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Partial Acetylation Studies on Benzyl 4-*O*-Methyl-β-D-xylopyranoside

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Partial acetylation studies on benzyl 4-*O*-methyl-β-D-xylopyranoside have shown that, with acetic anhydride and sodium acetate, the hydroxyl group on $C_{(2)}$ is the more reactive while the hydroxyl group on $C_{(3)}$ is the more reactive with acetic anhydride and perchloric acid. Other systems, such as acetic anhydride and pyridine, acetyl chloride and pyridine, and acetic anhydride and pyridine containing pyridine hydrochloride, give distributions of the acetyl groups showing lesser degrees of preferential reaction than these two extremes. The stability of the acetyl groups in benzyl 2-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside and benzyl 3-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside during methylation with methyl iodide and silver oxide in dimethylformamide, on treatment with silver oxide in dimethyl formamide, on carbamoylation with phenylisocyanate, and the stability concerning migration of the phenyl carbamoyl groups as well as the stability of the acetyl groups during treatment with monoethanolamine in ethanol, and during treatment with dimethyl sulphoxide have been examined. Previously reported work on the position of *O*-acetyl groups in birch xylan is discussed in the light of the results obtained.

So far, the isolation of two native acetylated polysaccharides from the cell wall of wood has been reported, an acetylated 4-*O*-methylglucuronoxylan from birch wood [1,2,3] and an acetylated glucomannan from pine [4,5] and spruce [6] wood. The problem of determining the exact position of attachment of the *O*-acetyl groups in native acetylated polysaccharides is complicated due to the alkali-lability of these groups and their ease of migration from one hydroxyl group to another given appropriate spatial arrangements. If an unequivocal method for the localisation of the *O*-acetyl groups can be found, the problem remains as to whether acetyl migration or selective removal of acetyl groups from some hydroxyl groups has occurred during the extraction and purification of the polysaccharide.

Various approaches to the determination of the position of the *O*-acetyl groups have been made. Periodate oxidations of the acetylated 4-*O*-methylglucuronoxylan [3] and of the glucomannan [4,5] have indicated that the *O*-acetyl

groups are located on the hydroxyl groups on $C_{(2)}$ and/or $C_{(3)}$ in the β-1,4-linked anhydroxylose residues and mainly on the hydroxyl groups on $C_{(2)}$ and/or $C_{(3)}$ in the β-1,4-linked anhydromannose residues respectively; the distribution of *O*-acetyl groups between these two positions could not be decided by this method. Methylation studies have been applied to the acetylated 4-*O*-methylglucuronoxylan [2,3], revealing that most of the *O*-acetyl groups in the acetylated, methylated xylan were attached to the hydroxyl group on $C_{(3)}$ and the remainder to the hydroxyl group on $C_{(2)}$. About 30 % of the *O*-acetyl groups were replaced by methoxyl groups during the methylations. A reasonably similar distribution was found by using phenylcarbamoyl groups as protective substituents [7], but rather more *O*-acetyl groups appeared to be attached to the hydroxyl groups on $C_{(2)}$ than found in the methylation study [2]. Less than 10 % of the *O*-acetyl groups were unaccounted for by this method.

Without attempting to elucidate the actual mechanism of biological acetylation in wood it was nevertheless of interest to acetylate under various conditions a model compound representative of the β-1,4-linked anhydroxyxylose units in the 4-*O*-methylglucuronoxylan to examine the relative reactivities towards acetylation of the hydroxyl groups on $C_{(2)}$ and $C_{(3)}$. As a suitable model compound benzyl 4-*O*-methyl-β-D-xylopyranoside was selected, the synthesis of this compound having been described earlier [8]. It was thought that after partial acetylation the benzyl group could be removed by catalytic hydrogenation [9] leaving the acetyl groups intact. In order to separate and identify the resulting partially substituted xylose derivatives it was desirable to have a method of performing paper electrophoresis in a buffer system which could form complexes with the substances in question at neutral pH where the acetyl groups are stable. Sulphonated phenylboronic acid was found to meet this requirement, as previously described [10].

