ABSOLUTE CONFIGURATION OF THE AMINO SUGAR MOIETY OF THE NEOCARZINOSTATIN CHROMOPHORE

KIYOTO EDO*, YURIKO AKIYAMA, KUNIHITO SAITO and MICHINAO MIZUGAKI

Department of Pharmaceutical Sciences, Tohoku University Hospital, 1-1 Seiryo-machi, Sendai 980, Japan

YOSHIO KOIDE

Kayaku Antibiotics Research Co., Ltd., 2-8-16 Funado, Itabashi-ku, Tokyo 174, Japan

NAKAO ISHIDA

Department of Bacteriology, Tohoku University School of Medicine, 2-1 Seiryo-machi, Sendai 980, Japan

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The neocarzinostatin chromophore (NCS-chr) has been isolated from the antitumor polypeptide antibiotic NCS by extraction methods[1~3]. NCS-chr is responsible for the biological activities of the parent compound NCS, such as growth inhibition of bacteria and tumor cells, as well as strand scission of DNA *in vivo* and *in vitro*[4,5]. We have recently elucidated the structure of native NCS-chr (**1a**, epoxide form) as a bicyclo[7,3,0]dodecadienediyne derivative, as well as that of its hydrochloride adduct (**1b**)[6] (Fig. 1).

Although the structures of a number of components have been investigated by spectroscopic means, the absolute configuration of the amino sugar moiety, *N*-methylfucosamine (2,6-dideoxy-2-methylaminogalactose, **2**) and the configuration of the five asymmetric carbons at positions 4, 5, 10, 11 and 13 remain unknown.

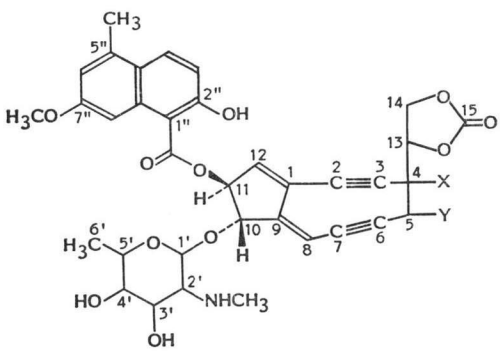
In this report, we demonstrate that the absolute configuration of **2** in **1a** and **1b** is D by direct comparison with a synthetic sample, and the anomeric configuration is assigned from spectroscopic data of analogous sugars.

Results

Isolation of **2** from **1b**

Direct hydrolysis of **1b** under acidic condi-

Fig. 1. Chemical structures of NCS-chr.



1a X, Y = −O−
1b X = OH Y = Cl

Scheme 1.

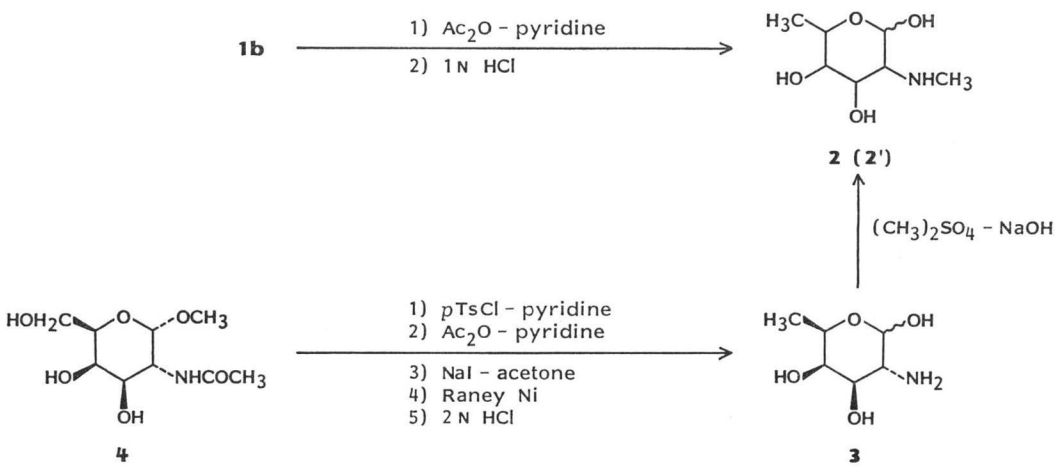


Table 1. ^{13}C NMR Data of **2** obtained from **1b**, **2'** and **2** in **1a** and **1b**.

