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Avoidance of degradation during the methylation of uronic acids

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ABSTRACT

The action of various strong bases during the methylation of uronic acids has been studied in order to establish conditions that cause a minimum of degradation. The system methyl iodode–sodium hydroxide–methyl sulphoxide gave good yields of methylated uronic acids if the methyl iodide was added before the base. The methylation products were analysed by g.l.c.–m.s.

INTRODUCTION

Methylation of carbohydrates is commonly performed in methyl sulphoxide with methyl iodide and a strong base such as sodium methanesulphinylmethanide[1,2], sodium hydride[3,4], potassium *tert*-butoxide[3], or alkali-metal hydroxide[3]. These methods can also effect simultaneous esterification of carboxyl groups.

Almost all of the methods for the methylation of carbohydrates that contain uronic acids involve prolonged contact between carbohydrate and base[4–6], which can cause extensive degradation *via* β-elimination reactions at uronic acid residues[4–9]. Although Lindberg *et al.*[7] concluded that β-elimination was insignificant, other workers[4,6,10] observed that methylation with sodium methanesulphinylmethanide in methyl sulphoxide caused significant degradation.

We now report conditions that cause minimum degradation during the methylation of uronic acids with methyl iodide and sodium hydroxide in methyl sulphoxide.

EXPERIMENTAL

Instrumentation. — Methylation was performed in thermostated mini-vials (Pierce) with magnetic stirring at 100 r.p.m.

G.l.c. was carried out on a Varian Model 3700 gas chromatograph equipped with an on-column injector[11], a flame-ionisation detector, and a fused-silica capillary column (25 m × 0.32 mm) with a 0.15-μm OV-1 cross-bonded film (Carlo–Erba). The temperature programme was 100°→250° at 4°/min. Nitrogen was used as carrier gas at 1.5

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mL/min. The peak areas were measured with a Minigrator integrator (Spectra-Physics).

E.i.-mass spectra were obtained with a Varian-Matt Model 311 mass spectrometer with an ionisation potential of 70 eV and an ionisation current of 300 μA.

Methylation. — To a solution of uronic acid (5 mg) in methyl sulphoxide (Me_2SO, 0.5 mL) were added methyl iodide (0.1 mL) and then powdered sodium hydroxide (5 mg, and a further 15 mg after 1 min). The mixture was stirred (20 min) in a closed vial at 25°, then poured into 0.5M sulphuric acid (5–10 mL), and extracted with chloroform (1 mL). The extract was washed with water (3 × 10 mL) and dried (Na_2SO_4), and an aliquot was analysed by g.l.c. For quantitative analysis, naphthalene (1 mg) was used as the internal standard. The yield of permethylation was calculated as the sum of the α and β pyranoside and furanoside forms of undegraded sugar. The precision of the assay was evaluated by repeated (5 ×) methylation and g.l.c. analyses (6 ×). When a polysaccharide was methylated, the product was purified by elution from a column (25 × 2 cm) of Sephadex LH-20 with 2:1(v/v) chloroform–acetone and the eluate was concentrated to dryness.

RESULTS AND DISCUSSION

The mechanism in the methylation of uronic acids is very similar to that of neutral carbohydrates[3] and polyalcohols[12], and the reactivity of the hydroxyl groups follows the sequence carboxyl > anomeric > secondary. The degradation reactions are caused by the base and are more pronounced than for neutral carbohydrates. They occur after the addition of the base both before and after reaction with methyl iodide. The products of the base-catalysed degradation process were methylated and analysed by g.l.c.–m.s. Fig. 1 shows the rate of degradation of various uronic acids with various strong bases in methyl sulphoxide. The highest yields of methylated uronic acids were obtained when the methyl iodide was added before the base. The yield of methylated uronic acids also depended on the base used. Methanesulphinylmethanide ($CH_3SOCH_2^-$) ion reacts exothermally with methyl iodide[3], so that the concentration of $CH_3SOCH_2^-$ will decrease rapidly when methyl iodide is added first, ionisation of the hydroxyl groups will be incomplete, and the yield of methylated uronic acid will be reduced. Prolonged reaction of the uronic acid with $CH_3SOCH_2^-$ does not increase the concentration of alkoxide ions but increases the extent of degradation. Prolonged reaction of the uronic acid with only sodium hydride subsquently gave a low yield of methylated uronic acid due to the formation of $CH_3SOCH_2^-$ in large excess. Sodium hydroxide cannot produce $CH_3SOCH_2^-$ and gave the best yield of methylated uronic acid.

