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12. Tsutomu Momose, Yo Ueda, and Masatake Iwasaki : Organic Analysis. VI.* Structure of the Dyes from Glucuronic Acid and Naphthoresorcinol.**

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In 1908, Tollens and Rorive[1] reported that glucuronic acid gives a violet coloration when heated with naphthoresorcinol in 5~6*N* hydrochloric acid. The usefulness of this reagent was proved by its wide use in the estimation of uronic acids. On the structure of the dye produced in the reaction two formulae have been presented.

Ogata and Nozaki[2] isolated a dye from the reaction mixture, and concluded that it was hydroxybis(naphthylfurfuryl)methane by the analytical data of its derivatives. As Machida[3] pointed out, this formula could not explain the fact that furfural and pentoses gave a different color with naphthoresorcinol, nevertheless penturonic acid, which produced no furfural by hydrochloric acid, gave a violet color with this reagent. Guerrero and Williams[4] found that the dye was also formed by the fusion of naphthoresorcinol with glucuronic acid, and supposed that the dye may be a xanthene or dinaphthylmethane derivative, without isolation of the dye. This paper describes the isolation of a new dye, and presents another reaction mechanism.

Isolation of Dyes When glucuronic acid was heated with naphthoresorcinol in 1~4*N* hydrochloric acid, the intensity of the violet color increased with the increasing concentration of the acid and prolongation of the reaction time (Fig. 1). It seemed to need two moles of naphthoresorcinol to one mole of glucuronic acid to give the maximum intensity as indicated by Ogata and Nozaki[2] (Fig. 2). To prepare the dye,

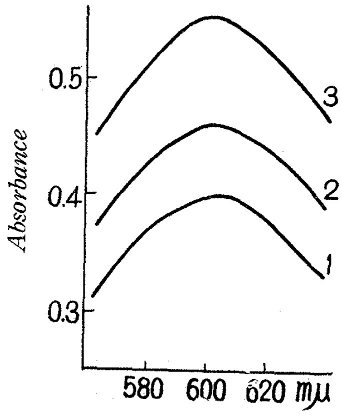

Fig. 1. Reaction time and Absorbances
(1) heated for 1 hr.
(2) heated for 2 hrs.
(3) heated for 3 hrs.

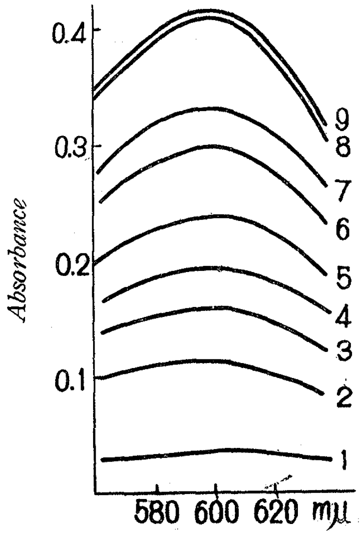

Fig. 2. Molar Ratio of Glucuronic Acid to Naphthoresorcinol, and Absorbances
Numbers are those in Table II.

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1) B. Tollens, F. Rorive : Ber., **41**, 1783 (1908).

2) A. Ogata, Y. Nozaki : J. Pharm. Soc. Japan, **63**, 416 (1942) ; **64**, 42 (1943).

3) S. Machida : J. Japan. Chem., **7**, 634 (1953).

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

as it was well known that the coloring matters were unstable, the reaction was carried out for 2~7 minutes in 3~4*N* hydrochloric acid, and the resulting mixture was extracted with ether. The ethereal solution of the coloring matters was methylated with diazomethane, and the substances that separated were collected. When the reaction was stopped in a short time (2~3 minutes) or carried out at low temperature (about 70°), the product consisted of crude greenish violet crystals. Chromatographic separation of their benzene solution gave seven fractions which were divided by their adsorption color on alumina (Table I).

