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Regio- and stereo-selective metabolism of 4-methylbenz[*a*]anthracene by the fungus *Cunninghamella elegans*

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Metabolism of 4-methylbenz[*a*]anthracene by the fungus *Cunninghamella elegans* was studied. *C. elegans* metabolized 4-methylbenz[*a*]anthracene primarily at the methyl group, this being followed by further metabolism at the 8,9- and 10,11-positions to form *trans*-8,9-dihydro-8,9-dihydroxy-4-hydroxymethylbenz[*a*]anthracene and *trans*-10,11-dihydro-10,11-dihydroxy-4-hydroxymethylbenz[*a*]anthracene. There was no detectable *trans*-dihydrodiol formed at the methyl-substituted double bond (3,4-positions) or at the 'K' region (5,6-positions). The metabolites were isolated by reversed-phase high-pressure liquid chromatography and characterized by the application of u.v.–visible-absorption-, ^{1}H-n.m.r.- and mass-spectral techniques. The 4-hydroxymethylbenz[*a*]anthracene *trans*-8,9- and -10,11-dihydrodiols were optically active. Comparison of the c.d. spectra of the *trans*-dihydrodiols formed from 4-methylbenz[*a*]anthracene by *C. elegans* with those of the corresponding benz[*a*]anthracene *trans*-dihydrodiols formed by rat liver microsomal fraction indicated that the major enantiomers of the 4-hydroxymethylbenz[*a*]anthracene *trans*-8,9-dihydrodiol and *trans*-10,11-dihydrodiol formed by *C. elegans* have *S,S* absolute stereochemistries, which are opposite to those of the predominantly 8*R*,9*R*- and 10*R*,11*R*-dihydrodiols formed by the microsomal fraction. Incubation of *C. elegans* with 4-methylbenz[*a*]anthracene under $^{18}O_2$ and subsequent mass-spectral analysis of the metabolites indicated that hydroxylation of the methyl group and the formation of *trans*-dihydrodiols are catalysed by cytochrome *P*-450 mono-oxygenase and epoxide hydrolase enzyme systems. The results indicate that the fungal mono-oxygenase–epoxide hydrolase enzyme systems are highly stereo- and regio-selective in the metabolism of 4-methylbenz[*a*]anthracene.

Methylbenz[*a*]anthracenes are ubiquitous environmental pollutants and are found in airborne particulates from cigarette-smoke condensate, stack gases, roofing-tar extracts and industrial effluents (Thomas *et al.*, 1978). The fate of methylbenz[*a*]anthracenes in the environment is of considerable interest, since certain isomers show moderate to high biological activity (Dunning & Curtis, 1960; Stevenson & Von Haam, 1965; Newman, 1976; Wislocki *et al.*, 1982). Although benz[*a*]anthracene is a weak carcinogen, substitution of a methyl group at the 7- and/or 12-position converts the parent hydrocarbon into 7-methylbenz[*a*]anthracene and 7,12-dimethylbenz[*a*]anthracene, both of which have been shown to be highly carcinogenic, mutagenic and tumorigenic (Dipple, 1976; Chouroulinkov *et al.*, 1977; Malaveille *et al.*, 1977; Wislocki *et al.*, 1981, 1982). Studies from several laboratories have suggested that metabolism of methyl-substituted benz[*a*]anthracene to 'bay'-region 3,4-dihydrodiol 1,2-epoxides, strong electrophiles, is an important pathway for eliciting their carcinogenic activities (Jerina *et al.*, 1977; Yang *et al.*, 1981).

In contrast with the attention that has been given to the mammalian metabolism of mono- or di-substituted methylbenz[*a*]anthracenes, very little is known about the microbial metabolism of these compounds. Wu & Wong (1981) reported that both

Abbreviation used: h.p.l.c., high-pressure liquid chromatography.

Penicillium notatum and *Pseudomonas aeruginosa* metabolized 7,12-dimethylbenz[*a*]anthracene to 7-hydroxymethyl-12-methylbenz[*a*]anthracene and 12-hydroxymethyl-7-methylbenz[*a*]anthracene. Neither phenolic nor dihydrodiol derivatives of 7,12-dimethylbenz[*a*]anthracene were found in either incubation. More recently, Cerniglia *et al.* (1982) reported that *Cunninghamella elegans* metabolized 7-methylbenz[*a*]anthracene primarily at the methyl group, this being followed by further metabolism at the 3,4- and 8,9-positions to form *trans*-3,4-dihydro-3,4-dihydroxy-7-hydroxymethylbenz[*a*]anthracene and *trans*-8,9-dihydro-8,9-dihydroxy-7-hydroxymethylbenz[*a*]anthracene. The metabolite pattern of 7-methylbenz[*a*]anthracene by *C. elegans* was qualitatively similar to that formed by rat liver microsomal fraction except that there was no metabolism at the 5,6-positions ('K' region) of the aromatic ring.

