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In Vitro Metabolism of Dibenzo[*a,l*]pyrene, 2-Chlorodibenzo [*a,l*]pyrene and 10-Chlorodibenzo[*a,l*]pyrene - Effects of Chloro Substitution

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Abstract: Stereoselective metabolism of dibenzo[*a,l*]pyrene (DB[*a,l*]P), 2-chlorodibenzo[*a,l*]pyrene (2-Cl-DB[*a,l*]P) and 10-chlorodibenzo[*a,l*]pyrene (10-Cl-DB[*a,l*]P) by rat liver microsomes was studied and effects of the chloro substituent on the metabolism were determined. All three compounds produced *trans*-8,9-dihydrodiol, *trans*-11,12-dihydrodiol, and the 7-hydroxyl derivative as major metabolic products and several other phenolic derivatives as minor metabolites. The *trans*-8,9- and 11,12-dihydrodiols of DB[*a,l*]P and 2-Cl-DB[*a,l*]P preferentially adopted a quasidiequatorial conformation, whereas 10-Cl-DB[*a,l*]P *trans*-8,9- and 11,12-dihydrodiols preferentially adopted a quasidiaxial conformation. The yields of the *trans*-11,12-dihydrodiol metabolites are: DB[*a,l*]P *trans*-11,12-dihydrodiol > 2-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol >> 10-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol. Circular dichroism (CD) spectral analysis indicates that the *trans*-8,9-dihydrodiol and *trans*-11,12-dihydrodiol metabolites from DB[*a,l*]P, 2-Cl-DB[*a,l*]P, and 10-Cl-DB[*a,l*]P are optically active. Furthermore, the major enantiomeric DB[*a,l*]P *trans*-11,12-dihydrodiol and 2-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol had R,R absolute configuration. Based on the fact that DB[*a,l*]P *trans*-11,12-dihydrodiol is the proximate tumorigenic metabolite of DB[*a,l*]P, our results suggest that DB[*a,l*]P exhibits the highest tumorigenic potency followed by 2-Cl-DB[*a,l*]P, and 10-Cl-DB[*a,l*]P exhibits the lowest tumorigenicity.

Keywords: Microsomal metabolism, dibenzo[*a,l*]pyrene, 2-chlorodibenzo[*a,l*] pyrene, 10-chlorodibenzo[*a,l*]pyrene.

Introduction

Chlorinated polycyclic aromatic hydrocarbons (PAHs) are a class of potent mutagenic and tumorigenic environmental contaminants [1-6]. These environmental compounds are predominantly formed by chlorination of PAHs. Since both chlorinated PAHs and PAHs are genotoxic, it is important to determine the role of chlorination reaction in the environment. It has been found that chlorinated PAHs exhibit mutagenicity and tumorigenicity either higher or lower than the parent PAHs [6]. For example, it was reported that 7-chlorobenz[*a*]anthracene exhibits higher tumorigenicity than the parent compound benz[*a*]anthracene in the neonatal mouse tumorigenicity boiassay [7]. Dibenzo[*a,l*]pyrene (DB[*a,l*]P) is an environmental contaminant exhibiting tumorigenicity much higher than benzo[*a*]pyrene. Its metabolic activation and DNA adduct formation have been widely studied [8-17]. A chemical with a molecular formula matched to that of chlorinated DB[*a,l*]P has been detected in incinerator fly ash [15]. Therefore, it is interesting to determine the biological activities of chlorinated DB[*a,l*]Ps. We report in this paper stereoselective metabolism of DB[*a,l*]P, 2-Cl-DB[*a,l*]P and 10-Cl-DB[*a,l*]P by rat liver microsomes and determination of the effects of the chloro substituent on metabolism. The mutagenicity of these in *Salmonella typhimurium* TA100 tested in the presence of S9 activation enzyme system was also determined.

Materials and Methods

Materials

Immature male Sprague-Dawley rats (150-200 g body weight) were given daily i.p. injections of 3-methylcholanthrene (3-MC) (25 mg/kg body weight) on 3 days and were killed one day after the last injection. Liver microsomes were prepared and the protein content was determined as previously described [18,19]. DB[*a,l*]P and 2-Cl-DB[*a,l*]P were synthesized following the procedures previously published by Vingiello *et al* with modification [20,21]. 10-Cl-DB[*a,l*]P was prepared by chlorination of DB[*a,l*]P with *N*-chlorosuccinimide in dimethylformamide [21]. The structures of the synthesized DB[*a,l*]P, 2-Cl-DB[*a,l*]P, and 10-Cl-DB[*a,l*]P were fully characterized by analysis of their UV-Visible absorption, mass and 500 MHz proton NMR spectral data [21]. NMR samples were measured in acetone-d6 and are reported in ppm downfield from tetramethylsilane. Their proton NMR assignments are shown below.

