[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Behavior of Xylan in Alkaline Solution: The Isolation of a New C5 Saccharinic Acid[1,2]

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A preparation of xylan from corn cob is stable to hot sodium hydroxide solution. Xylan oligosaccharides are readily degraded by lime water at 25° to a new C5 saccharinic acid, 2-hydroxymethyl-2,4-dihydroxybutyric acid. The isolation, structure and some derivatives of this acid are described.

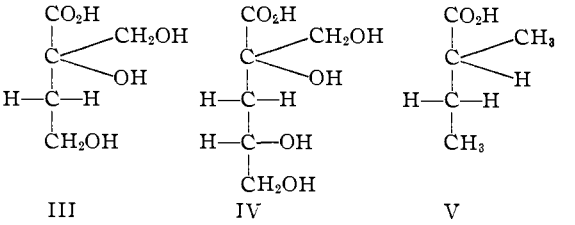
Several groups of workers are reinvestigating the action of alkali upon carbohydrates. Much of the work in progress is directed toward determination of the effect of alkali upon the monosaccharides and simple oligosaccharides in order to explain the mechanism by which these carbohydrates can be transformed to acidic products. An investigation of the action of alkali upon polysaccharides would be useful to provide a further understanding of changes which may occur when polysaccharides are isolated by means of alkaline solution. Although the occurrence of degradation would not invalidate the structures assigned to the polysaccharides, it may have significant effect on chain length determinations and biological activity. Thus, a polysaccharide I containing a reducing end group might undergo complete degradation of the main chain. All (1 → 6) branches joined to this chain would be left with a saccharinic acid end unit derived from the sugar unit of the main chain which formed the union with the branch II. The presence of a saccharinic acid end unit in the residues could have considerable effect on enzyme reactions which require the presence of a free reducing unit.

glycosyl-[anhydroglycose]$_z$-anhydroglycose II
|
glycosyl-[anhydroglycose]$_y$-anhydroglycose-[anhydroglycose]$_x$-glycose
I
glycosyl-[anhydroglycose]$_x$-anhydroglycose
|
II saccharinic acid
+ saccharinic acids, lactic acid, etc.

One polysaccharide which is isolated from numerous sources by extraction with sodium hydroxide is xylan. A sample of xylan, isolated from corn cob holocellulose which has been delignified by the sodium chloride procedure,[3] is quite stable to normal sodium hydroxide solution at 100° under oxygen-free conditions. The stability is shown by the non-formation of acidic products. In order to demonstrate that the stability of the xylan is not due to the type of linkage involved in the polysaccharide but to some special feature of the molecule itself, an examination was made of the effect of alkali upon xylotriose[4] at 25°. Calcium hydroxide was chosen as the alkali because degradation occurs more readily in lime water than in sodium hydroxide of similar normality[5] and because lime water directs the degradation of 1 → 3 and 1 → 4 linked glycans toward saccharinic acids which contain the same number of carbon atoms as the sugar units involved in the parent glycan. As anticipated, a solution of xylotriose in oxygen-free lime water is readily degraded to acidic products, the rate of formation of which is followed by the consumption of alkali. If the degradation occurs by a mechanism similar to that for cellotetraose[6] it would proceed through the following stages.

xylotriose ⟶ xylotriulose ⟶ xylobiose ⟶
xylobiulose ⟶ xylose + saccharinic acid

Since the xylose units in xylotriose are joined by 1 → 4 linkages, the degradation would be expected to give a new C5 saccharinic acid (III) corresponding to the known isosaccharinic acid (IV) of the hexose series.



The new acid is isolated from the calcium hydroxide degradation mixture of xylobiose, xylotriose and xylotetraose. The physical constants of the acid and its derivatives are very similar to those reported for the derivatives of β-D-hexoisosaccharinic acid given in Table I.

TABLE I

PROPERTIES OF PENTO- AND HEXOISOSACCHARINIC ACIDS AND THEIR DERIVATIVES[a]

	D-Hexoisosaccharinic acid α-Form	D-Hexoisosaccharinic acid β-Form	D-Pentoisosaccharinic acid (2-Hydroxymethyl-2,4-dihydroxybutyric acid)
Calcium salt	Insoluble[b]	$[\alpha]^{28}D$ +3.1°[c]	$[\alpha]^{25}D$ +1.0°
Soln. of lactone prepared by removal of Ca from calcium salt		$[\alpha]^{22}D$ +8.5°[c]	$[\alpha]^{25}D$ +2.5°
Brucine salt	M.p. 164°[b]	M.p. 200–210°[b]	M.p. 192–195°
	$[\alpha]^{20}D$ −26.1°[b]	$[\alpha]^{20}D$ −21°[b]	$[\alpha]^{25}D$ −27.6°
Lactone	M.p. 96°[b]		M.p. 96°
	$[\alpha]^{20}D$ +61.9°[b]	$[\alpha]^{22}D$ *ca.* +6°[b]	$[\alpha]^{25}D$ +1.1°

[a] All optical rotations are given for aqueous solutions. [b] J. U. Nef, *Ann.*, **376**, 52 (1910). [c] W. M. Corbett and J. Kenner, *J. Chem. Soc.*, 1789 (1954).

