Application of [13]C Chemical Shifts and Relaxation Times to the Study of Charge Distribution, Aggregation, and Conformation in Carbocyanine Dyes. Part I

By P. Mark Henrichs, Research Laboratories, Eastman Kodak Company, Rochester, New York 14650

[13]C N.m.r. spectra are reported for a variety of symmetrical carbocyanine dyes. Changes in concentration and solvent have minor effects on chemical shifts relative to the total spectral width; chemical shifts can be accounted for primarily in terms of internal dye properties. Qualitative agreement within each dye of chemical shifts with calculated charge densities was found, and the chemical shifts of C-2 could be ordered according to the Brooker dye basicities except for a reversal of 2,2'-diethyloxacarbocyanine and 2,2'-diethylthiacarbocyanine. Preferential tumbling of monomeric thiacarbocyanine about its long axis is indicated by a comparison of the spin–lattice relaxation times of carbons bearing one proton. Higher concentrations and lower temperatures shortened the relaxation times as would be expected for a higher degree of dye dimerization or aggregation. Possible implications about the structure of dye dimers are discussed. Single-frequency decoupling experiments permitted a reassignment of signals in the proton spectrum.

CYANINE dyes are important in the sensitization of photographic systems to desired wavelengths of light.[1] The most general formula is (I), but more commonly the nitrogen atoms are incorporated into heterocyclic rings. This paper concerns itself with dyes of the latter type in which the two rings are separated by a three-carbon chain. Such dyes are referred to as 'carbocyanines'. There is general interest in methods that give details about the charge distribution and the structure of these dyes. [1]H Chemical shifts have been roughly correlated with charge densities and reflect the alternation of charge density in the connecting chain.[2] [1]H N.m.r. has also been shown to give useful information about the structure of the aggregates formed by many cyanine dyes.[3] The ^{1}H spectra are complicated, however, by small chemical shift differences and by spin–spin splitting.

In spite of its lower sensitivity, the carbon spectrum of such compounds may be useful. It has already been demonstrated that carbon chemical shifts reflect charge densities in the connecting chain of dyes of type (I; R =

[1] C. E. K. Mees and T. H. James, 'The Theory of the Photographic Process,' Macmillan, New York, 1966, 3rd. edn., pp. 198—232.

[2] (a) G. Scheibe, W. Seiffert, H. Wengenmayr, and C. Jutz, Ber. Bunsengesellschaft Phys. Chem., 1963, 67, 560; (b) S. Dähne and J. Ranft, Z. phys. Chem. (Leipzig), 1963, 224, 65; (c) R. Radeglia, E. Gey, K.-D. Nolte, and S. Dähne, J. prakt. Chem., 1970, 312, 877.

[3] R. E. Graves and P. I. Rose, J. Phys. Chem., 1975, 79, 746.

alkyl).[4] [13]C Spin–lattice relaxation times may carry information about the structure of dye aggregates and conformations. The present study of the ^{13}C spectra of

$$R \diagdown N-[CH]_{2n+1}-N \diagdown R$$

$$(I)$$
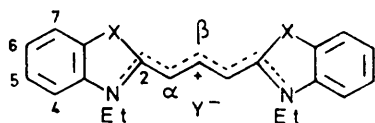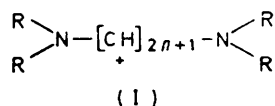
(II) X = NEt, Y = I
(III) X = S, Y = Cl
(IV) X = S, Y = p-MeC$_6$H$_4$SO$_3$; 6,6'-Me$_2$
(V) X = S, Y = p-MeC$_6$H$_4$SO$_3$; 5,5'-Me$_2$
(VI) X = S, Y = ClO$_4$; β-Bu^t
(VII) X = S, Y = Br; β-Et
(VIII) X = O, Y = p-MeC$_6$H$_4$SO$_3$
(IX) X = Se, Y = p-MeC$_6$H$_4$SO$_3$
(X) X = Me$_2$C, Y = I

dyes (II)—(X) was undertaken to learn how much information not available from the ^{1}H spectra [5] could be obtained from ^{13}C chemical shifts and relaxation times of dyes (II)—(X).

