*Asian Journal of Chemistry* Vol. 19, No. 7 (2007), 5688-5694

# Structural Studies of a Hemicellulose Fraction Isolated from Groundnut Shell (*Arachis hypogea*)

A. HANNAN KHAN†, SHAKILA RAHMAN* and M. SHAHJAHAN†
*Department of Chemistry, Bangladesh University of Engineering and Technology Dhaka-1000, Bangladesh*
*E-mail: shaki@chem.buet.ac.bd*

The groundnut shell was found to contain moisture (8.50 %), ash (3.01 %), lignin (23.60 %), protein (6.30 %), silica (0.69 %), crude fibre (65.94 %) and total carbohydrate (15.00 %). Hemicellulose fractions were isolated from groundnut shell by successive extractions with alkali of increasing concentration. A xylan fraction was isolated by extraction with 1 M aqueous sodium hydroxide. Sugar, methylation, specific rotation and IR spectral analyses of the purified hemicellulose revealed that the xylan obtained from groundnut shell was essentially (1→4)-β-linked and had a 4-O-methylglucuronopyranosyl unit substituted at position 2 of almost 13th xylose residues.

**Key Words: *Arachis hypogea, Fabaceae*, Hemicellulose, Silylation, Decarboxylation, Alditol acetates and Xylan.**

## INTRODUCTION

Groundnut, also called peanut, earthnut, monkeynut[1] or goober, despite its several common names is not a true nut but the pod or legume, of *Arachis hypogea* (family *Fabaceae*), which has the peculiar habit of ripening under ground. Groundnut shell has been reported[2] to be useful in the treatment of hypertension. The Chinese report described the isolation of β-sitosterol, luteolin, daucosterol and an unidentified saponin. Analysis of carbohydrate materials has also been reported[3] by Indian workers. Hemicellulose has recently shown the possibility of wide use in paper industry[4], food[5,6] and medicine[7]. Modified and sulphated xylan[8] is used as a blood-anticoagulant. This paper describes the isolation of a hemicellulose xylan from the groundnut shell.

## EXPERIMENTAL

Matured groundnuts (*Arachis hypogea*) were collected from the neighbourhood of Dhaka city. The outer shells of the nuts were separated manually, sun-dried and grounded into powder.

†Department of Chemistry, Jagannath University, Dhaka, Bangladesh.

Moisture, ash, lignin[9], protein[10], silica[11], crude fibre[10] and total carbohydrate[10] contents (Table-1) of the powdered groundnut shell were determined by following standard procedures. All evaporations were carried out under reduced pressure at bath temperatures not exceeding 40ºC.

TABLE-1
MOISTURE, ASH, LIGNIN, PROTEIN, SILICA, CRUDE FIBRE AND TOTAL CARBOHYDRATE CONTENTS OF THE POWDERED GROUNDNUT SHELL

| Constituents | Quantity (%) |
|---|---|
| Moisture | 8.50 |
| Ash | 3.01 |
| Lignin | 23.60 |
| Protein | 6.30 |
| Silica | 0.69 |
| Crude fibre | 65.94 |
| Total carbohydrate | 15.00 |

**Paper chromatography[12]:** Paper chromatograms were run on Whatmann No. 1 filter paper. The following solvent systems were used (v/v proportions): (a) ethyl acetate:pyridine:water (10:4:3)[13] and (b) 1-butanol:pyridine:water (6:4:3).

A saturated aqueous solution of aniline oxalate was used as spray reagent followed by heating at 120ºC for *ca.* 5 min.

Uronic acids were determined by a decarboxylation method[14]. IR[15] spectra (thin film) of hemicelluloses were recorded on a PYE UNICAM SP1000 Infra red spectrophotometer using nujol mull. Gas liquid chromatography (glc) was conducted with a Packard 427 instrument fitted with a flame ionization detector and quartz capillary column (28 m × 0.02 cm i.d.). Separations were performed on (a) OV-275 at 140-200ºC. 4ºC $min^{-1}$ (alditol acetates), (b) OV-225 at 170ºC, isotherm (partially methylated alditol acetates) and CP Sil 5 at 150-230ºC, 2ºC $min^{-1}$ (trimethylsilyl derivatives).

**Extraction of the plant material:** Powdered groundnut shell 200 g was refluxed with aqueous 80 % ethanol (5 × 900 mL, 0.5 h each time) followed by chloroform (3 × 600 mL, 0.5 h each time). The dried extractive free material was treated with water (3 × 800 mL, 5 h each time) on a boiling water bath under reflux. All the extractives were evaporated to dryness using rotary vacuum evaporator followed by freeze-drying and the extracts were 3.5, 1.25 and 4.68 %, respectively.

