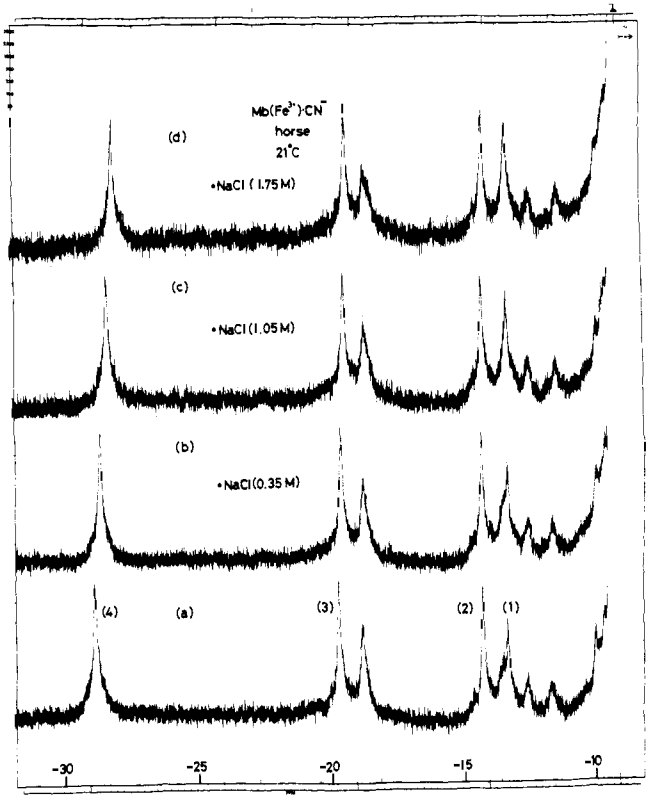

Figure 2. The effect of ionic additives to the nmr spectra (at 220 MHz) of horse Mb · CN^-. The spectra are taken at 21° in the absence (a) and presence (b-d) of NaCl. The heme concentration is 6 m*M* in D_2O.

In order to gain further insight into this restricted methyl rotation, we have examined the effect of some ionic additives on it. Addition of NaCl or $(NH_4)_2SO_4$ caused sharpening of the methyl signal(1) at 21° (see Figure 2), indicating that restricted rotation of this methyl group is released by the enhanced ionic strength of the horse myoglobin solution.[11a] Doubling of the methyl signal(1) in sperm whale $MbCN^-$, however, was not affected by the addition of NaCl or $(NH_4)_2SO_4$. Nonequivalence of these methyl proton signals in horse and sperm whale $MbCN^-$ also was not perturbed by the change of the pD value. These findings may allow us to expect that van der Waals contact at the methyl group(1) in horse $MbCN^-$ may be broken, presumably by perturbing the salt bridge, when NaCl is added and the ionic strength of the solution is increased.[11b]

In azido complexes of ferric horse and sperm whale myoglobin, there was no nmr spectral evidence for restricted rotation of heme side chain methyl group. For imidazole complex of sperm whale myoglobin, however, two of the four hyperfine-shifted methyl signals exhibited specific broadening, which could be interpreted in terms of restricted rotation.[12] The studies on the effect of some axial ligands on the hindered rotation of heme side chain methyl group are now under way.[13,14]

It is, therefore, tempting to expect that monitoring of rotational barrier of heme side chain methyl group could serve as a sensitive probe to detect some van der Waals contacts or steric interaction between heme side chain and apoprotein.[13]

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(9) The notation of pyrrole ring PL should be referred to ref 4.
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(11) (a) Addition of ionic additives also caused sizable upfield shift of the hyperfine shifted heme side chain signals. Details will be published in a separate paper (I. Morishima and T. Iizuka). (b) The effect of NaCl on COO^- group was confirmed by observation of an NaCl-induced specific shift[13] of the one methylene signal at +2.1 ppm from DSS and also by an NaCl-induced shift of the ^{13}C resonance of the COO^- group (I. Morishima, *et al.*, unpublished results).
(12) The proton nmr studies of ferric azido and imidazole, with attention to thermal equilibrium between high spin and low spin states, have been published (ref 1). Spectral features of these complexes are seen in these references.
(13) Details of the nmr study on the van der Waals interaction between heme side chain and apoproteins will be published in a separate paper (I. Morishima and T. Iizuka, submitted to *Biochim. Biophys. Acta.*)
(14) It has been anticipated by Otsuka that the slack of van der Waals contacts between heme side chain and apoprotein occurs with rising temperature, independently of the kind of axial ligands (see S. Otsuka, *Biochim. Biophys. Acta*, **214**, 233 (1970)).

