The synthesis of SQ-14225-^{14}C [N-[(S)-2-Methyl-3-mercaptopropanoyl-1-^{14}C] -L-proline]

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SUMMARY

The preparation of SQ-14225-^{14}C, anti-hypertensive drug, is described. The starting material, methacrylic acid-1-^{14}C, was prepared in a high yield by carbonation of the corresponding Grignard reagent with $^{14}CO_2$. The final product was obtained from the acid through a four step synthesis with specific activity of 3.20 mCi/mmol. The overall radiochemical yield was 8.0% based on $Ba^{14}CO_3$.

Key Words ; SQ-14225-^{14}C, Carbonation, Grignard reagent, Methacrylic acid-1-^{14}C

INTRODUCTION

SQ-14225, [N-[(S)-2-Methyl-3-mercaptopropanoyl]-L-proline] is a new anti-hypertensive drug which has been synthesized in Squibb & Sons Inc., USA (1). For use in metabolic studies, P. Egli of Squibb has synthesized SQ-14225-proline-^{14}C (2). However we attempted to label the drug with ^{14}C other than proline ring because L-proline-^{14}C is extremely expensive as the starting material.

RESULTS AND DISCUSSION

In order to obtain the SQ-14225 labelled at the side chain, methacrylic acid-1-^{14}C was chosen as the starting material. Urban (3) described

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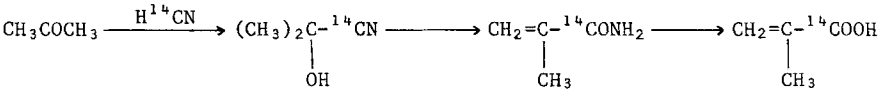
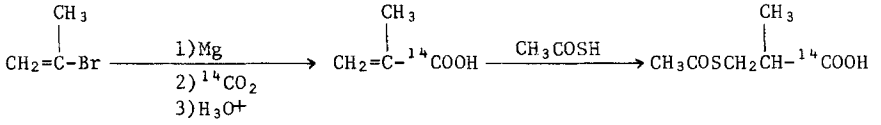
methacrylic acid-1-^{14}C synthesis from H^{14}CN according to the following scheme.

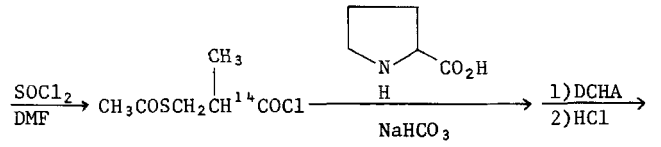


We have found, however, that his procedure was unsatisfactory since the yield was quite low due to polymerization by ^{14}C-radiation. Thus we have attempted the synthesis of methacrylic acid-1-^{14}C from Grignard reagent and $^{14}CO_2$ under a variety of reaction conditions, and found that the acid-1-^{14}C could be obtained in a 70% yield with a specific activity of 3 mCi/mmol.

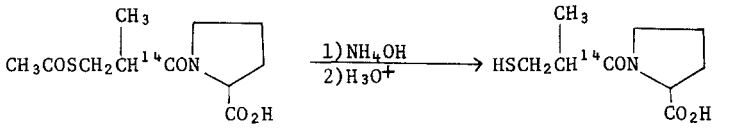
SQ-14225-^{14}C was synthesized from the acid by the method of P. Egli (2). To methacrylic acid-1-^{14}C was added an excess of thioacetic acid, and the solution was heated with stirring at 140°C for 2 h and distilled under reduced pressure to obtain 2-methyl-3-thioacetylpropionic acid-1-^{14}C (3) in quantitative yield. The acid (3) was converted to its acyl chloride (4) which was then reacted with L-proline in water to afford a mixture consisting of two diastereomeric isomers. In order to isolate the desired (S,S)-isomer, dicyclohexylamine was added to the mixture, and the white precipitate formed was recrystallized from isopropyl alcohol. Pure (S,S)-isomer of (5) obtained (17.4% from 3) from the salt by treatment with hydrochloric acid was converted to (6) with aqueous ammonia under an atmosphere of nitrogen in a 74.8% yield.



