HETEROCYCLES, Vol. 57, No. 8, 2002, pp. 1539 - 1553, Received, 10th April, 2002

POLYHYDROXYLATED ALKALOIDS WITH LIPOPHILIC MOIETIES AS GLYCOSIDASE INHIBITORS FROM HIGHER PLANTS

Makio Shibano, Daisuke Tsukamoto, and Genjiro Kusano*

Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka, 569-1094, Japan

Abstract — Two new types of polyhydroxylated alkaloids, broussonetines and radicamines, which have an aliphatic or an aromatic moiety, were isolated from *Broussonetia kazinoki* (Moraceae) and *Lobelia chinensis* (Campanulaceae), respectively, as glycosidase inhibitors. Feeding experiments using [1-^{13}C] glucose and ^{13}C-NMR spectroscopic studies showed that broussonetines are biosynthesized through routes similar to those of sphingosine and phytosphingosine.

1. INTRODUCTION

Many polyhydroxylated alkaloids mimicking monosaccharides (1-deoxynojirimycin, (2*R*,3*R*)-bis-hydroxymethyl-(3*R*,4*R*)-dihydroxypyrrolidine, swainsonine, australine, calystegines, etc.) have been isolated from higher plants and found to inhibit various glycosidases.[1] Modulation of the activity of carbohydrate-recognizing enzymes using these sugar mimics of the relevant carbohydrates has enormous therapeutic potential. Accordingly, inhibitors of various glycosidase will be a new generation of carbohydrate-controlled therapeutic agents for many diseases; thus the control of *N*-linked oligosaccharide biosynthesis to alter tumor cells displaying aberrant glycosylation or to prevent syncytium formation of HIV on lymphocytes has therapeutic implications.[2] For these sugar mimics to become drugs, a good inhibitor must satisfy a number of conditions such as stability in the stomach and membrane permeability, which often require the presence of lipophilic moieties.[3]

2. BROUSSONETINES

2-1. Isolation of Broussonetines

The deciduous tree *Broussontia kazinoki* (Japanese name *himekouzo*), is distributed throughout China, Taiwan, Korea, and Japan. Its cortex is a raw material for the Japanese paper called *washi*. Its branches, leaves, and fruits have been used as a diuretic, a tonic, and a suppressant of edema in Chinese folk medicine.

Dried branches of *B. kazinoki* (9.5 kg, collected in a mountainous area south of Osaka in 1995) were cut finely and then extracted with hot water (90°C, 40 L, 3 times) for 2 h. The extracted solution was chromatographed on an Amberlite CG-50 (H^+-form) column (i.d. 6.5×30 cm, repeated 8 times). After washing the column with water and then with 50% methanol, the adsorbed material was eluted with 50% methanol—28% ammonia solution (9 : 1). The eluted fraction was concentrated *in vacuo* to give a basic fraction (46 g). This fraction was chromatographed on a Dowex 50W-X4 column (200—400 mesh, 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 M formic acid), eluted with gradient elution [H_2O (2.0 L)→H_2O—28% ammonia solution (9 : 1, 2.0 L)]. The five fractions containing broussonetines were rechromatographed on silica gel (Chromatorex DM1020) using chloroform and methanol, and preparative HPLC [column: Asahipak ODP 5E (i.d.10×250 mm); solvent: CH_3CN—H_2O (12—17 : 88—83), adjusted to pH 12.0 with ammonia solution; flow rate: 1.5 mL/min; column temperature: ambient] was performed to provide purified broussonetines A—X, J_1, J_2, M_1, U_1, and broussonetinines A and B. These alkaloids were isolated as glycosidase inhibitors and have a common functionalized pyrrolidine ring system except for broussonetines N (pyrrolizidine ring), U, and U_1 (pyrroline ring) and a long hydrocarbon chain at the 5-alkyl position of the pyrrolidine ring (Figure 1).

2-2. Structural Elucidation of Broussonetines A and B, and Broussonetinines A and B [4, 5]

Broussonetine A (**1**) was obtained as a colorless powder, mp 164—166°C, $[\alpha]_D$ +32.1 ° (*c* = 0.21, MeOH) and appeared as a yellow spot on TLC upon spraying with Ninhydrin reagent followed by heating on a hot plate (Ninhydrin reaction). The molecular formula was determined to be $C_{24}H_{45}NO_{10}$ by positive high resolution-secondary ion mass spectrometry (pos. HR-SIMS) ($[M+H]^+$: *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, which was also isolated from the same tree and named broussonetinine A (**2**), and D-glucose $[\alpha]_D$ + 47.0 ° (*c* = 0.33, H_2O). The ^{1}H-NMR spectrum of **2**

