Biodegradation 7: 203–221, 1996.
© 1996 *Kluwer Academic Publishers. Printed in the Netherlands.*

Transformations of six isomers of dimethylbenzothiophene by three *Pseudomonas* strains

Kevin G. Kropp[1], Sanja Saftić[1,3], Jan T. Andersson[2] & Phillip M. Fedorak[1,*]
[1]*Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E9;* [2]*Department of Analytical Chemistry, University of Münster, D-48149 Münster, Germany;* [3]*present address: Stadtwerk Düsseldorf AG, Wasserwerk Holthausen, Wieldfeld 50, 40589 Düsseldorf, Germany (* Corresponding author)*

Accepted 6 February 1996

Key words: cometabolism, condensed thiophenes, dimethylbenzothiophenes, organosulfur compounds, *Pseudomonas*, sulfoxides

Abstract

Dimethylbenzothiophenes are among the sulfur heterocycles in petroleum that are known to be degraded by microbial activity. Six of the 15 possible isomers of dimethylbenzothiophene were synthesized and used in biotransformation studies with three *Pseudomonas* isolates that oxidize a variety of condensed thiophenes including methylbenzothiophenes and methyldibenzothiophenes. The isomers of dimethylbenzothiophene were chosen to have a variety of substitution patterns: both methyl groups on the thiophene ring (the 2,3- isomer); a methyl group on each of the rings (the 2,7-, 3,5- and 3,7- isomers); and both methyl groups on the benzene ring (the 4,6- and 4,7- isomers). Each isolate was grown on 1-methylnaphthalene or glucose in the presence of one of the dimethylbenzothiophenes and culture extracts were analyzed to identify nearly 30 sulfur-containing metabolites in total. Sulfoxides and sulfones were commonly found metabolites in culture extracts from the 2,3-, 2,7- and 3,7- isomers, whereas 2,3-diones, 3(2H)-ones and 2(3H)-ones were formed from the 4,6- and 4,7- isomers. High-molecular-weight products, some of which were tentatively identified as tetramethylbenzo[*b*]naphtho[1,2-*d*]thiophenes, were detected in the extracts of cultures incubated with 4,6- or 4,7-dimethylbenzothiophene. The methyl groups of all of the isomers, except 4,6-, were oxidized to give hydroxymethyl-methylbenzothiophenes and methylbenzothiophene-carboxylic acids, and these were the only products detected from the oxidation of 3,5-dimethylbenzothiophene.

Introduction

After carbon and hydrogen, sulfur is typically the third most abundant element in petroleum, ranging from about 0.04 to 5 weight percent in conventional oils (Speight 1980). Sulfur is found in several organic forms, including thiols, sulfides and thiophenes.

Biodegradation studies of organosulfur compounds have primarily focussed on those compounds found in the aromatic fraction of petroleum or coal-derived liquids. These include benzothiophene, alkylated benzothiophenes, dibenzothiophene and alkylated dibenzothiophenes. There have been numerous investigations with dibenzothiophene, which is commercially available to serve as a model condensed thiophene (Kodama et al. 1970, 1973; Hou & Laskin 1976; Laborde & Gibson 1977; Monticello et al. 1985; Crawford & Gupta 1990). Dibenzothiophene and its alkyl-substituted analogues have been shown to be removed from the aromatic fraction of petroleum by microbial activity (Atlas et al. 1981; Fedorak & Westlake 1983, 1984; Bayona et al. 1986; Hostettler & Kvenvolden 1994), although the identities of their metabolites were not determined in those studies.

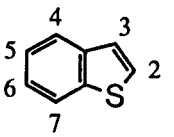
Analyses using gas chromatography-mass spectrometry (GC-MS) have demonstrated the presence of C_2-benzothiophenes in crude oils (Fedorak & Westlake 1983; Westlake 1983; Williams et al. 1986), shale oils (Willey et al. 1981; Andersson 1992) and coal tar (Burchill et al. 1982). These may be ethylbenzothiophenes or dimethylbenzothiophenes, which have the same molecular weight and are difficult to

distinguish by GC-MS analysis. Microbial activity has been shown to remove the C_2-benzothiophenes from crude oil (Fedorak & Westlake 1983, 1984; Westlake 1983; Williams et al. 1986), however, little is known about the metabolites of these compounds, since C_2-benzothiophenes are not commercially available.

Benzothiophene and the methylbenzothiophenes do not appear to serve as growth substrates for aerobic bacteria, but biotransformation products have been observed in cultures grown on other substrates. For example, Bohonos et al. (1977) used naphthalene as a growth substrate for mixed cultures and tentatively identified 2,3-dihydrobenzothiophene-2,3-diol, benzothiophene-2,3-dione and benzothiophene sulfoxide as metabolites of benzothiophene. The 2,3-dione was identified in the extracts of cultures of *Pseudomonas* strain BT1 grown on 1-methylnaphthalene, glucose or peptone (Fedorak & Grbić-Galić 1991). Eaton & Nitterauer (1994) grew *Pseudomonas putida* RE204, an isopropylbenzene-degrading bacterium, on succinate and yeast extract and identified *trans*-4-[3-hydroxy-2-thienyl]-2-oxobut-3-enoate as a product of cleavage of the homocyclic ring, and 2-mercaptophenylglyoxalate as a product of cleavage of the heterocyclic ring. They demonstrated that the latter compound cyclized to benzothiophene-2,3-dione when treated with acid.

Sulfoxides, sulfones and 2,3-diones were commonly found as metabolites of benzothiophene and six methylbenzothiophenes in the dichloromethane-extracts of acidified cultures (Fedorak & Grbić-Galić 1991; Saftić et al. 1992; Kropp et al. 1994a). Some of these cultures were also capable of oxidizing the methyl groups of the methylbenzothiophenes, yielding benzothiophene-methanols and benzothiophene-carboxylic acids (Kropp et al. 1994a). Benzo[*b*]naphtho[1,2-*d*]thiophenes were identified as products of the abiotic condensation of microbially produced benzothiophene sulfoxide and some methylbenzothiophene sulfoxides (Kropp et al. 1994b). The only report on the microbial metabolism of a dimethylbenzothiophene was by Saftić et al. (1992) who demonstrated that 2,3-dimethylbenzothiophene was oxidized to its sulfone and sulfoxide by *Pseudomonas* strain BT1.

The objective of this work was to detect and identify sulfur-containing metabolites of six isomers of dimethylbenzothiophene that were synthesized for this study. Three bacterial isolates were used for these investigations in which 1-methylnaphthalene or glucose served as the growth substrate. The ring numbering convention for benzothiophene is shown below.



Benzothiophene

Materials and methods

Chemicals

The methods for the syntheses of 2,3-, 2,7-, 3,5-, 3,7-, 4,6-, and 4,7-dimethylbenzothiophenes are given by Andersson (1986). These synthesized compounds were shown to be $\geq$99% pure by GC analyses. Sulfones of some of the dimethylbenzothiophenes were synthesized by boiling the dimethylbenzothiophene with H_2O_2 in acetic acid for 15 min. The structures of the sulfones were verified by GC-MS analyses which showed the correct molecular weight and by GC-FTIR analyses which showed strong absorptions in the regions of 1350–1300 cm^{-1} and 1160–1120 cm^{-1} that are characteristic of sulfones (Shriner et al. 1980). 3-Methylbenzothiophene, 5-methylbenzothiophene and 2,5-thiophenedicarboxylic acid were purchased from Lancaster Synthesis (Windham, NH), and 1-methylnaphthalene was purchased from Fluka (Buchs, Switzerland).

2-Methylbenzothiophene-3-carboxylic acid was synthesized from 2-methylbenzothiophene using a Friedel-Craft acylation with oxalyl chloride and aluminum chloride as catalyst, according to the procedure of Sokol (1973). 2-Methyl-3-hydroxymethylbenzothiophene was prepared by chloromethylating 2-methylbenzothiophene (Grummit & Buck 1955) and hydrolysing the resulting chloride by the procedure of Baciocchi & Mandolini (1968). 2-Hydroxymethyl-3-methylbenzothiophene was synthesized by bromination of 2,3-dimethylbenzothiophene and hydrolysis (Baciocchi & Mandolini 1968).

5-Methylbenzothiophene-2,3-dione and 7-methylbenzothiophene-2,3-dione were synthesized by the procedure of Hannoun et al. (1982). The structure and purity of the synthesized diones were confirmed by determination of the melting points, which were the same as literature values. Using the general methods of Dickinson & Iddon (1970) and Stridsberg & Allenmark (1974), authentic standards

of 4,7-dimethylbenzothiophene-2(3H)-one and 4,7-dimethylbenzothiophene-3(2H)-one were prepared. The 2(3H)-one was synthesized by treating 4,7-dimethylbenzothiophene (50 mg) with *n*-butyllithium, tri-(*n*-butyl)borate, and H_2O_2, whereas the 3(2H)-one was synthesized from 2 g of 2,5-dimethylthiophenol (Aldrich, Milwaukee, WI) by treating it with chloroacetic acid, thionyl chloride, and $AlCl_3$. Similarly, the 2(3H)-one and 3(2H)-one of 4,6-dimethylbenzothiophene were synthesized from 4,6-dimethylbenzothiophene (20 mg) and from 3,5-dimethylthiophenol (1 g, Aldrich), respectively. The 2(3H)-ones, present in analytical amounts, and the 3(2H)-ones of 4,6- and 4,7-dimethylbenzothiophene were recovered from reaction mixtures by extraction and maintained as solutions in dichloromethane to minimize the chance of air oxidation of the products (Friedländer 1906). GC-MS analyses of these solutions showed that extracts of reaction mixtures from each synthesis contained a single abundant compound with the molecular weight of the desired product.

Bacterial cultures, culture methods and media

The isolation and characteristics of *Pseudomonas* strain BT1 were described by Fedorak & Grbić-Galić (1991). The isolation of *Pseudomonas* strain W1 and *Pseudomonas* strain F has also been described previously (Saftić et al. 1993).

Growing cells of isolates BT1, W1 and F were used for biotransformation studies. Because these isolates would not grow on the benzothiophenes, the growth substrates used were 1-methylnaphthalene or glucose. Cultures were grown at 28°C in 500-mL shake-flasks containing 200 mL of liquid mineral medium supplemented with a trace metals solution (Kropp et al. 1994b). Often some of the metabolites of 1-methylnaphthalene interfered with the analysis of metabolites from the benzothiophenes. This problem did not arise when glucose was used. Each 200-mL portion of medium was supplemented with 50 mg of one of the growth substrates and 2 to 5 mg of the dimethylbenzothiophene. Unless otherwise noted, these cultures were incubated for 7 days prior to extraction. For each biotransformation experiment, appropriate sterile controls were incubated to account for any abiotic transformations.

