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Synthesis of $^{13}C_2$-benzo[*a*]pyrene and its 7,8-dihydrodiol and 7,8-dione implicated as carcinogenic metabolites

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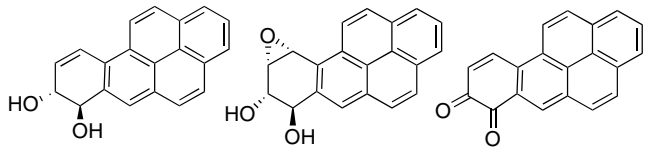
ABSTRACT

Synthesis of the $^{13}C_2$-labelled analogues of the carcinogenic polycyclic aromatic hydrocarbon benzo[*a*]-pyrene and its active metabolites is described. The method entails Pd-catalyzed Suzuki–Miyaura coupling of a naphthalene boronic acid with 2-bromobenzene-1,3-dialdehyde followed by Wittig reaction of the product with $^{13}CH_2{=}PPh_3$.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants that are produced in combustion of fossil fuels and other organic matter.[1–3] They occur commonly in auto and diesel engine exhaust,[4] tobacco smoke[5–7] and smoked and charbroiled meats.[1–3] PAHs have been designated as human carcinogens by the WHO,[2] and they are implicated in the causation of human lung cancer.[5–10]

Benzo[*a*]pyrene (BP) is the prototype PAH carcinogen. It is enzymatically activated to metabolites that react with DNA leading to mutations. The most studied activation path involves cytochrome P-450 [CYP] mediated formation of a dihydrodiol (BP-7,8-diol) followed by its oxidation to a *diol epoxide* (BPDE).[3,11] A second pathway entails aldo-keto reductase-mediated oxidation of BP-7,8-diol to a catechol that enters into a redox cycle with O_2 to form a quinone (BP 7,8-dione) and reactive oxygen species (ROS) that attack DNA.[12,13] A third pathway has also been proposed that entails peroxidase-mediated oxidation of BP to a radical-cation that reacts with DNA to form depurinated adducts.[14,15] However, the relative importance of these pathways for human cancer is not certain.



BP-7,8-diol BPDE BP-7,8-dione

Several advances have been made recently in understanding PAH activation in human cells susceptible to tumourigenesis. An investigation of metabolic activation of BP in human lung A549 cells has provided evidence that the AKR-mediated pathway to generate the BP quinone and ROS is operative in these cells.[16] And human bronchoalveolar H358 cells were recently introduced as a model for study of PAH metabolism in normal human lung cells.[17,18] In this connection, development of a stable isotope dilution liquid chromatography tandem mass spectrometric method for analysis of the metabolites of BP and its nucleoside adducts was also described.[19] In order to improve the scope and sensitivity of this methodology, ^{13}C-labelled analogues of BP and its active metabolites with at least two ^{13}C-atoms in the aromatic ring system are needed as authentic standards.

2. Results and discussion

We now report synthesis of $^{13}C_2$-benzo[*a*]pyrene ($^{13}C_2$-BP), $^{13}C_2$-BP-7,8-diol, and $^{13}C_2$-BP-7,8-dione required as standards for LC–MS/MS analysis of the patterns of BP metabolism and DNA adduct formation in human cells. Syntheses of all twelve of the mono-^{13}C-labelled isomers of BP with a ^{13}C-atom at each of the peripheral carbon atoms of the BP ring system were previously reported.[20] However, the classical synthetic methods employed were not adaptable to preparation of the $^{13}C_2$-labelled BP analogues.

