exposed by crushing the cells of fresh tissue. From 40 to 50% of the nitrogenous constituents appears to be included within the cell structural material, and are therefore metabolically inert.

Madison, Wisconsin

[Contribution from the Color Laboratory of the Bureau of Chemistry, United States Department of Agriculture]

# THE SPECTROSCOPY OF THE SULFONATED INDIGOTINS

By Walter C. Holmes

Received October 22, 1923

The potassium salts of the four indigotin sulfonic acids have been investigated with the purpose of defining the correlation between their constitution and various aspects of their absorption and general behavior, and of obtaining data bearing upon their spectrophotometric identification and evaluation. The materials examined were pure dyes prepared by M. X. Sullivan of the Hygienic Laboratory, United States Public Health Service. Their absorptions were measured throughout the visible spectrum by means of a commercial wave-length spectrometer provided with a standard photometer with such provisions as would demonstrate the influence of a variety of factors.

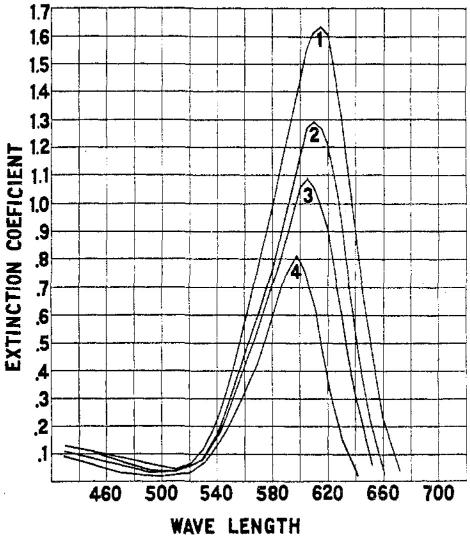

Fig. 1.—Potassium indigotin sulfonates. (Absorption of 40 parts of dye per million parts of 80% alcohol in a 1cm. cell.) (1) = Indigotin $SO_3K$; (2) = Indigotin $(SO_3K)_2$; (3) = Indigotin $(SO_3K)_3$; (4) = Indigotin $(SO_3K)_4$

The absorptions of the dyes in 80% alcohol are plotted in Fig. 1.

The dependence of the absorption upon constitution is clearly evident. With progressive sulfonation there occurs a displacement in the spectral location of the absorption maxima toward the violet together with a decrease in the intensities of absorption which is roughly proportional to the increase in molecular weights.

The absorptions of the dyes in distilled water are plotted in Fig. 2.

A relation between constitution and absorption similar to that noted in alcoholic solution is evident in the aqueous solutions of the di-, tri- and tetrasulfonic derivatives. The absorption of the monosulfonic derivative in aqueous solution, however, is abnormal in respect to intensity, to the location of the absorption maximum and to the relative breadth of the curve.

The ratios of the intensities of absorption at the maximum in water and in 80% alcohol vary with the degree of sulfonation as follows: indigotin monopotassium sulfonate, 0.73; dipotassium sulfonate, 0.99; tripotassium sulfonate, 1.04; tetrapotassium sulfonate, 1.10.

With progressive sulfonation the relative solubilities of the dyes increase in water and decrease in alcohol. The variation in the intensity ratios noted above is held to indicate that with progressive sulfonation the relative degree of association of solute dye molecules decreases in aqueous solutions and increases in alcoholic solution.

