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STUDIES OF THE ALKALINE DEGRADATION OF MONO-*O*-METHYL-SUCROSES

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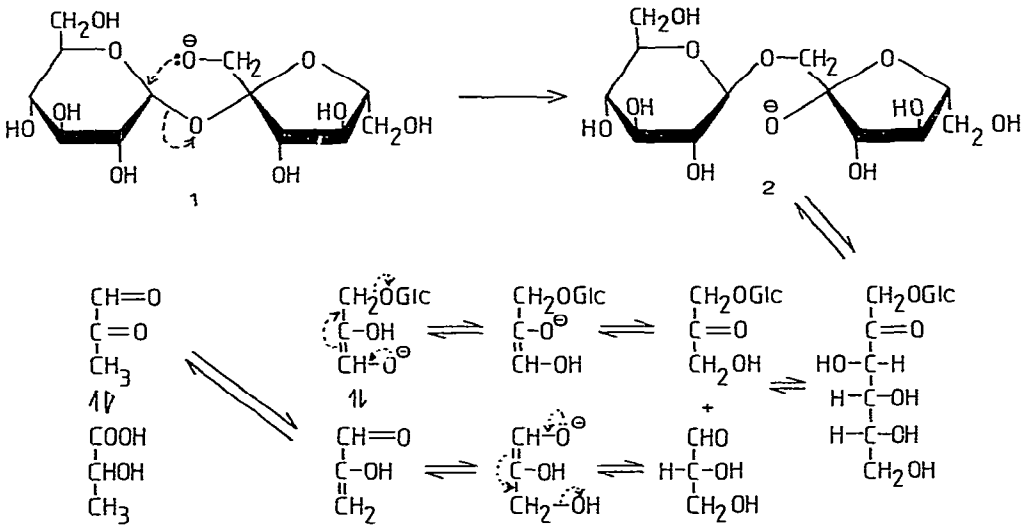
ABSTRACT

The alkaline degradation of a mixture of mono-*O*-methylsucroses has been studied. Some of the components of the mixture are degraded relatively rapidly at a rate similar to that of sucrose, but 1′- and 3′-*O*-methylsucrose are degraded much more slowly. It is suggested that the alkaline degradation of sucrose occurs *via* two initial, and competing, rate-determining steps, each involving $S_Ni cB$ mechanisms. These reactions involve substitution at C-1 by 1′- and 3′-oxyanions, to yield 1- and 3-*O*-α-D-glucopyranosyl-D-fructose, respectively. An analogous substitution by the 6′-oxyanion may also occur to a small extent. The products of the internal substitution reactions are extremely alkali-sensitive and are rapidly degraded mainly to lactic and D-glucometasaccharinic acids. The identification of the components of the mixtures of mono-*O*-methylsucroses before and after alkaline degradation is based on chromatographic and ^1H- and ^{13}C-n.m.r. data

INTRODUCTION

In a recent study of the alkaline degradation of some sucrose derivatives[1], we concluded that a first and rate-determining step in the surprisingly facile, alkaline degradation of sucrose (**1**) is an $S_Ni cB$ substitution effected at C-1 of the D-glucose moiety by an oxyanion derived from HO-1′, to produce 1-*O*-α-D-glucopyranosyl-D-fructose (**2**) (primed numbers refer to the D-fructose moiety). Very rapid degradation of **2** then occurs, mainly to lactic acid, by reactions such as those shown in Scheme 1. The possibility was also clearly stated that analogous, competing substitutions could also occur, involving the 3′- and 6′-oxyanions. We now report evidence that the 3′-oxyanion substitution is very important (together with the 1′ oxyanion) in the alkaline degradation of sucrose, whereas the analogous 6′-reaction, if it occurs, must be relatively unimportant.

The approach adopted in this work involved the alkaline degradation of a mixture of mono-*O*-methylsucroses. Some of the mono-*O*-methylsucroses were degraded very rapidly and it is concluded that these carry *O*-methyl groups on hydroxyl groups that are not involved as oxyanions in the rate-determining steps of



Scheme 1

the degradation of sucrose. Other mono-*O*-methylsucroses (notably 1′- and 3′-) were degraded relatively slowly and it is concluded that the oxyanions from HO-1′ and HO-3′ are necessary for the facile alkaline-degradation of sucrose.

RESULTS AND DISCUSSION

Sucrose was partially methylated by the Haworth method, to give a mixture of mono-, di-, tri-, and tetra-*O*-methylsucroses, together with unchanged sucrose. In preliminary experiments, the mixture was degraded with alkali until all of the sucrose had disappeared and the remaining, relatively alkali-stable mono-*O*-methylsucroses were separated from more highly methylated sucroses by preparative paper chromatography (p.c.). However, the product obtained by this method showed anomalous n.m.r. peaks, reflecting probable contamination with products of alkaline degradation of some of the higher methylated sucroses. It was therefore necessary to isolate the mixture of mono-*O*-methylsucroses before alkaline degradation and this was most simply effected by preparative p.c.

The composition of the mono-*O*-methylsucrose mixture was established on the basis of data obtained by p.c., borate t.l.c., and g.l.c., and by ^{1}H- and ^{13}C-n.m.r. spectroscopy after perdeuteriomethylation. The combined results of all of these methods are necessary for the unequivocal qualitative analysis. None of the methods was satisfactory for an accurate, quantitative analysis of the components, but it was possible to obtain an indication of the relative amounts.

