## Synthesis and Absolute Stereochemistry of (+)- and (-)-Benz[a]Anthracene 8,9-Oxide and Derived Mammalian Liver Metabolites of Benz[a]-Anthracene

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Benz[a]anthracene 8,9-oxide, (5), a major initial metabolite of benz[a]anthracene, has been synthesised in optically pure form. The (-)-enantiomer has been unequivocally assigned as [8S,9R] by configurational correlation with (+)-*trans*-9S-bromo-8S-(menthyloxyacetoxy)-8,9,10,11-tetrahydrobenz[a]anthracene (1B) whose absolute stereochemistry has been determined by X-ray crystal-structure analysis.

The major isolated mammalian liver metabolite of benz[a]anthracene, (-)-8,9-dihydroxy-8,9-dihydrobenz[a]anthracene, (11), has [8*R*,9*R*] stereochemistry. It is deduced that the latter (-)-(8*R*,9*R*)-dihydrodiol is enzymatically derived from benz[a]anthracene (+)-<math>(8*R*,9*S*)-oxide, and that it may be converted into*trans*-8*R*,9*S*-dihydroxy-10*S*,11*R*-epoxy-8,9,10,11-tetrahydrobenz[a]anthracene prior to being covalently bonded to cellular nucleicacids.

BENZ[a]ANTHRACENE (BA), the lowest molecular-weight member of the polycyclic aromatic hydrocarbon (PAH) series to be generally considered a carcinogen, occurs widely in the environment. The metabolism of BA by animal liver microsomes produces *trans*-dihydrodiols at the 1,2-, 3,4-, 5,6-, 8,9-, and 10,11-positions of BA, with 8,9-dihydroxy-8,9-dihydro-BA being the major isolated product.<sup>1.2</sup> From the latter diol is derived a further metabolite, 8,9-dihydroxy-10,11-epoxy-8,9,10,11-tetrausing a modification of the method reported earlier <sup>6</sup> and is similar to that used in the synthesis of (+)- and (-)-naphthalene and anthracene 1,2-oxides <sup>7</sup> (Scheme 2).

The key synthetic intermediate in Scheme 2, bromohydrin (2), was derived from 8-oxo-8,9,10,11-tetrahydro-BA as reported.<sup>6</sup> The isolation of the compounds (2)—(12) in optically pure form became possible after separation of the less soluble bromomenthyloxyacetate diastereoisomer (1B) (m.p. 128—130 °C,  $[\alpha]_{\rm p}$  +44°) by



hydro-BA, which was the first detected member of the epoxy-diol series in PAHs.<sup>3</sup> Diol-epoxide metabolites in turn have been shown to bind covalently to nucleic acids and some degree of correlation between the extent of bonding of PAHs to nucleic acids and their carcinogenicity has been noted.<sup>4</sup>

The stereochemistry of the metabolism of BA is determined by preferential addition of an oxygen atom from a mono-oxygenase enzyme to one face of the planar PAH structure during the primary metabolic step, *i.e.* arene oxide formation. This is exemplified by the stereochemical correlation established for metabolism at the 7,8-bond of benzo[a]pyrene (B[a]P) as shown in general form in Scheme 1.<sup>5</sup>

The synthesis of (-)-BA 8,9-oxide was carried out

recrystallization from dichloromethane-methanol (1:3). From the mother-liquors (1A), m.p. 118—119 °C,  $[\alpha]_{\rm p}$  —134°, was crystallised. The diastereoisomeric purity of these samples was determined directly from the n.m.r. spectrum of (1A) and (1B) (220 MHz, C<sub>6</sub>D<sub>6</sub>) where the diastereotopic exocyclic methylene protons H<sub>A</sub> and H<sub>B</sub> appeared as an AB quartet in (1B) (centred at & 4.05 and 3.93,  $J_{\rm AB}$  15.0 Hz) and as a singlet in (1A) (& 4.00). Further confirmation of the diastereoisomeric purity of (1A) and (1B) was obtained from h.p.l.c. analysis where (1A) was eluted before (1B). The above n.m.r. and h.p.l.c. characteristics of (1A) and (1B) are directly analogous to those reported for the corresponding diastereoisomers in the naphthyl,<sup>7</sup> anthryl,<sup>7</sup> and B[a]P <sup>5</sup> series. The chemical transformations of (1B) into the chiral derivatives (2), (3), (4), and (5) were carried out by standard methods <sup>7,8</sup> (Scheme 2) which did not involve any change in configuration at the benzylic C-8 chiral centre. The base-catalysed hydration of tetrahydro-epoxide (6) was assumed to occur with almost exclusive attack at C-8 as was found for the analogous reaction in the naphthalene,<sup>7</sup> anthracene,<sup>7</sup> and  $B[a]P^5$  series under identical experimental conditions.\* Since no change in absolute stereochemistry at C-8 occurred during the

corresponding 8-bromo-7-(menthyloxyacetoxy)-7,8,9,10tetrahydro-B[a]P <sup>5</sup> showed the bromine atom to be equatorial and the menthyloxyacetoxy-group to be quasi-equatorial. This would indicate that in the solid state the conformation is determined by crystal-packing forces and that there is no marked preference for either conformation in solution, as was concluded from a previous n.m.r. study.<sup>8</sup> Furthermore, as the molecule (1B) contained a (--)-menthyloxy-group of known absolute configuration, the absolute stereochemistry



