Experimental

Isolation of the Dye——To 5 g. of N-methyldiphenylamine dissolved in a mixture of 115 ml. of MeOH and 115 ml. of conc. HCl, 5 g. of fructose dissolved in 10 ml. of H_2O was added, and the mixture was refluxed on a water bath for 5 min. After cool at room temperature, 100 ml. of H_2O was added, and separated resinous precipitate was removed by filtration. To the filtrate, 200 ml. of H_2O was added, and allowed to stand overnight. The separated crude chloride of the dye (1.8 g.) was collected, and recrystallized from 80% MeOH in the presence of a small amount of HCl to golden prisms, m.p. 145° (decomp.). *Anal.* Calcd. for $C_{64}H_{56}O_3N_4Cl_2$: C, 76.86; H, 5.64; N, 5.60; Cl, 7.09; H, 5.88; N, 5.54; Cl, 6.88; N-CH_3, 5.48.

The sulfate of the dye was obtained as golden prisms, m.p. 155∼157° (decomp.), by the repeated recrystallization of the above chloride from 40% MeOH in the presence of a small amount of H_2SO_4. N-CH_3, 6.00. Found: C, 76.32; *Anal.* Calcd. for $C_{64}H_{58}O_{11}N_4S_2$: C, 68.43; H, 5.21; N, 4.99; S, 5.71; N-CH_3, 5.34. Found: C, 68.35; H, 5.55; N, 5.11; S, 5.52; N-CH_3, 4.93.

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 a Nujol mull with about 0.01 mm. thickness.

The authors extend their gratitude to Misses. Y. Hitaka and T. Shinkai for their technical help in these studies. They are also indebted to Misses. S. Tada and S. Indo, and Messrs. M. Shirozu and M. Shido for the microanalyses and to Messrs. H. Yano, H. Matsui, and K. Hikita for the measurement of infrared spectra.

Summary

The main dye of the color reaction between fructose and N-methyldiphenylamine was isolated in crystalline form as its chloride and sulfate. Its probable structure was presented and the reaction mechanism was assumed as the same as in the reaction of fructose with diphenylamine.

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87. Tsutomu Momose, Yo Ueda, and Masatake Iwasaki:
Organic Analysis. XXXIV[*1]. Reaction Mechanism of Glucuronic Acid with 1,3-Naphthalenediol.

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In a previous paper[1] of this series, the mechanism of the Tollens reaction,[2] in which glucuronic acid gave a violet coloration when heated with 1,3-naphthalenediol in moderately concentrated hydrochloric acid, was clarified by isolating the main dyes in crystalline form. On the other hand, Guerrero and Williams[3] found that the same coloration was also given by the fusion of glucuronic acid with 1,3-naphthalenediol, and stated that the dye formed in the reaction was difficult to purify. This paper describes

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[*1] Part XXXIII: This Bulletin, **10**, 544 (1962).

[*2] Katakasu, Fukuoka (百瀬 勉, 上田 陽, 岩崎正武).

[1] T. Momose, Y. Ueda, M. Iwasaki: This Bulletin, **4**, 49 (1956).

[2] B. Tollens, F. Rorive: Ber., **41**, 1783 (1908).

[3] A. H. Guerrero, R. T. Williams: Nature, **161**, 930 (1948).

the mechanism of the reaction, isolating the main dyes in crystalline form as their methyl ethers.

Isolation of the Dyes——Glucuronic acid and 1,3-naphthalenediol were mixed in the mole ratio of 1:2, and fused at 120∼130° for 20 minutes in an oil bath. The resultant reaction product was dissolved in ether, and methylated with diazomethane to stabilize the dyes. Chromatographic separation of the methylated dyes on alumina with benzene as the effluent gave ten fractions which were divided by their adsorption color as shown in Table I.

