A HIGH YIELD SYNTHESIS OF C-14-RING-LABELED AND C-14-METHYL-LABELED N-METHYL-N-NITROSOANILINE.

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SUMMARY

A synthetic procedure for C-14-labeled N-methyl-N-nitroso-aniline is presented. Separate labeling of the methyl and phenyl side chains was achieved by using C-14-labeled methyl iodide and aniline, respectively. The overall yield of the four reactions was 62% and the final products were stable as stored and chemically and radiochemically pure.

INTRODUCTION

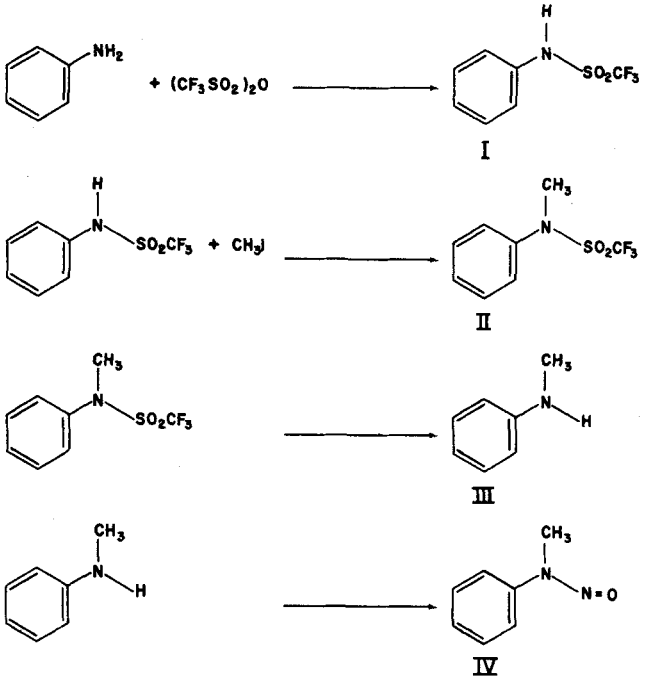
In order to study the metabolism of carcinogenic N-nitros-amines and their interaction with cellular macromolecules, it is necessary to have these compounds radio-labeled. Labeling with tritium is usually the method of choice because of economy and sometimes easier synthetic procedures. However, because of the lability in biological systems of the incorporated tritium in this class of compounds, the use of tritium-labeled N-nitros-amines for metabolic and biochemical experiments can lead to ambiguous results. For some compounds the labile tritium can be removed chemically, resulting in a compound of much lower activity.

Of interest here are the facts that the alpha-protons of N-nitrosamines are probably the most labile and that the alpha-position is likely the position involved in activation of these compounds to carcinogenic agents.[1,2] Therefore, for the methyl-alkyl N-nitrosamines, tritium labeling and subsequent chemical removal of labile tritium would severely limit the activity of the methyl group. Furthermore, residual activity in the methyl group, if any, would then be decreased even further because of the bio-lability of the tritium in the alpha-position.

Consequently, the synthesis of C-14-labeled N-methyl-N-nitrosoaniline (NMA) was developed. The structure of this compound and, in general that of other di-substituted N-nitrosamines, is such that it is of interest to specifically label each side chain separately. In biological systems this type of labeling of N-nitrosamines is useful in determining any differences in biological effectiveness of each side chain. For the carcinogenic NMA,[3,4] we wish to report the simple high yield synthesis of the C-14 ring-labeled and C-14 methyl-labeled compounds.

EXPERIMENTAL

The reaction scheme for the synthesis of C-14-labeled NMA is presented following this paragraph. All reagents were purified before use. Radioactive precursors (aniline hydrochloride for ring-labeled NMA, and methyl iodide for methyl-labeled NMA) were purchased from ICN Chemical and Radioisotope Division, Irvine. Trifluoromethanesulfonic anhydride was prepared from phosphorus pentoxide and trifluoromethanesulfonic acid at room temperature. The anhydride was purified by distillation (b.p. (atm.) 81-83° C).[5]



Reaction scheme for the synthesis of C-14-N-Methyl-N-Nitrosoaniline.

