4-DEOXY-4-FLUORO-D-FRUCTOSE: PREPARATION AND STRUCTURE

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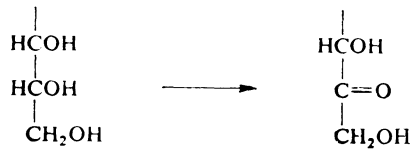
4-Deoxy-4-fluoro-D-fructose (*II*) was prepared by specific hydrogenation of 3-deoxy-3-fluoro-D-mannitol (*I*), using the enzymatic system of *Gluconobacter oxydans*. Using ^{1}H, ^{13}C and ^{19}F NMR spectra its structure and its existence in the pyranose conformation $^{2}C_{5}$(D) in aqueous solution was demonstrated.

One of the methods of study of the biochemistry of sugars consists in the preparation of their fluorinated derivatives, carried out by the replacement of some of the hydroxyl groups (with the exception of the semi-acetal group) by a fluorine atom which possesses similar sterical requirements as the original hydroxyl group. In fluoro derivatives of sugars relatively small conformational changes may be assumed with respect to the parent compounds, which are, however, accompanied by a change of chemical properties[1] and consequently by changed behaviour toward enzymes[2,3]. While these properties of fluoro sugars were exploited in the case of various fluoro derivatives of D-glucose[4], fluoro derivatives of D-fructose have not been investigated so far. In literature 1-deoxy-1-fluoro-D-fructose[5] and 1,6-dideoxy-1,6-difluoro-D-fructose[6] are well described, while only a short mention[7] has been published concerning the phosphates of various deoxyfluoro derivatives of D-fructose. Attempts at the preparation of 4-deoxy-4-fluoro-D-fructose (*II*) from 3,4-anhydro-1,3-O-isopropylidene-β-tagatopyranose were unsuccessful[8]. In this respect methyl 3,4-anhydro-β-D-tagatofuranoside seems promising as starting material, as is evident from the recently published paper by Guthrie and coworkers[9], concerning the synthesis of 4-deoxy- and 4-deoxyhalogen derivatives of D-fructose.

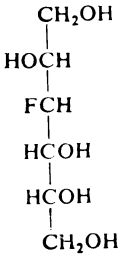
In this study we concentrated on the preparation of 4-deoxy-4-fluoro-D-fructose (*II*) which in view of its ability to exist in the furanose or the pyranose form and owing to the presence of the primary hydroxyl groups could be specifically phosphorylated by fructokinase and then converted enzymatically, (*cf.* refs[1,7,10]). For the preparation of fluorofructose *II* we utilized a similar method, which was found suitable

for the preparation of 5-deoxy-5-fluoro-L-sorbose and which consists in the dehydrogenation of 2-deoxy-2-fluoro-D-glucitol by means of the enzymes from *Acetomonas oxydans*[11]. As substrate 3-deoxy-3-fluoro-D-mannitol (*I*) was used, which was obtained by a multistep synthesis from 1,6-anhydro-β-D-glucopyranose[12]. The fermentation was carried out in the conventional manner after addition of the biomass of *Gluconobacter oxydans* to fluoromannitol *I*. The fermentation mixture which contained, according to paper chromatography, practically a single reducing hexose was worked up in a simple manner, affording fluorofructose *II* in a 79% yield. Dehydrogenation of fluoromannitol *I* took place in the position $C_{(5)}$ in agreement with Bertrand's and Hudson's rule[13] (*cf.* Kulhánek[14]), according to which D-*erythro*-diol arrangement in the neighbourhood of a hydroxymethyl group is dehydrogenated as shown in Scheme 1.

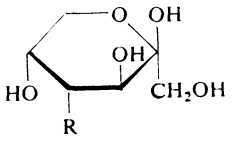


SCHEME 1

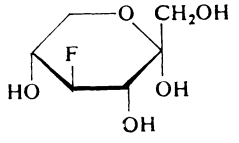
The structure of 4-deoxy-4-fluoro-D-fructose (*II*) was demonstrated by means of ^1H, ^{13}C and ^{19}F NMR spectra in ^2H_2O (see the data in Table I where the NMR data for D-fructose and 4-deoxy-4-fluoro-α-D-sorbopyranose (*IV*) from literature are



