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# CHARACTERISATION BY $^{1}H$- AND $^{13}C$-N.M.R. SPECTROSCOPY OF THE PRODUCTS FROM OXIDATION OF METHYL α- AND β-D-GALACTOPYRANOSIDE WITH PERIODIC ACID IN DIMETHYL SULPHOXIDE

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## ABSTRACT

Oxidation of methyl α- and β-D-galactopyranoside with periodic acid in dimethyl sulphoxide gave, in each case, one major, monomeric product, isolated as a diacetate. These syrupy products were characterised by m.s. and $^{1}H$- and $^{13}C$-n.m.r. spectroscopy as (1*S*,2*S*,4*R*,7*S*)-7-acetoxy-4-acetoxymethyl-2-methoxy-3,6,8-trioxabicyclo[3.2.1]octane and (1*S*,2*R*,4*R*,7*S*)-7-acetoxy-4-acetoxymethyl-2-methoxy-3,6,8-trioxabicyclo[3.2.1]octane, respectively. Chemical shifts and coupling constants for the products were determined partly from the experimental n.m.r. spectra, and partly by an iterative fitting-procedure with the computer program NEMEN.

## INTRODUCTION

Yu and Bishop[1] showed that several methyl glycopyranosides were oxidised by periodic acid in dimethyl sulphoxide, with consumption of only one mole of oxidant per mole of glycoside. The product from methyl β-L-arabinopyranoside was isolated as a crystalline monoacetate in a yield of 37%, and assigned a double, intramolecular, hemiacetal structure, (1*S*,2*S*,7*S*)-7-acetoxy-2-methoxy-3,6,8-trioxabicyclo[3.2.1]octane, on the basis of its $^{1}H$-n.m.r. spectrum. A more detailed investigation[2] confirmed this assignment.

The situation with hexopyranosides is potentially more complex than with pentopyranosides, because of the possibility of involvement of HO-6 in intramolecular hemiacetal formation. Yu and Bishop[1] showed that methyl α-D-galactopyranoside was selectively oxidised between C-3 and C-4, but even with this simplification, four different, double, intramolecular, hemiacetal structures (**1**–**4**, Scheme 1) could be formed. All of these products would yield diacetates.

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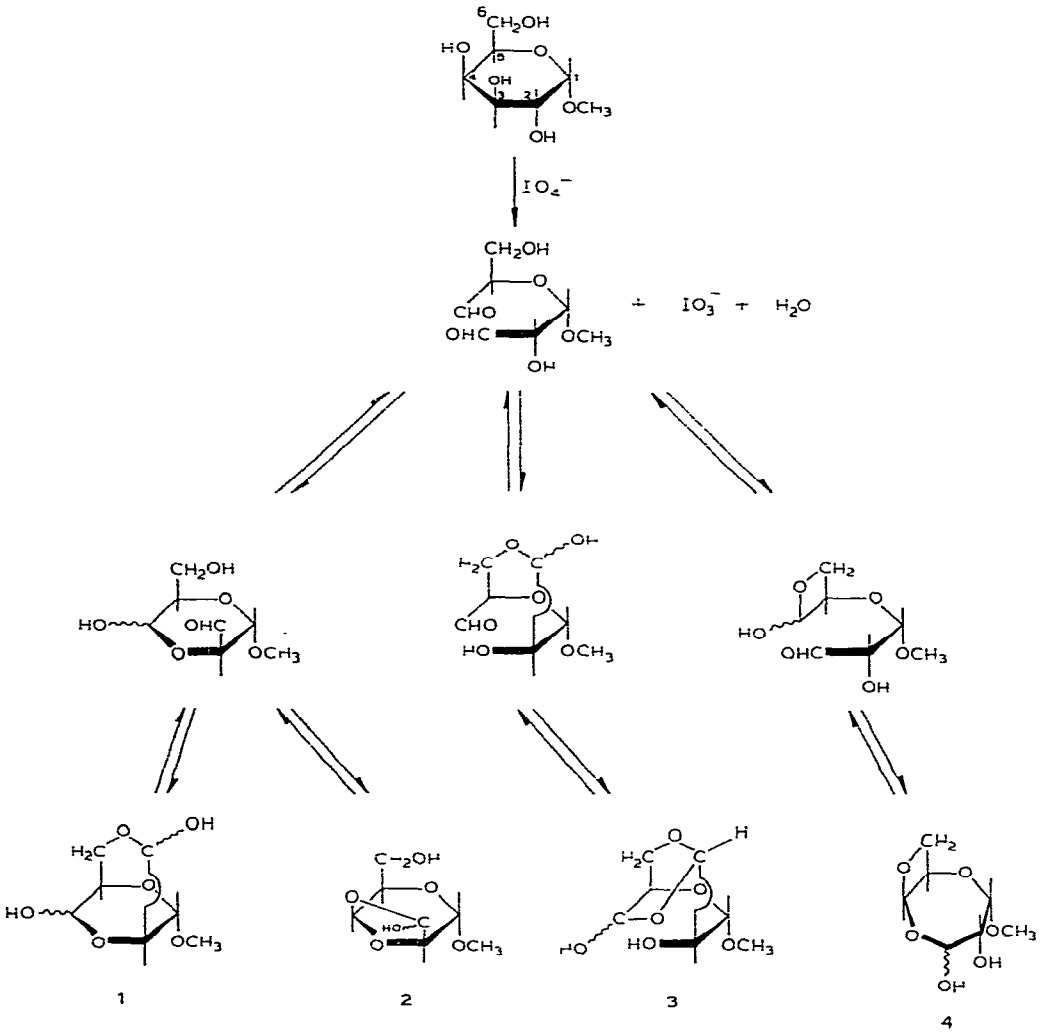

