295. Microcalorimetric Determination of ΔH^0, ΔG^0 and ΔS^0 for the Interaction of the Carrier Antibiotics Nigericin and Monensin with Sodium and Potassium Ions

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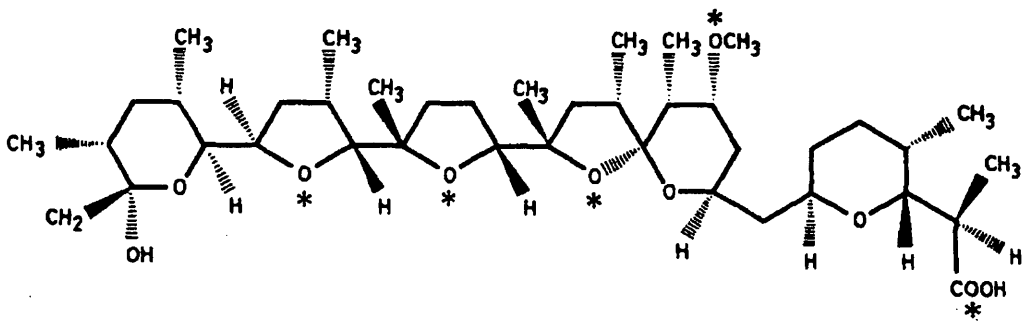
Summary. The thermodynamic parameters ΔH^0, ΔG^0 and ΔS^0 – and thereby the equilibrium constants – for the complexation of the carrier antibiotics nigericin and monensin with sodium and potassium ions in methanol at 25°C have been determined by microcalorimetry. The results are discussed in terms of the nature of the interaction between ligands and cations.

1. Introduction. – The antibiotics nigericin (I)[1] [1] and monensin (II)[1] [2] show specific alkali cation transport properties in rat liver mitochondria [3]. This is mainly due to selective complex (or salt) formation between the anions of these carrier antibiotics and metal cations [4]. As a prerequisite to an understanding of these selectiv-



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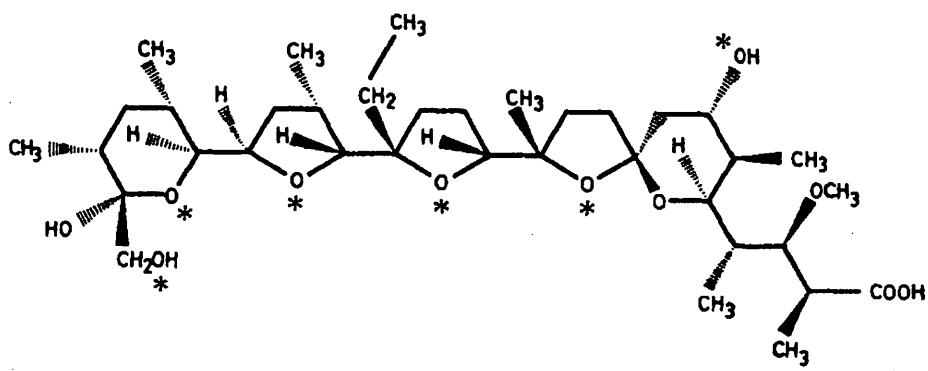

II

ities on a molecular level [5] a calorimetric study [6] of the relevant thermodynamic parameters has been undertaken.

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[1]) The starred oxygen atoms are involved in coordination to the metal ion.

The complexation reaction studied (1) is characterized by the relations (2).

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

$$K_c = \frac{c_{ML}}{c_{M^+} \cdot c_{L^-}}; \qquad K = K_c \cdot \frac{f_{ML}}{f_{M^+} \cdot f_{L^-}}. \tag{2}$$

M^+: metal cation
L^-: ligand anion
ML: complex salt
K: complex formation constant
c: concentrations
f: activity coefficients
K_c: concentration dependent complex formation constant

In order to have the antibiotic predominantly in the ionic form L^- all solutions of ligand and salt as well as the solvent for dilution contained a large excess of tributylamine.