(1) (2) (3)



(4)



(5) (6)

For the measurement of the optical purity of (5), we have found gas-liquid chromatography to be most convenient. Thus, (5) was first converted to its methyl ester by treatment with CH_2N_2 and then was subjected to GLC using 1.5% OV-17 as a column at 180 °C. The gas chromatogram (Fig.1) showed two peaks of almost the same peak area. The retention time of the second peak was identical with that of authentic (S,S) (5) methyl ester. The mass spectra of each peak were virtually identical and consistent with that of authentic (S,S) (5) methyl ester. Thus, we have concluded that the first peak in Fig.1 corresponded to (R,S)-isomer and the second one desirable (S,S)-isomer. After being purified with dicyclohexylamine, it showed a single peak in the gas chromatogram. As compared with more conventional measurement of the optical rotations, the GLC method is highly advantageous in its high sensitivity.

In the course of hydrolysis, (5) to (6), the product was always contaminated by a few percent of the corresponding disulfide. We have found a simple procedure for the removal of the disulfide in which a column chromatography was utilized. Thus, with a column of silica gel and a mixed solvent of

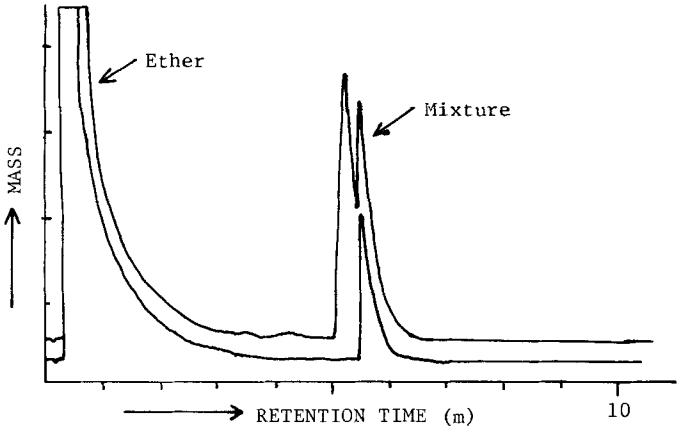


Fig. 1. Gas chromatograms of the mixture of methylated (5) and the authentic sample (5).

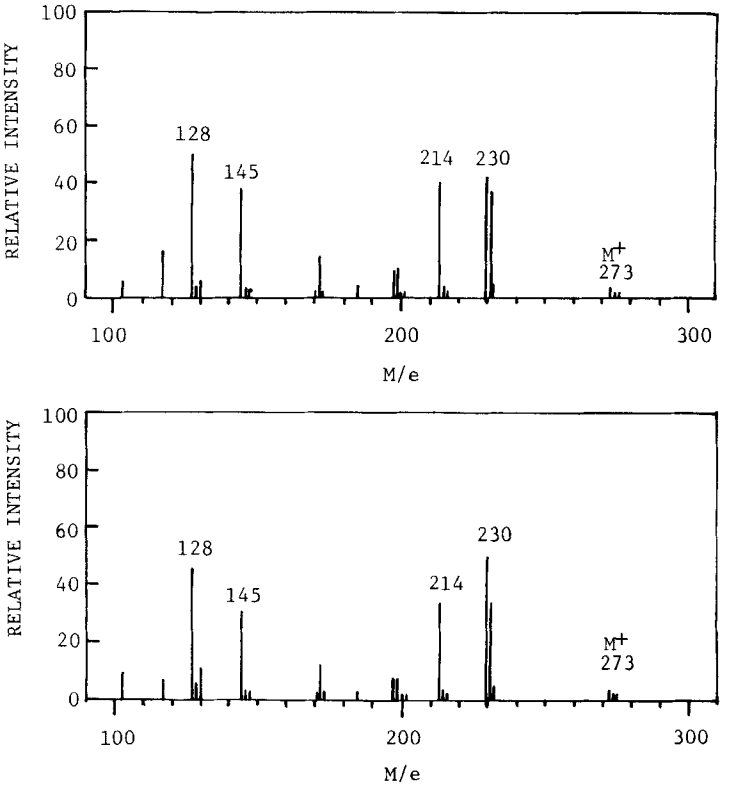


Fig. 2. Mass spectra of the second peak (top) and the authentic sample (5) (bottom).

chloroform : methanol : glac. acetic acid (90:5:1) containing 0.3% of 2-mercaptoethanol as an eluent, the disulfide was removed completely and the pure product (6) was recovered quantitatively.

