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FLUOROCARBOHYDRATES

PART XXV. SYNTHESIS AND STRUCTURE OF 2-DEOXY-2-FLUOROLACTOSE AND RELATED COMPOUNDS

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SUMMARY

Experimental details describing the addition of CF_3OF to hexa-O-acetyl-D-lactal (I) are presented. Four fluorinated disaccharides : trifluoromethyl 3,6-di-O-acetyl-2-fluoro-4-O-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-mannopyranoside (V), trifluoromethyl 3,6-di-O-acetyl-2-deoxy-2-fluoro-4-O-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (VI), 3,6-di-O-acetyl-2-deoxy-2-fluoro-4-O-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-mannopyranosyl fluoride (VII), and 3,6-di-O-acetyl-2-deoxy-2-fluoro-4-O-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl fluoride (VIII) were isolated from the product mixture. The profound changes in both the rate and the major products of the addition, compared to those reported for related monosaccharide glycals, are discussed in relation to the steric influence exerted by the presence of the non-reducing (galactoside-B) ring of the disaccharide glycals. The configuration and the conformation of the fluorinated portion of the adducts were assigned on the basis of ^{19}F.m.r. spectroscopic parameters and the structural

assignments confirmed by hydrolytic degradation and g.l.c. analysis of the resulting monosaccharides. In addition, the synthesis of β-D-lactosyl fluoride (IV) and of its heptaacetate (III) provided two important spectroscopic model compounds.

INTRODUCTION

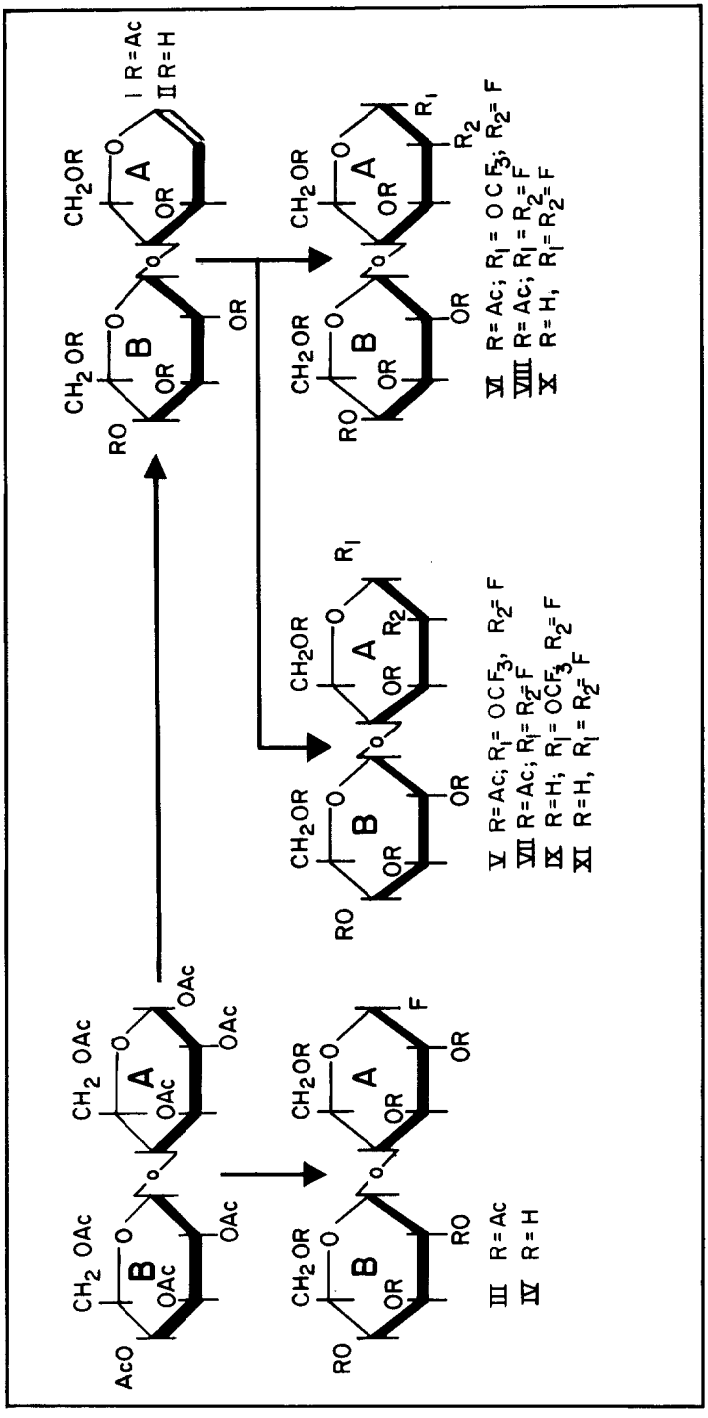
The interest in fluorinated carbohydrates has undobtedly been stimulated by a clearer understanding of the mode of action of such powerful anti-metabolites as fluorocitric acid[1], 9-α-fluorohydrocortisone[2], 5-fluorouracil[3], and a fluorine-containing antibiotic, nucleocidin[4]. The synthetic aspects of fluorination at the secondary positions of monosaccharides have encountered considerable obstacles, primarily due to poor nucleophilicity of F^- but the required range of suitable fluorinating agents has recently expanded greatly, and the resulting syntheses of a large number of monosaccharides, as well as their properties, have been the subject of two detailed reviews.[5,6].

