precursor activity that are not suitable to other analytical methods.

ACKNOWLEDGMENT

The author expresses appreciation to Dr. Derek Ball, Dr. Frank Bissett, and Leo Holmes for assistance during the research and interpretation of results.

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Received for review March 7, 1979. Revised manuscript received November 26, 1980. Accepted January 18, 1982. This paper reports research undertaken at the U.S. Army Natick Research and Development Command and has been assigned No. TP 2024 in the series of papers approved for publication. The findings in this paper are not to be construed as an official Department of the Army position.

Isolation, Purification, and Characterization of a Complex Heteroxylan from Industrial Wheat Bran

Jean-Marc Brillouet,* Jean-Paul Joseleau, Jean-Pierre Utille, and Dominique Lelièvre

A complex hemicellulosic heteroxylan was isolated from industrial wheat bran and was mainly constituted of equivalent amounts of arabinose and xylose. Minor quantities of galactose, glucose, and uronic acid were also present. Wheat bran heteroxylan showed a highly branched structure characterized by a β-(1→4)-xylan backbone branched by very short side chains of α-(1→2)- and α-(1→3)-linked arabinose and several doubly branched xylosyl residues carrying single terminal arabinosyl units. Glucuronic acid and its 4-*O*-methyl ether are also present in terminal nonreducing positions on side chains. This highly branched structure could be related to the strong water retention power exhibited by wheat bran.

In recent years, considerable attention has been given to dietary fibers, a fibrous constituent of human food. Dietary fiber rich materials are mainly constituted of cell wall polysaccharides, namely, cellulose and hemicelluloses. Wheat bran, one of the major sources of fiber, has been extensively studied for its physiological (Cummings et al., 1978) and nutritional properties (Southgate et al., 1976; Nomani et al., 1979). Furthermore physicochemical characteristics of wheat bran, mainly swelling and water retention capacity, have been investigated (Rasper, 1979) and compared to biochemical, physiological, and nutritional data (Bertrand et al., 1981).

Nevertheless, attempts to relate these various parameters to macromolecular structure of involved polysaccharides have been limited by the scarcity of fundamental data.

Most of previous biochemical studies on wheat bran concerned proximate analyses (Fraser and Holmes, 1959; Lee and Stenvert, 1973) or partial characterization of structural carbohydrates (D'Appolonia and Mac Arthur, 1976). More recently, some authors fractionated wheat bran into classes of various polysaccharides according to their solubility in different solvents (Schweitzer and Wursch, 1979; Theander and Åman, 1979; Ring and Selvendran, 1980; Anderson and Clydesdale, 1980). Adams (1955) isolated a complex arabinoxylan from bee-wing wheat bran and partially elucidated its structure (Adams and Bishop, 1956; Schmorak et al., 1957). This hemicellulose was characterized by permethylation technique, periodate oxidation, graded acid hydrolysis, and reducing power measurement. Moreover, aldobiouronic acids from the parent molecule were studied.

As part of a more general research program on industrial wheat bran, we have reexamined the isolation, purification, and structural characterization of the major hemicellulosic material of wheat bran with the purpose of bringing out possible relationships between structural data on bran polysaccharides and observed effects.

EXPERIMENTAL SECTION

Chemicals. Larchwood xylan and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide were purchased from Sigma Chemical Co. (St. Louis, MO). *m*-Phenylphenol was from Eastman Kodak Co. (Rochester, NY). α-Galactosidase from green coffee beans (EC 3.2.1.22; 50 units/mL),

I.N.R.A., Centre de Recherches Agro-Alimentaires, Laboratoire de Biochimie des Aliments, Chemin de la Géraudière, 44072-Nantes Cedex, France, and Centre de Recherches sur les Macromolécules Végétales, C.N.R.S., 53 X, 38041-Grenoble, France.

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β-galactosidase from *Escherichia coli* (EC 3.2.1.23; 150 units/mL), β-galactose dehydrogenase from *Pseudomonas fluorescens* (EC 1.1.1.48; 5 units/mL), and β-nicotinamide adenine dinucleotide (NAD) were purchased from Boehringer (Mannheim, Germany). Other chemicals were of the best quality.

Wheat Bran. Industrial wheat bran (*Triticum vulgare*) was provided by Grands Moulins de Paris (France) and was constituted of pericarp, aleurone layer, and part of the starchy endosperm. Its composition has been already mentioned (Brillouet and Mercier, 1979, 1981). Wheat bran was milled to pass 0.5-mm screen.

Preparation of Hemicellulose B from Wheat Bran. Fractionation procedure for preparation of hemicellulose B from wheat bran has been already described (Brillouet and Mercier, 1979, 1981; Lelièvre et al., 1980): it mainly consisted of chloroform–methanol (2:1) extraction, enzymatic destarching, oxalate extraction, chlorite delignification, and finally 1 N sodium hydroxide extraction of hemicelluloses. Hemicellulose A was precipitated by acidification to pH 4.8 of the alkaline extract and discarded by centrifugation. Hemicellulose B was recovered by ethanol precipitation of the dialyzed supernatant and dried by solvent exchange.

Hemicellulose B was further purified by redissolution in 1 N NaOH containing 1% $NaBH_4$ and acidification of the alkaline solution to pH 4.8 by glacial acetic acid while keeping the solution at 4 °C. After centrifugation at 10000*g* for 30 min and dialysis against distilled water of the supernatant, hemicellulose B was recovered by adding 5 volumes of ethanol, centrifuging, and drying by solvent exchange through ethanol, acetone, and ether. The entire purification procedure was repeated 3 times (~13% yield/starting bran).

