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Conformational Analysis of Carbocyanine Dyes with Variable-Temperature Proton Fourier Transform Nuclear Magnetic Resonance Spectroscopy

P. M. Henrichs* and S. Gross

Contribution from the Research Laboratories, Eastman Kodak Company, Rochester, New York 14650.

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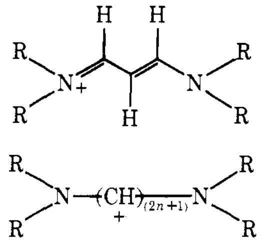
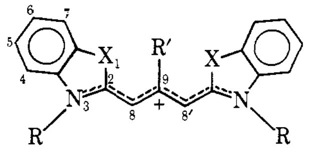
Abstract: The conformational properties of a series of meso-substituted carbocyanine dyes have been investigated by means of variable-temperature proton Fourier transform NMR spectroscopy. At equilibrium, the dyes in acetone-d_6 exist in varying proportions of two conformations differing in geometry about one of the 8-9 bonds in the connecting chain. The cis conformation is favored by meso substitution, the free-energy differences being 0.42 ± 0.1 (−9 °C), 0.65 ± 0.2 (−26 °C), and 0.70 ± 0.2 kcal/mol (−28 °C), respectively, for 3,3′-diethyl-9-methyloxacarbocyanine perfluorobutyrate, 3,3′,9-triethylthiacarbocyanine perfluorobutyrate, and 3,3′-diethyl-9-methylselenacarbocyanine perfluorobutyrate, with estimated errors indicated. The free energies of activation for conversion of the cis into the trans forms of the above three dyes were 14.6 ± 0.1 (12 °C), 12.6 ± 0.1 (−32 °C), and 12.2 ± 0.2 kcal/mol (−28 °C), respectively. For the sulfur- and selenium-containing dyes, exchange of the nonequivalent protons on the connecting chain occurred rapidly by means of a path not involving the trans conformation as an intermediate. The very strained di-cis conformation, which is cis at both bonds 8-9 and 8-9′ in the connecting chain, is proposed as an alternative intermediate. The free-energy barriers for conversion of the cis into the di-cis conformation are 8.4 ± 0.2 (−103 °C) and 8.0 ± 0.3 kcal/mol (−103 °C) for the sulfur- and selenium-containing dyes. The barrier is raised to 14.7 ± 0.1 kcal/mol (12 °C) for the oxygen-containing dye. A phenyl group is an effectively smaller substituent in 3,3′-diethyl-9-phenylthiacarbocyanine perfluorobutyrate than are the alkyl groups in the other dyes, the free-energy difference between the cis and trans conformations being 1.00 ± 0.30 kcal/mol (−27 °C). The free-energy barrier for the cis to di-cis process is much greater for the phenyl-substituted dye than for the other compounds. It is apparently larger even than the barrier for the cis to trans exchange of 13.8 ± 0.5 kcal/mol (−5 °C) and, thus, cannot be determined accurately. Correspondingly, very bulky meso substituents lower the barrier for conversion of the cis into the di-cis form through an increase of the energy of the ground-state conformations. The low barrier for 3,3′-dimethyl-9-*tert*-butylthiacarbocyanine perchlorate results in its having a temperature-independent NMR spectrum between 35 and −100 °C.

Cyanine dyes play an important role in the photographic process by sensitizing the silver halide emulsion to appropriate wavelengths of light.[1] In the simplest sense, any compound with the general structure I may be considered a cyanine, but the photographically important materials are terminated with heterocyclic rings as in II. Those dyes, such as II, containing a three-carbon bridge, are referred to as carbocyanines.

Space-filling models indicate that steric interactions are minimized in the extended or "all-trans" conformation of unsubstituted carbocyanine dyes and x-ray crystallography has shown 3,3′-diethylthiacarbocyanine (IIa) to be in the all-trans conformation in the solid state.[2] A general discussion of the relative stability of the various conformations of carbocyanine dyes has been given by West, Pearce, and Grum.[3] Dyes substituted in the 9-position show steric interaction of the substituent with the two heteroatoms in the "all-trans" (or simply trans) conformation. The interaction with one of the heteroatoms is removed by conversion of the dye into the "mono-cis" (or simply cis) conformation, although it is replaced by crowding involving the 8-proton. Carbocyanine dyes with 9-substituents have been found in both the trans[4] and cis[5] conformations in the solid state. Absorption spectroscopy has indicated that 9-substituted carbocyanine dyes exist in solution as a mixture of conformations;[6] the equilibrium can be shifted by irradiation into the absorption bands of either the cis or the trans form.[3] Even unsubstituted carbocyanines can be partially converted into the cis conformation in solution,[7] and the rate of cis–trans interconversion has been measured in a number

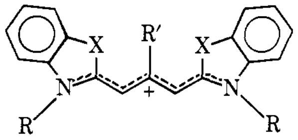


I

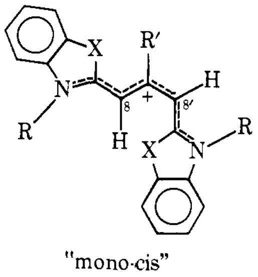


II

a, X = S; R = Et; R′ = H
b, X = S; R = R′ = Et
c, X = S; R = R′ = Me
d, X = O; R = Et; R′ = Me
e, X = Se; R = Et; R′ = Me
f, X = S; R = Me; R′ = Ph
g, X = Se; R = Et; R′ = Ph
h, X = S; R = Et; R′ = *t*-Bu



"all-trans"



"mono-cis"

of cases.[8,5b] The Arrhenius activation energies are generally between 12 and 16 kcal/mol for dyes of type II.

