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CONSTITUTION OF ERYTHROSIN AND RELATED DYES

By Walter C. Holmes and John T. Scanlan

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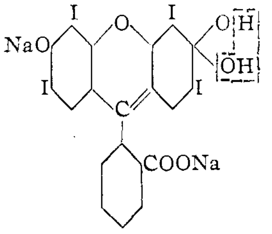
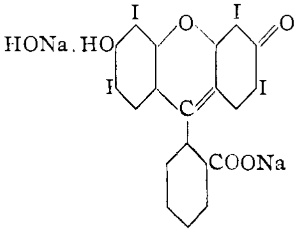
Recent investigations of Gomberg and Tabern,[1] Holmes[2] and Wales and Nelson[3] have established the fact that the composition of erythrosin, when dried by any ordinary means, may be represented as $C_{20}H_8O_6I_4Na_2$. This formula has the elements of a molecule of water in excess of the formula previously assigned to the anhydrous dye. The tenacity with which these elements are retained warrants the conclusion that their association with the dye molecule is more intimate than would be the case with water of hydration.

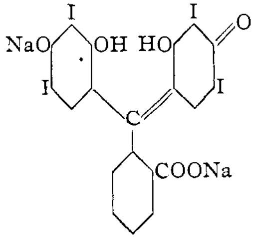
Gomberg and Tabern suggest the introduction of the elements of water at the quinoid-oxygen linkage, with the formation of hemiquinoid structure (I). Wales and Nelson consider it more probable that an addition product is formed by the union of one molecule of the monosodium salt of the dye with one molecule of sodium hydroxide (II). A third alternative is conceivable on the assumption that salt formation involves an opening of the pyrone ring (III).



I



II



III

In support of Formula I, Gomberg and Tabern cite the known tendency of diphenylfuchsone and analogous compounds to form similar products with water.

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[1] Gomberg and Tabern, *J. Ind. Eng. Chem.*, **14**, 1115 (1922).

[2] Holmes, *Color Trade J.*, **13**, 4 (1923).

[3] Wales and Nelson, This Journal, **45**, 1657 (1923).

No analogy exists, so far as we are aware, for a structural assumption of the particular type represented by Formula II. It appears improbable that a molecule of sodium hydroxide could be held in the additive state by a phenolic group.

Formula III is analogous to one which has been suggested as a possible formula for the "hydrated" form of the color acid of fluorescein. An analogy for the opening of the pyrone ring during salt formation is apparently supplied by certain data given by Moir. He records the absorption maxima of *o*-dihydroxy-aurine and *p*-hydroxy-resorcin benzein in alkaline aqueous solution as 545 and 544[4]mμ, respectively. If the identity of his materials is accepted, this agreement in color can be explained only on the assumption that salt formation with *p*-hydroxy-resorcin benzein involves scission of the pyrone ring.

It appears probable, however, that Moir's supposed *o*-dihydroxy-aurine was, in reality, *p*-hydroxy-resorcin benzein. It is doubtful if *o*-dihydroxy-triphenylmethane derivatives exist. If *o*-dihydroxy-aurine is capable of existence, the color of its alkali salt should be much deeper than that of its supposed salt as noted by Moir.

Similar objections may be offered to Formula III in the instance of erythrosin. If the structure was capable of existence, which appears doubtful, the color of this substance would almost certainly be much deeper than that of erythrosin. It would be an *o*-dihydroxytetra-iodophenolphthalein. The alkali salts of tetra-iodophenolphthalein are blue-violet (their maxima in alkaline aqueous solution are at 593mμ), and those of its *o*-dihydroxy derivative would be expected to be blue-green, or even green. It is hardly conceivable that they would be red, as is erythrosin.

A spectrophotometric investigation of the behavior of erythrosin with alkalies and with acids was undertaken to obtain further evidence on its structure.

