Bull. Environ. Contam. Toxicol. (1995) 55:251–257

Environmental Contamination and Toxicology

Antimicrobial, Phytotoxic, Nematicidal, Cytotoxic, and Mutagenic Activities of 1-Hydroxypyrene, the Initial Metabolite in Pyrene Metabolism by the Basidiomycete *Crinipellis stipitaria*

M. Lambert, S. Kremer, H. Anke

Department of Biotechnology, University of Kaiserslautern, Paul-Ehrlich-Straße 23, D-67663 Kaiserslautern, Germany

Received: 22 August 1994/Accepted: 16 January 1995

In recent years, there has been an increasing interest in the testing fungi for degradation or detoxification of various polycyclic aromatic hydrocarbons (PAHs) and elucidation of the pathways involved. Investigations on microbial metabolism of pyrene are limited to some bacteria (Heitkamp et al. 1988a,b, b, Walter et al. 1991) and the fungi *Cunninghamella elegans* (Cerniglia et al. 1986), *Phanerochaete chrysosporium* (Hammel et al. 1986), *Crinipellis stipitaria* (Lambert et al. 1994, Lange et al. 1994) and *Aspergillus niger* (Wunder et al. 1994). The fungal transformation products of pyrene, 1-hydroxypyrene, 1,6-dihydroxypyrene, 1,8-dihydroxypyrene, 1,6-pyrenequinone, 1,8-pyrenequinone and some glucoside conjugates of hydroxylated pyrenes were identified (Cerniglia et al. 1986, Hammel et al. 1986, Lambert et al. 1994, Lange et al. 1994, Wunder et al. 1994). Until now, only mutagenic activities of some of these metabolites towards *Salmonella typhimurium* have been reported (Okamoto and Yoshida 1981a,b). Nothing is known about additional biological activities of these compounds, especially their effects on soil organisms are of ecological importance. During bioremediation processes these compounds could accumulate.

In the present study we describe the antimicrobial, nematicidal, phytotoxic, cytotoxic and mutagenic activities of 1-hydroxypyrene, the initial transformation product of pyrene metabolization by *C. stipitaria* (Lambert et al. 1994) and comparise this, with the activities of pyrene and benzo[a]pyrene.

Correspondence to: H. Anke

MATERIALS AND METHODS

Pyrene was obtained from Sigma Chemical Co., St. Louis, Missouri and benzo[a]pyrene was purchased from Aldrich-Chemie, Steinheim, Germany. S9 mix (rat liver; arochlor 1254) was obtained from Organon Teknika Corp., Durham, North Carolina. The isolation of 1-hydroxypyrene formed by *C. stipitaria* was described by Lambert et al. (1994). Assays for the evaluation of the cytotoxic activity were described by Erkel et al. (1991). The estimation of the nematicidal activity was performed according to Stadler et al. (1993) and the estimation of antimicrobial and phytotoxic effects were accomplished as described by Anke et al. (1989). In the mutagenicity test (Ames et al. 1975), *Salmonella typhimurium* TA 98 and TA 100 were assayed according to Maron and Ames (1983) with and without S9 mix. Compounds to be tested were dissolved in methanol; tests were carried out twice as triplicates. The numbers given are the average revertants from six plates.

RESULTS AND DISCUSSION

1-Hydroxypyrene, pyrene and benzo[a]pyrene showed no cytotoxic effects towards L 1210 cells (ATCC CCL 219) up to 100 µg/mL. The effect of the compounds on HeLa S3 cells (ATCC CCL 240) is shown in Figure 1. Up to a concentration of 100 µg/mL, pyrene had no effect on the growth of the cells. Benzo[a]pyrene was weakly cytotoxic (IC_{50}: 100 µg/mL) and 1-hydroxypyrene was moderately cytotoxic (IC_{50}: 13 µg/mL).

