

## (+)- and (-)-Benzo[*a*]pyrene 7,8-Oxide: Synthesis, Absolute Stereochemistry, and Stereochemical Correlation with Other Mammalian Metabolites of Benzo[*a*]pyrene

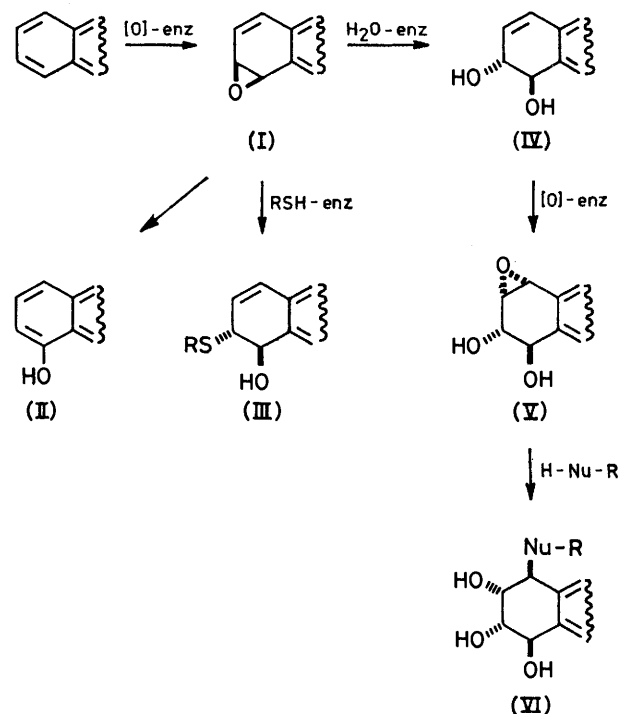
By Derek R. Boyd,\* Guru S. Gadaginamath, Anil Kher, and John F. Malone, Department of Chemistry, Queen's University of Belfast, Belfast BT9 5AG, N. Ireland  
Haruhiko Yagi and Donald M. Jerina, Laboratory of Bioorganic Chemistry, The National Institutes of Health, The National Institutes of Health, Bethesda, Maryland 20205, U.S.A.

Optically pure samples of (+)- and (-)-benzo[*a*]pyrene 7,8-oxide have been synthesized from the separated diastereoisomers of *trans*-8-bromo-7-methyloxyacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene. The latter separation was effected by short-column chromatography–recrystallization or high pressure liquid chromatography. The absolute stereochemistry of (+)-*trans*-8-bromo-7-methyloxyacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene has been assigned as (7*S*,8*S*) by an *X*-ray crystal structure analysis; this structure has in turn been unequivocally correlated with the stereochemistry of (7*S*,8*R*)-(-)-benzo[*a*]pyrene 7,8-oxide and a range of mammalian metabolites.

BENZO[*a*]PYRENE (B[*a*]P) is the most common of the polycyclic aromatic hydrocarbons (PAHs) which have been detected in the environment.<sup>1</sup> The omnipresence of B[*a*]P allied to its toxic, mutagenic, and carcinogenic effects in mammalian systems, has resulted in intensive investigations of the intermediates formed during metabolism of B[*a*]P and of the reactions of these intermediates with hepatic enzymes and cellular constituents.<sup>1</sup> The bioactivation of PAHs in mammalian systems (Scheme 1) involves the initial formation of arene oxides (I) by a mono-oxygenase-catalysed epoxidation. Subsequent arene-oxide transformations include spontaneous aromatization to yield a phenolic product (II), spontaneous or enzymatic addition of glutathione to form a glutathione conjugate (III), and epoxide-hydrase-catalysed addition of water to form a *trans*-dihydrodiol (IV). The latter product may undergo a further enzyme-catalysed epoxidation to yield mainly diol epoxide (V) which will attack nucleophilic groups including the amino<sup>2,3</sup> and phosphodiester<sup>2,4,5</sup> groups present in nucleic acids (Nu-R in Scheme 1). This provides a mechanism for the formation of covalent bonds between PAH metabolites and biopolymers and may [where the oxiran ring in (V) is located in the bay region of the PAH ring system<sup>6</sup>] be responsible for the mutagenicity and carcinogenicity of B[*a*]P, B[*a*]P 7,8-oxide, and derived mammalian metabolites.

