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EZOAMINUROIC ACID, 3-AMINO-3,4-DIDEOXY-D-XYLO-HEXOPYRANUROIC ACID, AS A CONSTITUENT OF EZOMYCINS A_1 AND A_2**

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Ezomycins are antifungal antibiotics produced by a Streptomyces. They inhibit specifically the growth of very limited species of phytopathogenic fungi such as Sclerotinia and Botrytis. Previously we elucidated that ezomycins A_1 and B_1, the main active components of the group, are new peptidyl nucleosides containing L-cystathionine.[1] In this paper we wish to report the structure of a novel aminodeoxyuronic acid named ezoaminuroic acid 1 which was isolated from ezomycins A_1 and A_2.

On hydrolysis with Dowex 50W (H^+) at 100° under a stream of N_2 for 5 hr, ezomycin A_1, $[\alpha]_D^{22}$ +13.5° (c=1.05, water), $C_{26}H_{40}N_8SO_{16}$ afforded L-cystathionine and ezomycin A_2, $[\alpha]_D^{16}$ +44.4° (c=1.0, 0.2N NaOH), $C_{19}H_{28}N_6O_{13}$, as main products. This suggests that ezomycin A_1 is composed of L-cystathionine and ezomycin A_2.

Then ezomycin A_2 was hydrolyzed with 3.2N HCl at 100° for 4 hr in vacuo, and the hydrolysate was evaporated to dryness. The residue was dissolved in a small volume of water and subjected to carbon column chromatography (water-acetone gradient elution) at pH 4. The first eluate with water, which gave positive colorations to both ninhydrin and aniline hydrogen biphthalate reagents, was concentrated and lyophilized. The solid was found to be mainly composed of 1 by gas chromatographic analysis (OV-17) after trimethylsilylation. The mass spectrum of the silyl ether gave M^+ at m/e 465 accounting for $C_6H_7NO_5(TMS)_4$. Without further

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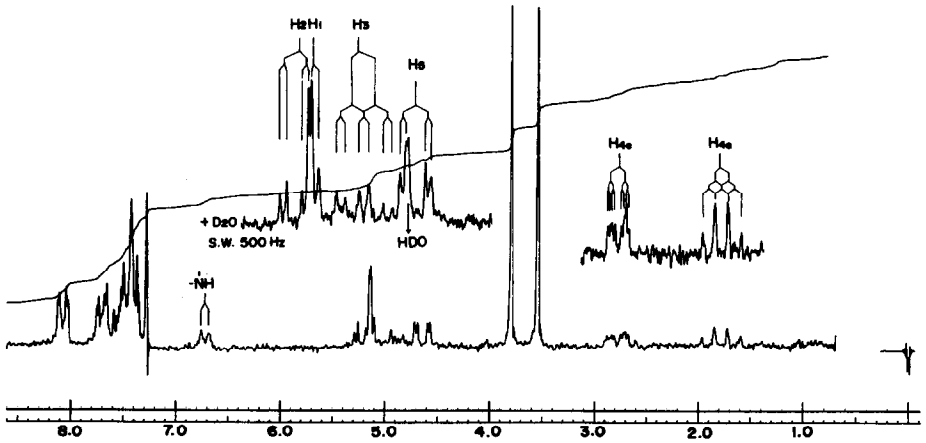


Fig. 1. Pmr Spectrum of 3 (100 MHz, $CDCl_3$)

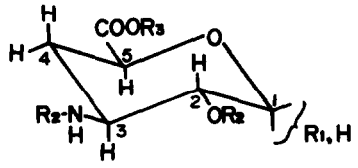
purification the lyophilized matter was heated in 5% methanolic HCl under reflux for 9 hr and applied to preparative TLC (Silica gel GF_{254}; $CHCl_3$-MeOH, 2:1) to afford hydrochloride of methyl (methyl ezoaminid)uronate 2, which was characterized by mass spectrometry as a trimethylsilyl derivertive: M^+ m/e 349 for $C_8H_{13}NO_5(TMS)_2$, 334 (M-15), 318 (M-31), 290 (M-59). The product was treated with benzoyl chloride in a mixture of trimethylamine and pyridine at room temperature. After usual work-up, the dibenzoyl derivertives were subjected to preparative TLC (Silica gel GF_{254}; benzene-EtOAc, 4:1) to give a 1:1 mixture (determined by pmr) of anomers 3 and 4. Subsequent preparative TLC (Silica gel GF_{254}; CCl_4-ether, 1:2) gave pure anomers: 3; amorphous powder, $[\alpha]_D^{18}$ +133° (c=0.91, MeOH), ν_{max} ($CHCl_3$) 1750, 1715, 1660, 1272, 1115 cm^{-1}, λ_{max} (MeOH) nm (ε) 228 (20,300), 274 (sh., 1300), 282 (sh., 820), and 4; fine needles (from EtOAc), m.p. 237.5-8°, $[\alpha]_D^{18}$ +58° (c=0.75, MeOH), ν_{max} ($CHCl_3$) 1755, 1716, 1658, 1270, 1115 cm^{-1}, λ_{max} (MeOH) nm (ε) 227.5 (15,900), 274 (sh., 1100), 282 (sh., 710). From high resolution mass spectrometry of 3 (obsd. 413.1491, calcd. 413.1473 for $C_{22}H_{23}NO_7$), the molecular formula of 1 was confirmed to be $C_6H_{11}NO_5$.