Isolation of Wheat Bran Heteroxylan. Hemicellulose B (1% in water) was further fractionated into nine fractions by stepwise increasing ethanol concentration (0–90%). Each fraction was weighed and analyzed for neutral sugars (GC) and uronic acid (colorimetric analysis) content as further described. Material precipitating from 60 to 90% ethanol was selected, redissolved in water, reprecipitated by ethanol (90% final concentration), and dried (~70% yield/hemicellulose B). This fraction was afterward named wheat bran hemicellulose or heteroxylan.

Preparation of Xylooligosaccharide Standards. The occurrence of β-(1→4)-linked xylan backbone in wheat bran hemicellulose was tested by comparing the chromatographic behavior of oligosaccharides released by partial acid hydrolysis (see below) to an homologous sery of β-(1→4)-linked xylooligosaccharides prepared as follows. Larchwood xylan was purified by barium chloride precipitation as described by Taiz and Honigman (1976): the entire procedure was repeated 5 times (~30% yield; xylose/uronic acid = 9). The purified xylan (20 mg) was partially hydrolyzed in 2 mL of 0.03 N trifluoroacetic acid (TFA) at 120 °C for 30 min. The hydrolysate was evaporated to dryness under vacuum at 40 °C, 4 mL of distilled water was added, and the sample was reevaporated. The procedure was repeated twice until there was no more acid. Acidic components were eliminated with Dowex 1X-8 resin (1 g in 4 mL of water, formate form); the sample was filtered on a glass crucible, and resin was washed with 10 mL of water. Then the sample was evaporated to dryness, and 0.1 mL of water was added before thin-layer (TLC) and high-pressure liquid chromatography (HPLC).

Partial Acid Hydrolyses of Wheat Bran Hemicellulose. The configuration and location of arabinosyl residues in the molecule were tested by heating samples of 5 mL of 0.1% hemicellulose in 0.01 N trifluoroacetic acid (pH ~2.0) at 100 °C for varying times. Then hydrolysates were evaporated to dryness under vacuum at 40 °C, 5 mL of distilled water was added, and samples were reevaporated. The procedure was repeated until there was no more traces of acid. After freeze-drying, 1 mL of water was added before TLC and HPLC analysis. Substantial amounts of acid-degraded polysaccharide were prepared in a similar way by heating 100 mL of 1% hemicellulose in 0.01 N trifluoroacetic acid at 100 °C for 6 h. After evaporation under reduced pressure, the solid residue was resuspended in distilled water and dialyzed. The suspension was then added with 5 volumes of ethanol and centrifuged. The resulting degraded polysaccharidic material was then dried by solvent exchange (yield ~23%/native hemicellulose) and used for methylation and NMR studies as compared to native hemicellulose.

Furthermore, with the aim to characterize some of the constitutive oligosaccharides in the molecule, 60 mg of the native hemicellulose was treated in a similar way as larchwood xylan (see above), and released oligosaccharides were analyzed by TLC and HPLC.

α- and β-Galactosidase Treatment of Wheat Bran Hemicellulose. The anomery of linkage between terminal galactosyl residues and the core of the molecule was checked by treating the polysaccharide either with α- or β-galactosidase in the following manner: 5 mL of 1% hemicellulose in 20 mM acetate buffer, pH 4.5, was incubated at 30 °C for 24 h after adding 20 μL of α-galactosidase suspension. β-Galactosidase treatment was achieved under same conditions by using 100 mM phosphate buffer (1.3 mM $MgSO_4$–0.02% sodium azide), pH 7.5. Occurrence of liberated galactose was tested by TLC or by using the β-galactose dehydrogenase–NAD system (Boehringer, 1978).

Analytical Methods. Moisture content was determined by drying at 130 °C for 2 h. All compositions are given on moisture-free basis. Uronic acids were measured as glucuronic acid by the *m*-phenylphenol method (Blumenkrantz and Asboe-Hansen, 1973). Polysaccharides were hydrolyzed by 2 N trifluoroacetic acid (1 h; 120 °C) (Albersheim et al., 1967) or with pretreatment by 72% sulfuric acid (2 h; 20 °C) and then dilution to 2 N and heating (2.5 h; 100 °C) (Saeman et al., 1954). After neutralization with saturated barium hydroxide, neutral sugars were reduced, acetylated (Sawardeker et al., 1965), and analyzed by gas–liquid chromatography as their alditol acetates as described further.

Sedimentation analysis was performed with a Beckman E analytical ultracentrifuge at a rotor speed of 40000 rpm at 25 °C (0.7% solution in 0.1 N NaOH). Viscosity measurements were made in a Fica viscosimeter (Versailles, France) at 25.00 ± 0.05 °C (in 0.05 N NaOH). Optical rotation was measured with a Schmidt Haensch Polartronic I polarimeter (Berlin, Germany) at 20 °C (c = 1% in 0.05N NaOH).

Chromatographic Methods. Mono- and oligosaccharides were identified by high-performance thin-layer chromatography (TLC) on 10 × 10 cm silica plates of Kieselgel 60 (Merck, Darmstadt, Germany) by using the solvent system acetonitrile–water (85:15). Samples were diluted twice with 2-propanol before spotting. Chromatoplates were developped 3 times, and sugars were detected with diphenylamine–aniline reagent (Buchan and Savage, 1952) by heating at 110 °C for 20 min.

