STRUCTURAL ALTERATION OF SN-07 CHROMOPHORE

KEN-ICHI KIMURA, SHŌJI NAKAYAMA, NOBUO MIYATA and GOSEI KAWANISHI

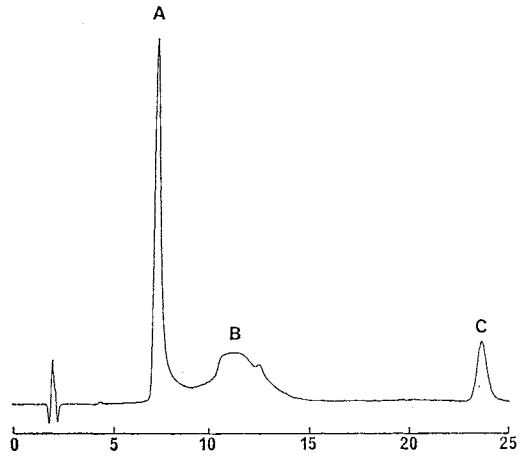
Research Institute of Life Science, Snow Brand Milk Products Co., Ltd., Ishibashi-machi, Shimotsuga-gun, Tochigi 329-05, Japan

(Received for publication April 30, 1988)

SN-07 chromophore[1,2] (barminomycin I)[3] is an anthracycline antibiotic and is the active constituent of the macromolecular antibiotic SN-07[4]. It is unique insofar as it contains an eight-membered ring with a carbinolamine structure. We showed that the carbinolamine structure changed to the imine structure in $CDCl_3$ during NMR measurement. We have defined the carbinolamine structure by the ^1H NMR spectrum and the imine structure by the ^{13}C NMR spectrum of the same sample, but the latter gave a complicated ^1H NMR spectrum. Since the SN-07 chromophore was labile in various solvents, especially under acidic conditions, we were unable to isolate the carbinolamine and imine structures and measure their ^1H NMR spectra separately. We describe here the isolation of the carbinolamine, imine and enamine structures of the SN-07 chromophore by HPLC and the identification of their structures by ^1H NMR. We report on the newly identified enamine structure that is the imine tautomer. Accordingly, we propose a new structure for the SN-07 chromophore.

We have already isolated the SN-07 chromophore as the carbinolamine structure from the mycelial cake of *Actinomadura roseoviolacea* var. *miuraensis* nov. var. Analytical HPLC showed that it initially gave a single peak with a retention time of 7.2 minutes (peak A, column: YMC R-ODS-5 (4.6×250 mm), solvent: CH_3CN - 0.1 M NaH_2PO_4 (40:60), flow rate: 1.5 ml/minute, detector: VIS (505 nm)). However the HPLC profile as well as the NMR spectrum in $CDCl_3$ of the SN-07 chromophore (carbinolamine structure) gradually changed with time. We recognized two new peaks that had retention times of 11.1 minutes (peak B) and 23.6 minutes (peak C) (Fig. 1). To elucidate the structure of these components, we have isolated them by preparative HPLC and determined their ^1H NMR spectra in $CDCl_3$.

Fig. 1. HPLC profile alteration of SN-07 chromophore.



Column: YMC R-ODS-5 (4.6×250 mm), solvent system: CH_3CN - 0.1 M NaH_2PO_4 (40:60), detector: VIS (505 nm), flow rate: 1.5 ml/minute.

The purification procedure used for peak A was that previously described[1]. Substances corresponding to peaks B and C were isolated with some modification of the same procedure. After Sephadex LH-20 column chromatography with MeOH, B and C rich fractions were combined separately and concentrated to a small volume. Then preparative HPLC was performed (column: Senshu Pak ODS-5251-N (20×250 mm), solvent: CH_3CN - 0.1 M NaH_2PO_4 (50:50), flow rate: 10 ml/minute, detector: UV (220 nm)). After removal of CH_3CN from B and C fractions, each was passed through a column of MCI-gel (CHP20P). The column was washed with deionized water and then eluted with MeOH. The eluates were concentrated to a small volume and lyophilized. NMR spectra were recorded on Jeol GX-400 or GSX-500 (400 MHz or 500 MHz) spectrometers using TMS as an internal standard.

