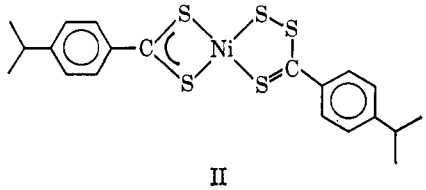


II

$k_2 = 0.91 \pm 0.03\ M^{-1}\ \text{sec}^{-1}$ at 300°K. The activation energy, 7.8 ± 1.0 kcal/mol, and entropy of activation, $\Delta S^{\pm} = -37 \pm 2$ eu, are consistent with a mechanism which involves kinetically controlled attack by the phosphine on the sulfur-rich complex, presumably at the sulfur atom of the disulfide linkage which is adjacent to the carbon. It has been established that this sulfur atom is the one removed[7] by triphenylphosphine from the sulfur-rich nickel(II) dithiobenzoate derivative in boiling $CHCl_3$. The sulfur abstraction from the analogous palladium(II) complex with triphenylphosphine is too rapid to be studied by the spectroscopic technique used here to study the nickel(II) derivative. Similarly the abstraction from ZnL_2S_2, where L = *p*-dithiocumate, is also too fast for this technique.

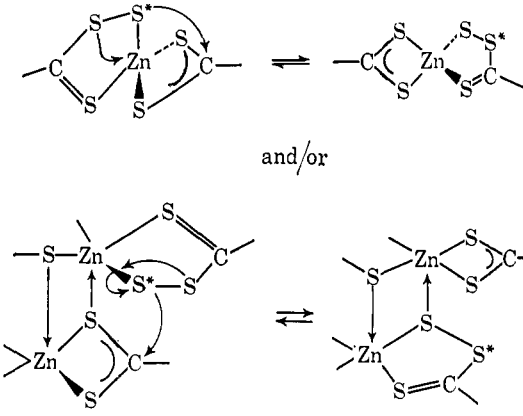
The kinetic lability of sulfur atoms in the zinc(II) sulfur-rich dithiolates is apparent from nuclear magnetic resonance studies comparing sulfur-rich dithiocumates[8] of zinc(II), palladium(II), platinum(II), and nickel(II) with a ML_2S stoichiometry. Figure 1 presents the proton magnetic resonance of these species in the aromatic region. The doubled AB pattern observed for the Ni(II), Pd(II), and Pt(II) species is the anticipated result for complexes containing two distinctly different ligands (II). The chemical shift difference observed in the Zn(II) complex for the H_a and H_b protons as a function of total sulfur content (Figure 1, A, B, and C) and the absence of splitting implicates a *sulfur atom exchange*[9] which makes the aromatic rings magnetically equivalent on the nmr time scale. To test this, solutions of ZnL_2S in 1:1 (v/v) CS_2:$(C_2H_5)_2O$ were cooled to −116° (Figure 2) at which temperature the doubled AB pattern associated with nonequvalent aromatic rings was observed.[9] Preliminary studies of this behavior as a function of temperature and the concentration of ZnL_2S have indicated an activation energy of ~5 kcal/mol for the exchange process. The reaction does not show a simple kinetic dependence on the concentration of complex, however. Extrapolation of the data to room temperature with $\sim 10^{-2}$ *M* concentration of ZnL_2S in the CS_2–ether solvent suggests a lifetime of $\sim 10^{-5}$ sec for a disulfide bond.

(7) See accompanying communication: Sulfur Chelates. X. J. P. Fackler, Jr., J. A. Fetchin, and J. A. Smith, *J. Amer. Chem. Soc.*, **92**, 2910 (1970).

(8) *Anal.* Calcd for $NiC_{20}H_{22}S_5$: C, 49.90; H, 4.61. Found: C, 49.75; H, 4.60. Calcd for $ZnC_{20}H_{22}S_5$: C, 46.18; H, 4.26. Found: C, 45.41; H, 4.67. Calcd for $ZnC_{20}H_{22}S_4$: C, 52.68; H, 4.86. Found: C, 53.01; H, 5.55. The ZnL_2S solutions are obtained from equimolar amounts of ZnL_2S_2 and ZnL_2. Calcd for $PdC_{20}H_{22}S_5$: C, 45.40; H, 4.19. Found: C, 45.81; H, 4.24. Calcd for $PtC_{20}H_{22}S_5$: C, 38.88; H, 3.59. Found: C, 38.61; H, 3.58.

