The structure of the ionophoric antibiotic Na-tetronasin (M139603) in solution

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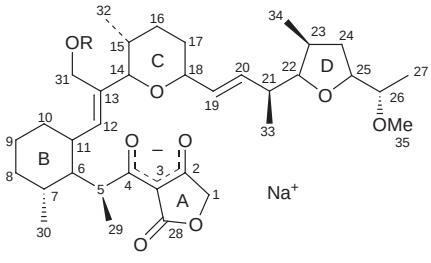
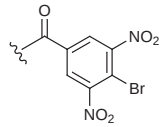
The structure of the ionophoric antibiotic sodium tetronasin (M139603) in chloroform solution has been determined using NMR spectroscopic methods and is shown to contain a water molecule bound to the sodium which is hydrogen bonded to oxygens in the molecule.

The ionophoric antibiotics are an intriguing series of compounds that have widespread biological action.[1] In particular they are efficient mediators of the transport of metal ions and H^+ through the limiting membranes of cells. This property is presumed to be responsible for their biological activity through the dissipation of trans-membrane ion concentration gradients. It is believed that in most cases the transport occurs by the ionophore forming a 1 : 1 complex with the metal ion which then diffuses through the membrane.



1 R = H [Tetronasin (or M139603)]

2 R = 

The discovery of M139603 **1** (subsequently called tetronasin) an ionophoric antibiotic from the aerobic fermentation of *Streptomyces longisporoflavus* NCIB 11426, was announced by ICI in 1980.[2] Its structure is rather different from those of the other common acidic ionophoric antibiotics in that it possesses a biosynthetically rare acid group in the form of an acyl tetronic acid moiety. Tetronasin and several of its derivatives have shown useful properties as coccidiostats and as growth promoters in ruminant animals, reducing methane production and increasing the propionate/acetate ratio in the rumen.[2–4]

A substantial amount of experimental work on the biosynthesis[5] *via* a polyketide mechanism and the synthesis[6–9] of tetronasin has been reported in recent years.

Experiments on the transporting ability of tetronasin for Li^+, Na^+ and K^+ through model biological membranes were performed by Riddell and Arumugam.[10] According to this work tetronasin is one of the faster transporting ionophoric antibiotics for the alkali metal ions. Interestingly, tetronasin displays the fastest association and dissociation rates in the membrane surface yet measured for the sodium salt of an ionophoric antibiotic.

The structure of tetronasin has been determined crystallographically from the 4-bromo-3,5-dinitrobenzoyl derivative **2** of the sodium salt.[11] The sodium ion was observed to be six-coordinated in the solid through five oxygen atoms from the molecule, two of which come from the tetronic acid, and a water molecule which occupies the sixth position. The sodium ion was observed to be at the centre of a very distorted octahedron.

An early report of the NMR spectra of tetronasin[12] suggested that the solution conformation of the ionophore is similar to that determined crystallographically. The solution conformation must, however, differ from that of the solid because ester formation at C(31) removes a hydroxy group which would undoubtedly be involved in hydrogen bonding in the original molecule. It seemed to us that modern NMR methods for the determination of the structure of biomolecules in solution would be appropriate to be applied to the sodium salt of tetronasin. The interest in the solution structure is two-fold. First, it would assist in explaining the rapid association and dissociation rates of the sodium salt during the transport process. Secondly, it might be possible to locate the bound water molecule, observed by crystallography, using NMR methods. Water molecules bound to biologically important molecules are potentially of functional importance and an ability to identify bound water molecules is of considerable significance. Other ionophoric antibiotics whose structures in solution have been solved by NMR methods include sodium salinomycin[13] and sodium monensin.[14]

The assignment of the ^1H NMR spectrum of sodium tetronasin[15] in $CDCl_3$ was straightforward. The resonances for H(12), H(19) and H(20) can readily be identified in the 1D ^1H NMR spectrum. Starting from these resonances the use of COSY and DQF COSY connectivities gave most of the assignments. The region between 1 and 2 ppm is rather crowded and so the chemical shifts of geminal hydrogen pairs were determined by a 2D HSQC experiment at natural abundance. Conformationally relevant scalar couplings were obtained from the 1D spectrum, from a *J*-resolved 2D spectrum and from an E. COSY spectrum.

NOESY spectra were obtained with mixing times of 100, 200, 300, 450 and 600 ms and NOE build up rates proved to be essentially linear in the 0–200 ms range. The NOESY cross peak intensities at 200 ms were used to establish distance restraints. The NOESY spectra contained cross peaks due to exchange between C(31)OH and a broad peak at around 1.77 ppm. At ambient temperature no additional NOESY cross peaks were observed from either of these peaks. However, as the temperature is lowered to −20 and −25 °C additional cross peaks of low intensity were observed from the 1.77 ppm peak which had shifted to 2.20 and 2.28 ppm respectively. Importantly, these cross peaks moved with changes in the position of the diagonal peak. At −25 °C these cross peaks are to $C(31)H_2$ (two cross peaks), C(19)H and $C(29)H_3$. The existence of these cross peaks clearly shows that the broad resonance in the 2.0 ppm region is from a bound water molecule. The absence of these cross peaks at ambient temperature is presumably due to rapid exchange of the bound water molecule.

