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ScienceDirect
Phytochemistry 69 (2008) 1261–1265
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Iminosugars from *Baphia nitida* Lodd.

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Received 13 April 2007; received in revised form 12 September 2007
Available online 14 January 2008

Abstract

Chromatographic separation of the 50% aqueous EtOH extract of the leaves of the African medicinal tree *Baphia nitida* resulted in isolation of 10 iminosugars. The plant contained 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP) as a major alkaloid. The structure of a new alkaloid was also elucidated by spectroscopic methods as the 1-*O*-β-D-fructofuranoside of DMDP, and this plant produced 3-*O*-β-D-glucopyranosyl-DMDP as well. DMDP is a potent inhibitor of β-glucosidase and β-galactosidase, whereas the other two derivatives lowered inhibition toward both of these enzymes and improved inhibitory activities toward rice α-glucosidase and rat intestinal maltase.

Keywords: *Baphia nitida*; Fabaceae; Polyhydroxyalkaloids; Iminosugar; 1-*O*-β-D-Fructofuranosyl-2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine; DMDP; Glycosidase inhibitor

1. Introduction

The realization that iminosugars (azasugars) might have enormous therapeutic potential in many diseases such as diabetes, viral infection, and lysosomal storage disorders has led to increasing interests and demand for them (Asano, 2003). They inhibit glycosidases by mimicking either the pyranosyl or furanosyl moiety of the corresponding substrate or the transition-state intermediates of glycosidases. 2*R*,5*R*-Dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP) (**7**) and 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) (**10**) can be regarded as β-fructofuranose analogues (Asano et al., 2000). DMDP (**7**) was first isolated from leaves of *Derris elliptica* (Fabaceae) (Welter et al., 1976) and removal of one hydroxymethyl group from DMDP (**7**) led to DAB (**10**), which was first found in the fruits of *Angylocalyx boutiqueanus* (Fabaceae) (Nash et al., 1985). These alkaloids were later shown to be present in many species of quite unrelated families (Watson et al., 2001). On the other hand, the occurrence of 2,5-dideoxy-2,5-imino-*glycero*-D-*manno*-heptitol (homoDMDP) appears limited to species in the Hyacinthaceae. Recently, we have reported the occurrence of DAB (**10**) derivatives bearing a long side chain, which were isolated from *Hyacinthoides non-scripta* (Hyacinthaceae) (Kato et al., 1999), *Adenophora triphylla* var. *japonica* (Campanulaceae) (Asano et al., 2000), and *Scilla peruviana* (Hyacinthaceae) (Asano et al., 2004). In the course of searching for novel polyhydroxylated pyrrolidine alkaloids, we found that polyhydroxylated pyrrolidines and piperidines and their glycosides are abundant in the African medicinal tree *Baphia nitida* (Fabaceae). Iminosugar-producing plants have also often been shown to contain many iminosugar glycosides, specifically α- and β-glucosides, α-galactosides, apiosides, β-xylosides, and β-mannosides (Asano et al., 2000, 2001, 2005; Watson et al., 2001; Yamashita et al., 2002; Kato et al., 2003).

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doi:10.1016/j.phytochem.2007.11.018

In this paper, we describe the first isolation of a β-fructofuranosyl-iminosugar, 1-*O*-β-D-fructofuranosyl-DMDP (**8**), together with six previously reported iminosugars and three iminosugar glucosides from the extract of *B. nitida*.

2. Results and discussion

The dried leaves (8.0 kg) of *B. nitida* were extracted with 50% aqueous EtOH, and the extract was subjected to a variety of ion-exchange resin chromatographic steps to afford alkaloids **1** (1.62 g), **2** (3 mg), **3** (213 mg), **4** (13 mg), **5** (32 mg), **6** (15 mg), **7** (1.89 g), **8** (32 mg), **9** (153 mg), and **10** (120 mg).

The alkaloids were determined to be 1-deoxynojirimycin (DNJ) (**1**), 3-*O*-β-D-glucopyranosyl-DNJ (**2**), 6-*O*-β-D-glucopyranosyl-DNJ (**3**), 1-deoxymannojirimycin (DMJ) (**4**), 1-deoxyallonojirimycin (**5**), 3-*epi*-fagomine (**6**), DMDP (**7**), 1-*O*-β-D-fructofuranoside of DMDP (**8**), 3-*O*-β-D-glucopyranosyl-DMDP (**9**), and DAB (**10**), respectively, from their ^{1}H NMR and ^{13}C NMR spectroscopic data. We have isolated DAB (**10**), 3-*epi*-fagomine (**6**), DNJ (**1**), and its glycosides from *Morus alba* (Moraceae) (Asano et al., 1994a,b) and DMDP (**7**) and its glycosides from *Connarus ferruginens* (Connaraceae) and *Albizia myriophlla* (Fabaceae) (Asano et al., 2005). Recently, we have enantiospecifically synthesized D- and L-enantiomers of fagomine and 3-*epi*-fagomine (**6**), and both enantiomers of DNJ (**1**) and its six epimers (Banba et al., 2001; Takahata et al., 2003; Kato et al., 2005). Furthermore, both enantiomers of DAB (**10**) and DMDP (**7**) have also been enantiospecifically synthesized (Fleet et al., 1985; Yu et al., 2004). These synthetic studies established that the iminosugars **1**, **4**–**7**, and **10** isolated from *B. nitida* are in the D-form. Fig. 1.

