Carbon and hydrogen determinations of the four lignin fractions gave the following results.

Anal. (Fraction A.) Subs., 0.1534, 0.1215: CO_2, 0.3517, 0.2783; H_2O, 0.0666, 0.0529. Found: C, 62.52, 62.46; H, 4.86, 4.87.

Anal. (Fraction B.) Subs., 0.0890, 0.0948: CO_2, 0.2043, 0.2171; H_2O, 0.0390, 0.0438. Found: C, 62.60, 62.45; H, 4.90, 5.17.

Anal. (Fraction C.) Subs., 0.1112, 0.0974: CO_2, 0.2614, 0.2298; H_2O, 0.0543, 0.0469. Found: C, 64.10, 64.34; H, 5.46, 5.39.

Anal. (Fraction D.) Subs., 0.0857, 0.1039: CO_2, 0.2139, 0.2597; H_2O, 0.0487, 0.0603. Found: C, 68.06, 68.16; H, 6.36, 6.49.

Summary

Lignin was fractionally extracted from corn cobs by a 2% alcoholic sodium hydroxide solution at room temperature, by 2% aqueous sodium hydroxide at 100° and at 135°, and finally by 4% aqueous sodium hydroxide at 180°. Each method of extraction was continued until no further lignin was obtained, before the next method in the series was employed. The results justify the conclusion that the lignin in corn cobs is unequally combined with the carbohydrates, part of it being loosely bound, possibly in the form of an ester, and the remainder being more firmly held, probably in the form of an ether-like combination.

WASHINGTON, D. C.

[149TH CONTRIBUTION FROM THE COLOR AND FARM WASTE DIVISION, BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE]

THE TAUTOMERISM OF BRILLIANT CRESYL BLUE

BY WALTER C. HOLMES

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Brilliant cresyl blue is an oxazine dye having metachromatic properties which finds important application in biological staining. The stain sold by the National Aniline and Chemical Company is the dimethyl homolog of the dye illustrated under No. 877 in the Colour Index.

With variation in concentration in aqueous solutions this dye undergoes a striking modification in color of the same type that all metachromatic dyes and the majority of aminated triphenylmethane and quinone-imide coloring matters in general undergo.[1] In relatively concentrated aqueous solutions it is present principally in a violet form, with maximum absorption at about 575mμ. The dilution of these solutions is accompanied by a progressive transition to a second dye form, which is blue, with maximum absorption at about 625mμ. Representative absorption curves are recorded in Fig. 1.

Considerable evidence has been obtained which indicates that color modification of this type does not arise from electrolytic or hydrolytic

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[1] Holmes, *Ind. Eng. Chem.*, **16**, 35 (1924).

dissociation, solvation or alteration in the state of molecular aggregation of the solute. It constitutes evidence of tautomerism in the dye, in which the modification in the dye molecule is probably the result of a rearrangement of affinities, brought about by the interplay of residual affinities of solute and solvent.[2,3]

