ELSEVIER

Journal of Chromatography A, 974 (2002) 213–222

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Study of fungal degradation products of polycyclic aromatic hydrocarbons using gas chromatography with ion trap mass spectrometry detection

Tomáš Cajthaml[a], Monika Möder[b], Petr Kačer[c], Václav Šašek[a], Peter Popp[b,*]

[a]*Department of Ecology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ-142 20 Prague 4, Czech Republic*

[b]*Department of Analytical Chemistry, UFZ – Centre for Environmental Research Leipzig-Halle, Permoserstr. 15, D-04318 Leipzig, Germany*

[c]*Faculty of Chemical Technology, ICT Prague, Technická 5, CZ-166 28 Prague 6, Czech Republic*

Abstract

Representatives of polycyclic aromatic hydrocarbons (PAHs) were degraded by ligninolytic fungus *Irpex lacteus*. The products were analyzed by GC–Ion trap mass spectrometry. The combination of full scan mass spectra, product ion scans (MS–MS) and derivatization of the degradation products of anthracene, phenanthrene, fluoranthene and pyrene provided further insight in the degradation mechanism initiated by *I. lacteus*. Particularly, the product ion scans enabled the interpretation of unknown degradation products, even though they were only produced at trace level. Most of the structures suggested were later confirmed with authentic standards.
© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Ligninolytic fungi; Polynuclear aromatic compounds

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) and some of their derivatives are a class of serious environmental pollutants which often have a high mutagenic and carcinogenic potential.

The natural occurrence of PAHs is relatively rare. PAHs occur in oil, coal, tar, some minerals, and have also been identified from certain geochemical processes. However, human activities are the prevailing source of these organopollutants, which are produced by burning fossil fuels and the high-temperature processing of carbon compounds (pyrolysis of oil fractions, etc.).

One way of removing PAHs from the environment is bioremediation using various types of organisms [1,2]. Microbial degradation by ligninolytic fungi has been intensively studied during the past few years [3,4]. Due to the irregular structure of lignin, ligninolytic fungi produce extracellular enzymes with very low substrate specificity, making them suitable for the degradation of different compounds. The ligninolytic system consists of three enzymes: lignin peroxidase, manganese dependent peroxidase and laccase [5]. In vitro experiments with purified enzymes proved that ligninolytic enzymes are able to

*Corresponding author.
E-mail address: popp@ana.ufz.de (P. Popp).

0021-9673/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(02)00904-4

extensively degrade PAHs [6]. But although the degradation process of PAHs by this type of organism has been intensively studied, the mechanism of degradation is not yet clear. It has been surmised that ligninolytic enzymes perform a one-electron radical oxidation, producing cation radicals from contaminants followed by the appearance of quinones [7]. However, the monooxygenase system of cytochrom P-450 generating epoxides may also be involved in degradation [8]. The epoxides can be rearranged into hydroxy derivatives or they may be hydrolyzed to vicinal dihydrodiols.

Although in the case of bacterial degradation the complete degradation pathways of several PAHs by bacteria have been already elucidated [9], the information available about fungal degradation products is limited. Using ^{14}C-labeled compounds, significant mineralization to carbon dioxide [10] was demonstrated. However, to our knowledge just two ring cleavage intermediates were identified: phthalic acid produced from anthracene and 2′,2-diphenic acid from phenanthrene [11,12].

A number of analytical techniques have been employed to study the microbial degradation of PAHs—mostly high-performance liquid chromatography (HPLC) with a diode array detector, and gas chromatography (GC) coupled with mass spectrometry [13]. Both these methods allow information to be acquired about the structure of products, which are finally identified by NMR or authentic standards [14]. However, using NMR requires fairly pure compounds in a quantity that is hardly available in degradation experiments with stationary incubated fungi.

In the case of ligninolytic fungi, usually quinones are accumulated, and the next step is rate limiting. Various sample preparation methods such as thin layer chromatography and solid-phase extraction have been applied to obtain enough material for the identification procedure [15].

The aim of this study was to apply GC–MS/MS in the study of PAHs degradation. This method combined with the technique of GC/chemical ionization (CI)–MS was used to interpret the structures of metabolites. Intermediates of several PAHs originating from their degradation by the ligninolytic fungus *Irpex lacteus* were elucidated by GC–MS/MS and later confirmed using authentic standards. The fungus has not yet been studied from this angle, although its ability to degrade PAHs has been already demonstrated [16,17].

Another reason why fungal remediation techniques have only slowly been introduced is the lack of detailed knowledge about mechanisms of the degradation or occurrence and toxicity of metabolites. The following investigations into the fungal degradation of anthracene, phenanthrene, fluoranthene and pyrene could improve understanding of the whole process of degradation.

