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Alkaline degradation of glucose: effect of initial concentration of reactants

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Abstract

The alkaline degradation of D-glucose in aqueous $Ca(OH)_2$ at 100°C resulted in a complex mixture of more than 50 compounds, including parasaccharinic acid, that were identified by GLC–MS. The effect of reactant concentrations on the alkaline reaction products was studied by varying the concentration of glucose (1.8–100%, w/w). Under the different reactant concentrations the same saccharinic acids were produced, which is also true when no water was added to the reaction mixture. The increase of glucose concentration favored the formation of C_6 acids (2-*C*-methylpentonic, hexametasaccharinic, and isosaccharinic acids) and decreased the formation of $< C_6$ acids (glycolic, lactic, glyceric, 2-*C*-methylglyceric, deoxytetronic, and deoxypentonic acids). The identification of parasaccharinic acid provides further support for the existence of the glucose-3,4-enediol.

Keywords: Alkaline degradation; Glucose

1. Introduction

Much research has been directed toward an understanding of the fundamental aspects of the alkaline degradation of monosaccharides in aqueous solution [1]. The reaction products consist principally of numerous saccharinic ($\leq C_6$) acids. Higher molecular-weight compounds ($> C_6$ acids), and miscellaneous non-acidic and cyclic unsaturated carbonyl compounds [2,3] are also formed in minor amounts.

The composition of the alkaline reaction products is influenced by several reaction parameters, such as temperature, the nature and concentration of the alkali, and the monosaccharides. A comprehensive literature review of reaction variables [4–7] may be

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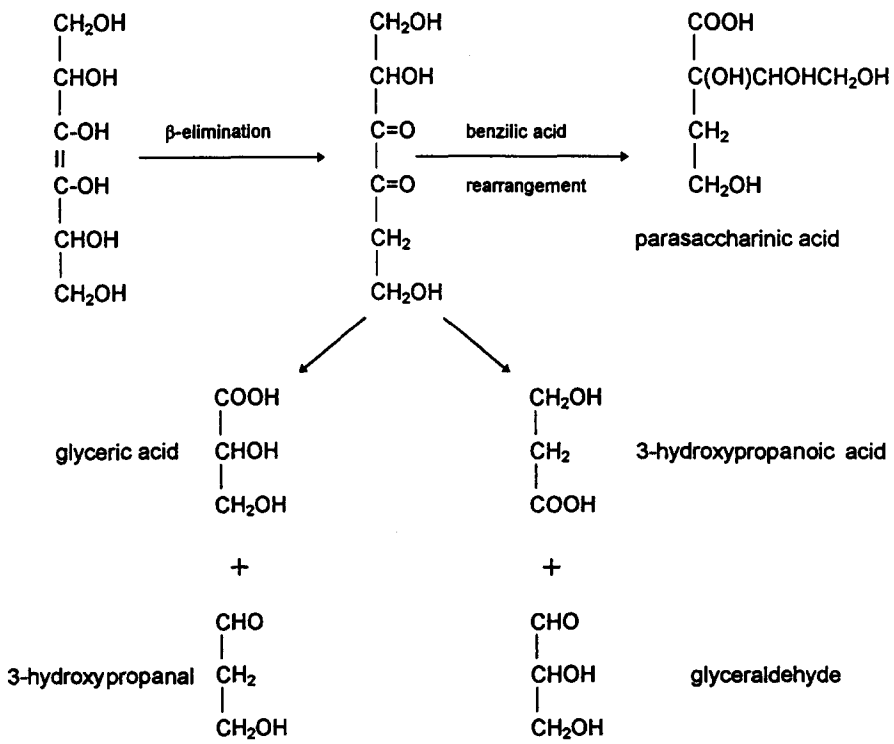
summarized for reducing monosaccharides as follows: the increase of hydroxyl ion concentration and the use of divalent cations favor the formation of lactic acid and decrease the total amount of C_1 (formic) to non-lactic C_3 (glyceric) acid products and the total amount of C_4–C_6 acid products; the composition of reaction products is independent of the reaction temperature at either 5 or 80°C; alkaline degradation of dilute solutions (1 mM) results in an almost complete conversion of the monosaccharides into $\leq C_6$ acids; high molecular-weight compounds are found to be increased at pH 11–12 and at high monosaccharide concentration (0.1 M).

As part of research on the alkaline degradation of carbohydrates, the effect of the concentration of reactants on the acids produced was studied by keeping an equimolar ratio of glucose and $Ca(OH)_2$ but varying the concentration of glucose from 1.8 to 100% (w/w).

2. Results and discussion

D-Glucose (0.1 M) in aqueous 0.1 M calcium hydroxide was completely degraded at 100°C after 30 min of reaction. The pH of the mixture gradually dropped to 8.3, which could be due to further degradation of reaction products.



Scheme 1. Saccharinic acids produced through 3,4-enediol formation of hexose.

