unpaired electron in the $d_{x^2-y^2}(t_{2u})$ MO.[25] Based on this bonding representation the over-all ground-state electronic configuration is T_{2u}. In order to attribute at least partly this crystallographic distortion of the $[Nb_6I_8]^{3+}$ ion from regular octahedral O_h symmetry to a *Jahn–Teller phenomena* (as differentiated from anisotropic coulombic forces), it is only necessary that the ground electronic state be degenerate. Such a situation would result either when one unpaired electron occupies a degenerate orbital (provided the other electrons are all spin paired) or when three spin-free electrons occupy a doubly degenerate orbital.

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(25) The Cotton–Haas molecular orbital metal atom cluster model applied to $[Mo_6X_8]^{4+}$ and $[M_6Cl_{12}]^{2+}$ (M = Nb, Ta) presumes an ordering of one-electron energy levels based on molecular orbital calculations involving only metal–metal interactions (with the perfect pairing approximation allowing separability with regard to metal–halogen interactions).[4] It should be noted that another molecular orbital calculation involving both metal–metal and metal–halogen interactions was applied to $[M_6X_{12}]^{2+}$ (M = Nb, Ta) by Robin and Kuebler,[5] and their resulting energy level pattern was considerably different from the Cotton–Haas energy level scheme for $[Nb_6Cl_{12}]^{2+}$. No doubt more rigorous MO treatments of these metal cluster systems will be necessary in order to clarify the orbital ordering for even the ground-state configuration (apart from the far more difficult problem of rationalizing the electronic spectra).

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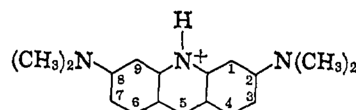
The Aggregation of Acridine Orange in Aqueous Solution[1]

Sir:

The optical properties of dye molecules in aqueous solution have been studied extensively for a number of years.[2] Of particular interest have been the changes observed in the visible region of the spectrum[3] for anionic dyes with a planar ring structure as the dye concentration is increased.[2b,e] These changes are generally interpreted in terms of an aggregation of the dye molecules to form dimers, trimers, and larger polymeric species at higher concentration.[1b,c,f] Although the structure of the aggregate cannot be established from the optical spectrum, the hypochromism and shift of λ_{max} can be interpreted theoretically by exciton theory[4] in terms of a parallel stacking of the adjacent rings in the aggregate (as opposed to a coplanar orientation of adjacent rings). In principle, it should be possible to confirm the most favored orientation of rings in the aggregate experimentally by following the proton chemical shifts as a function of dye concentration. If the rings are stacked parallel to each other, then a shift to low field would be noted for the ring protons upon dilution (disaggregation); if the rings are initially aggregated in a coplanar configuration, then dilution would produce a high-field shift. In this communication we wish to report the results of a study of the concentration dependence of the proton chemical shifts for the cationic dye, acridine orange (AO), in D_2O.



Dilute solutions of highly purified[5] AO cation were made up in 90% D_2O–10% H_2O, and the spectra were recorded with a Varian DA-60 spectrometer at room temperature (25 ± 1°). Because of the low concentrations (10^{-2}–10^{-4} *M*) of AO a Varian C-1024 time-averaging computer was used to enhance the signal-to-noise ratio. Figure 1 shows the spectrum obtained for the ring protons of a 10^{-2} *M* AO solution after 120 scans. The spectra were calibrated by superimposing audio side bands of the water signal on the spectrum in the final scan.

The analysis of the AO spectra is straightforward and a summary of the chemical shifts and coupling constants is given in Table I. The errors for these parameters are somewhat larger than normal and reflect an uncertainty in line positions due to the relatively large

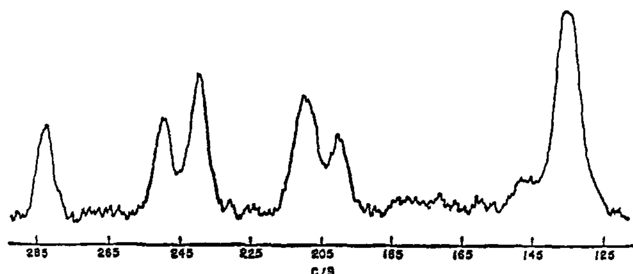


Figure 1. Nmr spectrum of the ring protons for acridine orange cation in D_2O (120 scans); 0.02 *M*.

Table I

Concn, *M*	No. of scans	δ_{CH_3},[a,b]	δ_5	$\delta_{1(9)}$	δ_{34},[c]	$J_{34(67)}$,[d] cps
1.0 × 10^{-2}	120	21.9	−282.2	−134.1	−224.9	9.7
5.0 × 10^{-4}	6700	3.5	−312.8	−178.7	−251.3	9.3

[a] ±2.0 cps. [b] δ values are relative to a trace of $(CH_3)_4NBr$ as internal reference. [c] Midpoint of AB spectrum. [d] ±0.5 cps.

(1) This work was supported by the U. S. Atomic Energy Commission.

(2) (a) S. E. Sheppard, *Proc. Roy. Soc.* (London), **A28**, 256 (1909); (b) V. Zanker, *Z. Physik. Chem.*, **199**, 225 (1952); (c) *ibid.*, **200**, 250 (1952); (d) G. R. Haugen and W. H. Melhuish, *Trans. Faraday Soc.*, **60**, 386 (1964); (e) M. K. Pal and M. Schubert, *J. Phys. Chem.*, **67**, 1821 (1963); (f) M. E. Lamm and D. M. Neville Jr., *ibid.*, **69**, 3872 (1965).

