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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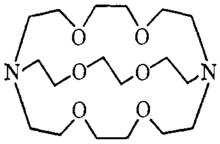
Cryptates. Cation Exchange Rates

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

$$[\mathrm{I}]_{aq} + [\mathrm{M}^{n+},m\mathrm{H_2O}] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\mathrm{I},\mathrm{M}^{n+}]_{aq} + m\mathrm{H_2O} \qquad K_s = k_1/k_{-1} \quad (1)$$

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 $[\mathrm{I},\mathrm{K}^+]\mathrm{F}^-$, 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 $[\mathrm{I},\mathrm{Tl}^+]\mathrm{Cl}^-$ (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 $[\mathrm{I},\mathrm{M}^{2+}]$ 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

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[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).

Table I. Spectral Parameters, Exchange Rates, and Free Energies of Activation for Cation Exchange in $[I,M^{n+}][X^-]_2$ Cryptates[a]

Cryptate	$\Delta\nu$ (temp, °C) ±1 Hz	T_c, °C ± 4°	k_c, sec^{-1}	$\Delta G_c^\ddagger$ ± 0.3 kcal/mol	Log K_s (20°)[2]	$-\Delta G°$ (20°), kcal/mol
$[I,Na^+]Cl^-$	12 (−9)	3	27	14.2	3.6	4.8
$[I,K^+]F^-$	17 (16)	36	38	15.8	5.1	6.8
$[I,K^+]Cl^-$	17 (16)	36	38	15.8	5.1	6.8
$[I,K^+]Br^-$	19 (−2)	35	42	15.7	5.1	6.8
$[I,Rb^+]Cl^-$	17 (−2)	9	38	14.4	3.7	4.9
$[I,Tl^+]Cl^-$	11.5[b] (12)	39	51	15.8		
	14.5[c] (12)	40	64	15.7		
$[I,Tl^+]NO_3^-$	11.5[b,d]	−6	51	13.5		
$[I,Ca^{2+}][Cl^-]_2$	9.0 (3)	>100	<20	>17.0	4.1	5.5
$[I,Sr^{2+}][Br^-]_2$	7.0 (33)	>100	<15	>17.0	13.0	17.4
$[I,Ba^{2+}][Cl^-]_2$	8.0 (33)	>100	<18	>17.0	⩾15.0	⩾20.1

[a] Spectra measured on an A-60 spectrometer. D_2O solutions are 10% in I and contain $(CH_3)_3Si(CH_2)_3SO_3Na$ as internal reference. The amount of salt added has been calculated so as to make up solutions containing 50% I and 50% cryptate. In the case of TlCl, pure cryptate has also been used. Abbreviations used are: $\Delta\nu$, separation of the CH_2–N signals of I and of cryptate at temperature indicated; T_c, coalescence temperature; k_c, exchange rate at coalescence; $\Delta G_c^\ddagger$, free energy of activation of coalescence; K_s(20°), stability constant at 20° (preliminary values taken from ref 2); $\Delta G°$ (20°), free energy difference calculated from K_s. [b] Tl–(CH_2–N) coupling. [c] Tl–(CH_2–O) coupling. [d] Tl–H coupling assumed to be the same as in $[I,Tl^+]Cl^-$.

calculated in the usual way from the spectral parameters and using the Eyring rate equation (Table I).

The exact mechanism of the cation exchange process may be very complex. I exists as an equilibrium mixture of three conformations: *o,o* (*out,out*), *o,i* (*out,in*), and *i,i* (*in,in*) and the same holds also for the cryptates;[2] exchange may occur in any one of these forms.

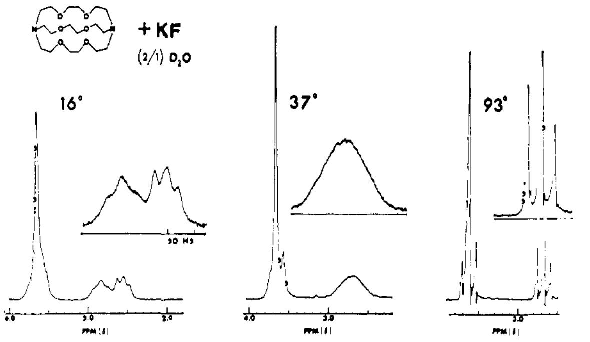
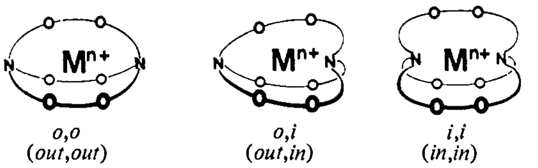


Figure 1. Temperature dependence of the 60-MHz pmr spectra of a D_2O solution containing compound I and the $[I,K^+]F^-$ cryptate in equal amounts.



