ACTA CHEMICA SCANDINAVICA 13 (1959) 2070–2074

Synthesis of Uracil-2, 4, 5, 6-^{14}C with High Specific Activity

Studies in Microsynthesis I

GÖSTA EHRENSVÄRD and JAN LILJEKVIST

Institute of Biochemistry, University of Lund, Lund, Sweden

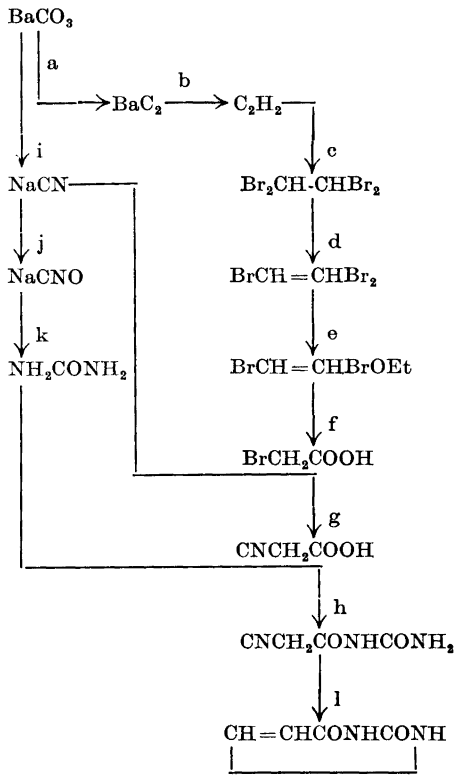
A method for preparing 2, 4, 5, 6-^{14}C-uracil in small scale from $BaCO_3$-^{14}C of highest possible specific activity is presented. The synthesis is carried out in the 100 μmole range giving a total yield of 10 % of 2, 4, 5, 6-^{14}C-uracil the activity of wich being unchanged from that of the starting material. The technique used may find applications in other fields of organic micro-synthesis.

In the course of an investigation on the chemical and biochemical effects of nuclear decay of some atom species incorporated into the molecular structure of organic compounds it was found necessary to develop procedures for multistep synthesis in the 25—250 μmole range. A special problem has been to synthesize uracil and thymine with a maximal ^{14}C activity (round 100 μC/μmole) in order to study the consequences of the decay of 5-^{131}I-uracil, and the overall effects of the $^{14}C \rightarrow {}^{14}N$ transition [1]. The starting material has been barium carbonate with an activity of 24.75 μC/μmole and the synthesis achieved according to the scheme in Table 1.

Each of the above conventional steps (for references see Table 1) has been modified to enable work in the 100 μmole range. The present procedure, as described below, gives a total yield of uracil from barium carbonate of 10 % with no dilution of the ^{14}C-activity. Of the different single steps the preparation of barium carbide has been considerably simplified, enabling the reduction of the barium carbonate with metallic barium to be carried out without the presence of helium [2]. Step *f*, the hydrolysis of 1,2-dibromovinyl-ethyl-ether to bromoacetic acid [5,6] was considerably difficult to run in small scale until a suitable closed micro vessel was employed. Step *l* involving the hydrogenation of cyanoacetylurea to uracil was found to give very poor yield as carried out according to the original procedure of Rupe *et al* [16]. In acetate buffer of pH 4.15, however, the yield was consistently 45 %.

As regards the technical aspect of the present work the aim has been to keep the number of transfers as low as possible. Reaction $c \rightarrow l$ are thus carried

Table 1.



Reaction steps	References
a	2
b	3
c	3
d	4
e	5, 6
f	5, 6
g	7, 8
h	9, 10
i	11, 12
j	13, 14, 15
k	15
l	16

out in the same reaction vessel, the construction of which enabling amount of solutions of around 500 μl to be effectively closed off, thus avoiding procedures involving heating under reflux. With regard to the latter, we have found that any reflux procedure in the 100 μmole range especially in the case of a two-phase system, involve considerable losses, and should be replaced with closed space procedures. The reaction vessel employed (see Fig. 1) could act as a micro-autoclave, standing a pressure up to 3 atm., as a microhydrogenation vessel, and as a centrifuge tube.

EXPERIMENTAL

Barium carbide-^{14}C. 49.3 mg barium carbonate (250 μmole, spec. act. 24.75 μC/μmole) was weighed on a small aluminum foil (weight about 50 mg) and a lump of ether washed barium metal, corresponding to the double theoretical amount was added. The foil was wrapped around the reactants to form a small pellet, which was put in a 4 ml Pyrex test tube. The tube was heated with a burner to red heat. The reaction was complete in a few seconds and the tube was allowed to cool. The tip containing the reaction mixture was broken off and crushed to a fine powder in a diamond mortar. This powder was used directly for preparing acetylene.

