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Biosynthesis of 1-deoxynojirimycin in *Commelina communis*: a difference between the microorganisms and plants

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Abstract

1-Deoxynojirimycin is a glycosidase-inhibitory alkaloid obtained from several plants and microorganisms. Administration experiments using [1-^{13}C] glucose in the higher plant *Commelina communis* and ^{13}C NMR spectroscopic analyses of products suggested that 1-deoxynojirimycin was biosynthesized through a different route compared with that in *Streptomyces* and *Bacilli* microorganisms.

Keywords: Commelinaceae: *Commelina communis*; 1-Deoxynojirimycin; Biosynthesis; NMR

1. Introduction

1-Deoxynojirimycin (DNJ), one of the simplest natural carbohydrate mimics, which was isolated from several higher plants and various strains of *Streptomyces* and *Bacilli* microorganisms, has been the focus of recent research (Asano et al., 2000; Robina et al., 2001; Saul et al., 1984; Watson et al., 2001; Winchester and Fleet, 2000; Zechel et al., 2003). Modulation of the activity of carbohydrate-recognizing enzymes using these sugar mimics of the relevant carbohydrates has enormous therapeutic potential, and therefore DNJ and its derivatives will become a new generation of carbohydrate-controlled therapeutic agents for many diseases. The control of *N*-linked oligosaccharide biosynthesis to alter tumor cells displaying aberrant glycosylation or to prevent syncytium format ion of HIV on lymphocytes also has therapeutic implications. Although numerous reports and reviews of various aspects of the syntheses and biochemistry of DNJ have been published, there is very little in the literature on the biosynthesis of DNJ. Biosynthetic enzymes can be useful in stereospecific synthesis, and the proposed pathway in *Streptomyces subrutilus* ATCC 27467 and *Bacillus subtilis* var. *niger* ATCC 9372 is shown in Scheme 1 (Hardick et al., 1991, 1992; Hardick and Hutchinson, 1993). This scheme describes a C2/C6 cyclisation of the original glucose molecule based on experimental results showing that the isotope label at C1 of glucose ends at C6 of DNJ. No suggest ion about the origins of DNJ in the higher plants has been made as yet. In the present study, we investigated the biosynthesis of DNJ in the dayflower, *Commelina communis* (Commelinaceae), which contains DNJ and (2*R*, 3*R*, 4*R*, 5*R*) 2,5-(bis)-hydroxymethyl 3,4-dihydroxypyrrolidine (DMDP) as the main polyhydroxylated alkaloids (Kim et al., 1999).

To clarify the biosynthetic route of DNJ in the dayflower, we grew the plant on aseptic medium and analyzed the enriched ^{13}C content of isolated DNJ after feeding with [1-^{13}C] glucose. In this communication, we report the primary results of labeling of DNJ in this plant after feeding with [1-^{13}C] glucose.

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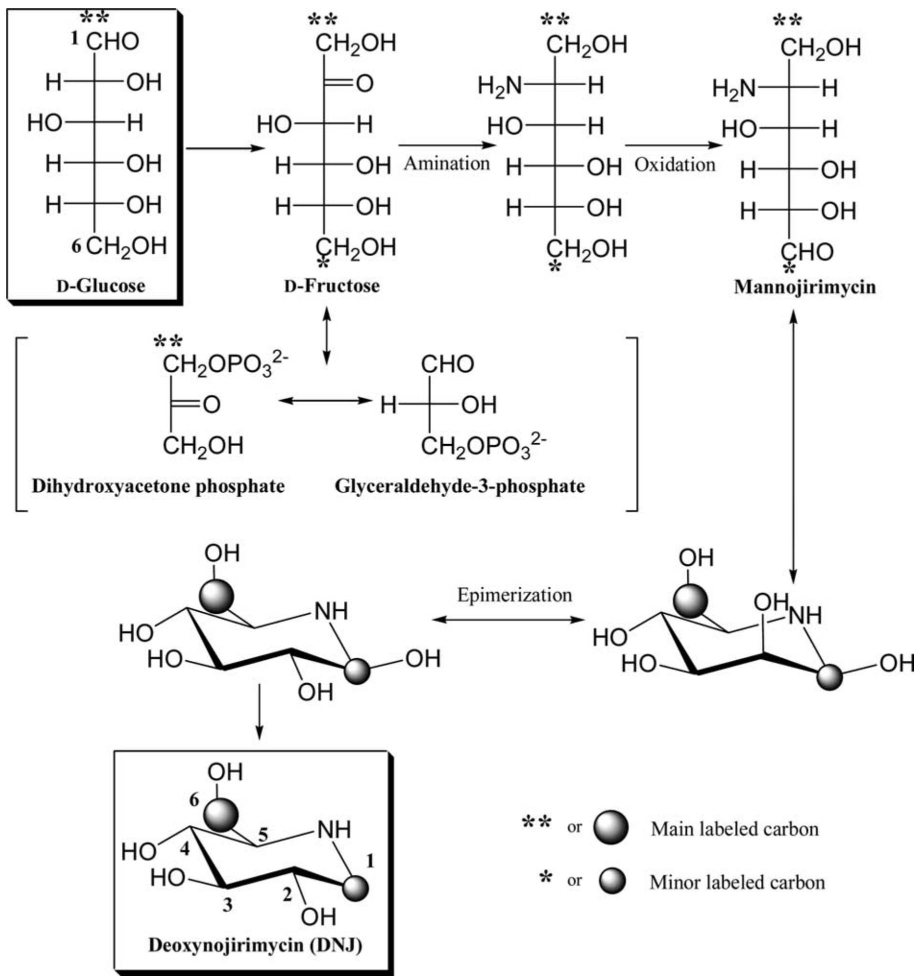