DB[*a,l*]P: 7.77-7.87 (m, 4, H2, H3, H12, H13), 7.99 (d, 1, $J_{8,9}$ = 9.0 Hz, H8), 8.09 (t, 1, $J_{5,6}$ = 7.7 Hz, H6), 8.11 (d, 1, H9), 8.21 (d, 1, $J_{6,7}$ = 7.5 Hz, H7), 8.40 (d, 1, $J_{11,12}$ = 7.3 Hz, $J_{11,13}$ = 2.4 Hz, H11), 8.70 (s, 1, H10), 9.08 (m, 2, H4 and H5) 9.16 (d, 1, $J_{1,2}$ = 7.7 Hz, $J_{1,3}$ = 1.9 Hz, H1), 9.27 (d, 1, $J_{13,14}$ = 7.3 Hz, $J_{12,14}$ = 1.9 Hz, H14).

2-Cl-DB[*a,l*]P: 7.82 (t, 1, H12 or H13), 7.84 (t, 1, H3), 7.87 (t, 1, H12 or H13), 8.00 (d, 1, $J_{8,9}$ = 9.0, H8), 8.11 (t, 1, $J_{6,7}$ = 7.2 Hz, H6), 8.12 (d, 1, H7), 8.43 (t, 1, $J_{11,12}$ = 8.2 Hz, $J_{11,13}$ = 1.5 Hz, H11),

8.75 (s, 1, H10), 9.07 (d, 1, $J_{5,6}$ = 8.0 Hz, H5), 9.10 (d, 1, $J_{3,4}$ = 8.8 Hz, H4), 9.12 (d, 1, $J_{1,3}$ = 2.4 Hz, H1), 9.20 (dd, 1, $J_{13,14}$ = 8.6 Hz, H14).

10-Cl-DB[*a,l*]P: 7.83 (t, 1, $J_{2,3}$ = 7.7 Hz, $J_{2,4}$ = 1.5 Hz, H2), 7.88 (t, 2, H3, H13), 7.95 (t, 1, $J_{12,13}$ = 7.7 Hz, $J_{12,14}$ = 1.5 Hz, H12), 8.15 (t, 1, $J_{6,7}$ = 7.7, H6), 8.18 (d, 1, $J_{8,9}$ = 9.5 Hz, H8), 8.29(d, 1, H7), 8.55 (d, 1, H9), 8.85 (d, 1, $J_{11,12}$ = 8.4 Hz, $J_{11,13}$ = 1.3 Hz, H11), 9.04 (d, 1, $J_{5,6}$ = 8.2 Hz, $J_{5,7}$ = 1.3 Hz, H5), 9.09 (d, 1, $J_{3,4}$ = 8.4 Hz, H4), 9.16 (d, 1, $J_{1,2}$ = 8.0 Hz, H1), 9.25 (d, 1, $J_{13,14}$ = 8.8 Hz, H14).

DB[*a,l*]P *trans*-11,12-dihydrodiol: 4.75 (dt, 1, $J_{12,13}$ = 10.8 Hz, $J_{12,14}$ = 2.4 Hz, H12), 4.88 (d, 1, $J_{11,12}$ = 11.0 Hz, $J_{11,13}$ = 2.2 Hz, H11), 6.35 (dd, 1, $J_{13,14}$ = 10.1 Hz, H13), 7.36 (dd, 1, H14), 7.69 (t, 1, $J_{2,3}$ = 7.7, $J_{2,4}$ = 1.3, H2), 7.76 (t, 1, $J_{3,4}$ = 8.4 Hz, H3), 8.02 (t, 1, $J_{6,7}$ = 7.7 Hz, H6), 8.10 (AB, 2, $J_{8,9}$ = 8.8, H8 and H9), 8.23 (d, 1, H7), 8.50 (s, 1, H10), 8.61 (dd, 1, $J_{1,2}$ = 8.2 Hz, $J_{1,3}$ = 1.3 Hz, H1), 8.94 (d, 1, H4), 8.97 (d, 1, $J_{5,6}$ = 7.7 Hz, $J_{5,7}$ = 0.9 Hz, H5).

2-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol: 4.77 (dt, 1, $J_{12,13}$ = 11.0 Hz, $J_{12,14}$ = 2.4 Hz, H12), 4.88 (dd, 1, $J_{11,12}$ = 10.8 Hz, $J_{11,13}$ = 2.2 Hz, H11), 6.43 (dd, 1, $J_{13,14}$ = 10.1 Hz, H13), 7.34 (dd, 1, H14), 7.76 (dd, 1, $J_{3,4}$ = 8.6, H3), 8.03 (t, 1, $J_{6,7}$ = 7.7 Hz, H6), 8.11 (AB, 2, $J_{8,9}$ = 8.8 Hz, H8 and H9), 8.26 (d, 1, H7), 8.53 (s, 1, H10), 8.60 (d, 1, $J_{1,3}$ = 2.2 Hz, H1), 8.95 (d, 1, $J_{5,6}$ = 8.0 Hz, H5), 8.96 (d, 1, H4).

