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Studies on Viomycin. XIV. Roles of Basic and Cyclic Moieties in the Antimicrobial Activity of Viomycin[1]

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Conformational analyses of acetylated derivatives of viomycin were performed by proton magnetic resonance (PMR) and circular dichroism (CD) spectroscopic methods. All of the acetylviomycins showed α-methine chemical shift values over a wide region from 4.21 to 4.98 ppm, suggesting that all of the acetyl derivatives possess a rigid conformation at the sixteen-membered ring, as in viomycin. The amino acid derivatives of viomycin showed similar profiles of PMR and CD spectra. These derivatives were thus assumed to retain the rigid conformation. 1-Guanylviomycin, with a strongly basic group at the 1-position, was prepared, and was also found to possess a similar rigid conformation. The antimicrobial activities of 1-guanylviomycin and the acylated derivatives of viomycin toward Gram-positive and Gram-negative bacteria suggested that the rigid conformation is a necessary but not sufficient condition for activity. 1-Guanylviomycin showed antimicrobial activity similar to that of viomycin, confirming the previous conclusion that the two basic groups in viomycin are important for the antimicrobial activity. It was also concluded that the distance between the two basic groups is not critical.

Keywords——viomycin; conformational analysis; NMR; shielding effect; CD; rigid ring; basic group; antimicrobial activity; 1-guanylviomycin

In our series of studies on viomycin, it has been observed that acylations of either or both of the two amino groups of the β-lysine residue in viomycin with acetyl groups[3,4] or neutral amino acids[5] result in inactivation or reduction of the antimicrobial activity of viomycin. However, derivatives of viomycin acylated with basic amino acids retained potency.[5] Viomycin possesses a conformationally rigid sixteen-membered ring.[6] Oxidative modifications of viomycin at the 3-ureidodehydroalanine residue caused conformational changes at the sixteen-membered ring together with loss of the antimicrobial activity.[6,7] These observations suggest that the rigid conformation of the sixteen-membered ring and the

1) Part XIII: T. Kitagawa, T. Fujitake, H. Taniyama, and T. Aikawa, *J. Biochem.*, **83**, 1493 (1978). This work was supported in part by a grant from the Scientific Research Fund of the Ministry of Education Science and Culture of Japan.
2) Location: *Bunkyo-machi, Nagasaki.*
3) T. Kitagawa, T. Miura, and H. Taniyama, *Chem. Pharm. Bull.* (Tokyo), **20**, 2176 (1972).
4) T. Kitagawa, T. Miura, T. Takaishi, and H. Taniyama, *Chem. Pharm. Bull.* (Tokyo), **23**, 2123 (1974).
5) T. Kitagawa, T. Miura, T. Takaishi, and H. Taniyama, *Chem. Pharm. Bull.* (Tokyo), **24**, 1324 (1975).
6) T. Kitagawa, T. Miura, and H. Taniyama, *J. Biochem.*, **81**, 1759 (1977).
7) T. Kitagawa, T. Miura, Y. Sawada, K. Fujiwara, R. Ito, and H. Taniyama, *Chem. Pharm. Bull.* (Tokyo), **22**, 1827 (1974).

basicity of amino groups of the β-lysine residue in viomycin are important for its antimicrobial activity.

We investigated the roles of the amino groups and the rigid ring in the activity of viomycin. A derivative of viomycin possessing a strongly basic substituent of the 1-amino group was prepared. It was confirmed that basicity of the amino groups of the β-lysine residue is necessary for the activity of viomycin.

Conformational analyses of acylated derivatives of viomycin were also performed. The rigid conformation of the sixteen-membered ring in viomycin is a necessary but not sufficient condition for the activity of viomycin.

Experimental

Materials——Viomycin,[3] acetylviomycin,[3] 1-monoacetylviomycin,[4] 6-monoacetylviomycin,[4] 1-lysylviomycin,[5] 1-arginylviomycin,[5] 1-glycylviomycin,[5] 1-ornitylviomycin[5] and 6-lysylviomycin[5] were prepared according to the method cited. The purity of these compounds was confirmed by paper partition chromatography, thin-layer chromatography, electrophoresis, proton magnetic spectroscopy and elemental analysis.

