Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVII, No. 9

SYNTHESIS OF 3-QUINUCLIDINOL-^{18}O, BENZILIC-d_5 ACID, AND 3-QUINUCLIDINYL-^{18}O BENZILATE-d_5

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SUMMARY

Isotopically labeled analogs of 3-quinuclidinol (Q), benzilic acid (BA), and 3-quinuclidinyl benzilate (BZ), for use as internal standards for isotope dilution measurements and the labeled intermediates 3-quinuclidinone and methyl benzilate used in their production, were synthesized. The isotopic purities of the 1-azabicyclo[2.2.2]octan-3-^{18}O-ol, α-hydroxy-α-phenyl-d_5-benzeneacetic acid, and 3-quinuclidinyl-^{18}O benzilate-d_5 were determined and the electron impact ionization mass spectrum was obtained for each.

Key Words: 3-quinuclidinol-^{18}O, benzilic-d_5 acid, 3-quinuclidinyl-^{18}O benzilate-d_5

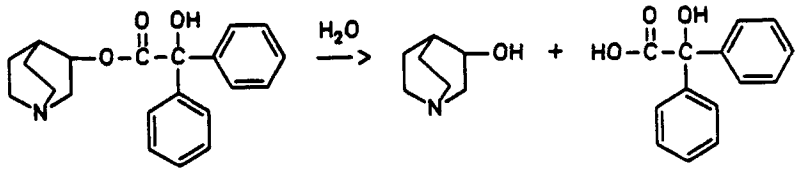
INTRODUCTION

The current military stockpiles of the incapacitating agent 3-quinuclidinyl benzilate (BZ) (1) are scheduled for demilitarization in the near future [1]. This process will create the potential for worker exposure to BZ and it is, therefore, desirable to develop a confirmatory test for human exposure to BZ prior to the demilitarization process.

BZ is a relatively nonvolatile ester that affects the brain and nervous system [2]. It is a specific binding antagonist for the muscarinic class of receptors [3] and can produce incapacitation at very small doses. Of the BZ that enters the body, 50 to 90% is excreted by the kidneys with most in the form of the metabolites 3-quinuclidinol (Q) (2) and benzilic acid (BA) (3).

0362–4803/89/090983–11$05.50

Received May 6, 1988
Revised March 1, 1989



(1) (2) (3)

The exact relationship between BZ dose and levels of Q, BA, and BZ in the urine is not well understood. However, based on the known no-effect level and the fact that most BZ will be hydrolyzed to Q and BA, the required detection limits of these analytes in urine have been set at 0.5 ng/mL for BZ and 5 ng/mL for Q and BA.

It was necessary to develop a method that could quantify either BZ or both Q and BA to achieve specificity for BZ exposure. To minimize the possibility of failing to detect exposure to BZ, a method that detected all three analytes was desirable. The method had to be highly specific to minimize interferences, and sensitive enough to detect low ng/mL levels of material in urine. The method selected was isotope dilution gas chromatography/mass spectrometry (GC/MS) with isotopically labeled analogs of Q, BA, and BZ to be used as internal standards. The Q was labeled with ^{18}O and the benzilic acid was labeled with five deuterium atoms on one of the phenyl rings. Both halves of the BZ molecule were labeled; the quinuclidinol moiety with oxygen-18 and one of the phenyl rings on the benzilic acid moiety with five deuteriums. The schemes for the synthesis of the labeled compounds are shown in Figure 1.

The quinuclidinol-^{18}O (6) was prepared by reduction of 3-quinuclidinone-^{18}O (5) which was synthesized by equilibration of the HCl salt of 3-quinuclidinone (4) with $H_2{}^{18}O$. When (4) is equilibrated with a large excess of $H_2{}^{18}O$, material which is 98 atom % ^{18}O can be obtained in a few hours. However, due to its high cost, a large excess of $H_2{}^{18}O$ was not used. Instead, repeated exchanges at a lower $H_2{}^{18}O$ to (4) ratio was used.

