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SYNTHESIS AND CONFORMATIONAL ANALYSIS OF THE N^1- AND N^3-5-FLUOROURACIL NUCLEOSIDES OF 4-DEOXY-L-*threo*-HEX-4-ENOPYRANURONIC ACID

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ABSTRACT

4′,5′-Unsaturated nucleosides are obtained by the action of 1,5-diazabicyclo-[5.4.0]undec-5-ene on N^1- and N^3-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl-uronate)-5-fluorouracil. The $^2H_1$ conformation of N^1- and N^3-(methyl 4-deoxy-α-L-*threo*-hex-4-enopyranosyluronate)-5-fluorouracil has been established by ^{1}H-n.m.r. and c.d. methods. Interaction of the heterocyclic base and the double bond of the sugar moiety is demonstrated.

INTRODUCTION

Nucleosides containing double bonds in the carbohydrate moieties can be used to study electron interactions through space. Peculiarities in the spectra of unsaturated nucleosides appear to be due to such interactions. The high polarisability of the π-electron clouds allows such interaction to be observed by c.d. and u.v. spectroscopy, the use of which has revealed some spectral anomalies for this class of compound.

The synthesis and properties of nucleosides having a double bond in different positions of the sugar moiety have been studied[1], and we have described[2] the synthesis of nucleosides of 4-deoxyhex-4-enopyranuronic acid.

We have now used ^{1}H-n.m.r., u.v., and c.d. spectroscopy to study the conformations and spectral characteristics of the unsaturated nucleosides obtained[3] from N^1- (**1**) and N^3-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-5-fluorouracil (**2**).

The β-elimination reaction used in the synthesis of α,β-unsaturated uronic acids proceeds smoothly when the acyl or alkoxyl group and the α-hydrogen are *trans*-diaxial[4]. When these functions are axial–equatorial, more drastic reaction conditions are necessary and the use of 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) as the base in chloroform or pyridine gives good results[5].

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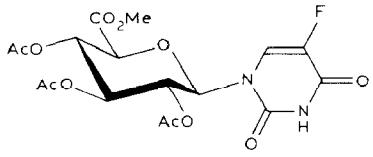
RESULTS AND DISCUSSION

Treatment of **1** and **2** severally with DBU in chloroform for 24 h at room temperature and column chromatography of the products gave N^1- (**3**) and N^3-(methyl 2,3-di-*O*-acetyl-4-deoxy-α-L-*threo*-hex-4-enopyranosyluronate)-5-fluorouracil (**4**). Deacetylation with methanolic hydrogen chloride then yielded the nucleosides **5** and **6**.



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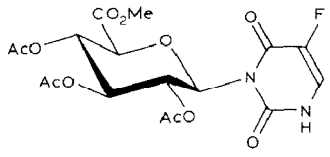

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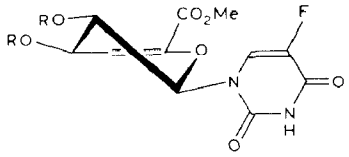

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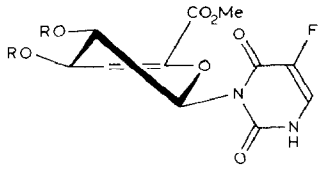
5 R = H



4 R = Ac
6 R = H

The structures of **3–6** followed from the ^{1}H-n.m.r. and u.v. data. In the ^{1}H-n.m.r. spectrum of the N^1-nucleoside **3**, the d for H-1′ (6.36 p.p.m., see Table I) reflected long-range coupling ($J_{1',F}$ 1.25 Hz), and $J_{1',2'}$ 9.5 Hz indicated H-1′,2′ to be *trans*-diaxial. The dd for H-2′ ($J_{2',3'}$ 7.5 Hz) is typical[6] for hex-4-enopyranuronosides in the $^2H_1$ half-chair conformation. Also, H-4′ was deshielded by the double bond and its signal was shifted downfield (6.12 p.p.m.). The value of the vinylallylic coupling $J_{3',4'}$ 2.75 Hz is characteristic of the quasi-axial orientation of H-3′. Thus, **3** is in the $^2H_1$ conformation with the heterocycle equatorial.

