ACTA CHEMICA SCANDINAVICA 24 (1970) 919–923

Synthesis of *O*-Acetyl Derivatives of Methyl 3,6-Dideoxy-α- and β-D-*xylo*-hexopyranosides (Abequosides)

HÅKAN F. G. BEVING, HANS B. BORÉN and PER J. GAREGG

Institute of Organic Chemistry, University of Stockholm, S-104 05 Stockholm 50, Sweden

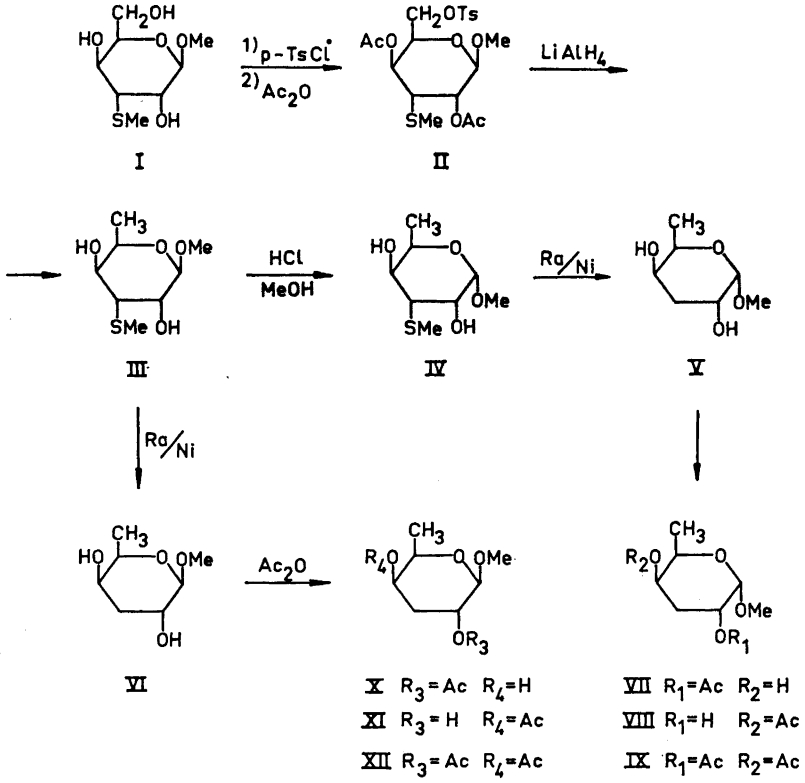
The methyl α- and β-pyranosides of 3,6-dideoxy-D-*xylo*-hexose (abequose) have been prepared. Partial acetylation of these glycosides yielded mixtures of mono- and di-*O*-acetyl derivatives, which have been separated and characterized.

Chemical and immunochemical studies on *Salmonella* group B lipopolysaccharides [1] have indicated that the repeating unit in their O-specific side chains contains a terminal 3,6-dideoxy-D-*xylo*-hexose (abequose) residue, α-pyranosidically linked to the 3-position in a mannose residue. This grouping is associated with the presence of O-factor 4. These results have been confirmed in recent studies,[2,3] in which it was also demonstrated that the *O*-acetyl groups, present in the O-specific side chains in some of these lipopolysaccharides, are linked to the 2-position of the abequose residues. These *O*-acetyl groups are associated with the presence of O-factor 5. Partially acetylated abequosides, and especially a 2-*O*-acetyl-α-abequoside, should therefore be of interest for immunochemical studies. The present communication reports the synthesis of such substances.

Abequose and some abequosides have previously been prepared by different routes.[4,5,9] We preferred to proceed *via* methyl 3,6-dideoxy-3-methylthio-β-D-gulopyranoside (III) with the hope that this might be a useful intermediate also in other synthetic work in this area.

Partial tosylation of methyl 3-deoxy-3-methylthio-β-D-gulo-pyranoside [6] (I), followed by acetylation, yielded methyl 2,4-di-*O*-acetyl-3-deoxy-3-methylthio-6-*O*-(*p*-tolylsulphonyl)-β-D-gulopyranoside (II). Reduction of II with lithium aluminium hydride yielded III, $[\alpha]_D$ −57° (*c* 0.47, chloroform). The corresponding α-anomer (IV) was obtained by treatment of (III) with methanolic hydrogen chloride followed by chromatography on silica gel. The methyl α- and β-abequosides (V and VI) were prepared from (IV) and (III) by Raney nickel desulphuration. All the glycosides but one, X, were amorphous but chromatographically homogeneous and their NMR spectra were consistent with the postulated structures. V was further characterized by transforma-

tion into crystalline tetra-*O*-acetyl-abequitol, indistinguishable from an authentic sample. The pyranosidic nature of VI was confirmed by transforming its fully methylated product into 1,5-di-*O*-acetyl-2,4-di-*O*-methyl-abequitol, indistinguishable from an authentic sample by GLC and by mass spectrometry.[2]