Scheme 1. Biosynthesis of deoxynojirimycin in *Bacilli*.

2. Result and discussion

C. communis was cultured on Murasige and Skoog gellan gum medium supplemented with 1% sucrose (hormone-free) under light (3000 lux) for 2 weeks at 25 °C. The plantlets cut at the joint were transplanted to culture tubes under the same growth condition, and [1-^{13}C] glucose 1 g was added to the medium. After 14 days, DNJ was isolated from 50% aqueous methanol extracts of the plantlets administered [1-^{13}C] glucose as described in Section 3.

The ^{13}C NMR spectrum of DNJ (Asano et al., 1998) showed the presence of clear enrichment of the C1 and C6 (normalized intensities: each 2.38 and 1.52) (Fig. 1, 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 DNJ.

The results were similar to those in *S. subrutilus* and *B. subtilis* var. *niger*, except for the C1/C6 enrich-

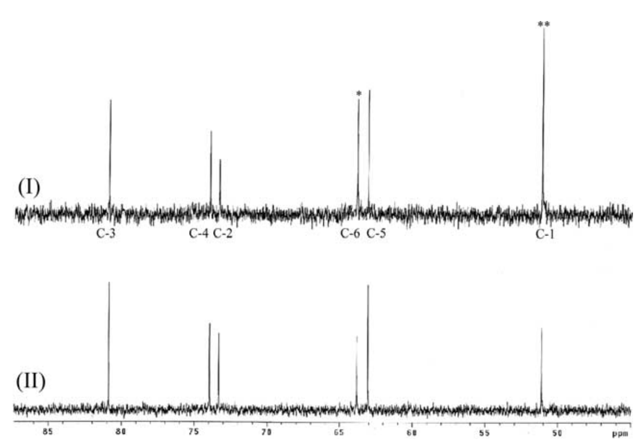


Fig. 1. ^{13}C NMR spectra of unlabeled DNJ (II) and DNJ derived from D-[1-^{13}C]glucose (I).

