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Metabolism of Polycyclic Aromatic Hydrocarbons by the Wood-Feeding Termite *Coptotermes formosanus* (Shiraki)

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Supporting Information

ABSTRACT: Polycyclic aromatic hydrocarbons (PAHs) are among the most prevalent and persistent pollutants in the environment. In this study, the wood-feeding termite (WFT) *Coptotermes formosanus* (Shiraki) was studied regarding the potential ability to degrade two selected low-molecular-weight PAHs, phenanthrene and anthracene. Pyrolysis−gas chromatography/mass spectrometry was employed for analysis of in vivo PAH degradation by three gut segments (fore-, mid-, and hindgut) of the WFT. The results revealed the capability of lower termite for PAH metabolism, which started from the foregut and mainly occurred in the midgut region. Remediation of phenanthrene by the termite has been proposed to be initiated via hydroxylation at the C-10 position. Anthracene metabolism first occurred at the C-3, C-5, and C-12 positions with the addition of aldehyde and carbonyl groups. Ring hydroxylation, methoxylation, esterification, carboxylation, and methylation were detected on both the PAHs for ring fission, suggesting the existence of effective PAH modification activity in the alimentary canal of *C. formosanus*. This new PAH degradation system of the WFT provides new insights for potential technologies for bioremediation of PAH-contaminated soil and sediment based on the related lingolytic enzymes.

KEYWORDS: *wood-feeding termite (WFT), phenanthrene, anthracene, pyrolysis−gas chromatography/mass spectrometry (Py−GC/MS), metabolism*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are highly toxic organic contaminants ubiquitously distributed in terrestrial and aquatic ecosystems as products of the incomplete combustion of solid and liquid fuels or derived from industrial activities.[1,2] They are of notable concern because of their mutagenicity and/or carcinogenicity[3] and related public health hazard.[1] PAHs are fused-ring aromatic compounds with high environmental persistence, and they are strongly resistant to nucleophilic attack.[1,4] Their chemical structures are a restrictive factor for natural biological degradation.[5]

The lower molecular weight unsubstituted PAH compounds, containing two to three rings, such as naphthalenes, phenanthrenes, and anthracenes, have significant acute toxicity to some organisms compared to the higher molecular weight four- to seven-ring aromatics, which do not.[6,7] In aquatic systems, these two- or three-ring PAHs tend toward increased toxicity with increased molecular weight.[6] Naphthalene (**N**) is a fused-ring bicyclic aromatic hydrocarbon and thus serves as an example for understanding the properties of a large class of environmentally prevalent PAHs. It is commonly found in crude oil or oil products and is proved to be a strong human carcinogen.[8] Phenanthrene (**P**) with three fused rings is commonly found as a pollutant in soils, estuarine waters and sediments, and other terrestrial and aquatic sites. **P** has been shown to be toxic to marine diatoms, gastropods, mussels, crustaceans, and fish by inducing cancers and also by affecting the nervous system.[9,10] This compound is so persistent that it requires a long period of time (2 years) for its degradation to nondetectable levels in soil litter after burning.[11] The biodegradation pathway of this consistent compound has been extensively studied in bacterial and fungal systems.[12−16] Anthracene (**A**) is an isomer of **P**, and its targets of impact are the skin, hematopoietic systems, lymphoid systems, and gastrointestinal tracts.[2] It is considered to be persistent, bioaccumulative, and toxic to freshwater and marine ecosystems.[17,18] Compound **A** is less stable than **P**, and some microorganisms have been reported to be able to degrade it.[19−21] Because of their higher polarity compared to most of the other PAHs with more rings, **N**, **P**, and **A** exhibit a higher solubility in water and lower adsorption coefficient.[22,23]

Recently, biological techniques based on microbial transformations or degradations of PAHs have attracted much attention in the treatment of soil, sediments, sludge, and wastewater. For these applications, numerous organisms have been used to achieve complete degradation of aromatic compounds, but without much success.[24,25] Since bacterial and fungal degradation of PAHs occurs in secondary metabolic pathways, appropriate growth conditions have to be accomplished by induction of the pathways by growth on similar chemicals. Meanwhile, the expression of the enzymes involved in phenol, aromatic amine, and dye degradation is not constant with time, but is dependent on the growth phase of the organisms and is influenced by inhibitors that may be present in the effluent.[26] In an attempt to overcome some of the problems associated with traditional chemical and biological waste-treatment systems, recent research has focused on the environmental application of certain enzymes. Due to a high specificity for individual species or classes of compounds,

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enzymatic processes can be developed to specifically target selected compounds that are detrimental to the environment.