2. Experimental

2.1. Chemicals

Anthracene (ANT), phenanthrene (PHE), pyrene (PYR), and fluoranthene (FLT) were used as substrates for degradation experiments and were purchased from Fluka, Germany. Labeled PHE and ANT with ^{13}C were kindly provided by Dr. Richnow, UFZ, Leipzig-Halle. All the compounds were applied without further purification to prepare stock solutions in dimethylformamide.

All solvents were purchased from Merck (Darmstadt, Germany) of p.a. quality, trace analysis quality or gradient grade.

The references of the structures identified by GC–MS are listed in Table 1.

2.2. Culture condition

Culture of *Irpex lacteus* (Fr., strain 617/93) was incubated stationary in 250 ml Erlenmeyer flasks in five parallels as described in detail elsewhere [16]. The liquid cultures were inoculated with 5% suspension of 1-week grown mycelia. Malt extract-glucose medium was contaminated at the time of inoculation. The samples were spiked with a solution of PAH in dimethylformamide such that the final amount of each PAH was 0.5 mg per flask. The samples were harvested several times up to day 50. Degradation was performed with all PAHs at once to examine degradation ability and also with individual compounds to clarify products' origin. The abiotic controls were performed using 1-week grown mycelia killed in an autoclave.

Table 1
Reference compounds used for identification of metabolites

Compound	Supplied by
Anthraquinone	Merck-Schuchardt, for synthesis
Anthrone	Fluka, p.a.
Phthalic acid	Fluka, pure
Dimethylphthalic acid	methylated phthalic acid with BF_3 in methanol
Phthalide	Merck-Schuchardt, for synthesis
9-Hydroxyphenanthrene	Aldrich, tech.
9-Methoxyphenanthrene	methylated 9-hydroxyphenanthrene with diazomethane [20]
9,10-Dihydrophenanthrenediol	hydrogenantion of phenanthrenequinone [21]
1,8-Naphthalenedicarboxylic anhydride	Merck-Schuchardt, for synthesis
2-Hydroxy benzoic acid	Fluka p.a.

2.3. Sample preparation

The whole content (mycelium with liquid) of each sample was homogenized with Ultraturrax and acidified to approximately pH 2. It was then extracted with five 10-ml portions of ethylacetate. Afterwards the extracts were dried with sodium sulfate and concentrated with a rotary evaporator.

To enable HPLC analysis, an aliquot of extract was mixed with acetonitrile at a ratio of 1:10, this mixture being used for injection.

In the case of gas chromatography (GC) coupled with electron impact and chemical ionization mass spectrometry as well as with MS–MS experiments, the extracts were directly injected without any derivatization. Moreover, the samples divided in three aliquots were treated separately with different derivatization reagents such as *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide (Merck, Germany), *N*-methyl-bis-trifluoroacetamide (Fluka, Germany), and BF_3/methanol (Fluka, Germany) [18].

2.4. Analysis

The degradation of PAHs was measured using a Hewlett-Packard 1040 HPLC (Amstelveen, Netherlands) equipped with diode array detector. An isocratic program was applied with 85% of acetonitrile and 15% of water, and PAHs were determined at 254 nm. PAHs were separated on a LichroCart-PAH column filled with LichroSphere (250 mm×5 mm, particle diameter 5 μm) provided by Merck (Darmstadt, Germany).

Intermediates were identified using GC–MS (GCQ, Finnigan, USA). The GC instrument was equipped with split/splitless injector and an HP-5 column was used for separation (30 m, 0.25 mm I.D., 0.25 μm film thickness). The temperature program started at 60 °C and was held for 1 min in splitless mode. Then the splitter was opened and the oven was heated to 150 °C at a rate of 25 °C/min. The second temperature ramp was up to 260 °C at a rate of 10 °C/min, this temperature being maintained for 20 min. The solvent delay time was set to 5 min. The transfer line temperature was set to 280 °C. Mass spectra were recorded at 1 scan s^{-1} under electron impact at 70 eV, mass range 50–350 amu. The excitation potential for the MS/MS product ion mode applied was 0.5 V, and 0.9 V in the case of more stable ions. Acetonitrile was used as a medium for CI, where the maximum time for ionization was 2000 μs and 40 μs for reaction.

