(3) E. Graf and M. El-Menshawy, *Pharm. Unserer Zeit*, **6**, 171 (1977).
(4) S. Garattini, F. Marcucci, and E. Musini, *Drug Metab. Rev.* 1, 291 (1972).
(5) G. H. Mandel in "Fundamentals of Drug Metabolism and Drug Disposition", B. N. La Du, G. H. Mandel, and A. L. Way, Eds., Williams and Wilkins, Baltimore, Md., 1972, pp 149–178.
(6) V. Ullrich, *Angew. Chem., Int. Ed. Engl.*, 11, 701 (1972).
(7) M. A. Schwartz, B. A. Koechlin, E. Postma, S. Palmer, and G. Krol, *J. Pharmacol. Exp. Ther.*, **149**, 424 (1965).
(8) L. De Angelis, M. Predominato, and R. Vertua, *Arzneim.-Forsch.*, **22**, 1328 (1972).
(9) S. A. Ravizza, German Patent 2 131 158; *Chem. Abstr.*, **76**, 72570c (1972).
(10) A. Nudelman, R. J. McCaully, and S. C. Bell, *J. Pharm. Sci.*, **63**, 1880 (1974).
(11) T. Kovač, F. Kajfež, V. Sunjič, and M. Oklobdžija, *J. Med. Chem.*, 17, 766 (1974).
(12) G. F. Tamagnov, R. Demaria, and F. Demarchi, *Arzneim.-Forsch.*, **25**, 720 (1975).
(13) F. Kajfež, A. Lisini, M. Oklobdžija, M. Mihalič, V. Sunji; and N. Blaževič, *Acta Pharm. Jugosyl.*, **26**, 199 (1976).
(14) J. Bešič, M. Grims, Z. Fuks, I. Beus, J. Rulnjević, V. Gjuirš, and B. Vrhovac, *Acta Pharm. Jugosyl.*, **26**, 107 (1976).
(15) J. Büchi, "Grundlagen der Arzneimittelforschung und der Synthetischen Arzneimittel", Birkhäuser Verlag, Basel, 1963, pp 226–230.
(16) W. A. Ritschel, "Angewandte Biopharmazie", Wissenschaftliche Verlagsgeselschaft Mbh, Stuttgart, 1973, pp 289.
(17) M. I. Gluckman, *Curr. Ther. Res., Clin. Exp.*, 7, 721 (1965).
(18) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).
(19) R. F. Banziger, *Arch. Int. Pharmacodyn. Ther.*, **154**, 131 (1965).
(20) L. O. Randall, C. L. Scheckel, and R. F. Banziger, *Curr. Ther. Res.*, **57**, 590 (1965).
(21) W. Schallek, J. Thomas, A. Kwehn, and F. Zabransky, *Int. J. Neuropharmacol.*, **4**, 317 (1965).
(22) W. P. Purcell, G. E. Boss, and J. M. Clayton, "Strategy of Drug Design", Wiley, New York, 1973, pp 126–143.

Synthesis and Biological Activities of Ftorafur Metabolites. 3′- and 4′-Hydroxyftorafur

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Four isomers of ftorafur were synthesized as authentic samples of possible ftorafur (FT) metabolites. 2,3-Dihydrofuran was treated with perbenzoic acid in MeOH to give 2-methoxy-3-hydroxytetrahydrofuran, which upon treatment with Ac_2O/pyridine yielded the key intermediate 2-methoxy-3-acetoxytetrahydrofuran. The other intermediate, 2-ethoxy-4-acetoxytetrahydrofuran, was prepared by acid hydrolysis (HCl/50% EtOH) of 1,1-diethoxy-3,4-dihydroxybutane, followed by acetylation (Ac_2O/pyridine). Treatment of 2,4-bis(trimethylsilyl)-5-fluorouracil with either 2-methoxy-3-acetoxytetrahydrofuran or 2-ethoxy-4-acetoxytetrahydrofuran in 1,2-dichloroethane at room temperature using $SnCl_4$ as catalyst afforded *cis*- and *trans*-3′-OAc-FT or 4′-OAc-FT, respectively. However, *trans*-3′-OAc-FT and *cis*-4′-OAc-FT were the major condensation products. In each case, separation of these cis and trans isomers was achieved by silica gel column chromatography. Treatment of 3′- or 4′-OAc-FT with NH_3/CH_3OH at 5 °C overnight yielded the desired hydroxylated FT. Both *trans*-3′-OH-FT and *cis*-4′-OH-FT showed no significant activity against L1210 up to 100 mg/kg. These two agents produced an inhibitory effect on HeLa cell growth equal to that of ftorafur, with ID_{50} = 200 μg/kg.

