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Na^+ Exchange Reactions of Monensin in Methanol and Methanol-Water Mixtures: A ^{23}Na NMR Study

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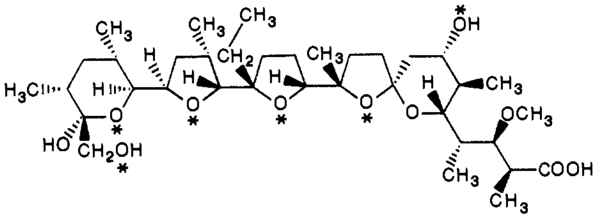
Abstract: Rate constants for exchange reactions of Na^+ with sodium monensin (NaMon) in methanol over a wide range of temperatures have been determined from ^{23}Na spin-lattice relaxation times, T_1. The rate-determining step is shown to be the dissociation of NaMon. The exchange rate is sensitive to solution pH, and measurements in methanesulfonic acid and acetate and chloroacetate buffer solutions show the reaction to be subject to specific acid catalysis. The results suggest exchange occurs predominantly via the protonated complex, $NaMonH^+$, at pH ca. 7 in methanol. The addition of water increases considerably the lability of NaMon, especially the dissociation rate constant.

A characteristic property of the carboxylic ionophores or polyether antibiotics (Nigericin group)[2-4] is their ability to promote cation-proton or cation-cation exchange across biological membranes. They possess multiple ether linkages, usually in the form of tetrahydrofuran or tetrahydropyran rings and a carboxylic acid group as in monensin (MonH, I), isolated from various strains of *streptomyces*. X-ray analysis of the ligands and their metal complexes shows them to have a macrocyclic structure stabilized by hydrogen-bond formation between the $-CO_2H$ (or $-CO_2^-$) group and appropriate -OH groups within the molecule.[5,6]



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The presence of the ionizable carboxylic acid group means that several equilibria and exchange processes may be important in homogeneous solution, or at a membrane/H_2O interface. These include the acid-base equilibria:

$$\text{MonH} \xrightleftharpoons{K_a(\text{MonH})} \text{Mon}^- + \text{H}^+ \qquad (1)$$

the complexation reactions of the anion and free acid forms of the ligand (eq 2 and 3), and exchange of cations and protons

$$\text{M}^+ + \text{Mon}^- \xrightleftharpoons{K_s(\text{M·Mon})} \text{M·Mon} \qquad (2)$$

$$\text{M}^+ + \text{MonH} \xrightleftharpoons{K_s(\text{M·MonH}^+)} \text{M·MonH}^+ \qquad (3)$$

between the various species. Evidence has been presented for complexes of the type $M{\cdot}MonH^+$ (eq 3) in solution[7-9] and in the solid state.[10] Kinetic studies have also shown that in acid solution direct replacement of Na^+ by H^+ on monensin occurs via $NaMonH^+$,[9] and that for the calcium-selective ionophore (calcimycin) dissociation of the calcium and magnesium is strongly catalyzed by H^+.[11]

Table I. Stability Constants of Monensin Complexes with Ag^+ and Na^+ in Methanol-Water Mixtures at 25 °C

vol % H_2O	x_{H_2O}	log K_s (mol dm^{-3})[a] Ag Mon	log K_s (mol dm^{-3})[a] Na Mon
0	0	7.86^b	6.34^c
10	0.169	7.4_2	6.1
20	0.354	6.7_1	5.4
30	0.484	6.4_3	5.1
40	0.590	5.9_2	4.7

[a] K_s values corrected to $I = 0$, log K_s ±0.1 (AgMon), ±0.2 (NaMon). [b] Reference 17. [c] Reference 17; cf. log K_s = 6.30 (ref 8).

The present paper describes a study of the kinetics of the exchange reaction between solvated and complexed Na^+ in methanol over a range of temperatures, eq 4. The reaction is

$$^*\text{Na}^+ + \text{NaMon} \rightleftharpoons {}^*\text{NaMon} + \text{Na}^+ \qquad (4)$$

shown to proceed via the dissociation of NaMon to give Na^+ and the free ligand, followed by rapid recombination, as in eq 5. The

$$\text{NaMon} \xrightarrow{k_d} \text{Na}^+ + \text{Mon}^-$$

$$^*\text{Na}^+ + \text{Mon}^- \xrightarrow{\text{fast}} {}^*\text{NaMon} \qquad (5)$$

exchange rate is sensitive to solution pH, and this is investigated quantitatively in the presence of a strong acid (methanesulfonic acid) and in acetate and chloroacetate buffers. Finally, the influence of water on the exchange reaction and on the stability of the Na^+ complex has also been studied. The addition of water decreases the stability of NaMon and increases considerably the lability, especially the dissociation rate constant. The exchange rate constants were determined from the influence of exchange on $^{23}Na^+$ spin-lattice relaxation times T_1.[12-14]