Synthesis of $^{13}C_2$-BP was carried out by a modified version of the new synthetic approach to BP recently reported.[21b] This method involves Pd-catalyzed Suzuki–Miyaura coupling of naphthalene 2-boronic acid (**1a**) with 2-bromobenzene-1,3-dialdehyde (**2**) to provide a dialdehyde product (**3a**) (Scheme 1). In the original approach the next step entailed double Wittig reaction of **3a** with (methoxymethylene)triphenylphosphine ($CH_3OCH{=}PPh_3$)[22] to provide the unlabelled di(methoxyvinyl compound (**4b**). However, the ^{13}C-labelled methoxymethyl halide ($CH_3O^{13}CH_2X$) needed as

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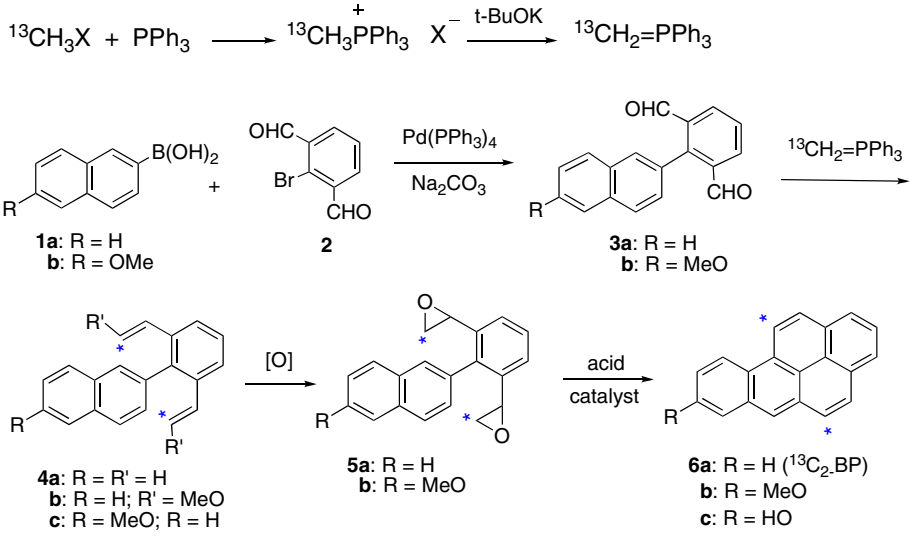


Scheme 1.

the starting compound for preparation of $CH_3O^{13}CH{=}PPh_3$ was unavailable. Fortunately, $^{13}CH_3I$ (99 atom%) was readily obtainable, and it was used for preparation of ^{13}C-methylene)triphenylphosphine ($^{13}CH_2{=}PPh_3$) by reaction with PPh_3 followed by treatment of the resulting phosphonium salt with *n*-BuLi.[23] Double Wittig reaction of $^{13}CH_2{=}PPh_3$ with **3a** afforded a diolefin (**4a**) with ^{13}C-atoms in both vinyl groups.[24] Although the di(methoxyvinyl) analogue (**4b**) was found previously to undergo cyclization smoothly in the presence of an acid catalyst,[21a] analogous reaction of **4a** gave principally polymeric products. This difficulty was avoided by conversion of **4a** to the bis-epoxide (**5a**) by treatment with Oxone/acetone followed by acid-catalyzed cyclization and dehydration to provide $^{13}C_2$-BP (**6a**).[25] The ^{1}H NMR spectrum of **6a** was in good agreement with that of unlabelled BP. Its ^{13}C NMR spectrum exhibited characteristic peaks at δ 122.1 and 128.1, confirming the presence of ^{13}C atoms at the C-5 and C-11-positions of the BP ring system.

Table 1
Effects of reaction variables on cyclization of **5a** to **6a**

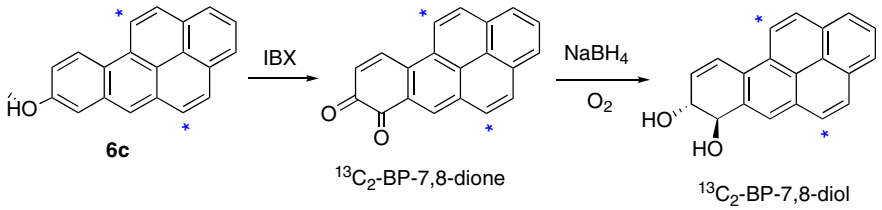
Catalyst[a]	25 °C		70 °C	
	Time (h)	Yield (%)	Time (h)	Yield (%)
$Hf(OTf)_4$	12	52	1.5	84
$Cu(OTf)_2$	12	<10	3.0	31
$In(OTf)_3$	12	<10	3.0	46
$Ce(OTf)_4$	12	13	1.5	51
$InCl_3$	12	<10	3.0	94
$BiOClO_4$	12	<10	6.0	<10
$ZnCl_4$	12	<10	6.0	<10
MSA	12	<10	3.0	80

[a] The molar ratio of the catalyst used was 5% of **5a**. MSA = methanesulfonic acid.

