SYNTHESIS OF ^{14}C-LABELLED 5-ETHYL DERIVATIVES OF PYRIMIDINE

M.Skakun-Todorović and V.Jezdić
Organic Chemistry Department
Boris Kidrič Institute
Vinča - Yugoslavia
Received on February 12, 1973

SUMMARY

A method for the synthesis of 5-ethylorotic acid-2-^{14}C (V),5-ethyluracil-2-^{14}C (VI) and 5-ethylytosine-2-^{14}C (VIII) starting from thiourea-^{14}C with the specific activity of 30 mCi/mM has been described. The labelled compounds obtained show satisfactory che--mical and radiochemical purity. The method was modified and adap--ted for work at the level 1-5 mM.

INTRODUCTION

In the recent time the synthesis of uracil derivatives has been carried out intensively with the aim to investigate their metabolic properties. Until recently 5-alkyl derivatives of uracil were neglected while in the recent years they attract more attention, especially after investigations of antimetabolic properties of 5-ethyluracil, 5-ethyluridine and 5-ethyldeoxyuridine. 5-Ethyluracil easily incorporates into DNA of bacteria[1] while 5-ethyldeoxyuridine readily substitutes thymidine in the bacteriophages of DNA[2]. Besides it has been found out that 5-ethyluracil and its nucleosides have the same pair formation property as similar compounds (uracil, thymine and their nucleosides)[3].

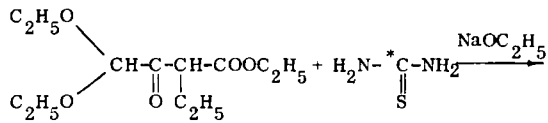
As labelled compounds are more suitable for biochemical and biological investigations we have developed a method for the synthesis of 5-ethylorotic acid, 5-ethyluracil and 5-ethylcytosine labelled with ^{14}C in the position 2.

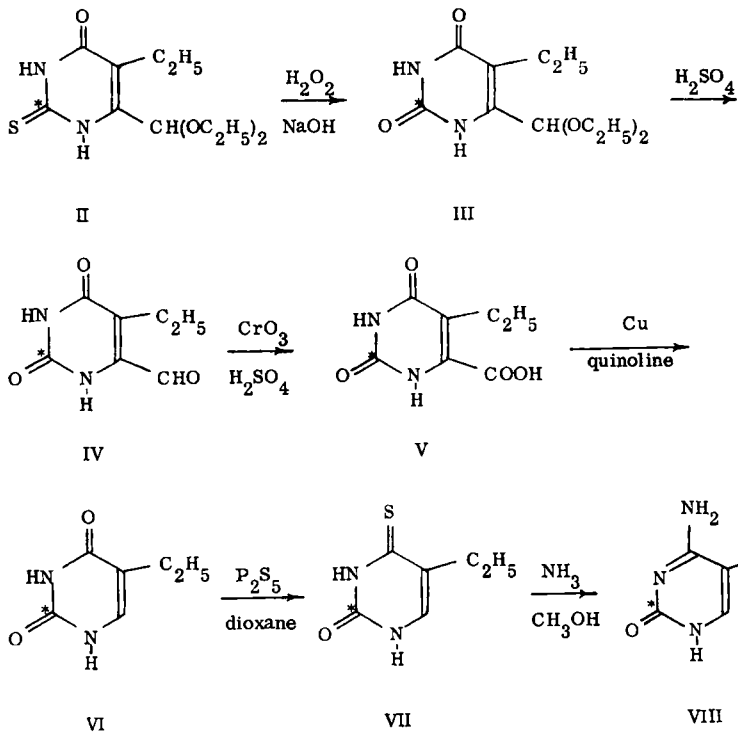
Two methods for the synthesis of 5-ethylorotic acid are described in the literature[4,5]. Clerc-Bory and Mentzer[4] condensed ethyl 3-ethyloxaloacetate with urea to obtain hydantoin which was then hydrolyzed to 5-ethylorotic acid. From ethyl 3-ethyloxaloacetate and S-alkylisothiouroniumsulphate Borodkin et al.[5] obtained 2-alkylthio-5--ethylorotic acid, from which they further obtained 5-ethylorotic acid by hydrolysis with hydrochloric acid. In both cases 5-ethylorotic acid was obtained in 35% yield based on the starting material.

