SYNTHESES OF DL-α-^{15}N-TRYPTOPHAN AND 2-^{15}N-PERLOLYRINE◆

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SUMMARY

DL-α-^{15}N-Tryptophan was synthesized by simple and convenient chemical reduction method, starting from α-^{15}N-glycine and benzoyl chloride via five steps. The yield of DL-α-^{15}N-tryptophan from α-^{15}N-glycine was 46.9%. 2-^{15}N-Perlolyrine was synthesized from DL-α-^{15}N-tryptophan and 5-acetoxymethyl-2-formylfuran via three steps. The yield of 2-^{15}N-perlolyrine from DL-α-^{15}N-tryptophan was 15.5%.
Keywords: DL-α-^{15}N-Tryptophan, 2-^{15}N-Perlolyrine, Synthesis

INTRODUCTION

An active alkaloid, perlolyrine, 1-(5-hydroxymethyl-furyl)-9H-pyrido [3,4-b] indole, has been isolated from *Lolium Perenne L.* (*Graminae*)[1] and *Ligusticum Wallichii Franchet*[2] and synthesized from tryptophan and 5-acetoxymethyl-2-formylfuran[1]. The alkaloid may be used to treat patients with coronary atherosclerotic heart disease by the confirmation of preliminary pharmacological experiments[3]. In order to investigate perlolyrine metabolic transformation in animals, preparation of 2-^{15}N-perlolyrine (1) is required. As perlolyrine is synthesized from tryptophan and 5-acetoxymethyl-2-formylfuran, DL-α-^{15}N-tryptophan (2) must be prepared.

In this report, synthesis of DL-α-^{15}N-tryptophan and 2-^{15}N-perlolyrine are described.

RESULTS AND DISCUSSION

Tryptophan was first synthesized by Elliger by the condensation of indole-3-aldehyde with hippuric acid by heating these together in the presence of acetic anhydride and sodium acetate to give azlactone, which was first hydrolyzed with sodium hydroxide to give α-benzamido-β-indole-3-acrylic acid, then reduced and hydrolyzed with sodium in alcohol

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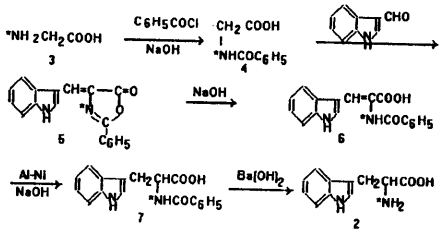
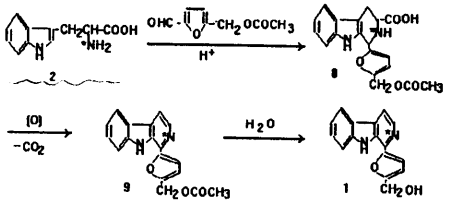
to give DL-tryptophan[4]. To improve this method of preparing tryptophan, Elks[5] used Raney nickel as a catalytic reduction agent and barium hydroxide as a hydrolytic agent in place of the Ellinger method of reduction and hydrolysis with sodium in alcohol. The yield of tryptophan from α-benzamido-β-indoie-3-acrylic acid by the Elks method was 72%, whereas Ellinger recored a 15% yield[5]. Using Al-Ni alloy in sodium hydroxide as a chemical reduction agent and barium hydroxide as a hydrolytic agent, we obtained the same yield of tryptophan as that of the Elks method. This method was simple and easy to operate. DL-α-^{15}N-Tryptophan was synthesized (scheme 1) by modification of the last procedures employed by Ellinger[4] and Elks[5]. By this method, we performed a satisfactory systhesis of the required labelled tryptophan. The yield of DL-α-^{15}N-tryptophan from α-^{15}N-glycine was 46.9%.

Scheme 1. Synthesis of DL-α-^{15}N-tryptophan (*N stands for ^{15}N)



2-^{15}N-Perlolyrine was synthesized from DL-α-^{15}N-tryptophan and 5-acetoxythyl-2-formylfuran by acid-catalysed ring-closure which was formally analogous to a Pictet-Spengler reaction, followed by oxidation where necessary and hydrolysis[1] (Scheme 2). DL-α-^{15}N-Tryptophan and 5-acetoxythyl-2-formylfuran reacted in acetic acid to give the ring-closed product (8), which was not isolated, but oxidised with acidified dichromate. This caused aromatisation of the newly formed ring and simultaneous loss of carbon dioxide. The process gave a mixture the acetyl derivative of 2-^{15}N-perlolyrine (9) and a smaller quantity of 2-^{15}N-perlolyrine (1). The mixture was not isolated but was hydrolysed with concentrated ammonia to give 2-^{15}N-perlolyrine. The operation method of not isolating the reaction mixture, which could save reagents and simplify operating steps, did not manifestly affect the yield of final product. The overall yield of 2-^{15}N-perlolyrine was 15.5% from DL-α-^{15}N-tryptophan.

Scheme 2. Synthesis of 2-^{15}N-perlolyrine (*N stands for ^{15}N)



EXPERIMENTAL

^{15}N-Glycine was from our laboratory. Indole-3-aldehyde was from Sigma Chemical Co., St Louis, USA. TLC and other reagents were from Beijing Chemical Reagents Co., China. Melting points were determined on a microscope melting pointing apparatus and were uncorrected. MS data were acquired with Hewlett-Packard 5971A/5890-II GC/MS. ^{1}H-NMR spectra were recorded with varian XL-300 spectrometer, TMS as the internal reference. UV spectra were detected by Beckman DU-600 and IR spectra determined by Beckman 4230 instrument.

