flask cooled with liquid nitrogen. This flask was then thawed, refrozen with Dry Ice, and the CO_2 distilled into a third vessel. The thawing and freezing were repeated and the CO_2 was transferred to a sample tube for mass-spectrometric analysis.

Isotopic analyses were conducted on a Nuclide Associates RMS 6-60 isotope-ratio mass spectrometer equipped with a double inlet system. Isotope ratios *m/e* 45:44 were measured alternately for the sample and the tank standard, at least six such cycles being used for calculation of the isotope ratio. All measurements were made at approximately the same pressure. In a particular determination the isotope ratio never varied by more than ±0.000002.

Acknowledgment. We are grateful to Mr. Gary Parr for the isotope ratio measurements, to Professor W. W. Cleland for several helpful discussions, and to Professor John Garver for supervising the growth of the bacteria.

Proton Magnetic Resonance Studies of the Cation-Binding Properties of Nonactin. II. Comparison of the Sodium Ion, Potassium Ion, and Cesium Ion Complexes[1]

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Received December 13, 1969

Abstract: A comparison of the ion-binding properties of the macrocyclic antibiotic nonactin to Na^+, K^+, and Cs^+ has been made. Pmr spectroscopy (220 MHz) has been used to study the complexation of nonactin to these alkali ions in anhydrous acetone-d_6 and acetone-d_6–water mixtures containing as much as 0.5 mol fraction of water. Complex formation constants of 7×10^4, 7×10^4, and 1×10^4 are obtained for the Na^+, K^+, and Cs^+ complexes, respectively, in dry acetone; in wet acetone, the respective binding constants are 210, 2×10^4, and 400. Thus, all three ions bind to nonactin with nearly equal affinity in dry acetone, but the binding constants are drastically reduced when the solvent system is altered by the addition of appreciable amounts of water. It is significant that the reduction is far greater for Na^+ and Cs^+ than for K^+ itself, making the binding of K^+ to nonactin highly favored in the more aqueous medium. It is shown that in wet acetone, the alkali ion must be stripped of its hydration shell prior to its accommodation in the nonactin cavity, and hence we surmise that hydration of the various ions in wet acetone must contribute significantly to the ion selective behavior of nonactin in the more aqueous media. It is felt that these results are pertinent to the selective potassium ion transport induced by nonactin in experimental lipid bilayers. Analysis of the pmr data also indicates that the nonactin ring undergoes sizable conformation changes on incorporation of these alkali ions. The extent of this conformational change is slightly different depending on the ion, but on the whole, the three complexes studied appear to exhibit quite similar structures.

Nonactin, the macrocyclic antibiotic depicted in Figure 1, has been shown to be influential in the regulation of metabolic behavior and is thought to act by selectively enhancing the transport of potassium ion through cell membranes.[4,5] Because of its structural simplicity and its potential importance as a lipid soluble ion carrier, it has become the object of a number of recent biophysical and biochemical studies, all aimed at obtaining an understanding, at the molecular level, of the action of this physiologically active agent.[6,7]

With this same objective in mind, we recently reported a proton magnetic resonance (pmr) study of the potassium ion binding properties of nonactin in dry acetone and in acetone–water mixtures.[8] Pmr spectroscopy has proven useful in the investigation of the conformational properties, and cation-binding properties of macrocyclic antibiotics, not only in this study but in studies of other ionophores as well. Several groups of workers, for example, have applied pmr spectroscopy to the study of valinomycin, a depsipeptide which exhibits an ion selectivity in metabolic behavior and membrane permeability similar to that observed for nonactin.[9-11]

In our recent study of nonactin the most significant among the results obtained were the conformational changes observed for the nonactin ring upon ion complexation and the implication of the solvent (H_2O) as an important factor in the origin of the ion selectivity of this antibiotic. The pmr data for the K^+–nonactin complex were shown to be in general consistent with the molecular structure determined earlier by crystallographic studies,[12] but in addition, comparison of the

(1) This work was supported in part by Grant No. GM 14523-02 and -03 from the National Institute of General Medical Sciences, U. S. Public Health Service, and Grant No. GP-8540 from the National Science Foundation.
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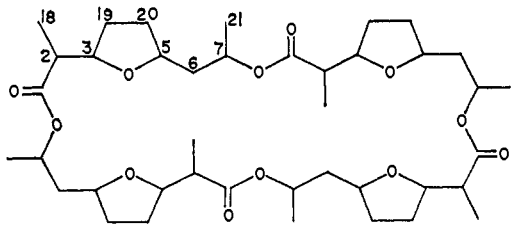

Figure 1. Nonactin.