The high field signals assigned to H_{4a} and H_{4e} in the pmr spectra of 3 and 4 (Fig. 1 and Table 1) suggest that 1 should be a deoxysugar derivertive. Large coupling constants between H_{4a} and its vicinal protons ($J_{4a,3}$=11,* $J_{4a,5}$=11.3-12)

* Chemical shifts are expressed in δ value and coupling constants in Hz.

Table 1. Pmr Spectra Data of 3 and 4 (100 MHz, $CDCl_3$)

	H_1	H_2	H_3	H_{4a}	H_{4e}	H_5	1-OMe	COOMe
3	5.12	5.20	4.87	1.79	2.78	4.64	3.54	3.79
	J=3.4	10.5	11	13	2.5			
				4.5				
				12				
4	4.71	5.09	4.52	1.86	2.77	4.36	3.59	3.79
	J=7.0	10	11	13.5	2.7			
				4.7				
				11.3				
Δδ	+0.41	+0.11	+0.35	-0.07	+0.01	+0.28	-0.05	0



1 : R_1=OH, R_2=R_3=H
2 : R_1=OCH_3, R_2=H, R_3=CH_3
3 : R_1=α-OCH_3, R_2=Bz, R_3=CH_3
4 : R_1=β-OCH_3, R_2=Bz, R_3=CH_3

show clearly that the both glycosides are present in a chair pyranose form with Cl (if they are D-isomers) or 1C (in another case) conformation. The amide proton observed at 6.72* as a broad doublet in the spectrum of 3 disappeared on addition of D_2O. An unresolved broad multiplet at 4.87 ascribed to H_{3a} changed to a double triplet ($J_{3,2}$=10.5, $J_{3,4a}$=11, $J_{3,4e}$=4.5) after this treatment. These data indicate that the benzamide and O-benzoyl groups are attached in equatorial at C-3 and C-2, respectively. Through comparison of the coupling constants of H_1 in 3 and 4 (3; J=3.4, 4; J=7.0), orientation of the anomeric proton of 3 was revealed to be equatorial and that of 4 to be axial. The signals assigned to H_{3a} and H_{5a} in the spectrum of 3 suffered significant downfield shifts by the anisotropic effect of the axial methoxyl group, and the anomeric proton signal appeared in lower field than that of 4. These observations are in good agreement with the results of extensive pmr spectrometric studies on various kinds of glycoside anomers.[2,3] Thus the relative stereostructures of these methyl glycoside anomers have been presented by the formula 3 and 4 or their antipodes.

The D-configuration of them were infered from the fact that 3 was more dextrorotatory than 4. This conclusion was further substantiated by CD spectra measurements of them.

The "dibenzoate chirality rule" has been known to be useful for determining the chiralities of optically active glycols.[4] Johnson et al. successively applied this rule to vicinal benzoate-benzamide derivertives and reported that N,O-dibenzoyl-α-L-vancosaminide gave negative first Cotton in its CD spectrum as ex-

pected from its negative chirality: $[\theta]_{237}$ -22,400, $[\theta]_{222}$ +10,600 (MeOH).[5]

The CD spectra of 3 and 4 showed two strong Cotton effects of opposite signs by Davidov splitting: 3; $[\theta]^{22}_{237.5}$ +47,100, $[\theta]^{22}_{222}$ -14,500 (MeOH), 4; $[\theta]^{20}_{237.5}$ +64,000, $[\theta]^{20}_{222}$ -23,600 (MeOH). These values are ascribable to the positive chirality, viz. clockwise in Newman's projection formula, of the benzoyl groups. The absolute structure of 3, therefore, was established to be methyl (methyl 3-benzamido-2-O-benzoyl-3,4-dideoxy-α-D-xylo-hexopyranosid)uronate, and that of 1 to be 3-amino-3,4-dideoxy-D-xylo-hexopyranuronic acid.

Up to the present, only several kinds of aminodeoxyuronic acids have been isolated as constituents of polysaccharides from microorganisms[6,7] and of antibiotics such as gougerotin, blasticidin S and polyoxins.[8] They are 2-amino-2-deoxy-, 4-amino-4-deoxy- or 5-amino-5-deoxy-D-hexuronic acid derivertives. Ezoaminuroic acid 1 is the first example of naturally occurring 3-amino-3-deoxy-hexuronic acids.

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References

1) K.Sakata, A.Sakurai and S.Tamura, Agr. Biol. Chem., 37, 697 (1973).
2) H.Maehr and C.P.Schaffner, J. Am. Chem. Soc., 92, 1697 (1970), S.Hanessian and T.H.Haskell, "The Carbohydrates, Chemistry and Biochemistry," Vol. IIA, ed. by W.Pigman and D.Horton, Academic Press, New York, 1970, p. 194.
3) R.U.Lemieux and J.D.Stevens, Can. J. Chem., 43, 2059 (1965), M.Matsui and M.Okada, Chem. Pharm. Bull., (Tokyo), 18, 2129 (1970).
4) N.Harada and K.Nakanishi, J. Am. Chem. Soc., 91, 3989 (1969).
5) A.W.Johnson, R.W.Smith and R.D.Guthrie, J. Chem Soc., Perkin I, 2153 (1972).
6) H.Heyn, G.Kiessling, W.Lindenberg, H.Paulsen and M.E.Webster, Chem. Ber., 92, 2435 (1959), A.R.Williamson and S.Zamenhof, J. Biol. Chem., 238, 2255 (1963), S.Hanessian and T.H.Haskell, ibid., 239, 2758 (1964).
7) H.R.Perkins, Biochem. J., 86, 475 (1963).
8) R.J.Suhadolnik, "Nucleoside Antibiotics," Wiley-Interscience, New York, 1970, p. 172, 189 and 218.