J. Membrane Biol. 1, 294–345 (1969)

The Effects of the Macrotetralide Actin Antibiotics on the Equilibrium Extraction of Alkali Metal Salts into Organic Solvents*

G. EISENMAN, S. CIANI**, and G. SZABO***

Department of Physiology, University of California, Medical Center, Los Angeles, California 90024

Received 15 August 1969

Summary. In order to clarify the mechanism by which neutral molecules such as the macrotetralide actin antibiotics make phospholipid bilayer membranes selectively permeable to cations, we have studied, both theoretically and experimentally, the extraction by these antibiotics of cations from aqueous solutions into organic solvents. The experiments involve merely shaking an organic solvent phase containing the antibiotic with aqueous solutions containing various cationic salts of a lipid-soluble colored anion. The intensity of color of the organic phase is then measured spectrophotometrically to indicate how much salt has been extracted. From such measurements of the equilibrium extraction of picrate and dinitrophenolate salts of Li, Na, K, Rb, Cs, and NH_4 into n-hexane, dichloromethane, and hexane-dichloromethane mixtures, we have verified that the chemical reactions are as simple as previously postulated, at least for nonactin, monactin, dinactin, and trinactin. The equilibrium constant for the extraction of each cation by a given macrotetralide actin antibiotic was also found to be measurable with sufficient precision for meaningful differences among the members of this series of antibiotics to be detected. It is noteworthy that the ratios of selectivities among the various cations were discovered to be characteristic of a given antibiotic and to be completely independent of the solvent used. This finding and others reported here indicate that the size and shape of the complex formed between the macrotetralide and a given cation is the same, regardless of the species of cation bound. For such "isosteric" complexes, notable simplifications of the theory become possible which enable us to predict not only the electrical properties of a membrane made of the same solvent and having the thinness of the phospholipid bilayer but also, and more importantly, the electrical properties of the phospholipid bilayer membrane itself. These predictions will be compared with experimental data for phospholipid bilayer membranes in the accompanying paper.

* This work was carried out largely at the University of Chicago with the support of U.S. Public Health Service Grant GM 14404-02/03 and of National Science Foundation Grant GB 6685.

** Present Address: Institute of Physics, University of Genoa, Genoa, Italy. Recipient of a Fulbright Traveling Scholarship.

*** Recipient of fellowships from the Canadian National Research Council and the University of Chicago.

In the first paper of this series (Ciani, Eisenman, & Szabo, 1969), referred to hereafter as I, a theoretical treatment was presented for the effects of neutral macrocyclic antibiotics, such as the macrotetralide actins, on the electrical properties of phospholipid bilayer membranes. This treatment was based on the reasonable supposition that such molecules solubilize cations within the membrane in the form of mobile charged complexes, thereby providing a "carrier" mechanism by which cations can cross the insulating hydrocarbon interior of the membrane. In the present paper, we deduce theoretically the salt extraction properties such molecules would be expected to confer on organic solvents and we measure these experimentally. The excellent quantitative agreement found between theoretical expectation and experimental observation not only verifies the correctness of a central supposition that such molecules act by solubilizing cations in low dielectric constant media, but also provides evidence that the chemistry for the macrotetralide actins is as simple as postulated. In addition, we deduce explicitly how the equilibrium extraction of salts by the macrotetralides into an arbitrarily chosen bulk solvent is related not only to the electrical properties of a thin membrane made of the same solvent but also to the electrical properties of phospholipid bilayer membranes of varied composition. These expectations will be compared with experimental data for phospholipid bilayer membranes in the third paper of this series (Szabo, Eisenman, & Ciani, 1969*a*), referred to hereafter as III.

Why study salt extraction if one is interested in membranes? We did so because such measurements for a given antibiotic are shown here and in paper I to yield the value of the particular combination of equilibrium parameters which theoretically should determine the effects of the antibiotic on the permeability ratios and conductance ratios of cations in phospholipid bilayer membranes. Moreover, such experiments are easy to perform and the results can be interpreted unambiguously. Indeed, despite the somewhat complex theoretical section that follows and the extensive analysis of the data necessary to verify the simplicity of the postulated chemistry, it is worth pointing out that only one very simple type of experiment is reported on in this paper. This experiment consists simply of shaking an aqueous solution containing various cationic salts of a colored anion (e.g., picrate) with an organic solvent phase containing the antibiotic and then measuring the color of the organic phase to indicate how much salt has been extracted.

The theory is presented first because the design and interpretation of the experiments rest on it. For those who wish to go directly to the results,

the principle theoretical point to be noted is that the salt extraction equilibrium corresponds to Eq. (7) over a wide range of conditions. The equilibrium constant, K_i, of this reaction can be evaluated from the measured concentrations of extracted picrate by using the first term of Eq. (28) together with the equilibrium concentrations deduced from the initial experimental conditions through Eqs. (29), (31), and (37).

Theory

We present first a thermodynamic description of the salt extraction properties expected to be conferred by a neutral lipophilic cation-binding molecule on a bulk organic solvent phase in contact with aqueous solutions. The initial theory makes no special assumptions about the properties (e.g., size and shape) of the complexes, yet it still enables one to predict the electrical properties of a given solvent, when studied as a thin membrane, from the salt extraction data. We then show the remarkably simple and powerful expectations that can be deduced as an immediate consequence of the postulate that the complex between cation and antibiotic is "isosteric" (i.e., the size, shape, and chemical properties of the complex are virtually independent of the particular cation bound). In particular, this postulate permits one to predict the electrical properties of phospholipid membranes of various compositions from the characterization of bulk phase extraction equilibria for a variety of solvents whose composition need no longer be the same as that of the membrane.

The model of paper I considered that a phospholipid bilayer membrane can be approximated by a thin liquid hydrocarbon phase whose dielectric constant is assumed to be sufficiently low that negligible quantities of free cations and anions are present within it in the absence of antibiotics. The neutral macrocyclic ion-binding molecules were assumed to be preferentially partitioned in the organic phase and to be capable of forming one-to-one complexes with cations, solubilizing them in the membrane and thereby rendering the membrane permeable to these species. For this model, explicit expressions were deduced for the dependence of membrane potential and membrane conductance on the concentrations of antibiotic and salt in the aqueous solutions, and the ratio of the permeabilities of two different ions was predicted to be equal to the ratio of the conductances of these ions. It was noted that the organic phase need not be studied as a membrane, since certain properties conferred on it by the antibiotics would remain if such a membrane phase were expanded into a bulk liquid phase and the effects of the neutral macrocyclic molecules on salt extraction equilibria were studied in the bulk system. This system differs from the bilayer in

that it is considerably thicker than the Debye length; also, the amount of salt extracted at equilibrium into a bulk organic solvent is measured instead of the electrical properties of a thin membrane.

Denoting monovalent cation and anion species by I^+ and X^- (i and x when used as subscripts), respectively, and denoting the neutral macrocyclic ion-binding molecule by S (s when used as a subscript), we assume, as in paper I, that a charged complex IS^+ can form between the cation and the neutral molecule according to the simple equilibrium:

$$I^+ + S \underset{K_{is}^+}{\rightleftharpoons} IS^+, \quad K_{is}^+ = \frac{a_{is}}{a_i a_s}. \tag{1}$$

The charged complex can further associate with the anion X^- to form the neutral species ISX through:

$$IS^+ + X^- \underset{K_{isx}}{\rightleftharpoons} ISX, \quad K_{isx} = \frac{a_{isx}}{a_{is} a_x}. \tag{2}$$

In Eqs. (1) and (2), a denotes the activity of the species in moles per liter of solution. Reactions (1) and (2) can occur not only in the organic solvent phase but also in the aqueous phase, although reaction (2) will generally be negligible in aqueous media. An asterisk (*) will be used to designate quantities characteristic of the organic solvent phase, usually referred to henceforth as the "solvent". Quantities not so designated will be understood to refer to the aqueous phase.

Classical partition equilibria for all species must also exist between the aqueous and solvent phases according to reactions of the type:

$$S \underset{k_s}{\rightleftharpoons} S^*, \quad k_s = \frac{a_s^*}{a_s}, \tag{3}$$

for uncharged species (e.g., S and ISX), and of the type

$$R^{\pm} \underset{k_r}{\rightleftharpoons} R^{\pm *}, \quad k_r = \frac{a_r \exp \dfrac{z_r F \psi^*}{RT}}{a_r \exp \dfrac{Z_r F \psi}{RT}} \tag{4}$$

for charged species (e.g., $R^{\pm} = I^+, IS^+, X^-$), where ψ is the electrostatic potential and z_r is the valance of $R^{\pm}$.

The set of Eqs. (1)–(4) is sufficient for a complete description of salt extraction *equilibria*, but the interrelationships between membrane and bulk systems can be seen more clearly by analyzing suitable combinations of these reactions.

The Cation Solubilization Reaction

For example, subtracting reaction (3) from Eq. (1) and adding Eq. (4) for the IS^+ species (i.e., $R^{\pm}=IS^+$), we get

$$I^+ + S^* \underset{\bar{K}_i}{\rightleftharpoons} IS^{+*}, \qquad \bar{K}_i = \frac{a^*_{is}}{a_i a^*_s} \exp \frac{Z_i F}{RT}(\psi^* - \psi), \tag{5}$$

whose equilibrium constant $\bar{K}_i$ is related to the parameters of reactions (1), (3), and (4) through:

$$\bar{K}_i = \frac{k_{is} K^+_{is}}{k_s}. \tag{6}$$

Eq. (5) is an heterogenous reaction, describing formally the process by which the IS^{+*} complex is formed within the solvent phase from a cation I^+ from the aqueous solution and an S^* molecule from the solvent phase[1]. $\bar{K}_i$ cannot normally be measured directly since its determination requires a knowledge of the (nonmeasurable) electrical potential difference $(\psi^* - \psi)$ between the two phases, but it is this reaction which in a thin membrane leads to the excess of the IS^{+*} complexes as a "space charge" determining the membrane's electrical properties as shown in paper I. Because $\bar{K}_i$ determines the number of cations solubilized in the membrane as such complexes, we will refer to it as the "membrane solubilization constant".

The Salt Extraction Reaction

On the other hand, by adding the reaction (4) for the anion X^- (i.e., $R^{\pm}=X^-$) to Eq. (5), we immediately obtain an expression for the extraction of a salt composed of monovalent ions I^+ and X^- from the aqueous phase into a bulk solvent phase.

$$I^+ + X^- + S^* \underset{K_i}{\rightleftharpoons} IS^{+*} + X^{-*}; \qquad K_i = \frac{a^*_{is} a^*_x}{a_i a_x a^*_s}. \tag{7}$$

Note that the electrical potential difference between the phases has cancelled, and thus need not be known, so that K_i, the equilibrium constant of this reaction:

$$K_i = \frac{k_{is} K^+_{is} k_x}{k_s}, \tag{8}$$

1 Of course, this particular process cannot be distinguished in an equilibrium system from an alternative reaction path by which the IS^+ complex is formed in the aqueous phase and is then partitioned into the solvent. [Considerations of reaction path will be found elsewhere (Szabo, Eisenman, & Ciani, 1969*b*)].

is experimentally measurable for a bulk phase. We will refer to K_i as the "bulk extraction constant", noting that this differs from the previous "membrane solubilization constant" only by the partition coefficient of the anion, k_x, since $K_i = \bar{K}_i k_x$.

Eq. (7) is our key reaction; however, before examining this reaction further, it will be useful to digress briefly to express explicitly the relationship expected theoretically between K_i and the electrical properties of thin membranes.

Comparison of Bulk and Membrane Properties

The relationships between K_i and $\bar{K}_i$ have been noted in paper I, but for clarity they will be examined more extensively here. Comparing the ratio of "bulk extraction constants", K_i/K_j, with the corresponding ratio of "membrane solubilization constants", $\bar{K}_i/\bar{K}_j$, for a membrane of the same solvent, it is seen that:

$$\frac{\bar{K}_j}{\bar{K}_i} = \frac{K_j}{K_i} = \frac{k_{js} K_{js}^+}{k_{is} K_{is}^+}, \tag{9}$$

since both k_x and k_s cancel in the case of a common anion. Therefore, the ratio of the equilibrium constants of the salt extraction reaction (7) for two different cations I^+ and J^+ is identical to the corresponding ratio for the cation solubilization reaction (5) when the same solvent is studied as a thin membrane.

Comparing Eq. (9) with the identity previously deduced [Eq. (63) of paper I] for the permeability ratios (P_j/P_i) and conductance ratios $[G_0(J)/G_0(I)]$ of a membrane made of the same solvent:

$$\frac{P_j}{P_i} = \frac{G_0(J)}{G_0(I)} = \frac{u_{js}^*}{u_{is}^*} \frac{k_{js} K_{js}^+}{k_{is} K_{is}^+}, \tag{10}$$

it should be clear that:

$$\frac{P_j}{P_i} = \frac{G_0(J)}{G_0(I)} = \frac{u_{js}^* K_j}{u_{is}^* K_i}. \tag{11}$$

Eq. (11) explicitly relates the ratio of equilibrium constants for salt extractions into a given solvent to the electrical properties of a thin membrane made of the same solvent. In principal, this equation offers a way to measure the mobility ratio, u_{js}^*/u_{is}^*, for these complexes by comparing the observed ratios of permeabilities (or conductances) with the equilibrium

constant ratios for bulk phase salt extraction. Any difference in these ratios would reflect the mobility ratio of the complexes according to the present theory.

Note that such characteristics as the size and shape of the complex could vary from cation to cation, and the above conclusions would still hold since no assumptions have been made in deducing Eqs. (10) or (11) as to independence of the physical properties of the complexes of the particular cation bound.

Simple Expectations for "Isosteric" Complexes

If the complexes with cations are "isosteric" (i.e., their overall size and shape as well as their externally viewed electron distribution is the same for all cations), the interaction energy of the complex with the solvent will not vary with the particular species bound[2]. The partition coefficients of all complexes are expected therefore to be the same so that:

$$\frac{k_{js}}{k_{is}}=1 \tag{12}$$

for all solvent (and phospholipid bilayer) compositions. This result immediately allows us to deduce, on inserting Eq. (12) in Eq. (9), that:

$$\frac{K_j}{K_i}=\frac{K_{js}^+}{K_{is}^+}, \tag{13}$$

which indicates that the ratios of the salt extraction equilibrium constants for different cation species, K_j/K_i, are expected to be independent of the solvent in which they are measured since K_{js}^+/K_{is}^+ is independent of the solvent (or membrane) composition. (Recall that K_{js}^+/K_{is}^+ is the ratio of stability constants for complex formation in aqueous solutions, a fact whose implications will be examined later.)

Similarly, the mobility ratios of "isosteric" complexes should also be independent of the particular cation bound so that:

$$\frac{u_{js}^*}{u_{is}^*}=1. \tag{14}$$

2 Of course this implies that the complex may be viewed as a large sphere with the cation sequestered deep inside. All details of electronic distribution, orientation of side chains, etc., are considered to be the same regardless of which (well-screened) cation species is at the center of the complex (*see* Fig. 1b of paper I). The Van der Waal's work will be the same for all complexes of the same size and shape if the detailed electronic distribution over the macrotetralide molecular complex is the same for all cations.

Again, this is expected to be true regardless of the composition of the membrane. Inserting Eq. (14) in Eq. (11), and recalling Eq. (13), we find the remarkably simple set of relationships:

$$\frac{P_j}{P_i}=\frac{G_0(J)}{G_0(I)}=\frac{K_j}{K_i}=\frac{K_{js}^+}{K_{is}^+}, \tag{15}$$

previously noted in Eq. (68) of paper I. Eq. (15) leads to the testable expectation that *the equilibrium constant ratios measured by salt extraction should be identical to the permeability and conductance ratios, regardless of the composition of membrane or solvent.* With this result, it is no longer critical that the solvent chosen be a "good prototype for the interior of the bilayer membrane", as we previously required (Eisenman, Ciani, & Szabo, 1968). Indeed, if it were possible to measure values of K_{is}^+ and K_{js}^+ accurately in water, such measurements would provide sufficient information to characterize the expected effects of antibiotic molecules on bilayers, as will be considered further in relation to Table 16 and Fig. 15.

General Description of Salt Extraction in Terms of Known or Measurable Quantities

Having related the equilibrium constant of the simple salt extraction reaction (7) to the properties expected for bilayer membranes, we now turn to the task of designing experiments to measure the value of this constant as well as to test the adequacy of the theory.

In addition to reaction (7), the possibility must also be considered of ion-pair formation between the IS^{+*} species and the anions X^{-*} according to the "neutralization" reaction:

$$IS^{+*}+X^{-*}\underset{K_{isx}}{\rightleftharpoons}ISX^*, \qquad K_{isx}^*=\frac{a_{isx}^*}{a_{is}^* a_x^*}, \tag{16}$$

particularly for solvents of low dielectric constant. In the usual experiments where the uptake of cation is followed by radioactive tracer (*see* Pressman, Harris, Jagger, & Johnson, 1967), or the uptake of the anion is measured by optical absorption (*see* Pedersen, 1968; Eisenman et al., 1968), only the total amount of salt extracted can be measured since it is generally impossible to distinguish between the uptake in the form of IS^{+*} and that in the form of ISX^*. Therefore, we will examine the expected salt extraction under conditions in which both reactions (7) and (16) can occur, noting that one of the tasks of the present theory is to guide the design of experiments so that K_i of reaction (7) can be measured accurately without complications due to the neutralization reaction (16).