Isao Morishima*
Department of Hydrocarbon Chemistry
Faculty of Engineering, Kyoto University
Kyoto 606, Japan
Tetsutaro Iizuka
Department of Biophysical Engineering
Faculty of Engineering Science, Osaka University
Osaka 560, Japan
Received July 8, 1974

Enantiomer Differentiation in Transport through Bulk Liquid Membranes[1]

Sir:

Transport in biological systems of amino acids through lipophilic cell walls up concentration gradients (active transport) is linked to H^+, Na^+, or K^+ transport down concentration gradients (passive transport).[2] Metal cation transport with natural or synthetic multiheteromacrocycles through thin synthetic membranes and organic bulk liquid membranes has been studied extensively.[3] Results have appeared of a study of amino acid and dipeptide transport by lipophilic anions or cations through bulk toluene from one aqueous solution to a second.[4]

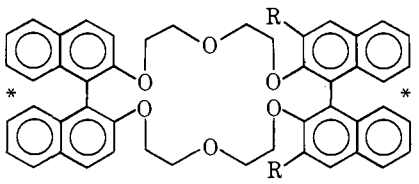
We report that *enantiomer differentiation* occurs when designed, neutral, lipophilic, and chiral host compounds[5] carry amino ester salts (guest compounds) from one aqueous solution through bulk chloroform to a second aqueous solution. Entropy of dilution and inorganic salt "salting out" of the organic salt provided the thermodynamic driving force for transport. Optically pure compounds **1, 2,** and **3** of established configurations were hosts,[5a] and **4, 5,** and **6** were guests.[5] Rate constants for transport were measured for the faster moving A enantiomer (k_A) and for the slower moving B enantiomer (k_B). Table I records the conditions and results. Transport of guest in the absence of host was at

Table I. Differential Transport of Enantiomers A (Faster Moving) and B (Slower Moving) of Guest by 0.027 *M* Host in Chloroform at 24°

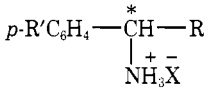
Run no.	Method[b]	Time, hr	Host	Initial guest α-phase: Compound	Initial guest α-phase: Concn (*M*)	Final guest β-phase: Concn (*M*)	Final guest β-phase: Isomer A: Config	Final guest β-phase: Isomer A: % opt purity	k_A/k_B	EDC[a] values K_A/K_B
1a	One	20	(*SS*)-**1**	(*R*)-**4**·HBr	0.05	0.007	*R*		1.5	1.5
1b	One	15	(*SS*)-**1**	(*S*)-**4**·HBr	0.05	0.005				
2a	One	15	(*SS*)-**1**	(*R*)-**4**·HBr	0.10	0.010	*R*		1.45	1.5
2b	One	4.5	(*SS*)-**1**	(*S*)-**4**·HBr	0.10	0.002				
3a	One	9	(*RR*)-**2**	(*S*)-**4**·HBr	0.10	0.008	*S*		2.3	
3b	One	10	(*RR*)-**2**	(*R*)-**4**·HBr	0.10	0.005				
4	Two	133	None	(*S,R*)-**4**·HBr	0.05	0.001		0	1.0	
5	Two	45	(*SS*)-**1**	(*S,R*)-**5**·HCl	0.21	0.036	*S*	35	2.2	2.5
6a	One	3	(*RR*)-**2**	(*R*)-**5**·HCl	0.05	0.009	*R*		10	12
6b	One	18	(*RR*)-**2**	(*S*)-**5**·HCl	0.05	0.005				
7	Two	19	(*RR*)-**2**	(*S,R*)-**5**·HCl	0.28	0.033	*R*	78	9	12
8	Two	12	(*RR*)-**3**	(*S,R*)-**5**·HCl	0.28	0.022	*R*	82	12	
9	Two	32	(*RR*)-**3**	(*S,R*)-**5**·HCl	0.28	0.034	*R*	77	8	
10	Two	66	None	(*S,R*)-**5**·HCl	0.28	0.005		0	1.0	
11	Two	182	(*RR*)-**2**	(*S,R*)-**6**·HCl	0.28	0.028	*R*	85	14	18[a]
12	Two	182	None	(*S,R*)-**6**·HCl	0.28	0.001		0	1.0	

