712. *The Degradation of Xylans by Alkali.*

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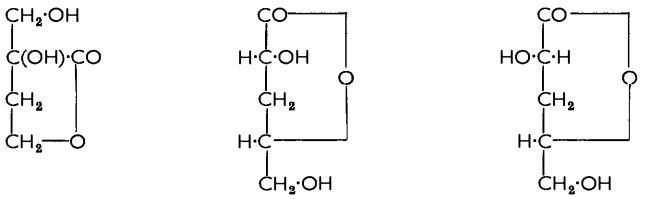
The degradation of some xylans by dilute alkali at room temperature has been followed by (i) analysis of the acids of low molecular weight formed and (ii) determination of the molecular weights. An alkali-stable polysaccharide is formed by reduction of rye-flour arabinoxylan with potassium borohydride. Only slight degradation occurs when this xylan is methylated under alkaline conditions. The results indicate that, under the conditions normally employed for their extraction from plant tissues, alkaline degradation of xylans is slow and proceeds only from the reducing end-group. The effect of side chains attached to position 3 of sugar residues in a 1,4-linked polysaccharide on the progress of the degradation reaction is discussed.

The majority of xylans from land plants require the use of alkaline solutions for their removal from the plant, but the extent to which these polysaccharides are structurally modified during their isolation has not yet been assessed. Alkaline degradation of polysaccharides [1,2] proceeds in a stepwise manner with formation of saccharinic acids [3] and exposure of new reducing groups until this "peeling" reaction is intercepted by a "stopping" reaction, as with cellulose [4] and amylose.[5] With certain exceptions [6] glycosides are stable to alkali under mild conditions, but under drastic conditions [7] may be degraded by alkali, and there is evidence that during the alkaline refining of wood pulp fission of glycosidic linkages in the middle of chains of cellulose [8] and hemicelluloses [9] leads to exposure of new reducing groups from which further "peeling" may ensue. In this

[1] Kenner and Richards, *J.*, 1957, 3019 and earlier papers.
[2] Whistler and BeMiller, *Adv. Carbohydrate Chem.*, 1958, **13**, 289.
[3] Sowden, *Adv. Carbohydrate Chem.*, 1957, **12**, 36.
[4] (*a*) Richards and Sephton, *J.*, 1957, 4492; (*b*) Machell and Richards, *J.*, 1957, 4500.
[5] Machell and Richards, *J.*, 1958, 1199.
[6] Ballou, *Adv. Carbohydrate Chem.*, 1954, **9**, 59.
[7] Janson and Lindberg, *Acta Chem. Scand.*, 1959, **13**, 138 and earlier papers.
[8] Samuelson and Wennerblom, *Svensk Papperstidn.*, 1954, **57**, 827; Franzon and Samuelson, *ibid.*, 1957, **60**, 720, 872.
[9] Hamilton, Partlow, and Thompson, *Tappi*, 1958, **41**, 803, 811.

paper a study of the alkaline degradation of some xylans under conditions normally employed for their isolation is reported. The majority of experiments were carried out on rye-flour arabinoxylan,[10,11] one of the few polysaccharides of this group which may be isolated without the use of alkaline reagents.

Xyloisosaccharinolactone (A) is the main product from the alkaline degradation of xylobiose and xylotriose,[12,13] but non-lactonisable acids are also formed, presumably by fragmentation of the sugars or of the 1,2-dicarbonyl compounds, which are intermediates in saccharinic acid formation.[14] Further information has now been obtained on the degradative reactions of 4-*O*-substituted D-xylose derivatives by a study of the action of alkali on 4-*O*-methyl-D-xylose. Although it has not been possible to account for all the acid products, the major product (0·65 mol. per mol.) was xyloisosaccharinic acid, isolated as the crystalline lactone (A). Traces of two other lactonisable acids were detected by paper chromatography. Formic acid was also characterised but no other non-lactonisable acid could be detected.



