The Degradation of Carbohydrates by Alkali. Part V. Lactulose, Maltose, and Maltulose.*

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The behaviour of lactulose, maltose, and maltulose towards lime-water at 25° accords with the scheme put forward earlier (*J.*, 1953, 2245).

It was shown in Part II (Corbett and Kenner, *J.*, 1953, 2245) that lactose suffers degradation by lime-water to a mixture of α- and β-*iso*saccharinic acid and galactose. Conversion of aldose into ketose was postulated as the first step. Accordingly lactulose has now been prepared by a method essentially that of Hudson and Montgomery (*J. Amer. Chem. Soc.*, 1930, **52**, 2101) but so reinforced that the product might satisfy chromatographic tests not then available.

The action of lime-water on lactulose has been studied by Isbell (*J. Res. Nat. Bur. Stand.*, 1941, **26**, 35), who attributed an observed increase in dextrorotatory power solely to partial reversion of the ketose to lactose. However, our examination of the various aspects of the reaction has shown it to correspond in detail with the degradation of lactose but to proceed, as expected, more rapidly.

In order further to substantiate the scheme of degradation put forward for lactose and lactulose, we also studied maltose and maltulose, with the expected results. Maltose gave glucose and α- and β-*iso*saccharinic acid as initial products, whilst the rate of decomposition of maltulose so substantially exceeded that of maltose that only in experiments on a larger scale could its formation be detected chromatographically during the degradation of maltose. Similarly no maltose, resulting from a Lobry de Bruyn transformation, was observable during the degradation of maltulose. By contrast the slower rates of degradation of lactose and lactulose permitted the detection of each during the degradation of the other.

Another difference between the reactivities of 4-*O*-α-glucosyl- and 4-*O*-β-galactosyl-fructose lay in that, whereas in the former case the observed decrease in alkalinity of its solution in lime-water in the early stages of the reaction afforded a measure of *iso*saccharinic

* Part IV, preceding paper.

acid formation consistent with the other experimental data, the value so deduced in the latter case was excessive. We shall later record a similar experience with cellobiose.

A time-lag in the onset of decomposition was observed with each aldose, corresponding to the necessary preliminary genesis of the ketoses and to the similarly delayed action on glucose (Kenner and Richards, preceding paper).

Finally, a detailed examination of the products from a large-scale experiment on the degradation of lactose showed it not to afford a satisfactory mode of preparing pure galactose owing to simultaneous formation of other hexoses not easily separable from it.

Experimental

Lactulose.—The amorphous product (18 g.) prepared from lactose (120 g.) by the adoption of Montgomery and Hudson's procedure (*J. Amer. Chem. Soc.*, 1930, **52**, 2101) described by the National Bureau of Standards (Circular C.440, Washington, 1942, p. 467) was freed from monoses by elution of these with water from the adsorbate on a charcoal–Celite column (Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677). The disaccharide was recovered by elution with 5% aqueous ethanol. After removal of ions by a mixture of resins [Amberlite IR-120(H) and IR-4B(OH)], the chromatographically satisfactory ketose was still amorphous, $[\alpha]_D^{23}$ (equil.) −49·1° (*c*, 1·140 in H_2O); values of −51·5° and −50·7° are recorded by the respective authorities cited above.

Table 1 summarises the observations made with a solution (50 ml.) of the ketose (0·3946 g.) in saturated oxygen-free lime-water (0·042N) at 25°, determinations of monoses and saccharinic acids being carried out as in the case of lactose (Corbett and Kenner, *loc. cit.*; Corbett, *Chem. and Ind.*, 1953, 1285) and calculated on the basis of formation of one molecular proportion of each from one of the ketose. As in all other cases studied the monose content reached a maximum and towards the end of the degradation was less than the disaccharide degraded owing to the further action of the lime-water on the former to give excess of acid.

