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^{13}C-Nuclear Magnetic Resonance Studies on Viomycin and Its Related Compounds[1]

Tsunehiro Kitagawa, Takako Miura, Koujirou Mori, Hyozo Taniyama,[2a] Keiichi Kawano, and Yoshimasa Kyogoku[2b]

Faculty of Pharmaceutical Sciences, Nagasaki University[2a] and Institute for Protein Research, Osaka University[2b]

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The ^{13}C-nuclear magnetic resonance spectra of viomycin, dihydroviomycin and broxoviomycin were measured. Most of the resonance lines in their spectra were assigned on the basis of the spectra of constituted amino acids. The spectra were reasonably interpreted by the proposed structures of viomycin and its derivatives.

Keywords——^{13}C-NMR study; partial and complete decoupling; spectral analyses of components; spectral analyses of viomycin and its derivatives; utility for unstable peptide

The current advances in the ^{13}C magnetic resonance spectroscopy have increased greatly the scope and utility of nuclear magnetic resonance (NMR) spectroscopy in the structural studies and in biogenetic problems. Thus, as a fundamental investigation for the structures of viomycin derivatives, ^{13}C NMR resonance signals of the antibiotic and its related compounds were studied. The present paper describes the spectral assignments of the signals of viomycin (**1**, VM), dihydroviomycin (**2**, 2HVM) and broxoviomycin (**3**, BroxoVM) in relation to their composed amino acids. The assignments are reasonably interpreted by the proposed structures for viomycin derivatives.

Materials and Methods

Procedures——Carbon-13 magnetic resonance spectra were recorded at frequency of 25.2 MHz by means of a JEOL PFT-100 pulse Fourier transform NMR system locked on D of deuterium oxide. In usual measurement 100—200 mg of the sample was dissolved in 0.8—1.2 ml of deuterium oxide. Chemical shifts were read relative to the ^{13}C signal of external tetramethyl silane (TMS).

Materials——Viomycin,[3] dihydroviomycin,[4] broxoviomycin[5] and α,β-diaminopropionic acid[6] were prepared according to the methods previously reported. Composed amino acids of viomycin such as β-lysine, tuberactidine and viomycidine were isolated from acid hydrolysates of viomycin according to similar methods of the reported ones.[7] Purity was confirmed by paper partition chromatography, electrophoresis, proton magnetic resonances and elemental analyses.

1) Part X of the series "Studies on Viomycin"; Part IX: T. Kitagawa, T. Miura, T. Takaishi, and H. Taniyama, *Chem. Pharm. Bull.* (Tokyo), **24**, 1324 (1976).
2) Locations: *a*) *Bunkyo-machi, Nagasaki*; *b*) *Suita, Osaka.*
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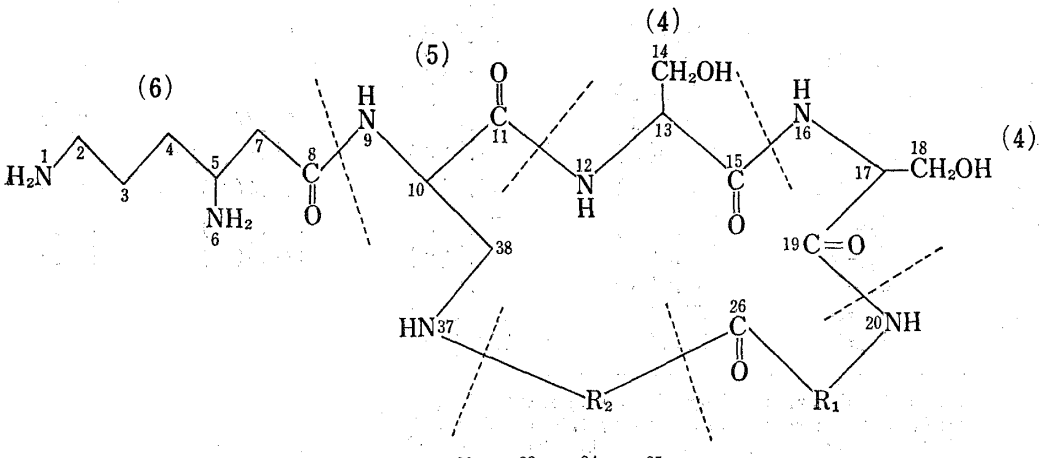
Results and Discussion

Spectral Analysis of Amino Acid Components of Viomycin

Viomycin consists of six amino acid residues, including four unusual ones and two serines. Isolated tuberactidine was unstable and easily converted to viomycidine during the time for signal accumulations.[8] Also, 3-ureidodehydroalanine was very unstable and could not be isolated. Thus, ^{13}C NMR spectra of α,β-diaminopropionic acid (**5**), β-lysine (**6**) and viomycidine (**7**) instead of tuberactidine (**8**) have been measured. The chemical shift of serine[9] and urea[10] were referred to the previously reported data.

