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PREPARATION OF TWO METHYL DEOXYFLUORO-β-D-GALACTOPYRANOSIDES, AND THEIR INTERACTION WITH GALACTAN-SPECIFIC IMMUNOGLOBULIN A J539 (FAB')

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ABSTRACT

Methyl 2-deoxy-2-fluoro-β-D-galactopyranoside (**2**) and methyl 4-deoxy-4-fluoro-β-D-galactopyranoside (**7**) have been prepared, and the possibility of their binding to (1→6)-β-D-galactopyranan-specific immunoglobulin A J539 (Fab') has been investigated. Compound **2** does not show binding, whereas **7** does. It appears that the 2-hydroxyl group of methyl β-D-galactopyranoside may take part in hydrogen bonding to the protein.

INTRODUCTION

The detailed coordinates of a computer-constructed, space-filling model of the variable region (F_v) of immunoglobulin J539 have recently been reported[1]. In that communication were suggested modes of binding between the immunoglobulin and (1→6)-β-D-galactotetraose that involve possible hydrogen-bonding. In order to pursue this matter further, we have prepared two methyl β-D-galactopyranosides in which a hydroxyl group was replaced by a fluorine atom. If hydrogen bonding indeed takes place, and if the protein were the hydrogen donor, in bonding with either, or both, of these two oxygen atoms (O-2 and O-4) in the sugar molecule, affinity would increase many-fold, due to the highly electronegative character of the fluorine atom. If, on the other hand, hydrogen bonding should occur through hydrogen donation by either the 2- or 4-hydroxyl group, or both, one or the other of the synthetic substrates **2** or **7** would probably show greatly diminished binding. Should hydrogen bonding not be mediated by either of these hydroxyl groups in the D-galactoside, the affinities would probably not be greatly affected.

RESULTS AND DISCUSSION

2-Deoxy-2-fluoro-α-D-galactopyranosyl fluoride (**1**) was prepared *via* 3,4,6-

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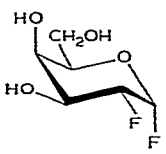
tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*lyxo*-hex-1-enitol by the method of Korytnyk and Valentekovic-Horvat[2]. Heating of **1** with methanolic hydrogen chloride gave[3] crystalline **2**, the ^{1}H-n.m.r. spectrum of which was consistent with its being the β anomer.

The preparation of **7** at first followed the known preparation of methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside[4]. Removal of the benzylidene group with aqueous acetic acid[5] yielded two products. The preponderant one was the expected methyl 2,3-di-*O*-benzyl-β-D-glucopyranoside (**3**), and the lesser, the corresponding 6-acetate. Compound **3** was benzoylated at O-6, and the resulting compound (**4**) was treated with methanesulfonyl chloride, to give methyl 6-*O*-benzoyl-2,3-di-*O*-benzyl-4-*O*-(methylsulfonyl)-β-D-glucopyranoside (**5**). Treatment of compound **5** with Amberlite A-26-F (3.5 meq. of F^-/g of resin) yielded methyl 6-*O*-benzoyl-2,3-di-*O*-benzyl-4-deoxy-4-fluoro-D-galactopyranoside (**6**). *O*-Deacylation, and reductive debenzylation of the product, then gave **7**.



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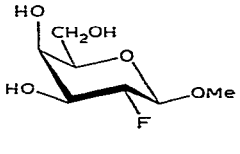

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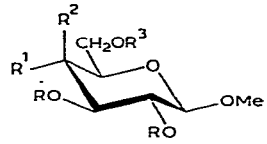

3 R = Bn, R^1 = OH, R^2 = R^3 = H
4 R = Bn, R^1 = OH, R^2 = H, R^3 = Bz
5 R = Bn, R^1 = OSO_2Me, R^2 = H, R^3 = Bz
6 R = Bn, R^1 = H, R^2 = F, R^3 = Bz
7 R = R^1 = R^3 = H, R^2 = F

Bn = $PhCH_2$
Bz = PhCO

Immunoglobulin J539 (Fab') shows ligand-induced changes in the tryptophanyl fluorescence[6]. We therefore explored the change in the fluorescence of the Fab' fragment of the immunoglobulin on addition of **2** or **7**. Whereas **2** did not cause a change, **7** did. Double diffusion in agar showed that **2** failed to inhibit the precipitation of whole J539 by pneumogalactan[7]. Quantitative estimation of the affinity between **7** and J539 Fab' showed a K_a of 750.

Previously, it had been found[8] that methyl β-D-galactopyranoside has, with J539, a K_a of 10^3. The diminution in affinity on going from the unsubstituted D-galactoside to the corresponding 4-deoxy-4-fluoro derivative **7** does not appear sufficiently large for us to propose that the 4-hydroxyl group in methyl β-D-galactopyranoside plays a significant role in hydrogen-bond formation. Rather, we expect that this small diminution in affinity may reflect a size effect.

