Carbohydrate Research, 80 (1980) 336–339

Note

Use of potassium *tert*-butoxide in the methylation of carbohydrates

JUKKA FINNE, TOM KRUSIUS, AND HEIKKI RAUVALA
Department of Medical Chemistry, University of Helsinki, Siltavuorenpenger 10 A, SF-00170 Helsinki 17 (Finland)

(Received February 16th, 1979; accepted for publication, July 23rd, 1979)

Hakomori methylation[1] of sugar derivatives and subsequent g.l.c.–m.s.[2] is widely used in structural studies of complex carbohydrates. A critical step in the procedure is the preparation of the methylation reagent, which is usually generated[3] from sodium hydride and methyl sulphoxide. However, moisture present during the preparation may inactivate the reagent[3] and increase interfering peaks in subsequent g.l.c.[4]. Commercial sodium hydride contains impurities that also interfere.

The use of potassium *tert*-butoxide in methyl sulphoxide has been reported[5,6] as an alternative methylation reagent. Potassium *tert*-butoxide dissolves rapidly to give a substantial proportion of methylsulphinyl carbanion in equilibrium with *tert*-butoxide anion[5]. Although permethylation could be effected readily using this reagent[6], 2-amino-2-deoxy-D-glucose penta-acetate was reported not to react. This result indicates that the butoxide reagent might be different from the sodium hydride reagent, since the latter readily *O*-deacetylates carbohydrates[2]. The possibility of under-methylation together with the lack of data on the reactions of non-acetylated saccharides has limited the use of potassium *tert*-butoxide in the methylation analysis of carbohydrates. We now report on the use of the potassium *tert*-butoxide reagent in methylation analysis.

In accord with the previous finding[6], treatment of 2-amino-2-deoxy-D-glucose penta-acetate with the *tert*-butoxide reagent yielded only a trace amount of the fully *O*- and *N*-methylated derivative (Fig. 1A). However, the same result was obtained with 2-acetamido-2-deoxy-D-glucose (Fig. 1B). Since reducing sugars isomerise and fragment under alkaline conditions[7,8], the behaviour of methyl 2-acetamido-2-deoxy-$\alpha\beta$-D-glucoside[9] towards the *tert*-butoxide reagent was examined. The fully methylated derivative was obtained from both the acetylated and the non-acetylated sugar in much-increased yield (Fig. 1, C and D). Similar results were obtained with the sodium hydride reagent. 2-Acetamido-2-deoxy-D-glucitol is also methylated without difficulty[10] using the *tert*-butoxide reagent.

The permethylation of samples of biological origin was studied next. Rat-brain glycopeptides and gangliosides, which contain 20 identified, differently substi-

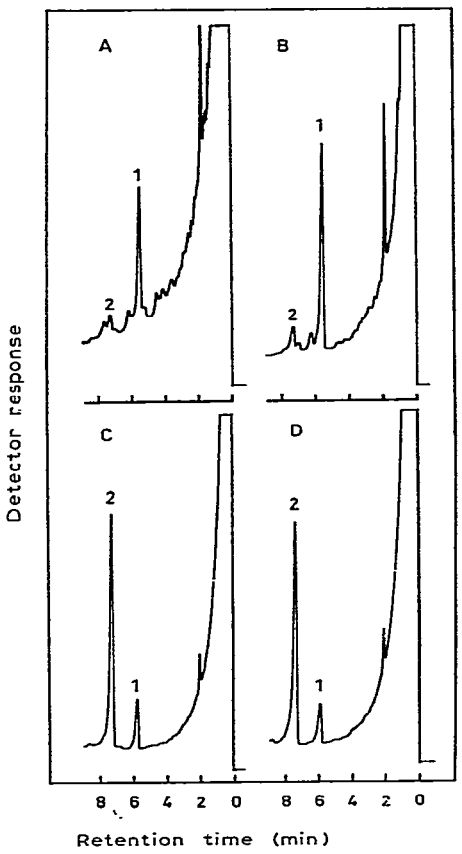

Fig. 1. Methylations using the *tert*-butoxide reagent: g.l.c. of products of methylation of 2-acetamido-2-deoxy-D-glucose tetra-acetate (A), 2-acetamido-2-deoxy-D-glucose (B), methyl 2-acetamido-2-deoxy-$\alpha\beta$-D-glucopyranoside triacetate (C), and methyl 2-acetamido-2-deoxy-$\alpha\beta$-D-glucoside (D) on 2.2% SE-30, 165→200° at 4°/min; peak 1, hexa-*O*-methyl-*myo*-inositol; peak 2, permethylated 2-acetamido-2-deoxy-D-glucose.

tuted hexose, hexosamine, and neuraminic acid derivatives[4,11,12], were used, together with mass fragmentography[11–13] to reveal any differences in methylation pattern. In contrast to the previous suggestion[6], the same results were obtained with hydride and *tert*-butoxide reagents even after per-*O*-acetylation, as shown in Fig. 2 for the per-*O*-acetylated gangliosides.