TABLE I. Chromatographic Separation of the Coloring Matters

Fraction No.	Color of effluent	Color on alumina
1	orange yellow	orange
2	pink	pink violet
3	yellowish green (with fluorescence)	yellowish green
4	pink violet	pink violet
5	violet	bluish violet
6	colorless	blue
7	colorless	brown

From the main fraction, No. 5, greenish violet prisms (I) of m.p. 241~242° (Dye-I methyl ether) were obtained.

When the reaction was carried out for a longer time or at higher temperature (above 80°), golden violet prisms (II) of m.p. 294~295° (Dye II-methyl ether) were obtained from the methylated mixture. The melting point and analytical data of this methyl ether agree with those of the methyl ether described by Ogata and Nozaki.[2]

The absorption spectra of these dyes are shown in Figs. 3 and 4. They have the absorption maxima at 572~574 mμ and 564 mμ, respectively, in benzene solution, indicating that the dyes are nothing but the main coloring matters of the reaction.

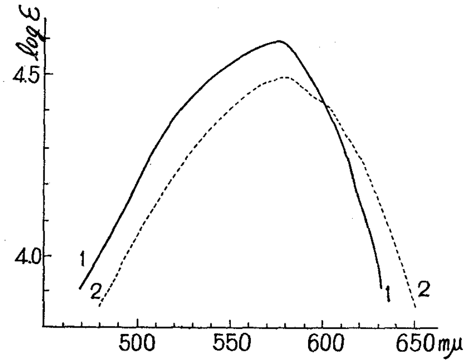


Fig. 3. Ultraviolet Absorption

(1) 1.047 mg. of Dye I-methyl ether dissolved in 100 cc. of benzene. λ_{max} 572~4 mμ (log ε = 4.62).

(2) 1.015 mg. of Dye I-methyl ether dissolved in 50 cc. of BuOH. λ_{max} 578 mμ (log ε = 4.49).

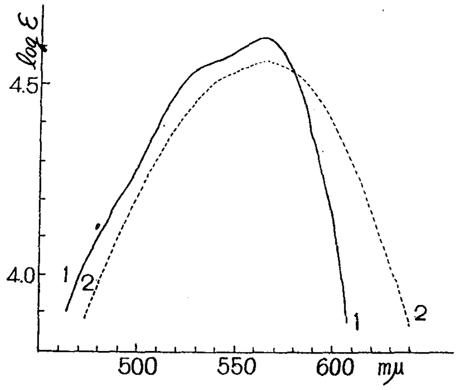


Fig. 4. Ultraviolet Absorption

(1) 1.984 mg. of Dye II-methyl ether dissolved in 500 cc. of benzene. λ_{max} 564 mμ (log ε = 4.63).

(2) 0.954 mg. of Dye II-methyl ether dissolved in 100 cc. of BuOH. λ_{max} 565 mμ (log ε = 4.57).

Structure of the Dyes and Reaction Mechanism. From the data of microanalyses and methoxyl determinations, it is more probable that (I) has the formula $C_{23}H_{12}O_4-(OCH_3)_2$, and (II) has the formula $C_{25}H_{12}O_4(OCH_3)$. As indicated by Guerrero and Williams,[4] these dyes are reduced by zinc and acetic acid or by sodium hydrosulfite to leuco compounds, which recover the original color by mild oxidation. Therefore, they must be quinones of xanthene derivatives.

The remaining two oxygens of the dyes may form lactones introduced from glucuronic acid, because the two methyl ethers are soluble in sodium hydroxide solution, and regenerate their color by hydrochloric acid. One methoxyl group of the methyl ethers must be in xanthene nucleus, and the other methoxyl group of (I) may be an enol ether of α-ketolactone, which loses a carbonyl group by heating with hydrochloric acid at higher temperature or in longer time.