RESULTS

Medium Effects.—The dyes that were available had several different counterions and varied widely in solubility. As a result, it was not always possible to prepare samples of sufficient concentration (0.1—0.125M) to give both coupled and decoupled ^{13}C spectra in reasonable periods of time. When solubility was low, therefore, decoupled spectra only were recorded. The different concentrations in different samples were a problem since there may be an equilibrium between significant amounts of both monomer and dimer in the concentration range involved,[6] and chemical shifts could be a function of the proportion of dimeric or higher aggregated material. The most practical overall solution was to demonstrate for one of the dyes that concentration actually has only a relatively small effect on the ^{13}C chemical shifts. As may be seen in Table 1 for dye (III), a change of concentration from 0.05 to 0.125M does change the chemical shifts of the unsaturated carbons by as much as 0.34 p.p.m. The changes are small, however, compared to the chemical shift differences for the various carbons. The largest effects seem to be in the side-chain where the CH$_3$ resonance changes by 1.28 p.p.m. Change of the counterion of (III) from Cl$^-$ to Br$^-$ produced no measureable effect at all on chemical shifts.

Methanol is a good n.m.r. solvent for cyanine dyes because of its low viscosity and because dyes are known to aggregate to a relatively low degree in it. Most of the

previous n.m.r. work, however, has involved dimethyl sulphoxide (DMSO) as solvent,[2,4] since many dyes that have low solubility in methanol dissolve readily in DMSO. The effect on ^{13}C chemical shifts of a change of solvent from methanol to DMSO was checked for a number of the dyes (Table 2). Peak positions were sometimes affected by >2 p.p.m., but the direction of change was the same at each position in the various dyes. The relative ordering of the dyes with respect to each other at the 2- and α-positions was unaffected, so that these positions may be used for comparison of different dyes.

Assignments.—The C-2 and -α peaks are convenient probes of dye properties since they, along with the C-β peak, are easily assigned. In each spectrum, C-α absorbed at <105 p.p.m. Its intensity decreased in highly deuteriated solvents owing to exchange of the attached proton.[3] The C-β signal was always to low field and was of reduced intensity. The lowest-field quaternary carbon signal was assigned to C-2. Quaternary carbon peaks in general were easily identified from the coupled spectrum.

Assignments of the aromatic peaks were more difficult, and only for thiacarbocyanine (III) were they unambiguous. It is known that methyl substitution in aromatic compounds causes small shifts *ortho* and *meta* to the site of attachment.[7] The 5- and 6-methyl substituted dyes (IV) and (V), therefore, allowed assignment of C-5 and -6. The furthest up-
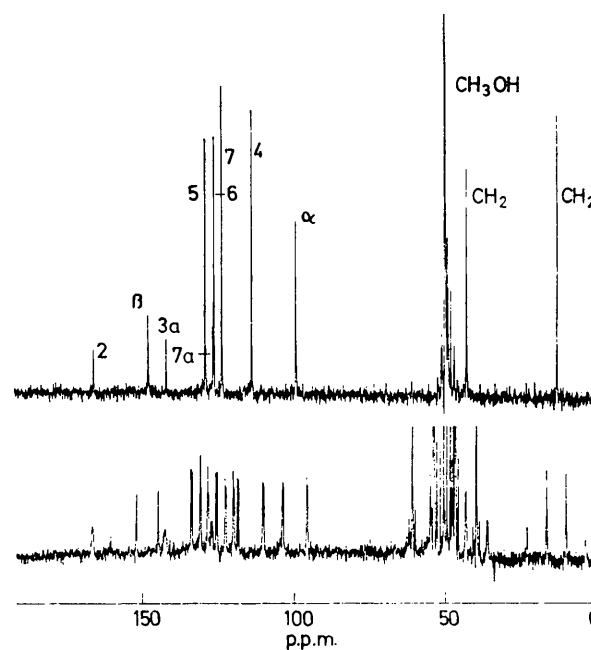

FIGURE 1 Decoupled (upper) and coupled (lower) [13]C n.m.r. of 0.125M-(III). Chemical shifts are from internal tetramethylsilane

field of the remaining aromatic signals belongs to C-4 by analogy with assignments made for benzofuran.[8]

A 'cross-referencing' of the assignments in the carbon

[4] (a) R. Radeglia, G. Engelhardt, E. Lippmaa, T. Pehk, K.-D. Nolte, and S. Dähne, *Org. Magnetic Resonance*, 1972, **4**, 571; (b) R. Radeglia, *J. prakt. Chem.*, 1973, **315**, 1121; (c) R. Radeglia, *ibid.*, 1974, **316**, 344.