The discovery and application of fluoroxy compounds[7-9], and fluoroxytrifluoromethane (CF_3OF)[10] in particular, rapidly established their usefulness as reagents of high reactivity towards a variety of olefinic compounds. Following the successful fluorination of steroids[11,12] using CF_3OF, it became clear that this reagent offered an interesting new route for the fluorination of the secondary positions of carbohydrates, via the unsaturated precursors. The use of CF_3OF as a source of "electrophilic fluorine" has been thus advantageously explored in both the hexose[13-14] and the pentose series[15,16] of the monosaccharides, and the course of such additions is well understood and accepted.

Recently, fluorinated carbohydrates have proved to be important vehicles as n.m.r. structural probes; ^{19}F.n.m.r. spectroscopic measurements of the binding modes of fluorinated substrates to enzymes and to antibodies enable the evaluation to be made of structure-activity relationships in solution. In addition to the possibility that fluorinated disaccharides may provide further probes for such studies, fluorination of disaccharides in itself presents a novel synthetic challenge due to the largely unknown influences arising from the presence of a second monosaccharide moiety. To date only the disaccharides in trehalose[17] and maltose[18] series, fluorinated at the primary positions, have been reported.

DISCUSSION

The procedure[19], which is generally successful for the synthesis of monosaccharide glycals, was found to be unsatisfactory in the lactose series. An alternative three-step sequence[20], however, gave hexa-O-acetyl-D-lactal (I) in good yield, though, as has been observed in the past[20b], not as a crystalline compound, even after chromatographic purification. Its chemical identity was confirmed by p.m.r. spectroscopy and deacetylation to the known, crystalline D-lactal (II)[20]. The treatment of octa-O-acetyl-α-D-lactose with HF/AcOH gave the expected[21,22], though hitherto uncharacterised hepta-O-acetyl-α-D-lactosyl fluoride (III), which was deacetylated ($MeOH/NH_3$) to α-D-lactosyl fluoride[23] (IV). Both the acetylated lactosyl fluoride (III) and the unsubstituted fluorosugar (IV) were important monofluorodisaccharide models of known configuration, containing the fluorine atom in the position which gives rise to a minimum number of heteronuclear interactions.


I R=Ac
II R=H
III R=Ac
IV R=H
V R=Ac; R1= OCF3, R2= F
VI R=Ac; R1 = OCF3; R2= F
VII R=Ac; R1= R2= F
VIII R=Ac; R1 = R2= F
IX R=H; R1= OCF3, R2= F
X R=H, R1= R2= F
XI R=H, R1 = R2= F

The addition of CF_3OF to the glycal (I), in $CFCl_3$ containing 20% $CHCl_3$ to aid solubility, proceeded at a satisfactory rate at 0° (no appreciable rate was observed at temperatures below 0°), and was complete upon the passage of 100% excess of the reagent. T.l.c. analysis of the product mixture (solvent 1) showed the presence of four components, which were particularly well-resolved on multiple elution, and showed negligible degradation. The resolution of the product mixture by column chromatography gave peracetylated adducts of fluoro-epilactose (V), (VII) and of fluorolactose (VIII) (R_F 0.45, 0.32 and 0.23 respectively), one of which, (V), was crystalline. The fourth adduct, trifluoromethyl fluorolactoside (VI, non-crystalline, R_F 0.36), was obtained by a repeat of the chromatographic treatment of the combined residual fractions. (see Reaction Scheme)

The ^{19}F.m.r. structural assignments of the fluorinated adducts and reference compounds are presented in Table 1.

As one of the objectives of the present work was to illustrate the suitability of fluorine as a rudimentary structural probe in molecules which give rise to p.m.r. spectra of considerable complexity, a more detailed discussion of the spectrometric data is pertinent at this stage. In Figure 1, the comparison of spectra (a) and (b) illustrates well the additional complications introduced by the presence of one fluorine atom at the anomeric position of a disaccharide molecule. The ^{19}F.m.r. spectrum of the compound (III) (shown on Fig.2 spectrum (a)), however, is a simple one and contains a considerable amount of information about the configuration and the conformation of the A ring in the molecule. The p.m.r. spectra of the fluoro derivatives of epilactose and lactose (V to VIII), and particularly those of the acetylated trifluoromethyl fluoroepilactoside (V) and acetylated fluorolactosyl fluoride (VIII) are very complex due to both multiplicity of hetero-