Several methods for the synthesis of 5-ethyluracil can also be found in the literature[6-8]. 5-Ethyluracil was obtained[6] in 70% yield by decarboxylation of 5-ethylorotic acid in presence of Cu powder in quinoline. Burckhalter and Scarborough[7] obtained 5-ethyl-2-thiouracil by condensation of the sodium salt of ethyl formylbutyrate with thiourea and by applying the usual method with monochloroacetic acid they converted it into 5-ethyluracil in 18% yield based on the starting thiourea. Shapira[8] synthesized 5-ethylbarbituric acid from urea and diethyl malonate and further in a series of reactions he obtained 5-ethyluracil in 28% yield, based on the starting urea. By using this method Gauri et al.[9] synthesized 5-ethyluracil labelled with tritium in the position 4.

Johnson and Menge[10] and Kulikowski and Shugar[11] synthesized 5-ethylcytosine by converting 5-ethyluracil by means of its chloro derivarive into 5-ethylcytosine in 43% yield.

In our paper the synthesis of the above mentioned 5-ethyl derivatives of pyrimidine labelled with ^{14}C in the position 2 was realized in a series of reactions:





The starting material, thiourea-^{14}C, was produced in our laboratory[12]. Ethyl 4,4-diethoxy-2-ethylacetoacetate (I) was synthesized by Claisen condensation of ethyl diethoxyacetate and ethyl butyrate[13,14]. Synthesis was further performed using a modified method described by. Holmes and Prusoff[15] for the synthesis of 5-methylorotic acid-2-^{14}C. By the cyclization of thiourea-^{14}C with compound I, 4-diethoxymethyl-5-ethyl-2-thiouracil--2-^{14}C (II) was obtained. In a series of reactions, II was converted into 5-ethylorotic acid--2-^{14}C (V) in 52% yield based on thiourea-^{14}C. 5-Ethyluracil-2-^{14}C (VI) was obtained by decarboxylation of V in 70% yield, i.e., 36% yield with respect to the starting thiourea-^{14}C. In order to obtain 5-ethylcytosine-2-^{14}C (VIII), the classical method was applied by introducing amino group by means of thio group. Thiation of 5-ethyluracil was performed by means of phosphorus pentaslphide in dioxane[16]. The obtained thio derivative VII was then converted (by means of ammonia in methanol) into 5-ethylcytosine-2-^{14}C in 65% yield, i.e.

in 22% over all-yield based on the starting thiourea-^{14}C.

Labelled compounds synthesized by this method show satisfactory chemical and radiochemical purity. Chemical purity was checked by paper chromatography, melting point and ultraviolet absorption spectrophotometry, while radiochemical purity was checked by radioscanning of the paper chromatogram. Specific activities of the labelled compounds were 28-30 mCi/mM.

EXPERIMENTAL

Anhydrous solvents and freshly distilled reagents were used for the synthesis. Ascending chromatography was performed on Whatman No.1 paper in the following solvent systems: n-butanol saturated with water, n-butanol-propionic acid-water (10:5:7) and isopropanol-ammonia-water (7:1:2). Melting points were not corrected.

Ethyl 4,4-diethoxy-2-ethylacetoacetate (I)

The mixture of 6.37 ml (50 mM) of ethyl butyrate and 6.3 ml (50 mM) of ethyl diethoxyacetate was added dropwise into a suspension of 50 mM of sodium ethoxide in 20 ml of benzene. The reaction mixture was then refluxed for 4 hours, with constant stirring. The cooled reaction mixture was then poured into a cold solution (ice bath) of 5 ml of glacial acetic acid in an equal volume of water. After all the solid material had been dissolved, the organic phase was separated and the aqueous layer extracted several times with ether. Ether extracts were combined with the organic phase and washed, first with water, then with saturated solution of sodium bicarbonate and finally, again with water. After washing the ethereal extracts were dried with sodium sulphate. Ether was removed by distillation and the residue was distilled in vacuum. There was obtained 4.9 g of fraction boiling at 118-120°C/5 mm Hg[17].

