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LIFETIMES OF COMPLEXES OF THE ANTIBIOTIC X-537A (LASALOCID A) WITH PHYSIOLOGICAL CATIONS IN METHANOL. DETERMINATION BY NMR USING A DISSOCIATIVE SHIFT REAGENT

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Using a magnetic resonance method we have determined the lifetimes of the following complexes of X-537A (HX) in methanol solution at 25°C, BaX^+, 132 μs; SrX^+, 34 μs; CaX^+, 24 μs, and KX, 15 μs, each with an estimated uncertainty of 30%. For NaX and LiX the lifetimes are too short to measure by the present method. The lifetime found for BaX^+ is considerably shorter than the result obtained by Patel and Shen using a different method. The present method depends upon the broadening of the resonance lines of certain protons in X^- due to reactions such as $X^- + Ba^{2+} = BaX^+$, when the resonance of the proton in the "free" X^- has been shifted from its normal frequency by Pr(III) which acts via the exchange $PrX_3 \rightleftharpoons PrX_2^+ + X^-$. To determine the lifetimes of interest the equations for a three-state spin system were derived; under the relevant conditions the final equations take the form of the Swift-Connick equations for a two-state system, with the characteristics of one of the states dependent upon the PrX_3 concentration. This dependence is used to extract the lifetimes from the data in a novel and simple way.

1. Introduction

Here we describe the results of a continuation of earlier work on solutions of X-537A (HX) in methanol in the presence of various ions which lead to results for the speciation and thermodynamics of the ion, X^- complexes [1], the kinetics of the MnX^+ and NiX^+ complexes [2], and the kinetics of the CoX^+ complex [3]. Originally it was planned to attempt to determine the kinetics of the complexes of X^- with some of the physiological cations *via* the line broadening in appropriate mixtures of Ni^{2+} with the cation of interest, and some of the resulting data are reported in a later section, but this program was abandoned when the advantages of using Pr^{3+} in place of Ni^{2+} were noticed.

In the meantime Shen and Patel [4] have reported results for the lifetime of BaX^+ in methanol solutions over a wide temperature range. Their work is further discussed below. The present work, which makes use of the special properties of the PrX_3 complex in methanol, was stimulated by studies by Chen and Springer which were recently reported [5]. The basic idea, of using a reactive paramagnetic ion to make the NMR spectrum of the bound X^- distinct from that of unbound X^-, has antecedents in the use of Co^{2+} by Jackson, Lemons, and Taube [6] as a shift reagent in the study of the labilities of the aquo complexes of ions such as Al^{3+} in water and the use of $Eu(fod)_3$ by Cheng and Gutowski [7] and Tanny et al. [18] as a shift reagent in the study of the conformational lability of ligands in solution.

2. Experimental

The circular dichroism and fluorescence measurements were carried out as in the work by Degani and Friedman [1] except that in the present work the CD measurements were made with a JASCO-J20A instrument. The NMR results reported here were all obtained with a Varian HFT80 instrument (80 MHz for protons). Methanol-d_4 (99% D) from Stohler Isotope Chemicals was used without further purification as solvent in all of the NMR studies. Anhydrous NaBr, KBr, $NiCl_2$, $CaBr_2$, $SrBr_2$, $BaCl_2$, and $Ba(ClO_4)_2$, obtained from Alfa Inorganics, were dried in vacuum before use. In early experiments $Pr(NO_3)_3 \cdot 5H_2O$

Table 1
Results of CD and fluorescence studies, solutions in methanol at 25°C

	Metal ion											
	Ni^{2+}		Ba^{2+}		Sr^{2+}			Ca^{2+}			Pr^{3+}	
CD spectra												
neutral solutions [a]												
$\Delta\epsilon$	−2.2	−0.9	−7.3	−4.3	−7.3	1.6	−3.7	−2.5	0.2	−1.1	−2.8	−1.0
λ, nm	290	240	290	237	295	252	236	290	252	236	290	242
acid solutions [b]												
$\Delta\epsilon$	−2.1	−0.4	−5.4	−1.7	−4.6	1.0	−1.9	−2.1	0.1	−0.4	−2.6	−0.7
λ, nm	290	240	290	237	295	252	236	288	248	236	290	242
CD composition dependence												
neutral solutions [a]												
complex formed	NiX^+		BaX^+ [e]		SrX^+ [e]			CaX^+ [e]			PrX^{2+} [g]	
pK for association [c]	−4.48 ± 0.05		(−6.46)		(−5.47)			(−4.57)			−7.22 ± 0.16	
acid solutions [b]												
apparent formula of complex	$NiH_2X_2^{++}$		$BaH_2X_2^{++}$		[f]			[f]				
pK for association [d]	−6.25 ± 0.25		−5.64 ± 0.08									
fluorescence composition dependence												
neutral solutions												
formula of complex	NiX^+											
pK for association [c]	−4.53 ± 0.21											

[a] 0.3 mM LiX and 0.3 mM M(II) or Pr(III).

[b] 0.3 mM HX and 0.03 mM M(II) or Pr(III).

[c] For the reaction $X^- + M^{n+} \rightarrow MX^{(n-1)+}$.

[d] For the reaction $2HX + M^{2+} \rightarrow M(HX)_2^{2+}$.

[e] Data from Degani and Friedman, 1974. Not redetermined.

[f] The variation of CD with composition could not be fit with a single mass-action constant.

[g] The complexes PrX_2^+ and PrX_3 also are formed [5].

