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Formation of a Helical Complex by a Sulfonphthalein Dye[1]

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When brom phenol blue is decolorized above *p*H 10 and then returned to neutral *p*H, it is only slowly reconverted to the blue form of the dye. The evidence suggests that this colorless form is a monovalent ion of the dye in which a hydroxyl group remains attached to the central carbon atom. Near *p*H 4 white fibers separate from this colorless solution, which are shown by X-ray diffraction to contain a helical arrangement of molecules. This is the second example of a helical complex forming from the aggregation of small molecules in a colloidal system.

It is well known that many phthalein dyes are decolorized on exposure to high *p*H, and this decolorization is generally ascribed to formation of a trivalent ion so that no quinoid groups remain.[2] La Mer and Amis[3] observed that if such a decolorized brom phenol blue solution was neutralized, an unusual cloudy precipitate formed, containing whitish strands of fibrous material. We wish to report X-ray diffraction studies carried out on this material, which show that the dye molecules have aggregated themselves into a complex of large dimensions, built up in a helical fashion. Tests carried out on numerous closely related compounds, including brom cresol green and tetraiodophenolsulfonphthalein, indicated that formation of the fibrous aggregate is specific to brom phenol blue. A similar phenomenon has been reported previously in the case of the steroid, deoxycholic acid.[4]

Method.—Brom phenol blue (La Motte Chemical Products) was used in an aqueous solution with no added salt. *p*H changes were brought about by the addition of NaOH or HCl at various concentrations depending upon the rapidity desired in the reaction being studied. Spectra of the solutions were recorded in a Cary Model 14 Recording Spectrophotometer, and *p*H measurements were made on the Beckman Model G *p*H meter.

The precipitate is in the form of white fibers which are macroscopic and appear to be very long. It proved possible to manipulate the fibrous precipitate into a form suitable for an X-ray diffraction study, by drawing and combing a moist, freshly prepared sample until the constituent fibers were oriented parallel to one another. The sample then was placed in a (Pantak) lithium glass capillary (1 mm. diameter), which was sealed at the ends with picein wax while the sample was still moist. A well oriented part of the sample, chosen by microscopic examination, was used for the X-ray diffraction. Filtered CuKα radiation from a Hilger Y25 semi-microfocus X-ray tube was used with a collimator 100 μ wide to obtain diffraction patterns.

Results

A solution of brom phenol blue slowly decolorizes just above *p*H 10, although the rate increases at higher *p*H values or at elevated temperatures. The decolorized solution showed the absorption band of the normal alkaline form of brom phenol blue at 312 mμ, but had lost the band at 594 mμ, which is responsible for the blue color. At *p*H below 10 and at room temperature, the decolorization reaction was slowly reversed, absorption in the visible region reappearing in a day or so at a weakly alkaline *p*H. Below *p*H 2 the usual absorption bands of the acid form, at 440 and 283 mμ, return within a few hours.

Some care must be taken in preparing the decolorized alkaline form of brom phenol blue. If a strongly basic solution is used (3 *M* NaOH) and the solution is heated, a yellow solution forms with strong absorption bands at 312 and 365 mμ. This solution was unchanged by subsequent lowering of the *p*H and no precipitate formed.

The flocculent precipitate described by La Mer and Amis[3] is formed at about *p*H 4, as the decolorized form of brom phenol blue is gradually acidified. This precipitate is also unstable and will gradually go back into solution as the supernatant becomes colored. These various reactions of brom phenol blue are summarized in Fig. 1. The acid form (I) has a lactone ring and exists as an uncharged molecule with a yellow color. Brom phenol blue is used as an indicator over the range 3.0–4.6 with the yellow color (I) gradually changing to the blue quinoid form (II) on the addition of hydroxyl ions. This divalent structure, with its resonance modifications, is stable between *p*H 4.6 and 10. On raising the *p*H above 10, the quinoid structure is slowly lost with the formation of the trivalent ion (III), which is characterized by the strong absorption band at 312 mμ.