The possibility that an oxygen atom will add stereoselectively to one face of a PAH during enzyme-catalysed arene oxide formation was initially proposed and investigated by the synthesis of optically active naphthalene 1,2-oxide.<sup>7,8</sup> Recent results<sup>9,10</sup> have suggested that epoxidation of B[*a*]P at the 7,8-position occurs primarily on one face of the B[*a*]P ring and that the subsequent hydration step under enzyme control occurs by attack of water exclusively at the 8-position. Thus the introduction of chirality during the asymmetric synthesis of B[*a*]P 7,8-oxide will determine the absolute con-

figuration of subsequent metabolites (II, III,† IV, V Scheme 1). The availability of resolved enantiomers of the 7,8-oxide of B[*a*]P is thus of importance for studies of their relative rates of enzymatic hydration in the presence of epoxide hydrase and of their relative mutagenic



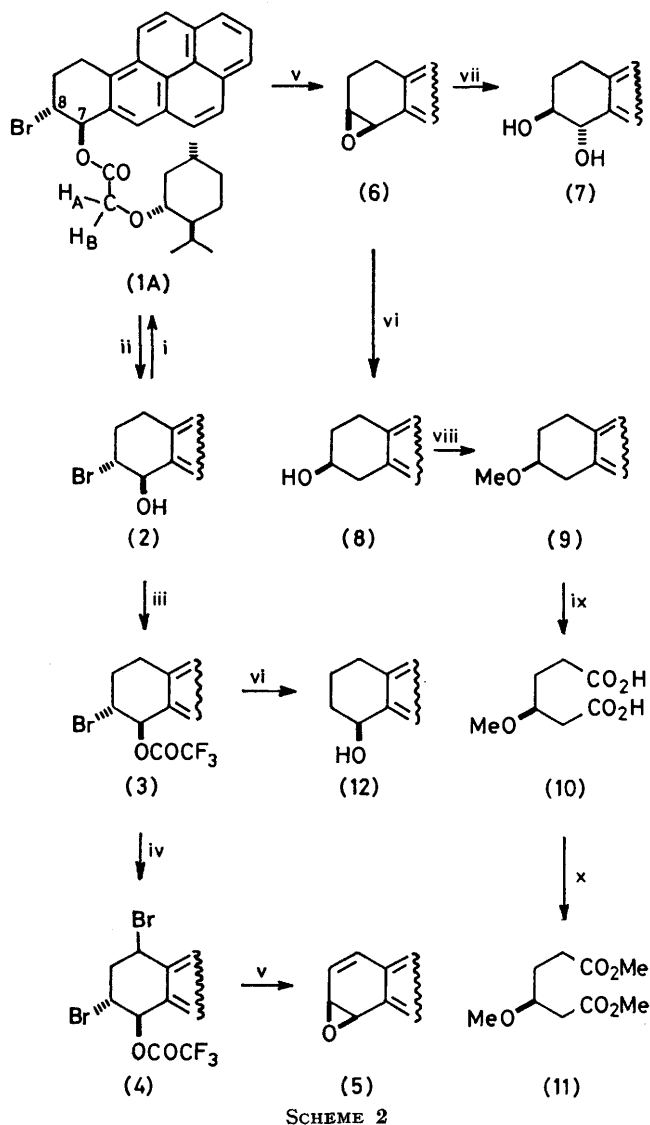
SCHEME 1

and carcinogenic effects. The latter investigations may ultimately provide valuable information about the nature of the active sites in enzymes responsible for drug metabolism and of the receptor sites for the carcinogens. The important role of the molecular shape of the PAH metabolites upon their biological action<sup>13</sup> requires that absolute stereochemistry be unequivocally established. Heretofore only a comparative method has been used, *i.e.* circular dichroism (c.d.) studies on the bis-*NN*-dimethyl-

† The hydroxy-group in general structure (III) is shown in a benzylic position as found in thiol adducts of naphthalene-<sup>11</sup> and anthracene 1,2-oxides.<sup>12</sup>

aminobenzoates of resolved *trans*-7,8-dihydroxy-7,8-dihydro B[*a*]P diol<sup>14</sup> and *trans*-7,8-dihydroxy-4,5,7,8,9,10,11,12-octahydro B[*a*]P.<sup>15</sup>

