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DEGRADATION OF ORGANIC SULFUR COMPOUNDS BY A COAL-SOLUBILIZING FUNGUS

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DEGRADATION OF ORGANIC SULFUR COMPOUNDS BY A COAL-SOLUBILIZING FUNGUS

ABSTRACT

Paecilomyces sp. TLi, a coal-solubilizing fungus, was shown to degrade organic sulfur-containing coal substructure compounds. Dibenzothiophene was degraded via a sulfur-oxidizing pathway to 2,2'-biphenol. No further metabolism of the biphenol was observed. Ethyl phenyl sulfide and phenyl sulfide were degraded to the corresponding sulfones. A variety of products were formed from benzyl sulfide, presumably via free radical intermediates. Phenyl disulfide and benzyl disulfide were cleaved to the corresponding thiols and other single-ring products. It was concluded that degradation of organic sulfur compounds by Paecilomyces involves an oxidative attack localized at the sulfur atom.

INTRODUCTION

Certain microorganisms, including both bacteria and fungi, have been demonstrated to transform low-rank coals to soluble products (1). The study of fungal coal solubilization has been focused primarily on the fate of the carbon ring structure of low-rank coal. Little attention has been paid to the fate of heteroatoms such as sulfur and nitrogen, which are known to be covalently incorporated into the coal polymer, during coal biosolubilization by these organisms.

Organic sulfur within coal is known to exist in a variety of forms, including thiophenes, sulfides, disulfides, and thiols (2). Various microorganisms (primarily bacteria) have been examined for their potential utility in organic sulfur removal from high-rank coals (2-4). (Since low-rank coals have a very low fuel value, efforts have focused on the treatment of high-rank -- generally bituminous -- coals exclusively.) Preliminary screens for biodesulfurization activity have focused on degradation of dibenzothiophene (DBT), a thiophenic coal substructure compound. DBT may be degraded via a localized, oxidative attack on the sulfur atom, with release of free sulfate; or via an undesirable ring-destructive pathway that does not achieve sulfur removal (Figure 1). Both sulfur-oxidizing (3-5) and ring-destructive (6-9) organisms have been described. These pathways are not, however, mutually exclusive: both have been shown to occur in a Pseudomonas sp. (10). There

is, however, little information on the fate of other forms of organic sulfur in cultures of organisms active against DBT. Thianthrene (Figure 2), a heterocyclic sulfide, was shown to be attacked by a purified fungal enzyme from Phanerochaete chrysosporium (11). Activity against thianthrene in vitro was localized at the sulfur atom with formation of the corresponding oxide. The fate of thianthrene in cultures was not determined. Intriguingly, P. chrysosporium was shown elsewhere to solubilize low-rank coal (12). The desulfurizing potential of other coal-solubilizing fungi has not been determined.

The experiments described here assessed the ability of a coal-solubilizing fungus to effect organic sulfur removal in vivo from compounds representative of coal substructures. The organism used in this work was Paecilomyces sp. TLi. The biosolubilizing activity of this organism has been well documented (13-15). Organic sulfur-containing test compounds used in this work included DBT, aryl sulfides, aryl disulfides, and thiophenol (Figure 2). These compounds apparently undergo an oxidative attack at the sulfur atom in the absence of ring cleavage to form relatively stable products.

METHODS

Organism and culture conditions

Paecilomyces sp. TLi, isolated from a Texas lignite coal in this laboratory (13) was used in this work. Stock cultures were maintained on potato dextrose agar slants. Experimental cultures (50-ml volume) were grown in submerged culture from conidial inocula as described previously (14). Initial spore density was 10^6/mL.

Spore inocula and growth media for experimental cultures were prepared in distilled deionized H_2O as delivered by a Milli-Q Water Purification System (Millipore Laboratories, Bedford, MA). Experimental cultures were grown in defined inorganic Czapek's medium (14) containing 0.1-5% maltose and 0.01% Tween 80. This medium typically contains 2 mM sulfate. In the present work, sulfate was deliberately omitted from the medium, i.e. sulfate-containing medium components were replaced with their nitrate salts. Nonetheless, at least 9 μM sulfur (measured as sulfate) was present as a medium contaminant. Organic sulfur compounds were dissolved in dimethylformamide (DMF), filter-sterilized, and added to the culture medium at the time of inoculation. The compounds formed a stable dispersion within the aqueous medium due to the presence of Tween 80. The test compounds were added to provide a final concentration of 2 mM sulfur in cultures. The final DMF concentration in cultures was 5%. DMF was included in control cultures to which no test compound was added. Uninoculated controls contained the test compound without spores. Cultures (controls and experimentals) were incubated at 30 °C with agitation for up to 30 d.

At the end of the incubation period, entire cultures (pooled duplicates) were extracted with an equal volume of methylene chloride. The aqueous fraction was acidified to pH 2 and reextracted with an equal volume of 2-butanol. The methylene chloride extraction was designed to isolate fairly nonpolar aromatic compounds. More polar compounds (including the bulk of normal cell metabolites) were extracted into 2-butanol. The acidification step prior to 2-butanol extraction was designed to protonate acidic products, thus enhancing their solubility in the latter solvent. The extracts were analyzed separately by gas and/or thin layer chromatography.

Gas chromatography (GC)

Capillary GC was carried out on a Hewlett-Packard Model 5890 instrument equipped with a DB-Wax column (J & W Scientific Co., Folsom, CA) and a split injector. Column dimensions were 60 m x 0.32 mm i.d. (0.5 μm film thickness). The carrier gas was He, supplied at 38 psi. Injector temperature was 240 °C. Column oven temperature ranged from 100 °C to 250 °C (5 °C/min). Products were detected by H_2 flame ionization at 320° C (20 psi). Chromatograms of experimental samples (culture extracts) were compared to those of authentic commerical standards. In some cases, product identification was confirmed by mass spectroscopy (MS) as described below.

GC/MS analyses were performed on a Hewlett-Packard 5985A instrument equipped with a DB-5 column (J & W Scientific Co.) and splitless injector. Column dimensions were 30 m x 0.32 mm i.d. (1.0 μm film thickness). The carrier gas was He, supplied at 16 psi. Injector temperature was 280 °C. Column oven temperature ranged from 100 °C to 280 °C. The GC was interfaced directly to the ion source; the GC/MS transfer line was maintained at 280 °C. MS analysis was performed at 70 eV and a source temperature of 200 °C.

Thin layer chromatography (TLC)

TLC was carried out Type 13181 silica gel plates (Eastman Kodak Co., Rochester, NY) containing a fluorescent indicator. Developing solvents were 1-heptane-acetone, (4:1) and (1:1). Phenolic products, which were colored, were detected under visible light. Other products were detected by viewing under short wavelength UV light. Products were identified by comparison of chromatograms of culture extracts to those of authentic commercial standards.

Reagents

The following compounds were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI); figures in parentheses indicate their purity: benzenesulfonic acid (90%), benzyl disulfide (98%), benzyl sulfide (98%), 2,2′-biphenol (99%), biphenyl (99%), DBT

(98%), DBT sulfone (97%), ethyl phenyl sulfide (97%), phenyl disulfide (99%), phenyl sulfide (98%), phenyl sulfone (97%), thianthrene (97%), thiophenol (99+%). All other chemicals were of reagent grade.

RESULTS

Paecilomyces grew quite well on trace amounts of sulfur: approximately one-fourth as much biomass was produced in the unsupplemented medium as was produced in medium containing 2 mM sulfate (data not shown). All forms of inorganic sulfur tested-- sulfate, sulfite, thiosulfate, elemental sulfur, and sulfide -- supported growth as well. The efficient scavenging of the nutrient complicated the assessment of the organism's ability to utilize the test compounds as sole sulfur source. Still, no clear evidence for utilization of these compounds was obtained. The compounds were not toxic to growth at the concentration used in these experiments (1-2 mM). *Paecilomyces* was clearly incapable of utilizing the organic sulfur compounds tested here as a sole carbon source when added at 1% final concentration (data not shown). Growth occurred only in the presence of an easily-metabolizable carbon source (here, maltose). Degradation of the test compounds as reported below was apparently promoted by high maltose concentrations. For this reason results of experiments conducted with cultures containing 5% maltose (nitrogen-limiting conditions) are presented here. Under these experimental conditions, growth ceased within 2.5 days' incubation.

The choice of method for the analysis of experimental culture extracts depended in part on the thermal stability of the test compound. Thermal degradation of DBT and its microbial metabolites during GC/MS analysis has been described elsewhere (10,16). This knowledge mandated the use of ambient-temperature TLC in analyses of DBT culture extracts. Similarly, organic sulfides were found to generate anomalous products during GC analysis. GC/MS results presented below describe products unique to biological extracts; TLC was performed to corroborate product identity when the appropriate authentic standards were available. TLC was also used to identify (underivatized) sulfonic acids and other acidic products, which would not be detected by the GC/MS system used here. (Phenols were detectable by GC.)

Cultures containing DBT remained colorless during the course of a 30-d incubation. TLC analysis of methylene chloride extracts of these cultures showed the appearance of an unidentified metabolite on day 3 (R_f=0.38) and of a compound co-eluting with DBT sulfone (R_f=0.70) on day 10 (Table 1). These compounds disappeared from cultures on further incubation. By day 30, a compound co-eluting with 2,2'-biphenol (R_f=0.49) had accumulated within cultures. Only DBT and biphenyl were detected through GC/MS analysis of these extracts, i.e., there was no evidence

for ring cleavage pathway metabolites such as 1,2-dihydroxy-DBT or 3-hydroxy-2-formyl benzothiophene. (Biphenyl -- presumably a thermal degradation product -- also appeared in gas chromatograms of the authentic DBT standard. Biphenyl was not detected by TLC of the DBT standard, nor did it co-elute with the unidentified bioproduct.) GC analysis of the 2-butanol extracts of these cultures revealed no compounds other than those mentioned above. TLC analysis of these extracts indicated the presence of a presumably acidic product that was not identified. No single-ring compounds (e.g., catechol) arising from further metabolism of 2,2′-biphenol were detected by TLC or GC. Cultures to which DBT-sulfone was added at the time of inoculation produced 2,2′-biphenol within 10 d; no further metabolism was observed over a total of 30 d. Preliminary results regarding the fate of 2,2′-biphenol in cultures when added at the time of inoculation were inconclusive, although acidic products were apparently formed.

GC/MS analysis was performed on the methylene chloride extracts of cultures incubated for 7 d with organic sulfide test compounds. Cultures containing ethyl phenyl sulfide generated the corresponding sulfone plus di-(2′-hydroxyethyl)-benzenes. Phenyl sulfide cultures yielded phenyl sulfone and phenyl disulfide. These products were unique to extracts of inoculated cultures, i.e. were clearly of biological origin. Cultures incubated with benzyl sulfide generated a variety of unique products, including substituted monoaromatic compounds (Figure 3). GC/MS analysis of methylene chloride extracts of thianthrene cultures indicated the presence of phenyl sulfide. TLC analysis of the same extract provided some corroborating evidence and indicated the presence of other, lower-molecular-weight and/or more polar products as well (Table 2). Benzenesulfonic acid was not detected. TLC analysis of the 2-butanol extracts of ethyl phenyl sulfide, phenyl sulfide, and thianthrene cultures indicated that benzenesulfonic acid was not formed.

GC/MS analysis was also performed on methylene chloride extracts of 7-d cultures containing organic disulfides. Phenyl disulfide generated thiophenol plus an alkyl- or alkenyl-substituted thiophenol. Benzyl disulfide generated benzenethiol plus alkylbenzenes. TLC analysis of the 2-butanol extract of phenyl disulfide indicated that benzenesulfonic acid was not formed.