Conformational isomerism has also been observed in other cyanine dyes, particularly those of type I.[9,10] For longer chain dyes, the barrier to internal rotation is apparently higher than for the carbocyanines, since separate components have been isolated by chromatography for the heptamethine dye 3,3′-diethylthiatricarbocyanine chloride. Each fraction, when redissolved, gave the original mixture.[11]

Because of its inherent time scale, NMR spectroscopy has been useful for the measurement of rate processes with barriers between 5 and 25 kcal/mol.[12] It is also a convenient method by which to measure conformational equilibrium constants, since differing extinction coefficients do not have to be considered as in other types of spectroscopy. A number of general studies of compounds of type I not containing heterocyclic rings have been made by NMR spectroscopy[13] and in some cases the barrier to rotation about the C–N bond was determined.[10] NMR spectroscopy has also been used for a few other investigations of other cyanine dyes,[14] but the requirement that spectra be obtained at low temperatures where solubilities are depressed has prevented detailed conformational studies.

We have found that by a careful choice of solvents and counterions to increase solubilities at low temperature and by the use of the Fourier transform method for sensitivity enhancement, we have been able to examine a variety of substituted carbocyanine dyes with variable-temperature proton NMR spectroscopy. Considerably more detailed information has been obtained than resulted from previous investigations with other methods. The study of the conformational behavior of compounds having extended conjugated chains is important in light of current interest in the conformations of retinal and related compounds, which play a role in the mechanism of vision.[15]

Results

Line Shape Analyses. The barriers for conformational interchanges in the dyes were determined through an analysis of various line broadenings and splittings resulting from sample cooling. The appropriate rate constants were obtained by line shape analysis with the computer program DNMR3.[16] In principle, at least, rate constants at several different temperatures can be used for the calculation of a complete set of thermodynamic parameters of activation. In practice, various systematic errors and experimental problems make accurate enthalpies and entropies of activation difficult to determine by NMR.[12c–e] On the other hand, free energies of activation may be obtained at a single temperature with the equation

$$\Delta G^{\ddagger} = -RT \ln (h/\kappa T)k$$

where R is the gas constant, T is the temperature, κ is Boltzmann's constant, h is Planck's constant, and k is the measured rate. Because of the logarithmic relation, an error of 20% in the rate constant leads to an error of only about 0.1 kcal/mol in the calculated $\Delta G^{\ddagger}$ value at −30 °C. Likewise, an error of ±2 °C in T leads to an error less than ±1%.

We have not attempted calculation of enthalpies and entropies of activation for the dyes described below because the spectral simulations were complicated by temperature-variant chemical shifts, by equilibrium constants as well as rate constants being variables, and, in some cases, by severe dipolar line broadenings at low temperature. Furthermore, possible small, long-range couplings were ignored. Nevertheless, we feel the rate constants resulting from a visual match of the calculated and experimental spectra are, except where noted, within the ±20% range mentioned above. In the absence of additional complications, such as peak overlap, the free energies of activation are believed to be accurate to within 0.1 kcal/mol. Special problems are described with respect to individual dyes. Equilibrium constants measured from integrated peak areas are believed to be accurate to roughly ±0.1 except where noted.

3,3′,9-Triethylthiacarbocyanine Perfluorobutyrate (IIb). The perfluorobutyrate counterion proved useful in enhancing the solubility of this and the other dyes to the point that 8.0×10^{-3} M solutions could be studied between 50 and −110 °C with supercooling.[17] Each spectrum required about 10 min of accumulation with the pulsed Fourier transform method. The results at various temperatures for IIb are shown in Figure 1. Chemical shifts for the nonaromatic protons are given in Table I.

The temperature effects were particularly pronounced for the 8-proton peaks. At room temperature, only a single line was observed owing to rapid averaging on the NMR time scale. Cooling caused this line first to broaden and then to split into two lines of unequal intensity. The smaller of these lines was assigned to the two equivalent 8- and 8′-protons in the trans conformation. Cooling of the sample to below −100 °C caused the larger line to split into two new peaks of equal intensity, which were assigned to the nonequivalent 8- and 8′-protons of the cis conformation. Changes in the NMR spectrum of the ethyl peaks and even the aromatic peaks also occurred with sample cooling.

