

(-)-4-Epiacorenone B (67). A mixture of 88 mg of 66, 0.3 g of lithium carbonate, and 0.15 g of lithium chloride in 5 mL of dimethylformamide was stirred at 140 °C for 15 min. The mixture was diluted with ether, washed twice with water and once with saturated brine, and dried (MgSO₄). Filtration, followed by removal of the solvent in vacuo, yielded an oil which was purified by preparative GLC (Carbowax column) to give 17 mg (30% from 54) of 67: $[\alpha]_D^{20}$ (ether) -50°; IR (film) 1670, 1445, 1425, 1360, 1235, 1140, 1115, 1080, 925 cm⁻¹; ¹H NMR (CDCl₃) δ 0.82 (3 H, d, *J* = 6.5 Hz), 0.88 (6 H, d, *J* = 3 Hz), 0.95-2.17 (7 H, m), 1.76 (3 H, d, *J* = 2 Hz), 2.17-2.57 (2 H, m), 2.36 (2 H, d, *J* =

Hz), 6.63-6.78 (1 H, m); MS *m/e* 220.180, calcd for C₁₅H₂₄O 220.183.

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Cation-Complexing Properties of Synthetic Macrocyclic Polyether-Diester Ligands Containing the Furan, Benzene, Tetrahydrofuran, and Thiophene Subcyclic Units¹

Jerald S. Bradshaw,*² Steven L. Baxter, John D. Lamb, Reed M. Izatt,* and James J. Christensen

Contribution from the Departments of Chemistry and Chemical Engineering and Contribution No. 224 from the Thermochemical Institute, Brigham Young University, Provo, Utah 84602. Received September 3, 1980

Abstract: Thirteen new macrocyclic polyether-diester ligands containing the furan, 3,4-dimethoxyfuran, tetrahydrofuran, and thiophene subcyclic units have been prepared by reacting a diacid dichloride with the appropriate glycol. The tetrahydrofuran ligands were also prepared by reducing the furan ring of the furano ligands. Certain of these ligands and corresponding macrocyclic polyether-diester ligands containing a benzene subcyclic unit have been shown to form complexes with alkylammonium and metal cations. Complexation with alkylammonium cations was accompanied by significant chemical-shift changes in the ¹H NMR spectra. Free energies of activation (ΔG^\ddagger) for the dissociation of the alkylammonium complexes were determined from their temperature-dependent ¹H NMR spectra. The relative kinetic stability (as measured by the ΔG^\ddagger values) for complexes between benzylammonium perchlorate and ligands containing an aromatic subcyclic unit increased with increasing ring size in the order 18- < 21- < 24-membered ring. Formation constants as well as ΔH and $T\Delta S$ values were determined in methanol by a calorimetric titration technique for the reaction of Na⁺, K⁺, Cs⁺, and Sr²⁺ with the tetrahydrofuran ligands. The complexes formed between the 18-membered ring containing a tetrahydrofuran subcyclic unit and the alkali-metal cations were about as stable as those formed from 2,6-diketo-18-crown-6 (2) but were less stable than those formed from the corresponding pyridine analogue (1). The 21- and 24-membered ring compounds containing a furan subcyclic unit were found to be effective carriers of Cs⁺ across a CHCl₃ liquid membrane separating aqueous phases.

We have reported the synthesis of macrocyclic polyether-diester compounds and compared their cation complexation properties to those of the crown ethers.³⁻¹¹ Generally, they do not complex metal cations as strongly as do the crown ethers.^{4,5,9,11} However, the diester ligands containing a pyridine subcyclic unit are an exception to this general rule. For example, the pyridino diester compound containing 18 ring members (1, Figure 1) forms complexes with metal cations and ammonium salts which are comparable in stability to those formed by any of the crown com-

pounds.^{8,9} It is significant that in addition to forming stable complexes with the various cations, compound 1 is relatively easy and inexpensive to prepare compared to most of the crown ether compounds.^{8,12}

Macrocyclic polyether compounds containing the 2,5-furan and tetrahydrofuran and the 3,4-thiophene and the 1,3-benzene subcyclic units have been studied in some detail by Cram and his co-workers¹³ and by Gray and Reinhoudt and their coworkers.¹⁴ Gray, Reinhoudt, and their co-workers have also studied macrocyclic polyether compounds containing 3,4-furan and thiophene and 1,2-benzene subcyclic units.¹⁵

We now report the synthesis of macrocyclic polyether-diester compounds containing the furan, tetrahydrofuran, and thiophene subcyclic units (compounds 3-15, Figure 1). Free energies of activation (ΔG^\ddagger) for the interaction of alkylammonium salts with these and some closely related ligands containing the benzene subcyclic unit (compounds 16-22) as well as those of the diester ligand 2,6-diketo-18-crown-6 (2) are reported. In addition, log *K*, ΔH , and $T\Delta S$ values are given for the reaction of several metal cations with some of these compounds (1-5, 10, 11, and 16). The use of several of these compounds to carry metals across a liquid