I



II, R = F
III, R = OH



IV

given for comparison). The proton decoupled ^{13}C NMR spectrum showed the presence of six carbon atoms of which each was split to a doublet (intensity ratio 1 : 1) by coupling with fluorine. The value of these interactions is considerably different (from 1·6 to 180 Hz), in dependence on the number of the bonds separating the interacting carbon and fluorine atoms. The number of protons directly bound to individual carbons was determined from the "attached proton test ^{13}C NMR spectra"[15]

TABLE I

NMR Parameters of 4-deoxy-4-fluoro-β-D-fructopyranose (*II*), D-fructose (*III*) and 4-deoxy-4-fluoro-α-D-sorbopyranose (*IV*) in ^2H_2O

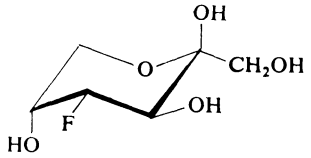
Compound	^{1}H NMR						
	H-1	H-1′	H-3	H-4	H-5	H-6(ax)	H-6′(eq)
II	3·716	3·555	4·064	4·783	4·258	4·028	3·746
	d	dd	dd	ddd	m	dt	ddd
	$J_{1,1'} = -11{\cdot}8$	$J_{1,1'} = -11{\cdot}8$	$J_{3,4} = 9{\cdot}75$	$J_{4,3} = 9{\cdot}75$	$J_{5,4} = 3{\cdot}5$	$J_{6',6} = -13{\cdot}0$	$J_{6',6} = -13{\cdot}0$
		$J_{1',F} = 1{\cdot}8$	$J_{3,F} = 12{\cdot}85$	$J_{4,5} = 3{\cdot}5$	$J_{5,6} = 1{\cdot}6$	$J_{6,5} = 1{\cdot}6$	$J_{6',F} = 7{\cdot}8$
				$J_{4,F} = 48{\cdot}95$	$J_{5,6'} = 2{\cdot}0$	$J_{6,F} = 1{\cdot}3$	$J_{6',5} = 2{\cdot}0$
					$J_{5,F} = 6{\cdot}5$		
III[a]	3·71	3·57	3·80	3·89	3·99	4·03	3·71
	d	d	d	dd	m	dd	dd
	$J_{1,1'} = -11{\cdot}8$	$J_{1',1} = -11{\cdot}8$	$J_{3,4} = 10{\cdot}0$	$J_{4,3} = 10{\cdot}0$	$J_{5,4} = 3{\cdot}2$	$J_{6,6'} = -12{\cdot}4$	$J_{6',6} = -12{\cdot}4$
				$J_{4,5} = 3{\cdot}2$	$J_{5,6} = 1{\cdot}3$	$J_{6,5} = 1{\cdot}3$	$J_{6',5} = 1{\cdot}8$
					$J_{5,6'} = 1{\cdot}8$		

Compound	^{13}C NMR					
	C-1	C-2	C-3	C-4	C-5	C-6
II[b]	63·58	98·62	66·17	91·58	67·49	62·69
	$J_{C,F} = 1{\cdot}6$	$J_{C,F} = 9{\cdot}7$	$J_{C,F} = 18{\cdot}3$	$J_{C,F} = 180{\cdot}1$	$J_{C,F} = 16{\cdot}6$	$J_{C,F} = 7{\cdot}0$
	$J_{C,H-1} = 144{\cdot}0$		$J_{C,H-3} = 146{\cdot}6$	$J_{C,H-4} = 153{\cdot}4$	$J_{C,H-5} = 148{\cdot}3$	$J_{C,H-6} = 144{\cdot}0$
	$J_{C,H-1'} = 144{\cdot}0$					$J_{C,H-6'} = 151{\cdot}2$
III[c]						
β-*p*	64·9	99·0	68·6	70·6	70·1	64·3
β-f	63·7	102·4	76·4	75·4	81·6	63·3
α-p	66·1	[d]	71·1	71·4	[d]	66·1
α-f	63·7	105·3	82·9	77·0	82·3	62·1
IV[e]	64·2	99·1	69·8	96·8	68·8	61·8
		$J_{C,F} = 10$	$J_{C,F} = 17$	$J_{C,F} = 178$	$J_{C,F} = 17$	$J_{C,F} = 10$

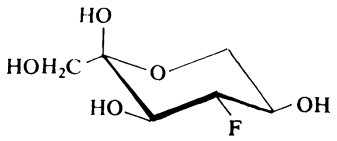
[a] Data for β-D-fructopyranose taken from ref.[20]. [b] Only one-bond carbon-proton coupling constants are presented. [c] Data taken from ref.[18]. [d] Chemical shift was not given in ref.[18]; it might be ~100 (ref.[19]). [e] Data are taken from ref.[8].