In the present paper we report on the metabolism of 4-methylbenz[*a*]anthracene by *C. elegans*. 4-Methylbenz[*a*]anthracene was selected for investigation from the 12 isomeric monomethylbenz[*a*]anthracenes to determine if the methyl substituent of 4-methylbenz[*a*]anthracene may block enzymic epoxidation at the methylated aromatic double bond and to determine if the presence of a methyl substituent on the aromatic ring affects the regio- and stereo-selectivity of the fungal cytochrome *P*-450 mono-oxygenase and expoxide hydrolase towards the metabolism of 4-methylbenz[*a*]anthracene by *C. elegans*.

Experimental

Chemicals

4-Methylbenz[*a*]anthracene was synthesized in accordance with known procedures (Yang *et al.*, 1981). H.p.l.c.-grade solvents were purchased from Fisher Chemical Co., Fair Lawn, NJ, U.S.A. All other chemicals were of reagent grade and in the highest available purity.

Micro-organisms and culture conditions

Stock cultures of *C. elegans* A.T.C.C. 36112 were maintained on Sabouraud dextrose agar (Difco, Detroit, MI, U.S.A.) slants and stored at 4°C. The spores and/or mycelia from several slants were used to inoculate ten 125ml Erlenmeyer flasks containing 30ml of Sabouraud dextrose broth. The flasks were incubated for 48h at 25°C on a rotary shaker at 150rev./min. After 48h incubation, the mycelia were removed by aseptic filtration and transferred to ten sterile 125ml Erlenmeyer flasks containing 30ml of Sabouraud dextrose broth. A 3mg portion of 4-methylbenz[*a*]anthracene dissolved in 0.3ml of dimethylformamide was added to each flask. Sterile control experiments were prepared by autoclaving the culture at 121°C for 40min before adding 4-methylbenz[*a*]anthracene. The flasks were incubated in the dark at 25°C for 24h as described above.

Isolation, detection and quantitative determination of 4-methylbenz[a]anthracene metabolites formed by C. elegans

After 24h, the flask contents were pooled and filtered to separate the broth from the mycelia. The broth and the mycelia were each extracted with 6vol. of ethyl acetate. The extracts were combined and dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure at 40°C in the dark. The residue was dissolved in methanol and analysed by h.p.l.c. Reversed-phase h.p.l.c. was performed with a Beckman system consisting of two model 100A pumps and a model 155-10 variable-wavelength absorbance detector adjusted at 254nm. A 5μ C_{18} Ultrasphere ODS column (4.6mm × 25cm) (Altex Scientific, Berkeley, CA, U.S.A.) was used to separate the parent hydrocarbon and the 4-methylbenz[*a*]anthracene metabolites. The separation was achieved with a programmed 30min linear gradient of methanol/water (1:1, v/v) to methanol at a solvent flow rate of 1.0ml/min.

After isolation by h.p.l.c., each metabolite formed from 4-methylbenz[*a*]anthracene by *C. elegans* was determined quantitatively by measurement of its u.v.-absorption spectrum. It was assumed that the u.v. molar extinction coefficients of 4-hydroxymethylbenz[*a*]anthracene *trans*-8,9-dihydrodiol and 4-hydroxymethylbenz[*a*]anthracene *trans*-10,11-dihydrodiol were similar to those of benz[*a*]anthracene *trans*-8,9-dihydrodiol (ε_{265} 71950 $M^{-1} \cdot cm^{-1}$ in ethanol) and benz[*a*]anthracene *trans*-10,11-dihydrodiol (ε_{274} 67280 $M^{-1} \cdot cm^{-1}$ in ethanol) respectively (Yang, 1982).

Incorporation of ^{18}O from $^{18}O_2$ in biotransformation experiments

Cells of *C. elegans* were incubated as described above except that a rubber stopper was used to seal each flask. The contents of the flask were degassed by a vacuum pump and then by flushing with N_2. $^{18}O_2$ (99.8atom%; Mound Facility, Monsanto Corp., Miamisburg, OH, U.S.A.) was introduced into the flask with a cannula. The isotopic composition of the atmosphere was monitored during the course of the experiment and was approx. 99atom% of ^{18}O. The $^{18}O/^{16}O$ ratio was determined by using a Varian–MAT CH-5-DF mass spectrometer. 4-Methylbenz[*a*]anthracene metabolites were isolated by h.p.l.c. and their isotopic abundance was calculated from the relative intensities of the molecular ions containing ^{16}O and ^{18}O species obtained by using a Finnigan model 4023 mass spectrometer.