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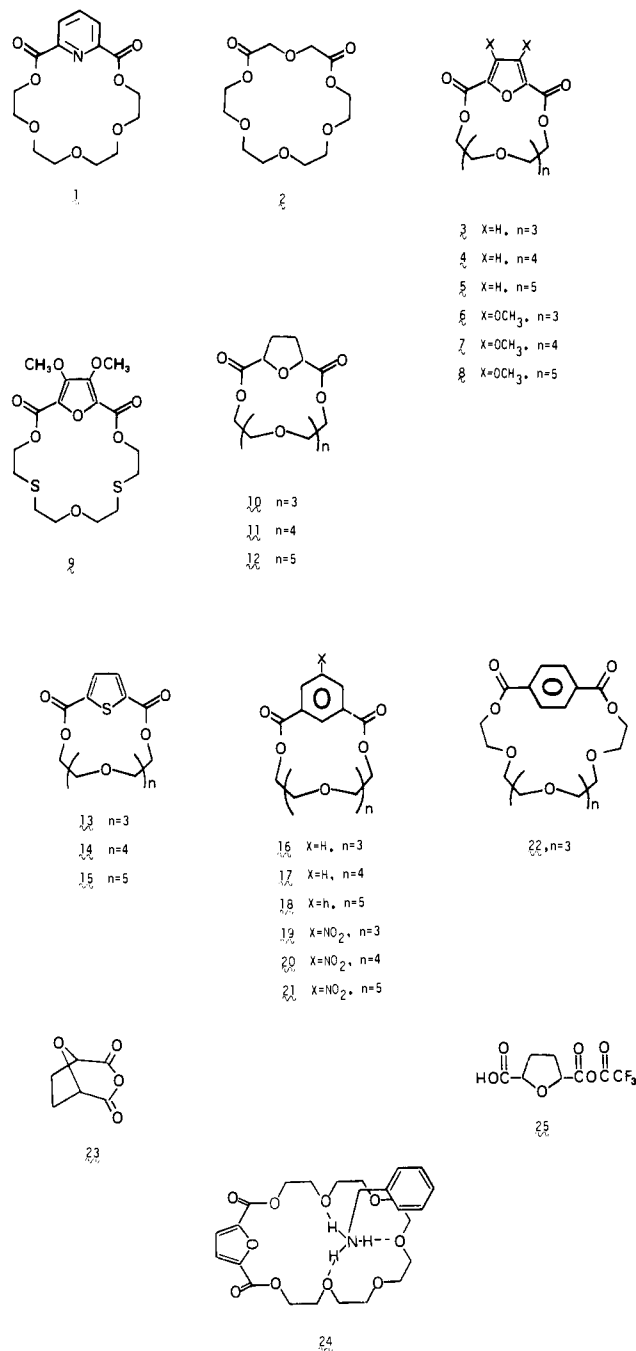
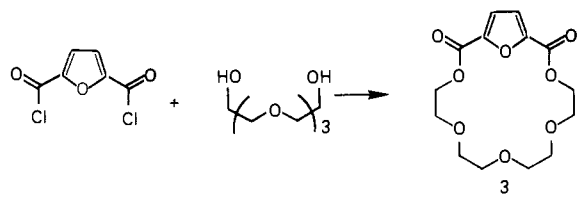


Figure 1. Structural formulas for compounds 1–25.

membrane separating aqueous phases is also included in this report. A preliminary paper describing the preparation and complexing properties of the furano and benzo ligands has been published.⁷

Results and Discussion

Preparation of Ligands. The various furano (3–9) and thiopheno (13–15) ligands were prepared from the appropriate diacid chloride and glycol.^{3,11} For example, compound 3 was prepared



from 2,5-furandicarbonyl dichloride and tetraethylene glycol. The yields of these reactions were generally good (25–50%).

The tetrahydrofurano ligands (10–12) were prepared both by using *cis*-2,5-tetrahydrofurandicarbonyl chloride and the appropriate glycol and by reducing the already formed furano ligand with the use of rhodium on carbon as the catalyst. The properties of benzo ligands (16–22) and diketo-18-crown-6 (2) were reported previously.^{4,16}

The starting 2,5-furandicarbonyl dichloride, 3,4-dimethoxy-2,5-furandicarbonyl dichloride, and 2,5-thiophenedicarbonyl dichloride were prepared according to published procedures.^{17–20} The *cis*-2,5-tetrahydrofurandicarbonyl acid needed for the preparation of ligands 10–12 was prepared by a catalytic reduction of the furan diacid with the use of rhodium on carbon.²¹ The crude product was purified by forming the cyclic anhydride (23)²² with a subsequent hydrolysis step.

We have prepared the penta- and hexaethylene glycols in our laboratory by reacting a monosodium salt of diethylene glycol with the appropriate dichloride.²³ A careful analysis of the silylation products from these glycols using a gas-liquid chromatograph (GLC) showed that a considerable amount of oligoethylene glycol with one more and one less ethylene glycol unit was also present in each case. These impurities are thought to be produced by an S_N² cleavage of the product glycol by basic material during the distillation step since no impurity glycols were observed when the reaction mixture was acidified with gaseous HCl prior to distillation.

The structural formulas proposed for the macrocyclic compounds are consistent with data derived from infrared (IR) and proton nuclear magnetic resonance (¹H NMR) spectra, combustion analyses, and molecular weight determinations. The aromatic portion of the ¹H NMR spectra exhibited the expected signals at δ 7.24 \pm 0.04 and 7.76 \pm 0.03 for compounds (3–5) (furan) and 13–15 (thiophene), respectively. The ¹H NMR spectral signals for the tetrahydrofuran moiety (δ 2.21 \pm 0.02 and 4.60 \pm 0.03) were present in the spectra for compounds 10–12. The remainder of the ¹H NMR signals were as reported for the macrocyclic polyether-diester compounds:^{8,16,24,25} δ 4.45 \pm 0.03 (3–9), 4.29 \pm 0.02 (10–12), 4.40 \pm 0.05 (13–15) (COOCH₂), 3.78 \pm 0.03 (COOCH₂CH₂), and 3.60 \pm 0.10 (OCH₂). The ¹H NMR signals for the complexes of all ligands with the various alkylammonium salts are given in the supplementary material.

Complexation Studies. The cation complexation properties of these macrocyclic polyether-diester compounds have been studied with the use of temperature-dependent ¹H NMR spectroscopy, calorimetry, and cation transport across liquid membranes. These studies will be discussed under separate headings.

Temperature-Dependent ¹H NMR Spectroscopy. Formation of complexes by these diester ligands with primary alkylammonium cations in an equimolar mixture of the two reactants in methylene-*d*₂ chloride was observed for all ligands except compounds 9, 13, 14, 16, 19, and 22. Complexation for the other ligands was accompanied by significant chemical-shift changes in the ¹H NMR spectra (see supplementary material and Figures 2 and 3). These chemical-shift changes were minor for the 18- and 21-membered ring compounds (see Figure 2) and were similar to those changes observed for the complexes of the 18- or 21-membered ring compounds containing a pyridine subcyclic unit.⁸ The chemi-

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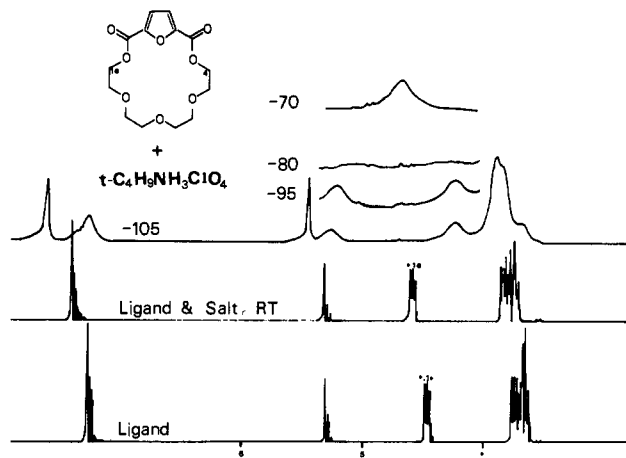


Figure 2. Proton NMR spectra for **3** and for the complex of **3** with *tert*-butylammonium perchlorate at room temperature (RT), $-105\text{ }^{\circ}\text{C}$, $-95\text{ }^{\circ}\text{C}$, $-80\text{ }^{\circ}\text{C}$ (coalescence temperature) and $-70\text{ }^{\circ}\text{C}$ in CD_2Cl_2 (solvent signal at δ 5.25).