TABLE 1

^{19}F.m.r. Parameters of some Fluorinated Disaccharides

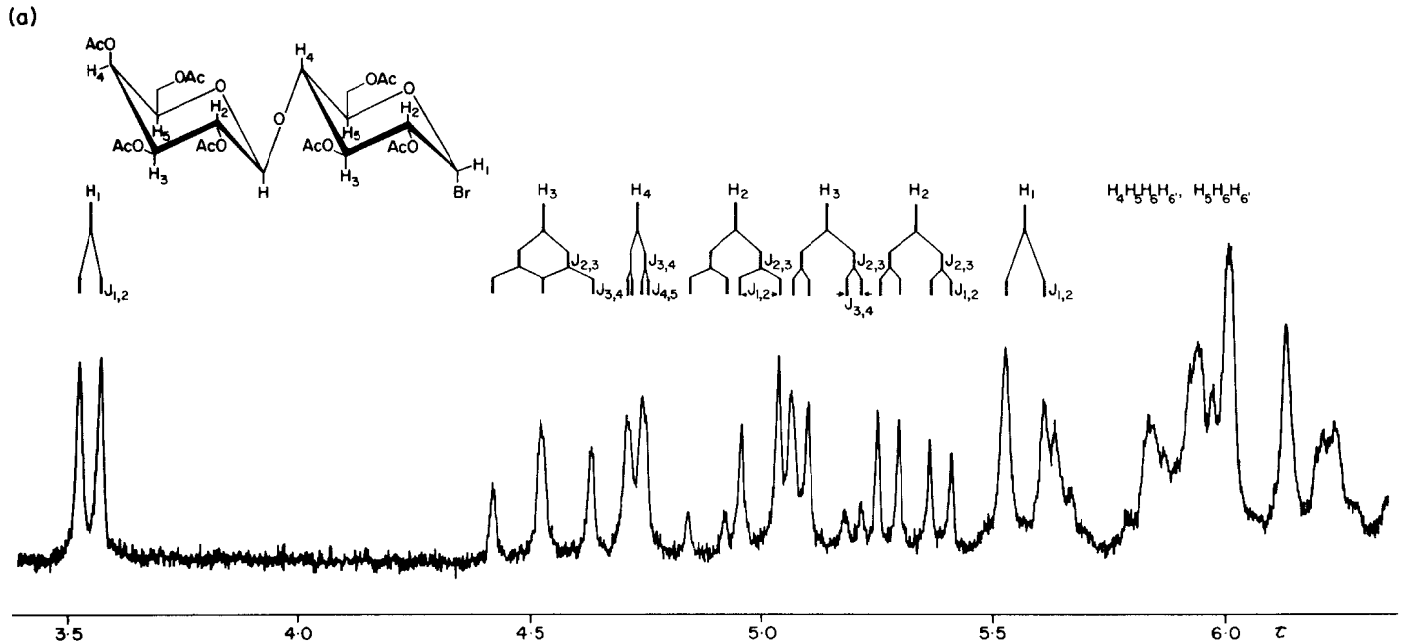
Compound	Configuration of A ring	Conformation of A ring	Coupling Constants (Hz)						Chemical Shifts (p.p.m.)			
			$J_{F_1H_1}$	$J_{F_1H_2}$	$J_{F_1F_2}$	$J_{F_2H_1}$	$J_{F_2H_2}$	$J_{F_2H_3}$	F_1	F_2	OCF_3	Solvent
(III)	α-D-Gluco	C-1	53.5	24.5	-	-	-	-	149.3	-	-	$CDCl_3$
(V)	β-D-Manno	C-1	-	-	-	16	51	27	-	219.9	59.5	"
(VI)	α-D-Gluco	C-1	-	-	-	*	48	12	-	202.0	58.9	"
(VII)	β-D-Manno	C-1	50	9	15	12	49	21	147.8	219.5	-	"
(VIII)	α-D-Gluco	C-1	53	23.7	19	*	48	12.2	151.2	203.9	-	"
(IV)	α-D-Gluco	C-1	54	25	-	-	-	-	166.5	-	-	10%$(CD_3)_2CO$ in D_2O)
(IX)	β-D-Manno	C-1	-	-	-	18	50	32	-	238.1	74.8	"
(X)	α-D-Gluco	C-1	52	22	18	*	48	13	166.3	219.8	-	"
(XI)	β-D-Manno	C-1	49	5	13.5	18	52	32.5	164.9	239.0	-	"

* not measured.

nuclear interactions and the superimposition of the ring protons of the two monosaccharide entities. The most complex of the ^{19}F.m.r. spectra, that of acetylated fluoroepilactosyl fluoride (VII) shown in Fig.2 spectrum (b), nevertheless provided a useful source of information about the configurational and conformational features of the A ring.

Considering the chemical shifts in the difluoro products, both F_1 and F_2 (and particularly F_2) in the spectra of the compounds (V) - (VIII) appear at the lower field consistent with fluorine being in equatorial rather than axial orientation. The chemical shifts of both fluorine atoms and the OCF_3 group were found to be of the same order of magnitude as those observed and reported [24] for a number of related monosaccharide structures. Even under the most careful inspection OCF_3 group resonated as a sharp singlet, thereforeby suggesting that there are no significant interactions between it and the adjacent nuclei. Although this appears to be a general feature of the relevant fluoromonosaccharides so far examined, a forthcoming publication from this laboratory[25] will disclose and discuss some interesting exceptions.

The α-D-gluco configuration and the C-1 conformation of the A ring in peracetylated lactosyl α-fluoride (III) was unequivocally established on the basis of the magnitude of vicinal H-H interactions.[26] The ^{19}F.m.r. data obtained for the acetylated derivative (III), and its deacetylated form (VI) and the acetylated fluorolactosyl α-fluoride (VIII) were also found to support strongly the α-D-gluco configuration of the A rings. Thus the magnitude of $J_{F_1H_1}$ (53.5Hz), (53.0Hz) and $J_{F_1H_2}$ (24.5Hz) (23.7Hz) in (III) and (VIII) respectively are identical within the limits of experimental error, as are $J_{F_2H_2}$ (48Hz) (48Hz and $J_{F_2H_3}$ (12.0Hz) (12.2Hz) in (VI)and (VIII) respectivcly. There is also


(a)
AcO
H4
OAc
O
H2
H5
AcO
AcO
H3
O
H
H4
OAc
O
H2
H5
AcO
AcO
H3
H1
Br
H1
J1,2
H3
H4
H2
H3
H2
H1
H4H5H6H6', H5H6H6'
J2,3
J3,4
J4,5
J1,2
3·5
4·0
4·5
5·0
5·5
6·0
τ

