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Alkaline degradation of fructofuranosides

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Abstract

The degradation of fructose with aqueous $Ca(OH)_2$ gives products similar to the same reaction with glucose. The fructofuranosyl linkage in glycosides cleaves at 130–140°C and the fructose so released degrades to products that are different in proportion to those from free fructose under similar reaction conditions. Non-reducing glycopyranosides are resistant to alkaline degradation at lower temperatures, methyl α-D-glucopyranoside and α,α′-trehalose at even 250°C. For purposes of comparison, the alkaline degradation of glucose, lactose, and xylose was also studied. The relative contributions of the intermediate enediols to the reaction pathways are discussed.

Keywords: Alkaline degradation; Fructofuranosides

1. Introduction

The alkaline degradation of reducing monosaccharides has been shown to proceed through 1,2-, 2,3-, and 3,4-enediol intermediates to give numerous products, principally the saccharinic acids [1–4]. This type of degradation is prevented by the absence of a reducing group in such compounds as α,α′-trehalose, methyl α-D-glucopyranoside, and gluconic acid, which are stable to alkaline degradation at temperatures up to 250°C. In reducing di- and oligo-saccharides, the degradation proceeds as expected, with β-elimination of the non-reducing residues by the well-established "peeling" process [5,6]. This process stops when the reducing residue rearranges without elimination of the glycosyloxy substituent on the penultimate sugar residue [7–10].

The stability of non-reducing sugars to alkaline degradation depends upon the resistance of the constituent glycosidic bonds to cleavage. That this does occur at 150–250°C has been known for some time in the study on the production of lactic acid

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from sucrose [11,12]. In this case the fructofuranosyl bond is broken, since under the same reaction conditions the glucopyranosyl bond is stable.

The consequence of the release of fructose at high temperature on the sequence of reactions is here studied in comparison to the degradation of free fructose.

2. Results and discussion

Under alkaline conditions, each of the 1,2-, 2,3-, and 3,4-enediols of reducing monosaccharides may undergo rearrangements that can be identified as primary pathways of degradation. Many products of the primary pathways retain the configurations of the initial sugar, for example the saccharinic acids from glucose and fructose have *ribo-*, *arabino-*, *threo-*, or *erythro*-configurations. Some of the intermediate products of these primary reactions are aldehydes that may undergo aldol condensation to give new sugars, the degradations of which are identified here as secondary reactions since the configurations of the resulting products may have lost the stereochemistry of the chiral centers of the primary sugar.

The primary and secondary reactions are similar and involve:

reaction *1*: enediol formation

reaction *2*: β-elimination of a hydroxyl or glycosyloxy group from the enediol to form a deoxy diketo derivative