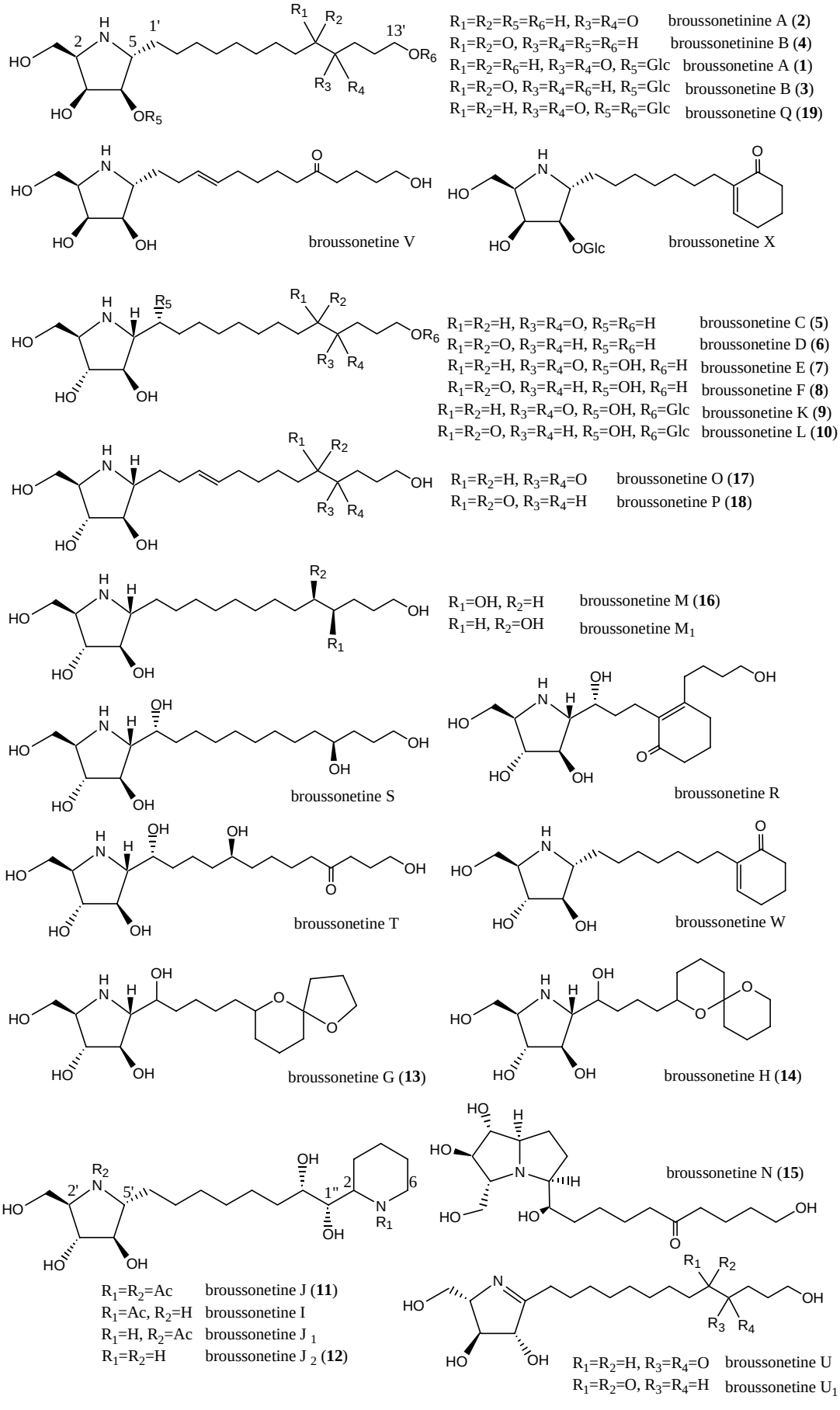


Figure 1. Structures of Broussonetines and Broussonetinines A and B

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 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 of **1** 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), and led to elucidation of the partial structures. The linkages among these partial structures were determined from the heteronuclear multiple bond correlation (HMBC) spectrum, as shown in Figure 2.

The relative stereochemistry of **2** was investigated by chemical conversion and nuclear Overhauser effect (NOE) experiments. NOE was observed between 2-H and 4-H in the NOE experiment of an acetate (**2a**) to establish a 2, 3-*cis*, and 3, 4-*cis* orientation (Figure 3).

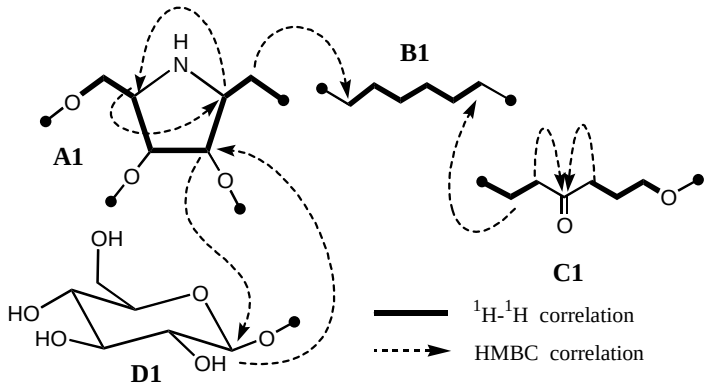


Figure 2. The Partial Structures and HMBC

Therefore **2** was formulated as 2β-hydroxymethyl-3β,4β-dihydroxy-5α-(10-oxo-13-hydroxytridecyl)pyrrolidine or its enantiomer. The structure of **1** was concluded to be the 4-*O*-β-D-glucopyranoside of **2**. The glucosylation shift was 10 ppm between the C-4 of **1** and that of **2**.

Broussonetine B (**3**) was obtained as a colorless powder, mp 154—156℃, $[\alpha]_D$ + 29.8 ° (*c* = 0.43, MeOH). 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-SIMS ($[M+H]^+$: *m/z* 508.3122). The IR spectrum showed a strong hydroxyl band at 3400 cm^{-1} and a carbonyl band at 1705 cm^{-1}.

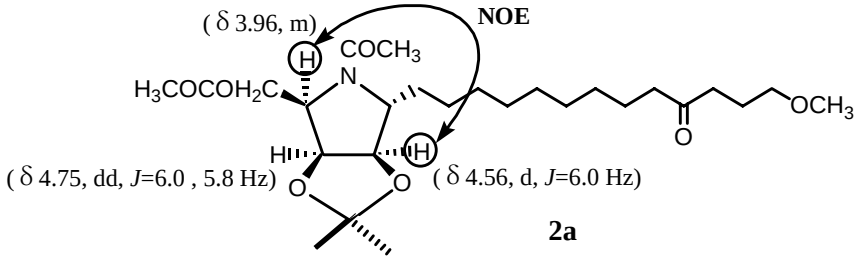


Figure 3. NOE of **2a**

The ^{1}H- and ^{13}C-NMR spectra were strikingly similar to those of **1**, and the ^{1}H-^{1}H COSY spectra of **3** and

its aglycone, which was prepared by acidic hydrolysis, also isolated from the same tree, and named broussonetinine B (**4**), showed the presence of the partial structure $COCH_2CH_2CH_2CH_2OH$ instead of $COCH_2CH_2CH_2OH$ as in **1** and **2**.

The absolute stereostructure of the pyrrolidine moiety of **2** was determined by a new version of Mosher's method.[6] The (*S*)- and (*R*)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA) esters (**2a***S*, **2a***R*) were prepared from **2**, and the ^1H-^1H COSY (500 MHz) spectra were analyzed. The $\Delta\delta$ ($=\delta_S-\delta_R$) values were determined, and these values for **2a** established the (*R*) configuration at C-4 of the pyrrolidine moiety (Figure 4).

