aminomethylated products were assayed either directly *via* the intermediate hydrochlorides, or by titration with perchloric acid.

Dimethylaminomethyl-dodecanes, b.p. 120–140°/10 Torr.

$C_{15}H_{33}N$ Calc. C 79.21 H 14.62 N 6.16% Found C 79.83 H 14.81 N 5.48%

Dimethylaminomethyl-tetradecanes, b. p. 120–140°/4 Torr.

$C_{17}H_{37}N$ Calc. C 79.92 H 14.59 N 5.48% Found C 80.48 H 14.59 N 5.22%

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150. Determination of ΔH^0, ΔG^0, and ΔS^0 of the Interaction of Ions with Carrier Antibiotics by Computerized Microcalorimetry

by **P. U. Früh, J. T. Clerc,** and **W. Simon**

Laboratorium für Organische Chemie, Eidgenössische Technische Hochschule Zürich

(12. VI. 71)

Summary. ΔH^0, ΔG^0, and ΔS^0 – and thereby the equilibrium constant of the interaction of carrier antibiotics with ions – are determined using a microcalorimeter on-line with a dedicated computer. Thermodynamic data of the interaction of monensin, macrotetrolides and valinomycin with sodium and potassium ions in methanol at 25°C are given.

1. Introduction. – In 1966 [1] we have shown that the ion selectivity of certain electrically neutral antibiotics in biological systems [2] is largely due to selective complex formation between the antibiotics and alkali metal cations. Considerable effort has been made to understand the carrier-mediated alkali cation transport across cellular membranes on the basis of the characteristics of such antibiotics [3] [4] and model compounds [5–8]. For a detailed study [7] the free energy (ΔG^0), enthalpy (ΔH^0), and entropy (ΔS^0) of complex formation with cations had to be measured. Because of sample limitations we have replaced the precision thermometric titration calorimeter [5] by a microcalorimetry system [9] with an on-line computer as suggested earlier [10].

2. Instrumentation. – The signal of the thermopile of a batch microcalorimeter[1]) is boosted by a DC amplifier[2]) and simultaneously fed into a recorder/integrator[3]) as

[1]) Model 10700-2, *LKB-Produkter AB*, Bromma, Sweden.

[2]) Microvolt Ammeter, 150 B, *Keithley Instruments, Inc.*, Cleveland, Ohio, USA.

[3]) Recorder model SRG (*E. H. Sargent & Co.*, Chicago, Ill., USA) equipped with an integrator (DISC model 204-DM, *Disc Instruments, Inc.*, Santa Ana, Calif. USA).

well as a 12-bit analog digital converter[4]) connected to a dedicated computer[5]) (see fig. 1). The signals (see fig. 2) are integrated numerically (integrals I) and either of two corrections, based on disparate assumptions, is applied. The results are thus printed by the teletype:

$$\text{Symmetric correction (S in fig. 2):} \quad I_S = F_2 - 3\,\frac{F_1 + F_3}{2}. \tag{1}$$

$$\text{Asymmetric correction (A in fig. 2):} \quad I_A = F_2 - 3F_3. \tag{2}$$

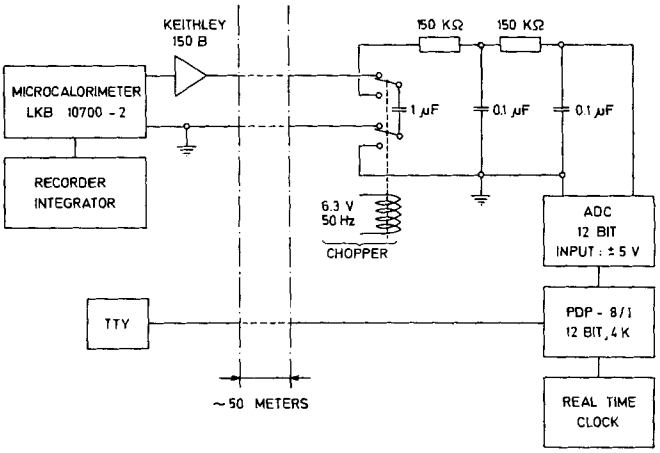

Fig. 1. *Computerized Microcalorimeter System*

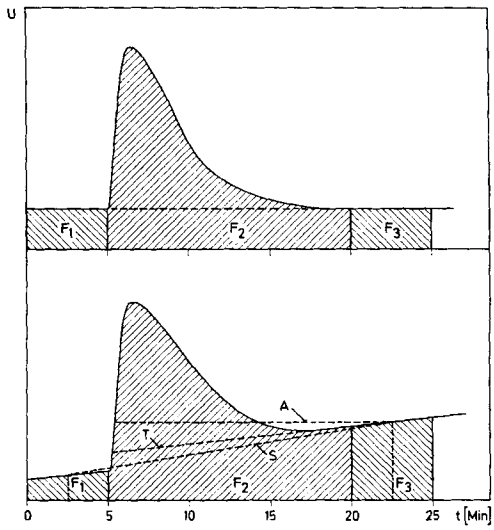

Fig. 2. *Integration of Typical Signals of Microcalorimeter*

[4]) Model AF01-A (*Digital Equipment Corporation*, Maynard, Mass., USA).