Table I. Chromatographic Separation of the Coloring Matters

Fraction No.	Color of effluent	Color on alumina
1	colorless (with fluorescence)	pale yellow
2	orange yellow	yellow
3	red violet	red violet
4	yellowish green (with fluorescence)	yellowish green
5	red violet	red violet
6	rose violet	rose violet
7	red violet	violet
8	violet	blue violet
9	colorless	blue
10	〃	brown

The main fractions which had violet colors in benzene consisted of four sections. After evaporation of the solvent, the fraction No. 8, left greenish violet prisms of m.p. 241∼242°, which were identified as Dye I-methyl ether (I) in the Tollens reaction by the melting point of admixture and also by the infrared spectrum. The fraction No. 7, gave golden violet prisms of m.p. 294∼295°. This substance was identified in the same way as Dye Ⅱ-methyl ether (Ⅱ) in the Tollens reaction.

Two new dyes were obtained from the other main fractions. Dye Ⅲ-methyl ether (Ⅲ), red violet needles of m.p. 268∼270° (decomp.), and Dye Ⅳ-methyl ether (Ⅳ), red violet needles of m.p. 258° (decomp.), were separated from the fractions Nos. 5 and 3, respectively. The absorption spectra of the new dyes are shown in Figs. 1 and 2. They have the absorption maxima at 558 mμ, in benzene solution, and indicate that they are also nothing but the main coloring matters of the reaction.

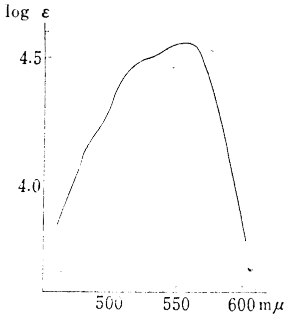


Fig. 1. Ultraviolet Absorption 1.075 mg. of Dye Ⅲ-methyl ether dissolved in 250 ml. of benzene λ_{max} 558 mμ (log ε 4.56)

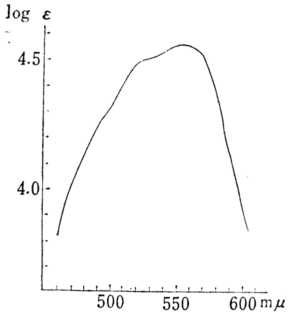


Fig. 2. Ultraviolet Absorption 1.186 mg. of Dye Ⅳ-methyl ether dissolved in 250 ml. of benzene λ_{max} 558 mμ (log ε 4.55)

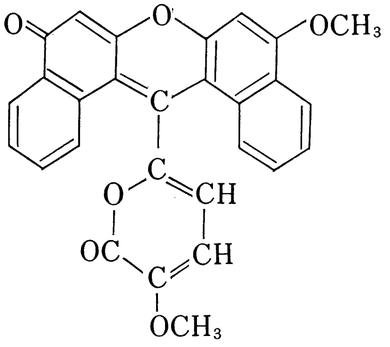
Structure of the Dyes and Reaction Mechanism——The nature of (Ⅲ) and (Ⅳ) quite resembles that of (I) and (Ⅱ). For example, they are reduced by zinc and acetic acid to leuco compounds, and recover their original color by the air oxidation. They are soluble in dilute alcoholic sodium hydroxide solution, from which they are regenerated by acidifying with hydrochloric acid. Therefore, they must also be quinones of xanthene derivatives.

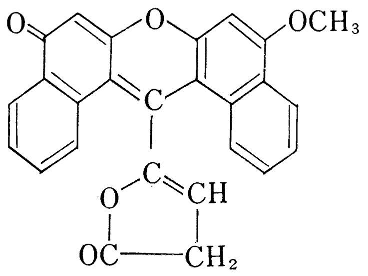
The data of microanalyses and methoxyl determination indicate that (III) may have the formula $C_{25}H_{15}O_4(OCH_3)$, and (IV) may have the formula $C_{26}H_{17}O_4(OCH_3)$. From the data of infrared spectra, it is more probable that both new dyes have a saturated γ-lactone ring with a carbonyl group of quinone (Table II). This assumption is also confirmed by the visible light absorption spectral data in the table, which indicates that (III) and (IV) may have no conjugated double bond to the quinone ring, showing a shift of 6 mμ from (II) to a shorter wave length region. This value of the shift may be reasonable when compared with the shift of 9 mμ of (II) from the most bathochromic compound (I), which has two conjugated double bonds.

