(15) T. Glonek and J. R. Van Wazer, *J. Magn. Reson.*, **13**, 390 (1974).
(16) J. F. McCullough, J. R. Van Wazer, and E. J. Griffith, *J. Am. Chem. Soc.*, **78**, 4528 (1956).
(17) T. Glonek, J. R. Van Wazer, R. A. Kleps, and T. C. Myers, *Inorg. Chem.*, **13**, 2337 (1974).
(18) A. Abragam, "The Principles of Nuclear Magnetic Resonance", Oxford University Press, London, 1962.
(19) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance", McGraw-Hill, New York, N.Y., 1955.
(20) D. W. Aksnes, M. Rhodes, and J. G. Powles, *Mol. Phys.*, 14, 333 (1968).
(21) P. S. Hubbard, *Phys. Rev.*, **131**, 1155 (1963).
(22) W. E. Morgan and J. R. Van Wazer, *J. Am. Chem. Soc.*, **97**, 6347 (1975).
(23) See eq 24′ on p 274 of A. Abragam, "The Principles of Nuclear Magnetism", Clarendon Press, Oxford, 1961. In this equation $A = \mathcal{H}(t) = -\nu_n h \Sigma_q H_q(t) I_q$.
(24) M. M. Crutchfield, C. F. Callis, R. R. Irani, and G. C. Roth, *Inorg. Chem.*, **1**, 813 (1962).
(25) R. R. Irani and C. F. Callis, *J. Phys. Chem.*, **65**, 934 (1961).
(26) T. Glonek, R. A. Kleps, E. J. Griffith, and T. C. Myers, *Phosphorus*, **5**, (1975).
(27) T. Glonek and J. R. Van Wazer, *J. Phys. Chem.*, **80**, 639 (1976).
(28) G. T. Rossetti and K. E. Van Holde, *Biochem. Biophys. Res. Commun.*, **26**, 717 (1967).
(29) A. Szent-Gyorgi, "Bioenergetics", Academic Press, New York, N.Y., 1957.

Ion Binding to Nucleosides. A ^{35}Cl and ^{7}Li NMR Study

Albert C. Plaush[1a] and Robert R. Sharp*[1b]

Contribution from the Departments of Chemistry, Saginaw Valley State College, University Center, Michigan 48710, and University of Michigan, Ann Arbor, Michigan 48109. *Received January 23, 1976*

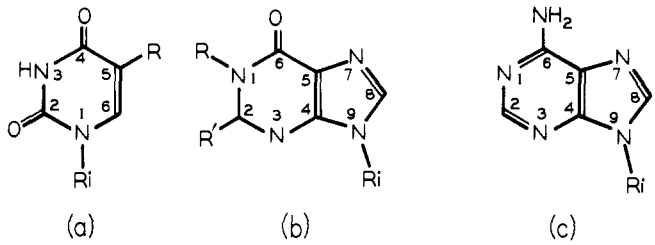
Abstract: Large variations in ^{7}Li and ^{35}Cl nuclear magnetic relaxation times are observed when nucleosides, bases, and ribose are added to LiCl solutions in DMSO. The molar shortening of T_1 is very sensitive to the presence of specific functional groups in the solutes studied and is shown to reflect binding by Cl^- and Li^+ to specific sites on the solutes. Binding to guanosine, 1-methylguanosine, inosine, adenosine, uridine, *d*-thymidine, cytidine, adenine, thymine, uracil, cytosine, and ribose has been studied. Three binding sites have been found: (1) Cl^- binds strongly to the (N_1–H, C_2–NH_2) region of guanosine, and more weakly to the corresponding regions of its analogues, 1-methylguanosine and inosine; (2) Li^+ binds to the N_3 site of cytidine and cytosine hindering rotation of the adjacent amino group; (3) LiCl binds, probably as an ion pair, to the furanose ring of all ribosides studied; *d*-thymidine shows no evidence of this binding in the ion relaxation data. Uracil, thymine, and adenine show no evidence of binding in the T_1 data although proton resonances of NH and NH_2 groups in these bases are chemically shifted. In all other cases proton chemical shifts generally confirm inferences drawn from relaxation data. Relations between the equilibrium constants, relaxation rates, and total concentrations have been derived and used to estimate the association constant and relaxation rate for ^{7}Li in the ribose site.

A substantial body of experimental evidence has shown that divalent metal ions bind to nucleosides, nucleotides, and nucleic acids (this subject has been reviewed by Izatt et al.[2a] and Phillips[2b]). The binding of many metal ions to phosphate esters of nucleotides and nucleic acids has been extensively documented by ^{31}P NMR, potentiometry, and IR and Raman spectroscopy. NMR (^{1}H and ^{15}N) and potentiometric measurements have also shown that certain divalent metals (e.g., Cu^{2+} [3]) bind to specific sites on purine and pyrimidine bases. Paramagnetic ions produce particularly striking effects on resonances of protons near the binding site. These resonances are selectively broadened or shifted by through-space dipolar couplings.[3,4] Diamagnetic ions generally produce much smaller NMR effects except at NH and OH groups that are directly involved in coordination to the ions. Protons in these groups are not visible in NMR spectra of aqueous solutions due to rapid exchange with the solvent protons but can be observed in solvents that have no exchangeable protons, such as DMSO.

Several studies of the shifts induced in ribonucleosides by group 2A and group 2B chlorides in DMSO solutions have been reported.[5-13] All of these salts selectively shift certain NH, OH, and NH_2 proton resonances downfield by 0.2–0.5 ppm, and the shifts have been used to infer sites of ion binding. In solutions of the group 2A chlorides substantial shifts have been observed at (1) the ribose hydroxyl protons of inosine and adenosine;[5] (2) at the C_4–NH_2 location on the pyrimidine ring of cytidine,[6,13] at which position ion association causes a splitting of the amino protons; (3) at the (N_1–H, C_2–NH_2) positions of the purine base of guanosine[7,9,14] and at the corresponding site in inosine.[5] Numbering of the rings is shown in Figure 1. The group 2B chlorides act differently in certain respects.[5,7,11-13] In particular, Hg(II) and Zn(II) appear to bind at the N-7 position of guanosine and the C_6–NH_2 position of adenosine (where the group 2A chlorides cause small if any chemical shift), while $ZnCl_2$ and $CdCl_2$ produce no shifts in the ribose hydroxyl protons. Nearly all of these concentration-dependent shifts have been attributed to site-specific binding by the divalent metal cations.

Quite recently, Chang and Marzilli[14] have argued that the downfield shifts observed in the base protons of guanosine are more probably caused by binding of the chloride anion rather than the metal cation. To support this view they have shown that the shift is essentially independent of the cation but varies markedly in solutions of different halides and falls to zero for nitrates and perchlorates. Thus for at least one purine binding site the anion, rather than the cation, is responsible for the proton shifts. In our view, previous interpretations are subject to further ambiguity because of the possibility of complex ion formation. The stability of stepwise halide complexes of the group 2B elements and of many divalent transition metal ions is well known in aqueous media.[15] Although data are relatively scarce, halide complexes of even greater stability seem to be the rule in DMSO solution.[16,17] These complexes raise the possibility that interactions reflected in the chemical shift data involve various species, both charged and uncharged. Ion binding as monitored by ^{1}H and ^{13}C chemical shifts has been shown to be sensitive to both anion and cation in a complicated way,[13] quite possibly due to the influence of complex equilibria.