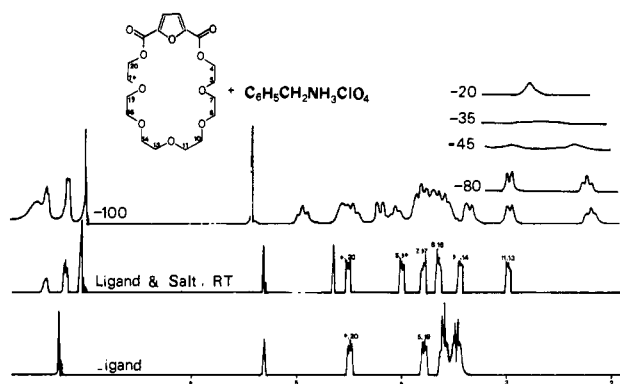


Figure 3. Proton NMR spectra for **5** and for the complex of **5** with benzylammonium perchlorate at room temperature (RT), $-100\text{ }^{\circ}\text{C}$, $-80\text{ }^{\circ}\text{C}$, $-45\text{ }^{\circ}\text{C}$, $-35\text{ }^{\circ}\text{C}$ (coalescence temperature), and $-20\text{ }^{\circ}\text{C}$ in CD_2Cl_2 (solvent signal at δ 5.25 and benzyl CH_2 signal at δ 4.60).

cal-shift changes in the ^1H NMR spectra for the complexes of benzylammonium perchlorate with the 24-membered ring compounds containing furan and benzene subcyclic units (**5**, **8**, **18**, and **21**) were most dramatic (see Figure 3). The signals for the ether methylene hydrogen atoms changed from two or three sets of multiplets (δ 3.46–3.65) to four distinctive sets at δ 3.04 \pm 0.06, 3.45 \pm 0.01, 3.65 \pm 0.01, and 3.79 \pm 0.03. The methylene hydrogen atoms β to the ester oxygen shifted downfield from δ 3.81 \pm 0.03 to 4.02 \pm 0.04. The assignment for all these hydrogens was accomplished with use of proton decoupling in the ^1H NMR spectrum. Upfield shift changes were observed for all aromatic hydrogen atoms of these complexes and also for the methyl hydrogen atoms of the methoxy group for the complex of compound **8**. Similar chemical-shift changes (but less dramatic) were observed in the ^1H NMR spectrum for the benzylammonium perchlorate complex with thiophene compound **15**. Such dramatic chemical-shift changes were not observed for the complexes of (β -phenylethyl)ammonium and *tert*-butylammonium perchlorates with compounds **5**, **8**, **18**, and **21**. We believe that the dramatic chemical shift changes in the ^1H NMR spectra for the benzylammonium perchlorate complexes of compounds **5**, **8**, **18**, and **21** as outlined above are suggestive of the structure of the complex. The 200-Hz upfield shift (to δ 2.98) of the protons on the macroring at positions 11 and 13 (the carbons on the macroring opposite the furan ring) in the ^1H NMR spectrum (see Figure 3) suggests that the benzene ring of the benzylammonium cation is centered over the ether part of the macrocycle (see compound **24**). Indeed, at very low temperatures, the macroring protons at positions 11 and 13 that are on the same side of the macroring as the benzene ring of the ammonium salt are shifted even further upfield to δ 2.19 (see Figure 3). The absence of any change in

the frequency of the carbonyl infrared band or the maximum in the furan ultraviolet spectrum upon complexation also indicates that the furan (or benzene) dicarbonyl group is not involved in complexation. Supporting the above structural features of the benzylammonium perchlorate complex of compounds **5**, **8**, **18**, and **21** is the knowledge that the furan oxygen is a weak hydrogen-bond acceptor¹³ and the fact that compounds **18** and **21** do not have a heteroatom on the aromatic ring. Thus, one would expect complexation to take place in the polyether portion of the macroring.

The ^1H NMR spectra for the benzylammonium perchlorate complexes of compound **12** did not exhibit the dramatic chemical-shift changes that were observed for the other 24-membered ring compounds. Cram and co-workers found the tetrahydrofuran oxygen to be an excellent hydrogen-bond acceptor¹³ so that a different pattern of alkylammonium complexation would be expected for compounds **10**–**12** than that observed for compounds **3**–**8**. The thiophene macrocyclic compounds (**13**–**15**) are probably sterically similar to those prepared from terephthalic acid (**22**)¹⁶ which did not form complexes with alkylammonium salts. Compounds **13** and **14** likewise did not form complexes. Compound **15** did form a complex with benzylammonium perchlorate but with a lower stability (see below) than those found for corresponding complexes with furan compounds **5** or **8**.

The temperature dependencies of the ^1H NMR spectra for the complexes of compounds **2**–**21** with benzyl-, (β -phenylethyl)- and *tert*-butylammonium perchlorates have been examined. For the 18- and 21-membered ring compounds where a complex formed, the ^1H NMR signal attributable to the ester methylene hydrogen atoms separated into two multiplets of equal intensities at low temperature (Figure 2). The ^1H NMR signal attributable to the methylene hydrogens at positions 11 and 13 of the 24-membered ring compounds (**5**, **8**, **15**, **18**, and **21**) also separated into two multiplets of equal intensities at low temperatures (Figure 3). These signals can be attributed to the hydrogen atoms on one side of the complex vs. those on the other side when the exchange rate has become slow at low temperatures (see structure **24**). In some cases, complexes formed but no separation of the ^1H NMR signals was observed at $-100\text{ }^{\circ}\text{C}$. Only broadening of the ^1H NMR signals was observed for all uncomplexed macrocyclic compounds at low temperatures.

