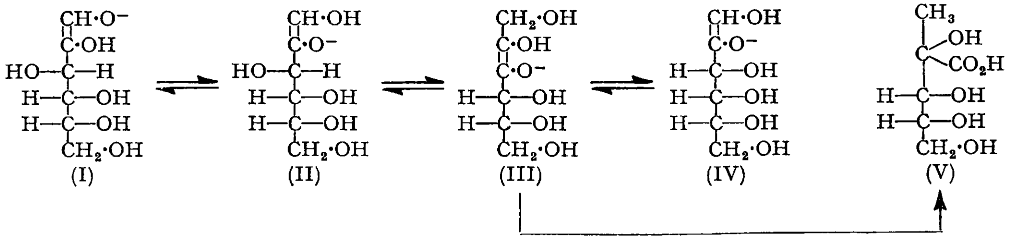
*The Degradation of Carbohydrates by Alkali. Part IV.** 1-O-*Methylfructose, Glucose, and Fructose.*

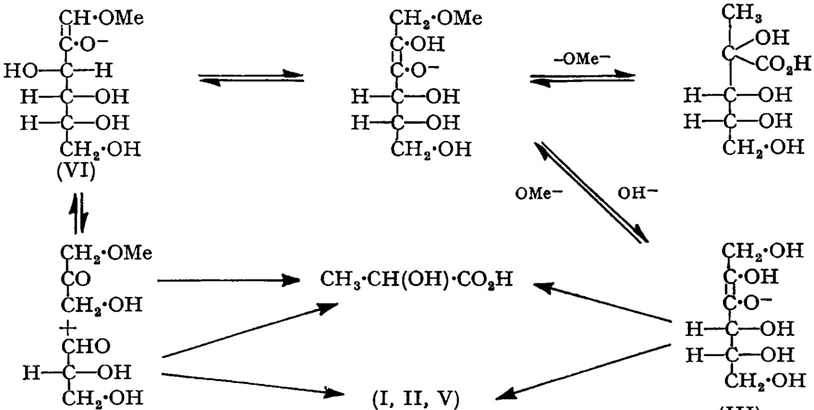
By J. Kenner and G. N. Richards.

[Reprint Order No. 5054.]

1-*O*-Methylfructose is shown to yield α-D-glucosaccharinic acid on treatment with lime-water. The behaviour of glucose and fructose towards this reagent has also been examined.

It was emphasised in Part III * of this series that the presence of an *O*-alkyl or *O*-glycosyl group in the 3- or the 4-position of a glucose chain determines the type of saccharinic acid formation occasioned by the action of lime water on the compound. Further, Kiliani obtained D-glucosaccharinic acid (V) as the chief product of the action of lime-water on invert sugar at the room temperature (*Ber.*, 1882, **15**, 2953). This, as we may reasonably infer from the β-alkoxy- or -hydroxy-carbonyl mechanism operative in this type of reaction (cf. Corbett and Kenner, *J.*, 1953, 2245), arises from the anion of the 3-ketohexose (III), which is in equilibrium with those of glucose (I), fructose (II), and psicose (IV), but loses a hydroxyl ion more readily than these do because the electronic flux towards $C_{(1)}$ is impeded less by the hydrogen atoms attached to it than are those towards $C_{(3)}$ and $C_{(4)}$ by the groups respectively attached to them.





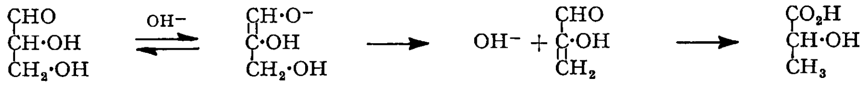
Observations on the presence of psicose in alkaline solutions of glucose have been recorded by Wolfrom, Lew, and Goepp (*J. Amer. Chem. Soc.*, 1946, **68**, 1443), Hough, Jones, and Richards (*J.*, 1953, 2005), and Schneider and Erlemann (*Naturwiss.*, 1952, **39**, 160), and are also evidence of the presence of (III) in such solutions.

