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## Note

# Methylation analysis of non-glycosidic phosphoric diesters of carbohydrates*

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Methylation of synthetic[1] α,β-D-glucose 2-[α,β-D-glucose 6-(sodium phosphate)] (**1**), α,β-D-glucose 3-[α,β-D-glucose 6-(sodium phosphate)] (**2**), and α,β-D-glucose 6-[α,β-D-glucose 6-(sodium phosphate)] (**3**) according to Hakomori[2] was not satisfactory. After borodeuteride-reduction of **1–3** (yielding **4, 6**, and **8**, respectively) followed by methylation[2], the formation of **5, 7**, and **9**, respectively, was expected. However, g.l.c.–m.s. revealed that large proportions of each of the latter phosphoric triesters had been cleaved, mainly into the corresponding glucose dimethyl phosphates (molar ratios of di- to mono-glucose triesters, 1:2–1:10) together with smaller amounts of other products, including anhydro forms (data not shown).

Esterification of **4, 6**, and **8** with diazomethane in ether followed by methylation[4] gave the phosphoric triesters **5, 7**, and **9**, which were identified by g.l.c. and g.l.c.–m.s. (Table I). Only small amounts of mono-glucose phosphoric triesters were formed. Cleavage of **5** with $LiAlH_4$ in ether[5] followed by acetylation gave 2-*O*-acetylpenta-*O*-methylhexitol-*1-d* (**10**) and 6-*O*-acetylpenta-*O*-methylhexitol-*1-d* (**11**). Likewise, **7** gave 3-*O*-acetylpenta-*O*-methylhexitol-*1-d* (**12**) and **11**, and **9** gave **11**. Compounds **10–12** were identified by g.l.c.–m.s. The ratio of **10** and **12** to **11** was ~1:1. Using splitless injection, 150 μg were sufficient for the derivatisation procedure described.

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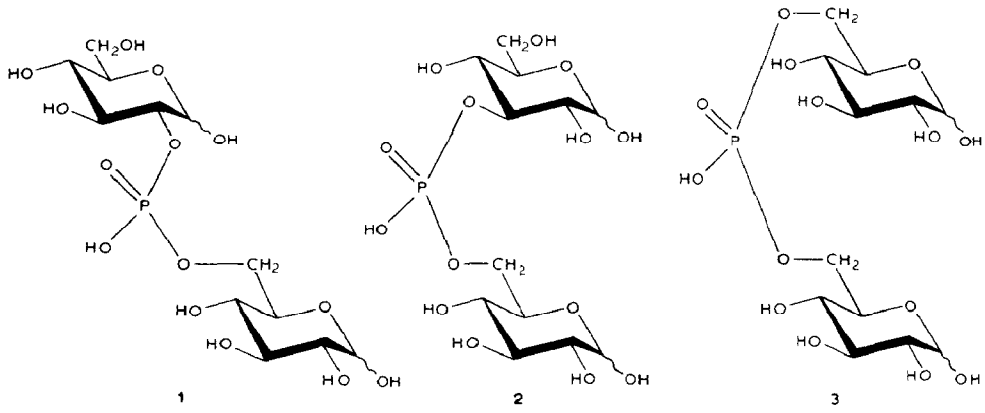
TABLE I