The β-glycosides of 4-deoxy-L-*threo*-hex-4-enopyranuronates exist[6] in the $^2H_1$, and the α-glycosides in the $^1H_2$, half-chair conformation. In the $^2H_1$ conformation, destabilising factors are absent (MeO-1 is axial, no anomeric effect[7], other substituents are equatorial). The $^1H_2$ conformation is stabilised by the allylic effect[8], which counters the destabilising effect of the axial substituents at C-2 and C-3, and MeO-1 is axial. Thus, the conformational preference is determined by the anomeric or allylic effects.

The existence of the α-L-nucleoside **3** in the $^2H_1$ conformation is in contrast to the $^1H_2$ conformation of α-L-glycosides and may be due to a low value of the anomeric effect of the equatorially oriented heterocyclic base and/or the anomeric

TABLE I

^{1}H-N M R SPECTRAL DATA FOR DERIVATIVES OF D-GLUCOPYRANURONIC AND 4-DEOXY-L-*threo*-HEX-4-ENOPYRANURONIC ACIDS

Compound	*Solvent*	*Chemical shifts*								*Coupling constants*					
		H-1'	*H-2'*	*H-3'*	*H-4'*	*H-5'*	*H-6*	*COO*Me	*OCO*Me	*1,2*	*2,3*	*3,4*	*6,F*	*1',F*	*6,NH*
1	$CDCl_3$	6.04dd	5.42dd	5.56dd	5.30dd	4.26d	8.00d	3.72	2.04 2.00	8.5	9.0	9.0	6.5	1.0	—
2	$CDCl_3$	6.30d	6.04dd	5.54dd	4.98dd	4.68d	7.86dd	3.68	2.00 1.98 1.90	9.0	9.0	9.0	6.0	—	6.0
3	$CDCl_3$	6.36dd	5.42dd	5.82dd	6.12d	—	7.46d	3.86	2 12	9.5	7.5	2.75	5.5	1.25	—
4	$CDCl_3$	5.62d	6.20dd	5.76dd	6.00d	—	7.36dd	3.84	2.08 2.00	9.5	7.5	2.5	5.5	—	5.5
5	D_2O	6.08dd	3.92dd	4.56dd	6.08d	—	8.00d	3.80	—	10.0	7 5	2.5	6.0	1.5	—
6	D_2O	6.32d	4.66dd	4.48dd	6.06d	—	7.70d	3.80	—	9.5	7.0	2.25	5.0	—	—
7	D_2O	5.85d	3.90dd	4.36dd	5.90d	—	7.90d	3.80	—	9.0	7.5	2.5	5.6	—	—
8	D_2O	5.10d	3.84dd	4.34dd	6.13d	—	—	—	—	2.5	7.2	3.0	—	—	—
9	$CDCl_3$	5.10–5.24m		5.60dd	6.06d	—	—	3.80	2.12 2.08	2.5	8.0	2.8	—	—	—

effect being countered by the interaction of the heterocycle and the 4,5-double bond conjugated with the methoxycarbonyl group.

In the ^{1}H-n.m.r. spectrum of the N^3-nucleoside **4**, the J values (see Table I) characteristic of the N^1-nucleoside **2** are mainly retained. Both the N^1- and N^3-isomers exist in the $^2H_1$ conformation.

The signal for H-1′ in the spectrum of the N^3-isomer **4** was a d (no coupling with fluorine) and that for H-6 was a dd owing to coupling with the fluorine and the proton of the NH-group (each J is 5.5 Hz).

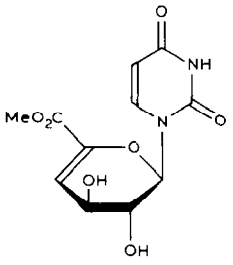
Comparison of the chemical shifts of the signals for the protons in **3–6** reveals that, in the N^1-isomers, the signal for H-2′ is shifted upfield in relation to that for H-3′, whereas the reverse is true for the N^3-isomers. A similar situation is found for **1** and **2** (see Table I). The relative shifts of the signals for H-2′ and H-3′ in the isomeric N^1- and N^3-nucleosides may reflect the deshielding effect of the 2-ketone group of the pyrimidine heterocycle. In N^3-nucleosides of the pyrimidine series, there is no preferential *syn*- or *anti*-conformation at the glycoside linkage, since both ketone groups are *ortho* to the glycosidic nitrogen.

