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

SYNTHESIS OF 5-SUBSTITUTED URACILS DERIVED FROM 5-ACETYLURACIL

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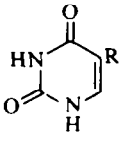
Abstract—5-Acetyluracil (**1**) has been converted into 5-(bromoacetyl)-uracil (**2**) by an established procedure. Reduction of **2** with sodium borohydride gave 5-(2-hydroxyethyl)uracil (**4**) in low yield. Treatment of 5-vinyluracil (**7**), obtained from **1** by published methods, with 1 molecular proportion of bromine followed by heating to 100°, gave *E*-5-(2-bromovinyl)uracil (**8**). Reaction of **8** with potassium t-butoxide gave 5(7)H-furano[2,3,d]pyrimidin-6-one (**10**) and upon reduction with sodium in liquid ammonia, **8** gave 5-ethyluracil (**11**). Compound **2** showed low antibacterial activity against *Staphylococcus aureus*, *Streptococcus faecalis* and *Escherichia coli* in nutrient broth and in a medium containing only inorganic salts, glucose and thymine, appreciable activity (~6 μg/ml) against *E. coli*. Compound **2** was not incorporated into the DNA of *E. coli*.

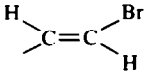
Currently there is much interest in the synthesis of 5-substituted uracils because of the possibility of their incorporation into DNA and because some of their deoxyribonucleosides are antiviral agents.[1-7] In previous papers we have described the synthesis of 5-vinyluracil and shown that it is incorporated into the DNA of *Escherichia coli* and *Mycoplasma mycoides* subsp. *capri* PG3.[8-11] The starting material for this synthesis was 5-acetyluracil. In continuing this work we have carried out a number of reactions on 5-acetyluracil and have converted it into other 5-substituted uracil derivatives.

Treatment of 5-acetyluracil (**1**) with bromine in methanol by the method of Ross *et al.*[12] gave 5-(bromoacetyl)uracil (**2**). Results given later indicated that this compound might be incorporated into the DNA of *Escherichia coli*, so in order to establish whether this was so, the synthesis of this compound labelled with tritium in the 5-substituent was carried out. This was achieved by the action of bromine on the appropriately tritium-labelled 5-acetyluracil.[8] Reduction of **2** with an excess of sodium borohydride in aqueous sodium hydroxide under conditions which gave a high yield of 5-(1-hydroxyethyl)uracil (**3**) from 5-acetyluracil, gave in low yield, 5-(2-hydroxyethyl)uracil (**4**). The assignment of this structure to the compound was based upon elemental analysis, NMR spectrum (two triplets at 2.6 δ and 3.7 δ which were assigned to the two side chain methylene groups) and UV spectrum. This compound had been obtained previously by other workers by the condensation of urea with α-*hydroxymethylene*-γ-butyrolactone in the presence of sodium methoxide.[13]



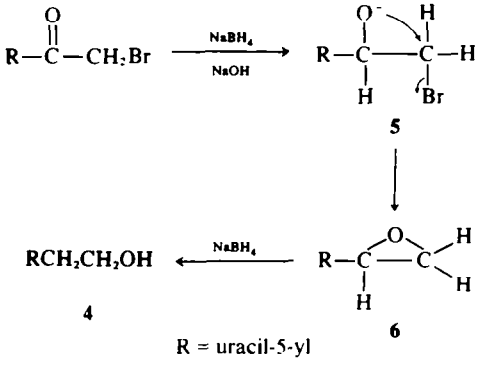
1: R = –COCH$_3$
2: R = –COCH$_2$Br
3: R = –CHOHCH$_3$
4: R = –CH$_2$CH$_2$OH

7: R = –CH=CH$_2$

8: R = 

9: R = –CHBr–CH$_2$Br
11: R = –CH$_2$–CH$_3$

A possible pathway for the formation of **4** from **2** is via

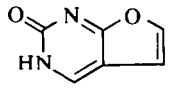


an epoxide (**6**). This epoxide could then be further reduced by sodium borohydride to give either a primary or secondary alcohol. The formation of the primary alcohol (**4**) appears to be favoured. In an analogous case it has been found that the action of a methyl Grignard reagent on styrene oxide gives the primary alcohol.[14]