The calcium salt of the acid is reduced by phosphorus and hydriodic acid to calcium α-methylbutyric acid (V). The isolation of this acid proves the branched structure of the saccharinic acid.

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(2) Paper presented before the joint Divisions of Carbohydrate and Cellulose Chemistry at the 128th Meeting of the American Chemical Society at Minneapolis, Minn., September, 1955.

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Periodate oxidation of D-pentoisosaccharinolactone produces 0.83 mole of formaldehyde in contrast to D-hexoisosaccharinolactone and D-hexosaccharinolactone which produce 1.34 and 0.91 mole, respectively.

Although xylotriose is readily degraded by lime water at 25°, a sample of xylan is quite stable to normal sodium hydroxide at 100°. The stability of the latter carbohydrate must be ascribed to the absence of aldehyde groups which are a prerequisite for rapid alkaline degradation. The possibility of xylan being a non-reducing polysaccharide is difficult to imagine since it would require the presence of an odd non-reducing terminal glycosidic linkage or a terminal unit other than a normal sugar unit. It is most likely that the aldehyde group of the natural xylan molecule undergoes transformation during the isolation of the polysaccharide. Transformation is possible during delignification by the sodium chlorite–acetic acid procedure used for the isolation of the corn cob holocellulose. This procedure is capable of oxidizing aldehydes[7] to carboxylic acids which would make the xylan stable to alkali. Destruction of alkali lability by oxidation aldehyde groups with chlorous acid has already been shown to occur with periodate oxidized cellulose.[8]

Experimental

Paper Chromatography.—Separations were made on Whatman No. 1 filter paper at room temperature using butanol-1–pyridine–water in the volume ratio of 6:4:3. The components were detected by spraying with *p*-anisidine hydrochloride[9] or silver nitrate and sodium hydroxide.[10]

Treatment of Xylan with Sodium Hydroxide.—A 100-ml. solution of 1.0055 g. of xylan, prepared from corn cob,[3] in 0.8 *N* sodium hydroxide was freed from oxygen by streaming with nitrogen. Samples of 5 ml. were heated at 100° in an atmosphere of nitrogen. The samples were acidified by the addition of 5 ml. of *N* sulfuric acid and, after storing at room temperature for 1/4 hr., the excess acid was titrated with 0.10 *N* sodium hydroxide to the first semi-permanent end-point of phenolphthalein. The results are as follows:

Time of heating, hr.	0	0.5	1	2.25	7
Titer (ml. of NaOH)	1.11	1.10	1.11	1.19	1.15

Degradation of Xylotriose by Lime Water. (a) Quantitative Results.—A 500-ml. solution of 1.808 g. of xylotriose[4] in 0.042 *N* lime water was freed of oxygen and maintained at 25°. Periodically, 2-ml. samples were withdrawn and run into 10 ml. of 0.01 *N* sulfuric acid. After 1/4 hr. the excess acid was back titrated with 0.01 *N* sodium hydroxide to the first semi-permanent end-point of phenolphthalein. The results are given in Table II, together with the results of paper chromatographic analysis. The presence of xylotriulose and xylobiulose were suggested by their positions with respect to the corresponding aldo-sugars.

TABLE II

THE DEGRADATION OF XYLOTRIOSE BY LIME WATER AT 25°

Time (hr.)	Moles acid produced/ mole of xylotriose	Initial appearance of substances on chromatograms	Time (hr.)	Moles acid produced/ mole of xylotriose
0	0.000	Xylotriose	32	1.548
0.5	.069	Xylotriulose	48	1.855
1	.114	Xylobiose	73	2.230
2	.160		96	2.445
3.5	.195	Xylobiulose	121	2.615
8	.464	Xylose	169	2.740
24	1.215		224	2.845

(b) **Isolation of Calcium Salt of 2-Hydroxymethyl-2,4-dihydroxybutyric Acid.**—A mixture of 8.564 g. of xylobiose, xylotriose and xylotetraose[4] was dissolved in 2 l. of saturated lime water, freed of oxygen and maintained at 25° for 6 days. The excess calcium hydroxide was precipitated as calcium carbonate by the addition of solid carbon dioxide followed by boiling and the filtered solution was concentrated to 100 ml. On standing there was deposited 0.448 g. of calcium lactate identified by its *p*-bromophenacyl ester. The filtrate was diluted and the calcium precipitated by the requisite amount of oxalic acid. After filtration, the solution was concentrated to 6.800 g. of sirup. The dried sirup was extracted with acetone, and concentration of the extract gave 5.325 g. of brown sirup. This was neutralized with calcium hydroxide whereby 7.312 g. of calcium salts was obtained.