Calculation of kinetic parameters for the formation of the complexes was done with use of eq 1²⁶ and 2 (the Eyring equation)

$$k_c = \pi\Delta\nu/2^{1/2} \quad (1)$$

$$-\Delta G_c^*/RT_c = \ln(k_c h/kT_c) \quad (2)$$

where k_c = exchange rate at the coalescent temperature (T_c), $\Delta\nu$ = ^1H NMR signal separation, ΔG_c^* = free energy of activation, h = Planck constant, k = Boltzmann constant, and R = molar gas constant. The observed $\Delta\nu$ and T_c values as well as the calculated k_c and ΔG_c^* values for all complexes are given in the supplementary material. The ΔG_c^* values are given in Table I. The ΔG_c^* values where the molar ratio of crown to salt, C:S, is 1:1 can be equated to the free energy of activation (ΔG_{d+ri}^*) for an equilibrium of the ammonium salt from one face of the ligand to the other face with both dissociative and ring-inversion components.^{27–30} In solutions where C:S = 2:1, the measured ΔG_c^* probably involves an equilibrium between complexed and uncomplexed ligands and thus can be equated to the free energy of activation for dissociation, ΔG_d^* . The differences in these free energy of activation values ($\Delta G_{d+ri}^* - \Delta G_d^*$) (compare salt A vs. salt D values in Table I) for the benzylammonium perchlorate

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Table I. Free Energies of Activation (ΔG^\ddagger_c) for the Complexation of Equal Molar Ratios of Primary Alkylammonium Salts with Compounds 2-21^a

salt ^b	compd	ΔG^\ddagger_c ^c	compd	ΔG^\ddagger_c ^c	
A	2	9.3	11	<8	
	3	9.7	12	<8	
	4	10.7	15	8.5	
	5	10.8	17	9.3	
	6	10.2	18	10.5	
	7	10.8	20	9.1	
	8	11.0	21	10.1	
	10	<8			
	B	3	9.5	6	9.6
		4	10.4	7	10.5
5		10.3	8	10.3	
3		8.7	7	<8	
C	4	<8	8	<8	
	5	<8	17	<8	
	6	9.0	18	<8	
	3	9.1	8	11.0	
D	5	10.5	18	10.3	
	6	9.6			

^a Specific details of the parameters for calculating these values are given in the supplemental materials. Complexes did not form for compounds 9, 13, 14, 16, 19, and 22. ^b A = benzylammonium perchlorate; B = (β -phenylethyl)ammonium perchlorate; C = *tert*-butylammonium perchlorate; D = benzylammonium perchlorate where the ratio of crown to salt (C:S) is 2:1. ^c ± 0.3 kcal/mol.

complexes are very small (0.5 ± 0.1 for complexes of 18-membered ring ligands 3 and 6 and 0.17 ± 0.04 for complexes of 24-membered ligands 5, 8, and 18) indicating a high degree of rigidity in these macrocyclic systems.

The ΔG^\ddagger_c values are a measure of the kinetic stabilities of the complexes. Thus, the effects of substituents, ring size, and different subcyclic units on ligand-alkylammonium cation complexation can be studied. As shown in Figure 4, methoxy substituents enhanced the kinetic stability of the complex of the 18-membered ring containing a furano unit (6) with benzylammonium perchlorate ($\Delta G^\ddagger_c = 10.2$ kcal/mol for X = OCH₃ and 9.7 kcal/mol for X = H). Neither methoxy nor nitro substituents affected the kinetic stability of complexes formed from the larger macrorings (Figure 4) ($\Delta G^\ddagger_c = 10.7 \pm 0.2$ kcal/mol for 4 and 7 and 10.9 ± 0.1 kcal/mol for 5 and 8). These results are different from those of the pyridino crown ester (1) complexes where the substituent stability order was OCH₃ > H > Cl for all macroring sizes.⁸ Alkylammonium ion complexation to the furano ligand with 18-ring members must involve the furan ring oxygen so that electron-donating substituents would enhance complexation. Complexation in the larger rings would not involve the furan ring as we have already discussed, and therefore the substituents would not effect the complex stability.

We observed previously that there is a significant decrease in the stabilities of crown ligand-metal or ammonium cation complexes when ether oxygens in the crown are replaced by ester groups (for example, the log *K* for the formation of a potassium ion-18-crown-6 complex is over 6 while that for a potassium ion-2 complex is 2.79 ± 0.02).⁹ This drop in stability may be a result of the electron-withdrawing effect of the carbonyl oxygen which would make the adjacent macroring oxygen less electronegative. Although the effect is not great, we observed that when a furan ring is placed between the two carbonyl groups of 2 to form 3, the stability of the ammonium complex is increased (ΔG^\ddagger_c changes from 9.3 to 9.7 kcal/mol). The electron-rich aromatic system restores the electronegativity of the macroring oxygen atoms next to the carbonyl groups.

The effects of the type of subcyclic unit and macroring size on the kinetic stability of alkylammonium cation complexes can be seen in Figure 5. The pyridino crown ether-diester ligands of 18- and 21-membered rings form more stable complexes with both benzyl- and *tert*-butylammonium perchlorates than do the ligands with furan or benzene subcyclic units. Whereas the complexes

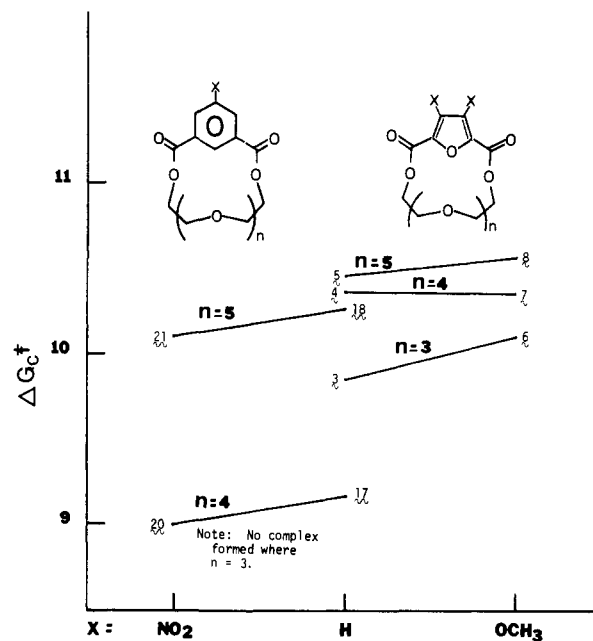


Figure 4. Free energies of activation (ΔG^\ddagger_c , kcal/mol) for the dissociation of benzylammonium perchlorate complexes of the furano (3-8) and benzo (17, 18, 20, and 21) ligands vs. the substituent.

