NOTES

RELATIONSHIPS BETWEEN ANTIMICROBIAL ACTIVITIES AND CHEMICAL STRUCTURES OF REDUCED PRODUCTS OF VIOMYCIN*

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In the preceding paper, we reported the novel selective partial hydrolysis of peptide bonds in viomycin and its derivatives at N-sides of hydroxy amino acid serine residues by using N→0 acyl migrative reactions followed by methanolysis of resulting ester linkages. The following sequential analysis of the products led to the conclusion on one hand, that the structure of viomycin is formulated as **I**[1]. On the other hand, these studies have provided us with a number of chemically modified derivatives of the antibiotic.

Until now, our knowledge on structure-activity relationships of this antibiotic still remained meager, due to the lack of systematic investigations of this problem. It is well known that the most peptide antibiotics are cyclic and contain D-form amino acid constituent(s)[2]. The recent hypothesis suggested that dehydroamino acid residue is supposed to be a precursor of D-form amino acid[3].

Since viomycin is found to be a cyclic peptide and all of its constitutive amino acids are L-form[4] except 3-ureidodehydroalanine residue[5], the characteristic chromophore group of viomycin, modifications of this dehydroaminoacid moiety and investigations of potencies of modified products might give us useful informations from the view point of structure-activity relationships.

Preparations of Reduced Products of Viomycin

Standard viomycin sulfate was purified according to the method reported previously[6]. The catalytic hydrogenation products, tetrahydroviomycin hydrochloride (**II**) and perhydroviomycin hydrochloride (**III**) were obtained according to the modified method of corresponding acetyl derivate preparations[1]. Another reduced product named dihydroviomycin hydrochloride** (**IV**) was prepared as purified form by means of sodium borohydride reduction of viomycin*** followed by repeated Sephadex column chromatographies[6].

Experimental

All melting points were taken with a Yanagimoto MP-82 type micro-melting point apparatus and were uncorrected. IR spectra were determined from KBr discs with Japan Spectroscopic DS-301 spectrophotometer, NMR spectra on Hitachi Perkin-Elmer H-60 instrument in D_2O, and optical rotations on Yanagimoto Direct Recording polarimeter Model OR-20 (*c* 1 %, cell length: 5 cm, H_2O). The purity of preparations was determined using the Hitachi KLA-3 type amino acid analyser and by paper chromatography with Toyo filter paper No. 51 UH and Rf values refer to the following solvent systems; Rf_1, *n*-BuOH-*t*-BuOH-Pyridine-AcOH-H_2O (15 : 4 : 10 : 3 : 12, solvent 1); Rf_2, *t*-BuOH-AcOH-H_2O (2 : 1 : 1), and on thin-layer chromatography with Merck silica gel G and Rf_{11} refer to the solvent 1, Rf_{12} to 10% NH_4OAc-acetone-10 % NH_4OH (10 : 9 : 0.5). Electrophoresis was performed at 500 V, 3～5 mA using Toyo C type instrument. Rm values were obtained with reference to viomycin defining the electrophoresis distance of viomycin as 1, using pyridine-AcOH-H_2O (5 : 0.2 : 95, pH 6.3) and ninhydrin, SAKAGUCHI or RYDON-SMITH reagent for the detections in these tests. Abbreviations used are ser, dpr, β-lys, dihydrovio for serine, α, β-diaminopropionic acid, β-lysine and dihydroviomycidine, respectively.

Preparation of dihydroviomycin hydrochloride (**IV**).

To a solution of viomycin (2 g) in 11 ml of water, was added with sodium borohydride (0.5 g) and