A solution of 5.960 g. of the calcium salts in 10 ml. of water was kept at 0° whereby 0.126 g. of calcium salts was deposited. To the filtrate was added alcohol to give the following fractions: to 66% alcohol no precipitation; 73% alcohol gave 2.317 g. of amorphous calcium salt, $[\alpha]^{25}D$ +4.0° (*c* 1.0, water); 81.5% alcohol gave 1.326 g. of amorphous calcium 2-hydroxymethyl-2,4-dihydroxybutyrate, $[\alpha]^{25}D$ +1.0° (*c* 1.0, water) which on treatment with Amberlite IR-120(H) resin had $[\alpha]^{25}D$ +2.5° (*c* 0.8 calcd. for lactone, water); 85% alcohol gave 0.328 g. of amorphous powder, $[\alpha]^{25}D$ +1.9° (*c* 1.1, water). Concentration of the mother liquors and digestion of the residue with alcohol gave 1.541 g. of amorphous powder, $[\alpha]^{25}D$ +1.9° (*c* 1.1, water) Samples of the fractions were treated with Amberlite IR-120(H) resin and chromatographed. Each fraction gave only one lactone with R_f 0.79, together with traces of xylose.

Brucine Salt of 2-Hydroxymethyl-2,4-dihydroxybutyric Acid.—Two tenths gram of calcium 2-hydroxymethyl-2,4-dihydroxybutyrate was dissolved in 50 ml. of water and the solution was stirred for 1/2 hr. with excess Amberlite IR-120(H) resin. The filtered solution was heated at 100° for 1 hr. with excess brucine, and when cool the solution was filtered. Concentration of the filtrate gave an amorphous powder which was extracted with 10 ml. of cold water. Evaporation of the extract gave 0.578 g. of amorphous powder. It crystallized from a mixture of acetone and alcohol and after two recrystallizations from ethanol it had m.p. 192–195°, $[\alpha]^{25}D$ −27.6 (*c* 1.7, water).

Anal. Calcd. for $C_{23}H_{36}O_9N_2$: N, 5.15. Found: N, 5.17.

Lactone of 2-Hydroxymethyl-2,4-dihydroxybutyric Acid.—Two tenths gram of the above recrystallized brucine salt was dissolved in 20 ml. of water and stirred for 1 hr. with excess Amberlite IR-120(H) resin. The filtered solution was concentrated at 35° under reduced pressure and the resultant sirup partially crystallized on long standing. The sirup was drained on a sintered glass funnel and the crystals thus obtained were plated. On recrystallization from ethyl acetate the lactone crystallized in prisms having m.p. 95–96°, $[\alpha]^{25}D$ +1.0° (*c* 5.4, water).

Anal. Calcd. for $C_5H_8O_4$: C, 45.45; H, 6.11. Found: C, 45.63; H, 6.19.

Samples of 3.64 mg. of the lactone in 2 ml. of water were oxidized by sodium metaperiodate and the formaldehyde estimated by the method of Reeves.[11] After 2 hr., 0.83 and after 6 hr., 0.87 mole of formaldehyde per mole of lactone were produced. Under similar conditions D-hexoisosaccharinolactone gave 1.34, 1.39 and 1.34 moles of formaldehyde after 1, 2 and 3 hr., respectively, whereas D-hexosaccharinolactone gave 0.92, 0.90 and 0.91 mole for the same periods of time.

Reduction of Calcium 2-Hydroxymethyl-2,4-dihydroxybutyrate.—A mixture of 2.310 g. of the calcium salt, 0.9 g. of red phosphorus and 30 ml. of hydriodic acid (d. 1.97) was refluxed for 20 hr. The reaction mixture was diluted with an equal volume of water and exhaustively extracted with ether. Evaporation of the ether gave a dark oil which was decolorized by stirring with 5 ml. of *N* sulfuric acid and

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(11) R. E. Reeves, THIS JOURNAL, **63**, 1476 (1941).

1 g. of zinc dust. The solution was then steam distilled and the distillate neutralized with calcium carbonate and concentrated to give 0.775 g. of a white calcium salt, $[\alpha]^{25}D$ +0.7° (*c* 1.4, water). A solution of 0.421 g. of the calcium salt in 30 ml. of water was stirred with Amberlite IR-120(H) resin to give a solution of the free acid, $[\alpha]^{25}D$ +1.8° (*c* 1.1, water), which was neutralized with sodium hydroxide to give 0.404 g. of the sodium salt.

