Studies on the Constituents of *Broussonetia* Species. II.[1] Six New Pyrrolidine Alkaloids, Broussonetine A, B, E, F and Broussonetinine A and B, as Inhibitors of Glycosidases from *Broussonetia kazinoki* SIEB.[2]

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Six new pyrrolidine alkaloids called broussonetine A, B, E, F, and broussonetinine A and B were isolated from the branches of *Broussonetia kazinoki* SIEB. (Moraceae). Broussonetine A, B, E and F were formulated as 2β-hydroxymethyl-3β-hydroxy-5α-(10-oxo-13-hydroxytridecyl)-pyrrolidine-4-*O*-β-D-glucopyranoside (1), 2β-hydroxymethyl-3β-hydroxy-5α-(9-oxo-13-hydroxytridecyl)-pyrrolidine-4-*O*-β-D-glucopyranoside (2), 2β-hydroxymethyl-3α,4β-dihydroxy-5α-(1,13-dihydroxy-10-oxo-tridecyl)-pyrrolidine (3), and 2β-hydroxymethyl-3α,4β-dihydroxy-5α-(1,13-dihydroxy-9-oxo-tridecyl)-pyrrolidine (4), respectively. Broussonetinine A and B (5 and 6) were also isolated and identified as the aglycones of 1 and 2. 3 and 4 exhibited a strong inhibition of α-glucosidase, β-glucosidase, β-galactosidase and β-mannosidase, while 5 and 6 showed a strong inhibition of β-galactosidase and α-mannosidase.

Key words pyrrolidine alkaloid; glycosidase inhibitor; *Broussonetia kazinoki*; broussonetine; broussonetinine

Inhibition of glycosidases has a number of potential therapeutic uses, including the treatment of cancer, diabetes and AIDS.[3] In a previous paper we reported the absolute structures of two new pyrrolidine alkaloids, broussonetine C and D, isolated from *Broussonetia kazinoki* SIEB., shown to be inhibitors of β-galactosidase and β-mannosidase.[1] In this paper we deal with the isolation of six new pyrrolidine alkaloids, called broussonetine A (**1**), B (**2**), E (**3**), F (**4**) and broussonetinine A (**5**) and B (**6**) from the same tree, and describe their structural elucidation and the inhibitory activity on some glycosidases.

The branches of this tree were extracted with hot water and the alkaloidal constituents concentrated as follows (Fig. 1): The extract solution was subjected to chromatography on an Amberlite CG-50 column. The adsorbed fraction was eluted with an ammonia solution (28%NH_3: H_2O=1:9) to provide an alkaloid fraction which was chromatographed on a Dowex 50W column. Elution with