Alkaloid **8** was determined to have the molecular formula $C_{12}H_{23}NO_9$ by HRFABMS. The positive response (red color) to the naphthoresorcinol–sulfuric acid reagent on silica gel 60F_{254} TLC plates and a characteristic carbon signal (δ 106.3) in the ^{13}C NMR spectrum (Table 2) suggested that **8** 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. After washing the resin with H_2O, the alkaloid part was displaced from the resin with 0.5 M NH_4OH, concentrated to dryness, and confirmed as DMDP (**7**) from the ^{13}C NMR spectroscopic data. The R_f value and red spot of the filtrate on TLC, and the absence of the anomeric proton signal in the ^{1}H NMR specturm of **8** (Table 1) implied that the sugar part was D-fructose. The COSY and HMBC spectra established that the carbon signals of δ 63.2, 64.6, 77.3, 79.9, 83.8 and 106.3 are derived from the sugar part, and these carbon chemical shifts were in good accordance with those of β-D-fructofuranoside (Bock et al., 1984). DMDP (**7**) gives very simple NMR spectra (three peaks in the ^{13}C NMR spectrum, four spin systems in the ^{1}H NMR spectrum) due to its symmetrical structure. Introduction of a β-fructofuranosyl group into DMDP (**7**) leads to breakdown of its symmetrical structure and gives six carbon peaks (δ 63.3, 64.6, 64.7, 64.9, 80.7, 80.8). However, no significant glycosylation shift is observed in the chemical shifts of DMDP compared to those (δ 64.4, 64.9, 80.7) of DMDP as a free base. This means that the sugar linkage site is on either of the two hydroxymethyl groups of DMDP. As noted in the literature (Bock et al., 1984), introduction of a β-fructofuranosyl group into the hydroxymethyl group (C-6) of D-glucose causes no significant glycosylation shift at the linkage site. Hence, alkaloid **8** was characterized as 1-*O*-β-D-fructofuranosyl-DMDP (**8**).

The IC_{50} values of polyhydroxylated pyrrolidine alkaloids toward various glycosidases are shown in Table 3. DNJ, piperidine type glycosidase inhibitor, was used as positive control. DMDP (**7**) is known to be a potent

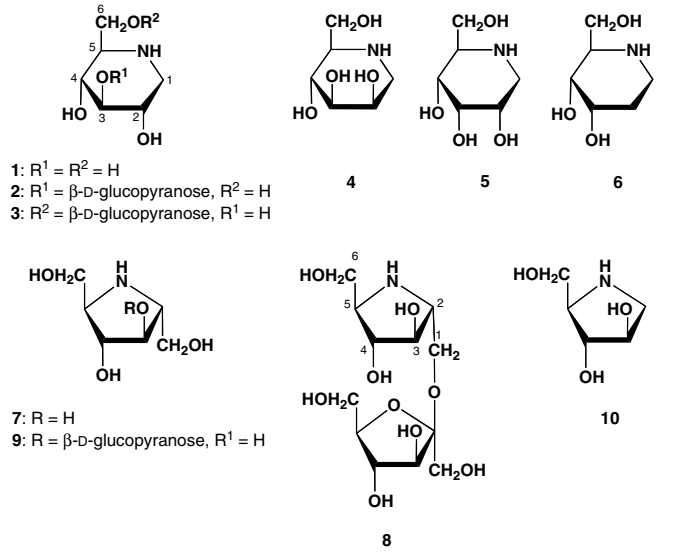

Fig. 1. Structures of the polyhydroxylated alkaloids.

