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Microwave promoted methylation of plant polysaccharides

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Abstract—*O*-Methylation is of outstanding importance in structural polysaccharide chemistry. A novel method for the methylation of polysaccharides using microwave (MW) irradiation is described. Seed gum from *Cyamopsis tetragonolobus* (Guar) was fully methylated with dimethyl sulphate and sodium hydroxide using 100% microwave power for 4 min in 68% yield. The completely methylated seed gum thus obtained was hydrolyzed by 70% formic acid followed by 0.5N H_2SO_4 under full microwave power for 1.16 and 1.66 min, respectively. The partially methylated monosaccharides were separated and identified.

Methylation is a laborious and time consuming method for getting important information about the structural units that make up a polysaccharide. The procedure involves the preparation of an exhaustively methylated polysaccharide, hydrolysis to a mixture of monomers, and the separation, identification, and quantitative estimation of the components of this mixture. The original procedure, as used by Denham and Woodhouse,[1] and by Haworth,[2] in which the polysaccharide in 25–30% aqueous sodium hydroxide is treated with dimethyl sulphate (DMS) is still the standard method and has been modified from time to time for better results. In general the method does not give complete methylation and the product has to be methylated by some other method, for example treatment of the partially methylated material with silver oxide in gently boiling methyl iodide, according to Purdie and Irvine,[3] is often carried out. However, the Purdie methylation is not always very effective, and generally has to be repeated many times, and also has been reported to have a depolymerization action.[4] A better method by Hakomori[5] involves the use of the strong base methylsulphinylmethyl sodium, and methyl iodide in dimethylsulphoxide (DMSO). However, polysaccharides not soluble in DMSO usually have to be partially methylated[6] by Haworth's method first before applying the Hakomori methylation. A more recent method[7] involves dissolution of the polysaccharide in DMSO by sonication followed by treatment with NaOH and methyl iodide. MW irradiation[8] as an efficient source of thermal energy is becoming a standard technique in various fields of chemistry including carbohydrate chemistry. Hydrolyses of starch[9] and plant seed gums[10,11] have been done under MW irradiation under very mild reaction conditions in very short reaction times. Synthesis of alkyl glycosides[12] and graft copolymerization[13] of acrylic acid with starch has been efficiently carried out recently under MW irradiation. In the present work we report the complete methylation of a representative seed gum (Guar gum) and its hydrolysis to methylated monosaccharides under MW irradiation.

The seed gum was fully methylated with dimethyl sulphate and aqueous sodium hydroxide using MW irradiation within 4 min. The completely methylated product had no absorption in the IR at 3600–3400 cm^{-1}. The fully methylated gum was hydrolyzed in 2.83 min using MW irradiation, using 70% HCOOH acid, followed by 0.5N H_2SO_4. Thus, under MW irradiation the polysaccharide can be fully methylated in good yield and thereafter hydrolyzed very efficiently under mild conditions into a mixture of the methylated monosaccharides within a very short reaction time. The hydrolyzates of the methylated guar gum, methylated conventionally and by the MW method, were co-chromatographed in solvent-A; three methylated monosaccharides namely 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-mannose and 2,3-di-*O*-methyl-D-mannose were detected. The products were fractionated by paper chromatography on Whatman no 3 MM paper (solvent-A). The methylated sugars were identified[14] by their mp, optical rotation, and by forming their crystalline derivatives.

Keywords: Guar gum; methylation; microwave irradiation; hydrolysis; methylated monosaccharides.

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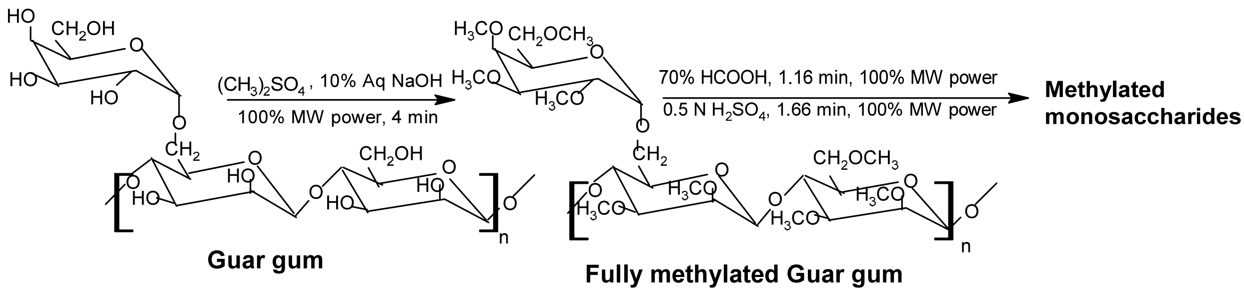