GC/MS analysis of 2-butanol extracts of cultures containing thiophenol indicated that the test compound completely disappeared from cultures during a 7-d incubation. Products were not detected by GC. TLC analysis revealed the presence of a presumbly acidic product which did not coelute with benzenesulfonic acid and was thus not identified.

DISCUSSION

This work was designed to assess the ability of Paecilomyces to degrade organic sulfur-containing coal model compounds under cultural and environmental conditions shown previously to support the production of coal-solubilizing activity (14,15). Results presented in this paper suggest that Paecilomyces is capable of transforming DBT and other organic sulfur compounds via cometabolism. DBT oxidation by Pseudomonas spp. was shown elsewhere to require the presence of a cosubstrate (8,17); but in contrast to the present work, an easily-metabolizable carbohydrate (glucose) inhibited activity. Carbon catabolite repression was apparently not a factor in the regulation of the degradative activities produced by the nitrogen-limited cultures used here.

A DBT degradation product from Paecilomyces was first detected immediately after the end of the active growth phase. Another product, DBT sulfone, was first detected several days later. This kinetic profile suggests either that the unknown product was a precursor of DBT-sulfone, or that two DBT transformation pathways are operative in this organism. The former possibility suggests that this unidentified product was DBT-sulfoxide. The unidentified product was not detectable by GC/MS and presumably degraded at high temperatures. Sulfoxides are known to be similarly thermolabile (16). The latter possibility suggests a potentially biphasic fungal attack on DBT. Mormile and Atlas (10) described a Pseudomonas sp. that exhibited both a growth-related ring-destructive activity and a secondary metabolic, or idiophasic, sulfur-oxidizing activity toward DBT. These authors were able to identify intermediates in both pathways by GC/MS analysis. They also detected another product by TLC that reverted to DBT during GC/MS analysis. This product, which was red, is probably the ring-cleavage product described elsewhere (9). The unidentified product generated in the present work was similarly thermolabile, but a red color was not detected in cultures. These observations suggest that this unknown metabolite is not the same product.

The presence of the unidentified metabolite makes it impossible to rule out the possibility of a ring-destructive or other DBT transformation activity. However, the absence of evidence of other, thermostable metabolites arising from the ring-destructive pathway suggests that metabolic flux in this organism proceeds primarily via sulfur oxidation. The idiophasic transformation of DBT (or its metabolite) apparently proceeded via direct oxidative (oxygenative) attack at the sulfur atom with formation of the corresponding sulfone. That compound was ultimately cleaved to form 2,2′-biphenol, presumably with release of free sulfate.

Results discussed above suggest that attack on organic sulfides, like that on DBT, would proceed at least in part via a sulfur-

specific (oxygenative) oxidation. Indeed, ethyl phenyl sulfide and phenyl sulfide were transformed to the corresponding sulfones. But neither sulfonic acids nor phenolic compounds, which would be predicted to arise from sulfate release, were observed over the course of this 7-d incubation. It is not known whether these more-oxidized products ultimately would have appeared. Benzyl sulfone was not detected, although the corresponding cleavage product (benzyl alcohol) did appear in cultures (although not necessarily by the proposed pathway; see below).

The variety of products generated from nonheterocyclic organic sulfides in general and benzyl sulfide in particular suggests an underlying mechanism for degradation of organic sulfur compounds by this organism, namely a sulfur-specific attack by a biological catalyst with generation of free radicals. A possible reaction mechanism would involve an initial one-electron oxidation of the sulfur atom with formation of a thiyl radical. A fairly straightforward subsequent cleavage and condensation would result in the formation of a disulfide, as observed in the case of phenyl sulfide. However, the thiyl radical would also be expected to undergo rearrangement to a variety of resonance forms. Localization of the unpaired electron on an adjacent carbon atom would yield aryl and/or alkyl radicals that would be free to undergo rapid condensations, with formation of substituted benzenes. Benzylic radicals are particularly stable (16), perhaps explaining the proliferation of products from benzyl sulfide. Thiyl radical-mediated oxidations of this type have been implicated in the biological degradation of nonphenolic lignin model compounds by a manganese peroxidase of *P. chrysosporium* (18).

In the present system, thianthrene was transformed to phenyl sulfide. The mechanism underlying this activity is not known. However, this apparent attack on only one of the two sulfur atoms is similar to that observed during treatment of thianthrene with *P. chrysosporium* lignin peroxidase (11). Thianthrene was oxidized *in vitro* to its corresponding monooxide via a sulfur-specific, oxygenative attack. However, the formation of cation radicals -- which are characteristically produced during reactions with this enzyme (19) -- was not detected during the reaction with thianthrene. The relevance of this observation to the involvement of thiyl radicals in fungal oxidation of organic sulfur compounds, as proposed here, is not known.

Transformations of organic disulfides in cultures appeared to be somewhat less complex than those of sulfides. Thiol formation is consistent with a simple cleavage across the sulfur-sulfur bond; the underlying mechanism, however, cannot be determined from this work. The appearance of substituted alkylbenzenes (particularly in the case of benzyl disulfide) suggests that involvement of thiyl radicals as described above.

The present work provides little insight into the kinetics of the attack on sulfur by this organism or the identity of the catalyst(s) involved. Production and breakdown of DBT sulfone were clearly independent of growth, as was further metabolism of the unidentified material. This behavior is consistent with prior reports that described the production of an oxidative coal-solubilizing activity by fungi as a secondary metabolic or idiophasic function (12,20). Paecilomyces was shown previously in this laboratory to carry out an oxidative degradation of soluble coal components (15). This activity occurred slowly over a prolonged (120-d) incubation period that extended well past the active growth phase. It is possible that some of the same oxidative, idiophasic catalysts involved in that degradative activity contribute to the transformation of DBT and its metabolites. A similar hypothesis would explain too the persistence of thiophenol (produced from phenyl disulfide) in cultures, a somewhat surprising event in light of the apparent disappearance of thiophenol from cultures when added at the time of inoculation. It is possible that thiophenol is degraded only by growing cells, and that the attack on phenyl sulfide is strictly idiophasic.

In the present work, oxygenative and nonoxygenative oxidation reactions contributing to the transformation of nonheterocyclic organic sulfides compounds appeared to proceed in tandem. Factors determining the relative importance of oxygenative and nonoxygenative mechanisms were not determined. The cultures described here were grown in submerged mode in the absence of forced aeration. It is possible that increased accessibility to oxygen would promote oxygenative activity leading to release of free sulfate ion. Oxygen has been proposed elsewhere to act as a scavenger of thiyl radicals, with formation of sulfonated products (18). Indeed, the sulfones observed in this work may have been formed in this manner.

The results of this work suggest that Paecilomyces has potential utility in the desulfurization of coal-related compounds. This organism may form the basis for the design of a combined process for solubilization of low-rank coal and for the cleaning of the biosolubilized product in situ. An alternative application would be in the desulfurization of high-rank coal, which is not solubilized by Paecilomyces. This organism's demonstrated ability to catalyze a surface attack on coal during solubilization, coupled with the current findings, suggest that it may catalyze sulfur removal from the surface of finely-ground coal particles. Paecilomyces and other sulfur-oxidizing organisms may be particularly active at the interface between organic and inorganic regions within coal, where "immature", relatively labile sulfur-containing structures are thought to be localized (21). A sulfur-specific attack designed to preserve the substrate's carbon content or caloric value would require operating conditions that discourage ring cleavage reactions. The use of pregrown biomass may achieve this end. The relative yields of oxygenated and nonoxygenated products, and of sulfur-

containing and non-sulfur-containing products, will be important determinants of the practical utility of coal desulfurization processes based on this organism.

ACKNOWLEDGMENTS

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Table 1

PRODUCTS FORMED BY PAECILOMYCES FROM DBT*

	Rf values				
Standards:					
DMF	----	----	0.62	----	----
DBT	0.88	----	0.62	----	----
DBT sulfone	----	0.69	0.62	----	----
2,2′-biphenol	----	----	0.61	0.50	----
Uninoculated controls					
DBT**	0.89	----	0.63	----	----
Experimental cultures					
Control (DMF only)***	----	----	0.62	----	----
DBT (3-d culture)	0.85	----	0.62	----	0.38
DBT (10-d culture)	0.89	0.70	0.63	----	0.38
DBT (30-d culture)	0.90	----	0.62	0.49	----

* Thin layer chromatographic analysis of methylene chloride extracts of cultures incubated with DBT . Solvent = 1-heptane:acetone, 1:1. DMF, which was present in all cultures, was used as an internal standard.

** Results are presented for a 3-d culture; similar results were obtained through day 30.

*** Results are presented for a 10-d culture; similar results were obtained through day 30.

Table 2

PRODUCTS FORMED BY PAECILOMYCES FROM THIANTHRENE*

	R_f values				
Standards:					
DMF	----	----	----	0.60	----
Thianthrene	----	0.90	----	0.62	----
Phenyl sulfide	0.95	----	----	0.62	----
Experimental cultures					
Control (DMF only)**	----	----	----	0.62	----
Thianthrene	0.93	0.90	0.74	0.61	0.45

* Thin layer chromatographic analysis of methylene chloride extracts of cultures incubated with thianthrene . Solvent = 1-heptane:acetone, 1:1. DMF, which was present in all cultures, was used as an internal standard.

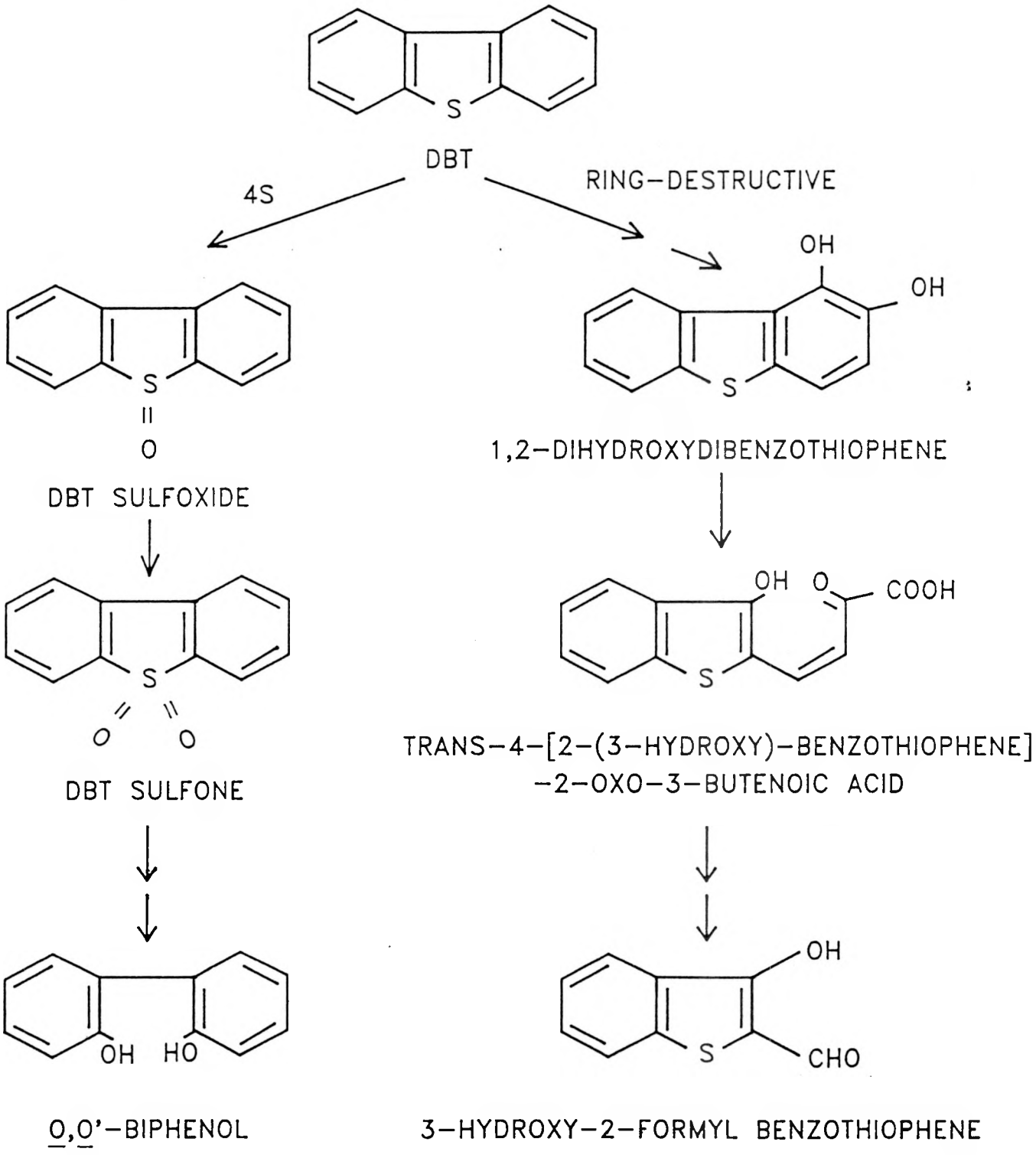
** Results are presented for a 10-d-old culture. Thianthrene was stable in uninoculated controls through day 10.