(*R*,*S*)-1-(Tetrahydro-2-furanyl)-5-fluorouracil (ftorafur or FT), a pyrimidine antimetabolite, has shown significant antitumor activity in several adenocarcinomas with a spectrum of activity similar to, but less toxic than, 5-fluorouracil (5-FU).[1-3] It was considered a prodrug of 5-FU,[4-7] and microsomal enzymes[8] were probably involved in the conversion of FT to 5-FU in vivo. However, studies of the pharmacologic fate of FT in several species suggested the presence of other metabolites in addition to 5-FU.[9,10] Although, Smolyan-Skaya and Tugarino[7] first demonstrated the in vivo formation of a microbiologically active metabolite from FT, we were the first to report the detection and isolation of hydroxylated FT metabolites from patients' urine after FT administration.[10] These findings received support from other investigators, who described similar metabolites in the urine of rabbits treated with FT.[11] Further, these authors suggested that the structures of the metabolites were 3′-OH- and 4′-OH-FT, based on NMR evidence. However, in their work no authentic samples were available for comparison and the absolute configurations of the hydroxyl group in these metabolites remained undetermined.

The synthesis of the hydroxylated FT metabolites received added impetus from observation in our clinical pharmacological studies of FT. In man, apparently a single intravenous administration of FT at a nontoxic therapeutic dose elicits a sustained plasma concentration of 5-FU several times higher than that achievable by the direct continuous infusion of 5-FU at its maximum tolerated dose, without causing serious effects, particularly serious mucositis.[12] Clearly, FT is not merely a depot form of 5-FU, but most important, our clinical studies suggest that perhaps FT, and more likely its hydroxylated metabolite(s), may selectively protect the gastrointestinal mucosa from 5-FU toxicity.[12] This hypothesis must be verified biochemically, enzymatically, and pharmacologically with synthetic FT metabolites. We now describe the synthesis of the four pairs of diastereoisomers of 3′- and 4′-OH-FT.

Chemistry. Since four pairs of diastereoisomers of hydroxylated FT metabolites may exist, a nonstereospecific approach was used in the synthesis of these isomers. The synthesis of the two isomeric 3′-OH-FT is outlined in Scheme I.

The preparation of *trans*-3-hydroxy-2-methoxytetrahydrofuran (**3**) followed a previously described procedure[13] that involved treatment of 2,3-dihydrofuran with perbenzoic acid in methanol at 5 °C. The acetylation of compound **3** was achieved by using Ac_2O/pyridine as the acetylating agent at room temperature to give compound **4** in good yield. Treatment of 2,4-bis(trimethylsilyl)-5-fluorouracil with compound **4** in 1,2-dichloroethane under the catalysis of stannic chloride[14] at room temperature yielded a mixture of compounds **5** and **6** which were separated on a silica gel column using THF–petroleum

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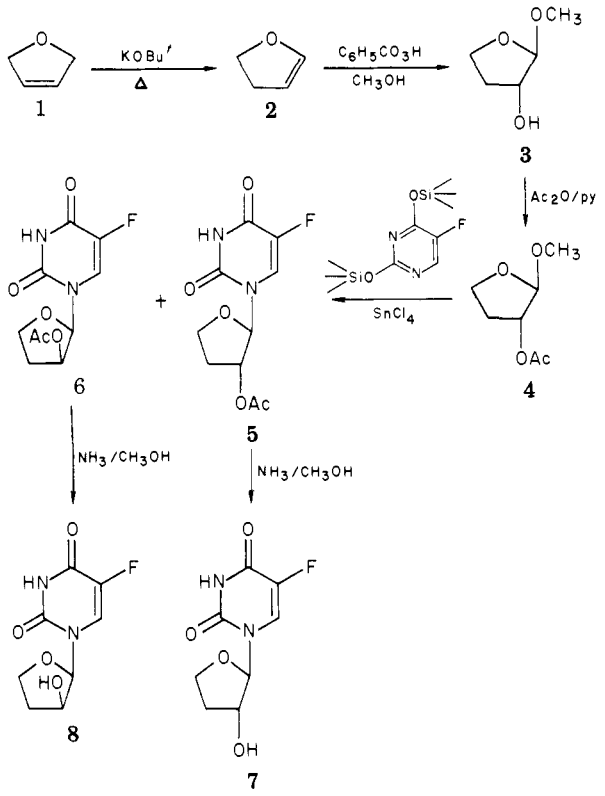
Scheme I



