ASSOCIATION OF ALKALI METAL SALTS WITH POLYENE MACROLIDES

IN METHANOL SOLUTION

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Abstract ^{23}Na NMR spin-lattice relaxation times and ^{1}H NMR chemical shift
changes demonstrate weak association between alkali metal ions and polyene
macrolides in methanol solution.

Amphotericin B (1) and Nystatin (2) are clinically useful antifungal agents.[1] Their
site of action is the cytoplasmic membrane of the microorganism, and they are selectively
toxic to sterol-rich membranes, particularly when ergosterol is a major component.
Incorporation of the polyene into sterol-containing membranes leads to leakage of
monovalent ions, particularly K^{+}, and of small molecules.[2] This is in part responsible
for their toxic action although the precise mechanism is still subject to controversy.[3]
It is generally assumed that aggregation of the polyene and sterol occurs, possibly in a
rather specific fashion[4] to produce a half-pore in the bilayer. Ion and molecule transport
may then be effected across two such half-pores, so that the mode of action is believed to
be different from carrier ionophores[5] like valinomycin or monensin which bind strongly and
specifically to the alkali metal ion to give a complex which has trans-membrane mobility.
We report evidence for weak complexation between these ions and polyene macrolides, not
previously considered to be significant for their biological action.

The ^{23}Na NMR spin-lattice relaxation time T1 of NaI (2.5 - 5 mM) in methanol was
measured by standard techniques.[6,7] Addition of nystatin (1 mM) consistently led to
shorter T1 values, both at ambient temperature and at 0°. In these experiments there
was no significant alteration of the ^{23}Na NMR line-shape but at enhanced nystatin
concentration (10 mM, solubilised by 0.07 mL. AcOH/1.0 ml CH_3OH) the ^{23}Na NMR line-width
of 2.5 mM NaI was increased from ω = 20 to ω = 30 Hz. Taken together, these observations

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FIGURE 1 ^{1}H NMR SPECTRUM OF NYSTATIN IN CD$_3$OD – 2DCOSY OF OLEFINIC REGION INSET, 500 MHz.
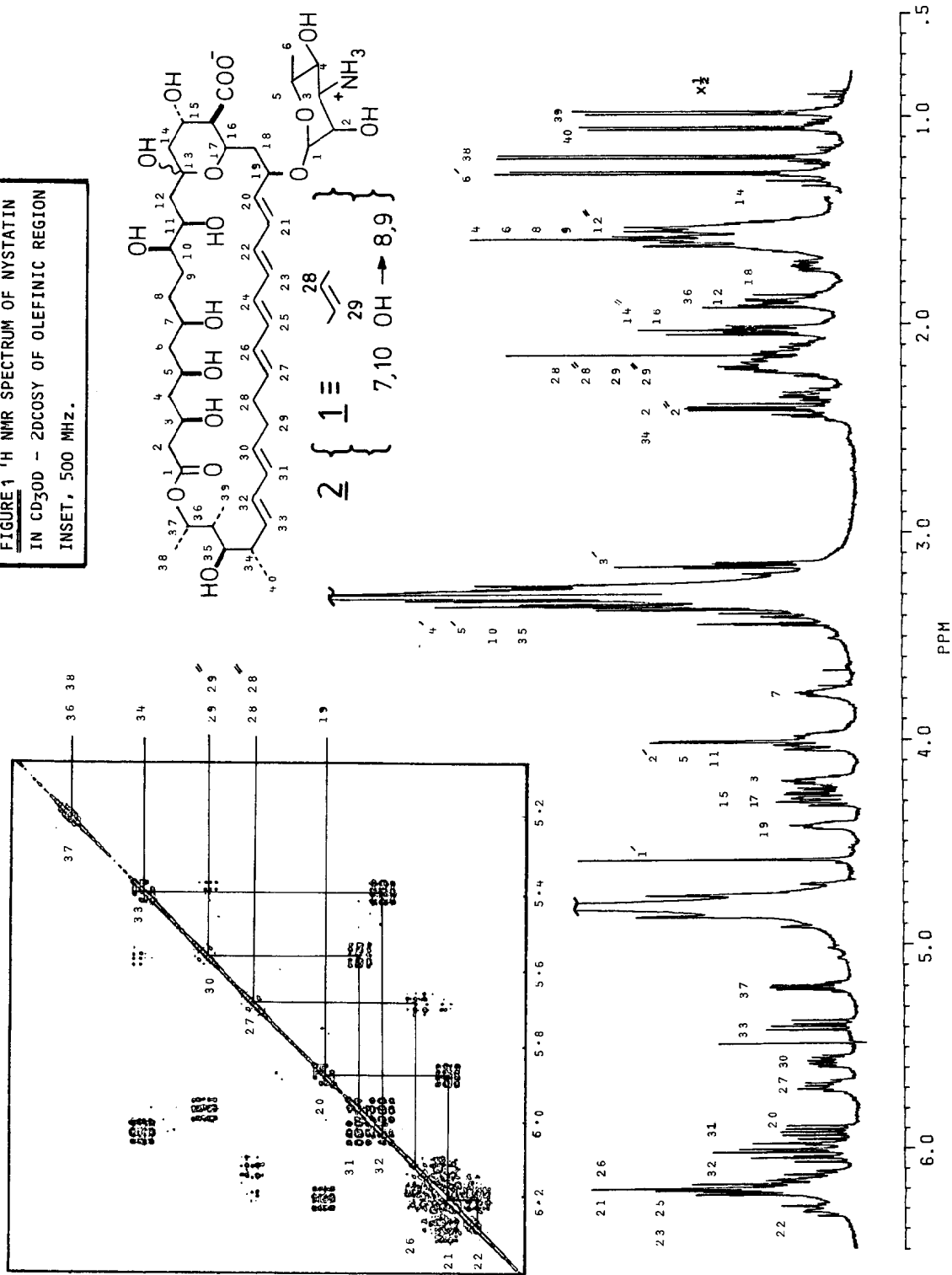
are consistent with weak reversible association between the polyene and alkali-metal salt, decreasing the rotational correlation time of ^{23}Na. Results are recorded in the Table.