[5] (a) S. Dähne and J. Ranft, *Z. phys. Chem. (Leipzig)*, 1966, **232**, 259; (b) E. Kleinpeter, R. Borsdorf, G. Bach, and J. v. Grossmann, *J. prakt. Chem.*, 1973, **315**, 587.

[6] O. Wörz and G. Scheibe, *Z. Naturforsch.*, 1969, **24b**, 381.

[7] (a) J. B. Strothers, 'Carbon-13 N.M.R. Spectroscopy,' Academic Press, New York, 1972, p. 97; (b) G. C. Levy and G. L. Nelson, 'Carbon-13 Nuclear Magnetic Resonance for Organic Chemists,' Wiley–Interscience, New York, 1972, p. 81.

[8] N. Platzer, J.-J. Basselier, and P. Demerseman, *Bull. Soc. chim. France*, 1974, 905.

spectrum of (III) with assignments in the proton spectrum may be made by single-frequency decoupling experiments.[9] Previous reports have shown that the resonance pattern of one of the aromatic protons (assigned to 5-H) is >0.2 p.p.m. upfield from the rest of the aromatic signals.[5]

equal, possibly due to a small difference in ¹H chemical shifts.[11] The 'fingerprint' method has been developed specifically for symmetrical systems,[12] and was consequently of no help for assignments in the other dyes. Some doubt remains, therefore, in the assignments for dye (VIII). The

TABLE 1

¹³C Chemical shifts of carbocyanine dyes in methanol ^{a} (J_{OH}/Hz in parentheses)

Compound	Concentration	2	4	5	6	7	α	β	3a	7a	CH₂	CH₃
(II)	Saturated	149.64	110.49 (165)	124.97 (163)7	124.97 (1643)	110.49 (165)	84.50	143.10	133.49	133.49	40.86	14.05 (128)
(III)	0.125M	165.96	(113.96 (166)	129.21 (164)	126.31 (164)	123.73 (168)	99.37	147.93	142.04	126.70	44.08	14.25
(III)	0.05M	166.31	114.10	129.36	126.50	123.84	99.41	148.26	142.22	126.84	42.84	12.97
(IV)	Saturated		113.73	130.34		123.74					42.80	12.97, 21.62
(V)	Saturated	166.23	114.25	140.32	127.65	123.38	99.36	147.60	142.37		42.71	12.98, 21.63
(VI)	Saturated	164.36	113.58	129.18	126.70	123.79	97.77	178.34	140.99	126.34	43.38	12.98
(VII)	Saturated		114.11	129.73	126.27	123.85	100.14				43.06	12.72
(VIII)	0.1M	163.26	111.84	126.31 ^{b}	127.18 ^{b}	111.84	85.92 (165)	148.71 (153)	132.12 ^{b}	148.28 ^{b}	40.34	13.21 (129)
(IX)	Saturated	170.61	115.56	129.28	127.03	126.41	103.75	151.79			43.78	12.92
(X)	0.1M	175.44	112.16	129.94	126.65	123.53	103.52	152.18	142.83 ^{b}	142.23 ^{b}	40.37	12.76, 28.24

^{a} P.p.m. from internal tetramethylsilane. ^{b} Might be interchanged.

TABLE 2

¹³C Chemical shifts of carbocyanine dyes in [²H₆]DMSO ^{a}

Compound	Concentration	2	4	5	6	7	α	β	3a	7a	CH₃
(II)	0.035M	147.52	109.52	123.41	123.41	109.52	83.57	141.37	131.68	131.68	13.39
(III)	0.1M	163.98	113.24	127.98	125.03	123.03	98.51	146.31	140.66		12.53
(VIII)	0.1M	161.19	111.10 ^{b}	124.90 ^{c}	125.79 ^{c}	110.80 ^{b}	84.82	146.39	130.71 ^{b}	146.00 ^{b}	12.66
(IX)	0.1M	168.68	114.54	127.93 ^{b}	126.29 ^{b}	124.86 ^{b}	103.02	150.57	142.11	125.28	12.45
(X) ^{d}	0.045M	173.34	111.23	128.59	125.11	122.50	102.30	149.86	141.36 ^{b}	140.64 ^{b}	12.19, 27.29

^{a} P.p.m. from internal tetramethylsilane. ^{b} Might be interchanged. ^{c} Might be interchanged. ^{d} Quaternary carbon in (X) 48.79.