Scheme II

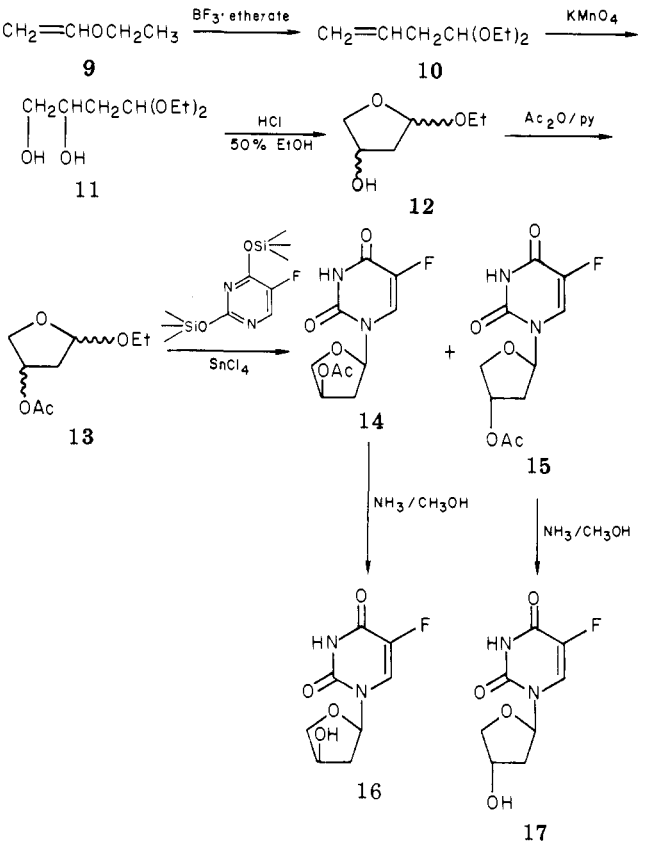


Table I. Physical Parameters of Hydroxyftorafur

compd	UV λ_{max}, nm (ϵ)[a]	^{1}H NMR chem shift, ppm[b] (J, Hz) C-2′ H	C-6 H
trans-3′-OH-FT (**7**)	270 (9300)	5.58 (t; J_t = 1.5)	7.75 (d, J_d = 7)
cis-3′-OH-FT (**8**)	270 (7000)	6.21 (d; J_d = 3.7)	7.62 (d, J_d = 5)
trans-4′-OH-FT (**17**)	268 (9200)	6.09 (t, d; J_t = 7, J_d = 2)	7.90 (d, J_d = 7)
cis-4′-OH-FT (**16**)	270 (8700)	6.05 (d, t; J_d = 8, J_t = 2)	8.10 (d, J_d = 7)

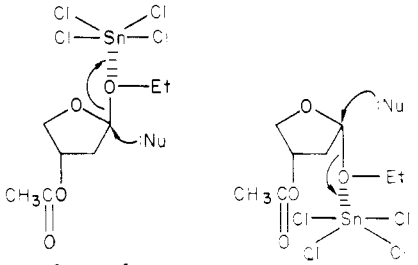
[a] In methanol. [b] In Me_2SO-d_6.

ether (2:3) as the eluent. The major product, compound **5**, was assigned the trans isomer, while the minor product, **6**, was assigned the cis isomer. Treatment of compound **5** or **6** with NH_3/CH_3OH at 5 °C overnight yielded the desired final product **7** or **8**, respectively.

The assignments of the structures were based on NMR spectra (Table I) and the "trans rule" of nucleoside synthesis. It has been reported[15] that the spacings of anomeric proton NMR signals of ribofuranosyl nucleosides do not aid in the stereochemical assignment of anomeric pairs because both have similar coupling constants. However, the resonance of the anomeric proton of α anomers has been found to occur at a lower field than that of β anomers, the α/β chemical shift difference being about 15 Hz. This difference was due to the shielding effect of the α-hydroxy substituent. In contrast, both the α- and the β-anomeric proton of deoxyribonucleosides have similar resonance positions, and in every case the β-anomeric proton forms a pseudotriplet (J = 6.5 to ~7 Hz) and the α, a doublet of doublets (J = 7 and 3–4 Hz). The anomeric proton (C-2′) of **5** (Table I) appears as a triplet with J = 1.5 Hz indicating coupling with C-3′ H and a long-range coupling with C-5 F. A similar long-range coupling between the anomeric proton and fluorine of 5-fluorodeoxyuridine was reported.[11] In contrast, the anomeric proton (C-2′ H) of compound **6** appears as a doublet (J = 2.5 Hz) and comes to resonance at a lower field than compound **5**, the chemical-shift difference between **5** and **6** being about 24 Hz, in agreement with the observation of α and β isomers of ribonucleosides.[16] Therefore, compound **5** was assigned the trans isomer and compound **6** the cis isomer. The difference in anomeric proton chemical shift between cis and trans isomers was probably due to the shielding effect of the 3′-acetoxy group.[16] The C-6 H signals of **8** were slightly shielded by 3′-OH, and the chemical-shift difference of C-6 H in trans/cis isomers is about 8 Hz. The chemical-shift difference for the anomeric proton between **7** and **8** is 40 Hz, again due to the shielding effect of 3′-OH. From the NMR data, the structure of compound **7** was assigned *trans*-1-(3-hydroxy-2-tetrahydrofuranyl)-5-fluorouracil and that of compound **8**, *cis*-1-(3-hydroxy-2-tetrahydrofuranyl)-5-fluorouracil.

