SYNTHESIS OF TAURINE-$^{13}C_2$

A POTENTIAL PROBE FOR HUMAN $^{13}CO_2$ BREATH TESTS

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SUMMARY

The synthesis of a 2% mixture of taurine-$^{13}C_2$, in nonlabelled taurine is presented. Using 1,2-dibromoethane-$^{13}C_2$, labelled taurine was prepared in two steps as a new and potentially useful probe for $^{13}CO_2$ breath tests in the diagnosis of intestinal malabsorption. Unique analytical data are presented, including gas chromatographic and mass spectral data of a derivative of taurine, N-carbobenzyloxytaurine amide. These new results were essential for the characterization and determination of isotopic enrichment of the 2% labelled taurine probe. Considerations for human feeding experiments with this stable, isotopically labelled mixture are also discussed.

Key Words: Taurine-$^{13}C_2$, $^{13}CO_2$ Breath Tests, and Mass Spectral Data.

INTRODUCTION

It is often highly desirable to utilize chemical probes for the diagnosis of disease states in man. Generally, radioactive probes are administered and measurements of radioactivity originating from various body fluids, tissues or the breath are made. These measurements of radioactivity then allow the determination of a great many parameters, such as the elucidation of biochemical pathways, the certainty of cellular metabolism, or observation of abnormal cell growth. Use of these probes in evaluating gastrointestinal function, especially allows decreased dependence upon studies requiring intestinal intubation and/or fecal collections (1,2).

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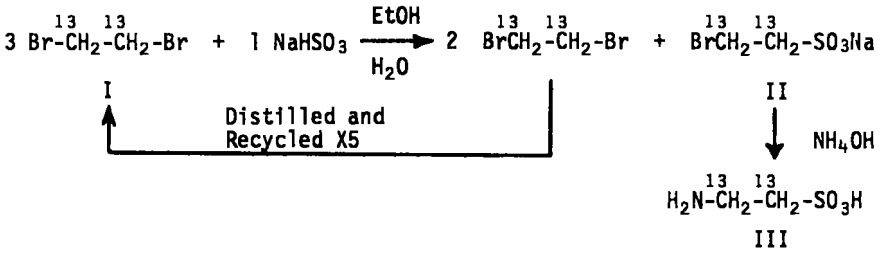
Recent work (3) with experimental rats, possessing an intestinal bacterial overgrowth, has demonstrated an excessive amount of $^{14}CO_2$ in the breath following the oral administration of ^{14}C-taurine. This new probe appears unique because of its very limited catabolism by control animals and man (4) and specificity for detecting the intestinal blind loop syndrome (5,6).

In order to utilize the extreme specificity of this labelled taurine breath test in detecting small intestine bacterial overgrowth without incurring potential long-term deposition of radioactive amino acids into body protein pools, the preparation of a ^{13}C-labelled taurine probe for use in a human $^{13}CO_2$ breath test was desired. This probe would also allow the safe utilization of a nonradioactive test in the evaluation of children and reproductive-age females with possible small intestine bacterial overgrowth (2,7).

The preparation of taurine from dibromoethane has been reported previously (8). This synthesis requires the monosulfonation of dibromoethane with sodium sulfite followed by the amination of the intermediate, sodium 2-bromoethanesulfonate, with concentrated ammonium hydroxide. The first step of this synthesis requires an excess amount of 1,2-dibromoethane. Following these procedures, initial attempts to prepare nonlabelled sodium 2-bromoethanesulfonate resulted in low yields (relative to dibromoethane) and loss of significant amounts of starting material. However, greatly increased synthetic yields were realized when unreacted dibromoethane was distilled from the sulfonation reaction mixture and collected. The starting material was then reacted an additional five times to generate more intermediate, sodium 2-bromoethanesulfonate. Reaction of this intermediate with excess ammonium hydroxide yielded taurine (65% overall from dibromoethane).

In order to circumvent the problems associated with small reaction sizes and volatile starting materials, 1,2-dibromoethane-$^{13}C_2$ (90%) was added to nonlabelled 1,2-dibromoethane to produce approximately a 2% ^{13}C-enriched mixture. This labelled starting material was then used to generate labelled

sodium 2-bromoethanesulfonate (2%-^{13}C), II, and taurine (2%-^{13}C), III, following the pathway outlined in Scheme 1.



Scheme 1: Preparation of taurine-$^{13}C_2$.

