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Tryptophan Moiety of Rufomycin Homologs*1,2

Amino acid sequences of rufomycin homologs have been shown in the previous paper,[1] and also we suggested the presence of a substituent in the tryptophan moiety. While, T. Takita[2,3] isolated a tryptophan derivative from the acidic hydrolysate of ilamycin B_1*3 and established the structure of it as II, and also indicated the structure of ilamycin B_1.

The present communication deals with the structure of tryptophan derivatives obtained from rufomycin A and rufomycin B_1. Also, the structure of rufomycin B_1 is described.

Degradation of rufomycin B_1 with a saturated barium hydroxide solution at 100° for 96 hr. yielded a new tryptophan derivative (III), UV : $\lambda_{max}^{80\%EtOH}$ 282.5 mμ, IR ν_{max}^{KBr} cm^{-1} : 920, 995 ($-CH=CH_2$), NMR (D_2O) 6 protones of *gem*-methyl (8.3 τ singlet). Catalytic hydrogenation of III over platinum gave a dihydro compound (IV), UV : $\lambda_{max}^{80\%EtOH}$ 285 mμ, and the infrared spectrum of IV showed that the end vinyl group of III was saturated in IV. Treatment of IV with acetic acid-hydrochloric acid (1:1) at 100° for 30 min. gave tryptophan (VI) quantitatively. From these results, the structures of III and IV are estimated as 1-(2-methyl-3-buten-2-yl)tryptophan and 1-(1,1-dimethylpropyl)-tryptophan, respectively. On the other hand, a tryptophan derivative was isolated from a hydrolysate of rufomycin B_1 with acetic acid-hydrochloric acid (1:1) at 100° for 3 hr.[3] by the chromatography on an Amberlite CG-50 column with 15% acetic acid. The IR spectrum of the derivative was identical with that of II isolated from ilamycin B_1.[3] Peaks for II, III, IV and VI which emerged from a 15 cm. column with pH 5.28 buffer by an amino acid autoanalyser (Beckmann) are obtained respectively at positions about 100 ml., 113 ml., 105 ml. and 47 ml. Structural changes between II, III and IV were confirmed by the amino acid analysis. Treatment with acetic acid-hydrochloric acid (1:1) at 100° for 30 min. or 70% fromic acid at 100° for 2.5 hr. converted most of III to II. Substance IV yielded quantitatively VI by the same treatment. Moreover II was recovered without any change by a treatment either with a barium hydroxide solution at 100° for 96 hr. or with acetic acid-hydrochloric acid (1:1) at 100° for 18 hr. These results suggest that III is a original component in rufomycin B_1. To confirm this assumption, hydrorufomycin B_1 (V) obtained by a catalytic reduction of rufomycin B_1 over platinum[4] was submitted to hydrolysis. Degradation of V with a barium hydroxide solution at 100° for 96 hr. gave IV while treatment of V with acetic acid-hydrochloric acid (1:1) at 100° for 4 hr. yielded L-tryptophan. These results are shown in the Chart 1. Consequently, the structure of rufomycin B_1 is estimated as shown in Fig. 1.

Meanwhile, degradation of rufomycin A and it's derivatives gave a new tryptophan derivative. Namely, rufomycin A (VII), dihydrorufomycin A[1] (XI) obtained by the reduction

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*1 This paper forms part of the series by Sueo Tatsuoka "Studies on Antibiotics."

*2 Part of this paper on rufomycin A and report about tryptophan derivatives from ilamycin and ilamycin B_1 were presented at the 139th meeting of Japan Antibiotics Research Association (J. A. R. A.) by M. Fujino, *et al.* and T. Takita, *et al.*, independently.

*3 Rufomycin A and rufomycin B_1 are resemble to ilamycin and ilamycin B_1, respectively.

1) J. Ueyanagi, M. Fujino, T. Kamiya, H. Iwasaki, A. Miyake, S. Tatsuoka : Abstracts of the 7th Symposium on the Chemistry of Natural Products, p. 109 (Oct. 1963).

2) T. Takita : The amino acid sequence of ilamycin and ilamycin B; J. Antibiotics (Japan) Ser. A, **16**, 211 (1963) [The 135th meeting of J. A. R. A. July 27 (1963)].

3) T. Takita, H. Naganawa, K. Maeda, H. Umezawa : A new amino acid from ilamycin B_1 and the structure of ilamycin B_1; J. Antibiotics (Japan) Ser. A, **17**, 90 (1964).

4) J. Ueyanagi, H. Iwasaki, M. Fujino, T. Kamiya, A. Miyake, S. Tatsuoka : Abstracts of the 6th Symposium on the Chemistry of Natural Products, p. 53 (1962).

