Thermodynamics of Reaction in Heterogeneous Systems (Water–Organic Phases) between the Ionophore Monensin and Alkali-Metal Cations[†]

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Gibbs functions, enthalpies, entropies, and volumes of reaction for the reaction of the bacterial ionophore monensin with alkali-metal cations in various water-organic biphasic solvent systems are obtained by using various experimental methods. In addition, weak but perceptible solubilization of the complexes formed is shown to occur in the aqueous phase. Their dissociation in water is studied. All the data are discussed in terms of structure and solvation of the various species. The selectivity sequence is not solvent dependent though the enthalpic and entropic contributions for different cations vary with the solvent system involved. On the basis of these data, both biological functional aspects and analytical aspects relevant to the testing of such ionophores are discussed.

Introduction

Monensin is a naturally occurring ionophore isolated from strains of Streptomyces cinamonensis.¹ It is widely used for improvement of cattle feed and treatment of poultry coccidiosis and is one of the most extensively studied members of the now large class of carboxylic acid ionophores of bacterial origin. These substances facilitate the translocation of cations across biological membranes through their complexing ability allied to their lipophilic character. Ligand properties of monensin toward monovalent cations have been widely investigated in protic and polar aprotic solvents²⁻¹⁰ in which the ionophore, its complexes, and the inorganic salts are all soluble. In methanol, particularly, formation constants of complexes and corresponding enthalpies, entropies, and volumes have been obtained and kinetics of complexation have also been studied.^{8,11,12} However, results in homogeneous media are not necessarily representative of what happens between biological aqueous media and membranes. Direct measurements of transport of cations by monensin across natural and artificial (i.e., bimolecular lipid) membranes^{13,14} have been reported. A frequently used accepted approximation to the membrane is achieved by an organic phase "immiscible" with water. Measurements of cation flux facilitated by the presence of monensin were reported for such systems¹⁵ as were studies on quasi-stationary states in electrochemicals cells so constituted.¹⁶⁻¹⁸ This last method is one of the ways of studying the selectivity of the ionophore for the various cations in water-organic systems.

The present paper reports direct studies of equilibria between alkali-metal cations M⁺ in an aqueous phase and the ionophore AH in an organic "immisicible" phase. For monensin and alkali-metal cations some qualitative results have been reported by Pressman,¹⁹ and data limited to certain systems were published by Caffarel-Mendez²⁰ and Gaboyard.²¹ A systematic determination of the thermodynamic quantities (G, H, S, V) associated with reaction 1, namely, substitution of the metal cation for the proton of the ionophore in the organic phase, was undertaken. (Hereafter, a species I will be designated I in water and I in the organic phase.)

$$AH + M^+ \rightleftharpoons AM + H^+$$
 (1)

In some cases, partitioning of the ionophore complex salt AM between the aqueous and the organic phases was found to be nonnegligible. An attempt was then made to determine the partition constant K_2 corresponding to reaction 2:

$$\overline{\mathrm{AM}} \rightleftharpoons \mathrm{AM}$$
 (2)

It has been shown⁸ that, though the solubility of monensin in water is very low, that of its tetraalkylammonium salts is high. This suggests that the monensin anion is well accommodated by water and that dissociation of AM according to reaction 3 may therefore be important:

$$AM \rightleftharpoons A^- + M^+ \tag{3}$$

A systematic study of the solubility of AM in water as a function of anion A⁻ concentration would afford the corresponding dissociation constant K_3 .

Dissociation of AM in a weakly polar organic phase can be neglected. Equations 1-3 would then allow the whole system to be described provided that other species such as A₂M₂, AHAM, etc., are not formed in the organic phase. Assuming that the solvent systems used are accepted membrane models, such determinations would be expected to give some insight into those reactions involving monensin and monovalent cations that occur between an aqueous solution and a membrane: species involved, selectivity for the various cations, occurrence of the exchange reaction at the interface or in the aqueous phase, and conformational flexibility of the ionophore as related to entropic effects.

Two types of organic phase were chosen: chloroform, previously used in transport experiments,¹⁵ and 1-butanol-toluene 3:7 mix-

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Figure 1. Monensin structure showing the carbon and oxygen numbering schemes.

ture, the so-called "Pressman phase" widely used by experimenters in this field. Octanol, which was also selected, unfortunately did not dissolve some of the ionophore salts and showed a tendency to form a third phase with water in partition experiments.

Experimental Section

Chemicals. Monensin was prepared from its commercial sodium salt (Sigma Co.); 5 g of this salt was washed with 15 cm³ of acetone, then filtered, and dried in a rotary evaporator. The salt was then dissolved in 200 cm³ of chloroform, and this solution was washed three times with 200 cm³ of a 0.5 M HCl solution in water (from HCl, 31% pro analysi, Merck Co., Darmstadt, Germany) and then three times with 200 cm³ of triply distilled water. The chloroform phase was evaporated off and 10-15 cm³ of acetone added. After dissolving, the acetone was evaporated off. This operation, intended to carry away traces of water, was repeated once. A white powder was obtained which was dried overnight in a vacuum oven at 50 °C on a poly(tetrafluoroethylene)-coated flat dish. No attempt was made to eliminate small amounts of monensin B from monensin A given their very similar properties. (Monensin B differs only in bearing a methyl group in place of the ethyl on the furan ring.)

The monensin alkali-metal salts were prepared in methanol by exact neutralization of the acid ionophore with corresponding methoxides. The methanol was evaporated off, and the salts were dried as described for the acid form. Lithium, sodium, and potassium methoxides were obtained by reaction with methanol of freshly cut cubes of the pure alkali metal, under a stream of argon. Rubidium and cesium methoxides were prepared electrochemically, using their amalgams as previously described.¹⁰ The methanol solutions thus obtained were titrated by potentiometry and then appropriately diluted.

The tetramethylammonium salt of monensin was obtained and purified by using the same procedure, the corresponding methoxide solutions in methanol being prepared and standardized as previously reported.²²

The solvents used were of the best commercial grade: toluene (Fluka puriss p.a.), 1-butanol (Prolabo normapur) redistilled to eliminate traces of sodium, chloroform (Merck pro analysi) stabilized with ethanol, and methanol as previously described²² (Merck pro analysi).

The alkali-metal chlorides and cesium carbonate were Merck suprapur products; rubidium carbonate was an Alfa-Ventron 99.993% pure product. Absence of other cations, particularly sodium (<0.02%), was checked by atomic spectroscopy in the concentrated hydroxide solutions: LiOH (R.C. normapur) and KOH (normatom) from Prolabo Co.; CsOH and RbOH from Aldrich Co.

The amines used for the buffer solutions were tris (Merck pro analysis), Bis-Tris (Sigma), and dimethylethanolamine (Fluka) redistilled. An 85% aqueous solutions of phosphoric acid was obtained from the Merck Co. (grade pro analysi).

Extraction and Partition. All the experiments were carried out in an air-conditioned room at 25 °C. Extractions were performed by shaking a 2×10^{-3} mol dm⁻³ organic monensin solution with a 10^{-2} mol dm⁻³ aqueous alkali-metal solution (from metal chloride or metal hydroxide), in which the pH was maintained by buffering, the ionic strength being kept at 2×10^{-2} . The following buffer solutions were used: Bis-Tris + HCl (pH from

(22) Woznicka, J.; Lhermet, C.; Morel-Desrosiers, N.; Morel, J. P.; Juillard, J. J. Chem. Soc., Faraday Trans. 1 1989, 85, 1709. 5.0 to 7.4), Tris + HCl (pH from 7.0 to 9.5), N,N-dimethylethanolamine + HCl (pH from 9.0 to 9.8), alkali-metal carbonate + HCl (pH from 8.0 to 10.3), phosphoric acid + alkali-metal hydroxide (pH from 10 to 11), and if necessary metal hydroxide solutions (pH from 11 to 12). A 3-cm³ sample of each solution was put in a tube on a vibrating stirrer; after 1 min of stirring, the mixture was centrifuged for 1 min at 3000 rpm. The two phases were then separated. The pH of the aqueous phase was measured with a Tacussel TB 11HA combined glass-calomel electrode. A 2-cm³ portion of the organic phase was stirred with 2 cm³ of 0.5 M HCl solution in water. After centrifugation a known volume of the aqueous phase was appropriately diluted to determine the alkali-metal concentration by atomic spectroscopy. Variation of phase volume was taken into account in the calculations. Measurements were made using a Perkin-Elmer 420 spectrometer, either by absorption, for Na and K, or by emission for Li, Cs, and Rb, standardization being achieved using solutions in triply distilled water of the corresponding chloride.