However, the finding that replacement of the 2-hydroxyl group by fluorine caused complete cessation of binding of methyl 2-deoxy-2-fluoro-β-D-galactopyranoside to J539 Fab' indicates that the 2-hydroxyl group *is* involved in hydrogen bonding (as a hydrogen donor) to the protein. Previous work had shown the importance of the

2-hydroxyl group when it was found that 2-deoxy-D-*lyxo*-hexose appears not to bind[8]. Also, it had been found that *o*-nitrophenyl β-D-galactopyranoside shows lower affinity for J539 than the corresponding *p*-nitrophenyl D-glucoside[9]. This agrees with the present finding, because intramolecular hydrogen-bonding of the 2-hydroxyl group to the closely situated *o*-nitro group would lessen the availability of that hydroxyl group for binding to the protein.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter at 20 ± 1°. Solvents were of analytical grade. Thin-layer chromatography was performed on precoated plates of silica gel GF (250 μm, Analtech), and compounds were detected by spraying with 5% sulfuric acid followed by charring. Amberlite A-26-F was obtained from Fluka AG Chem. Fabrik, FRG. ^{1}H-N.m.r. and ^{19}F-n.m.r. spectra were recorded with a Jeol FX-100 spectrometer. Chemical shifts are given relative to internal tetramethylsilane. Chemical-ionization (c.i.) mass spectra were recorded with a Finnigan-1015D instrument.

Methyl 2-deoxy-2-fluoro-β-D-galactopyranoside (**2**). — 2-Deoxy-2-fluoro-α-D-galactopyranosyl fluoride (**1**) (184 mg), prepared by a known method[2], in 2% of hydrogen chloride in methanol (1 mL), was boiled under reflux. After 2 h, t.l.c. (1:4 methanol–ether) showed the reaction to be essentially complete, and the solution was cooled to room temperature, made neutral with Amberlite IR-45 anion-exchange resin, and evaporated to a syrup which was added to the top of a column of silica gel, and eluted with 49:1 ether–methanol, to yield crystalline **2** (96 mg), m.p. 151–152°, $[\alpha]_{589}^{20}$ +43° (*c* 2.5, water); c.i.m.s.: m/CH$^+$ = 214. (This spectrum is nearly identical to that for **7** shown in Fig. 1.)

Anal. Calc. for $C_7H_{13}FO_5$: C, 42.85; H, 6.63. Found: C, 43.24; H, 6.89.

The ^{1}H-n.m.r. spectrum (D_2O) of **2** was consistent with the β-pyranose form: δ 4.65 (d of d, 1 H, H-1, $J_{H\text{-}1,F\text{-}2}$ ~3, $J_{H\text{-}1,H\text{-}2}$ 9 Hz), and 4.38 (t of d, $J_{F\text{-}2,H\text{-}2}$ 52, $J_{H\text{-}2,H\text{-}3}$ 9 Hz). The ^{19}F-n.m.r. spectrum of this solution (external reference, C_6F_6) contained a peak at −40.99 p.p.m.

Methyl 6-O-benzoyl-2,3-di-O-benzyl-β-D-glucopyranoside (**4**). — Methyl 2,3-di-*O*-benzyl-β-D-glucopyranoside (3.74 g) in dry pyridine (20 mL) was cooled to −46°, and benzoyl chloride (1.41 g; equimolar amount) was added dropwise under a stream of nitrogen. The temperature was allowed to reach −20°, and was kept there overnight. The solution was poured onto ice–water, and the resulting, white solid was collected by filtration (4.12 g, 90%). This crude material was added to the top of a column of silica gel, and the mixture was eluted with 1:5 ether–hexane, to yield two products. The first one (minor) was identified by its ^{1}H-n.m.r. spectrum as methyl 4,6-di-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-glucopyranoside, m.p. 118–120°. The second compound to be eluted was methyl 6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-glucopyranoside (**4**) (2.92 g), m.p. 96–98°, $[\alpha]_D^{20}$ −8.9° (*c* 0.9, $CHCl_3$).

Anal. Calc. for $C_{28}H_{30}O_7$: C, 70.27; H, 6.32. Found: C, 70.21; H, 6.41.

