The Synthesis of 2-^{14}C-N-Nitrosothiazolidine

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SUMMARY

2-^{14}C-N-Nitrosothiazolidine was synthesized with a chemical purity of 99.6%, a radio chemical purity of 99.4%, and an overall yield of 30.7%. Unlabelled N-nitroso-thiazolidine, synthesized using the same method, resulted in a purity of 99.85% by capillary GC and 99.7% by HPLC. Analysis of N-nitrosothiazolidine by GC was found to require a low injection port temperature of 160°C to prevent denitrosation in the injection port. During the nitrosation of thiazolidine, if the pH was reduced below 4 with hydrochloric acid, ring hydrolysis occurred, and chlorinated disulfide compounds formed.

Key words: 2-^{14}C-N-nitrosothiazolidine, nitrosamine, byproducts, carbon 14

INTRODUCTION

N-Nitrosothiazolidine is a nitrosamine recently found to occur in many smoke-cured meats (Skrypec et al., 1985). A structure-activity analysis by Rosenkranz & Klopman (1987) determined N-nitrosothiazolidine to have a high probability of being moderately carcinogenic. Comparison of this compound to other structurally related N-nitrosamines would tend to support their analysis. Two chemical analogs, N-nitrosopyrrolidine and N-nitrosooxazolidine, are carcinogenic in rats (Lijinsky and Reuber, 1981; Wiessler, M. and Schmahl, D., 1976). A homolog, N-nitrosothiomorpholine, has also tested positive for carcinogenicity (Garcia et al, 1973). Yet, a recent bioassay in rats has shown N-nitrosothiazolidine to be non-carcinogenic.

Lijinsky and coworkers (1988) found that over an average life span of 110 weeks, 20 female Fisher 344 rats showed no statistical increase in cancer when exposed to approximately 413 mg N-nitrosothiazolidine per rat. The reasons for the lack of correlation between the animal bioassay

0362–4803/89/070777–05$05.00

Received July 18, 1988

and the structure-activity analysis are not evident, but may be due to differential metabolism of the compounds.

In the present study, to determine the pharmacokinetic and metabolic fate of N-nitroso-thiazolidine, we synthesized the ^{14}C-labelled compound. Because the electron withdrawing influence of N and S would tend to make oxidation at the number 2 position a likely biological pathway, the compound was synthesized with this position labelled.

METHODS

Chemicals. ^{14}C-Formaldehyde (99% radiochemical purity, 50 mCi/mmole) was obtained from ICN Radiochemicals (Irvine, CA). Formaldehyde, 2-aminoethanethiol-HCl, sodium nitrite (99.9%) and were purchased from Aldrich Chemical (Milwaukee, WI).

Synthesis of Nitrosamine. ^{14}C-Formaldehyde (250 μCi @ 50 mCi/mmole), mixed with 4.29 mMoles formaldehyde, and 4.29 mMoles 2-aminoethanethiol-HCl, were dissolved in 60 ml of distilled water (Figure 1). The pH was adjusted to 8 with the addition of 5 N sodium hydroxide and the solution allowed to stir overnight. The resulting thiazolidine was extracted from the mixture with dichloro-methane (DCM) and the solvent was removed by rotary evaporation. The thiazolidine was redissolved in 80 ml ice-cold distilled water and 5.58 mMoles sodium nitrite was added. The pH was reduced to 4.5 with the addition of 8 N sulfuric acid and then the mixture stirred overnight. The resulting N-nitrosothiazolidine was extracted with DCM and then the organic layer was washed once with 1 N NaOH, and 3 times with distilled water. The washed solution was filtered through magnesium sulfate and then the DCM was removed by rotary evaporation. Residual amounts of solvent were removed by continued rotary evaporation with the flask immersed in hot water (50°C). The 2-^{14}C-N-nitrosothiazolidine formed a yellow oil on the bottom of the flask.

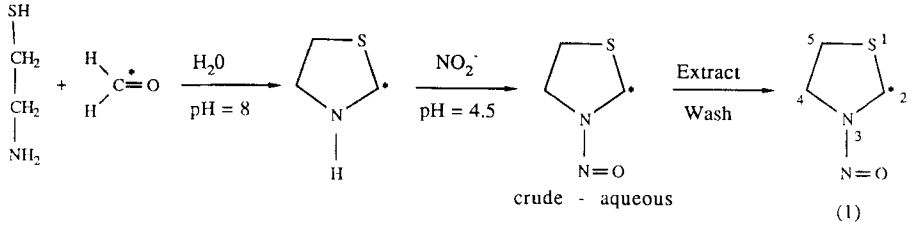

Figure 1. The Synthesis of 2-^{14}C-N-Nitrosothiazolidine (1)

The 2-^{14}C-N-nitrosothiazolidine was dissolved in ethanol. Radiochemical purity was determined to be 99.4% +/-.2 with thin layer chromatography using a solvent system of ethyl acetate : pentane : dichloromethane (1:2:7) and silica gel TLC plates (Eastman Kodak, Rochester, NY). The plates were

scanned for radioactivity with a Bioscan System 200 Imaging Scanner equipped with a Bioscan Autochanger 3000 (Bioscan, Washington, DC). Unlabelled N-nitrosothiazolidine was synthesized as previously described. Purity was determined to be 99.85% +/-.03 by capillary GC (FID) and 99.7% +/-.1 by HPLC (uv @365 nm). Structure of the reference was determined by GC/MS, GC/FT-IR, and NMR (Table 1). Concentration of the 2-^{14}C-N-nitrosothiazolidine in ethanol was determined by capillary GC (FID) using methyl acetamide as the internal standard. The standard nitrosamine concentrations ranged from 4 to 12 mg/ml and the methyl acetamide concentration was a constant 28.2 mg/ml. An aliquot of the 2-^{14}C-N-nitrosothiazolidine was diluted with DCM and the methyl acetamide was added. The concentration of this sample was estimated to be 3.97 +/-.06 mg/ml. Yield of the product was 30.7%. In addition, aliquots of the 2-^{14}C-N-nitrosothiazolidine were added to Liquiscint scintillation fluid (National Diagnostics, Somerville, NJ) and counted on a Tricarb 2000CA Liquid Scintillation Counter (Packard Instruments, Downers Grove, IL); the specific activity was determined to be 41.55 μCi/mMol.

