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STRUCTURES OF EZOMYCINS A_1 AND A_2*

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Ezomycins A_1 $C_{26}H_{38}N_8O_{15}S \cdot H_2O$ (I) and A_2 $C_{19}H_{26}N_6O_{12} \cdot H_2O$ (II) are antifungal nucleoside antibiotics produced by a strain of Streptomyces.[1,2] Positive colorations to a p-dimethylaminobenzaldehyde test**,[3] suggest the presence of a ureido group in both I and II. On acid hydrolysis, I liberated cytosine.[1] Hydrolysis of I with Dowex 50W (H^+) afforded L-cystathionine and II as main products. II was further acid-hydrolyzed to give ezoaminuroic acid (III), 3-amino-3,4-dideoxy-D-xylohexopyranuroic acid.[4]

Hydrolysis of I with 1.5N NaOH at 90° gave anhydronucleoside A (IV): $C_{13}H_{14}N_4O_8$#; needles, mp >280° (dec.); $[\alpha]_D^{25}$ +21.8° (c=1.2, N NH_4OH); $\lambda_{max}^{0.05N\ HCl}$ nm (ε) 211 (8400), 238 (7700), 257 (7700); $\lambda_{max}^{0.05N\ NaOH}$ 225 (sh., 10,700), 261 (5700); pKa' 3.8, 9.9; M^+ m/e 714 for $C_{13}H_9N_4O_8(TMS)_5$. All of the signals in the pmr spectrum of IV were assigned by spin-decoupling experiments as shown in Table 1. The UV (λ_{max} 261 nm) and pKa' (9.9) data indicated that the cytosine moiety in I was converted to uracil in IV. IV was negative to ninhydrin but still positive to DMA. On esterification with methanolic HCl, IV gave monomethyl ester (V): $C_{14}H_{16}N_4O_8$#; needles, mp >300°; M^+ m/e 656 for $C_{14}H_{12}N_4O_8(TMS)_4$. Acetylation of V with acetic anhydride-pyridine afforded mainly monoacetate (VI): fine needles, mp >256° (dec.); λ_{max}^{MeOH} nm (ε) 212.5 (21,200), 244 (23,200). Chemical shift of H-2' of VI (Table 1) shows that C-2' hydroxyl was acetylated. Spin-decoupling experiments showed that the broad multiplet (4.7, H-5', $W_{1/2}$=18) is coupled with an amide proton (6.48, d., 9.0) as well as with H-4' and H-6'. This indicated that the ureido is attached to C-5'. Thus, four nitrogens of IV were attributed to a uracil moiety and a ureido group. Six of

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* This paper comprises part IV of the series, "Studies on Ezomycins, Antifungal Antibiotics." Preceding paper, see Reference 2.

** Abbreviated as DMA hereafter.

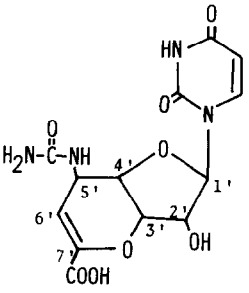
\# This formula was confirmed by elemental analysis.

Table 1. PMR Spectral Data* of II, IV, VI, VIII and IX

	H-1'	H-2'	H-3'	H-4'	H-5'	H-6'	H-7'	H-5	H-6	other protons
II (2% ND_3 in D_2O)	5.88 s	4.4 ∫ 4.6	3.91 dd, 11.0, 5.0	4.4 ∫ 4.6	4.4 ∫ 4.6	(4.98) br. t	4.4 ∫ 4.6	6.13 d, 8.0	7.69 d, 8.0	1.55(H-4"a, br. q, 11), 2.33(H-4"e, br. d, 11), 3.2(m, H-2", 3"), 4.18 (H-5", br. d, 11), 4.74 (H-1", d, 6.5)
IV (2% ND_3 in D_2O)	5.94 s	4.55 d, 4.9	3.98 dd, 10.8, 4.9	4.23 dd, 10.8, 4.6	4.74 dd, 5.9, 4.6	5.86 d, 5.9		5.81 d, 7.6	7.60 d, 7.6	
VI ($DMSO-d_6$)	5.85 s	5.57 d, 5.0	3.96 dd, 11,	4.17 dd, 11,	4.7 br. m	6.04 d, 6.0		5.68 d, 8.0	7.62 d, 8.0	2.12, 3.74(3H, each s), 5.60(2H, s) } -NH 6.48(1H, d, 9.0) }
VIII (2% ND_3 in D_2O)	5.81 s	(4.47)	3.81 dd, 10.5 5.0	(4.47)	(4.61)	4.30 dd, 2.5, 1.8	(4.40)	6.07 d, 8.0	7.62 d, 8.0	
IX ($DMSO-d_6$)	5.83 s	5.49	4.12	4.12	4.51 m	5.49	4.70 d, 1.8	7.21 d, 8.0	7.95 d, 8.0	2.06(6H, s), 2.12, 2.16, 3.64(3H, each s), 8.80(1H, d, 6.5, -NH)
IX ($CDCl_3$-MeOH-d_4 =3:1)	5.87 s	5.55 d, 5.6	4.07 dd, 11, 5.6	4.34 dd, 11, 4.0	4.65	5.65 d, 1.8, 3.0	4.65	7.47 d, 8.0	7.92 d, 8.0	2.13, 2.18, 2.22, 2.24, 3.74(3H, each s)