Removal of PAHs has been demonstrated by various means, including chemical oxidation, photooxidation, volatilization, and bioremediation.[27] Bioremediation seems to be the most promising method by utilizing the metabolic versatility of microorganisms to degrade hazardous pollutants into metabolites or to totally mineralize them.[28] Enzymes involved in PAH degradation are lignolytic enzymes, oxygenases, and dehydrogenases. Due to the heterogeneity of lignin, the lignolytic enzymes are nonspecific, nonstereoselective, and very effective against a broad spectrum of aromatic compounds,[29,30] making them suitable for degradation of different PAHs.[3] Among the enzymes from white rot fungi, the lignolytic enzymes were proved to play a pivotal role in PAH degradation.[30−32] One-electron oxidations of PAHs are catalyzed by laccase (Lac), manganese peroxidase (MnP), lignin peroxidase (LiP), and H_2O_2-generating enzymes.[33−35]

Besides the wood-rot fungi, lignolytic enzymes are also demonstrated to exist in the termite gut and to be responsible for lignin modification prior to efficient cellulose utilization.[36−38] Vu et al. revealed ferrous iron reduction in the termite gut, which provided indirect evidence for lignin oxidation by the termite.[39] Meanwhile, Brune et al.[40,41] verified a gradient oxygen profile in termite guts to be a critical cosubstrate for aromatics degradation. By collecting the respired $^{14}CO_2$ after feeding termites natural and synthetic ^{14}C-labeled lignins and analogues, Butler and Buckerfield[42] and Cookson[43] demonstrated the disruption of synthetic and native lignin by the wood-feeding termite (WFT). There are also direct observations of structural modifications on aromatic compounds in termite guts from our previous study.[44] Furthermore, characterization of the native lignin framework after WFT digestion provided considerable insights into the selective lignin modification by the WFT.[37,38,45,46] Despite the fact that the lignolytic genes currently are only reported to exist in symbiont-free tissue of WFTs, a consortium of microorganisms are still thought to be involved in lignin modifications.[36,47−49] Some microbial studies showed lignolytic activities in the gut of xylophagous termites by isolating monophenyl, biphenyl, or straw lignin degrading bacteria.[48−51] On the basis of the ability of the WFT to efficiently modify lignin and degrade aromatic compounds,[38,44] we propose that the lower termite will be able to digest PAHs with the aid of its unique lignolytic enzyme system. In this study, the metabolism of two- and three-ring PAHs along the alimentary canal of *Coptotermes formosanus* has been explored with the aid of pyrolysis−gas chromatography/mass spectrometry (Py−GC/MS).

■ MATERIALS AND METHODS

Sample Collection and Preparation. Colonies of *C. formosanus* termites, collected in Poplarville, MS, were kept in the laboratory in 5.6 L covered plastic boxes containing blocks of Southern pine wood and moist sand in 90% humidity at 28 ± 1 °C until they were used in the experiments. Before PAH feedings, the termite workers were first fed Whatman filter paper for 3 days in complete darkness for gut content evacuation. To make the PAH distribution on the filter paper uniform and reproducible, 1 g samples of naphthalene, phenanthrene, and anthracene were suspended in 10 mL of deionized (DI) water and stirred at room temperature. Ten pieces of 1 cm × 2 cm filter paper were discarded into the suspensions of the three separately dissolved compounds to absorb each of them uniformly. Later, all the pieces of filter paper containing PAH compounds were air-dried for 1 h and fed to the termites for 7 days. The gut segments of the termite workers feeding on filter paper for 7 days served as negative controls, while the PAH compound which was set in darkness at 28 °C in 90% humidity for 7 days was employed as a positive control for each experiment. Prior to gut segment collection, the termite body was externally washed with ethanol and later with Ringer's solution three times to prevent contaminants. Gut samples were collected by removal of the body tissue with fine forceps to isolate three whole guts, which were then separated into three parts (foregut, midgut, and hindgut) under a dissection microscope in a sterile condition. On the basis of the larger hindgut volume and requirement of small sample size by Py−GC/MS, three foreguts, three midguts, and two hindguts composed the samples for each gut section. All triplicate samples were rapidly frozen in liquid nitrogen to halt further substrate digestion and then placed directly into a quartz sample tube for Py−GC/MS analysis.

Py−GC/MS Analysis of PAHs Metabolized in Each Gut Segment of the Termite. Modifications of the PAHs that occurred during passage through each gut segment were determined using Py−GC/MS at 250 °C. The small sample size and thermal stability of PAH compounds at this temperature[52] make Py−GC/MS an ideal technique for detection of PAH degradation in vivo in termites. The pyrolysis process was performed with a CDS 5000 pyrolysis autosampler attached to a Thermo Trace GC 6890N/MSD 5975B gas chromatography/mass spectrometry system (Agilent Technologies, Inc., Bellevue, WA). The samples were first pretreated at 210 °C for 3 min, then pyrolyzed at 250 °C for 1 min, and finally held in the pyrolysis zone for 56 min. The volatile products were separated on a 30 m × 0.25 μm inner diameter (5% phenyl) methylpolysiloxane nonpolar column with helium as the carrier gas (17.3 mL/min). The pyrolysis interface was kept at 210 °C, the GC/MS interface was kept at 280 °C, and the GC/MS system was programmed from 40 °C (1 min) to 280 °C (15 min) at a rate of 6 °C/min. The mass spectrometer was operated in electron ionization (EI) mode (70 eV) at a source temperature of 230 °C. The eluted pyrolysates were identified by EI mass spectrometry using the NIST MS Search 2.0 electronic libraries to provide information for PAH metabolites in the termite gut.[44,53,54] Furthermore, the pure standards for four available and critical metabolites, which will be mentioned later, α-methylbenzeneethanol (Aldrich), 1-phenanthrenol (Aldrich), 1,4,6-trimethylnaphthalene (3B Scientific Corp.), and 1,1-diphenylheptane (3B Scientific Corp.), were also run through Py−GC/MS with the same procedure. Each analysis was replicated three times using three different pieces of each sample collected at a different time.

