Synthesis of [13C]- and [14C]-Labeled Methanesulfinic and Methanesulfonic Acids

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SUMMARY

The enzyme catalyzed displacement of the methyl sulfonyl group from penta-chlorophenyl methyl sulfone, the m-chloroperoxybenzoic acid oxidation of methyl mercaptan and the reaction of methyllithium with sulfur dioxide are methods described for the synthesis of isotopically labeled methanesulfinic acid. The first two methods can be carried out on a milligram scale and are suitable for high specific activity preparations while the latter method is suitable for preparations on a gram scale. Conditions are also given for the preparation of isotopically labeled methanesulfonic acid.

Keywords: [13C]Methanesulfinic acid, [14C]methanesulfinic acid and [14C] methanesulfonic acid.

INTRODUCTION

Mammalian metabolism of many xenobiotics involves a complex series of steps, including conjugation with glutathione either by direct reaction at an electrophilic site or at a site that has been metabolically activated such as an arene oxide.[1,2,3] One metabolic pathway of the glutathione conjugate involves conversion to the cysteine conjugate, cysteine-conjugate β-lyase cleavage of the cysteine conjugate to the thiol, methylation of the thiol, oxidation to the methyl sulfonyl compound and replacement of the methyl sulfonyl group with glutathione to reinitiate the sequence.[4,5,6] The removal of the methylsulfonyl group results in the formation of methanesulfinic acid and probably methanesulfonic acid by subsequent oxidation. Both [13C]- and [14C]-labeled acids were required to determine endogenous levels and fate of these acids. We present here our procedures used in their preparation.

EXPERIMENTAL

Ion exchange chromatography was done on an 8 mm x 30 cm column of analytical grade AG 2-X8 (Bio-Rad Laboratories, Richmond, CA 94804) and was monitored with a Packard Tri-Carb RAM 7500 radioactivity monitor using No. 6010700 scintillation beads. Reversed phase chromatography was done with C_{18} Sep-Paks, Waters Associates, Milford, MA 01757. Chemical sources were as follows: Sepharose, Sigma Chemical Co., St. Louis, MO 63178; methyl[14C] mercaptan, Pathfinder Laboratories, St. Louis, MO 63178; methyl[13C] iodide, Cambridge Isotope Laboratories, Woburn, MA 01801. NMR spectra were obtained in D_2O with a JEOL FX 90Q spectrometer. Proton spectra were referenced to sodium 3-(trimethylsilyl)-2,2,3,3-tetradeuteropropionate and carbon spectra were referenced to internal CH_3CN at δ 1.30. Negative ion fast atom bombardment (FAB) mass spectra were obtained with a Varian-MAT Model CH-5 DF mass spectrometer interfaced with an SS-200 data system, and equipped with an AMD Intectra combination source and post accelerator. An Ion Tech saddle-field gun operated at 7 kV with xenon gas provided the beam of fast atoms to bombard glycerol solutions of the compounds.

Method A. Microsomal enzymes from fresh rat livers were immobilized onto cyanogen bromide activated Sepharose by the method of Pallante et al.[7] Glutathione-S-transferase activity was 184 nmol/min/ml of beads using a spectrophotometric assay with 1-chloro-2,4-dinitrobenzene as a substrate.[7] A typical reaction mixture contained the following: 5.0 mg of pentachlorophenyl [14C]methyl sulfone in 100 μl of absolute ethanol, 15 mg reduced glutathione, 15 ml Sepharose beads bound with enzymes and 15 ml 0.1 M pH 7.4 phosphate buffer. The mixture was stirred slowly for 90 min at 38°C. The Sepharose beads were removed by filtration and washed with water. The combined aqueous extracts (approximately 13% of the starting activity) were concentrated at reduced pressure and applied to a C_{18} Sep-Pak. At the pH of the applied extract, lipophilic components were not eluted with water. Elution with 5.0 ml of water yielded a fraction (37% of the aqueous activity) that contained the desired methanesulfinic acid (shown by the formation of p-bromophenacyl methyl sulfone by a crown ether catalyzed reaction with p-bromophenacyl bromide). The methanesulfinic acid was derivatized without purification by a modified method of Durst et al.[8] using p-bromophenacyl-8 reagent (1 mmol/ml p-bromophenacyl bromide, 0.005 mmol/ml crown ether in acetonitrile, Pierce Chemical Co., Rockford, IL 61105) at 80° for 2 hr. Chromatography on AG 2-X8 with a step gradient of water, 0.015 N HCl and 0.1 N HCl yielded 2.5% of the radioactivity in the water eluate, 92.5% in the 0.015 N HCl fraction (methanesulfinic acid), and 5% in the 0.1 N HCl fraction (methanesulfonic acid).