In addition, different exchange mechanisms may be operative: for instance a dissociation–complexation process corresponding to eq 1 or a bimolecular process of the following type.

$$[I]_{aq} + [I,M^{n+}]_{aq} \rightleftharpoons [I,M^{n+}]_{aq} + [I]_{aq} \qquad (2)$$

The present results favor mechanism 1; as the cryptates are much more stable than the dissociated state ($K_s > 10^3$, $k_1 > 10^3k_{-1}$)[2], the exchange rates k_c and the $\Delta G_c^\ddagger$ values correspond to the rate k_{-1} for the cation leaving the molecular cavity of I. In the solid state, the cryptates exist in conformation *i,i*;[3,4] as a first approximation one may then assume that the measured quantities correspond to eq 1 where $[I]_{aq}$ and $[I,M^{n+}]_{aq}$ are in the *i,i* conformation.

The results in Table I show that the exchange rates and the $\Delta G_c^\ddagger$ values for Na^+, K^+, and Rb^+ are in the reverse order of the stability constants K_s: exchange becomes slower ($K^+ < Rb^+ < Na^+$) as the stability of the cryptate increases. The change in $\Delta G_c^\ddagger$ is of the same order than the change in $\Delta G°$ (at 20°) when comparing the results for two cations. In $CDCl_3$ solution, although temperature-dependent chemical shift changes are observed, exchange is slow up to 60° ($\Delta G^\ddagger > 15$ kcal/mol) for the Na^+, K^+, Rb^+, Ca^{2+}, Sr^{2+}, and Ba^{2+} cations but not for the TlCl cryptate. In this case splittings due to Tl–H coupling are observed at low temperature; coalescence occurs at *ca.* 33° yielding $\Delta G_c^\ddagger = 15.5$ kcal/mol. In the case of the $TlNO_3$ cryptate (see Figure 2) the coalescence temperature is above the boiling point of the $CDCl_3$ solvent ($\Delta G_c^\ddagger > 16.5$ kcal/mol).

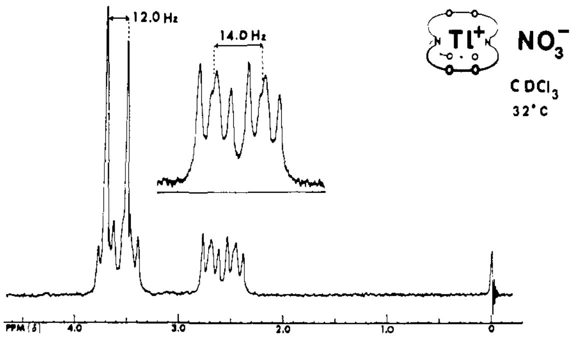


Figure 2. Pmr spectrum (60 MHz) of the $[I,Tl^+]NO_3^-$ cryptate in $CDCl_3$ solution. The indicated splittings are due to Tl–H spin–spin coupling.

Although the nature of the bonding (ion–dipole, ion-induced dipole, and dispersion interactions between the cation and the heteroatomic complexation sites, as in hydrates[6]) is presumably similar in cryptates formed from various systems of type I,[1] cation selectivities[7] and thermodynamic and kinetic data[7] are strongly influenced by the nature of the macrobicyclic ligand. A more de-

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(6) J. A. A. Ketelaar, "Chemical Constitution," Elsevier Publishing Co., Amsterdam, 1953; F. Cotton and G. Wilkinson, "Advanced Inorganic Chemistry," Interscience Publishers, New York, N. Y., 1966.

(7) B. Dietrich, J. M. Lehn, and J. P. Sauvage, in preparation.

tailed study (including medium effects) will be given at a later date.[8]

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(8) Note Added in Proof. Recent pH-metric studies lead to dissociation rates k_{-1} of the order of 10^{-5} and 10^{-4} sec^{-1} for the barium and strontium cryptates, respectively, and to log K_s values of the order of 10 for these complexes. More accurate values will be given in the final account of this work.

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On the Volume of Diels–Alder Transition States

Sir:

We wish to report the first case of a bimolecular reaction for which the transition state is smaller than the product. The Diels–Alder additions of three different dienes to maleic anhydride have activation volumes which are significantly more negative than the volume changes on reaction.