Scheme 1. Different possibilities for the formation of a double, intramolecular hemiacetal after oxidation of methyl α-D-galactopyranoside between C-3 and C-4.

The work now described confirms that methyl α-D-galactopyranoside is cleaved selectively between C-3 and C-4, shows that this is also true for the corresponding β anomer, and identifies the major oxidation product from each anomer.

## EXPERIMENTAL

N.m.r. spectra were recorded with a JEOL FX 100FT spectrometer for 5% (w/v) solutions in $CDCl_3$ (internal tetramethylsilane) and $D_2O$ [internal sodium 3-(trimethylsilyl)propionate-$d_4$] at 25°. G.l.c. was performed with 2-μl samples of solutions (1 mg/ml) in $CH_2Cl_2$, and a Hewlett–Packard 5840A gas chromatograph

operated in conjunction with a Hewlett–Packard 5985 mass spectrometer, which also served as the chromatographic detector. The ion-chamber temperature was 200°. The SE-54 column was 20 m × 0.35 mm, the carrier gas was helium (2 ml/min), and the temperature program was 60 (2 min)→250° at 15°/min. For electron-impact (e.i.) mass spectrometry (m.s.), the energy was 70 eV, and for chemical-ionisation (c.i.) m.s., it was 200 eV, with methane at $1.8 \times 10^{-5}$ mmHg as the ionising gas. E.i.–m.s. was also performed on an AEI MS902 instrument, with an ion-chamber temperature of 100°. T.l.c. was performed on Merck DC-Alufolien plates (silica gel 60; 0.2-mm layer) with benzene–acetone (9 : 1), and detection with an ammonium sulphate dipping-reagent[3].

Following the procedure of Yu and Bishop[1], samples (7.5 mmol) of galactoside were oxidised with periodic acid (9.0 mmol) in $Me_2SO$ (10 ml) for 1.5 h at 5°, and the products were acetylated and isolated as yellow syrups. The components were separated by preparative t.l.c., 10 mg of syrup in chloroform (0.15 ml) being applied to each plate (20 × 20 cm). The bands were located with dipping reagent[3] on strips (1 cm) cut from each side and the centre of the plate. The silica gel was scraped from the appropriate areas, and extracted with hot ethanol (3 × 15 ml). After filtration and evaporation of solvent, the yield of the major component from each plate was ~5 mg.

## RESULTS AND DISCUSSION

On oxidation with periodic acid in dimethyl sulphoxide, followed by acetylation, methyl α- and β-D-galactopyranoside each gave one major and two minor products. The latter gave very complex $^1$H-n.m.r. spectra, and were inferred to arise by dimerisation or polymerisation of the major component. They were not further investigated. The major components were non-crystalline, but were homogeneous by g.l.c. They were also clearly separable by g.l.c., showing retention times of 12.4 and 12.2 min, respectively, for the products from the α and β anomers.