After water extraction, dried extractive free powder 100 g was treated with aqueous sodium hydroxide (1 M, 2 × 700 mL) for 20 h at room temperature with stirring in an atmosphere of nitrogen. The combined extracts

were neutralized with aqueous 50 % acetic acid. The neutral extract was dialyzed, concentrated to a suitable volume and freeze-dried, affording hemicellulose $P_1$ (5.2 g). Successive extraction of the residual material with 2.5 and 4.5 M sodium hydroxide gave hemicellulose fractions $P_2$ (3.7 g) and $P_3$ (0.25 g), respectively.

**Analysis of aqueous 80 % ethanol extract:** A small portion (100 mg) of aq. 80 % ethanol extract was dissolved in ethanol and examined by paper chromatography in solvents A and B using spray solution followed by heating at 120ºC for *ca.* 5 min. It indicated the presence of D-xylose in large quantity. Again a part of the neutral fraction (5 mg) was analyzed by trimethylsilylation method where *myo* inositol (1 mg) was used as the internal standard. The resulting trimethylsilyl derivatives were analyzed by GLC (Table-2).

TABLE-2
RELATIVE COMPOSITION OF SUGAR CONSTITUENTS OF 80 % ETHANOL EXTRACT

| Sugar residues | Sugar constituents (%) |
|---|---|
| L-Rhamnose | 2.8 |
| L-Xylose | 60.9 |
| D-Mannose | 14.7 |
| D-Glucose | 21.6 |

**Fractionation of hemicellulose $P_1$**

**Ion-exchange chromatography:** A small portion (400 mg) of $P_1$ was dissolved in 30 mL of water containing a few drops of 1 M aqueous sodium hydroxide and applied on a DEAE-Sephadex A-50 column (2.7 × 68cm) in phosphate buffer (0.1 M, pH 6.5). The column was first eluted with the same buffer and the fractions were monitored by the phenol-sulphuric acid method[16]. No carbohydrate-containing fraction was obtained. Later the column was eluted with the buffer containing 1 M sodium chloride (2 L, pH 5.8). Absorbance of the coloured solutions resulting from the treatment of the fractions with phenol sulphuric acid was measured at 485 nm with reference to a blank test. Fractions 85-120 had maximum concentrations of carbohydrates. These fractions were combined, dialyzed and freeze-dried which afforded the hemicellulose $P_4$ (205 mg).

**Gel filtration:** The purified hemicellulose $P_4$ (190 mg) in phosphate buffer (12 mL, pH 7.0) was applied to Sepharose CL-2B gel column. The column was eluted with the same buffer and the fractions (3 mL) were tested by the phenol-sulphuric acid method. Only a single elution curve was obtained. The fractions containing polysaccharide were combined,

dialyzed and freeze-dried $P_4$ (150 mg). The specific rotation of purified hemicellulose $P_4$ was measured and found to be -92º.

**Analysis of hemicellulose $P_4$:** A small portion (100 mg) of purified hemicellulose fraction $P_4$ was hydrolyzed with 0.5 M sulphuric acid and the hydrolyzate was examined by paper chromatography in solvents A and B using the spray reagent followed by heating. The syrupy hydrolyzate revealed spots corresponding to xylose and 4-O-methyl-glucuronic acid with trace amounts of rhamnose, arabinose, galactose and glucose. Again hemicellulose fraction $P_4$ (5 mg) was hydrolyzed with 0.5 M trifluoroacetic acid 2 mL with *myo*-inositol (1 mg) as internal standard. The resulting neutral sugars were analyzed by GLC as their alditol acetates[17] (Table-3).

TABLE-3
RELATIVE COMPOSITION OF THE SUGAR CONSTITUENTS OF HEMICELLULOSE $P_4$

| Sugar residues | Sugar constituents (%) |
|---|---|
| L-Rhamnose | 6.2 |
| L-Arabinose | 6.3 |
| D-Xylose | 70.8 |
| D-Mannose | 2.0 |
| D-Galactose | 3.9 |
| D-Glucose | 3.0 |
| Uronic acid | 7.8 |

**Carboxyl-reduction[18]:** Purified hemicellulose $P_4$ (25 mg) was dissolved in water (5 mL) and the pH was adjusted to 4.75 by addition of 0.1 M hydrochloric acid. N-(3-dimethyl aminopropyl)-N′-ethyl carbodiimide hydrochloride (EDC, 200 mg) was added to the solution and the pH was kept at 4.75 for 0.5 h. Aqueous sodium borohydride (500 mg in 5 mL of water) was added to the solution drop-wise while maintaining the pH at 7.0 by adding 2 M hydrochloric acid. The solution was kept at pH 7.0 for 2 h. Later the excess borohydride was destroyed with aqueous 50 % acetic acid and the reaction mixture was dialyzed for 48 h against running distilled water. The dialysate was concentrated and freeze-dried (20 mg, $CRP_4$). A small portion (5 mg) of $CRP_4$ was hydrolyzed with 0.5 M, TFA with *myo*-inositol as internal standard. The resulting hydrolyzate was reduced by sodium borohydride, converted to alditol acetates and analyzed by GLC (Table-4).

