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ORIGINAL PAPER

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# Formation of sulfate and glucoside conjugates of benzo[*e*]pyrene by *Cunninghamella elegans*

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**Abstract** Benzo[*e*]pyrene is a pentacyclic aromatic hydrocarbon, which, unlike its structural isomer benzo[*a*]pyrene, is not a potent carcinogen or mutagen. The metabolism of benzo[*e*]pyrene was studied using the filamentous fungus *Cunninghamella elegans* ATCC 36112. *C. elegans* metabolized 65% of the [9, 10, 11, 12-$^{3}$H]benzo[*e*]pyrene and unlabeled benzo[*e*]pyrene added to Sabouraud dextrose broth cultures after 120 h of incubation. Three major metabolites of benzo[*e*]pyrene were separated by reversed-phase high- performance liquid chromatography. These metabolites were identified by $^{1}$H and $^{13}$C NMR, UV-visible, and mass spectral analyses as 3-benzo[*e*]pyrenylsulfate, 10-hydroxy-3-benzo[*e*]pyrenyl sulfate, and benzo[*e*]pyrene 3-*O*-*β*-glucopyranoside.

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) are frequently studied because of their ubiquitous nature. Some are carcinogenic in various biological systems and metabolites of such PAHs have been implicated as ultimate carcinogens (Ayrton et al. 1990). Many PAHs are considered environmental pollutants with risk to human health (Grimmer 1983). Benzo[*e*]pyrene, a pentacyclic aromatic hydrocarbon and an isomer of the carcinogen benzo[*a*]pyrene, is marginal in carcinogenic and mutagenic activity in mammalian systems (MacLeod et al. 1980). Benzo[*e*]pyrene has been detected in mainstream cigarette smoke, marijuana smoke, smoked and charcoal-broiled food, vegetable oil, and wastewater. However, crude oil and dried sediment from lakes contain the highest amounts of benzo[*e*]pyrene, ranging from 1.2 to 28.9 mg/kg in oil and from 2.0 to 2110 μg/kg in sediment (IARC 1983). PAHs, being low in aqueous solubilities, tend to adsorb to particle surfaces in the environment. As a result, they may persist under many natural conditions and become problematic environmental contaminants. Bioremediation of such PAHs involves the understanding of basic biochemical and microbiological principles of biodegradation and the applicability of these processes is the key to bioremediation of PAH-contaminated environments (Cerniglia 1992).

A wide variety of fungi are known to metabolize PAHs (Cerniglia 1992). The white rot fungus *Phanerochaete chrysosporium* oxidizes PAHs by extracellular peroxidases (Hammel et al. 1986, 1992; Sutherland et al. 1991) while a soil fungus, *Aspergillus niger*, metabolizes pyrene via a cytochrome P-450 monooxygenase enzyme system, to hydroxylated and conjugated products (Wunder et al. 1994). Also, the basidiomycete *Crinipellis stipitaria* strains JK375 and JK364 metabolized pyrene to hydroxylated products, quinones, and sulfate conjugates which have been implicated as detoxification products of pyrene (Lambert et al. 1994; Lange et al. 1994).

Similarly the zygomycete *Cunninghamella elegans* metabolizes several PAHs via cytochrome P-450 monooxygenase to derivatives that are generally less mutagenic than the parent compounds (Cerniglia 1992; Pothuluri and Cerniglia 1994; Sutherland 1992). In comparison with the bioactivation pathways of PAHs found more commonly in mammals, the fungal metabolic pathways are usually directed toward detoxification (Cerniglia 1992; Pothuluri and Cerniglia 1994; Sutherland 1992).

Even though microbial metabolism of benzo[*a*]pyrene has been studied extensively (Cerniglia 1992), to our knowledge, no studies have reported on the

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microbial metabolism of benzo[*e*]pyrene. In this study, we report the metabolism of benzo[*e*]pyrene to sulfate and glucoside conjugates by *C. elegans*.