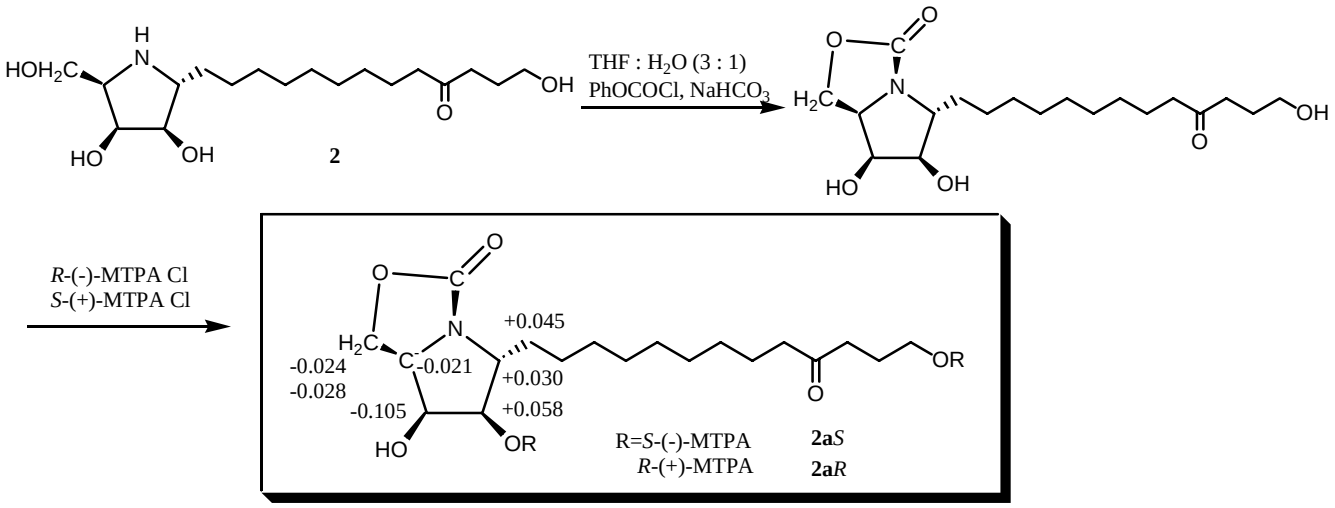


Figure 4. $\Delta\delta$ Values Obtained for the MTPA Esters of **2**

Thus the absolute stereostructure of **1** was determined to be (2*R*, 3*S*, 4*R*, 5*R*)-2-hydroxymethyl-3-hydroxy-5-(10-oxo-13-hydroxytridecyl)pyrrolidine-4-*O*-β-D-glucopyranoside. Similarly, **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.

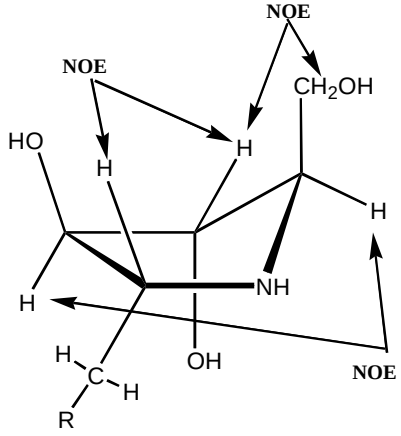


Figure 5. NOEs of **5**

2-3. Structural Elucidation of Broussonetines C—F, K, and L [4, 7, 8]

Broussonetine C (**5**) was obtained as a colorless powder, mp 147—149 °C, $[\alpha]_D$ + 25.0° (*c* = 0.96, MeOH), and the molecular formula was determined to be $C_{18}H_{35}NO_5$ by pos. HR-SIMS ($[M+H]^+$, *m/z*: 346.2579). The IR specrum showed a strong hydroxyl band at 3370 cm^{-1} and a carbonyl band at 1706 cm^{-1}. The ^1H- and ^{13}C-NMR spectra were strikingly similar to those of **2**. When **5** was treated with *p*-toluenesulfonic acid in acetone to

prepare an acetonide, no acetonide was obtained. **5** and **2** were epimeric compounds with the molecular formula $C_{18}H_{34}NO_5$. The relative stereostructure of the pyrrolidine ring moiety (2β-hydroxymethyl-3α,4β-dihydroxy-5α-alkylpyrrolidine structure) was revealed by NOEs in the nuclear Overhauser and exchange spectroscopy (NOESY) experiments, as shown in Figure 5.

The absolute stereostructure was determined to be (2*R*,3*R*,4*R*,5*R*) using the following benzoate chirality method. A diacetylacetoamide (**5a**) was prepared selectively from **5** by treatment with acetic anhydride in pyridine at room temperature, and then a dibenzoate (**5b**) was obtained by benzoylation of **5a** and purification of the products in preparative HPLC. The circular dichroism (CD) curve of **5b** showed a negative Cotton effect ($\Delta\varepsilon_{237}$ -15.9) and a positive effect ($\Delta\varepsilon_{223}$ +16.4) to establish the chiral arrangement in a counterclockwise manner as depicted in the stereochemical drawing of Figure 6.[9,10]

Broussonetine D (**6**) was obtained as a colorless powder, mp 136—138 ℃, $[\alpha]_D$ + 22.9 ° (*c* = 0.31, MeOH). It appeared as a yellow TLC spot by in the Ninhydrin reaction, and the molecular formula was determined to be $C_{18}H_{35}NO_5$ by pos. HR-SIMS ($[M+H]^+$, *m/z* 346.2586). The IR spectrum showed a strong hydroxyl band at 3405 cm^{-1} and a carbonyl band at 1704 cm^{-1}. The ^1H-^1H COSY spectrum suggested the presence of the partial structure $COCH_2CH_2CH_2CH_2OH$ instead of $COCH_2CH_2CH_2OH$ as in **5**. These results led to the conclusion that **6** is a structural 9'-oxo isomer to **5** (10'-oxo compound) .