MATERIALS AND METHODS

Sodium 2-bromoethanesulfonate (2% $^{13}C_2$), II. Into a round bottom flask fitted with a reflux condenser, magnetic stirrer, and a dropping funnel were placed 49.0 g (0.260 moles) unlabelled 1,2-dibromoethane, 1.0 g (5.2 mmoles) 1,2-dibromoethane-$^{13}C_2$, 100 ml ethanol, and 37 ml water. This solution was stirred and heated to reflux at which point 11.0 g (0.087 mmoles) of sodium sulfite in 37 ml H_2O was added dropwise over a period of 2 hours. This solution was stirred at reflux for an additional 4 hours after which the ethanol and 1,2-dibromoethane were distilled over and collected for reuse. The water fraction was rotary evaporated to dryness leaving white crystals of NaBr and sodium 2-bromoethanesulfonate. The residue of sodium 2-bromoethanesulfonate was extracted twice with 100 ml hot ethanol and the insoluble NaBr filtered off. The resulting filtrate was evaporated to dryness leaving pure sodium 2-bromoethanesulfonate (containing 2% $^{13}C_2$). The initial ethanol distillate, containing 1,2-dibromoethane (2% $^{13}C_2$), was again reacted with sodium sulfite in water. The amount of sodium sulfite added to this new reaction mixture was determined by difference from the molar amount of sodium 2-bromoethanesulfonate(2% $^{13}C_2$) generated in the previous step. This experimental procedure was repeated until very little 1,2-dibromoethane (2% $^{13}C_2$)

could be detected (by gas chromatography) in the ethanol distillate. After five such reactions, 45.78 g (218 mmoles), 81.25% (calculated from 1,2-dibromo-ethane, 2% $^{13}C_2$), of sodium 2-bromoethanesulfonate (2% $^{13}C_2$) was obtained. M.p. 283-285°C (dec.); IR (cm^{-1}, KBr): 2941 and 2857 ($-CH_2-$), 1470 ($-CH_2-$), 1204 and 1063 ($-SO_3-$), and 810 and 870 ($-SO_3-$).

2-Amino-ethanesulfonic acid (2% $^{13}C_2$), III. The 45.78 g (218 mmoles) of sodium 2-bromoethanesulfonate (2% $^{13}C_2$) was dissolved in 500 ml of 58% ammonium hydroxide and the mixture allowed to stand for one week as described previously (8). The final yield of labelled taurine was 21.8 g (0.174 moles), 79.82% (from sodium 2-bromo-ethanesulfonate, 2% $^{13}C_2$) and 64% overall. M.p. >300°C (dec.); IR (cm^{-1}, KBr): 3450 (NH_2), 3200-2900 ($-SO_3H$ and $-CH_2-$), 1605 and 1505 ($-NH_2$), 1200 ($-SO_3-$), 1040 and 740 ($-SO_3-$); TLC (Si-gel, E.M. Corp.): R_f = 0.75 (ethanol: acetone: 17.4 N acetic acid: H_2O, 2:2:1:2), ninhydrin/heat positive.

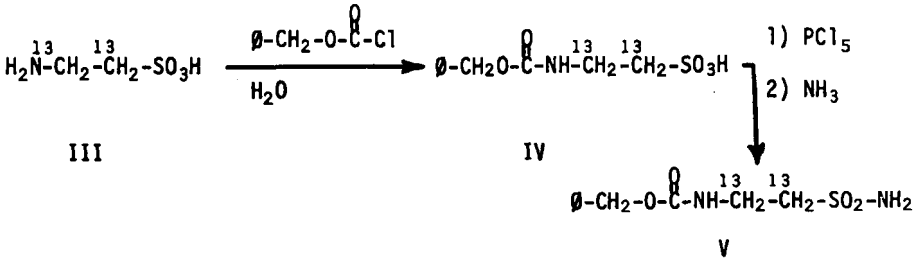
Sodium N-carbobenzyloxytaurine (2% $^{13}C_2$), IV. One gram (7.8 mmoles) of 2-amino-ethanesulfonic acid (2% $^{13}C_2$) was dissolved in 5 ml warm water. This solution was cooled with ice and 1.4 g (170 mmoles) $NaHCO_3$ and 3.6 g (210 mmoles) benzylchloroformate were added following known procedures (9). Work up of the reaction yielded 2.5 g of a mixture of sodium chloride and sodium N-carbobenzyloxytaurine (2% $^{13}C_2$). IR (cm^{-1}, KBr): 3300-2900 (broad $-SO_3H$, -NH, and CH_2),3030 (aromatic C-H), 1680 (sharp -O-CO-NH-), and 1200 ($-SO_3-$).

