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SOLUTION STRUCTURE AND CATION-BINDING ABILITIES OF TWO QUASI-ISOMORPHOUS ANTIBIOTIC IONOPHORES, M 139603 AND TETRONOMYCIN

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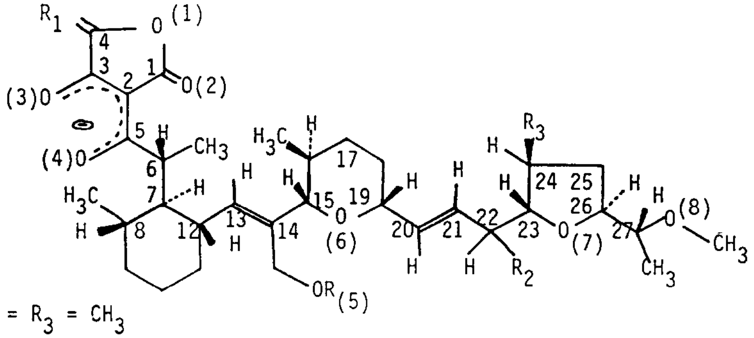
Abstract : The 200 and 400 MHz ^1H nmr spectra of the title antibiotics are fully attributed and analyzed with the help of COSY and DEPT sequences. The solution conformation, the same for the free acid and the sodium salt, is very similar to that in the solid.

NATURE offers numerous surprises : one of which is the remarkable structural similarity between two antibiotic ionophores, M139603 and tetronomycin 1 and 2. Apart from slight structural differences[1], the two molecules share very similar constitutions but have opposite configurations at each of the 12 chiral centers[2] : the formula below represents the configurations at the various asymmetric carbons of 1.



(1) $R_1 = H_2$; $R_2 = R_3 = CH_3$

(2) $R_1 = CH_2$; $R_2 = R_3 = H$

We have discovered also that both of these ionophores display a marked synergism for transport of Pr^{3+} across lipid bilayers, when used jointly with lasalocid (X-537 A)[3]. The first step in cation transport is complexation by the antibiotic. Therefore, we have determined, using standard methods[6], the binding constants of a number of cations, in competition with Na^+, by 1. We also report upon the solution conformation of 1 and 2, as deduced from ^1H nmr (200 and 400 MHz), using COSY 45 and COSY 90 techniques[4,5] for unambiguous spectral attributions. We find, not only virtually identical conformations for the free acid and for the sodium salt, but also a solution geometry extremely reminiscent of the solid-state structure.

cation	binding constant K (1:1, M^{-1}, ± σ)
Li^+	(too small to be detected)
Na^+	500 ± 50
K^+	340 ± 30
Rb^+	54 ± 6
Mg^{++}	1,300 ± 150
Ca^{++}	$> 10^4$
L-norepinephrine	40 ± 4

Table 1 : Binding Constants between 1 and Various Perchlorates, or Norepinephrine, in Methanol-Water Binary Mixtures (70:30), at 305 K.

In solvents mimicking a lipophilic membrane environment[7], these antibiotic display a selectivity for Na^+ and Ca^{++}, two cations with very similar ionic radii, 0.95 and 1.0 Å, respectively (Table 1). We had earlier shown that lasalocid (X-537 A) displays, in methanol-hexane mixtures, competitive binding of Na^+ ions and biogenic amines[8]. Here, the binding of L-norepinephrine is weaker by one order of magnitude to that of sodium ions (Table 1).

Our proton assignments (Table 2), which result unambiguously from two-dimensional nmr experiments of the COSY type, further support those earlier made after selective irradiation[1]. In some cases, the high-field spectra (400 MHz) allowed resolution of hitherto coincident signals; for instance, H-26 occurs at $\delta = 4.14$ while H-23 is at $\delta = 4.05$, for 2. In order to characterize unambiguously the methylene group (1) in position 4 (not present in 2), we have used both ^1H and ^{13}C correlated [9-10] and DEPT ^{13}C [11] spectra : they occur at 71.5 ppm (^{13}C) and at $\delta = 4.27$ (^1H; accidentally isochronous).

The ^1H-^1H coupling constants for 2 (Table 2) have been obtained from the spectrum at 400 MHz. The results, very similar to those for M 139603 (1), correspond to strictly superimposable spectra for 2 (or 1) as the free acid or as the sodium salt. Hence, the conformation remains the same when ionophores bind a sodium cation, oxygens 3,4,6,7 and 8 serving as coordinating atoms[1,2]. Furthermore, this unique conformation is strikingly reminiscent of that found by X-ray for an Ag^+ salt of an acetylated derivative of 2.[1] We have indicated in Table 2 approximate values for some of the relevant dihedral angles in the solution conformation of 2, as estimated from a Karplus-type relationship[12]. Thus, we find, as previously reported by Anteunis[13] for other antibiotics, a pre-formed geometry of these two ionic receptors 1 and 3 towards sodium binding.

	δ	^3J (Hz, ± 0.1 Hz)	dihedral angles
H-6	3.73 d.q	6.9 (CH_3)	-
		3.5 (H-7)	55°
H-13	5.05 d	9.8 (H-12)	160°
H-15	3.20 d	∿ 9 (H-16)	150°
		(9.8 in 1)	(160°)
H-19	3.80 d.d.d	8.8 (H-20)	150°
		10.8 (H-18a)	170°
		2.2 (H-18e)	65°
H-20	5.56 d.d	8.8 (H-19)	150°
		15 (H-21)	180°
H-21	6.04 d.d.d	15 (H-20)	180°
		10.5 (H-22)	165°
		4.2 (H-22')	50°
H-23	4.05 m		
	3.62 d.d (1)	10.3 (H-22)	165° (or 0°)
		4 (H-24)	120° (or 50°)
H-26	4.14 d.d.d	9.1 (H-25)	150°
		6.5 (H-25')	30°
		2.6 (H-27)	60°

Table 2. Vicinal Coupling Constants and Estimated Dihedral Angles for Selected Protons of 2 (sodium salt, 20 mM in CH_3OH solution).

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