1-Guanylviomycin——Viomycin sulfate (0.3 g, 0.35 mmol) and 0.26 g (1.4 mmol) of 1-guanyl-3,5-dimethylpyrazole nitrate, which was prepared from acetylacetone and aminoguanidine nitrate according to the method of Bannard *et al.*,[8] were dissolved in 4 ml of 1 M triethylamine–bicarbonate buffer (pH 10.0) then stirred for 18 hr at room temperature. The reaction mixture was washed three times with 10 ml each of ether to remove unreacted 1-guanyl-3,5-dimethylpyrazole, and the aqueous layer was neutralized with 0.1 N hydrochloric acid then evaporated to dryness.

The residue was chromatographed on a Sephadex LH-20 column (2.0×130 cm), eluting with distilled water. Fractions (10 g/tube) in peak 1 (No. 22—24) showed a positive Sakaguchi color test and gave crude

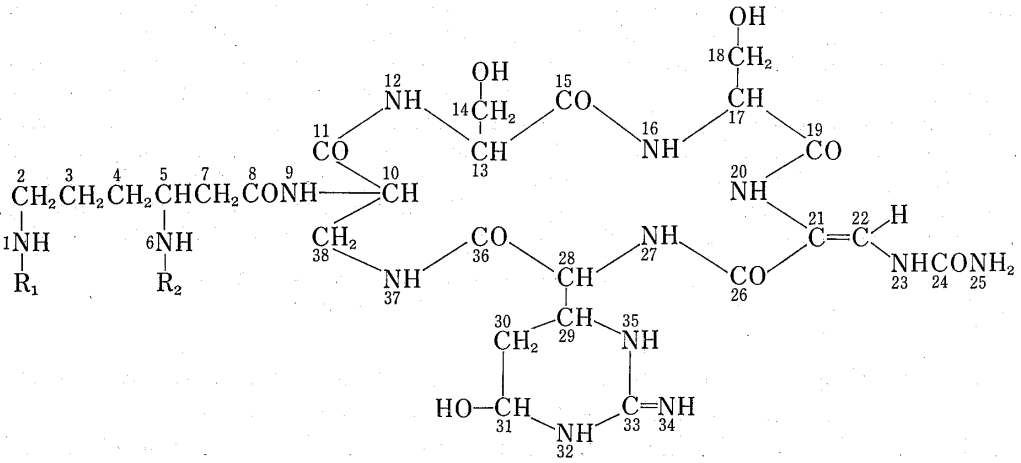

Fig. 1. Chemical Structures of Viomycin and Its Derivatives with Numbering

	R_1	R_2
Viomycin	H	H
Acetylviomycin	CH_3CO–	CH_3CO–
1-Monoacetylviomycin	CH_3CO–	H
6-Monoacetylviomycin	H	CH_3CO–
1-Glycylviomycin	H_2NCH_2CO–	H
1-Arginylviomycin	H_2N–C(=NH)–NH–$(CH_2)_3$–CH(NH_2)–CO–	H
1-Ornitylviomycin	H_2N–$(CH_2)_3$–CH(NH_2)–CO–	H
1-Lysylviomycin	H_2N–$(CH_2)_4$–CH(NH_2)–CO–	H
6-Lysylviomycin	H	H_2N–$(CH_2)_4$–CH(NH_2)–CO–
1-Guanylviomycin	H_2N–C(=NH)–	H

8) R.A.A. Bannard, A.A. Casselman, W.F. Cockburn, and G.M. Brown, *Can. J. Chem.*, **36**, 1541 (1958).