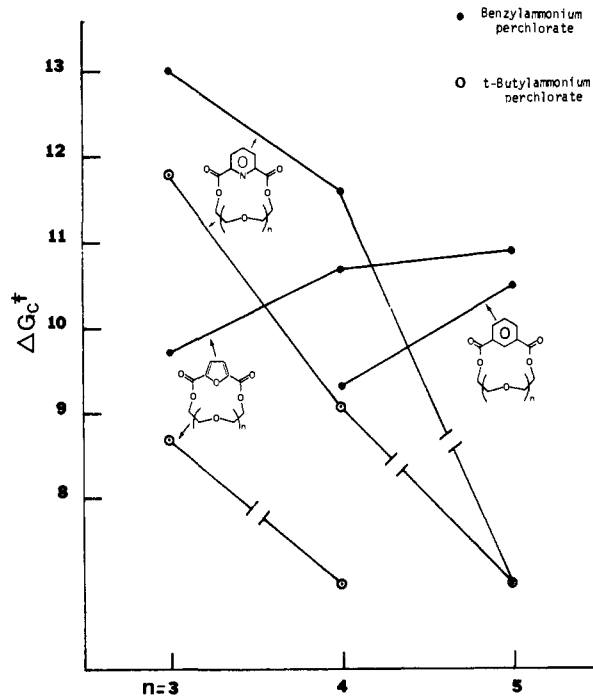


Figure 5. Free energies of activation (ΔG^\ddagger_c , kcal/mol) for the dissociation of benzyl- and *tert*-butylammonium perchlorate complexes of pyridino (1), furano (3-5), and benzo (17, 18) ligands vs. the macroring size.

of the pyridino ligands exhibit a kinetic stability order of 18-membered > 21-membered > 24-membered ring,⁸ the furano and benzo ligands show the opposite stability order with the benzylammonium perchlorate complexes of the 24-membered ring being the most stable (Figure 5). Complexes of the furano ligands with (β -phenylethyl)ammonium perchlorate show the same kinetic stability order as those with benzylammonium perchlorate but those with *tert*-butylammonium perchlorate do not (see Table I). These latter results show the steric nature of the complexes formed from the 24-membered rings containing furano and benzo subcyclic units. The ¹H NMR spectra of the benzylammonium perchlorate complex with these 24-membered ring ligands as discussed above suggest that the benzene ring of the ammonium salt is over the protons at positions 11 and 13 (see structure 24).

Table II. $\log K$, ΔH (kcal/mol), and $T\Delta S$ (kcal/mol) Values for the Interaction of Several Macrocyclic Ligands with Metal Ions in Methanol at 25 °C and $\mu = 0.005$

ligand	value	Na ⁺	K ⁺	Cs ⁺	Sr ²⁺
10	log <i>K</i>	2.29 ± 0.06	2.79 ± 0.02		1.75
	ΔH	-4.4 ± 0.4	-8.4 ± 0.3		~-4
	$T\Delta S$	-1.28	-4.6		-1.6
2	log <i>K</i>	2.50	2.79	2.55	
	ΔH	-2.27	-5.87	-1.52	
	$T\Delta S$	1.14	-2.06	2.0	
18-C-6 ^{b,c}	log <i>K</i>	4.36	6.06	5.32	>5.5
	ΔH	-8.36	-13.41	-12.09	-8.61
	$T\Delta S$	-2.41	-5.14	-4.83	
11	log <i>K</i>		3.03 ± 0.05	2.64 ± 0.05	
	ΔH		-7.4 ± 0.4	-9.6 ± 0.6	
	$T\Delta S$		-3.3	-6.0	
21-C-7 ^d	log <i>K</i>	1.73	4.22	5.01	1.77
	ΔH	-10.37	-8.59	-11.18	-7.10
	$T\Delta S$	-8.0	-2.83	-4.34	-4.69
1	log <i>K</i>	4.29	4.66		
	ΔH	-6.19	-9.3		
	$T\Delta S$	-0.34	-3.0		
16		<i>a</i>		<i>a</i>	

^a No measurable heat other than heat of dilution, indicating that ΔH and/or $\log K$ are very small. ^b 18-C-6 = 18-crown-6.

^c Reference 9. ^d 21-C-7 = 21-crown-7.

The fact that the ¹H NMR spectra for the (β -phenylethyl)ammonium perchlorate complexes of compounds **5** and **8** do not exhibit dramatic chemical shift changes indicates that the benzene ring of the (β -phenylethyl)ammonium salt is *not* close to the macrocyclic hydrogen atoms. Indeed, molecular models show that the benzene ring of the benzylammonium salt of these complexes is directly over the macrocyclic hydrogens in positions 11 and 13, that the benzene ring of the (β -phenylethyl)ammonium salt is away from the macrocyclic ring, and that the tert-butyl group of *tert*-butylammonium perchlorate is sterically hindered when the ammonium ion is hydrogen bonded to macrocyclic oxygen atoms at positions 6, 12, and 18 (see structure 24). Thus, one would expect complexes formed from the less bulky benzyl- and (β -phenylethyl)ammonium salts to be more stable than those from *tert*-butylammonium salts. Alkylammonium ion complexation with the pyridino crown esters on the other hand takes place at the pyridine nitrogen so that complex stability should decrease as the macrocyclic size increases.