Methyl 6-O-benzoyl-2,3-di-O-benzyl-4-O-(methylsulfonyl)-β-D-glucopyranoside (**5**). — A solution of **4** (0.965 g) in dry pyridine (5 mL) was cooled in ice, and methanesulfonyl chloride (0.256 g, 10% molar excess) was added dropwise under a stream of nitrogen. Stirring was continued overnight at 5°, the solution was poured into ice–water, and the mixture extracted with chloroform (3 × 100 mL). The extracts were combined, successively washed with water, dilute aqueous hydrochloric acid, and water, dried (sodium sulfate), and evaporated. The residue was triturated with cold ether, to afford white prisms in 87% yield, m.p. 134–136°, $[\alpha]_{589}^{20}$ +29.3° (*c* 0.75, $CHCl_3$).

Anal. Calc. for $C_{29}H_{32}O_9S$: C, 62.57; H, 5.79; S, 5.76. Found: C, 62.95; H, 5.90; S, 6.03.

Methyl 6-O-benzoyl-2,3-di-O-benzyl-4-deoxy-4-fluoro-β-D-galactopyranoside (**6**). — A solution of **5** (0.278 g) in dry benzene (10 mL) was boiled under reflux with Amberlite A-26-F ion-exchange resin (1 g, 3.5 meq. of F^-/g) until t.l.c. failed to show any further progress of the reaction. The resin was filtered off and washed with benzene, and the filtrate and washings were combined, and evaporated, to give a residue that was added to the top of a column of silica gel. The column was eluted

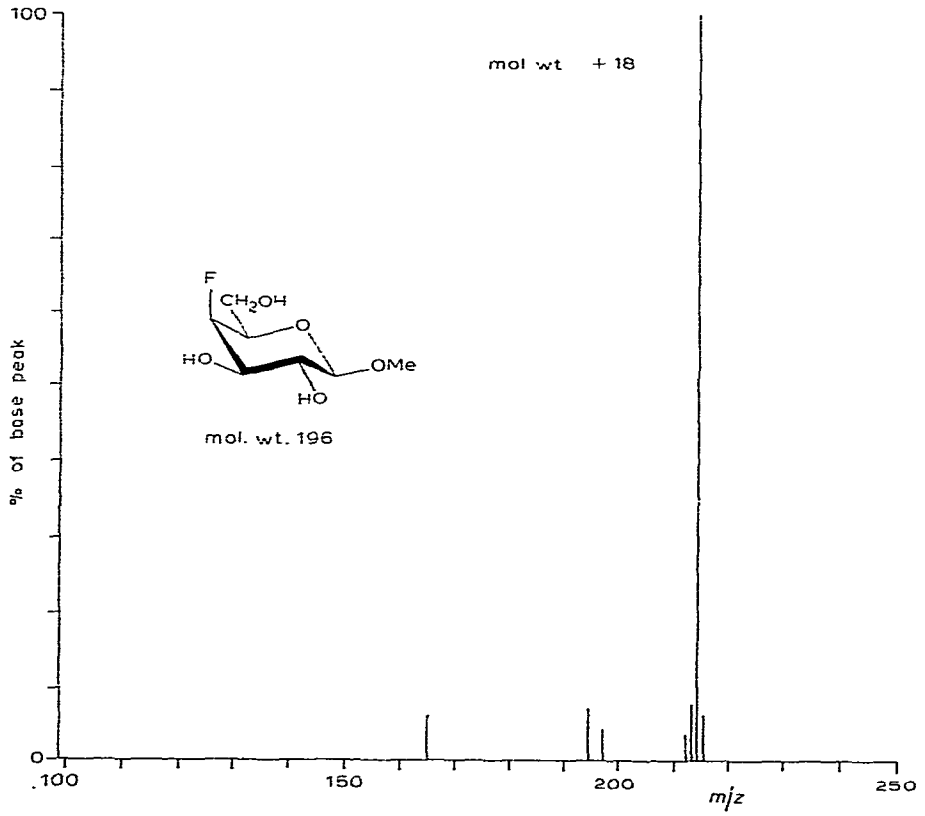

Fig. 1. Chemical-ionization, mass spectrum (NH_4^+) of **7**.

with a gradient of hexane–ether in which the proportion of the latter was gradually increased from 20 to 50%. The product (**6**, 0.105 g) was recrystallized from ether–hexane, to give white needles m.p. 119–120°, $[\alpha]_D^{20}$ −12° (*c* 1.56, $CHCl_3$), and 146 mg of **5** was recovered.

Anal. Calc. for $C_{28}H_{29}FO_6$: C, 69.99; H, 6.09; F, 3.95. Found: C, 69.87; H, 5.90; F, 3.94.

The ^{19}F-n.m.r. spectrum of this material (external reference C_6F_6) was consistent with the *galacto* configuration: δ −54.44 (ddd, $J_{F\text{-}4,H\text{-}3} = J_{F\text{-}4,H\text{-}5} = 27$ Hz, $J_{F\text{-}4,H\text{-}4}$ 51 Hz).