Partition experiments were carried out using the same procedure. Originally, the organic phase contained the monensin alkali-metal salt at concentrations from 5×10^{-3} to 10^{-2} mol dm⁻³; the aqueous phase contained a Tris-HCl buffer to maintain a pH of about 9.

Solubilities. The monensin alkali-metal salt was stirred (using a magnetic stirrer) for at least 2 h in an aqueous solution containing 10^{-6} mol dm⁻³ of tetraalkylammonium hydroxide (to repress any hydrolysis of the salt) in the presence of varying amounts of monensin tetramethylammonium salt. The solution was held in a jacketed vessel and its temperature maintained at 25.0 °C by circulation of water from a thermostated tank. The solution so obtained was filtered at 25 °C on a Whatman type GF/F filter. After exact dilution the metal content was determined by atomic spectroscopy.

Calorimetry. The experiments consisted in measuring heats of solutions of monensin (AH) and its various alkali-metal salts (AM) either in methanol or in the organic solvent (chloroform or toluene-1-butanol mixture) equilibrated beforehand with an equal volume of water. Enthalpies of solution were measured by the method of the broken ampule using an LKB 8700 solution calorimeter. The salt was placed in a 1-cm³ ampule and the solvent in a 25-cm³ cell. In the case of methanol, 10⁻⁶ mol dm⁻³ tetrabutylammonium methoxide was added to prevent hydrolysis of the salt. A major problem in these measurements was closing the ampules. Fire sealing was ruled out because of the danger of degrading the salt. "Viton" taps were used, solvent tightness being secured by covering col and tap with a thin coat of LKB sealing wax for methanol and gelatin for chloroform and toluene-butanol solvents. Gelatin (Merck für die mikrobiologie) was added to a small quantity of water; after swelling and cooling this gel was coated on to the tap and left overnight to dry.

Densimetry. Densities of monensin and monensin salt solutions in various solvents were measured with a digital flow densimeter (Sodev) with a precision of at least $\pm 5 \times 10^{-6}$ g cm⁻³.

Analysis of Phase Composition. Vapor-phase chromatographic analysis was performed using a Delsi 700 chromatograph with an FID detector and an SE 52 capillary column. Chloroform in aqueous phases was extracted, volume to volume, with pentane containing 0.5 wt % 1,2-dichloroethane as internal standard; pentane solutions were dried on magnesium sulfate. Standardization was performed using chloroform solutions of various concentrations in pentane equilibrated with water and dried as above.

UV analysis of toluene in the aqueous phase was at $\lambda = 260$ nm, using a Beckman DU⁸R spectrometer, standardization being performed with toluene solutions of various concentrations in water-1-butanol (97:3 wt %).

Water analysis in organic phases was performed in the usual way using a Methrom Karl Fischer biamperometric titration apparatus and Fluka K.F. reagent.

Methods and Results

Solvent-Phase Composition. All the extraction and partition

data were obtained by using equal volumes at 25 °C of aqueous and organic solutions. In order to obtain the calorimetric and densimetric data under like conditions, the organic phases were equilibrated with one volume of water to one volume of the organic mixture, either chloroform with a small amount of ethanol or toluene 70% + 1-butanol 30% by weight (density $\rho = 0.8438$). The exact composition of the phases so obtained was then determined by using various analytical methods: water content in the organic phases was obtained by Karl Fischer biamperometric titration, alcohol content in chloroform, chloroform content in water, and composition of toluene-butanol mixture by gas chromatography, and toluene content in water by UV spectroscopy.

The following results were obtained (composition in weight percent at 25 °C). For the first system, starting with commercial chloroform 99.10 stabilized with ethanol 0.90 (density $\rho = 1.4685$), the composition of the organic phase was chloroform 99.84, ethanol 0.11, water 0.05, ($\rho = 1.4771$), and the composition of the aqueous phase water 98.05, ethanol 1.14, chloroform 0.81 (solubility in pure water 0.82 at 20 °C; $\rho = 0.9976$).

For the second system the composition of the two phases at equilibrium was toluene 72.02, 1-butanol 26.24, water 1.74 ($\rho = 0.8484$) and water 96.34, 1-butanol 3.63, toluene 0.60 ($\rho = 0.9906$).

The phase volumes at equilibrium were calculated by using density for initial and final compositions and confirmed by direct volume measurements. The ratios of the initial-to-final volumes of the aqueous phase ζ and organic phase $\overline{\zeta}$ were found to be $\zeta = 0.981$ and $\overline{\zeta} = 1.019$ for the first system (water-chloroform) and $\zeta = 0.962$ and $\overline{\zeta} = 1.050$ for the second one (water-toluene, butanol).

It was further assumed that, given the low electrolyte and ionophore concentrations, the phase volume and composition were unaffected by the presence and reaction of the solutes.

Equilibrium Constant for the Heterogeneous Exchange Reaction. The Gibbs function for reaction 1 could be accessed by a classical extraction procedure. This method was first used for carboxylic ionophores by Pfeiffer.²³ It consists in equilibrating two solutions: one in water containing the metal salt at concentration c°_{M} plus agents to stabilize the pH, the other in an organic phase containing the ionophore at concentration \bar{c}°_{A} . At equilibrium the pH of the aqueous phase and the concentration of the metal in the organic phase \bar{c}_{M} are measured. Reactions other than the simple exchange reaction 1, such as formation of A_2M_2 or A_2HM , can also occur; but exclusive formation of AM was assumed to occur first. If the values of K_1 so obtained from experiments in various conditions (pH, concentration of reactants) were not constant, then other assumptions would be made.

Assuming then only reaction 1

$$K_1 = \frac{a_{\mathrm{H}^+}a_{\overline{\mathrm{A}\mathrm{M}}}}{a_{\overline{\mathrm{A}\mathrm{H}}}a_{\mathrm{M}^+}} = \frac{a_{\mathrm{H}^+}}{[\mathrm{M}^+]y_{\pm}} \frac{[\mathrm{A}\mathrm{M}]}{[\overline{\mathrm{A}\mathrm{H}}]} \tag{4}$$

in which [I] and [I] stand for molar concentration of species I respectively in the aqueous phase and in the organic phase. If the concentrations in the organic phase are low, they can be likened to activities. Mean molar activity coefficient y_{\pm} in the aqueous phase can be estimated by using the Debye-Hückel extended law. If there is no significant partition of AM in the aqueous phase, then eq 4 becomes

$$K_{1} = \frac{a_{\mathrm{H}^{+}}}{y_{\pm}} \frac{\overline{\zeta} \overline{\zeta} c_{\mathrm{M}}}{\zeta (\overline{\zeta} c^{\circ}{}_{\mathrm{M}} - \overline{c}_{\mathrm{M}}) (\overline{\zeta} \overline{\zeta} c^{\circ}{}_{\mathrm{A}} - \overline{c}_{\mathrm{M}})}$$
(5)

in which the total actual concentration of metal and ionophore are respectively c_M and c_A in the aqueous phase and \bar{c}_M and \bar{c}_A in the organic one, the c° corresponding to initial concentrations and the ζ to initial-final volume ratios of the two phases.