[a] See ref 5a. [b] See footnote 6.

least one order of magnitude slower than in the presence of host (compare run 1 with 4, run 6 or 7 with 10, and run 11 with 12).[6]



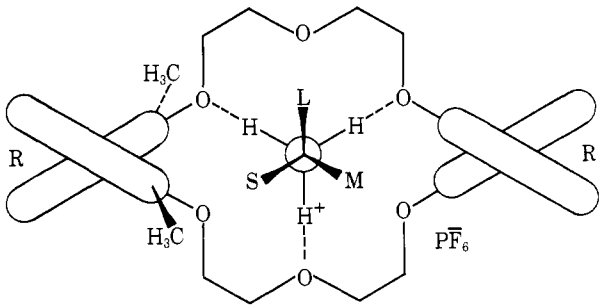
1, R = H
2, R = CH_3
3, R = CH_2Cl



4, R = CH_3; R′ = H
5, R = CO_2CH_3; R′ = H
6, R = CO_2CH_3; R′ = OH

The k_A/k_B values when host was present ranged from 1.5 to 14, and varied with the structures of both the host and guest. Runs 1-3 involved α-phenylethylammonium salt (**4**). In run 3, host **2** that contains two additional methyl groups gave k_A/k_B = 2.3, as compared to 1.5 obtained with parent host **1** in runs 1 and 2. The two methyl groups extend the chiral barrier of the binaphthyl units and increase the chiral recognition of the host by guest. A more dramatic increase from k_A/k_B = 2.2 (run 5) to k_A/k_B = 10 (run 6) was observed for phenylglycine ester salt (**5**) when the two methyl groups were present in the host. The presence of two additional chloromethyl groups in (*RR*)-**3** gave similar values (8-12, runs 8 and 9). The highest value, k_A/k_B = 14 (run 11), was obtained when *p*-hydroxyphenylglycine ester salt (**6**) was employed, with (*RR*)-**2** as host.

The symbols L, M, and S stand for the large, medium, and small groups attached to the asymmetric center of the guest salts. The observed chiral recognition in transport by the host of the guest is rationalized in terms of structure **7** as the more stable diastereomer when (*RR*)-**2** is the host for the three salts. The cycle itself possesses a C_2 axis, and the same complex is formed when the alkylammonium ion is bound to either face of the macroring. The large aryl groups occupy one of the two cavities between the walls formed by the two naphthalenes on the complexed side of the cycle. The small and medium sized groups occupy the other cavity. The small hydrogen in Corey-Pauling-Koltun molecular models of **7** is thrust against the methyl-extended chiral barrier, and the medium sized methyl or carbomethoxy groups are aligned parallel to the face of the naphthalene ring.



7

In prior experiments, enantiomers of racemic amine salts **4-6** were distributed between aqueous inorganic salt solutions, and chloroform solutions of optically pure hosts, **1** or **2**. The results provided enantiomer distribution constants, EDC = D_A/D_B, where D_A is the distribution coefficient of the more, and D_B that of the less complexed enantiomer drawn into the chloroform phase.[5a] The data of Table I indicate that $k_A/k_B \sim$ EDC. The relationship, $k_A/k_B = D_A/D_B$ has been derived[7] and applies when the following conditions are fulfilled: (1) all guest in the organic phase is essentially completely complexed by host, which is soluble only in the organic phase; (2) the mixing in the organic layer (the slow step in the transport) provides a uniform concentration of host and a concentration gradient of guest throughout the medium; and (3) at the interfaces,[6] the enantiomeric organic salts continually equilibrate between the two phases. The kinetic evaluation used here requires that at the β-interface,[6] the organic salts pass essentially irreversibly into the β-phase, a condition that applies strictly only at zero time. The data suggest these conditions were approached. Transport experiments of this kind can be used to determine EDC values, which in turn can be used to determine separation factors in chromatographic resolution.[5b] The method is particularly valuable in cases where D_A and D_B values are extremely low. In the transport experiments, longer times can be exchanged for low *D* values.

