U.D.C. *547.672.5 : 547.457.1*

8. **Tsutomu Momose, Yo Ueda, Kei Sawada, and Ayako Sugi** : Organic Analysis. VIII.* Reaction Mechanism of Anthrone with Sugars. (1).**

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The mechanism of the color reaction of anthrone with sugars introduced by Dreywood[1] has been studied spectrophotometrically by several investigators. Sattler and Zerban[2] concluded that the color was due to formation of furfural compounds from sugars by strong sulfuric acid. This conclusion was confirmed when Shriver, Webb, and Swanson[3] revealed that the spectral curves of anthronated glucose and xylose were almost identical with those of hydroxymethylfurfural and furfural, respectively, with the reagent. Furthermore, Yemm and Wills[4] pointed out that the rate of formation of 5-hydroxymethylfurfural from different hexoses was parallel to the rate of development of the anthrone color. This paper presents evidence of another reaction mechanism from analytical data.

Reaction conditions

The most suitable conditions for the preparation of the dye formed by anthrone with glucose were first examined. When anthrone was heated with glucose at 85~90° in sulfuric acid, the variation of concentration of the acid gave different absorption curves. The maximum absorption around 620 mμ shifted to a longer wave length region with the increasing concentration of the acid and held the maximum value of absorption coefficient at 630 mμ when the reaction was carried out in 70% (v/v) acid (Fig. 1). The reaction time also changed the value even when the concentration of the acid was the same and gave the maximum value when the mixture was heated for 20 minutes (Fig. 2). Another

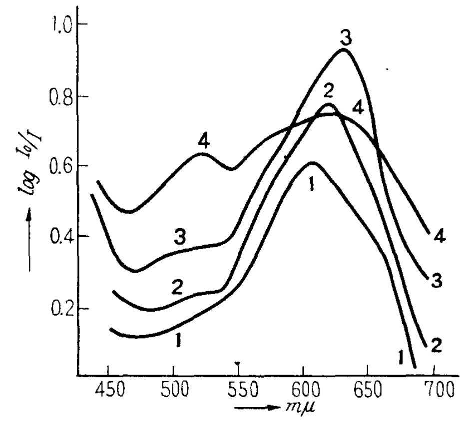

Fig. 1.

Absorption Curve of the Reaction Mixture

0.25 mg. of glucose heated for 20 mins. with 12 mg. of anthrone at 85~90° in 15 cc. of (1) 60%, (2) 65%, (3) 70%, and (4) 75% (v/v) H_2SO_4.

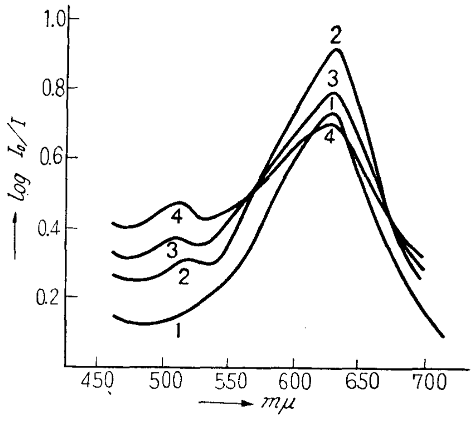

Fig. 2.

Absorption Curve of the Reaction Mixture

0.25 mg. of glucose heated with 12 mg. of anthrone and 15 cc. of 65% by volume of H_2SO_4 at 85~90° for (1) 10, (2) 20, (3) 30, and (4) 40 mins.

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* Part Ⅶ : This Bulletin, **4**, 209(1956).

** A short communication was published in this Bulletin, **3**, 323(1955).

*** Katakasu, Fukuoka (百瀬 勉, 上田 陽, 沢田 啓, 杉 絢子).

1) R. Dreywood : Ind. Eng. Chem., Anal. Ed., **18**, 499(1946).

2) L. Sattler, F. W. Zerban : Science, **108**, 207(1948).

3) E. H. Shriver, M. B. Webb, J. W. Swanson : TAPPI, **33**, 578(1950).