A comparison of ^1H NMR spectra and chemical shifts for compounds A, B and C are shown in Fig. 2 and Table 1. The ^1H NMR spectra of the aglycone moieties are superimposable. Remarkable differences in chemical shifts were only observed in the acetal and 3′-H of the daunosamine moieties. Mild acid hydrolysis

Fig. 2. ^1H NMR spectra of SN-07 chromophore (400 or 500 MHz, $CDCl_3$).
(A) Carbinolamine, (B) imine, (C) enamine.

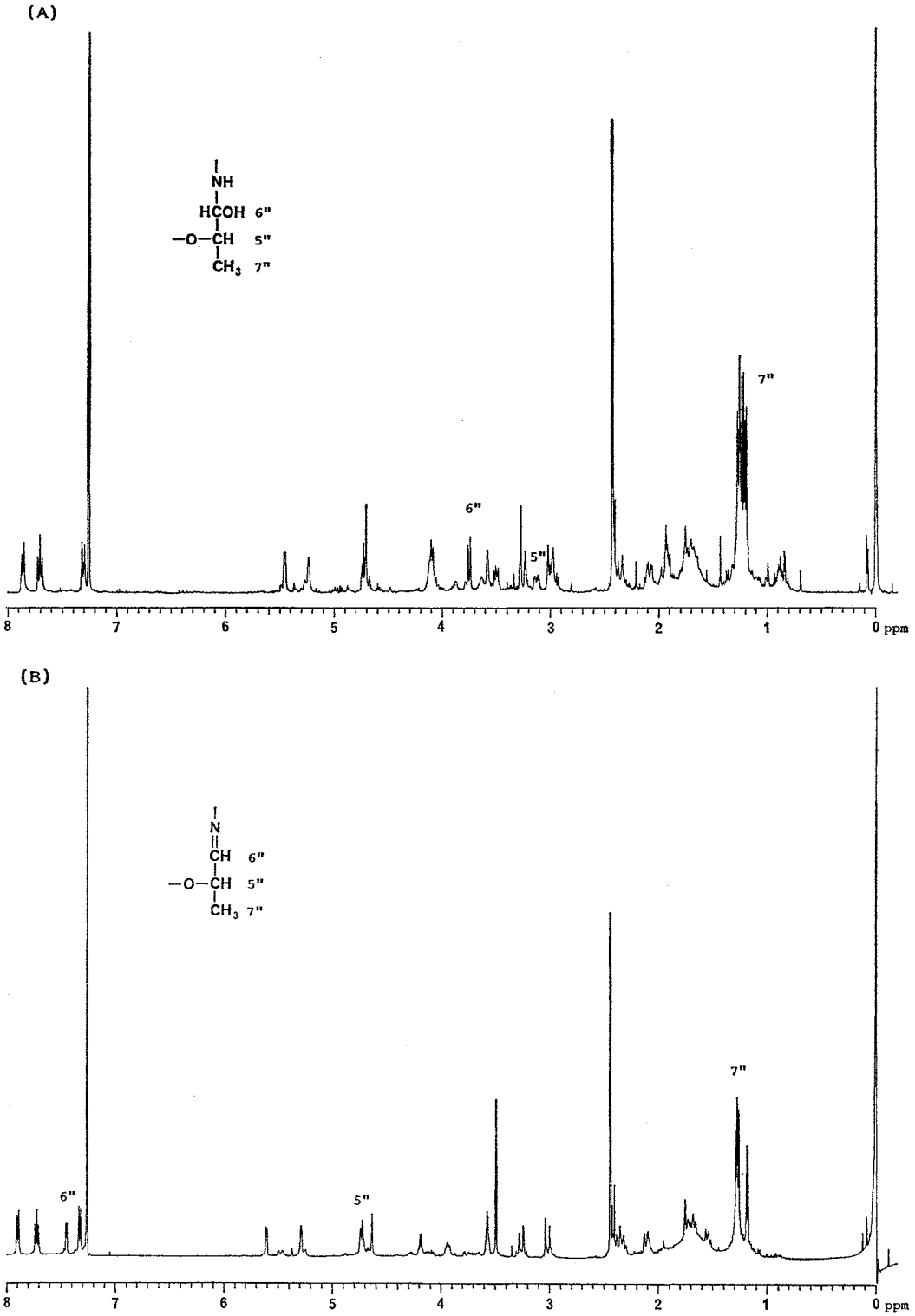


Fig. 2. (Continued)

