

If the "real  $D$ " in Simpson's sense<sup>3</sup> 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.<sup>4</sup>

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#### THE IDENTIFICATION OF CYTIDYLIC ACIDS *a* AND *b*<sup>1</sup>

Sir:

The identification of the isomeric cytidylic acids *a* and *b*<sup>2</sup> with known cytidine-2'- or 3'-phosphates has received a considerable amount of attention<sup>3,4,5</sup> but thus far these efforts have been unsuccessful. It is certain, however, that the isomerism is positional,<sup>4</sup> *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.<sup>6</sup> 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 pH 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.<sup>2,6</sup> Specific rotation,  $[\alpha]_D$ , for *a* was  $-3^\circ$ ; for *b*,  $+50^\circ$ ,  $c = 1$ , aqueous sodium hydroxide, pH 10.<sup>6</sup>

We have carried out high-precision density measurements on aqueous solutions (*ca.* 0.15–0.5%) of

the isomers.<sup>7</sup> 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<sup>8</sup> 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 pH titration curves obtained with the aid of a Cambridge pH meter.<sup>9</sup> The  $pK'_a$  of the *a* isomer was found to be 4.36; for the *b*, 4.28.<sup>10</sup> Since the  $pK'_a$  will be lower for the isomer with the greater separation of charge<sup>11</sup> these data also show that the *b* isomer exhibits the greater separation of charge.

The detailed structure of cytidine has been presented by Furberg<sup>12</sup> 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.

(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 pH 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.*,<sup>6</sup> report 6.2 and 6.0, respectively. In the zwitterionic form (at *ca.* pH 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)*, **A168**, 68 (1937).

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

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(2) C. E. Carter and W. E. Cohn, *Federation Proc.*, **3**, 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).

#### 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