

88. Tsutomu Momose and Yosuke Ohokura : Organic Analysis. XXXV.\*1  
Mechanism of the Color Reaction between Hexose and Thymol. (1).

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Thymol gave a sensitive orange red coloration when heated with a carbohydrate material in sulfuric acid,<sup>1)</sup> and was used in the estimation of hexose,<sup>2)</sup> blood sugar,<sup>3)</sup> and carbohydrate in biological fluid.<sup>4)</sup> Prof. Kiyoshi Takiura suggested that the coloring matter of the reaction might be isolated in crystalline form,<sup>\*3</sup> but the mechanism of the reaction has remained unknown. By his favour, the present authors had the chance to study the mechanism, and isolated two leuco dyes from the reaction mixture.

**Isolation of the Leuco Dyes**—Thymol was heated with glucose or fructose at 50° in 70 v/v% of sulfuric acid and allowed to stand at room temperature overnight. Higher temperature was avoided to prevent coagulation of the coloring matter into clots. The resultant colored mixture was poured into ice water, the separated substance was collected, and extracted with ethyl acetate.

Chromatographic separation of the extract on alumina with ethyl acetate as eluant gave two fractions which were divided by their adsorption colors on alumina. From the first fraction, which showed a faint yellow band on alumina, two colorless compounds appeared in crystalline form. One of them was in prisms of m.p. 281°, which gave an acetate as colorless needles, m.p. 184°, and was identified as tri(6-thymolyl)methane (III),<sup>5)</sup> which was synthesized in the experimental part. The other compound was in leaflets of m.p. 161° and this gave an acetate as colorless needles, m.p. 97°, and was identified as dithymolymethane (II).<sup>6)</sup>

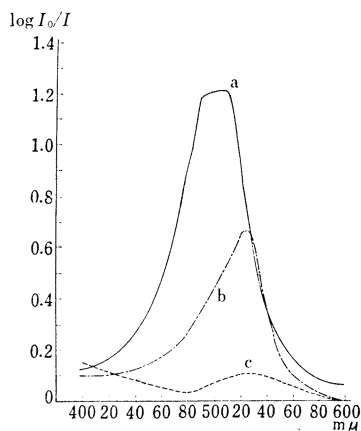


Fig. 1. Absorption Spectra of the Reaction Mixture and the Leuco Dyes

- a : 50  $\gamma$  of Glucose heated with 3.01 mg. of Thymol at 100° for 20 min. in 5 ml. of 60% H<sub>2</sub>SO<sub>4</sub>  
 b : 4.40 mg. of Tri(6-thymolyl)methane in 1 L. of 60% H<sub>2</sub>SO<sub>4</sub> [ $\lambda_{\max}$  m $\mu$  (log  $\epsilon$ ) : 525 (4.83)]  
 c : 3.12 mg. of Di(6-thymolyl)methane in 100 ml. of 60% H<sub>2</sub>SO<sub>4</sub> [ $\lambda_{\max}$  m $\mu$  (log  $\epsilon$ ) : 525 (2.90)]

\*1 Part XXXIV : This Bulletin, 10, 546 (1962).

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Tri(6-thymolyl)methane gave a strong orange red coloration with sulfuric acid, and its absorption spectrum in 60% sulfuric acid is shown in Fig. 1. Di(6-thymolyl)methane also gave a weak orange red coloration with sulfuric acid, and its absorption spectrum is also shown in Fig. 1. Both compounds had the absorption maxima at 525  $m\mu$ , and indicated that they were the leuco dyes of some part of the main coloring matters, because the reaction mixture of thymol and hexose had the maximum at 510~515  $m\mu$ <sup>4)</sup> as shown in Fig. 1.

The second fraction, which showed a brown band on alumina, seemed to be a mixture of several dyes which gave a blue violet coloration with sulfuric acid, and were difficult to separate. The alumina column held a large quantity of resinous matter on the top, which could not be eluted with ethyl acetate.