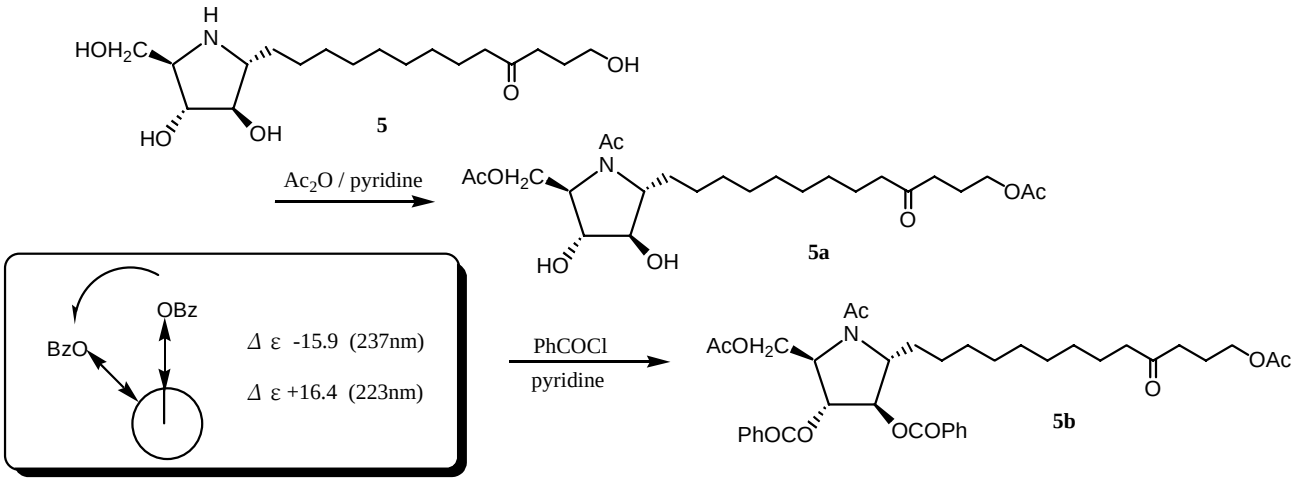


Figure 6. Chiral Arrangement of **5**

Broussonetine E (**7**) was obtained as a colorless powder, mp 103—105℃, $[\alpha]_D$ + 4.9 ° (*c* = 1.06, MeOH), and the molecular formula was determined to be $C_{18}H_{35}NO_6$ by pos. HR-SIMS ($[M+H]^+$: *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 ^1H-NMR spectrum was strikingly similar to that of **5**, 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 **5** (δ 4.44) as shown Figure 7. Thus **7** was formulated as 2 β -hydroxymethyl-3 α ,4 β -dihydroxy-5 α -(1,13-dihydroxy-10-oxotridecyl)pyrrolidine or its enantiomer.

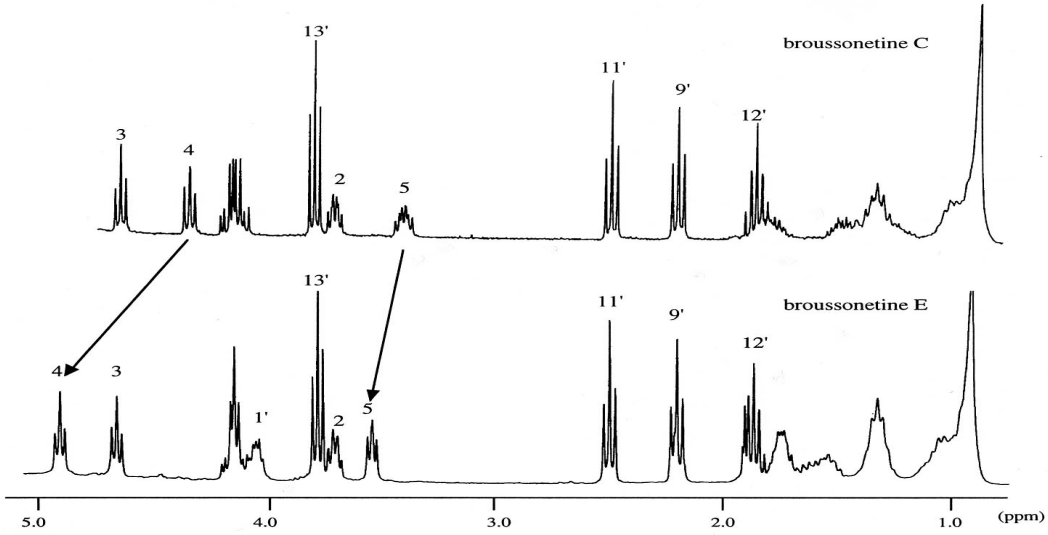


Figure 7. Comparison of ^{1}H-NMR Spectra of Broussonetines C (**5**) and E (**7**)

Broussonetine F(**8**) was obtained as a colorless powder, mp 105—107 ℃, $[\alpha]_D$ + 13.5 ° (*c* = 0.93, MeOH), and the molecular formula was determined to be $C_{18}H_{35}NO_6$ by pos. HR-SIMS ($[M+H]^+$: *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 suggested that the same structural relationships were found between **7** and **8** as between **1** and **3**, and **5** and **6**. Thus **8** was formulated as 2 β -hydroxymethyl-3 α , 4 β -dihydroxy-5 α -(1, 13-dihydroxy-9- oxotridecyl)pyrrolidine or its enantiomer.

Broussonetines K (**9**) and L (**10**) were 13'-*O*- β -D-glucopyranosylated derivatives of **7** and **8**, respectively. The absolute stereochemistry of the pyrrolidine ring moiety of **8** was determined to be (2*R*, 3*R*, 4*R*, 5*R*) using the following benzoate chirality method. Cyclic carbamate (**8a**) was prepared from **8** by reaction with phenyl chloroformate in THF : H_2O (7 : 3), and monoacetate (**8b**) and diacetate (**8c**) were prepared from **8a** with acetic anhydride in pyridine at room temperature, and dibenzoate (**8d**) was obtained by benzolation of **8c** and purification of the products by preparative HPLC. The CD curve of **8d** showed a negative Cotton effect ($\Delta\varepsilon_{237}$ -30.9) and a positive Cotton effect ($\Delta\varepsilon_{223}$ +15.9) to establish the chiral arrangements in a counterclockwise manner (Figure 8).

