Proton Magnetic Resonance Studies of the Cation-Binding Properties of Nonactin. I. The K^+–Nonactin Complex*

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ABSTRACT: In an effort to elucidate the cation specificity of certain macrocyclic antibiotics in metabolic behavior, particularly in ion transport through membranes, the nature of the binding of K^+ ion to the macrotetrolide nonactin and the molecular structure of the K^+–nonactin complex have been investigated by 220-MHz proton magnetic resonance spectroscopy. Studies were made in dry acetone-d_6 and an acetone-d_6–water mixture containing 0.34 mole fraction of D_2O as a function of varying $KClO_4$ concentrations. Salt-induced chemical shifts were observed for all the nonactin protons except H_2 and H_{21}, and these spectral observations were also accompanied by changes in the vicinal coupling constants between the H_2 and H_3 protons and between the H_5 and H_6, $H_{6'}$ protons. Analysis of the salt-induced shifts yielded apparent formation constants of 7×10^4 and 1.7×10^4 (concentrations expressed in mole fractions) for the K^+–nonactin complex in dry and wet acetone, respectively. The smaller formation constant observed in wet acetone was attributed to the reduced activity of the K^+ ion in the solvent mixture as a consequence of hydration. Since the magnetic parameters deduced for the complex indicated that the identical complex is formed in the two solvent systems, it was concluded that the K^+ ion is bound without its water of hydration, and if hydrated prior to complex formation it must be stripped of its hydration shell before entering the central aperture of the nonactin ring. The observed coupling constant changes were readily interpreted in terms of conformational changes induced in the nonactin ring upon the formation of the complex. These and other possible conformational changes in the nonactin ring upon complex formation, as well as the nature of the coordination of the K^+ ion in the complex, are discussed in the light of the X-ray structure of the crystalline complex recently reported by Kilbourn *et al.* The implications of these findings on the origin of the cation specificity are also discussed.

Certain macrocyclic antibiotics, such as nonactin, enniatin, valinomycin, etc., are known to exhibit a high degree of cation specificity in metabolic behavior (Graven *et al.*, 1966; Pressman *et al.*, 1967). The macrotetrolides, nonactin and monactin, for example, possess highly specific K^+ and Rb^+ transport properties (Mueller and Rudin, 1967). In studies with synthetic membranes, these antibiotics have been shown to enhance the transport of K^+ ion to a significantly greater extent than Na^+ ion, and selectivity constants as high as 750 have been reported (Štefanac and Simon, 1967). Numerous theories have been advanced to account for the action of these cyclic antibiotics (Pressman, 1968; Eisenman *et al.*, 1968), but there is as yet insufficient experimental evidence to precisely define the mechanism of transport enhancement and cation selectivity. In general, the proposed theories ascribe ion transport and selectivity either to the ability of the macrocycle to bind a given ion in its central aperture or to the ability of the macrocycle to pass a given ion through this aperture. In view of these theories, it has become important to answer questions concerning the relative affinity of the antibiotic molecule for various ions, about the effective size of the ion when it is in the bound state, and about the conformation of the macrocycle before and after the formation of the ion complex. Answers to these questions can be obtained from nuclear magnetic resonance studies.

In this paper, we present a preliminary proton magnetic resonance study of the cation-binding properties of nonactin (Figure 1). Nonactin was chosen since this macrotetrolide contains several hydrogens which are in reasonably close proximity to the potential coordination sites in the macrocycle, and it was felt that the proton magnetic resonance spectrum of these hydrogens would then provide a sensitive monitor of the coordination of the cation as well as the conformational changes induced in the macrocycle upon the formation of the complex. The results described in this paper are concerned with the K^+–nonactin complex. In a separate communication, we shall compare the cation-binding properties of nonactin with Na^+, K^+, and Cs^+.

Experimental Section

The nonactin used in this study was generously provided by Dr. B. Stearns of the Squibb Institute for Medical Research, New Brunswick, N. J. The $KClO_4$ was a Mallinckrodt reagent grade chemical. Both were used without further purification. Solutions were prepared by weight in acetone-d_6 or D_2O–acetone-d_6 mixtures. The acetone-d_6 was obtained from Chemi Standards, Inc., New Castle, Del. The D_2O was supplied by Columbia Organic Chemicals, Columbia, S. C. In the K^+-binding studies, the solutions were all 0.0055 *m* in nonactin and were of varying $KClO_4$ concentration.

The proton magnetic resonance spectra of these solutions were recorded at 17° on a Varian HR-220 nuclear magnetic resonance spectrometer, operating at a magnetic field of 51.7

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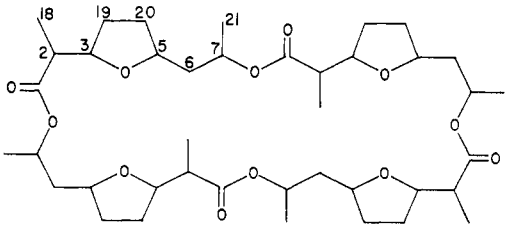


FIGURE 1: Nonactin.

kgauss. This magnetic field was produced by a superconducting solenoid immersed in liquid helium. The advantages of the greater dispersion of chemical shifts possible at the higher magnetic fields as well as the high-grade performance of the spectrometer both in sensitivity (90:1) and in resolution (two parts in 10^9) were clearly evident in this work. Tetramethylsilane was used as an internal standard and chemical shifts were measured relative to this standard by side-band modulation techniques. Where additional signal-to-noise was necessary, a Varian C-1024 time-averaging computer was employed.