The absorption band of the disodium salt of erythrosin has its maximum at approximately 524mμ in aqueous solutions and at approximately 531mμ in alcoholic solutions. The addition of considerable excesses of alkali gave no indication of further salt formation in either aqueous or alcoholic solution. In conjunction with the difficulty of reconciling Formula III with the color of the dye already referred to, this fact is taken as conclusive evidence against that formula. If the dye (disodium salt) contained free hydroxyl groups, further salt formation would be expected with an excess of alkali, attended by appreciable modifications in color.

The reactions of the dye with acid may be followed with the spectrophotometer to best advantage when alcohol and acetic acid are employed as solvents. The band of the dye in 95% alcohol has its maximum absorption at approximately 531mμ. (It is advisable to add a trace of alkali

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[4] Moir, *Trans. Roy. Soc. S. Africa,* [4], **10,** 233; [3], **10,** 159 (1922).

to insure complete exclusion of the monosodium salt.) With the addition of a very small quantity of acetic acid, the band of the disodium salt disappears and is replaced by that of the monosodium salt, which has its maximum at approximately 541mμ. When an excess of acid is avoided, this transformation is attended by little or no decrease in color intensity. Further additions of acid result in a progressive transformation of the monosodium salt into the (yellow) color acid which has a band with maximum absorption at approximately 490mμ. The color and absorption of the color acid are much less intense than those of its salts.

The difference in color and absorption between the disodium and monosodium salts is a strong argument against the validity of Formula II and in favor of Formula I. Addition products of triphenylmethane dyes are known[5] and do not differ appreciably in color from the dyes from which they are prepared. By analogy it would be expected that the color of a compound of the structure of Formula II would be the same as that of the monosodium salt of the dye. On the other hand, the observed difference in color and absorption between the monosodium and disodium salts of erythrosin is readily accounted for on the basis of salt formation at the phenolic group. Data are available on a number of triphenylmethane dyes containing a phenolic group, such as Patent Blue A, Patent Blue V, Cyanole Extra and Fast Green FCF, which indicate that salt formation at such a group invariably has a hypsochromic effect upon color, of the same general order of magnitude as that observed in the present instance.

A small quantity of the monosodium salt of erythrosin was prepared by the addition of 1 molecular equivalent of acid to an alcoholic-aqueous solution of the disodium salt and the evaporation of the solution. The residue was pulverized and extracted repeatedly with water and ether to remove the disodium salt and color acid present. The resulting product was sparingly soluble in water and gave the spectrum of the monosodium salt. (The absorption maximum in alcohol was 541mμ.) After drying at 110° for four days it reached approximately constant weight. Two weighed portions were then dissolved in water, with the addition of a little alkali, and the color acid was precipitated by the addition of acid, dried and weighed. From 0.6637 and 0.4522 g. of monosodium salt, 0.6354 and 0.4333 g. of color acid were obtained, respectively. These values indicate that the dried salt contained approximately 0.83 of a molecule of water. The monosodium salt of erythrosin, accordingly, may be represented as $C_{20}H_9O_6I_4Na$ rather than $C_{20}H_7O_5I_4Na$. It retains the elements of a molecule of water as does the disodium salt. The hypothesis of Wales and Nelson predicates an anhydrous monosodium salt.

For these reasons we favor the Gomberg and Tabern formula for the

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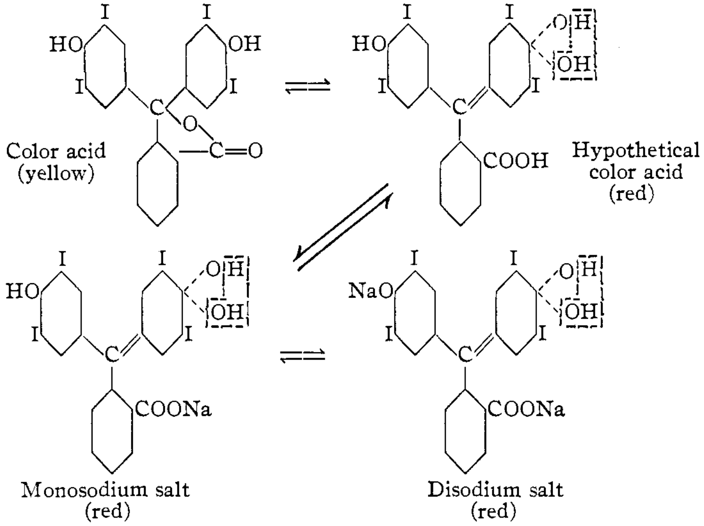
[5] Holmes and Hann, unpublished results.