The mass spectra of the two products were essentially identical, except for small differences in the relative intensities of the different peaks. The molecular ions (*m/z* 276) were not obtained by either e.i.– or c.i.–m.s., but e.i. gave peaks due to the loss of $CH_3O\cdot$ at *m/z* 245 (~1%), $CH_3CO\cdot$ at 233 (~7%), $CH_3CO_2\cdot$ at 217 (~5.5%), $2CH_3CO_2\cdot$ at 158 (~1.4%), $CH_3CO_2\cdot$ + $CH_3CO_2H$ at 157 (~12.6%), and $2CH_3CO_2H$ at 156 (~1.3%), as well as a fragment at *m/z* 43 (100%) characteristic of acetate groups. The spectra therefore accorded well with the formulation of monomeric, di-*O*-acetylated, methyl acetals.

As judged by $^1$H- and $^{13}$C-n.m.r. spectroscopy, the major component from the α anomer was identical with one of the two major components[4] isolated in the same way after oxidation of methyl α-D-glucopyranoside. Similarly, the major component from the β anomer was identical with one of the two major components[4] isolated after oxidation of methyl β-D-glucopyranoside. This proved that the products from the galactosides had originated from selective cleavage between C-3 and C-4, because the

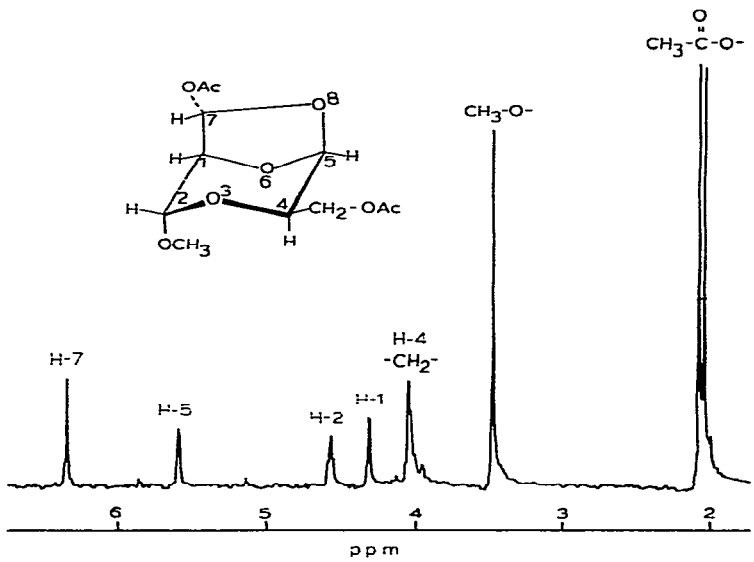


Fig. 1. $^1$H-N.m.r. spectrum (100 MHz, $CDCl_3$) of the diacetate 5 derived from methyl α-D-galactopyranoside.

products obtained by cleavage between C-2 and C-3 could not be obtained from the glucosides.

*(1S,2S,4R,7S)-7-Acetoxy-4-acetoxymethyl-2-methoxy-3,6,8-trioxabicyclo[3.2.1]-octane* (**5**). — The diacetate derived from methyl α-D-galactopyranoside was assigned structure **5** on the basis of the following data. The $^1$H-n.m.r. spectrum (Fig. 1) shows two signals at 2.07 and 2.11 p.p.m., each of intensity three, due to the methyl protons in the two acetoxyl groups. The signal at 3.49 p.p.m. of intensity three comes from the methoxyl group. The total intensity of the other signals amounts to seven, and is due to the ring and methylene protons.

The $^1$H-n.m.r. spectrum of the crude mixture of oxidation products, before acetylation and separation, is shown in Fig. 2, and there is no difficulty in identifying corresponding signals in Figs. 1 and 2. The most striking effects of acetylation are as follows: (*i*) to move the singlet at 5.58 p.p.m. in Fig. 2 downfield to 6.36 p.p.m. in Fig. 1; this singlet must be due to H-7, because it is the only signal of unit intensity that moves downfield; moreover, it shows no coupling to H-1, which indicates a dihedral angle of ~90° and, hence, that AcO-7 has the *exo*-orientation (*cf.* Gelas *et al.*[2]); (*ii*) to move the multiplet at ~3.50 p.p.m. in Fig. 2 downfield to ~4.05 p.p.m.; the total intensity of this multiplet is three, and it must correspond to H-4 together with the two methylene protons (the multiplet between 3.60 and 4.00 p.p.m. in Fig. 2 is due mainly to unreacted galactoside, as can also be seen from the two methoxyl signals).

