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A SIMPLE AND RAPID METHOD FOR THE PERMETHYLATION OF CARBOHYDRATES

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ABSTRACT

As a result of a study of the permethylation of sugars in such dipolar aprotic solvents as methyl sulphoxide, the hitherto accepted role of the $CH_3SOCH_2^-$ anion is questioned. The HO^- and H^- ions appear to be the effective basic agents in these methylation reactions. This conclusion suggested a new method for the permethylation of sugars involving methyl iodide, a solid base (NaOH, KOH, or *tert*-BuOH/NaOH), and methyl sulphoxide. Excellent yields (98 ±2%) of permethylation products were obtained in a remarkably short (6–7 min) reaction time. The non-sugar peaks that appear in gas chromatograms of the products of Hakomori methylation were absent when the new reagent was used.

INTRODUCTION

Methylation is a commonly used derivatisation method for the g.l.c. of carbohydrates. Such strong bases as sodium hydride or potassium *tert*-butoxide in dipolar aprotic organic solvents are now used instead of the earlier methods of Purdie[1] or Haworth[2]. Using methyl iodide in *N,N*-dimethylformamide with silver oxide[3,4], barium hydroxide[5], or sodium hydride[6] as the basic reagent, the reaction rates increase but full methylation is not achieved.

In the Hakomori[7] method, the $CH_3SOCH_2^-$ anion, generated from methyl sulphoxide and sodium hydride, is considered[8] to be the effective basic agent. In spite of the low yields (0.3 mol of permethylated derivative per mol of sugar), the Hakomori method has been used extensively in structural investigations of carbohydrates. The use of potassium *tert*-butoxide[9] instead of sodium hydride improved the stability of the reagent but did not substantially increase the yield of permethylated product[10,11].

We now report the results of a detailed study of these techniques. The conclusion that HO^- or H^- must be more important basic agents than $CH_3SOCH_2^-$ suggested a new method for the methylation of sugars, involving a solid hydroxide, methyl sulphoxide, and methyl iodide. The method is characterised by short reaction times, high yields, and clean gas chromatograms.

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EXPERIMENTAL

Reagents. — Methyl sulphoxide, *N,N*-dimethylacetamide and *N,N*-dimethylformamide were analytical-grade solvents with a water content of 0.1%. Before use, sodium and potassium *tert*-butoxide and NaH were washed with hexane. Naphthalene or anthracene was used as the internal standard in g.l.c.

Instrumentation. — A Chromatron (Berlin) GCHF 18.3 chromatograph was used with a non-vaporising on-column injection system, a flame-ionisation detector, a Minigrator (Spectra Physics) peak-area integrator, and silanised glass columns (1.5 m × 3 mm i.d.) filled with 3% of SE 30 on Gas Chrom Q (80–100 mesh).

Methylations. — Thermostated mini-vials (Pierce) with magnetic stirrer were used.

To a solution of the carbohydrate sample (4–5 mg) in Me_2SO (0.3–0.5 mL) were added finely powdered NaOH (20 mg; or 30 mg of KOH, or 12 mg of NaH, or 40 mg of *tert*-BuONa + 20% of NaOH) and methyl iodide (0.1 mL). Each mixture was stirred (100 r.p.m.) for 6 min in a closed vial at 25° (7 min for KOH or *tert*-BuONa + 20% NaOH, and 15 min for NaH). Water (1 mL) and chloroform (1 mL) were then added, and the chloroform layer was washed with water (3 × 10 mL) and dried (Na_2SO_4). For quantitative analysis, naphthalene or anthracene (2 mg) was added to the sample.

RESULTS AND DISCUSSION

The methylation of carbohydrates occurs through successive, base(B)-catalysed ionisation of the hydroxyl groups followed by reaction with the methylating agent (MeI)

$$R(OH)_n + B^- \rightleftharpoons R(OH)_{n-1}O^- + BH \qquad (1)$$

$$R(OH)_{n-1}O^- + MeI \rightarrow R(OH)_{n-1}OMe + I^- \qquad (2)$$

. . . . . .