Taking into account the above data, the methylation of the uronic acids was optimised for sodium hydroxide added after methyl iodide. For full *O*-methylation of uronic acids, a minimum excess of 2.5 equiv. of sodium hydroxide per mol of replaceable hydrogen was necessary. However, a large excess of sodium hydroxide decreased the time of reaction but increased the extent of degradation. Similar results were obtained with sodium hydride, but the use of sodium hydroxide was more convenient.

The optimal base:solvent ratio was also investigated. Complete methylation was

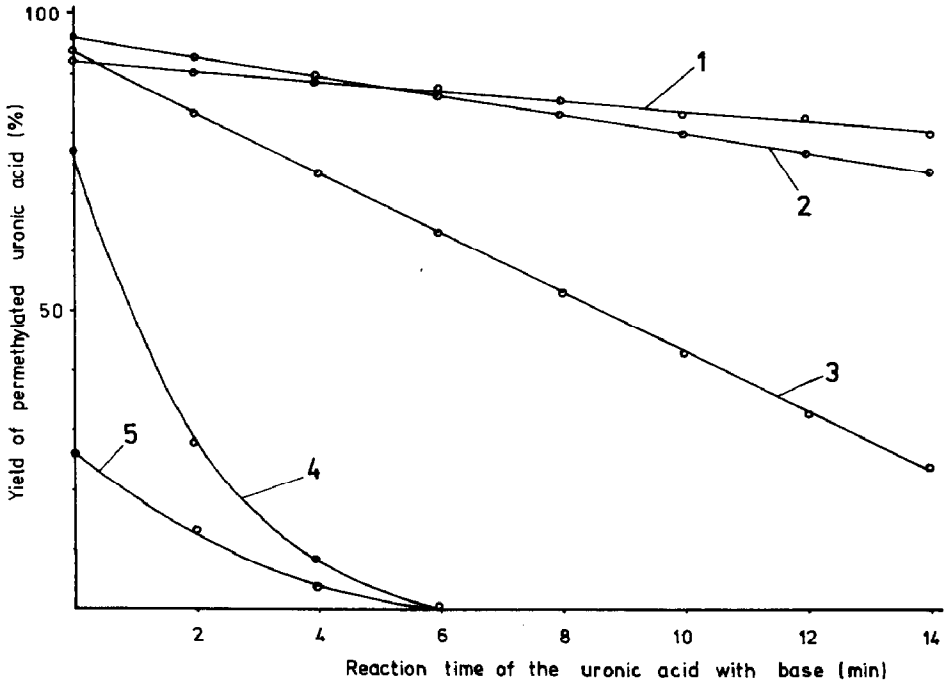

Fig. 1. The degradation of uronic acids with various strong bases (2 mmol of base per mL of Me_2SO; 3 equiv. of base per mol of replaceable H; 25°; after degradation with base, methylation was effected for 15 min with 4.5 equiv. of methyl iodide per mol of replaceable H): 1, pectic acid with NaOH; 2, D-galacturonic acid with NaOH; 3, D-glucuronic acid with NaH; 4, D-glucofuranurono-6,3-lactone with NaOH; 5, D-galacturonic acid with $CH_3SOCH_2^-$.