10-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol: 4.55 (m, 1, $J_{12,13}$ = 6.0, H12), 5.56 (m, 1, $J_{11,12}$ < 2 Hz, $J_{11,13}$ = 1.1 Hz, H11), 6.53 (dd, 1, $J_{13,14}$ = 9.7 Hz, H13), 7.57 (d, 1, H14), 7.71 (t, 1, $J_{2,3}$ = 7.5, $J_{2,4}$ = 1.3 Hz, H2), 7.80 (t, 1, $J_{3,4}$ = 8.2 Hz, H3), 8.10 (t, 1, $J_{6,7}$ = 7.7 Hz, H6), 8.25 (d, 1, $J_{8,9}$ = 8.8, H8), 8.31 (d, 1, H7), 8.54 (d, 1, H9), 8.57 (d, 1, $J_{1,2}$ = 8.2 Hz, $J_{1,3}$ = 1.3 Hz, H1), 8.94 (d, 1, H4), 9.04 (d, 1, $J_{5,6}$ = 7.7 Hz, H5).

DB[*a,l*]P *trans*-8,9-dihydrodiol: 5.00 (d, 1, $J_{8,9}$ = 9.7 Hz, H8), 5.12 (d, 1, H9), 7.65 (t, 1, $J_{12,13}$ = 7.5 Hz, $J_{12,14}$ = 1.5 Hz, H12), 7.68 (t, 1, $J_{13,14}$ = 7.5 Hz, H13), 7.74 (t, 1, $J_{2,3}$ = 7.7 Hz, H2), 7.76 (t, 1, $J_{3,4}$ = 7.5 Hz, H3), 7.79 (t, 1, $J_{6,7}$ = 7.7 Hz, H6), 8.00 (d, 1, H7), 8.13 (d, 2, $J_{11,12}$ = 7.5 Hz, $J_{11,13}$ = 1.9 Hz, H11), 8.28 (s, 1, H10), 8.76 (d, 1, $J_{5,6}$ = 8.4 Hz, $J_{5,7}$ = 1.1 Hz, H5), 8.91 (d, 1, H4), 8.97 (d, 1, H14), 8.99 (d, 1, $J_{1,2}$ = 7.5 Hz, H1).

2-Cl-DB[*a,l*]P *trans*-8,9-dihydrodiol: 5.00 (d, 1, $J_{8,9}$ = 9.7 Hz, H8), 5.12 (d, 1, H9), 7.68 (t, 1, $J_{12,13}$ = 7.5 Hz, H12), 7.74 (m, 2, H3 and H13), 7.80 (t, 1, $J_{6,7}$ = 7.7 Hz, H6), 8.02 (dt, 1, H7), 8.16 (d, 1, $J_{11,12}$ = 7.7 Hz, $J_{11,13}$ = 1.3 Hz, H11), 8.32 (s, 1, H10), 8.74 (d, 1, $J_{5,6}$ = 8.2 Hz, $J_{5,7}$ = 0.9 Hz, H5), 8.92 (d, 1, $J_{13,14}$ = 8.6 Hz, H14), 8.93 (d, 1, $J_{3,4}$ = 9.0 Hz, H4), 8.96 (d, 1, $J_{1,3}$ = 2.4 Hz, H1).

10-Cl-DB[*a,l*]P *trans*-8,9-dihydrodiol: 5.18 (d, 1, $J_{8,9}$ = 3.2 Hz, H8), 5.89 (d, 1, H9), 7.74 (t, 1, $J_{2,3}$ = 7.7 Hz, $J_{2,4}$ = 1.3 Hz, H2), 7.76-7.81 (m, 4, H3, H6, H12, H13), 8.58 (m, 1, H11), 8.81 (dd, 1, $J_{5,6}$ = 7.1 Hz, $J_{5,7}$ = 1.3 Hz, H5), 8.87 (d, 1, $J_{1,2}$ = 8.2 Hz, $J_{1,3}$ = 1.5 Hz, H1), 8.90 (d, 1, $J_{3,4}$ = 8.2 Hz, H4), 8.97 (m, 1, H14).

7-OH-DB[*a,l*]P: 7.69 (d, 1, H6), 7.71 (t, 1, $J_{2,3}$ = 7.5 Hz, $J_{2,4}$ = 1.3 Hz, H2), 7.75 (m, 2, H12 and H13), 7.77 (t, 1, H3), 8.02 (d, 1, H9), 8.31 (d, 1, $J_{8,9}$ = 9.2 Hz, H8), 8.36 (m, 1, H11), 8.62 (s, 1, H10), 8.93 (d, 2, $J_{3,4}$ = 8.8 Hz, $J_{5,6}$ = 8.8 Hz, H4 and H5), 9.07 (d, 1, $J_{1,2}$ = 8.4 Hz, H1).9.21 (m, 1, H14).