Extraction and analytical methods

After incubation, the cultures were acidified with sulfuric acid to pH<2 and extracted with dichloromethane (4 times 20 mL) to recover substrates and products. To screen for the presence of sulfur-containing metabolites, the extracts were analyzed by capillary GC using a 30-m DB-5 capillary column in a Hewlett-Packard model 5890 equipped with a flame ionization detector and a sulfur-selective flame photometric detector (FPD). Details of the operating conditions were given by Fedorak & Grbić-Galić (1991). Details of the methods for GC-MS and GC-FTIR analyses have been described previously (Saftić et al. 1993).

To facilitate GC-MS identification of some of the metabolites, trimethylsilyl (TMS) derivatives of compounds in culture extracts were made by silylating with N,O-bis(trimethylsilyl)acetamide (BSA) in acetonitrile according to the manufacturer's instructions (Pierce, Rockford, IL; method 5).

Some culture extracts containing compounds suspected of being carboxylic acids (based on GC-MS analysis) were methyl-esterified using BF_3 in methanol (Aldrich). Specifically, a portion of the extract was evaporated to dryness under nitrogen. The residue was then dissolved in 1.0 mL of BF_3-methanol reagent and heated at 65°C for 9 min. After cooling, the mixture was added to 10 mL of dichloromethane and washed with 10 mL of 4 M aqueous NaCl solution. The aqueous phase was separated and extracted with 10 mL of dichloromethane. The two organic extracts were pooled, dried over anhydrous sodium sulfate, concentrated and analyzed by GC.

Results

Nearly 30 sulfur-containing metabolites, from the six isomers of dimethylbenzothiophenes, were detected and identified during this study. None of the transformation products described below were detected in the sterile controls, thus they were all products of bacterial oxidations. Active growth of the cultures on 1-methylnaphthalene and glucose, as indicated by turbidity increases, occurred within the first 3 days of incubation. However, most of the cultures were incubated for 7 days prior to extraction, and those sulfur-containing metabolites that remained were identified. With the exception of the cultures of isolate BT1 grown in medium containing 3,5-dimethylbenzothiophene, all of the extracts of the 7-day-old cultures contained residual di-

methylbenzothiophene that had not been oxidized by the *Pseudomonas* strains.

Metabolites from 2,3-dimethylbenzothiophene

GC-FPD analysis of an extract of a culture of isolate W1 grown on glucose in liquid medium containing 2,3-dimethylbenzothiophene revealed several sulfur-containing metabolites. GC-MS analysis showed that the most abundant of these gave a mass spectrum that was the same as that of 2,3-dimethylbenzothiophene sulfoxide identified by Saftić et al. (1992). Eluting immediately after the sulfoxide was a smaller peak which gave a mass spectrum with a molecular ion of m/z 194 and base peak at m/z 151. This spectrum was the same as that of an authentic standard of 2,3-dimethylbenzothiophene sulfone.

Two other sulfur-containing metabolites eluted before the sulfoxide of 2,3-dimethylbenzothiophene. These two products eluted within 0.5 min of each other and gave identical mass spectra (Figures 1a and 1b). The molecular ion for each of these was at m/z 178 which was consistent with the structures of hydroxy-substituted 2,3-dimethylbenzothiophenes. Loss of OH $(M-17)^+$ would give the fragment at m/z 161. An authentic standard of 2-methyl-3-hydroxymethylbenzothiophene had an identical mass spectrum (Figure 1c) to those of the metabolites (Figures 1a and 1b). The retention time of this standard matched that of the metabolite that was first to elute, indicating that the hydroxy substitution was on the methyl group at position 3. The metabolite that was later to elute is likely 2-hydroxymethyl-3-methylbenzothiophene. We refer to these types of compounds as methanols.

TMS-derivatives were prepared using BSA, and the mass spectrum and retention time of the derivatized methanol with the shorter retention time matched that of the TMS-derivative of an authentic standard of 2-methyl-3-hydroxymethylbenzothiophene. The other methanol metabolite also reacted to give a TMS-derivative.

In the extract of a second glucose-grown culture of isolate W1, the sulfoxide of 2,3-dimethylbenzothiophene was the most abundant product, and the sulfone and two methanols were again detected. However, when this extract was treated with BSA to derivatize the two methanols, two new derivatized metabolites were detected by GC-MS analysis. These were present in small amounts and both eluted after the sulfone of 2,3-dimethylbenzothiophene. The molecular ion of these products was at m/z 264 (Figures 2a and 2b). The fragmentations to give the abundant ions are shown in Figure 2 except for the ion at m/z 205 which is the result of the loss of a methyl group from the TMS substituent, followed by skeletal rearrangement with the loss of CO_2. This is a characteristic fragmentation pattern for TMS-esters of carboxylic acids (Pierce 1968). The fragmentation pattern of these derivatized methylbenzothiophene-carboxylic acid metabolites closely matched that of a TMS-derivatized authentic standard of 2-methylbenzothiophene-3-carboxylic acid (Figure 2c).

GC-MS analyses of the extracts of cultures of isolate F grown in medium containing 1-methylnaphthalene and 2,3-dimethylbenzothiophene showed that they contained 2,3-dimethylbenzothiophene sulfoxide and sulfone, both methanol isomers and a carboxylic acid that yielded a TMS-dervative that gave the same mass spectrum as that shown in Figure 2b.

An extract of a culture of isolate F grown on glucose in the presence of 2,3-dimethylbenzothiophene was also analyzed by GC-MS. Again, 2,3-dimethylbenzothiophene sulfoxide was the most abundant metabolite, and a methanol with the same retention time as authentic 2-methyl-3-hydroxymethylbenzothiophene was detected. In this extract, there was enough of a carboxylic acid present that it was detected in its free form, despite the fact that it was very poorly chromatographed. The mass spectrum (Figure 3a) showed the molecular ion at m/z 192 and abundant fragments at m/z 174 and 147 from the loss of H_2O and COOH, respectively. This methylbenzothiophene-carboxylic acid loses H_2O to give the ion at m/z 174 rather than losing OH to give an abundant fragment at m/z 175 because regardless of which methyl group is oxidized to a carboxy group by isolate F, there will be a methyl group ortho to it on the thiophene ring. By proton transfer from the methyl group and elimination of H_2O from the carboxy group, the abundant ion $(M-18)^+$ is formed (Silverstein et al. 1991). This is consistent with the mass spectrum that is observed for an authentic standard of 2-methylbenzothiophene-3-carboxylic acid as shown in Figure 3b.

Extracts from isolate BT1 grown on glucose or 1-methylnaphthalene in the presence of 2,3-dimethylbenzothiophene contained the sulfoxide as the major metabolite and the sulfone as a minor metabolite. Small amounts of both methanol isomers were found in the extract of the glucose-grown culture.

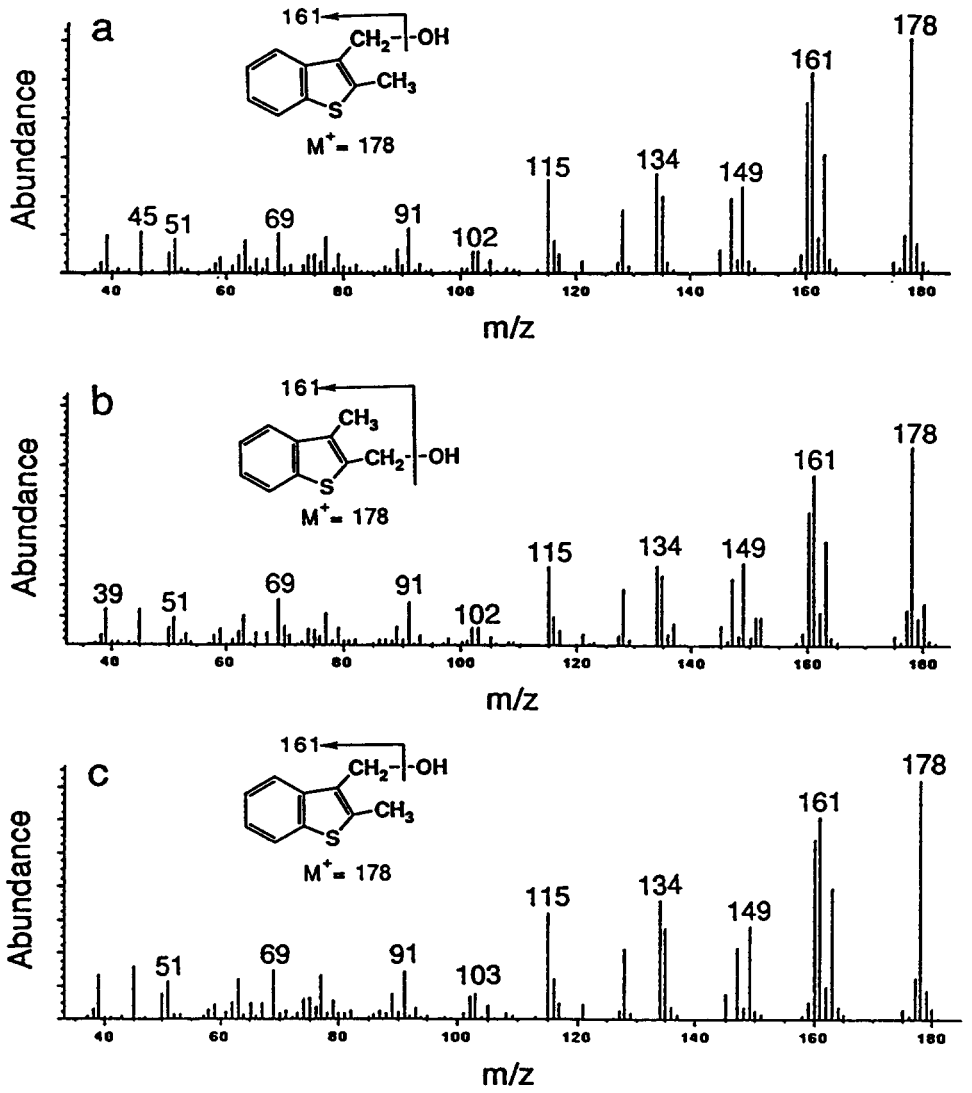


Figure 1. From GC-MS analyses, the mass spectra of two sulfur-containing metabolites from a culture of isolate W1 grown on glucose in the presence of 2,3-dimethylbenzothiophene. The metabolite with the shorter retention time (*a*) and the metabolite with the longer retention time (*b*). The mass spectrum of authentic 2-methyl-3-hydroxymethylbenzothiophene (*c*).

