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135. Tsutomu Momose, Yo Ueda, and Masaru Nakamura: Organic Analysis. XXV.[*1] Mechanism of the Reaction between Fructose and Diphenylamine.[*2]

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The mechanism of the color reaction of fructose with diphenylamine introduced by Ihl[1] has been studied by several investigators. Schoorl, *et al.*[2] concluded that 5-hydroxymethylfurfural might be an intermediate of the reaction, because the compound gave the same coloration as the sugar when heated with diphenylamine in a mixture of ethanol and hydrochloric acid. On the other hand, Radlberger[3] supposed that the dye produced in the reaction might be diphenylbenzidine violet, which gave diphenylbenzidine on reduction with zinc powder. More recently, Thies and Kallinich[4] separated the dye as an amorphous powder and assumed that it might be a derivative of triphenylmethane dye, because its absorption spectrum resembled that of methoxymalachite green. This paper presents a more probable structure of the dye.

Isolation of the dye

The crude dye prepared according to the literature[4] was extracted with a mixture of 80% methanol and a small amount of hydrochloric acid. When the solvent was concentrated, a crystalline dye separated which was repeatedly recrystallized from 80% methanol in the presence of a small amount of hydrochloric acid. The dye came as golden prisms of m.p. 178°(decomp.), soluble in ethanol, methanol, and acetone, giving a deep violet solution. It was insoluble in water, benzene, and ether, and sparingly soluble in ethyl acetate.

The sulfate of the dye was prepared by using sulfuric acid in the above extraction instead of hydrochloric acid. It was recrystallized from 80% methanol in the presence of a small amount of sulfuric acid to golden prisms of m.p. 198°(decomp.). Its solubility in usual solvents was similar to that of the chloride.

The absorption curve of the dye as sulfate and chloride is shown in Fig. 1. It has an absorption maximum at 633 mμ and the curve agrees with that of the developed color (Fig. 2) in the shape and maximum, indicating that this dye is the main coloring matter of the reaction.

Structure of the Dye and Reaction Mechanism

From the data of microanalyses it is more probable that the dye has the formula of $C_{60}H_{48}O_3N_4\cdot Cl_2$ or $(HSO_4)_2$, which indicates that two moles of fructose may react with four moles of diphenylamine as follows:

$$2C_6H_{12}O_6 + 4C_{12}H_{11}N + 2HCl + O_2 \longrightarrow C_{60}H_{48}O_3N_4Cl_2 + 11H_2O$$

The molecular weight of the dye cannot successfully be determined as a chloride or sulfate, though Thies and Kallinich already gave a molecular weight of about 1160 to the free amorphous dye.

[*1] Part XXIV: This Bulletin, 8, 514(1960).

[*2] A short communication was published in this Bulletin, **7**, 853(1959).

[*3] Katakasu, Fukuoka (百瀬 勉, 上田 陽, 中村 優).

1) A. Ihl: Chemiker-Ztg., **9**, 451(1885).

2) N. Schoorl, P.C.J. van Kalmthout: Ber., **20**, 181(1887).

3) L. Radlberger: Österr.-ungar. Z. Zuckerind. u. Landwirtsch., **44**, 261(1915).

4) H. Thies, G. Kallinich: Chem. Ber., **85**, 438(1952).

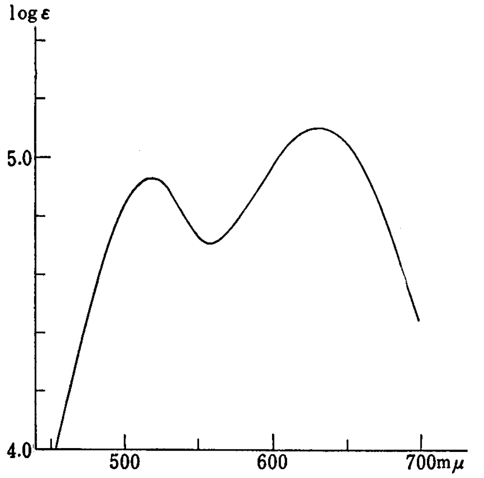


Fig. 1. Absorption Spectrum of the Dye as Sulfate and Chloride (Solvent same as in Fig. 2)