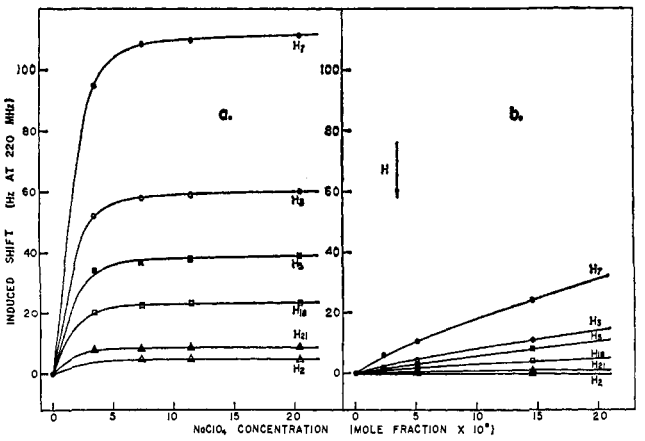

Figure 2. Effect of $NaClO_4$ on the chemical shifts of the nonactin H_7, H_8, H_5, H_2, H_{18}, and H_{21} protons in (a) dry acetone-d_6 containing minimal water (1×10^{-3} mol fraction), and (b) wet acetone-d_6 containing 0.39 mol fraction of D_2O. Nonactin concentration is 3.1×10^{-4} mol fraction; temperature, 17°.

results for the complex with those for the free molecule indicated that the nonactin ring system is relatively flexible and possibly capable of binding ions within a reasonable range of ionic radii. The complex formation constant of the potassium complex was shown to decrease markedly from its value in anhydrous acetone when the ion was hydrated prior to entering the nonactin aperture, implying that ion hydration, rather than any steric or electronic property of the nonactin cavity itself, may be the predominant factor in determining the ion selectivity in aqueous media.

As further confirmation of these ideas, we have extended similar measurements to the Na^+ and Cs^+ complexes and in this paper we wish to compare the properties of these complexes with the results which we have previously reported for the K^+ complex. Because of the different sizes of the ions involved (Na^+, 0.98; K^+, 1.33; Cs^+, 1.67 Å), these studies can provide some insight into the range of allowable nonactin conformations as well as the intrinsic affinities of the nonactin ring for the various ions in their anhydrous or hydrated states.

Experimental Section

The pmr spectrum of nonactin was studied as a function of Cs^+ and Na^+ perchlorate concentration in both anhydrous and wet acetone solutions. The solutions were in general 0.005 *M* in nonactin and of varying salt concentration. The nonactin was a generous gift from Dr. B. Stearns of the Squibb Institute for Medical Research, New Brunswick, N. J. The Na^+ and Cs^+ salts were obtained from the G. Frederick Smith Chemical Co., Columbus, Ohio, and Research Inorganic Chemicals, Sun Valley, Calif., respectively. The acetone-d_6 was supplied by Dia-Prep, Atlanta, Ga. For the studies in anhydrous solutions, special precautions

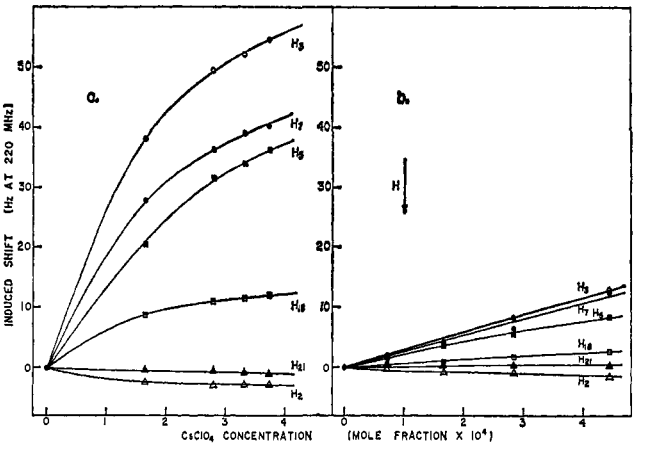

Figure 3. Effect of $CsClO_4$ on the chemical shifts of the nonactin H_7, H_8, H_5, H_2, H_{18}, and H_{21} protons in (a) dry acetone-d_6 containing minimal water (3×10^{-3} mol fraction), and (b) wet acetone-d_6 containing 0.55 mol fraction of D_2O. Nonactin concentration is 3.7×10^{-4} mol fraction; temperature: 17°.

were taken to exclude water from the system: the salts were dried under high vacuum at elevated temperatures, and the acetone-d_6 was distilled from anhydrous $CaSO_4$[13] and sealed under vacuum. Subsequently, solutions were prepared by weight in a water-free atmosphere. After preparation, residual water as detected by pmr spectroscopy was found to be less than 3×10^{-3} mol fraction. For the studies in wet acetone solutions, sufficient D_2O (Columbia Organic Chemicals, Columbia, S. C.) was added to make up solutions 0.39 to 0.55 mol fraction in water. The pmr spectra of these solutions were run at 17° on a Varian HR-220 spectrometer and a C1024 time-average computer was used to enhance the signal to noise. Chemical shifts were measured relative to a TMS internal standard to 0.2 Hz by standard side band modulation techniques. The shifts reported in this paper are given in hertz at a magnetic field of 51.7 kG.

