LABELED METABOLITES OF POLYCYCLIC AROMATIC HYDROCARBONS IV. 7-HYDROXYBENZO[a]PYRENE-7-^{13}C

W. P. Duncan, W. C. Perry, and James F. Engel

Midwest Research Institute, 425 Volker Boulevard

Kansas City, Missouri 64110

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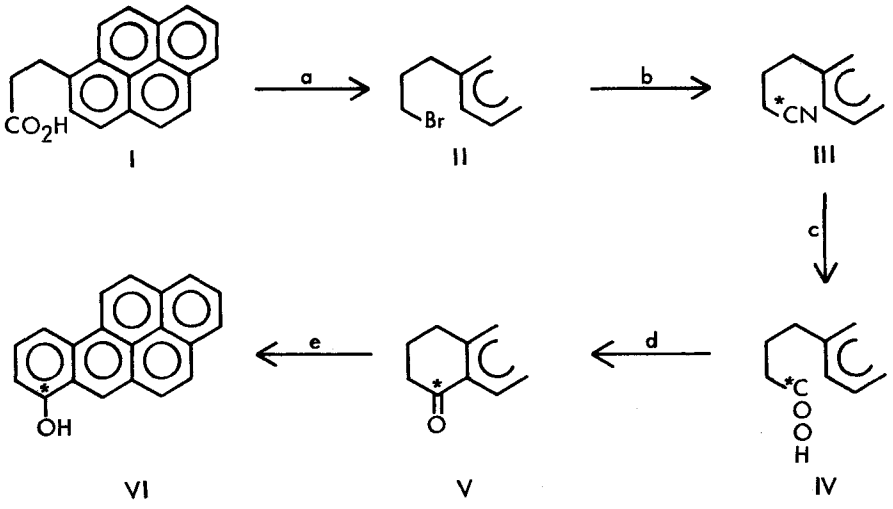
The potential for utilizing carbon-13 labeled compounds in biosynthesis and metabolism studies is well documented [1]. However, to our knowledge specifically carbon-13 labeled derivatives of benzo[a]pyrene (BP) have not been used to study the oxidative metabolism of this biologically important molecule because such labeled materials have not been available.

To facilitate an evaluation of the usefulness of carbon-13 labeled BP derivatives for such investigations we now report the synthesis and carbon-13 nmr spectral data of 7-hydroxybenzo[a]pyrene-7-^{13}C. The synthesis pathway, outlined in Scheme I, uses the known β-1-pyrenylpropionic acid (I) obtained in 56% yield from commercially available 1-pyrenecarboxaldehyde [2] by the malonic ester synthesis [3]. Diborane reduction [4] of I gave a 77% yield of the corresponding alcohol which in turn was converted to the bromide (II) in 68% yield [5]. Reaction of II with potassium cyanide-^{13}C in DMSO [6] followed by hydrolysis [3], cycli-

zation [7], and aromatization [8] afforded 7-hydroxybenzo[a]pyrene-7-^{13}C (VI) in an overall yield of 20% (based on II).

The natural abundance carbon-13 nmr spectrum of benzo[a]pyrene, 0.24 M in chloroform-d, has been reported [9]. The resonance at δ 128.77 was assigned to C-7. We have observed a chemical shift of 154.95 for C-7 of VI using a 0.30 M solution in acetone-d_6 [10].

SCHEME I



I II III IV V VI

[a] B_2H_6, THF, reflux; H_3O^+; ϕ_3P, CBr_4, Et_2O/THF. [b] K*CN, DMSO, 70°.

[c] HOAc/HCl, reflux. [d] HF. [e] MeNaph, reflux.

EXPERIMENTAL

Potassium cyanide-^{13}C was obtained from Pathfinder Laboratories, Inc., St. Louis, Missouri, at an isotopic purity of 98%. IR spectra were determined with a Beckman Acculab I, using Nujol. UV spectra were recorded with a Cary 118 spectrophotometer. Mass spectra were obtained using an Atlas CH-4B medium-resolution mass spectrometer equipped with a Varian 620/i computer. Microanalyses were performed by Galbraith Laboratories,

Inc., Knoxville, Tennessee. All experimental operations were conducted under a nitrogen atmosphere.