SCHEME 2 (a) (-)-Menthyloxyacetyl chloride-pyridine; (b) diborane-THF; (c) AcCl-pyridine; (d) N-bromosuccinimide; (e) NaOMe-THF; (f) KOH-aqueous Bu<sup>t</sup>OH; (g) PhCOCl-pyridine; (h) 1,5-diazabicyclo[4.3.0]non-5-ene; (i) LiAlH<sub>4</sub>

interconversions  $(7) \longrightarrow (8) \longrightarrow (9) \longrightarrow (10) \longrightarrow (11)$ the stereochemical correlation between structures (1)---(12) shown in Scheme 2 obtained.

In order unequivocally to assign absolute stereochemistry to compounds (2)—(12) an X-ray structure analysis on (1B) was carried out. In the crystal all molecules of (1B) adopted the same conformation. The C-7a—C-11a ring had the normal half-chair cyclohexene conformation but it was noteworthy that the bromine atom and the (—)-menthyloxyacetoxy-group adopted axial and quasi-axial positions, respectively.

By comparison, an X-ray structure analysis of the

at the C-8 and C-9 chiral centres is unequivocally established. Thus (1B) and (1A) have (8S,9S) and (8R,9R) configurations respectively. A projection of (1B) is shown in the Figure. The specific rotations and configurations of molecules (1)—(12), along with the yields and physical properties, are shown in Table 1.

A recent <sup>2</sup> liver microsomal metabolism study of BA using rats pre-treated with 3-methylcholanthrene showed the almost exclusive (96% optical purity) formation of (--)-BA 8,9-dihydro-8,9-diol which is now established as being of the (8R,9R) configuration. On the assumption that (as in all previously studied arene oxides) the epoxide hydrolase enzyme present in the liver microsomal fraction attacks exclusively at the non-benzylic (C-9) position, and that (as might be expected from earlier

<sup>\*</sup> This conclusion regarding absolute stereochemistry is supported by independent c.d. studies on (11) carried out by Professor Koreeda (ref. 2).

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results <sup>2,6</sup>) the arene oxide (5) is a good substrate for the epoxide hydroase enzyme, then it appears probable that the optically pure sample of (-)-[8R,9R]-dihydrodiol (11) was derived from optically pure (+)-(8R,9S)-arene oxide (5).

Recent fluorescence spectral studies<sup>9</sup> have indicated that the major DNA-BA adduct formed *in vivo* or in



**FIGURE** A projection of the molecule down the +c axis

cell culture may be the *trans*-BA-8,9-diol 10,11-oxide. This result is further supported by the observation <sup>10</sup> that the major DNA–BA adduct formed during metabolism by liver microsomes pre-treated with 3-methylcholan-threne shows identical h.p.l.c. characteristics to the *trans*-BA-8,9-diol 10,11-oxide. Since the (-)-BA-8,9-di-hydro-(8R,9R)-diol was a major liver microsomal meta-

#### TABLE 1

Physical properties and microanalytical data

	-	<b>1</b> 1		2	
	Yield		Lit.		
	(%)	M.p. (°C)	m.p.ª (°C)	$[\alpha]_{589}$ (°)	Configuration
(1B)	90 <sup>b</sup>	128 - 131	b, c		8 <i>S</i> , 9 <i>S</i>
(2)	60	144 - 146	151—152 d	-80	8S, 9S
(3)	65	166 - 168	е	+102	8 <i>S</i> , 9 <i>S</i>
(4)	62	118 - 120	f	+345	8S, 9S
(5)	46	106 - 110	112 - 115 d	-115	8S, 9R
(6)	81	143 - 144	158-160 d	-130	8S, 9R
(7)	<b>74</b>	208 - 210	190—191 <sup>d</sup>	+55'	8R, 9R
(8)	63	124 - 125	153 - 154	-93	8R, 9R
(9)	<b>79</b>	145 - 146	137 - 139 <sup>h</sup>	-457	8R, 9R
10) -	23	195 - 196	167—168 <sup><i>h</i></sup>	-322	8R, 9R
J1)	73	185 - 187	$168 - 170^{h}$	-297 g	8R, 9R
12)	62	151 - 152	122 - 123 d	+56	9R
	T) /	1	5 B K . 4	C (1A)	

