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PRELIMINARY COMMUNICATIONS

STRUCTURE OF TWO HYDROXYLATED METABOLITES OF FTORAFUR

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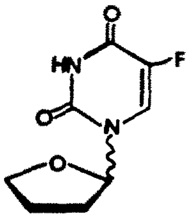
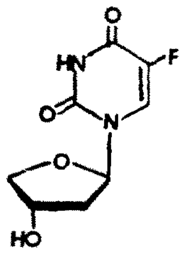
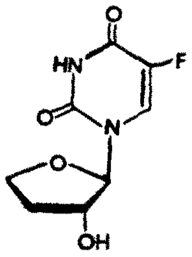
Recent investigations by Sadee and coworkers on the mechanism of activation of the anti-cancer drug 5-fluoro-1-(tetrahydrofuran-2-yl)-uracil (ftorafur) in rats and rabbits [1] and in humans [2] have shown the existence of some hydroxylated metabolites. We now report the total stereospecific synthesis of 1-(2-deoxy-β-D-*glycero*-tetrafuranosyl)-5-fluorouracil (I) and 1-(3-deoxy-β-D-*glycero*-tetrofuranosyl)-5-fluorouracil (II), their equivalence with the ftorafur metabolites by chromatographic and spectral comparison, and the absolute configuration of one of the metabolites.



Ftorafur

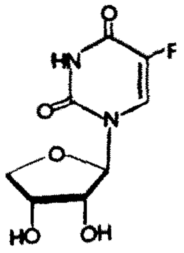


I

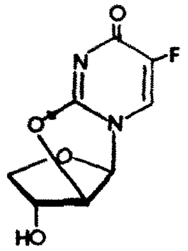


II

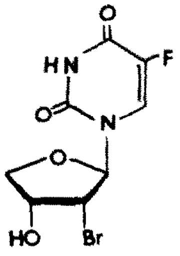
Chemistry.* D-Erythrose was prepared by the method of Perlin [3] and converted to 1,2,3-tri-*O*-acetylerythrofuranose by the method of Andersson *et al*. [4]. Condensation of this sugar with the bis(trimethylsilyl) derivative of 5-fluorouracil in dry acetonitrile with $SnCl_4$ catalyst [5] gave, after NaOMe deacetylation and silica gel chromatography, blocked 1-β-D-erythrofuranosyl-5-fluorouracil (III, 19%), m.p. 183-185°, which was obtained upon its crystallization from the column fractions. UV λ_{max} (pH 1) 269.5 nm (ε = 9010); (pH 13) 270 (6790); $[\alpha]_D$ - 46.0 (c 3.0 w/v, H_2O).



III



IV



V

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[*] Compounds I-IV gave satisfactory elemental analyses within ±0.4%. Ultraviolet spectra were run on a Cary 118, n.m.r. spectra were run on a Varian FT-80 at 80 MHz, and c.d. spectra on a Jasco J-15. Melting points are uncorrected. Preparative t.l.c. was on AnalTech Anasil HF plates.

When III was treated with diphenyl carbonate in N,N-dimethylformamide in the presence of $NaHCO_3$ at 150° [6] and the resultant solution poured into ether, 2,2'-anhydro-5-fluoro-β-D-threofuranosyluracil (IV) was obtained in 75% yield after recrystallization from methanol; m.p. 252-254° (d); UV λ_{max} (pH 1, 7) 254.5 nm (ε = 9020), (pH 13) 256.5 (7300). Treatment of IV with HBr/DMF[7] gave the intermediate 1-(2-bromo-2-deoxy-β-D-erythrofuranosyl)-5-fluorouracil (V) which was purified by silica gel column chromatography (ethyl acetate). A yield of 57% of chromatographically homogeneous V was obtained which was used directly for the next step. A solution of V in 1:2 H_2O/methanol (v/v) and sodium acetate was shaken with hydrogen at 50 p.s.i. over a $Pd/BaSO_4$ catalyst [8] to give, after preparative silica gel thin-layer chromatography (ethyl acetate), 35% of I, m.p. 173-175° (recrystallization from ethanol); UV λ_{max} (pH 1, 13) 269.5 nm.