reaction *3*: benzilic acid-type rearrangements of the deoxy-diketo derivative

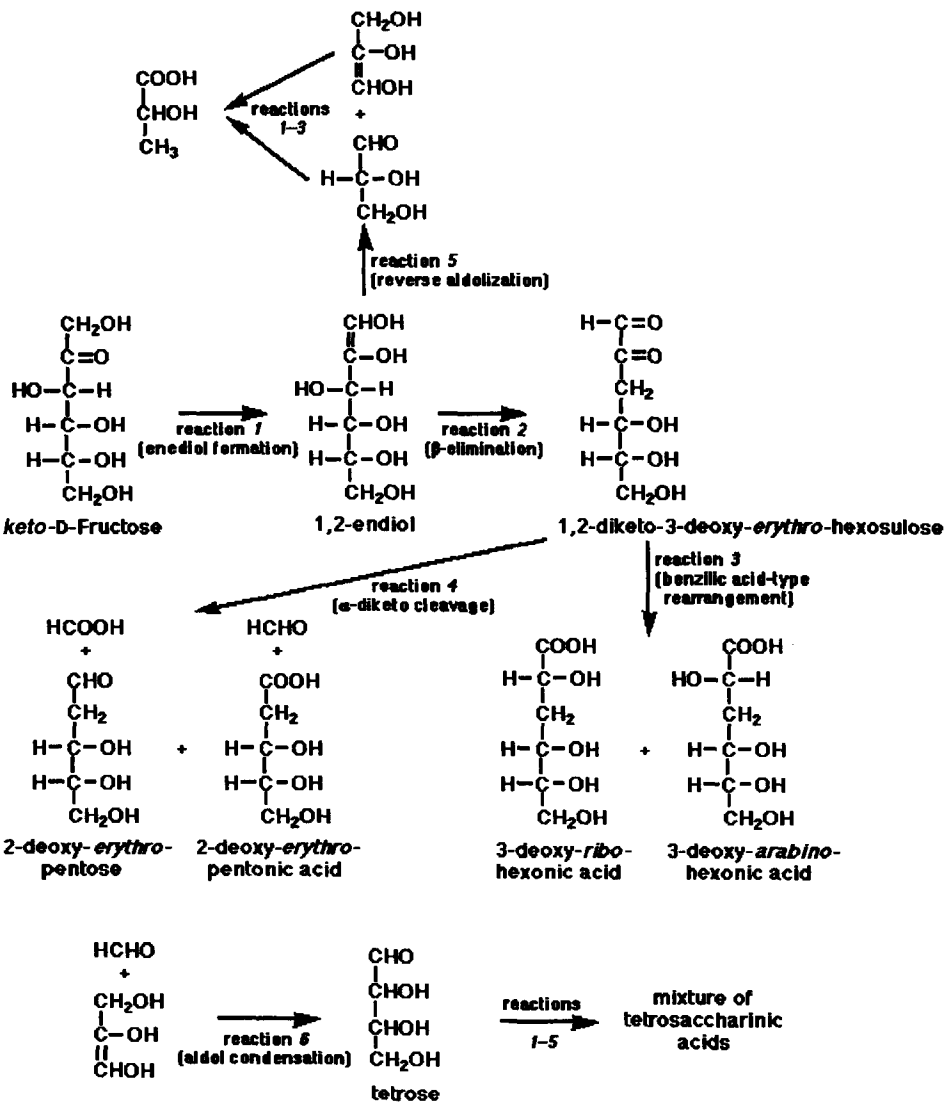
reaction *4*: α-diketo cleavage to give potentially two pairs of products, each pair including an acid and an aldehyde

reaction *5*: reverse aldolization of the enediol to give two reducing fragments

reaction *6*: aldol condensation of two aldehydes to give new reducing sugars.

Reactions *1–5* reflect the primary pathways; reaction *6* initiates the secondary pathways that proceed by way of reactions *1–6*. These may be illustrated for D-fructose 1,2-enediol by Scheme 1, in which reaction *6* (realdolization) is one example of many. Characteristic, therefore, of the primary degradation of the 1,2-enediol of D-fructose would be formation of 3-deoxy-*ribo-* and *arabino*-hexonic acids, lactic acid, and 2-deoxy-*erythro*-pentonic acid. The 2,3-enediol intermediate would be characterized by the formation of 2-*C*-methyl-*ribo*-pentonic acid and 2-*C*-(hydroxymethyl)-*threo* and *erythro*-pentonic acids, 3,4-dihydroxybutanoic acid, glycolic acid, and erythronic acid by these same type reactions. The 3,4-enediol of D-fructose would form parasaccharinic acid isomers, glyceric acid, and 3-hydroxypropanoic acid.

As noted by several workers [13–15], realdolization of intermediate aldehydo-fragments (reaction *6*) to initiate secondary pathways is a significant component in the complex reaction mechanism of the alkaline degradation of monosaccharides. Since newly-formed reducing sugars may then degrade in the manner of the original monosaccharide (reactions *1–5*), an analysis of the total products would show the predominance, if any, of a favored pathway. Indications of such a favored pathway will depend principally upon identification of products that can only arise by realdolization, such as 3-deoxypentonic acid from fructose. Some of the products may be derived from either pathway, such as 3-deoxy-*ribo*-hexonic acid from D-fructose.



Scheme 1.