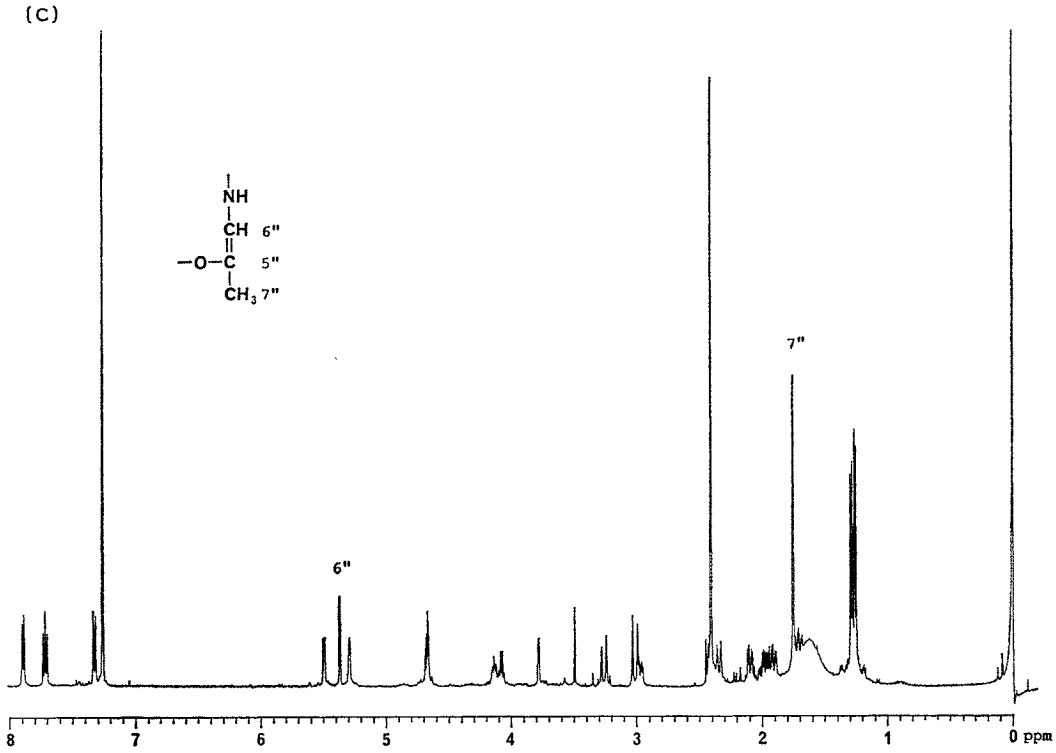
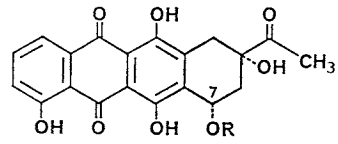


Table 1. ^{1}H NMR chemical shifts of SN-07 chromophore.

