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INCORPORATION OF 5-SUBSTITUTED URACIL DERIVATIVES INTO NUCLEIC ACIDS—I

SYNTHESIS OF SOME 5-SUBSTITUTED URACILS

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Abstract—5-(1-Hydroxyethyl)uracil, prepared by the reduction of 5-acetyluracil with sodium borohydride, has been dehydrated with formic acid to give 5-vinyluracil and a dimer of 5-vinyluracil, *trans* 1,3-bis (uracil-5-yl)but-1-ene. These compounds have also been prepared with a tritium label in an identified position and with a high specific activity such that their incorporation into nucleic acids can be monitored.

It is known that some 5-substituted uracil derivatives, particularly 5-bromouracil, can replace a large amount of the thymine in the DNA of thymine-dependent mutants of *Escherichia coli*[1] and that such bacteria are killed more easily by UV radiation than are bacteria containing only thymine.[2] Preparation of the corresponding deoxynucleoside enabled 5-bromouracil to be incorporated also into phage[3] and mammalian cell DNA.[4] Many other 5-substituted uracil analogues have also been incorporated and are normally those which contain a 5-substituent (Cl, I, CF_3, NH_2) which appreciably modifies both the electronic and H-bonding properties of the pyrimidine ring. 5-Ethyluracil, which differs from thymine principally by the larger van der Waal's radius of the 5-substituent, has also been incorporated into bacterial[5] and phage DNA.[6] This paper describes the synthesis of some 5-substituted uracils, particularly 5-vinyluracil, whose 5-substituent has a van der Waal's radius in between that of the Me and Et groups but which modifies the electronic structure of the molecule such that the UV absorption maximum of the base in neutral conditions is 288 nm. If incorporated into DNA, this would appear to enable those cells with nucleic acid molecules which contain the analogue to be selectively irradiated and hence killed by UV radiation.

Although the preparation of 5-acetyluracil has been previously described[7,8] we required a method of obtaining tritiated material with a high specific activity so that the incorporation of the compound into nucleic acids could be followed. It was found that the Me protons could be exchanged for deuterium by dissolving 5-acetyluracil in alkaline D_2O and the specificity and position of labelling could be confirmed from the NMR spectrum of the product. Similarly 5-acetyluracil could be tritiated in high yield using tritiated water to obtain a product with a very high specific activity. The tritium would not exchange under normal physiological conditions but the action of both strong acid or alkali resulted in exchange.

5-Acetyluracil could be readily reduced by sodium borohydride under strongly alkaline conditions to give 5-(1-hydroxyethyl)uracil (2). Under the more usual reduction conditions at pH 9·9 in borate buffer, the reaction was much slower and considerable ring cleavage was obtained as evidenced by the presence of a urea on a chromatogram of the reaction products and the yield of the alcohol produced accounted for only 45% of the original 5-acetyluracil used. It would appear that this reaction is very similar to that known to occur[9] when N^4-acetylcytidine is reduced with sodium borohydride to give N^4-acetyl-3,4,5,6-tetrahydrocytidine. In 5-acetyluracil, the 5,6-double bond is in conjugation with two CO groups thus facilitating attack at C-6 by hydride ion. Once reduced to the dihydrouracil derivative, ring cleavage and further reduction would follow.[10] In 0·1N NaOH both N-protons will have been removed and the resulting negative charge on the heterocyclic ring may discourage attack by hydride at C-6. 5-(Hydroxyethyl)uracil was easily obtained with a tritium label by using tritiated sodium borohydride. The position of labelling was confirmed to be the 1′ position of the side chain, by analogy with the product isolated from the reduction with sodium borodeuteride and obtaining its NMR spectrum.

The dehydration of 5(1-hydroxyethyl)uracil to give the olefin, 5-vinyluracil (3) was investigated under a variety of acidic conditions. The action of 98% formic acid at 175° in a sealed tube resulted in cleavage of the side chain and the production of uracil although after an hour some starting material was still present. Formic acid (98%) treatment of 5-(1-hydroxyethyl)uracil at 100° for 30 min resulted in the production of a compound which was identified by NMR spectroscopy to be a

dimer of 5-vinyluracil (**4**), which is thought to originate according to Scheme 1.

Using an aqueous solution of formic acid (80%), it was established that the reaction soon contained a constant amount of a compound thought to be 5-vinyluracil (**3**) and further heating of the solution only resulted in a diminution of the amount of starting material and the production of more dimer. Thus for ease of separation of the products it was found to be better to allow the reaction to proceed under reflux for only 5 min, to isolate the compound which was identified as 5-vinyluracil and to retreat the mixture of unchanged starting material and 5-vinyluracil dimer with 80% formic acid to produce more 5-vinyluracil. The compound could also be easily produced with a tritium label and with the same specific activity as the tritiated starting material, 5-(1-hydroxyethyl)uracil, as no exchange takes place under the conditions of the reaction.