N-Carbobenzyloxytaurine amide (2% $^{13}C_2$), V. A part, 0.5 g, of the sodium N-carbobenzyloxytaurine (2% $^{13}C_2$) mixture was converted to 0.15 g (5.9 mmoles) of N-carbobenzyloxytaurine (2% $^{13}C_2$) following known procedures (9). M.p. 131-132°C; IR (cm^{-1}, KBr): 3350 and 3250 (NH) 3030 (aromatic C-H), 1690 (O-CO-NH-), 1550 (phenyl) 1325-1275 (multiplet, $-SO_2NH_2$), 1145 ($-SO_2-$), and 750 and 700 (phenyl-H); gas chromatography (175°-260°C at 6°/min., methylene units):

7% OV-7 (29.0) and 5% OV-1 (22.7); TLC (Si-gel, E.M. Corp.): R_f = 0.14 ($CHCl_3$: acetone, 4:1) and R_f = 0.20 (ethanol: acetone: 17.4 N acetic acid: H_2O, 2:2:1:2), diphenylcarbazone (1% in $CHCl_3$)/$HgSO_4$ (4% in 1N H_2SO_4) positive.

RESULTS

The infrared spectrum of the ^{13}C-labelled taurine mixture was identical to the spectrum published previously for taurine (10). In order to determine the amount of ^{13}C incorporated into the taurine probe, mass spectral analysis was attempted. However, the taurine mixture, containing both taurine-$^{13}C_2$ and nonlabelled taurine, would not volatilize, even directly into the ion source of a mass spectrometer. Therefore a volatile derivative was needed in order to ascertain isotopic enrichments. A review of the literature concerning gas chromatographic or mass spectral data of taurine, or its analogues, revealed that no pertinent information had been presented regarding volatile derivatives of the parent compound. Therefore we needed to prepare a suitable compound. Conversion of the labelled taurine to a trimethylsilyl or an acetyl derivative yielded very unstable products containing many impurities. These materials were poorly suited for isotopic enrichment measurements. However, following modifications of a procedure previously described (9) for the preparation of antibacterials, the labelled taurine mixture was converted to N-carbobenzyloxytaurine amide (2% $^{13}C_2$), V, (Scheme 2).



III IV V

Scheme 2: Preparation of a volatile derivative for mass spectral analysis.

This derivative was then amenable to gas chromatographic and mass spectral analysis. Figure 2 reveals the first reported mass spectrum of this ^{13}C-enriched derivative. This new gas chromatographic and mass spectral data should be very useful in the identification of taurine in biological samples. Calculations of isotopic enrichments, when compared to nonlabelled taurine, following published statistical procedures (11), confirmed that the percent molar excess of ^{13}C was 1.95% ± 0.09% (an average of 10 scans).

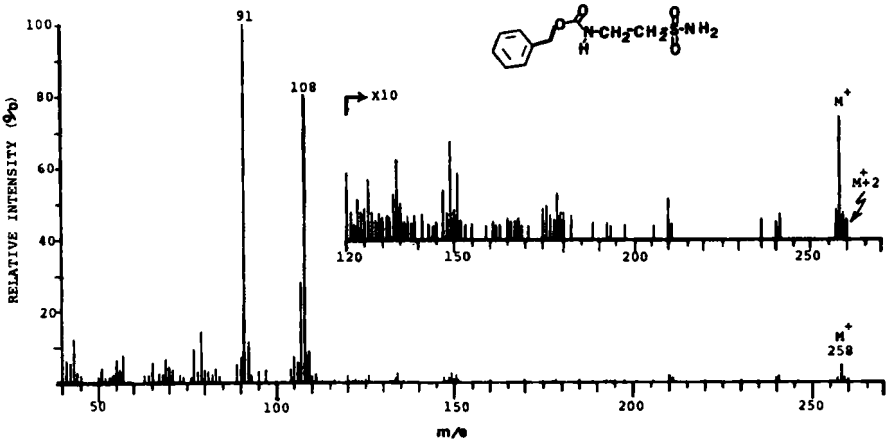


Figure 2: Mass spectrum of N-carbobenzyloxytaurine amide enriched with 2% ^{13}C. Spectral data was obtained by probe distillation directly into the ion source of the mass spectrometer (DuPont 490-F single focusing magnetic sector instrument; 70eV, EI mode).

CONCLUSION

The synthesis presented here reveals a improved procedure for the preparation of nonlabelled taurine, enriched with a $^{13}C_2$-labelled taurine probe. The synthetic scheme used, generated only a slightly enriched ^{13}C-labelled

mixture. However, because the percent isotopic enrichment could be determined accurately utilizing an important volatile derivative of the labelled taurine, the amount of material to be administered to patients can easily be calculated. Previously it was shown (7) that at least 0.28-1.4 μ moles/Kg (body weight)-hour of a ^{13}C-labelled probe must be administered in order to produce detectable amounts of $^{13}C_2$ in the breath. For example, from this data a typical, 5 μ moles/Kg single oral dose (12), for a 70 Kg human would require the administration of 2.2 g of the new labelled mixture of taurine-$^{13}C_2$ in nonlabelled taurine. Approximately 10 malabsorption tests could be performed utilizing the procedure described here in which 22 g of taurine (2% $^{13}C_2$) were obtained (65% yield) from 1 g of 1,2-dibromoethane-$^{13}C_2$.

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