Results

As in the case of the potassium–nonactin studies, the addition of $NaClO_4$ and $CsClO_4$ to acetone solutions of nonactin results in changes in the chemical shifts of many of the nonactin protons. Several coupling constants between these protons are also modified. These changes in themselves give insight into the conformational changes in the macrocyclic ring upon the incorporation of the alkali metal ion as well as the nature of the ion coordination. A detailed study of the chemical shift changes as a function of salt concentration and solvent system provides a convenient method for following the relative affinities of the nonactin aperture for various ions under a variety of experimental conditions. Details for the methods of analysis of the pmr data as well as the determination of binding constants have been described in our previous paper.

Chemical Shifts. The salt-induced shifts observed upon the addition of $NaClO_4$ and $CsClO_4$ to acetone solutions of nonactin are summarized in Figures 2 and 3. The results for dry acetone are shown in Figures 2a and 3a, and those for wet acetone in Figures 2b and 3b. As in the case of the K^+–nonactin complex, the most pronounced shifts were observed for the H_3, H_5, and H_7 protons. In the K^+–complex, we noted that these protons are in close proximity either to one of

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Table I. Chemical Shifts of the Nonactin Protons in the Na^+, K^+, and Cs^+ Complexes[a]

	H_7	H_3	H_5	H_{18}	H_{21}	H_2
Na^+	−113 ± 5	−62 ± 3	−39 ± 2	−23 ± 1	−8.8 ± 1	−4.4 ± 1
K^+	−115 ± 5	−103 ± 4	−56 ± 2	−20 ± 1	−1.9 ± 1	2.2 ± 1
Cs^+	−67 ± 13	−90 ± 18	−60 ± 12	−20 ± 4	1.8 ± 1	4.8 ± 1

[a] Relative to their corresponding values in free nonactin; in Hz.

the coordinating keto oxygens or to one of the coordinating ether oxygens of the tetrahydrofuran rings.

For the Na^+ and K^+ complexes in dry acetone solution, the limiting shifts could be effectively reached experimentally and should be highly accurate. However, in the case of Cs^+, the limiting shifts had to be determined by least-squares fit to the theoretical chemical shift equation because solubility problems prevent attaining more than 60% complex formation. As a result, these shifts are more susceptible to error. The limiting shifts for the protons in the Na^+ and Cs^+ complex are presented in Table I along with those previously reported for the potassium complex and the free molecule. Values for H_6, $H_{6'}$, H_{19}, $H_{19'}$, H_{20}, and $H_{20'}$ are not presented; this region of the spectrum is quite complex, and in addition is complicated by the presence of a multiplet due to the residual protons in the incompletely deuterated solvent (acetone-d_6).

Meaningful trends in the data for the three complexes are difficult to detect because of the interplay of a number of effects on the chemical shifts. However, if one considers the ratios of the H_3 to H_7 shifts or the H_5 to H_7 shifts rather than their absolute magnitudes, there appears to be a notable increase in these ratios as one increases the size of the central ion. For H_3 the ratios are 0.55, 0.89, and 1.34 for Na^+, K^+, and Cs^+, respectively, and for H_5 they are 0.35, 0.49, and 0.90. The salt-induced shifts of the other protons are too small to interpret with any degree of confidence.

Similar results were obtained in solutions containing significant amounts of D_2O. For potassium it was previously shown that the same limiting shifts were reached for complexes formed in dry or wet acetone solution. For Na^+ and Cs^+, the limiting shifts could not be reached experimentally in wet acetone solution because of solubility problems and low complex formation constants. The relative shifts of the various protons, however, could be measured, and were found to be characteristic of the ion regardless of the solvent system used. For example, in the acetone–water mixture, the ratios of the H_3 and H_5 shifts to the H_7 shift for Cs^+ are 1.2 and 0.8, respectively, and the corresponding ratios for Na^+ are 0.5 and 0.3. These can be compared to the ratios of 1.3 and 0.9 for the H_3 and H_5 protons in the case of Cs^+, and 0.6 and 0.4 in the case of Na^+ in dry acetone solution. Thus, these ratios could change dramatically with the size of the central ion, but are essentially the same for the complexes formed in dry and wet acetone. Since at any point in the equilibrium, the ratios of the shifts for any two protons must be equal to the ratios of their limiting shifts, it seems reasonable to conclude that the limiting shifts are also very similar in the two solvent systems.