TABLE I

Methylation of uronic acids, lactones, and neutral carbohydrates in methyl sulphoxide with methyl iodide added before sodium hydroxide

Sample	*Equiv. of NaOH per mol of replaceable H*	*Mmol of NaOH per mL of Me_2SO*	*Reaction time (min)*[a]	*Yield* (%)
D-Galacturonic acid	2.5	1	15	97 ±2.2
	3.5	2	6	92 ±2.8
D-Glucuronic acid	2.5	1	15	95 ±2.7
D-Glucofuranurono-6,3-lactone	2.5	1	5	77 ±6.1
D-Galactose	2.5	1	18	98 ±1.5
	3.5	2	6	98 ±1.5
D-Glucose	2.5	1	18	98 ±1.5
Pectic acid	2.5	1	15	95 ±3.1

[a]Time required to give the maximum yield

achieved with 1 mmol of sodium hydroxide per mL of methyl sulphoxide. At molar ratios >2, degradation was significant.

The low yields of methylated uronic acids caused by the saponification of ester groups can be eliminated if a 30% excess of methyl iodide was used in relation to the base introduced.

Prolonged exposure of methylated uronic acids to base causes degradation by elimination of alcohol molecules[13]. Under the optimal methylation conditions, the yield of methylated uronic acid was practically unchanged after 48 h of methylation but, for ethyl and propyl derivatives, the extent of degradation increased.

The optimal temperature was 20–25° and the reaction mixtures were agitated vigorously in order to diminish side reactions on the surface of the solid base.

The degradation of lactones during methylation compared to the corresponding uronic acids and neutral carbohydrates is shown in Table I. The lactones were more rapidly methylated than the corresponding uronic acids, but the yields of methylated derivatives were lower because degradation was accelerated. Therefore, the formation of lactones before methylation should be avoided. The neutral carbohydrates gave very good yields of methylated products and reacted more slowly than the corresponding uronic acids. The methylation of pectic acid (poly-galacturonic acid) was nearly quantitative and the yield was close to that obtained with galacturonic acid.

Figure 2 shows the gas chromatograms of the products of methylation of glucuronic acid and glucofuranurono-6,3-lactone under the optimal conditions. Separation of the α and β anomers on a packed column was difficult and a capillary column was necessary. The components in the peaks were identified by e.i.-m.s. and the mass spectra of peaks 1–7 are given in Table II.

The interpretations of mass spectra were based on the known fragmentation of methylated neutral monosaccharides[14] and uronic acids[15,16].

The mass spectrum of the component in peak 1 (intense fragment ion at m/z 141 and characteristic ions at m/z 126 and 98) was compatible with the structure methyl (methyl 2,4-dideoxy-3-*O*-methyl-D-*glycero*-hex-1,3-dienopyranosid)uronate. The abundant ion at m/z 141 is due to $[M - MeOCO]^+$ and indicates the presence of a pyran ring.

The components in peaks 2 and 2′ gave identical mass spectra and were identified as the α and β anomers of methyl (methyl 2,4-dideoxy-3-*O*-methyl-hex-2,4-dienopyranosid)uronate. The presence of the pyran ring was indicated by the intense fragment ion at m/z 169 due to $[M - MeO]^+$

The component in peak 3 gave the same fragment ions as those in peaks 1 and 2, but differed from these by the intense fragment ion at m/z 155, the characteristic fragment ion at m/z 97, and the absence of the fragment ion at m/z 101. These data indicate the component to be methyl (methyl 2,3-dideoxy-5-*O*-methyl-D-*glycero*-hex-1,3-dienofuranosid)uronate. The presence of the furan ring was indicated by the characteristic fragment ion at m/z 97.

The components in peaks 4 and 4′ gave identical mass spectra with weak peaks at m/z 187, 169, 159, 143, 141, 127, 115, 100, 95, 83, and 75, and an intense fragment ion at

