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SEPARATION OF AN UNKNOWN SUGAR FROM THE HYDROLYZATE OF A HEMICELLULOSE OF BROAD-BEAN SEEDS.

A PRELIMINARY REPORT.

Teiiti NARASAKI (Laboratory of Agricultural Products Technology) (Received July 31, 1957)

The detection of a ribose-like sugar was reported by KAWAMURA & NARASAKI[1] and KAWAMURA, et al.[2] in the hydrolyzate of a hemicellulose B_1 of a dehulled broad-bean seeds of variety "Nagasaya". The detection and the identification were made only by the Rf values on paper chromatograms by three sets of different solvent systems, but no description was made on the color reaction of the spot on chromatograms.

If the Rf values are identical in at least three different solvents and in color reactions with various spray reagents, good evidence can be said to be provided that the two sugars are identical. However, the present opinions are such that paper chromatography cannot give a conclusive evidence at all, and the unknown should be separated and characterized as crystalline derivatives.

The present experiments were conducted to separate the unknown sugar free from the other component sugars for the further studies of its identification. Some results contradicting former presumption were obtained and these results are presented in the present paper preliminarily.

EXPERIMENTAL

1. *Preparation of a hemicellulose B_1.*

A hemicellulose B_1 (2g) was obtained from 10 kg of dehulled broad-bean seeds of variety "Nagasaya" by a procedure shown in Fig. 1.

The hemicellulose B_1 preparation showed m. p. 230–50°C (decomposition), well coinciding with the preceeding results ([1,2]). The preparation could be hydrolyzed almost quantitatively by heating in a sealed glass tube in a boiling-water bath with 2 *N* H_2SO_4 for 4 hours, but a very small amount of an insoluble residue was noted after hydrolysis when a larger amount of the preparation (100—500mg) was taken for hydrolysis. The insoluble residue showed positive MOLISH reaction and negative biuret reaction, and it seemed to be an acid-resistant polysaccharide.

2. *Two-dimensional ascending paper chromatography of the hydrolyzate of hemicellulose B_1.*

Preparation of the hydrolyzate. The hemicellulose B_1 (0.2g) was hydrolyzed with 20 ml of 2 *N* H_2SO_4 in a sealed glass tube by heating in boiling water for 4 hours. The cooled filtrate was neutralized with $BaCO_3$, centrifuged, and the neutralized supernatant was concentrated to syrup below 40°C. The syrup was extracted with 10 ml of 50% ethanol, and the extract was concentrated to 1 ml for the application to paper chromatography.

Solvents. Chromatography was carried out by using phenol : water (PhOH) (4:1, v/v) for the first run, and *n*-butanol:acetic acid : water (BuOH : AcOH) (4:1:2, v/v) and *n*-butanol : pyridine : water (BuOH:C_5H_5N) (3:1:1.5, v/v) for the second run. Double development was made with BuOH:AcOH and BuOH:C_5H_5N, while simple development was employed with PhOH.

Color reagents. A. Ammoniacal $AgNO_3$. A mixture of equal volumes of 0.1 *N* $AgNO_3$ and 5 *N* NH_4OH were sprayed and the color was developed by heating at 105° for 5–10 minutes.