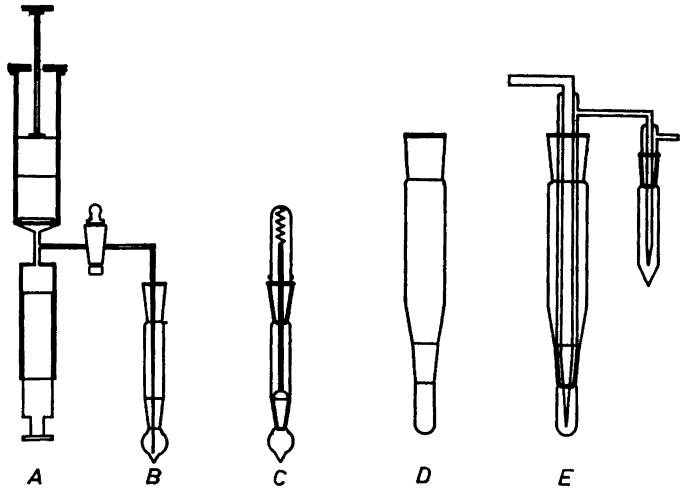


Fig. 1. Gas generator and reaction vessel for small-scale synthesis.

The gas generator A consist of two connected ordinary all-glass syringes, one holding 25 ml, the other 10 ml. In the bottom part of the upper, larger syringe a close-fitting porcellain filter plate is placed, covered with a filter paper disc, cut to exact size. Lubricants were avoided; the stopcock of the assembly was of non-lubricated Teflon-type. The arrangement permits a quantitative generation of acetylene from barium carbide in a closed system, see text.

The receiver tube B fulfills a multiple function as reaction vessel, autoclave and centrifuge tube. The bottom compartment with a volyme of 1 000 μl can be closed off by fitting a stopper into the lower joint, the shaft of the stopper extending 1 cm above the top opening of the vessel. Stopper and joint could be of standard B5- or B7-type, carefully ground. The upper joint of the vessel, a B 10, is closed with a ground stopper, containing a spring housed in the top part, thus enabling the exertion of a pressure upon the shaft of the lower stopper. The tube and its upper stopper are held together with a clip for spherical joints. By this means the small 1 000 μl compartment could act as a micro-autoclave standing a pressure up to 3 atm. Hydrogenation is likewise easy to carry out by connection the vessel to a hydrogen tank *via* an arrangement enabling the flushing out of air. Centrifugation could be carried out in a swing-out head centrifuge at 1 000 $\times$ *g*.

Sodium cyanide-^{14}C was prepared from barium carbonate by a modification of the procedure of Jeanes [11]. After 2.5 hours heating of an intimate mixture of barium carbonate 39.4 mg, metallic sodium 40 mg and zink powder 200 mg in a porcelain combustion boat housed in a quartz tube in ammonia atmosphere, the contents are worked up as follows. The porcelain boat was transferred to a distillation unit, 5 ml 20 % perchloric acid added and 10 ml of distillate collected in 240 μl 1 N sodium hydroxide in a receiver vessel. The solution was now divided into two equal parts and transferred to two vessels of the type shown in Fig. 1 D. The alkaline solution was titrated with 0.1 N silver nitrate solution to a faint turbidity thus enabling a quantitative estimation of the yield of cyanide. Then 250 mg sodium hydrogen carbonate was added and the silver cyanide was precipitated by addition of an equal amount of 1 N silver nitrate solution as titrated before. The silver cyanide was centrifuged off into the smaller compartment of the vessel. After washing with water and acetone, twice with each, the dry silver cyanide samples could be stored for further working up to sodium cyanide.

The conversion of silver cyanide into sodium cyanide was accomplished by inserting into the lower joint a unit (see Fig. 1 E) enabling the sweeping out of hydrogen cyanide with nitrogen. 400 μl 1 N hydrochloric acid was added above the now closed lower joint and the stream of nitrogen accordingly adjusted. After a momentary lifting of the cone,

the hydrochloric acid was allowed to react with silver cyanide. The hydrogen cyanide swept out is absorbed in 120 μl 1 N sodium hydroxide thus yielding a concentrated solution of sodium cyanide, to be immediately utilized whether for making cyanoacetic acid or urea. The time for sweeping out the hydrogen cyanide could be kept as low as 15 min by occasional gentle heating of the reaction mixture. A yield of more than 90 % of concentrated sodium cyanide solution from barium carbonate is obtained.

