Syntheses of Carbon-14 and Sulfur-35 Labeled 2-(Morpholinothio)benzothiazoles and Carbon-14 Labeled 2-(Cyclohexylaminothio)benzothiazoles

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SUMMARY

Some vulcanizing accelerators, mercaptobenzothiazole derivatives labeled with carbon-14 or sulfur-35 were prepared. 2-(Morpholinothio)benzothiazole labeled with carbon-14 or sulfur-35 of the sulfhydryl group at position 2 was synthesized by oxidative condensation with sodium hypochlorite from a mixture of morpholine and 2-mercaptobenzothiazole-2-^{14}C or 2-mercaptobenzothiazole-2-^{35}S. The same method was applicable to the synthesis of 2-(morpholino-U-^{14}C-thio)-benzothiazole using morpholine-U-^{14}C as starting material.

2-(Cyclohexylaminothio)benzothiazole-2-^{14}C was prepared, by oxidation with a mixture of iodine and potassium iodide, from cyclohexylamine and 2-mercapto-benzothiazole-2-^{14}C, which was synthesized from carbon-^{14}C disulfide and 2-mercaptoaniline in the presence of trace sodium sulfide in dimethylformamide. 2-(Cyclohexyl-U-^{14}C-aminothio)benzothiazole was also obtained from cyclohexyl-amine-U-^{14}C and 2-mecaptobenzothiazole.

Key Words: vulcanizing accelerators, mercaptobenzothiazole derivatives, oxidation condensation

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INTRODUCTION

The use of chemicals in practically every aspect of life has grown rapidly and evaluation of their biological hazard has become imperative. In relation to a basic need for the assessment of the toxicity and/or safety of consumer products, we prepared five kinds of labeled vulcanizing accelerators, mercaptobenzothiazole derivatives, which are necessary for metabolic studies with laboratory animals.

EXPERIMENTAL

Starting Materials:

Carbon-^{14}C disulfide (59 mCi/mmol), carbon disulfide-$^{35}S_2$ (13.6 mCi/mmol) and morpholine-U-^{14}C hydrochloride (23.6 mCi/mmol) were purchased from Amersham International LTD. Cyclohexylamine-U-^{14}C hydrochloride (1.71 mCi/mmol) was obtained from Mallincrodt Chemical Works. Other chemicals were of reagent grade commercially available.

Wakogel C-100 (Wako Chemical industries) was used for silica gel column chromatography. Silica gel spot films containing fluorescence indicator were used for thin-layer chromatography (TLC) of synthetic products unless otherwise specified. Spots were visualized by their quenching of fluorescent background under an UV lamp. Radiochromatograms were scanned with a radioscanner TRM-1B (Aloka). Decay of sulfur-35 compounds was corrected in the measurement of radioactivity. All melting-points are uncorrected.

Synthesis of 2-(Morpholinothio)benzothiazole-2-^{14}C:

2-Mercaptobenzothiazole (468 mg) was added to 2-mercaptobenzothiazole-2-^{14}C (38 mg, 89.3 μCi) in 2-propanol (3 ml) and then mixed with redestilled morpholine (3 ml). The mixture was stirred for 90 min at 45-55°C. After the salt formation was complete, 10% sodium hypochlorite solution (4 ml) was slowly added to the

mixture during 3 hr with constant stirring at 45-55°C. Stirring was continued further 5 hr. Then 0.64% sodium sulfite solution (25 ml) was added to the mixture, which was allowed to stand overnight at 5°C. The collected precipitate (688 mg) was washed thoroughly with cold water. It melted at 88-89°C after being dried at room temperature. The crystals were treated with warm methanol (13 ml) to remove insoluble 2,2'-dithiobis(benzothiazole-2-^{14}C). The methanol-soluble fraction was found to be pure 2-(morpholinothio)benzothiazole-2-^{14}C (650 mg, 85.1% yield). The product showed a single radioactive spot with an R_f of 0.67 on a thin-layer chromatogram with a solvent system of benzene:ethyl acetate:acetone (10:4:0.1). Its R_f value was identical to that of an authentic sample (melting point 89-89.5°C), which was prepared by a known method(1). The structure of 2-(morpholinothio)benzothiazole was supported by ^{13}C-NMR signals at 174.4, 155.0, 135.2, 126.0, 124.0, 122.0, 121.0, 67.9 and 56.6 ppm, and ^{1}H-NMR signals at 3.28(2H,t,J=4.64 Hz), 3.81(2H,t,J=4.64 Hz), 7.28(1H,td,J=7.81, 1.22 Hz), 7.41(1H,td,J=7.77, 1.46 Hz), 7.81(1H,dd,J=7.94, 0.73 Hz) and 7.85 ppm(1H, dd,J=8.06, 0.73Hz). Radiochemical yield was 83.4 %.