Assuming ideal behavior of all species in the solvent phase and of the neutral species in the aqueous phase, we can replace activities of these species by their concentrations so that the equilibrium constants K_i and K^*_{isx} of reactions (7) and (16) can be rewritten:

$$K_i = \frac{C^*_{is} C^*_x}{a_i a_x C^*_s}, \tag{17}$$

and

$$K^*_{isx} = \frac{C^*_{isx}}{C^*_{is} C^*_x} = \frac{C^*_{isx}}{K_i a_i a_x C^*_s}, \tag{18}$$

respectively.

The extraction of a single cation species, I^+, into a bulk solvent phase (i.e., one considerably thicker than the Debye length) requires, by electroneutrality, that the amount of IS^{+*} complex taken up by the solvent be accompanied by an equal amount of anions, so that in a system containing a lipid-compatible anion X^- (e.g., picrate$^-$) and a nonlipophilic anion A^- (e.g., OH^- or Cl^-) present for experimental convenience:

$$C^*_{is} = C^*_x + C^*_a, \tag{19}$$

where C^*_a refers to the concentration of A^{-*}.

By Eq. (3):

$$\frac{C^*_a}{C^*_x} = \frac{k_a a_a}{k_x a_x}. \tag{20}$$

Substituting this result in Eq. (19) gives:

$$C^*_{is} = C^*_x \left(1 + \frac{k_a a_a}{k_x a_x}\right); \tag{21}$$

and substituting Eq. (21) in Eq. (17), we find

$$C^{*2}_x = \frac{K_i}{1 + \frac{k_a a_a}{k_x a_x}} a_i a_x C^*_s. \tag{22}$$

In the experiments to be presented in this paper, what will be measured is C^{Tot*}_x, the total uptake of a lipid-compatible chromophore anion X^- (typically picrate or dinitrophenolate) from mixtures of alkali metal hydroxides (or chlorides):

$$C^{Tot*}_x = C^*_x + C^*_{isx}. \tag{23}$$

Expressing C_x^* and C_{isx}^* through Eqs. (18) and (22) and inserting into Eq. (23) gives:

$$C_x^{\mathrm{Tot}*}=\left[\frac{K_i}{1+\frac{k_a a_a}{k_x a_x}} a_i a_x C_s^*\right]^{1/2}+K_{isx}^* K_i a_i a_x C_s^*. \tag{24}$$

For the usual range of X^- and A^- concentrations studied, the partition coefficient ratio k_x/k_a is so large that $k_a a_a/k_x a_x \ll 1$ or $C_a^* \ll C_x^*$. Eq. (19) may therefore be written approximately as

$$C_{is}^*=C_x^*. \tag{25}$$

Since in this approximation (and considering as negligible the concentration of the salt IX^* in the solvent in the absence of the antibiotic) we can equate the total uptake of species I^+ with that of the species X^-,

$$C_i^{\mathrm{Tot}*}=C_{is}^*+C_{isx}^*=C_x^{\mathrm{Tot}*}. \tag{26}$$

Eq. (24) can therefore be reduced to the simpler form:

$$C_i^{\mathrm{Tot}*}=C_x^{\mathrm{Tot}*}=(K_i a_i a_x C_s^*)^{1/2}+K_{isx}^*(K_i a_i a_x C_s^*). \tag{27}$$

The aqueous activities, a_i and a_x, can be expressed in terms of the aqueous concentrations, C_i and C_x, and (known) activity coefficients, y_i and y_x, through the definition: $a_i a_x = C_i C_x y_i y_x$. Then Eq. (27) can be rewritten in the form which we will test:

$$C_i^{\mathrm{Tot}*}=C_x^{\mathrm{Tot}*}=(K_i C_i C_x C_s^* y_i y_x)^{1/2}+K_{isx}^*(K_i C_i C_x C_s^* y_i y_x). \tag{28}$$

Eq. (28) expresses the measurable quantity $C_x^{\mathrm{Tot}*}$ in terms of C_i and C_x, the equilibrium concentrations of I^+ and X^- in the aqueous phase, and C_s^*, the equilibrium concentration of antibiotic in the organic solvent phase. It is seen to consist of two terms corresponding to the contributions of reactions (7) and (16), respectively. The first term describes the uptake of the dissociated anion X^{-*}, whereas the second describes that of the anion-associated neutral complex ISX^*. Eq. (28) shows that the concentration of the chromophore anion X^- extracted is a function of the single variable $(C_i C_x C_s^* y_i y_x)$; the experimental data of this paper will be presented in terms of this variable[3].

3 Note that Eq. (28) also gives the expectations for experiments where the cation uptake ($C_i^{\mathrm{Tot}*}$) is measured (e.g., by radioactive tracers).

The Equilibrium Values for C_i, C_x, and C_s^* in Terms of the Initial Experimental Conditions and the Measured Extraction of Chromophore Anion

The extraction experiments to be presented here are typically carried out by equilibrating a known volume, V^*, of solvent containing initially a known concentration of antibiotic, $C_s^{In^*}$, with a known volume, V, of aqueous solution containing an initially known concentration of salt. We then measure $C_x^{Tot^*}$, the equilibrium concentration of the chromophore anion in the solvent phase. From this value, by taking into account the conservation of mass of the species transferred between the phases, it is possible to calculate the individual concentrations at equilibrium of C_i, C_x, and C_s^* as shown below.

Let us denote by C_x^{In} the initial concentration of chromophore anion in the aqueous solution and by $C_s^{In^*}$ the initial concentration of antibiotic in the solvent phase.

For the range of experimental conditions to be explored, the initial amounts of the cation I^+ present are always so much larger than the amounts extracted that the equilibrium concentration of I^+ will remain equal to its initial concentration for all extractions to be analyzed in this paper, so that:

$$C_i = C_i^{In}. \tag{29}$$

On the other hand, for the chromophore anion X^-, the amount extracted ($V^* C_x^{Tot^*}$) must equal that initially present in the aqueous phase ($V C_x^{In}$), minus that remaining in the aqueous phase ($V C_x + V C_{ix}$), so that

$$V^* C_x^{Tot^*} = V C_x^{In} - V C_x - V C_{ix}. \tag{30}$$

If virtually all the X^- in the aqueous phase is dissociated, as is expected for the alkali picrates and dinitrophenolates, the last term of Eq. (30) is negligible, so that

$$C_x = C_x^{In} - \frac{V^*}{V} C_x^{Tot^*}, \tag{31}$$

in good approximation.

The total amount of antibiotic in the solvent at equilibrium ($V^* C_s^{Tot^*}$) must equal that present initially ($V^* C_s^{In^*}$) minus that which is lost to the aqueous phase ($V C_s^{Tot}$) so that

$$V^* C_s^{Tot^*} = V^* C_s^{In^*} - V C_s^{Tot}. \tag{32}$$

The total antibiotic concentration in the solvent consists of the amounts in the various forms:

$$C_s^{\mathrm{Tot}*} = C_s^* + C_{is}^* + C_{isx}^* ; \tag{33}$$

whereas in the aqueous solution (allowing for the possibility that some IS^+ complex may form but assuming that formation of ISX will be negligible), the total concentration of S is:

$$C_s^{\mathrm{Tot}} = C_s + C_{is} . \tag{34}$$

Introducing Eqs. (33) and (34) into Eq. (32), we find:

$$V^*(C_s^* + C_{is}^* + C_{isx}^*) = V^* C_s^{\mathrm{In}*} - V(C_s + C_{is}); \tag{35}$$

and recognizing that $(C_{is}^* + C_{isx}^*)$ equals $C_x^{\mathrm{Tot}*}$ by Eqs. (23) and (25), Eq. (35) can be rearranged as:

$$C_s^* = C_s^{\mathrm{In}*} - C_x^{\mathrm{Tot}*} - (C_s + C_{is})\, V/V^* . \tag{36}$$

The last term of Eq. (36) will be negligible for most of the experiments to be presented; so that C_s^* can usually be approximated by:

$$C_s^* = C_s^{\mathrm{In}*} - C_x^{\mathrm{Tot}*}, \tag{37}$$

which expresses C_s^* in terms of the initial conditions and the measured value of $C_x^{\mathrm{Tot}*}$.

All the information needed to analyze the salt extraction equilibria is contained in Eq. (28) together with Eqs. (29), (31), and (37). Before turning to the experiments, however, it will be helpful to deduce explicitly certain expectations for salt extraction for the particular situation in which the complexes are "isosteric" (i.e., have the same size and shape regardless of the particular cation bound). This will make it easier to see, as the data are presented, the extent to which not only the general consequences of reactions (1)–(4) are in accord with experimental observation but also the extent to which the observations in bulk solvents support the simplifications previously noted in Eqs. (12)–(15), which follow directly when the complex is "isosteric".

Particular Expectations for Extraction Equilibria When the Complexes Are "Isosteric"

Effects of Varying the Solvent. The expectations for the effects of varying the solvent are easily assessed in those situations for which reaction (16), and hence the second term of Eq. (28), is negligible. In this case,

only the effects on reaction (7) are important. K_i, defined in Eq. (8), can be written explicitly for two different solvents (′) and (″) by denoting by (′) and (″) those parameters whose values depend on the solvents:

$$K_i' = \frac{k_{is}' k_x'}{k_s'} K_{is}^+, \tag{38}$$

and

$$K_i'' = \frac{k_{is}'' k_x''}{k_s''} K_{is}^+. \tag{39}$$

Notice that although k_{is}, k_x, and k_s are all expected from their definitions in Eqs. (3) and (4) to depend on the solvent, K_{is}^+ is totally independent of the solvent.

Taking the ratio K_i/K_j between two cations (for a given anion) in each of these solvents, we obtain from Eqs. (38) and (39) the ratios:

$$\frac{K_j'}{K_i'} = \frac{k_{js}'}{k_{is}'} \frac{K_{js}^+}{K_{is}^+}, \tag{40}$$

and

$$\frac{K_j''}{K_i''} = \frac{k_{js}''}{k_{is}''} \frac{K_{js}^+}{K_{is}^+}, \tag{41}$$

respectively. From Eqs. (40) and (41), it should be apparent that the ratios of K_j/K_i measured for two different solvents are expected to differ only to the extent that k_{js}/k_{is}, the ratio of partition coefficients of the IS^+ and JS^+ complexes, differs in the two solvents. When the overall size of the complex in a given solvent is the same for all cations, the partition coefficients of IS^+ and JS^+ are expected to be equal to each other, as has been noted in Eq. (12), so that

$$\frac{k_{js}'}{k_{is}'} = \frac{k_{js}''}{k_{is}''} = 1. \tag{42}$$

Substituting Eq. (42) in Eqs. (40) and (41), and recalling that K_{js}^+/K_{is}^+ does not depend on the solvent, we obtain the important expectation for "isosteric" complexes:

$$\frac{K_j'}{K_i'} = \frac{K_j''}{K_i''}. \tag{43}$$

Eq. (43) signifies that for "isosteric" complexes the ratio of salt extraction constants should be independent of the solvent in which it is measured. The satisfactory extent to which this expectation is verified will be demonstrated in Table 5.

A very convenient alternative way of expressing the theoretical expectations of varying the solvent follows from rearranging Eq. (13) in the form:

$$K_i = \frac{K_{is}^+}{K_{js}^+} K_j, \tag{44}$$

which, in logarithmic form, for the case of $J^+ = K^+$ is:

$$\mathrm{Log}\, K_i = \mathrm{Log}\, K_K + \mathrm{Log} \frac{K_{is}^+}{K_{Ks}^+}. \tag{45}$$

Eq. (45) indicates that a plot of $\log K_i$ for the various solvents as a function of $\mathrm{Log} K_K$ for these solvents should yield straight lines of slope equal to 1 and y-intercept equal to $\log(K_{is}^+/K_{Ks}^+)$. Such a plot will be presented in Fig. 8.

Effects of the Neutralization Reaction

Another consequence of the complex having the same size and shape for all cations is that K_{isx}^*, the equilibrium constant of the neutralization reaction (16), should be independent of the species of the cation bound if the neutralization is predominantly due to electrostatic ion pairing of the Bjerrum type, as seems likely. Therefore, we expect:

$$K_{isx}^* = K_{jsx}^*. \tag{46}$$

A measurement of K_{isx}^* in solvents of low dielectric constant for various cations provides an independent way to test the assumption that the size of the complex is independent of the species of cation bound. This will be verified in Fig. 4 [4].

Methods

Following the procedure introduced by Pedersen (1968) for the cyclic polyethers, the amounts of salt extracted into various organic solvents by the macrotetralide actins were characterized by equilibrating a known volume, V^*, of solvent containing initially

4 Eq. (46) has interesting implications for the selectivity measured for salt extraction even when the neutralization reaction [Eq. (16)] is not negligible. In the limit where all of the picrate extracted is in the form of the neutralized ISX^* complex, the equilibrium constant of the overall reaction is given by $K_i K_{isx}^*$. Taking the ratio of this for two different cations and recalling Eq. (46), it is clear that

$$\frac{K_{jsx}^* K_j}{K_{isx}^* K_i} = \frac{K_j}{K_i}, \tag{47}$$

indicating an alternative way of measuring the desired K_j/K_i ratio which should apply in the limit of complete neutralization.

a known concentration of antibiotic, C_s^{In*}, with a known volume, V, of aqueous solution containing an initially known concentration of the hydroxides or chlorides of Li, Na, K. Rb, Cs, NH_4, Ca, Mg, and Th in the presence of a known concentration of a lipophilic chromophore (e.g., picrate or dinitrophenolate). Then the equilibrium concentration of the chromophore anion in the solvent phase, C_x^{Tot*}, was measured optically. Equilibrium was reached easily and rapidly by shaking the solutions vigorously in Pyrex tubes with Teflon-lined screw caps, and the phases were separated by centrifugation at moderate speeds. Controls in which solutions were shaken three times at 5-min intervals and then subsequently at 1 hr, 24 hr, and several weeks later, showed that extraction was complete after the first few shakings. Standard spectrophotometric techniques (Matsen, 1956) were found to be convenient and adequate for characterizing the extractions; usually only the optical absorbance of the organic phases was measured since a series of comparisons with measurements on the aqueous phase gave virtually identical results. From these measurements, it is possible to calculate the concentrations C_i, C_x, and C_s^* which appear in Eq. (28) and which correspond to the concentrations at equilibrium of the cation and chromophore anion in the aqueous phase and of the unreacted antibiotic molecule in the organic solvent phase.

All salt solutions were prepared from analytical grade chemicals of better than 99.9% purity as described elsewhere (Eisenman, 1965). Aqueous picrate and dinitrophenolate solutions were prepared by adding appropriate volumes of standard solutions of picric acid or 2,4-dinitrophenol (Eastman) to the salt solutions. When necessary, the aqueous solutions were neutralized with slight excesses of LiOH. Distilled tap water, redistilled using a Corning Pyrex glass still, was used for preparing all aqueous solutions, except those for which water of especially low NH_4^+ content was prepared by also deionizing the distilled water with a Barnstead mixed-bed ion exchanger (to a conductivity of less than 0.1 ppm as NaCl) and then by redistilling it. The total NH_3 content of the redistilled-distilled water was calculated from extraction experiments to be 10^{-6} M, whereas that of the specially purified water was less than 3×10^{-7} M. Baker (reagent grade) dichloromethane and Eastman (spectral purity) n-hexane were used as solvents without further purification.

Monactin, dinactin, and trinactin were generously supplied by Dr. Hans Bickel of CIBA and were used without further purification; nonactin (SQ 15,859) was a gift from Miss Barbara Stearns of the E.R. Squibb Company. A Cahn electrobalance was used for weighing these. All solutions were stored at +2 °C when not in use, but all experiments were performed at 23 ± 1 °C. The solutions were found to be stable over a period of at least 6 months under these conditions. All solutions were prepared with an estimated accuracy of better than 1%.

The concentration of picrate (or dinitrophenolate) in the organic and aqueous phases was determined from the optical absorbance measured with a Beckman DBG Spectrophotometer for a variety of path lengths (0.1 to 4.0 cm) of the absorption cell, depending on the optical density of the sample. All absorbances were measured relative to 100% transmission at 520 mμ compared to the pure solvent in the reference beam. In all cases, the expected linearity of the absorption with path length and concentration was verified experimentally. For the low extractions when n-hexane was the solvent, it was necessary to use the maximum possible sensitivity by measuring the percent transmission and expanding the 90 to 100% range of transmission to full scale. Control measurements of the uptake of salt in the absence of added antibiotic established that such uptake was negligible except as described for the data for n-hexane in Tables 1 and 2 where corrections were necessary using the values of blanks for zero picrate and zero monactin.

The absorbance was measured at the wave length of maximum absorption, which was verified for every measurement by recording the spectrum in the region of the

peak. The wave length for maximum absorption was found to be a constant characteristic of the solvent and independent of species or concentration of cation or antibiotic. (For 2,4-dinitrophenolate, there are two absorption maxima.) For the macrotetralide actins, there was no discernible shift in the absorption spectrum of picrate between its dissociated form (X^{-*}) and the neutral pair (ISX^*) formed by interaction with IS^{+*}. Therefore, only the total amount of picrate taken up in the organic phase could be measured, and no distinction as to its state of dissociation could be made from these studies. (An apparent exception to this is the absorption due to the acid form of picric acid, which occurs at shorter wave lengths than picrate.)