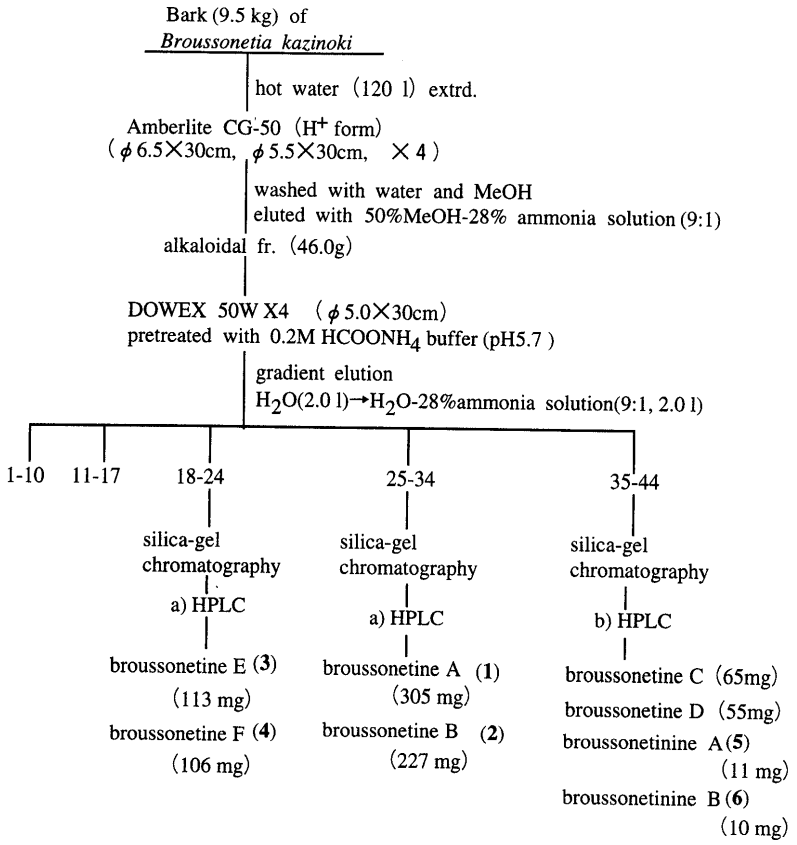

Fig. 1

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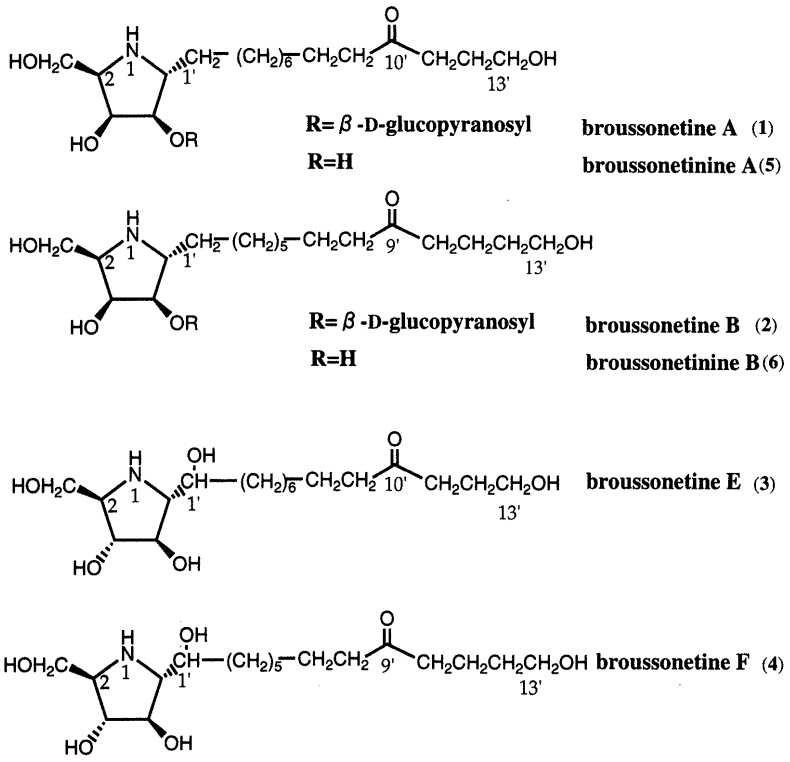

Fig. 2

serial dilutions of ammonia provided five fractions, which were subjected to silica-gel column chromatography and semi-HPLC to provide purified alkaloids (**1**—**6**) (Fig. 2).

Broussonetine A (**1**) was obtained as a colorless powder, mp 164—166 °C, $[\alpha]_D$ +32.1°, it appeared as a yellow spot on TLC on spraying with ninhydrin reagent followed by heating on a hot plate (ninhydrin reaction), and the molecular formula was determined to be $C_{24}H_{45}NO_{10}$ by positive high resolution secondary ion mass spectrometry (pos.HR-SI-MS) ($[M+H]^+$: *m/z* 508.3119). The IR spectrum showed a strong hydroxyl band at 3400 cm^{-1} and a carbonyl band at 1705 cm^{-1}.

The ^{1}H-NMR spectrum (pyridine-d_5) showed an anomeric proton [δ 5.04 (1H, d, J=7.8 Hz)]. Hydrolysis of **1** with 1.5% HCl provided a genuine aglycone (**5**), which was also isolated from the same tree and named broussonetinine A, and D-glucose ($[\alpha]_D$ +47.0°). The ^{1}H-NMR spectrum of **5** suggested the presence of nine methylene groups [δ 1.10—1.65 (18H, m)], two oxymethylene groups [δ 4.26 (1H, dd, J=11.5, 5.0 Hz), δ 4.32 (1H, dd, J=11.5, 5.0 Hz), δ 3.90 (2H, t, J=7.3 Hz)], two oxymethine groups [δ 4.60 (1H, t, J=6.2 Hz), δ 4.10 (1H, dd, J=6.2, 6.6 Hz)], two methylene groups attached to a carbonyl group, and two methine groups attached to a nitrogen atom [δ 3.78 (1H, m), δ 3.56 (1H, m)]. The ^{1}H- and ^{13}C-NMR signals were assigned by ^{1}H–^{1}H correlated spectroscopy (^{1}H–^{1}H COSY), heteronuclear single quantum coherence (HSQC), and distortionless enhancement by polarization transfer (DEPT), as shown in Tables 1 and 2. These data on above groups led to the partial structures.

The linkages among these partial structures were determined from the heteronuclear multiple bond correlation (HMBC) spectrum as shown in Fig. 3.