Experimental and Results

Sodium monensin (Sigma) was purified by recrystallization from methanol/water as previously described.[15] Nmr T_1 measurements were

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also carried out on a sample of sodium monensin purified by flash chromatography to remove any traces of monensin B,[16] with identical results within experimental error. Bu_4NMon was prepared from NaMon, via the acid form MonH, as previously described.[15] Methanol (Fisons HPLC grade, 0.05% water) was used without further purification. Inorganic salts, methanesulfonic acid, acetic acid, and chloroacetic acid were high-purity commercial samples used without further purification. Acetate and chloroacetate buffers in methanol were prepared by partial neutralization of the acid with standardized (ca. 1 M) solutions of Bu_4NOH in methanol (Aldrich).

Stability Constant Measurements. Stability constants for AgMon and NaMon were determined in methanol and methanol-water mixtures containing up to 40% v/v water ($x_{H_2O} = 0.590$). K_s(AgMon) values were determined by direct titrations of Ag^+ with an excess of Bu_4NMon using pAg^+ potentiometry. K_s(NaMon) values were determined by disproportional reaction of the cations with the corresponding Ag^+ complex (eq 6). Full details of the procedure have been reported previously.[17] Solution concentrations used in the various potentiometric titrations were $2 \times 10^{-4} \leq [AgNO_3]/M \leq 5 \times 10^{-4}$, $4 \times 10^{-4} \leq [Bu_4NMon]/M \leq 1.2 \times 10^{-3}$, $7 \times 10^{-4} \leq [NaClO_4]/M \leq 5 \times 10^{-3}$ M. The results are listed in Table I.

$$\text{AgMon} + \text{Na}^+ \overset{K_e}{\rightleftharpoons} \text{NaMon} + \text{Ag}^+ \qquad (6)$$

The pK_a(MonH), eq 1, in methanol has been reported previously (10.24[18] and 10.30[7]) as has the stability constant of $NaMonH^+$, eq 3 (log $K_s(NaMonH^+) = 2.5$[7] and 2.3[18]).

NMR Kinetic Measurements. The exchange reaction represented by eq 4 may be characterized by two observable spin-lattice relaxation times T'_{1A} and T'_{1B} which are a function of the T_1 values of free and complexed Na^+ in the absence of exchange (T_{1A} and T_{1B}, respectively), together with τ_A and τ_B, the lifetimes of the nuclei in the free and complexed states, respectively.[12-14] The slower of the two relaxations T'_{1A} (which may be determined with the greatest accuracy) is given by

$$1/T'_{1A} = \tfrac{1}{2}\{(1/T_{1B} + 1/T_{1A} + 1/\tau_B + 1/\tau_A) - [(1/T_{1B} - 1/T_{1A} + 1/\tau_B - 1/\tau_A)^2 + 4/\tau_A\tau_B]^{1/2}\} \qquad (7)$$

Rearrangement of eq 7, and introduction of the condition $P_A/\tau_A = P_B/\tau_B$, where P_A and P_B are the proportions of free and complexed Na^+, leads to eq 8 for the exchange rate under equilibrium conditions. Derivations of eq 7 and 8 are given in ref 12 and 13, and a full discussion of the application of the T_1 measurements to determine the exchange rate constants for systems such as in eq 4 is also given earlier.[14]

$$\frac{1}{\tau_A} = \frac{(1/T_{1B} - 1/T'_{1A})(1/T'_{1A} - 1/T_{1A})P_B}{P_A/T_{1A} + P_B/T_{1B} - 1/T'_{1A}} \qquad (8)$$

