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Note

Methylation of carbohydrates with lithium methylsulphinyl carbanion

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In methylation analysis of oligosaccharides and polysaccharides, the critical step is the formation of alkoxide ions from the hydroxyl groups of sugar residues[1]. In the Hakomori procedure[2], which is now almost universally used, the methylsulphinyl carbanion base[3] is used to promote alkoxide formation. The carbanion is usually generated from Me_2SO by using sodium hydride[4], but potassium hydride has also been used[5–7]. The carbanion has also been made from alkali metal amides[8] and butyllithium[9]. The choice of method for the preparation of carbanion is governed by several considerations: (*1*) the convenience of the method of preparation, (*2*) the nature of the counter ion, since, because of ion pairing, it determines the concentration of free carbanion available for reaction[8,10], (*3*) the availability of pure starting reagents, and (*4*) the extent of generation of side products during carbanion formation. These considerations are most important when small amounts of polysaccharides are to be analysed, as minor amounts of partially methylated alditol acetates may be lost in the background of contaminant peaks during gas chromatography[7,11].

Pure, dry dimethyl sulphoxide is necessary for the synthesis of methylsulphinyl carbanion, as the carbanion reacts rapidly with oxygen, carbon dioxide, and water[9]. Carbon dioxide and oxygen, for which dimethyl sulphoxide is a good solvent[12], may be removed by purging with nitrogen or argon[13]. Water, which is usually present in commercial samples, may be removed with molecular sieves[14] or by distillation from calcium hydride or sodium hydride[8,13]. Organic contaminants may be removed by fractional distillation under vacuum or by freezing[13,14].

Alkali metal hydrides are normally only available as commercial grades. The preparation of methylsulphinyl carbanion from sodium hydride requires[9] 45 min of heating at 70–75° or[15] several h at 50–60°, with concomitant thermal decomposition of the anion[15]. Lithium hydride reacts with dimethyl sulphoxide even more slowly than sodium hydride, so that extensive thermal decomposition of the anion occurs before the reaction is complete[9,16]. However the carbanion generated with

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potassium hydride can be prepared at room temperature[6,7], and when used for methylation produces fewer contaminants than the carbanion generated with sodium hydride[6]. The carbanion solutions produced from alkali metal hydrides are usually grey-green[1], green, or yellow[9] and contain solid particles that may be removed by centrifugation[7]. Exner and Steiner[8], using high purity, alkali metal amides produced colourless solutions of carbanion, indicating that the colouration of carbanion solutions produced from metal hydrides is due to impurities. Rose *et al.*[17] have recently described a micro-procedure for the permethylation of acylated peptides. They stress the necessity of using pure, dry dimethyl sulphoxide, vacuum distilled from sodium hydride under oxygen-free nitrogen, and pure, oil-free sodium hydride to produce clear, colourless solutions of the anion. They noted that, if the anion solution is yellow or darker, the colour is due to the entry of air or overheating and recommended that such preparations be discarded.

In this paper we report the results of methylation with methyl sulphinyl carbanion prepared from butyllithium. This reagent is widely used in organic synthesis, but requires careful handling as it is pyrophoric[18]. However, under an inert atmosphere, solutions of <20% (w/v) butyllithium in hexane are stable at room temperature[18] and may be readily handled by using equipment designed for air-sensitive compounds[19]. In the experiments reported, lithium methylsulphinyl carbanion was produced by adding a cold solution of butyllithium in hexane to dry dimethyl sulphoxide under argon. Purging with argon removed the hexane and butane formed during the reaction, yielding a clear, pale-yellow solution that contained no sediment.

Lithium methylsulphinyl carbanion prepared from butyllithium was compared with sodium and potassium methylsulphinyl carbanions, produced from their respective hydrides, by direct gas chromatography of methylation mixtures of sucrose and perseitol (Fig. 1). The chromatograms obtained when the lithium carbanion was used contained fewer contaminant peaks than those obtained with carbanions generated from sodium and potassium hydrides. Presumably this was due to the superior purity of the starting reagent.

The extent of methylation obtained when lithium methylsulphinyl carbanion was used was tested by using maltoheptaose and pullulan. Both samples were completely methylated in a single methylation, based on measurement of the incorporation of [^{14}C]methyl iodide[7]. Methylation analysis of a wide range of oligosaccharides and polysaccharides was successfully achieved by using the lithium methylsulphinyl carbanion. The time of exposure to methyl iodide was increased when the lithium carbanion was used, because some samples were slow to dissolve completely. This may be due to a slower reaction of the lithium alkoxide with methyl iodide. The chromatograms from each gave the expected ratio of partially methylated alditol acetates, and gave clean base lines with few contaminant peaks. The gas chromatography–mass spectrometry total-ion chromatograms for barley β-D-glucan and wheat arabinoxylan are shown in Fig. 2.