Because the yield of **6a** was found to be dependent upon reaction conditions, a brief study of the influence of conditions on yield was undertaken using unlabelled **5a**. The findings are summarized in Table 1. Significantly higher yields were obtained from reactions carried out at 70 °C than from those conducted at ambient temperature. The optimum yield of BP (94%) was obtained from reaction of **5a** at 70 °C with $InCl_3$ (5%) as the catalyst. Good yields of BP were also obtained from reactions carried out with $Hf(OTf)_4$ and methanesulfonic acid catalysts (84% and 80%, respectively).

$^{13}C_2$-8-Methoxy-BP (**6b**), needed as the starting compound for synthesis of the oxidized metabolites of BP, was prepared by an analogous sequence (Scheme 1). The dialdehyde **3b** needed for this purpose was prepared by Pd-catalyzed Suzuki coupling of 6-methoxynaphthalene-2-boronic acid (**1b**) with **2**, as previously described.[21a] Reaction of **3b** with $^{13}CH_2{=}PPh_3$ furnished $^{13}C_2$-2-methoxy-6-(2,6-divinylphenyl)naphthalene (**4b**). The ^{1}H NMR spectrum of **4b** was in good agreement with that of its unlabelled analogue **4a**,[21a] and its ^{13}C NMR spectrum exhibited a peak at δ 114.6, confirming the presence of ^{13}C-atoms in the vinyl groups. Compound **4b** was converted to the bis-epoxide (**5b**) by treatment with Oxone/acetone.[25] Cyclization of **5b** took place smoothly in the presence $InCl_3$ at 70 °C to provide $^{13}C_2$-8-MeO-BP (**6b**). The ^{1}H NMR spectrum of **6b** was in good agreement with this structural assignment.[21a] Its ^{13}C NMR spectrum contained peaks at δ 122.0 and 128.1, closely similar to the signals found for ^{13}C-BP with ^{13}C in the C-5 and C-11-positions. Demethylation of **6b** with HI/HOAc took place smoothly to furnish $^{13}C_2$-8-HO-BP (**6c**) (97%).[26]

The $^{13}C_2$-labelled oxidized metabolites of $^{13}C_2$-BP were synthesized from **6c** via procedures analogous to those employed for synthesis of the unlabelled analogues (Scheme 2). Thus, oxidation of **6c** with *o*-iodoxybenzoic acid (IBX) in DMF[21a] took place smoothly to furnish the *o*-quinone, $^{13}C_2$-BP-7,8-dione (80%).[27] Reduction of



Scheme 2.

$^{13}C_2$-BP-7,8-dione with $NaBH_4/O_2$[21a,28] furnished $^{13}C_2$-*trans*-7,8-dihydro-7,8-dihydroxybenzo[*a*]pyrene ($^{13}C_2$-BP-7,8-diol).[29] This dihydrodiol may be readily converted to the $^{13}C_2$-*anti*- and *syn*-diol epoxide isomers by the procedures previously described for synthesis of the corresponding unlabelled compounds.[30]

The syntheses described in preceding paragraphs provide convenient access to the $^{13}C_2$-labelled analogues of BP and its presumed carcinogenic metabolites. Synthesis of the $^{13}C_2$-labelled analogues of the potent carcinogenic PAH dibenzo[*def*,*p*]chrysene and its corresponding active metabolites by a different synthetic approach was recently reported by us.[31] In principle, these methods are potentially applicable to synthesis of $^{13}C_2$-labelled analogues of a wide range of other PAH carcinogens and their oxidized metabolites.