Since the remaining signals change their chemical shifts very little upon acetylation, these features strongly indicate that one acetoxyl group must be bound to the

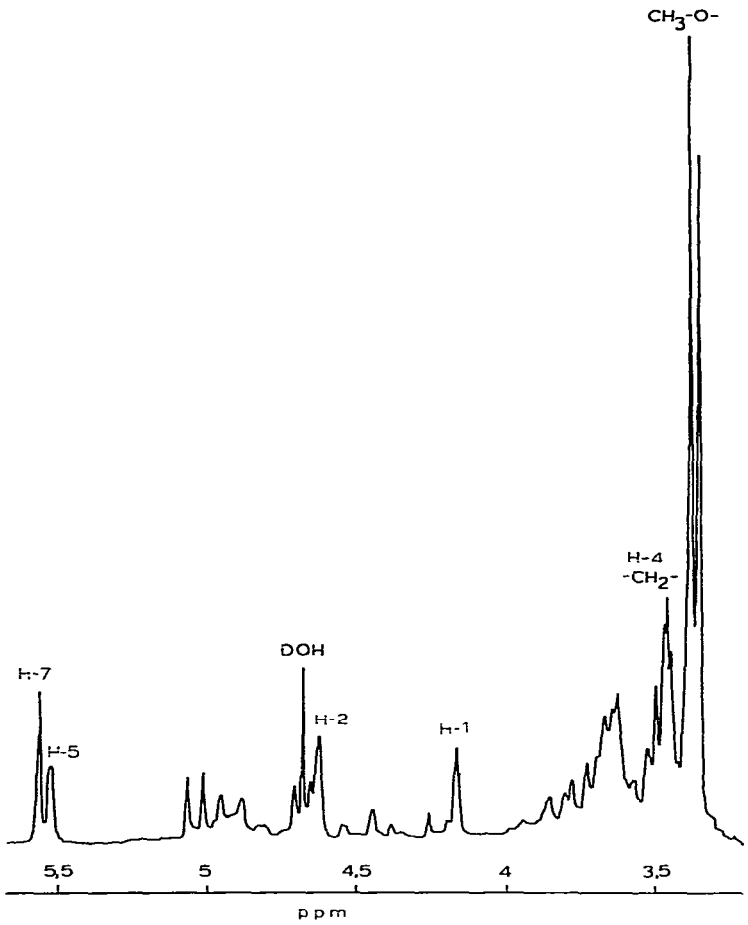


Fig. 2. $^1$H-N.m.r. spectrum (100 MHz, $D_2O$) of the crude oxidation-product from methyl α-D-galactopyranoside.

carbon atom corresponding to C-6 in the parent glycoside. The methylene group must therefore be exocyclic, and the only possible structure is **2**. Since the multiplet at 4.05 p.p.m. in Fig. 1 constitutes a rather complex part of the spectrum, it was analysed by an iterative fitting procedure[5], which gave $\nu_{meth.1}$ 409.98, $\nu_{meth.2}$ 400.95, and $\nu_{H-4}$ 400.36 Hz, and $J_{meth.1,meth.2}$ −11.4, $J_{H-4,meth.1}$ 6.2, and $J_{H-4,meth.2}$ 6.4 Hz.

The signals at 5.61, 4.59, and 4.33 p.p.m. can be assigned to H-5, H-2, and H-1, respectively, because of the deshielding effect of the oxygen atoms bound to the corresponding carbon atoms[1]. This also agrees well with the observed coupling constants, $J_{1,2} = J_{2,5} = 1.0$ Hz. Coupling between H-2 and H-5 is well known when both are equatorial[6].

The completely decoupled $^{13}$C-n.m.r. spectrum of the acetylated product is shown in Fig. 3. Apart from the signals for $Me_4Si$ and $CDCl_3$, it shows eleven reso-

