Analysis of Benzo(a)pyrene:DNA Adducts Formed in Cells in Culture by Immobilized Boronate Chromatography

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ABSTRACT

A chromatographic procedure using boronic acid residues linked to a cellulose support (||N-N'||-[m-(dihydroxyboryl)phenyl]succinamyl[amino][ethyl cellulose], used by Sawicki et al. (Cancer Res., 43: 3212-3218, 1983) for analysis of 7,12-dimethylbenz(a)anthracene:DNA adducts, was modified to allow the analysis of benzo(a)pyrene (BaP):DNA adducts formed in cells in culture. Adducts resulting from reaction of 7,8,9,10a-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (anti-BaPDE) contain cis-vicinal hydroxyl groups that complex with the boronic acid residues; adducts resulting from 7,8,9-epoxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (syn-BaPDE) do not. A mixture of [3H]-syn-BaPDE:deoxyguanosine (dGuo) adduct and [14C]-anti-BaPDE:dGuo adduct was completely resolved on a column of boronate:cellulose.

Early-passage cultures of Sencar mouse, Syrian hamster, and Wistar rat embryo cells and a culture of a human hepatoma cell line (Hep G2) were exposed to [3H]BaP, and the BaP:DNA adducts were resolved by boronate chromatography and high-performance liquid chromatography. The Hep G2 cells and mouse embryo cells contained two major adducts, a (+)-anti-BaPDE:dGuo adduct and a syn-BaPDE:dGuo adduct. Boronate chromatography permitted the resolution of an additional minor syn-BaPDE:deoxyribonucleoside adduct in the mouse embryo cells. The hamster and rat embryo cells contained a number of major BaP:DNA adducts that were resolved by boronate chromatography followed by high-performance liquid chromatography. The rat embryo cells contained three syn-BaPDE:deoxyribonucleoside adducts and approximately equal amounts of two adducts tentatively identified as dGuo adducts of the (+) and (−) enantiomers of anti-BaPDE. The boronate chromatography-high-performance liquid chromatography procedure improves the separation of the BaP:DNA adducts formed in biological systems and facilitates the identification of the BaP metabolite(s) responsible for the formation of these adducts.

INTRODUCTION

Determination of the role of individual as well as specific combinations of carcinogenic polycyclic aromatic hydrocarbon:DNA interaction products in the induction of neoplasia depends on the ability to separate and identify the individual DNA adducts formed in cells in culture and in vivo. Studies with other classes of carcinogens, such as methylating agents, have demonstrated the importance of specific alkylated bases in the induction of biological effects (reviewed in Ref. 9). DNA adducts formed by reaction of syn- or anti-BaPDE or 9-hydroxy-BaP 4,5 oxide have also been shown to differ in relative mutagenic potency in a Salmonella typhimurium assay (8). Brookes and Osborne (5) have demonstrated that the (+) and (−) enantiomers of BaPDE differ in mutagenic potency per DNA adduct formed in the V79 Chinese hamster cell line and that the types of DNA adducts formed by these enantiomers differ. The presence of the (+)-anti-BaPDE:dGuo adduct has been shown to correlate with mutation induction in human cells (19), but these cells were able to repair this adduct by an error-free process (27, 28). The biological effects induced by the reaction of hydrocarbon metabolites with DNA may depend upon the specific adducts formed; therefore, it is essential to be able to analyze the specific hydrocarbon adducts present in the DNA.

BaP:deoxyribonucleoside adducts from DNA of BaP-treated cells were initially analyzed by Sephadex LH-20 chromatography (1). The subsequent use of reverse-phase HPLC (11) allowed the separation of many of the individual deoxyribonucleoside adducts. The major BaP:deoxyribonucleoside adducts in a number of cell systems have been shown to result from the binding of the syn (epoxide on the same face of the molecule as the benzylic hydroxyl group) and anti (epoxide on the opposite face of the molecule from the benzylic hydroxyl group) isomers of BaPDE with DNA (reviewed in Ref. 6, Table 5, and in Ref. 21). Anti-BaPDE has been shown to react with the 2-amino group of dGuo (13, 17, 18, 20), the 6-amino group of dAdo (12), and dCyd (14). However, neither Sephadex LH-20 chromatography nor reverse-phase HPLC has the ability to completely resolve all of the BaP:deoxyribonucleoside adducts formed by reaction of anti- and syn-BaPDE with DNA (11). King et al. (15) separated the BaP:deoxyribonucleoside adducts formed from the reaction of syn- and anti-BaPDE with DNA by eluting Sephadex LH-20 columns with methanol containing sodium borate. The borate complexed with the cis-vicinal hydroxyl groups present on the adducts formed from anti-BaPDE, and they eluted earlier than adducts formed from syn-BaPDE. This technique has been used by several research groups to determine the proportions of the deoxyribonucleoside adducts formed by the syn and anti isomers of BaPDE in cells in culture treated with BaP (2, 22, 24); however, it does not allow the resolution of the individual adducts. Attempts to analyze the individual deoxyribonucleoside adducts present in the syn- and anti-BaPDE adduct fractions by subsequent reverse-phase HPLC were unsuccessful (2).