(9) The chemical shift difference between the two room temperature aromatic ring AB doublets of the ZnL_2S_x is a linear function of x over the complete range from $x = 0$ to $x = 2$. This establishes the fact that the averaging process involves added sulfur. Furthermore, the isopropyl methyl protons show a splitting in the nickel, palladium, and platinum ML_2S species, which is not observed with ZnL_2S. If x is different from 1 for ZnL_2S_x, the intensities of the four peaks centered near 8 ppm at −116° (Figure 2) reflect the unequal concentrations of trithioperoxy and dithiocumate ligands.

Intra- or intermolecular sulfur atom exchange leads directly to the sulfur atom scrambling results observed mass spectrophotometrically with the sulfur-rich zinc(II) dithiobenzoate.[7] The nmr results described here can reflect a sulfur atom exchange and scrambling by path ssimilar to III. Also, exchange



III

between the complex and "free" sulfur may produce the nmr results without causing sulfur atom scrambling.[10] These various possibilities will be considered thoroughly in a full report of this work.[11] However, it is clear from the results reported here that sulfur atom exchange occurs with considerable speed in these zinc(II) dithiolates.

(10) The sulfur atom exchange process occurring rapidly in the ZnL_2S system but not detected by nmr at room temperature with nickel, palladium, or platinum is consistent with ^{35}S atom exchange data reported by I. V. Khodzhoeva and Yu. V. Kissen, *Russ. J. Phys. Chem.*, **37**, 412 (1963). These authors report an activation energy for the sulfur atom exchange in some metal diethyldithiocarbamates to be lowest with zinc(II). However, their estimated activation energy is larger than we observe for the nmr process by about a factor of 4. The origin of this difference, if real, has not been established but clearly bond rupture must be accompanied by bond formation in any mechanism suggested. Since C–S and S–S bond energies are similar (~60–65 kcal/mol) a mechanism involving concerted S–S bond rupture with C–S bond formation could account for the data provided no net change in metal–sulfur bonding occurs. Such mechanisms may be written, but since detailed molecular geometries are presently unknown with sulfur-rich zinc complexes, we feel such speculation is presently unwarranted. Furthermore, the concentration dependence of the process studied by nmr does not permit a direct interpretation of the activation energy observed. In fact, sulfur atom exchange without position scrambling could lead to the nmr results. A rapid secondary process could produce sulfur atom scrambling.[7]

(11) We acknowledge the support of the National Institutes of Health, Grant No. AM-13558-01, and the NSF, Grant No. GP-11701, for this work.

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Stereospecificity of the Enzymic Synthesis of the *o*-Xylene Ring of Riboflavin

Sir:

The biochemical conversion of two molecules of 6,7-dimethyl-8-(1′-D-ribityl)lumazine (**1**) to riboflavin (**2**) and 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine (**3**) proceeds by the donation of a 4-carbon moiety from one lumazine (**1**, donor) to the 6- and 7-methyl groups of a second substrate molecule (**1**, acceptor) with the accompanying loss of two hydrogens from each of the