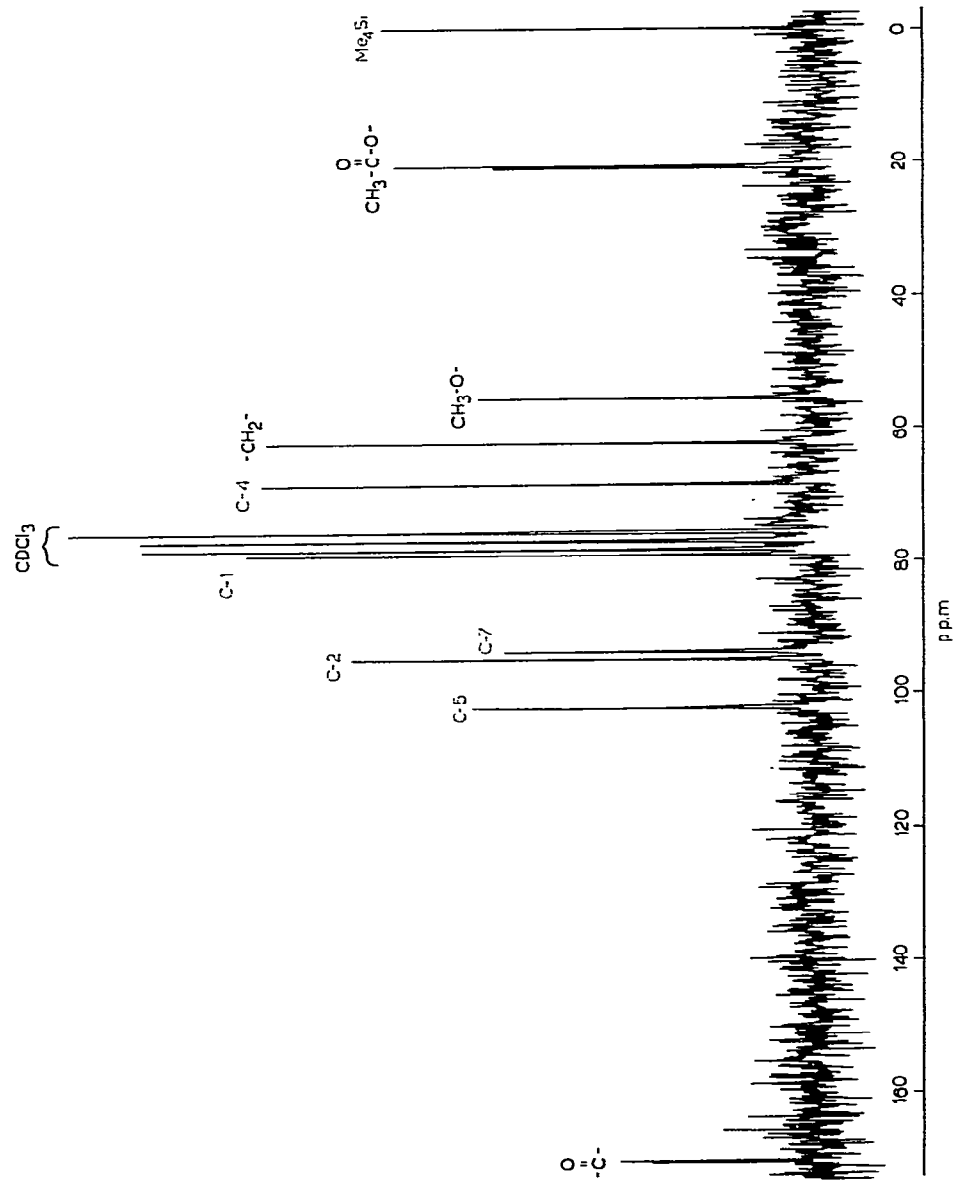


Fig. 3. Proton-noise decoupled $^{13}$C-n.m.r. spectrum (25.05 MHz, $CDCl_3$) of the diacetate **5** from methyl α-D-galactopyranoside.

nances as expected. By selective decoupling of the different proton resonances, the corresponding carbon resonances were identified, as shown in Table II. They correspond fairly closely to those reported for (1*S*,2*S*,7*S*)-7-acetoxy-2-methoxy-3,6,8-trioxabicyclo[3.2.1]octane[2].

The two acetyl methyl groups, the methoxyl group, and the two carbonyl

TABLE I

$^1$H-N.M.R. AT 100 MHz FOR SOLUTIONS OF 5 (Fig. 1) and 6 (Fig. 4) IN DEUTERIOCHLOROFORM

| *Proton* | *Chemical shifts*[a] | | *Coupling constant (Hz)* | 5 | 6 |
|---|---|---|---|---|---|
| | 5 | 6 | | | |
| $CH_3$-COO- | 2.07 | 2.07 | $J_{1,2}$ | 1.0 | 1.7 |
| | 2.11 | 2.09 | | | |
| | | | $J_{2,5}$ | 1.0 | 0.0 |
| $CH_3$-O- | 3.49 | 3.51 | | | |
| | | | $J_{H-4,meth.1}$ | 6.2 | 6.0 |
| H-4 | 4.02 | 3.83 | | | |
| | | | $J_{H-4,meth.2}$ | 6.4 | 6.8 |
| -$CH_2$- | 4.03 | 4.04 | $J_{meth.1,meth.2}$ | −11.4 | −11.4 |
| | 4.12 | 4.15 | | | |
| H-1 | 4.33 | 4.22 | | | |
| H-2 | 4.59 | 4.69 | | | |
| H-5 | 5.61 | 5.56 | | | |
| H-7 | 6.36 | 6.50 | | | |

