Absolute Stereochemistry of the Dihydroanthracene-*cis*- and -*trans*-1,2-diols produced from Anthracene by Mammals and Bacteria

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(+)-1,2-Dihydroanthracene-*trans*- and -*cis*-1,2-diols have been isolated as major anthracene metabolites from rabbits and the bacterium *Beijerinckia* B-836, respectively. The absolute stereochemistry and optical purity of each diol has been related to that of 1,2,3,4-tetrahydroanthracen-2-ol. The latter was resolved *via* the diastereoisomeric (−)-menthyloxyacetates, whose optical purity and absolute stereochemistry was determined by a range of techniques including kinetic resolution, asymmetric synthesis, and c.d. (exciton chirality method).

THE metabolic formation of vicinal dihydro-diols is one of the principal pathways by which both mammals and bacteria oxidize aromatic rings.[1,2] The dihydro-diols of mammalian origin have *trans* relative stereochemistry and result from the enzymic hydration of arene oxides; those of bacterial origin have *cis* relative stereochemistry and result from the action of dioxygenases.

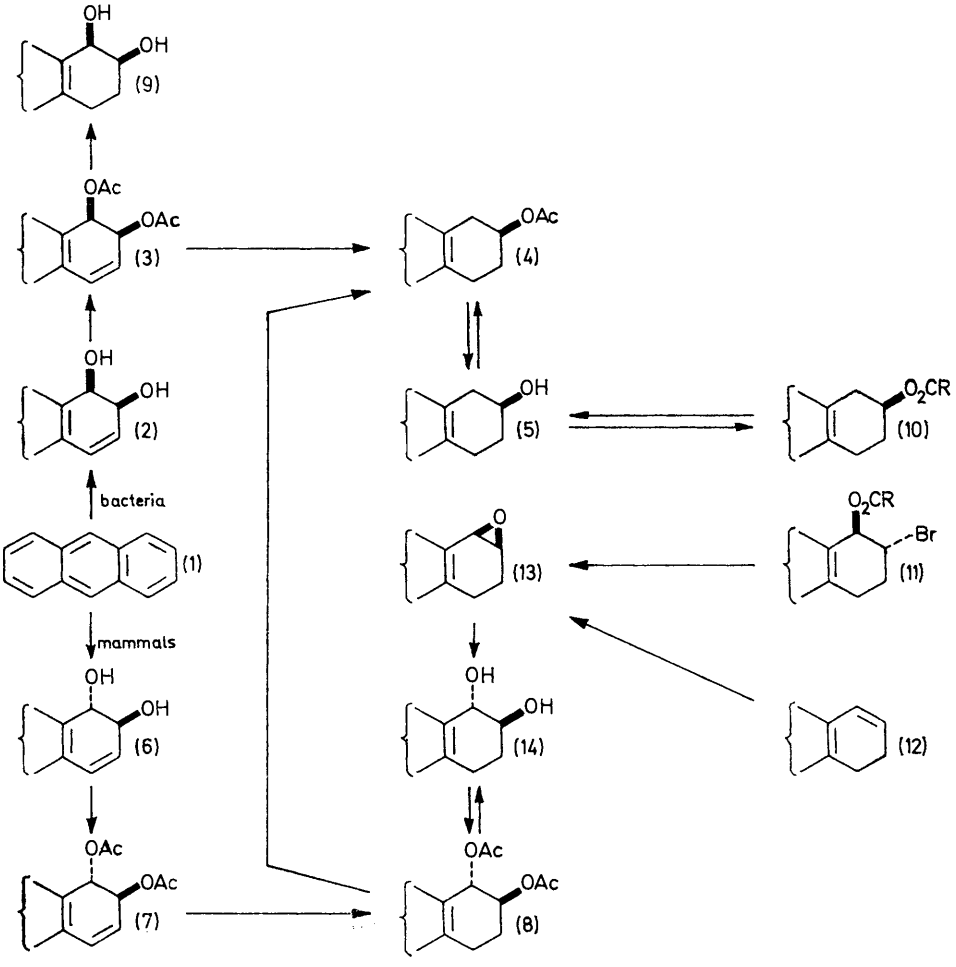
Relatively little is known about the absolute stereochemistry of arene dihydro-diols. The sign of optical rotation has been related to the absolute stereochemistry of the *trans*-1,2-dihydro-diol metabolite from benzene by comparison with the known *trans*-cyclohexane-1,2-diol stereochemistry.[3] The absolute stereochemistries of the *trans*-1,2-dihydro-diol of naphthalene and the *trans*-9,10-dihydro-diol of phenanthrene have been deduced by oxidation to a derivative of tartaric acid.[4] The absolute configuration of 3-methylcyclohexa-3,5-diene-1,2-diol, the metabolite of toluene, has been related to that of 2-methyladipic acid by chemical methods[5] and has been confirmed by *X*-ray crystallography.[6] The *cis*-1,2-dihydro-diol metabolite of naphthalene was related to 1,2,3,4-tetrahydronaphthalen-2-ol.[7] In the present study also, this last method was used for assigning absolute stereochemistry to the *cis*- and *trans*-1,2-dihydro-diols formed from anthracene, by chemical conversion into 1,2,3,4-tetrahydroanthracen-2-ol which has now been resolved and assigned absolute stereochemistry.