Physical and chemical analyses

U.v.–visible-absorption spectra of the metabolites were determined in methanol on a Beckman model 25 recording spectrophotometer. Mass spectra were obtained with a Finnigan model 4023 mass spectrometer operated at 70eV ionizing voltage with a solid probe. Direct-probe mass spectrometry was performed on samples that had been dissolved in 5 μl of methanol and dried in glass sample cups. Spectra were recorded as the probe temperature was increased ballistically from 30°C to 300°C with monitoring of the ion-source temperature at 270°C. C.d. spectra were determined with a quartz cell of 1cm path-length at room temperature on a Jasco 500A spectropolarimeter equipped with a Jasco DP-500 data processor. C.d. spectra are expressed by ellipticity (ψ_λ in millidegrees) for methanol solutions that read 1.0 absorbance in a u.v.–visible spectrophotometer at the wavelength of maximum absorption in a quartz cell of 1cm path-length. The ellipticity and molar ellipticity ($[\theta]_\lambda$ in $deg \cdot cm^2 \cdot dmol^{-1}$) are related to molar absorption coefficient ($\varepsilon_{max.}$ in $M^{-1} \cdot cm^{-1}$) as follows:

$$[\theta]_\lambda = 0.1\, \varepsilon_{max.} \cdot \psi_\lambda$$

C.d.-spectral data obtained from different laboratories are more conveniently çompared if they are expressed by ψ_λ, because the $\varepsilon_{max.}$ values of polycyclic aromatic hydrocarbon dihydrodiols are either not known or are difficult to determine accurately.

The ^{1}H-n.m.r. spectra were recorded on a Bruker WM500 spectrometer. The data were acquired under the following conditions: data number, 32000; sweep width, 7042Hz; filter width, 17800Hz; temperature, 305K; flip angle, 68°. The spectra were recorded in [$^2H_6$]acetone. The chemical shifts (δ) are reported in p.p.m. downfield from the internal standard tetramethylsilane. Assignments were made via homonuclear decoupling experiments and shielding effects.

Results

Cells of *C. elegans* were incubated with 4-methylbenz[*a*]anthracene in Sabouraud dextrose broth for 24h. H.p.l.c. analysis of the ethyl acetate-soluble material is shown in Fig. 1. Each compound was collected, and, after repeated injections of the 4-methylbenz[*a*]anthracene-metabolite extract, fractions of similar compositions and h.p.l.c. retention times were pooled and concentrated and their u.v.–visible-absorption- and mass-spectral properties determined. Metabolites II (14.0min) and III (14.8min) were major metabolites of 4-methylbenz[*a*]anthracene formed by *C. elegans*. The u.v.–visible-absorption-spectral properties of metabolites II and III (Fig. 2) are similar

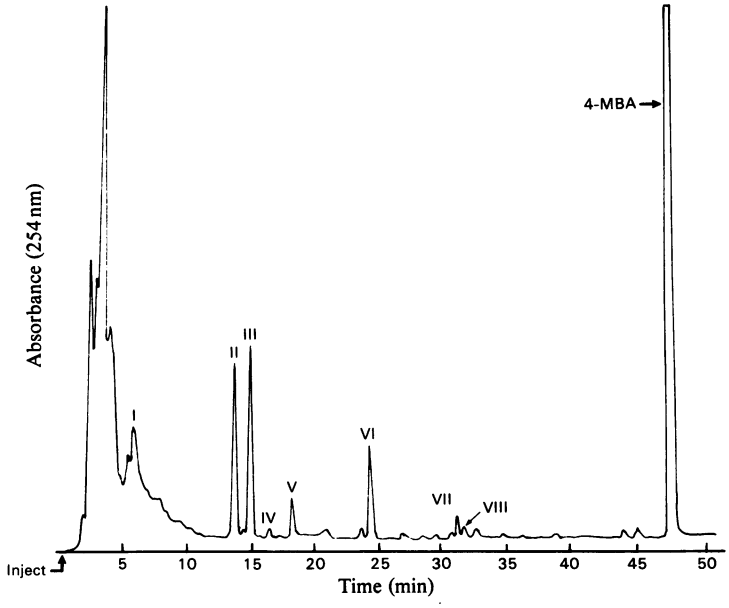


Fig. 1. *H.p.l.c. elution profile of metabolites formed from 4-methylbenz[a]anthracene by C. elegans*
The metabolites were separated with a 5μ C_{18} Ultrasphere ODS column with a 30min linear gradient of methanol/water (1:1, v/v) to methanol at a flow rate of 1.0ml/min. Compounds were collected and identified as described in the text. Abbreviation: 4-MBA, 4-methylbenz[*a*]anthracene.