1-guanylviomycin on lyophilization. Repeated chromatographies of the lyophilized product of the peak 1 fractions on a Sephadex LH-20 column followed by lyophilization furnished purified 1-guanylviomycin (0.22 g) as a white amorphous powder; positive to the ninhydrin test and Sakaguchi color reaction; mp above 280°; UV,[9] $\lambda_{max}^{H_2O}$ 268 nm (log ε=4.39): IR, ν_{max}^{KBr} cm^{-1} 3400, 1650, 1500, 1320, 1220, 1070 (broad); PMR, δ ppm, 8.0 (s, 1H), 5.20 (t, 1H), 3.28 (m, 3H). Elemental *Anal.* Calcd. for $C_{26}H_{60}N_{15}O_{22}S_{1.5}$,($C_{26}H_{45}N_{15}O_{10}\cdot 3/2H_2SO_4\cdot 6H_2O$): C, 31.77; H, 6.15; N, 21.38; S, 4.89. Found: C, 31.99; H, 5.88; N, 21.54; S. 4.97.

The chemical structures of viomycin and its derivatives are shown in Fig. 1.

Procedures——A JASCO ORD/UV-5 spectrophotometer with a CD attachment was used for measurements of CD spectra in distilled water. PMR spectra were recorded on a JEOL JMN PS-100 spectrophotometer with JES-VT-3B temperature control equipment. Sample solutions in heavy water measured in tubes of 5 mm o.d. using the external H_2O lock method at 20° or 70° Heavy water containing 20% trifluoroacetic acid solution was also used for PMR measurements. Chemical shifts are given as δ values (ppm) from sodium 4,4-dimethyl-4-silapentane sulfonate in heavy water. UV spectra were recorded on a Hitachi 124 spectrophotometer.

Antimicrobial activities were measured by the twofold tube dilution method using a nutrient bouillon as the medium; the minimum inhibitory concentrations obtained are given in μg/ml.

Results and Discussion

Conformational Analyses of the Acylated Derivatives of Viomycin

Conformational analyses of the acylated derivatives of viomycin were performed by the method of Kitagawa *et al.*[6] using PMR and CD spectroscopic studies in the same way as viomycin, dihydroviomycin and broxoviomycin. PMR spectroscopic studies of the unexchanged protons: PMR spectra of acetylviomycin and 1-and 6-monoacetylviomycin were

Table I. Chemical Shifts of Viomycin and Its Derivatives in D_2O

		VM	AcVM	1-AcVM	6-AcVM	BrVM
Lys	N_1–$COCH_3$		1.99(3H, s)	1.99(3H, s)		
	C_2–H_2	3.04(2H, m)	3.18(2H, m)	3.17(2H, m)	2.91(2H, m)	3.05(2H, m)
	C_3–H_2	1.8 (2H, m)	1.5 (2H, m)	1.7 (2H, m)	1.6 (2H, m)	1.8 (2H, m)
	C_4–H_2	1.8 (2H, m)	1.5 (2H, m)	1.7 (2H, m)	1.6 (2H, m)	1.8 (2H, m)
	C_5–H	3.7 (1H, m)	3.7 (1H, m)	3.7 (1H, m)	3.7 (1H, m)	3.7 (1H, m)
	N_6–$COCH_3$		1.99(3H, s)		1.96(3H, s)	
	C_7–H_2	2.63(1H, dd)	2.38(1H, dd)	2.62(1H, dd)	2.43(1H, dd)	2.71(1H, dd)
		2.83(1H, dd)	2.46(1H, dd)	2.75(1H, dd)	2.65(1H, dd)	2.87(1H, dd)
Dpr	C_{10}–H	4.7 (1H)[a]	4.7 (1H)[a]	4.7 (1H)[a]	4.7 (1H)[a]	4.5—4.7[b]
	C_{38}–H_2	3.2 (1H)	3.2 (1H)	3.2 (1H)	3.2 (1H)	3.7 (2H)
		3.8 (1H)	3.8 (1H)	3.8 (1H)	3.8 (1H)	
Ser(1)	C_{13}–H	4.76(1H, t)[a]	4.75(1H, t)[a]	4.78(1H, t)[a]	7.73(1H, t)[a]	4.5—4.7[b]
	C_{14}–H_2	3.89(2H, d)	3.94(2H, d)	3.92(2H, d)	3.91(2H, d)	3.9 (2H)
Ser(2)	C_{17}–H	4.23(1H, dd)	4.22(1H, dd)	4.21(1H, dd)	4.22(1H, dd)	4.5—4.7[b]
	C_{18}–H_2	3.9 (1H)	3.9 (1H)	3.9 (1H)	3.9 (1H)	3.9 (2H)
		4.12(1H, dd)	4.11(1H, dd)	4.15(1H, dd)	4.14(1H, dd)	
Uda	C_{21}–H					5.35(0.3H, s)[7]
	C_{22}–H	8.00(1H, s)	8.07(1H, s)	8.04(1H, s)	8.09(1H, s)	
Tbd	C_{28}–H	5.01(1H, d)	4.97(1H, d)	4.98(1H, d)	4.98(1H, d)	4.5—4.7[b]
	C_{29}–H	4.7 (1H)[a]	4.7 (1H)[a]	4.7 (1H)[a]	4.7 (1H)[a]	4.5—4.7[b]
	C_{30}–H_2	1.6 (1H)	1.6 (1H)	1.6 (1H)	1.6 (1H)	1.8 (1H)
		2.0 (1H)	2.0 (1H)	2.0 (1H)	2.0 (1H)	2.0 (1H)
	C_{31}–H	5.16(1H)	5.15(1H)	5.20(1H)	5.17(1H)	5.24(1H)

