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THE SYNTHESIS OF E-β-[2-^{14}C] INDOL-3-YLACRYLIC ACID

H. Morrison, Taj Mohammad and B. Severns

Department of Chemistry
Purdue University
West Lafayette, IN 47907

SUMMARY

We report a two step synthesis of indoleacrylic acid with a ^{14}C-label in the 2-position of the indole ring. [2-^{14}C] Indole was formylated at the 3-position with phosphorous oxychloride and DMF. Subsequent condensation with malonic acid provided the title compound with specific activity and radiochemical purity of 112 μCi/mmol and 93% respectively.

Key words: indoleacrylic acid, ^{14}C, synthesis

INTRODUCTION

The photolytic binding of the histidine metabolite, urocanic acid (β-imidazol-4-ylacrylic acid), to DNA has been a subject of considerable recent interest because of the presence of this compound in the skin and because of its light-absorption properties(1). Indoleacrylic acid, a tryptophane metabolite, is also found in animals, and even more extensively in plants(2). The two acrylic acid derivatives may well have the common photobiological role of providing surface defense against foreign organisms by photoinactivating DNA and, in fact, preliminary studies in our laboratory suggest that both agents are capable of photoinactivating phage(3). We have therefore been interested in the possibility that indoleacrylic acid may also undergo light-initiated covalent binding to DNA, and to probe that possibility have developed the synthesis of ^{14}C-labeled substrate described below. Though there are previous reports of the synthesis of indoleacrylic acid labeled with ^{14}C in the β-position of the side chain(4,5), the possibility of chain cleavage made it desirable to have the marker within the indole nucleus itself, so that any covalent attachment of the indole residue

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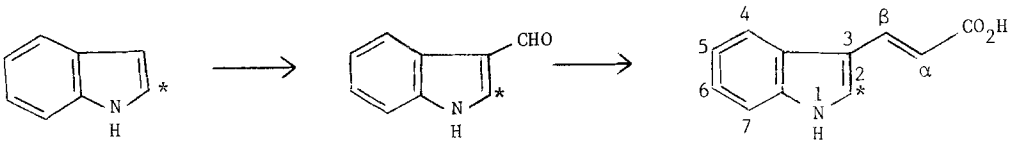
would be detectable. Our synthesis thus complements the previous reports in that the label is incorporated at the 2-position of the ring.

DISCUSSION

[2-^{14}C] Indole was formylated with phosphorous oxychloride and N, N-dimethylformamide at the 3-position by following the literature method(7) reported for the nonlabeled aldehyde, 1. The product was obtained in 76.7% radiochemical yield having chemical and radiochemical purity of 97 and > 99% respectively.



1 2

*Position of ^{14}C-label

The aldehyde was condensed with malonic acid in pyridine/piperidine according to the known procedure(8) with several modifications. The acid 2 was isolated in 43% radiochemical yield having 96 and 93% chemical and radiochemical purity respectively. A product of > 99% radiochemical purity (based on the formation of ~5% Z-isomer during purification) could be obtained by high performance liquid chromatography. An important modification of the condensation step was that the reaction provided the nonlabeled 2 in comparable yield when equimolar quantities of nonlabeled 1 and malonic acid were utilized. This modification can be exploited in the preparation of 2 with a ^{14}C-label in the α-position using ^{14}C-labeled malonic acid and nonlabeled 1. However, we have not pursued this in the present study.

Our initial studies indicate that indoleacrylic acid photolytically binds with DNA, and these results will be published elsewhere.

EXPERIMENTAL

Melting points (uncorrected) were determined on a Fisher-Johns melting point apparatus. Thin layer chromatography (TLC) was performed on pre-coated fluorescent plates of 0.2 mm thickness (Kieselgel $60F_{254}$) using solvent systems A, chloroform:95% ethanol::4:1(v/v); B, tert-butanol:acetic acid:water:::

2:1:0.5(v/v/v); C, chloroform:acetone::9:1(v/v). The spots were visualized under shortwave UV light and/or iodine vapor. Ultraviolet (UV) spectra in 0.1M sodium phosphate buffer, pH 7, were measured on a Hewlett-Packard 8451A Photodiode Array and/or Gilford modified Beckman DU spectrophotometers. Infrared (IR) spectra in KBr disks were recorded on a Perkin-Elmer 1800 Fourier transform spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a General Electric QE-300 spectrometer in acetone-d_6 solutions, and chemical shift values are expressed in δ units relative to internal tetramethylsilane (TMS) at δ 0.00 ppm. Electron impact mass spectra (EIMS) of probe samples were measured on a Finnegan 4000 spectrometer; relative intensity is noted in parentheses after each fragment. Radioactivity measurements were performed by liquid scintillation counting (LSC) on a Packard 300C liquid scintillation spectrometer using ACS® cocktail (Amersham Corp., Arlington Heights, IL), employing appropriate dilutions or fractions collected from the HPLC column. High performance liquid chromatography (HPLC) analysis was effected on a Varian 5000 Liquid Chromatograph using Alltech C-8 reverse phase stainless steel column (4.6 mm x 250 mm), employing an isocratic solvent program using 0.05M sodium phosphate buffer pH 7:HPLC grade methanol::70:30(v/v) for compound 1, and 75:25(v/v) for compound 2, at a flow rate of 1.0 ml min^{-1} with a Varian 2050 variable wavelength detector set at 254 and 280 nm for compound 1 and 2 respectively. [2-^{14}C] Indole (specific activity 49 mCi/mmol) was purchased from Research Products International Corp.