The relative stereochemistry of **5** was studied by chemical conversion and nuclear Overhauser effect (NOE) experiments. The methyl ether (**5a**) was treated with *p*-toluenesulfonic acid in acetone to provide an acetonide (**5b**), which was followed by acetylation with acetic anhydride in pyridine to provide an acetate (**5c**) (Fig. 4). NOE was observed between 2-H and 4-H in the NOE experiment to establish a 2,3-*cis* and 3,4-*cis* orientation.

Therefore, **5** was formulated as 2β-hydroxymethyl-3β,4β-dihydroxy-5α-(10-oxo-13-hydroxytridecyl)-pyrrolidine. The structure of **1** was concluded to be the 4-*O*-β-D-glucopyranoside of **5**: An NOE was observed between the anomeric 1″-H and 4-H, and the glucosylation shift was 10 ppm between the C-4 of **1** and that of **5** (Table 1) to suggest the glucosylation site. The coupling constant (J) of the anomeric proton signal was 7.8 Hz, and the chemical shifts of other carbinyl protons (2″,3″,4″,5″-H) and carbons (2″,3″,4″,5″-C) were identical to those reported in the literature.[4,5]

Broussonetine B (**2**) was obtained as a colorless powder, mp 154—156 °C, $[\alpha]_D$ +29.8°, it appeared as a yellow spot on TLC after the ninhydrin reaction, and the molecular formula was determined to be $C_{24}H_{45}NO_{10}$ by pos.HR-SI-MS ($[M+H]^+$: *m/z* 508.3122). The IR spectrum showed a strong hydroxyl band at 3400 cm^{-1} and a carbonyl band at 1705 cm^{-1}.

The ^{1}H- and ^{13}C-NMR spectra were strikingly similar to those of **1** and each signal was attributed as shown in Tables 1 and 2. The ^{1}H–^{1}H COSY spectra of **2** and its aglycone (**6**), which was prepared by acidic hydrolysis, also isolated from the same tree, and named broussonetine B, showed the presence of the partial structure, $COCH_2CH_2CH_2CH_2OH$ instead of $COCH_2CH_2CH_2OH$ in **1** and **5** as exemplified by the ^{1}H–^{1}H COSY spectrum

Table 1. ^{1}H-NMR Spectral Data for **1**, **2**, **3**, **4**, **5** and **6**

Proton	**1**	**5**	**3**	Proton	**2**	**6**	**4**
2	3.78 m	3.78 m	3.85[a] m	2	3.71 m	3.82 m	3.82 m
3	4.82 t (6.3)	4.60 t (6.2)	4.72 t (6.4)	3	4.76 t (6.3)	4.65 t (6.3)	4.71 t (6.4)
4	4.10[a]	4.10 dd (6.2, 6.6)	4.96 t (6.4)	4	4.30[a]	4.14 m	4.96 t (6.4)
5	3.71 m	3.56 m	3.68 t (6.4)	5	3.67 m	3.52 m	3.65 t (6.4)
1′	1.98[a]	1.95[a], 1.65[a]	4.15[a] m	1′	1.90[a]	1.95[a], 1.68[a]	4.15[a] m
2′—7′	0.70—1.65	1.10—1.65	1.15—2.00	2′—6′	1.15—1.65	1.20—1.70	1.15—1.70
				7′	1.56[a]	1.60[a]	1.55[a]
8′	1.60[a]	1.60[a]	1.55[a]	8′	2.35 t (6.7)	2.39 t (8.0)	2.35 t (7.3)
9′	2.44 t (7.3)	2.40 t (7.3)	2.61 t (7.3)	9′			
10′				10′	2.48 t (6.7)	2.51 t (8.0)	2.49 t (7.3)
11′	2.71 t (7.3)	2.69 (7.3)	2.71 t (7.3)	11′	1.90 quintet (6.7)	1.93[a]	1.75[a]
12′	2.11 quintet (7.3)	2.08 quintet (7.3)	1.95 quintet (7.3)	12′	1.75 quintet (6.7)	1.80[a]	1.87[a]
13′	3.92 t (7.3)	3.90 t (7.3)	3.90[a] t (7.3)	13′	3.87 t (6.7)	3.90 t (8.0)	3.87 t (7.3)
CH_2OH	4.25[a]	4.32 dd (11.5, 5.0)	4.22[a] m	CH_2OH	4.25[a]	4.40 dd (11.3, 5.0)	4.27[a] m
		4.26 dd (11.5, 5.0)					
1″	5.04 d (7.8)			1″	5.00 d (7.3)	4.32 dd (11.3, 5.0)	
2″	4.05[a]			2″	4.05[a]		
3″	4.30[a]			3″	4.30[a]		
4″	4.35[a]			4″	4.35[a]		
5″	4.35[a]			5″	4.35[a]		
6″	4.40[a], 4.60			6″	4.40[a], 4.60		

δ in pyridine-d_5 at 300 MHz, ppm (Hz). *a*) Overlapped signals.

