The Degradation of Carbohydrates by Alkali. Part VI. Laminaribiose and Turanose.*

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The observed approximate agreement in the rates of degradation of laminaribiose and of turanose by lime-water to glucometasaccharinic acid and glucose is attributed to their having a common doubly charged enediol ion. Earlier formulations, in this series, of similar reactions are correspondingly revised and a general interpretation of the behaviour of glucose towards varying strengths of alkali is provided.

It is implicit in the β-alkoxycarbonyl mechanism of the alkaline degradation of disaccharides and of alkylated monoses that such degradation should be especially easy when the glycoside or alkyl group is attached in the β-position to the carbonyl group of the monose. Details are now communicated in regard to laminaribiose (3-*O*-β-D-glucopyranosyl-D-glucopyranose), the first case in which this inference was confirmed (Corbett, Kenner, and Richards, *Chem. and Ind.*, 1953, 154). As was further expected, the products of its treatment with lime-water proved to be essentially a mixture of the α- and the β-form of metasaccharinic acid (II) with glucose and fructose. Graphs of the respective courses of biose decomposition, monose formation, and acid formation are closely concordant until, in the later stages of the reaction, some degradation of monose asserts itself.

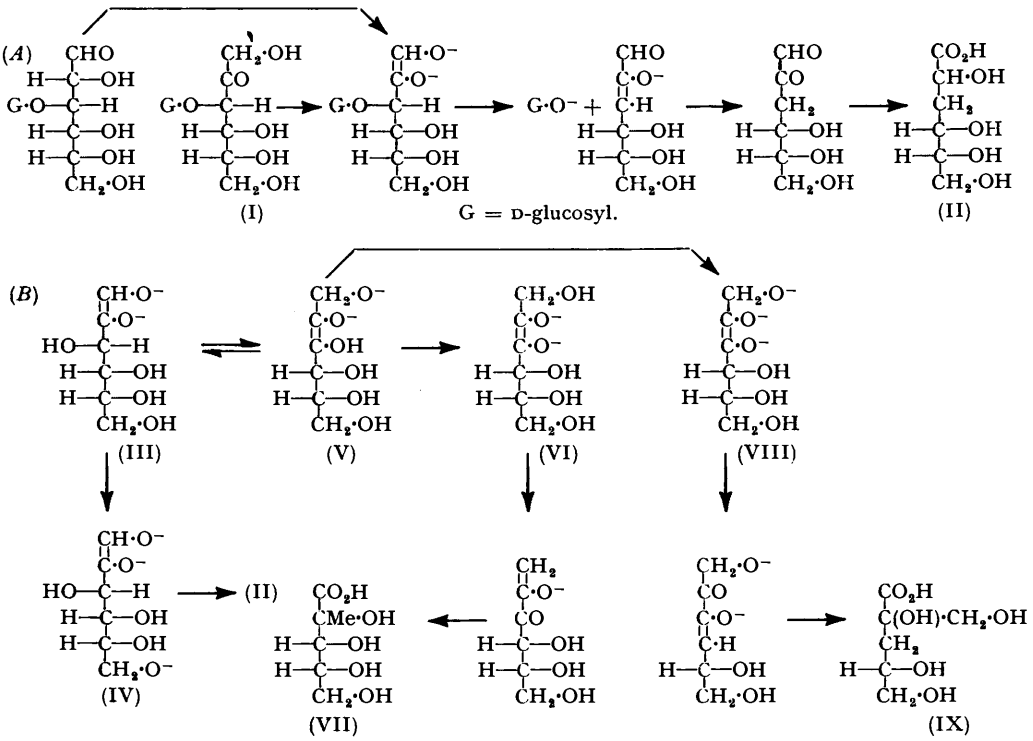
Turanose is the ketose, 3-*O*-α-D-glucopyranosyl-D-fructose (cf. I) (Isbell, *J. Res. Nat. Bur. Stand.*, 1941, **26**, 35), and Isbell interpreted the results of his study of its behaviour towards N-potassium hydroxide in terms of hydrolysis to equimolecular proportions of fructose and glucose, citing, as evidence of formation of monosaccharide, the isolation of potassium arabonate after oxidation of the resulting alkaline solution. However, our experiments with lime-water demonstrated the formation, as in the case of laminaribiose, of glucometasaccharinic acid as the other main product. Some lactic acid was also formed, as it was from 3-*O*-methylfructose (Kenner and Richards, *J.*, 1954, 278).

The degradation of reducing sugars by anion-exchange resins, observed by Rebenfeld and Pacsu (*J. Amer. Chem. Soc.*, 1953, **75**, 4370; cf. Hulme, *Nature*, 1953, **171**, 610) occurs by a mechanism analogous to that for lime-water, and their observation that the degradation of turanose by the carbonate form of Amberlite IRA-400 resin liberates only glucose (and no fructose) is in agreement with our results. With more basic reagents (*e.g.*, calcium hydroxide or the hydroxide form of IRA-400) fructose is produced at a later stage by a Lobry de Bruyn–van Ekenstein transformation of glucose (*Rec. Trav. chim.*, 1895, **14**, 203; 1897, **16**, 251, 274, 278).