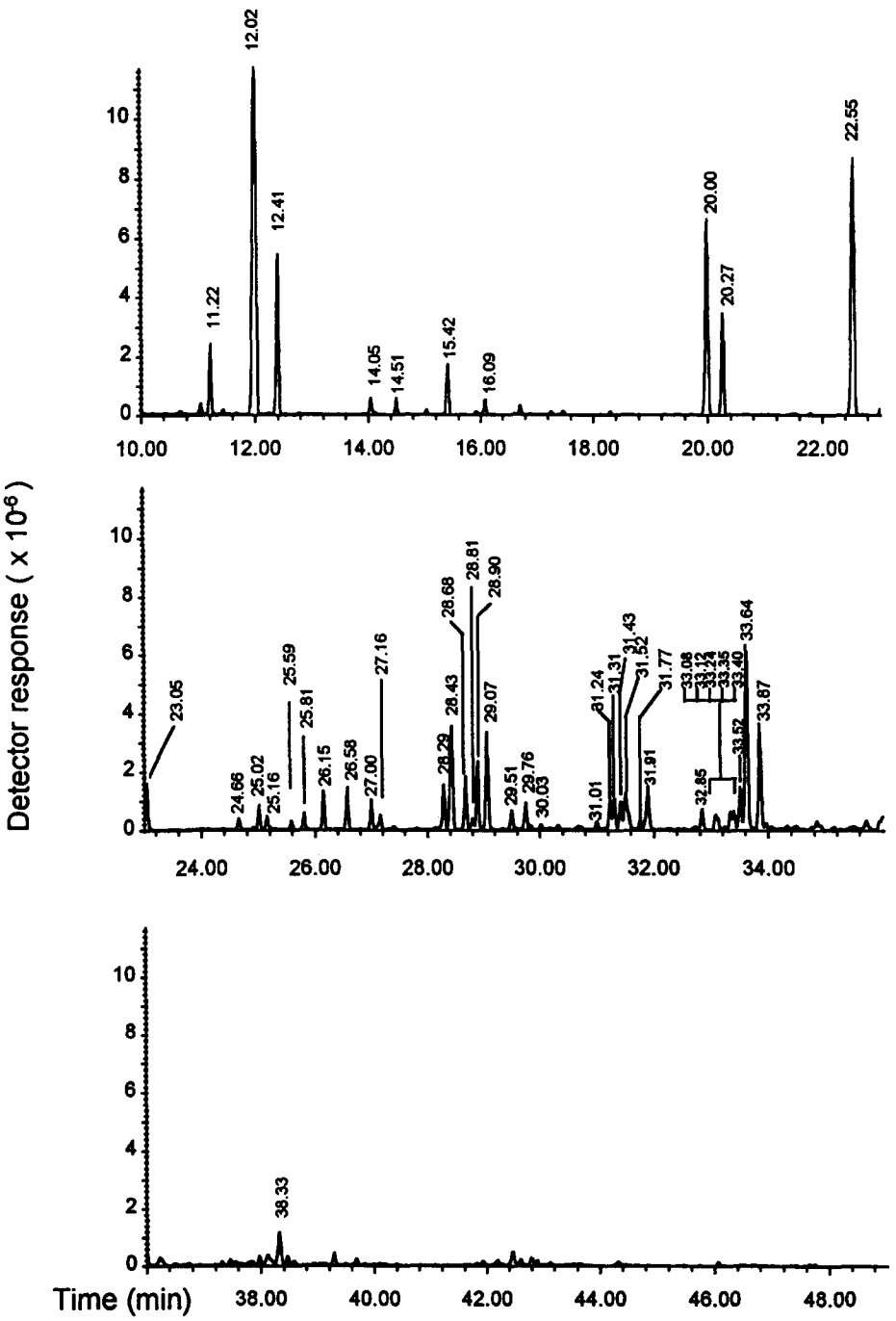


Fig. 1. GLC–MS chromatogram of anionic fractions, as trimethylsilyl derivatives, from the degradation of glucose in $Ca(OH)_2$ solution at 100°C. Standard temperature program (Method, *ia*) used. The peaks are identified in Table 1.

Table 1
Reaction products from the degradation of glucose in $Ca(OH)_2$ solution at 100°C

Peak no	T_R [a]	Rel/glc [b]	Rel/std [c]	Products [d]	Concn (μmol/mmol Glc)
1	11.05			Unknown	
2	11.22			Reagent peak	
3	12.02	1.00	1.00	Propanoic acid, 2-hydroxy-(lactic acid)	255
4	12.41	1.03	1.03	Ethanoic acid, 2-hydroxy-(glycolic acid)	62
5	14.05	1.17	1.17	Butanoic acid, 2-hydoxy-	5
6	14.51	1.21	1.21	Propanoic acid, 3-hydroxy-	5
7	15.00	1.25		Reagent peak	
8	15.42	1.28		Unknown	
9	16.09	1.34		Reagent peak	
10	20.00	1.66	1.67	Propanoic acid, 2-methyl-2,3-dihydroxy-(2-*C*-methylglyceric acid)	65
11	20.27	1.69	1.70	Propanoic acid, 2,3-dihydroxy-(glyceric acid)	25
12	22.55	1.88	1.89	Butanoic acid, 2,4-dihydroxy-	66
13	23.05	1.92	1.93	Butanoic acid, 3,4-dihydroxy-	8
14	24.66	2.05	2.05	3-Deoxypentono-1,4-lactone	5
15	25.02	2.08		3,4-Dideoxy-pentonic acid	17
16	25.16	2.09		2-Deoxypentono-1,4-lactone	12
17	25.59	2.13		Tetrono-1,4-lactone	3
18	25.81	2.15		Unknown	
19	26.15	2.18	2.19	Erythronic acid	8
20	26.58	2.21	2.21	Threonic acid	9
21	27.00	2.25		2-*C*-Methylerythronic acid	4
22	27.16	2.26		2-*C*-Methylthreonic acid	2
23	28.29	2.35		3-Deoxy-2-*C*-(hydroxymethyl) tetronic acid	9
24	28.43	2.37	2.38	2-*C*-Methylribono-1,4-lactone	33
25	28.68	2.39	2.40	3-Deoxy-*erythro*-pentonic acid	10
26	28.81	2.40	2.42	2-Deoxy-*threo*-pentonic acid	1
27	28.90	2.40		2-Deoxy-*erythro*-pentonic acid	5
28	29.07	2.42	2.43	3-Deoxy-*threo*-pentonic acid	15
29	29.51	2.46	2.47	3-Deoxy-2-*C*-(hydroxymethyl)-*erythro*-pentono-1,4-lactone	7
30	29.76	2.48		3-Deoxy-2-*C*-(hydroxymethyl)-*threo*-pentono-1,4-lactone	8

31	30.03	2.50		2-Deoxyhexono-1,4-lactone	2
32	31.01	2.58		Isomer of 31	2
				and parasaccharino-1,4-lactone	< 1
33	31.24	2.60	2.58	Ribono-1,4-lactone	9
34	31.31	2.60		Isomer of 33	11
35	31.43	2.61	2.63	3-Deoxy-*arabino*- and -*ribo*-hexono-1,4-lactones	13
36	31.52	2.62	2.64	3-Deoxy-*xylo*-hexono-1,4-lactone	15
37	31.77	2.64	2.66	Ribonic acid	2
38	31.91	2.65		Isomer of 37	4
				and 3-deoxy-*lyxo*-hexono-1,4-lactone	6
39	32.85	2.73	2.75	2-*C*-Methylribonic acid	4
40	33.08	2.75		2-Deoxyhexonic acid	2
41	33.12	2.76		Parasaccharinic acid	2
42	33.24	2.77		Isomer of 40	< 1
43	33.35			Isomer of 41	2
44	33.40	2.78	2.80	3-Deoxy-*lyxo*-hexonic acid	2
45	33.52	2.79	2.81	3-Deoxy-*xylo*-hexonic acid	6
46	33.64	2.80	2.82	3-Deoxy-*ribo*- and -*arabino*-hexonic acids	36
47	33.87	2.82	2.83	3-Deoxy-2-*C*-(hydroxymethyl)-*erythro*-pentonic acid	20
48	38.33	3.19		Unknown	
				Formic acid [e]	252
				Acetic acid [e]	65
				Propanoic acid [e]	1
				Threitol	< 1
				Erythritol	1
				D-Glucitol	< 1

[a] Retention time (min) in GLC–MS (Fig. 1).
[b] Relative retention time of glucose degradation components to lactic acid.
[c] Relative retention time of authentic and synthesized acids to lactic acid.
[d] Isomers (*erythro-, threo-, lyxo-, ribo-, arabino-*) are designated with reference to elution characteristics described elsewhere [24] when reference compound not available.
[e] Quantitated by HPLC.

The alkaline degradation of sugars produces a complex mixture of compounds, the majority of which can be derived from a sequence of reactions that are initiated by the formation of an enediol from the reducing sugar and elimination of the β-hydroxyl group from the enediol in question, resulting in a diketodeoxyglycitol. The latter undergoes either a benzilic acid rearrangement to give a deoxyglyconic acid (a saccharic acid) or the diketo intermediate cleaves to give acid and aldehyde fragments. This is illustrated by the 3,4-enediol of hexoses (Scheme 1). The second sequence of reactions involves an aldol condensation of the aldehyde fragments produced in the cleavage of the diketo intermediate in the first sequence of reactions, whereby tetroses, pentoses and $> C_6$ sugars may be formed. The deoxy derivatives of these sugars may also be produced, and all such new reducing sugars can undergo the first sequence of reactions.