Several methods were tried to reduce (5) [4]. In each method the yield and purity of the product were checked. Reduction of (5) with sodium in ethanol produced an excellent yield of (6) but only 79% of the ^{18}O label was

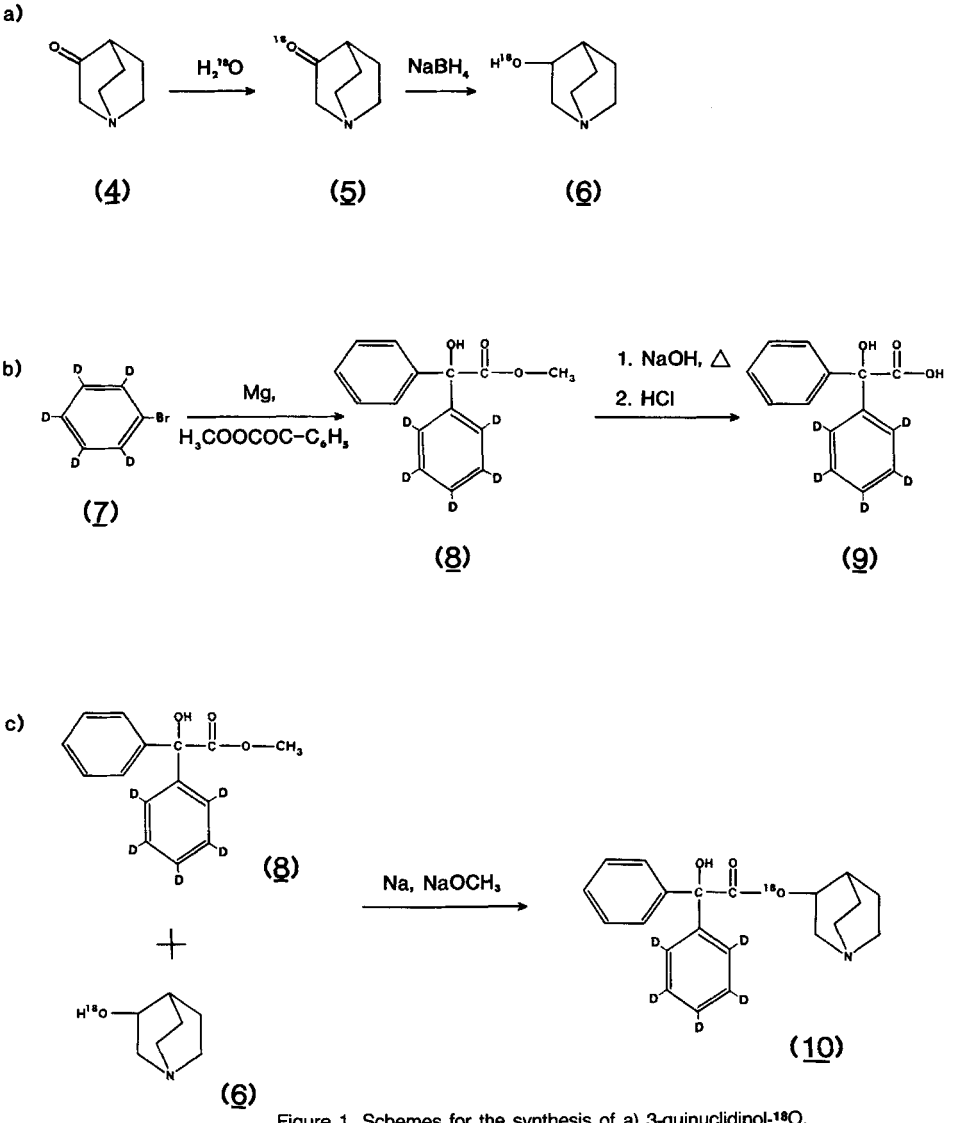


Figure 1. Schemes for the synthesis of a) 3-quinuclidinol-^{18}O, b) benzilic acid-d_5, and c) 3-quinuclidinyl-^{18}O-benzilate-d_5.

retained. Catalytic reduction with palladium in carbon at a pressure of 1 to 2 atmospheres produced little or no Q. Reduction of (5) with either lithium aluminum hydride or sodium borohydride gave satisfactory results with little loss of the ^{18}O label. The reduction with sodium borohydride was used since the product appeared more nearly pure by GC/MS. This method gave a yield of 91.5% of a final product containing 94 atom % ^{18}O.

