SYNTHESIS OF ^{14}C-METHYL BENZYL NITROSAMINE AND METHYL ^{14}C-BENZYL NITROSAMINE

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SUMMARY

The synthesis of ^{14}C-labelled methyl benzyl nitrosamine is reported. Two forms were prepared, one with a label in the methyl group and one with label in the methylene portion of the benzyl group. Specific activities were approximately 5 mCi/mmol.

Key Words: ^{14}C-Methyl benzyl nitrosamine, Methyl-^{14}C-benzyl nitrosamine, Trifluoromethanesulfonamides, Gabriel synthesis.

INTRODUCTION

During our investigation of the potent esophageal carcinogen methyl benzyl nitrosamine (1), we required radiolabelled versions of this compound for experiments on its metabolism and distribution in rat tissues, as well as on its in vitro binding to DNA and other polynucleotides. No radiolabelled version of this compound was available commercially, so we undertook the syntheses described in this communication--our efforts being directed by the putative metabolic pathway for nitrosamines and certain features of the planned experiments. The metabolism of nitrosamines is believed to involve α-oxidation (2) and, with the unsymmetrical methyl benzyl nitrosamine, quite different products would be observed, depending on the site of oxidation. We thus chose to have available isomers with a benzyl label as well as a methyl label. A specific activity of about 5 mCi/mmol was necessary for whole-body autoradiography, and this was to be achieved with ^{14}C as label. Tritium was deemed unacceptable, since protons adjacent to a nitrosamino function are susceptible to exchange (3).

The synthesis of dialkyl nitrosamines is primarily an exercise in secondary amine synthesis; the subsequent nitrosation

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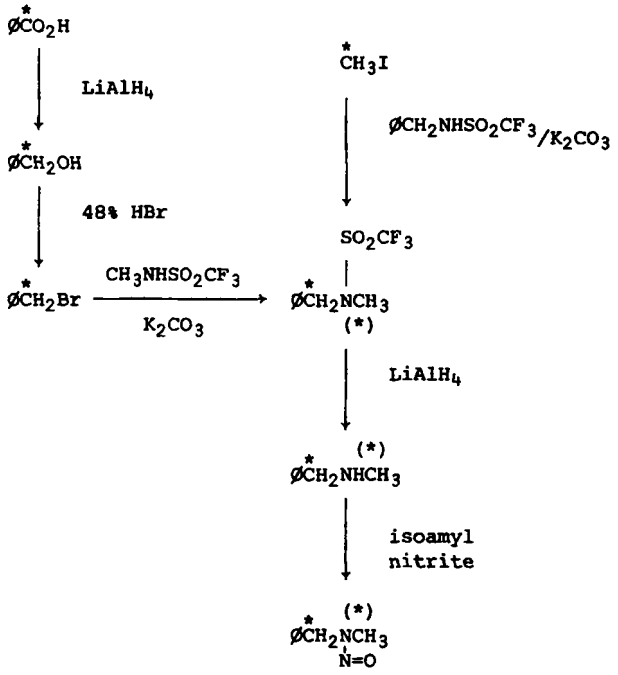
presents no real challenge. We were thus presented with one of the classic difficulties* of organic chemistry, which we resolved with a modern approach--a variant of the Gabriel synthesis employing the trifluoromethylsulfonyl (trifyl) group in place of the original phthalimide (5)--the following scheme outlines the process:



This method is eminently suitable for use with small quantities and offers considerable ease of reagent manipulation and reaction work-up with no need to isolate intermediates. In addition, it provided duplication in the latter half of the two synthetic paths, thus reducing the amount of pilot work.

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*In the case of cyclic or α-functionalized amines, different approaches are generally needed. Interesting examples of radiosyntheses may be found in reference (4).