The hydrolysis products of the mono-*O*-methylsucrose mixture were examined by p.c. Mild conditions were used in order to minimise acid decomposition of the

fructose derivatives. The hydrolysates contained glucose, fructose, and their mono-*O*-methyl derivatives. The mono-*O*-methylhexose mixture was isolated by preparative p.c. and examined by analytical p.c. with a range of solvents and detection reagents. Apart from the conclusion that comparable amounts of *O*-methyl-glucoses and -fructoses were present, it was not possible to obtain more-exact evidence of composition by this method, because of the complexity of the mixture. However, the same approach was much more productive with the alkali-stable mono-*O*-methylsucroses (see below). Similar results were obtained by borate t.l.c.

The same mixture of mono-*O*-methylhexoses was *O*-trimethylsilylated and then examined by g.l.c., which gave a complex of peaks (*T* 6–9 min) corresponding to mono-*O*-methylfructoses, a pair of major peaks corresponding to anomers of 2-*O*-methyl-D-glucose (*T* 11.0 and 13.4 min), and a minor peak corresponding to 6-*O*-methyl-D-glucose (*T* 14.2 min). Experiments with authentic compounds showed that all of the possible mono-*O*-methyl-D-fructoses from mono-*O*-methylsucroses were eluted between 6 and 10 min, while all of the possible mono-*O*-methyl-D-glucoses were eluted between 10 and 14 min.

The *O*-methyl-singlet region of the ^{1}H-n.m.r. spectrum of the mono-*O*-methylsucrose mixture after perdeuteriomethylation is shown in Table I and Fig. 1. These results are not easy to analyse, because the *O*-methyl singlets occur in the same region as the complex signals of the ring protons; for most of the individual components of this mixture, the contribution of the *O*-methyl singlet is virtually lost in this background. One *O*-methyl singlet at 3.37 p.p.m. (X in Fig. 1) exceeds all of the others, but unfortunately this singlet is given by two different *O*-methyl groups in *O*-methylsucroses and neither has previously been identified, although we have shown that it must represent any two of the 2, 3′, and 4′ positions[2]. Since g.l.c. of the hydrolysate from the mono-*O*-methylsucrose mixture showed that 2-*O*-methylsucrose is a major component, peak X in Fig. 1 must include the 2-*O*-methyl singlet. It is tentatively concluded that it also represents the 3′- rather than the 4′-*O*-methyl for the

TABLE I

^{1}H-N.M.R. CHEMICAL-SHIFTS OF SINGLETS FOR METHYL GROUPS IN THE ORIGINAL (*A*) AND ALKALI-STABLE (*B*) MONO-*O*-METHYLSUCROSE MIXTURES AFTER PERDEUTERIOMETHYLATION[a]

Peak	*Product* (A)	*Product* (B)	*Assignment*
I	3.168 ±0.004	3.174 ±0.004	1′[b]
II	3.201 ±0.0008	N.d.[d]	6′[b]
III	3.231 ±0.002	N.d.	6[b]
IV	3.251 ±0.001	N.d.	4′[c]
V, VI	3.373 ±0.005	3.368 ±0.003	2, 3′[c]
VII	N.d.	N.d.	4[b]
VIII	N.d.	N.d.	possibly 3[b]

[a]Shifts in p.p.m. downfield from internal Me_4Si, in benzene-d_6. [b]From ref. 2. [c]Tentative assignment, this paper. [d]N.d., not detected.

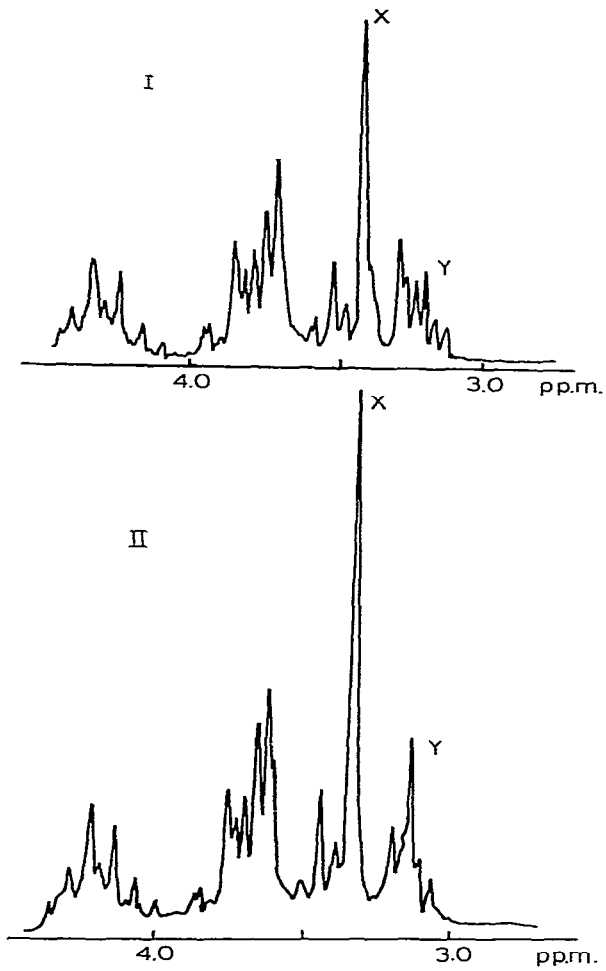


Fig. 1. ^{1}H-N.m.r. spectra of original (I) and alkali-stable (II) mono-*O*-methylsucrose mixtures after perdeuteriomethylation.