5-Acetyluracil was converted into 5-vinyluracil (**7**) via 5-(1-hydroxyethyl)uracil by the procedure previously described.[9] The action of bromine on 5-vinyluracil was investigated. In view of the reactivity of the 5:6 double bond in uracil and thymine there was the possibility that addition would occur at this bond as well as at the exocyclic double bond. However, treatment of **7** with 1 molecular proportion of bromine in dimethylformamide at room temperature resulted in an almost instantaneous reaction to give only one major product. This had a UV spectrum which is typical of a 5-substituted uracil with a saturated side chain. This compound could not be isolated, however, because it decomposed during the isolation procedure. When the reaction mixture was subsequently heated to 100° however, a compound was produced which had a UV spectrum typical of a 5-substituted uracil with an unsaturated side chain. This product was isolated and identified as *E*-5-(2-bromovinyl)uracil (**8**). Its structure was established from its elemental analysis and NMR and UV spectra. The assignment of the *E* configuration around the exocyclic double bond was based on the coupling constant (J = 13 Hz) of the vinylic protons. It seems reasonable to assume that the unstable compound

initially produced at room temperature is 5-(1,2-dibromoethyl)uracil (**9**). This compound is sterically crowded, so that its ready dehydrobromination on heating is satisfactorily explained. The formation of the 2-bromovinyl derivative, as opposed to the 1-bromovinyl derivative appears to be preferred for both steric and electronic reasons. The most stable *E* stereoisomer was the only product detected. No work on the mechanism of this elemination has been carried out, but the formation of the product can be equally well explained on the basis of either an E_1 or an E_2 reaction.

Treatment of 5-(2-bromovinyl)uracil with potassium t-butoxide gave the fluorescent bicyclo compound, 5(7)H-furano[2,3,d]pyrimidin-6-one (**10**) which appeared to have been formed by the nucleophilic displacement of bromide by a negatively charged oxygen at C-4 of the pyrimidine ring. Sodium in liquid ammonia brought about the reduction of **8** to 5-ethyluracil (**11**).



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Our main interest in 5-substituted uracils is in their possible biological activity and incorporation into DNA and polynucleotides. 5-Ethyluracil and its deoxyribonucleoside are incorporated into DNA of some organisms[7] and the deoxyribonucleoside has been shown to have antiviral activity.[7] In addition to showing that 5-vinyl uracil is incorporated into the DNA of *E. coli* and *M. capri*[10] we have also shown that 5-acetyluracil is incorporated to a small extent into the DNA of *M. capri* but not into that of *E. coli* or T_3 bacteriophage.[11] The two bromine-containing uracil derivatives prepared in this work, namely 5-(bromoacetyl)uracil (**2**) and 5-(2-bromovinyl)uracil (**8**) were tested for growth inhibitory activity against a number of bacteria and fungi; **8** showed no activity but **2** showed a low activity (~60–120 μg/ml) against *Staphylococcus aureus*, *Streptococcus faecalis*, and *E. coli* in a nutrient broth medium. In a synthetic medium containing only inorganic salts, glucose and thymine, the compound was appreciably active (~6 μg/ml) against a thymine-dependent and a thymine-independent strain of *E. coli*. It was thought possible that **2** might be incorporated into the DNA of the thymine-dependent strain but experiments in which the tritium-labelled 5-(bromoacetyl)uracil was used, showed that this was not the case.

EXPERIMENTAL

5-Acetyluracil. This was prepared by a three-stage synthesis from diketene and ethyl carbamate as described.[13] The overall yield was 26%. The product had λ_{max} 284 nm (pH 6) ε, 12,000.

5-(Bromoacetyl)uracil. This was obtained from 5-acetyluracil by reaction with bromine in methanol as described.[12] The product was obtained in 73% yield (Found: C, 31.1; H, 2.2; N, 12.0. Calc. for $C_6H_5BrN_2O_3$: C, 30.9; H, 2.2; N, 12.0%), λ_{max} 227 nm (ε, 10,500), 288 nm (ε, 12,000) in ethanol. δ, 4.64 (2H, s, $-COCH_2Br$), 8.20 (1H, s, H-6), 11.70 ppm (2H, b, -NH). 5-(Bromoacetyl)uracil labelled with tritium in the $-COCH_2Br$ group was prepared in a similar manner starting with the appropriately-labelled 5-acetyluracil which had been prepared by the method previously described.[8] The product had a specific activity of 1.65 mCi/mmole.

Reaction of 5-(bromoacetyl)uracil with sodium borohydride. 5-(Bromoacetyl)uracil (500 mg) was dissolved in aqueous 0.1 M NaOH (10 ml) to which was added $NaBH_4$ (500 mg). The mixture was stirred at room temp. for 26 hr and then acidified with a 10-fold excess of Zeocarb 225 resin (H^+ form). The resin was filtered off and washed thoroughly with water. The filtrate was evaporated to dryness and borate was removed by co-evaporation with MeOH. The resulting yellow solid was crystallised from aqueous MeOH and then from MeOH to give 5-(2-hydroxyethyl)uracil (50 mg) (Found: C, 45.1; H, 5.0; N, 17.6. Calc. for $C_6H_8N_2O_3$: C, 46.2; H, 5.1; N, 17.9%), λ_{max} 264 nm at pH 6, 292 nm at pH 13. δ, 2.6 (2H, t, H-1') 3.7 ppm (2H, t, H-2'). *m/e* 156.

