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Short communication

Hydrolysis of plant seed gums by microwave irradiation

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Abstract

Under microwave irradiation (MW), the seed gums, guar and *Ipomoea quamoclit* were hydrolyzed to constituent monosaccharides and oligosaccharides in very mild conditions and short reaction time. Under MW both the seed gums could be completely hydrolyzed using very dilute acid (0.00625N H_2SO_4) within two minutes. Hydrolysis occurs in 2 min and 20 s even in absence of acid under the MW irradiation. Thus hydrolytic fragmentation under MW provides an efficient tool in structural elucidation of polysaccharides.

Keywords: Seed gums; Acid hydrolysis; Microwave irradiation; Polysaccharides

1. Introduction

The conventional method of determining the sugar residues, present in a polysaccharide is to hydrolyze the material and identify the sugars obtained after purification. Polysaccharides require hydrolysis under the mildest condition (Kowkabany, 1954) to avoid any possible degradation of the sugars. It has been reported that the hydrolysis of the seed gums require either prolonged (>40 h) heating with 2N H_2SO_4 (Kapoor, Chanzy, & Travel, 1995) or it takes 4 h with 1 M trifluoroacetic acid (Singh, Mishra, Khare, & Gupta, 1997). No literature is available about the use of microwave irradiation to hydrolyze polysaccharides except starch (Xia, Li, Cao, & Xi, 2000). Complete hydrolysis of a polysaccharide into constituent monosaccharides is reported to either involve strong acids or sufficiently prolonged hydrolysis time (Kapoor et al., 1995; Singh et al., 1997).

Microwave irradiation to efficiently apply thermal energy is becoming a standard technique in various fields of chemistry. In the present communication we report on the use of microwave irradiation in the acid catalyzed fragmentation of guar and *Ipomoea quamoclit* seed gums. Microwave assisted acid hydrolysis provided a quick method for the identification of component monosaccharides and oligosaccharides, under very mild conditions eliminating the chances of any possible degradation of the sugars during the hydrolysis.

2. Experimental

A Kenstar (Model No. MOW 9811, 1200 W) domestic microwave oven was used for all the experiments. The average bulk temperature at the end of the reaction was measured by inserting thermometer in the reaction mixture. Solutions were concentrated at diminished pressure at 60–62°. Paper chromatography was carried out at room temperature with solvent system A, 1-butanol–2-propanol–water (Rizvi, Gupta, & Kaul, 1971), (11:6:3); B, ethylacetate–pyridine–water (Aspinall, Begbie, & Mackay, 1962) (10:4:3); C, ethylacetate–pyridine–water (Meier, 1960), (2:1:2), with detection using aniline hydrogen phthalate. A Neukon 5700 Gas Chromatograph equipped with flame ionization detector, at 190° with a Superleco S P 2380 column (3.0×0.53 mm^2) was used for GLC, the carrier gas being nitrogen. Seeds were supplied by Himani Seed stores, Dehradun, and identified by the Botanical Survey of India, Allahabad, India.

2.1. Isolation of the seed gums

Dried crushed seeds were extracted successively with light petroleum and ethanol to defat and decolorize,

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respectively, then extracted with 1% aqueous acetic acid and extracts were added slowly, with stirring to large excess of ethanol. The crude gums were collected, washed with ethanol and dried.

2.2. Purification of the seed gum

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

2.3. Hydrolysis of the seed gums under microwave irradiation

The pure seed gums were hydrolyzed with varying concentrations of sulphuric acid for different exposure time under full microwave power to monitor the partial and complete hydrolysis of the seed gums. Oligosaccharides and monosaccharides were detected with paper chromatography using solvent systems A–C.

Two grams of the guar gum was dissolved in 150 ml of 0.1N H_2SO_4 and 20 ml of the gum solutions were exposed to 100% microwave power for different exposure times. The experiment was repeated with 0.05, 0.025, 0.0125, 0.00625N H_2SO_4 and also without sulphuric acid.