[a]In p.p.m. downfield from internal $Me_4Si$.

TABLE II

$^{13}$C-CHEMICAL SHIFTS AT 25.05 MHz FOR SOLUTIONS OF 5 (FIG. 3) AND 6 (FIG. 6) IN DEUTERIOCHLOROFORM

| *Carbon* | 5[a] | 6[a] |
|---|---|---|
| $CH_3$-COO- | 20.62 | 20.67 |
| | 20.96 | 20.96 |
| $CH_3$-O- | 55.50 | 56.37 |
| -$CH_2$- | 62.02 | 61.97 |
| C-4 | 68.44 | 73.20 |
| C-1 | 78.94 | 78.36 |
| C-7 | 93.53 | 92.46 |
| C-2 | 94.75 | 96.40 |
| C-5 | 102.00 | 100.73 |
| >C=O | 169.80 | 169.46 |
| | 170.09 | 170.09 |

[a]In p.p.m. downfield from internal $Me_4Si$.

carbons are easily identified. Decoupling of the proton resonances now interpreted as due to H-4, H-1, H-2, H-5, and H-7, respectively, gave single $^{13}$C-resonances at 62.02, 68.44, 78.94, 94.75, 102.00, and 93.53 p.p.m. This is to be expected, because of the deshielding effect of the oxygen atoms bound to those carbon atoms. This supports the assignment of the signals for H-1 and H-2 in the proton spectrum, which otherwise may be difficult because of a rather small difference in chemical shift. Thus, as

shown in Table II, and in agreement with the interpretation now given for the proton spectrum (Table I), C-2 resonates ~16 p.p.m. downfield from C-1, because of the deshielding effect of two neighbouring oxygen atoms on C-2, compared to one on C-1.

The diacetate **5** had $[\alpha]_D^{20}$ +11° (*c* 1.9, chloroform), corresponding to a molecular rotation[7] of $[M]_D^{20}$ +30°. For the product from methyl β-L-arabinopyranoside, which should be homomorphous with **5**, Yu and Bishop[1] reported $[\alpha]_D^{22}$ +44° (chloroform), which corresponds to $[M]_D^{22}$ +90°. According to empirical rules that are valid for pyranosides[7], substitution of C-4 in Yu and Bishop's compound with an equatorial acetoxymethyl group should diminish the $[M]_D$ value by, maximally, 55°. This maximal, negative contribution would be expected when the ester oxygen atom is antiperiplanar to O-3. This is therefore its most probable conformation in **5** (in chloroform).

*(1S,2R,4R,7S)-7-Acetoxy-4-acetoxymethyl-2-methoxy-3,6,8-trioxabicyclo-[3.2.1]octane* (**6**). — The structure of the diacetate derived from methyl β-D-galactopyranoside is assigned as **6** on the basis of the following data. The n.m.r. results shown in Figs. 4–6, and in Tables I and II, for **6** are parallel to those given for **5**, and lead by similar arguments to the conclusions that AcO-7 has the *exo*-orientation, and

