CCXXX.—*Polysaccharides. Part IV. The Constitution of Xylan.*

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In these studies, emphasis has been placed on the need for quantitative experiments connecting the preparation of derivatives of the polysaccharides with the formation of their cleavage products. Equally important also is the application of methods which are designed to elucidate the structural character of the latter through the isolation of crystalline derivatives of their oxidation products.

In the growing plant, glucose units in cellulose are to a considerable extent converted into xylose residues with the consequent formation of xylan. This link with cellulose invests the subject of xylan with a special interest.

The chemistry of the pentosans owes much to the pioneer experiments of Tollens and his co-workers, who isolated the so-called wood gum from various cellulosic materials and recognised that a constituent sugar in this pentosan was xylose. The early controversies on the question whether xylan contained only xylose residues have persisted almost to the present time. Difficulties arise in the ultimate analysis of specimens of xylan, and some recent papers have been devoted to the problem of elucidating the empirical formula, and the expressions $(C_5H_8O_4)_n$ and $(C_{10}H_{18}O_9)_n$ have been advanced (Schorsch, *Papier-Fabr.*, 1927, **25**, 576; Heuser and Schorsch, *Cellulose Chem.*, 1928, **9**, 109). Under certain conditions xylan retains sodium hydroxide, and appears to form the compound $(C_5H_8O_4)_2,NaOH$, corresponding to the formula of the hydrate $(C_5H_8O_4)_2,H_2O$ which is involved in the expression $(C_{10}H_{18}O_9)_n$. There is no evidence that sodium hydroxide combines with xylan other than in an exceedingly labile form of union, since the alkali can be eliminated by various solvents. Similarly a specimen of anhydrous xylan can be prepared by desiccation at 112° in a vacuum. But the analysis of this anhydrous specimen is complicated by the fact that it rapidly regains moisture from the atmosphere during subsequent operations (Johnson, *J. Amer. Chem. Soc.*, 1896, **18**, 215). By the method of estimating separately the carbon and hydrogen, and then the moisture content of an air-dried sample of xylan, we have reached the conclusion that the empirical formula $(C_5H_8O_4)_n$ is substantiated.

The whole of the present work has been conducted on specimens of xylan extracted from esparto cellulose by the application of the usual reagent, dilute alkali (Thomsen, *J. pr. Chem.*, 1879, **19**, 146; Wheeler and Tollens, *Annalen*, 1889, **254**, 307; Irvine and Hirst, J., 1924, **125**, 15). The xylan content was 31%, and the yield of pure xylan from this source 25%; under average conditions of atmospheric humidity it retained about 11% of moisture. Specific tests for lignin and furan compounds showed that these were absent. Complete hydrolysis with dilute nitric acid (Heuser and Jayme, *J. pr. Chem.*, 1923, **105**, 232) gave 93% of the theoretical yield of crystalline xylose. It is evident, therefore, that xylan is composed entirely of xylose residues. The highest yield previously obtained was 85% (Heuser and Jayme, *loc. cit.*).

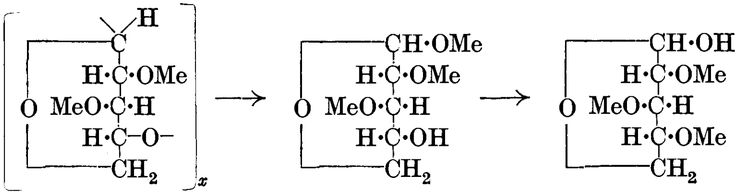
Hitherto the attempts to prepare *dimethyl xylan* appear to have resulted in the formation of products which have little relation to the original xylan, since the doubtful operation of heating in sealed vessels under pressure with silver oxide and methyl iodide was introduced (Heuser and Ruppel, *Ber.*, 1922, **55**, 2084; Komatsu, Inoue, and Nakai, *Mem. Coll. Sci., Kyoto Imp. Univ.*, 1923, **6**, 25). This procedure leads to syrupy or low-melting substances having a dextro-rotation, and these we have found (see experimental section) to be mixtures of degraded products.

A review of the methods and of the experimental results of these authors convinced us that they were not in possession of a genuine dimethyl xylan. The specific rotation of their product, obtained in poor yield, is given as + 30·4°, whereas xylan is strongly lævorotatory. Hydrolysis of such a product could not be expected to lead to a satisfactory decision on the constitution of xylan, inasmuch as it was clearly a mixture of substances which had suffered profound modification in structure. We have observed that on hydrolytic cleavage this dextrorotatory substance gives rise to trimethyl, as well as to dimethyl and monomethyl xyloses.

The earlier claim to have determined the distribution of the free hydroxyl groups in xylan (Komatsu, Inoue, and Nakai, *loc. cit.*) is based on the examination of a cleavage fragment regarded as 2 : 3-dimethyl xylose, which the authors omitted to characterise. Their oxidation of this fragment led to the isolation of gums of doubtful homogeneity, the identification of which was attempted by methods that were inherently incapable, owing to the variety of possible interpretations, of attaining a decisive result.