The two signals at the highest field (in δ 62·69 and 63·58) belong to the carbons of the CH_2 type. The values of their coupling constants with the fluorine in position 4 can be utilized for the following assignments: the signal with $J_{C,F} = 7{\cdot}0$ Hz belongs to the carbon $C_{(6)}$ (J over three bonds), while the signal with $J_{C,F} = 1{\cdot}6$ Hz belongs to the carbon $C_{(1)}$ (J over four bonds). The observed values of $^1J_{C,H}$ in the proton nondecoupled ^{13}C NMR spectrum are in agreement with this assignment: for the $C_{(1)}$ carbon of the more mobile CH_2OH group with both directly attached hydrogens the J values are equal (144 Hz), while for the $C_{(6)}$ carbon, which is a part of a rigid cycle and the hydrogens of which have an axial or an equatorial character, the J values differ (144·0 or 151·2 Hz, respectively). Three further signals at lower field (δ 66·17, 67·49 and 91·58) belong to the methine carbons. From the known distinct deshielding effect of the fluorine in the position α (ref.[16]) (about 63 ppm in contrast to the OH group which gave about 41 ppm) and from the value of $J_{C,F} = 180{\cdot}1$ Hz the signal at 91·58 can be assigned unambiguously to the carbon $C_{(4)}$, carrying fluorine. The assignment of the carbons $C_{(3)}$ and $C_{(5)}$ with similar shifts and $J_{C,F}$ values, equal to 18·3 and 16·6 Hz, respectively, is more difficult. On comparison with the chemical shifts of these carbons in β-D-fructofuranose[17,18] ($C_{(3)}$ at a slightly higher field than $C_{(5)}$) and under the assumption of an approximately equal effect of the substitution of the hydroxyl by fluorine in β-position (about -2 ppm)[16] we assigned the signal at δ 66·17 to the carbon $C_{(3)}$ and the signal at δ 67·49 to the carbon $C_{(5)}$. The last signal in the spectrum, at δ 98·62, belongs to the quaternary tetrasubstituted carbon. Its coupling constant is $J_{C,F} = 9{\cdot}7$ Hz and it can be assigned unambiguously to the carbon $C_{(2)}$. In contrast to D-fructose, where it was found by means of ^{13}C NMR in addition to the dominant β-pyranose (70%)[17–19] also 23% of β-furanose and a small amount of α-pyranose (2%) and α-furanose (5%), we demonstrated in 4-fluoro derivative *II* only a single form, *i.e.* the β-pyranose. The β-configuration of the OH group on $C_{(2)}$ follows from the observed values of $^3J_{C-1,H-3} = 1{\cdot}6$ which is in agreement with the gauche arrangement of $C_{(1)}$ and $H_{(3)}$ and which excludes the α-anomer, where $C_{(1)}$ and $H_{(3)}$ would be *trans*-diaxial with a value of $^3J_{C-1,H-3}$ 6 Hz (ref.[16], p.56). This is also true for $^2C_5$(D) conformation which was demonstrated by means of ^{1}H NMR spectra. The chemical shifts and the coupling constants $J_{C,F}$ in the ^{13}C NMR spectrum of fluorofructose *II* correlated very well with the coresponding parameters[8] of 4-deoxy-4-fluoro-β-D-sorbopyranose (*IV*). Both compounds, *i.e.* fluorofructose *IIa* in the conformation $^2C_5$(D) and fluorosorbose *IVa* in the conformation $^5C_2$(D), disregarding the chirality at $C_{(5)}$, are enantiomeric, which will be understandably reflected in the similarity of their ^{13}C NMR spectra.

The ^{1}H NMR spectrum of compound *II* (Fig. 1*a*) also shows that this compound exists in ^2H_2O in practically a single conformation, $^2C_5$(D). Repeated measurements confirmed that the part of the conformers in the solution does not change with time. The spectrum has a pseudo first-order character with a partial overlapping of the



IIa, $^2C_5$(D)



IVa, $^5C_2$(D)