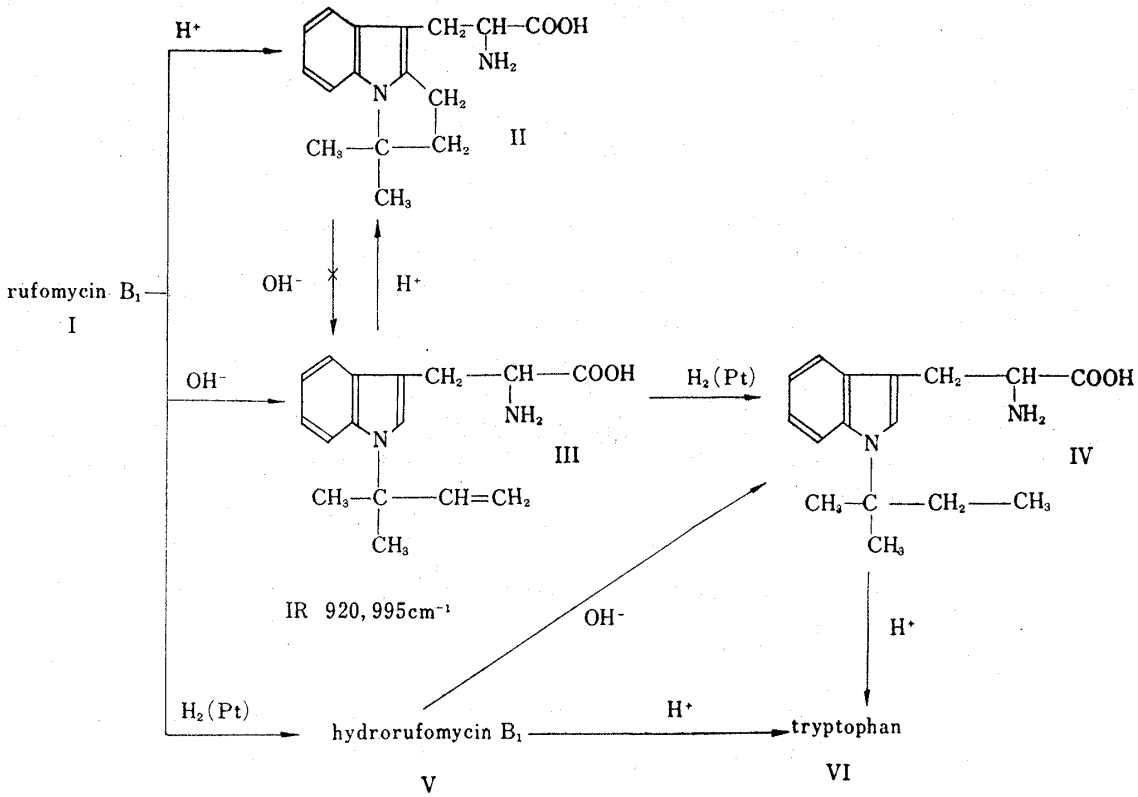

Chart 1.

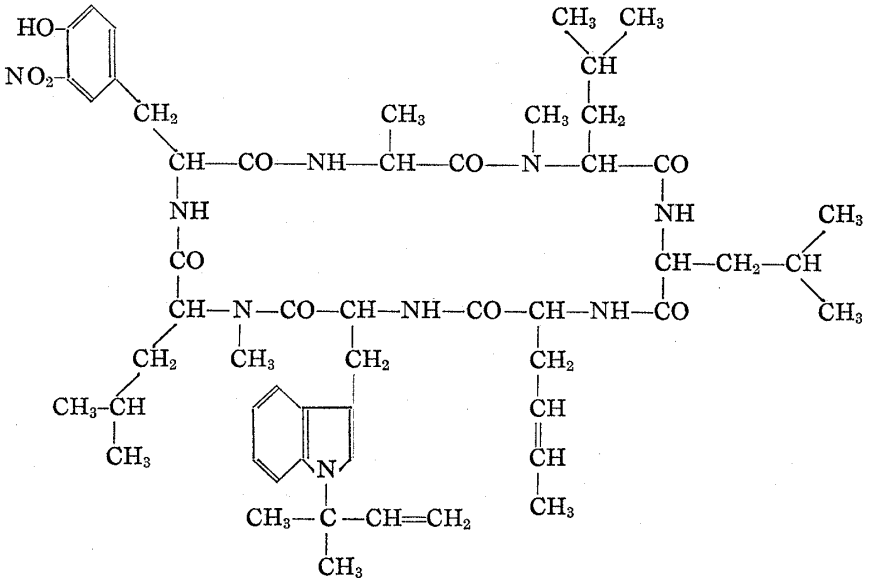

Fig. 1. Structure of Rufomycin B_1

with sodium borohydride[5] and the partial hydrolysate[1] (XII) were respectively treated with barium hydroxide solutions at 100° for 96 hr. to give a new tryptophan derivative (VIII), *Anal.* Calcd. for $C_{16}H_{22}O_4N_2 \cdot H_2O$ (VIII): C, 59.24; H, 7.46; O, 24.66; N, 8.63. Found: C, 59.51; H, 7.15; O, 24.37; N, 8.40. UV: $\lambda_{max}^{H_2O}$ 285 mμ, IR: ν_{max}^{KBr} 3300 cm^{-1} (broad: OH), NMR (D_2O) 6 protons of the *gem*-CH_3 (8.31 τ singlet). Treatment of VIII with 2*N* HCl at 105° for 4 hr. yielded VI, but more drastic conditions with acid converted VIII to a black