dye. The probable mechanism of salt formation may be represented in the following manner.



The assumption of hemiquinoid structure in the dye salts apparently affords a satisfactory explanation of their relatively moderate depth of color in comparison with the color acid. In the phenolphthalein and phenolsulfonephthalein series salt formation, which is accepted as involving transition between lactonoid and quinoid structure, results in an extreme modification in color. The formation of the sodium salt of phenolsulfonephthalein, for example, shifts the absorption band in the direction of longer wave length by approximately 120mμ. If salt formation in the instance of erythrosin also involved transition between lactonoid and quinoid structure it should result in a corresponding displacement of the band. The formation of the monosodium salt of erythrosin, however, shifts the band by only about 50mμ. The color of the salts of erythrosin, accordingly, appears inconsistent with quinoid structure.

Preliminary data on nine other dyes of the erythrosin group have also been obtained. Although the purity of these products was not established with sufficient thoroughness to warrant absolute conclusions, the results were definitely indicative of analogy with erythrosin. It is considered highly probable, therefore, that the halogenated derivatives of fluorescein, in general, retain the elements of a molecule of water in excess of their anhydrous composition and should be represented by formulas analogous to those which have been advanced for erythrosin.

Summary

A spectrophotometric investigation of salt formation with erythrosin has been made and the monosodium salt prepared and tested.

The evidence obtained is held to support the hemiquinoid formula for the dye advanced by Gomberg and Tabern and an analogous formula for the monosodium salt.

It appears probable that the halogen derivatives of fluorescein, in general, are of analogous structure.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF TUFTS COLLEGE]

THE ADDITION OF AMINO AND HYDRAZINO BASES TO NITROSTYRENE[1]

BY DAVID E. WORRALL

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While the properties and reactions of such unsaturated substances as benzalacetophenone or ethyl cinnamate have been extensively investigated, much less is known concerning the chemistry of ethylene derivatives containing a nitro group; yet the latter are tractable solids easily prepared and purified, provided an aromatic group is present. Accordingly, nitrostyrene, $C_6H_5CH{=}CHNO_2$, a typical unsaturated nitro compound of this class, has been studied with regard to its chemical behavior toward some representative substances known to react with α, β unsaturated ketones and esters and which, therefore, by inference might be expected to combine with nitrostyrene. This paper contains the results obtained with ammonia and various organic bases derived from ammonia and hydrazine containing an amino group. Posner[2] in a systematic investigation of the action of hydroxylamine on α,β unsaturated acids and esters derived from cinnamic acid found that the same base reacts with nitrostyrene, forming a saturated nitro compound. With this exception, apparently no work has been done previously in this particular field.

It has been found in this Laboratory that such common reagents as aniline and phenylhydrazine react very readily indeed with nitrostyrene, forming colorless crystalline addition products. Nitrostyrene is an α,β unsaturated nitro compound containing a conjugated unsaturated system and accordingly is capable of 1,4 addition. There seems no reason to doubt that these amino derivatives react with nitrostyrene in this manner. The reaction starts through the mobile hydrogen of the amino group which, because of its chemical affinity for oxygen, becomes linked to that element. The rest of the molecule attaches itself to carbon (of the ethylene group), more remote from the nitro group.

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[1] This problem was suggested by Professor A. Michael, to whom the writer is greatly indebted, and much of the work was done in his Laboratory.

[2] Posner, *Ann.*, **389**, 114 (1912).