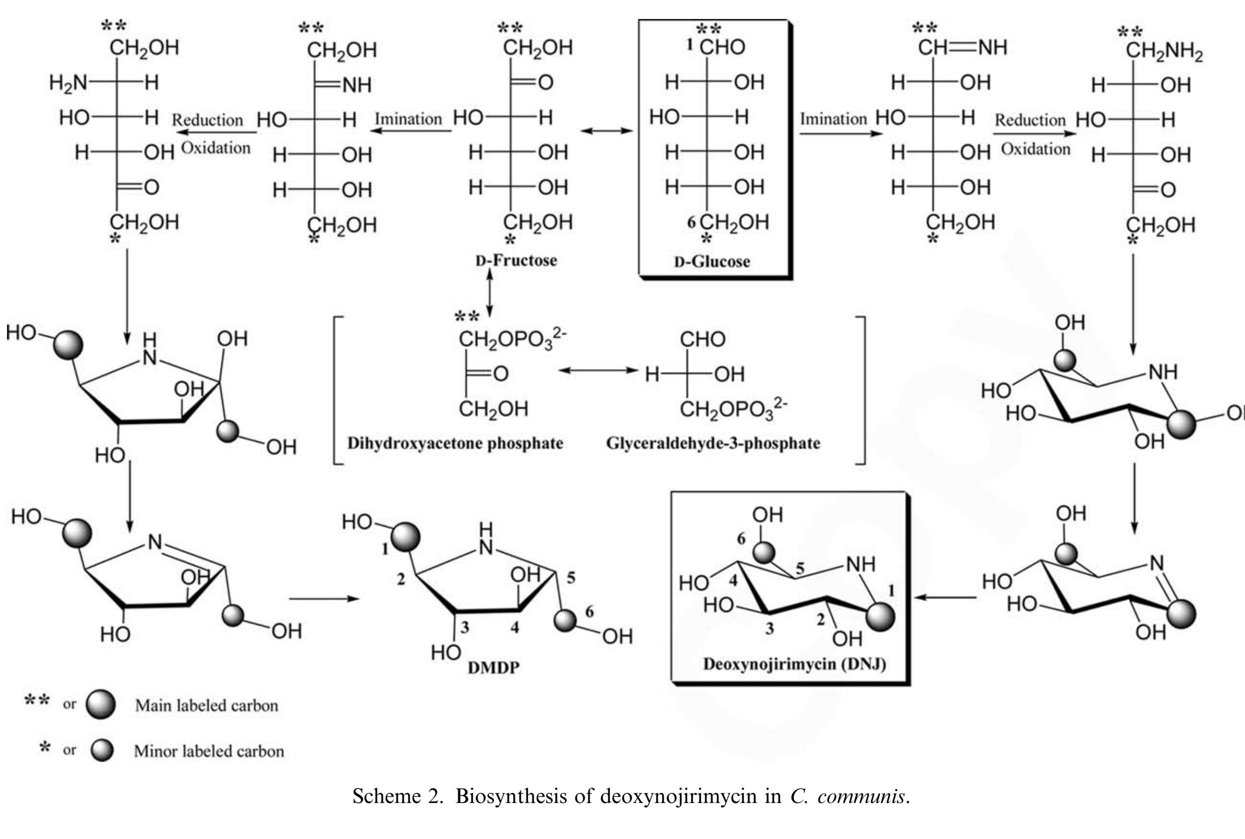
Table 1
^{13}C NMR data of labeled DNJ, fructose, and DMDP

–	DNJ		β-D-Fructopyranose		DMDP	
	δ (ppm)	Peak height[a]	δ (ppm)	Peak height[a]	δ (ppm)	Peak height[a]
1	51.14	2.38	64.51	3.05	62.77	2.52
2	73.32	0.84	98.71	1.21	62.97	1
3	80.87	0.91	68.18	1.02	78.83	1.24
4	73.96	0.97	70.31	1.08	78.83	–
5	63.04	1	69.83	1	62.97	–
6	63.82	1.58	64	1.66	62.77	–

[a] The signal intensities were normalized to C5 and compared with those of unlabeled DNJ, fructose and DMDP.

ment ratio. The significant ^{13}C label was located at C6 in DNJ from the microorganisms, while that from the plant was at C1. The difference in the enrichment at C1 and C6 suggested a different biosynthetic route between the microorganisms and the higher plants. This resultled to the hypothesis on the biosynthesis of DNJ in *C. communis* indicated in Scheme 2, which describes C1/C5 cyclization of the original glucose molecule without the inversion in which the isotope label introduced at C1 of glucose finishes at C6 of DNJ during biosynthesis. Thus, not C2/C6 cyclization, but C1/C5 cyclization occurs in *C. communis*. The relative difference in the enrichment at C1 and C6 was also observed in fructose obtained from administration of [1-^{13}C] glucose as well as in DNJ (Fig. 2, Table 1). Therefore, when the isotope label is at C1 of glucose, this will become C1 of dihydroxyacetone phosphate and consequently C3 of glyceraldehyde-3-phosphate. The latter will mostly continue into other C3 metabolites via glycolysis, but as the aldol reaction is reversible, it is possible that the ^{13}C label will find its way into C6 of glucose via fructose-1,6-diphospate. This hypothesis was also supported by the finding that under the same experimental conditions we also obtained DMDP and its labeling patterns corresponded with those of DNJ, as shown in Table 1.

