# COMMUNICATIONS TO THE EDITOR

## SOLVENT EFFECTS IN MEROCYANINE SPECTRA

*Sir:*

Brooker and his collaborators[1,2] have recently described remarkable solvent effects in the spectra of highly dipolar merocyanine dyes such as I and II below. (Our dye I is Brooker's I in ref. 2). In solvents such as pyridine–water mixtures, the absorption is strongly displaced to higher frequency as the polarity (*i.e.*, the water content) of the solvent is increased. To explain this displacement, Brooker, *et al.*, suggest that the dipolar resonance structures Ib and IIb, which are major contributors to the ground states of dyes of this class, are increasingly stabilized as the solvent polarity (*i.e.*, the dielectric constant) is increased. The energy of the ground state is thus lowered relative to the excited state, and the transition energy is correspondingly increased. The problem has been treated in quantum mechanical terms by Simpson,[3] who adopts the same view and introduces the solvent dielectric constant $D$ into his expression for the energy of charge separation in the dipolar mesomers.

This assumption raises an important theoretical question, since *all* the terms that contribute to intramolecular potential energies are fundamentally electrostatic charge separation terms such as $-e^2/r$, where $e$ is the electronic charge and $r$ is the distance between two charges. If these are all to be replaced in the extreme case by terms such as $-e^2/Dr$ in solution, one would expect $D$ to enter as a scale factor into all treatments of solution spectra. This is not the case in all simple spectra, where the molecular state energies, and the energy differences as revealed by spectra, are relatively insensitive to the solvent dielectric constant, or indeed to whether the absorbing molecule is in solution or in the gas state. It is not impossible that there may be some gradation in the solvent effect in that $D$ may modify the electrostatic energy of widely separated charges on the "outside" of a solute molecule much more than in the case of charges that are deep down "within" it. Simpson[3] is careful to state that "the real $D$ experienced by the merocyanines in highly polar solvents may be an order of magnitude smaller than the solvent $D$." However Simpson's work implies proportionality between the "real" $D$ and the solvent $D$.

As part of a general study of solvent effects in spectra,[4] we have obtained evidence bearing on this point in the spectra of dyes I and II (samples kindly supplied by Dr. Brooker). The following solvents were used, their designations and dielectric constants being given in parentheses: Chloroform (C, 5.2), pyridine (P, 12), acetone (A, 21), ethanol (E, 28), nitrobenzene (N, 35), water (W, 79), formamide (F, 109). Figure 1 shows the results for dye II; those for dye I are quite similar.

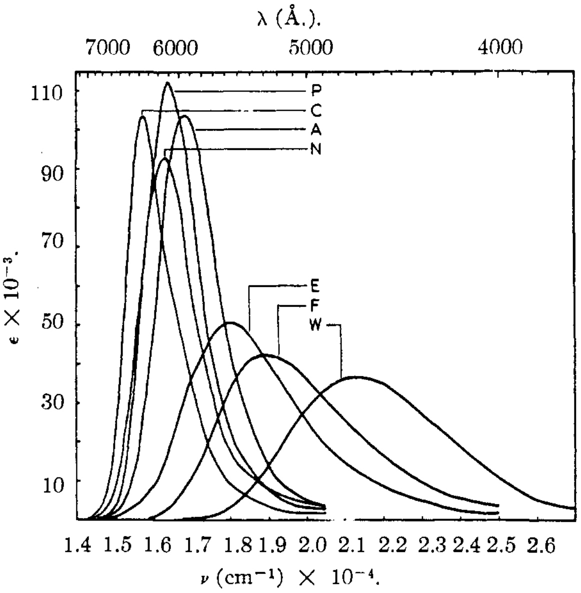
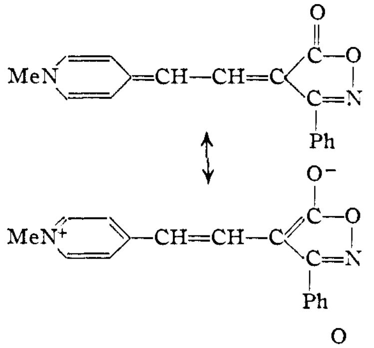


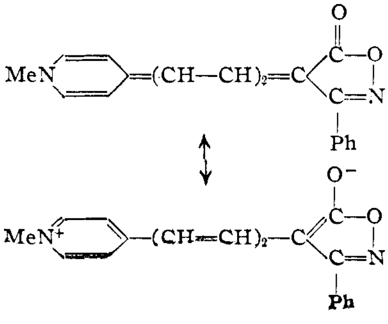
Fig. 1.

It will be seen that there is no simple correlation between the spectra and the solvent dielectric constants. The spectra in chloroform, pyridine, acetone and nitrobenzene are almost identical in frequency, even though $D$ in these solvents ranges from 5 to 35. The highest $D$ is that of formamide (109), yet the spectrum in this medium is close to that in ethanol ($D$ = 28) and roughly midway between the spectra in the first group of solvents and the spectrum in water ($D$ = 79).



Ia

Ib



IIa

IIb

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[1] L. G. S. Brooker, *et al.*, This Journal, **73**, 5332 (1951).

[2] L. G. S. Brooker, *et al.*, *ibid.*, **73**, 5350 (1951).

[3] W. T. Simpson, *ibid.*, **73**, 5359 (1951).

[4] To be submitted to the *Australian Journal of Chemistry*.

If the "real $D$" in Simpson's sense[3] is the determining factor, then this real $D$ bears no obvious relation to the macroscopic solvent $D$.