## Materials and methods

### Chemicals

[9, 10, 11, 12-$^3$H]Benzo[*e*]pyrene (specific activity, 17 GBq/mmol or 460 mCi/mmol) was provided by Dr. Peter Fu (NCTR). Benzo [*e*]pyrene (purity, 98%) was purchased from Aldrich (Milwaukee, Wis., USA). Solvents, high-performance liquid chromatography (HPLC) grade, were purchased from Fisher Scientific (Pittsburgh, Pa., USA). All other chemicals were reagent grade and the highest purity available.

### Microorganism and culture conditions

The fungal incubation conditions reported previously (Pothuluri et al. 1990) were modified for this study. After growth of *C. elegans* ATCC 36112 cultures in 30 ml of Sabouraud dextrose broth for 48 h, 5 mg of benzo[*e*]pyrene was dissolved in 0.5 ml dimethyl sulfoxide and added to each culture. All flasks were incubated for an additional 96 h, after which, the contents of the flasks were pooled and filtered to separate the broth from the mycelia. The mycelia and the broth were then extracted with six equal volumes of ethyl acetate. Sterile control flasks were also incubated with benzo[*e*]pyrene.

Kinetic experiments were conducted as described above, with [9, 10, 11, 12-$^3$H]benzo[*e*]pyrene (specific activity, 62.2 kBq or 1.68 μCi) and 5 mg of unlabeled benzo[*e*]pyrene added to duplicate flasks. The percentage metabolism to various products was quantified by liquid scintillation methods (Pothuluri et al. 1990).

### Physical and chemical analyses

Benzo[*e*]pyrene metabolites were separated on a Perkin-Elmer series 10 HPLC equipped with an LC-95 UV-visible absorbance detector (Perkin-Elmer, Norwalk, Conn., USA), at 254 nm. An Altex Ultrasphere $C_{18}$ column (25 cm by 4.6 mm; Altex Scientific, Berkeley, Calif., USA) was used to separate benzo[*e*]pyrene metabolites. A 40-min linear gradient of methanol-water [from 30:70 to 95:5 (vol:vol)], at a flow rate of 1.0 ml/min, eluted the metabolites, which were collected from repeated injections. Fractions with similar HPLC retention times were pooled and concentrated. The metabolites then were purified isocratically with methanol:water ratios of either 40:60 (vol:vol) or 48:52 (vol:vol) at a flow rate of 0.8 ml/min.

UV-visible absorption spectra of the metabolites were determined in methanol with a Shimadzu UV-2101 PC spectrophotometer. Mass spectral (MS) analyses were conducted as described previously (Pothuluri et al. 1990) except that the mass spectrometer was upgraded from a Finnigan 4000 to a 4500. The samples were analyzed on a Finnigan rhenium wire direct exposure probe (DEP), using a current ramp of 5 mA/s from 0 to 650 mA. The ion source temperature was 150°C, the electron energy was 70 V and the quadrupole analyzer was scanned continuously from 50 to 750 daltons with a cycle time of 1.2 s.

One metabolite was acetylated by the addition of 1.0 ml pyridine and 1.5 ml acetic anhydride (Pothuluri et al. 1990) and incubation at 50°C for 24 h. The sample was analyzed for possible glucoside conjugates by MS. The electron impact (EI) mass spectral conditions were the same as those reported previously (Pothuluri et al. 1990).

Deconjugation of sulfate conjugates was performed as described previously (Pothuluri et al. 1994), and also with the following modifications: 5 U (350 μl) of arylsulfatase (type V, Sigma) was added to each tube containing 2 ml of TRIS-HCl buffer and incubated for 96 h at 37°C. The deconjugated metabolites were also analyzed by nuclear magnetic resonance (NMR) spectroscopy.

