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An α-glucoside of 1,4-dideoxy-1,4-imino-D-lyxitol with an eleven carbon side chain

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ABSTRACT

The distribution of pyrrolidine-type iminosugars with a long-side chain appears to be restricted to the relatively unrelated plant families Moraceae, Campanulaceae, and Hyacinthaceae. In a search for glycosidase inhibitors in these plant families, we isolated the 1,4-dideoxy-1,4-imino-D-lyxitol (DIL) glucoside bearing the 1,2,11-trihydroxyundec-4-ene side chain at the C-1α position from the roots of *Adenophora triphylla*. This iminosugar was a powerful and selective inhibitor of coffee bean α-galactosidase, with an IC_{50} value of 8 μM.

1. Introduction

Glycosidase-inhibiting iminosugars are currently of great interest as potential therapeutic agents for diseases such as diabetes, obesity, viral infections, and therapeutic agents for some genetic disorders (Asano, 2009). For example, the α-glucosidase inhibitor *N*-hydroxyethyl-1-deoxynojirimycin (Seibule) has been introduced onto the market for the treatment of type-2 diabetes (Mitrakou et al., 1998), and a potent lysosomal α-galactosidase inhibitor 1-deoxygalactonojirimycin (Amigal) has finished in Phase II clinical trials for the treatment of Fabry disease (Fan et al., 1999). The therapeutic application of the α-glucosidase inhibitor *N*-nonyl-1-deoxynojirimycin as an antiviral agent against human hepatitis viruses C (HCV) is also under investigation (Woodhouse et al., 2008). These iminosugars can inhibit various glycosidases because of a structural resemblance to the sugar moiety of the natural substrate. They are classified into five major structural classes: polyhydroxylated derivatives of pyrrolidine, piperidine, indolizidine, pyrrolizidine, and nor-tropane (Asano et al., 2000a). Among them, the polyhydroxylated pyrrolidine alkaloids (2*R*,5*R*)-bis(dihydroxymethyl)-(3*R*,4*R*)-dihydroxypyrrolidine (DMDP) and 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1) would appear to be fairly widespread secondary metabolites (Watson et al., 2001). On the other hand, the distribution of pyrrolidine derivatives with a long-side chain appears to be restricted to the families Moraceae, Campanulaceae, and Hyacinthaceae. α-1-*C*-(1-Hydroxypentyl)-D-AB1 has been isolated from *Adenophora triphylla* var. *japonica* (Regel) H. Hara (Campanulaceae) (Asano et al., 2000b) and α-1-*C*-(1,10,13-trihydroxytridecyl)-D-AB1 from *Broussonetia kajinoki* (Moraceae) (Tsukamoto et al., 2001). We also have isolated α-1-*C*-(3,6-dihydroxyheptyl)-D-AB1 from *Scilla campanulata* (Kato et al., 1999) and *Scilla socialis* bulbs (Kato et al., 2007). In this paper, we describe the isolation and structural determination of a new polyhydroxylated pyrrolidine glycoside with a C_{11} side chain from *A. triphylla*. Furthermore, we report its inhibitory activities toward glycosidases.

2. Results and discussion

The roots (5 kg) of *A. triphylla* were extracted three times with hot water for 1 h, and the extract was subjected to a variety of ion-exchange resin chromatographic steps to give alkaloids **1** (20 mg), **2** (11 mg), **3** (366 mg), and **4** (241 mg). The ^1H NMR and ^{13}C NMR spectra of alkaloids were in accord with those of 1-deoxynojirimycin **1**, 1-deoxymannojirimycin **2**, and DMDP **3**, respectively (Asano et al., 1998).