RESULTS AND DISCUSSION

Biosynthetic Dihydro-diols from Anthracene.—The mammalian metabolism of anthracene has been investi-

[1] J. W. Daly, D. M. Jerina, and B. Witkop, *Experientia*, 1972, **28**, 1129.
[2] D. T. Gibson, *Crit. Rev. Microbiol.*, 1972, **1**, 199.
[3] D. M. Jerina, H. Ziffer, and J. W. Daly, *J. Amer. Chem. Soc.*, 1970, **92**, 1056.
[4] R. Miura, S. Honmaru, and M. Nakazaki, *Tetrahedron Letters*, 1968, 5271.
[5] H. Ziffer, D. M. Jerina, D. T. Gibson, and V. M. Kobal, *J. Amer. Chem. Soc.*, 1973, **95**, 4048.
[6] V. M. Kobal, D. T. Gibson, R. E. Davis, and A. Garza, *J. Amer. Chem. Soc.*, 1973, **95**, 4420.
[7] A. M. Jeffrey, H. T. C. Yeh, D. M. Jerina, T. R. Patel, J. F. Davey, and D. T. Gibson, *Biochemistry*, 1975, **14**, 575.

gated previously,[8-12] but to date the absolute configuration of the *trans*-1,2-dihydro-diol metabolites has remained unknown. In the present work (+)-1,2-dihyroanthracene-*trans*-1,2-diol was isolated from the urine of rabbits to which anthracene had been administered. Formation of this dihydro-diol by mammals does not occur in a totally stereoselective manner. In general the rabbit tends to produce an excess of the (+)-enantiomer whereas the rat produces an excess of the (−)-form. The optical purity of the dihydro-diol



isolated depends upon the particular mammalian species and the degree of fractional crystallization used during purification (Table).

(+)-1,2-Dihydroanthracene-*cis*-1,2-diol was obtained through the action of the bacterium *Beijerinckia* B-836 on anthracene.[13] This bacterium is a mutant strain which is deficient in dihydro-diol dehydrogenase activity.

Resolution of 1,2,3,4-*Tetrahydroanthracen-2-ol* (5).—The racemic form of the alcohol (5), obtained in quantitative yield by reduction with lithium aluminium hydride of the epoxide (±)-(13) gave crystalline (−)-menthyloxyacetate diastereoisomers of (10; R = 3-menthyloxymethyl) on reaction with (−)-menthyloxyacetyl chloride. Multiple recrystallization of the (−)-menthyloxyacetates from light petroleum (b.p. 60—80°) to constant optical rotation produced two diastereoisomers from the racemic alcohol, $[\alpha]_{589}$ −72°, m.p. 85—86°, and $[\alpha]_{589}$ −27°, m.p. 152—153° (less soluble). Hydrolysis with methanolic potassium hydroxide gave the enantiomeric alcohols (5), $[\alpha]_{589}$ −47° and $[\alpha]_{589}$ +52°. The slightly lower rotation value for (−)-(5) was due to the difficulty of crystallizing and purifying the residual more soluble diastereoisomer. Two lines of evidence suggested that the optical purity of the resolved samples of the alcohol (5) was high (>90%). (*a*) Attempts to increase the optical rotations above 52°, by using several batches of racemic alcohol and repeated crystallizations of the optically active alcohol or the (−)-menthyloxyacetates, were unsuccessful. (*b*) The observation in the n.m.r. spectrum of differing degrees of non-equivalence of the diastereotopic methylene protons in menthyloxyacetate