Host compound **2** carried the faster moving enantiomer of salts **4** and **5** through the chloroform layer more rapidly

by factors of 2 and 3, respectively, than did host compound **1** without the two methyl groups. Apparently although more sterically hindering, the methyl group's inductive effect makes the aryl oxygens of **2** more basic than those of **1**, and **2** is therefore a better binder. Host **2** carried the faster moving enantiomers of the three racemic salts through chloroform at rates that varied with structure as follows: **5** ~ **4** > **6**. Without host present, the orders of relative rates of transport also were **5** ~ **4** > **6**. These orders probably reflect both the hydrophilicity-lipophilicity of the organic salts and the binding capacity of the salts for the hosts.

Although these results describe passive transport, active transport experiments involving chiral recognition by charged and noncharged hosts have been designed and are in progress.

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(6) In a "U-tube" of 14 mm internal diameter was placed 10 ml of spectral grade chloroform that was 0.027 *M* in optically pure host compound. Water, 0.80 *M* in $LiPF_6$ and 0.08 *M* in HCl (5.0 ml) containing the guest amine salt, was placed in the α-arm. The β-arm contained 5.0 ml of 0.10 *M* HCl solution in water. The α- and β-interfaces were about 1.5 cm^2 each, and the average chloroform path length was about 6.5 cm. A small magnetic stirrer in the chloroform mixed (constant rate) that phase, and less well the aqueous phases. Transport rates of the guest salts were followed with the absorbence of the β-phases in the uv spectrum at 256 nm for **4**, 265 or 272 nm for **5**, and 291 nm for **6**. Host compound was undetectable (uv) in the aqueous phases. In method one individual transport rates for each optically pure guest salt (ref 5) were measured in separate runs under identical conditions through ~10% transport. Essentially linear plots of ten or more points of absorbance *vs.* time for the β-phase extrapolated to zero time gave zero-order rate constants for transport of each enantiomer. Method two involved racemic guest salt. After ~10% transport, amine in the β-phase was isolated. From the signs and magnitudes of rotations, and the established maximum rotations and configurations of the amines, k_A and k_B values (one point first-order rate constants) were estimated. Control runs on the isolation procedures with known starting solutions were made and established the validity of the procedure.

(7) M. Newcomb and D. J. Cram, to be submitted for publication.

Martin Newcomb, Roger C. Helgeson, Donald J. Cram*

Contribution No. 3373, Department of Chemistry
University of California at Los Angeles
Los Angeles, California 90024

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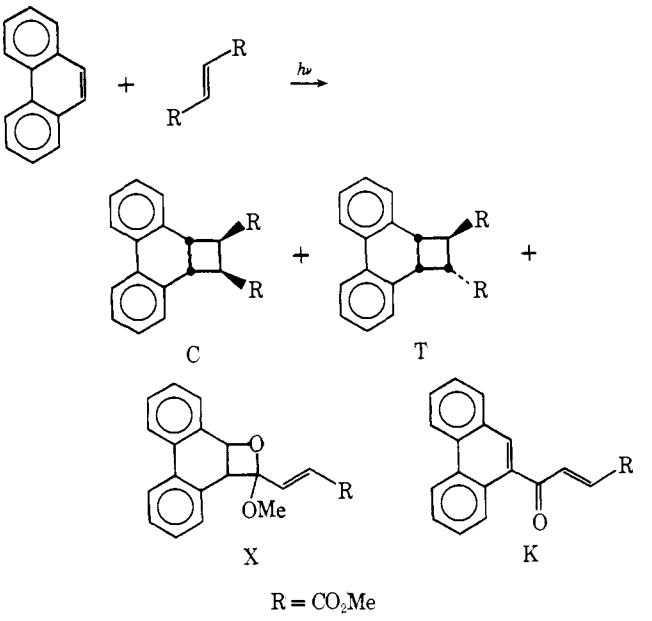
Role of a Singlet Exciplex in the Photocycloaddition of Phenanthrene to Dimethyl Fumarate