Synthesis of 2-(Mercapto-^{35}S)benzothiazole:

Carbon disulfide-$^{35}S_2$ (5.5 mg, 912 μCi) was diluted with carbon disulfide (0.08 ml) in N,N-dimethylformamide (DMF)(5 ml) at -50°C. 2-Mercaptoaniline (177 mg) in DMF (5 ml) was added to the CS_2 solution, which also contained a trace of Na_2SO_3. The reaction mixture was allowed to stand overnight and then heated on a steam bath for 2 hr. The reaction solution was acidified with 10% HCl (40 ml) and allowed to stand in a refrigerator to precipitate the desired compound. Crude 2-(mercapto-^{35}S)benzothiazole was purified by dissolution with 1N NaOH and precipitation with 10% HCl. The purity of the precipitated crystals was determined on a thin-layer plate (Merck Kiesel gel 60F-254) in solvent systems of n-hexane:$CHCl_3$ (1:2) (R_f=0.37), $CHCl_3$ (R_f=0.53) and benzene:dioxane:acetic acid (30:5:1) (R_f=0.84). The yield was 150.0 mg and its specific activity showed 2.438 μCi/mg. Radiochemical yield was 40.1%.

Synthesis of 2-(Morpholinothio-^{35}S)benzothiazole:

A mixture of 2-(mercapto-^{35}S)benzothiazole (120.9 mg) and 2-mercaptobenzothiazole (80.9 mg) was suspended in 2-propanol (1 ml). A solution of morpholine (129 mg) in 2-propanol (0.6 ml) was added to the above suspension and the resulting mixture was stirred for 1 hr at 45-50°C. Aqueous 10% NaClO solution (1.5 ml) was added to the reaction solution over a period of 2 hr. Stirring was continued for 1 hr at 45-50°C. To the reaction solution was added a solution of Na_2SO_3 (25 mg) in water (10 ml). The reaction solution was allowed to stand overnight in an ice-box. The precipitate was separated into the methanol-soluble and methanol-insoluble fractions as described above. The methanol-soluble fraction gave 2-(morpholinothio-^{35}S)benzothiazole (207.9 mg, 1.096 μCi/mg). Chemical yield and radiochemical yield were 68.3% and 77.3%, respectively. The methanol-insoluble fraction gave 2,2'-dithio-^{35}S-bis(benzothiazole) (17.8 mg, 1.183 μCi/mg).

Formation of 2,2'-Dithiobis(benzothiazole-2-^{14}C) from 2-(Morpholinothio) benzothiazole-2-^{14}C:

2-(Morpholinothio)benzothiazole-2-^{14}C (obtained by mixing 40.2 mg of 0.11 μCi/mg material and 78.5 mg of 6.71 μCi/mg material) was dissolved in dimethyl sulfoxide (DMSO)(10 ml). Sulfur trioxide-pyridine complex (50.5 mg) was added to the DMSO solution. The mixture was heated on a steam bath for 3 hr and a crystalline compound appeared while heating. After cooling, the reaction solution was diluted with water (50 ml) to give a precipitate which was identified as 2,2'-dithiobis(benzothiazole-2-^{14}C) 75.5 mg, 0.699 μCi/mg.

Synthesis of 2-(Morpholino-U-^{14}C-thio)benzothiazole:

Morpholine-U-^{14}C hydrochloride (1.3 mg, 250 μCi) was diluted with morpholine hydrochloride (272 mg) in water (2.3 ml) and extracted thoroughly with ether after alkalization with 5N NaOH. Total ether-extract solution (15 ml) was evaporated to give 231 mg (67 %) of the residual oil which then was mixed with

81 mg of morpholine as carrier to obtain finally 312 mg of morpholine-U-^{14}C.

A mixture of 2-mercaptobenzothiazole (426 mg) and morpholine-U-^{14}C (260 mg) in 2-propanol (3 ml) was oxidized with 10% NaClO solution as described above. After the oxidation 0.25% aqueous Na_2SO_3 solution (20 ml) was added to the reaction mixture, which was allowed to stand in an ice box overnight. The precipitate was collected on a glass filter and washed well with cold water to give crude crystals (469 mg). The crystals were treated with ether to remove an ether-insoluble fraction (33 mg). The radiochemical purity of the 2-(morpholino-U-^{14}C-thio)benzothiazole obtained from the ether-soluble fraction (432 mg) was found to be higher than 99% by TLC. The radiochemical yield was 77.4%.