■ RESULTS AND DISCUSSION

In Vivo Degradation of Phenanthrene by *C. formosanus*. Figure 1 shows the pyrograms of gut samples from termites fed **P**. The distributions of **P** and its metabolites are shown in Table 1. The results were compared with the negative control of filter paper fed termite guts (Supporting Information, Supplementary Figure 1, pyrograms from filter paper fed termite guts to serve as the negative control). The pyrograms obtained from each gut segment with the presence of **P** indicated the ingestion of the compound by the termites. Careful analysis of the termite guts showed the appearance of compounds **1P**, **2P**, **4P**, **6P**, **7P**, **8P**, **9P**, **10P**, **12P**, **14P**, and **16P** in the foregut sample, among which **16P** was the major metabolite (Table 1), implying the hydroxylation on the C-10 position of the substrate started in the foregut. Likewise, the methoxylation reaction was evidenced by the presence of the **14P** compound. In addition to this, metabolism was indicated by further hydroxylation (**9P**, **14P**), methylation (**1P**, **4P**), and methoxylation (**7P**, **12P**, **14P**) on the ring, as well as ring fission (**4P**, **7P**, **8P**). It has already been reported that the reaction of hydroxylation is a common detoxification property of the biological system for PAH compounds.[29,55,56] In this regard, portions of the substrates were metabolized at several

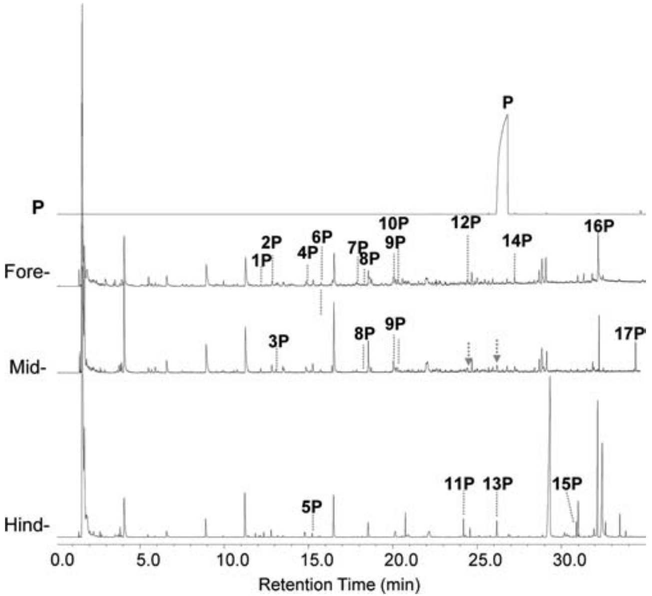


Figure 1. Phenanthrene modification in each gut segment. The unlabeled arrows indicate where the compounds appeared in the control. The pyrograms obtained from each gut segment with the presence of **P** indicated the ingestion of the compound by the termites. The appearance of 11 new compounds in the foregut pyrogram implied that modification of the substrate started in the foregut. Further metabolism of the coproducts in the midgut was indicated by the absence of six pyrolysates in the foregut pyrogram and the appearance of two new ones in the midgut pyrogram. The appearance of three pyrolyzed compounds and disappearance of four in the hindgut pyrogram implied coproduct rearrangement and further remediation in the hindgut.

different positions as they appeared not only in the foregut (**8P**, **9P**, **10P**, **12P**). Two new aromatic pyrolysates (**3P** and **17P**) were detected from the midgut region, while **17P** was the dominant one (66.7% of all the aromatic pyrolysates). This aromatic nitrogen compound is speculated to be generated from the pyrolysis reaction of aromatic compounds (with a similar structure) and amino compounds (enzyme or cellular protein) from termite tissues. However, it is still possible that the compound came from the termite digestion, which was strongly supported by the pyrograms given by the negative and positive controls. Hence, the two new structures indicated ring fission, hydroxylation, methylation, and esterification of the side ring happened in the termite midgut. Meanwhile, disappearance of **1P**, **2P**, **4P**, **6P**, **7P**, **14P**, and **16P** in this region indicated further metabolism of the coproducts or absorption/utilization by the gut tissue. It is worthwhile to mention here that no aromatic derivatives were detected from pyrolysis of the termite body without gut tissue, indicating complete aromatics degradation with no accumulation in the body tissue. With continued passage of **P** metabolites through the dilated region of the hindgut, some monomeric/dimeric coproducts (**8P**, **9P**, **10P**, **12P**, **17P**) observed in the midgut disappeared, and new ones (**5P**, **11P**, **13P**, and **15P**) appeared. Their presence not only implied coproduct rearrangement, but also provided further evidence for side ring carboxylation, carbonylation, methylation, and hydroxylation. The chromatograms of the commercial pure compounds, **3P** and **16P** (Figure 2), which were identified as metabolites of **P** in the termite gut, were in accordance with the retention time and fragmentation pattern of the related peaks appearing in Figure 1.

