6‴				4.31[a], 4.48[a]

a) Overlapped signals.

Table 2. ^{13}C-NMR Spectral Data for **1**—**4** and **4a** (Pyridine-d_5)

	1[a]	**2**[a]	**3**[a]	**4**[a]	**4a**[b]
2	64.71	64.98	64.57	61.42	62.12
3	80.02	80.22	79.77	73.18	73.42
4	84.01	84.24	83.76	88.95	79.93
5	62.55	62.24	61.90	59.83	61.48
1′	35.44	35.68	35.17	35.36	35.32
2′		30.40	30.13		
3′	30.10, 30.07	130.83	130.69	30.10, 29.85	30.05, 29.88
4′	29.94, 29.88	130.48	130.05	29.69, 29.65	29.76, 29.61
5′	29.83, 27.22	32.71	32.45	29.40, 27.56	29.35, 27.61
6′	26.29	29.63	29.12		
7′		28.96	23.43		
8′		24.02	42.33	24.06	24.07
9′	38.27	42.78	210.79	42.78	42.72
10′	70.94	210.75	42.37	210.99	210.67
11′	34.93	39.52	20.69	39.31	39.40
12′	30.17	27.81	32.69	24.44	27.42
13′	62.34	61.24	61.44	68.98	61.06
CH_2OH	63.45	63.54	63.14	62.13	61.84
1″				105.35	
2″				74.92[c]	
3″				78.16[c]	
4″				71.47[c]	
5″				78.28[c]	
6″				62.56[c]	
1‴				104.41	
2‴				74.62[c]	
3‴				77.97[c]	
4‴				71.18[c]	
5‴				78.22[c]	
6‴				62.23[c]	

a) Measured at 125 MHz. *b*) Measured at 75 MHz. *c*) Assignments may be interchangeable.

Table 3. Concentration of Inhibitor Required to Produce 50% Inhibition of Enzyme Activity

	Inhibitors						
	1	**2**	**3**	**4**	DNJ	DGJ	DMJ
α-Glucosidase (from yeast)	NI	NI	NI	NI	0.93	—	—
β-Glucosidase (from sweet almond)	NI	1.4	2.4	1.4	0.58	—	—
β-Galactosidase (from bovine liver)	8.1	0.17	0.20	0.60	—	0.13	—
α-Mannosidase (from Jack beans)	NI	NI	NI	NI	—	—	0.94
β-Mannosidase (from snail acetone powder)	NI	8.2	7.6	20.0	—	—	0.81

NI: up to 100 μM or no inhibition. (μM)

The inhibitory activities of **1**—**4**, 1-deoxynojirimycin (DNJ),[8,9] 1-deoxygalactonojirimycin (DGJ), and 1-deoxymannojirimycin (DMJ)[8,9] were assayed with respect to α-glucosidase, β-glucosidase, β-galaclosidase, α-mannosidase and β-mannosidase by the methods described in the Experimental section and the results are summarized in Table 3.

Broussonetines M (**1**) inhibited β-galactosidase and broussonetines O (**2**), P (**3**), and Q (**4**) inhibited β-glucosidase, β-galactosidase and β-mannosidase.

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 enzyme assay).

Column chromatography was carried out on an 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 using 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 in a $CHCl_3$–MeOH–AcOH–H_2O (20 : 10 : 7 : 5) solvent system, and detection was carried out with the ninhydrin reagent followed by heating.

Isolation of 1—4 Dried branches of *Broussonetia kazinoki* (7.5 kg, collected in Takatsuki City (Osaka) in 1998) were cut finely and then extracted with hot water (40 l×3) for 2 h. 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 (35.3 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), with gradient elution (H_2O(2.0 l)→H_2O–28% ammonia solution (9 : 1, 2.0 l)). The fractions containing **1**, **2**, **3** and **4** were 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 (15 : 85), adjusted to pH 12.0 with ammonia solution; flow rate: 1.5 ml/min; column temperature : ambient]. **1** (20 mg), **2** (10 mg), **3** (20 mg) and **4** (10 mg) were finally obtained.

Broussonetine M (**1**): Colorless powder, ninhydrin reaction: positive (a yellow spot on TLC), $[\alpha]_D$ +5.9° (c=0.30, MeOH), $C_{18}H_{37}NO_5$, pos. HR-SI-MS m/z: 348.2741 ($[M+H]^+$) error: −0.7 mmu, IR ν (KBr) cm^{-1}: 3275 (OH, NH), ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1, 2.

Broussonetine O (**2**): Colorless powder, ninhydrin reaction: positive (a brownish spot on TLC), $[\alpha]_D$ +22.7° (c=0.27, MeOH), $C_{18}H_{33}NO_5$, pos. HR-SI-MS m/z: 344.2435 ($[M+H]^+$) error: 0.0 mmu, IR ν (KBr) cm^{-1}: 3406 (OH, NH), 1706 (CO), ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1, 2.

Broussonetine P (**3**): Colorless powder, ninhydrin reaction: positive (a brownish spot on TLC), $[\alpha]_D$ +28.8° (c=0.96, MeOH), $C_{18}H_{33}NO_5$, pos. HR-SI-MS m/z: 344.2442 ($[M+H]^+$) error: +0.7 mmu, IR ν (KBr) cm^{-1}: 3308 (OH, NH), 1705 (CO), ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1, 2.