The molar extinction coefficients, ε, which define the absorbance per mole of chromophore were measured for picrate and dinitrophenolate ions in the various solvents as well as in water in order to calculate the concentrations from the measured absorbances. The extinction coefficient of picrate in aqueous solutions was measured to be 13,700 for H_2O (at 356 mμ) and 18,300 for dichloromethane (at 378 mμ). It has been estimated to be 15,000 at 345 mμ in n-hexane (Eisenman et al., 1968), which is sufficiently accurate for our purposes. For the mixed solvent, 64% hexane – 36% dichloromethane (V/V), the absorption maximum was found to occur at 377 mμ, as in pure dichloromethane, and the extinction coefficient was therefore taken to be 18,300, as in pure CH_2Cl_2, which is sufficiently accurate for our purposes. For 2,4-dinitrophenolate, the extinction coefficient in water was found to be 13,600 (at 364 mμ); in dichloromethane, there are absorption maxima at 373 and 422 mμ with extinction coefficients of 16,150 and 15,900, respectively. In all cases, the units of ε are in 1,000 cm^2/mole.

Because the macrotetralide actins have a significant absorption at 214 mμ, it was possible, since n-hexane is sufficiently transparent at this wave length, to check directly that losses of these from n-hexane into the aqueous phase were negligible. Further controls that such losses are also negligible in the case of CH_2Cl_2 are provided by the agreement in results for two series of experiments performed for ratios of aqueous to organic phases differing by a factor of five (*compare* Tables 13 and 14).

Values for the activity coefficient product ($y_i y_x$) were calculated from tabulated values for the mean activity coefficients of the salts (Harned & Owen, 1958; Robinson & Stokes, 1959), assuming the activity coefficient for picrate to equal that of the OH^- or Cl^- anion. Additional experimental details will be given, where appropriate, in the text.

Results

In the first part of this section, we examine the ability of the typical macrotetralide antibiotic, monactin, to extract salts of monovalent cations and lipid-compatible anions (typically picrate and dinitrophenolate) into solvents of dielectric constant varying from as low as 2.023 for n-hexane (Weissberger, Proskauer, Riddick, & Toops, 1955) to as high as 9.08 for dichloromethane (Weissberger et al., 1955). These studies will verify the correctness and completeness of reactions (1)–(4) to describe the chemistry of the interactions of the macrotetralides with cations. In particular, the experimental results will verify that complex formation occurs according to the 1:1 stoichiometry of reaction (1), that the neutralization reaction (2) can occur to a significant extent in solvents of low dielectric constant, that the chemistry is remarkably ideal, that the equilibrium constant K_i of the

salt extraction reaction (7) can be characterized accurately, and that an estimate can be made of the value of K_{isx}^{*} of reaction (16).

The experimental results also demonstrate that the values of K_i depend markedly on the dielectric constant of the solvent (as well as on the partition coefficient of the lipophilic anion) in the manner theoretically expected. Nevertheless, the data show that the ratio of K_i/K_j is independent of these variables, a finding expected only if the complexes are "isosteric". This characterization lays the basis for the examination in the second part of this section of the effects of varying the molecular composition in the macrotetralide actin series: nonactin, monactin, dinactin, and trinactin.

Before presenting the results, it will be helpful to the reader unfamiliar with experiments of the present type to comment on their reproducibility and accuracy. Since the experimental system is so simple (as well as non-living), measurements can be made from minute to minute, day to day, or month to month with repeatabilities quite common in analytical chemistry but quite unfamiliar to those accustomed to dealing with biological preparations. Indeed, except for the difficult series of experiments where the solvent was pure n-hexane, which were only marginally satisfactory because of the extremely small concentrations of picrate extracted, the accuracy with which the picrate could be measured spectrophotometrically was never the limiting factor. For example, not only was the reproducibility of a given absorbance measurement always better than 0.2%, but also duplicate determinations of the absorbance for separate samples of a given solution always agreed this well. Indeed, the accuracy of the measurement is only limited by the accuracy with which the solutions can be prepared, provided the same absorption cell is used for all measurements and calibrations. Duplicate samples of solutions prepared by volumetric methods had absorbances which agreed to better than 1% in all cases (corresponding to better than 2.5% in terms of concentration). Even when gravimetric methods were necessary, the accuracy was better than 5% in terms of concentration.

Because spot checks using duplicate solutions in each experiment always agreed to better than 5% in terms of concentration, it was not necessary to carry out a further analysis of the errors, which are negligible for the purpose of the paper. All deviations from the theoretical curves represent real and reproducible phenomena. At low salt concentrations, these deviations are principally due to traces of such species as NH_4^+ in the distilled water; at high concentrations, they represent the effects of the small losses of antibiotic to the aqueous phase, which are usually neglected when using the approximate Eq. (37) instead of the more precise form

of Eq. (36). Since neither of these effects is significant over the middle range of our data, they were not analyzed further, although the data are sufficiently reproducible that they could have been if it had been felt worthwhile.

The internal consistency of the present results can be seen by comparing all of the data to be presented from point to point and from table to table (for the most reliable middle range of experimental conditions). To emphasize this consistency, it is worth noting such examples as the K_i values calculated in Table 3 for hexane-dichloromethane where K_i is seen to be 0.00227 and 0.00228 for 0.0045 M and 0.0090 M RbOH, respectively (as well as 0.0104 and 0.0107 for 0.0045 M and 0.009 M KOH, respectively). Similarly, good agreement can be seen for pure dichloromethane in Table 4 by comparing the italicized values of K_i. Even for the difficult experiments with pure n-hexane, the differences seen between duplicate determinations in Table 2 do not preclude the semiquantitative analysis of the data.

1. General Characteristics of the Equilibrium Extraction of Salts by the Typical Macrotetralide, Monactin

n-Hexane

The following section is presented in small print because of the difficulty in making reliable measurements on the barely detectable salt extractions produced by the macrotetralide actins in a solvent having as low a dielectric constant as n-hexane. The data will nevertheless be presented not only because this solvent has been considered to be an appropriate model for the interior of a phospholipid membrane (Eisenman et al., 1968), but also because the results are consistent with those observed in hexane-dichloromethane mixtures and in pure dichloromethane. The data for n-hexane can even be analyzed semiquantitatively despite the large experimental errors.

The ability of a neutral macrocyclic molecule such as monactin to extract the picrate salts of the alkali metal cations into n-hexane is demonstrated by the data of Tables 1 and 2 and Figs. 1 and 2. Table 1 and Figs. 1 and 2 present the results of a typical experiment in which the uptake of picrate was studied by equilibrating 10-ml aliquots of 2×10^{-4} M monactin in n-hexane with 10-ml volumes of aqueous solutions containing 2×10^{-4} M picric acid and the indicated concentrations of KOH. In the absence of added monactin, n-hexane shows no detectable uptake of picrate from any of the solutions in this experiment, as indicated by the row labelled "zero monactin" in the table and the curves labelled "zero monactin" of Figs. 1 and 2. However, 2×10^{-4} M monactin produces the measurable, albeit very small, extraction of picrate indicated in the column labelled "A^*_{348}", for the optical absorbance at 348 mμ, from which the concentrations of picrate tabulated in the column labelled "C_x^{Tot*}" were calculated.

Table 1. *Extraction of picrate into hexane by monactin*[a] *(Initial conditions:* $C^{In*}_{Monactin} = C^{In}_{Picrate} = 2 \times 10^{-4}$ M; $V^* = V = 10$ ml)

C^{In}_{KOH}	A^*_{348}	C_x^{Tot*} ($\times 10^{-7}$)	C_x ($\times 10^{-4}$)	C_s^* ($\times 10^{-4}$)	$y_i y_x$	K_i ($\times 10^{-6}$)
Zero monactin	0.000000	0.0000				
0.00988	0.0000	0.00	2.000	2.000	0.81	—
0.0988	0.000800	0.533	1.9995	1.9995	0.62	1.2
0.1976	0.000875	0.583	1.9994	1.9994	0.57	0.75
0.3952	0.00182	1.123	1.9988	1.9988	0.54	—
0.5928	0.00194	1.293	1.9987	1.9987	0.53	—
0.790	0.00237	1.580	1.9984	1.9984	0.55	—
0.988	0.00281	1.873	1.9981	1.9981	0.57	—

[a] The tabulated values of absorbances per cm were calculated from the percent transmission measured at 348 mμ using a 4.0-cm path length. These absorbances have been corrected for the slight reduction in transmittance to 99.25% at 348 mμ relative to 520 mμ in the absence of picrate but in the presence of monactin. This corresponds to an absorbance per cm due to the monactin of 0.000822 at 348 mμ. The values of K_i in the last column were calculated by the first term of Eq. (28) for the experimental points for which the effects of traces of NH_4^+ as well as the neutralization reaction are negligible. Picrate concentrations were calculated from them assuming the molar extinction coefficient to be 15,000 (1,000 cm²/mole). All concentrations in this and subsequent tables are given in moles per liter of solution. Note that the maximum picrate extracted (1.873×10^{-7} M) is less than 0.1% of the initial concentrations of both picrate or monactin (2×10^{-4} M).

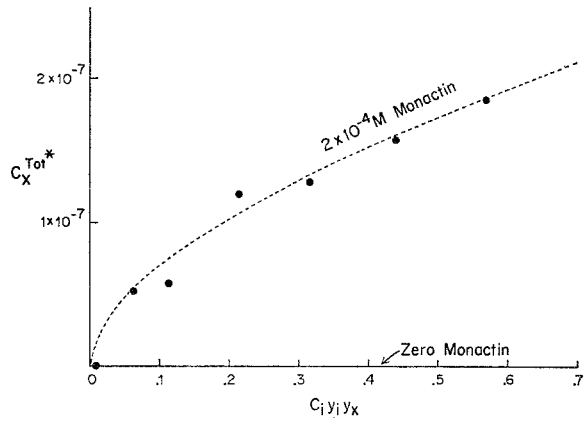

Fig. 1. Extraction of potassium picrate into n-hexane by monactin. The experimentally observed concentrations in moles per liter of picrate extracted by 2×10^{-4} M monactin are plotted as points as a function of $(C_i y_i y_x)$. For comparison, the dashed curve represents the theoretical expectations of Eq. (28) for $K_i = 0.99 \times 10^{-6}$ and $K^*_{isx} = 0.17 \times 10^7$. Note that $C_x C_s^*$ has an essentially constant value of 4×10^{-8} for this experiment so that the abscissa is proportional to $(C_i C_x C_s^* y_i y_x)$

From the values of C_x^{Tot*}, the equilibrium concentrations of C_x (the picrate remaining in the aqueous phase) and C_s^* (the uncomplexed monactin in the hexane phase) were calculated using Eqs. (*31*) and (*36*) to yield the values in the fourth and fifth columns

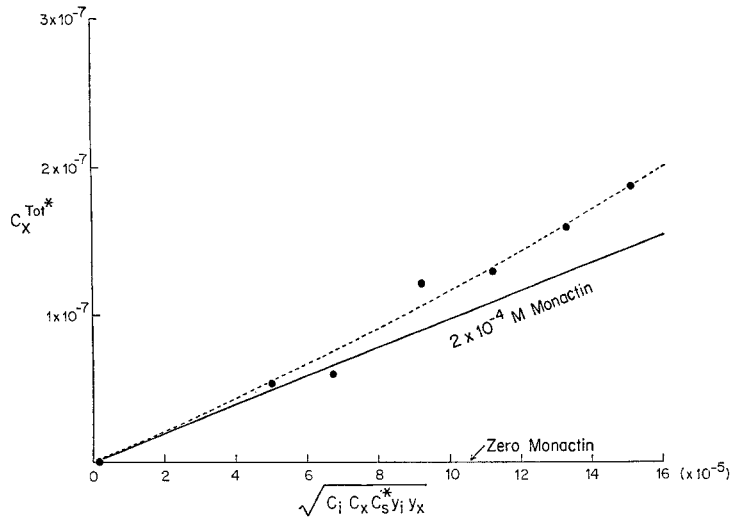

Fig. 2. Square root dependence of the extraction of potassium picrate into n-hexane by monactin. The picrate extracted in moles per liter is plotted as a function of $\sqrt{C_i C_x C_s^* y_i y_x}$ to illustrate the square root dependence at low extractions expected from the first term of Eq. (28) as indicated by the solid line. The slope of this line signifies that $K_i = 0.99 \times 10^{-6}$ moles/liter; the dashed curve gives the theoretical expectations of Eq. (28) when $K_{isx}^* = 0.17 \times 10^7$

of Table 1, which differ only slightly from their initial values since so little salt is extracted into hexane[5].

To compare the salt extraction observed with that expected theoretically from Eq. (28), it is only necessary to plot C_x^{Tot*}, the concentration of picrate extracted, against $(C_i C_x C_s^* y_i y_x)$, as in Fig. 1, and against $(C_i C_x C_s^* y_i y_x)^{1/2}$, as in Fig. 2. The data of Fig. 1 show the curvilinear uptake expected from Eq. (28); the square root plot of Fig. 2 demonstrates the expected linear dependence in the limit of sufficiently low concentrations such that the second term is negligible.

From the limiting slope at low concentration of Fig. 2, K_i is calculated to be 1.0×10^{-6} liters/mole (in agreement with the values of 0.87×10^{-6} and 1.32×10^{-6} which were obtained under different experimental conditions in the data to be presented in Table 2). From the higher concentration data, K_{isx}^* is estimated to be approximately 0.17×10^7 liters/mole. Using these values, the theoretical expectations of Eq. (28) are indicated by the dashed curves for comparison with the experimental data.

Values of K_i and K_i/K_j. Because the extraction of the picrates of the other alkali metal cations by monactin is even smaller than that for K^+, it is even more difficult to characterize the equilibria for these. Nevertheless, by quadrupling the concentration of monactin in one series of experiments and by doubling both the monactin and the picrate in another, estimates were obtained for the values of K_i for the other cations. These are summarized in Table 2. Approximate values of K_i and K_i/K_K have been

5 The maximum concentration of picrate extracted by monactin in this experiment (i.e., from 0.988 M KOH) is only 1.87×10^{-7} M, which is nearly 100 times lower than the amount extracted by a cyclic polyether under the same conditions (*see* Fig. 8 of Eisenman et al., 1968).

Table 2. *Extraction of alkali metal picrates into hexane by monactin*[a]

	C^{In}_{MCl}	A^{*}_{348}	C_x^{Tot*} ($\times 10^{-7}$)	C_x ($\times 10^{-4}$)	C_s^{*} ($\times 10^{-4}$)	K_i ($\times 10^{-6}$)	K_i/K_K
(Initial conditions: $C^{In*}_{Monactin} = 8 \times 10^{-4}$ M; $C^{In}_{Picrate} = 2 \times 10^{-4}$ M; $V^* = V = 10$ ml)							
LiCl	0.1976	0.00025	0.167	2	8	0.0035	0.004
		0.000218	0.145	2	8	0.0027	0.003
NaCl	0.1976	0.000700	0.467	2	8	0.028	0.032
		0.000575	0.383	2	8	0.019	0.022
CsCl	0.1976	0.0017	1.133	2	8	0.16	0.19
		0.00165	1.100	2	8	0.15	0.18
RbCl	0.1976	0.00288	1.1920	2	8	0.47	0.54
		0.00280	1.867	2	8	0.45	0.51
KCl	0.1976	0.00390	2.600	2	8	0.87	1.0
(Initial conditions: $C^{In*}_{Monactin} = C^{In}_{Picrate} = 4 \times 10^{-4}$ M; $V^* = V = 10$ ml)							
LiCl	0.0988	0.000217	0.145	4	4	0.022	0.016
NaCl	0.0988	0.000475	0.317	4	4	0.102	0.078
		0.000325	0.217	4	4	0.035	0.026
CsCl	0.0988	0.000900	0.60	4	4	0.37	0.28
RbCl	0.0988	0.00120	0.80	4	4	0.65	0.50
		0.00120	0.80	4	4	0.65	0.50
KCl	0.0988	0.00170	1.113	4	4	1.32	1.0

[a] The upper portion presents the results of the extraction of picrate by 8×10^{-4} M monactin from 0.1976 M chloride solutions containing 2×10^{-4} M picric acid alkalinized with 4×10^{-4} M LiOH. The lower portion presents the results of the extractions by 4×10^{-4} M monactin from 0.0988 M chloride solutions containing 4×10^{-4} M picric acid alkalinized with 8×10^{-4} M LiOH. Duplicate results represent duplicate measurements. Absorbances were measured as percent transmission at 348 mμ using a 4.0-cm path length as in Table 1. These absorbances have been corrected for a small absorption due to monactin at zero picrate concentration which corresponds to 99.5% transmittance in the experiment with 8×10^{-4} M monactin and 99.7% transmittance in the experiment containing 4×10^{-4} M monactin. $y_i y_x$ was taken to be 0.52 for the upper portion and 0.59 for the lower portion, using the values from Robinson and Stokes (1959), Appendix 8.10, for KCl for all cations.

calculated from Eq. (28), assuming the last term to be negligible, and are given in the last two columns of the table where duplicate results represent duplicate measurements. That the second term of Eq. (28) is negligible is a reasonable assumption for picrate extractions less than 0.6×10^{-7} M, since the K^+ data of Fig. 2 indicate that the second term is negligible below this concentration.

Examining Table 2, it can be seen that monactin extracts the alkali metal picrates in the sequence K > Rb > Cs > Na > Li, which is the same as the sequence of permeability ratios (and conductance ratios) characteristic of monactin-induced cation permeation of phospholipid bilayers as noted previously (Eisenman et al., 1968). From this correspondence, it was concluded that n-hexane was an appropriate solvent to use as a model for the interior of the bilayer membrane. However, since much more accurate

measurements of K_i can be made by increasing the dielectric constant of the solvent phase, and the value of the K_i/K_j ratio will be shown to be quite independent of the solvent, we move on to salt extraction equilibria in solvents of higher dielectric constant before examining cation selectivity further.

