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SYNTHESIS OF RACEMIC [2'-^{13}C] TRYPTOPHAN

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SUMMARY

[2-^{13}C] Indole was synthesized in 39% yield by a three-step procedure starting with K^{13}CN. The labeled indole was converted to D,L[2'-^{13}C] tryptophan in three subsequent steps and the overall yield from K^{13}CN was 13.5%. Proton and ^{13}C-NMR, and GC/mass spectral analyses confirmed the position and extent of ^{13}C incorporation.

INTRODUCTION

The existence of rapid motions in proteins is now broadly accepted (for reviews see references 1-3). NMR relaxation measurements in particular offer effective methods for detecting and quantifying protein dynamics over a broad time range (4, 5). In principle, measurements of the spin-lattice relaxation times (T_1) of proton resonances could also be used to study picosecond dynamics in proteins, but in fact the averaging of protein relaxation rates due to spin-diffusion (6) markedly limits the potential usefulness of this method. In contrast, ^{13}C-T_1 measurements are sensitive to picosecond motions but are of limited value in many proteins because of the low natural abundance of ^{13}C. However, the method is of

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particular value with ^{13}C-enrichment, especially when unique and therefore readily assignable residues are incorporated (7-10).

Other methods have been used to provide data on the existence and nature of protein motions. For example, measurements of time resolved fluorescence anisotropy decay have provided data supporting the existence of subnanosecond motions of intrinsic protein fluorophores (11-14). For a study designed to correlate mobility data on one moiety in a protein using fluorescence and ^{13}C-NMR techniques, it was necessary to prepare a fluorescent amino acid containing a ^{13}C-enriched aromatic ring carbon. To this end, a synthesis of racemic [2'-^{13}C]tryptophan was designed. Starting with ^{13}C-labeled potassium cyanide, C2-enriched indole was prepared. The synthetic indole was subsequently converted into racemic ^{13}C-ring labeled tryptophan by the sequence of reactions shown in Figure 1.