Abbreviations in Table 1: VM, viomycin; AcVM, acetylviomycin; 1-AcVM, 1-monoacetylviomycin; 6-AcVM, 6-monoacetylviomycin; BrVM, broxoviomycin; β-Lys, β-lysine; Dpr, α,β-diaminopropionic acid; Ser, serine; Uda, ureidodehydroalanine; Tbd, tuberactidine; s, singlet; d, doublet; dd, double-doublet; m, multiplet.

a) Chemical shift measured at 70°.

b) Chemical shifts measured in 20% trifluoroacetic acid solution.

9) Abbreviations used are: UV, ultraviolet; CD, circular dichroism; PMR, proton magnetic resonance; ppm, parts per million; s, singlet; t, triplet; m, multiplet; ORD, optical rotatory dispersion.

measured in heavy water at 20° and 70°, since some methine protons were overlapped by HOD signals at room temperature. On raising the sample temperature to 70°, the HOD signals shifted to higher field and all of the masked signals in the spectra of the viomycin derivatives could be observed separately, as reported previously.[6] The measurement of PMR spectra in heavy water containing 20% trifluoroacetic acid[6,10] was also used for comparison, since the HOD signal was shifted to lower field, and the masked signals could again be observed.

The PMR spectra of the three acetylviomycins showed good correspondences to that of viomycin except for the protons on the N-acetylated β-lysine residues, as reported in the previous papers.[4,5] Consequently, the assignments of the proton signals of the acetylated derivatives of viomycin were easily performed by comparisons of the chemical shift values and splitting patterns with those of the corresponding protons of viomycin. Some decoupling experiments were carried out to confirm the assignments. The results are summarized in Table I, together with those for viomycin and broxoviomycin for comparison.

All of the assigned chemical shift values of the α-methine protons of the acylated derivatives of viomycin at C_{10}–H, C_{13}–H, C_{17}–H and C_{28}–H were between 4.21 and 4.98 ppm. All the values assigned were consistent with those of the corresponding protons in viomycin but were not consistent with those of broxoviomycin, which appeared at 4.5—4.7 ppm.

As discussed previously,[6] viomycin and dihydroviomycin are assumed to possess a rigid conformation at their sixteen-membered ring, whereas broxoviomycin was thought to possess a flexible sixteen-membered ring, judging from the chemical shift values of the α-methine protons. The finding that all the acetyl derivatives of viomycin exhibit α-methine chemical shifts over a wide region, like viomycin, provides direct evidence that these acetyl derivatives