Metabolites from 2,7-dimethylbenzothiophene

Three abundant sulfur-containing metabolites were detected during the GC-FPD analysis of an extract of a culture of isolate W1 grown on glucose in medium containing 2,7-dimethylbenzothiophene. The metabolite that eluted last gave the mass spectrum shown in Figure 4. The molecular ion was at m/z 192 with a major fragment at m/z 147 $(M-45)^+$, corresponding to the loss of COOH, suggesting that one of the methyl groups had been oxidized to a carboxylic acid. The TMS-derivative was prepared and its mass spectrum was very similar to that shown in Figure 2b with the molecular ion at m/z 264, and abundant ions at m/z 249 $(M-15)^+$, 175 $(M-89)^+$, and 147 $(M-117)^+$. There was also an abundant fragment at m/z 205 $(M-59)^+$, a char-

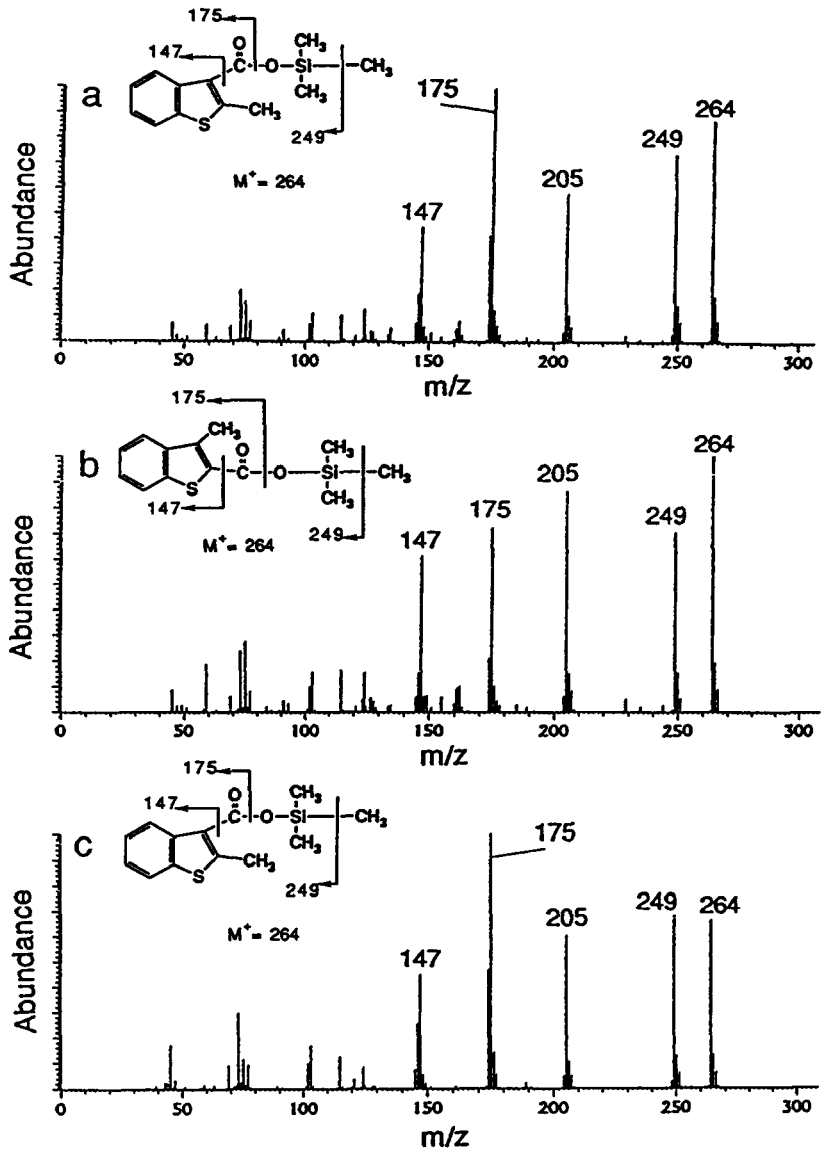


Figure 2. From GC-MS analyses, the mass spectra of the TMS-derivatives of two metabolites of 2,3-dimethylbenzothiophene (*a,b*) from a culture of isolate W1 grown on glucose. The mass spectrum of the TMS-derivative of authentic 2-methylbenzothiophene-3-carboxylic acid (*c*).

acteristic fragmentation of TMS esters of carboxylic acids.

GC-MS analysis revealed that a second sulfur-containing metabolite in the extract of this culture gave a mass spectrum (Figure 5a) with the molecular ion at m/z 178, consisent with the metabolite being the sulfoxide of 2,7-dimethylbenzothiophene. The fragments at m/z 163, 162 and 161 correspond to the losses of CH_3, O and OH, respectively. The base peak at m/z 135 may be the result of the loss of the oxygen atom, the C-2 atom and the C-2 methyl group $(M-43)^+$. Porter (1967) demonstrated the loss of the C-2 atom and the C-2 methyl group $(M-27)^+$ from 2-methylbenzothiophene. GC-FTIR analysis of this extract showed that the metabolite gave a strong absorption at 1089 cm^{-1} (Figure 5b) which is characteristic of a sulfoxide (Shriner et al. 1980).

GC-MS analysis showed that the third sulfur-containing metabolite in this extract had a molecular ion, which was the base peak, at m/z 178. Other abundant ions were at m/z 161, 149, 134 and 115. These ions were found in the spectrum of 2-methyl-3-hydroxymethylbenzothiophene (Figure 1), suggesting that this metabolite was the result of the microbial oxidation of one of the methyl groups of 2,7-dimethylbenzothiophene to yield a methanol. GC-MS analysis

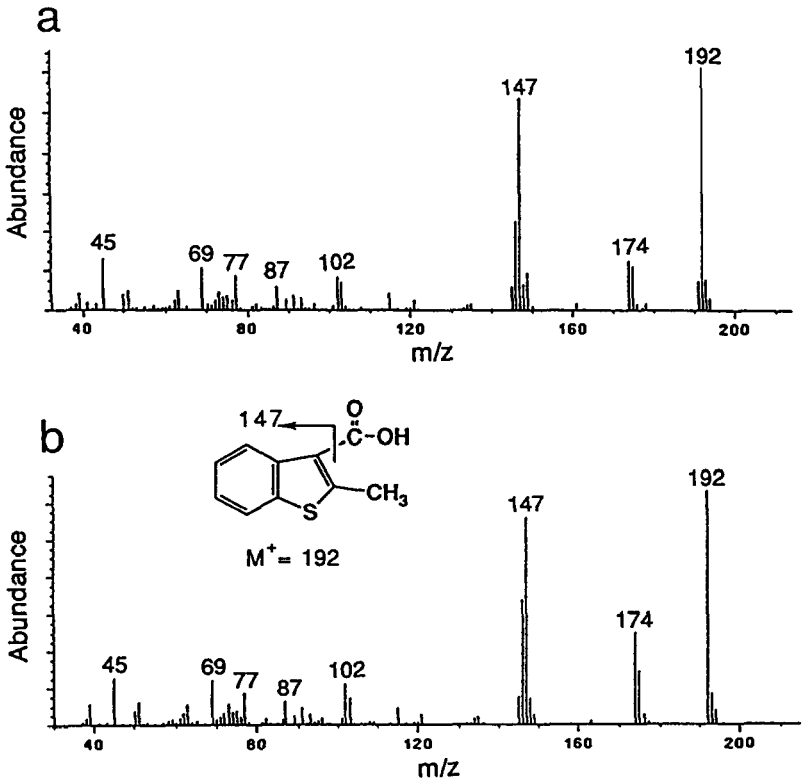


Figure 3. From GC-MS analyses, the mass spectra of a sulfur-containing metabolite (*a*) from a culture of isolate F grown on glucose in the presence of 2,3-dimethylbenzothiophene and authentic 2-methylbenzothiophene-3-carboxylic acid (*b*).

of this extract after it was treated with BSA showed the loss of this peak and the apearance of a new peak with a molecular ion at m/z 250, and a base peak at m/z 161, resulting from the loss of $OSi(CH_3)_3$. This is consistent with the formation of the TMS-derivative of a hydroxymethyl-methylbenzothiophene.

GC-FPD analysis of the dichloromethane extract of isolate BT1 grown on 1-methylnaphthalene with 2,7-dimethylbenzothiophene showed a single, major sulfur-containing metabolite. The mass and FTIR spectra of this compound were nearly identical to those shown in Figure 5. Thus, the metabolite was 2,7-dimethylbenzothiophene sulfoxide. GC-FTIR analysis showed two different spectra existed within the peak of the sulfoxide. Closer examination showed that a second compound eluted on the tail of the sulfoxide peak. Its FTIR spectrum had two strong absorptions at 1327 and 1160 cm^{-1} which are the same as an authentic standard of 2,7-dimethylbenzothiophene sulfone. The sulfone could not be detected by GC-MS analysis of this extract. Isolate BT1 also produced a small amount of a single isomer of methylbenzothiophene-carboxylic acid with a mass spectrum similar to that of the acid product from isolate W1 shown in Figure 4.

GC-MS analysis of an extract of a culture of isolate F grown on 1-methylnaphthalene in liquid medium containing 2,7-dimethylbenzothiophene showed four sulfur-containing products. The metabolites were: a methylbenzothiophene-carboxylic acid; 2,7-dimethylbenzothiophene sulfoxide; and two isomers of the hydroxymethyl-methylbenzothiophenes. The same metabolites were detected when isolate F was grown on glucose in the presence of 2,7-dimethylbenzothiophene. The sulfone of 2,7-dimethylbenzothiophene was not detected in any of the extracts of isolates F or W1.

Metabolites from 3,5-dimethylbenzothiophene

Four metabolites were detected by GC-FPD analysis of an extract of a culture of isolate W1 grown in medium with glucose and 3,5-dimethylbenzothiophene. GC-MS analysis showed that two of these compounds had virtually identical mass spectra (Figure 6), with the molecular ion at m/z 192. The major ions at

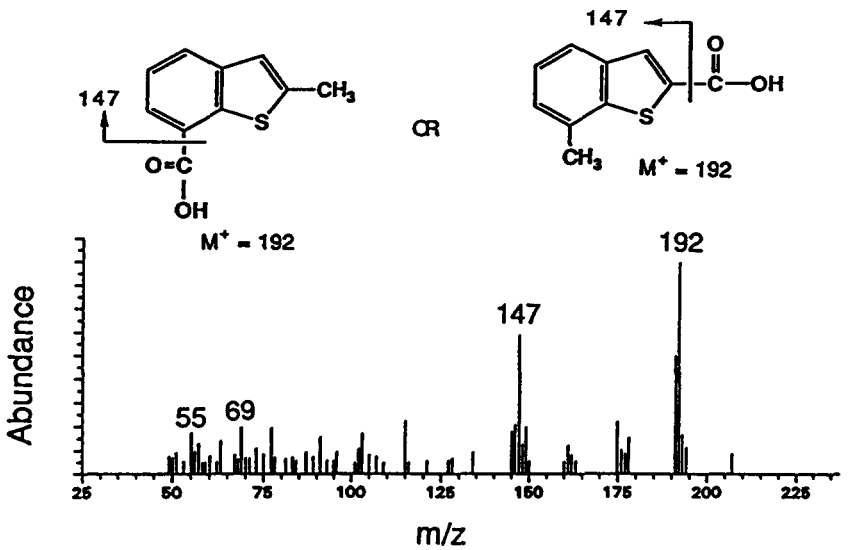


Figure 4. From GC-MS analysis, the mass spectrum of a sulfur-containing metabolite from a culture of isolate W1 grown on glucose in the presence of 2,7-dimethylbenzothiophene.

m/z 175 and 147 are the $(M-17)^+$ and $(M-45)^+$ ions, corresponding to the loss of OH and COOH, respectively. These data suggested that the metabolites were the results of oxidation of each of the two methyl substituents to carboxyl groups resulting in two methylbenzothiophene-carboxylic acids. The ion at m/z 147 was observed in Figures 3, 4 and 6, however the ion at m/z 175 was observed in the carboxylic acids from 3,5-dimethylbenzothiophene (Figure 6) and 2,7-dimethylbenzothiophene (Figure 4), but was not as abundant as the ion at m/z 174 $(M-18)^+$ in the acids from 2,3-dimethylbenzothiophene (Figure 3). The methyl group adjacent to the carboxy group in the acids from 2,3-dimethylbenzothiophene facilitated the loss of H_2O, rather that OH.