In the present study we have used relaxation time measurements (T_1) of the ^{7}Li and ^{35}Cl isotopes as an ion-specific



(a) (b) (c)

Figure 1. Nucleosides: (a) uridine (R = H) and thymidine (R = CH_3 and deoxyribose sugar); (b) guanosine (R = H, R′ = NH_2), inosine (R = H, R′ = H), and 1-methylguanosine (R = CH_3, R′ = NH_2); (c) adenosine.

probe of binding in LiCl/DMSO solutions. Binding of the two nuclei to various bases, ribosides, one deoxyribonucleoside (*d*-thymidine), and ribose, has been studied. LiCl offers the great advantage of participating in only a single ion-association equilibrium, which limits to three the species that can be involved in binding to the nucleoside. The equilibrium can be treated quantitatively using an association constant previously inferred by Dunnett and Gasser[18] from activity measurements. The ^{7}Li and ^{35}Cl resonances provide independent cation and anion binding probes and thus permit an assessment of the binding affinity of a univalent metal cation. Alkali metals have not previously been reported to bind nucleosides, although an association with the phosphate esters of nucleotides and nucleic acids has been inferred from a study of Donnan equilibria.[19]

The technique of using halogen relaxation times to monitor halide binding to heavy metals was developed by Hertz[20] and has since found wide application in the study of metalloproteins.[21-33] Nuclei such as ^{35}Cl and ^{7}Li possess nuclear quadrupole moments and are relaxed by interactions between the quadrupole moment and electric field gradients arising from asymmetry in the surrounding electronic charge distribution. When the nucleus is located in a chemical bond with quadrupole coupling constant (e^2qQ/h), the relaxation rate is[34]

$$\frac{1}{T_1} = \frac{1}{T_2} = \frac{3}{40}\frac{2I+3}{I^2(2I-1)}(e^2qQ/h)^2\tau_c$$

where τ_c is the rotational correlation time of the chemical bond (the extreme narrowing limit and cylindrical bond symmetry are assumed). An unassociated ion in aqueous or DMSO solution is very symmetrically solvated. Consequently electric field gradients, which vanish in cubic or higher symmetry, are typically two orders of magnitude or more smaller for simple ions than for nuclei in covalent bonds. Thus T_1 for ^{35}Cl is about four orders of magnitude longer for unassociated chloride ion than for ^{35}Cl in complexes such as $HgCl_4^{2-}$ [33] or in covalent tetrahalides.[35] Rapid exchange of Cl^- between free solution and an asymmetric bound environment results in a relaxation rate that is a weighted average of the rates in the two sites:

$$(T_1)^{-1}_{obsd} = \frac{f_A}{T_{1A}} + \frac{f_B}{T_{1B}}$$

where $f_{A,B}$ are fractional site populations. According to this relation, a very small bound fraction in a site of very short T_{1B} can dominate $(T_1)^{-1}_{obsd}$ of a much larger pool of free ions. Baldeschwieler[21] has called this effect "chemical amplification".

Thus electric quadrupole relaxation of ion resonances provides a very sensitive probe of binding in electrolyte solutions. However, it is necessary to distinguish T_1 shortening associated with ion binding to specific chemical sites from shortening due to viscosity changes or to relatively nonspecific interactions with the solute that may simply reflect lower average symmetry of the solution environment. A modest concentration dependence of the relaxation time does not of itself imply chemical binding at specific sites, as has been shown by Hertz and Holz[36]

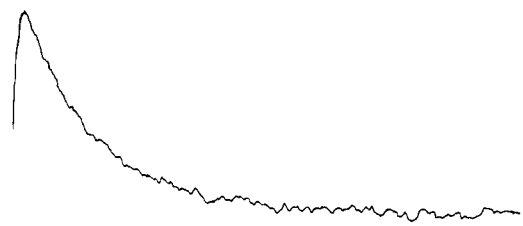


Figure 2. Typical decay of ^{35}Cl in 0.5 M LiCl (four scans) from the automatically stepped 180°-τ-90° sequence described in the text.

who demonstrated that ^{79}Br T_1's are strongly affected by nonspecific interactions with the hydrophobic regions of amphipolar solutes. Binding may, however, be inferred from a comparative study of similar ligands. In the present experiments, which involve six nucleosides, four bases, one deoxyribonucleoside (*d*-thymidine), and ribose, we have observed large chemically specific effects on the relaxation of ^{7}Li and ^{35}Cl. Effects of various ligands differ by an order of magnitude, and the chemical specificity of binding is qualitatively different for Li^+ and Cl^-. The largest effect is seen for ^{35}Cl in guanosine solutions, where the T_1^{-1} increases by a factor of 30 per (M^{-1}) of ligand. The smallest effect occurs for several bases, for which the molar increase in $(T_1)^{-1}$ is approximately fourfold. Fractional changes in the ^{7}Li T_1's are much smaller than for ^{35}Cl and range between 25 and 400% for different ligands. These ligands still have effects that differ among themselves by an order of magnitude, however, and clearly indicate that chemically specific interactions are involved. By comparing ^{7}Li and ^{35}Cl relaxation data with chemical shift data of the ligand protons, we have inferred an internally consistent picture of the LiCl binding sites.

Experimental Section

Samples were prepared from reagent grade DMSO (Baker analyzed reagent) that had been dried for 24 h over Linde 3A molecular sieves. Nucleosides, bases, and ribose were obtained from Sigma Chemical Co., checked by NMR, and used without further purification or drying. Reagent grade LiCl (J. T. Baker Co.) was dried for several hours prior to use in a vacuum oven. The solid reagents were weighed directly in the sample tubes. Known aliquots of DMSO were transferred to the tubes in a drybox under nitrogen atmosphere and the tubes were then sealed. Each of the samples is very nearly 0.5 M in LiCl. Precise concentrations quoted in the tables and figures are given in molalities. Viscosities were measured at 25.00 ± 0.05% C using Ostwald viscometers. The viscosities, relative to zero ligand concentration, were fitted to a polynomial of the form $\eta_r = 1 + c_1m + c_2m^2$ by linear regression analysis, and the smoothed values were used to correct the T_1 data.

Relaxation times were measured on a Bruker B-KR 322S variable frequency pulsed spectrometer using an external NMR field lock and data sampling system described elsewhere.[37,38] All measurements were made at 25 ± 1 °C. T_1 for ^{7}Li was measured by a modified triplet sequence,[37] 90_0-t-$(90_0$-180_π-$90_0)_n$. T_1 for ^{35}Cl was obtained by the inversion-recovery sequence, 180°-τ-90°, using a modification of a procedure described elsewhere.[39] τ is automatically stepped along the decay in 100 increments up to a maximum delay of about $5T_1$. The F.I.D. following the 90° pulse is sampled, integrated, and signal averaged in a CAT. A typical decay on 0.5 M LiCl is shown in Figure 2. The relaxation data plotted in the figures are an average of the three to five separate measurements. Uncertainty in T_1 is about 3% for ^{7}Li and 6% for ^{35}Cl.

Proton spectra were obtained using a Varian T-60A MHz spectrometer. Chemical shifts were measured relative to the DMSO peak as internal standard.

Results and Discussion

A detailed analysis of relaxation appropriate to the present experiments is given in the Appendix. Analytical expressions for $T^{-1}_{1,obsd}$ are derived as a function of total added concentration, N_T, of nucleoside, ribose, or base at constant total LiCl.