α-^{15}N-Hippuric acid(4). To a stirred solution of α-^{15}N-glycine (3, 1.055g, 14.3mmol) in 10% sodium hydroxide (11mL) was added gradually a mixture of benzoyl chloride (4.8mL) and 10% sodium hydroxide (22.5mL). The mixture was stirred until the excess benzoyl chloride was hydrolyzed; then the product was precipitated by acidification with hydrochloric acid. Purification was accomplished by repeated extraction of the benzoic acid with ether, and gave pure α-^{15}N-hippuric acid(4) 2.463g(97.8%), mp: 190-192 ℃.

3-^{15}N-4-[3-Indolylmethylene]-2-phenyl-2-oxazolin-5-one(azlactone, 5). The indole-3-aldehyde(0.972g, 6.7mmol), α-^{15}N-hippuric acid(4, 1.000g, 5.6mmol), anhydrous sodium acetate(0.492g, 6.0mmol) and acetic anhydride (1.8mL) were heated for 15 minutes on the water bath. The azlactone began to separate when the solution was still hot. The reaction product was extracted with boiling water, the solid was collected and dried. Recrystallisation from glacial acid gave the brownlish red prismatic plates (5) 1.352g(84%), mp: 200-202 ℃.

α-^{15}N-α-Benzamido-β-indole-3-acrylic acid(6). By repeated boiling with 100 times its weight of 1% sodium hydroxide, the azlactone (5, 0.864g, 2.8mmol) was hydrolysed to the acid(6). The solution was cautiously acidified with hydrochloric acid, the precipitated solid was obtained. Recrystallisation from 70% alcohol gave the pale yellow prismatic needles solid(6) 0.685g(74.6%), mp: 226-228 ℃.

DL-α-^{15}N-α-Benzoyltryptophan(7). To a stirred solution of α-^{15}N-α-benzamido-β-indole-3-acrylic acid(6, 0.600g, 1.9mmol) and 1N sodium hydroxide(7mL) was added gradually Al-Ni alloy(0.322g). When reaction was complete in 6 hours, the mixture was filtered, the residual washed with a little 1N sodium hydroxide and water, and the filtrate cautiously acidified with dilute hydrochloric acid. Recrystallisation from aqeous alcohol gave the pink solid(7) 0.580g(96%), mp: 190-192 ℃. Difficulty was exprienced in obtaining a pure sample of this substance, for it rapidly became pink on heating in solvents.

DL-α-^{15}N-Tryptophan(2). DL-α-^{15}N-α-Benzoyltryptophan (7, 0.500g, 1.0mmol), barium hydroxide(2.750g) and water(12.8mL) were mixed and refluxed for 24 hours. After removal of barium ions with 2N sulphuric acid, the filtered solution was evaporated to dryness in a vacuum, and the residue washed with alcohol to remove benzoic acid. Recrystallisation from hot water gave the pale yellow product(2) 0.264g(79.7%), mp: 280(decomp.)

with darkening from 260 ℃; homogeneous and identical with authentic DL-tryptophan upon paper chromatography (n-butyl acohol-acetic acid-water, 12: 3: 5) Rf=0.5; UV (H_2O): λ_{max}=279nm.

2-^{15}N-1-(5-Hydroxymethyl-2-furyl)-9H-pyrido[3,4-b]indole (2-^{15}N-perloly rine, 1). Stock solution were made of (i) potassium dichromate (50g), concentrated sulphuric acid (40mL), water (to 500mL); (ii) oxalic acid dihydrate (50g) in water (to 250mL), (this solution has to be used warm as it is super-saturated at room temperature); and (iii) sodium disulphite (50g) in water (to 300mL). DL-α-^{15}N-Tryptophan (2, 0.264g, 1.3mmol), 5-acetoxymethyl-2-formylfuran (0.264g, 1.6mmol), and glacial acetic acid (6.6mL) were heated on a steam bath for 20 minutes and continued for 20 minutes, the liquid was usually dark brown. It was then poured into a boiling mixture of water (130mL) and the dichromate solution (10.6mL). Boiling was continued for 1 minutes, then the disulphite solution (6.6mL) was added, followed by oxalic acid solution (13.2mL). This was cooled and anhydrous sodium carbonate (13.2g) in water (52.8mL) was added, and the liquid was shaken with ether (30mL × 6). The ether solution was washed with water until the washings were colourless, dried over anhydrous sodium sulfate, and evaporated to dryness. The residual was dissolved methanol (5mL), then an equal volume of concentrated ammonia was added, and the reaction mixture was kept in the dark for a few days. It was then isolated and crystallized from ethyl acetate to give crystals (1) 0.053g (15.5%), mp: 185-190; CIMS: m/z265 (M^+); UV (MeOH): 237, 253, 272, 291, 366, 382nm; ^{1}H-NMR (CH_3COCH_3, TMS) δ (ppm): 8.32 (1H, d, 3-H), 7.88 (1H, d, 4-H), 7.2 ~ 8.3 (4h, m, H-Ar), 7.22 (1H, d, 3'-H), 6.53 (1H, d, 4'-H), 4.69 (2H, s, 6'-H); IR (KBr) cm^{-1}: 3365 (NH), 1500 ~ 1700 (C=C + C=^{15}N) 1632 (s), 1608 (w), 1563 (s), 1528 (w), 1490 (s), 1016 (s), 748 (s); homogeneous upon basic aluminum oxide TLC (chloroform - methyl alcohol, 9: 1) Rf=0.9.

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