The NMR spectra were recorded in the $^1$H and $^{13}$C configurations at 500.13 MHz and 125.77 MHz, respectively, using a Bruker AM500 spectrometer (Bruker Instruments, Billerica, Mass., USA). Samples were dissolved in acetone-$d_6$ and chemical shifts are reported on the $\delta$ scale by assigning the methyl resonance to 2.05 ppm or 29.80 ppm. Spectra were recorded at approximately 30°C. General conditions and procedures for acquisition of one-dimensional $^1$H NMR spectra have been reported previously (Evans et al. 1994). $^{13}$C NMR spectra were obtained with $^1$H decoupling (final data point resolution 1.02 Hz/point). Two-dimensional (2-D) $^{13}$C-$^1$H chemical shift correlation spectra were obtained with delays adjusted for detection of one-bond coupling constants (160 Hz). For benzo[*e*]pyrene, an additional 2-D experiment was performed with delays adjusted for detection of long-range coupling constants (7.5 Hz) (Cho et al. 1991).

## Results

The reversed-phase HPLC elution profile of the ethyl-acetate-extractable metabolites formed by incubation of [9, 10, 11, 12-$^3$H]benzo[*e*]pyrene and 5 mg of unlabeled benzo[*e*]pyrene for 96 h with *C. elegans* is shown in Fig. 1. Benzo[*e*]pyrene was metabolized to three principal compounds, metabolites I–III, which eluted at 9.5, 14.0, and 30.0 min, respectively. Benzo[*e*]pyrene eluted at 46.5 min (Fig. 1).

These metabolites were identified by UV-visible absorption, mass spectral and NMR analyses. Structural characterization was done by NMR spectroscopy. The $^1$H NMR spectrum of metabolite I contained 10 aromatic resonances (Table 1). This indicated a di-substituted benzo[*e*]pyrene. From examination of the coupling constant pattern and several decoupling experiments, it was readily apparent that the substitutions had to be at either C1 or C3 and at either C10 or

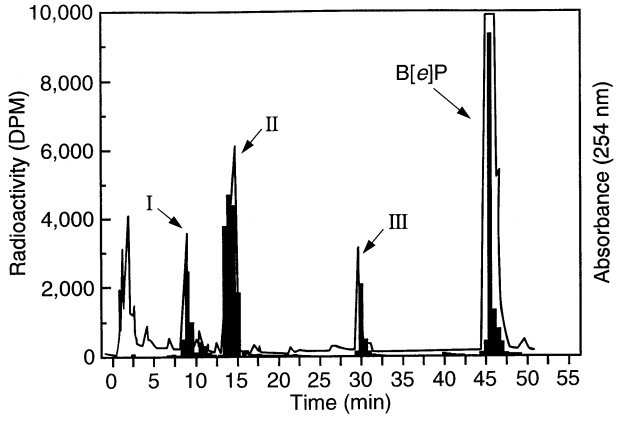


**Fig. 1** HPLC elution profile and radioactivity of the ethyl acetate-soluble metabolites formed from [9, 10, 11, 12-$^3$H]benzo[*e*]pyrene by *Cunninghamella elegans*. Fractions eluting from the chromatograph were collected at 0.5-min intervals and their radioactivities were measured by liquid scintillation counting

**Table 1** $^{1}$H NMR spectral parameters for benzo[*e*]pyrene (B[*e*]P), metabolites and related compounds formed from *Cunninghamella elegans*[a]

| Assignment | Compound | | | | | |
|---|---|---|---|---|---|---|
| | B[*e*]P | 10-OH-3-Benzo[*e*]-pyrenylsulfate (I) | 3,10-DihydroxyB[*e*]P | 3-Benzo[*e*]-pyrenylsulfate (II) | 3-OH-B[*e*]P | B[*e*]P 3-*O*-*β*-glucopyranoside[c] (III) |
| | Chemical shift (ppm) | | | | | |
| 1 | 9.05 | 8.80 | 8.74 | 8.95 | 8.89 | 8.99 |
| 2 | 8.09 | 8.35 | 7.60 | 8.42 | 7.65 | 7.96 |
| 3 | 8.27 | – | – | – | – | – |
| 4 | 8.13 | 8.59 | 8.44 | 8.61 | 8.46 | 8.63 |
| 5 | 8.13 | 8.01 | 8.03 | 8.04 | 8.06 | 8.11 |
| 6 | 8.27 | 8.18 | 8.19 | 8.21 | 8.22 | 8.26 |
| 7 | 8.09 | 7.99 | 8.01 | 8.03 | 8.05 | 8.08 |
| 8 | 9.05 | 8.79 | 8.80 | 8.97 | 8.98 | 9.02 |
| 9 | 8.98 | 8.26 | 8.26 | 8.92 | 8.91 | 8.94 |
| 10 | 7.79 | – | – | 7.70[b] | 7.69 | 7.73 |
| 11 | 7.79 | 7.31 | 7.30 | 7.75[b] | 7.73 | 7.77 |
| 12 | 8.98 | 8.76 | 8.69 | 8.92 | 8.84 | 8.91 |