5-Vinyluracil. This was prepared in an overall yield of 60%, from 5-acetyluracil via 5-(1-hydroxyethyl)uracil by the method previously described.[9]

E-5-(2-Bromovinyl)uracil. To a solution of 5-vinyluracil (450 mg, 3.3 mmole) in dry DMF (50 ml) there was added with shaking a freshly prepared soln of Br_2 (263 mg, 3.3 mmole) in dry DMF (10 ml). (Examination of the soln at this stage by TLC showed that there was present one major product which had λ_{max} 264 nm at pH 6 and 292 nm at pH 13). The bulk of the soln was heated at 100° for 1 hr and then evaporated to give a brown oil. To this, water (25 ml) was added and the resulting yellow ppt filtered off and crystallised from MeOH to give *E-5-(2-bromovinyl)uracil* (600 mg, 85% yield) m.p. 220° (d) (Found: C, 33.0; H, 2.5; N, 12.7. $C_6H_5BrN_2O_2$ requires C, 33.2; H, 2.3; N, 12.9%), λ_{max} 239 nm (ε, 12,300), 288 nm (ε, 7000); λ_{min} 264 nm (ε, 4750) at pH 1. λ_{max} 267 nm (ε, 15,400), 305 nm (ε, 11,400); λ_{min} 290 nm (ε, 10,500) at pH 13, δ, 6.8 and 7.3 (2H, two doublets, H-1' and H-2', $J_{1',2'}$ 13 Hz), 7.7 (1H, d, H-6, J = 6 Hz), 11.2 ppm (2H, bd, -NH).

5(7)H-Furano[2,3,d]pyrimidin-6-one. 5-(2-Bromovinyl)uracil (250 mg) was dissolved in dry DMF (50 ml), t-BuOK (1.7 g) was added and the mixture heated at 100° for 5 hr with stirring. The resulting suspension was filtered off and the filtrate evaporated to dryness and the residue dissolved in hot MeOH. The MeOH soln was acidified with glacial AcOH and the resulting yellow ppt filtered off and crystallised from MeOH to give *5(7)H-furano[2,3,d]pyrimidin-6-one* (90 mg, 58% yield), m.p. 260° (Found: C, 52.6; H, 3.2; N, 20.4. $C_6H_4N_2O_2$ requires: C, 52.9; H, 3.0; N, 20.6%), λ_{max} 243 nm (ε, 10,200), 320 nm (ε, 4400); λ_{min} 234 nm (ε, 8800), 267 nm (ε, 0) at pH 6. λ_{max} 244 nm (ε, 13,000), 310 nm (ε, 6500); λ_{min} 230 nm (ε, 8000), 270 nm (ε, 0) at pH 13. δ, 6.70 (1H, d, H-3), 7.65 (1H, d, H-2, $J_{2,3}$ = 3 Hz), 8.30 ppm (1H, s, H-4).

Reaction of 5-(2-bromovinyl)uracil with sodium in liquid ammonia. *E*-5-(2-Bromovinyl)uracil (300 mg) was dissolved in anhyd ammonia (50 ml) and Na added in small pieces until the soln remained blue. The ammonia was allowed to evaporate off at room temp. and the white solid which remained dissolved in water (25 ml). The soln was neutralised with 1 M HCl, evaporated to dryness and the residue extracted with hot EtOH (200 ml). Examination of the extract by TLC in EtOH–$CHCl_3$ (1:9) and water–acetonitrile (1:9) showed that all of the starting material had disappeared and that only one major UV absorbing product was present. From the UV absorption of the soln it was calculated that the yield was 200 mg. The ethanolic extract was concentrated to a small volume and allowed to stand at 0° overnight. This gave a crystalline sample (50 mg) of the product, 5-ethyluracil (Found: C, 51.4; H, 5.4; N, 19.5. Calc. for $C_6H_8N_2O_2$: C, 51.4; H, 5.7; N, 20.0%), λ_{max} 265 nm (ε, 7900); λ_{min} 236 nm (ε, 2100) in ethanol. δ, 1.0 (3H, t, H-2'), 2.2 (2H, q, H-1', $J_{1',2'}$ = 4 Hz), 7.2 ppm (1H, s, H-6).

Antibacterial activities and incorporation of 5-(bromoacetyl)uracil into DNA of E. coli. These were carried out as previously described,[10] the study on the incorporation into DNA being carried out with the tritium-labelled 5-(bromoacetyl)uracil.

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