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Alkaloid **4** was determined to have the molecular formula $C_{22}H_{41}NO_{11}$ by HRFABMS. The ^{13}C NMR spectroscopic data revealed the presence of nine methylene (δ 61.8, 60.5, 60.2, 31.2, 30.8, 28.8, 28.6, 26.7 and 24.0) and thirteen methine (δ 133.5, 124.9, 102.8, 83.4, 75.7, 75.7, 73.5, 72.9, 72.1, 71.0, 69.3, 60.4 and 60.1) carbon atoms. The connectivity of the carbon and hydrogen atoms was defined from COSY and HMBC spectroscopic data. Two methine signals (δ 60.1 and 60.4) with relatively high-field chemical shifts were suggestive of being bonded to the nitrogen of the pyrrolidine ring. The methylene signal at δ 60.2 (C-6′) was attributed to the hydroxymethyl carbon, this showed HMBC correlations to δ 60.1 (C-5′) and 72.1 (C-4′). The methine signal at δ 72.1 (C-4′) showed HMBC correlations to δ 60.1 (C-5′) and 83.4 (C-3′), and the methine signal at δ 83.4 (C-3′) showed HMBC correlations to δ 60.4 (C-2′), 73.5 (C-1) and 102.8 (C-1″). This confirms the pyrrolidine ring structure and also shows that a glucose moiety is linked to C-3′. The positive response (red color) to the naphthoresorcinol-sulfuric acid regent and a characteristic carbon signal (δ 102.8) in ^{13}C NMR suggested that **4** was a glycoside of an alkaloid. A small amount of this glycoside was subjected to acid hydrolysis (100°C, 8 h) using Dowex 50W-X2 (H^+ form) resin. The sugar part of **4** in the water eluent fraction was confirmed as D-glucose by the D-glucose oxidase-peroxidase method using the glucose CII-test. The coupling constant of 7.9 of the 1H″ doublet at 4.53 is strongly suggestive this is the alpha anomer. The relative configurations of the stereogenic centres were assigned by the NOE effects and $^3J_{H,H}$ coupling constants. The coupling pattern of H-4′ ($J_{4,5} = J_{3,4} = 4.1$ Hz) and the NOE correlations observed between H-3′ and H-4′ and H-5′ imply that H-3′, H-4′ and H-5′ are on the same side of the ring. The large coupling constant (7.3 Hz) observed between H-2′ and H-3′ suggests that they are in a pseudo-*trans*-axial position, confirmed by the small NOE interaction observed between H-2′ and H-3′. This together with the lack of NOE interactions observed between H-2′ and H-5′, and H-2′ and H-4′ suggests the ring configuration to be 2′*R*,3′*R*,4′*S*,5′*R*. The coupling pattern of H-1 ($J_{1,2'} = 6.3$ Hz, $J_{1,2} = 3.8$ Hz) and the NOE correlations observed between H-2′, H-1 and H-2 are consistent with the tentatively proposed relative configuration of the side chain diol. An unambiguous chemical synthesis of the aglycone will firmly establish the overall relative and absolute stereochemistry. An NOE correlation was observed between H-4 and H-5 suggesting a *cis* double bond. There was also an NOE interaction observed between H-1, H-2 and H-4 confirming the *cis* orientation of H-1 and H-2. Thus, the structure of alkaloid **4** was assigned as (1*S*,2*S*,*Z*)-1-((2′*R*,3′*R*,4′*S*,5′*R*)-3′-*O*-α-D-glucopyranosyl-4′-hydroxy-5′-(hydroxymethyl)pyrrolidin-2-yl)undec-4-ene-1,2,11-triol, or its enantiomer (Fig. 1).

