

SYNTHESIS OF $\underline{D,L-1-^{13}C}$ -METHIONINE

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SUMMARY

The stable, isotopically labelled amino acid $\underline{d,l-1-^{13}C}$ -methionine, was prepared synthetically from 90% $K^{13}CN$ and 3-(methylthiopropionaldehyde). Chromatographic, infrared and mass spectrometric properties are presented which aid in the identification of this compound in body fluids and tissues.

KEY WORDS: Methionine- $1-^{13}C$, Thin layer chromatography, Infrared spectrometry, Mass spectrometry

INTRODUCTION

The need for stable, isotopically labelled compounds as probes for both in vitro and in vivo studies in biochemistry and medicine has recently become more apparent (1,2). Because radioactive compounds for in vivo testing in man, and in particular, pediatric patients or women capable of childbirth, possess the characteristics of a potential health hazard, the preference for and use of stable, isotopically labelled compounds as probes

in the diagnosis of diseased states is mandatory. However, the lack of suitable, stable isotopically labelled compounds has often precluded research into the clinical diagnosis of diseased states.

One area of extensive clinical investigation has dealt with homocystinuria, an inborn error of sulfur amino acid metabolism (3). In an attempt to circumvent the exposure to radioactive hazards or possible percutaneous liver biopsy (4) encountered during the diagnosis of this disease, the preparation of a new stable, isotopically labelled analog of methionine, d,l-1-¹³C-methionine, was needed. This paper reports the synthesis of this compound which augments one other ¹³C-labelled methionine, ¹³CH₃-S-(CH₂)₂-CHNH₂CO₂H, (5) and may be very beneficial in the diagnosis of significant inborn errors of metabolism. Analytical data is also presented to aid in the identification of this new ¹³C-labelled methionine, isolated from body fluids and tissues.

EXPERIMENTAL

Infrared (IR) - Infrared analysis was performed using a Beckman 4210 IR spectrometer with ordinate expansion.

Mass Spectrometry (MS) - Mass spectral analysis was performed using a DuPont 490-F single focusing mass spectrometer (70 ev, electron impact mode). Samples were probe distilled directly into the ion source of the mass spectrometer.

Preparation of d,l-1-¹³C-Methionine - Anhydrous ammonia was bubbled into 40 ml of absolute ethanol, until the solution was saturated. To this solution was added 35 ml of 14.8 N NH₄OH, 1.7 g (0.026 moles) of K¹³CN, and 1.37 g (0.026 moles) of dry NH₄Cl. At room temperature the homogenous

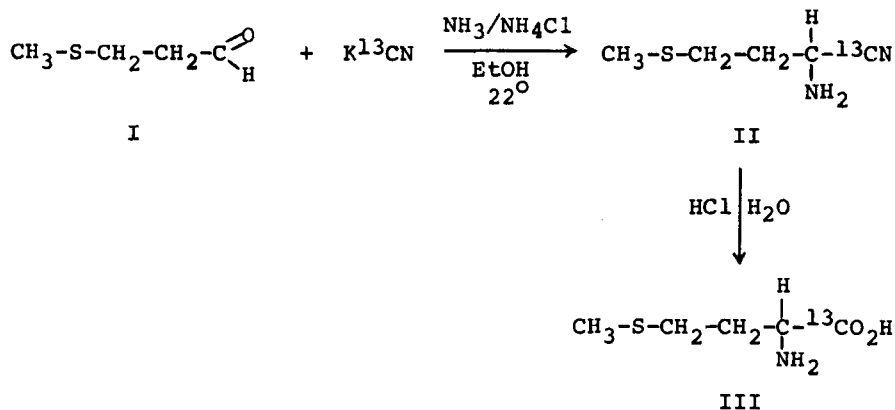
and clear solution was stirred and 2.68 g (0.026 moles) of 3-(methylthio)-propionaldehyde (Pfaltz and Bauer) was added dropwise over a five minute period. The reaction flask was stoppered and allowed to stir at room temperature (22°) for 12 hrs. At the end of that time, the ethanol and ammonia were removed under reduced pressure by a rotary evaporator. Evaporation was continued until a light yellow oil was observed in the reaction flask. To this flask was added 20 ml of 12.1 N HCl and the solution heated to 100° for 4 hours in the hood. After that time the dark brown solution was cooled and 100 ml of distilled water added and rotary evaporated to a small volume to remove HCl vapor. The removal of HCl was repeated three times. The fourth time 100 ml of distilled water was added and 1 gram of activated charcoal. The resulting solution was heated to boiling and filtered hot to yield a clear solution. Neutralization of this solution with 2 N NaOH and setting at 10° overnight yielded the first batch (2.6 g) of pure 1-¹³C-methionine. Removal of most of the water from the mother liquor followed by crystallization at 10° yielded an additional 0.7 g of crystals. These crystals were combined to yield 3.2 g (84%) of d,l-1-¹³C-methionine: m.p. = 282° (decomposes).