Methyl benzilate-d_5 (8) was prepared by the reaction of the Grignard reagent phenyl-d_5 magnesium bromide, prepared from bromobenzene-d_5 (7) [5], with methyl benzoylformate followed by acid hydrolysis [6]. The yield was 25.2% of (8) containing more than 99 atom % deuterium. Benzilic-d_5 acid (9) was prepared in 96% yield by basic hydrolysis [7] of (8).

The labeled 3-quinuclidinyl benzilate (10) was synthesized by the sodium methoxide-catalyzed transesterification of (8) with (6) [8,9]. A 44.3% yield of (10) containing 94 atom % ^{18}O and more than 99 atom % d_5 was obtained.

EXPERIMENTAL

3-Quinuclidinone-^{18}O (5). A glass vacuum manifold was used for the exchange reactions. A sample of (4) (2.35 g, 14.5 mmoles) was weighed into a 50-mL sidearm flask. A magnetic stirring bar, $H_2{}^{18}O$ (0.6 mL, 30 mmoles, 98.3 atom %) and chloroform (1 mL) were added. The mixture was placed under nitrogen and stirred magnetically. After one hour, the chloroform was removed under vacuum [approximately 65 Pa (0.5 Torr)] and the $H_2{}^{18}O$ was distilled into a second flask held at 77 °K with liquid nitrogen. The second flask contained an additional 2.35 g of (4). Analysis of the material in the first flask by mass spectrometry showed 36 atom % ^{18}O incorporation. An additional 0.6 mL of $H_2{}^{18}O$ and 2 mL of chloroform were added to this material, and the mixture was stirred and warmed to 50 °C. After 2.5 hours the chloroform was removed under vacuum and the $H_2{}^{18}O$ was distilled into the second flask as before. This process was repeated three more times. After the last exchange, (4) in the first flask contained 94 atom % ^{18}O label and that in the second flask 78 atom % ^{18}O.

3-Quinuclidinol-^{18}O (6). Compound (6) was prepared by weighing sodium borohydride (0.20 g, 5.3 mmoles) into a 100-mL round bottom flask and adding 25 mL absolute ethanol, a stirring bar, and (5) (0.5 g, 3.1 mmoles, 94 atom % ^{18}O). The flask was closed with a drying tube and stirred overnight. The ethanol was removed under reduced pressure. The residue was acidified with 3 mL of 3 mol/L hydrochloric acid and heated at 90 °C for ten minutes to hydrolyze the borate complex. The solution was made alkaline with 5 mL of 10%

sodium carbonate, concentrated to dryness, and the residue was extracted four times with 50 mL chloroform-10% ethyl acetate. The yield was 0.36 g (91.5%). MP 208-210 °C, lit. MP 221-223 °C [4]. The final product contained 94 atom % ^{18}O.

Methyl benzilate-d_5 (8). A 500 mL flask was equipped with a Claisen connecting tube to which a Friedrichs reflux condenser and a dropping funnel with pressure equalizer were attached. A drying tube was attached to the condenser. Magnesium turnings (4.16 g, 0.17 moles) were placed in the flask and the apparatus was dried by flaming. The flask was allowed to cool to room temperature. Compound (7) (25.03 g, 0.15 mole, 99.5 atom %) was placed in a dropping funnel along with 20 mL of diethyl ether. Diethyl ether (42 mL) was added to the flask containing the magnesium turnings and the mixture was stirred with a magnetic stirrer. About 5 mL of the (7)/ether mixture was added dropwise. Since there was no sign of reaction, a crystal of iodine was added. The reaction began and the rest of the (7)/ether mixture was added dropwise over 20-25 minutes. A water bath was placed around the flask, and the solution was refluxed with stirring for four hours. Methylbenzoylformate (24.89 g, 0.15 mole) was placed in a one-liter flask that had been cooled with a drying tube in place after removing it from the oven. A magnetic stirring bar and 200 mL of diethyl ether were added and the flask was placed in an ice bath and the contents stirred. The Grignard reagent was transferred to a dropping funnel and added dropwise to the flask. A precipitate of the bromomagnesium salt of (8) formed. An additional 50 mL of ether was added so that stirring was possible and the mixture was stored in the refrigerator overnight. The precipitate was filtered off, washed with ether, and transferred to a beaker. Water (25 mL) was added followed by a 6 mol/L sulfuric acid (13 mL) to a slight acidity. The compound (8) separated as an oil and was taken up in 50 mL of ether. Both layers were transferred to a separatory funnel. The aqueous layer was extracted two times with 50 mL of ether. The combined ether layers were washed two times with 25 mL water, dried over sodium sulfate, filtered, and concentrated under reduced pressure. Hexane was added and (8) crystallized. The crystals were collected by filtration, washed with cold hexane, and