The methyl esters of these acids from 3,5-dimethylbenzothiophene were prepared by treating the culture extract with BF_3 in methanol. GC-MS analysis of these methyl esters showed that they had a molecular ion at m/z 206, and major fragments at m/z 175 $(M-CH_3O)^+$, and 147 $(M-CH_3OCO)^+$. These results are consistent with the metabolites being 3-methylbenzothiophene-5-carboxylic and 5-methylbenzothiophene-3-carboxylic acids. No standards of these two acids were available, but the methyl ester of authentic 2-methylbenzothiophene-3-carboxylic acid was prepared and its mass spectrum showed a molecular ion at m/z 206, and abundant ions at m/z 175 and 147.

GC-MS analysis showed the other two sulfur-containing metabolites from 3,5-dimethylbenzothiophene had very similar mass spectra. The molecular ion was at m/z 178, and the loss of OH gave an $(M-17)^+$ fragment at m/z 161. These results suggested that the metabolites were the products of oxidation of a methyl group to the corresponding methanol. Other abundant ions were at m/z 149, 134 and 115. These ions were observed in the spectrum of authentic 2-methyl-3-hydroxymethylbenzothiophene (Figure 1). TMS-derivatives of these metabolites were prepared and their mass spectra showed a molecular ion at m/z 250, and the fragments at m/z 235, from the loss of CH_3, and m/z 161, from the loss of $OSi(CH_3)_3$. Although these two products may be phenols, it is more likely that they are the products of the oxidation of the two methyl groups to methanols because two methylbenzothiophene-carboxylic acids were detected as metabolites from 3,5-dimethylbenzothiophene (Figure 6).

Sulfur-containing metabolites detected from 3,5-dimethylbenzothiophene in a 1-methylnaphthalene-grown culture of isolate F were the two methanols and a monocarboxylic acid. In the extract from a glucose-grown culture of isolate F (with 3,5-dimethylbenzothiophene in the medium), a single methanol was detected, along with two carboxylic acids arising from the oxidation of each of the two methyl groups.

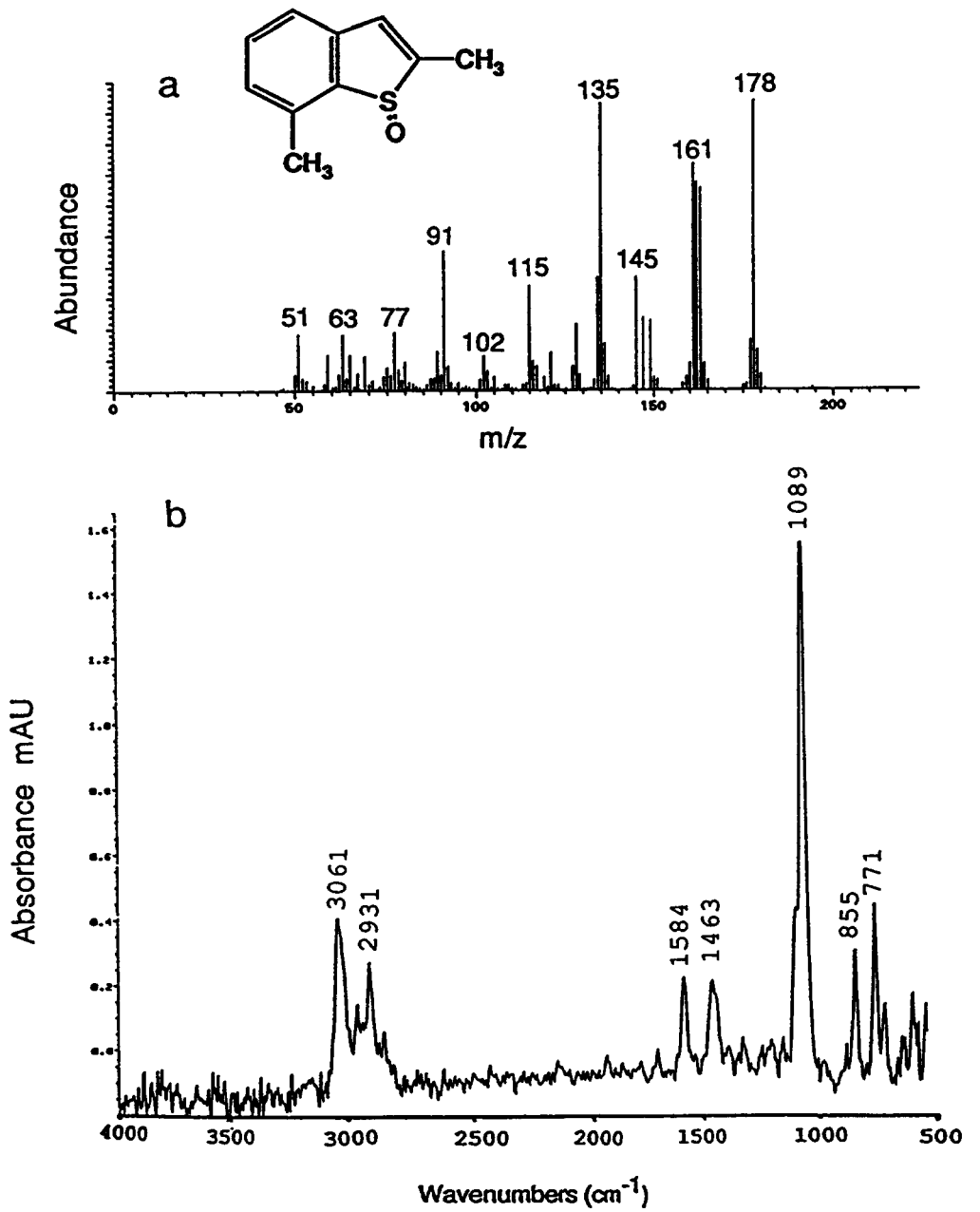


Figure 5. Mass spectrum (*a*) and FTIR spectrum (*b*) of a sulfur-containing metabolite from a culture of isolate W1 grown on glucose in the presence of 2,7-dimethylbenzothiophene.

Surprisingly, no sulfur-containing metabolites were found in the extracts of isolate BT1 grown for 7 days in the presence of 3,5-dimethylbenzothiophene. In addition, only trace amounts of residual 3,5-dimethylbenzothiophene were found in these extracts.

Metabolites from 3,7-dimethylbenzothiophene

GC analysis of an extract of a culture of isolate W1 grown in glucose-containing medium with 3,7-dimethylbenzothiophene showed five sulfur-containing metabolites. The last two of these to elute gave mass spectra with base peaks at m/z 192, which were the molecular ions. The next most abundant frag-

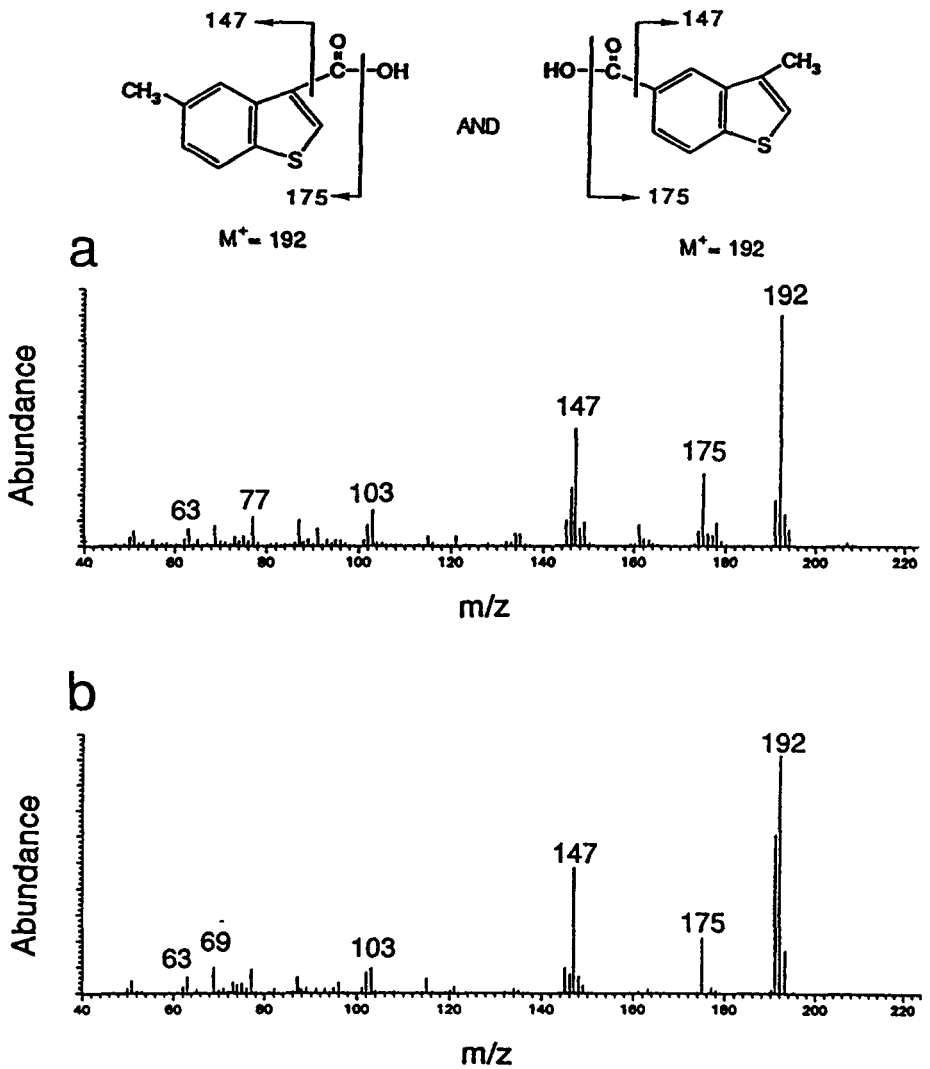


Figure 6. From GC-MS analysis, the mass spectra of two sulfur-containing metabolites from a culture of isolate W1 grown on glucose in the presence of 3,5-dimethylbenzothiophene. The metabolite with the shorter retention time (*a*) and the metabolite with the longer retention time (*b*).

ments were at m/z 147 and 175. These were consistent with the metabolites being methylbenzothiophenecarboxylic acids. The TMS-derivatives were prepared and the GC-MS analysis showed molecular ions at m/z 264 with major fragments at 249, 205 (base peak), 175 and 147. With the exception of the mass to charge ratio of the base peak, these spectra matched those of the TMS-derivatives of the methylbenzothiophenecarboxylic acids from 2,3-dimethylbenzothiophene (Figure 2).