following reasons. 3′-*O*-Methylsucrose should be a major product of partial methylation of sucrose, because, for inductive reasons, HO-3 should (with HO-2′) be one of the most acidic groups in sucrose. Furthermore, it is argued below that 3′-*O*-methylsucrose was a major component of the alkali-stable mono-*O*-methylsucroses and that 2-*O*-methylsucrose was absent. Since peak X in Fig. 1 survived the alkaline degradation, it must have contained both the 3′-*O*-methyl and 2-*O*-methyl singlets.

The ^{1}H-n.m.r. spectrum (Table I) therefore provides unequivocal evidence for the presence in the mono-*O*-methylsucrose mixture of 6-, 1′-, and 6′-*O*-methylsucroses and, less directly, for the presence of the 2, 3′, and 4′ isomers. The 3- and 4-*O*-methylsucroses may have been present in small proportions, but could not be detected by n.m.r. spectroscopy.

The ^{13}C-n.m.r. spectrum of the *O*-methyl region for the perdeuteriomethylated mono-*O*-methylsucrose mixture is shown in Table II and Fig. 2. The ^{13}C spectrum is quantitatively less reliable than the ^{1}H spectrum, but the *O*-methyl signals are subject to less interference. The major peak (B at 51.83 p.p.m.) has not previously been assigned and is now tentatively assigned to MeO-2, on the grounds that 2-*O*-methylsucrose was a major component of the mixture, which almost disappeared on alkaline degradation. Earlier work[2] unequivocally identified ^{13}C-n.m.r. peaks for *O*-methyl groups at positions 4, 6, 1′, and 6 of sucrose. From these assignments, it is seen from Fig. 2 that 1′- and 6′-*O*-methylsucroses were also present in significant amount in the mono-*O*-methylsucrose mixture. 6-*O*-Methylsucrose was probably also present (peak E), but 4-*O*-methylsucrose[2] at 53.73 p.p.m. could not be distinguished from the background. The major peak (C) at 51.90 p.p.m. has not previously been assigned, but must represent the 2-, 3-, 3′-, or 4′-*O*-methyl group (see ref. 2). Since Fig. 2 shows that this peak survived alkaline degradation and on arguments similar to those used for the ^{1}H-n.m.r. spectra, this peak is now tentatively assigned to MeO-3′. Peak A, also present in significant amount, must therefore be due to MeO-3 or MeO-4′, and since it has been concluded from the ^{1}H spectra that 4′-*O*-methylsucrose was present in significant amount, whereas 3-*O*-methylsucrose could not be detected, peak A in the ^{13}C spectrum is tentatively assigned to the 4′-*O*-methyl group. The ^{13}C- and ^{1}H-n.m.r. evidence therefore agree in failing to detect either 3- or 4-*O*-methylsucrose in the mono-*O*-methylsucrose mixture.

The above methods of analysis were applied to the mono-*O*-methylsucrose mixture which had been treated with alkali under conditions that would completely degrade sucrose. In all cases, the relatively alkali-stable mixture of mono-*O*-methylsucroses was purified by preparative p.c. before analysis. P.c. results for the mono-*O*-methylhexoses produced on hydrolysis of the alkali-stable product are shown in Table III. The components (two major and one trace) reacted with anisidine and with

TABLE II

^{13}C-N.M.R. CHEMICAL-SHIFTS FOR METHYL GROUPS IN THE ORIGINAL (*A*) AND ALKALI-STABLE (*B*) MONO-*O*-METHYLSUCROSE MIXTURES AFTER PERDEUTERIOMETHYLATION[a]

Peak	*Product* (A)	*Product* (B)	*Assignment*
A	51.76	N.d.[d]	4′[c]
B	51.83	51.83 (trace)	2[c]
C	51.90	51.90	3′[c]
D	52.27	N.d.	6′[b]
E	N.d.	N.d.	6[b]
F	52.76	52.76	1′[b]
G	N.d.	N.d.	4[b]
H	N.d.	N.d.	3[c]

[a]Shifts in p.p.m. downfield from internal Me_4Si, in acetone-d_6. [b]From ref. 2. [c]Tentative assignment, this paper. [d]N.d., Not detected.