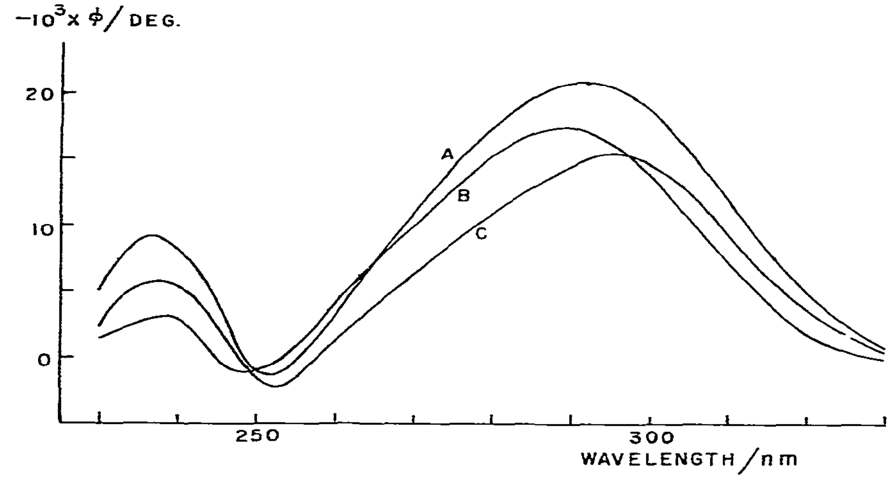

Fig. 1. CD spectra of Ca-X537A solutions in methanol at 25°C, ellipticity as a function of wavelength. The stoichiometric concentration of X-537A is 0.256 mM in all cases. Other stoichiometric concentrations: For curve A, [LiOH] = 0.31 mM and [$CaBr_2$] = 25 mM. For curve B, [$CaBr_2$] = 0.75 mM. For curve C, [$CaBr_2$] = 750 mM.

was used and assay of Pr^{+3} was made by using a cation exchange resin and titrating the liberated acid. Subsequently $PrCl_3$ was prepared from Pr_6O_{11} dissolved in concentrated HCl and dried in vacuum at room temperature; material dried at 100°C solvolyzed in methanol.

For the final rate studies a stock solution containing the desired diamagnetic ions and X^- was mixed with varying amounts of a stock solution containing the paramagnetic ion in its fully complexed form; thus containing Pr as PrX_3 and Ni as NiX^+, where we abbreviate X537A as HX. All stock solutions containing HX or ions derived from it were stored in a refrigerator and used within a few days of preparation.

3. Preliminary studies

In order to find suitable compositions for the kinetic studies, earlier results [1] were supplemented by new studies of the species formed by the interaction of X^- with metal ions in methanol. In one series the chlorides or bromides of selected metals were mixed with LiX in methanol. In the other series HX was used in place of LiX. The acid solution studies were motivated by the expectation that solutions of Pr(III) compounds with no added acid might be unstable with respect to solvolysis in methanol.

3.1. CD and fluorescence studies

The CD observations in neutral solutions (table 1) mostly confirm those reported earlier. The CD spectra in fig. 1 show the characteristic features of the CD of X537-A and its compounds with extrema near 235, 250, and 290 nm. The intensities and locations of these extrema are given in table 1. It may be remarked that the small differences of the coefficients in between neutral and acid solutions reported in the table correspond to changes that are clearly evident in the spectra themselves, as illustrated by fig. 1.

New data for the composition dependence of the CD of solutions of Ni(II) complexes lead to a somewhat different (table 1) binding constant for NiX^+ than the value pK = −3.95 reported earlier [1]. Consequently, the binding constant was also determined from the composition dependence of the fluorescence, the new result confirming the new CD value. Finally, the composition dependence of the fluorescence in mixtures of $NiCl_2$ with KX gave pK = 0.90 ± 0.10 for the process

$$KX + Ni^{2+} = K^+ + NiX^+. \quad (1)$$

Combined with the formation constant for KX [1] this gives a value

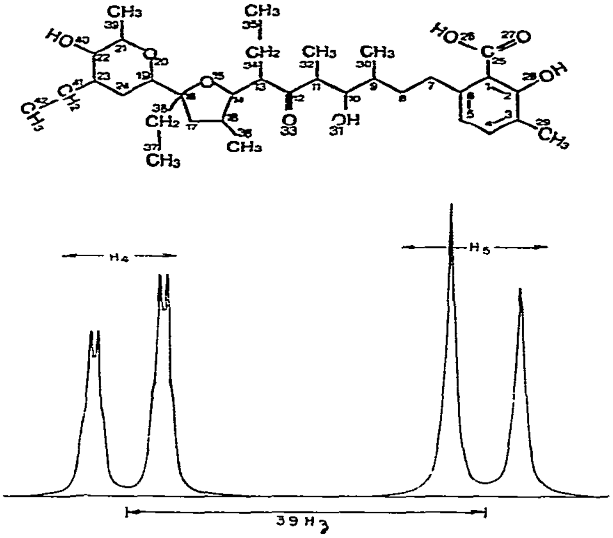

Fig. 2. 80 MHz proton NMR spectrum of the H_4-H_5 region of X-537A. 20 mM in methanol at 25°C. The structure and numbering convention are shown in the inset.

$$pK = -4.50 \pm 0.20$$

for the formation constant of NiX^+, in good agreement with the other two determinations in the present work, shown in table 1.

The formation constant for PrX^{2+} was determined from the dependence of the CD upon composition in mixtures of Ba^{2+}, Pr^{3+}, and LiX under conditions such that there is uncomplexed Pr^{3+} ion at equilibrium. The composition dependence is accounted for by the reaction

$$BaX^+ + Pr^{3+} \rightarrow Ba^{2+} + PrX^{2+} \qquad (2)$$

with $pK = -0.76 \pm 0.11$. Combined with the formation constant for BaX^+ [1] this leads to the formation constant for PrX^{2+} given in table 1. This indirect determination is more precise than one yielding $pK = -7$ based on the study of the composition dependence of the CD of mixtures of Pr^{3+} with LiX [5] because the CD changes are much smaller in the latter case. Chen and Springer [5] have shown that PrX_2^+ and PrX_3 are formed under conditions in which the activity of X^- is greater.

