4. *The Action of Lime-water on* 3-O-*Methyl*-D-*xylose.*

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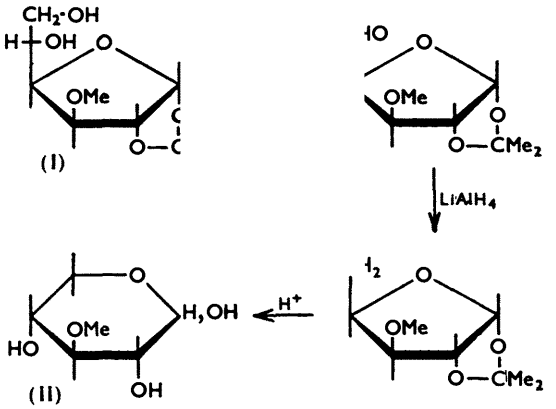
3-*O*-Methyl-D-xylose has been synthesised by a new method. Its degradation by oxygen-free lime-water has been studied and the products (3-deoxy-D-xylonic and 3-deoxy-D-lyxonic acid) have been isolated.

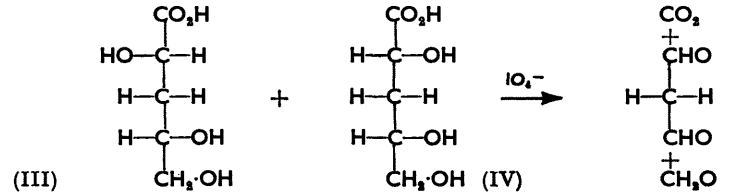
DURING work on the structure of the neutral oligosaccharides isolated from the partial hydrolysis of maize fibre hemicellulose,[1] a study of the action of lime-water on the oligosaccharides gave valuable preliminary information on their structure. Thus, 2-*O*-D-xylopyranosyl-L-arabinose could be easily distinguished from 3-*O*-D-xylopyranosyl-L-arabinose because the former [2] in lime-water solution at 25° gave no acidic products or monosaccharide whereas the latter [1] gave acidic products and xylose. In view of the importance of the saccharinic acids as reference compounds in the elucidation of the structures of oligosaccharides,[3] we have now examined the effect of lime-water on a 3-*O*-substituted pentose. Because of the limited quantity of 3-*O*-D-xylopyranosyl-L-arabinose available, and in order to avoid subsequent alkaline degradation of primary products, it was decided to use 3-*O*-methyl-D-xylose (II). This was conveniently synthesised from the known 3-*O*-methyl-1 : 2-*O*-*iso*propylidene-D-glucose (I) by the method indicated in the scheme, and was shown to be identical with a sample prepared from 1 : 2-*O*-*iso*propylidene-D-xylose by Levene and Raymond's [4]



In accordance with earlier generalisations,[3] 3-*O*-methyl-D-xylose was readily degraded by oxygen-free lime-water at 25° to acidic products. The rate of degradation was rather greater than that observed for 3-*O*-methyl-D-glucose,[5] but no explanation can be offered at present for this difference. The saccharinic acids were isolated as their calcium salts and appeared to contain mainly one component. The properties of derivatives of this component correspond approximately to those of Nef's "(—)-*threo*-1 : 3 : 4-trihydroxyvaleric acid" [6] which, in more modern nomenclature,[7] is D-*threo*-$\alpha\gamma\delta$-trihydroxyvaleric acid or 3-deoxy-D-lyxonic acid (III). Therefore the (—)-acid may have the D-lyxose configuration (III). The minor component isolated from the fractional precipitation would then be 3-deoxy-D-xylonic acid (IV) [Nef's "(+)-*erythro*trihydroxyvaleric acid"). The structure

[1] Whistler and Corbett, *J. Amer. Chem. Soc.*, 1955, **77**, 6328.
[2] Whistler and Corbett, *ibid.*, p. 3822.
[3] See Kenner, *Chem. and Ind.*, 1955, 727.
[4] Levene and Raymond, *J. Biol. Chem.*, 1933, **102**, 331.
[5] Kenner and Richards, *J.*, 1954, 278.
[6] Nef, *Annalen*, 1910, **376**, 46.
[7] Sowden, personal communication.

of the main product was verified by oxidation with periodate which gave malondialdehyde together with an almost theoretical yield of formaldehyde.



