Chemical Synthesis of the (1*R*,2*S*) and (1*S*,2*R*) Arene Oxide Metabolites of Acridine

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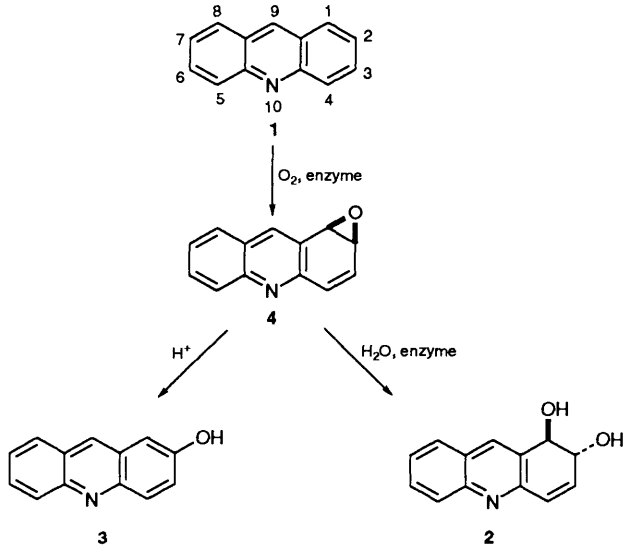
Enantiopure samples of (+)-(1*R*,2*S*) and (−)-(1*S*,2*R*)-1,2-epoxy-1,2-dihydroacridine **4** have been obtained from the corresponding *trans*-2-bromo-1,2,3,4-tetrahydroacridin-1-ol MTPA esters **7a** and **b**. Absolute configurations were deduced by stereochemical correlation to (+)-(1*R*,2*R*)-*trans*-2-bromo-1-(2-methoxy-2-phenyl-2-trifluoroacetoxy)-1,2,3,4-acridine **7a** which was unequivocally assigned by X-ray crystal structure analysis. (−)-(1*R*,2*R*)-*trans*-1,2-Dihydroacridine-1,2-diol **2** was obtained by alkaline hydrolysis of (+)-(1*R*,2*S*)-acridine 1,2-oxide **4**.

Acridine **1** is a member of the aza-polycyclic aromatic hydrocarbon (APAH) series and is distributed widely in the environment[1] due to partial combustion of fossil fuels (*e.g.* motor vehicle exhaust emissions)[2] and plant material (*e.g.* tobacco smoke).[3] Acridine **1** has been reported to be weakly mutagenic using *S. typhimurium*[4] cultures and to produce chromosomal abberations in Chinese hamster cell cultures.[5] In view of the biological activity associated with acridine **1** and the derived metabolites, animal metabolism studies have been carried out.[6,7] Both *trans*-1,2-dihydroacridine-1,2-diol **2** and acridin-2-ol **3** metabolites were formed due to enzyme-catalysed oxidation at the 1,2-bond of acridine using 3-methylcholanthrene-induced rat liver enzymes. Although acridine 1,2-oxide **4** has not been identified among the metabolites, it is probable that both the *trans*-dihydro diol **2** and phenolic product **3** were formed *via* the initial arene oxide metabolite **4** (see Scheme 1). Recent studies from these laboratories[8] have



Scheme 1

shown how racemic samples of acridine 1,2-oxide **4** and *trans*-1,2-dihydroacridine-1,2-diol **2** may be chemically synthesised. As part of a wider programme to study the metabolism of simpler members of the APAH series[9-12] we have recently developed synthetic routes to enantiopure arene oxide and dihydro diol metabolites of quinoline. This report describes how (−)-(1*S*,2*R*)- and (+)-(1*R*,2*S*)-acridine 1,2-oxides **4** and (−)-(1*R*,2*R*)-*trans*-dihydro diol **2** can be synthesised and configurationally assigned.

