THE STRUCTURE OF MINIMYCIN, A NOVEL CARBON-LINKED NUCLEOSIDE ANTIBIOTIC RELATED TO β-PSEUDOURIDINE

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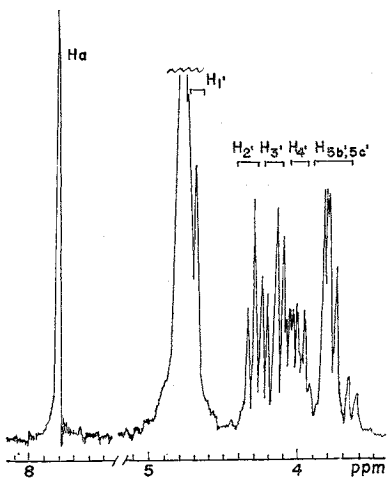
Structural elucidation of minimycin, a novel C-ribonucleoside antibiotic, which was established as 5-β-D-ribofuranosyl-1,3-oxazin-2,4-dione (I), and structural relationship to β-pseudouridine are described.

Minimycin was first isolated from the fermentation broth of *Streptomyces* strain sp. 80,432 by KUSAKABE *et al.*[1] Minimycin is a colorless crystalline antibiotic of empirical formula $C_9H_{11}NO_7$ (M.W. 245 by mass spectrometry) which is active against both gram-positive and gram-negative bacteria, and also possesses significant antitumor activity against mouse ascites and som solid tumor cells *in vivo*[1]. Minimycin has a melting point of 164~166°C with decomposition, a specific rotation of $[\alpha]_D^{25}$ +18° (*c* 1, water), ultraviolet end-absorption with an inflection at 230 nm in methanol, and a pK'a value of 7.55 by the potentiometric titration method.

Treatment of minimycin with methanolic ammonium hydroxide at room temperature or with ammonium carbonate in aqueous methanol at 60~70°C afforded in poor yield pseudouridine which showed ultraviolet absorption maxima at 208 and 264 nm (ε 12,850 and 8,850), and was identified with authentic β-pseudouridine (purchased from Carbiochem, Los Angels, Calif., U.S.A.) by comparisons of their mass spectra, ultraviolet spectra, and cellulose thin-layer chromatographic behaviors.

Fig. 1. Minimycin.
Solvent : D_2O. Int. std. : DSS



The 100 MHz pmr spectrum of minimycin was much closely similar to that of β-pseudouridine except for the chemical shift of the vinyl proton (Fig. 1). The spectral assignment and a

Table 1. Proton chemical shifts and coupling constants (J) of minimycin and β-pseudouridine

Proton	Minimycin		β-Pseudouridine	
	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
Ha	7.78 (s)		7.63 (s)	
$H_{1'}$	4.65 (d)	$J_{1'2'}$ 5.0	4.67 (d)	$J_{1'2'}$ 5.0
$H_{2'}$	4.27 (t)	$J_{2'3'}$ 5.0	4.27 (t)	$J_{2'3'}$ 5.0
$H_{3'}$	4.13 (t)	$J_{3'4'}$ 5.2	4.13 (t)	$J_{3'4'}$ 5.2
$H_{4'}$	4.00 (ddd)	$J_{4'5b'}$ 3.0	4.00 (ddd)	$J_{4'5b'}$ 3.5
$H_{5b'}$	3.81 (dd)	$J_{4'5c'}$ 4.3	3.82 (dd)	$J_{4'5c'}$ 4.5
$H_{5c'}$	3.71 (dd)	$J_{5b'5c'}$ −12.5	3.73 (dd)	$J_{5b'5c'}$ −12.5

(s) singlet ; (d) doublet ; (t) triplet ; (dd) double doublet ; (ddd) doubling double doublet.

computer analysis of ribose-ring protons of β-pseudouridine have been reported in detail by HRUSKA *et al.*[2] Proton chemical shifts and coupling constants of both minimycin and β-pseudouridine determined in deuterium oxide were presented in Table 1, and the spectral pattern of the region, 3.6~4.8 ppm, was virtually agreed with the calculated spectrum which was computer-analyzed as a 6-spin system[2].

