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7. Tsutomu Momose and Yosuke Ohkura: Organic Analysis. XIII.* Estimation of Hexose with 5-Hydroxy-1-tetralone.

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In the previous papers[1,2] of this series, it was shown that 5-hydroxy-1-tetralone gave a sensitive fluorescence reaction selectively with hexose when heated with sulfuric acid, and that a fluorescent compound produced by this reaction was isolated in crystalline form. A probable structure of the compound might be benzonaphthenedione, and the reaction mechanism was also assumed. This paper describes the estimation of hexose with the reagent.

Nature of the Fluorescent Compound

First, a preliminary study on the nature of the fluorescent compound was carried out to find colorimetric conditions for the estimation. When the fluorescent compound dissolved in 20% by volume of sulfuric acid was excited by ultraviolet light of 365 mμ, it gave a strong green fluorescence, which had the maximum intensity at 532 mμ. It gave no fluorescence in a neutral or alkaline medium.

The longest band of absorption spectrum and the fluorescence spectrum of the compound are shown in Fig. 1, and an approximate mirror-image relation, already observed in a number

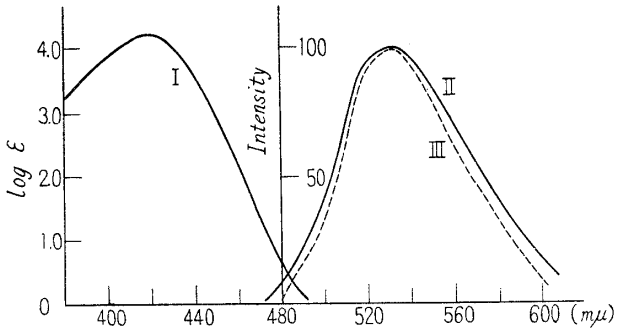

Fig. 1.
I : Absorption spectrum of fluorescent compound in EtOH
II : Fluorescence spectrum of fluorescent compound
III : Fluorescence spectrum of reaction mixture of 5-hydroxy-1-tetralone and glucose

of fluorescent compounds,[3] is also shown in these two spectra. The intensity of the fluorescence obeyed the Beer's law at 532 mμ in a lower concentration of the compound than $3\times10^{-6}M$, but it was weakened by a longer irradiation of the ultraviolet light, and lost about 80% of strength after 10 minutes. The concentration of sulfuric acid which dissolved the compound affected the fluorescence intensity (Fig. 2) and gave the maximum intensity in 20% by volume. An attempt to extract with an organic solvent from the sulfuric acid solution

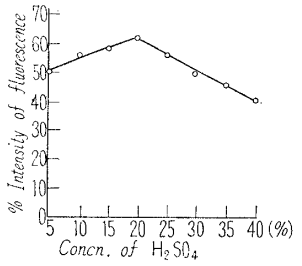

Fig. 2. Effect of Concentration of Sulfuric Acid in Measurement of Fluorescence
($2.5\times10^{-6}M$ dye)

* Part XII: This Bulletin, **6**, 669 (1958).
** Katakasu, Fukuoka (百瀬 勉, 大倉洋甫).
1) Part VII: T. Momose, Y. Ohkura: This Bulletin, **4**, 209 (1956).
2) Part X: T. Momose, Y. Ohkura: *Ibid.*, **6**, 412 (1958).
3) cf. R. Shoental, E. J. Y. Scott: J. Chem. Soc., **1949**, 1683.

and read the fluorescence were unsuccessful in the usual solvents. Those involved benzene, toluene, benzyl alcohol, butanol, chloroform, carbon tetrachloride, and ethyl acetate. Therefore the intensity should be read in a dilute sulfuric acid solution.

Conditions for Estimation of Glucose

A solution of a definite concentration of 5-hydroxy-1-tetralone was heated with an aqueous glucose solution and sulfuric acid in boiling water bath, diluted with water, irradiated by ultraviolet light of 365 mμ, and the resulting fluorescence was read at 532 mμ. When alcohol was used as the solvent, the fluorescence became less intense and less stable with increasing concentration of alcohol. Sulfuric acid solution of the reagent gave the highest intensity of the fluorescence which was fairly stable under irradiation of ultraviolet light. This reagent solution was faint yellow and was quite stable enough for a long storage when stored in a refrigerator. The fluorescence spectrum produced by the reagent solution and glucose in the presence of sulfuric acid is also shown in Fig. 1. It is almost identical with that of the fluorescent compound dissolved in dilute sulfuric acid, having the maximum intensity at 532 mμ. This fact might confirm the assumption that only one fluorescent compound was produced in the reaction.