2. Experimental. – The instrumentation used has been described in detail [6].

ΔH^0: For the determination of ΔH^0 the heat of reaction between 2 ml 10^{-3} m (mole/kg) antibiotic and 2 ml $5 \cdot 10^{-2}$ m salt solution was measured by simultaneous dilution of 2 ml salt solution with 2 ml solvent in the reference cell. With an assumed complex formation constant of 10^4 kg/mole this excess of metal ions corresponds to 99.8% complexation.

K_c: For the determination of K_c equal concentrations (10^{-3} m) of antibiotic and salt were used. The heat evolved, as a fraction of ΔH^0, yields directly the concentration c_{ML} of the complex formed.

K: For the correction of K_c to K the mean activity coefficients $f_{M^+SCN^-}$ were determined by vapour pressure osmometry [7]. The activity coefficients f_{L^-} were estimated from the *Debye-Hückel* equation

$$\log f_{\pm} = -\frac{A\sqrt{\mu}}{1 + B \cdot a \cdot \sqrt{\mu}}$$

assuming an average radius of the antibiotic $a = 6$ Å; the values of A and B were taken as 1.6864 and 0.4522 respectively for the ionic strength μ given in molal (mole/kg) concentrations in methanol at 25°C [8]. The activity coefficients for concentrations of $5 \cdot 10^{-4}$ m (after mixing the two solutions of 10^{-3} m) are:

$$f_{NaSCN} = f_{KSCN} = 0.917; f_{Nig^-} = f_{Mon^-} = 0.921.$$

The activity coefficient f_{ML} of the neutral complex was taken as 1. Any change in the ionic strength caused by incomplete complexation influencing activity coefficients and heats of dilution was neglected.

ΔG^0 and ΔS^0 were calculated using the formulae:

$$\Delta G^0 = -R \cdot T \cdot \ln K \text{ and } \Delta S^0 = (\Delta H^0 - \Delta G^0)/T.$$

The errors (relative and absolute standard deviations of a single determination for ΔH^0 and $\log K$, respectively) given in the Table are calculated by variation of all independent parameters as discussed elsewhere [9], except for the nigericin sodium complex, where the experimental errors are bigger than the calculated ones.

The heats of dilution obtained in the sample and reference cells differ systematically by 2.5%; the reason for this is unknown. The values for the monensin potassium complex obtained earlier [6] were correspondingly adjusted (Table).

Solvent: Methanol (*puriss. p.a., Fluka AG*, Buchs) was dried by refluxing with magnesium and distillation. All solutions prepared were $5 \cdot 10^{-2}$ m in tributylamine (TBA) (*puriss., Fluka AG*, Buchs). The molalities given for ligand- and salt-solutions refer to the number of moles of the corresponding components dissolved in 1 kg of methanolic TBA-solution.

Thermodynamic Parameters for 1:1 Complexes of Nigericin- and Monensin-Anion with Sodium- and Potassium-Cation

(Microcalorimetry; Methanol; 25°C = 298.16 K) 1 J ≙ 0.239 cal

Antibiotic	Cation	ΔH^0 [kJ/mole]	ΔG^0 [kJ/mole]	ΔS^0 [J/mole · K]	log K	K [kg/mole]	References
Nigericin$^-$	Na^+	$+6.9 \pm 11\%$	-22.2	$+98$	3.9 ± 0.1	$8 \cdot 10^3$	
		$+9.6$	-22.0	$+106$		$7 \cdot 10^3$ [a]	[12]
						$2 \cdot 10^4$ [a]	[11]
	K^+	$-4.1 \pm 7\%$	-32.0	$+93$	5.6 ± 0.7	$4 \cdot 10^5$	
						$1 \cdot 10^5$ [a]	[11]
Monensin$^-$	Na^+	$-16.2 \pm 2\%$	-34.3	$+61$	6.0 ± 0.5	$1 \cdot 10^6$	
						$6 \cdot 10^5$ [a]	[11]
						$> 8 \cdot 10^5$ [a]	[12]
	K^+	$-15.6 \pm 2\%$ [b]	-26.0 [b]	$+35$ [b]	4.6 ± 0.1 [b]	$4 \cdot 10^4$ [b]	
		-16.2	-25.6	$+31$		$3 \cdot 10^4$	[6]
						$7 \cdot 10^4$ [a]	[11]
						$2 \cdot 10^4$	[6]