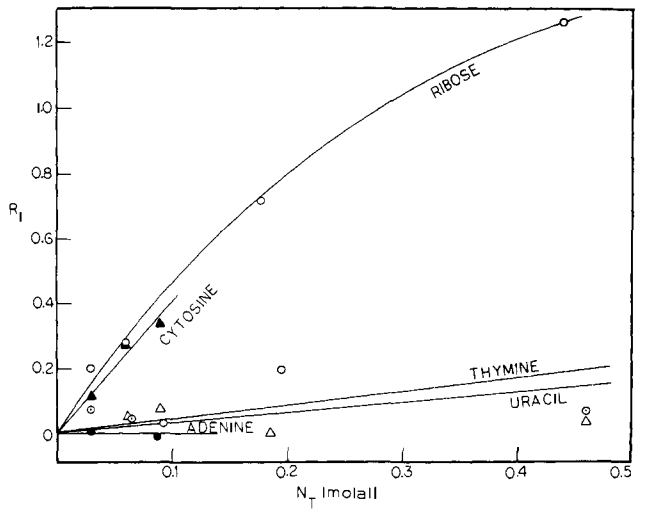


Figure 3. Incremental relaxation rates of ^{7}Li in LiCl/DMSO solutions of adenine, various pyrimidine bases, and ribose.

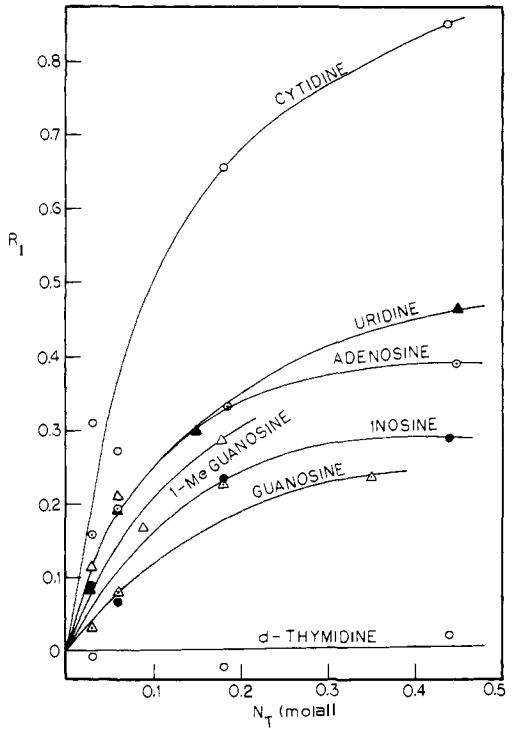


Figure 4. Incremental relaxation rates of ^{7}Li in LiCl/DMSO solutions of various purine and pyrimidine nucleosides.

Simultaneous equilibria of the type

$$Li^+ + Cl^- = LiCl$$

followed by one of the reactions

$$Li^+ + N = LiN^+$$

$$Cl^- + N = ClN^-$$

or

$$LiCl + N = LiClN$$

are considered. The relaxation data are expressed as the fractional increment in relaxation rate, R_1, after correction for changes in the relative viscosity,

$$R_1 \equiv [(T_1\eta_r)^{-1}/(T_{1,0})^{-1}] - 1$$

$(T_{1,0})^{-1}$ is the relaxation rate at fixed concentration of LiCl (0.458 *m* in the present experiments) in the absence of added ligand. The concentration dependence of R_1 has been written as a power series in N_T, and the series was truncated after the quadratic term:

$$R_1 = (T_{1,0})^{-1}\{\alpha N_T + \beta N_T^2\}$$

Expressions for α and β in terms of equilibrium constants and the total added concentrations of solutes are given in eq A-10 and A-11 of the Appendix.

Table I. Relaxation Data of ^{7}Li and ^{35}Cl in DMSO Solutions of Nucleosides, Bases, and Ribose[a]

Solute in 0.458 *m* LiCl/DMSO	Concn, *m*	η_r	^{35}Cl T_1 (av), ms	^{35}Cl R_1	^{7}Li T_1 (av), s	^{7}Li R_1
Adenine	0.029	1.023	1.23	0.256	3.84	0.009
	0.059	1.050	1.02	0.475		
	0.087	1.078	0.886	0.655	3.72	−0.009
Cytosine	0.030	1.025	1.51	0.021	3.46	0.119
	0.061	1.053	1.19	0.261	2.94	0.282
	0.091	1.082	1.04	0.405	2.74	0.339
Thymine	0.031	1.023	1.42	0.087	3.58	0.082
	0.066	1.049	1.15	0.302	3.61	0.048
	0.093	1.068	1.02	0.450	3.60	0.033
	0.195	1.136	0.724	0.920	2.91	0.201
	0.464	1.287	0.344	2.57	2.87	0.073
Uracil	0.032	1.028	1.53	0.005		
	0.062	1.053	1.11	0.351	3.56	0.058
	0.090	1.076	1.06	0.385	3.41	0.081
	0.185	1.142	0.730	0.895	3.46	0.004
	0.462	1.260	0.410	2.06	3.03	0.040
Ribose	0.028	1.023	1.04	0.485	3.22	0.202
	0.058	1.049	0.822	0.832	2.96	0.279
	0.178	1.155	0.449	2.05	2.00	0.718
	0.441	1.414	0.246	3.54	1.24	1.26
Adenosine	0.032	1.059	1.03	0.448	3.22	0.160
	0.060	1.113	0.794	0.788	2.98	0.194
	0.185	1.377	0.351	2.27	2.19	0.303
	0.448	2.057	0.148	4.19	1.35	0.391
Cytidine	0.032	1.059	1.00	0.492	2.86	0.311
	0.060	1.114	0.708	1.00	2.80	0.272
	0.182	1.384	0.339	2.37	1.73	0.657
	0.438	2.118	0.104	6.17	1.01	0.855
Guanosine	0.030	1.059	0.707	1.11	3.63	0.031
	0.062	1.126	0.408	2.44	3.26	0.082
	0.182	1.412	0.184	5.08	2.28	0.234
	0.357	1.933	0.096	7.51	1.66	0.236
1-Methyl-guanosine	0.027	1.053	1.02	0.471	3.38	0.115
	0.060	1.121	0.652	1.16	2.92	0.212
	0.090	1.187	0.489	1.72	2.86	0.168
	0.178	1.402	0.298	2.78	2.20	0.285
Inosine	0.032	1.056	0.818	0.825	3.44	0.090
	0.060	1.108	0.565	1.52	3.36	0.067
	0.182	1.363	0.247	4.16	2.36	0.234
	0.441	2.065	0.086	7.90	1.49	0.289
Thymidine	0.030	1.051	1.32	0.140	3.81	−0.010
	0.062	1.105	0.940	0.522		
	0.178	1.303	0.510	1.38	3.12	−0.023
	0.441	1.766	0.282	2.18	2.20	0.023
Uridine	0.032	1.052	0.937	0.603	3.47	0.086
	0.060	1.099	0.782	0.838	3.03	0.191
	0.148	1.256	0.441	1.85	2.42	0.304
	0.448	1.885	0.155	4.40	1.43	0.465
LiCl	0.458	1.000	1.58	0.0	3.97	0.0

[a] Relative viscosities are given with respect to the 0.458 *m* LiCl/DMSO reference solution.