More generally, the measurements are made at variable pH but at constant initial ionophore concentration in the organic phase and at constant ionic strength and concentration of the metal cation in the aqueous phase. The pH of the aqueous solution can



Figure 2. Extraction of alkali-metal cations by monensin in waterchloroform system $r = \bar{c}_M/\bar{c}_A^o$ as a function of the pH of the aqueous phase ($c_M^o = 10^{-2} \text{ mol dm}^{-3}$, $\bar{c}_A^o = 2 \times 10^{-3} \text{ mol dm}^{-3}$, equal volumes of aqueous and chloroform phase, 25 °C): \blacktriangle , Li⁺; \blacklozenge , Na⁺; \circlearrowright , K⁺; \square , Rb⁺; \triangle , Cs⁺.

TABLE I: Logarithm of the Cation-Exchange Constant (Reaction 1) in Water-Chloroform (1) and Water-Toluene, Butanol (2) Systems (Molar Concentration in mol dm^{-3} at 25 °C)

	system 1	system 2	
	system 1	system 2	
Li	-6.4 ± 0.2	-5.7 🛳 0.1	
Na	-4.2 ± 0.1	-3.8 单 0.2	
K	-5.8 🏚 0.1	-4.95 ± 0.10	
Rb	-6.8 🛥 0.2	-5.95 🛳 0.10	
Cs	-8.5 单 0.1	-6.6 ± 0.2	

be set with buffering agents or strong acids or bases. The partition of these substances has to be strongly in favor of the aqueous phase, and they must not be extracted, in any form, by the ionophore. In addition, concentrations of cations other than the one being investigated must be very low. Providing this is so, it follows from relation 5 that, y_{\pm} being constant, the shape of the curve r = $\bar{c}_{\rm M}/\bar{\zeta}c^{\circ}{}_{\rm A} = f(\rm pH)$ (see Figure 2) is independent of the value of K_1 , which only determines the location of this curve on the pH scale. This can be used as a rapid check for the absence of any notable formation of complexes other than AM as well as the absence of significant partition of AM with the aqueous phase. These curves have a typical sigmoid shape with an asymptote r= 1. The r = f(pH) curves obtained from the extraction measurements in the water chloroform system are given, as an example, in Figure 2. These curves (except that for Li⁺) agree well with the theoretical shape obtained assuming formation only of AM, according to reaction 1. Moreover, calculations give, for Na⁺ K⁺, Rb⁺, and Cs⁺ in the two systems, constant K_1 values for 6-8 points, distributed all along the curve except at the ends, each from three concurrent determinations. These values along with the corresponding maximum deviations are reported in Table I.

If there is partition of AM between the aqueous and organic phases, the r = f(pH) curves present an asymptote r = 1/(1 + Q) (instead of r = 1), Q being the partition ratio of the ionophore salt, $Q = ([AM] + [A^-])/[\overline{AM}]$. Such a situation occurred here for Li⁺. A notable partition of ALi is observed, the partition ratio Q being in our experimental conditions of the order of 0.47 in the water-chloroform system and 0.29 in the water-toluene, butanol system. In such a case determination of K_1 would strictly need values of K_2 and K_3 to be known. An approximate value of K_1 can nevertheless be obtained if some assumptions are made. Given the values fixed for concentrations c°_{M} and \bar{c}°_{A} , it was assumed here for the sake of simplicity that $K_3 = 0$ and $Q = K_2$. Equation 4 then becomes

$$K_{1} = \frac{a_{\mathrm{H}^{+}}}{y_{\pm}} \frac{\zeta \zeta \bar{c}_{\mathrm{M}}}{\left[\bar{\zeta} \zeta \bar{c}^{\circ}_{\mathrm{M}} - (\zeta + \bar{\zeta} Q) \bar{c}_{\mathrm{M}}\right] \left[\bar{\zeta} \zeta \bar{c}^{\circ}_{\mathrm{A}} - (\zeta + \bar{\zeta} Q) \bar{c}_{\mathrm{M}}\right]} \tag{6}$$

⁽²³⁾ Pfeiffer, D. R.; Lardy, H. A. Biochemistry 1976, 15, 935 and references therein.

TABLE II: Determination, Given as an Example, of the Standard Enthalpy of Reaction 1 for Monensin-Lithium Ion in Water-Chloroform System at 25 °C^a

	chloroformic phase			ase methanol							
	n, 10 ⁻⁵ mol	ΔH _{exp} , J	Δ H^e sol, kJ mol ^{−1}		n, 10 ⁻⁵ mol	$\Delta H_{\rm exp}, { m J}$	x, 10 ⁻⁵ mol	$x\phi_{\rm L}, 10^{-3} {\rm J}$	x∆H ^e _{3m} , J	$\Delta H_{\rm corr}, J$	∆ <i>H</i> ^e _{sol} , kJ mol ^{−1}
1	3.535	-1.116	-46.85	1'	3.018	-0.362	0.830	1.9	-0.020	-0.342	-20.30
2	6.778	-2.648	-46.94	2′	5.747	0.973	1.197	3.2	-0.029	-0.944	-21.08
3	8.086	-3.266	-46.95	3′	8.953	-1.653	1.528	4.6	-0.037	-1.617	-21.00
4	14.437	-6.256 mean	-46.91 -46.9 ± 0.1	4′	11.994	-2.271	1.791	5.7	-0.043	-2.228 mean	-20.74 -20.8 ● 0.5

^aConstant values used (see text for notations): k = -0.476 (chloroformic phase) and k = -0.229 (methanol) from Figure 3, $v \simeq 1.15$ cm³, $\rho \simeq 1.5$; $\Delta H^{\Theta}_{1m} = 19.2$ kJ mol⁻¹, $\Delta H^{\Theta}_{3m} = -2.4$ kJ mol⁻¹, and $pK_{3m} = 3.9$ (methanol¹⁰); $\Delta H^{\Theta}_{b} - \Delta H^{\Theta}_{a} = \Delta H_{t}(H^{+}) - \Delta H_{t}(Li^{+})$ [methanol \rightarrow water] = -6.32 kJ mol⁻¹;²¹ $\Delta H^{\Theta}_{c} = \Delta H_{t}(AH)$ [methanol \rightarrow chloroformic phase] = -50.2 kJ mol⁻¹ (from an analogous determination). $\Delta H^{\Theta}_{d} = \Delta H_{t}(AM)$ [methanol \rightarrow chloroformic phase] = -26.1 \pm 0.6 kJ mol⁻¹; $\Delta H^{\Theta}_{1} = \Delta H^{\Theta}_{1m} + \Delta H^{\Theta}_{d} - \Delta H^{\Theta}_{c} + (\Delta H^{\Theta}_{b} - \Delta H^{\Theta}_{a}) = 37.0$ kJ.

Values thus obtained are also given in Table I. Using these values of K_1 for Li⁺ later allowed K_2 and K_3 to be determined from solubility and partition experiments. A more accurate calculation of K_1 could then be made. It showed that errors introduced via eq 6 were less than the maximum deviation reported in Table I.

Standard Enthalpy of the Heterogeneous Exchange Reaction. No reliable means was found of directly measuring the enthalpy of reaction 1. It was therefore obtained from the standard enthalpy of the same reaction in homogeneous medium, methanol, and the standard enthalpies of transfer of the reactants and products of the reaction from methanol to water (H^+ and M^+) or from methanol to the organic phase considered (AH and AM) according to the following thermodynamic scheme

$$M^{+} + \overline{AH} \longrightarrow H^{+} + \overline{AM} \qquad (1)$$

$$\downarrow a \qquad \downarrow c \qquad \downarrow b \qquad \downarrow d$$

$$^{m}M^{+} + ^{m}AH \longrightarrow {}^{m}H^{+} + ^{m}AM \qquad (1m)$$

where the superscript m stands for methanol. Then

$$\Delta H^{\Theta}_{1} = \Delta H^{\Theta}_{1m} + (\Delta H^{\Theta}_{d} - \Delta H^{\Theta}_{c}) + (\Delta H^{\Theta}_{b} - \Delta H^{\Theta}_{a})$$
(7)

The ΔH^{Θ}_{lm} values had been obtained previously.¹⁰ The $(\Delta H^{\Theta}_{b} - \Delta H^{\Theta}_{a})$ values were taken from tables of enthalpies of transfer of ions or electrolytes from water to methanol.²⁴ In doing this, it was assumed that the enthalpies of transfer of alkali-metal cations and the hydrogen ion from water to our aqueous phases were either negligible or identical. Though no data have been reported for water-1-butanol systems, results in other water-alcohol systems, such as those involving *tert*-butyl alcohol,²⁵ support this assumption.

