

The Use of Radioactive Elements. (I)*

The Synthesis of β -Diethylaminoethyl Xanthene-9-Carboxylate [Carboxyl- C^{14}] Methobromide

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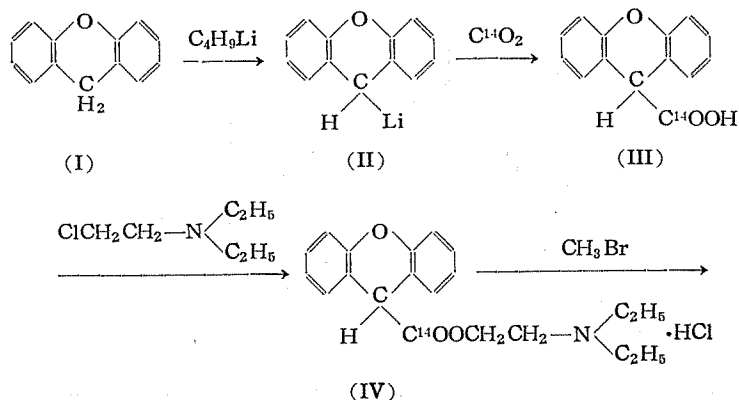
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A procedure is described for the synthesis of β -diethylaminoethyl xanthene-9-carboxylate [carboxyl- C^{14}] methobromide, starting with $BaC^{14}O_3$ and involving the preparation of xanthene-9-carboxylic acid [carboxyl- C^{14}], and β -diethylaminoethyl xanthene-9-carboxylate [carboxyl- C^{14}] hydrochloride.

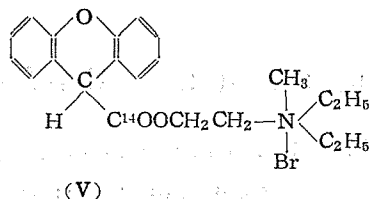
A considerable number of radioactively labeled drugs have already been synthesized and provided a modern and convenient method for following the fate of the compound in metabolic process. For pharmacological purpose, we synthesized C^{14} -carboxyl-labeled β -diethylaminoethyl xanthene-9-carboxylate methobromide, an anticholinergic drug "Banthine" which has been utilized recently in the successful treatment of gastric ulcers. The synthesis of Banthine was described by Cusic et al¹⁾, but the synthesis of labeled Banthine has not been previously reported in the literature.

Our experimental procedures were partly similar to that previously described though with some modifications. The process of synthesis is outlined by the following equations :



* This is a translation of the same article which appeared in Japanese in Journal of the Pharmaceutical Society of Japan, 75, 1014 (1955).

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Xanthene-9-carboxylic acid [carboxyl-C¹⁴] (III) was synthesized from xanthene (I), by the carbonation of its lithium derivative (II) with C¹⁴O₂ in vacuum system. The acid (III) was converted to β-diethylaminoethyl xanthene-9-carboxylate [carboxyl-C¹⁴] hydrochloride, refluxing with β-diethylaminoethyl chloride in isopropanol. β-Diethylaminoethyl xanthene-9-carboxylate [carboxyl-C¹⁴] methobromide (V) was prepared from (IV) and methylbromide in alcoholic solution in a closed vessel.

For the determination of specific radioactivity, the labeled compounds were burned in a wet combustion apparatus described by Claycomb et al²⁾, and derived to barium carbonate which was counted at infinite thickness by a G-M tube with a thin mica window. The activities of synthesized compound were compared with that of the standard BaC¹⁴O₃ sample* under identical conditions.

EXPERIMENTAL

Xanthene-9-carboxylic acid [carboxyl-C¹⁴] (III)

Synthesis was carried out in a vacuum system which was designed and constructed as Fig. 1³⁾** and was performed in a well-ventilated hood. The apparatus has five components: a carbon dioxide generator G, an open-arm manometer

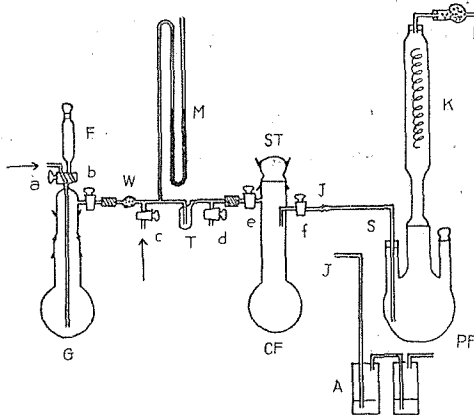


Fig. 1.

* The standard BaC¹⁴O₃ sample for the counting was prepared as follows. BaC¹⁴O₃ distributed from A.E.C. of U.S.A., specific activity 0.0295 mc/mg., was diluted with a calculated amount of BaCO₃ carrier and it was treated with perchloric acid in Claycomb's apparatus and derived to BaCO₃ by precipitation and plating at infinite thickness.

** Modification of Langham's carbonation apparatus for lithium alkyl.

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M, a trap T, a carbonation flask CF, and a *n*-butyllithium preparation flask PF. For the purpose of shaking flask by hand, G and CF were connected to the line with rubber tubing. W is a wad of glass wool for filtering the solid particles spattered by the evolution of gas.

