[Chem. Pharm. Bull. 12 (6) 706 ~ 709]

UDC 543.854.72-4

100. Ryoji Sawamura and Takashi Koyama: Color Reaction of Pentose with Anthrone. III.*1 Fluorescence formed by the Reaction of Pentose with Anthrone.

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Since Dreywood[1] originally reported the color reaction of hexose with anthrone in strong sulfuric acid, the reaction has been used for the determination of various kinds of sugars. According to Shriver, Webb and Swanson,[2] and to Kohler,[3] the reaction is available for the determination of methylpentose. Bridges[4] applied the reaction to xylose, arabinose, and ribose. Bailey[5] investigated the reaction conditions for pentoses.

In previous papers of this series,*1,[6] we reported that furfural gave a specific blue color with anthrone when the reaction mixture was carefully cooled, and the reaction mechanisms were also investigated. When the reaction mixture of furfural with anthrone was heated, a fluorescence was observed on irradiation of the ultraviolet ray. In the present work, the fluorescence formed by the reaction of pentose or furfural with anthrone was investigated.

The reaction conditions were identical to that reported by Bailey.[5] Ten milliliters of anthrone reagent containing 0.01 w/v% of anthrone in 70 v/v% sulfuric acid was added with cooling to 1 ml. of sugar solution containing 0.1~2.0 mg. of pentose. On

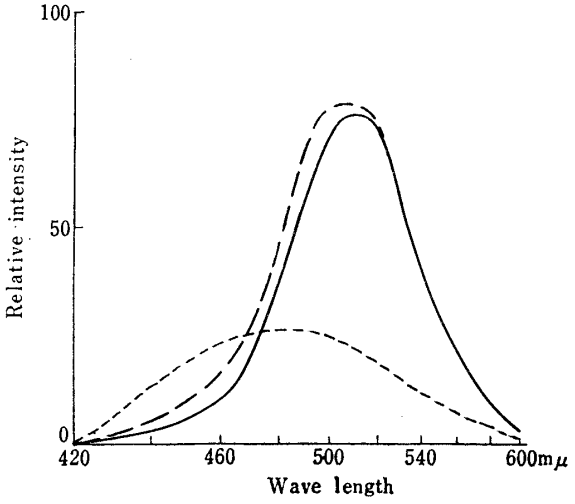

Fig. 1. Fluorescence Spectra formed by the Reaction of Pentose with Anthrone (Pentose 0.5 mg./ml.)

——— Xylose
— — — Arabinose
----- Blank

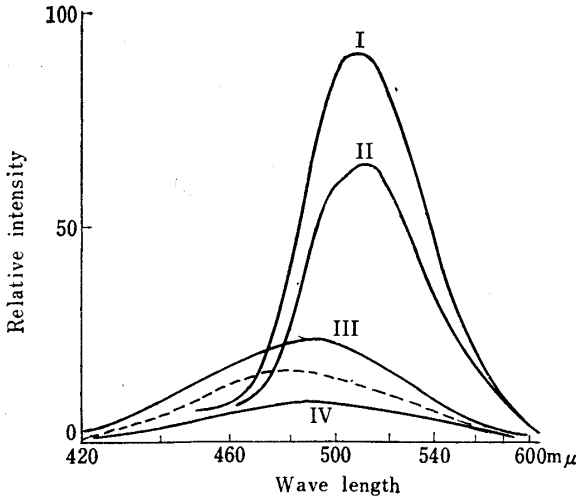

Fig. 2. Fluorescence Spectra formed by the Reaction of Furfural and Glucose with Anthrone

I : Furfural 0.1 mg./ml.
II : Furfural 0.01 mg./ml.
III : Glucose 0.1 mg./ml.
IV : Glucose 0.5 mg./ml.
----- Blank

*1 Part II. R. Sawamura, T. Koyama: Yakugaku Zasshi, **84**, 82 (1964).