Partial acetylations of V and VI, followed by chromatographic separations afforded the mono- and di-*O*-acetyl derivatives (VII–XII). In both the acetylations the 2-*O*-acetyl derivative was formed preferentially to the 4-*O*-acetyl derivative. The position of the *O*-acetyl group in each of the four monoacetates was clearly revealed by NMR.

As is generally found,[7] the axial acetoxy protons (4-*O*-acetyl derivatives) were more strongly deshielded than were the equatorial acetoxy protons (2-*O*-acetyl derivatives). Furthermore, whereas the signal from the anomeric proton in each of the two unacetylated glycosides appeared at lower fields than the other signals, those given by the proton attached to carbon carrying acetoxy groups in the acetates were the more strongly deshielded. The relative intensities of the two peaks in the doublet given by the anomeric proton therefore revealed whether coupling occurred to a proton giving a signal at higher field (4-acetate) or to one giving a signal at lower field (2-acetate).

EXPERIMENTAL

General methods. Evaporations were carried out under reduced pressure at a bath temperature below 50°. Melting points are corrected. Thin layer chromatography was performed on silica gel GF_{254} (Merck). Sulphuric acid was used as spray reagent. Gas liquid chromatography was performed with a Perkin-Elmer Model F 11 gas chromatograph equipped with double glass columns and flame ionization detectors. The column material consisted of 3 % ECNSS-M on Gas-Chrom Q, 100/120 mesh (supplied by Applied Science Laboratories U.S.A.). An LKB 9000 combined gas chromatograph-mass spectrometer was used. IR spectra were recorded with a Perkin-Elmer Model 137 Grating Spectrophotometer. NMR spectra were obtained from a Varian A-60 A spectrometer. Tetramethylsilane was used as an internal reference and deuterochloroform as solvent throughout. Chemical shifts are given in ppm downfield from tetramethylsilane. Optical rotations were measured at room temperature (20—22°) with a Perkin-Elmer Model 141 photoelectric polarimeter.

*Methyl 2,4-di-*O*-acetyl-3-deoxy-3-methylthio-6-*O*-(*p*-tolylsulphonyl)-β-*D*-gulopyranoside (II).* To a solution of methyl 3-deoxy-3-methylthio-β-D-gulopyranoside [6] (I) (10.5 g) in pyridine (200 ml), kept at −20°, small amounts of *p*-toluenesulphonyl chloride were added. After each addition the reaction mixture was allowed to attain room temperature. The reaction was followed by TLC (water-saturated butanone). After optimal reaction, acetic anhydride (18 ml) was added at −20° and the reaction mixture was kept at room temperature over-night. The mixture was poured into ice-water and extracted with chloroform. The combined chloroform extracts were washed several times with 0.25 M aqueous sulphuric acid, saturated sodium hydrogen carbonate and water, dried over magnesium sulphate, filtered and concentrated. The resulting crude material (17.8 g) was purified by chromatography on a silica gel column using ethyl ether-benzene (2:5) as eluent. The fractionation was followed by TLC in the same solvent. Chromatographically pure methyl 2,4-di-*O*-acetyl-3-deoxy-3-methylthio-6-*O*-(*p*-tolylsulphonyl)-β-D-gulopyranoside (II) was obtained as a syrup, 9.9 g (46 %), $[\alpha]_D$ −42° (*c* 0.27, chloroform). (Found: C 49.4; H 5.99; O 30.9; S 13.7. Calc. for $C_{19}H_{26}O_9S_2$: C 49.3; H 5.67; O 31.1; S 13.9).

NMR, δ 7.78 (2-proton doublet, *J* 8 Hz, aromatic), δ 7.33 (2-proton doublet, *J* 8 Hz, aromatic), δ 4.8—5.1 (2-proton multiplet, H-2 and H-4), δ 4.53 (1-proton doublet, low-field peak dominating, *J* 8 Hz, H-1), δ 4.29—4.45 (1-proton multiplet, H-3 or H-5), δ 4.0—4.2 (2-proton multiplet, H-6, H′-6), δ 3.42 (3-proton singlet, methoxyl), δ 3.2—3.5 (1-proton multiplet, H-3 or H-5), δ 2.41 (3-proton singlet, *S*-methyl), δ 2.17, 2.08, and 2.00 (each 3-proton singlets, two *O*-acetyl, one methyl attached to aromatic nucleus).

