A Convenient and Economical Preparation of 
L-Methionine-methyl-d₃

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The use of isotopically labeled methionine in determining the course of biosynthetic alkylation is well established (1-5). The incorporation of a deuterium, rather than a tritium, label enables direct qualitative and quantitative determination of the label by NMR and mass spectroscopic techniques, but such methods require a higher incorporation of deuterium than is required in the classical methods using tritium. Moreover, while the extent of incorporation of a labeled substrate is dependent upon a number of factors, it is apparent that, within certain limits, the higher the concentration of labeled substrate the higher is the extent of incorporation. These considerations have necessitated the use of large quantities of L-methionine-methyl-d₃ in these laboratories and the present communication reports a convenient and inexpensive synthesis of this material.

METHODS

It has been shown that trimethylsulfoxonium iodide is readily prepared from dimethylsulfoxide and methyl iodide, and that this salt, upon pyrolysis, is converted back to the starting materials (6). It is also known that trimethylsulfoxonium nitrate exchanges its hydrogen atoms for deuterium under neutral conditions in heavy water (7). These observations suggested a route to deuteriomethyl iodide and a synthesis based upon these reactions has been reported (8).

There are however, certain disadvantages to the reported procedure (8): thus the dry pyrolysis at 200° in a vacuum system necessitates the use of a highly efficient trapping system to collect the volatile methyl iodide; in addition, the pyrolysis when carried out on a large scale (>40 gm) gives irreproducible results and impure products.

Contrary to this, we have found that trimethylsulfoxonium iodide,
when moistened with water, can be decomposed back to starting materials at low temperatures and in high yields. Accordingly, the salt when mixed with half its weight of water and heated at 110–120° smoothly decomposes to give a 94% yield of methyl iodide.

It is sometimes, however, more convenient to have a partly labeled compound (particularly when working with tritium) and for this purpose the recrystallization of the salt from isotopic water is not practical, simply because the procedure requires such a large excess of the isotope. In these cases, the isotope exchange was accomplished by dissolving the salt in dry nonlabeled dimethylsulfoxide mixed with a limited amount of the isotopic water and a catalytic amount of base. Recovery of the labeled salt followed by dry pyrolysis gave the appropriately labeled methyl iodide.

Methyl-labeled methionine is usually prepared in a two-step synthesis which involves the preparation and isolation of either homocystine (9) or S-benzylhomocysteine (10) from methionine, followed by their reduction and realkylation with a suitably labeled reagent. Since, however, these reactions, which are normally carried out in liquid ammonia, are both irreversible and quantitative, it was expected that realkylation of the homocysteine anion by labeled methyl iodide, immediately after a reductive cleavage of methionine, should give labeled methionine in high yield.

Thus when L-methionine was reacted with a 3 mole equivalent of sodium in liquid ammonia, followed by the addition of 1 mole of ammonium chloride (to neutralize sodium amide) and 1 mole of labeled methyl iodide, an over-all yield of 87% of optically pure (11) labeled L-methionine was obtained.

**EXPERIMENTAL**

*Trimethylsulfoxonium Iodide (6)*

A mixture of freshly distilled dimethylsulfoxide (128 gm) and methyl iodide (240 ml) was gently refluxed for 7 days, in the absence of light, in an oil bath at 70–80°C. The excess methyl iodide was then removed by distillation (115 ml of distillate) and the residue diluted with 100 ml of acetone. The mixture was cooled in dry ice-acetone and the crystalline product collected by filtration, washed with acetone, and air dried to give 189 gm (52.4%).

The combined filtrate and acetone washings were fractionally distilled to give unreacted dimethylsulfoxide (60 gm). The corrected yield of the salt was then 98.5% for dimethylsulfoxide and 42% for methyl iodide.
**Nonadeuterio Trimethylsulfoxonium Iodide**

Trimethylsulfoxonium iodide (240 gm) was recrystallized from 800 ml of deuterium oxide containing 100 mg of anhydrous sodium bicarbonate. A second identical recrystallization gave 220 gm (90% recovery) of the perdeuterated salt as colorless prisms.

The addition of a small amount of the base catalyzed the exchange as well as preventing decomposition of the salt. Such decomposition is presumably catalyzed by the formation of hydroiodic acid:

\[(\text{CH}_3)_3\text{SOI} + \text{H}_2\text{O} \rightleftharpoons (\text{CH}_3)_3\text{SOOH} + \text{HI}\]
\[(\text{CH}_3)_3\text{SOI} + 2\text{HI} \rightarrow (\text{CH}_3)_2\text{SI} + \text{I}_2 + \text{H}_2\text{O}\]

Thermal decomposition of the salt on the direct inlet probe of a mass spectrometer gives methyl iodide and dimethyl-sulfoxide, thereby enabling the D:H ratio to be measured.