(A) (B) (C)

A number of xylans were then treated with dilute aqueous sodium hydroxide at room temperature in the absence of oxygen. The solutions were kept until no further acidic degradation products were formed. The degraded polysaccharides were isolated and the carboxylic acids of low molecular weight were examined (see Tables 1 and 2). As with simple 4-*O*-substituted xylose derivatives, xyloisosaccharinolactone (A) was detected as an important product in each case. However, as in the alkaline degradation of cellulose [4] and amylose,[5] non-lactonisable acids, including formic, lactic, and probably glycollic acid, were also formed in appreciable amounts. Hydrolysis of the alkali-degraded rye-flour arabinoxylan gave, in addition to neutral sugars, a mixture of two lactonisable acids which were chromatographically indistinguishable from xylometasaccharinolactones [3-deoxy-*erythro*- and 3-deoxy-*threo*-pentonic acid lactones (B and C)]. Termination of the alkaline degradation of this polysaccharide, therefore, results from a re-arrangement of a reducing end group to give a metasaccharinic acid residue (as with cellulose [4] and amylose [5]).

TABLE 1. *Paper chromatography of acidic degradation products from xylans.*

	Saccharinic					
Xylan degraded	Iso	Meta	Glycollic	Lactic	Lactyl-lactic	Others
Rye flour B	++	Tr.	++	+++	++	++
Reduced rye flour B ...	—	—	—	—	—	—
Barley husks	++	Tr.	++	+++	+	++
Oat straw A	++	Tr.	++	+++	+	++
Oat straw B	++	Tr.	++	++	+	++

(Iso = xyloisosaccharinolactone; meta = xylometasaccharinolactone.)

Number-average molecular weights of the xylans and the derived alkali-stable polysaccharides were determined on their acetylated derivatives by isothermal distillation in

[10] Preece and Hobkirk, *J. Inst. Brewing*, 1953, **59**, 385.
[11] (*a*) Aspinall and Sturgeon, *J.*, 1957, 4469; (*b*) Aspinall, Cairncross, Wilkie, and Sturgeon, *J.*, 1960, 3881; (*c*) Aspinall and Cairncross, *J.*, 1960, 3998.
[12] Whistler and Corbett, *J. Amer. Chem. Soc.*, 1956, **78**, 1003.
[13] Aspinall, Carter, and Los, *J.*, 1956, 4807.
[14] Machell and Richards, *J.*, 1960, 1924, 1932, 1938.

1,3-dioxolan. Benzene was preferred as a solvent for methylated polysaccharides. The values obtained are shown in Table 3.

TABLE 2. *Analysis of acidic degradation products from xylans.*

Xylan degraded	Terminal L-arabinofuranose residues (approx. %)	Total acid	Formic acid	Lactic acid
Rye flour B	30	170	17·6	57·7
Reduced rye flour B	30	Nil	Nil	Nil
Barley husks	5 *	86	16·1	10·8
Oat straw A	6	129	10·8	19·5
Oat straw B	3	117	22·8	4·8

(Quantities of acids are expressed as mequiv. per pentose residue taken.)

* The xylan from barley husks also contains some 2-*O*-substituted L-arabinofuranose residues which would not be degraded further under the conditions employed.

TABLE 3. *Number-average molecular weights of acetylated and methylated polysaccharides.*

Polysaccharide	Derivative	Mol. wt.	Degree of polymern.
Rye arabinoxylan A	Acetate	13,200 ± 500	64 ± 3
Rye arabinoxylan A	Methylated (i) *	9,100 ± 500	57 ± 3
Rye arabinoxylan B	Acetate	15,700 ± 500	72 ± 3
Rye arabinoxylan B	Methylated (i) *	10,800 ± 500	67 ± 3
Rye arabinoxylan B	Methylated (ii) *	10,400 ± 500	65 ± 3
Alkali-degraded rye arabinoxylan B	Acetate	12,500 ± 500	58 ± 3
Reduced rye arabinoxylan B	Acetate	15,100 ± 500	70 ± 3
Alkali-degraded reduced rye arabinoxylan B	Acetate	15,100 ± 500	70 ± 3
Barley-husk xylan	Acetate	18,200 ± 500	84 ± 3
Alkali-degraded barley-husk xylan	Acetate	14,500 ± 500	67 ± 3

* Prepared by (i) direct methylation of the polysaccharide and (ii) simultaneous methylation and deacetylation of the acetylated polysaccharide.

The results show that the alkaline degradation of the arabinoxylans from rye flour [10,11] and from barley husks [15] caused a reduction of *ca.* 20% in molecular weight before the alkaline erosion was terminated. Reaction, however, is slow under these conditions and the period of reaction (20—25 days) considerably longer than those normally used in the extractions of xylans. It is probable, therefore, that relatively little degradation occurs when xylans are extracted from plant tissues with dilue alkali at room temperatures.