Glucose and fructose.—It may be noted from Table 1 that the principal organic acids from the degradation of both glucose and fructose at 100 and 250°C are similar at the respective temperatures. The principal product is lactic acid, with significant amounts also of glycolic acid, 2,4-dihydroxybutanoic acid, 2-*C*-methylglyceric acid, 3-deoxy-*erythro*- and *threo*-pentonic acid, 2-*C*-methylribonic acid, and 3-deoxy-*ribo*- and 3-deoxy-*arabino*-hexonic acids. The latter C_6 saccharinic acids are indicative of the 1,2-enediol intermediate and, together with lactic acid, demonstrate the primary pathway.

Table 1
Reaction products from selected carbohydrates in $Ca(OH)_2$ solution

Products [a]	Fructose				Glucose				Furanosides					
									Sucrose [b,d]					
	100 [c]		250 [d]		100 [c]		250 [d]		150 [e]		200 [e]		250 [e]	
	(mmol	mol%)	(mmol	mol%)	(mmol	mol%)	(mmol	mol%)	(mmol	mol%)	(mmol	mol%)	(mmol	mol%)
Saccharinic acids														
C_2 Ethanoic acid, 2-hydroxy-(glycolic acid)	66	8	75	15	63	8	80	10	13	14	72	9	85	11
C_3 Propanoic acid, 2-hydroxy-(lactic acid)	317	39	353	71	258	33	457	60	78	84	604	79	571	74
Propanoic acid, 3-hydroxy-	4	$\ll 1$	4	1	5	1	2	$\ll 1$	0	0	4	$\ll 1$	2	$\ll 1$
Propanoic acid, 2,3-dihydroxy-(glyceric acid)	19	2	6	1	25	3	20	3	0	0	8	1	6	1
C_4 Propanoic acid, 2-methyl-2,3-dihydroxy-(2-*C*-methylglyceric acid)	32	4	4	1	66	8	30	4	0	0	6	1	6	1
Butanoic acid, 2-hydroxy-	5	1	4	1	5	1	9	1	0	0	7	1	7	1
Butanoic acid, 2,4-dihydroxy-	62	8	26	5	66	8	80	10	1	1	74	10	61	8
Butanoic acid, 3,4-dihydroxy-	7	1	0	0	8	1	0	0	0	0	0	0	0	0
Erythronic acid	5	1	0	0	9	1	0	0	0	0	0	0	0	0
Threonic acid	5	1	0	0	11	1	0	0	0	0	0	0	0	0
C_5 2-*C*-methylerythronic acid	3	$\ll 1$	$\ll 1$	$\ll 1$	4	1	$\ll 1$	$\ll 1$	0	0	0	0	0	0
2-*C*-methylthreonic acid	2	$\ll 1$	$\ll 1$	$\ll 1$	2	$\ll 1$	$\ll 1$	$\ll 1$	0	0	0	0	0	0
Ribonic acid	25	3	< 1	$\ll 1$	23	3	< 1	$\ll 1$	0	0	0	0	1	$\ll 1$
2-Deoxy-*erythro*-pentonic acid	4	< 1	0	0	3	< 1	0	0	0	0	0	0	0	0
2-Deoxy-*threo*-pentonic acid	7	1	0	0	14	2	0	0	0	0	0	0	0	0
3-Deoxy-*erythro*-pentonic acid	17	2	6	1	12	2	29	4	0	0	0	0	1	$\ll 1$
3-Deoxy-*threo*-pentonic acid	29	4	6	1	18	2	32	4	0	0	0	0	1	$\ll 1$
3,4-Dideoxy-pentonic acid	5	1	< 1	$\ll 1$	15	2	10	1	0	0	0	0	1	$\ll 1$
3-Deoxy-2-C-(hydroxymethyl)tetronic acid	7	1	< 1	$\ll 1$	9	1	< 1	$\ll 1$	0	0	0	0	0	0
C_6 2-*C*-methylribonic acid	45	6	< 1	$\ll 1$	34	4	< 1	$\ll 1$	0	0	0	0	< 1	$\ll 1$
Parasaccharinic acid	3	$\ll 1$	0	0	4	1	0	0	0	0	0	0	0	0
2-Deoxy-hexonic acid	0	0	0	0	6	1	0	0	0	0	0	0	0	0
3-Deoxy-*lyxo* and *xylo*-hexonic acid	12	1	2	$\ll 1$	12	2	5	1	< 1	$\ll 1$	22	3	9	1
3-Deoxy-*ribo* and *arabino*-hexonic acid	79	10	11	2	56	7	13	2	0	0	49	6	22	3
3-Deoxy-2-C-(hydroxymethyl)-*erythro*- and *threo*-pentonic acid	44	6	< 1	$\ll 1$	53	7	< 1	$\ll 1$	0	0	0	0	0	0
Total amounts of acids (mmol)	804		498		782		766		93		845		775	
Unreacted carbohydrate [b]	0		0		0		0		810		61		39	