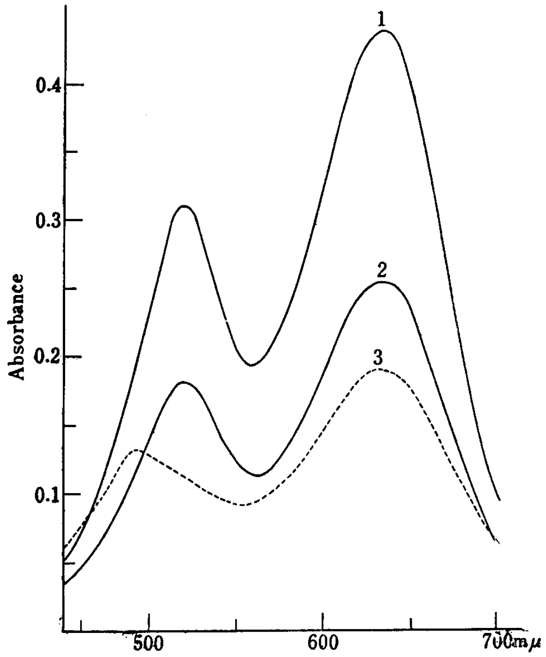
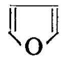
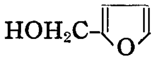
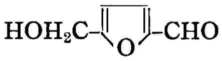
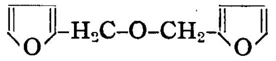
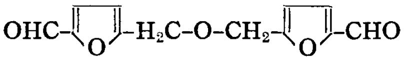
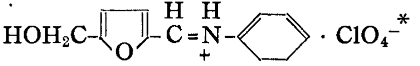


Fig. 2. Absorption Spectra of the Developed Color
1. 1/1000 mole of 5,5′-diformyldifurfuryl ether
2. 2/1000 mole of 5-hydroxymethylfurfural
3. 2/1000 mole of fructose
The above compd. was refluxed with a little excess of diphenylamine in a mixture of 2 volumes of EtOH, 2 volumes of conc. HCl, and 1 volume of H_2O for 15 min., and diluted to 5 volumes with the mixture.

The infrared spectra of the chloride and sulfate of the dye show neither hydroxyl nor carbonyl absorption band. On the other hand, both spectra show five absorption bands which may be caused by the furan ring. These bands are found in the six regions which were proposed by Yamaguchi[5] as the characteristic absorption of furan deriva-

Table I.

Absorption region (in μ) Compd.	3.18~ 3.20	6.37~ 6.41	6.62~ 6.67	9.70~ 9.95	11.30~ 11.45	13.0~ 13.5
	3.16	6.33	6.71	9.32	11.47	13.11
	3.16 m.	6.40	6.82	9.80 s.	11.32 s.	13.22 s.
	3.15 m.	6.40 w.	6.64 s.	9.90 s.	11.31 s.	13.40 s.
	3.15 m.	6.42 v.s.	6.53 s.	9.35 m.	—	13.20 m.
	3.10 m.	6.28 w.	6.62 s.	9.40 s.	11.30 m.	13.50 s.
	3.12 m.	—	6.54 m.	9.70 m.	11.26 m.	13.56 m.
	3.10 w.	6.54 s.	6.69 m.	9.71 s.	—	13.10 s.
Dye (chloride)	3.21 w.	6.38 s.	6.76 s.	9.65 w.	—	13.35 s.
Dye (sulfate)	3.10 w.	6.40 s.	6.75 v.s.	9.60 s.	—	13.32 s.

w = weak; m = medium; s = strong; v = very.
* G. Kallinich : Arch. Pharm., **63**, 274(1958).