When rye-flour arabinoxylan was treated with potassium borohydride an alkali-stable polysaccharide was formed since treatment of the reduced polysaccharide with alkali resulted in neither production of acid nor decrease in molecular weight. It follows that under these conditions the observed degradations proceeded solely from the reducing groups of the polysaccharides, and that there was no evidence for random chain scission arising from alkaline hydrolysis of glycosidic linkages. It is possible that the cleavage of glycosidic linkages by alkali might not be readily detected by analysis of acids of low molecular weight, but the number-average molecular weights of the resulting polysaccharide provide a much more sensitive test for a reaction involving the breaking of only a few bonds per molecule. It may be noted that modification of reducing end groups in cellulose by reduction [16-18] and selective oxidation [18] has increased the alkali-stability of the polysaccharide.

It is of interest that the oat-straw xylans [19] which were extracted from the straw after removal of lignin with chlorous acid were still susceptible to the action of alkali. Whistler and Corbett [12] have reported that maize-cob xylan, isolated in a similar manner, is stable to alkali and have suggested that the reducing groups of the polysaccharide had been oxidised

[15] Aspinall and Ferrier, *J.*, 1957, 4188.
[16] Richtzenhain, Lindgren, Abrahamsson, and Holmberg, *Svensk Papperstidn.*, 1954, **57**, 363.
[17] Head, *J. Textile Inst.*, 1955, **46**, T584.
[18] Meller, *Tappi*, 1951, **34**, 171; 1952, **35**, 72; 1953, **36**, 366; *Holzforschung*, 1960, **14**, 78, 129.
[19] Aspinall and Wilkie, *J.*, 1956, 1072.

to aldonic acids with chlorous acid. Our results suggest that not all the reducing end groups are necessarily accessible to the reagent in this heterogeneous reaction.

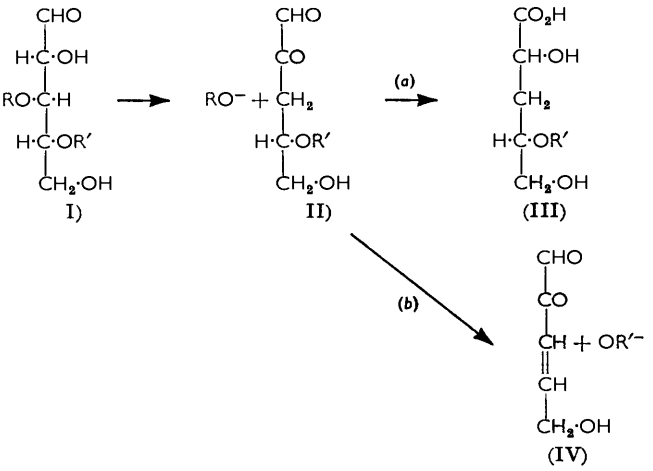
In order to assess the extent of alkaline degradation which may take place when polysaccharides are methylated under alkaline conditions, rye-flour arabinoxylan was converted into the methylated derivative with methyl sulphate and sodium hydroxide, both by direct reaction and by simultaneous deacetylation and methylation of the acetylated polysaccharide. Molecular-weight determinations (Table 3) showed that the methylation procedure caused only a small decrease in degree of polymerisation. It is probable that glycosidation of the reducing groups takes place rapidly to give an alkali-stable polysaccharide before the alkaline erosion has proceeded far: stabilisation of cellulose towards alkali by glycosidation has been observed by Reeves *et al.*,[20] Meller,[18] and Machell and Richards.[21]

The main features of the alkaline degradation of linear 1,4-linked polysaccharides by erosion from the reducing end have been established for cellulose[4] and amylose.[5] It is clear from this and earlier[12,13] studies that 1,4-linked xylans are also degraded in a stepwise manner with the formation in this case of xyloisosaccharinic acid as the major product. The xylans studied in this investigation, however, contained varying proportions of other sugar residues attached as side chains, mostly single L-arabinofuranose end groups linked to position 3 of D-xylose residues in the main chains. Whistler and BeMiller[2] have suggested that in a 1,4-linked polysaccharide the action of alkali on a reducing group which carried a branch at $C_{(3)}$ would result in the elimination of the side chain together with rearrangement of the reducing group to an alkali-stable metasaccharinic acid residue. Hitherto direct experimental evidence on this point has not been available. Two observations from these experiments suggest that the alkaline erosion of arabinoxylans is not necessarily arrested at such branching points. First, the decrease in degree of polymerisation of rye-flour arabinoxylan on treatment with alkali was considerably greater than would be expected if degradation were stopped at the first branching point, as this polysaccharide contains L-arabinofuranose side chains attached on the average to every second D-xylose residue in the main chain. Secondly, the rye arabinoxylan with a higher proportion of arabinose side chains gave relatively larger quantities of lactic acid (see Table 2). Lactic acid is known to be formed by the action of alkali on L-arabinose,[22] but has not been detected when 4-*O*-methyl-D-xylose, which may be regarded as a model compound for a 1,4-linked xylan, is treated with alkali. Since the molecular sizes of the xylans examined were of the same order of magnitude, and as the polysaccharides differed in the proportions rather than in the nature of the structural units present, it seems that larger amounts of lactic acid are formed from xylans containing higher proportions of L-arabinofuranose side chain because the alkaline erosion by-passes the branching points so that more than one arabinose residue per polysaccharide molecule could be eliminated.