**Reaction Mechanism**—Isolation of the two leuco dyes described above indicated that hexose first formed 5-hydroxymethylfurfural in the reaction, from which formaldehyde was liberated by the action of sulfuric acid. This fact was also confirmed by the experiment that 5-hydroxymethylfurfural gave the same coloration with thymol in sulfuric acid, and tri(6-thymolyl)methane was isolated in relatively high yield from the reaction mixture. Again the same compound was also obtained from the reaction mixture of formaldehyde and thymol in sulfuric acid.

Now, the problem remained if tri(6-thymolyl)methane was oxidized to the corresponding methylenequinone (IV) in the reaction mixture or not. The compound, (IV), was obtained from tri(6-thymolyl)methane by the oxidation of ferric chloride as brown red prisms, m.p. 265° (decomp.), and its structure was identified by the elemental analysis and by the infrared spectrum, which showed a hydroxyl band at 3268  $cm^{-1}$ , and a carboxyl band at 1623  $cm^{-1}$ . It showed an orange red color when dissolved in sulfuric acid, and a violet coloration in alkali. The absorption spectra of these colorations are shown in Fig. 2. On the other hand, the methylenequinone was not successfully isolated in crystalline form from the reaction mixture of thymol and hexose, but its occurrence might be proved by the following study.

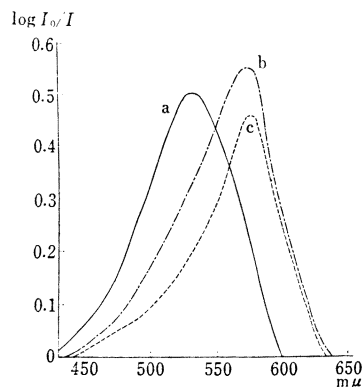
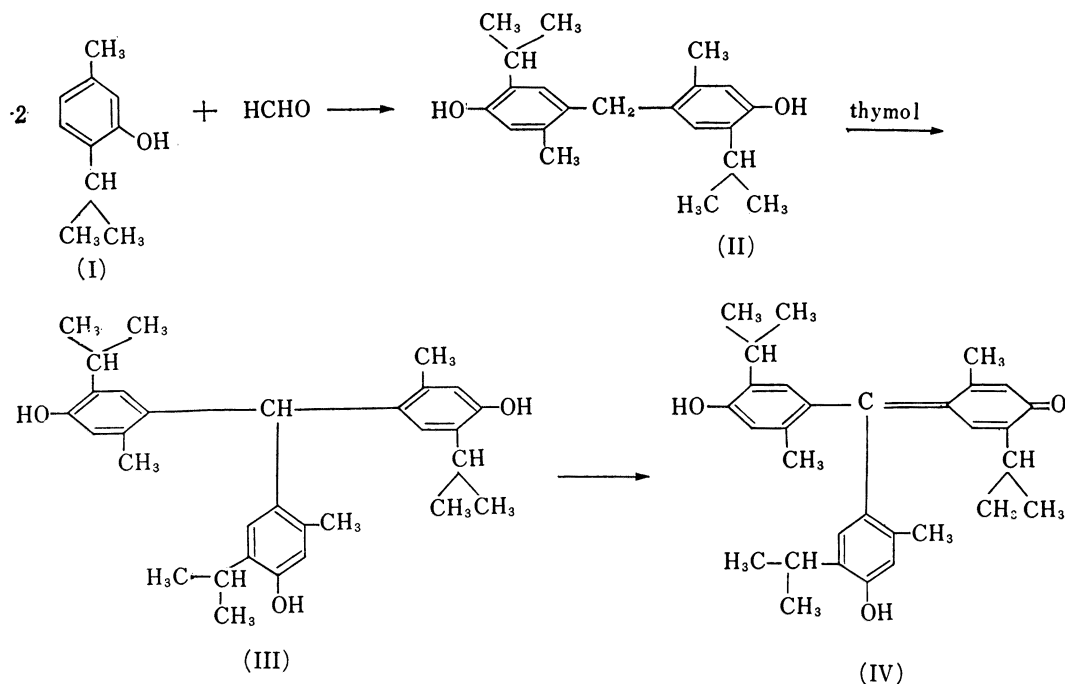