The metabolite that eluted just prior to the carboxylic acids had a molecular ion at m/z 178. This molecular weight results from the incorporation of a single oxygen atom into 3,7-dimethylbenzothiophene. However, this metabolite did not react with the TMS-derivatizing reagents so it was not an alcohol or phenol. GC-FTIR analysis showed a strong absorption at 1069 cm^{-1} which is characteristic of a sulfoxide. Thus, the metabolite was 3,7-dimethylbenzothiophene sulfoxide. Interestingly, no sulfone was detected.

The first two metabolites to elute from the GC-MS also had base peaks that were the molecular ions at m/z 178 and very similar mass spectra with major fragments at m/z 161, 149, 134, and 115, like those in Figure 1. Both of these products reacted with BSA giving products with molecular ions at m/z 250. The data sug-

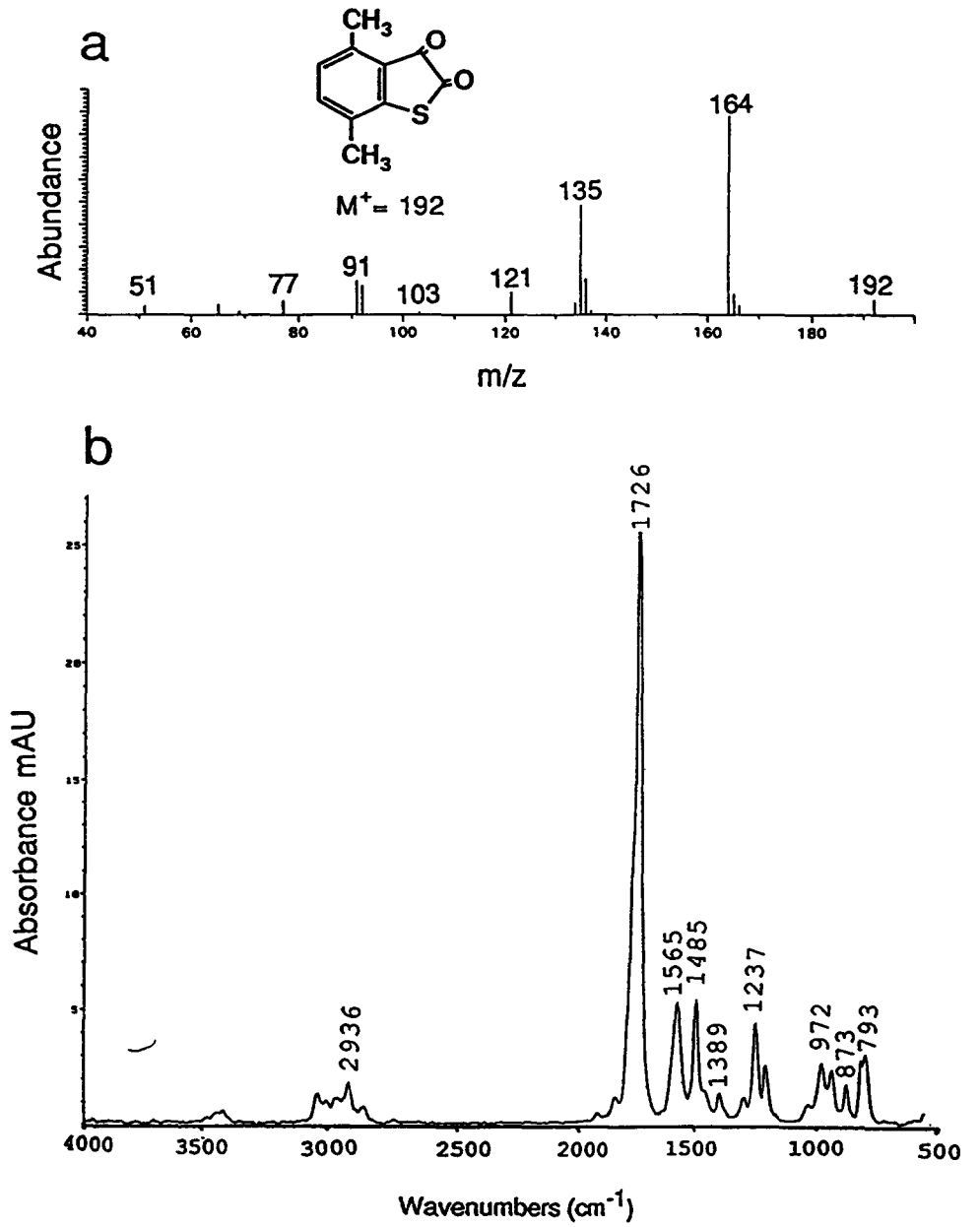


Figure 7. Mass spectrum (*a*) and FTIR spectrum (*b*) of a sulfur-containing metabolite from a culture of isolate BT1 grown on 1-methylnaphthalene in the presence of 4,7-dimethylbenzothiophene. The metabolite was identified as 4,7-dimethylbenzothiophene-2,3-dione.

gest that these two products are hydroxy-substituted 3,7-dimethylbenzothiophenes, presumably methanols.

GC-MS analyses of extracts of cultures of isolate F grown on glucose or 1-methylnaphthalene in medium containing 3,7-dimethylbenzothiophene showed that it produced the same five metabolites as isolate W1. Under the same growth conditions, the major metabolite produced by isolate BT1 was 3,7-dimethylbenzothiophene sulfoxide. In addition, a small amount of a metabolite with the same mass spectrum and retention time as authentic 3,7-dimethylbenzothiophene sulfone (M^+ = 194) was detected in these extracts. When grown on glucose, isolate BT1 oxidized the methyl groups producing the two methanols from 3,7-dimethylbenzothiophene in addition to the sulfoxide and sulfone.

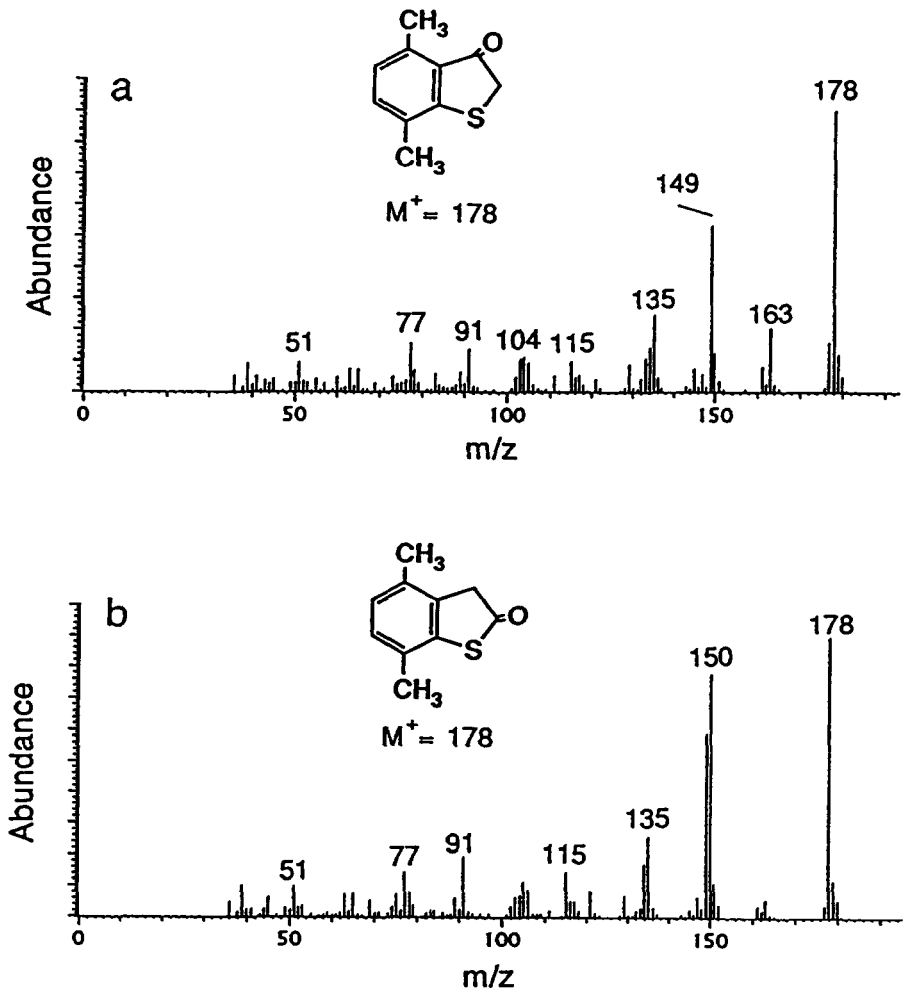


Figure 8. From GC-MS analysis, the mass spectra of two sulfur-containing metabolites from a culture of isolate BT1 grown on 1-methylnaphthalene in the presence of 4,7-dimethylbenzothiophene. The metabolite with the shorter retention time (*a*) and the metabolite with the longer retention time (*b*).

Metabolites from 4,7-dimethylbenzothiophene

GC-FPD analysis of an extract of a culture of isolate BT1 grown on 1-methylnaphthalene in the presence of 4,7-dimethylbenzothiophene showed an abundant sulfur-containing metabolite and several other minor products. The mass spectrum of the most abundant metabolite (Figure 7a) showed a weak molecular ion at m/z 192. This molecular weight was consistent with the structure of 4,7-dimethylbenzothiophene-2,3-dione. The base peak (m/z 164) resulted from the loss of CO $(M-28)^+$. Subsequent loss of CHO gave the abundant fragment at m/z 135 $(M-57)^+$. Fragments at $(M-28)^+$ were found in the mass spectra of benzothiophene-2,3-dione (Fedorak & Grbić-Galić 1991) and 5-methylbenzothiophene-2,3-dione (Saftić et al. 1992). Similarly, the $(M-57)^+$ was observed in the mass spectra of 5-methyl- and 7-methylbenzothiophene-2,3-diones (Saftić et al. 1992). GC-FTIR analysis of this metabolite (Figure 7b) showed a single strong absorption at 1726 cm^{-1}, similar to the absorptions at 1737 cm^{-1} and 1735 cm^{-1} observed for 5-methyl- and 7-methylbenzothiophene-2,3-diones, respectively. These data indicate that the most abundant metabolite was 4,7-dimethylbenzothiophene-2,3-dione.