[8] E. Boyland and A. A. Levi, *Biochem. J.*, 1935, **29**, 2679.
[9] J. Booth and E. Boyland, *Biochem. J.*, 1949, **44**, 361.
[10] E. Boyland and C. W. Shoppee, *J. Chem. Soc.*, 1947, 801.
[11] P. Sims, *Biochem. J.*, 1964, **92**, 621.
[12] E. Boyland, M. Kimura, and P. Sims, *Biochem. J.*, 1964, **92**, 631.
[13] H. Selander, H. Yagi, D. M. Jerina, M. C. Wells, J. F. Davey, V. Mahadevan, and D. T. Gibson, *Arch. Biochem. Biophys.*, in the press.

diastereoisomers of cyclic secondary alcohols and application of this difference as a monitor of enantiomeric homogeneity has been reported.[14] Non-equivalence of the analogous methylene peaks in the (−)-menthyloxyacetates of each enantiomer of the alcohol (5) are shown in Figure 1. The sensitivity of this 220 MHz

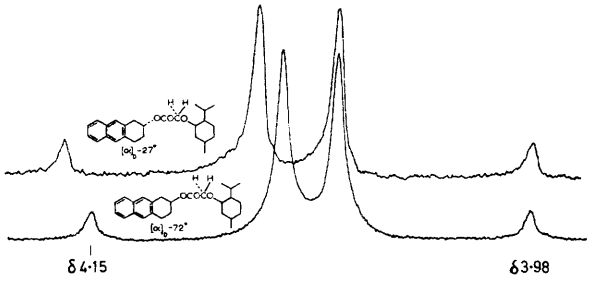
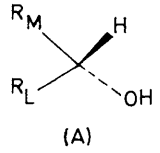


FIGURE 1 220 MHz N.m.r. spectrum of the (−)-menthyloxyacetates of the enantiomeric alcohols (5)

n.m.r. technique in the latter example should permit the detection of the minor diastereoisomeric constituent at a concentration of >10%.

Correlation of Absolute Stereochemistry. The absolute stereochemistry of the enantiomers of the alcohol (5) was deduced from the following evidence.

(i) The technique of kinetic resolution has been successfully used to deduce absolute stereochemistry of a wide range of both cyclic and acyclic secondary alcohols.[15,16] The Mislow method,[16] involving preferential formation of one sulphinate ester diastereoisomer from optically pure secondary alcohol and toluene-*p*-sulphinyl chloride followed by stereospecific conversion into optically active methyl *p*-tolyl sulphoxide, has now been applied to (+)-1,2,3,4-tetrahydroanthracen-2-ol [(+)-(5)]. Preference for the (+)-form of methyl *p*-tolyl sulphoxide, as found in the present example, suggests that the original alcohol had the configuration (A). On the assumption that the cyclohexene ring prefers to exist in a half-chair conformation and that the axial and equatorial hydrogen atoms on the benzylic carbon atom at position 4 have a slightly greater steric requirement (1,3-diaxial interactions) [15]



(A)

R_M medium bulk group (CH_2Ar)

R_L large bulk group ($CH_2{\cdot}CH_2Ar$)

than the aryl ring in this fixed conformation, then (+)-1,2,3,4-tetrahydroanthracen-2-ol should have the *R*-configuration [the formula shows (5) with (2*S*)-configuration]. The fine distinction between the 'effective bulk' of R_M and R_L allied to the low optical purity of methyl *p*-tolyl sulphoxide isolated (*ca.* 7%) demanded configurational correlation by independent methods.

(ii) Epoxidation of 1,2-dihydroanthracene (12) with (+)-peroxycamphoric acid produced (−)-1,2-epoxy-1,2,3,4-tetrahydroanthracene, which gave (+)-1,2,3,4-tetrahydroanthracen-2-ol on reduction ($LiAlH_4$). The isolation of (+)-(2*R*)-2-tetralol after a similar epoxidation–reduction sequence with 1,2-dihydronaphthalene [17] implies that the analogous anthracene analogue, (+)-(5), has the *R*-configuration. It is improbable that substitution of 1,2-dihydroanthracene (12) for 1,2-dihydronaphthalene will result in reversal of stereochemistry during the asymmetric oxygen atom transfer step.