2-Cl-7-OH-DB[*a,l*]P: 7.73 (d, 1, H6), 7.75 (d, 1, $J_{3,4}$ = 9.0 Hz, H3), 7.78 (t, 1, $J_{12,13}$ = 7.5 Hz, $J_{12,14}$ = 1.5 Hz, H12), 7.82 (t, 1, H13), 8.02 (d, 1, H9), 8.32 (d, 1, $J_{8,9}$ = 9.2 Hz, H8), 8.36 (d, 1, $J_{11,12}$ = 7.7 Hz, $J_{11,13}$ = 1.5 Hz, H11), 8.66 (s, 1, H10), 8.90 (d, 1, $J_{5,6}$ = 8.6 Hz, H5), 8.92 (d,1, H4), 9.03 (d, 1, $J_{1,3}$ = 2.4 Hz, H1).9.15 (d, 1, $J_{13,14}$ = 8.6 Hz, H14).

Metabolism of DB[a,l]P, 2-Cl-DB[a,l]P and 10-Cl-DB[a,l]P by rat liver microsomes

Following the previously published procedure with modification [21], metabolism of DB[*a,l*]P, 2-Cl-DB[*a,l*]P and 10-Cl-DB[*a,l*]P was each conducted in a 100 mL reaction mixture containing 5 mmol Tris-HCl**,** pH 7.4, 300 μmol magnesium chloride, 12 μmol $NADP^+$, 200 μmol glucose-6-phosphate, 10 units glucose-6-phosphate dehydrogenase, 100 mg mouse liver microsomal protein, and 8 μmol DB[*a,l*]P, 2-Cl-DB[*a,l*]P, or 10-Cl-DB[*a,l*]P (dissolved in 2 mL acetone). After shaking at 37 ^oC for 1 hr, the reaction was quenched with an equal volume of acetone and the metabolites and residual substrate were partitioned with ethyl acetate (2 x 2 volumes). To stabilize the metabolites, 1% triethylamine was added to the ethyl acetate fraction. The organic phase was collected and the solvent evaporated under reduced pressure. The residue dissolved in 200 μL methanol was separated by reversed-phase HPLC employing a Prodigy 5 μ ODS column (4.6 x 250 mm, Phenomenex, Torrance, CA). The solvent systems used for metabolism of DB[*a,l*]P and 2-Cl-DB[*a,l*]P were: a 70-min linear gradient of 70-100% methanol in water at 1 mL/min; and for 10-Cl-DB[*a,l*]P, a 60-min linear gradient of 40-100% methanol in water at 1 mL/min. Repeated incubations under identical conditions were conducted to obtain sufficient metabolites for structural identification.

Mutagenicity assays

Reversion to prototrophy was measured using *Salmonella typhimurium* histidine auxotrophic strains TA100 as described previously by Maron and Ames [22]. The post-mitochondrial supernatant fraction (S9) was prepared from liver homogenates of Aroclor 1254-pretreated male Sprague-Dawley rats as described by Maron and Ames [22] and used at a concentration of 50 □l/plate. The variability in assays conducted in triplicate was generally $<\pm$20%. All test chemicals were assayed on at least two separate occasions with similar results.

Physiochemical properties of metabolites

UV-Visible absorption spectra of metabolites in methanol were measured on a Beckman DU-65 spectrophotometer. CD spectra of the *trans*-dihydrodiol metabolites were determined in a quartz cell of 1 cm path length on a Jasco 500A spectropolarimeter. CD spectra are expressed as ellipticity for

methanol solutions that read 1.0 Å unit in a UV-Visible spectrophotometer at the wavelength of maximum absorption in a quartz cell of 1 cm length.

Results

HPLC separation and identification of metabolites

Metabolism of DB[*a,l*]P, 2-Cl-DB[*a,l*]P, and 10-Cl-DB[*a,l*]P by liver microsomes of rats pretreated with 3-MC (3-MC-microsomes) was conducted under similar incubation conditions. The resulting metabolites were separated by reversed-phase HPLC. The HPLC profile of DB[*a,l*]P is shown in Figure 1A. The chromatographic peak eluted at 58 min was the recovered substrate, DB[*a,l*]P. Based on comparison of their UV-Visible absorption, mass, and NMR spectra with the previously published data [11,21], the chromatographic peaks *1*, *2*, and *3*, eluted at 23.2, 27.3, and 42.4 min were identified as DB[*a,l*]P *trans*-8,9-dihydrodiol, DB[*a,l*]P *trans*-11,12-dihydrodiol, and 7-OH-DB[*a,l*]P, respectively. Their 500 MHz proton NMR spectral data are listed in Material and Method Section.