Table 2. ^{13}C-NMR Spectral Data for **1**, **2**, **3**, **4**, **5** and **6**

Carbon	**1**	**5**	**3**	Carbon	2	6	4
2	62.14	61.40	65.99	2	61.79	61.99	65.66
3	73.18	72.64	80.72	3	73.60	73.93	80.36
4	89.46	78.18	80.37	4	89.33	79.49	80.19
5	60.22	60.73	67.68	5	60.05	62.36	67.32
1′	35.69	34.38	74.14	1′	35.70	35.71	73.78
2′—7′	29.74, 29.99	28.93, 28.65	35.15, 29.95	2′—6′	29.48, 29.73	27.58, 29.51	34.79, 29.92
	29.99, 30.17	28.48, 28.48	30.39, 29.71		29.82, 27.66	29.74, 29.82	29.72, 26.61
	30.39, 28.17	28.24, 26.68	30.06, 28.10		30.14	30.18	29.40
				7′	24.17	24.17	24.04
8′	24.44	22.90	24.42	8′	42.60	42.72	42.60
9′	43.09	41.60	43.06	9′	210.64	210.66	210.57
10′	211.02	209.46	210.98	10′	42.71	42.63	42.48
11′	39.79	38.29	39.74	11′	20.99	21.00	20.85
12′	27.95	26.32	26.94	12′	33.13	33.16	32.99
13′	61.16	60.09	61.59	13′	61.79	61.79	61.68
CH_2OH	62.78	61.10	63.68	CH_2OH	62.55	62.75	63.33
1″	106.00			1″	105.65		
2″	75.18			2″	74.91		
3″	78.87			3″	78.39		
4″	71.70			4″	71.48		
5″	78.69			5″	78.57		
6″	62.78			6″	62.63		

δ in pyridine-d_5 at 75 MHz, ppm (Hz).