The mass spectrum of minimycin showed molecular ion peak at *m/e* 245 and characteristic fragmentation peaks at *m/e* 227 ($M-H_2O$), 209($M-2H_2O$), 197, 184, 172, and 112 (base peak due to $C_4H_2NO_3$), these were the peaks one mass unit larger than those from β-pseudouridine.

Treatment of minimycin with aqueous hydrazine at 100°C gave D-ribose which was detected on paper chromatograms by the use of aniline phthalate spray[3]. This procedure has been employed for the detection of D-ribose in pseudouridine[4]. These data strongly indicated the presence of ribosyl function, $C_5H_9O_4$, attached to $C_4H_2NO_3$ moiety having three possible partial structures, suggesting that minimycin is a novel D-riboside antibiotic.

Fig. 2.

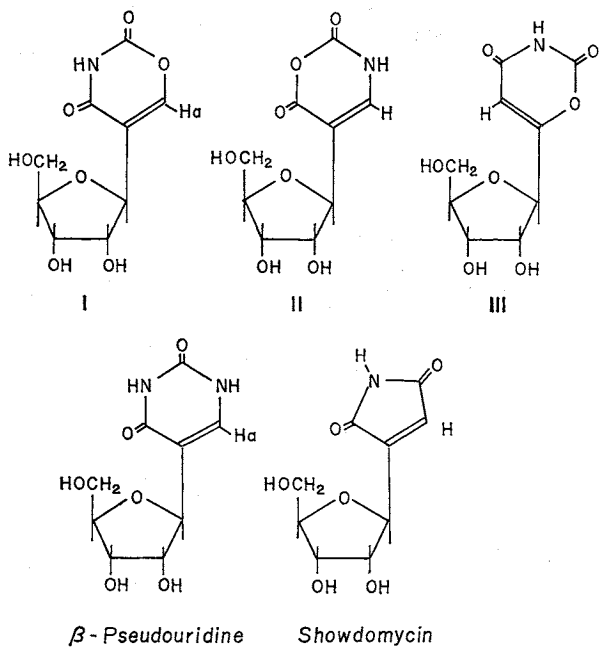
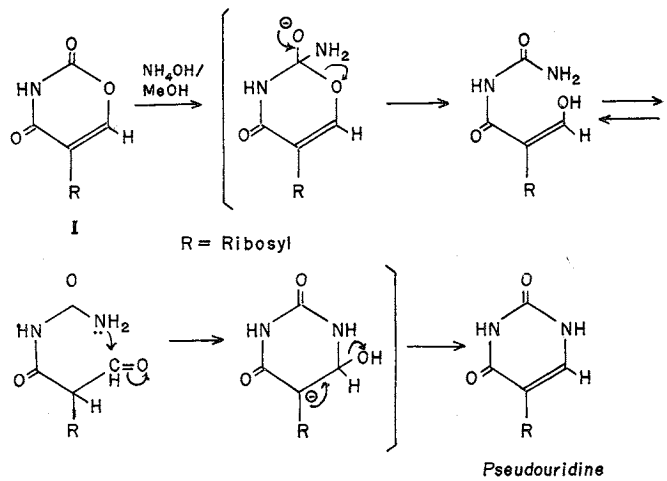


Fig. 3.



Examination of the PMR spectrum of minimycin revealed the anomeric proton centered at 4.65 ppm which is deciding proof of the attachment of D-ribose moiety on carbon. β-Pseudouridine under similar condition exhibits the anomeric proton at 4.67 ppm as compared to 5.82 ppm for that of uridine in D_2O[2]. Other naturally occurring carbon-substituted ribonucleoside antibiotics are showdomycin[5], formycin[6], and laurusin[6]. Showdomycin (3-β-D-ribofuranosylmaleimide) exhibits the anomeric proton at 4.82 ppm in D_2O and deuteroacetic acid-d_4[5]. The vinyl proton of mini-

mycin at 7.78 ppm is a singlet centered at 0.15 ppm downfield from that of β-pseudouridine (7.63 ppm). The difference of these chemical shifts between vinyl protons of minimycin and β-pseudouridine can be accounted by the deshielding effect due to the stronger electronegativity of oxygen atom in the former than that of nitrogen atom in the latter, suggesting that minimycin has the structure I rather than II (Fig. 2).