The same experiments were performed with *I. quamoclit* seed gum. Paper chromatography (solvent-A) of the complete hydrolyzates after 120 s (with acid under MW) and after 140 s (without acid) revealed the presence of galactose (R_f 0.15) and mannose (R_f 0.21). A portion of the hydrolyzate obtained by the hydrolysis of the gum solutions using MW was dissolved in aqueous methanol and adsorbed on a cellulose column and separation of the constituent sugars was done using 1-butanol–ethanol–water (5:1:4) as a solvent. Identities and configurations of the monosaccharides were confirmed by co-chromatography with authentic samples and preparations of derivatives. D-galactose, mp 163 °C, $[\alpha]_D^{30} + 80°$ (water); D-galactose phenyl hydrazone, mp 153 °C; D-mannose, mp 131°, $[\alpha]_D^{30} + 14°$ (water); D-mannose phenyl hydrazone, mp 198°. The ratio of the constituent monosaccharides was determined by GLC (Kapoor et al., 1995). The complete hydrolyzates of the seed gums (after 120 s under MW) were evaporated, the residues were reduced with sodium borohydride and the products acetylated with pyridine-acetic anhydride (1:1 v/v, 1 h at 100°). The resulting alditol acetates were analyzed by GLC. To ensure the stability of the constituent monosaccharides to microwaving, a control experiment was performed in which a known mixture (1:2) of the galactose and the mannose in 20 ml of water was estimated gravimetrically by treating with excess of Fehling's solution and then filtering off, followed by washing with water and alcohol and weighing the Cu_2O, before and after exposure under full microwave power for 2.33 min. To ensure that hydrolysis of the seed gums to monosaccharides is complete under microwave conditions galactose and mannose were estimated gravimetrically in the complete hydrolyzate of the seed gums, hydrolyzed conventionally (with 2N H_2SO_4 for 48 h) and by microwaves (with 0.1N H_2SO_4 under 100% mw power for 2 min).

3. Results and discussions

Two plant seed gums namely *guar* gum and *I. quamoclit* seed gums were subjected to hydrolytic fragmentation under the microwave irradiation. When seed gums were hydrolzsed with 0.1N H_2SO_4 at 100% microwave power, the oligosaccharides were detected after an exposure time of 10 s and the seed gums could be fully hydrolzsed within 100 s, where no oligosaccharides could be detected. While with 0.05 and 0.025N H_2SO_4 oligosaccharides could be detected just after 10 s and complete hydrolysis was observed after 120 s. The gums could be fully hydrolzsed within 120 s even when the acid strength was reduced to 0.0125 and 0.00625N H_2SO_4. Under full microwave power even in the absence of sulphuric acid the seed gums could be hydrolzsed in 140 s. Identical results were obtained for the seed gum from *I. quamoclit* also. The ratio of D-galactose to D-mannose was found to be 1.00:2.01 in *guar gum* and 1.02:2.06 in *I. quamoclit*) by the GLC of the complete hydrolyzate obtained after the hydrolysis of the seed gums with 0.00625N H_2SO_4 and without any H_2SO_4 under microwave irradiation for 2 min and for 2.33 min, respectively. Hydrolysis of the seed gums by conventional method using 2N H_2SO_4 for 48 h also showed the same ratio of galactose to mannose by GLC. Identities and configurations of the monosaccharides were confirmed by co-chromatography with authentic samples and preparation of derivatives

$$\text{Galactomannan} \xrightarrow[\text{2 min, 0.00625N } H_2SO_4]{\text{100\% MW Power}} \text{Galactose} + \text{mannose}$$

$$\text{Galactomannan} \xrightarrow[\text{2.33 min}]{\text{100\% MW Power}} \text{Galactose} + \text{mannose}$$

In a control experiment, gravimetric estimation of the monosaccharides, galactose and mannose (mixed in 1:2 ratio), before and after exposure gave the same results revealing that they do not degrade under the microwave

conditions. Gravimetric estimation of galactose and mannose in the complete hydrolyzate of the seed gums, hydrolzsed by conventional method (with 2N H_2SO_4 for 48 h) and under microwaves (with 0.1N H_2SO_4 under 100% mw power for 2 min) also gave the same results showing hydrolysis to monosaccharides is complete under microwave conditions.

The hydrolysis under microwave irradiation was observed even in the absence of acid indicating that quick hydrolysis under mild conditions is not merely the temperature effect but it is also microwave effect. Under the influence of microwave radiation glycosidic linkages in a polysaccharide molecule were observed to hydrolyze under very mild reaction conditions in a very short reaction time, thus providing an efficient tool for the structural elucidation of plant polysaccharides.

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