Absolute Stereochemistry of the Highly Mutagenic 7,8-Diol 9,10-Epoxides Derived from the Potent Carcinogen *trans*-7,8-Dihydroxy-7,8-dihydrobenzo[*a*]pyrene

Sir:

The ubiquitous environmental carcinogen benzo[*a*]pyrene (BP) is now believed to exert most of its biological activity via covalent interaction between critical cellular entities and a particular class of reactive metabolites of the hydrocarbon, the diastereomeric 9,10-epoxides (**1, 2**) of the *trans*-7,8-dihydrodiol (**3**).[1] This pathway for the metabolism of BP (Figure 1) proceeds by initial oxidation to the carcinogen BP 7,8-oxide[2a] which is enzymatically hydrolyzed to the even more carcinogenic[2b] BP 7,8-dihydrodiol (**3**). Further metabolic oxidation of **3** by the hepatic cytochrome P-450 system results mainly in epoxidation of the 9,10-double bond to form **1** and **2**, the ratio of which is highly dependent upon the enantiomer of **3** used as substrate.[3] Both **1** and **2** are highly mutagenic[4] and bind to nucleic acid.[1b,5] Since the specific enantiomers of these diol epoxides may have different toxic, mutagenic, and carcinogenic activities, we have synthesized and assigned the absolute configuration of the enantiomers of **1** and **2** in order to assess their individual biological activity.

We had previously resolved **3** into its (+)- and (−)- enantiomers via chromatographic separation[3a] of the diastereomeric bis-(−)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA)[6] esters. Instability and probable carcinogenicity of these esters of **3** limit their usefulness for the larger scale separations required here. In contrast, the diastereomeric 7-mono-, 8-mono, and 7,8-bis-MTPA esters of (±)-*trans*-7,8-dihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (**4**) proved to be quite stable[7] and are not anticipated to be carcinogenic. Of these, the (+)- and (−)-diastereomers of 7-MTPA-**4** were the easiest to separate chromatographically. Complete resolution of the 7-MTPA esters of (+)- and (−)-**4** was readily effected either by preparative TLC on silica gel (75 mg per 1000 μ × 20 cm × 20 cm plate, Analtech) after several developments with THF/hexane (1/4), by HPLC on a Whatman Magnum 9 Partisil column (25 mg per injection, $k' = 15$ [7-MTPA-(+)-**4**] and 20 [7-MTPA-(−)-**4**]) eluted with 3% THF and 0.5% isopropyl alcohol in hexane, or by preparative liquid chromatography on a Waters' PrepLC/System 500 eluted with 0.5% THF in CH_2Cl_2 (4 g per injection, recycled through two cartridges). After hydrolysis with 1 N NaOH in MeOH (1/1) to remove the MTPA residue, conversion of the (+)- and (−)-**4** into optically active diol epoxides **1** and **2** via **3** was achieved by procedures established for racemic material.[1c,8] Specific rotations of the intermediates and products are given in Table I.

Assignment of absolute configuration for each series (Figure 1 and Table I) was achieved by application of the exciton chirality circular dichroism (CD) method.[9] Initial attempts to establish the configuration of (−)-**4** as its bis-*p*-*N*,*N*-dimethylaminobenzoate were unsatisfactory due to multiple interactions between the electric transition dipoles of the two benzoate chromophores and the tetrahydrobenzo[*a*]pyrene chromophore as can be seen from the extremely unsymmetrical shape of two Cotton effects (CE) in the 280–330 nm region (Figure 2). The Cotton effects were too different in intensity to be certain which interactions were causing the apparent exciton splitting CD. Such an effect is expected because of the several strong UV absorptions for the 7,8,9,10-tetrahydrobenzo[*a*]pyrene chromophore[10] near the long axis intramolecular charge transfer UV absorption of the *p*-*N*,*N*-dimethylaminobenzoyloxy chromophore at 312 nm (ε 29 000). Hence, although the strong CE at 320 nm (Δ +56.0) may include a first CE due to a positive chiral interaction between two ben-

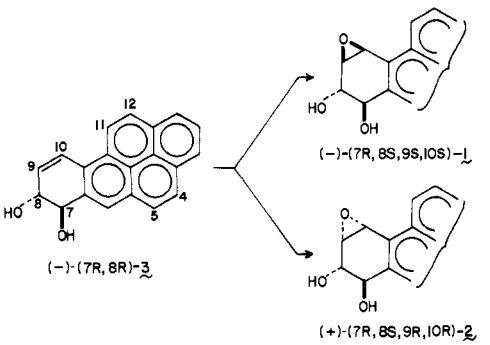

Figure 1. Metabolic conversion of (−)-(7*R*,8*R*)-dihydrodiol ((−)-**3**) into the diastereomeric 7,8-diol 9,10-epoxides ((−)-**1** and (+)-**2**, Table I).