Method B. Methyl[13C] iodide (1.0 ml, 0.12 mol) was added with stirring to a suspension of 0.3 g (0.043 g atm) of lithium chips in 25 ml of dry ether at a rate that yielded a gentle reflux. After the addition was completed, the reaction was stirred for 15 min, cooled to -20° and the unreacted lithium was removed. Sulfur dioxide (300 ml, measured by filling a balloon inside a 300 ml flask) was slowly bubbled into the methyllithium solution with vigorous stirring. The sulfur dioxide was diluted with a sufficient amount of nitrogen prior to introduction to control the reaction and to prevent plugging of the syringe needle used for the introduction. After the addition was completed, the reaction was allowed to warm to room temperature and the solvent was removed at reduced pressure. The solid was dissolved in methanol and filtered to remove a small amount of insoluble material. Addition of diethyl ether yielded 470 mg of light tan solid. Addition of more ether yielded an additional 180 mg of white solid. Both fractions showed large carbon NMR peaks for methanesulfinic acid, δ

48.2 in D$_2$O, and small peaks for methanesulfonic acid, δ 38.8, (1.4% for the first fraction and 0.5% for the second fraction, assuming identical relaxation times). Freeman and Angeletakis[9] report values of 44.30 for methanesulfinic acid and 39.06 for methanesulfonic acid in CDCl$_3$ with TMS as an internal standard. We obtained 44.7 and 38.9 for the acids in D$_2$O with internal CH$_3$CN, but conducted most measurements on sodium or lithium salts because salts of methane sulfinic acid were more stable. Proton NMR yielded a doublet at δ 2.30, J$_{HC}$ = 134 Hz; comparative integration with a sample of natural abundance lithium methanesulfinate prepared from stoichiometric amounts of lithium carbonate and pure methanesulfinic acid[10] indicated purities of 87.8 and 90.9% respectively for the first and second fractions. Negative ion fast atom bombardment (FAB) mass spectrometry showed the presence of methanesulfonate, iodide, and hydrogen sulfate ions.

Method C. Reaction time, temperature and stoichiometry were modified from the method of Filby et al.[11] A solution of 155 mg (0.9 mmol) of m-chloroperoxy-benzoic acid in 5 ml of benzene was added to a solution of 13.6 mg (0.28 mmol), 2.0 mCi, methyl mercaptan in 5 ml of benzene and the solution was stirred for 5 hours. When a precipitate resulted, the mixture was filtered and the solution was extracted five times with 1 ml portions of water. Since precipitation of m-chlorobenzoic acid resulted in the coprecipitation of radioactive products, the m-chlorobenzoic acid was dissolved in a minimum amount of ethanol and water was added. The precipitated m-chlorobenzoic acid was removed by filtration, the filtrate was added to the aqueous extracts and the combined extracts were chromatographed on AG 2-X8 using a step gradient of water, 0.015 N HCl and 0.1 N HCl. The eluates were monitored with a radioactive flow monitor. Water (125 ml) eluted two peaks, one sharp peak at approximately one column volume and one broad peak. The 0.015 N HCl (175 ml) usually eluted one peak, the desired methanesulfinic acid, during the later two thirds of the elution volume. Occasionally another compound eluted during the early portion of the elution volume. To remove this compound, the eluate was neutralized with sodium bicarbonate, the water was removed at reduced pressure, the residue was extracted with a small amount of anhydrous ethanol and the extracts were again chromatographed on AG 2-X8 to yield a similar profile of neutral products, methanesulfinic acid and methanesulfonic acid. Once pure, methanesulfinic acid yielded only small amounts of methanesulfonic acid on further manipulation and chromatography. Methanesulfonic acid was eluted with 0.1 N HCl. Yields of methanesulfinic and methanesulfonic acids were 68% and 12% respectively. Yields varied greatly depending on amount of peracid used, reaction time and manipulative procedures. Increasing the amount of m-chloroperoxybenzoic acid to 207 mg (4.3 to 1 mole ratio) and the reaction time to 48 h yielded 4.3% methanesulfinic acid and 70% methanesulfonic acid. The benzene layer contained volatile ¹⁴C activity under all reaction conditions tried. A 4.3/1 mole ratio of peracid to mercaptan yielded 12% of the activity in the benzene layer while a 2/1 ratio yielded 40%.