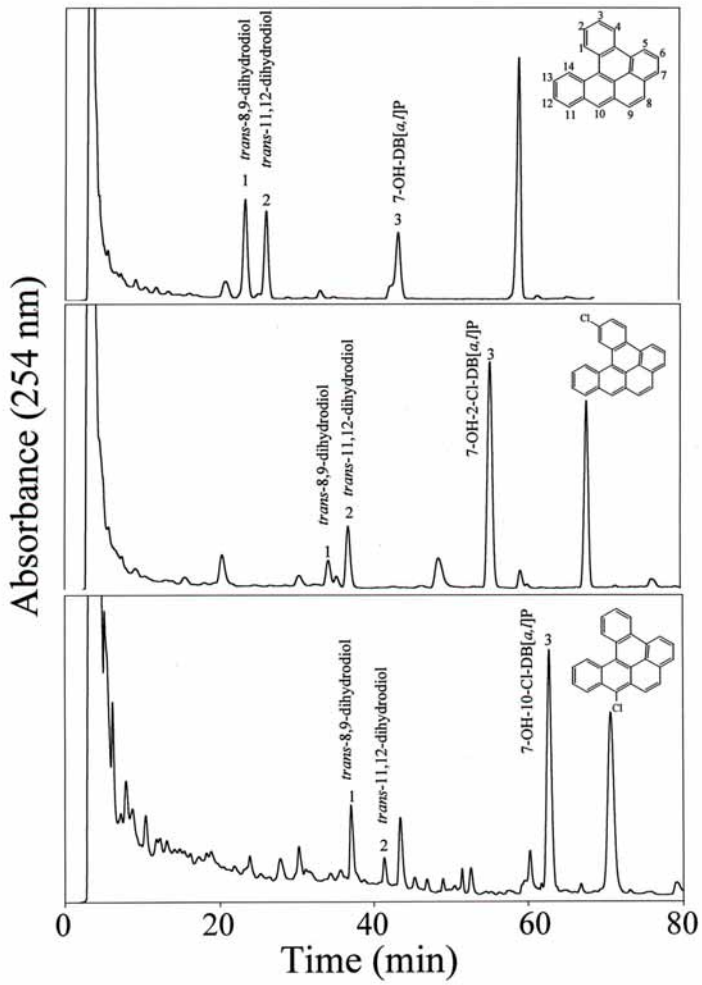

Figure 1. Reversed-phase HPLC profiles of the organic solvent extractable metabolites obtained from incubation of (A) DB[*a,l*]P, (B) 2-Cl-DB[*a,l*]P and (C) 10-Cl-DB[*a,l*]P with liver microsomes from rats pretreated with 3-MC.

The metabolites of 2-Cl-DB[*a,l*]P were separated by HPLC (Figure 1B). Again, based on UV-Visible absorption, mass, and NMR spectral analysis, metabolites contained in chromatographic peaks *1*, *2*, and *3* were identified as 2-Cl-DB[*a,l*]P *trans*-8,9-dihydrodiol, 2-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol, and 7-OH-2-Cl-DB[*a,l*]P, respectively. Similar results were obtained from metabolism of 10-Cl-DB[*a,l*]P (Figure 1C). The materials contained in the chromatographic peaks *1*, *2*, and *3* were identified as 10-Cl-DB[*a,l*]P *trans*-8,9-dihydrodiol, 10-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol, and 7-OH-10-Cl-DB[*a,l*]P, respectively. The metabolite in chromatographic peak eluted at 60.1 min had a mass matched to a OH-10-Cl-DB[*a,l*]P metabolite. Due to lack of sufficient quantity for NMR spectral assignment, the position of the hydroxyl group could not be determined.

Quantification of metabolite formation

No attempts were made to determine the exact yield of each metabolite from metabolism of these three substrates. However, the relative yield of the metabolites was compared based on NMR spectral measurements of the metabolites using an internal standard of known quantity. Thus, it was determined that the relative amounts of DB[*a,l*]P *trans*-11,12-dihydrodiol, 2-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol, and 10-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol are at the ratio of 6 to 3.5 to 1. The relative quantities of DB[*a,l*]P *trans*-8,9-dihydrodiol, 2-Cl-DB[*a,l*]P *trans*-8,9-dihydrodiol, and 10-Cl-DB[*a,l*]P *trans*-8,9-dihydrodiol are about 7 to 4 to 1. However, 7-OH-2-Cl-DB[*a,l*]P and 7-OH-10-Cl-DB[*a,l*]P are about equal while the yield of 7-OH-DB[*a,l*]P is much lower.