Synthesis of 2-Mercaptobenzothiazole-2-^{14}C:

2-Mercaptobenzothiazole-2-^{14}C was prepared according to the modified method of Hofmann (2, 3). To a DMF solution (5 ml) containing carbon-^{14}C disulfide (0.8 ml, 1 mCi) in a breakseal tube was added a solution containing 2-mercaptoaniline (0.15 ml) and sodium sulfide (10 mg) in DMF (5 ml). The mixture was allowed to stand for 30 min and then heated on a steam bath for about 30 min. On cooling, 5%HCl (50 ml) was added to the reaction mixture and the precipitate was collected. The precipitate was dissolved in 4 ml of 5% NaOH containing 16 mg of 2-mercaptobenzothiazole as carrier and precipitated with 5%HCl. The precipitate was filtered, washed with 5%NaOH (15 ml), and then water. The dried crystals (89 mg) showed only one spot on the thin-layer chromatogram: R_f=0.73, 0.68 and 0.27, for ethanol, chloroform:THF (17:3) and chloroform solvent systems, respectively. The radiochemical yield of 2-mercaptobenzothiazole-2-^{14}C was 61.3%.

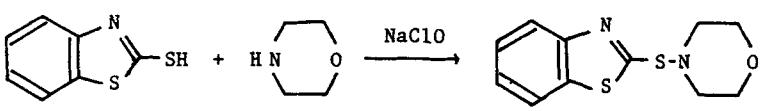
Synthesis of 2-(Cyclohexylaminothio)benzothiazole:

2-(Cyclohexylaminothio)benzothiazole was prepared by the method of Carr, et al.,(3). The product was recrystallized from dichloromethane and melted at 105-106.5°C (lit. (4) mp 102°C). Calcd. C 59.08, H 6.10, N 10.60. Found C 58.95, H 6.12, N 10.72. ^{13}C-NMR: 174.4, 155.1, 135.1, 125.8, 123.5, 121.5, 121.0 60.3,

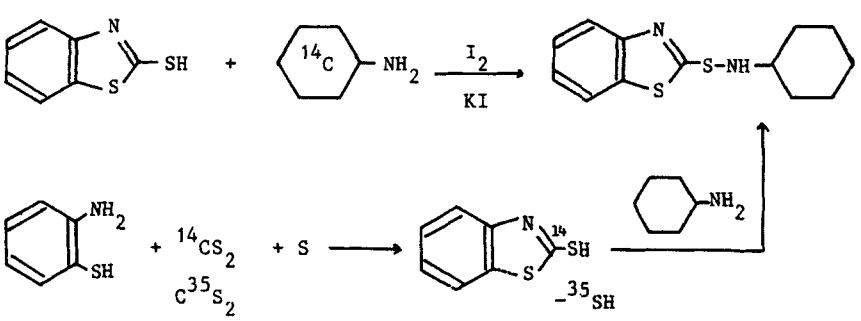
33.8, 25.7 and 24.9 ppm, and ^{1}H-NMR: 1.22(5H), 1.64(1H), 1.76(1H), 2.09(1H), 2.90(1H),3.29(1H, d,J=6.29 Hz), 7.25(1H, d,J=6.34 Hz), 7.35(1H,d,J=8.06 Hz), 7.76(1H,d,J=6.83 Hz) and 7.80 ppm(1H,d,J=7.33 Hz).

Synthesis of 2-(Cyclohexyl-U-^{14}C-aminothio)benzothiazole:

A mixture of 2-mercaptobenzothiazole (177 mg) and cyclohexylamine-U-^{14}C) (160 mg, 150 μCi) was dissolved in 3.85% NaOH solution (4 ml) and oxidized with potassium iodide solution (280 mg KI and 267 mg I_2 in 3.3 ml of water). After reaction, the precipitate was dissolved in dichloromethane and subjected to column chromatography (silica gel, 10g, 15 x 1.4 cm), and eluted with dichloromethane (each 20 ml/fraction). The eluates (fractions 1 and 2) were combined and removal of the solvent gave a crystalline compound. The crystals were purified further by silica gel column chromatography under the same condition as mentioned above. The contaminating colored materials were completely removed. The yield of pure 2-(cyclohexyl-U-^{14}C-aminothio)benzothiazole was 131 mg with total radioactivity of 63.8 μCi (radiochemical yield, 42.5%).