Additionally, a new version of Mosher's method was applied to determine the absolute configuration of C-1' in **8**. The di (*S*)- and (*R*)-MTPA esters and tri (*S*)- and (*R*)-MTPA esters prepared from **8a** were

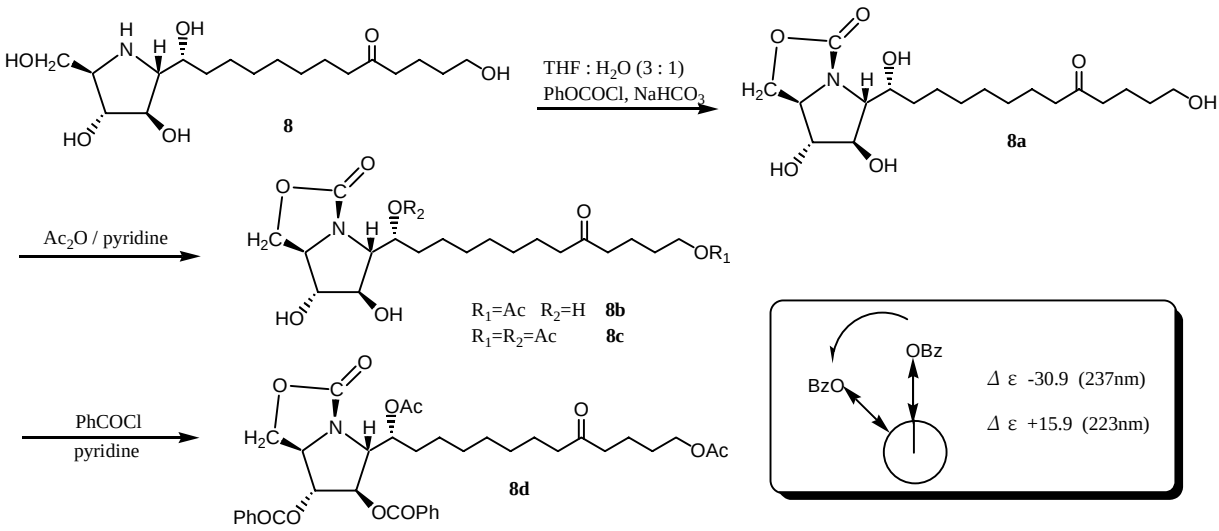


Figure 8. Chiral Arrangement of **8**

assigned by ^{1}H-^{1}H COSY (500 MHz) and the $\Delta\delta$ ($=\delta_S-\delta_R$) values were determined. These values thereby established the (*R*) configuration at C-1' of **8a** by comparison between di-MTPA esters and tri-MTPA esters. Thus the absolute stereostructure of **10** was formulated as (2*R*,3*R*,4*R*,5*R*)-2-hydroxy-methyl-3,4-dihydroxy-5-[(1*R*)-1-hydroxy-9-oxo-13-(β-D-glucopyranosyloxy) tridecyl]pyrrolidine, and **9** was concluded to be (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(1*R*)- 1-hydroxy-10-oxo-13-(β-D-glucopyranosyloxy)tridecyl]pyrrolidine by comparison of the $\Delta\delta$ values patterns of tri-MTPA esters and those of **8**. [8]

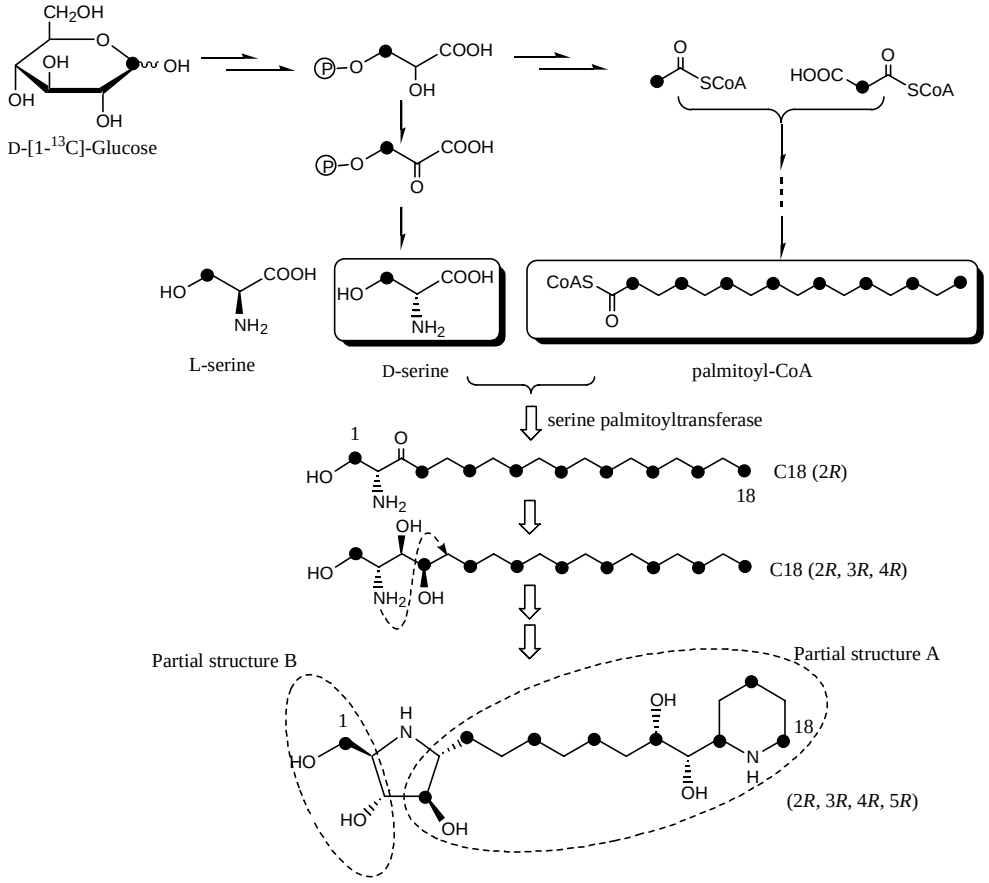
2-4. Other Broussonetines

We isolated many other broussonetines and reported their structures. Broussonetines I, J (**11**), J_1, and J_2 (**12**) have a piperidine ring system at the end of the C-5 alkyl chain,[11,12] and broussonetines G (**13**) and H (**14**) have a unique spiroacetal functionality.[13] Broussonetine N (**15**) is the first pyrrolizidine alkaloid from Moraceae plants.[14] Broussonetines R, W, and X have a cyclohexanone moiety at the side chain, and broussonetines U and U_1 are pyrroline alkaloids.[12,15] All broussonetines proved to have 18-carbon chain skeletons with characteristic structures.