Exhaustive experiments which we have instituted with the view of determining the conditions of methylation of xylan have shown that non-homogeneous mixtures are frequently encountered unless the appropriate conditions herein described are adhered to. In agreement with Heuser and Ruppel we have not found it possible to complete the methylation with methyl sulphate and sodium hydroxide in a reasonable number of operations without introducing drastic treatment. On the other hand xylan has now been completely methylated in two operations, with potassium hydroxide and methyl sulphate in the cold, to give a dimethyl xylan in almost quantitative yield, and this product is formed as a white solid having a specific rotation of — 92° in chloroform as compared with the rotation of — 109·5° for anhydrous xylan dissolved in dilute alkali. It is insoluble in water, stable to alkali, and non-reducing.

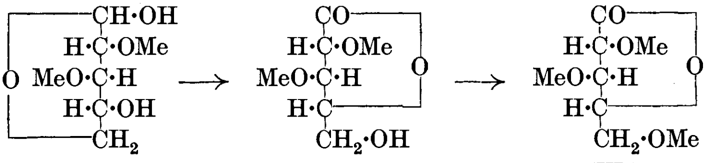
Hydrolysis of the dimethyl xylan (I) with methyl-alcoholic hydrogen chloride led to the isolation of a *dimethyl methylxyloside* in a yield of 90%. The proof that this was, indeed, 2 : 3-dimethyl methylxylopyranoside (II) was developed in the following way. First, the substance was submitted to further methylation, and the resulting trimethyl methylxyloside was hydrolysed to give crystalline 2 : 3 : 4-trimethyl xylose (III) (xylopyranose), which was identical with a specimen previously prepared from methylxyloside (Hirst and Purves, J., 1923, **123**, 1352; Haworth and Jones, J., 1927, 2349; Haworth and Morton, in the press) and has been shown to possess the six-atom ring. The yield of this sugar was 81%, slight losses having occurred through the formation of furan derivatives by secondary changes introduced by the mineral acid.


(I.) (II.) (III.)

Secondly, the 2 : 3-dimethyl methylxyloside was hydrolysed to give 2 : 3-dimethyl xylose (IV) (yield, 85%), which, although not yet obtained in a crystalline condition, gave a crystalline *anilide.*

From a further specimen of the 2 : 3-dimethyl methylxyloside the corresponding lactone was prepared by hydrolysis and oxidation with bromine water. The 2 : 3-dimethyl γ-xylonolactone (V), although a liquid, gave in high yield the crystalline phenylhydrazide of 2 : 3-dimethyl xylonic acid. Similarly the p-*bromophenylhydrazide* of 2 : 3-dimethyl xylonic acid was isolated as a crystalline substance.

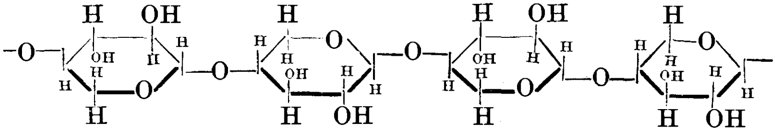
The dimethyl xylose failed to give an osazone, but since it gave rise to 2 : 3 : 4-trimethyl xylose on complete methylation it could not have been 2 : 5-dimethyl xylose. Again, the ready formation of a γ-lactone from the corresponding dimethyl xylonic acid showed that position 4 was not occupied by a methyl group, and inasmuch as the above phenylhydrazide was not identical with the 3 : 5-dimethyl derivative obtained by one of us in the course of previous work (Haworth and Porter, J., 1928, 611) it now became evident that the methyl groups could only be situated in the 2 : 3-positions.


(IV.) (V.) (VI.)

Complete confirmation of these views on the structure of the lactone derived from the 2 : 3-dimethyl xylose was supplied by the following observations. The rate of hydrolysis of the lactone, followed polarimetrically, was exceedingly slow and equilibrium in aqueous solution was approached only after 400 hours. This behaviour (see earlier papers) is characteristic of γ-lactones. The corresponding change of 2 : 3-dimethyl xylonic acid to the lactone was similarly studied, and it appeared that at equilibrium an aqueous solution contained approximately 58% of unchanged lactone. Furthermore the 2 : 3-dimethyl γ-xylonolactone gave rise on complete methylation to the 2 : 3 : 5-trimethyl γ-xylonolactone (VI). The properties of this compound were in complete agreement with those already determined by Haworth and Porter (*loc. cit.*), and moreover the lactone gave the corresponding crystalline phenylhydrazide identical with that which had been prepared by those authors. It was, therefore, evident that positions 4 and 5 were unmethylated in the original 2 : 3-dimethyl xylose derived from dimethyl xylan, and that, although the sugar assumed the pyranose form, the lactone derived from it passed into the furanose type by ring displacement, a behaviour which is common to such partly methylated sugars.

An endeavour was made to isolate other dimethylxylosides from partly methylated xylan, but in all cases the only dimethyl sugar obtained was the 2 : 3-isomeride. Monomethyl and unsubstituted xylose were also present in the products, and it is evident that, as with starch and cellulose, there is no preferential methylation of the hydroxyl groups in xylan.