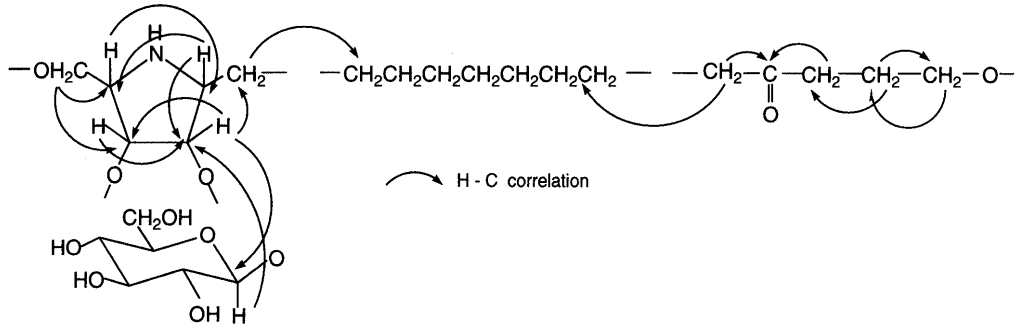

Fig. 3. The Partial Structures and HMBC

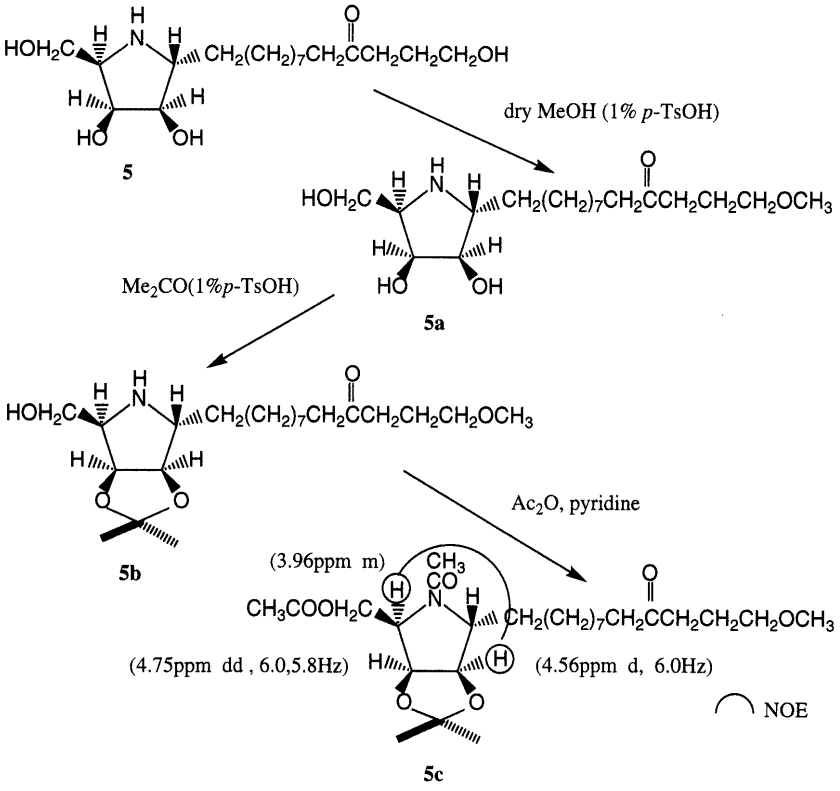

Fig. 4

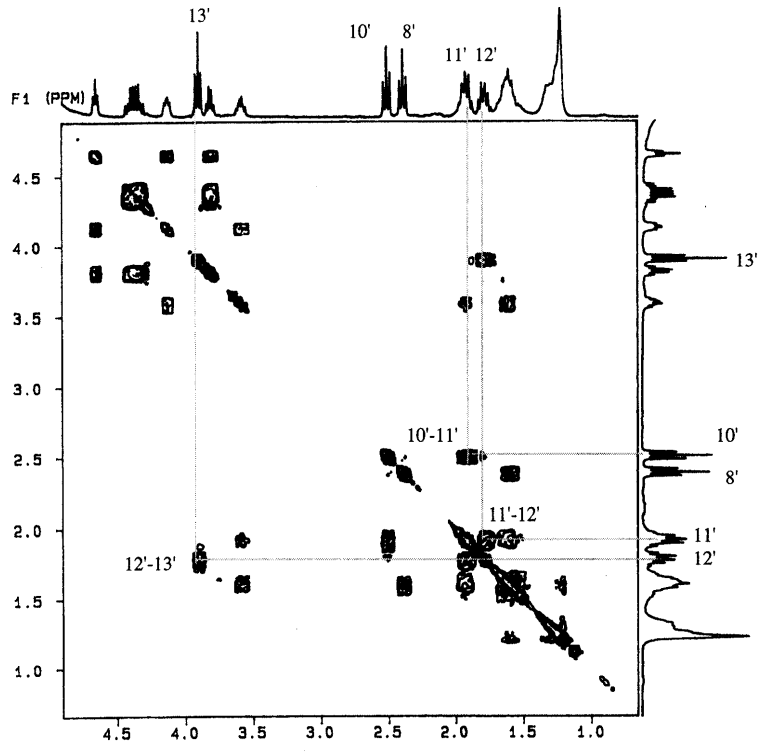

Fig. 5. ^{1}H–^{1}H COSY Spectrum of **6** in Pyridine-d_5

of **6** (Fig. 5). The same relationship was recognized between broussonetine C and D.[1] Thus, **6** was formulated as 2β-hydroxymethyl-3β,4β-dihydroxy-5α-(9-oxo-13-hydroxytridecyl)-pyrrolidine. The structure of **2** was concluded to be the 4-*O*-β-D-glucopyranoside of **6**, similarly to **1**.

Broussonetine E (**3**) was obtained as a colorless powder, mp 103—105 °C, $[\alpha]_D$ +4.9°, it appeared as a yellow spot on TLC after the ninhydrin reaction, and the molecular formula was determined to be $C_{18}H_{35}NO_6$ by pos.HR-SI-MS ($[M+H]^+$: *m/z* 362.2537). The IR spectrum showed a strong hydroxyl band at 3369 cm^{-1} and a carbonyl band at 1703 cm^{-1}.