EXPERIMENTAL

Melting points were uncorrected. Radioactivity was measured with an Aloka Model LSC 601 liquid scintillation spectrometer. Thin layer chromatography (TLC) was conducted with precoated silica gel glass plates (E. Merck 5714/0100) which were developed in the following solvent systems.

1) Chloroform : Methanol : Acetic acid (45:5:1 v/v)

2) Benzene : Acetic acid (3:1 v/v)

Spots were detected by UV light at 254 nm and by exposure to I_2 vapor. Radiochemical purity was determined on thin-layer chromatograms with an Aloka Model TRM-IB radiochromatogram scanner system. Authentic sample of SQ-14225 was supplied by Sankyo Co., Ltd., Tokyo. Nuclear magnetic resonance (NMR) spectra were taken on a Hitachi R-24B instrument in deuteriochloroform with tetramethylsilane as an internal standard. GC-MS were taken on a Hitachi RMU-6MG model using a 1 m, 3 mm i.d. glass column packed with 1.5% OV-17.

Methacrylic acid-1-^{14}C (2)

Grignard reagent was prepared from 1.10 g (45 mmol) of magnesium turnings and 4.84 g (40 mmol) of 2-bromopropene in 150 ml of anhydrous tetrahydrofuran. Carbon dioxide-^{14}C liberated from 3.77 g (382 mCi, 19.1 mmol) of barium carbonate-^{14}C and 60% perchloric acid solution (20 ml) was introduced into 26.7 mmol of the Grignard reagent, and stirred at -30 ∽ -40 °C for 1 h. Unreacted carbon dioxide-^{14}C was purged by a nitrogen stream and was absorbed into 1M NaOH solution. To this mixture, 10 ml of 3M H_2SO_4 and 30 ml of ether

containing 6.57 g (76.3 mmol) of cold methacrylic acid were added dropwise with cooling by an ice bath and after vigorous stirring for 1 h, the reaction mixture was extracted with ether (30 ml × 3). The combined ether extract was washed with water, then dried (Na_2SO_4) and filtered. After removal of the ether, the residual oil was purified by distilling under reduced pressure to give (2), 258 mCi, 5.63 g (80.3 mmol) as a colorless oil.

2-Methyl-3-thioacetylpropionic acid-1-^{14}C (3)

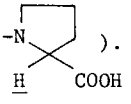
The acid (2), 258 mCi, 5.63 g (80.3 mmol) was added dropwise to 22.9 g of thioacetic acid with stirring under an atmosphere of nitrogen at 140 °C. The mixture was stirred for 2 h at the same temperature and excess thioacetic acid was evaporated in vacuo at 40 °C to leave an oily residue. The residual oil was distilled to give (3), 252 mCi, 12.70 g (78.4 mmol) : 125 ∽ 127 °C/1 mmHg. The purified (3) was estimated as having a radiochemical purity of greater than 99% by TLC and GLC. NMR ($CDCl_3$) δ 1.25 (d, 3H, J=7Hz, $-\underset{\underset{CH_3}{|}}{CH}-$), 2.30 (s, 3H, CH_3COS-), 2.75 (m, 1H, $-\underset{\underset{CH_3}{|}}{CH}-$), 3.10 (d, 2H, $-CH_2-\underset{\underset{CH_3}{|}}{CH}-$), 11.20 (s, 1H, $-\underset{\underset{CH_3}{|}}{CH}-COOH$).

2-Methyl-3-thioacetylpropanoyl chloride-1-^{14}C (4)

To 9 ml of thionyl chloride containing 0.4 ml of dimethylformamide was added gradually (3), 252 mCi, 12.70 g (78.4 mmol) with stirring under an atmosphere of nitrogen at 40 °C. The mixture was stirred for 2 h at the same temperature and excess thionyl chloride was evaporated in vacuo at 30 °C, to leave an oily residue. The residual oil was distilled to give (4), 13.20 g (73.0 mmol) : bp 85 °C/9 mmHg.

