The Degradation of Carbohydrates by Alkali. Part VIII. Melibiose.*

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The action of lime-water on melibiose is shown to yield lactic acid and galactose, and, by a parallel reaction, galactosyl meta- and *iso*-saccharinic acids. This behaviour is contrasted with that of glucose.

WE now present the results of our study of the action of lime-water on melibiose as a complement to that of Hough, Jones, and Richards (*J.*, 1954, 295) on the behaviour of the disaccharide towards ammonia.

Degradation of this 6-*O*-galactosylglucose by alkali would be expected to correspond to that of glucose itself which, under similar conditions, yields essentially lactic and saccharinic acid. Thus we should have as alternative modes of decomposition :

(*a*) 1 mol. of melibiose ⟶ 2 mols. of lactic acid + 1 mol. of galactose

and

(*b*) 1 mol. of melibiose ⟶ 1 mol. of galactosylsaccharinic acid(s)

whence (on the assumption that galactose is only slowly attacked under the conditions of the experiment at any time), in molar concentrations, lactic acid is equivalent to two galactose molecules, and galactosylsaccharinic acid is equivalent to the melibiose decomposed

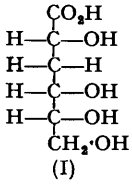
Degradation of melibiose by saturated lime-water at 25°.

Time (hr.)	α_D	Melibiose decompd. (equiv.)	Monoses produced (equiv.)	Lactic acid (calc. equiv.)	Gal.-sacch. acids (calc. equiv.)	Total acid (calc. equiv.)	Total acid (found equiv.)	Substances found on paper
1	+0·16°	0·000	0·000	0·000	0·000	0·000	0·022	Melibiulose
2	0·16	0·000	0·000	0·000	0·000	0·000	0·023	
3	0·16	0·000	0·000	0·000	0·000	0·000	0·053	Galactose
4	0·15	0·000	0·000	0·000	0·000	0·000	0·073	
5	0·15	0·000	0·000	0·000	0·000	0·000	0·102	
6	0·14	0·063	0·063	0·126	0·000	0·126	0·128	
24	0·11	0·218	0·202	0·405	0·016	0·421	0·398	
30	0·11	0·244	0·202	0·405	0·041	0·446	0·499	Acids and tagatose
48	0·10	0·344	0·289	0·577	0·055	0·632	0·648	
54	0·09	0·375	0·256	0·511	0·120	0·631	0·708	
120	0·07	0·525	0·409	0·819	0·116	0·935	0·924	Further acids and talose
144	0·07	0·552	0·427	0·853	0·126	0·975	0·975	
192	Turbid	0·597	0·457	0·917	0·134	1·051	1·043	
216		0·617	0·442	0·917	0·175	1·092	1·108	
288		0·700	0·558	1·118	0·142	1·260	1·202	

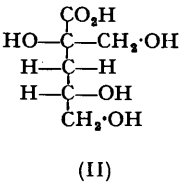
minus the galactose formed. The annexed Table of analytical results shows how, after the early stages of reaction, they broadly confirm this deduction.

* Part VII, preceding paper.

On the other hand, and in striking contrast with the behaviour of glucose, the saccharinic acids obtained from melibiose are essentially of the meta- (I), with some of the *iso*-type (II), whereas the main product of the action of lime-water on glucose is saccharinic acid (III) (Kiliani, *Ber.*, 1882, **15**, 2953). Our findings accord rather with Nef's (*Annalen*, 1910, **376**, 89) on the action of 8N-sodium hydroxide on glucose, which we have interpreted in terms of attack by the alkali on the primary alcoholic group (*J.*, 1954, 3274). This group



(I)



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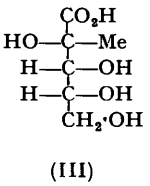

(III)

is of course not available in melibiose and it will be desirable to ascertain whether other compounds of this type behave similarly.

Experimental

Isolation of the Degradation Products.—A solution [5 l.; $\alpha_D = 2{\cdot}77°$ ($l = 4$) after 5 min.] of melibiose (28·45 g.) in saturated oxygen-free lime-water (0·033N) was kept at approx. 21—22° until the observed optical rotatory power was constant, values being :

Time (days)	1	4	5	6	7	8	11	12	15	19
α_D (4 dm.)	2·27°	1·70°	1·68°	1·44°	1·41°	1·30°	1·20°	1·16°	1·06°	0·98°

The calcium ions were then completely removed by addition of the theoretical amount of oxalic acid before the solution was concentrated under reduced pressure to 100 ml., and exhaustively extracted with ether for 5 days, the extract being kept neutral by the presence of an aqueous suspension of zinc carbonate (Nadeau, Newlin, and Evans, *J. Amer. Chem. Soc.*, 1933, **55**, 4957). Zinc DL-lactate (5·673 g.) was thus obtained and identified by conversion into the (−)-brucine salt, m. p. and mixed m. p. 190—200°. The extracted aqueous solution was filtered from inorganic material and stirred with Amberlite IR-4B resin (50 g.) for 20 hr. After filtration and washing of the resin with water (2 × 100 ml.) concentration of the combined filtrate and washings yielded a syrup (10·705 g.) which was shown by paper chromatography to be essentially a mixture of mono- and di-saccharides; it gave spots of R_F (BuOH–C_5H_5N–H_2O; 6 : 4 : 3) 0·057 (melibiose), 0·10 (melibiulose), 0·21 (galactose), 0·255, 0·31, 0·34, and 0·39 (tagatose, talose, sorbose ?), 0·49, 0·55, and 0·70 (saccharins). The resin was then stirred for 24 hr. with 0·1M-sodium carbonate (400 ml.), filtered, and washed with water (2 × 100 ml.), and the combined filtrate and washings were then stirred for a further 24 hr. with Amberlite IR-120 resin (100 g.). After filtration the resin was washed with water (2 × 100 ml.), the combined filtrate and washings were concentrated under reduced pressure to a syrup (3·862 g.) and neutralised with lime-water. Concentration afforded the amorphous calcium salts (4·023 g.).