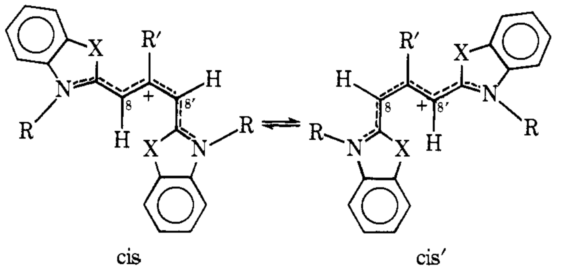
The chemical shifts, especially for the 8-proton peak in the trans conformation, were affected by a change in the counterion from perfluorobutyrate to perchlorate as shown in Table I. The addition of 10% D_2O caused similar changes. The effect of the counterion suggests that ion pairing may be responsible for the shifts, since the nature of the counterion reportedly affects ion pairing and chemical shifts in other related nitrogen-containing aromatic cations.[18]

The higher temperature change in the NMR spectrum of the 8-protons is a result of slowing on the NMR time scale of cis–trans interconversion. The lower temperature change is due to additional slowing of the cis to cis′ interconversion shown in eq 1. Spectral simulation was done separately for the two

Table I. Chemical Shifts (δ) of Nonaromatic Protons of Carbocyanine Dyes in Acetone-d_6

Dye	Temp, °C	Position 8-H	 9-Substituent	 N-Substituent
IIa	35	6.78 d (J = 13.0)	*a*	4.53 q, 1.48 t (J = 7.0)
IIb	34	6.63	3.06, 1.42 (J = 7.0)	4.67 q, 1.58 t (J = 7.0)
	−61	7.18 6.55	2.98,[b] 1.39[b]	4.71,[b] 1.54[b]
	−107	7.14, 6.52, 6.37	*a*	*b*
IIb perchlorate	35	6.61	3.06, 1.43	4.65 q, 1.57 t
	−61	6.99 6.53	2.97,[b] 1.38[b]	4.69, 1.54[b]
IIb[c]	−61	7.01 6.53	*b*	*b*
IId	50	6.29	2.78	4.43 q, 1.54 t (J = 7.0)
	−9	6.83, 6.37, 5.82	2.92, 2.68	*b*
IIe	34	6.86	2.64 s	4.63 q, 1.54 t (J = 7.0)
	−65	7.40 6.70	*b*	*b*
	−107	6.77,[d] 6.44[d]	*b*	*b*
IIf	39	7.05	*a*	8.03
	−39	7.20 6.24	*a*	8.03
IIg	35	7.42		4.61 q, 1.51 t (J = 7.0)
IIh	35	6.44 1.41		4.66 q, 1.59 t (J = 7.0)

[a] Obscured by aromatic peaks. [b] Broadened. [c] 90% acetone-d_6, 10% D_2O. [d] Peaks are not completely separated.



(1)

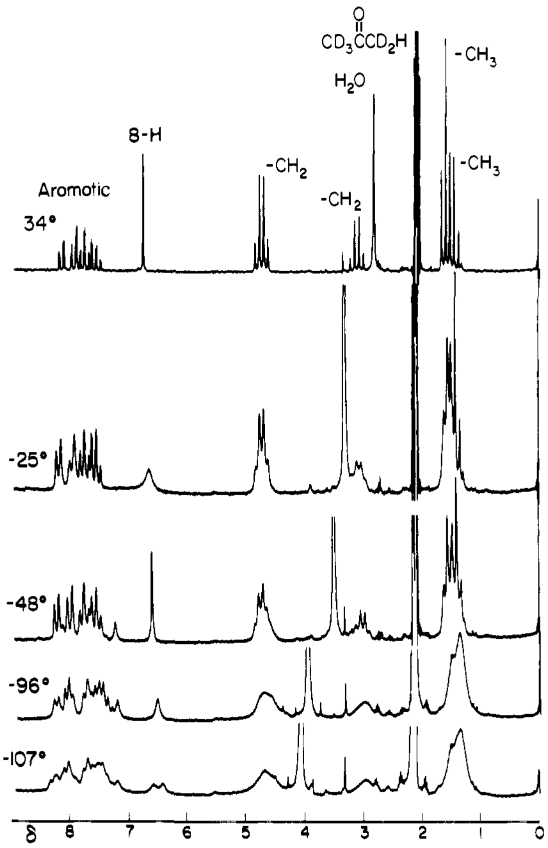


Figure 1. Variable-temperature spectra of 3,3′,9-triethylthiacarbocyanine perfluorobutyrate (IIb). Spectra are scaled to maintain a constant area in the aromatic region except for the top spectrum, which is scaled to 0.3 of the others.

processes with an arbitrarily large rate constant inserted for the cis–cis′ process at the higher temperatures and with a rate of zero for the cis–trans interchange at the low temperatures (Figure 2). The free energy of activation of the cis–cis′ interchange was calculated on the basis that an intermediate is involved and that the overall rate of conversion of the cis into the cis′ conformation is only half that for conversion into the intermediate. The results are given in Table II. Because of the severe line broadening at low temperatures, the calculated barrier is only estimated to be reliable to ±0.3 kcal/mol. Within the error of our measurements, the barrier for cis–trans interchange is unaffected by 10% D_2O in the sample. Equilibrium constants are given in Table III.