Comparison of the CD data (table 1) in acid solutions with those in neutral solutions shows that the metal-X-537A complex is not the same in any case. While the CD differences are small, they are well outside the experimental errors. The variation of CD with change in composition in the acid mixtures can be accounted for by assuming that, in the case of nickel, a complex of formulation $Ni(HX)_2^{2+}$ with association constant $pK = -6.25$ is present. The corresponding result in the case of barium is a complex of formulation $Ba(HX)_2^{2+}$ with association constant $pK = -5.64$. These formulas, although perhaps surprising, fit the data better than others (NiX^+, $NiHX_2^+$) which were tried. More extensive work would be required to learn whether $M(HX)_2^{2+}$ complexes actually are formed or whether there is a mixture of several different complexes. For the remaining metal ions in the table it was not possible to interpret the composition dependence of the CD spectra in the acid media in terms of the formation of a *single* metal-X-537A complex.

3.2. Proton NMR

In the work reported here the signals of H_{29}, H_5, and H_4, previously [2,3] designated H_{mm}, H_m, and H_p, respectively, were used because they are most sensitive to the binding of a paramagnetic ion by X-537A [2]. In the present work no use was made of H_{10} which was shown by Patel and Shen to be shifted measurably when Ba^{2+} is bound by X-537A [4].

The H_4 and H_5 spectrum of X-537A in MeOH is shown in fig. 2. A small splitting of the H_4 resonance due to $J_{4-29} = 0.75$ Hz is evident. Working at 300 MHz Anteunis [8] found $J_{4-29} = 0.6$ Hz for X-537A in C_6D_6 and 0.8 Hz in $CDCl_3$. He also reports $J_{4-5} = 7.5$ Hz in both cases, a value in agreement with that found in the present work by simulation of the spectrum in fig. 2 using eight Lorentzians for H_4 and two for H_5. The H_{29} line always is very nearly Lorentzian with no evident effect of the J_{4-29} splitting so it was not necessary to simulate it to extract shift and T_2 data. For the H_4 and H_5 lines the simulations with the coupling parameters specified above were used for this purpose.

Small shifts are measured when X-537A forms complexes with various diamagnetic species, as summarized in table 2. The reference solution, 40 mM

Table 2
Upfield shifts (Hz) from X^- in methanol solutions at 25°C and 80 MHz [a]

	H_4	H_5	H_{10}	H_{29}
reference [b] X^-	0	0	0	0
HX [c]	−17.0	−12.3	6	−2.9
	(−16.8)	(−12.0)	(6.4)	
HX, LiX [c]	−7.0	−4.8	2.8	−1.0
HX, LiX, CaX^+ [c]	−10.2	−6.8	1.2	−1.4
HX, LiX, SrX^+ [c]	−9.5	−5.8	−21.2	−1.2
HX, LiX, BaX^+ [c]	−9.5	−5.8	−34.8	−1.2
LiX, BaX^+ [c]	−2.2	0.0		−0.3
NaX [c]	−1.5	1.0	(−20.0)	−0.4
	(−1.6)	(4.8)		
NaX, CaX^+ [c]	−4.5	−2.3		−0.5
NaX, SrX^+ [c]	−3.2	−0.3		−0.3
NaX, BaX^+ [c]	−3.0	0.2		−0.2

[a] Values in parentheses are 80 MHz values calculated from the 360 MHz values given by Shen and Patel [4].
[b] Actual measurements in 40 mM LiX. The contribution of the small amount of undissociated LiX is neglected.
[c] 20 mM of each listed species.

LiX, contains effectively free X^- in methanol because LiX is so loosely bound [1].

These shifts all seem too small for use in determining the rates of formation of the various complexes by lineshape measurements at 80 MHz. Indeed no extra broadening attributable to slow exchange reactions is evident in any of the mixtures in table 2.

Very large shifts are observed when Pr(III), even in small amounts, is added to solutions containing HX, X^-, or X^- complexes. It is convenient to define coefficients for the shift $\omega - \omega_0$ and the increased relaxation rate of a given proton species due to added Pr(III) as follows

$$\bar{s} \equiv (\omega - \omega_0)[X]/3[Pr], \tag{3}$$

$$\bar{k} \equiv (1/T_2 - 1/T_{2,0})[X]/3[Pr], \tag{4}$$

where [X] is the stoichiometric concentration of X-537A, [Pr] is the stoichiometric concentration of Pr(III), and the subscript 0 specifies the absence of Pr(III). Coefficients defined in this way have the following significance: In the case that all Pr(III) is present as PrX_3 and all HX is present either as X^- or PrX_3, and in the fast exchange limit, $\bar{s}$ is the shift of the proton (H_4, H_5, or H_{29}) frequency in PrX_3 relative to X^- and $\bar{k}$ is the corresponding difference in relaxation rates.

These coefficients were determined for several solutions with [Pr] in the range from 0 to 2.5 mM. Typical results are given in table 3. It is found that $\bar{s}$ is accurately constant in this range, showing only random variations of ±3% with changing [Pr]. These variations are attributed to uncertainties in the solution compositions. Therefore in analyzing the data for the relaxation rates, it is often convenient to employ the shifts rather than the concentrations as the independent variables which measure the concentration of added Pr(III), as in fig. 3.

Table 3
PrX_3 as shift reagent. Shift coefficients $\bar{s}$ and relaxation coefficients $\bar{k}$ in various solutions in methanol at 25°C [a]

	H_4		H_5		H_{29}	
Solution stoichiometric composition	$\bar{s}$	$\bar{k}$	$\bar{s}$	$\bar{k}$	$\bar{s}$	$\bar{k}$
[HX] = 34.6 mM, [LiOH] = 14.6 mM	1047	5.7	1323	9.3	723	5.0
[LiX] = 37.5 mM [b]	1104	7.0	1340	10.7	752	5.7
	1104	7.3	1351	10.7	768	5.7
					718	4.7
[HX] = 36.5 mM					447	600
[NaX] = 39.7 mM	1047	14.5	1300	15.2	691	7.7
[NaX] = 40.2 mM, $[SrBr_2]$ = 20.5 mM	1067	20.7	1330	25.3	695	22.8
[NaX] = 41.4 mM, $[BaCl_2]$ = 20.2 mM	1080	31.8	1333	36.2	726	12.4

[a] Measurements at 80 proton MHz. $\bar{k}$ in units of s^{-1} and $\bar{s}$ in units of rad/s.
[b] Results of three independent determinations.