eight oxygens of IV are ascribed to a uracil, a ureido, a carboxyl and a hydroxyl, and the remaining two are supposed to constitute ether linkages.

The chemical shift of the olefinic proton (5.86, H-6', d., 5.9) and the p*Ka'* value (3.8) of IV suggest that the alkaline hydrolysis of I caused cleavage of L-cystathionine from I accompanying β-elimination of III to give IV having α-alkoxy-α,β-unsaturated carboxylic acid moiety. This was further substantiated by well consistence of the λ_{max} 238 nm of IV with the calculated value (232-237 nm).[5] Hydrogen deficiency 9 in IV demands that the sugar part should be bicyclic. The planar structure of IV, therefore, is formulated as the following.



IV

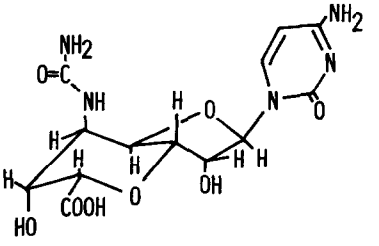
Oxidation of I with 2 molar equiv. of $NaIO_4$, followed by mild acid-hydrolysis furnished glyoxal (identified as bisphenylhydrazone), lactam-aminomemiacetal (VII) and nucleoside A (VIII): $C_{13}H_{17}N_5O_8$#; prisms, mp >255° (dec.); $[\alpha]_D^{27}$ +114° (c=0.51, 0.1N NaOH), $\lambda_{max}^{0.05N\ HCl}$ nm (ε) 212.5 (9800), 278.5 (13,000); $\lambda_{max}^{0.05N\ NaOH}$ 230 (sh., 7200), 271 (8400), positive to DMA. Esterification of VIII with methanolic HCl followed by acetylation afforded monomethyl ester-tetraacetate (IX): $C_{22}H_{27}N_5O_{12}$#; mp >280° (dec.); $[\alpha]_D^{27}$ +68.7° (c=0.15, $CHCl_3$-MeOH=1:1); λ_{max}^{MeOH} nm (ε) 212 (20,700), 249 (18,700), 297 (8400), negative to DMA. In the spectrum of IX (Table 1) the signals due to H-2' and H-6' moved downfield by more than 1 ppm as compared with those fo VIII. This shows the presence in VIII of two hydroxyls at C-2' and C-6', which were acetylated in IX. The negative coloration with DMA and the UV (λ_{max} 297 nm) of IX indicated

* Otherwise stated the spectra were measured at 100 MHz and chemical shifts are expressed in δ values and coupling constants in Hz.

that the remaining two acetyls were introduced to the ureido and cytosine functions. The signal due to H-5' and H-7' (4.65, 2H, in $CDCl_3$-MeOH=3:1) was separated to 4.51 (m., $W_{1/2}$=11) and 4.70 (d., 1.8) in DMSO-d_6. The broad multiplet ascribed to H-5' became narrow ($W_{1/2}$=6.5) on addition of D_2O. This confirmed that the ureido group is attached to C-5'. To nucleoside A was assigned the structure VIII containing an unusual novel sugar moiety.[9] Accordingly, II was postulated to have a structure connecting III to C-6' of VIII through a glycosyl linkage.

In the spectra of II, IV, VI, VIII and IX the anomeric proton appeared as sharp singlet.[6] This suggest that the furanose ring is fixed in C-2' exo- or C-3' endo-conformation.[6c,7] H-3' and H-4' are present in 1,2-trans-diaxial relationship because of their large coupling constant ($J_{3',4'}$=11). From the consideration with Dreiding model, only the following relative stereo-structure can be formulated for VIII.