Several authors[1] have previously measured activation volumes for Diels–Alder reactions, but there has been considerable controversy concerning the accuracy of these data.[2] In particular, the data for the dimerization of isoprene have been interpreted to support either a two-step diradical mechanism[1b] or a concerted multicenter molecular mechanism[2a] for the Diels–Alder reaction. This situation has led to skepticism regarding any interpretation of these data in recent reviews on the mechanism of the Diels–Alder reaction.[3]

The new data for activation volumes come from precise determinations of reaction rate constants at elevated pressures, using an improved technique involving *in situ* mixing to avoid errors due to heat of compression. This technique[4] should yield rates appreciably more accurate than those which could be achieved in previous high-pressure kinetic studies of Diels–Alder reactions. Reactant and product volumes were measured as partial molal volumes by an accurate dilatometric method, similar to that used by McCabe, *et al.*[5]

The reactions studied included maleic anhydride with isoprene, with 1,3-cyclohexadiene, and with *trans*-1-methoxy-1,3-butadiene, and dimethyl acetylenedicarboxylate with cyclopentadiene. Rate constants based on mole fractions were measured at 50, 2500, 5000, 7500, 10,000, 15,000, and 20,000 psi. Initial reactant concentrations were approximately 0.01 mol fraction. Activation volumes at zero pressure were determined from a least-squares fit to the equation $\ln k = a + bP + cP^2$ and from the relationship

$$\left(\frac{\partial \ln k}{\partial P}\right)_{T,P=0} = -\frac{\Delta \bar{v}_0^{\ddagger}}{RT} = b$$

which is rigorously correct only when rate constants are based on pressure-independent concentration units.[6] The activation volumes and the differences in partial molal volumes between reactants and products are listed in Table I.[7]

Table I. Comparison of Activation Volumes with the Volume Change on Reaction for Several Diels–Alder Reactions

Reaction	Solvent	Temp, °C	Actn vol, $\Delta \bar{v}_0^{\ddagger}$, cc/g mol	Vol change on reactn,[a] cc/g mol
Maleic anhydride–isoprene	Acetone	35	-39.0 ± 0.8	-35.9 ± 0.9
Maleic anhydride–*trans*-1-methoxy-1,3-butadiene	1,2-Dichloroethane	35	-43.9 ± 2.0	-30.4 ± 0.9
Maleic anhydride–1,3-cyclohexadiene	Dichloromethane	35	-39.6 ± 0.8	-30.3 ± 0.9
Dimethyl acetylenedicarboxylate–cyclopentadiene	Ethyl acetate	10	-30.2 ± 0.7	-33.8 ± 0.8

[a] $\bar{v}^{\infty}$(product) $-$ $\bar{v}^{\infty}$(diene) $-$ $\bar{v}^{\infty}$(dienophile).

Comparison of the activation volumes and volume changes on reaction indicates that the transition state is smaller than the product for the three maleic anhydride reactions. Measurements of activation volumes and volume changes in eight additional solvents for the maleic anhydride–isoprene reaction showed that the transition state was consistently smaller than the product, indicating that neither electrostriction nor specific solute–solvent interactions were responsible for the effect.

If the diene and maleic anhydride are coplanar in the transition state, some increase in volume might be expected after the new bonds are completely formed, due to conformational changes in the product. A similar difference in position of the anhydride entity is found between the *endo* and *exo* adducts of maleic anhydride and cyclopentadiene. The *exo* adduct was prepared by the method of Craig[8] and the partial molal volumes of the *endo* and *exo* adducts were measured in nitromethane and dichloromethane. The partial molal volumes at infinite dilution are shown in Table II. The volume difference between the two is an order of

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(1) (a) B. Raistrick, R. H. Sapiro, and D. M. Newitt, *J. Chem. Soc.*, 1761 (1939); (b) C. Walling and J. Peisach, *J. Amer. Chem. Soc.*, **80**, 5819 (1958); (c) C. Walling and H. J. Schugar, *ibid.*, **85**, 607 (1963).

(2) (a) S. W. Benson and J. A. Berson, *ibid.*, **84**, 152 (1962); (b) C. Walling and D. T. Tanner, *ibid.*, **85**, 612 (1963); (c) S. W. Benson and J. A. Berson, *ibid.*, **86**, 259 (1964).

(3) (a) J. Sauer, *Angew. Chem.*, **79**, 76 (1967); (b) S. Seltzer, *Advan. Alicyclic Chem.*, **2**, 1 (1968).

(4) R. A. Grieger and C. A. Eckert, *AIChE J.*, in press.

(5) J. R. McCabe, R. A. Grieger, and C. A. Eckert, *Ind. Eng. Chem. Fundamentals*, **9**, 156 (1970).

(6) W. J. LeNoble, *Progr. Phys. Org. Chem.*, **5**, 207 (1967).

(7) As a check on these results, the alternate scheme of Benson and Berson[2a] for getting activation volumes from a modification of the Tait equation was applied. Because of a numerical approximation in the development, this method is applicable only to points taken at pressures above about 2000 atm. The rate constants have been measured to 6000 atm for the maleic anhydride–isoprene reaction and for the dimethyl acetylenedicarboxylate–cyclopentadiene reaction, and the activation volumes found from the high-pressure points by this method are within experimental error of the activation volume from the quadratic fit of the low-pressure points.

(8) D. Craig, *J. Amer. Chem. Soc.*, **73**, 4889 (1951).