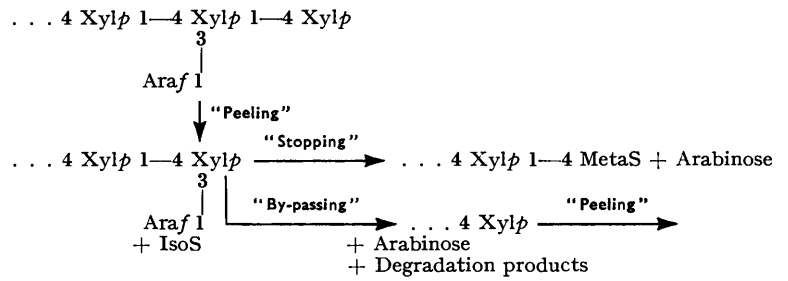
A possible pathway for the alkaline degradation of 3,4-di-*O*-substituted pentoses (I) involves the initial formation of an intermediate (II). If this intermediate undergoes a benzilic acid type of rearrangement the product (route *a*) would be a 4-*O*-substituted metasaccharinic acid (III). The intermediate (II), however, is also a β-alkoxycarbonyl compound, which, under alkaline conditions, might be expected to undergo β-elimination of alkoxide ion OR'^- (route *b*) with the formation of (IV) or some similar compound. Although such degradation products have not yet been characterised, evidence in favour of route (*b*) as one of the possible stages in the degradation of 3,4-di-*O*-substituted pentoses by alkali has now been obtained by the isolation of methanol and D-xylose after treatment of 3-*O*-methyl-4-*O*-(β-D-xylopyranosyl)-D-xylose (I; R = Me, R′ = β-D-xylopyranosyl) with alkali.[23] As far as we are aware the only previous evidence for the alkaline

[20] Reeves, Schwartz, and Giddens, *J. Amer. Chem. Soc.*, 1946, **68**, 1383.
[21] Machell and Richards, *Tappi*, 1958, **41**, 12.
[22] Nef, *Annalen*, 1910, **376**, 1.
[23] Aspinall and Ross, following paper.

degradation of a 3,4-di-*O*-substituted aldose involving elimination of both substituents has been reported by Kuhn and Gauhe.[24] 2-*O*-α-L-Fucopyranosyl-D-galactose and L-fucose were detected chromatographically as alkaline degradation products from " lacto-difucotetraose," 3-*O*-α-L-fucopyranosyl-4-*O*-(2-*O*-α-L-fucopyranosyl-β-D-galactopyranosyl)-D-glucose.



Three reactions may now be recognised as being involved in the alkaline degradation of arabinoxylans: (1) the " peeling " reaction which exposes a new reducing group in the main chain and is accompanied by the formation of xyloisosaccharinic acid; (2) the " by-passing " of the branching point which exposes new reducing groups in both the main chain and the side chain and is accompanied by the formation of unidentified intermediates; and (3) the " stopping " reaction which exposes a new reducing group in the side chain only and is accompanied by rearrangement of the reducing xylose residue to give an alkali-stable metasaccharinic acid end group. The intermediate (II), which may undergo benzilic acid rearrangement by route (*a*) or β-elimination by route (*b*), would also be formed by elimination of hydroxyl ion from 4-*O*-substituted pentoses (I; R = H,



(IsoS = isosaccharinic acid; MetaS = metasaccharinic acid.)