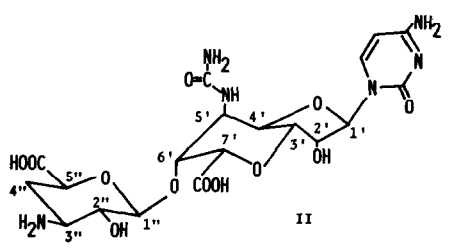
In the structure the furanose moiety takes a C-3' endo-conformation ($\phi_{1',2'}$=95-110°, $\phi_{3',4'}$=180°). Since $J_{4',5'}$ is 4-5 Hz, the ureido group on C-5' is in β-axial. β-Elimination of III from I suggests that the hydroxyl on C-6' (α-axial) and H-7' (β-axial) in VIII are present in 1,2-trans-diaxial. The coupling constants ($J_{5',6'}$=2.5-3.0, $J_{6',7'}$=1.8) in the spectra of VIII and IX support this assumption.



VIII

From CD spectra of I, II and VIII (Fig. 1), the cytosine nuclei in these compounds are deduced to have β-configuration.[8] Thus the structure of VIII was determined as 1-(3',7'-anhydro-5'-deoxy-5'-ureido-D-threo-β-D-allooctofuranosyluronic acid)-cytosine. In the spectrum of II (Table 1), H-1" appeared at 4.74 as a doublet ($J_{1",2"}$=6.5). This large coupling constant favored β-glycoside linkage.[10] Accordingly, the structure of ezomycin A_2 was elucidated as II.

Hydrogenation of I with Raney Ni liberated L-alanine, suggesting that L-cystathionine is attached to II at the homocysteine part.[1] Reduction of VII: $[\alpha]_D^{27}$ -43.2° (c=0.50, H_2O); δ(D_2O)



II

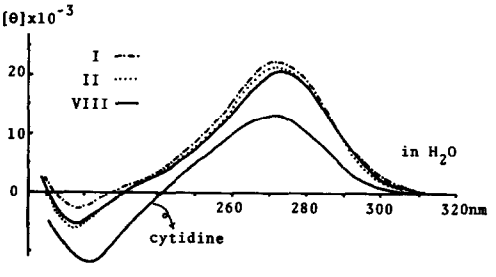
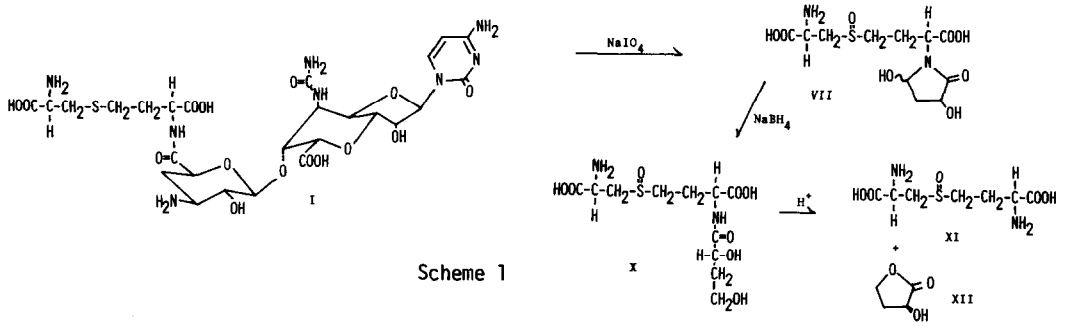


Fig. 1. CD Spectra of I, II, VIII and cytidine.



Scheme 1

2.2-3.0 (4H), 3.46 (2H, m.), 3.82 (2H), 4.6-5.1 (3H), 5.60 (0.5H, t., 6.0), 5.80 (0.5H, d., 6.0) with $NaBH_4$ afforded N-(2',4'-dihydroxybutyryl)-L-cystathionine sulfoxide (X): $\delta(D_2O)$ 2.3 (2H, m.), 2.65 (2H, m.), 3.45 (2H, t., 7.5), 3.82 (2H, d., 6.0), 4.15 (2H, t., 6.5), 4.72 (2H, m.). A pair of anomeric proton signals (5.60 and 5.80) in VII disappeared and a triplet (4.15) ascribable to a hydroxymethyl having two vicinal hydrogens was observed. This allows the assignment of a lactam-aminohemiacetal structure to VII as depicted in Scheme 1.

X was readily hydrolyzed with 0.5N HCl at 90° for 30 min. to afford L-cystathionine sulfoxide (XI): $C_7H_{14}N_2O_5S$#; *amorphous*, mp >210° (dec.); $[\alpha]_D^{27}$ +10.2° (c=0.52, 0.1N NaOH) and α-hydroxybutyrolactone (XII): M^+ m/e 102 for $C_4H_6O_3$; CD (MeOH) $[\theta]_{222}^{25}$ +5800. The positive Cotton effect at 222 nm shows S-configuration of XII[11] and, consequently, confirms D-configuration of III.[4] Thus, the structure of ezomycin A_1 was proposed as I.

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