Results

Spectral Assignment. The 220-MHz proton magnetic resonance spectrum of nonactin is shown in Figure 2. The numbering system used to identify the various protons in this paper is that depicted in Figure 1. Although the nonactin macrotetrolide consists of four basic subunits, two optical isomers of the subunit are involved and these are arranged alternately in the nonactin ring. Despite this geometrical nonequivalence, there is no *a priori* reason to expect magnetic nonequivalence of the protons attached to analogous carbon atoms of the two optically related subunits. In fact, if the backbone of the macrocycle assumes an over-all configuration which possesses an S_4 symmetry axis, as it nearly does in the K^+–nonactin crystalline complex (Kilbourn *et al.*, 1967), the magnetic environments of the two sets of protons of the two types of subunits would be identical. We observe this magnetic equivalence in all of our proton magnetic resonance spectra, and hence in the following discussion we shall merely refer to the protons in the first subunit.

The assignment of the spectrum in Figure 2 is based on correlation of intensities, chemical shifts, and coupling constants with the chemical environment of each proton. It was later confirmed by double-irradiation experiments. The H_{18} and

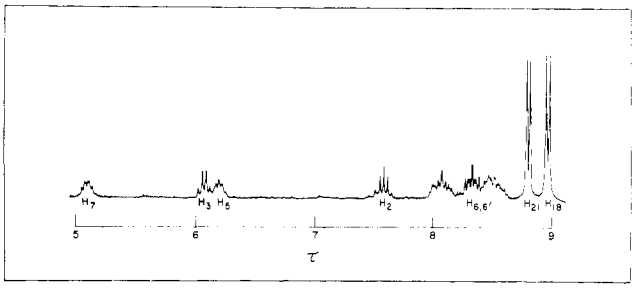


FIGURE 2: The 220-MHz proton magnetic resonance spectrum of nonactin in CCl_4. Nonactin concentration: 0.026 *m;* temperature: 17°.

TABLE I: Observed Proton Chemical Shifts and Coupling Constants of Nonactin in Acetone-d_6.

Chemical Shifts[a] (τ)	Coupling Constants[b] (Hz)
H_7 5.039	$J_{H_7,H_{21}} = 6.4 \pm 0.2$
H_3 6.013	$^1/_2(J_{H_7,H_6} + J_{H_7,H_6'}) = 6.4 \pm 0.4$
H_5 6.129	$J_{H_3,H_2} = 7.6 \pm 0.4$
H_2 7.513	$^1/_2(J_{H_3,H_{19}} + J_{H_3,H_{19}'}) = 7.0 \pm 0.4$
H_{21} 8.785	$J_{H_2,H_{18}} = 7.1 \pm 0.2$
H_{18} 8.946	$^1/_2(J_{H_5,H_6} + J_{H_5,H_6'}) = 6.2 \pm 0.4$
H_6, $H_{6'}$ 8.260	$^1/_2(J_{H_5,H_{20}} + J_{H_5,H_{20}'}) = 6.8 \pm 0.4$
	$J_{H_6,H_{6'}} = 11.7 \pm 0.4$

[a] ± 0.005. [b] Absolute values.

H_{21} methyl resonances are readily identified by their intensities, their positions at high field, and their coupling to a single proton. The three groups of resonances at low field are expected to arise from the H_7, H_3, and H_5 protons, these protons being adjacent to ether oxygens (Pople *et al.*, 1959). Irradiation of the spin multiplet furthest downfield was found to collapse the downfield methyl doublet to a singlet. These resonances must therefore be assigned to H_7 and H_{21}, since neither H_3 nor H_5 is expected to be coupled to a methyl proton. Irradiation of the remaining methyl doublet (H_{18}) was shown to collapse the quintet at τ 7.58 to a doublet, thus assigning the H_2 multiplet. Similarly, irradiation of the H_2 spin multiplet collapses the quartet at τ 6.08 to a triplet, identifying the H_3 multiplet, since the H_2 and H_3 protons are expected to be coupled. By the process of elimination, we therefore assign the multiplet centered at τ 6.20 to H_5 and the remaining resonances in the spectral region between τ 8.64 and 7.96 to the various methylene hydrogens. On the basis of similar double-irradiation experiments, the methylene multiplet centered at τ 8.33 can be assigned to the H_6, $H_{6'}$ protons with reasonable certainty. This tentative assignment was later verified by computer simulation of the methylene H_6, $H_{6'}$ spin multiplet.

