SYNTHESIS OF L-METHIONINE-$^{13}CH_3S$

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L-Methionine-$^{13}CH_3S$ was obtained via reaction of $^{13}CH_3I$ with the sodium salt of L-homocysteine prepared from sodium-liquid ammonia reduction of L-homocystine.

MATERIALS AND METHODS

DL-Homocystine, obtained from Sigma Chemical Company, St. Louis, Mo., was dried by azeotropic distillation with benzene followed by evaporation of residual solvent at 0.1 mm for 12 hr. A melting point of 275-280 C (e.s.t.-evacuated sealed tube), was observed; a value of 260-265 C has been reported (1, p. 1626). L-Homocystine, obtained from the same source, had a melting point of 284-286 C and an optical rotation of $[\alpha]^{26}_{D} = +71.7°$ in 1 N HCl. Values reported (1, p. 2100) were melting point 281-284 and optical rotation $[\alpha]^{26}_{D} = +77°$ in 1 N HCl. Methyl iodide-^{13}C, purchased from Stohler Isotope Chemicals, Inc., Waltham, Mass., was used without further purification.

L-Methionine-$^{13}CH_3S$ preparation

To vigorously stirred, liquid ammonia (125 ml) was added sodium (1.8 g, 0.078 g atom) and L-homocystine (3.4 g, 0.0126 mole) alternately in small portions. The progress of the reaction could easily be followed since sodium and liquid ammonia produce a blue color which is discharged upon addition of homocystine. After adding the specified quantities of sodium and homocystine, the reaction mixture had a pale blue color which indicated a slight excess of sodium and, hence, the completion of the reaction. Methyl iodide-^{13}C (4.0 g, 0.028 mole; slightly brown colored) was added in one portion with caution to avoid spattering. Stirring was continued until the ammonia had evaporated. The flask was connected to a high vacuum through another flask containing concentrated H_2SO_4; the system was left overnight.

The residue was dissolved in 19.5 ml of water, and the solution was treated with Norit (100 mg) at room temperature for 20 min and then filtered. The filtrate was acidified with HCl to pH 5.5 and the solution was chilled with cold water. Crystals of L-methionine were filtered off, washed (5 ml of ice water, 4 ml of absolute C_2H_5OH), and dried. Purification via four fractional crystallizations from water (with Norit) gave 1.0 g of our product. It had a melting point of 277-279 C which compared favorably with melting points reported previously (1, p. 2100; 2). An additional 0.35 g of material was obtained from the mother liquors by concentration and purification for a total yield of 36.3%. Mass spectral analysis on the LKB-9000 unit established that the ^{13}C content of the methionine-$^{13}CH_3$-S was 83% of the theoretical value.

In the model experiment with DL-homocystine (3.35 g, 0.0125 mole), ammonia (150 ml) and sodium (1.15 g, 0.05 g atom), DL-methionine was obtained, 1.8 g (48%), m.p. 268-270 C. An authentic sample of DL-methionine melted at 271-273 C.

RESULTS AND DISCUSSION

L-Methionine is an important amino acid utilized in the *in vivo* synthesis of many proteins. Since ^{13}C could be detected readily by NMR spectroscopy (3), even in macromolecules (4), we sought simple and efficient ways to synthesize L-methionine-$^{13}CH_3S$. A search of the literature did not reveal such a preparation although a number of procedures are available for nonlabeled methionine. A lone report (2) on the synthesis of S-ethyl-L-cysteine from homocystine prompted us to attempt the preparation of L-methionine-$^{13}CH_3S$ with considerable modifications. To develop the techniques required for high efficiency, because of the cost of ^{13}C starting materials, model experiments were conducted with DL-homocystine.

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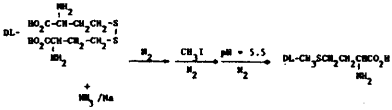
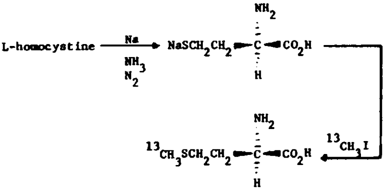


The presence of an N_2 atmosphere and careful adjustment of pH to 5.5 in the workup was crucial to obtain a product of highest purity. Since sodium iodide was produced, high acidity may yield HI which could be oxidized to iodine. Brown-colored DL product from the model experiment appeared to contain iodine.

Treatment of L-homocystine with sodium in liquid ammonia followed by addition of a slight excess above 2 equivalents of $^{13}CH_3I$ (containing 87 atom % of ^{13}C according to the supplier) gave L-methionine (4, 5) in 36.3% yield. Melting point, infrared analysis, NMR analysis, and a mass spectral (83 atom % of ^{13}C) determination confirmed its identity (6, 7). In summary, an analytical sample of L-methionine-$^{13}CH_3S$ can be obtained within one day by the route described and is stable in storage indefinitely.



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