$$R(OMe)_{n-1}O^- + MeI \rightarrow R(OMe)_n + I^- \qquad (3)$$

$$B^- + MeI \rightarrow MeB + I^- \qquad (4)$$

Dipolar aprotic solvents, for example, methyl sulphoxide (μ 4.3), *N,N*-dimethylformamide (μ 3.82), and *N,N*-dimethylacetamide (μ 3.79), solvate cations but not small anions[12]. Larger and polarisable anions, for example I^-, are solvated. Thus, in reactions *1–3*, the occurrence of solvation will be $B^- < R(OH)_{n-1}O^- < I^-$. Such side-reactions as *4* may generate non-sugar peaks in g.l.c. The reactivity of hydroxyl groups in carbohydrates follows the sequence glycosidic > primary >

TABLE I

METHYLATION OF CARBOHYDRATES[a] WITH METHYL IODIDE IN DIPOLAR APROTIC SOLVENTS + A STRONG BASE

Method	*Base*	*Solvent*	*Base/solvent (mmol/mL)*	*Temp. (degrees)*	*Time (min)*	*Yield[d] (%)*
Alkali-metal hydride	NaH[b]	Me_2SO	1.5	20	80	28 ±5.5
	NaH	$HCONMe_2$	2	25	240	79 ±2.3
	NaH	CH_3CONMe_2	2	25	120	91 ±1.5
	NaH	Me_2SO	2	25	15	97.8 ±1.8
Akali-metal *tert*-butoxide	*tert*-BuOK[c] +1% KOH	Me_2SO	2	25	60	38 ±2.6
	tert-BuONa +1% NaOH	Me_2SO	2	25	60	46 ±1.2
	tert-BuONa +20% NaOH	Me_2SO	2	25	7	97.3 ±2.0
Alkali-metal hydroxide	NaOH	$HCONMe_2$	2	25	200	72 ±4
	NaOH	CH_3CONMe_2	2	25	100	80 ±3
	NaOH	Me_2SO	2	25	6	98.8 ±1.1
	KOH	Me_2SO	2	25	6.5	98.2 ±1.5

[a]D-Glucose, D-galactose, L-arabinose, L-sorbose, D-xylose, D-mannose, L-rhamnose, D-ribose, maltose, lactose, sucrose, and raffinose. [b]As described in ref. 7. [c]As described in ref. 11. [d]Calculated for the fully methylated D-glucose; similar values were obtained for the other carbohydrates investigated.

secondary; once a methyl group is introduced, it will lower the reactivity of the vicinal hydroxyl groups. This explains the poor reactivity in the final stages of methylation (reaction *3*). The results of applying the methylation procedures described by Hakomori[7], Lindberg[10], and Finne *et al.*[11], with some modifications, to various mono-, di-, and tri-saccharides are given in Table I, together with the data for the new method now reported.

Sodium hydride method. — Sodium hydride reacts (20 min, 50°) with methyl sulphoxide, generating the methylsulphinylmethanide ($CH_3SOCH_2^-$) ion. It is generally believed[13] that this ion, easy detectable with a simple colour reaction, is the effective basic agent (B^-) in the Hakomori methylation. The methylsulphinylmethanide ion is sensitive[14] to moisture and CO_2, and reacts exothermally with methyl iodide. This undesired side-reaction can be overcome by adding the sodium hydride and methyl iodide at room temperature to the solution of the carbohydrate sample in methyl sulphoxide. The effect of increasing the amount of sodium hydride on the methylation of D-glucose is shown in Fig. 1. The yield increased up to 3 equiv. of sodium hydride per mol of replaceable H of the saccharide. In the range 3–6 equiv., the yields remained constant; with larger excesses of sodium hydride, the yields declined due, perhaps, to the formation of $CH_3SOCH_2^-$ ion in larger excess which reacted with MeI. This decreasing trend was absent for solutions in *N,N*-dimethylformamide. Analysis of the side products in the Hakomori methylation by g.l.c.–m.s. indicates the formation of ethyl methyl sulphoxide, disulphur derivatives, and further condensation and methylation derivatives.

For solutions in $HCONMe_2$ or CH_3CONMe_2, the methylation yields were

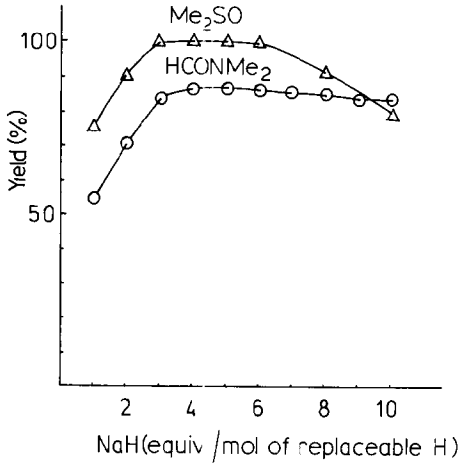

Fig. 1. The dependence of the yield of permethylated D-glucose on the solvent and the equiv. of NaH per mol of replaceable H (2 mmol of NaH per mL of solvent; 3 equiv. of methyl iodide per mol of replaceable H).

lower than those for methyl sulphoxide, due to the lower polarity of these solvents[15] and the corresponding poorer solvation of the reactants. The optimal amount of sodium hydride was 3–4 equiv. per mol of replaceable H. For methyl iodide, an equimolar ratio to sodium hydride is recommended.