The chemical shifts of the various protons and the coupling constants deduced from analysis of the various spin multiplets are summarized in Table I. These results do not include data for the methylene protons of the tetrahydrofuran rings, since the spin multiplets corresponding to these protons were not analyzed in detail.

Ion-Binding Studies. The addition of $KClO_4$ to a dilute solution of nonactin (3×10^{-4} mole fraction) in acetone containing a minimal amount of water ($<7 \times 10^{-3}$ mole fraction) results in downfield shifts of all the nonactin resonances except those of H_2 and H_{21}. The salt-induced shifts observed for the H_7, H_3, H_5, H_{18}, H_{21}, and H_2 protons are presented as a function of salt concentration in Figure 3a. It is noted that the induced shifts are quite abrupt, being essentially complete at a salt concentration of 5×10^{-4} mole fraction. The largest shifts are observed for the H_7 and H_3 protons with limiting shifts of 115 and 105 Hz, respectively.[1] Somewhat smaller shifts are observed for the H_5, H_6, H_{19}, H_{20}, and H_{18} protons. The limiting shifts for the H_5 and H_{18} protons are 56 and 20

[1] Method of determining the limiting shifts is given in the Discussion.

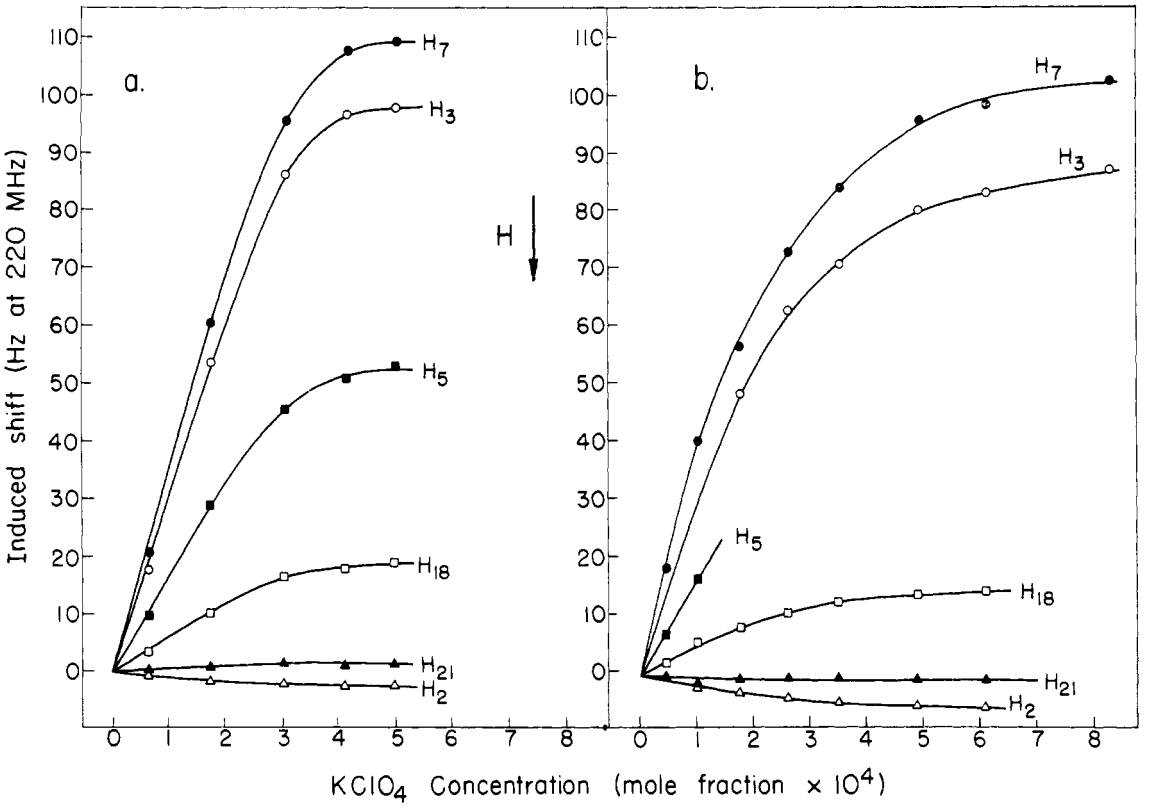


FIGURE 3: Salt-induced shifts of the nonactin H_7, H_3, H_5, H_2, H_{18}, and H_{21} protons as a function of $KClO_4$ concentration observed in (a) dry acetone-d_6 containing minimal water ($<7 \times 10^{-3}$ mole fraction), and (b) wet acetone-d_6 containing 0.34 mole fraction of D_2O. Nonactin concentration: 3.0×10^{-4} mole fraction; temperature: 17°.

Hz, respectively, and although the remaining methylene resonances could not be analyzed in detail at all levels of salt concentration, none were shifted more than 50 Hz downfield. The H_2 and H_{21} resonances are not noticeably shifted upon the addition of salt. Insofar as we were able to ascertain, the two sets of protons corresponding to the two optically isomeric subunits remain magnetically equivalent over the entire range of salt concentrations investigated.

