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Note

Permethylation of polysaccharides by a modified Hakomori method

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Polysaccharides generally have a resistance to complete *O*-methylation, probably due to their three-dimensional structures whose hydroxyl groups are highly restricted with inter- and intra-molecular hydrogen-bonds. As urea has been used to denature protein molecules by splitting hydrogen bonds[1], 1,1-dimethyl-, 1,3-dimethyl-, and 1,1,3,3-tetramethyl-urea have now been applied to polysaccharides to accelerate *O*-methylation by the Hakomori method[2] in order to cause relaxation of the hydrogen bonding.

For selection of the most suitable ratio of dimethyl sulfoxide (Me_2SO) to dimethyl- or tetramethyl-urea in order to give the best results in permethylation, amylose ["Amylose B" (Nakarai), mol. wt. 16,000] was used as the standard polysaccharide for the present study. From the experimental results, pretreatment of amylose with a mixture of Me_2SO and 1,1,3,3-tetramethylurea (Me_4U) in the ratio of 1:1 is recommended for modification of Hakomori's method in order to afford per-*O*-methylamylose in high yield within a short reaction-time. These conditions were established as being applicable to other polysaccharides, and to the oligosaccharide portions of saponins, with satisfactory results, overcoming the difficulties that often arise in permethylation.

EXPERIMENTAL

Permethylation of amylose. — "Amylose B (Nakarai)" (6 g) was divided into halves (3 g each); one part (A) was mixed with Me_2SO (150 mL), and the other (B) with Me_2SO (100 mL). Each mixture was placed in a flask fitted with a magnetic stirrer, and stirred to afford a homogeneous solution. Solution A was used as a control (without any additive), and to solution B was added Me_4U (100 mL).

To each flask was added methylsulfinyl carbanion solution, prepared from Me_2SO (240 mL) and NaH (30 g), and, after 30 min, a large excess of methyl iodide (60 mL) was added dropwise to the mixture under vigorous stirring, and cooling to keep the reaction temperature below 50°.

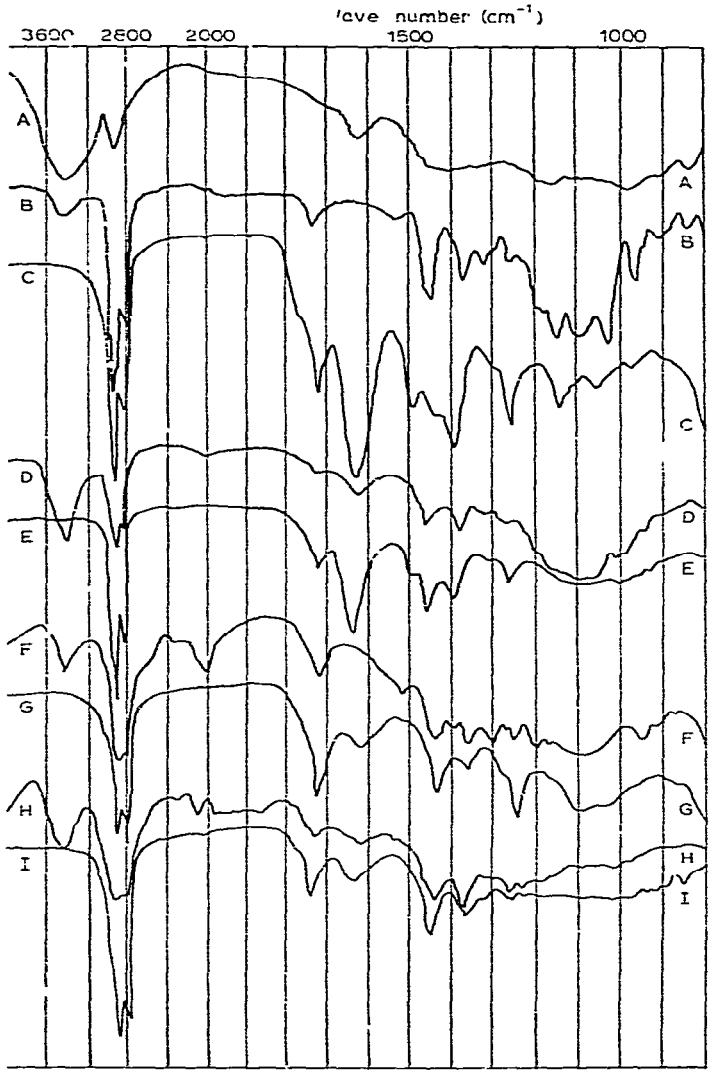

Fig. 1. The infrared absorption spectra of amylose B and of polysaccharides methylated by single treatment by the Hakomori method and by its modified procedure. [These spectra were recorded with a JASCO DS-701 G model spectrometer by the capillary method in CCl_4 on a NaCl plate (*), or as a KBr pellet (**). Key: A**, amylose B; B*, methylated amylose B by the Hakomori method; C*,permethylated amylose B by the present procedure; D**, methylated GE-3 by the Hakomori method; E*, permethylated GE-3 by the present procedure; F*, methylated cellulose by the Hakomori method; G*, permethylated cellulose by the present procedure; H*, methylated dextran A by the Hakomori method; and I*, permethylated dextran A by the present procedure.]

Exactly 5 min after completion of the addition of methyl iodide, the first aliquot (~1/6th of the total volume) of the reaction mixture was removed with a pipet, and dropped into water to stop the reaction. Samplings of the same size were removed at 10, 60, 120, 180, and 240 min during the methylation, in order to check

the i.r. spectra of the products; complete disappearance of the hydroxyl band in the region of 3700–3200 cm^{-1} from the spectra indicated the completion of permethylation.

After the methylation was completed, the mixture was diluted with water, and dialyzed against running water to remove the excess of methyl iodide and Me_4U, as well as the sodium iodide formed. The inner portion (the dialyzate) was extracted with chloroform, and the extract was washed with water, dried, and evaporated. The residue was dissolved in ether–petroleum ether mixture, and treated as already described; the product was dried *in vacuo* at 60°, to give per-*O*-methylamylose, yield ~80%.

RESULTS AND DISCUSSION

I.r.-spectral analysis of the product obtained by the original Hakomori method (without using Me_4U) showed that free hydroxyl groups still remain after reaction for 240 min. On the other hand, the present, modified method using 1:1 Me_2SO–Me_4U for the permethylation of amylose is complete within only 5 min (giving no i.r. absorption for free hydroxyl on the first sampling). By the ordinary Hakomori method, complete methylation of amylose can only be achieved by repeated treatment, or by subsequent methylation by the Kuhn method.

Permethylation of other polysaccharides. — Pustulan (GE-3), a polysaccharide[3] from the lichen *Gyrophora esculenta* Miyoshi, shows strong resistance to permethylation by the ordinary Hakomori method, where, on addition of methylsulfinyl carbanion, the reaction mixture forms an insoluble gel that prevents smooth progress of the reaction.

Using 1:1 Me_2SO and Me_4U, GE-3 did not form any gel on addition of methylsulfinyl carbanion, and permethylation was complete within 30 min. Permethylation by the present, modified method was also applied to the following polysaccharides: amylopectin, agarose, cellulose, glycogen types II and III, dextran A (mol. wt. 177,000) and dextran B (mol. wt. 5,000,000–40,000,000), and pachyman (mol. wt. 180,000–370,000), to give a satisfactory result in each case.

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