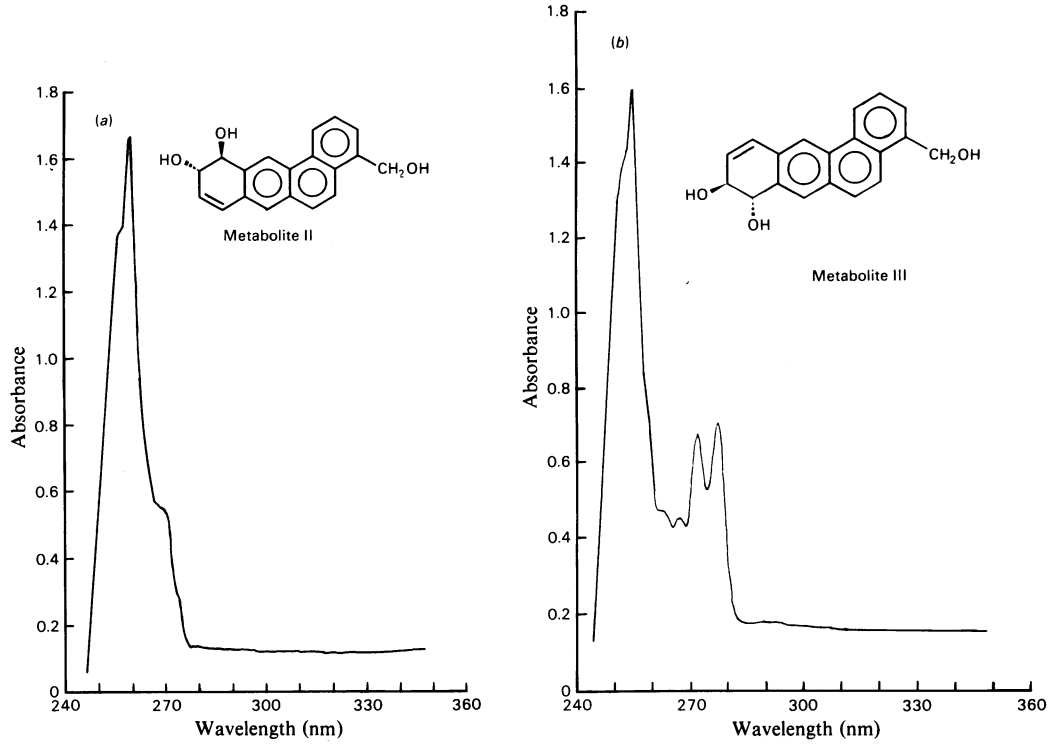


Fig. 2. *U.v.–visible-absorption spectra of metabolite II (a) and metabolite III (b) identified as 4-hydroxymethylbenz[a]anthracene trans-10,11-dihydrodiol and trans-8,9-dihydrodiol respectively*

Metabolites were collected as described in the text, and spectra were measured in methanol in a Beckman model 25 recording spectrophotometer.

to those of *trans*-10,11-dihydro-10,11-dihydroxybenz[*a*]anthracene and *trans*-8,9-dihydro-8,9-dihydroxybenz[*a*]anthracene respectively (Lehr *et al.*, 1977). Mass-spectral analysis (Fig. 3) revealed, however, that each compound had a molecular ion (M^+) at *m/z* 292 and the characteristic fragment ions at *m/z* 274 ($M^+ - 18$) due to the loss of a water molecule and *m/z* 246 ($M^+ - 46$) (H_2O, CO loss). The u.v.-absorption- and mass-spectral data indicate that metabolites II and III are *trans*-10,11-dihydro-10,11-dihydroxy-4-hydroxymethylbenz[*a*]anthracene (4-hydroxymethylbenz[*a*]anthracene *trans*-10,11-dihydrodiol) and *trans*-8,9-dihydro-8,9-dihydroxy-4-hydroxymethylbenz[*a*]anthracene (4-hydroxymethylbenz[*a*]anthracene *trans*-8,9-dihydrodiol) respectively. The structures of metabolites II and III were further confirmed by high-resolution 500 MHz ^{1}H-n.m.r. spectroscopy. The 500 MHz ^{1}H-n.m.r. spectra of 4-hydroxymethylbenz[*a*]anthracene *trans*-10,11-dihydrodiol and 4-hydroxymethylbenz[*a*]anthracene *trans*-8,9-dihydrodiol were determined and the assigned chemical shifts and coupling constants are given as follows: for 4-hydroxymethylbenz[*a*]anthracene *trans*-10,11-dihydrodiol (metabolite II), δ (p.p.m.) ([$^2H_6$]acetone) 4.51 (1H, dd, 10-C*H*), 4.94 (1H, d, 11-C*H*), 5.15 (2H, s, CH_2), 6.08 (1H, dd, 9-C*H*), 6.66 (1H, d, 8-C*H*), 7.67 (1H, dd, 2-C*H*), 7.68 (1H, s, 7-C*H*), 7.72 (1H, d, 3-C*H*), 7.83 (1H, d, 5-C*H*), 8.08 (1H, d, 6-C*H*), 8.74 (1H, d, 1-C*H*) and 8.97 (1H, s, 12-C*H*), $J_{1,2} = 7.7$ Hz, $J_{2,3} = 7.7$ Hz, $J_{5,6} = 9.5$ Hz, $J_{8,9} = 9.5$ Hz, $J_{8,10} = 2.6$ Hz, $J_{9,10} = 1.7$ Hz and $J_{10,11} = 10.3$ Hz; for 4-hydroxymethylbenz[*a*]anthracene *trans*-8,9-dihydrodiol (metabolite III), δ (p.p.m.) ([$^2H_6$]acetone) 4.50 (1H, d, 8-C*H*), 4.90 (1H, dd, 9-C*H*), 5.15 (2H, s, CH_2), 6.07 (1H, dd, 10-C*H*), 6.75 (1H, dd, 11-C*H*), 7.64 (1H, dd, 2-C*H*), 7.72 (1H, d, 3-C*H*), 7.86 (1H, d, 5-C*H*), 8.09 (1H, d, 6-C*H*), 8.12 (1H, s, 7-C*H*), 8.53 (1H, s, 12-C*H*) and 8.73 (1H, d, 1-C*H*), $J_{1,2} = 8.2$ Hz, $J_{2,3} = 6.5$ Hz, $J_{5,6} = 9.0$ Hz, $J_{8,9} = 10.3$ Hz, $J_{9,10} = 2.2$ Hz, $J_{9,11} = 2.2$ Hz and $J_{10,11} = 9.5$ Hz. The coupling constants between the carbinol protons of 4-hydroxymethylbenz[*a*]anthracene 8,9-dihydrodiol and 10,11-dihydrodiol are large, with both values being 10.3 Hz. In addition, the coupling constants between the non-benzylic carbinol and the non-benzylic olefinic protons of these dihydrodiols are