*Methyl 3,6-dideoxy-3-methylthio-β-*D*-gulopyranoside (III).* II (7.5 g) and lithium aluminium hydride (6 g) in tetrahydrofurane (450 ml) was refluxed for 1 h. Excess of hydride was destroyed by adding ethyl acetate and then water. The reaction mixture was neutralized with phosphoric acid (85 %), filtered and concentrated to an amorphous mass, III, 3 g (87 %), $[\alpha]_D$ −57° (*c* 0.47, chloroform), which was chromatographically pure (TLC/water-saturated butanone). (Found: C 46.0; H 7.67; O 30.9; S 15.5. Calc. for $C_8H_{16}O_4S$: C 46.1; H 7.74; O 30.7; S 15.4).

*Methyl 3,6-dideoxy-3-methylthio-α-*D*-gulopyranoside (IV).* A solution of III (2 g) in 0.05 M methanolic hydrogen chloride (250 ml) was refluxed for 20 h. The reaction was followed polarimetrically and was interrupted when the optical rotation had reached a constant value. The reaction mixture was cooled and poured into water, neutralized with Dowex 3 (free base) and concentrated. The product was purified by chromatography on a silica gel column using ethyl acetate as eluent. The fractionation was followed by TLC using the same solvent. Pure methyl 3,6-dideoxy-3-methylthio-α-D-gulopyranoside (IV) was obtained as a syrup (370 mg).

Samples of III and IV were acetylated and shown to be pure by GLC.

*Methyl 3,6-dideoxy-α-*D*-xylo-hexopyranoside (Methyl α-abequoside) (V).* A mixture of IV (370 mg) and Raney nickel (10 g) in 70 % aqueous ethanol (50 ml) was refluxed over-night. The reaction mixture was filtered. Concentration yielded methyl 3,6-dideoxy-α-D-*xylo*-hexopyranoside (V) as a syrup, 259 mg (90 %), $[\alpha]_D$ +108°, $[\alpha]_{5461}$ +128° (*c* 0.5, chloroform); $[\alpha]_D$ +89°, $[\alpha]_{5461}$ +100° (*c* 0.8, methanol); Stirm *et al.*[4] $[\alpha]_{5461}^{25}$ +102°

(*c* 0.8, methanol); Capek *et al.*[5] $[\alpha]_{5461}^{20}$ +180° (*c* 0.34, methanol); Siewert and Westphal [9] $[\alpha]_D^{25}$ +148° (*c* 1.04, methanol).

NMR, δ 4.69 (1-proton doublet, high-field peak dominating, J 3.5 Hz, H-1), δ 3.6—4.2 (3-proton multiplets, H-2, H-4, H-5), δ 3.45 (3-proton singlet, methoxyl), δ 1.7—2.3 (two 1-proton multiplets and broad 2-proton singlet at δ 1.95, H-3, H'-3, and hydroxyls), δ 1.22 (3-proton doublet, low-field peak dominating, J 6.5 Hz, H-6 protons).

*Methyl 3,6-dideoxy-β-*D-xylo-*hexopyranoside (Methyl β-abequoside) (VI).* III (400 mg) was treated with Raney nickel (12 g) as above. The reaction mixture was filtered. Concentration yielded methyl 3,6-dideoxy-β-D-*xylo*-hexopyranoside (VI) as a syrup, 298 mg (95 %), $[\alpha]_D$ −60°, $[\alpha]_{5461}$ −69° (*c* 0.5, chloroform); $[\alpha]_D$ −69°, $[\alpha]_{5461}$ −81° (*c* 0.4, methanol); Stirm *et al.*[4] $[\alpha]_{5461}^{25}$ −90° (*c* 0.4, methanol).

NMR, δ 4.13 (1-proton doublet, high-field peak dominating, J 8 Hz, H-1), δ 3.4—4.0 (3-proton multiplets, H-2, H-4, H-5), δ 3.52 (3-proton singlet, methoxyl), δ 2.90 (2-proton broad singlet, hydroxyls), δ 2.03—2.48 and δ 1.52—1.80 (each 1-proton multiplets, H-3, H'-3), δ 1.27 (3-proton doublet, low-field peak dominating, J 6.5 Hz, H-6 protons).