Eluting before the 2,3-dione, and within 0.5 min of each other, were two sulfur-containing metabolites that each had a molecular weight of 178. The product with the shorter retention time fragmented to give ions at

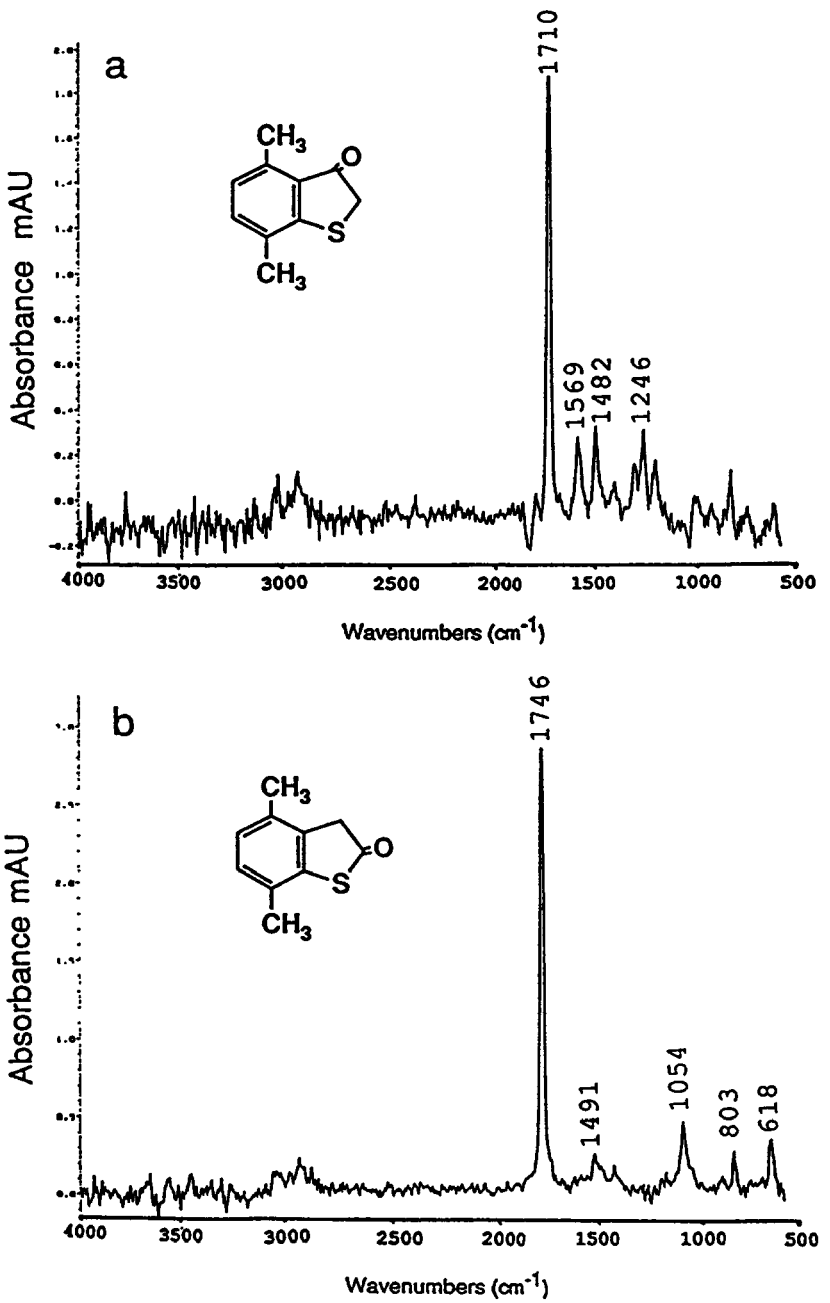


Figure 9. From GC-FTIR analysis, the FTIR spectra of two sulfur-containing metabolites from a culture of isolate BT1 grown on 1-methylnaphthalene in the presence of 4,7-dimethylbenzothiophene. The metabolite with the shorter retention time (*a*) and the metabolite with the longer retention time (*b*).

m/z 163 $(M-15)^+$ and 149 $(M-29)^+$(Figure 8a), whereas the product with the longer retention time showed an abundant fragment ion at m/z 150 $(M-28)^+$(Figure 8b). Although these products had a molecular weight that was consistent with the incorporation of a single atom of oxygen into 4,7-dimethylbenzothiophene, they did not show fragmentation patterns that were commonly observed with hydroxy-substituted dimethylbenzothiophenes, such as the loss of OH $(M-17)^+$ (Figure 1). Furthermore, GC-FTIR analysis showed that the product with the shorter retention time gave a strong absorption at 1710 cm^{-1} (Figure 9a) whereas the other prod-

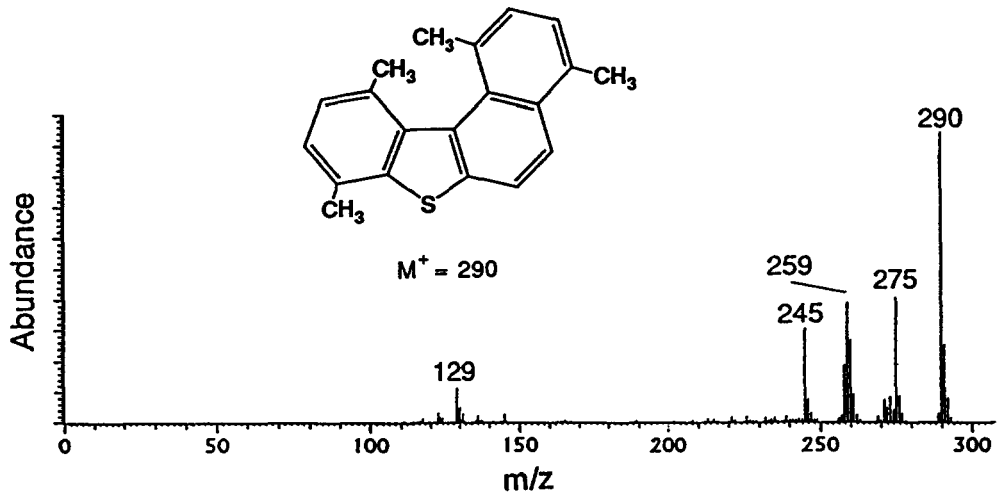


Figure 10. From GC-MS analysis, the mass spectrum of a high-molecular-weight sulfur-containing metabolite from a culture of isolate BT1 grown on 1-methylnaphthalene in the presence of 4,7-dimethylbenzothiophene.

uct gave a strong absorption at 1746 cm^{-1} (Figure 9b). Thus, the oxygen atom in each of these products was in a carbonyl group.

It was speculated that these two products were 4,7-dimethylbenzothiophene-3(2H)-one and 4,7-dimethylbenzothiophene-2(3H)-one, and these two compounds were synthesized. The former compound had the same retention time as the metabolite with the shorter retention time, and the same mass and FTIR spectra as shown in Figures 8a and 9a, respectively. The second metabolite (Figures 8b and 9b) had identical characteristics to the authentic standard of 4,7-dimethylbenzothiophene-2(3H)-one. Thus, these two products were the keto tautomers of 3-hydroxy-4,7-dimethylbenzothiophene and 2-hydroxy-4,7-dimethylbenzothiophene. The equilibrium for the keto-enol tautomerism lies strongly toward the keto form so that the oxygen atom is observed to be in a carbonyl group during GC-FTIR analysis.

In addition to these metabolites, there were several high-molecular-weight, sulfur-containing products in this extract that could not be identified. One had a molecular weight of 290 (Figure 10), and was likely a tetramethylbenzo[*b*]naphtho[1,2-*d*]thiophene ($C_{20}H_{18}S$) which could arise from the condensation of two molecules of 4,7-dimethylbenzothiophene in an analogous manner to that by which benzo[*b*]naphtho[1,2-*d*]thiophene was formed from benzothiophene (Kropp et al. 1994b).

Other high-molecular-weight compounds had molecular ions at m/z 292, 308, 322, 324, 340, and 342. Many of these products gave spectra in which the loss of methyl groups from the molecular ion was an abundant fragmentation, suggesting that these were some forms of condensation products with numerous methyl groups arising from the dimethylbenzothiophene. These products may be intermediates in the condensation reaction which leads to formation of the tetramethylbenzonaphthothiophene. They may also be products of bacterial oxidation of the tetramethylbenzonaphthothiophene. We did not pursue the identification of these minor products.

When strains F and W1 were grown on 1-methylnaphthalene in the presence of 4,7-dimethylbenzothiophene, they produced 4,7-dimethylbenzothiophene-2,3-dione, 4,7-dimethylbenzothiophene-3(2H)-one, 4,7-dimethylbenzothiophene-2(3H)-one and high-molecular-weight sulfur-containing products with molecular ions at m/z 290 (corresponding to $C_{20}H_{18}S$), 292, 308, 322, 340, and 342. In addition, each strain produced two metabolites that had mass spectra similar to those shown in Figure 6, with M^+ = 192, and major fragments at m/z 175 $(M-17)^+$ and 147 $(M-45)^+$. These fragments corresponded to the loss of OH and COOH, respectively, suggesting that the metabolites were the two possible monocarboxylic acids. To verify this, the TMS-esters were prepared and GC-MS analysis gave the mass spectra with M^+ =

264 and the fragmentation pattern: m/z 249 $(M-15)^+$, 205 $(M-59)^+$, 175, and 147 as observed in Figure 2.

Metabolites from 4,6-dimethylbenzothiophene

In the extract of a 1-methylnaphthalene-grown culture of isolate BT1 incubated with 4,6-dimethylbenzothiophene was a metabolite with a weak molecular ion at m/z 192, a base peak at m/z 164 $(M-28)^+$ and another abundant fragment at m/z 135 $(M-57)^+$. This spectrum suggested that the metabolite was 4,6-dimethylbenzothiophene-2,3-dione because it was very similar to that of 4,7-dimethylbenzothiophene-2,3-dione identified by GC-MS and GC-FTIR analyses (Figure 7) of extracts of cultures of isolates W1, BT1, and F when grown on 1-methylnaphthalene in the presence of 4,7-dimethylbenzothiophene.

