Tetrahedron Letters No. 37, pp 3191 - 3194, 1975. Pergamon Press. Printed in Great Britain.

Structures of Ezomycins B_1, B_2, C_1, C_2, D_1, and D_2*

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(Received in Japan 23 June 1975; received in UK for publication 5 August 1975)

Ezomycin complex is a mixture of antifungal antibiotics produced by a strain of Streptomyces.[1-5] The structural elucidation of ezomycins A_1 (1) and A_2 (2) has been preliminarily reported.[4,5] In this paper we wish to present the structures of other components of the complex: ezomycins B_1 (3), B_2 (4), C_1 (5), C_2 (6), D_1 (7), and D_2 (8).

The ezomycin B_1, reported in the previous paper,[2] was proved to be a mixture (ca. 2.5:1) of ezomycins B_1 (3) and C_1 (5). In addition, new components, ezomycins C_2 (6), D_1 (7), and D_2 (8) were isolated from the complex. 3: $C_{26}H_{37}N_7SO_{16}\cdot H_2O$; amorphous, mp >200°(dec.); $[\alpha]_D^{22}$ -5.5° (c= 0.83, H_2O); $\lambda_{max}^{pH\ 2}$ nm (ε) 262 (7000), $\lambda_{max}^{pH\ 12}$ 286 (6100), A_{280}/A_{260} (pH 12) 1.81; δ** 4.04, (H-3', dd, 10.5, 5.0), 5.01 (H-1', s), 7.74 (H-6, s); $[\Theta]_{274}^{25}$ (H_2O) -660. 4: $C_{19}H_{25}N_5O_{13}\cdot H_2O$; prisms, mp >205°(dec.); $[\alpha]_D^{16}$ +10.8° (c=1.02, 0.2N NaOH); $\lambda_{max}^{pH\ 2}$ 261.5 (6300), $\lambda_{max}^{pH\ 12}$ 286 (4900), A_{280}/A_{260} 1.88; 4.00 (H-3', dd, 11.0, 5.0), 5.06 (H-1', s), 7.75 (H-6, s); $[\Theta]_{275}^{25}$ (H_2O) -1900. 5: $C_{26}H_{37}N_7SO_{16}\cdot H_2O$; amorphous, mp >200°(dec.); $[\alpha]_D^{22}$ -76.4° (c=0.94, H_2O); $\lambda_{max}^{pH\ 2}$ 263 (6400), $\lambda_{max}^{pH\ 12}$ 287 (4900), A_{280}/A_{260} 1.50; δ 4.35 (H-3', dd, 11.0, 4.5), 5.63 (H-1', d, 3.5), $[\Theta]_{272}^{25}$ (H_2O) +3800. 6: $C_{19}H_{25}N_5O_{13}\cdot H_2O$; needles, mp >200°(dec.); $[\alpha]_D^{18}$ -100° (c=0.52, 0.5N NH_4OH); $\lambda_{max}^{pH\ 2}$ 263.5(6200), $\lambda_{max}^{pH\ 12}$ 287 (4800), A_{280}/A_{260} 1.53; δ 4.21 (H-3', dd, 11.3, 4.0), 5.47 (H-1', d, 3.5), 7.82 (H-6, s); $[\Theta]_{272}^{25}$ (H_2O) +2900. 7: $C_{26}H_{39}N_7SO_{17}$; amorphous, mp >200°(dec.); $[\alpha]_D^{22}$ -62.1° (c=0.84, H_2O); $\lambda_{max}^{pH\ 2}$ 263.5 (6900), $\lambda_{max}^{pH\ 12}$ 287 (5200), A_{280}/A_{260} 1.52; δ 5.20 (H-1', d, 4.0), 7.87 (H-6, s). 8: $C_{19}H_{27}N_5O_{14}$; needles, mp >208°(dec.); $[\alpha]_D^{18}$ +58.8° (c=0.87, 0.5N NH_4OH); $\lambda_{max}^{pH\ 2}$ 264 (6700), $\lambda_{max}^{pH\ 12}$ 287 (4900), A_{280}/A_{260} 1.50; δ 3.90 (H-3', dd, 10.0, 4.0), 5.22 (H-1', d, 4.0), 7.91 (H-6, s).

* This paper is Part VI of the series, "Studies on Ezomycins, Antifungal Antibiotics." Preceding paper, see Reference 5.

** Otherwise stated, spectra were measured in 2% ND_3 in D_2O. Chemical shifts are expressed in δ value and coupling constatns in Hz.

