938. *The Degradation of Xylobiose and Xylotriose by Alkali.*

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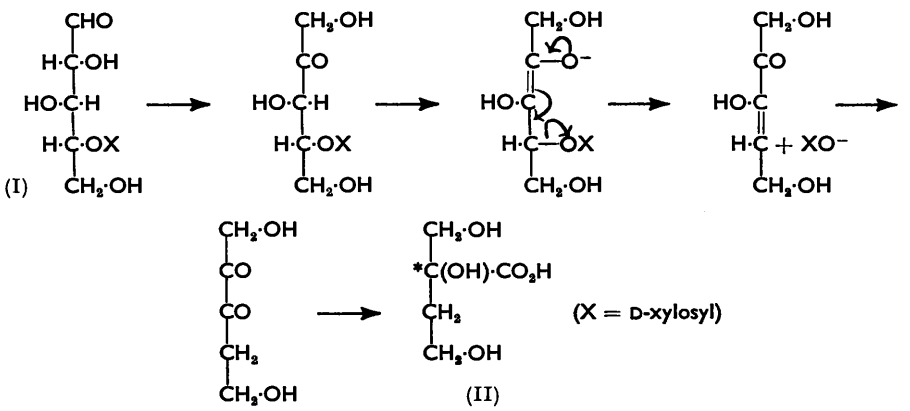
The 1 : 4-lactone of (±)-2 : 4-dihydroxy-2-hydroxymethylbutanoic acid (xylo*iso*saccharinolactone) has been synthesised from 1 : 4-diacetoxybutan-2-one. The alkaline degradation of xylobiose and xylotriose has been studied, and xylo*iso*saccharinolactone has been identified as a major degradation product.

The xylans, which constitute the major fraction (other than cellulose) of the cell-wall polysaccharides of many land plants, normally require the use of alkaline solutions for their removal from the plant. Structural studies [1] have shown that all the xylans so far examined from land plants contain a backbone of 1 : 4-linked β-D-xylopyranose residues, but that the several xylans differ in the number, nature, and mode of linkage of the sugar residues attached as side-chains. We have little knowledge, however, of the extent to which these polysaccharides may have undergone structural modification during their isolation by a process analogous to the alkaline degradation of reducing sugars.[2] We now report on the alkaline degradation of 4-*O*-β-D-xylopyranosyl-D-xylopyranose (xylobiose) (I) and xylotriose, oligosaccharides chosen as model compounds containing the 1 : 4-linkages typical of the xylans. Since the appearance of a preliminary account [3] of some of the following results, Whistler and Corbett [4] have published the results of a similar investigation.

It would be expected from work by Corbett, Kenner, and Richards [2] that 1 : 4-linked xylo-oligosaccharides [*e.g.* xylobiose (I)] would be degraded by alkali with the formation of xylo*iso*saccharinic acid (II). This acid contains only one asymmetric carbon atom, marked *, and if degradation proceeds by the type of mechanism indicated involving a benzilic acid rearrangement in the final stage a racemic product would be expected. To facilitate subsequent studies this acid was synthesised. 1 : 4-Diacetoxybutan-2-one, prepared by the hydration of butyne-1 : 4-diol diacetate,[5] was converted into the cyano-



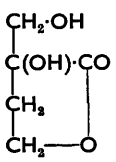
hydrin; hydrolysis of the crude cyanohydrin, which was accompanied by removal of the acetyl groups and lactonisation, yielded the 1 : 4-lactone (III) of (±)-2 : 4-dihydroxy-2-hydroxymethylbutanoic acid (II). The structure of the lactone was confirmed by the isolation of formaldehyde and α-tetronic acid [6] on periodate oxidation.

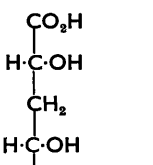
[1] Hirst, *J.*, 1955, 2974.
[2] Corbett, Kenner, and Richards, *J.*, 1955, 1810, and previous papers in the series "The Degradation of Carbohydrates by Alkali."
[3] Aspinall, Carter, and Los, *Chem. and Ind.*, 1955, 1553.
[4] Whistler and Corbett, *J. Amer. Chem. Soc.*, 1956, **78**, 1003.
[5] Lozac'h, *Bull. Soc. chim. France*, 1944, **11**, 514.
[6] Schinz and Hinder, *Helv. Chim. Acta*, 1947, **30**, 1349.