Conformation analysis of the trans-8,9-dihydrodiol metabolites

The magnitude of the NMR coupling constants between the carbinol protons has long been used for determination of the conformation of *trans*-dihydrodiols [23-26]. The coupling constants between the carbinol protons of DB[*a,l*]P *trans*-11,12-dihydrodiol and 2-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol are 11.2 and 10.8 Hz, respectively, indicating that these two dihydrodiols have a *trans*-configuration and preferentially adopt a quasidiequatorial conformation [23-26]. The coupling constant between the carbinol protons of 10-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol is small, with a value of about 1.0 Hz, indicating that this compound is a *trans*-dihydrodiol preferentially with a quasidiaxial conformation. Similarly, the coupling constants between the carbinol protons of both DB[*a,l*]P *trans*-8,9-dihydrodiol and 2-Cl-DB[*a,l*]P *trans*-8,9-dihydrodiol are 9.7 Hz, indicating that these two dihydrodiols have a *trans*-configuration and preferentially adopt a quasidiequatorial conformation. The coupling constant between the carbinol protons of 10-Cl-DB[*a,l*]P *trans*-8,9-dihydrodiol is 3.2 Hz, which indicates that this compound is a *trans*-dihydrodiol preferentially with a quasidiaxial conformation.

Optical property of metabolites

The CD spectra of the *trans*-8,9-dihydrodiol and *trans*-10,11-dihydrodiol metabolites of DB[*a,l*]P, 2-Cl-DB[*a,l*]P, and 10-Cl-DB[*a,l*]P were measured under an identical concentration. The CD spectra

of DB[*a,l*]P *trans*-11,12-dihydrodiol and 2-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol are identical, with the only exception that the CD spectrum of DB[*a,l*]P *trans*-11,12-dihydrodiol had a slightly higher ellipticity (Figure 2). Comparison of their CD spectra with those of DB[*a,l*]P 11R,12R-dihydrodiol and DB[*a,l*]P 11S,12S-dihydrodiol [17] indicates that both the predominant enantiomers of the DB[*a,l*]P *trans*-11,12-dihydrodiol and 2-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol metabolites are in an 11R,12R absolute configuration.

On the other hand, the CD spectrum of 10-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol showed a different spectral pattern. These results are in agreement with the previous findings that the CD spectral pattern of a dihydrodiol is dependant on both the configuration and the conformation of the dihydrodiol [23].

The DB[*a,l*]P *trans*-8,9-dihydrodiol, 2-Cl-DB[*a,l*]P *trans*-8,9-dihydrodiol, and 10-Cl-DB[*a,l*]P *trans*-8,9-dihydrodiol metabolites are all optically active, as evidenced by their CD spectra shown in Figure 3, although the absolute configuration has not been determined.

Mutagenicity of DB[a,l]P, 2-Cl-DB[a,l]P, and 10-Cl-DB[a,l]P

The mutagenicity of DB[*a,l*]P, 2-Cl-DB[*a,l*]P, and 10-Cl-DB[*a,l*]P in *salmonella typhimurium* TA100 was determined in the presence of S9 activation enzymes (Table 1). The results indicate that DB[*a,l*]P exhibits the highest mutagenicity, followed by 2-Cl-DB[*a,l*]P. 10-Cl-DB[*a,l*]P is essentially inactive.

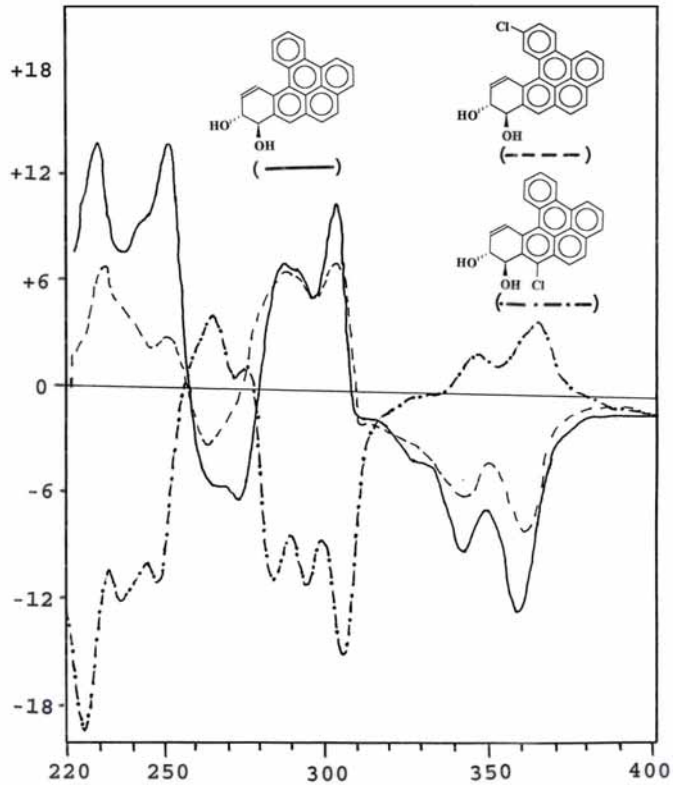

Figure 2. CD spectra of DB[*a,l*]P *trans*-11,12-dihydrodiol, 2-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol, and 10-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol.