N-Phenyltrifluoromethanesulfonamide (I). To a methylene chloride solution of trifluoromethanesulfonic anhydride [4.4 mmol] in an acetone-Dry Ice bath was added an equimolar amount of aniline dissolved in methylene chloride. The aniline was or was not radioactive, depending on the side chain being labeled. The reaction mixture was allowed to warm to room temperature slowly and then stirred for 16 hr. The mixture was then washed twice with water, dried with $MgSO_4$, filtered and taken to dryness with a dry nitrogen stream. The resulting amber solid (4.0 mmole, yield 91%) had a sharp melting point of 60-61°C (reported, 66-67°C[6]) and no detectable radioactive impurities, as determined by thin-layer chromatography on silica gel G and subsequent autoradiography. A single spot with an R_f of 0.21 in the solvent system of hexane-ethyl ether-methylene chloride (4:3:2 v/v) was observed.

N-Methyl-N-Phenyltrifluoromethanesulfonamide (II).[6] The dried N-phenyltrifluoromethanesulfonamide (4 mmol) was dissolved in acetone and an equimolar amount of methyl iodide was added. The methyl iodide was radioactive or not, again depending on the side chain being labeled. The reaction mixture was shaken for 24 hr at room temperature. A precipitate formed, which was removed by filtration and washed well with acetone. The resulting filtrate and washings were combined, and the solvent evaporated with a gentle stream of dry nitrogen. Analysis of the highly concentrated product by thin-layer chromatography (same solvent system as above) and subsequent autoradiography revealed only one product with an R_f of 0.51 (yield, 92%).

N-Methylaniline (III). The N-methyl-N-phenyltrifluoromethanesulfonic acid (3.7 mmol) was taken up in diethyl ether and added slowly to an ethereal solution of lithium aluminum hydride (2-molar excess) at 0°C. Upon completion of the addition, the reaction mixture was refluxed gently for 4 hr. Unreacted lithium aluminum hydride was disposed of by addition of distilled water to the reaction mixture, which was then made basic by adding 50% NaOH. The ether layer, combined with three ether washes of the aqueous layer, was evaporated again with a gentle stream of dry nitrogen. Thin-layer chromatography and autoradiography, as presented above, revealed once more a single radioactive product with an R_f of 0.57. Further analysis of the product by gas-liquid chromatography (GLC) on a one-meter column of 28% Pennwalt containing 4% KOH at 150°C showed the presence of an apparent non-radioactive compound. The chemical impurity was less than 1% of the total product (yield, 84%).

N-Methyl-N-Nitrosoaniline (IV). Two milliliters of distilled water was added to the above product (3.1 mmol) (III), and the solution was cooled to 0°C, after which a 0.1 molar excess of concentrated HCl was added. To the cold acidic solution was added, dropwise, an aqueous solution of sodium nitrite (equimolar amount as HCl). The reaction was continued at 0°C for an additional 30 min and then warmed slowly to room temperature. Extraction of the product was made with several 3-ml portions of methylene chloride, and the extracts were combined, dried with magnesium sulfate and evaporated with dry nitrogen (yield, 88%).

DISCUSSION

The product, N-nitroso-N-methylaniline (NMA), either labeled with C-14 in the methyl group or generally in the ring, was analyzed for chemical impurities and compared to authentic NMA by GLC on a 1-meter column containing 8% DEGS on 80-100 mesh Chromasorb W at 140°C. Analysis for radioactive impurities was performed by TLC on silica gel using the solvent system given in the experimental part above. The chromatograms were then analyzed by autoradiography and by a radiochromatogram scanner after fixing the chromatogram with iodine vapor. No radioactive impurities were found and a single chemical impurity of <1% was detected which was not a nitrosamine and therefore not removed. The final product of C-14 NMA had an R_f of 0.50 in this solvent system.

The separately C-14-labeled side-chain products were stored as dilute solutions in hexane at 10°C. Final purity is maintained for long periods of time in this solvent, which is easily removed by dry nitrogen evaporation before use in biological systems.

ACKNOWLEDGMENT

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