[CONTRIBUTION FROM WESTERN REGIONAL RESEARCH LABORATORY[1]]

Esters of Lima Bean Pod and Corn Cob Hemicelluloses

BY J. F. CARSON AND W. DAYTON MACLAY

This paper describes the preparation of the acetate, propionate and butyrate of lima bean pod hemicellulose and the acetate, propionate, butyrate, caprate, laurate, myristate, palmitate and benzoate of corn cob hemicellulose. It also reports studies on the fractionation of some of these esters by extraction with organic solvents.

Husemann[2] obtained molecular weights of xylans from wheat straw and beechwood by osmotic and viscometric measurements of the corresponding methyl ethers, mixed methyl ether acetates, and benzyl ether acetates. His results indicated that undegraded xylans from wheat straw and beechwood were homogeneous, with chain lengths of approximately 150 repeating anhydroxylose units. Both Husemann[2] and Heuser and Schlosser[3] reported that xylan diacetates were poorly soluble in organic solvents, hence the application of the more soluble mixed ether acetates. Recently Millatt and Stamm[4] have prepared acetates of aspen hemicellulose from which molecular weights were determined.

Numerous investigators[5] have studied the xylan–glucuronide type of hemicellulose by fractional precipitation from alkaline solutions and by copper precipitation. In most cases, convincing evidence of the homogeneity of the various fractions was lacking. Many of the fractions contained varying amounts of hexosan either chemically combined in the xylan chain or simply as mixtures. O'Dwyer[6] and Anderson and co-workers[7] have presented evidence that some hemicellulose fractions, particularly from the sapwood of English oak, lemon wood, and black locust sapwood contain anhydroglucose units chemically united to anhydroxylose units. These fractions were observed to give violet to blue colors with iodine. Digestion with taka-diastase or saliva produced polysaccharides free of hexose no longer giving the color with iodine, but the enzymatic hydrolysis required a considerably longer time than expected for a physical mixture of xylan and starch.

The hemicelluloses used for esterification were obtained by alkaline extraction of corn cob and lima bean pod holocellulose. The holocellulose was extracted successively with boiling water, 2% sodium carbonate, and 5 and 24% potassium hydroxide solutions. The yields and composition of the two fractions of corn cob and lima bean pod hemicelluloses obtained by extraction with potassium hydroxide are recorded in Table I.

TABLE I

HEMICELLULOSE FRACTIONS

Holocellulose	Concn. of potassium hydroxide	Yield[a]	$[\alpha]^{25}D$ (2% KOH)	Xylan,[b] %	Uronic anhydride[c]	Methoxyl[d]
Corncob	5	22	−98.2	89.3	6.2	0.7
Corncob	24	8	−91.1	83.4	4.5	.7
Lima bean pod	5	17	−44.7	74.8	7.9	1.4
	24	8	−58.9	71.8	4.7	0.7

[a] Yields are based on dry weight of the hollocellulose. [b] Xylan was determined by furfural distillation and precipitation as the phloroglucide and was corrected for the furfural equivalent of the uronic anhydride. [c] Uronic anhydride analyses were performed by the procedures of Lefevre and Tollens, as modified by McCready, Swenson and Maclay (*Ind. Eng. Chem., Anal. Ed.*, 18, 290 (1946)). [d] Methoxyl was determined by Clark's modification of the Vieböch-Schwappach method (*J. Assoc. Off. Agr. Chem.*, **22**, 100, 622 (1939)). The samples contained no adsorbed alcohol, as was demonstrated by humidification in a desiccator over water.

The esters, except the benzoate, were prepared by reaction with pyridine and acid anhydride or acid chloride under relatively mild conditions. Benzoylation was performed by reaction with benzoyl

TABLE II

HEMICELLULOSE ESTERS

Source of xylan	Ester	Acyl, % Calcd.[a]	Found[b]
Lima bean pod	Acetate	38.8	39.3
Lime bean pod	Propionate	45.1	45.8
Lima bean pod	Butyrate	50.9	51.0
Corncob	Acetate	39.0	39.5
Corncob	Propionate	45.2	45.5
Corncob	Butyrate	51.3	50.8
Corncob	Caprate	70.0	69.9
Corncob	Laurate	73.0	70.0
Corncob	Myristate	75.7	72.2
Corncob	Palmitate	77.9	76.4
Corncob	Benzoate	60.9	57.5