5) T. Takita: J. Antibiotics (Japan), Ser. A, **16**, 175 (1963).

gummi substance. Ⅷ was also obtained by a hydrolysis of Ⅶ with 70% formic acid at 100° for 5~24 hr. To confirm positions of OH-groups, Ⅷ was oxidized with sodium periodate at 5° for 30 min. to formaldehyde (identified as formaldimethone about 40% yield) and Ⅸ, UV : $\lambda_{max}^{H_2O}$ 282 mμ ($E_{1cm}^{1\%}$ 175), IR : ν_{max}^{KBr} 1720 cm^{-1} (carbonyl), NMR 6 protons of the *gem*-CH_3 (τ 8.29). Ⅸ was converted to a 2,4-dinitrophenylhydrazone and treatment of the hydrazone with 2*N* HCl at 100° for 40 min. gave quantitatively Ⅵ and a 2,4-dinitrophenylhydrazone of the substituent (X), *Anal.* Calcd. for $C_{10}H_{10}O_4N_4$: C, 47.61; H, 4.79; N, 22.21. Found : C, 47.80; H, 4.40; N, 21.77. Melting point of X (194° (decomp.)) was not depressed by admixture with the authentic 2,4-dinitrophenylhydrazone of 2-methylacrolein. The UV spectrum of X ($\lambda_{max}^{CHCl_3}$ 368 mμ) exhibited characterisitics of that of the authentic sample. From these results, the structure of Ⅷ was estimated as 1-(1,1-dimethyl-2,3-dihydroxypropyl)tryptophan.

The reactions described in this communication are shown in the Chart 1 and 2. Further studies on the structure of the original tryptophan moiety of rufomycin A is now under investigations.

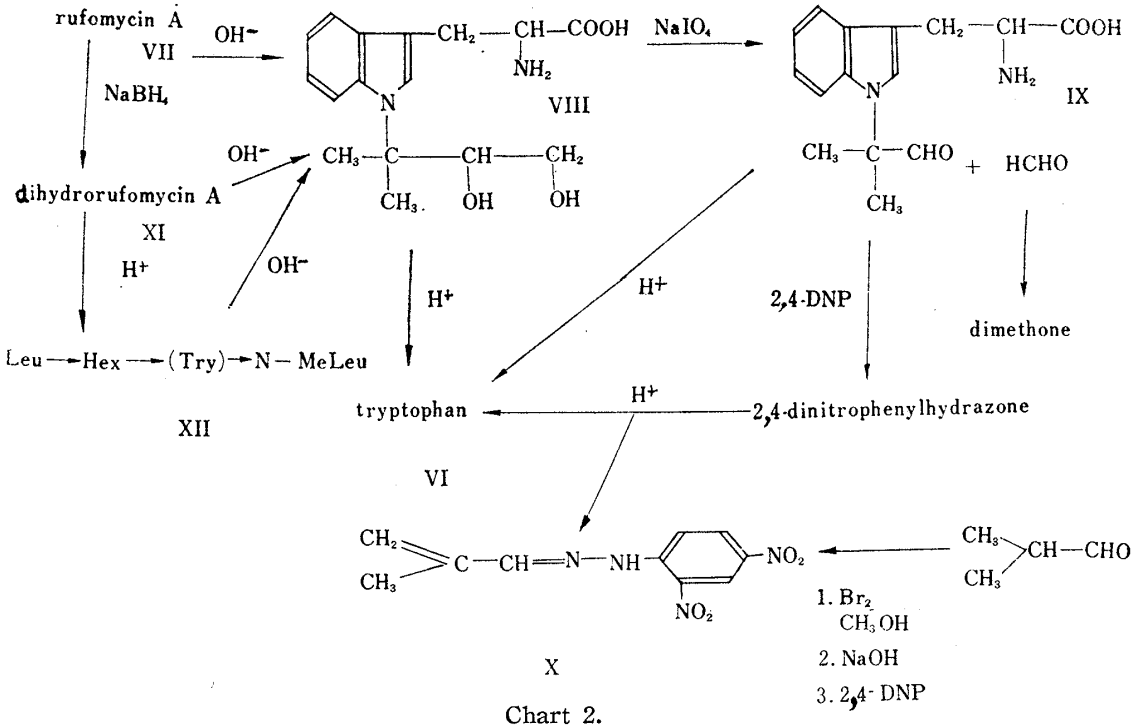

Chart 2.

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