Values of T_{1A}, T_{1B}, and T'_{1A} for $^{23}Na^+$ were obtained by inversion–recovery techniques performed on a Bruker WP80 FT NMR spectrometer operating at 21.19 MHz. The spectrometer was field/frequency locked on the ^2H resonance of ^2H_2O or $C^2H_3O^2H$ in the inner compartment of a coaxial tube. Typically eight delay times varying from 0.6 ms to about $2T_1$ and a measured infinity value ($\geq 10T_1$) were used. The T_1 values were obtained from a normal first-order plot of the approach of the intensity (signal height) of the free induction decay to the infinity value. Depending upon the line width and hence signal-to-noise ratio, between 128 and 10 000 free induction decays were collected. The concentration of Na^+ (as NaBr and NaMon) was normally about 0.04 M. The temperature control system on the spectrometer was calibrated to ±0.5 °C using chrome–alumel thermocouples. Results were reproducible to within ±3% except where otherwise indicated.

Kinetic measurements were made in three series: (i) in methanol, over a range of temperatures (210–330 K); (ii) in methanol solutions of methanesulfonic acid, and of acetate and chloroacetate buffers at 302 K; and (iii) in methanol-water mixtures at 302 K. These are considered separately below.

(i) Na^+/NaMon in Pure Methanol. Relaxation times were measured over a wide range of temperatures for 0.04 M NaBr ($240 \leq T/K \leq 332$), 0.04 M NaMon ($301 \leq T/K \leq 332$) and 0.04 M NaBr/0.04 M NaMon ($212 \leq T/K \leq 332$). The temperature range for NaMon was limited by the minimum delay time of the spectrometer (0.6 ms), which became comparable to the relaxation time at temperatures below about 300 K. Detailed results are given in Table M1 in the microfilm edition, and they are illustrated in Figure 1 in the form of a plot of ln $(1/T_1)$ vs. $1/T$.

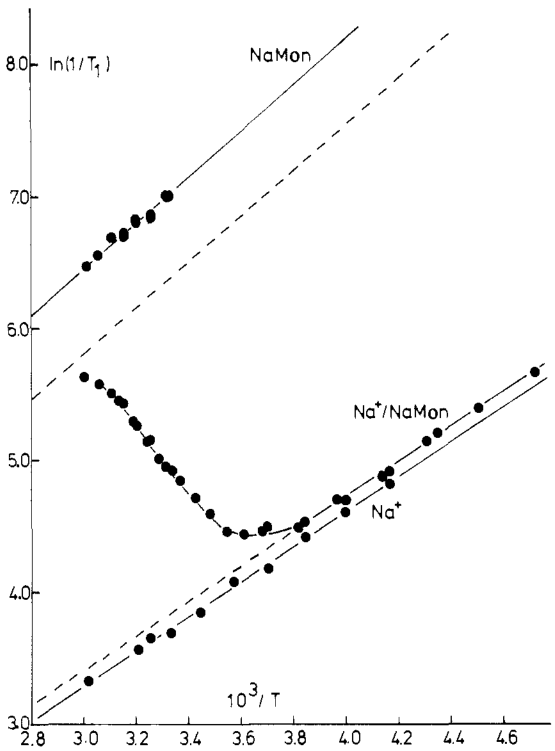

Figure 1.

The results in Figure 1 show that for both Na^+ and NaMon ln $(1/T_1)$ varies linearly with $1/T$, and may be analyzed according to the normal Arrhenius equation (Figure 1) or via transition state theory as in the equation:

$$1/T_1 = \frac{kT}{h} e^{\Delta S^{\ddagger}/R} e^{-\Delta H^{\ddagger}/RT} \qquad (9)$$

Linear regression analysis according to eq 9 gives the following results:

$$T_{1A}(\text{Na}^+, 298\text{ K}) = 23.4\ (\pm 1.2)\text{ ms};\ \Delta H^{\ddagger} = -13.2\ (\pm 0.3)\text{ kJ mol}^{-1};\ \Delta S^{\ddagger} = -258\ (\pm 1.0)\text{ J K}^{-1}\text{ mol}^{-1} \qquad (10)$$

$$T_{1B}(\text{NaMon}, 298\text{ K}) = 0.88\ (\pm 0.04)\text{ ms};\ \Delta H^{\ddagger} = -15.8\ (\pm 0.9)\text{ kJ mol}^{-1};\ \Delta S^{\ddagger} = -240\ (\pm 2.9)\text{ J K}^{-1}\text{ mol}^{-1} \qquad (11)$$

Relaxation times for Na^+/NaMon at low temperatures (slow exchange) are slightly shorter (10%) than those of complex-free Na^+ solutions. This difference has been observed previously in related systems and has been shown to correlate with the influence of the complexes on solvent viscosity;[13,14,19] it becomes negligible at NaMon concentrations lower than ca. 10^{-2} M. In calculating exchange rates (eq 1), $1/\tau_A$ from eq 8, we have used $1/T_{1A}$ values extrapolated from the low-temperature Na^+/NaMon system (Figure 1, lower dotted line), and $1/T_{1B}$(NaMon) calculated from eq 9 using the activation parameters given in eq 11. Detailed results are listed in Table M1 in the microfilm edition.