The reaction of sulphuric acid on 5-(1-hydroxyethyl)uracil resulted in considerable charring. Syrupy phosphoric acid resulted in the production of a compound which was identified as the dimer of 5-vinyluracil as it was identical to the compound isolated from the treatment of 5-(1-hydroxyethyl)uracil with formic acid, by which route the compound could be more easily prepared and characterised.

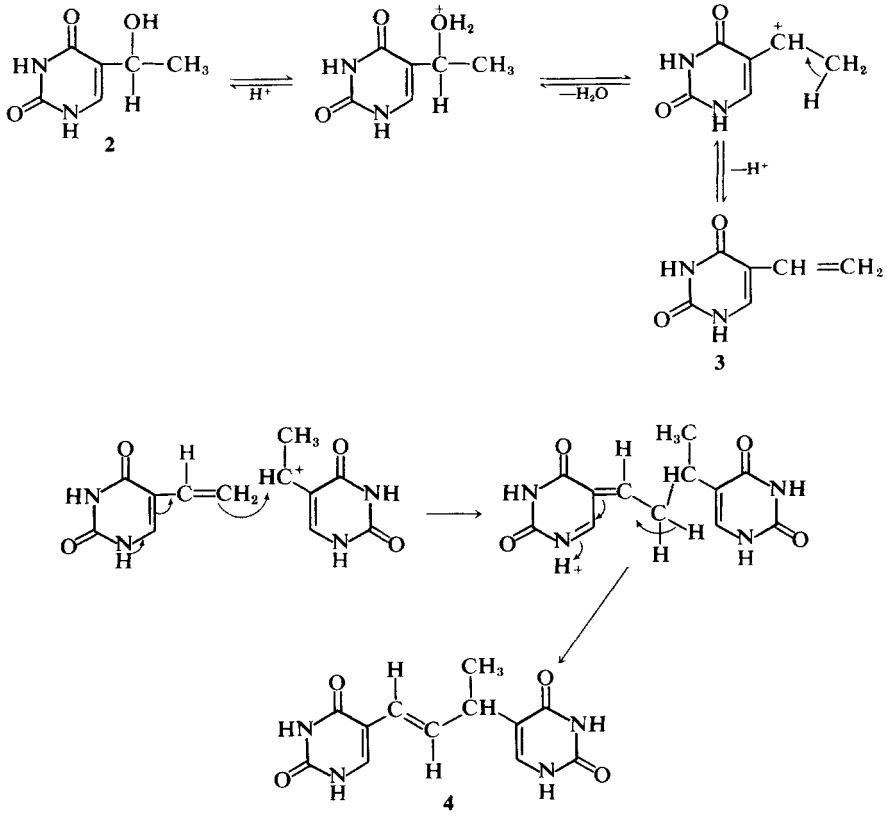
5-Vinyluracil was found to react rapidly with water under aqueous mineral acid conditions to give quantitative yields of the 5-(1-hydroxyethyl)-uracil at 100° in 1 hr. The compound was much more stable under alkaline conditions, but rather more drastic conditions with refluxing alkali caused the production of the alcohol.

Thus 5-vinyluracil has been prepared and characterised in a labelled form suitable for following its incorporation into deoxynucleosides and DNA. The maximum UV absorption of the molecule (288 nm) should be far enough removed from that of the other constituent bases of DNA, such that selective irradiation should be possible. The uptake of this compound into DNA will be reported elsewhere.

EXPERIMENTAL

General. D_2O (> 99·7 atom % D), NaOD (> 98 atom % D) and $NaBD_4$ (> 98 atom % D) were obtained from Koch-Light Laboratories Ltd., [^{3}H] H_2O (5Ci/ml) and [^{3}H] $NaBH_4$ (800 mCi/mmole) were obtained from the Radiochemical Centre, Amersham.

Chromatography. Paper chromatography was carried out with the following solvents: (1) n-BuOH-EtOH-



SCHEME 1. **2**: 5-(1-hydroxyethyl)uracil; **3**: 5-vinyluracil; **4**: 5-vinyluracil dimer.

water (4:1:5, organic phase); (2) t-BuOH-methyl ethyl ketone-ammonia (d. 0·88)-water (40:30:30:10); (3) i-PrOH-HCl-water (136:33:31); (4) n-BuOH saturated with water-ammonia (d. 0·88) (100:1). The R_F values of the compounds in these solvents are shown in Table 1.