Intuitively, one would expect that ethyl and methyl substituents would have approximately the same effective sizes, since the methyl in the ethyl group can usually be accommodated in an uncrowded position.[19] We have found, in fact, that the variable-temperature spectra of the methyl-substituted dye IIc were similar to those of IIb. Unfortunately, however, a pure sample of IIc was not available, so that a detailed study of it was not attempted.

3,3′-Diethyl-9-methyloxacarbocyanine Perfluorobutyrate (IId). The peak of the 8-protons for this dye was broad even at room temperature. With cooling to −9 °C, however, it split into three peaks of almost equal intensity (Figure 3). Further cooling produced differential broadening of the three signals, particularly that in the center, but there was no further splitting.

The almost equal areas of the 8-proton peaks for IId hindered their assignment on that basis alone. Careful integration (see Experimental Section) at several temperatures did, however, indicate that the central peak was slightly smaller than the two outer peaks (Table III). Furthermore, at −42 °C the central peak moved upfield by 15 Hz upon addition of about 10% D_2O to the sample, whereas the upfield peak was shifted downfield by only 3 Hz and the low-field peak was unaffected. By comparison with the results for IIb, the central peak would, therefore, be assigned to the trans isomer and the outer peaks to the cis isomer.

The different degrees of broadening of the 8-proton lines provided another clue to assignments. Although long-range coupling could contribute in part to the observed line widths at low temperature, at least part of the effect is ascribed to dipolar broadening, since the widths become progressively greater at lower temperature. For an isotropically tumbling

Table II. Rate Constants and Free Energies of Activation for Conformational Interchange of Carbocyanine Dyes in Acetone-d_6

Dye	Temp (±2 °C)	$k_{\text{cis-trans}}$[a]	$\Delta G^{\ddagger}$	$k_{\text{cis-cis}'}$	$k_{\text{cis-di-cis}}$[b]	$\Delta G^{\ddagger}$
IIb	−26	40	12.6 ± 0.1	10 000[c]		
	−32	20	12.6 ± 0.1	10 000[c]		
	−103	0[c]		32	64	8.4 ± 0.3
	−107	0[c]		6	12	8.7 ± 0.3
IIb[d]	−32	18	12.6 ± 0.1	∞[c]		
IId	19	80	14.6 ± 0.1	70	60	14.7 ± 0.1
	12	38	14.6 ± 0.1	35	32	14.7 ± 0.1
	7	24	14.6 ± 0.1	24	24	14.6 ± 0.1
	1	10	14.8 ± 0.1	11	12	14.7 ± 0.1
IIe	−28	40–60[e]	12.2 ± 0.3	∞[c]		
	−103	0[c]		80	160	8.0 ± 0.3
IIf	−5	30[e]	13.8 ± 0.5[e]	15[c]	0[c]	

[a] For cis → trans. [b] For cis → di-cis. See text. [c] Assumed values. [d] 90% acetone-d_6, 10% D_2O solution. [e] Of lower accuracy owing to additional assumptions made. See text.

Table III. Conformational Equilibrium Constants and Free Energy Differences of Carbocyanine Dyes in Acetone-d_6

Dye	Temp, °C	K[a]	ΔG, kcal/mol
IIb	−18	0.25[b] ± 0.10	0.71 ± 0.20
	−26	0.27[b] ± 0.10	0.65 ± 0.20
	−32	0.28[b] ± 0.10	0.60 ± 0.20
	−61	0.39[b] ± 0.10	0.40 ± 0.20
IId	−9	0.45 ± 0.10	0.42 ± 0.10
	−80	0.48 ± 0.10	0.28 ± 0.10
	−103	0.45 ± 0.10	0.27 ± 0.10
IIe	−28	0.24[c] ± 0.10	0.70 ± 0.20
IIf	−27	7.8 ± 2.0	−1.00 ± 0.30

[a] For the reaction cis ⇄ trans. Errors are estimated. [b] Best fits of spectra and a plot of ln K vs. $1/T$. [c] Estimated.

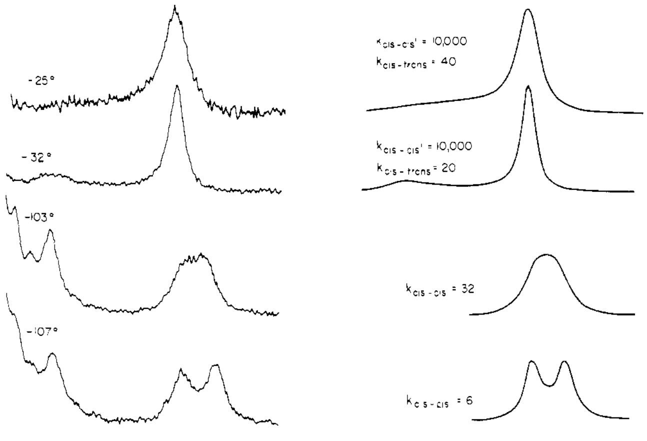


Figure 2. Comparison of experimental and computer-calculated spectra of 3,3′,9-triethylthiacarbocyanine perfluorobutyrate (IIb).

molecule, dipolar broadening should be most severe for those protons in close proximity to other protons. A broader line might thus be expected for the 8-protons in the trans conformation, owing to their interaction with each other, and for the 8′-proton in the cis conformation, owing to its interaction with the methyl group.