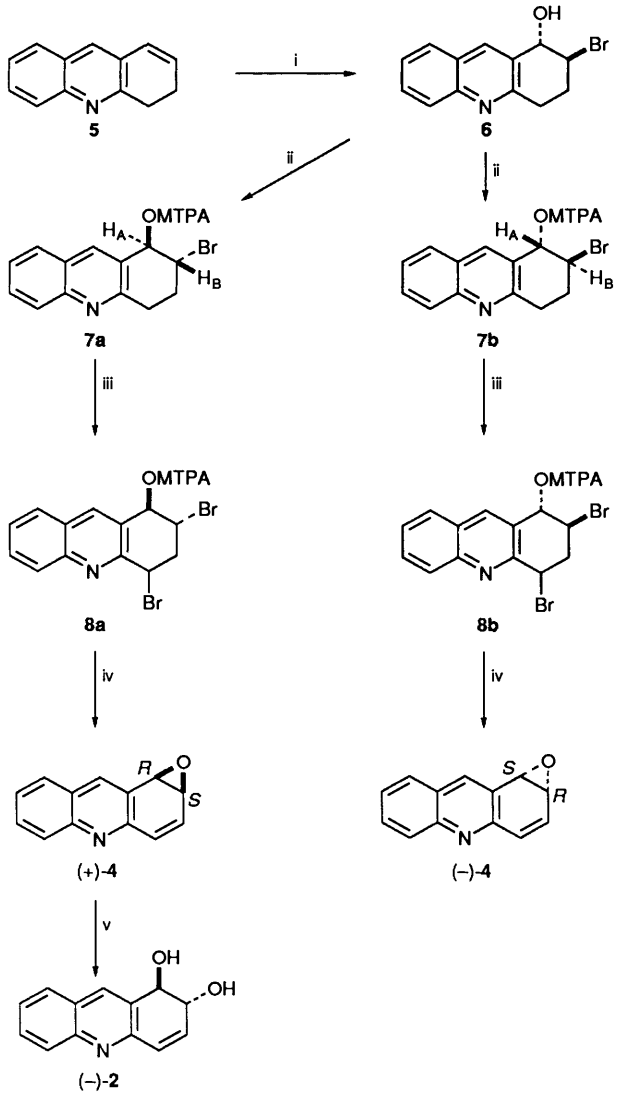
Results and Discussion

3,4-Dihydroacridine **5** was synthesised using the method reported previously.[8] Treatment of the dihydroacridine **5** with *N*-bromoacetamide (NBA) in aqueous THF gave the racemic *trans*-2-bromo-1,2,3,4-tetrahydroacridin-1-ol **6** (78%) (see Scheme 2). Esterification using (+)-2-methoxy-2-phenyl-2-trifluoromethylacetyl (MTPA) chloride, followed by preparative TLC separation on silica gel, yielded the corresponding bromo MTPA diastereoisomers **7a** (low R_f; $[\alpha]_D$ +63, MeOH) and **7b** (high R_f; $[\alpha]_D$ −8, MeOH). The diastereoisomeric purity of the bromo MTPA esters **7a** and **b** obtained from the preparative TLC separation, was confirmed as >99% by analytical HPLC [Microsorb, ethyl acetate–hexane (6:94), selectivity factor α = 1.6] and ^{1}H NMR spectral analysis.

Previous studies on bromo-MTPA esters of similar structure to compounds **7a** and **b**, in the PAH series,[13] using (+)-MTPA chloride have provided an empirical method for absolute configuration determination based upon ^{1}H NMR spectroscopy. Thus, the high-R_f isomer, having a smaller positive δ_H value for the proton H_B, and a smaller coupling constant J_{AB} (Hz), will have an S_A,S_B configuration. Conversely, the low-R_f isomer with a larger positive δ_H value for H_B, and a larger coupling constant J_{AB}, must have an R_A,R_B configuration.

The diastereoisomer **7b** of higher R_f, showing a lower positive δ_H value for proton H_B (δ 4.56) and a lower coupling constant J_{AB} (4.0 Hz), may thus be tentatively assigned the 1*S*,2*S* configuration and conversely the lower-R_f diastereoisomer **7a** (H_B δ 4.59 and J_{AB} 4.9 Hz) the 1*R*,2*R* configuration, on the basis of this empirical method. A more rigorous determination of absolute configuration resulted from X-ray crystallographic analysis of the lower-R_f bromo MTPA ester **7a** ($[\alpha]_D$ +63, MeOH) (see Fig. 1). In the crystalline state the cyclohexene ring in compound **7a** adopted a half-chair conformation with the bromine atom and the MTPA group having a *trans*-axial–quasiaxial relationship. Since the (+)-MTPA group was of *R* configuration, the absolute configuration of the bromo MTPA ester **7a** can thus be unequivocally established as 1*R*,2*R*. This assignment was in agreement with the earlier prediction based only on ^{1}H NMR analysis.

