43. Complex Formation of Macrotetrolide Carrier Antibiotics with Cations Studied by Microcalorimetry and Vapour Pressure Osmometry

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Summary. Complex formation parameters of macrotetrolide antibiotics with alkali and alkaline earth metal cations are given. The stability constants for the complexes in methanol and ethanol at 30°, as determined by vapour pressure osmometry, and ΔH^0, ΔG^0, and ΔS^0 for some interactions in methanol and ethanol at 25°, measured by microcalorimetry, are compared and discussed.

1. Introduction. – Macrotetrolide antibiotics [1] show an ion specific behaviour in biological systems [2] [3] which is mainly due to selective complex formation [3] [4]. The determination of formation constants by different authors using various methods yielded a considerable amount of data [5]–[8] containing large discrepancies for apparently identical systems. These discrepancies suggested the application of new methods using identical experimental parameters whenever possible for the determination of such formation constants.

Microcalorimetry, which has been shown to be a suitable method for such investigations [9], gives the ΔH^0 and ΔS^0 values in addition to the formation constants. A differential method of vapour pressure osmometry [10] together with corrections for ion pair formation and the application of independently determined activity coefficients of the metal salts [11] gave an additional set of complex formation constants.

The formal complexation reaction studied

$$M^+ + L \rightleftharpoons ML^+ \tag{1}$$

is characterized by the concentration dependent constant K_c and the thermodynamic constant K.

$$K_c = \frac{c_{ML^+}}{c_{M^+} \cdot c_L} \; ; \quad K = K_c \cdot \frac{f_{ML^+}}{f_{M^+} \cdot f_L} \tag{2}$$

K_c, K:	[kg/mol]	M^+:	metal cation
c:	concentrations [mol/kg]	L:	ligand
f:	activity coefficients	ML^+:	complex cation

2. Experimental

Solvent. Methanol (puriss. p.a., *Fluka AG*, Buchs) was dried by refluxing over magnesium and destillation. Ethanol (puriss., *Fluka AG*, Buchs) was dried by refluxing a part of the solvent over magnesium and traces of CCl_4, then adding the rest of the solvent, refluxing and destillation. *Inorganic Salts.* The following salts were all used after drying for at least 12 h at 70°/10^{-2}–10^{-3} Torr: sodium thiocyanate (*Fisher* Certified Reagent, 99.7%, *Fisher Scientific Company*, Fair Lawn, N.J., USA), potassium thiocyanate (pro analysi, >99%, *E. Merck AG*, Darmstadt, Germany), rubidium chloride and cesium chloride (both pro analysi, >99.5%, *E. Merck AG*,

Darmstadt, Germany), barium perchlorate (pro analysi, >98%, *E. Merck AG*, Darmstadt, Germany).

Measurements by vapour pressure osmometry. The apparatus [12] used and the method [10], have been described elsewhere. Solutions approximately aequimolal in ligand and salt were measured with the pure salt solution as reference. The concentration range of the solutions was $1–4 \cdot 10^{-3}$ M (mol/kg). The measurements were carried out at 303.2 K. The signals corresponding to the free ligand concentration c_L were corrected by the separately determined osmotic coefficients of the salts [11] and by the osmotic coefficients of the charged complexes calculated by a first order *Debye-Hückel* approximation [13]. The correction of the resulting K_c values to the thermodynamic constants K was similarly carried out and was found to be within experimental error.

For the uncertainties in the vapour pressure osmometry measurements see [11].

Measurements by microcalorimetry. The instrumentation used has been described in detail [9]. All measurements of complexation reactions were carried out with simultaneous salt dilution in the reference cell at 298.2 K. The concentration range of the solutions used was $1–2 \cdot 10^{-3}$ M (ligand), $1–2 \cdot 10^{-3}$ M and $\sim 5 \cdot 10^{-2}$ M (salt) for the ΔG- and ΔH-determinations respectively. The correction factor linking the determined K_c to K was neglected because the activity coefficients f_{M^+} and f_{ML^+} are approximately equal and thus compensate.

All uncertainties of microcalorimetric measurements given are expressed as standard deviations of data calculated in the following manner. The experimental parameters are simultaneously varied using a random procedure with the standard deviations

s (differential weighing, microbalance) = 7 μg

s (differential weighing, macrobalance) = 0.7 mg

s (microcalorimeter) = 2% or 0.3 mJ

The standard deviations of ΔH^0 and K are then obtained from a set of 1000 values calculated with these varied parameters. For ΔH^0 the standard deviation is defined exclusively by the error of the measured heat of reaction if the simultaneous dilution procedure is chosen [9]. The standard deviation of K depends on the standard deviation of the mean of the ΔH^0-determination and on the actual value of K. It is a minimum for K in the range of $10^3–10^4$ kg/mol and increases for smaller and larger values.