Sawicki et al. (23) employed a chromatographic technique based upon the use of immobilized dihydroxyboryl groups (10, 26) to separate the deoxyribonucleoside adducts formed from...
the syn and anti diol-epoxides of DMBA. In this paper, we describe a modified procedure which allows the separation of BaP:deoxyribonucleoside adducts formed by the syn and anti isomers of BaPDE and its application to the analysis of BaP:deoxyribonucleoside adducts formed in cells in culture.

MATERIALS AND METHODS

Preparation of [3H]BaP-DNA Adducts. Primary embryonic cell cultures were prepared from 13th-day Sencar mouse embryos (Harlan Sprague-Dawley, Inc., Indianapolis, IN), 13th-day Syrian hamster embryos (Engle Laboratory Animals, Inc., Farmersburg, IN), and 17th-day Wistar rat embryos (Harlan Sprague-Dawley, Inc., Indianapolis, IN) as described previously (4). Tertiary monolayer cultures were prepared in 175-cm² culture flasks with 50.0 ml minimum Eagle's medium (Grand Island Biological Co., Grand Island, NY) or basal medium Eagle's (M. A. Bioproducts, Walkersville, MD) containing 10% fetal bovine serum (Reheis Chemical Co., Phoenix, AZ). The Hep G2 cell line, a human hepatoma cell line which has retained a number of differentiated characteristics including the ability to metabolize BaP (16), was obtained from Dr. Barbara Knowles, The Wistar Institute, and grown as described previously (4). [G-3H]BaP (Amersham, Arlington Heights, IL) was diluted with BaP (Gold Label; Aldrich Chemical Co., Milwaukee, WI) to the specific activities stated in “Results” and was added to the cultures at a final concentration of 0.5 μg/ml medium. After 24 hr, the cells were harvested with trypsin:Versene, and the nuclei were isolated by homogenization of the pellet in a hypotonic buffer containing Triton-X (3) and stored at −80°. The DNA was isolated by treatment with RNase A (Sigma Chemical Co., St. Louis, MO) and proteinase K (Sigma) followed by CHCl₃:isoamyl alcohol (24:1, v/v) extraction and ethanol precipitation. After 3 repetitions, the DNA was precipitated first with 2-ethoxyethanol and then with ethanol. The DNA was enzymatically degraded to deoxyribonucleosides (1). Cation-exchange HPLC analysis of the deoxyribonucleosides demonstrated that the DNA was essentially free of RNA. BaP:deoxyribonucleoside adducts were isolated by chromatography on a Quik-Sep Sephadex LH-20 column (Isolab, Inc., Akron, OH) (3), an aliquot was reserved for HPLC analysis, and the remainder was used for boronate chromatography.

HPLC Analysis of BaP-DNA Adducts. The samples were chromatographed by HPLC on a 25-cm x 4.6-mm Ultrasphere octyl reverse-phase column (Beckman Instruments, Inc., St. Louis, MO). The column was eluted with methanol:water (46:54) for 34 min at a flow rate of 1.0 ml/min, then for 10 min with a linear gradient of methanol:water (46:54 to 55:45), followed by 24 min with methanol:water (55:45). Fifteen 1.0-ml fractions were collected, each 3 min each were collected in scintillation vials. Radioactivity was determined by liquid scintillation counting using ACS (Amersham, Arlington Heights, IL). An aliquot of [3H]-anti-BaPDE:dGuo marker, prepared by reaction of [3H]-anti-BaPDE (National Cancer Institute, Radiochemical Repository) with calf thymus DNA as described previously (3), was added to each sample as a marker.