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References and notes

1. International Agency for Research on Cancer *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. In *Polynuclear Aromatic Compounds, Part 1, Chemical, Environmental and Experimental Data*; IARC: Lyon, France, 1983; Vol. 32.
2. Straif, K.; Boan, R.; Grosse, Y.; Secretan, B.; Ghissassi, F. E.; Cogliano, V. *Nat. Oncol.* **2005**, *6*, 931–932.
3. Harvey, R. G. *Polycyclic Aromatic Hydrocarbons*; Cambridge University Press: Cambridge, UK, 1991.
4. Marr, L. C. et al *Environ. Sci. Technol.* **1999**, *3*, 3091–3099.
5. International Agency for Research on Cancer *Monographs on the Evaluation of Carcinogenic Risk to Humans*. In *Tobacco Smoke and Involuntary Smoking*; IARC: Lyon, France, 2004; Vol. 83.
6. Pfiefer, G. P.; Denissenko, M. F.; Olivier, M.; Tretyakova, N.; Hecht, S.; Hainaut, P. *Oncogene* **2002**, *21*, 7435–7451.
7. World Health Organization *Tobacco or Health: A Global Status Report*; WHO: Geneva, 1997. pp 10–48.
8. Boffetta, P.; Jourenkova, N.; Gustavsson, P. *Cancer Causes Control* **1997**, *8*, 444–472.
9. Armstrong, B.; Hutchinson, E.; Unwin, J.; Fletcher, T. *Environ. Health Perspect.* **2004**, *112*, 970–978.
10. Rubin, H. *Carcinogenesis* **2001**, *22*, 1903–1930.
11. Baird, W. M.; Hooven, L. A.; Mahadevan, B. *Environ. Mol. Mutagen.* **2005**, *45*, 106–114.
12. Palackal, N. T.; Burczynski, M. E.; Harvey, R. G.; Penning, T. M. *Biochemistry* **2001**, *40*, 10901–10910.
13. Penning, T. M.; Onishi, S. T.; Onishi, T.; Harvey, R. G. *Chem. Res. Toxicol.* **1996**, *9*, 84–92.
14. Cavalieri, E. L.; Rogan, E. G. *Xenobiotica* **1995**, *25*, 677–688.
15. Melendez-Colon, V. J.; Luch, A.; Seidel, A.; Baird, W. M. *Carcinogenesis* **1999**, *20*, 1885–1891.
16. Park, J.; Mangal, D.; Tacka, K. A.; Quinn, A. M.; Harvey, R. G.; Blair, I. A.; Penning, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 6846–6851.
17. Jiang, H.; Gelhaus, S. L.; Mangal, D.; Harvey, R. G.; Blair, I. A.; Penning, T. M. *Chem. Res. Toxicol.* **2007**, *20*, 1331–1341.
18. Ruan, Q.; Gelhaus, S.; Penning, T. M.; Harvey, R. G.; Blair, I. A. *Chem. Res. Toxicol.* **2007**, *2*, 424–431.
19. Ruan, Q.; Kim, H. Y. H.; Jiang, H.; Penning, T. M.; Harvey, R. G.; Blair, I. A. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1369–1380.
20. Bodine, R. S.; Hylarides, M.; Daub, G. H.; VanderJagt, D. L. *J. Org. Chem.* **1978**, *43*, 4025–4028; Simpson, J. E.; Daub, G. H.; VanderJagt, D. L. *J. Labelled Compd. Radiopharm.* **1980**, *17*, 895; Klassen, S. E.; Daub, G. H.; VanderJagt, D. L. *J. Org. Chem.* **1983**, *48*, 4361–4366; Bodine, R. S.; Daub, G. H.; VanderJagt, D. L. *J. Labelled Compd. Radiopharm.* **1984**, *22*, 475–485.
21. (a) Harvey, R. G.; Dai, Q.; Ran, C.; Penning, T. M. *J. Org. Chem.* **2004**, *69*, 2024–2032; (b) Harvey, R. G.; Lim, K.; Dai, Q. *J. Org. Chem.* **2004**, *69*, 1372–1373.
22. $CH_3OCH{=}PPh_3$ was prepared by reaction of CH_3OCH_2Br with PPh_3 and treatment of the resulting phosphonium salt with *t*-BuOK (Ref. 21a).