R′ ≠ H). It follows, therefore, that although the " stopping " reaction, which has been previously demonstrated in the alkaline degradation of linear 1,4-linked polysaccharides (cellulose [4] and amylose [5]), is presumably preceded by the formation of the C_6-analogue of (II), the formation of such an intermediate is not necessarily followed by rearrangement giving an alkali-stable metasaccharinic acid end group. The alternative reaction pathway involving β-elimination would lead to the exposure of a new reducing end group and thus permit the " peeling " reaction to proceed further down the polysaccharide chain.

[24] Kuhn and Gauhe, *Annalen*, 1958, **611**, 241.

Experimental

The following solvents and sprays were used for paper chromatography on Whatman No. 1 paper. Solvents: (A) propan-1-ol–ammonia (*d* 0·88) (3 : 2); (B) pentan-1-ol–ethyl acetate–formic acid–water (4 : 2 : 1 : 3); (C) ethyl acetate–pyridine–water (10 : 4 : 3); (D) butan-2-one saturated with water. Sprays: (*a*) [25] aqueous aniline oxalate for reducing sugars; (*b*) [26] hydroxylamine–ferric chloride for lactones; (*c*) [27] potassium iodate–potassium iodide–starch and (*d*) [28] Bromocresol Green for acids; (*e*) [29] aqueous mercuric chloride for formic acid.

4-*O*-Methyl-D-xylose was prepared by the method of Hough and Jones [30] and was purified by chromatography on cellulose, with butan-1-ol–light petroleum (b. p. 100—120°), saturated with water, as eluant. After recrystallisation from methanol–water the sugar had m. p. 103—105° and $[\alpha]_D$ +44° ⟶ +6° (equil.) (*c* 1·01 in H_2O) (Found: OMe, 18·5. Calc. for $C_6H_{12}O_5$: OMe, 18·9%). The sugar had $R_{rhamnose}$ 1·86 in solvent D and was readily distinguishable from the 2- and the 3-methyl ether of D-xylose which had $R_{rhamnose}$ 2·00 and 2·28 respectively. The derived phenylosazone had m. p. 160° (Hough and Jones [30] report m. p. 161° for 4-*O*-methyl-D-xylosazone).

The following polysaccharide samples were used: rye-flour arabinoxylan A, some of which was used in previous investigations,[11] was kindly provided by Professor I. A. Preece; [10] methylated rye arabinoxylan A, $[\alpha]_D$ −121° (*c* 0·5 in $CHCl_3$), was prepared previously; [11a] rye-flour arabinoxylan B was isolated as described by Preece and Hobkirk [10] and purified by precipitations from aqueous solution with ammonium sulphate and with acetone; oat-straw xylan A was unfractionated hemicellulose isolated by alkaline extraction of the chlorite holocellulose; [19] oat-straw xylan B, some of which was used in an earlier investigation,[19] was obtained from oat-straw xylan A after several precipitations of the copper complex; barley-husk xylan, some of which was used in an earlier investigation,[15] was obtained by direct alkaline extraction.

Alkaline Degradation of 4-O-*Methyl-*D*-xylose and Examination of Products.*—4-*O*-Methyl-D-xylose (200·7 mg.) in oxygen-free 0·88N-sodium hydroxide (5 ml.) was set aside for 14 days. Sodium ions were removed with Amberlite resin IR-120(H) and the solution was made up to 50 ml.

(a) *Qualitative.* A sample (5 ml.) was evaporated to a syrup under reduced pressure, the receiver being cooled with ice to trap volatile products. Methanol was identified in the first few drops of the distillate by oxidation to formaldehyde, which was detected by the chromotropic acid test.[31] The rest of the distillate was neutralised with 0·018N-sodium hydroxide and taken to dryness. Chromatography of the sodium salts in solvent A showed formic acid (sprays *d* and *e*) only. Formic acid was identified by conversion into the 4-bromophenacyl ester, m. p. and mixed m. p. 140°. Chromatography of the residual non-volatile syrup in solvent C showed a lactone, which was indistinguishable from xyloisosaccharinolactone, together with small amounts of two other lactones, $R_{isosaccharinolactone}$ 0·76 and 1·57, but no non-lactonisable acids.

The lactones obtained from the degradation of a further quantity (50 mg.) of sugar were separated on filter sheets by using solvent C, and the main component was identified as xyloisosaccharinolactone, m. p. and mixed m. p. 94—96°.