The two monoacetates of benzyl 4-*O*-methyl-β-D-xylopyranoside, which were obtained pure as described below, were distinguished from one another and their structures assigned on the basis of their oxidation with lead tetraacetate [11] and their paper electrophoretic behaviour after hydrogenation. The hydrogenations were performed with palladium on carbon [12] in methanol or ether solution, and complete removal of benzyl groups with retention of virtually all the acetyl groups was achieved. 2-*O*-Acetyl-4-*O*-methyl-D-xylose consumed 0.08 mole lead tetraacetate and was immobile on paper electrophoresis in sulphonated phenylboronic acid at pH 7.4. 3-*O*-Acetyl-4-*O*-methyl-D-xylose consumed 0.95 mole lead tetraacetate and had a mobility similar to that of 3,4-dimethylxylose on paper electrophoresis in the same buffer. Under the conditions used for the oxidation 4-*O*-methylxylose consumed 2.2 mole lead tetraacetate and 2,3-di-*O*-acetyl-4-*O*-methyl-D-xylose prepared by hydrogenation of the corresponding benzyl β-D-xyloside did not consume any oxidant.

Benzyl 4-*O*-methyl-β-D-xylopyranoside was partially acetylated with acetic anhydride and sodium acetate, with acetic anhydride in pyridine, with acetyl chloride in pyridine, with acetic anhydride in pyridine containing 1.5 % by weight of hydrogen chloride combined as pyridine hydrochloride, and finally with acetic anhydride and perchloric acid. A chromatographic investigation

by thin layer chromatography [13] of the course of the reaction with acetic anhydride and sodium acetate indicated that the ratio of acetyl groups on the $C_{(2)}$ hydroxyl group to that on $C_{(3)}$ was of the order of 2:1 estimated by visual examination of the chromatograms. The acetylation mixtures from the partial acetylation with 1.1 mole acetic anhydride in pyridine and 1.1 mole acetyl chloride in pyridine were separated into the four components by chromatography on silicic acid columns and the ratio of the fractions determined by weighing. The molar ratio of benzyl 2,3-di-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside: benzyl 2-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside: benzyl 3-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside: benzyl 4-*O*-methyl-β-D-xylopyranoside was 2.5:4.0:2.3:1 with acetic anhydride as the acetylating agent and 2.0:1.1:1:1.4 with acetyl chloride. A visual examination of the thin layer chromatograms of the acetylation with 1.1 mole of acetic anhydride in pyridine containing 1.5 % by weight of hydrogen chloride indicated a ratio of about 1:1 of the two monoacetates, a semiquantitative estimation by thin layer chromatography by cutting out the appropriate areas from the plate, extracting and weighing gave a ratio of 1:1. The acetylations with acetic anhydride and perchloric acid were followed by thin layer chromatography. It was found that the hydroxyl on $C_{(3)}$ was by far the more reactive towards this type of acetylation. The ratio of the two monoacetates was in this case from 1:3 to 1:5, estimated by visual examination of the plates. Table 1 gives the ratio of the two monoacetates obtained in the various acetylations.

Jeanloz *et al.* in an investigation of the partial acylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside [14] concluded that on acetylation of this compound with acetic anhydride in pyridine the acylation occurred preferentially at the $C_{(3)}$ hydroxyl. With other acylating agents such as acetyl chloride, benzoic anhydride, benzoyl chloride, methanesulphonic anhydride, methanesulphonyl chloride, *p*-toluenesulphonic anhydride and *p*-toluenesulphonyl chloride, all in pyridine solution, the preferential attack was at the $C_{(2)}$ hydroxyl. Acetic anhydride in pyridine containing a little pyridine hydrochloride gave the 2-*O*-acetyl derivative as the primary product. Bourne, Stacey *et al.* prepared the trifluoroacetyl derivative of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside by controlled methanolysis of the bistrifluoroacetyl compound [15]. From the trifluoroacetyl derivative were prepared derivatives of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside with substituents at either of the $C_{(2)}$ and $C_{(3)}$ hydroxyls depending on the reaction medium. The authors concluded that the remaining trifluoroacetyl group probably was on the $C_{(3)}$

Table 1. Ratios of benzyl 2-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside: benzyl 3-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside obtained in partial acetylations.