2-5. Biosynthesis of Broussonetines[16]

All broussonetines have 18-carbon chain skeletons. To clarify the biosynthetic route of these alkaloids, we grew the plant on aseptic medium and analyzed the enriched ^{13}C of the isolated alkaloids after feeding with [1-^{13}C] glucose. Broussonetine J (**11**) was isolated from 50% methanol extracts of the plantlets fed

with [1-^{13}C] glucose. The ^{1}H- and ^{13}C-NMR spectra of **11** were too complicated to analyze, suggesting the presence of rotational isomers of two amides. Therefore **11** was hydrolyzed with 1 N HCl to yield **12**. The ^{13}C-NMR spectrum of **12** showed the presence of clear enrichment of nine signals (C1, C4, C6, C8, C10, C12, C14, C16, and C18). These enrichment signals were found regularly in every carbon, but C2 and C3 were enriched irregularly. The labeling pattern indicated that a partial structure A (Scheme 1) was formed *via* palmitoyl CoA through the acetate-malonate pathway, whereas the structure formed by C1, C2, and C3 (partial structure B) was *via* serine from 3-phosphoglyceric acid. Thus the 18-carbon chain of **11** was assumed to be formed initially by condensation of serine and palmitoyl-CoA. This assumption was supported by the biosynthesis of sphingosine (or sphinganine) and phytosphingosine (or 4-hydroxysphinganine).



Scheme 1

A number of sphingosine-related metabolites have been isolated from marine microorganisms and sponges.[17] Sphingosine is a long-chain amino alcohol that generally has 18 carbon atoms. The committed step in *de novo* sphingolipid synthesis proved to begin with the condensation of serine and palmitoyl-CoA to produce an 18-carbon unit such as sphingosine and phytosphingosine. Recently, enzymes of

sphingolipid metabolism in plants have been investigated,[18] although these sphingosine-related compounds as secondary metabolites have not been isolated from higher plants to our knowledge. If broussonetines are biogenetically related to sphingosine derivatives, they would be formed *via* them by serine-palmitoyltransferase with several other hydroxylation, reduction, cyclization, and other reactions (Scheme 1). This hypothesis was also supported by the facts that under the same experimental conditions we also obtained broussonetines C and E and their labeling patterns corresponded with those of **11**.[16] Because broussonetines appear to be biosynthesized through intermediates related to sphingosines, which play important roles in biological processes in animals and marine organisms, further research is ongoing to determine whether broussonetines are biosynthesized through sphingosine-related metabolites in higher plants.

Table 1. Concentration of Inhibitor Required to Produce 50% Inhibition of Enzyme Act

	α-Glucosidase (Yeast)	β-Glucosidase (Sweet Almond)	β-Galactosidase (Bovine Liver)	α-Mannosidase (Jack Beans)	β-Mannosidase (Snail)
DNJ	9.3×10^{-7}	5.8×10^{-7}	NI	NI	NI
DMJ	NI	NI	NI	9.4×10^{-7}	8.1×10^{-7}
DGJ	NI	NI	1.3×10^{-7}	NI	NI
1	NI	NI	NI	NI	NI
2	NI	NI	1.6×10^{-7}	3.0×10^{-7}	NI
3	NI	NI	NI	NI	NI
4	NI	NI	1.0×10^{-7}	2.9×10^{-7}	NI
5	NI	NI	3.6×10^{-8}	NI	1.0×10^{-7}
6	NI	NI	2.9×10^{-8}	NI	1.0×10^{-7}
7	3.3×10^{-6}	5.5×10^{-8}	2.0×10^{-9}	NI	2.3×10^{-8}
8	1.5×10^{-6}	1.0×10^{-8}	4.1×10^{-9}	NI	2.8×10^{-8}
9	NI	2.6×10^{-8}	5.0×10^{-9}	NI	3.0×10^{-7}
10	NI	1.7×10^{-8}	4.0×10^{-9}	NI	2.0×10^{-7}
11	NI	NI	NI	NI	NI
13	NI	2.4×10^{-8}	2.0×10^{-9}	NI	7.6×10^{-7}
14	NI	3.6×10^{-8}	3.2×10^{-9}	NI	3.2×10^{-7}
15	NI	5.8×10^{-7}	2.9×10^{-7}	NI	3.3×10^{-7}
16	NI	NI	8.1×10^{-6}	NI	NI
17	NI	1.4×10^{-6}	1.7×10^{-7}	NI	8.2×10^{-6}
18	NI	2.4×10^{-6}	2.0×10^{-7}	NI	7.6×10^{-6}
19	NI	1.4×10^{-6}	6.0×10^{-7}	NI	2.0×10^{-5}

NI, no inhibition. (M)

2-9. Glycosidase Inhibitory Activities

The inhibitory activities of broussonetines, 1-deoxynojirimycin (DNJ), 1-deoxymannojirimycin (DMJ), and 1-deoxygalactonojirimycin (DGJ) were assayed with respect to α-glucosidase (from yeast), β-

glucosidase (from sweet almond), β-galactosidase (from bovine liver), α-mannosidase (from jack bean), and β-mannosidase (from snail). The results are shown in Table 1.

3. RADICAMINES

3-1. Isolations of Radicamines

Lobelia chinensis (Campanulaceae) (Japanese name *azemushiro*), is distributed throughout China, Taiwan, Korea, and Japan. The whole plants have been used as a diuretic, an antidote, hemostat, and as carcinostatic agents for stomach cancer in Chinese folk medicine. Various alkaloids, for example, lobeline, lobelanine, lobelanidine, and others are known constituents of this herb. Dried *L. chinensis* (0.6 kg, collected in Takatsuki City, Osaka, in 2000) were cut finely and then extracted with 50% MeOH (20 L) for 2 h. The extracted solution was chromatographed on an Amberlite CG-50 (H^+-form) column (i.d. 6.5 ×30 cm). After washing the column with water and 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 (2.5 g). This fraction was chromatographed on a Dowex 50W-X4 column (200－400 mesh, i.d. 5.0×15 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 (1.0 L)→H_2O－28% ammonia solution (9 : 1, 1.0 L)]. The fraction containing radicamines A and B was rechromatographed on HPLC (column: Shodex NH_2-P (i.d. 4.6×250 mm); solvent: CH_3CN－H_2O (80 : 20), 1.0 ml/min; column temperature: ambient. Radicamine A (**20**) (15 mg) and radicamine B (**21**) (1.2 mg) were finally obtained (Figure 9).

