ANTHRONE REACTION OF THE UNIDENTIFIED SUGAR IN A HEMICELLULOSE FROM BROAD-BEAN SEEDS

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In 1956 KAWAMURA and NARASAKI[1] reported preliminarily that the hydrolyzate of the so-called hemicellulose B_1 prepared from dehulled seeds of broad bean consisted of glucose, xylose, galactose, and ribose in approximate molar ratios of 46:35:14:5, as determined by the SOMOGYI method after separation by paper chromatography. The detailed report was made in 1957[2], where the authors emphasized the detection of ribose in plant polysaccharides. NARASAKI[3] separated the fraction corresponding to ribose as a chromatographically pure solution and showed that the ribose-like sugar seems to be neither pentose nor hexose by various qualitative examinations. Further examinations[4 5] revealed that the unidentified sugar is of the methylpentose type, probably being fucose.

In the present paper, the anthrone reaction of the unidentified sugar will be described. This reaction also suggested that the unidentified sugar is of the methylpentose type.

The anthrone reagent was originally described by DREYWOOD in 1946[6] for the qualitative test of carbohydrates, and first adapted for the quantitative use by MORRIS[7]. MORSE[8] reported that the anthrone-carbohydrate color is sensitive to heating temperature, and various modifications[9-11] in the procedure were adopted to control this factor. TREVEYLAN and HARRISON[10] minimized the heat of mixing by chilling either the sample solution or the anthrone reagent prior to mixing the two and the full color was then developed by heating the mixture in a boiling-water bath. Finally, the heat of mixing was completely eliminated by the use of 60% sulfuric acid as solvent for both anthrone and carbohydrate sample[11]. With the elimination of the heat of mixing, the study of the influence of the duration of applied heat upon the color became possible. The work of KOEHLER[12], who published data for the anthrone-reaction rates of a number of the common classes of monosaccharides as well as some related polymers and derivatives, indicated that three classes of sugars can be distinguished by their anthrone-reaction rates. SCOTT and MELVIN[13] revealed the influence of temperature intensity upon the anthrone color of glucose. The work of SCOTT and MELVIN[13] was extended by HELBERT and BROWN[14] to methylpentoses, hexoses, pentoses and uronic acids. These results of KOEHLER[12] and HELBERT and BROWN[14] seem to be applicable for the differentiation of microgram amounts of carbohydrates in both pure and mixture states. Thus the anthrone reactions were adopted for the characterization of the unidentified sugar in the hemicellulose B_1 from broad-bean seeds.

EXPERIMENTAL

1. *Characterization of the unidentified sugar from hemicellulose B_1 by anthrone-reaction rate*

Sample. A pure solution of the unidentified sugar prepared in the previous experiments[5] was employed. The solution was proved to be chromatographically pure and contained the carbohydrate equivalent to 40γ of glucose as determined by the phenol-sulfuric acid method[15].

Authentic carbohydrates. The various monosaccharides employed in this experiments were from commercial sources and gave single spot on each paper chromatogram having Rf value well coinciding with published data. Carbohydrate solution was prepared by dissolving a quantity of solid carbohydrate in redistilled water so that each milliliter of solution contained 50γ of carbohydrate.

Sulfuric acid. Commercially available *G.R.* grade reagent was used without diluting with water.

Anthrone. A commercial product of Mann Research Laboratories, Inc., New York, N. Y., U. S. A., was dissolved in sulfuric acid so as to make 2% solution of the reagent. The reagent solution was usually made up 1 to 2 hours prior to use.

Procedure. Two milliliters of the sugar solution was pipetted into a test tube, cooled in an ice-water bath, and 4 ml of the ice-cooled anthrone reagent was added with vigorous shaking in an ice-water bath. The reaction mixture was then transferred to a tap-water bath for a minute, and finally into a boiling-water bath for various lengths of time to obtain a time cure of color development for each of the sugars. After heating, the reaction mixtures were cooled in an ice-water bath to stop the color development, and then equilibrated at room temperature for 20 to 25 minutes to be measured by Beckman spectrophotometer, Model DU. Absorbance measurements were made at 620 mμ by the use of 1-cm glass cells.

Results. Relationships between the heating time and the absorbance are delineated in Figure 1. These results are very similar to those of KOEHLER[12] and of HELBERT and BROWN[14]. Three classes were distinguished, *i. e.*, the aldohexose type, the ketohexose and methylpentose type, and the pentose type. The unidentified sugar showed an undistinguishable behavior from that of the ketohexose and methylpentose type.

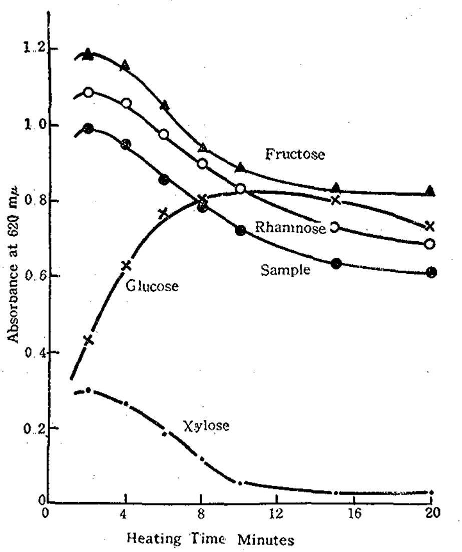

Figure 1. Relationship between the heating time and the absorbance.