Position	**2** obtained from **1b**[a] and **2'**		**2** in **1a**[b] (ppm)	**2** in **1b**[c] (ppm)
	α (ppm)	β (ppm)		
1	88.7 d	93.1 d	95.4 d	95.6 d
2	59.1 d	61.7 d	59.5 d	59.2 d
3	67.5 d	71.6 d	68.2 d	68.1 d
4	72.3 d	72.3 d	72.4 d	72.2 d
5	69.2 d	72.0 d	69.1 d	68.9 d
6	16.6 q	16.6 q	16.6 q	16.6 q
NCH_3	32.0 q	32.0 q	32.7 q	32.3 q

[a] Spectra of the anomeric mixture (α: β=10: 3) were measured at 25 MHz in D_2O at room temp.
[b] Taken at 100 MHz in CD_3COOD - CD_3OD (1: 1) from reference[6].
[c] Taken at 100 MHz in $^{12}CD_3OD$ from reference[6].

Table 2. ^1H NMR Data of **2** obtained from **1b**, **2'** and **2** in **1a** and **1b**.

Position	**2** obtained from **1b**[a] and **2'**[a]		**2** in **1a**[b] (ppm)	**2** in **1b**[c] (ppm)
	α (ppm)	β (ppm)		
1	5.93 (1H, d, J=4 Hz)	5.39 (1H, d, J=9 Hz)	5.75 (1H, d, J=3.0 Hz)	5.63 (1H, d, J=3.8 Hz)
2	ND	ND	3.65 (1H, dd, J=3.0, 10.5 Hz)	3.43 (1H, dd, J=3.8, 11.0 Hz)
3	ND	ND	4.21 (1H, dd, J=10.5, 2.5 Hz)	3.99 (1H, dd, J=11.0, 3.0 Hz)
4	ND	ND	3.90 (1H, d, J=2.5 Hz)	3.76 (1H, d, J=3.0 Hz)
5	ND	ND	4.07 (1H, q, J=6.5 Hz)	4.07 (1H, q, J=6.5 Hz)
6	1.65 (3H, d, J=7 Hz)	1.71 (3H, d, J=7 Hz)	1.26 (3H, d, J=6.5 Hz)	1.26 (3H, d, J=6.5 Hz)
NCH_3	3.23 (3H, s)	3.28 (3H, s)	3.02 (3H, s)	2.92 (3H, s)

[a] Spectra of the anomeric mixture (α: β=10: 3) were measured at 100 MHz in D_2O at room temp.
[b] Taken at 400 MHz in CD_3COOD - CD_3OD (1: 1) from reference[3].
[c] Taken at 400 MHz in $^{12}CD_3OD$ from reference[6].
ND: Not determined.

tions gave little amino sugar moiety (**2**). After acetylation of **1b**, **2** was isolated by 1 N HCl acid hydrolysis. Crude **2** was purified by ion exchange column chromatography (Dowex 50 (H^+)). The ninhydrin positive fractions were pooled and then purified by preparative Avicel cellulose TLC (Scheme 1).

Structure of **2** Obtained from **1b**

The molecular formula ($C_7H_{15}NO_4$) and molecular weight (177) of **2** were established by fast atom bombardment mass spectrum (FAB-MS) and high resolution mass spectrum (HR-MS). The optical rotation of **2** obtained from **1b** was +73.3° in water. The ^{13}C NMR signals of the α-anomer of **2** showed very similar chemical shifts and coupling patterns to those of amino sugar moiety in **1a** and **1b** (Table 1), clearly indicating **2** as a structural component of **1a** and **1b**. The above results and ^1H NMR data of **2**, and **2** in **1a** and **1b** (Table 2) suggest that this amino sugar moiety obtained from **1b** is methylfucosamine as described before[6,7].

Identification of **2** as D-Methylfucosamine

The absolute configuration of **2** was suggested to be D because of the similarity of its optical rotation value to that of the known *N*-demethylated derivative (+81°)[8].

In order to confirm this assumption, we syn-

Table 3. Identification **2** obtained from **1b** with **2'**.

	2'	**2** obtained from **1b**
TLC[a] Rf value	0.32	0.32
FAB-MS (MH^+) m/z	178	178
GC-MS[b,c]		
retention time (minutes)	2.6, 3.0	2.6, 3.0
(M^+) m/z	393, 393	393, 393
$[\alpha]_D^{25}$ (c 0.1, H_2O)	+73.1°	+73.3°

[a] Avicel cellulose plate (Funakoshi), solvent system; BuOH - EtOH - H_2O (4: 1: 1).

[b] Trimethylsilylation; hexamethyldisilazane - trimethylchlorosilane - pyridine (2: 1: 10), room temp, 20 minutes.