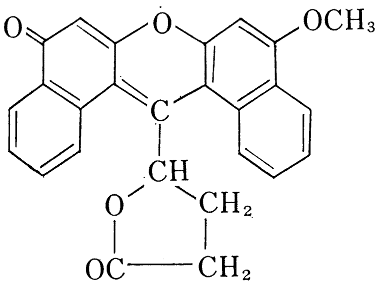
TABLE II. Spectral Data of the Dyes

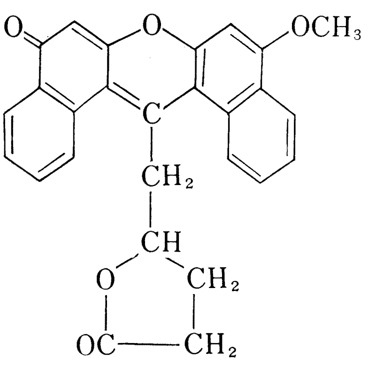
Dye	λ_{max} (mμ)	log ε	$\nu_{C=O}$ in quinone (cm^{-1})	$\nu_{C=O}$ in lactone (cm^{-1})
(I)	573	4.62	1634	1727
(II)	564	4.49	1650	1792
(III)	558	4.56	1639	1783
(IV)	558	4.55	1645	1783

From the above conclusion, it is clear that Dye I-methyl ether and Dye II-methyl ether may be formed by the same mechanism as in the Tollens reaction. Dye III-methyl ether may have the structure of (III), and its original dye may be formed by the


(I)


(II)


(III)


(IV)

intra-molecular oxidation and reduction in the absence of the air in the fused reaction. Dye IV-methyl ether may be assumed to have the structure of (IV), but the mechanism of the reaction is not successfully explained in the present state, except it may be said that some reducing reaction may occur in the vigorous reaction of fusion.

Experimental

Isolation of the Dyes——Mixture of 5 g. of 1,3-naphthalenediol and 3.8 g. of glucuronic acid was heated at 120~130° for 20 min. with stirring. At the end of the reaction the mixture turned to bluish resinous substance. After cool, it was dissolved in 800 ml. of Et_2O, and methylated in an ice bath with CH_2N_2 produced from 20 g. of nitrosomethylurea. After 2 days, the excess CH_2N_2 was decomposed with AcOH, and the solvent was removed by distillation. The violet brown residue was dissolved in benzene, poured onto an alumina column, and eluted with benzene as shown in Table I.

After evaporation of the solvent, violet prisms of m.p. 241~242° and m.p. 294~295° were obtained from the fractions No. 8 and 7, and identified as Dye I-methyl ether (I) Dye II-methyl ether (II), respectively. The fraction No. 5, left red violet needles when the solvent was removed by distillation. This substance (Dye III-methyl ether) was recrystallized from benzene-petr. benzine, m.p. 268~270°(decomp.). Yield, 20 mg. *Anal.* Calcd. for $C_{26}H_{18}O_5$: C, 76.09; H, 4.42; O, 19.49; OCH_3, 7.56. Found: C, 76.15; H, 4.03; O, 19.20; OCH_3, 7.80.

Dye IV-methyl ether was obtained from the fraction No. 3, and recrystallized from benzene-petr. benzine to red violet needles, m.p. 258°(decomp.). Yield, 25 mg. *Anal.* Calcd. for $C_{27}H_{20}O_5$: C, 76.40; H, 4.75; O, 18.85; OCH_3, 7.31. Found: C, 76.54; H, 4.38; O, 18.47; OCH_3, 7.17.

Light Absorption Spectra——The spectra in visible region were measured by a Beckman Model DK-2 ratio recording spectrophotometer in a 10-mm. cell. The infrared spectra were measured by a Koken Model DS-301 recording infrared spectrophotometer in Nujol mull in about 0.01 mm. thickness.

The authors are indebted to Messrs. M. Shido, and Y. Sato for the microanalyses, and to Messrs. H. Matui, and K. Hikita for the light absorption spectra.

Summary

The main coloring matters of the fused reaction of glucoronic acid with 1,3-naphthalenediol were isolated in crystalline form as their methyl ethers. Two of them were identified as the dyes produced in the Tollens reaction. The structures of two new dyes were presented, and the reaction mechanism was discussed.

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