TABLE-4
SUGAR ANALYSIS OF $CRP_4$

| Sugar | Relative mole (%) of $CRP_4$ |
|---|---|
| L-Rhamnose | 6.0 |
| L-Arabinose | 4.5 |
| D-Xylose | 72.9 |
| D-Galactose | 4.0 |
| D-Glucose | 2.9 |
| D-Mannose | 1.7 |
| 4-O-Methylglucose | 8.0 |

**Methylation analysis:** The carboxyl-reduced hemicellulose $CRP_4$ (5 mg) was methylated with dimsyl sodium (2 M, 3 mL) and methyl iodide (4 mL) in DMSO (5 mL) following the Hakomori[19] procedure. The methylated polysaccharide was hydrolysed and the partially methylated sugars were analyzed as their alditol acetates with glc[20] (Table-5).

TABLE-5
METHYLATION ANALYSIS OF $CRP_4$

| Sugar and location of methoxyl groups | Retention time* | Mole (%) | Mode of Linkage |
|---|---|---|---|
| 2, 3, 4-tri-O-Methylxylose | 0.65 | 0.6 | Xyl*p* $(\overset{1}{\rightarrow}$ |
| 2, 3-di-O-Methylxylose | 1.19 | 82.8 | $\overset{4}{\rightarrow}$ )Xyl*p* $(\overset{1}{\rightarrow}$ |
| 3-O-Methylxylose | 1.95 | 8.6 | $\overset{4}{\rightarrow}$ )Xyl*p* $(\overset{1}{\rightarrow}$ <br> $\uparrow^{2}$ |
| 2,3,4,6-tetra-O-Methylglucose | 1.00 | 8.0 | 4-O-methyl-Glc*p* A $(\overset{1}{\rightarrow}$ |

*Retention time of the corresponding alditol acetate relative to that of 1,5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-glucitol on a OV-225 glass capillary column at 170ºC.

## RESULTS AND DISCUSSION

Moisture, ash, lignin, silica, crude fibre and carbohydrates contents (Table-1) of the sun-dried and powdered groundnut shell were determined by following standard procedures. Low-molecular weight materials and lipids were extracted from the shell by extraction with aq. 80 % ethanol and chloroform, respectively. Water-soluble polysaccharide was separated from the shell by extraction with boiling water.

Silylation (Table-2) of the 80 % ethanol extracts showed that it contained the highest amount of L-xylose (60.9 %).

Hemicellulose fraction from the extractive free groundnut shell were isolated by successive extraction with 1.0, 2.5 and 4.5 M aqueous sodium hydroxide. Hemicellulose $P_1$ on passage through DEAE-Sephadex A-50 ion-exchange column did not produce any neutral fraction and gave only one acidic fraction. This acidic polysaccharide was further purified on Sepharose CL-2B gel column where it produced a symmetrical elution curve of the purified hemicellulose $P_4$.

Infrared absorption spectrum of hemicellulose, $P_4$ showed absorption at 3420 $cm^{-1}$ for O-H str., 2920 $cm^{-1}$ for C-H str. and band at 1600 $cm^{-1}$ was due to C-O str. of COOH group. The aforesaid absorption bands clearly indicate the presence of uronic acid in the polymer, $P_4$.

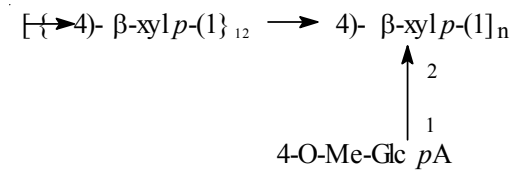
Sugar analysis of the purified hemicellulose $P_4$ showed that it contained 7.8 % of uronic acid. Again sugar analysis of the carboxyl-reduced hemicellulose ($CRP_4$) showed the presence of 8.0 % of 4-O-methyl-glucose. This clearly indicated that the uronic acid was 4-O-methylglucuronic acid. As the D-galactose and D-glucose contents did not increase in any significant proportion, it may be said that all the glucuronic acid was 4-O-methylated and there was no free uronic acid residue in the polymer.

Purified and carboxyl-reduced polymer ($CRP_4$) fromgroundnut shell was subjected to methylation by the Hakomori[19] method. Analysis of the fully methylated polymer by GLC (Table-5) showed the presence of 82.8 % of 2, 3-di-O-methylxylose indicating that the main chain was comprised of (1→4)-linked D-xylose. The presence of 8.6 % of 3-O-methylxylose indicated branching of D-xylose residues at position 2.