Table 1
^{1}H NMR spectroscopic data of sucrose and 1-*O*-β-D-fructofuranosyl-DMDP (**8**) at 500 MHz

Position	Sucrose[a]	**8**[a]
1a	5.42 *d* (3.7)[b]	3.67 *dd* (5.0, 12.4)
1b		3.82 *dd* (2.7, 12.4)
2	3.56 *dd* (3.7, 9.6)	3.13 *m*
3	3.76 *t* (9.6)	3.90 *t* (6.9)
4	3.47 *t* (9.6)	3.86 *t* (6.9)
5	3.80–3.91	3.06 *m*
6a	3.80–3.91	3.60 *dd* (6.9, 10.1)
6b	3.80–3.91	3.84 *dd* (3.7, 10.1)
1′a	3.68 *s*	3.72 *d* (12.4)
1′b	3.68 *s*	3.75 *d* (12.4)
2′		
3′	4.22 *d* (8.7)	4.17 *d* (8.3)
4′	4.05 *t* (8.7)	4.12 *t* (8.3)
5′	3.80–3.91	3.84–3.88 *m*
6′a	3.80–3.91	3.65 *dd* (6.5, 11.5)
6′b	3.80–3.91	3.72 *dd* (4.1, 11.5)

[a] Chemical shifts are expressed in ppm downfield from TSP in D_2O.
[b] *J* in Hz.

Table 2
^{13}C NMR spectroscopic data of DMDP (**7**), 1-*O*-β-D-fructofuranosyl-DMDP (**8**), and sucrose at 125 MHz

Carbon	7	8	Sucrose
1	64.9[a]	64.9[a]	95.0[a]
2	64.4	63.3	73.9
3	80.7	80.8	75.4
4	80.7	80.7	72.1
5	64.4	64.6	75.2
6	64.9	64.7	63.0
1′		63.2	64.2
2′		106.3	106.5
3′		79.9	79.3
4′		77.3	76.8
5′		83.8	84.2
6′		64.6	65.2

[a] Chemical shifts are expressed in ppm downfield from TSP in D_2O.

inhibitor of yeast α-glucosidase and mammalian β-glucosidase and β-galactosidase (Scofield et al., 1986; Asano et al., 1994a,b). On the other hand, DAB (**10**), which lacks one hydroxymethyl group from DMDP (**7**), was a better inhibitor of yeast α-glucosidase, rat intestinal isomaltase and porcine kidney trehalase with no significant inhibitory activities toward other β-glycosidases. DAB (**10**) has been found to be a potent inhibitor of glycogen phosphorylase both in vitro and in vivo (Andersen et al., 1999; Fosgerau et al., 2000). Recently, the first glycoside of DMDP (**7**) was isolated from *Stemona tuberosa* (Stemonaceae) and the structure was determined to be 3-*O*-β-D-glucopyranosyl-DMDP (**9**) (Asano et al., 2005). In the present work, this glucoside was found to have no inhibition of yeast α-glucosidase and lower inhibition of bovine liver β-glucosidase ($IC_{50} = 850$ μM) and β-galactosidase ($IC_{50} = 465$ μM), but had instead potent inhibitory activity toward rice α-glucosidase ($IC_{50} = 0.79$ μM) and rat intestinal maltase ($IC_{50} = 4.7$ μM) and sucrase ($IC_{50} = 5.0$ μM). The introduction of the β-D-fructofuranosyl residue to C-1 (or C-6) of DMDP (**7**) to give **8** also markedly lowered its inhibitory activity toward β-glucosidase and β-galactosidase but enhanced its inhibitory potential toward porcine kidney trehalase ($IC_{50} = 26$ μM) by about 10-fold. Furthermore, alkaloid **8** was also more inhibitory toward rice α-glucosidase and rat intestinal maltase than DMDP (**7**). The fructofuranoside of DMDP (**8**) is, however, less potent than the glucopyranoside (**9**) against the α-glucosidase as might be expected for a glucose specific enzyme. Compound (**9**) is also a more potent inhibitor of rat intestinal maltase, isomaltase and sucrase than **8**. It is interesting to note that the glucoside of DMDP (**9**) has a similar glucosidase inhibition profile to the glucose analogue DNJ (**1**) with the yeast α-glucosidase and bovine liver β-glucosidase being weakly inhibited, whereas the other α-glucosidases are strongly inhibited. DMDP (**7**) itself is most potent against the glucosidases not strongly inhibited by DNJ (**1**).

Table 3
Concentration of pyrrolidine alkaloids (**7**–**10**) giving 50% inhibition of glycosidases

Enzyme	IC_{50} (μM)				
	7	**8**	**9**	**10**	DNJ
α-Glucosidase					
Rice	NI[a]	22	0.79	250	0.05
Yeast	0.71	NI	NI	0.15	300
Rat intestinal maltase	NI	65	4.7	55	0.36
Rat intestinal isomaltase	91	136	12	5.8	0.3
Rat intestinal sucrase	40	57	5.0	16	0.21
β-Glucosidase					
Bovine liver	9.7	NI	850	NI	210
β-Galactosidase					
Bovine liver	3.3	NI	465	NI	NI
Invertase					
Candida utilis	356	NI	NI	NI	NI
Trehalase					
Porcine kidney	200	26	NI	4.8	41

[a] NI: No inhibition (less than 50% inhibition at 1000 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. Naphthoresorcinol–sulfuric acid reagent was used for detection of glycosides. Optical rotations were measured with a Jasco DIP-370 digital polarimeter (Tokyo, Japan). ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a JEOL ECP-500 spectrometer (Tokyo, Japan). Chemical shifts were expressed in ppm downfield from sodium 3-(trimethylsilyl)-propionate (TSP) in D_2O as an internal standard. The assignment of proton and carbon signals in the NMR spectra was determined from extensive homonuclear decoupling experiments, DEPT, ^1H-^{13}C COSY, HMQC, and HMBC spectroscopic data. FABMS were measured using glycerol as a matrix on a JEOL JMS-700 spectrometer (Tokyo, Japan).