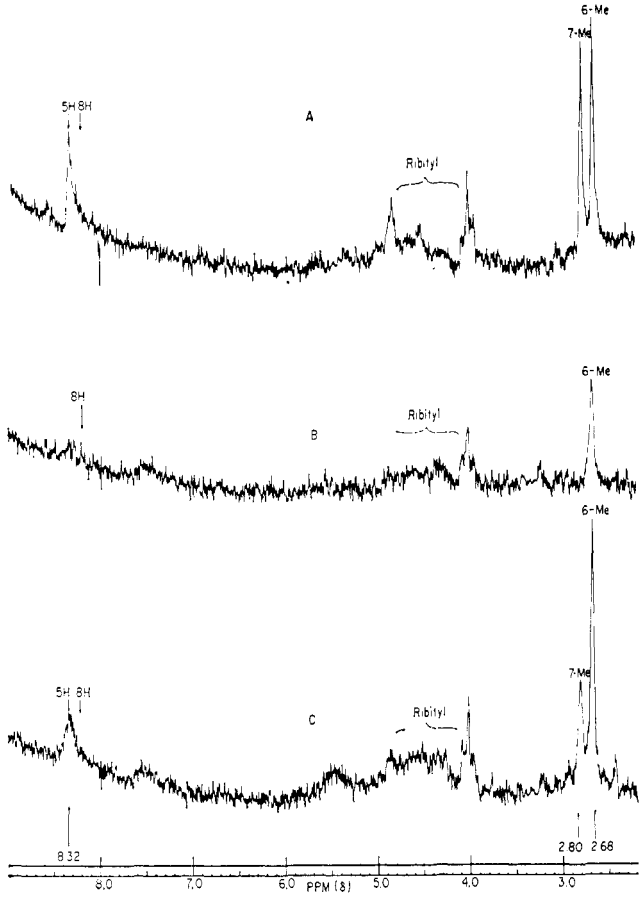
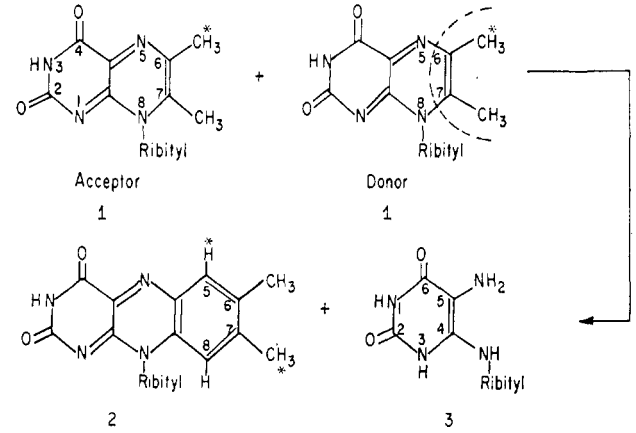


Figure 1. Nuclear magnetic resonance spectra of unlabeled and deuterium-labeled riboflavin. The nmr spectra were recorded in an HA 100 spectrometer using trimethylsilane as the locking signal and assigned a chemical sift of 0.00 ppm. The sweep width is 1000 Hz: A, 12.5 mg of riboflavin/ml of trifluoroacetic acid; B, 12.5 mg of deuterated riboflavin/ml of trifluoroacetic acid; C, 12.5 mg of riboflavin + 12.5 mg of deuterated riboflavin/ml of trifluoroacetic acid. Deuterium-labeled riboflavin was formed from 6-deuteriomethyl-7-methyl-8-(1′-D-ribityl)lumazine[7b] in the presence of purified yeast riboflavin synthetase.[4]

methyl groups of the acceptor molecule (see Scheme I).[1-3]

Scheme I



Recent studies have established that in the enzymic conversion of the lumazine to riboflavin, hydrogen elimination from the 7-methyl precedes that from the 6-methyl group of the acceptor lumazine.[4]

Paterson and Wood[5] have shown by nmr spectroscopy that the *chemical* synthesis of riboflavin[6ab], from 6-methyl-7-deuteriomethyl-8-(1′-D-ribityl)lumazine, formed *in situ* from the unlabeled compound in D_2O, results in formation of riboflavin containing deuterium in positions 8 and the 6-methyl group of the *o*-xylene ring. They suggested that formation of the aromatic ring occurs by condensation of the 6- and 7-methyl groups of the lumazine acceptor (**1**, acceptor) with carbon atoms 7 and 6, respectively, of the donor molecule (**1**, donor).

The same stereospecific mode of transfer of the 4-carbon moiety occurs *enzymically*, demonstrated here by the conversion of 6-deuteriomethyl-7-methyl-8-(1′-D-ribityl)lumazine to form riboflavin (**2**) containing deuterium at carbon 5 and the 7-methyl group (Figure 1). 6-Deuteriomethyl-7-methyl-8-(1′-D-ribityl)lumazine was chosen for the present study since, in contrast to the 7-deuteriomethyl analog, isotope dilution due to exchange with solvent protium does not occur.[7a,b]

The group assignments and chemical shifts for unlabeled riboflavin (Figure 1A) observed here (6-methyl at 2.68, 7-methyl at 2.80, 5-H at 8.32, and 8-H at 8.23 ppm) are in accord with the nmr spectra of riboflavin 5-phosphate originally determined by Bullock and Jardetsky[8] and confirmed for riboflavin.[5] The absorption at 2.80 and 8.32 ppm is absent from deuterated riboflavin formed enzymically from 6-deuteriomethyl-7-methyl-8-(1′-D-ribityl)lumazine (Figure 1B), indicating that the 7-methyl group and carbon 5 are substituted with deuterium. Absorption at these positions appears again in a mixture of equal amounts of the deuterated and nonlabeled riboflavin (Figure 1C). The absorption at 2.80 ppm is now one-half that at 2.68 ppm; this is expected, since the mixture should contain equal quantities of deuterium and protium at the 7-methyl group of riboflavin, while only protium substituents should be present at the 6-methyl group.