3. Results and Discussion. – The results obtained are given in Tables 1 and 2. There is perfect agreement between the selectivity sequence

$$K^+ \geqslant Rb^+ > Cs^+ > Na^+ > Ba^{2+} \tag{3}$$

found here and the sequence obtained by relaxation techniques [6], nuclear magnetic resonance [8], EMF studies [7] [14], conductance measurements on lipid bilayer membranes [14], extraction techniques, and measurements in biological systems [15].

Similarly the sequence of the extraction constants for alkali and alkaline earth metal cations of the macrotetrolides [14]

$$\text{trinactin} > \text{dinactin} > \text{monactin} > \text{nonactin} \tag{4}$$

is consistent with the formation constants presented here, within experimental error.

All formation constants in ethanol are larger by a factor 3–10 than the corresponding values in methanol, but the above mentioned sequences are unchanged. This increase of the formation constants with decreasing dielectric constant ε of the solvent might, apart from a possible difference in the free energies of solvation of the uncomplexed cation, be due to the interaction of the polar groups of the uncomplexed carrier with the solvent molecules [16]. The selectivity sequence (3) is in agreement with calculations using an electrostatic model [16]. The complex formation constant is a maximum for the cation fitting best into the cavity formed by this kind of ligand [6].

Table 1. *Thermodynamic Complex Formation Constants for the Interaction of Macrotetrolide Antibiotics with Ions Determined by Vapour Pressure Osmometry at 30° = 303 K* [kg/mol] [a])

Antibiotic	Na^+	K^+	Rb^+	Cs^+	Ba^{2+}	Solvent
Nonactin	$2.1 \pm 0.2 \cdot 10^2$	$3.9 \pm 1.7 \cdot 10^3$	$3.3 \pm 0.9 \cdot 10^3$	$7.3 \pm 0.4 \cdot 10^2$	$4.1 \pm 4.0 \cdot 10^1$	MeOH
Monactin	$3.3 \pm 0.7 \cdot 10^2$	$1.1 \pm 0.6 \cdot 10^4$	$3.3 \pm 0.7 \cdot 10^3$	$1.1 \pm 0.2 \cdot 10^3$	$1.5 \pm 0.8 \cdot 10^2$	MeOH
Dinactin	$7.6 \pm 0.4 \cdot 10^2$	$5.3 \pm 1.4 \cdot 10^3$	$4.2 \pm 1.5 \cdot 10^3$	$1.7 \pm 0.3 \cdot 10^3$	$1.2 \pm 1.2 \cdot 10^2$	MeOH
Trinactin			$7.1 \pm 3.7 \cdot 10^3$	$2.2 \pm 0.2 \cdot 10^3$		MeOH
Nonactin	$1.8 \pm 0.2 \cdot 10^3$	$4.1 \pm 0.8 \cdot 10^4$			$2.0 \pm 0.6 \cdot 10^2$	EtOH
Monactin	$3.0 \pm 0.5 \cdot 10^3$	$2.9 \pm 0.7 \cdot 10^4$			$2.1 \pm 0.2 \cdot 10^2$	EtOH
Dinactin	$4.3 \pm 1.6 \cdot 10^3$					EtOH
Trinactin	$3.5 \pm 0.4 \cdot 10^3$					EtOH

[a]) For the anions of the metal salts see experimental section.

Table 2. *Thermodynamic Parameters for the Interaction of Macrotetrolide Antibiotics with Ions Determined by Microcalorimetry and Relaxation Methods* [6] *at 25° = 298 K*

Antibiotic	Cation	$\Delta H°$ [kJ/mol]	$\Delta G°$ [kJ/mol]	$\Delta S°$ [J/mol·K]	log K	K [kg/mol]	Sol.	Lit.
Nonactin	Na^+	-11.1 [a]) ± 0.2	-15.5	14.6	2.71 ± 0.03	$5.2 \cdot 10^2$	MeOH	
		~ -18.8					MeOH	[6] [b])
	K^+	-43.6 [a]) ± 0.9	-25.6 [a])	-60.3 [a])	4.49 [a]) ± 0.08	$3.1 \cdot 10^4$ [a])	MeOH	
Monactin	Na^+	-22.4					MeOH	[9]
		-25.1	-14.8	-34	2.6	$4 \cdot 10^2$	MeOH	[6] [b])
Dinactin	Na^+	-27.6	-16.5	-37	2.9	$8 \cdot 10^2$	MeOH	[6] [b])
Trinactin	Na^+	-30.5	-18.3	-41	3.2	$2 \cdot 10^3$	MeOH	[6] [b])
Nonactin	Na^+	-27.4 ± 0.5	-18.7	-29.4	3.27 ± 0.03	$1.9 \cdot 10^3$	EtOH	
	K^+	-52.2 ± 1.0	-30.0	-74.4	5.26 ± 0.23	$1.8 \cdot 10^5$	EtOH	

[a]) Values of reference [9] corrected for difference of salt dilution heats in sample and reference cell as discussed elsewhere [18].