The antimicrobial effects of the three compounds are given in Table 1. Several important soil inhabitants were included in the test. The results show, that pyrene and benzo[a]pyrene had no antimicrobial effects up to concentrations of 100 µg/mL. In contrast, 1-hydroxypyrene showed significant antibacterial and antifungal activities. Growth of most bacteria was inhibited at 2.5 µg/mL. Growth was not restored after replacing the medium with 1-hydroxypyrene-free medium. Among the fungi tested, only *Fusarium oxysporum* was not affected by 1-hydroxypyrene up to concentrations of 100 µg/mL.

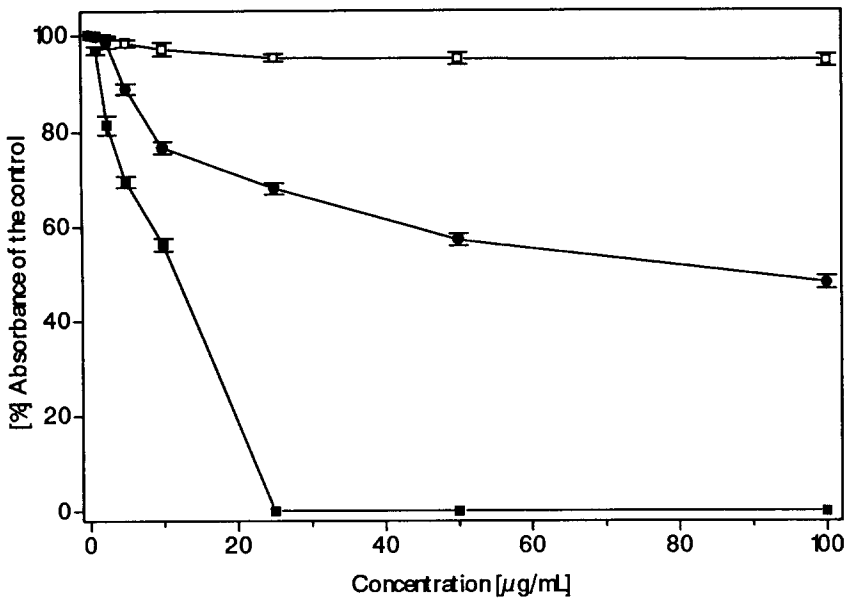

Figure 1. Cytotoxic effect of 1-hydroxypyrene (■), pyrene (□) and benzo[a]pyrene (●) towards HeLa S3-cells. Each point is the mean of three determinations. The error bars indicate standard deviation from the mean.

The results of the phytotoxicity test are summarized in Table 2. 1-Hydroxypyrene and pyrene had no significant effects towards *Lepidium sativum* and *Setaria italica*, but benzo[a]pyrene impaired growth of the tested plants starting at concentrations between 25 and 50 µg/disk (13 mm diameter). Phytotoxic effects reported for PAHs include reduced growth of wheat and barley (Sims and Overcash 1983) in the presence of 3,4-benzofluoranthene. Additional effects of 1-hydroxypyrene towards *Setaria italica* and of benzo[a]pyrene towards *Lepidium sativum* and *Setaria italica* can now be added.

Activities towards the saprophytic soil-inhabiting nematode *Caenorhabditis elegans* are shown in Table 3. 1-Hydroxypyrene and benzo[a]pyrene showed very strong nematicidal activities. Whereas the effects of 1-hydroxypyrene were already visible after 1 hr, immobilization of the nematodes by 1 µg/mL of

Table 1. Minimal inhibitory concentrations (MIC, for viable cell counts) of 1-hydroxypyrene, pyrene and benzo[a]pyrene in the serial dilution assay towards bacteria and fungi. Concentration tested: 1; 2.5; 5; 10; 15; 20; 25; 50 and 100 µg/mL.