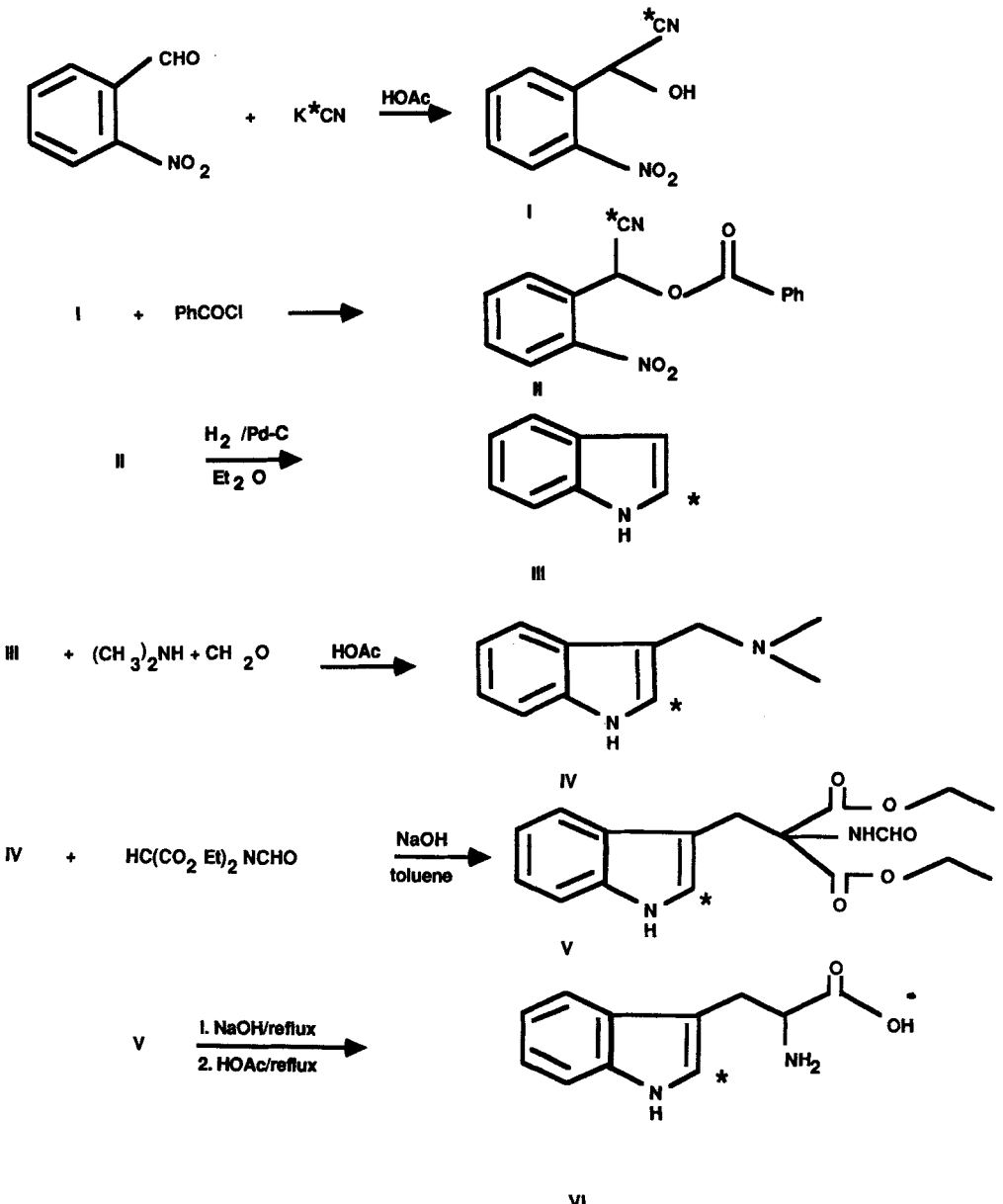
EXPERIMENTAL

Materials and Methods. All of the following chemicals were purchased from Aldrich Chemical Co.: 2-nitrobenzaldehyde, anhydrous pyridine, 37% formaldehyde, 40% dimethylamine and diethyl formamidomalonate. ^{13}C-Potassium cyanide (99%) was obtained from Stohler Isotope Chemicals, Inc. IR spectra were taken with a Perkin Elmer Model 1320 Spectrophotometer. A Carlo-Erbe gas chromatograph/Kratos 500 mass spectrometer was used for GC/MS measurements. ^{1}H and ^{13}C-NMR spectra of purified ^{13}C-labeled indole and tryptophan were recorded on Varian XL-400 and Nicolet (GE) 300 MHz spectrometers, respectively. The chemical shifts reported are in ppm downfield from tetramethylsilane. Melting points are uncorrected and were obtained with a Mel-Temp apparatus.

o-Nitromandelo[^{13}C]nitrile (I). 2-Nitrobenzaldehyde (9.5 g, 62.8 mmol) and ^{13}C-potassium cyanide (4.5 g, 68.1 mmol) were reacted according to the method of Alford and Schofield (15). The crude product was washed with cold water until the wash was neutral (litmus). The pale yellow solid was dried *in vacuo* over phosphorus pentoxide for 8 hr to yield product (9.3 g, 51.9 mmol, 83%: mp = 87-90° C (lit. (15) mp = 92-93° C); IR (KBr) 3500(s), 3480(s), 3120(m), 2260(m), 1525(s), 1390(s), 1180(s), 790(s), and 735 cm^{-1}(s).

o-Nitromandelo[^{13}C]nitrile Benzoate (II). In a modified version of the method of White (16), benzoyl chloride (2.3 mL, 19.8 mmol) was added dropwise with ice-bath cooling to a solution of o-nitromandelo[^{13}C]-nitrile (2.92 g, 16.3 mmol) in 13 mL anhydrous pyridine at 5° C. The deep burgundy solution was stirred at 5-10° C for 0.5 hr, 15 mL of water was added,

FIGURE 1


CHO
NO2
+ K*CN
HOAc
*CN
OH
NO2
I
I + PhCOCl
*CN
O
O
Ph
NO2
II
II
H2 /Pd-C
Et2 O
N
H
*
III
III + (CH3)2NH + CH2O
HOAc
N
N
H
*
IV
IV + HC(CO2 Et)2 NCHO
NaOH
toluene
O
O
NHCHO
O
O
N
H
*
V
V
1. NaOH/reflux
2. HOAc/reflux
O
OH
NH2
N
H
*
VI
* =13 C

and a yellow-brown precipitate formed as the mixture was kept at 5-10° C for 0.5 hr (without stirring). The crude product was collected by filtration, dried *in vacuo* over phosphorus pentoxide for 4 hr, recrystallized from absolute ethanol, and dried *in vacuo* over phosphorus pentoxide for 5 hr to yield product (4.1 g, 14.5 mmol, 90%): mp = 86-87° C (lit. (16) mp = 87-89° C); IR (KBr) 3080(w), 1735(s), 1525(s), 1340(s), 1250(s), 1095(s), 1070(s), 790(s), and 725 cm^{-1}(s).