(iii) During independent studies [18] it was found that the corresponding *trans*-2-bromo-1,2,3,4-tetrahydro-1-naphthyl menthyloxyacetate diastereoisomers were separable by column chromatography. A similar chromatographic separation was effected with the analogous tetrahydroanthracene diastereoisomers of (11; R = 3-menthyloxymethyl). Treatment of the first eluted diastereoisomer of *trans*-2-bromo-1,2,3,4-tetrahydro-1-naphthyl menthyloxyacetate with base followed by reduction ($LiAlH_4$) gave (−)-(2*S*)-1,2,3,4-tetrahydronaphthalen-2-ol. It is thus probable that identical treatment of the first eluted diastereoisomer of *trans*-2-bromo-1,2,3,4-tetrahydro-1-anthryl menthyloxyacetate (11) to yield (−)-(5) affords further confirmation of the (2*S*)-configuration in the latter enantiomer.

(iv) The exciton chirality method [19–21] was applied to the *p*-chlorobenzoyl derivative of (+)-1,2,3,4-tetrahydroanthracen-2-ol. Both 1,2,3,4-tetrahydroanthracene and *p*-chlorobenzoyloxy chromophores have strong π–π^* transitions at 230 (ε *ca.* 100 000; $^1A - {}^1B_b$ band) and 240 nm (ε 24 000; intramolecular charge-transfer band), respectively. Furthermore, the directions of the electric transition dipoles due to these two absorptions are firmly established by MO calculations.[21,22] The c.d. spectrum of the *p*-chlorobenzoate (Figure 2) showed a typical exciton interaction pattern of two Cotton effects with opposite signs ($\Delta\varepsilon_{235}$ −32.3; $\Delta\varepsilon_{225}$ +32.8). The negativity of the first Cotton effect defines the absolute spatial dispositions of the two chromophores, *i.e.*, (2*R*)-configuration. This application can be compared with the known example [22] of a naphthalene chromophore; thus 17β-dihydroequilenin 3-methyl ether 17-benzoate (Figure 3) showed $\Delta\varepsilon$ +34.0 and −14.3 at 235 and 220 nm, respectively.

The four independent methods discussed above all support the assignment of the *S*-configuration to the (−)-enantiomer of 1,2,3,4-tetrahydroanthracen-2-ol (5).

Absolute Configuration of Anthracene Metabolites.—The assignment of absolute stereochemistry to the (+)-*trans*-dihydro-diol obtained from the urine of rabbits relies on

[14] D. R. Galpin and A. C. Huitric, *J. Org. Chem.*, 1968, **33**, 921.
[15] A. Horeau and J. K. Sutherland, *J. Chem. Soc.* (*C*), 1966, 247 and references therein.
[16] M. M. Green, M. Axelrod, and K. Mislow, *J. Amer. Chem. Soc.*, 1966, **88**, 861.
[17] D. R. Boyd, D. M. Jerina, and J. W. Daly, *J. Org. Chem.*, 1970, **35**, 3170.
[18] M. S. Akhtar and D. R. Boyd, *J.C.S. Chem. Comm.*, in the press.
[19] N. Harada and K. Nakanishi, *Accounts Chem. Res.*, 1972, **5**, 257 and references therein.
[20] M. Koreeda, N. Harada, and K. Nakanishi, *J. Amer. Chem. Soc.*, 1974, **96**, 266.
[21] S. F. Mason, R. H. Seal, and D. R. Roberts, *Tetrahedron*, 1974, **30**, 1671.
[22] N. Harada and K. Nakanishi, *J. Amer. Chem. Soc.*, 1969, **91**, 3989.

the well established [8-12] sequence: (+)-dihydro-diol (6) ⟶ (+)-dihydro-diol diacetate (7) ⟶ (+)-tetrahydro-diol diacetate (8) ⟶ (−)-tetrahydro-diol (14).

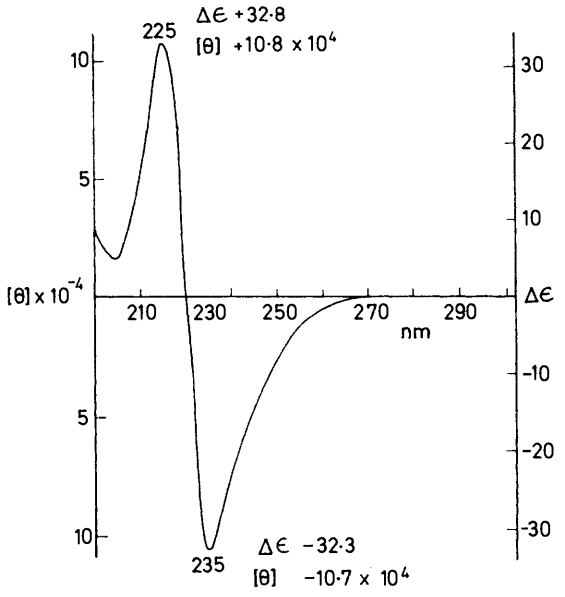


FIGURE 2 C.d. spectrum of (2*R*)-1,2,3,4-tetrahydro-2-anthryl *p*-chlorobenzoate in 10% dioxan–methanol