Mono- and oligosaccharides from partial acid hydrolyses studies were analyzed by high-pressure liquid chromatography (HPLC) with a Waters Associates chromatograph

Table I. Yield and Sugar Composition of Fractions from Stepwise Ethanol Fractionation of Hemicellulose B

	ethanol, %								
	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90
yield[a]	1.9	4.0	12.2	4.4	3.7	2.9	44.3	22.2	4.4
sugar									
arabinose[b]	0.09	0.18	0.21	0.31	0.42	0.56	1.09	1.14	1.14
xylose[b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
galactose[b]	0	0	0	0	0	0.02	0.06	0.06	0.06
glucose[b]	0.7	1.30	4.24	1.13	0.70	0.30	0.04	0.02	0.02
uronic acid[c]	1.2	1.9	1.1	2.1	1.6	1.4	5.4	5.6	5.7

[a] Percent of hemicellulose B. [b] Molar ratios relative to xylose. [c] Percent of dry weight.

Table II. Analytical Composition of Original and Acid-Degraded Wheat Bran Hemicellulose

	%[a]					
hemicellulose	Ara	Xyl	Gal	Glc	4-OMeGlc	uronic acid[b]
original	48	41	3.5	2.5		5.9
partially degraded	12.5	64	3.2	10		11
partially degraded and carboxyl reduced	14.4	60.1	3.3	21.8	3	

[a] Percent of dry weight. [b] Determined colorimetrically.

(Milford, MA) equipped with a Waters 6000 A pump and fitted with a (250 × 4.6 mm i.d.) column packed with Lichrosorb NH_2 (particle size 5 μm) (Merck). Elution conditions were as follows: solvents were acetonitrile–water mixtures, 85:15 for monosaccharides and 70:30 for oligosaccharides; flow rate was 2 mL/min; ambient temperature. Sugars were detected with a Waters R401 differential refractometer.

Gel filtration of the purified wheat bran hemicellulose was conducted on a 900 × 26 mm i.d. glass column filled with Sepharose CL-2B (Pharmacia, Upsala, Sweden) at room temperature in 0.3% NaCl; the flow rate was 18 mL/h; polysaccharide was detected in the eluate by the phenol–sulfuric acid method (Dubois et al., 1956).

Alditol acetates of monosaccharides from complete acid hydrolyses of polysaccharides were analyzed by gas–liquid chromatography (GC) with a Girdel 300 chromatograph (Girdel, Puteaux, France) fitted with a (180 cm × 2 mm i.d.) glass column packed with 3% SP 2340 coated on 100–120-mesh Supelcoport (Supelco, Bellefonte, PA). Operating parameters were as follows: nitrogen carrier gas, 20 mL/min; hydrogen, 20 mL/min; air, 400 mL/min; column temperature, 225 °C; injector temperature, 250 °C; flame ionization detector temperature, 250 °C. Inositol was used as the internal standard.

Carboxyl Reduction of Polysaccharides. This was performed according to Taylor and Conrad (1972). To an aqueous solution of polysaccharide (100 mg) adjusted at pH 4.75, 50 mg of solid 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide was added progressively. The pH was maintained at 4.75 by addition of 0.1 M hydrochloric acid. A solution of 3 M sodium borohydride ($NaBH_4$) was then added to a final concentration of 2 M and the pH maintained at 7.0 during the reduction. After dialysis, the reduced polysaccharide was freeze-dried.

Methylation of Polysaccharides. Native and acid-degraded polysaccharides were methylated by the method of Hakomori (1964). The material recovered by lyophilization was submitted to two additional methylation reaction according to Purdie and Irvine (1903). The permethylated polysaccharides were then hydrolyzed with 90% formic acid at 100 °C for 1 h and then with 2 M trifluoroacetic acid for 3 h at 100 °C, and the partially methylated sugars were determined by GC–MS of their alditol acetate derivatives on a Girdel 3000 chromatograph coupled to a AE I MS-30 mass spectrometer (Jansson et al., 1976). Spectra were recorded at 70 eV with an ionization current of 100 μA and an ion-source temperature of 100 °C.

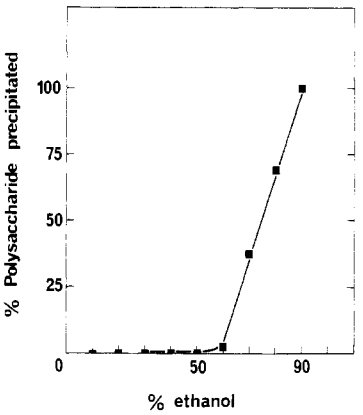

Figure 1. Solubility of wheat bran hemicellulose in ethanol–water mixtures.

NMR Analysis. Proton-decoupled, ^{13}C NMR spectra were recorded at 62.86 MHz with a CAMECA 250 spectrometer equipped with Fourier transform (pulse width, 13 μs (≃90 °C); acquisition time, 0.6 s; spectral windows, ~200 ppm). Original and partial hydrolyzed polysaccharide samples were examined as solutions in D_2O at 80 °C (150 mg in 1.5 mL, with tubes of 8-mm o.d.); chemical shifts were measured relative to internal nondeuterated acetone and converted into values relative to tetramethylsilane, according to the equation $\delta(Me_4Si) = \delta(\text{acetone}) + 30.4$ ppm (Stothers, 1972).