Saccharinic acids												
C_2 Ethanoic acid, 2-hydroxy- (glycolic acid)	11	15	51	9	73	13	22	13	46	8	92	9
C_3 Propanoic acid, 2-hydroxy- (lactic acid)	61	84	452	78	413	73	143	85	518	85	813	84
Propanoic acid, 3-hydroxy-	0	0	0	0	2	≪1	0	0	3	≪1	2	≪1
Propanoic acid, 2,3-dihydroxy- (glyceric acid)	<1	≪1	5	1	6	1	1	≪1	6	1	7	1
C_4 Propanoic acid, 2-methyl-2,3-dihydroxy- (2-*C*-methylglyceric acid)	<1	≪1	7	1	5	1	1	≪1	3	≪1	4	<1
Butanoic acid, 2-hydroxy-	<1	≪1	7	1	6	1	1	≪1	5	1	7	1
Butanoic acid, 2,4-dihydroxy-	1	1	36	6	29	5	1	≪1	18	3	23	2
Butanoic acid, 3,4-dihydroxy-	0	0	0	0	<1	≪1	0	0	0	0	1	≪1
Erythronic acid	0	0	0	0	0	0	0	0	0	0	≪1	≪1
Threonic acid	0	0	0	0	0	0	0	0	0	0	≪1	≪1
C_5 2-*C*-methylerythronic acid	0	0	0	0	0	0	0	0	0	0	0	0
2-*C*-methylthreonic acid	0	0	0	0	0	0	0	0	0	0	0	0
Ribonic acid	0	0	0	0	1	≪1	0	0	0	0	1	≪1
2-Deoxy-*erythro*-pentonic acid	0	0	0	0	0	0	0	0	0	0	0	0
2-Deoxy-*threo*-pentonic acid	0	0	0	0	0	0	0	0	0	0	0	0
3-Deoxy-*erythro*-pentonic acid	0	0	0	0	1	≪1	0	0	0	0	1	≪1
3-Deoxy-*threo*-pentonic acid	0	0	0	0	1	≪1	0	0	0	0	2	≪1
3,4-Dideoxy-pentonic acid	0	0	0	0	1	≪1	0	0	0	0	<1	≪1
3-Deoxy-2-C-(hydroxymethyl)tetronic acid	0	0	0	0	0	0	0	0	0	0	0	0
C_6 2-*C*-methylribonic acid	0	0	0	0	0	0	0	0	0	0	4	≪1
Parasaccharinic acid	0	0	0	0	0	0	0	0	0	0	0	0
2-Deoxy-hexonic acid	0	0	0	0	0	0	0	0	0	0	0	0
3-Deoxy-*lyxo* and *xylo*-hexonic acid	0	0	7	1	11	2	0	0	1	≪1	1	≪1
3-Deoxy-*ribo* and *arabino*-hexonic acid	0	0	12	2	20	4	0	0	12	2	12	1
3-Deoxy-2-C-(hydroxymethyl)-*erythro*- and *threo*-pentonic acid	0	0	0	0	0	0	0	0	0	0	0	0
Total amounts of acids (mmol)	73		578		567		169		612		969	
Unreacted carbohydrate [b]	583		52		57		640		72		73	

[a] *mmol of product per mol of initial carbohydrate*; <1 and ≪1 indicate less than 0.5 mmol (or %) and trace amounts respectively. [b] mmol of product per mol of anyhydrous hexose equivalent. [c] Reactions held at 100°C for 2 h. [d] Reactions held at the reaction temperatures for 10 min. [e] Reaction temperature.