Isolate BT1 also oxidized 4,6-dimethylbenzothiophene to give two minor products which nearly coeluted during GC analysis. These metabolites had identical mass spectra to the 3(2H)-one and 2(3H)-one of 4,7-dimethylbenzothiophene (see Figure 8). To prove the identities of these two metabolites, 4,6-dimethylbenzothiophene-3(2H)-one and 4,6-dimethylbenzothiophene-2(3H)-one were synthesized. The metabolite that eluted first had the same retention time and mass spectrum as the authentic 3(2H)-one. Similarly, the GC retention time and mass spectrum of the second metabolite matched those of the authentic 2(3H)-one.

This extract from isolate BT1 also contained several high-molecular-weight, sulfur-containing products that were not identified. Among these were products with abundant molecular ions at m/z 290, 322, 324 and 340. The product with a molecular ion at m/z 290 is consistent with the structure of a tetramethylbenzo[*b*]naphtho[1,2-*d*]thiophene ($C_{20}H_{18}S$) and had a mass spectrum similar to the analagous high-molecular-weight product from 4,7-dimethylbenzothiophene (Figure 10).

4,6-Dimethylbenzothiophene-2,3-dione was the major metabolite found in extracts of isolates F and W1 grown on 1-methylnaphthalene in the presence of 4,6-dimethylbenzothiophene. Also found in these extracts were minor amounts of the 3(2H)-one and 2(3H)-one of 4,6-dimethylbenzothiophene. A trace amount of the high-molecular-weight product with a molecular weight of 290 ($C_{20}H_{18}S$) was detected in the extract from isolate F, but not in that of isolate W1.

Additional investigations with isolate BT1

Further studies with strain BT1 were prompted by the observations that this bacterium completely removed 3,5-dimethylbenzothiophene from its growth medium after 7 days of incubation and that no oxidation products were detected. When a culture was incubated for 1 day, its extracts contained two isomers of hydroxymethyl-methylbenzothiophene and two isomers of methylbenzothiophene-carboxylic acid. No metabolites were found in a culture that was incubated for 3 days. Thus, isolate BT1 oxidized the methyl groups of 3,5-dimethylbenzothiophene to methanols and carboxylic acids which were transient metabolites.

It was hypothesized that this strain might oxidize both methyl groups, yielding a dicarboxylic acid that was too polar to be recovered by the extraction method used. Thus, 3-, 5-, and 7-day-old cultures were adjusted to pH 12, freeze-dried and the residue from each culture was refluxed with methyl alcohol and a catalytic amount of sulfuric acid to form the methyl esters of any carboxylic acids present. However, the hypothesized product, 3,5-benzothiophenedicarboxylic acid, was not detected by GC-MS analyses in any of the cultures. The validity of this method was verified using 2,5-thiophenedicarboxylic acid, which is too polar to extract into dichloromethane from an aqueous solution at pH 2. Indeed, the dimethyl ester of this acid was readily detected by GC analysis after the freeze-drying and esterification procedures.

To determine whether strain BT1 could oxidize the methyl groups of 3-methylbenzothiophene and 5-methylbenzothiophene, it was also grown on either 1-methylnaphthalene or glucose with one of these condensed thiophenes in the growth medium for 1, 3, or 14 days. Interestingly, neither the methanol nor the carboxylic acid of these two isomers was detected after any incubation period.

The ability of isolate BT1 to grow on 1-naphthalenemethanol and 1-naphthoic acid (50 mg per 200-mL culture) was tested. Lag periods of 2 days and 6 days, respectively, were observed with these substrates. Stationary phase (OD_{600} = 0.3) was reached after 5 days of incubation with 1-naphthalenemethanol, and after 8 days of incubation with 1-naphthoic acid. In contrast, stationary phase was reached after 1 day of incubation with 1-methylnaphthalene.

Table 1. Summary of the sulfur-containing products found in the extracts of three bacterial cultures after incubation with various dimethylbenzothiophenes for 7 days. The products were found in cultures grown on 1-methylnaphthalene or glucose.

Substrate	Products found in cultures of *Pseudomonas* strain		
	BT1	W1	F
2,3-dimethyl-benzothiophene	sulfoxide sulfone methanols	sulfoxide sulfone methanols carboxylic acids	sulfoxide sulfone methanols carboxylic acids
2,7-dimethyl-benzothiophene	sulfoxide sulfone carboxylic acid	sulfoxide methanol carboxylic acid	sulfoxide methanols carboxylic acid
3,5-dimethyl-benzothiophene	none	methanols carboxylic acids	methanols carboxylic acids
3,7-dimethyl-benzothiophene	sulfoxide sulfone methanols	sulfoxide methanols carboxylic acids	sulfoxide methanols carboxylic acids
4,6-dimethyl-benzothiophene	2,3-dione[a] 3(2H)-one 2(3H)-one $C_{20}H_{18}S$[b]	2,3-dione[a] 3(2H)-one 2(3H)-one	2,3-dione[a] 3(2H)-one 2(3H)-one $C_{20}H_{18}S$
4,7-dimethyl-benzothiophene	2,3-dione[a] 3(2H)-one 2(3H)-one $C_{20}H_{18}S$[b]	2,3-dione[a] 3(2H)-one 2(3H)-one carboxylic acids $C_{20}H_{18}S$[b]	2,3-dione[a] 3(2H)-one 2(3H)-one carboxylic acids $C_{20}H_{18}S$[b]

[a] likely a dimethyl-substituted 2-mercaptophenylglyoxalate at neutral pH
[b] and several other high-molecular-weight products

Discussion

Six of the possible 15 isomers of dimethylbenzothiophene were used in this investigation. The isomers were chosen to have a variety of substitution patterns: both methyl groups on the thiophene ring (the 2,3- isomer); a methyl group on each of the rings (the 2,7-, 3,5- and 3,7- isomers); and both methyl groups on the benzene ring (the 4,6- and 4,7- isomers).

Table 1 summarizes the sulfur-containing metabolites detected in the extracts of 7-day-old cultures of the three *Pseudomonas* strains studied. The plural entries 'methanols' and 'carboxylic acids' indicate that two isomers of these compounds were found in the extracts.

Sulfoxides and sulfones were detected from only those compounds with a methyl group on the thiophene ring (ie. the 2,3-, 2,7- and 3,7- isomers). In contrast, 2,3-diones were detected from those compounds with no methyl groups on the thiophene ring (ie. the 4,6- and 4,7- isomers). These findings are consistent with the predictions of Fedorak & Grbić-Galić (1991) and observations of Saftić et al. (1992) with monomethylbenzothiophenes, although in the latter study and in that of Kropp et al. (1994a) sulfoxides and sulfones were observed from some methylbenzothiophenes with unsubstituted thiophene rings. The 2,3-diones detected in these cultures (Table 1) likely existed as dimethyl-substituted 2-mercaptophenylglyoxalates in the culture medium at neutral pH. Indeed, Eaton & Nitterauer (1994) showed that benzothiophene was microbially oxidized to 2-mercaptophenylglyoxalate which cyclized to benzothiophene-2,3-dione when acidified. In our study, the cultures were routinely acidified prior to extraction.

Although no sulfoxides were detected in the cultures incubated with 4,6- or 4,7-dimethylbenzothiophenes, they were very likely produced, and then subsequently reacted to give the high-molecular-weight products with the empirical formula $C_{20}H_{18}S$. Kropp et al. (1994b) demonstrat-

ed that two molecules of benzothiophene sulfoxide undergo an abiotic Diel-Alder-type condensation to form benzo[*b*]naphtho[1,2-*d*]thiophene. Kropp et al. (1994a) detected dimethylbenzo[*b*]naphtho[1,2-*d*]thiophenes in cultures incubated with 4-, 5-, 6- or 7-methylbenzothiophene. However, no high-molecular-weight products were detected from 2- or 3-methylbenzothiophene because the methyl group on the thiophene ring sterically hinders the condensation reaction. The empirical formula $C_{20}H_{18}S$ is consistent with tetramethylbenzo[*b*]naphtho[1,2-*d*]thiophenes, and these products were not found in the extracts of the cultures incubated with isomers of dimethylbenzothiophene with a methyl group on the thiophene ring (2,3-, 2,7-, 3,5- and 3,7-dimethylbenzothiophenes). The sulfoxides of these isomers, with the exception of the 3,5- isomer, were detected in culture extracts (Table 1).

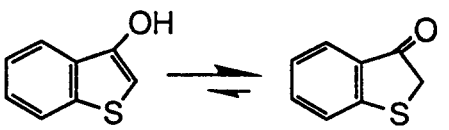
The metabolism of 3,5-dimethylbenzothiophene was unique among the six isomers studied (Table 1). In the 7-day-old cultures of strains W1 and F, methanols and carboxylic acids were found, but no sulfoxide was detected. No metabolites were found in the extract of a 7-day-old culture of strain BT1. Methanols and carboxylic acids were transient intermediates in cultures of strain BT1.

With the exception of 4,6-dimethylbenzothiophene, methyl group oxidation products were found in culture extracts from isolates W1 and F (Table 1). These isolates were known to produce methanols and carboxylic acids from methylbenzothiophenes (Kropp et al. 1994a) and methanols from some isomers of methyldibenzothiophene (Saftić et al. 1993). In addition, 1-naphthalenemethanol and 1-naphthoic acid were found to accumulate in cultures of strains W1 and F grown on 1-methylnaphthalene (Kropp et al. 1994a), and neither of these oxidation products serve as growth substrates for these two isolates. In contrast, methanols or carboxylic acids were found in fewer extracts from 7-day-old cultures of isolate BT1 (Table 1). Furthermore, 1-naphthalenemethanol and 1-naphthoic acid were produced by isolate BT1 but they only transiently accumulated in cultures of this isolate grown on 1-methylnaphthalene before they were further degraded. Indeed, 1-naphthalenemethanol and 1-naphthoic acid serve as growth substrates for strain BT1.

Studies on the bacterial metabolism of dimethylnaphthalenes have shown that the methyl groups are also susceptible to oxidation. For example, Dean-Raymond & Bartha (1975) showed that although dimethylnaphthalenes would not support growth of their bacterial isolates that grew on naphthalene and methylnaphthalenes, 1,5- and 2,6-dimethylnaphthalenes were oxidized to their corresponding monocarboxylic acids by naphthalene-grown resting cells. Similarly, Barnsley (1988) identified 2-hydroxymethyl-6-methylnaphthalene and 6-methyl-2-naphthalenecarboxylic acid as metabolites from 2,6-dimethylnaphthalene. In addition to the metabolites found by Barnsley (1988), Miyachi et al. (1993) detected 2,6-naphthalene dicarboxylic acid by HPLC analysis of fluids from cultures grown on 2,6-dimethylnaphthalene.

Because of the transient nature of the methanols and carboxylic acids produced from 3,5-dimethylbenzothiophene by strain BT1, attempts were made to detect the corresponding dicarboxylic acid. Since the methanols and carboxylic acids were present after 1 and 2 days incubation but had been depleted from the medium by the third day, we tried to detect the dicarboxylic acid over incubation periods of 3, 5 and 7 days. The dicarboxylic acid was not detected, nor were any other sulfur-containing metabolites. Thus, the fates of the methanols and carboxylic acids from 3,5-dimethylbenzothiophene are unknown, and further investigations are required to determine the identities of subsequent metabolites.

