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Note

Methylation of carbohydrates with dimsyl potassium in dimethyl sulfoxide

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The elucidation of the structure of oligosaccharides is now frequently accomplished by mass spectrometry (m.s.). The native compounds are generally plagued with problems of thermal lability and low volatility, thus rendering them unsuitable for direct, mass-spectrometric observation. However, developments and procedural refinements in the permethylation of oligosaccharides[1–5] usually afford a derivative of sufficient thermal stability and volatility to allow the investigative use of mass spectrometry[6].

A general methylation method may be evaluated by ease of preparation of the reagent(s), a short reaction-time, ease of reaction processing, purity of the product, and completeness of methylation. Although many methylation methods have been reported[1–5], the Hakomori methylation[3] has gained indisputable popularity through common usage*. Treatment of a carbohydrate with sodium methylsulfinylmethide (dimsyl sodium[7]) in dimethyl sulfoxide, followed by reaction with iodomethane, yields a completely methylated product. The method essentially satisfies the previously mentioned criteria for methodology; however, significant improvements in the ease of preparation of the reagent and in the purity of the product can be obtained by the replacement of dimsyl sodium with dimsyl potassium.

Dimsyl potassium[8] was prepared by addition of dimethyl sulfoxide to dry potassium hydride powder at room temperature. The evolution of hydrogen subsided within a few minutes, to yield a pale yellow-green solution of dimsyl potassium. Thus, an eight-millimole batch of reagent may be freshly prepared for immediate use in less than thirty minutes. When stored at 0°, unused reagent demonstrated no appreciable change in activity after one month. Alternatively, the use of potassium *tert*-butoxide has been reported[4]; however, the resultant reagent is a mixture of dimsyl and butoxide bases[9].

Raffinose, stachyose, sucrose, and 2-acetamido-2-deoxy-D-galactose were

*According to *Curr. Contents (Life Sci.)*, 23 (June 9, 1980), the Hakomori methylation (ref. 3) "has been cited over 780 times since 1964".

TABLE I

G.L.C.–M.S. ANALYSIS OF REACTION PRODUCT

Compound used	*Permethylated product in reaction mixture after processing*[a] (%)[b]	
	Dimsyl sodium	*Dimsyl potassium*
Sucrose	42	94
Raffinose	82	94
Stachyose	82	94
2-Acetamido-2-deoxy-D-galactose	35[c]	91[c]
(*N*-Acetyl-L-alanyl)-phenyl-L-alanine	44[c]	74[c]

[a]Two equal portions of each compound were prepared. One portion was treated with dimsyl sodium, and the other, with dimsyl potassium. Reaction of each with iodomethane was followed by identical processing. [b]Percentage of permethylated product was determined by peak integration of the gas–liquid chromatogram. [c]The mass-spectrometric data were consistent with *N,O*-permethylated structures.

arbitrarily chosen to be methylated through utilization of dimsyl potassium. Each sample was dissolved in dimethyl sulfoxide, treated with the dimsyl potassium reagent, and then allowed to react with iodomethane. Extraction of the reaction mixtures with chloroform, followed by several washings with water, usually yielded an almost colorless product. Subsequent analysis by gas–liquid chromatography (g.l.c.)–m.s. revealed completely *N,O*-permethylated products of moderate to high purity (see Table I). Substituting dimsyl sodium for dimsyl potassium, the same general procedure again yielded completely *N,O*-permethylated products; however, the purity of the products was significantly less than before (see Table I). A simple dipeptide, (*N*-acetyl-L-alanyl)-phenyl-L-alanine, yielded similar results when methylated in the same way.

It was observed that potassium iodide displays very limited solubility in chloroform, whereas sodium iodide is freely soluble therein. It seemed probable that the presence of the latter salt in the organic phase during processing of the reaction mixture would carry impurities and undesired by-products along with the desired, permethylated product, and such a condition invokes a necessity for more-rigorous purification-procedures, such as some form of chromatography. Therefore, the absence of the salt should help to minimize the problem of contaminating the desired product with impurities. This can, in part, be avoided through the use of dimsyl potassium, because of the very limited solubility of the product salt, potassium iodide, in chloroform.