On the basis of the results, the degradation of phenanthrene in the termite gut is proposed to occur as follows: (i) metabolism of **P** by hydroxylation at the C-10 position beginning in the foregut (Scheme 1), (ii) transformation of the substrate into related monomeric aromatic compounds in the fore- and midgut, indicating the existence of an aromatic metabolism system in these regions of the termite gut, and (iii) structural rearrangement of residual monomeric aromatics in the hindgut. Our previous studies on the modification of native wood lignin and other aromatics in termite guts revealed that the lignin modification was initiated in the foregut and intensified in the midgut region.[38,44] Meanwhile, Tartar et al.[57] and Coy et al.[36] identified laccase, peroxidase, phenoloxidase, and cytochrome oxidase in termite guts which are active in modifying and/or degrading aromatic compounds, including lignin. Therefore, these enzymes are speculated to contribute to the rapid degradation of **P** in the fore- and midgut regions.

Several lignolytic fungi have also been reported to be able to modify or metabolize compound **P**.[3] In currently reported fungal systems, the compound was hydroxylated at the C-12 and C-13 positions, which are located on the center aromatic ring, and later the fungi manage the ortho cleavage of the compound to generate *trans*-9,10-phenanthrenedihydrodiol.[3,58] Methoxylation at the C-1 position was also reported as a novel metabolite in phenanthrene transformation.[41] It has been reported that some bacteria also can accomplish hydroxylation on the side ring of phenanthrene for meta cleavage and ultimately mineralize it.[28,59] The double hydroxylation of the bay region by a dioxygenase to form *cis*-3,4-phenanthrenedihydrodiol is then converted by the action of dihydrodiol dehydrogenase to 3,4-dihydroxyphenanthrene, which undergoes meta cleavage, and in subsequent steps the ring cleavage product is converted to 1-hydroxy-2-naphthoic acid. This acid is considered to further undergo oxidative decarboxylation to form 1,2-dihydroxynaphthalene, which is subsequently metabolized by the classical naphthalene degradation pathway via salicylic acid and catechol.[60,61] Compared to the reported microbial systems, the wood-feeding termite system proved to be quite different. Furthermore, due to the flow rate of the gut content, the digestion cycle of the termite is reported to be within 24 h;[62] therefore, it is possible that the PAH degradation by the termite is shorter than that of other microbial systems.

In Vivo Degradation of Anthracene by *C. formosanus*. Figure 3 and Table 2 showed in vivo degradation of anthracene during passage through each gut segment of *C. formosanus*. Ingestion of the target substrate (**A**) by termites was confirmed by the appearance of **A** in the fore- and midgut segments, after which it was further modified during the digestion process. Initial carbonylation on the side aromatic ring and oxidation on the center ring (**11A**) occurred in the foregut as an initial metabolism step. Further metabolism of the conjugated ring structure was detected in the fore- and midgut, which is supported by the appearance of monomeric/dimeric aromatics, together with structures formed by methylation, oxidation, methoxylation, and esterification on the ring (**3A**, **5A**, **9A**, **10A**, **12A**, **13A**, **15A**). The ring fission occurred not only on the side ring, but also on the center aromatic ring (**10A**, **13A**). Further oxidation (introduction of carbonyl functional groups) on the side ring (**9A**), methoxylation (**15A**), and hydroxylation (**12A**) on the center aromatic ring, as well as methylation on the side and center rings (**3A**) was also observed. Interestingly, the target substrate (**A**) was absent from the hindgut, which indicated the complete degradation of anthracene before the

Table 1. Pyrolysis Products from Phenanthrene before and after Termite Digestion[a]

No.	Compounds	Ion pairs	RT[#], min	% of total (Probability %)			
				Control	Foregut	Midgut	Hindgut
1P		118 119 133	12.171	–[†]	0.2±0.0* (51.7)	–	–
2P		117 90 116	12.843	–	6.9±0.0 (63.5)	–	–
3P		92 91 45	13.169	–	–	0.1±0.0 (76.1)	0.5±0.0 (78.9)
4P		119 91 192	14.858	–	0.4±0.0 (63.9)	–	–
5P		91 178 92	15.225	–	–	–	7.4±0.0 (88.5)
6P		240 225 239	15.751	–	0.3±0.0 (56.8)	–	–
7P		121 326 161	17.641	–	0.7±0.0 (63.5)	–	–
8P		167 166 168	18.167	–	0.1±0.0 (58.1)	0.1±0.0 (48.3)	–
9P		152 137 151	20.285	–	1.8± (41.4)	0.7± (53.1)	–
10P		137 42 152	20.294	–	0.2 (38.3)	0.2 (43.8)	–
11P		238 152 195	24.196	–	–	–	40.4±0.6 (68.3)
12P		41 39 79	24.690	–	17.5±0.3 (38.3)	31.9±0.3 (45.6)	–
P		178 176 179	26.173	100.0±0.0 (88.9)	0.1±0.0 (56.6)	0.3±0.0 (61.5)	–
13P		178 176 76	26.176	–	–	–	28.4±0.2 (46.3)
14P		154 125 65	27.226	–	3.0±0.0 (63.5)	–	–
15P		239 253 240	30.893	–	–	–	23.3±0.1 (44.2)
16P		194 165 195	31.933	–	69.0±0.1 (79.8)	–	–
17P		208 209 250	34.405	–	–	66.7±0.1 (59.1)	–

