CONVENIENT PREPARATION OF DEUTERATED URACILS AND DIHYDROURACILS

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SUMMARY

The reaction of [3-$^{2}H_1$]propiolic acid with urea provides a simple route to [6-$^{2}H_1$]uracil, and further exchange with DCl affords [5,6-$^{2}H_2$]uracil. Reaction of the meso and d,l-isomers of [2,3-$^{2}H_2$]succinamide with lead tetraacetate leads to the cis and trans isomers of [5,6-$^{2}H_2$]5,6-dihydrouracil.

Key words: [6-$^{2}H_1$]uracil; [5,6-$^{2}H_2$]uracil; [5,6-$^{2}H_2$]5,6-dihydrouracil.

Samples of the nucleic acid pyrimidine bases specifically labeled with hydrogen isotopes have proven valuable in metabolic studies.[1] The original syntheses of [5-$^{2}H_1$], [6-$^{2}H_1$] and [5,6-$^{2}H_2$] uracil (**1-3**) by Parkanyi and Sorm[2] are not completely satisfactory; preparation of **1** by hydrogenolysis of 5-bromouracil requires relatively expensive deuterium gas, and the decarboxylation of deuterated orotic acid is a low-yield route to **2**. Subsequent research has resulted in improved syntheses of the 5-labeled uracils: electrolytic reduction of 5-bromouracil in tritiated water leads to [5-$^{3}H_1$]uracil[3], and a particularly convenient route to **1** is the exchange of the 5-H of uracil by refluxing 6N DCl[4]. No corresponding improvement in the preparation of **2** has been reported. The exclusive exchange of H-6 in 2,4-dimethoxypyrimidine by Pt in tritiated water[5] does not appear to have been applied to the preparation of a labeled uracil.

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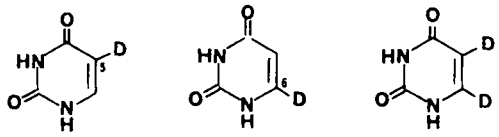


1 2 3

We report a new, simple route to [6-$^2H_1$]uracil using D_2O as the source of the isotope. Our method is based on the reported synthesis of uracil from propiolic acid and urea[6], and is similar to a synthesis of deuterated thiouracils reported by Mercer and Warrener.[7] [3-$^2H_1$]Propiolic acid is readily available from the decarboxylation of potassium acetylenedicarboxylate[8] in D_2O. Use of this deuterated propiolic acid in the reaction with urea proceeds without appreciable loss of isotope to afford **2** in 50-60% yield. The isotopic purity is easily monitored by proton NMR: **2** shows a singlet for H-5 at δ5.42, and any residual hydrogen at C-6 appears as a doublet at δ7.33.

[5,6-$^2H_2$]Uracil can then be prepared easily from **2** by exchange of H-5 with DCl.[4]

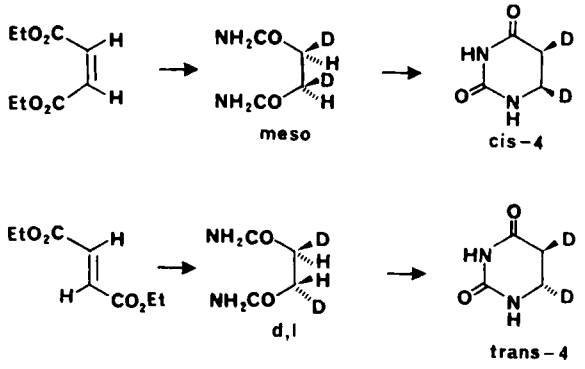
$$KO_2C\text{-}C{\equiv}C\text{-}CO_2H \xrightarrow[\Delta]{D_2O} D\text{-}C{\equiv}C\text{-}CO_2H \xrightarrow[\substack{H_2SO_4\\ \Delta}]{urea} \mathbf{2} \xrightarrow{DCl} \mathbf{3}$$

[5,6-$^2H_2$]5,6-Dihydrouracil. Parkanyi and Sorm[2] reported the preparation of a [5,6-$^2H_2$]dihydrouracil (**4**) by catalytic deuteration of uracil. Even after exchange of the N-D bonds with water, however, the product contained almost 50% more deuterium than calculated for **4**, indicative of significant exchange of C-H bonds. No evidence was provided as to possible stereospecificity in the reduction.

We have synthesized the separate *cis* and *trans* isomers of **4** by an unambiguous route in which the key step is the lead tetraacetate variation of the Hofmann rearrangement of succinamide.[9] Following the strategy

of Tchen and van Milligan,[10] the meso and d,l-isomers of diethyl [2,3-$^2H_2$]succinate were prepared by catalytic deuteration of diethyl maleate and diethyl fumarate, respectively, and converted to the amides with ammonium hydroxide. Oxidation with lead tetraacetate then converted the deuterated succinamides to the deuterated dihydrouracils **4** in one step.