[5]) PDP-8/I, 12 bit, 4K with real time clock (*Digital Equipment Corporation*, Maynard, Mass., USA); program by *F. Erni* and *P. U. Früh*.

To eliminate errors due to drift and/or shift of the base-line, values are accepted only if I_S and I_A do not differ by more than 1.5% (above 50 mJ) for actual runs and 1.0% for electric calibration. For signals below 50 mJ the deviations may be larger; a check of the recorded trace reveals the most adequate value (closest to T in fig. 2): I_S, I_A or $(I_S + I_A)/2$.

3. Calibration, Accuracy, and Reproducibility of the Computerized Microcalorimeter. – The reproducibility and the accuracy of the system were studied using the protonation of α-picoline in water (constant ionic strength $\mu = 0.1$ (KNO_3)) [11] (results of electrical calibration see tab. 1). Obviously the computer is superior to the DISC integrator except for signals around 5 mJ where the reproducibility of the calorimeter becomes poorer than that of the DISC integrator. For signals larger than 20 mJ numerical integration gives differences between I_S and I_A that are 5–6 times smaller than those obtained with the DISC integrator, therefore the latter represents the overriding source of error. The protonation of α-picoline (2 ml) with nitric acid (4 ml, both same molality; control by titration) in water at 25°C, gave relative standard deviations of 0.6, 0.9, 2.5, and 1.7% for measured heats of 5000, 500, 50, and 5 mJ respectively. Values of ΔH^0 of -26.37, -26.31, and -24.60 kJ · mole^{-1} for sample concentrations between 10^{-1} to 10^{-3} M were obtained showing deviations of -0.6, -0.4, and $+6.1$% from the value $\Delta H^0 = -26.2$ kJ · mole^{-1} found by *Anderegg & Wenk* [11]. Below 50 mJ use of calibration materials of the electrically neutral ligand type [5] [12] is suggested[6]).

Table 1. *Reproducibility of Electrical Calibrations of the Computerized Microcalorimeter Performed before and after the Reaction Run*

Integration		Reproducibility for signals of approx.				
		5000 mJ	500 mJ	50 mJ	5 mJ	
DISC	I_S	1.6	3.2	1.4	1.0	Relative standard deviation of single calibration integral (%)
	I_A	0.8	0.8	0.6	1.2	
Computer	I_S	0.3	0.4	0.6	1.8	
	I_A	0.5	0.6	0.5	1.8	
DISC		1.2	3.0	1.5	2.5	Relative difference between mean values $\bar{I}_S$ and $\bar{I}_A$ (%)
Computer		0.2	0.5	0.2	2.2	

Repeated runs with equal amounts of pure solvent in both cells to determine the heat of friction generated in mixing show (even after 3 h of thermostation) that upon subtraction of this term (final constant value) there remains a 'hidden' contribution (0–1 mJ) probably due to temperature gradients. Although extrapolations are possible, uncertainties up to 0.3 mJ have to be expected.

4. Measurements of Thermodynamic Data on Carrier Antibiotics. – 4.1. ΔH^0: The enthalpy ΔH of the reaction of a ligand L (antibiotic) with a metal cation M

[6]) Due to uncontrollable parameters the protonation of α-picoline is not suited for testing the range below 50 mJ.

[equ. (3)] with the concentration dependent formation constant K' [equ. (4)] can be

$$L + M \underset{\Delta H^0,\ \Delta G^0,\ \Delta S^0}{\rightleftharpoons} ML \tag{3}$$

$$K = \frac{a_{ML}}{a_L \cdot a_M} = K' \frac{f_{ML}}{f_L \cdot f_M} \tag{4}$$

a: activities; f: activity coefficients.

obtained directly through a measurement with an excess of M[7]).

4.2. K': An additional measurement is necessary for K' [equ. (5)] where q is the measured heat per kg of solvent. Fig. 3 shows that optimal information is provided by

$$K' = \frac{q/\Delta H_0}{(c_{M,TOT} - q/\Delta H^0)\,(c_{L,TOT} - q/\Delta H^0)} \tag{5}$$

$c_{M,TOT} = c_{ML} + c_M$; c: concentrations [mole kg^{-1}]; $c_{L,TOT} = c_{ML} + c_L$; $c_{ML} = q/\Delta H^0$,

measurements with approximately equal amounts of ligand and cation, the deviation from the curve with infinite formation constant there being a maximum.

K may be obtained by extrapolation of a set of K'-values to $\mu = 0$ or by applying estimated or independantly determined activity coefficients (for instance by vapour pressure osmometry [13]).

ΔG^0, ΔS^0, are given by $\Delta G^0 = -RT \ln K$ (6)

and $$\Delta S^0 = (\Delta H^0 - \Delta G^0) \cdot \frac{1}{T}. \tag{7}$$

To avoid too large metal salt concentrations, incomplete complexation and therefore too small values of ΔH may be corrected by iteration.