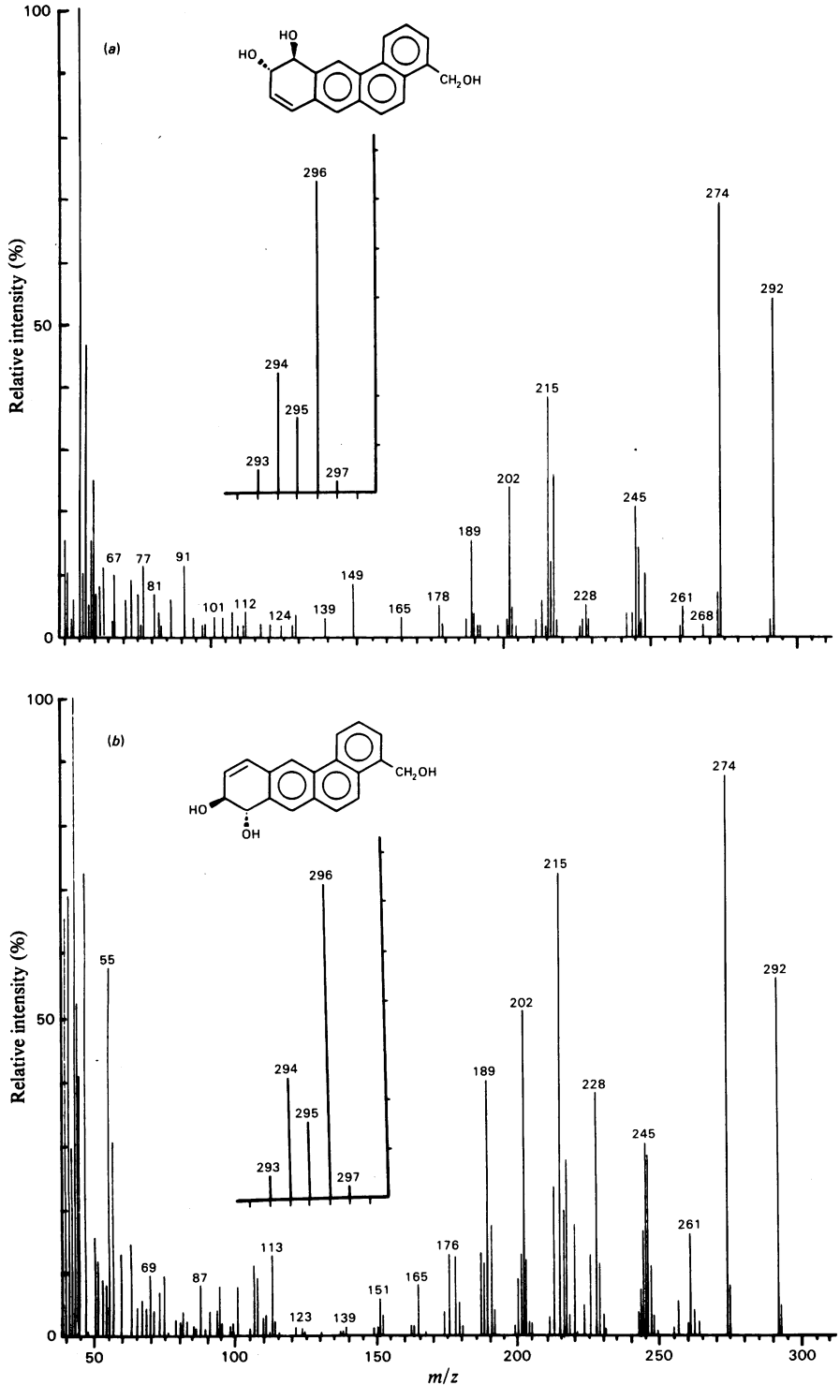


Fig. 3. *Mass spectra of metabolite II* (*a*) *and metabolite III* (*b*)
^{18}O incorporation into each of these compounds is shown in the insets.