Calorimetry. In Table II are listed $\log K$, ΔH , and $T\Delta S$ values for the reactions of **10**, **11**, and **16** with Na⁺, K⁺, Cs⁺, and Sr²⁺ in methanol as determined by titration calorimetry. Corresponding results are also presented for other macrocyclic ligands for comparison purposes. Compound **10** forms complexes with Na⁺ and K⁺ which are very similar in thermodynamic parameters to compound **2**.⁹ The ΔH values for formation of complexes with **10** are somewhat more exothermic than for those with **2**; however, this effect is offset by the $T\Delta S$ terms so that the stability constants are similar, indeed, identical in the case of K⁺. It has been demonstrated⁹ that the crown ether-diester form less stable cation complexes than crown ethers, as illustrated by comparing results for **10** and **2** with those for 18-crown-6 in Table II. The lower stabilities of complexes of the diesters has been attributed to the lower basicity of the ester oxygens compared to ether oxygens rather than to ring conformational rigidity. This explanation is further borne out in the similar results for **10** and **2**, ligands which differ considerably in flexibility but which are very similar in donor atom basicities.

Unlike 21-crown-7, compound **11** is not selective for Cs⁺, which best fits the ligand cavity, over Rb⁺ (Table II). As with previously studied crown ether-diester, the complexes of **11** are less stable than those of the corresponding crown ether, 21-crown-7. The stabilities of complexes of pyridino-substituted crown ether diester **1** are lost when the pyridine nitrogen atom is replaced by carbon to give **16**.

Table III. $\log K$, ΔH , and $T\Delta S$ for the Reaction of Compounds **3**, **4**, and **5** with Ammonium, Methylammonium, and Benzylammonium Chlorides in a 50% v/v Chloroform/Methanol Mixture at 25 °C^{a,b}

ligand	value	NH ₄ ⁺	CH ₃ NH ₃ ⁺	PhCH ₂ NH ₃ ⁺
3	log <i>K</i>	1.61 ± 0.01	1.12 ± 0.03	0.5
	ΔH	-6.4 ± 0.8	-7.0 ± 0.8	
	$T\Delta S$	-4.2	-4.9	
4	log <i>K</i>	2.51 ± 0.01	1.76 ± 0.01	1.36 ± 0.12
	ΔH	-4.9 ± 0.2	-5.5 ± 0.4	-8.3 ± 1.0
	$T\Delta S$	-1.6	-3.1	-6.4
5	log <i>K</i>	1.29 ± 0.12	1.60 ± 0.11	1.40 ± 0.07
	ΔH	-2.8 ± 0.5	-4.2 ± 0.6	-10.2 ± 0.7
	$T\Delta S$	-1.0	-2.0	-8.3

^a Error limits are given as the average deviation from the mean determined from two or more independent experiments. ^b ΔH and $T\Delta S$ given in kcal/mol.

In Table III are given $\log K$, ΔH , and $T\Delta S$ values for reaction of the furano crown ether-diester ligands with ammonium, methylammonium, and benzylammonium cations in 50% by volume chloroform/methanol solvent. Unfortunately, these ligands were not sufficiently soluble in methanol to give results with metal cations comparable to those in Table II. Binding constants were determined previously for a number of primary ammonium cations with 18-crown-6.³¹ The drop in stability constants through the series NH₄⁺, CH₃NH₃⁺, and PhCH₂NH₃⁺ with ligands **3** and **4** (Table III) is comparable to the trend in methanol for binding with 18-crown-6. Binding constants presented in Table III are considerably lower than those for 18-crown-6 measured in methanol, even though binding constants in 50% chloroform/methanol ought to be much higher than in methanol since the former solvent has a lower dielectric constant.³² We thus observe that ester groups are responsible for loss in complex stability with ammonium cations as well. Unlike ligands **3** and **4**, the largest ligand, **5**, in Table III follows the stability sequence CH₃NH₃⁺ > PhCH₂NH₃⁺ > NH₄⁺. In addition, in methanol, 21-crown-7 binds primary ammonium cations less strongly than 18-crown-6, whereas as shown in Table III the larger ligand **4** binds the ammonium cations more strongly. Indeed, benzylammonium cation is bound most strongly by the 24-membered ring (**5**) just as was shown by the temperature-dependent ¹H NMR studies given earlier. These peculiarities in the binding constant data pose interesting problems for future investigation.

Like the furano crown ether-diester compounds, the thiophene ligands (**13**–**15**) were not sufficiently soluble in methanol to perform calorimetric experiments.

Membrane Transport. We have measured the transport rates of several cations through a chloroform membrane³³ which contained one of several of the ligands studied and which separated two aqueous phases. The results are shown in Table IV. In general, the crown ether-diester ligands transport cations at slower rates than the crown ethers, due to the lower cation binding constants of these ligands.³³ Indeed, a correlation has been made³³ between $\log K$ in methanol and transport data such as those in Table IV. In cases where the transport rates are less than $\sim 2 \times 10^{-7}$ mol/24 h in Table IV, it may be assumed that $\log K_{\text{MeOH}}$ is less than 4. As previously reported with the pyridino ligands (**1**),⁸ the transport rates for K⁺, Rb⁺, and Cs⁺ increased when methoxy groups were substituted onto the furan ring (compare ligands **3** and **6** in Table IV). This order is also consistent with the stability order for the organic ammonium ions described above for the 18-membered ring compounds.

Table IV shows that the nature of the ligand subcyclic group is also important in affecting transport rate. The transport rate order for most metal ions with the 18-membered ring ligands is

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Table IV. Rates^a of Transport of Various Metal Nitrate Salts (mol/24 h × 10⁷) through a Stirred Chloroform Membrane Containing 10⁻³ M Ligand

ligand	Li ⁺	Na ⁺	K ⁺	Rb ⁺	Cs ⁺	Mg ²⁺	Ca ²⁺	Sr ²⁺	Ba ²⁺
3	<i>b</i>	0.67 ± 0.06	2.0 ± 0.4	0.7 ± 0.2	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>
6	<i>b</i>	<i>b</i>	4.4 ± 1.2	2.4 ± 1.4	2.05 ± 0.12	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>
7	<i>b</i>	1.5 ± 0.6	2.8 ± 1.0	7.9 ± 2.1	53 ± 2	<i>b</i>	<i>b</i>	<i>b</i>	8 ± 4
8	<i>b</i>	2.4 ± 0.5	0.90 ± 0.01	2.0 ± 0.4	13 ± 1.5	<i>b</i>	<i>b</i>	<i>b</i>	8 ± 4
10	<i>b</i>	0.9 ± 0.1	2.8 ± 1.4	0.7 ± 0.1	0.6 ± 0.2	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>
11	<i>b</i>	1.0 ± 0.2	2.3 ± 0.8	4 ± 2	4 ± 2	0.34 ± 0.07	0.5 ± 0.1	<i>b</i>	<i>b</i>
12	<i>b</i>	1.38 ± 0.04	2.0 ± 0.3	5.6 ± 0.7	1.7 ± 0.1	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>
2	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>
1	<i>b</i>	2.7 ± 0.2	43 ± 11	31 ± 3	1.8 ± 0.5	<i>b</i>	1.07 ± 0.17	15 ± 5	<i>b</i>

^a Reported as average of three independent determinations and standard deviations from the average. ^b Measured transport rate corresponds to that of "blank" experiment with no ligand in the membrane (<0.3 × 10⁻⁷ mol/24 h).