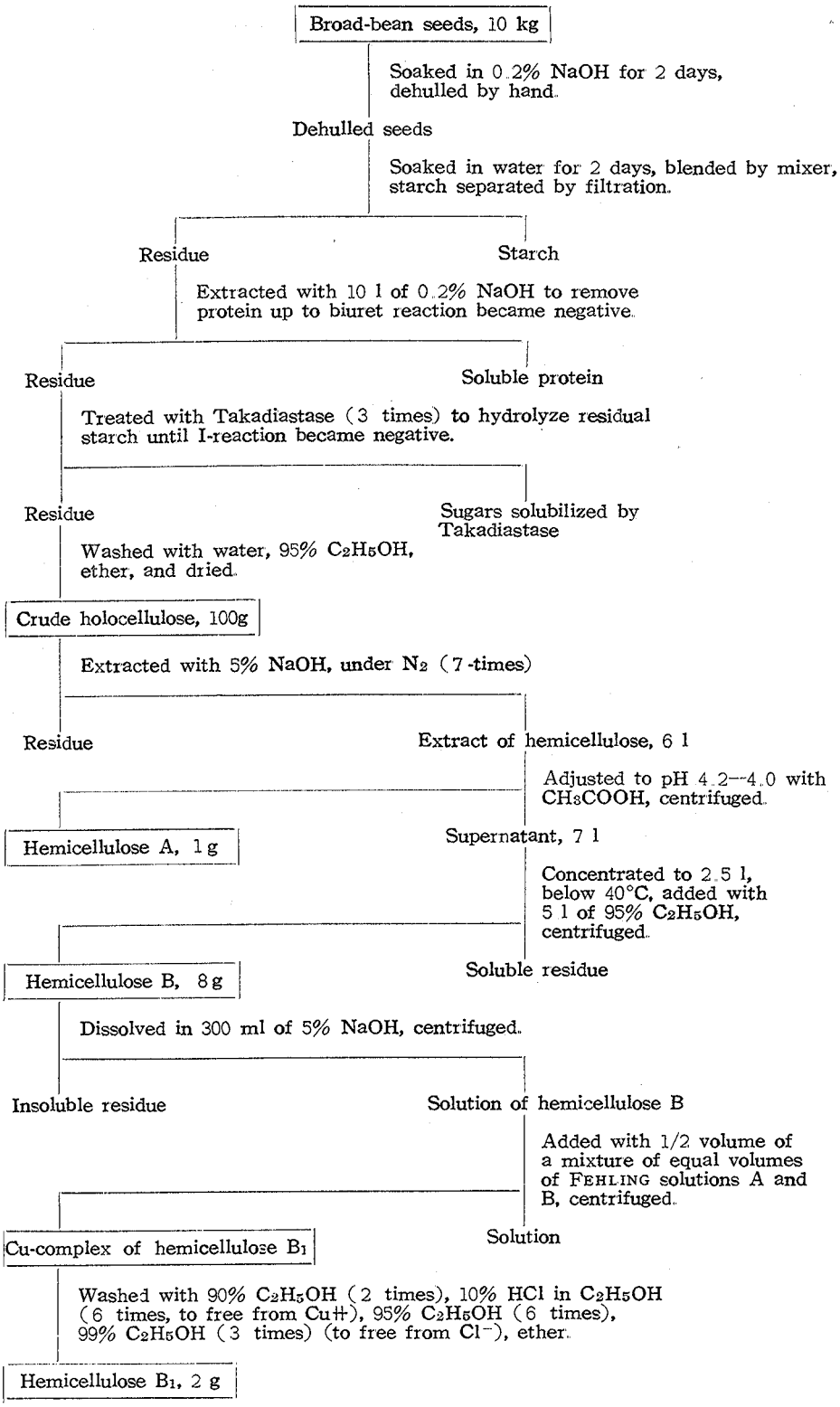

Fig. 1. Procedure of preparing hemicellulose B_1

The developed chromatograms were then washed with 0.1 *N* $Na_2S_2O_3.5H_2O$, followed by washing with water for permanent preservation of the developed chromatograms. The spots on paper chromatograms were somewhat enlarged, but the washed chromatograms were well preserved without any change even after one year of storage.

B. Aniline hydrogen phthalate. The reagent was made by adding 930 mg of aniline (freshly distilled) and 1.6g of phthalic acid to 100 ml of water-saturated *n*-butanol. The dried paper sheets were sprayed with this reagent and heated for 5 minutes at 105°.

Chromatography. The hydrolyzate was applied on Tōyō No. 50 filter paper sheets (20 × 20 cm), and the paper sheets were run with PhOH one time, after development the paper was washed with ether to remove residual phenol on paper, dried, and then run with BuOH:AcOH or BuOH:C_5H_5N by double development for second direction. Washing with ether markedly improved the uniformity of second solvent development.

Results. Typical chromatograms are shown in Figs. 2 and 3. White spots in the chromatograms were those of supplemented standard sugars. Colors of the spots were given with aniline hydrogen phthalate as described in these figures. All the spots gave positive reaction with ammoniacal $AgNO_3$, but no characteristic difference was noted in the colors.