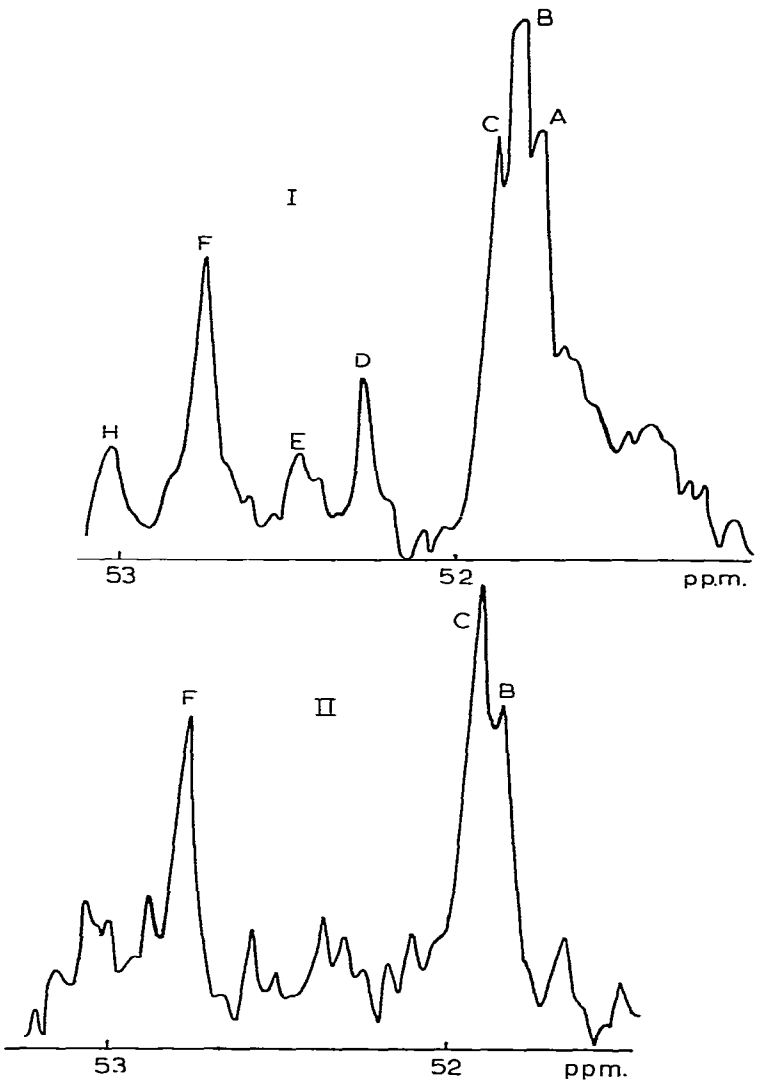


Fig. 2. ^{13}C-N.m.r. spectra of original (I) and alkali-stable (II) mono-*O*-methylsucrose mixtures after perdeuteriomethylation.

naphthoresorcinol sprays, and since the latter is very insensitive to aldoses, it was concluded that all three products were mono-*O*-methylfructoses. Co-chromatography with authentic compounds suggested that they were 1- and 3-*O*-methyl-D-fructose (major components) and 6-*O*-methyl-D-fructose (trace). Borate t.l.c. revealed two ketoses in similar amounts. The first behaved identically with 1-*O*-methyl-D-fructose, and the second corresponded to 3- and/or 6-*O*-methyl-D-fructose.

The mono-*O*-methylhexoses derived from the relatively alkali-stable product were also examined by g.l.c. as the trimethylsilyl ethers, and the results are shown in

TABLE III

PAPER CHROMATOGRAPHY[a] OF THE HYDROLYSIS PRODUCTS FROM THE ALKALI-STABLE, MONO-*O*-METHYLSUCROSE MIXTURE[b] AND OF MONO-*O*-METHYLFRUCTOSES

Sample	R_{FRU}[c]
1-*O*-Methyl-D-fructose	1.38
3-*O*-Methyl-D-fructose	1.47
4-*O*-Methyl-D-fructose	1.41
6-*O*-Methyl-D-fructose	1.53
Mono-*O*-methylsucrose mixture	1.37, 1.45, 1.54 (trace)

[a]Solvent *B*, spray *B*. Double-developed × 2 overnight. [b]After removal of D-glucose. [c]Averages of triplicate papers.

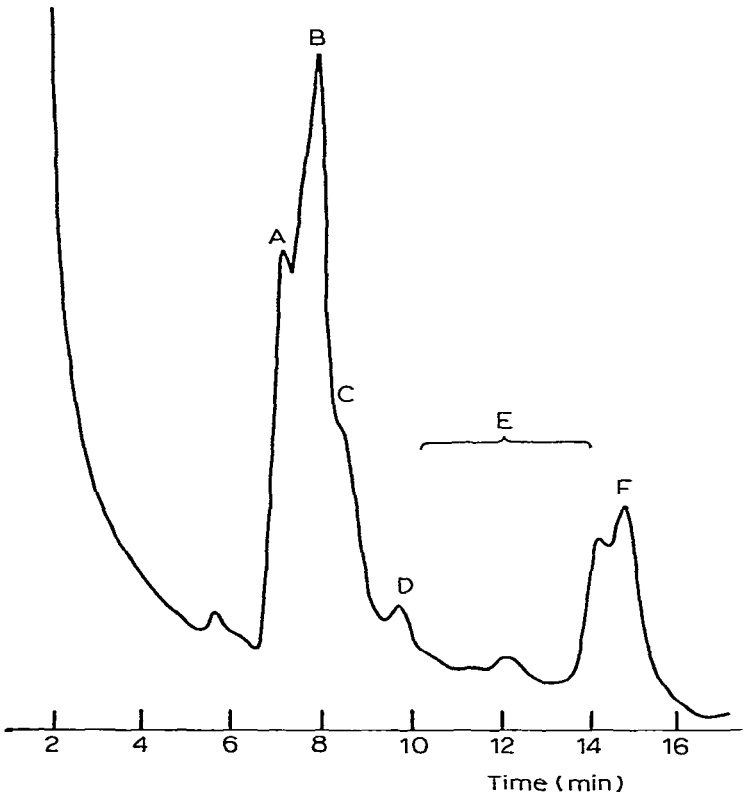


Fig. 3. G.l.c. of trimethylsilyl ethers of mono-*O*-methylhexoses derived from the alkali-stable, mono-*O*-methylsucrose mixture.