EXPERIMENTAL

Methyl triflamide ($CH_3NHSO_2CF_3$)

Methylamine (2.3 g, 0.075 mol) was condensed into a flask at -78° and diluted with CH_2Cl_2 (15 ml). A solution of $(CF_3SO_2)_2$ (10 g, 0.036 mol) in CH_2Cl_2 (15 ml) was added dropwise followed by gradual warming to room temperature. After 3 hr, it was worked up by washing with 5 N H_2SO_4, drying, and evaporating the solvent to yield a light-yellow oil (4.1 g). This was distilled at reduced pressure (water aspirator) to yield a colorless oil as the major fraction, which was collected at 78-80° [lit. ref. (6) 86-94° at 20 mm]. The yield was 3.5 g (60%).

Methyl benzyl nitrosamine, methyl-^{14}C

Labelled methyl iodide (1 mCi, 7.1 mg, 0.050 mmol) was vacuum-transferred into a flask containing benzyl triflamide (5) ($\phi CH_2NHSO_2CF_3$) (24.8 mg, 0.104 mmol), anhydrous K_2CO_3 (15.2 mg, 0.110 mmol), and acetone (1 ml). The reaction mixture was stirred for 5 hr at room temperature followed by addition of cold methyl iodide via syringe (0.050 mmol). This final mixture was then stirred for 4 days at room temperature. Acetone was boiled off; 1 N NaOH (1 ml) and ether (2 ml) were added. The contents of the flask were vigorously stirred, then transferred to another flask, and the process was repeated. The aqueous phase from the combined washings was discarded. The ether phase was dried ($MgSO_4$), filtered, and added slowly to a suspension of $LiAlH_4$ (100 mg) in ether (2 ml). This was stirred for 24 hr at room temperature followed by addition of $Na_2SO_4 \cdot 10\ H_2O$ (0.6 g) to destroy excess hydride. After 4 hr, the reaction was filtered; the solids were rinsed several times with ether. The combined ether extracts were reduced to about 1 ml by boiling and were treated with isoamyl nitrite (0.15 ml, 1.4 mmol) for 2 days at room temperature. The

volume of the reaction mixture was then reduced as far as possible by heating in a water bath and applied to a column of silica gel (10 g) packed in acetone. Elution with acetone provided the product as the first component; it was greater than 99% radiochemically pure by TLC (silica gel/acetone). The total activity of the product was 255 μCi (25.5% of starting activity) as determined by liquid scintillation counting of a diluted sample. The yield (6.5 mg, 0.043 mmol) was determined by chromatographic (HPLC) comparison with a standard. Thus a specific activity of 5.9 mCi/mmol was obtained for the product.

Methyl benzyl nitrosamine, methylene-^{14}C

A solution of benzoic acid-7-^{14}C (1.5 mCi, 13.5 mg, 0.111 mmol) in ether (10 ml) was added slowly to a suspension of $LiAlH_4$ (80 mg) in ether (2 ml). The mixture was heated at reflux for 2 days and worked up with $Na_2SO_4 \cdot 10\ H_2O$ as described above. Cold benzyl alcohol (10 μl, 0.099 mmol) was added to the filtrate, and the ether was removed by boiling. The residue was dissolved in 48% HBr (1 ml) which, after 3 hr, was neutralized with $NaHCO_3$ and extracted 3 times with ether. The ether was dried ($MgSO_4$) and concentrated as much as possible. The residue was dissolved in acetone (1 ml) and added to a mixture of methyl triflamide (42 mg, 0.26 mmol) and K_2CO_3 (37 mg, 0.27 mmol). This was stirred for 2 days at room temperature, worked up, and reduced with $LiAlH_4$ as described above. The ether solution (filtrate) obtained from the work up (described above) of the $LiAlH_4$ reduction was extracted with 1 N HCl (2 ml). The acid phase was extracted twice with ether, rendered basic with 3 N NaOH, and extracted 3 times with ether. The combined ether extracts were boiled down to about 1 ml and treated with isoamyl nitrite as described above. Column chromatography yielded a radiochemically pure (99%) product with a total activity of 364 μCi (24% of initial activity). Precise

determination of yield by HPLC was not possible due to the presence of UV-absorbing impurities, but it was possible to obtain an upper limit of the yield (13 mg, 0.087 mmol), which gives a lower limit to the specific activity of 4.2 mCi/mmol. The upper limit of 6.75 mCi/mmol is readily calculated from the amount of dilution with cold benzyl alcohol.

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