4) E. W. Yemm, A. J. Willis : Biochem. J.(London), **57**, 508(1954).

absorption maximum around 510 mμ became more distinct by the increased concentration of the acid and the reaction time, and finally, the original blue color turned to red as the absorption around 620 mμ decreased gradually.

The molar ratio of anthrone to glucose also affected the absorption maximum without changing the shape of the curve (Fig. 3). It seemed to require at least three moles of anthrone to one mole of glucose to develope the maximum coloration.

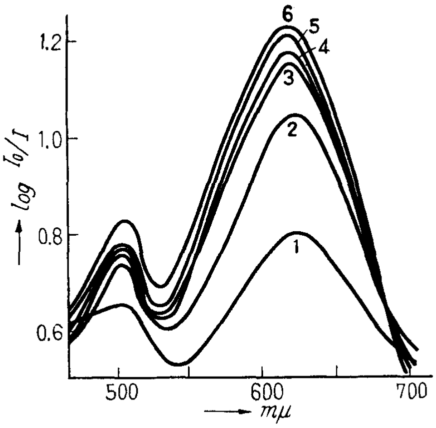

Fig. 3.
Absorption Curve of the Reacion Mixture

18 mg. of glucose heated for 20 mins. at 85～90° with (1) 20 mg., (2) 40 mg., (3) 60 mg., (4) 80 mg., (5) 100 mg., and (6) 120 mg. of anthrone in 7 cc. of 68.5%(v/v) H_2SO_4, to which 3 cc. of AcOH was added and diluted to 550 cc. with a mixture of 6 volumes of 80% (v/v) H_2SO_4 and 3 volumes of AcOH.

Isolation of a dye

From the results mentioned above, the reaction was carried out under controlled conditions in 70% (v/v) sulfuric acid, to which glacial acetic acid was added to prevent coagulation of the dye into clots. The mixture was poured into water, separated amorphous brown substance was collected, and extracted with hot benzene. Insoluble substances still gave a blue color with sulfuric acid, but those could not be treated successfully.

Chromatographic separation of the benzene solution on alumina gave five fractions which were divided by their adsorption color on alumina (Table I).

TABLE I. Chromatographic Separation of the Dye

Fraction number	Adsorption color on alumina	Coloration with 80% H_2SO_4
1	yellow	orange
2	orange	green
3	orange red	brownish green
4	red	blue
5	brown	red

After evaporation of the solvent, the second fraction left a very small amount of oily dye, which gave a green coloration with sulfuric acid. The third fraction seemed to be a mixture of several dyes which were difficult to separate. The main fraction, the fourth, which gave a blue coloration with sulfuric acid, gave red needles when the solvent was concentrated.

The dye, m.p. 290°(decomp.), was sparingly soluble in organic solvents and was recrystallized with difficulty from benzene, glacial acetic acid, or nitrobenzene. Sulfuric acid solution of the dye had a blue color and the wave length of the maximum absorption shifted to a longer wave length region with the increased concentration of the acid, its coefficient increasing gradually as shown in Table II.

This variation of the wave length agreed with that of the maximum absorption given by anthrone with glucose which shifted with the increasing concentration of

Table II. Absorption Maximum of the Dye in H_2SO_4

Concn. H_2SO_4(%)	Wave length (mμ)	Absorption coefficient (log ε)
65	620	4.50
68	625	4.70
70	630	4.71
73	635	4.73
75	640	4.74
80	655	4.76

sulfuric acid.

The intensity of the blue color produced by the reagent with glucose, lactose, glycogen, or maltose was shown by Morris[5] to obey the Beer's law at 620 mμ. For routine estimation of hexoses or hexose units the reaction had been carried out in about 65~70% (v/v) of sulfuric acid, and the intensity of the color had been read at 620~630 mμ. Therefore, the dye isolated was no doubt one of the coloring matters which took part in the estimation of the sugar.

The alumina column held a reasonably large quantity of resin at the top, which could not be eluted with benzene, and still gave a blue coloration with sulfuric acid, suggesting that other dyes might be present.