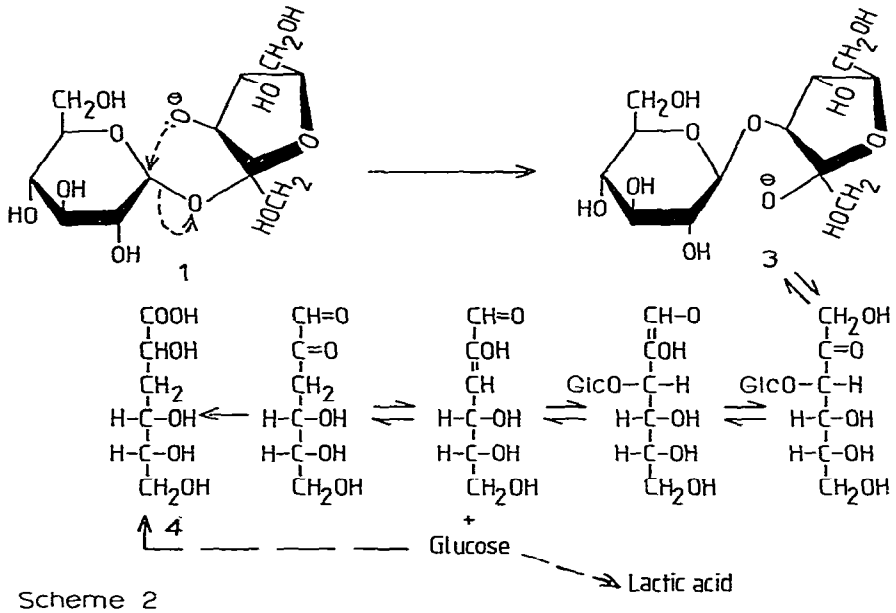
Fig. 3. The region E denotes the range of retention times for all of the possible mono-*O*-methyl-D-glucoses. The peaks found therefore represent mono-*O*-methyl-D-fructoses. They are compatible in general profile with a mixture of 1- and 3-*O*-

methyl-D-fructose, but are not highly diagnostic in this respect. Therefore, the most-definite conclusion from this experiment was that all mono-*O*-methylsucroses carrying the methyl group on the glucose moiety were rapidly degraded by alkali. The double peak at $T \sim 14$ min was not identified.

The ^{1}H-n.m.r. spectrum of the relatively alkali-stable mono-*O*-methylsucroses after perdeuteriomethylation (Fig. 1, Table I) showed one major and one minor peak due to *O*-methyl singlets. One corresponded well with the chemical shift unequivocally assigned[2] to MeO-1′ (peak Y, 3.17 p.p.m.), and the other coincided with that which has now been tentatively assigned to MeO-3′ (peak X, 3.37 p.p.m.). Other evidence shows that the coincident 2-*O*-methylsucrose is removed by the alkali treatment. There was evidently much more 3′- than 1′-*O*-methylsucrose present in the alkali-stable mixture. However, the corresponding spectrum before alkaline degradation shows that there was originally very much less 1′-*O*-methylsucrose present than the combined total of 2- and 3′-*O*-methylsucroses. The ratio of the latter two components is not known and therefore it is not possible to deduce from this evidence the relative (low) rates of alkaline degradation of 1′- and 3′-*O*-methylsucroses.

The ^{13}C-n.m.r. spectrum from the same alkali-stable product is shown in Fig. 2 and Table II. The chemical shifts of the two major methoxyl peaks correspond exactly with the 1′-position (52.76 p.p.m., previously assigned[2]) and the 3′-position (51.90 p.p.m., tentatively assigned in this report). A small amount remains of the 2-*O*-methylsucrose (51.83 p.p.m.) that was a major component of the mixture before alkaline degradation. This material was not present in sufficient amount for detection by other methods of analysis. The difference in the relative sizes of the 1′- and 3′-peaks in the ^{1}H- and ^{13}C-n.m.r. spectra after alkaline degradation is due to nuclear Overhauser effects in the ^{13}C spectrum.