3. Results and discussion

In all degradation experiments, the intermediates and products merely appeared at trace (μg/ml) level and did not show any accumulation (except anthraquinone). The intermediates were identified by comparing the mass spectra with data in the NIST 98 library, and independently by interpreting the fragmentation pattern. Additionally, unknown structures of metabolites were explored using MS/MS (product ion scan) to clarify the fragmentation sequence. Due to mostly low concentration of the metabolites or insufficient gas chromatographic separation of the complex mixtures, the use of MS–MS and several derivatization procedures was required to enhance selectivity and to increase the probability of correct

structural assignment. Product ion scans are commonly used to study the successive m.s. decomposition of selected ions. Starting from the molecular ion structure elucidation of unknown compounds is facilitated although some time is needed for MS–MS studies of mixture components since the combination with GC requires for every single product ion scan experiment a new GC run. Mostly, the resulting product ion spectra contain only few fragmentation and the full structural information is not opened up until the last precursor ion was investigated. In order to simplify the interpretation of mass spectra and to concentrate the results we have shown only full scan spectra including fragmentation schemes related from MS–MS experiments. Subsequent derivatization resulted in additional information, e.g. on functional groups. The so obtained information could be combined to some novel metabolic structures that supplement the degradation mechanisms of the selected PAHs. Despite all efforts not all theoretically expected intermediates and metabolites could be detected, probably caused by too low concentration and incomplete derivatization reactions.

After all, the moiety of the suggested structures was later confirmed by authentic standards.

3.1. Degradation of anthracene

As recently described for the degradation of anthracene by other white rot fungi [8], one of the major metabolites is anthraquinone. In our experiments using *I. lacteus*, anthraquinone proved to be the main and most stable intermediate. It was accumulated until day 30 (186 μg in flask), after which the concentration decreased again (98 μg in flask at day 50). Certain toxic properties of anthraquinone [19] make its final removal highly significant. Some of the metabolites were only found in the last sampling period, probably being caused by depleting nutrients and the suppressed metabolic activity of the fungi.

Fig. 1 also shows the recovery of degraded PAHs including ANT from samples with living fungus and from abiotic controls. The figure indicates that less than 5% of the original amounts of ANT remained after 30 days. The analysis of corresponding controls showed about 85% recovery of ANT, which emphasizes the secondary role of analyte loss by evaporation and adsorption/absorption by biomass or active surfaces inside the experimental setup.

Table 2 lists retention data and mass spectral

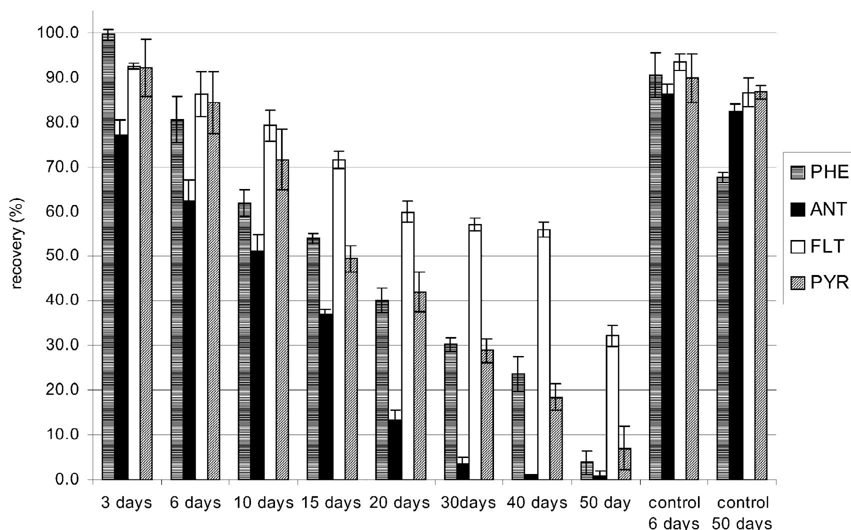

Fig. 1. Degradation of PAHs by *I. lacteus*. The controls are abiotic with heat-killed mycelium of the fungus.

Table 2
Retention data and electron impact mass spectral characteristics of ANT metabolites