TABLE 3

Chemical shifts of (XII) ^{a}

| 2	2′	4, 4′	5,5′, 6,6′, 7,7′	α	α′	β	3a, 3a′	7a, 7a′
164.70	159.13	112.33 114.93	122.28, 123.04, 123.58 125.87, 127.61, 128.33	111.51	93.38	160.06	142.03 140.98	124.88 126.96

^{a} P.p.m. from internal tetramethylsilane.

Single-frequency irradiation of this pattern produced sharpening primarily of the C-6 signal, however. Apparently, the previous assignments of C-5 and -6 were interchanged. Reversal of the assignments brings them into agreement with those made for a related dye by Graves and Rose.[3]

Data in the literature show that most substituents on aromatic rings cause relatively small changes in chemical shifts *ortho* and *para* to the site of attachment.[7b] The principal exceptions are those substituents in which an oxygen or a nitrogen atom is attached directly to the ring. It was, therefore, assumed that the pattern in the aromatic portion of the spectrum would be similar at least for dyes (III), (IX), and (X), and assignments were made accordingly.

Of the remaining dyes, the upfield signal of the nitrogen-containing dye was assigned to C-4 by analogy to the previous assignments. In principle, this assignment could be confirmed by the 'fingerprint' method in which characteristic differences in bandwidths in the coupled spectrum of symmetrically distributed benzenes have been found.[10] For dye (II), however, the bandwidth of C-4 and -5 were

overall assignments for the various dyes are in good agreement with those that have been made for the related compounds (XI).[13]
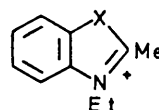
(XII)   X = NR, O, S, Se, or CMe₂

Conformational Effects.—The presence of the β-substituent in the thiacarbocyanine derivatives (VI) and (VII) produced only minor changes in chemical shifts, especially for the benzenoid carbons. Larger differences would have been reasonable, however, since thiacarbocyanine (III) is believed to exist largely in the all-*trans*-conformation shown in ref. 14, whereas the substituted dyes should be mostly in the mono-*cis*-conformation produced by rotation about the C-8–C-9 bond.[15] The spectrum of dye (XII), which is rigidly locked

[9] Ref. 7b, pp. 9—10.
[10] H. Günther, H. Schmickler, and G. Jikeli, *J. Magnetic Resonance*, 1973, **11**, 344.
[11] G. Jikeli, W. Herrig, and H. Günther, *J. Amer. Chem. Soc.*, 1974, **96**, 323.
[12] H. Günther, personal communication.
[13] E. Kleinpeter and R. Borsdorf, *J. prakt. Chem.*, 1973, **315**, 765.
[14] J. Q. Umberger, *Phot. Sci. Eng.*, 1967, **11**, 392.
[15] (a) W. West, S. Pearce, and F. Grum, *J. Phys. Chem.*, 1967, **71**, 1316; (b) L. G. S. Brooker, D. W. Heseltine, and L. L. Lincoln, *Chimia (Switz.)*, 1966, **20**, 327; (c) R. Steiger, R. Kitzing, R. Hagen, and H. Stoeckli-Evans, *J. Phot. Sci.*, 1974, **22**, 151.

in the *cis*-conformation, reveals that there are, in fact, substantial differences in chemical shifts of corresponding carbons in the two benzenoid rings (Table 3). The differences are particularly pronounced for the carbons *ortho* and

normally decoupled spectrum with one in which the decoupler was gated on only during the acquisition period. As expected,[16] the values for the aromatic carbons are equal to the theoretical maximum (1.99) within experimental error,
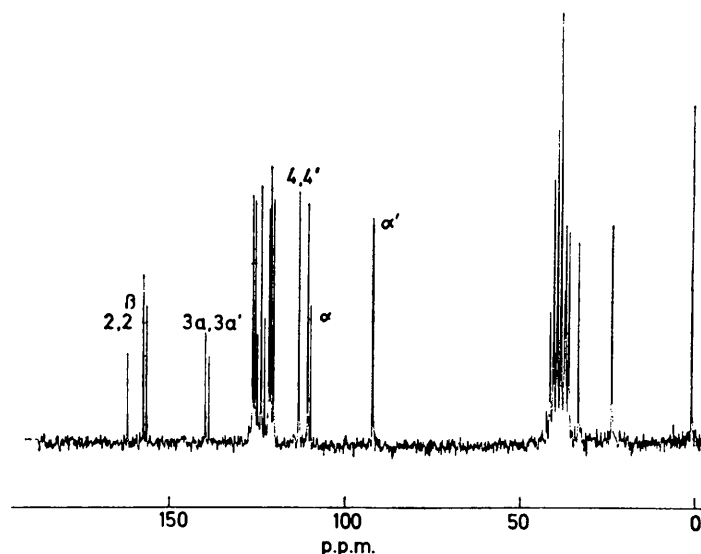