[c] Electron impact, accelerating voltage; 3 kV, ionizing potential; 70 eV, 2% OV-17 (1 m), 150~200°C.

thesized D-*N*-methylfucosamine (**2'**). First, D-fucosamine (**3**) was prepared according to ZEHAVI's method[8], using methyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**4**). Namely, treatment of **4** with *p*-toluenesulfonyl chloride (*p*TsCl) in pyridine gave the 6-*O*-*p*-tolylsulfonyl derivative. After acetylation with acetic anhydride in pyridine, this *p*-tolylsulfonyl derivative was allowed to react with sodium iodide in acetone. Hydrogenolysis of the obtained iodosugar by Raney nickel, followed by acid hydrolysis, yielded **3**. Then, **2'** was obtained by methylation of **3** with dimethyl sulfate in the presence of sodium hydroxide according to literature[9] (Scheme 1).

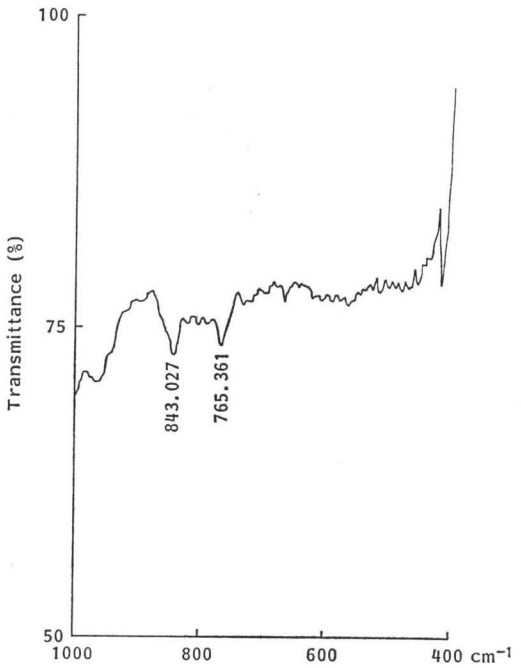
The properties of **2'** are identical with those of **2** as shown in Table 3. Compound **2'** exhibited the same molecular weight by FAB-MS and Rf value on TLC as **2**. In addition, the mass chromatogram of the trimethylsilyl derivatives of **2** and **2'** presented two peaks, with mass spectra of the first and second peaks corresponding to α- and β-anomer forms being essentially the same.

The optical rotation of **2** was +73.3° as described above, while that of **2'** was +73.1°. These data confirm that **2** obtained from NCS-chr was identical with **2'**.

The Configuration of C-1 Position of Methylfucosamine in **1a** and **1b**

In order to clarify the configuration of the anomeric position of **2** in **1a** and **1b**, ^1H NMR, ^{13}C NMR and IR spectral data of **2** in **1a** and **1b** were compared with those of the α- and β-anomers of **2'**. ^{13}C and ^1H NMR assignments

Fig. 2. Fourier transform infrared spectrum of **1b** in KBr pellet at room temp.
The spectra represents 32 scans.

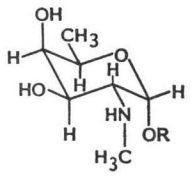


of both α- and β-anomers of **2'** and **2** in **1a** and **1b** are summarized in Tables 1 and 2, respectively.

The coupling constants between the protons on C-1 and C-2 ($J_{1,2}$) of **2** in **1a** and **1b** were 3.0 and 3.8 Hz, respectively, which were closer to that of α-anomer of **2'** (4.0 Hz) than that of β-anomer of **2'** (9.0 Hz). ^{13}C Chemical shifts of **2** in **1a** and **1b** except for the C-1 position, also showed close similarity to that of α-anomer of **2'** as shown in Table 1.

It has already been established that alkylation of the hydroxyl group at the anomeric (C-1) position on a glycopyranose causes downfield chemical shifts of both α- and β-anomeric carbons of 8~11 ppm. In the case of galactose, the shifts of the α- and β-anomers, caused by conversion to the methyl glycoside, are 6.97 and 7.21 ppm, respectively[10]. As shown in Table 1, the ^{13}C NMR spectral data show that the differences of chemical shifts between **2** in **1a** and **1b** (glycoside form) and α-anomer of **2'** (glycose form) are 6.7 and 6.9 ppm, respectively, and those between **2** in **1a** and **1b** (glycoside form) and β-anomer of **2'** (glycose form) are 2.3 and 2.5 ppm, respectively. These differences of

Fig. 3. Conformational structure of α-D-*N*-methylfucosamine in **1a** and **1b**.



$^4C_1$

chemical shifts between **2** in **1a** (**1b**) and the α- (or β-) anomer clearly indicate that it is reasonable to regard **2** in **1a** (**1b**) as the α-anomer.

This assumption was supported by the IR spectral data of **1b**. In the Fourier transform infrared spectrum of **1b** (Fig. 2), characteristic absorption bands of α-galactopyranose (type 2a; $844\pm8\ cm^{-1}$ and type 3; $766\pm10\ cm^{-1}$) as reported by BARKER *et al.*[11] were found at 843 and 765 cm^{-1} but no absorption band of type 2b ($891\pm7\ cm^{-1}$) of β-galactopyranose was observed.