H-3 and H-6 or H-1 and H-6′ signals. On analysis of the spectra the values of the chemical shifts and the coupling constants of all hydrogens were obtained and the parameters were made more accurate by the simulation-iterative procedure (calculated spectrum – Fig. 1*b*). The values of the chemical shifts of the hydrogens H-1, H-1′, H-6′ and of all proton-proton interactions are very close to the values described for β-D-fructofuranose. The signals of H-4 and – to a lesser extent – of H-3 andH-5 are shifted downfield by the substitution with fluorine. The value $J_{3,4} = 9{\cdot}75$ Hz indicates an antiperiplanar orientation of the H-3 and H-4 protons and hence the $^2C_5$(D) conformation. All the hydrogens, with the exception of H-1, interact with fluor-

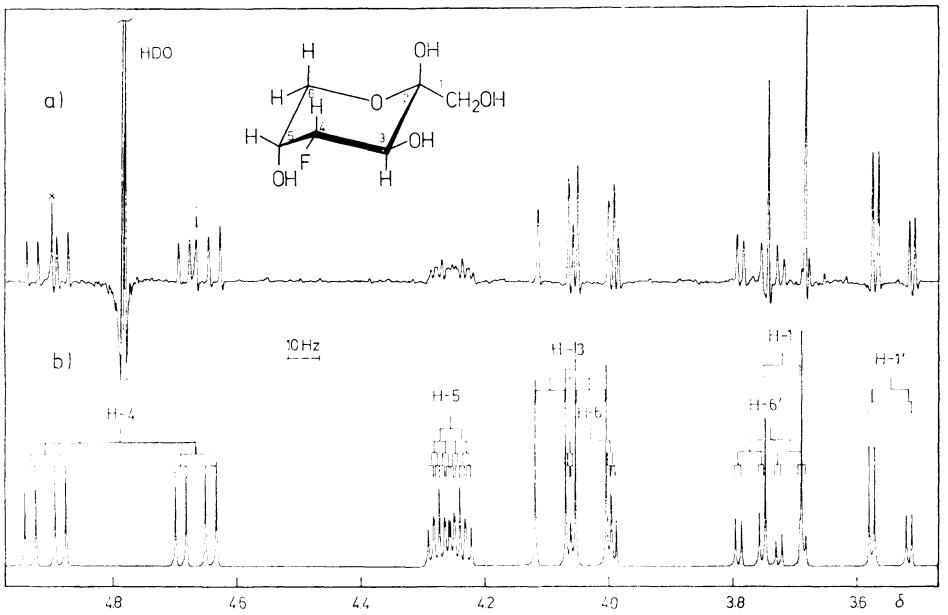


FIG. 1

^{1}H NMR spectrum of 4-deoxy-4-fluoro-β-D-fructopyranose (*II*): *a*) experimental spectrum in ^2H_2O; *b*) simulated spectrum calculated from the parameters given in Table I

rine. The $J_{H,F}$ couplings were determined from the hydrogen spectrum and confirmed by the measurement of the ^{19}F NMR spectrum. The proton decoupled ^{19}F NMR spectrum affords a singlet at δ 201·9 while a complex multiplet with splittings corresponding the $J_{H,F}$ couplings determined from the ^1H NMR spectrum is observed without a proton decoupling. The geminal coupling constant $J_{H,F}$ = = 48·95 Hz confirms the position of fluorine on $C_{(4)}$. The long-range interactions of fluorine over four bonds, with H-6 and H-6', the values of which 1·7 and 7·8 Hz permit an unambiguous assignment of H-6 to the axial and H-6' to the equatorial hydrogen are of importance. Of them only the second has a planar zig-zag coupling pathway leading to a high $J_{H,F}$ value. The non-zero $^5J_{H,F}$ (1·3 Hz) coupling constant for one of the hydrogen atoms on $C_{(1)}$ indicates a restricted rotation around the $C_{(1)}$—$C_{(2)}$ bond, evidently in consequence of the intramolecular H-bridge between $C_{(1)}$—OH and $C_{(3)}$—OH or $O_{(6)}$.

The ^1H NMR spectrum of compound *II* in hexadeuteriodimethyl sulfoxide indicates the existence of several forms at equilibrium, the proportion of which could not be determined. Similarly as in D-fructose[20–22] a different behaviour may thus be found for 4-deoxy-4-fluoro-D-fructose (*II*) in ^2H_2O and hexadeuteriodimethyl sulfoxide solutions.