Considering these reactions for glucose in its 1,2- and 2,3-enediol forms, all of the possible compounds that would be predicted are found in the analysis of the degradation products (Fig. 1 and Table 1). The principal product is lactic acid, which has been the basis of many studies to commercialize the process from such starting materials as cane or beet sugar molasses [8]. The other principal acids, together with their lactones, are the

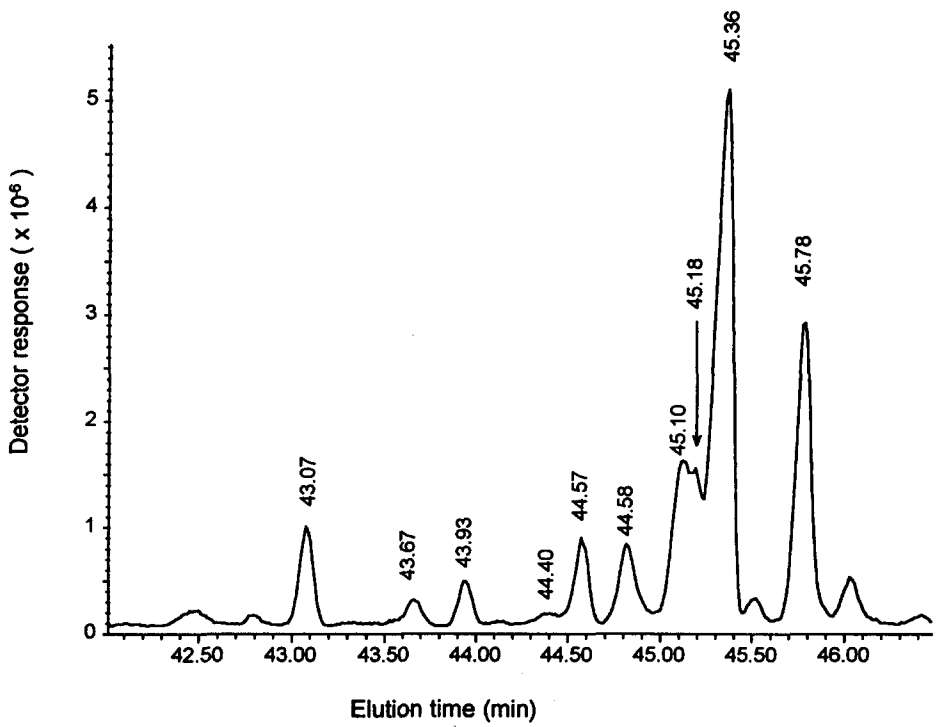


Fig. 2. GLC–MS chromatogram for the separation of parasaccharinic acid by modified temperature program (Method, *ib*): 2-*C*-methylribonic acid, 43.07; 2-deoxyhexonic acid isomers, 43.67 and 44.40; parasaccharinic acid isomers, 43.93 and 44.57; 3-deoxy-*lyxo*-hexonic acid 44.58; 3-deoxy-*xylo*-hexonic acid 45.10; 3-deoxy-2-*C*-(hydroxymethyl)-*threo*-pentonic acid 45.18; 3-deoxy-2-*C*-(hydroxymethyl)-*erythro*-pentonic acid 45.78; 3-deoxy-*ribo*- and -*arabino*-hexonic acids, 45.36.

various saccharinic acids. Some compounds, present in smaller amounts, such as propanoic acid, 2-hydroxybutanoic and the 2- and 3-deoxy-hexonic and -pentonic acids, call for further condensation and rearrangement of secondary products in order to account for their presence in the complex mixture of products from the alkaline degradation of glucose.

One of the new compounds identified was parasaccharinic acid [2-*C*-(hydroxyethyl)tetronic acid], which had been proposed earlier [9] as a rearrangement product of a 3,4-enediol. The presence of the latter has also been invoked to account for some sugar interconversions in alkali [10]. Further support for the generation of a glucose 3,4-enediol isomer is derived from the present work. The standard temperature program (analytical methods for GLC and GC–MS, *ia*) for the trimethylsilyl (Me_3Si) derivatives of the alkaline degradation products demonstrated two isomers of parasaccharinic acid eluting from a DB5 column close to one isomer of 2-deoxyhexonic acid (T_R 33.12 min) and 3-deoxy-*lyxo*-hexonic acid (T_R 33.40 min). Only one isomer of parasaccharino-1,4 lactone was observed eluting close to a 2-deoxyhexono-1,4-lactone (T_R 31.01 min). A modified temperature program (Methods, *ib*) separated the two isomers of Me_3Si-parasaccharinic acid from the 2-deoxyhexonic acid and 3-deoxy-*lyxo*-hexonic acid, and another isomer of 3-deoxy-2-*C*-(hydroxymethyl)-*threo*-pentonic acid (Fig. 2).

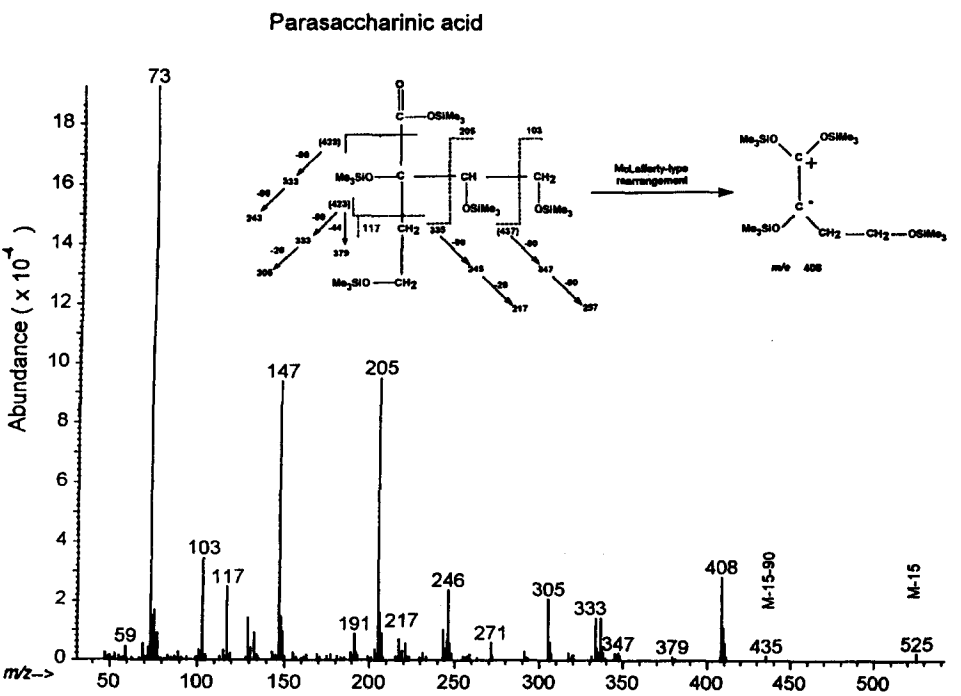


Fig. 3. Mass spectrum of parasaccharinic acid, 2-*C*-(hydroxyethyl)tetronic acid, as its trimethylsilyl (Me_3Si) derivative.