 ΔH_d and ΔH_a , the enthalpies of transfer from methanol to the organic phase, were obtained here from the differences between the enthalpies of solution of AH or AM in methanol and in the organic phase. These standard enthalpies of solution ΔH^{σ}_{sol} were determined in an isoperibolic calorimeter by using the ampule breaking method. Solid samples of AH or AM were put in the ampule, and the solvent was put in the vessel. The experimental enthalpies corresponding to the solution of *n* moles of solid AH or AM is

$$\Delta H_{\rm exp} = n \Delta H^{\Theta}_{\rm sol} + \Delta H_{\rm B} + (x \Delta H^{\Theta}_{\rm 3m} + x L_{\phi}) \qquad (8)$$

 $\Delta H_{\rm B}$ is related to the so-called "bubble effect", i.e., the saturation by the solvent of the free air in the ampule when it is broken. It is proportional to the air volume in the ampule

$$\Delta H_{\rm B} = -k(v - nM/\rho) \tag{9}$$

in which v is the internal volume of the ampule, M the molar mass of the solid, and ρ its density. k depends on the nature of the solvent. It is related to its heat of vaporization. The last two terms of eq 8 account for any dissociation of AH or AM in the solvent. This can reasonably be supposed not to occur in our weakly polar organic phases but must be considered in methanol. ΔH^{Θ}_{3m} is



Figure 3. Determination, given as an example, of the enthalpy of solution of monensin lithium salts in methanol (\odot) and in the chloroform phase (\Box) . Experimental or corrected enthalpies as a function of the number of moles, *n*, of salt dissolved, according to eq 10.

the enthalpy of dissociation (reaction 3m analogous to reaction 3, but in methanol) calculated from the previously reported values of the enthalpies of dissociation of both AH and AM.¹⁰ x is the number of moles of the solute dissociated calculated from its dissociation constant.¹⁰ L_{ϕ} is the enthalpy of dilution of 1 mol of solute from the final concentration in the vessel to infinite dilution. It was calculated by using the equation previously obtained,²⁶ deriving as a function of the temperature the Debye-Hückel extended expression of the excess Gibbs functions, using Bjerrum q as distance parameter. It can be seen from the example given in Table II that correction is slight and can be neglected.

For monensin and each of the alkali-metal salts at least four ampules containing varying quantities (number of moles n) were broken in each solvent phase (chloroform, 1-butanol-toluene, and methanol). The standard enthalpies of solution were obtained via relation 8, which was rearranged as follows:

$$\Delta H_{\exp} - (x \Delta H^{\Theta}_{3m} + xL_{\phi}) = \Delta H_{\text{corr}} = n(\Delta H^{\Theta}_{\text{sol}} + kM/\rho) - kv (10)$$

 ΔH_{corr} was plotted as a function of *n*: examples of such plots are given in Figure 3 for the monensin-lithium system in methanol and in the chloroform phase. *k* was obtained from the ordinate at the origin and ΔH^{Θ}_{sol} from the slope. The value of *k* was also determined by breaking empty ampules in the solvent. Agreement was generally good. For example, for the system cited here the values of *k* obtained from plots in Figure 3 and those independently determined were respectively -0.229 and -0.249 for methanol and

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TABLE III: Transfer Enthalpies of Monensin and Monensin Salts at 25 °C from Methanol to the Chloroform (1) and Toluene, Butanol (2) Phases in kJ mol⁻¹ (Estimated Accuracy ±0.5 kJ mol⁻¹)

	system 1	system 2		system 1	system 2
AH	-50.2	-16.0	AK	-35.2	-10.7
ALi	-26.1	-11.7	ARb	-20.2	-8.9
ANa	-33.4	-11.8	ACs	-46.3	-15.2

TABLE IV: Thermodynamic Functions of the Cation Heterogeneous Exchange Reaction 1 in the Two Solvent Systems (Molar Scale in Each Solvent)

	ΔG^{Θ} , kJ mol ⁻¹	ΔH^{Θ} , kJ mol ⁻¹	ΔS^{Θ} , J K ⁻¹ mol ⁻¹
	Water-C	hloroform System	
Li	36.5	37.0	+1.7
Na	24.0	16.6	-24.8
K	33.1	18.3	-49.6
Rb	38.8	35.4	-11.4
Cs	48.5	7.9	-136.0
	Water-Buta	nol. Toluene Systen	n
Li	32.5	17.2	-51.3
Na	21.7	3.9	-59.7
K	28.3	8.7	-65.7
Rb	34.0	12.4	-72.4
Cs	37.7	4.7	-110.7
а	±1.0	±1.0	±5.0

"Estimated accuracy.

-0.476 and -0.440 for the chloroform phase.

Given the unknown state of the AM salt in the solid, the enthalpies of solution obtained here do not have a clear meaning and they are not therefore reported here. However, the calculated enthalpies of transfer of monensin AH and its alkali-metal salts AM from methanol to the chloroform or toluene, butanol phases do not depend on their solid state and can give some insight on the variation with the solvent of the solvation of these species. They are accordingly given in Table III.

By use of relation 7 the standard enthalpies of reaction 1 for the two systems, water-chloroform and water-toluene, butanol, could then be calculated.

Apparent Molal Volumes of Monensin and Monensin Alka*li-Metal Salts.* These were determined in methanol and in the chloroform phase resulting, as previously, from equilibration with an equal volume of water. Apparent molal volumes V_{ϕ} of these solutes of molar mass M were calculated in the usual way from the density ρ of their solutions at molality *m* in the respective "solvents" of density ρ_0 .

$$V_{\phi} = \frac{1}{m} \left(\frac{1000 + mM}{\rho} - \frac{1000}{\rho_0} \right)$$

Measurements were made for each compound in each "solvent" at four concentrations from 1×10^{-2} to 5×10^{-2} mol kg⁻¹. A small correction was applied to the experimental data in methanol to take into account partial dissociation of the salt, using previously reported dissociation constants¹⁰ and molal volumes of cations²⁷ and of the monensin anion⁵ in methanol.

In no case was any systematic variation with the concentration observed, as might have been expected with such neutral species. Mean apparent molal volumes at the working concentrations can thus be considered as identical to the standard molal volumes at the precision of the experiment. Values obtained are reported in Table V. Agreement is fairly good with values previously reported for monensin and its potassium and sodium salts in methanol.⁵ Using standard molal volumes of proton and alkali-metal cations in water,²⁸ it was then possible to reach reaction 1 volumes. In doing so it was assumed that the differences between the molal

TABLE V: Volumes for Alkali-Metal Cations and Monensin Salts and for Reaction 1 in Water-Methanol and Water-Chloroform Systems at 25 °C

	water		methanol		chlorof pha	ormic se
	V ^e M+ ^a	V ^e M+ ^b	V ^e AM ^c	ΔV_1^d	V ^e AM ^c	ΔV_{i}^{d}
Н	-5.8	-17.0	561		563	
Li	-6.6	-17.9	545	-15.2	557	-4.7
Na	-7.0	-17.1	563	+3.2	566	+4.2
K	3.4	-7.3	570	-0.2	571	-1.0
Rb	8.5	-1.7	567	-8.3	574	-3.2
Cs	15.7	4.8	565	-17.5	579	-5.3

"Standard molal volume of cations in water according to ref 28. ^bStandard molal volume of cations in methanol according to ref 27. ^cApparent molal volumes $(10^{-2} < m < 5 \times 10^{-2} \text{ mol kg}^{-1})$ taken as standard molal volumes of monensin acid or alkali-metal salts in methanol and in the chloroformic phase in cm³ mol⁻¹ (estimated accuracy ± 1 cm³ mol⁻¹). ^d Volume of heterogeneous reaction 1 in watermethanol and water-chloroform systems in cm³ mol⁻¹ (estimated accuracy $\pm 2 \text{ cm}^3 \text{ mol}^{-1}$).