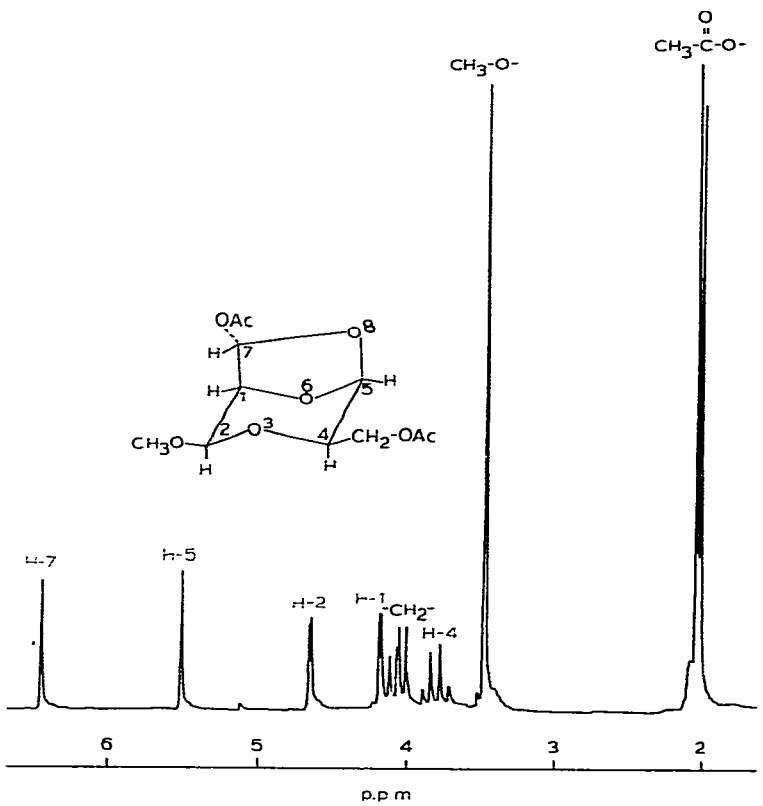


Fig. 4. $^1$H-N.m.r. spectrum (100 MHz, $CDCl_3$) of the diacetate **6** from methyl β-D-galactopyranoside.

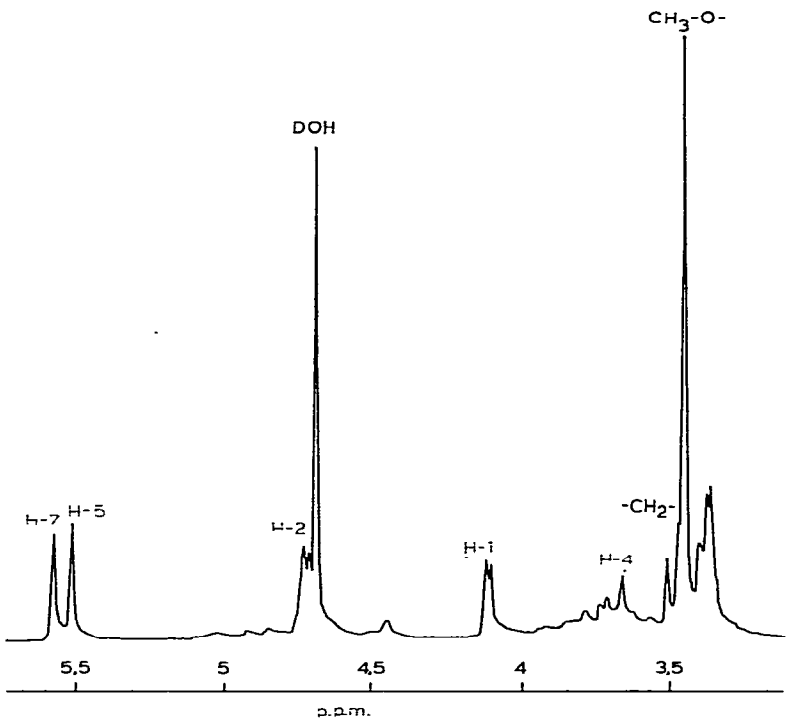


Fig. 5. $^1$H-N.m.r. spectrum (100 MHz, $D_2O$) of the crude oxidation-product from methyl β-D-galactopyranoside.

that the original hydroxymethyl group is *O*-acetylated, rather than incorporated into a hemiacetal ring. The multiplet at ~4.10 p.p.m., with an intensity of two (Fig. 4), is due to methylene protons, and that at ~3.80 p.p.m., with an intensity of unity, is due to H-4. This part can be described as nearly an ABC spectrum, and it was analysed by an iterative fitting procedure[5], which gave $\nu_{meth.1}$ 413.70, $\nu_{meth.2}$ 402.61, and $\nu_{H\text{-}4}$ 381.25 Hz, and $J_{meth.1,meth.2}$ −11.4, $J_{H\text{-}4,meth.1}$ 6.0, and $J_{H\text{-}4,meth.2}$ 6.8 Hz.