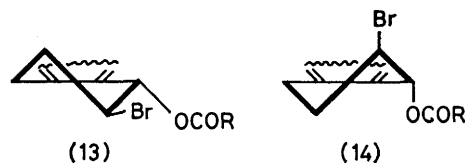
The synthetic methods used for (+)- and (-)-benzo[*a*]pyrene 7,8-oxide (as indicated in a preliminary report<sup>16</sup>) were based upon the previously reported route to the racemic compounds<sup>17</sup> (Scheme 2). Resolution



**Reagents:** (i) (-)-menthyloxyacetyl chloride-pyridine; (ii) diborane-THF; (iii)  $(CF_3CO)_2O-CHCl_3$ ; (iv) *N*-bromosuccinimide; (v) NaOMe; (vi)  $LiAlH_4$ ; (vii) KOH-aq·Bu<sup>t</sup>OH; (viii) MeI-NaH; (ix)  $O_3-HCO_2H$ ; (x)  $CH_2N_2$

of the bromohydrin (2) racemate was effected *via* the (-)-menthyloxyacetyl derivatives. A partial separation of the diastereoisomers of *trans*-8-bromo-7-menthyloxyacetoxy-7,8,9,10-tetrahydro B[*a*]P (1A and 1B) was achieved by passage of the mixture through a short column of silica gel. Total resolution was obtained by recrystallization of either the initial or terminal chromatography fractions. The efficiency of this resolution could be determined by analytical high pressure liquid

chromatography (h.p.l.c.). Preparative h.p.l.c. proved to be a more efficient method in terms of the total yield of fully resolved material. The diastereoisomer (1A) which was less polar (eluted initially by both chromatographic procedures) showed a specific optical rotation



( $[\alpha]_D -112^\circ$ ) greater in magnitude and of opposite sign to that of the more polar (1B) ( $[\alpha]_D +36^\circ$ ). A further characteristic feature of (1A) was evident from the n.m.r. spectrum (220 MHz,  $C_6D_6$ ) where the diastereotopic methylene protons ( $H_A, H_B$ ) appeared as a singlet ( $\delta$  4.12). By contrast protons  $H_A$  and  $H_B$  in (1B) appeared as an AB quartet centred at  $\delta$  3.98 and 4.20 with a coupling constant ( $J_{AB}$ ) of 16.3 Hz. A comparable pattern for the sign and magnitude of  $[\alpha]_D$  values related to the elution sequence from silica gel, or the degree of non-equivalence in the n.m.r. spectrum has previously been noted in the naphthalene,<sup>8</sup> anthracene,<sup>8</sup> and phenanthrene<sup>18</sup> ring systems. While the absolute configuration of diastereoisomers (1A) and (1B) may be deduced by this comparative method such an empirical correlation was not considered totally satisfactory in the present studies. A previous analysis of the conformation of (3) by n.m.r. spectroscopy suggested that the two half-chair conformations (13) and (14) were present in approximately equal proportions at equilibrium.<sup>17</sup> It was also proposed that benzylic bromination to form (4) would occur by axial attack exclusively on the equatorial-quasiequatorial conformation (13). In the present study an analogous conformation has been found by an X-ray analysis of the structure of (1B). In the crystal, all molecules adopt the same conformation.

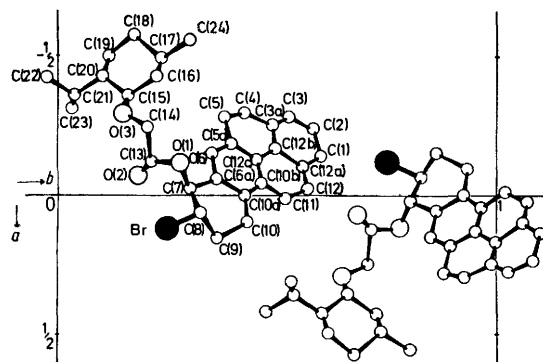


FIGURE Numbering of atoms, and absolute stereochemistry in (+)-1B

The C(6a)—C(10a) ring has the anticipated half-chair cyclohexene conformation with the bromine atom equatorial and the menthyloxyacetoxy-group in a quasi-equatorial position (13; R = menthyloxy). A projection of the contents of one unit cell (two symmetry-related molecules) is shown in the Figure. Furthermore,

the absolute configuration at the C-7 and C-8 chiral centres is thus established unequivocally by determination of configuration *relative* to the known<sup>19</sup> absolute configuration of the menthyloxy-group in 1B,  $[\alpha]_D +36^\circ$ . The configuration is (7S,8S) [Scheme 2 shows the 7R,8R configuration for (1A)].