64% Hexane – 36% Dichloromethane

It is possible to increase greatly the amount of salt extracted into the solvent phase, and at the same time to decrease the undesirable effects of association between the complex and picrate, by adding the more polar solvent, dichloromethane (CH_2Cl_2), to n-hexane. Indeed, such an addition to some extent mimics the more polar nature of the normally present double bonds of the unsaturated fatty acids of the phospholipid membrane. In any event, since the ratio of K_i/K_j will be shown presently to be independent of the solvent, it is unnecessary that the model solvent exactly imitate the interior of the membrane in order for us to measure meaningful values of K_i/K_j for comparison with phospholipid bilayers.

Table 3. *Extraction of picrates into 64% hexane – 36% dichloromethane (v/v) by monactin*[a] *(Initial conditions:* $C^{In*}_{Monactin} = 0.2 \times 10^{-4}$ M; $C^{In}_{Picrate} = 10^{-4}$ M; $V^* = 14$ ml, $V = 2$ ml*)*

	C^{In}_{MOH}	A^*_{377}	C_x^{Tot*} ($\times 10^{-6}$)	C_x ($\times 10^{-4}$)	C_s^* ($\times 10^{-4}$)	$y_i y_x$	K_i	K_i/K_k
Zero monactin		0.000000	0.00000					
LiOH	0.09	0.000108	0.00590	0.9996	0.1999	0.52	*0.00000037*	0.000034
NaOH	0.09	0.001295	0.07076	0.9950	0.1993	0.58	*0.000048*	0.0045
CsOH	0.09	0.002075	0.1134	0.9921	0.1989	0.65	*0.00011*	0.0103
RbOH	0.0045	0.002412	0.1318	0.9908	0.1987	0.86	*0.00227*	0.21
	0.009	0.003305	0.1806	0.9874	0.1982	0.81	*0.00228*	0.21
	0.09	0.01192	0.6514	0.9544	0.1935	0.62	—	—
KOH	0.0009	0.001743	0.0952	0.9933	0.1990	0.93	—	—
	0.00225	0.003305	0.1806	0.9874	0.1982	0.90	—	—
	0.0045	0.005105	0.2790	0.9805	0.1972	0.86	*0.0104*	0.97
	0.009	0.007060	0.3858	0.9730	0.1961	0.81	*0.0107*	1.0
	0.09	0.02614	1.428	0.9000	0.1857	0.60	—	—
NH_4OH	0.01	0.01048	0.5683	0.9602	0.1943	1.0	*0.420*	39.2

[a] Absorbances were measured as % transmittance at 377 mμ for a 4.0-cm path length. In the absence of monactin, there was no measurable picrate absorbance at any salt concentration, as indicated in the row labelled "zero monactin". The concentration of dissociated NH_4^+ ion is calculated from the dissociation constant of NH_4OH to be 0.000412 moles/liter for 0.01 M NH_4OH. Values for K_i, calculated according to the first term of Eq. (28), are given for all extractions for which the second term of Eq. (28) is expected to be negligible (i.e., extractions lower than 0.4×10^{-6} M except for NH_4^+ which was slightly higher). Values of K_i for solutions more dilute than 0.0045 M have not been presented because these are influenced by traces of NH_4^+ in the water. Italicized values are considered to be the most reliable.

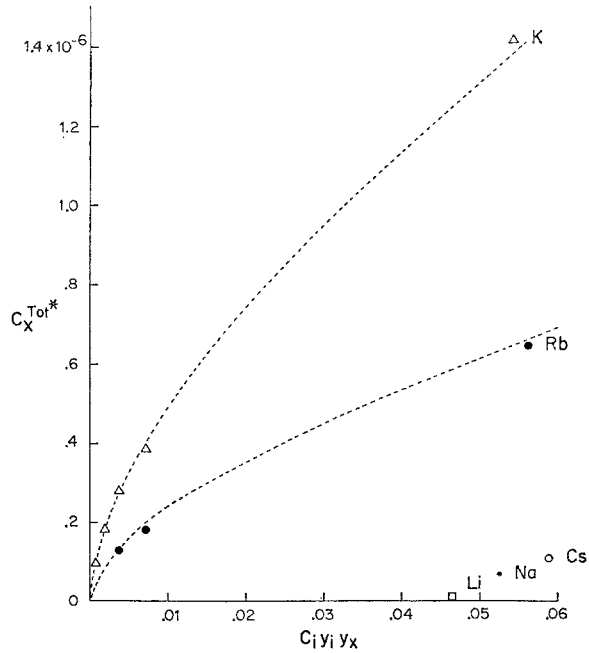

Fig. 3. Extraction of alkali metal picrates into 64% hexane — 36% dichloromethane by monactin. The experimentally observed extractions in moles per liter due to 0.2×10^{-4} M monactin are plotted as points as a function of the aqueous hydroxide concentrations, corrected for activity coefficient effects ($C_i y_i y_x$). In the absence of monactin, there is no detectable extraction. For K^+ and Rb^+, the dashed curves give the theoretically expected extractions calculated by Eq. (28) for $K_K = 0.010$, $K^*_{Ksx} = 5.0 \times 10^5$; $K_{Rb} = 0.0023$, $K^*_{Rbsx} = 6.9 \times 10^5$ assuming for ease of calculation that C_x and C_s^* differ negligibly from their initial values

The extraction by monactin of the picrates of monovalent cations into the mixed solvent 64% hexane and 36% dichloromethane (V/V) is summarized in Table 3 and Figs. 3 and 4. Considerably larger extractions of salt are observed for the hexane-dichloromethane mixture than was the case for pure n-hexane (the highest extraction is 7% in Fig. 3 compared to 0.1% in Fig. 1); the increased accuracy of the data makes the demonstration of the contributions of the two terms of Eq. (28) more convincing. The square root dependence at low extractions, expected from the first term of Eq. (28), is seen particularly clearly in Fig. 4. Since the linearity of uptake with $(C_i C_x C_s^* y_i y_x)^{1/2}$ holds to extractions as high as 0.4×10^{-6} M, these data indicate that the picrate anion and the complexed cation are still dissociated in this solvent at concentrations as high as this.

Values of K_i and K_i/K_j. Values of K_i and of the ratio K_i/K_j can be accurately assessed for the mixed solvent and are given in the last two

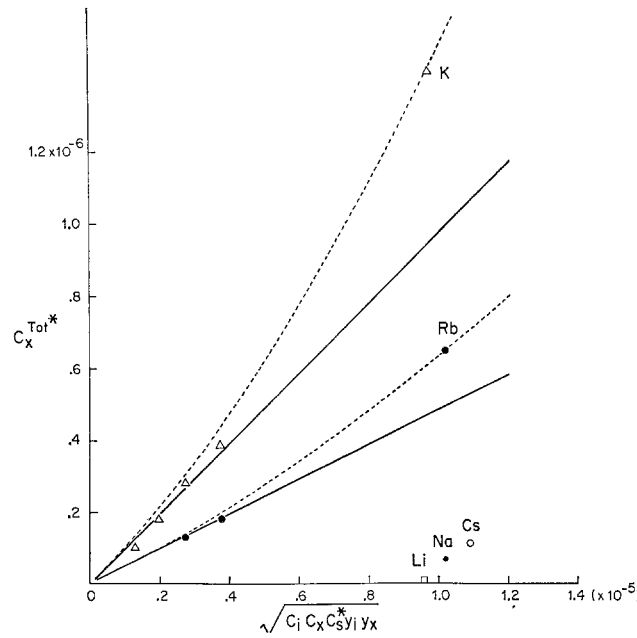

Fig. 4. Square root dependence at low extractions in 64% hexane – 36% dichloromethane. The picrate extractions of Fig. 3 are plotted as a functions of $\sqrt{C_i C_x C_s^* y_i y_x}$ to illustrate the square root dependence at low extractions indicated by the solid lines. The values for K^*_{Ksx} and K^*_{Rbsx} were calculated numerically to be 5.0×10^5 and 6.5×10^5, respectively. The dashed curves give the theoretical extractions calculated by Eq. (28) for $K_K = 0.010$, $K^*_{Ksx} = 5.0 \times 10^5$; $K_{Rb} = 0.0023$, $K^*_{Rbsx} = 6.9 \times 10^5$

columns of Table 3. Comparing the K_i values of Table 3 with those of Table 2 for the corresponding cation species, the magnitudes of K_i are seen to be much larger in hexane-dichloromethane than in hexane (e.g., by four orders of magnitude in the case of K^+). This increase in the equilibrium constant for salt extraction with increasing dielectric constant is exactly what is expected through Eq. (8) from the increase of the partition coefficients k_x and k_{is} of the charged species due to the decrease in the electrical work of taking the charged IS^+ and X^- species from water into the solvent (the effects on the partition coefficient k_s of the neutral species are expected to be less important).

Despite the large change in magnitudes of K_i, the ratios K_i/K_k of Table 3 are in the same selectivity sequence (K > Rb > Cs > Na > Li) as was the case for n-hexane. Thus, these ratios are seen to be independent of the solvent, at least qualitatively. The quantitative aspects of this independence will be examined in Fig. 8, but further comparison of the values of K_i

between hexane and hexane-dichloromethane will be deferred until the salt extraction equilibria have been described for dichloromethane.

Estimated Value of K_{isx}^*. From the data for the highest extractions of K^+ and Rb^+ in Table 3 and Fig. 4, values of K_{isx}^* can be calculated numerically. K_{isx}^* is found to be 5.0×10^5 for K^+ and 6.9×10^5 for Rb^+, which are sufficiently close that we can consider Eq. (46) to be verified. This result provides supporting evidence for the postulate that the complex is "isosteric". Moreover, the value of K_{ksx}^* is seen to be smaller than that measured for n-hexane, which is in the proper direction for the decreased interactions between IS^{+*} and X^{-*} expected to result from the increase in dielectric constant. For comparison with the experimentally observed data points, the theoretical expectations of Eq. (28) calculated for the indicated values of K_i and K_{isx}^* are presented as dashed curves in Figs. 3 and 4.

Dichloromethane

Typical data for the extraction by monactin into dichloromethane of the picrates of Li, Na, K, Rb, Cs, and NH_4 are summarized in Table 4 and Figs. 5–7. Except for the difference of solvent, the experimental conditions of Table 4 and Fig. 5 are similar to those of Table 3 and Fig. 3; thus, these figures and tables can be easily compared. In the absence of monactin, no significant uptake of picrate by the dichloromethane phase was measurable from any of the solutions studied, as indicated by the data in the table labelled "zero monactin". However, in the presence of 0.2×10^{-4} M monactin, the large extractions of Fig. 5 and Table 4 are observed. These data indicate that the salt extraction by monactin is very efficient in this solvent. Indeed, 93% of the picrate is extracted at the highest KOH concentration of Fig. 5. [For comparison with the observed data points, theoretical curves are drawn according to the expectations of the first term of Eq. (28) for the values of K_i calculated from Fig. 7.]

For dichloromethane, the extraction is sufficiently large that a detailed comparison between experimental observation and theoretical expectation is possible for all cations. This is conveniently done by expressing Eq. (28) in logarithmic form which, in the limiting case when the neutralization reaction (16) is negligible, reduces to

$$\log C_x^{\mathrm{Tot}*} = \tfrac{1}{2} \log (C_i C_x C_s^* y_i y_x) + \tfrac{1}{2} \log K_i , \tag{48}$$

or, in the extreme case when reaction (16) goes to completion, becomes

$$\log C_x^{\mathrm{Tot}*} = \log (C_i C_x C_s^* y_i y_x) + \log K_{isx}^* K_i . \tag{49}$$

Table 4. *Extraction of the picrates into dichloromethane by monactin*[a]
(Initial conditions: $C^{In}_{Picrate} = 1.0 \times 10^{-4}$ M; $C^{In*}_{Monactin} = 0.20 \times 10^{-4}$ M; $V^* = 10$ ml, $V = 2$ ml*)*

	C^{In}_{MOH}	A^*_{378}	C_x^{Tot*} ($\times 10^{-4}$)	C_x ($\times 10^{-4}$)	C_s^* ($\times 10^{-4}$)	$y_i y_x$	K_i
Zero monactin		0.000	0.0000				
LiOH	0.0009	(0.078)	(0.0426)	(0.787)	(0.1574)	0.93	—
	0.00225	(0.035)	(0.0191)	(0.9045)	(0.1809)	0.90	—
	0.0045	(0.026)	(0.0142)	(0.929)	(0.1858)	0.86	—
	0.009	(0.028)	(0.0153)	(0.9235)	(0.1847)	0.81	(0.19)
	0.09	0.050	0.0273	0.8635	0.1727	0.52	*0.11*
NaOH	0.009	(0.088)	(0.0481)	(0.7595)	(0.1519)	0.93	—
	0.00225	(0.087)	(0.0475)	(0.7625)	(0.1525)	0.90	(9.6)
	0.0045	0.104	0.0568	0.716	0.1432	0.86	*8.1*
	0.009	0.129	0.0705	0.6475	0.1295	0.81	*8.1*
	0.09	0.211	0.1153	0.4235	0.0847	0.58	7.1
CsOH	0.0009	(0.125)	(0.0683)	(0.6585)	(0.1317)	0.93	—
	0.00225	(0.126)	(0.0688)	(0.656)	(0.1312)	0.90	(27)
	0.0045	0.151	0.0825	0.5875	0.1175	0.86	*25*
	0.009	0.179	0.0978	0.511	0.1022	0.81	*25*
	0.09	0.264	0.1443	0.2785	0.0557	0.65	23
RbOH	0.0009	(0.208)	(0.1137)	(0.4135)	(0.0863)	0.93	—
	0.00225	0.233	0.1273	0.3635	0.0727	0.90	*300*
	0.0045	0.261	0.1426	0.287	0.0574	0.86	*320*
	0.009	0.280	0.1530	0.235	0.0470	0.81	*290*
	0.09	0.320	0.1749	0.1255	0.0251	0.62	170
KOH	0.009	(0.248)	(0.1355)	(0.3225)	(0.0645)	0.93	—
	0.00225	0.275	0.1503	0.2485	0.0497	0.90	*900*
	0.0045	0.293	0.1601	0.1995	0.0399	0.86	*830*
	0.009	0.310	0.1694	0.153	0.0306	0.81	*840*
	0.09	0.340	0.1858	0.071	0.0142	0.60	630
NH_4OH	0.01	0.312	0.1705	0.1475	0.0295	1.0	16,000

[a] Parenthesized values are influenced by the trace amounts of NH_4^+. The italics values of K_i, calculated according to the first term of Eq. (28), are considered the most reliable. Note that the concentration of dissociated NH_4^+ in 0.01 M NH_4OH is calculated to be 0.000412 M. 4.0-cm path length.

Plotting $\log C_x^{Tot*}$ vs. $\log(C_i C_x C_s^* y_i y_x)$, a linear dependence with slope $\frac{1}{2}$ is therefore predicted if the neutralization reaction is negligible, whereas a slope of 1 is expected if the neutralization reaction goes to completion.

Fig. 6 presents the data of Table 4 in this manner, and a linear dependence with slope $\frac{1}{2}$ is seen. Therefore, Eq. (48) suffices to describe the data; we can conclude that the extraction in this solvent is due to reaction (7)

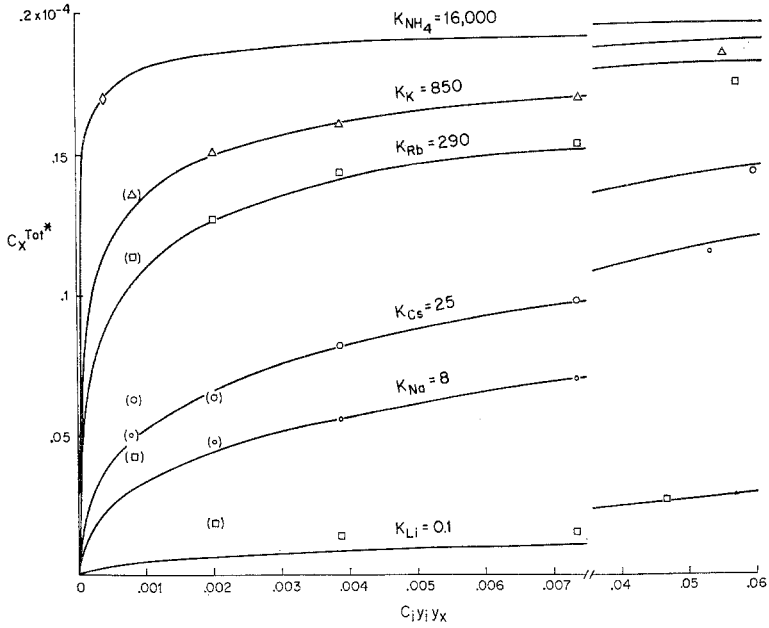

Fig. 5. Extraction of picrates into dichloromethane by monactin. The concentration of picrate extracted is plotted as a function of the aqueous hydroxide concentration corrected for activity coefficient effects. The units of the ordinate and abscissa are moles per liter. The points are experimentally observed, the curves theoretically calculated from the first term alone of Eq. (28). Note that the abscissa is interrupted at the right and also that the extractions for the most preferred ions approach the maximum possible of 0.2×10^{-4} moles/liter under these experimental conditions

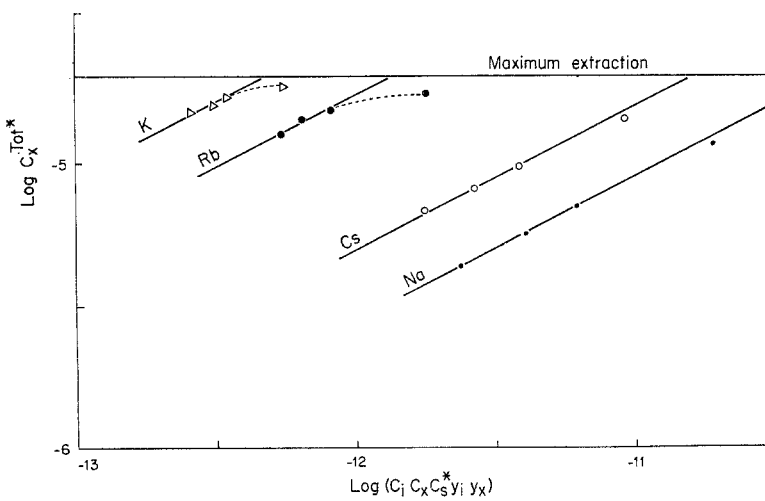

Fig. 6. Demonstration of the square root dependence of picrate extracted on $(C_i C_x C_s^* y_i y_x)$. The logarithms of the experimentally measured picrate concentrations in dichloromethane are plotted as points, whereas the solid lines are drawn with slope $\frac{1}{2}$ as expected from Eq. (48). Note that the extractions approach the maximum possible of 0.2×10^{-4} moles/liter, indicated by the horizontal line, so that the deviations indicated by the dashed lines are not considered to be significant

alone, ion-pairing in this solvent being entirely negligible[6]. Such a decrease in ion-pairing compared to that in hexane-dichloromethane is as expected theoretically from the higher dielectric constant of pure dichloromethane. We therefore conclude that K^*_{isx} is sufficiently small in dichloromethane that values of K_i can be measured directly and precisely in this solvent over the range of extractions explored here.