Experimental

3-O-*Methyl*-D-*xylose.*—An aqueous solution (300 ml.) of 3-*O*-methyl-1 : 2-*O*-*iso*propylidene-D-glucose [8] (28·42 g.) was stirred under cooling with sodium metaperiodate (30·0 g.) until dissolution was complete (15 min.). The solution was kept at room temperature for 1 hr., then evaporated to dryness, and the dry residue was extracted with boiling anhydrous ether. Evaporation of the extracts yielded a colourless syrupy aldehyde (23·7 g., 97%) which gave an immediate positive reaction with Schiff's reagent. A solution of this aldehyde (18·4 g.) in anhydrous ether (250 ml.) was stirred at room temperature while a solution of lithium aluminium hydride (*ca.* 4 g.) in ether (250 ml.) was added during 30 min. The resulting mixture was heated under reflux for 30 min. and then kept at room temperature overnight. To the mixture was added, dropwise and with stirring, ethyl acetate (10 ml.), followed by water (200 ml.). Organic solvents were removed by distillation and the remaining aqueous suspension was neutralised (pH 8) with sulphuric acid. After filtration the aqueous solution was extracted with chloroform (6 × 100 ml.) and the extracts were dried (Na_2SO_4) and evaporated to a colourless syrup (13·25 g., 72%). A solution of this product (11·65 g.) in 0·05N-sulphuric acid (250 ml.) was heated on a boiling-water bath for 1 hr. ($[\alpha]_D^{18}$ $-38° \longrightarrow +10°$ const.). The solution was neutralised with Amberlite IR-4B resin and evaporated to dryness, to give a residue (7·96 g., 85%) which crystallised readily. It recrystallised from ethanol–ethyl acetate as colourless prisms, m. p. 99·5—101°, $[\alpha]_D^{18}$ +51° (5 min.) $\longrightarrow$ +15° (18 hr. const.) (*c* 2·0 in H_2O). The m. p. was not depressed on admixture with an authentic sample of 3-*O*-methyl-D-xylose prepared by the method of Levene and Raymond.[4]

Degradation of 3-O-*Methyl*-D-*xylose by Lime-water.*—(a) *Measurement of the acids produced.* A solution of 3-*O*-methyl-D-xylose (0·2004 g.) in 0·0387N-lime-water (50 ml.) was freed from oxygen by passage of nitrogen and then kept at 25°. Samples (2 ml.) were withdrawn periodically and run into 0·01N-sulphuric acid (10 ml.). The solution, after standing for 15 min., was back-titrated with 0·01N-sodium hydroxide to the first semi-permanent end-point of phenolphthalein. The results are given in the annexed Table.

Degradation of 3-O-*methyl*-D-*xylose by lime-water at* 25°.

Time (hr.)	0	0·5	1	2	2·75	4·25	5	6	7	7·75	12	24
Equivs. of acid produced	0·000	0·089	0·205	0·394	0·486	0·661	0·729	0·774	0·846	0·875	1·020	1·090

(b) *Isolation of the acids produced.* A solution of 3-*O*-methyl-D-xylose (1·406 g.) in saturated oxygen-free lime-water was kept at room temperature for 2 days. The solution was then saturated with carbon dioxide, boiled for 10 min., and filtered. The filtrate was concentrated to dryness to give amorphous calcium salts (1·693 g.) which were dissolved in water (4·8 ml.). Ethyl alcohol was added portionwise to give the following fractions: calcium carbonate (0·033 g.) (from 53·4% ethanol); calcium carbonate combined with calcium salts of an organic acid (0·078 g.) (from 67·5% ethanol); amorphous salts (0·706 g.), $[\alpha]_D^{25}$ −7·9° (*c* 2·1 in H_2O) (from 80·8% ethanol); amorphous salts (0·186 g.), $[\alpha]_D^{25}$ −4·0° (*c* 1·0 in H_2O) (from 86·2% ethanol); 0·008 g. (from 92·5% ethanol). Concentration of the mother-liquors and digestion of the residue with ethanol gave more calcium salts (0·027 g.). An aqueous solution of the main fraction (0·210 g.) was stirred with Amberlite IR-120 (H) resin for 10 min., and then heated at 100° for 1 hr. with excess of brucine. The solution was cooled, filtered, and concentrated under reduced pressure and the residue extracted with 5 ml. of water. Concentration of the extract