2. *Characterization of the unidentified sugar from hemicellulose B_1 by absorption spectra of the anthrone-carbohydrate color developed at various lengths of heating.*

Materials and methods. The materials and the anthronation procedure were exactly the same as those described above. Spectral measurments were made promptly after 20-min equilibration at room temperature and completed within 30 minutes.

Results. Figures 2 through 6 show the absorption spectra for representative monosaccharides as references and the unidentified sugar after 2-, 10-, and 40-min heating.

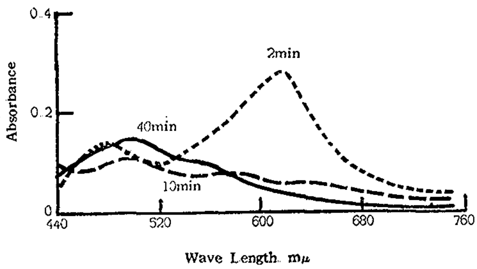

Figure 2. Absorption spectra of xylose.

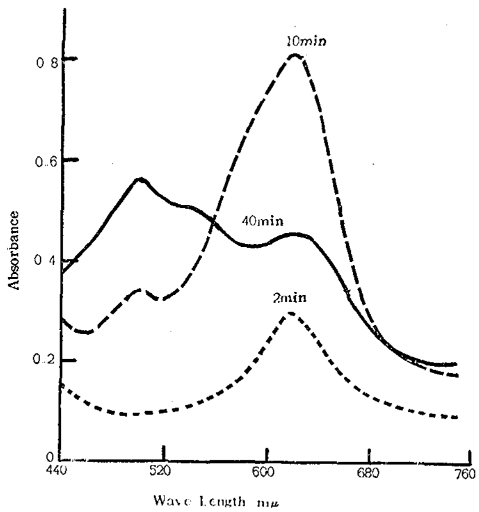

Figure 3. Absorption spectra of glucose.

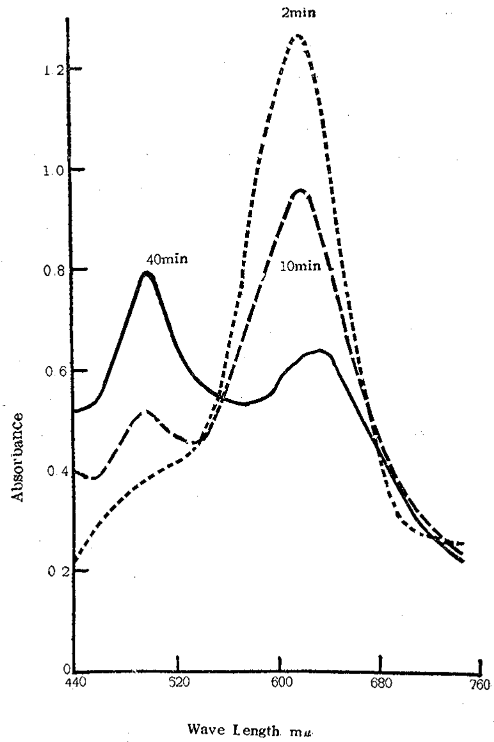

Figure 4. Absorption spectra of fructose.

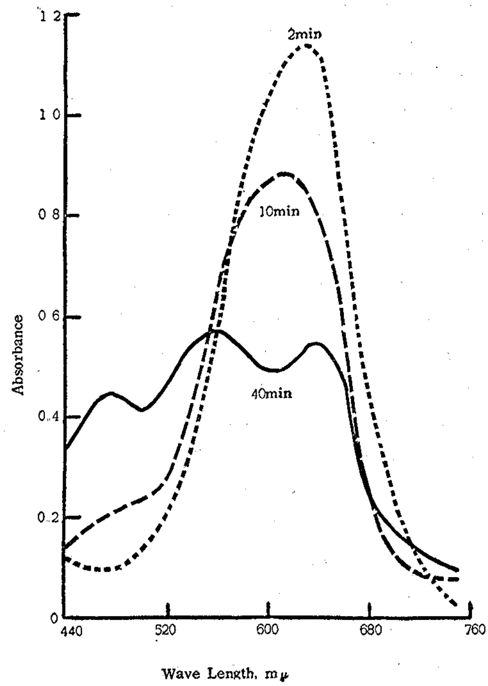

Figure 5. Absorption spectra of rhamnose.