[a] The theoretical per cent. acyl was calculated from the composition of the original hemicellulose. The original hemicelluloses were analyzed for xylan, methoxyl and uronic anhydride, and the residual unaccounted-for material was assumed to be hexosan. The calculated values include the contribution of each of these, assuming no loss on esterification. [b] % acyl was measured by alkaline saponification of the esters by the procedure of Genung and Mallatt (*Ind. Eng. Chem., Anal. Ed.*, **13**, 369 (1941)). The caprate, laurate, myristate, palmitate and benzoate were also analyzed by saponification, followed by acidification, extraction of the free organic acid and titration.

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) Husemann, *J. prakt. Chem.*, **263**, 13 (1940).

(3) Heuser and Schlosser, *Ber.*, **56**, 392 (1923).

(4) Millatt and Stamm, *J. Phys. Chem.*, **51**, 134 (1947).

(5) (a) Norris and Preece, *Biochem. J.*, **24**, 59, 973 (1930); (b) Preece, *ibid.*, **25**, 1304 (1931); (c) Angell and Norris, *ibid.*, **30**, 2159 (1936); (d) Buston, *ibid.*, **29**, 196 (1935); Anderson and Kaznarich, *J. Biol. Chem.*, **111**, 549 (1935).

(6) O'Dwyer, *Biochem. J.*, **28**, 2116 (1934); **31**, 254 (1937); **33**, 712 (1939).

(7) Anderson, Seeley, Stewart, Redd and Westerbike, *J. Biol. Chem.*, **135**, 189 (1940).

TABLE III
FRACTIONATION OF LIMA BEAN POD HEMICELLULOSE ESTERS

Ester	Fractionation Solvent	Insoluble %	Composition of hemicellulose regenerated from insoluble fraction, % Xylan	Uronic anhydride	Methoxyl	$[\alpha]^{25}D$ (2% KOH)
Acetate	Chloroform-acetone (1:1) 100 parts	92	76	8.1	1.3	....
Propionate	Chloroform-acetone (1:1) 100 parts	90	77	8.0	1.2	....
Butyrate	Chloroform-acetone (1:1) 100 parts	85	75	9.1		....
Butyrate[a]	Dioxane, 100 parts	88	77	6.4		−45.1
Acetate	Pyridine, 50 parts	86	77	8.0	1.2	−44.9
Propionate	Pyridine, 50 parts	82	75	5.4	1.4	−44.4
			Composition[b] of original hemicellulose (5% KOH extraction)			
			75	7.9	1.4	−44.7

[a] A second fractionation of the dioxane-insoluble butyrate under the same conditions yielded an insoluble portion amounting to 90%. Analysis of the regenerated polysaccharide indicated no change in composition. [b] Determination of L-arabinose by the diphenylhydrazine method of Wise and Peterson (*Ind. Eng. Chem.*, **22**, 362 (1930)) showed arabinose to be absent. Tests for mannose (phenylhydrazine) and for galactose (mucic acid) were negative.

chloride and aqueous sodium hydroxide. The esters and their analyses are recorded in Table II.

All of the esters had very low solubilities in organic solvents such as chloroform, dioxane, acetone, nitrobenzene and *m*-cresol. For example, only 25% of the dipropionate and 30% of the dibutyrate of corn cob hemicellulose were soluble in two hundred parts of dioxane, and only 8, 10 and 15% of lima bean pod hemicellulose diacetate, dipropionate and dibutyrate, respectively, were soluble in one hundred parts of chloroform–acetone (1:1). Since hexosan triesters, if not of too high molecular weight, are usually more soluble in organic solvents than are the xylan esters, it was thought that extraction of the esters with organic solvents and regeneration of the polysaccharide from the insoluble ester fraction would enrich the xylan content, if the non-pentosan components were merely present in a mixture. Lima bean pod diacetate, dipropionate, and dibutyrate were fractionated with a 1:1 chloroform–acetone mixture; the dibutyrate was also fractionated with dioxane, and the diacetate and dipropionate were fractionated with pyridine. In each case, the insoluble fraction was saponified and the regenerated polysaccharide analyzed for xylan and uronic anhydride. These results are compiled in Table III for comparison with the original hemicellulose.