On neutralizing the alkaline form (III) of the molecule, very little change is initially observed in the solution, and the absorption band at 312 mμ remains. Neither the band at 594 mμ nor at 440 mμ reappears, hence the molecule is not in the quinoid form (II) or in the form with the closed lactone ring (I). It is likely that the molecule exists as a monovalent ion (IV) which is colorless. This form, however, is unstable, reverting to the blue form (II) in the course of a day, or at lower *p*H to the yellow form (I) in a few hours. It is the monovalent ion (IV) which produces the insoluble white fibrous precipitate when the *p*H is lowered to about 4. It is possible that this precipitate is brought about by the addition of a proton to the sulfonic acid groups during the complex formation.

It should be mentioned that Fig. 1 is somewhat oversimplified. A freshly made solution of (I), brought to *p*H 5, shows a slight absorption peak at 312 mμ and this peak is still found in solutions which have been standing for long periods. Thus the reactions (IV) → (I) and (IV) → (II), indicated in Fig. 1, are reversible, though the equilibrium favors (II) in neutral solution. It may well be that the two absorption peaks shown by (I) are due to two different forms in equilibrium, one lacking the lactone ring.

The X-ray diffraction pattern obtained from the precipitated fibers is sketched in Fig. 2 directly

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[1] This work was supported by a Research Grant from the National Cancer Institute, Bethesda, Md.

[2] G. W. Wheland, "Resonance in Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1955.

[3] V. K. La Mer and E. S. Amis, *Science*, **90**, 90 (1939).

[4] A. Rich and D. M. Blow, *Nature*, **182**, 423 (1958).

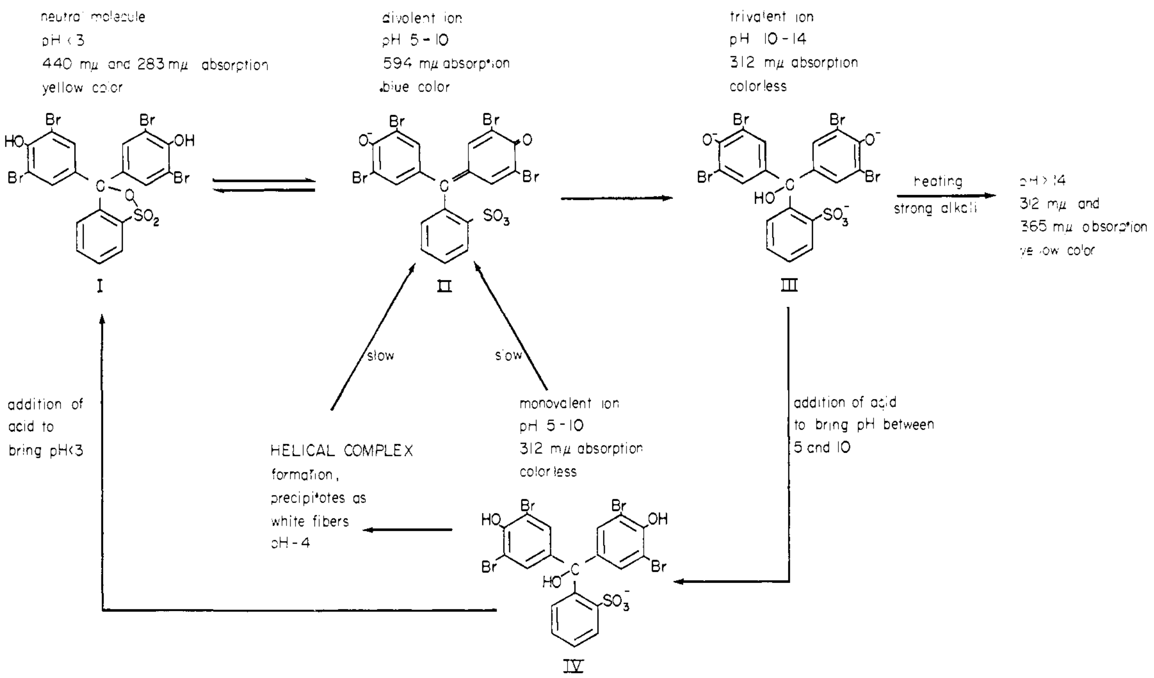

Fig. 1.—The reactions of brom phenol blue as a function of *p*H.