Structure of the Dye and Reaction Mechanism

The determination of molecular weight of the dye was carried out with difficulty in phenol by the Beckmann's method. The value obtained was about 530. Acetylation of the dye with acetic anhydride in the presence of zinc dust gave a leucoacetate as light yellow needles, m.p. 207°. From the data of elementary analyses and acetyl determination it seems probable that the dye has the formula $C_{47}H_{30}O_3$ and the leucoacetate $C_{53}H_{42}O_6$. Therefore it may be supposed that three moles of anthrone reacts with one mole of glucose as in the following :

$$3C_{14}H_{10}O + C_6H_{12}O_6 \longrightarrow C_{47}H_{30}O_3 + 5H_2O + CH_2O$$

Above formula indicates that the dye has three carbonyl groups, but no hydroxyl

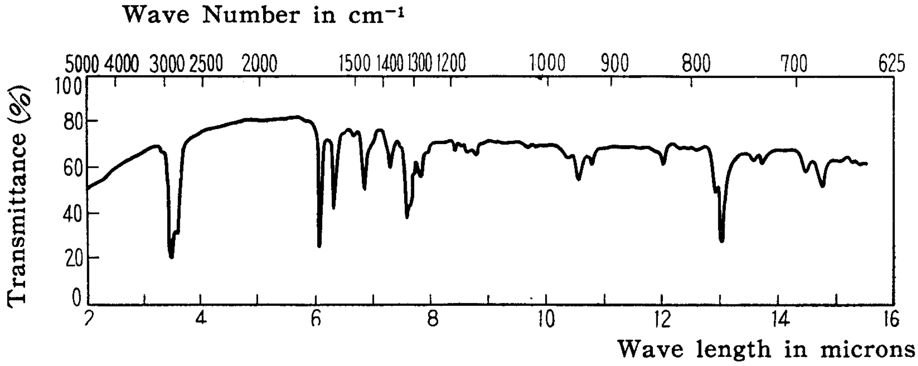

Fig. 4-1.

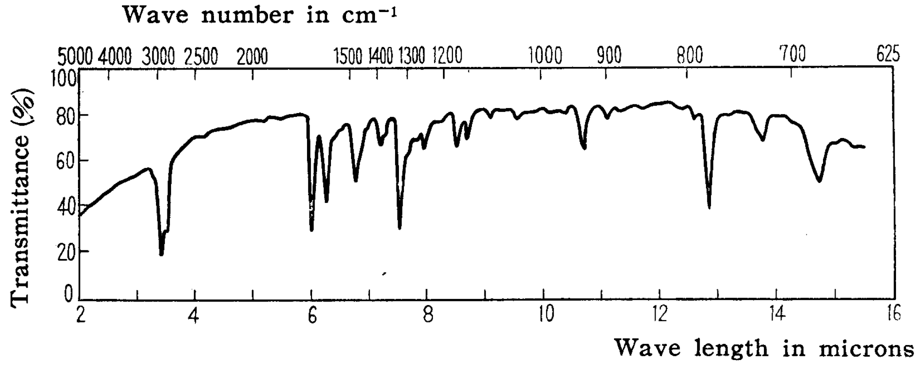

Fig. 4-2.

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[5] D. L. Morris : Science, **107**, 254(1948).

or ether linkage. The infrared spectrum of the dye shows only one carbonyl band at 6.0 μ, indicating that the three groups are in the same conditions in the molecule (Fig. 4–1). On the other hand, the infrared spectrum of dianthronylideneëthane (Ⅲ) resembles that of the dye, having also one carbonyl band at the same wave length (Fig. 4–2). The absorption spectrum of the dye in benzene in the visible region is shown in Fig. 5 (1). It has the maximum at 464~465 mμ (log ε 4.66) and resembles that of dianthronylideneëthane (Fig. 5(2)). in shape and intensity. This substance also has a blue color in sulfuric acid and its absorption curve is very similar to that of the dye dissolved in sulfuric acid (Fig. 6). Thus, a probable structure of the dye may be expressed as 1,2,5– (I) or 1,3,5–trianthronylidenepentane (Ⅱ) in relation to the following reaction mechanism.