The presence of the 2,3-enediol intermediate is shown, particularly at 100°C, by identification of 3,4-dihydroxybutanoic acid, erythronic acid, 2-*C*-methyl-*ribo*-pentonic acid, and 2-*C*-(hydroxymethyl)-*erythro*-pentonic acid and its *threo*-isomer. As in the case of lactic acid, although glycolic acid is a primary product it can also derive from many other sugars formed by realdolization of aldehydo intermediates.

The involvement of fructose 3,4-enediol is indicated by the presence of glyceric acid and 3-hydroxypropanoic acid as fragmentation products of the 3,4-dicarbonyl-(2)5-deoxy intermediate and parasaccharinic acid as a rearrangement product. Parasaccharinic acid is produced in minor amounts at 100°C from both glucose and fructose, but not in detectable amounts at 250°C, which, together with the significant yields of the above C_3 acids, suggests that fragmentation and not a benzilic acid-type rearrangement of the 3,4-diketo-(2)5-deoxy intermediate predominates at higher temperatures. Alternatively, by aldol condensation of glycolaldehyde and glyceraldehyde, which are reverse aldolization products in the primary reactions of 1,2- and 2,3-enediols of the hexoses, these C_3 acids could derive from the resulting pentose in secondary reactions.

As noted in earlier studies [16], Ca^{2+} enhances realdolization reactions, which are seen in the present work by the presence of *xylo*- and *lyxo*-isomers of the C_6 saccharinic acid and the 3-deoxy-*erythro*-pentonic acid and its *threo*-isomer from both glucose and fructose.

Differences in the relative yields of acids from glucose and fructose are principally reflected in their response to elevated reaction temperatures. The total yield of non-volatile acids at 250°C is 62% lower than at 100°C for fructose, but the proportion of lactic acid is nearly double at 250°C on a mol% basis. Also, at 250°C, fructose degrades to a lesser extent to several of the C_5 and C_6 acids than at 100°C (Table 1). This is in contrast to the results with glucose. However, these differences are relatively small and the overall observation in both sugars would be that fragmentation reactions and subsequent realdolization of the aldehydo-fragments take greater emphasis at higher temperatures than formation of the primary C_5 and C_6 saccharinic acids.

Sucrose, raffinose, and inulin.—These sugars represent fructofuranosides in non-reducing oligosaccharides where alkaline degradation cannot occur without prior cleavage of some glycosidic bonds. The breakdown occurs at elevated temperatures, becoming apparent at 130–140°C (from the recovery of unreacted starting sugars; data not shown), due to the splitting of the fructofuranosyl linkage; methyl α-D-glucopyranoside and α,α′-trehalose were stable in 0.1 M $Ca(OH)_2$ at 250°C. At 150°C the residual sugars, as determined by the phenol–sulfuric acid method [17], were 81% for sucrose, 58% for raffinose, and 64% for inulin. Cleavage of the fructofuranosyl linkage at elevated temperatures immediately exposes the released reducing sugars, fructose and glucose from sucrose, melibiose and fructose from raffinose, and fructose from inulin, to the high reaction temperatures. Consequently, the degradation pathways may differ from those seen where the reducing sugar is gradually heated to 100 or 250°C, the final reaction temperatures.