The results of a similar set of experiments for a solution of nonactin in acetone containing 0.339 mole fraction of D_2O are illustrated in Figure 3b. Here again, large downfield shifts of the H_7 and H_3 resonances are observed upon the addition of salt. The limiting shifts observed for these protons as well as for the remaining nonactin protons are comparable with those observed in essentially dry acetone. For the H_7, H_3, and H_{18} protons, for example, the limiting shifts are, respectively, 113, 96, and 18 Hz. Due to interference by the HDO resonance, the H_5 resonance cannot be observed over the entire salt concentration range; however, on the basis of the limited data, it also appears to have undergone a similar downfield shift in the two solvent systems. In contrast to the rather abrupt shifts observed in dry acetone, the variation of the salt-induced shifts with salt concentration observed in wet acetone is somewhat more gradual, approaching their limiting values only when a salt concentration of 8×10^{-4} mole fraction or more is attained. Again, there is no evidence for any salt-induced magnetic nonequivalence between the two sets of protons corresponding to the two optical isomers making up the four subunits of the nonactin molecule.

The salt-induced shifts observed upon the addition of K^+ are also accompanied by other spectral changes. The vicinal coupling constant $|J_{H_2,H_3}|$, for example, increases from 7.6 ± 0.4 to 9.3 ± 0.4 Hz upon saturation of the system with $KClO_4$, and this coupling constant change is essentially the same whether the solvent system is dry or wet acetone. The patterns of the H_7 and H_5 spin multiplets also vary with salt concentration (Figures 4 and 5), and these spectral changes are due either to a change in magnetic nonequivalence of the two methylene H_6 hydrogens to which both H_5 and H_7 are coupled, or to changes in the spin–spin coupling between these hydrogens (J_{H_5,H_6}, $J_{H_5,H_6'}$, J_{H_7,H_6}, and $J_{H_7,H_6'}$).

The observed spectral changes in the H_7 multiplet most likely arise from the increased magnetic nonequivalence of the methylene H_6, H_6' hydrogens, since the over-all width of this spin multiplet remains unchanged over the entire range of salt concentrations investigated (Figure 4). The H_7 multiplet constitutes the X spectrum of an ABP_3X system,[2] where A, B refer to the nearly chemical shift equivalent H_6,H_6' protons, and P denotes the three magnetically equivalent H_{21} methyl protons. Its over-all width is expected to be given by $3|J_{H_7,H_{21}}| + |J_{H_7,H_6} + J_{H_7,H_6'}|$. Since $J_{H_7,H_{21}}$ is not altered by complex formation, $|J_{H_7,H_6} + J_{H_7,H_6'}|$ must also remain unchanged. The details of the H_7 multiplet also depend upon the quantities (Emsley *et al.*, 1965): $D_\pm = {}^1/_2\{[\delta_{AB} \pm {}^1/_2(J_{AX} - J_{BX})]^2 + J_{AB}{}^2\}^{1/2}$, specifically $|D_+ - D_-|$, where δ_{AB} is the chemical

[2] For a description of the nomenclature used to characterize nuclear spin systems, see Emsley *et al.* (1965).

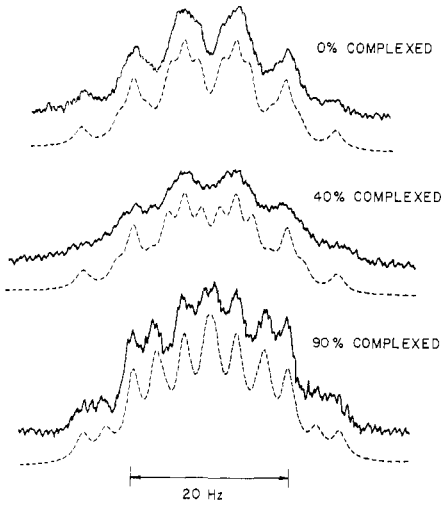


FIGURE 4: Comparison of the observed and calculated spectral the H_7 multiplet upon complexation.

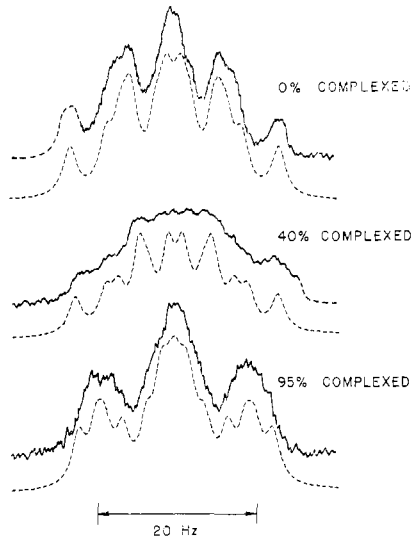


FIGURE 5: Comparison of the observed and calculated spectral changes in the H_5 multiplet upon complexation.

shift difference between the methylene H_6, H_6' protons in Hz. As the geminal spin–spin coupling constant $|J_{H_6,H_6'}|$ is not expected to be altered by the formation of the complex, the observed spectral changes in the H_7 multiplet must be due to changes in $(J_{H_7,H_6} - J_{H_7,H_6'})$ and/or δ_{AB}. In view of the constancy of $(J_{H_7,H_6} + J_{H_7,H_6'})$, changes in the magnetic nonequivalence between the H_6, H_6' protons appear to be more likely.