Reaction medium	Ratio 2-acetate : 3-acetate
Acetic anhydride, perchloric acid	approx. 1 : 3
Acetyl chloride, pyridine	1.1 : 1
Acetic anhydride, pyridine-pyridine hydrochloride	approx. 1 : 1
Acetic anhydride, pyridine	1.7 : 1
Acetic anhydride, sodium acetate	approx. 2 : 1

hydroxyl and that in pyridine media facile migration of the trifluoracetyl group occurred allowing the incoming group to take up its more favoured position, which was at the $C_{(2)}$ hydroxyl for the *p*-toluene sulphonyl group on acylation with the acid chloride in pyridine and at the $C_{(3)}$ hydroxyl for the acetyl group on acylation with the anhydride in pyridine.

That the configuration at the glycosidic carbon atom is of relatively little importance in determining the substitution pattern in partial acylations in this type of molecule is indicated by the work of Newth on the partial *p*-toluenesulphonation in pyridine solution of 1,5-anhydro-4,6-*O*-benzylidene-D-glucitol [16] where the $C_{(2)}$ hydroxyl again reacted preferentially and by the work reported by Jeanloz *et al.* on partial benzoylation and *p*-toluenesulphonation in pyridine of 1,6-anhydro-β-D-glucopyranose [17] where the $C_{(2)}$ hydroxyl again was more reactive than the $C_{(3)}$ hydroxyl. Substitution occurred preferentially at the $C_{(2)}$ and $C_{(4)}$ hydroxyl groups.

The results obtained for the partial acetylation of benzyl 4-*O*-methyl-β-D-xyloside indicating that the $C_{(2)}$ hydroxyl is the more readily acetylated in pyridine as well as with sodium acetate as catalyst and the $C_{(3)}$ hydroxyl with perchloric acid catalyst, therefore do not fall into the pattern described above for methyl 4,6-*O*-benzylidene-α-D-glucopyranoside, and it seems doubtful that the difference can be ascribed to the different confgiuration at the glycosidic carbon atom. The differences between the xyloside and the above compounds that govern its pattern of substitution on acetylation might be the conformational freedom of the xyloside as compared to the more rigid conformations in the other compounds.

The possibility of acetyl migration in the two monoacetates occurring in pyridine solution was checked by thin layer chromatography. Under the conditions used during the acetylations no migration was observed. The distribution of *O*-acetyl groups found in the partial acetylations in pyridine is therefore kinetically controlled.

The possibility of migration of the acetyl groups in the two monoacetates was also examined under the conditions pertinent to the previous studies on the position of the *O*-acetyl groups in birch xylan [2,3,7].

Methylation of each of the two monoacetates with methyl iodide and silver oxide in dimethyl formamide [2,3], removal of *O*-acetyl groups and hydrolysis led to a mixture of dimethylxylose and 2,3,4-tri-*O*-methylxylose. The composition of the dimethylxylose fraction which predominated was the same in each case and contained about 35 % 3,4-di-*O*-methylxylose and 65 % 2,4-di-*O*-methylxylose. This closely corresponds to the finding that 70 % of the *O*-acetyl groups in the previously reported methylated acetylated xylans were attached to the hydroxyl group on $C_{(3)}$ [2,3], which presumably represents a similar redistribution. A definite assignment of the position of the *O*-acetyl groups by this method is therefore improbable.