A further and very significant relation between the results obtained with the 3-*O*-

* Part V, *J.*, 1954, 1789.

methyl- and the 3-*O*-glucosyl-hexoses lay in the approximately equal rates of degradation of aldose and ketose in each case. It thus appears that a common anion is concerned and compliance with this is secured by postulating charges on both the 1- and the 2-oxygen atom of the common enediol form. First and second dissociation constants of glucose and fructose have been recorded by various workers (Hirsch and Schlago, *Z. physikal. Chem.*, 1929, **41**, 387; Stearn, *J. Phys. Chem.*, 1931, **35**, 2226; Shaffer and Harned, *J. Biol. Chem.*, 1931, **93**, 311; Urban and Shaffer, *ibid.*, 1932, **94**, 697; Urban and Williams, *ibid.*, 1933, **100**, 237) and a third constant asserts itself at pH 13·6 (Urban and Williams, *loc. cit.*). Although calculation on this basis indicates that under our experimental conditions only about 4% of glucose would be present in the doubly charged form as against 70% in the singly charged form, it is nevertheless evident that the former is much more prone to extrude an anion and so, when it is available, is the rate-determining organic reactant. We thus have the annexed reactions (*A*), with corresponding amendment of reaction schemes in earlier papers of this series. In particular, the scheme (*B*) expresses the behaviour of glucose and fructose towards dilute and concentrated alkali respectively, though there can obviously be no sharp line of distinction between the two. Since a primary is more acidic than an isomeric secondary alcohol, the anion (III), common to glucose and fructose, may be expected in concentrated alkali to furnish (IV) from which meta-



saccharinic acid will be derived; on the other hand, the anion (III) is convertible by prototropy into (V) which leads, in relatively weak alkali, through (VI) to saccharinic acid (VII) but in concentrated alkali through (VIII) to *iso*saccharinic acid (IX). Nef obtained meta- with a small proportion of *iso*-saccharinic acid from glucose by the action of 8N-sodium hydroxide (*Annalen*, 1910, **376**, 89).

Experimental

Laminaribiose.—The Table records observations made when a solution of laminaribiose (Bächli and Percival, *J.*, 1952, 1243) {0·2869 g.; m. p. 182—186°; $[\alpha]_D^{21}$ +18·1° (*c*, 3·21 in H_2O)} in oxygen-free lime-water (50 ml.; 0·0383N) was kept at 25°. The course of the reaction

was followed by determining at intervals (*a*) biose and monose (cf. Corbett, *Chem. and Ind.*, 1953, 1285), (*b*) saccharinic acid (Bamford, Bamford, and Collins, *Proc. Roy. Soc.*, 1950, *A*, **204**, 85), and (*c*) total acidity (by direct titration). The general agreement of the experimental results in regard to (*a*) and (*b*) demonstrates the general accuracy of these determinations but the earlier results under (*c*) in relation to low concentrations are manifestly unsatisfactory.

Degradation of laminaribiose by saturated lime-water at 25°.

Time (hr.)	α_D	Monoses formed (%)	Laminaribiose decompd. (%)	Saccharinic acids formed (%)	Total acids formed (%)	Paper chromatography *			
						Laminaribiose	Glucose	Fructose	Sacc. acids
0·1	+0·03°	7·0	4·3	7·7	3·3	3	1	—	—
0·5	0·03	19·8	17·6	11·6	11·9	3	1	—	1
1	0·04	31·5	29·4	31·1	21·5	3	1	—	1
2	0·05	54·3	51·1	49·0	42·7	2	2	1	1
3	0·04	65·8	64·4	62·3	55·3	2	2	1	1
4	0·03	74·6	74·7	71·6	65·7	1	2	1	1
5	0·02	75·7	77·0	73·9	72·3	1	2	1	1
6	±0·00	81·5	81·3	79·8	77·1	1	2	1	1
12	±0·00	88·4	87·7	84·2	92·2	1	2	2	2
24	−0·02	86·6	92·1	99·2	104·5	—	2	2	2
27	−0·02	86·0	93·5	102·9	118·5	—	2	2	2
48	−0·04	84·0	100·0	114·4	125·8	—	2	1	2

* Numerals denote relative intensity, 3 being the greatest.

The saccharinic acid obtained was chromatographically identical with the metasaccharinic acid prepared, as will be described in a later paper, directly and as sole acidic product, by similar treatment of "insoluble" laminarin, and thus available in the quantity adequate for the separation into α- and β-isomerides requisite for its proper characterisation (cf. Nef, *loc. cit.*).