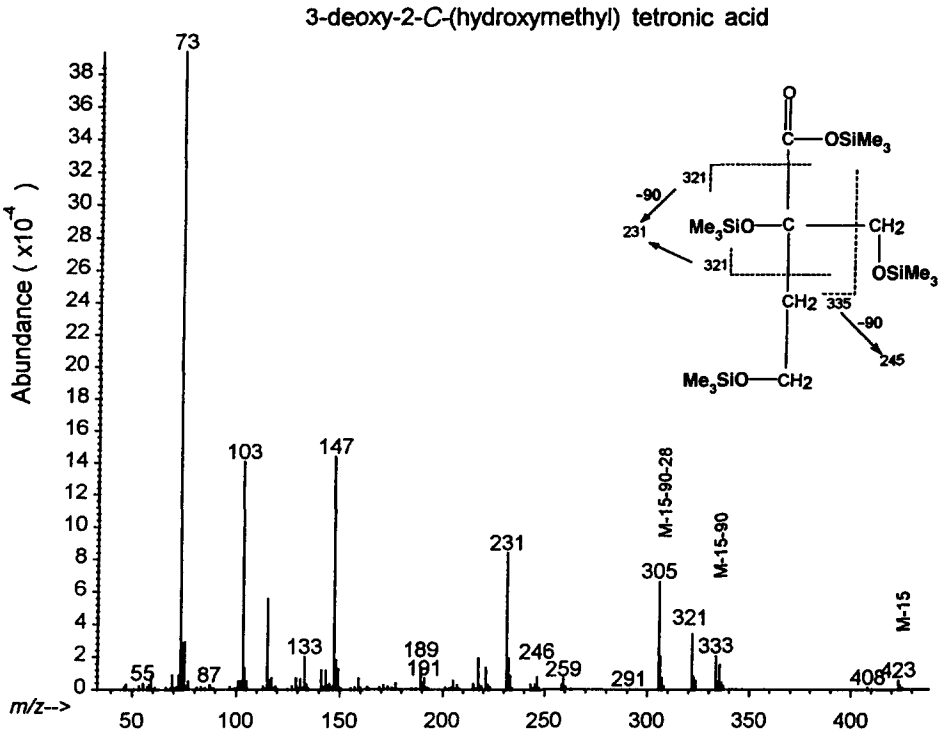


Fig. 4. Mass spectrum of 3-deoxy-2-*C*-(hydroxymethyl)tetronic acid as its trimethylsilyl (Me_3Si) derivative.

The parasaccharinic acids can be distinguished from the other saccharinic acids by mass spectrometry. The C_6 saccharinic acids are characterized by the molecular ion (or the M − 15 ion) and in particular the parasaccharinic acids give a McLafferty-type rearrangement ion [11], m/z 408 (Fig. 3). The fragmentation patterns of the parasaccharinic acids can be deduced from those of the 3-deoxyhexonic acids, 2-*C*-methyltetronic acid, and 3-deoxy-2-*C*-(hydroxymethyl)tetronic acid [12], the latter also producing the same McLafferty-type rearrangement ion (m/z 408) as the parasaccharinic acids (Fig. 4).

The fragmentation patterns of 1,4-lactones of parasaccharinic acids (Fig. 5) and the four isomers of the 3-deoxyhexonic acids are similar, but the latter fragment with a strong McLafferty-type rearrangement ion, m/z 246. Differentiation of these lactones can be made by the relative abundance of the m/z 205 and 246 ions, the ratio for which in the present work is found to be for these 1,4-lactones, parasaccharinic acid 3.1, 3-deoxy-*arabino*-hexonic acid 1.3, 3-deoxy-*ribo*-hexonic acid 1.1, 3-deoxy-*xylo*-hexonic acid 0.8, and 3-deoxy-*lyxo*-hexonic acid 0.7 (Fig. 6).

Similarly complex in reaction mechanism and relatively small in amount (2% of the initial glucose) are those polycyclic compounds that are lipophilic. These are listed in Table 2. The major components were derivatives of catechol and acetophenone, which had been reported earlier from the degradation of glucose or xylose with sodium

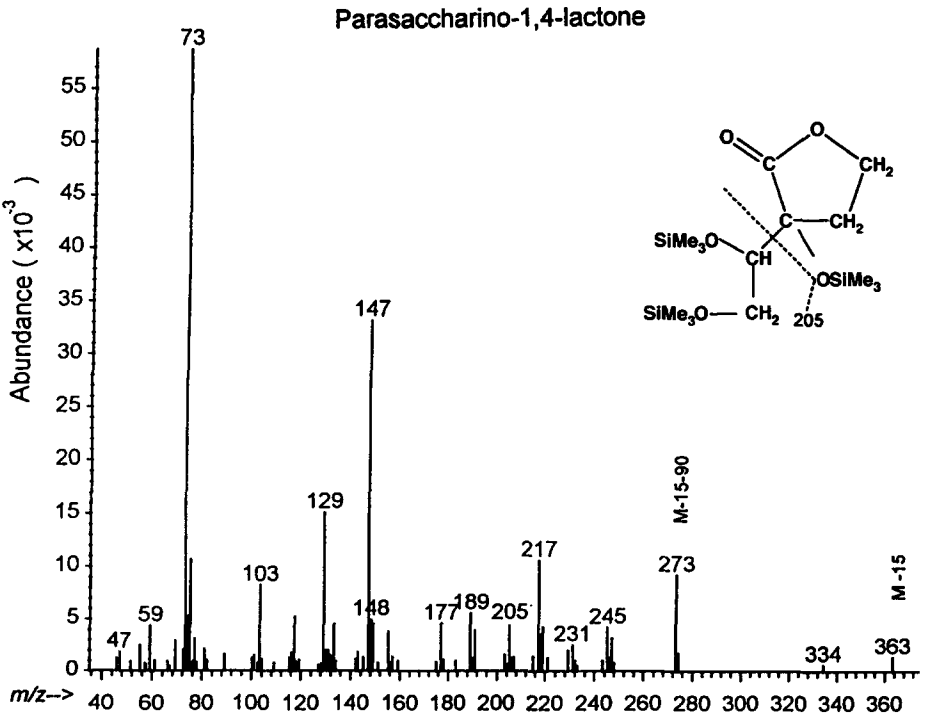


Fig. 5. Mass spectrum of parasaccharino-1,4-lactone as its trimethylsilyl (Me_3Si) derivative.

hydroxide [3]. Of the compounds extracted from the degradation reaction mixture, approximately 20%, by weight, are accounted for by GLC analysis, the remainder being of higher molecular weight [1].

Preliminary analyses of the higher molecular-weight products by ion-exchange chromatography resulted in three fractions (5% w/w), which did not give trimethylsilyl derivatives sufficiently volatile to be eluted from GLC columns and did not dialyze through 1000 or 3500 MW cut-off membranes. Examination by NMR, UV, and IR showed some indication of COOH-, CH_3-, CH_2-groups, and alkyl ether, but failed to give support for any complete structures.

The effect of aqueous reactants concentration (1.8–50%) using $Ca(OH)_2$ on the acid products.—Aqueous alkaline degradations of glucose in $Ca(OH)_2$ were performed by increasing the amount of glucose from 1.8 to 50% (w/w) while maintaining a 1:1 molar ratio of glucose to $Ca(OH)_2$. The reaction products were freeze-dried. The resulting dried salts were trimethylsilylated for gas–liquid chromatography (GLC). Since the calcium salts were not readily soluble in the derivatizing reagents they were sonicated for 1 h, followed by further heating at 80°C for another hour.