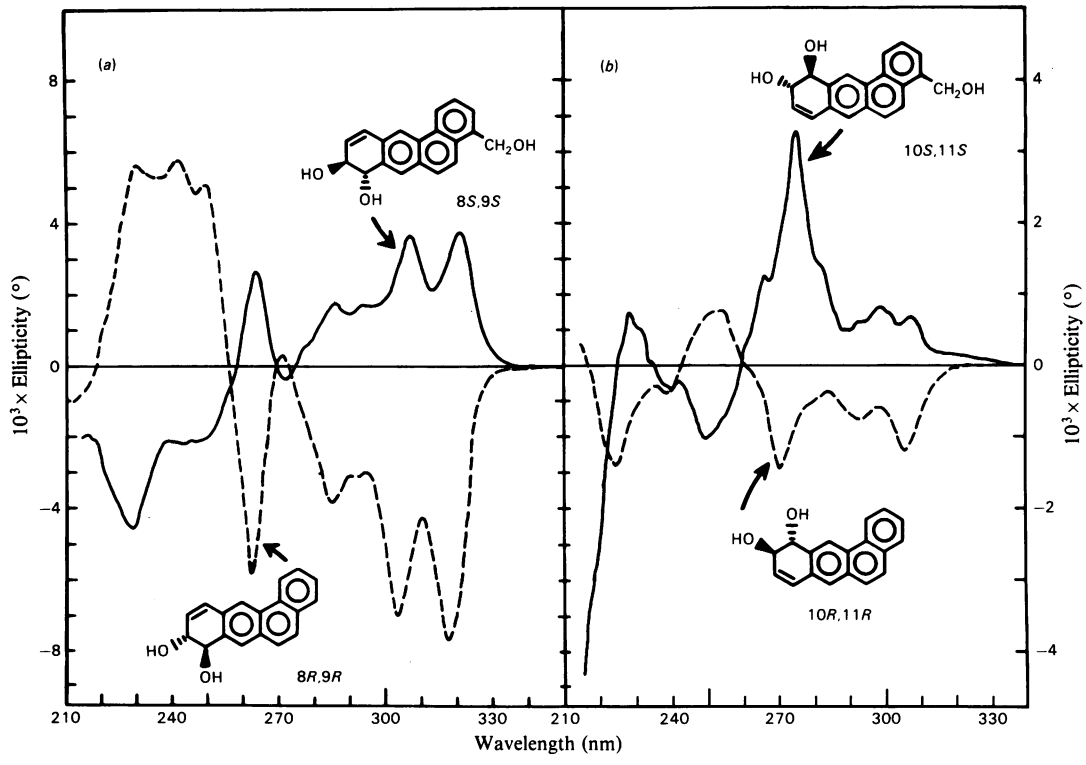


Fig. 4. *C.d. spectra of benz[a]anthracene 8R,9R-dihydrodiol* (---- *in a,* $\varepsilon_{max.}$ *at 264 nm; optical purity 96%) and benz[a]anthracene 10R,11R-dihydrodiol* (---- *in b,* $\varepsilon_{max.}$ *at 272 nm; optical purity* 96%) *formed from metabolism of benz[a]anthracene by rat liver microsomal fraction (Thakker et al., 1979; Yang, 1982), and of the 4-hydroxymethylbenz[a]anthracene trans-8,9-dihydrodiol* (—— *in a,* $\varepsilon_{max.}$ *at 306 nm) and trans-10,11-dihydrodiol* (—— *in b,* $\varepsilon_{max.}$ *at 274 nm) formed from the metabolism of 4-methylbenz[a]anthracene by C. elegans*

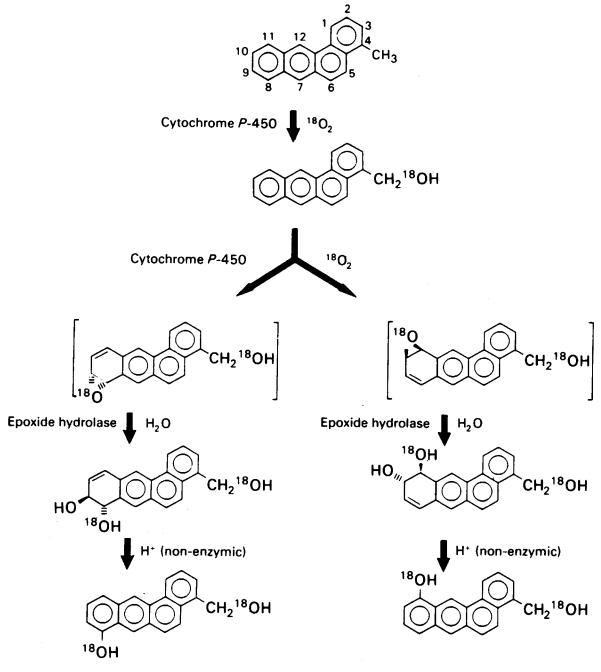
The ellipticities of the dihydrodiols are expressed for methanol solutions that read 1.0 absorbance unit at the wavelength of $\varepsilon_{max.}$.

2.2 Hz ($J_{9,10}$) and 1.7 Hz ($J_{9,10}$) respectively. These data indicate that the two dihydrodiols are *trans* isomers with both hydroxy groups preferentially at the quasi-equatorial positions (Zacharias *et al.*, 1979).

