

# SPECTROPHOTOMETRIC IDENTIFICATION OF FUCOSE

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KAWAMURA and NARASAKI<sup>(1)</sup> found the presence of a methylpentose as one of the constituents of hemicellulose B<sub>1</sub> from broad-bean seeds. This methylpentose, isolated by paper chromatography and cellulose column chromatography, was assumed to be fucose by spectrophotometric observations of various color reactions<sup>(2, 3)</sup>, determination of R<sub>f</sub> on paper chromatogram<sup>(1, 2)</sup>, and synthesis of 2,4-dinitrophenylhydrazone by the solvent diffusion method<sup>(2)</sup>.

It was deemed useful to apply characteristic spectrophotometry in order to identify a minute amount of a sugar separated by such means as paper chromatography. In the course of such studies the authors discovered that the phenol-sulfuric acid reaction<sup>(4)</sup> could identify fucose and rhamnose, the two methylpentoses relatively widely distributed in nature as the polysaccharide constituents.

## EXPERIMENTAL

### 1. Spectrophotometric examinations of some color reactions of fucose and rhamnose.

The spectrophotometer used was of the Beckman DU model of the Shimadzu Co., Ltd., Kyôto. The light source was a tungsten lamp. The photoelectron multiplier IP28 and the quartz cells of 10mm width were used.

#### 1.1. The specific cysteine-sulfuric acid reaction.

The sugar solutions of about 25  $\gamma$ /ml were used for this reaction<sup>(5)</sup>. The time of heating was 10 min. The absorption spectra (Fig. 1-A) were obtained after allowing the reaction mixture to stand for 24 hours at the room temperature. Both fucose and rhamnose showed strong absorption at 400m $\mu$  characteristic to methylpentoses. The spectra were entirely similar for the two sugars.

#### 1.2. The thioglycolic acid-sulfuric acid reaction.

As shown in Fig. 1-B, the two methylpentoses showed almost the same spectra again with maximum absorptions at 400m $\mu$  in this reaction<sup>(6)</sup>.

#### 1.3. The Molisch reaction as revised by Dische.

The sugar solutions of about 50  $\gamma$ /ml were used. The classical reaction with 1-naphthol was examin-

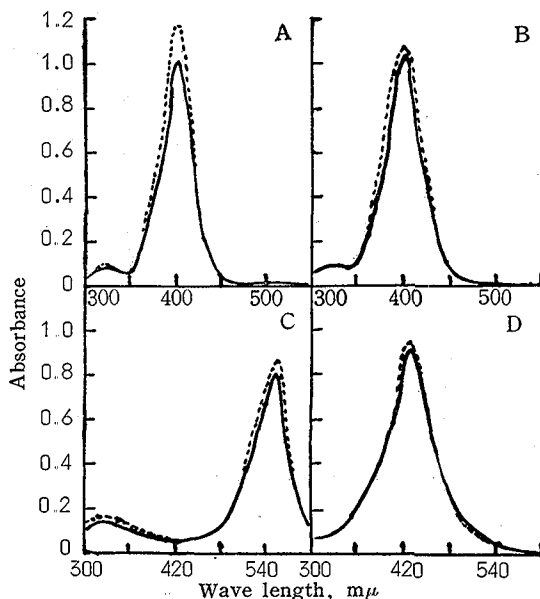


Figure 1. Absorption spectra of fucose and rhamnose in A) cysteine-sulfuric acid, B) thioglycolic acid, C) 1-naphthol, and D) orcinol reactions.

— fucose, - - - - - rhamnose

ed according to DISCHE (7). The spectra (Fig.1-C) were again very similar for fucose and rhamnose with the maximum absorption at 565m $\mu$ .

1.4. The orcinol-sulfuric acid reaction.

By this method(8) only ketoses develop color by very short heating, but aldoses also do by longer heating. Fig. 1-D shows almost the same absorption spectra for the two methylpentoses of about 50  $\gamma$ /ml by 15-min heating.

1.5. The phenol-sulfuric acid reaction.

In this case(4) a clear difference was discovered, as shown in Fig.2. Fucose showed three absorption maxima at 330, 400, and 480m $\mu$ , the absorbance at 330m $\mu$  being higher than that at 480m $\mu$ . Rhamnose showed only two absorption maxima at 330 and 480m $\mu$ , the absorbance at 330m $\mu$  being much lower than that at 480m $\mu$ .

