The Biosynthesis of Broussonetines: Origin of the Carbon Skeleton

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Broussonetines are glycosidase-inhibitory alkaloids obtained from *Broussonetia kazinoki*. 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.

Key words biosynthesis; *Broussonetia kazinoki*; broussonetine J; broussonetine C; broussonetine E

Several structurally related monocyclic and bicyclic polyhydroxy pyrrolidines have been shown to be biologically active alkaloids, such as competitive inhibitors of glycosidases,[1—4] antiviral agents,[5] and acaricides.[6] In the course of our survey of biologically active constituents extracted from crude drugs with hot water, we reported 20 pyrrolidine alkaloids, broussonetines A—H, K—T, U, V, broussonetinines A and B, 2 pyrrolidiny piperidine alkaloids, broussonetines I and J, one pyrrolizidine, one pyrroline alkaloid, and broussonetines N and U from *Broussonetia kazinoki* SIEB. (Moraceae).[7—14] All broussonetines proved to have 18-carbon chain skeletons with characteristic structures (Fig. 1). 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. In this communication, we report the primary results of labeling of the alkaloids of this plant after feeding with [1-^{13}C]glucose.

Petioles of *Broussonetia kazinoki* were cultured on Murasige and Skoog (MS) gellan gum medium supplemented with 3% sucrose and 2,4-dichlorophenoxy acetic acid (2, 4-D) 1 mg/l.[15] Callus obtained after one week of culture was propagated on MS gellan gum medium containing 3% sucrose and 6-benzyladenine (6-BA) 1 mg/l. Multiple shoots were obtained after the callus was cultured on 1/3 MS gellan gum medium containing 1% sucrose and 6-BA 1 mg/l under light (3000 lux) for 7 weeks at 25 °C. The plantlets were grown on hormone-free 1/3 MS gellan gum medium containing 1% sucrose under light (3000 lux) for 2 weeks at 25 °C and [1-^{13}C]glucose 2 g was added to the medium.[16] After 20 d, broussonetine J (**1**) was isolated from 50% methanol extracts of the plantlets fed with [1-^{13}C]glucose by previously reported methods. The ^{1}H- and ^{13}C-NMR spectra of **1** were too complicated to analyze, suggesting the presence of rotational isomers of two amides.[10] Therefore compound **1** was hydrolyzed with HCl 1 N to yield compound **2**, which showed homogenous NMR spectra as described in a previous report.[10]

The ^{13}C-NMR spectrum of **2** showed the presence of clear enrichment of the nine signals (C1, C4, C6, C8, C10, C12, C14, C16, and C18) (Fig. 2, Table 1). The relative ^{13}C signal intensities of the native and ^{13}C-enriched product were compared and analyzed to determine the degree of isotopic enrichment. These values were obtained by first normalizing all ^{13}C resonance intensities to the intensity of the ^{13}C signal of C5. The degree of enrichment was then determined by calculating the ratio between each normalized resonance intensity in the labeled sample and its counterpart in the intensities from the native compound **2**. These enrichment signals were

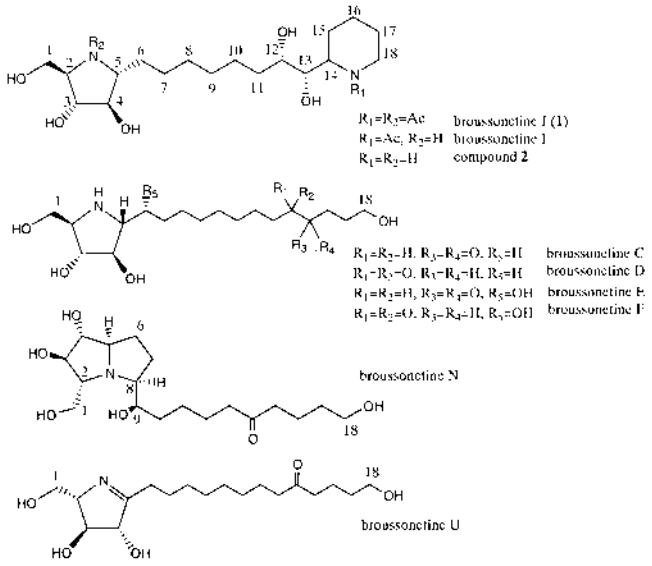

Fig. 1. Structures of Broussonetines

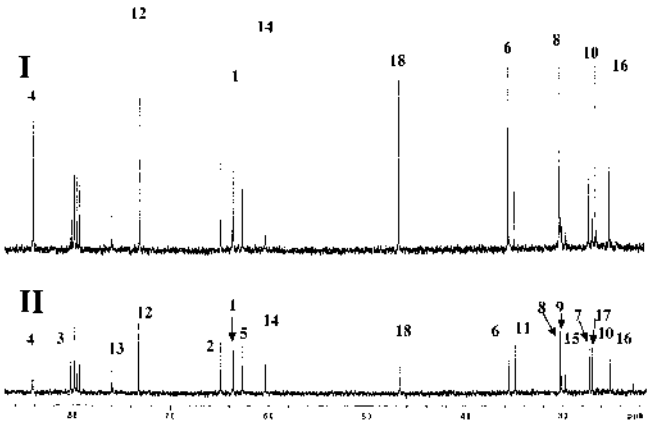

Fig. 2. ^{13}C-NMR Spectra of Unlabeled **2** (II) and **2** Derived from D-[1-^{13}C]Glucose (I)

Table 1. ^{13}C-NMR Data of Labeled **2**

	δ (ppm)	Peak height[a)]		δ (ppm)	Peak height[a)]
1	63.55	21.5	10	26.43	24.3
2	64.83	5.1	11	34.82	−0.6
3	80.12	1.4	12	73.09	28.1
4	84.10	19.8	13	75.87	0.0
5	62.61	0.0	14	60.28	27.4
6	35.48	25.7	15	29.63	−2.1
7	27.19	2.0	16	25.03	22.6
8	30.20	25.0	17	26.81	−0.7
9	30.16	0.3	18	46.62	22.1

a) The signal intensities were normalized to C5 and compared with those of unlabeled **2**.