	(2)　　NaI	(2)　　NaI	(2)　　NaI	(2)*　　NaI
[mM]	1　　5	1　　2.5	1　　5	10　　2.5
T 1, mS	20	13	9 (0°)	14
control T1	23	23	14 (0°)	21

Table ^{23}Na T1 measurements in CH$_3$OH (*0.07 ml AcOH/ml CH$_3$OH added)

At 500 MHz, the ^{1}H NMR spectrum of nystatin in CD$_3$OD was fully assignable with the aid of a 2D COSY experiment. The limiting line-widths are ca. 1Hz, precluding significant aggregation (Figure 1). By contrast with Amphotericin B[8](1), the olefinic region is much easier to analyse since the saturated linkage C28 – C29 leads to improved dispersion. In preliminary work it was noted that the addition of alkali metal salts selectively shifted some resonances in the olefin and mycosamine regions, with KI and NaI producing similar effects. A quantitative experiment was carried out employing nystatin (1 mg. to 0.5 ml) and NaI (100 mM) in CD$_3$OD. Changes in the resulting chemical shifts of 5 Hz or greater are recorded in Figure 2; overlapping resonances were analysed by means of a further 2D COSY experiment. It is clear that only a rather specific region of the molecule is affected, in proximity to the mycosamine and the related terminus of the olefinic chain. Nystatin exists as a zwitterion in solution and the observed results are consistent with formation of an ion-quartet in which the averaged orientation of polyene and salt gives rise to the observed shieldings and deshieldings. It is interesting to note that the 18 and 18" protons shift in opposite directions indicating that the interaction is face-specific.
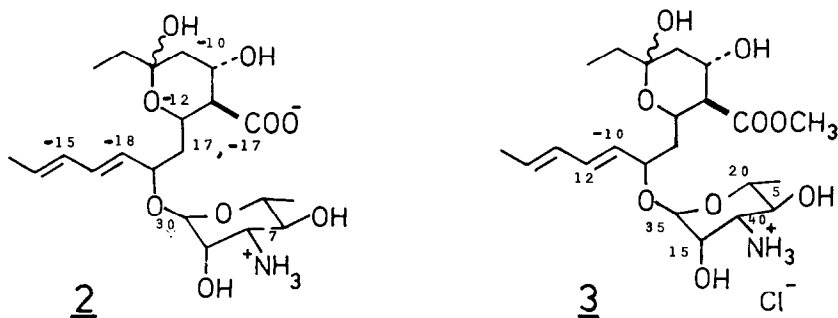

Figure 2 Chemical shift changes (Hz at 500 MHz) following NaI complexation.

Amphotericin B (1) is itself insufficiently soluble in methanol for comparison but the methyl ester hydrochloride (3)[9] is, and the results of similar experiments are recorded in Figure 2. Only the mycosamine resonances are strongly affected in this case, and since nystatin methyl ester behaves similarly it is evident that the change in charge type is responsible, rather than the minor differences in polyene structure. The results give further credence to the existence of polyene: salt complexes.

In the apolar environment of a bilayer membrane interior, the binding of sodium ion or potassium ion may be enhanced, particularly in a sterol-rich hydrophobic region. This will contribute to the efficiency of trans-membrane ion-transport, and provides a model for the process which does not require highly organised aggregation of sterol and polyene.[10]

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