Organism	MIC [µg/mL]		
	1-Hydroxy-pyrene	Pyrene	Benzo[a]-pyrene
Bacteria:			
Agrobacterium rubi	10	>100	>100
Arthrobacter citreus	2.5	>100	>100
Bacillus brevis	2.5	>100	>100
Pseudomonas fluorescens	2.5	>100	>100
Streptomyces spec.	2.5	>100	>100
Fungi:			
Aspergillus niger	10	>100	>100
Fusarium oxysporum	>100	>100	>100
Mucor miehei	5	>100	>100
Penicillium notatum	10	>100	>100
Rhodotorula glutinis	10	>100	>100
Saccharomyces cerevisiae	10	>100	>100
Trichoderma viride	25	>100	>100

Table 2. Phytotoxic activities of 1-hydroxypyrene, pyrene and benzo[a]pyrene towards *Lepidium sativum* and *Setaria italica*. Concentration tested 10; 25; 50 and 100 µg/disk (13 mm diameter).

Test plant	Compound	Concentration [µg/disk]			
		10	25	50	100
Lepidium sativum	1-Hydroxypyrene	-*	-	-	-
	Pyrene	-	-	-	-
	Benzo[a]pyrene	-	+/-	+	++
Setaria italica	1-Hydroxypyrene	-	-	-	+/-
	Pyrene	-	-	-	-
	Benzo[a]pyrene	-	-	+/-	+

*: - = no phytotoxic effect; +/- = shoot-/root-length ≤50 % of the control; + = shoot-/root-length >50 % of the control; ++ = no germination

Table 3. Nematicidal activities of 1-hydroxypyrene, pyrene and benzo[a]pyrene towards *Caenorhabditis elegans* after 1 and 48 hr of incubation. Concentration tested: 1; 2.5; 5; 10; 15; 20; 25; 50 and 100 µg/mL.

Tested compound	Incubationperiod	ND50*[µg/mL]
1-Hydroxypyrene	1 hr	2.5
	48 hr	1
Pyrene	1 hr	>100
	48 hr	>100
Benzo[a]pyrene	1 hr	50
	48 hr	1

*ND50: concentrations causing immotility of 50 - 55 % of the worms

benzo[a]pyrene came into effect only after 48 hr. This indicates, that a transformation product rather than benzo[a]pyrene itself may be responsible for the nematicidal effects.

The results of the mutagenicity test carried out as "plate incorporation test" are summarized in Table 4. As aspected, pyrene and 1-hydroxypyrene showed mutagenic effects neither with S9 mix nor without microsomal activation. This is in agreement with the results of Okamoto and Yoshida (1981b), who reported that 1-hydroxypyrene is not mutagenic towards strain TA 98 in the presence of S9 mix. TA 100 was not tested. The control mutagens daunomycin and ethyl methylsulfonate significantly increased the number of revertants of the appropriate strain. As reported by other authors (Cerniglia et al. 1985), benzo[a]pyrene caused an increase in the number of revertants in both strains after metabolic activation by S9 mix.

The data presented here indicate, that 1-hydroxypyrene has toxic effects towards many organisms, whereas pyrene was not toxic in any test. The greater solubility of 1-hyroxypyrene as compared to pyrene could not solely account for the drastic increase of the various biological activities. Ecologically important are the strong antimicrobial and nematicidal activities of 1-hydroxypyrene since it has been shown

Table 4. Mutagenic effects of 1-hydroxypyrene (OH-Pyr), pyrene (Pyr) and benzo[a]pyrene (B[a]P) in the "plate incorporation test" after 48 hr. Daunomycin (DM) and ethyl methanesulfonate (EMS) were used as control mutagens. The values are the means of the values obtained from six replicates. Standard deviations are given in parenthesis.

Strain	S9mix	Number of revertants/plate					
	[µL]	C[a]	Pyr[b]	OH-Pyr[b]	DM[c]	EMS[d]	B[a]P[c]
TA 98	-	7 (±1.1)	7 (±1.4)	8 (±1.4)	2370 (±162.2)	n.t.	6 (±1.1)
	10	7 (±0.9)	14 (±1.4)	7 (±0.9)	13 (±1.9)	n.t.	56 (±2.8)
	25	8 (±1.2)	13 (±1.1)	7 (±1.4)	15 (±2.1)	n.t.	104 (±4.2)
TA 100	-	93 (±3)	94 (±2.6)	99 (±4.6)	n.t.	5230 (±233.4)	96 (±3.3)
	10	96 (±2.8)	116 (±3.2)	117 (±5.1)	n.t.	118 (±5.5)	179 (±4.6)
	25	107 (±3.8)	128 (±5.9)	138 (±4.8)	n.t.	109 (±3.9)	369 (±12.4)