[a]Definition of symbols: –, compound did not appear; #, retention time; †, no appearance in the pyrograms; *, calculated proportions of pyrolyzed lignolytic compounds from their percentages in the total pyrolysates (g/g, mean of three replicate analyses). **1P** = 2,5-dimethylbenzyl 2,5-dimethylbenzoate, **2P** = α-(hydroxyimino)benzenepropanoic acid, **3P** = α-methylbenzeneethanol, **4P** = 4-(4-methylphenyl)-4-oxobutanoic acid, **5P** = α-ethylhydrocinnamic acid, **6P** = 2-ethyl-9*H*-thioxanthen-9-one, **7P** = 2,4-dimethoxy-5-[(2*E*)-3-(4-methoxyphenyl)-2-propenoyl]benzaldehyde, **8P** = 9-nitrosocarbazole, **9P** = 2,3,5-trimethyl-1,4-benzenediol, **10P** = 2-(2-hydroxyhex-1-enyl)-3-methyl-5,6-dihydropyrazine, **11P** = 2-hydroxy-5-methylanthracene-1,4-dione, **12P** = 4,4a,5,6,7,8-hexahydro-1-methoxy-2(3*H*)-napthalenone, **13P** = 9-methylene-9*H*-fluorene, **14P** = 3,5-

Table 1. continued

dimethoxyphenol acetate, **15P** = phthalic acid 3,4-dimethylphenyl 3-methylphenyl diester, **16P** = 1-phenanthrenol, and **17P** = 1-(4,4-dimethyl-4*H*-benzo[*h*]quinolin-1-yl)ethanone.

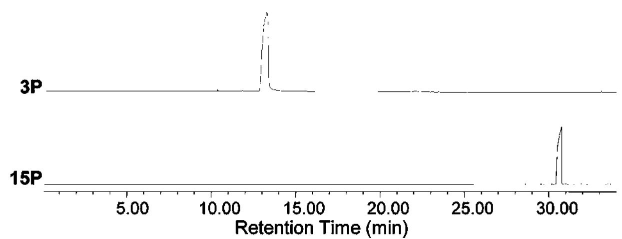
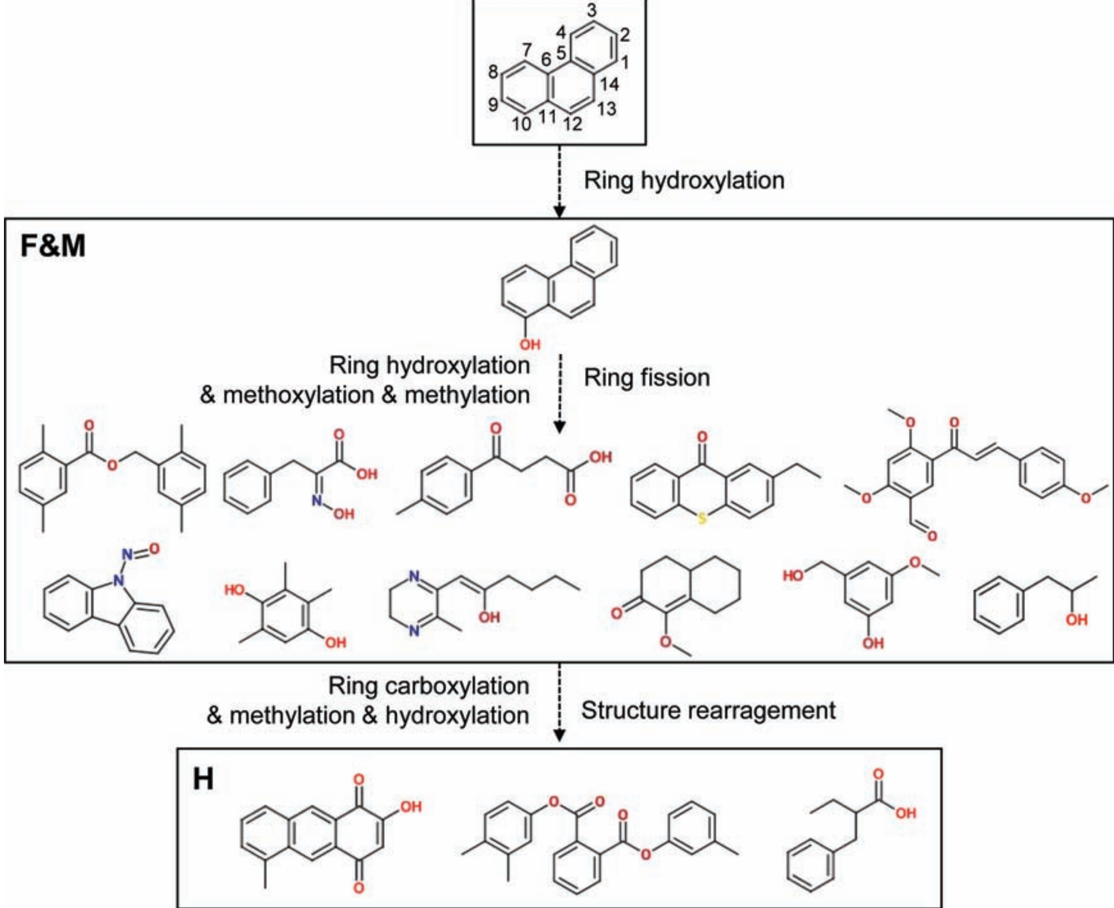


Figure 2. Standard chromatograms of pyrolysates **3P** and **15P** in the termite gut.

hindgut. The possible degradation pathway for anthracene is proposed in Scheme 2. Being different from phenanthrene metabolism, anthracene degradation is likely initiated from the C-3, C-5, and C-12 positions with the addition of aldehyde and carbonyl groups. The substrate is then speculated to be transformed to the related simple structures (**6A**, **9A**, **10A**, **12A**, **15A**) in the fore- and midgut and further metabolized and rearranged in the hindgut. The chromatograms of the commercial pure compounds, **3A** and **5A** (Figure 4), which were identified as metabolites of **A** in the termite gut, were in accordance with the retention time and fragmentation pattern of the related peaks appearing in Figure 3.