The exchange process may, in principle, occur by two pathways;[14,19,20] viz, a rate-determining dissociation of NaMon to give $Na^+ + Mon^-$ as in eq 5, or a direct exchange reaction:

$$^*\text{Na}^+ + \text{NaMon} \xrightarrow{k_1} \text{Na}^+ + {}^*\text{NaMon} \qquad (12)$$

In the general case the average lifetime of Na^+ in the presence of NaMon is given by:

$$1/\tau_A = k_1[\text{NaMon}] + k_d[\text{NaMon}]/[\text{Na}^+] \qquad (13)$$

In the present case, however, a plot of $1/\tau_A$ against $1/[Na^+]$ at constant [NaMon] (0.04 M) is linear with a negligible intercept (Figure 2). In fact, a linear regression analysis of the data shown in Figure 2 gives a

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Table II. Exchange Rates for Na^+/NaMon[a] in Buffer Solutions in Methanol at 29 °C

CH_3SO_3H		CH_2ClCO_2H			CH_3CO_2H		
10^3[HA]/M	$(1/\tau_A)/s^{-1}$ [b]	10^3[HA]/M	r [c]	$(1/\tau_A)/s^{-1}$ [b]	10^3[HA]/M	r [c]	$(1/\tau_A)/s^{-1}$ [b]
0	107	0		107	0		107
0.512	192	0.814	0.075	121	7.46	1.71	119
1.013	338	1.732	0.161	166	15.2	3.51	157
1.013	332	2.633	0.247	220	22.8	5.32	175
1.545	478	3.517	0.333	263	33.9	8.03	223
1.987	578	4.385	0.419	334	44.8	10.7	266
2.925	824	5.236	0.505	383	58.7	14.4	302
3.827	1140	6.071	0.591	407	72.2	18.0	339
		7.696	0.763	537			
		9.263	0.935	616			

[a] $[Na^+]$ = [NaMon] = 0.04 M. [b] $1/\tau_A$ (eq 8) is the lifetime of Na^+ in the exchanging system (eq 4). [c] r = [HA]/[A^-].

Table III. $^{23}Na^+$ Spin-Lattice Relaxation Times (T_1) for Na^+,[a] NaMon,[a] and Na^+/NaMon[a] in Methanol-Water Mixtures at 29 °C

vol % H_2O	x_{H_2O}	$1/T_{1A}(Na^+)$ (s^{-1})	vol % H_2O	x_{H_2O}	$1/T_{1B}$(NaMon) (s^{-1})[b]	vol % H_2O	x_{H_2O}	$1/T'_{1A}(Na^+$/NaMon) (s^{-1})
0	0	38.1	0	0	1060	0	0	137
4.76	0.101	43.8	4.76	0.101	1280	2.44	0.063	198
9.09	0.184	47.9	9.09	0.184	1470	4.76	0.101	268
13.0	0.252	47.4	13.0	0.252	1870	6.98	0.144	341
16.7	0.310	49.3	16.7	0.310	2150	9.09	0.184	360
20.0	0.360	51.2	20.0	0.360	2180	11.1	0.219	448
23.1	0.403	51.7	23.1	0.403	2510	13.0	0.252	518
44.4	0.643	49.8				14.9	0.282	566
56.5	0.745	44.8				16.7	0.310	700
75.3	0.873	27.5				18.4	0.336	750
100	1.0	16.1				20.0	0.360	805
						21.6	0.382	842

[a] $[Na^+]$ = [NaMon] = 0.04 M. [b] ±5% (1060) to ±10% (2510).