RESULTS AND DISCUSSION

Isolation and Purification of Wheat Bran Hemicellulose. Industrial wheat bran was chosen as the starting material since this rough and complex material was more representative of available french fiber rich products than bee-wing bran (Adams, 1955; Ring and Selvendran, 1980). Hemicellulose B extracted from this material was shown to be constituted of at least two polysaccharidic populations; the major one, corresponding to ~50% of total hemicelluloses of wheat bran, precipitated in the range 60–90% ethanol and was found homogeneous for neutral and acidic sugars composition in the whole alcohol fractionation range as indicated in Table I. Carbohydrate composition of this purified wheat bran hemicellulose is reported in Table II. The isolated po-

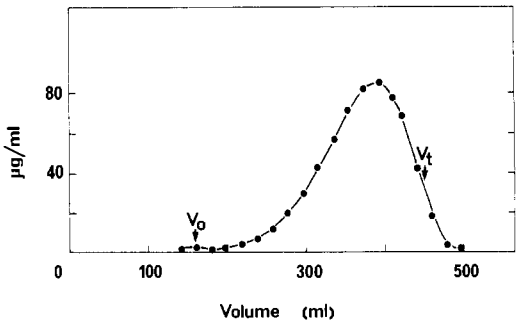

Figure 2. Gel filtration profile of wheat bran hemicellulose on Sepharose 2B.

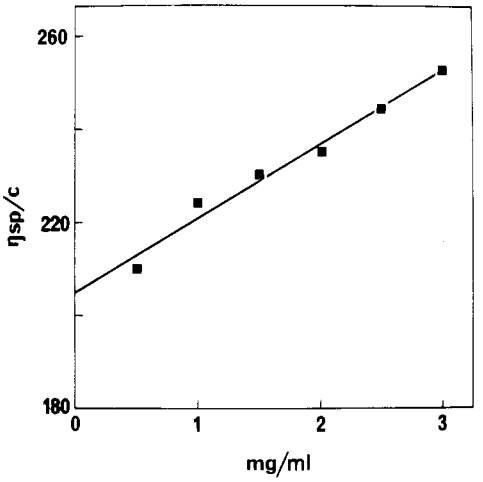

Figure 3. Reduced viscosity (η_{sp}/c)/concentration curve for wheat bran hemicellulose.

lysaccharide is mainly constituted of xylose and arabinose with a molar ratio of 0.85:1 (relative to arabinose); minor amounts of galactose, glucose, and uronic acid are also present.

Solubility of wheat bran hemicellulose in ethanol–water mixtures is extremely high as indicated by Figure 1 and is likely indicative of a strongly branched polymeric structure. Furthermore, the very high hydrophilicity of this hemicellulosic material could be related with the strong water retention capacity exhibited by wheat bran (Rasper, 1979). The gel filtration chromatography profile of wheat bran hemicellulose on Sepharose 2B is shown in Figure 2. The polysaccharide is eluted in a single peak between the void volume (V_0) and total bed volume (V_t). The purified hemicellulosic material gave a single and symmetric peak on ultracentrifugation analysis. The viscosity/concentration curve is presented in Figure 3: a linear relationship was obtained, giving an intrinsic viscosity of 208 mL/g; no attempt was made to calculate the degree of polymerization from coefficients measured by Husemann (1940) for beech xylan since structural features indicate a very different shape for wheat bran hemicellulose as compared to that for more linear xylans. Optical rotation, $[\alpha]^{20}_D$, was −1.6°, which indicates a rough equivalence between α and β linkages in the molecule.

Partial Acid Hydrolysis Studies. Results of hydrolysis of wheat bran hemicellulose by very dilute trifluoroacetic acid (0.01 N) are indicated in Figure 4. Arabinose was released rapidly during the first 6 h (~40%/total arabinosyl residues) with practically no liberation of xylose (~3–4%/total xylosyl residues). Then the rate of splitting of arabinose was strongly decreased while liberation of xylose still proceeded at a constant speed. Moreover, galactosyl component was also released

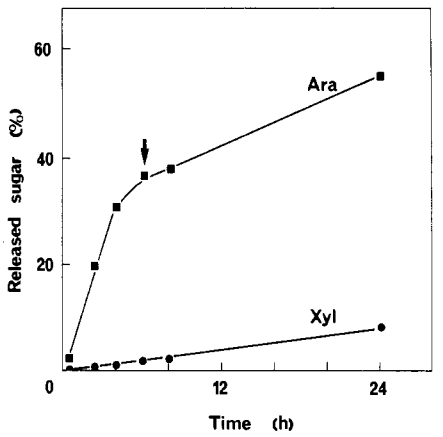

Figure 4. Progressive acid hydrolysis of wheat bran hemicellulose by dilute trifluoroacetic acid. Released sugars, (Ara) arabinose and (Xyl) xylose, are expressed as percent of maximum sugar content in the polysaccharide. The arrow indicates the choosen time for preparation of acid-degraded wheat bran hemicellulose.

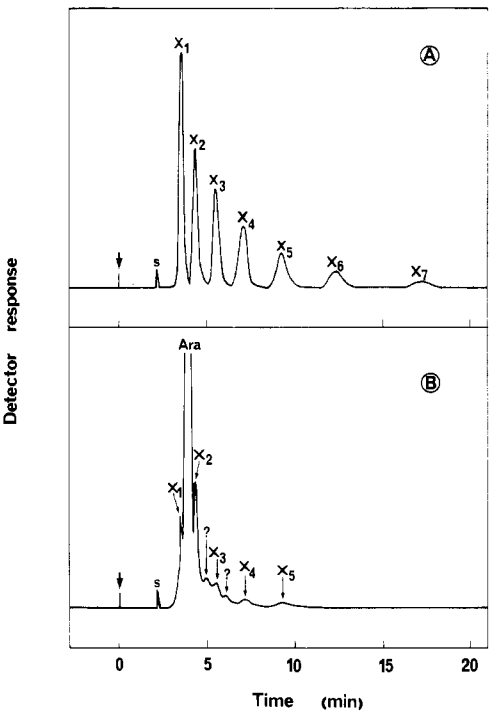

Figure 5. High-pressure liquid chromatograms of partial acid hydrolysates of (A) larchwood xylan and (B) wheat bran hemicellulose. (S) Solvent; (?) unknown oligosaccharides.

in small amounts. Oligosaccharide release was negligible. Consequently, at least 40% of arabinosyl residues were present in the usual furanoside form as readily split end groups which corresponds to previously observed data on bee-wing bran hemicellulose (Adams, 1955). The reduced rate of arabinose release observed after 6 h of hydrolysis indicates that the remaining portion of arabinose units could be on a nonterminal position. Considerable differences of dilute acid sensitivity of xylose units exist between polysaccharide purified by Adams (1955) and our hemicellulosic material: the xylosyl moiety seems to be far more sensitive in the former since ~30% is released after 6 h of hydrolysis under similar conditions.