For sucrose at 200 and 250°C in 0.1 M $Ca(OH)_2$, as compared to glucose and fructose at 250°C, the yield of lactic acid is increased at the expense of the pentonic acids and 2-*C*-methylglyceric acid. The yield of 3-deoxy-*ribo*-hexonic acid is similar at all temperatures. The same observations are generally true for raffinose and inulin,

except that the yield of lactic acid at 250°C is 28% less from raffinose and 42% more from inulin than from sucrose. The results are consistent with the predominance of reactions involving the 1,2-enediol intermediate, that is, reverse aldolization and rearrangement gave lactic acid and benzilic acid-type rearrangement of the 1,2-diketo-3-deoxy intermediate gave 3-deoxy-*ribo*-(or *arabino*-)-hexonic acid. Cleavage of the 1,2-diketo-3-deoxy intermediate to give pentonic acids was low.

The nearly equimolar presence of glycolic acid and 2,4-dihydroxybutanoic acid, with relatively small amounts, if any, of 3,4-dihydroxybutanoic acid speaks to the degradation of the 2,3-diketo-4-deoxy intermediate. The α-dicarbonyl cleavage to glycolaldehyde and 3,4-dihydroxybutanoic acid was not favored, whereas the alternative cleavage to glycolic acid and 3,4-dihydroxybutanal explains in part the presence of glycolic acid in the alkaline degradation. This sequence of reactions does not account for the significant amount of 2,4-dihydroxybutanoic acid, a product of the benzilic acid-type rearrangement of the 1,2-diketo-3-deoxy intermediate of tetroses. The latter could arise from the hexoses by either the reverse aldolization of the fructose 2,3-enediol intermediate to give tetrose and glycolaldehyde, the latter by realdolization giving another tetrose. The two tetrose intermediates would then rearrange as their 1,2-enediol to give 2,4-dihydroxybutanoic acid or 3-hydroxypropanoic acid, both of which are found in the reaction products.

In summary, high-temperature alkaline cleavage of sucrose results in a predominance of primary reactions of the 1,2-enediol intermediate from the released glucose and fructose to produce lactic acid and 3-deoxy-*ribo* and *arabino*-hexonic acids. There is a less significant involvement of the 2,3-enediol intermediate from which glycolic, 2,4-dihydroxybutanoic, 2-*C*-methylglyceric, and 3-hydroxypropanoic acids are derived by a 2,3-dicarbony cleavage. No 2-*C*-methyl or 2-*C*-(hydroxymethyl) pentonic acids are detected and there is minimal evidence of primary products from the 3,4-enediol intermediate.

Inulin gives results very similar to sucrose, as does raffinose, the latter however producing the 3-deoxy-*xylo* and *lyxo*-hexonic acids from the galactose residue in the released melibiose. Melibiose, a (1 → 6)-α-D-galactopyranoside, reacts in a "peeling" manner where the leaving group at C-6 plays little role in directing the saccharinic acid formation of the reducing glucosyl residue [18].

Lactose.—In contrast to melibiose, the (1 → 4)-β-D-galactosyl residue in lactose plays a significant role in the pathway of alkaline degradation [19]. At 100°C the favored saccharinic acids are 3-deoxy-2-*C*-(hydroxymethyl)-*threo* and *erythro*-pentonic acids with, however, significant 3-deoxy-*lyxo*- and *xylo*-hexonic acids (Table 2). With increased reaction temperatures the total yield of acid decreases, mainly at the expense of the C_6 saccharinic acids but also with reduction of lactic acid. The mole percentage of acids in the mixture does not change greatly. The difference between the reaction products of glucose and lactose at similar reaction temperatures clearly support the "peeling" reaction as the process of glycosidic cleavage, as opposed to a hydrolysis as seen in the fructofuranosides.