The metabolism mechanism of introducing a carbonyl group to the aromatic ring for oxidation in *C. formosanus* is similar to the initial bioremediation step performed by the bacterial strain of *Rhodococcus opacus* 412, which was able to transform anthracene in a liquid mineral medium to anthraquinone and 6,7-benzocoumarin.[12] Although the two specific metabolites were not detected in the present work, the detected ones were within the same class. van Herwijnen et al.[63] and Evans et al.[59] demonstrated hydroxylation by different bacterial strains on the side ring of anthracene for destabilization, which could also be demonstrated in the termite hindgut. *Mycobacterium* sp. strain RJGII-135 grown in the presence of benz[*a*]anthracene formed five metabolites, two of which were characterized and identified as *cis*-5,6-benz[*a*]anthracenedihydrodiol and *cis*-10,11-benz[*a*]-anthracenedihydrodiol.[64] On the basis of the degradation behavior of various PAHs, the *Mycobacterium vanbaalenii* PYR-1 strain is speculated to possess multiple monooxygenases and dioxygenases to catalyze the initial steps in various degradation

Scheme 1. Postulated Catabolic Pathway for Phenanthrene by *C. formosanus*[a]



[a]The main reaction that happened in the foregut is ring hydroxylation. Ring methylation, hydroxylation, and destruction happened in both the foregut and midgut. Further ring carboxylation and structural rearrangement happened in the hindgut.

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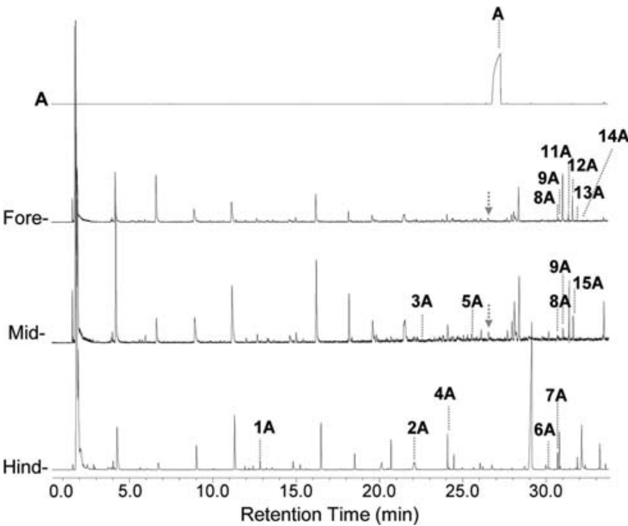


Figure 3. Anthracene modification in each gut segment. The ingestion of **A** by the termites was demonstrated by its appearance in the fore- and midgut segments. Its absent in the hindgut indicated the degradation before the hindgut. The appearance of **11A** in the foregut pyrogram showed the initial degradation step by the termites. The pyrolysates of each gut segment were totally different from each other, implying different roles each segment played in the whole remediation process.

pathways, in addition to a number of other enzymes that perform subsequent aromatic ring cleavage steps in those pathways.[65,66]

Most notably, the ability of *C. formosanus* to transform phenanthrene and anthracene suggests a partially new metabolism pathway for PAHs in the fore- and midgut which is different from that reported for PAH-utilization microbes. Continued degradations of related coproducts from phenanthrene and anthracene were also detected in the hindgut region of the termite. Although the termites used for the PAH degradation study were fed filter paper before PAH administration, the involvement of gut symbionts cannot be avoided, and those symbionts are also regarded to play a role in PAH degradation. Interestingly, the degradation mechanisms (both the reaction and reaction sequence) in *C. formosanus* proved to be different for the two isomers of phenanthrene and anthracene. The surmised explanation is the difference in the cyclic conjugation of their π-electrons, leading to more stability of phenanthrene than anthracene.[67] It is a well-known fact that, among isomers, the stability of benzenoid compounds increases with the number of Kekulé structures.[68,69] In comparison to the two three-ring PAHs, naphthalene showed a detrimental effect on the survival of the termites, which is speculated to be a result of a high concentration of volatile naphthalene affecting termite respiration or poisoning via skin penetration or production of toxic metabolites during transformation of naphthalene in this termite system. The latter supports previous reports that such a degradation pathway is specific for naphthalene and is not involved in the degradation of larger PAHs such as phenanthrene and anthracene.[70−72] Wilcke et al.[73] demonstrated that termites of the wood-feeding genus *Nasutitemes* concentrated naphthalene from the wood for nest construction; naphthalene was also reported as a defensive compound for the *C. formosanus* termite.[74] Both are in accordance with our finding that the wood-feeding termite did not tend to degrade naphthalene.