Although most recent studies of the alkaline degradation of carbohydrates [2] have involved the use of lime-water, we have investigated the action of *ca.* N-sodium hydroxide (in the absence of oxygen) on xylobiose (I) and xylotriose in order to simulate the conditions normally employed in the extraction of xylans from lignified tissues. Preliminary experiments showed that the reaction patterns were similar with both reagents and we have already reported the isolation of the lactone (III) from the reaction of lime-water on xylobiose.[3] Paper chromatography showed that xylobiose when treated with alkali produced four lactones, one of which travelled at the same rate as the synthetic lactone (III). The other three lactones were also detected as products of the alkaline degradation of D-xylose and it is probable that these were the lactones of the isomeric "*erythro*"- and "*threo*"-D-2 : 4 : 5-trihydroxypentanoic acids (IV and V) and of 2 : 4-dihydroxybutanoic acid (VI) isolated by Nef from the action of 8N-sodium hydroxide on D-xylose.[7] The *iso*lactone (III) could not be detected amongst the degradation products of D-xylose. When an acid-degraded esparto xylan was treated with N-sodium hydroxide, chromatographic examination of the products showed the presence of the *iso*lactone and an unknown substance which moved more slowly on the chromatogram; the three lactones known to be formed from xylose could not be detected. The unknown substance was an ester or lactone which yielded xylose and the *iso*lactone on hydrolysis; insufficient of this compound was available for a full characterisation, so that its importance as a product cannot be assessed. It is possible, however, that the compound was a reversion product derived from xylose and the *iso*lactone as a chromatographically similar substance was shown to be produced when an alkaline solution of the acid (II) and D-xylose was de-ionised with cation-exchange resin and concentrated; as no lactones of the acids (IV), (V), and (VI) were detected during the xylan degradation it is unlikely that xylose was formed as a reaction intermediate, but it is possible that xylose might have been produced by the hydrolysis of unchanged xylan during the concentration of the acidic products. The absence of the lactonisable acids (IV), (V), and (VI) during the xylan degradation suggests that these straight-chain acids arise only from xylose, and that their presence amongst the degradation products of xylobiose and xylotriose indicates the formation of xylose in the course of the reaction of these oligosaccharides with alkali—xylose, indeed, was detected chromatographically in these reactions. This suggests that the degradation of xylobiose and xylotriose with alkali involves a "peeling" reaction [2] of the type

$$\text{xylotriose} \longrightarrow \text{xylobiose} \longrightarrow \text{xylose}$$

in which each successive reducing sugar residue gives rise to acidic products with the simultaneous exposure of a new reducing group.


(III)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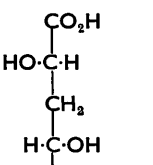
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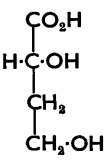
(VI)

Xylo*iso*saccharinolactone (III) was isolated by chromatography from the products of the action of *ca.* N-sodium hydroxide on both xylobiose and xylotriose, and was identical with the synthetic lactone. In neither case was optical activity observed. It is possible that the low optical activity observed for the similar product isolated by Whistler and Corbett [4] either arose from a contaminant or resulted from a partial resolution during its separation *via* the brucine salt.

Quantitative measurements of the acids produced during the alkaline degradations were carried out by a modification [8] of Bamford, Bamford, and Collins's method,[9] in which

[7] Nef, *Annalen*, 1910, **376**, 1.
[8] Kenner and Richards, *J.*, 1954, 1784.
[9] Bamford, Bamford, and Collins, *Proc. Roy. Soc.*, 1950, *A*, **204**, 85.

the lactonisable and non-lactonisable acids were differentiated. From the quantities of lactonisable acids formed from xylose, xylobiose, and xylotriose (0·35, 0·75, and 1·16 mol., respectively), taken together with the quantities of xylo*iso*saccharinolactone isolated from xylobiose and xylotriose (0·38 and 0·82 mol.), it is reasonable to conclude that the same quantities of acids (IV), (V), and (VI) are produced in each case, and that the additional quantities of lactonisable acid formed from xylobiose and xylotriose can be accounted for in terms of the *iso*saccharinolactone. Such an observation is again consistent with a "peeling" mechanism for the alkaline degradation of xylobiose and xylotriose. It is clear, however, from these quantitative measurements that the degradations also result in the formation of non-lactonisable acids and that formation of saccharinic acid is not the only reaction in which glycosidic bonds are broken with the exposure of new reducing groups.