recrystallized from hexane-ether (95:5). The crystals were separated by filtration, washed with hexane-ether (98:2), and dried under vacuum. Yield 9.61 g (25.2%). MP 73-74.5 °C, lit. MP 75 °C [10]. Mass spectrometry showed an isotope incorporation of >99 atom %. The mother liquor yielded a second crop of 0.625 g (1.6%).

Benzilic-d_5 acid (9). To 0.625 g (2.53 mmoles) of (8) in a round bottom 100-mL flask was added 10 mL of 10% sodium hydroxide solution. The mixture was refluxed for an hour until the layer of (8) disappeared and a clear solution resulted. The solution was acidified with 3 mol/L hydrochloric acid. The precipitate (9) was collected by filtration and washed with water. The yield was 0.564 g (95.8%). The material was recrystallized from hot water. Yield 0.355 g (60.2%). MP 139-141 °C, lit. MP 150 °C [10]. No impurities were observed by mass spectrometric analysis and the final product contained more than 99 atom % d_5.

3-Quinuclidinyl-^{18}O-benzilate (d_5) (10). A 100-mL round bottom flask was equipped with a Dean-Stark trap and a condenser. In the flask were put 100 mg (0.78 mmoles) of (6) and 4 mL of heptane. Sodium (7.4 mg) and sodium methoxide (3.7 mg) were added and the mixture was heated to reflux. Compound (8) (191 mg, 0.77 mmole) in 8 mL of boiling heptane was added dropwise and the flask containing the (8) rinsed with additional hot heptane. Additional heptane was added for sufficient volume (about 10 mL) for refluxing from the Dean-Stark trap. After one hour of refluxing an additional 7.4 mg of sodium and 3.7 mg of sodium methoxide were added, and refluxing was continued for an additional three hours. The hot supernatant was poured off into a flask. Compound (10) crystallized. Analysis by GC/MS of a derivatized solution of the product showed the crystals were (10) and contained about 10% of (9). The material was recrystallized from 5 mL heptane, the crystals were washed with heptane, and dried in a vacuum desiccator. Yield 118 mg (44.3%) MP 159-162 °C, lit. MP 166-168 °C [11]. GC/MS of the derivatized material showed 10% impurity of (9). The final product contained 94 atom % ^{18}O and more than 99 atom % d_5.

Mass Spectrometric Determinations. Mass spectrometric determinations of sample purity and isotope incorporation were carried out on a quadrupole mass

spectrometer system equipped with a 30 m DB-5* fused silica capillary column connected directly to the ion source. The split flow injector port was maintained at a temperature of 250 °C. The temperature of the column was initially held at 140 °C for two minutes, and then programmed to 300 °C at a rate of 8 °C/min. Exact masses were determined by peak matching on a double focusing magnetic mass spectrometer at a resolution of 10,000. Analysis of (9) was carried out on the bis(trimethylsilyl) derivative formed by dissolving the solid in a solution of 2:1 (v/v) acetonitrile:N-methyl-N-(trimethylsilyl)-trifluoroacetamide.