Table 1. R_F Values of some 5-substituted uracils

Compound	Solvent 1	2	3	4
5-Acetyluracil (1)	0·53	0·53	0·73	0·45
5-(1-Hydroxyethyl)uracil (2)	0·55	0·45	0·61	0·32
5-Vinyluracil (3)	0·66	0·75	0·95	0·22
5-Vinyluracil dimer (4)	0·19	0·35	0·56	0·22

5-Acetyluracil (1). This was prepared by the method of Shaw and coworkers.[7,8]

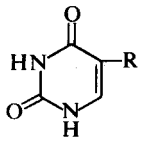
Synthesis of 5-[^{2}H] *acetyluracil.* 5-Acetyluracil (100 mg) suspended in D_2O (1·1 ml) containing NaOD (41 mg) was heated in a sealed tube at 100° for 3 hr. 1N AcOH (3 ml) was added to the cooled mixture and the resulting ppt was removed by centrifugation, washed with glacial AcOH and dried *in vacuo* to give a product (yield 61·7 mg, m.p. 283–5° (d)) which cochromatographed with an authentic sample of 5-acetyluracil; (λ_{max} (pH 1) 277 nm, ϵ 11,300; λ_{max} (pH 13) 253 nm, ϵ 9,700, 308 nm, ϵ 15,100. An NMR spectrum in $(CD_3)SO$ was identical to that of 5-acetyluracil: δ 10·96 (2H, s, 5-H), 8·07 (1H, s, H-6), 2·44 ppm (3H, s, MeC=O) except the resonance due to the acetyl protons was absent.

Synthesis of 5-[^{3}H] *acetyluracil.* 5-Acetyluracil (100 mg) was suspended in [^{3}H] H_2O (0·2ml, 1Ci) and 1·25N NaOH (0·8 ml) and the reaction allowed to proceed and the product isolated as described above. The yield was 73 mg and the product had the same physical characteristics and had a specific activity of 30 mCi/mmole. The supernatant liquid from this preparation was added to 1·25N NaOH (20 ml) and used to tritiate a further sample of 5-acetyluracil (2·5 g). A pure product was obtained (1·73 g) with a specific activity of 1·16 mCi/mmole.

Synthesis of 5-(1-*hydroxyethyl*)*uracil* (2). 5-Acetyluracil (2 g) was dissolved in 0·1M NaOH (170 ml) and $NaBH_4$ (1·9 g) added. The mixture was left at room temp in the dark for 22 hr, when the UV spectrum of the soln confirmed the absence of starting material. The excess of reducing agent was destroyed and the soln neutralised with Zeo Karb 225 resin (H^+ form). The filtrate was evaporated to dryness, borate was removed by repeated co-evaporation with MeOH and the product recrystallised from MeOH (1·3 g); λ_{max} (pH 1) 263 nm, ϵ 7, 650: λ_{max} (pH 13) 287 nm ϵ 9,200. (Found: C, 45·9; H, 5·0; N, 17·8. $C_6H_8N_2O_3$ requires: C, 46·1; H, 5·1; N, 17·9%); δ (CD_3SO): 10·95 (2H, s, N-H), 7·23 (1H, s, H-6), 4·95 (1H, s, OH), 4·55 (1H, q, CH-Me), 1·22 ppm (3H, d, Me). Reduction of 5-acetyluracil at pH 9·9 (0·1M borate buffer) with $NaBH_4$ resulted in the production of compounds obviously formed by the opening of the pyrimidine ring. Chromatography of the soln showed the presence of a urea and only 45% of the starting material could be accounted for on the chromatogram in the form of UV-absorbing compounds.

Synthesis of 5-(1-*hydroxy*-1-[^{2}H]*ethyl*)*uracil.* The deuterated material was prepared as described above for the preparation of 5-(1-hydroxyethyl)uracil, except that $NaBD_4$ was used. The products were identical except that the NMR spectrum of the deuterated material had the peak at δ 4·55 ppm missing and the peak at δ 1·22 ppm was a singlet for three protons, showing that the tertiary proton of the side chain had been replaced by deuterium.

Synthesis of 5-(1-*hydroxy*-1-[^{3}H]*ethyl*)*uracil.* This compound was prepared as described above except that [^{3}H]-$NaBH_4$ (100 mCi, 4·5 mg) was used to reduce 5-acetyluracil (3 g). A pure product was isolated with a specific activity of 750 μCi/mmole.