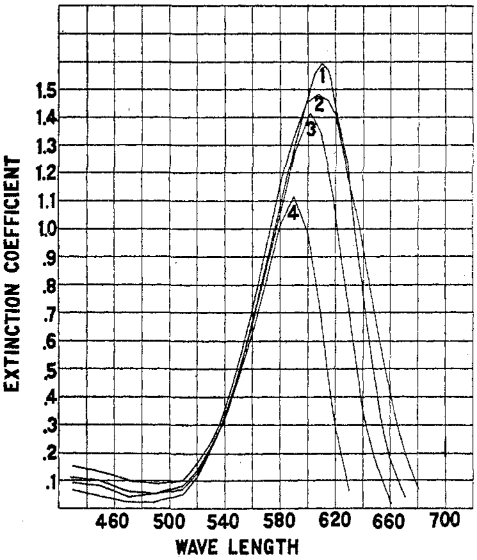

Fig. 2.—Potassium indigotin sulfonates. (Absorption of 50 parts of dye per million parts of water in a 1cm. cell.) (1) = Indigotin $(SO_3K)_2$; (2) = Indigotin $SO_3K$; (3) = Indigotin $(SO_3K)_3$; (4) = Indigotin $(SO_3K)_4$

The relatively minor displacement of the absorption maximum of the monosulfonic derivative in aqueous solution is indicative of an incipient transition in absorption form. By the addition of acids or salts to the aqueous solution, and the removal of the portion of the dye thereby precipitated by means of filtration, it is possible to obtain highly colloidal solutions of residual dye which exhibit two distinct and widely separated bands in varying degrees of ascendency, of which one is that characteristic of the normal dye solution. The second band thus developed may be obtained to the complete exclusion of the first by means of drying a solution of the dye upon a microscope slide. The phenomenon is an instance of a radical alteration in color and absorption associated with change in degree of molecular aggregation of the general type first noted by Sheppard[1] with certain dyes of the cyanine group.

Absorption curves obtained with dry samples of the other indigotins were less well defined. It was established, however, that the maximum of the trisulfonic derivative is displaced in drying from approximately 603$\mu\mu$ to 560$\mu\mu$. No decided shifts were observed in the instances of di-

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[1] Sheppard, *Proc. Roy. Soc.* (London), **82A,** 256 (1909).

and tetra-derivatives. It appears probable that the opposite behavior under the conditions in question of the dyes of unsymmetrical constitution to that of the dyes of symmetrical constitution may indicate that the modification in the absorption of the former arises from a type of tautomeric alteration possible only in instances of unsymmetrical structure.

The approximate locations of the absorption maxima of the dyes in the solvents employed are as follows.

| Indigotin | $(SO_3K)$ | $(SO_3K)_2$ | $(SO_3K)_3$ | $(SO_3K)_4$ |
|---|---|---|---|---|
| Water, μμ | 608 | 610 | 603 | 590 |
| 80% alcohol, μμ | 615 | 610 | 605 | 598 |

For purposes of identification these data afford a means of ready differentiation between the individual dyes of the group. From dyes of other groups which may have identical absorption maxima they may be distinguished by means of the characteristic form of their absorption curves and the equally characteristic manner of the modification of their absorption under various conditions to be outlined.

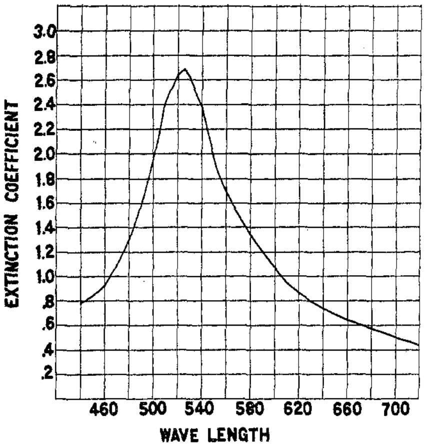

Fig. 3.—Potassium indigotin monosulfonate (dry dye)

With acids the behavior of the di-, tri- and tetrasulfonic derivatives is very similar, consisting in a decrease in intensity of absorption without any decided alteration in the form of the absorption curves. Although the effects of slight acidity are appreciable, the decrease in intensity in 20% sulfuric acid is only about 20%. The original loss in intensity is partially regained on prolonged standing. Small quantities of acid have a decided effect upon the absorption of the monosulfonic derivative, while larger quantities result in the partial precipitation of the dye together with the modification in the form of the absorption of the residual solution to which reference has already been made.

