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SYNTHESIS OF STABLE ISOTOPE-LABELLED ANALOGS OF THE CYSTEINE AND N-ACETYLCYSTEINE CONJUGATES OF TETRACHLOROETHYLENE

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SUMMARY

Stable isotope-labelled analogs of the cysteine and N-acetylcysteine conjugates of tetrachloroethylene have been prepared. S-(1,2,2-Trichlorovinyl)-DL-cysteine-3,3-$^2H_2$ was synthesized in a rapid, one-step procedure from tetrachloroethylene and DL-cysteine-3,3-$^2H_2$. Unlabelled S-(1,2,2-trichlorovinyl)-L-cysteine was prepared in a similar fashion. The corresponding ^{13}C-N-acetyl-S-(1,2,2-trichlorovinyl)cysteine compounds were then prepared via acetylation of the deuterated and unlabelled cysteine conjugates with ^{13}C-acetyl chloride.

Key words: Tetrachloroethylene, Cysteine, N-Acetylcysteine, Metabolites, Stable Isotope, Internal Standard.

INTRODUCTION

Tetrachloroethylene and other haloalkenes are converted to glutathione conjugates in the rat and/or the mouse (1-4). The amount of glutathione conjugation is highly variable for the haloalkenes, ranging from a minor route of metabolism for tetrachloroethylene and trichloroethylene to a major metabolic pathway for tetrafluoroethylene (2-4). These tripeptide conjugates are subsequently cleaved to the corresponding cysteine conjugates. These cysteinyl-metabolites are then thought to undergo conversion to nephrotoxic metabolites via the cysteine conjugate ß-lyase enzyme (1, 4, 5-7). The amount of glutathione conjugation occurring for these compounds is often estimated from the levels of cysteine or N-acetylcysteine (mercapturic acid) conjugates formed *in vivo* or *in vitro* (2-4, 7-8).

To aid in the quantitative, mass spectral determination of the cysteine and N-acetylcysteine metabolites of tetrachloroethylene, stable isotope labelled analogs of these compounds were prepared. These materials contained two deuterium atoms at the 3-position of the cysteine moiety and/or a ^{13}C-label in the 1-carbon of the N-acetyl group.

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RESULTS AND DISCUSSION

The deuterium labelled cysteine conjugate of tetrachloroethylene, 1, was prepared via reaction of excess tetrachloroethylene (9 equivalents) and the labelled cysteine in DMSO, with the base 1,5-diazabicyclo-[4.3.0]non-5-ene (Figure 1). The desired product was obtained after 30 min (room temp.) as a crystalline solid in 61% yield, with a purity of 92%. The use of fewer tetrachloroethylene equivalents resulted in significant levels of disubstituted reaction products. The unlabelled cysteine conjugate 2 was prepared in a similar manner (yield 66%). This reaction scheme is a modification of that used in the preparation of N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine (3), which utilized equimolar amounts of tetrachloroethylene and N-acetyl-L-cysteine. This synthetic route may be preferable to previously reported methods involving the use of sodium/ammonia or an N-t-butoxycarbonyl-protected cysteine reagent (9-10).

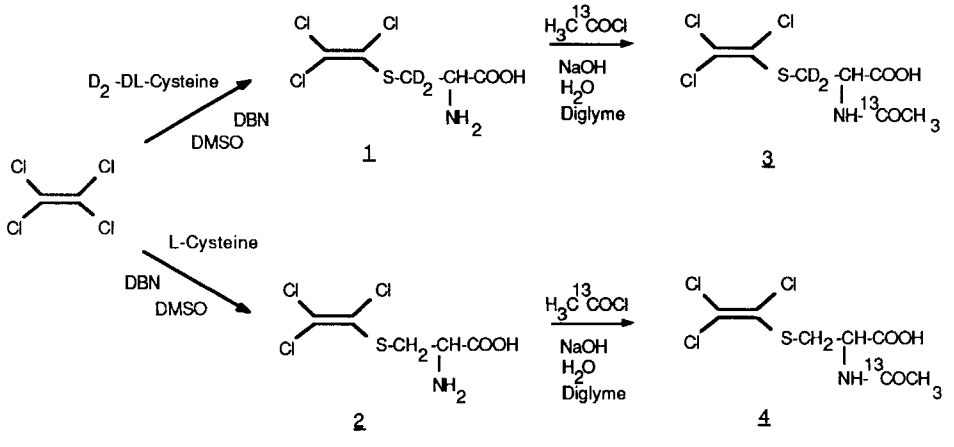

Figure 1. Synthesis of 1-4.