Product no.	t_R (min)	MW according to CI	m/z of fragment ions (relative intensity)	Structural suggestion
1	6.37	166	148 (2.3), 104 (100), 76 (41.2), 50 (20.4)	phthalic anhydride[a]
1	9.92	310	310 (54.8), 295 (48.3), 265 (6.7), 220 (20.1), 193 (12.2), 149 (100), 147 (98,6), 73 (17,6)	phthalic acid di-TMS
2	6.63	134	134 (12.7), 105 (100),77 (40.9), 51 (9.0)	phthalide[a,b]
3	7.5	194	194 (3.0), 163 (100),133 (15.8), 77 (9.7)	dimethyl phthalic acid[a]
4	12.57	194	194 (100), 165 (98.4), 139 (49.6), 81 (37.1)	anthrone[a]
5	12.75	208	208 (100), 180 (64.2),152 (58.8), 126 (4.4), 76 (5.9)	9,10-anthracenedione[a]
6	12.95	224	224 (100), 208 (26.8), 196 (66.1), 168 (71.9), 139 (38.3)	?-hydroxy-9,10-anthracenedione
7	13.37	224	224 (100), 196 (80.1), 168 (63.1), 139 (79.9)	?-hydroxy-9,10-anthracenedione
8	14.13	210	210 (100), 193 (50.2), 180 (72.8), 165 (73.1), 152 (60.1)	?-hydroxyanthrone
9	14.61	242	242 (14.3), 224 (100), 196 (41), 168 (32.8), 139 (14.1)	2-(2′-hydroxybenzoyl)-benzoic acid

[a] Structures were later identified with authentic standard.
[b] Dehydrated form of the metabolite.

characteristics of major metabolites found. The structural assignment of ANT degradation products was supported by additional experiments using ^{13}C-labeled substrate. The time-dependent presence of metabolites in the culture solution provides some idea of the probable succession of degradation reactions. Fig. 2 shows the related pathway proposed for the ANT degradation by *I. lacteus*, where enzymatic system probably responsible for degradation is also mentioned. The results of MS/MS investigations of compounds 1 and 2 indicate phthalic acid and 2-hydroxymethyl-benzoic acid as structures which typically underwent dehydratation during injection into GC–MS. In order to confirm the surmised structures, the extracts were derivatized. Using trimethylsilylation (TMS) the phthalic acid was characterized as di-TMS-derivative, whose fragmentation is included in Table 2. Finally, the structures were confirmed by references (Table 1).

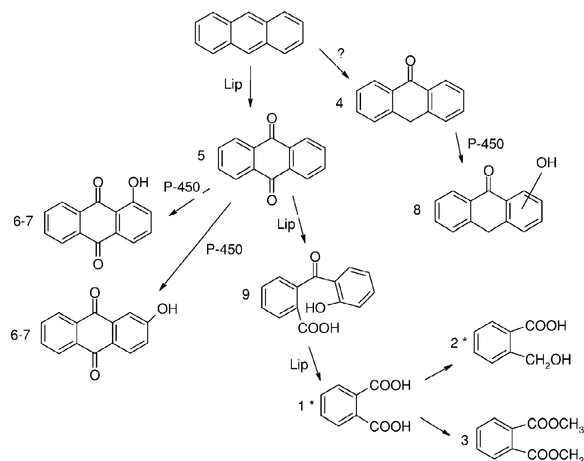

Fig. 2. Pathway proposed for ANT degradation by *I. lacteus*. The structures labeled with asterisks were detected as dehydrated forms caused by hot GC injection.

Despite the slower GC temperature program, pyrocatechol was not detected as a complementary intermediate to phthalic acid. However, this particular compound is known to be a very good substrate for LiP and can be immediately transformed.

Fig. 3 represents a spectrum of metabolite 9 which is proposed as 2-(2-hydroxy-benzoyl)-benzoic acid. The successive fragmentation of the molecule was explained by product ion scans starting with the molecular ion of 242 amu. The fragmentation pathways of MS–MS-generated product ions are indicated by arrows in the figure. The loss of water from the molecular ion ($M-H_2O=m/z$ $224]^{+\cdot}$) indicates the presence of two closely adjacent hydroxyl or carboxyl groups. The resulting ion at m/z 224 predominates the full scan spectrum (Fig. 3) as the base peak. Furthermore, it is the main ion formed in the product ion scan of the molecular ion at 242 amu. The high abundance of this fragment ion and MS–MS experiment implies a stable structure. The further stepwise loss of a carbonyl group and COH