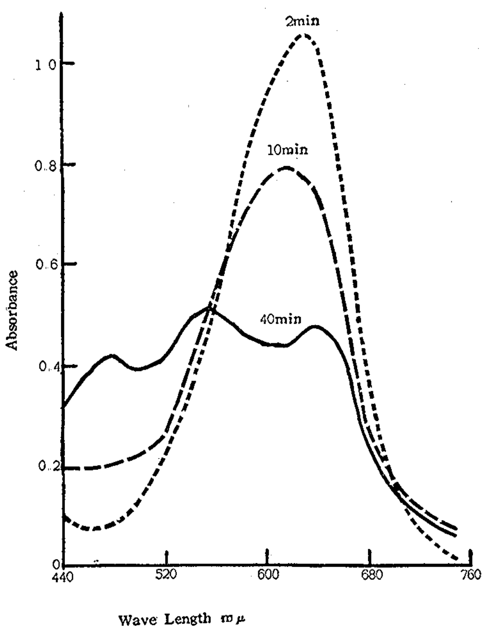

Figure 6. Absorption spectra of the unidentified sugar.

Influences of the heating time upon absorption spectra are very characteristic for each type of sugars. Xylose showed the absorption maximum at 620 mμ only at 2 min heating, showing far lower maximum at 500 mμ after 10-min heating. The glucose maximum at 620mμ lowered after 10 min, while that at 500mμ rose. Fructose showed the analogous hypochromic shift to that of glucose. Glucose, fructose, and xylose showed the maxima of various intensities at 500mμ after 40-min heating while rhamnose showed the absorption minimum at that wave length. Rhamnose showed three maxima at 480, 560, and 640 mμ after 40-min heating. The unidentified sugar showed the perfectly similar absorption spectra to those of rhamnose at any times of heating examined.

DISCUSSION

As KOEHLER[12] described, carbohydrates can be classified into the three types, *i. e.*, the ketohexose type, to which methylpentoses are included, the aldohexose type, and the pentose type, by the relationships between the heating time and the color intensity of the anthrone reaction developed in a boiling-water bath. The unidentified sugar from the hemicellulose B_1 seems to belong to the ketohexose and methylpentose type (Figure 1). More classes could be distinguished by HELBERT and BROWN[14] by changing the heating temperature and they differentiated the methylpentose type from the ketohexose type and the other types by heating at 60° C. All the works in this regard indicate that monosaccharides in a given type tend to behave in an analogous manner. Therefore, the anthrone procedure can be applicable for the differentiation of considerably pure samples of monosaccharides and their polymers.

The 40-min heating was employed for the detection of deoxysugars by KOEHLER in 1954[16], who described that deoxyglucose can be detected in a 1:1 mixture with glucose by the absorption spectra of the anthrone color developed after 40-min heating. No description was made on the behaviours of other types of sugars. The present data are the first on the absorption spectra of representative monosaccharides after 40-min heating. These spectra at 40-min heating (Figs. 2−5) indicate that the differentiation of each type of monosaccharides is possible by the spectra, if the sugars are in pure state. The absorption spectra of the unidentified sugar (Fig. 6) seem to suggest that the sugar belongs to the methylpentose type.

The anthrone reaction was originally described for the general reaction of carbohydrates. The specificity described above is concluded to be applicable for the characterization of a considerably pure sugar. However, the specificity appears not to be so high that the detection of each component sugar is possible in their mixtures.

SUMMARY

(1) Representative monosaccharides were differentiated into the three classes, *i. e.*, the aldohexose type, the ketohexose and methylpentose type, and the pentose type, by the relationships between the heating time and the color intensity of anthrone reaction.

(2) Each type of monosaccharides showed very characteristic absorption spectrum of the anthrone carbohydrate color developed after 40-min heating. Glucose fructose, and xylose rose the absorption maxima at 500 mμ with various intensities, while rhamnose showed three absorption maxima at 480, 560, and 640mμ.

(3) The unidentified sugar in the hemicellulose B_1 of broad-bean seeds showed the perfectly similar behaviours to those of rhamnose throughout the present experiments.

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ソラマメのヘミセルロースから分離された未知糖のアントロン反応

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われわれが先に(5)ソラマメのヘミセルロース B_1 から分離した未知糖についてアントロン反応を試みた．KOEHLER(12) の反応速度による糖の分別法によると，この糖はケトヘキソース・メチルペントース型に属する．HELBERT 及び BROWN(14) は反応温度を60～40°Cに下げることによって，ケトヘキソース型とメチルペントース型を分けたが，本実験において沸騰水中で発色させた場合は，ケトヘキソースとメチルペントースの間に，はっきりした相違を認められなかった (Fig. 1)．吸収曲線の反応時間による変化 (Figs 2 6) も糖の型によって特徴がある．特に40分加熱発色した時の吸収曲線によると，メチルペントースは他の糖から容易に区別できる．ケトヘキソース，アルドヘキソース，ペントースは 500mμに強さの異なる吸収極大を示すが，メチルペントースは 500mμには吸収の谷を示し，480，560，640mμ に三つの極大吸収を示す．しかし，これらの差異は十分特異的であるとはいえないから，混合物中の構成糖を混合状態のまゝで検出することは不可能であると思われるが，比較的純粋に分離された糖の推定には有効であろう．この吸収曲線測定の結果では，未知糖は完全にメチルペントースと一致する．

従って，アントロン反応の結果からも未知糖が一種のメチルペントースであるという推定(4 5)が確認された．

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