[a] Samples dissolved in acetone-$d_6$. First-order measurements of coupling constants ($J$) are as follows: for3-benzo[*e*]pyrenylsulfate, $J_{1,2} = 8.6$; $J_{4,5} = 9.1$; $J_{6,7}7.7 = 7.7$; $J_{6,8} = 1.1$; $J_{7,8} = 8.2$; $J_{9,10} = 8.2$; $J_{9,11} = 1.7$; $J_{10,11} = 7.1$; $J_{10,12} = 1.5$; and $J_{11,12} = 8.2$ Hz. For 10-OH-3-benzo[*e*]pyrenylsulfate, $J_{1,2} = 8.6$; $J_{4,5} = 9.2$; $J_{6,7} = 7.7$; $J_{6,8} = 0.8$; $J_{7,8} = 8.0$; $J_{9,11} = 2.6$; and $J_{11,12} = 8.8$ Hz. The corresponding hydroxy compounds and the glucoside have the same $J$ values within experimental error

[b] Assignments may be reversed

[c] Chemical shifts of glucose protons are as follows: 1′, 5.37; 2′, 3.79; 3′, 3.65; 4′, 3.56; 5′, 3.68; 6a′, 3.95, and 6b′, 3.77 ppm. Coupling constants are as follows: $J_{1',2'} = 7.7$; $J_{2',3'} = 9.2$; $J_{3',4'} = 8.9$; $J_{4',5'} = 9.3$; $J_{5',6a'} = 2.7$; $J_{5',6b'} = 5.6$ Hz; and $J_{6a',6b'} = 11.9$ Hz. Measurements of sugar resonances were carried out with a trace of $D_2O$ added

C11. A key experiment in elucidating the structure involved saturation of the H9 resonance, since this was determined from its *meta* coupling constant pattern to be adjacent to one of the sites of substitution. A nuclear Overhauser effect (NOE) to only one resonance, H8, was observed. In addition, saturation of the H4 resonance resulted in only a single NOE to the H5 resonance, and saturation of the H6 resonance resulted in NOEs to the H5 and H7 resonances. These experiments demonstrated that the sites of substitution were C3 and C10. The lack of other nonexchangeable resonances suggested hydroxyl or sulfate substitution, consistent with our earlier results from several other PAH metabolites (Pothuluri et al. 1994). The nature of the substituents was investigated from analysis of the chemical shifts (see below).

The $^{1}$H NMR spectrum of metabolite II contained 11 aromatic resonances (Table 1), which indicated substitution at only a single site on the benzo[*e*]pyrene ring system. Decoupling and several NOE experiments showed that substitution was at the C3 atom and that the chemical shifts of the protons adjacent to the substituent were similar to those of metabolite I. This strongly suggested that metabolites I and II had the same substituent at the C3 position. The main chemical shift changes from the C3 substituent for metabolite II were as follows: *ortho*, 0.33; *meta*, −0.10; and *peri*, −0.48 ppm.

A larger amount of the metabolites were collected for analysis by natural abundance $^{13}$C NMR spectroscopy. The $^{13}$C NMR spectra did not reveal any additional carbon atoms which might be expected from a substituent, although sensitivity was low. A 2-D heteronuclear chemical shift correlation experiment enabled assignment of the resonances from carbons with a directly attached proton for both metabolites I and II. A similar analysis of the parent compound was conducted (Table 2) to enable calculation of $^{13}$C chemical shift substituent effects. The main substituent effects for metabolite II were as follows: *ortho*, −7.56; *meta*, 0.20; and *peri*, −4.62 ppm. The results for metabolite I were similar. Both the $^{13}$C and $^{1}$H chemical shift substituent effects are inconsistent with hydroxy substitution at C3.