Broussonetine Q (**4**): Colorless oil, ninhydrin reaction: positive (a brownish spot on TLC), $[\alpha]_D$ +8.8° (c=0.25, MeOH), $C_{30}H_{55}NO_{15}$, pos. HR-SI-MS m/z: 670.3664 ($[M+H]^+$) error: +1.7 mmu, IR ν (KBr) cm^{-1}: 3402 (OH, NH), 1705 (CO), ^{1}H- and ^{13}C-NMR (pyridine-d_5): Tables 1, 2.

Carbamate (1a) **1** (10 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 subject-ed to HPLC [column, Asahipak ODP-5E (i.d. 6.0×250 mm); solvent, CH_3CN–H_2O (30 : 70), 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 (8 mg).

(*S*)-(−)-MTPA Ester (1b*S*) **1a** (4 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). **1b***S*: $C_{59}H_{63}NO_{14}F_{12}$ pos. SI-MS m/z: 1238 $(M+H)^+$, 189 (base peak). ^{1}H-NMR ($CDCl_3$) δ: 1.150*—1.730* (CH_2), 1.545* (9′-H), 1.606* (12′-H), 1.651* (11′-H), 1.688* (12′-H), 3.488 (3H, s, OCH_3), 3.510*—3.547* (9H, OCH_3), 3.962 (1H, m, 2-H), 4.020 (1H, m, 5-H), 4.200* (1H, 13′-H), 4.231 (1H, C<u>H</u>$_2$O), 4.279* (1H, 13′-H), 4.586 (1H, m, C<u>H</u>$_2$O), 4.870 (1H, m, 3-H), 5.034 (1H, m, 10′-H), 5.202 (1H, m, 4-H), 7.350—7.550 (20H, m, MTPA-Ar H). *: overlapped signals.

(*R*)-(+)-MTPA Ester (1b*R*) **1b** (4 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]. **1b***R* was obtained as a colorless oil (1.5 mg). **1b***R*: $C_{59}H_{63}NO_{14}F_{12}$ pos. SI-MS m/z: 1238 $(M+H)^+$, 189 (base peak). ^{1}H-NMR ($CDCl_3$) δ: 1.130*—1.720* (CH_2), 1.538* (11′-H), 1.596* (12′-H), 1.650* (9′-H), 1.686* (12′-H), 3.466 (3H, s, OCH_3), 3.503*—3.543* (9H, OCH_3), 3.886 (1H, m, 2-H), 3.961 (1H, m, 5-H), 4.200* (1H, 13′-H), 4.278 (1H, m, 13′-H), 4.385 (1H, dd, J=9.7, 4.2 Hz, C<u>H</u>$_2$O), 4.592 (1H, m, C<u>H</u>$_2$O), 5.010* (1H, 3-H), 5.032* (1H, 10′-H), 5.245 (1H, m, 4-H), 7.350—7.540 (20H, m, MTPA-Ar H). *: overlapped signals.

Hydrolysis of 4 with 1 N HCl **4** (8 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 chromatographed on a Sep-Pak C-18 column (Waters), and elution with water afforded D-glucose (1.3 mg), $[\alpha]_D$ +40.5° (c=0.13, H_2O), which was identified by HPLC (t_R=12.2 min) [column, Shodex NH_2 P-50 4E (i.d. 4.6×250 mm); solvent, CH_3CN–H_2O (75 : 25); flow rate, 0.8 ml/min; detection, RI; column temperature, 40 °C], ^{1}H- and ^{13}C-NMR. Elution with MeOH afforded an aglycone, broussonetinine A (**4a**) (3 mg), as a colorless powder.

Enzyme Assays Materials α-Glucosidase (from Bakers yeast), β-galactosidase (from bovine liver), α-mannosidase (from Jack beans), and β-mannosidase (from snail acetone powder) were obtained from Sigma Chemical Company (St. Louis, U.S.A.), and β-glucosidase (from sweet almond) was obtained from Toyobo Company (Osaka, Japan). *p*-Nitrophenyl-α-D-glucopyranoside, -β-D-glucopyranoside, -α-D-mannopyranoside, and -β-D-galactopyranoside were obtained Nacalai Tesque, Inc (Osaka, Japan), *p*-nitrophenyl-β-D-mannopyranoside from Sigma Chemical Company, and DNJ, DGJ, and DMJ from Funakoshi Company (Tokyo, Japan).

Assay of β-Galactosidase Inhibition The reaction mixture consisted of 475 μl 0.1 M acetate buffer (pH 5.0), 250 μl 20 mM *p*-nitrophenyl-β-D-glucopyranoside and 250 μl β-galactosidase solution (a stock solution of 1.0 mg/ml in 10 mM acetate buffer (pH 5.0) was diluted 5 times with the same buffer, pH 5.0, just before assay), with the substrates **1**, **2**, **3**, **4** or DGJ (25 μl solution, concentration: 20—0.1 mg/ml). After incubation for 20 min at 37 °C, the reaction was interrupted by the addition of 1 ml 0.2 M sodium carbonate, and the amount of *p*-nitrophenol liberated was measured colorimetrically at 400 nm (ODtest). The rates of inhibition (%) were calculated from the formula 100−100×(ODtest−ODblank)/(control ODtest−control ODblank) and the IC_{50} values were obtained from the inhibition curves . The IC_{50} values were 8.1 μM for **1**, 0.17 μM for **2**, 0.20 μM for **3** and 0.60 μM for **4**, while that of DGJ was 0.13 μM. Assays of α-glucosidase, β-glucosidase, β-mannosidase and α-mannosidase were carried out as above using *p*-nitro-

phenyl-α-D-glucopyranoside, -β-D-glucopyranoside, -β-D-mannopyranoside and -α-D-mannopyranoside as substrates. The IC_{50} values are shown in Table 3.

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