Barium carbonate [C^{14}] 1.037 g. with an activity of 1.091 mc was weighed into generator G and 15 ml. of concentrated sulfuric acid was added to funnel F. Stopcocks a, b, e, and f were opened and c and d were closed. Air in the whole apparatus was swept out through calcium chloride tube D with dry nitrogen supplied through stopcock a. When the stopcocks a and b were closed, air in the generator G was replaced perfectly with nitrogen. Further supply of nitrogen to the system was fulfilled through the stopcock c.

A solution of *n*-butyllithium was prepared in PF under an atmosphere of nitrogen by refluxing a mixture of 2 g. of lithium, cut into 255 pieces, and 13 g. of *n*-butylbromide dissolved in 100 ml. of ether, and its concentration was determined by differential titration⁴⁾. Stopcock c was closed. Then 25 ml. (12.5 m. mol) of *n*-butyllithium solution was transferred from the preparation flask PF to the carbonation flask CF which was provided with mark at 25 ml. volume. The transfer was made through siphon S by a negative pressure caused by slow aspiration from stopcock d. Stopcocks f and d were closed and the stopper ST was replaced by the condenser K. The flask PF was removed from the joint J. Opening stopcock c and 2 g. of xanthene was added to *n*-butyllithium solution in the flask CF under the atmosphere of nitrogen, and deep red solution was refluxed for half an hour. Before carbonation the flask CF was cooled by an ice-salt bath, the condenser was removed, and replaced by the stopper ST. Stopcock c was closed, and the reaction system was connected to a vacuum pump at d, then stopcocks b and d were opened and evacuated to the boiling of ether. The stopcock d was closed and the apparatus was allowed to stand for a few minutes and tested for leaks.

Carbon dioxide [C^{14}] was generated in G, by adding 10 ml. of concentrated sulfuric acid drop by drop to $BaC^{14}O_3$. Quantitative evolution of $C^{14}O_2$ was attained by warming and shaking. The carbonation flask CF was simultaneously shaken vigorously by hand, and the absorption of $C^{14}O_2$ was observed by the fall of pressure in the manometer M. Meanwhile the colour of the reaction mixture changed from red to orange red. When pressure became constant, the apparatus was filled with dry nonisotopic carbon dioxide which was generated from the second generator (Kipp's apparatus) connected to the stopcock a, with the caution of the inner pressure not to exceed the atmospheric. The carbonation flask CF was shaken again, until the colour of the solution changed to yellow. It took about an hour to complete the reaction.

With the stopcock a opened, nitrogen gas was admitted into the system equal to atmospheric pressure. The joint J was connected to the absorber A which contained

2N-NaOH solution. Stopcock f was opened and unabsorbed $C^{14}O_2$ was swept out with nitrogen gas, which was led out through the stopcocks a, b, e, f, J and the absorber A. Removing the carbonation flask CF, 50 ml. of water was added to the residue in flask CF, and insoluble substances filtered off, and the filtrate was acidified with 10 % hydrochloric acid. The crystalline product was collected and washed with water. Yield, 1.45 g. Crude acid product was redissolved in 30 ml. of 2 % NaOH solution, filtered, and the filtrate was reprecipitated with 10 % hydrochloric acid, again washed with water. The final pure product, white needles, melting at 215-216° were obtained in a yield of 1.354 g. and had a specific activity of 0.407 μc per mg. Activity yield was 50.5 % of barium carbonate [C^{14}] used.

β -Diethylaminoethyl Xanthene-9-carboxylate [$carboxyl-C^{14}$] HCl (IV)

Sixty-three-hundredths gram of β -diethylaminoethyl chloride was added to the solution of 1.043 g. of (III) in 6 ml. of isopropanol and the solution refluxed on a steam-bath for 3 hours. The reaction mixture was cooled, filtered and the filtrate was evaporated on a steam-bath, residual viscous oil was allowed to stand overnight in a desiccator. The precipitated white crystals were collected, washed with ether and ethyl acetate. Yield, 1.032 g. Almost white crystals, melting at 140-143°, had a specific activity of 0.247 μc per mg.

β -Diethylaminoethyl Xanthene-9-carboxylate [$carboxyl-C^{14}$] methobromide (V)

Synthesis was carried out in a 40 ml. heavy walled, roundbottom closed vessel. On weighing 0.856 g. of (IV) in a closed vessel, it was dissolved in 2 ml. of absolute alcohol on a steambath and to this solution added 2.76 ml. of 0.862 N-alcoholic KOH. After cooling the vessel with ice-salt mixture, 10 ml. of 20 % methylbromide alcoholic solution was added. The reaction vessel was allowed to stand overnight at room temperature. The precipitated white crystals (KCl) were filtered off and the filtrate was treated as described in (IV). The precipitated crystalline products were filtered, washed with ether. Yield, 0.888 g. After recrystallization from alcohol-ether mixture, 0.759 g. of almost white crystals melting at 170-173° were obtained and had a specific activity of 0.210 μc per mg.

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