The above assumption will be confirmed by the infrared spectra of the dyes (Fig. 5). (I) exhibits strong absorption bands at $5.79\,\mu$($1727\,\mathrm{cm}^{-1}$) and $6.12\,\mu$($1634\,\mathrm{cm}^{-1}$), which correspond to a δ-lactone and a quinone, respectively. (II) has also two bands at $5.58\,\mu$($1792\,\mathrm{cm}^{-1}$) and $6.06\,\mu$ ($1650\,\mathrm{cm}^{-1}$), indicating a β,γ-unsaturated γ-lactone and a quinone, respectively.

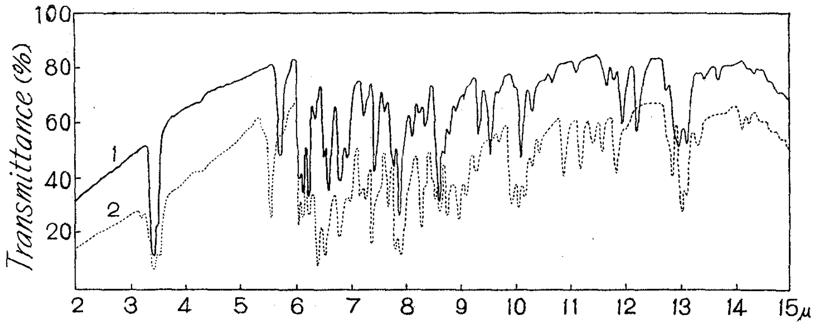
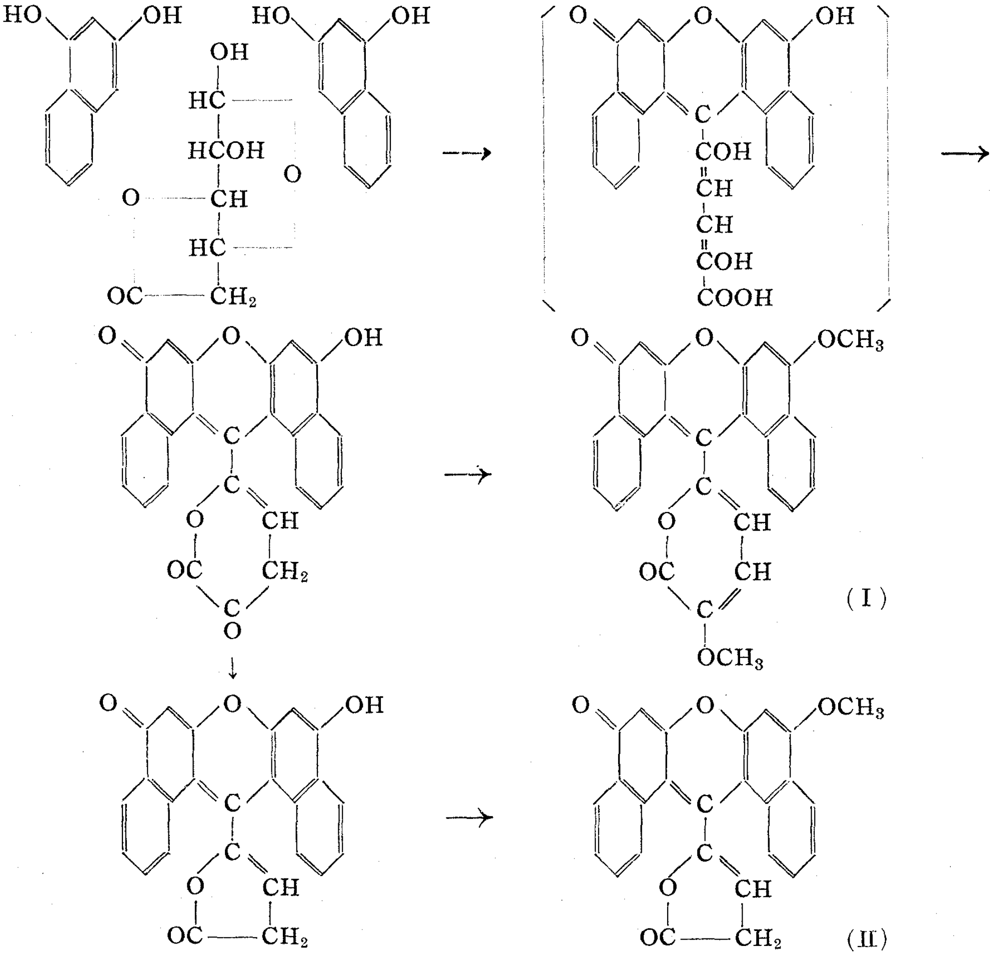