After these had been heated with water (7 ml.) the mixture was cooled and filtered, to give an insoluble calcium salt (0·812 g.) of which a sample (0·148 g.) gave brucine DL-lactate (0·608 g.), m. p. and mixed m. p. 190—200°, $[\alpha]_D^{24}$ −35·4° (*c*, 2 in H_2O). Alcohol was added in portions to the filtrate to give fractions : (*a*) (from 59% ethanol) white amorphous powder (1·205 g.), $[\alpha]_D^{22}$ +58·6° (*c*, 1·01 in H_2O) ⟶ +68·7° (*c*, 0·80 in H_2O, corrected for removal of calcium ions) on treatment with Amberlite IR-120 resin; (*b*) (from 72% ethanol) white amorphous powder (0·310 g.), $[\alpha]_D^{22}$ +65·2° (*c*, 1·10 in H_2O) ⟶ +68·8° (*c*, 0·99 in H_2O) (Found : C, 39·0; H, 6·7. Calc. for $C_{24}H_{42}O_{22}Ca$: C, 39·9; H, 5·9%); (*c*) (from 85% ethanol) straw-coloured amorphous powder (0·077 g.), $[\alpha]_D^{21}$ +72·6° (*c*, 0·77 in H_2O), and (*d*) straw-coloured amorphous powder (0·228 g.), $[\alpha]_D^{21}$ +66·3° (*c*, 0·77 in H_2O). These fractions proved to contain mixtures of 6-*O*-galactopyranosylsaccharinic acids. Thus, when a sample of fraction (*a*) (0·767 g.) in water (50 ml.) was heated with Amberlite IR-120 resin (5 g.) at 75° for 120 hr. the following periodical observations were made :

Time (hr.)	0	1	20	26	43	49	100	120
α_D (2 cm.)	1·81°	1·80°	1·75°	1·73°	1·66°	1·64°	1·51°	1·34°

Paper chromatography with butanol–water–acetic acid (4 : 2 : 1) followed by the hydroxylamine–ferric chloride spray of Abdel Akher and Smith (*J. Amer. Chem. Soc.*, 1951, **73**, 5859) then

yielded two main spots, with R_F 0·19 and 0·29 (cf. authentic β-metasaccharin 0·28), and faint spots with R_F 0·35 and 0·43 (cf. authentic α-*iso*saccharin 0·37). With butanol–pyridine–water (6 : 4 : 3) (silver nitrate spray; Trevelyan, Proctor, and Harrison, *Nature*, 1950, **166**, 444) the main components had R_F 0·17 (authentic galctose 0·17) 0·53, 0·65 (authentic β-metasaccharin 0·62), 0·70 (authentic α-*iso*saccharin 0·68), 0·76 (authentic saccharin 0·74), and 0·80, with streaks of approx. values 0·29 and 0·37. The filtered solution was concentrated, and the residue dried at 45°/0·01 mm. for 1 hr. (0·651 g.) and extracted with dry acetone. The acetone-insoluble residue (0·250 g.) was shown by paper-chromatography to be essentially galactose and from it was prepared galactosazone, m. p. and mixed m. p. 193—195°. The syrup (0·276 g.) obtained by concentration of the acetone extract was essentially a mixture of saccharins.

An aqueous solution of the mixture (0·241 g.) was heated on a boiling-water bath for 1 hr. with excess of brucine, cooled, filtered, and concentrated to a syrup (0·768 g.) which slowly crystallised to give a white product (0·201 g. from 0·536 g.). It was essentially brucine α-metasaccharinate, m. p. 110—127°, $[\alpha]_D^{20}$ −30·5° (*c*, 0·786 in H_2O), and when its aqueous solution was stirred with Amberlite IR-120 (H) resin it gave a lactone, $[\alpha]_D^{20}$ +22·5° (*c*, 0·222 in H_2O). Nef (*loc. cit.*) gives m. p. 145—150°, $[\alpha]_D$ −23·1° and $[\alpha]_D^{20}$ +25·3° for brucine α-metasaccharinate and α-metasaccharin respectively.

Determination of the Degradation Products.—A solution (100 ml.) of melibiose (0·8704 g.) in saturated oxygen-free lime-water (0·0313N) was kept at 25°. Samples (2 ml.) were withdrawn periodically and run into excess of 0·01N-sulphuric acid (10 ml.). After the solution had been titrated with 0·01N-sodium hydroxide (phenolphthalein), it was made up to 50 ml., the optical rotatory power was observed, and the mono- and di-saccharides were estimated in samples of 1 ml. by the charcoal–Celite column method (Corbett, *Chem. and Ind.*, 1953, 1285). Samples of the neutralised solutions were submitted to paper chromatography with butanol–pyridine–water (6 : 4 : 3) (silver nitrate and naphtharesorcinol sprays for development; Hough, Jones, and Wadman, *J.*, 1950, 1702). As above, the ketoses were identified by the latter spray and by comparison of their R_F values with those of the corresponding aldoses. The results are shown on p. 3281.

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