Boronate Chromatography. Each BaP:deoxyribonucleoside adduct sample was evaporated to 0.5 ml, diluted with 1.5 ml of 1 M morpholine (pH 9.0), and applied to a 1.0-g (0.9-x 3-cm) column of [N-9-M-β-Dihydroxyboryl]phenyl]-succinamyl]aminol]ethyl cellulose as described by Ho et al. (10) and Weith et al. (26). The column was eluted with 40 ml of 1 M morpholine (pH 9.0), followed by 40 ml of 1 M morpholine:10% sorbitol, pH 9.0. Eighty 1.0-ml fractions were collected, and 0.1-ml aliquots of all fractions were removed and placed in scintillation vials. Radioactivity was determined by liquid scintillation counting in Ready-Solv HP (Beckman Instruments, Inc., Fullerton, CA). Morpholine buffer fractions containing radioactivity were pooled and applied to a Quik-Sep Sephadex LH-20 column. After elution of the buffer with water, the BaP-modified deoxyribonucleosides were eluted with methanol. BaP:deoxyribonucleoside adducts eluted in the morpholine:sorbitol buffer fractions were prepared by the same procedure. The BaP:deoxyribonucleosides were then chromatographed by HPLC as described above. The boronate column packing material was regenerated by washing with 30 ml 0.05 M ammonium acetate (pH 4.5) and stored in 95% ethanol.

RESULTS

Boronate Chromatography of BaPDE:DNA Adduct Markers. To determine if the BaP:deoxyribonucleoside adducts formed from the syn and anti isomers of BaPDE could be separated by boronate chromatography, a mixture of [3H]-syn-BaPDE:dGuo adduct and [14C]-(+)-anti-BaPDE:dGuo adduct was applied to a column of boronate:cellulose. Elution with morpholine removed greater than 98% of the tritiated material, and subsequent elution with buffer containing 10% sorbitol removed essentially all of the [14C]-labeled material (Chart 1). Thus, boronate chromatography allowed complete resolution of the syn- and anti-BaPDE:dGuo adducts.

To verify the resolution of this technique and to determine if the syn- and anti-BaPDE:dGuo adducts isolated by boronate chromatography were suitable for subsequent HPLC analysis of the individual adducts, the morpholine buffer fractions and the morpholine:sorbitol buffer fractions were pooled, and the adducts were concentrated by Sephadex LH-20 chromatography and then analyzed by reverse-phase HPLC. The morpholine buffer fractions contained one peak of radioactive material that eluted in the same position as did a syn-BaPDE:dGuo marker. The morpholine:sorbitol buffer fractions also contained only one peak of radioactive material that eluted in the same volume as an (+)-anti-BaPDE:dGuo marker. The morpholine buffer fractions did not contain any detectable anti-BaPDE:deoxyribonucleoside adduct, and the morpholine:sorbitol buffer fractions contained no detectable syn-BaPDE:deoxyribonucleoside adduct. Thus,

4 E. Pfannkoch, personal communication.
BaP:DNA Adducts in Sencar Mouse Embryo Cell Cultures. Sencar mouse embryo cell cultures were exposed to [G-3H]BaP (0.5 µg/ml medium; specific activity, 5.68 Ci/mmol) for 24 hr. The BaP:deoxyribonucleoside adducts present in the DNA were isolated, and an aliquot was analyzed by reverse-phase HPLC (Chart 2A). The sample contained 2 major adduct peaks; the larger eluted in the same position as did the (+)-anti-BaPDE:dGuo marker, and the smaller eluted later in the same relative position to this marker as a [3H]-syn-BaPDE:dGuo adduct. The remainder of the BaP:deoxyribonucleoside adducts was analyzed by boronate chromatography (Chart 3A). The morpholine buffer fractions contained 17.7% of the radioactivity, and the morpholine:sorbitol buffer fractions contained 82.3%. The radioactive material in each buffer was analyzed by reverse-phase HPLC (Chart 2B and C). The morpholine buffer fractions (Chart 2B) contained 2 adduct peaks, the larger of which eluted in the same relative position as a marker of syn-BaPDE:dGuo. A small amount of another BaP:deoxyribonucleoside adduct eluted prior to the [14C]-(+)-anti-BaPDE:dGuo marker. The morpholine:sorbitol buffer fractions (Chart 2C) contained essentially a single adduct peak that coeluted with the [14C]-(+)-anti-BaPDE:dGuo marker. The relative ratio of the syn- to anti-BaPDE:deoxyribonucleoside adducts calculated from the total BaP:deoxyribonucleoside adduct HPLC (Chart 2A) was 1:4, a value in agreement with the ratio calculated from the boronate chromatogram (Chart 3A).