From these experimental results the conclusion is drawn that, in xylan, the hydroxyl positions 2 and 3 are not involved in the mutual union of the conjugated xylose residues, and that positions 4 and 5 are occupied either in the linking of units or in ring formation. Although the evidence for the pyranose structure (six-atom ring) is still not definite, yet the properties and especially the stability of the polysaccharide incline us to the view that it is the pyranose form of xylose which is present in xylan. It appears evident that β-xylose residues only are concerned in the xylan complex, since the specific rotation of pure xylan (— 109°) is analogous to that of β-methylxyloside (— 67°) but very different from that of α-methylxyloside (+ 153°). The contiguous units of xylan may be represented by the following scheme :

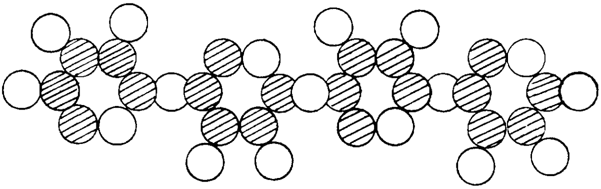


When this is compared with the structural plan for cellulose which is based upon the constitution of cellobiose, it will be seen that xylan differs from cellulose only in the absence of the side chain $-CH_2{\cdot}OH$ from each xylopyranose unit.

The projection diagram of a model of this scheme for xylan is shown below (Fig. 1), the hydrogen atoms being omitted. The shaded circles represent carbon atoms, and the unshaded, oxygen atoms.

A more compact conformation of this model may be attained by the simple device of rotating the hexagon units round the valency positions at the glucosidic oxygens. Several ways of accomplishing this change of conformation are possible whilst maintaining the hexagons in approximately the same plane. One of these forms is indicated in Fig. 2.

FIG. 1.



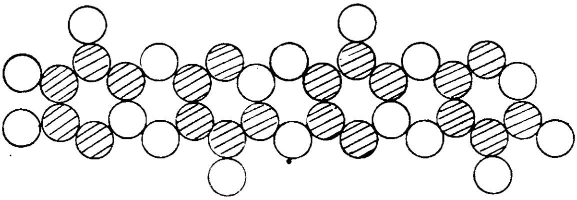


FIG. 2.

At this stage we are not able to advance definite views on the molecular complexity of xylan, but experiments are being directed to this end.

EXPERIMENTAL.

Preparation of Xylan from Oran Esparto.—Esparto cellulose was specially prepared for us from Oran esparto grass by Mr. A. J. C. Aikman, B.Sc., in the laboratories of the Guardbridge Paper Company, Ltd. After removal of the lignin from the wax-free esparto with dilute sodium hydroxide solution under the mildest possible conditions, the material was bleached, again under mild conditions, and then thoroughly washed with water. The resulting esparto cellulose was colourless, retained when air-dry about 8% of moisture, and when boiled with 12% aqueous hydrochloric acid yielded furfuraldehyde equivalent in amount to a pentosan content of 31%.

Esparto cellulose (500 g.) was extracted with boiling 12% aqueous sodium hydroxide (5000 c.c.) for 12 hours. The hot mixture was then poured through a Buchner filter funnel. A layer of cellulose soon formed which acted as an efficient filter bed and a second filtration through this gave a clear brown liquid free from cellulose fibres. To the filtrate, cooled to 35°, an equal volume of methylated spirit was added cautiously with continuous stirring. The granular powder obtained was kept for 12 hours before being separated from the supernatant liquor by decantation, after which it was triturated with a mixture of equal volumes of glacial acetic acid and alcohol, then with cold water, alcohol and ether in quick succession. The xylan was dried at room temperature in a vacuum desiccator (yield, 125 g.).

Xylan prepared in this manner was usually colourless, but was occasionally yellowish. In the latter case it was dissolved in 12% aqueous sodium hydroxide and recovered from the alkaline solution by precipitation with alcohol and trituration with acetic acid, water, alcohol and ether in the manner first described.

Xylan from esparto cellulose retained 10·7% of moisture when air-dry under average conditions of humidity. It was neutral to litmus, without action on boiling Fehling's solution, insoluble in cold water, but soluble in dilute aqueous sodium hydroxide: $[\alpha]_D^{22^\circ}$ − 109·5° in 2·5% aqueous sodium hydroxide ($c = 0{\cdot}89$, calculated as moisture-free material). The same rotation was observed in 8% aqueous sodium hydroxide. A Zeisel estimation revealed that no methoxyl groups were present in xylan. On analysis 5·151 mg. gave 7·609 mg. CO_2, 2·94 mg. H_2O and 0·01 mg. ash. The moisture content, estimated by drying in a vacuum at 110°, was 10·7%. From these figures the percentage composition of dry ash-free xylan was calculated to be C, 45·2; H, 5·8 ($C_5H_8O_4$ requires C, 45·4; H, 6·1%).