Consideration of these facts made a study of the behaviour of 1-*O*-methylfructose desirable. The compound is available by methylation and subsequent hydrolysis of 2 : 3-4 : 5-di-*O*-*iso*propylidenefructose (Ohle, *Ber.*, 1925, **58**, 2577; Glen, Myers, and Grant, *J.*, 1951, 2568), and on treatment with lime-water yielded α-D-glucosaccharinic acid (IV),

---

* Part III, *J.*, 1954, 278.

thus indicating wider validity for the generalisation of Part III. The acid was accompanied, however, by a considerably larger proportion of lactic acid. In addition, there was chromatographic evidence of the formation of very small quantities of other acids, with R_F values corresponding to those of *iso*- and *meta*-glucosaccharinic acid. Similarly, and confirmatory of the above scheme, there was evidence of the presence at intermediate stages of the anions, not only of 1-*O*-methylfructose (VI) but also of psicose (and possibly of its 1-*O*-methyl derivative), glucose, fructose, and mannose. The alternative possible modes of formation are shown in the scheme. The predominance of lactic acid over saccharinic acid here recorded is also familiar in the degradation of glucose and fructose by alkali and is perhaps due to the greater simplicity of a reversible aldol reaction in comparison with the elimination of anion on which saccharinic acid formation depends, though it must be realised that this latter is exactly reproduced in the ultimate stages of lactic acid formation :

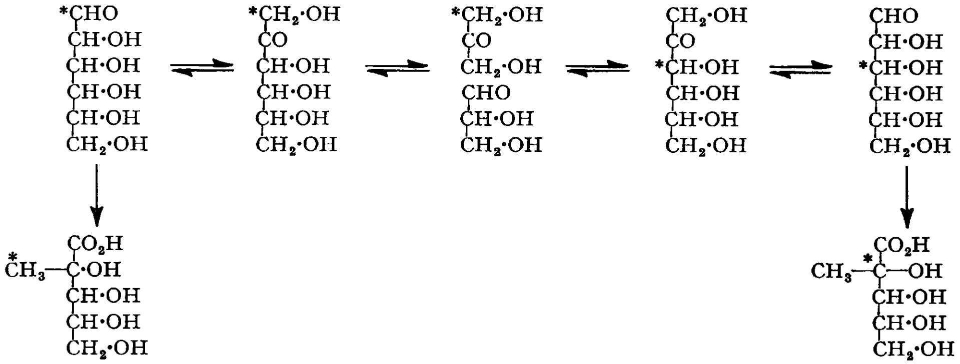


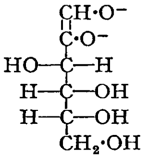
We have now also followed the decomposition of glucose and fructose themselves by lime-water. The apportionment of the total acidic products, as measured by decrease in alkalinity of the solutions, between lactic and saccharinic acids depended on lactonisation

Time (hr.)	Acid equivs. per mole of hexose	Total hexoses (mg.)	Ketose (mg.)	Aldose (mg.)	Paper chromatography †				
					G	F	P	T	Sacc. acids
Glucose (0·316 *g.*) *in* 0·0375N-*lime-water* (100 ml.).									
0	0	316	0	316	3	—	—	—	—
0·25	0	316	9·0	307	3	0·5	—	—	—
0·55	0	316	10·2	306	3	0·5	—	—	—
1·0	0	315	14·4	302	3	0·5	—	—	—
2·0	0·017	318	22·6	293	3	1	—	—	—
3·0	0·014	317	30·4	286	3	1	—	—	—
7·25	0·057	308	64·4	244	3	2	0·5	—	—
10	0·100	304	90·0	214	3	2	0·5	—	0·5
24	0·279	267	111	156	2	3	1	0·5	1
27	0·328	259	105	154	2	3	1	0·5	1
31	0·367	250	105	145	2	3	1	0·5	1
48	0·580	203	89·0	114	2	2	1	0·5	1
72	0·789	161	71·0	90	2	2	1	0·5	2
168	1·24	83	23·4	60	1	1	0·5	—	3
218	1·37	55	14·4	41	1	0·5	0·5	—	3
338	1·525	31	8·0	23	1	0·5	—	—	3
Fructose (0·316 *g.*) *in* 0·0404N-*lime-water* (100 ml.).									
0	0	316	316	0	—	3	—	—	—
0·25	0·014	316	307	9	0·5	3	—	—	0·5
0·5	0·023	317	297	18	0·5	3	—	—	0·5
0·75	0·034	314	288	26	0·5	3	—	—	0·5
1·0	0·037	311	278	33	0·5	3	—	—	0·5
1·5	0·051	311	258	53	1	3	—	—	0·5
2·0	0·071	306	248	58	1	3	—	—	0·5
3·0	0·100	302	217	85	2	3	—	—	0·5
4·0	0·116	296	208	88	2	2	0·5	—	0·5
5·5	0·159	292	189	103	2	2	0·5	—	0·5
7·5	0·219	280	162	118	2	2	0·5	—	0·5
24	0·593	199	74	125	2	1	1	0·5	1
31·5	0·684	176	60·5	116	2	1	1	0·5	1
48	0·860	140	43	97	2	1	0·5	0·5	2
131	1·25	70	18	52	1	0·5	—	0·5	3
179	1·39	52	12·5	39	1	0·5	—	0·5	3
299	1·57	22	6	16	0·5	0·5	—	—	3
400	1·615	15	*	*	0·5	0·5	—	—	3
525	1·63	8	*	*	0·5	—	—	—	3