The results on at least these two dyes thus do not support Brooker's contention that the factor determining the displacement of the spectrum is the stabilization of the dipolar resonance forms Ib and IIb. There are more obvious correlations with other solvent properties than the dielectric constant. Firstly, the order of the spectra in Fig. 1 is qualitatively the same as the order of the sizes of the solvent molecules. Secondly, the spectra in Fig. 1 seem to divide themselves into two groups—(a) the spectra in pyridine, chloroform, acetone and nitrobenzene, and (b) those in ethanol, formamide and water. Group (b) are just those solvents whose molecules contain hydrogen atoms with the hydrogen bonding property, suggesting that hydrogen bond formation may play a part in the spectral displacement. These various effects are the subject of current study in this laboratory, and will be reported shortly.[4]

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## THE IDENTIFICATION OF CYTIDYLIC ACIDS *a* AND *b*[1]

*Sir:*

The identification of the isomeric cytidylic acids *a* and *b*[2] with known cytidine-2′- or 3′-phosphates has received a considerable amount of attention[3,4,5] but thus far these efforts have been unsuccessful. It is certain, however, that the isomerism is positional,[4] *i.e.*, that the 2 and 3 positions of the D-ribose portion are involved. In this preliminary communication we report the results of measurements which allow a decision to be made based on the distances between the 4-amino and phosphate groups.

Cytidylic acids *a* and *b* were isolated according to the procedure of Loring, Bortner, Levy and Hammell.[6] The samples were recrystallized alternately as their ammonium salts and free acids to constancy with respect to ultraviolet spectral characteristics, optical rotation values, density measurements and $p$H titration curves. For the *a* isomer the 250/260, 280/260 and 290/260 values were 0.47, 1.80 and 1.21; for the *b* isomer, 0.43, 2.01 and 1.43, respectively.[2,6] Specific rotation, $[\alpha]_D$, for *a* was $-3°$; for *b*, $+50°$, $c = 1$, aqueous sodium hydroxide, $p$H 10.[6]

We have carried out high-precision density measurements on aqueous solutions (*ca.* 0.15–0.5%) of the isomers.[7] Measurements were made on two different samples of each isomer; the samples used represented the final and penultimate samples from the recrystallization procedure. To provide a check on the determinations, appropriate dilutions of each of the four samples were made in such a way that equal concentrations of the various solutions could be compared. This crisscross technique thus provided a check both for the weighings and the different fractions. The *b* isomer was found to have a greater density at all concentrations. For example, in 0.3% solutions *b* had a density greater by 18 parts per million. Since it has been firmly established[8] that as the distance between the charged groups of a zwitterion is increased the density of its aqueous solutions increases (because of increased electrostriction) we conclude that the *b* isomer exhibits the greater separation of charge.

We have also determined the apparent dissociation constants of the 4-ammonium group from $p$H titration curves obtained with the aid of a Cambridge $p$H meter.[9] The $pK'_a$ of the *a* isomer was found to be 4.36; for the *b*, 4.28.[10] Since the $pK'_a$ will be lower for the isomer with the greater separation of charge[11] these data also show that the *b* isomer exhibits the greater separation of charge.

The detailed structure of cytidine has been presented by Furberg[12] on the basis of X-ray crystallographic analysis. One of the essential features of the structure is that the glycosidic bond ($C_{1'}$–$N_1$) lies in the plane of the pyrimidine ring and is symmetrically disposed with respect to the 2 and 6 carbon atoms of the pyrimidine ring. Thus, regardless of the angle of rotation of the sugar ring about the pyrimidine ring the 3 position of the D-ribose will always be further from the 4-amino group than the 2 position. We conclude, therefore, that the cytidylic acid isomer showing the greater separation of charge (*i.e.*, the *b* isomer) is cytidine-3′-phosphate.

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[1] This investigation was supported by grants from the National Cancer Institute, National Institute of Health, United States Public Health Service, and from the Atomic Energy Commission, Contract No. At(30-1)-910. The author wishes to acknowledge the continued interest of Dr. George Bosworth Brown.

[2] C. E. Carter and W. E. Cohn, *Federation Proc.*, **8**, 190 (1949); W. E. Cohn, THIS JOURNAL, **72**, 2811 (1950).

[3] D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 52 (1952).

[4] H. S. Loring, M. L. Hammell, L. W. Levy and H. W. Bortner, *J. Biol. Chem.*, **196**, 821 (1952).

[5] R. Markham and J. D. Smith, *Nature*, **168**, 406 (1951).

[6] H. S. Loring, H. W. Bortner, L. W. Levy and M. L. Hammell, *J. Biol. Chem.*, **196**, 807 (1952).

[7] The falling drop method used gave results reproducible to within 1 to 2 parts per million.

[8] See, for example, Cohn and Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, p. 155.

[9] The reproducibility of this instrument was 0.006 $p$H unit.

[10] The $pK'_a$ of the secondary phosphoryl dissociation for the cytidylic acids was 6.17 for the *a* and 6.00 for the *b*. Loring, *et al.*,[6] report 6.2 and 6.0, respectively. In the zwitterionic form (at *ca.* $p$H 4) the secondary phosphoryl dissociation is negligible and $pK'_a$ values for these potential hydrogen ions are not pertinent to the question of separation of charge.

[11] A. Neuberger, *Proc. Roy. Soc.* (*London*), **A158**, 68 (1937).

[12] S. Furberg, *Acta Chem. Scand.*, **4**, 751 (1950).

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## THIOUREA ADDUCTS OF *n*-PARAFFINS

*Sir:*

In applying the reasonably well-defined principles of urea and thiourea adduction to the development of a systematic, analytical fractionation of petrolatums and other high boiling petroleum stocks, it was found by analysis that certain thiourea adducts contained considerable amounts