EXPERIMENTAL

The melting points were determined on a micromelting point apparatus according to Boëtius. The optical rotation was measured on a Bendix–Ericsson ETL 143A polarimeter. The ^1H and ^{13}C NMR spectra were measured on a pulse FT- NMR spectrometer Varian XL-200 using solutions of about 20 mg of substance in 0·4 ml of ^2H_2O and sodium 4,4-dimethyl-4-silapentane--1-sulfonate as internal reference (δ-scale, coupling constants in Hz). Conditions of the measurement: ^1H NMR (200 MHz); spectral width 2000 Hz, pulse width 4 μs (flip angle 45°), acquisition time 4 s, data points 16 000, 20 transients accumulated. For resolution enhancement the exponential multiplication and Gaussian apodization function with parameters RE = 0·3 and AF = 0·9 was used. The computation of the spectrum of compound *II* was carried out using the standard program SPINI (LAME version), which is a component of the spectrometer software. In view of the limitation of the program to a maximum of 6 non-equivalent spins the simulation was carried out in two parts for ABCDEF systems: *1*) H-1, H-1', H-3, H-4, H-5 and F, *2*) H-6, H-6', H-5, H-4, H-3 and F. Fig. 1*b* shows the part *2*) for the hydrogens H-3, H-4, H-5 and H-6, and the summation of the parts *1*) and *2*) for the hydrogens H-1, H-1' and H-6'. The input values were obtained by a 1st order analysis and the parameters are made accurate by the iterative procedure. The resulting values are given in Table I.

^{13}C NMR (50·31) MHz: spectral width 3 200 Hz, pulse width 10 μs (flip angle 60°), acquisition time 5 s, 32 000 data points.

a) For proton decoupled spectrum 500 transients were accumulated without any sensitivity enhancement function. *b*) For attached proton test described pulse sequence[15] was used and 3 000 transients accumulated without weighting of FID. *c*) For proton nondecoupled spectrum the irradiation of protons during 5 s out of acquisition time (for NOE establishment) was used and 2 600 transients accumulated with sensitivity enhancement (exponential multiplication — line broadening 0·4 Hz).

^{19}F NMR spectrum was measured on a Varian XL-100 (94·1 MHz) spectrometer and referenced to the external CF_3COOH. The chemical shift was calculated to $CFCl_3$ using the equation $\delta CF_3COOH = -78{\cdot}5$ ppm[23].

4-Deoxy-4-fluoro-D-fructose (*II*)

3-Deoxy-3-fluoro-D-mannitol[12] was fermented at 32°C under aerobic conditions by means of *Gluconobacter oxydans* CCM 2370 (kept for a long period in lyophilized form[24]). Before use the culture was reinoculated twice on a slanted agar medium containing 5 g D-glucose, 2 g of calcium carbonate, an autolysate from 2·5 g of baker's yeast and 2·5 g of agar in 100 ml of water; growth duration: 2 days. The culture obtained in this manner was used for inoculation of 80 ml of a medium containing 4 g D-glucitol and an autolysate from 2 g of baker's yeast. After two days of cultivation on a longitudinal shaker at 2 Hz frequence and 9 cm amplitude the grown biomass was centrifuged at 3 000 *g* and washed twice by stirring with 80 ml of sterile distilled water and centrifuging. The washed biomass was added into 17 ml of a solution containing 319 mg of 3-deoxy-3-fluoro-D-mannitol (*I*) and 3·2 mg of D-chloramphenicol in sterile distilled water, and put in a flask provided with a cotton wool stopper onto a shaker. The fermentation course was checked by paper chromatography on Whatman paper No 1 impregnated with 3% aqueous metaphosphoric acid in water saturated 2-butanone; detection was carried out with sodium periodate and ammoniacal silver nitrate solution or aniline hydrogen carbonate. After one-day fermentation the mixture contained only the reducing substance *II* (R_F 0·18; R_F of the starting compound *I* is 0·12). After filtration and concentration under reduced pressure the syrup obtained (0·27 g, 85%) was dissolved in 10 ml of methanol, refiltered, the solution evaporated and the residue (0·25 g, *i.e.* 79%) was dissolved in a mixture of methanol and ethyl acetate and the solution was evaporated again. The residual syrup crystallized. It was recrystallized twice from ethanol to give 187 mg (59%) of 4-deoxy-4-fluoro-D-fructose, m.p. 123–126°C, $[\alpha]_D$ −116° (*c* 0·45, water). The substance reduces Fehling's reagent on heating. For $C_6H_{11}FO_5$ (182·2) calculated: 39·56% C, 6·08% H, 10·43% F; found: 39·56% C, 5·88% H, 10·55% F.

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