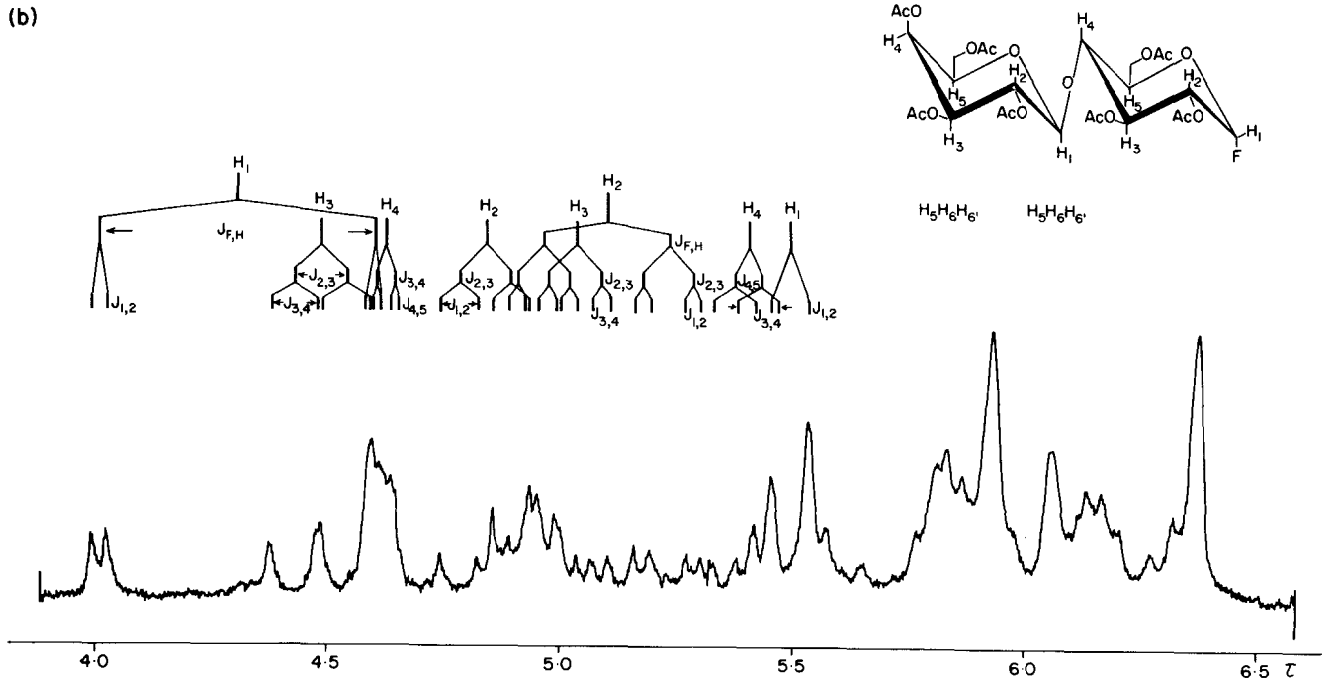


Fig. 1 . Proton magnetic resonance spectra of hepta-O-acetyl-D-lactosyl-α-bromide (spectrum a) and hepta-O-acetyl lactosyl-α-fluoride (III) (spectrum b). Measurements were made in $CDCl_3$ at 84.7MHz at 33° in the frequency sweep mode with tetramethylsilane as reference.