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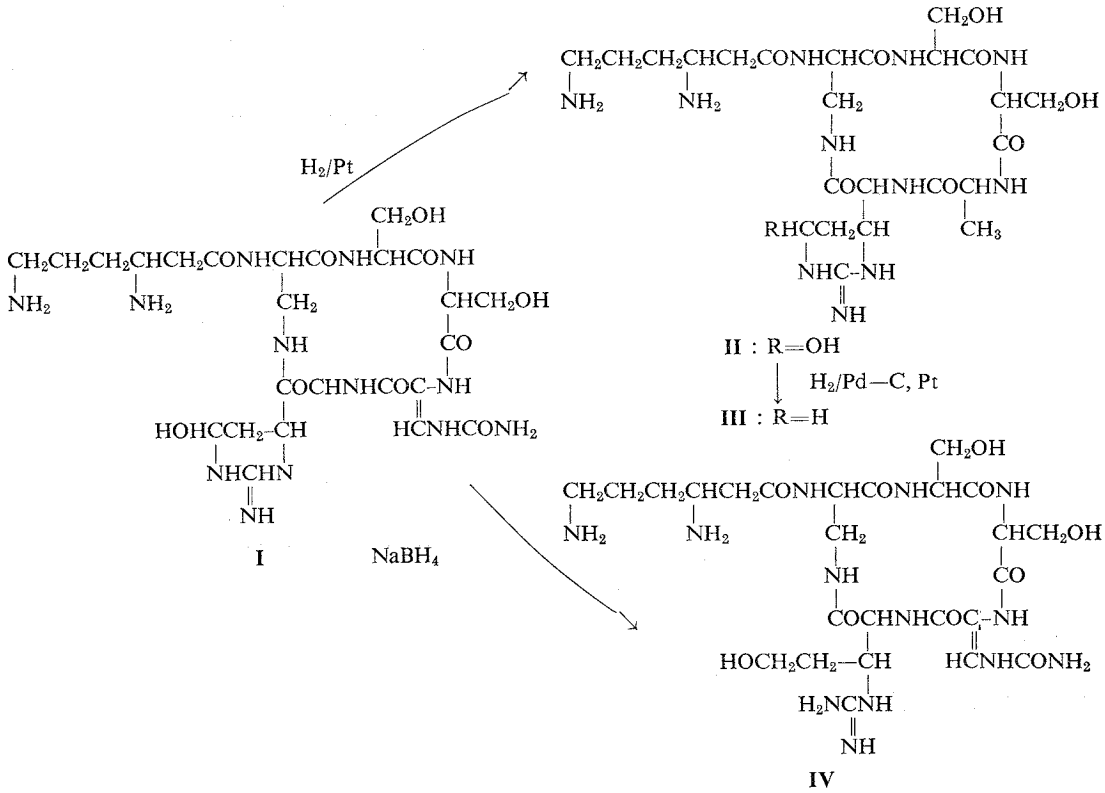
* This present part VII' of "Studies on viomycin".

** TAKITA *et al.*[7] gave names deoxyviomycin and deoxyviomycidine to this compound and its hydrolysate. However the latter name was revised as dihydroviomycidine[7b].

*** TAKITA *et al.*[7] reported this reaction without isolating and characterizing the purified product, but with detailed discussion of its constituent dihydromycidine.

Chart 1.



the mixture was stirred at room temperature for overnight. An excessive reagent was decomposed with acetic acid (*ca.* 0.5 ml) and the solution was condensed to 3 ml *in vacuo* at below room temperature. The residue was chromatographed on a column of Sephadex LH-20 (2×150 cm) using 10% methanolic solution as the eluent. Fractions (15 g/fraction) Nos. 16~18 contained two ninhydrin-positive substances, Rf_1, 0.11 and 0.32 (950 mg), and Nos. 19~25 contained one component, dihydroviomycin as sulfate, Rf_1, 0.32 (520 mg). The products in the fractions Nos. 16~18 were combined and then subjected the same chromatography to give another crop of dihydroviomycin sulfate, mp 242~246°C (decomp.), $[\alpha]_D^{15°}$ +2.2 (*c* 1%, H_2O), yield (970 mg) 47.5%, Rf_1, 0.32, Rm, 0.80, UV λ_{max}^{nm} ($\log\varepsilon$); 268 (4.22 in H_2O), 268(4.23 in 0.1 N HCl), 287(4.14 in 0.1 N NaOH): IR_{max}^{KBr} cm^{-1}, 3400 (OH, NH), 1660, 1500 (CONH); NMR δ ppm, 8.0 (1H, s): positive to ninhydrin, SAKAGUCHI and RYDON-SMITH tests. Amino acid analysis: ser (1.9), dpr (1.0), β-lys (0.9), dihydrovio (0.5).

A conversion of dihydroeiomycin sulfate to its hydrochloride was achieved by addition of an equivalent amount of barium hydrochloride to the solution of the sulfate and after removal of the precipitate the filtrate was lyophilized. Then the residue was chromatographed on a column of Sephadex LH-20 (1.5×150 cm) using water as the eluent, and the ninhydrin-positive fractions were combined and lyophilized. For elementary analysis the same procedure was repeated for three times to give dihydro. viomycin hydrochloride, mp above 270°C, $[\alpha]_D^{15°}$ +15.8°, UV λ_{max}^{nm} ($\log\varepsilon$); 268 (4.3 in H_2O or 1N HCl), 287 (4.1 in 0.1 N NaOH), NMR δ ppm, 8.0 (1H, s), positive to ninhydrin, SAKAGUCHI and RYDON-SMITH reeactions, Rf_1, 0.33, Rf_2, 0.60, Rf_{11}, 0.10, Rf_{12}, 0.65, Rm 0.91. Anal. Calc. for $C_{25}H_{45}O_{10}N_{13}\cdot 3HCl\cdot 3H_2O$: C, 35.28; H, 6.39; N, 21.39; Cl, 12.50. Found: C, 35.29; H, 6.35; N, 21.41; Cl, 12.47.

