A Nonmutagenic Thymidine Analog with Antiviral Activity. 5-Ethyldeoxyuridine[1]

M. ŚWIERKOWSKI AND D. SHUGAR

Department of Radiobiology, Institute of Oncology, and Institute of Biochemistry and Biophysics, Academy of Sciences, Warsaw, Poland

Received February 4, 1969

It has been previously shown that 5-ethyluracil may undergo incorporation into bacterial DNA,[2] and that 5-ethyldeoxyuridine readily replaces thymidine (5-methyldeoxyuridine) in bacteriophage DNA.[3] Particular interest attaches to 5-ethyluracil and its glycosides, since these would be expected to exhibit the same base-pairing properties as the parent compounds, *i.e.*, uracil, thymine, and their glycosides.[4]

The syntheses of 5-ethyluridine,[5,6] its 5′-mono and pyrophosphates, and poly-5-ethyluridylic acid[4,6] have already been reported. The present communication describes the preparation of 5-ethyldeoxyuridine (V) and some of its properties; preparation of its 5′-triphosphate is under way with a view to its subsequent polymerization with either DNA polymerase or deoxynucleotidyl transferase. The photochemical behavior of the nucleoside in aqueous medium is also of special interest.[7]

The deoxynucleoside has been synthesized according to standard procedures[8] by the condensation of the monomercury salt of 5-ethyluracil (II) with 3,5-di-*O*-*p*-chlorobenzoyl-2-deoxy-α,β-D-ribofuranosyl chloride (III) to give 1-(3,5-di-*O*-*p*-chlorobenzoyl-2-deoxy-α,β-D-ribofuranosyl)-5-ethyluracil (IV), in about 50% yield with respect to 5-ethyluracil.

The mixture of the anomers of IV was initially debenzoylated to give a mixture of the anomers of 5-ethyldeoxyuridine, which was deposited on a strongly basic Dowex 1 × 2 column as described by Dekker,[9] and eluted with NH_4HCO_3 to give two reasonably well-defined peaks. Slow evaporation of these two fractions gave crystalline residues. One of these, but not the other, proved capable of sustaining growth of the thymine-less strain *Escherichia coli* 15T⁻ and of some of the T coliphages[2,3] and was therefore assigned the configuration β.

However, subsequent attempts to effect the above separation on a preparative scale, with the aid of larger columns, were unsuccessful. Preparative thin layer chromatography was then tried and the two benzoylated anomers of IV, but not the free nucleosides, were readily separated and obtained in highly purified, crystalline form. Nmr spectroscopy was then applied to establish the configurations of each of the anomers, by reference to the corresponding anomers of thymidine,[10] and, as might have been anticipated, they were found to correspond to those previously determined from their ability to support bacterial and phage growth.

As was to be expected, the uv spectra of 5-ethyldeoxyuridine were very similar to those of thymidine.[11] Of special interest was the fact that the pK_a (9.9) for dissociation of the ring N^3 hydrogen, determined spectrophotometrically,[11] was pracitcally identical with that for thymidine.[11]

The possible mutagenic activity of 5-ethyldeoxyuridine was investigated by Dr. Irena Pietrzykowska, with 5-bromodeoxyuridine as a control, by means of the spot test of Freese,[12] using 16 rII mutants of phage T_4 and looking for reversions to r^+. Under conditions where 5-bromodeoxyuridine gave the expected frequency of reversions, 5-ethyldeoxyuridine was quite inactive. Analogous results were obtained by Professor C. Heidelberger with a sample of V supplied to him.

The potential antiviral activity of V was examined by Professor Heidelberger, who found that the α anomer was inactive, as expected, while the β anomer was almost as active as 5-iododeoxyuridine and 5-bromodeoxyuridine in inhibition of vaccinia viral replication in HeLa cells. It is of particular interest in this connection that Guari and Malorny[13] have reported V to be an effective inhibitor of herpes simplex virus, although the preparation and properties of their compound were not described. The simultaneous presence of antiviral activity, and absence of mutagenic activity, is rather striking and suggests the advisability of synthesizing additional 5-alkyl-substituted pyrimidine nucleoside analogs. The preparation and properties of 5-ethylcytidine and 5-ethyldeoxycytidine will be reported shortly.[14]