The approach used for the synthesis of *cis*- and *trans*-1-(4-hydroxy-2-tetrahydrofuranyl)-5-fluorouracil is in Scheme II. 1,1-Diethoxy-3,4-dihydroxybutane (**11**) was prepared[17] by $KMnO_4$ oxidation of 1,1-diethoxy-3-butene (**10**), which in turn was prepared from ethyl vinyl ether by a previously reported procedure.[18] Treatment of compound **11** with diluted HCl in 50% EtOH at 5 °C overnight gave 2-ethoxy-4-hydroxytetrahydrofuran (**12**) in moderate yield. Acetylation of compound **12** in 1:1 Ac_2O–pyridine gave nearly quantitative yield of 2-ethoxy-4-acetoxytetrahydrofuran (**13**). Treatment of **13** with 2,4-bis(trimethylsilyl)-5-fluorouracil in dichloroethane under the catalysis of stannic chloride gave a mixture of *cis*-1-(4-acetoxy-2-tetrahydrofuranyl)-5-fluorouracil (**14**) (major) and *trans*-1-(4-acetoxy-2-tetrahydrofuranyl)-5-fluorouracil (**15**) (minor). The separation of cis (**14**) and trans (**15**) isomers was achieved by silica gel column chromatography, using THF–ligroin (1:1) as the eluent.

Scheme III



favored less favored

The structural assignment of cis and trans isomers was again based on the NMR studies. The anomeric proton (C-2′ H) of cis isomer **14** appears as a triplet of doublets (J_d = 7 Hz, J_t = 2 Hz). The doublets were due to the coupling of the anomeric proton with one of the C-3′ H and the triplet was apparently due to a combination of coupling with another C-3′ H proton and a long-range coupling with C-5 F. The anomeric proton (C-2′ H) of trans isomer **15** appears as a doublet of triplets (J_t = 7 Hz, J_d = 1.5 Hz). The small J_d may be due to long-range coupling with C-5 F. The chemical shift of the anomeric protons of both cis and trans isomers is about 6.45 ppm, consistent with the reported chemical shift for both the α- and β-anomeric protons of deoxyribonucleosides.[19]

While the predominant formation of *trans*-1-(3-acetoxy-2-tetrahydrofuranyl)-5-fluorouracil (**5**) could be explained by the "trans rule" or neighboring group participation, the predominant formation of cis-1-(4-acetoxy-2-tetrahydrofuranyl)-5-fluorouracil (**14**) observed in this study is interesting. One possible explanation is that the rate-limiting step of the nucleoside formation in the latter case involves the complex formation between stannic chloride and the ethoxy function of 2-ethoxy-4-acetoxytetrahydrofuran (**13**). The *trans*-2-ethoxy-4-acetoxytetrahydrofuran forms a complex with stannic chloride much easier than does the cis isomer (Scheme III), due to steric effects. Consequently, the SN_2 coupling reaction between 2,4-bis(trimethylsilyl)-5-fluorouracil and the stannic chloride–compound **13** complex leads to cis-1-(4-acetoxy-2-tetrahydrofuranyl)-5-fluorouracil (**14**) as the major product.

Treatment of compound **14** or **15** with methanolic ammonia gave the final product **16** or **17**, respectively, in good yield. The NMR data (Table I) on **16** and **17** are as expected.

From the NMR studies of the compounds synthesized in this study, it appears that the structural assignment of Sadee's metabolites isolated from rabbit urine is incorrect. They reported[11] that the anomeric proton of metabolite M_1 appears as a triplet of doublets (6.15 ppm, J_d = 8 Hz, J_t = 2 Hz) and assigned structure 3′-OH-FT, which is in no way close to either the trans (**7**) or the cis (**8**) isomer of 3′-OH-FT synthesized in this study. In addition, they reported that the metabolite M_2 with an anomeric proton (6.21 ppm) appears as a doublet of triplets (J_t = 7 Hz, J_d = 1.5 Hz) and assigned the structure 4′-OH-FT. They did not determine the configuration of the 4′-hydroxyl group. Our NMR data showed (Table I) that the anomeric proton of compound **17** appears as a triplet of doublets (6.07 ppm, J_d = 8 Hz, J_t = 2 Hz), in agreement with the NMR data of Sadee's metabolite M_1. Therefore, the correct structure of the M_1 metabolite reported by Sadee should be *cis*-1-(4-hydroxy-2-tetrahydrofuranyl)-5-fluorouracil (**16**). Our NMR data on *trans*-1-(4-hydroxy-2-tetrahydrofuranyl)-5-fluorouracil (**17**) indicated the anomeric proton was a doublet of triplets (6.09 ppm, J_t = 7 Hz, J_d = 2 Hz), with chemical shift and coupling constant close to the reported value of the anomeric proton of M_2. Therefore, the structure of M_2 reported by Sadee should be *trans*-1-(4-hydroxy-2-tetrahydrofuranyl)-5-fluorouracil (**17**). The slight difference in chemical shift between our compound and the metabolites reported by Sadee may be due to the difference in instrument and solvent used in the studies. In our work spectra were taken in Me_2SO-d_6, while Sadee used acetone-*d* as the solvent. We find the solubility of these compounds in acetone-*d* to be very poor.