Preliminary deconjugation experiments using methods described previously (Pothuluri et al. 1994) did not indicate that a sulfate conjugate of benzo[*e*]pyrenol was present. Therefore, based on $^{13}$C NMR results, the effect of sulfatase activity on metabolites I and II was reexamined. This method was modified by adding larger amounts of sulfatase and longer incubation conditions. The hydrolysis of the metabolites was observed (see below) and the resulting compounds had $^{1}$H NMR spectral parameters that were characteristic of hydroxy substitutions. The HPLC elution profile and UV-visible spectrum of metabolite I,

**Table 2** $^{13}C$ NMR spectral parameters for benzo[*e*]pyrene (B[*e*]P) and two sulfate conjugate metabolites formed from *C. elegans*[a]

| Assignment | Compound | | |
|---|---|---|---|
| | B[*e*]P[b] | 10-OH-3-benzo[*e*]pyrenylsulfate (I)[c] | 3-Benzo[*e*]pyrenylsulfate (II)[d] |
| | Chemical shift (ppm) | | |
| 1 | 121.47 | 120.96[e] | 121.67 |
| 2 | 127.30 | 120.02 | 119.74 |
| 3 | 127.24 | – | – |
| 4 | 128.31 | 123.69 | 123.65 |
| 5 | 128.31 | 126.84 | 126.92 |
| 6 | 127.24 | 126.71 | 126.78 |
| 7 | 127.30 | 126.83 | 127.09 |
| 8 | 121.47 | 120.62[e] | 120.96 |
| 9 | 124.78 | 108.98 | 124.65[f] |
| 10 | 128.71 | – | 127.77[e] |
| 11 | 128.71 | 118.12 | 128.58[e] |
| 12 | 124.78 | 126.05 | 124.41[f] |

[a] Samples dissolved in acetone-$d_6$
[b] Assignments for quaternary carbons are as follows: 3a, 5a, 132.64; 3b, 5b, 124.94; 8a, 12b, 130.05; and 8b, 12a, 131.06 ppm
[c] Tentative list of chemical shifts of quaternary carbons: 157.69, 149.40, 132.79, 132.96, 130.03, 125.76, 125.38, 125.04, 124.82, and 124.33 ppm
[d] Tentative list of chemical shifts of quaternary carbons: 150.68, 132.86, 131.49, 130.34, 125.76, 125.38, 125.04, 124.82, and 124.33 ppm
[e,f] Assignments may be reversed

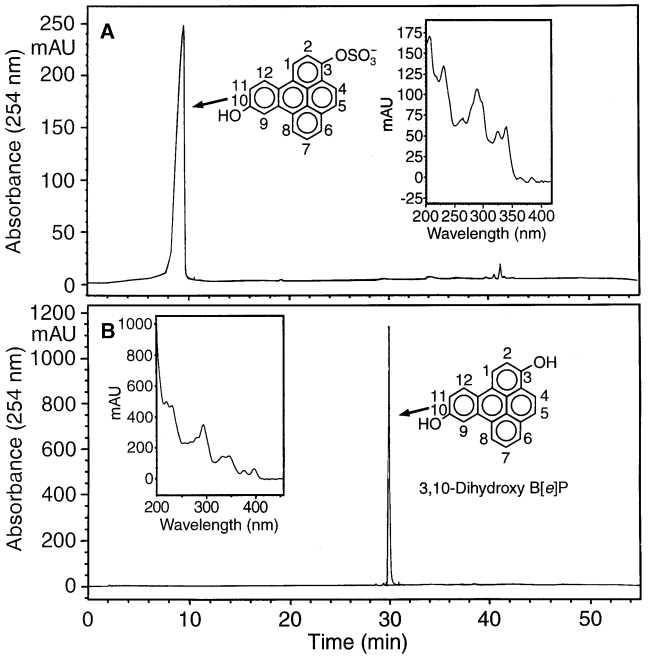


**Fig. 2** **A** HPLC elution profile of ethyl acetate-soluble metabolite I (10-hydroxy-3-benzo[*e*]pyrenylsulfate). **B** 10-Hydroxy,3-substituted phenolic derivative of benzo[*e*]pyrene (*B*[*e*]*P*) after deconjugation. *Inset* shows the UV-visible absorption spectrum obtained with a diode array detector