The analyses in Table III show that, although in two cases the uronic anhydride content was decreased by treatment with organic solvents, the xylan content was increased only slightly and remained practically constant. The material unaccounted-for as xylan, uronic anhydride, and methoxyl in the original hemicellulose fraction was approximately 16.5% and for the saponified insoluble fractions the smallest figure obtained for this value was approximately 14.5%. Apparently very little extraction of hexosan was accomplished. Moreover, the specific rotation of the regenerated fractions were close to that of the original. Further, when the saponified pyridine-insoluble fractions of the lima bean pod hemicellulose diacetate and dipropionate were re-dissolved in alkali and precipitated by the copper method,[8] the polysaccharides were recovered in practically quantitative yields with the same specific rotations and xylan and uronic anhydride contents as the original. The lima bean pod hemicelluloses isolated by 5 and by 24% potassium hydroxide extraction, and all of the fractions isolated as described gave a violet to blue color with iodine. That the blue color was not due to starch carried through the fractionations was shown by the fact that digestion with saliva for five days did not change this property, and extraction with boiling calcium chloride solution[9] (density 1.3) left a residue still colored blue or violet when treated with iodine. The corn cob hemicelluloses did not give the colors. The failure to remove hexosan constituents by extraction with organic solvents furnishes an independent confirmation of the findings of O'Dwyer[6] and Anderson and co-workers[7] that anhydrohexose units are probably chemically combined with xylan in certain types of hemicelluloses.

Experimental

Preparation of Corn Cob and Lima Bean Pod Holocelluloses.—Corncobs and dried lima bean pods of normal canning maturity were reduced to twenty mesh and extracted successively with benzene–ethanol (2:1) for twelve hours in a large Soxhlet extractor, twice with thirty parts of boiling 0.5% ammonium oxalate solution for two-hour periods, and twice with thirty parts of boiling water. Delignification was accomplished by the sodium chlorite procedure of Wise, Murphy and D'Addieco[10] as follows: Two hundred grams of air-dried extracted material was heated to 70° with four liters of water in a six-liter flask in a fume hood. Glacial acetic acid (15 ml.) and technical sodium chlorite (30 g.) were added and the suspension held at 68–70° for an hour. Addition of acetic acid and sodium chlorite was repeated and heating was continued for another hour. This treatment produced a white fibrous holocellulose from the lima bean pods. A third addition

(8) The hemicelluloses were precipitated according to the directions of Angell and Norris (*Biochem. J.*, **30**, 2155 (1936)).

(9) The hemicellulose was treated according to the directions of Steiner and Guthrie (*Ind. Eng. Chem., Anal. Ed.*, **16**, 736 (1944)), who showed that quantitative extraction of starch from various plant materials can be accomplished with boiling calcium chloride solution.

(10) Wise, Murphy and D'Addieco, *Paper Trade J.*, **122** [2], 35 (1946).

of reagents and an additional hour of heating was required to produce a white product from the corn cobs. The suspension was cooled to room temperature, filtered on a cloth mat in a Buchner funnel, and the white holocellulose was washed repeatedly with large quantities of distilled water, 95% ethanol and acetone. Corncob and lima bean pod holocelluloses were obtained in yields of 81 and 90%, respectively. The yields were based on the dry extractive-free material.

Extraction of Hemicellulose Fractions from the Holocelluloses.—Four hundred grams of air-dried holocellulose was heated for two hours with six liters of boiling water, filtered, and then steeped for forty-eight hours in eight liters of 2% sodium carbonate (in the absence of oxygen), refiltered and washed with a liter of distilled water. The filter cake from the carbonate extraction was steeped for two hours in eight liters of 5% potassium hydroxide at 20–22°. Nitrogen was bubbled through the mixture to assist in mixing and removal of oxygen. The pale amber-colored suspension was filtered on hardened paper, and the filter cake was washed with two liters of distilled water without drawing air through the funnel. The hemicellulose fraction was isolated as a white curdy precipitate when the filtrate was poured into two volumes of 95% ethanol. The suspension was acidified (*p*H 4.5–5.0) with acetic acid and filtered. The hemicellulose was washed successively with 70% ethanol—5% acetic acid, 70, 85, and 95% ethanol and acetone, and air dried.