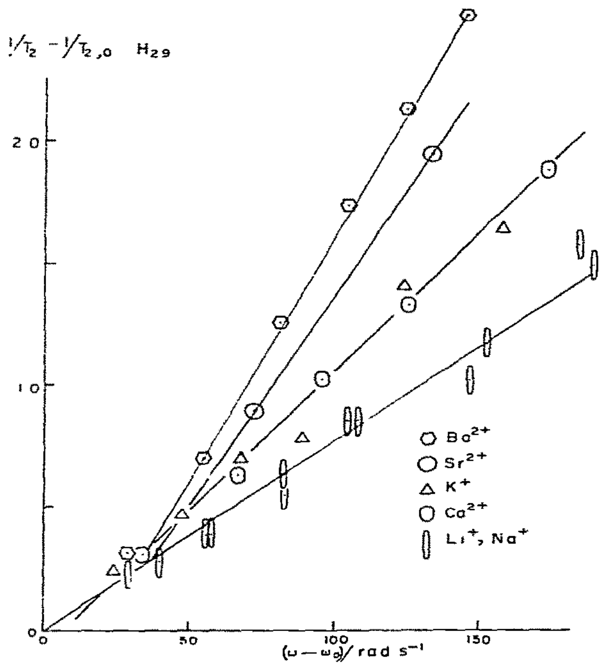

Fig. 3. Relaxation rates as functions of shift due to adding PrX_3 to solutions containing X^-, a small amount of HX, and the complex of X^- with Li^+, Na^+, K^+, Ca^{2+}, Sr^{2+}, or Ba^{2+}. In all cases the stoichiometric X-537A concentration is near 40 mM and the free X^- concentration exceeds 1 mM.

From fig. 3 one deduces that $\bar{k}$ is quite accurately constant in solutions of LiX or NaX and moreover is the same in the two cases. In the other cases $\bar{k}$ is larger and is dependent upon the Pr(III) concentration; in these cases the $\bar{k}$ values in table 3 are averages over the range of concentrations studied. (It may be noted that the straight lines that do not pass through the origin do not give constant $\bar{k}$ values.) The data for systems with increased and nonconstant $\bar{k}$ can be used to determine the lifetimes of the relevant complexes as described in sections 5 and 6. For the rest, in this section and in section 4, consideration is limited to solutions made by adding Pr(III) to methanol solutions of LiX, HX, and their mixtures.

For solutions made by adding Pr(III) to LiX or LiX, HX mixtures the simplest behavior is found when there is an appreciable concentration of free X^-, at least 1 mM, at equilibrium. In 40 mM LiX in methanol the concentration of free X^- is roughly 20 mM [1]. Addition of HX to the solution does not greatly change the concentration of free X^-. The same is true of addition of PrX_3, but addition of Pr(III) as $PrCl_3$ or $Pr(NO_3)_3$ tends to tie up three X^- per Pr^{3+} [5]. As long as there is an appreciable concentration of free X^- the observed shift and broadening effects may be attributed to the reaction

$$PrX_3 \rightleftharpoons PrX_2^+ + X^- \tag{5}$$

rather than to

$$X^- + PrX_3 \rightleftharpoons PrX_4^- . \tag{6}$$

The latter reaction would imply that $\bar{s}$ and $\bar{k}$ would be quite sensitive to the concentration of free X^-. It may be noted that eq. (6) is analogous to the process

$$LnK_3 + S \rightleftharpoons LnK_3S \tag{7}$$

by which the complex LnK_3 of a lanthanide ion with three β-diketonate ligands produces shifts in associating substrates S in non-coordinating solvents [9]. The large size of X^- and the ability of methanol to coordinate to metal ions both disfavor eq. (6) compared to (5). It may be remarked that in shifting the resonance of a pool of X^- *via* reaction (5) the shift reagent acts by a dissociative mechanism in contrast to the associative mechanism by which LnK_3 shifts the resonance of a pool of S in eq. (7).

In solutions of Pr^{3+} and HX in methanol without added X^- different and more complicated behavior is found. The different behavior may be expected on the basis of the CD data (table 1) which show that different complexes are present in acid than in neutral solutions. Other data show that small amounts of base (OH^- or MeO^-) in the solutions at equilibrium have profound effects on the shifts and linewidths of H_4, H_5, and H_{29} in the presence of Pr^{3+} while the same additions do not noticeably affect the CD and the fluorescence.

In view of all these complications the kinetic studies are best made in a composition regime in which, at equilibrium, there is some HX and some free X^- in the solutions; i.e. each should be at least at the millimolar level.

4. PrX_3–X^- interaction

The effect of Pr^{3+} on the relaxations and shifts of the X^- protons in mixtures of X^- with small amounts of PrX_3 may be described by the Swift-Connick equations [10] which we write as

$$\bar{k} = Y_{21} \frac{k_1(k_1 + Y_{21}) + \delta_{21}^2}{(k_1 + Y_{21})^2 + \delta_{21}^2}, \tag{8a}$$

$$\bar{s} = \frac{\delta_{21} Y_{21}^2}{(k_1 + Y_{21})^2 + \delta_{21}^2}, \tag{8b}$$

where

Y_{21} = probability per unit time for transfer of an X^- ion to state 2 (free X^-) from state 1 (in PrX_3). Thus $1/Y_{21}$ is the mean lifetime of X in PrX_3.

δ_{21} = $\omega_2 - \omega_1$ where ω_j is the resonance frequency (rad/sec) in state j.

k_j = $1/T_2$ in state j, the transverse relaxation rate in state j in the absence of exchange.