The ^{1}H-NMR spectrum was strikingly similar to that

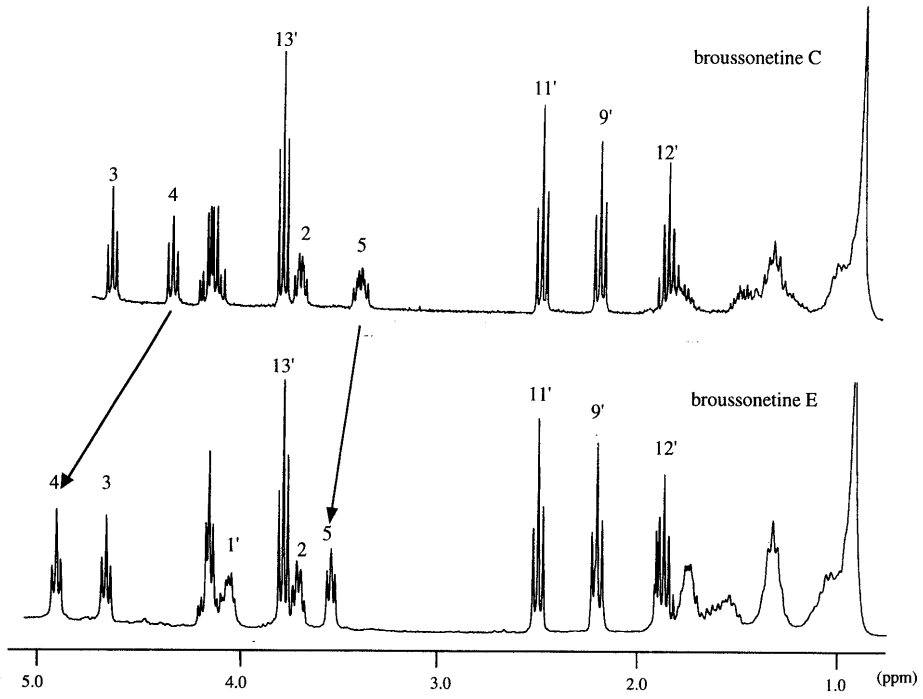

Fig. 6. Comparison of ^{1}H-NMR Spectra of Broussonetine C and **3** (in Pyridine-d_5)

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

	Inhibitor					
	1	**2**	**3**	**4**	**5**	**6**
α-Glucosidase (from yeast)	NI	NI	3.3	1.5	NI	NI
β-Glucosidase (from sweet almond)	NI	NI	0.055	0.010	NI	NI
β-Galactosidase (from bovine liver)	NI	NI	0.002	0.004	0.016	0.010
α-Mannosidase (from Jack beans)	NI	NI	NI	NI	0.30	0.29
β-Mannosidase (from snail acetone powder)	NI	NI	0.023	0.028	NI	NI

NI: up to 100 μM or no inhibition.

of broussonetine C,[1] except for an additional oxymethine signal [δ 4.15 (1H, m)] and that of 4-H, which appeared at δ 4.96 and was deshielded by 0.52 ppm from that of broussonetine C (δ 4.44) as shown Fig. 6. The ^{1}H- and ^{13}C-NMR signals of **3** were assigned as in **1**, **2**, **5** and **6**, and summarized in Tables 1 and 2. Thus, **3** was formulated as 2β-hydroxymethyl-3α,4β-dihydroxy-5α-(1,13-dihydroxy-10-oxotridecyl)pyrrolidine.

Broussonetine F(**4**) was obtained as a colorless powder, mp 105—107 °C, $[\alpha]_D$ + 13.5°, it appeared as a yellow spot on TLC after the ninhydrin reaction, and the molecular formula was determined to be $C_{18}H_{35}NO_6$ by pos.HR-SI-MS ($[M+H]^+$: m/z 362.2541). The IR spectrum showed a strong hydroxyl band at 3397 cm^{-1} and a carbonyl band at 1707 cm^{-1}.

The ^{1}H-NMR spectrum was strikingly similar except for the signals due to a partial structure, $COCH_2CH_2$-CH_2CH_2OH instead of $COCH_2CH_2CH_2OH$ in **3**. The same relationships were found in **1** and **2**, and **5** and **6** as shown above and in broussonetine C and D.[1] The ^{1}H- and ^{13}C-NMR signals were assigned as above and summarized in Tables 1 and 2. Thus, **4** can be formulated as 2β-hydroxymethyl-3α, 4β-dihydroxy-5α-(1,13-dihydroxy-9-oxotridecyl)pyrrolidine.

Broussonetinine A and B (**5** and **6**) were isolated from the extract as described above and identified by direct comparison with authentic specimens. The absolute stereochemistry of **1**—**6** is being studied.

The inhibitory activity of **1**—**6** on α-glucosidase, β-glucosidase, β-galactosidase, α-mannosidase and β-mannosidase as assayed by the methods described in the experimental section and the results are summarized in Table 3. It is interesting that **3** and **4** strongly inhibited α-glucosidase, β-glucosidase, β-galactosidase and β-mannosidase, while **5** and **6** strongly inhibited β-galactosidase and α-mannosidase. The IC_{50} values of broussonetine C and D were more than 100 μM for β-glucosidase, 0.036 and 0.029 μM for β-galactosidase and 0.32 and 0.34 μM for β-mannosidase,[1] while those of **3** and **4** were 0.055 and 0.010 μM for β-glucosidase and 0.002 and 0.004 μM for β-galactosidase, 0.023 and 0.028 μM for β-mannosidase. These results suggest that introduction of a hydroxy group on C-1′ increases the inhibitory potency by over an order of magnitude. Because the IC_{50} values of **5** and **6** were 0.016 and 0.010 μM for β-galactosidase, and 0.30 and 0.29 μM for α-mannosidase, 1′-hydroxylated congeners of **5** and **6** are being sought as more potent inhibitors of glycosidases.