Results and Discussion

Antimicrobial Activities

The antimicrobial activities of viomycin and its reduced products against gram-potitive and negative microorganisms were investigated and the results obtained are summarized in Table 1. The antimicrobial activities sometimes were reduced one fifth to one tenth of the original, although no decomposition was observed with samples by paper chromatographic experiments.

Table 1. Antimicrobial spectra of viomycin and its reduced products

	Minimum inhibitory concentration (mcg/ml)			
	I	II	III	IV
Staphylococcus aureus FDA 209P SM-STH-R[a]	100	>100	>100	>100
S. aureus Terajima	10	30	>100	30
Bacillus subtilis NRRL 3411	3.1	12.5		25
B. subtilis K-02	12.5	25		25
Proteus vulgaris OX-19	25	200	>100	200
Escherichia coli NIHJ	50	200	>100	400
E. coli K-12	10	100	>100	30
Mycobacterinm 607	1.6	3.1		6.2
M. tuberculosis $H_{37}Rv$	10	30		100
Pseudomonas aeruginosa Tsuchijima	100	400	>100	>30
Candida albicans ATCC 10257	>100	>30	>100	>30
Trichophyton mentagrophytes QM 248	>100	>30	>100	>30
Trichomonas vaginalis 4F	>100	>30	>100	>30

I: Viomycin sulfate, **II**: Tetrahydroviomycin hydrochloride, **III**: Perhydroviomycin hydrochloride, **IV**: Dihydroviomycin sulfate, a): SM-STOH-R: streptomycin and streptothricin resistant, Medium, Culture: Bouillon dilution method, Nutrient bouillon (pH 7.0, 1 ml/test tube), Inoculum: 1 drop of $1 : 10^5$ dilution of an overnight culture in the broth per test tube, incubation temperature and time: 37°C for 48 hours

To avoid such deviations, samples were maintained in complete dryness.

Discussion

Viomycin possesses four chemically reactive functions: primary amino groups of the N-terminal amino groups of β-lysine residue, hydroxyl functions of serine moieties, chromophoric 3-ureidodehydroalanine residue and the reactive tuberactidine constituent.

Acylations of the N-terminal amino groups nulified the antimicrobial activities of mother molecule while, alkylations of hydroxyl function on tuberactidine residue made no significant changes on their potency was already reported in the previous paper[6].

However, judging from the observed antimicrobial activities intactness of the six-membered ring on tuberactidine residue seemed to be necessary for full expression of the activity, since dihydroviomycin in which the ring reductically opened showed only less than one fourth potency of original antibiotic.

On the contrary to the expectation that the chromophore group, the dehydroamino acid residue, might be an important factor for the antimicrobial activity of viomycin, the tetrahydro derivative in which the chromophore group was destructively hydrogenated to alanine with loss of urea unit still possessed half to one fourth potency of the parent antibiotic. On the other hand, perhydroviomycin in which both the chromophore group and tuberactidine residue were reduced showed no activity.

Further investigations of viomycin derivatives from the view point of structure-activity relationships are now in progress.

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References

1) KITAGAWA, T.; T. MIURA, K. FUJIWARA & H. TANIYAMA: The total structure of viomycin by sequential analysis. Chem. Pharm. Bull. (Tokyo) 20 : 2215~2225, 1972
2) BODANSZKY, M. & D. PERLMAN: Peptide antibiotics. Do they represent only a few of many yet undiscovered microbial peptides? Science 163 : 352~358, 1969
3) BYCROFT, B.W.: Structural relationships in microbial peptides. Nature 224 : 595~597, 1969
4) HASKELL, T. H.; S. A. FUSARI, R. P. FROHARDT & Q. R. BARTZ: The chemistry of viomycin.

J. Am. Chem. Soc. 74 : 599~602, 1952

5) BYCROFT, B. W.; D. CAMERON, L. R. CROFT, A. HASSANALI-WALJI, A. W. JOHSON & T. WEBB: The chromophore and partial structure of viomycin. Tetrahedron Letters 1968 : 5901~5904, 1968

6) KITAGAWA, T.; T. MIURA & H. TANIYAMA: Characterization of viomycin and its acyl derivatives. Chem. Pharm. Bull. (Tokyo) 20 : 2176~2184, 1972

7) a) TAKITA, T. & K. MAEDA: The structure of guanido amino acid moiety of viomycin. J. Antibiotics 21 : 512~516, 1968; b) The structure of dihydroviomycidine. J. Antibiotics 22 : 39, 1969