Figure 4. Overall solubility c_{Na} of the sodium monensin complex salt in water at 25 °C, as a function of the tetramethylammonium monensin salt concentration $c_{\rm T}$. s is the actual solubility of ANa.

volumes of a given alkali-metal cation and the hydrogen ion were identical in water and in the aqueous phases. From what is known of the variation of these molal volumes in water-organic mixtures at low organic solvent content, for example in tert-butyl alcohol-water systems,²⁸ this is a reasonable assumption, mean variations observed being less than 1 cm³ mol⁻¹ up to 5 wt % alcohol. Data thus obtained for the water-chloroform system and calculated for the water-methanol system are given in Table V.

Solubility and Dissociation of the Ionophore Salts in Water. The solubility of the alkali-metal cation complex salts of monensin, AM, was found to be nonnegligible in water. In order to determine simultaneously the solubility of AM in its molecular form s, its ionic solubility product K_s , and thereby its dissociation constant K_3 , its overall solubility was determined for various concentrations $c_{\rm T}$ of a tetraalkylammonium salt of monensin, A⁻, NR⁺₄, which is highly soluble and dissociated in water. What was measured was the analytical concentration $c_{\rm M}$ of alkali metal in the saturated solutions.

Provided that no hydrolysis of the ionophore salt occurs, ensured by adding a small amount of hydroxide, the system is governed by the two mass balance equations

$$c_{\rm M} = [{\rm M}^+] + [{\rm A}{\rm M}] = [{\rm M}^+] + s$$
 (11)

$$c_{\rm M} + c_{\rm T} = [{\rm A}^-] + [{\rm A}{\rm M}] = [{\rm A}^-] + s$$
 (12)

and the expression of the solubility product

$$K_s = [A^-][M^+]y_{\pm}^2 \tag{13}$$

A plot of $c_{\rm M}$ vs $c_{\rm T}$, here for sodium, is presented in Figure 4. These curves are, as $c_{\rm T}$ increases, asymptotic to $c_{\rm M} = s$. For $c_{\rm T} = 0$ the metal concentration would in absolutely pure water be $c_{M} = s +$

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TABLE VI: Solubility s, Ionic Solubility Product K_r, and Dissociation Constant K_3 of the Alkali-Metal Complex Salts of Monensin AM in Water at 25 °C

		K ₃	pK ₃	pK _{3m} ^a
s, mol dm ⁻³	<i>K</i> ,, mol ² dm ^{−6}		molar scale	
$(1.5 \pm 0.2) \times 10^{-3}$	$(5.2 \pm 1.0) \times 10^{-6}$	3.5×10^{-3}	2.4 ± 0.2	3.9
$(8.3 \pm 1.5) \times 10^{-5}$	$(1.6 \pm 0.4) \times 10^{-7}$	1.9×10^{-3}	2.7 ± 0.2	6.3
$(1.25 \pm 0.5) \times 10^{-4}$	$(1.6 \pm 0.4) \times 10^{-6}$	1.3×10^{-2}	1.9 ± 0.3	5.2
	$(5 \pm 2) \times 10^{-5}$	>10 ⁻¹	<1	4.4
	$(3 \pm 1) \times 10^{-5}$	>10 ⁻¹	<1	3.7
	s, mol dm ⁻³ (1.5 \pm 0.2) × 10 ⁻³ (8.3 \pm 1.5) × 10 ⁻⁵ (1.25 \pm 0.5) × 10 ⁻⁴	s, mol dm ⁻³ K_{μ} , mol ² dm ⁻⁶ $(1.5 \pm 0.2) \times 10^{-3}$ $(5.2 \pm 1.0) \times 10^{-6}$ $(8.3 \pm 1.5) \times 10^{-5}$ $(1.6 \pm 0.4) \times 10^{-7}$ $(1.25 \pm 0.5) \times 10^{-4}$ $(1.6 \pm 0.4) \times 10^{-6}$ $(5 \pm 2) \times 10^{-5}$ $(3 \pm 1) \times 10^{-5}$	$ \begin{array}{c cccc} & & & & & & & & & & & \\ \hline s, \mbox{ mol dm}^{-3} & K_s, \mbox{ mol}^2 \mbox{ dm}^{-6} & & & & & & \\ \hline & & & & & & & & & \\ \hline & & & &$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

"In methanol from ref 10. b Assuming $K_s^{1/2} \gg s$.

 $K_s^{1/2}/y_{\pm}$. s and K_s being determined, the dissociation constant of a complex salt of the ionophore in water could then be obtained:

$$K_3 = \frac{[A^-][M^+]y_{\pm}^2}{[AM]} = \frac{K_s}{s}$$
(14)

This method was applied to all the alkali-metal cation salts of monensin AM in water. Their solubilities were measured in pure water and in aqueous solutions of monensin tetramethylammonium salt with concentrations varying from 2×10^{-4} to 10^{-2} mol dm⁻³. Processing of data gave reliable values of K_s and s only for Li, Na, and K. They are reported in Table VI. No satisfactory solution was found for Rb and Cs. The actual solubilities of ARb and ACs were found to be quite high, although extraction data showed that the partition ratio was small. It was therefore concluded that the solubility of their complex form AM was poor and that the dissociation in water of these two salts was high. Only the ionic solubility products could be roughly calculated. These are also given in Table VI. It must be underlined that such solubility determinations are difficult and that the confidence allowed such measurements is, as stated in Table VI, rather poor. Calculated values of the dissociation constant of AM complexes in water, K_3 , are hence somewhat approximate, and results given must be considered with some caution.

Partition of the Monensin Complex Salts between Aqueous and Organic Phases. Measurement of partition ratio Q of AM between aqueous and organic phases (using equal volumes of each phase) allow K_2 to be determined, provided K_3 is known. Since dissociation equilibria are slightly shifted going from pure water to aqueous phases, pK_3 values used in the calculations were arbitrarily fixed at $pK_3 = pK_3(w) + 0.5$ for the water-butanoltoluene aqueous phase and $pK_3 = pK_3(w) + 0.2$ for the waterethanol-chloroform aqueous phase. Ideally, the partition ratio Q would then be determined at high pH, so as to be in the flat part of the r + f(pH) curve (see Figure 2) and to have no presence of AH to consider. Then

$$Q = K_2(1 + K_3 / [M^+] y_{\pm}^2)$$
(15)

Frequently, such high pH values can only be reached with hydroxides. Q is obtained through measurement of the M⁺ concentration in water at equilibrium. Alkali-metal hydroxide solutions cannot be used, nor can tetraalkylammonium hydroxide solutions which are generally not free from traces of alkali metal. In addition, formation of a third phase was observed in waterchloroform systems. It is preferable to uses amines for preparing buffer solutions, but then high pH values cannot be reached. In these experimental conditions, the calculation of K_2 is only possible if both K_1 and K_3 are known. Provided this is so, it is possible to solve the equation system, obtained from equilibria (1)-(3) and mass conservation of ionophore and metal coming from the initial amount of AM in the organic phase.