Table V. Yields and Other Physical Properties for Compounds 3–15^a

product	% yield	mp, °C (bp, °C)
3	60	117–118
4	36	133–134
5	30	93.5–95
6	67	167–167.5
7	31	84–85
8	14	67–67.5
9	3	102.5–104
10	82 ^b	(180–184 (1 mm))
11	14.5 ^c	(200 (1 mm))
11	70 ^b	(205 (1 mm))
12	52 ^b	(210 (1 mm))
13	17	111–113
14	18	96–97
15	14	63–64.5

^a Complete details are given in the supplementary material.

^b Prepared by reducing the corresponding furano compound.

^c Prepared from the acid chloride.

1 (pyridino) > 6 (dimethoxy furano) > 3 (furano) > 10 (tetrahydrofurano) > 2 (no subcyclic group). This order is similar to the ligand-alkylammonium cation stability order as measured by the temperature-dependent ¹H NMR and calorimetric techniques given above.

The transport selectivity of 6 for K⁺ is as one would expect for 18-membered ring ligands. However, both the 21- and 24-membered ring ligands (7 and 8) exhibited transport selectivity for Cs⁺. In both cases the selectivity of Cs⁺ over Rb⁺ is about 6 to 1.

Experimental Section

All infrared (IR) spectra were obtained on a Hilger and Watts H-1200 infragraph or a Beckman AccuLab 2 spectrometer. The proton nuclear magnetic resonance (¹H NMR) spectra were obtained on a Varian EM-390 and a Perkin-Elmer R-34 spectrometer and are given in the supplementary material. The carbon-13 nuclear magnetic resonance (¹³C NMR) spectra were obtained on a JEOL FX-90Q spectrometer at 22.5 MHz. Molecular weights were determined by osmometry with use of a Hitachi Perkin-Elmer 115 molecular weight apparatus. Combustion analyses were performed by M-H-W Laboratories, Phoenix, AZ. Melting points were determined on a Thomas-Hoover capillary type apparatus and are uncorrected. All new compounds reported gave satisfactory ¹H and ¹³C NMR, IR, molecular weight, and combustion analyses. Complete details are given in the supplementary material.

Starting Materials. 2,5-Furandicarbonyl dichloride, 3,4-dimethoxyfuran-2,5-dicarbonyl dichloride, and 2,5-thiophenedicarbonyl dichloride were prepared according to published procedures.^{17–20} An attempt to prepare *cis*-tetrahydrofuran-2,5-dicarboxylic acid as a precursor to the acid chloride was made following a known method involving catalytic hydrogenation of 2,5-furandicarboxylic acid over Rh on C.²¹ The product diacid had a broad melting range (mp 109–122 °C, lit.²¹ 128–129 °C). The impurity was most likely the trans form of the diacid. Consequently, the crude diacid was treated with trifluoroacetic anhydride to yield the mixed anhydride 25,²² which upon heating gave the cyclic anhydride 23. Hydrolysis of 23 gave *cis*-tetrahydrofuran-2,5-dicarboxylic acid (mp 127–128 °C). This diacid was readily converted into the acid chloride (bp 85–87 °C (4 mm)) by thionyl chloride by using a catalytic amount of dimethylformamide.

We prepared the penta- and hexaethylene glycols using our reported procedure.²³ A convenient method to determine the purity of the oligo-

ethylene glycols has been developed. The oligoethylene glycol was treated with an excess of *bis*-*N,O*-trimethylsilylacetamide (BSA) for 15 min at 80 °C. The resulting silylated glycol was analyzed on a gas-liquid chromatograph (GLC) with use of a 1/8 in. × 2 ft. column packed with 10% SE-30 on 100/120 mesh Chromosorb G. This technique separated each silylated glycol up to heptaethylene glycol. The penta- and hexaethylene glycols prepared in our laboratory²³ were found to contain small amounts of the oligoethylene glycol with one more and one less ethylene oxide unit. These glycol impurities probably resulted from a substitution or cleavage reaction catalyzed by the residual alkoxide during the distillation step. When the crude reaction mixture was treated with gaseous HCl before distillation, the desired glycol was not contaminated with the other glycols.

General Procedures for the Preparation of Compounds 3–12. Equimolar amounts of the diacid chloride and glycol each dissolved in 200-mL portions of benzene were simultaneously dripped into 1 L of rapidly stirring benzene at 50 °C. The solution was stirred at 50 °C for 48 h, and the solvent was then removed under reduced pressure. Solid products were extracted from the polymeric residue by a continuous liquid-liquid extraction with hot hexane. Decolorization with carbon followed by recrystallization in absolute ethanol gave white crystalline solids. Liquid products were isolated by direct distillation from the polymeric residue. The liquid products were very hygroscopic, and satisfactory combustion analyses could be obtained only by refluxing the product in excess acetic anhydride followed by redistillation. Less rigorous drying techniques were not effective. Compounds 10–12 were prepared by reducing the corresponding furano compound (3–6) with use of 5% Rh on C. Compound 11 was also prepared from the acid chloride.

General Synthetic Procedure for Compounds 13–15. Equimolar amounts of 2,5-thiophenedicarbonyl dichloride and glycol were dissolved in separate portions of methylene chloride and simultaneously dripped into 1 L of rapidly stirring methylene chloride containing excess triethylamine. The solution was stirred at room temperature for 48 h and then concentrated to precipitate the triethylamine hydrochloride. The amine salt was removed by filtration and the remaining methylene chloride evaporated under reduced pressure. The product was isolated by a continuous liquid-liquid extraction with hot hexane followed by recrystallization from absolute ethanol or methanol.

