THE SYNTHESIS OF SOME TRITIATED 5-SUBSTITUTED URACIL DERIVATIVES

P.J. Barr, L. Boháček*, A.S. Jones and R.T. Walker
Chemistry Department, Birmingham University, Birmingham B15 2TT, U.K.
*Institute for Research, Production and Use of Radioisotopes, Praha 7, Přístavní 24, Czechoslovakia.

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SUMMARY

The synthesis of 5-[^{3}H-ethynyl]uracil and 5-acetyl[6-^{3}H]uracil in good yield and of a medium specific activity (>40 mCi/mmol) is described. The compounds are necessary for following the incorporation of these and other derived 5-substituted uracils into DNA and also for studying the metabolism and mode of action of their deoxynucleosides, some of which possess antiviral properties.

Key Words: 5-[^{3}H-ethynyl]uracil, 5-acetyl[6-^{3}H]uracil

INTRODUCTION

We have been involved for some time in the synthesis of thymine and thymidine analogues possessing potential radiation-sensitive substituents in the 5-position of the pyrimidine ring.[1-3] The incorporation of some of these analogues into DNA has been studied[4] and it has also been found that several of the deoxynucleosides have an antiviral activity, particularly against herpes virus.[5] In order to study the incorporation of these analogues into nucleic acid and also to study their metabolism and mode of action as antiviral compounds, it has been found necessary to have the compounds radioactively labelled in the heterocyclic moiety. We here describe the preparation of 5-ethynyluracil (1) labelled in the ethynyl group with tritium. This compound was our original target in the synthesis of thymine analogues with radiation-sensitive side chains and the deoxynucleoside is a thymidylate synthetase inhibitor.[3]

We also describe the synthesis of 5-acetyl[6-^{3}H]uracil. 5-Acetyluracil (2) is the precursor of all our analogues.[1] Previously, we have described the preparation of 5-[^{3}H-acetyl]uracil[1] but this method involved the use of rather large quantities of tritiated water and resulted in a product with only a low specific activity (<5 mCi/mmol).

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RESULTS AND DISCUSSION

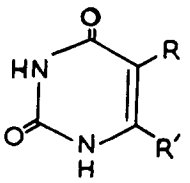
Three attempts were made to prepare a tritiated sample of 5-ethynyluracil (1) of medium specific activity. It was known that addition of HCl across the triple bond of 5-ethynyluracil occurred under very mild conditions to give 5-(1-chlorovinyl)uracil (3) and that this latter compound could be converted quantitatively under mild conditions back to 5-ethynyluracil by the action of dilute alkali (unpublished results, see also ref. 5). However, preliminary experiments (data not shown) using deuterated HCl and analysing the product by NMR or using tritiated HCl and monitoring the specific activity of the products, confirmed that both the addition and elimination of HCl were stereospecific and thus the product was identical in all respects to the starting material.

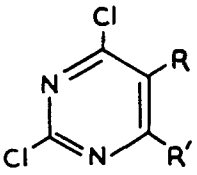
A second attempt involved adding sodium methoxide to an aqueous solution of 5-ethynyluracil (1) and boiling the resulting solution under reflux for 45 min. in the presence of tritiated water. Some exchange of the ethynyl proton took place and 5-[^{3}H-ethynyl]uracil could be recovered in 77% yield but only 12% of the theoretical number of the ethynyl protons could be exchanged for tritium before unacceptable levels of chemical decomposition of the 5-ethynyluracil occurred. Even then, 0.5 Ci of tritiated water was used to obtain just over 100 mg of product having a specific activity of 2.16 mCi/mmol.

However, if 5-ethynyluracil (0.5 mmoles) was dissolved in dimethylsulphoxide, tritiated water (5 mmoles) and 1,5-diazabicyclo [4,3,0] non-5-ene (1.5 mmoles, DBN) added and the solution left at 20° for 96h, then 5-ethynyluracil, labelled in the side chain, could be isolated having a specific activity of 40 mCi/mmol.

The preparation of 5-substituted uracil derivatives labelled at position 6 of the heterocyclic nucleus has some interest for incorporation and metabolic studies even though the corresponding derivatives labelled in the side chain are now readily available, as one can follow the metabolic fate of the heterocyclic ring and compare it with that of the side chain.