Experimental

Paper-partition chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems (v/v): (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) butan-1-ol–formic acid–water (500 : 115 : 385; top layer); and (C) ethyl acetate–acetic acid–water (10 : 1·3 : 1).

1 : 4-*Lactone of* (±)-2 : 4-*Dihydroxy*-2-*hydroxymethylbutanoic Acid* (*Xylo*iso*saccharinolactone*).—1 : 4-Diacetoxybutan-2-one [5] (15·9 g.) was shaken with a solution of sodium metabisulphite (16 g.) in water (40 ml.), and a solution of potassium cyanide (10 g.) in water (25 ml.) then added slowly with shaking during 0·5 hr., the temperature being maintained at 0°. The mixture was extracted with ethyl acetate and the extract was evaporated. The resulting syrup was hydrolysed with a mixture of concentrated hydrochloric acid (20 ml.) and water (30 ml.) at 60° for 2 hr. and set aside overnight at room temperature. Acid was removed by distillation, the dry residue was extracted with ethyl acetate, and the extract filtered; after partial removal of solvent the 1 : 4-*lactone* of 2 : 4-*dihydroxy*-2-*hydroxymethylbutanoic acid* (6·2 g.) crystallised on cooling. After recrystallisation from ethyl acetate the lactone (5·8 g.) had m. p. 95·5—96·5° (Found: C, 45·6; H, 6·2. $C_5H_8O_4$ requires C, 45·5; H, 6·1%).

*Periodate Oxidation of Xylo*iso*saccharinolactone.*—(a) *Identification of formaldehyde.* Sodium metaperiodate solution (5 ml.; *ca.* 0·3M) was added to a solution of the lactone, and set aside overnight. Excess of periodate was destroyed according to Bell's procedure [10] and addition of dimedone (160 mg.) in ethanol (2 ml.) yielded the formaldehyde derivative, m. p. and mixed m. p. 189—190°.

(b) *Identification of α-tetronic acid.* Sodium metaperiodate solution (1·78 g. in 175 ml. of water) was added slowly during 6 hr. to a solution of the lactone (1·11 g.) in water (25 ml.). Next morning the solution was extracted with ether for 6 hr. Evaporation yielded a solid which, after four sublimations and two recrystallisations from benzene, had m. p. 109—110° and mixed m. p. 106—109° (with α-tetronic acid, synthesised by Schinz and Hinder's method; [6] m. p. 105—106°).

Preparation of Xylobiose and Xylotriose.—Esparto hemicellulose (50 g.) was shaken in water (750 ml.) for 24 hr., water (3·25 l.) and N-sulphuric acid (1 l.) were added, and the mixture was heated on a boiling-water bath for 100 min. The cooled solution was neutralised with sodium hydrogen carbonate and set aside for 48 hr. at 4°. A small quantity of degraded esparto xylan separated and was used in a subsequent experiment. The solution was concentrated and poured on charcoal–Celite (1 : 1, 56 × 7 cm.); elution with water removed monosaccharides (xylose together with small amounts of arabinose) and inorganic salts. Elution with ethanol–water (1 : 19; 13 l.) and ethanol–water (3 : 17; 6 l.) yielded fractions 1 (4·2 g.) and 2 (2·5 g.), consisting largely of xylobiose and xylotriose respectively. Final purifications were effected by chromatography on filter sheets (Whatman 4MM), solvent A being used. A sample of xylobiose was crystallised from aqueous methanol containing a little light petroleum (b. p. 60—80°) and had m. p. and mixed m. p. 183—187°. The xylotriose was characterised by conversion into the octa-acetate, m. p. 110° (Whistler and Tu [11] report m. p. 109—110°).