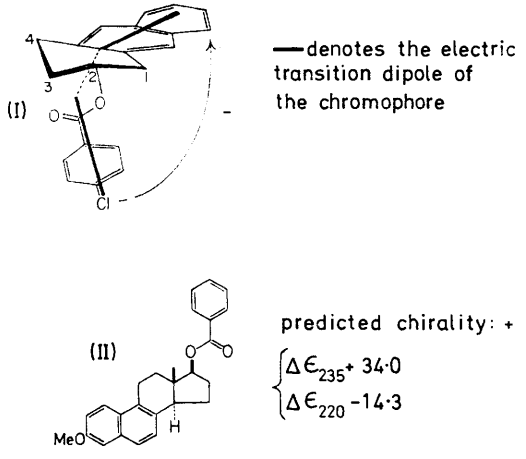


FIGURE 3 (I) (2*R*)-1,2,3,4-Tetrahydro-2-anthryl *p*-chlorobenzoate; (II) 17β-dihydroequilenin 3-methyl ether 17-benzoate

On the assumption that (as with analogous epoxides [23]), nucleophilic attack (OH^-) on the epoxide (13) will occur at the benzylic position to form the *trans*-diol (14), then the stereochemistry of the diol can be related to that of the alcohol (5). The (−)-enantiomer of the epoxide (13) [(1*S*,2*R*), as shown previously] thus gave the (+)-(2*R*)-alcohol (5) by reduction ($LiAlH_4$) and the (+)-*trans*-(1*R*,2*R*)-tetrahydro-diol (14) by addition of hydroxide. In addition, the (+)-tetrahydro-diol diacetate (8) was reduced to (−)-(4) of >50% optical purity. On the basis of the above correlations the metabolite (6) must have the absolute configuration (1*S*,2*S*) as shown.

The (+)-*cis*-dihydro-diol (2) produced by *Beijerinckia* B-836 was converted into the diacetate (3), which was converted into the acetate (4) of (5) by catalytic reduction and hydrogenolysis. Since change in configuration at C-2 is not possible in this series of reactions, and since the sequence of signs of optical rotation (+)-dihydro-diol (2) ⟶ (−)-(4) ⟶ (−)-(5) applies, the absolute configuration of the (+)-metabolite (2) is (1*R*,2*S*). The magnitude of the optical rotation for the acetate (4) from the metabolite equals that of the chemically resolved and acetylated alcohol (5). The bacterial metabolite thus appears to be optically pure.

The c.d. spectra of the free diols as well as the diacetate of the *trans*-diol and of the acetonide of the *cis*-diol were determined in order to confirm the absolute stereochemistry (Figure 4). The spectra display several bands and are not readily interpreted. Most notable are the large changes resulting from alterations in the conformations of the diols through formation of derivatives.[13]

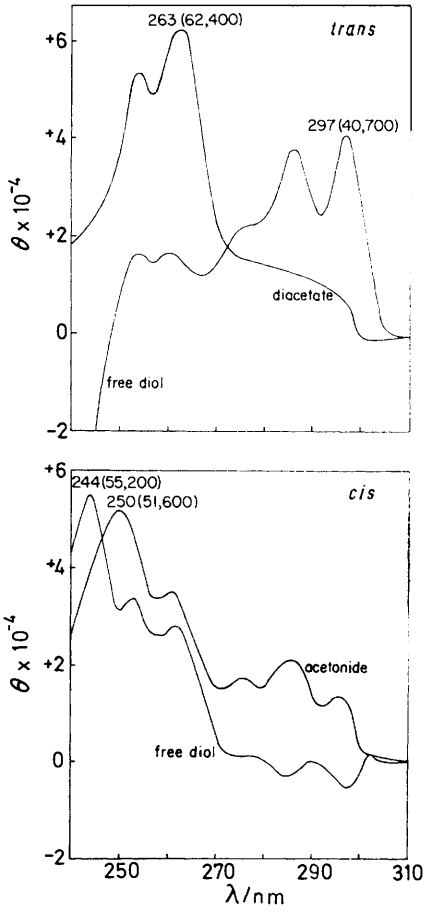
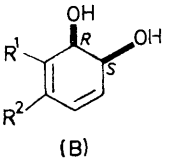