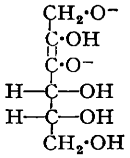
* Not determined. † Numbers denote relative intensity, 3 denoting the greatest.
G, glucose; F, fructose + mannose; P, psicose; S, saccharinic acids; T, supposed triose.

of the latter and ascription of the residual acidity to lactic acid (cf. Bamford, Bamford, and Collins, *Proc. Roy. Soc.*, 1950, *A*, **204**, 85). Alternatively, but less reliably owing to the need to use an additional set of experimental results, the relative proportions were deduced from the consideration that each molecule of hexose can afford either one of saccharinic acid, or two of lactic acid, the amount of $(\pm)$-$\alpha\gamma$-dihydroxybutyric acid formed being very small. As in earlier papers of this series the actual consumption of hexose was determined by the Hagedorn–Jensen method, and, in addition, the proportion of ketose present at any time was estimated colorimetrically by means of resorcinol and hydrochloric acid (cf. Gray, *Analyst*, 1950, **75**, 314). Finally, chromatographic analysis was applied to the reactant solution at each stage. The Table shows the results obtained at 25°. The lag observed in the development of lactic acid from glucose, as compared with fructose, supports the generally accepted view that fission of the six-carbon chain proceeds, not as was suggested by Wohl (*Z. angew. Chem.*, 1907, **20**, 1169) independently in the case of glucose through 3-deoxyglucosone, but in each case through the ketose. As an evident consequence of the reversibility of the reaction, an isotopic carbon atom originally in position 1 can appear in the equilibrium mixture partly in position 1 and partly in position 3, so that in the D-glucosaccharinic acid ensuing from the further action of lime-water, the isotope will occur in one or other of the positions shown. This has been recently confirmed by Sowden (*J. Amer. Chem. Soc.*, 1953, **75**, 2788), but his inference, that the mechanism of the saccharinic acid formation is thereby rendered doubtful, is clearly not permissible. Rather the results support the suggestion that conversion of glucose into galactose through fission into three-carbon units is possible (Kenner, *Nature*, 1935, **135**, 506). On the other hand such a process is inhibited by substitution in the 4-*O*-position, and the occurrence of this in maltose, lactose, cellobiose, and the corresponding oligosaccharides may be Nature's device for safeguarding the storage of glucose until its due use is required.