Two mechanisms have been proposed recently to explain the chemical conversion of 6,7-dimethyl-8-(1′-D-ribityl)lumazine to riboflavin.[5,7a,b] Both proposals have abandoned the initial ring opening followed by an aldol condensation originally suggested by Rowan and Wood,[6a,b] since this reaction sequence is unlikely under certain chemical conditions (*e.g.*, acid media)[7a,b] and for the enzyme-catalyzed transformation.[3] The mechanism advanced to explain the present results (Figure 2) is similar to that proposed previously for the enzymic[4] conversion of 6,7-dimethyl-8-(1′-D-ribityl)-lumazine to riboflavin, but differs in that the 6-methyl group of the lumazine (**1**) becomes the 7-methyl group and carbon 5 of riboflavin (**2**), respectively. In contrast to the proposal of Paterson and Wood,[5] this mechanism avoids carbonyl intermediates, since previous attempts to trap such compounds in the enzyme-catalyzed reaction were unsuccessful.[3] Binding of the substrate in Figure 2 is visualized to occur at two sites, one leading to donation of a 4-carbon unit (donor site)

(1) (a) G. W. E. Plaut, *J. Biol. Chem.*, **235**, PC 41 (1960); (b) G. W. E. Plaut, *ibid.*, **238**, 2225 (1963).
(2) H. Wacker, R. A. Harvey, C. H. Winestock, and G. W. E. Plaut, *ibid.*, **239**, 3493 (1964).
(3) R. A. Harvey and G. W. E. Plaut, *ibid.*, **241**, 2120 (1966).
(4) G. W. E. Plaut, R. L. Beach, and T. Aogaichi, *Biochemistry*, **9**, 771 (1970).
(5) T. Paterson and H. C. S. Wood, *Chem. Commun.*, 290 (1969).
(6) (a) T. Rowan and H. C. S. Wood, *Proc. Chem. Soc.*, 21 (1963); (b) *J. Chem. Soc., C*, 452 (1968).
(7) (a) R. L. Beach and G. W. E. Plaut, *Tetrahedron Lett.*, **40**, 3489 (1969); (b) R. L. Beach and G. W. E. Plaut, *Biochemistry*, **9**, 760 (1970).
(8) F. Bullock and O. Jardetsky, *J. Org. Chem.*, **30**, 2056 (1965).