A small sample was hydrolyzed with 0.25 M aqueous sulphuric acid (25 min, 100°), reduced with sodium borohydride and acetylated. The crystalline material, m.p. 67.5—68.5°, was indistinguishable from the abequitol acetate prepared from authentic abequose (mixed m.p., IR, GLC). (Found: C 52.8; H 6.89; O 40.4. Calc. for $C_{14}H_{22}O_8$: C 52.8; H 6.97; O 40.2).

Samples of V and VI were methylated by treatment with methylsulphinyl carbanion and methyl iodide in methyl sulphoxide,[8] hydrolyzed with 0.25 M aqueous sulphuric acid, reduced with sodium borohydride and acetylated. The obtained 1,5-di-*O*-acetyl-2,4-di-*O*-methyl-abequitol was indistinguishable from the substance obtained by Hellerqvist *et al.*[3] by GLC-mass spectrometry.

*Acetylation of methyl 3,6-dideoxy-α-*D-xylo-*hexopyranoside (VII—IX).* To a solution of V (250 mg) in pyridine (20 ml), kept at room temperature, portions of acetic anhydride in chloroform were added. The reaction was followed by TLC (ethyl acetate). When the formation of mono-acetates was optimal, water was added and the reaction mixture concentrated to dryness. Fractionation by chromatography on a silica gel column using ethyl acetate-toluene (3:1) yielded methyl 2,4-di-*O*-acetyl-3,6-dideoxy-α-D-*xylo*-hexopyranoside (IX), 78 mg, $[\alpha]_D$ +81°, $[\alpha]_{5461}$ +95° (*c* 0.5, chloroform), Stirm *et al.*[4] $[\alpha]_{5461}^{25}$ +98° (*c* 0.5, chloroform). (Found: C 53.8; H 7.37. Calc. for $C_{11}H_{18}O_6$: C 53.6; H 7.37). Methyl 2-*O*-acetyl-3,6-dideoxy-α-D-*xylo*-hexopyranoside (VII), 46 mg, $[\alpha]_D$ +142°, $[\alpha]_{5461}$ +167° (*c* 0.5, chloroform) and methyl 4-*O*-acetyl-3,6-dideoxy-α-D-*xylo*-hexopyranoside (VIII), 27 mg, $[\alpha]_D$ +87°, $[\alpha]_{5461}$ +103° (*c* 0.5, chloroform) (contains 10 % of methyl 2-*O*-acetyl-3,6-dideoxy-α-D-*xylo*-hexopyranoside). The substances were not distilled and might therefore contain traces of solvent.

NMR, VII, δ 4.96—5.37 (1-proton multiplet, H-2), δ 4.80 (1-proton doublet, low-field peak dominating, J 3.5 Hz, H-1), δ 3.7—4.2 (2-proton multiplets, H-4, H-5), δ 3.40 (3-proton singlet, methoxyl), δ 1.9—2.3 (3-proton multiplets containing broad singlet, H-3, H'-3, and hydroxyl), δ 2.05 (3-proton singlet, *O*-acetyl), δ 1.21 (3-proton doublet, low-field signal dominating, J 6.5 Hz, H-6 protons). NMR, VIII, similar to that of VII, δ 4.69 (1-proton doublet, each peak of similar intensity, J 3.5 Hz, H-1), δ 3.44 (3-proton singlet, methoxyl), δ 2.09 (3-proton singlet, *O*-acetyl), δ 1.30 (3-proton doublet, low-field peak dominating, J 6.5 Hz, H-6 protons). About 10 % of VII was present as shown by the presence of weak signals corresponding to H-1, methoxyl, *O*-acetyl and H-6 protons for VII at fields described above. NMR, IX, δ 4.88—5.24 (2-proton multiplets, H-2, H-4), δ 4.71 (1-proton doublet, low-field peak dominating, J 3.5 Hz, H-1), δ 4.0 (1-proton octet, $J_{4,5}$ 2 Hz, $J_{5,6}$ 6.5 Hz, H-5), δ 3.41 (3-proton singlet, methoxyl), δ 1.9—2.3 (2-proton multiplets, H-3, H'-3), δ 2.12 and δ 2.05 (3-proton singlets each, *O*-acetyl), δ 1.10 (3-proton doublet, low-field peak dominating, J 6.5 Hz, H-6).