The combined gas chromatographic–mass spectrometric (GLC–MS) analysis of the trimethylsilyl derivative of the salts showed the same saccharinic acids at all initial reactant concentrations. The trimethylsilylated derivatives of the calcium salts avoid possible lactonization of acids (especially 1,4-lactones) and thus simplify the GLC

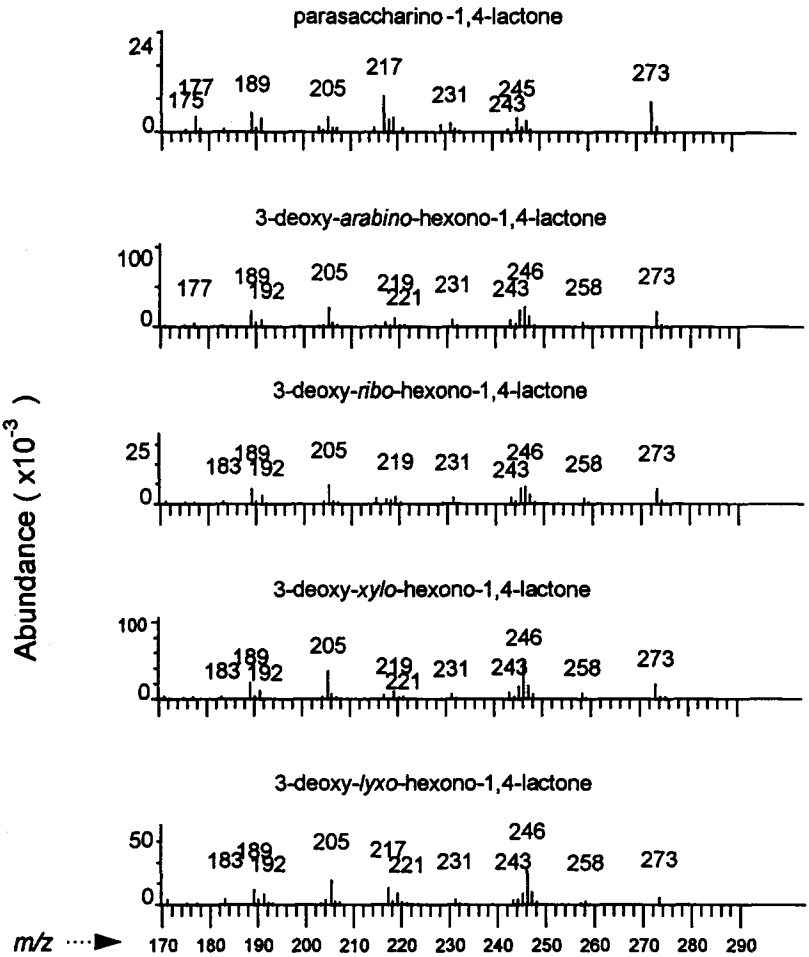


Fig. 6. MS between m/z 177–273 of trimethylsilylated 1,4-lactones of parasaccharinic acid, 3-deoxy-*arabino*-, 3-deoxy-*ribo*-, 3-deoxy-*xylo*- and 3-deoxy-*lyxo*-hexonic acids (from top to bottom).

analysis. The major components (12 acids) from the reaction products accounted for 90% of the total area integrated in GLC.

Increasing the glucose concentration generally decreased the formation of $< C_6$ saccharinic acids (glycolic to deoxy-pentonic) whereas C_6 acids (2-*C*-methylribonic, glucometasaccharinic, and glucoisosaccharinic acids) initially increased and then decreased (Table 3 and Figs. 7a and 7b). The C_3 acids, especially lactic acid, were found to be the principal component of the reaction products (41%) at lower reactant concentration (1.8% glucose) whereas C_6 acids, such as 2-*C*-methylribonic (27%) and glucometasaccharinic acids (25%) are major components at higher reactant concentration (50% glucose). The higher concentrations of glucose appear to decrease the secondary reactions noted in the more dilute solutions.

Table 2
Lipophilic compounds from the degradation of glucose in $Ca(OH)_2$ solution at 100°C

Peak no	T_R [a]	Compounds	Concn (nmol/mmol Glc)
1	8.78	Phenol	89
2	10.10	3-Methyl-1,2-cyclopentanedione (2-hydroxy-3-methyl-2-cyclopenten-1-one)	1124
3	10.97	3,5-Dimethyl-cyclopentane-1,2-dione [b]	114
4	11.56	2,4-Dimethyl-1,3-cyclopentanedione [b]	306
5	12.96	Catalized products [c]	713
6	13.66	Unknown [c]	210
7	13.83	Unknown [c]	179
8	14.40	Isomer of peak 9	157
9	14.84	2,4-Dihydroxybenzaldehyde	175
10	15.43	Catechol (1,2-benzendiol)	1867
11	16.39	Unknown [b]	193
12	17.28	3-Methylcatechol (3-methylbenzenediol)	160
13	17.78	Dihydroxyacetophenone (isomer of peak 17) [c] (possible 2′,3′-dihydroxy-)	449
14	18.12	4-Methylcatechol (4-methylbenzenediol)	528
15	19.61	2-Methylresorcinol (2-methyl-1,4-benzenediol)	61
16	21.65	3-Methoxy-2-methylphenol [c]	166
17	24.03	2′,5′-Dihydroxyacetophenone	62

[a] Retention time of the compound in GLC.
[b] Expressed as 3-methyl-1,2-cyclopentanedione equivalent.
[c] Expressed as catechol.
[d] Expressed as 2′,5′-dihydroxyacetonephenone equivalent.

Degradation of glucose in the aqueous mixture of $Ca(OH)_2$ and $Mg(OH)_2$.—The possible use of $Ca(OH)_2Mg(OH)_2$ mixtures for the alkaline degradation of glucose was studied. Aqueous reaction products of glucose (1.8%) were prepared using the mixture of $Ca(OH)_2$ and $Mg(OH)_2$ (1:2 molar ratio). The characterization of the degradation products as trimethylsilylated derivatives showed a similar GLC profile as that shown for $Ca(OH)_2$ solution, with however glucometasaccharinic acid being a more significant component. The total amount of acids produced with the mixture was significantly decreased (611 μmol $mmol^{-1}$ glucose in $Ca(OH)_2$ (Table 3, col. A) and 369 μmol $mmol^{-1}$ glucose in the mixture of $Ca(OH)_2$ and $Mg(OH)_2$ (Table 3, col. B), in part due to the reaction products still containing a portion of undegraded glucose (30 μmol $mmol^{-1}$ glucose), fructose (27 μmol $mmol^{-1}$ glucose), and mannose (17 μmol $mmol^{-1}$ glucose).