Urea-^{14}C. The concentrated sodium cyanide solution (120 μl) prepared as above was transferred to a 4 ml centrifuge tube containing freshly prepared copper hydroxide (precipitated from 13.10 mg cryst. copper sulphate). The mixture was cooled in an ice bath and the cyanide was oxidized to cyanate by the dropwise addition of a potassium permanganate solution (12.18 mg in 400 μl of water), excess being destroyed with hydrogen peroxide. After the manganese dioxide formed was separated off by centrifugation and washed three times with water the supernatants were combined and transferred to reaction vessel C (see Fig. 1) and evaporated to round 200 μl *in vacuo*. After addition of 13.2 mg ammonium sulphate and 50 μl concentrated aqueous ammonia the tube was immediately closed off as shown in Fig. 1 C and heated under gentle shaking at 70° for 40 min. The tube was allowed to cool, opened and the contents evaporated to dryness *in vacuo*. The dry residue was extracted 4 times with *n*-butanol with shaking at 70° for 20 min each time. The extracts were evaporated *in vacuo* to dryness at 50°. The dry urea was taken up in 50 μl of water immediately before its further conversion to cyanoacetyl-urea (see below).

Bromoacetic acid- 1,2-^{14}C from barium carbide-^{14}C. The smaller syringe of the plunger arrangement (see Fig. 1, A, B) was separately filled with 4 ml of 1 N hydrochloric acid and attached to the T-tube, carefully avoiding any entrance of the acid into the system. The stopcock should at this stage be open. Freshly prepared barium carbide from 49.3 mg of barium carbonate was carefully deposited on the filter paper at the bottom part of the 25 ml syringe. The plunger was put in place as tightly to the bottom as possible. After this the stopcock is closed, the small vessel B is charged with 100 μl of bromine and 100 μl of water and is attached to the rest of the assembly, so that the capillary outlet safely dips under the surface of the bromine. The plunger of the acid-filled syringe is now slowly pressed up allowing not more acid than to moisten the filter paper disc into the 25 ml syringe, thus starting the reaction. When the gas evolution has started the plunger of the lower syringe is manipulated downwards thereby sucking back most of the acid from the upper system. Excess pressure caused by occasional friction of the plunger is to be avoided. The stopcock is now cautiously opened enabling the gas to pass through the bromine at round 1 bubble per second. Conveniently the upper plunger could now be pressed down with a micrometer screw. When all gas is driven out of the system a small amount of acid is again allowed into the upper compartment — the stopcock being closed at this stage — and the procedure being repeated until no more gas is evolved.

The reaction vessel D, now containing *1, 1, 2, 2, -tetrabromoethane* plus excess bromine and water is centrifuged for a few minutes enabling all droplets of the reaction procedure to collect in the tip. 100 μl of concentrated formic acid is added [17], the mixture gently heated at the water bath to 60° until decolorization, cooled to 10° and recentrifuged. The water solution above the tetrabromoethane droplet is carefully sucked away with a capillary pipette as complete as possible; the remainder of water is removed by storing the open tube over phosphorus pentoxide in a desiccator over night at ordinary pressure.

To the reaction vessel, now containing around 15 μl of dry 1,1,2,2,-tetrabromoethane, 150 μl of a sodium ethoxide solution (50 mg of sodium per 1 000 μl of ethanol) is added, corresponding to 315 μ-atomes of sodium. The bottom part of the reaction vessel is then closed with the stopper arrangement (see Fig. 1) and the content heated to 60° in a small oilbath under shaking (a "Microid flask shaker" is used throughout). After 1 h the vessel is allowed to cool off, is opened and 300 μl of water added. After centrifugation for 10 min a droplet slightly colored, has separated out, representing mainly *1,2-dibromovinyl-ethyl ether*. A check should be made that actually all oil is separated from the alcohol-water supernatant by adding around 50 μl of additional water. In case of cloudiness the vessel should be recentrifuged. The supernatant alcohol-water solution is now removed as complete as possible with a capillary pipette, 300 μl of water added, and the vessel closed again and heated in an oilbath under shaking at 110° for 1 h. At this stage the dibromovinyl-ethyl ether has become completely hydrolysed; no cloudiness should appear upon cooling of the content of the tube. This solution represents *bromoacetic acid-1,2-^{14}C*.