Hydrolysis of Xylan to Xylose.—Xylan (2·32 g. dry-weight) was boiled for exactly one hour with 3% nitric acid (111 c.c.) according to the method of Heuser and Jayme (*loc. cit.*). The xylan had then dissolved, and the reaction was stopped by cooling the solution to 0° and neutralising the acid with barium carbonate. The neutral solution was evaporated to dryness at 60°/15 mm., and the residue extracted several times with boiling absolute alcohol. After removal of most of the alcohol under diminished pressure, pure xylose crystallised (2·30 g.), and from the mother-liquor a further 0·15 g. was obtained, making in all 2·45 g. or 92·7% of the theoretical quantity. The properties of the xylose thus obtained were identical with those recorded for the pure sugar, m. p. 142°. An aqueous solution ($c = 1{\cdot}27$) showed: $[\alpha]_D^{21^\circ}$ + 72° (7 minutes after dissolution in water), 62·6° (10 mins.), 51° (15 mins.), 43·2° (20 mins.), 37·2° (25 mins.), 31·8° (30 mins.), 26·3° (40 mins.), 21·0° (60 mins.), 19·3° (equilibrium value after 70 mins.); whence $k_1 + k_2 = 26{\cdot}9 \times 10^{-3}$ (in minutes and decimal logarithms) and $[\alpha]_D^{21^\circ} = + 100^\circ$ (initial value). The latter figure is in exact agreement with the observations of Parcus and Tollens (*Annalen*, 1890, **257**, 175).

Methylation of Xylan with Methyl Sulphate and Potassium Hydroxide.—When xylan (2·5 g.) was heated with 45% aqueous potassium hydroxide (220 c.c.) it was usually found that complete solution did not take place unless a small quantity of water (15—20 c.c.) was added to the mixture. This alkaline solution of xylan was treated with methyl sulphate (200 c.c.) at room temperature, with continuous mechanical stirring, the addition occupying 5 hours; thereafter the temperature was raised to 100° for 1 hour. The hot, slightly alkaline solution was filtered through fine muslin, which retained the partly methylated xylan (OMe, 33%) in the form of a white solid contaminated with potassium sulphate. It was found to be inadvisable, owing to the tendency of the methylated xylan to become gummy, to attempt further purification at this stage, except in certain experiments where, for the purpose of obtaining a specimen for analysis, the method described below was employed. The crude product was therefore re-methylated immediately by treating it under the same conditions as before with 45% aqueous potassium hydroxide (220 c.c.) and methyl sulphate (200 c.c.). During this process *dimethyl xylan* separated as small white nodules, which, after the final heating for 1 hour at 100°, were separated from the boiling solution by filtration, washed several times with boiling water until free from sulphate, and dried by heating for 2 hours at 100°/10 mm. The product was a white solid fibrous in structure, which had no action on boiling Fehling's solution and showed no tendency to become gummy when treated with either hot or cold water (yield, 95% of the theoretical, calculated on ash-free material).

The ash content (1·4%, consisting mainly of iron oxide and silica) could be reduced considerably by dissolving the substance in a small volume of chloroform, filtering the solution, and precipitating the dimethyl xylan by the addition of an excess of dry ether. The precipitate was washed with ether and dried in a vacuum desiccator. Dimethyl xylan was thus obtained as a white powder, m. p. 194—196° (slight decomp.), $[\alpha]_D^{18.5^\circ} - 92^\circ$ in chloroform ($c = 0{\cdot}5$). It was easily soluble in cold chloroform, tetrachloroethane, benzene or glacial acetic acid, soluble in warm carbon tetrachloride, very slightly soluble in boiling methyl alcohol, and insoluble in water, alcohol, ether, acetone or light petroleum (Found : C, 52·1; H, 7·3; OMe, 38·7; ash, 0·9. $C_7H_{12}O_4$ requires C, 52·5; H, 7·6; OMe, 38·75%).

Hydrolysis of Dimethyl Xylan.—Dimethyl xylan (4·03 g., ash-free) was hydrolysed by boiling it with 1·2% methyl-alcoholic hydrogen chloride (250 c.c.). The dimethyl xylan was almost insoluble in acid methyl alcohol and the rate of dissolution varied with the physical condition of the material used. Very finely divided material dissolved completely in about 7 hours, whereas coarser particles required at least 15 hours. The boiling was continued until the resulting solution attained a constant rotation. In the present experiment this was found to be $[\alpha]_D^{20^\circ} + 54^\circ$ (calculated as dimethyl methylxyloside), but at other times, when different concentrations of hydrogen chloride had been used, slightly different values were recorded (compare Hess, Micheel, and Littmann, *Annalen*, 1928, **466**, 115). In every case, however, the equilibrium rotation of the resulting dimethyl methylxyloside in methyl alcohol containing 0·8% of hydrogen chloride was found to be $[\alpha]_D^{20^\circ} + 43^\circ$. The acid was next neutralised with silver carbonate, and the filtered solution evaporated to a syrup at 60°/12—15 mm. This was dissolved in ether and filtered to remove a minute mineral residue. After removal of the ether the resulting syrup (4·35 g. or 90% of the theoretical amount) was distilled under diminished pressure, giving 3·96 g. of 2 : 3-*dimethyl methylxyloside*, b. p. about 80°/0·04 mm., $n_D^{17^\circ}$ 1·4581, $[\alpha]_D^{21\cdot5^\circ} + 61\cdot8^\circ$ in methyl alcohol ($c = 1\cdot2$); $[\alpha]_D^{22^\circ} + 43^\circ$, equilibrium value after being heated in a sealed tube for 5 hours at 100° with 0·8% methyl-alcoholic hydrogen chloride (Found : OMe, 47·9. Calc. for $C_8H_{16}O_5$: OMe, 48·2%).