Maltose and Maltulose.—Tables 2 and 3 in respect of the behaviour of maltose (0·8616 g.) in oxygen-free lime-water (100 ml.; 0·044N) and of maltulose (0·4266 g.) in oxygen-free lime-water (50 ml., 0·041N) are appended. The preparation of the ketose in a chromatographically

Table 1. *Degradation of lactulose by saturated lime-water at 25°.*

Time (hr.)	α_D	Lactulose decompd. (%)	Monoses formed (%)	Sacc. acids (%)	Paper-chromatography *				
					lactulose	lactose	galactose	tagatose	sacc. acids
0·1	−0·06°	1·7	1·2	0·0	3	—	—	—	—
0·5	0·06	2·5	2·4	6·9	3	—	1	—	—
1	0·05	6·4	7·2	9·1	3	—	1	—	—
2	0·03	15·5	13·2	16·2	3	1	1	—	1
3	0·01	18·3	22·8	14·7	3	1	1	—	1
5	+0·01	25·0	33·7	29·2	3	1	1	1	1
6	0·01	29·5	36·7	37·4	3	1	1	1	1
24	0·03	66·8	58·8	63·8	2	1	2	1	2
30	0·03	74·4	66·7	71·1	2	1	2	1	2
48	0·01	81·7	67·5	84·5	1	1	2	1	2
54	0·01	83·9	69·3	82·4	1	1	2	1	2
72	0·01	88·1	68·1	85·5	1	1	2	1	2
96	0·01	89·2	65·0	81·2	1	1	2	1	2
168	0·01	89·9	63·2	82·4	1	1	2	1	2

* Numerals here and in Tables 2 and 3 denote relative intensity, 3 denoting the greatest intensity.

Table 2. *Degradation of maltose by saturated lime-water at 25°.*

Time (hr.)	α_D	Maltose decompd. (%)	Monoses formed (%)	Total acids produced (%)	Paper chromatography				
					maltose	glucose	fructose	mannose	sacc. acids
0·5	+0·48°	0·0	0·0	0·0	3	—	—	—	—
1	0·46	0·0	0·0	0·0	3	—	—	—	—
2	0·43	7·5	4·8	7·6	3	1	—	—	—
4	0·36	16·5	8·6	21·0	3	1	—	—	—
6	0·31	36·0	22·4	31·4	3	1	—	—	1
24	0·10	83·4	71·5	100·0	1	2	1	1	2
48	0·04	91·7	78·2	126·8	1	2	1	1	2
120	±0·00	100·0	65·5	171·0	—	1	1	1	2
144	±0·00	100·0	67·5	174·0	—	1	1	1	2
192	±0·00	100·0	61·5	188·0	—	1	1	1	2
288	±0·00	100·0	56·2	199·0	—	1	1	—	2

TABLE 3. *Degradation of maltulose by saturated lime-water at 25°.*

Time (hr.)	α_D	Maltulose decompd. (%)	Monoses formed (%)	Total acids produced (%)	Paper chromatography: maltulose	glucose	fructose	mannose	sacc. acids
0·25	+0·06°	12·9	18·4	5·2	3	1	—	—	—
1	0·06	31·5	36·1	19·9	3	1	—	—	1
2	0·05	50·6	50·2	37·7	3	1	—	—	1
3	0·06	59·3	56·3	53·5	3	2	—	—	1
4	0·04	70·7	68·6	61·8	2	2	—	—	1
5	0·03	78·0	77·5	66·1	2	2	—	—	2
6	0·04	80·5	75·2	71·5	2	2	—	—	2
7	0·03	82·5	78·0	73·4	1	2	1	—	2
24	0·01	95·5	88·7	94·3	1	2	2	1	2
31	±0·00	97·0	87·0	99·0	1	2	2	1	2
48	0·00	100·0	88·0	108·2	—	2	2	1	2
72	0·00	100·0	73·0	122·0	—	2	2	1	2

satisfactory state followed the lines indicated in the case of lactulose and the product had $[\alpha]_D^{19}$ (equil.) +56·2° (*c*, 0·499 in H_2O). Peat, Roberts, and Whelan (*Biochem. J.*, 1952, **51**, xvii) and Hough, Jones, and Richards (*J.*, 1953, 2005) respectively observed $[\alpha]_D$ +52·8° (in H_2O) and +58—64° (*c*, 1·58 in H_2O).