Experimental Section

5-Ethyluracil was prepared according to the method of Burckhalter and Scarborough[15] as modified by Shapira.[5]

N-1-Acetyl-5-ethyluracil (I) was obtained as described by Spector and Keller[16] for other diketopyrimidines. 5-Ethyluracil (1.4 g, 10 mmoles) in 3 ml of Ac_2O containing 25–50 mg of pyridine was heated for 15–20 min under reflux. Several minutes of heating sufficed to completely dissolve the ethyluracil. The solution was brought to room temperature and the crystalline precipitate was filtered off and washed (Ac_2O) to give 1.41 g (78%) of **I**, mp 142–145°.

Monomercury Salt of 5-Ethyluracil (II).—To a solution of 2.46 g of $Hg(OAc)_2$ in 77.5 ml of anhydrous MeOH, heated under reflux and constantly stirred, was added 1.41 g of **I**. Heating was continued for 2 hr and the mixture then was left overnight. The resulting precipitate was filtered off and dried to give 2.65 g (100%) of **II**, mp >360.°

3,5-Di-O-*p*-chlorobenzoyl-2-deoxy-α,β-D-ribofuranosyl chloride (III) was prepared according to Fox, *et al.*,[8] by chlorination of methyl α,β-2-deoxy-D-ribofuranoside.[17] The product, following prolonged washing with Et_2O, had mp 113°, as compared to a literature[8] value of 118–120°. Other observers[18] have also

(1) This investigation was supported in part by the Wellcome Trust, the World Health Organization, and the Agricultural Research Service, U. S. Department of Agriculture [UR-E21-(30)-32].

(2) M. Piechowska and D. Shugar, *Biochem. Biophys. Res. Commun.*, **20**, 768 (1965).

(3) (a) I. Pietrzykowska and D. Shugar, *ibid.*, **25**, 267 (1966); (b) I. Pietrzykowska and D. Shugar, *Acta Biochim. Polon.*, **14**, 165 (1967).

(4) D. Shugar, M. Świerkowski, M. Fikus, and D. Barszcz, 7th International Congress of Biochemistry, Tokyo, 1967, Vol. I, Symposium 1, pp 59–60.

(5) J. Shapira, *J. Org. Chem.*, **27**, 1918 (1962).

(6) M. Świerkowski and D. Shugar, in preparation (see also ref 4).

(7) I. Pietrzykowska and D. Shugar, *Science*, **161**, 1248 (1968).

(8) J. J. Fox, N. Yung, I. Wempen, and M. Hoffer, *J. Am. Chem. Soc.*, **83**, 4066 (1961).

(9) C. A. Dekker, *ibid.*, **87**, 4027 (1965).

(10) R. U. Lemieux, *Can. J. Chem.*, **39**, 116 (1961).

(11) J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, **9**, 369 (1952).

(12) E. Freese, *Proc. Natl. Acad. Sci. U. S.*, **45**, 622 (1959).

(13) K. K. Gauri and G. Malorny, *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.*, **257**, 21 (1967).

(14) T. Kulikowski and D. Shugar, in preparation.

(15) J. H. Burckhalter and H. C. Scarborough, *J. Am. Pharm. Assoc.*, **44**, 545 (1955).

(16) L. B. Spector and E. B. Keller, *J. Biol. Chem.*, **232**, 185 (1958).

(17) R. E. Deriaz, W. G. Overend, M. Stacey, and L. F. Wiggins, *J. Chem. Soc.*, 2836 (1949).

(18) H. J. Minnemeyer, P. B. Clarke, and H. Tieckelmann, *J. Med. Chem.*, **7**, 567 (1964).

reported difficulties in obtaining a product with mp 118–120°. It is therefore of interest that, during chlorination, the resulting precipitate of III consisted initially of needles, followed by tiny platelets; prior to extensive washing with Et_2O, the precipitate melted in two steps under a microscope hot stage, mp 94 (platelets) and 102° (needles). These are obviously the two anomers of III and the melting point of the final product will clearly depend on the ratio of the two anomers which, in turn, determines the ratio of the two anomeric nucleosides in the condensation reaction.[19] Because of the lability of III, the freshly prepared mixture of anomers was used as such immediately in the subsequent condensation step.