The action of propionyl bromide on 5-fluorouridine has been reported to give 2'-bromo-2'-deoxy-5-fluorouridine, presumably via a 2,2'-anhydro derivative [8]. We have found that the tetrofuranose nucleoside III did not react in the same way, however. When III was treated with propionyl bromide [9] and the resulting mixture of products subjected to catalytic hydrogenation over 5% $Pd/BaSO_4$, followed by sodium methoxide deacylation, five compounds were observed on t.l.c. (silica gel, ethyl acetate). These were unreacted III, 5-fluorouracil, I, II, and IV. The major product, the 3'-deoxyisomer II, was isolated in 22% yield by preparative t.l.c. (silica gel, methanol/chloroform, 1/7); m.p. 168-170° (d) (recrystallization from methanol); UV (pH 1) 270.5 nm (ε = 9260), (pH 13) 270 (6525); $[\alpha]_D^{25}$+ 22.9 (c, 1.5 H_2O).

Structures of the products. The structure of diol III as the β-D-erythro isomer is evident both from the method of synthesis [5] and from the subsequent formation of IV from it. The differentiation of the structures of the two deoxy nucleosides, I and II, was made by examination of the n.m.r. spectra.

The pertinent shifts and coupling constants are given in Table 1. All compounds except anhydro nucleoside IV gave a small long-range coupling of the $H_{1'}$ to F of 1.3-1.9 Hz. The structure of IV was readily assigned as the 2,2'-anhydro isomer due to the distinct downfield location of the signal for $H_{2'}$, and is in accord with spectra of similar anhydro compounds in the pentofuranoside series [9]. Compound I exhibits the strong coupling of the anomeric proton with two 2' protons, as is commonly observed in 2'-deoxy-β-nucleosides [10].

The identity of II was at first perplexing because of its unexpected appearance and its unusual n.m.r. spectrum. It has been noted, however, that trans-2,3-disubstituted-tetrahydrofurans frequently show $J \leq 1$ Hz between the 2 and 3 protons [11-13]. Furthermore, certain 3'-deoxyribonucleosides have $J_{1',2'}$ of 1 Hz [15]. This and the small long-range fluorine coupling give rise to the observed closely spaced pseudotriplet. The appearance of II is most likely due to the formation of a 2,3'-anhydronucleoside from the 2',3'-acyloxonium ion [8] formed in the propionyl bromide reaction. Bromide attack on this intermediate would give a 1-(3-bromo-3-deoxy-β-D-erythrofuranosyl)-5-fluorouracil intermediate, which should give II after hydrogenation.

Table 1. Nuclear magnetic resonance spectra data of the Tetrofuranosylnucleosides*

Compound	$H_{1'}$	$H_{2'}$	$J_{1',2'}$	$J_{F,1'}$
I	6.12 (td)	2.13 (dd) (2 H)	6.9,6.9	1.9
I(D_2O)	6.02 (t)	2.33 (qq), 2.10 (dq)	6.5,7.1	1.3
II	5.53 (t)	4.24 (m)	1.7	1.7
II($CDCl_3$)	5.64 (t)	4.39 (td)	1.3	1.3
III	5.73 (dd)	4.12 (t)	6.0	1.7
IV	6.31 (d)	5.22 (d)	5.3	0
V	6.05 (dd)	4.68 (dd)	7.0	1.6

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*Values are in p.p.m. (δ) using, as internal standard, either tetramethylsilane (δ=0) in $CDCl_3$, acetonitrile (δ =2.00, 159.08 Hz) in D_2O, or d_5-Me_2SO (δ = 2.49, 198.055 Hz) in d_6-Me_2SO. Coupling constants are given in Hz. The solvent is d_6-Me_2SO unless otherwise specified.

Comparison of I and II with the ftorafur metabolites. The ^{1}H-n.m.r. data of compound I are equivalent to that reported by Wu *et al.* [1] for the ftorafur metabolite M_2 whose structure was correctly assigned by those authors. This is also the metabolite MH-1 isolated from the urine of human patients by Au *et al.* [2]. The spectrum of compound II is equivalent with that reported by Au *et al.* [2] for metabolite MH-3 (and repeated by us), isolated from the urine of human patients receiving ftorafur. This metabolite may now be assigned the 1-(3-deoxy-β-D- or α-L-*glycero*-tetrofuranosyl)-5-fluorouracil structure. The metabolite called M_1 in Ref. 1 and MH-2 in Ref. 2 is,therefore, not equivalent to our compound I as originally suspected. A very recent report by Lin *et al.*[14] describes the synthesis of racemic I and its *cis* analog and of racemic II and its *cis* analog. This group also assigned the *cis*-2'-deoxy-isomer of I as the structure of M_2. A difficulty with that work, however, is the ambiguity in the assignment of the *cis* and *trans*-isomers of the diasteromeric 1-(2-deoxytetrofuranosyl)-5-fluorouracils. Their assignment of the geometrical isomers was on the basis of the coupling constants of the anomeric proton, which depend strongly on ring conformation (note, for instance, that $J_{1',2'}$ of 1.7 Hz shown in II is greatly different from the $J_{1',2'}$ of 6.0 Hz in compound III). Our unambiguous synthesis of I confirms both the correct geometry of M_2 and the tentative assignments of Lin *et al.* [14].