For each proton species H_4, H_5, and H_{29} one has a pair of Swift-Connick equations, each with its characteristic k_1 and δ_{21}, but of course with the same Y_{21} because these proton species do not exchange appreciably with other protons in the system.

The application of the Swift-Connick equations to the H_4 and H_5 resonances may be questioned because sufficiently fast longitudinal relaxation of these protons tends to remove the splittings and give lineshape changes that cannot be accounted for accurately by means of the generalized Bloch equations (from which the Swift-Connick equations are derived) [11]. In the present case these complications are not expected to be serious because k_1 is rather small (and $1/T_1$ in the complex must be at least as small) compared to the relevant coupling J_{4-5} = 7.5 Hz = 47 rad/s.

It may be noted from table 3 that we find $\bar{k}/|\bar{s}| \ll 1$ in each case. Also, because eqs. (8) may be combined to give

$$\bar{k}/|\bar{s}| = k_1^2/|\delta_{21}|Y_{21} + k_1/|\delta_{21}| + |\delta_{21}|/Y_{21}, \tag{9}$$

we may conclude that in these systems we have

$$k_1 \ll |\delta_{21}| \ll Y_{21}.$$

and eqs. (8) may be approximated by

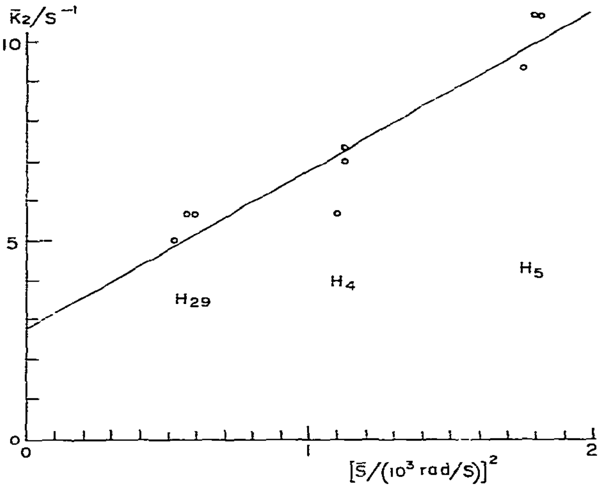

Fig. 4. Graphs of eq. (10a) using the data for LiX in table 3. The line is a least-squares fit.

$$\bar{k} \approx k_1 + \delta_{21}^2/Y_{21}, \quad \bar{s} \approx \delta_{21} \tag{10a,b}$$

where the neglected terms are smaller by a factor k_1/δ_{21} or δ_{21}/Y_{21} or some product of these fractions.

By using the data in table 3 we may evaluate the parameters in eqs. (10) as shown in fig. 4. We find

$$k_1 = 2.8 \text{ s}^{-1} \quad \text{and} \quad 1/Y_{21} = 4.0\ \mu\text{s}$$

for LiX-HX solutions.

It is the short lifetime of the PrX_3 complex with respect to exchange as well as the large shift δ_{21} that make Pr^{3+} very suitable for the present studies. If the lifetime were longer then $\bar{k}$ would be bigger, making it more difficult to measure the additional broadening produced by the addition of other ions that complex X^-. Thus, when Ni^{2+} is used in place of Pr^{3+} the desired rates are much more difficult to measure (section 7).

For later reference we note the least squares values of δ_{21} obtained from eq. (8b) and the LiX data in table 3, namely δ_{21} = 1075 rad s^{-1}, 1330 rad s^{-1}, and 725 rad s^{-1} for H_4, H_5 and H_{29} respectively. These results as well as the value for Y_{21} are in excellent agreement with those determined independently in another way [5].

5. Equations for transverse relaxation and shift in a three-state model

To extract the rate information from the additional broadening of the resonance of a proton species in X, such as that produced by Ba^{2+} in the last row of table 3, we consider a model in which X can be in one of three states:

state 1 = PrX_3, state 2 = $X^- + HX$,

state 3 = $MX^{(n-1)+}$

with the following parameters (cf. discussion following eq. (8)),

Y_{ab} = probability of transfer of any given X molecule to state a from state b per unit time.
k_a = $1/T_2$ of X in state a in the absence of exchange.
ω_a = resonance frequency (rad/s) in state a in the absence of exchange.
x_a = the fraction of X in state a.

We assume that HX and X^- as well as any other X-species in state 2 are in rapid exchange equilibrium so that no appreciable line broadening results from mixing them in the absence of states 1 and 3.

The following results are obtained from the generalized Bloch equations [11–13] for the three-state model under conditions such that a single nearly lorentzian line is observed for a given proton species, the line having negligible direct contribution from the magnetization of state 1.

$$1/T_2 = f_2 k_2^{\ddagger} + f_3 k_3 + f_2^2 f_3 Y_{23}^{-1} [(\omega_2^{\ddagger} - \omega_3)^2 - (k_2^{\ddagger} - k_3)^2], \tag{11a}$$

$$\omega_0 = f_2 \omega_2^{\ddagger} + f_3 \omega_3, \tag{11b}$$

where we define

$$k_2^{\ddagger} \equiv k_2 + (x_1/f_2) Y_{21} \frac{k_1(k_1 + Y_{21}) + (\omega_1 - \omega_0)^2}{(k_1 + Y_{21})^2 + (\omega_1 - \omega_0)^2}, \tag{12a}$$

$$\omega_2^{\ddagger} \equiv \omega_2 + (x_1/f_2) \frac{(\omega_1 - \omega_0) Y_{21}^2}{(k_1 + Y_{21})^2 + (\omega_1 - \omega_0)^2}, \tag{12b}$$

$$f_a \equiv x_a/(x_2 + x_3), \tag{13}$$

and where ω_0 is the center frequency of the one observed line and $1/T_2$ its linewidth. Eqs. (11) are derived by the method of Sack [14] under the following assumptions.