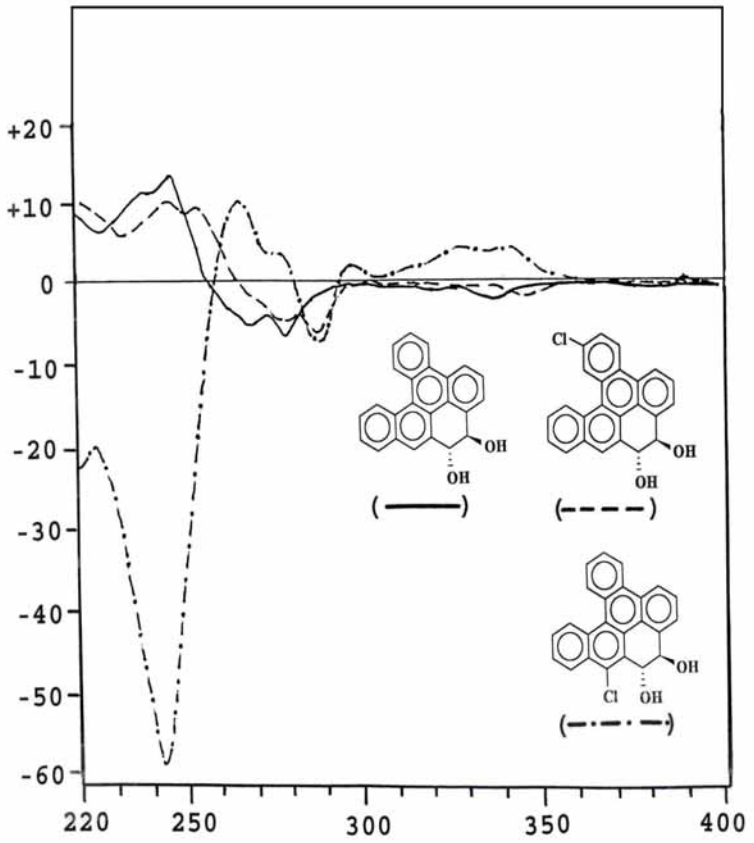

Figure 3. CD spectra of DB[*a,l*]P *trans*-8,9-dihydrodiol, 2-Cl-DB[*a,l*]P *trans*-8,9-dihydrodiol, and 10-Cl-DB[*a,l*]P *trans*-8,9-dihydrodiol.

Table 1. Mutagenicity of DB[*a,l*]P, 2-Cl-DB[*a,l*]P, and 10-Cl-DB[*a,l*]P in *Salmonella typhimurium* TA100 in the presence of S9 enzyme system

Compound	Dose µg/plate	Revertants/plate (mean ± S.D.)
DMSO		280 ±76
Benzo[*a*]pyrene	1 µg	1049 ± 112
DB[*a,l*]P	0.2 µg	591 ± 89
2-Cl-DB[*a,l*]P	1 µg	353 ± 55
10-Cl-DB[*a,l*]P	25 µg	380 ± 48

Discussion

We present in this paper the metabolism profiles of DB[*a,l*]P and its two chlorinated derivatives, 2-Cl-DB[*a,l*]P and 10-Cl-DB[*a,l*]P. These compounds provide a similar metabolism profile, all producing the 7-hydroxyl metabolite as the predominant product and *trans*-11,12-dihydrodiol formed in considerable amounts. However, the relative yields of the same type of metabolites vary. For

example, the relative amount of the *trans*-11,12-dihydrodiol metabolites formed is: DB[*a,l*]P *trans*-11,12-dihydrodiol > 2-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol >> 10-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol, with a ratio of 6 to 3.5 to 1.

It has been demonstrated that metabolism of diequatorial *trans*-dihydrodiols produces the corresponding vicinal diol-epoxides as a predominant metabolism pathway. On the other hand, formation of vicinal diol-epoxide is not favoured from metabolism of a *trans*-dihydrodiol preferentially adopting a diaxial conformation [26].