Results from HPLC analysis of partial acid hydrolysates of larchwood xylan and wheat bran hemicellulose are indicated in Figure 5. After elimination of acidic components by anion-exchange resin, the larchwood xylan hydrolysate showed the presence of homologous β-(1→4)-linked xylooligosaccharides (xylose X_1 → xyloheptaose X_7),

which were well separated under conditions used for HPLC analysis; no traces of arabinose were found, contrary to Aspinall and McKay (1958). Wheat bran hemicellulose gave on partial hydrolysis mainly arabinose which confirms the above statement about end group location under furanosidic form. Xylose (X_1) to xylopentaose (X_5) were identified in the hydrolysate of our hemicellulosic fraction as compared to the elution volume of xylo-oligomers from larchwood xylan. X_1 and X_2 were produced in noticeable amounts, whereas very small quantities of higher oligomers were observed as compared to larch hydrolysate. Occurrence of a β-(1→4)-xylan backbone in wheat bran hemicellulose could therefore be inferred from above observations. Moreover, two additional unknown oligosaccharides were produced and might be related to various branched xylose residues occurring in the molecule.

Structural Analysis. Structural study was carried out on the purified wheat bran hemicellulose and the material resulting from the selective hydrolysis of arabinose with dilute trifluoroacetic acid. Total acid hydrolysis of the original and trifluoroacetic acid degraded material showed that the ratio of xylose to arabinose which was 0.85 in the wheat bran hemicellulose was raised to 5.07 in the degraded xylan. This decrease of the arabinose content was accompanied by an increase in the uronic acid content from about 6% in the intact hemicellulose to 11% in the degraded polysaccharide (Table II). Hydrolysis of the carboxyl-reduced partially hydrolyzed hemicellulose (Taylor and Conrad, 1972) showed the presence of a peak corresponding to 4-*O*-methylglucitol in the chromatogram of the alditol acetate derivatives. By comparison of the proportion of this peak to that of the glucitol coming from the carboxyl-reduced uronic acid, it could be deduced that about 40% of the glucuronic acid of the hemicellulose was present as its 4-*O*-methyl ether. The simultaneous presence of the two forms of uronic acids is a general trend in the hemicellulose from Graminae (Wilkie, 1979).

The wheat bran heteroxylan, the partially acid degraded xylan, and the carboxyl-reduced degraded xylan were subjected to methylation by the method of Hakomori (1964). Permethylation of the polysaccharides was ensured by remethylating them with methyl iodide–silver oxide (Purdie and Irvine, 1903).

The hydrolysates were examined by gas–liquid chromatography of the alditol acetate derivatives, and identification of the peaks was made by comparison to authentic partially methylated standards. Confirmation of the identity of the methylated derivatives was achieved by GC–MS (Jansson et al., 1976). From the results in Table III, it can be seen that the arabinoglucuronoxylan from wheat bran has a highly branched structure, as indicated by the great proportion of terminal nonreducing sugars. The ratios of 2,3-di-*O*-methylxylose, mono-*O*-methylxylose, and xylose in the hydrolysate of the original methylated polysaccharide of 15.4, 14.3, and 15.9, respectively, show that there are about as many unsubstituted xylose residues as there are of monosubstituted and doubly branched xylose residues. This substitution pattern on the 1→4-linked xylosyl backbone agrees with the high proportion of terminal residues: 2,3,5-tri-*O*-methylarabinose, 2,3,4-tri-*O*-methylxylose, and 2,3,4,6-tetra-*O*-methylgalactose in the native wheat bran hemicellulose and 2,3,4,6-tetra-*O*-methylglucose in the carboxyl-reduced TFA-treated hemicellulose. Contrary to the results described previously by Adams (1955), no branching through arabinose residues could be observed since there was no peak corresponding to the monomethylarabinose derivative in the chromatogram. The rather bad correspondence between branched and terminal residues reported by Adams (1955) might be likely due to undermethylation of arabinosyl components. Therefore, most of the branchings take place through xylosyl residues on O-2 and/or on O-3 by arabinofuranosyl groups. Terminal xylosyl, galactosyl, and glucuronosyl (or 4-*O*-methyl ether) also could be possible substituents. From the respective proportions of tri-*O*-methyl- and di-*O*-methylarabinose derivative, it can be concluded that most of the substitutions involve single units of arabinose. When arabinose does not occupy a terminal position, it is 1→2- or 1→3-linked, as indicated by the presence of 3,5- and 2,5-di-*O*-methylarabinose, likely forming short chains branched on the xylosyl backbone. It is noteworthy that in the methylation analysis of the trifluoroacetic acid degraded xylan, a great part of the unmethylated xylose is missing by comparison to the analysis of the original polysaccharide. This is to relate to the nearly total disappearance of 2,3,5-tri-*O*-methylarabinose and the proportional increase in 2,3-di-*O*-methylxylose. It is thus clear that nearly all of the doubly branched xylose residues were substituted with single-terminal arabinose units. Also, it can be seen that the 1→3-linked arabinofuranosyl units were more susceptible to the partial acid hydrolysis treatment than the 1→2-linked residues. This is indicative of a difference in the rate of hydrolysis of the arabinofuranosyl residues which depends on the mode of interglycosidic linkage (Joseleau and Gancet, 1981) and would thus explain the resistance of a part of the arabinose during the trifluoroacetic acid treatment (Figure 3). Another likely explanation for the remaining of some arabinose residues after mild acid hydrolysis is in the presence of a trisaccharide *O*-xylosyl-(1→2)-*O*-arabinofuranosyl-(1→2)-arabinofuranosyl as a side chain. Such a trisaccharide is expected to hydrolyze into xylosyl-(1→2)-*O*-arabinose and leave an arabinosyl residue linked on the main chain of the xylan (Wilkie and Woo, 1977).