All of the above evidence suggests that 1′- and 3′-*O*-methylsucroses are much more stable to alkali than the other mono-*O*-methylsucroses. It therefore seems possible that ionisation of HO-1′ and HO-3′ is necessary for facile alkaline-degradation of sucrose. A comparison of the rates of alkaline degradation of sucrose and of the mono-*O*-methylsucrose mixture (Fig. 4) suggests that some of the mono-*O*-methylsucroses were degraded at a rate similar to that of sucrose, whereas others (the 1′- and 3′-ethers) were degraded much more slowly, but still following pseudo-first-order kinetics. We therefore propose the hypothesis that there are two major, and competing, rate-determining steps in the alkaline degradation of sucrose. One is the S_NicB substitution at C-1 by the 1′-oxyanion, and this has already been detailed[1] (Scheme 1). In addition, it is now concluded that a similar substitution at C-1 by the 3′-oxyanion occurs, as shown in Scheme 2. In this pathway, the first product is 3-*O*-α-D-glucopyranosyl-D-fructose (3). This product is analogous to 3-*O*-methyl-D-fructose, whose alkaline degradation has already been studied[3], and it must be subject to very rapid degradation by such mechanisms as those shown in Scheme 2. The present demonstration of the prominent involvement of the 3′-oxyanion in alkaline degradation of



Scheme 2

sucrose is fully compatible with the observation[1,4] that melezitose is relatively stable to alkali.

Fig. 4 suggests that the relatively alkali-labile mono-*O*-methylsucroses (curve sector A) comprise ~25% of the total weight of mono-*O*-methylsucroses. The rate of degradation of the relatively labile mono-*O*-methylsucroses may be estimated from the difference in slope between sectors A and B, since the latter represents the rate of degradation of the relatively stable mono-*O*-methylsucroses after removal of the labile isomers. This difference is $\sim 0.7 \times 10^{-5}\text{sec}^{-1}$, which must be multiplied by four to allow for the fact that the relatively labile components comprise only 25% of the total material. The resulting rate of $2.8 \times 10^{-5}\text{sec}^{-1}$ for the degradation of the relatively labile mono-*O*-methylsucroses compares well with that ($2.91 \times 10^{-5}\text{sec}^{-1}$) previously published[1] for sucrose in 1.9M sodium hydroxide and with the sucrose rate ($3.6 \times 10^{-5}\text{sec}^{-1}$) derived from Fig. 4.

Our hypothesis requires that 1′-*O*-methylsucrose is degraded *via* 3′-displacement and that 3′-*O*-methylsucrose is degraded *via* 1′-displacement, whereas sucrose is degraded at a rate that would be the sum of the two types of displacement (*i.e.*, $k_{\text{sucrose}} = k_{1'} + k_{3'}$). The rate of degradation of the relatively stable mono-*O*-methylsucroses derived from sector B in Fig. 4 is $0.3 \times 10^{-5}\text{sec}^{-1}$, and this must approximate to the rate of degradation of 3′-*O*-methylsucrose, which is the major component at this stage of the reaction (Fig. 1). However, it is likely that the rate of degradation of 3′-*O*-methylsucrose is less than the 1′-displacement component ($k_{1'}$) in the degradation of sucrose itself, because of steric hindrance between MeO-3′ and HO-2.

There remains the possibility that an analogous $S_Ni cB$ substitution at C-1 by the 6′-oxyanion may also occur in the alkaline degradation of sucrose. This might be

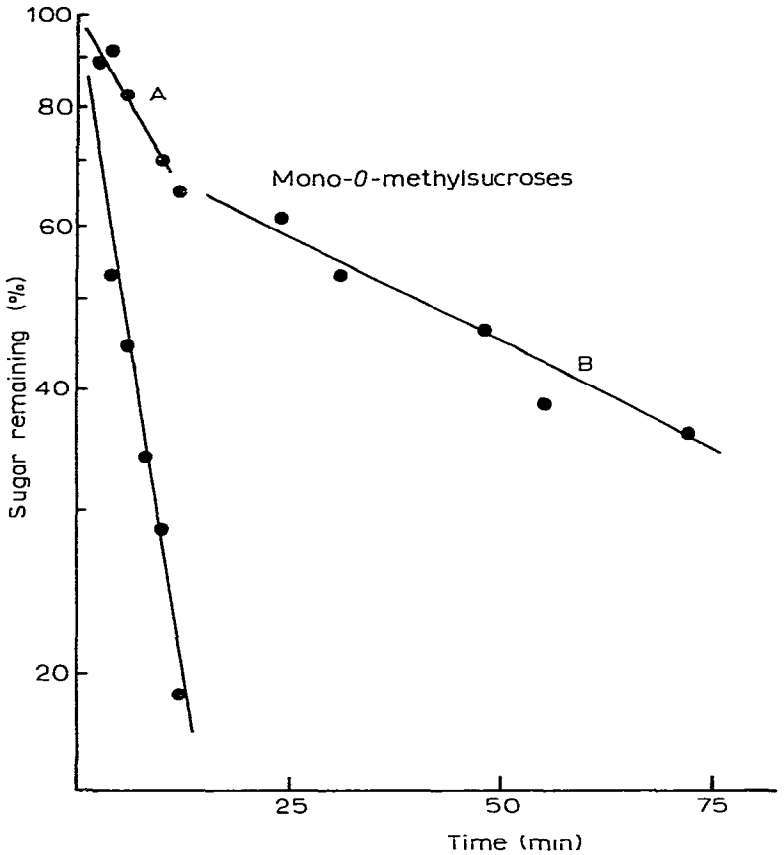


Fig. 4. Alkaline degradation of sucrose and mono-*O*-methylsucrose mixture in 2M sodium hydroxide at 100°.

expected to be less favoured than the 1′- and 3′-mechanisms, because it involves a 6- rather than a 5-membered-ring transition-state. The evidence reported herein is inconclusive in this matter. The ^{1}H- and ^{13}C-n.m.r. spectra show the presence of 6′-*O*-methylsucrose in the original mixture, but it was evidently present in relatively small amount and was not detected after alkaline degradation. In support of a 6′-oxyanion substitution mechanism, there is only the very tentative evidence of a trace component detected by p.c., which may correspond to 6-*O*-methyl-D-fructose derived from the alkali-stable mono-*O*-methylsucroses.