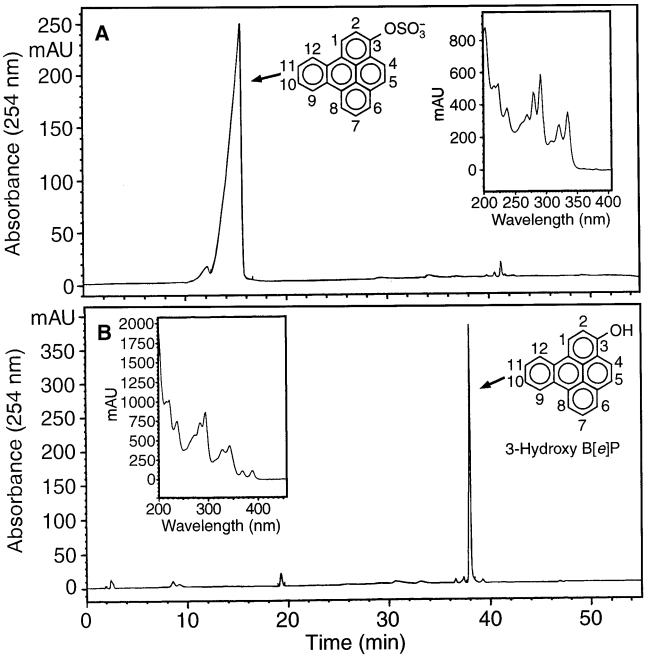


**Fig. 3** **A** HPLC elution profile of ethyl acetate-soluble metabolite II (3-benzo[*e*]pyrenylsulfate). **B** 3-Substituted phenolic derivative of benzo[*e*]pyrene (*B*[*e*]*P*) after deconjugation. *Inset* shows the UV-visible absorption spectrum obtained with a diode array detector

obtained using a diode array detector, are shown in Fig. 2A. When metabolite I was treated with sulfatase, deconjugation of sulfate occurred and the diphenol formed is evidenced by the shift in the HPLC elution time (Fig. 2B). Similarly, metabolite II was hydrolyzed with sulfatase enzyme, as reported for metabolite I. Figure 3A shows the HPLC elution profile and UV-visible spectrum of metabolite II, and when the

metabolite was treated with sulfatase, sulfate deconjugation occurred and the phenol formed is seen by the shift in the HPLC elution time (Fig. 3B). The $^1$H NMR spectral parameters and resonance assignments for these two metabolites and their deconjugated forms are given in Table 1. The deconjugated form of metabolite II has NMR spectral parameters that are the same as those previously reported for 3-OH-benzo[*e*]pyrene (Lee et al. 1981). Some minor changes in resonance assignments for 3-OH-benzo[*e*]pyrene were made from those reported previously (Table 1). Metabolite I is identified as 10-hydroxy-3-benzo[*e*]pyrenylsulfate and that metabolite II is 3-benzo[*e*]pyrenylsulfate.

The UV-visible absorption spectra for metabolites I and II showed absorption maxima at 259, 279, 289, 299, 326, and 340 nm and at 236, 269, 279, 292, 321, and 335 nm, respectively. The EI mass spectrum for benzo[*e*]pyrene/metabolite I revealed a molecular ion at *m/z* 284 and a characteristic fragment ion at *m/z* 255 $[M-\cdot HCO]^+$. The EI mass spectrum of metabolite I is consistent with thermal decomposition of the conjugate to dihydroxybenzo[*e*]pyrene with $SO_2$ with a characteristic ion at *m/z* 64 as the base peak. Metabolite II showed a molecular ion at *m/z* 268 with a characteristic fragment ion at *m/z* 239 $[M-\cdot HCO]^+$. Similarly, the mass spectrum of metabolite II is consistent with thermal decomposition to hydroxybenzo[*e*]pyrene with some $SO_2$ at *m/z* 64. The mass spectral data indicated that metabolite I and II were consistent with sulfate conjugates of benzo[*e*]pyrene diphenol and phenol, respectively.