When 0.195 g. of the sodium salt was dissolved in 1 ml. of water and refluxed for 1 hr. with 0.5 g. of *p*-bromophenacyl bromide in 5 ml. of alcohol, crystals of the *p*-bromophenacyl ester separated. After one recrystallization from ethanol, the ester had m.p. 52–53°, undepressed on admixture with the *p*-bromophenacyl ester of α-methylbutyric acid.

Anal. Calcd. for $C_{13}H_{15}O_3Br$: C, 52.17; H, 5.06. Found: C, 52.01; H, 5.03.

The *p*-phenylphenacyl ester was similarly prepared. Recrystallized once from ethanol, the crystals had m.p. 68–68.5°, undepressed on admixture with the *p*-phenylphenacyl ester of α-methylbutyric acid.

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[CONTRIBUTION FROM RESEARCH AND DEVELOPMENT DEPARTMENT, U. S. NAVAL POWDER FACTORY]

Spectrophotometric Studies on the Action of Sulfuric Acid on Reducing Sugars and the Isolation and Identification of the Ether-soluble Substances Produced from Pentoses under Acid Conditions[1]

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Ultraviolet spectrophotometric studies on reducing sugars in solutions of sulfuric acid–water over a wide range of temperature and acid concentration indicate no differences in the reaction products formed from individual sugars in the same series. Observed differences in the ultraviolet absorption spectra of a series of hexoses or pentoses at the end of a specified interval of time are attributable to the difference in the rate of development of both the absorption maxima and the final apparent equilibrium reached by the individual sugars. In the pentose series under all conditions investigated, the relative rates of development of the ultraviolet absorption and apparent final maxima were: D-lyxose > D-ribose > D-xylose > D-arabinose. The rates at which compounds having absorption in the ultraviolet region were formed from a sugar increased with both increased acid concentration and increased temperature. The compounds responsible for the characteristic ultraviolet absorption curve of a reducing sugar in sulfuric acid solution are ether soluble. However, weak absorption observed between 220 and 260 mμ after ether extraction suggests that small amounts of unsaturated compounds remain in the aqueous phase. Examination of the ether extract has shown that not only is furfuraldehyde formed in the reaction of a pentose with aqueous H_2SO_4, but acetaldehyde, formaldehyde and crotonaldehyde are produced as well. The aldehydes were separated and identified as their 2,4-dinitrophenylhydrazones. Separation was accomplished by means of chromatography on silicic acid.

When reducing sugars are dissolved in acid solution at room temperatures or lower (6–10°), the observed optical rotations are found to increase[2] as the concentration of acid is increased,[3] and although the monosaccharide can be recovered in almost quantitative yield,[2] a certain proportion condenses to form disaccharides.[4] It is possible that an equilibrium reaction is involved.[3] The increase in observed optical rotation is probably due to an increase in the concentration of the *aldehydo* form of the sugar.[5]

Both *anhydro* sugars[6] and disaccharides[7] are formed when a reducing sugar is treated with hot, dilute (0.2–0.5 *N*) mineral acids.

Heat and moderately concentrated mineral acids (2–5 *N*) lead to extensive degradation of the sugar molecule. Hexoses yield 5-(hydroxymethyl)-2-furaldehyde,[8] while the pentoses produce furfuraldehyde.[9] Quantitative yields, however, are obtained only under specific conditions[10] of temperature and acid concentration.

It is possible that mineral acid is not essential for the transformation hexose → 5-(hydroxymethyl)-2-furaldehyde[11] or pentose → furfuraldehyde. Haworth and Jones[12] found that while D-fructose formed considerable quantities of 5-(hydroxymethyl)-2-furaldehyde when treated with a hot oxalic acid solution, D-glucose was recovered unchanged. That limited quantities of 5-(hydroxymethyl)-2-furaldehyde are formed at *p*H 6.5 has been shown definitely by the ultraviolet studies of Wolfrom, Schuetz and Cavalieri.[13] The effect of *p*H in relation to the browning reaction has been studied by Kroner and Kothe[14] who found the rate of degradation to have only slight dependence on *p*H above 3.

Spectrophotometric studies of the behavior of carbohydrates in 79% (weight/volume) sulfuric acid[15] show that after 2 hours at 25° or 15 minutes at 100°, each of the series of hexoses studied had a characteristic ultraviolet absorption curve, which was similar, but not superimposable, upon the curve of 5-(hydroxymethyl)-2-furaldehyde. Bandow[16] also found that a pentose after one day in

(1) Published with permission of the Bureau of Ordnance, Navy Department. The opinions and conclusions are those of the authors.

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