DISCUSSION OF MASS SPECTRA

The mass spectra obtained by GC/MS of the three compounds, Q, BA (as the bis(trimethylsilyl) derivative), and BZ, and the isotopically labeled compounds are shown in Figures 2-4. BA did not chromatograph and required derivatization to make it suitable for chromatography. The Q spectrum in Figure 2a has a peak for the molecular ion at m/z 127, an $(M-CH_3)^+$ peak at m/z 112, and a peak at m/z 98 which is about 60% $(M-C_2H_5)^+$ and 40% $(M-HCO)^+$ as determined from measurement at high resolution. These, except that part of m/z 98 which results from loss of an oxygen atom containing fragment, are shifted up by 2 u in the spectrum of the labeled material shown in Figure 2b. The bis(trimethylsilyl) derivative of BA shows no peak for the molecular ion at m/z 372 in its spectrum shown in Figure 3a but has an $(M-CH_3)^+$ ion at m/z 357 and a slightly larger $(M-CH_3-CO)^+$ ion at m/z 329. Both of these ions are shifted up by 5 u in the spectrum of the labeled material in Figure 3b. The base peak is the $[(C_6H_5)_2CO\text{-}TMS]^+$ ion at m/z 255 and m/z 260 in the labeled material. In Figure 4a, the BZ mass spectrum, BZ shows a molecular ion at m/z 337 which is shifted in the spectrum of the labeled material in Figure 4b to m/z 344. The series, m/z 183, 105, and 77, is characteristic of the benzilate function and verifies the presence of the d_5 label by shifting to m/z 188, 110, and 82 in the labeled

*Identification of any commercial product does not imply recommendations or endorsement by the National Bureau of Standards, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

material. The base peak is the $[(C_6H_5)_2CO\text{-}TMS]^+$ ion at m/z 183 in Figure 4a and m/z 188 in Figure 4b. The quinuclidinoxy fragment at m/z 126 shifts to m/z 128 in the labeled material.