Starting from the structure derived from X-ray crystallography and employing standard methods[16] including simulated annealing with the use of 49 NOE distance constraints, applied as ±7% of the measured NOE distance, a structure of the molecule was derived without the additional bound water

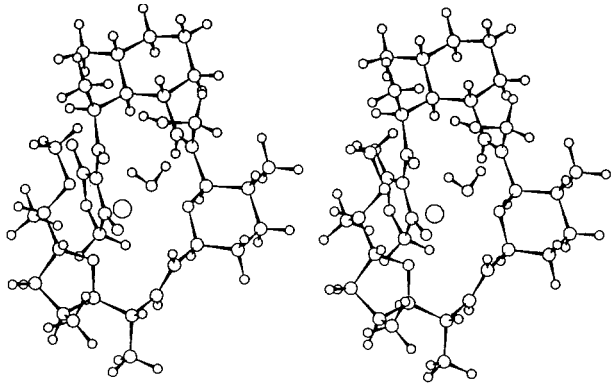


Fig. 1 Stereoview of the finalised structure of sodium tetronasin in solution incorporating the bound water molecule found from molecular modelling and incorporating the NMR distance constraints

molecule. The C(31) hydroxy group is found to hydrogen bond to the C(4) oxygen. The sodium is surrounded by five oxygen atoms. At this point the additional water molecule was introduced which was held close to the sodium with a 5 Å tether. No NOE constraints from the four cross peaks due to the bound water were introduced because the quantification of the NOE intensities was complicated by chemical exchange. The final structure, shown in Fig. 1, emerged after initial minimisation followed by restrained simulated annealing from 400 to 10 K and a final minimisation.

To test the goodness-of-fit a back calculation of the NOE intensities was performed on the model without the bound water molecule, which gave a relative root mean square deviation of 0.16.[17]

Molecular modelling without the NOE constraints led to the molecule unfolding after 100 ps of molecular dynamics at 303 K. A similar calculation on the coupling constants, which had not been used as restraints in the molecular modelling, gave a root mean square deviation of 0.05.[18] The model is thus seen to be in very satisfactory agreement with the experimental data.

The sodium is seen to be at the centre of a distorted octahedron of oxygen atoms with sodium to oxygen distances in the region 2.42 to 2.67 Å. The C(31) hydroxy group remains hydrogen bonded to the C(4) oxygen and the additional water molecule, whose oxygen is 2.67 Å from the sodium, is hydrogen bonded at one end to the C(31) hydroxy group and at the other to the C(26) (methoxy) oxygen.

In contrast to the other ionophoric antibiotics such as monensin, tetronasin has no tail to head hydrogen bond to 'close the circle' within the ligand. The only hydrogen bond involving a hydroxy group occurs across the molecule near the head between the C(31) hydroxy group and the C(4) oxygen. The water forms an additional 'brace' in a 'head to tail' fashion but this is expected to be less strong than a direct hydrogen bond. The C(31) hydroxy group thus has a defined role of structural importance for the sodium tetronasin complex. The design of other ionophoric materials with five oxygen ligands may well require water to be incorporated in the structure in a similar manner to the water in tetronasin. Also, unlike the other ionophoric antibiotics tetronasin takes a water molecule with it into the hydrophobic environment of a chloroform solution and, by implication, into the fatty interior of the membrane. Finally, during the initial series of simulated annealing all of the conformations observed had the acid head group of the molecule close to the sodium ion but in some of these conformations the oxygens remote from the tetronic acid had broken free. These three factors suggest why the on–off rates for sodium with tetronasin are the fastest yet observed for the ionophoric antibiotics. The lack of a direct head to tail hydrogen bond and the presence throughout of one molecule of water will lead to fewer steps in the association/dissociation processes. The lower number of steps in turn could lower the activation energies for these recognition processes.

Notes and References

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15 All spectra were obtained at 300 K on a Varian Unity-plus 500 spectrometer operating at 500.3 MHz for ^{1}H, using standard pulse sequences unless otherwise stated.
16 All molecular mechanics simulations were carried out on Silicon Graphics O2, Origin 200 and INDY computers using MSI/Biosym's CDiscover (Discover 95) software package in an Insight II environment. For the energy calculation the extensible Systematic Force Field (ESFF) was used with a 9.5 Å cut off for van der Waals and Coulomb interactions. The effect of solvent was taken into account by application of a distance dependent dielectric constant varying from 1 to an upper limit of 4.5. During the molecular dynamics calculations the velocity Verlet integrator and 1 fs timesteps were used. The temperature was controlled by direct velocity scaling with a 10 K temperature window. Minimisation was applied after every simulated annealing in cascade manner; steepest-descent, conjugate gradient (Fletcher algorithm), Newton method (BFGS algorithm). The iteration limit was 1500 steps, and the final convergence criterion was 0.0001 as maximum derivative.
17 No allowance was made in this unweighted calculation for the relatively larger errors that occur with the smaller NOE observations.
18 The root mean square deviation (rmsd) is here defined as:

$$\text{rmsd} = \sqrt{\frac{\sum_n \left(\frac{J_{(obs)} - J_{(calc)}}{J_{(obs)}} \right)^2}{n}}$$

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