Earlier reports have indicated that WFTs prefer a diet containing lignin over pure cellulose or fungus-infected wood, suggesting the importance of lignin-modifying biofactors in termite guts.[75] The related enzymes are considered to be involved in the PAH metabolism processes. Recently, laccase transcripts and phenoloxidase activity in symbiont-free salivary gland and foregut tissue[36] have been detected in the termite species *Reticulitermes flavipes*. This genetically supports our finding of the PAH degradation capability of the termite, as well as helps explain the modification sites (fore- or midgut) of PAHs by leaching of the lignolytic enzymes from the foregut into the midgut. The microorganisms hosted in the termite guts are also considered to play a role in the degradation of aromatic related compounds, which makes it plausible to say that there is collaboration between the termite-originated ligninlytic enzymes and the related microorganisms responsible for PAH digestion.[76] Meanwhile, the transformation cycle of phenanthrene and anthracene within termites most likely occurs at high rates attributed to the high flow rate of the gut contents (within 24 h).[3,62,77,78] This is a key factor for the commercialization of the termite bioremediation system. As such, the identification and isolation of the potential PAH-degrading enzymatic system in termite guts, described herein, for effective bioremediation of the environmentally hazardous PAHs are promising.

At the same time, the use of Py−GC/MS for metabolite analysis is somewhat discussable, since there is the possibility that the observed compounds might not be metabolites from exposure but products of reactions occurring during the pyrolysis process in the inlet of the gas chromatograph. However, low-temperature Py−GC/MS is proved to a better choice for in situ structural analysis of a small amount of metabolite without pretreatment and has already been successfully applied for characterization of various compounds, including PAHs.[44,53,54] Furthermore, careful comparison of the metabolites with negative and positive controls indicated the most plausible PAH metabolism event occurring in the termite gut.

In conclusion, the results acquired in this study extended our understanding of biological degradation of PAHs. PAH degradation occurs throughout the entire gut of the lower termite *C. formosanus*, indicating a collaborative digestion behavior of termite- and symbiont-originated lignolytic enzymes. The remediation of phenanthrene in the termite gut was initiated by hydroxylation at the C-10 position. However, metabolism of anthracene is initiated by addition of aldehyde and carbonyl groups at the C-3, C-5, and C-12 positions. The metabolites were further transformed into related monomeric aromatics in the fore- and midgut, indicating the function of the lignolytic degradation system in the termite fore- and midgut. Selective methoxylation, esterification, and methylation on phenanthrene and anthracene suggested a specific remediation mechanism for three-ring PAHs by *C. formosanus*. The existing lignolytic enzyme(s) responsible for lignin unlocking during original wood digestion are proposed to contribute to PAH metabolism in the termite gut. In light of this, commercialization of the related enzyme(s) proved to have potential for dealing with the environmentally hazardous PAHs. We anticipate that further elucidation of the functional mechanisms of ligninolytic enzymes in the termite gut will contribute to our understanding of the biochemical routes of PAH remediation by the termite, as well as technically

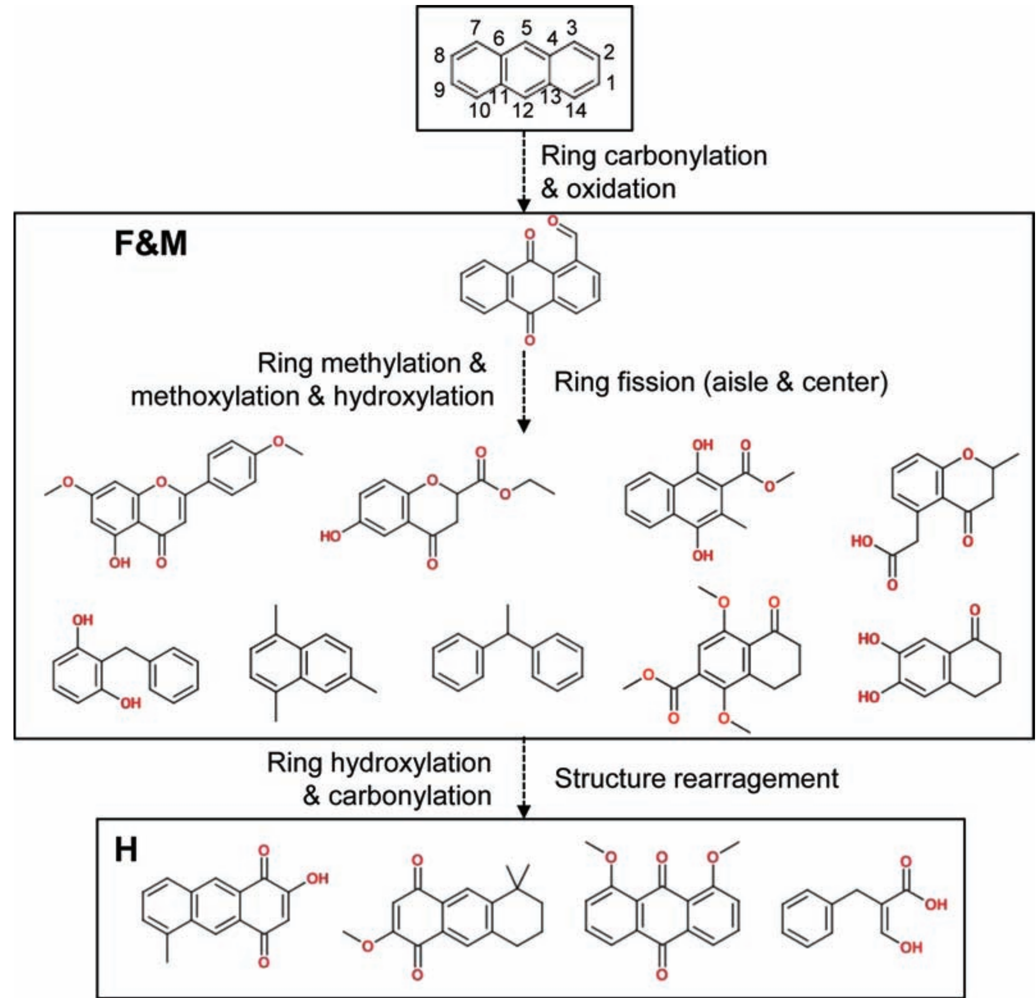
Table 2. Pyrolysis Products from Anthracene before and after Termite Digestion[a]