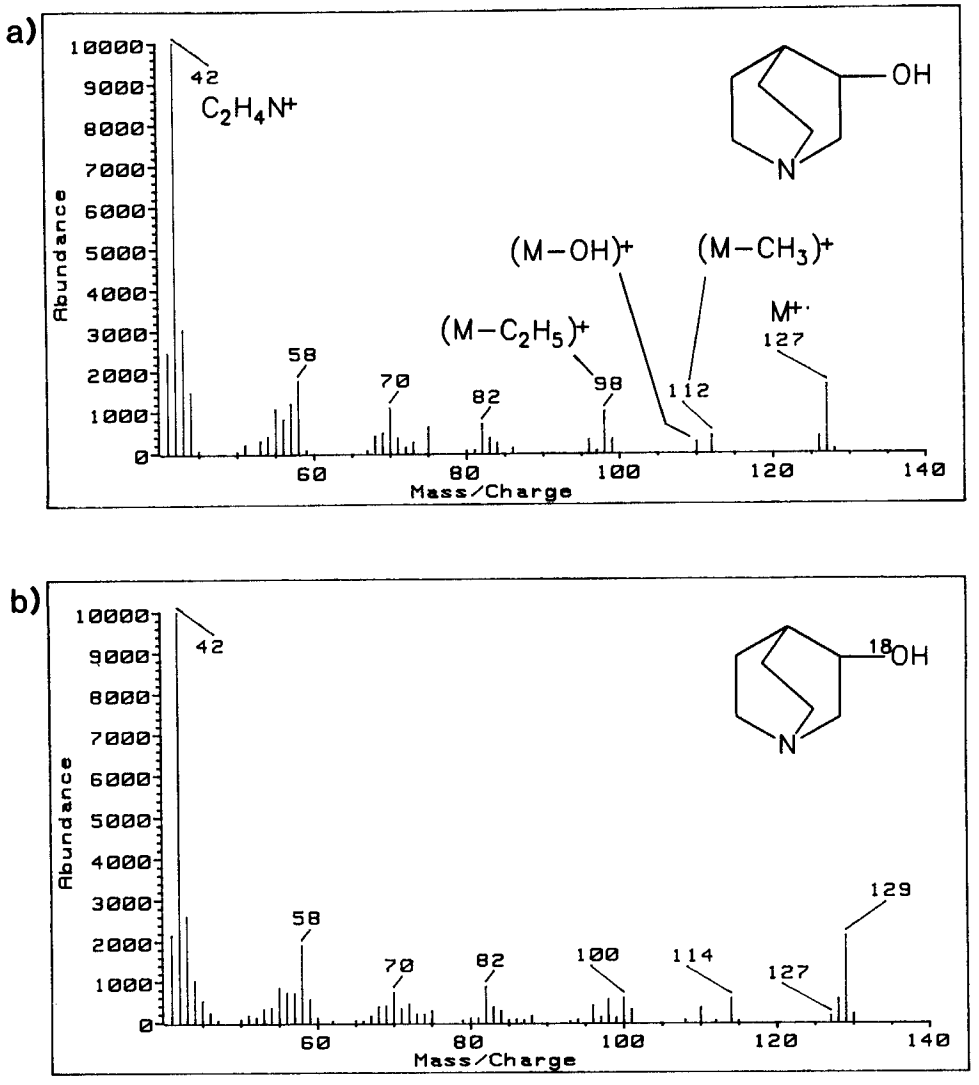


Figure 2. Mass spectrum of a) unlabeled 3-quinuclidinol and b) 3-quinuclidinol-^{18}O.

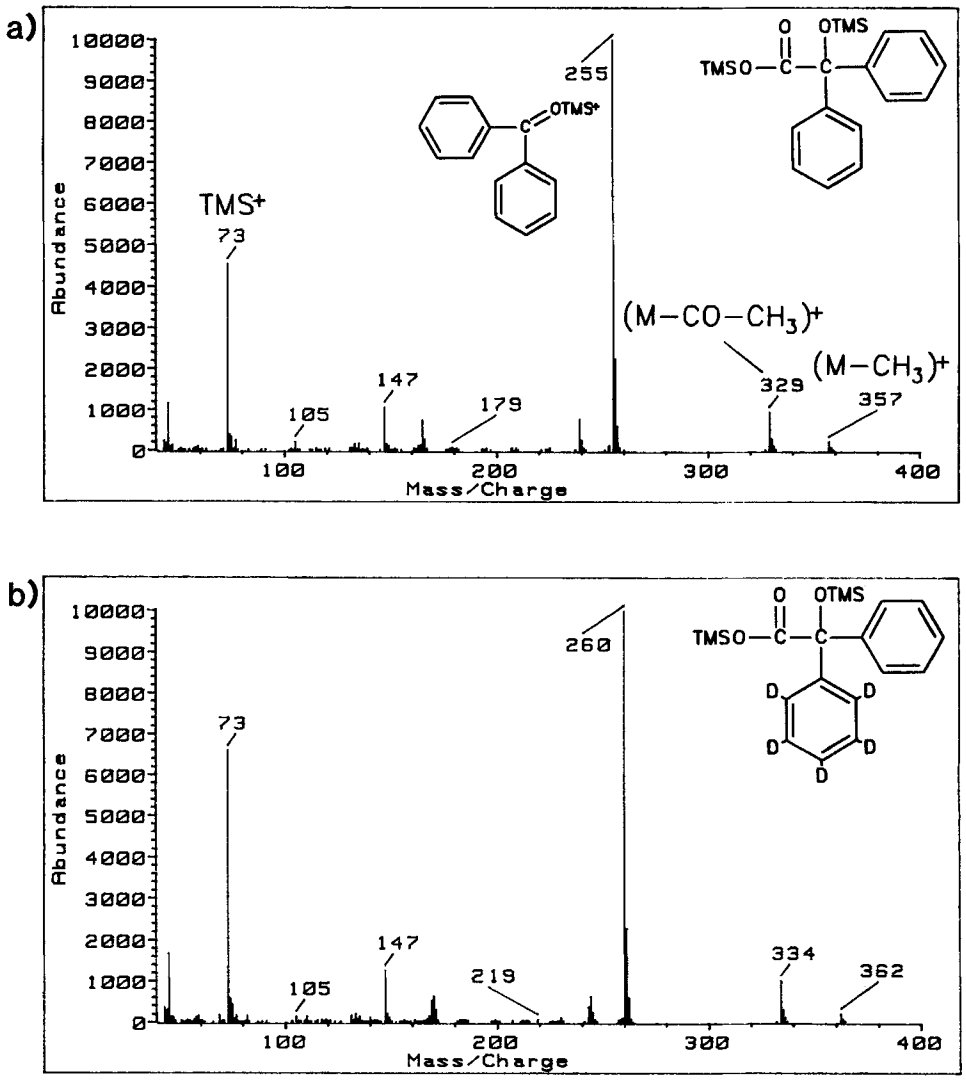


Figure 3. Mass spectrum of the bis(trimethylsilyl) derivative of a) unlabeled benzilic acid and b) benzilic acid-d_5.

