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SYNTHESIS AND NMR CHARACTERIZATION OF (^{15}N)TAURINE
[2-(^{15}N)Aminoethanesulfonic acid]

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SUMMARY

The title compound was prepared in three steps with 55% overall yield starting from potassium (^{15}N)phthalimide. The synthetic route involved reaction with 1,2-dibromoethane, hydrolysis of the resulting N-(2-bromoethyl)(^{15}N)phthalimide with HBr and treatment of the 2-bromoethyl(^{15}N)amine thus formed with sodium sulphite. The product was characterized by ^{13}C, ^{1}H and ^{15}N NMR spectroscopy. The absolute coupling constants of ^{15}N with the ^{13}C nuclei and the non-exchanging protons were determined and an unambiguous assignment of the proton signals obtained.

KEYWORDS : taurine, ^{15}N labelling, biomarker, NMR data.

INTRODUCTION

Taurine is recognized as a conditionally essential amino acid for the human and many other species (1). In the cat, dietary taurine deficiency has been associated with retinopathy and even blindness (2,3) and more recently, with dilated cardiomyopathy and vascular changes (4). For our studies on the metabolism and the bioavailability of dietary taurine in the cat, the use of the ^{15}N labelled compound was the method of choice.

This paper describes the synthesis and NMR characterization of (^{15}N)taurine. To our knowledge, this is the first reported synthesis of taurine labelled on the nitrogen atom. Previous published preparations of labelled taurine involved deuterated (5), tritiated (6,7), ^{13}C (8,9) and ^{35}S (10) analogues.

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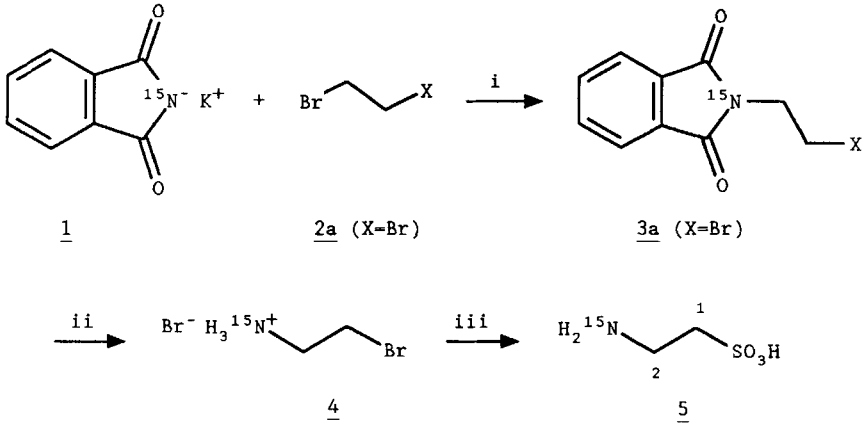
RESULTS AND DISCUSSION

Synthesis

The synthesis of (^{15}N)taurine was carried out in a 3-step sequence hitherto unreported *in extenso* for unlabelled taurine. Although reference was made to documented preparations for each step (11,12,13), major variations were introduced into the described methods to account for isotopic labelling.

The synthetic pathway is shown in **Figure 1**. Potassium (^{15}N)phthalimide offered a low cost commercially available starting material, and synthesis proceeded through a 2-stage Gabriel amine synthesis of 2-bromoethyl(^{15}N)amine, followed by nucleophilic substitution of the bromine atom with the sulphite anion.



1 2a (X=Br) 3a (X=Br)

4 5

(i)DMF, 90°C, 1h; (ii)HBr/CH_3COOH, reflux, 9h; (iii)aq.Na_2SO_3, reflux, 24h

Figure 1

Reacting potassium (^{15}N)phthalimide (1)(99% isotopic enrichment) with a threefold excess of 1,2-dibromoethane (2a) in dimethylformamide (DMF) resulted in the intermediate 3a in 94% yield. Attempts to perform the same reaction with potassium 2-bromoethanesulfonate (2b, X=$SO_3^-K^+$) instead of 2a did not lead to significant formation of the corresponding compound 3b (X=$SO_3^-K^+$) under a variety of solvent and temperature conditions. Hydrolysis of 3a by heating in 33% HBr in acetic acid gave the amine hydrobromide 4 in 70% yield. Treatment of 4 with a threefold molar excess of sodium sulphite in boiling water, followed by filtering the reaction mixture through Dowex cation exchanger to remove sodium ions, afforded (^{15}N)taurine (5) in 84% yield after crystallization from aqueous ethanol. The overall yield of the synthetic pathway was 55%.