(A) Li^+ Binding to Nucleosides and Bases. The T_1 data for ^{7}Li are plotted in Figures 3 and 4 and are recorded in Table I. As pointed out above, the different solutes have effects on the relaxation rate that differ by an order of magnitude. The smallest effect is observed for three of the bases studied, thymine, uracil, and adenine, which produce about a 35% increase

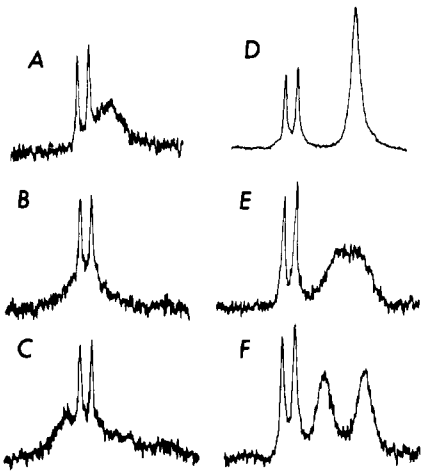
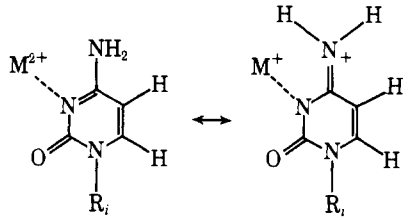


Figure 5. The H_6 and NH_2 resonances of cytosine and cytidine in DMSO in the presence and absence of LiCl: (A) 0.1 M cytosine, 18 °C; (B) 0.1 M cytosine + 0.5 M LiCl, 35 °C; (C) as in (B), but 18 °C; (D) 0.5 M cytidine, 18 °C; (E) 0.5 M cytidine + 0.5 M LiCl, 35 °C; (F) as in (E), but 18 °C.

in T_1^{-1} in a 0.45 *m* solution. This effect is accounted for entirely by viscosity changes in the media, since R_1, the viscosity corrected increment to the relaxation rate, is constant within experimental error. Thus, the T_1 data indicate that Li^+ does not bind to specific chemical sites on these bases. It is noteworthy that although the ^7Li T_1's do not in themselves suggest specific binding, proton spectra of all three bases (see Table II) do show significant downfield shifts in the NH and NH_2 resonances. These shifts appear not to reflect cation binding, however, and will be discussed further below.

The fourth base studied, cytosine, shows an anomalously large effect on R_1, for which the limiting slope at low concentration is approximately 3.41 m^{-1}. Similarly, cytidine has a substantially larger effect on R_1 than do the other ribosides, which produce similar effects that are intermediate between cytidine and the A-T-U "baseline". These data support the existence of a Li^+ binding site on the cytosine ring. Effects of Li^+ binding are also clearly apparent in the proton spectra from the substantial shift of H_5 ($\Delta(\delta)$ = 0.20 ppm) and from the large temperature dependent splitting of the amino protons at C_4. Figure 5 shows the spectral region about H_6 and C_4-NH_2 for cytosine and cytidine at different temperatures. In the presence of 0.5 M LiCl, the NH_2 resonance of cytidine splits into two peaks due to hindered rotation about the C_4-N bond (Figure 5D-F). The amino resonance of cytosine is also shifted by 0.5 M LiCl and at 18 °C shows an asymmetry that may be related to incipient doublet structure (Figure 5A-C). Yokono et al.[6] have observed similar behavior but with somewhat larger chemical shifts in DMSO solutions of cytidine with added Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} chlorides. Li and co-workers[8,11] have also observed the effect with $HgCl_2$ (but not with $ZnCl_2$) and attributed the splitting to stabilization of the imino resonance structure by the metal ion:



Li^+ is the first univalent cation observed to bind cytidine at position N_3 and evidently forms a complex sufficiently long lived at 18 °C to prevent coalescence of the amino protons. The amino protons do coalesce at higher temperature and are in the intermediate exchange region at 38 °C. At this temperature, the mean interval between rotations about the C_4-N bond can be computed from the relation[40]

$$\tau^{-1} = \frac{2\pi}{2^{1/2}} |\nu_A - \nu_B|$$

where $|\nu_A - \nu_B|$ = 24 Hz is the difference in chemical shift of the two amino protons in resonance structure B. τ is computed to be approximately 9.3 ms at 38 °C.

The remaining ribosides (adenosine, inosine, guanosine, 1-methylguanosine, and uridine) also exert large effects on R_1 of ^7Li (Figure 4). Since the various bases examined, with the exception of cytosine, show no relaxation effects attributable to binding, the R_1 increments of the nucleosides are most readily explained by Li^+ binding to the ribose moiety. Ribose itself has an even larger effect on R_1 than do the ribosides in the intermediate group in Figure 4. At first sight this may be surprising since ribose has about half the molecular volume and consequently half the rotational correlation time of the nucleosides. Thus for equal binding affinity, ribose should have about half the effect on R_1 as do the nucleosides. The relatively large effect of ribose probably results from the furanose $\rightleftharpoons$ aldose equilibrium of the free sugar which is not present for the ribosides.

Binding sites at the ribose hydroxyl protons have previously been inferred from proton chemical shifts in DMSO solutions of the group 2A metal chlorides.[5] Proton spectra of the LiCl solutions also show large shifts of the hydroxyl protons as indicated in Table II. Overlapping of resonances is serious at 60 MHz, however, and we have indicated these effects in only a qualitative manner. As a rough indication of magnitude, the

Table II. ^1H NMR Shifts of Bases and Nucleosides in 0.458 *m* LiCl/DMSO[a]

Solute	δ	
	Base moiety	Ribose moiety[b]
Adenine	0.00 (H_2), 0.00 (H_8), 0.10 ± 0.05 (C_6-NH_2)	
Adenosine	0.00 (H_2), 0.05 (H_8), 0.02 (C_6-NH_2)	+
Cytosine	0.20 (H_5), 0.04 (H_6)	
Cytidine	0.10 (H_5), 0.00 (H_6)	+
Guanosine	0.37 (H_1), 0.43 (C_2-NH_2) 0.00 (H_8)	+
1-Methylguanosine	0.10 (C_2-NH_2), 0.00 (H_8)	+
Inosine	0.17 ± 0.05 (N_1-H), 0.00 (H_2), 0.00 (H_8)	+
Thymine	0.00 (H_6), 0.10 (N_3-H)[c]	
Thymidine	0.06 (H_6), 0.03 (N_3-H)	+
Uracil	0.15 (N_3-H), 0.00 (H_5), 0.00 (H_6)	
Uridine	0.00 ± 0.04 (N_3-H), 0.00 (H_5), 0.00 (H_6)	+

[a] Chemical shifts are accurate to ±0.02 ppm unless otherwise specified and are uniformly downfield with respect to salt-free solutions. [b] A substantial downfield shift in the ribose hydroxyl region is indicated by +. [c] See text.

shifts span a range of 0.17–0.27 ppm downfield in 0.5 M LiCl. Thus the proton shifts and ^{7}Li T_1's consistently indicate a Li^+ binding site on ribose.

In order to locate the specific functional groups responsible for cation binding to ribose we have examined one deoxynucleoside, *d*-thymidine. Here the absence of a hydroxyl group in the 2′ position suppresses the ligand effect on R_1 and eliminates all evidence of cation binding. Evidently, the 2′-hydroxyl group is involved in Li^+ binding, but consideration of both the ^{35}Cl and ^{7}Li data led us to conclude that cation binding directly to the hydroxyl oxygen probably does not occur. We will return to the nature of the binding site and bound species below.