Uracil-2,4,5,6-^{14}C from bromoacetic acid-1,2,-^{14}C. The acid solution containing the bromoacetic acid-1,2-^{14}C is now titrated under gentle shaking with 1 N sodium hydroxide, using an Agla micro-syringe with a fine glass capillary entering the solution, thymol blue being used as an indicator. As an average of 20 experiments 120 μl of 1 N sodium hydroxide solution is required for neutralization. 120 μl of the concentrated sodium cyanide solution prepared as above is now added, the vessel closed and heated for 20 min in an oilbath at 100°. After cooling, the vessel is opened, 150 μl of 1 N hydrochloric acid added and the content evaporated to dryness. This procedure is carried out by attaching the vessel *via* the upper joint to a B 10 male joint, connected by a flexible arrangement to a water aspirator pump. During the evaporation procedure the vessel is vigorously shaken and heated to 30°; at sufficient shaking frequency splashing is prevented. A pilot test of this procedure is recommended. To the dry residue, now representing *cyanoacetic acid-1,2,3-^{14}C* plus salts 15 μl of a water solution of urea prepared as above is added with a micro pipett followed by the addition of 500 μl of acetic anhydride. The solids are suspended in the anhydride and the mixture now heated to 60° for 20 min under gentle shaking; the vessel could at this stage be open. After cooling down the content is evaporated to dryness, as previously described, yet at 50°. The dry residue representing *cyanoacetylurea*, plus salts, is dissolved in 500 μl of sodium acetate buffer pH 4.15 ionic strength 0.1 and approximately 20 mg of Raney nickel (calculated dry weight) is added as a thick paste from an ordinary alcoholic suspension. The upper joint of the reaction vessel is safely connected to a hydrogen tank *via* an arrangement enabling the flushing out of air by intermittent suction and application of hydrogen. The subsequent hydrogenation of cyanoacetyl urea is carried out at a hydrogen pressure of 2.5 atm under vigorous shaking at 65° during 1 h. After release of the hydrogen pressure the reaction vessel is centrifuged and the supernatant is withdrawn with a micro pipett from the catalyst, the latter washed once with 100 μl of water and again centrifuged. The combined solutions are chromatographed on a 23 cm wide strip of Whatman 3 MM paper, the solvent system being methylethylketone-acetone-water-formic acid (100 %) 80:4:12:2 (Reio [17]). R_F for uracil in this system is 0.25; for localization of the compound the paper was photographed after drying in UV-light. After repeated elution of the uracil-containing paper region with a solution of acetone-1 N hydrochloric acid 4:1, totally 40 ml, the evaporated eluate was rechromatographed and again eluted in the same way. Careful evaporation to dryness gives a residue of *uracil-2,4,5,6-^{14}C*, the purity of which was checked by its UV-absorption curve. Yield of uracil from barium carbonate was 10 % with a specific activity of around 100 μC per μmole.

REFERENCES

1. Ehrensvärd, G., Liljekvist, J., Mosbach, K. and Fritzson, P. *Acta Chem. Scand.* **12** (1958) 485.
2. Cheronis, N. D. *Micro and Semimicro Methods*, in Weisberger *Technique of Organic Chemistry*, Vol. VI, 3966 Interscience New York 1954.
3. Sabanejeff, A. *Anal. Chem.* **178** (1875) 113.
4. Lemoult, M. *Bull soc. chim. France*, Sér. 3 **33** (1905) 194.
5. Crompton, H. and Vanderstichele, P. L. *J. Chem. Soc.* **117** (1920) 691.
6. *cf Beilsteins Handbuch der Organischen Chemie*, 1. Erg.w. **1**, p. 377.
7. Fiquet, E. *Bull. soc. chim. France*, Sér. 3 **25** (1901) 593.
8. Fritzson, P. *Scand. J. Clin & Lab. Invest.* **4** (1952) 375; *Chem. Abstr.* **47** (1953) 9917 d.
9. Traube, W. *Ber.* **33** (1900) 1380.
10. Baum, F. *Ber.* **41** (1908) 530.
11. Jeanes, J. K. *Science* **118** (1953) 719.
12. Prelog, V., Urech, H. J., Bothner-By, A. A. and Wuersch, J. *Helv. Chim. Acta* **38** (1955) 1106.
13. Smith, L. H., Yates, P. *J. Am. Chem. Soc.* **76** (1954) 6080.
14. Gall, H. and Lehman, G. *Ber.* **61** (1928) 670.
15. Haley, E. E. and Lambooy, J. P. *J. Am. Chem. Soc.* **76** (1954) 2926.
16. Rupe, H., Metzger, A. and Vogler, H. *Helv. Chim. Acta* **8** (1925) 850.
17. Reio, L. *J. Chromatography* **1** (1958) 338.

Received August 12, 1959.