The $^1$H NMR spectrum of metabolite III contained 11 aromatic resonances, and unlike the other two metabolites, it contained aliphatic resonances as well. The site of substitution at C3 was determined, and a comparison between the NMR spectral parameters of the three metabolites provided further support. Seven aliphatic resonances were present, which suggested substitution of a sugar. The chemical shifts of the aliphatic protons were similar to those reported for 1-phenanthryl *β*-D-glucopyranoside with one exception (H1′) (Cerniglia et al. 1989). The coupling constants can be used to determine the nature of the sugar (Cerniglia et al. 1989). The results were essentially the same as those previously reported for the glucoside (Table 1).

The UV-visible absorption for metabolite III showed absorption maxima at 236, 271, 282, 293, 325, and 340 nm. The EI mass spectrum of metabolite III is consistent with a hydroxybenzo[*e*]pyrene since the glucose moiety has been lost thermally to yield hydroxybenzo[*e*]pyrene. No molecular ion was observed for the intact glucoside (Fig. 4A). However, analysis of acetylated metabolite III showed that the glucoside was intact. Figure 4B illustrates the spectrum of acetylated metabolite III with a molecular ion at *m/z* 598, with typical acetylated glucose fragment ions at *m/z* 331, 169, and 109. The ion at *m/z* 268 indicated is from the hydroxybenzo[*e*]pyrene portion of the conjugate.

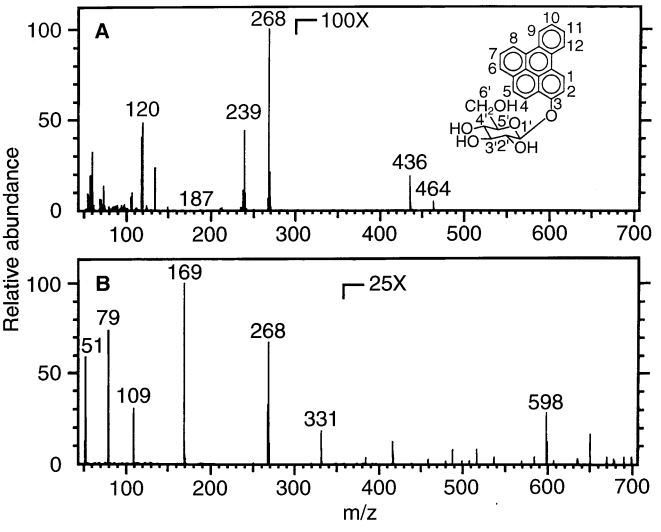


**Fig. 4** **A** Mass spectra of metabolite III (glucose conjugate) produced from *C. elegans* cultures exposed to benzo[*e*]pyrene and, **B** acetylated derivative of benzo[*e*]pyrene

Therefore, the spectrum is consistent with an acetylated benzo[*e*]pyrene glucoside. It is concluded that metabolite III is benzo[*e*]pyrene 3-*O*-*β*-glucopyranoside (Fig. 4).

Experiments with $^3$H-labeled benzo[*e*]pyrene showed that about 40% of the total radioactivity added to the culture was recovered in the organic phase, and that remaining was bound to the mycelia. At the time zero extraction, 83% of the absolute recovered radioactivity in the organic phase was benzo[*e*]pyrene. At 120 h, benzo[*e*]pyrene decreased to about 17%, while the three metabolites together accounted for about 66% of the total recovered radioactivity. Metabolite II (3-benzo[*e*]pyrenylsulfate) was the major metabolite and accounted for 42% of the recovered radioactivity. Metabolites I (10-hydroxy-3-benzo[*e*]pyrenylsulfate) and III (benzo[*e*]pyrene 3-*O*-*β*-glucopyranoside) accounted for 14% and 10.0% of the radioactivity, respectively. No metabolites were found in sterile control flasks containing benzo[*e*]pyrene (data not shown).