Their spectral assignments were performed from consideration of their chemical structures and chemical shift rules[11] with the aids of the spectra at partially decoupled conditions. The assignments are summarized in Table I.



Vid : viomycidine (**7**) Tbd: tuberactidine (8) Dvd : dihydroviomycidine (**9**)

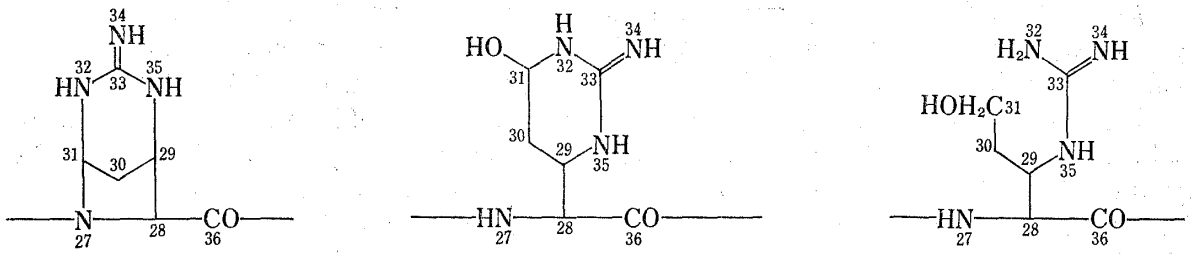


Fig. 1. Structures and Numbering of Viomycin (**1**), Dihydroviomycin (**2**), Broxoviomycin (**3**) and Their Amino Acid Constituents

8) Carbon-13 NMR spectrum of tuberactidine acetate, whose structure was confirmed by proton magnetic resonance (PMR) spectrum, was measured a week after the confirmation, but the obtained spectrum was identical with that of viomycidine.
9) P. Keim, R.A. Vigna, J.S. Morrow, R.C. Marsjall, and F.R.N. Furd, *J. Biol. Chem.*, **248**, 7811 (1973).
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11) P. Viglino, C. Franconi, A. Lai, E. Brosio, and F. Conti, *Org. Magnetic Resonance*, **4**, 237 (1972).

TABLE I. ^{13}C Chemical Shifts of Viomycin and Its Derivatives

	A	B	VM	2HVM	BroxoVM
β-Lys	C_2–H_2	38.5(t)	37.5(t)	38.0(t)	37.6
	C_3–H_2	23.3(t)	24.1(t)	24.1(t)	23.8
	C_4–H_2	29.4(t)	30.2[a](t)	30.4(t)	30.0[a]
	C_5–H	49.1(d)	49.5(d)	49.4(d)	49.4
	C_7–H_2	39.3(t)	40.2(t)	40.2(t)	40.1
Dpr	C_{10}–H	54.8(d)	53.3[b](d)	53.3[b](d)	54.2[b]
Ser	C_{13}–H	55.8(d)	55.5[b](d)	55.9[b](d)	56.5[b]
	C_{14}–H_2	61.2(t)	60.2[c](t)	60.4[c](t)	62.4[c]
	C_{17}–H	55.8(d)	55.6[b](d)	56.6[b](d)	56.8[b]
	C_{18}–H_2	61.2(t)	63.7[c](t)	63.0[c](t)	63.2[c]
Uda	C_{21}=C		106.0(s)	106.1(s)	85.4(C_{21}–H)
	C_{22}–H		136.2(d)	136.1(d)	
	C_{24}=O	161.2(s)	157.6(s)	157.7[d](s)	
	C_{26}=O		168.4(s)	168.2(s)	171.1—176.5
Vid	C_{28}–H	67.4(d)	57.5(d)	57.2(d)	58.1
	C_{29}–H	52.2(d)	47.2(d)	51.3(d)	47.1
	C_{30}–H_2	32.2(t)	30.6[a](t)	35.1(t)	30.6[a]
	C_{31}–H	70.4(d)	71.2(d)	58.7(t)	71.2
	C_{33}=N	154.6(s)	154.7(s)	158.2[d](s)	155.0
Dpr	C_{38}–H_2	42.8(t)	41.6(t)	41.4(t)	41.6
C=O[e]	C_8=O	170.8(s)	171.8(s)	172.2(s)	171.1
	C_{11}=O	172.3(s)	172.2(s)	172.9(s)	171.9
	C_{15}=O	172.3(s)	172.5(s)	172.9(s)	172.4
	C_{19}=O	177.0(s)	173.2(s)	173.6(s)	173.0
	C_{36}=O	178.1(s)	173.8(s)	174.1(s)	176.5