(B) Cl^- Binding to Nucleosides and Bases. Figures 6 and 7 show R_1 vs. N_T for the ^{35}Cl resonance in the LiCl solutions studied. As was the case for ^{7}Li, the smallest effect is seen for the four bases, three of which have virtually identical slopes. Thymine, uracil, and cytosine produce about a fourfold increase in the relaxation rate, after application of viscosity corrections, per mole liter^{-1} of ligand concentration. Adenine appears to produce a somewhat larger molar increment in R_1 than do C-T-U, although data on this base are limited to concentrations below 0.1 *m*. The question of whether these relaxation increments reflect site-specific ion binding or simply a lowering of the average solvation symmetry of the Cl^- ion is difficult to resolve with the present data and is discussed further in the following section.

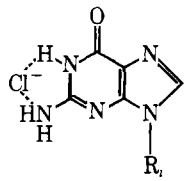
The nucleosides exhibit a range of effects on the ^{35}Cl R_1, varying from a 30-fold molar shortening for guanosine down to an effect for *d*-thymidine that is no larger than the effects produced by C-T-A-U. Here the various ligands do not cluster into easily identifiable classes as was true for Li^+. However, the relaxation data are consistent with the following binding scheme:

(i) Guanosine, inosine, and to a lesser extent 1-methylguanosine bind Cl^- through hydrogen bonded interactions at N_1-H and C_2-NH_2 sites on the purine rings.

(ii) Cl^-, in the form of a LiCl ion pair, binds to the furanose moiety of all nucleosides. The deoxyribonucleoside *d*-thymidine shows no evidence of this interaction.

(iii) The cytidine-Li^+ complex has some affinity for Cl^- binding through simple ionic association.

A strong interaction of Cl^- with the guanine ring has been proposed by Marzilli and Chang[14] on the basis of proton chemical shifts. They suggested that anions bind through hydrogen-bonded interactions to N_1-H and C_2-NH_2:



Guanosine produces a dramatic shortening of the ^{35}Cl R_1 but produces no specific effect on the ^{7}Li R_1. Thus our data confirm the suggestion that guanine binds only the anion. The stability of the hydrogen-bonded complex is attested both by the large proton shifts of N_1-H and NH_2 (see Table II) and by the magnitude of the effect on the ^{35}Cl R_1.

We have examined two analogues of guanosine in which the N_1-H and C_2-NH_2 sites are selectively blocked in order to determine whether the two hydrogen-bonding groups are independently able to bind Cl^-. The N_1-H position is blocked in 1-methylguanosine, and the data in Figure 7 show that the effect of this riboside on R_1 is considerably reduced relative to guanosine. Some residual binding to the NH_2 group probably remains, however, since the absolute effect of 1-methylguanosine on R_1 for ^{35}Cl remains significantly larger than that for uridine and adenosine, to which Cl^- probably binds through the ribose but only weakly if at all through the base (vide infra).

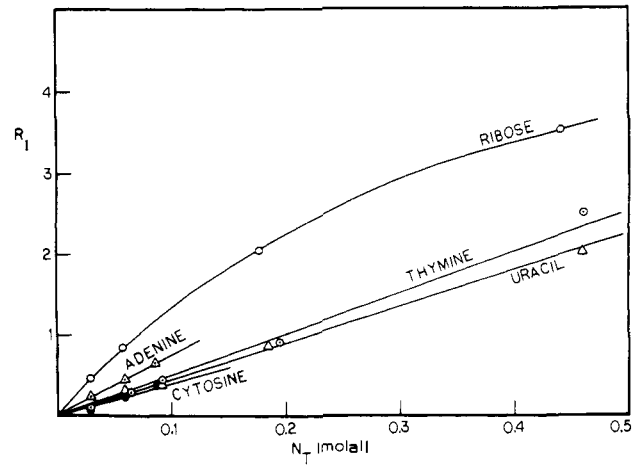


Figure 6. Incremental relaxation rates of ^{35}Cl in LiCl/DMSO solutions of adenine (▲), thymine (⊙), uracil (△), cytosine (●), and ribose (○).

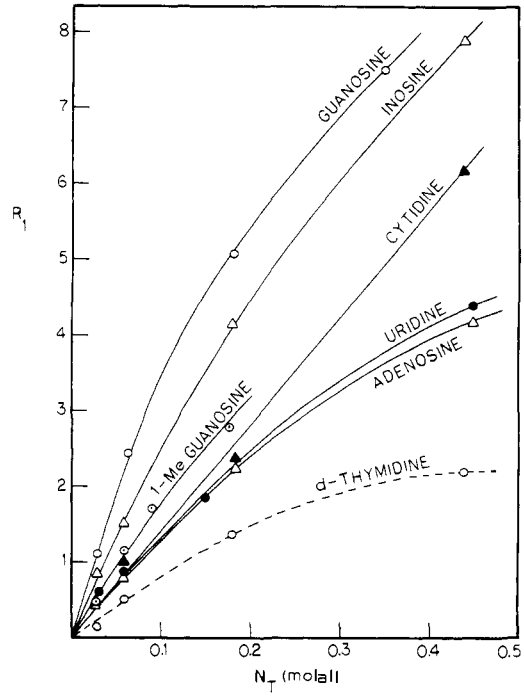


Figure 7. Incremental relaxation rates of ^{35}Cl in LiCl/DMSO solutions of various purines and pyrimidine nucleosides.

Also, the NH_2 protons still experience a modest downfield shift of 0.1 ppm (Table II).

Inosine is a guanosine analogue that lacks an amino group at the 2 position. This riboside exerts a substantially larger effect on R_1 for ^{35}Cl than does any other nucleoside except guanosine, which indicates that fairly strong binding at the N_1-H site remains when the C_2 position is blocked. The N_1-H proton experiences a downfield shift of 0.2 ppm in 0.5 M LiCl consistent with binding at this site, although the much larger shift of the same proton in guanosine indicates a substantial difference in binding strength. Sohma et al.[5] observed shifts and broadening of the N_1-H proton in DMSO solutions of all the group 2A and 2B chlorides. They attributed these perturbations to the binding of divalent metals, but our results show clearly that a hydrogen-bonded interaction to Cl^- is involved. Inosine exerts no specific effect on the ^{7}Li resonance (other than effects attributable to ribose binding), and the magnitude of its effect on the ^{35}Cl and ^{1}H resonances is entirely consistent with the presence of the Cl^- binding site at N_1-H.