1-(3,5-Di-O-*p*-chlorobenzoyl-2-deoxy-α,β-D-ribofuranosyl)-5-ethyluracil (IV).—II (1.49 g, 4.4 mmoles) was suspended in about 130 ml of anhydrous PhMe, vigorously stirred, and dried azeotropically by removal of about one-third of the solvent; 3.8 g of III (8.8 mmoles), previously dried, was added rapidly, and the mixture was heated 3 min, then cooled and filtered through glass wool. The precipitate was dissolved ($CHCl_3$), the solution was washed (30% KI, H_2O), and the orgnic phase was dried (Na_2SO_4). The salt was filtered off, the $CHCl_3$ solution was brought to dryness, and the residue was dissolved in hot anhydrous EtOH. Crystallization occurred on cooling to give 1.1 g (47%) of IV, melting at 154–178° and exhibiting under a microscope hot stage two types of crystals.

Attempted Separation of the Anomers of V by Ion-Exchange Chromatography.—A Dowex 1-X2 (200–400 mesh) (OH^-) column, 23 × 2 cm, was washed with 500 ml of 30% aqueous MeOH, and 15 mg of V in the same solvent deposited on the column. The latter was then washed with 250 ml of 60% aqueous MeOH and 250 ml of 90% aqueous MeOH. The nucleoside was then eluted with 0.05 *M* NH_4HCO_3 at a flow rate of about 1.5 ml/min and fractions of 13 ml were collected. The nucleoside appeared in two fairly-well-defined peaks: fraction 13 (4.9 mg, mp 172–174.5°) and fraction 16 (5.2 mg, mp 143–147°). Fraction 16, but not fraction 13, supported bacterial and phage multiplication and is therefore the β anomer of V.

Separation of anomers of IV was achieved on GF_{254} silical gel, deposited as 1.0-mm layers on 20 × 15 cm glass plates, with the solvent system $CHCl_3$–Et_2O (8:2, v/v). It was necessary to run each plate three or four times to obtain adequate separation, the final R_f values for the α and β anomers being about 0.85 and 0.95. The gel containing each of the spots was deposited on a sintered-glass filter and eluted with $CHCl_3$. The eluates were brought to dryness and the residues crystallized from EtOH to give the pure α and β anomers of IV with mp 186–187.5° and 196–197°, respectively.

2′-Deoxy-α(β)-D-ribofuranosyl-5-ethyluracil (V).—The α and β anomers of IV were debenzoylated according to Prystas and Šorm[20] and each was recrystallized (EtOH) to give the α and β anomers of V with mp 177–179° and 152–153°, respectively. The spectral data of the α anomer was as follows: $\lambda_{max}^{pH_2}$ 268 mμ (ϵ_{max} 9.78 × 10^3), $\lambda_{min}^{pH_2}$ 235 mμ (ϵ_{min} 2.42 × 10^3), $\lambda_{max}^{pH_{12}}$ 268 mμ (ϵ_{max} 7.40 × 10^3), $\lambda_{min}^{pH_{12}}$ 245 mμ (ϵ_{min} 4.27 × 10^3); pK_a = 9.86. The spectral data of the β anomer was as follows: $\lambda_{max}^{pH_2}$ 267.5 mμ (ϵ_{max} 9.61 × 10^3), $\lambda_{min}^{pH_2}$ 235 mμ (ϵ_{min} 2.35 × 10^3), $\lambda_{max}^{pH_{11}}$ 267.5 mμ (ϵ_{max} 7.28 × 10^3), $\lambda_{min}^{pH_{12}}$ 245 mμ (ϵ_{min} 4.62 × 10^3); pK_a = 9.98.

Nmr spectra were determined in D_2O for each of the anomers at 30° with a Varian HA-100 spectrometer and Me_4Si as external standard. Except for the added signals due to the presence of a 5-Et in place of a 5-Me substituent, the spectra were similar to those of the corresponding anomers of thymidine;[10] for α-5-ethyldeoxyuridine, 6.51 ppm (H_1, quartet), $J_{H_1'-H_2'}$ = 8.0 cps, $J_{H_1'-H_2''}$ = 3.6 cps; for the β anomer, 6.58 ppm (H_1, triplet) $J_{H_1'-H_2'}$ = $J_{H_1'-H_2''}$ = 7.0 cps.