The principal differences that indicate an *R* configuration at C-2 are (*i*) the absence of any coupling between H-2 and H-5, which are now no longer di-equatorial; (*ii*) the fact that H-4 resonates at a higher field than in **5** (due to the deshielding effect[8] of the methoxyl group upon H-4 when they are 1,3-*syn*-axial, as in **5**); and (*iii*) the fact that C-4 resonates at a lower field than in **5**. Comparison with results[9] for simple hexopyranosides suggests that this is a general phenomenon.

The diacetate had $[\alpha]_D^{20}$ −94° (*c* 0.6, chloroform), corresponding to $[M]_D^{20}$ −259°. The difference between this and the $[M]_D$ value (+30°) for **5** is thus 289°, which agrees well with the value (+290°) obtained by a direct application of the parameters of Lemieux and Martin[7], on the assumption that the methyl carbon atom is antiperiplanar with respect to C-1 in both **5** and **6**. This conformation is expected both on steric grounds and because of the exo-anomeric effect[7].

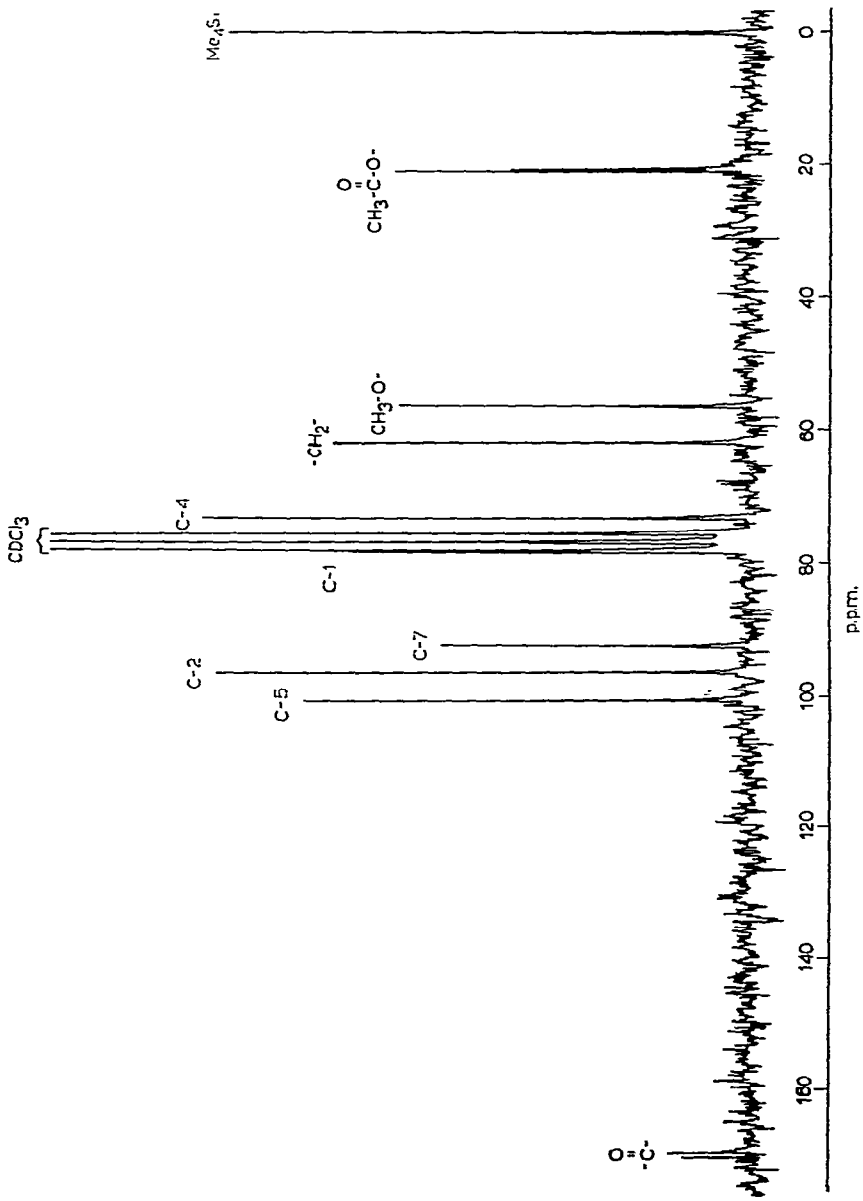


Fig. 6. Proton-noise decoupled, $^{13}$C-n.m.r. spectrum (25.05 MHz, $CDCl_3$) of the diacetate **6** from methyl $\beta$-D-galactopyranoside.

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