Figure 1. Proposed pathways for microbial metabolism of DBT.

Figure 2. Organic sulfur-containing coal model compounds.

Figure 3. Products formed by Paecilomyces from benzyl sulfide.

ORNL DWG 90A-382R



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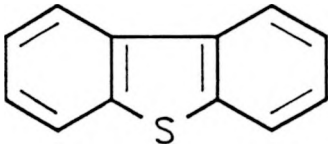
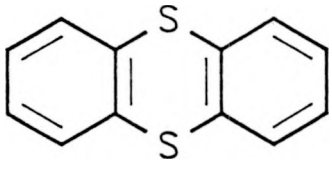


DIBENZOTHIOPHENE
(DBT)



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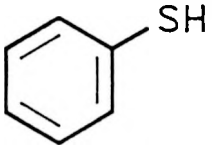

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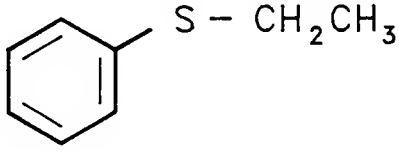

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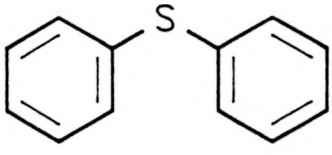

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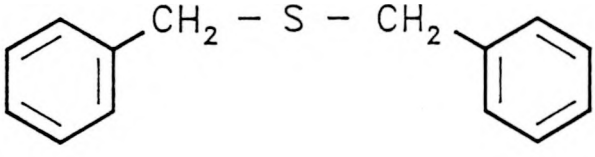
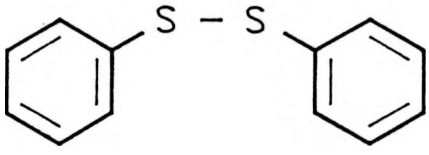
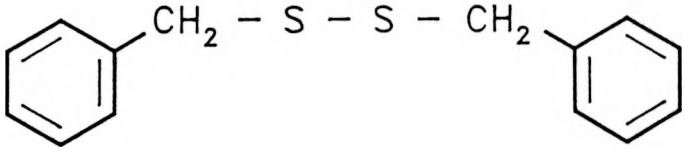


BENZYL SULFIDE

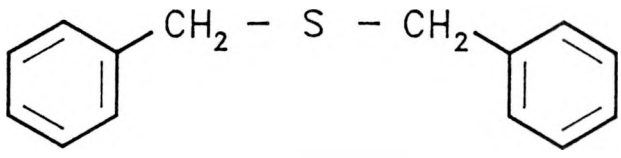


PHENYL DISULFIDE



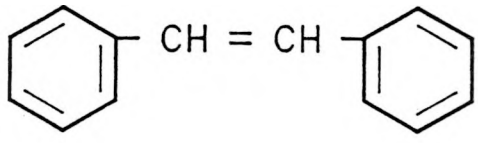
BENZYL DISULFIDE

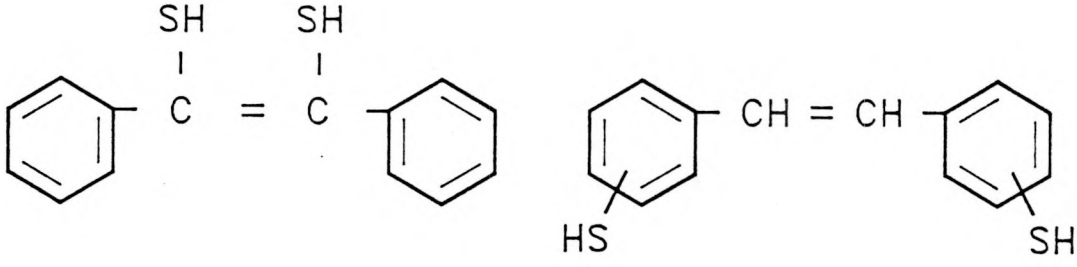
ORNL DWG 90A-434R

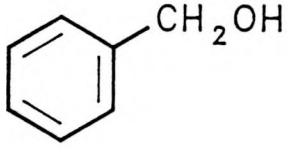


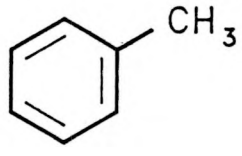
Benzyl Sulfide
(substrate)

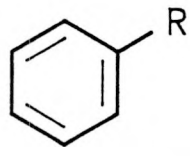
Products:

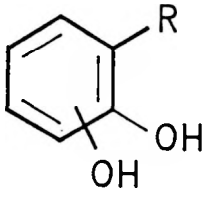


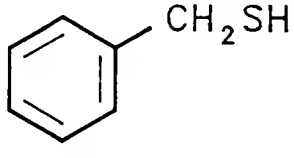


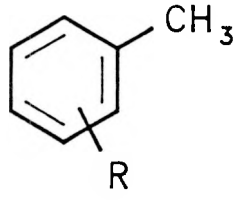












R = alkyl

CONF-900531--2 (1)