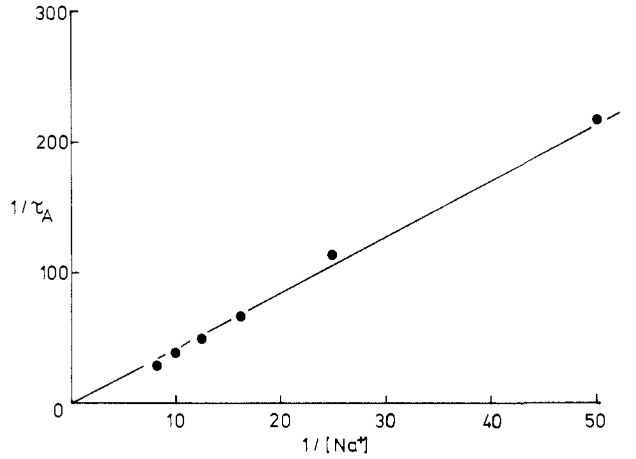

Figure 2.

slightly negative intercept (-7 ± 4 s^{-1}). Thus the dominant mechanism for exchange involves dissociation of NaMon as in eq 5, and $1/\tau_A$ calculated from the results in Figure 1 may be identified directly with k_d, the rate constant for dissociation of NaMon. Analysis of the results according to transition state theory as in eq 7 gives the following:

$$k_d(\text{NaMon, 298 K}) = 83\ (\pm 4)\ s^{-1};\ \Delta H^{\ddagger} = 46.8\ (\pm 0.7)\ \text{kJ mol}^{-1};\ \Delta S^{\ddagger} = -51.1\ (\pm 2.5)\ \text{J K}^{-1}\ \text{mol}^{-1} \quad (14)$$

(ii) Na^+/NaMon Exchange in Buffer Solutions. The exchange rate in methanol was accelerated considerably by the addition of strong acid (CH_3SO_3H) or in the presence of buffers ($CH_3CO_2H/CH_3CO_2^-$ or $CH_2ClCO_2H/CH_2ClCO_2^-$). Exchange rate constants obtained in the various solutions are listed in Table II, and the measured relaxation times in the different solutions are given in Table M2 of the microfilm edition. In the buffer solutions, the exchange rate constants (k_d values) were independent of the total buffer concentration at *constant buffer ratio r* = [HA]/[A^-]; e.g., in acetic acid buffers with [HA]/[A^-] = 0.802, a 20-fold variation in [HA] from 1.8 × 10^{-3} to 32 × 10^{-3} M produced no change in exchange rate. In all cases, however, $1/\tau_A$ (k_d) varied linearly with the buffer ratio as illustrated, for example, in Figure 3 for reactions in acetic acid buffers.

(iii) Influence of Water on the Exchange of Na^+/NaMon. T_1 values for Na^+ (0.04 M) (T_{1A}), NaMon (0.04 M) (T_{1B}), and Na^+/NaMon

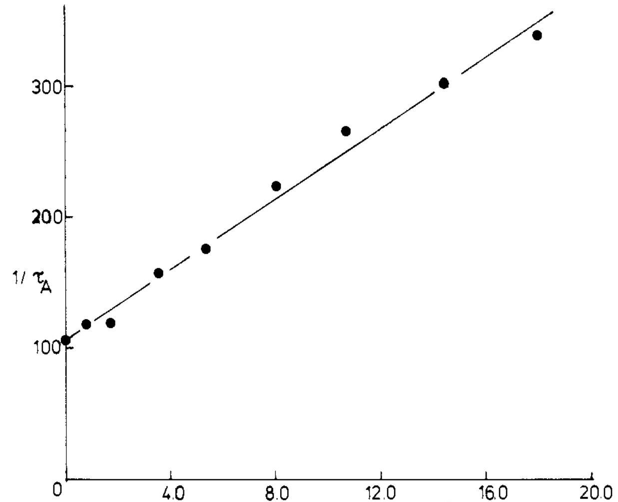

Figure 3.

