A HIGH-YIELD SYNTHESIS OF ^{14}C-LABELLED NITROSOPROLINE AND NITROSOSARCOSINE

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Introduction

The carcinogenic activity of N-nitroso compounds is well established[1,2]. These compounds may represent environmental hazards, since there is evidence that certain nitrosamines are present in some foods. Two such compounds are: nitrosodimethylamine (NDMA), which has been reported to occur in fish[3] and meat[4] products, and nitrosopyrrolidine (NP), which occurs in fried bacon[5,6]. The mechanism of formation of these nitrosamines has not been established. One suggestion is that the amino acids proline and sarcosine are nitrosated and subsequently decarboxylated to yield NP and NDMA respectively[7]. Ender and Ceh have demonstrated the formation of NP from proline and NDMA from sarcosine in a model system by heating the amino acids in the presence of nitrite[8]. Radioactively-labelled nitrosoproline and nitrososarcosine are required in order to facilitate development of procedures for their analysis and investigation of conditions under which they would react to yield NDMA and NP in foods.

For preparation of ^{14}C-labelled nitrosoproline and nitrososarcosine, we required a method which made efficient use of expensive radioactive precursors. Our method is a modification of that given

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by Lijinsky, Keefer and Loo[9]. In order to maximize yields, nitrite concentration and reaction time were increased, and the pH was adjusted to close to the optimum for nitrosation[10].

Experimental

Uniformly labelled L-proline-^{14}C and sarcosine-methyl-^{14}C were obtained from New England Nuclear (Boston, MA). The proline (500 μCi; 0.22 mg) was used without further purification. The sarcosine (600 μCi; 14 mg), which was described by the supplier as 90% pure with dimethylamine as the probable major impurity, was dissolved in water at neutral pH and evaporated to dryness under reduced pressure in an attempt to remove dimethylamine. Activity of the remaining material was 552 μCi. The following reaction procedure was the same for both compounds.

To a solution of the radioactive amino acid in 25 ml water was added 500 mg unlabelled amino acid. A tenfold excess of sodium nitrite was then added and the solution was acidified to pH 3 with hydrochloric acid. The reaction was allowed to proceed in the dark, while being stirred at room temperature for 18 hours. The mixture was then acidified to pH 1 to protonate the acid and facilitate solvent extraction. The water was evaporated under reduced pressure and the residual solids extracted with two 25-ml portions of methylene chloride. The extract was dried with sodium sulfate, filtered and finally methylene chloride was removed under a stream of nitrogen.

Nitrosoproline is a white solid, m.p. 95-96°C, but tends to form a yellow oil unless the extracting solvent is thoroughly dry.

We recovered 560 mg (90%) and 434 μCi (87%). Nitrososarcosine is a yellow oil after removal of solvent, but crystallizes to a white solid, m.p. 62-63°C, on standing in a dry atmosphere. We recovered 641 mg (96%) and 351 μCi (64%). The nitrosoamino acids can be recrystallized from chloroform/carbon tetrachloride, but spectroscopically and chromatographically pure compounds were obtained by our procedure without this step.

In an attempt to improve the radiochemical yield of nitrososarcosine, 144 μCi (26% of initial activity) of activity were located in the solid residue following methylene chloride extraction. Further extraction, however, failed to increase recovery of labelled nitrososarcosine. Since the chemical recovery was almost quantitative, we conclude that the labelled sarcosine used as starting material probably contained impurities in addition to dimethylamine.

Nitrosoproline and nitrososarcosine were characterized by IR, UV, and NMR spectroscopy. Spectra were in good agreement with those previously reported[9]. The purity of the nitrosoamino acids was also assessed by thin layer chromatography on silica gel using 95% ethanol:benzene:water (4:1:1) as developing solvent[11]. Visualization with iodine vapor revealed only one spot for each compound having the same R_f values as authentic material (nitrosoproline, $R_f = 0.57$; nitrososarcosine, $R_f = 0.62$). Zone scraping of the TLC plates followed by scintillation counting showed activity only at the position of the nitrosoamino acid. Both nitrosoproline and nitrososarcosine were stable either in the solid state, or in aqueous solution, when stored in the dark at 5°C. Decomposition to the parent amino acid can be detected by the TLC method (proline, $R_f = 0.34$; sarcosine, $R_f = 0.29$).

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T. Hansen, W.T. Iwaoka and M.C. Archer*

* Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

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