BaP:DNA Adducts in Hep G2 Cell Cultures. Hep G2 cell cultures were exposed to [G-3H]BaP (0.5 µg/ml medium; specific activity, 5.0 Ci/mmol) for 24 hr. The BaP:deoxyribonucleoside adducts were isolated from the DNA, and an aliquot was analyzed by reverse-phase HPLC (Chart 4A). The sample contained 2 major adduct peaks: a large peak of BaP:deoxyribonucleoside adduct which coeluted with the [14C]-(+)-anti-BaPDE:dGuo marker, and a small peak of material that eluted in the same position as a syn-BaPDE:dGuo marker. Chromatography of the remainder of the BaP:deoxyribonucleoside adduct sample on a
boronate column resulted in the elution of 23.0% of the radioactivity in the morpholine buffer and 76.9% in the morpholine:sorbitol buffer. The morpholine buffer fractions (Chart 4B) contained 2 adducts that were resolved by HPLC: a major adduct peak that eluted in the same position as a syn-BaPDE:dGuo marker, and a small amount of a second syn-BaPDE:deoxyribonucleoside adduct that eluted prior to the \([^{14}C]^{-}\text{anti-BaPDE:dGuo}\) marker. The morpholine:sorbitol buffer fractions (Chart 4C) contained essentially one adduct peak which coeluted with the \([^{14}C]^{-}\text{anti-BaPDE:dGuo}\) marker.

**BaP:DNA Adducts in Syrian Hamster Embryo Cell Cultures.** Syrian hamster embryo cell cultures were exposed to [G-\(^3\)H]BaP (0.5 \(\mu\)g/ml medium; specific activity, 5.0 Ci/mmol) for 24 hr. The BaP:deoxyribonucleoside adducts were isolated, and an aliquot was analyzed by reverse-phase HPLC (Chart 5A). The sample contained 4 major adduct peaks (Chart 5A, a, b, c, and d), the largest of which, c, coeluted with the \([^{14}C]^{-}\text{anti-BaPDE:dGuo}\) marker. Analysis of the remainder of the sample by boronate chromatography resulted in the elution of 39.4% of the radioactivity in the morpholine buffer fractions and 60.6% in the morpholine:sorbitol buffer fractions. The radioactive material in each buffer was analyzed by reverse-phase HPLC (Chart 5, B and C). The morpholine buffer fractions (Chart 5B) contained 2 major adduct peaks, the larger of which, d, eluted in the same position relative to the \([^{14}C]^{-}\text{anti-BaPDE:dGuo}\) marker. The smaller adduct peak, b, eluted prior to the \([^{14}C]^{-}\text{anti-BaPDE:dGuo}\) marker. The morpholine:sorbitol buffer fractions (Chart 5C) contained a major peak that coeluted with the \([^{14}C]^{-}\text{anti-BaPDE:dGuo}\) marker, c, and a small earlier-eluting adduct peak, a, that has tentatively been identified as the adduct formed from the reaction of \(-\text{anti-BaPDE}\) with dGuo. Thus, both enantiomers of anti-BaPDE as well as syn-BaPDE are involved in the binding of BaP to DNA in these cells.