(both 0.04 M) (T'_{1A}) were measured in water-methanol mixtures. Beyond 21.6 vol % H_2O (x_{H_2O} = 0.382) the solubility of NaMon was too low to permit convenient kinetic measurements. The relaxation times are given in Table III. In order to calculate $1/\tau_A = k_d$ values using eq 8, $1/T_{1A}(Na^+)$ and $1/T_{1B}$(NaMon) at the mole fraction of water were obtained from smoothed plots of $1/T_1$ (Table III) vs. x_{H_2O} and combined with $1/T_{1A}$ (Na^+/NaMon) from Table III. For the $1/T_{1A}$ values it was assumed that the 10% correction applied to directly measured Na^+ values was required in the methanol-water mixture as in pure methanol; in fact, calculated $1/\tau_A$ values are insensitive to the exact value of $1/T_{1A}$ as the exchange rates are well above the slow exchange limit. Rate constants for the exchange reaction in the solvent mixtures are listed in Table IV. Also given in Table IV are formation rate constants for Na^+ + Mon^- obtained by combining $1/\tau_A$ (k_d) with stability constants for NaMon ($K_s = k_f/k_d$) interpolated from the results in Table I. The k_f values are subject to a significant uncertainty because of combined uncertainties in k_d and especially K_s values.

Discussion

In pure methanol the rate of exchange of free and complexed Na^+ (eq 4) is controlled by the rate of dissociation of NaMon

Table IV. Formation and Dissociation Rate Constants for NaMon in Methanol-Water Mixtures at 29 °C

vol % H_2O	x_{H_2O}	$k_d{}^a/s^{-1}$	$10^{-8}k_f{}^b/M^{-1}\ s^{-1}$
0	0	102	2.1
2.44	0.053	176	3.0
4.76	0.101	277	3.9
6.98	0.144	397	4.9
9.09	0.184	413	4.1
11.1	0.219	683	5.7
13.0	0.252	689	4.9
14.9	0.282	812	4.8
16.7	0.310	1260	6.2
18.4	0.336	1290	5.4
20.0	0.360	1480	5.0
21.6	0.382	1550	4.5

[a] $k_d = 1/\tau_A$ (eq 8), ±10%. [b] $k_f = K_s k_d$, ±30%.

Table V. Kinetic and Thermodynamic Parameters for Complex Formation between Na^+ and Mon^- in Methanol at 25 °C

(i)	dissociation	k_d/s^{-1}	$\Delta H_d{}^{\ddagger}/kJ\ mol^{-1}$	$\Delta S_d{}^{\ddagger}/J\ K^{-1}\ mol^{-1}$
		83 (±4)	46.8 (±0.7)	−51.1 (±2.5)
(ii)	formation	$k_f/M^{-1}\ s^{-1}$	$\Delta H_f{}^{\ddagger}/kJ\ mol^{-1}$	$\Delta S_f{}^{\ddagger}/J\ K^{-1}\ mol^{-1}$
		$1.8\ (\pm 0.3) \times 10^8$	32.2 (±1.2)	29 (±5)
(iii)	equilibrium	$\log (K_s/M^{-1})^{a,b}$	$\Delta H_c{}^{\circ}/kJ\ mol^{-1\,b}$	$\Delta S_c{}^{\circ}/J\ K^{-1}\ mol^{-1}$
		6.34	−14.6	80

[a] Reference 17. [b] Reference 18.

to give Na^+ and Mon^-. Thus the measured exchange rate constant, $1/\tau_A$, may be identified with the dissociation rate constant, k_d, for the complexation reaction:

$$Na^+ + Mon^- \underset{k_d}{\overset{k_f}{\rightleftharpoons}} NaMon \qquad (15)$$

Combination of k_d and its associated activation parameters with the thermodynamic properties for the equilibrium (K_s and the enthalpy, ΔH_c, and entropy ΔS_c, of complexation)[17,18] gives the rate constant and activation parameters for the formation reaction. The values are listed in Table V.

Equilibrium entropies of complexation of the carboxylate ionophores such as monensin are notable for being some 100 J K^{-1} mol^{-1} more positive than those of neutral macrocyclic ionophores and also of their neutral protonated forms.[21] The most likely explanation for the *difference* is that in solvents such as methanol the uncomplexed carboxylate ion, probably in an open-chain form, is heavily solvated, and the solvent molecules involved (along with those associated with the cation) are largely released upon complexation.[22] Similar effects may be expected in the kinetic activation parameters, and it is of interest to compare the results here with corresponding values for other ionophores.

The large negative entropy of activation for the dissociation reaction of NaMon (−51 J K^{-1} mol^{-1}) is typical for (Na^+) complexes of ionophores, although the available data are somewhat limited. $\Delta S_d{}^{\ddagger}$ values for various Na^+ cryptates in methanol vary between −35 and −70 J K^{-1} mol^{-1},[23,24] and for $NaMonH^+$ in ethanol, $\Delta S_d{}^{\ddagger} = -71$ J K^{-1} mol^{-1}.[9] For the cryptates of the higher charge-density Ca^{2+}, $\Delta S_d{}^{\ddagger}$(water) values between −50 and −200 J K^{-1} mol^{-1} have been reported.[25] These large, negative $\Delta S_d{}^{\ddagger}$ values suggest significant resolvation of the emerging cation in the transition state, with a corresponding loss of translation entropy of solvent molecules and also a relatively low activation enthalpy for dissociation.