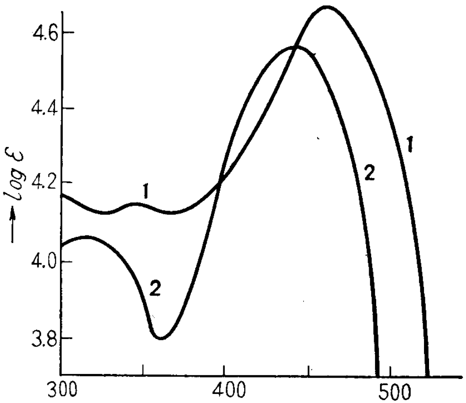


Fig. 5. Absorption Curve of the Dye

(1) 0.646 mg. of the dye dissolved in 100 cc. of benzene. (2) 0.232 mg. of dianthronylideneëthane dissolved in 50 cc. of benzene.

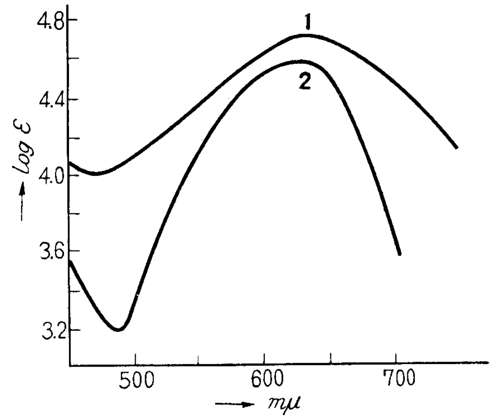
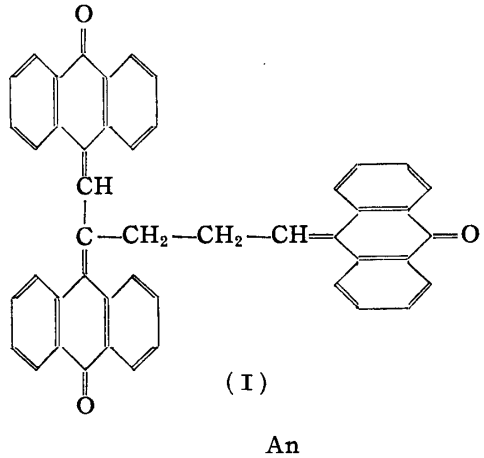
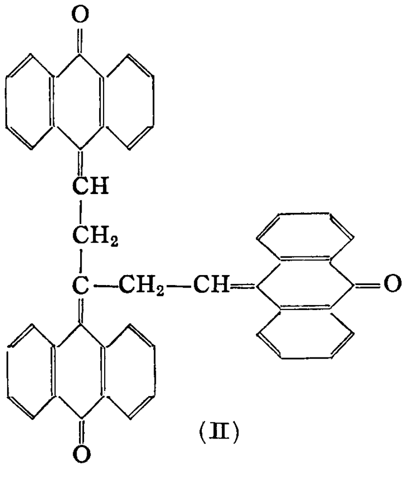


Fig. 6. Absorption Curve of the Dye

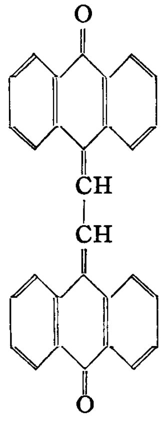
(1) 0.869 mg. of the dye dissolved in 100 cc. of 70% (v/v) H_2SO_4. (2) 0.784 mg. dianthronylideneëthane dissolved in 100 cc. of 70% (v/v) of H_2SO_4.