Proof of the Constitution of 2 : 3-*Dimethyl Methylxyloside.*—The experiments described in the following paragraphs were designed to provide evidence concerning the constitution of the dimethyl methylxyloside obtainable from methylated xylan. They were carried out with (*a*) dimethyl methylxyloside from dimethyl xylan, (*b*) dimethyl methylxyloside from partly methylated xylan (see below), and (*c*) dimethyl methylxyloside from the methylated and partly hydrolysed xylan obtained by following the instructions of Heuser and Ruppel (*loc. cit.*, see below). In all cases the experiments followed a precisely similar course and it will be sufficient to describe in detail one typical series.

Methylation of 2 : 3-*dimethyl methylxyloside.* Dimethyl methylxyloside (3·9 g.) was treated in the usual manner with silver oxide and methyl iodide (yield, 3·9 g.). The operation was repeated and the product then distilled, giving trimethyl methylxyloside (3·6 g.), b. p. about 65°/0·02 mm., $n_D^{18^\circ}$ 1·4403 (Found : OMe, 58·5. Calc., 60·2%). The distillate was treated with 3% aqueous hydrochloric acid (1·02 g. of substance in 25 c.c. of solution) for 1 hour at 100° in order to hydrolyse the trimethyl methylxyloside. During the hydrolysis a trace of furfuraldehyde was detected by means of the colour reaction with aniline acetate. (This is usual, but the amount of decomposition is very small when the reaction is carried out under the mild conditions prescribed.) The acid was neutralised with barium carbonate, the solution evaporated to dryness, and the trimethyl xylose extracted from the residue by means of boiling ether. Removal of the ether left a solid crystalline mass (0·77 g. or 81% of the theoretical). One recrystallisation from ether sufficed to give large rectangular prisms of 2 : 3 : 4-trimethyl xylose, m. p. 89—92°, alone or when mixed with an authentic specimen prepared from xylose (Carruthers and Hirst, J., 1922, **121**, 2299). No evidence could be obtained of the presence of any substance other than 2 : 3 : 4-trimethyl xylose.

2 : 3-*Dimethyl xylose and its anilide.* When the 2 : 3-dimethyl methylxyloside was heated at 100° with 3% aqueous hydrochloric acid, the initial specific rotation decreased regularly to a constant value $[\alpha]_D^{20°}$ + 25° ($c = 1{\cdot}1$, calculated as free sugar). During the hydrolysis a detectable quantity of furfuraldehyde was formed (aniline acetate test). The solution was neutralised with barium carbonate, evaporated to dryness at 60°/15 mm. and the product extracted from the solid residue with boiling ether. On evaporation of the ether 2 : 3-dimethyl xylose was left as a viscid syrup (yield, 85%) which readily reduced warm Fehling's solution; $n_D^{20°}$ 1·4783, $[\alpha]_D^{20°}$ + 22·6° in water 6 minutes after dissolution ($c = 3{\cdot}5$), $[\alpha]_D^{22°}$ + 24° (constant value). It could not be crystallised, but a crystalline *anilide* was obtained from it by boiling for 2½ hours a dilute alcoholic solution of the sugar (1 part) and aniline (7 parts). When the solvent was evaporated, the anilide slowly crystallised. After 3 days the excess of aniline was removed by washing with cold dry ether and the solid residue was recrystallised from ethyl acetate containing a little light petroleum, forming long needles, m. p. 146° (yield, 50% of the theoretical) (Found : C, 61·7; H, 7·5; N, 5·6; OMe, 24·5. $C_{13}H_{19}O_4N$ requires C, 61·6; H, 7·6; N, 5·5; OMe, 24·5%). The value $[\alpha]_D^{19°}$ + 185° (in ethyl acetate, $c = 0{\cdot}76$) was constant for 24 hours; but mutarotation proceeded rapidly in ethyl acetate containing 4·3 g. of acetic acid per 100 c.c. (compare Baker, J., 1928, 1979), and after 60 minutes the rotation was constant at + 65·5°. Mean $k_1 + k_2 = 4{\cdot}7 \times 10^{-2}$ (in mins., and logarithms to the base 10).