The main difference between the reactions of the monensin anion and reactions of neutral ionophores appears in the overall formation reaction provided a one-step complex formation reaction is postulated. The absolute magnitude of the formation rate constant (1.8×10^8 M^{-1} s^{-1}) is not unusual, being similar in magnitude to values reported for several different neutral macrocyclic ionophores in methanol: Na^+(monactin), $k_f = 3 \times 10^8$ M^{-1} s^{-1};[26] Na^+(trinactin), $k_f = 7.2 \times 10^7$ M^{-1} s^{-1};[26] Na^+(valinomycin), $k_f = 1.3 \times 10^7$ M^{-1} s^{-1};[27] Na^+(2,2,1), $k_f = 8.7 \times 10^7$ M^{-1} s^{-1}.[23] However, compared with $Na(2,2,1)^+$, the most stable cryptate complex of Na^+, $\Delta S_f{}^{\ddagger}$ is 71 J K^{-1} mol^{-1} more positive and $\Delta H_f{}^{\ddagger}$ is larger by 17 kJ mol^{-1}. Both of these are consistent with a larger degree of desolvation on forming the transition state for NaMon than $Na(2,2,1)^+$. The differences are probably associated with the charge on the carboxylate group in Mon^- rather than the overall ligand structure, as results (in ethanol) for reaction of the neutral MonH with Na^+ ($\Delta H_f{}^{\ddagger} = 13.8$ kJ mol^{-1}, $\Delta S_f{}^{\ddagger} = -71$ J K^{-1} mol^{-1})[9] are close to those for (2,2,1) + Na^+.

Monensin and other carboxylate ionophores are known to promote M^+/H^+ exchange reactions across membranes,[3,4] and it is of some interest therefore to consider the influence of pH upon the dissociation (exchange) of Na^+ from NaMon. Acids could assist the exchange by a direct displacement of Na^+ (general acid catalysis) or through formation of a protonated intermediate, $NaMonH^+$ (specific acid catalysis). Earlier results[9] for the reaction between NaMon and the acid 2,4-dinitrophenol in ethanol suggested that the latter mechanism (eq 16 and 17) is dominant,

$$NaMon + HA \underset{(fast)}{\overset{K}{\rightleftharpoons}} NaMonH^+ + A^- \qquad (16)$$

$$NaMonH^+ \xrightarrow{k_d'} MonH + Na^+ \qquad (17)$$

and the present results are also consistent with such a mechanism. In particular, the exchange rate increases with increasing $[H^+]$ in strong acid or in buffers but is independent of the total buffer concentration, *at fixed buffer ratio.*

It may readily be shown that provided the fraction of NaMon in the form of $NaMonH^+$ is small, the exchange rate in buffer solutions (HA/A^-) is given by eq 18 or 19, where $K = K_{HA}/$

$$1/\tau_A = k_d + k_d'(K[HA]/[A^-]) \qquad (18)$$

$$1/\tau_A = k_d + (k_d'[H^+]/K_{NaMonH}) \qquad (19)$$

K_{NaMonH} and K_{HA} and K_{NaMonH} are the acid dissociation constants of HA and $NaMonH^+$, respectively. In strong acid it is more convenient to write eq 19 in the form:

$$1/t_A = k_d + (k_d'[NaMonH^+]_T/[NaMon]_T) \qquad (20)$$

where $[NaMonH^+]_T = [H^+]_T$ equals the stoichiometric $[H^+]$ added to the solution, and $[NaMon]_T$ is the total concentration of NaMon present ($[H^+]_T < [NaMon]_T$). In the present case the fraction of $NaMonH^+$ was always small (≤3% in buffer, ≤9% in CH_3SO_3H solutions), but higher concentration of $NaMonH^+$ could be allowed for in eq 18–20 by multiplying all terms on the right by $K_{NaMonH}/([H^+] + K_{NaMonH})$.