The *O*-acetyl groups also migrated in dimethyl formamide containing silver oxide albeit much more slowly than under the actual methylation conditions as indicated by thin layer chromatography [13]. Some deacetylation was observed in this case as well.

The *O*-acetyl groups did, however, appear quite stable towards treatment with hot dimethyl formamide under the conditions previously reported for

the carbanilation of the acetyl 4-*O*-methylglucuronoxylan [7]. Each of the two monoacetates was carbanilated with phenylisocyanate and the products were subjected to methanolysis to remove the *O*-acetyl groups, followed by methylation with methyl iodide and silver oxide in dimethyl formamide, removal of the phenylcarbamoyl groups by lithium aluminium hydride reduction and acid hydrolysis. Benzyl 2-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside on this treatment yielded essentially 2,4-di-*O*-methyl-xylose and benzyl 3-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside yielded essentially 3,4-di-*O*-methylxylose indicating that the phenylcarbamoyl groups were sufficiently stable towards the methylation conditions to be used in the assignment of the position of *O*-acetyl groups in compounds with the spatial arrangement of the hydroxyl groups on $C_{(2)}$ and $C_{(3)}$ in benzyl 4-*O*-methyl-β-D-xylopyranoside and hence probably in β-1,4-linked xylans.

Treatment of each of the two monoacetates in 3 % ethanolic monoethanolamine under the conditions used for the preparation of chlorine-monoethanolamine holocellulose [1-3] caused acetyl migration to near an equilibrium where the 3-*O*-acetyl isomer predominated somewhat over the 2-*O*-acetyl isomer as indicated by thin layer chromatography [13]. Some deacetylation also occurred. In the preparation of chlorine-monoethanolamine holocellulose the medium is heterogeneous, reservations for *O*-acetyl migration during the treatments with monoethanolamine must, however, be made. Bouveng [7] used delignification with chlorite and this is probably to be preferred to treatments with boiling monoethanolamine solutions.

No acetyl migration was observed on treating the 3-*O*-acetyl isomer with dimethyl sulphoxide under the conditions used for the extraction of acetyl 4-*O*-methyl-glucuronoxylans whereas with the 2-*O*-acetyl isomer a slight migration occurred. No deacetylation was observed. The extraction of native acetylated xylan with this solvent therefore should not give rise to any serious migration of *O*-acetyl groups.

EXPERIMENTAL

All melting points are corrected. Evaporations were carried out under reduced pressure at a bath temperature below 40°.

Chromatography. Paper chromatography: Papers: Whatman No. 1, water eluted Schleicher und Schüll 602 hP for preparative chromatography. Solvent: Butanol-ethanol-water 10:3:5. Thin layer chromatography [13]. Absorbent: Kieselgel G nach Stahl, E. Merck AG. Solvent: Ethyl ether.

Paper electrophoresis. Papers: Whatman No. 3 MM. Buffers: 0.1 M borate buffer at pH 10, 0.05 M sulphonated phenylboronic acid [10] at pH 7.4. Spray reagents: Anisidine hydrochloride for reducing sugars, potassium periodate-cuprate [18] for non-reducing, acetylated sugars. The spots on the thin layer chromatograms were located either with iodine vapour or with potassium periodate-cuprate.

Partial acetylation of benzyl 4-O-methyl-β-D-xylopyranoside with acetic anhydride in pyridine. The xyloside (1.333 g) was dissolved in anhydrous pyridine (2 ml), acetic anhydride (0.55 ml) was added and the solution was allowed to stand at room temperature for 3 days. The solution was poured into ice-water in a separating funnel and the mixture was extracted several times with chloroform. The combined chloroform extracts were successively washed four times with ice-cold 2 N sulphuric acid, three times with ice-cold saturated aqueous sodium bicarbonate and four times with water, dried over anhydrous sodium sulphate, filtered and concentrated to a syrup which was dried over phosphorus

pentoxide in a vacuum to yield 1.389 g syrup. Thin layer chromatography gave four spots, the slowest with a mobility corresponding to that of the starting material and the fastest to benzyl 2,3-di-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside. Of the two spots of intermediate mobility the slower, monoacetate B, predominated over the faster-moving monoacetate A. The mixture was added to the top of a silicic acid column (Mallinckrodt silicic acid, A.R., 100 mesh, activated by heating overnight at 180° and kept out of contact with moist air; 3.5 × 50 cm). The column was irrigated with mixtures of dry ethyl ether and benzene.