Thin Layer Chromatographic (TLC) Analysis - Using hard coated silica gel - 60 TLC plates (E.M. Corporation) the d,l-1-¹³C-methionine revealed the following: R_f = 0.50 (MeOH), R_f = 0.52 (Ethanol), R_f = 0.00 (ethylacetate: methanol: 14.8 N NH₄OH: 85:10:5), R_f = 0.66 (n-butanol: acetone: 17.4 N acetic acid: H₂O: 2:2:1:2). ¹³C-labelled methionine, after reaction with ninhydrin, yielded purple spots on TLC with UV light or red spots with heat. Brown spots were observed on TLC with iodoplatinate (K₂PtCl₆+KI).

RESULTS AND DISCUSSION

The synthesis of d,l-1-¹³C-methionine followed essentially the same

procedures as previously reported (6) with the exception that 90% $K^{13}CN$ (Stohler Isotope Chemicals) was used as a convenient source of labelled ^{13}C -carbon.



The addition of an equivalent amount of $K^{13}CN$ to an ethanolic solution of ammonia and 3-(methylthio)-propionaldehyde, I, resulted in the formation of the amino-nitrile, II. Acid hydrolysis of this nitrile, II, resulted in a high yield (84%) of d,l- l - ^{13}C -methionine, III.

Figure 1 reveals the infrared spectrum of 150 μg of d,l- l - ^{13}C -methionine. This spectrum is representative of typical free amino acids (7) and is similar to the published data (8) of d,l-methionine. Characteristic ammonium ($-\text{NH}_3^+$) absorption bands are observed at $3530\text{-}2925\text{ cm}^{-1}$, 1618 cm^{-1} , 1505 cm^{-1} , 1205 cm^{-1} , and 1175 cm^{-1} . Additional IR absorption bands at 1597 cm^{-1} , 1555 cm^{-1} and 1412 cm^{-1} reveal the carboxylate ($-\text{CO}_2^-$) of the labelled methionine. Absorption bands at 1385 cm^{-1} , 1335 cm^{-1} , and $1060\text{-}1047\text{ cm}^{-1}$ reveal the hydrocarbon portion of the labelled molecule.

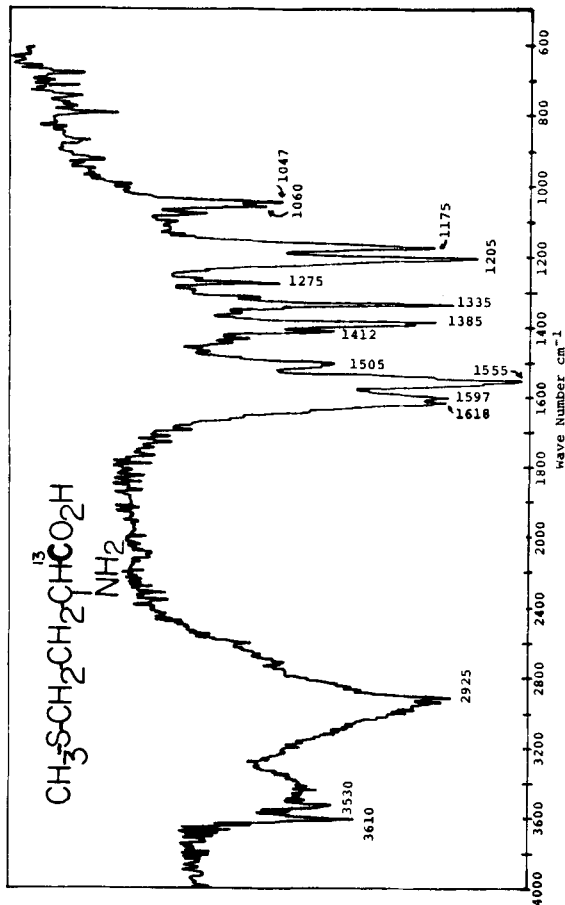


Figure 1: Infrared spectrum (KBr window) of d,1-¹³C-methionine. The spectrum represents 150 μg of material.