It was found that methylation could be performed in $HCONMe_2$ or CH_3CONMe_2 solution where the $CH_3SOCH_2^-$ anion does not exist, and that the methylation yields were higher and the products were cleaner if the formation of $CH_3SOCH_2^-$ was hindered. These observations do not accord with the generally accepted role of the methylsulphinylmethanide anion as the basic agent in the Hakomori methylation. It seemed more reasonable to assume that, instead of the $CH_3SOCH_2^-$ ion, another anion, for example H^-, was the basic agent (B^-) of the reaction. This hypothesis accorded with the experimentally observed formation of hydrogen when sodium hydride was added directly to the carbohydrate solution.

The alkaline tert-*butoxide method.* — The use of potassium *tert*-butoxide in methyl sulphoxide has been proposed as a methylation reagent[10,11]. In methyl sulphoxide solution, *tert*-BuOK generates the methylsulphinylmethanide anion[9] identified by its colour reaction with triphenylmethane[14]. The role of $CH_3SOCH_2^-$ as the basic agent was accepted for this method.

The preparation of the *tert*-BuOK reagent is quite simple and fewer non-sugar peaks appeared in g.l.c. than for the Hakomori procedure. However, the yields (40–50%) of fully methylated products were not high, and the results were not easily reproducible probably because of the variable content of potassium hydroxide in the reagent. A water content of 0.1% in the starting *tert*-butyl alcohol will result in a 0.25% content of potassium hydroxide in the *tert*-butoxide. However, the *tert*-butoxide prepared from dry *tert*-butyl alcohol had a very low activity, which suggested that HO^- ions were the effective basic agent (B^-).

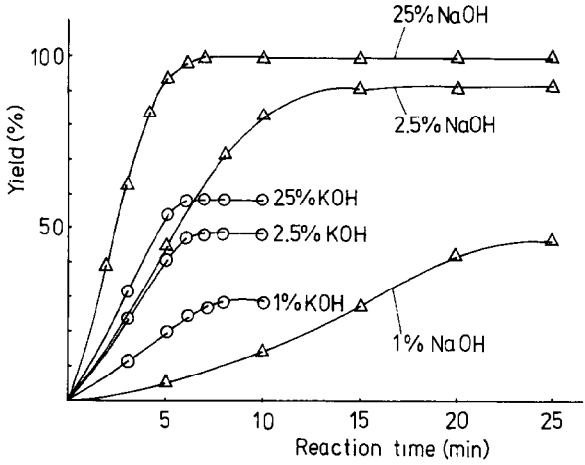

Fig. 2. The dependence of the yield of permethylated D-glucose on the amount of alkali-metal hydroxide added to alkaline *tert*-butoxide in methyl sulphoxide (2 mmol of alkaline *tert*-butoxide/mL of Me_2SO; 3 equiv. of (*tert*-BuOK + KOH) per mol of replaceable H; 3 equiv. of (*tert*-BuONa + NaOH) per mol of replaceable H; 3 equiv. of MeI per mol of replaceable H; ○, *tert*-BuOK; △, *tert*-BuONa).

This hypothesis was checked by adding various amounts of alkali-metal hydroxide to the parent *tert*-butoxide having a 1% original content of potassium or sodium hydroxide. Fig. 2 gives the yields for the permethylation of D-glucose in methyl sulphoxide + alkali-metal *tert*-butoxide with various contents of hydroxide. An increase of potassium hydroxide content from 1 to 2.5% considerably increased the yield. The effect of a more substantial amount (25%) of potassium hydroxide was smaller, but was also evident.

The use of *tert*-BuONa with similarly increasing contents of sodium hydroxide produced a marked increase in the yield of permethylated product. The differences between the effects of potassium and sodium hydroxides are noteworthy. One reason must be the 550-fold higher solubility[12] of *tert*-BuOK than *tert*-BuONa in methyl sulphoxide.