2. The phenol-sulfuric acid reactions of some monosaccharides.

The phenol-sulfuric acid reaction of DUBOIS et al. (4) was applied to eighth common monosaccharides in about 50 $\gamma$ /ml solutions. Hexoses showed high absorption maxima at 490m $\mu$ , and pentoses showed those at 480m $\mu$ ; both groups of sugars showed lower absorption maxima at 330m $\mu$ . Their spectra were similar to the spectrum for rhamnose shown in Fig. 2.

The absorbances for various sugars as well as the ratio of the absorbance at 330m $\mu$  to that at 480 or 490 m $\mu$  are given in Table I.

Table I. Characteristics of common monosaccharides in the phenol-sulfuric acid reaction.

Sugars	Absorbance at 330m $\mu$ (A)	Absorbance at $\lambda$ max. in longer wave region		A/B
		$\lambda$ max., m $\mu$	Absorbance (B)	
D-Galactose	0.402	490	0.770	0.52
D-Glucose	0.528	490	0.810	0.65
D-Mannose	0.346	490	1.02	0.34
L-Arabinose	0.294	480	0.836	0.35
D-Ribose	0.298	480	1.19	0.25
D-Xylose	0.354	480	1.18	0.30
L-Fucose	0.498	480	0.378	1.32
L-Rhamnose	0.400	480	0.888	0.45

Thus this ratio higher than 1 would show the presence of fucose.

3. The effect of heating time in the phenol-sulfuric acid reaction.

In some color reactions of sugars the heating time affects so largely the absorption spectra of the reaction products that the qualitative and quantitative analyses become thereby possible (3 5 8). By the original method of DUBOIS et al. (4), the heating was not controlled, but it was carried out by utilizing the heat produced by dropwise addition of sulfuric acid into the aqueous medium. Now an experiment was made to see the effect by varying the heating time on this reaction.

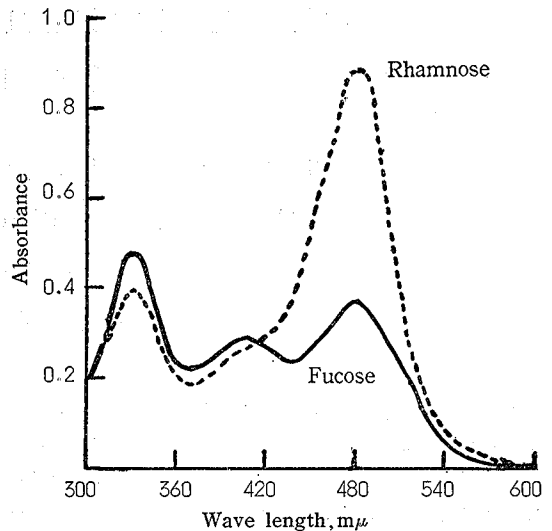


Figure 2. Absorption spectra of fucose and rhamnose in the phenol-sulfuric acid reaction.

Sulfuric acid was added under ice cooling. The reaction mixture was kept for 2, 8, or 32 min in a boiling-water bath. The spectra are shown in Fig. 3. As the heating time was lengthened, the absorbance at 330m $\mu$  increased, while that at 480m $\mu$  did not so much increase. In the case of fucose longer heating than 8 min led to the disappearance of the maximum at 480m $\mu$ . Anyhow no conditions were found to be more effective in identifying fucose from rhamnose than the conditions of the original method.

**4. Detection of fucose as a constituent of hemicellulose B<sub>1</sub> from broad-bean seeds.**

The hydrolyzate of the hemicellulose B<sub>1</sub> was purified in respect to the methylpentose fraction step by step, and the following four samples, A, B, C, and D, were prepared :

A -- the hydrolyzate was chromatographed on a large sheet of paper (T $\ddot{o}$ y $\ddot{o}$  No. 51A, 40cm $\times$ 40cm) with phenol-water (4:1) as the solvent,

B -- sample A was chromatographed with butanol-acetic acid-water (4 : 1 : 2) as the solvent,

C -- sample B was again chromatographed with phenol-water as the solvent, and

D -- sample C was chromatographed on a cellulose column with butanol-ethanol-water (4 : 1 : 5, the upper layer).

The results of the phenol-sulfuric acid reaction of these four sample solutions are shown in Table II. Fig.4 shows the absorption spectra for the samples A and D (spectra for the other intermediate samples are omitted for simplicity sake). Fig. 4 and Table II show instantly that the unknown sugar isolated and purified is fucose. This paper confirms the former assumption<sup>(1-3)</sup> that the hemicellulose B<sub>1</sub> from broad bean seeds contains fucose as a constituent.