**Methyl-\(d_2\) Iodide**

Nonadeuterio trimethylsulfoxonium iodide (104 gm) and deuterium oxide (50 ml) were placed in a 250 ml distillation flask mounted with a column (2 × 25 cm) packed with glass helices. The mixture was heated in an oil bath at 120° and the distillate collected, in a receiver protected from moisture and surrounded by a dry ice-acetone bath, as a pale yellow oil 61.8 gm (94%). This material was redistilled from molecular sieves (4A) and the fraction boiling at 42–43° was collected and stored over a piece of bright copper wire in the absence of light. Mass spectrum (70 eV) \(m/e\) (relative intensity), 146 (1.5), 145 (100), 144 (5.5), 143 (13.0), 142 (1.0), 141 (3.0), 139 (4.0), H/D less than 0.02. Cf. \(\text{CH}_3\text{I}\) 143 (1.4), 142 (100), 141 (16.0), 140 (4.0), 139 (5.0).

The nonvolatile residue of the distillation (which was dark reddish brown due to \(\text{I}_2\)) was decolorized by addition of anhydrous sodium thiosulfate, followed by distillation under reduced pressure. This gave 37.6 gm (75% recovery) of \(\text{D}_2\text{O}\), 15 gm of an intermediate fraction, and 31.4 gm (82%) of dimethylsulfoxide-\(d_6\), which was dried over \(\text{CaH}_2\) and redistilled. Mass spectrum (70 eV) \(m/e\) (relative intensity), 85 (3), 84 (59), 83 (7), 82 (1), 66 (100), H/D less than 0.02. Cf. \((\text{CH}_3)_2\text{SO}\), 79 (3.1), 78 (85), 77 (1.6), 76 (0.6), 63 (100).

**Partial Exchange Reaction**

A solution of trimethylsulfoxonium iodide (2 gm) in dry dimethyl-sulfoxide (15 ml) was mixed with 0.5 ml of \(\text{D}_2\text{O}\) and 10 mg of sodium bicarbonate and stirred for 2 hours at room temperature. The mixture
was diluted with acetone (30 ml) and the salt was recovered by cooling, followed by filtration. By mass spectrometry the deuterium content was shown to be 36%, corresponding to 71% of the theoretical.

A similar treatment with tritium oxide yielded a radioactive salt (65% yield). The specific activity corresponding to 46% of the theoretical.

L-Methionine-methyl-d₃

L-Methionine (72.5 gm, 0.49 mole) was dissolved in 2.5 liters of dry liquid ammonia, and into this stirred solution was added small pieces of metallic sodium (34.5 gm, 1.50 moles). The stirring was continued for a further hour (the solution was still blue) and then solid ammonium chloride (27.0 gm, 0.5 mole) followed by methyl-d₃ iodide (33 ml, 0.52 mole) was added. The solution was left to stand overnight at room temperature to let the solvent evaporate. The residue was dissolved in 500 ml of water, the solution filtered, and the filtrate acidified with HCl to litmus (but still basic to Congo red) to give a crystalline precipitate. This precipitate was redissolved by gently warming the solution, 1.5 liters of hot 95% ethanol was then added, and the solution was slowly chilled to −5°C. The crystalline precipitate was collected by filtration and washed with cold ethanol and then ether. Air drying gave 51.1 gm (69%) of silky shining plates. The filtrate was reduced in volume to 200 ml, yielding a heavy precipitate that was collected by filtration. This residue was extracted with hot 20% aqueous ethanol. The ethanolic solution was taken to dryness and the resultant solid extracted with hot methanol. The methanolic solution was cooled in a dry ice-acetone bath, and the precipitate collected by filtration and reccrystallized from 25% aqueous methanol to afford a second crop (13.3 gm).

The combined product (64.4 gm) represents an over-all yield of 87% of L-methionine-methyl-d₃, m.p. 275°C (dec.); [α]D²⁰ = −8.1° in water and 21.5 in 1N HCl, for an authentic sample [α]D²⁰ = −8.8° in water and 21.6° in 1N HCl. No exchange occurred in this reaction and the deuterium content of the S-methyl group is the same as that of the methyl-d₃ iodide.

SUMMARY

The thermal decomposition of nonadeuterio trimethylsulfoxonium iodide, prepared from the nondeuterated salt and deuterium oxide, gives a high yield of methyl-d₃ iodide. Direct alkylation of the L-homocysteine anion, generated by the sodium-liquid ammonia reduction of L-methionine, by methyl-d₃ iodide gives optically pure L-methionine-methyl-d₃. A procedure for the preparation of tritiated compounds is also developed.
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REFERENCES