Values of K_i and K_i/K_j. Although values for K_i can be read directly from the y-intercepts of the lines of Fig. 6, they are more conveniently obtained by plotting $\log(C_x^{\mathrm{Tot}*2}/C_x C_s^*)$ vs. $\log(C_i y_i y_x)$ according to the theoretical expectation for the first term of Eq. (28), when rearranged as:

$$\log(C_x^{\mathrm{Tot}*2}/C_x C_s^*)=\log(C_i y_i y_x)+\log K_i. \tag{50}$$

Eq. (50) predicts a straight line of slope 1 and y-intercept of $\log K_i$. Such plots for the data of Table 4 are presented in Fig. 7 and show that Eq. (50) represents the observed data quite well[7]. It is from the values of K_i so obtained that the theoretical curves of Fig. 5 were drawn. The excellent agreement seen in Figs. 5–7 between the experimental data and the expectations of the first term of Eq. (28) will be shown later (*see* Fig. 10) to hold over an extremely wide range of experimental conditions.

Analysis of the Effects of Varying the Solvent on the Values of K_i and K_i/K_j

Values of K_i extracted from the data of Tables 2–4 (together with Figs. 4 and 7) are summarized in the upper portion of Table 5. Comparing the values for a given cation species for the three solvents, it is clear that

6 The deviations seen for Rb^+ and K^+ at the highest extractions are in the opposite direction from those expected from the neutralization reaction. These are probably due to small losses of monactin into the aqueous phase, which could be analyzed further by using Eq. (36) in place of the approximate Eq. (37) used here. Extractions for concentrations lower than 0.00225 M have not been plotted in Fig. 6 because these are affected by traces of NH_4^+ in the Pyrex-redistilled distilled water used for these experiments. For the same reason, the data for Li^+ have not been plotted because only the highest concentration point is unaffected by the presence of NH_4^+.

7 Deviations from theoretical expectations (indicated by the dashed lines in Fig. 7) are seen at the lowest concentrations in Figs. 5 and 7 (particularly for the most poorly extracted cations, Li^+ and Na^+). These deviations are attributable to traces of ammonia (10^{-6} M) present in the Pyrex-redistilled (but not deionized) distilled water used in this experiment. The effects of NH_4^+ become more negligible at higher concentrations not only because of the increase in concentration of the cation relative to NH_4^+ but also through suppression of the NH_4^+ ionization by the increasing OH^- concentration. For clarity, all data in Table 4 which are significantly influenced by NH_4^+ have been parenthesized and will be thus indicated in subsequent tables also. This effect can be decreased by deionizing the distilled water prior to redistilling; data for Li^+ and Na^+ using such especially purified H_2O are given in Fig. 13 for dinactin and trinactin.

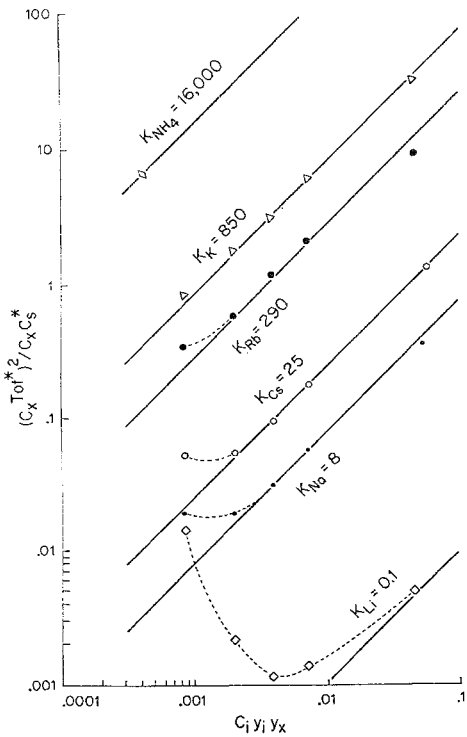

Fig. 7. Equilibrium extraction of the picrate salts of Li, Na, K, Rb, Cs, and NH_4 into a dichloromethane phase containing 2×10^{-5} M monactin. The data are presented in logarithmic form according to the expectations of Eq. (50). The ordinate is dimensionless; the abscissa is in moles per liter of solution

Table 5. *Effects of solvent on the equilibrium constants for alkali picrate extraction by monactin*[a]

Equilibrium constant	Solvent	Li	Na	K	Rb	Cs	NH_4
K_i	CH_2Cl_2	0.10	8.0	850	290	25	16,000
	64% hexane – 36% CH_2Cl_2	0.00000037	0.000048	0.0104	0.0023	0.00011	0.42
	hexane	0.0031 $\times 10^{-6}$	0.024 $\times 10^{-6}$	[0.87 $\times 10^{-6}$]	[0.46 $\times 10^{-6}$]	[0.16 $\times 10^{-6}$]	–
K_i/K_K	CH_2Cl_2	0.00012	0.0094	1.0	0.34	0.029	18.8
	64% hexane – 36% CH_2Cl_2	0.000036	0.0046	1.0	0.22	0.011	40.4
	hexane	[0.0035]	[0.027]	[1.0]	[0.53]	[0.19]	–

[a] The K_i values for CH_2Cl_2 are from Fig. 7, for hexane-dichloromethane from Table 3, and for n-hexane from the upperpart of Table 2. Brackets have been used to indicate those values for which the effects of the second term of Eq. (28) may not be entirely negligible.

the magnitudes of K_i depend greatly on the solvent, increasing nine orders of magnitude in the case of K^+ with the increase of dielectric constant from 2.023 to 9.08 between hexane and dichloromethane! This is as expected theoretically according to Eq. (8), since the predominant effect of increasing the dielectric constant of the solvent should be to increase the values of both k_{is} and k_x, the partition coefficients of the charged species, thereby increasing K_i; the effect on k_s for the neutral species should be much smaller.

In view of the immense changes in the magnitude of K_i when the solvent is varied, the ratios K_i/K_j are seen in the lower portion of Table 5 to be remarkably independent of the solvent, even though they are not precisely the same. Notice, in particular, the closeness of the agreement for these ratios for given pairs of ions for dichloromethane and for the hexane-dichloromethane mixture. Even for the considerably less reliable data for hexane, the agreement is surprisingly good. Since there is no systematic trend in the values of the ratios with decreasing dielectric constant, we attribute the apparent differences for hexane to the experimental difficulty of measuring K_i accurately in this solvent.

An alternative way of illustrating the effect of varying the solvent on K_i and K_i/K_j is through Fig. 8, which plots $\log K_i$ vs. $\log K_K$ for each of these solvents to test the expectations of Eq. (45). The straight lines of unit slope show the theoretical expectations, the dashed lines show deviations from these. The parallelism between the curves indicates that the K_i/K_K ratio is remarkably constant, considering the large variation in K_i encompassed in these experiments (note the logarithmic scales).

We therefore reach the important conclusion that, although the magnitudes of the equilibrium constants of reaction (7) are highly sensitive to the dielectric constant of the solvent, as indeed they are expected to be from the present theory, *the ratios of these equilibrium constants are essentially independent of the solvent.* This finding indicates that the interaction between the complex and the solvent is essentially the same regardless of which cation is sequestered in the interior of the molecule. It therefore provides striking experimental support for the "isosteric" postulate that the size and shape of the complex is independent of the particular cation bound. This particular property of the macrotetralide actin antibiotics leads to drastic simplifications in the theoretically expected effects of these molecules on bulk phases and on membranes, as was noted in the theory section. Such a property, however, is not necessarily a characteristic of all neutral sequestering molecules. Indeed, these "isosteric" complexes are not expected for the cyclic polyethers, nor are the ratios of K_i/K_j found

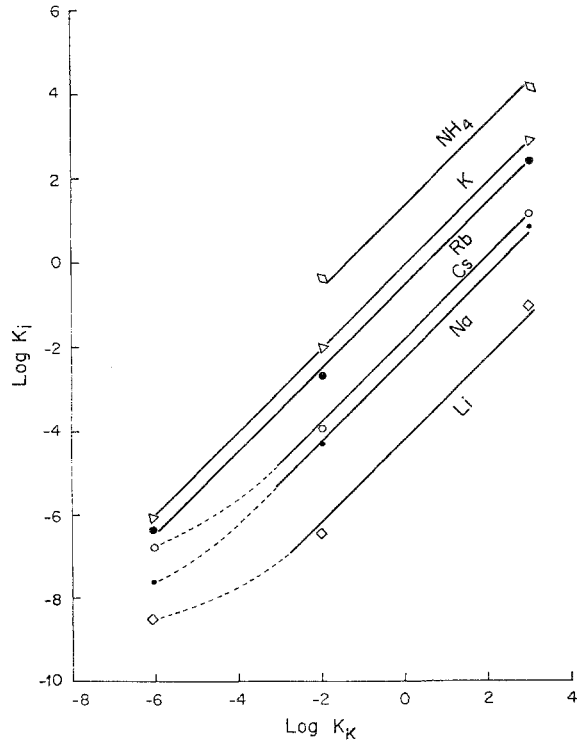

Fig. 8. The effect of varying the solvent on the value of K_i. The values of $\log K_i$ for the indicated cations observed in n-hexane, in a 64% hexane — 36% dichloromethane mixture, and in pure dichloromethane are plotted from left to right as a function of the value of $\log K_K$ for each of these solvents. The solid lines of unit slope indicate the expectations of Eq. (45); the dashed lines represent deviations from this. Note that such deviations are important only for n-hexane and for Cs^+, Na^+, and Li^+, which are the least reliable of the data

to be independent of the solvent (Eisenman et al., 1968, note the change of Na:Cs ratio in Tables 2 and 4; McLaughlin, Szabo, Eisenman, & Ciani, unpublished results).

Effects of Varying the Anion Species Used as a Chromophore

By carrying out studies such as those of Figs. 5 – 7, but using 2,4-dinitrophenolate as the lipid-soluble chromophore anion in place of picrate, it is possible to show that, although the value of K_i changes with the anion used, the ratio of K_i/K_j is independent of the anion, as is expected from Eq. (9). The salt extraction into dichloromethane produced by monactin, using 2,4-dinitrophenolate instead of picrate, is summarized in Table 6 and Fig. 9, for which the experimental conditions were otherwise identical to those of Table 4 and Fig. 7. Comparing the columns labelled $C_x^{\mathrm{Tot}*}$ in

Table 6. *Extraction of the 2,4-dinitrophenolates into dichloromethane by monactin*[a] *(Initial conditions:* $C^{In}_{DNP} = 1.0 \times 10^{-4}$ M; $C^{In*}_{Monactin} = 0.20 \times 10^{-4}$ M; $V^* = 10$ ml, $V = 2$ ml)

	C^{In}_{MOH}	A^*_{373}	A^0_{373}	C_x^{Tot*} ($\times 10^{-4}$)	C_x ($\times 10^{-4}$)	C_s^* ($\times 10^{-4}$)	$y_i y_x$	K_i
LiOH	0.08	0.00965	<0.002	0.005975	0.9701	0.1940	0.52	(0.0046)
NaOH	0.008	0.01775	<0.002	0.01099	0.9451	0.1890	0.81	0.104
	0.08	0.04694	<0.002	0.02907	0.8547	0.1709	0.59	0.123
CsOH	0.004	0.0162	<0.002	0.01003	0.9499	0.1900	0.86	(0.162)
	0.008	0.02688	<0.002	0.01664	0.9168	0.1834	0.81	0.252
	0.08	0.0620	<0.002	0.03839	0.8081	0.1616	0.66	0.214
RbOH	0.0008	0.02352 #	0.00625	0.02352	0.8824	0.1765	0.93	(4.75)
	0.002	0.03095 #	0.00325	0.03095	0.8453	0.1691	0.90	*3.72*
	0.004	0.04148 #	0.00200	0.04148	0.7926	0.1585	0.86	*3.97*
	0.008	0.05325 #	0.00225	0.05325	0.7338	0.1468	0.81	*4.04*
	0.08	0.09783 #	0.00225	0.09783	0.5109	0.1022	0.63	3.66
KOH	0.0008	0.03715 #	0.00175	0.03715	0.8143	0.1629	0.93	13.9
	0.002	0.05263 #	0.00175	0.05263	0.7369	0.1474	0.90	*14.2*
	0.004	0.06563 #	0.00150	0.06563	0.6719	0.1344	0.86	*13.8*
	0.008	0.07987 #	0.00225	0.07987	0.6007	0.1201	0.81	*13.6*
	0.08	0.1276 #	0.00200	0.1276	0.3620	0.0724	0.60	12.9
NH_4OH	0.01	0.133	<0.002	0.08235	0.5883	0.1177	1.0	*238*

[a] Parenthesized values are influenced by the trace amounts of NH_4^+. The italicized values of K_i, calculated according to the first term of Eq. (28), are considered the most reliable. Note that the concentration of dissociated NH_4^+ in 0.01 M NH_4OH is calculated to be 0.000412 M. Values indicated by (#) have been corrected for the absorbances measured in the absence of monactin given in the column labelled "A^0_{373}".

Tables 4 and 6, it is clear that, under corresponding conditions, the extractions of the dinitrophenolates are considerably smaller than those of the picrates. This is reflected in the smaller values of K_i in Fig. 9 than in Fig. 7. The differences in values of K_i for dinitrophenolate compared to picrate are summarized in the upper portion of Table 7 and are consistent with a partition coefficient for picrate some 70 times larger than for dinitrophenolate[8].

8 From Eq. (8), it is seen that the ratio of partition coefficients of picrate to dinitrophenolate in the H_2O:CH_2Cl_2 system is equal to the ratio of K_i measured for picrate vs. dinitrophenolate [K_i(picrate)/K_i(DNP)] using a common cation, I^+, according to:

$$\frac{K_i(\text{picrate})}{K_i(\text{DNP})} = \frac{k_{\text{picrate}}}{k_{\text{DNP}}}, \tag{51}$$

because k_{is}, k_s, and K_{is}^+ all cancel for a given cation and antibiotic molecule. The values of $k_{\text{picrate}}/k_{\text{DNP}}$ so calculated from the data of Table 7 are: 67, 63, 73, and 73 for NH_4^+, K^+, Rb^+, and Na^+, respectively, and 22 and 109 for Li^+ and Cs^+, respectively.

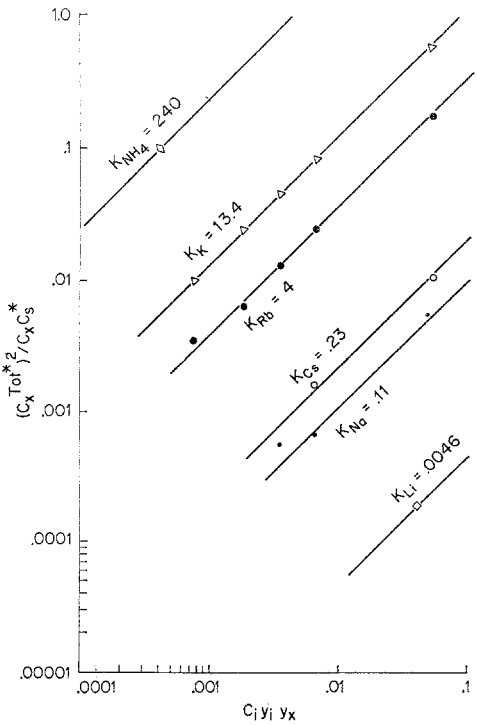

Fig. 9. Equilibrium extractions of the 2,4-dinitrophenolate salts of Li, Na, K, Rb, Cs, and NH_4 into a dichloromethane phase containing 2×10^{-5} M monactin. These data are to be compared to Fig. 7, obtained under otherwise identical conditions

Table 7. *Effects of dinitrophenolate vs. picrate on the salt extraction by monactin into dichloromethane*

Equilibrium constant	Anion species	Li	Na	K	Rb	Cs	NH_4
K_i	2,4-DNP	(0.0046)	0.11	13.4	4.0	0.23	240
	Picrate	(0.10)	8.0	850	290	25	16,000
K_i/K_K	2,4-DNP	(0.00034)	0.0082	1.0	0.30	0.017	18
	Picrate	(0.00012)	0.0094	1.0	0.34	0.029	19

Of more importance is the finding (lower portion of Table 7) that the ratios of K_i/K_j for each cation relative to K^+ are essentially independent of whether they are measured using dinitrophenolate or picrate. We may therefore conclude that the measured ratios of K_i/K_j are indeed independent of the common anion, as expected theoretically.