Fig. 2. Absorption Spectra of the Methylenequinone

- a : 4.58 mg. of the Methylenequinone in 1 L. of 60%  $H_2SO_4$  [ $\lambda_{max}$   $m\mu$  ( $\log \epsilon$ ): 530 (4.70)]
- b : 4.78 mg. of the Methylenequinone in 1 L. of *N*/10 alcoholic NaOH [ $\lambda_{max}$   $m\mu$  ( $\log \epsilon$ ): 573 (4.84)]
- c : 20 mg. of Tri(6-thymolyl)methane treated with  $H_2SO_4$ , dissolved in 100 ml. of *N*/10 alcoholic NaOH

Tri(6-thymolyl)methane was heated at 100° in 70% sulfuric acid for 2 hours. The ethereal extract of the mixture was separated on alumina, and the coloring matter which gave a violet coloration with alkali was collected. The absorption spectrum of the coloring matter dissolved in alcoholic sodium hydroxide resembled that of the methylenequinone in the shape and maximum as shown in Fig. 2. Above results might reveal the schema of the reaction as follows :



### Experimental

**Isolation of the leuco dyes**—To a solution of 10 g. of thymol in 45 ml. of conc.  $\text{H}_2\text{SO}_4$ , 10 g. of glucose or fructose dissolved in 20 ml. of  $\text{H}_2\text{O}$  was added under cooling, heated at  $50^\circ$  for 10–20 min., and allowed to stand overnight. The mixture was poured into ice water, the separated substance was collected, and washed with  $\text{H}_2\text{O}$ . After drying in a vacuum desiccator, it was extracted with AcOEt, and the extract was successively washed with  $\text{Na}_2\text{CO}_3$  solution and saturated solution of NaCl. The extract was concentrated, poured into alumina column, and eluted with AcOEt. From first fraction crystals were separated when the solvent was concentrated and benzene was added. First recrystallization from EtOH gave colorless prisms, m.p.  $281^\circ$ . Yield, 350 mg. from glucose, and 450 mg. from fructose. The acetate was obtained by the acetylation of AcCl in pyridine and recrystallization from EtOH as needles, m.p.  $184^\circ$ . The leuco dye and this acetate were identified, respectively, as tri(6-thymolyl)methane and its triacetate synthesized below, by the melting point of admixture and by the infrared spectra.

The mother liquid of the leuco dye was extracted with 10% NaOH solution. The extract was treated with active carbon, acidified with HCl, and then extracted with benzene. From benzene extract crystals were separated when the solvent was concentrated. Recrystallization from benzene gave colorless leaflets, m.p.  $161^\circ$ , which were identified as di(6-thymolyl)methane.<sup>9</sup> Acetylation with  $\text{Ac}_2\text{O}$  and  $\text{H}_2\text{SO}_4$  gave diacetate, which was recrystallized from MeOH to needles, m.p.  $97^\circ$ . *Anal.* Calcd. for  $\text{C}_{25}\text{H}_{32}\text{O}_4$ : C, 75.72; H, 8.13. Found: C, 75.74; H, 8.12.

In the case of 5-hydroxymethylfurfural, 2 g. of it was dissolved in 8 ml. of  $\text{H}_2\text{O}$ , and 2 g. of thymol dissolved in 18 ml. of conc.  $\text{H}_2\text{SO}_4$  was added under cooling. The mixture was warmed at  $30^\circ$  for 1 hr. with stirring, and allowed to stand overnight. The mixture was treated as above, and separated crystals were recrystallized from EtOH to prisms, m.p.  $281^\circ$ , which showed no depression of melting point on admixture with tri(6-thymolyl)methane. Yield, 100 mg.