NMR characterization

In the *carbon spectrum* in D_2O at ca. 23°C, two signals were found at 50.29 ppm (s, C(1)) and at 38.21 ppm (d, C(2)). With Gaussian resolution enhancement, the singlet at 50.29 ppm could be resolved into a narrow doublet, and the following coupling constants derived:

$$|^1J^{15}NC(2)| = 5.7 \text{ Hz} \qquad |^2J^{15}NC(1)| = 0.3 \text{ Hz}$$

These coupling constants, which compare favourably with the literature values for amines (14), confirmed the signal assignment first given by Norton (15). The shifts are in agreement with those of Evanochko et al.(16), while other authors give different values, depending upon pH, ionic strength, temperature, and methods of referencing (15,17,18).

In the *proton spectrum* in D_2O at 30°C, two groups of signals were obtained at ca. 3.45-3.41 ppm (CH_2(2)) and 3.29-3.24 ppm (CH_2(1)). The line patterns resembled those of an AA'BB' system with additional ^{15}NH couplings of ca. 0.7 Hz and 2.75 Hz, respectively. The proton resonance assignment, which agrees with that of earlier work (16,19,20), was derived from the carbon signals by two selective heteronuclear proton decoupling experiments. Despite the simplicity of the molecule, this assignment seems to be non-trivial, since the opposite interpretation can be found even in the most recent literature (21,22).

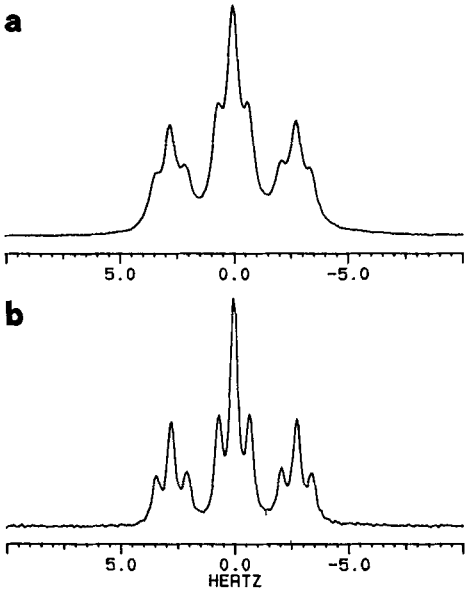


Figure 2 : ^{15}N-NMR spectrum of (^{15}N)taurine

The ^{15}NH coupling constants were confirmed by the *^{15}N spectrum* which, without any filtering, suggested a triplet-of-triplets structure (**Figure 2a**). With some Gaussian resolution enhancement (**Figure 2b**), the following coupling constants could be obtained:

$$|^{2}J^{15}NH| = 0.67 \text{ Hz} \qquad |^{3}J^{15}NH| = 2.75 \text{ Hz}$$

This agrees well with the values tentatively derived from the proton spectrum. These ^{15}NH coupling constants are an additional piece of evidence for the assignment of the proton resonances, since absolute three-bond coupling constants greater than two-bond couplings are often found in amino acids (table 7.3 in ref.14).

EXPERIMENTAL

General

Potassium (^{15}N)phthalimide (99% ^{15}N) was purchased from ICN Stable Isotopes, Innerberg, Switzerland. Other reagents and solvents were from Fluka AG, Buchs, Switzerland, or from Merck AG, Darmstadt, FRG, and were reagent grade or of the best available purity. Reactions were monitored on Merck HPTLC silicagel plates (Si 60 F_{254}); elution system A: ethyl acetate/ethanol/water/25% ammonia solution 60:20:10:3; elution system B: propanol/water 70:30; revelation: UV 254nm or ninhydrin. Elemental analysis was carried out by G. Nein, private laboratory, Basle, Switzerland. Fast atom bombardment (FAB) mass spectra were obtained using a Finnigan/MAT 8430 double-focusing mass spectrometer equipped with a saddle-field FAB gun (Ion Tech, Teddington, UK); for analysis, (^{15}N)taurine was dissolved in water and mixed with glycerol matrix directly on the target.