Both *trans*-dihydrodiol metabolites were found to be optically active. Their c.d. spectra are given in Fig. 4. The c.d. spectra of the 4-hydroxymethylbenz[*a*]anthracene *trans*-8,9-dihydrodiol and 4-hydroxymethylbenz[*a*]anthracene *trans*-10,11-dihydrodiol formed from 4-methylbenz[*a*]anthracene by *C. elegans* (Fig. 4) are close to mirror images of those of benz[*a*]anthracene 8*R*,9*R*-dihydrodiol and 10*R*,11*R*-dihydrodiol respectively formed from benz[*a*]anthracene by rat liver microsomal fraction (Thakker *et al.*, 1979; Yang, 1982). This clearly indicates that the major enantiomers of the 4-hydroxymethylbenz[*a*]anthracene *trans*-8,9-dihydrodiol and *trans*-10,11-dihydrodiol metabolites have the *S*,*S* absolute configurations. These results indicate that there are differences in the stereoselectivity of the fungal cytochrome *P*-450 monooxygenase–epoxide hydrolase enzyme systems from those observed in rat liver systems.

Peak I (Fig. 1) is due to a natural product formed by *C. elegans*. This material was also found in control samples. Metabolites IV, V and VI each gave a mass spectrum that had a molecular ion at *m/z* 274, suggesting that these compounds are either monohydroxylated derivatives of 4-hydroxymethylbenz[*a*]anthracene or dihydroxylated derivatives of 4-methylbenz[*a*]anthracene. These metabolites have not been further characterized because of insufficient material. Metabolite VII (31.0 min) gave a mass spectrum that had a molecular ion at *m/z* 258, suggesting that it is a phenolic derivative of 4-methylbenz[*a*]anthracene. Metabolite VIII (31.8 min), identified as 4-hydroxymethylbenz[*a*]anthracene, had an h.p.l.c. retention time, a molecular ion at *m/z* 258, and a u.v.-visible-absorption spectrum identical with those given by authentic 4-hydroxymethylbenz[*a*]anthracene.

After 24 h incubation, *C. elegans* metabolized 32% of the added 4-methylbenz[*a*]anthracene.



Scheme 1. *Major pathways for the oxidative metabolism of 4-methylbenz[a]anthracene to 4-hydroxymethylbenz[a]anthracene trans-dihydrodiols by C. elegans*
Compounds in brackets were not isolated.

Approx. 80% of the added 4-methylbenz[*a*]anthracene was ethyl acetate-extractable. The compounds 4-hydroxymethylbenz[*a*]anthracene *trans*-8,9-dihydrodiol and 4-hydroxymethylbenz[*a*]anthracene *trans*-10,11-dihydrodiol accounted for 44% and 25% respectively of the total metabolites.

C. elegans was incubated with 4-methylbenz[*a*]anthracene under molecular $^{18}O_2$ for 24h. The resulting metabolites were separated by reversed-phase h.p.l.c. and then analysed for ^{18}O incorporation by mass-spectral analyses. 4-Hydroxymethylbenz[*a*]anthracene was found to contain ^{18}O (M^+, *m/z* 260). Each of the 4-hydroxymethylbenz[*a*]anthracene *trans*-8,9- and *trans*-10,11-dihydrodiols contained two atoms of ^{18}O/molecule (M^+, *m/z* 296) (Fig. 3 insets). In order to determine which hydroxy group in the *trans*-dihydrodiols was derived from molecular oxygen, both dihydrodiols were converted into phenolic products by acid-catalysed dehydration and these were isolated by h.p.l.c. Acid-catalysed dehydration of 4-hydroxymethylbenz[*a*]anthracene *trans*-8,9-dihydrodiol yielded predominantly 8-hydroxy-4-hydroxymethylbenz[*a*]anthracene. Mass-spectral analysis indicated that two atoms of ^{18}O/molecule were contained in 8-hydroxy-4-hydroxymethylbenz[*a*]anthracene (M^+, *m/z* 278). The results indicate that the oxygen atoms at both the $C_{(8)}$–OH and the C_4–CH_2OH of 4-hydroxymethylbenz[*a*]anthracene *trans*-8,9-dihydrodiol were derived from molecular O_2 (Scheme 1). Acid-catalysed dehydration of 4-hydroxymethylbenz[*a*]anthracene *trans*-10,11-dihydrodiol yielded predominantly 11-hydroxy-4-hydroxymethylbenz[*a*]anthracene. Mass-spectral analysis indicated that two atoms of ^{18}O/molecule were contained in 11-hydroxy-4-hydroxymethylbenz[*a*]anthracene (M^+, *m/z* 278). The results indicate that the oxygen atoms at both the $C_{(11)}$–OH and the $C_{(4)}$–CH_2OH of 4-hydroxymethylbenz[*a*]anthracene *trans*-10,11-dihydrodiol were derived from molecular O_2 (Scheme 1). The *trans* addition of water at the C-9-position of 4-hydroxymethylbenz[*a*]anthracene 8,9-epoxide and at the C-10-position of 4-hydroxymethylbenz[*a*]anthracene 10,11-epoxide (Scheme 1) is consistent with the available evidence, which indicates that epoxide hydrolase-catalysed hydration occurs by attack at the least-hindered epoxide carbon atom (Hanzlik *et al.*, 1976; Lu & Miwa, 1980).

