THE SYNTHESIS OF [2-$^3H_2$] TAURINE AND [2-$^3H_2$] HYPOTAURINE

KEY WORDS

Taurine, Hypotaurine, Tritium.

SUMMARY

The synthesis of [2-$^3H_2$]-2-aminoethanesulfonate [2-^3H]-taurine by the reduction of cyanomethanesulfonic acid with tritium gas is described. The conversion of [2-^3H]-taurine and its ^{14}C and ^{35}S isotopic forms to 2-aminoethanesulfinate (hypotaurine) was accomplished by converting taurine to its corresponding sulfonyl chloride and reducing the latter with metallic zinc.

INTRODUCTION

The metabolic origin, fate, and function of taurine has currently become a topic of intense interest (1). The biosynthesis of taurine from hypotaurine has yet to be clearly established, thus radioisotopic forms of hypotaurine are useful for such studies. The synthesis of ^{35}S-hypotaurine has been reported from the alkaline oxidation of ^{35}S-cystamine (2). We have developed a synthesis of [2-$^3H_2$]-2-aminoethanesulfonate ([2-^3H]-taurine) and [2-$^3H_2$]-aminoethanesulfinate ([2-$^3H_2$]-hypotaurine) which provides a product of high specific activity, by synthetic routes hitherto not reported. This labeled position was specifically chosen because we were also concerned with the possible transamination of both taurine and hypotaurine by mammalian tissue, followed by measuring the rate of appearance of tritiated water as transamination proceeds (3). After successfully completing the synthesis of these radiolabeled substrates as set forth below, we investigated.the transamination of both taurine and hypotaurine in mammalian tissue and microorganisms. We observed several microorganisms capable of transaminating both substrates. However, only hypotaurine is transaminated by mammalian tissue (4).

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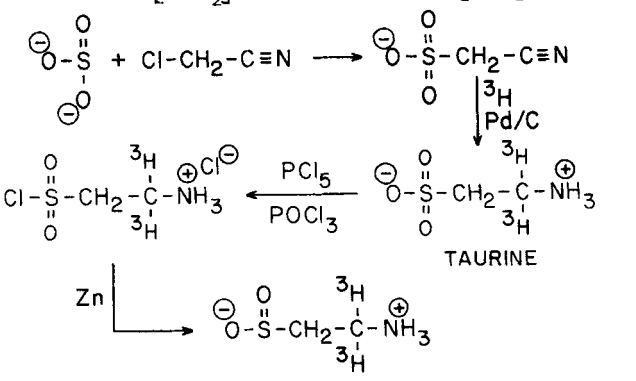
SYNTHESIS OF $[2\text{-}^3H_2]$-2-AMINOSULFONATE $[2\text{-}^3H]$-TAURINE



HYPOTAURINE

Sodium sulfite (1.4 g) dissolved in 12 ml of water was allowed to react overnight with 0.8 g of chloroacetonitrile at steam bath temperature in a stoppered tube. A crude crystalline product was obtained by the addition of 70 ml of ethanol and cooling. The residue was extracted with 20 ml of hot methanol and after filtration, 240 mg of sodium cyanomethylsulfonate was obtained as white crystals from methanol-ether. Infrared spectrum CH_2 = 2975^{cm-1}, 2935^{cm-1}, C≡N = 2265^{cm-1}, SO = 1280^{cm-1}, and 1060^{cm-1} (KBr pellet).

The sodium cyanomethanesulfonate was converted to taurine by the following procedure: 75 mg of sulfonitrile dissolved in 2.4 ml of 1 N HCl was hydrogenated at 20 psi pressure using 10% palladium/charcoal catalyst for 3 h at room temperature. The clear filtrate obtained from the reaction mixture was evaporated to dryness and the product recrystallized from water/methanol. The crystalline product was identified as taurine by co-chromatography with an authentic sample of taurine on silica gel thin layer chromatography with isopropyl alcohol ammonia (8:1) as solvent and on AG-50 and Bio-Rex 5 ion exchange column chromatography. The infrared spectrum was identical to that obtained with authentic taurine. The yield was 30%. Tritiation of sodium cyanomethanesulfonate was carried out by arrangement with New England Nuclear using the procedure described above. The specific activity of the product taurine was 13.7 Ci/mmol. The material obtained was purified on a 15 cm x 0.7 cm Bio-Rex 5 ion exchange resin

column. The fraction eluting with the void volume contained the taurine and was used for the synthesis of hypotaurine.

SYNTHESIS OF [2-$^3H_2$]-HYPOTAURINE

One microcurie of 2-aminoethanesulfonate-2-^{3}H was added to 1.25 mg of taurine in 1 ml of 1 N HCl in a 100 x 10 mm Pyrex test tube fitted with a screw top cap having a Teflon seal. The solution was evaporated to dryness and 0.3 ml of a solution containing 250 mg PCl_5 and 250 mg $AlCl_3$ dissolved in 5 ml of freshly distilled $POCl_3$ was added. The tube was capped and heated at 70°C for 90 minutes in a controlled heating block. The volatile excess $POCl_3$ and PCl_5 was evaporated under a gentle stream of dry nitrogen. The residue was dissolved in 2 ml of dry methanol and 180-700 mg of powdered zinc was added. The mixture was shaken for a few min. and filtered. The methanol was evaporated; the residue dissolved in 1 ml of water and placed on a 7 cm x 0.9 cm AG50 ion exchange resin column. The column was washed with 14 ml of water followed by 14 ml of 2 N NH_4OH. The latter contained all of the hypotaurine. Both ^{14}C and ^{35}S isotopically labeled hypotaurine were also synthesized from the corresponding labeled taurine precursors. The yield was consistently greater than 90% conversion of taurine to hypotaurine and in this example 100 mCi/mmol specific activity.

The hypotaurine thus obtained, co-chromatographed in several solvent systems on thin layer chromatographic plates (cellulose; n-butanol acetic acid water (4:1:1) and silica gel; isopropyl alcohol water (5:1). It co-chromatographed on cation ion exchange resin columns with hypotaurine and was oxidized as expected, to taurine under suitable oxidizing conditions, such as by treatment with dilute hydrogen peroxide (5).

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