PREPARATION OF ℓ-[1-(P-N-BUTOXYBENXYL-1'^{3}H) HYOSCYAMINIUM BROMIDE.

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[3α-Hydroxy-8-(p-n-butoxybenzyl)-1αH,5αH-tropanium-(-) tropate] bromide (ℓ-[1-(p-n-butoxybenzyl) hyoscyaminium] bromide, abbreviated as BHB) has been recognized to have anticholinergic action[1]. The radioactive BHB was required to investigate the metabolic fate of BHB in animals. This paper is concerned with the preparation of ℓ-[1-(p-n-butoxybenzyl-1'-^{3}H) hyoscyaminium] bromide (^{3}H-BHB).

p-n-Butoxybenzyl alcohol-1-^{3}H

A ethanol solution of p-n-butoxybenzaldehyde (208 mg) was partially reduced with tritium gas (1.5 Ci) diluted by hydrogen (6.3 ml) in the presence of palladium on charcoal (5 %, 25 mg) and completely reduced with hydrogen gas (22 ml) in succession. After removal of solvent the residue was dissolved in methanol and the solvent was evaporated in vacuo. This procedure to remove the labile tritium was repeated several times. 177 mg of p-n-butoxybenzyl alcohol-^{3}H with a specific radioactivity of 5.55 mCi/mg was obtained. This labeled compound showed a radioactive peak at the same position as the authentic p-n-butoxybenzyl alcohol (Rf : 0.37) on a thin layer chromatogram of Kieselgel G developed with a solvent system of benzene/ethylacetate, 2/1. The labeling position of tritium in p-n-

butoxybenzyl alcohol was confirmed as follows. A solution of 1.5 g of potassium permanganate in 50 ml of acetone and 5 ml of 3 % NaOH aqueous solution was added to the mixture consisting of the tritium labeled compound (41.7 μCi) and 500 mg of p-n-butoxybenzyl alcohol at 4°C, and then the reaction mixture was stirred for 48 hours at room temperature. After evaporation of the solvent, the residue was suspended in 50 ml of water and filtered. The filtrate was extracted with ethylether to remove the starting material. Following acidification with 2 N H_2SO_4, the resultant solution was extracted with ethylether four times. 91 mg of crude product obtained by evaporation of the ethereal extract showed only one spot at the same position as the authentic p-n-butoxybenzoic acid[2] on thin layer chromatograms under UV-lamp (2536 A). The chromatograms were run on Kieselgel GF_{254} (250 μ in thickness) using two solvent systems of ethylacetate/methanol/5 N NH_4OH, 50/8/5 (Rf : 0.20) and n-hexane/dioxane/acetic acid, 20/5/1 (Rf : 0.75). This crude product was repeatedly recrystallized from benzene. The fact that 32.1 mg of p-n-butoxybenzoic acid, of which specific radioactivity was 0.022 pCi/mg, was obtained indicates that p-n-butoxybenzyl alcohol was labeled with tritium at the C-1 position alone.

l-[1-(p-n-Butoxybenzyl-1'-^{3}H) hyoscyaminium] bromide

To 106 mg of p-n-butoxybenzyl alcohol-1-^{3}H (46 mCi) in 1 ml of benzene, 0.5 ml of 48 % hydrogen bromide was added dropwise in an ice-water bath under stirring. After standing for 1.5 hours, the reaction mixture was extracted three times with 25 ml of benzene. The benzene layer was washed with 7 % NaCl aqueous solution and dried over granulated anhydrous $MgSO_4$.

The residue, which was obtained following evaporation of benzene, was dissolved in 1.5 ml of acetone and added to a solution of 98 mg of 3-α-hydroxy-1αH,5αH-tropan-(-)tropate (*l*-hyoscyamine) in each 1 ml of acetone and ethanol. The mixture was stirred for 1 hour in ice-water bath. After removal of solvent from the reaction mixture at room temperature in vacuo, the residue was dissolved in 5 ml of acetone and stood at -20°C for 2 hours. The ^{3}H-BHB obtained in a yield of 40 % was confirmed to be pure by thin layer chromatograms. Thin layer chromatography was carried out using Kieselgel GF_{254} (250 μ in thickness) with two solvent systems of acetic acid/n-butanol/water, 1/4/5 (upper layer) (Rf : 0.52) and ethylacetate/acetic acid/water, 9/2/2 (Rf : 0.35). The product showed a specific radioactivity of 146 μCi/mg and to be stable in the two year's storage at -20°C.

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