The bromo MTPA ester **7a** ($[\alpha]_D$ +63, MeOH) was converted into the corresponding dibromo MTPA compound **8a** using *N*-bromosuccinimide (NBS) in CCl_4. Because of its instability, compound **8a** was converted directly into the acridine 1,2-oxide **4** ($[\alpha]_D$ +22, $CHCl_3$) by treatment with sodium methoxide in THF. The arene oxide **4** proved to be a remarkably stable crystalline compound whose structure was assigned by X-ray crystallography[8] and whose enantiopurity was confirmed by chiral stationary-phase HPLC (CSPHPLC) analysis. A baseline separation of enantiomers was observed



Scheme 2 *Reagents and conditions:* i, NBA, THF; ii, MTPA-Cl, pyridine; iii, NBS, CCl_4; iv, NaOMe, THF; v, KOH, Bu^{t}OH

(CHIRALCEL OB, $\alpha = 2.2$), since the (1*R*,2*S*)-enantiomer was retained more strongly and hence was eluted later. Using the bromo MTPA diastereoisomer **7b** ($[\alpha]_D$ −8, MeOH), the arene oxide **4** ($[\alpha]_D$ −22, $CHCl_3$) was formed *via* the dibromo ester **8b**. Recent kinetic studies on the racemic acridine 1,2-oxide **4** suggest that this arene oxide is much more stable toward hydrolysis (aqueous dioxane, 25 °C) compared with acridine 3,4-oxide or anthracene 1,2-oxide.[8] (1*R*,2*S*)-Acridine 1,2-oxide **4** ($[\alpha]_D$ +22, $CHCl_3$), when heated with potassium hydroxide in aqueous *tert*-butyl alcohol at 45 °C, yielded *trans*-1,2-dihydroacridine-1,2-diol **2** ($[\alpha]_D$ −59, MeOH, 51%) after preparative TLC purification on silica gel. Previous experiments from these laboratories, using $H_2{}^{18}O$–Bu^{t}OH–KOH in the hydrolysis of quinoline 5,6- and 7,8-oxides, to yield the corresponding *trans*-dihydro diols[9] showed that *ca.* 88 ± 5% attack of hydroxide anion occurred at the allylic position. On the assumption that a similar preferential attack at the C-2 position of (1*R*,2*S*)-acridine-1,2-oxide **4** occurs, then *trans*-1,2-dihydroacridine-1,2-diol **2** ($[\alpha]_D$ −59, MeOH) will have a 1*R*,2*R* configuration and an optical purity of *ca.* 75%.

Acridine 1,2-oxide **4** showed no evidence of spontaneous racemization at ambient temperature and thus appears to be similar to anthracene 1,2-oxide which was predicted[14] (and later observed)[13] to be configurationally stable. Based upon the comparable configurational stability of the arene oxides of quinoline (5,6- and 7,8-),[10] and now acridine (1,2-), with the corresponding arene oxides of naphthalene (1,2-)[13] and anthracene (1,2-),[13] it appears that the PMO calculations, previously used to predict the ease of racemization (*via* an unstable oxepine intermediate), may also be applicable to other arene oxides in the APAH series.

The availability of enantiopure samples of the stable oxide of acridine **4** and of sensitive methods for the determination of both absolute configuration and optical purity (CSPHPLC) from the present study, should now allow both the structure and the stereochemistry of this arene oxide metabolite to be determined using crude liver microsomal enzyme systems (with inhibition of the epoxide hydrolase enzyme)[15] or pure enzymes. The assignment of absolute configuration to the *trans*-dihydro diol metabolite of acridine **2**[6,7] should also be possible by comparison with the authentic sample of (−)-(1*R*,2*R*)-*trans*-1,2-dihydroacridine-1,2-diol **2**.

Experimental

^{1}H NMR spectra were obtained using a 300 MHz General Electric QE300 instrument and $CDCl_3$ solvent with tetramethylsilane as reference; *J* values are given in Hz. Optical rotations were determined using a Perkin-Elmer Model 241 polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. HPLC analysis were carried out using a Perkin-Elmer Series 3B liquid chromatograph coupled to a Hewlett Packard 33805 integrator and UV detector. 3,4-Dihydroacridine **5** was obtained from 1,2,3,4-tetrahydroacridine-1-one using the method reported previously.[8] (+)-2-Methoxy-2-phenyl-2-trifluoromethylacetyl chloride ($[\alpha]_D$ +128, CCl_4) was obtained from (+)-2-methoxy-2-phenyl-2-trifluoromethylacetic acid (Aldrich Chemical Co.) after treatment with thionyl chloride.