The molecular formulae suggest that the relationship between 3 and 4, 5 and 6, and 7 and 8 is identical with that between 1 and 2: 1 is composed of 2 and L-cystathionine.[4] Positive coloration of 3, 4, 5, 6, 7, and 8 in the p-dimethylaminobenzaldehyde test shows the presence of a ureido group in each compound.[5] The UV maxima together with the olefinic proton signals (7.68-8.02, 1H, s) indicate that the chromophoric group is a 5-substituted uracil in 3 - 8.[6]

Acid hydrolysis of 3 with 3N HCl gave L-cystathionine (9) $C_7H_{14}N_2O_4S$[1] and ezoaminuroic acid (10) $C_6H_{11}NO_5$, 3-amino-3,4-dideoxy-D-xylohexopyranuroic acid.[3,5] Oxidation of 4 with 2 molar equivalents of $NaIO_4$, followed by treatment with phenylhydrazine at pH 1, gave glyoxal bisphenylhydrazone (11), α-hydroxybutyrolactol (12) $C_4H_6O_4$, and cucleoside B (13): $C_{13}H_{16}N_4O_9$; $[\alpha]_D^{22}$ +60.1° (c=1.05, 0.5N NH_4OH); M^+ 876 for $C_{13}H_9N_4O_9(TMS)_7$; $\lambda_{max}^{pH\ 2}$ nm (ε) 261.5 (5400), $\lambda_{max}^{pH\ 12}$ 286 (4600), A_{280}/A_{260} (pH 12) 1.76; $[\theta]_{275}^{25}$ (H_2O) -1200. The pmr spectrum of 13 and mass spectrum of its TMS-derivative indicated that the anhydrooctose uronic acid in 13 is identical with that of nucleoside A (14): diagnostic signals, H-1' (4.96, s) and H-3' (3.96, dd, 11.0, 5.0).[4] Further, biogenetical consideration suggests the identity in the absolute configuration of the both sugar parts. The value of A_{280}/A_{260} (1.76) as well as the CD spectrum confirmed β-configuration of the 5-substituted uracil in 13.[6,7] Accordingly, the structure of 13 was deduced to be 5-(3',7'-anhydro-5'-deoxy-5'-ureido-D-threo-β-D-allooctofuranosyluronic acid)-uracil.

As in the case of 4, periodate oxidation of a mixture of 3 and 5 gave, in addition to 11 an 13, lactam-aminohemiacetal (15) and nucleoside C (16): $C_{13}H_{16}N_4O_9$; $[\alpha]_D^{22}$ -107° (c=0.79, 0.5N NH_4OH); M^+ 876 for $C_{13}H_9N_4O_9(TMS)_7$; $\lambda_{max}^{pH\ 2}$ nm (ε) 263.5 (6400), $\lambda_{max}^{pH\ 12}$ 286.5 (4800), A_{280}/A_{260}(pH 12) 1.52; δ 4.23 (H-3', dd, 11.0, 4.0), 5.52 (H-1', d, 3.0), 7.93 (H-6, s); $[\theta]_{273}^{25}$ (H_2O) +2800. The mass spectrum of TMS-derivative of 16 revealed the same molecular ion peak as that of 13. The coupling constant of H-1' in 16 is consistent with $J_{1',2'}$=3.0 of α-pseudouridine, which assumes preferably C-3'-endo conformation.[8] The furanose moiety of the anhydrooctose uronic acid also takes C-3'-endo conformation.[4] Positive Cotton effect at 273 nm in the CD spectrum of 16 proved an α-configuration at C-1': 16 is an α-anomer at C-1' of 13. Therefore, 5 and 6 should be α-anomers at C-1' of 3 and 4, respectively.[7]

The lactam-aminohemiacetal (15) was identical with that derived from 1.[4] Accordingly, the binding site of L-cystathionine in 3 and 5 has been determined.

Alkaline hydrolysis of 4 with 1N NaOH at 95°C for 1.5 hrs yielded anhydronucleoside B (17): $C_{13}H_{14}N_4O_8$; $\lambda_{max}^{pH\ 2}$ nm (ε) 265 (sh., 7500), 237 (10,000); $\lambda_{max}^{pH\ 12}$ 254 (6000); M^+ 786 for $C_{13}H_9N_4O_8(TMS)_6$. The UV difference spectrum between 17 and 4 at pH 2 showed a λ_{max} at 233 nm, indicating

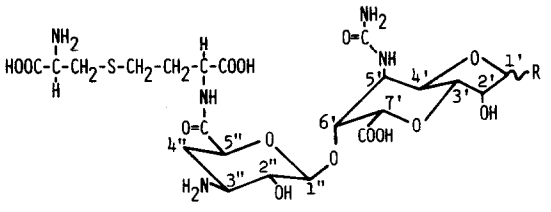
the presence of α-alkoxy-α,β-unsaturated carboxylic acid moiety in 17.[4,9] The coupling constant of H-1" (4.84, d) in 4 was 6.5. Accordingly, ezoaminuroic acid (10) must be attached to C-6' through β-glycosyl linkage.[10] Therefore, the structures of ezomycins B_1 and B_2 have been deduced to be 3 and 4, respectively.