The proton NMR spectra of cis and trans-**4** show H-6 as a doublet at δ3.7 and the H-5 signal at 2.9 (doublet in trans-**4**, multiplet in cis-**4**). The coupling constants $J_{H,H}$ are 3.3 Hz (cis) and 7.8 Hz (trans). Coupling constants have been reported for several substituted dihydrouracils which exist predominantly in one conformation:[11] $J_{5a,6a}$ = 10.3; $J_{5e,\ 6e}$ = 3.4;



$J_{5a,6e}$ = 6.1; $J_{5e,6a}$ = 3.4 Hz. Assuming that cis and trans **4** are each a rapidly interconverting mixture of half-chair conformations, an average J can be calculated for each: J_{cis} = 4.8 and J_{trans} = 6.8 Hz, in relatively good agreement with the observed values.

EXPERIMENTAL SECTION

Melting points were determined on a Thomas Hoover capillary apparatus and are uncorrected. Proton NMR spectra were recorded on a Varian EM-390 spectrometer and ^{13}C spectra were run on a JEOL PF-100 instrument, in both cases using tetramethylsilane as an internal standard. Mass spectra were obtained on a Finnigan 4023 gas chromatograph-mass spectrometer by Mr. Courtney Pape.

[3-$^2H_1$]Propiolic acid was prepared in 59% yield by the decarboxylation of acetylenedicarboxylic acid monopotassium salt (Aldrich) in D_2O following the literature procedure.[12] The deuterium content was determined by conversion with ethereal diazomethane to methyl 3-pyrazolecarboxylate;[13] integration of the NMR signal at 6.9 showed a minimum of 96% ^2H.

[6-$^2H_1$]Uracil (2). Following the procedure of Harada and Suzuki[6], 1.1 g of urea was ground in a mortar and added to 20 mL of conc H_2SO_4 at 0°C. After dissolution, [3-$^2H_1$]propiolic acid (1.3 g) was added and the mixture was heated at 90-105°C under nitrogen for 7 h. After cooling, the mixture was poured into 80 mL of ice water and kept in the refrigerator overnight. The brown solid which had separated was collected and dried; in four runs the yield was consistently 1.1-1.2 g (51-56%). Recrystallization from water (charcoal) gave pure uracil. The NMR spectrum (DMSO-d_6) showed H-5 as a sharp singlet at 5.42 and only a trace of the H-6 signal at 7.33; minimum ^2H content = 96%.

[5,6-$^2H_2$]Uracil (3). A suspension of 0.60 g of 2 in 15 mL of 20% DCl in D_2O was heated under reflux under a nitrogen atmosphere for 4.5 h, then cooled at 0°C until precipitation was complete. The solid was collected, washed with ice water, and dried in vacuo for two days to give 0.54 g (90%) of 3. The NMR spectrum (DMSO-d_6) showed only a broad N-H signal.

d,l-[2,3-$^2H_2$]Succinamide. A solution of 1.72 g of diethyl fumarate in 100 mL of cyclohexane was stirred with 200 mg of Adams catalyst in an atmosphere of deuterium gas (Matheson, 99.5 atom%) at 25°C and 1 atm for 4 h, at which time the calculated amount of deuterium had been absorbed. After filtration through Celite the solvent was removed under reduced pressure to leave 2.0 g of d,l-diethyl[2,3-$^2H_2$]succinate; NMR ($CDCl_3$) δ1.2 (t, 6H), 2.6 (s, 2H), 4.1 (q, 4H).

A mixture of 1.5 g of this ester and 25 mL of concentrated ammonium hydroxide was allowed to stir at room temperature for 24 h. The solid was filtered, washed with cold water, recrystallized from water, and dried at 80°C under vacuum to give 0.81 g of d,l-[2,3-$^2H_2$]succinamide, mp 250°; NMR (CF_3CO_2H) δ3.1 (s, 2H), 7.0-9.3 (br s, 4H).

meso-[2,3-$^2H_2$]Succinamide. The procedure above was followed, substituting diethyl maleate for diethyl succinate. The NMR spectrum was identical with that of the d,l-isomer.

trans-[5,6-$^2H_2$]5,6-Dihydrouracil (4). Following the procedure of Beckwith and Hickman,[9] lead tetraacetate (1.8 g, Fluka) was added to a suspension of 0.5 g of d,l-[2,3-$^2H_2$]succinamide in 6.0 mL of dry dimethylformamide. The solution was stirred at 50-60°C for 20 min, cooled, and filtered. Recrystallization of the residue from acetic acid afforded 0.45 g (92%) of trans-4, mp 273-275°C; NMR (CF_3CO_2H) δ 2.9 (d, 1H, J = 7.8 Hz), 3.7 (d, 1H, J = 7.8 Hz), 7.3 (s, 1H), 9.4 (s, 1H); mass spectrum: m/z 116 (100), 73 (35), 43 (80).

cis-[5,6-$^2H_2$]5,6-Dihydrouracil (4). The procedure above was followed, substituting the meso isomer of [2,3-$^2H_2$]succinamide, to afford 0.42 g (86%) of cis-4, mp 273-275°C; NMR (CF_3CO_2H) δ2.9 (m, 1H), 3.7 (d, 1H, J 3.3 Hz), 7.3 (s, 1H), 9.4 (s, 1H); ^{13}C NMR (CF_3CO_2H) 30 (t, C-5), 37 (t, C-6), 159 (s, C-2), 177 (s, C-4); mass spectrum: m/z 116 (78), 73 (35), 43 (100).

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