Because of its higher solubility in methyl sulphoxide, *tert*-BuOK will generate methylsulphinylmethanide anion to a greater extent, which was proved by the colour reaction with triphenylmethane. For all the carbohydrates investigated, short reaction times, but low permethylation yields, resulted from the use of potassium *tert*-butoxide. In contrast, only low concentrations of $CH_3SOCH_2^-$ were detectable in the *tert*-BuONa + $NaOH/Me_2SO$ system, but high permethylation yields were obtained at the higher contents of sodium hydroxide (97.3% in 7 min). Thus, HO^- ion must be a more effective basic agent (B^-) and the $CH_3SOCH_2^-$ ion, apparently, has only a catalytic effect on undesirable side-processes.

The alkali-metal hydroxide methods. — The next step was to investigate the permethylation of sugars in methyl sulphoxide containing only sodium or potassium hydroxide. Finely powdered hydroxide was added with continuous stirring to the solution of the carbohydrate sample in methyl sulphoxide. The solubility is

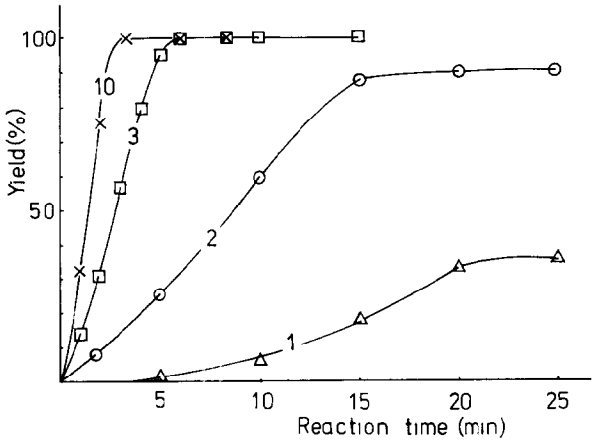

Fig. 3. Methylation of D-glucose at various (1–10) equiv. of sodium hydroxide per mol of replaceable H (2 mmol of NaOH/mL of Me_2SO).

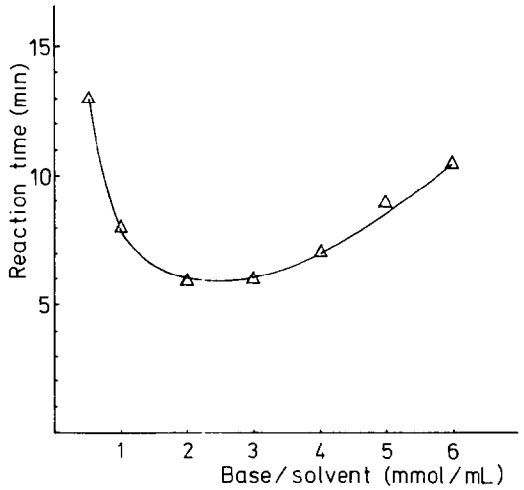

Fig. 4. The reaction time for the permethylation of D-glucose as a function of the NaOH/Me_2SO ratio (mmol/mL) (3 equiv. of NaOH per mol of replaceable H).

quite low, but sufficient to rapidly replace the base consumed in the reaction with the carbohydrate. The hydroxide also retains the water formed in the reaction.

Larger amounts of sodium hydroxide increased the permethylation yields, as shown in Fig. 3. With 3 mol of sodium hydroxide per mol of replaceable H in the sugar, quantitative permethylation was achieved in 6 min. Larger excesses of hydroxide were not necessary, although they had no disturbing effect. Methylation was slower when sodium hydroxide was replaced by potassium hydroxide, due, perhaps, to the lower solubility[12] of potassium hydroxide in methyl sulphoxide.