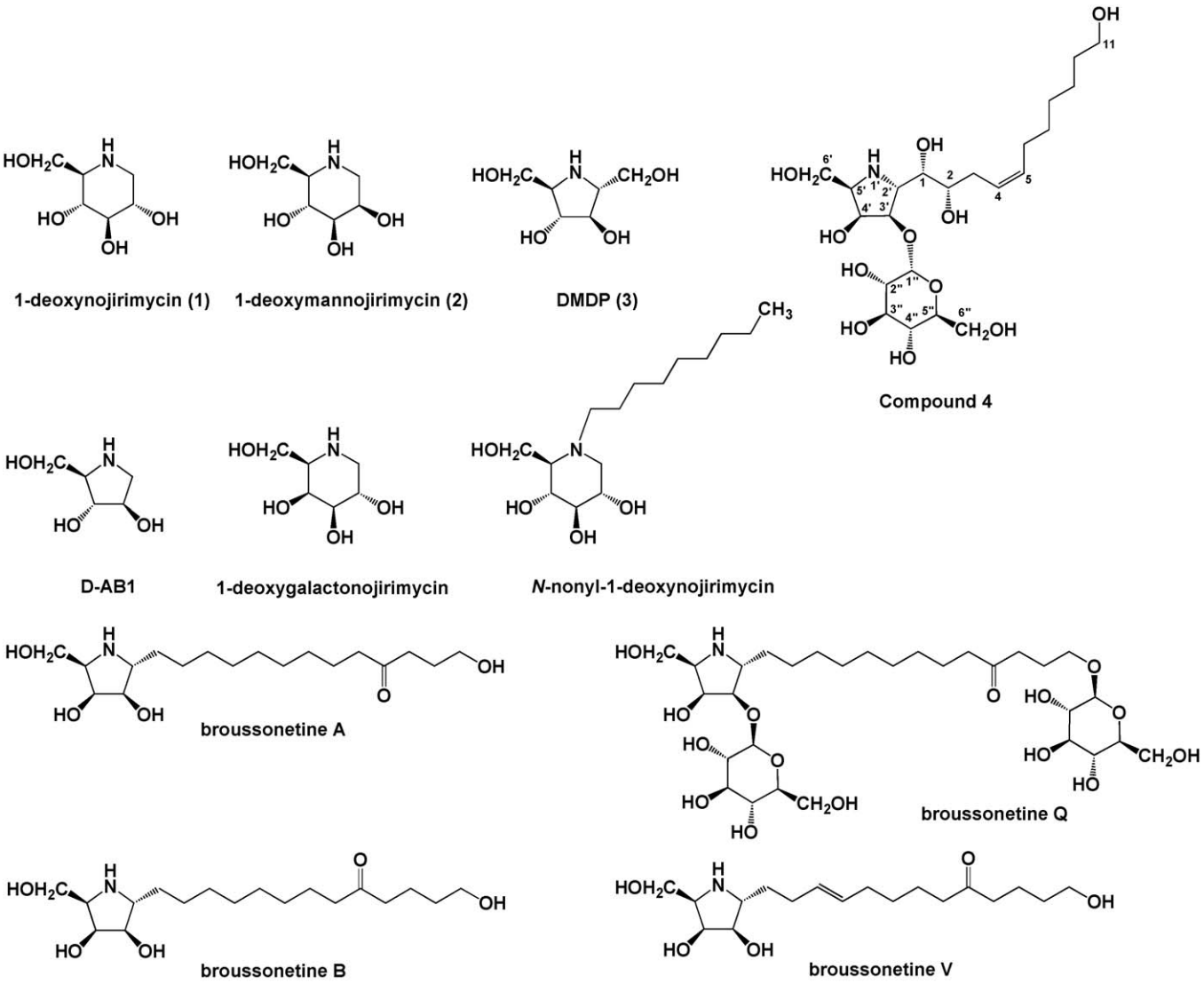

Fig. 1. Structures of iminosugars isolated from the roots of *Adenophora triphylla* and their related iminosugars.

Table 1
Concentration of iminosugars giving 50% inhibition against glycosidases.

Enzyme	IC_{50} (μM)	
	4	DIL[a]
α-Glucosidase		
Rice	NI[b]	845
Yeast	NI	NI
β-Glucosidase		
Almond	NI	329
Bovine liver	222	NI
α-Galactosidase		
Coffee bean	8	0.5
β-Galactosidase		
Bovine liver	NI	NI
α-Mannosidase		
Jack bean	NI	39
β-Mannosidase		
Snail	NI	NI
α-L-Fucosidase		
Bovine epididymis	NI	98

[a] Ref.: Mercer et al. (2009).
[b] NI: no inhibition (less than 50% inhibition at 1000 μM).

Previously, a variety of 1,4-dideoxy-1,4-imino-D-lyxitol (DIL) derivatives with long-side chains have been isolated from *B. kajinoki* (Moraceae) and were designated as broussonetines A, B, Q, and V (Shibano et al., 1997, 2000; Tsukamoto et al., 2001). Interestingly, all these broussonetines were characterized with C_{13} side chains at C-1α position of DIL, while our isolated new alkaloid **4** has a C_{11} side chain. *B. kajinoki* (Moraceae) and *A. triphylla* (Campanulaceae) belong to quite unrelated families. Thus, we need more investigation of the biosynthetic pathways of these plant families.

The IC_{50} values of compound **4** and DIL toward various glycosidases are shown in Table 1. We have previously reported that DIL was a potent inhibitor of coffee bean α-galactosidase and a moderate inhibitor of jack bean α-mannosidase, with IC_{50} values of 0.5 and 39 μM, respectively (Mercer et al., 2009). Compound **4** gave no inhibition toward rice α-glucosidase, almond β-glucosidase, and jack bean α-mannosidase, while it still kept strong α-galactosidase inhibition, with IC_{50} value of 8 μM.

3. Experimental

3.1. General

The purity of samples was checked by HPTLC on silica gel $60F_{254}$ (E. Merck) using the solvent system PrOH–AcOH–H_2O (4:1:1), and a chlorine-*o*-tolidine reagent or iodine vapor was used for detection. Optical rotations were measured with a Jasco DIP-370 digital polarimeter (Tokyo, Japan). Structure elucidation was carried out using NMR (Bruker DRX500, 500 MHz) for full ^1H and ^{13}C assignments. Chemical shifts were expressed in ppm downfield from tetramethylsilane in D_2O as an internal standard. The assignment of proton and carbon signals in the NMR spectra were determined from extensive homonuclear decoupling experiments, DEPT, COSY, HMQC, and HMBC spectral data. FABMS were measured using glycerol as a matrix on a JEOL JMS-700 spectrometer (Tokyo, Japan).