Fig. 5. Infrared Spectra
(1) Dye I-methyl ether. (2) Dye II-methyl ether.



From the above conclusion, the raction mechanism is supposed as follows : Two moles of naphthoresorcinol may first combine with the aldehyde group of glucuronic acid and dehydrations at 2–3 and 4–5 positions of glucuronic acid would result in α-oxo-γ,δ-unsaturated δ-lactone (Dye I), which gives an enolic methyl ether (I) with diazomethane. Dye I may lose a carbonyl group by hydrochloric acid, changing to a β,γ-unsaturated γ-lactone (Dye II), which gives a methyl ether (II) by methylation.

The authors extend their thanks to Mr. T. Hattori for the microanalyses. They are also indebted to Dr. A. Watanabe of Takeda Research Laboratory for the infrared spectra, and to Chugai Pharmaceutical Co., Ltd. for the gift of glucuronic acid and naphthoresorcinol.

Experimental

Examinations of Reaction Conditions—One cc. of 0.1% aq. solution of glucuronic acid was mixed with 1 cc. of 0.1% aq. solution of naphthoresorcinol and 2 cc. of 30% HCl, and heated for a definite time in boiling water. After cooling in an ice bath, resulting mixture was extracted with 3 cc. BuOH. One cc. of the clear supernatant was diluted to 5 cc. with BuOH and its absorbance was read. The results are shown in Fig. 1.

The molar ratio of glucuronic acid to naphthoresorcinol was varied as shown in Table II, the resultant mixture was heated for 3 hrs., and its absorbance was read as above. The results are shown in Table II and Fig. 2.

TABLE II. Molar Ratio of Glucuronic Acid to Naphthoresorcinol and Absorbance at 600 mμ

Expt. No.	G*(cc.)	N*(cc.)	H_2O(cc.)	Mol. ratio(G/N)	Absorbance at 600 mμ
1	1	0.2	1.8	1/0.25	0.034
2	1	0.4	1.6	1/0.5	0.113
3	1	0.6	1.4	1/0.75	0.158
4	1	0.8	1.2	1/1	0.192
5	1	1.0	1.0	1/1.25	0.239
6	1	1.2	0.8	1/1.5	0.297
7	1	1.4	0.6	1/1.75	0.331
8	1	1.6	0.4	1/2	0.406
9	1	1.8	0.2	1/2.25	0.415

* G=0.1% aq. solution of glucuronic acid.
N=0.1% aq. solution of naphthoresorcinol.

Dye I-Methyl Ether (I)—To a solution of 0.75 g. of glucuronic acid in 200 cc. of 15% HCl, 1 g. of naphthoresorcinol was added and heated for 5 mins. at 70° or 2~3 mins. in boiling water. The resulting mixture was cooled in an ice bath and extracted with 300 cc. of ether. The ethereal solution was washed successively with 5% $NaHCO_3$ and water, and methylated in an ice bath with CH_2N_2 produced from 3.5 g. of nitrosomethylurea. After 2 days greenish violet crystals appeared. The excess CH_2N_2 was decomposed with AcOH, the solvent was evaporated, and the reddish brown residue was dissolved in benzene with the crystals. This solution was poured through 100 g. of alumina column, and eluted with benzene alone or the same solvent to which 3% of acetone was added. From the main fraction greenish violet prisms were obtained by condensation of the solvent. Recrystallization from benzene-EtOH gave pure crystals of m.p. 241~242°(microscopic determination). Yield, 10 mg. This substance is soluble in benzene, glacial AcOH, pyridine, and conc. H_2SO_4, and sparingly soluble in EtOH. *Anal.* Calcd. for $C_{28}H_{18}O_6$: C, 74.66; H, 4.03; OCH_3, 13.78. Found : C, 74.34; H, 4.12; OCH_3, 13.48.