Experimental

General The instruments used in this work were as follows: Yanagimoto micro-melting apparatus (for melting points, uncorrected); JASCO digital polarimeter (for specific rotation, measured at 25 °C), Perkin–Elmer 1720X-FTIR spectrometer (for IR spectra); Hitachi M-80 spectrometer (for MS spectra); Varian Gemini-200, Varian XL-300, General electric DMX-500 (for NMR spectra, measured in pyridine-d_5, on the δ scale using tetramethylsilane as an internal standard); Shimadzu spectrophotometer UV 1200 (for enzyme assay).

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 DM 1020/Fuji Silysia Chemical Ltd.) columns. HPLC was conducted using a Gilson 305 pump equipped with a JASCO 830-RI detectors. Silica-gel 60 F_{254} (Merk) precoated TLC plates were used and detection was carried out by spraying with ninhydrin reagent followed by heating on a hot plate.

Isolation of 1, 2, 3, 4, 5 and 6 Dried branches of *Broussonetia kazinoki* (9.5 kg, collected in a mountainous area of 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 in gradient mode (H_2O (2.0 l)→(H_2O–28% ammonia solution (9 : 1, 2.0 l)). The fractions 18—24, 25—34 and 35—44 were respectively rechromatographed on silica-gel (Chromatorex DM1020) using $CHCl_3$ and MeOH, followed by preparative a) 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] or b) HPLC [column: Asahipak ODP 5E (i.d. 10 × 250 mm); solvent: CH_3CN–H_2O (17 : 83), adjusted to pH 12.0 with ammonia solution; flow rate: 1.5 ml/min; column temperature: ambient]. **1** (305 mg), **2** (227 mg), **3** (113 mg), **4** (106 mg), **5** (11 mg) and **6** (10 mg) were finally obtained.

Broussonetine A (**1**): Colorless powder, ninhydrin reaction: positive (a yellow spot on TLC), mp 164—166 °C, $[\alpha]_D$ +32.1° (c = 0.21, MeOH). $C_{24}H_{45}NO_{10}$, pos.HR-SI-MS: m/z 508.3119 ($[M+H]^+$), error: 0.0 m mass. IR ν (KBr) cm^{-1}: 3400 (OH, NH), 1705 (C=O). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Table 1, 2.

Broussonetine B (**2**): Colorless powder, ninhydrin reaction: positive (a yellow spot on TLC), mp 154—156 °C, $[\alpha]_D$ +29.8° (c = 0.43, MeOH). $C_{24}H_{45}NO_{10}$, pos.HR-SI-MS: m/z 508.3122 ($[M+H]^+$), error: 0.3 m mass. IR ν (KBr) cm^{-1}: 3400 (OH, NH), 1705 (C=O). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Table 1, 2.

Broussonetine E (**3**): Colorless powder, ninhydrin reaction: positive (a yellow spot on TLC), mp 103—105 °C, $[\alpha]_D$ +4.9° (c = 1.06, MeOH). $C_{18}H_{35}NO_6$, pos.HR-SI-MS: m/z 362.2537 ($[M+H]^+$), error: −0.4 m mass. IR ν (KBr) cm^{-1}: 3369 (OH, NH), 1703 (C=O). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Table 1, 2.

Broussonetine D (**4**): Colorless powder, ninhydrin reaction: positive (a yellow spot on TLC), mp 105—107 °C, $[\alpha]_D$ +13.5° (c = 0.93, MeOH). $C_{18}H_{35}NO_6$, pos.HR-SI-MS: m/z 362.2541 ($[M+H]^+$), error: 0.0 m mass. IR ν (KBr) cm^{-1}: 3397 (OH, NH), 1707 (C=O). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Table 1, 2.

Broussonetinine A (**5**): Colorless powder, ninhydrin reaction: positive (a yellow spot on TLC), mp 126—128 °C, $[\alpha]_D$ +25.8° (c = 0.57, MeOH). $C_{18}H_{35}NO_5$, pos.SI-MS: m/z 346($[M+H]^+$) (100%), IR ν (KBr) cm^{-1}: 3405 (OH, NH), 1707 (C=O). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Table 1, 2.