Acknowledgments.—We are indebted to Professor M. Anteunis (Service de Chimie Organique, Universite de Gent, Belgium) for the nmr spectra, to Professor C. Heidelberger (University of Wisconsin) for the tests of antiviral activity, and to Dr. I. Pietrzykowska and Dr. M. Piechowska for the tests on the growth-supporting properties of the anomers and the possible mutagenic activity of β-5-ethyldeoxyuridine.

(19) B. R. Baker, J. P. Joseph, E. E. Schaub, and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954).

(20) M. Prystas and F. Šorm, *Collection Czech. Chem. Commun.*, **31**, 1035 (1966).

4,4′,6,6′-Tetrabromo-2,2′-biphenyldiol Mono(dihydrogen phosphate). A New Agent for Combating Distomatosis[1]

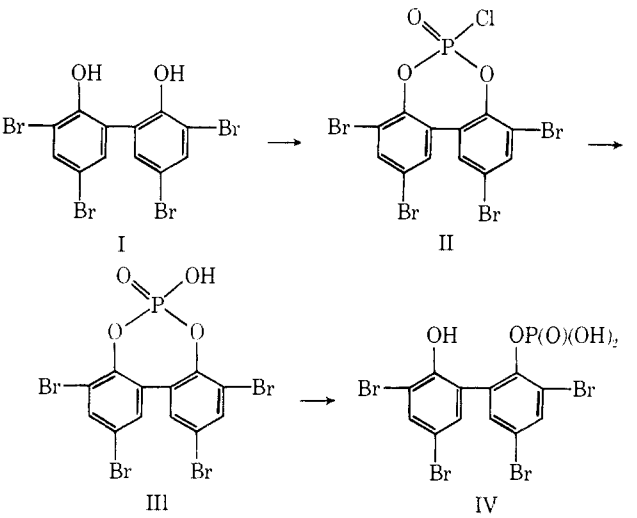
S. VAN DER MEER AND H. POUWELS

ACF Amsterdam Chemie Farmacie N. V., Research Laboratories, Amsterdam, The Netherlands

Received October 25, 1968

In the course of investigations on agents for combating distomatosis, a disease of sheep and cattle caused by the liver fluke, *Fasciola hepatica*, the title compound (IV) was synthesized according to Scheme I.

SCHEME I



The starting material I was obtained by bromination of 2,2′-biphenyldiol.[2] Treatment with $POCl_3$ in toluene provided the cyclic phosphorochloridate (II). Hydrolysis of II was carried out best by dissolving the crystalline compound in toluene, adding this solution to an aqueous EtOH solution of NaOH, and refluxing the mixture. In this way the poor solubility of the sodium salt of the intermediate III did not interfere. Protracted boiling of the alkaline solution results in complete loss of the acid group. The few per cent of I formed along with the open phosphate IV is easily removed by the purification process described in the Experimental Section.

Pharmacology.—The title compound has been found to be a potent agent in controlling distomatosis. Therapeutic doses of 16 and 12 mg/kg, respectively, in sheep and cattle would require doses of 20 and 16 mg/kg of the standard drug 2,2′-methylenebis(3,4,6-trichlorophenol) to obtain comparable results.[3] The acute toxicity in mice (LD_{50} > 150 mg/kg) was lower than that of the standard. A dose of 36 mg/kg may be safely administered to cattle. Laboratory tests with mice and rats[4] and field trials with some thousands of cattle[5] indicate also activity against immature liver

(1) S. van der Meer, W. Kruyt, and H. Pouwels, Dutch Patent Appl. 65.05635 (1966); corresponding foreign applications are pending.

(2) O. Diels and A. Bibergeil, *Ber.*, **35**, 306 (1902).

(3) W. Kruyt and E. J. van der Steen, *Tijdschr. Diergeneesk.*, **94**, 308 (1969).

(4) W. Kruyt and E. J. van der Steen, to be published.

(5) J. S. Reinders, *Tijdschr. Diergeneesk.*, **94**, 324 (1969).