The preparation of methylsulphinyl carbanion from butyllithium is both rapid

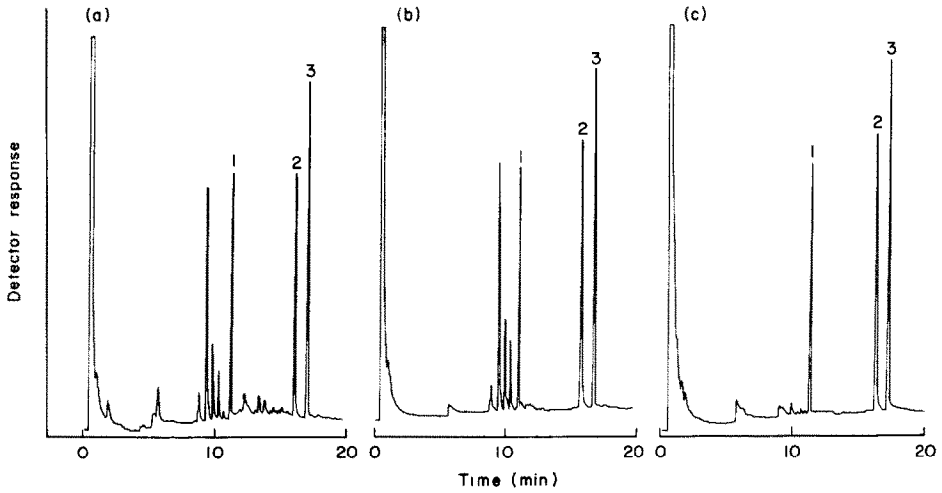

Fig. 1. Separation of permethylated derivatives of perseitol and sucrose on a Silar 10C glass-capillary column. The temperature was kept at 100° for 4 min and then increased to 230° at 8°/min. (*a*) Sodium anion; (*b*) potassium anion; (*c*) lithium anion; 1, perseitol; 2, sucrose; 3, *myo*-inositol hexa-acetate (internal standard).

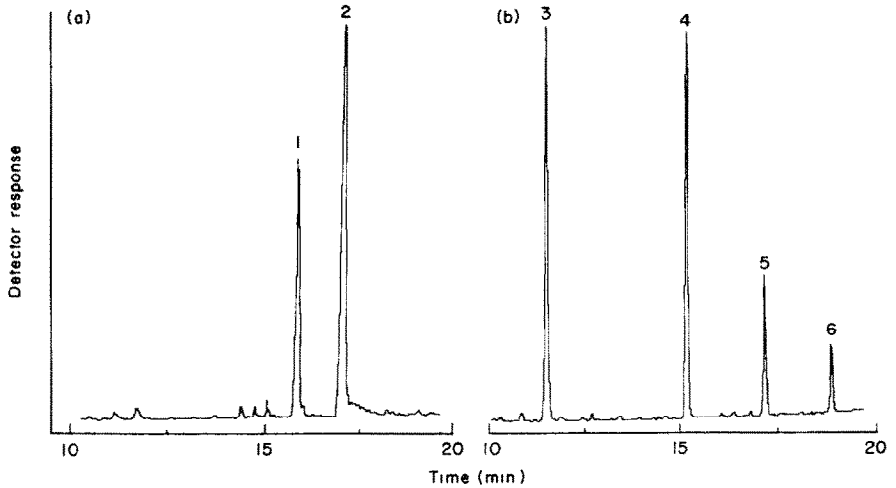

Fig. 2. Total-ion chromatograms of partially methylated alditol acetates formed in methylation analysis with lithium methylsulphinyl carbanion (*a*) (1→3,1→4)-β-D-glucan from barley endosperm; (*b*) arabinoxylan from wheat endosperm. 1, 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-glucitol; 2, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol; 3, 1,4-di-*O*-acetyl-2,3,4-tri-*O*-methylarabinitol; 4, 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylxylitol; 5, 1,3,4,5-tetra-*O*-acetyl-2-*O*-methyl xylitol; 6, xylitol penta-acetate.

and convenient. The carbanion produced gave complete methylation of all carbohydrates tested and produced for, gas chromatography, clean methylation mixtures low in contaminants. When stored under argon at −20° the concentration remained the same for at least two months.

Pure sodium amide is available commercially as a suspension in toluene and, after removal of the toluene with hexane[10], also provides a convenient means of producing pure sodium methylsulphinyl carbanion.

EXPERIMENTAL

Reagents. — Methyl iodide (Puriss, inhibited with silver), butyllithium (1.6M in hexane), and formanilide were obtained from Fluka A.G., Buchs, Switzerland; "Insta-gel" from Packard Instruments, Melbourne, Australia; Blue Dextran 2000 and Sephadex LH-20 from Pharmacia Fine Chemicals AB, Uppsala, Sweden; and [^{14}C]methyl iodide from Amersham Australia Pty Ltd., Sydney. All other reagents were the purest grade commercially available. Argon, high purity (<10 p.p.m. O_2) was from Commonwealth Industrial Gases Ltd., Melbourne, Australia. Prior to use it was freed of any residual oxygen and water by passing it through an oxygen trap (Oxy-trap, Alltech Associates, Melbourne, Australia) and a drying tube, filled with Linde molecular sieve 5Å.

