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SYNTHESIS OF ^{13}C AND ^{15}N LABELED (S)-TRYPTOPHAN[1]

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ABSTRACT: (R,S)-serine-1-^{13}C was incubated in a culture of Escherichia coli cells to produce (S)-tryptophan-1-^{13}C. Bromoacetyl bromide-2-^{13}C was converted to bromoacetanilide and cyclization of the anilide, followed by reduction and dehydrogenation furnished indole-3-^{13}C. Indole-^{15}N was synthesized by known sequences. These ^{13}C and ^{15}N isotomers of indole were converted by commercially available, lyophilized E. coli to furnish (S)-tryptophan-γ-^{13}C and (S)-tryptophan-indole-^{15}N, respectively.

INTRODUCTION

Various ^{13}C and ^{15}N labeled isotomers of tryptophan have been utilized for the biosynthetic studies of quinine[2], streptonigrine[3], anthramycin[4] and vindoline[5]. (S)-Tryptophan-γ-^{13}C also has been incorporated into protein for conformational studies[6]. The experimental sections in these studies generally describe the preparation of ^{13}C and ^{15}N isotomers of racemic (R,S)-tryptophan by laborious, multistep syntheses.

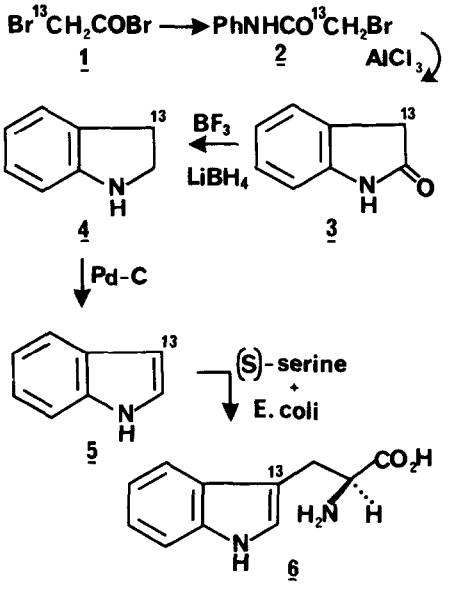
(S)-Tryptophan now has been synthesized in one step from indole and serine by incubation in an aqueous suspension of commercially available Escherichia coli cells containing high levels of tryptophanase[7]. By reacting (R,S)-serine-1-^{13}C, indole-3-^{13}C or indole-^{15}N in this medium, we were able to obtain (S)-tryptophan-1-^{13}C, γ-^{13}C or indole-^{15}N in 40 to 50% yield. This biosynthetic approach can also accommodate other labeling patterns by the proper choice of starting materials and represents the simplest route for the preparation of optically active products.

RESULTS AND DISCUSSION

(R,S)-serine-1-^{13}C was synthesized from glycine-1-^{13}C (90% ^{13}C) following the procedure developed by Shemin[8]. Along with the (S)-tryptophan-1-^{13}C, we also isolated (R)-serine-1-^{13}C (70% yield) from the incubation brew, and this unnatural serine could be racemized to produce (R,S)-serine-1-^{13}C for recycling.

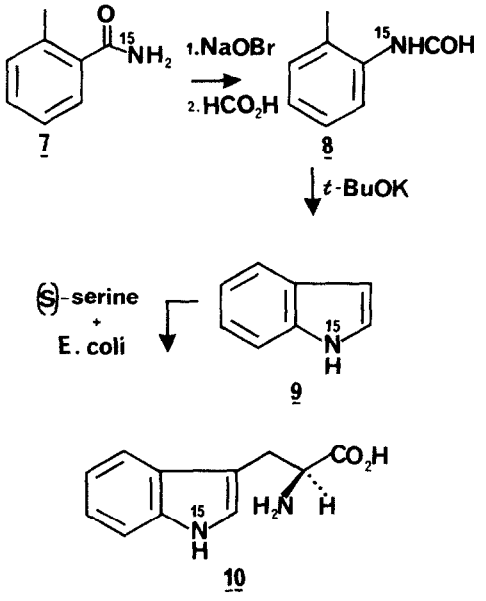
Indole-3-^{14}C was synthesized by Heidelberger[9] starting with the nitration of toluene-methyl-^{14}C to give a mixture of ortho, para and meta isomers of nitrotoluene from which only the ortho isomer would give indole-3-^{14}C in a three-step sequence. Later, Leete et al[10] converted pyruvic acid-3-^{14}C to its phenylhydrazone and then in four steps, arrived at indole-3-^{14}C.