N-[(S)-2-Methyl-3-thioacetylpropanoyl-1-^{14}C]-L-proline (5)

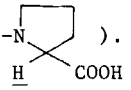
L-Proline, 11.50 g (94.8 mmol) was dissolved in 45 ml of distilled water

containing 12.30 g (146 mmol) of sodium bicarbonate and 13.20 g (73.0 mmol) of (4) was added dropwise with vigorous stirring at 0 °C. After the addition was complete, the ice bath was removed and the mixture was stirred for 2 h at room temperature with occasional addition of sodium bicarbonate to keep the pH 6.7. The pH of the mixture was adjusted to 2.4 by addition of concentrated hydrochloric acid and the mixture was extracted with methylene chloride. The combined methylene chloride extract was washed twice with water, then dried (Na_2SO_4) and filtered. After removal of methylene chloride, the residual oil was dissolved in 20 ml of ether. To this solution was added gradually 27 ml of dicyclohexylamine with stirring at 0 °C and the precipitate formed was collected by filtration, washed with ether and recrystallized from isopropyl alcohol. To the mother liquor was added 0.31 g of cold (5) and a second crop of crystals was recovered. The crystals were combined and dissolved in 20 ml of water. To the solution was added 3 ml of concentrated hydrochloric acid and extracted with methylene chloride. The combined methylene chloride extract was washed with water, dried (Na_2SO_4) and evaporated to give (5), 44.1 mCi, 3.99g (15.4 mmol), mp 80 ∽ 81.5 °C. The radiochemical purity was shown to be 99% by radio-thin layer chromatography on silica gel with chloroform : methanol : acetic acid (45:5:1 v/v) as a developing solvent and its optical purity was also shown to be 99% by GLC. NMR ($CDCl_3$) δ 1.30 (d, 3H, J=7Hz, $-\underset{\underset{CH_3}{|}}{CH}-$), 2.35 (s, 3H, CH_3COS-), 2.75 (m, 1H, $-\underset{\underset{CH_3}{|}}{CH}-$), 3.10 (d, 2H, $-CH_2-\underset{\underset{CH_3}{|}}{CH}-$), 4.60 (t, 1H, J=5Hz, ).

N-[(S)-2-Methyl-3-mercaptopropanoyl-1-^{14}C]-L-proline (6)

To (5), 44.1 mCi, 3.99 g (15.4 mmol) dissolved in 7.7 ml of oxygen-free distilled water was added dropwise 6 ml of 28% aqueous ammonia with stirring under an atmosphere of nitrogen at 0 °C. After addition was complete, the ice

bath was removed and then stirred for 1 h at room temperature. The pH of the mixture was adjusted to 7.5 by addition of concentrated hydrochloric acid and the mixture was washed twice with methylene chloride. The aqueous layer was acidified with concentrated hydrochloric acid and extracted with methylene chloride. The combined methylene chloride extract was washed with water, dried (Na_2SO_4) and the solvent evaporated. The residual oil was purified with a column of 60 g of silica gel using chloroform : methanol : acetic acid : 2-mercaptoethanol (90:5:1:0.3 v/v) as an eluent. The resulting colorless oil was recrystallized from ethyl acetate — n-hexane to give (6), 33 mCi, 2.25 g (10.4 mmol) as prisms, mp 108 ∽ 110 °C : specific activity, 3.20 mCi/mmol. The purified (6) was estimated as having a radiochemical purity of greater than 99% by thin-layer chromatography (TLC) and reversed dilution analysis.

NMR ($CDCl_3$) δ 1.20 (d, 3H, J=7Hz, $-\underset{\underset{CH_3}{|}}{CH}-$), 2.70 (m, 1H, $-\underset{\underset{CH_3}{|}}{CH}-$), 3.10 (d, 2H, $-CH_2-\underset{\underset{CH_3}{|}}{CH}-$), 4.60 (t, 1H, J=5Hz, ).

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