The Extraction of Picrate is Independent of Non-Chromophore Anions such as OH^- and Cl^-

When the partition coefficient ratio of the chromophore anion to non-chromophore anion is sufficiently large, the concentration of non-lipid-compatible anions such as OH^- or Cl^-, which appear in Eq. (24), can be neglected. That this has been the case under the experimental conditions examined so far is indicated by the following observations. First, the values of K_i are the same whether OH^- or Cl^- is present (compare the data for K^+ in Tables 1 and 2). Second, precise agreement has been seen between the data and Eq. (28) over wide variations in OH^- concentration and will also be found in the next section when the picrate concentration is varied widely. Third, the equilibria measured with 2,4-dinitrophenolate are in agreement with those measured using picrate, despite the fact that the partition coefficient for dinitrophenolate is 1/70 that of picrate so that effects due to OH^- would be 70 times more prominent. Fourth, the extraction of picrate will be shown below to obey perfect 1:1 stoichiometry, which would not be the case if any significant amount of OH^- were also entering the solvent phase to balance the charge of the complexes.

Despite these indications that OH^- and Cl^- per se have negligible effects, we decided to test this further by examining the effect of adding large excesses of these ions. This was done by adding the hydroxide or chloride of a cation which is poorly extracted (e.g., Li^+) to a solution containing a cation which is well extracted (e.g., K^+). Any observed effect would then be due to the anion alone. The results of such an experiment are presented in Table 8, which shows the negligible effects of high Cl^- and OH^- concentrations on the amount of picrate extracted under experimental conditions otherwise identical to those of Table 4 [9].

Table 8. *Lack of effect of large amounts of Cl^- or OH^- on the equilibrium extraction of picrate by monactin into CH_2Cl_2*

Aqueous solution	A^*_{378}	C_x^{Tot*}
0.0009 M KOH	0.248	0.136×10^{-4} M
0.0009 M KOH + 0.01 LiOH	0.244	0.133×10^{-4} M
0.0009 M KOH + 0.1 LiOH	0.242	0.132×10^{-4} M
0.0009 M KOH + 0.1 LiCl	0.247	0.135×10^{-4} M
0.0009 M KOH + 1.0 LiCl	0.247	0.135×10^{-4} M

9 In fact, the small suppression of picrate extractions expected from ionic strength effects per se are partly compensated by the small extractions of picrate expected to be due to the Li^+ ion (recall from Table 4 that 0.027×10^{-4} M picrate is extracted in the presence of 0.09 M LiOH.

Studies Under Widely Varied Initial Concentrations of Macrotetralide in the Solvent Phase and of Picrate in the Aqueous Phase

Experiments have been carried out under extensively varied monactin and picrate concnetrations in order to define the range of conditions over which the present theory is adequate. These experiments provide the best test of the postulated 1:1 stoichiometry of complex formation between the macrotetralides and cations. They also verify that the behavior in the solvent phase is ideal over a wide concentration range, as postulated in Eqs. (17) and (18).

The picrate extraction by monactin into dichloromethane under two sets of experimental conditions, quite different from each other and from those of Table 4, is summarized in Tables 9 and 10. In Table 9, the monactin concentration is 10 times higher and the picrate concentration is double that of Table 4; in Table 10, the monactin concentration is half that of Table 4 and the picrate concentration is the same. The data are compared most easily with the aid of Table 11, where representative data from Table 14 under comparable volume conditions ($V^* = V = 10$ ml) are also included for comparison. It can be seen that not only are the initial conditions very different, but also so are the concentrations at equilibrium. Despite these differences, the values of K_i in the last column of Table 11,

Table 9. *Extraction of picrate into dichloromethane by monactin at ten times higher concentration of monactin and twice the picrate concentration than the usual experiment*[a]

(Initial conditions: $C^{In^*}_{Monactin} = C^{In}_{Picrate} = 2 \times 10^{-4}$ M; $V^* = V = 10$ ml*)*

	C^{In}	A^*_{378}	$C_x^{Tot^*}$	C_x	C_s^*	$y_i y_x$	K_i
LiOH	0.0988	0.40	(0.208×10^{-4})	1.792×10^{-4}	1.792×10^{-4}	0.52	(0.25)
NaOH	0.0988	1.74	0.906×10^{-4}	1.094×10^{-4}	1.094×10^{-4}	0.58	*12*
CsOH	0.0988	2.16	1.13×10^{-4}	0.87×10^{-4}	0.87×10^{-4}	0.65	*26*
RbOH	0.0988	3.12	1.63×10^{-4}	0.37×10^{-4}	0.37×10^{-4}	0.62	*314*
KOH	0.0988	3.38	1.76×10^{-4}	0.24×10^{-4}	0.24×10^{-4}	0.60	*910*
LiCl	0.0988	0.68	(0.35×10^{-4})	1.65×10^{-4}	1.65×10^{-4}	0.63	(4.2)
NaCl	0.0988	1.88	0.974×10^{-4}	1.021×10^{-4}	1.021×10^{-4}	0.60	15
KCl	0.0988	3.33	1.73×10^{-4}	0.27×10^{-4}	0.27×10^{-4}	0.59	771

[a] Note that the path length of the absorption cell was 0.1 cm and the molar absorption coefficient measured for this cell was 19,200. Absorbances are expressed per cm. Excellent agreement was found between the measurements of the table and those made on the aqueous phase for LiOH, NaOH, and KOH for which C_x was directly measured to be 1.82, 1.09, and 0.22×10^{-4} M, in comparison with the calculated values of 1.792, 1.094, and 0.24×10^{-4} M of the table. The chloride solutions have not been neutralized with LiOH. Parenthesized and italicized values have their usual meaning.

Table 10. *Extraction of picrate into dichloromethane by monactin at half the usual concentration*[a]

(Initial conditions: $C_{\text{Monactin}} = 10^{-5}$ M; $C_{\text{Picrate}} = 10^{-4}$ M; $V^* = V = 10$ ml*)*

	$C_{\text{MOH}}^{\text{In}}$	A_{378}^*	$C_x^{\text{Tot}*}$	C_x	C_s^*	$y_i y_x$	K_i
Zero monactin							
NaOH	0.00225	0.063	0.0367×10^{-4}	0.9633×10^{-4}	0.0633×10^{-4}	0.90	*9.9*
CsOH	0.00225	0.093	0.0508×10^{-4}	0.9492×10^{-4}	0.0492×10^{-4}	0.90	*27.2*
RbOH	0.00225	0.155	0.0847×10^{-4}	0.9153×10^{-4}	0.0153×10^{-4}	0.90	*252*
KOH	0.00225	0.170	0.0929×10^{-4}	0.9071×10^{-4}	0.0071×10^{-4}	0.90	660

[a] Path length 4.0 cm.

Table 11. *Salt extraction into CH_2Cl_2 under widely varied initial concentrations of monactin and picrate*

Data source	Aqueous solution	Initial conditions			Equilibrium concentrations			
		$C_i y_i y_x$	C_x^{In} ($\times 10^{-4}$)	$C_s^{\text{In}*}$ ($\times 10^{-4}$)	$C_x^{\text{Tot}*}$ ($\times 10^{-4}$)	C_x ($\times 10^{-4}$)	C_s^* ($\times 10^{-4}$)	K_i
Table 9	NaOH	0.0577	2	2	0.906	1.094	1.094	12.0
Table 10	NaOH	0.00203	1	0.1	0.0367	0.9633	0.0633	9.9
Table 14	NaOH	0.00203	1	0.2	0.0530	0.947	0.147	10.0
Table 9	CsOH	0.0646	2	2	1.13	0.87	0.87	26.0
Table 10	CsOH	0.00203	1	0.1	0.0508	0.9492	0.0492	27.2
Table 14	CsOH	0.00203	1	0.2	0.0787	0.9213	0.1213	27.3
Table 9	RbOH	0.0617	2	2	1.63	0.37	0.37	314
Table 10	RbOH	0.00203	1	0.1	0.0847	0.9153	0.0513	252
Table 14	RbOH	0.00203	1	0.2	0.1552	0.8448	0.0448	313
Table 9	KOH	0.0595	2	2	1.76	0.24	0.24	910
Table 10	KOH	0.00203	1	0.1	0.0929	0.9071	0.0071	660
Table 14	KOH	0.00203	1	0.2	0.1820	0.8180	0.0180	1100

calculated from these data by the first term of Eq. (28), are seen to agree well from experiment to experiment (and, incidentally, with the values of Table 4). This result, encompassing a concentration range of uncomplexed macrotetralide in the solvent from 7.1×10^{-7} to 1.09×10^{-4} M and of complexed cations (and picrate anions) from 3.7×10^{-6} to 1.76×10^{-4} M, demonstrates the remarkably ideal behavior of all species in the solvent phase.

The data of Table 11 can also be used to test if the second term of Eq. (28) is negligible over a much wider range of conditions than was previously examined in Fig. 6. This is illustrated in Fig. 10, which plots as solid points the logarithm of the picrate extracted as a function of $\log(C_i C_x C_s^* y_i y_x)$. The agreement between the data points and lines of

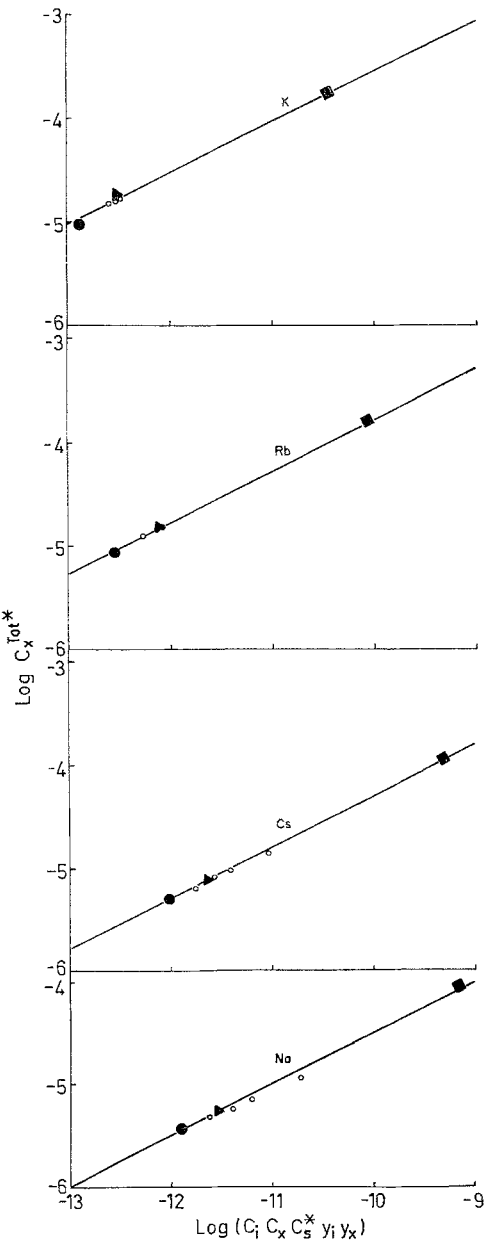

Fig. 10. Demonstration that reaction (7) satisfactorily describes the salt extraction equilibrium over a wide range of experimental conditions. The logarithm of the picrate extracted into dichloromethane by 2×10^{-4} M monactin from solutions containing initially 2×10^{-4} M picrate is plotted as a solid square; that extracted by 2×10^{-5} M monactin from solutions containing initially 10^{-4} M picrate is plotted as a solid triangle; and that extracted by 10^{-5} M monactin from 10^{-4} M picrate solutions is plotted as a filled circle. In all cases, $V = V^* = 10$ ml. For comparison, the open circles replot the data of Fig. 6 obtained with a five times smaller volume ($V = 2$ ml) of the aqueous phase. The lines are all drawn with slope $\frac{1}{2}$

slope $\frac{1}{2}$ is exceedingly good, indicating that over the entire range of concentrations studied the salt extraction equilibrium corresponds solely to reaction (7).

Evidence for 1:1 Stoichiometry

The data of these experiments provide a particularly clear demonstration that the stoichiometry of the complex formation is indeed 1:1. This is implicit in the agreement with the lines of slope $\frac{1}{2}$ in Fig. 10, but the ease with which such a plot distinguishes between alternative stoichiometries is best illustrated in Fig. 11, where the data points from Table 11 are compared

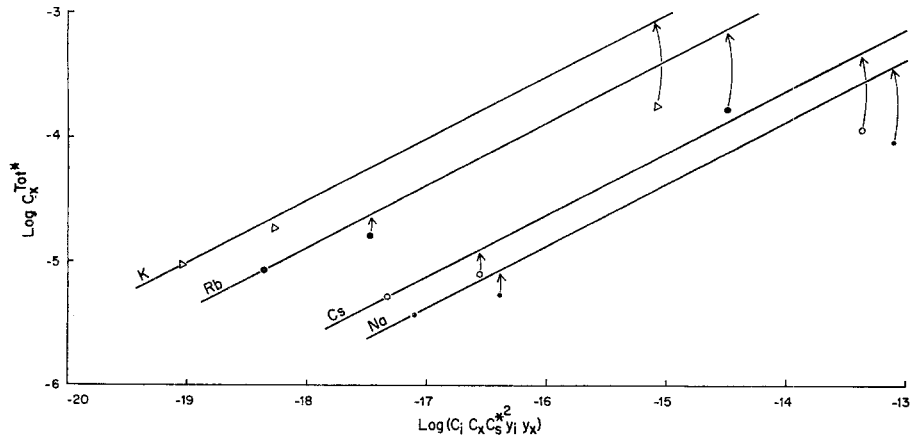

Fig. 11. Demonstration that the stoichiometry is not one cation per two monactin molecules

with the solid lines expected for 1:2 cation:macrotetralide stoichiometry, according to Eq. (55)[10]. The large deviations of the points from the lines (recall the logarithmic scale) clearly exclude this alternative.

10 For stoichiometry of the type

$$n I^+ + m S^* + n X^- \underset{K_i^{n,m}}{\rightleftharpoons} I_n^n S_m^{+*} + n X^{-*} \underset{K^*_{i_n s_m x_n}}{\rightleftharpoons} I_n S_m X_n^*, \tag{52}$$

we have:

$$K_i^{n,m} = \frac{C_x^{*n} C^*_{i_n s_m}}{a_i^n C_s^{*m} a_x^n} \quad \text{and} \quad K^*_{i_n s_m x_n} = \frac{C^*_{i_n s_m x_n}}{C_x^{*n} C^*_{i_n s_m}} = \frac{C^*_{i_n s_m x_n}}{K_i^{n,m} a_i^n C_s^{*m} a_x^n}. \tag{53}$$

Since electroneutrality requires that

$$C_x^* = n\, C^*_{i_n s_m}, \tag{54}$$

we find:

$$C_x^{Tot*} = C_x^* + n\, C_{i_n s_m x_n} = (n K_i^{n,m} a_i^n C_s^{*m} a_x^n)^{\frac{1}{n+1}} + n K_i^{n,m} K_{i_n s_m x_n} a_i^n C_s^{*m} a_x^n, \tag{55}$$

which reduces to Eq. (28) only when $n = m = 1$.

Negligible Effects of Ionic Strength and of Ionic Species such as Th^{4+}, Ca^{2+}, Mg^{2+}

In paper III, it will be shown that the conductance of a phospholipid bilayer membranes is markedly dependent on the ionic strength of the aqueous solutions to which it is exposed, as well as on the concentrations of ions such as Ca^{2+} and Th^{4+}. There, this dependence will be attributed to effects on the physical properties of the lipid. That such effects are not on the antibiotic molecules themselves is easily shown in the present system by studying the picrate extraction for those aqueous concentrations at

Table 12. *Lack of effect of Ca^{2+}, Mg^{2+}, and Th^{4+} on the extraction of K picrate by monactin into CH_2Cl_2*[a]

Aqueous solution	A^*_{378}	C_x^{Tot*}
0.0009 KOH	0.248	0.136×10^{-4}
0.0009 KOH + 10^{-4} $CaCl_2$	0.259	0.1415×10^{-4}
0.0009 KOH + 10^{-2} $CaCl_2$	0.256	0.140×10^{-4}
0.0009 KCl	0.266	0.145×10^{-4}
0.0009 KCl + 10^{-2} $MgCl_2$	0.259	0.1415×10^{-4}
0.0009 KCl + 10^{-2} $CaCl_2$	0.259	0.1415×10^{-4}
0.0009 KCl + 10^{-5} $ThCl_4$	0.270	0.1475×10^{-4}
0.0009 KCl + 10^{-4} $ThCl_4$	0.267	0.146×10^{-4}

[a] The upper portion of the table gives the extraction into 10 ml of dichloromethane containing 0.2×10^{-4} moles/liter monactin of picrate from 2-ml volumes of aqueous solutions at 10^{-4} M. The lower portion of the table examines the effects of the addition of Mg^{2+}, Ca^{2+}, and Th^{4+} to chloride solutions, used to ensure that the low solubilities of the hydroxides of Th^{4+} and Mg^{2+} would not complicate the interpretation of the results. The apparent picrate uptakes from such solutions at the neutral pH range are slightly complicated by the extraction of a small amount of picric acid, which, however, does not interfere with the interpretation of the experiment.

which the effects on the bilayer are marked. The data of Table 8 show that effects of ionic strength in the bulk system are negligible at concentrations at which the effects on the bilayer are marked (*see* Fig. 9 of paper III). Table 12 demonstrates that there is also no significant effect of Th^{4+}, Mg^{2+}, or Ca^{2+} at concentration levels which produce striking alterations in the properties of phospholipid bilayers (*see* Fig. 12 of paper III).