The reaction of glucose (100%, w/w) in the mixture of CaO and MgO (1:2 molar ratio).—The reaction products of glucose (100%: finely powdered, no water added) failed to be derivatized directly in the trimethylsilyl reagents even after the longer sonication and heating as done before. Therefore the fine powdered products were suspended in water and stirred with cation-exchange resin for 2 h. The products were filtered, the residue washed twice with water, and combined filtrate and the washings

Table 3
Major acidic products from the degradation of glucose at different reactant concentrations at 100°C

Acids	Reaction conditions																											
	A (Glucose: $Ca(OH)_2$ = 1:1 molar ratio)																						B (Glucose:$Ca(OH)_2$:$Mg(OH)_2$ = 3:1:2 molar ratio)					
	1.8%			3.6%			6.7%			12.6%			22.4%			36.5%			50.0%			1.8%			100%		
	μmol [a]	C [b]	%	μmol	C	%	μmol	C	%	μmol	C	%	μmol	C	%	μmol	C	%	μmol	C	%	μmol	C	%	μmol	C	%
C_2 Glycolic	27	27	4	35	35	5	27	27	4	23	23	4	19	19	3	15	15	4	10	10	3	26	26	7	18	18	3
C_3 Lactic	249			242			180			154			123			102			70			93			125		
Glyceric	7	256	42	7	249	35	5	185	27	4	158	26	4	127	21	2	104	25	1	71	21	7	100	27	< 1	125	19
C_4 2-*C*-methyl-glyceric	39			40			33			30			21			19			11			12			20		
3-Deoxytetronic	69			71			63			55			44			37			24			47			48		
2-Deoxytetronic	4	112	18	5	116	16	4	100	15	3	88	14	4	69	11	1	57	14	3	38	11	2	61	17	< 1	68	10
C_5 3-Deoxy-*erythro*-pentonic	17			21			21			19			17			13			8			8			16		
2-Deoxypentonic	7			7			8			7			6			3			2			4			4		
3-Deoxy-*threo*-pentonic	29	53	9	30	59	8	29	58	9	24	50	8	20	43	7	16	32	8	10	20	6	19	31	8	16	36	5
C_6 2-*C*-methyl-ribonic	55			109			143			136			168			96			93			40			167		
Glucometa-saccharinic	83			110			122			114			132			84			86			70			184		
Glucoiso-saccharinic	25	163	27	34	253	36	43	308	45	39	289	48	42	342	57	30	210	50	26	205	60	42	152	41	62	413	63
Total	611			713			678			607			600			418			343			369			660		

[a] μmol of acid per mmol of initial glucose.
[b] Total amount of acid with same number of carbon.

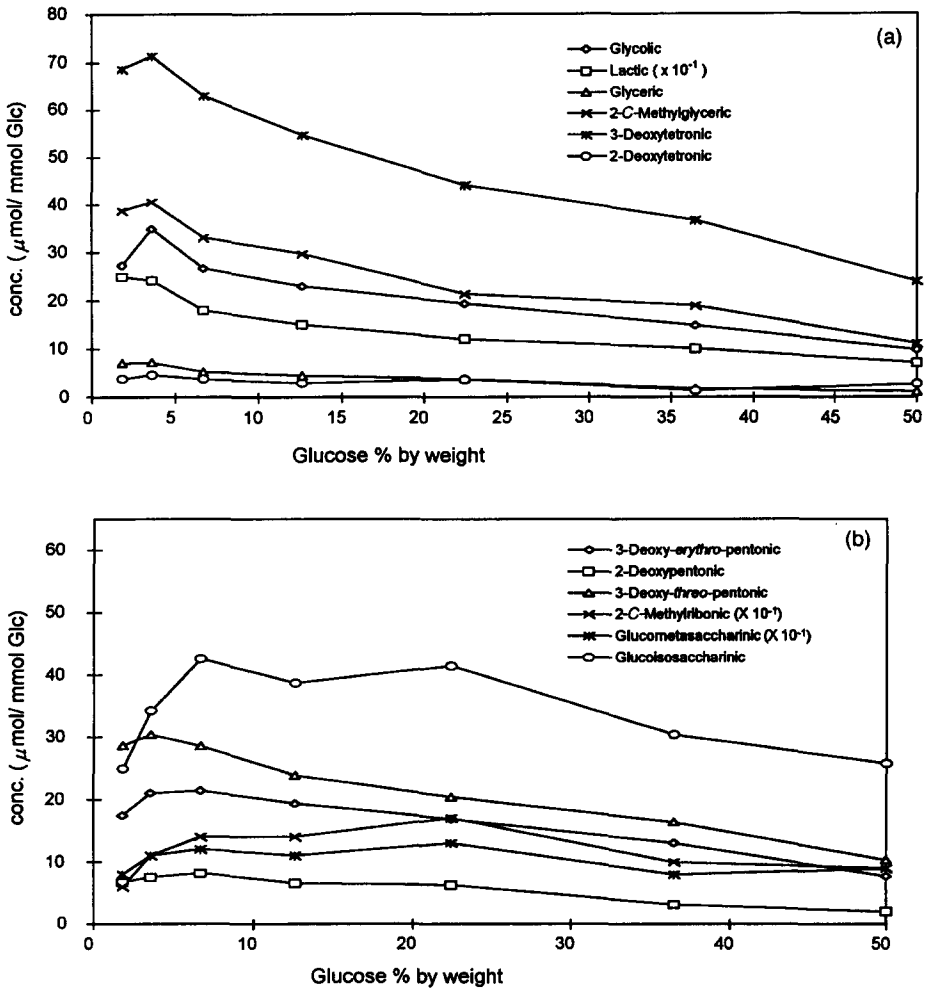


Fig. 7. (a) The formation of C_2, C_3, and C_4 saccharinic acids at different reactant concentrations. (b) The formation of C_5 and C_6 saccharinic acids at different reactant concentrations.

were passed through an anion-exchange resin. The anionic fraction, as the trimethylsilylated derivatives, was subjected to GLC–MS analysis, which showed a similar GLC profile of saccharinic acids as was seen in the aqueous reaction products. Glucometasaccharinic and 2-*C*-methylribonic acids were the major components (Table 3, col. B).

3. Summary

In order to account for the variety of acids produced by the degradation of D-glucose with calcium hydroxide, it is necessary to involve the 1,2-, 2,3-, and 3,4-enediol

intermediates, the reverse aldol reactions and benzilic acid-type rearrangements of each intermediate. The 3,4-enediol intermediate is further substantiated by the identification of the parasaccharinic acids. Additional complexity is introduced by the aldol condensation of the transitory C_6, C_4 and C_5 aldehyde products, thus accounting for the $> C_6$ products and the presence of the galacto-saccharinic acids. It is also clear that the secondary reactions are reduced with the increase in the concentration of glucose in the reaction.