(b) *Quantitative.* Samples (2 ml.) were withdrawn and titrated with carbonate-free 0·018N-sodium hydroxide. Acid formed corresponded to 1·12 mole per mole of sugar. Samples (10 ml.) were evaporated to dryness under reduced pressure (bath temp. 60°), the distillate being collected in an ice-cooled receiver. Water (2 × 5 ml.) was added and the contents of the flask were again evaporated to dryness. The combined distillates were titrated with carbonate-free 0·005N-sodium hydroxide. Volatile acid corresponded to 0·13 mole per mole of sugar. A further quantity of distillate was boiled with freshly prepared yellow mercuric oxide for 3 hr. to ensure complete oxidation of formic acid to carbon dioxide.[32] Inorganic salts were removed from the cooled solution and titration with alkali indicated no volatile acids other than formic

[25] Partridge, *Nature*, 1949, **164**, 443.
[26] Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.
[27] Long, Quayle, and Stedman, *J.*, 1951, 2197.
[28] Cheftel, Munier, and Machebouef, *Bull. Soc. Chim. biol.*, 1953, **35**, 1095.
[29] Klein and Wenzl, *Mikrochemie*, 1932, **11**, 73.
[30] Hough and Jones, *J.*, 1952, 4349.
[31] Eegriwe. *Mikrochim. Acta*, 1937, **2**, 329.
[32] Evans and Hass, *J. Amer. Chem. Soc.*, 1926, **48**, 2708; see also ref. 4*a*.

acid. Samples (0·05 ml.) were evaporated to dryness to remove volatile acids, and the residual syrup was made up to 2 ml. with methanol–water. The solution was treated with hydroxylamine and ferric chloride as described by Kaye and Kent.[33] Optical densities were measured at 505 mμ and compared with those similarly measured from standard solutions of xyloisosaccharinolactone. Lactonisable acid formed corresponded to 0·75 mole per mole of sugar.

Lactonisable acids produced from 4-*O*-methyl-D-xylose (100 mg.) were separated on filter sheets by using solvent C and afforded xyloisosaccharinolactone (53·4 mg.), corresponding to 0·65 mole per mole of sugar.

Reduction of Rye Arabinoxylan B.—Rye-flour arabinoxylan B (500 mg.) was dissolved in water (30 ml.), potassium borohydride (100 mg.) was added, and the solution was kept overnight at room temperature. Excess of hydride was destroyed by the addition of acetic acid and the reduced polysaccharide was isolated by precipitation with ethanol.

Methylation of Rye Arabinoxylan B.—(i) Direct methylation of the polysaccharide with methyl sulphate and sodium hydroxide, followed by treatment with methyl iodide and silver oxide, afforded methylated polysaccharide, which was fractionated by dissolution in boiling chloroform–light petroleum (b. p. 60—80°) to give: (i) Methylated rye arabinoxylan (40 mg.) soluble in chloroform–light petroleum (b. p. 60—80°) (35 : 65), $[\alpha]_D$ −118° (in $CHCl_3$) [Found: OMe, 38·5%]. (ii) Methylated rye arabinoxylan B (40 mg.), soluble in chloroform–light petroleum (b. p. 60—80°), $[\alpha]_D$ −116° (in $CHCl_3$) (Found: OMe, 38·6%), was prepared in a similar manner by methylation of acetylated rye arabinoxylan B (300 mg.).

Acetylation of Xylans.—Xylan samples (*ca.* 160 mg.) were dispersed in formamide (3 ml.) at 50°, freshly distilled pyridine (3 ml.) was added to the cooled solution during 30 min., and freshly distilled acetic anhydride (3 ml.) was added during 1 hr. The mixture was shaken overnight and poured into ice-water (100 ml.). The precipitate was washed with water (3 × 50 ml.) and dissolved in chloroform, the solution was dried and concentrated, and the acetylated xylan was precipitated by the addition of light petroleum (b. p. 60—80°) and dried at 70°/0·5 mm. In each case analysis [34] indicated complete acetylation.

Alkaline Degradation of Xylans and Examination of Acidic Products.—Xylan (1 g.) was stirred overnight with water (25 ml.), and oxygen-free 1·76N-sodium hydroxide (25 ml.) was added. Nitrogen was bubbled through the solution, and the flask was stoppered and kept at room temperature for 25 days (complete reaction). The solution was passed through a column of Amberlite resin IR-120(H) to remove sodium ions, and the eluate and washings were made up to 250 ml. Aliquot parts of this solution were used for both qualitative and quantitative examinations. Degraded polysaccharide was precipitated from the remaining solution by the addition of ethanol (2 vol.).