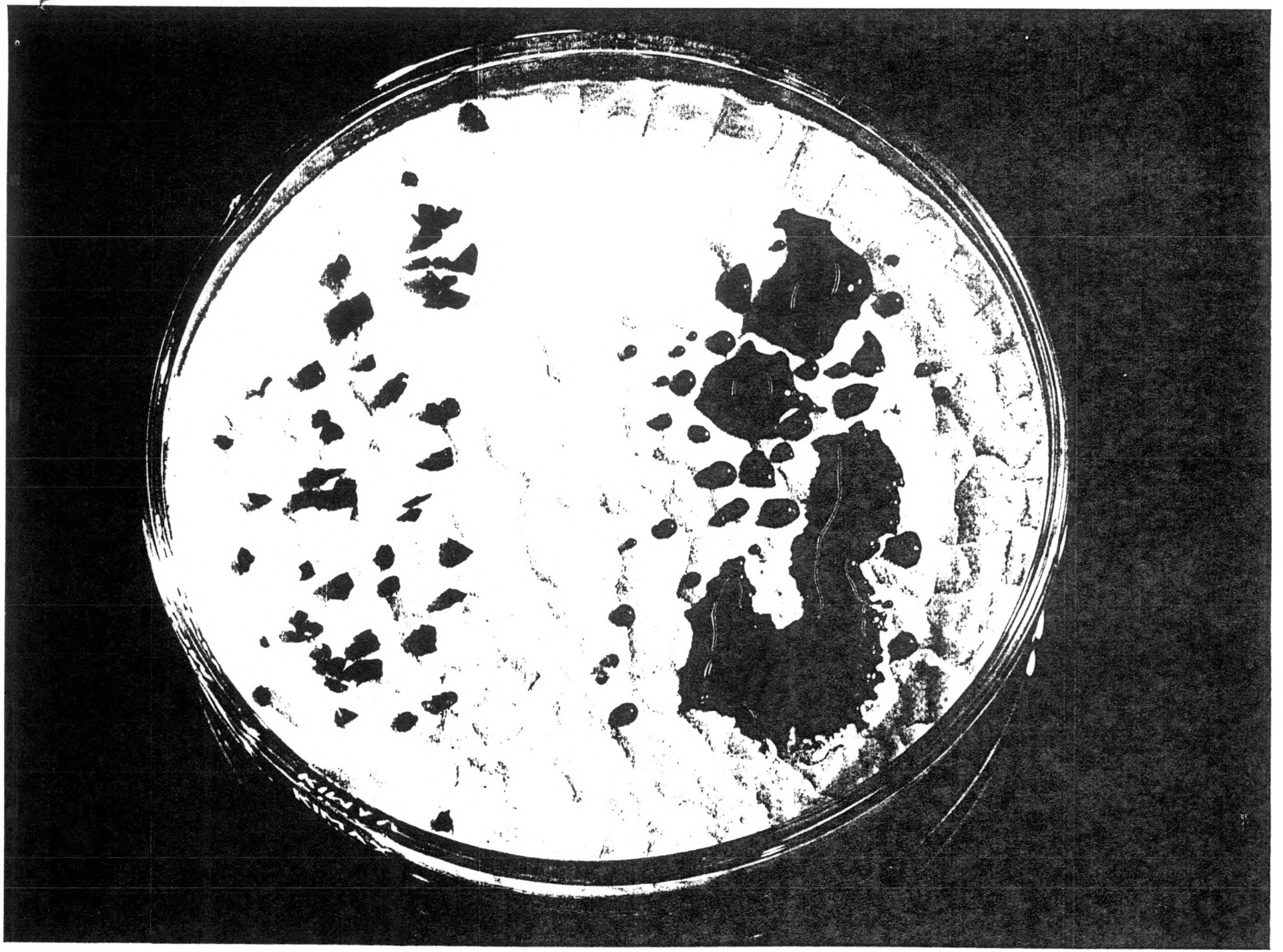
ORNL WS 14258

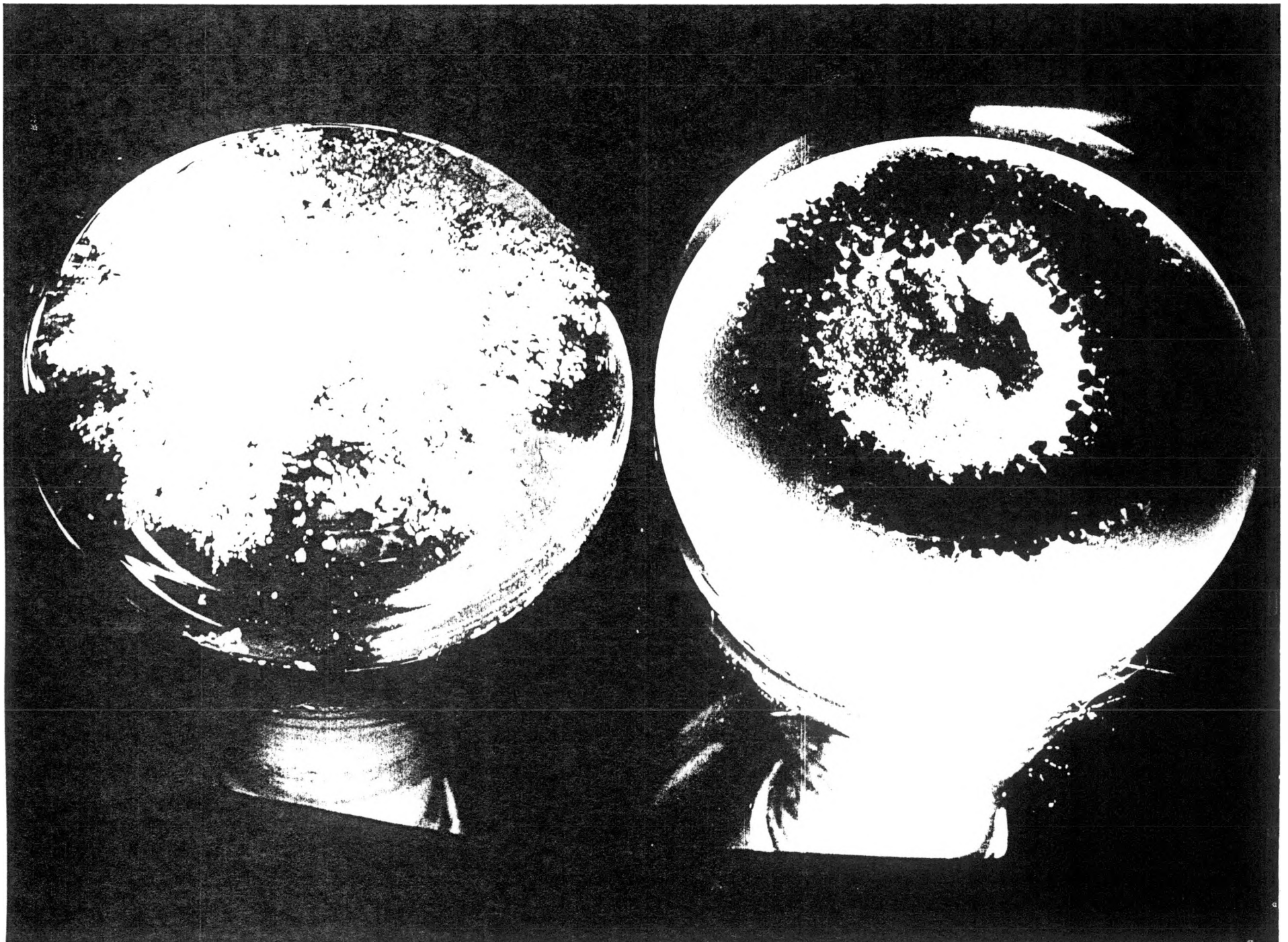
Degradation of Organic Sulfur Compounds by a Coal-Solubilizing Fungus*

B. D. Faison, T. M. Clark, S. N. Lewis, D. M. Sharkey, and C. A. Woodward

Chemical Technology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37831-6194

*Research supported by Fossil Energy Advanced Research and Technology Program, managed by the Pittsburgh Energy Technology Center, U.S. Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

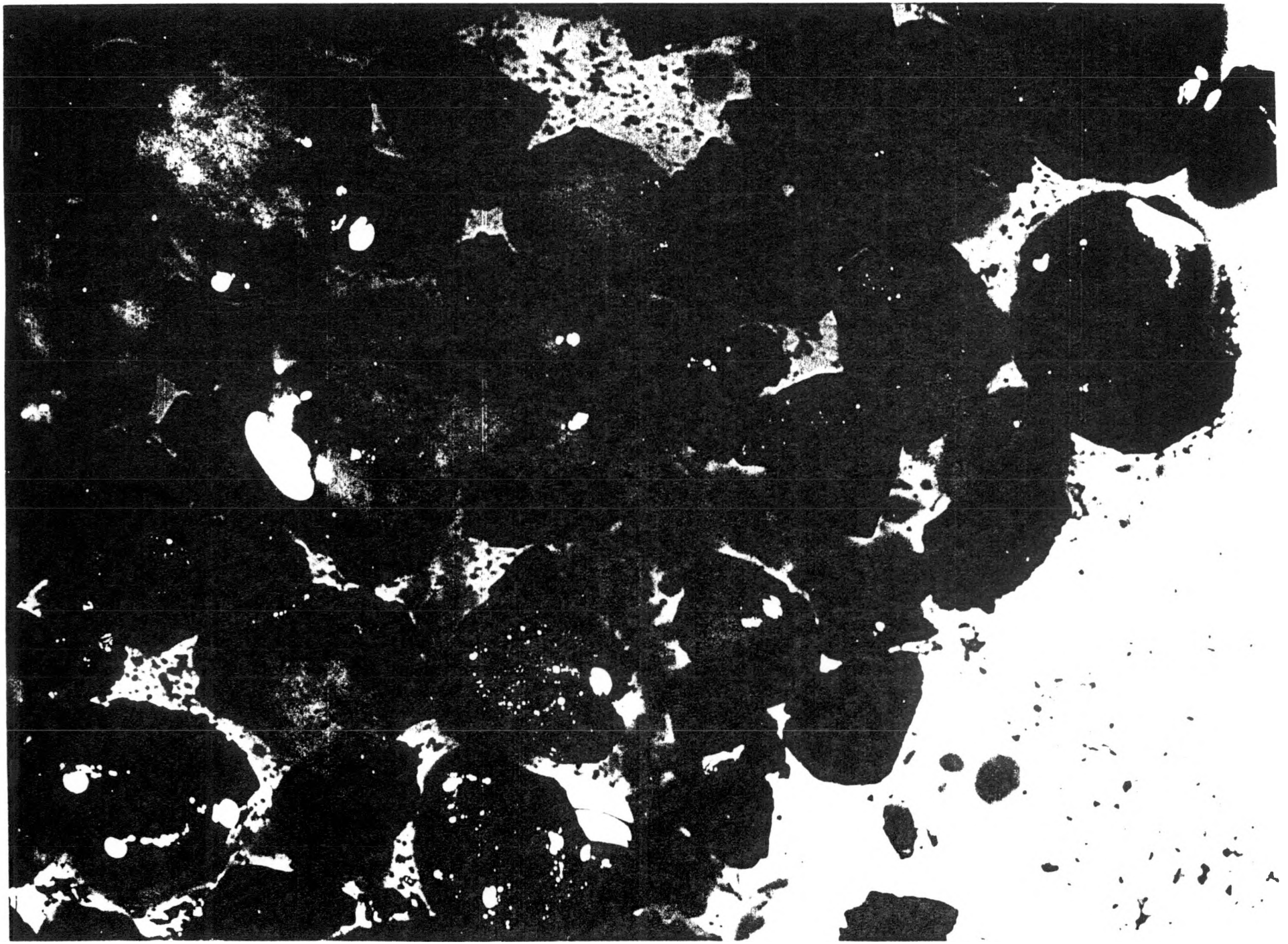




Fungal Coal Solubilization: Process Characteristics

- Low-rank coals are more susceptible than higher-rank coals
- A gross correlation between rate and extent of solubilization and particle size has been noted


0
1
2
millimeters



Fungal Coal Solubilization: Process Characteristics (continued)

- Process is oxidative and oxygenative
  - Product is water-miscible
  - Polyphenolic and acidic compounds are generated

Fungal Coal Solubilization: Process Characteristics (continued)

- Some bond cleavage occurs
  - Molecular weight of product decreases over time
  - Enzymes (peroxidase, laccase) have been implicated

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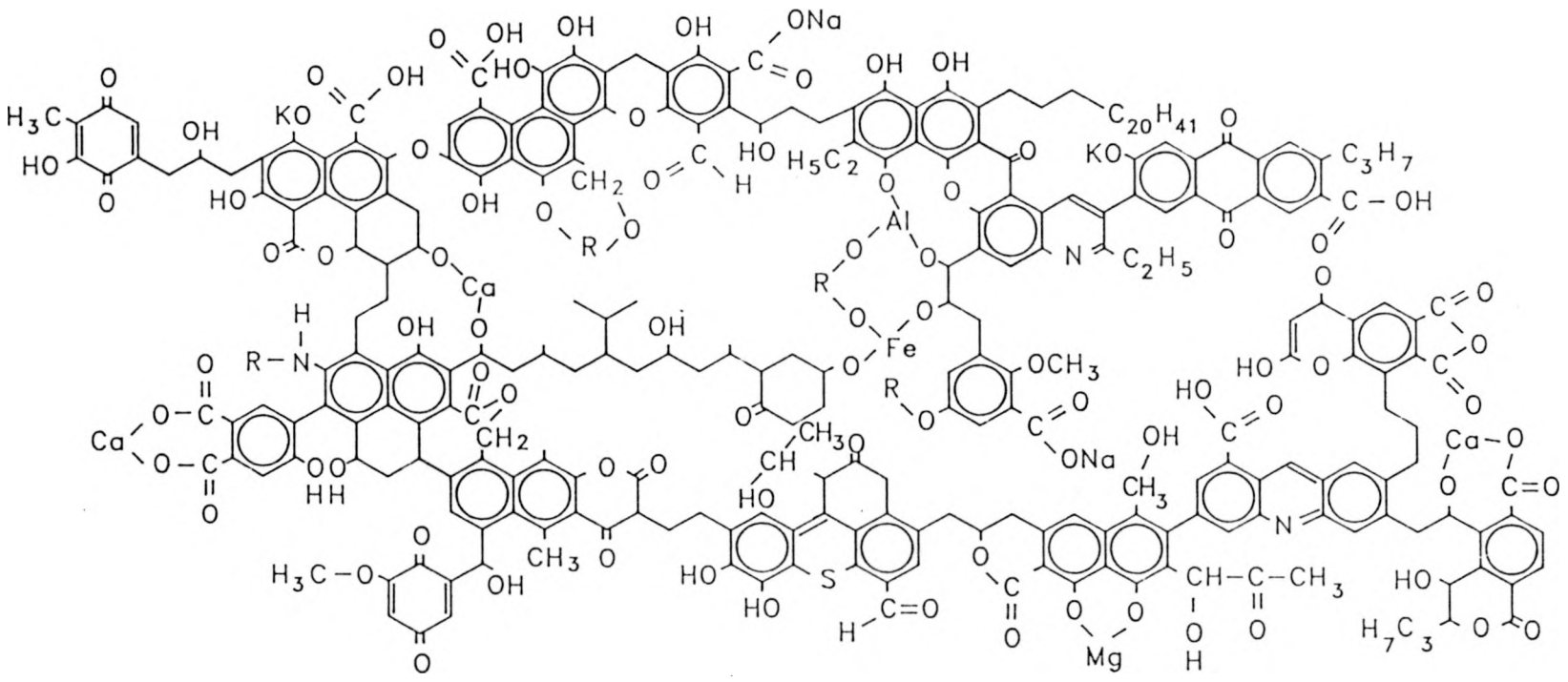


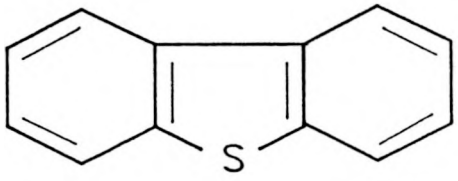
PROPOSED STRUCTURAL UNIT OF A BROWN COAL