Discussion

The NCS-chr possesses α-D-methylfucosamine (Fig. 3) as a partial structure according to the above data. The only conformation of **2** in **1a** and **1b** compatible with the observed coupling constants of vicinal hydrogens on the methylfucosamine ring is the $^4C_1$ type conformation of the α-anomer under the NMR conditions (in deutero methanol and deutero methanol - deutero acetic acid at 8°C), based on the conformational nomenclature mentioned by IUPAC-ICB joint commission[12].

Compound **2** showed no antimicrobial activity for *Micrococcus luteus* in the presence of the apoprotein of NCS (apo-NCS) at the level of the parent substances **1a** and **1b**[13]. However, **1a** and **1b** are bound to apo-NCS not only by a hydrophobic bond but also by an ionic bond between the basic aminosugar center of NCS-chr and acidic residue(s) of apo-NCS[14]. In addition, it is assumed that when NCS-chr intercalates with DNA, electrostatic interaction between the positively charged 2-methylamino group of the amino sugar residue and the negatively charged oxygens of the phosphate in the DNA backbone is probably required before the interaction of the naphthoic residue between base pairs[15]. These findings suggest that methylfucosamine of NCS-chr plays important roles in binding of NCS-chr with apo-NCS or DNA.

Experimental

Chemicals

NCS was a product of Kayaku Antibiotics Research Co., Ltd., Tokyo. All other chemicals were of the highest grade commercially available.

Spectrometrical Measurements

Optical rotations were determined with a DIP-140 digital polarimeter. IR spectra were recorded using a FT-IR Nicolet 60SX spectrometer. 100 MHz ^1H NMR and 25 MHz ^{13}C NMR spectra were measured by Jeol-FX 100 instrument at room temp in D_2O using TMS as internal standard. MS were obtained on a Jeol-DX 303 spectrometer.

Isolation of **1b** from NCS

NCS powder (1 g) was suspended in glacial acetic acid (50 ml) at 4°C for 1 hour in the dark. The resulting suspension was centrifuged at 3,000 rpm for 15 minutes. The supernatant was added 0.3 ml of concentrated hydrochloric acid and lyophilized. A brown powder was obtained and yield was about 100 mg.

Hydrolysis of **1b** under Acidic Conditions and Purification of **2**

1b (70 mg) was treated with acetic anhydride - pyridine (1 : 1) mixture (7 ml) for 2 days at room temp in the dark. The solvent was evaporated *in vacuo*, and acetylated **1b** was extracted with $CHCl_3$. Acetylated **1b**, free from solvent by evaporation under reduced pressure, was dissolved in 70 ml of 1 N HCl and left for 2 days in the dark at room temp. Then the reaction mixture was filtered through glass wool, washed with diethylether, and purified by TLC (Avicel cellulose TLC plate, Funakoshi, Tokyo, Japan) with BuOH - EtOH - H_2O at 4 : 1 : 1 ratio. The Rf value of **2** was 0.32, which was the same as that of **2'**, and the yield was 6.6 mg (25%). HR-MS of tetratrimethylsilyl derivative of **2**: *m/z* 465.2589; calcd for $C_7H_{15}NO_4\cdot(TMS)_4$ 465.2580; $[\alpha]_D^{25}$ +73.3° (final, *c* 0.07, H_2O).

Synthesis of **2'**

Hydrochloride of **3** was prepared by the method described in literature[8], using **4** (Nakarai Chemical Ltd., Tokyo Japan) as starting material. **2'** was synthesized by the modified method of KUEHL *et al.*[9] as follows: Dimethyl

sulfate, 6 μl, was added to 16 mg of **3** dissolved in 50 μl of 1 N NaOH, and the solution was shaken at room temp for 1.5 hours. The reaction mixture was diluted with 1 ml of ice-cold water and passed through a column (0.5 × 5 cm) of Dowex 50(H^+). The column was washed with about 10-bed volumes of water, and the adsorbed **2'** was eluted with 10 ml of MeOH - 3 N NH_4OH - H_2O (2: 5: 3). Eluted **2'** was freed from solvent under reduced pressure and purified by TLC (Avicel cellulose TLC plate, Funakoshi, Tokyo, Japan) with BuOH - EtOH - H_2O (4: 1: 1). The Rf value of **2'** was 0.32 and yield was 11.3 mg (80%). The purified **2'** was obtained as a colorless needles: MP 158~161°C (dec); $[\alpha]_D^{25}$ +73.1° (final, *c* 0.1, H_2O).

Anal Calcd for $C_7H_{15}NO_4 \cdot HCl$:
C 39.35, H 7.55, N 6.56.
Found: C 39.21, H 7.80, N 6.31.

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