from the flat film on which the pattern was registered. Four layer lines are easily seen together with several reflections which are on the equator. Although orientation is far from perfect, it is sufficient to permit a qualitative interpretation of the pattern. The sharpness of both meridional and equatorial reflections indicates that the aggregates are many hundreds of ångströms in length and are laterally packed together over large areas. The equatorial reflections can be indexed on a hexagonal lattice with a = 31.5 Å. The hkl indices are indicated in Fig. 2, together with the reflections, which are drawn with lengths which provide an indication of the intensity of the reflection.

The four layer lines spacings are 9.8, 6.2, 3.8 and 3.08 Å. which are not simply related to each other. There is a strong meridional reflection at 3.8 Å., but the other reflections are off the meridian. The second layer line occurs at a reciprocal lattice spacing ζ ($= \lambda/d$) of 0.248, while the fourth layer line appears at twice this value ($\zeta = 0.50$). However, in addition, the first layer line occurs at a position in reciprocal space which is 0.245 unit below the meridional reflection at $\zeta = 0.403$. These features are characteristic of helical symmetry.[5] The primitive helix represented by this diffraction pattern has a pitch of 9.8 Å., with a residue every 3.8 Å. of translation along the helix axis.

The upper layer lines in the photograph do not appear to have sharp reflections on them but rather a continuous blur, which probably represents the continuous Fourier transform. It can be shown that the structure factor for a given layer line is the sum of a series of terms, each of which contains a Bessel function $J_n(2\pi rR)$, where r = radius of an atom, R = radial distance of the reflection in reciprocal space ($= \xi/\lambda$) and n is the order of the Bessel function. By measuring the R coördinates of the most intense part of the layer line we can obtain a "weighted" average radius r for the helical structure. Since half the scattering power of brom phenol blue is due to the four bromine atoms, it is quite likely that this average radius represents

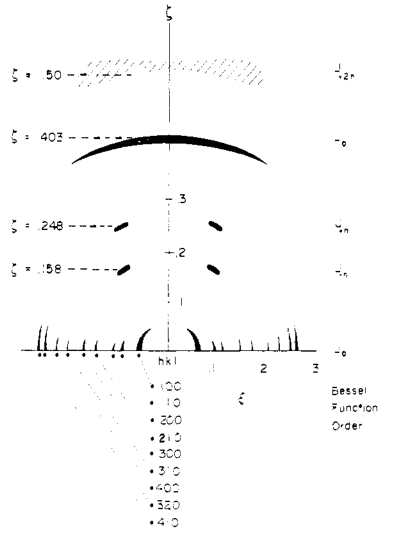

Fig. 2.—Sketch of the X-ray diffraction pattern of brom phenol blue. The meridian is vertical and the fiber has been tilted to show the upper layer lines. The reciprocal space coordinates ξ and ζ are indicated in units of λ/d where λ = 1.5416 Å. The equatorial reflections index accurately on a hexagonal lattice and their indices are shown. In the sketch, the length of the various arcs is an indication of the intensity of the corresponding reflections. The shading on the upper layer line indicates a diffuse, relatively weak reflection. On the right hand side, the orders of the Bessel functions contributing to each layer line are indicated, assuming an n-fold symmetry axis parallel to the fiber axis.