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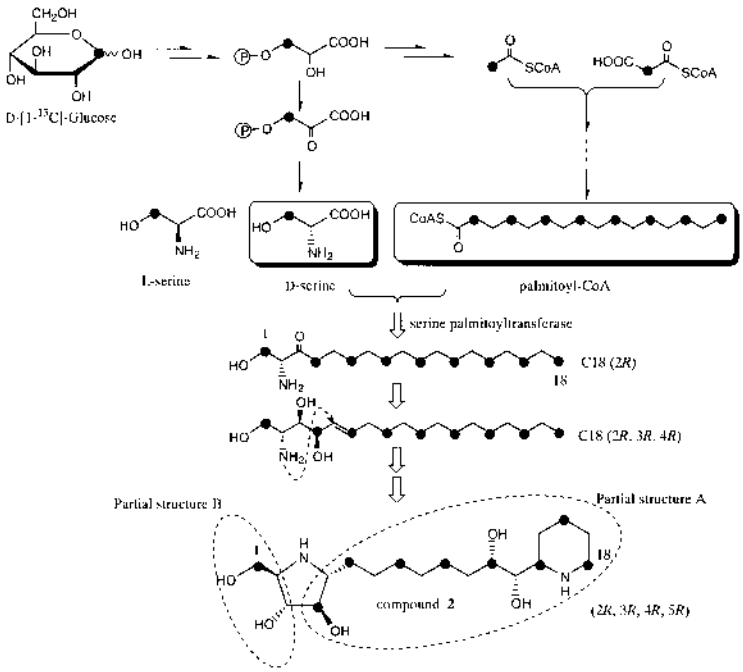
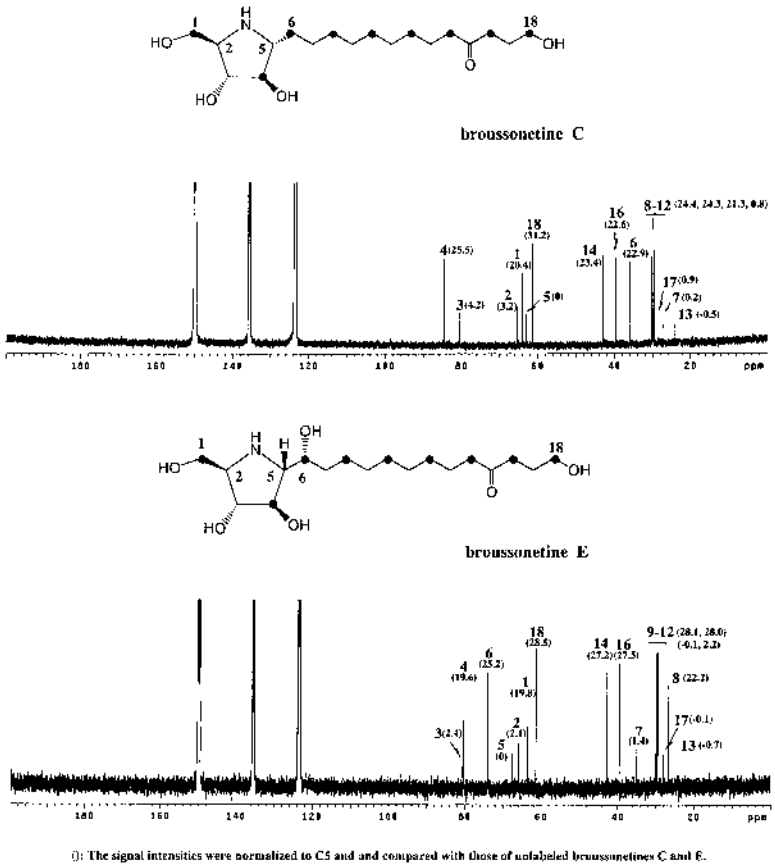


Chart 1



†: The signal intensities were normalized to C5 and and compared with those of unlabeled broussonetines C and E.

Fig. 3. ^{13}C-NMR Spectrum of Broussonetines C and E Derived from D-[1-^{13}C]Glucose

found regularly in every other carbon, but C2 and C3 were enriched irregularly, as shown in Fig. 2. The labeling pattern indicated that a partial structure A (Chart 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 **1** 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-hydroxysphin-

ganine).

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 other several hydroxylation, reduction, cyclization, and other reactions (Chart 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 **1**, as shown in Fig. 3.

As shown in Chart 1, the absolute stereostructures of the pyrrolidine moieties of broussonetines are related to D-serine, and that of the pyrroline moiety of broussonetine U is related to L-serine. Similar biosynthetic routes were proposed for pseudodistomins A and B (L-serine) and pseudodistomin C (D-serine).[17] 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 on going to determine whether broussonetines are biosynthesized through sphingosine-related metabolites in higher plants.

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