DISCUSSION

In vivo metabolism studies of pentachlorothioanisole in rats[4,5,6] led us to investigate the metabolism of its oxidation products. After methanesulfinic acid was determined to be one of the metabolic products,[4] we prepared ¹⁴C-labeled methanesulfinic acid using immobilized glutathione-S-transferase enzymes and pentachlorophenyl methyl sulfone (method A). The method is convenient for preparation of milligram quantities of methanesulfinic acid and arylglutathione conjugates if the enzyme system and an isotopically labeled methyl sulfonyl com-

pound are available. Pentachlorophenyl methyl sulfone is the only compound we
have used for the synthesis of labeled methanesulfinic acid; however, other com-
pounds have been used in the synthesis of glutathione conjugates[7] and these
would likely be suitable substrates for enzymic synthesis of methanesulfinic
acid. Yields were poor as only about 13% of the substrate reacted and of this
only 37% was methanesulfinic acid; however, reaction conditions were likely not
optimum. The unreacted pentachlorophenyl methyl sulfone could be recovered and
used in subsequent reactions. The methanesulfinic acid was characterized by
reaction with p-bromophenacylbromide to form p-bromophenacyl methyl sulfone.
        When larger amounts (mass and/or radioactivity) of methanesulfinic acid
were required, alternate methods were developed. A variety of methods have been
used for the synthesis of sulfinic acids.[12] In our hands, the reaction of
methyllithium (prepared from lithium and methyl iodide) and sulfur dioxide
(method B) proved most convenient for gram quantities of methanesulfinic acid
because of ease of purification, and this method was used for the preparation of
the ^{13}C-labeled compound. Recrystallization of the lithium salt yielded
material that was of high purity except for the presence of lithium hydrogen
sulfate and lithium iodide. These impurities were not detrimental in our appli-
cations and were not removed.
        The methyllithium-sulfur dioxide method was unsuitable for preparation of
milligram quantities of methanesulfinic acid because of the difficulty in pre-
paring milligram quantities of methyllithium; therefore the oxidation of methyl
mercaptan with m-chloroperoxybenzoic acid (method C)[11] was chosen for the
preparation of millicurie amounts of methanesulfinic acid at moderately high
specific activities. We obtained mixtures of products under all conditions
investigated. Figure 1 is a composite chromatogram of several trials since not
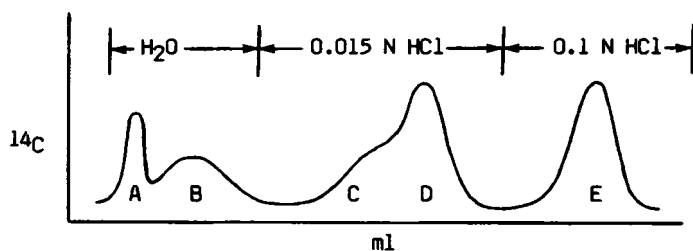all compounds were encountered in every reaction. Compounds D and E were

Figure 1.

methanesulfinic and methanesulfonic acid respectively as shown by negative ion
FAB mass spectrometry, proton and carbon NMR, and isotope dilution. Compounds A
and B were probably dimethyl disulfide and methyl mercaptan respectively based
on ion exchange behavior and volatility. Decreasing the amount of peracid
caused an increase in A and B and a decrease in D and E, while placing the
sample under vacuum prior to chromatography caused decreases in both A and B,
especially in B. Compound C was only encountered occasionally, and was found to
be unstable yielding A, B, D and E when the solution was neutralized with sodium
bicarbonate, the solvent removed and the residue rechromatographed. Negative
ion FAB mass spectrometry on fraction C showed the presence of methanesulfonic
acid. These data suggest that C was methanesulfenic acid. A large excess of
peracid yielded mostly methanesulfonic acid, but also yielded some neutral
product(s).

Characterization and yield determination of methanesulfinic acid was dif-
ficult because of decomposition during manipulations required in purification.
Wolf et al. reported similar difficulties with chloromethanesulfinic acid.[13] We
found carbon NMR and negative ion FAB mass spectrometry especially useful in
identification. FAB mass spectrometry and proton NMR were useful in purity
determinations of the ^{13}C analog by comparison with a sample of lithium methane-
sulfinate prepared by neutralizing pure methanesulfinic acid obtained by the
method of Wudl et al.[10] with lithium carbonate. Proton NMR was also used in
purity determinations by comparison to methanol as an internal standard. Salts
of methanesulfinic acid were more stable than the free acid and long term
storage was best done as the dry sodium or lithium salt. Solvent removal at
reduced pressure and ion exchange chromatography generated small amounts of
methanesulfonic acid which was suppressed by the addition of 0.05% dithio-
threitol to the eluting solvents; however, this was detrimental to analysis by
FAB mass spectrometry.

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