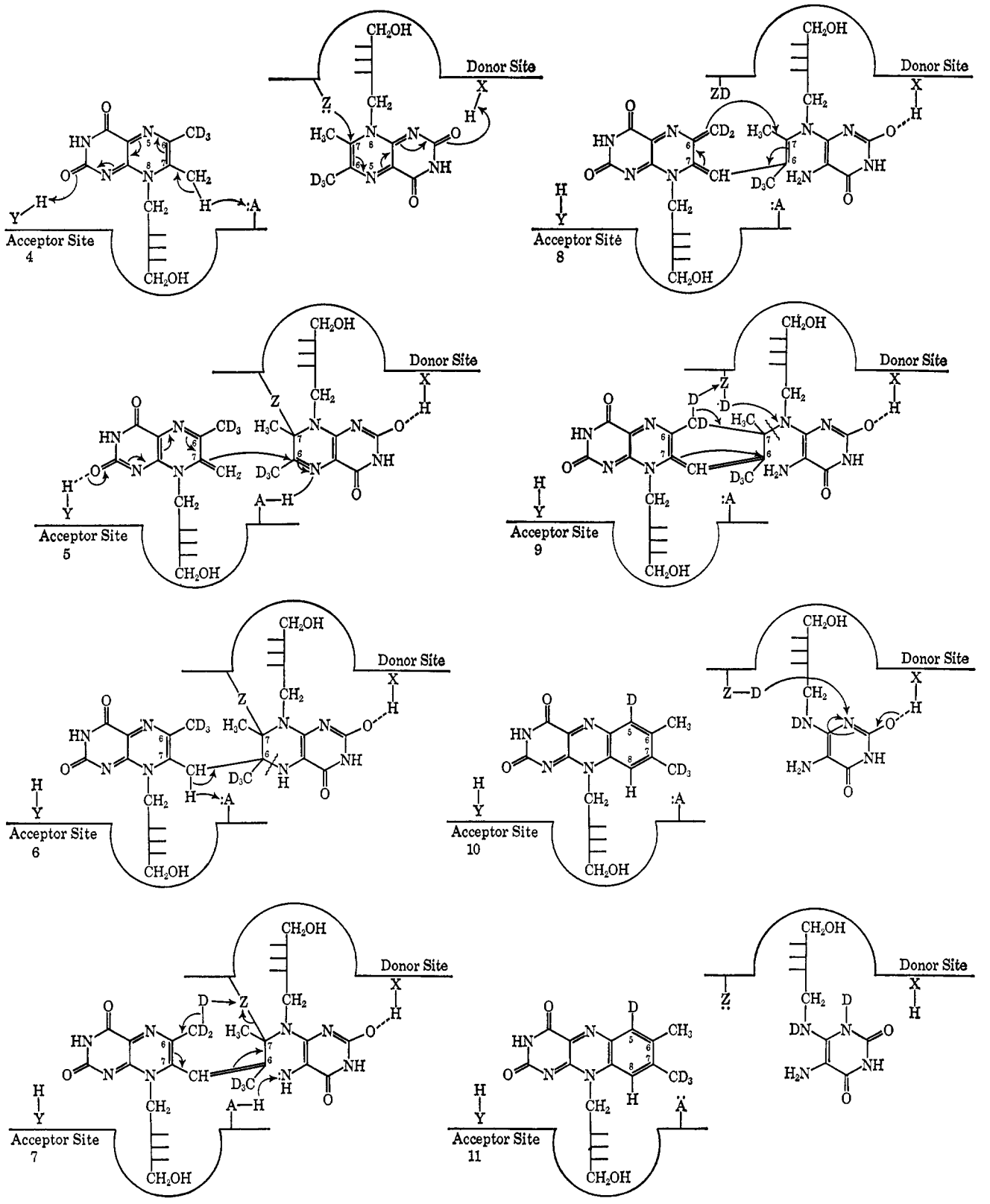


Figure 2. Proposed mechanism of riboflavin synthetase.

and the other to its acceptance (acceptor site).[3] The sterically exact attachment of the ribityl groups[9] to the protein is represented in the scheme in Figure 2 by the indentations accommodating these functions. Groups A: and Z: are proton acceptors which may be sulfhydryl[3,4] and/or imidazole groups on the enzyme. A close spatial alignment of the lumazines at the 4-carbon donor and acceptor sites has been indicated in the diagram as a possible explanation of the 50–100-fold higher substrate concentration needed for chemical formation of riboflavin at 100°[6a,b] than for enzymic synthesis at 37°.[3]

(9) C. H. Winestock, T. Aogaichi, and G. W. E. Plaut, *J. Biol. Chem.*, **238**, 2866 (1963).

The first step of the reaction (**4**) involves the removal of a proton from the 7-methyl group of the lumazine at the acceptor site by group A:. The developing carbonium center at carbon 7 of the lumazine at the donor site is stabilized simultaneously by interaction with group Z: on the enzyme. The methylene developed at the acceptor site (**5**) leads to an attack at carbon 6 of the lumazine at the donor site causing localization of the electron pair followed by protonation of nitrogen 5 by A-H. Proton removal from carbon 7 of the acceptor molecule by A: (**6**) causing rupture of the covalent bond between nitrogen 5 and carbon 6 at the donor site is followed by loss of a deuteron from the 6-deuteriomethyl group (**7**), resulting in a tautomeric shift and cleavage of the covalent bond between carbon 7 and the group Z. Cyclization occurs by attack of the electron pair of the 6-deuteriomethylene at the carbonium center (carbon 7) accompanied by a tautomeric shift of electrons (**8**). Elimination of a second deuteron from the 6-deuteriomethyl group of the acceptor site (**9**) and cleavage of the covalent bond between nitrogen 8 and carbon 7 at the donor site leads to formation of 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine and riboflavin (**10** and **11**) labeled with deuterium in the positions expected from the experimental results, *i.e.*, carbon 5 and the 7-methyl group. The enzyme is regenerated and can catalyze another transformation of substrate to products (Figure 2, *cf.* **4** and **11**). Thus, enzymic formation of the aromatic ring of riboflavin occurs by condensation of the 6- and 7-methyl groups of one molecule of 6,7-dimethyl-8-(1′-D-ribityl)lumazine with carbons 7 and 6, respectively, of a second molecule of the substrate.