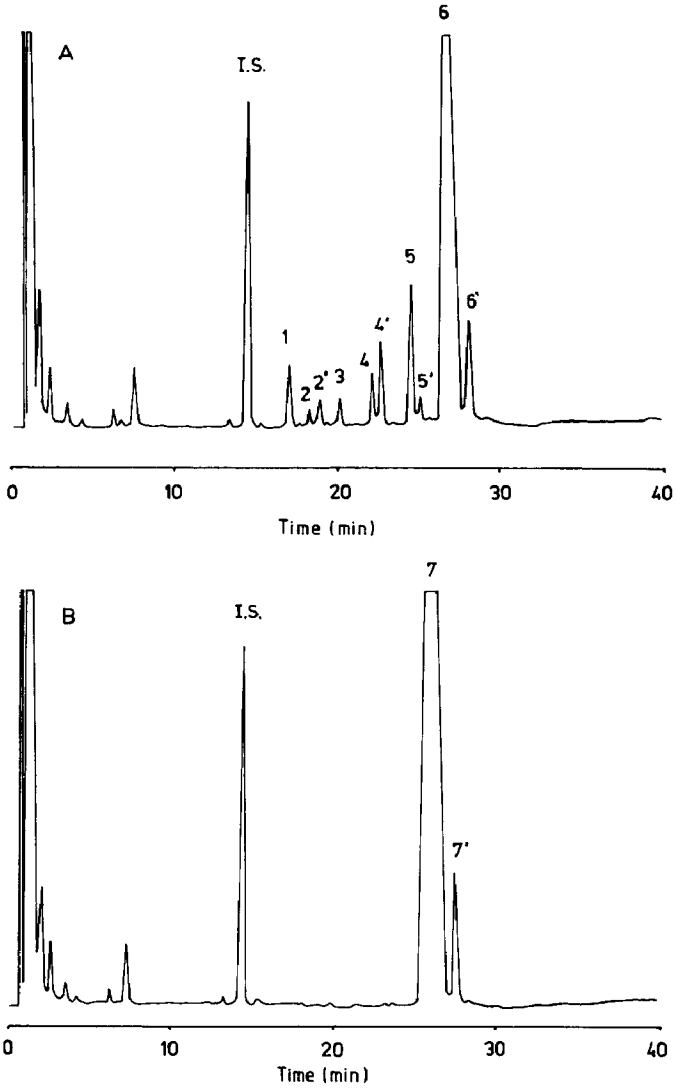

Fig. 2. Gas chromatograms (see Experimental) of the products of methylation of A, D-glucofuranurono-6,3-lactone; and B, D-glucuronic acid in methyl sulphoxide with methyl iodide added before sodium hydroxide (4 equiv. of methyl iodide and 2.5 equiv. of NaOH per mol of replaceable H, 1 mmol of NaOH per mL of Me_2SO, 15 min at 25°): 1, methyl (methyl 2,4-dideoxy-3-*O*-methyl-D-*glycero*-hex-1,3-dienopyranosid)uronate; 2 and 2', methyl (methyl 2,4-dideoxy-3-*O*-methyl-α(β)-hex-2,4-dienopyranosid)uronate; 3, methyl (methyl 2,3-dideoxy-5-*O*-methyl-D-*glycero*-hex-1,3-dienofuranosid)uronate; 4 and 4', methyl (methyl 4-deoxy-2,3-di-*O*-methyl-α(β)-L-*threo*-hex-4-enofuranosid)uronate; 5 and 5', methyl (methyl 3-deoxy-2,5-di-*O*-methyl-α(β)-D-*erythro*-3-enofuranosid)uronate; 6 and 6', methyl (methyl 2,3,5-tri-*O*-methyl-α(β)-D-glucofuranosid)uronate; 7 and 7', methyl (methyl 2,3,4-tri-*O*-methyl-α(β)-D-glucopyranosid)uronate; I.S., naphthalene.

TABLE II

Partial e.i.-mass spectra of the products of methylation of D-glucofuranurono-6,3-lactone (**1–6**) and D-glucuronic acid (**7**)