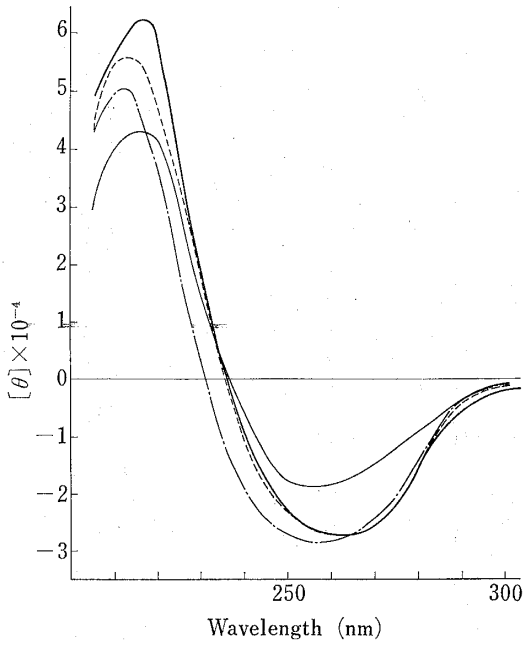

Fig. 2. Circular Dichroism Spectra of Viomycin and Acetylviomycins

——, viomycin.
——, acetylviomycin.
--------, 1-monoacetylviomycin.
——, 6-monoacetylviomycin.

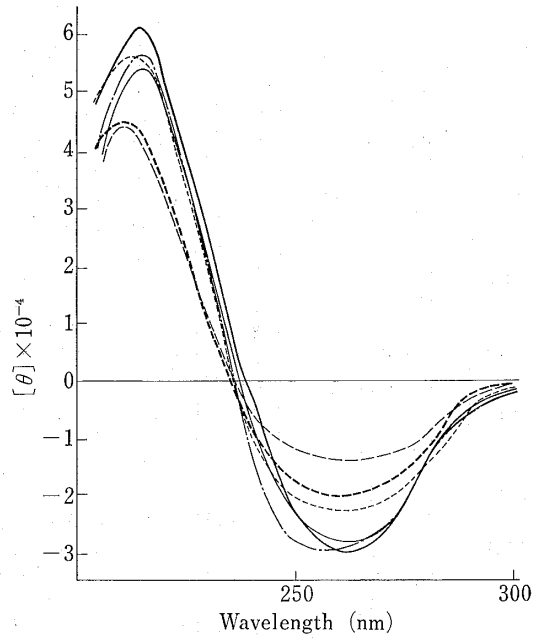

Fig. 3. Circular Dichroism Spectra of the Derivatives of Viomycin

——, 1-guanylviomycin.
——, 1-arginylviomycin.
-------, 1-lysylviomycin.
-------, 6-lysylviomycin.
– – –, 1-ornitylviomycin.
——, 1-glycylviomycin.

10) T. Wakamiya and T. Shiba, *Bull. Chem. Soc. Japan*, **48**, 2502 (1975).

retain a rigid conformation of the sixteen-membered ring. The diversity of shift values for α-methine protons of acetylviomycin, compared with those of broxoviomycin, can only be explained by differences in shielding or deshielding effects on the α-methine protons by the neighboring amide carbonyl double bond (or bonds), owing to the rigid conformation. In addition, the similarities in the PMR profiles of acetylviomycin and viomycin indicate that the conformations of the sixteen-membered rings of the acetyl derivatives of viomycin are similar to that of viomycin, since similar shielding and/or deshielding effects on each corresponding proton would only be expected in similar conformational states. CD spectroscopic study: As reported previously, viomycin and related compounds assumed to possess a rigid conformation on the basis of PMR studies showed a peak near 215 nm, whereas the derivatives which were assumed to be flexible did not exhibit this peak in their CD spectra.[6] All of the acetylviomycins showed this peak in their CD spectra, as shown in Fig. 2.

It was concluded that acetylation at the free amino groups of the β-lysine residue, which is located outside the sixteen-membered ring, did not cause conformational change in the cyclic part of viomycin. This also seems to be the case for amino acid derivatives of viomycin which were prepared by a similar method and under the same conditions used for the preparation of acetylviomycins. Similar CD spectra with a peak at 215 nm were obtained for all the amino acid derivatives of viomycin, as shown in Fig. 3.