The $^{2}H_{2}$-^{13}C-N-acetylcysteine conjugate of tetrachloroethylene, 3, was prepared by acetylation of 1 with ^{13}C-acetyl chloride via the Schotten-Baumann procedure (11). A crystalline product was obtained in 47% yield (purity 97%). Although similar results may be obtained by the use of ^{13}C-acetic anhydride/pyridine, the acetyl chloride was preferred due to its lower cost. The ^{13}C-labelled analog 4 was prepared in the same manner, affording the desired product in 63% yield (purity 97%).

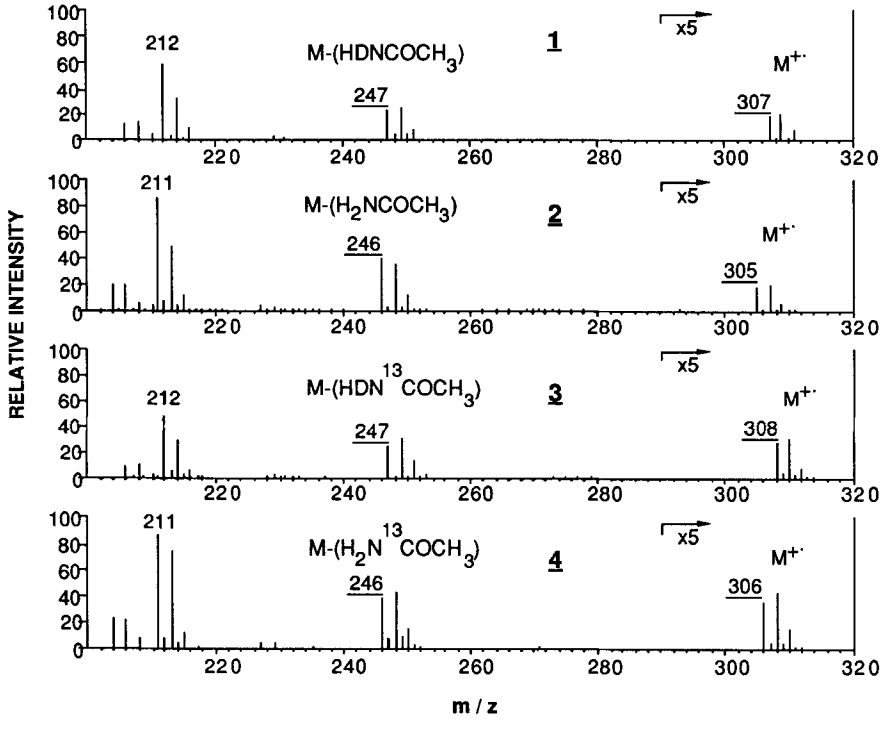

Figure 2. Partial mass spectra for the methyl esters of 1-4 (1 and 2 acetylated with acetic anyhydride/pyridine).

Mass spectral and NMR analyses indicate complete retention of the deuterium label of the cysteine moiety for compounds 1 and 3. Futher examination of the partial mass spectra in Figure 2 show that the major M-59 fragment ion of the unlabelled, methylated mercapturic acid of tetrachloroethylene is due to loss of the acetamide moiety of the molecule. This *α,i* rearrangement is accompanied by a selective loss of one of the 3-carbon protons, as seen by the M-60 and M-61 fragment ions of the derivatives of 1 and 3, respectively.