(a) *Qualitative.* Samples (25 ml.) were evaporated to dryness under reduced pressure (bath-temp. 60°) and the distillate was collected in an ice-cooled receiver. Water (2 × 5 ml.) was added to the residue, the contents were again evaporated, and the distillate was collected. The combined distillates were neutralised with 0·02N-sodium hydroxide and the solution was taken to dryness. Formic acid (as sodium salt was identified by chromatography in solvent A and characterised by conversion into the 4-bromophenacyl ester, m. p. and mixed m. p. 140°.

Ethanol (50 ml.) was added to samples (25 ml.) of the degradation products, and the precipitated alkali-stable polysaccharides were removed. The filtrates were concentrated and the resulting syrups were examined chromatographically in solvent B, with sprays *c* and *d* for acids and *b* for lactones. The acids and lactones, which were identified, are shown together with their relative proportions in Table 1.

The mixture of acids and lactones from the degradation of rye arabinoxylan B (1 g.) was placed on a column of Amberlite resin IRA-400 (formate form). Elution with water removed lactones (not absorbed) and elution with 15% formic acid removed non-lactonisable acids. The main component of the mixture of non-lactonisable acids was characterised as lactic acid by conversion into the 4-bromophenacyl ester, m. p. and mixed m. p. 113°.

(b) *Quantitative.* Total acids were determined by direct titration of aliquot parts of the reaction mixture after removal of sodium ions. Volatile acids from degradations were obtained on distillation, and titrations before and after boiling with an excess of freshly prepared yellow mercuric oxide for 3 hr. showed that formic acid was the only volatile acid. Samples (25 ml.)

[33] Kaye and Kent, *J.*, 1953, 81.
[34] Belcher and Codbert, "Quantitative Organic Microanalysis," Longmans, Green & Co., London, 1945, p. 123.

of degradation mixtures were treated as before to remove alkali-stable polysaccharide, 20% w/v copper sulphate solution (1 ml.) was added to each non-volatile residue, and the volume was made up to 25 ml. with water. Lactic acid in aliquot portions (0·2—0·5 ml.) was determined colorimetrically by means of 4-hydroxybiphenyl according to Barnett's procedure,[35] optical densities being compared with those from standard lactic acid solutions. The results are recorded in Table 2.

Examination of Alkali-stable Degraded Rye Arabinoxylan B.—0·05M-Sodium acetate buffer (2 ml.; pH 5), " Hemicellulase " enzyme preparation (L. Light and Co.) (20 mg.), and toluene (2 ml.) were added to alkali-stable degraded rye arabinoxylan B (200 mg.) in water (10 ml.), and the mixture was incubated at 35° for 48 hr. to effect complete hydrolysis. The enzyme was inactivated by heating the solution at 100° for 2 min., sodium ions were removed by passage through Amberlite resin IR-120(H), and the solution was concentrated to a syrup. Chromatography in solvent C showed xylose and arabinose (spray *a*). Non-volatile acids were neutralised with sodium hydroxide, and the mixture of reducing sugars and salts was poured on a column of Amberlite resin IRA-400 (formate form). Elution with water removed reducing sugars, and elution with 10% formic acid desorbed acids. The acid eluate was concentrated and chromatography in solvent B showed two lactones (spray *b*) with the same mobilities as the metasaccharinolactones formed from D-xylose.[22]

Determination of Molecular Weights.—The number-average molecular weights of the xylan samples were determined by isothermal distillation. The method used has been found to be particularly satisfactory for hemicellulose samples [36] and will be reported in detail elsewhere. The technique adopted was essentially that described by Gee [37] in which the rate of distillation of solvent into the solution is measured. The dynamic method requires calibration. For this purpose, triolein (*M*, 885) and tristearin (*M*, 891) were used. Benzene was used as the solvent for methylated polysaccharides and 1,3-dioxalan for acetylated polysaccharides. The calibration constants for different mole-fractions of the calibrating solutes were in agreement within ±5%, and this represents the limiting accuracy of the molecular-weight results shown in Table 3.

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[35] Barnett, *Biochem. J.*, 1951, **49**, 527.
[36] Broatch and Greenwood, unpublished results; Broatch, Ph.D. Thesis, Edinburgh, 1956.
[37] Gee, *Trans. Faraday Soc.*, 1940, **36**, 1163.