1: R = C(=O)—Me
2: R = CHOH—Me
3: R = CH=CH_2

Dehydration of 5-(1-*hydroxyethyl*)*uracil*

(a) *With* 98% *formic acid.* 5-(1-Hydroxyethyl)uracil (100 mg) was dissolved in 98% formic acid (5 ml) and the soln heated in a sealed tube at 100° for 30 min. The formic acid was removed, the product extracted several times with water (3 × 10 ml) at 90° and the remaining solid dried *in vacuo* to give a product which was homogeneous by paper chromatography and was identified as a dimer of 5-vinyluracil; *trans* 1,3-bis-(uracil-5-yl)but-1-ene (4), yield 53 mg, m.p. 320° (d); λ_{max} (pH 1) 246 nm (sh 270 nm), ϵ 9,600; λ_{max} (pH 14) 254 nm, ϵ 8,100; 296 nm, ϵ 6,200. (Found: C, 52·2; H, 4·7; N, 19·7. $(C_6H_6N_2O_2)_2$ requires: C, 52·2; H, 4·39; N, 20·3%); δ (CD_3SO): 11·18–10·76 (4H, m, N-H), 7·60 (1H, s, H-6′), 7·18 (1H, s, H-6″), 6·61 (1H, 2d, H-2; $J_{2,3}$ 7 Hz, $J_{1,2}$ 17 Hz), 6·17 (1H, d, H-1), 1·22 ppm (3H, d, CH_3). The peak due to H-3 is masked by traces of water in the solvent but its presence was confirmed by decoupling experiments.

(b) *With* 80% *formic acid.* 5-(1-Hydroxyethyl)uracil (1 g) was dissolved in 80% formic acid (25 ml) and added to 80% formic acid (475 ml) which was boiling under reflux. The soln was boiled for 5 min, rapidly cooled and the formic acid removed under reduced pressure. The product was dissolved in a mixture of EtOAc (80 ml) and water (40 ml) which was placed in the first four tubes of a counter current apparatus. After 120 transfers. the contents of tubes 65–90 were pooled evaporated to dryness and a chromatographically homogeneous compound identified as 3 was isolated, yield 130 mg, m.p. 248° (d); λ_{max} (pH 1) 239 nm, ϵ 12,300; 288 nm, ϵ 6,900, λ_{max} (pH 14) 252 nm, ϵ 12,400; 309 nm, ϵ 7,900. (Found: C, 52·3; H, 4·6; N, 20·05. $C_6H_6N_2O_2$ requires: C, 52·2; H, 4·39; N, 20·3%); δ (CD_3SO) 11·20 (2H, s, N-H), 7·65 (1H, s, H-6), 6·48 (1H, q, H-1′; $J_{1',2'\,trans}$ 18 Hz, $J_{1',2'\,cis}$ 11 Hz), 6·02 (1H, 2d, H-2′; $J_{2',2'}$ 2·6 Hz), 5·14 ppm (1H, 2d, H-2′). 5-(1′-[^{3}H]vinyl)uracil (120 mg) was prepared in an identical manner starting from 5-(1-hydroxy-1-[^{3}H]ethyl)uracil (1 g). The specific activity of the starting material and product was identical at 750 μCi/mmole.

Acidic hydrolysis of 5-*vinyluracil.* 5-Vinyluracil (1 mg) was dissolved in 1N HCl (100 ml) and the rate of hydrolysis followed at 20°, 37° and 100°. UV spectra of the solns taken at timed intervals had isobestic points at 256 nm and 274 nm which is indicative of the decomposition of one UV-absorbing compound into *one* other UV-absorbing compound. Chromatography of the solns after 24 hr showed that only 5-(1-hydroxyethyl)uracil and the starting material was present. Thus it was possible to

determine the rate of production of 5-(1-hydroxyethyl) uracil. At 37° and 100°, hydrolysis was complete in 1 hr and 30 hr respectively. At 20° only 40% hydrolysis had occurred after 30 hr.

Alkaline hydrolysis of 5-vinyluracil. 5-Vinyluracil (2 mg) in 1N NaOH (100 ml) was stable at 37° for a period of at least 3 days. When a soln of 5-vinyluracil (2 mg) was heated under reflux in 0·1N NaOH, the presence of isobestic points in the UV spectra of the soln taken at timed intervals confirmed chromatographic evidence that only 5-(1-hydroxyethyl)uracil was being produced; 9% after 2 hr, 21% after 4 hr, 30% after 7 hr and 80% after 24 hr.

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