The absolute configurations of *trans*-bromomenthyloxy-esters in the naphthalene,<sup>20</sup> anthracene,<sup>21</sup> and phenanthrene<sup>22</sup> members of the PAH series have previously been unequivocally determined by formation of the methyl ethers which on ozonolysis yielded dimethyl  $\beta$ -methoxyadipate (11) of known configuration. A similar sequence of interconversions, (1A)→(6)→(8)→(9)→(10)→(11) was carried out under identical conditions in the present work but resulted in a much more complex mixture of products including (11) (g.l.c.-mass spectrometric analysis). Thus it was not possible to isolate (11) by the preparative g.l.c. method used earlier.<sup>21,22</sup> Preparative t.l.c. yielded (11) (*ca.* 90% pure), along with a minor impurity which appeared to be similar to dimethyl adipate. The sign and magnitude of  $[\alpha]_D$  for this impure sample of (11) was as anticipated assuming a configuration of (7R,8R) for (1A). The

TABLE 1

Yields, physical properties, and microanalytical data for optically active compounds (1)–(12) derived from (1A)

Compound	Yield (%)	M.p. (°C)	Lit. m.p. (°C) <sup>a</sup>	$[\alpha]_D$ (°)	Absolute configuration
(1A)	86 <sup>b</sup>	143–144	<i>c</i>	–112	(7R, 8R)
(2)	70	170–171	158–159 <sup>20</sup>	+68	(7R, 8R)
(3)	70	132–133	130–132 <sup>17</sup>	–122	(7R, 8R)
(4)	50	181–182	176–178 <sup>17</sup>	–712	(7R, 8R)
(5)	60	<i>d</i>	<i>d</i>	+175 (+100 <sup>e</sup> )	(7R, 8S)
(6)	89	140–141	157–160 <sup>20</sup>	+144	(7R, 8S)
(7)	50	215	234–235 <sup>20</sup>	–63 <sup>e</sup>	(7S, 8S)
(8)	83	188–189	<i>f</i>	–48	(8S)
(9)	76	78–79	<i>g</i>	–25 <sup>h</sup>	(8S)
(11)	25	<i>i</i>	<i>i</i>	–3.2	(8S)
(12)	85	134	141–142 <sup>20</sup>	+52	(7S)

<sup>a</sup> M.p.s refer to racemic compounds. <sup>b</sup> A mixture of (1A) and (1B). <sup>c</sup> Found: C, 70.5; H, 6.45. C<sub>22</sub>H<sub>26</sub>BrO<sub>2</sub> requires C, 70.2; H, 6.4%. <sup>d</sup> Indefinite m.p. due to decomposition on heating. <sup>e</sup> In tetrahydrofuran solvent. <sup>f</sup> Found: C, 88.4; H, 5.7. C<sub>20</sub>H<sub>18</sub>O requires C, 88.2; H, 5.9%. <sup>g</sup> Found: C, 87.9; H, 6.4. C<sub>21</sub>H<sub>18</sub>O requires C, 88.1; H, 6.3%. <sup>h</sup> Derived from (–)-(8)  $[\alpha]_D -23^\circ$ . <sup>i</sup> Quantities isolated were too small to permit measurement of b.p.; the sample showed identical spectral characteristics to an authentic sample.

ozonolysis method for determination of absolute stereochemistry proved to be more difficult for this higher molecular weight member of the PAH series where a greater range of oxidation products was found.

The chemical transformation of (1A) and (1B) into the range of structures shown in Scheme 2 was carried out using previously reported methods.<sup>8,21</sup> The  $[\alpha]_D$  values and absolute configurations of all chiral samples are given in Table 1.