Figure 2 shows a mass spectral comparison between nonlabelled (A) and ^{13}C -labelled (B) methionine. These comparisons reveal those fragment ions of d,l- ^{13}C -methionine which contain the ^{13}C -label and give added insight into the pathways of mass spectral fragmentation of methionine. The significant fragment ions in the mass spectrum of the labelled methionine

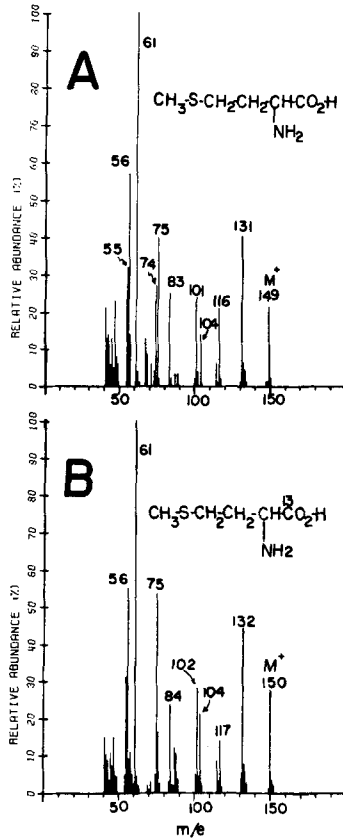
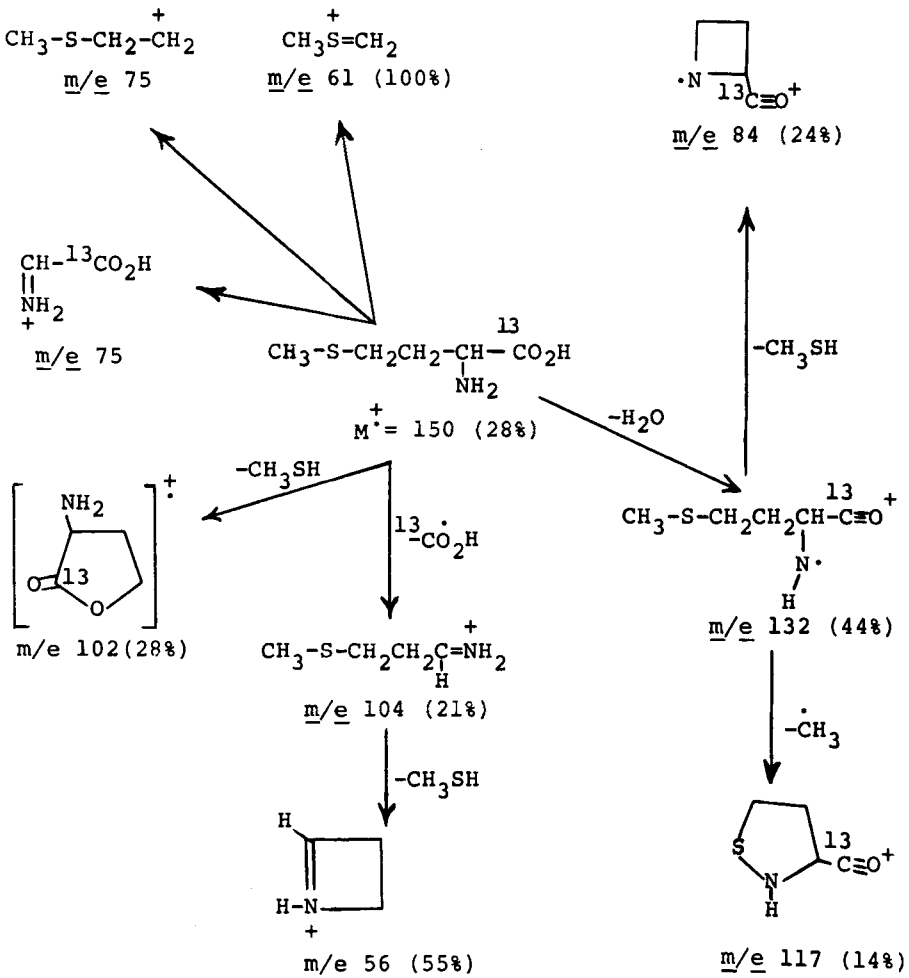


Figure 2 (A): Mass spectrum of methionine.

(B): Mass spectrum of d,l- ^{13}C -methionine.

(Figure 2-B) can be explained and follow the rationale previously outlined for the mass spectral fragmentation of methionine and deuterium labelled analogs of methionine (9). Using these data, the formation of significant mass spectral fragment ions of d,l-1-¹³C-methionine can then be proposed and are shown in Scheme 1.



Scheme 1: Significant fragment ions of d,l-1-¹³C-methionine in Figure 2-B. Percent relative abundance in parentheses.

CONCLUSION

A new analog of methionine, d,l-1-¹³C-methionine, has been prepared and its characteristic chromatographic and spectral data presented which may aid in the identification of this compound in body fluids or body tissues when used in the diagnosis of errors of sulfur amino acid metabolism. Significant mass spectral fragment ions of d,l-1-¹³C-methionine have been explained which aid in understanding the fragmentation of the nonlabelled amino acid. Finally a ¹³C-labelled compound of methionine has been prepared which may circumvent problems associated with radioactive probes of methionine used for in vivo studies in man. This compound may also find applications in the diagnosis and treatment of diseases of methionine metabolism through the monitoring of labelled ¹³CO₂ in the breath.

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