An experiment to test the above hypothesis was possible, since the 8-protons in carbocyanine dyes exchange readily; a sample of IId in acetone-d_6 was about 90% deuterated after 5 weeks, as was indicated by the size of the 8-proton peak relative to those of the aromatic signals. At low temperature, the central 8-proton line of the partially deuterated dye was considerably sharper than that of a freshly prepared dye solution. The other peaks were little affected. A reduction of long-range coupling between the 8- and 8′-protons in the cis conformation would cause peak sharpening, but it would affect two lines rather than one. We again assign the central line to the trans conformation. It is sharpened because deuterium, with its lower gyromagnetic ratio, is much less effective in causing dipolar broadening of nearby nuclei than is a proton.[20]

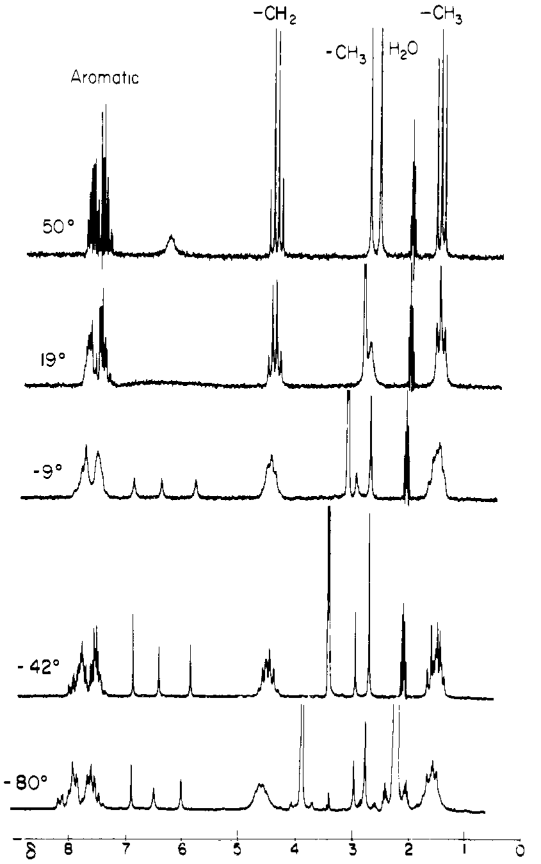


Figure 3. Variable-temperature spectra of 3,3′-diethyl-9-methyloxacarbocyanine perfluorobutyrate (IId).

Results of spectral simulation for IId are shown in Figure 4. The free energies of activation in Table II were calculated on the assumption that cis–cis′ interchange can occur by way of the trans conformation as an intermediate at half the rate for cis–trans conversion itself. The remaining cis–cis′ conversion occurs by way of another route, as is described above and in the Discussion, and for which the free energy of activation is given in Table II. Several test calculations indicated that if

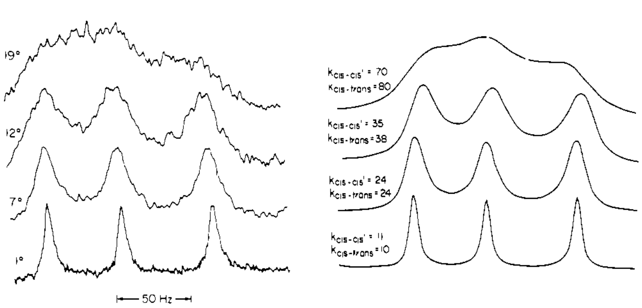


Figure 4. Comparison of experimental and computer-calculated spectra of 3,3′-diethyl-9-methyloxacarbocyanine perfluorobutyrate (IId).

the downfield line were reassigned to the trans conformation, the ultimate rate constants giving the best fit would be within a factor of 2 to those in Table II. The corresponding free energies of activation would be affected by less than 0.3 kcal/mol, so that even a misassignment of peaks would not invalidate our analysis.

3,3′-Diethyl-9-methylselenacarbocyanine Perfluorobutyrate (IIe). The spectral results for this compound were qualitatively very similar to those for the thiacarbocyanine IIb in that cooling first produced splitting of the 8-proton peak into two peaks of unequal area followed by further splitting of the larger peak at lower temperatures. The downfield signal of the trans conformation overlapped the resonances of the aromatic protons, however. Its relative area was crudely estimated by extrapolation of the position of the averaged line above the coalescence point *vs.* $1/T$ to −28 °C. We then have

$$P_{\text{trans}} = P_{\text{cis}}[(\nu_{\text{cis}} - \nu_{\text{avg}})/(\nu_{\text{avg}} - \nu_{\text{trans}})]$$

where P_{trans} and P_{cis} are the relative areas of the trans and cis signals, ν_{cis} and ν_{trans} are the cis and trans chemical shifts, and ν_{avg} is the calculated average shift. The broadening in the cis resonance was simulated with the computer program based on the calculated population. Because of the assumptions involved, the free energy of activation found is, of course, less reliable than those for IIb or IId.