The possible involvement of 3- and 4-oxyanions cannot be eliminated on the available evidence, since 3- or 4-*O*-methylsucrose was not detected in the mono-*O*-methylsucrose mixture. However, the possibility of involvement of the 4-oxyanion has been eliminated previously by demonstrating that 4,6-*O*-isopropylidenesucrose undergoes alkaline degradation at a rate similar to that of sucrose[1], and the use of molecular models reveals no facile, internal substitution mechanisms that would involve the 3-oxyanion.

TABLE IV

YIELD OF D-GLUCOMETASACCHARINIC ACIDS FROM ALKALINE DEGRADATION IN 5.69M SODIUM HYDROXIDE AT 100°

Substrate	*Yield*	
	By weight (%)	*Theoretical[a] (%)*
D-Glucose	17	18
D-Fructose	14	15
3-*O*-Methyl-D-glucose	53	56
3-*O*-Methyl-D-fructose	47	50
Sucrose	14	15

[a]Assuming total carbon return as the acid.

The studies[3] of the alkaline degradation of 3-*O*-methyl-D-fructose might be regarded as models for the mechanisms shown in Scheme 2, although the conditions used were very different, *viz.*, lime water at 25° compared with conc. sodium hydroxide at 100°. 3-*O*-Methyl-D-fructose gave high yields of D-glucometasaccharinic acids (**4**) by mechanisms analogous to those shown in Scheme 2, and the products of alkaline degradation of sucrose were therefore analysed for these acids. The results are shown in Table IV. D-Glucometasaccharinic acid(s) was the only type of six-carbon saccharinic acid detected, but only in yields similar to that given by alkaline degradation of D-glucose and D-fructose. Much higher yields were obtained from 3-*O*-methyl-D-glucose and 3-*O*-methyl-D-fructose, although it should be noted that these yields were lower than those obtained[3] in lime water at 25°. It has previously been shown that the benzylic acid rearrangements involved in such alkaline degradations are specifically catalysed by calcium ions: in sodium hydroxide, other types of reactions compete more effectively with those that yield six-carbon saccharinic acids[5,6]. Evidently such effects mask any detectable increase in yield of the D-glucometasaccharinic acids (in comparison with that from D-glucose) associated with the intermediate formation of **3** in the alkaline degradation of sucrose. It is also interesting to note that D-glucosaccharinic acid was not detected in the degradation of D-glucose, D-fructose, or sucrose in hot, concentrated sodium hydroxide, despite the fact that this saccharinic acid is a significant product from low-temperature, lime-water degradation of the hexoses[6,7].

EXPERIMENTAL

General methods. — Methods used for permethylation and for n.m.r. spectroscopy have been described previously[2].

P.c. was performed at room temperature in the descending mode with Whatman No. 1 paper for analytical, and No. 17 (46 × 57 cm) for preparative, runs. The following solvents and sprays were used: solvents *A*, 1-butanol–ethanol–water (5:1:4,

upper layer): *B*, 1-butanol–benzene–pyridine–water (5:1:3:3, upper layer); sprays *A*, *p*-anisidine hydrochloride[8]: *B*, naphthoresorcinol–phosphoric acid[9]. R_{Glc} represents rate of movement relative to D-glucose, and R_{Fru} relative to D-fructose.

T.l.c. was performed on Kieselgel (Merck, precoated plastic sheets that had been briefly immersed in a 1% solution of boric acid in 95% ethanol[10] and then dried at room temperature) in the ascending mode with 1-butanol–acetone–water (4:5:1), using sprays *A* and *B*.

G.l.c. of the trimethylsilyl ethers of mono-*O*-methylhexoses was performed on a glass column (2 m × 3 mm) packed with OV-17 (1.5%) and QF-1 (2%) on 80/100 Gas-Chrom Q, using a flame-ionisation detector, nitrogen carrier-gas, and a temperature programme of 140° +0.5°.min^{-1}. In the mixtures, peaks were identified by co-chromatography with added, authentic compounds. For trimethylsilyl ethers of sucrose, mono-*O*-methylsucroses, and trehalose, a steel column (1.8 m × 2.4 mm) packed with 3% of SE-30 on 80/100 Chromosorb W-HP was used isothermally at 230°. The mono-*O*-methylsucrose mixture gave a peak with an inflexion on the trailing edge; during alkaline degradation of the mixture, the main peak decreased more rapidly than the inflexion. In the rate analyses detailed below, the total, complex peak was integrated. The same SE-30 column was also used for g.l.c. of the trimethylsilyl ethers of saccharinic acid lactones, isothermally at 170° (*cf.* ref. 11).