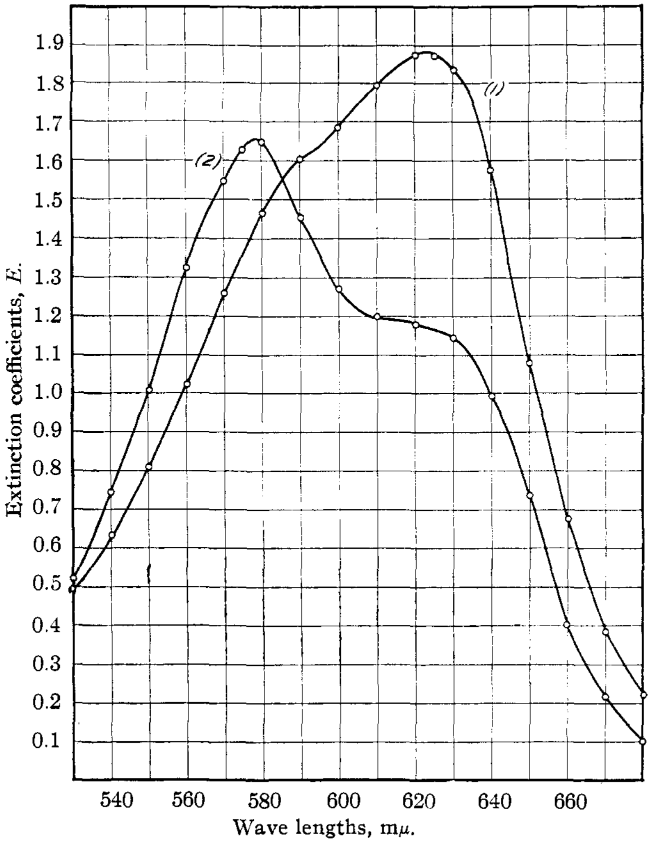

Fig. 1.—Brilliant cresyl blue. (1) Approximately 40 mg. of dye per liter of distilled water in a 1-cm. layer. (2) Approximately 1000 mg. of dye per liter of distilled water in a 0.0432-cm. layer.

Means have been found of obtaining the violet form of brilliant cresyl blue in a medium in which its properties may be investigated to better advantage than is possible in aqueous solutions. The writer is indebted to Dr. Marian Irwin of the Rockefeller Institute for suggestions which led to this discovery.

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[2] Holmes, This Journal, **46**, 2118 (1924).

[3] Holmes, *Am. Dyestuff Reporter*, **16**, 429 (1927).

A 0.1% solution of the dye is prepared in a $M/15$ acetate buffer solution of 0.6 M sodium chloride content, having a *P*H value of 3.42. (The solution should be distinctly acid, but the precise degree of acidity is relatively immaterial.) The solution is shaken thoroughly with an equal volume of chloroform and the chloroform is diluted 1:5 to obtain a suitable intensity of color for spectrophotometric measurements in 1-centimeter cells. The absorption curve of such a solution is recorded in Fig. 2, Curve 1.

In this solution most of the dye is present in its violet form. The curve gives evidence, however, of a small proportion of dye in its blue form. The modification of this solution upon saturation with ammonia is illustrated by Fig. 2, Curve 2.

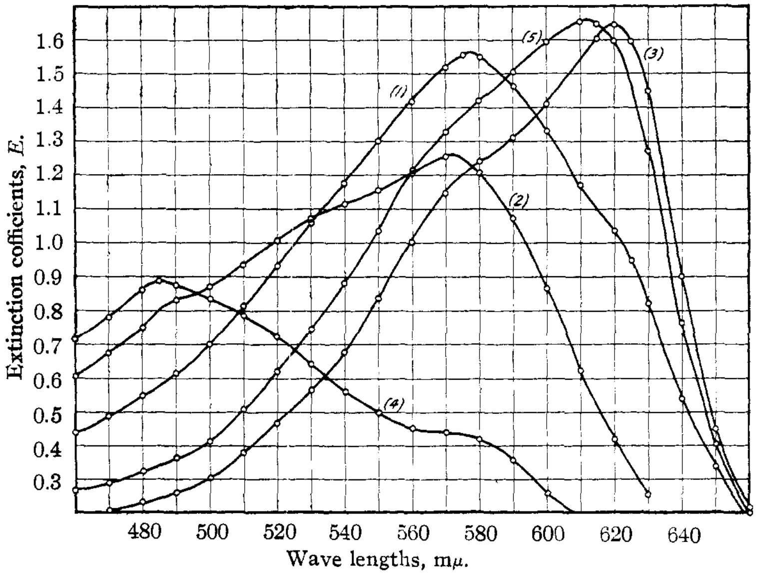

Fig. 2.—Brilliant cresyl blue.

By direct solution of the solid dye in chloroform a solution which gives the absorption represented by Fig. 2, Curve 3, may be obtained. In this solution the greater part of the dye is present in its blue form, although there is definite evidence of the presence of a small proportion of the violet form. The modification of this solution upon saturation with ammonia is recorded in Fig. 2, Curve 4, and the subsequent modification of the ammoniacal solution upon the addition of 2% glacial acetic acid is shown in Fig. 2, Curve 5.

The principal interest of these data lies in the evidence which they supply respecting the relative reactivity of the various forms of the dye.

The saturation of the solution of the violet form of the dye with ammonia results in only a minor degree of conversion to the orange dye-base (see Curves 1 and 2, Fig. 2). The actual degree of conversion of violet dye to base is much less than the apparent degree, owing to the presence in the original solution of appreciable quantities of the blue form of the dye, which undergoes complete conversion to base. On the other hand, the saturation of the solution of the blue form of the dye with ammonia results in its immediate and complete conversion to the dye-base (see Curves 3 and 4, Fig. 2). The resulting solution still contains the dye originally present in the violet form, apparently in an unaltered state. The blue form of the dye is very reactive to alkali, whereas the violet form of the dye is highly inactive.