a = Control with solvent (methanol); b 5 µg/1 mL top agar; c = 2 µg/1 mL top agar; d = 2 µl/1 mL top agar; n.t. = not tested

to be produced by bacteria as well as by fungi during metabolization and degradation of pyrene. This is an example of the conversion of a non-toxic compound into a biologically active one and shows the necessity of toxicologic surveys during soil treatment in addition to analytical ones in order to optimize bioremediation techniques.

Acknowledgments. This work was supported by special grants (Forschungsschwerpunkt Umweltschutz und Energietechnik an der Universität Kaiserslautern) from the government of Rheinland-Pfalz, Germany.

REFERENCES

Ames BN, McCann J, Ymasaki, E (1975) Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mut Res 31:347-364

Anke H, Bergendorff HO, Sterner O (1989) Assays of the biological activities of guaiane sesquiterpenoids

isolated from the fruit bodies of edible *Lactarius* species. Food Chem Tox 27:393-397
Cerniglia CE, White GL, Heflich RH (1985) Fungal metabolism and detoxification of polycyclic aromatic hydrocarbons. Arch Microbiol 143:105-110
Cerniglia CE, Kelly DW, Freeman, JP, Miller DW (1986) Microbial metabolism of pyrene. Chem Biol Interact 57:203-216
Erkel G, Anke T, Velten R, Steglich W (1991) Podoscyphic acid, a new inhibitor of avian myeloblastosis virus and Moloney murine leukemia virus reverse transcriptase from a *Podoscypha* species. Z Naturforschung 46c:442-450
Hammel KE, Kalyanaraman B, Kirk TK (1986) Oxidation of PAHs and dibenzo[p]dioxins by *Phanerochaete chrysosporium* ligninase. J Biol Chem 261:16948-16952
Heitkamp MA, Franklin W, Cerniglia CE (1988a) Microbial metabolism of polycyclic aromatic hydrocarbons: Isolation and characterization of pyrene-degrading bacterium. Appl Environ Microbiol 54:2549-2555
Heitkamp MA, Freeman JP, Miller DW, Cerniglia CE (1988b) Pyrene degradation by a *Mycobacterium* spec.: Identification of ring oxidation and ring fission products. Appl Environ Microbiol 54:2556-2565
Lambert M, Kremer S, Sterner O, Anke A (1994) Metabolism of pyrene by the basidiomycete *Crinipellis stipitaria* and identification of pyrenequinones and their hydroxylated precursors in strain JK375. Appl Environ Microbiol 60:3597-3601
Lange B, Kremer S, Sterner O, Anke H (1994) Pyrene metabolism in *Crinipellis stipitaria*: Identification of *trans*-4,5-dihydro-4,5-dihydroxypyrene and 1-pyrenylsulfate in Strain JK364. Appl Environ Microbiol 60:3602-3607
Maron MM, Ames BN (1983) Revised methods for the *Salmonella* mutagenicity test. Mut Res 113:173-215
Sims RC, Overcash MR (1983) Fate of polynuclear aromatic compounds (PNAs) in soil-plant systems. Residue Reviews 88:1-68
Stadler M, Arendholz WR, Anke H, Hansske F, Anders U, Bergquist KE, Sterner O (1993) Lachnumon and lachnumol A, new metabolites with nematicidal and antimicrobial activities from the ascomycete *Lachnum papyraceum* (Karst.) Karst. J Antibiotics 46:961-967
Walter U, Beyer M, Klein J, Rehm HJ (1991) Degradation of pyrene by *Rhodococcus* spec. UW1. Appl Microbiol Biotechnol 34:671-676
Wunder T, Kremer S, Sterner O, Anke H (1994) Metabolism of the polycyclic aromatic hydrocarbon pyrene by *Aspergillus niger* SK 9317. Appl Microbiol Biotechnol, in Press