2. Characterization of Salt Extraction Equilibria for the Series of Macrotetralides: Nonactin, Monactin, Dinactin, and Trinactin

Chemical formulas and space-filling models for nonactin, monactin, dinactin, and trinactin have been given in Fig. 1 of paper I, where it was noted that the members of this series of molecules differ solely by the

Table 13. *Extraction of picrates into dichloromethane by nonactin, dinactin, and trinactin*
(Initial conditions: $C^{In}_{Picrate}=10^{-4}$ M, $C^{In*}_{Macrotetralide}=0.2\times10^{-4}$, $V^*=10$ ml, $V=2$ ml*)*

		Nonactin			Dinactin			Trinactin		
	C^{In}_{MOH}	C_x^{Tot*}	C_x	C_s^*	C_x^{Tot*}	C_x	C_s^*	C_x^{Tot*}	C_x	C_s^*
LiOH	0.0009	(0.0224)	(0.888)	(0.178)	(0.0137)	(0.9315)	(0.1863)	(0.02034)	(0.899)	(0.1798)
	0.00225	(0.0131)	(0.934)	(0.187)	(0.0137)	(0.9315)	(0.1863)	(0.0164)	(0.918)	(0.1836)
	0.0045	(0.0109)	(0.946)	(0.189)	(0.0148)	(0.926)	(0.1852)	(0.0169)	(0.9155)	(0.1831)
	0.009	(0.0104)	(0.948)	(0.190)	(0.0175)	(0.9125)	(0.1825)	(0.0208)	(0.896)	(0.1792)
	0.09	0.0197	0.902	0.180	0.0328	0.836	0.1672	0.0377	0.8115	0.1623
NaOH	0.0009	(0.0322)	(0.839)	(0.168)	(0.0503)	(0.7485)	(0.1497)	(0.0607)	(0.6965)	(0.1393)
	0.00225	(0.0322)	(0.839)	0.168	0.0667	0.6665	0.1333	0.0792	0.604	0.1208
	0.0045	0.0399	0.800	0.160	0.0825	0.5875	0.1175	0.0956	0.522	0.1044
	0.009	0.0492	0.754	0.151	0.0984	0.508	0.1016	0.1109	0.4455	0.0891
	0.09	0.0874	0.563	0.112	0.1421	0.2895	0.0579	0.1519	0.2405	0.0481
CsOH	0.0009	(0.0470)	(0.765)	(0.153)	(0.0776)	(0.612)	(0.1224)	(0.0880)	(0.560)	(0.112)
	0.00225	0.0540	0.730	0.146	0.0885	0.5575	0.1115	0.0973	0.5135	0.1027
	0.0045	0.0656	0.672	0.134	0.1005	0.4975	0.0995	0.1098	0.451	0.0902
	0.009	0.0776	0.612	0.122	0.1131	0.4345	0.0869	0.1251	0.3745	0.0749
	0.09	0.118	0.410	0.082	0.1443	0.2785	0.0557	0.1525	0.2375	0.0475
RbOH	0.0009	(0.0820)	(0.590)	(0.118)	(0.1328)	(0.336)	(0.0672)	(0.1421)	(0.2895)	(0.0579)
	0.00225	0.0989	0.505	0.101	0.1475	0.2625	0.0525	0.1562	0.219	0.0438
	0.0045	0.114	0.430	0.086	0.1607	0.1965	0.0393	0.1661	0.1695	0.0339
	0.009	0.126	0.370	0.074	0.1683	0.1585	0.0317	0.1727	0.1365	0.0213
	0.09	0.157	0.215	0.043	0.1798	0.101	0.202	0.1825	0.0875	0.0175
KOH	0.0009	(0.108)	(0.460)	(0.092)	(0.1525)	(0.2375)	(0.0475)	(0.1628)	(0.186)	(0.0372)
	0.00225	0.122	0.390	0.078	0.1656	0.172	0.0344	0.1727	0.1365	0.0273
	0.0045	0.132	0.340	0.068	0.1721	0.1395	0.0279	0.1798	0.101	0.0202
	0.009	0.144	0.280	0.056	0.1792	0.1040	0.0208	0.1836	0.0820	0.0164
	0.09	0.164	0.180	0.036	0.1809	0.0955	0.0191	0.1858	0.0810	0.0142
NH_4OH	0.001	0.143	0.285	0.057	—	—	—	—	—	—
	0.01	0.160	0.200	0.040	0.1743	0.1285	0.0257	0.1809	0.0955	0.0191

All concentrations are understood to be multiplied by 10^{-4} moles per liter.

Table 14. *Extraction of picrates into*
(Initial conditions: $C^{In}_{Picrate} = 10^{-4}$ M,

	C^{In}_{MOH}	Nonactin			Monactin	
		C_x^{Tot*}	C_x	C_s^*	C_x^{Tot*}	C_x
LiOH	0.0009	(0.0273)	(0.9727)	(0.1727)	(0.0317)	(0.9683)
	0.00225	(0.0180)	(0.982)	(0.182)	(0.0246)	(0.9754)
	0.0045	(0.0169)	(0.9831)	(0.1831)	(0.0240)	(0.9760)
	0.009	(0.0180)	(0.982)	(0.182)	(0.0219)	(0.9781)
	0.09	0.0268	0.9732	0.1732	0.0339	0.9442
NaOH	0.0009	(0.0350)	(0.965)	(0.165)	(0.0448)	(0.9552)
	0.00225	0.0377	0.9623	0.1623	0.530	0.9470
	0.0045	0.0464	0.9536	0.1536	0.0661	0.9339
	0.009	0.0601	0.9399	0.1399	0.0836	0.9164
	0.09	0.1109	0.8891	0.0891	0.1508	0.8492
CsOH	0.0009	(0.0514)	(0.9486)	(0.1486)	(0.0656)	(0.9344)
	0.00225	0.0628	0.9372	0.1372	0.0787	0.9213
	0.0045	0.0781	0.9219	0.1219	0.0984	0.9016
	0.009	0.0962	0.9038	0.1038	0.1148	0.8852
	0.09	0.1492	0.8508	0.508	0.1694	0.8306
RbOH	0.0009	(0.1022)	(0.8978)	0.978	(0.1300)	(0.8700)
	0.00225	0.1268	0.8732	0.0732	0.1552	0.8448
	0.0045	0.1448	0.8552	0.0552	0.1710	0.8290
	0.009	0.1607	0.8393	0.0393	0.1814	0.8186
	0.09	0.1798	0.8202	0.0202	0.1956	0.8044
KOH	0.0009	(0.1317)	(0.8683)	(0.0683)	(0.1667)	(0.8333)
	0.00225	0.1530	0.8470	0.0470	0.1820	0.8180
	0.0045	0.1634	0.8366	0.0366	0.1891	0.8109
	0.009	0.1716	0.8284	0.0284	—	—
	0.09	0.1819	0.8181	0.0181	0.1989	0.8011
NH_4OH	0.01	—	—	—	—	—

[a] All concentrations are understood to be multiplied by 10^{-4} moles/liter. $y_i y_x$ are the same as in Table 4. Values in parentheses are influenced by traces of NH_4^+. For

successive addition of methyl groups. Having established that K_i can be measured precisely in dichloromethane and that the value of the ratio K_i/K_j is independent of the solvent as well as of the chromophore anion, we are now in a position to examine the effects on K_i and K_i/K_j of the systematic variation in molecular composition of this series of antibiotics.

Tables 13–15 summarize the salt extraction produced by these antibiotics. Table 13 represents experiments carried out under conditions identical to those of Table 4 except that especially purified distilled water (*see* Methods) was used for the Li and Na solutions in the case of dinactin

dichloromethane by the macrotetralide actins[a]
$C^{In*}_{Macrotetralide} = 0.2 \times 10^{-4}$, $V^* = V = 10$ ml)

	Dinactin			Trinactin		
C_s^*	C_x^{Tot*}	C_x	C_s^*	C_x^{Tot*}	C_x	C_s^*
(0.1683)	(0.0208)	(0.9792)	(0.1792)	(0.0273)	(0.9727)	(0.1727)
(0.1754)	—	—	—	—	—	—
(0.1760)	0.0219	0.9781	0.1781	0.0295	0.9705	0.1705
(0.1781)	—	—	—	—	—	—
0.1442	—	—	—	—	—	—
(0.1552)	(0.0628)	(0.9372)	(0.1372)	(0.0781)	(0.9219)	(0.1219)
0.1470	—	—	—	—	—	—
0.1339	0.0106	0.9894	0.1894	0.1197	0.8803	0.0803
0.1164	—	—	—	—	—	—
0.0492	—	—	—	—	—	—
(0.1344)	(0.0907)	(0.9093)	(0.1093)	(0.1066)	(0.8934)	(0.1934)
0.1213	—	—	—	—	—	—
0.1016	0.1273	0.8727	0.0727	0.1372	0.8628	0.0628
0.0852	—	—	—	—	—	—
0.0306	—	—	—	—	—	—
(0.1700)	(0.1656)	(0.8344)	(0.0344)	(0.1743)	(0.8257)	(0.0257)
0.0448	—	—	—	—	—	—
0.0290	0.1896	0.8104	0.0104	0.1929	0.8071	[0.0071]
0.0186	—	—	—	—	—	—
0.0044	—	—	—	—	—	—
(0.0333)	(0.1896)	(0.8104)	[(0.0104)]	(0.1934)	(0.8066)	[(0.0066)]
0.0180	—	—	—	—	—	—
0.0109	0.1978	0.8022	[0.0022]	0.1989	0.8011	[0.0011]
—	—	—	—	—	—	—
[0.0011]	—	—	—	—	—	—
—	0.1973	0.8027	[0.0027]	0.1995	0.8005	[0.0005]

those values of C_s^* which are bracketed, less than 5% of the initial concentration is uncomplexed at equilibrium.

and trinactin. Table 14 represents experiments carried out under the same conditions except that the volume of the aqueous phase was increased five times to test that the loss of antibiotic to the aqueous phase was negligible and also to verify that not more than one cation was extracted per antibiotic molecule.

The results of these experiments are summarized graphically in Fig. 12, where $\log(C_x^{Tot*})^2/C_x C_s^*$ is plotted against $\log(C_i y_i y_x)$ in the manner of Fig. 7. From the agreement between experimental points and theoretical straight lines, particularly over the most reliable middle concentration

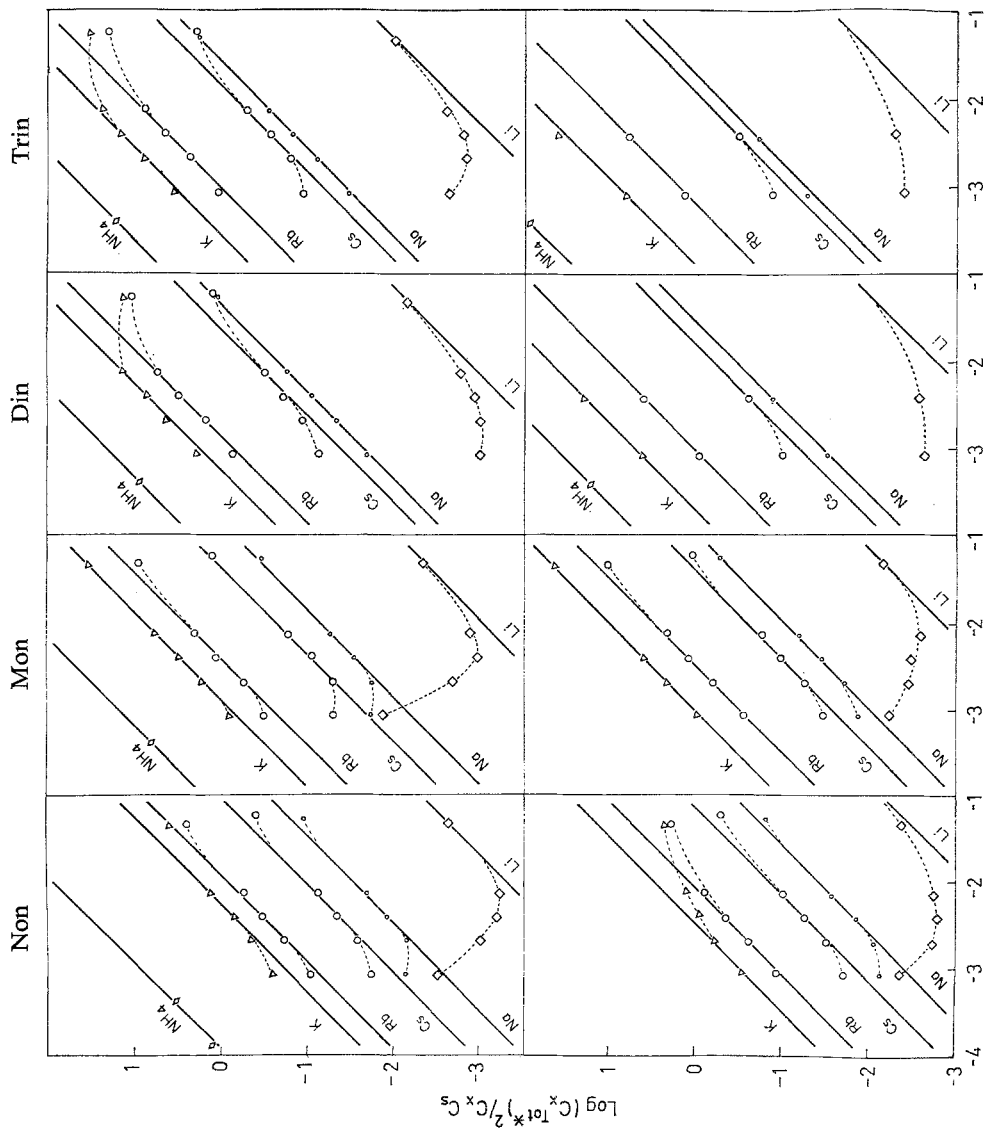

Fig. 12. Summary of salt extraction into dichloromethane by the macrotetralide actin antibiotics (from left to right, Non-, Mon-, Din-, Trin-). The upper half of the figure presents the data of Table 13, the lower the data of Table 14. The solid lines are those theoretically expected by Eq. (50). Dashed lines at low concentrations indicate deviations due to traces of NH_4^+ in the distilled water

range, it is clear that our previous conclusions for monactin apply to the other macrotetralides as well. Furthermore, the agreement between the two sets of experiments in Fig. 12 verifies that losses of the antibiotic molecules to the aqueous phase are negligible[11].

Table 15. *Selectivity parameters for the equilibrium extraction of picrates by the macrotetralide actin antibiotics*[a]

Equilibrium constant	Macrotetralide actin and data source	Li	Na	K	Rb	Cs	NH_4
K_i	Nonactin						
	Table 13	0.05	3.2	190	90	11.5	9,000
	Table 14	0.07	3.7	310	120	14	—
	Monactin						
	Table 13	0.10	8.0	850	290	25	16,000
	Table 14	0.14	9.7	1,030	324	26	—
	Dinactin						
	Table 13	0.15	25	2,000	800	46	24,000
	Table 14	0.17	34	[5,300]	1,100	65	[45,000]
	Trinactin						
	Table 13	0.23	42	4,000	1,170	75	46,000
	Table 14	0.3	54	[8,600]	[1,700]	88	[230,000]
K_i/K_K	Nonactin						
	Table 13	0.00026	0.017	1.0	0.47	0.061	47
	Table 14	0.00023	0.012	1.0	0.39	0.045	—
	Monactin						
	Table 13	0.00012	0.0094	1.0	0.34	0.029	19
	Table 14	0.00014	0.0094	1.0	0.31	0.025	
	Dinactin						
	Table 13	0.000075	0.013	1.0	0.40	0.023	12
	Table 14	[0.000032]	[0.0064]	[1.0]	[0.21]	[0.017]	[8.5]
	Trinactin						
	Table 13	0.000058	0.011	1.0	0.29	0.019	12
	Table 14	[0.000035]	[0.0063]	[1.0]	[0.20]	[0.010]	[27]

[a] For values indicated by brackets, more than 95% of the antibiotic is in the form of the charged complex, and less than 5% is in the neutral form. These values are subject to large experimental errors since a small difference is used in the calculations.

11 The only important differences between the two experiments are the apparently higher values of K_i in the experiment of Table 14 for K^+ and NH_4^+ in the case of dinactin and trinactin and for Rb^+ for trinactin. Because of the nearly complete conversion of neutral antibiotic to the complex (*see* values of C_s^* in Table 14), the calculations are subject to large error, and values dependent on these are bracketed in the tables to indicate their lesser reliability.