We decided to use the more easily accessible bromoacetyl bromide-2-^{13}C (90% ^{13}C)[10] as our starting material for the synthesis of indole-3-^{13}C.

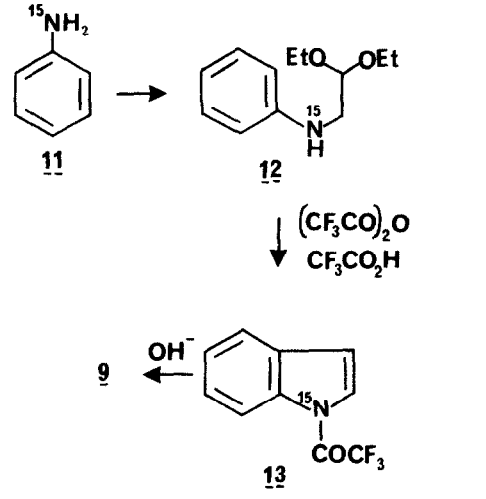


Condensation of 1 with aniline gave 2 in 86% yield[11]. Cyclization of 2 using $AlCl_3$[12] produced oxindole-3-^{13}C (3) in 58% yield. Reduction (45%) and dehydrogenation (90%) furnished the desired indole-3-^{13}C (5)[13].

For the synthesis of indole-^{15}N (99% ^{15}N), we first followed the scheme developed by Leete et al[14]:



We also utilized the indole synthesis scheme of Nordlander et al[15], starting with aniline-^{15}N (99% ^{15}N), although our yields were much lower than reported for unlabeled aniline[16].



The ^{13}C-indole, 5, and the analog, 9, were incubated in separate trials with E. coli cells[7] to give correspondingly labeled (S)-tryptophan 6 and 10.

EXPERIMENTAL

General: ^1H-NMR and ^{13}C-NMR spectra were obtained using Varian EM360A and Brucker HFX-100 NMR Spectrometers. Isotope labeled starting materials were prepared according to either Murray and Williams[17] or Ott[18].

(R,S)-Serine-1-^{13}C (90%): ^1H-NMR (D_2O): δ4.1 (2H) and 4.3 (1H) ppm (AB type complex mutiplets than unlabeled system).

(S)-Tryptophan-1-^{13}C (90%): Incubation was carried out using the literature procedure[7], except that the E. coli cells we used were commercially available, lyophilized Crooke's Strain in trytophanase (Sigma Chemicals Catalog No. EC-8739). ^1H-NMR (DCl-D_2O): δ3.5 (2H, t, J=4Hz, J_{CCCH}=4Hz, β-H), 4.5 (1H, q, J=4Hz, J_{CCH}=4Hz, α-H), 7.2-7.9 (5H, m, indole). $[\alpha]_D^{25°}$ (C=1, H_2O).

Bromoacetanilide-2-^{13}C (2): was prepared according to (10). ^1H-NMR (d_6): δ4.0 (2H, d, J_{CH}=154Hz, CH_2Br, gration showed 90% ^{13}C), 7.0-7.7 m, C_6H_5) and 10.2ppm (1H, bs, NH).