Secondly, effect of the concentration of the reagent and of sulfuric acid, and of the reaction time on the fluorescence intensity were studied under the same conditions as those of the standard procedure described in the Experimental Part. Fig. 3 shows that a 0.1%

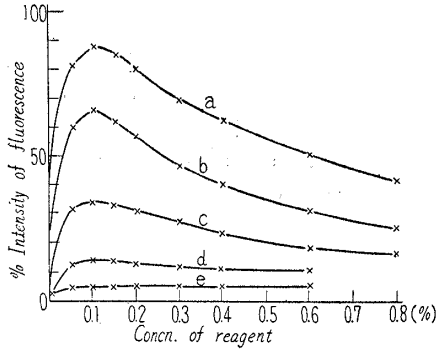

Fig. 3. Effect of Concentration of Reagent
a: 100 γ/cc. glucose
b: 50 γ/cc. glucose
c: 25 γ/cc. glucose
d: 10 γ/cc. glucose
e: No 〃

solution of the reagent gave a higher intensity of the fluorescence than other solutions in all the concentrations of glucose tested, and the increased concentration of the reagent diminished the intensity, indicating that a large excess of the reagent might absorb the fluorescence. However, increased concentration of glucose required a larger amount of the reagent. For example, 500 γ/cc. of glucose required 0.15% solution of the reagent to develop the maximum intensity.

The concentration of sulfuric acid in the reaction mixture also affected the intensity and 80% by volume of sulfuric acid gave the strongest fluorescence in all concentrations of glucose (Fig. 4). Fig. 5 shows that the fluorescence reached a constant intensity after heating the reaction mixture at 100° for 40 minutes in any concentrations of glucose and of the reagent.

It is well known that a fluorescence intensity was, in general, influenced by temperature of the solution at the time of measurement. In the case of this fluorescence, the intensity increased gradually at the rate of 1~2% with the rising temperature between 15° and 25°, and increased rapidly at a temperature above 30° (Fig. 6). Therefore, the fluorescence intensity should be read at a definite temperature in the range of 15~25°.

The calibration curves of glucose measured by the standard procedure are shown in Fig. 7. The Beer's law holds at 532 mμ in a concentration of glucose below 40 γ/cc. with a 0.1% solution of the reagent, and below 70 γ/cc. with a 0.2% solution. A satisfactory result

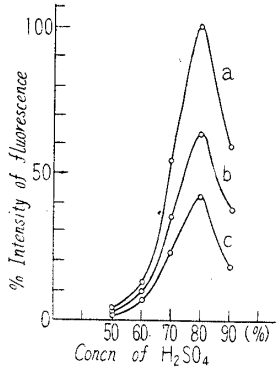

Fig. 4. Effect of Concentration of Sulfuric Acid in Reaction Mixture
a: 100 γ/cc. glucose
b: 50 γ/cc. glucose
c: 25 γ/cc. glucose

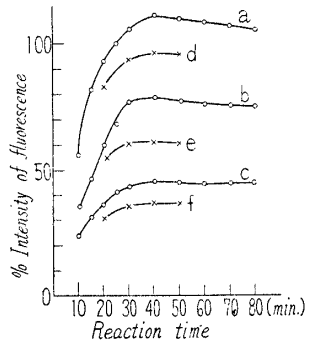

Fig. 5. Effect of Reaction Time
a: 100 γ/cc. glucose
b: 50 γ/cc. glucose } 0.1% reagent
c: 25 γ/cc. glucose
d: 100 γ/cc. glucose
e: 50 γ/cc. glucose } 0.2% reagent
f: 25 γ/cc. glucose

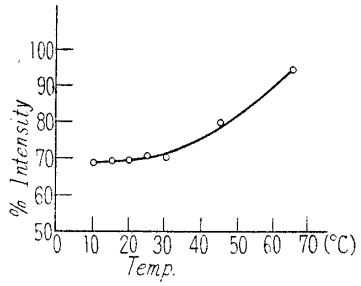

Fig. 6. Effect of Temperature in Measurement of Fluorescence
(50 γ glucose)