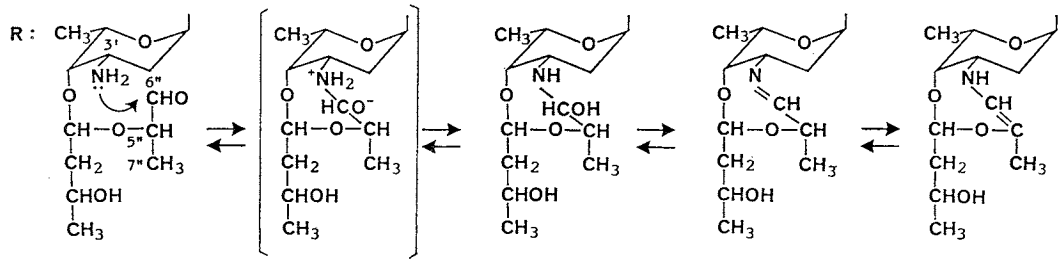
Proton	Substance A (carbinolamine, δ)	Substance B (imine*, δ)	Substance C (enamine*, δ)	Remarks
1-H	7.87 (dd)	7.90 (dd)	7.89 (dd)	Aglycone moiety
2-H	7.71 (dd)	7.72 (dd)	7.72 (dd)	
3-H	7.31 (dd)	7.33 (dd)	7.33 (dd)	
7-H	5.24 (br s)	5.29 (br s)	5.30 (br s)	
8-H_{eq}	2.36 (br d)	2.37 (br d)	2.35 (br d)	
8-H_{ax}	2.09 (dd)	2.11 (dd)	2.10 (dd)	
10-H_{eq}	3.26 (br d)	3.26 (dd)	3.26 (dd)	
10-H_{ax}	3.00 (d)	3.02 (d)	3.02 (d)	
$COCH_3$	2.43 (s)	2.44 (s)	2.41 (s)	
1′-H	5.46 (br d)	5.61 (d)	5.49 (d)	Daunosamine moiety
2′-H_2	1.6~1.8 (m)	2.3~1.6 (m)	1.9, 1.7 (m)	
3′-H	3.13 (m)	3.57 (m)	2.97 (m)	
4′-H	3.58 (br s)	3.57 (br s)	3.78 (br s)	
5′-H	4.10 (m)	4.19 (dq)	4.07 (dq)	
6′-H_3	1.27 (d)	1.28 (d)	1.29 (d)	
1″-H	4.73 (dd)	4.73 (dd)	4.68 (dd)	Acetal moiety
2″-H_2	1.9~2.0 (m)	1.6~1.8 (m)	1.9~2.0 (m)	
3″-H	4.10 (m)	3.93 (m)	4.13 (m)	
4″-H_3	1.23 (d)	1.19 (d)	1.26 (d)	
5″-H	3.50 (m)	4.73 (m)	—	
6″-H	3.75 (d)	7.45 (d)	5.37 (s)	
7″-H_3	1.20 (d)	1.27 (d)	1.75 (s)	

Spectra were measured in $CDCl_3$ using TMS as an internal reference (400 MHz, * 500 MHz).

Fig. 3. Structural alteration of SN-07 chromophore.



Carminomycinone



Aldehyde
$C_{33}H_{39}NO_{13}$
(MW 657)

Carbinolamine
$C_{33}H_{39}NO_{13}$
(MW 657)

Imine
$C_{33}H_{37}NO_{12}$
(MW 639)

Enamine
$C_{33}H_{37}NO_{12}$
(MW 639)

with 0.4 N HCl at room temperature for 2 hours converted SN-07 chromophore to carminomycin I[5,6] identified by direct comparison with an authentic sample using ^{1}H NMR, HPLC and TLC. In the ^{1}H NMR spectrum of substance A, the signal at δ 3.75 (1H, d, J=8.3 Hz, 6″-H) coupled with the signal at δ 3.50 (1H, m, 5″-H) and this coupled with the signal at δ 1.20 (3H, d, J=6.1 Hz, 7″-CH_3) by the two-dimentional (2D) proton-proton shift correlation spectrum (COSY). The field desorption mass spectrum (FD-MS) of substance A did not show a molecular ion peak m/z 657 ($C_{33}H_{39}NO_{13}$), but an intensive fragment peak m/z 639 (M−18) with the loss of H_2O. Elemental analysis (*Anal* Calcd: C 58.67, H 6.07, N 2.07. Found: C 58.81, H 5.92, N 2.03) gave a molecular formula $C_{33}H_{39}NO_{13}\cdot H_2O$. Therefore we propose the carbinolamine structure for substance A.

The characteristic signal of substance B was the methine signal at δ 7.45 (1H, d, J=4.0 Hz, 6″-H) and correlated to a signal at δ 164.8 in ^{13}C NMR spectrum on the basis of the ^{1}H-^{13}C COSY spectrum. In $CDCl_3$, the carbinolamine signal at δ 3.75 (6″-H) in substance A was gradually converted to the signal at δ 7.45. Low field shift of the signals at δ 3.13 (1H, m, 3′-H) and δ 3.50 (1H, m, 5″-H) in substance A to the signals at δ 3.57 (1H, m, 3′-H) and δ 4.73 (1H, m, 5″-H) respectively seemed to form the imine structure through C-3′- and C-6″.

In the ^{1}H NMR spectrum of substance C, the carbinolamine signal at δ 3.75 (6″-H) in substance A changed to a singlet with a signal at δ 5.37 (1H, s, 6″-H). The doublet signal at δ 1.20 (7″-CH_3) in substance A also changed to singlet with a signal at δ 1.75. Signal for the 5″-H was not observed in substance C. The FD-MS spectrum of substance C gave the molecular ion peak m/z 640 $(M+H)^+$. These data indicated the existence of a double bond between C-5″ and C-6″ and the enamine structure.