Oxindole-3-^{13}C (3): was prepared cording to (12). ^1H-NMR ($CDCl_3$): (2H, d, J_{CH}=134Hz, 90% ^{13}C), 6. (4H, m, C_6H_4) and 9.9ppm (1H, bs, N

Indoline-3-^{13}C (4): To a solution of oxindole-3-^{13}C in 200mL of tet drofuran under nitrogen was added of lithium borohydride in 200mL o over 30 min. Then 24mL of boron tri ide etherate in 100mL of THF was over 1.5 h. The mixture was stirre 24 h and the solvent was remove vacuo. Methanol (150mL) was added ly and the mixture was refluxed f min. The solution was evaporatec the residue was taken up in wate basified with sodium hydroxide. The duct was extracted into methylene ride, dried and evaporated. Dist tion, bp 65-70o-/0.3torr, gave 2.

product (45%). ^{1}H-NMR (CCl_4): δ2.9 (2H, dt, J=8Hz, J_{CH}=134Hz, 3-C$\underline{H}_2$, 90% ^{13}C), 3.5 (2H, dt, J=8Hz, J_{CCH}=4Hz, 2-C$\underline{H}_2$) and 6.2-7.0ppm (4H, m, $C_6\underline{H}_4$).

Indole-3-^{13}C (5): Indoline-3-^{13}C was aromatized by catalytic dehydrogenation according to (12). ^{1}H-NMR (CCl_4): δ6.4 (1H, dt, J=2Hz, J_{CH}=192Hz, 3-C$\underline{H}$, 90% ^{13}C), 6.7 (1H, dt, J=2Hz, J_{CCH}=61Hz, 2-C$\underline{H}$), and 6.9-7.7ppm (5H, m, $C_6\underline{H}_4$ and N$\underline{H}$).

(S)-Tryptophan-γ-^{13}C (6): ^{1}H-NMR (DCl-D_2O): δ3.5 (2H, t, J=5Hz, J_{CCH}=5Hz, β-C$\underline{H}_2$), 4.4 (1H, t, J=5Hz, α-$\underline{H}$) and 7.2-7.8ppm (5H, m, 7.4 and 7.6 peaks were more complex than unlabeled, aromatic protons). $[\alpha]_D^{25°}$ = -30.3° (C=1, H_2O).

Indole-^{15}N (9): ^{1}H-NMR ($CDCl_3$): δ6.3 (1H, t, J=2Hz, 3-C$\underline{H}$), 6.7 (1H, quintet, J=2Hz, J_{NCH}=4Hz, 2-C$\underline{H}$), 7.1 (1H, t, J=2Hz, J_{NH}=96Hz, N$\underline{H}$) and 6.9-7.6ppm (4H, m, $C_6\underline{H}_4$).

^{15}N-trifluoroacetylindole (13): Our yield for this material was only 50% from 12 (1 run) and its hydrolysis to indole yielded 75%. ^{1}H-NMR ($CDCl_3$): δ 6.7 (1H, dd, J=4Hz, J_{NCH}=5Hz, 2-C$\underline{H}$), 7.2-7.7 (4H, m, C_3-C_6 protons) and 8.3-8.6ppm (1H, m, C_7-H).

(S)-Tryptophan-indole-^{15}N (10): ^{1}H-NMR (DCl-D_2O): only the 7.2 peak showed some ^{15}N coupling. ^{13}C-NMR (NaOD-D_2O): δ 24 (β-C), 49 (α-C), 107 (d, J_{NCC}=16Hz, 3'-C), 109 (7'-C), 111 (6'-C), 111.5 (4'-C), 114 (d, J_{NCCCC}=16Hz, 5'-C), 117 (d, J_{NC}=96Hz, 2'-C, integration showed 99% ^{15}N), 120 (d, J_{NCC}=32Hz, 9'-C), 129 (d, J_{NC}=104Hz, 8'-C, 99% ^{15}N) and 170ppm ($\underline{C}O_2$). $[\alpha]_D^{25°}$ = -30.0° (C=1, H_2O).

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