THE STRUCTURE OF NAGSTATIN, A NEW INHIBITOR OF *N*-ACETYL-β-D-GLUCOSAMINIDASE

Sir:

In the preceding paper[1], we have described the taxonomy, isolation, physico-chemical properties and biological activities of nagstatin (Fig. 1), a novel inhibitor of *N*-acetyl-β-D-glucosaminidase (NAG-ase). In this paper, we describe the structure determination of nagstatin.

The molecular weight and formula of nagstatin were elucidated as $C_{12}H_{17}N_3O_6$ (MW 299.3) by the SI-MS peak at *m/z* 322 $(M+Na)^+$, elemental analysis (found: C 48.58, H 5.80, N 13.96, O 31.99; calcd for $C_{12}H_{17}N_3O_6$: C 48.16, H 5.73, N 14.04, O 32.08) and ^1H and ^{13}C NMR spectra (Table 1). UV spectrum showed the maximum at 225 nm (log ε 3.52) in H_2O. IR spectrum (KBr) showed the presence of hydroxy group (3412 cm^{-1}), an amide bond (1670 cm^{-1}) and a carboxylate anion (1590 and 1376 cm^{-1}) which were supported by ^{13}C NMR signals at δ_C 175.9 (C-12) and 176.8 (C-10) ppm. Furthermore, ^{13}C NMR spectrum revealed three signals of sp^2 carbon except those of two carbonyl carbons and seven signals of sp^3 carbon in which three signals (C-6, C-7 and C-11) appeared in lower field (δ_C 69.7, 70.4 and 61.6 ppm) indicating the oxygen-bearing carbons, and also the signal (C-8) at δ_C 47.2 ppm indicating the nitrogen-bearing carbon.

In the ^1H-^1H COSY spectrum and proton decoupling NMR experiments, an *allyl*-spin spin coupling between an aromatic proton at δ_H 7.52 (3-H) ppm and methylene protons at δ_H 3.62 (9-H_2) ppm was observed, and *vicinal*-spin spin couplings between the signals at δ_H 4.16, 4.20 (11-H_2) ppm and the signal at δ_H 4.50 (5-H) ppm, δ_H 4.50 (5-H) and 4.54 (6-H) ppm, δ_H 4.54 (6-H) and 4.25 (7-H) ppm, δ_H 4.25 (7-H) and 5.23 (8-H) ppm were also observed. The methyl protons at δ_H 2.14 (13-H_3) ppm suggested the presence of acetyl moiety from its chemical shift. From the above results, the presence of three partial structures (Fig. 2A, B and C) were revealed.

As shown in Fig. 3, in the HMBC (Heteronuclear Multiple Bond Connectivity) spectrum, the methine proton at δ_H 5.23 (8-H) ppm coupled to three carbons at δ_C 70.4 (C-7), 143.3 (C-8a) and 175.9 (C-12) ppm, and the aromatic proton at δ_H 7.52 (3-H) ppm coupled to two carbons at δ_C 131.7 (C-2) and 143.3 (C-8a) ppm. The observation of NOE

Fig. 1. Structure of nagstatin.

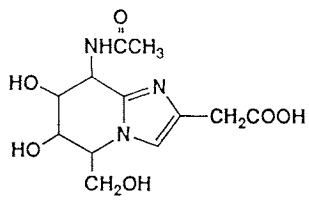


Table 1. ^{13}C and ^1H NMR data of nagstatin in D_2O.

Carbon	δ_C ppm (100 MHz)	δ_H ppm (*J* in Hz, 400 MHz)
2	131.7 (s)	
3	118.6 (d)	7.52 (1H, br s)
5	61.1 (d)	4.50 (1H, m)
6	69.7 (d)	4.54 (1H, dd, 2.1, 2.1)
7	70.4 (d)	4.25 (1H, dd, 10.0, 2.1)
8	47.2 (d)	5.23 (1H, d, 10.0)
8a	143.3 (s)	
9	33.9 (t)	3.62 (2H, br s)
10	176.8 (s)	
11	61.6 (t)	4.16 (1H, dd, 11.7, 5.1), 4.20 (1H, dd, 11.7, 5.9)
12	175.9 (s)	
13	23.0 (q)	2.14 (3H, s)

Fig. 2. Partial structures of nagstatin.

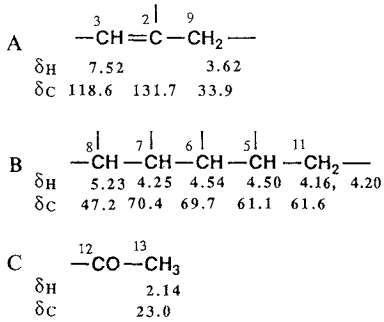


Fig. 3. ^1H-^{13}C correlation by HMBC experiment.

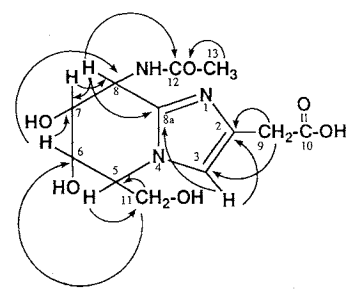
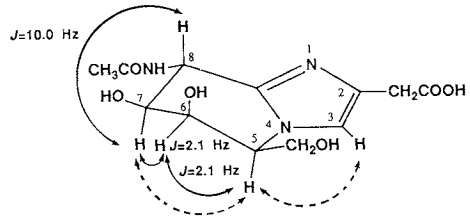


Fig. 4. Relative configuration of nagstatin.

Proton spin spin couplings as solid-line arrows and NOEs as dotted-line arrows.



enhancement between 3-H (δ_H 7.52) and 5-H (δ_H 4.50) established the position of substituent on the imidazole ring. Therefore, the structure of nagstatin was determined to be 8-(acetylamino)-5,6,7,8-tetrahydro-6,7-dihydroxy-5-(hydroxymethyl)-imidazo-[1,2-*a*]pyridine-2-acetic acid (Fig. 1).

The relative stereochemistry of nagstatin was presumed on the following grounds. In the ^{1}H NMR spectrum, the coupling constant between 7-H and 8-H ($J=10.0$ Hz) suggests these protons to be in *trans* diaxial relationship. Since the coupling constant between 6-H and 7-H was small ($J=2.1$ Hz), 6-H was assigned to be equatorial. Regarding the relative configuration of the remaining C-5, the NOE enhancement was observed between 5-H and 7-H, suggesting that the six membered ring takes a half-chair form in which these protons exist on the same side of the ring. The relative configuration of nagstatin has thus been deduced to be as illustrated in Fig. 4. The similar structure has been found in kifunensine[2].

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