m/z	*Relative intensity* (%)						
	1	*2 + 2'*	*3*	*4 + 4'*	*5 + 5'*	*6 + 6'*	*7 + 7'*
45	9.4	23.2	25.2	18.2	43.3	56.4	38.2
53	15.0	21.6	41.8	7.1	5.5	6.2	3.5
55	22.8	32.1	27.6	11.5	12.8	8.5	9.6
57	9.2	37.4	32.5	2.8	7.2	11.3	11.2
59	21.2	43.2	50.1	11.2	11.6	3.3	11.8
61	–	–	–	8.3	2.3	1.2	2.2
69	18.1	17.2	42.3	6.5	9.8	3.5	–
71	1.7	20.4	32.8	9.6	6.3	7.5	11.5
73	1.2	8.7	17.1	1.3	31.2	1.2	22.8
75	20.5	25.5	41.6	11.6	35.4	87.6	49.6
81	10.8	12.8	27.5	1.5	5.5	1.6	–
83	7.8	26.6	12.8	8.3	4.6	1.5	–
85	15.4	27.3	29.1	5.8	20.2	10.8	12.8
87	–	7.5	9.5	2.2	6.5	1.5	13.8
88	–	3.9	2.7	0.8	69.2	18.3	69.6
89	1.6	2.6	8.6	0.5	13.6	8.8	7.5
91	–	7.8	5.3	1.2	1.5	0.8	0.6
95	1.8	5.4	13.2	3.8	1.4	0.5	–
97	1.6	10.3	49.7	1.8	2.2	1.2	0.8
98	36.2	20.5	15.6	2.2	1.2	3.5	2.2
99	5.1	36.2	31.5	100.0	9.6	4.8	1.2
100	1.8	3.1	0.8	8.5	1.3	–	–
101	12.8	14.5	–	1.2	100.0	100.0	100.0
102	–	–	–	–	6.8	6.7	5.8
111	4.1	18.3	10.2	3.2	11.5	4.3	3.9
113	17.2	16.5	12.5	4.3	6.3	3.2	1.2
115	–	8.6	26.2	9.4	1.7	3.2	2.6
117	1.2	2.3	3.1	–	2.5	8.2	1.5
125	3.4	27.1	24.3	1.3	2.8	2.3	2.2
126	21.6	8.5	11.2	1.5	–	0.8	–
127	4.2	40.7	31.3	8.3	3.2	2.4	0.5
129	3.1	2.9	3.5	1.6	4.5	15.3	1.6
141	100.0	47.2	42.2	7.8	9.6	3.3	2.8
143	4.5	5.3	–	11.5	4.8	1.8	1.2
145	–	2.2	–	0.5	0.8	8.5	2.3
155	2.2	38.1	100.0	0.8	3.4	2.6	0.8
159	–	39.5	7.8	3.4	2.6	0.5	0.5
161	–	–	–	–	–	22.6	0.5
163	–	–	–	–	–	–	16.6
169	1.8	100.0	10.5	7.4	1.8	11.3	0.5
173	2.3	2.5	1.8	1.5	3.2	5.6	1.9
178	–	–	–	–	–	2.5	0.5
187	–	–	–	8.2	2.3	0.8	0.8
201	–	–	–	–	2.2	1.8	3.8
205	–	–	–	–	–	2.8	1.4
233	–	–	–	–	–	0.2	0.2

m/z 99, and were identified as the α and β anomers of methyl (methyl 4-deoxy-2,3-di-*O*-methyl-L-*threo*-hex-4-enofuranosid)uronate.

The identical mass spectra of the components in peaks 5 and 5′, with fragment ions at *m/z* 201, 187, 173, 155, 141, 129, 111, 101, 88, 75, and 73, indicated the presence of the α and β anomers of methyl (methyl 3-deoxy-2,5-di-*O*-methyl-D-*erythro*-hex-3-enofuranosid)uronate.

The identical mass spectra of the components in peaks 6 and 6′ contained the same fragment ions as the components in the peaks 5 and 5′, and characteristic fragment ions at *m/z* 205 and 161. The fragments at *m/z* 161 $[M - 103]^+$ and 129 $[M - 103 - 32]^+$ indicated a furanoid ring and the components were identified as the α and β anomers of methyl (methyl 2,3,5-tri-*O*-methyl-D-glucofuranosid)uronate.

The mass spectra of the components in peaks 7 and 7′ were identical with those reported[16] and were consistent with the α and β anomers of methyl (methyl 2,3,4-tri-*O*-methyl-D-glucopyranosid)uronate.

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