The PMR spectra of the amino acid derivatives of viomycin were also quite similar to that of acetylviomycin and were different from that of broxoviomycin. The chemical shift values of their α-methine protons were distributed between 4.20 and 5.02 ppm.

Thus, all of the amino acid derivatives of viomycin were assumed to possess a rigid sixteen-membered ring similar to that of viomycin.

A strongly basic derivative of viomycin possessing a guanidyl substituent at the 1-position was prepared by applying the method of Bannard *et al.*[8] for guanidylation of an amino group. Thus, viomycin dissolved in triethylamine-bicarbonate buffer was incubated with 1-guanyl-3,5-dimethylpyrazole nitrate. The 1-guanylviomycin obtained showed a peak at 215 nm in its CD spectrum, as shown in Fig. 3. The PMR spectrum of 1-guanylviomycin showed signals of its α-methine protons over a wide range between 4.22 and 5.01 ppm. Therefore the product was assumed to possess the same rigid conformation at the sixteen-membered ring as viomycin.

The antimicrobial activity of the 1-guanyl derivative against Gram-positive and Gram-negative strains of bacteria was measured in comparison with those of viomycin, acetylviomycins and amino acid derivatives of viomycin; the results are shown in Table II.

Table II. Antimicrobial Spectra of Viomycin and Its Derivatives towards Gram-positive and Gram-negative Bacteria

	Minimum inhibitory concentration (μg/ml)							
	VM	AcVM	1-AcVM	GlyVM	ArgVM	1-LysVM	6-LysVM	GuaVM
E. coli Q-13	25	>1600	1600	200	50	25	50	25
E. coli NIHJ	25	>3200	3200	200	100	50	50	50
My. smegmatis 607	3.2	3200	800	100	12.5	3.2	6.3	3.2
S. aureus Terajima	30			500	10	10	30	30
S. aureus 209P	200	>3200	3200		200			200
P. vulgaris OX 19	200	>3200	>3200		200			200
B. subtilis PCI 219	50	>3200	>3200		50			50

Abbreviations: VM, viomycin; AcVM, 1,6-diacetylviomycin (acetylviomycin): 1-AcVM, 1-monoacetylviomycin; GlyVM, 1-glycylviomycin; ArgVM, 1-arginylviomycin; 1-LysVM, 1-lysylviomycin; 6-LysVM, 6-lysylviomycin; GuaVM, 1-guanylviomycin:
E. coli, *Escherichia coli*; *My. smegmatis*, *Mycobacterium smegmatis*; *S. aureus*, *Staphylococcus aureus*; *P. vulgaris*, *Proteus vulgaris*; *B. subtilis*, *Bacillus subtilis*.

Acetyl derivatives of viomycin, shown to possess the same rigid conformation at the sixteen-membered ring as viomycin, had lost the antimicrobial activity. It is concluded, therefore, that the presence of the rigid conformation in viomycin is a necessary but not sufficient condition for antimicrobial activity.

Acylations at either of the two amino groups in viomycin with basic amino acids essentially did not affect the antimicrobial activity.[5] The α-amino groups of the introduced basic amino acid residue, however, does not seem to replace the role of the original amino group in the activity, since the derivatives acylated with neutral amino acids, such as glycine, possessing an α-amino group showed reduced potency. The ε-amino group of the introduced lysine residue or the ω-guanidine function of arginine in the modified viomycins seemed, therefore, to be important for the activity. Although, the distance between the two basic 1- and 6-amino groups in viomycin and that between the 6-amino group and ε-amino group of the introduced lysine residue or ω-guanidine group of the introduced arginine residue in the modified viomycins at 1-position are rather different the modified viomycins still retained full activity. The introduction of a guanidine group, which is a strongly basic function, at the 1-position of viomycin provides useful information on the structure-activity relationship of viomycin as regards the roles of basicity and the distance between the two basic groups.

1-Guanylviomycin showed antimicrobial activity similar to that of viomycin. This supports the previous conclusion that the two basic groups in viomycin are important for the antimicrobial activity. It can also be concluded that the distance between the two basic groups is not critical.