Synthesis of 2-(Morpholinothio)benzothiazole



Syntheses of 2-Mercaptobenzothiazole-2-^{14}C, 2-(Mercapto-^{35}S)benzothiazole, and 2-(Cyclohexyl-U-^{14}C-aminothio)benzothiazole

RESULTS AND DISCUSSION

Although 2-benzothiazylsulfenamides belong to a class of the most popular vulcanizing accelerators, few papers have been reported concerning the syntheses of these labeled compounds. Thirty years ago, Russian chemists reported the synthesis of 2-(mercapto-^{35}S)benzothiazole-1-^{35}S in good yield from phenylisothiocyanate, sulfur-35 and a small amount of water at 250-260°C in a sealed tube(5). We used carbon disulfide-$^{35}S_2$ as a starting material because labeling with sulfur-35 was necessary only for the sulfhydryl group attached at the position 2 of the 2-mercaptobenzothiazole molecule. 2-Mercaptobenzothiazole labeled with sulfur-35 or carbon-14 was synthesized in moderate yields from carbon disulfide-$^{35}S_2$ and carbon-^{14}C disulfide, respectively. A sealed vial containing labeled starting material was used directly as a reaction vessel to prevent loss by evaporation of carbon disulfide.

When the syntheses of labeled 2-mercaptobenzothiazole, 2-(morpholinothio)benzothiazole, and 2-(cyclohexylaminothio)benzothiazole were carried out, a small amount of 2,2'-dithiobis(benzothiazole) was always detectable as a by-product on the thin-layer chromatogram. Removal of the disulfide was achieved by treatment of the crude product with methanol. Carr, et al.(4) reported that 2-(cyclohexylaminothio)benzothiazole was entirely ether-soluble and that 2,2'-dithiobis(benzothiazole) was ether-insoluble. In our studies, however, methanol was a prefered solvent for removal of 2,2'-dithiobis(benzothiazole) as pointed out by Luecken and Sullivan(6). This method is hereon being used for determination of the purity of 2-(morpholinothio)benzothiazole and 2-(cyclohexylaminothio)benzothiazole.

There are some important factors for the preparation of sulfenamides. One of the most commonly used methods for their preparation is dehydrohalogenation between sulphenyl chlorides and N-H compounds, amines. An addition-elimination mechanism for the reaction has been known(7). However, in the case of benzothiazylsulfenamides, benzothiazole-2-sulphenyl chloride also reacts rapidly

with nucleophiles. Since 2-mercaptobenzothiazole is much greater than morpholine (or cyclohexylamine) in nucleophilicity, 2,2'-dithiobis(benzothiazole) was predominantly formed on chlorination of 2-mercaptobenzothiazole to the sulphenyl chloride. Accordingly the synthesis of 2-(morpholinothio)benzothiazole by condensation of benzothiazole-2-sulfenyl chloride with the amine(8) was not tried. On the other hand, condensation of chloromorpholine and 2-mercaptobenzothiazole has been carried out in liquid ammonia in the presence of potassium hydroxide(9), but the yield of the desired sulfenamide was low because of the use of unstable chloromorpholine and formation of a yellowish product which needed further decolorization. In the case of the sulfenamide from carbon-14 labeled morpholine, a heavy loss of the labeled compound was anticipated.

Therefore, we chose a mild method(1) with 2-step reaction; salt-formation from 2-mercaptobenzothiazole and morpholine followed by oxidative condensation with NaClO. Two important factors in the reaction were solubility of the salt and retardation of disulfide production. Dioxane, tetrahydrofuran, tetrahydropyran and 2-propanol were considered to be suitable solvents to dissolve both the salt-like 2-mercaptobenzothiazole-amine complex and the aqueous NaClO solution. 2-Propanol was selected owing to a high solubility, a low toxicity and proper boiling point.

After the formation of 2-mercaptobenzothiazole-morpholine complex was complete, slow addition of NaClO solution was essential to minimize the formation of the disulfide in oxidative condensation reaction, or else only the formation of the disulfide would prevail. Reaction temperature of 45-55°C was also an important factor to proceed the reaction.

Oxidation reagents such as NaClO, I_2, Cl_2 (air), KI-I_2 and H_2O_2 are often used for oxidative condensation of 2-mercaptobenzothiazole and amines. In our work NaClO and KI-I_2 were used for the syntheses of labeled 2-(morpholinothio)benzothiazole and 2-(cyclohexylaminothio)benzothiazole, respectively.

Since a 97.2% yield of 2-(cyclohexylaminothio)benzothiazole was obtained by starting with 3 moles of non-radioactive cyclohexylamine, an excess of cyclohexylamine was used in the non-labeled 2-(cyclohexylaminothio)benzothiazole

synthesis. However, an excess of the amine was not used for the synthesis of 2-(cyclohexyl-U-^{14}C-aminothio)benzothiazole.

We tried to synthesize a urinary metabolite of 2-(morpholinothio)benzothiazole, the sulfate ester of 2-mercaptobenzothiazole, from 2-mercaptobenzothiazole and sulfur trioxide-pyridine complex, a mild sulfonating agent. But no desired compound was obtained, and only 2,2'-dithiobis(benzothiazole) was isolated in this reaction.

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