With the exception of the *trans*-diol (7), none of the polycyclic structures in Scheme 2 have previously been resolved, and the maximum  $[\alpha]_D$  values given in Table 1 are considered to be from optically pure samples. The

sample of diol (7) obtained from alkaline hydrolysis of an enantiomerically homogeneous sample of (6) appeared to be *ca.* 80% optically pure in comparison with the previously<sup>15</sup> resolved sample of (7). In all other transformations between chiral structures in Scheme 2, total configurational integrity was maintained. The degree of racemization resulting from hydration of the epoxide (6) may be rationalized in terms of selective (*ca.* 90%) attack at the benzylic position. The stereochemical relationship between the *trans*-bromomenthyloxyacetate (1) and the *trans*-diol (7) parallels that found in the naphthalene,<sup>8</sup> anthracene,<sup>23</sup> and phenanthrene<sup>18,22</sup> series. In all cases the *trans*-bromo-ester retains the same configuration at the oxygen-substituted benzylic position during base-catalysed ring closure to form the tetrahydro-epoxide; substitution of the OH group during base-catalysed opening of the oxiran ring occurs almost exclusively at the benzylic carbon atom in all cases examined to date.\* Thus it is now possible to assign unequivocally the absolute configuration of the metabolites B[a]P 7,8-oxide, *trans*-7,8-dihydroxy-7,8-dihydro B[a]P, 9 $\beta$ ,10 $\beta$ -epoxy-7 $\beta$ ,8 $\alpha$ -dihydroxy-7,8,9,10-tetrahydro B[a]P, 9 $\alpha$ ,10 $\alpha$ -epoxy-7 $\beta$ ,8 $\alpha$ -dihydroxy-7,8,9,10-tetrahydro B[a]P, and the adducts formed between the latter dihydroxy-epoxides with cellular nucleophiles *in vivo* or *in vitro*.<sup>25,26</sup>

The chiral arene oxide (5), while chemically unstable in the crystalline state at and above room temperature, proved to be configurationally stable in solution over 24 h. Thus during this period no change in the  $[\alpha]_D$  value or n.m.r. spectrum was observed. The 7,8-oxide of B[a]P, like the 1,2-oxides of naphthalene and anthracene, does not appear to equilibrate with the valence tautomeric oxepin form, in accord with predictions.<sup>19</sup> (+)-B[a]P 7,8-oxide is the metabolic precursor<sup>13</sup> of (+)-9 $\alpha$ ,10 $\alpha$ -epoxy-7 $\beta$ ,8 $\alpha$ -dihydroxy-7,8,9,10-tetrahydro B[a]P, an ultimate carcinogen of B[a]P.<sup>27</sup> Studies on tumours (W. Levin, unpublished data), including both initiation promotion on mouse skin and intraperitoneal injection into new-born mice, have established that (+)-B[a]P 7,8-oxide is from three- to eight-fold more active than the (–)-enantiomer as required by the above metabolic relationship.

#### EXPERIMENTAL

M.p.s were determined using a Reichert Kofler hot-stage apparatus. N.m.r. spectra on optically active molecules were obtained for comparison with racemic samples using JEOL JNM-PMX60, Bruker WH90, and Varian HR-220 instruments with deuteriochloroform as solvent and tetramethylsilane as reference unless otherwise stated. Specific rotations were measured for solutions in chloroform using a Perkin-Elmer 141 automatic polarimeter.

Silica gel (Kieselgel G type 60) used in short-column chromatography was supplied by Merck. Light petroleum (b.p. 40–60 °C) and ether were purified by distillation.

\* Experiments on the hydration of 1,2-epoxy-1,2,3,4-tetrahydronaphthalene using <sup>18</sup>O-labelled water indicate that attack of the OH<sup>–</sup> nucleophile occurs almost exclusively at the benzylic carbon atom (>90%, C(1); ref. 24).

Analytical h.p.l.c. was carried out using a 6.2 mm  $\times$  25 cm Du Pont Zorbax Sil column while preparative h.p.l.c. separations were obtained using a 1 in  $\times$  4 ft column of 10 $\mu$  silica gel.

The bromohydrin (2) was obtained<sup>17</sup> in several steps from 9,10-dihydrobenzo[*a*]pyrene-7(8*H*)-one (Aldrich). Since full experimental details are given for the analogous transformations in the naphthalene<sup>28</sup> and anthracene<sup>28</sup> series and for the racemic B[*a*]P analogues<sup>17</sup> the reaction conditions, yields and physical properties are summarized in Scheme 2 and in Table 1. N.m.r. spectra were identical to those previously reported for the racemic compounds and have not been included.