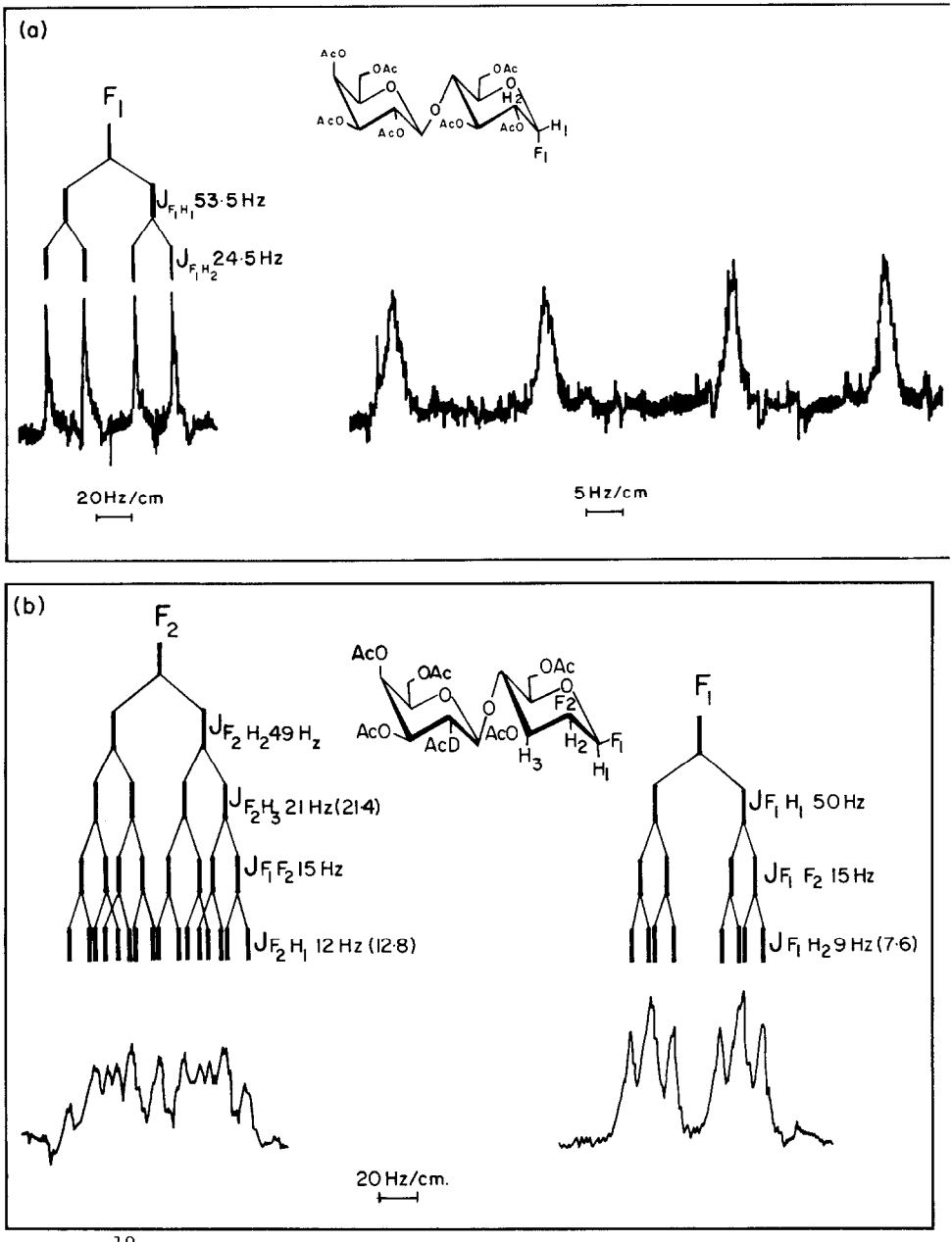


Fig. 2 . ^{19}F-Magnetic resonance spectra of hepta-O-acetyl lactosyl-α-fluoride (III) (spectrum a) and its 2-deoxy-2-fluoro derivative (VIII) (spectrum b). Measurements were made in $CDCl_3$ at 87.4MHz at 33° with $CFCl_3$ as reference.

a close correlation between the above values and those observed and published for closely related fluoromonosaccharide structures. C-1 conformation is expected to be preferred, due to the stabilisation by four equatorial substituents and a large anomeric effect[27] observed to operate in glycopyranosyl fluorides.

The manno configuration and C-1 conformation of the A ring in the epilactose compounds (V) and (VII) is supported by the magnitude of $J_{F_2H_3}$ (20-25Hz) in both being characteristic of the trans-diaxial relationship between F_2 and H_3. The β-D-orientation at the anomeric centre gives rise to the relatively high value of $J_{F_1H_2}$ (9.0Hz) for the 1,2 difluoride (VII), and a significant overall decrease in the values of $J_{F_2H_1}$ and $J_{F_2H_3}$ in (VII), as compared to those in the trifluoromethyl glycoside (V); both the observations are consistent with the presence of an equatorial fluorine at the anomeric centre of the difluoride (VII). The latter effect may be considered as a time-averaged consequence of a partial conformational destabilisation of (VII), arising from a large anomeric effect [28,29] of a glycosidic fluorine atom (Table 1). It has been shown that similar conformational destabilisation causes conformational changes in the related fluoro-pentopyranosides [15,16a] in which 1-C conformation predominates in order to accommodate the aglycone in the preferred axial orientation.

It may be seen from the discussion so far that two marked differences between the addition of CF_3OF to the disaccharide glycal (I) and the comparable reaction in the monosaccharide series have emerged. Significant deactivation of the olefinic disaccharide towards the reagent (CF_3OF) has only one parallel in the monosaccharide series, namely the reaction of 2-acetoxy-3,4-di-O-acetyl-D-arabinal [16a], which only reacts at 0°. All other glycals, curiously enough, even 3,4,6-

tri-O-acetyl-2-fluoro-D-glucal [13c], react readily at -70°. The preponderance of the epilactosyl adducts (V) and (VII), having β-D-mannoconfiguration of the A ring, is indicative of cis-addition (cis to the substituent at C-3, A ring), and is in marked contrast to the results in the monosaccharide series where, in accordance with the stereo-electronic rationalisations [29], cis addition takes place predominantly trans to the C-3 substituent.

These observations suggest that the presence of the second monosaccharide ring (B ring) exerts a major steric influence. It would also seem possible that the second ring (B), in the disaccharide glycal (1), may be "folded" in such a way that its C-6 substituent is brought into a close proximity of the olefinic site in the A ring, so causing considerable hindrance to the approach of the attacking reagent from the α-D-gluco side, so facilitating the alternative attack from the β-D-manno side. At temperatures below 0°, it may be possible that the reduced rotational mobility about the glycosidic bond between A and B rings hinders the formation of an initial 2-fluoro-C-1 carbonium ion, so that the overall reaction rate is diminished. Furthermore, the presence of the second hexosyl residue B at C-4 of the glucal ring A must impose considerable restraint on the conformational flexibility $^{C1}_{1C}$ of the latter ring.

The fluorinated epilactose derivatives (V, VII) and the fluorolactose derivatives (VIII), which were produced in sufficiently high yields to allow degradative studies, were deacetylated ($MeOH/NH_3$), to give the corresponding crystalline de-esterified derivatives (IX), (X) and (XI). Some caution was necessary when assessing the completeness of deacetylation by t.l.c., and a complete deacetylation was confirmed by the absence of methyl group signals in p.m.r. spectra.

J_{F_1H} values obtained from ^{19}F.m.r. spectra of the de-esterified compounds (IX) - (XI) further supported the initial structural assignments of their peracetylated precursors. An overall increase in the magnitudes of J_{F_2H} for the fluorolactosyl fluoride (X) suggested an overall reduction in conformational strain; the relevant parameters correlated closely with the reported J_{F_1H} values for the corresponding anomers of 2-deoxy-2-fluoro-D-glucose and -mannose. [13b]

Selective hydrolytic removal of the aglycone in the fluoroepilactoses (IX, XI) and the fluorolactose derivative (X), using aqueous mineral acid, could not be achieved in adequate yield without a substantial cleavage of the disaccharidic linkage, even using conditions considerably milder than those employed for monosaccharide glycosides. Attempts to isolate the pure monofluorodisaccharides from the products of hydrolysis of fluorolactose and epilactose derivatives were seriously hindered by close similarity in chromatographic properties. In view of the suitability of the glycosyl fluorides as substrates for glycosidases [30], a more fruitful and realistic approach towards the synthesis of 2-deoxy-2-fluoro disaccharides as free sugars may well rest with the enzymatic rather than chemical procedures.