EMPIRICAL FORMULA $C_{270}H_{240}N_3S_1O_{90}$

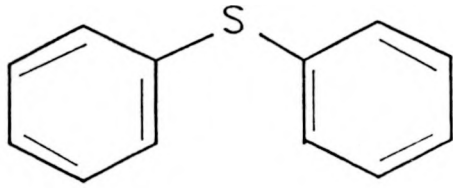
HUTTINGER AND MICHENFELDER, 1987

ORNL DWG 90A-379

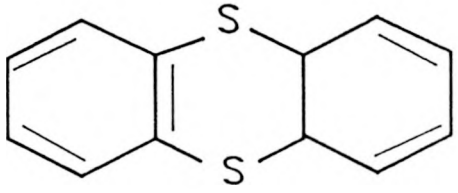
Organic Sulfur-Containing Coal Model Compounds



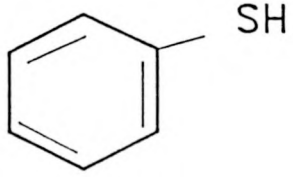
DIBENZOTHIOPHENE
(DBT)



PHENYL SULFIDE



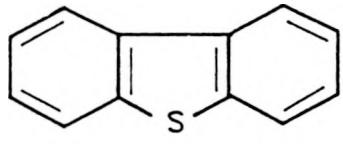
THIANTHRENE



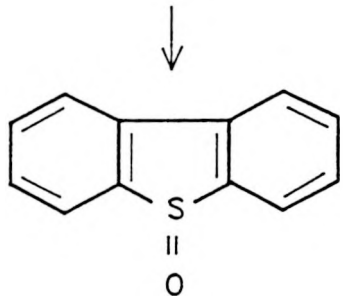
THIOPHENOL

ORNL DWG 90A-383

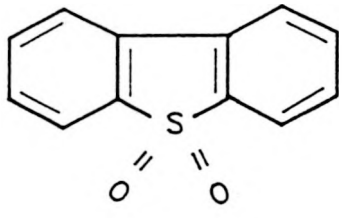
Microbial Transformations of DBT
"4S" Pathway



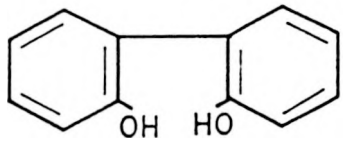
DBT



DBT SULFOXIDE



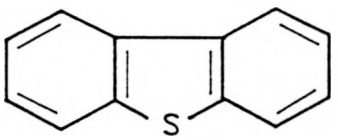
DBT SULFONE



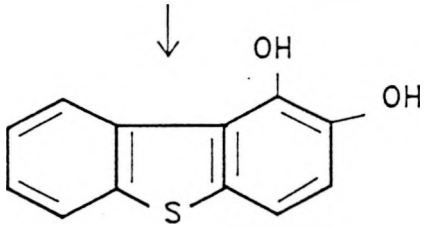
O,O'-BIPHENOL

ORNL DWG 90A-384

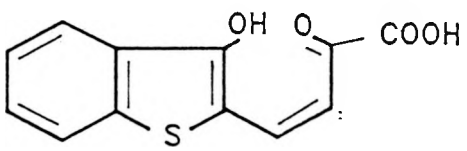
Microbial Transformations of DBT
Ring-Destructive Pathway



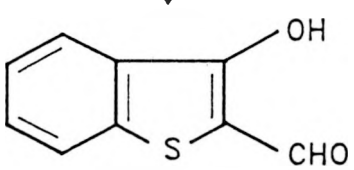
DBT



1,2-DIHYDROXYDIBENZOTHIOPHENE



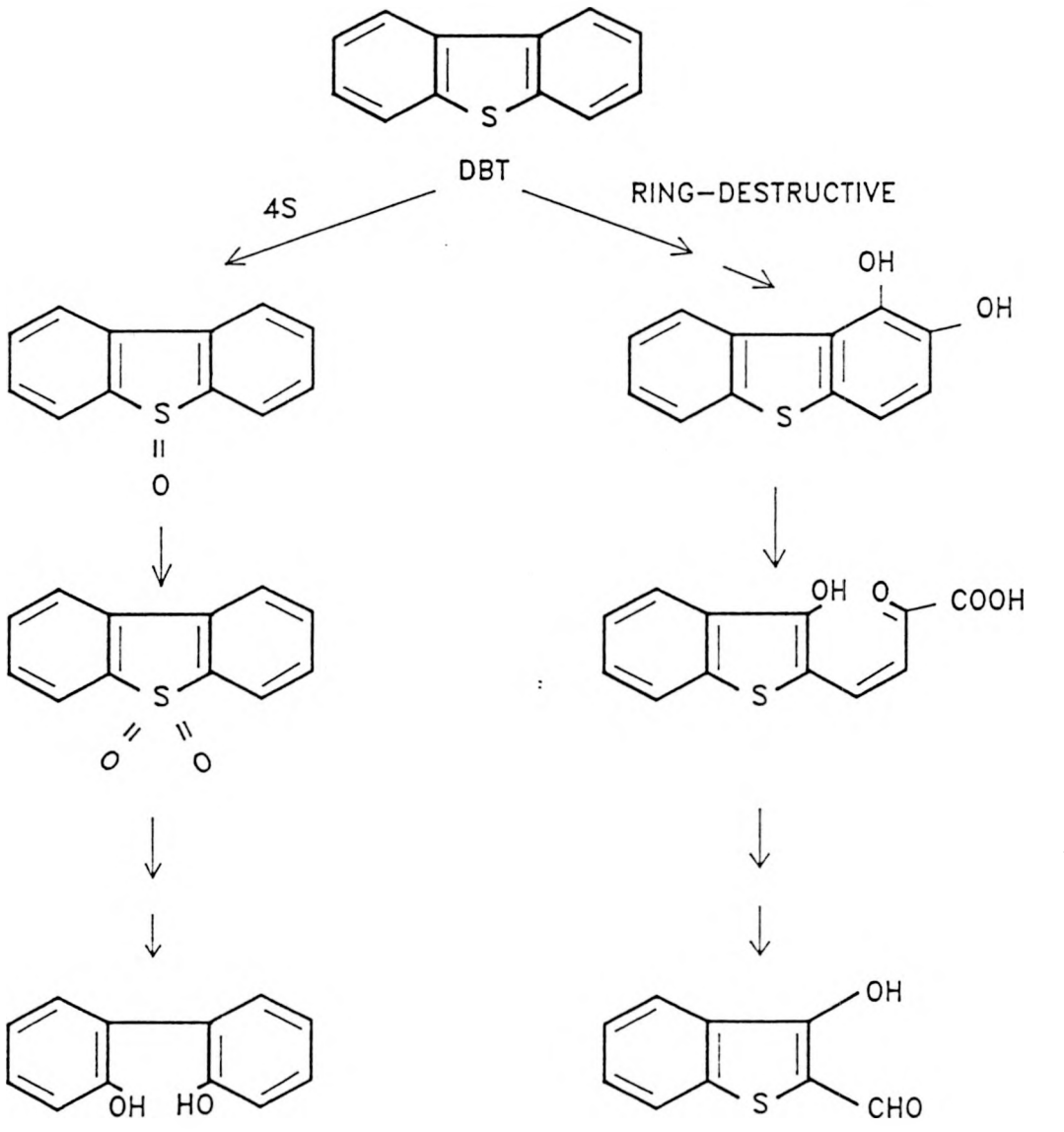
TRANS-4-[2-(3-HYDROXY)-BENZOTHIOPHENE]-2-OXO-3-BUTENOIC ACID



3-HYDROXY-2-FORMYL BENZOTHIOPHENE

ORNL DWG 90A-382

Microbial Transformations of DBT



0,0'-BIPHENOL

3-HYDROXY-2-FORMYL BENZOTHIOPHENE

(19)

ORNL WS 14262

Degradation of Organic Sulfur Compounds By *Paecilomyces*

Attack on sulfur atom

vs

Attack on aromatic moiety

Utilization of Inorganic Sulfur Compounds By *Paecilomyces*

Sulfur Source	Biomass yield (g dry wt/mL)	Oxidation State
None (control)	0.97	N/A
SO_4^{2-}	4.51	+6
SO_3^{2-}	7.08	+4
$S_2O_3^{2-}$	3.50	+2
S^0	2.06	0
S^{2-}	2.35	-2

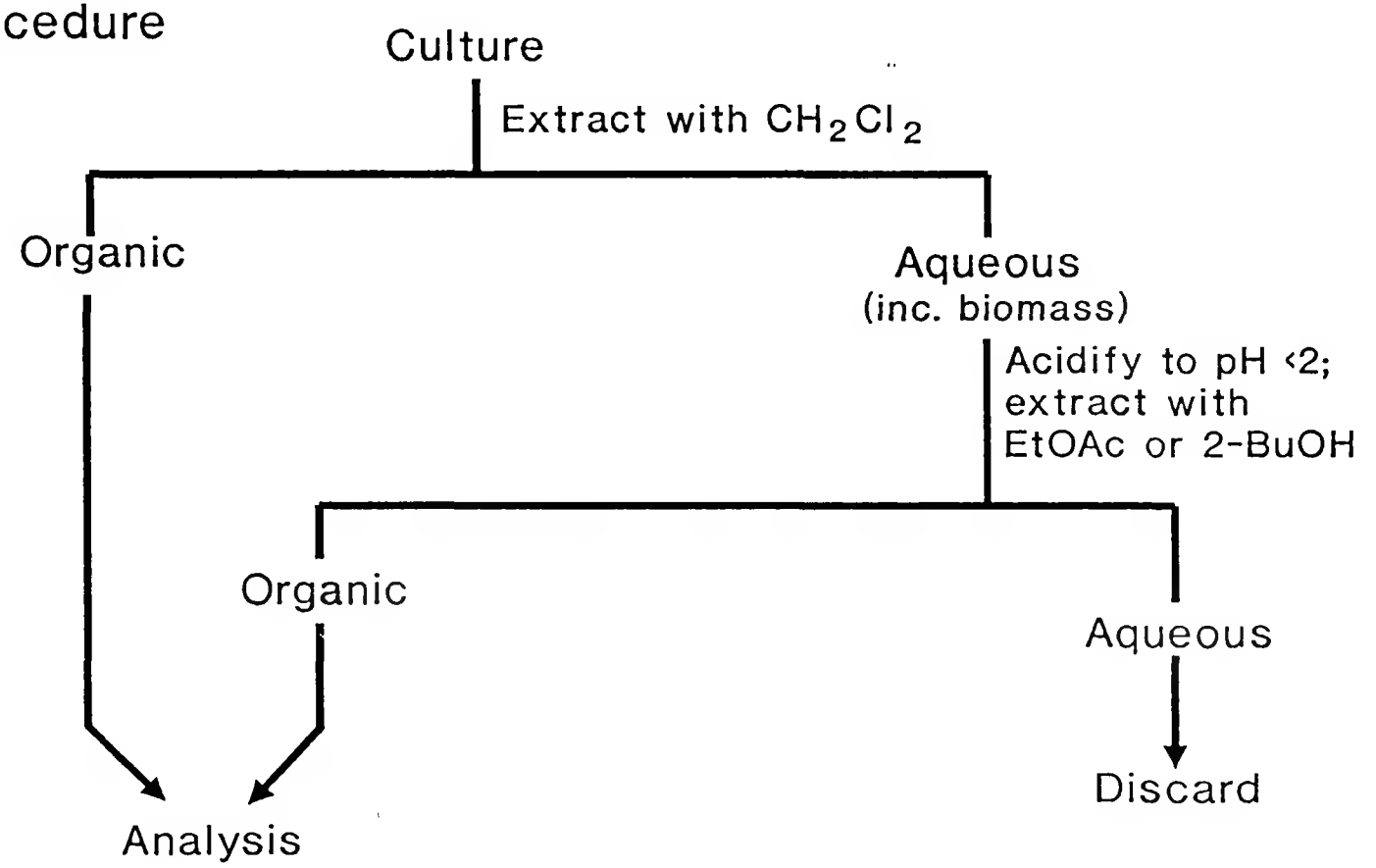
Degradation of Organic Sulfur Compounds By *Paecilomyces*