*Resolution of (-) and (+)-trans-8-Bromo-7-(menthyl-oxyacetoxy)-7,8,9,10-tetrahydrobenzo[*a*]pyrene (1A,1B).*—A

TABLE 2

Final atomic co-ordinates and temperature factors, with estimated standard deviations in parentheses

	<i>x</i>	<i>y</i>	<i>z</i>	<i>U</i> <sub>iso</sub>
Br	0.122(1)	0.250(0)	0.437(1)	*
O(1)	-0.113(3)	0.277(2)	0.147(8)	0.04(1)
O(2)	-0.114(5)	0.182(3)	-0.121(12)	0.12(2)
O(3)	-0.293(4)	0.146(3)	0.123(11)	0.09(2)
C(1)	-0.139(5)	0.600(4)	-0.935(14)	0.06(2)
C(2)	-0.228(7)	0.584(5)	-1.103(17)	0.09(3)
C(3)	-0.284(5)	0.525(4)	-1.065(14)	0.04(2)
C(3a)	-0.251(6)	0.486(5)	-0.877(16)	0.08(3)
C(4)	-0.297(6)	0.423(4)	-0.842(16)	0.06(2)
C(5)	-0.286(6)	0.379(4)	-0.642(14)	0.06(2)
C(5a)	-0.182(6)	0.393(4)	-0.498(16)	0.08(3)
C(6)	-0.148(6)	0.352(4)	-0.275(16)	0.07(3)
C(6a)	-0.053(6)	0.361(4)	-0.111(15)	0.05(2)
C(7)	-0.020(4)	0.300(3)	0.053(12)	0.03(2)
C(8)	0.066(5)	0.328(4)	0.200(14)	0.05(2)
C(9)	0.156(4)	0.364(3)	0.137(11)	0.02(2)
C(10)	0.096(4)	0.432(2)	0.027(10)	0.01(1)
C(10a)	-0.001(5)	0.412(3)	-0.187(14)	0.05(2)
C(10b)	-0.021(5)	0.455(3)	-0.352(13)	0.04(2)
C(11)	0.016(6)	0.520(4)	-0.352(16)	0.07(3)
C(12)	0.000(6)	0.569(4)	-0.526(14)	0.05(2)
C(12a)	-0.100(5)	0.559(3)	-0.758(12)	0.02(2)
C(12b)	-0.168(6)	0.495(4)	-0.702(14)	0.04(2)
C(12c)	-0.129(6)	0.453(4)	-0.524(15)	0.06(2)
C(13)	-0.130(7)	0.210(4)	0.007(17)	0.16(3)
C(14)	-0.242(6)	0.204(4)	0.242(17)	0.08(3)
C(15)	-0.362(5)	0.160(3)	-0.105(13)	0.03(2)
C(16)	-0.424(6)	0.224(3)	-0.041(15)	0.06(3)
C(17)	-0.484(6)	0.241(6)	-0.278(17)	0.10(3)
C(18)	-0.575(10)	0.169(7)	-0.333(24)	0.16(5)
C(19)	-0.500(6)	0.114(4)	-0.430(14)	0.05(2)
C(20)	-0.431(6)	0.100(4)	-0.133(16)	0.07(2)
C(21)	-0.362(6)	0.037(4)	-0.168(14)	0.06(2)
C(22)	-0.415(10)	-0.026(7)	-0.159(24)	0.16(5)
C(23)	-0.305(5)	0.033(4)	-0.402(14)	0.05(2)
C(24)	-0.557(8)	0.299(5)	-0.266(18)	0.10(3)

\*  $U_{11} = 0.055(4)$ ,  $U_{22} = 0.063(4)$ ,  $U_{33} = 0.040(4)$ ,  $U_{23} = 0.028(6)$ ,  $U_{31} = -0.005(3)$ ,  $U_{12} = 0.026(6)$ .

partial separation of (1A) and (1B) was obtained using light petroleum-ether (9 : 1 v/v) to elute a mixture (10g) from a column of silica gel (1 kg; 120 mm diam.). Preparative h.p.l.c. of (1A) and (1B) (1g) on silica gel was completed using cyclohexane-ether (49 : 1 v/v) as eluant at a flow rate of 40 ml min<sup>-1</sup>. In the latter method, two fractions >95% diastereoisomerically pure were recovered in >90% total yield. The efficiency of separation was checked by analytical h.p.l.c. Elution from a Zorbax Sil column with ether-cyclohexane (1 : 19 v/v) occurred with baseline separation [(1A),  $k' = 1.68$ ; (1B),  $k' = 2.04$ ]. Recrystallization of fractions highly enriched in the less polar diastereoisomer

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

gave pure (1A), m.p. 143–144 °C,  $[\alpha]_D -112^\circ$  (CHCl<sub>3</sub>), while the more polar isomer yielded pure (1B), m.p. 134–135 °C,  $[\alpha]_D +34^\circ$  (CHCl<sub>3</sub>).