2 : 3-*Dimethyl γ-xylonolactone.* A solution of dimethyl methylxyloside (3·06 g.) in 3% aqueous hydrobromic acid (41 c.c.) was heated for 1 hour at 85° and then maintained at 75° during the addition of bromine (3·8 c.c.). After 16 hours the reducing action towards Fehling's solution had disappeared and the excess of bromine was removed by aeration. The solution was neutralised with silver oxide, and the dissolved silver precipitated by addition of the exact quantity of hydrochloric acid. The silver chloride coagulated on warming and the solution could then be filtered readily. Removal of the water under diminished pressure left a syrup which,

after being heated for several hours at 100°/15 mm., was dissolved in ether. The filtered solution was evaporated to a syrup (yield, 2·59 g.), which was distilled, giving 2 : 3-dimethyl γ-xylonolactone as a colourless uncrystallisable liquid (2·44 g.), b. p. about 115°/0·02 mm., $n_{D}^{16\cdot5^\circ}$ 1·4640, $[\alpha]_{D}^{22\cdot8^\circ} + 97^\circ$ (initial value in water, $c = 1\cdot2$) (Found : C, 47·8; H, 6·9; OMe, 34·4. Calc. for $C_7H_{12}O_5$: C, 47·7; H, 6·9; OMe, 35·2%).

Phenylhydrazide. When the lactone (1 mol.) was heated on the water-bath with an ethereal solution of phenylhydrazine (1 mol.), the corresponding phenylhydrazide was obtained. This crystallised after repeated trituration with dry ether (yield, 79%) and then separated from ethyl acetate, containing a little light petroleum (b. p. 40—60°), in rosettes of needles, which were insoluble in ether or light petroleum but readily soluble in water, alcohol, or ethyl acetate; m. p. 107—108°; m. p. 86—89°, when mixed with a sample, m. p. 96°, of the phenylhydrazide of 3 : 5-dimethyl xylonic acid (Haworth and Porter, *loc. cit.*); $[\alpha]_{D}^{23^\circ} + 30^\circ$ in alcohol ($c = 0\cdot5$) (Found : C, 55·2; H, 7·0; N, 10·1; OMe, 21·3. Calc. for $C_{13}H_{20}O_5N_2$: C, 54·9; H, 7·0; N, 9·9; OMe, 21·8%).

The corresponding p-*bromophenylhydrazide* was prepared in a similar manner. It crystallised readily when triturated with dry ether and was recrystallised from ethyl acetate containing a little light petroleum, forming long silky needles, m. p. 150—151° (yield, 80%) (Found : C, 43·0; H, 5·4; N, 8·0; OMe, 16·3. $C_{13}H_{19}O_5N_2Br$ requires C, 43·0; H, 5·3; N, 7·70; OMe, 17·1%).

Hydrolysis of 2 : 3-*dimethyl* γ-*xylonolactone.* The specific rotation of 2 : 3-dimethyl xylonic acid, when determined in the usual manner, was found to be $[\alpha]_{D}^{22^\circ} + 30\cdot4^\circ$ in water ($c = 1$, calculated as lactone), and the conversion of the acid into the lactone was followed polarimetrically. The reaction velocities were very small and the behaviour throughout was exactly similar to that observed with other γ-lactones. The equilibrium value $[\alpha]_{D}^{20^\circ} + 69^\circ$ was reached in about 400 hours from the lactone side, and the proportion of lactone then present was approximately 58%. For the conversion of the lactone into the acid at 20° the value of the velocity coefficient $k_1 + k_2$ was approximately $3\cdot0 \times 10^{-3}$ (in hours and decimal logarithms).

Lactone to acid.			Acid to lactone.		
t (hrs.).	$[\alpha]_{D}^{20^\circ}$.	% Lactone.	t (hrs.).	$[\alpha]_{D}^{20^\circ}$.	% Lactone.
0	+97°	100	0	+30·4°	0
24	89	86·5	24	36·8	9·6
48	85·7	84·5	72	46·1	23·6
96	81·4	76·6	96	50·5	30·2
144	77·2	70·3	144	56·2	38·8
264	71·3	61·5	264	60·5	45·3
384	69	58	500	62·1	48·6
500	69	58	700	63	49

Conversion of 2 : 3-dimethyl γ-xylonolactone into 2 : 3 : 5-trimethyl xylonolactone. The dimethyl lactone (1·3 g.) was treated twice with methyl iodide and silver oxide and then distilled, giving 2 : 3 : 5-trimethyl γ-xylonolactone as a mobile colourless liquid (1·1 g.), b. p. about 84°/0·03 mm., $n_D^{20°}$ 1·4426, $[\alpha]_{5461}^{19°} + 100°$ (initial in water, $c = 1·5$). The corresponding acid gave $[\alpha]_{5461}^{20°} + 41°$ in water ($c =$ 1·3, calculated as lactone). Further proof of the identity of this lactone was obtained by preparing from it in good yield the corresponding phenylhydrazide. This crystallised from benzene in rosettes of needles, m. p. 89°, alone or when mixed with an authentic specimen of the phenylhydrazide of 2 : 3 : 5-trimethyl xylonic acid prepared by Haworth and Porter (*loc. cit.*).