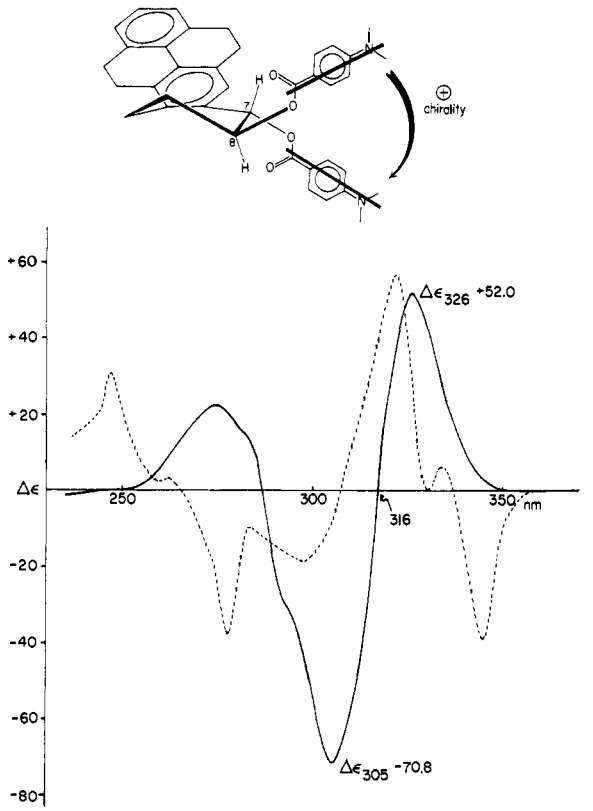

Figure 2. CD spectra (MeOH) of the bis-*p*-*N*,*N*-dimethylaminobenzoates of *trans*-7,8-dihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (compound **4**, - - -) and *trans*-7,8-dihydroxy-4,5,7,8,9,10,11,12-octahydrobenzo[*a*]pyrene (diester of **5** shown, —). Both compounds were derived from (−)-**4** (Table I) which has 7*S*,8*S* absolute configuration.

Table I. Specific Rotations ($[\alpha]^{23}_D$) of the Optically Pure Enantiomers

Compound[a]	7*S*,8*S*-Series	7*R*,8*R*-Series
Diacetate of **5**[b] (octahydro-BP)	+93° (THF)	—
4 (tetrahydro-BP)[c]	−80° (THF)	+83° (THF)
Diacetate of **4**	+141° ($CHCl_3$)	—
Dibenzoate of **4**	+84° ($CHCl_3$)	−78° ($CHCl_3$)
7-Mono-MTPA of **4**	−70° ($CHCl_3$)	+33° ($CHCl_3$)
3 (BP-7,8-dihydrodiol)	+432° (THF)	−410° (THF)
	+409° (acetone)	−405° (acetone)
Dibenzoate of **3**	+385° ($CHCl_3$)	−372° ($CHCl_3$)
Diol epoxide **1**	+123° (THF)	−127° (THF)
Diol epoxide **2**	−68° (THF)	+72° (THF)

[a] Structures of compounds **1**, **2**, and **3** are shown in Figure 1. [b] The bis-*p*-*N*,*N*-dimethylaminobenzoate is shown in Figure 2. [c] Identical with **3** except that the 9,10-double bond is reduced.

zoate chromophores, it does not provide a conclusive assignment in the absence of a rigorous theoretical treatment. The chiral effect of the tetrahydrobenzo[*a*]pyrene chromophore on that of the *N,N*-dimethylaminobenzoyloxy groups would be eliminated if the 4,5- and 11,12-double bonds in **4** were reduced to produce an octahydrobenzo[*a*]pyrene (biphenyl) chromophore (**5**). The resulting isolated exciton chirality interaction between the two *N,N*-dimethylaminobenzoyloxy chromophores in such a diester of **5** would provide an unambiguous basis for configurational assignment. After examination of a wide variety of reduction conditions on **4** and its esters, catalytic hydrogenation of the diacetate of (−)-**4** (70 mg) in THF with 50 psi of H_2 in the presence of 10% Pd–C and a trace amount of concentrated HCl for 7 days, followed by reacetylation of the crude product, allowed isolation of 12 mg of *trans*-7,8-dihydroxy-4,5,7,8,9,10,11,12-octahydrobenzo[*a*]pyrene (**5**) as its diacetate.[11] The diacetate was subsequently converted to the bis-*p*-*N,N*-dimethylaminobenzoate of **5** (12 mg) for which a clear pair of CE centering at 316 nm was observed (Figure 2). This requires 7*S*,8*S*-absolute stereochemistry as shown and provides an unequivocal assignment for each series (Table I).