4-Diethoxymethyl-5-ethyl-2-thiouracil-2-^{14}C (II)

Thiourea-^{14}C (0.106 g, 1.4 mM) and 10 ml of sodium ethoxide in ethanol (0.035 g of sodium, 1.52 mM) were heated on a steam bath at the temperature of 95-100°C with stirring under anhydrous conditions until all the thiourea was dissolved. Then 0.59 g (2.4 mM) of ethyl 4,4-diethoxy-2-ethylacetoacetate was quickly added to the hot solution and the reaction mixture was refluxed for 6 hours with constant stirring. The solvent was

then evaporated and the residue dissolved in 15 ml of water, treated with activated charcoal and evaporated to a small volume. The ice-cooled solution was then carefully neutralized with hydrochloric acid (3 N), up to pH = 6. After cooling, the precipitated crystals were filtered off, washed with cold water and dried.

It was obtained 0.338 g of 4-diethoxymethyl-5-ethyl-2-thiouracil-2-^{14}C (93.5% based on the thiourea-^{14}C), m.p. 126-8°C.

4-Diethoxymethyl-5-ethyluracil-2-^{14}C (III)

Sodium hydroxide solution (0.68 ml, 5N) was slowly added dropwise, with constant stirring, to the ice-cooled suspension of 0.274 g (1.06 mM) of the compound II, 1.6 ml of water and 0.68 ml of 30% hydrogen peroxide. After addition the cooling was continued for another 10 minutes and the solution was stirred for 2 hours at room temperature. The excess of hydrogen peroxide was removed by heating the reaction mixture on the boiling water bath, then 0.34 ml of concentrated sulphuric acid was slowly added dropwise with constant stirring to the thoroughly cooled reaction mixture (ice bath). 4-Diethoxymethyl-5-ethyluracil-2-^{14}C which immediately appeared as a white precipitate was used without isolation for the next step of the synthesis.

5-Ethyl-4-uracilcarboxaldehyde-2-^{14}C (IV)

The reaction mixture with the precipitated compound III from the preceding phase of the synthesis was heated to boiling (bath temperature 130-135°C) and allowed to boil for 2 minutes to perform hydrolysis and remove sulphur dioxide formed. The solution was then cooled in an ice bath until, after longer time, 5-ethyl-4-uracilcarboxaldehyde-2-^{14}C was separated as a white precipitate. The product was used for further synthesis without isolation.

5-Ethylorotic acid-2-^{14}C (V)

The reaction mixture from the preceding phase with precipitated aldehyde IV, was thoroughly cooled. The solution of 0.318 g (3.18 mM) of chromium trioxide in 0.45 ml of water and 0.34 ml of concentrated sulphuric acid were added successively to the reaction mixture dropwise with constant stirring. The precipitate separated quickly after removal of the ice bath and the reaction mixture was first stirred for 3 hours at room temperature

then heated for 10 minutes on the bath at the temperature of 80–85°C to achieve complete oxidation. After standing overnight in the refrigerator the precipitate was filtered off, washed with cold water and dried.

The yield of 5-ethylorotic acid-2-^{14}C was 0.110 g (52% based on the thiourea-^{14}C) with m.p. 313–314°C (reported[5] m.p. 311–313°C); λ_{max} = 272,5 nm, λ_{min} = 238 nm (aqueous solution).

5-Ethyluracil-2-^{14}C (VI)

5-Ethylorotic acid-2-^{14}C (0.100 g, 0.54 mM), 0.02 g of copper powder and 1 ml of quinoline were refluxed for 1 hour on the metallic bath at the temperature of 260–265°C. A solution of sodium hydroxide (2.5 ml, 2N) was added to the cooled reaction mixture and the quinoline was removed by extraction with ether. The aqueous layer was filtered and acidified with hydrochloric acid (2N). 5-Ethyluracil-2-^{14}C was separated by continuous extraction with ethyl acetate for 5 hours. Ethyl acetate was then evaporated, the residue was dissolved in water, treated with activated charcoal and evaporated to smaller volume. The white crystalline precipitate of 5-ethyluracil-2-^{14}C was filtered off, washed with ice cold water and dried.