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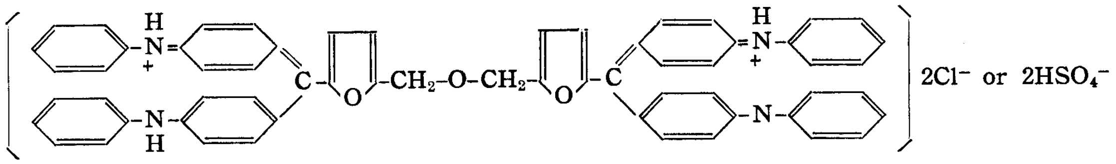
[5] M. Yamaguchi : Bunseki Kagaku, **7**, 210(1958).

tives, and are shown in Table I with the corresponding bands of some related furan derivatives. Thus, a probable structure of the dye may be written as follows :



This assumption may be confirmed by the fact that 5,5′-diformyldifurfuryl ether[6] gives a higher absorption curve in the developed color than fructose and 5-hydroxymethylfurfural (Fig. 2). The same dye is also isolated in higher yield from the reaction product of the ether with diphenylamine in a mixture of methanol and hydrochloric acid.

Now it seems clear that fructose first forms 5-hydroxymethylfurfural in the color reaction and then dehydrated to the ether in an acid medium. This ether may combine with four moles of diphenylamine and the resultant substance may be oxidized by the air to the above derivative of triphenylmethane dye.

Experimental

Isolation of the Dye—Two g. of the crude dye was extracted with a mixture of 100 cc. of 80% MeOH and 0.5 cc. of HCl at 50°. The filtrate was concentrated to about 40 cc., and 1 cc. of HCl was added. The separated crystals (140 mg.) were repeatedly recrystallized from 80% MeOH in the presence of a small amount of HCl to golden prisms, m.p. 178°(decomp.). *Anal.* Calcd. for $C_{60}H_{48}O_3N_4Cl_2$: C, 76.33; H, 5.13; N, 5.94; Cl, 7.52. Found : C, 76.39; H, 5.33; N, 5.80; Cl, 7.97.

The sulfate was prepared by extraction of 2.0 g. of the crude dye with a mixture of 150 cc. of 80% MeOH and 0.5 cc. of H_2SO_4. It was recrystallized from a mixture of 80% MeOH and a very small amount of H_2SO_4 to golden prisms, m.p. 198°(decomp.). *Anal.* Calcd. for $C_{60}H_{50}O_{11}N_4S_2$: C, 67.51; H, 4.73; O, 16.50; N, 5.25; S, 6.01. Found : C, 67.46; H, 5.24; O, 16.44; N, 4.96; S, 5.80.

The same dye was also isolated from the reaction product of 5,5′-diformyldifurfuryl ether with diphenylamine. In this case, 0.7 g. of the ether was refluxed with 2.0 g. of diphenylamine in a mixture of 17.5 cc. of MeOH and 17.5 cc. of HCl for 5 min. The mixture was diluted with the same volume of water, the separated substance was collected, and washed with water. This amorphous crude dye was treated in the same way as mentioned above to give the crystalline dye. The sulfate (130 mg.) thus formed was identified as the above dye by the m.p. of admixture and infrared spectrum.

Absorption Spectra—The visible-light spectra were measured by a Hitachi EPU-2 Spectrophotometer in a cell of 10 mm. optical length. The infrared spectra were measured by a Koken DS-301 Infrared Spectrophotometer in Nujol mull with about 0.01 mm. thickness.

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Summary

The main dye produced in the color reaction of fructose with diphenylamine was isolated in crystalline form as chloride and sulfate. Its probable structure was presented and the reaction mechanism was assumed.

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[6] J. Kiermayer : Chemiker-Ztg., **19**, 1003(1895).