**BaP:DNA Adducts in Wistar Rat Embryo Cell Cultures.** Wistar rat embryo cell cultures were exposed to [G-\(^3\)H]BaP (0.5 \(\mu\)g/ml medium; specific activity, 10.0 Ci/mmol) for 24 hr. Reverse-phase HPLC analysis of an aliquot of the BaP:deoxyribonucleoside adducts demonstrated the presence of 5 major BaP:deoxyribonucleoside adduct peaks (Chart 6A, a, b, c, d, and e). Two partially resolved adduct peaks, b and c, constituted a large portion of the adducts, but quantitation of the individual adducts was impossible. Analysis of the adducts by boronate chromatography (Chart 3B) showed that 49.1% of the radioactivity eluted in the morpholine buffer and 49.9% eluted in the morpholine:sorbitol buffer. The morpholine buffer fractions contained 3 adduct peaks that were completely resolved by reverse-phase HPLC (Chart 6B, b, d, and e). The largest peak had an elution position similar to that of a syn-BaPDE:deoxyribonucleoside adduct observed in the Syrian hamster embryo cells, but this adduct represented only a minor portion of the total syn-BaPDE:deoxyribonucleoside adducts present in those cells (Chart 5B, Peak b). The Wistar rat embryo cells contained a smaller amount of the syn-BaPDE:dGuo adduct (Peak d) which was the major syn adduct present in the Hep G2, mouse embryo, and hamster embryo cell cultures. An additional late-eluting adduct peak, e, was also present in the Wistar rat embryo cell cultures. Reverse-phase HPLC of the morpholine:sorbitol buffer fractions demonstrated the presence of 2 major adduct peaks (Chart 6C, a and c). The later-eluting peak, c (representing 54% of the radioactivity), coeluted with the \([^{14}C]^{-}\text{anti-BaPDE:dGuo}\) marker. The earlier-eluting adduct peak, a (representing 46% of the radioactivity), eluted in the same position relative to the \([^{14}C]^{-}\text{anti-BaPDE:dGuo}\) marker as a marker of \(-\text{anti-BaPDE}\).
Chart 5. HPLC profile of BaP:deoxyribonucleoside adducts present in Syrian hamster embryo cells after exposure to 0.5 µg [3H]BaP (5.0 Ci/mmol) per ml medium for 24 hr. The BaP:deoxyribonucleoside adducts were prepared and analyzed as described in Chart 2. A, aliquot of BaP:deoxyribonucleoside adducts prior to boronate chromatography; B, BaP:deoxyribonucleoside adducts present in 1 M morpholine fractions; C, BaP:deoxyribonucleoside adducts present in 1 M morpholine:10% sorbitol fractions. Arrow, elution position of [14C]-(+)-anti-BaPDE:dGuo adduct marker.

Chart 6. HPLC profile of BaP:deoxyribonucleoside adducts present in Wistar rat embryo cells after exposure to 0.5 µg [3H]BaP (10 Ci/mmol) per ml medium for 24 hr. The BaP:deoxyribonucleoside adducts were prepared and analyzed as described in Chart 2. A, aliquot of BaP:deoxyribonucleoside adducts prior to boronate chromatography; B, BaP:deoxyribonucleoside adducts present in 1 M morpholine fractions; C, BaP:deoxyribonucleoside adducts present in 1 M morpholine:10% sorbitol fractions. Arrow, elution position of [14C]-(+)-anti-BaPDE:dGuo adduct marker.
BaPDE:dGuo. The combined boronate chromatography reverse-phase HPLC procedure resolved this very complex BaP:deoxyribonucleoside adduct profile into individual adducts and allowed the identification of 2 enantiomeric anti-BaPDE:dGuo adduct peaks.

DISCUSSION

The theoretical basis for the separation of the BaP:deoxyribonucleoside adducts formed by the anti and syn isomers of BaPDE by boronate chromatography is illustrated in Chart 7. The terminal boronic acid group contains 2 hydroxyls that can complex with cis-vicinal hydroxyl groups, but not with trans-hydroxyl groups (26). The adducts formed from syn-BaPDE contain trans-diols and do not complex with the immobilized dihydroxyboryl groups (Chart 7, right); therefore, these adducts are not retained by the column. The adducts formed from anti-BaPDE contain cis-vicinal hydroxyl groups (on positions 8 and 9) which can complex with the immobilized dihydroxyboryl groups (Chart 7, left). The boronate complex formed with the anti-BaPDE:deoxyribonucleoside adducts can be reversed upon the addition of 10% sorbitol to the eluting buffer. This allows elution of the adducts formed from anti-BaPDE from the column. We have demonstrated that the boronate chromatography technique is capable of resolving a [3H]-syn-BaPDE:dGuo adduct from a [14C]+)-anti-BaPDE:dGuo adduct and that the separated adduct fractions are suitable for subsequent analysis by reverse-phase HPLC (Chart 1).