Compounds 3, 5, and 7 are partially interconvertible in acidic conditions (0.2N HCl, 80°C, 30 min). Such conversion was also observed among 4, 6, and 8 even under more mild conditions (0.1N HCl, r.t., 24 hrs). Therefore, 5, 6, 7, and 8 are considered to be artifacts.

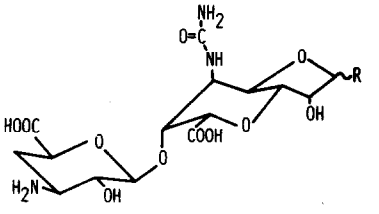
Periodate oxidation of 7 yielded 11, 15, and 5-formyl uracil (18). Compound 8 also gave 18 following the same treatment. This shows the presence of an additional hydroxyl at C-1' in both 7 and 8, each of which is presumably a 1:1 anomeric mixture at C-1'. The structures 7 and 8 were, therefore, assigned to ezomycins D_1 and D_2, respectively.

Moffatt et al. reported that under mild conditions (1N HCl, r.t.) some 5-(pentahydroxypentyl) uracils cyclized to pseudouridines accompanying isomerisation between α- and β-pseudouridines.[11] Based on this observation as well as the isomerisation mechanism of pseudouridines proposed by Chambers et al.,[6] we postulate a mechanism (Fig. 1) for the interconversion of ezomycins, which occurrs more easily than that of pseudouridines. The reaction is easy because a strain in the furanose part of 3, 4, 5, and 6 easily opens the ring. Because the 6-membered ring in 7 and 8 prevents the nucleophilic attack of the C-4' hydroxyl on the C-1', compounds 7 and 8 occurr as hydrates.

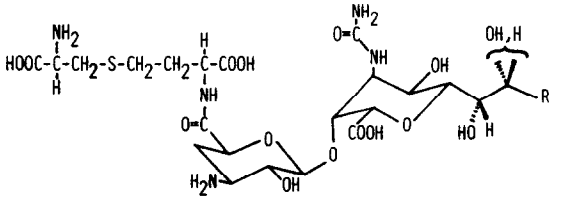
Several C-C nucleoside antibiotics such as formycins, pyrazomycin, showdomycin, and mini-



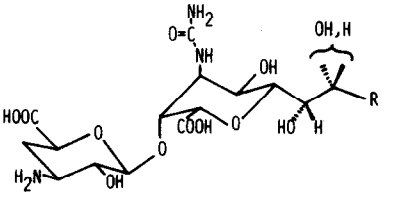
3 : R=β-5-uracil
5 : R=α-5-uracil



4 : R=β-5-uracil
6 : R=α-5-uracil



7 : R=5-uracil



8 : R=5-uracil

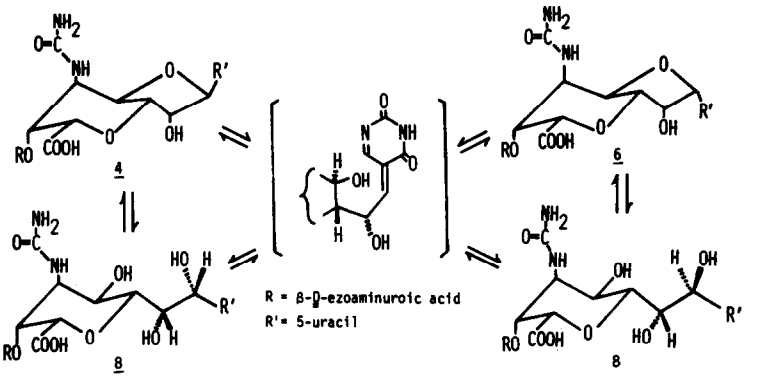


Fig. 1.

mycin have been isolated.[12,13] Ezomycins B_1, B_2, C_1, C_2, D_1, and D_2 are the first pseudouridine nucleoside antibiotics to be isolated. Occurrence of both cytidine- and pseudouridine-type nucleosides containing the same complex sugar parts suggests a plausible biosynthetic pathway of 3 and 4 through deamination of cytosine nuclei of 1 and 2, followed by rearrangement. This remindes us of a possible biosynthetic route of pseudouridines.[14]

Acknowledgments. The authors are indebted to Dr. Y. Mase and his coleagues of Fermentation Laboratories of Sankyo Co., Ltd. for production of ezomycins. They also thank Dr. J. Uzawa and Mrs. Y. Sugita of this institute for determining pmr and ms spectra, respectively.

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