To a solution of 4 ml. of 37% HCHO in 26 ml. of  $\text{H}_2\text{O}$ , 7 g. of thymol dissolved in 130 ml. of conc.  $\text{H}_2\text{SO}_4$  was added under cooling. The mixture was heated at  $50^\circ$  for 30 min., allowed to stand overnight, and treated as above. The separated crystals, m.p.  $281^\circ$ , were identified as tri(6-thymolyl)methane.

**Tri(6-thymolyl)methane**—This compound had been already synthesized by the condensation of *p*-thymolaldehyde to thymol with HCN,<sup>9</sup> but it was easily prepared by the oxidative condensation of di(6-thymolyl)methane to thymol with  $\text{FeCl}_3$  as follows.

To a solution of 10 g. of di(6-thymolyl)methane and 5 g. of thymol dissolved in 50 ml. of AcOH, 23 g. of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  dissolved in 30 ml. of AcOH was added, and heated on a water bath for 2 hr. The mixture was diluted with  $\text{H}_2\text{O}$ , and extracted with  $\text{Et}_2\text{O}$ . The  $\text{Et}_2\text{O}$  extract was successively washed with  $\text{NaHCO}_3$  solution and  $\text{H}_2\text{O}$ , and dried over  $\text{Na}_2\text{SO}_4$ . After removal of the solvent, the residue was dissolved in benzene, and the separated crystals were recrystallized from EtOH to prisms, m.p.  $281^\circ$ . Yield, 600 mg. *Anal.* Calcd. for  $\text{C}_{31}\text{H}_{40}\text{O}_3$ : C, 80.83; H, 8.75. Found: C, 80.54; H, 8.61. The triacetate, m.p.  $184^\circ$ , was obtained by the usual method.

**Oxidation of Tri(6-thymolyl)methane**—To a solution of 1 g. of tri(6-thymolyl)methane in 100 ml. of AcOH, 2.4 g. of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  dissolved in 10 ml. of AcOH was added, and heated on a water bath for 6.5 hr. The mixture was poured into  $\text{H}_2\text{O}$ , and separated substance was extracted with  $\text{Et}_2\text{O}$ . The  $\text{Et}_2\text{O}$  extract was successively washed with  $\text{NaHCO}_3$  solution and  $\text{H}_2\text{O}$ , dried over  $\text{Na}_2\text{SO}_4$ , and concentrated. Then the solution was passed through an alumina column, and eluted with  $\text{Et}_2\text{O}$ . The starting material was recovered from the first fraction, and the second fraction which showed an orange red band on alumina left an oily residue, which was recrystallized from benzene-EtOH to brown red prisms with metallic luster, m.p.  $265^\circ$  (decomp.). *Anal.* Calcd. for  $\text{C}_{31}\text{H}_{38}\text{O}_3$ : C, 81.18; H, 8.35. Found: C, 81.00; H, 8.71.

**Absorption Spectra**—The visible light spectra were measured by a Beckman DK-2 ratio recording 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

Tri(6-thymolyl)methane and di(6-thymolyl)methane were isolated from the colored reaction mixture of hexose with thymol in sulfuric acid. The former compound was also isolated from the reaction mixture of thymol with 5-hydroxymethylfurfural or formaldehyde. The mechanism of the color reaction was discussed.

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### 89. Tsutomu Momose and Masaru Nakamura : Organic Analysis. XXXVI.\*<sup>1</sup> Identification of Amines as the Salts of 3,6-Dinitrophthalic Acid.

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Several carboxylic acids were used in the identification and separation of amines as their salts because of easiness of the operation and simplicity of recovering the original amines. These included 3,5-dinitrobenzoic acid,<sup>1)</sup> 2,4-dinitrobenzoic acid,<sup>2)</sup> and  $\beta$ -resorcylic acid.<sup>3)</sup> In the authors' laboratory 3,6-dinitrophthalic acid proved to be much useful in the same purpose, giving crystalline salts readily with wide ranges of aliphatic and aromatic amines.

\*<sup>1</sup> Part XXXV : This Bulletin, 10, 550(1962).

\*<sup>2</sup> Katakasu, Fukuoka (百瀬 勉, 中村 優).

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