(3) Hypochromism and shift of λ_{max} to shorter wavelength.

(4) (a) H. Devoe, *J. Chem. Phys.*, **41**, 393 (1964); (b) M. Kasha, *Radiation Res.* **20**, 55 (1963).

(5) Kindly supplied by Dr. L. Bunville of Argonne National Laboratory.

line widths.[6] A comparison of the chemical shifts for the two concentrations shows that the ring and N-methyl protons shift to low field very markedly upon dilution. The magnitude of the deshielding is much larger, *ca.* 50-fold, than would be expected on the basis of simple concentration effects known to influence the shifts of aromatic solutes.[7] Nor can the deshielding be explained in terms of an electric field effect due to dissociation of ion pairs[8,9] since this would not account for the large changes for the 3, 4, and $N-CH_3$ protons. The most plausible explanation is one which invokes a disaggregation of polymeric AO species (in which the AO molecules are stacked with their planes parallel) into simpler oligomeric, dimeric, and monomeric forms. Although the existence of a monomer $\rightleftharpoons$ dimer equilibrium in the concentration range 10^{-4} to 10^{-6} M has been well established by optical measurements,[2b,g] it is likely that the deshielding observed in the present case is due largely to a dissociation of higher aggregates.[10] The present results also suggest that the most favored orientation of adjacent AO molecules in the stack is one in which the 1,9 (and $N-CH_3$) protons are located more nearly over the center of adjacent rings than the protons in positions 4, 5, and 6.

(6) The relatively broad line widths (3–5 cps) are due to a combination of unresolved long-range couplings and small spectrometer instabilities.
(7) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, Chapter 16.
(8) W. F. Reynolds and T. P. Schaefer, *Can. J. Chem.*, **41**, 2339 (1963).
(9) G. Fraenkel, *J. Chem. Phys.*, **39**, 1614 (1963).
(10) Preliminary calculations of $\Delta\sigma$, using McConnell's dipole approximation, indicate that the shift changes calculated for a dimer–monomer dissociation are insufficient to account for the observed values: H. M. McConnell, *J. Chem. Phys.*, **27**, 226 (1957).

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Book Reviews

The Proteins. Composition, Structure, and Function. Volume III. Second Edition. Edited by Hans Neurath, Department of Biochemistry, University of Washington, Seattle, Wash. Academic Press Inc., 111 Fifth Ave., New York, N. Y. 1965. x + 585 pp. 16 × 23.5 cm. $21.00, list; $18.50, subscription.

As the editor states, the third volume of this extensive treatise on "The Proteins" extends the central theme of the relationship between composition, structure, and function to four specific groups of proteins: the plasma proteins, antibodies and antigens, the proteins of the blood clotting system, and the virus proteins. Two general aspects are included: the fractionation of proteins and the interaction of proteins with radiation.

The first chapter, Chapter 12, on "Fractionation of Proteins" was written by H. A. Sober, R. W. Hartley, Jr., W. R. Carroll, and E. A. Peterson. They review some newer methods that have been used for the separation of proteins in solution; however, little is said about the extraction of proteins from natural sources or of older methods such as fractionation by neutral salts, organic solvents, or heavy metals. Recent work on protein fractionation by solubility, partition, chromatography, dialysis or ultrafiltration, electrophoresis, and sedimentation is discussed. There are included sections on immunological methods and auxiliary techniques such as methods for concentrating, desalting, and detection of proteins, as well as general recommendations on the criteria of protein purity.

Chapter 13 is entitled "Structure and Function of Virus Proteins and Viral Nucleic Acid" and was written by H. Fraenkel-Conrat. He first reviews his own work and that of others on tobacco mosaic virus and on the protein coats of virus which have helical symmetry. The proteins of viruses of cubic symmetry are discussed more briefly. Then the structure of bacteriophage proteins or viruses with complex structure is described in relationship to their supposed function. Finally a section on effects of viral mutation in terms of protein structure relates the amino acid exchange in chemically evoked mutants to the corresponding change in nucleic acid structure.

Chapter 14 by F. W. Putnam is entitled "Structure and Function of the Plasma Proteins." The plasma protein system is described and the problem of resolution, identification, fractionation, purification, and characterization of the different components discussed. The use of fractionation procedures such as electrophoresis, ultracentrifugal analysis, chromatography, or gel filtration for the characterization of plasma proteins is reviewed. Then data on the molecular structure and function, molecular properties, chemical composition, end groups, and terminal sequence of the plasma protein are presented. Current knowledge of the structure and function of the major plasma proteins is summarized. This section includes discussions of the structure and function of serum albumin, glycoproteins, α- and β-lipoproteins, transferrin, haptoglobulins, ceruloplasmin, fetuin, clotting components, and γ-globulins.

Considerable progress has been made in the last decade on the "Structure and Function of the Antibody Proteins," which is reviewed in Chapter 15 by S. J. Singer. The chemical properties of antigens and antibodies are described, and the antigen–antibody reaction is discussed in detail. This is followed by sections on the structure of protein antigens including antigenic determinants and the new concepts of the structure of antibodies with a discussion of the structure and chemistry of the antibody-combining sites. Finally, current theories of antibody formation are reviewed.