An examination of Figure 7 shows that three other nucleo-

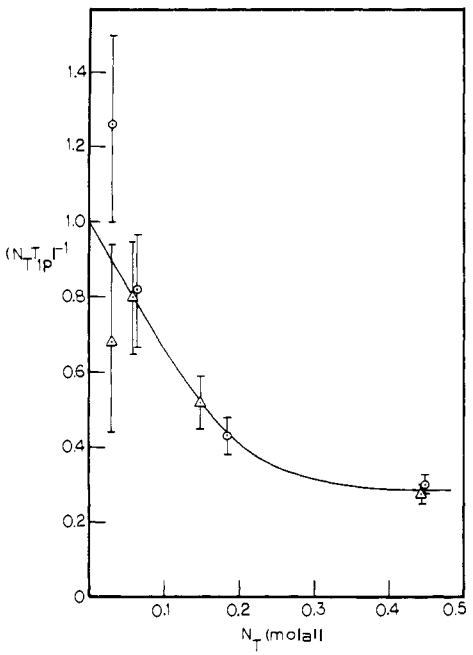


Figure 8. Plots of $(N_T T_{1p})^{-1}$ vs. N_T for uridine (△) and adenosine (○). The slope and intercept of these plots determine the LiCl binding constant and ^{7}Li relaxation rate in the ribose site as discussed in the text.

sides (adenosine, uridine, and cytidine) that do not possess potent hydrogen bonding sites to Cl^- on the base moiety produce essentially the same effect on R_1 over a concentration range of 0–0.2 *m*. Above 0.2 *m* uridine and adenosine remain very similar, while the relative effect of cytidine increases. Similarly, up to 0.2 *m* (the limit of solubility of cytosine), the corresponding bases, uracil, adenine, and cytosine, produce a relatively small effect on R_1 that is essentially equal for all three bases. This systematic difference in effects of the ribonucleosides and their corresponding bases is consistent with an interaction of Cl^- with the furanose ring rather than with sites on the bases. In support of this hypothesis, we note that proton spectra of these compounds also show substantial shifts in the ribose hydroxyl region. Furthermore, ribose itself produces a considerably larger increment in R_1 for ^{35}Cl than do any of the bases.

Deoxythymidine exerts a relatively small effect on the ^{35}Cl R_1 compared to the ribosides. Obviously, this difference results from the absence of the 2′-hydroxyl group and it provides a further indication of the involvement of the ribose moiety in anion binding. The same pronounced difference in behavior between *d*-thymidine and the ribosides was discussed above for the ^{7}Li resonance. It is a striking fact that the modification of a single functional group lessens the affinity of ribose for both Li^+ and Cl^- and does so to the same relative degree for both ions (that is, the effect of thymidine is reduced to that of the base thymine). It is highly unlikely that a single hydroxyl directly participates as an independent binding site for both anions and cations. Thus the similarity suggests that an ion pair may be involved. A reasonable structure for the binding site would have Cl^- hydrogen bonded to 2′-OH and/or 3′-OH and Li^+ coordinated to the furanose ring oxygen. INDO charge density calculations[41] on the purine nucleosides indicate that this oxygen bears a negative charge of approximately 0.3 e^-, which makes it a reasonable site for cation binding. Hydrogen bonding of Cl^- to ribose hydroxyl groups is also consistent with the previously described affinity of Cl^- for hydrogen-bonding sites on guanosine and its analogues. Significant downfield shifts of 0.16–0.28 ppm occur in the hydroxyl protons (Table II), and although it is often difficult to resolve and assign the $OH_{2'}$, $OH_{3'}$, and $OH_{5'}$ resonances at 60 MHz, it is clear in certain cases (uridine and cytidine) that almost all of the shift occurs in peaks corresponding to two of the protons. It is tempting to conclude that these represent the $OH_{2'}$ and $OH_{3'}$ protons, but a careful study of the proton shifts at higher fields than we have used would be required for confident assignments of the protons involved.

As noted above, the three ribosides cytidine, adenosine, and uridine produce the same relaxation increment at concentrations of 0.2 M and below, but at the highest concentration (0.5 M) R_1 for cytidine increases significantly relative to the other two ligands. This increase may reflect additional binding by Cl^- to the pyrimidine ring. Cytidine is known to bind Li^+ very strongly in contrast to all the other bases, and the positively charged Li^+-cytidine complex could have an affinity for Cl^-.

Summary and Conclusions

A summary of binding sites inferred from the T_1 and proton chemical shift data is given below:

(i) Li^+ binds to the N_3 position of cytidine and cytosine and hinders rotation of the adjacent amino group.

(ii) Cl^- binds to the (C_2-NH_2, N_1-H) region of guanosine, to the C_3-NH_2 site of 1-methylguanosine, and to the N_1-H site of inosine.

(iii) LiCl binds to a pair of sites on the ribose moiety of all ribonucleosides examined. *d*-Thymidine, a deoxyribonucleoside, shows no evidence of this binding in the ion relaxation data.

(iv) Cl^- may, at high concentrations, bind to the N-3 region of the Li^+-cytidine complex.

The main area of uncertainty lies in the interpretation of the proton shifts of the bases adenine, uracil, and thymine. The N_3-H resonances of uracil and thymine are shifted downfield by about 0.1 ppm, and adenine exhibits a similar shift in the C_6-NH_2 resonance. These shifts are reduced or absent in the corresponding nucleosides. It is very unlikely that the shifts arise from Li^+ binding, since these bases cause no discernible change in R_1 for ^{7}Li. The shifts could arise from weak Cl^- binding and if so would explain the relatively small relaxation increments of these bases in Figure 6. On the other hand, composition-dependent shifts in the tautomer equilibria could be involved. In support of this possibility, we note that the N_3-H resonance of thymine, which is broad and structured in the absence of LiCl, becomes a sharp, slightly shifted singlet in salt solution as would be consistent with rate enhancement of a tautomer equilibrium. Thus our present data do not permit an unambiguous conclusion concerning the possibility of weak anion binding to uracil, thymine, and adenine.

***K*$_2$ and T_1^{-1} for ^{7}Li in the Furanose Binding Site.** Plots of R_1 vs. N_T for the ^{35}Cl and ^{7}Li data (Figures 4 and 7) show pronounced curvature tending toward zero slope at high values of N_T. The curvature is expected when a large fraction of the ions are bound and is consistent with the negative value of β given in the Appendix. In principle, the slope β and intercept α of a plot of $(N_T T_{1p})^{-1}$ vs. N_T can be used to compute the association constant K_2 for ion binding as well as T_1^{-1} in the bound site, $(T_{1e})^{-1}$, through eq A-7,8,10,11. The concentration dependence of T_{1p}^{-1} is not well defined by the data, but rough estimates of K_2 and T_{1e} can be obtained. Binding of ^{7}Li to uridine and adenosine is considered, since these nucleosides possess only a single ion binding site. Figure 8 contains an appropriate plot of the data and gives the approximate values $\alpha = 1.0\ m^{-1}\ s^{-1}$ and $\beta = 3.5\ m^{-2}\ s^{-1}$. Equations A-7,8 for the binding of an ion pair are used in conjunction with Dunnett and Gasser's[18] value, $K_1 = 5.3\ m^{-1}$, for the ion association constant of LiCl in DMSO. Activity coefficients for the ions are also taken from graphical interpolations of the data in Dunnett and Gasser's paper. Their measured stoichiometric activity coefficients, $\gamma_\pm$, were converted to mean ion activity coefficients,

$f_{\pm}$, using methods described by Davies.[42] Equations A-10,11 of the Appendix then give the following values for $(1/T_{1e})$ and K_2: $K_2 = 7 \pm 3\ m^{-1}$ and $(T_{1e}) = 1.0 \pm 0.5$ s. The value T_{1e} is given in the limit where $\eta_r = 1$.

The computed association constant and relaxation rate are only estimates since they depend on the poorly defined slope in Figure 7. Nevertheless, the magnitudes of both quantities are reasonable in terms of the expected binding strength of LiCl to the ribose moiety. The association constant is quite similar to the value of 5.9 M^{-1} obtained by Kan and Li[8] for ion binding to the purine sites of guanosine in $HgCl_2$ solution.