FIGURE 2   Decoupled ^{13}C n.m.r. spectrum of a saturated solution of (XII) in [$^2H_6$]dimethyl sulphoxide.   The peaks are labelled in pairs except for the group between 120 and 130, for which only the signals of C-7a and C-7a' can be assigned with certainty

TABLE 4

Spin lattice relaxation times (s) of thiacarbocyanine (III)

Concentration	$T/°C$	4	5	6	7	α	β	CH_2	CH_3
0.1M [a]	40	0.75	0.54	0.66	0.77	0.72	0.71	0.35	1.30
0.2M [b]	40	0.53	0.42	0.48	0.54	0.50	0.49	0.25	1.02
0.2M [c]	27	0.26	0.24	0.28	0.27	0.27	0.30	0.14	0.58
		(1.90)	(1.95)	(2.05)	(1.95)	(1.75)	(1.77)		

[a] Varian XL-100-15.   Average of two determinations.   [b] Varian XL-100-15.   [c] Varian CFT-20.   NOE values in parentheses

para to the nitrogens. Apparently, the close agreement of chemical shifts of (III), (VI), and (VII) is produced by chemical shift averaging.

(XII)
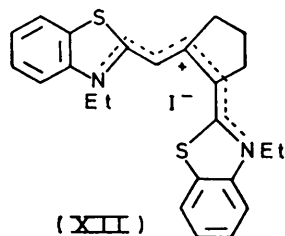
Relaxation Times.—Spin–lattice relaxation times were measured only for thiacarbocyanine (III) since each determination required substantial instrument time owing to the low concentrations involved. It may be seen from Table 4 that the relaxation times of all the carbons of (III) are considerably shorter at 0.2 than at 0.1M. The relaxation times of C-5 are shorter than are those of C-4 and -7, and those of C-6 are intermediate, at least for the spectra at 40°. The ratio of the relaxation time of C-5 to those of C-4 and -7 is 1.4 for a 0.1M solution at 40° but only 1.3 for a 0.2M solution at 40°. The ratio for the 0.2M solution at 27° was 1.1.

The nuclear Overhauser enhancements (NOE) of the unsaturated carbons were measured by comparison of a

indicating that these carbons relax exclusively by the dipolar mechanism. Some care in interpretation is in order, however, since somewhat less than the full NOE was measured for the bridge carbons suggesting possible competition by a second mechanism for those atoms.

DISCUSSION

^1H Chemical shifts of cyanine dyes depend strongly on concentration owing to the formation of dye aggregates. The direction and size of the shifts have allowed conclusions about the structure of a dye aggregate to be made.[3] A change in concentration from 0.05 to 0.125M of dye (III) in methanol affected the ^{13}C chemical shifts of the unsaturated carbons by <1 p.p.m., however, even though the carbon spin–lattice relaxation times and the optical spectrum suggest that dimerization and/or aggregation of the dye does occur (see below). Chemical shifts produced by formation of aggregates are presumably a result of diamagnetic shielding by the aromatic rings of one dye molecule on the protons in adjacent

[16] The dipolar mechanism is found to be dominant for monoprotonated carbons in most aromatic systems, even in undegassed samples. See, for example, R. E. Echols and G. C. Levy, *J. Org. Chem.*, 1974, **39**, 1321.

molecules. Diamagnetic shielding is relatively important for ^{1}H chemical shifts but is of minor importance in the determination of carbon chemical shifts.[17]

Solvent effects on chemical shifts were slightly larger than concentration effects, some changes produced by the change in solvent from dimethyl sulphoxide to methanol being >2 p.p.m. The direction of change was always downfield and the relative order of different dyes in terms of chemical shifts was affected only for the β-position. Overall, it may be concluded that ^{13}C chemical shifts reflect internal properties rather than environmental effects more closely than do ^{1}H chemical shifts.