Results obtained are given in Table VII. No reliable values of the partition ratio could be obtained for the rubidium and cesium salts which means that given the accuracy of the method they must be of the order of 10^{-3} or less. From K_1 , K_2 , and K_3 it is possible to calculate the heterogeneous dissociation constant of monensin K_a as given by reaction 16

$$\overline{\mathbf{A}\mathbf{H}} \rightleftharpoons \mathbf{A}^- + \mathbf{H}^+ \tag{16}$$

$$pK_a = -(\log K_1 + \log K_2 + \log K_3)$$
(17)

TABLE VII: Partition Constant K_2 of the Alkali-Metal Complex Salts of Monensin AM between the Aqueous and Organic Phases (in Terms of pK_2) at 25 °C As Related to Molar Concentrations in mol dm⁻³

system	ALi	ANa	AK	ARb ACs
water-chloroform	0.51	1.9	2.1	>3
water-toluene, butanol	0.64	1.8	1.8	>3
assumed accuracy	±0.03	±0.1	±0.2	

TABLE VIII: Heterogeneous Dissociation Constant K_3 Corresponding to Eq 18 of the Alkali-Metal Salt of Monensin in the Two Solvent Systems at 25 °C in Terms of pK_3

	chloroform	toluene, butanol	
ALi	3.2	3.6	
ANa	4.8	5.0	
AK	4.2	4.2	
ARb	2.6	3.2	
ACs	0.9	2.4	

Data in Tables I, VI, and VII give, using values corresponding to reactions with Li, Na, and K, respectively, 9.6, 9.0, and 10.0 (mean 9.4 \pm 0.6 in the water-chloroform system) and 9.3, 8.8, and 9.2 (mean 9.0 \pm 0.3) in the water-toluene, butanol system. The distribution of these results obtained from independent measurements with these three cations gives some indication of the consistency of our data which would seem fairly acceptable. These pK_a values in heterogeneous systems can be compared to the previously reported¹⁰ pK_a value in methanol, 10.24. The dissociation constant of monensin in water could not be obtained owing to both the very low solubility of free acid form and its instability in acidic media. Assuming an open extended conformation, it can be estimated to be of the order of 4.7. From what is known for simple carboxylic acids, the pK_a in the aqueous phases cannot be very different, say 4.8 with 1% ethanol and 5.0 with 3% butanol. The partition constant K_2 of the free acid AH can thus be estimated by $pK_2(AH) = p\overline{K}_a - pK_a$, which yields pK_2 = 4.2-5.2 for the water-chloroform system and $pK_2 = 3.8-4.3$ for the water-toluene, butanol system. The solubility of monensin acid was found to be 0.35 mol dm⁻³ in the organic phase of the water-toluene, butanol system. The solubility of monensin in the aqueous phase would thus be the order of 4×10^{-5} mol dm⁻³. A solubility of 10⁻⁶ in water was proposed⁹ by Cox et al. on the basis of precipitation data by HCl solution in monensin tetrabutylammonium salt solutions. Although high, such a difference of solubility between water and water-3% butanol is not unlikely. Heterogeneous dissociation of the complex salts of monensin

AM, corresponding to reaction 18, can also be determined.

$$\overline{AM} \rightleftharpoons A^- + M^+ \tag{18}$$

The corresponding constant is $\bar{K}_3 = K_2 K_3$. Values thus calculated are given in Table VIII for the two systems. In the case of rubidium and cesium, the product $K_2 K_3$ was estimated from the corresponding mean value of \bar{K}_a , the acid heterogeneous dissociation constant, using eq 17.

Discussion

Alkali-Metal Salts of Monensin in Water. Equilibria between a carboxylic ionophore of bacterial origin in an organic phase and metal cations in an aqueous phase are currently only described in terms of cation exchange at the interface of these two phases. Both the presence of free cation in the organic phase and presence of ionophore and ionophore metal complexes in the aqueous phase are left out of account. The present experiments show that, although slight, the solubility of the alkali-metal salts of monensin is never negligible. This raises two questions. (i) Does the exchange reaction occur only at the interface or partially in the aqueous phase? (ii) Can monensin be eliminated through dissolution in aqueous biological phases? No definitive answer can yet be given to these questions in the absence of appropriate results, but it is noteworthy that the solubility of monensin in the aqueous phase is governed by the sodium ion, which is the most abundant free cation in the extracellular fluid. Of the biological alkali-metal cations, it is the one which forms the least soluble complex in water and has the smallest ionic solubility product as indicated in Table VI. In addition, the partition constant must be strongly in favor of the lipid phases as observed here for the organic phases used (Table VII). Therefore, the actual total concentration of the ionophore under any form must be, in any case, very low in the aqueous biological phases.

Both solubilities of the complex form and ionic solubility products increase in the order Na < K < Li (Table VI). Though the solubility of the complex is not only related to its state in solution but also to its state in the solid, it can be supposed that the solvation of the complex in water increase in the same order Na < K < Li. This determines, along with the order of hydration of the free cation, K < Na < Li, the order observed for the dissociation of the complex Na < Li < K (Table VI). It can also be assumed that the hydrophilicity of the complex varies as the partition constant between water and the organic phases reported in Table VII: Na $\sim K < Li$. The sodium monensin complex salt is therefore the most stable in water and the most hydrophobic; the potassium salt is as hydrophobic but less stable, and the lithium salt is, like the free cation, the most solvated by water which gives it an unexpected appreciable stability and solubility in this solvent.

The structure of the complex salt AM in water is unknown but can be inferred from what is known in another protic solvent, methanol. From NMR studies, the structure of the monensin sodium salt in methanol is, according to Anteunis,³⁰ very close to its structure in aprotic solvents,³⁰ i.e., not too different from its solid-state structure:³¹ the monensin anion is folded to form a shell wrapping the cation, the buttoning being through O_{10} -C-OO, O_{11} -COO, and O_5 - O_{10} hydrogen bonding and the coordination of the cation by O_5 , O_6 , O_7 , O_8 , O_9 , and O_{11} . Analogous structures have been found for the silver³² and potassium³³ salts of monensin in the solid state with small angular torsion.

The low solubility of the monensin sodium salt in water as well as its strong hydrophobicity may be related to a good fit of the envelope to the sodium ion, which implies both a mainly external orientation of the hydrophobic alkyl group and a strong shielding of the cation electric field. These two effects would result in a poor affinity of this complex for the surrounding water molecules. On the contrary, the hydrophobicity of the lithium complex salt of monensin is weak, which can be attributed to a strong remaining solvation of the lithium ion and possibly to a greater accessibility of the oxygen salts, of the ligand to the surrounding water molecules. Comparing crystallographic data on lithium monensin B³⁴ and sodium monensin B,³⁵ Walba et al. noted³⁴ that the former corresponds to a more "collapsed" structure. As compared to the sodium salt, torsion of backbone angles so as to better accommodate the smaller lithium ion would result in less protection of the cation. When the size of the cation increases beyond sodium, which seems to be placed in a sort of optima hidden state, enlargement of the envelope also results in weaker protection of the cation. Accordingly, both solvation of the complex salt through interaction of water molecules with the trapped cation and dissociation of the complex increase. A strong dissociation was postulated for rubidium and cesium salts, implying high ionic solubility products, perhaps corresponding to instability of the complex through dilation of the envelope.

Selectivity of Monensin for the Alkali-Metal Cations in Water-Organic Heterogeneous Systems. Data on equilibria (1) can be discussed in terms of the selectivity of monensin for the various alkali-metal cations. The selectivity constant K_{ii} , say for lithium, $K_{\text{Li}^+,\text{M}^+}$ corresponds to reaction 19 and $K_{\text{Li}^+,\text{M}^+} = K_1$ - $[M^+]/K_1[Li^+].$

$$\overline{ALi} + M^+ \rightleftharpoons \overline{AM} + Li^+$$
(19)

The selectivity scale of monensin for the various alkali-metal cations can also be obtained from emf measurements involving liquid membrane electrodes. The selectivity constant arising from such determinations, the so-called " K_{ij}^{pot} ", is a function of the heterogeneous equilibrium constant K_{ij} but also of the mobility of the ions in the membrane u_i and u_{j}^{36} Such emf data have been obtained by Lutz et al.¹⁶ for water-decanol systems and by Suzuki et al.¹⁷ for water-dibenzyl ether insert in PVC membranes.