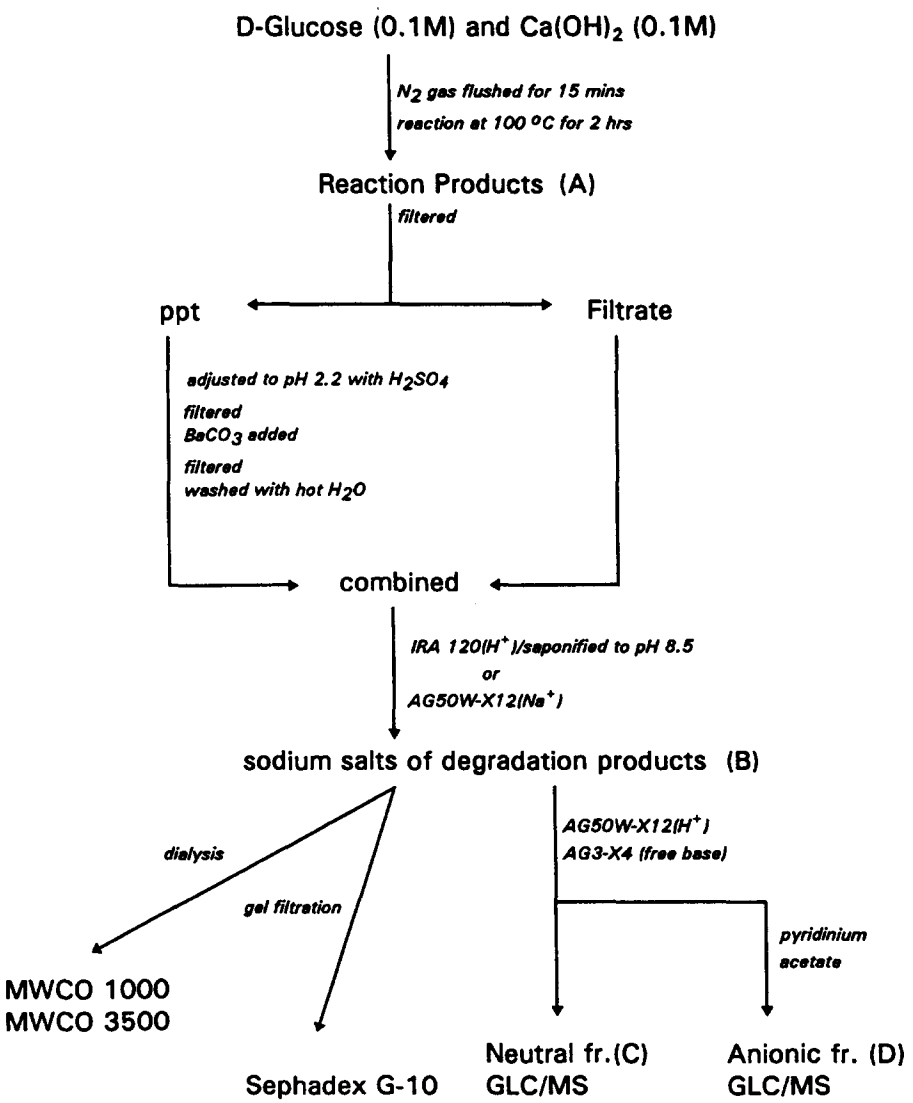
4. Experimental

Materials.—Anhydrous D-glucose, D-galactose, D-xylose, and CaO were purchased from Fisher Scientific Company. Sodium lactate and ribono-1,4-lactone were purchased from Sigma. Chloroacetone, 3-hydroxypropanonitrile, chloroacetone, vinylacetic acid, sodium cyanide, sodium formate, α-hydroxy-γ-butyrolactone, D-erythronic-1,4-lactone, 2-deoxy-D-*erythro*-pentose, α-lactose, and D_2O (100 atom%) were purchased from Aldrich. 3-*O*-Methyl-D-glucose, L-arabinose, L-rhamnose, L-fucose, 2-deoxy-D-*arabino*-hexose, and 2-deoxy-D-*lyxo*-hexose were purchased from Pfanstiehl. Bromine was purchased from EM Science. Ion-exchange resins (AG50W-X12/H^+, 200–400 mesh and AG3-X4/free base, 100–200 mesh) were purchased from Bio-Rad and Amberlite IRA-120/H^+) from Aldrich. Sephadex G-10 (40–120 μm) and dialysis membranes (MWCO, 1000 and 3500) were purchased from Phamacia Fine Chemicals and Spectrum, respectively.

Analytical methods.—Hexose determination. The glucose in the reaction products (A, Scheme 2) was analyzed by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD) on a Dionex BioLC (Sunnyvale, CA) fitted with a CarboPac PA1 anion-exchange column. The gradient was established as follows: the first 20 min of the gradient was isocratic at 20 mM NaOH, followed by a 5-min gradient that increased the NaOH concentration to 100 mM, after which followed a 30-min gradient in which a sodium acetate concentration from 0–500 mM was applied while the sodium hydroxide concentration remained constant at 100 mM.

Preparation of the acidic or neutral reaction products for GLC or GLC–MS analysis. An aliquot of the reaction solution (converted to the sodium salts) was applied sequentially to cation (AG50W-X12/H^+) and anion (AG3-X4/free base) exchange columns and the neutral substances were eluted with distilled water. The acids (D in Scheme 2) were eluted with 1.5 M pyridinium acetate, the eluent freeze-dried and dissolved in an appropriate volume of water. The neutral fraction (C in Scheme 2) was freeze-dried and dissolved in an appropriate volume of water. An aliquot of the resulting aqueous solution was evaporated under a flow of N_2 gas. Dichloromethane was added and evaporated to remove all water. The dried residues were converted to their oxime/trimethylsilyl derivatives [13,14] for GLC or GLC–MS analysis as described below.

In the case of pulverized samples of alkaline degradation products, the resulting fine powder other than 100% glucose reaction products was redisolved in water and sonicated for 1 h. An aliquot of the supernatant (100 μL) after centrifugation was derivatized as the trimethylsilyl esters and ethers. The fine powder from the 100%



Scheme 2. Schematic diagram for the analysis of alkaline degradation products.

glucose reaction products was suspended in water and stirred with the cation-exchange resin (AG50W-X12/H^+) for 2 h, filtered and washed with water. The combined filtrate and washings was applied to an anion-exchange column (AG3-X4/free base) and the anionic fraction eluted with pyridinium acetate (1.5 M). After freeze-drying it was trimethylsilylated. The resulting trimethylsilyl derivatives were analyzed by GLC or GLC–MS as described below.

GLC and GLC–MS. The trimethylsilyl derivatives or organic extracts 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 the trimethylsilyl derivatives and 250°C for organic extracts. A DB5 capillary column (0.25 mm i.d. × 30 m, J&W Scientific, Folson, CA) was temperature-programmed as follows: (*ia*) for the analysis of trimethylsilyl derivatives, 60°C for 3 min followed by an increase in temperature to 300°C at 5°C min^{-1}; (*ib*) for the analysis of the parasaccharinic acid, the temperature was held 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 200°C at 2°C min^{-1}. After 1 min at 220°C, the temperature was increased to 300°C at 10°C min^{-1}. (*ii*) for the analysis of organic extracts, 60°C for 3 min followed by an increase to 130°C at 5°C min^{-1}, then to 300°C at 2.5°C min^{-1}.

High-performance liquid chromatographic analysis (HPLC). The samples were disolved in 10 mM HCl or 5 mM H_2SO_4 and analyzed by HPLC [15] (HPX 87H strong cation-exchange column, 300 × 7.8 mm, column temperature 60°C, aqueous 5 mM H_2SO_4 as the eluent, flow 0.6 mL min^{-1}, and UV-detection at 210 nm).

^{13}C NMR and ^{1}H NMR analysis. The ^{13}C NMR and ^{1}H NMR spectra of the sodium salts of reaction products (B) (Scheme 2) in D_2O were recorded on a 360 MHz (Bruker WM-360) spectrometer.

UV and IR spectroscopy. UV spectrum of dialyzed products (Scheme 2) was recorded at 25°C and pH 7.0 on a AVIV spectrophotometer Model 14DS UV-VIS-IR. KBr tablets of dry samples were prepared to record IR spectra on a Beckman IR 4210 Infrared Spectrometer.