I) $Y_{13} = 0$ and $Y_{31} = 0$: there is no direct exchange between states 1 and 3.

II) Y_{23} is much larger than any of the following,

$$|\omega_2^{\ddagger} - \omega_2|, \quad |\omega_2 - \omega_3|, \quad k_2^{\ddagger}, \quad k_3.$$

These requirements together with the observation of only one resonance line will usually require also that $x_1 \ll 1$ so that $f_2 \approx x_2$ and $x_1/f_2 \approx x_1/x_2$.

Eq. (11a) has the same form as a classical result by Meiboom et al. [15], recently rederived by McLaughlin and Leigh [16], for a two state (2 and 3) system, if the differences between k_2 and $k_2^{\ddagger}$ and between ω_2 and $\omega_2^{\ddagger}$ are overlooked, and if the term in eq. (11a) in $(k_2^{\ddagger} - k_3)^2$ is neglected. Thus the effect of state 1 upon the standard two-state equation is to replace the parameters k_2 and ω_2 by effective parameters $k_2^{\ddagger}$ and $\omega_2^{\ddagger}$ that are determined by the state 1 : state 2 exchange by eqs. (12). Eqs. (12) bear a striking resemblance to the Swift-Connick equations (eqs. (8)). Thus these results, which are derived from the generalized Bloch equations under the stated assumptions, coincide with the simplest guess one might make about the behavior of the three-state system.

In most chemical kinetics studies using the Meiboom-Luz-Gill [15] or the Swift-Connick [10] equations only the T_2 data are fully exploited. Occasionally T_1 data may be combined with the T_2 data to increase the reliability of interpretation, but the shift data are not often used for this purpose, even when the broadening is not too great to permit accurate measurements of the shifts. In the present study the shifts can be measured accurately and they have been exploited as follows.

Both $1/T_2$ and ω_0 are measured for a series of solutions of fixed f_2 and f_3 but varying x_1; solutions made, for example, by adding various amounts of PrX_3 to aliquots of a given mixture of states 2 and 3. For each x_1 the quotient

$$Q(x_1) \equiv [(1/T_2)_{x_1} - (1/T_2)_0] / [(\omega_0)_{x_1} - (\omega_0)_0] \tag{14}$$

is calculated from the data. From eqs. (11) we find

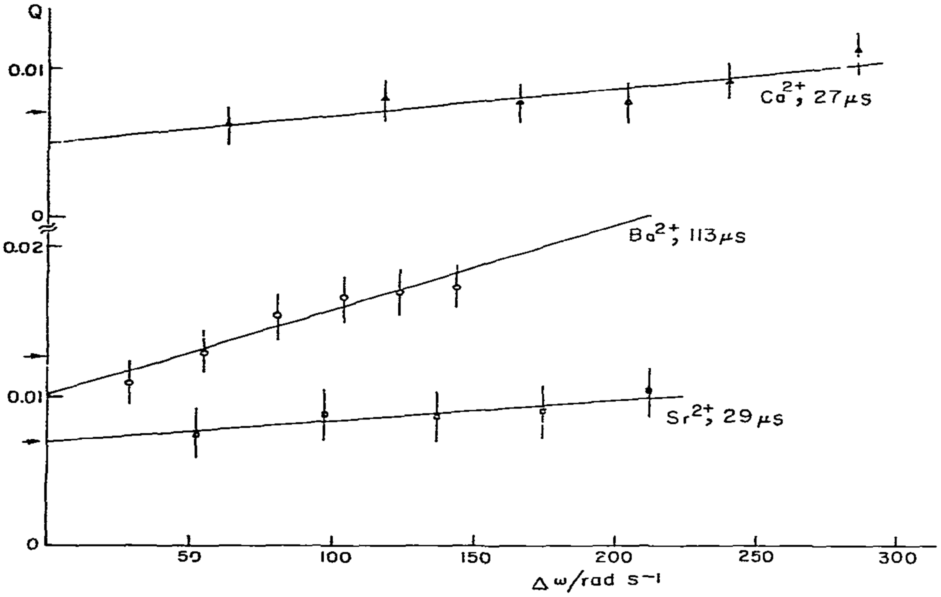

Fig. 5. Graphs of eq. (15a) for several systems. Data for H_{29} in each case. The arrows show the independently calculated intercept. The straight lines are least squares fits to the data. The indicated lifetimes serve to key the data to table 4 where the solution compositions are given.

$$Q = A_+ + [(1 - A_+^2) f_3/Y_{23} + A_-/\delta_{12}]\Delta\omega + \ldots, \quad (15a)$$

where we define

$$\Delta\omega \equiv (\omega_0)_{x_1} - (\omega_0)_0, \quad (15b)$$

$$A_\pm \equiv [\pm k_1(k_1 + Y_{21}) + \delta_{12}^2]/\delta_{12}Y_{21}, \quad (15c)$$

and where the terms omitted in (15a) can be neglected if, in addition to the conditions for the validity of eq. (11), we have

$$|k_2 - k_3|/Y_{23} \ll 1, \quad |\delta_{32}/\Delta\omega| \ll 1, \quad |\Delta\omega/\delta_{21}| \ll 1,$$

where the value of Δω may be taken anywhere in the experimental range. It seems that these conditions are satisfied in the systems studied here as well as in any two state system (2,3) to which one would wish to add a shift reagent to study the 2,3 interconversion rate. Moreover in many cases the bracketed factor in (15a) will reduce to f_3/Y_{23}. Examples of the data treated according to eq. (15a) are given in fig. 5, and the results for the lifetime $1/Y_{23}$ obtained in this way are collected, along with others, in table 4. The coefficients A_+ and A_- in table 4 were calculated from the results in section 4.

In order to employ eq. (14) the composition must be varied by adding PrX_3 to solutions of the complex being studied. For other composition variations one may use a less exact result obtained as follows. As noted above (section 3.2) the broadening depends nearly linearly on $\rho \equiv [Pr]/[X]$, where [...] means stoichiometric concentration.