Scheme IV

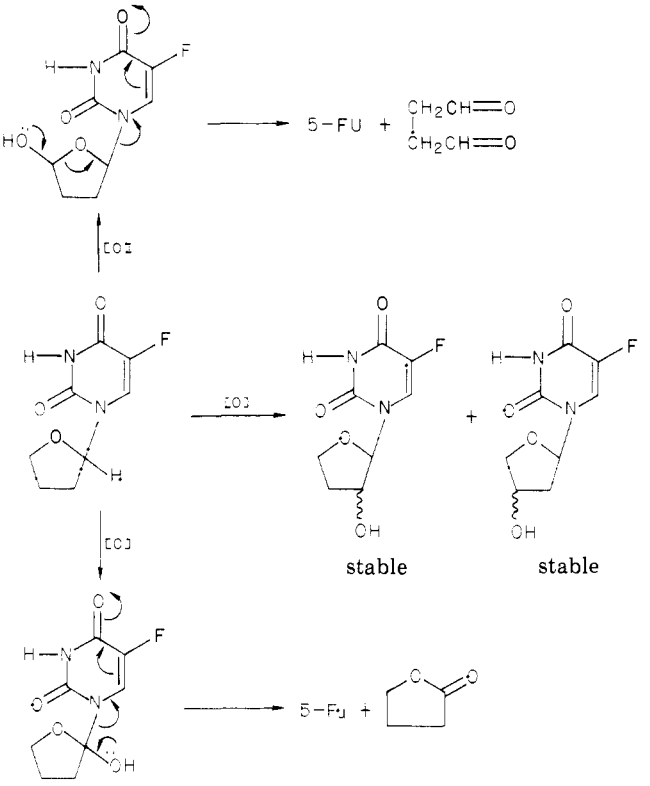


Table II. Antitumor Effects against L1210

compd	dosage, mg/kg	% T/C
cis-4′-OH-FT	100	114
	80	91
	60	106
trans-3′-OH-FT	100	96
	80	98
	60	108
5-FU	20	153
FT	80	102

The isolation of 4′-OH FT as FT metabolites does not necessarily rule out the possibility that 2′- and 5′-OH-FT are also formed as part of the hydroxylation products of FT in vivo. However, since 5′- and 2′-OH-FT are hemiacetal and hemiketal, respectively, they would be too unstable to be isolable. Although the mechanism of transformation of FT to 5-FU remains unclear, it could be mediated through the formation of unstable 2′- and 5′-OH-FT, which decompose to generate 5-FU, as shown in Scheme IV.

Antitumor Studies. Compounds **7** and **16** were tested against L1210 according to the NCI protocol and were found to be inactive up to 100 mg/kg dosage. Under the same testing, FT was also found to be inactive up to 80 mg/kg (Table II).

Inhibition of HeLa Cell Growth in Vitro. Studies on the effect of hydroxylated ftorafur on the progression of HeLa cells through the cell cycle indicated that 3′- and

Table III. Effect on HeLa Cell Cycle Traverse[a]

treatment (μg/mL)	cells in mitosis	cells in interphase	total	mitotic index, %
5-FU (50)	95	205	300	31.7
5-FU (25)	78	141	218	35.8
5-FU (12.5)	137	164	300	45.7
FT (200)	108	192	300	36.0
FT (100)	181	119	300	60.3
trans-3′-OH-FT (200)	104	196	300	34.7
trans-3′-OH-FT (100)	192	108	300	64.0
cis-4′-OH-FT (200)	132	168	300	44.0
cis-4′-OH-FT (100)	190	110	300	63.3
control, colcemid (0.05)	194	106	300	64.7

[a] The cells were incubated for about 16 h with medium containing the drug and colcemid (0.05 μg/mL). At the end of incubation, mitotic indices were determined. The lower the mitotic index, the greater the inhibition of cell cycle traverse by the drug.