3.2. Plant material

Roots of *A. triphylla* var. *japonica* were purchased at a herbal medicine shop (Uchida Wakanyaku Co., Tokyo, Japan) in July 2008. A voucher specimen (No. Nash 2009/2) is deposited at the herbarium of the Institute of Biological, Environmental and Rural Sciences, Aberystwyth, UK.

3.3. Extraction and isolation

The roots (5 kg) of *A. triphylla* were extracted three times with hot water for 1 h. The filtrate was applied to a column of Amberlite IR-120B (1000 mL, H^+ form). The 0.5 M NH_4OH eluate was concentrated to give a brown syrup (30.8 g). This syrup was applied to Dowex 1-X2 (OH^- form) short column to remove amino acids and pigments, and eluted with H_2O. This pool treated with Dowex 1-X2 was further chromatographed with Amberlite CG-50 (2.2 cm × 56 cm, NH_4^+ form) with H_2O as eluant and/or Dowex 1-X2 (OH^- form) with H_2O as eluant to give 1-deoxynojirimycin (20 mg), 1-deoxymannojirimycin (11 mg), DMDP (366 mg), and the DIL glucoside (**4**) (241 mg).

3.4. (1S,2S,Z)-1-((2′R,3′R,4′S,5′R)-3′-O-α-D-glucopyranosyl-4′-hydroxy-5′-(hydroxymethyl)pyrrolidin-2-yl)undec-4-ene-1,2,11-triol

Colorless syrup; $[\alpha]_D$ + 11.8 (*c* 1.89, H_2O); ^1H NMR (500 MHz, D_2O) δ/ppm 5.55–5.60 (1H, m, H-5), 5.39–5.44 (1H, m, H-4), 4.53 (1H, d, *J* = 7.9 Hz, H-1″), 4.29 (1H, t, *J* = 4.1 Hz, H-4′), 4.23 (1H, dd, *J* = 4.1, 7.3 Hz, H-3′), 3.85 (1H, d, *J* = 12.6 Hz, H-6′a), 3.75 (1H, dd, *J* = 6.3, 11.4 Hz, H-6′a), 3.75 (1H, m, H-2), 3.71 (1H, dd, *J* = 4.4, 12.6 Hz, H-6″b), 3.62 (1H, dd, *J* = 6.6, 11.4 Hz, H-6′b), 3.55 (2H, t, *J* = 6.3 Hz, H-11), 3.51 (1H, dd, *J* = 3.8, 6.3 Hz, H-1), 3.38–3.48 (3H, m, H-3″, 4″, 5″), 3.34 (1H, dd, *J* = 6.3, 7.4 Hz, H-2′), 3.29 (1H, dd, *J* = 7.9, 9.1 Hz, H-2″), 3.23 (1H, ddd, *J* = 4.1, 6.3, 6.6 Hz, H-5′), 2.21–2.37 (2H, m, H-3), 1.95–2.08 (2H, m, H-6), 1.47–1.55 (2H, m, H-10), 1.23–1.38 (6H, m, 3x CH_2-7, 8, 9); ^{13}C NMR (126 MHz, D_2O) δ/ppm 133.5 (C-5), 124.9 (C-4), 102.8 (C-1″), 83.4 (C-3′), 75.7 (C-3″), 75.7 (C-5″), 73.5 (C-1), 72.9 (C-2″), 72.1 (C-4′), 71.0 (C-2), 69.3 (C-4″), 61.8 (C-11), 60.5 (C-6″), 60.4 (C-2′), 60.2 (C-6′), 60.1 (C-5′), 31.2 (C-3), 30.8 (C-10), 28.8 (C-8), 28.6 (C-7), 26.7 (C-6), 24.0 (C-9). HRFABMS *m*/*z* 496.2763 $[M+H]^+$ ($C_{22}H_{42}NO_{11}$ requires 496.2759).

3.5. Enzyme assays

The enzymes α-glucosidase (from rice, assayed at pH 5.0; from yeast, assayed at pH 6.8), β-glucosidase (from almond, assayed at pH 5.0), α-galactosidase (from coffee bean, pH 6.5), β-galactosidase (from bovine liver, assayed at pH 6.8), α-mannosidase (from Jack bean, pH 4.5), β-mannosidase (from snail), α-L-fucosidase (from bovine kidney), *p*-nitrophenyl glycosides, and disaccharides were purchased from Sigma–Aldrich Chemical Co. (St. Louis, Mo. USA). Glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as substrate. The reaction was stopped by adding 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

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