In summary, synthetic routes have been described for the synthesis of various stable isotope-labelled analogs of the cysteine and N-acetylcysteine conjugates of tetrachloroethylene. The unlabelled and deuterium-labelled cysteine conjugates were prepared via a rapid, one-step synthesis which afforded a signficant yield of the desired products with acceptable purities. Subsequent acetylation of these compounds with ^{13}C-acetyl chloride yielded the desired mercapturic acids. The three labelled compounds (1, 3 & 4) should be useful as internal standards for quantitative mass spectral analysis of the unlabelled tetrachloroethylene metabolites. The deuterated compound 1 should also be useful in the

investigation of any isotope effects for the cysteine conjugate ß-lyase enzyme. Finally, these synthetic routes may prove useful for the synthesis of cysteine and N-acetylcysteine conjugates of other related haloalkenes.

EXPERIMENTAL

Melting points were determined on an Exothermal capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet 60SX FT-IR spectrometer. Mass spectra were obtained on a Finnigan MAT TSQ-70 GC/MS. NMR spectra were recorded on an IBM AF300 spectrometer using TMS as an internal standard. Purities were calculated assuming equivalent molecular weights for products and impurities.

S-(1,2,2-Trichlorovinyl)-DL-cysteine-3,3-$^2H_2$ (1). 1,5-Diazabicyclo[4.3.0]non-5-ene, 1.01 g (8.1 mmol), and 0.5 g (4.1 mmol) DL-cysteine-3,3-$^2H_2$ (Cambridge Isotope Laboratories, Woburn, MA; 98% isotopic purity) were added to 10 ml dimethylsulfoxide. The solution was deoxygenated (N_2 stream) for 45 min at room temp. To this solution was then added 5.83 g (35.1 mmol) tetrachloroethylene. The resulting mixture was stirred under a nitrogen blanket for 30 min, diluted to 40 ml (water), and adjusted to pH 4-5 (acetic acid). The resulting mixture, containing a white precipitate, was cooled to -10°C for 1 hr and filtered to afford 0.636 g (61%) 1 as a white crystalline solid, mp 148-149°C; ir (KBr) 3435 (broad), 2955 (broad), 1585, 1400, 1345, 885 cm^{-1}; ^{1}H-nmr (^2H_2O, NaO^{2}H) δ 3.35 (s, 1, C<u>H</u>); ^{13}C-nmr (^2H_2O, NaO^{2}H) δ 180.8 (<u>C</u>=O), 127.4 (<u>C</u>=C), 122.9 (C=<u>C</u>), 56.1 (<u>C</u>H), 39.5 (m, <u>C</u>$^2H_2$); EI mass spectrum of the N-acetylated methyl ester m/z, 310, 309, 308, 307 (M^+, 0.5, 4.1, 0.5, 3.9), 249, 248, 247 (24.7, 5.3, 23.2), 214, 213, 212, 211 (33.4, 3.9, 59.3, 0.6), 146 (100), 88 (55.6). Purity via ^{1}H-nmr 92%.

S-(1,2,2-Trichlorovinyl)-L-cysteine (2). S-(1,2,2-Trichlorovinyl)-L-cysteine (2) was prepared in the same manner from 5.8 g (35.2 mmol) tetrachloroethylene and 0.656 g (5.4 mmol) L-cysteine to afford 0.888 g (66%) 2 as a white crystalline solid, mp 153.5-155.5°C; ir (KBr) 3435 (broad), 2945 (broad), 1595, 1515, 1395, 885 cm^{-1}; ^{1}H-nmr (^2H_2O, NaO^{2}H) δ 3.30 (dd, 1, C<u>H</u>), 3.16 (dd, 1, C<u>H</u>H), 3.01 (dd, 1, CH<u>H</u>)($J_{H\text{-}2,H\text{-}3}$=4.9 and 6.9 Hz, $J_{H\text{-}3,H\text{-}3}$=13.8 Hz); ^{13}C-nmr ($^2H_2O$, NaO^{2}H) δ 180.2 (<u>C</u>=O), 127.2 (<u>C</u>=C), 122.3 (C=<u>C</u>), 55.8 (<u>C</u>H), 40.0 (<u>C</u>H_2); EI mass spectrum of the N-acetylated methyl ester m/z, 310, 309, 308, 307, 306, 305 (M^+, 0.1, 1.2, 0.4, 3.8, 0.4, 3.7), 249, 248, 247, 246 (2.6,

35.8, 2.6, 38.6), 214, 213, 212, 211 (3.9, 48.5, 7.0, 78.2), 144 (77.9), 88 (100). Purity via ^{13}C-nmr 90%.