The total width of the H_5 multiplet, on the other hand, decreases somewhat with the addition of salt. The H_5 multiplet is best characterized as the X spectrum of an ABPQX system,[2] arising from coupling of the H_5 proton to the H_6, H_6' methylene protons (A, B) and to the H_{20}, $H_{20'}$ tetrahydrofuran ring protons (P, Q). Theoretically, its over-all width should be given by $|J_{H_5,H_6} + J_{H_5,H_6'}| + |J_{H_5,H_{20}} + J_{H_5,H_{20'}}|$. Since conformational changes in the tetrahydrofuran ring are rather restricted, we suspect that the observed decrease in the over-all width of the H_5 multiplet has its origin in a small reduction in $|J_{H_5,H_6} + J_{H_5,H_6'}|$ from about 12.4 to 11.0 Hz upon the binding of the cation. If this is the case, the spectral changes observed for the H_5 multiplet upon the addition of salt to the solution reflect both a change in magnetic nonequivalence of the methylene H_6,H_6' protons and a change in the spin–spin coupling between H_5 and the individual H_6, H_6' protons in the complex.

The above interpretation of the observed spectral changes in the H_5 and H_7 multiplets has been in general confirmed by computer simulation of the methylene H_6, H_6' spectral region and curve fitting of the H_5 and H_7 multiplets. For the uncomplexed nonactin molecule, the following parameters were found to reproduce the methylene H_6, H_6' region quite well.

$$|\delta_{AB}| = 19 \text{ Hz}$$

$$|J_{H_6,H_6'}| = 11.7 \text{ Hz}$$

$$|J_{H_7,H_6} + J_{H_7,H_6'}| = 12.8 \text{ Hz}$$

$$|J_{H_7,H_6} - J_{H_7,H_6'}| = 4.8 \text{ Hz}$$

$$|J_{H_5,H_6} + J_{H_5,H_6'}| = 12.4 \text{ Hz}$$

$$|J_{H_5,H_6} - J_{H_5,H_6'}| = 4.0 \text{ Hz}$$

In the complex, curve fitting of the H_5 multiplet indicates that $|J_{H_5,H_6} + J_{H_5,H_6'}|$ has indeed decreased by $\sim$1.4 Hz, while curve fitting of the H_7 multiplet confirms that only the value of $|D_+ - D_-|$ for the ABX part of this multiplet has increased, although it has increased somewhat beyond what one would expect on the basis of an increase in $|\delta_{AB}|$ alone. In Figures 4 and 5 we have reproduced calculated spectra illustrating the effect of the above changes on the H_7 and H_5 multiplets. The agreement between calculated and observed spectra can be seen to be satisfactory in every case.

As expected, the vicinal coupling constants $|J_{H_2,H_{18}}|$ and $|J_{H_7,H_{21}}|$ remain virtually unchanged over the range of salt concentrations studied.

Discussion

Determination of Ion-Binding Constants. In acetone solution, the K^+ ions exchange rapidly between the solvent and the nonactin cavity,[3] and as a result the salt-induced shifts, summarized in Figure 3, reflect the distribution of nonactin molecules between the free and complexed states. The data can therefore be interpreted in terms of the following simple equilibrium and can be used to determine the affinity of the potassium ion for the central aperture of the nonactin ring (eq 1).

$$\text{nonactin} + K^+ \rightleftharpoons \text{complex} \qquad (1)$$

Under conditions of rapid chemical exchange, the salt-induced shifts observed for a given proton represent a weighted average of the chemical shifts in the free and complexed environments, and it is readily shown that

$$\delta = \frac{1}{2}\delta_c\{(1 + \phi + \eta) - [(1 + \phi + \eta)^2 - 4\phi]^{1/2}\} \qquad (2)$$

[3] Slow chemical exchange has recently been reported for the K^+–valinomycin system in $CHCl_3$ by Haynes *et al.* (1969).