[2-^{13}C] Indole (III). A crude sample of [2-^{13}C] indole was prepared according to Snyder (17) and White (16) by catalytic hydrogenation of a mixture of o-nitromandelo[^{13}C]nitrile benzoate (4.4 g, 15.5 mmol), 1.8 mL triethylamine, 5.2 g anhydrous magnesium sulfate, 6.5 g 10% Pd/C, and 75 mL ether at 56 p.s.i. for 24 hr. The reduced mixture was acid and base washed, and the product extracted into ether. An ether solution of crude indole was transferred to a silica gel column (2.54 x 21 cm) and eluted with petroleum ether-ethyl ether (1:1 v/v). The fractions containing product were pooled, dried over anhydrous magnesium sulfate, and evaporated *in vacuo*. The residue obtained was sublimed to yield a white solid (706 mg, 6.0 mmol, 39%): mp = 49.5-51° C (lit. (18) mp = 52° C). ^{1}H-NMR ($CDCl_3$) δ 6.58 (pair of t, 1H, H-3), 7.15 (t, 1H, H-6), 7.22 (t, 1H, H-7), 7.41 (d, 1H, H-8), 7.21 (pair of t, 1H, H-2, J_{C-H} = 183 Hz), 7.68 (d, 1H, H-5) and 8.13 (broad S, 1H, NH). ^{13}C-NMR ($CDCl_3$) δ 102.6 (C-3, d, J_{C-C} = 68 Hz), 111.0 (C-7), 119.8 (C-6), 120.7 (C-4), 122.0 (C-5), 124.1 (C-2, ^{13}C-enriched), 127.8 (C-8) and 135.7 (C-9).

3-(Dimethylaminomethyl)-[2-^{13}C]-indole (IV). According to Snyder's (19) modification of the method of Kuhn (20), [2-^{13}C] indole (0.70 g, 5.92 mmol), acetic acid (0.8 mL), 40% dimethylamine (0.78 mL, 6.2 mmol), and 37% formaldehyde (0.445 mL, 5.93 mmol) were mixed together at 5° C and stirred at room temperature for 6 hr. The product was dried overnight *in vacuo* over phosphorus pentoxide to yield a solid (1.03 g, 5.88 mmol, 99%): mp = 126-128° C, some solid remained unmelted, (lit. (19) mp = 127-128° C); IR (KBr) 3100(b), 2940(s), 2820(2), 1455(s), 1410(s), 1350(s), 1240(s), and 740 cm^{-1}(s).

Diethyl formamido(3-[2-^{13}C]indolylmethyl) malonate (V). 3-(Dimethylaminomethyl)-[2-^{13}C]indole (0.5 g, 2.85 mmol), diethylformamidiomalonate (0.6 g, 2.87 mmol), and 35 mg of sodium hydroxide were refluxed for 0.5 hr in toluene according to the procedure of Hellmann (21). The crude product was recrystallized from ethanol-water, and dried overnight *in vacuo* over phosphorus pentoxide to yield a white solid (0.67 g, 2.0 mmol, 70%): mp = 178-180° C (lit. (21) mp = 179° C); IR (KBr) 3410(s), 3465(s), 3060(w), 2980(m), 2910(w), 1740(s), 1710(s),

1660(s), 1490(s), 1240(s), and 1070 cm^{-1}(s).