Synthesis

1. N-(2-Bromoethyl)(^{15}N)phthalimide (3a)

A mixture of 17.0 g (91.3 mmoles) potassium (^{15}N)phthalimide (1) (99% ^{15}N) (TLC, system A, UV 254nm: Rf 0.14) and 51.5 g (0.274 mole) 1,2-dibromoethane (2a) in 90 ml DMF was heated at 90°C for 1h. After removal of the solvent under reduced pressure, the residue was taken up in 90 ml water and the product extracted with 3 portions of 50 ml dichloromethane. The pooled organic phases were dried over sodium sulfate, filtered and the solvent removed. The pasty residue was triturated in 90 ml petroleum ether (b.p. 40-60°C) and the resulting solid was dried, giving 21.96 g (86.1 mmoles, 94.3%) of nearly colorless crystals. TLC, system A, UV 254nm: Rf 0.95.

2. 2-Bromoethyl(^{15}N)amine hydrobromide (4)

A solution of 21.94 g (86.0 mmoles) 3a in a mixture of 115 ml 33% HBr in acetic acid and 45 ml water was heated at reflux for 9h. After removal of the solvent under reduced pressure, the residue was taken up in 18 ml water and the resulting suspension cooled at 0°C for 1h to precipitate out phthalic acid. The mixture was filtered and the filtrate concentrated *in vacuo*. The pasty residue was triturated in acetone and the resulting solid was quickly collected and dried (1 mBar, 60°C), giving 12.37 g (60.1 mmoles, 69.9%) of colorless hygroscopic crystals. TLC: ninhydrin, system A: Rf 0.63; system B: Rf 0.11.

3. (^{15}N)Taurine (5)

A solution of 12.33 g (60.1 mmoles) 4 and 23.0 g (0.183 mole) sodium sulphite in 120 ml water was heated at reflux for 24h. The mixture was concentrated to half the volume and poured onto a chromatographic column filled with 275 ml Dowex 50W-X12 50-100 mesh (H^+ form). The product was eluted with water and collected in 150 ml fractions, each of which was checked for the presence of taurine by the ninhydrin test and for the absence of sodium ion by the flame test. The fractions containing taurine (fractions 1-8) were pooled and concentrated to about 25 ml until precipitation began. Then, 135 ml ethanol were slowly added while stirring to complete the crystallization. After cooling down the mixture to 0°C, the precipitate was collected, washed with absolute ethanol and dried, giving 6.53 g (51.8 mmoles) of colorless crystals. The above crystallization procedure was repeated and the resulting product was thoroughly dried over P_2O_5 (24h, 60°C, 0.01 mBar) to provide 6.37 g (50.5 mmoles, 84.0%) of pure material. TLC, system B, ninhydrin: Rf 0.45. The overall yield of 5 from potassium (^{15}N)phthalimide (1) was 55.0%.

Elemental analysis : Calc. for $C_2H_7{}^{15}NO_3S$ (MW 126.14): C 19.04; H 5.59; N 11.89; S 25.42. Found: C 19.18; H 5.67; N 12.00; S 25.49.

MS analysis (estimation of isotopic content) : The positive and negative ion FAB spectra of 5 showed the expected $[M+H]^+$ ion at m/z 127 and $[M-H]^-$ ion at m/z 125, respectively. ^{15}N content was estimated by the relative abundance of the ion at m/z 126 in the positive ion spectrum. By averaging 60 spectra, a value of 1.8% was obtained (m/z 127: 100%), thus indicating an isotopic purity better than 98% (lower limit of estimate). No upper limit is given, because of the uncertainty about the homogeneity of the peak at m/z 126.

NMR experiments

All NMR spectra were recorded on a Bruker AM 360 instrument. ^1H and composite pulse decoupled ^{13}C spectra with respect to internal 3-(trimethylsilyl)tetra-

deuteriopropionic acid sodium salt (TSP) were acquired with a digital resolution of 0.044 and 0.118 Hz/point, respectively, using 5 mm o.d. sample tubes. For ^{15}N spectra, a 10 mm o.d. sample tube (Wilmad 513-7PP) containing 83.5 mg of 5, 4.584 g D_2O and 0.6 mg TSP was measured at 22°C. The proton frequency of TSP (internal standard) being 360.1348430 MHz, the ^{15}N signal shown in figure 2 was found at 36.4963491 MHz, or, in the absolute shift scale, at 10.1340789 MHz for 100.000 MHz proton frequency. For easier comparison with literature values, a 1.5 mm i.d. concentric capillary tube (Wilmad type 519 inner) containing neat (^{15}N)nitromethane was added for another measurement, and a shift of -351.56 ppm obtained for the signal of 5, assuming the external standard to be 0.0 ppm (no proton decoupling, center of a quartet signal, no susceptibility correction). Resolution enhancement parameters of the spectrum shown in figure 2b were LB=-0.3 and GB=0.4.

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