

NOTE

A NOVEL SYNTHESIS OF α -N-METHYL- ^{14}C -DL-ASPARAGINE BY GENERATION OF METHYLAMINE- ^{14}C IN SITU

SUMMARY

A method is described by which methylamine- ^{14}C is generated from its hydrochloride by *N,N*-diisopropylethylamine in the presence of maleamic acid to produce the title compound in high yield.

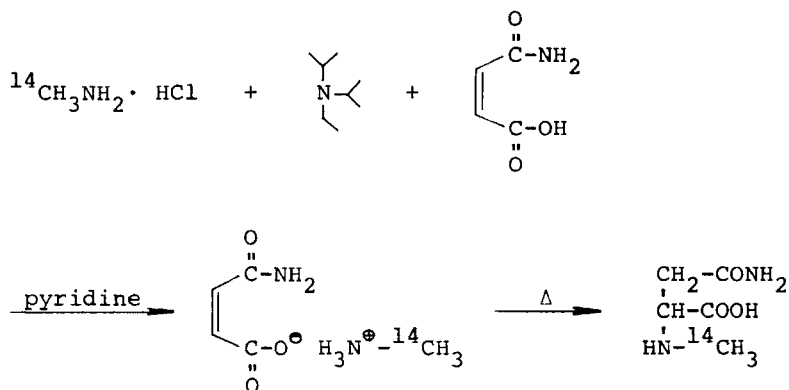
Key words: Synthesis of α -N-Methyl- ^{14}C -DL-Asparagine

INTRODUCTION AND DISCUSSION

During the course of studies on the induction of ornithine decarboxylase [E.C. 4.1.17] (ODC) it became apparent that this enzyme could be induced in a salts-glucose medium by L-asparagine (1). An extended study showed that α -N-methyl-DL-asparagine would also induce ODC (2). It therefore became apparent that a detailed understanding of the mechanism of induction of ODC would necessitate the synthesis of radioactive α -N-methyl-DL-asparagine.

The synthesis of α -N-alkyl-DL-asparagine from maleamic acid, an alkylamine and pyridine has been reported (3). Application of this method to the synthesis of α -N-methyl- ^{14}C -DL-asparagine presents difficulties because only the hydrochloride salt of methylamine- ^{14}C which is insoluble in pyridine, is commercially available. These difficulties were circumvented by using *N,N*-diisopropylethylamine, a sterically hindered proton scavenger to free the methylamine from its hydrochloride in the presence of maleamic acid, while the nucleophilic attack by the sterically hindered amine was prevented (4). This permitted the methylamine-

^{14}C to react with maleamic acid as it was being generated. Addition of a slight amount of ethanol enhanced the solubility of methylamine hydrochloride without incurring appreciable hydrolysis of maleamic acid to maleic acid under reflux condition. After purification by preparative TLC and identification by autoradiography, α -N-methyl- ^{14}C -DL-asparagine was obtained with a yield of 67% according to the equation:



EXPERIMENTAL

To an ethanolic solution of methylamine- ^{14}C hydrochloride (0.34 mg, 4.9 μmole , 0.25 mCi) were added methylamine hydrochloride (1.43 mg, 21.2 μmole) and maleamic acid (3 mg, 26 μmole). The ethanol was removed by distillation, and a mixture of N,N-diisopropylethylamine (10 mg, 78 μmole), pyridine (1 ml) and absolute ethanol (20 μl) was added to the residue in the flask.

The reaction mixture was refluxed at 110 $^\circ\text{C}$ for 3 hours, and cooled. The residue was collected and washed with acetone (5x6 ml). This was applied to a preparative cellulose plate (Avicel, 1000 μ , 20x20 cm) and developed in a solution (n-BuOH:py:H₂O =

15:13:12) for 8 hours. Autoradiography showed one major band with R_f value of 0.18, along with four minor bands with R_f values of 0.06, 0.29, 0.44 and 0.53. The major band of the cellulose plate was extracted repeatedly with absolute methanol. α -N-Methyl- 14 C-DL-asparagine (0.168 mCi) was obtained with a yield of 67%. Rechromatography of the product on an analytical cellulose plate gave clearly a single spot, as shown in the autoradiography.

Non-radioactive α -N-methyl-DL-asparagine was prepared by the same procedure: mp 205-207 °C; Anal. Calcd for $C_5H_{10}N_2O_3$: C, 41.09; H, 6.90; N, 19.17. Found: C, 41.05; H, 7.02; N, 18.94; NMR (D_2O) δ 2.7 (s, 3 H), 2.88 (d, 2 H, $J = 5.5$ Hz), 3.8 (t, 1 H, $J = 5.5$ Hz).

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