3,3′-Diethyl-9-phenylthiacarbocyanine Perfluorobutyrate (IIf). The 8-proton line for this compound was broad even at room temperature. Cooling caused a sharpening and the appearance of a very small peak to higher field, which was assigned to one of the cis 8-proton peaks (Figure 5). No further splitting of the small peak was seen with additional cooling and, as a result, we believe that cis-cis′ interconversion slows on the NMR time scale in the same temperature range as does cis-trans interconversion, but that one of the signals occurs in the aromatic region and is obscured. The diamagnetic shielding of the phenyl ring might very well cause a substantial downfield shift of the proton adjacent to it in the cis conformation. A position −15.0-Hz downfield from the trans peak was arbitrarily chosen and used in the simulation, since it is not known exactly where in the aromatic region the peak actually does occur. The uncertainty in the position of this line is another source of possible error in the calculations. However, in the slow-exchange region, broadening of the larger line should be insensitive to the exact position of the smaller lines and it was found that adequate broadening of the trans signal was produced by the calculations only when the cis–trans interconversion was faster than the cis-cis′ interconversion. As a result, the rate constant for the former was set equal to twice that of the latter in the final calculations, as would be expected if cis-cis′ interconversion occurred entirely by a pathway involving the trans conformation as an intermediate.

3,3′-Dimethyl-9-phenylselenacarbocyanine Perfluorobutyrate (IIg). The 8-proton line occurred in the midst of the aromatic peaks and was broad even at 50 °C. It was found to sharpen with sample cooling, however, and new small peaks appeared in the aromatic region. Because of the peak overlap, no detailed analysis was made.

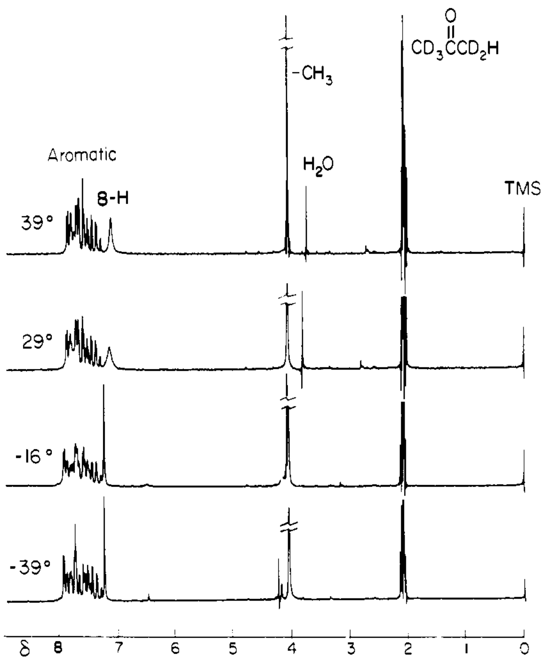


Figure 5. Variable-temperature spectra of 3,3′-diethyl-9-phenylthiacarbocyanine perfluorobutyrate (IIf).

3,3′-Diethylthiacarbocyanine Perfluorobutyrate (IIa) and 3,3′-Diethyl-9-*tert*-butylthiacarbocyanine Perchlorate (IIh). No effect of cooling the samples to −110 °C other than line broadening and slight upfield shifts in line positions was observed.

Discussion

Although only free energies are given in Tables II and III, we would expect the entropy change associated with simple bond rotation to be small, so that free-energy differences should not differ greatly from enthalpy differences. We would expect further that entropy effects would be similar for the various dyes studied. We have made no effort to elucidate fully all contributions to the conformational energy differences and energies of activation, but have concentrated on the changes caused by modifications in the heteroatoms or substituents and have assumed these changes are caused primarily by steric effects.

It may be concluded from Table III that bulkier substituents in the 9-position of carbocyanines favor more of the cis conformation relative to the trans. For example, the unsubstituted thiacarbocyanine dye IIa is believed to exist entirely in the trans conformation,[3] whereas the phenyl-substituted dye IIf has a small amount of cis. The phenyl group has a small effective size in these dyes, since it is known to exist perpendicular to the main plane of the dye, where it has very little conjugative interaction with the chromophore.[21] The much larger ethyl group shifts the equilibrium over to a predominance of the cis conformation. In light of the trend, it would be expected that the *tert*-butyl dye IIh would be entirely in the cis conformation and two 8-proton peaks should be observed at low temperatures. A very low barrier for cis-cis′ interchange precludes this observation within our temperature range, however, as will be shown below.