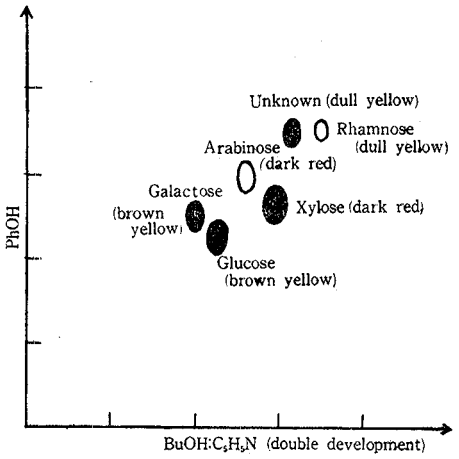

Fig. 2. Two-dimensional paper chromatogram of sugars in the hydrolyzate of a hemicellulose B_1 by PhOH-BuOH:AcOH system.

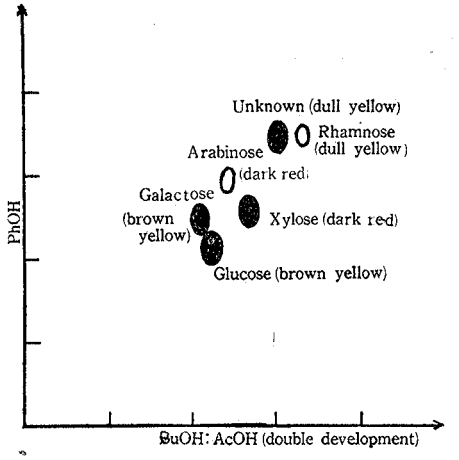

Fig 3. Two-dimensional paper chromatogram of sugars in the hydrolyzate of a hemicellulose B_1 by PhOH-BuOH:C_5H_5N system.

3. *Separation of the unknown sugar in the hydrolyzate of a hemicellulose B_1 by paper chromatography.* A hemicellulose B_1 (1g) was hydrolyzed as mentioned above, and the hydrolyzate was subjected to paper-chromatographic separation of the unknown sugar.

The hydrolyzate was introduced along a horizontal line 5cm from the botom of Tōyō No. 50 filter paper sheet 40 × 40 cm. The sheet was irrigated with BuOH:AcOH solvent by the triple development technique. Three strips were cut from the dried sheet as a guide strip, and developed with aniline hydrogen phthalate. The area containing the unknown sugar was cut by referring to the three developed strips and extracted by refluxing with 50 ml of water.

The extract of unknown sugar showed negative BIAL, phloroglucinol and orcinol reactions, and only one spot having Rf values of 0.55 with BuOH:AcOH, 0.56 with BuOH:C_5H_5N, and 0.59 with PhOH was detected by paper chromatography.

4. *Preparation of osazone.* The unknown sugar solution (20 ml). was concentrated to about 2 ml *in vacuo* below 40°C. The concentrated sugar solution was added with 50 mg phenylhydrazine hydrochloride

and 75 mg sodium acetate, and the mixture was heated on a boiling-water bath for about 1 hour. The resulting yellow crystalline product was filtered, washed with water, dissolved into 0.5 ml of hot ethanol, and recrystallized by adding about 2 ml of cold water. Yellow needles obtained showed m.p. 157—9° (uncorrected). D-Ribose phenylosazone was prepared from standard D-ribose by the similar procedure, which showed m.p. 160—2° (uncorrected). Mixed melting point was 155° (uncorrected) of the two osazones, showing a clear depression of the melting point.

5. *Cysteine-sulfuric acid reaction of the separated unknown sugar.* DISCHE found[3] that hexoses, pentoses, methylpentoses, 2-deoxypentose, and heptoses produce substances with very characteristic absorption spectra in his cysteine-sulfuric acid reaction[4]. These sugars seem to be differentiated from each other due to the fact that the development of the colors, as well as their ensuing destruction, depends upon the types of sugars. Thus the cysteine-sulfuric acid reaction by DISCHE was applied to the separated unknown sugar.

The unknown sugar solution (0.1 ml) was diluted to 1 ml, added with 4 ml of conc. H_2SO_4 while cooling in tap water. The mixture was kept at room temperature for 1 hour with frequent shaking. Then 0.1 ml of 3% cysteine hydrochloride was added and the mixture was shaken. Color intensities were read at 5 minutes-interval in the first 30 minutes after the addition of cysteine solution at room temperature, and then from time to time up to the 24th hour by a photoelectric colorimeter at 430mμ.

The results are shown in Table I, which showed that the greatest color intensity was developed after about 6 hours after the addition of cysteine solution at room temperature.

A ratio of an absorption at 430 mμ to that at 530 mμ was about 5.0 at the begining, and it decreased to as low as 1.1 in 6 hours.