Under these circumstances the main effect of the lifetime of state 3 is to make the broadening coefficient $\bar{k}$ defined in eq. (4) bigger than it would otherwise be. Therefore it is interesting to see whether the lifetime $1/Y_{23}$ can be estimated directly from the extra broadening.

Writing

$$\phi(\rho) \equiv (1/T_2)_\rho - (1/T_2)_0 \quad (16)$$

for the extra broadening produced by the Pr (cf. eq. (4)), the mean broadening coefficient becomes

$$3\bar{k} = (\phi(\rho_{max}) - \phi(\rho_{min}))/(\rho_{max} - \rho_{min}), \quad (17)$$

Table 4
Lifetime results, MeOH solutions at 25°C

	Y_{23}^{-1} lifetime, μs [a]				Stoichiometric concentration, mM			
complex	H_4	H_5	H_{10} [b]	H_{29}	HX	LiX	NaX	Other
BaX^+				108		37.7		$BaCl_2$, 19.2
			190			22.2		$BaCl_2$, 7.24
			190			14.5		$BaCl_2$, 7.24
			300			10.63		$BaCl_2$, 7.24
			340 ± 50		6.8	6.8		$BaCl_2$, 6.8
			450 ± 50			15.2		$BaCl_2$ = 7.6 $NaNO_3$ = 7.6
				91	16.5	21.5		$BaCl_2$, 11.4
	133	128		109	1.7		40.8	$BaCl_2$, 22.4
				50 [c]	1.7		40.8	$BaCl_2$, 22.4
				134			39.3	$BaCl_2$, 11.5
				113	3.2	40.0		$BaCl_2$, 26.2
SrX^+	31	41		29	1.7		40.8	$SrBr_2$, 19.5
	38 [c]	33 [c]		142 [c]			40.2	$SrBr_2$, 20.5
				29	3.2	40.0		$SrBr_2$, 22.5
CaX^+	19 [c]	20 [c]		36			36.8	$CaBr_2$, 23.9
	23	19		30	1.7		40.8	$CaBr_2$, 27.1
				27	3.2	40.0		$CaBr_2$, 23.8
KX	9 [c]	5 [c]					39.3	KBr, 25.1
	12	19		15	3.2	40.0		KBr, 23.4
$10^3 A_+$	6.9	7.4		6.8	see section 4			
$10^3 A_-$	1.7	3.2		−1.0				

[a] Lifetimes measured by broadening of the spectrum of the indicated proton species. Results of present work using eq. (14) except as otherwise indicated.

[b] Results of Shen and Patel [5] who observed the broadening at 360 MHz without added paramagnetic species. In some cases they did not completely specify the compositions of the solutions. Then we made the simplest assumption to complete this table.

[c] Results of present work using eq. (18).

where ρ_{min} and ρ_{max} define the range of ρ spanned by the data. If we define the broadening coefficient in the absence of state 3 by $\bar{k}_2$ and if we assume $\rho \approx x_1$ and $\bar{k}_2 \ll |s|$, then eq. (11a) can be written in the form

$$\bar{k} = \bar{k}_2 + 3(f_3/Y_{23})\bar{s}^2(\rho_{max} + \rho_{min}), \qquad (18)$$

where $\bar{s}$ is the shift coefficient defined by the eq. (3) and where we have used the fact that $\bar{s}$ is practically the same whether or not state 3 is present.

While eq. (18) involves many more approximations than eq. (11a) it provides an alternative method for estimating the lifetime that is useful when the extra broadening due to the lifetime of state 3 is too small to determine accurately.

6. Results of experiments with praseodymium shift reagent

The present results are collected in table 4 together with those reported by Shen and Patel [4].

For BaX^+ the mean (rms) value of the lifetime Y_{23}^{-1} determined in the present work is 132 μs. The difference of this value from the mean value, 327 μs, of the results of Shen and Patel [4], table 4, who used a different method, is somewhat greater than we would expect on the basis of the internal consistency of either method.

The other complexes for which we can determine lifetimes are SrX^+ (34 μs), CaX^+ (24 μs), and KX (15 μs), where the root mean square values based on table 4 are given. For NaX and LiX one can only say that on the basis of the present studies the lifetimes are less than 10 μs. The uncertainties in these results are best gauged by examining the ranges of lifetimes reported in table 4.

While the present results are not very precise they seem rather reliable since the results are about the same whether based upon the data for H_4, H_5, or H_{29}. The last has been most fully studied because it is far enough from other lines so it is very nearly lorentzian and need not be simulated for determination of the shift and broadening parameters.

The most likely systematic error in the present study is neglect of reactions such as

$$\mathrm{Li}^+ + \mathrm{BaX}^+ \rightleftharpoons \mathrm{Ba}^{2+} + \mathrm{LiX}$$

which can shorten the lifetime of the complex studied. However, if this reaction and its Na^+ analogue were important one would not expect the lifetimes in table 4 to be so nearly independent of whether LiX or NaX was used as the source of X^- in the solutions. Another class of reactions that has been neglected is represented by the reaction

$$\mathrm{PrX}_3 + \mathrm{Ba}^{2+} \underset{k_{13}}{\overset{k_{31}}{\rightleftharpoons}} \mathrm{PrX}_2^+ + \mathrm{BaX}^+ \tag{19}$$

If this reaction is important then the assumption $Y_{13} = Y_{31} = 0$ made in section 5 is not realistic; without this assumption the formulas for the shift and broadening become much more complicated. However, we may proceed in the following way. Using the known equilibrium constants [1,5] we find

$$Y_{31} \approx (10^{-5}\ \mathrm{M}) k_{13} [\mathrm{Ba}]/3(\mathrm{X}^-), \tag{20a}$$

$$Y_{13} \approx (10^{-5}\ \mathrm{M}) k_{13} [\mathrm{Pr}]/(\mathrm{X}^-), \tag{20b}$$

where we used the fact that in the solutions in which the rates were studied nearly all the Ba is present as BaX^+ and nearly all the Pr as PrX_3 and where [...] means stoichiometric concentration. We notice that k_{13} cannot exceed the diffusion controlled limit which is estimated to be about $2 \times 10^8\ \mathrm{s}^{-1}\ \mathrm{M}^{-1}$. Then under the conditions of these experiments Y_{31} is much less than $Y_{21} = 250 \times 10^3\ \mathrm{s}^{-1}$; the flow of spins from state 1 to state 3 is quite small compared to the flow from state 1 to state 2. Also Y_{13} is quite small compared to $Y_{23} = 8 \times 10^3\ \mathrm{s}^{-1}$; the flow of spins from state 3 goes almost completely to state 2. In view of these considerations the approximation $Y_{13} = Y_{31} = 0$ seems quite realistic.

