HEMICELLULOSES OF CORNSTALKS

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INTRODUCTION

Although the hemicelluloses may be considered as one of the three major groups of constituents of such materials as cornstalks, cereal straws, hulls, cobs, hays, and similar agricultural materials, they have not been studied to a great extent.

Browne and Tollens (2) hydrolyzed the pith of cornstalks with 6-percent sulfuric acid and identified d-xylose and l-arabinose in the hydrolysate. Also, by extracting the pith of cornstalks with warm 5-percent aqueous sodium hydroxide solution and then adding alcohol to the clarified alkaline extract, they obtained a crude hemicellulosic product that amounted to 17.4 percent of the weight of the pith. This product contained 4.16 percent of ash and 60.75 percent of pentosans and on hydrolysis with hydrochloric acid yielded d-xylose and l-arabinose.

Peterson and Hixon (10) digested cornstalks (previously extracted with a 1-percent aqueous ammonium hydroxide solution) with 5-percent aqueous sodium hydroxide solution at room temperature for 12 hours. The reaction mixture was filtered, and the digestion with 5-percent aqueous sodium hydroxide solution was repeated twice, each extraction lasting 4 hours. To the combined alkaline extract, ethanol was added until precipitation ceased. The precipitate was redissolved in 1-percent aqueous sodium hydroxide solution and re-precipitated with ethanol. The hemicellulose preparation thus obtained was very impure. It contained 13.1 percent of lignin and 8.1 percent of ash. No attempt was made to determine the composition of the hemicellulose.

METHODS AND RESULTS

DEPECTINIZATION

A portion of the cornstalks used in this investigation was ground in a Wiley mill, and the following constituents were determined (in percent): Ash, 10.62; pentosans, 22.94; uronic acids (as anhydrides), 6.81. All results were calculated on the moisture-free basis.

The cornstalks used for the isolation of the hemicelluloses were cut into pieces about one-half inch long and extracted for 30 hours with a 1:2 ethanol-benzene solution in a large continuous copper extractor. This extractor was built on the same principle as the well-known Soxhlet extractor. The extracted cornstalks were ground in a Wiley mill fine enough to pass a 60-mesh sieve, and a portion of this material

1 Received for publication July 29, 1941. A résumé of this paper was presented on September 11, 1941, at the one hundred and second meeting of the American Chemical Society, held at Atlantic City, N. J.
2 Italic numbers in parentheses refer to Literature Cited, p. 406.
3 Determined by methods described in a previous publication (11).
(765 gm.) was digested with 8 liters of hot (85° C.) water for 3 hours. The mixture was stirred manually from time to time during this heating operation. The product was filtered and the above-described extraction with hot water was repeated twice. In the last two extractions, however, only 600 cc. of hot water was used for each extraction. The three aqueous extracts were combined and evaporated to dryness on the steam bath. The dry extract weighed 50.6 gm., which was 6.6 percent of the weight of the dry ethanol-benzene-extracted cornstalks. The dry extract was analyzed for pectin by the Emmett and Carré (5) modification of the Carré and Haynes (3) method. It yielded a calcium pectate precipitate that weighed 0.5 percent of the weight of the ethanol-benzene-extracted moisture-free cornstalks.

The ground cornstalks, which had been extracted with hot water, were treated with 6 liters of 0.5-percent aqueous ammonium oxalate solution, and the mixture was digested at 85° C. for 4 hours, with manual stirring at frequent intervals. It was then filtered, and the residual material was digested twice with 0.5-percent aqueous ammonium oxalate solution, the procedure given above being followed. The insoluble material was filtered off, and it was then digested with two 6-liter portions of hot water, the digestion period being in each case approximately 1 hour. The 0.5-percent ammonium oxalate and hot water extracts were combined and concentrated under reduced pressure. To the concentrated solution four times its volume of 95-percent ethanol containing some hydrochloric acid was added, and the precipitate, after drying, was analyzed for pectin by the method already referred to. The yield of pectin (as calcium pectate) was only 0.11 percent.

PARTIAL DELIGNIFICATION

To the pectin-free cornstalks a sufficient quantity of a 2-percent solution of sodium hydroxide in 60-percent ethanol was added to cover the cornstalks completely, and the mixture was digested at room temperature for 24 hours. During this digestion period, the reaction mixture was stirred mechanically. The plant material was filtered off, and the extraction with alcoholic sodium hydroxide solution was repeated twice. The combined alcoholic extract was neutralized with hydrochloric acid, and the ethanol was distilled under reduced pressure. The residual solution was made acid with hydrochloric acid, and the lignin was filtered. Neither the lignin nor the filtrate from it (treated as described in a previous publication (11)) afforded any furfural when distilled with 12-percent hydrochloric acid, thus indicating that in all probability this method of delignification did not bring about any loss of hemicellulose (9).