β-Lys, β-lysine (6); Dpr, α,β-diaminopropionic acid (5); Ser, serine (4); Vid, viomycidine (7); Uda, 3-ureidodehydroalanine; A, numbering and type of the carbon; B, the chemical shifts of amino acids and urea. The assignments with the same superscript, a, b, c, or d, are interchangeable. The signals of the group e were not assigned to individual C=O group.
(s) indicates singlet, (d), doublet, and (t), triplet measured at partially decoupled conditions.

Spectral Analysis of Viomycin and Its Modified Products

From the point of view of the spectral analysis, the signals studied can be classified into three groups those in the fields from 23 to 72 ppm, 85—140 ppm and 150—177 ppm. Table I summarized the assignments for viomycin and its modified products.

The high-field part of the spectra from 23 to 72 ppm.

β-Lysine Residue: Assignments of the resonances of C_2, C_3, C_5 and C_7 of viomycin, dihydroviomycin and broxoviomycin were straight forward by comparison with those of β-lysine as shown in Table I. Two pairs of the very close shift values such as 30.2 and 30.6 ppm are seen in the spectrum of viomycin, and 30.0 and 30.6 ppm in that of broxoviomycin. The down field peaks were tentatively assigned to C_4, since the C_4 peak of β-lysine was 29.4 ppm and C_{30} signal of viomycidine was 32.2, but the assignment still remains ambiguous. The resonance signal at 30.4 ppm in the spectrum of dihydroviomycin was assigned to C_4, and the shifted signal at 35.1 ppm was deduced to C_{30}. It is reasonable because dihydroviomycin was chemically modified at C_{31}.

α,β-Diaminopropionic Acid: The C_{38} signal in the spectra of the tested compounds were performed unambiguously by comparison with that of the original amino acid. The C_{10} resonances were assigned as shown in the table, but a little ambiguity is left for the conversion to the C_{13} peaks.

Serine: Viomycin and its derivatives contain two serine residues in their molecules. The assigned resonance peaks for C_{13} and C_{17} are 55.5 and 55.6 ppm for viomycin, 55.9 and 56.6 ppm for dihydroviomycin, 56.5 and 56.8 ppm for broxoviomycin, respectively. These pairs of as-

signed carbons could be reversed. Also, the assigned peak of either C_{14} or C_{18} was 60.2 or 63.7 ppm for viomycin, 60.6 or 63.0 ppm for dihydroviomycin and 62.4 or 63.2 ppm for broxoviomycin.

Tuberactidine or Dihydroviomycidine Residue (**9**): As the result of chemical modification, the carbon signals assigned to dihydroviomycin show distinct shifts at the peaks of C_{29}, C_{30}, C_{31} and C_{33} compared with those of viomycin and broxoviomycin. Although, viomycin and broxoviomycin possess tuberactidine residue, assignment of resonances for C_{28}, C_{29}, C_{30}, C_{31} and C_{33} in the spectra of these two compounds were performed by taking the corresponding ^{13}C shift values of viomycidine and dihydroviomycin into account. Thus, the shift values of C_{28}, C_{29} and C_{30} of viomycin and broxoviomycin do not well correspond to those of viomycidine as shown in Table I. Equivocality in the assignment of C_{30} of viomycin and broxoviomycin has been discussed already. A discussion for assignment of C_{33} as 158.2 ppm for dihydroviomycin will be done below.

The mid-field region from 85 to 140 ppm.

The signals at 106.0 and 106.1 ppm were assigned to C_{21} of viomycin and dihydroviomycin, while, 136.2 and 136.1 ppm to C_{22}, since the former signals appeared as singlet and the latter resonances were doublet in their spectra of partially decoupled conditions. The assignments were confirmed by comparison with the spectrum of broxoviomycin where 3-ureidodehydroalanine is replaced by α-oxyglycine. It shows C_{21} signal at 85.4 ppm, but no resonance is found near 106 and 136 ppm.

The lower-field region from 150 to 177 ppm.

The lower field part of the spectra contains the resonances due to seven amide carbonyls at C_8, C_{11}, C_{15}, C_{19}, C_{24} and C_{36} besides C_{33} guanidino carbon.