Dye II-Methyl Ether (II)—When the reaction was carried out for 5~7 mins. at above 80°, golden violet prisms separated after methylation. Recrystallization from benzene gave pure crystals of m.p. 294~295°(microscopic determination). Yield, 15 mg. This substance is soluble in glacial AcOH, pyridine, and conc. H_2SO_4, slightly soluble in benzene, and sparingly soluble in EtOH. *Anal.* Calcd. for $C_{26}H_{16}O_5$: C, 76.46; H, 3.95; OCH_3, 7.60. Found : C, 76.15; H, 3.73; OCH_3, 7.65.

Light Absorption Spectra—The spectra in visible region were measured by a Shimadzu Photoelectric Spectrophotometer type QB-50 in a 10-mm. cell, with the reagent as a blank. The infrared spectra were measured by a Perkin-Elmer Infrared Spectrophotometer as a Nujol mull in about 0.01 mm. thickness.

Summary

The coloring matter of naphthoresorcinol reaction with glucuronic acid was shown to consist of two main dyes, one of which was isolated by the present authors. The new dye was supposed to change to the Ogata and Nozaki's dye at a higher temperature or by a longer reaction time by hydrochloric acid. The newly presented structure of the dyes may explain all the analytical data and the reaction mechanism.

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13. Minoru Kawashima, Akira Miyake, Teruji Hemmi, and Shizuko Fujii :

Biochemical Studies on Acidomycin. III. Antibiotin Activity of Acidomycin and Its Related Compounds.*

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Recently, it was reported that acidomycin, 2-(5-carboxypentyl)-4-thiazolidone, was antagonistic to biotin on cultures of *Mycobacterium tuberculosis typus avium* and several lactic acid bacteria.[1] Miyake previously announced the antitubercular activity of various compounds related to acidomycin and discussed the relationship between their antitubercular activity and chemical structures.[2]

As necessary prerequisites for the compounds to show antitubercular activity he revealed that; (1) the side-chain attached to the 2-position of the thiazolidone ring must contain a carboxyl or a group derivable from carbonyl, (2) the alcohol derivatives obtained by the reduction of the carboxyl still have some antitubercular activity, but when the $-CH_2OH$ group is further reduced to a methyl group the activity disappears, (3) the side-chain must be a straight pentamethylene chain, (4) the side-chain is not a sole factor of the activity, (5) modifications at 2- and 3-positions of the thiazolidone ring cause disappearance of the activity, and (6) the carbonyl group at 4-position as well as the bivalent sulfur at 1-position is essential.

This report describes experiments designed to obtain some insight into the relationship between the chemical structures, antitubercular activity, and properties antagonistic to biotin of these compounds.

Material and Methods

The microörganism employed in these experiments was *Mycobacterium tuberculosis typus avium*. This was regularly maintained in the form of slant cultures containing 1% meat extract, 1%

TABLE I. Composition of the Basal Medium

Na_2HPO_4	3.0 g.
KH_2PO_4	4.0 〃
$MgSO_4$	0.6 〃
Sodium citrate	2.5 〃
Asparagine	5.0 〃
Glycerol	20.0 〃
Tween 80 (in 25% solution)	20.0 cc.
Distilled water	1,000.0 〃 (pH 7.0)

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[1] M. Kawashima, Y. Hamada, S. Fujii : This Bulletin, **1**, 94 (1953).

[2] A. Miyake : *Ibid.*, **1**, 89 (1953).