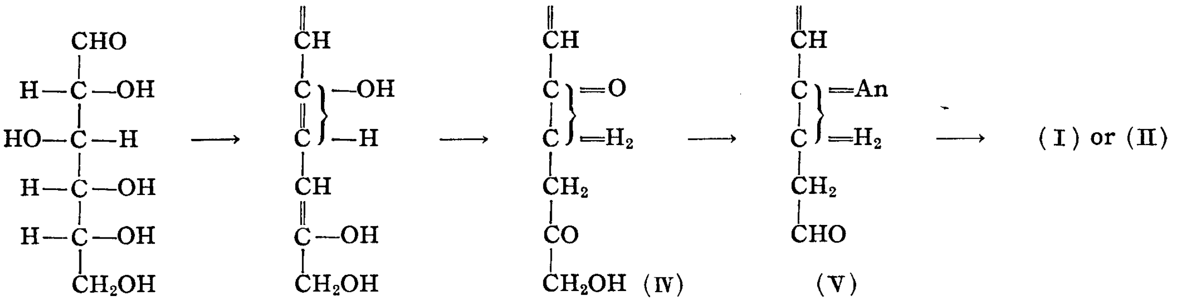
(I)



(Ⅱ)



(Ⅲ)



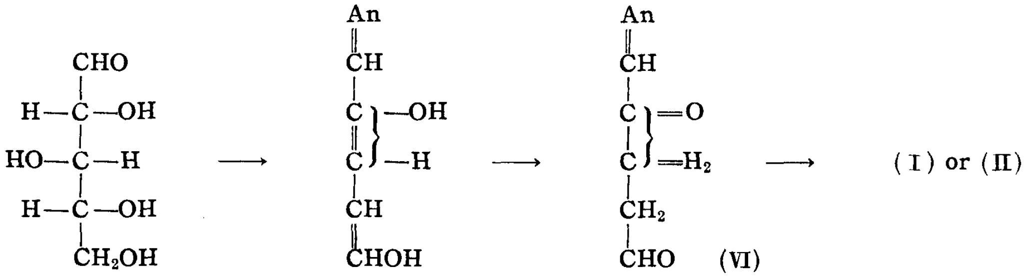
An = Anthronylidene residue

Anthrone may combine first with the aldehyde group of glucose, and simultaneous dehydrations may occur at 2–3 and 4–5 positions to form a ketole (IV). This ketole may be converted with the liberation of formaldehyde to an aldehyde (V), which may give the dye by further combination with anthrone.

The above assumption will be confirmed by the fact that the same dye is obtained from xylose and anthrone. It had been known[3] that the blue-green color produced by pentose changes rapidly to amber, but Bridges[6] found that the Beer's law also held at 620 mμ for D-xylose, L-arabinose, and D-ribose on cooling the reaction mixture immediately after the reaction was completed. As Johanson[7] pointed out, anthrone color with pentose is very weak when the reaction is carried out in a more dilute sulfuric acid, and it gives the maximum intensity when heated for 10 minutes in about 73% sulfuric acid, showing the maximum absorption at 675～700 mμ. Therefore, the preparation of the coloring matter from xylose was carried out in this concentration of the acid.

The brown substance which separated on diluting the reaction mixture with water dissolved almost wholly in benzene. Chromatographic separation of the solution on alumina was carried out in the same way as mentioned before and red needles of m.p. 290° were obtained from the main fraction. This dye was identified as the dye obtained from glucose by the melting point of admixture and also by the infrared spectra.

As for the reaction mechanism, the dehydration may occur to convert the primary alcohol group of the pentose to an aldehyde (VI), which may combine with anthrone to give the dye (I) or (II):



The authors extend their gratitude to Mr. T. Hattori for the microanalyses. They are also indebted to Dr. A. Watanabe of Takeda Research Laboratory for the infrared spectra.

Experimental

Isolation of the Dye—Anthrone solution was prepared by dissolving 20 g. of anthrone in 600 cc. of 80% H_2SO_4, and 300 cc. of glacial AcOH was added. To the resulting solution 20 g. of glucose dissolved in 100 cc. of water was added at a time and heated for 20 mins. at 85～90°. The mixture was poured into 2 L. of water, separated substance was collected, and washed with water. After drying in a vacuum dessicator the substance was refluxed for 1 hr. with 1 L. of benzene and filtered while hot. The benzene solution was concentrated to about 100 cc., passed through 250 g. of alumina column and eluted with benzene. The eluate was divided into five fractions as shown in Table I.