Coupling Constants. In addition to inducing changes in the chemical shifts of certain protons, the addition of Na^+, K^+, and Cs^+ salts also modifies several vicinal proton–proton coupling constants. With the exception of H_{18} and H_{21}, all the spin multiplets involve spin coupling of the proton in question to at least two vicinal protons. The H_2 multiplet results from coupling of the H_2 proton to H_3 and H_{18}. The effects of the H_2–H_{18} coupling can be ascertained by measuring this coupling constant directly from the H_{18} methyl resonance; the residual H_2,H_3 coupling can then be readily determined, albeit with some uncertainty. The H_7 proton is coupled to the H_{21} methyl protons as well as to the two nearly chemical-shift equivalent methylene H_6,$H_{6'}$ protons. As a result of this latter coupling, the details of the multiplet are complicated by second-order effects, and we have resorted to computer simulation for analysis (see Figure 4). Since the overall width of the H_7 multiplet is expected to be given by $3|J_{H_7,H_{21}}| + |J_{H_7,H_6} + J_{H_7,H_{6'}}|$, and $|J_{H_7,H_{21}}|$ can be measured directly from the H_{21} resonance, $|J_{H_7,H_6} + J_{H_7,H_{6'}}|$ can be deduced from the overall width of the multiplet, and this preliminary value can be used as the starting point of the computer simulation. Similar second-order effects complicate the analysis of the H_5 multiplet, where coupling exists between the H_5 and the H_{19},$H_{19'}$ protons of the tetrahydrofuran ring as well as the methylene H_6,$H_{6'}$ protons. Again we have resorted to spectral simulation on a digital computer (see Figure 5). Theoretically the overall width of the H_5 multiplet should be given by $|J_{H_5,H_6} + J_{H_5,H_{6'}}| + |J_{H_5,H_{19}} + J_{H_5,H_{19'}}|$, and the observed changes in line width of this multiplet could be due to modifications in either coupling. In our computer simulation it was not possible to distinguish between changes in $|J_{H_5,H_6} + J_{H_5,H_{6'}}|$ and $|J_{H_5,H_{19}} + J_{H_5,H_{19'}}|$ by simple curve fitting However, the tetrahydrofuran ring is expected to be relatively rigid and little change in $|J_{H_5,H_{19}} + J_{H_5,H_{19'}}|$ is likely. Therefore the value of $|J_{H_5,H_6} + J_{H_5,H_{6'}}|$ was determined holding $|J_{H_5,H_{19}} + J_{H_5,H_{19'}}|$ constant at 13.6 Hz for all three complexes studied.

Limiting values of the coupling constants for the various complexes are compared to those for the free nonactin molecule in Table II. One notes that within the limits of experimental error, $|J_{H_2,H_3}|$ is constant at 9.7 Hz for all three complexes, while the value of $|J_{H_2,H_3}|$ in the free molecule (7.6 Hz) is significantly

Table II. Vicinal Proton–Proton Coupling Constants Observed for Nonactin and Its Complexes[a]

	$\lvert J_{H_2,H_3}\rvert$	$\lvert J_{H_5,H_6} + J_{H_5,H_{6'}}\rvert$	$\lvert J_{H_7,H_6} + J_{H_7,H_{6'}}\rvert$
Nonactin	7.6 ± 0.4	12.4 ± 0.4	13.0 ± 0.4
Na^+ complex	10.0 ± 0.4	10.9 ± 0.4	13.2 ± 0.4
K^+ complex	9.4 ± 0.4	11.0 ± 0.4	13.0 ± 0.4
Cs^+ complex	9.7 ± 0.8	9.3 ± 0.8	13.0 ± 0.8

[a] Values in Hz.

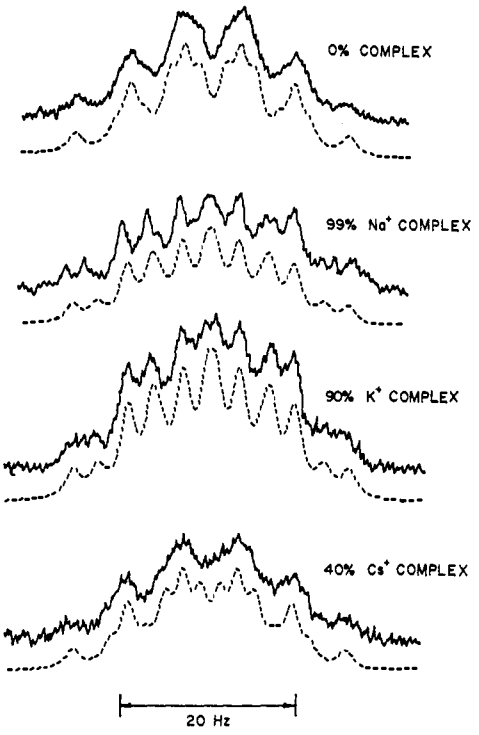


Figure 4. Comparison of the observed and calculated spectral changes in the H_7 multiplet of nonactin upon ion complexation: ----, computer simulated.