The signals at 154.7 and 155.0 ppm in the spectra of viomycin and broxoviomycin were assigned to C_{33}, six membered cyclic type guanidino carbons, by comparison with the C_{33} shift of viomycidine at 154.6 ppm. While, for dihydroviomycin having the terminal guanidine group, the peak at 158.2 ppm is favored to be assigned to C_{33} rather than that at 157.7 ppm as discussed below.

Concerning the assignment of C_{24}, the carbon signal of urea was observed at 161.2 ppm.[10] Taking the shift value into account, the observed peak at 157.6 ppm was assigned to C_{24} of viomycin. Dihydroviomycin possesses the same 3-ureidodehydroalanine residue as viomycin. Thus, the 157.7 ppm signal was assigned to C_{24} rather than the peak at 158.2 ppm. Those two values assigned to C_{24} and C_{33} in dihydroviomycin, however, show such a small shift difference that the possibility for reversed assignments can not be excluded.

The resonance signals at 168.4 and 168.2 ppm in the spectra of viomycin and dihydroviomycin, respectively were assigned to C_{26}, judging from the fact that the carbon is the only one of the α,β-unsaturated amide carbonyl type and its chemical shift should be different from that of normal amide carbon resonances around 170—177 ppm. This resonance cannot be seen for broxoviomycin. It is reasonable because the α,β-double bond is saturated and the carbonyl group has become to be an ordinal one. Its resonance should move to 170—177 ppm. The other five signals in the spectra of viomycin, dihydroviomycin and broxoviomycin deduced to the carbonyl carbons, C_8, C_{11}, C_{15}, C_{19} and C_{36} in the field between 171 and 177 ppm could not be assigned to each residue.

In 1972, Viglino, *et al.*[11] reported PMR study of viomycin together with the spectral assignments of ^{13}C resonances of the antibiotic. Several discrepancies are found in our present and their previous assignments of ^{13}C resonances of viomycin. Their work lacks the resonance data of some of the constituents, β-lysine, α,β-diaminopropionic acid, urea and those of the related compounds of viomycin like dihydroviomycin and broxoviomycin. Their assignments were mainly performed by considering Grant's resonance shift rule.[12] We also performed the

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measurement of partially proton decoupled spectra and the results were quite useful for distinguishing the type of each carbons, *i. e.* how many protons are bounded to the carbons.

The chemical structure of viomycin was first proposed by Shiba and his collaborators.[13] Our sequential analysis[14] and also, X-ray crystalographic investigation of Bycroft[15] get the same conclusion. The structures of dihydroviomycin and broxoviomycin were proposed by Kitagawa, *et al.*[4,5]

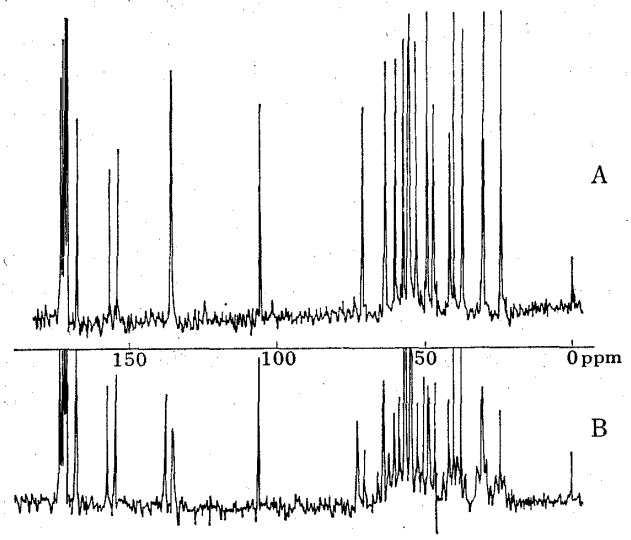

Fig. 2. ^{13}C NMR Spectra of Viomycin measured at Proton Decoupled (A) and partially Decoupled (B) Conditions

Viomycin and its derivatives such as broxoviomycin and dihydroviomycin are very unstable compounds. This nature had made difficult to purify them or elucidate their structures. All of the compounds examined in this report give well resolved ^{13}C NMR spectra, in contrast to ^{1}H NMR spectra which show serious overlap of signals. Also the spectra were found to be sensitive to the slight change in chemical structure. The present results on their ^{13}C NMR spectra are well explained by these structures and supported the proposed structures of viomycin derivatives. These evidences are useful for us to follow the modification in the chemical structure of viomycin and the process of its biosynthesis by ^{13}C NMR spectroscopy.

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