- Growth medium
  - Inorganic salts medium containing trace levels of SO_4^{2-}
  - Maltose (0.1-5%) provided as primary carbon source
  - Organic compounds (2 m*M*) added in 5% dimethylformamide solution, in the presence of 0.01% Tween 80

ORNL DWG 90-378

Degradation of Coal Model Compounds by *Paecilomyces*

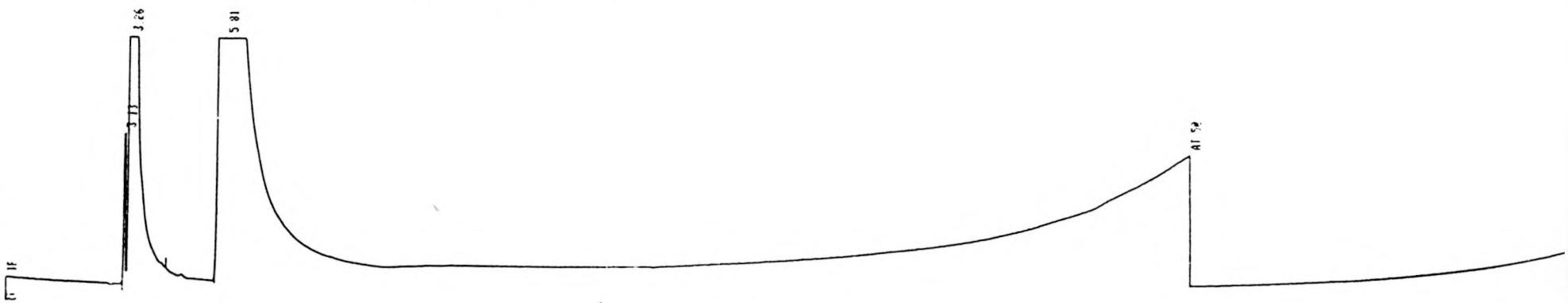
Fractionation procedure



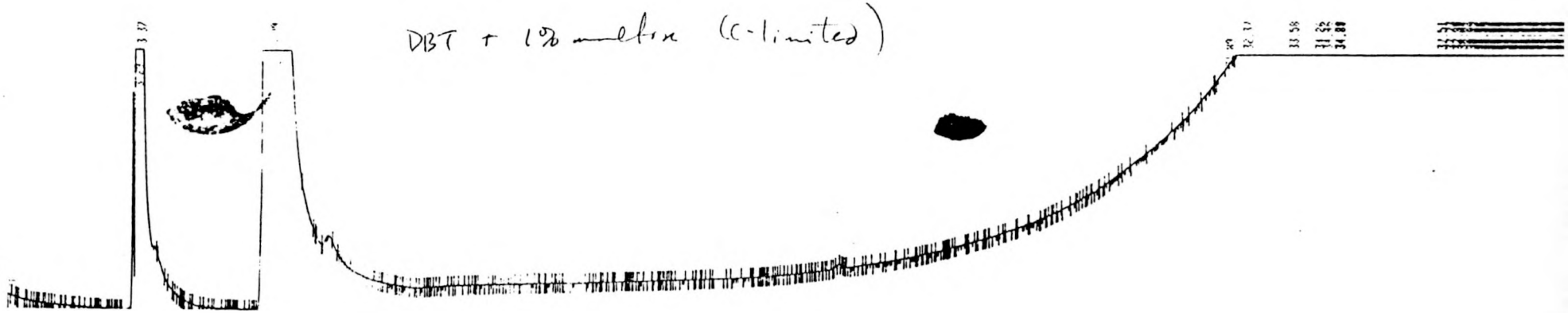
structures of organic S cpds

= 90 A 379

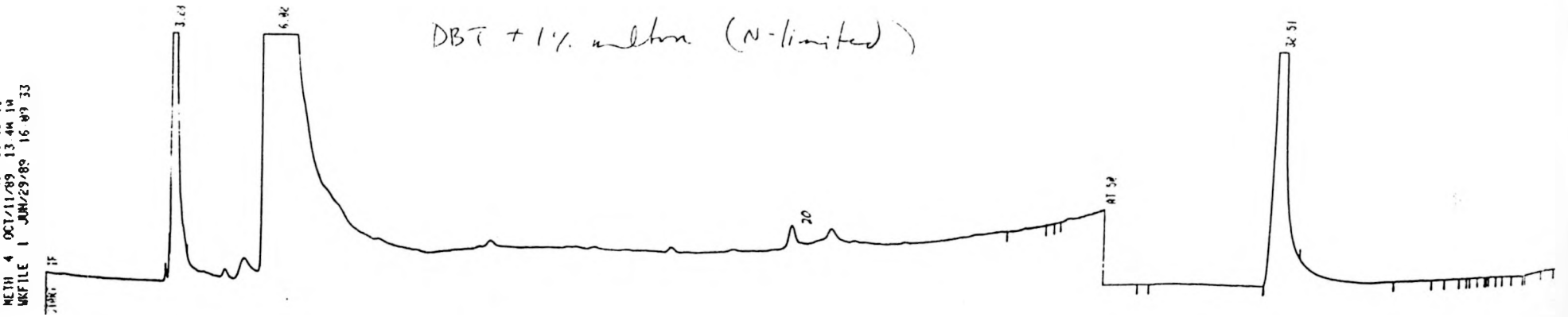
control culture



DBT + 1% culture (C-limited)



DBT + 1% culture (N-limited)



Degradation of Dibenzothiophene by *Paecilomyces*

TLC solvent - *n*-heptane:acetone (1:1)

	R_f values				
Standards					
DMF	–	–	0.62	–	–
DBT	0.88	–	0.62	–	–
DBT sulfoxide/sulfone	–	0.69	0.62	–	–
o,o'-biphenol	–	–	0.61	0.50	–
Catechol	–	0.67	0.62	–	–
Uninoculated controls					
DBT, day 3	0.89	–	0.63	–	–
DBT, day 10	0.87	–	0.61	–	–
Experimental cultures					
DMF, day 10 (control)	–	–	0.62	–	–
DBT, day 3	0.85	–	0.62	–	0.38
DBT, day 10	0.89	0.70	0.63	–	0.38
DBT, day 30	0.90	–	0.62	0.49	–

Degradation of Dibenzothiophene by *Paecilomyces*

TLC solvent - *n*-heptane:acetone (4:1)

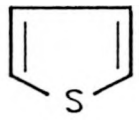
	R_f values			
Standards				
DMF	–	0.39	–	–
DBT	0.65	0.39	–	–
DBT sulfoxide/sulfone	–	0.40	0.27	–
Catechol	–	0.39	–	0.14
Experimental cultures				
DMF, day 10 (control)	–	0.39	–	–
DBT, day 10	0.63	0.39	0.26	–

Degradation of Organic Sulfur Compounds By *Paecilomyces*

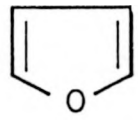
- Dibenzothiophene degradation
  - Occurs in cultures limited for nitrogen but not in cultures limited for carbon
  - Products are oxidized

ORNL DWG 90A-380

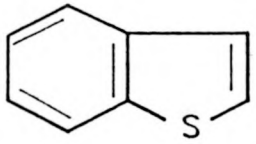
Organic Sulfur-Containing Coal Model Compounds
Thiophenes and Analogs