The fourth eluate from about 3 L. of benzene was concentrated to about 100 cc. and the residue crystallized after several days. Recrystallization from benzene, glacial AcOH, or nitrobenzene gave red needles, m.p. 290°(decomp.). Yield, 300 mg. *Anal.* Calcd. for $C_{47}H_{30}O_3$: C, 87.83; H, 4.71; mol. wt., 642. Found : C, 88.13; H, 4.37; mol. wt., 530.

In the case of xylose, 10 g. of the sugar was dissolved in 30 cc. of water and 300 cc. of anthrone solution diluted with 150 cc. of glacial AcOH was added. The mixture was heated 10 mins. at 80～85° and treated as above. Chromatographic separation and recrystallization from benzene gave red needles, m.p. 290°(decomp.), which showed no depression of m.p. on admixture with the dye obtained from glucose. Yield, 120 mg.

Leucoacetate—To a mixture of 100 mg. of the dye and 5 cc. of Ac_2O, 1 g. of Zn dust was added and refluxed for 30 mins. The hot solution was filtered from the unreacted Zn and the latter was

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6) R. R. Bridges : Anal. Chem., **24**, 2004(1952).
7) R. Johanson : *Ibid.*, **26**, 1331(1954).

washed with 5 cc. of glacial AcOH. To the combined filtrate and washing, 30 cc. of water was added and the separated substance was recrystallized from glacial AcOH to light yellow needles, m.p. 207° (decomp.). Yield, 40 mg. *Anal.* Calcd. for $C_{53}H_{42}O_6$: C, 82.15; H, 5.46; CH_3CO, 16.65. Found : C, 82.67; H, 5.34; CH_3CO, 16.5.

Absorption spectra—The ultraviolet spectra were measured by a Shimadzu Spectrophotometer type QB-50 in a cell of 10 mm. optical depth with the reagent blank. The infrared spectra were measured by a Perkin-Elmer Infrared Spectrophotometer in Nujol mull with about 0.01 mm. thickness.

Summary

The anthrone color of glucose was shown to have the maximum intensity when the reaction was carried out for 20 minutes at 85~90° in 70%(v/v) of sulfuric acid. The absorption maximum of the blue-green color shifted to a longer wave length region with the increasing concentration of the acid. It seemed to require three moles of anthrone to one mole of glucose in the reaction.

The soluble part of coloring matter of the reaction in benzene was separated on alumina and was shown to consist of several dyes which gave different coloration with sulfuric acid. One of the main dyes was isolated in crystalline form, and its probable structure might be 1,2,5- or 1,3,5-trianthronylidenepentane from its analytical data and infrared spectrum, and its reaction mechanism was considered. The same dye was also isolated from anthronated xylose and the reaction mechanism with the pentose was discussed.

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9. Masao Shimizu, Genkichi Ohta, Shin-ichi Kitahara,* Genzo Tsunoo, and Shin-ichiro Sasahara : Studies on the Constituents of Rice Bran Oil. I. Isolation of Phenolic Substances.

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During their studies on vegetable oils, Tsuchiya and Kaneko1) found that rice bran oil exhibited a characteristic absorption in the ultraviolet region and searching for its source, isolated a crystalline substance, which they named oryzanol. Accoring to their report, oryzanol melts at 137.5~138.5°, shows absorption maxima (in heptane) at 231, 290 and 315 mμ; colors yellow with alkali hydroxides, is negative to sterol color reactions, and presence of a hydroxyl and carbonyl groups is suggested from its infrared absorption spectrum.

The present writers attempted the extraction of this substance from rice bran oil, through the kind permission of the foregoing workers, and a few observations were gained, which are reported in the present paper.

Tsuchiya and Kaneko removed free fatty acids from rice bran oil with sodium carbonate, esterified the fats, and fatty acid ester were removed by low-pressure distillation. Repeated chromatography of the residue and recrystallization afforded oryzanol.

Under the detailed instructions of the foregoing workers, oryzanol was successfully

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1) R. Kaneko, T. Tsuchiya : J. Soc. Chem. Ind. Japan, **57**, 526(1954).