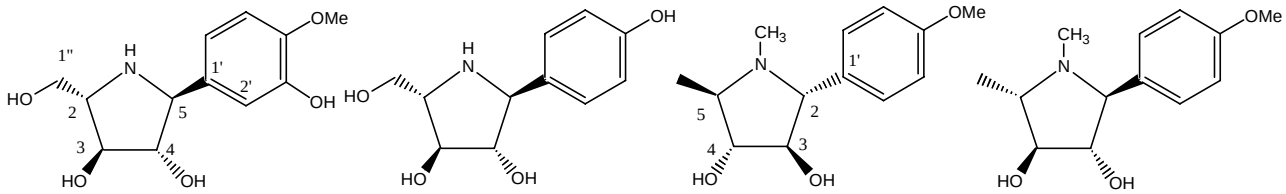


Figure 9. Structures of **20**, **21**, (-)-Condonopsinine, and (+)-Condonopsinine

3-1. Structural Elucidation of Radicamines A and B [19]

Radicamine A (**20**) was obtained as a pale yellow oil, $[\alpha]_D$ +43.7° (*c* = 0.13, H_2O), showing a brownish spot on TLC after the Ninhydrin reaction, and the molecular formula was determined to be $C_{12}H_{17}NO_5$ on the basis of pos. HR-SIMS (*m/z*: 256.1188, $[M+H]^+$, error, +0.4 mmu). The IR spectrum showed a

strong OH and NH band at 3386 cm^{-1} and benzenoid bands at 1596 and 1516 cm^{-1}. The ^{1}H-NMR spectrum of **20** showed the presence of an oxymethyl group (δ 3.84 [3H, s]), an oxymethylene group (δ 3.67 [1H, dd, *J* =6.4, 11.6 Hz], δ 3.73 [1H, dd, *J* =4.3, 11.6 Hz]), two oxymethine groups (δ 3.93 [1H, t, *J*=7.3 Hz], δ 4.06 [1H, dd, *J*=7.3, 8.9 Hz]), two methine groups attached to a nitrogen atom (δ 3.24 [1H, m], δ 3.86 [1H, d, *J*=8.9 Hz]), and a 1, 3, 4-trisubstituted benzene ring (δ 6.94 [1H, br.s], δ 6.93 [1H, dd, *J*=2.0, 8.6 Hz], δ 7.02 [1H, d, *J*=8.6 Hz]). Partial structures were obtained by tracing ^{1}H-^{1}H COSY cross-peaks and they were connected on the basis of the HMBC spectrum and characteristic mass fragment ion (*m/z* 131) to establish the planar structure (Figure 10). The relative stereochemistry of the pyrrolidine moiety in **20** was determined based on NOEs [between H-2 and H-4, H-3 and H-5, *O*-Me and H-5'] in the NOESY spectrum to establish the structure as 2 α -hydroxymethyl-3 β ,4 α -dihydroxy-5 β -(3-hydroxy-4-methoxyphenyl)pyrrolidine (Figure 11).

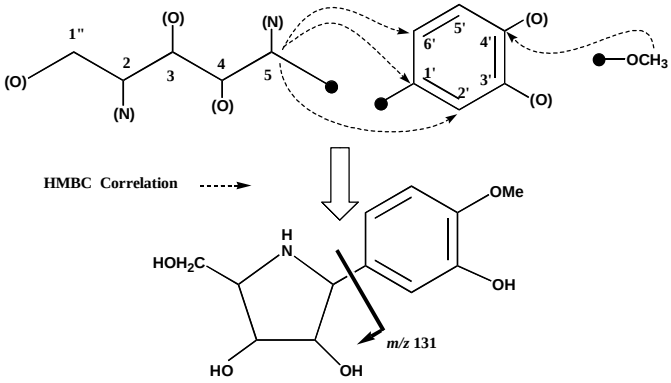


Figure 10. The Planar Structure of **20**

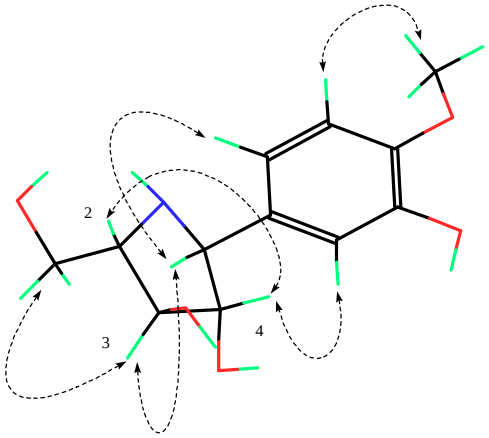


Figure 11. NOEs of **20**

Radicamine B (**21**) was obtained as a pale yellow oil, [α]$_D$ +72.0° (*c* = 0.10, H_2O), showing a brownish spot on TLC by ninhydrin reaction. The molecular formula was determined to be $C_{11}H_{15}NO_4$ on the basis of pos. HR-SIMS (*m/z*: 226.1073, $[M+H]^+$, error, -0.5 mmu). The IR spectrum showed a strong OH and NH band at 3402 cm^{-1} and benzenoid bands at 1615 and 1519 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra were similar to those of **20**. These spectra suggested the presence of a 1,4-disubstituted benzene ring partial structure, instead of a 1,3,4-trisubstituted benzene ring.

The absolute configuration of the pyrrolidine moiety of **20** and **21** was derived from the [α]$_D$ value by comparison with (+)-codonopsinine 〔[α]$_D$ +12.5° (*c*= 2.55, MeOH)〕 and (-)-codonopsinine 〔[α]$_D$ –11.8° (*c*= 0.69, MeOH)〕.[20, 21] *N*-Methylradicamines A (**20a**) and B (**21a**) were prepared from **20** and **21**

by treatment with H_2, 10% Pd-C, and HCHO in MeOH at room temperature. The $[\alpha]_D$ values of **20a** and **21a** were $[\alpha]_D$ +6.3° (*c*= 0.80, MeOH) and $[\alpha]_D$ +8.3° (*c*= 0.05, MeOH), respectively. Therefore, radicamines A and B were concluded to be (2*S*,3*S*,4*S*,5*S*)-2-hydroxymethyl-3,4-dihydroxy-5-(3-hydroxy-4-methoxyphenyl)pyrrolidine and (2*S*,3*S*,4*S*,5*S*)-2-hydroxymethyl-3,4-dihydroxy-5-(4-hydroxyphenyl)pyrrolidine, respectively. The absolute stereostructures were supported by the results of the following benzoate chirality method. A tribenzoate (**20d**) and two dibenzoates (**20b**, **20c**) were obtained by benzoylation of **20a** and purification of the products by preparative HPLC. The CD difference curve between **20b**, **20c**, and **20d** showed a positive Cotton effect ($\Delta\varepsilon_{235}$ +7.9) and a negative Cotton effect ($\Delta\varepsilon_{218}$ -1.0) to establish the chiral arrangement in a clockwise manner (Figure 12).