Preliminary studies of the metabolism-induced mutagenicity[3a] of (+)- and (−)-**3** as well as their individual tumorogenicity[12] indicate that these enantiomers have different biological activity. Racemic diol epoxides **1** and **2** alkylate the exocyclic N^2-amino group of guanosine[5b,d] in poly(G) by cis and trans addition at C-10 and alkylate the phosphate backbone.[5d] Exposure of bovine bronchial explants to BP has allowed identification of a single enantiomer of diol epoxide **2** as a trans-adduct at the N^2-amino group of guanine in the RNA of these cells as one of the hydrocarbon adducts formed.[5c] Comparison of the chromatographic mobility of the guanosine adducts from (+)- and (−)-diol epoxides **1** and **2** has allowed assignment of absolute stereochemistry to the eight possible adducts.[13] Examination of the RNA from the skins of mice treated topically with BP has provided evidence that both cis and trans adducts of (+)-diol epoxide **1** and (+)-diol epoxide **2** are important metabolites in the binding of BP to RNA in vivo.[13] Interestingly, both of these diol epoxides formed by skin have the 9*R*,10*R*-configuration at the epoxide moiety.

Note Added in Proof. Nakanishi et al.[15] have examined the CD spectrum of the bis-*p*-*N,N*-dimethylaminobenzoate of (−)-**3** and have assigned the same absolute configuration as does the present study. Kapitalnik et al.[16] have found that racemic diol epoxide **2** is highly carcinogenic when compared to BP.

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(7) Esterification of (±)-*trans*-7,8-dihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (**4**) with 1.1 mole equiv of (−)-MTPA chloride in pyridine/CH_2Cl_2 at room temperature gave a ca. 1:1 mixture of 7- and 8-mono-(−)-MTPA esters which were separated by column chromatography on silica gel with 5–20% THF in hexane as eluent. The diastereomeric pair of 7-mono-MTPA esters of **4** (isomer with k' = 15, H_7 δ 6.59 and H_8 4.30 with $^3J_{7,8}$ = 6.0 Hz; isomer with k' = 20, H_7 δ 6.59 and H_8 4.30 with $^3J_{7,8}$ = 7.0 Hz) was readily distinguished from the 8-mono-MTPA diastereomers (mixture, H_7 δ 5.20 and H_8 5.48) by NMR spectroscopy (220 MHz, $CDCl_3$). The order of elution of the 7-MTPA diastereomers of (±)-**4** inverts in CH_2Cl_2 based chromatography systems.

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(10) The diacetate of **4** has UV absorptions (MeOH) at 343, 329, 313, and 277 nm with extinction coefficients of 40 800, 29 400, 12 100, and 35 900, respectively.

(11) The structure of the (+)-diacetate of **5** (mp 186–187 °C) was assigned from its mass spectrum (M^+ 376 by CI with NO–N_2) and NMR spectrum (220 MHz, CCl_4): δ (ppm) 1.96 (3 H, s, OAc), 2.04 (3 H, s, OAc), 2.10 (2 H, m, H_9), 2.60–2.95 (10 H, br s, H_4, H_5, H_{10}, H_{11}, and H_{12}), 5.00 (1 H, m, H_8), 5.90 (1 H, d, H_7), and 6.80–7.20 (4 H, m, aromatic) with $^3J_{7,8}$ = 6.0 Hz. The UV spectrum (MeOH) showed λ_{max} at 275, 285, and 297.5 nm at long wavelength.

(12) Studies in collaboration with Drs. A. H. Conney, W. Levin, A. W. Wood, and R. L. Chang, Hoffmann La Roche, Nutley, N.J.

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H. Yagi,[14a] H. Akagi,[14b] D. R. Thakker[14a]
H. D. Mah,[14a] M. Koreeda,*[14b] D. M. Jerina*[14a]
Laboratory of Chemistry
National Institute of Arthritis, Metabolism and Digestive Diseases National Institutes of Health
Bethesda, Maryland 20014
Department of Chemistry, The Johns Hopkins University
Baltimore, Maryland 21218
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Solvolysis of 2-Aryl-2-bicyclo[2.1.1]hexyl *p*-Nitrobenzoates. Evidence for the Absence of σ-Participation by the Application of the Tool of Increasing Electron Demand

Sir:

Solvolysis of 2-aryl-2-bicyclo[2.1.1]hexyl *p*-nitrobenzoates provides a value for ρ^+ of −4.31, even more negative than the ρ^+ observed in the solvolysis of 2-aryl-*endo*-norbornyl *p*-nitrobenzoates (ρ^+ = −3.72), a system where σ-participation is believed to be absent. Consequently, application of the tool of increasing electron demand to the bicyclo[2.1.1] system does not support the presence of significant σ-participation. Moreover, extrapolation of the data from the tertiary 2-aryl-2-bicyclo[2.1.1]hexyl derivatives to the parent secondary system fails to reveal any enhancement in rate of the secondary derivative attributable to σ-participation. It is concluded that