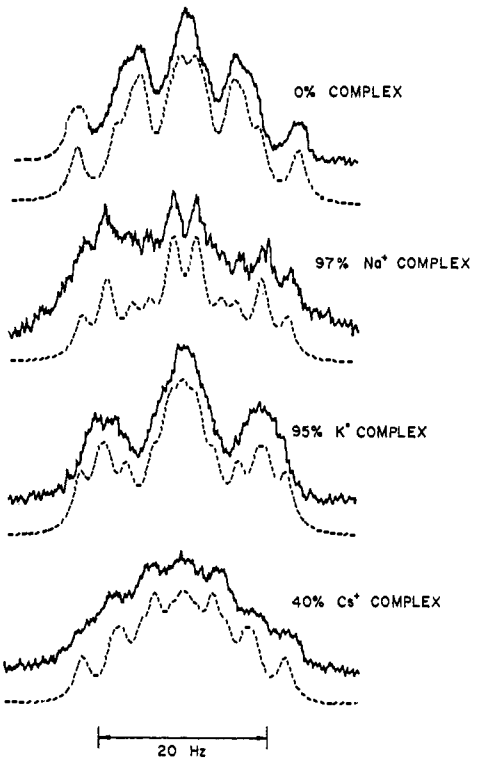


Figure 5. Comparison of the observed and calculated spectral changes in the H_5 multiplet of nonactin upon ion complexation: ----, computer simulated.

smaller. $|J_{H_5,H_6} + J_{H_5,H_6'}|$ changes continually throughout the series, ranging from 12.4 for the free molecule to 9.3 for the Cs^+ complex. $|J_{H_7,H_6} + J_{H_7,H_6'}|$, however, remains unchanged from its value of 13.0 Hz in the uncomplexed molecule.

Spectral changes in the H_7 and H_5 multiplets upon the formation of the various alkali ion–nonactin complexes are presented in Figures 4 and 5. The agreement between the observed and calculated spectra can be seen to be satisfactory in every case. In the case of H_7, where $|J_{H_6,H_7} + J_{H_6',H_7}|$ is the same for the three complexes as well as for the free molecule, the observed spectral changes are primarily the result of changes in the second order effects which, we believe, arise from differences in the magnetic nonequivalence of the methylene H_6,H_6' protons to which H_7 is coupled. The spectral changes in the H_5 multiplet, however, are due to changes in the coupling between the H_5 and H_6,H_6' protons as well as the aforementioned differences in the magnetic nonequivalence of the H_6 and H_6' protons.

Complex Formation Constants. Although the limiting shifts and coupling constants do not appear to be affected by the solvent system, the apparent formation constants deduced from the data for the various complexes were found to be very sensitive to small amounts of water. These formation constants are summarized in Table III. In dry acetone complex formation constants are very large for all three ions and are remarkably and surprisingly similar, being the same within experimental error for Na^+ and K^+ and somewhat smaller for Cs^+. The effect of 0.3 to 0.5 mol fraction of water, however, is dramatic. The effect is largest for Na^+ and Cs^+, where binding constants are reduced by factors of 300 and 30, respectively. The reduction in the binding constant in the case of the K^+ complex, however, was only a factor of 4.

Table III. Formation Constants of the Various Alkali Ion–Nonactin Complexes in Dry and Wet Acetone Solutions at 17°

Salt	D_2O, mol fraction	K, mol fraction units
$NaClO_4$	1×10^{-3}	$7 \pm 2 \times 10^4$
$NaClO_4$	0.39	210 ± 10
$KClO_4$	7×10^{-3}	$7 \pm 2 \times 10^4$
$KClO_4$	0.34	$1.7 \pm 0.2 \times 10^4$
$CsClO_4$	3×10^{-3}	$15 \pm 12 \times 10^3$
$CsClO_4$	0.55	400 ± 80

Discussion

On the basis of our earlier work with potassium ion it was conjectured that the nonactin ring system is quite flexible and should be capable of expanding or contracting to accommodate ions having ionic radii within a certain range. Our present experiments with the Na^+ and Cs^+ salts confirm this contention. The similarity of the binding constants determined for $NaClO_4$, $KClO_4$, and $CsClO_4$ in dry acetone is particularly noteworthy, and this result, we believe, reflects directly the relative affinity of the nonactin cavity for the cations in question in this solvent system. Since the equilibrium involves the interaction of a univalent cation with a neutral species to form a charged complex, interionic effects on the activities of the charged species are expected to cancel largely in the equilibrium expression, except at high ionic strengths. We have also considered the effect of ion pairing. However, on the basis of reasonable ion-pairing constants, the salts

can be shown to be largely dissociated under the conditions of our experiments, and any ion pairing which may exist was shown to influence the apparent binding constants in a fairly uniform manner within the series and hence does not alter the above conclusion.[14]