The compound required is 5-acetyl-6-chlorouracil (4) which can then be dehalogenated using tritium gas in the presence of a palladium catalyst to 5-acetyl[6-^{3}H]uracil. The preparation of 5-acetylbarbituric acid from barbituric acid has already been recorded.[6] This





1 R = C≡CH, R'=H

2 R = $C(=O)-CH_3$, R'=H

5 R = $CHCl=CH_2$, R'=H

3 R = $CHCl=CH_2$, R'=H

4 R = $C(=O)-CH_3$, R'=Cl

6 R = $CHCl=CH_2$, R'=Cl

7 R = C≡CH, R'=Cl

product when treated with phosphoryl chloride in the presence of diethylaniline under conditions similar to that used for the preparation of 5-(1-chlorovinyl)-2,4-dichloropyrimidine (5) from acetyluracil,[3] gave 5-(1-chlorovinyl)-2,4,6-trichloropyrimidine (6) in fair yield. This could then be treated under basic conditions to give 6-chloro-5-ethynyluracil (7). Unfortunately it was not possible to prepare 5-ethynyl[6-^{3}H]uracil from 6-chloro-5-ethynyluracil as it was known from other work (Gough, Jones and Walker, unpublished results) that the standard conditions used to replace the 6-chloro group with hydrogen resulted in the reduction of the triple bond to give 5-ethyluracil. It is of interest to note that 2'-deoxy-5-ethyluridine has antiviral properties and this preparation would give a route to a labelled compound which might be useful for metabolic studies.[7] Other attempts to find conditions which would replace the chlorine with hydrogen, but leave the triple bond untouched, failed.

Thus, 6-chloro-5-ethynyluracil (7) was treated with HCl to give 5-acetyl-6-chlorouracil (4) which when reacted in the presence of 5% palladium-on-barium sulphate with tritium gas, gave 5-acetyl[6-^{3}H]uracil having the same specific activity as the tritium gas used for the dehalogenation. The product was characterised by chromatography with an authentic sample.

EXPERIMENTAL

Chromatography

Paper chromatography was carried out using the solvent propan-2-ol, ammonia (d. 0.88), water (7:1:2). TLC was carried out on silica plates (Merck, Kieselgel 60 F_{254}) in the solvents ethanol, chloroform (1:4) and the organic phase of butan-1-ol, ethanol, water (4:1:5). Radioactive compounds were treated using a Radiochromatogram Spark Chamber (Birchover Instruments). Non-radioactive markers were located by photography under UV light. The R_f values of 5-acetyluracil in the three systems listed (in order) were: 0.66, 0.75, 0.47 and for 5-ethynyluracil were 0.36, 0.41 (streaking) and 0.30 (streaking).

5-[^{3}H-Ethynyl]uracil (i)

Sodium methoxide (135 mg, 2.5 mmoles) was dissolved in water (0.4 ml) and tritiated water (0.1 ml, 5 Ci/ml) added. To this solution was added 5-ethynyluracil (150 mg, 1.1 mmoles) and the resulting solution was heated under reflux for 45 min. The solution was allowed to cool, neutralised by addition of glacial acetic acid (0.15 ml) and then stood at 4° for 18h. The solid which precipitated was collected by centrifugation, washed with water and then repeatedly evaporated with water to remove any rapidly-exchangeable tritium atoms. The product, 5-[^{3}H-ethynyl]uracil was obtained in 77% yield (116 mg, 2.16 mCi/mmol) and was identified by comparison on chromatography in the three systems listed with an authentic specimen of unlabelled material and was shown to possess the correct UV absorption data including ϵ values.[3]

5-[^{3}H-Ethynyl]uracil (ii)

5-Ethynyluracil (60 mg, 0.44 mmoles) was dissolved in a mixture of dimethylsulphoxide (0.6 ml) and DBN (0.18 ml, 1.32 mmoles) and tritiated water (0.09 ml, 5 mmoles, 90 mCi/mmol) added. The reaction mixture was kept at 20° for 96h and was then evaporated to dryness under reduced pressure and repeatedly co-evaporated with methanol. 5-Ethynyluracil was separated by preparative layer chromatography on silica gel using the solvent system propan-2-ol, ammonia (d. 0.880), water (7:1:2) and recovered by elution with water to give 5-[^{3}H-ethynyl]uracil in 80% yield (48 mCi/mmol) which was identified by comparison on

chromatography in the three systems listed with an authentic specimen of unlabelled material and was shown to possess the correct UV absorption data including ϵ values.[3]

5-Acetyl [6-^{3}H]uracil

(i) 5-Acetylbarbituric acid This compound was prepared by the method of Vul'fson and Zhurin[6] to give 5-acetylbarbituric acid (52 g) in 65% yield.