The proportion of the cis conformation is apparently also increased by larger heteroatoms. The changes in the conformational equilibrium constant are smaller than those produced by substituent changes, since the increased steric interaction of the heteroatom with the group in the 9-position in the trans

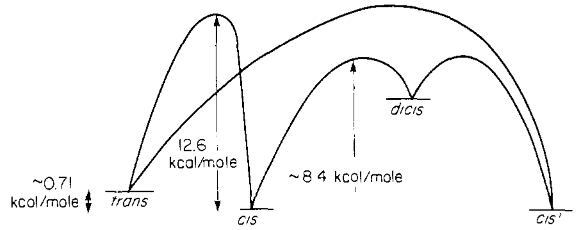
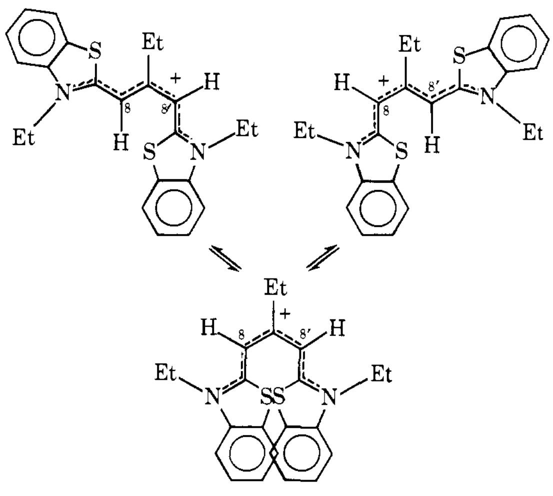


Figure 6. Proposed free-energy diagram for 3,3′,9-triethylthiacarbocyanine perfluorobutyrate (IIb).



conformation is partly balanced by an interaction of the heteroatom with the 8-proton in the cis conformation.

Interestingly, our data indicate that even the oxacarbocyanine IId is largely in the cis conformation. It was previously concluded from the absorption spectrum that this dye was almost entirely trans.[3] Recent molecular orbital calculations have suggested that the two conformations may absorb at almost the same wavelength, and experimental data have confirmed the prediction, however.[22] Thus, a resolution of signals in the absorption spectrum for the two conformations may not be possible.

The conformational equilibrium constant for IId is intriguingly close to that predicted solely by the lower symmetry of the cis compared with the trans conformation.[23] The equilibrium constant was invariant with temperature within the accuracy of our measurements, consistent with an enthalpy difference close to zero. On the other hand, lower temperatures shifted the thiacarbocyanine IIb strongly toward the trans conformation. The temperature effect for this dye might thus be interpreted in terms of a lower enthalpy for the trans conformation. Dimerization of the dye at low temperatures is another possible explanation, though, since it is known that the trans form aggregates more readily than does the cis.[24] The net effect would be an increase in the total concentration of the trans conformation at lower temperatures.

The same factors that control the conformational equilibrium constants also determine the rate constants of conformational interchange. A priori, it might be expected, for example, that the cis–cis′ interchange shown in eq 1 would occur preferentially by way of the symmetrical trans conformation as an intermediate. The overall rate for cis–cis′ interchange would then be one-half that for cis–trans exchange, since every cis dye that passes into the trans conformation has equal probability of returning to the cis conformation or passing over into the cis′ conformation.

Contrariwise, at least for IIb and IIe, cis–cis′ exchange occurs much more rapidly than does cis–trans exchange, indicating that there is a lower energy path not involving the trans conformation. Molecular models do, in fact, suggest such a path. In both the cis and trans conformations, there are two sites of considerable steric interaction. In the cis form, one of these involves one of the heteroatoms and the 8-proton and the other involves the other heteroatom and the 9-substituent. In the trans form, both heteroatoms interact with the substituent. Rotation about the 8′–9 bond of the cis conformation leading to the trans relieves only one of the interactions. Rotation about the 8–9 bond, on the other hand, relieves both steric problems even though it leads ultimately to the "di-cis" conformation. This conformation is so crowded that it probably cannot assume a completely planar geometry. Nevertheless, it provides a nice explanation of the experimental results.

The di-cis conformation is a symmetrical conformation and passage through it ensures interchange of the 8- and 8′-protons. In spite of its inherent strain, we have assumed in all of the calculations that it is a true intermediate since, as rotation around the 8–9 bond proceeds, some of the conjugative energy that is originally lost should be regained even though the di-cis conformation must necessarily be highly distorted from planarity. If instead the di-cis merely represents a transition state, the calculated free energies of activation would be changed slightly, but the overall conclusion would be the same. Indirectly, the calculations provide an upper limit of about 8.4 kcal/mol for the energy of the di-cis form of IIb relative to the cis conformation. The appropriate free-energy diagram is given in Figure 6.

A reduction of the size of either the heteroatom or the 9-substituent raises the barrier for both cis–trans and cis–cis′ conversion (Table II). The effect on the cis–cis′ barrier is particularly dramatic. The steric environment of the transition state between the cis and di-cis forms is less demanding than that of the cis conformation itself. Therefore, introduction of smaller groups should lower the energies of the ground states more than that of the transition state, resulting in a higher overall barrier.