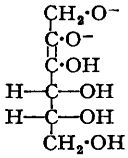




(VII)



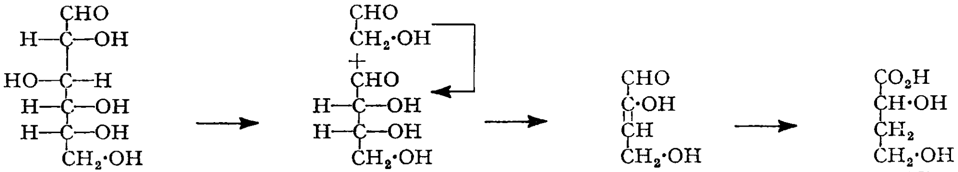
(VIII)



(IX)

There can evidently be no sharp line of division between the effects of strong and of dilute alkali and we have already referred to indications of the presence of the meta- and *iso*-acids among the products of the action of lime-water on methylfructose. These were more pronounced in the experiments on fructose itself, and Nef found them to be the chief saccharinic products when glucose is treated with 8N-sodium hydroxide. These conditions should favour the existence of doubly charged anions, among which (VII) might be expected to predominate and yield metasaccharinic acid; (VIII) should be unreactive, but (IX) could furnish *iso*saccharinic acid.

Lactic acid was the chief product of the reaction and is generally regarded as arising primarily from dealdolisation between $C_{(3)}$ and $C_{(4)}$ on the glucose chain. Clearly, however, a similar process could occur between $C_{(2)}$ and $C_{(3)}$, yielding glycollaldehyde and a tetrose, which by further action of the alkali could be converted into the four-carbon metasaccharinic system, (±)-αγ-dihydroxybutyric acid (X), which was also an important product.



(X)

Nef (*loc. cit.*) demonstrated that the deoxytetronic acid is also formed from glycollaldehyde, presumably, as indicated, through the tetrose. Mention should also be made of a kinetic study of these reactions by Bamford, Bamford, and Collins (*loc. cit.*).

EXPERIMENTAL

1-*O*-Methylfructose (Ohle, *loc. cit.*; Glen, Myers, and Grant, *loc. cit.*) was freed from traces of fructose on a cellulose column (cf. Kenner and Richards, *loc. cit.*). The syrupy product, $[\alpha]_D^{20}$ −50·2° (*c*, 1 in MeOH), was characterised by its *p-nitrophenylhydrazone*, orange needles, m. p. 166° (Found : N, 12·8. $C_{13}H_{19}O_7N_3$ requires N, 12·8%).

A solution of the ketose (6·03 g.) in oxygen-free water (100 ml.) was kept at room temperature with calcium hydroxide (5 g.) for 14 days, with occasional agitation. After filtration the solution was freed from calcium ions by means of Amberlite resin IR-120, and evaporated under reduced pressure to a thin syrup which was extracted continuously with ether for 24 hr. Concentration of the extract yielded long needles of α-D-glucosaccharinolactone (1·25 g.); recrystallised from acetone, this had m. p. 164·5—165°, $[\alpha]_D^{21}$ +92·8° (*c*, 2 in H_2O) (Found : C, 44·3; H, 6·3. Calc. for $C_6H_{10}O_5$: C, 44·4; H, 6·2%). Nef (*loc. cit.*) reported m. p. 160°, $[\alpha]_D^{20}$ +93·28°.

The remainder of the ethereal extract, after removal of the saccharinolactone, was evaporated to dryness and part of the residual syrup (0·242 g. from 3·98 g.) was dissolved in water, neutralised with sodium hydroxide (final vol. 10 ml.), and heated under reflux with a solution of 4-bromophenacyl bromide (0·7 g.) in ethanol (10 ml.) for 1 hr. Fractional precipitation of the product by water from the resulting solution yielded 4-bromophenacyl lactate, m. p. and mixed m. p. 111—113°.

In a kinetic study of the decomposition of 0·708 g. in oxygen-free lime-water (250 ml.; 0·0419N) at 25°, the total acidic products were estimated by back-titration of excess of sulphuric acid, and the saccharinic acid proportion by a development of the method of Bamford, Bamford, and Collins (*loc. cit.*) whereby the neutralised solution was brought to 0·02N with sulphuric acid and boiled for 15 min., and the decrease in acidity determined by titration with potassium hydroxide (for correction factor, see below). Identification and rough visual estimation of carbohydrates by paper chromatography, with butanol–pyridine–water (3 : 2 : 1·5) solvent (A), gave results as follows :

(*a*) Glucose (R_F 0·28), responsive to *p*-anisidine, and silver nitrate–sodium hydroxide, but not to naphtharesorcinol spray. These tests gave negative results after a deionised solution of the mixture (1 ml. at 40° for 20 hr.) had been treated with glucosedehydrogenase (for which we were indebted to Dr. M. V. Lock). This treatment, however, yields a product corresponding to gluconic acid (R_F < 0·1).