Discussion

We have shown that the fungus *Cunninghamella elegans* oxidizes 4-methylbenz[*a*]anthracene primarily at the methyl group with further oxidation and enzymic hydration to form hydroxymethylbenz[*a*]anthracene *trans*-8,9- and *trans*-10,11-dihydrodiols. Both dihydrodiols were shown to be preferentially in the quasi-diequatorial conformation. In contrast with previous studies on the metabolism of benz[*a*]anthracene and 7-methylbenz[*a*]anthracene by *C. elegans* (Cerniglia, 1981; Cerniglia *et al.*, 1982), there was no detectable 3,4-dihydrodiol formed from the metabolism of 4-methylbenz[*a*]anthracene by *C. elegans*, indicating that the 4-methyl substituent blocks the enzymic epoxidation reaction at the methyl-substituted 3,4-double bond. In contrast, 4-methylbenz[*a*]anthracene 3,4-dihydrodiol is a detectable metabolite from the metabolism of 4-methylbenz[*a*]anthracene by rat liver microsomal fraction (Yang *et al.*, 1981). Comparison of the results of the present study with those reported previously for the fungal metabolism of 7-methylbenz[*a*]anthracene indicates that, whereas the 3,4- and 8,9-double bonds were major sites of attack in 7-methylbenz[*a*]anthracene metabolism, 4-methylbenz[*a*]anthracene is metabolized predominantly at the 10,11- and 8,9-double bonds. Thus the fungal enzymes show considerable variation in the regio-selective metabolism of methylbenz[*a*]anthracenes. Similarly to our previous findings on the fungal metabolism of polycyclic aromatic hydrocarbons (Cerniglia, 1981), there was a lack of metabolism in the 'K'-region 5,6-positions of 4-methylbenz[*a*]anthracene. Interestingly, the 'K' region is a major site of enzymic attack in the mammalian metabolism of methyl-substituted benz[*a*]anthracene (Yang *et al.*, 1981).

In the present study the c.d. spectra of the 4-hydroxymethylbenz[*a*]anthracene *trans*-8,9- and *trans*-10,11-dihydrodiols were determined. The major enantiomers of both 4-hydroxymethylbenz[*a*]anthracene *trans*-8,9-dihydrodiol and 4-hydroxymethylbenz[*a*]anthracene *trans*-10,11-dihydrodiol had *S*,*S* absolute configurations. Since the major enantiomers of benz[*a*]anthracene 8,9- and 10,11-dihydrodiols formed from metabolism of benz[*a*]anthracene by rat liver microsomal fraction have *R*,*R* absolute configurations (Thakker *et al.*, 1979), our results suggest that there is a difference in the stereoselective preference of the fungal cytochrome *P*-450 mono-oxygenase–epoxide hydrolase enzyme systems towards the 8.9- and 10,11-double bonds of 4-methylbenz[*a*]anthracene in the formation of *trans*-8,9- and *trans*-10,11-dihydrodiols from that reported for rat liver enzyme systems. The ^{18}O-incorporation and acid-catalysed dehydration experiments with the *trans*-8,9- and *trans*-10,11-dihydrodiols of 4-hydroxymethylbenz[*a*]anthracene formed by *C. elegans* (Scheme 1) indicate that, similarly to the epoxide hydrolase from mammalian liver microsomal fraction, the fungal epoxide hydrolase also catalyses the enzymic hydration of the arene oxide metabolites, through a *trans* addition of a water molecule from the less-hindered side of the arene oxide (Hanzlik *et al.*, 1976; Lu & Miwa, 1980). Therefore the different enantiomeric *trans*-8,9- and *trans*-10,11-dihydrodiols formed by *C. elegans* are due to the epoxidation by cytochrome *P*-450 on different faces of the 8,9- and 10,11-double bonds of 4-hydroxymethylbenz[*a*]anthracene. Yang *et al.* (1982) have demonstrated that different forms of cytochrome *P*-450 may catalyse the epoxidation reaction preferentially at different sides of the methyl-substituted double bond of a planar polycyclic hydrocarbon molecule. Also, we have reported in a study on the fungal metabolism of naphthalene that *C. elegans* formed predominantly (+)-1*S*,2*S*-naphthalene dihydrodiol, which is opposite in stereochemistry to the major enantiomer reported for mammalian enzyme systems (Cerniglia *et al.*, 1983).

Further studies are necessary on the fungal metabolism of polycyclic aromatic hydrocarbons to determine the regio- and stereo-selective preference of the fungal cytochrome *P*-450–epoxide hydrolase enzyme systems in the metabolic formation of *trans*-dihydrodiols.

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