G.L.C. AND E.I.-M.S. DATA FOR **5**, **7**, AND **9**

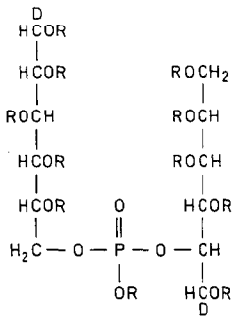
| *Compound* | *Mol. wt.*[a] | *Retention time*[b] | m/z *(% of base peak)* |
|---|---|---|---|
| **5** | 582 | 3.66 | 45 (22.2), 46 (12.2), 60 (11.1), 71 (24.4), 72 (18.9), 75 (20.0), 88 (20.0), 89 (24.4), 90 (22.2), 101 (100), 102 (32.2), 116 (20.0), 127 (8.9), 146 (28.9), 172 (26.7), 236 (48.9) |
| **7** | 582 | 3.65 | 45 (11.1), 46 (8.9), 60 (10.0), 71 (13.3), 72 (7.8), 75 (15.6), 88 (10.0), 89 (13.3), 90 (17.8), 101 (100), 102 (14.4), 116 (26.7), 146 (14.4), 172 (30.0), 236 (27.8) |
| **9** | 582 | 4.22 | 45 (17.8), 46 (13.3), 60 (13.3), 71 (40.0), 72 (16.7), 75 (24.4), 88 (28.9), 89 (15.6), 90 (27.8), 101 (100), 102 (25.6), 113 (20.0), 116 (15.5), 127 (10.0), 146 (17.8), 172 (12.2), 236 (12.2) |

[a]Determined by c.i. (ammonia)-m.s. on the basis of peaks at $m/z$ for $(M + 1)^+$ and $(M + 18)^+$. [b]Relative to that of α-D-glucose penta-acetate; see Experimental.

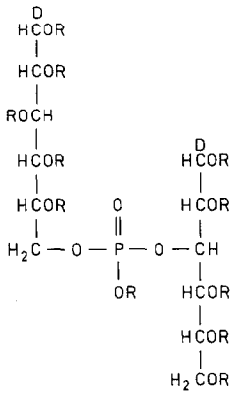



EXPERIMENTAL

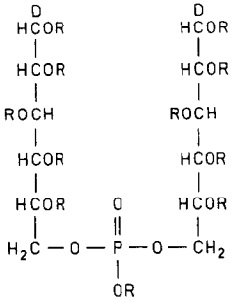
Compounds **1–3** were synthesised as described[1] and conventional reduction with $NaB^2H_4$ afforded **4**, **6**, and **8**, respectively, separate solutions (1–2 mg in 0.1 mL) of which in methanol were treated with ethereal diazomethane (1 mL) for 30 min at room temperature. After concentration, each residue was dried over $P_2O_5$ and dissolved in dry $Me_2SO$ (1 mL), and finely powdered dry NaOH (~25 mg) and MeI (1 mL) were added[4]. Each mixture was kept for 15 min at room temperature,



4 R = H
5 R = Me



6 R = H
7 R = Me



8 R = H
9 R = Me

then diluted with water (2 mL) and extracted twice with $CH_2Cl_2$. The combined extracts were concentrated, and a solution of the residue in $Me_2SO$ (0.2 mL) was diluted with water (0.2 mL) and purified on a $C_{18}$ cartridge[3] (SEP-PAK) by washing in sequence with water and acetonitrile–water (1:10) followed by elution with acetonitrile–water (1:5). A solution of each methylated phosphoric diester in ether (0.5 mL) was treated (30 min, room temperature)[5] with $LiAlH_4$ (~5 mg). After evaporation of the solvents, the residue was kept under nitrogen and water was added. The resulting salts were removed by centrifugation, the supernatant solution was concentrated, and the residue was acetylated conventionally (acetic anhydride–pyridine).

G.l.c. was performed on a Varian 3700 gas chromatograph equipped with a flame-ionisation detector and a fused-silica capillary column (25 m × 0.32 mm i.d.) with chemically bonded SE-54 (0.2 $\mu$m) (Weeke, Mühlheim), $H_2$ as the carrier gas (1.5 bar), and temperature programmes of 160° for 3 min, 5°/min → 300° (for **5**, **7**, and **9**), and 130° (for partially methylated hexitols).

G.l.c.–m.s. was performed on a Hewlett–Packard 5985 instrument equipped with an SE-54 column and an HP-1000 data system. E.i.-mass spectra were recorded at 70 eV and c.i.-mass spectra were obtained with ammonia as reactant gas. The ion-source temperature was 200°.

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