It has thus been shown that two benefits can be derived from substitution of dimsyl potassium for dimsyl sodium in Hakomori methylation of carbohydrates. First, preparation of dimsyl potassium reagent is a much faster operation than the preparation of dimsyl sodium reagent; in addition, the preparation of dimsyl

potassium reagent is performed at ambient temperature and requires no heating. Second, reaction products tend to be significantly freer from impurities when dimsyl potassium reagent is used.

EXPERIMENTAL

Gas–liquid chromatography–mass spectrometry. — Mass spectra were recorded with an LKB 2091 Gas Chromatography–Mass Spectrometer equipped with an LKB 2130 Data System (DEC PDP 11/34 minicomputer employing the RT-11 disk-based operating system). Electron-impact, mass spectra were scanned from samples introduced *via* a 25-m, glass capillary column, wall-coated with SE-30 as the stationary phase (LKB 2101-202). A 2-μL sample was injected onto the column through a glass-lined splitter at 300°, set at a 1:25 split ratio. The column temperature was either maintained at 300° (stachyose), or initially set at 225° for a 2-min isothermal period, and then elevated at 8°.min^{-1} to a final temperature of 330° (raffinose, sucrose, and 2-acetamido-2-deoxy-D-galactose). The linear velocity of helium through the column was 40 cm.sec^{-1}. The single-stage, jet separator was at 275°, and the ion-source temperature was 180°; the ionizing potential was 70 eV, and the ionizing current was 50 μA.

Reagents. — Reagents were obtained from the following sources, and used without further purification: raffinose, stachyose, sucrose, and 2-acetamido-2-deoxy-D-galactose (Pfanstiehl Laboratories, Inc., Waukegan, IL); potassium hydride (23% in oil) [Thiokol/Ventron Division (Alpha Products), Danvers, MA]; and iodomethane (Aldrich Chemical Company, Milwaukee, WI). Dimethyl sulfoxide (Sigma Chemical Company, St. Louis, MO) was dried by distillation from calcium hydride, and then stored over molecular sieves.

Preparation of dimsyl potassium. — The following procedure was performed at room temperature under a nitrogen or argon atmosphere. Commercially available potassium hydride (23% by weight in oil) was washed three times with hexane, to give 0.45 g of dry potassium hydride, and dimethyl sulfoxide (6.8 mL) was slowly added, with stirring, during 5 min. Vigorous evolution of hydrogen subsided in a few minutes, and ceased entirely after 10 min. A slightly turbid, pale yellow-green solution, with a small amount of white precipitate, was thus obtained. The concentration of the dimsyl anion was determined to be 1.3M by titration with methanol, using triphenylmethane as the indicator. The reagent was stored in a serum-capped (Teflon-lined), glass vial at 0°.

Methylation procedure. — The following procedure was performed in screw-capped (Teflon-lined), conical, glass vials at room temperature under a nitrogen atmosphere. The samples (10 μmol) were dissolved in dimethyl sulfoxide (250 μL), and the dimsyl potassium reagent (150 μL) was slowly added with brief vortexing. Each resulting mixture was allowed to stand for 30 min, with occasional, ultrasonic agitation. Iodomethane (100 μL) was then added with brief vortexing at ice-bath temperature. The resulting mixtures were allowed to stand for 1 h at room tempera-

ture, with occasional agitation, diluted with water (2 mL), and extracted with chloroform (2 mL). The extracts were combined, washed with six 2-mL portions of water, and evaporated to dryness under a stream of nitrogen. The residues were dissolved in a mixture of chloroform (50 μL) and methanol (50 μL), and subjected to analysis by g.l.c.–m.s.

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