Methylation of Xylan with Sodium Hydroxide and Methyl Sulphate.—Methyl sulphate (130 c.c.) was added slowly with efficient stirring to a solution of xylan (8·67 g.) in 30% aqueous sodium hydroxide (225 c.c.) maintained at 85°. After the addition of sufficient sulphuric acid (50%) to neutralise almost the whole of the residual alkali, the solution was boiled; methylated xylan then separated and was collected by filtering the hot liquid through muslin. The amount of methylated xylan remaining in the filtrate was usually negligible. The crude methylated xylan was dried and powdered by trituration under cold ether (yield, 13·1 g.). It contained methylated xylan (8·23 g.; yield, 83% of the theoretical), sodium sulphate (2·75 g.), sodium methyl sulphate (1·22 g.), silica (0·47 g.), and moisture (0·53 g.). The amount of silica was estimated by oxidising a weighed portion with nitric acid and weighing the insoluble residue. The sodium sulphate was determined by precipitating with barium chloride an aliquot portion of the filtrate from the silica estimation. The sodium methyl sulphate remaining in the filtrate from the last operation was converted into sodium sulphate by boiling with concentrated alkali solution and then estimated in the usual way. Alternatively, an aliquot portion of the filtrate from the silica estimation was boiled with alkali and the total sulphate estimated, the amount of sodium methyl sulphate being obtained by difference. The same value was obtained by both methods.

The crude methylated xylan (5 g.) was freed from sodium methyl sulphate by boiling for 3 hours with 7% sodium hydroxide solution (120 c.c.). After neutralisation with 50% sulphuric acid the solution was evaporated to dryness on the water-bath. The residue, after being dried for several hours at 60°/10 mm., was repeatedly extracted with boiling chloroform. The filtered chloroform solution when evaporated under diminished pressure gave a glassy solid (2·7 g.), which was again dissolved in chloroform. Addition of ether now yielded a white flocculent precipitate of purified methylated xylan, which was collected, washed with ether, and dried in a vacuum desiccator (yield, 2·3 g.). Ash content, 1·2% (Found : OMe, 29·2%); $[\alpha]^{22°}_{D}$ — 85° in chloroform (c = 0·27), $[\alpha]^{20°}_{D}$ — 116° in water (c = 0·33).

The partly methylated xylan was treated on numerous occasions under very varied conditions with methyl sulphate and sodium hydroxide, but in no case could the methoxyl content be raised beyond 30%.

Hydrolysis of methylated xylan (OMe, 29%). When the above partly methylated xylan was boiled with 1·2% methyl-alcoholic hydrogen chloride, solution was completed in 26 minutes. At this stage the high lævorotation of the original product had fallen to zero and after a further 108 minutes a constant value $[\alpha]^{22°}_{D}$ + 74° was reached, equivalent to $[\alpha]^{22°}_{D}$ + 62° calculated on the amount of hydrolysed material. This value is intermediate between the equilibrium values, now determined under similar conditions, for 2 : 3-dimethyl methylxyloside, + 43°, and methylxyloside, + 76°.

An experiment made with 10·9 g. of methylated xylan yielded 12·1 g. (92% of the theoretical yield) of hydrolysed product in the form of a mobile liquid which gave on distillation (*a*) 0·12 g., b. p. 85—95°/0·01 mm. (Found : OMe, 54·0%)—this was probably decomposed material; (*b*) 7·0 g. of 2 : 3-dimethyl methylxyloside, b. p. 110—118°/0·03 mm., $n^{16°}_{D}$ 1·4570, $[\alpha]^{20°}_{D}$ + 43° (equilibrium in methyl alcohol containing 1% of hydrogen chloride; c = 4·7) (Found : OMe, 47·8%); (*c*) 3·2 g. of monomethyl methylxyloside, b.p. about 140°/0·03 mm., $n^{18°}_{D}$ 1·4753, $[\alpha]^{21°}_{D}$ + 63° (equilibrium value in methyl alcohol containing 0·8% of hydrogen chloride; c = 1·6) (Found : OMe, 34·0. Calc., 34·9%); (*d*) undistillable residue, 1·1 g. These figures indicate that the methylated xylan was an inseparable mixture or solid solution containing, in effect, dimethyl xylan (60%), monomethyl xylan (25%), and unmethylated xylan (15%).

Treatment of Partly Methylated Xylan with Methyl Iodide and Silver Oxide.—Attempts to obtain dimethyl xylan by treating the partly methylated substance with methyl iodide and silver oxide under slight pressure (10—15 cm. of mercury) were unsuccessful (compare Heuser and Ruppel, *loc. cit.*). Surprising and unexpected results were, however, obtained in experiments in which these reagents were employed at 90—100°, the reaction being conducted in sealed tubes as described by Heuser and Ruppel. Finely divided methylated xylan (4 g.; OMe, 29%) was mixed with silver oxide (20 g.) and placed in a Carius tube with methyl iodide (32 c.c.), special care being taken to ensure thorough mixing of solid and liquid. After 3 days at 100° the considerable gas pressure was released. The contents of the tube were then extracted with boiling chloroform and on removal of the solvent a stiff syrup remained which vigorously reduced Fehling's solution (yield, 4 g. Found: OMe, 35·1%). After one further methylation with methyl iodide and silver oxide (yield, 4·2 g. from 3·9 g.) the reducing action had disappeared and the methoxyl content had risen to 43·8%. When this material was heated in a vacuum, a considerable portion of it (9·6 g. from 16·9 g.) was found to be volatile. Distillation commenced when the bath temperature was 73°/0·1 mm., continued steadily while the temperature was raised slowly to 150°, and ceased entirely at 175/0·1 mm. A clear transparent gum remained, which set when cold to a hard glass.