The optimal base/solvent ratio was also investigated (Fig. 4). Complete methylation was achieved with a base/solvent ratio between 2–3 mmol of sodium

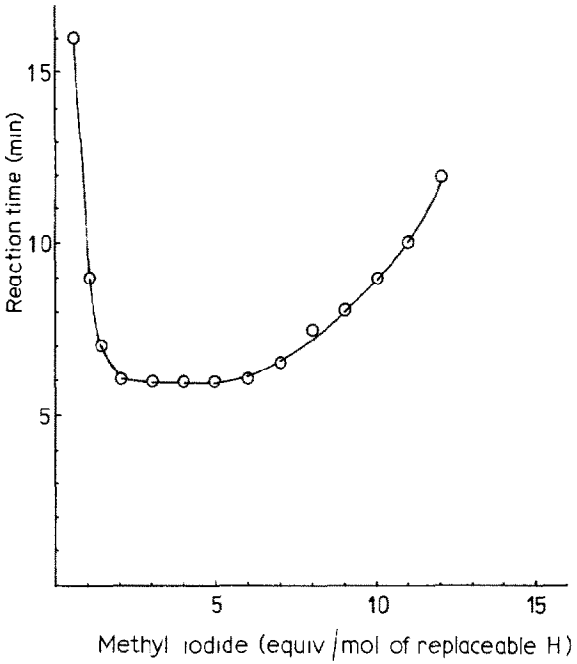

Fig. 5. The dependence of the reaction time for the permethylation of D-glucose on the amount of methyl iodide (3 equiv. of NaOH per mol of replaceable H, 2 mmol of NaOH/mL of Me_2SO).

hydroxide per mL of methyl sulphoxide. At molar ratios higher than 6 mmol per mL of methyl sulphoxide, secondary reactions with colour formation occurred. Methyl iodide must also be added in excess due to its consumption in side reaction *4*. An optimal ratio of 3–5 mol of methyl iodide per mol of replaceable H was established (Fig. 5). The reaction rates were lower for solution in $HCONMe_2$ or CH_3CONMe_2, and the permethylation yields were lower due to the lower polarity[15] of these solvents as compared to that of methyl sulphoxide. All of the reactions were performed at 25° with magnetic stirring at 100 r.p.m. in order to minimise side reactions accompanied by colour formation.

The conformational equilibria of sugars in solution in methyl sulphoxide are similar to those in aqueous media. The gas chromatogram of the fully methylated carbohydrates will therefore reflect the composition of these equilibria. The addition of alkali-metal hydroxide will influence the rate of mutarotation, but no evidence for such chemical transformations as epimerisation or β-elimination was obtained. A solution of α-D-glucose in methyl sulphoxide retained its $[\alpha]_{578}^{25}$ value of +120.5° (*c* 10.1). When solid alkali-metal hydroxide was added, the value changed rapidly to +56.5°. The same equilibrium value was reached in acidic media after 120 min.

Fig. 6 shows a gas chromatogram of the products formed on permethylation of D-glucose in methyl sulphoxide + sodium hydroxide, which reflects the equilib-

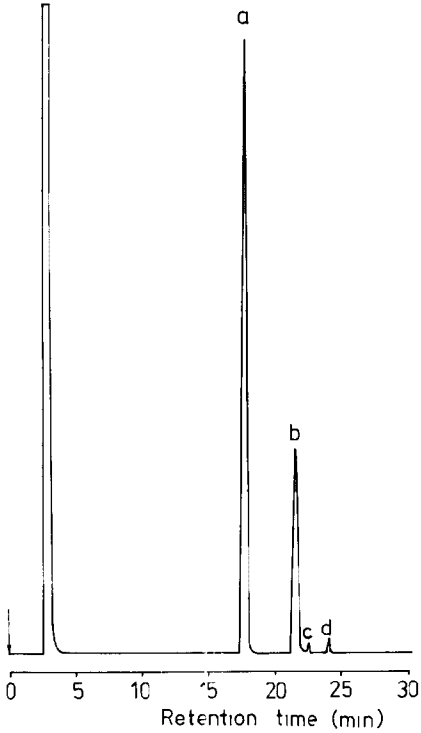

Fig. 6. Gas chromatogram of D-glucose permethylated in methyl sulphoxide + sodium hydroxide (25-m capillary column with SE-30; 140 → 220° at 4°/min): a, β-pyranoside; b, α-pyranoside; c, β-furanoside; d, α-furanoside.

rium composition of D-glucose in this system. All the peaks (a–d) were identified by m.s. as penta-*O*-methyl-D-glucoses. The proportions computed from peak areas are in agreement with those estimated from polarimetric measurements on solutions in water and methyl sulphoxide, namely, β-pyranoside, 72.2; α-pyranoside, 25.3; α-furanoside, 0.8; and β-furanoside, 1.7%.

Thus, the combination methyl iodide, solid alkali-metal hydroxide, and methyl sulphoxide is excellent for the permethylation of carbohydrates. The method is rapid and gives high yields without the formation of the non-sugar products that are characteristic of the Hakomori or other methods.

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