SYNTHESIS OF ETHAMBUTOL-^{14}C DIHYDROCHLORIDE [(+)-N, N'-BIS(1-HYDROXY-2-BUTYL)-ETHYLENE-U-^{14}C-DIAMINE DIHYDROCHLORIDE]

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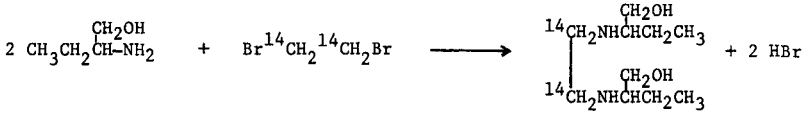
Despite the increasing use of ethambutol dihydrochloride (Myambutol®) in the clinical treatment of primary tuberculosis (1), the precise mechanism by which this drug inhibits the growth of mycobacteria has yet to be clearly defined (2). Peets and Buyske (3) compared the *in vivo* metabolism of ethambutol and its less active (-)-isomer with the aid of the ^{14}C-labeled compounds; but a macro-scale synthetic procedure for the unlabeled drugs (4) was referenced to, and the details of the radioactive synthesis were not presented. Recently, Beggs and Auran (5) utilized a ^{14}C-labeled ethambutol dihydrochloride that we had synthesized for *in vitro* studies designed to elucidate the mechanism of action of this drug. The proliferation of such studies (6,7,8,9) requiring the use of ethambutol-^{14}C dihydrochloride prompts us to report this semimicro-scale synthesis, which is a modified version of the macro synthesis of Wilkinson *et al.* (4). Our procedure allows for maximal recovery of the radiolabeled drug



of the correct optical configuration, i.e. (+) -- by addition of unlabeled (+)-ethambutol as carrier to cocrystallize with the labeled compound from the mother liquor after initial isolation of a first crop of crystals. Although the specific activity of this carrier-added product is reduced by approximately one-half, many *in vitro* studies which do not require high specific activity samples can still be carried out with this product.

The optical activity of the ^{14}C-labeled ethambutol dihydrochloride was not

determined; however, the products from numerous "cold" runs gave values for $[\alpha]_D^{28°}$, viz., +6.01° ± 0.29 (c = 1.0 in H_2O), which compare favorably with that reported (10) for the active (+)-isomer ($[\alpha]_D^{25°}$ +5.5 ± 0.4 in H_2O). This ^{14}C-labeled product exhibited microbiological activity identical to that of the unlabeled drug and was radiochemically homogeneous by thin layer chromatography (5).

EXPERIMENTAL

(+)-N,N'-bis(1-hydroxy-2-butyl)ethylene-U-^{14}C-diamine Dihydrochloride -- A mixture of ethylene-U-^{14}C dibromide (Amersham-Searle) (2.0 mCi, 0.375 g, 1.37 mmole) and (+)-2-aminobutanol (Aldrich Chemical Company) (0.947 g, 10.6 mmole) in approximately 3.0 ml of glass-distilled acetonitrile was heated under reflux for 4 hours. The reaction mixture was concentrated to dryness in vacuo, the residue was redissolved in propyl alcohol and reconcentrated, and 1.4 ml of propanolic KOH (1.3 g of KOH in 10 ml of propyl alcohol) was added to neutralize the HBr. The KBr which precipitated on cooling was removed by filtration and washed with propyl alcohol (3x). The combined filtrate was concentrated in vacuo, the residue taken up in propyl alcohol and the solution filtered. The filtrate was acidified with 7.8 N ethanolic HCl, whereupon 72.1 mg of crystals were isolated after cooling overnight at -15° C in the freezer compartment of a refrigerator. Concentration of the filtrate followed by work-up as above gave a second crop of crystals, 161.1 mg, giving a total of 233.2 mg (65% yield) of crude product. The combined product was extracted with absolute ethanol in a soxhlet apparatus for 3 hours to remove traces of inorganic salts, the extract was concentrated to dryness in vacuo, and the solids were dissolved in a minimal amount of hot ethanol. Addition of ethanolic HCl to the warm solution followed by cooling overnight gave 148.8 mg of white crystals, m.p. 197-201° C; specific activity, 5.12 μCi/mg.

A lower specific activity sample was obtained by adding 101 mg of unlabeled (+)-ethambutol to the mother liquor from above, concentrating the solution to dryness and recrystallizing the residue from ethanol-ethanolic HCl as above; 122.7 mg, m.p. 196-201° C; specific activity 2.83 μCi/mg.

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F.N. Shirota and H.T. Nagasawa

Medical Research Laboratories,
Veterans Administration Hospital, and
Department of Medicinal Chemistry,
University of Minnesota,
Minneapolis, Minnesota 55417