(*b*) Fructose + mannose (R_F 0·31); mainly the former as judged by relative responses to naphtharesorcinol, *p*-anisidine, and silver nitrate–sodium hydroxide sprays.

(*c*) Psicose (R_F 0·375 in agreement with an authentic sample, kindly supplied by Professor J. K. N. Jones), yielding pink colours with *p*-anisidine and naphtharesorcinol sprays.

(*d*) 1-*O*-Methylfructose (R_F 0·475), coloured brown by *p*-anisidine and pink by naphtharesorcinol sprays.

(*e*) 1-*O*-Methylpsicose (?) (R_F 0·54), with colour reactions as (*d*).

(*f*) A monomethyltriose (?) (R_F 0·74); for methylglyoxal R_F 0·85 was found.

The annexed table summarises the results.

A sample of the solution remaining after completion of the reaction with lime-water was shaken with excess of Amberlite resin IR-120 and then subjected to paper chromatography with butanol–ethanol–acetic acid–water (45 : 5 : 1 : 49) (solvent B.) Lactones were subsequently detected by spraying the paper with hydroxylamine followed by acidic ferric chloride as described by Abdel-Akher and Smith (*J. Amer. Chem. Soc.*, 1951, **73**, 5859). Authentic samples of β-glucometasaccharinolactone, α-glucoisosaccharinolactone, and α-D-glucosaccharinolactone gave R_F values 0·35, 0·455, and 0·56 respectively; lactic acid gave a faint reaction (R_F 0·71). The solution from the treatment of 1-*O*-methylfructose was shown in this way to contain a large amount of α-D-glucosaccharinolactone and lactic acid, together with a faint trace of the α-gluco*iso*saccharinolactone and β-glucometasaccharinolactone and also three other lactones in similar amount, thought to be β-glucoisosaccharinolactone (R_F 0·42), β-D-glucosaccharinolactone (R_F 0·61), and α-gluco*meta*saccharinolactone (R_F 0·28).

Time (hr.)	Total acid (eq./mole)	Sacc. acid (eq./mole)	Lactic acid (eq./mole)	Paper chromatography						
				a	*b*	*c*	*d*	*e*	*f*	Sacc. acids
0·55	0·082	0·015	0·067	—	—	—	4	—	—	—
1·0	0·127	0·023	0·104	—	—	—	4	0·5	—	—
2·0	0·211	0·037	0·174	0·5	0·5	0·5	4	0·5	0·5	—
3·0	0·310	0·053	0·257	×	×	×	×	×	×	×
4·0	0·401	0·060	0·341	0·5	1	0·5	3	1	0·5	—
5·0	0·466	0·068	0·398	×	×	×	×	×	×	×
6·0	0·531	0·075	0·456	0·5	1	1	3	1	1	1
7·5	0·628	0·119	0·509	×	×	×	×	×	×	×
24·5	1·18	0·189	0·99	1	1	1	2	1·5	0·5	2
55	1·41	0·191	1·22	—	0·5	—	1	0·5	—	2
97	1·54	0·197	1·34	×	×	×	×	×	×	×
121	1·60	0·222	1·38	×	×	×	×	×	×	×
144	1·61	0·229	1·38	×	×	×	×	×	×	×
169	1·65	0·242	1·41	×	×	×	×	×	×	×
193	1·66	0·268	1·39	×	×	×	×	×	×	×
318	1·67	0·264	1·41	×	×	×	×	×	×	×
482	1·67	0·268	1·40	—	—	—	—	—	—	3

— Not detected. × Not examined.