## Discussion

Our studies have documented that in the metabolism of PAHs by *C. elegans*, the metabolic pathways and biochemical reactions for the biotransformation of PAHs follow the phase I (oxidation) and subsequent phase II (conjugation) pathways (Cerniglia 1992). In this study, *C. elegans* oxidized benzo[*e*]pyrene mainly to hydroxylated products, which subsequently formed conjugated products. The hydroxylated products identified were 3,10-dihydroxybenzo[*e*]pyrene (Fig. 2B) and 3-OH-benzo[*e*]pyrene (Fig. 3B) which were subsequently conjugated to form 10-hydroxy-3-benzo

[*e*]pyrenylsulfate (Fig. 2A) and 3-benzo[*e*]pyrenylsulfate (Fig. 3A). 3-OH-benzo[*e*]pyrene also undergoes glycosylation to form benzo[*e*]pyrene 3-*O*-*β*-glucopyranoside (Fig. 4).

The formation of sulfate and glucoside conjugates shows the benzo[*e*]pyrene detoxification potential of this fungus, because conjugated products are generally less mutagenic than the parent compound. This has been demonstrated previously in our laboratory with other PAHs where the fungus *Cunninghamella elegans* has shown promise, since it was able to oxidize potentially carcinogenic PAHs predominantly to detoxified products rather than the proximate or ultimate carcinogens typically found in mammalian metabolism (Cerniglia 1992; Pothuluri and Cerniglia 1994; Sutherland 1992). A classic study with the potentially carcinogenic PAH benzo[*a*]pyrene showed that *C. elegans* transformed this genotoxic compound into detoxified derivatives. Metabolism of benzo[*a*]pyrene by *C. elegans* occurred via cytochrome P-450 monooxygenase and epoxide hydrolase reactions, to form 3-OH-benzo[*a*]pyrene, 9-OH-benzo[*a*]pyrene, 7,8- and 9,10-*trans*-dihydrodiols, dihydrodiol epoxides, tetraols, and quinones. These primary metabolites were further transformed to sulfate and glucoronide conjugates which have been shown to be the detoxification products (Cerniglia and Gibson 1980; Cerniglia et al. 1980).

Recent studies using other fungi, like *Aspergillus niger* and *Crinipellis stipitaria* have also found formation of conjugated products of PAHs which have been implicated as detoxification products (Lambert et al. 1994; Lange et al. 1994; Wunder et al. 1994). The fungus *A. niger* SK9317 metabolizes pyrene (a four-fused-ring PAH) by cytochrome P-450 monooxygenase systems, to 1-pyrenylsulfate and 1-hydroxy-8-pyrenylsulfate; this is implicated as the predominant mechanism of detoxification of pyrene by *A. niger* (Wunder et al. 1994). A basidiomycete, *Cr. stipitaria* JK375, also metabolizes pyrene to 1-hydroxypyrene, 1,6- and 1,8-dihydroxypyrene, 1,6- and 1,8-pyrenequinone (Lambert et al. 1994). A different strain, *Cr. stipitaria* JK364, forms *trans*-4,5-dihydro-4,5-dihydroxypyrene and 1-pyrenyl-sulfate as detoxification products of pyrene (Lange et al. 1994). Similarly, *C. elegans* hydroxylated pyrene predominantly at the 1,6- and 1,8-positions, with subsequent glycosylation to form glucoside conjugates of 1-hydroxypyrene, 1,6- and 1,8-dihydroxypyrene (Cerniglia et al. 1986). These results substantiate further the usefulness of fungi in metabolizing PAHs into conjugated products which have been previously shown to be detoxification products. Therefore, fungi may have potential in the bioremediation of PAHs in aqueous and terrestrial ecosystems.

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