Examination of the volatile portion. This was divided into three portions by fractional distillation: (*a*) 4·46 g., mainly 2 : 3 : 4-trimethyl methylxyloside, b. p. 65°/0·1 mm., $n_D^{18°}$ 1·4420, $[\alpha]_D^{20°} + 19·5°$ in water ($c = 1·44$). This was neutral to litmus and non-reducing until hydrolysed with acid. Treatment of a weighed sample with excess of hot *N*/10-sodium hydroxide solution showed that lactones or esters were present, if at all, in negligible amounts (Found: OMe, 56·6. Calc. for $C_9H_{18}O_5$: OMe, 60·2%).

(*b*) 3·72 G., mainly 2 : 3-dimethyl methylxyloside, b. p. 95—105°/0·1 mm., $n_D^{17°}$ 1·4551, $[\alpha]_D^{22°} + 35·2°$ in water ($c = 1·37$). This was neutral to litmus, non-reducing until after hydrolysis with acid, and contained negligible quantities of oxidation products (Found: OMe, 48·2. Calc. for $C_8H_{16}O_5$: OMe, 48·2%).

(*c*) 0·9 G., b. p. 120—160°/0·1 mm., $n_D^{17°}$ 1·4630. This was very viscous, reduced Fehling's solution, and was evidently an incompletely methylated portion.

Fractions (*a*) and (*b*) together constituted 91% of the volatile portion of the methylated xylan. That they were in reality xylose derivatives was proved by obtaining from them in good yield crystalline trimethyl xylose. For this purpose the remainder of (*a*) and (*b*) (7·7 g. in all) was methylated twice in the usual way by Purdie's reagents. The product (7·2 g.) was a colourless mobile liquid having the properties of 2 : 3 : 4-trimethyl methylxyloside, b. p. 115°/17 mm., $n_D^{15°}$ 1·4407 (Found: C, 51·7; H, 8·8; OMe, 58·2. Calc. for $C_9H_{18}O_5$: C, 52·5; H, 8·8; OMe, 60·2%).

The distillate was hydrolysed with 3% aqueous hydrochloric acid for 1 hour at 100°. When the product was extracted in the usual way, crystalline 2 : 3 : 4-trimethyl xylose was obtained in good yield, m. p. 90—93° alone or when mixed with an authentic specimen. When it was heated with 1% methyl-alcoholic hydrogen chloride, the equilibrium rotation, calculated as trimethyl methylxyloside, was $[\alpha]_D^{20°} + 50°$, in agreement with the value previously recorded.

It was thus apparent that treatment of methylated xylan in sealed tubes at 90° with methyl iodide and silver oxide resulted in extensive hydrolysis, unaccompanied, however, by oxidation. An explanation was thus afforded of the statements of Heuser and Ruppel and of Komatzu, Inoue, and Nakai that the specimens of dimethyl xylan prepared by them became solid only after being heated for some days at 80° in a vacuum. It is obvious that hydrolysis had occurred in the course of their experiments and that during the drying process a portion of the volatile products escaped.

Examination of the solid residue. The residue left after removal of the volatile portions of the methylated xylan was a clear transparent glass, which was very hygroscopic. It corresponded exactly to the substances described by Heuser and Ruppel and by Komatzu, Inoue, and Nakai as dimethyl xylan, and the analytical figures were in fortuitous agreement with those required for dimethyl xylan (Found: C, 51·8; H, 7·9; OMe, 38·2. Calc. for $C_7H_{12}O_4$: C, 52·5; H, 7·6; OMe, 38·8%). The specific rotation, $[\alpha]_D^{20^\circ}$ + 20° in chloroform ($c = 1{\cdot}1$), was slightly lower than that recorded by the earlier authors and this indicated that the material now described had been separated more completely from the accompanying hydrolysis products. In confirmation of this view it was found that in another experiment, after very prolonged heating in a high vacuum, the solid residue showed $[\alpha]_D^{20^\circ}$ + 14·5° in chloroform ($c = 1{\cdot}6$). In the latter case little or no trace of trimethyl xylose was obtained on hydrolysis, whereas the specimen with $[\alpha]_D^{20^\circ}$ + 20° gave rise to some 12% of trimethyl xylose, and therefore could not have been completely free from hydrolysed products. The hydrolyses were carried out and the products identified by the methods described above. The solid material ($[\alpha]_D^{20^\circ}$ + 20°) gave after treatment with methyl-alcoholic hydrogen chloride 2 : 3 : 4-trimethyl methylxyloside (14% by weight), 2 : 3-dimethyl methylxyloside (57%), monomethyl methylxyloside (22%), and a glassy residue (15%) (total yield, 85% of the theoretical). The solid ($[\alpha]_D^{20^\circ}$ + 14°) gave under similar conditions 2 : 3-dimethyl methylxyloside (50%), monomethyl methylxyloside (28%), and a solid residue (20%).

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