FIGURE 4 C.d. spectra of the *trans*- and *cis*-dihydro-diol metabolites of anthracene and the corresponding diacetate and acetonide (in ethanol)

To date, the absolute stereochemistry has been assigned for only three *cis*-dihydro-diols resulting from the

[23] E. E. van Tamelen, G. Van Zyl, and G. D. Zuidema, *J. Amer. Chem. Soc.*, 1950, **72**, 488.

bacterial metabolism of aromatic hydrocarbons: (+)-(1*S*,2*R*)-3-methylcyclohexa-3,5-diene-1,2-diol (*Pseudomonas putida*, strain 39/D),[5] (+)-(1*R*,2*S*)-1,2-dihydronaphthalene-1,2-diol (*Pseudomonas putida*, strain 119),[7] and (+)-(1*R*,2*S*)-1,2-dihydroanthracene-1,2-diol (*Beijerinckia*, strain B-836). In all cases the sign of rotation is positive and the exclusively preferred enantiomer will have the general configuration shown in (B). The



(B)

R^1 = aryl or methyl
R^2 = aryl or hydrogen

validity of this correlation for larger aromatic hydrocarbons is under current investigation.

In contrast, the *trans*-dihydro-diols produced by

Optical rotations {$[\alpha]_{589}$ (°) in dioxan}

trans-Diol (6) *	*trans*-Diacetate (7)	Ref.
−100 [a]	−362 [a] †	8
+16 [b]	+309 [b]	8
0 ⟶ −149 [a,c]	−342 [a,c]	9
0 ⟶ −154 [a,c]	−345 [a,c]	9
−80 [a]	−240 [a]	10
0 [b]		10
0, [a] −160 [a,d]		11
0 ⟶ +140 [b,e]	+254 [b]	

* An arrow denotes the isolation of several fractions of optical rotation within this range. † Based on the reduction of (+)-7 to (−)-4. In the present study this value corresponds to an optical purity of >95%.

[a] Rat. [b] Rabbit. [c] After multiple recrystallization. [d] After hydrolysis of the glucosiduronic acid. [e] Present work.

hydration of arene oxides in mammalian systems (Table) show a wide range both in magnitude of optical purity and in absolute configuration, and no simple correlation is apparent.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. Optical rotations were obtained with a Perkin-Elmer 141 automatic polarimeter; values quoted were the average of several readings and showed a deviation of ±0.002°. N.m.r. spectra were determined at 100 MHz with a Varian HA-100 or at 220 MHz with a Varian HR-220 spectrometer.

(±)-1,2-*Epoxy*-1,2,3,4-*tetrahydroanthracene* (13).—A solution of *m*-chloroperbenzoic acid (6 g, 0.033 mol) in chloroform (300 ml) was added to an ice-cooled, stirred mixture of 1,2-dihydroanthracene [24] (5 g, 0.028 mol) and anhydrous sodium carbonate (6.5 g) in chloroform (250 ml) over 1 h. Stirring was continued for 2.5 at 0 °C and finally for 30 min at room temperature. The solution was washed (2N-Na_2SO_3, 2N-$NaHCO_3$, and water), dried ($MgSO_4$), and concentrated and the residue was recrystallized from ether–light petroleum (b.p. 40—60°) to give crystals of the *epoxide* (4.5 g, 90%), m.p. 146—150° (decomp.) (Found: C, 85.9; H, 6.3. $C_{14}H_{12}O$ requires C, 85.7; H, 6.1%).

Use of (+)-peroxycamphoric acid instead of *m*-chloroperbenzoic acid yielded (−)-1,2-epoxy-1,2,3,4-tetrahydroanthracene, $[\alpha]_{589}$ −3.6° (*c* 5 in $CHCl_3$).

(±)-1,2,3,4-*Tetrahydroanthracen*-2-*ol* (5).—1,2-Epoxy-1,2,3,4-tetrahydroanthracene (5.5 g; 0.028 mol) was refluxed with an excess of lithium aluminium hydride (3 g, 0.08 mol) for 8 h. Decomposition with water, extraction with ether, and drying and concentration of the extract afforded a residue which was purified by chromatography on active alumina. Recrystallisation from light petroleum (b.p. 60—80°) yielded the (±)-*alcohol* (5), m.p. 144—146° (Found: C, 84.1; H, 7.0. $C_{14}H_{14}O$ requires C, 84.3; H, 7.1%).