The results in Figure 3 for acetate buffers, and similar plots for the data for chloroacetate buffers and CH_3SO_3H solutions (Table II), show eq 18–20 to be obeyed in all cases. The measurements in CH_3SO_3H solution yield directly a value for the rate constant for dissociation of $NaMonH^+$ of $k_d' = 1.03 \times 10^4$ s^{-1} (29 °C); this may be compared with k_d(NaMon, 29 °C) = 107 s^{-1}). Less certain estimates of k_d' may be obtained from the reactions in chloroacetate and acetate buffers, where the slopes ($k_d' K_{HA}/K_{MaMonH}$) of $1/\tau_A$ against $r = [HA]/[A^-]$ are 560 and 13.5 s^{-1}, respectively. Combining these with the known pK_a values for CH_2ClCO_2H ($pK_a = 7.76$),[28] CH_3CO_2H (9.56),[28] and $NaMonH^+$ (6.3 ± 0.3), estimated in using eq 21, gives $k_d' = 1.5 \times$

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$$\mathrm{p}K_a(\mathrm{NaMonH}^+) = \mathrm{p}K_a(\mathrm{MonH}) + \log K_s(\mathrm{NaMonH}^+) - \log K_s(\mathrm{NaMon}) \quad (21)$$

10^4 and 2.3×10^4 s^{-1}, respectively. In view of the large uncertainty in estimating k_{HA}/K_{NaMonH} in methanol, the agreement is satisfactory and confirms that the same exchange mechanism is operating in all three systems. The relatively modest increase in k_d on protonation ($k_d' \sim 100k_d$) means that pH values near pK_a(NaMonH$^+$) are required before significant increases in exchange rates (Na$^+$ for Na$^+$ or Na$^+$ for H$^+$) beyond those obtained from the dissociation rate constant for NaMon will be observed. This in turn will mean pH values substantially below the pK_a value of the free acid (MonH).

Finally, we note the large increase in the dissociation rate constant upon addition of water to methanol (Table IV). Part of the reason for this increase almost certainly comes from the more favorable solvation of Na$^+$ by water than methanol,[29,30] and it is generally observed that k_d values for complexes of ionophores in water are higher than those in methanol. However, it has been argued that an important property of water in these reactions is the ability to interact also with the ligand donor atoms via hydrogen-bond formation.[31] In the case of monensin complexes this would seem to be especially relevant as, even from "anhydrous" methanol, salts such as AgMon crystallize as the monohydrate[5] and the hydrogen-bonded water molecule appears to be in an ideal position to function as one of the water molecules solvating the cation in the complexation or decomplexation step.

Two pieces of evidence, apart from the increase in k_d with added water, support the suggestion of a water molecule or other H-bond donor solvent molecules, playing a more important role in the complexation kinetics of NaMon. The first is that the formation rate constant, k_f, also increases (slightly) with increasing water content of the solvent. This is in sharp contrast to the behavior of valinomycin complexes in methanol where, for example, k_f for K(valinomycin)$^+$ decreases steadily on addition of water and is reduced by a factor of 27 on going from methanol to 30 vol % methanol/water.[32] For other neutral ionophores k_f values in pure water are also ca. 10^2 lower than in methanol,[31] although the behavior in methanol/water mixtures has not been reported. In these cases the simplest explanation is that a significant contribution to the rate decrease comes from a competition between water and cations for the ligand donor sites, and indeed there is some evidence for this beginning to occur at higher water concentrations for NaMon (Table IV). The second is that while for simple neutral ligands such as the bicyclic cryptands k_d values for Na$^+$ complexes are much larger (10–100 times) in dipolar aprotic solvents which strongly solvate Na$^+$ (dimethyl sulfoxide and dimethylformamide) than in methanol, the opposite is true for NaMon. Thus k_d for NaMon in methanol is 500 times that in dimethylformamide and 20 times that in dimethyl sulfoxide.[33] This rather dramatic difference in the kinetic behavior of complexes of neutral and carboxylate ionophores points to very strong and specific interactions between the protic solvents and the anionic ionophores, presumably involving hydrogen bonding between the carboxylate group and the solvent molecules.

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Registry No. NaMon, 22373-78-0.

Supplementary Material Available: Tables of relaxation times for Na$^+$/NaMon in methanol and for NaBr (0.04 M) + NaMon (0.04 M) in buffer solutions in methanol (4 pages). Ordering information is given on any current masthead page.

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