DB[*a,l*]P *trans*-11,12-dihydrodiol has been demonstrated to be the carcinogenic proximate metabolite of DB[*a,l*]P [15,16]. Since 2-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol preferentially adopts a quasidiequatorial conformation but with lower metabolism yield, it is anticipated that 2-Cl-DB[*a,l*]P exhibits slightly lower biological activities compared with DB[*a,l*]P. On the other hand, 10-Cl-DB[*a,l*]P *trans*-11,12-dihydrodiol adopts a quasidiaxial conformation and is formed in a much lower yield from metabolism, it is likely that this dihydrodiol is inactive biologically. Therefore, these results suggest that 10-Cl-DB[*a,l*]P should be weakly tumorigenic or not tumorigenic at all. This can be supported by the mutagenicity potency of DB[*a,l*]P, 2-Cl-DB[*a,l*]P, and 10-Cl-DB[*a,l*]P, of which DB[*a,l*]P is a potent mutagen and 10-Cl-DB[*a,l*]P is not mutagenic. Thus, based on the results described above, we propose that the biological activities, possibly including tumorigenicity, of the parent compounds are: DB[*a,l*]P > 2-Cl-DB[*a,l*]P >> 10-Cl-DB[*a,l*]P.

Based on the chlorinated PAHs so far identified from the environmental samples, they are the structural isomers identical to those obtained from direct chlorination of the parent PAHs. Since 10-Cl-DB[*a,l*]P is prepared from direct chlorination of DB[*a,l*]P and has been determined to be much less mutagenic than the parent DB[a,l]P, we propose that chlorination of DB[*a,l*]P in the environment is a detoxification pathway. This is opposite to our previous finding that 7-chlorobenz[*a*]anthracene, a chemical recently detected in the environment, exhibits higher tumorigenic activity than the parent PAH, benz[a]anthracene [7]. The proposed metabolic activation pathway of DB[*a,l*]P, 2-Cl-DB[*a,l*]P, and 10-Cl-DB[*a,l*]P leading to tumor induction is shown in Scheme 1.

In conclusion, our results have demonstrated that a chloro substituent can affect: (i) regio- and stereo-selectivity of the metabolism of DB[*a,l*]P; (ii) the conformation of the *trans*-dihydrodiols *peri* to the chloro substituent; and (iii) the mutagenicity of the compound.

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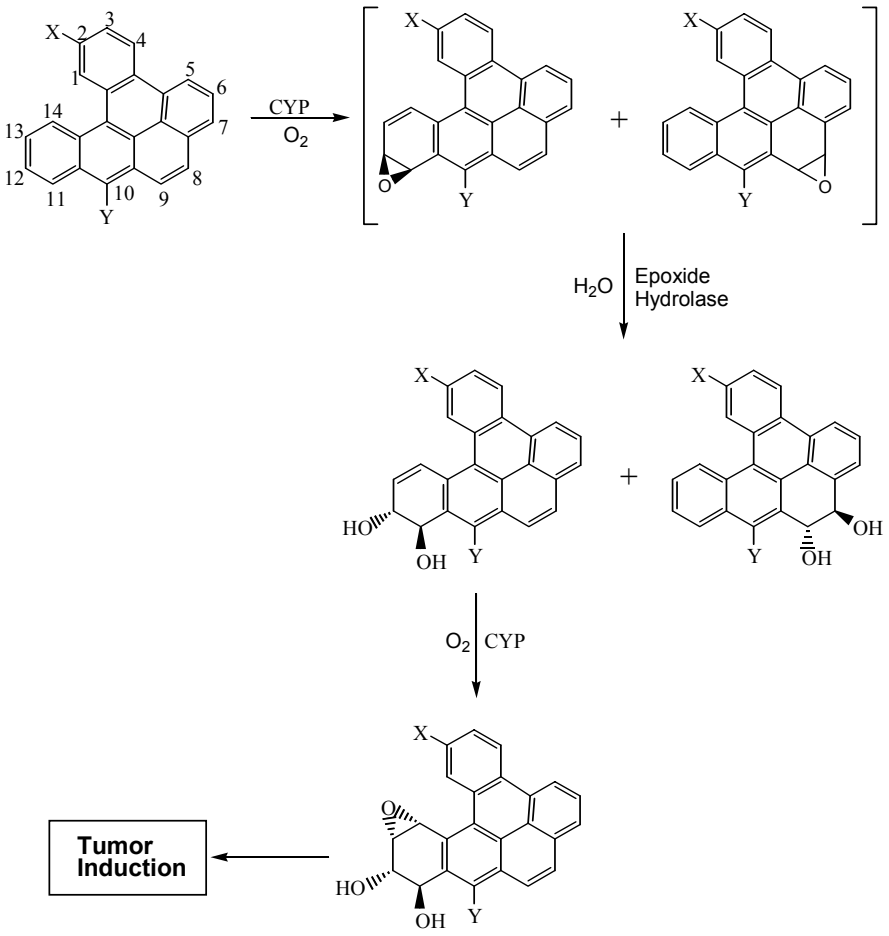

Scheme 1. Proposed metabolic activation of DB[*a,l*]P, 2-Cl-DB[*a,l*]P, and 10-Cl-DB[*a,l*]P leading to tumorigenicity.

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