Preparation of mono-O-methylsucroses. — An aqueous solution (1 L) containing sucrose (65 g) and sodium hydroxide (56.1 g) was stirred at room temperature while dimethyl sulphate (50 mL) was added dropwise during 60 min. Stirring was continued overnight, and the solution was then neutralised with sulphuric acid and evaporated to dryness. The dried residue was extracted with boiling, absolute ethanol (3 × 100 mL) and the extracts were evaporated, to give a pale-yellow syrup (29.1 g). Analytical p.c. (solvent *A*) showed spots at R_{Glu} 0.63 (sucrose) and at 1.10, 1.79, 2.33, and 3.07, corresponding to mono-, di-, tri-, and tetra-*O*-methylsucrose mixtures, respectively.

A sample (0.687 g) of the partially methylated sucrose was subjected to preparative p.c. (solvent *A*), and the mono-*O*-methylsucrose mixture was isolated as a colourless glass (0.073 g). All such preparations were checked for homogeneity by p.c. (solvent *A*) before combining.

Alkaline degradation of mono-O-methylsucroses. — A solution of the mono-*O*-methylsucrose mixture (0.725 g) in M sodium hydroxide (71.2 mL) contained in a stainless-steel tube was flushed with nitrogen, sealed with a rubber stopper, and heated in an oil bath at 100 ±0.1° for 65 h. The solution was cooled, diluted with water (80 mL), and then neutralised and deionised by stirring with Amberlite resins IRC-50(H^+) (80 mL) and IR-45(HO^-) (10 mL). Evaporation of the filtered solution yielded the crude, alkali-stable mono-*O*-methylsucrose (ASMS) as a brown glass (0.423 g). The whole of this product was subjected to preparative p.c. (solvent *A*), to yield the ASMS mixture as a colourless glass (0.110 g).

Acid hydrolysis of the alkali-stable mono-O-methylsucroses. — The mono-*O*-methylsucrose mixture (0.1 g) was heated in 25mM sulphuric acid (5 mL) at 55 ±0.05°

for 11 h, and then neutralised with barium carbonate, filtered, and evaporated to dryness. Preparative p.c. (solvent *A*) of the product yielded the mixed mono-*O*-methyl-D-glucose and -fructose derivatives free from D-glucose and D-fructose. The mono-*O*-methyl derivatives were compared with authentic compounds by p.c. (solvent *B*). All of the three components detected reacted readily with sprays *A* and *B*, indicating that they were fructose rather than glucose derivatives; the results are summarised in Table III. T.l.c. gave two spots at R_{Glc} 0.9 and 1.3, corresponding in rate of movement and spray response to authentic 1-*O*-methyl-D-fructose and 3- and/or 6-*O*-methyl-D-fructose, respectively.

Measurement of rate of alkaline degradation of the mono-O-methylsucroses. — The sucrose ethers (~10 mg) were dissolved in 2M sodium hydroxide (~1 g) in a steel tube. Samples (~50 μL) of the solution were transferred by syringe to a glass vial, and an aqueous solution (5 μL) of α,α-trehalose was added. To this point, all aliquot calculations were based on weight rather than volume. The solution was then neutralised (pH 5.5–6) by addition of acetic acid (6 μL), and evaporated to dryness at 40°/0.1 mmHg. To the white, solid residue was added dry, redistilled dimethyl sulphoxide (100 μL) followed, after ultrasonication for 15 min, by *N*-trimethylsilylimidazole (50 μL). The mixture was shaken at room temperature for 45 min and then hexane (100 μL) was added followed by further shaking for 5 min. The resulting, upper layer was used to obtain a calibration factor relating trehalose response in g.l.c. to that of the mono-*O*-methylsucroses. When performed on pure sucrose, this procedure gave a coefficient of variation of 3.7 ($n = 5$) for the factor.

The remainder of the alkaline solution was flushed with nitrogen, and the tube was closed with a rubber septum and heated in an oil bath at 100 ±0.1°. At intervals, 50-μL samples were removed by syringe *via* the septum, α,α-trehalose was added, and the samples were analysed as above. The procedure was also repeated with sucrose.

Analysis of D-glucometasaccharinic acids in alkaline-degradation products. — The appropriate sugar (60 mg) was dissolved in 5.69M sodium hydroxide (1.0 mL) at 100° and kept at that temperature for 1 h (monosaccharides) or 24 h (sucrose). After cooling, an aliquot portion (~50 μL) of the solution was removed and weighed, and then a 0.99% aqueous solution (50 μL) of D-glucitol was added followed by water (250 μL) and Amberlite IR-120 (H^+) resin (0.3 g). After shaking at room temperature for 5 min, a portion (250 μL) of the solution was filtered, and dried for 1 h at 40°/1 mmHg over phosphoric oxide. Tri-sil (150 μL, Pierce Chemical Co.) was added and g.l.c. was performed as described above.

Calibration was performed with the same internal standard (D-glucitol) and with authentic samples of α- and β-D-glucometasaccharinic acids. The β isomer was eluted slightly more rapidly than the α isomer (2.75 and 2.8 min, isothermally at 170°), but mixtures were not resolved. Authentic samples of α-D-glucosaccharinolactone and α-D-glucoisosaccharinolactone gave single peaks at 4.7 and 5.4 min ($120° + 8°.min^{-1}$) [*cf.* the meta isomers: 6.3 min (α) and 6.2 min (β)]. The meta-isomer peak was the only six-carbon saccharinic acid detected in the alkaline degradations studied.

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