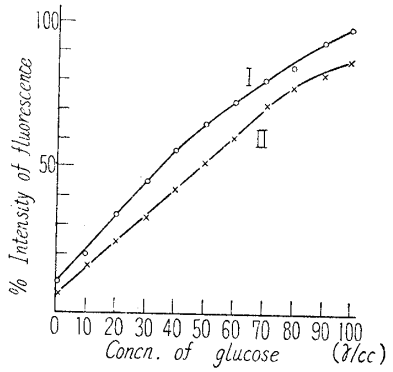

Fig. 7. Calibration Curves of Glucose
I: 0.1% reagent
II: 0.2% reagent

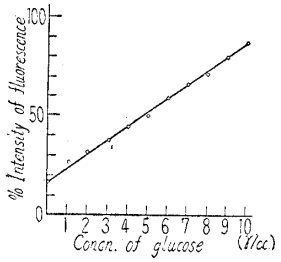

Fig. 8. Calibration Curve of Glucose with 0.1% reagent below 10 γ/cc. glucose

was also obtained in a concentration of glucose lower than 10 γ/cc. with 0.1% solution (Fig. 8), and therefore, this new reagent might be used in a micro-estimation of the sugar.

Effect of Pentose

Pentoses tested gave no fluorescence under the same conditions. Those involved xylose, ribose, arabinose, and rhamnose. A very large amount of xylose mixed in glucose showed a tendency to decrease the fluorescence intensity produced by glucose, but in actual estimation, the effect of pentose was negligible as shown in Table I which indicated that even 10 times of xylose gave no effect on the fluorescence intensity in the estimation of 25 γ/cc. of glucose.

TABLE I. Effect of Xylose on Intensity of Glucose

Ratio of Xylose/Glucose		0	1	2	3	4	5	6	8	10
Glucose 100 γ/cc.	Xylose (γ/cc.)	0	100	200	300					
	Intensity (%)	100.0	99.1	85.0	81.0					
Glucose 50 γ/cc.	Xylose (γ/cc.)	0	50	100	150	200	250		400	
	Intensity (%)	66.0	64.8	64.8	65.0	64.5	65.2		53.6	
Glucose 25 γ/cc.	Xylose (γ/cc.)	0	25	50	75	100	125	150		250
	Intensity (%)	28.0	27.7	28.0	28.3	27.5	27.3	28.3		27.5

Fluorescence Intensity of Other Hexoses

The other hexoses tested under the same conditions gave intensities different from that of glucose, though they gave constant intensities after being heated at 100° for 40 minutes. When the fluorescence intensity of glucose was taken as a standard at 100.0, the intensities of fructose, galactose, and mannose were 104.3, 49.0, and 37.7, respectively.

Disaccharides (sucrose, maltose, and lactose) and trisaccharide (raffinose) gave constant fluorescence intensities after heating at 100° for 50 minutes. The values observed were 102.5 for sucrose, 100.0 for maltose, 71.0 for lactose, and 77.0 for raffinose, when the value of glucose was taken as a standard. These values almost coincide with the additive values of the individual sugars which composed the above-mentioned di- or trisaccharides.

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Experimental

Reagent Solution—Prepared by dissolving 100 mg. of 5-hydroxy-1-tetralone in 100 cc. of H_2SO_4 and stored in a refrigerator.

Sulfuric Acid—J.I.S. 1st grade (sp. gr. 1.84).

Standard Solutions of Sugars—Prepared by dissolving each sugar in distilled water.

Fluorescence Intensity—Measured by the Hitachi L-3 fluorometer attached to a Type EPU-2 spectrophotometer with a quartz cell of 10-mm. optical length. The ultraviolet light source was a high-pressure mercury lamp and the light was passed through a filter of 365 mμ.

Standard Procedure—To 1 cc. of the test solution which contained 1~40 γ of hexose and placed in a test tube of 25~30-cc. capacity, 1 cc. of the reagent solution and 3 cc. of H_2SO_4 were added under cooling in an ice-water bath. The mixture was agitated, heated for 40 mins. in a boiling water bath, cooled rapidly in an ice-water bath, and diluted with 15 cc. of H_2O. The resulting solution was allowed to stand for about 30 mins. at room temperature (15~25°), and its fluorescence was read at 532 mμ, exciting with ultraviolet light of 365 mμ. 1 cc. of H_2O was treated as above and used as a reference.

Summary

The fluorescence produced by 5-hydroxy-1-tetralone and hexose in sulfuric acid had the maximum intensity at 532 mμ. The fluorescence intensity of glucose obeyed the Beer's law in a concentration of 1~40 γ/cc. of the sugar and a standard procedure of the estimation was established. The other hexoses and oligosaccharides which contained hexose units in their molecule gave individual intensities. This method was not interfered by pentoses.

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