Sawicki et al. (23) utilized a similar chromatographic method for the separation of deoxyribonucleoside adducts formed from DMBA. They found that the addition of 2 M urea to the 1 M morpholine eluting buffer achieved optimum peak sharpness and elution position for the DMBA:deoxyribonucleoside adducts and that the adducts formed from each isomer of DMBA diol-epoxide eluted in 20 ml of buffer. This elution procedure was not successful in separating the deoxyribonucleoside adducts of the 2 isomers of BaPDE, because chromatography of a mixture of [14C]+)-anti-BaPDE:dGuo adduct and [3H]+)-syn-BaPDE:dGuo adduct resulted in elution of some of the 14C-labeled material as well as 3H-labeled material prior to the addition of sorbitol to the eluting buffer. Complete resolution of [14C]+)-anti-BaPDE:dGuo could not be obtained even by doubling the amount of immobilized boronate material used. We found that the BaPDE isomer adducts could be completely resolved by elution with buffers that did not contain urea. In addition, these elution conditions also successfully resolved the isomeric DMBA diol-epoxide:deoxyribonucleoside adducts.5

The combined technique of boronate chromatography followed by reverse-phase HPLC demonstrated the differences in the BaP:deoxyribonucleoside adducts formed in 3 species of rodent embryo cells and a human hepatoma cell line (Hep G2). In some cases, reverse-phase HPLC is sufficient to resolve the adduct peaks; in Hep G2 cells, only 2 major adduct peaks are present, and these are completely resolved by HPLC of the total adduct mixture (Chart 4A). Similarly, in the Sprague-Dawley mouse embryo cells (Chart 4A), 2 well-resolved BaP:deoxyribonucleoside adducts are observed, one of which coelutes with the [14C]+)-anti-BaPDE:dGuo adduct and the other of which elutes in the same position relative to [14C]+)-anti-BaPDE:dGuo as does a syn-BaPDE:dGuo adduct. However, boronate chromatography demonstrates the presence of a smaller syn-BaPDE adduct peak (Chart 2B, Fractions 65 to 75) which appears as a minor shoulder on the major adduct peak in the sample analyzed prior to boronate chromatography (Chart 2A). Thus, the use of boronate chromatography permits the detection of minor BaP:DNA adducts which would be missed by conventional HPLC analysis.

A very complex mixture of BaP:DNA adducts is formed in the BaP-treated rat and hamster embryo cell cultures. Analysis of BaP:deoxyribonucleoside adducts formed in Syrian hamster embryo cells by HPLC resulted in the separation of 4 adduct peaks, 2 of which, b and c, were poorly resolved (Chart 5A). Ivanovic et al. (11) have demonstrated previously the complexity of the BaP:DNA adduct profile formed in Syrian hamster embryo cells and have shown that adducts corresponding to the 2 anti-BaPDE isomers were present; however, the syn isomer adduct which elutes between these 2 peaks (Chart 5, Peak b) was apparently not resolved by this procedure. By using boronate chromatography followed by reverse-phase HPLC analysis, Peak b can be resolved from Peak c and identified.

The most complex pattern of BaP:DNA adducts was that present in the Wistar rat embryo cells. In this case, HPLC alone gave only limited resolution of Peaks b and c, and accurate quantitation of the material in these peaks would be difficult (Chart 6A). The boronate chromatography-HPLC procedure allowed the complete resolution of the individual BaP:deoxyribonucleoside adducts present in rat embryo cells (Chart 6, B and C). In addition, the release of the 2 major adducts by sorbitol helped to establish that both resulted from binding of anti-BaPDE to DNA (Chart 6B). The finding that similar proportions of adducts were formed by the (+) and (−) enantiomers of anti-BaPDE suggests that the stereocchemical pathway of metabolic activation of BaP in these cells differs from that observed in most other cells (Ref. 6, Table 5). This result is also surprising in view of the high stereospecificity of metabolism of BaP to the (+)-anti-BaPDE in rat liver enzyme preparations (25). The reasons for the formation of the high proportion of (−) enantiomer and the role of enzyme induction in determining the profile of adducts formed in cells are being investigated. The use of boronate

5 T. Smolarek and W. M. Baird, unpublished results.

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chromatography followed by reverse-phase HPLC will facilitate the further identification of the BaP:deoxyribonucleoside adducts and studies of the role of individual adducts in the induction of biological effects.

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