Methyl 4-deoxy-4-fluoro-β-D-galactopyranoside (7). — A solution of **6** (99.2 mg) in methanol (10 mL) containing freshly prepared sodium methoxide (20 mg) was kept overnight at room temperature, made neutral with Amberlite 120 resin, evaporated, and the residue purified by chromatography on a column of silica gel, with 1 : 1 ether–hexane as the eluant. The product, methyl 2,3-di-*O*-benzyl-4-deoxy-4-fluoro-β-D-galactopyranoside, was recrystallized; m.p. 90–96°, $[\alpha]_{589}^{21}$ −5.9° (*c* 0.34,

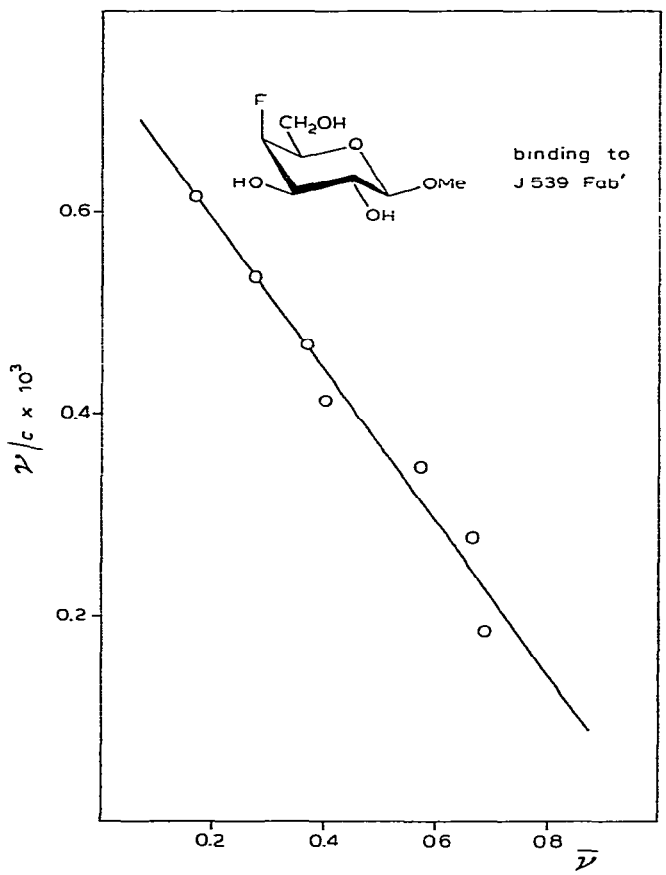

Fig. 2. Scatchard plot of $\bar{\nu}/c$ *versus* $\bar{\nu}$ for immunoglobulin J539 Fab′ and **7** in phosphate-buffered-saline, pH 7.4.

$CHCl_3$). This material (19.6 mg) was reductively debenzylated in the presence of freshly reduced palladium chloride during 12 h at room temperature; t.l.c. then showed complete reaction. The mixture was filtered, the solids were washed with methanol, and the filtrate and washings were combined, and evaporated under diminished pressure. The residue was purified by t.l.c. on a preparative plate (0.5 mm × 20 × 20 cm) with 1:8 methanol–ethyl acetate, to yield **7** (7 mg), m.p. 129–130°. Another batch (9.5 mg), similarly prepared, had $[\alpha]_{589}^{20}$ −30.8° (*c* 2.04, CH_3OH); it was recrystallized from 1:3 ethanol–hexane to remove a small proportion of fluorescent material. The NH_3-c.i. mass spectrum showed an essentially single peak at 214 (mol. wt. +18) (see Fig. 1).

Affinity measurements. — Immunoglobulin J539 Fab′ was prepared as previously described, and titrations using changes in the ligand-induced fluorescence were performed as before[6].

When $Ab_{Fab'} + L \rightleftharpoons Ab_{Fab'}L$, $K_a = C_{Ab_{Fab'}L}/C_{Ab_{Fab'}} \times C_L = \bar{\nu}/(1 - \bar{\nu}) \times c$, where $\bar{\nu}$ is the fraction of the antibody Fab′ bound, and *c* is the concentration of free ligand (L). From this, it follows that $\bar{\nu}/c = (1 - \bar{\nu})K_a$, and Fig. 2 shows the Scatchard plot of these data, where the negative slope equals the value of K_a.

Inhibition experiments. — Ouchterlony double-diffusion[10] was performed in agar, using a circular well-system (7 mm diam.). Compound **2** was spotted at 0.5M concentration, and the concentration of the pneumogalactan was 0.1%. Ascites was used for J539.

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