Action of Lime-water on Glucose and Fructose.—As a supplement to Kiliani's work (*loc. cit.*), a solution of glucose (25·5 g.) in saturated oxygen-free lime-water (500 ml.), with calcium hydroxide in suspension, was maintained with occasional shaking for 500 hr. at 20° ± 1°. The filtered solution (then only slightly reducing) was again filtered after treatment with an aqueous solution (200 ml.) of oxalic acid dihydrate (20 g.). After concentration of this filtrate under reduced pressure to 500 ml., residual calcium ions were removed by means of Amberlite resin IR-120 (150 g.), and the solution, with washings, was concentrated to a thin syrup. The distillate at this stage contained $7{\cdot}6 \times 10^{-3}$ equiv. of volatile acid including $5{\cdot}6 \times 10^{-3}$ equiv. of formic acid (determined according to Evans and Hass, *J. Amer. Chem. Soc.*, 1926, **48**, 2705). Continuous extraction of the syrup with ether for 48 hr. over a mixture of ether, water, and zinc carbonate furnished zinc lactate (14·4 g.) (cf. Evans and Hass, *loc. cit.*) (Found: H_2O, 30·6. Calc. for $C_3H_5O_3Zn_{\frac{1}{2}},3H_2O$: H_2O, 30·5. Found, on anhyd. salt, Zn, 27·0. Calc. for $C_3H_5O_3Zn_{\frac{1}{2}}$: Zn, 26·9%). The recovered residual yellow syrup (8·3 g.), with slight reducing properties, partly crystallised after several weeks at 0°. The separated solid, twice recrystallised from acetone (yield 1·05 g.), showed m. p. 164·5—165°, $[\alpha]_D^{20}$ +92·8° (*c*, 2 in H_2O) (Found: C, 44·3; H, 6·3. Calc. for $C_6H_{10}O_5$: C, 44·4; H, 6·2%). Data from quantitative experiments are recorded in the introduction. Acidic products were determined as in the case of 1-*O*-methylfructose, and aldose–ketose determinations as described in Part III. Chromatographic identification of carbohydrates depended on comparison with known materials in respect of R_F values. That of a supposed triose was 0·74 (solvent A). Similarly the lactones present in an equilibrium solution of glucose, or of fructose, in lime-water, after shaking with Amberlite resin IR-120, were identified (solvent B) as chiefly α-D-glucosaccharinolactone (R_F 0·56), with α-D-gluco*iso*saccharinolactone (R_F 0·42), and β-D-glucometasaccharinolactone (R_F 0·28), together with the isomers tentatively identified in the product from 1-*O*-methylfructose.

In addition to the quantitative studies cited in the introduction, others were made, based on the modified lactonisation procedure of Bamford, Bamford, and Collins (*loc. cit.*) with a correction factor of 1·15 based on a preliminary experiment with authentic α-D-glucosaccharinolactone. The same factor was applied to the corresponding experimental results obtained with 1-*O*-methylfructose. The following results were thus obtained with solutions of glucose (0·788 g.) and fructose (0·783 g.) respectively, each in oxygen-free lime-water (250 ml.; 0·0396N) at 25°.

	Glucose.			*Fructose.*		
Time (hr.)	Total acid (eq./mole)	Sacc. acid (eq./mole)	Lactic acid (eq./mole)	Total acid (eq./mole)	Sacc. acid (eq./mole)	Lactic acid (eq./mole)
0·25	0	*	0	0·013	0	0·013
0·5	0	*	0	0·023	0	0·023
1·0	0	*	0	0·046	0·003	0·043
2·0	0·003	*	0·003	0·072	0·013	0·059
3·0	0·007	*	0·007	0·01	0·02	0·08
4·0	0·023	0	0·023	0·13	0·03	0·10
5·0	0·030	0·003	0·027	0·16	0·03	0·13
6·0	0·047	0·007	0·040	0·20	0·04	0·16
7·0	0·064	0·013	0·051	0·22	0·045	0·18
24	0·37	0·05	0·32	0·56	0·10	0·46
49	0·69	0·10	0·59	0·82	0·13	0·69
193	1·34	0·28	1·06	1·34	0·32	1·02
529	1·58	0·28	1·30	1·52	0·33	1·19
625	1·62	0·28	1·34	1·52	0·32	1·20

* Not determined

This work forms part of the programme of fundamental research undertaken by the Council of the British Rayon Research Association.

British Rayon Research Association,
Barton Dock Road, Urmston, Nr. Manchester.

[*Received, January* 23*rd*, 1954.]