trans-2-*Bromo*-1,2,3,4-*tetrahydroacridin*-1-*ol* **6**.—Freshly recrystallized *N*-bromoacetamide (3.4 g, 24.6 mmol) was added to a solution of 3,4-dihydroacridine **5** (4.0 g, 22.1 mmol) in THF (300 cm^3) and water (200 cm^3) and the mixture was stirred at room temperature for 4 h. The THF was removed under reduced pressure and the product was extracted using ethyl acetate (3 × 150 cm^3). The extract was dried ($MgSO_4$) and the solvent removed to yield the product **6** (4.8 g, 78%), m.p. 149–151 °C (dichloromethane) (Found: C, 55.6; H, 4.3; N, 5.0. $C_{13}H_{12}BrNO$ requires C, 56.1; H, 4.3; N, 5.0%); δ_H 2.48 (1 H, m, 3-H), 2.73 (1 H, m, 3′-H), 3.31 (2 H, m, 4-H), 4.40 (1 H, m, 2-H), 5.07 (1 H, d, $J_{1,2}$ 7.8, 1-H), 7.50 (1 H, m, 7-H), 7.70 (1 H, m, 6-H), 7.82 (1 H, d, $J_{7,8}$ 8.1, 8-H), 8.00 (1 H, d, $J_{5,6}$ 8.4, 5-H) and 8.37 (1 H, s, 9-H).

(+)-(1R,2R)- *and* (−)-(1S,2S)-trans-2-*Bromo*-1-(2-*methoxy*-2-*phenyl*-2-*trifluoroacetoxy*)-1,2,3,4-*tetrahydroacridine* **7a** *and* **7b**.—(+)-MTPA-chloride (1.9 g, 7.5 mmol) was added to a solution of *trans*-2-bromo-1,2,3,4-tetrahydroacridin-1-ol **6** (2.0 g, 7.14 mmol) and 4-dimethylaminopyridine (0.2 g) in dry pyridine (12 cm^3) and the solution was stirred under nitrogen at 0 °C for 3 h and at room temperature for 0.5 h. The pyridine was removed under reduced pressure and the residue was treated with aqueous sodium carbonate (10%; 25 cm^3). The product was extracted using dichloromethane (3 × 50 cm^3) and the extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give the product **7a**, **b** as a crude oil (3.1 g, 87%). Preparative TLC on silica-gel [hexane–diethyl ether (1:1), two elutions] was used to separate the diastereoisomers **7a** (low R_f) and **b** (high R_f). Analytical HPLC using a Microsorb column (100 × 4.6 mm), ethyl acetate–hexane (6:94) as eluent at a flow rate of 1.5 cm^3 min^{-1} was found to provide a convenient method for checking the purity of the separated