The eluate was divided into fractions of approximately 500 ml, the dry contents of which were weighed and examined by thin layer chromatography. Similar fractions were combined, concentrated to dryness and dried over phosphorus pentoxide in a vacuum. Benzene and ethyl ether, 9:1 (6700 ml), eluted benzyl 2,3-di-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside (381 mg) which crystallised on standing. Recrystallisation from isopropyl ether gave 304 mg crystals with m.p. 79.5—80°; $[\alpha]_D^{20}$ −104° (c = 1.0 in chloroform). (Found: C 60.1; H 6.56; O 33.2. Calc. for $C_{17}H_{22}O_7$: C 60.3; H 6.56; O 33.1). Benzene and ethyl ether, 4:1 (4500 ml), eluted monoacetate A (302 mg) which did not crystallise; $[\alpha]_D^{20}$ −84° (c = 1.0 in chloroform). Benzene and ethyl ether, 2:1 (6700 ml), eluted monoacetate B (528 mg) which did not crystallise; $[\alpha]_D^{20}$ −81° (c = 1.0 in chloroform). Benzene and ethyl ether; 2:1 (1700 ml), and finally ethyl ether eluted benzyl 4-*O*-methyl-β-D xylopyranoside (115 mg) which crystallised on standing. Two recrystallisations from isopropyl ether gave 68 mg with m.p. 73—76° undepressed on admixture with authentic material.

Partial acetylation of benzyl 4-O-methyl-β-D-xylopyranoside with acetyl chloride in pyridine. The xyloside (1.297 g) was dissolved in pyridine (2 ml) and cooled to −4°. Acetyl chloride (0.40 ml) at the same temperature was added with stirring. The mixture was allowed to stand for 24 h at −4° and was worked up as described above. Yield 1.260 g. Thin layer chromatography gave four spots, the slowest corresponding to the starting material. Of the two intermediate spots, the slower-moving, monoacetate B, predominated only slightly over the faster-moving, monoacetate A. Separation on a silicic acid column as described above yielded crystalline diacetate (525 mg) with m.p. 78—80° after recrystallisation from isopropyl ether, syrupy monoacetate A (234 mg), monoacetate B (262 mg), and crystalline starting material (278 mg) with m.p. and mixed m.p. 74—76.5° after recrystallisation from isopropyl ether.

Identification of the two monoacetates. Monoacetate A (54.8 mg) was hydrogenated at a pressure slightly over the atmospheric in abs. methanol (5 ml) with palladium on carbon (from 100 mg 5 % palladium chloride on carbon, prepared according to Mozingo [12], hydrogenated in methanol and washed free from hydrochloric acid on sintered glass [9]). The uptake was 4.5—5 ml in 2 h. The catalyst and carbon were filtered off on kiselguhr and washed with methanol. The filtrate was concentrated to dryness and dried over phosphorus pentoxide and silica gel in a vacuum. Yield 38.2 mg. Paper chromatography indicated complete removal of benzyl groups but retention of virtually all the acetyl-groups. The amount of reducing sugar was estimated by hypoiodite oxidation and found to be 34.9 mg. Oxidation with lead tetraacetate in abs. acetic acid according to Perlin [11] gave a net consumption of 0.95 mole oxidant per mole aldose. The aldose (14.3 mg) was dissolved in acetic acid (10 ml) and 10 ml of oxidant (1.0 g potassium acetate and 1.0 g lead tetraacetate in 50 ml acetic acid). Samples (5 ml) were withdrawn after 10, 30, 60 and 90 min, added to 10 ml "stopping solution" (20 g potassium iodide and 100 g sodium acetate in 200 ml water). The iodine formed was titrated with thiosulphate. The oxidation was complete in about 5 min. The carbon dioxide produced during the oxidation was measured in a Warburg apparatus and represented about 2—3 mole % of the oxidant used. Under the same conditions 4-*O*-methylxylose consumed 2.2 mole lead tetraacetate. 2,3-Di-*O*-acetyl-4-*O*-methylxylose, prepared by hydrogenating benzyl 2,3-di-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside as described above, but with dry ethyl ether as solvent during the hydrogenation of the xyloside, did not consume any lead tetraacetate under these conditions.