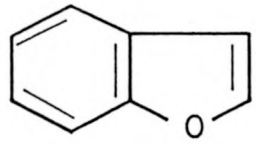
THIOPHENE



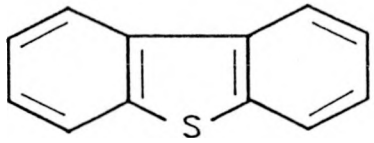
FURAN



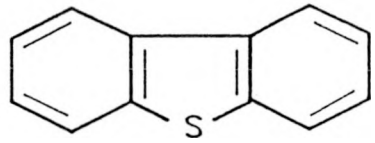
BENZOTHIOPHENE



BENZOFURAN



DIBENZOTHIOPHENE

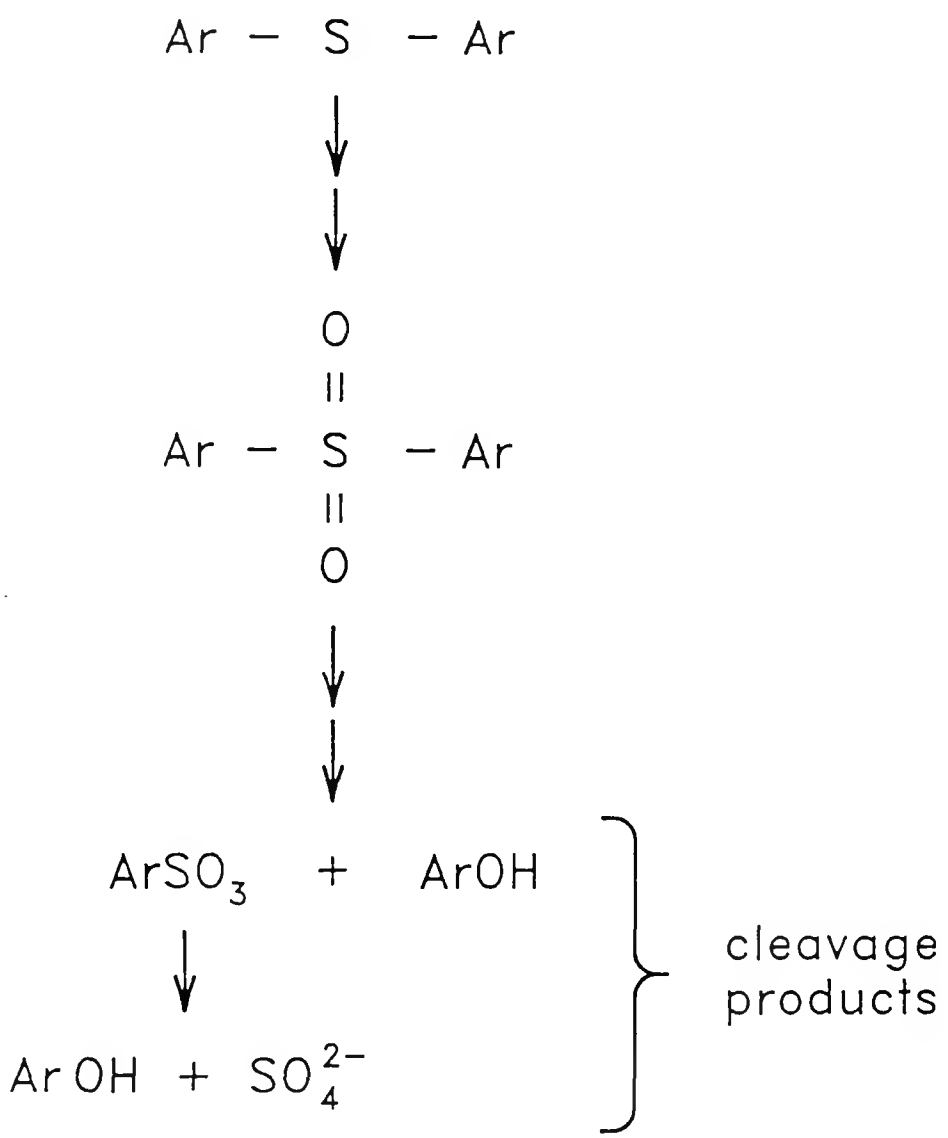


DIBENZOFURAN

ORNL DWG 90A-428

Degradation of Aryl Sulfides by Paecilomyces

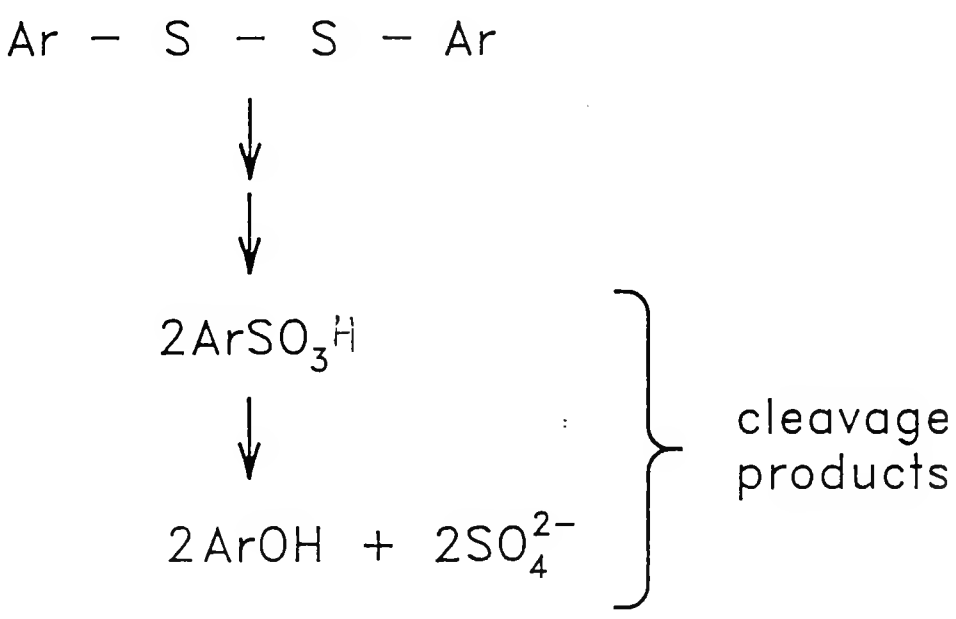
Proposed pathway:



ORNL DWG 90A-429

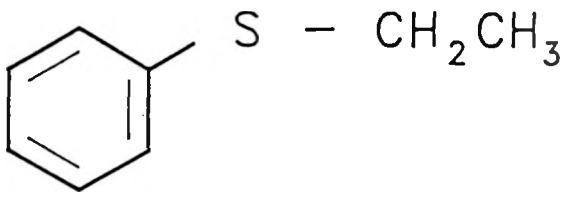
Degradation of Aryl Disulfides by *Paecilomyces*

Proposed pathway:

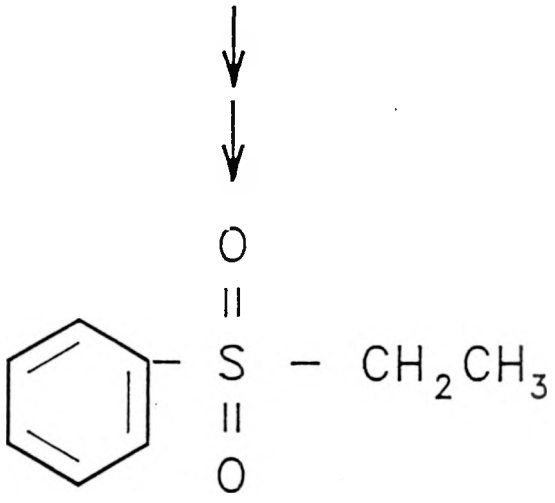


ORNL DWG 90A-433

Degradation of Aryl Sulfides by Paecilomyces*



Ethyl Phenyl Sulfide

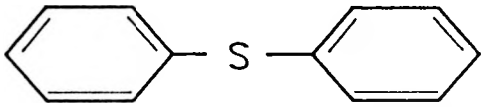


Ethyl Phenyl Sulfone

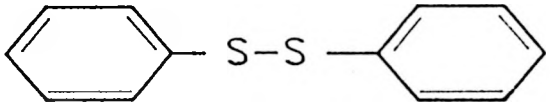
* CH_2Cl_2 extraction

ORNL DWG 90A-381

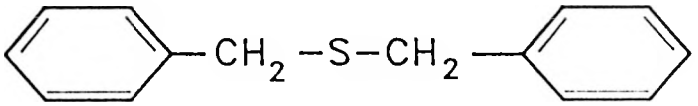
Organic Sulfur-Containing Coal Model Compounds
Aryl Sulfides and Aryl Disulfides



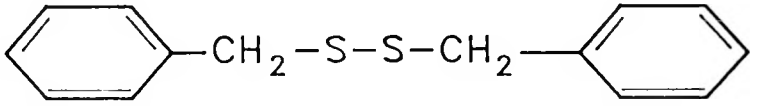
PHENYL SULFIDE



PHENYL DISULFIDE



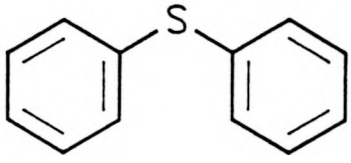
BENZYL SULFIDE



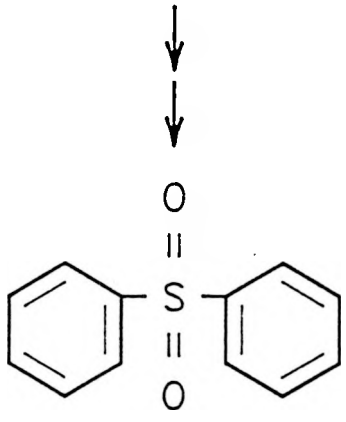
BENZYL DISULFIDE

ORNL DWG 90A-432

Degradation of Aryl Sulfides by Paecilomyces*

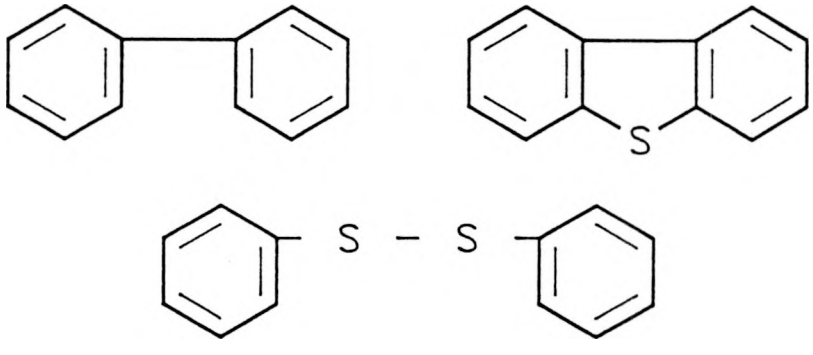


Phenyl Sulfide



Phenyl Sulfone

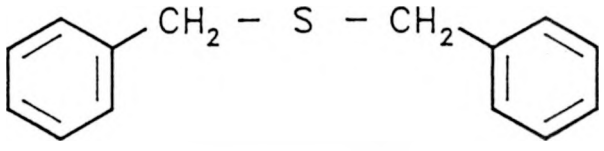
Other products:



* CH_2Cl_2 extraction

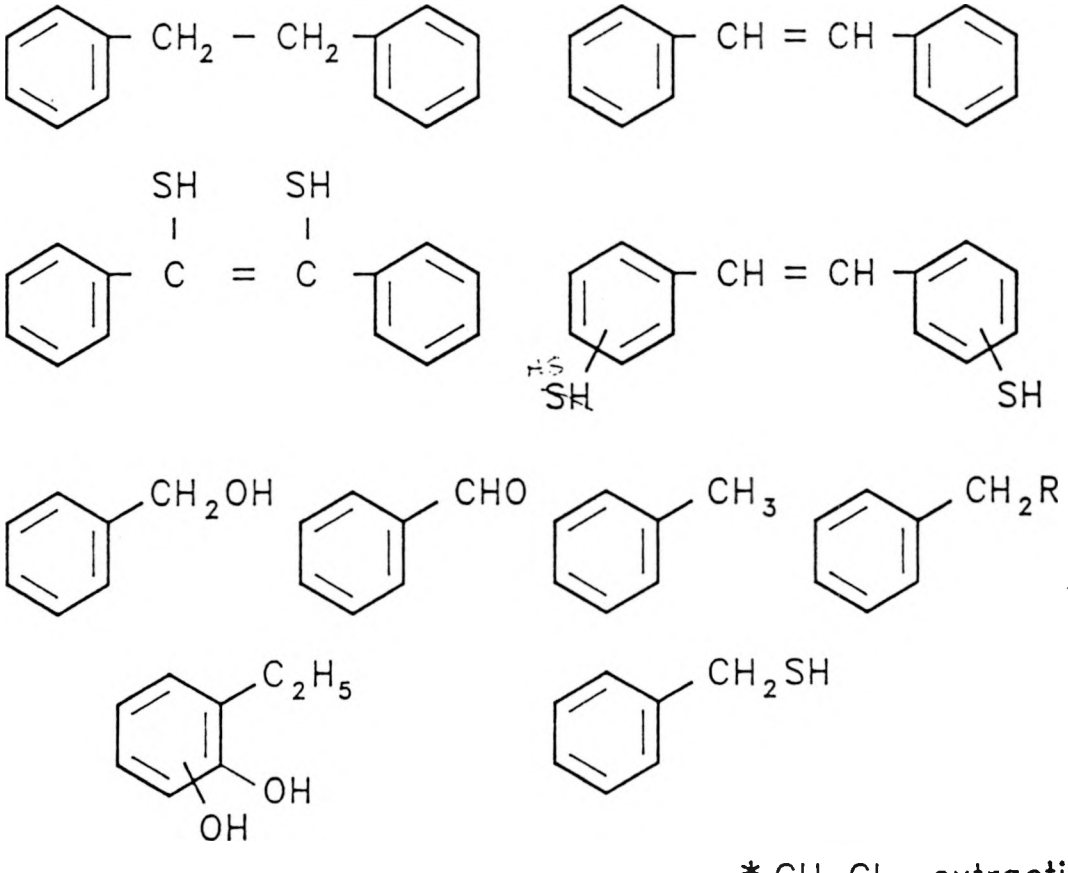
ORNL DWG 90A-434

Degradation of Aryl Sulfides by Paecilomyces*



Benzyl Sulfide

Products:



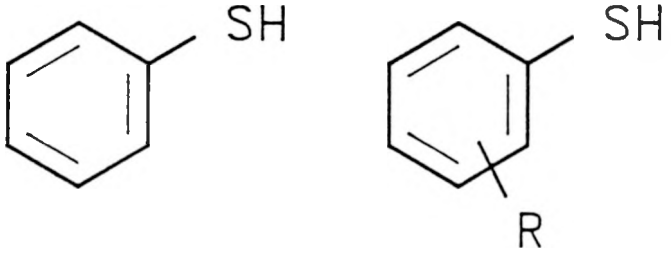
* CH_2Cl_2 extraction

ORNL DWG 90A-431

Degradation of Aryl Disulfides by Paecilomyces*

Phenyl Disulfide

Products:



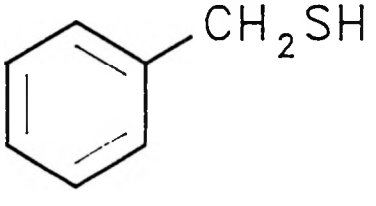
* CH_2Cl_2 extraction

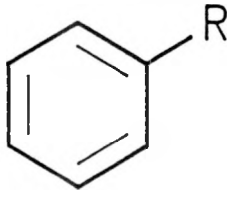
ORNL DWG 90A-430

Degradation of Aryl Disulfides by Paecilomyces*

Benzyl Disulfide

Products:

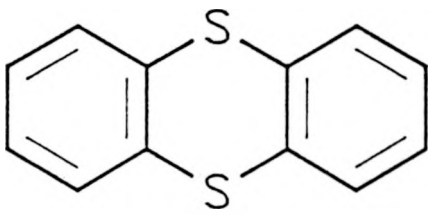




* CH_2Cl_2 extraction

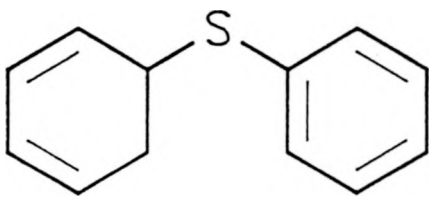
ORNL DWG 90A-446

Degradation of Aryl Sulfides by Paecilomyces *



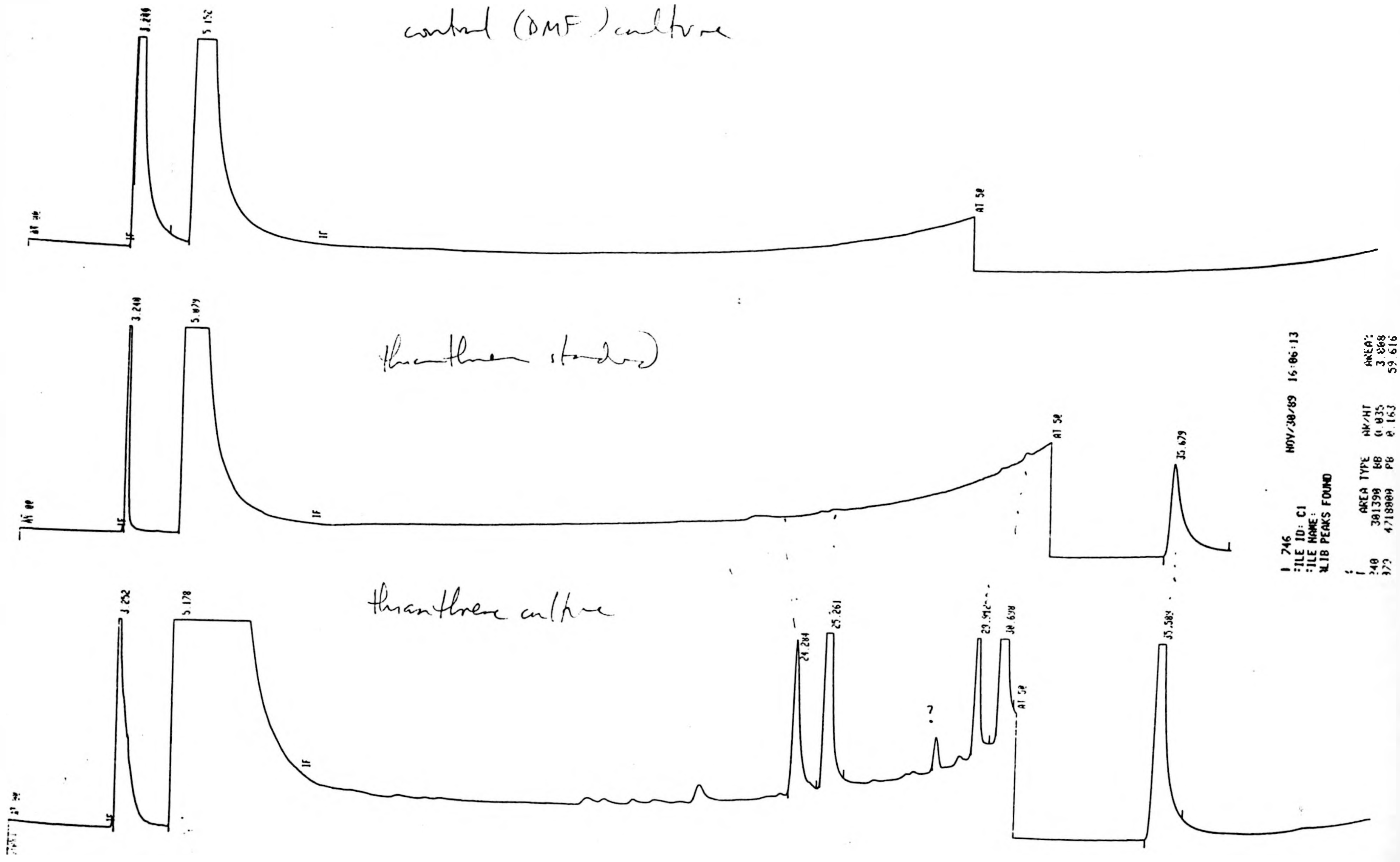
Thianthrene

Product:



Phenyl Sulfide

* CH_2Cl_2 extraction


control (DMF) culture
thianthrene standard
thianthrene culture
NOV/30/89 16:06:13
?

Degradation of Thianthrene by *Paecilomyces*

TLC solvent - *n* -heptane:acetone (1:1)

	R_f values				
Standards					
DMF	–	–	–	0.60	–
Thianthrene	–	0.90	–	0.62	–
Phenyl sulfide	0.95	–	–	0.62	–
Experimental cultures					
DMF, day 10 (control)	–	–	–	0.62	–
Thianthrene, day 10	[0.93]	0.74	0.61	0.45	

Degradation of Organic Sulfur Compounds By *Paecilomyces*

Summary

- DBT is degraded via a sulfur-oxidizing pathway
  - Contribution of ring cleavage to DBT degradation is not known
- Degradation of aryl sulfides and disulfides may also involve sulfur oxidation
  - Free radical mechanisms have been implicated
  - Both oxidized and reduced products are formed

Degradation of Organic Sulfur Compounds By *Paecilomyces*

Conclusions

- Degradation occurs via cometabolism and may represent a secondary metabolic activity
- General mechanism is oxidative (± oxygenative)
- Fate of ring systems is not known

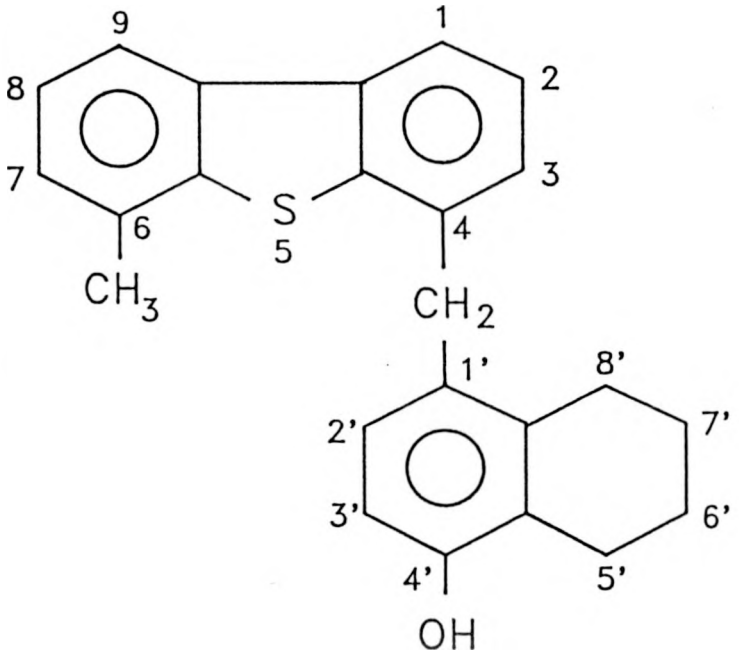
Degradation of Organic Sulfur Compounds By *Paecilomyces*

Potential Utility

- Beneficiation of high-rank coal
- Beneficiation of biosolubilized low-rank coal

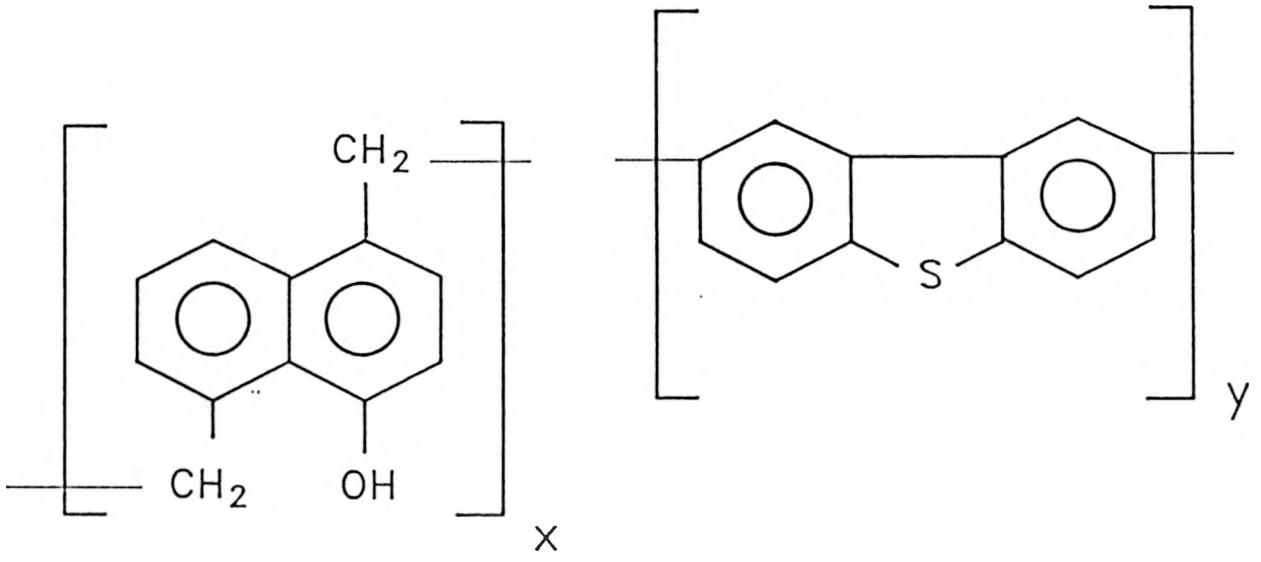
ORNL DWG 90A-385

4(4'-Hydroxy-5',6',7',8'-Tetrahydro-1'-naphthylmethyl)-6-methyldibenzothiophene



ORNL DWG 90A-386

A 1-hydroxynaphthalene-dibenzothiophene Polymer Linked by Methylene Bonds



2-3% S CONTENT
Mol. wt ~10,000-30,000

Degradation of Organic Sulfur Compounds By *Paecilomyces*

Future Work

- Fate of homocyclic ring systems
- Fate of organic sulfur atom
- Fate of inorganic sulfur forms