A New Pyrrolizidine Alkaloid, Broussonetine N, as an Inhibitor of Glycosidase, from *Broussonetia kazinoki* SIEB. and Absolute Stereostructures of Broussonetines A and B[1)]

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A new pyrrolizidine alkaloid, broussonetine N, was isolated from the branches of *Broussonetia kazinoki* SIEB. (Moraceae). Broussonetine N was formulated as (1*R*,2*R*,3*R*,5*S*,8*R*)-1,2-dihydroxy-3-hydroxymethyl-5-[(1*R*)-1,10-dihydroxy-6-oxo-decyl] pyrrolizidine (1) by spectroscopic and chemical methods. 1 inhibited β-glucosidase, β-galactosidase, and β-mannosidase. Absolute stereostructures of broussonetines A (2) and B (3) as well as that of 1 were also determined by a new version of Mosher's method.

Key words pyrrolizidine alkaloid; glycosidase inhibitor; *Broussonetia kazinoki*; broussonetine N; broussonetine A; broussonetine B

Several structurally related monocyclic and bicyclic polyhydroxy pyrrolidines have been shown to be biologically active alkaloids such as competitive inhibitors of glycosidases,[2—5)] antiviral agents,[6)] and acaricides.[7)] In the course of our survey for biologically active constituents extracted from crude drugs with hot water, we reported 14 pyrrolidine alkaloids, broussonetines A—L and broussonetinines A and B from *Broussonetia kazinoki* SIEB. (Moraceae).[8—11)] The continuing search led us to isolate a new pyrrolizidine alkaloid, broussonetine N (**1**). This communication deals with the structural elucidation and its inhibitory activities against β-glucosidase, β-galactosidase, β-mannosidase, and some glycosidases, along with the absolute stereostructures of broussonetines A (**2**) and B (**3**).

The branches of this tree were extracted with hot water and the alkaloidal constituents were concentrated as reported in previous papers. Compound **1** was isolated by preparative HPLC (column: Asahipak ODP 5E [i.d. 10×250 mm]; solvent: CH_3CN–H_2O [10 : 90], adjusted to pH 12.0) of the concentrated alkaloids.

Compound **1** was obtained as a colorless oil, $[\alpha]_D$ +5.4° (MeOH, c= 0.17), 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 to be $C_{18}H_{33}NO_6$ on the basis of positive high-resolution secondary ion mass spectroscopy (pos. HR-SIMS) (*m/z*: 360.2392, $[M+H]^+$, error, +0.8 mmu). The IR spectrum showed a strong OH and NH band at 3436 cm^{-1} and a carbonyl band at 1699 cm^{-1}.

The ^{1}H-NMR spectra of **1** showed the presence of seven methylene groups (δ 1.38—2.30 [14H, m]), two oxymethylene groups (δ 4.23 [2H, m], δ 3.82 [2H, t, J=6.0 Hz]), three oxymethine groups (δ 4.75 [1H, t, J=6.0 Hz], δ 4.49 [1H, t, J=6.0 Hz], δ 4.40 [1H, m]), two methylene groups attached to a carbonyl group (δ 2.43 [2H, t, J=7.0 Hz], δ 2.37 [2H, t, J=7.0 Hz]), and three methine groups attached to a nitrogen atom (δ 4.17 [1H, m], δ 3.96 [1H, ddd, J=6.0, 6.0, 6.0 Hz], δ 3.40 [1H, ddd, J=6.5, 6.5, 2.0 Hz]).

Partial structures **A**—**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 bond correlation (HMBC) spectrum to establish the planar structure (Fig. 2).

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

The relative stereochemistry of the pyrrolizidine moiety in **1** was disclosed by the vicinal coupling constants ($J_{1,2}$= $J_{2,3}$=$J_{1,8}$=6.0 Hz) and nuclear Overhauser effects (NOEs) in the nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum, that is, NOEs were observed between H-1 and H-3, H-2 and H-8, and H-5 and H-8 to establish the 3α-hydroxymethyl-1α,2β-dihydroxy-5β-alkyl-8α-pyrrolizidine structure.

The absolute stereostructure of the pyrrolizidine moiety

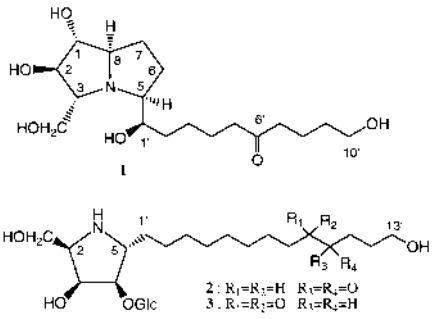

Fig. 1. Structures of Broussonetines N (**1**), A (**2**), and B (**3**)

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for **1**

	Broussonetine N (**1**)	
	Proton	Carbon
1	4.49 t (6.0)	81.45
2	4.75 t (6.0)	80.52
3	4.17 m	64.07
5	3.40 ddd (6.5, 6.5, 2.0)	65.36
6	1.95 m, 2.30 m	25.85
7	2.02 m, 2.20 m	29.06
8	3.96 ddd (6.0, 6.0, 6.0)	69.57
CH_2OH	4.23 m	63.72
1′	4.40 m	67.98
2′	1.52[a)], 1.70[a)]	35.95
3′	1.42 m, 1.69[a)]	26.03
4′	1.62 m	23.67
5′	2.37 t (7.0)	42.16
6′		211.30
7′	2.43 t (7.0)	41.95
8′	1.77[a)]	20.22
9′	1.72[a)]	32.09
10′	3.82 t (6.0)	61.05

ppm (Hz)

δ in pyridine-d_5. ^{1}H-NMR at 500 MHz. ^{13}C-NMR at 125 MHz. *a*) overlapped signals.