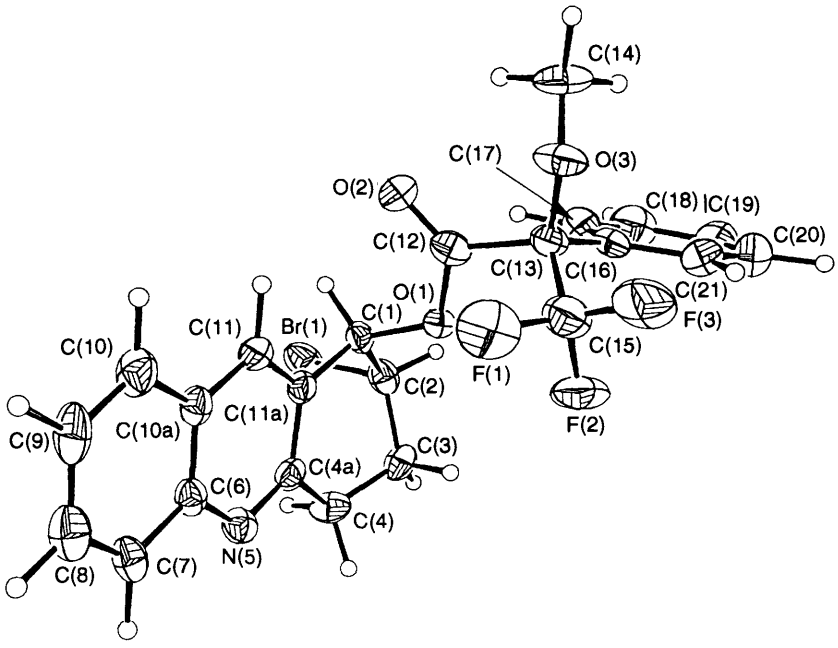


Fig. 1 ORTEP drawing of compound **7a**

diastereoisomers (**7a**/**b**, $\alpha = 1.6$). Crystallisation of the resolved diastereoisomers from diethyl ether–hexane yielded analytically pure samples: **7a** (low R_f), m.p. 101–103 °C (Found: C, 56.0; H, 3.8; N, 2.4. $C_{23}H_{19}BrF_3NO_3$ requires C, 55.9; H, 3.85; N, 2.8%); $[\alpha]_D$ +63 (*c* 1.1 in MeOH); δ_H 2.43 (1 H, m, 3-H), 2.63 (1 H, m, 3′-H), 3.22 (1 H, m, 4-H), 3.34 (1 H, m, 4′-H), 3.55 (3 H, s, OCH_3), 4.59 (1 H, m, 2-H), 6.54 (1 H, d, $J_{1,2}$ 4.9, 1-H), 7.46 (1 H, m, Ar-H), 7.69 (1 H, m, 6-H and 8-H), 7.95 (1 H, s, 9-H) and 7.99 (1 H, d, $J_{5,6}$ 8.7, 5-H).

7b (high R_f), m.p. 108–109 °C (Found: C, 55.8; H, 3.8; N, 3.2. $C_{23}H_{19}BrF_3NO_3$ requires C, 55.9; H, 3.85; N, 2.8%); $[\alpha]_D$ −8 (*c* 0.9 in MeOH); δ_H 2.35 (1 H, m, 3-H), 2.50 (1 H, m, 3′-H), 3.21 (1 H, m, 4-H), 3.40 (1 H, m, 4′-H), 3.46 (3 H, s, OCH_3), 4.56 (1 H, m, 2-H), 6.53 (1 H, d, $J_{1,2}$ 4.0, 1-H), 7.34 (1 H, m, Ar-H), 7.53 (1 H, m, 7-H), 7.76 (2 H, m, 6-H and 8-H), 8.04 (1 H, d, $J_{5,6}$ 8.5, 5-H) and 8.16 (1 H, s, 9-H).

Crystal data for **7a**. $C_{23}H_{19}NO_3F_3Br$, $M = 478.3$, monoclinic, space group $P2_1$ (No. 4), $a = 9.346(1)$, $b = 10.316(2)$, $c = 10.969(2)$ Å, $\beta = 92.63(1)°$, $U = 1056.5$ Å^3, $Z = 2$, μ(Mo-Kα) = 19.2 cm^{-1}, $D_c = 1.50$ g cm^{-3}, F(000) = 484, λ(Mo-Kα) = 0.710 73 Å, crystal size 1.0 × 0.5 × 0.3 mm.

Data collection, analysis and refinement. Siemens P3/V2000 diffractometer, $\theta/2\theta$ scan, scan range $3 < 2\theta < 50°$, scan width 1°, 1981 unique data measured; Patterson and difference Fourier solution (SHELXS-86),[16] full-matrix least squares refinement (SHELX-76),[17] anisotropic vibration parameters for non-hydrogen atoms, hydrogens included at geometrically calculated positions with common isotropic temperature factors for methyl, methylene, tertiary CH and benzene-type hydrogens which refined to $U = 0.11(2)$, 0.07(1), 0.05(1) and 0.07(1) Å^2, respectively. The 1435 data with $I > 3\sigma(I)$ were used in the final cycles and yielded $R = 0.039$, $R_w = 0.040$ with $w = 0.96/[\sigma^2(F_o) + 0.0013\ F_o^2]$; maximum residual electron density 0.21 e Å^{-3}. An ORTEP[18] picture of the molecule is shown in Fig. 1. Tables of atomic coordinates, temperature factors, bond lengths and angles have been deposited with the Cambridge Crystallographic Data Centre.*

