NOTES

SYNTHESIS OF ^{14}C-LABELED STEPHOLIDINE

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SUMMARY

(8-^{14}C)Stepholidine was synthesized by Mannich condensation of 7-benzyloxy-1-(4-benzyloxy-3-hydroxybenzyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline (2) with (^{14}C)formaldehyde at pH 6.4 followed by methylation and debenzylation in 32% radiochemical yield.

Key words: Stepholidine, Tetrahydroprotoberberine, ^{14}C-labeled compound, Mannich condensation

INTRODUCTION

L-Tetrahydroprotoberberine (THPB) alkaloids are the dopamine-receptor antagonists[1]. Stepholidine (1)[2] has been shown to possess the strongest pharmacological effects[3,4] among the THPB alkaloids studied. In order to studt its metabolism and the mode of action of the drug, a radiolabeled stepholidine was required. We report here the synthesis of ^{14}C-labeled stepholidine by Mannich condensation of 7-benzyloxy-1-(4-benzyloxy-3-hydroxybenzyl)-6- methoxy-1,2,3,4-tetrahydroisoquinoline (2) with (^{14}C)formaldehyde followed by methylation and debenzylation in 32% radiochemical yield(Scheme 1).

RESULTS AND DISCUSSION

In a radiochemical synthesis it is desirable to incorporate the isotope into

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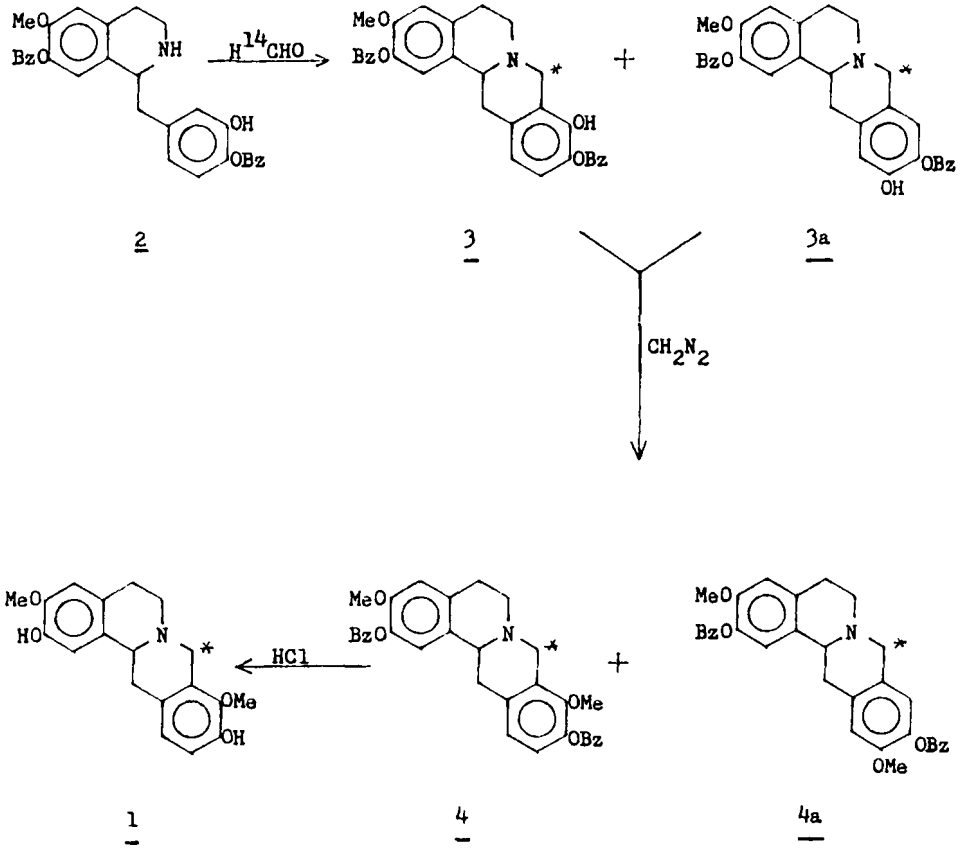

* : Position of ^{14}C-label

Scheme 1

the molecule at the final stage of the synthesis in order to obtain an efficient overall yield. Stepholidine has been synthesized by Rajeswari[5] and Hsuch-ching Chiang[6]. The synthesis of ^{14}C-labeled (1) is based on that of Rajeswari's method with some modifications. The advantages of this method are that ^{14}C-label is introduced at a late step by Mannich condensation and that ^{14}C-label is in position C_8 of stepholidine ring which is expected to be metabolically stable.