Paper electrophoresis of the hydrogenated monoacetate A run in sulphonated phenylboronic acid at pH 7.4 for 3 h at tap water temperature and about 20 V/cm gave a rate of migration similar to that of 3,4-dimethylxylose which had M_G 0.1.

Monoacetate B (37.8 mg) was hydrogenated as described above, but with dry ethyl ether as solvent during the hydrogenation of the xyloside. The yield of material dried over phosphorus pentoxide and silica gel in a vacuum was 21.0 mg. Paper chromatography indicated complete hydrogenation of benzyl groups and little (less than 5 %) deacetylation. Previous attempts to hydrogenate in methanolic solutions led to considerable deacetylation. The monoacetyl 4-*O*-methylxylose was oxidised with lead tetraacetate as described above. The net consumption of lead tetraacetate was 0.08 mole oxidant per mole aldose. The monoacetyl 4-*O*-methylxylose did not migrate on paper electrophoresis in sulphonate phenylboronic acid at pH 7.4 as described above.

These results prove that monoacetate A is benzyl 3-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside and monoacetate B is benzyl 2-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside.

Chromatographic investigations of acetylations of benzyl 4-O-methyl-β-D-xylopyranoside. (i) Acetic anhydride-sodium acetate. The xyloside (50 mg) was dissolved in acetic anhydride (0.25 ml) and anhydrous sodium acetate (25 mg) was added. The mixture was heated at 100°. The solution was spotted on to thin layer chromatograms at time intervals of 1 min. After 1 min both monoacetates were formed, after 3 min the diacetate appeared while starting material virtually disappeared. The proportion of diacetate increased rapidly and was the major component after 10 min. Throughout, the slower-moving monoacetate, monoacetate B, predominated over monoacetate A; the ratio was visually estimated as approx. 2:1 by comparison with a standard chromatogram containing known ratios of the two monoacetates.

(ii) Acetic anhydride-pyridine containing pyridine hydrochloride. Benzyl 4-*O*-methyl-β-D-xylopyranoside (120 mg) was dissolved in pyridine (0.2 ml) containing 1.5 % by weight of dry hydrogen chloride combined as pyridine hydrochloride. Acetic anhydride (0.05 ml) was added and the mixture allowed to stand overnight. Visual examination of thin layer chromatograms indicated a ratio of about 1:1 for the two monoacetates. The reaction mixture was streaked on to a thin layer plate which was run as described above. The streaks of the four components were located by brief exposure to iodine vapour, cut from the plate and the powder extracted with ethanol. The extracts were filtered, concentrated to dryness and the residues dried in a vacuum over phosphorus pentoxide and silica gel and weighed. The proportions found for diacetate, monoacetate A, monoacetate B, and starting material were 3:2:2:1. Thin layer chromatography of the four components showed each to be free from contamination by the others.