(1R,2R)- *and* (1S,2S)-2,4-*Dibromo*-1-(2-*methoxy*-2-*phenyl*-2-*trifluoroacetoxy*)-1,2,3,4-*tetrahydroacridine* **8a** *and* **8b**.—The bromo MTPA ester **7a** (1.0 g, 2.0 mmol) was dissolved in carbon tetrachloride (50 cm^3) containing azoisobutyronitrile (*ca.* 20 mg). Freshly recrystallized *N*-bromosuccinimide (0.4 g, 2.2 mmol) was added to the reaction mixture which was then heated at 60–70 °C until the reaction was complete (*ca.* 0.7 h). The solution was cooled to *ca.* 0 °C, the succinimide was filtered off and the filtrate was evaporated under reduced pressure to give an oil which appeared to be a single isomer of the dibromo ester **8a** by ^{1}H NMR analysis (1.0 g, 86%). Attempted purification of compound **8a** resulted in decomposition so it was identified on the basis of its ^{1}H NMR spectrum and used immediately without purification. The diastereoisomeric dibromo ester **8b** was obtained in a similar manner (1.1 g, 95%).

8a: δ_H 3.00 (1 H, m, 3-H), 3.11 (1 H, m, 3′-H), 4.91 (1 H, m, 2-H), 5.63 (1 H, m, 4-H), 6.70 (1 H, d, $J_{1,2}$ 8.9, 1-H), 7.52 (6 H, m, ArH), 7.77 (3 H, m, ArH) and 8.03 (1 H, d, $J_{5,6}$ 8.6, 5-H).

8b: δ_H 2.99 (1 H, m, 3-H), 3.10 (1 H, m, 3′-H), 3.57 (3 H, s, OCH_3), 4.89 (1 H, m, 2-H), 5.65 (1 H, m, 4-H), 6.64 (1 H, d, $J_{1,2}$ 8.2, 1-H), 7.44 (3 H, m, ArH), 7.68 (5 H, m, ArH), 8.00 (1 H, s, 9-H) and 8.09 (1 H, d, $J_{5,6}$ 8.2, 5-H).

(+)-(1R,2S)- *and* (−)-(1S,2R)-1,2-*Epoxy*-1,2-*dihydroacridine* **4**.—The dibromo MTPA ester **8a** (1.1 g, 1.9 mmol) was stirred with sodium methoxide (1.1 g) in dry THF (100 cm^3) at 0 °C for 1 h under nitrogen and for 3 h at ambient temperature. The solvent was removed under reduced pressure and water (20 cm^3) was added to the residue. The product was extracted with dichloromethane and the extracts were dried ($MgSO_4$), filtered and evaporated under reduced pressure to give the crude arene oxide **4**. Recrystallization of this from dichloromethane–hexane gave colourless crystals of (1*R*,2*S*)-1,2-epoxy-1,2-dihydroacridine **4** (0.24 g, 64%), m.p. 136–138 °C (decomp.), $[\alpha]_D$ +22 (*c* 0.9 in $CHCl_3$). By a similar procedure (1*S*,2*R*)-1,2-epoxy-1,2-dihydroacridine **4** (0.22 g, 65%), m.p. 146–148 °C (decomp.), $[\alpha]_D$ −22 (*c* 0.8 in $CHCl_3$) was obtained from the dibromo MTPA ester **8b**. The samples of (+)- and (−)-1,2-epoxy-1,2-dihydroacridine **4** were spectrally indistinguishable from a racemic sample.[8] The enantiopurity of the (+)- and (−)-samples of arene oxide **4** was found to be >99% using the CSPHPLC

* Details of the deposition scheme are available in 'Instructions for Authors', *J. Chem. Soc., Perkin Trans. 1*, Issue 1, 1994.

method [Chiralcel OB, 250 × 4.6 mm; propan-2-ol–hexane (20:80), flow rate of 0.5 cm^3 min^{-1}, α = 2.2].

(−)-(1R,2R)-trans-1,2-*Dihydroacridine*-1,2-*diol* **2**.—(1*R*,2*S*)-1,2-Epoxy-1,2-dihydroacridine **4** (36 mg, 0.18 mmol) was heated in a mixture of aqueous sodium hydroxide (2 mol dm^{-3}; 3 cm^3) and *tert*-butyl alcohol (3 cm^3) using the method previously outlined for a racemic sample of the arene oxide **4**.[8] Purification by preparative TLC on silica gel [MeOH–$CHCl_3$ (5:95)], followed by recrystallization from ethyl acetate yielded (−)-(1*R*,2*R*)-*trans*-1,2-dihydroacridine-1,2-diol **2** (20 mg, 51% yield); m.p. 204–212 °C (decomp.); $[\alpha]_D$ −59 (*c* 0.6 in MeOH). This sample was spectrally indistinguishable from the racemic sample of *trans*-dihydro diol **2**.[8]

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