The ^{13}C nuclear magnetic resonance spectra were obtained on a Varian XL-100-12 spectrometer with Fourier transform and a 16K 620/L computer. Samples (0.03 M in acetone-d_6) were examined at an ambient temperature of 28° in a 5 mm probe with proton noise decoupling. The ^{13}C spectra represent an average of 84,000 transients. An internal solvent deuterium lock was used, and all chemical shifts are expressed in ppm relative to internal TMS.

3-(1-Pyrenyl)-1-bromopropane (II)

To a stirred solution of 7.1 g (26 mmol) of I and 50 ml of dry THF was slowly added 40 ml of a 1M solution of diborane in THF. After refluxing the resulting reaction mixture overnight, the excess diborane was hydrolyzed by the dropwise addition of 25 ml of 5.5 N HCl to the cooled mixture. An additional 100 ml of water was added and the resulting suspension was extracted with 3 x 100 ml of ether. The extracts were combined, washed with water and 5% sodium bicarbonate solution, dried (Na_2SO_4), filtered and concentrated (in vacuo) to give a yellow-green viscous residue. Column chromatography of this residue on silica gel (E. Merck), 5 cm x 20 cm (packed using cyclohexane), eluting first with 750 ml of benzene followed by 1,500 ml of benzene:ethyl acetate (5:1), gave a light brown solid which upon crystalization from ethanol afforded 5.2 g (73%) of white crystalline alcohol suitable for the next step. The IR was consistent with expected results, i.e., -OH band at 3,300 cm^{-1}.

In a well-dried, 250 ml, round-bottom flask equipped with a magnetic stirrer, reflux condenser and drying tube was placed 5.2 g (20 mmol) of the 3-(1-pyrenyl)-1-propanol, 5.7 g (22 mmol) of triphenylphosphine, 80 ml of anhydrous ether and 20 ml of dry THF. To this stirred solution

was slowly added (dropwise) a solution of 7.3 g (22 mmol) of carbon tetrabromide in 20 ml of THF. An immediate reaction occurred with concomitant precipitation of triphenylphosphine oxide. After refluxing the resulting reaction mixture for 3 hr the cooled solution was filtered and concentrated (in vacuo) to give a light yellow viscous oil. Column chromatography of this residue on silica gel (E. Merck), 5 cm x 20 cm (packed using hexanes), eluting with hexanes, gave a buff solid which upon crystallization from ethanol gave 4.9 g (76%) of II, mp 62-64°C; TLC, R_f 0.68 silica gel/hexanes:acetone (4:1). Anal. Calc. for $C_{19}H_{15}Br$: C, 70.60; H, 4.68; Br, 24.72. Found: C, 70.72; H, 4.74; Br, 24.69; mass spectrum (70eV) m/e (rel. intensity) 324(17), 323(4), 322(18), 242(44), 241(22), 239(20), 227(12), 226(13), 216(28), 215(100), 213(14), 120(12), 119(12), and 94(12).

3-(1-Pyrenyl)-1-cyanopropane-(cyano-^{13}C) (III)

To a well-stirred mixture of 500 mg (7.56 mmol) of potassium cyanide-^{13}C and 120 ml of dimethylsulfoxide at 70° was added 2.45 g (7.56 mmol) of II over a 10-min period. The resulting reaction mixture was heated at 70° for an additional 40 min, cooled, diluted with water (about 200 ml) and extracted with ether. The ether solution was dried (Na_2SO_4), filtered and concentrated (in vacuo) yielding a yellow solid which upon recrystallization from ethanol gave 1.41 g (70%) of III as a light yellow crystalline solid, mp 93-95°; TLC, R_f 0.35 silica gel/hexanes:acetone (4:1). The IR showed a characteristic nitrile band at 2,260 cm^{-1}.