5. Experimental Details. – *Solvent:* Methanol (*puriss. p.a., Fluka AG,* Buchs), dried by refluxing with magnesium and distillation.

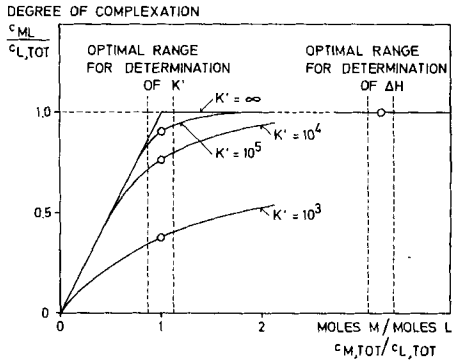

Fig. 3. *Optimal Ranges for the Determination of Thermodynamic Parameters of 1:1 Complexes* ($c_{L,TOT} = 10^{-3}$ *m*)

[7]) Since the concentration dependence of ΔH is small, the determined values ΔH can be assumed to be equal to the thermodynamic ΔH^0 within the accuracy of the method.

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: Nonactin[8]), monactin[8]), and commercial valinomycin (A grade, *Calbiochem*, Los Angeles, Calif., USA). Monensin: prepared form the $CHCl_3$ solution of the sodium salt[8]) with 0.1 M HCl, crystallized from water/acetone, and dried 12 h at 25°/10^{-3} Torr. The calorimetric measurements on monensin were made using solutions in methanolic $5 \cdot 10^{-2}$ *m* (*m*: molal) tributylamine (*puriss.*, *Fluka AG*, Buchs).

α-Picoline: Commercial product (*purum*, *Fluka AG*, Buchs), doubly distilled.

Measurements. – Determination of ΔH: a ratio $c_{M,TOT}/c_{L,TOT}$ of 100 or 10 depending on the value K expected was selected using approx. 2 ml of 10^{-3} *m* antibiotic solution.

In determining K' the degree of complexation should remain as small as possible; therefore $c_{M,TOT} = c_{L,TOT}$, with concentrations chosen so as to obtain a signal of at least 20 mJ. The concentration was about $1–5 \cdot 10^{-3}$ *m* (approx. 2 ml solution each). The enthalpy of dilution for the ligand was determined separately while the corresponding value for the salt was either obtained in such a separate experiment or compensated for by simultaneous dilution in the reference cell. The quantities of solvent or solution were determined by weighing the liquids in the syringe. The measurements were effected at 25°C.

Table 2. *Thermodynamic Parameters for 1:1 Complexes with Carrier Antibiotics* (Methanol, 25°C)

Antibiotic	Cation	ΔH^0 [kJ/mole]	ΔG^0 [kJ/mole]	ΔS^0 [kJ/mole K]	K[a]) [kg/mole]	Literature
Nonactin	K^+	−45.9	−24.8	−0.071	$2 \cdot 10^4$	
	K^+				$5 \cdot 10^3$	[8] [14]
	Na^+	−14.2				
Monactin	Na^+	−22.4				
	Na^+	−25.1				[3]
Monensin	K^+	−16.2	−25.6	+0.031	$3 \cdot 10^4$	
	K^+				$2 \cdot 10^4$ [b])	[15]
Valinomycin	K^+	−19				

[a]) Activity coefficients: determined by vapour pressure osmometry [13] for the inorganic salts; estimated for the charged ligand or complex by the *Debye-Hückel* approximation.

[b]) Redetermined using a carefully prepared and handled solvent.

6. Results; Error Analysis. – The results (given in table 2 together with some literature data) will be discussed elsewhere.

Error analysis for the determination of ΔH and K: If X is a function F of the mutually independent parameters A_i

$$X = F(A_i) \tag{8}$$

then its variance $V(X)$ is given by [16]

$$V(X) = \sum_i \left(\frac{\partial F}{\partial A_i}\right)^2 \cdot V(A_i) \tag{9}$$

where $V(A_i)$ is the variance of the parameter A_i.

[8]) We are indepted to Dr. *H. Bickel, CIBA-GEIGY AG*, Basel, and to *ELI LILLY & Co.*, Indianapolis, USA, for generous gifts of macrotetrolides and monensin sodium salt (370-559-AD-291), respectively.

The evaluation of the errors due to the different parameters shows that for ΔH only the errors in the heats Q (measured) and Q (dilution, salt) contribute appreciably to the total error. If the correction for Q (dilution, salt) is made by the simultaneous dilution method, the total error decreases by a factor of about 10, Q (measured) now being in the same order of magnitude as Q (complexation). Therefore the precision of the microcalorimeter is the factor limiting the precision of ΔH, Q (reaction), and K. For approximate values of ΔH and K (-40 kJ · mole^{-1} and 10^4 kg · mole^{-1}) relative standard deviations of 4 and 20% respectively have been calculated. Comparison of several independent sets of experimental values suggests errors originating from uncontrolled sources (humidity of solvent, purity of reagents, etc.).

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