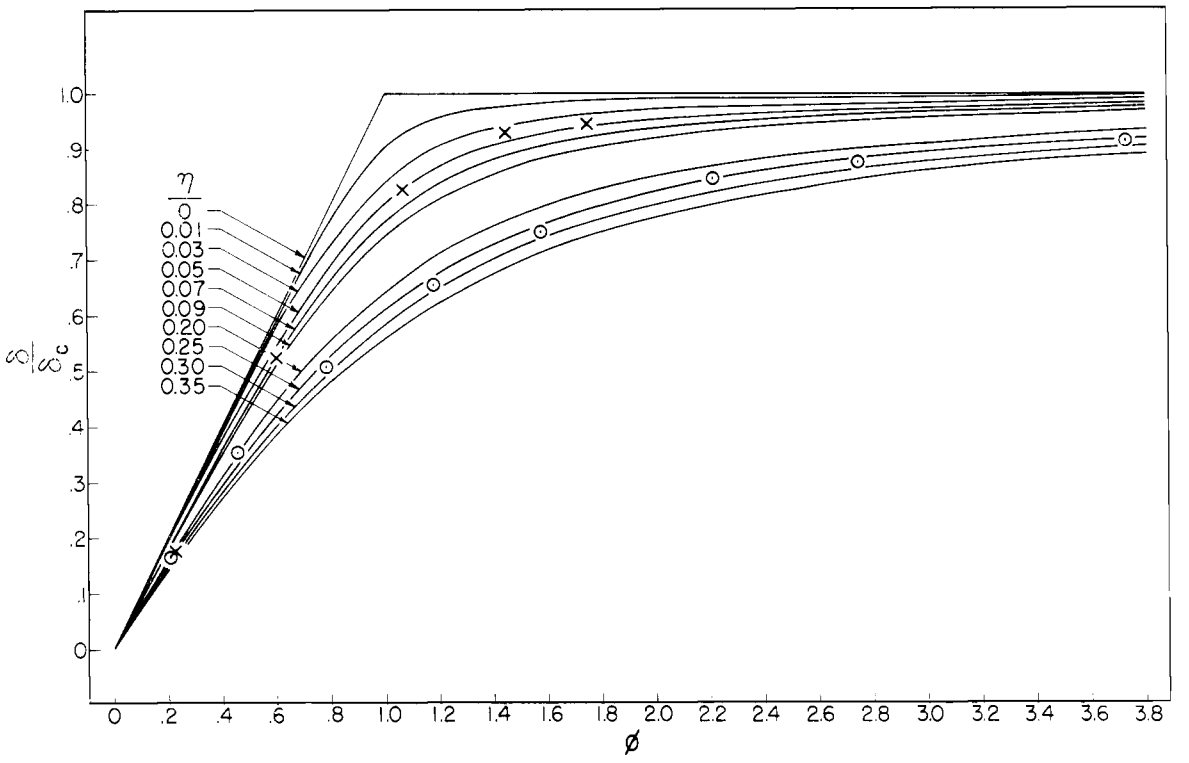


FIGURE 6: Theoretical curves of δ/δ_c *vs.* ϕ for various values of η, and fitting of the salt-induced shifts observed for the nonactin H_7 proton in dry acetone (×) and wet acetone (⊙).

where δ is the observed salt-induced chemical shift, δ_c is the chemical shift of the proton in the complexed state relative to that in the uncomplexed environment, ϕ is the stoichiometric concentration ratio of K^+ to nonactin, and η is the reciprocal of the product of the apparent formation constant K and the stoichiometric nonactin concentration. Since the stoichiometric concentration of nonactin is fixed in our experiments (changes in the mole fraction of nonactin upon the addition of $KClO_4$ are negligible owing to the low salt concentration), and the apparent formation constant K is not expected to vary significantly over the range of salt concentration investigated, η may be taken to be a constant in our treatment of the data. Equation 2 then suggests a graphical procedure for the determination of K. In Figure 6 we have plotted theoretical curves of δ/δ_c *vs.* ϕ for various values of η. This family of curves can be compared with the experimentally observed variations of δ/δ_c with ϕ, and in this manner K can be obtained. Since values of the limiting shifts can only be determined to within 10–20% visually, further refinement of the δ_c's and K's was made by least-square fitting of the data to the theoretical expression.

The salt-induced shifts observed for the nonactin H_7 proton on the binding of K^+ to nonactin in both dry acetone and the acetone–D_2O mixture are plotted *vs.* ϕ in Figure 6. Comparison of the data with the theoretical curves yields the apparent ion-binding constants of $(7 \pm 2) \times 10^4$ and $(1.7 \pm 0.2) \times 10^4$ (concentrations expressed in mole fractions) for dry and wet acetone, respectively. Examination of Figure 6 indicates that the binding of K^+ to nonactin in dry acetone is almost stoichiometrically complete, and hence the extent to which nonactin molecules are complexed, as expressed by δ/δ_c, is not particularly sensitive to the binding constant. In wet acetone, on the other hand, the smaller binding constant can be determined with considerably higher precision. Thus, in addition to facilitating the determination of K, the method of data treatment outlined above provides some visual assessment of the accuracy of the binding constant determined.

The State of the K^+ Ion in the Complex. Considerations of the relative affinities of nonactin for K^+ ion in the two solvent systems investigated and the magnetic parameters deduced from the proton magnetic resonance spectra for the complex in both cases provide some insight into the nature of the complex formed.