Selectivity sequences for complexation of alkali-metal cations by monensin were previously obtained not only in homogeneous media and in methanol¹⁰ but also in other protic and dipolar aprotic solvents.⁹ Constants of cation-exchange reactions in homogeneous media can be compared to constants obtained here for reaction 19 in heterogeneous systems. A better term of comparison would be obtained by using constants of the hypothetical reaction 19 between water and the solvent concerned. These constants can be calculated from the corresponding homogeneous constants and the Gibbs functions of transfer of the cations from water to the solvent involved.37

This was done, and results corresponding to two of these hypothetical systems are reported in Figure 5 along with data for real water-organic systems, those studied here and those from emf measurements.

Variation of the selectivity of monensin with the cations presents the same trend in all the water-organic systems so far known; a monotonous decrease from sodium to cesium and a decrease from sodium to lithium. The two processes appear independent, which places the selectivity for lithium ion somewhere between the selectivity for potassium and cesium, the exact location depending on the organic solvent concerned.

Considering first phase equilibrium data, real or hypothetical, i.e., data for the four systems at the top of Figure 5, it appears that the variation with the cation of the selectivity constant is very close for these systems except for cesium, for which a more sensitive solvent dependence is observed.

Considering now data from emf measurements using liquidphase membrane monensin electrodes and recalled here in Figure 5 for comparison, the variation of the selectivity with cation thus obtained is analogous to the preceding ones but weaker. This method of estimating selectivity of ionophores is thus less sensitive than direct extraction measurements. Though extraction data are reported for the two systems involved, it can be expected that variations of K_{ii} would be like those obtained with other solvent systems and that observed weaker variation of K_{ij}^{pot} would be due to differences in the mobility of various alkali-metal cationmonensin complexes in the organic phase. Since some proportionality is observed, this mobility would be inversely related to the solvation of the complex, which might be expected.

A practical question concerning the determination of the selectivity for the various cations of a given ionophore in waterorganic systems is what determines pH ranges for the extraction of the various cations. Data obtained here for the waterchloroform systems are given Figure 2. Results for the water-

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Figure 5. Selectivity of the ionophore monensin for the various alkalimetal cations according to the solvent system used, water/toluene, 1butanol (\bullet), water/chloroform (\bullet), hypothetical water/methanol (O), hypothetical water/propylene carbonate (\blacktriangle), using values in ref 9, water/decanol (\blacksquare) from Figure 3 of ref 16, and water/dibenzyl ether (\Box) in PVC membranes from Figure 1 of ref 18, in terms of the logarithm of the selectivity constants K_{ij} or K_{ij}^{oot} . Representation is shifted vertically for clarity.

toluene, butanol system in the same concentration conditions showed that extraction occurs at lower pH (0.4 units for sodium, 1.9 units for rubidium) and that the extraction curves are nearer one another as reflected by equilibrium constant K_1 in Table I. Formation of AM salts in heterogeneous systems can be considered as a two-step process: heterogeneous acid dissociation of the ionophore

$$\overline{\mathbf{A}\mathbf{H}} \rightleftharpoons \mathbf{A}^- + \mathbf{H}^+ \tag{16}$$

and heterogeneous formation of the salt

$$A^- + M^+ \rightleftharpoons \overline{AM}$$

this last reaction being the reverse of reaction 18. Given the estimated acidity constants K_a reported for the two solvent systems, the first step corresponds to a shift of about 0.5 pH between the two systems. The second step, as appearing on the equilibrium constant \tilde{K}_3 in Table VIII, corresponds to shift of 0–0.5 pH units depending on the cation concerned, except for cesium for which the step is of the order of 1.5 pH units. Thus, insofar as the data obtained here can be generalized, they suggest that using a less protic organic phase for selectivity determination through extraction measurements affords a better discrimination between the various alkali-metal cations and globally shifts the extraction curves to more basic pH.

Thermodynamics of the Heterogeneous Exchange Reaction 1. Standard thermodynamic functions associated with the reaction of monensin in the organic phases with the cation in the aqueous phase (reaction 1) are reported in Tables IV and V:

$$\overline{AH} + M^+ \rightleftharpoons \overline{AM} + H^+$$
 (i)

 $\Delta G^{\Phi}, \Delta H^{\Phi}, \Delta S^{\Phi}$, and ΔV^{Φ} of hypothetical reaction 1 involving water and methanol were also estimated by using previously reported¹⁰ dissociation constants and enthalpies of AH and AM in methanol, molal volume obtained here, and differences between Gibbs functions,²⁴ enthalpies,²⁴ and volumes^{27,28} of transfer of H⁺ and M⁺ from water to methanol. Enthalpies and volumes of reaction 1 for the solvent systems investigated are plotted against ionic radius in Figures 6 and 7.



Figure 6. Standard enthalpy of reaction 1 for the solvent systems water-methanol (O), water-toluene, butanol (\bullet), and water-chloroform (\bullet) as a function of the cation size.



Figure 7. Standard volumes of reaction 1 for water-chloroform (Θ) and water-methanol (O) systems in cm³ mol⁻¹ as a function of the ionic radius of the cation.

It must first be noted that, as seen in Figures 5–7, the variations with the cation of a given thermodynamic function for reaction 1 are somewhat analogous in the three water-organic systems, though their amplitudes differ. Formation, by this reaction, of the sodium salt as compared to the formation of other alkali-metal salts corresponds to an extreme case: minimum of Gibbs functions, minimum of enthalpies (with the exception of cesium when going to more aprotic organic phase), and maximum of volume. Corresponding values for potassium are never far from those of sodium. Higher Gibbs functions and enthalpies and lower reaction volumes are found for lithium and rubidium. Hence, readier formation of the sodium salt and to a lesser extent of the potassium salt of monensin is confirmed in certain systems. Such a selectivity



Figure 8. Variations (referred to lithium) of the crystallographic volume of the cation⁴⁴ (\Box), of the volume of reaction 1, $\delta \Delta V^{\Theta}_{1}$ (\Rightarrow), of minus the molal volume of M⁺ in water, $-\delta V^{\Theta}_{M^{+}}$ (\oplus), and of the molal volume of AM in chloroform, δV^{Θ}_{AM} (O), and as a function of the crystallographic volume of the cation.

for sodium is governed by enthalpy, not entropy, changes. As observed in Table IV, the entropy of reaction 1 decreases as the size of the cation increases (except for rubidium in chloroform-water systems).

The thermodynamic functions of the heterogeneous reaction 1 are governed by both the state of the cation (and of the proton) in the aqueous phase and the state of the monensin salt (and of the monensin acid) in the organic phase. Considering, in a given water-organic system, the cation-exchange reaction e.g., reaction 19, enables us to remove those effects that are related to the proton in the aqueous phase and the monensin acid in the organic phase and to compare reaction 1 for the various cations.

$$\overline{ALi} + M^+ \rightleftharpoons \overline{AM} + Li^+$$
(19)

Our data can thus be discussed in terms of contributions to the trend observed for reaction 1, i.e., to the thermodynamic function X of reaction 19, of the corresponding thermodynamic functions of the cation in water and of the alkali-metal salt in the organic phase:

$$\delta \Delta X_{1} = \Delta X_{19} = \delta X^{\Theta}_{\overline{AM}} - \delta X^{\Theta}_{M^{+}} = (X^{\Theta}_{\overline{AM}} - X^{\Theta}_{\overline{ALi}}) - (X^{\Theta}_{M^{+}} - X^{\Theta}_{Li^{+}})$$
(20)

The standard molal volumes of the salt AM in the organic phase were directly measured and are reported in Table V. The ionic standard molal volumes of the cations M⁺ in water are known.²⁸ The absolute entropies of the cations in water are taken from tables³⁸ which allows δS^{Θ}_{AM} to be determined via relation 20. Standard molal enthalpies of the alkali-metal cations in water are not accessible, but it can be assumed that for such monatomic cations their variation with the cation involved is equal to the variation of the corresponding enthalpies of hydration³⁹ for which numerical values are taken from tables;³⁸ δH^{Θ}_{AM} is thus obtained via relation 20. $\delta \mu^{\Theta}_{AM}$ and $\delta \mu^{\Theta}_{M^+}$ are accessed from the corre-



Figure 9. Variation as a function of the ionic radius of the cation of the entropy of reaction 1, $\delta \Delta S^{\Theta}_{1}$ (\Box), and of minus the entropy of M⁺ in water, $-\delta S^{\Theta}_{M^{+}}$ (Θ), and of the entropy AM in chloroform δS^{Θ}_{AM} (O).

sponding enthalpies and entropies.