ISOLATION OF THE CRUDE HEMICELLULOSES

The material that had been extracted with alcoholic sodium-hydroxide solution was placed on the steam bath, and the alcohol was removed by evaporation. It was then mixed with sufficient 5-percent aqueous sodium hydroxide solution to make a thin suspension, and was allowed to digest at room temperature for 24 hours. It was then

* All yields recorded in this paper were calculated on the basis of moisture-free and ethanol-benzene-extracted stalks.
filtered, and the above-described digestion with 5-percent aqueous sodium hydroxide solution was repeated three times. The combined alkaline extract amounted to 18.3 liters. To this was added sufficient 95-percent ethanol to make a 60 percent by volume ethanol solution. After being thoroughly mixed, the solution was allowed to stand at room temperature for 24 hours. The supernatant solution was drawn off, and the precipitate of hemicelluloses was mixed with sufficient 70-percent ethanol to make a thin suspension, from which the hemicelluloses were separated with the aid of the centrifuge. This treatment with 70-percent ethanol was repeated twice. The hemicellulose precipitate was then mixed with a fresh portion of 70-percent ethanol, and sufficient concentrated hydrochloric acid was added to make the mixture slightly acid. This was allowed to stand for several hours in order to insure complete neutralization, after which the acidified ethanol solution was removed with the aid of the centrifuge. The precipitate of crude hemicelluloses was then washed with three portions of 70-percent ethanol and with several portions of 95-percent ethanol. It was finally washed successively with two portions of absolute ethanol and one portion of anhydrous ether. It was dried in vacuo at 50° C. The yield amounted to 21 percent.

Analysis of the product by the method of Goss and Phillips (6) showed that it contained 2.73 percent of lignin.

DELIGNIFICATION OF THE HEMICELLULOSES

The delignification of the hemicelluloses was accomplished by a modification of the procedure described in a previous publication (13). The crude hemicellulose preparation was placed in centrifuge bottles surrounded with ice. A current of chlorine was passed through the bottles for 2 hours. The bottles were then stoppered and allowed to remain in the refrigerator at a temperature of 8° C. for 4 to 5 hours. The material was washed twice with 95-percent ethanol and then extracted with a 3-percent ethanolamine solution in 95-percent ethanol. The product was washed successively with 95-percent ethanol, absolute ethanol, and anhydrous ether, and then dried in vacuo at 50°. The yield was 17 percent.

Analysis of the delignified product gave the following results (in percent): Pentosans, 83.51; uronic acids (as anhydrides), 7.73; ash, 1.95; methoxyl, 0.63; lignin, none.

HYDROLYSIS OF THE LIGNIN-FREE HEMICELLULOSES

To 45 gm. of lignin-free hemicelluloses, 2.5 liters of 2.5-percent sulfuric acid was added, and the mixture was boiled on an electric hot plate under a reflux condenser for 15 hours. After the reaction mixture had cooled, the dark precipitate was filtered on a weighed filter paper and dried at 105° C. The dried material weighed 0.70 gm., which was 1.5 percent of the weight of the hemicelluloses. To the filtrate approximately nine-tenths of the calculated quantity of barium hydroxide solution was added slowly, with stirring, while the temperature of the reaction mixture was kept at 40°. An excess of barium carbonate was then added, and the mixture was heated at 70° to 80° until neutral to litmus. Some Norit and Filter Cel were added to this, and after standing overnight, the mixture was filtered. The filtrate was concentrated to a volume of approximately 100 cc.
under reduced pressure at a temperature not exceeding 45°. The concentrated solution was poured slowly, with stirring, into 4 volumes of absolute ethanol, and the precipitated barium salt was separated with the aid of the centrifuge. The barium salt precipitate was dissolved in 25 cc. of water; the solution was decolorized with Norit and filtered; and the filtrate was poured into 4 volumes of absolute ethanol. The precipitate was again separated with the aid of the centrifuge, was washed successively with absolute ethanol and with anhydrous ether, and was dried in vacuo at 50°. The barium salt weighed 1.7 gm.

The supernatant alcoholic solution from the barium salt was concentrated under reduced pressure at 50° C. to remove the ethanol, and the thin sirup was transferred quantitatively to a 500-cc. volumetric flask and made up to the mark with distilled water. The total reducing sugars in this solution as determined by the method of Munsen and Walker (1) amounted to 33.5 gm. (calculated as glucose). Five cubic centimeters of the sugar solution, when distilled with 12-percent hydrochloric acid according to the procedure used for the determination of pentosans (1), afforded 0.3413 gm. of phloroglucide precipitate. This sugar solution contained a total of 8.48 gm. of arabinose, as determined by the Wise and Peterson (14) modification of the method of Neuberg and Wohlgemuth (8).

The remainder of the sugar solution was concentrated under reduced pressure to a thin sirup, which was allowed to remain in a desiccator over anhydrous calcium chloride until a considerable quantity of the sugar had crystallized. When filtered off and recrystallized from dilute ethanol, the sugar weighed 7.4 gm. It was identified as \(d\)-xylose by Bertrand's (7) method. The refractive indices of the double cadmium salt were found to be identical with those of the salt prepared by the same method from a known specimen of pure \(d\)-xylose.5

The sirup remaining after the separation of the crystals of \(d\)-xylose contained \(l\)-arabinose (identified as the diphenylhydrazone). No mannose, galactose, or fructose could be detected.