Table I. Change of color intensity of the unknown sugar by the cysteine-sulfuric acid reaction with time.

Time (hr)	0	0.5	1.0	2.0	4.0	8.0	18	24
Optical density at 430 mμ	0.26	0.46	0.60	0.75	0.86	0.88	0.78	0.78

DISCUSSION

The unknown sugar, which was reported as ribose in the preceeding papers[1,2], was separated into a pure solution. The purity of the solution was proved by paper chromatography, showing only one spot of Rf values coinciding with those of unknown sugar in the preceeding reports by the three sets of solvent systems. The color reaction of the spot was dull yellow with aniline hydrogen phthalate. The result suggests that the unknown sugar cannot be ribose, for ribose shows dark red color if it appears on paper chromatograms at all. The color of the spot was very similar to that of methylpentoses. The spot, which was reported as ribose by HONMA & AKEIAGAWA[5] detected from fermented soybean paste, showed also dull yellow color. Various color reactions for pentoses were all negative with the separated unknown sugar solution. Therefore, it seems to be very improbable that the unknown sugar can be ribose.

Measurement of melting point of the phenylosazone of unknown sugar could not give any conclusive evidence for the identification of the sugar, but the results also seem to unfavor the former assumption that the unknown sugar is ribose.

The reaction product of pentoses by the cysteine-sulfuric acid reaction shows the greatest absorption after 15—20 minutes after the addition of cysteine solution at room temperature at 390 mμ, and then it begins to decrease slowly. Hexoses develop maximum color intensities more rapidly at 412—4 mμ, and the color is stable at room temperature. Therefore, the present results coincide with those of neither pentoses nor hexoses. While, heptoses produce an orange color with an absorption maximum

around 430 mμ, and the compound is unstable and transformed slowly into a purple compound with an absorption maximum at 510 mμ ; the greatest color intensity is reached after several hours. The color reaction of the unknown sugar seems to show a very similar behavior with heptoses described above. Methylpentoses give the maximum color intensity only after 24 hours, and 2-deoxypentoses give the final reaction product only after 24 hours. Therefore, it seems less probable that the unknown sugar is one of these 2 kinds of sugars by the results of cysteine-sufuric acid reaction.

SUMMARY

(1) A hemicellulose B_1 was prepared from debullled broad-bean seeds of variety "Nagasaya", and hydrolyzed with sulfuric acid to study the included unknown sugar.

(2) The unknown sugar was separated from the hydrolyzate of a hemicellulose B_1 by paper chromatography with BuOH:AcOH solvent on a large paper sheet (40 × 40 cm), and extracted with water to obtain an unknown sugar solution.

(3) The separated sugar showed negative BIAL, phloroglucinol, and orcinol reactions. The phenylosazone of the unknown sugar melted at 157—9°C. (decomposition).

(4) Cysteine-sulfuric acid reaction suggested that the unknown sugar is most probable to be one of heptoses. Some possibilities seemed to remain that the sugar can be methylpentose or 2-deoxypentose, but the sugar seemed to be neither pentose nor hexose.

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ソラマメのヘミセルロースB_1加水分解物からの未知糖の分離（予報）

楢崎丁市

ソラマメヘミセルロースB_1の構成糖類を検索するため，その加水分解物のペーパークロマトグラフイーを試みた所，クロマトグラム上でフコースに接近した新しい斑点を認めた。スポットのアニリン・フタール酸試薬による呈色は淡黄色であるが，そのRf値は標準のリボースと極めてよく一致したので仮にリボースとして報告した[1,2]。このヘミセルロースB_1加水分解物中からペーパークロマトグラフ法により未知糖をクラマトグラフ的に純粋な溶液として分離しその二三の性質を調べた。この糖はBIAL，フロログルシノール，オルシノール等のペントースの特性反応を示さず，DISCHE[4]のシステイン-硫酸反応はヘプトースのそれと傾向がよく似ていた。オサゾンの融点は157〜9°で，リボースのオサゾン（m.p. 160〜2°）と混融すると融点降下を示した。以上の点から，この未知糖がリボースであるとの推定は支持できないもののように思われる。

この研究の経費の一部分は文部省科学研究費（川村信一郎：多糖類成分としてのリボースの研究）により支払われた。この研究を行う機会を与えられ，終始御指導下さつた本学生物化学研究室の川村信一郎教授に深く感謝する。