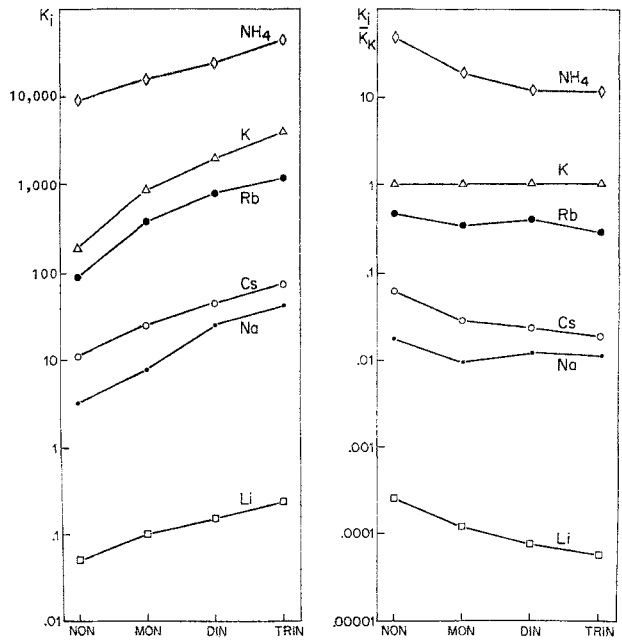

Fig. 13. Dependence of K_i and K_i/K_K on the composition of the macrotetralide actin antibiotics. The left-hand portion of the figure plots the dependence of K_i for the indicated cations as a function of the increasing number of methyl groups as one proceeds from nonactin through trinactin. The right-hand portion plots the ratios of K_i/K_K in the same manner. Note that the ordinate is logarithmic

The values of K_i from the y-intercepts of Fig. 12 are summarized in the upper portion of Table 15; the K_i/K_j ratios of these relative to K^+ (i.e., K_i/K_j) are given in the lower portion of this table. Comparing the values of K_i and K_i/K_K as the antibiotic molecule is varied, systematic differences in the values of K_i and of K_i/K_K are clearly apparent as the number of methyl groups is increased. These effects can be seen in Fig. 13. A systematic increase of K_i is observed with increasing methylation, and the ratios of K_i/K_K are also seen to change, although less markedly. The effects of methylation are in the direction expected theoretically, as will be examined in the Discussion, where we will conclude that the primary effect of methylation is to increase the dipole moment of the ligand oxygens.

Within the framework of the present theory which assumes that the macrotetralide actins produce their characteristic effects on phospholipid bilayers and bulk solvent phases by forming lipid-soluble molecular complexes with cations, the values of K_i and K_i/K_K of Table 15 and Fig. 13 suffice for the detailed prediction of the effects of each of these antibiotics

on the electrical properties of phospholipid bilayer membranes (*see* Discussion). The success with which this can be done is seen in the results of the following paper III.

Discussion

Evidence that the Size and Shape of the Cation-Macrotetralide Complex is Independent of the Particular Cationic Species Sequestered

The "isosteric" postulate that the size and shape of the macrotetralide-cation complex should be independent of the particular cation bound was initially made from examining space-filling models of the molecules (*see* Fig. 1B of paper I) in the configuration expected [on simple energetic considerations and the known structure in crystals (Kilbourn et al., 1967) in solvents of low dielectric constant. The results of two independent sets of measurements made here support this postulate. The main experimental support is provided by the observation that over a range of solvent dielectric constants, from 2 to 9, the K_j/K_i ratios are constan teven though the absolute magnitudes of K_i and K_j vary by more than nine orders of magnitude. This is predictable a priori if the partition coeffcients of the IS^+ and JS^+ complexes are independent of the solvent which, on purely electrostatic energy considerations, is only expected if the overall size of the complex is the same regardless of the particular cation species bound. Subsidiary evidence is the finding that K^*_{isx}, the equilibrium constant for the reaction between the picrate ion and the charged complex, is the same for K^+ and Rb^+ in hexane-dichloromethane. Neither of these results is expected if the configuration or dimensions of the complex differed significantly for different cations; both results, however, are predictable from the present theory if the size and shape of the complex is the same for all cations (provided also that the details of electronic distribution over the macrotetralide molecule also are essentially the same as viewed from the outside, regardless of the particular cation species at the center of the complex).

Independent evidence in support of this conclusion is provided by comparing the K_j/K_i ratios measured here with the stability constants for complex formation between nonactin and monactin and K^+ and Na^+ in methanol, which have been measured by Simon and his colleagues (Pioda, Wachter, Dohner, & Simon, 1967). Recall that in Eq. (13) we deduced for "isosteric" complexes that K_j/K_i should be identically equal to K^+_{js}/K^+_{is} measured in aqueous solution. Assuming that the ratio of K^+_{js}/K^+_{is} (which has yet to be measured directly in aqueous solutions) is

Table 16. *Comparison of K_i/K_j for picrate extraction into CH_2Cl_2 from H_2O with $[K_{is}^+(CH_3OH)/K_{js}^+(CH_3OH)]$ measured in methanol by Pioda et al. (1967)*

Macrotetralide actin and ion	$\frac{K_i}{K_j}$	$\frac{K_{is}^+(CH_3OH)}{K_{js}^+(CH_3OH)}$
Nonactin		
Na	0.017	0.026
K	1.0	1.0
Monactin		
Na	0.0094	0.0043
K	1.0	1.0

approximately equal to the value of the ratio $K_{js}^+(CH_3OH)/K_{is}^+(CH_3OH)$ which has been measured in methanol[12], we expect:

$$\frac{K_j}{K_i}=\frac{K_{js}^+}{K_{is}^+}\cong\frac{K_{js}(CH_3OH)}{K_{is}(CH_3OH)}. \tag{56}$$

The satisfactory agreement with which the expectations of Eq. (56) are borne out is seen in Table 16.

Lastly, this postulate is supported by the precise correspondence which will be demonstrated in paper III between the K_j/K_i ratios of the present paper and the permeability ratios (as well as conductance ratios) of lecithin (and lecithin-cholesterol) membranes. According to Eq. (11), such correspondence is expected only if the mobility ratio of the complexes in the membrane phase is unity, which in turn is expected only if the complexes have the same overall size and shape regardless of the particular cation bound.

Predicted Effects of the Macrotetralide Actin Antibiotics on Phospholipid Bilayers

The data of Table 15, together with Eq. (15) deduced for "isosteric" complexes, suffice to predict the main effects of the macrotetralide actin antibiotics on phospholipid bilayers. For convenience, Eq. (15) is rewritten here:

$$\frac{K_j}{K_i}=\frac{P_j}{P_i}=\frac{G_0(J)}{G_0(I)}. \tag{57}$$

12 This approximation is reasonable since the differences of solvation energies of cations in methanol are approximately the same as those in water (Conway, 1952). Note that the value of K_{js}^+/K_{is}^+ [or $K_{js}^+(CH_3OH)/K_{is}^+(CH_3OH)$] is determined by the differences of hydration (or solvation) energies of the ions vs. the differences of their binding energies to the macrotetralide molecule.

The agreement between these expectations and the experimental observations will be seen in Fig. 17 and Table 5 of paper III.

It is also possible to predict the dependence of the absolute magnitude of the membrane conductance on the absolute value of K_i as follows. Eq. (59) of paper I can be written as:

$$G_0(I)=A_i K_{is}^+, \tag{58}$$

where A_i is a constant, characteristic of each cation for conductances measured at a given concentration of antibiotic and activity of salt, defined by:

$$A_i=\frac{F^2}{d} u_{is}^* k_{is} C_s^{\mathrm{Tot}} a_i, \tag{59}$$

for a given lipid composition and antibiotic molecule.

On the other hand, Eq. (8) can be written:

$$K_i=B_i K_{is}^+ \tag{60}$$

where B_i is a constant for each cation, defined by:

$$B_i=\frac{k_{is} k_x}{k_s}, \tag{61}$$

for a given solvent, anion, and antibiotic molecule.

Both Eqs. (58) and (60) are directly proportional to K_{is}^+, which the reader will recall is independent of the properties of the solvent or the composition of lipid. Comparing these equations, we therefore find:

$$G_0(I)=\frac{A_i}{B_i} K_i, \tag{62}$$

signifying that a direct proportionality is expected between the observed conductance and the salt extraction equilibrium constant for a given cation and antibiotic molecule. Moreover, the value of the proportionality constant (A_i/B_i) should be independent of the cation for "isosteric" complexes since k_{is} and u_{is}^* are the same for all cations in this case. The proportionality expected from Eq. (62) is seen in the data of Fig. 19 of paper III, which verifies that the proportionality constant (A_i/B_i) is indeed the same for all cations for a given macrotetralide.

Predicted Relationships When the Antibiotic Molecule is Varied

The macrotetralide actins differ only by the successive additions of methyl groups to a much larger molecule. It is therefore reasonable to assume that the resultant changes in size and shape are small. In this case, the mobilities of the complexes should not differ greatly among these molecules, nor should the values of k_{is} and k_s. Therefore, A_i and B_i should be virtually the same for all the macrotetralides. That this is so will be seen in Fig. 20 of paper III.

From the same reasoning, if the nonactin and monactin complexes are similar in external size and shape, the proportionality constant B_i of Eq. (60), between the values of K_i measured in a given solvent and the value of K_{is}^+, should be the same for all cations and macrotetralides. Assuming that the association constant measured in methanol, $K_{is}^+(CH_3OH)$, is proportional to the association constant in water, K_{is}^+, this expectation can be tested by plotting our measurements of $\log K_i$ for Na^+ and K^+ and for nonactin and monactin against Simon's measurement of $\log K_{is}^+$ in methanol. This is done in Fig. 14, where the agreement with the line of slope 1 indicates that this expectation is fulfilled.

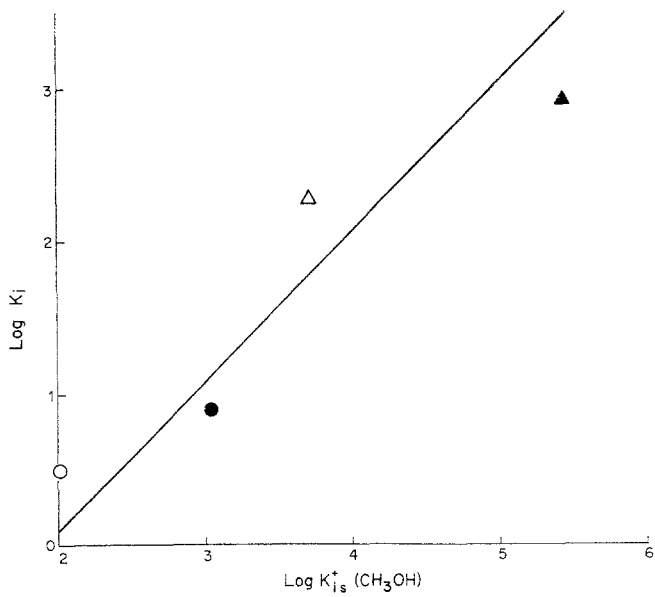

Fig. 14. Correlations between values of salt extraction equilibrium constants (K_i) of the present paper and the constants for complexation of cations in methanol of Pioda et al. (1967). Data for Na^+ are indicated by circles, for K^+ by triangles, for nonactin by open symbols, and for monactin by solid symbols. The straight line of slope 1 is expected from Eq. (60) for "isosteric" complexes, as discussed in the text

The Chief Effect of Methylation is on the Dipole-Moment of the Ligand Oxygens

In the light of the single proportionality constant demonstrated in Fig. 20 of paper III between the observed data and the expectations of Eq. (62), we are forced to conclude that the principle effect of the additional methyl groups in the macrotetralide actin series is on the value of K_{is}^{+}, and that they do not significantly alter the value of k_s. A_i/B_i can only be constant if k_s is not altered [since, recalling the definitions of A_i and B_i in Eqs. (59) and (61), $\frac{A_i}{B_i}=\left[\frac{F^2 u_{is}^{*} C_s^{\mathrm{Tot}} a_i}{d k_x}\right] k_s$, where the bracketed quantity is independent of varying the macrotetralide]. Thus the predominant effect of methylation must be from the electron repelling action of the methyl group, increasing the dipole moments of the ligand oxygens within the center of the antibiotic complex. This increases the value of K_{is}^{+}, with the consequent increase in K_i.

Interestingly, even such details as the tendency, with increasing methylation, of Na^+ to become more comparable to Cs^+ (and of K^+ to be increasingly preferred relative to Cs^+) are as expected from such an increase in the dipole moments of the ligand groups[13].

Conclusions

The principal significance of the experimental results is their detailed confirmation of the simple chemistry postulated in reactions (1)–(4) of paper I for the interaction between cations and the macrotetralide actin antibiotics. The following conclusions have been reached:

(1) Lipophilic neutral molecules typified by the macrotetralide actin antibiotics form stoichiometric 1:1 complexes with monovalent cations, thereby solubilizing them in media of low dielectric constant. This leads to the observed ability of such neutral molecules to extract appropriate salts of monovalent cations into organic solvents.

(2) The detailed agreement over wide concentration ranges between the theoretically expected and experimentally observed extractions verifies the postulate that the behavior of the complexes and antibiotic molecules in the solvent is highly ideal.

13 The reader who is interested in pursuing this in further detail is referred to the selectivity patterns of Eisenman (1962) from which it can be seen that there is a tendency for the selectivity of rank order IV characteristic of nonactin to tend toward rank order V at the "higher field strengths" expected for trinactin. That the field strength concept, as originally used for monopolar charged sites, can be applied to the present neutral dipolar sites has been shown elsewhere (Eisenman, 1969).

(3) In addition, and equally important, a wide variety of observations, when interpreted within the present theoretical framework, point to the conclusion that the complexes formed between the macrotetralide actins and the cations studied are "isosteric" (i.e., have the same size and shape for all cations).

(4) Incorporation of this "isosteric" property of the complex within the general theoretical framework of paper I leads to important simplifications of the theoretical expectations. In particular, it makes possible the quantitative prediction of the effects of the macrotetralide actins on phospholipid bilayer membranes from their measured ability to extract salts into an arbitrarily chosen solvent.

Thanks are due to Dr. Elizabeth S. Low for her able technical assistance with many of the extraction experiments. It is a pleasure to thank Drs. Stuart McLaughlin and Robert Eisenberg for reading the manuscript and for their perceptive and valuable comments.

References

Ciani, S., G. Eisenman, and G. Szabo. 1969. A theory for the effects of neutral carriers such as the macrotetralide actin antibiotics on the electric properties of bilayer membranes. *J. Membrane Biol.* **1**:1.

Conway, B. E. 1952. Electrochemical Data. Elsevier, Amsterdam.

Eisenman, G. 1962. Cation selective glass electrodes and their mode of operation. *Biophys. J.* **2**:pt. 2, 259.

— 1965. The electrochemistry of cation selective glass electrodes. In: Advances in Analytical Chemistry and Instrumentation, vol. 4. C. N. Reilley, editor. p. 213. Wiley-Interscience, New York.

—, S. M. Ciani, and G. Szabo. 1968. Some theoretically expected and experimentally observed properties of lipid bilayer membranes containing neutral molecular carriers of ions. *Fed. Proc.* **27**:1289.

— 1969. Theory of membrane electrode potentials: An examination of the parameters determining the selectivity of solid and liquid ion exchangers and of neutral ion-sequestering molecules. Chapter 1 in: *Ion-Selective Electrodes.* R. A. Durst, editor. Natl. Bureau of Standards Special Publ. 314. U.S. Govt. Printing Office, Washington.

Harned, H. S., and B. B. Owen. 1958. The Physical Chemistry of Electrolytic Solutions. Reinhold Publ. Corp., New York.

Kilbourn, B. T., J. D. Dunitz, L. A. R. Pioda, and W. Simon. 1967. Structure of the K^+ complex with nonactin, a macrotetralide antibiotic possessing specific K^+ transport properties. *J. Mol. Biol.* **30**:559.

Matsen, F. A. 1956. Chemical Applications of Spectroscopy. Vol. 9 of Technique of Organic Chemistry. Interscience Publ., Inc., New York.

Pedersen, C. J. 1968. Ionic complexes of macrocyclic polyethers. *Fed. Proc.* **27**:1305.

Pioda, L. A. R., H. A. Wachter, R. E. Dohner, and W. Simon. 1967. Komplexe von Nonactin und Monactin mit Natrium-, Kalium- und Ammonium-Ionen. *Helv. Chim. Acta* **50**:1373.

Pressman, B. C., E. J. Harris, W. S. Jagger, and J. H. Johnson. 1967. Antibiotic-mediated transport of alkali ions across lipid barriers. *Proc. Nat. Acad. Sci.* **58**:1949.

Robinson, R. A., and R. H. Stokes. 1959. Electrolyte Solutions. Butterworth, Inc. London.

Szabo, G., G. Eisenman, and S. Ciani. 1969*a*. The effects of the macrotetralide actin antibiotics on the electrical properties of phospholipid bilayer membranes. *J. Membrane Biol.* **1**:346.

— — — 1969*b*. Ion distribution equilibria in bulk phases and the ion transport properties of bilayer membranes produced by neutral macrocyclic antibiotics. In: *Proc. Coral Gables Conference on the Physical Principles of Biol. Membranes, Dec.* 18—20, 1968. Gordon and Breach, Science Publ., New York (*in press*).

Weissberger, A., E. S. Proskauer, J. A. Riddick, and E. E. Toops, Jr. 1955. Organic solvents. *In*: Technique of Organic Chemistry, vol. VII. A. Weissberger, editor. Interscience Publ., Inc., New York.