N-Acetyl(1-^{13}C)-S-(1,2,2-Trichlorovinyl)-DL-cysteine-3,3-$^{2}H_2$ (3). A solution containing 109 mg (0.43 mmol) 1 in 3 ml dimethoxyethane was added to 5 ml 1 M aq. NaOH. This solution was flushed with a nitrogen stream for 10 min. To the solution was added dropwise a solution of 220 mg (2.8 mmol) acetyl-1-^{13}C-chloride (Cambridge Isotope Laboratories, Woburn, MA; 99% isotopic purity) in 3 ml dimethoxyethane, under a nitrogen blanket. The resulting solution was allowed to stand at room temp. for 2.25 hr. The solution was then acidified to pH 1-2 (conc. HCl), concentrated (N_2) to approx. 6 ml, diluted to 10 ml (water) and extracted with ethyl ether (4 x 10 ml). The combined ethyl ether extracts were washed with water (1 x 5 ml), dried ($MgSO_4$) and evaporated (N_2) to afford 59 mg (47%) 3 as a tan solid, mp 158-159°C; ir (KBr) 3415 (broad), 3330, 1715, 1560, 1515, 1265, 1205, 875 cm^{-1}; ^{1}H-nmr (MeOH-$^{2}H_4$) δ 4.58 (d, 1, CH), 1.99 (d, 3, CH_3)($J_{H\text{-}2,13C}$=3 Hz, $J_{CH_3,13C}$=6 Hz); ^{13}C-nmr (MeOH-$^{2}H_4$) δ 173.3 (^{13}C=O), 172.7 (COOH), 128.2 (C=C), 122.8 (C=C), 53.4 (CH), 35.7 (m, $C^{2}H_2$), 22.4 (d, 1, CH_3)($J_{CH_3,13C}$=51 Hz); EI mass spectrum of the methyl ester m/z, 310, 309, 308, 307 (M^+, 6.1, 0.8, 5.7, 0.2), 249, 248, 247 (30.2, 2.2, 24.1), 214, 213, 212, 211 (29.3, 6.0, 48.9, 0.8), 147 (100), 88 (78.0). Purity via ^{13}C-nmr 97%.

N-Acetyl(1-^{13}C)-S-(1,2,2-Trichlorovinyl)-L-cysteine (4). N-Acetyl(1-^{13}C)-S-(1,2,2-Trichlorovinyl)-L-cysteine (4) was prepared in the same manner from 101 mg (0.4 mmol) 2 and 220 mg acetyl-1-^{13}C-chloride to afford 74 mg (63%) of 4 as a light tan solid, mp 155-156°C; ir (KBr) 3400 (broad), 3385, 1715, 1575, 1500, 1300, 1210, 865 cm^{-1}; ^{1}H-nmr (MeOH-$^{2}H_4$) δ 4.59 (ddd, 1, CH), 3.57 (dd, 1, CHH), 3.23 (dd, 1, CHH), 1.99 (d, 3, CH_3)($J_{H\text{-}2,13C}$=3.1 Hz, $J_{H\text{-}2,H\text{-}3}$=4.6 Hz and 8.5 Hz, $J_{H\text{-}3,H\text{-}3}$=14.0 Hz, $J_{CH_3,13C}$=6.1 Hz); ^{13}C-nmr (MeOH-$^{2}H_4$) δ 173.3 (^{13}C=O), 172.7 (COOH), 128.2 (C=C), 122.8 (C=C), 53.6 (CH), 36.2 (CH_2), 22.4 (d, CH_3)($J_{CH_3,13C}$=50 Hz); EI mass spectrum of the methyl ester m/z, 310, 309, 308, 307, 306 (M^+, 3.3, 1.2, 8.6, 1.1, 7.4), 249, 248, 247, 246 (8.7, 44.2, 8.2, 39.1), 214, 213, 212, 211 (5.0, 75.0, 8.1, 87.1), 145 (100), 88 (85.3). Purity via ^{13}C-nmr 97%.

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