One internal property of particular interest in cyanine dyes is the charge distribution, and ^{13}C chemical shifts are known to correlate at least qualitatively with calculated charge densities in a variety of aromatic systems.[18] Charge densities for π electrons in a variety of the carbocyanines have been calculated by Sturmer by a modified

charge distribution in different dyes. The ordering of dyes from top to bottom in Table 3 is according to the Brooker deviations,[22] which have long been used as a measure of the ability of a dye heterocycle to donate electronic charge. The chemical shifts of C-2 are found to fall in the same order except for an interchange of thiacarbocyanine (III) and oxacarbocyanine (VIII). The sequence of chemical shifts that we have observed for C-2 is exactly the same as for the chemical shifts for C-2 in the related compounds (XI),[13] for which the chemical acidities of the methyl protons corresponded.[23]

The observed close agreement of the chemical shifts of dyes (VI) and (VII) with those of (III) is apparently due to averaging of the shifts in the two different kinds of rings in the cis-conformation. The chemical shifts of dye (XII), which is rigidly locked in the cis-conformation, suggest, however, that there is a real chemical difference in the ends of that conformation. Since the biggest effects are for those carbons ortho and para to the nitrogen

TABLE 5

Calculated charge densities

Compound	2	4	5	6	7	α	β	3a	7a
(II)	1.022	1.023	0.979	0.979	1.023	1.167	0.970	1.071	1.071
(III)	0.992	1.025	0.983	0.981	1.015	1.149	0.961	1.042	1.067
(VIII)	1.021	1.014	0.971	0.974	1.015	1.143	0.949	1.089	1.062
(IX)	0.931	1.008	0.971	0.973	0.979	1.094	0.889	1.043	1.056

Pariser–Parr–Pople (SCF-CI) method[19] and are listed in Table 5. Qualitatively, Table 2 predicts the ordering of the aromatic peaks for each dye. The calculations also anticipate the close agreement in chemical shifts of C-4 and -7 and C-5 and -6 for oxacarbocyanine (VIII). Various attempts at quantitative correlation of the observed chemical shifts with the calculated charge densities failed, however, to give satisfactory results.

It should be noted that the previously reported ^{1}H chemical shifts of thiacarbocyanine (III)[5] (with reassignment as described in the Results section) do not correlate with either the ^{13}C chemical shifts or the calculated charge densities even though such a correspondence has been reported for other aromatic compounds.[20] In fact, the chemical shifts of 5- and 6-H are always upfield from those of 4- and 7-H, opposite to the order observed for the carbon peaks. The particularly upfield position for 6-H has also been observed for other heteroatomic compounds,[21] but is not reflected in an unusual chemical shift for C-6.

As expected,[4] the chemical shifts of C-2, -α, and -β reflect the alternation of charge density within the connecting chain. It might also be expected that the carbons in the chain would be sensitive to variations in

atoms, the difference is possibly due to a different charge distribution in the two ends of the dye.

In addition to chemical shifts, spin–lattice relaxation times (T_1) of carbon nuclei can be informative about properties of organic materials in solution.[24] Because C–H bond lengths are fairly constant, the dipolar relaxation times of carbons of most materials in non-viscous solutions relate simply to the number of attached hydrogens and the correlation time for tumbling of the C–H bonds. The full NOE measured for the aromatic carbons suggests that the measured relaxation times may be interpreted in terms of the dipolar mechanism alone. Qualitative conclusions about the anisotropy of the tumbling of the material in solution may be made from simple inspection. Further quantitative analysis of the relaxation times in terms of the rotational diffusion tensor may also be possible.[25] The striking effect of concentration and temperature on the relaxation times of dye (III) is most easily explained in terms of changes in the degree of dimerization or aggregation of the dye. Indeed, the optical spectrum of the 0.2M solution revealed a substantial concentration of dimeric material, although the presence of higher aggregated dye in addition could

[17] Ref. 7a, pp. 102—106.
[18] Ref. 7a, pp. 239—269.
[19] D. M. Sturmer, personal communication. These π-electronegativity and σ-polarization corrections are described by H. A. Hammond, *Theor. Chim. Acta*, 1970, 239. The atomic coordinates used for the dyes were based on C–C bond lengths of 1.40 Å for the methine chains and X-ray crystal structures for the heterocyclic rings.
[20] P. Lazzeretti and F. Taddei, *Org. Magnetic Resonance*, 1971, 3, 283.