<sup>*a*</sup> Racemic compounds. <sup>*b*</sup> Mixture of (1A) and (1B); Found: C, 68.8; H, 6.7.  $C_{30}H_{35}BrO_3$  requires C, 68.8; H, 6.7%. <sup>*c*</sup> Compound (1A) had m.p. 118 –119 °C,  $[\alpha]_{589} - 134^\circ$ , and 8R,9R configuration. <sup>*d*</sup> Ref. 6. <sup>*c*</sup> Found: C, 65.2; H, 4.8.  $C_{20}H_{17}BrO_2$  requires C, 65.1; H, 4.6%. <sup>*f*</sup> Found: C, 53.7; H, 3.6.  $C_{20}H_{16}Br_2O_2$  requires C, 53.6; H, 3.6%. <sup>*u*</sup> In acetone solvent.

bolite, the derived *trans*-BA-8,9-diol 10,11-oxide must have (8R,9S,10S,11R) stereochemistry. Thus the absolute stereochemistry and optical purity of the liver metabolites of B[a]P at the 7,8,9,10-ring shown in Scheme 1 are identical to those now determined for the BA metabolism at the 8,9,10,11-ring.

The similarity in stereochemistry and mechanism of metabolism at the 7,8,9,10-ring of  $B[a]P^{11}$  and the 8,9,10,-11-ring of BA (Scheme 1) is not reflected in their carcinogenic activity. In accordance with the 'bay region theory of carcinogenicity' these 'bay-region' metabolites of B[a]P of specified stereochemistry are highly tumourogenic.<sup>12</sup> Despite the formation of these 'non-bay-region' nucleic acid-BA adducts (of similar stereochemistry) as the major products, they show very weak carcinogenic activity.<sup>12</sup>

One further aspect of the stereochemistry of the optically pure enantiomers of BA 8,9-oxide considered was the possibility of racemization via a contribution from the valence tautomeric oxepin form. The observed configurational stability over an extended period (>12 h) was in concurrence with predictions based upon chemical and biochemical evidence,<sup>6</sup> and with a prediction made on the basis of PMO calculations.<sup>13</sup> Thus racemization of the initial metabolite, (+)-BA 8,9- oxide, *in vivo*, is probably also precluded.

#### EXPERIMENTAL

N.m.r. spectra were recorded using Bruker WH90 and Varian HR-220 instruments,  $\text{CDCl}_3$  or  $\text{C}_6\text{D}_6$  solvents, and tetramethylsilane as internal reference. Specific optical rotations ([ $\alpha$ ]) were recorded at 589 nm in CHCl<sub>3</sub> solvent using a Perkin-Elmer 141 digital polarimeter unless otherwise stated.

Analysis of the diastereoisomers (1A) and (1B) was carried out using a 6.2 mm  $\times$  25 cm Du Pont Zorbax Sil column and cyclohexane-ether (95:5) as eluant [(1A) k' = 1.21; (1B), k' = 1.40.]

Optically pure compounds (2), (5), (6), (7), (8), (9), (10), (11), and (12) were prepared by similar methods to those previously reported for racemic samples.<sup>6,8,14</sup> These chiral compounds were found to have identical i.r. and n.m.r. spectra to the racemic compounds.

Diastereoisomers (1A) and (1B) were prepared in the normal method 7 using bromohydrin 2 and (-)-menthyloxy-acetyl chloride in pyridine.

The total separation of diastereoisomers was achieved in low yield (<10%) by fractional recrystallizaton as indicated, but could be carried out in high yield (>90%) by a combination of preparative h.p.l.c. (using conditions outlined in ref. 5) and recrystallization. While the data reported refer to only one enantiomeric series, in practice both series have been examined and were found to have opposite signs of  $[\alpha]_{\rm p}$  and identical properties.

Crystal Data for (1B).— $C_{30}H_{35}BrO_3$ , M = 523.5. Orthorhombic, space group  $P2_12_12_1$ , a = 7.014(7), b = 49.383(50), c = 7.494(7) Å, U = 2595.7 Å<sup>3</sup>,  $D_m = 1.336$  g cm<sup>-3</sup>, Z = 4,  $D_c = 1.339$  g cm<sup>-3</sup>, F(000) = 1.096,  $\mu(Cu-K_{\alpha}) = 26.0$  cm<sup>-1</sup>. Crystals were pale yellow, well-formed, elongated flat plates. The crystal used for data collection had dimensions  $0.45 \times 0.22 \times 0.07$  mm.