The final structural proof of the fluorodisaccharide adducts (V) - (XI) was provided by methanolysis, followed by g.l.c. examination of the resulting methyl glycosides. This type of analysis was equally applicable to both the acetylated derivatives (V)-(VIII) and deacetylated fluorides (IX) - (XI). As may be seen from the results presented in the Experimental section, pairs of disaccharides (V) and (VII), and (IX) and (XI) gave only methyl glycosides of 2-deoxy-2-fluoro-D-mannose and D-galactose (consistent with the epilactose structures proposed), whereas (VI) and (VIII) and (X) gave only methyl glycosides of 2-deoxy-2-fluoro-D-galactose (consistent with "the lactose" structure proposed).

The reference standards for this analysis were the adducts of CF_3OF to 3,4,6-tri-O-acetyl-D-glucal, which were subjected to the same degradative procedure.

EXPERIMENTAL

Melting points were determined on a Townsend and Mercer hot-stage apparatus and are uncorrected. Thin-layer chromatography (t.l.c.) we performed on glass plates (20 x 5 cm and 20 x 20 cm), coated with Keiselgel PF_{254} and developed in the following solvent systems: ethyl acetate : petroleum ether b.p. 60-80° (1;1, v/v, solvent 1), ether : petroleum ether b.p. 40-60° (1;9 v/v, solvent 2) and ethyl acetate: methanol (4:1 v/v, solvent 3). The carbohydrate components were detected by spraying the plates with 10% solution of concentrated sulphuric acid in ethanol, followed by heating at 120° for several minutes. Column chromatography was performed on Keiselgel PF_{254}, (wet packed under pressure [31]) of approximate length 30 cm. Gas-liquid chromatography (g.l.c.) was carried out using trimethylsilyl derivatives of carbohydrates on a Pye series 104 chromatograph (model 24) fitted with dual circular columns ($6\frac{1}{2}$ft) of diataport S (80-100 mesh) (Hewlett-Packard Inc) and 3% S.E. 30 (Applied Science Laboratories Inc). The nitrogen flow was 40 ml/min. Proton magnetic resonance (p.m.r.) spectra were determined on a Brucker spectrometer, operating at 84.7mHz, and 33° in the frequency sweep mode, using deuteriochloroform solutions containing tetramethylsilane as the internal standard. Fluorine magnetic resonance spectra (^{19}F.m.r.) were determined on the same instrument, operating in the frequency sweep mode. Solutions of fluorinated derivatives were prepared in deuteriochloroform or in deuterium

oxide containing 10% of deuterioacetone, with fluorotrichloromethane as the internal standard. Optical rotations were measured on Bellingham and Stanley Ltd (London) polarimeter. Elemental analyses were carried out by the University of Durham analytical laboratory and Bernhardt Microanalytical Laboratories.

1,2,3,6-Tetra-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranose. Concentrated perchloric acid (0.5ml) [32] was added to freshly distilled acetic anhydride (90ml) at 0°. D-Lactose (14.0g) was introduced in portions over a period of 1 hour, at such a rate that the temperature was between 0° and 5°, and the resulting solution was maintained at 5°, with stirring, overnight. The reaction mixture was then poured into ice and water, and the resulting syrup was extracted with chloroform. The combined chloroform extracts were washed thoroughtly with alternating portions of water and sodium hydrogen carbonate solution (saturated, aqueous) until all the excess acetic anhydride was removed. After being dried over anhydrous magnesium sulphate, the solution was concentrated under diminished pressure, and the last traces of solvent were removed under high vacuum. The syrup, yield 20g (72%) crystallised from acetone/petroleum ether. The product had m.p. 153°, $[\alpha]_D^{20}$ + 52.5° (c,1.0, $CHCl_3$). Lit. [33] m.p. 152°, $[\alpha]_D^{20}$ + 53.54° (c, $CHCl_3$).

The above octaacetate was converted to the lactosyl bromide hepta-acetate m.p. 143° dec. (from $CHCl_3$/petroleum ether b.p. 40-60°), $[\alpha]_D^{20}$ + 107.5° (c, 1.0, $CHCl_3$); Lit [20a] m.p. 145° dec. (from $CHCl_3$/petroleum ether b.p. 40-60°), $[\alpha]_D^{23}$ + 108° in 70% yield, according to the published procedure, and further into D-lactal hexaacetate (I)

in 60% yield. D-lactal hexaacetate failed to crystallise even after purification by column chromatography, and was characterised by deacetylation with methanolic ammonia (saturated solution at 0°), to the well-defined D-lactal (II) m.p. 193-4°, $[\alpha]_D^{20}$ + 27° (c, 1.5, H_2O) Lit. [20b] m.p. 191-2°, $[\alpha]_D^{23}$ + 27.7° (c, 1.6, H_2O)
Analysis : Found : C, 46.5 ; H,6.6%. $C_{12}H_{20}O_9$ requires C, 46.8 ; H,6.5%.