Sir:

Many 2 + 2 photocycloadditions are thought[1] to proceed *via* the intermediacy of exciplexes,[2] but direct evidence for this mechanistic pathway is sparse.[3] Both singlet[4] and triplet[4,5] exciplexes have been suggested as intermediates in the photoaddition of phenanthrene (P) to dimethyl fumarate (F) and maleate (M), and there is strong evidence[3,6] that photoadditions of 9-cyanophenanthrene to "electron-rich" olefins proceed *via* singlet exciplexes. Kaupp[7] has recently questioned the intermediacy of an exciplex in the P + F and M photocycloadditions and has suggested an alternative singlet biradical pathway. We now report the quenching of the P + F singlet exciplex by electron donors. The results confirm the mechanism originally suggested by Farid[4] for the P + F photocycloaddition and indicate that charge transfer is an important factor in the quenching of singlet exciplexes.

Irradiation (347 nm) of P in the presence of F in outgassed benzene afforded[4] the isomeric cyclobutanes C and T and the oxetane X (isolated as the ketoester K following acid treatment). The ratio T/C was 2.2 and X/(C + T) was 2.3 at [F] = 0.26 *M*. The fluorescence of P in benzene was quenched by F (k_F = 7.5 × 10^9 l. mol^{-1} sec^{-1}) and a broad, weak, emission (λ_{max} = 452 nm) was observed at increasing [F]. This new emission is red shifted (λ_{max} = 467 nm) and diminished in intensity[8] on addition of methanol (5% by volume). These results confirm Farid's observation[4] of a singlet exciplex.[2,8] The weakness of the exciplex fluorescence may explain Kaupp's failure[7] to observe it.



R = CO_2Me

An exciplex can be simply regarded as an excited donor: acceptor pair, *e.g.*, $(P^{\delta+}- - -^{\delta-}F)^*$, and ought to be susceptible to further charge-transfer perturbation. Thus the P + F exciplex fluorescence intensity is dramatically attenuated by electron donors (see Figure 1) with no concomitant effect on the residual P fluorescence. The quenching efficiencies ($k_Q{}^F\tau_x$) parallel[9] the ionization potentials (Ip) of the donors (Table I). P itself quenches the exciplex fluorescence (see Table I) but F does not. Formation of ground state

Table I. Quenching of Exciplex Fluorescence and Photoproduct Formation by Electron Donors[a]

Quencher, Q	*Ip*,[b] eV	$k_Q{}^F\tau_x$, M^{-1} [d]	$k_Q{}^R\tau_x$, M^{-1} [f]
2-Methylbut-2-ene	8.89	<0.1	
Ethyl vinyl ether	8.49[c]	0.7	
2,3-Dimethylbut-2-ene	8.30	2.4	2.3[e]
Dihydropyran	8.34	3.9	4.2
Phenanthrene	8.10	5.0[g]	
Triethylamine	7.50	9.4[e]	8.7[e]

[a] [P] = 8 × 10^{-3} *M*, [F] = 0.1 *M*. [b] Adiabatic *Ip*'s from J. L. Franklin, J. G. Dillard, H. M. Rosenstock, J. T. Herron, K. Fraxl, and F. H. Field, *Nat. Stand. Ref. Data. Ser., Nat. Bur. Stand.* No. 26 (1969). [c] M. P. Niemczyk, N. E. Schore, and N. J. Turro, *Mol. Photochem.*, **5**, 69 (1973). [d] Precision ±5% air saturated benzene. [e] Outgassed benzene. Air saturated values are the same within experimental error. [f] Precision ±10%. [g] From [P] dependence of $k_Q\tau$ values for quenching by 2,3-dimethyl-2-butene.