[21] O. Sciavelli and W. von Philipsborn, *Org. Magnetic Resonance*, 1971, 3, 339.
[22] L. G. S. Brooker, A. L. Sklar, H. W. J. Cressman, G. H. Keyes, L. A. Smith, R. H. Sprague, E. Van Lare, G. Van Zandt, F. L. White, and W. W. Williams, *J. Amer. Chem. Soc.*, 1945, 67, 1875.
[23] E. Kleinpeter, R. Borsdorf, and F. Dietz, *J. prakt. Chem.*, 1973, 315, 600.
[24] G. C. Levy, *Accounts Chem. Res.*, 1973, 6, 161.
[25] R. R. Becker, S. Berger, D. K. Dalling, D. M. Grant, and R. J. Pugmire, *J. Amer. Chem. Soc.*, 1974, 96, 7008.

not be ruled out. Measurement of the optical spectrum of such concentrated solutions is difficult, however, and carbon spin–spin lattice relaxation times may present an attractive alternative method for the study of dimerization or aggregation.

The unequal relaxation times of the 0.1M solution are easily explained since inspection of a model shows that the C–H bond of C-5 lies close to the long axis of the molecule about which rotation would preferentially occur.[26] Higher concentrations and lower temperature apparently decrease the preference for rotation about this axis, however. In the event that higher order aggregates may be ignored, this change in the anisotropy of molecular reorientation may contain information about the structure of dye dimers. It seems unlikely that formation of a dimer in which there is only partial overlap of the constituent dyes, such as in (XIII), would result in more isotropic rotational motion. On the other hand, (XIV) should tumble almost as a sphere. Of course, the formation of large amounts of even higher aggregates might also account for the results regardless of structure. It should be noted, however, that the crystal structure of solvated (III) consists entirely of structures corresponding to (XIV),[27] even though equal amounts of (XIII) and (XIV) were present in an unsolvated crystal.[28]

(XIII)   (XIV)
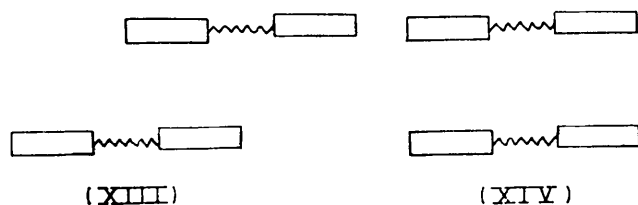
The less than full NOEs measured for C-α and -β are puzzling, but conceivably could be due to interaction with trace paramagnetic impurities at specific dye sites which affect the chain carbons but have little or no influence on the relaxation of the aromatic carbons. Additional studies to clarify the mechanisms of spin–lattice relaxation and to determine how reliable conclus-

ions from them can be made are in progress and will be reported separately.

EXPERIMENTAL

The dyes used were synthesized and characterized in the Research Laboratories of Eastman Kodak Company by standard procedures.[29] Most of them are common carbocyanines and were used without additional purification. In no instance were lines observed in the carbon spectra that might have been due to an impurity.

Chemical shifts were measured from internal tetramethylsilane on a Varian CFT-20 spectrometer operating at 20 MHz with 8 K words of memory. A spectral width of 4 000 Hz was standard, and chemical shifts were reproducible to within 0.1 p.p.m. Solvents were used without special purification. The methanol samples were 4 : 1 MeOH–CD_3OD (v/v).

Relaxation measurements were made by the inversion–recovery method. A Varian CFT-20 instrument was used for the 0.2M solution, but a Varian XL-100 instrument equipped with disc memory was used for the 0.1M solution. A least-squares analysis with a quadratic weighting of points was used with the XL-100. Pulse delays on both instruments were chosen to be more than five times the longest relaxation times. The relaxation times are believed to be accurate to within ±10%, and least-squares analysis indicated an absolute error of ≤0.04.

Off-resonance decoupling experiments were made with the CFT-20 spectrometer. The 100 Hz signal used in the synthesis of the decoupling frequency was replaced by a signal from a crystal oscillator for these experiments. The oscillator frequency could be continuously varied from 90 to 100 Hz so that the resulting decoupling signal could be arbitrarily set at any desired frequency. Details of the construction are available upon request. Coupled spectra were recorded by the gated decoupling technique.

[26] D. Chandler, Accounts Chem. Res., 1974, 7, 246.
[27] P. J. Wheatley, J. Chem. Soc., 1959, 4096.
[28] P. J. Wheatley, J. Chem. Soc., 1959, 3245.
[29] F. M. Hamer, ' The Cyanine Dyes and Related Compounds,' Interscience, New York, 1964.