Xylose.—Alkaline degradation of xylose proceeds in a manner similar to glucose (Tables 1 and 2) and it permits a facile way to demonstrate the importance of the secondary pathways and aldol condensation of intermediate C_3-aldehyde products. The

Table 2
Reaction products from lactose and xylose in $Ca(OH)_2$ solution

Products [a]	Lactose [b]								Xylose	
	100 [c]		150 [d]		200 [d]		250 [d]		200 [d]	
	(mmol	mol%)	(mmol	mol%)	(mmol	mol%)	(mmol	mol%)	(mmol	mol%)
Saccharinic acids										
C_2 Ethanoic acid, 2-hydroxy- (glycolic acid)	41	5	34	10	27	7	34	9	41	7
C_3 Propanoic acid, 2-hydroxy- (lactic acid)	234	29	186	52	135	36	182	45	320	57
Propanoic acid, 3-hydroxy-	0	0	0	0	0	0	1	$\ll 1$	3	< 1
Propanoic acid, 2,3-dihydroxy- (glyceric acid)	10	1	3	1	5	1	4	1	8	1
C_4 Propanoic acid, 2-methyl-2,3-dihydroxy- (2-*C*-methylglyceric acid)	9	1	2	< 1	2	< 1	3	< 1	18	3
Butanoic acid, 2-hydroxy-	2	$\ll 1$	2	< 1	2	< 1	2	$\ll 1$	6	1
Butanoic acid, 2,4-dihydroxy-	19	2	13	4	14	4	18	4	56	10
Butanoic acid, 3,4-dihydroxy-	7	1	0	0	0	0	0	0	1	$\ll 1$
Erythronic acid	1	$\ll 1$	0	0	0	0	$\ll 1$	$\ll 1$	3	< 1
Threonic acid	1	$\ll 1$	0	0	0	0	$\ll 1$	$\ll 1$	3	< 1
C_5 2-*C*-methylerythronic acid	0	0	0	0	0	0	0	0	5	1
2-*C*-methylthreonic acid	0	0	0	0	0	0	0	0	3	< 1
Ribonic acid	10	1	5	1	6	2	8	2	< 1	$\ll 1$
2-Deoxy-*erythro*-pentonic acid	0	0	0	0	0	0	0	0	0	0
2-Deoxy-*threo*-pentonic acid	0	0	0	0	0	0	0	0	0	0
3-Deoxy-*erythro*-pentonic acid	10	1	4	1	7	2	6	1	27	5
3-Deoxy-*threo*-pentonic acid	12	2	4	1	9	2	8	2	39	7
3,4-Dideoxy-pentonic acid	0	0	0	0	0	0	4	1	6	1
3-Deoxy-2-C-(hydroxymethyl)tetronic acid	2	$\ll 1$	0	0	0	0	1	$\ll 1$	7	1
C_6 2-*C*-methylribonic acid	7	1	3	< 1	0	0	0	0	0	0
Parasaccharinic acid	0	0	0	0	0	0	0	0	0	0
2-Deoxy-hexonic acid	0	0	0	0	0	0	0	0	0	0
3-Deoxy-*lyxo* and *xylo*-hexonic acid	130	16	28	8	47	12	37	9	11	2
3-Deoxy-*ribo* and *arabino*-hexonic acid	0	0	0	0	0	0	0	0	5	1
3-Deoxy-2-C-(hydroxymethyl)-*threo*- and *erythro*-pentonic acid	302	38	70	20	123	33	97	24	2	$\ll 1$
Total amounts of acids (mmol)	798		355		377		405		563	
Unreacted carbohydrate [b]	22		111		0		45		10	

[a,b,c,d] Refer to Table 1.

formation of the hexonic acids, principally the 3-deoxy derivatives, arises by the reverse aldolization reaction of the xylose-1,2-enediol to give glyceraldehyde, two molecules of which undergo realdolization to give hexose, which as the 1,2-enediol produced principally 3-deoxy-*lyxo* and *xylo*-hexonic acids indicating preferred *galacto*- or *talo*-configurations of the new hexoses. The presence of 3-deoxy-2-*C*-(hydroxymethyl)-*threo*- and *erythro*-pentonic acids suggests the formation also of the 2,3-enediol of the new hexoses.