2,3,4,6-Tetra-O-methylglucose originated from carboxyl-reduced 4-O-methylglucuronic acid residues. Small amount of 2,3,4-tri-O-methylxylose resulted from the non-reducing end groups of the xylan polymer.

From the relative amounts of non-reducing terminal xylose residues, revealed by the methylation analysis 2,3,4-tri-O-methylxylose, the molecular weight of the hemicellulose from groundnut shell was calculated to be 22,700. Again this value of molecular weight was probably too high as acetylated 2,3,4-tri-O-methylxylose may be lost during evaporation.

From the sugar and methylation analyses and other evidences it was concluded that the xylan obtained by 1 M alkali extraction of the extractive free groundnut shell was essentially (1→4)-β-linked xylan that had a 4-O-methylglucuronopyranosyl unit substituted at position 2 of almost 13th xylose residues. The average repeating unit of xylan is tentatively shown below.



Thus the xylan obtained by 1 M alkali-extraction of the extractive-free groundnut shell resembled xylan from jute stem[21] and med rib of tobacco stalks[22]. The polymer, $P_4$ contained lower uronic acid content. Therefore, it may be useful for chemical modification leading to biologically important products[23]. The protecting effects of xylans have been utilized in reducing intraluminal pressures in diverticular disease[24].

## ACKNOWLEDGEMENTS

The authors thank Dr. Muzaffar Husain and Mr. S.K. Roy of BCSIR, Dhaka, for GLC analyses of the samples.

## REFERENCES

1. J.D. Hooker, *Flora of British India*, 161 (1973).
2. Chun-Hsien Wei, Shuching Yu, Chih-Tung Kou, Hsing-Hsing Ho and Chu-Fen Mou, *Yao Hsuch tung pao*, **15**, 44 (1980); *Chem. Abstr.*, **95**, 49262n (1981).
3. B.R. Murthy and V.R. Srinivasan, *J. Sci. Ind. Res. (India)*, **16C**, 59 (1957).
4. L.E. Wise and K.H. Lauer, in ed.: C.E. Libby, Cellulose and Hemicelluloses in Pulp and Paper Science and Technology, Vol. 1, p. 67 (1962).
5. R.J. Ferrier and P.M. Collins, Monosaccharide Chemistry, Penguin Books Ltd., Harmondsworth, Middlesex, England, p. 249 (1972).
6. R.C. Torres, J.B. Monalo and F.E. Anzaldo, Abstract Papers, The First Princess Chulabhorn Science Congress, Bangkok, Thailand, p. 162 (1987).
7. S. Svensson, A. Lindberg and A. Lundblad, *Chem. Abstr.*, **103**, 16846m (1985).
8. G. Kindness, F.B. Williamson and W.F. Long, *Biochem. Biophys. Res. Commun.*, **88**, 1062 (1979).
9. A.J. Mian and T.E. Timell, *Tappi*, **44**, 48 (1990).
10. A Manual of Laboratory Techniques, National Inst. of Nutrition, Indian Council of Medical Research, Hyderabad-500007, India (1976).
11. L. Banos, Manual of Physiological Studies of Rice, IRRI, Philippines (1976).
12. R. Stock and C.B.F. Rice, Chromatographic Methods, edn. 3 (1974).
13. A.R. Archibald and D.J. Manners, *Biochem. J.*, **73**, 292 (1959).
14. M. Bylund and A. Donetzhuber, *Sven. Papperstidn*, **15**, 505 (1968).
15. W. Kem, Organic Spectroscopy, edn. 3, p. 63, 66 (1991).
16. M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, *Anal, Chem.*, **28**, 350 (1955).
17. J.S. Sawardeker, J.H. Sloneker and A. Jeanes, *Anal. Chem.*, **37**, 1602 (1956).
18. R.L. Taylor and H.E. Conrad, *Biochemistry*, **11**, 1383 (1972).
19. S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
20. J.A. Lomax, A.H. Gordon and A. Chesson, *Carbohydr. Res.*, **138**, 177 (1985).
21. Q.N. Haq, O. Larm, M. Mosihuzzamn, N. Nahar and M. Nizamuddin, *Swedish J. Gri. Res.*, **17**, 63 (1999).
22. S. Eda, F. Watanabe and K. Kato, *Agric. Biol. Chem.*, **40**, 424 (1976).
23. J. Hoffman, O. Larm, L.O. Andersson and G. Soderstrom, *Carbohydr. Polym.*, **2**, 115 (1982).
24. S.S. Stivala, V. Crescenzi and I.C.M. Dea, Industrial polysaccharides, The Impact of Biotechnology and Advanced Methodologies (2000).

(*Received*: 20 January 2007; *Accepted*: 20 June 2007) AJC-5756