2,3,6-Tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galacto-pyranosyl)-α-D-glucopyranosyl fluoride (III) α-D-Lactose octaacetate (3.4g., 5m moles) was dissolved, at 0° and with stirring, in 45% solution of HF in glacial acetic acid (35ml) and the stirring continued for 6 hr at 0° under anhydrous conditions. The reaction mixture was then poured into a mixture of ice and sodium hydrogen carbonate solution (saturated, aqueous)/chloroform (total volume 500ml) and stirred vigorously. The chloroform layer was separated quickly and washed successively with sodium hydrogen carbonate solution (saturated, aqueous) (4 x 100ml), water (4 x 100ml) dried (anhydrous $MgSO_4$). The solvent was removed under diminished pressure to leave a yellow syrup, which was purified by chromatography on a column of 100 gm of Keiselgel PF_{254}. The product (III) was obtained in an amorphous form, (2.0g) (62%), $[\alpha]_D^{20}$ + 43° (c, 1.0, $CHCl_3$).*
Analysis: Found: C, 49.2; H. 5.7, F, 2.5; $C_{26}H_{35}FO_{17}$ requires C, 48.9; H, 5.5; F, 3.0%.

Structures were assigned for peracetylated α-D-lactose, α-D-lactosyl bromide, α-D-lactosyl fluoride and D-lactal on the basis of p.m.r. data (Table 2).

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* Predicted by Hudson and Kunz [34] to be + 42°

TABLE 2

P.M.R. Data for Peracetylated Lactose Derivatives (at 100 MHz, all solutions in $CHCl_3$ with TMS as internal standard)

GLUCOSE RING

	Chemical Shifts (τ)							Coupling Constants (Hz)				
	H_1	H_2	H_3	H_4	H_5	H_6	H_6^1	$J_{1,2}$	$J_{1,3}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
A	3.78	5.04	4.57	5.6			6.5	3.2	-	10.0	8.0	-
B	5.03	5.35	4.53	5.6			6.5	4.0	-	10.0	9.5	-
C	4.3	5.1	4.48	5.4	5.6		6.5	3.0	-	9.6	8.0	5.0
D	3.58	5.18	4.63	5.5			6.4	5.5	1.2	3.0	7.3	-

GALACTOSE RING

	Chemical Shifts (τ)							Coupling Constants (Hz)			
	H_1	H_2	H_3	H_4	H_5	H_6	H_6^1	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
A	5.5*	4.9	5.1	4.68	5.6		6.5	7.0	10.0	3.5	1.5
B	5.56	4.9	5.14	4.72	5.6		6.5	7.5	10.5	3.0	1.3
C	5.47	4.8	5.03	4.62	5.05		6.5	7.5	10.0	3.5	2.0
D	5.35	4.8	5.02	4.65	5.5		6.4	7.5	10.0	3.5	2.0

A. α-D-lactose octaacetate;

B. α-D-lactosyl bromide heptaacetate;

C. α-D-lactosyl fluoride heptaacetate;

D. D-lactal hexaacetate (I)

* Chemical shift of this portion in α and β-lactose octaacetates are reported relative to $CHCl_3$ peak (35)

Reaction of hexaacetyl-D-lactal with fluoroxytrifluoromethane. A solution of hexaccetate (I) (5.6g. 10mmols) in fluorotrichloromethane (200ml) containing dry chloroform (20%) was stirred with calcium oxide (2.0g) and purged thoroughly with nitrogen. Fluoroxytrifluoromethane (20mmols), diluted with nitrogen, was then passed into the solution, while the temperature was maintained at 0^{o}, during a period of 4 hrs, and the reaction was monitored by observing the response of small aliquots of the reaction mixture to alkaline permanganate. The excess reagent was then removed by purging the reaction mixture with nitrogen for 30min. The reaction mixture was filtered into sodium hydrogen carbonate solution (saturated, aqueous, 200ml), and the residue was washed thoroughly with chloroform (200ml). The combined organic extracts were separated, washed with water (3 x 200mls), dried (anhydrous $MgSO_4$), and the solvent was removed under diminished pressure. The resulting pale yellow syrup (6.0g) was shown by t.l.c. (Solvent 1), to be a mixture containing four components, Rf 0.45, 0.36, 0.32 and 0.23.

A concentrated solution of the mixed products in system A was applied slowly and under pressure to a column of Keiselgel PF_{254}(200g). Elution with the solvent 1, at a flow rate of 0.5ml/min gave the four following products. The fluoroepilactoside (V) (nc) (1.9g) (28.6%) had m.p. 203-5^{o} (crystallised from ethyl acetate/petroleum ether b.p. 40-60^{o}), $[\alpha]_D^{22}$ + 4^{o} (c, 3.0, $CHCl_3$)
Analysis: Found : C, 45.1; H, 4.8; F, 11.0%. $C_{25}H_{32}F_4O_{16}$ requires C, 45.2 ; H, 4.9 ; F, 11.4%.

The fluoroepilactosyl fluoride (VII), (nc) (1.4g. 23.4%), as an amorphous powder $[\alpha]_D^{22}$ + 20^{o} (c, 2.0, $CHCl_3$). Analysis: Found : C,47.7; H, 5.4; F, 6.6%. $C_{24}H_{32}F_2O_{15}$ requires C, 48.2 ; H, 5.4 ; F, 6.4%.

α-D-Lactosyl fluoride (IV) The peracetate (III) (3.2g, 5mmols) was dissolved in a solution of methanolic ammonia (saturated at 0°, 30 mls), and the resulting mixture kept at room temperature for 24 hrs. The ammonia and methanol were then evaporated under diminished pressure whereupon the product began to crystallise from the residual syrup. The semi-crystalline residue was co-evaporated with methanol several times, then suspended in ethyl acetate, filtered and washed thoroughly with ethyl acetate (to remove acetamide). The product (IV) so obtained, yield 1.4g (80.9%), was pure by t.l.c. (Solvent 3), m.p. 185° dec., $[\alpha]_D^{22}$ + 90° (c, 2.0, H_2O) Lit., [23] m.p. 180-195°, $[\alpha]_D^{15}$ + 83.2° (H_2O). Analysis: Found : C, 42.1; H, 6.1%. $C_{12}H_{21}FO_{10}$ requires C, 41.9; H. 6.2%.