[b]) Values converted to [kg/mol] respectively [kJ/mol].

Although its ionic radius is comparable to the one of K^+ and Rb^+, Ba^{2+} occupies the last position in the sequence (3). The discrimination of Ba^{2+} relative to K^+ and Rb^+, however, depends to a large extent on the thickness s of the ligand sphere and the dielectric constant of the solvent used [16]. A high preference of K^+ relative to Ba^{2+} is to be expected for the values of s involved here [16].

The entropy changes ΔS^0 for the reaction of nonactin with Na^+ and K^+ in the two solvents methanol and ethanol obtained by microcalorimetry can be discussed on the basis of equation (5). The S^0 values on the right hand side of (5) are understood to be standard absolute entropies of ML^+ and M^+, and the standard partial entropy of L respectively, all in the one molal standard state.

$$\Delta S^0 = S^0(ML^+) - S^0(M^+) - S^0(L) \qquad (5)$$

Table 3 lists the entropies $S^0(M^+)$ which are the values published by *Criss et al.* [17] converted to the one molal state, corresponding to an assignment of -20.9 J/mol·K $= -5$ eu for $S^0(H^+)$.

Table 3. *Standard Absolute Ionic Entropies [J/mol·K] at 25° = 298 K* [17] (one molal standard state)

Cation	MeOH	EtOH	S^0(MeOH)–S^0(EtOH)
H^+	−63.4	−85.3	+21.9
Li^+	−45.4	−68.5	+23.1
Na^+	−15.3	−39.7	+24.4
K^+	+17.3	− 7.9	+25.2

There are two contributions to the standard absolute entropies which should be considered in this context. An entropy increase results on disruption of the solvent structure. This is more significant for methanol than for ethanol because methanol probably has a more pronounced H-bonding structure. On the other hand an entropy decrease results on building up a solvation shell, which is more significant for the cation with the smaller ionic radius [17].

The difference of the ΔS^0 values of the complexation reactions with Na^+ and K^+ can now be calculated with (5) using the numerical values of table 3, yielding the relation

$$S^0(NaNon^+) > S^0(KNon^+) \tag{6}$$

with $S^0(NaNon^+) - S^0(KNon^+) = 42$ J/mol · K in methanol and 13 J/mol · K in ethanol. If it is assumed that the complexing ligand forms a cavity of fixed dimension, the diameter of the complex cation should be the same for K^+ and smaller metal cations. Therefore the standard entropies for the two complexes should also be nearly equal. A possible explanation for relation (6) might be that Na^+, which is too small to fill the cavity, still has a certain degree of translatoric freedom. This entropy gain, however, should be independent of the solvent. Thus the much more positive entropy of the Na^+-complex in methanol remains unaccounted for.

The difference of the ΔS^0 values of the complexation reactions in the two solvents can be calculated analogously. Assuming, that the standard partial entropy of the electrically neutral ligand might be nearly equal in the two solvents, substitution of numerical values yields the relation

$$S^0(MNon^+, MeOH) > S^0(MNon^+, EtOH) \tag{7}$$

with $S^0(MNon^+, MeOH) - S^0(MNon^+, EtOH) = 68$ J/mol · K for the Na^+ complex and 39 J/mol · K for the K^+ complex. The corresponding differences of S^0 values for the metal cations in Table 3 are smaller and show an increase parallel to the ionic radius and the concurrent decrease in interaction with the solvent. This suggests that the complexes do not build up a solvation shell which is also to be expected because of thorough shielding of the cation by the spherical ligand.

Despite nearly identical experimental conditions some of the new complex formation constants determined by vapour pressure osmometry and by microcalorimetry

differ somewhat from one another. In a preliminary investigation, the temperature dependence of the formation constants was determined by using supplementary vapour pressure osmometry measurements of the systems nonactin/K^+ and monactin/K^+ at 333 K. For a rise in temperature of 5°, a decrease of less than 0.2 logarithmic units was found. This is verified by calculating the temperature dependence from the calorimetrically determined ΔH^0 of nonactin/K^+ in methanol, which gives -0.13 logarithmic units difference in K between 30° and 25°. Corrections of osmotic coefficients for ion pair association of the salt have been investigated [11]. Estimates of this influence show that it could explain the difference of the nonactin/K^+ value in ethanol, the VPO value being too small. The discrepancy of the formation constant for nonactin/K^+ in methanol cannot, however, be fully explained.

Nevertheless, the sequences (3) and (4) for the complex formation constants could be confirmed. Microcalorimetry is shown to yield additional information which helps to elucidate the ion selectivity of such ligands.

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