Isolation of the Degradation Products from Maltose.—A solution (5 l.) of maltose (50·0 g.) in saturated oxygen-free lime-water was kept at 20—23° for 65 hr. before it was neutralised with cold aqueous oxalic acid, filtered, and concentrated at 60° to *ca.* 500 ml. The sparingly soluble calcium α-*iso*saccharinate (7·20 g., 24·7%) which separated was converted quantitatively into α-*iso*saccharin, having m. p. 94—96°, $[\alpha]_D^{20}$ +60·3° (*c*, 4·21 in H_2O), after one crystallisation from ethyl acetate (Found: C, 44·3; H, 6·3. Calc. for $C_6H_{10}O_5$: C, 44·5; H, 6·2%). Nef (*Annalen*, 1910, **376**, 52) gave m. p. 96°, $[\alpha]_D^{20}$ +61·9°.

After quantitative precipitation of calcium, as oxalate, from the mother-liquors these were eluted from a column of Amberlite IR-4B resin (100 g.). The combined eluate and aqueous washings (1 l.) were concentrated to a syrup (24·67 g., 94·0% for monoses), shown by paper chromatography to contain essentially glucose and fructose, with small amounts of maltose, maltulose, and psicose (?), and traces of saccharinic acids. The dried syrup (from which glucose phenylosazone, m. p. and mixed m. p. 203—205°, was prepared in good yield) was extracted with dry acetone; concentration of the extract afforded a mixture of *iso*saccharins (1·56 g.).

Finally the basic resin was eluted with 0·1M-sodium carbonate (750 ml.) followed by water (500 ml.), the combined eluates then being stirred for several hours with Amberlite IR-120 resin (200 g.) and filtered. The acidic resin was washed with water (3 × 250 ml.), and the washings were combined with the filtrate and concentrated under reduced pressure to a dark syrup (3·487 g., 15%). This, when neutralised with lime-water, treated with charcoal, filtered and concentrated, yielded a syrup which solidified on trituration with alcohol. The calcium salts (2·831 g.) thus obtained were heated with water (5 ml.) and then kept at 0° for several hours before removal of the sparingly soluble calcium salt (0·548 g.). This was calcium lactate from which was prepared the brucine salt, m. p. 192—199°, $[\alpha]_D^{22}$ −29·0° (*c*, 1·79 in H_2O) (Found: N, 5·9. Calc. for $C_{26}H_{32}O_7N_2$: N, 5·8%). Nef (*loc. cit.*) gives m. p. 210°, $[\alpha]_D^{20}$ −29·05°. To the filtrate alcohol was added in portions to give the following fractions: from 54% alcohol, a white amorphous powder (0·049 g.), $[\alpha]_D^{23}$ −2·1° (*c*, 0·49 in H_2O); from 61% alcohol, 0·031 g., $[\alpha]_D^{23}$ ±0·00° (*c*, 0·31 in H_2O); from 70% alcohol, *calcium* β-iso*saccharinate* (0·365 g.), $[\alpha]_D^{23}$ +3·1° (*c*, 0·96 in H_2O) (Found: Ca, 10·7. $C_{12}H_{22}O_{12}Ca$ requires Ca 10·1%); from 82% alcohol, further calcium β-*iso*saccharinate (0·334 g.), $[\alpha]_D^{23}$ +2·2° (*c*, 1·39 in H_2O), from which was prepared brucine β-*iso*saccharinate, m. p. and mixed m. p. 190—198°, $[\alpha]_D^{21}$ −22·0° (*c*, 1·81 in H_2O) [Nef (*loc. cit.*) gives m. p. 200—210°, $[\alpha]_D^{20}$ −21°]; from 100% alcohol, further calcium β-*iso*saccharinate (0·863 g.), $[\alpha]_D^{20}$ +2·8° (*c*, 1·39 in H_2O) (Found: Ca, 10·1%). A solution of this salt, after the removal of calcium ions by Amberlite IR-120 resin, gave $[\alpha]_D^{22}$ +8·5° (*c*, 0·93 in H_2O), calculated for β-*iso*saccharin; Nef (*loc. cit.*) gives $[\alpha]_D^{22}$ *ca.* +6° for a crude sample of the β-*iso*saccharin. Crystalline β-*iso*saccharin could not be obtained from the purified calcium salt.

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