The barium salt obtained in the hydrolysis of the hemicellulose preparation described above gave Tollens' (12) naphthoresorcinol test for uronic acids. On oxidation with either nitric acid or bromine water, it did not form mucic acid, thus indicating the absence of galacturonic acid. The cinchonine salt as well as the brucine salt was prepared from the barium uronate according to the procedure described in a previous publication (4). The melting points of these two salts indicated that the uronic acid was \(d\)-glucuronic acid.

The analytical data (calculated on the moisture-free and ash-free basis) and the percentage composition of the hemicellulose preparation calculated from these data are as follows (the calculations were made as described in a previous publication (13)): Uronic acid (as anhydride), 7.88; total furfural, 49.81; furfural from uronic acid, 1.50; \(l\)-arabinose, 23.00; furfural from \(l\)-arabinose, 11.04; furfural from \(d\)-xylose (by difference), 37.27; \(d\)-xylose (calculated from furfural), 64.70; molar ratio of uronic acid to \(l\)-arabinose and \(d\)-xylose, 2:7:19.

The cellulosic material which remained after the exhaustive extraction with 5-percent aqueous sodium hydroxide solution and which still contained furfural-yielding constituents (10.4 percent, calculated as pentosans) was next treated with 5 liters of 10-percent aqueous

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5 All identifications by optical methods were made by G. L. Keenan of the Microanalytical Division of the Food and Drug Administration, Federal Security Agency.
sodium hydroxide solution, and the mixture was stirred mechanically at room temperature for 7 hours. The mixture was then filtered, and the extraction operation described above was repeated twice. The residual cellulose material obtained from the third extraction was washed free of alkali and dried. It contained 5.87 percent of pentosans and 1.40 percent of uronic acid (as anhydride).

To the alkaline filtrate sufficient 95-percent ethanol was added to make a 60-percent alcohol solution. The hemicellulose precipitate was separated with the aid of the centrifuge and washed successively with neutral 70-percent ethanol, with 70-percent ethanol acidified with acetic acid, with graded strengths of ethanol, and finally with ether. After it was dried in the vacuum oven at 50° C., it weighed 13.85 gm. An analysis of this material gave the following results (in percent): Pentosans, 64.45; uronic acid (as anhydride), 5.09; methoxyl, 1.12; ash, 0.54.

A portion (172 gm.) of the cellulosic residue remaining from the extraction with the 10-percent aqueous sodium hydroxide solution was treated with 2 liters of 17-percent aqueous sodium hydroxide solution and allowed to remain at room temperature for 2 days. From time to time the reaction mixture was stirred manually. It was filtered, and the residual cellulosic material was again digested with 17-percent aqueous sodium hydroxide solution for 2 days. The residual material from the second extraction was thoroughly washed with cold and hot water and then dried at 105° C. It weighed 38.5 gm. and contained 2 percent of pentosans and 1.20 percent of uronic acids (as anhydride).

The alkaline extracts and washings from the two extractions with 17-percent aqueous sodium hydroxide solution were combined, and the hemicelluloses were isolated, the procedure already described being used. The yield amounted to 10.7 gm. The uronic acids (as anhydrides) and the furfural-yielding constituents, calculated as pentosans, in this hemicellulose fraction amounted to 3.62 and 49.34 percent, respectively.

HYDROLYSIS OF THE HEMICELLULOSE FRACTIONS FROM THE 10-AND 17-PERCENT AQUEOUS SODIUM HYDROXIDE EXTRACTIONS

The hydrolysis of the hemicellulose fractions from the 10- and 17-percent aqueous sodium hydroxide extractions was carried out in the manner already described. In both cases, the only sugars identified were D-xylose and L-arabinose. Because of the small quantity of barium uronate obtained, it was not possible to identify the uronic acid in these two hemicellulose fractions.

SUMMARY

A hemicellulose fraction was isolated from cornstalks, which had previously been freed of fatty and waxy materials, sugars, and pectic substances, by extracting them exhaustively at room temperature with a 5-percent aqueous sodium hydroxide solution and precipitating with ethanol. The product was delignified by treatment with chlorine and extraction with an ethanolamine solution in ethanol. On hydrol-
ysis with dilute sulfuric acid, the lignin-free hemicellulose fraction afforded D-glucuronic acid, L-arabinose, and D-xylose in the approximate molar ratio of 2:7:19.

The cellulosic material remaining after the removal of the first hemicellulose fraction was successively extracted in the cold with 10- and 17-percent aqueous sodium hydroxide solutions, and two additional hemicellulose fractions were obtained. These differed quantitatively from the first hemicellulose fraction, although on hydrolysis they both yielded D-xylose and L-arabinose.

LITERATURE CITED

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