Similar conclusions follow for the importance of the reaction

$$\mathrm{PrX}_3 + \mathrm{BaX}^+ = \mathrm{PrX}_4^- + \mathrm{Ba}^{2+}$$

in view of similar arguments. Moreover the neglect of the direct exchange of spins between states 1 and 3 seems equally justified for the other systems for which rates are determined here.

According to eq. (11) and the parameters determined in the present study one would expect to be able to measure lifetimes as small as 1 μs by the present method. However without further work it does not seem safe to conclude that the lifetimes of LiX and NaX are less than 1 μs; with results having the precision attainable in the present study it only is sure that they are less than 10 μs.

The sequences of lifetimes $\mathrm{CaX}^+ < \mathrm{SrX}^+ < \mathrm{BaX}^+$ and LiX, NaX < KX are contrary to what is expected on the basis of elementary considerations as well as comparison with some data for lifetimes of complexes with simple ligands [17]. However, the present sequences are what one might expect for complexes with a rigid multidentate ligand such as a crown ether for which binding strength as a function of cation size is expected to peak at the size that best fits into the cage presented by the ligand. In the case of a less rigid multidentate ligand such as X-537A such an effect is less expected, but is indeed found in the thermodynamic data: in hexane the stability constants increase in the sequence $\mathrm{MgX}_2 < \mathrm{CaX}_2 < \mathrm{SrX}_2 < \mathrm{BaX}_2$ [1] and presumably would decrease again if the series could be extended to large enough M^{2+}. On the other hand in methanol solution the metal ions in MX^+ are still relatively strongly solvated by the methanol [1], the X-537A can participate in hydrogen bonding with the solvent, and it seems quite likely that the observed trends in lifetimes indicate that the conformation of the ligand is different in different complexes, a suggestion that has other support [1,5].

7. Experiments with nickel shift reagent

The shift and broadening coefficients are now defined as in eqs. (3) and (4) but with [Ni] in place of 3[Pr]. Data for a number of systems in which NiX^+

Table 5
Shift coefficients $\bar{s}$ and relaxation coefficients $\bar{k}$ for various solutions in methanol, 25°C. NiX^+ as shift reagent [a)]

H_4		H_5		H_{29}		Stoichiometric concentration, mM			
$\bar{s}$	$\bar{k}$	$\bar{s}$	$\bar{k}$	$\bar{s}$	$\bar{k}$	HX	LiX	NaX	Other
411	283	1919	1450	192	152			39.65	
265	196	760	640	110	113	16.6	24.5		
213	247	709	1083	50	138			39.65	$Ba(ClO_4)_2$, 18.3
−30	97	−60	320	−98	57	17.7	16.7		$Ba(ClO_4)_2$, 18.9 [b)]

[a)] $\bar{s}$ in rad/s, $\bar{k}$ in s^{-1}.

[b)] There is no appreciable concentration of free X^- in this solution. The strange results suggest that for Ni as shift reagent, as for Pr as shift reagent (section 3.2), simple kinetic results cannot be obtained without some free X^- in the solutions.

rather then PrX_3 is the shift reagent, are reported in table 5. It is remarkable that the substitution of Li by Na makes such a big difference in both $\bar{k}$ and $\bar{s}$. Even more remarkable is the effect of added $Ba(ClO_4)_2$ which *reduces* both $\bar{k}$ and $\bar{s}$.

It may be recalled that Degani and Friedman determined the rate constant Y_{21} = 240/s for the reaction

$$NiX^+ \rightarrow Ni^{2+} + X^- \tag{21}$$

in methanol solution at 25°C. From the Swift-Connick equations, eqs. (8) with NiX^+ identified as state 1, we find in all generality:

$$\bar{k} \leqslant Y_{21} \quad \text{and} \quad \bar{s} < Y_{21}. \tag{22}$$

These conditions apparently are violated by many of the data in table 5 if Y_{21} = 240/s.

These contradictions and other strange features suggest that eq. (19) is not the only mechanistic step that contributes to Y_{21}, the rate constant for the transfer of X to state 2 from state 1. For example the process

$$NiX^+ + X^- \rightleftharpoons NiX_2 \tag{23}$$

also may contribute. It could be negligible in the systems investigated by Degani and Friedman but important in the systems in table 5 because in the former systems the equilibrium concentration (X^-) was at most 4 mM while in many of the systems in table 5 it is much larger. But other processes such as

$$NiX^+ + NaX \rightarrow \text{exchange} \tag{24}$$

also may contribute to Y_{21}. Bimolecular reactions such as those in eqs. (23) and (24) may be much more important than the corresponding processes for the systems in which praseodymium is used as the shift reagent because the unimolecular decomposition of NiX^+ is so slow (240/s) compared to the unimolecular decomposition of PrX_3 (750 000/s) (section 4).

An extensive investigation would be required to learn enough about the mechanisms in the nickel systems to use NiX^+ as a shift reagent for determining lifetimes of complexes in the way we were able to use PrX_3. On the other hand it may turn out at some stage that it is interesting to pursue the investigation of the systems in table 5 in order to learn more about reactions like those in eqs. (23) and (24).

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