A similar reduction of (−)-(13) ($[\alpha]_{589}$ −3.6°) yielded (+)-(5), $[\alpha]_{589}$ +1.2°, $[\alpha]_{436}$ +2.8° (*c* 5 in $CHCl_3$).

Resolution of (±)-1,2,3,4-*Tetrahydroanthracen*-2-*ol*.—Racemic 1,2,3,4-tetrahydroanthracen-2-ol (10.5 g; 0.053M) was dissolved in pyridine (120 ml) and stirred while (−)-menthyloxyacetyl chloride (12.4 g, 0.053 mol) was added dropwise over 1 h. Stirring was continued for 24 h before addition of water and ether. The ether layer was washed (2N-HCl, 2N-$NaHCO_3$, and water), dried, and concentrated to yield a mixture of (−)-2-methyloxyacetate diastereoisomers. Fractional crystallization of the least soluble component to constant optical rotation yielded *crystals*, m.p. 152—153°, $[\alpha]_{589}$ −27° ($CHCl_3$) (Found: C, 79.4; H, 8.8. $C_{26}H_{34}O_3$ requires C, 79.2; H, 8.6%).

Alkaline hydrolysis (refluxing for 3 h with 2.5% aqueous methanolic potassium hydroxide) of the less soluble diastereoisomer ($[\alpha]_{589}$ −27°) yielded the alcohol (+)-(5), $[\alpha]_{589}$ +52° ($CHCl_3$). Similar treatment of the other diastereoisomer, $[\alpha]_{589}$ −72° ($CHCl_3$), m.p. 85—86°, gave the alcohol (−)-(5), $[\alpha]_{589}$ −47° ($CHCl_3$). The optically active alcohols had i.r. spectra identical with that of the racemic alcohol and both crystallized from light petroleum (b.p. 60—80°) with m.p. 136—138° (Found: C, 84.7; H, 7.1%).

1,2,3,4-*Tetrahydro*-2-*anthryl Acetate*.—(+)-1,2,3,4-Tetrahydroanthracene-2-ol, $[\alpha]_{589}$ +50° ($CHCl_3$), was dissolved in dry pyridine (2 ml) and heated with acetic anhydride (6 ml) for 2 h on a steam-bath. The product was extracted with dichloromethane and the extract was washed, dried ($MgSO_4$), and concentrated. The crystalline residue afforded the *acetate*, m.p. 93—94° (from pentane), $[\alpha]_{589}$ +56° (EtOH) (Found: C, 79.8; H, 6.6. $C_{16}H_{16}O_2$ requires C, 80.0; H, 6.7%).

1,2,3,4-*Tetrahydro*-2-*anthryl* p-*Chlorobenzoate*.—(+)-1,2,3,4-Tetrahydroanthracen-2-ol (0.015 g), $[\alpha]_{589}$ +50° ($CHCl_3$), was dissolved pyridine (0.3 ml) and treated with dry *p*-chlorobenzoyl chloride (0.15 ml) at ambient temperature for 15 h. The product was extracted with dichloromethane and subjected to preparative t.l.c. (20 × 20 cm, 1000 μm silica gel GF_{254}; elution with 2% MeOH–CH_2Cl_2). The resulting crystalline solid (0.016 g), on recrystallization from ether–light petroleum, gave the p-*chlorobenzoate*, m.p. 167—168°, $[\alpha]_{589}$ −39° ($CHCl_3$) (Found: C, 74.4; H, 5.2. $C_{21}H_{17}ClO_2$ requires C, 74.9; H, 5.1%).

Synthesis of Toluene-p-*sulphinate Ester Derivatives of* (+)-1,2,3,4-*Tetrahydroanthracen*-2-*ol*.—The reaction of toluene-*p*-sulphinyl chloride (in excess) at −78 °C with (+)-1,2,3,4-tetrahydroanthracen-2-ol (0.25 g) in pyridine (10 ml) according to the literature procedure [16] yielded a crude diastereoisomeric mixture of toluene-*p*-sulphinate ester derivatives, identified by i.r. [$\nu_{max.}$ 1010 cm^{-1} (−OSO−)] and n.m.r. spectroscopy. Further purification was avoided to prevent any change in the proportion of diastereoisomers and the crude toluene-*p*-sulphinate ester mixture was refluxed for 30 min with the Grignard reagent prepared from magnesium (0.064 g) and methyl bromide (0.25 g). After

[24] J. Rigaudy and N. K. Cuong, *Compt. rend.*, 1959, **248**, 262.

chromatography on deactivated alumina, the eluted methyl *p*-tolyl sulphoxide, m.p. 50—54°, showed $[\alpha]_{589}$ −12.3° ($CHCl_3$) (7% optical purity [16]).