Dimethyl sulphoxide (Merck, Darmstadt, Germany, high purity) was dried over molecular sieve type 4Å and then fractionally distilled under vacuum. *myo*-Inositol hexa-acetate (m.p. 217°) was prepared as previously described[20].

Samples for methylation. — Samples were obtained from the following commercial sources: Cellulose (Avicel, a micro-crystalline preparation) from Macherey Nagel and Co., Düren, Germany; sucrose from BDH Ltd., Poole, U.K.; cellobiose, perseitol, pullulan and stachyose from Sigma Chemical Co., St. Louis, MO, U.S.A.; and maltoheptaose from Boehringer Mannheim Sydney, Australia. (1→3,1→4)-β-D-Glucan was isolated from barley endosperm[21] and arabinoxylan was isolated from wheat endosperm[22].

Preparation of methylsulphinyl carbanion. — Sodium and potassium methylsulphinyl carbanions were prepared from sodium and potassium hydrides as described previously[7].

Lithium methylsulphinyl carbanion (about 2M) was prepared from butyllithium as follows: Dimethyl sulphoxide (80 mL) was transferred by using a glass syringe to a dry, 3-necked, 500-mL round-bottomed flask containing argon and a magnetic stirrer-bar. The flask was evacuated and purged three times with dry, oxygen-free argon. Argon purging of the flask was continued throughout the reaction. A 150-mL pressure-compensating dropping funnel was fitted to the flask which, was also purged with argon. Cold (4°) butyllithium (100 mL, 1.6M in hexane) was transferred by syringe to the dropping funnel and added to the stirred dimethyl sulphoxide solution over a 10-min period. Any spills of butyllithium were covered with mineral oil, which decreases its reactivity[18]. The solution warmed to 40° during the reaction, became cloudy, and then cleared to yield a pale-yellow solution. Stirring and purging with dry, oxygen-free argon was continued for 30 min to remove butane and hexane. Vacuum evaporation at 40° provided a successful alternative method of removing butane and hexane.

The same batch of dry dimethyl sulphoxide was used to prepare all three methylsulphinyl carbanions. The concentration of each carbanion was determined by titration with formanilide under argon, using triphenylmethane as the indicator[9,23]. Each methylsulphinyl carbanion preparation was then diluted to 1.6M with dimethyl sulphoxide before use. All methylsulphinyl carbanion preparations were stored under argon in Teflon-sealed, screw-capped borosilicate glass tubes at −20° until required.

Methylation procedures. — Carbohydrate samples were methylated by the procedure of Harris *et al.*[7] except that, where lithium carbanion was used, the time of exposure to methyl iodide was increased to 40 min. Some samples were recovered after methylation by using Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA, U.S.A.) as described by Mort *et al.*[24]. The extent of methylation of pullulan and maltoheptaose was determined by using [^{14}C]methyl iodide and Sephadex LH-20 gel-permeation chromatography as previously described[7].

To compare the extent of contamination from the reagents or arising during the methylation procedure, sucrose and perseitol were methylated using each of the three carbanions. After reaction with methyl iodide, water (20 mL) was added to the methylation mixture. Dichloromethane (2 mL), containing 1 mg of *myo*-inositol hexa-acetate as an internal standard, was then added. After thorough mixing and phase separation 2 μL of the dichloromethane phase was injected directly into the gas chromatograph.

Gas chromatography. — Permethylated compounds and partially methylated alditol acetates were separated on a 28 m × 0.5 mm (i.d.) Silar 10C SCOT glass capillary column[19] (S.G.E. Pty. Ltd., Melbourne, Australia) in a Hewlett–Packard 5710A chromatograph equipped with a flame-ionization detector and a modified S.G.E. "Unijector" capillary injection system, used in the split mode. High-purity hydrogen was used as the carrier gas at a flow rate of 72 cm/sec (determined by using pentane). Two temperature programmes were used: (*a*) 100° for 4 min followed by an 8°/min rise to 230° for the direct analysis of permethylated compounds, and (*b*) 150° for 4 min followed by a 4°/min rise to 230° for partially methylated alditol acetates. The injection port and detector were heated to 250 and 300°, respectively. Peak areas were recorded with a Hewlett–Packard model 3386A reporting integrator. The identity of the peaks was confirmed by using a modified Jeol (Japanese Electron Optics Co., Tokyo, Japan) JCG-20K gas chromatograph equipped with a modified SGE unijector and linked to a Jeol JMS-D100 double-focussing mass spectrometer used in the electron-impact mode. The gas chromatograph was fitted with a 25 m × 0.2 mm (i.d.) vitreous-silica WCOT capillary column coated with the bonded phase[25] BP-75 (S.G.E.), which was inserted directly into the ion source of the mass spectrometer. Ultrahigh-purity helium was used as the carrier gas.

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