Turanose.—The material used, supplied by L. Light and Co., was chromatographically uniform towards butanol–pyridine–water (3 : 2 : 1·5) (alkaline silver nitrate spray) and had m. p. 168°, $[\alpha]_D^{21}$ +74·7° (*c*, 2·60 in H_2O). Bates (Nat. Bur. Stand. Circular 6440, p. 758, Washington 1942) gives m. p. 157°, $[\alpha]_D^{20}$ +75·8°. A solution of turanose (1·230 g.) in saturated oxygen-free lime-water (0·043N) (250 ml.) was kept at 25° for 48 hr. After quantitative removal of calcium as oxalate, the filtered solution was concentrated and exhaustively extracted with ether, the extract being kept neutral by the presence of an aqueous suspension of zinc carbonate (see Nadeau, Newlin, and Evans, *J. Amer. Chem. Soc.*, 1933, **55**, 4957). From the extract was obtained zinc lactate (0·231 g.) from which was prepared (−)-brucine DL-lactate, m. p. and mixed m. p. 190—200°.

The residual aqueous solution was passed through Amberlite IR-4B (OH) resin (5 g.), the column then being washed with water (25 ml.) until free from reducing material. Concentration of the eluate and washings afforded a syrup (0·839 g.) which was extracted with dry acetone (3 × 20 ml.). The residue (0·693 g., 107%) was shown by paper chromatography to be essentially a mixture of glucose and fructose with traces of psicose. The acetone extract yielded a mixture (0·136 g., 23·4%) of α- and β-metasaccharin. The resin column was eluted with 0·1M-sodium carbonate (50 ml.) and then with water (100 ml.). The combined eluates were freed from sodium ions by Amberlite IR-120 (H) resin (100 g.) and concentrated, to give a mixture of metasaccharins (0·130 g., 22·3%) from which was prepared (−)-brucine β-metasaccharinate, m. p. and mixed m. p. 145—168°, $[\alpha]_D^{22}$ −38·0° (*c*, 0·21 in H_2O). Nef (*loc. cit.*) gives m. p. 130—150° (decomp.), $[\alpha]_D$ −33·1°.

Tests preliminary to a kinetic study of the above reaction showed the charcoal–Celite column techniques used in previous instances to be inadequate in this case owing to elution of some turanose with the monosaccharides. Another method, based on reducing power determined by the Hagedorn–Jensen procedure was therefore evolved [though it was subsequently found possible to separate glucose and turanose by using charcoal–Celite columns of increased length (Corbett, *loc. cit.*)]. In this way 0·342 mg. of turanose and 0·360 mg. of glucose were found to be equivalent to 1·98 and 4·72 ml. respectively of 0·002N-thiosulphate. Hydrolysis of an aqueous solution (2 ml.) of the ketose (0·012 g.) in N-sulphuric acid (5 ml.) having been shown to be complete after 3 hr. at 100°, the procedure was applied to mixtures of glucose and turanose equivalent to x and y ml. of thiosulphate solution respectively. Hence before hydrolysis, titre $A = (x + y)$ ml., and after hydrolysis $B = (x + 2{\cdot}38y)$ ml., whence $B - A = 1{\cdot}38y$ ml.

Then a solution (50 ml.) of turanose (0·2845 g.) in saturated oxygen-free lime-water (0·0333N) was kept at 25°; at noted periods aliquot portions (2 ml.) were withdrawn and run into 0·01N-sulphuric acid (10 ml.); after ¼ hr. the solution was back-titrated with 0·01N-sodium hydroxide to the first semi-permanent end-point of phenolphthalein. The solution was diluted to 50 ml., the rotation observed, and the total reducing power before and after acid hydrolysis estimated on samples of 2 ml. The tabulated data were thus obtained.

Degradation of turanose by saturated lime-water at 25°.

Time (hr.)	α_D	Turanose decompd. (%)	Monoses formed (%)	Total acids formed (%)	Paper chromatography *			
					Turanose	Glucose	Fructose	Sacc. acids
0·1	+0·06°	15·0	14·2	3·0	3	1	—	—
1	0·04	32·7	28·4	39·4	3	1	—	1
2	0·01	48·3	41·7	64·8	2	2	—	1
3	±0·00	52·5	50·0	74·4	1	2	—	2
4	±0·00	61·9	59·2	79·5	1	2	1	2
5	±0·00	75·1	65·8	87·0	1	2	1	2
6	±0·00	80·1	76·6	93·0	—	2	1	2
7	±0·00	89·1	84·3	96·8	—	2	1	2
24	±0·00	90·0	86·7	106·0	—	2	2	2
31	±0·00	94·0	85·0	112·5	—	2	2	2
48	±0·00	98·6	78·4	131·8	—	2	2	2

* Numerals denote relative intensity, 3 being the greatest.

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