Broussonetinine B (**6**): Colorless powder, ninhydrin reaction: positive (a yellow spot on TLC), mp 128—130 °C, $[\alpha]_D$ +15.3° (c = 0.29, MeOH). $C_{18}H_{35}NO_5$, pos.SI-MS: m/z 346 ($[M+H]^+$) (100%), IR ν (KBr) cm^{-1}: 3400 (OH, NH), 1704 (C=O). ^{1}H- and ^{13}C-NMR (pyridine-d_5): Table 1, 2.

Hydrolysis of 1 and 2 with 1.5% HCl **1** (15 mg) was dissolved in 1.5% HCl (5.0 ml) and the solution was refluxed on a water bath for 4 h. After cooling, the reaction mixture was passed through an Amberlite IRA-35 (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 (5.3 mg), $[\alpha]_D$ +47.0° (c = 0.33, H_2O), which was identified by TLC and ^{1}H-NMR. The elution with MeOH afforded an aglycone, broussonetinine A (**5**) (10 mg) as a colorless powder. Similarly, D-glucose (2.5 mg), $[\alpha]_D$ +46.7° (c = 0.25, H_2O) and an aglycone, broussonetinine B (**6**) (7.5 mg) were obtained from **2** (10 mg).

Chemical Conversion of 5 **5** (10 mg) was dissolved with 1.0% *p*-toluenesulfonic acid in dry MeOH and the solution was refluxed for 5 h. The reaction mixture was neutralized by Amberlite IRA-35 (OH^- form) column (i.d. 2.0 × 5.0 cm) chromatography. Evaporation of the solvent from the resulting fraction provided the methyl ether (**5a**) (10 mg). **5a** was treated with 1.0% *p*-toluenesulfonic acid in acetone to provide an acetonide (**5b**) (11.9 mg), which was undergone acetylation with acetic anhydride (1.0 ml) in pyridine (3.0 ml) at r.t. to provide an acetate (**5c**) (9.1 mg). **5c**: colorless oil, pos.SI-MS: m/z 424 ($[M-CH_3COOH]^+$, 21.1%). ^{1}H-NMR (pyridine-d_5): δ 1.32, 1.45 (3H, s, acetonide), 2.05, 2.10 (3H each, s, 2 × $COCH_3$), 3.32 (3H, s, OCH_3), 3.85 (1H, m, 5-H), 3.96 (1H, m, 2-H), 4.56 (1H, d, J = 6.0 Hz, 4-H), 4.75 (1H, dd, J = 6.0, 5.8 Hz, 3-H), 4.35 (1H, t, J = 11, 2 Hz, $-C\underline{H}_2OCO-CH_3$), 5.10 (1H, dd, J = 11.2, 6.0 Hz, $-C\underline{H}_2OCOCH_3$).

Enzyme Assays: Materials α-Glucosidase (from Bakers yeast, lot 83H8000), β-galactosidase (from bovine liver, lot 54H7025), α-mannosidase (from Jack beans, lot 48F95454) and β-mannosidase (from snail acetone powder, lot 45H3826) were obtained from Sigma Chemical Company (St. Louis, U.S.A.), and β-glucosidase (from sweet almond, lot 25240) was obtained from Toyobo Company (Osaka, Japan). *p*-Nitrophenyl-α-D-glucopyranoside, -β-D-glucopyranoside, -α-D-mannopyranoside and -β-D-galactopyranoside were obtained from Nacalai Tesque, Inc. (Osaka, Japan), *p*-nitrophenyl-β-D-mannopyranoside was from Sigma Chemical Company.

Assay of Inhibition of α-Glucosidase The reaction mixture consisted of 475 μl 0.1 M phosphate buffer (pH 7.0), 250 μl 250 mM *p*-nitrophenyl-β-D-glucopyranoside and 250 μl α-glucosidase solution (a stock solution of 1.0 mg/ml in 10 mM phosphate buffer (pH 7.0) was diluted 40 times with the same buffer, pH 7.0, just before the assay), for the substrates **1**, **2**, **3**, **4**, **5** and **6** (25 μl of solutions of concentration, 200—0.1 mg/ml). After incubation for 20 min at 37 °C and interrupting the reaction by addition of 1 ml 0.2 M sodium carbonate, the amount of *p*-nitrophenol liberated was measured colorimetrically at 400 nm (OD test). The inhibition rates (%) were calculated from the formula 100 − 100 × (OD test − OD blank)/(control OD test − control OD blank) and IC_{50} values were obtained from the inhibition curves. Assays for β-glucosidase, β-galactosidase, α-mannosidase and β-mannosidase were carried out as above using *p*-nitrophenyl-β-D-glucopyranoside, -β-D-galactopyranoside, -α-D-mannopyranoside and -β-D-mannopyranoside as substrates. The IC_{50} values are shown in Table 3.

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