Table 1

Spectra Data of N-Nitrosothiazolidine

instrument	spectral data
low-resolution MS	M+ = 118 (17), 88 (100), 60 (32), 59 (15), 45 (12), 42 (26),
IR (vapor)	2951 (m), 1492 (s), 1251 (s), 1086 (s), 987 (m), 803 (m) cm^{-1}
^{1}H-NMR	Z isomer: δ 5.20 (s, C_2-H), δ 4.50 (t, C_4-H), δ 3.09 (t, C_5-H)
	E isomer: δ 5.22 (s, C_2-H), δ 3.72 (t, C_4-H), δ 3.06 (t, C_5-H)

Instruments. Gas chromatography (GC) was carried out on an HP 5890 gas chromatograph (Hewlett Packard, Santa Clara CA) equipped with an flame ionization detector and a 30 m x 0.25 mm i.d. DB-1 fused silica capillary column (J & W Scientific, Inc., Rancho Cordova CA). Helium (30 cm/sec) was used as the carrier gas. To reduce the possible hazard from N-nitrosothiazolidine vaporized in the injection port, the splitter gas was vented to hood exhaust. The oven temperature was programmed at 4°C/min from 80°C to a final temperature of 220°C where it was held for 10 min. The injection port temperature was 160°C and the detector temperature was 280°C. Tandem GC/MS was performed on a VG ZAB-2F MS (VG Micromass, Manchester England) coupled with an HP 5790 gas chromatograph equipped with the capillary column used for GC analysis. Tandem GC/FT-IR was completed with an HP 59970C IRD Chem Station, which had an HP 5965A infrared detector, and an HP 5890 GC, which had a J&W 15 m X 0.25 mm i.d. DB-1 column. Injection port temperature was 160°C and light pipe temperature was 200°C.

High performance liquid chromatography was performed on Waters Associates HPLC equipment using a Model 680 automated gradient controller, a Model U6K injector, a Model 501 solvent delivery system, a Lambda-Max Model 481 variable-wavelength spectrophotometer (@365 nm) (Water Associates, Milford, MA), and a Supelco (Belafonte, PA) 4.6 mm X 25 cm LC-18 column. The linear solvent gradient was programed from 100% hexane to 100% dichloromethane over a 15 minute interval, with a flow rate of 1 ml/min.

The ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR) spectra were obtained using a Varian EM-390 Continuous Wave NMR (Varian, Walnut Creek, CA). The solvent was $CDCl_3$ with tetramethylsilane as a reference.

RESULTS AND DISCUSSION

The synthesized 2-^{14}C-N-nitrosothiazolidine was determined to be 99.6% chemically pure by GC, and of 99.4% radiochemical purity by TLC. The overall yield was 30.7%. Purity of the unlabelled nitrosamine was 99.85% by GC (FID) and 99.7% by HPLC (@365 nm). Analysis of N-nitrosothiazolidine by capillary GC was found to require a relatively low injection port temperature of 160°C. Higher injection port temperatures caused denitrosation of the nitrosamine. We also determined 2 factors that had a great influence on the synthesis of N-nitrosothiazolidine.

Nitrosation of thiazolidine both required an atypical pH and the use of sulfuric acid for the adjustment of pH instead of the use of hydrochloric acid. The optimal pH for nitrosation of most secondary amines is 2.5 to 3.5 (Mirvish, 1975). Hydrochloric acid is commonly used to reduce the pH for the nitrosation of amines (Ray, 1978). Yet, both of these conditions were found to cause many side reactions and result in an impure final product. Thiazolidine, when nitrosated at a pH of 3 in dilute hydrochloric acid, formed many byproducts. The byproducts constituted up to 66% of the peak area of the GC chromatogram. Analysis of these compounds by GC/MS tentatively determined the byproducts to be disulfide compounds (Table 2). The disulfide compounds were the

Table 2

Summary of Byproducts formed during N-Nitrosothiazolidine Synthesis.

Parent Compound (tentative)	Structure	Base (m/z)	M+ (m/z)
1-Chloroethyl(1'-chloroethyl)disulfide	$(ClCH_2CH_2S)_2$	190	190
1-Chloroethyl(1'-hydroxy-ethyl)disulfide	$ClCH_2CH_2SSCH_2CH_2OH$	172	45
1-Chloroethyl(ethenyl)disulfide	$H_2C{=}CHSSCH_2CH_2Cl$	154	154
1-Chloroethyl(methyl)disulfide	$ClCH_2CH_2SSCH_3$	142	142
1-Hydroxy-ethyl(ethenyl)disulfide	$H_2C{=}CHSSCH_2CH_2OH$	136	136

likely result of acid hydrolysis of the thiazolidine ring. We found that a reduction of the pH below 4 promoted the formation of the disulfide compounds. Many of the byproducts were chlorinated, and thereby, a reduction in the chloride concentration, by using sulfuric acid instead of hydrochloric acid, reduced their formation.

ACKNOWLEDGEMENTS

This work was supported in part by NIEHS Training Grant # T32 -ES07059

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