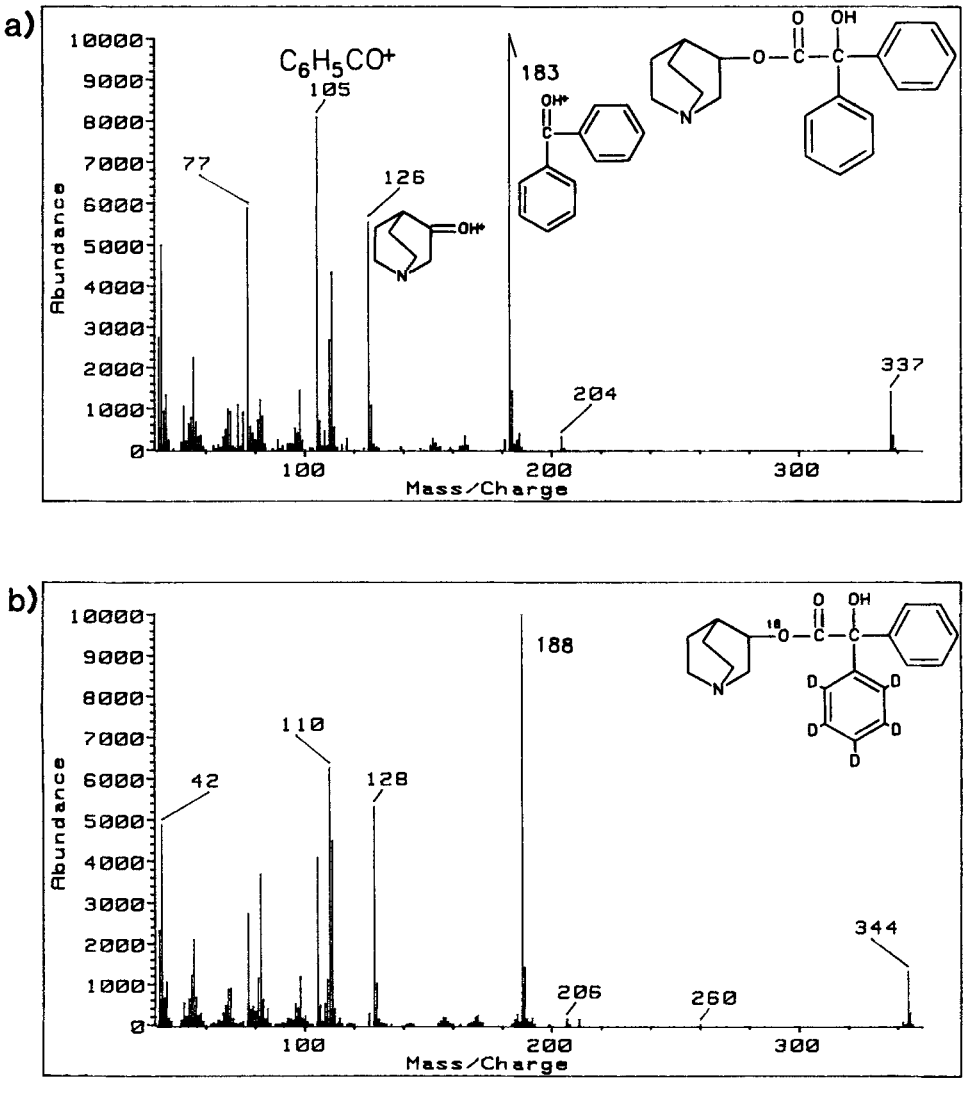


Figure 4. Mass spectrum of a) unlabeled 3-quinuclidinylbenzilate and b) 3-quinuclidinyl-^{18}O-benzilate-d_5.

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