When the blue form of the dye is converted to dye-base and the dye is subsequently regenerated, the absorption curve of the recovered dye does not coincide in spectral location with that of the original dye (see Curves 3 and 5, Fig. 2). The recovered dye is not brilliant cresyl blue but its monomethyl homolog, as indicated by the agreement between its absorption and that of the monomethyl homolog prepared by direct synthesis at this Laboratory. It is well known that various quinone-imide dyes, such as methylene blue, are so susceptible to alkaline oxidation that their conversion to the dye-base may be attended by the loss of an alkyl substituent group. The blue form of brilliant cresyl blue is so readily oxidized in ammoniacal chloroform solutions that the reaction is practically instantaneous.

The orange dye-base is readily and completely converted into the dye by acids (see Curves 4 and 5, Fig. 2). The dye-base is so reactive, in fact, that the mere exposure of its unprotected chloroform solutions to air results in a comparatively rapid conversion into dye carbonate.

Both the orange dye-base and the blue form of the dye, accordingly, are highly reactive, whereas the violet form of the dye is relatively inert.

In accordance with the hypothesis recently advanced by the writer[4] to account for the tautomerism observed with variation in concentration of aminated dyes in aqueous solution, the transition concerned is one between structure of addition product type, in which the amino nitrogen atom involved is essentially trivalent, and structure of the ammonium salt type in which it is pentavalent. In the instance of brilliant cresyl blue the two color forms may be represented in the following manner

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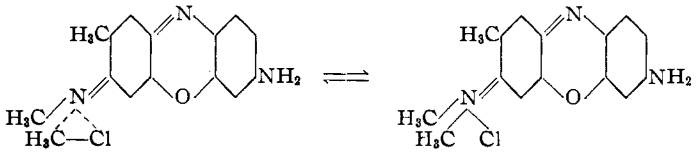

II

(violet) (blue)

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[4] Holmes, *Stain Technology*, **1**, 116 (1926).

This hypothesis was based upon color considerations and apparently affords a thoroughly adequate explanation of the observed differences in color in tautomeric forms of aminated dyes. Direct evidence of structure of the type illustrated in Formula I is lacking, although a closely analogous structure has been demonstrated in the complexes which basic dyes form with cyclic derivatives containing phenolic groups.[5]

A compound of the structural type represented by Formula I would be expected to be relatively inert. The writer's hypothesis, accordingly, supplies an explanation of the observed lack of chemical reactivity in the violet form of brilliant cresyl blue, and this inactivity, in turn, supports the validity of the hypothesis.

Summary

The violet and blue forms of brilliant cresyl blue, which exist in tautomeric equilibrium in aqueous solutions of the dye, have been isolated. The former tautomer is chemically inert whereas the latter is reactive. These facts are held to support the author's hypothesis that the tautomerism is one between structure of addition product type and structure of ammonium salt type.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE CHEMISTRY LABORATORY OF CORNELL COLLEGE]

THE WURTZ REACTION. FACTORS INVOLVED IN THE PREPARATION OF OCTANE

BY HARRY F. LEWIS, ROBERT HENDRICKS AND G. ROBERT YOHE

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The work herein described is concerned with an attempt to determine the factors which govern the successful carrying out of a Wurtz reaction. The preparation of octane from butyl bromide offers such an opportunity.

The preparation of 2,5-dimethylhexane from *iso*butyl iodide and sodium as described by Würtz[1] opened up to the organic chemist a valuable method for the preparation of the higher aliphatic hydrocarbons. Fittig and others soon adapted the reaction for use in the synthesis of mixed aromatic aliphatic and higher aromatic hydrocarbons.

Schorlemmer,[2] using butyl iodide and sodium, was the first to use the reaction in the preparation of normal octane. He compared his product with those octanes formed through the hydrogenation of methylhexylcarbinol and suberic acid and proved their identity.

The mechanism of the Würtz-Fittig reaction has received much study

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[5] Holmes and Hann (awaiting publication). This paper will probably be published in the October, 1928, issue of *Stain Technology.*

[1] Wurtz, *Ann.*, **96**, 365 (1855).

[2] Schorlemmer, *Ann.*, **161**, 280 (1872).