Compound (11) was identified by g.l.c.–mass spectrometric analysis as reported previously<sup>21</sup> and purified by preparative t.l.c. on silica gel using light petroleum-ether (4 : 1 v/v).

*Crystal Data for (1B).*—C<sub>32</sub>H<sub>35</sub>BrO<sub>3</sub>, *M* 547.5. Monoclinic, space group *P*2<sub>1</sub>, *a* = 12.838(13), *b* = 20.270(20), *c* = 5.310(5) Å,  $\beta = 95.2(2)^\circ$ , *U* = 1375.3 Å<sup>3</sup>, *Z* = 2, *D*<sub>c</sub> = 1.32, *F*(000) = 572.

The crystals were extremely small thin plates which diffracted X-rays very weakly. The crystal used for collection of data had dimensions 0.6  $\times$  0.07  $\times$  0.03 mm. 1490 Independent diffraction intensities were measured on an Enraf-Nonius CAD3 automatic diffractometer, using Cu-*K* $\alpha$  radiation. Of these only 258 had *I* > 3 $\sigma$ (*I*), 340 had *I* > 2 $\sigma$ (*I*), while 547 had *I* >  $\sigma$ (*I*) and accordingly the latter criterion was used as it allowed use of data to  $\theta_{\max}$  ca. 50°, equivalent to a resolving power of ca. 1.0 Å. Intensities were corrected for Lorentz and polarization factors. The Br atom was located in a Patterson synthesis and the C and O atoms were found in a subsequent difference Fourier synthesis. Refinement of the structure by least squares with allowance for anisotropic vibration for Br and isotropic vibrations for all other atoms produced convergence at *R* = 0.13. Atomic scattering factors were taken from ref. 31. Final atomic co-ordinates and temperature factors with estimated standard deviations are given in Table 2. Predictably, with a data : parameter ratio of ca. 3.6, all e.s.d.s are high, but while no claims are made on the basis of molecular dimensions the independent location of each atom and subsequent refinement establishes the stereochemistry unequivocally as [7*S*, 8*S*]. Tables of bond lengths and angles and of observed and calculated structure factors have been deposited as Supplementary Publication No. SUP 22815 (7 pp.).†

We thank Messrs. B. McKnight and W. J. Swindall for microanalytical data and the N.I.H. for postdoctoral funds (to G. S. G.).

[9/1614 Received, 11th October, 1979]

## REFERENCES

- 1 'Carcinogenesis, Vol. 3: Polynuclear Aromatic Hydrocarbons,' eds. P. W. Jones and R. I. Freudenthal, Raven Press, New York, 1978.
- 2 M. Koreeda, P. D. Moore, H. Yagi, H. J. C. Yeh, and D. M. Jerina, *J. Amer. Chem. Soc.*, 1976, **98**, 6720.
- 3 A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, I. B. Weinstein, F. A. Beland, R. G. Harvey, H. Kasai, I. Miura, and K. Nakanishi, *J. Amer. Chem. Soc.*, 1976, **98**, 5714.
- 4 D. W. Nebert, A. R. Boobis, H. Yagi, D. M. Jerina, and R. E. Kouri, in 'Biological Reactive Intermediates,' eds. D. J. Jallow, J. J. Kocsis, R. Snyder, and H. Vainio, Plenum Press, 1977, pp. 125–145.
- 5 H. B. Gamper, A. S. C. Tung, K. Straub, J. C. Bartholomew, and M. Calvin, *Science*, 1977, **197**, 671.
- 6 D. M. Jerina and R. E. Lehr, in 'Microsomes and Drug Oxidations,' eds. V. Ullrich, I. Roots, A. G. Hildebrandt, R. W. Estabrook, and A. H. Conney, Pergamon Press, Oxford, 1977, pp. 709–720.
- 7 D. R. Boyd, D. M. Jerina, and J. W. Daly, *J. Org. Chem.*, 1970, **92**, 1056.
- 8 M. N. Akhtar and D. R. Boyd, *J.C.S. Chem. Comm.*, 1975, 916.
- 9 D. R. Thakker, H. Yagi, W. Levin, A. Y. H. Lu, A. H. Conney, and D. M. Jerina, *J. Biol. Chem.*, 1977, **252**, 6328.
- 10 S. K. Yang, P. P. Roller, and H. V. Gelboin, *Biochem.*, 1977, **16**, 3680.