Thus, we conclude that in dry acetone, all three ions, Na^+, K^+, and Cs^+, bind with nearly equal affinity to nonactin. The somewhat lower constant for Cs^+ may indicate the approach of a steric limit to ion size. The standard free energy of ion solvation (relative to the free gaseous ion) in a well-behaved solvent is expected to decrease monotonically with decreasing ionic radius,[15] and barring steric restrictions, there is no *a priori* reason not to expect a similar free-energy dependence for the complexation of the various alkali ions to nonactin. Since the apparent binding constants determined in this work correspond to differences in the standard free energies of ion solvation and ion complexation for each ion, our observations in dry acetone would suggest a rather similar dependence of the free energy of ion coordination on ion size for both nonactin and the solvent, at least over the range of ionic radii investigated. This result is perhaps not surprising if the nonactin ring is reasonably flexible, since the keto groups of the acetone molecules could mimic rather well the coordinating carbonyl oxygens of the macrocycle. This apparent adaptability of the nonactin ring system, however, fails to explain its high selectivity in ion transport through lipid bilayers, where K^+ transport is highly favored over the transport of either Na^+ or Cs^+.[16] These observations point to the possible importance of the solvent in contributing to ion selectivity.

The apparent binding constants for Na^+, K^+, and Cs^+ are seen to be drastically reduced when the solvent system is altered by the addition of appreciable amounts of water. It is significant that the reduction is far greater for Na^+ and Cs^+ than for K^+ itself, making the binding of K^+ highly favored in the more aqueous medium. This apparent reduction, we believe, arises from a decrease in the activity of the ion as a result of hydration and the necessity of removing a hydration shell from the ion prior to its accommodation in the central cavity of the nonactin ring. We note that the apparent reduction in binding constant in itself is not sufficient evidence for this hypothesis, since a hydrated ion may also bind to the nonactin ring, possibly with a lower binding constant. It is therefore also necessary to compare the magnetic environments of complexes formed in the two solvent systems.

Chemical shifts observed for the various protons are the result of small differences in their magnetic environments which in turn are sensitive to conformational changes. Changes in geometry necessary to accommodate the larger hydrated ion should therefore result in differences in the limiting chemical shifts of the nonhydrated and hydrated complexes. In the case of potassium ion, where the limiting shifts for the complexes formed in both wet and dry acetone were measured directly, no such differences were noted. In the case of Cs^+ and Na^+, where the effect of hydration is more pronounced, the limiting shifts of H_7, H_3, and H_5 could not be reached experimentally. However, the relative shifts of H_7, H_3, and H_5 here are equally indicative of changes in the properties of the complex formed, as evidenced by the changes in the ratios of these shifts as the central ion is changed. We have summarized these ratios in Table IV. The ratio of the H_3 to H_7 shifts, for example, changes from 0.55 to 1.34 on substituting Cs^+ for Na^+. In comparison to this gross dependence on ion size, the small changes in the ratios observed between complexes formed in anhydrous and wet acetone solutions (see Table IV) are insignificant. We therefore conclude that only the unhydrated ion enters the nonactin cavity regardless of its state of hydration before complex formation, and the apparent reduction in the complex formation constant when water is added to the system is the result of reduction in ion activity.

Table IV. Ratios of the Salt-Induced Shifts for the Various Nonactin Complexes in Dry and Wet Acetone Solutions

		Na^+	K^+	Cs^+
H_3/H_7	Dry	0.55	0.89	1.34
	Wet	0.47[a]	0.85	1.16[a]
H_5/H_7	Dry	0.35	0.49	0.90
	Wet	0.33[a]	0.44	0.75[a]

[a] Experimental error, ±10%; otherwise, ±5%.

On the basis of the relative solvation energies of the alkali ions in water and acetone, it is not surprising that there should be a reduction in the activity of the ions in wet acetone. That this reduction does not vary monotonically with ion size is, perhaps, surprising. But this result, we believe, arises merely from subtle differences in the dependence of the ion solvation free energy on ion size in the two solvent systems. To illustrate this point, we have depicted schematically in Figure 6 possible dependences of the standard free energies of ion complexation and ion solvation for nonactin and the two solvent systems under consideration. Here, the standard free energies of hydration of the alkali ions relative to the free gaseous ions have been used to provide a meaningful scale to the free energy diagram, and the standard solvation free energies of the ions in our water–acetone mixtures were assumed to be the same as in bulk water.[15] The curves for dry acetone and nonactin were included on the basis of the binding constant data reported in this paper. Since differences in the standard free energies of ion coordination can be seen to be small compared with the total free energy involved for each particular ion, it is perhaps not too surprising that subtle variations in the radius dependence of the free energy curves for ion complexation and ion solvation (hydration) can lead to the dramatic ion selectivity of nonactin.

From the apparent reduction of binding constants in wet acetone, one can calculate the activities of the various ions relative to their standard states in dry acetone. ($\Delta G^{\circ\prime} = -RT \ln a$; see Figure 6). For Na^+, K^+, and Cs^+ these are 0.003, 0.25, and 0.03, respectively. Interestingly enough, there is a qualitative correspondence of these activities with the transport activities observed for these ions in experimental lipid bilayers where K^+ transport is highly favored.[16]

(14) C. W. Davies, "Ion Association," Butterworths, Washington, D. C., 1962, p 98.
(15) J. D. Bernal and R. H. Fowler, *J. Chem. Phys.*, 1, 515 (1933).
(16) P. Mueller and D. O. Rudin, *Biochem. Biophys. Res. Commun.*, 26, 398 (1967).