1,2,3,4-*Tetrahydroanthracene*-trans-1,2-*diol*.— (−)-1,2-Epoxy-1,2,3,4-tetrahydroanthracene {0.5 g, 0.003 mol; $[\alpha]_{589}$ −3.6° ($CHCl_3$)} was refluxed in a mixture of N-potassium hydroxide (50 ml) and t-butyl alcohol (50 ml) for 10 h. The alcohol was removed under vacuum and the aqueous residue was extracted with ether; the extract was washed with water, dried ($MgSO_4$), and concentrated. Recrystallization of the residue from ether–light petroleum (b.p. 40—60°) yielded the *trans*-diol, m.p. 156—158°, $[\alpha]_{589}$ +0.5°, $[\alpha]_{436}$ +1.1° ($CHCl_3$) [lit.,[25] m.p. 162—163° (rac.)].

Isolation of the trans-*Dihydro-diol from Rabbit Urine*.— Twelve white New Zealand female rabbits (2 kg) were injected (IP) with anthracene (4.8 g) in peanut oil (196 ml). Daily injections (25 mg of anthracene per ml of peanut oil) were continued for 4 days, during which period urine was collected in ice. The urine was concentrated by lyophilization (6 l ⟶ 2 l) and adjusted to pH 5 with acetate buffer. β-Glucuronidase/sulphatase (1 000 000 units) was added and the urine was incubated for 18 h at 37 °C under toluene. The sodium chloride-saturated urine was subjected to continuous extraction for 8 h with ethyl acetate and an additional 8 h with ether. The combined extracts, after washing (NaOH) and drying, were further purified by preparative t.l.c. [500 μm silica gel, developed with benzene–ethanol (9 : 1)] and by recrystallization from benzene. The *trans*-dihydro-diol isolated (0.09 g) was racemic. The mother liquors were acylated with pyridine (2 ml) and acetic anhydride (6 ml). Purification of the product by recrystallisation (2 ml of ethanol, −20 °C) gave the *trans*-1,2-dihydro-1,2-diacetate (7), $[\alpha]_{589}$ +254° (dioxan). Catalytic reduction (3 h; 1 atm H_2; 0.02 g 5% Pd–C) of the (+)-*trans*-1,2-dihydro-1,2-diacetate (7) (0.076 g) in tetrahydrofuran (11 ml) followed by preparative t.l.c. (250 μm silica gel eluted with 15% ether–cyclohexane) provided the *trans*-1,2,3,4-tetrahydro-1,2-diacetate, $[\alpha]_{589}$ +40.6° (dioxane), in 93% yield.

A solution of this (+)-diacetate (0.012 g) in acetic acid (2.0 ml) was subjected to further reduction with hydrogen over 10% Pd–C (0.030 g) for 3.5 h. The desired 1,2,3,4-tetrahydro-2-anthryl acetate was isolated by preparative t.l.c. ($CHCl_3$); $[\alpha]_{589}$ −38° (EtOH).

Isolation of the cis-*Dihydro-diol produced by* Beijerinckia *B*-836.—The isolation of the *cis*-dihydro-diol, $[\alpha]_{589}$ +255° (CH_3OH), and preparation of the acetonide, $[\alpha]_{589}$ +284° (CH_3OH), were performed as previously described.[13] Catalytic hydrogenation (18 h; 1 atm H_2, 0.16 g of 5% Pd–C) of the diacetate (0.16 g) in acetic acid (10 ml) gave a range of reduction products. Preparative t.l.c. [silica gel, 250 μm developed (*a*) with benzene, (*b*) with 10% ether–cyclohexane, and (*c*) with 5% ether–hexane] finally yielded pure 1,2,3,4-tetrahydro-2-anthryl acetate (4), $[\alpha]_{589}$ −55° (EtOH), identical with that obtained by acetylation of resolved optically pure (+)-1,2,3,4-tetrahydroanthracen-2-ol (5) except for the sign of optical rotation {$[\alpha]_{589}$ +56° (EtOH)}.

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