The fluorolactosyl fluoride (VII), (nc) (1.0g, 16.7%) was an amorphous powder $[\alpha]_D^{22}$ + 62°(c, 2.0, $CHCl_3$). Analysis: Found : C, 48.4; H, 5.5; F, 6.3%. $C_{24}H_{32}F_2O_{15}$ requires C,48.2; H, 5.4; F, 6.4%.

Overlapping fractions of the components having R_f 0.36 and 0.32 (i.e. derivatives (VI) and (VII) were combined and rechromatographed on 100g of Keiselgel PF_{254} as previously described elsewhere, using Solvent 2. This gave, in addition to (VII) (yield incorporated in the figure quoted above), the pure fluorolactoside (VI), (nc) (0.4g, 6.0%) also as an amorphous powder, $[\alpha]_D^{22}$ + 168° (c, 0.55, $CHCl_3$). Analysis : Found : C, 45.0; H, 5.0; F, 10.7%. $C_{25}H_{32}F_4O_{16}$ requires C, 45.2; H, 4.9; F, 11.4%.

Trifluoromethyl 2-deoxy-2-fluoro-β-D-"epilactoside" (IX).

3.32g (5mmols) of the epilactoside (V) were de-acetylated as described above, for 36hrs. The crystalline residue was suspended in a mixture of ethyl acetate and methanol, filtered and washed thoroughly with

ethyl acetate. The product obtained was homogeneous on t.l.c. (Solvent 3) (nc) (1.5g, 72%) m.p. 136-8° (dec. above 140°), $[\alpha]_D^{22}$ - 19° (c, 2.0, H_2O). Analysis: Found : C, 37.9; H, 4.9%. $C_{13}H_{20}F_4O_{10}$ requires C, 38.0; H, 4.7%.

2-deoxy-2-fluoro-β-D-epilactosyl fluoride (XI) 1.5g (2.5mmols) of the compound (VII) were deacetylated as described above giving t.l.c. pure product, (nc) (0.5g, 63%), m.p. 165°., $[\alpha]_D^{22}$ - 9° (c,2.0, H_2O) Analysis: Found : C, 41.6; H,5.5%. $C_{12}H_{20}F_2O_9$ requires C, 41.6; H, 5.8%.

2-deoxy-2-fluoro-β-D-lactosyl fluoride (X) 1.5g (2.5mmols) of the peracetylated compound (VIII) were treated as described above, to give t.l.c. pure crystalline product, (nc) (0.6g, 69%) m.p. 170° dec., $[\alpha]_D^{22}$ + 23° (c, 2.0, H_2O) Analysis: Found : C, 41.8; H, 5.5%. $C_{12}H_{20}F_2O_9$ requires C, 41.6; H, 5.8%.

G.l.c. analysis of fluorinated disaccharides Samples (10mg) of each of the acetylated fluoro adducts (III) to (XI), were treated with 3% methanolic HCl solution (anhydrous, 3mls), in a sealed tube at 80° for 24 hrs. After neutralisation with silver carbonate, the solutions were filtered and the solvent removed under diminished pressure. The resulting mixtures of methyl glycosides were silylated using trimethylsilyl chloride/hexamethyl disilizane/pyridine mixture, and aliquots 1μl examined using a temperature programmed gradient of 1°/min from 140° to 228°, with persilylated mannitol as the internal standard. The retention times obtained are shown in Table 3 below, and are compared with those obtained for reference compounds: D-lactose, D-glucose, D-galactose and fluorinated monosaccharides obtained from the reaction of CF_3OF with 3,4,6-tri-O-acetyl-D-glucal.

TABLE 3

GLC - Analysis of Products of Methanolysis of Fluorolactose and Fluoroepilactose Derivatives, compared with Fluorine-Free Analogues.

Substrate	No. of Peaks	Retention Times* of Methanolysis Products	Ratio of Peak Heights** of Products
D-Glucose	2	0.85, 0.89	2.5 : 1
D-Galactose	3	0.68, 0.75, 0.81	1 : 2.3 : 1
D-Lactose	5	0.68, 0.75, 0.81; 0.85, 0.89	1 : 2.3 : 1; 2.5 : 1
Peracylated α- lactosyl Fluoride (III) & Lactosyl Fluoride (IV)	5	0.68, 0.76, 0.81; 0.85, 0.88	1 : 2.3 : 1; 2.5 : 1
A and B (see below)	2	0.50, 0.54	1 : 2.3
Fluorolactosyl Derivatives (VI), (VIII) and (X)	5	0.70, 0.75, 0.81, 0.51, 0.55	1 : 2.3 : 1, 1 : 2.3
C and D (see below)	2	0.46, 0.57	7.5 : 1
Epifluorolactosyl Derivatives (V),(VII),(IX) & (XI)	5	0.68, 0.74, 0.80; 0.47, 0.58	1 : 2.3 : 1; 1.5 : 1

* Relative to persilylated D-mannitol ** Peak Height ratios of sharp symmetrical peaks observed are indicative of ratios of areas under peaks.

A. Trifluoromethyl, 3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-α-D-glucopyranoside. B. 3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl fluoride. C. Trifluoromethyl 3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-mannopyranoside. D. 3,4,6-tri-O-acetyl-2-fluoro-2-deoxy-β-D-mannopyranosyl fluoride.

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