The key intermediate (2) was prepared through some modifications of the general route[5]. Mannich condensation of 2 with ^{14}C-formaldehyde solution at pH 6.4 gave a mixture of 3 and 3a. Methylation of 3 and 3a yielded a

mixture of 4 and 4a. Instead of chromatographic separation of 3 and 3a the required 4 was separated from 4a by recrystallization from methanol and their structures were confirmed by MS and H-NMR. Debenzylation of 4 with HCl gave ^{14}C-labeled stepholidine (1) found to be identical with authentic sample. The overall radiochemical yield of 1 was 32% and the radiochemical purity was 95%.

EXPERIMENTAL

Melting points were uncorrected. H-NMR spectra were determined on a JEOL-PS-100 spectrometer (solvent:$DCCl_3$, chemical shifts in ppm downfield from TMS), MS spectra on MAT-44S and IR on PE-599B. Radioactivity was determined using a liquid scintillation counter YSJ-78, radiochemical purity was performed on a radiochromatogram scanner FJ-2109. For TLC silica gel Merck 60F254 was used. ^{14}C-Formaldehyde solution was prepared by reduction(7) of $^{14}CO_2$ generated from $Ba^{14}CO_3$.

2,10-Dibenzyloxy-3,9-dimethoxy-(8-^{14}C)-berbine (4)

To a solution of the hydrochloride salt of 2 (350mg) in methanol (50ml) whose pH was adjusted to 6.4 with diluted $NaHCO_3$, was added a solution of ^{14}C-formaldehyde (640 μCi, 28ml) in THF-water (1:2). The pH of the solution was adjusted again to 6.4 with diluted $NaHCO_3$. After stirring for 2 hr at room temperature unlabeled formaldehyde (0.05ml, 36%) was added. The mixture was stirred overnight at room temperature and then was evaporated *in vacuo* to dryness. Ethyl acetate and diluted $NaHCO_3$ were added. The organic layer was washed with water and dried (Na_2SO_4). After removal of solvent the residue was dissolved in methanol and an ethereal solution of diazomethane was added. The mixture was allowed to stand overnight. Evaporation of the solvent and recrystallization from methanol gave pure 4 (95 mg, 28%, cold run 42%), M.P. 57-58°C. MS (cold run): 507 (M^+), 416, 149 (base peak), 121. H-NMR (cold run): 3.58 (1H, d, J=16Hz, C_8-H), 3.88 (6H, S $2OCH_3$), 4.26 (1H, d, J=16Hz, C_8-H), 5.10, 5.12 (4H, 2S, $2OCH_2Ph$), 6.60-6.67 (4H, aromatic), 7.3 (10H, aromatic). The residue of methanolic mother liquid gave white crystal 4a, (36 mg, 10.5%), M.P. 128-129°C. MS (cold run) 507 (M^+), 416, 266, 241, 150. H-NMR (cold run): 3.6 (1H, b, C_8-H), 3.74

(6H, S, $20CH_3$), 4.04 (1H, b, C_8-H), 4.90, 4.94 (4H, 2S, $20CH_2Ph$), 6,46-6.6o (4H, aromatic), 7.2-7.4 (10H, aromatic).

($8-^{14}C$) stepholidine hydrochloride (1)

Compound 4 (95 mg) was refluxed under nitrogen in a solution of ethanol (4ml) and concentrated hydrochloric acid (4ml) for 10 hr. After evaporation of the solvent, the residue was recrystallized from ethanol to give ($8-^{14}C$)-stepholidine hydrochloride (1), (52 mg, 76%). specific activity 4 μCi/mg (59.6MBq/mmole), M.P. 210°C The free base was identical with that from natural source (M.P.,TLC, MS,H-NMR and IR). The radiochemical purity was 95% as determined by TLC (silica gel, chloroform:methanol=9:1) and radio-chromatogram scanning.

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