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[5] W. Cochran, F. H. C. Crick and V. Vand, *Acta Cryst.*, **5**, 581 (1959).

their position. If $n = 1$ for the first and second layer line (Fig. 2) then r is about 4.4 Å. This is probably too small, since the dye molecules must complex together to make a structure which is over 30 Å. in diameter. Furthermore, the two sets of bromine atoms in the molecule are about 9 Å. apart and there is no obvious arrangement which would put them at an average radius of 4.4 Å. This suggests that there is additional symmetry in the molecule in the form of a two or three fold axis of rotation parallel to the helix axis which would increase the weighted radius by approximately two or three times, respectively. This means that the helical pitch of the molecule is $9.8 \times n$ where $n = 2$ or 3. Equivalent units are then related to each other by a translation of 3.8 Å. along the helix axis, and a rotation of $360° \times 3.8/9.8n$ which is equal to 70° if $n = 2$ or 47° if $n = 3$. Consideration of density suggests that each asymmetric unit related in this way probably contains two or three dye molecules. The structure is thus a complicated one.

Discussion

This is the second example which we have found of a small molecule which can be caused to form an oriented, high particle weight complex showing helical symmetry. The earlier example, the bile salt sodium deoxycholate,[4] provided a system much more amenable to detailed study. Studies of that system indicated that the complexing phenomenon depends strongly on electrostatic influences such as *p*H and ionic strength, and it seems clear that there is a close relationship with the well known micelle-forming properties of the bile salt. The particles of the helical complex differ from normal micelles in their much greater size, as indicated by their viscosity, sedimentation constant and turbidity and by their evident internal crystallinity, indicated by a very detailed wide-angle X-ray diffraction pattern. As in the present case a number of structurally similar compounds were found to show no comparable effect. It was thus concluded that the forces of aggregation were enhanced by a specific internal structure favoring van der Waals and hydrogen bond interaction.[6]

It is well known that dye molecules constitute another group of colloidal electrolytes, and there can be little doubt that a similar phenomenon is taking place in the case of brom phenol blue. Of the internal forces which stabilize the structure, there is in this case the interesting possibility that charge transfer forces are involved between the bromine atoms and adjacent aromatic nuclei. In addition, the hydroxyl groups may be involved in hydrogen bonds, and the unsaturated rings are capable of a substantial van der Waals interaction. The behavior is evidently specific to this structure, since attempts to produce the same effect in several allied molecules produced no results.[7]

As this is now the second example of this type of behavior, it is unlikely that they are unique cases. It seems entirely probable that other systems which show greatly exaggerated micellar properties may be made to form macroscopic fibers which will exhibit helical internal structures, since this is the most general way of generating an elongated regular structure. In this connection, it might be worthwhile investigating the structure of other elongated complexes which form in solution. Although this phenomenon of helical aggregation may become more widely recognized, it is also quite clear that many micellar systems do not behave in this way.

In some respects, the growth of a helical complex in solution is analogous to the mechanism of crystal growth provided by screw dislocations.[8]

Both of these rely on the ability of helical packing to produce unlimited growth. In the case of crystals, lateral growth also occurs, but Frank[9] showed in connection with the tin "whiskers" that when the diameter is limited by other influences, a much greater growth rate is possible. In the case of a helix of non-crystallographic symmetry, lateral growth is prohibited by geometrical considerations, and mechanical stability is achieved by packing the elongated rods together, usually in a hexagonal manner to produce a fibrous substance.

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[6] D. M. Blow and A. Rich, THIS JOURNAL, **82**, 3566 (1960).

[7] The materials tested include tetraiodosulfonphthalein, brom cresol green, brom cresol purple, brom thymol blue, phenol red and eosin.

[8] F. C. Frank, *Discussions Faraday Soc.*, **5**, 48 (1949); W. K. Burton, N. Cabrera and F. C. Frank, *Phil. Trans. Roy. Soc. London, Ser. A*, **243**, 299 (1951).

[9] F. C. Frank, *Phil. Mag.*, **44**, 853 (1954).