Even after the purification process, some galactose was still present and is certainly part of the heteroxylan from wheat bran as indicated by its appearance only in the 60–90% ethanol fractionation range (Table I). This galactose which amounts to about 3% in the original hemicellulose was essentially found under the form of 2,3,4,6-tetra-*O*-methylgalactose with traces of 2,3,6-tri-*O*-methylgalactose. This shows that like many heteroxylans from Gramineae, galactose occupies mainly a terminal position on side chains (Wilkie and Woo, 1977). Only α-galactosidase released galactose from wheat bran hemicellulose after prolonged incubation. Consequently, terminal galactosyl residues seemed to be on the α-pyranoside form. Structural localization of glucuronic acid and its 4-*O*-methyl ether was achieved by methylation analysis of the carboxyl-reduced polysaccharide. Best results were obtained by using the partially degraded hemicellulose (Table III). The occurrence of 2,3,4,6-tetra-*O*-methylglucose in the chromatogram of the carboxyl-reduced material indicates that part of the uronic acids occupies a terminal position either directly attached to the xylan backbone or through xylosyl side chains. Part of the glucuronic acid must be 3 linked due to the appearance of 2,4,6-tri-*O*-methylglucose in carboxyl-reduced TFA-treated hemicellulose (Table III). This very unusual structural feature would need more work to be confirmed but could partly explain the relative resistance of a fraction of terminal arabinosyl (partial acid hydrolysis studies) if it is linked to glucuronosyl moieties.

In the fraction under study, a hemicellulosic glucan must be associated with the heteroxylan as shown by the 2,3,6-

Table III. Methylation Analysis of Native and Acid-Degraded Wheat Bran Hemicellulose

methylated sugar derivatives[a]	T^b	native wheat bran hemicellulose[c]	TFA-treated hemicellulose[c]	carboxyl-reduced TFA-treated hemicellulose[c]
2,3,5-Me_3Ara	0.59	30.6	3.5	3.8
2,3,4-Me_3Xyl	0.77	9.8	11.9	8.7
3,5-Me_2Ara	0.95	4.5	5.9	4.9
2,3,4,6-Me_4Glc	1.00			7.3
2,5-Me_2Ara	1.07	6.7	1.3	1.8
2,3,4,6-Me_4Gal	1.14	1.0	1.1	1.4
2,3-Me_2Ara	1.22	t	t	t
2,3-Me_2Xyl	1.35	15.4	43.7	31.3
2,4,6-Me_3Glc	1.46	t	3.6	12.1
2,3,6-Me_3Gal	1.70		t	t
2,3,6-Me_3Glc	1.76	1.8	9.5	8.4
2(3)-MeXyl	1.88	14.3	16.6	16.5
Xyl	2.40	15.9	2.8	3.6

[a] Alditol acetate derivatives: 2,3,5-Me_3Ara = 2,3,5-tri-*O*-methylarabinose, etc. [b] Retention time relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. [c] Data expressed as relative mole percent.

Table IV. ^{13}C NMR Data of Wheat Bran Hemicellulose: Assignments of the Main Identified Peaks

residues	carbons	references
α-L-arabinofuranosyl	C-1, 98.9–108.0; C-5, 61.01	Joseleau et al. (1977)
β-(1→4)-D-xylopyranosyl (main chain)	C-1, 101.84; C-2, C-3, and C-4, 72.90, 73.97, and 76.64; C-5, 63.22	Utille (1979b)
terminal β-D-xylopyranosyl	C-1, ~101.84; C-2, C-3, and C-4, 73.15, 75.91, and 69.46; C-5, 65.45	Utille (1979b)
α-D-glucopyranosyl	C-1, 98.15; OMe, 59.30; C-5, 63.11	Joseleau and Utille (1979)
β-(1→4)-D-glucopyranosyl	C-1, 102.50; C-6, 60.48	Gagnaire et al. (1978)

and 2,4,6-tri-*O*-methylglucose found among the products from methylation analysis. The amount of glucan is very small in the original polysaccharide but is proportionally greater in the degraded xylan. As expected (Buchala and Wilkie, 1971), there are more 1→4- than 1→3-glucosidic linkages in this accompanying glucan.

NMR Study. The highly branched structure of the heteroxylan from wheat bran represented a good substrate for our studies of the correlation between chemical analysis and ^{13}C NMR spectroscopy (Joseleau et al., 1977; Utille, 1979a) of hemicelluloses. Examination of the ^{13}C NMR spectrum of the original hemicellulose from wheat bran shows the complexity of the structure exhibited by the numerous peaks present in the anomeric region between δ 98.9 and 108.0 (Figure 6). However, two main groups of signals could be distinguished, one centered at δ 106.4 and one centered at δ 100.4. By comparison to the spectra of reference compounds of known structures, it was possible to identify these two groups of signals and relate them to the dominant analytical features of the heteroxylan from wheat bran.