(iii) Acetic anhydride-perchloric acid. Benzyl 4-*O*-methyl-β-D-xylopyranoside (50 mg) was dissolved in acetic anhydride (0.25 ml) containing perchloric acid (0.3 %), and the solution was allowed to stand at room temperature. The solution was spotted on thin layer chromatograms at 1 min intervals. After 1 min the starting material had disappeared, both monoacetates as well as a small amount of diacetate were present. Monoacetate A predominated throughout and the ratio of the two monoacetates was visually estimated as described above to be about 3:1 at first, increasing to about 5:1 or more after about 10 min. The formation of diacetate was relatively slow and after 40 min an appreciable amount of monoacetate A was still left in the solution.

Treatment of the two monoacetates with pyridine. Each of the two monoacetates (5 mg) was dissolved in pyridine (0.1 ml) at room temperature and allowed to stand for 3 days at room temperature. Thin layer chromatography indicated that no acetyl migration had occurred.

Methylation studies on the monoacetates. Monoacetate A (116 mg) was treated for 20 h with silver oxide (1 g) and methyl iodide (2 ml) in dimethyl formamide (5 ml) [19] and worked up as previously described [20]. Yield 125 mg syrup. Deacetylation was performed by dissolving the syrup in 5 ml abs. methanol, adding 1 ml 10 % ammoniacal methanol, allowing the solution to stand overnight and concentrating to dryness. The resulting syrup was hydrolysed by heating with 8 % aqueous sulphuric acid at 100° for 2 h, neutralised with barium carbonate, filtered and concentrated to dryness to yield 66.2 mg material. Paper chromatography indicated about 70—80 % dimethylxylose, 20—30 % 2,3,4-tri-*O*-methylxylose and traces of 4-*O*-methylxylose to be present. The dimethylxylose fraction was separated from the other components by chromatography on thick paper, the yield being 31.5 mg. Paper electrophoresis in borate buffer indicated the presence of 2,4- and 3,4-di-*O*-methylxylose, the former predominating noticeably. Lead tetraacetate oxidation as described above gave a consumption of 0.37 mole oxidant per mole aldose, the quantity of aldose in the sample being previously determined by hypoiodite oxidation.

Monoacetate B (107 mg) was methylated, deacetylated and hydrolysed as described above to yield 61 mg syrup. Paper chromatography indicated a composition of methyl ethers very similar to that derived from monoacetate A, and the dimethylxylose fraction was obtained free from the other components by chromatography on thick paper; yield 36.6 mg. Lead tetraacetate oxidation of a sample gave a consumption of 0.33 mole oxidant per mole aldose.

The efficiency of the methylation treatments was checked by methylating benzyl 4-*O*-methyl-β-D-xyloside as described above. After hydrogenation with palladium on carbon in methanol solution as described above only traces of dimethylxylose were observed on paper chromatography, the practically pure 2,3,4-trimethyl-D-xylose crystallised, m.p. 88—91°, undepressed on admixture with an authentic specimen.

Treatment of the monoacetates with silver oxide in dimethyl formamide. Monoacetate A, homogeneous on thin layer chromatograms (10 mg) was dissolved in anhydrous dimethyl formamide (1 ml). Silver oxide (0.2 g) was added and the mixture shaken in a sealed ampoule for 18 h at room temperature. The silver oxide was removed by filtration on Kiselguhr, and washed with dimethyl formamide. The filtrate was poured into excess chloroform, and the chloroform solution was extracted ten times with water to remove the dimethylformamide. The chloroform layer was finally dried over sodium sulphate, filtered and concentrated to dryness. Thin layer chromatography revealed a slight degree of migration but no deacetylation. No spot corresponding to dimethyl formamide was observed.

Monoacetate B was treated in the same way. Again acetyl migration occurred to a slight extent.

On repeating this treatment of the two monoacetates for 72 h rather more migration was observed on thin layer chromatography (of the order of 10 %) and deacetylation also occurred. Monoacetate B (5 mg) was treated with dimethyl formamide (1 ml containing 1 % water) and silver oxide (0.2 g) for 20 h. Thin layer chromatography indicated that the rate of deacetylation had increased to some extent, but that the rate of acetyl migration was about the same as before.