4′-OH-FT are about equally active as that of ftorafur in the inhibition of HeLa cell growth. At a concentration of 200 μg/mL, both FT and the metabolites reduced the mitotic accumulation to about 50% of the control value (Table III). Under the same conditions, 5-FU is about eight to ten times more effective than ftorafur and its hydroxylated derivatives. This experiment suggested that the action of 3′- and 4′-OH-FT, like FT, may also be mediated through the formation of 5-FU during the incubation.

Experimental Section

All melting points were taken on a calibrated Thomas-Hoover capillary melting point apparatus. Analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga. Spectral data were obtained using Perkin-Elmer Infrared Model 727B and Varian T-60A spectrometers. The latter used Me_4Si as an internal standard. The NMR and IR spectra were as expected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements are within ±0.4% of the theoretical values.

3-Acetoxy-2-methoxytetrahydrofuran (4). 3-Hydroxy-2-methoxytetrahydrofuran[13] (9.5 g) in 40 mL of a pyridine and Ac_2O (1:1) mixture was stirred at room temperature for 2 days. The solvent was removed under reduced pressure. The oil was dissolved in 50 mL of chloroform, washed twice with H_2O, and dried over Na_2SO_4. The solvent was removed using a rotary evaporator at a temperature below 40 °C. The resulting oily residue was distilled in vacuo [ca. 94–96 °C (2.7 mm)] to give 9.2 g of the desired product. Anal. ($C_7H_{12}O_4$) C, H.

***cis*- and *trans*-1-(3-Acetoxy-2-tetrahydrofuranyl)-5-fluorouracil (5 and 6).** 5-Fluorouracil (3 g, 0.023 mol) was refluxed in 15 mL of hexamethyldisilazane with a catalytic amount of ammonium sulfate until a clear solution was obtained. The excess hexamethyldisilazane was removed under reduced pressure. The oily product was dissolved in 30 mL of dried dichloroethane. To the solution was added 3-acetoxy-2-methoxytetrahydrofuran (4; 3.8 g, 0.023 mol) and $SnCl_4$ (5.3 g, 0.023 mol). After the solution was stirred at room temperature overnight, a saturated $NaHCO_3$ solution (20 mL) was added, and the mixture was extracted with tetrahydrofuran (THF) several times. The THF extracts were combined, dried over Na_2SO_4, and evaporated to dryness. The residue was dissolved in a minimum amount of THF and applied to a silica gel column, using THF–petroleum ether (2:3) as the eluent. The fractions were monitored by TLC (silica gel N-HR/UV 254, 0.2 mm, Macherey–Nagel + CO), using THF–ligroin (1:1) as a solvent. The fractions with R_f 0.5 were pooled and evaporated to dryness, and the white powder was recrystallized from EtOH to give 2 g (33%) of trans isomer **5**, mp 179–180 °C. Anal. ($C_{10}H_{11}FN_2O_5$) C, H, N.

Evaporation of the pooled fractions with R_f 0.38 gave 150 mg of an oil that was identified as cis isomer **6**. The oil failed to crystallize from various solvent systems and was used for the next reaction without further purification.

***trans*-1-(3-Hydroxy-2-tetrahydrofuranyl)-5-fluorouracil (7).** Compound **5** (700 mg, 2.7 mmol) in 20% NH_3–CH_3OH (65 mL) was kept overnight at 5 °C. The solvent was evaporated to dryness. The oil was crystallized from CH_3OH to give 400 mg of colorless crystals. Another crop of crystals (100 mg) was obtained by concentration of the mother liquor to give a total yield of 500 mg (85%), mp 214–216 °C (MeOH). Anal. ($C_8H_9FN_2O_4$) C, H, N.

***cis*-1-(3-Hydroxy-2-tetrahydrofuranyl)-5-fluorouracil (8).** Compound **6** (0.5 g) wad dissolved in 10 mL of a 20% NH_3–MeOH solution and kept at 5 °C overnight. The solvent was evaporated to dryness, yielding a white powder that was recrystallized from EtOH to give white crystals (0.25 g, 57%), mp 154–156 °C. Anal. ($C_8H_9FN_2O_4$) C, H, N.

2-Ethoxy-4-hydroxytetrahydrofuran (12). 1,1-Diethoxy-3,4-dihydroxybutane[17] (24.2 g, 0.17 mol) was dissolved in 50 mL of 50% aqueous ethanol. To the solution was added 24 mL of concentrated HCl and the solution was kept in a refrigerator overnight. Sodium bicarbonate powder was added to neutralize the acid, and the whole mixture was then extracted three times with chloroform. The chloroform extracts were combined, dried over Na_2SO_4, and evaporated to give an oil. Vacuum distillation [70 °C (0.1 mm)] of the oil gave 12 g (53%) of compound **12**.