The effect of salt upon the absorption of the dyes is very similar in essential respects to that of acids. With the di-, tri- and tetrasulfonic derivatives the decrease in intensity of absorption with 2% of salt is comparatively moderate. With the monosulfonic derivative the addition of 1% of salt is followed by the immediate precipitation of a considerable portion of the dye.

The effects observed with both acids and salt are attributed to resulting modifications in the state of molecular aggregation of the dyes.

Buffered solutions of the dyes with a Sörensen (*P*H) value of 6.5 undergo immediate oxidation upon the addition of potassium permanganate solution. With limited quantities of permanganate the relative decrease in intensity of absorption increases with the degree of sulfonation of the dye. The character of the modification in absorption is illustrated in the curves obtained with a sample of technical indigotin (sodium indigotin disulfonate) recorded in Fig. 4.

The influence of alkalies upon the color and absorption of the dyes is in some measure dependent upon the strength of the alkali. Under slightly alkaline conditions (in the presence of 0.1% of sodium carbonate or 0.01% of sodium hydroxide) no decided immediate change occurs, but the absorptions of the solution undergo a gradual decrease accompanied by only relatively slight alteration in the general form of the absorption curves and in the color of the solutions. With more decidedly alkaline conditions the decrease in the absorption is much more rapid, while the form of the absorption curve and color of the solutions are modified in a considerable degree.

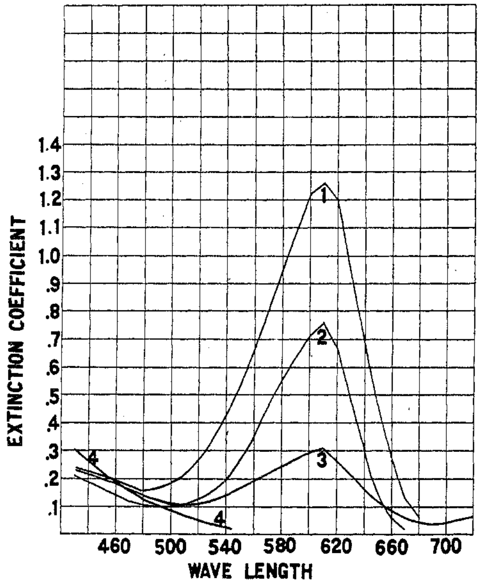

Fig. 4.—Technical indigotin. (Absorption of 50 parts of technical indigotin per million parts of buffered aqueous solution (*P*H 6.5) in a 1cm. cell.) (1) = 0.4 parts of $KMnO_4$ per million parts of solvent; (2) 4.0 parts; (3) 10 parts; (4) 20 parts

The general character of the modification of the absorptions of the indigotins under the influence of alkalies is illustrated in the curves obtained with technical indigotin recorded in Fig. 5.

The alterations in the form of the absorption under decidedly alkaline conditions bear a close resemblance to those effected by oxidation with potassium permanganate.

Employing the standard buffer solutions of Clark, solutions of the dyes with Sörensen values of 11.70 and 10.79 exhibit, respectively, the two types of behavior which have been described as characteristic of the effects of decided and of slight alkalinity. Solutions with a Sörensen value of 8.08 give no indications of alkalinity effects even upon prolonged standing. The employment of buffer reagents is attended by appreciable salt effects.

In Table I are included data bearing upon the relative stabilities of the dyes to the action of permanganate and of various degrees of alkalinity. The recorded values are ratios of the intensities of absorptions at the maxima under the conditions specified to those of solutions in distilled water.