A second fraction was isolated by extracting the filter cake from the 5% potassium hydroxide extraction in the same general way but with four liters of 24% potassium hydroxide solution.

Hemicellulose Diacetate, Dipropionate and Dibutyrate.—Twelve grams of hemicellulose from corn cob or lima bean pod (dried *in vacuo* at 70°) was gelatinized by stirring for one hour at 63–65° with 250 g. of formamide. Pyridine (250 ml.) was added and the mixture was cooled to 25°. The appropriate acid anhydride (0.7–0.9 mole) was added in four equal portions over a four-hour period. The reaction mixture was stirred for six hours at 25–30° and allowed to stand overnight at room temperature. The esters were isolated by pouring the reaction mixture into 3 liters of water containing a kilogram of chopped ice. The esters, which precipitated either as flocs or as grainy precipitates, were filtered and washed successively with cold 2% aqueous hydrochloric acid, distilled water, 95% ethanol and ether. They usually had acyl contents one or two per cent. below the theoretical and were esterified a second time by treatment with pyridine and acid anhydride for several days at room temperature to yield the diesters in quantitative yields with maximum esterifications.

Hemicellulose Benzoate.—Eight grams of corncob hemicellulose (anhydrous) was stirred for one hour in 100 ml. of water at 80–85° and a smooth suspension was obtained. To the cooled mixture 100 ml. of 25% sodium hydroxide solution was added and the resulting clear amber solution was cooled to 5° with an ice-bath. Forty grams of benzoyl chloride was added in small quantities with vigorous stirring over a three-hour period and stirring was continued for two more hours at 7–10°. Xylan benzoate which precipitated as a white granular material was filtered and washed successively with 2% aqueous hydrochloric acid, hot (70°) distilled water, 95% ethanol and ether. Hemicellulose benzoate, 17.5 g., was obtained which had a benzoyl content of 55.8%, equivalent to 1.7 benzoyl groups per anhydroxylose repeating unit. A second esterification with pyridine and benzoyl chloride for two days at 22–25° yielded a benzoate containing approximately 1.8 benzoyl groups per repeating unit.

Hemicellulose Caprate, Laurate, Myristate and Palmitate.—These esters could not be prepared satisfactorily in formamide dispersion because of extensive side reactions between the acid chlorides and formamide. In a typical preparation, ten grams of hemicellulose was dispersed to a smooth paste by heating for fifteen minutes at 90° with 70 ml. of water in a 3-neck liter, round-bottom flask. To the paste 150 ml. of anhydrous pyridine was added, and the mixture was stirred mechanically and heated while pyridine and water were distilled off azeotropically. Fresh pyridine was added at intervals and the distillation continued until the distillation temperature reached 114° at which point the polysaccharide was practically free of water. The reaction mixture was cooled to 40°, additional pyridine was added to give a total volume of approximately 400 ml. and 0.3 mole of acid chloride was added in three equal quantities over a three-hour period. The reaction mixture was stirred for a total of six hours at 43–45°, allowed to stand overnight, and the esters recovered as waxy or resinous aggregates by pouring into two liters of ice water. In order to remove completely excess fatty acid, it was usually necessary to swell and partially dissolve the ester in dioxane and to re-precipitate into ethanol.

Acknowledgment.—The authors are grateful to Miss Nylan Jeung for analysis of methoxyl and uronic anhydride.

Summary

The acetate, propionate, butyrate, caprate, laurate, myristate, palmitate and benzoate of a corncob hemicellulose and the acetate, propionate, and butyrate of a lima bean pod hemicellulose have been prepared and their solubility characteristics studied.

Fractionation of the acetate, propionate and butyrate of the lima bean pod hemicellulose with organic solvents into soluble and insoluble fractions failed to accomplish an appreciable change in xylan content of the regenerated hemicellulose, which indicated that the non-pentosan part is probably chemically combined with the xylan in agreement with earlier observations of hemicelluloses extracted from sapwood tissue.

ALBANY, CALIFORNIA RECEIVED JULY 26, 1947