2-Ethoxy-4-acetoxytetrahydrofuran (13). Acetylation of compound **12** by the same procedure as that of the acetylation of 3-hydroxy-2-methoxytetrahydrofuran (**3**) gave compound **13** in 80% yield, bp ca. 108–110 °C (2.7 mm). Anal. ($C_8H_{14}O_4$) C, H.

***cis*- and *trans*-1-(4-Acetoxy-2-tetrahydrofuranyl)-5-fluorouracil (14 and 15).** 4-Acetoxy-2-ethoxytetrahydrofuran (**13**; 3.6 g, 0.02 mol), 2,4-bis(trimethylsilyl)-5-fluorouracil (0.024 mol, prepared from 3 g of 5-FU), and stannic chloride (5 g, 0.02 mol) in 30 mL of dried 1,2-dichloroethane were stirred at room temperature for 36 h. To the clear, greenish solution was added 100 mL of saturated $NaHCO_3$ solution. The mixture was extracted three times with 150 mL of THF. The extracts were combined, dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The oily residue was separated on a silica gel column and eluted with THF–ligroin (1:1) as the solvent. The fractions were monitored with TLC (silica gel 60F-254, 0.2 mm, EM reagents) using THF–ligroin (1:1) as the solvent. Fractions with R_f 0.56 and 0.52 were separately pooled and evaporated to give 40 (mp 143–144 °C) and 800 mg (mp 165–167 °C), respectively. The compound with R_f 0.58 was identified by NMR as *trans*-1-(4-acetoxy-2-tetrahydrofuranyl)-5-fluorouracil (**15**) and the compound with R_f 0.52 as the cis isomer **14**.

***cis*-1-(4-Hydroxy-2-tetrahydrofuranyl)-5-fluorouracil (16).** Deacetylation of compound **14** was achieved by the same method described in the synthesis of compound **7** and **8** to give an 80% yield of the desired product **16**. It was recrystallized from ethanol to give white crystals, mp ca. 204–205 °C dec. Anal. ($C_8H_9FN_2O_4$) C, H, N.

***trans*-1-(4-Hydroxy-2-tetrahydrofuranyl)-5-fluorouracil (17).** The method for the synthesis of **16** was adapted for compound **17**, mp 181–182 °C dec. Anal. ($C_8H_9FN_2O_4$) C, H, N.

Determination of Antineoplastic Effect. Compounds prepared in this study were tested for antineoplastic effect against L1210. The NCI protocol was followed during the study. The results are shown in Table II.

Effect of Hydroxylated FT on HeLa Cell Cycle Traverse. The day before the experiment, 3.5×10^5 HeLa cells were plated into 35-mm plastic dishes with 2 mL of Eagle's MEM containing 10% fetal calf serum and antibiotics. The next day the cultures were treated with various concentrations of drug in the same medium in the presence of 0.05 μg/mL colcemid and reincubated. Sixteen hours later, the cells were trypsinized from the dishes and deposited on slides using the cytocentrifuge, fixed in 3:1 absolute MeOH–AcOH. The cells were stained with acetoorcein and scored for mitotic index. The results are shown in Table III.

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References and Notes

(1) N. G. Blokhina, E. K. Vozny, and A. M. Garin, *Cancer*, **30**, 390–392 (1972).

(2) N. I. Karev, N. G. Blokhina, E. K. Vozny, and M. P. Pershin, *Neoplasma*, **19**, 347–350 (1972).
(3) N. Valdivieso, G. P. Bodey, J. A. Gottlieb, and E. J. Freireich, *Cancer Res.*, **36**, 1821–1824 (1976).
(4) A. M. Cohen, *Drug Metab. Dispos.*, **3**, 303 (1975).
(5) S. Fihii, H. Okuda, A. Akazawa, Y. Yasuda, Y. Kawaguchi, Y. Fukunaga, and H. Nishikawa, *Yakugaku Zasshi*, **95** 732–740 (1975).
(6) S. Fujimoto, A. Akao, B. Itoh, I. Koshizuka, K. Kayano, Y. Kitsukawa, M. Takahashi, T. Minami, H. Ishigami, Y. Nomura, and K. Itoh, *Cancer Res.*, **36**, 33–36 (1976).
(7) A. Z. Smolyan-skaya and O. A. Tugarinov, *Neoplasma*, **19**, 341–345 (1972).
(8) J. A. Benvenuto, K. Lu, S. W. Hall, R. S. Benjamin, and T. L. Loo, *Cancer Res.*, **38**, 3867–3870 (1978).
(9) A. T. Wu, H. J. Schwandt, C. Finn, and W. Sadee, *Res. Commun. Chem. Pathol. Pharmacol.*, 14, 89–102 (1976).
(10) J. A. Benvenuto, D. Farquhar, E. J. Freireich, and T. L. Loo, *Proc. Am. Assoc. Cancer Res.*, 18, 145 (1977).
(11) A. T. Wu, J. L. Au, and W. Sadee, *Cancer Res.*, **38**, 210–214 (1978).
(12) T. L. Loo, R. S. Benjamin, K. Lu, J. A. Benvenuto, S. W. Hall, and E. M. McKelvey, *Drug Metab. Rev.*, **8**, 137–150 (1978).
(13) F. Sweet and R. K. Brown, *Can. J. Chem.*, **44**, 1571–1576 (1966).
(14) U. Niedballa and H. Vorbruggen, *Angew. Chem., Int. Ed. Engl.*, **9**, 461 (1970)
(15) A. F. Casy, "PMR Spectroscopy in Medicinal and Biological Chemistry", Academic Press, London and New York, 1971.
(16) T. Nishimura and B. Shimizu, *Chem. Pharm. Bull.*, **13**, 803 (1965).
(17) S. Hattori and K. Matsumoto, *Yuki Gosei Kagaku Kyokai Shi*, **19**, 461–463 (1961).
(18) S. Hattori, *Yuki Gosei Kagaku Kyokai Shi*, **19**, 453–460 (1961).
(19) M. J. Robins and R. K. Robins, *J. Am. Chem. Soc.*, **87**, 4934 (1965).