The N^1-nucleosides have a preferential *anti*-conformation around the glycosylic linkage which does not exclude local energy minima for the *syn*-conformer and dynamic equilibrium between the *anti*- and *syn*-conformations. Thus, in the N^3-nucleosides, the deshielding action of the ketone groups on H-2′ may occur in each conformation whereas, in the N^1-nucleosides, such deshielding is possible only in, but is not typical for, the *syn*-conformation. Also, H-3′ is remote from the pyrimidine heterocycle and is less deshielded.

The degree of deshielding of H-1′ in the N^3-isomers must also be greater than in N^1-isomers since the probability of populating the *anti*-conformation in the latter is not equal to one, and in the N^3-isomers the ketone groups of the heterocycle in each conformation may deshield H-1′.

The above interpretation accords with the experimental data. The signals for H-1′ of the N^3-isomer of each pair of nucleosides have a large chemical shift in comparison with those of the N^1-isomers, whereas the chemical shifts for the signals for H-3′ in each pair are similar. Comparison of the chemical shifts of these protons in nucleoside **5**, N^1-(methyl 4-deoxy-α-L-*threo*-hex-4-enopyranosyluronate)uracil[2] (**7**), and methyl (methyl 4-deoxy-β-L-*threo*-hex-4-enopyranosid)uronate[5,6] (**8**) (see Table I) reflects the deshielding of H-1′ by the nucleoside ketone groups. Moreover, the chemical shifts for the signals of H-2′ and H-3′ of **5**, **7**, and **8** are similar, which points to the existence of the N^1-nucleosides **3** and **7** being mainly in the *anti* conformation.

In the u.v. spectra of 3′,4′-unsaturated nucleosides of pentofuranuronic acid[1,9], there is a hypsochromic shift (5–12 nm) of the λ_{max} of the pyrimidine chromophore and the spectra are not simple superpositions of those of the related saturated nucleoside and the unsaturated sugar[1]. This difference may reflect the interaction of the heterocycle and the double bond conjugated with the alkoxycarbonyl group.



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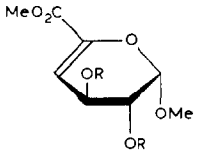

8 R = H
9 R = Ac

In the u.v spectra of nucleosides **1** and **2** (see Table II), the λ_{max} of the 5-fluorouracil chromophore is observed at 262 and 272 nm, respectively, and in the unsaturated nucleosides **3** and **4** it is at 258 and 273 nm, respectively. The hypsochromic shift (4 nm) of λ_{max} occurs only in the N^1-series, which may be a result of different orientation of the π-electron clouds of the 5-fluorouracil chromophores in the N^1- and N^3-isomers in relation to the double bond of the sugar moiety.

The second λ_{max} in the spectra of **3** and **4** in the region of 246 nm appears to belong to the electron transition of the heterocycle interacting through space with the double bond conjugated with the methoxycarbonyl group.

Methyl (methyl 2,3-di-*O*-acetyl-4-deoxy-β-L-*threo*-hex-4-enopyranosid)uro-

TABLE II

U V AND C D SPECTRAL DATA FOR DERIVATIVES OF D-GLUCOPYRANURONIC AND 4-DEOXY-L-*threo*-HEX-4-ENOPYRANURONIC ACIDS

Compound	*Solvent*	*U.v.*		*C.d.*
		max ($\varepsilon \times 10^{-3}$)	*min* ($\varepsilon \times 10^{-3}$)	($[\theta] \times 10^{-3}$)
1	MeOH	262 (9.3)		268 (10.0)
2	MeOH	272 (6.0)		259 (2.2)
3	MeOH	208 (9.7)	227 (7.6)	215 (−2.8)
		246 (8.4)		242 (15.3)
		258 sh (7.8)		265 sh (5.1)
4	MeOH	208 (6.5)	230 (4.5)	215 (−2.3)
		246 (5.0)	258 (4.9)	242 (11.4)
		273 (5.5)		270 (−2.3)
5	MeOH	211 (8.7)	228 (6.1)	242 (12.2)
		255 (10.3)		265 (0)
				280 (2.0)
6	MeOH	212 (5.2)	228 (4.4)	242 (17.4)
		253 (7.6)		255 (0)
		270 sh (6.7)		265 (−9.4)
7	H_2O	250 (14 4)		215 (−4.0)
				242 (20.5)
9	EtOH	233 (5.2)		230 (18.0)

nate[5] (**9**, the u.v. spectrum is not given in ref. 5) was synthesised in two stages from methyl (methyl 2,3,4-tri-*O*-benzoyl-α-D-galactopyranosid)uronate[10] and had λ_{max} 233 nm. Since **3**, **4**, and **9** have similar conformations and the main distinction is the absence of the second chromophore in **9**, the bathochromic shift (13 nm) of the λ_{max} of the unsaturated sugar chromophore in **3** and **4** reflects the interaction of two chromophores through space.