In contrast to the sodium hydride reagent, the *tert*-butoxide reagent tended to give less background in g.l.c. This was the usual, but not invariable, situation. The somewhat cleaner chromatograms obtained after using the *tert*-butoxide reagent may be important when small amounts of sample are analysed. In contrast to sodium hydride, potassium *tert*-butoxide is available commercially as a crystalline powder of $\geq$ 97% purity and is amenable to further purification.

Thus, the methylation reagent prepared from potassium *tert*-butoxide is as

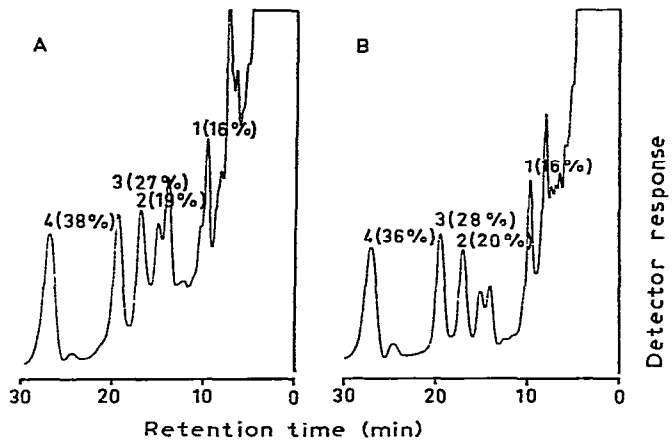

Fig. 2. Gas chromatograms (1% OV-225, 165°) of methylated hexose derivatives from per-*O*-acetylated, rat-brain gangliosides (50 nmol), obtained by using A, sodium hydride reagent; B, potassium *tert*-butoxide reagent: 1, 2,3,4,6-Me_4-Gal; 2, 2,4,6-Me_3-Gal; 3, 2,3,6-Me_3-Glc; 4, 2,6-Me_2-Gal. The relative proportions of the derivatives are indicated in parentheses.

efficient as that prepared from sodium hydride. Moreover, it is more rapidly, conveniently, and safely prepared, and can afford less interfering impurities in the analysis of methylated carbohydrates by g.l.c.–m.s.

EXPERIMENTAL

Gangliosides and glycopeptides were prepared from rat brain[14,15]. Methylsulphinyl carbanion was prepared (*a*) from sodium hydride[3], and used as a 2M solution (titrated with water using triphenylmethane as indicator); and (*b*) by dissolving (30 min, 60°) potassium *tert*-butoxide (Merck) in methyl sulphoxide (Merck, 0.03% of water) to 3M. A clear, yellow liquid, with a small amount of white precipitate, was obtained; the base concentration of the reagent was 1.9M. The reagents were stable for months when stored in Teflon-lined, screw-cap tubes in a desiccator at 4°. Before use, the reagents were thawed at room temperature, and centrifuged.

Methylation procedure. — Methylation was carried out in Teflon-lined, screw-cap tubes (14 × 100 mm) at room temperature. At each step, the tubes were flushed with nitrogen in order to exclude moisture. The dried samples (containing between 5 nmol and 5 μmol of carbohydrate) were dissolved in methyl sulphoxide (50–250 μl) and kept for 30 min in a sonicating bath. An equal volume of the carbanion reagent was added, and the samples were sonicated for 1 h. The excess of base was then ascertained by testing[4] a small aliquot from each sample with triphenylmethane. Methyl iodide (150 μl) was then added with cooling. After sonication for 1 h, each sample was diluted with water to 2 ml and partitioned with 2 ml of chloroform. The chloroform phase was extracted thrice with water and concentrated.

Each sample was degraded by acetolysis–acid hydrolysis[16], and the neutral and amino sugar derivatives were identified[11,12] by g.l.c.–m.s. The neuraminic acid derivatives were identified after methanolysis[17] by mass fragmentography[12]. Methylated methyl 2-acetamido-2-deoxy-$\alpha\beta$-D-glucopyranoside was identified[12] by g.l.c.–m.s.

ACKNOWLEDGMENTS

We thank Mrs. Liisa Kuivalainen, Mrs. Maire Ojala, Mrs. Hilkka Rönkkö, and Mrs. Kristiina Heinonen for skilful technical assistance. This work was supported by the Sigrid Jusélius Foundation.

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