Mechanism of the Dealkylation of Tertiary Amines by Hepatic Oxygenases. Stable Isotope Studies with 1-Benzyl-4-cyano-4-phenylpiperidine

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The microsomal oxidative dealkylation of 1-benzyl-4-cyano-4-phenylpiperidine has been studied and the source of oxygen shown to be molecular oxygen. The rate of debenzylation was decreased by substituting deuterium for hydrogen in the methylene portion of the benzyl group. The isotope effect was measured by comparison of the reaction rates of the d_0 and d_2 compounds **1a** and **1b** and also of the d_5 and d_7 compounds **1c** and **1d**. Determination of the reaction rates for various mixtures of labeled and unlabeled species allowed the rates for 0 (k_H) and 100 mol % (k_D) to be accurately obtained. A primary isotope effect of 1.46 was observed when the methylene hydrogens of benzyl were replaced by deuterium. No secondary isotope was observed when the aromatic hydrogens of benzyl were replaced by deuterium. The results of this study are consistent with a mechanism involving direct hydroxylation at the benzyl methylene position in a rate-determining step.

One of the important reactions mediated by the membrane-bound oxygenases of liver is the oxidative N-dealkylation of amines. The products of the conversion, the dealkylated amine and an aldehyde, are thought to arise from the spontaneous dissociation of an intermediate carbinolamine [$R_2NCHOHR \rightarrow R_2NH + RCHO$].[1,2] The formation of the carbinolamine has been considered to occur by direct hydroxylation of the α-carbon atom;[1] i.e., oxidative dealkylation is really another example of hydroxylation at an aliphatic carbon atom. Cytochrome P 450 mediated aliphatic hydroxylation, as typified by the conversion of ethylbenzene to predominantly (*R*)-methylphenylcarbinol, occurs by direct oxygen insertion (without inversion). Molecular oxygen serves as a source of the oxygen atom and a deuterium isotope effect of about 1.8 can be demonstrated.[3] In an early study, Elison, Elliott, Look, and Rapoport[4] found an isotope effect for the demethylation of *N*-(trideuteriomethyl)morphine of 1.4, consistent with a hydroxylation mechanism. The possibility that microsomal dealkylation might occur via intermediate *N*-oxides has been discussed but is considered less likely, since the dealkylation of *N*-oxides by cytochrome P 450 has been shown to involve an initial step in which *N*-oxide is reduced to tertiary amine, which in turn is dealkylated by the hydroxylation mechanism.[5] In contrast to tertiary amines, dealkylation of secondary amines may involve more than one mechanism. Prough and Ziegler[6] have recently presented data which suggest that certain secondary amines, such as benzphetamine, may undergo dealkylation via an *N*-hydroxy intermediate.

In the present study, the debenzylation of 1-benzyl-4-cyano-4-phenylpiperidine (**1a**) was investigated with respect to both source of oxygen and deuterium isotope effects. The study of deuterium isotope effects has become of increasing importance not only because of mechanistic implications but also because of the great increase in the use of deuterium labeling in biological research. It becomes important to know when primary and secondary deuterium isotope effects can be expected and when they would be unlikely.

The primary isotope effect of the debenzylation of **1a** would normally be measured by comparison of the reaction rates of **1a** and **1b**, in which deuterium has been substituted for hydrogen in the *methylene* portion of the benzyl group. In order to increase the accuracy of the measurement of the primary isotope effect, it was also determined by a second method, comparing the reaction rates of **1c** and **1d**, which correspond to **1a** and **1b** with the addition of having deuterium substituted for hydrogen in the *aromatic* portion of the benzyl moiety. The presence

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