Quantitation of GLC responses. Sodium lactate, sodium 2-hydroxy-butanoate, calcium glycerate, sodium 2,4-dihydroxybutanoate, erythronic acid, ribonic acid, sodium glucometasaccharinate, and calcium isosaccharinate were derivatized (as trimethylsilyl ethers and esters and their GLC responses in FID were obtained) at three different molar concentrations. A regression equation was obtained (slope 1.00, constant 0.06, r^2 0.87) and used to quantitate the GLC peaks of the acids in reaction mixtures.

Preparation of reference compounds.—The following reference compounds were prepared for characterization of their GLC and MS behavior.

3-Hydroxypropanoic acid was prepared by hydrolysis of 3-hydroxypropanonitrile with cold 2 M NaOH according to the procedure of Reed [16].

2-*C*-Methylglyceric acid was prepared essentially by the esterification of chloroacetone [17], followed by cyanohydrin synthesis [18] and hydrolysis to the free acid [19].

3,4-Dihydroxybutanoic acid was prepared from vinylacetic acid by the procedure of Fieser and Fieser [20].

2,4-Dihydroxybutanoic acid and ribonic acid were prepared by hydrolysis of the corresponding lactones.

Deoxyglyconic acids [21] were prepared by oxidization, with aqueous bromine, of 2-deoxy-D-*erythro*-pentose, 2-deoxy-D-*arabino*-hexose, 2-deoxy-D-*lyxo*-hexose, rhamnose, and fucose.

2-*C*-Methyl-D-ribonic acid (glucosaccharinic acid) was prepared by the action of calcium hydroxide on invert sugar [22].

3-Deoxy-erythronic and -threonic acids [9] were prepared by the degradation of D-xylose or L-arabinose by 8 N NaOH at 80°C.

3-Deoxy-*ribo*- and *arabino*-hexonic (glucometasaccharinic) acids, and 3-deoxy-*xylo*- and *lyxo*-hexonic (galactometasaccharinic) acids were prepared by the action of calcium hydroxide on 3-*O*-methyl-D-glucose [22] and on galactose [9], respectively.

3-Deoxy-2-(hydroxymethyl)-*erythro*-pentonic acid (glucoisosaccharinic acid) was obtained by alkaline degradation of lactose [22].

Saccharinic acid lactones. Each saccharinic acid was acidified by the addition of hydrochloric acid (6 M HCl) and then evaporated to dryness to give the lactone [23].

Reaction conditions. The procedures for the alkaline degradations of glucose are summarized in Scheme 2.

Procedure 1. An aqueous solution of 0.1 M D-glucose in 0.1 M $Ca(OH)_2$ was heated at 100°C with stirring. After 2 h, the mixture was filtered. The precipitate was washed with approximately 5 mM H_2SO_4 solution and filtered. $BaCO_3$ was added to the resulting filtrate and the resulting mixture was filtered; the filtrate was combined with the original filtrate. The combined solution was converted to the sodium salts (B in Scheme 2) either by passage through a column of cation exchange column (AG50W-X12/Na^+) or by passage through a column of IRA(H^+) followed by neutralization and saponification with NaOH.

Procedure 2. Aqueous glucose solutions [1.8, 3.6, 6.7, 12.6, 22.4, 36.5, 42.8, and 50% (w/w)] containing equimolar $Ca(OH)_2$, were heated at 100°C for 1.5 h, cooled, neutralized with 0.1 M hydrochloric acid and freeze-dried. The resulting material was pulverized to a fine powder.

Procedure 3a. An aqueous solution of 1.8% glucose was mixed with $Ca(OH)_2$ and $Mg(OH)_2$ in a 3:1:2 molar ratio. The resulting solution was heated at 100°C for 1.5 h, cooled, neutralized with 0.1 M hydrochloric acid and processed further as described in Procedure 2.

Procedure 3b. Anhydrous glucose, CaO, and MgO (3:1:2 molar ratio) were stirred with a magnetic stirring-bar overnight in a 50-mL round-bottom flask. The resulting dry mixture was heated at 100°C for 1 h and then at 125°C for a further 2 h in an open system. The mixture was pulverized to a fine powder while it was still hot.

Analysis of reaction products.—(a) Volatile acids. The alkaline degradation reaction solution of glucose (A in Scheme 2) was adjusted to pH 2.1 at room temperature by adding H_2SO_4. The acidified products were distilled at 40°C with a rotary evaporator under diminished pressure to dryness, and the operation was repeated twice more by adding water. The volatile acids were collected at cold trap (−6°C) and analyzed by HPLC as described before.

(b) Sephadex G-10 chromatography. An aliquot (1 mL) of sodium salts (B in Scheme 2) of degradation products was applied to a Sephadex G-10 (40–120 μm) column (1.0 × 50 cm) and eluted with de-ionized water (0.5 mL min^{-1}). The elution was followed by refractometry. Maltoheptaose, sodium gluconate, and sodium lactate were used to calibrate the column.

(c) Dialysis. Sodium salts of reaction products (B) were dialyzed sequentially against NH_4HCO_3 and NaCl buffer solution, stepwise decreasing the concentration from 0.2 M to distilled water. Any possible chloride in the retentate was tested by silver nitrate. The retentate was freeze-dried.

(d) Lipophilic compounds. Aqueous alkaline reaction products from glucose (13.5 g), CaO (4.21 g), and de-ionized water (750 mL) were prepared as described in Procedure 1. The final reaction solution was brought to pH 6.0 with 0.1 M HCl, saturated with NaCl and extracted with EtOAc (2×250 mL). The black emulsion was broken by centrifugation (2500 *g*). The resulting organic phase was separated and dried over anhydrous $MgSO_4$ and Na_2SO_4. The lipophilic compounds (249 mg) were obtained after removal of the EtOAc at 40°C. The aqueous phase after the first EtOAc extraction was further acidified to pH 3.5 and extracted with a mixture of EtOAc and ether (1:1 v/v, 2×150 mL). These additional lipophilic compounds (11 mg) were isolated as before. The resulting two organic extracts, without any derivatization, were analyzed by GLC and GLC–MS.

(e) High molecular-weight compounds. Aqueous alkaline reaction products of glucose (1.80 g of glucose, 0.56 g of CaO and 100 mL of deionized water) were prepared as described in Procedure 1. The reaction solution, while still hot, was acidified to pH 2.2 with H_2SO_4 and filtered. The precipitate was washed twice with hot water. The filtrates were pooled and treated with $BaCO_3$ to remove sulfate. One third of the resulting solution (600 mg of glucose equivalent) was chromatographed on an anion-exchange column (AG3-X4/free base, 0.45×40 cm). The column was washed with water (100 mL), followed by sequential elution with 0.35 M H_2SO_4 (75 mL), and a series of concentrations of pyridinium sulfate (0.15 M, 100 mL), 0.75 M (50 mL), and 1.5 M (100 mL). Elution with 1.5 M pyridinium acetate was continued until the colored materials were completely eluted from the column. Each fraction (2.3 mL) was treated with $BaCO_3$, filtered and lyophilized. Lyophilized fractions were dissolved in 2 mL of 10 mM HCl or water and analyzed by refractive index. The resulting fractions of high molecular-weight compounds were submited to HPLC analysis as described before.

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