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Revised Structure of a Homonojirimycin Isomer From *Aglaonema Treubii*: First Example of a Naturally Occurring α-Homoallonojirimycin

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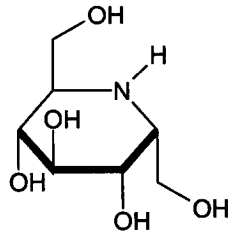
Abstract: The structure of a homonojirimycin isomer isolated from *Aglaonema treublii* and originally proposed as α-3,4-di-*epi*-homonojirimycin was revised to α-4-*epi*-homonojirimycin **3** ("α-homoallonojirimycin") on the basis of NMR analysis and synthetic studies. Its activity as a glycosidase inhibitor is compared to that of other homonojirimycin isomers.

Azasugars as competitive inhibitors of glycosidases and glycosyltransferases have been recently the subject of much interest owing to their high potential in a number of therapeutic areas such as diabetes, cancer and viral infection (HIV).[1] Homoazasugars,[2] piperidine azasugars bearing a hydroxymethyl substituent at C-1, form a class of "aza-*C*-glycosyl" compounds which were shown in several instances to exhibit greater selectivity as glycosidase inhibitors compared to the parent azasugars.[3] It is noteworthy that the synthesis of the first reported homoazasugar, α-homonojirimycin **1**,[4] was followed shortly by the discovery of its natural occurrence.[5]

Very recently, various homoazasugars have been isolated from a 50% aqueous EtOH extract of *Aglaonema treublii* Engl. (Araceae).[6] In addition to known compounds such as α-homonojirimycin **1**, a new natural product was reported and characterized as α-3,4-di-*epi*-homonojirimycin **2**. In this paper, we wish to report the revision of the structure of that compound to α-4-*epi*-homonojirimycin **3** ("α-homoallonojirimycin") as a result of synthetic, biological and spectroscopic evidence.



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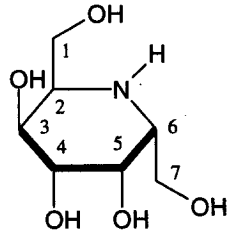

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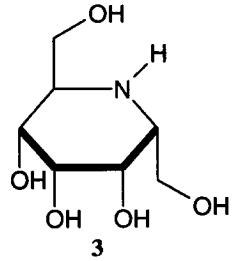

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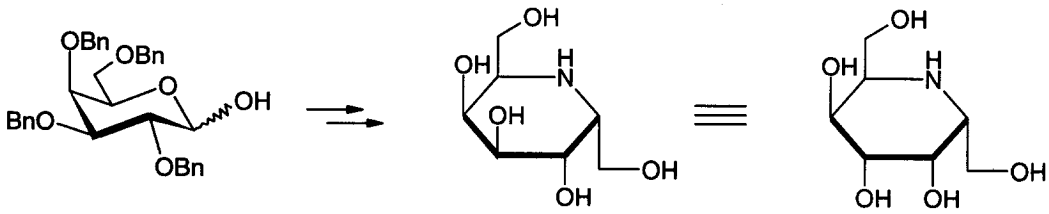
In 1995, the synthesis of α-homogalactostatin **5** was realized in 10 steps from 2,3,4,6-tetra-*O*-benzyl-*D*-galactose **4**.[7] The structure of **5** was determined unambiguously on the basis of mass and NMR spectral data (^1H and ^{13}C), of the structure of its synthetic precursor **4**, and from synthetic studies.[8] Within the context of a collaboration concerning a chemical chaperone therapy for Fabry disease by using 1-deoxygalactonojirimycin derivatives,[9] we had an opportunity to compare spectral data for α-homogalactostatin **5** and the natural product isolated from *Aglaonema treublii*. Examination of the structure of **5** and that of α-3,4-di-*epi*-homonojirimycin **2** revealed that the two compounds should be identical! However, the spectral data for the two products were clearly different (Table 1). In addition, the measured IC_{50} value against coffee bean α-galactosidase for compound **5** (0.022 μM) is nearly 4000 times smaller than that of the natural product (80 μM)[10].



4 **5** **2**

Careful re-examination and comparison of NMR data led us to propose the alternative structure **3** having an α-D-*allo* configuration (α-4-*epi*-homonojirimycin). The ^1H NMR spectral data, combined with extensive decoupling experiments and 2D ^1H-^{13}C COSY spectral data, allowed complete assignment of all carbons and hydrogens for compound **5** and **3** (Table 1).

	5		Natural	homoazasugar **3**
Position	$^{13}C^a$	$^1H^b$	$^{13}C^a$	$^1H^b$
1a	64.5	3.67 (dd, *J* = 7.5, 11.0)	63.5	3.14 (dd, *J* = 7.6, 11.5)
1b		3.72 (dd, *J* = 5.8, 11.0)		3.79 (dd, *J* = 4.9, 11.5)
2	55.8	3.05 (m)	57.2	3.12 (ddd, *J*= 4.9, 6.5, 7.6)
3	71.9	4.00 (dd, *J* = 2.6, 3.0)	72.0	3.68 (br dd, *J* = 2.9, 6.5)
4	73.8	3.70 (dd, *J* = 3.0, 9.8)	72.1	3.90 (t, *J* = 2.9, 2.9)
5	71.8	4.04 (dd, *J* = 5.9, 9.8)	72.2	3.92 (m)
6	59.3	3.30 (m)	58.1	3.06 (dt, *J* = 4.6, 4.6, 8.1)
7a	59.6	3.78-3.85 (m)	62.7	3.73 (dd, *J* = 4.6, 11.5)
7b				3.85 (dd, *J* = 8.1, 11.5)