We first comment on the binding constants obtained in the two solvent systems studied. In this laboratory, R. T. Iwamasa, H. Lütje, and S. I. Chan (unpublished results) have recently shown that a monomeric water molecule in acetone binds to sodium ion with a binding constant of about 40 (in mole fraction units). A similar binding constant has not been determined for K^+, but one would expect it to be lower. Thus in dry acetone, with less than 7×10^{-3} mole fraction water present, no more than 20% of the potassium ions would be associated with a water molecule in the solvent, and the measured apparent K of 7×10^4 should very nearly represent the binding constant of potassium ion to nonactin in anhydrous acetone. In wet acetone containing 0.34 mole fraction of D_2O, an appreciable percentage, perhaps as much as 90% of the K^+ ion in solution would be expected to be hydrated to some extent. Thus, the smaller apparent formation constant for the complex obtained in this solvent system either reflects the reduced activity of the K^+ ion as a result of more favorable solvation of the ion in the solvent, or else indicates that the nonactin ring

can incorporate a hydrated K^+ ion, but has less tendency to do so. It is of course not possible to distinguish between these two possibilities on the basis of the binding constant data alone.

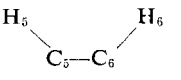
The magnetic parameters deduced from the proton magnetic resonance spectra for the K^+–nonactin complex, however, provide evidence that only the unhydrated ion is bound in the central aperture of the nonactin ring. This is true for complexes formed in both solvent systems. First, the limiting chemical shifts of all the nonactin protons monitored are the same (within 10%) in both acetone and the acetone–D_2O mixture. Since these proton chemical shifts characterize the magnetic environment of the various protons, which in turn is sensitive to the interactions between the cation and the nonactin ring, one can conclude that the complexes formed in the two solutions have identical magnetic and electronic environments for their corresponding protons. Secondly, the vicinal coupling constants $|J_{H_2,H_3}|$, $|J_{H_5,H_6} + J_{H_5,H_6'}|$, and $|J_{H_7,H_8} + J_{H_7,H_6'}|$, which are extremely sensitive to the conformation of the nonactin ring, are similar for the limiting complexes formed in the two solvent systems. Since it is inconceivable that the central aperture of the nonactin ring could accommodate the large hydrated potassium ion without grossly distorting the ring conformation from that observed for the complex containing only the unhydrated ion, and without modifying in the process the aforementioned vicinal coupling constants as well as the magnetic environment of the H_3, H_5, and H_7 nonactin protons, we conclude that only the unhydrated potassium ion is bound in the central aperture of the nonactin ring and that the same complex is formed in the two solvent systems.

Nature of the Coordination of the Potassium Ion and Conformation of the Nonactin Ring in the Complex. On the basis of the spectral changes observed upon complex formation, it is possible to draw certain conclusions regarding the nature of the coordination of the potassium ion and the conformation of the nonactin ring in the complex.

In the absence of any noticeable salt-induced magnetic nonequivalence between similar protons on the four subunits, one must conclude that the potassium ion is coordinated symmetrically to the four subunits of the nonactin molecule. Upon examination of a molecular model of the nonactin molecule constructed using CPK atomic models and the crystal structure data of Kilbourn *et al.* (1967), we note that the protons whose resonances are shifted to the greatest extent upon complex formation, namely, H_7 and H_3, are those which are geometrically in close proximity to one of the four centrally directed carbonyl groups, each located at the corners of an approximate tetrahedron enclosing the central cavity of the nonactin ring. This observation would suggest tetrahedral coordination of the potassium ion to the four carbonyl oxygens. The H_5 proton also experiences a fairly large downfield shift upon complex formation. Since this proton is not in close proximity to a carbonyl group, but is instead adjacent to the ether oxygen of its tetrahydrofuran ring, one might infer from the sizeable H_5 shifts some participation of the ether oxygens in the coordination of the K^+ ion. Approximate eightfold cubic coordination has been indicated by the crystallographic studies of Kilbourn *et al.* (1967), and in the Kilbourn structure, these ether oxygens occupy the remaining corners of the cube which encompass the tetrahedron of carbonyl oxygens, at very similar K^+–O distances. This cursory interpretation of the proton magnetic resonance data, without due consideration of contributions arising from direct electrostatic polarization of the various C–H bonds by the bound positively charged cation, is, however, misleading. The ion-induced polarization shifts can be estimated using the standard formula for electric field effects (Buckingham, 1960), and a calculation based upon the geometry of the crystalline complex shows that direct polarization of the C_5–H bond by the central ion can adequately account for the salt-induced shifts observed for the H_5 proton without the necessity of invoking any indirect effects resulting from possible coordination of the K^+ ion to the ether oxygens. The calculated polarization shifts for the H_7 and H_3 protons, on the other hand, are not large enough to account for the salt-induced shifts observed for these protons. In the case of H_7, for example, ion-induced polarization accounts for a mere 10% of the observed limiting shift. Thus, unless the geometry of the complex assumed in these calculations is grossly in error, we are forced to conclude that complexation of the K^+ ion results in some modification of the electronic distribution of the carbonyl groups and/or is accompanied by a conformational change about the ester linkages which brings the carbonyl oxygens in close proximity to these protons. We take this as positive evidence for the participation of the carbonyl groups in the coordination of nonactin to the centrally bound potassium ion. On the basis of our proton magnetic resonance results, it is not possible to ascertain the extent of involvement of the ether oxygens in the coordination of the K^+ ion. Therefore, at present we would prefer to describe the electronic structure of the K^+–nonactin complex in terms of coordination by two interlocking sets of unequally coordinated tetrahedra of oxygens.