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1) R. Dreywood: Ind. Eng. Chem., Anal. Ed., **18**, 499 (1946).
2) E. H. Shriver, M. B. Webb, J. W. Swanson: Tappi, **33**, 578 (1946).
3) L. H. Kohler: Anal. Chem., **24**, 1577 (1952); *Ibid.*, **26**, 1941 (1954).
4) R. R. Bridges: *Ibid.*, **24**, 2004 (1952).
5) R. W. Bailey: Biochem. J., **68**, 669 (1958).
6) R. Sawamura, T. Koyama: Yakugaku Zasshi, **81**, 1689 (1961).

heating for 7 minutes at 100°, and cooling for 30 minutes in dark, the reaction mixture colored greenish blue and fluoresced with the ultraviolet ray. A blank test was performed using 1 ml. of water and 10 ml. of anthrone reagent. The fluorescence spectra of xylose, arabinose, and a blank are shown in Fig. 1. The ranges of the fluorescence spectra of pentose were from 470 to 600 mμ and the maxima of the spectra were about 510~520 mμ. The anthrone reagent itself gave a weak flat fluorescence spectrum from 420 to 600 mμ apparently owing to the partial isomerization to anthranol.[7] The shape of its spectrum was, however, quite different from that of pentose. Solutions of furfural (0.01~1.0 mg./ml.) and of glucose (0.02~5.0 mg./ml.) were also reacted with anthrone (Fig. 2). The shape of the fluorescence spectrum of furfural was similar to that of pentose. The fluorescence of glucose was very weak. Relative fluorescence intensity of these reaction mixtures was recorded at each wave length. The differences between the fluorescence intensity at 510 and at 470 mμ: (fluorescence intensity at 510 mμ)−(fluorescence intensity at 470 mμ)=$Fi_{\Delta 510:470}$*3 are shown in Table I. $Fi_{\Delta 510:470}$ was around

Table I. Relative Fluorescence Intensity of Sugars and Furfural reacted with Anthrone (Meter Readings)

Sugars (mg./ml.)		Relative intensity		Intensity increment
		470 mμ	510 mμ	$Fi_{\Delta 510:470}$*3
Blank		26.8	23.2	− 3.6
Xylose	0.1	25.0	44.0	+19.0
	0.2	16.5	52.5	+36.0
	0.5	18.3	77.7	+59.4
	1.0	23.0	79.5	+56.5
	2.0	9.0	52.5	+43.5
Arabinose	0.1	23.0	49.0	+26.0
	0.2	24.5	54.7	+30.2
	0.5	24.5	80.7	+56.2
	1.0	25.5	85.3	+59.8
	2.0	15.5	59.0	+43.5
Blank		17.0	16.5	− 0.5
Ribose	0.1	16.0	40.0	+24.0
	0.2	14.5	50.0	+35.5
	0.5	14.5	78.6	+64.1
	1.0	17.8	79.3	+61.5
	2.0	14.0	72.0	+58.0
Blank		15.5	13.5	− 2.0
Frufural	0.01	12.0	65.5	+53.5
	0.1	17.0	90.6	+73.6
	1.0	21.5	92.4	+70.9
Glucose	0.02	21.7	20.7	− 1.0
	0.05	13.5	12.0	− 1.5
	0.1	19.0	19.5	+ 0.5
	0.5	8.5	9.5	+ 1.0
	2.5	4.3	6.0	+ 1.7
	5.0	0.5	1.0	+ 0.5
Xylose	0.5	11.5	58.5	+47.0
Xylose 0.5 +	Glucose 0.02	11.0	61.0	+50.0
	0.1	16.5	78.5	+62.0
	0.5	6.8	31.5	+24.7
	2.5	2.2	5.2	+ 3.0

*3 Fi=Relative fluorescence intensity.

7) K. H. Meyer: Ann., **379**, 57 (1911).