Table V lists the yields and physical properties for compounds 3–15. A more complete description including spectra for these compounds is given in the supplementary materials.

Temperature-Dependent ¹H NMR Spectra. The ¹H NMR spectrum of the macrocyclic compound (about 20 mg) in methylene-d₂ chloride was first obtained. Then the solution was mixed with an equimolar amount of the alkylammonium salt (or half-molar in the case of the 2:1 complex) and another ¹H NMR spectrum was obtained. The probe temperature was then lowered until one or more sets of peaks separated (usually -60 to -105 °C; the specific temperatures are in the supplementary material). Successive ¹H NMR spectra were taken while the temperature was raised to about 20 °C above the coalescence temperature. The calculated free energy of activation (ΔG[‡]) values are listed in Table I.

Determination of log K, ΔH, and TΔS in Methanol. Log K, ΔH, and TΔS values for the interaction of several ligands with the cations shown in Table II were determined in methanol (Fisher reagent, <0.05% H₂O) at 25 °C by isoperibol titration calorimetry in a 25-mL reaction vessel as described previously.³⁴ Those values shown in Table III were determined in 50% v/v chloroform (Mallinckrodt reagent)/methanol under the same conditions as those carried out in methanol. The sources and purities of metal salts used are given elsewhere.⁹ Doubly recrystallized

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portions of the ligand were used to prepare titrant ligand solutions. For experiments in the chloroform/methanol solvent, special attention was paid to sealing the calorimeter vessel against leaks; the head above the vessel was flushed with chloroform during the pre-equilibration period, which was longer than usual (approximately 1 h).

Cation Transport Measurements. The rates of transport of metal nitrate salts through stirred chloroform membranes containing the carriers listed in Table IV were determined by using a method described previously.³³ A 3-mL stirred chloroform membrane containing 1.0 mM ligand separated a 1.0 M source phase solution of metal nitrate (0.33 M in the case of Ba(NO₃)₂) and a distilled water receiving phase. The moles of cation transferred into the receiving phase in 24 h was determined by ion chromatography (Rb and Cs) or by atomic absorption spectroscopy (all other cations).

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Supplementary Material Available: Complete details on the syntheses and physical properties for compounds 3-15, a table of ¹H NMR spectra of compounds 3-15 and the various alkylammonium complexes with compounds 2-21 (Table VI), and a table of temperature-dependent ¹H NMR spectral data and kinetic parameters for the complexation of primary alkylammonium salts with compounds 2-21 (Table VII) (18 pages). Ordering information is given on any current masthead page.

Solvent Effects on the Deacylation of Acyl-chymotrypsins: A Critical Comment on the Charge-Relay Hypothesis

Susan E. Hamilton and Burt Zerner*

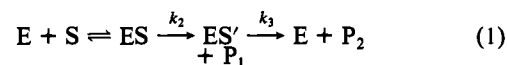
Contribution from the Department of Biochemistry, University of Queensland, St. Lucia, Australia 4067. Received July 25, 1980

Abstract: The pH dependencies for deacylation of a series of acyl-chymotrypsins, prepared by using peptide-free enzyme, are reported. The pK_a' values exhibit considerable variation, from 7.63 for α-benzamido-*trans*-cinnamoyl-chymotrypsin to 6.6 for 3,5-dinitrobenzoyl-chymotrypsin. It is proposed that the variation results from an interaction of variable strength between the carboxylate ion of Asp-102 and protonated His-57 and, further, that the kinetically determined values of the pK_a' are attributable in all cases to the ionization of His-57. High values for the pK_a' controlling deacylation are therefore indicative of a strong electrostatic interaction and low values reflect a relatively weak interaction. The effect of 20.4% (w/w) dioxane on the pK_a' associated with the deacylation of a number of acyl-chymotrypsins is reported. The measured shifts in the value of the pK_a' provide strong evidence for this description of the Asp-His pair in acyl-chymotrypsins. In the hydrolysis of the specific substrate *N*-acetyl-L-tryptophan ethyl ester, the ionizing group controlling deacylation behaves as a simple cationic acid. The results clearly indicate that a "charge relay" is not involved in the hydrolysis of specific substrates and argue against a kinetically important role for aspartic acid acting as a base in the mechanism of action of the serine proteinases.

A simple approach to the problem of characterizing catalytically important acidic groups in enzymes makes use of the differential effect of the dielectric constant of the solvent on the acidity of neutral and cationic acids. Simple neutral acids (RH) decrease in acidity and cationic acids (RH⁺) increase slightly in acidity as the dielectric constant of the solvent is lowered; these effects are qualitatively accounted for on the basis of simple electrostatic models.¹⁻⁴ A decrease in the dielectric constant of the solvent thus affects the pH dependence for an enzyme-catalyzed reaction in a manner which depends on the charge type of the dissociating group or groups participating in the reaction.

Rabin's group first used the approach to characterize the ionizing groups involved in the mechanism of action of bovine pancreatic ribonuclease.⁵ A refinement of their procedure resulted from the development by Hui Bon Hoa and Douzou⁶ of a simple method for determining the hydrogen ion activity of buffered solvent-water systems. The buffers were used to determine directly the effect of solvent dielectric constant on the pH dependencies

for several enzyme-catalyzed reactions.^{7,8} Serine proteinases catalyze the hydrolysis of esters and amides via an acyl-enzyme intermediate (ES', eq 1) in which the active-site serine residue



is acylated. The rate constants for acylation (*k*₂) and deacylation (*k*₃) exhibit similar pH dependencies, suggesting the participation of a single ionizable group, of pK_a' ≈ 7, active in the free base form. Maurel's group determined the effect of a decrease in solvent dielectric constant on the pH dependence for the trypsin-catalyzed hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester⁷ and for the α-chymotrypsin-catalyzed hydrolysis of *N*-acetyl-L-tryptophan ethyl ester.⁸ A small decrease in pK_a' was observed in the former case and negligible change in the latter, consistent in each case with the ionization of a cationic acid. There exists a considerable body of independent evidence which suggests that the group is a histidine residue (His-57 in α-chymotrypsin) and that it acts as a general base in the catalytic mechanism.

In 1969, Blow et al.⁹ published a model of the active site of α-chymotrypsin that was based on an electron density map of the

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