[a] Recorded at 100 MHz in D_2O (ppm from TSP[c] as internal standard)
[b] Recorded at 400 MHz in D_2O (ppm from TSP[c] as internal standard, *J* = coupling constants in Hz)
[c] TSP = sodium 3-(trimethylsilyl)propionate

Table 1: ^{13}C and ^1H NMR data for compound **3** and **5** in D_2O

In the case of the homoazasugar **3**, irradiation of H-2 enhanced the intensity of H-7b. This confirmed the configuration at C-6 of the homoazasugar. Another crucial NOE effect was distinctly observed between H-3 and H-5 and confirmed the configuration at C-3 (Figure 1). Furthermore, no significant NOE effects were seen between H-2 and H-4 and between H-5 and H-7a or H-7b implying that H-4 and H-5 are equatorial and axial, respectively. The coupling constants are also in good agreement with the proposed structure **3**.

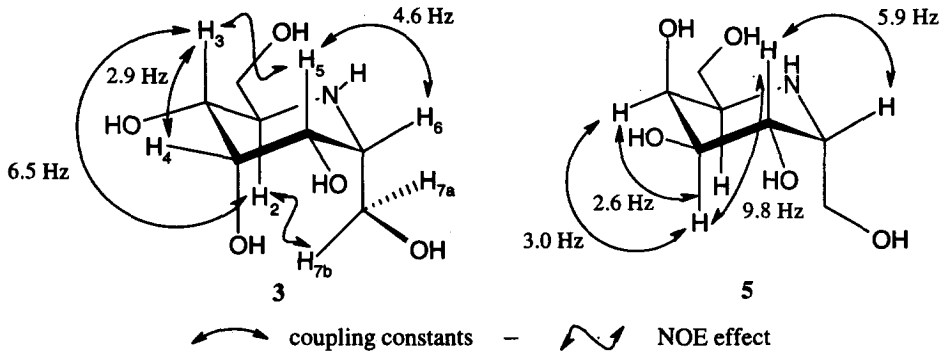
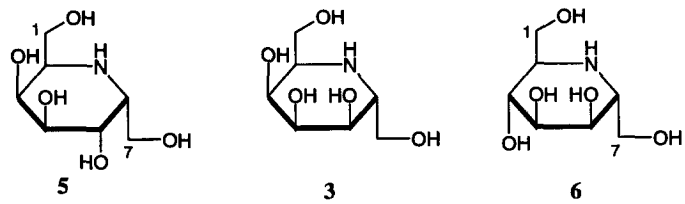


Figure 1 : Selected coupling constants and NOE effects in **3** and **5**

While the $^{3}J_{H,H}$-coupling constants observed for the "α-D-*galacto*" compound **5** show clearly that this compound exists predominantly in a chair conformation, the coupling constants in the "α-D-*allo*" epimer **3** indicate that the conformation of this compound deviates from a chair form as a result of 1,3 *syn*-diaxial interactions between the substituents at C-4 and C-6. The coupling constants could be consistent with a skew-conformation near the $^{N}S_{2}$ form.

The availability of the homonojirimycin epimer having the α-D-*allo* configuration[11] is of particular interest in connection with the structure-activity relationship studies of this family of iminoheptitols.[10,12] Compound **3** is indeed the C-4 epimer of α-homonojirimycin **1**, the C-5 epimer of α-homogalactostatin **5**, and the C-3 epimer of α-homomannojirimycin **6**.[3b]



Results of enzymatic assays on selected glycosidases are reported in Table 2. In addition, compounds **1** and **3** are not inhibitors of α-mannosidases (from Jack bean and rat liver lysosome).[13] It is remarkable that, in most cases, the change of configuration at a single site abolishes the activity of the product as an inhibitor or dramatically reduces its activity. These results reveal that the OH group corresponding to HO-3 in the parent glucosides and to HO-2 in galactosides are essential and that it is necessary to conserve these groups in the proper orientation in order to design efficient inhibitors. The importance of the relative orientation of the OH group in the 1-deoxynojirimycin series has already been documented.[14]

Enzyme	IC_{50} (μM)			
	1	**3**	**5**	**6**
α-glucosidase (rat intestinal maltase)	0.34[b]	NI[b,c]	NI	46[b]
α-galactosidase (coffee bean)	NI[b]	80[b]	0.022	NI[b]
α-galactosidase (rat epididymis)	NI	67	4.5	NI
β-galactosidase (bovine liver)	NI	160	NI	NI
α-L-fucosidase (bovine epididymis)	NI[b]	NI[b]	NI	30[b]

[a]Experimental methods are described in ref 10. [b]Taken from ref. 10.
[c]NI =Less than 50% inhibition at 1 mM.

Table 2: IC_{50} values towards selected glycosidases[a]

In conclusion, we have revised the structure of a homonojirimycin isomer isolated from *Aglaonema treubii* to α-4-*epi*-homonojirimycin **3** ("α-homoallonojirimycin"). The biological activity of this compound could be reinterpreted in light of its correct structure and provided useful information on structure-activity relationships in the family of homoazasugars.

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12. Compounds **1**, **3** and **5** are, strictly, 2,6-dideoxy-2,6-imino-heptitols having the D-*glycero*-L-*gulo*, D-*glycero*-L-*allo*, and D-*glycero*-L-*galacto* configurations.
13. Comp. **6** is a very weak inhibitor of the Jack bean enzyme (IC_{50} = 980 μM); it does not inhibit the rat liver enzyme.
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