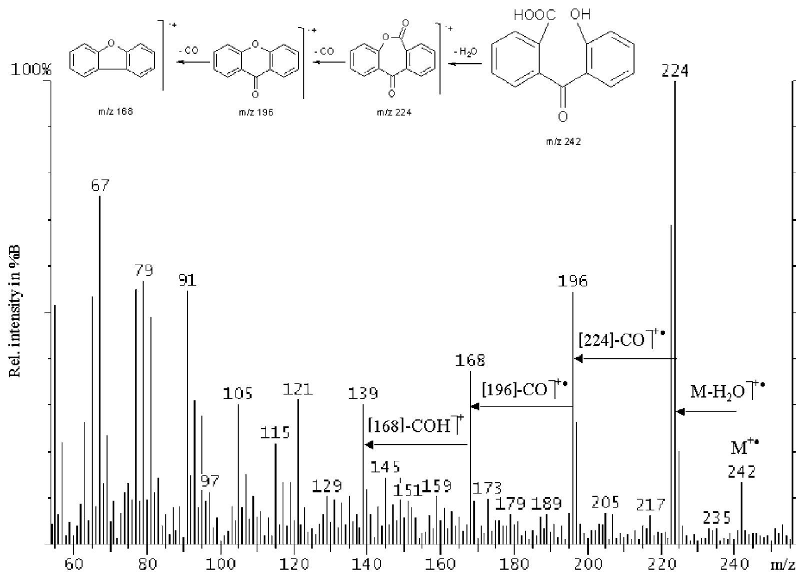

Fig. 3. Full scan electron impact mass spectrum of metabolite 9. The fragmentations marked amid the spectrum by arrows describe the m.s. decompositions found in corresponding product ion scans successively performed.

tally with mass spectrometric fragmentation typical of aromatic carbonyl as well as phenol and benzofurane derived compounds. The product ion scan of fragment ion m/z 168 corresponds exactly to the fragmentation pattern of dibenzofurane, with m/z 139—one of the most abundant ions (relative intensity 29%)—being produced by COH loss. The ion m/z 139 is quite stable, and not even increasing the collision activation potential stimulated extensive decomposition.

In summary, the degradation pathway of ANT coincides with previously reported results using ligninolytic fungi [11]. The newly found metabolite 9 fits the transformation pathway of ANT via anthraquinone generating phthalic acid. Reduction of phthalic acid to 2-hydroxymethyl-benzoic acid corresponds with proposed pathway for phenanthrene degradation by *P. chrysosporium* [12]. Since the degraded compound was anthracene the resulting compound was phthalic acid (not diphenic acid), though similar type of reduction was involved in the process. Some more intermediate structures (nos. 6, 7, 8) detected indicate that cytochrom P-450 may also play a role in ANT degradation. Product 3 is also initially described in the degradation of ANT, although its presence is not surprising due to the known methylation abilities of this group of fungi. The production of anthrone (no. 4) seems to use another but not uncommon pathway of ANT transformation. Probably, the primarily formed 9-hydroxy-anthracene isomerizes under thermal stress fast to anthrone which is a well known process. Therefore, the first stage of hydroxylation of ANT is not subsumable as stable intermediate [22].

Generally, it has to be pointed out that the anhydride and lactone structures shown in Fig. 2 were found by GC–MS–MS and derived unerringly from the corresponding 1,2 dicarboxylated as well as ortho-substituted hydroxymethyl benzoic acid.

3.2. Degradation of phenanthrene

I. lacteus is also able to degrade phenanthrene significantly. Despite abiotic control indicating 70% recovery at the end of the experiment (Fig. 1), the reduction of phenanthrene was significant (4%) and in the same range as anthracene degradation. Table 3 presents mass spectral characteristic and related structures of the intermediates found. As for ANT, metabolic structures were supported by parallel experiments using ^{13}C-labeled PHE.

A scheme of the degradation pathway of PHE is

Table 3
Retention data and electron impact mass spectral characteristics of PHE metabolites

Product no.	t_R (min)	MW according to CI	m/z of fragment ions (relative intensity)	Structural suggestion
10	11.45	198	198 (80.1), 154 (87.9), 126 (100)	2,3-naphthalic anhydride[b]
11	12.6	196	196 (100), 168 (61.5), 139 (42.6)	3,4-benzocoumarine[b]
12	12.63	208	208 (77.9), 193 (40.3), 165 (100)	9-methoxyphenanthrene[a]
13	13.88	212	212 (100), 194 (33), 181 (56.2), 165 (67.8), 153 (14.1), 152 (13.4), 77 (21.1)	phenanthrene-9,10-dihydrodiol[a]
13	9,88	404	404 (27.3), 355 (4.6), 290 (100), 245 (5.5), 193 (34.1), 177 (20.1), 165 (48), 152 (6.7)	phenanthrene-9,10-dihydrodiol di-TFA ester

[a] Structures were later identified with authentic standard.
[b] Dehydrated form of the metabolite.

proposed in Fig. 4. The assignment of 9-methoxyphenanthrene appeared unusual because further experiments to locate traces of the supposed precursor 9-phenanthrol failed, but 9-methoxyphenanthrene was confirmed with a standard. The product of degradation of PHE via cytochrom P-450 9-phenanthrol is usually generated together with phenanthrene-9,10-dihydrodiol. The latter was detected and the structure was also confirmed by derivatization with MBTFA.