(This paper was presented before the 170th meeting of the Kansai Branch of the Agricultural Chemical Society of Japan on June 4, 1960, at the

Table II. Phenol-sulfuric acid reaction of methylpentose fractions separated from the hydrolyzate of hemicellulose B<sub>1</sub> of broad-bean seeds.

Samples	Absorbance at 330m $\mu$ (A)	Absorbance at 480m $\mu$ (B)	A/B
A	0.342	0.542	0.63
B	0.374	0.474	0.78
C	0.398	0.406	0.98
D	0.458	0.382	1.20

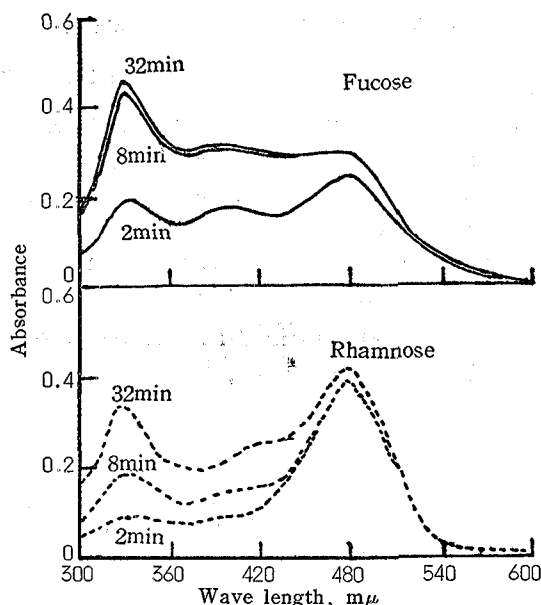


Figure 3. Effect of heating time on the absorption spectra of fucose and rhamnose in the phenol-sulfuric acid reaction.

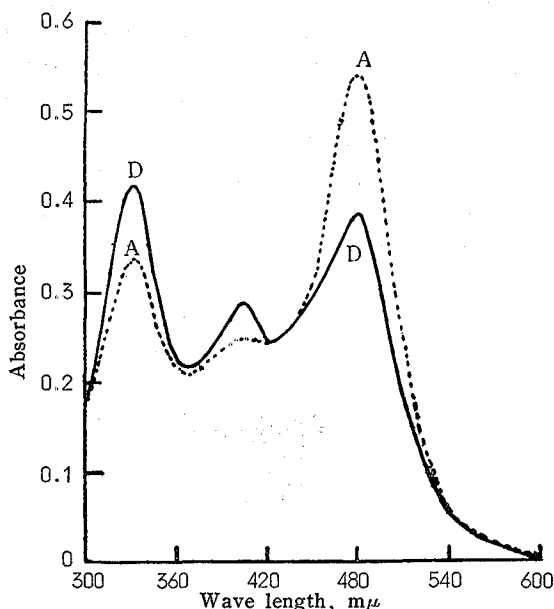


Figure 4. Absorption spectra of the samples A and D in the phenol-sulfuric acid reaction.

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## 分光光度計によるフコースの検出

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糖類の呈色反応は一般にペントース, ヘキソース, メチルペントースなどの群によりことなる。ところが同じメチルペントースでもラムノースとフコースとでは明らかに分光光度計による観察で区別ができる反応があることを発見した。それはDUBOISら<sup>(4)</sup>のフェノール・硫酸反応である。

DISCHE, SHETTLES<sup>(6)</sup>のシステイン・硫酸反応(図1-A), チオグリコール酸・硫酸反応<sup>(6)</sup>(図1-B), 1-ナフトール反応<sup>(7)</sup>(図1-C), オルシノール・硫酸反応<sup>(8)</sup>(図1-D)ではフコースとラムノースとはほとんど同様な吸収スペクトルを与える。これに反しフェノール・硫酸反応<sup>(4)</sup>の吸収スペクトル(図2)を短波長にひろげて検討するとフコースはラムノースとちがっている。ヘキソース(ガラクトース, グルコース, マンノース), ペントース(アラビノース, リボース, キシロース)とも全然ちがう(表I)。つまり短波長(330m $\mu$ )の真吸光度が長波長(480または490m $\mu$ )の真吸光度より高いのはフコースのみであった。

この反応の加熱時間を変えて見たが, 図3に示すとおり, 原法よりすぐれた条件は見つからなかった。

この反応によりソラマメのヘミセルローズB<sub>1</sub>に含まれているメチルペントースがフコースであるとの従来の推定<sup>(1-3)</sup>を確認した(図4, 表II)。

(1960年6月4日西宮市武庫川女子大学における日本農芸化学会大会(第170回講演会)で発表した。)