The low-field group of signals is typical of the chemical shifts observed for the anomeric carbons of α-L-arabinofuranosyl residues (Joseleau et al., 1977). From the value of the chemical shifts, it can be concluded that all these arabinose residues are in their α configuration. Similarly, the group of signals at δ 100.4 corresponds to the β-D-xylopyranosyl residues of the heteroxylan (Utille, 1979a). When this spectrum was compared to that of the partially hydrolyzed hemicellulose, it was clear that the degraded polysaccharide had a structure resembling to that of a true arabinoxylan (esparto grass) (Utille, 1979b) as shown by the great similarity between their spectra (Figure 6).

The anomeric region of the C-1 of the arabinosyl units shows multiple peaks. This denotes that the anomeric carbons of the differently linked arabinosyl residues undergo a slight shift which depends on their mode of linkage. As can be seen from the methylation analysis, three different kinds of partially methylated arabinose derivatives were obtained. This shows that arabinose exists in the hemicellulose in at least three types of situations. However, it is not surprising to see more than three peaks in this region since it is well established that the chemical shifts of the anomeric carbons are not only sensitive to the substitution pattern of the sugar residue but also are influenced by the neighboring glycosidically linked sugars. It is nevertheless tempting to assign the main peak at δ 77.532 to the terminal arabinosyl residue, the predominent type present in the hemicellulose. The second main anomeric group of signals belongs to the β-D-xylosyl residues. Here again, the multiplicity of the signals centered at δ 100.4 is characteristic of a highly substituted xylan structure. No peak is really dominant among this group of signals. This is in agreement with the fact that there are very few "open regions" in the structure, that is, no long part of contiguous unsubstituted β-(1→4)-linked xylosyl residue, as it is the case in the reference esparto grass xylan (Figure 6). On the other hand, this type of structure clearly becomes predominant after mild acid hydrolysis of the heteroxylan, and its spectrum affords characteristic peaks for the C-1, C-2, C-3, C-4, and C-5 of a β-(1→4)-linked backbone of unsubstituted xylan residue (Utille, 1979b) (Table IV).

Another evidence of the numerous terminal xylose residues in the original hemicellulose is given by the presence of signals at 65.452, 69.457, 73.147, and 75.914 ppm corresponding respectively to C-5, C-4, C-2, and C-3 of end residues as observed for the terminal nonreducing β-xylopyranosyl unit of xylobiose and for the linear xylose oligomers (Utille, 1979a). This was also confirmed by the methylation analysis, as it was anticipated by the isolation of branched oligosaccharides in HPLC analysis (Figure 5).

Aside from the main structural characteristics of highly branched structure, the ^{13}C NMR spectrum of the degraded wheat bran xylan shows a peak at δ 59.216 which was assigned to a methyl carbon atom and was indicative of the presence of 4-*O*-methylglucuronic acid in the molecule. The spectra of the original and degraded hemicellulose showed also the presence beside the heteroxylan

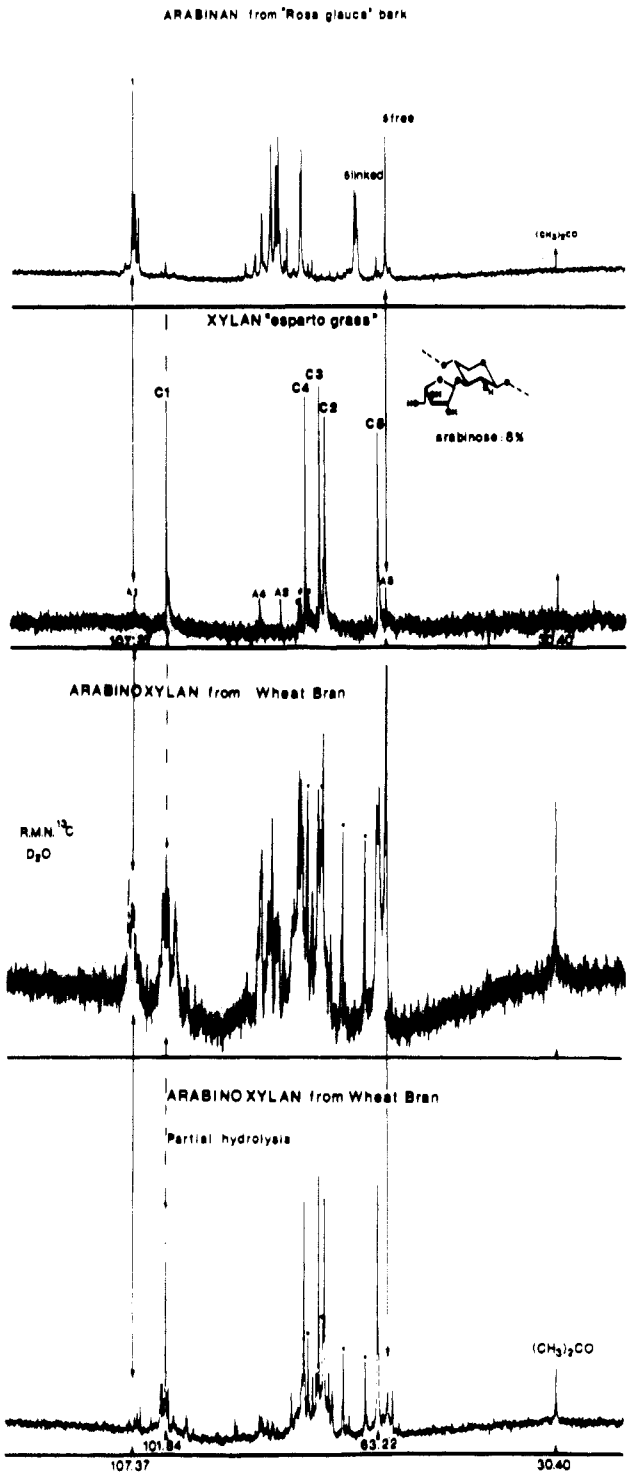

Figure 6. NMR spectra of arabinan (*Rosa glauca* bark) (Joseleau et al., 1977), xylan (esparto grass) (Utille, 1979), native arabinoxylan (wheat bran), and acid-degraded arabinoxylan (wheat bran).

of a contaminant β-D-glucan. This glucan is a β-(1→3),-(1→4)-linked heteroglucan as indicated by the results of methylation analysis (Table III).