2-Amino-3,3'-[2-^{13}C]indole propanoic acid (VI). Diethyl formamido (3-[2-^{13}C]indolylmethyl) malonate (1.44 g, 4.32 mmol) was hydrolyzed and decarboxylated according to the method of Hellmann (21) to yield a white solid (654 mg, 3.19 mmol, 74%): mp = 268-270° C(dec). The product was recrystallized from acetic acid and dried overnight *in vacuo* over phosphorus pentoxide to yield D,L-[2'-^{13}C] tryptophan acetate (22) (773 mg, 2.91 mmol, 67%): mp = 278° C(dec); one ninhydrin positive spot on TLC (cellulose, isopropanol-water-ammonium hydroxide, 7:2:1 v/v), Rf = 0.45 (identical to authentic material). Mass (M^+) 653 for the di-N-heptafluorobutyryl isobutyl ester derivative of the ^{13}C-enriched product. ^{1}H-NMR (D_2O, pH2.0) δ 2.08 (s, 3H, acetate-CH_3), 3.34 (m, 1H, β_a), 3.49 (m, 1H, β_b), 4.07 (dd, 1H, α, $J_{\alpha-\beta}$ = 4.2 Hz avg.), 7.31 (d, 1H, indole-2, J_{C-H} = 181.3 Hz), 7.73 (d, 1H, indole-4, J_{H-H} = 7.9 Hz), 7.19 (t, 1H, indole-5, J_{H-H} = 7.4 Hz avg.), 7.28 (t, 1H, indole-6, J_{H-H} = 7.6 Hz avg.) and 7.53 (d, 1H, indole-7, J_{H-H} = 8.1 Hz). ^{13}C-NMR (D_2O, pH2.0) δ 125.8 (d, indole C-2, ^{13}C-enriched).

RESULTS AND DISCUSSION

The synthetic strategy for the preparation of the desired D,L-[2'-^{13}C] tryptophan was to first prepare [2-^{13}C]indole. The indole synthesis was accomplished in three steps in 39% overall yield starting with K ^{13}CN (Figure 1). Confirmation of the specific incorporation of ^{13}C at position 2 of indole was obtained from ^{13}C-NMR data, which included enhancement of the signal at 124.1 ppm and $J_{C_2-C_3}$ equal to 68 Hz. Additionally, J_{C2-H} was determined to be 183 Hz, further corroborating the assigned incorporation.

[2-^{13}C]indole was subsequently converted into D,L-[2'-^{13}C] tryptophan in three steps (Figure 1) using reactions previously described (19-22). The overall yield of labeled tryptophan was 13.5% based on K^{13}CN as starting material. The yields of all reactions except the conversion of o-nitromandelo[^{13}C]nitrile benzoate into [2-^{13}C]indole ranged from 67 to 99%. This reduction-based preparation of [2-^{13}C]indole followed by sublimation gave product in 55% yield, however, a contaminant was present that caused polymerization to occur during attempts to convert [2-^{13}C]indole into 3-(dimethylaminomethyl)-[2^{13}C]indole (IV). Purification of crude [2-^{13}C]indole by silica gel chromatography prior to sublimation lowered the yield of pure [2-^{13}C]indole to 39%, but was necessary to insure the successful preparation of compound IV.

Absorption and fluorescence emission spectra of the labeled product were identical to that of unlabeled commercial D,L-tryptophan. Further, there was no evidence of extraneous materials. The results of GC/MS analysis of the synthetic product showed that only one ^{13}C-carbon atom was incorporated into the indole moiety and that the enrichment was > 95%. Proton and ^{13}C-NMR spectra confirmed that the synthetic product was tryptophan. As noted in the Experimental section, the final product was recrystallized from acetic acid and therefore appears as the acetate salt of the zwitterionic free tryptophan, hence the acetate in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectra showed the chemical shift of the ^{13}C in the decoupled spectrum at 125.8 ppm. The coupling constant for the proton coupled to the enriched carbon at C2 of the indole moiety was 181.3 Hz. Through-bond coupling of the ^{13}C to the β-a and β-b protons of the aminopropanoyl ("alanyl") side chain is also evident, and the average coupling constant is 4.6 Hz. Thus, NMR and GC/MS data demonstrate exclusive and > 95% enrichment of ^{13}C of the 2 position of the indole moiety of D,L-tryptophan.

Additionally, the availability of pure [2-^{13}C]indole from the procedure described here makes possible the synthesis of a wide variety of ^{13}C-enriched natural products containing the important heterocyclic nucleus. Moreover, the preparation of enantiomeric L-[2'-^{13}C]tryptophan may be accomplished from [2-^{13}C]indole by the one step enzymatic method of Marconi and coworkers (23, 24).

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