GLC of the partially methylated alditol acetates[6] obtained by reduction with $NaBH_4$ and acetylation of the hydrolysate of methylated seed gum, showed that 2,3,4,6-tetra-*O*-methyl-D-mannose, 2,3-di-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-mannose are present in 1.00:1.01:1.02 molar ratio[15]

Experimental

A Kenstar (Model No. MOW 9811, 1200W) domestic microwave oven was used for all the experiments. Solutions were concentrated at diminished pressure at 60–62°C. Paper chromatography was carried out at room temperature with solvent system A, 1 butanol-ethanol–water[16] (5:14); B, benzene:ethanol:water[17] (169:47:15); C, butanone–water[18] (10:1), with detection using aniline hydrogen phthalate. Infra red (IR) spectra were recorded on a Brucker Vector-22 Infra red spectrophotometer using KBr pellets. A Neukon 5700 Gas Chromatograph equipped with flame ionization detector, at 190°C with a Superleco S P 2380 column (3.0×0.53 mm) was used for GLC, the carrier gas being nitrogen. Seeds were supplied by Himani Seed stores, Dehradun and identified by Botanical Survey of India, Allahabad, India.

Isolation and purification of the seed gum[14]

Dried crushed seeds were extracted successively with light petroleum and ethanol to defat and decolorize, respectively, then extracted with 1% aqueous acetic acid and the extract was added slowly, with stirring to a large excess of ethanol. The crude gum was collected, washed with ethanol and dried.

The crude seed gum was purified through barium complexing by preparing 2.5% (w/v) solutions of the gum by continuous stirring for 12 h at 60°C and precipitating with a saturated barium hydroxide solution. The complex was separated by centrifugation and taken in 1 M acetic acid, stirred for 8 h, centrifuged, precipitated with ethanol and was washed with 70, 80, 90, 95% ethanol. The sample was finally purified by dialysis and filtration through 0.45 μm membranes. The pure seed gum was a non-reducing, white, amorphous material.

Methylation of Guar gum under microwave irradiation

The pure seed gum (1.0 g) was dissolved in 25 ml 10% aqueous NaOH, stirring vigorously on a magnetic stirrer. 40 ml of 30% aqueous NaOH and 20 ml of DMS were added to the above solution in four equal installments followed by 1 min exposure to full MW power after each addition. On the addition of each installment of the reagents, the pH of the reaction mixture was observed to be 12, which decreases to 3.5 after the first exposure, to 6–7 after the second exposure and to 11 after the third exposure, while no change in pH after the last fourth exposure was observed which in turn indicated the completion of the reaction. After the removal of the sodium sulphate formed during the reaction by filtration, the reaction mixture was extracted with $CHCl_3$ thoroughly, the extract was dried over anhydrous sodium sulphate and the solvent distilled off under reduced pressure. The methylated product was obtained as a crispy light yellow product, yield 680 mg, $[\alpha]_D$ +40 (*c* 1.2, $CHCl_3$).

Hydrolysis of methylated gum using MW irradiation

300 mg of the methylated seed gum were dissolved in 20 ml of 70% aqueous formic acid and the solution was exposed to 100% MW power for 1.16 min. The solution was then concentrated and the last traces of formic acid were removed under vacuum. It was then dissolved in 15 ml of 0.5N H_2SO_4 and exposed to full MW power for 1.66 min. The hydrolyzate was cooled, neutralized with barium carbonate, filtered and concentrated under reduced pressure to a light yellow syrup. Three partially methylated monosaccharides were identified by paper chromatography (pc) in solvent A, and GLC of their alditol acetates. The partially methylated alditol acetates were obtained by reduction of the hydrolyzate with $NaBH_4$ followed by acetylation with acetic anhydride in pyridine.[19]

In conclusion we have developed a short and efficient technique for methylation of polysaccharides using MW irradiation. Besides being rapid the method has the advantage of having water as a solvent for both the reactants and the reagents, which is not only cost effective but also eco-friendly. Further reaction condition optimization for the methylation of other classes of polysaccharides is in hand.

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