This accompanying glucan is a common aspect of many hemicelluloses from Gramineae (Wilkie and Woo, 1977). Through the ^{13}C NMR spectrum, it could be determined that the β-(1→4) linkages are dominant since no individual signals for the β-(1→3)-glucosyl linkages could be identified. The structural information gained through the spectroscopic study is important since this technique is nondestructive and allows a direct observation of the polysaccharide. This is particularly evidenced in the comparison between the original heteroxylan and partially degraded heteroxylan.

ACKNOWLEDGMENT

Thanks are due to the Centre Grenoblois de Résonance Magnétique Nucléaire de Haute Résolution for the use of the CAMECA NMR facilities. We also thank C. Bosso, C.E.R.M.A.V.-C.N.R.S., for recording the mass spectra.

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Received for review June 1, 1981. Revised manuscript received November 3, 1981. Accepted January 6, 1982.

Direct Characterization of Nutmeg Constituents by Mass Spectrometry-Mass Spectrometry

Dean V. Davis and R. Graham Cooks*

Constituents of *Myristica fragrans* (nutmeg) in the molecular weight range 100–700 amu are characterized via MS–MS (mass spectrometry–mass spectrometry). The nutmeg sample is analyzed directly; no extraction, derivatization, or other sample preparation is necessary. The analysis is facilitated by temperature profiling and by utilizing different reagent gases in the chemical ionization (CI) source of the mass spectrometer. Data were taken with both a sector type and a triple quadrupole MS–MS instrument. Compounds were characterized by recording the mass spectrum of fragments formed by collision-induced dissociation of particular ions, often the protonated molecules. Groups of compounds with particular structural units were characterized by scans of all precursor ions which yield mass-selected fragment ions. This also was used to pinpoint instances where MS–MS spectra contained contributions from more than one structural isomer. In this study, one new compound, a dehydrodiphenylpropanoid derivative of myristicin, was identified. Correlation of the results with literature data demonstrates that analysis of foodstuffs by MS–MS is rapid and provides detailed structural information.

Mass spectrometry–mass spectrometry (MS–MS) is a developing technique for the identification and quantitation of organic constituents present in complex mixtures (Hunt et al., 1980; Kondrat and Cooks, 1978; Unger and Cooks, 1979; Shushan and Boyd, 1980). Due to the large variety of samples which have been analyzed by MS–MS in the last several years, the methodology is fast becoming established as an alternative and/or supplement to gas chromatography–mass spectrometry (GC–MS). This study on the identification of constituents in the spice *Myristica fragrans* (nutmeg) was undertaken to explore some recent refinements in the MS–MS technique, including comparisons between data taken on a high energy sector instrument and a triple quadrupole device.

Up until now most MS–MS work on complex mixtures has been targeted at one or just a few prespecified components; however, the need exists for more complete characterization of such mixtures. The capability of MS–MS for such detailed characterization has not yet been demonstrated, with the possible exception of recent work on coal liquids (Zakett et al., 1981, 1979b; Wilson et al.; 1981; Hunt and Shabanowitz, 1981). This particular aim is here facilitated by using temperature profiling in conjunction with MS–MS. The complexity of the mixture actually present in the ion source at any time is reduced by repetitively recording mass spectra as a function of probe temperature. In other words, selective volatilization allows more complex mixtures to be studied without increasing the spectral complexity.

Department of Chemistry, Purdue University, West Lafayette, Indiana 47907.

Most of the data in this paper consist of daughter ion spectra as shown in eq 1. In addition, most of these data

$$m_1^+ \rightarrow m_2^+ + m_3 \quad (1)$$

fix m_1^+, scan m_2^+: daughter spectrum

fix m_2^+, scan m_1^+: parent spectrum

are recorded at high collision energy. Using a triple quadrupole instrument which utilizes much lower energy collisions, one can compare the effects of collision energy on MS–MS spectra and also access two other types of scans (Yost and Enke, 1979). Referring to reaction 1, one can fix m_2^+ and scan m_1^+, the so-called parent scan (Gallegos, 1976), or fix m_3 and scan m_1^+, termed a neutral loss scan (Lacey and MacDonald, 1979; Zakett et al., 1979a; Haddon, 1980). Both of these latter spectra have potential in identification of all individual compounds which contain a particular functional group. Investigation of the practical utility of these newer types of scans was one of the objectives of this study.

A further motivation behind this work was to test the potential of MS–MS in the area of foods analysis. Background on current techniques in this growing area of analysis is provided is recent reviews by Self (1979) and Kolor (1979). Analysis of foods by MS–MS is expected to be particularly attractive not only because it has the necessary speed and sensitivity but also because it should require little derivatization or other sample workup prior to analysis.

The complex mixture of organic species which make up nutmeg has been studied previously by both standard extraction techniques and by gas chromatography–mass spectrometry. Extraction and characterization of the

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