[a] Value converted to kg/mole units.

[b] Values of reference [6] corrected for difference in salt dilution heats as discussed in the experimental section.

Inorganic salts: Sodium thiocyanate (*Fisher* Certified Reagent, 99.7%, *Fisher Scientific Company*, Fair Lawn, N. J., USA) and potassium thiocyanate (*pro analysi*, > 99%, *E. Merck AG*, Darmstadt, Germany), both dried 12 h at 70°/10^{-3} Torr.

Antibiotics: Nigericin was prepared by shaking a chloroform solution of the sodium salt[2]) with 0.1 M HCl. The product was crystallized from acetone/water and dried at 25°/10^{-3} Torr. Microanalysis of the oxygen content: 24.59% (calc.: 24.28%). Monensin was prepared in the same way from the sodium salt[2]). Oxygen content of the monohydrate [10]: 27.68% (calc.: 27.87%).

3. Results and Discussion. – The thermodynamic parameters for the interaction of nigericin and monensin with sodium and potassium ions are given in the Table.

The ΔG^0 and K-values obtained are in good agreement with the data found by EMF.- [11] and relaxation [12] techniques. They confirm the selectivity order $K^+ > Na^+$ for nigericin and $Na^+ > K^+$ for monensin observed in biological systems [13] and obtained by other physico-chemical methods [11] [14]. These ion selectivities are reproduced by model calculations which will be described in detail elsewhere [15].

ΔS^0: Assuming that the complexation between two oppositly charged species is due mainly to Coulombic interaction the *Bjerrum* electrostatic model [16] leads to the relation [17]

$$\Delta S = -\frac{k}{\varepsilon}\left(\frac{\delta \ln \varepsilon}{\delta T}\right)$$

k: constant for a given ion pair; ε: dielectric constant of the medium

Because of the negative temperature dependence of the dielectric constant ε of the solvent, ΔS is positive, in agreement with the values in the Table. The observed trends in ΔS can be interpreted in terms of the translation entropies of ligand, cation and com-

[2]) We are indebted to *Eli Lilly & Co.*, Indianapolis, USA, for the generous gift of samples of nigericin- and monensin-Na-salt (189-380B-171-A and 370-559-AD-291, resp.).

plex. The former two, as charged species, are likely to be more strongly solvated than the electrically neutral complex. Complexation would then liberate a certain number of solvent molecules, thus increasing the entropy of the system. The carboxylate anion of nigericin is involved in coordination to the metal ion whereas the carboxylate anion of monensin is not, leading to a more pronounced charge separation and hence presumably to a more extended solvation shell around the monesin complex. In this connection it is striking that all known crystalline monensin salts contain two water molecules whereas nigericin salts crystallized under similar conditions, are anhydrous [2]. We may therefore assume that the number of solvent molecules liberated by complexation is smaller in the monensin than in the nigericin case, thus accounting qualitatively for the less positive ΔS^{o}-value for the monensin system. Entropy changes due to alteration in the conformational degrees of freedom of the ligands are small since free acid and complex have similar macrocyclic conformations in both cases [10] [11].

Differences in the ΔS^{o}-values for sodium and potassium complexations can be explained in the same way. Sodium, as the smaller ion, has a stronger solvation shell so that its complexation yields a more positive ΔS^{o}.

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