23. $^{13}CH_2{=}PPh_3$ was prepared via reaction of $^{13}CH_3I$ (99 atom%) with PPh_3 and treatment of the phosphonium salt with *n*-BuLi. Thus, to a suspension of $^{13}CH_3PPh_3I$ (2.0 g, 4.9 mmol) in anhydrous THF (20 mL) at −78 °C under argon was added a solution of *n*-BuLi (2.0 mL of a 2.5 M solution in THF, 5.0 mmol). The solution was stirred at −78 °C for 20 min, then the cooling bath was removed, and stirring was continued for an additional 30 min.
24. $^{13}C_2$-2-(2,6-divinylphenyl)naphthalene (**4a**) was synthesized by reaction of **3a** with $^{13}CH_2{=}PPh_3$ prepared as described above. To the solution of $^{13}CH_2{=}PPh_3$ was added a solution of **3a** (0.70 g, 2.45 mmol) in THF (10 mL), and the solution was stirred for 30 min when TLC indicated reaction to be complete. Conventional work-up and flash chromatography on silica gel eluted with hexane/EtOAc (15:1) gave **4a** (0.68 g, 98%) as a colourless oil; the ^{1}H NMR spectrum was in agreement with that for unlabelled **4a**; ^{13}C NMR ($CDCl_3$) δ 114.7. This compound was unstable and deteriorated on standing.
25. $^{13}C_2$-Benzo[*a*]pyrene (**6a**) was synthesized from **4a** via conversion to the bis-epoxide (**5a**) and acid-catalyzed cyclization. To a solution of **4a** (0.29 g, 1.0 mmol) in EtOAc/acetone/H_2O (20:10:10) was added $NaHCO_3$ (0.63 g, 7.5 mmol), and to this solution was added dropwise a solution of Oxone (5.4 g, 9.0 mmol) in (30 mL) over a 4 h period. Stirring was continued for an additional 3 h, then the solvent was evaporated. The product was dissolved in EtOAc and purified by flash chromatography to provide **5a** (290 mg). This was dissolved in $CHCl_3$ (7.2 mL), $InCl_3$ (11 mg) was added, and the mixture was heated at reflux for 12 h. Flash chromatography of the product provided **6a** (70%); the ^{1}H NMR spectrum was in generally good agreement with that of unlabelled BP; ^{13}C NMR ($CDCl_3$) δ 122.1 and 128.1.
26. A suspension of **6b** (40 mg, 0.14 mmol) in 57% HI (5 mL) and HOAc (5 mL) was stirred at 140 °C until TLC showed reaction to be complete (1.5 h). Then the solution was cooled to room temperature, and poured into ice water (50 mL) to afford **6c** (37 mg, 97%) which was used directly in the next step.
27. The ^{13}C NMR spectrum of $^{13}C_2$-BP-7,8-dione exhibited characteristic signals at δ 122.9 and 128.8, corresponding to the ^{13}C-atoms in the C-5 and C-11-positions.
28. Sukumaran, K. B.; Harvey, R. G. *J. Org. Chem.* **1980**, *45*, 4407–4413.
29. The ^{13}C NMR spectrum of $^{13}C_2$-BP-7,8-diol showed signals at δ 122.5 and 127.6 corresponding to the ^{13}C-atoms in the C-5 and C-11-positions.
30. Harvey, R. G. In *Polycyclic Hydrocarbons and Carcinogenesis*; Harvey, R. G., Ed.; ACS Symp. Series, No. 283; Amer. Chem. Soc.: Washington, DC, 1985; pp 35–62.
31. Xu, D.; Duan, Y.; Blair, I. A.; Penning, T. M.; Harvey, R. G. *Org. Lett.* **2008**, *10*, 1058–1062.