Together with the above considerations, determination of pKa of minimycin was of considerable assistance in further structure elucidation. The pK'a value of 7.55 could account for significantly increased acidity probably due to the more inductive effect of oxygen atom on vinyl carbon in the structure I than the effect of nitrogen atom on vinyl carbon of pseudouridine, since pKa value of pseudouridine is 9.1[7], 9.29 for showdomycin[5], and 9.17 for uridine[8]. Furthermore, it can not expect the pKa value of 7.55 from the structure II since the above pKa values are arising from the -CONHCO- grouping in the molecule.

The structure III (Fig. 2) is easily excluded by the evidence of the chemical shift of the vinyl proton at 7.78 ppm when compared to 6.74 ppm for the vinyl proton of showdomycin[5], and to 5.90 ppm for that of uridine[9].

The structure proposed for minimycin based on present studies is 5-β-D-ribofuranosyl-1,3-oxazin-2,4-dione (I). According to the descriptions by DARNALL[5] it follows that assignment of the configuration is β since a study of the PMR spectrum of the C-3', C-4' and C-5' proton region, 3.6~4.8 ppm, reveals that this region showed an absorption pattern virtually identical to that for β-pseudouridine[5]. COHN suggests that α-pseudouridine (pseudouridine B) shows the anomeric proton 0.33 ppm downfield from that of the β-isomer[10]. The C-3', C-4' and C-5' proton region, 3.7~4.8 ppm, of the PMR spectrum of α-pseudouridine is distinctly different from that of the β-derivative[5]. These data provide strong support for the assignment of the β-configuration.

The mechanism of the formation of β-pseudouridine from minimycin can be depicted reasonably as shown in Fig. 3.

Minimycin bears a similar structural relationship to β-pseudouridine, and can be regarded as β-pseudouridine which is displaced an -NH- group by an -O- group.

Experimental

Ultraviolet spectra were recorded with a Shimazu UV-200 spectrometer, and PMR spectra were taken with a JEOL JNM-4H-100 (100 MHz) spectrometer in D_2O with DSS as internal references. The mass spectra were recorded with a Hitachi JMS-4 spectrometer.

Formation of pseudouridine from minimycin

Procedure A: A mixed solution of 204 mg of minimycin in 8 ml of methanol and 9 ml of conc. ammonium hydroxide was stirred at room temperature for 6 hours. The reaction mixture was then evaporated to dryness under reduced pressure, and the resulting oily residue was digested with 95% ethanol to give a precipitate. The material showed at least three spots on silica gel thin-layer chromatography developed with *n*-BuOH - AcOH - water (4 : 1 : 2), one of which was the main spot having a similar Rf value to that of β-pseudouridine, and was chromatographed on silica gel with the same solvent system, affording crude pseudouridine from the fractions 7~11. The crude product was then rechromatographed on a preparative silica gel plate, and the band corresponding to pseudouridine was taken off, and eluted with 50% aq. methanol followed evaporation *in vacuo* into a

colorless powder (25 mg) which showed the same Rf value of 0.26 with the same solvent system on cellulose thin-layer chromatography as that of authentic β-pseudouridine : λ_{max}^{MeOH} 208 and 264 nm (ε 12,850 and 8,850); mass spectrum at *m/e* 244 (M^+), 226, 208, 183, 171, 154, 141 (base peak), 126, 112, 98, 82, 73, 60, 57 and 55.

Procedure B : To a solution of 50 mg of minimycin in 1 ml of water and 9 ml of methanol was added 115 mg of ammonium carbonate monohydrate. The reaction mixture wsa refluxed for 1 hour, and then evaporated to dryness under reduced pressure into a colorless oil which showed, in fact, the same thin-layer chromatography behaviors on silica gel as the one obtained in the Procedure A. The oil was preparatively purified on a silica gel plate as described in (A) giving a small amount of a colorless powder of pseudouridine.

Treatment of minimycin with hydrazine

A mixture of 20 mg of minimycin, 1 ml of water, and 80 ml of hydrazine (80 %) was heated at 100°C for 5 hours. After cooling the solution, benzaldehyde (10 ml) was added, and the mixture was extracted repeatedly with ether. The aqueous solution was then used for paper chromatography. The chromatograms were sprayed with aniline hydrogen phthalate[3] and then heated at 100°C for 5 minutes.

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