1 650 Independent diffraction intensities were measured on an Enraf-Nonius CAD3 automatic diffractometer, using Cu- $K_{\alpha}$  radiation. The 1 315 intensities with  $I > \sigma(I)$ were corrected for Lorentz and polarisation factors and were used in the subsequent structure analysis and refinement. The Br atom was located in a Patterson synthesis and the carbon and oxygen atoms were found in subsequent Fourier syntheses. The enantiomer was fixed by choosing the set of atomic co-ordinates consistent with the known absolute stereochemistry of the (-)-menthyloxy-group. The structure was refined by least squares with allowance for anisotropic vibrations for all non-hydrogen atoms. The refinement converged at a final R value of 0.11. The final weighting scheme was  $w = (10 + |F_0|)^{-1}$ . Atomic scatter-

TABLE 2 Final atomic co-ordinates, with estimated standard deviations in parentheses

		1	
	x	у	z
Br	0.033 9(4)	0.1101(1)	0.0324(6)
O(1)	$0.527\ 2(19)$	$0.110\ 3(3)$	-0.255 9(22)
O(2)	$0.357 \ 1(21)$	$0.136\ 5(3)$	-0.4406(32)
O(3)	$0.677 \ 8(19)$	0.1648(3)	-0.517 9(30)
C(1)	$0.590\ 9(25)$	-0.0177(4)	0.192 6(46)
C(2)	$0.627 \ 3(31)$	-0.0454(4)	$0.231\ 0(42)$
C(3)	$0.607\ 7(28)$	-0.0648(5)	0.098 5(50)
C(4)	$0.557 \ 4(29)$	-0.0580(4)	-0.0806(40)
C(4a)	$0.520 \ 5(30)$	$-0.029\ 2(4)$	-0.1286(47)
C(5)	$0.462 \ 4(32)$	$-0.023 \ 2(4)$	-0.3150(39)
C(6)	$0.426\ 6(29)$	$0.002\ 1(5)$	-0.332 2(41)
C(6a)	$0.431 \ 8(27)$	0.023 9(4)	-0.187 4(43)
C(7)	$0.387 \ 0(28)$	$0.051 \ 0(4)$	$-0.245 \ 3(40)$
C(7a)	$0.398 \ 6(25)$	0.070 8(3)	-0.135 2(40)
C(8)	$0.347 \ 0(30)$	$0.099\ 5(4)$	$-0.190\ 5(43)$
C(9)	$0.313\ 1(34)$	$0.119\ 0(5)$	$-0.021 \ 0(54)$
C(10)	$0.440\ 3(41)$	$0.116\ 4(5)$	$0.124 \ 9(69)$
$C(\Pi)$	$0.436\ 3(39)$	$0.086 \ 0(4)$	$0.202 \ 1(47)$
C(11a)	$0.449\ 0(27)$	$0.064 \ 3(4)$	$0.049\ 3(44)$
C(12)	0.499 5(29)	$0.039\ 3(4)$	$0.093\ 6(44)$
C(12a)	0.492 8(24)	0.0175(4)	-0.023 3(33)
C(12D)	0.535 4(24)	-0.0099(4)	0.008 8(38)
C(13)	$0.504 \ 1(30)$	0.1287(4)	-0.3839(43)
C(14)	0.707 7(30)	0.138 9(4)	-0.4519(49)
C(10)	0.848 0(29)	0.170 0(4)	0.399 9(40)
C(10)	0.9814(32)	0.1837(4)	-0.4400(42)
C(17)	1.100 4(30)	0.1997(0)	-0.495 9(50)
C(10)	0.079.5(40)	0.223 8(5)	-0.595 5(50)
C(19)	0.572 5(40)	0.210 8(0)	-0.703 8(48)
C(20)	0.639.8(41)	0.202 0(4)	-0.0380(42) -0.840 3(40)
C(22)	0.558 0(53)	0.223 3(5)	
C(23)	0.717 (53)	0.178 1/6	-0.901.6(54)
C(24)	12927(43)	0.207.7(6)	-0.340.3(50)
~(~ <b>1</b> )	1.000 (40)	0.201 1(0)	- 0.040 0(09)

ing factors were taken from ref. 15. Final atomic fractional co-ordinates are listed in Table 2. Tables of anisotropic temperature factors, bond lengths and angles, and of observed and calculated structure amplitudes have been deposited as Supplementary Publication No. SUP 22897

\* For details see Notice to Authors No. 7, J.C.S. Perkin I, 1979, Index issue.

(19 pp.).\* A projection of the molecule is shown in the Figure. The known absolute stereochemistry of the (-)-menthyloxy-moiety thus establishes the absolute configuration of the molecule as [8S,9S].

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