The synthetic samples of I and II were also compared with the metabolites by reverse phase h.p.l.c. With 5% (v/v) MeOH in 0.01 M sodium acetate buffer, pH 4.2, compound II and MH-3 coeluted with a retention time of 6.5 min. Using 15% MeOH in the same buffer, compound I, M_2, and MH-1 coeluted at 2.4 min.

The above discussion, of course, makes reference only to the identification of the geometrical structure of the metabolites. It is of great interest to assign the stereochemistry for the following reasons. Ftorfur is administered as an *R*,*S* mixture. The metabolites produced from the *R* isomer would have the β-D-configuration and could be substrates for uridine and/or thymidine phosphorylase, as pointed out by Wu *et al.* [1]. The urinary metabolites from rats and rabbits [1] and humans [2], however, may be comprised mostly of the α-L-isomers if the phosphorylases have cleaved the isomers with β-D-stereochemistry.

Since our syntheses of I and II were stereospecific, the determination of the absolute configuration of the metabolites is possible. We had available to us an authentic sample of MH-3. The circular dichroism spectra of II gave $[\theta]_{273}$ + 13600 and $[\theta]_{249}$ 0 (1.19×10^{-4} M, H_2O). The c.d. of MH-3 was $[\theta]_{273}$ -4700 and $[\theta]_{252}$ 0 (1.22×10^{-4} M, H_2O). From this data it appears that this sample of MH-3 contained the α-L- and β-D-enatiomers in a 2.05:1 proportion.

The greater amount of α-L-enantiomer could be due to (a) differential rates of hydroxylation of *R* and *S*-ftorafur, (b) cleavage of the β-D-enantiomer by uridine or thymidine phosphorylase (or a similar activity), or (c) differential rates of excretion of the compounds. Au *et al.* [2] found that MH-3 did not release 5-fluorouracil when incubated with uridine or thymidine phosphorylase.

We are currently investigating the action of the pyrimidine nucleoside phosphorylase on I, II, and III, and studying the cytotoxicity of the compounds in cell culture. We are also attempting to obtain a c.d. spectrum of M_2 and MH-1 for the determination of their enantiomeric composition.

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REFERENCES

1. A. T. Wu, J. L. Au and W. Sadee, *Cancer Res.* *38*, 210 (1978).
2. J. L. Au, A. T. Wu and W. Sadee, *Cancer Treat. Rep.* *63*, 343 (1979).
3. A. S. Perlin, *Meth. Carbohyd. Res.* *1*, 64 (1962).
4. R. Andersson, O. Theander and E. Westerlund, *Carbohyd. Res.* *61*, 501 (1978).
5. U. Niedballa and H. Vorbruggen, *J. Org. Chem.* *39*, 3625 (1974).
6. A. Hampton and A. W. Nichol, *Biochemistry* *5*, 2076 (1966).
7. J. E. Codington, I. L. Doerr and J. J. Fox, *J. Org. Chem.* *29*, 558 (1964).

8. S. Ozaki, T. Katakami and M. Saneyoshi, Bull. Chem. Soc. Japan 50, 2197 (1977).
9. S. Greenberg and J. G. Moffatt, J. Am. Chem. Soc. 95, 4016 (1973).
10. R.V. Lemieux and M. Hoffer, Can. J. Chem. 39, 110 (1961).
11. T. E. Stone and G. D. Daves, Jr., J. Org. Chem. 42, 2152 (1977).
12. M. Holik and I. Borkovcova, Chemicke Zvesti 28, 374 (1974).
13. J. P. Decor and G. Descotes, Bull. Chem. Soc. France 6, 2370 (1970).
14. A.T. Lin, R.S. Benjamin, P. N. Rao and T.L. Loo, J. Med. Chem. 22, 1096 (1979).
15. J.T. Witkowski, M. Fuertes, P.D. Cook and R.K. Robins, J. Carbohyd. Nucleosides Nucleotides 2, 1 (1975).