In the c.d. spectrum of **3** (see Table II) the B_{2u}-band of the 5-fluorouracil residue appears as a shoulder at 265 nm (positive Cotton effect). The optically active absorption band at 242 nm (positive Cotton effect) belongs to the double bond of the sugar moiety interacting with the electrons of the heterocycle. In the c.d. curve of the unsaturated sugar **9**, the absorption band of the conjugated double bond is in the region of 230 nm (positive Cotton efect). In the spectrum of **4**, the B_{2u}-band at 270 nm has a negative and that at 242 nm has a positive Cotton effect. The c.d. curves of the members of the pairs of nucleosides **3,5** and **4,6** are similar.

In the unsaturated nucleosides **3–7** and the sugar derivative **9**, the configuration at C-3 is dextrorotatory according to the rules of Brewster[11] and Mills[12]. Therefore, the optically active absorption band in the c.d. spectra of **3–7** and **9** are at 242 and 230 nm, respectively, and have a positive Cotton effect.

EXPERIMENTAL

^{1}H-N.m.r. spectra (internal Me_4Si) were recorded with a JNM PS-100 spectrometer (Jeol). U.v. spectra were recorded with a Zeiss Specord UV-Vis spectrophotometer. C.d. spectra and optical rotations were determined on a JASCO-20 spectropolarimeter (JASCO). Reactions were monitored, and the purity of products was assessed, by t.l.c. on Silufol 254 with chloroform–methanol (95:5, 4:1). Melting points were measured on a Boetius hot-stage unit.

N^1-(**3**) *and* N^3-*(methyl 2,3-di-*O*-acetyl-4-deoxy-α-*L*-threo-hex-4-enopyranosyluronate)-5-fluorouracil* (**4**). — 1,5-Diazabicyclo[5.4.0]undec-5-ene (0.6 g, 4 mmol) was added to a solution of *N*^{1}-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-5-fluorouracil[3] (0.9 g, 2 mmol) in chloroform (25 mL). The mixture was kept at room temperature for 24 h and concentrated. A solution of the residue in a small volume of chloroform was added to a column of silica gel L (100/160 μ, 50 g). Elution with chloroform–methanol (95:5) gave **3** (0.6 g, 77.7%), isolated as an amorphous powder having $[\alpha]_D^{20}$ +104° (*c* 1, ethanol).

Anal. Calc. for $C_{15}H_{15}FN_2O_9$: C, 46.63; H, 3.88; N, 7.25. Found: C, 46.32; H, 3.60; N, 6.98.

In a similar way, the *N*^{3}-isomer[3] (0.9 g) was converted into **4** (0.56 g, 72.5%), $[\alpha]_D^{20}$ +25° (*c* 2.6, ethanol).

Anal. Found: C, 46.25; H, 3.69; N, 6.92.

N^1-(**5**) *and* N^3-*(methyl 4-deoxy-α-*L*-threo-hex-4-enopyranosyluronate)-5-fluorouracil* (**6**). — Acetyl chloride (0.5 mL) was added to a solution of **3** (0.39 g; 1 mmol) in anhydrous methanol (10 mL). The mixture was kept at room tempera-

ture for 3 days, then neutralised with pyridine, and concentrated to dryness. A solution of the residue in a small volume of acetone was placed on a column of silica gel (25 g) and eluted with chloroform–methanol (4:1) to yield **5** (0.26 g, 85%), m.p. 205–210° (from ethanol, $[\alpha]_D^{20}$ +30° (*c* 1, water).

Anal. Calc. for $C_{11}H_{11}FN_2O_7$: C, 43.70; H, 3.64; N, 9.27. Found: C, 43.46; H, 3.46; N, 9.09.

In the same way, **4** (0.39 g) was converted into **6** (0.24 g, 80%), m.p. 133–135°, $[\alpha]_D^{20}$ −7° (*c* 1, water).

Anal. Found: C, 43.40; H, 3.36; N, 9.00.

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