Determination of Acids Produced by the Action of Alkali on Xylose, Xylobiose, and Xylotriose.—Chromatographically pure sugars were dissolved in carbonate-free sodium hydroxide solution (25 ml.) under oxygen-free nitrogen. The mixture was set aside at room temperature (16—18°),

[10] Bell, *J.*, 1948, 992.
[11] Whistler and Tu, *J. Amer. Chem. Soc.*, 1952, **74**, 4334

and samples (2 ml.) were withdrawn periodically and were de-ionised by passing through a column (35 × 1·2 cm.) of Amberlite resin IR-120(H) (*ca.* 25 g.). The column was then washed with water (30 ml.), and the eluate was titrated potentiometrically with carbonate-free sodium hydroxide (*ca.* 0·1N). After the electrodes had been washed, the total solution was acidified with hydrochloric acid (0·5 ml.; *ca.* N) and concentrated to *ca.* 15 ml. The solution was rapidly

Sugar degraded	Time (days)	Acid (equiv./mole) total	lacton-isable	non-lacton-isable
Xylose (0·079M in 1·1N-NaOH)	3	0·74	0·26	0·48
	5	1·10	0·30	0·80
	7	1·14	0·35	0·79
Xylobiose (0·032M in 0·88N-NaOH)	1	0·55	0·31	0·24
	3	1·30	0·65	0·65
	5	1·80	0·75	1·05
	7	2·15	0·67	1·47
Xylotriose (0·0569M in 0·88N-NaOH)	1	0·78	0·59	0·19
	3	1·50	0·69	0·81
	5	2·12	0·96	1·16
	7	2·54	1·08	1·45
	9	2·84	1·10	1·74
	16	3·03	1·16	1·87

cooled and kept at room temperature for 15 min., and the non-lactonisable acids were titrated potentiometrically with carbonate-free sodium hydroxide. All operations were carried out in a nitrogen atmosphere.

Paper Chromatography of Saccharinic Acids.—Alkaline solutions were de-ionised by treatment with Amberlite resin IR-120(H) and the resulting solutions were evaporated. Saccharinic acids were detected on the chromatogram as lactones by spraying the paper with hydroxylamine followed by acidic ferric chloride as described by Abdel-Akher and Smith.[12] The lactones derived from D-xylose had R_x values of 0·62, 0·81, and 1·09 in solvent C and 0·74, 0·86, and 1·07 in solvent B (**x** = xylo*iso*saccharinolactone). These three lactones in addition to the *iso*-lactone were detected as degradation products from xylobiose and xylotriose.

When acid-degraded esparto xylan was treated with 1·03N-sodium hydroxide in an atmosphere of nitrogen for 28 days, paper chromatography showed the presence of two substances, giving a colour with hydroxylamine and ferric chloride and having R_x values of 0·51 and 1·0 in solvent B. A small sample of the slower-moving component was separated on filter sheets, solvent B being used; hydrolysis with 0·5N-sulphuric acid yielded the *iso*lactone and xylose, both detected chromatographically. An alkaline solution of D-xylose and the synthetic lactone was de-ionised with Amberlite resin IR-120(H) and concentrated to a syrup; chromatography showed substances having R_x values of 0·51 and 1·0.

*Isolation of Xylo*iso*saccharinolactone from the Alkaline Degradation of Xylobiose and Xylotriose.*—A solution of xylobiose (0·88 g.) in 1·1N-sodium hydroxide (carbonate-free) (35 ml.) was set aside at room temperature for 10 days in an atmosphere of nitrogen. The solution was de-ionised by passing it through Amberlite resin IR-120(H) (75 g.) and concentrated to a syrup which was fractionated on filter sheets with solvent B. Extraction of the appropriate sections of the papers yielded a chromatographically pure syrup (0·171 g.) which was crystallised from ethyl acetate to give xylo*iso*saccharinolactone, m. p. and mixed m. p. 94°, $[\alpha]_D$ 0° (*c*, 2·8 in H_2O). In a similar experiment xylotriose (0·414 g.) yielded xylo*iso*saccharinolactone (0·134 g.), m. p. and mixed m. p. 94—96°, $[\alpha]_D$ 0° (*c*, 1·8 in H_2O).

The authors thank Professor E. L. Hirst, F.R.S., for his interest and advice, Messrs. Alex. Cowan and Sons Ltd. for financial assistance (to M. E. C.), and the Rockefeller Foundation for a grant.

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[12] Abdel-Akher and Smith, *ibid.*, 1951, **73**, 5859.