We could not detect the aldehyde structure by HPLC and ^{1}H NMR. However we succeeded in isolating the acyclic amino alcohol (carminomycin III) by reduction of SN-07 chromophore[2], and this supports the tautomeric equilibrium of aldehyde-carbinolamine in the SN-07 chromophore. Carminomycin III was already determined to have the 3″*S* and 5″*S* configuration[7], so the SN-07 chromophore (aldehyde structure) should have the same configuration. From these results, we propose a new structure for the SN-07 chromophore as shown in Fig. 3. The optical rotation $[\alpha]_D$ of the carbinolamine structure was positive ($[\alpha]_D^{25}$ +120° (*c* 0.030, $CHCl_3$)) in contrast with our previous paper[2]. $[\alpha]_D$ also changed according to structural alteration of SN-07 chromophore.

We propose that *A. roseoviolacea* var.

miuraensis nov. var. initially biosynthesizes the SN-07 chromophore as the aldehyde structure. However an intermolecular nucleophilic reaction between amino group at C-3′ and aldehyde group at C-6″ should occur immediately. In our isolation procedure, we obtained mainly the carbinolamine structure. On standing in $CHCl_3$, the carbinolamine structure forms a Shiff base and converts to the imine structure. A trace of acid in the $CDCl_3$ could catalyze the elimination of water from the carbinolamine structure giving rise to a Shiff base. The enamine structure is a tautomer of the imine structure. This mechanism is analogous to that proposed for the reaction of the pyrrolo[1,4]benzodiazepines[8] and the saframycins[9].

Acknowledgment

The authors are grateful to Drs. K. ISONO, M. URAMOTO and J. UZAWA, RIKEN, the Institute of Physical and Chemical Research, for their powerful discussions and measurement of NMR.

References

1) KIMURA, K.; S. NAKAYAMA, T. KOYAMA, S. SHIMADA, N. KAWAGUCHI, N. MIYATA, Y. TAKESHITA & G. KAWANISHI: SN-07 chromophore: An anthracycline antibiotic from the macromolecular antibiotic SN-07. J. Antibiotics 40: 1353～1355, 1987
2) KIMURA, K.; S. NAKAYAMA, N. MIYATA, Y. TAKESHITA & G. KAWANISHI: The structure of SN-07 chromophore. J. Antibiotics 41: 411～414, 1988
3) UCHIDA, T.; M. IMOTO, Y. TAKAHASHI, A. ODAGAWA, T. SAWA, K. TATSUTA, H. NAGANAWA, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: New potent anthracyclines, barminomycins I and II. J. Antibiotics 41: 404～408, 1988
4) KIKUCHI, Y.; M. NIWANO, N. YAJIMA, G. NAKAMURA & N. MIYATA: A novel macromolecular antibiotic, SN-07. Taxonomy of producing organism, isolation, characterization and biological activities. J. Antibiotics 38: 1670～1676, 1985
5) BRAZHNIKOVA, M. G.; V. B. ZBARSKY, V. I. PONOMARENKO & N. P. POTAPOVA: Physical and chemical characteristics and structure of carminomycin, a new antitumor antibiotic. J. Antibiotics 27: 254～259, 1974
6) BRAZHNIKOVA, M. G.; V. B. ZBARSKY, D. TRESSELT & K. ECKARDT: Stereochemistry of the antibiotic carminomycin. J. Antibiotics 29: 469～471, 1976
7) OGAWA, Y.; H. MORI, N. YAMADA & K. KON: The absolute structures of rubeomycins A and A_1 (carminomycins II and III) and rubeomycins B and B_1 (4-hydroxybaumycinols A_1 and A_2). J. Antibiotics 37: 44～56, 1984
8) THURSTON, D. E. & L. H. HURLEY: A rational basis for development of antitumor agents in the pyrrolo[1,4]benzodiazepine group. Drugs Future 8: 957～971, 1983
9) LOWN, J. W.; A. V. JOSHUA & J. S. LEE: Molecular mechanisms of binding and single-strand scission of deoxyribonucleic acid by the antitumor antibiotics saframycins A and C. Biochemistry 21: 419～428, 1982