Appendix

Previous uses of the halide binding probe experiment have been based on measurements of T_2, either indirectly from line widths in CW experiments, or in one instance[33] involving $^{78,81}Br$, from the free induction decay in a pulsed spectrometer. T_1 is measured in the present experiments. In the fast exchange limit, T_1 is determined by a weighted average of relaxation rates in the various environments,

$$\frac{1}{T_1} = \sum_i \frac{f_i}{T_i} \qquad \text{(A-1)}$$

Chemical shifts between bound and free sites can produce large effects on T_2 when the shift $(2\pi\delta)$ and inverse lifetime of the bound ion are comparable.[20] T_1 is independent of chemical shifts, which provides an important advantage in studies of weak binding where the difference between relaxation rates in different sites is not necessarily large.

In the present experiments the total chloride concentration, $[Cl_T] = [LiCl] + [Cl^-]$, is maintained constant and the ligand concentration, N_T (N = nucleoside, ribose, or base concentration), is varied. At constant $[Cl_T]$ the concentrations of ionic species remain approximately constant, and to first approximation the slow variation of activity coefficients with concentration of uncharged solutes can be neglected. The following equilibria are considered:

$$Li^+ + Cl^- = LiCl$$

$$K_1 = [LiCl]/[Li^+][Cl^-]f_{\pm}^2$$

followed by $X + N = XN$

$$K_2 = [XN]/[X][N]$$

where X = Li^+, Cl^-, or LiCl. (Activity coefficients are assumed to cancel in the expression for K_2.) The bound fractions in eq A-1 can be calculated as a function of $[Cl_T]$, N_T, K_1, and K_2 in the following way. From the expression for K_2 and the mass balance for N, we have

$$K_2[X] = [XN]/(N_T - [XN]) \qquad \text{(A-2)}$$

[X] is expressed as a power series in [XN],

$$[X] = A + B[XN] + C[XN]^2 + \ldots \qquad \text{(A-3)}$$

using the first equilibrium expression and the mass balance for X. Substituting (A-2) into (A-3) and retaining terms to second order in [XN], eq A-3 can be solved to give

$$[XN] = \frac{-(N_TB - A - K_2^{-1}) - \{(N_TB - A - K_2^{-1})^2 - 4N_TA(N_TC - B)\}^{1/2}}{2(N_TC - B)}$$

A. B. and C are functions only of $[Cl_T]$ and K_1 and thus are constant at constant $[Cl_T]$. We seek a solution for [XN] in the form of a power series in N_T. Such a series can be obtained by expanding both the square root and the denominator as power series in N_T. Retaining terms only to the quadratic in the final result gives

$$f_b = \frac{[XN]}{[Cl_T]} = \frac{N_T}{[Cl_T]}\left\{\frac{A}{(A + K_2^{-1})} + \left[\frac{AB}{(A + K_2^{-1})^2} + \frac{A^2B}{(A + K_2^{-1})^3}\right]N_T\right\} \qquad \text{(A-4)}$$

This expression is independent of the constant C in the quadratic term in eq A-3 so that explicit expressions need be derived only for A and B. The expansion A-3 can be obtained from the mass balance equation for X, the expression defining K_1, and the equation of charge balance (to be specific we let $X = Cl^-$),

$$[Cl^-] = [Cl_T] - [LiCl] - [ClN^-]$$

$$[LiCl] = f_{\pm}^2K_1[Cl^-]\{[Cl^-] + [ClN^-]\}$$

Eliminating [LiCl], these expressions can be solved for $[Cl^-]$ using the quadratic formula, and the resulting square root expanded as a power series in $[ClN^-]$. The final result is

$$A = [Cl^-]_{N_T=0} = \frac{1}{2K_1'}\{-1 + (4K_1'[Cl_T] + 1)^{1/2}\} \qquad \text{(A-5)}$$

$$B = -\tfrac{1}{2}\{1 + (4K_1'[Cl_T] + 1)^{1/2}\} \qquad \text{(A-6)}$$

where $K_1' = f_{\pm}^2K_1$. The binding of Li^+ to N leads to identical expressions. LiCl binding is described by different constants,

$$A' = [LiCl]_{N_T=0} = [Cl_T] + \frac{1}{2K_1}(1 - [1 + 4K_1'[Cl_T]]^{1/2}) \qquad \text{(A-7)}$$

$$B' = -1 + (1 + 4K_1'[Cl_T])^{-1/2} \qquad \text{(A-8)}$$

Having expressed the bound fraction f_B as a power series in N_T, we can compute the two unknowns, K_2 and T_{1b}, directly from a linear plot of the relaxation rate vs. N_T. The most convenient form for plotting the relaxation data (corrected for viscosity) is obtained by subtracting the background relaxation rate:

$$T_{1p}^{-1} \equiv (T_1\eta_r)^{-1} - T_{1,0}^{-1} = \frac{\Delta f^{Cl}}{T_{1A}} + \frac{\Delta f^{LiCl}}{T_{1B}} + \frac{f^{ClN}}{T_{1C}} \cong f^{ClN}\left\{\frac{1}{T_{1C}} + \frac{B}{T_{1A}} + \frac{B'}{T_{1B}}\right\} \qquad \text{(A-9)}$$

T_{1A}, T_{1B}, and T_{1C} are relaxation times in Cl^-, LiCl and LiClN, respectively, in the limit $\eta_r = 1$. The latter two terms in braces are normally much smaller than $1/T_{1C}$ and can be neglected, but to preserve generality we retain all three terms and refer to the quantity in braces as $1/T_{1e}$. The bound fraction, f^{ClN}, is given by eq A-4 in conjunction with (A-5,6) or (A-7,8). Dividing eq A-9 by N_T, it is evident that at low concentration $(N_TT_{1p})^{-1}$ is linear in N_T with an intercept

$$\alpha = \frac{A}{[Cl_T](A + K_2^{-1})}(1/T_{1e}) \qquad \text{(A-10)}$$

and slope

$$\beta = \alpha\left\{\frac{B}{A + K_2^{-1}} + \frac{AB}{(A + K_2^{-1})^2}\right\} \qquad \text{(A-11)}$$

These plots uniquely determine K_2 and $(1/T_{1e})$ when K_1 and $\gamma_{\pm}^2$ are known.

References and Notes

(1) (a) Saginaw Valley State College; (b) University of Michigan.
(2) (a) R. M. Izatt, J. J. Christensen, and J. H. Rytting, *Chem. Rev.*, **71**, 439 (1971); (b) R. Phillips, *ibid.*, **66**, 501 (1966).
(3) G. L. Eichhorn, P. Clark, and E. D. Becker, *Biochemistry*, **5**, 245 (1966).
(4) C. D. Barry et al., *Nature (London)*, **232**, 236 (1971).