The intermediate number 11 is originally 2′-hydroxy-2-carboxy biphenyl and 3,4-benzocoumarine appears in dehydrated form after GC injection. Unfortunately, none of the derivatization reagents used could supply an additional confirmation of the originally open structure.

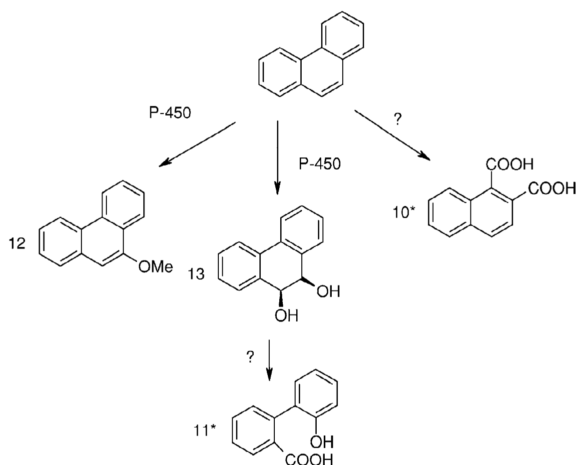

Fig. 4. Pathway proposed for the PHE degradation by *I. lacteus*. The structures labeled with asterisks were detected as dehydrated forms.

The mass spectrum of compound 10 exhibits neutral losses of 44 and 28 amu, respectively. Applying MS–MS enabled the fragmentation sequence of the molecule to be elucidated and assisted the structural proposal shown in Fig. 5. The fragment m/z 126 is stable to collision activation, probably indicating a naphthalene residue. The proposed structure of naphthalene-1,2-dicarboxylic anhydride is almost certainly derived from the dicarboxylic acid and indicates that PHE is not only attacked at the K-region as usual but also at the side ring, where the structure was probably opened. This observation needs to be supported by further experiments because the resulting naphthalene residues differ totally from the biphenyl structures remaining after K-region attack, leading to differences in toxicity assessment.

3.3. Degradation of fluoranthene and pyrene

There is only a limited number of publications describing the degradation of pyrene and fluoranthene by ligninolytic fungi. The fungus *I. lacteus* was able to reduce the concentration of FLT to 32.2% and PYR to 7.1%. The degradation results are presented in Fig. 1 and the metabolites detected are characterized in Table 4.

Two major products were found in FLT degradation. Structure 14, 1,8-naphthalic anhydride compound was classified as the dehydrated form of dicarboxylic acid. The structure confirmed with

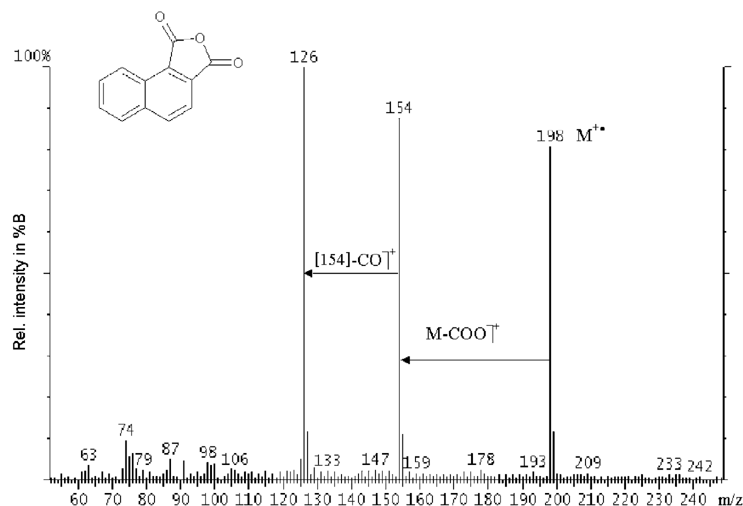

Fig. 5. Full scan electron impact mass spectrum of metabolite 10 (detected dehydrated form). The marked decompositions are derived from product ion scan experiments.

authentic standard points to the complete decomposition of one of the aromatic rings.

The full scan mass spectrum of structure 15 is shown in Fig. 6. Product ion scans of the molecular ion indicated the loss of a methyl group as well as of the methylated carboxylic group followed by splitting off the carbonyl group. The last stable ion in the spectrum is m/z 152, which describes the remaining acenaphthylene structure. The combination of mass spectrometric information denotes a structure such as 2-formyl-acenaphthene-1-carboxylic acid methylester.