3-[2-^{14}C] Formylindole (1): The pentane solution of [2-^{14}C] indole (54 μCi) was added to the carrier indole (55 mg, 0.47 mmol) (Aldrich Chemical Co.) and the solvent was evaporated under a slow stream of argon. Phosphorous oxychloride (47 μl, 0.51 mmol) was added slowly to N, N-dimethylformamide (dried over molecular sieves type 4A)(0.16 ml, 2 mmol) with stirring at ice-water temperature. A solution of the above diluted [2-^{14}C] indole in N, N-dimethylformamide (40 μl, 0.5 mmol) was added dropwise while maintaining the reaction temperature at 20-30°C. The resultant yellow solution was stirred at 35°C for 1h. Cold distilled water was added to the opaque, canary yellow paste, and the obtained clear yellow solution was treated with 4.8N NaOH (0.47 ml)

dropwise with stirring and cooling until one-third of the NaOH solution had been added. The remaining two-thirds was added rapidly. The resultant suspension was quickly heated to boiling and then allowed to cool to room temperature and stored in the refrigerator overnight. The off-white crystals were collected by filtration, washed with cold water and dried in air to give the aldehyde, 1 (41.4 μCi, specific activity 112 μCi/mmol, 76.7% radiochemical yield). The product exhibited predominantly a single UV and iodine vapor visible spot, R_f 0.68 (solvent A) and 0.24 (solvent C). HPLC analysis (R_t 20.90 min.) indicated a 97% chemical purity, and the combined HPLC-LSC analysis showed > 99% radiochemical purity.

A large-scale cold synthesis of 1 was carried out using the literature procedure(7) to confirm the chemical identity of [2-^{14}C]-1. The product (> 99% pure by HPLC) isolated in 97% yield was crystallized from 95% ethanol as an off-white crystalline solid, mp 196-197°C (lit.(7) mp 197-199°C); UV: λ_{max} nm(ε): 210(21,750), 246(11,480), 262(10,875), 302(11,780); IR: 3160(N-H), 1635(C=O), 1520, 1440, 1390, 1240, 1125, 760 cm^{-1}; ^{1}H NMR: δ 7.22-7.65(m, 3H, ArH), 7.98-8.24 (m, 2H, ArH), 10.03 (s, 1H, CHO), 11.24 (br s, 1H, NH); EIMS: m/z 145 (79,$M^{+\cdot}$), 144(100), 116(27), 89(30), 63(18).

E-β-[2-^{14}C]Indol-3-ylacrylic acid(2): A stirred mixture of [2-^{14}C]-1 (39.2 μCi, 0.35 mmol) and malonic acid (0.107 g, 1.05 mmol) in pyridine (0.35 ml) containing piperidine (7 μl) was maintained at 40°C for 48h. The reaction mixture was cooled and cold distilled water (0.75 ml) was added, followed by basification with 4.8N NaOH at 0°C. The solution was extracted with ether (4 x 1 ml) and the aqueous layer was treated with activated carbon KB(ICI Americans, Inc.). The filtered, light yellow solution was carefully acidified with 5N HCl. The precipitated yellow solid was refrigerated for 1h, filtered, washed with cold water and air dried. The solid was extracted with anhydrous ether (3 x 4 ml) and the ether evaporated to provide [2-^{14}C]-2 (16.8 μCi, specific activity 112 μCi/mmol, 43% radiochemical yield), R_f 0.59 (solvent A), 0.79 (solvent B). HPLC analysis (Rt 7.11 min.) showed a 96% chemical purity and combined HPLC-LSC analysis indicated a 93% radiochemical purity.

A large-scale cold synthesis of 2 was carried out with the modified

literature method(8) to confirm the chemical identity of [2-^{14}C]-2. The product (> 99% pure by HPLC) was obtained after one recrystallization from ethyl acetate in 46% yield as a pale yellow solid. A second recrystallization gave a solid with mp 192-193°C [lit.(8) mp 195°C]; UV: λ_{max} nm(ϵ) 226(19,800), 276(11,400), 316(17,500); IR: 3429, 1690(C=O), 1656(C=C), 1607, 1528, 1416, 1284, 1105, 740 cm^{-1}; ^{1}H NMR: δ 6.44(d, J=16 Hz, 1H, α-H), 7.20-7.27(m, 2H, ArH), 7.50-7.53 (m, 1H, ArH), 7.84-7.99 (m, 3H, ArH, β-H), 10.87 (br s); EIMS: m/z 187(100,$M^{+\cdot}$), 170(38), 159(2), 158(3), 143(4), 141(18), 115(43).

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