Of the remaining nonactin protons, direct electrostatic polarization of the respective C–H bonds by the centrally bound ion can be shown to produce shifts of the proper magnitude and direction, except in the case of H_2. Here the calculated polarization shift is some 50 Hz downfield, whereas the observed salt-induced shift is small and upfield. Since the H_2 proton is adjacent to both the ether oxygen of the tetrahydrofuran ring and the carbonyl oxygen of the ester linkage, it is possible that the small shifts here reflect also a compensating contribution originating from conformational changes in this part of the nonactin ring. In fact, there is evidence for a salt-induced conformation change about the C_2–C_3 bond which could easily decrease the net deshielding effect of the ether oxygen on the H_2 proton. These conformation changes will be discussed next.

On the basis of the limited coupling constant data which we have obtained, it is of course not possible to deduce any detailed structural information on the conformation of the nonactin ring either before or after complex formation. However, certain conformational changes are apparent upon the binding of the K^+ ion. The increase in $|J_{H_2,H_3}|$ from 7.6 to 9.3 Hz, for instance, indicates a change in the dihedral angle between the C_2–H and C_3–H bonds. According to theoretical estimates of vicinal coupling constants (Karplus, 1959), the value of 9.3 Hz observed for the complex would correspond to a dihedral angle near 180°. This is approximately the angle predicted from the crystal structure data of Kilbourn *et al.* (1967). A departure from 180° to a dihedral angle of 155° would account for the coupling constant of 7.6 Hz observed for the free nonactin molecule. Likewise, the apparent decrease in $|J_{H_5,H_6} + J_{H_5,H_6'}|$ from 12.4 to 11.0 Hz indicates a small increase in the



dihedral angle, of perhaps 10 or 15°. Manipulation of a molecular model of the nonactin ring indicates that the above change in dihedral angles would produce a change in the size of the central cavity without requiring further conformational changes in the remaining parts of the nonactin ring. A large conformational change, involving rotations about the C_6–C_7 single bond, seems unlikely since the sum of the vicinal coupling constants between the H_7 and H_6 protons remains unaltered upon formation of the complex. Unfortunately, on the basis of the present preliminary proton magnetic resonance study, it is not possible to rule out conformational changes about the ester linkages, and hence we cannot ascertain whether the nonactin ring expands or contracts when the K^+ ion enters the central aperture. There is little question, however, that the backbone of the nonactin ring is quite flexible, and therefore we suspect that the central aperture of nonactin can readily accommodate cations of various sizes within a reasonable range of ionic radii.

On the whole, the proton magnetic resonance results which we have obtained in this study are consistent with the structure of the complex deduced from the X-ray data by Kilbourn *et al.* (1967) who described the conformation of the 32-membered nonactin ring in the complex as resembling the seam of a tennis ball, with the carbonyl and ether oxygens directed at the K^+ ion in the center and with the methyl substituents and methylene groups of the tetrahydrofuran rings on the outside. Our proton magnetic resonance data indicate that on the average the ring maintains S_4 symmetry. The crystal structure does indicate approximate S_4 symmetry, but minor differences in the conformations of the optically related subunits were observed. The apparent higher symmetry observed in solution may reflect either the inability of our method to resolve these minor structural differences or the result of rapid averaging over the long proton magnetic resonance time scale of observation. It is also possible that the minor structural differences are inherent in the crystalline complex as a result of distortions by crystal forces not present in solution.

Conclusions

The present proton magnetic resonance study of the potassium–nonactin complex in acetone solution and in an acetone–water mixture indicates that nonactin has a very high affinity for K^+ ion, that the K^+ ion is bound without its water of hydration even when it is hydrated prior to entering the central aperture of the nonactin ring, and that the conformation of the nonactin ring in the complex is somewhat different from that of the free molecule. Insofar as we can ascertain, the spectral results obtained for the complex are consistent with the X-ray structure of the crystalline complex recently reported by Kilbourn *et al.* (1967).

If ion specificity does indeed arise from the tendency of a cation to complex with nonactin, the above conclusions may have rather important implications concerning the origin of the specificity. Since the nonactin is quite flexible, it seems unlikely that the size of the aperture would be the controlling factor in discriminating between various cations of comparable sizes. On the other hand, the fact that the ion must be stripped of its water of hydration before formation of the complex suggests that hydration energy of the cation may contribute to the cation specificity observed in metabolic behavior. Experiments are presently under way to test these suggestions.

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