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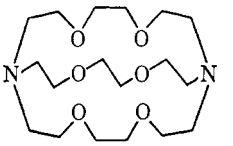
Cryptates. Cation Exchange Rates

Sir:

Cryptates, a new type of metal cation complexes, have been described recently.[1,2] It was found that the macroheterobicyclic diamine I[1] showed a very strong tendency to form remarkably stable complexes with various metal cations. The results (see ref 2 and results presented here) lead to the formulation of these complexes as inclusion compounds in which the cation is contained within the central molecular cavity of the macrobicyclic ligand (I).[3]



I

Preliminary pH-metric titrations[2] lead to the stability constants K_s for the equilibrium

$$[I]_{aq} + [M^{n+},mH_2O] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [I,M^{n+}]_{aq} + mH_2O \qquad K_s = k_1/k_{-1} \quad (1)$$

We found that temperature-dependent nmr spectra may be observed in certain circumstances, and we present here some results of these studies concerning a new type of cation exchange process.[5]

A solution of I in D_2O shows a clearly resolved triplet at 2.83 ppm for the CH_2–N protons. When an equimolar quantity of KF is added, a new well-resolved triplet located at 2.58 ppm is obtained for these protons; this new spectrum is due to formation of the potassium cryptate. However, when the quantity of KF added is such that the solution contains equimolar amounts of I and of the corresponding potassium ion cryptate $[I,K^+]F^-$, a broad unresolved signal is obtained for the CH_2–N protons at normal probe temperature. When the solution is cooled to 16° the CH_2–N signal splits into two triplets at 2.83 and 2.55 ppm which correspond respectively to the free diamine (I) and to the potassium cryptate. On heating to 93° a single sharp triplet is obtained at 2.67 ppm (see Figure 1). Similar spectra are observed when KCl or KBr is used. Furthermore temperature dependent spectra are also obtained when potassium salts are replaced by sodium and rubidium salts.

In the case of thallium chloride, the pure cryptate shows at 7° a spectrum where all signals are doubled. The observed splitting is the same at 60 and at 100 MHz, and is due to spin–spin coupling of the $^{203,205}Tl$ nuclei (which have nearly equal magnetic moments) to all the protons of the ligand (see below and Figure 2). This also indicates that the ion is in the center of the molecular cavity.[3] Heating the solution leads to coalescence of the signals. The coalescence temperature is very different for the sparingly soluble thallium chloride (39°) and for the more soluble thallium nitrate (−6°). The origin of this effect is not yet clear but might be related to the solubility and to the tendency of thallium salts to form ion pairs. The same coalescence temperature is obtained for a solution containing I and $[I,Tl^+]Cl^-$ (1/1), as for a solution containing only the cryptate.

When alkali metal salts are replaced by alkaline earth metal salts (Ca^{2+}, Sr^{2+}, Ba^{2+}) separate signals are observed for I and for the $[I,M^{2+}]$ cryptates from room temperature up to 95–100°. In the case of calcium salts line broadening is setting in at *ca.* 100°.

We interpret the temperature-dependent changes described above as being due to the variations of the exchange rate of the cryptated cation with temperature. The corresponding exchange rates k_c and free energies of activation $\Delta G_c^{\ddagger}$ at coalescence temperature may be

(1) B. Dietrich, J. M. Lehn, and J. P. Sauvage, *Tetrahedron Lett.*, 2885 (1969).
(2) B. Dietrich, J. M. Lehn, and J. P. Sauvage, *ibid.*, 2889 (1969).
(3) This has been recently confirmed by an X-ray crystallographic study of a rubidium cryptate, which further shows that the conformation of the cryptate is *i,i*.[4] The same is true for the potassium cryptate which is isostructural with the rubidium cryptate.
(4) B. Metz, D. Moras, and R. Weiss, *Chem. Commun.*, 217 (1970).
(5) Encapsulation of anions in macrobicyclic diamines has been described recently by C. H. Park and H. E. Simmons, *J. Amer. Chem. Soc.*, **90**, 2431 (1968).