[8] Freudenberg, Dürr, and Hochstetter, *Ber.*, 1928, **61**, 1739.

gave a brucine salt (0·572 g.) which, after two recrystallisations from ethanol, had m. p. 133—135°, $[\alpha]_D^{25}$ −32·0° (*c* 2·28 in H_2O) (Found : N, 5·1. Calc. for $C_{28}H_{36}O_9N_2$: N, 5·15%).

When the calcium ions were removed with resin from an aqueous solution of the calcium salt and the solution concentrated under reduced pressure, the lactone was obtained as syrup, $[\alpha]_D^{25}$ +41·6° (*c* 2·72 in H_2O). The lactone was heated with phenylhydrazine. After two recrystallisations from alcohol, the phenylhydrazide had m. p. 137—138°, $[\alpha]_D^{25}$ −35·6° (*c* 0·31 in EtOH) (Found : C, 55·1; H, 6·9. Calc. for $C_{11}H_{16}O_4N_2$: C, 55·0; H, 6·7%).

Nef [6] gives "(−)-*threo*-1 : 3 : 4-trihydroxyvaleric acid" as having a brucine salt, m. p. 145—150°, $[\alpha]_D^{20}$ −34·1°, and a phenylhydrazide, m. p. 110—112°, $[\alpha]_D^{20}$ −25·43°.

A further 4·3 g. of the calcium salts when fractionally precipitated as before yielded, from 61% ethanol, the salt (3·5 g.) corresponding to the main fraction above. The fraction (0·851 g.) obtained from 72% ethanol had $[\alpha]_D^{21}$ −12·3° (*c* 1·7 in H_2O) and gave a mixture of brucine salts which was separated by fractional crystallisation. The first component had m. p. 195—198° (decomp.), and $[\alpha]_D^{21}$ −23·4° (*c* 1·15 in H_2O). This may be the brucine salt of 3-deoxy-D-xylonic acid which would correspond to Nef's [6] "(+)-*erythro*-2 : 4 : 5-trihydroxyvaleric acid," quoted as having m. p. 200—202°, $[\alpha]_D^{20}$ −22·7° (Found : C, 59·9; H, 6·7; N, 4·7. Calc. for $C_{28}H_{36}O_9N_2,H_2O$: C, 59·8; H, 6·8; N, 5·0%). The second component isolated had m. p. 131—138°, undepressed in admixture with the above brucine salt of similar m. p., and $[\alpha]_D^{21}$ −32·6° (*c* 1·14 in H_2O).

Periodate Oxidation of the Calcium Salts.—An aqueous solution of the main fraction of the above calcium salts (0·25 g.) was passed through Amberlite IR-120 (H) resin, and to the resulting solution, together with washings, was added metaperiodic acid (0·55 g.). The solution was distilled to dryness below 50°, water (25 ml.) added, and the solution again distilled to dryness. To the combined distillates was added a saturated solution (200 ml.) of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid and the mixture kept at room temperature for 20 hr. The resulting orange precipitate was separated by filtration, washed with water, and then extracted with boiling ethanol. Evaporation of the extracts yielded a pale orange residue (0·29 g., 94%), m. p. 160—163°, undepressed on admixture with the formaldehyde derivative. The alcohol-insoluble residue recrystallised from nitrobenzene as an orange powder, m. p. 309° (decomp.), not depressed on admixture with malondialdehyde bis-2 : 4-dinitrophenylhydrazone prepared from 6-*O*-methylmetasaccharinic acid.[9]

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[9] Kenner and Richards, *J.*, 1956, 2916.