Treatment of the monoacetates with hot dimethyl formamide. Each of the monoacetates as well as benzyl 2,3-di-*O*-acetyl-β-D-xylopyranoside (*ca.* 5 mg) were treated with dimethyl formamide (0.5 ml) in sealed ampoules at 100° for 3 h. The solutions were poured into excess chloroform, the chloroform was extracted ten times with water, dried over sodium sulphate, filtered and concentrated. Thin layer chromatography did not indicate any migration of the acetyl groups. No deacetylation was observed.

Carbanilation, hydrolysis, methylation, reduction, and hydrolysis of the two monoacetates. Monoacetate A (91 mg) in anhydrous dimethyl formamide (0.5 ml) was treated with phenylisocyanate (0.055 ml) in a sealed ampoule at 100° for 3 h [7]. The solution was concentrated to a syrup. Thin layer chromatography indicated complete reaction, only one spot with faster mobility than that of monoacetate A being observed. The syrup was dissolved in 2 % methanolic sulphuric acid (10 ml) and the solution boiled under reflux overnight to remove the acetyl groups. The solution was neutralised with 10 % methanolic ammonia [7], the salts were removed by filtration and the solution concentrated to a syrup which was dried over phosphorus pentoxide and silica gel in a vacuum. The syrup was methylated twice with methyl iodide (3 ml) and silver oxide (2 g) in dimethyl formamide (5 ml) as previously described [19,20]. The product was treated with an excess of lithium aluminium hydride in boiling dry tetrahydrofuran for 3 h. to remove the carbamoyl groups [7]. The excess lithium aluminium hydride was destroyed with ethanol and the alcoholates decomposed with water. After neutralisation with phosphoric acid [7] and filtration the solution was concentrated to dryness. The resulting syrup was hydrolysed with 0.5 N sulphuric acid (5 ml) at 100° overnight, neutralised with saturated aqueous barium hydroxide, filtered and concentrated to a syrup (17 mg). Paper chromatography and paper electrophoresis in borate indicated the presence of 3,4-di-*O*-methylxylose (M_G 0.33) with only traces of 4-*O*-methylxylose (M_G 0.19) and 2,4-di-*O*-methylxylose. (M_G 0.00). Monoacetate B (64 mg) was subjected to the same sequence of reactions as described above to yield 19 mg of a syrup. Paper chromatography and paper electrophoresis in borate indicated that 2,4-di-*O*-methylxylose was present along with only traces of 4-*O*-methylxylose and 3,4-di-*O*-methylxylose.

Treatment of the monoacetates with refluxing 3 % monoethanolamine in ethanol. Each of the two monoacetates (5 mg) was dissolved in 3 % monoethanolamine in 95.5 %

ethanol (0.5 ml), the solutions were sealed in ampoules and heated at 80° for 30 min. The solutions were poured into excess chloroform and the chloroform solutions extracted ten times with water, dried over sodium sulphate, filtered and concentrated. Thin layer chromatography indicated that acetyl migration reached a state of equilibrium. There was no difference between the mixtures obtained from the two monoacetates. Monoacetate A predominated over monoacetate B; the ratio was visually estimated to about 2:1. Considerable deacetylation (about 20–30 %) had occurred.

Treatment of the monoacetates with dimethyl sulphoxide at room temperature. Each of the monoacetates (*ca.* 5 mg) was dissolved in dimethyl sulphoxide (0.1 ml) and allowed to stand for 20 h in a sealed ampoule. The solutions were added to excess chloroform, the chloroform solutions were extracted ten times with water to remove the dimethyl sulphoxide. No spot corresponding to dimethylsulphoxide was observed on subsequent thin layer chromatograms. The chloroform solutions were dried over sodium sulphate, filtered and concentrated. Thin layer chromatograms indicated that no deacetylation of either of the two monoacetates had occurred. The acetyl groups in monoacetate A had not migrated; a slight migration of the acetyl groups had occurred in monoacetate B.

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