3.2. Plant material

Leaves of the legume *B. nitida* Lodd. were collected and dried in Koforidua in Ghana in 2006. A voucher specimen prepared by Josephine Antwi of the Ghana Herbarium has been deposited at the herbarium of the Institute of Grassland and Environmental Research, UK.

3.3. Extraction and isolation

The dried leaves (8.0 kg) of *B. nitida* were extracted with H_2O–EtOH(1:1,v/v). The filtrate was applied to a column of Amberlite IR-120B (2000 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 an Amberlite CG-50

column (3.6 × 48 cm, NH_4^+ form) with H_2O as eluant (fraction size 15 ml). Fractions were divided into three pools: I (fractions 14–22, 9.0 g), II (fractions 23–36, 16.7 g), and III (fractions 37–70, 3.0 g). The 0.5 M NH_4OH eluate from the same column was designated pool IV (346 mg). Each pool was applied to Dowex 1-X2 (OH^- form) short columns to remove amino acids and pigments, and eluted with H_2O. Each pool, treated with Dowex 1-X2, was further purified with either Dowex 1-X2 (OH^- form) with H_2O as eluant and/or Amberlite CG-50 (2.2 × 56 cm, NH_4^+ form) with H_2O as eluant to give 6-*O*-β-D-glucopyranosyl-DNJ (**3**) (213 mg) and DNJ (**1**) (1.62 g) from pool I, 3-*O*-β-D-glucopyranosyl-DMDP (**9**) (153 mg) and 1-*O*-β-D-fructofuranosyl-DMDP (**8**) (32 mg) from pool II, 1-deoxymannojirimycin (**4**) (13 mg), 3-*O*-β-D-glucopyranosyl-DNJ (**2**) (3 mg), and 3-*epi*-fagomine (**6**) (15 mg) from pool III. Pool IV (312 mg) was further chromatographed with Amberlite CG-50 (2.0 × 56 cm, NH_4^+ form) with 0.1 M NH_4OH as eluant to give DMDP (**7**) (1.89 g), DAB (**10**) (120 mg), and 1-deoxyallonojirimycin (**5**) (32 mg).

3.4. 1-O-β-D-Fructofuranosyl-DMDP (8)

Colorless hygroscopic powder; $[\alpha]_D$ −3.1 (*c* 1.1, H_2O); For ^1H NMR and ^{13}C NMR spectra, see Tables 1 and 2; HRFABMS *m/z* 326.1451 $[M + H]^+$ ($C_{12}H_{24}NO_9$ requires 326.1451).

3.5. Acid hydrolysis of 1-O-β-D-fructofuranosyl-DMDP (8)

Alkaloid **8** (5.8 mg) was heated at 100 °C with Dowex 50W-X2 (1 ml, H^+ form) in H_2O for 8 h. The resin was filtered out and packed into a short column. The alkaloid moiety was eluted with 0.5 M NH_4OH and concentrated, then subjected to a Dowex 1-X2 (0.6 × 5 cm, OH^- form) column with H_2O as eluant to give the purified alkaloid fraction (2.1 mg). From its NMR spectroscopic data, the alkaloid moiety of **8** was identified as DMDP.

3.6. Enzyme assays

The enzymes α-glucosidase (from rice, assayed at pH 5.0; from yeast, assayed at pH 6.8), invertase (from *Candida utilis*, assayed at pH 4.0), trehalase (from porcine kidney, assayed at pH 6.8), β-glucosidase (from bovine liver, assayed at pH 6.8), β-galactosidase (from bovine liver, assayed at pH 6.8), *p*-nitrophenyl glycosides, and disaccharides were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Brush border membranes prepared from rat small intestine according to the method of Kessler et al. (1978) were assayed at pH 5.8 for rat intestinal maltase, isomaltase and sucrase using maltose, isomaltose and sucrose. The released D-glucose was determined colorimetrically using the Glucose CII-test Wako (Wako Pure Chemical Ind., Osaka, Japan). Other 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.

Acknowledgments

This research was partially supported by the Ministry of Education, Culture, Sports, Science and Technology, Grant-in-Aid for Young Scientists (B), 18790086 (A.K.) and Cooperative Research Center of Life Science (N.A.).

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