In conclusion, we have clarified the biosynthetic pathway of the carbon skeleton of DNJ in *C. communis*, and discovered a very interesting difference between the pathways in microorganisms and a higher plant. This synthesis pathway appears to be a brief and rational route in comparison with that in



Scheme 2. Biosynthesis of deoxynojirimycin in *C. communis*.

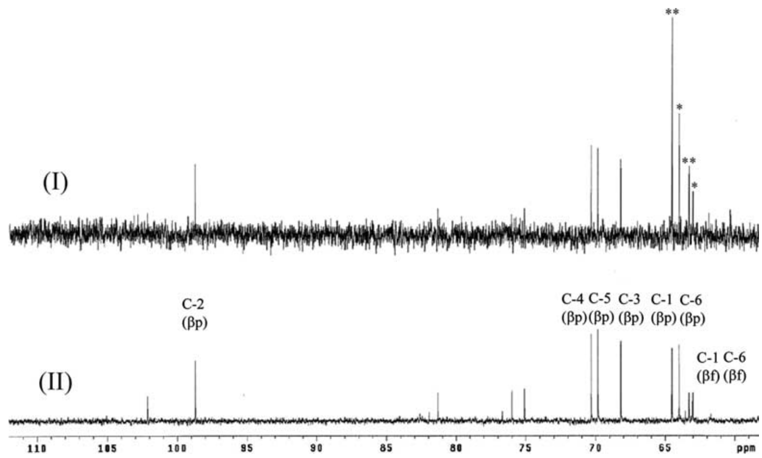


Fig. 2. ^{13}C NMR spectra of unlabeled fructose (II) and fructose derived from D-[1-^{13}C]glucose (I).

microorganisms. Moreover, it is interesting that such a major difference was recognized in a plant and microorganisms in the biosynthetic pathway of the same compound. Further investigation to clarify the biosynthetic enzymes in each step is now in progress. These enzymes can be useful for an enzymatic synthetic approach for DNJ and its derivatives and their biomimetic synthesis.

3. Experimental

3.1. General

The instruments used in this work were a Hitachi M-80 spectrometer (for MS spectra) and a Varian Mercury 300, unity Inova-500 (for NMR spectra measured in D_2O, DSS as an internal standard).

HPLC was conducted with a JASCO PU 980 equipped with a JASCO 830-RI as a detector. Silica gel 60 F_{254} (Merck) precoated TLC plates were used, developed with a $CHCl_3$–MeOH–AcOH–H_2O (20:10:7:5) solvent system, and detection was carried out by ninhydrin reagent followed by heating.

3.2. Plant material

The plant material used in this study, *C. communis* (Commel inaceae) was collected at medicinal plant garden of the Osaka University of Pharmaceutical Sciences in April 2002.

3.3. Isolation of DNJ, DMDP and fructose derived from [1-^{13}C] glucose

The dried aerial parts of *C. communis* (5.5 g) were cut finely and then extracted with 50% aqueous methanol (100 ml) for 2 h. The solution so obtained was then applied to an Amberlite CG-50 (H^+-form) column (2.1 i.d. × 6.0 cm). After washing the column with water and then with H_2O–MeOH (1:1), the adsorbed material was eluted with 50% MeOH-28% ammonia solution (9:1). The eluted fraction was concent rated in vacuo to give the basic fraction. This fraction was applied to a Dowex 50W-X4 column (200–400 mesh, 2.1 i.d. × 5.0 cm) pretreated with formic acid–ammonium formate buffer (0.2 M ammonia formate, adjusted to pH 5.7 with 1 N formic acid), with stepwise elution (H_2O, H_2O–28% ammonia solut ion (9:1)). The fraction (H_2O–28% ammonia solution (99:1)) was subjected to preparative HPLC under the following conditions: column, Asahipak NH2P (4.6 i.d. × 250 mm), solvent, CH_3CN–H_2O (80:20), flow rate, 1.0 ml/min, column temperature, ambient. DNJ (1.2 mg) and DMDP (1.1 mg) were finally obtained. Fructose (1.1 mg) was obtained from the water fract ion of Amberlite CG-50 chromatography by HPLC.

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