TABLE I

| Conditions | Indigotin $SO_3K$ | Indigotin $(SO_3K)_2$ | Indigotin $(SO_3K)_3$ | Indigotin $(SO_3K)_4$ |
|---|---|---|---|---|
| 4 Parts $KMnO_4$ per million | 0.87 | 0.77 | 0.73 | 0.70 |
| 0.1% NaOH | .76 | .62 | .37 | .34 |
| Buffered solution (*P*H 11.70) | [a] | .80 | .61 | .43 |
| 0.1% $Na_2CO_3$ (after 18 hours) | .64 | .61 | .58 | .76 |
| 0.01% NaOH (after 24 hours) | .70 | .55 | .50 | .78 |
| Buffered solution (*P*H 10.79) (after 24 hours) | [a] | .71 | .75 | .92 |

[a] Precipitation of dye occurred.

The stability of the dyes to the action of permanganate and to that of decided alkalinity decreases with progressive sulfonation. Under slightly alkaline conditions, on the other hand, the stability of the tetrasulfonic derivative is even greater than that of the monosulfonic derivative.

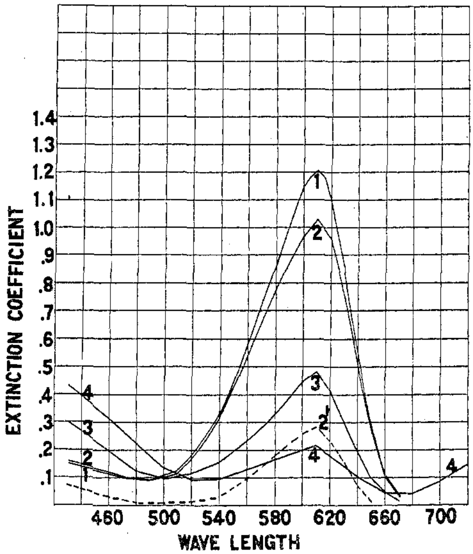

Fig. 5.—Technical indigotin. (Absorption of 50 parts of dye per million parts of solvent in a 1cm. cell.) (1) Distilled water; (2) 0.02% NaOH; (2') 0.02% NaOH (after 20 hrs.); (3) 0.10% NaOH; (4) 0.20% NaOH

The investigations of Chilikin and Milanovskii[2] have shown that oxygen rather than the hydroxyl ion is the reactive agent in the changes which occur in alkaline solutions of indigotin and that the rate of the oxidation is dependent upon hydroxyl-ion concentration. The spectrophotometric evidence obtained in the present investigation supports those conclusions but indicates, furthermore, that the hydroxyl-ion concentration influences the course which the reaction follows apart from determining its rate. A decided alteration in the mechanism of the reaction and in the products formed occurs in varying the alkalinity of the solution between the Sörensen values of 10.79 and 11.70.

In the evaluation of the dyes on the basis of quantitative absorption measurements, a rigid regulation of hydrogen-ion concentration is neces-

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[2] Chilikin and Milanovskii, *J. Russ. Phys. Chem. Soc.*, **44**, 359 (1912).

sary and a standardized technique advisable. Standard absorption data for the pure dyes are submitted in Table II. The extinction coefficients recorded were obtained in one-cm. cells with solutions prepared by dissolving 0.002 g. of dye in 250 cc. of distilled water, adding 25 cc. of a stock buffer solution containing 1.785 g. of sodium phosphate and 0.24 cc. of glacial (99.5%) acetic acid[3] and diluting to 500 cc.

It was found necessary to resort to heating in order to insure the complete solution of the monosulfonic derivative prior to the addition of the buffer reagent. The solution of the other dyes was effected at room temperature. The tabulated values are averages of three series of determinations in which the extreme variations in absorption at the maxima were in all instances less than 2%.