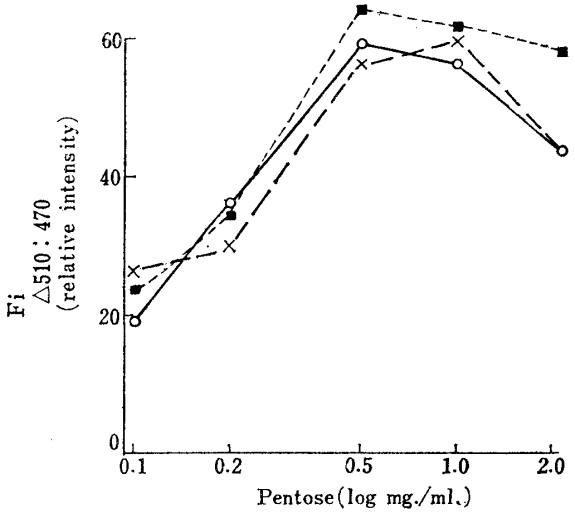

Fig. 3. Relationship between $Fi_{\Delta 510:470}$*3 and the Concentration of Pentose

○——○ Xylose
×---× Arabinose
■-·-■ Ribose

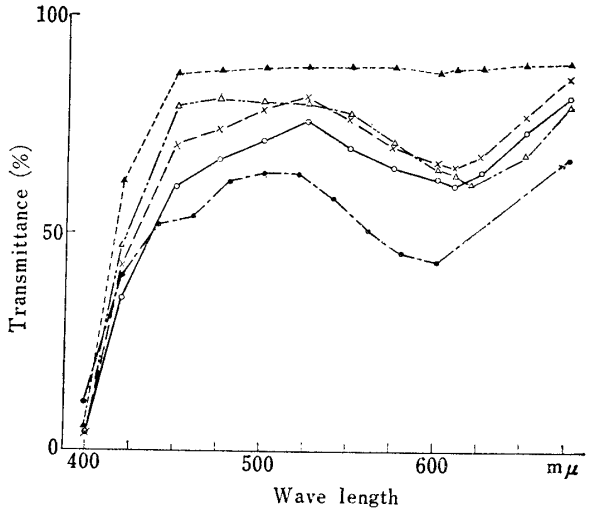

Fig. 4. Absorption Spectra of the Reaction Mixture of Sugar or Furfural reacted with Anthrone (each containing sugar or furfural 0.1 mg./ml.)

○——○ Xylose
×----× Arabinose
●---● Furfural
△----△ Glucose
▲---▲ Blank

zero for glucose and a blank, but it was markedly positive for xylose, arabinose, ribose, and furfural. The relationship between $Fi_{\Delta 510:470}$ and the concentration of pentose is shown in Fig. 3. The fluorescence intensity showed its maximum at approximately 0.5~1.0 mg./ml. concentration of pentose and decreased at lower and higher concentrations.

The absorption curves of the reaction mixtures, each containing 0.1 mg. of each sugar or furfural, and of the blank test are shown in Fig. 4. Absorption spectra of a reaction mixture containing sugar or furfural had maxima at 610~620 mμ. In the case of pentose and furfural the absorbance decreased from 450 to 525 mμ and increased from 525 to 610 mμ, and in the case of glucose the curve was flat between 450 and 525 mμ and increased toward 620 mμ. The blank had no absorption between 450 and 675 mμ. The absorption spectra of pentose and furfural between 450 and 600 mμ resembled their fluorescence spectra.

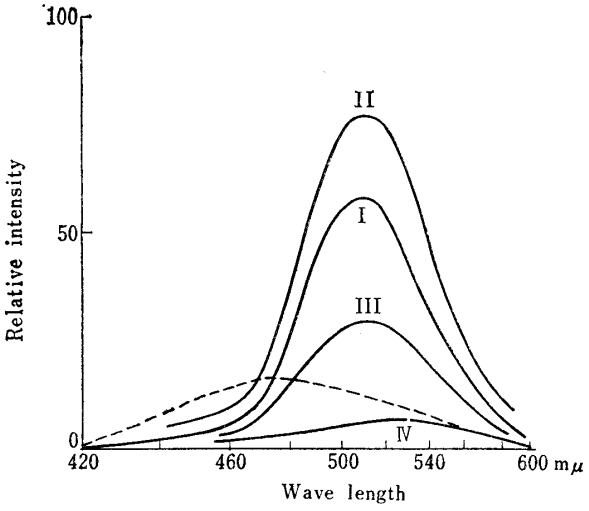

Fig. 5. Fluorescence Spectra formed by the Reaction of Xylose with Anthrone in the Presence of Various Amounts of Glucose

I : Xylose 0.5 mg./ml.
II : Xylose 0.5 mg./ml.+Glucose 0.1 mg.
III : Xylose 0.5 mg./ml.+Glucose 0.5 mg.
IV : Xylose 0.5 mg./ml.+Glucose 2.5 mg.