(ii) 5-(1-Chlorovinyl)-2,4,6-trichloropyrimidine (6) To a suspension of 5-acetylbarbituric acid (20 g) in freshly distilled N,N-diethylaniline (32 ml) was added freshly distilled phosphoryl chloride (132 ml) and the mixture was heated to 115 - 120° for 20h. The excess of phosphoryl chloride was then removed by distillation and the residue poured onto ice/water (400 ml) and stirred. The resulting solid was filtered off, dissolved in diethylether (200 ml) washed with water (2 x 100 ml), saturated sodium carbonate (2 x 100 ml) and with water (2 x 100 ml), dried over anhydrous magnesium sulphate, the ether removed by distillation and the residue distilled at 100 - 105° (1 mm) to give 5-(1-chlorovinyl)-2,4,6-trichloropyrimidine (17.4 g, 60%) m.p. 39°. Found: C, 29.8; H, 1.0; N, 11.8; Cl, 58.4. $C_6H_2Cl_4$ requires C, 29.6; H, 0.82; N, 11.5; Cl, 58.1%. λ_{max} 227 nm, ϵ, 8.500; 257 nm, ϵ, 4,500, λ_{min} 251 nm, ϵ, 3,900 in ethanol; δ ($CDCl_3$) 5.93 (1-H, d, H-2' cis to ring, J = 3Hz), 5.60 ppm (1-H, d, H-2' trans to ring, J = 3Hz).

(iii) 6-Chloro-5-ethynyluracil (6) 5-(1-Chlorovinyl)-2,4,5-trichloropyrimidine (4 g) was dissolved in dioxan (16 ml), 2 M KOH (50 ml) added and the solution boiled under reflux for 1 hr. The reaction mixture was then neutralized by the addition of 1N HCl to pH 6 and evaporated to dryness under reduced pressure and the last traces of dioxan removed by coevaporation with water. The residue was triturated with water (25 ml) and the resulting solid was filtered off, washed with water and recrystallised from methanol to give 6-chloro-5-ethynyluracil (1.8 g, 64%) m.p. > 300° (d). Found: C, 38.2; H, 2.8; N, 14.6; Cl, 18.5. $C_6H_3ClN_2O_2.H_2O$ requires C, 38.2; H, 2.7; N, 14.8; Cl, 18.8%. λ_{max} 231 nm, ϵ, 9,100; 286 nm, ϵ, 8,900; λ_{min} 253 nm, ϵ, 2,700 at pH 1; λ_{max} 250 nm, ϵ, 10,200; 296 nm, ϵ, 9,900; λ_{min} 270 nm, ϵ, 2,800 at pH 13; δ $(CD_3)_2$ SO 3.60 ppm (s, acetylenic H).

(iv) 5-Acetyl-6-chlorouracil (4) Instead of isolating the product from the previous reaction, after neutralization of the dioxan solution to pH 6, a further amount (15 ml) of 1N HCl was added before the solution was taken to dryness. Traces of dioxan were removed as before, the resulting solid was triturated with water (10 ml), the pH of the suspension adjusted to 6 with 1N KOH and the solid filtered off and recrystallised from methanol to give 5-acetyl-6-chlorouracil (1.6 g, 52% overall yield) m.p. > 300° (d). Found: C, 38.1; H, 2.7; N, 14.5; Cl, 18.6. $C_6H_5ClN_2O_3$ requires C, 38.2; H, 2.67; N, 14.8; Cl, 18.8%. λ_{max} 236 nm, ϵ, 5,700; 277 nm, ϵ, 10,000; λ_{min} 251 nm, ϵ, 4,600 at pH 1; λ_{max} 261 nm, ϵ, 4,900; 304 nm, ϵ, 7,400; λ_{min} 273 nm, ϵ, 4,400 at pH 13, δ $(CD_3)_2SO$ 2.30 ppm (s, CH_3CO).

(v) 5-Acetyl[6-^{3}H]uracil 5-Acetyl-6-chlorouracil (10 mg, 0.05 mmol) was hydrogenated at room temperature and atmospheric pressure in the presence of 5% palladium on barium sulphate (40 mg) and 1N ammonium hydroxide (0.7 ml). Tritium (specific activity 8 mCi/mmol, 1.1 ml, 0.05 mmol) was consumed in 25 min. The catalyst was removed and the product isolated by paper chromatography using the solvent butan-1-ol, acetic acid, water (4:1:5) to give 5-acetyl[6-^{3}H]uracil (4.2 mg, 50% yield, 8 mCi/mmol) which was identified by comparison on chromatography in the three systems listed with an authentic specimen of unlabelled material and was shown to possess the correct UV absorption data including ϵ values.[1]

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