- <sup>11</sup> A. M. Jeffrey and D. M. Jerina, *J. Amer. Chem. Soc.*, 1975, **97**, 4427.
- <sup>12</sup> M. N. Akhtar, J. G. Hamilton, D. R. Boyd, A. Braunstein, H. E. Seifried, and D. M. Jerina, *J.C.S. Perkin I*, 1979, 1442.
- <sup>13</sup> D. M. Jerina, H. Yagi, D. R. Thakker, J. M. Karle, H. D. Mah, D. R. Boyd, G. Gadaginamath, A. W. Wood, M. Buening, R. L. Chang, W. Levin, and A. H. Conney, *Adv. Pharmacology and Therapeutics*, 1978, **9**, 53.
- <sup>14</sup> K. Nakanishi, H. Kasai, H. Cho, R. Harvey, A. Jeffrey, K. Jennette, and I. Weinstein, *J. Amer. Chem. Soc.*, 1977, **99**, 258.
- <sup>15</sup> H. Yagi, H. Akagi, D. R. Thakker, H. D. Mah, M. Koreeda, and D. M. Jerina, *J. Amer. Chem. Soc.*, 1977, **99**, 2358.
- <sup>16</sup> D. R. Boyd, G. S. Gadaginamath, R. Hamilton, H. Yagi, and D. M. Jerina, *Tetrahedron Letters*, 1978, 2487.
- <sup>17</sup> H. Yagi and D. M. Jerina, *J. Amer. Chem. Soc.*, 1975, **97**, 3185.
- <sup>18</sup> D. R. Boyd, J. D. Neill, and M. E. Stubbs, *J.C.S. Chem. Comm.*, 1977, 873.
- <sup>19</sup> W. Klyne and J. Buckingham in 'Atlas of Stereochemistry,' Chapman and Hall, London, 1978, vol. 2, p. 78.
- <sup>20</sup> H. Arakawa, N. Torimoto, and Y. Masui, *Tetrahedron Letters*, 1968, 4115.
- <sup>21</sup> M. N. Akhtar and D. R. Boyd, *J.C.S. Perkin I*, 1976, 676.
- <sup>22</sup> M. Koreeda, M. N. Akhtar, D. R. Boyd, J. D. Neill, D. T. Gibson, and D. M. Jerina, *J. Org. Chem.*, 1978, **43**, 1023.
- <sup>23</sup> M. N. Akhtar, D. R. Boyd, N. J. Thompson, M. Koreeda, D. T. Gibson, V. Mahadevan, and D. M. Jerina, *J.C.S. Perkin I*, 1975, 2506.
- <sup>24</sup> H. E. Audier, J. F. Dupin, and J. Jullien, *Bull. Soc. chim. France*, 1968, 3844.
- <sup>25</sup> M. Koreeda, P. D. Moore, P. G. Wislocki, W. Levin, A. H. Conney, H. Yagi, and D. M. Jerina, *Science*, 1978, **199**, 778.
- <sup>26</sup> I. B. Weinstein, A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, R. G. Harvey, C. Harris, H. Autrup, H. Kasai, and K. Nakanishi, *Science*, 1976, **193**, 592.
- <sup>27</sup> M. K. Buening, P. G. Wislocki, W. Levin, H. Yagi, D. R. Thakker, H. Akagi, M. Koreeda, D. M. Jerina, and A. H. Conney, *Proc. Nat. Acad. Sci. U.S.A.*, 1978, **11**, 5358.
- <sup>28</sup> M. N. Akhtar, D. R. Boyd, and J. G. Hamilton, *J.C.S. Perkin I*, 1979, 2437.
- <sup>29</sup> J. F. Waterfall and P. Sims, *Biochem. J.*, 1972, **128**, 265.
- <sup>30</sup> P. Sims, *J. Chem. Soc. (C)*, 1968, 32.
- <sup>31</sup> International Tables for X-Ray Crystallography, Vol. III, Kynoch Press, Birmingham, 1962.