*Acetylation of methyl 3,6-dideoxy-β-*D-xylo-*hexopyranoside (X—XII).* VI (365 mg) was partially acetylated and the reaction mixture fractionated as described above for the anomeric α-glycoside yielding methyl 2,4-di-*O*-acetyl-3,6-dideoxy-β-D-*xylo*-hexopyranoside (XII), 53 mg, $[\alpha]_D$ −71°, $[\alpha]_{5461}$ −84° (*c* 0.5, chloroform), methyl 2-*O*-acetyl-3,6-dideoxy-β-D-*xylo*-hexopyranoside (X), 177 mg, m.p. 122—123°, $[\alpha]_D$ −65°, $[\alpha]_{5461}$ −76° (*c* 0.5, chloroform) (Found: C 53.1; H 7.71; O 39.2. Calc. for $C_9H_{16}O_5$: C 52.9; H 7.90; O 39.2), and methyl 4-*O*-acetyl-3,6-dideoxy-β-D-*xylo*-hexopyranoside (XI), 11 mg, $[\alpha]_D$ −72°, $[\alpha]_{5461}$ −84° (*c* 0.5, chloroform) (contains 5 % of methyl 2-*O*-acetyl-3,6-

dideoxy-β-D-*xylo*-hexopyranoside). The amorphous substances were not distilled and might therefore contain traces of solvent. NMR, X, δ 4.96 (1-proton octet, $J_{1,2}$ 8 Hz, $J_{2,3}$ 5 Hz and 11 Hz, H-2), δ 4.32 (1-proton doublet, low-field peak dominating, J 8 Hz, H-1), δ 3.6—3.9 (2-proton multiplets, H-4, H-5), δ 3.49 (3-proton singlet, methoxyl), δ 2.25—2.59 (1-proton multiplet, H-3), δ 2.22 (1-proton broad singlet, hydroxyl), δ 2.06 (3-proton singlet, *O*-acetyl), δ 1.53—1.87 (1-proton multiplet, H′-3), δ 1.29 (3-proton doublet, low-field peak dominating, J 6.5 Hz, H-6 protons). NMR, XI, similar to that of X, δ 4.14 (1-proton doublet, high-field peak dominating, J 8 Hz, H-1), δ 3.54 (3-proton singlet, methoxyl), δ 2.08 (3-proton singlet, *O*-acetyl), δ 1.30 (3-proton doublet, low-field peak dominating, J 6.5 Hz, H-6 protons). About 5 % of X was present as shown by the presence of weak signals corresponding to methoxyl and *O*-acetyl at fields described above. NMR, XII, δ 4.7—5.2 (2-proton multiplets, H-2, H-4), δ 4.38 (1-proton doublet, low-field peak dominating), H-1, δ 4.80 (1-proton octet, $J_{4,5}$ 2 Hz, $J_{5,6}$ 6.5 Hz, H-5), δ 3.51 (3-proton singlet, methoxyl), δ 2.20—2.56 (1-proton multiplet, H-3), δ 2.14 (3-proton singlet, *O*-acetyl), δ 2.04 (3-proton singlet, *O*-acetyl), δ 1.46—1.92 (1-proton multiplet, H′-3), δ 1.21 (3-proton doublet, low-field peak dominating, J 6.5 Hz, H-6 protons).

Acknowledgement. The authors are indebted to Prof. Bengt Lindberg for his interest and helpful advice and to *Statens Naturvetenskapliga Forskningsråd* for financial support.

REFERENCES

1. Lüderitz, O., Staub, A. M. and Westphal, O. *Bacteriol. Rev.* **30** (1966) 192.
2. Hellerqvist, C. G., Lindberg, B., Svensson, S., Holme, T. and Lindberg, A. A. *Carbohyd. Res.* **8** (1968) 43.
3. Hellerqvist, C. G., Lindberg, B., Svensson, S., Holme, T. and Lindberg, A.A. *Carbohyd. Res.* **9** (1969) 237.
4. Stirm, S., Lüderitz, O. and Westphal, O. *Ann.* **696** (1966) 180.
5. Čapek, K., Němec, J. and Jarý, J. *Collection Czech. Chem. Commun.* **33** (1968) 1758.
6. Dahlgard, M., Chastin, B. H. and Lee Han, Ru-Jen. *J. Org. Chem.* **27** (1962) 932.
7. Hall, L. D. *Advan. Carbohydrate Chem.* **19** (1964) 51.
8. Hakomori, S. *J. Biochem. (Tokyo)* **55** (1964) 205.
9. Siewert, G. and Westphal, O. *Ann.* **720** (1968) 171.

Received September 16, 1969.