No.	Compounds	Ion pairs	RT#, min	% of total (Probability %)			
				Control	Foregut	Midgut	Hindgut
1A		117 90 116	12.782	–†	–	–	22.4±0.1* (68.8)
2A		210 182 211	21.795	–	–	–	0.1±0.0 (49.4)
3A		170 155 167	22.783	–	–	2.8±0.0 (48.3)	–
4A		238 210 169	24.196	–	–	–	38.1±0.2 (57.1)
5A		167 168 165	25.673	–	–	6.3±0.1 (29.4)	–
A		178 176 179	26.192	100.0±0.0 (88.3)	25.0±0.1 (59.9)	5.9±0.0 (63.5)	–
6A		270 227 255	30.169	–	–	–	1.0±0.0 (57.1)
7A		267 249 268	30.893	–	–	–	38.4±0.3 (42.2)
8A		134 174 176	30.954	–	1.4±0.0 (33.2)	3.2±0.2 (52.1)	–
9A		178 122 150	30.975	–	0.8±0.0 (38.3)	32.7±0.1 (61.8)	–
10A		234 206 105	30.984	–	1.3±0.0 (45.6)	–	–
11A		236 180 152	31.333	–	4.7±0.1 (56.1)	–	–
12A		200 115 232	31.398	–	2.7±0.0 (34.4)	–	–
13A		298 135 299	31.640	–	59.4±1.0 (44.6)	–	–
14A		199 200 122	31.709	–	4.8±0.0 (48.3)	–	–
15A		264 235 233	31.866	–	–	49.1±0.5 (39.2)	–

[a]Definition of symbols: –, compound did not appear; #, retention time; †, no appearance in the pyrograms; *, calculated proportions of pyrolyzed lignolytic compounds from their percentages in the total pyrolysates (g/g, mean of three replicate analyses). **1A** = α-(hydroxyimino)-benzenepropanoic acid, **2A** = 9-methyl-1-phenazinyl acetate, **3A** = 1,4,6-trimethylnaphthalene, **4A** = 2-hydroxy-5-methylanthracene-1,4-dione, **5A** = 1,1-diphenylheptane, **6A** = 5,6,7,8,-tetrahydro-2-methoxy-5,5-dimethyl-1,4-anthracenedione, **7A** = 1,8-diethoxyanthracene-9,10-dione, **8A** = 3,4-dihydro-2-methyl-4-oxo-2*H*-1-benzopyran-5-acetic acid, **9A** = 6,7-dihydroxy-3,4-dihydro-1(2*H*)-naphthalenone, **10A** = 6-hydroxy-4-oxo-4*H*-1-benzopyran-2-carboxylic acid ethyl ester, **11A** = 9,10-dioxo-9,10-dihydroanthracene-1-carbaldehyde, **12A** = methyl 1,4-dihydroxy-3-methyl-2-naphthoate, **13A** = 4′,7-dimethylapigenin, **14A** = 2-benzylresorcinol, and **15A** = 6-Carbomethoxy-5,8-dimethoxy-1-tetralone.

Table 2. continued

Scheme 2. Postulated Catabolic Pathway for Anthracene by *C. formosanus*[a]



[a]The main reaction that happened in the foregut is ring carbonylation and oxidation. Ring methylation, methoxylation, oxidation, and destruction happened in both the foregut and midgut. Further ring hydroxylation, oxidation, and structural rearrangement happened in the hindgut.

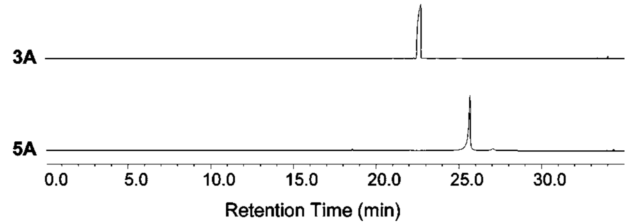


Figure 4. Standard chromatograms of pyrolysates **3A** and **5A** in the termite gut.

support remediation engineering of PAH-contaminated soils and sediment.

■ ASSOCIATED CONTENT

Supporting Information

Pyrograms of filter paper fed termite gut segments. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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