In addition to shedding light on the origin of ion specificity, our pmr data indicate certain changes in the geometry of the complex and the nature of the coordination as the central ion is varied. For the potassium complex the pmr data were shown to be consistent with the X-ray crystal structure which shows the molecule to possess a near-S_4 symmetry axis with the ion at the center of the four centrally directed carbonyl groups, and the four centrally directed ether groups occupying the corners of two staggered tetrahedra. Judging from the magnetic equivalence of the corresponding protons in the four subunits of the molecule throughout our work, we can conclude that the S_4 symmetry axis is maintained in all three complexes studied, but minor variations in coordination as well as geometry are also apparent.

Coupling constants between vicinal protons along the backbone of the nonactin ring are sensitive to rotations about the various carbon–carbon bonds. In nonactin, for example, J_{H_6,H_7}, J_{H_6,H_5}, and J_{H_2,H_3} would be sensitive to such rotations. For simple aliphatic compounds a theory developed by Karplus is probably adequate to relate these coupling constants to the appropriate dihedral angles.[17] The present situation, however, is somewhat more complex both because of the polar substituents on the ring and because of possible electrostatic effects when the ion is bound to the nonactin ring. It is therefore only possible to interpret the coupling constant changes in a qualitative manner.

In comparison to the relatively large changes in these coupling constants observed upon complex formation[8] the alterations which occur on changing the size of the central ion are relatively minor. In fact, $|J_{H_2,H_3}|$ and $|J_{H_7,H_6} + J_{H_7,H_{6'}}|$ are essentially identical for the three complexes. $|J_{H_5,H_6} + J_{H_5,H_{6'}}|$ is also invariant within experimental error for the K^+ and Na^+ complexes, although it decreases from 11.0 to 9.3 Hz in the case of the Cs^+ complex. Thus it appears that the three complexes are quite similar, at least as far as their geometries about the C_2–C_3, C_6–C_7, and C_5–C_6 bonds are concerned. Small differences do exist, and in the case of the Cs^+ complex the smaller $|J_{H_5,H_6} + J_{H_5,H_{6'}}|$ coupling constant indicates that the C_{19}–C_5–C_6–C_7 dihedral angle is less than its value of near 180° in the free nonactin molecule and the Na^+ and K^+ complexes. An examination of a CPK molecular model of nonactin indicates that such a decrease in dihedral angle can contribute to a slight cavity expansion in the case of the Cs^+ complex. It is important to realize, however, that because of the limitations of the Karplus theory and the fact that we can monitor rotations about only 12 of the many ring linkages, it is not possible to characterize the conformational differences among the complexes more precisely.

Variations in the limiting chemical shifts observed for analogous protons of the three complexes provide some additional insight into the conformational differences among the complexes. The interpretation of these shifts unfortunately is not straightforward, since they are the result of the interplay of several effects. The least complicated of these effects is that due to the direct electrostatic polarization of the various C–H bonds by the bound positive ion.[18] This effect depends not only on the distance between the polarizing ion and the proton in question but also on the relative orientation of the polarizing field and the bond axis. It may be that part of the observed differences in the limiting chemical shifts for the various complexes reflect modifications of one or both of these parameters. This could come about through contraction and expansion of the nonactin cavity with changes in the size of the ion or some more subtle conformational change to achieve more effective interaction between the central ion and the two types of ligands. The more difficult effects to take into consideration are those which have their origins in polar substituents or magnetically anisotropic groups within the nonactin molecule. Several of the protons of interest, *e.g.*, H_7, H_3, and H_5, are in close proximity to the ligands of coordination, and it is likely that part of the differences in the limiting shifts observed for these protons in the various complexes also reflect somewhat different interactions of the metal ions with their ligands, or some conformational change which alters the spatial relationships of these protons relative to the ligands.

The limiting shifts observed for the hydrogens on the periphery of the nonactin ring such as those attached to C_{18} or C_{21}, which are relatively far removed from the polar substituents of the ring as well as from the coordinating ligands, are largely determined by direct electrostatic polarization of the C–H bonds by the central ion or by indirect effects due to polar or magnetically anisotropic groups in the surrounding solvent medium. Although the observed trends are qual-