In conclusion, the release of reducing sugars from sucrose, raffinose, and inulin at high temperatures (200–250°C) results in an alkaline degradation that is directed more through the 1,2-enediol intermediates than at 100°C. The 2,3-enediol intermediate proceeds principally through either reverse aldolization to give a tetrose, which degrades to 2,4-dihydroxybutanoic acid, or the 2,3-diketo-4-deoxyhexose intermediate, which cleaves to give glycolic acid and a 2-deoxytetrose. The importance of realdolization of the aldehydo-intermediates is demonstrated by the formation of hexosaccharinic acids from xylose, which degrades in a manner similar to glucose. The stability of the glycopyranosides to alkaline hydrolysis is seen in the stability of α,α'-trehalose and methyl α-D-glucopyranoside at 250°C in 0.1 M $Ca(OH)_2$, and the "peeling" pattern of lactose and melibiose in reactions at 200 and 250°C.

3. Experimental

Materials.—D-Xylose, D-glucose, raffinose, inulin, and methyl α-D-glucopyranoside were purchased from Pfanstiehl (Waukegan, IL). D-Fructose, sucrose, and calcium oxide are from Fisher Scientific Company. Ion-exchange resins (AG50W-X12/ H^+, 200–400 mesh and AG3-X4A/free base, 100–200 mesh) were purchased from Bio-Rad (Richmond, CA).

GLC and GLC–MS.—Analysis and quantitation of the reaction products followed precisely that given in an earlier paper [4]. In summary, Me_3Si derivatives were analyzed by GLC (Hewlett Packard 5890 series II) fitted with either a flame-ionization detector (FID) or a Mass-Selective Detector (Hewlett Packard 5971A) with He as carrier gas (25 cm s^{-1}). The injection temperature was maintained at 270°C for analysis of the trimethylsilyl derivatives. A DB5 capillary column (0.25 mm i.d. × 30 m, J&W Scientific, Folson, CA) was temperature programmed as follows: (*i*) for analysis of trimethylsilyl derivatives, 60°C for 3 min followed by an increase in temperature to 300°C at 5°C min^{-1}; (*ii*) for the analysis of parasaccharinic acid, the temperature was kept at 60°C for 3 min, followed by an increase at 5°C min^{-1} to 130°C for 1 min, after which the temperature was further increased to 220°C at 2°C min^{-1}. After 1 min at 220°C, the temperature was increased to 300°C at 10°C min^{-1}.

Reaction conditions.—Aqueous solution of carbohydrate (0.1 mol monosaccharide equivalent) in an equimolar amount of $Ca(OH)_2$ (0.1 M) was heated from room temperature at 4°C min^{-1} to 100, 150, 200, 240, or 250°C with stirring, as summarized in Tables 1 and 2.

At the 100°C reaction temperature, an aqueous reaction solution (glucose, fructose or lactose) was maintained for 2 h at 100°C with stirring and filtered while hot. The

precipitate was dissolved in the minimum of 5 mM H_2SO_4 and filtered. Barium carbonate was added to the washings until they were neutral and the resulting suspension was filtered. The filtrate was combined with the original filtrate. An aliquot of the combined solution was applied sequentially to cation-(AG50W-X12/H^+) and anion-(AG3-X4A/free base) exchange columns. The neutral substances were eluted with distilled water and determined by the phenol–sulfuric method [17]. The acids were eluted with 1.5 M pyridinium acetate. The eluent was freeze dried and dissolved in an appropriate volume of water. An aliquot of the resulting aqueous solution was evaporated at room temperature under a flow of N_2. Dichloromethane was added and evaporated to remove all water. The dried residues were converted to their trimethylsilyl derivatives for GLC or GLC–MS analysis as described previously [4].

At the higher temperatures (> 100°C), the reaction was performed in a High Pressure–High Temperature Autoclave (Autoclave Engineers, Inc., Erie, PA). The aqueous solution of carbohydrate (0.1 mol monosaccharide equivalent) in an equimolar amount of $Ca(OH)_2$ (0.1 mol) in a glass ampoule was flushed for 15 min with nitrogen. The ampoule was immediately sealed and attached to the stirring rod in the autoclave containing water. The reaction mixture was brought to the desired temperature at 4°C min^{-1}, held at the temperature for 10 min and cooled to 60°C. The reaction solution was treated as described for the lower reaction temperature.

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