In PYR degradation, only one product was attributed to coupled mechanisms occurring ligninolytic and via complex of cytochrom P-450 monooxygenase. The first generates 1,6- and 1,8-quinones while the second chiefly forms *trans*-4,5-pyrendihydrodiol. Unfortunately, such initial oxidation products were not detected. The structure thought to be lactone of 4-hydroxy-5-phenanthrencarboxylic acid represents the dehydrated form of the original intermediate (Fig. 7). Characteristic fragments at m/z 220, 192 and 163 indicate losses of neutral CO and radical COH. Corresponding MS–MS experiments exhibited a highly stable ion of 163 amu. Although the structural suggestion is not completely unambiguous, an attack of PYR at positions 4 and 5 is quite logical.

Table 4
Retention data and electron impact mass spectral characteristics of FLT and PYR metabolites

Product no.	t (min)	MW according to CI	m/z of fragment ions (relative intensity)	Structural suggestion	Parent compound
14	13.38	198	198 (69.6), 154 (100), 126 (70.6), 74 (9.6)	1,8-naphthalic anhydride[a,b]	FLT
15	14.53	240	240 (80.9), 225 (15.8), 208 (78.9), 180 (100), 152 (69.2)	2-formyl-acenaphthen-1-carboxylic acid methylester	FLT
16	15.72	220	220 (100), 192 (30.2), 163 (42.7)	lacton of 4-hydroxy-5-phenanthrenecarboxylic acid[b]	PYR

[a] Structures were later identified with authentic standard.
[b] Dehydrated form of the metabolite.

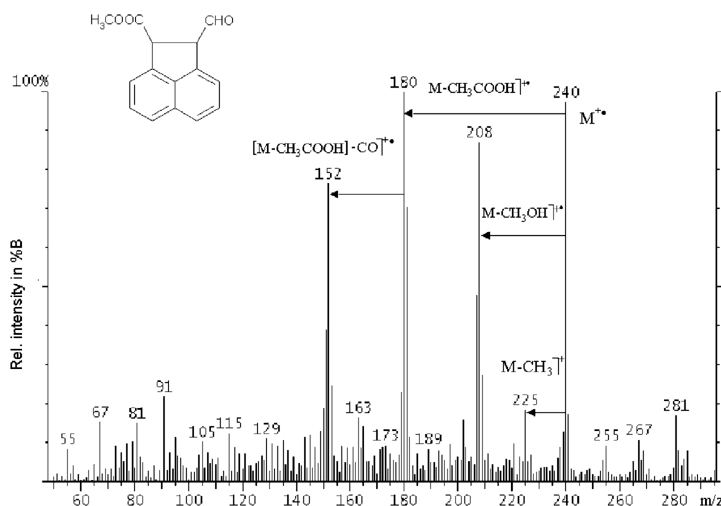

Fig. 6. Full scan electron impact mass spectrum of metabolite 15 including information obtained from appropriate product ion scans.

4. Conclusions

GC–MS and especially GC–MS–MS techniques coupled with derivatization and chemical ionization provide a valuable tool for the metabolic study of PAH degradation. Some new metabolic structures were established, completing degradation pathways and giving an understanding of the degradation mechanisms used by *Irpex lacteus*. New intermediates were detected that were formed by the opening of aromatic rings of PAHs and in some cases the partial removal of aromatic rings. Completing the pathways of FLT and PYR in particular is a matter for further study.

The correct assignment of structures is especially important for explaining and assessing the toxicity of metabolite mixtures produced during complex degradation processes. Although the entire spectrum of possible metabolites could probably not be identified, it was confirmed that none of the intermediates were

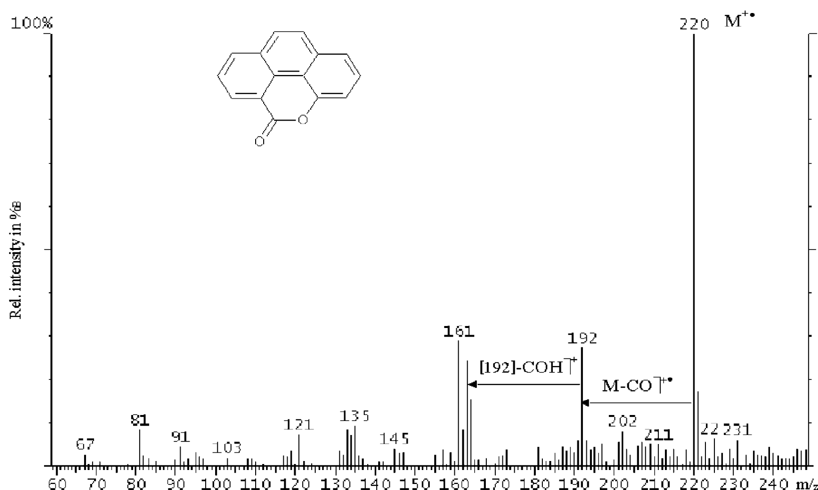

Fig. 7. Full scan electron impact mass spectrum of metabolite 16 (detected dehydrated form) and related fragmentation detected by product ion scan experiments.

accumulated during degradation. Naturally, the polarity and water solubility of products increased, which might create a potential risk for groundwater quality.