Results thus obtained are given in Figures 8 and 9 for volumes and entropies in chloroform and in Table IX for Gibbs functions, enthalpies, and entropies in the three systems. Properties of alkali-metal cations in water are well-known and have been amply discussed. We will therefore focus on the relative contribution of δX_{M^+} and δX_{AM} to $\delta \Delta X_1$ and on the properties of AM in the organic phase.

Relevant volume data in the chloroform-water system are presented in Figure 8. From sodium to cesium, the variation as a function of the cation size of its molal volume in water is the main contribution to the variation of the volume of reaction 1. The molal volume of the alkali-metal salt of monensin increases more smoothly with the size of the cation, only a little more than the crystallographic volume of the cations. This variation seems to be weaker than the corresponding variation observed in the solid state at least from sodium to potassium for which Ashton reported⁴⁰ volumes in their crystals of 578 and 595 cm³ cm⁻¹, respectively, as compared to values cited here in chloroform, 557 and 568 cm³ mol⁻¹. From sodium to lithium the variation of the reaction volume is determined by the marked decrease in the volume of the AM salt in both solvents, about 20 cm³ mol⁻¹ in methanol and 10 cm³ mol⁻¹ in chloroform. This decrease can be caused by two types of volume effect, either contraction of the envelope around a smaller cation or contraction of the solvent through variation of its forces of interaction with the complex salt. The fact that the corresponding volume change is still marked in an apolar solvent such as chloroform would suggest that the constriction of the molecule plays an important role here.

As shown in Figure 9, the entropy of reaction 1 in the waterchloroform system is mainly governed from lithium to potassium by the entropy of the cation in water which increases with the size of the ion more than the entropies of the AM salt in chloroform. For rubidium and cesium, sharp changes are observed. These correspond to sharp changes in the variation of the entropy of the AM salt in chloroform, the variation of the entropy of the cation in water being smooth. Results concerning the entropy of the alkali-metal monensin salt in the three organic phases are reported in Table IX. It can be seen that, whatever the solvent

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TABLE IX: Variation from Lithium to Other Cations of the Enthalpies, Chemical Potentials, and Entropies of the Cation M⁺ in Water and of the Monensin Alkali-Metal Salt AM in the Organic Phase According to Assumptions and Calculation Ways Reported in the Text

	Li ⁺	Na ⁺	K+	Rb ⁺	Cs ⁺
δμ ^θ M ⁺ , kJ mol ⁻¹	0	101	173	188	209
$\delta \mu \Theta_{\overline{AM}}$, kJ mol ⁻¹					
methanol	0	92	172	192	216
toluene-butanol	0	90	169	189	214
chloroform	0	89	170	190	221
δH ^e M+, kJ mol ⁻¹	0	115	199	221	244
δH^{Θ}_{AM} , kJ mol ⁻¹					
methanol	0	102	189	213	235
toluene-butanol	0	102	191	216	232
chloroform	0	95	180	219	215
δS ^e M+, J K ⁻¹ mol ⁻¹	0	46	88	110	119
δS^{Θ}_{AM} , J K ⁻¹ mol ⁻¹					
methanol	0	31	66	72	64
toluene-butanol	0	38	74	89	60
chloroform	0	20	37	97	-19

system, entropy of ACs is less than that of ARb and that entropy regularly decreases from AK to ALi, this last fact agreeing with the picture previously suggested of a more constricted structure of ALi. Both $\delta \Delta G^{\Theta}_{1}$ and $\delta \mu^{\Theta}_{AM}$ are for a given cation only weakly dependent on the organic solvent involved, except in the case of the cesium ion. Therefore, it can be suggested that the $\delta \mu^{\Theta}_{AM}$ value is mainly governed by the "internal" or "intrinsic" effects in the AM salt. The "external" or "medium" effects may be important but vary little for the monensin salts of the various alkali-metal cations, though appreciable differences are observed for the cesium ion. This is not so for the enthalpies. Significant though comparatively small differences with the solvent system involved are observed in both the ΔH^{Θ}_{1} in Figure 6 and the δH^{Θ}_{AM} in Table IX, particularly for rubidium and cesium. That these differences increase with the aproticity of the solvent would suggest that, in addition to the internal enthalpic effects, external enthalpic effects are being observed here, resulting from variation of the interactions of the salt with the solvent. It is noteworthy that these external enthalpic effects are fairly well balanced by corresponding entropic effects. This results in weak solvent effects in the Gibbs function, except for cesium ion.

The question arises of what determines solvent effects on the thermodynamic functions of AM in the organic phase. Roughly similar closed structures are expected for the monensin salts in the three organic solvents. As regards chloroform and methanol, there is some evidence of this from previous NMR data on the sodium salt.^{29,30} The first results of an ongoing ¹H and ¹³C NMR study of all the alkali-metal salts of monensin in these two solvents^{41,42} confirm and extend this finding, but variations of some of the ¹³C and ¹H chemical shifts and ¹H-¹H coupling constants are also observed, showing that depending on the cation and to a smaller extent the solvent, angular torsions of the backbone and

the heterocycles occur, according to cation size, the strength of its interactions with the various coordination sites, and the solvent concerned. Torsions of the backbone and consequent modification of the orientation and accessibility of both polar and apolar groups of the ionophore complex salt would give rise to changes in both the dipolar and the van der Waals interactions with the solvent molecules. The former, which probably cause the lowering of volume observed for ALi in methanol, are hardly involved in chloroform. Therefore, the effects observed in both enthalpy and entropy of AM in this solvent, particularly for ARb and ACs, may be ascribed to weaker alkyl-alkyl interactions between salt and solvent for ARb than AK and markedly stronger ones for ACs than for the other AM salts. This last observation may denote a better fit of the ionophore in the wrapping of the cesium ion, which could be related to the well-known template effect of this cation. This was recently interpreted⁴³ in terms of the composition of its periodic zero-potential surfaces (POPS) and tangential-field surfaces (TFS) which determine for this cation a better structure-modeling ability than for the other cations. Such a building effect is favored in aprotic solvents but can be balanced in protic solvent by strong interactions between the solvent molecules and the oxygen sites of the ligand. This could explain the wide variability of the cesium effect observed here and the strong dissociation of the cesium complex in water.

In conclusion, selectivity of monensin for the various alkali-metal cations in water-immiscible organic solvent systems seems to be fairly independent of the system involved. It results from small differences between two strongly cation-dependent parameters: the chemical potential of the cation in water and the chemical potential of the lasalocid alkali-metal salt in the organic phase. Changes with cation of the Gibbs functions of formation from the monensin acid of the complex neutral salt, which determines this selectivity, are mainly governed by enthalpic effects. Some specific effects, depending on the organic phase and the cation concerned, are observed in both enthalpy and entropy of formation. They are mainly medium effects, and since they are well balanced they do not intervene appreciably in the selectivity sequence.

Though some structural variations in the complex salt do certainly arise when the cation changes, thermodynamic data reported here cannot yet be unequivocally linked to precise well-defined structure changes. More spectroscopic data on these complex salts in the organic phase involved are needed.

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Registry No. Na, 7440-23-5; K, 7440-09-7; Rb, 7440-17-7; Cs, 7440-46-2; Li, 7439-93-2; monensin, 17090-79-8; methanol, 67-56-1; chloroform, 67-66-3; toluene, 108-88-3; 1-butanol, 71-36-3; monensin sodium salt, 22373-78-0; monensin potassium salt, 28643-78-9; monensin rubidium salt, 28643-79-0; monensin cesium salt, 133042-94-1; monensin lithium salt, 133042-95-2.

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