MOLECULAR STRUCTURE AND GLYCOSIDASE-INHIBITORY ACTIVITY OF NOJIRIMYCIN BISULFITE ADDUCT

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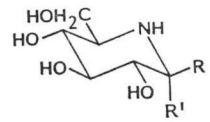
Nojirimycin is a potent inhibitor against glucosidases and glucoamylase[1~3], but is very unstable, especially under acidic conditions. However, it can be stored as its stable bisulfite adduct (**1**), which is quantitatively interconvertible to the free base (**2**)[4].

Since the first report on the preparation of 5-aminosugar bisulfite adduct in 1964[5], the structures of most 5-aminosugar bisulfite adducts including **1** have been considered to be acyclic[6]. Recently, we found that nojirimycin B bisulfite adduct was not acyclic but cyclic on the basis of ^{1}H NMR spectroscopy[7]. Furthermore, nojirimycin B bisulfite adduct showed stronger inhibitory activity than the parent nojirimycin B against apricot emulsin[7]. These results led us to examine the molecular structure and enzyme-inhibitory activity of **1**, and the results are described in this paper.

The structure of **1** was elucidated by X-ray structural analysis. Compound **1** was crystallized as monohydrate, and the crystal data are shown in Table 1. Fig. 1 shows the molecular framework of **1** illustrating the cyclic structure with a nitrogen in the ring. The torsion angle of C(2)–C(3)–C(5)–N is 0°, which means that these four atoms are coplanar. The two other ring atoms, C(1) and C(4), lie on opposite sides of this plane, with vertical distance of 0.68Å. This result indicated that **1** takes the C_1 conformation. The sulfonate group at C(1) is oriented β with equatorial substitution. The present compound is the first example of an 5-aminopyranose sugar, for which the conformation has been determined by X-ray analysis.

Table 2 gives the chemical shifts and coupling constants of **1** in deuterium oxide. The observed coupling constants of $J_{1,2}$, $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$ were 10.0~11.2 Hz, which, when the Karplus equation was applied[8], are in accord with the dihedral angles determined by X-ray analysis: H(N)–N–C(1)–H(1), 173.5°; H(1)–C(1)–C(2)–H(2), 166.3°; H(2)–C(2)–C(3)–H(3), 179.1°; H(3)–C(3)–C(4)–H(4), 175.4°; H(4)–C(4)–C(5)–H(5), 179.7°. This consistency indicated that **1** has a similar chair ring form with predominant β-configuration both in the crystal and in aqueous solution. No NMR signal corresponding to the α-epimer was observed when the aqueous solution was kept at room temperature for 24 hours.

Table 3 shows the 50% inhibition concentration of **1** against three glycosidases, and compares them with those of **2**. Compound **1** showed



1 R=SO_3OH, R′=H
2 R,R′=OH,H

Table 1. Crystal data of nojirimycin bisulfite adduct.

Composition:	$C_6H_{13}NO_7S\cdot H_2O$
Crystal system:	Triclinic
Space group:	P1 (z=2)
Lattice constant:	a=8.997 (Å)
	b=9.322
	c=6.045
	α=88.5°
	β=88.6°
	γ=77.0°
Lattice volume:	493.7 (Å^3)
Density:	1.75

Fig. 1. Perspective drawing of the molecule of nojirimycin bisulfite adduct (**1**).

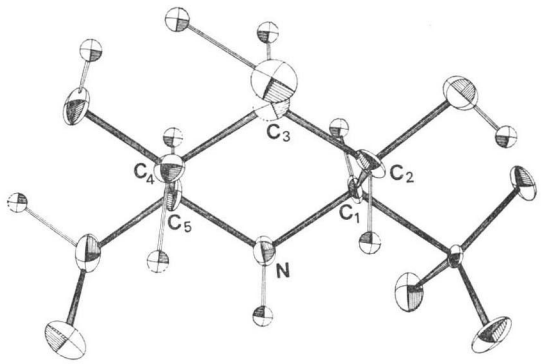


Table 2. Chemical shifts (δ) and coupling constants (J) of nojirimycin bisulfite adduct (**1**) in D_2O.

δ Values were determined by decoupling experiment.

	δ	J (Hz)
H_1	4.23	$J_{1,2}=10.5$
H_2	3.95	$J_{2,3}=10.0$
H_3	3.64	$J_{3,4}=10.0$
H_4	3.75	$J_{4,5}=11.2$
H_5	3.32	$J_{5,6}=4.4$
H_6	3.97	$J_{5,6'}=3.6$
$H_{6'}$	4.05	$J_{6,6'}=12.0$

Table 3. Inhibitory activities of nojirimycin bisulfite adduct (**1**) and nojirimycin free base (**2**) against apricot emulsin, *Trichoderma* β-glucosidase and rat epididymal α-mannosidase.

Compound	Concentrations required to cause 50% inhibition (I_{50})		
	β-Glucosidase		α-Mannosidase
	Emulsin	*Trichoderma*	Rat epididymal
1	2.0×10^{-5} M	1.4×10^{-5} M	$>10^{-2}$ M
2	7.9×10^{-6} M	5.0×10^{-6} M	3.8×10^{-4} M

potent activity against apricot emulsin and *Trichoderma viride* β-glucosidase, though it was 2.5 to 3.5 fold less active than **2**. The potent β-glucosidase-inhibitory activity of **1** may be related to the cyclic structure, which is much closer to that of the substrate than the open-chain structure once predicted, because the Lineweaver-Burk plot showed the inhibition of **1** to be competitive against apricot emulsin, showing an inhibition constant of 1.3×10^{-5} M.

However, **1** did not show inhibitory activity against rat α-mannosidase, while **2** showed potent activity. This result indicated that the potent β-glucosidase-inhibitory activity found for **1** may not be due to the formation of **2** by partial hydrolysis of sulfonate group, since if this were so, **1** should also show activity against α-mannosidase, against which **2** is active. The formation of the α and β epimers of **2** in solution is probably responsible for the inhibitory activity of **2** against α and β-glycosidases.

Therefore, we conclude that the nojirimycin bisulfite adduct with a cyclic structure and the bisulfite group having the β-configuration is responsible for the β-glucosidase-inhibitory activity.

Experimental

The preparation of **1** has been described[4]. The ^1H NMR spectrum was recorded on a Jeol 400 MHz spectrometer at room temperature.

X-Ray Structural Analysis

A single crystal of **1** was grown from an aq solution. Three dimensional intensity data were collected on a Philips PW-1100 automatic four-circle diffractometer with CuKα radiation. A total of 1,805 of independent reflections were collected.

The crystal structure was solved by the multi-solution method (MULTAN). The location of a sulfur atom was determined by the heavy atom procedure. The positional parameters of the 16 non-hydrogen atoms were obtained from a difference Fourier synthesis and refined by least-squares methods. Final refinement was performed by block-diagonal least-square method with inclusion of anisotropic thermal parameters for the non-hydrogen atoms and with isotropic thermal parameters for the hydrogen atoms. This gave a final R value of 0.057. The positional parameters are deposited with the Cambridge Crystallographic Data Center.

Enzyme Assay

Apricot emulsin and *Trichoderma* β-glucosidase used were the same preparations previously reported[1]. Rat epididymal α-mannosidase was prepared as described separately[7]. Activities of β-glucosidases and α-mannosidase were assayed using *p*-nitrophenyl β-D-glucopyranoside and *p*-nitrophenyl α-D-mannopyranoside as the respective substrates[1,7].

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