Acknowledgements

This work was kindly supported by the Saxony State Ministry of Science and Art, Grant No. 526/99/0519 of the Grant Agency of the Czech Republic, and also Institutional Research Concept No. AVOZ020903. Furthermore, the authors are grateful to Dr. P. Erbanová of the Institute of Microbiology, Prague, for her technical assistance in preparing fungal cultures.

References

[1] C.E. Cerniglia, Adv. Appl. Microbiol. 30 (1984) 31.
[2] C.E. Cerniglia, M.A. Heitkamp, in: U. Varanasi (Ed.), Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment, CRC Press, Boca Raton, FL, 1989, p. 41.
[3] J.A. Field, E. de Jong, F.C. Gumersino, J.A.M. de Bont, Appl. Environ. Microbiol. 58 (1992) 2219.
[4] T. Cajthaml, V. Pacáková, V. Šašek, Chem. Listy 95 (2001) 404.
[5] C. Namhyun, I.-S. Lee, H.-S. Song, W.-G. Bang, J. Microbiol. Biotechnol. 10 (2001) 737.
[6] M. Hofrichter, K. Schneibner, I. Schneegaß, W. Fritzche, Appl. Environ. Microbiol. 64 (1998) 399.
[7] B.R.M. Vyas, S. Bakowski, V. Šašek, M. Matucha, FEMS Microbiol. Ecol. 14 (1994) 65.
[8] L. Bezalel, Y. Hadar, C.E. Cerniglia, Appl. Environ. Microbiol. 63 (1997) 2495.
[9] M. Kästner, in: H.-J. Rehm, G. Reed, A. Pühler, P. Stadler (Eds.), Biotechnology, Vol. 11b, Wiley-VCH, Weinheim, 2000, p. 212, Ch. 9.
[10] M. Wolter, F. Zadrazil, R. Martens, M. Bahadir, Appl. Microbiol. Biotechnol. 48 (1997) 398.
[11] K.E. Hammel, B. Green, W.Z. Gai, Protl. Natl. Acad. Sci. USA 88 (1991) 10605.
[12] K.E. Hammel, W.Z. Gai, B. Green, M. Moen, Appl. Environ. Microbiol. 58 (1992) 1832.
[13] A. Majcherczyk, C. Johannes, A. Hüttermann, Enzyme Microb. Technol. 22 (1998) 335.
[14] L. Bezalel, Y. Hadar, P.P. Fu, J.P. Freeman, C.E. Cerniglia, Appl. Environ. Microbiol. 62 (1996) 2554.
[15] J.B. Sutherland, A.L. Selby, J.P. Freeman, F.E. Evans, C.E. Cerniglia, Appl. Environ. Microbiol. 57 (1991) 3310.
[16] Č. Novotný, P. Erbanová, T. Cajthaml, N. Rothschild, C. Dosoretz, V. Šašek, Appl. Microbiol. Biotechnol. 54 (2000) 850.
[17] S.-S. Hwang, H.-G. Song, J. Microbiol. Biotechnol. 10 (2000) 344.
[18] Fluka, Silylating Agents, ISBN 3-905617-0702, 1995.
[19] R.J. Lewis, Sr., R.L. Tatken (Eds.), Registry of Toxic Effects of Chemical Substances. On-line Ed. National Institute for Occupational Safety and Health. Cincinnati, OH. CB4725000, 1989.
[20] Org. Synthesis 15/4 (1935) in: Org. Synthesis, Coll. Vol. 2, Revised ed. Ann. Vols. X–XIX, A.H. Blatt (Ed.), J. Wiley, N.Y. London Sydney, 1943, p. 165.
[21] K.L. Platt, F. Oesch, Synthesis 3 (1982) 459.
[22] S. Hauptmann, J. Graefe, H. Remane, Lehrbuch der Organischen Chemie, in: VEB Deutscher Verlag für Grundstoffindustrie, Leipzig, 1976, p. 312.