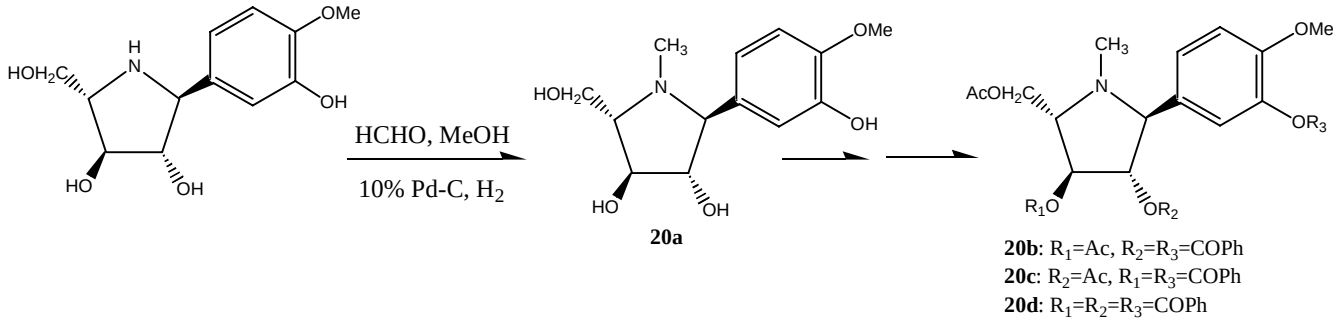


Figure 12. Structures of **20a**, **b**, **c**, and **d**

3-2. α-Glycosidase Inhibitory Activities

The inhibitory activities of **20**, **21**, and (2*R*,3*R*)-bishydroxymethyl-(3*R*,4*R*)-dihydroxypyrrolidine (DMDP) were assayed with respect to α-glucosidase (from yeast) by the modified method of Dahlquist.[22]

The IC_{50} values were found to be 6.7×10^{-6} M for **20**, 9.3×10^{-6} M for **21**, and 4.9×10^{-6} M for DMDP, respectively. These two new compounds, which are polyhydroxy alkaloids with an aromatic ring, could show interesting biological activities, similar to those of 1-deoxynojirimycin. We will synthesize and survey related compounds.

4. DISCUSSION

Over one hundred polyhydroxylated alkaloids have been isolated from plants and microorganism. These alkaloids, which can be potent and highly selective glycosidase inhibitors, promise a new generation of carbohydrate-based therapeutic for the control of various diseases including diabetes, cancer and viral

infections. Accordingly, polyhydroxylated alkaloids with lipophilic moieties (broussonetines and radicamines) can gain a reputation as a new class of glycosidase inhibitor from natural products.

REFERENCES

1 a) N. Asano, R.J. Nash, R. J. Molyneux, and G. W. J. Fleet, *Tetrahedron: Asymmetry*, 2000, **11**, 1645. b) A.A. Watson, G. W. J. Fleet, N. Asano, R. J. Molyneux, and R.J. Nash, *Phytochemistry*, 2001, **56**, 265.

2 a) R. Saul, J.P. Chambers, R.J. Molyneux, and A.D. Elbein , *Arch. Biochem. Biophys*., 1983, **221**, 593. b) R. Saul, R.J. Molyneux, and A.D. Elbein, *Arch. Biochem. Biophys*., 1984, **230**, 668. c) B. Winchester and G.W. Fleet, *J. Carbohydrate Chem.*, 2000, **19**, 471.

3 I. Robina, A.J. Moreno-Vargas, J.G. Fernandes-Bolanos, J. Fuentes, R. Demange, and P. Vogel, *Bioorg. Med. Chem. Lett*., 2001, **11**, 2555.

4 M. Shibano, S. Kitagawa, and G. Kusano, *Chem. Pharm. Bull*., 1997, **45**, 505.

5 M. Shibano, D. Tsukamoto, and G. Kusano, *Chem. Pharm. Bull*., 1999, **47**, 907.

6 I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Am. Chem. Soc*., 1991, **113**, 4092.

7 M. Shibano, S. Kitagawa, S. Nakamura, N. Akazawa, and G. Kusano, *Chem. Pharm. Bull*., 1997, **45**, 700.

8 M. Shibano, S. Nakamura, N. Motoya, and G. Kusano, *Chem. Pharm. Bull*., 1999, **47**, 472.

9 M. Koreeda, N. Harada, and K. Nakanishi, *Chem. Commun*.,**1969**, 548.

10 N. Harada, H. Sato, and K. Nakanishi, *Chem. Commun*.,**1970**, 1691.

11 M. Shibano, S. Nakamura, M. Kubori, K. Minoura, and G. Kusano, *Chem. Pharm. Bull*., 1998, **46**, 1416.

12 D. Tsukamoto, M. Shibano, and G. Kusano, *Chem. Pharm. Bull*., 2001, **49**, 1487.

13 M. Shibano, S. Nakamura, N. Akazawa, and G. Kusano, *Chem. Pharm. Bull*., 1998, **46**, 1048.

14 M. Shibano, D. Tsukamoto, R. Fujimoto, Y. Masui, H. Sugimoto, and G. Kusano, *Chem. Pharm. Bull*., 2000, **48**, 1281.

15 D. Tsukamoto, M. Shibano, R. Okamoto, and G. Kusano, *Chem. Pharm. Bull*., 2001, **49**, 492.

16 M. Shibano, D. Tsukamoto, T. Inoue, Y. Tasase, and G. Kusano, *Chem. Pharm. Bull*., 2001, **49**, 504.

17 J. Kobayashi and M. Ishibashi, *Heterocycles*, 1996, **42**, 943.

18 D.V. Lynch, *Methods Enzymol.*, 2000, **311**, 130.

19 M. Shibano, D. Tsukamoto, A. Masuda, Y. Tanaka, and G. Kusano, *Chem. Pharm. Bull*., 2001, **49**,

1362.

20 H. Iida, N. Yamazaki, and C. Kibayashi, *J. Org. Chem.*, 1987, **52**, 1956.

21 H. Yoda, T. Nakajima, and K. Tanabe, *Tetrahedron Lett.*, 1996, **37**, 5531.

22 A. Dahlqvist, *Anal. Biochem.*, 1964, **7**, 18.