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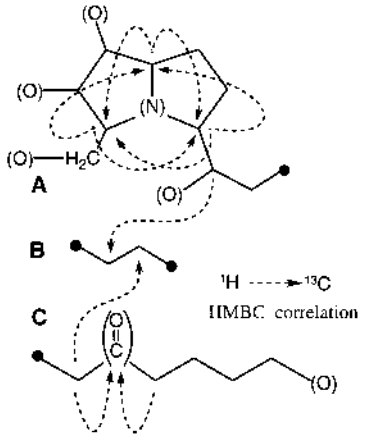

Fig. 2. Partial Structures (**A**—**C**) of **1** and HMBC Correlation

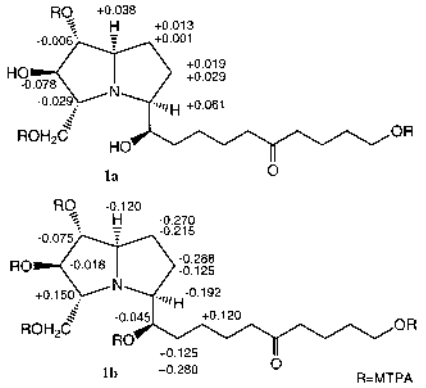

Fig. 3. $\Delta\delta$ Values Obtained for the MTPA Esters (**1a**, **1b**) of **1**

and C-1′ in **1** were determined by a new version of Mosher's method.[12] The tri (*S*)- and (*R*)-2-methoxy-2-phenyl-2-(trifluoromethyl) acetic acid (MTPA) esters (**1a***S*, **1a***R*) and penta (*S*)- and (*R*)-MTPA esters (**1b***S*, **1b***R*) were prepared from **1** and ^1H–^1H COSY (500 MHz) spectra were analyzed.[13] The $\Delta\delta$ ($=\delta_S-\delta_R$) values were measured, respectively; these values from **1a** established the (*R*) configuration at C-1 of the pyrrolizidine moiety and the values from **1b** built up the (*R*) configuration at C-1′ (Fig. 3).

Thus the structure of **1** was formulated as (1*R*,2*R*,3*R*,5*S*,8*R*)-1,2-dihydroxy-3-hydroxymethyl-5-[(1*R*)-1,10-dihydroxy-6-oxo-decyl] pyrrolizidine. Similarly, the absolute stereostructure of the pyrrolidine moiety of **2** was determined by a new version of Mosher's method, as shown in Fig. 4. The $\Delta\delta$ values from **2a** established the (*R*) configuration at C-4 of the pyrrolidine moiety. Thus the structure of **2** was formulated as (2*R*,3*S*,4*R*,5*R*)-2-hydroxymethyl-3-hydroxy-5-(10′-oxo-13′-hydroxytridecyl)-pyrrolidine-4-*O*-β-D-glucopyranoside. The $[\alpha]_D$ of **3** was similar to that of **2** and the relative stereostructures were the same.[9] Thus the absolute stereostructure of **3** was formulated as (2*R*,3*S*,4*R*,5*R*)-2-hydroxymethyl-3-hydroxy-5-(9′-oxo-13′-hydroxytridecyl)-pyrrolidine-4-*O*-β-D-glucopyranoside.

The inhibitory activities of **1** and 1-deoxynojirimycin (DNJ)[14,15] were assayed with respect to α-glucosidase, β-glucosidase, β-galactosidase, α-mannosidase, and β-mannosidase. **1** inhibited β-glucosidase (IC_{50} 6.7×10^{-6} M), β-galactosidase (IC_{50} 2.9×10^{-6} M), and β-mannosidase (IC_{50} 3.3×10^{-6} M), while DNJ inhibited β-glucosidase (IC_{50} 5.8×10^{-6} M).

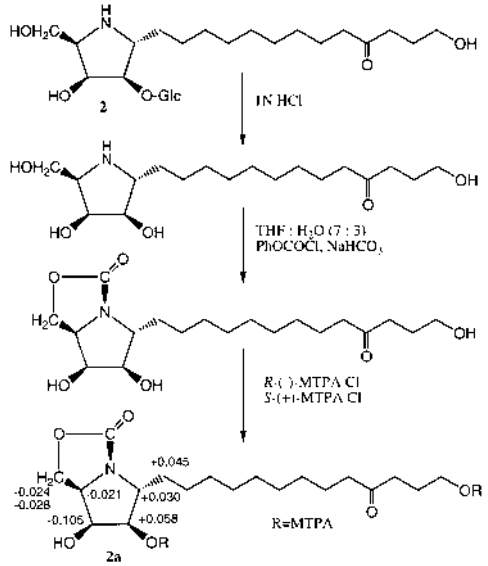

Fig. 4. $\Delta\delta$ Values Obtained for the MTPA Esters of **2**

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13) **1** (6 mg) and **2** (6 mg) were treated with (*R*)-(−)-, (*S*)-(+)-MTPA-Cl (20 μl) in pyridine (300 μl) at room temperature for 30 min, respectively, 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).
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