(5) J. Sohma, S. Shimokawa, and K. Hotta, "Recent Development of Magnetic Resonance in Biological Systems", S. Fujiwara and L. H. Piette, Ed., Hirokawa Publishing Co., Tokyo, Japan, 1968, p 57.
(6) T. Yokono, S. Shimokawa, H. Fukui, and J. Sohma, *Nippon Kagaku Zasshi*, **2**, 201 (1973).
(7) S. Shimokawa, H. Fukui, J. Sohma, and K. Hotta, *J. Am. Chem. Soc.*, **95**, 1777 (1973).
(8) L. S. Kan and N. C. Li, *J. Am. Chem. Soc.*, **92**, 4823 (1970).
(9) F. Jordan and B. Y. McFarquhar, *J. Am. Chem. Soc.*, **94**, 6557 (1972).
(10) J. A. Happe and M. Morales, *J. Am. Chem. Soc.*, **88**, 2077 (1966).
(11) S. M. Wang and N. C. Li, *J. Am. Chem. Soc.*, **90**, 5069 (1968).
(12) L. S. Kan and N. C. Li, *J. Am. Chem. Soc.*, **92**, 281 (1970).
(13) T. Yokono, S. Shimokawa, and J. Sohma, *J. Am. Chem. Soc.*, **97**, 3827 (1975).
(14) C. H. Chang and L. G. Marzilli, *J. Am. Chem. Soc.*, **96**, 3656 (1974).
(15) L. Sillén, Ed., *Chem. Soc. Spec. Publ.*, **No. 17** (1964).
(16) N. A. Rambaut and H. L. Peeters, *Bull. Soc. Chim. Belg.*, **76**, 33 (1967).
(17) D. C. Luehrs and K. Abate, *J. Inorg. Nucl. Chem.*, **30**, 549 (1968).
(18) J. S. Dunnett and R. P. H. Gasser, *Trans. Faraday Soc.*, **61**, 922 (1965).
(19) U. P. Strauss, C. Helfgott, and H. Pink, *J. Phys. Chem.*, **71**, 2550 (1967).
(20) H. G. Hertz, *Z. Elektrochem.*, **65**, 36 (1961).
(21) T. R. Stengle and J. D. Baldeschwieler, *Proc. Natl. Acad. Sci. U.S.A.*, **55**, 1020 (1966).
(22) R. P. Haugland, L. Stryer, T. R. Stengle, and J. D. Baldeschwieler, *Biochemistry*, **6**, 498 (1967).
(23) G. Gillberg-LaForce and S. Forsen, *Biochem. Biophys. Res. Commun.*, **38**, 137 (1970).
(24) M. Zeppezauer, B. Lindman, S. Forsen, and I. Lindqvist, *Biochem. Biophys. Res. Commun.*, **37**, 137 (1969).
(25) J. L. Sudmeier and J. J. Pesek, *Anal. Biochem.*, **41**, 39 (1971).
(26) R. G. Bryant, *J. Am. Chem. Soc.*, **91**, 976 (1969).
(27) R. G. Bryant, *J. Am. Chem. Soc.*, **89**, 2496 (1967).
(28) H. Csopak, B. Lindman, and H. Lilja, *FEBS Lett.*, **9**, 189 (1970).
(29) A. G. Marshall, *Biochemistry* **7**, 2450 (1968).
(30) C. F. Springgate, A. S. Mildvan, R. Abramson, J. L. Engle, and L. A. Loeb, *J. Biol. Chem.*, **248**, 5987 (1973).
(31) W. D. Ellis, H. B. Dunford, and J. S. Martin, *Can. J. Biochem.*, **47**, 157 (1969).
(32) R. G. Bryant, *Biochem. Biophys. Res. Commun.*, **37**, 603 (1969).
(33) T. R. Collins, Z. Starcuk, A. H. Burr, and E. J. Wells, *J. Am. Chem. Soc.*, **95**, 1649 (1973).
(34) A. Abragam, "The Principles of Nuclear Magnetism", Oxford, London, 1961, p 314.
(35) K. J. Johnson, J. P. Hunt, and H. W. Dodgen, *J. Chem. Phys.*, **51**, 4493 (1969).
(36) H. G. Hertz and M. Holz, *J. Phys. Chem.*, **78**, 1002 (1974).
(37) R. R. Sharp, *J. Chem. Phys.*, **57**, 5322 (1972).
(38) R. M. Hawk, R. R. Sharp, and J. W. Tolan, *Rev. Sci. Instrum.*, **45**, 96 (1974).
(39) R. R. Sharp and J. W. Tolan, *J. Chem. Phys.*, in press.
(40) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance", McGraw-Hill, New York, N.Y., 1959, Chapter 10.
(41) S. Kang, *Jerusalem Symp. Quantum Chem. Biochem.*, **5**, 271 (1973).
(42) C. W. Davies, "Ion Association", Butterworths, Washington, D.C., 1962, Chapter 3.

Molecular Rydberg Transitions. 3. A Linear Combination of Rydberg Orbitals (LCRO) Model for the Two-Chromophoric System 2,2,4,4-Tetramethylcyclobutane-1,3-dione (TMCBD)[1]

P. Brint, K. Wittel, P. Hochmann, W. S. Felps, and S. P. McGlynn*

Contribution from the Coates Chemical Laboratories, The Louisiana State University, Baton Rouge, Louisiana 70803. *Received March 19, 1976*

Abstract: The Rydberg absorption spectra of acetone and 2,2,4,4-tetramethylcyclobutane-1,3-dione (TMCBD) have been obtained and analyzed. TMCBD is treated as a composite of two acetone molecules and its Rydberg spectrum is analyzed by comparison with that of acetone. In order to retain the categorization of molecular Rydberg levels as s, p, d, . . . (or, equivalently, to retain the assignment power of empirical quantum defect ranges), we are forced to develop a linear combination of Rydberg orbitals (LCRO) model. The LCRO model leads to a facile analysis of the TMCBD spectrum, whereas a one-center model, in which the Rydberg orbital is positioned on the inversion center of TMCBD, does not. The implications of the LCRO model with respect to Rydberg intensities, precursor orthogonality requirements, charge delocalization in Rydberg states, the completeness or incompleteness of Rydberg series, and the empirical content of the s, p, d, . . . labeling are discussed briefly.

Introduction

A major goal of molecular spectroscopy is the transcription of chemical relatedness onto the spectroscopic data. An outstanding example of this approach is furnished by photoelectron spectroscopy, where a large amount of data (i.e., ionization potentials) has been correlated by using the molecular orbital approximation in varying degrees of sophistication.[2] The MO method, bolstered, as required, by configuration interaction, can also account for most of the valence excitations observed in electronic spectroscopy. Extravalence or "Rydberg" excitations, on the other hand, have been discussed, almost exclusively on the basis of the Rydberg equation

$$h\nu = I - [R/(n + \delta)^2] \qquad (1)$$

where $h\nu$ is the energy of the Rydberg transition; I is the ionization energy to which the Rydberg series of interest converges; R is the Rydberg constant; δ is the so-called quantum defect; and n is a serial index, $n = 1, 2, 3, \ldots$, which identifies individual members of a given series. This equation has been used mostly for correlating different electronic transitions within one atom or molecule.[3a] Recently, however, eq 1 has also been applied to the correlation of related transitions within different molecules.[3]

The present work represents an attempt to carry the idea of intermolecular correlation somewhat further. Specifically, we view 2,2,4,4-tetramethylcyclobutane-1,3-dione (TMCBD) as a composite of two acetone moieties and attempt an interpretation of the vacuum uv spectrum of the former by exploiting fully the knowledge available for the vacuum uv spectrum of the latter. Although this approach is not totally alien to vacuum uv spectroscopy,[4,5] it does seem appropriate to review some traditional ideas about Rydberg states before presentation of the model.

Acetone and TMCBD are shown in Figure 1. If acetone be denoted A, it does not seem inappropriate to denote TMCBD as A_LA_R, where the subscripts L/R denote left/right, respectively. If the association $A \leftrightarrow A_LA_R$ is a valid one with respect to the acetone $\leftrightarrow$ TMCBD dyad, it follows that the vacuum uv spectrum of TMCBD should exhibit some sort of