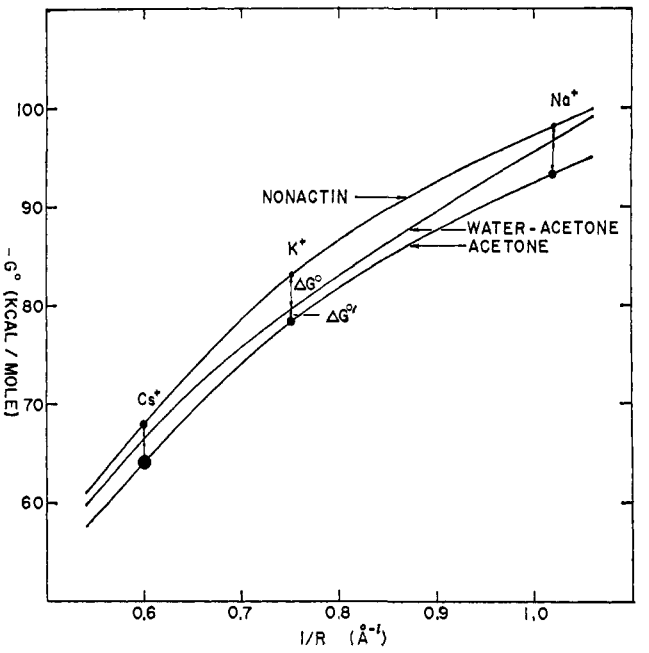
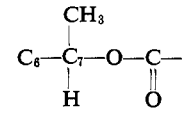


Figure 6. Possible variations of the standard free energy of ion coordination with ion size for nonactin, dry acetone, and wet acetone. $\Delta G°$ and $\Delta G° + \Delta G°'$ refer to the standard free energies of complexation of the alkali ions to nonactin in wet acetone and dry acetone, respectively. The errors in $\Delta G°$ and $\Delta G°'$ are indicated by the size of the data points.

(17) M. Karplus, *J. Chem. Phys.*, 30, 11 (1959).

(18) A. D. Buckingham, *Can. J. Chem.*, 38, 300 (1960).

itatively consistent with a slight expansion of the nonactin ring as the size of the bound ion is increased, these shifts are in general too small to permit meaningful interpretation. In the case of H_5, however, the limiting shifts are larger, and since the orientation of the C_5–H bond with respect to the ether oxygen of the tetrahydrofuran ring is relatively fixed, to a first approximation, differences in the limiting shifts for this proton can be interpreted on the basis of direct ion polarization and indicate changes in the stereochemistry of the tetrahydrofuran ring relative to the centrally bound ion. The observed trend can, for example, be accounted for by a reorientation of the tetrahydrofuran ring, which points the ether oxygen more directly into the central cavity and tips the C_5–H bond to a more obtuse angle with respect to the M^+–H_5 vector in the case of the larger ion. Examination of a molecular model of nonactin indicates that this conformational change would lead to the change in the C_{19}–C_5–C_6–C_7 dihedral angle suggested earlier by the coupling constant data and the accompanying expansion of the central aperture necessary to accommodate the larger ion. This conformational change possibly brings the tetrahydrofuran oxygens into more effective coordination with the central ion, which may also contribute to the increased downfield limiting shifts in the case of the K^+ and Cs^+ complexes. The H_7 limiting shifts do not readily lend themselves to a similar interpretation. In fact, in the potassium complex, the ion-induced polarization of the C_7–H bond could only account for 10% of the large downfield shift observed for the H_7 resonance. Since, in the complex, the H_7's are in close proximity to the coordinating carbonyl groups, it is likely that the major part of the salt-induced shift observed here results from some modification of the electronic distribution of the keto groups and/or an accompanying conformational change about the ester linkages which brings the carbonyl oxygens into closer proximity with these protons. The observed differences in the H_7 limiting shifts for the three complexes, *e.g.*, probably reflect differences in the stereochemistry about this ester linkage. Thus the smaller H_7 limiting shift for the Cs^+ complex may indicate that the



group has been rotated about the ester linkage so that the C_7–H bond points more directly into the central cavity and is less eclipsed with the carbonyl group. Examination of the molecular model indicates that this conformational change would also lead to a slight expansion of the nonactin aperture. The interpretation of the H_3 salt-induced limiting shifts is further complicated by the proximity of the H_3 proton to both the carbonyl and ether oxygens, and a discussion of these shifts will not be attempted here.

Thus, in spite of inherent limitations of the pmr method for detailed conformational analysis, it is nevertheless apparent from this work that the method is sensitive enough to indicate that the nonactin ring is quite flexible, and that certain minor structural differences do exist among the nonactin complexes associated with the ions of various sizes.

Conclusions

The results of this research indicate that nonactin is a flexible molecule, capable of binding a range of alkali ions with nearly equal affinity, at least in acetone solution, leaving it with little inherent propensity for selective binding. The inability of the nonactin ring to accommodate an ion with its hydration sphere and subtle differences in the variations of the standard free energies of ion hydration and ion complexation with ion size were shown to provide the differences necessary to allow selective binding in less anhydrous media.

Acknowledgment. The authors wish to thank Dr. B. Stearns of the Squibb Institute for Medical Research for her generous gift of nonactin. One of us (J. H. P.) would also like to thank Professor Max Delbrück (Caltech) for several enlightening discussions during the course of this work.