There was obtained 0.053 g of 5-ethyluracil-2-^{14}C (36% based on the thiourea-^{14}C) with m.p. 308–309°C (reported[6] m.p. 308–309°C); λ_{max} = 265 nm, λ_{min} = 234 nm (aqueous solution).

5-Ethyl-6-thiouracil-2-^{14}C (VII)

5-Ethyluracil-2-^{14}C (0.100 g, 0.71 mM), 0.078 g of phosphorus pentasulphide and 15 ml of dioxane were refluxed with constant stirring under anhydrous conditions for 4 hours. The solvent was then evaporated, the residue dissolved in water and methanol, the solution treated with activated charcoal and evaporated to smaller volume. After cooling, the yellow crystalline precipitate was filtered off, washed with ice cold water and dried.

The yield was 0.103 g of 5-ethyl-6-thiouracil-2-^{14}C (93% based on 5-ethyluracil-2-^{14}C) with m.p. 305–306°C (decomp.).

5-Ethylcytosine-2-^{14}C (VIII)

5-Ethyl-6-thiouracil-2-^{14}C (0.118 g, 0.75 mM) and 15 ml of methanolic ammonia (previously saturated at 0°C) were heated in a sealed tube at 100°C for 18 hours. After the tube had been opened the reaction mixture was heated to boiling (methanol was added to clarify the solution), treated with activated charcoal and left in the refrigerator to crystallize. The white crystalline precipitate was filtered off the next day, washed with a small quantity of chilled methanol and dried.

There was obtained 42 mg of 5-ethylcytosine-2-^{14}C with m.p. 275-278°C. Mother liquor was concentrated to smaller volume and after standing overnight in the refrigerator additional 46 mg of compound VIII were obtained with m.p. 268-270°C. By recrystallization from methanol the total quantity of 68 mg 5-ethylcytosine-2-^{14}C was obtained (60% based on the 5-ethyluracil-2-^{14}C) with m.p. 275-278°C (reported[11] m.p. 274-277°C); λ_{max} = 284 nm, λ_{min} = 242 nm (0.1 N hydrochloric acid).

REFERENCES

1. Piechowska, M. and Shugar, D. - Biochem. Biophys. Res. Commun., 20 : 768 (1965).

2. Pietrzikowska, J. and Shugar, D. - Biochem. Biophys. Res. Commun., 25 : 267 (1966).

3. Shugar, D., Swierkowski, M., Fikus, M. and Barszcz, D. - 7 th International Congress of Biochemistry, Tokyo, 1967, Vol. I, Symposium 1, pp. 59-60.

4. Clerc-Bory, C. and Mentzer, C. - Bull. Soc. Chim. France, 436 (1958).

5. Borodkin, S., Jonsson, S., Cocolas, G. H. and Mc Kee, R. L. - J. Med. Chem., 10 : 290 (1967).

6. Guyot, A., Chopin, J. and Mentzer, C. - Bull.Soc.Chim.France, 1596 (1960).

7. Burckhalter, J. H. and Scarborough, M. C. - J. Am. Pharm. Assoc., 44 : 545 (1955).

8. Shapira, J. - J. Org. Chem., 27 : 1918 (1962).

9. Gauri, K. K., Pflughaupt, K. W. and Müller, R. - Z. Naturforsch., 24 b : 833 (1969).

10. Johnson, T. B. and Menge, G. A. - J. Biol. Chem., 2 : 105 (1906).

11. Kulikowski, T. D. and Shugar, D. - Acta Biochim. Pol., 18 : 209 (1971).

12. Jezdić, V. and Rajnvajn, J. - Bull. Inst. Nucl. Sci. Boris Kidrič, 12 : 127 (1961).

13. Johnson, T. B. and Critcher, L. H. - J. Biol. Chem., 26 : 29 (1916).

14. Royals, E. E. and Robinson, A. G. - J. Am. Chem. Soc., 78 : 4161 (1956).

15. Holmes, W. L. and Prusoff, W. H. - J. Biol. Chem., 206 : 817 (1954).

16. Škarić, V., Gašpert, B., Jerkunica, J. and Škarić, Dj. - Croat. Chem. Acta, 37 : 199 (1965).

17. Henze, H. R. and Carroll, D. W. - J. Am. Chem. Soc., 76 : 4580 (1954).