The steric interaction between one of the heteroatoms and the substituent is preserved in the transition state between the cis and trans conformations. Therefore, although sterically smaller substituents and heteroatoms do lower the energy of the ground state, the energy of the transition state is also substantially reduced. Smaller groups are, as a result, much less effective in lowering the barrier for cis–trans interconversion than that for the cis to di-cis process.

The absence of a temperature effect on the spectrum of 3,3′-diethyl-9-*tert*-butylthiacarbocyanine (IIh) is now readily explained. The very large substituent raises the energy of the ground-state cis conformation to the point where the barrier to cis–cis′ interconversion is so low that no line broadenings or resolution of coalesced peaks in the NMR spectrum are observed even at −110 °C.

Additional experimentation in which the equilibria of these dyes are perturbed by irradiation into their optical absorption bands would be interesting. Unfortunately, the intensities of monochromatic light that would be required have so far prevented the successful completion of such an experiment.

Experimental Section

All spectra were recorded on a Varian HA-100 spectrometer, modified and equipped with a Varian 620/i 8K computer to allow Fourier transform operation. The proton-observe signal was derived from a master oscillator also used in the generation of a deuterium field-frequency lock signal.

Experimentally, we found that relative intensities of the 8-proton peaks were not changed even when flip angles as high as 90° were used. For the final spectra compared with those calculated by computer, a more conservative 60° angle was used, however. It has been shown theoretically that the Fourier transform of the free induction decay of chemically exchanging systems is equivalent to the CW spectrum,[25] particularly for small flip angles.[26] The spectra of the oxacarbocyanine IId were accumulated with a 2-s acquisition time corresponding to one data point every 0.25 Hz in the transformed spectrum. Other spectra were recorded with a 1-s acquisition time. Digital filtering was applied when lines were broad, but not to the extent that line shapes were significantly changed.

Integrations were made by weighing tracings of the relevant peaks. Baseline drift made digital integration less satisfactory. For the thiacarbocyanine IIb, the equilibrium constants in Table III were also adjusted to give the best fit in the calculated spectra at a variety of temperatures consistent with a linear plot of $\ln K$ vs. $1/T$. The equilibrium constant for the selenacarbocyanine IIe was estimated by comparison of chemical shifts as described in the text. The value within the range of estimation that gave the best spectral fit is reported in Table III.

Temperatures were measured, both before and after the recording of a spectrum, by means of a calibrated copper–constantan thermocouple in an NMR tube containing the same quantity of acetone as the dye samples. Variations of less than 0.5 °C were normally found. Small-gauge wire (28) was used to minimize heat conduction. Better stability in temperature above -30 °C was obtained when dry ice-acetone, rather than liquid nitrogen, was used as the coolant.

All simulation was done on an IBM 370 Model 158 computer with the program DNMR3.[16] Spectra were plotted with an Calcomp 563 plotter for comparison with the experimental results. Line widths in the absence of exchange were estimated from those of nonexchanging protons. In some cases, there was considerable doubt as to the accuracies of these estimates, but the spectra were compared when possible only in regions at which the natural line widths were small compared with the line widths due to exchange. At low temperatures, the uncertainties in line widths constitute a source of error, especially since we have evidence that they may differ for different lines in the spectrum. Chemical shifts were measured above or below the coalescence points and were extrapolated to the temperatures of the spectra to be simulated by plotting vs. $1/T$. In some cases, there were indications that the actual chemical shifts differ slightly from those obtained by extrapolation, but we believed that variation of them would not improve the overall determination of rate constants.

The dyes used in this study were originally prepared in the dye synthesis laboratory of Eastman Kodak Company by D. W. Heseltine and associates. Purity is routinely checked during synthesis by thin-layer and ionographic methods, and no impurities were detected in the proton NMR spectra.

Perfluorobutyrates were normally prepared from the iodides by reaction for 1 h of 100 mg of dye with 1.0 g of heptafluorobutyric acid (Eastman) and 25.0 g of propylene oxide with gentle warming. The solution was then evaporated to about 5 ml, to which 50 ml of diethyl ether was added. After overnight cooling, the dye was collected by filtration, washed with ether, and dried under vacuum at 65 °C for 1 h. Perfluorobutyrates could also be prepared with an ion exchange column, but residual amounts of perfluorobutyric acid tended to be left in the dye. The acid could be detected at low temperature (-100 °C) by fluorine NMR spectroscopy, since its signals were separated from those of the anion. Excess acid was removable in a vacuum oven, but was not found to have an appreciable effect on the variable-temperature proton NMR spectra.

Where necessary, iodides were prepared from the bromides by precipitation from a solution of the iodide in methanol with a saturated solution of sodium iodide in methanol.

Sample concentrations were 8.0×10^{-3} M in either Stohler 99.5% acetone-d_6 or Bio-Rad "100.0%" acetone-d_6. In some experiments solvent was dried with Fisher 4A molecular sieves, although it was important that such solutions be filtered carefully to remove small particles of the sieves so that the solutions could be supercooled to -110 °C. The use of new NMR tubes also helped in this respect. Except where noted, samples were used within a few days of preparation before extensive deuteration could occur.

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