Pentose apparently produced a fluorescent substance, but hexose seemingly did not. There is a possibility that hexose also produced a fluorescent substance and the fluorescence emitted by the substance was absorbed by the color substance in the reaction mixture and, hence, only a weak fluorescence was observable. Since the light absorption of the reaction mixture of hexose and anthrone at 450~525 mμ was very weak, this possibility was excluded. This was further confirmed by the following experiment.

The reaction mixture was diluted with 2 volumes of water and the greenish blue dye present was extracted with petroleum benzin. In the case of pentose, the aqueous layer gave an intense fluorescence, but only a faint fluorescence was observed in the case of hexose and blank. If the hexose produced a fluorescent substance and the fluorescence emitted was absorbed by the dye, the reaction mixture should show a distinct fluorescence upon removal of the light-absorbing materials. Therefore, it is conclusive that a fluorescent substance was produced not from hexose but from pentose.

The mixtures of pentose and various amounts of hexose were reacted with anthrone reagent, and the fluorescence spectra of their products are shown in Fig. 5 ($Fi_{\lambda 510:470}$ of these fluorescence are shown in Table I). Fluorescence intensity of the reaction mixture containing 0.5 mg./ml. of xylose was weaker than that of the mixtures containing 0.02~0.1 mg. of glucose and 0.5 mg. of xylose, and stronger than that of the mixtures containing 0.5~2.5 mg. of glucose and 0.5 mg. of xylose. But the shape of these fluorescence spectra formed from xylose in the presence of glucose was similar to that formed from xylose only.

Accordingly, this specific reaction could be used for the characterization of pentose in the presence of hexose, but an excess of glucose decreased the pentose fluorescence. The pentose fluorescence may be due to the furfural fluorescence.

Experimental

Sugar and Furfural——The sugars used were D-xylose, L-arabinose, D-ribose, and D-glucose. All of them were recrystallized from MeOH, dried, and shown to be chromatographically pure by the usual techniques. Furfural was freshly distilled *in vacuo*.

Anthrone Reagent——Anthrone (10 mg.) was dissolved in H_2SO_4 solution, prepared by adding concentrated acid (70 ml.) to H_2O (30 ml.).

Reaction of Anthrone and Sugar——Aqueous solution (1 ml.) of sugar or furfural was pipetted into a test tube (200×24 mm.). Each tube was placed in a bath of cold water and agitated while anthrone reagent (10 ml.) was slowly added. The tubes were fitted with glass stoppers after their contents were throughly mixed and immersed in a boiling water bath for 7 min. Then they were placed in a cold water bath and stored in the dark for 30 min. before the intensity of fluorescence was measured. A blank consisting of H_2O (1 ml.) and anthrone reagent (10 ml.) was included in each batch of reactions.

Fluorescence Spectrum——The fluorescence spectra were observed with a Hitachi Photo-electric Spectrophotometer, type EPU-2A, having an L-3 Fluorophotometry Attachment, and recorded by an S-1 Automatic Recording Apparatus with 10 mm^2 cell. The intensity of fluorescene from 420 to 600 mμ was recorded in relative intensity.

The authors are indebted to Mrs. K. Tanaka (née Miyama), Mr. M. Okamoto, and Mr. A. Hasegawa for their technical assistance in the experimental works.

Summary

Fluorescence was observed in the reaction of pentose with anthrone in strong sulfuric acid. The range of its fluorescence spectrum was from 470 to 600 mμ and the maximum was at about 510 mμ.

The anthrone reagent itself had a flat fluorescence spectrum from 420 to 600 mμ for it contains anthranol, but the shape of its spectrum was quite different from pentose fluorescence, and, furthermore, the intensity was far weaker than pentose fluorescence. In the case of glucose the fluorescence spectrum resembled that of the blank.

This reaction was specific for pentose in the presence of hexose, but excess glucose decreased the pentose fluorescence.

(Received January 13, 1964)