γ-1-Pyrenylbutyric acid-(carboxyl-^{13}C) (IV)

To a stirred mixture of 1.31 g (4.86 mmol) of IV and 20 ml of glacial acetic acid was added 10 ml of concentrated hydrochloric acid. After refluxing this solution overnight, water was added (100 ml) to precipitate the product. The resulting solid was filtered, washed with water, dried (in vacuo) at 30-40° and crystallized from ethyl acetate to yield

1.1 g (77%) of IV as mica-like crystals, mp 184-187°, lit. 187-188° [11]; TLC, R_f 0.24 silica gel/cyclohexane:dioxane (3:2).

9,10-Dihydrobenzo[a]pyrene-7(8H)-one-7-^{13}C (V)

To 1.01 g (3.52 mmol) of IV in a 100 ml polyethylene beaker was added 40 ml of anhydrous HF. The mixture was covered and stirred for 1.5 hr. The HF was evaporated under a slow stream of nitrogen and the resulting solids taken up in benzene. The benzene solution was filtered, washed with water, dried (Na_2SO_4), filtered and concentrated (in vacuo) yielding a yellow-brown residue. Crystallization from benzene followed by recrystallization from xylene afforded 701 mg (74%) of V as yellow plates, mp 173-174°, lit. 174-174.5° [7,8]; TLC, R_f 0.57 silica gel/cyclohexane:dioxane (3:2).

7-Hydroxybenzo[a]pyrene-7-^{13}C (VI)

A mixture of 701 mg (2.60 mmol) of V, 150 mg of 10% Pd/C and 10 ml of methylnaphthalene was heated at reflux for 18 hr. The resulting reaction mixture was cooled, diluted with 15 ml of boiling xylene, filtered to remove the catalyst, and the filter cake was washed with an additional 15 ml of hot xylene. The xylene solutions were combined, concentrated (in vacuo) to near dryness and the resulting solid was recrystallized from xylene to yield 350 mg (50%) of VI as fine yellow needles, mp 216-218° (decomp.), lit. 218-219° [8]; TLC, R_f 0.42 silica gel/benzene:ethanol (19:1); mass spectrum (70eV) m/e (rel. intensity) 270(24), 269(100), 268(7), 240(15), 239(70), 238(8), 237(13).

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REFERENCES

1. (a) Sequin, U., and A. I. Scott, Science, 186, 101 (1974).

   (b) Matwiyoff, N. A., and D. G. Ott, Science, 181, 1125 (1973).

2. Aldrich Chemical Company, Milwaukee, Wisconsin.

3. Bachmann, W. E., and M. Garmack, J. Amer. Chem. Soc., 63, 2494 (1941).

4. Brown, H. C., and B. C. Subba Rao, ibid., 82, 681 (1960).

5. Friederang, A. W., and D. S. Tarbell, J. Org. Chem., 33, 3797 (1968).

6. (a) Friedman, L., and H. Shechter, ibid., 25, 877 (1960).

   (b) Smiley, R. A., and C. Arnold, ibid., 25, 257 (1960).

7. Buchte, F., and P. Vincke, Chem. Ber., 98, 208 (1965).

8. Fieser, L. F., E. B. Hershberg, L. Long, and M. S. Newman, J. Amer. Chem. Soc., 59, 475 (1937).

9. Buchanan, G. W., and R. S. Ozubko, Can. J. Chem., 53, 1829 (1975).

10. This downfield shift is in good agreement with the carbon-13 substituent effect observed for the hydroxyl-bearing carbon of phenol relative to benzene, cf. Levy, G. C., and G. L. Nelson, Carbon-13 Nuclear Magnetic Resonance for Organic Chemists, John Wiley and Sons, Inc., New York, New York, p. 81 (1972).

11. (a) Cook, J. W., and C. L. Hewett, J. Chem. Soc., 398 (1933).

    (b) Fieser, L. F., and M. Fieser, J. Amer. Chem. Soc., 57, 782 (1935).