TABLE II

| | Extinction Coefficients | | | |
|---|---|---|---|---|
| Wave length μμ | Indigotin $SO_3K$ | Indigotin $(SO_3K)_2$ | Indigotin $(SO_3K)_3$ | Indigotin $(SO_3K)_4$ |
| 430 | 0.14 | 0.13 | 0.12 | 0.105 |
| 440 | .14 | .13 | .115 | .10 |
| 450 | .135 | .125 | .11 | .09 |
| 460 | .13 | .115 | .09 | .07 |
| 470 | .125 | .10 | .07 | .055 |
| 480 | .115 | .09 | .06 | .05 |
| 490 | .12 | .09 | .06 | .05 |
| 500 | .13 | .10 | .08 | .07 |
| 510 | .15 | .12 | .105 | .09 |
| 520 | .195 | .165 | .15 | .135 |
| 530 | .26 | .235 | .215 | .20 |
| 540 | .365 | .325 | .305 | .285 |
| 550 | .495 | .45 | .425 | .395 |
| 560 | .65 | .60 | .565 | .52 |
| 570 | .81 | .77 | .705 | .64 |
| 580 | .975 | .925 | .85 | .77 |
| 590 | 1.13 | 1.075 | .995 | .855 |
| 600 | 1.205 | 1.22 | 1.08 | .74 |
| 610 | 1.215 | 1.28 | 1.005 | .495 |
| 620 | 1.135 | 1.205 | 0.795 | .225 |
| 630 | 0.97 | 0.94 | .56 | .055 |
| 640 | .77 | .655 | .235 | ... |
| 650 | .565 | .345 | .095 | ... |
| 660 | .36 | .16 | ... | ... |
| 670 | .205 | .05 | ... | ... |
| 680 | .11 | ... | ... | ... |
| 690 | .05 | ... | ... | ... |
| 700 | .02 | ... | ... | ... |

The accuracy of the spectrophotometric method of estimation in the instance of the indigotins is probably but slightly inferior to that of the method of reduction with titanous chloride. It possesses the important

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[3] *J. Assoc. Off. Agr. Chem.*, [2] **II**, 166.

advantage over chemical methods of applicability in the direct determination of the dyes in the presence of many other dyes.

The spectroscopy of the sodium salts of the indigotin sulfonic acids is unquestionably closely analogous to that of the potassium salts. In the spectrophotometric evaluation of technical indigotin various anomalies are encountered which may be attributed in part to the presence of varying quantities of monosulfonate. Comprehensive data on the absorption of the pure dye throughout the visible spectrum, obtained with particular consideration to the practical requirements of the analysis of mixtures of food dyes, are in course of preparation by H. Wales of the Color Laboratory.

## Summary

The absorption of the pure potassium salts of the sulfonated indigotins in the visible spectrum has been measured under a variety of conditions.

Data are supplied for their spectrophotometric identification and evaluation.

WASHINGTON, D. C.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF BARNARD COLLEGE]

# THE ISOMERIC ESTERS OF PARA-METHOXY-BENZOYLACRYLIC ACID

BY GRACE POTTER RICE

RECEIVED OCTOBER 26, 1923

In a recent communication[1] the transformation in the sunlight of the yellow methyl and ethyl esters of benzoylacrylic acid into the corresponding colorless isomers and the reactions of these esters were described; in a continuation of the investigation of unsaturated ketonic esters the isomeric methyl and ethyl esters of the *p*-methoxy derivative of benzoylacrylic acid have now been prepared and studied. It has been found that the stable yellow esters when exposed to the sunlight in benzene solution are changed into colorless isomers and that the reverse transformation of the colorless into the yellow esters is brought about by exposing solutions of the colorless solids containing a crystal of iodine to the sunlight. The yellow esters of *p*-methoxy-benzoylacrylic acid, like those of the unsubstituted acid, are doubtless the stable forms; as is usually the case with stereo-isomers of this type, the stable methyl *p*-methoxy-benzoylacrylate has the higher melting point, but the reverse is true with ethyl *p*-methoxy-benzoylacrylate whose labile form melts 4° higher than its isomer. The behavior of the two yellow esters when exposed to the sunlight in the solid state is very different; the ethyl ester, like the two esters of benzoylacrylic acid, liquefies in the sunlight and is transformed into its colorless isomer. The methyl ester, with its melting point of 72°, does not liquefy when

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[1] THIS JOURNAL, 45, 222 (1923).