The 3(2H)-ones and 2(3H)-ones of 4,6- and 4,7-dimethylbenzothiophene (Table 1) are novel products. 3-Hydroxybenzothiophene (the enol form shown below) and 2-hydroxybenzothiophene are known to exist almost exclusively as their keto tautomers (Iddon & Scrowston 1970) and therefore are more appropriately called benzothiophene-3(2H)-one and benzothiophene-2(3H)-one, respectively.



enol form keto form

These compounds react as if they have a hydroxyl group on the thiophene ring. For example, the methyl ethers of the 2(3H)-one and the 3(2H)-one can be formed by the methods of Dickinson & Iddon (1970) and Friedländer (1907), respectively. In this study, the 3(2H)-ones and 2(3H)-ones of the dimethylbenzothiophenes tautomerized to the enol forms which reacted with BSA giving TMS derivatives.

In their studies on bacterial metabolism of benzothiophene, Eaton & Nitterauer (1994) identified 2-hydroxybenzothiophene and 3-hydroxybenzothiophene. They proposed that these phenols were the result of dehydration of 2,3-dihydroxy-2,3-dihydrobenzothiophene. Phenol formation resulting from dehydration of dihydrodiols under acidic conditions has been observed in studies with dibenzothiophene (Laborde & Gibson 1977), phenanthrene and anthracene (Jerina et al. 1976) and carbazole (Resnick et al. 1993). Thus, the 2(3H)-ones and the 3(2H)-ones found in the extracts of cultures incubated with 4,6- and 4,7-dimethylbenzothiophene are likely the dehydration products of undetected 2,3-dihydrodiols.

During this investigation, quantitative analyses of the sulfur-containing metabolites were precluded for several reasons. For example, routine analyses were done with a FPD which gives nonlinear response and is susceptible to quenching. In addition, authentic standards of some products, such as the sulfoxides and the tetramethylbenzo[*b*]naphtho[1,2-*d*]thiophenes, were not available, and the quantities of other reference compounds that were synthesized, such as the 3(2H)- and 2(3H)-ones, were not sufficient for the preparation of calibration curves for quantitation using a flame ionization detector. The analyses of sulfoxides is also complicated by their decomposition in GC injection port liners (Fedorak & Andersson 1992).

The focus of these investigations was the identification of sulfur-containing metabolites that could be partitioned from an acidified culture into dichloromethane and that were amenable to GC analysis. Other metabolites may have been produced but not detected if they were too polar to be extracted or chromatographed. Nonetheless, nearly 30 sulfur-containing products were identified during this survey. The metabolism of 3,5-dimethylbenzothiophene by *Pseudomonas* strain BT1 deserves further study because of its complete removal of this compound from the medium and the absense of metabolites after 7 days of incubation.

Whether microbial oxidations of C_2-benzothiophenes in petroleum- or creosote-contaminated environments lead to the same metabolites that were identified in this laboratory study is yet to be determined. Similarly, the fates of the identified oxidation products in diverse microbial populations found in the environment have not been determined.

Acknowledgments

Funding for these studies was provided by Environment Canada, through the Groundwater and Soil Remediation Program, contract KA168-2-2191; by the Canadian Petroleum Products Institute; and by the Natural Sciences and Engineering Research Council of Canada. Financial support for the synthetic work was given by the Fonds der chemischen Industrie. An award from the NATO Collaborative Research Grants Programme is also gratefully acknowledged. K.G. Kropp was supported by a University of Alberta PhD Scholarship. We thank D. Coy for technical assistance, and S.E. Hrudey and L. Harrower for the use of their GC-MS systems for analyses.

References

Andersson JT (1986) Gas chromatographic retention indices for all C_1- and C_2-alkylated benzothiophenes and their dioxides on three different stationary phases. J. Chromatogr. 354: 83–98

Andersson JT (1992) Polycyclic aromatic sulfur heterocycles. I. Use of hydrogen peroxide oxidation for the group separation of polycyclic aromatic hydrocarbons and their sulfur analogs. Intern. J. Environ. Anal. Chem. 48: 1–15

Atlas RM, Boehm PD & Calder JA (1981) Chemical and biological weathering of oil, from the Amoco Cadiz spillage, within the littoral zone. Estuarine, Coastal Shelf Sci. 12: 589–608

Baciocchi E & Mandolini L (1968) Products, kinetics, and mechanism of the chlorination of 2,3-dimethylbenzothiophene in acetic acid. J. Chem. Soc. (B). 397–400

Barnsley EA (1988) Metabolism of 2,6-dimethylnaphthalene by Flavobacteria. Appl. Environ. Microbiol. 54: 428–433

Bayona JM, Albaigés J, Solanas AM, Parés R, Garrigues P & Ewald M (1986) Selective aerobic degradation of methyl-substituted polycyclic aromatic hydrocarbons in petroleum by pure microbial cultures. Intern. J. Environ. Anal. Chem. 23: 289–303

Bohonos N, Chou T-W & Spanggord RJ (1977) Some observations on biodegradation of pollutants in aquatic systems. Jpn. J. Antibiot. 30(suppl): 275–285

Burchill P, Herod AA & Pritchard E (1982) Identification of sulphur heterocycles in coal tar and pitch. J. Chromatogr. 242: 51–64

Crawford DL & Gupta RK (1990) Oxidation of dibenzothiophene by *Cunninghamella elegans*. Current Microbiol. 21: 229–231

Dean-Raymond D & Bartha R (1975) Biodegradation of some polynuclear aromatic petroleum components by marine bacteria. Dev. Ind. Microbiol. 16: 97–110

Dickinson RP & Iddon B (1970) Condensed thiophen ring systems. Part III. A new synthesis of benzo[b]thiophen-2(3H)-and -3(2H)-ones and some reactions of benzo[b]thiophen-2(3H)-one with dimethylsulfate in the presence of base. J. Chem. Soc. (C). 1926–1928

Eaton RW & Nitterauer JD (1994) Biotransformation of benzothiophene by isopropylbenzene-degrading bacteria. J. Bacteriol. 176: 3992–4002

Fedorak PM & Andersson JT (1992) Decomposition of two methylbenzothiophene sulphoxides in a commercial GC injection port liner. J. Chromatogr. 591: 362–366

Fedorak PM & Grbić-Galić D (1991) Aerobic microbial cometabolism of benzothiophene and 3-methylbenzothiophene. Appl. Environ. Microbiol. 57: 932–940

Fedorak PM & Westlake DWS (1983) Microbial degradation of organic sulfur compounds in Prudhoe Bay crude oil. Can. J. Microbiol. 29: 291–296

Fedorak PM & Westlake DWS (1984) Degradation of sulfur heterocycles in Prudhoe Bay crude oil by soil enrichments. Water, Air Soil Pollut. 21: 225–230

Friedländer P (1906) Ueber schwefelhaltige Analoga der Indigogruppe. Chem. Ber. 39: 1060–1066

Friedländer P (1907) Ueber Thionaphtenderivate und Thioindigo. Annalen. 351: 390–420

Grummitt O & Buck A (1955) Organic Syntheses Coll. Vol. 3. John Wiley & Sons, Inc., New York. pp. 195–197

Hannoun M, Blazević N, Kolbah D, Mihalić M & Kajfez F (1982) *alpha*-Phenylpropionic acid derivatives. Synthesis and dethiation of 5-benzoylbenzo[b]thiophene-3-carboxylic acid. J. Heterocyclic Chem. 19: 1131–1136

Hostettler FD & Kvenvolden KA (1994) Geochemical changes in crude oil spilled from the *Exxon Valdez* supertanker into Prince William Sound, Alaska. Org. Geochem. 21: 927–936

Hou CT & Laskin AI (1976) Microbial conversion of dibenzothiophene. Dev. Ind. Microbiol. 17: 351–362

Iddon B & Scrowston RM (1970) Recent advances in the chemistry of benzo[b]thiophenes. In: Katritzky AR & Boulton AJ (Eds) Advances in Heterocyclic Chemistry, Vol. 11 (pp. 177–381) Academic Press, New York

Jerina DM, Delander H, Yagi H, Wells MC, Davey JF, Mahadevan V & Gibson DT (1976) Dihydrodiols from anthracene and phenanthrene. J. Am. Chem. Soc. 98: 5988-9596

Kodama K, Nakatani S, Umehara K, Shimizu K, Minoda Y & Yamada K (1970) Microbial conversion of petro-sulfur compounds. III. Isolation and identification of products from dibenzothiophene. Agric. Biol. Chem. 34: 1320–1324

Kodama K, Umehara K, Shimizu K, Nakatani S, Minoda Y & Yamada K (1973) Identification of microbial products from dibenzothiophene and its proposed oxidation pathway. Agric. Biol. Chem. 37: 45–50

Kropp KG, Gonçalves JA, Andersson JT & Fedorak PM (1994a) Bacterial transformations of benzothiophene and methylbenzothiophenes. Environ. Sci. Technol. 28: 1348–1356

Kropp KG, Gonçalves JA, Andersson JT & Fedorak PM (1994b) Microbially mediated formation of benzonaphthothiophenes from benzo[*b*]thiophenes. Appl. Environ. Microbiol. 60: 3624–3631

Laborde AL & Gibson DT (1977) Metabolism of dibenzothiophene by a *Beijerinkia* species. Appl. Environ. Microbiol. 34: 783–790

Miyachi N, Tanaka T, Suzuki T, Hotta Y & Omori T (1993) Microbial oxidation of dimethylnaphthalene isomers. Appl. Environ. Microbiol. 59: 1504–1506

Monticello DJ, Bakker D & Finnerty WR (1985) Plasmid-mediated degradation of dibenzothiophene by *Pseudomonas* species. Appl. Environ. Microbiol. 49: 756–760

Pierce AE (1968) Silylation of Organic Compounds (pp. 33–39). Pierce Chemial Company, Rockford, IL

Porter QN (1967) Mass-spectrometric studies. I. Benzo[*b*]thiophen, some alkyl and aryl derivatives, and the 1,1-dioxide and 2,3-dihydro-1,1-dioxide. Aust. J. Chem. 20: 103-116

Resnick SM, Torok DS & Gibson DT (1993) Oxidation of carbazole to 3-hydroxycarbazole by naphthalene 1,2-dioxygenase and biphenyl 2,3-dioxygenase. FEMS Microbiol Lett. 113: 297–302

Saftić S, Fedorak PM & Andersson JT (1992) Diones, sulfoxides and sulfones from the aerobic cometabolism of methylbenzothiophenes by *Pseudomonas* strain BT1. Environ. Sci. Technol. 26: 1759–1764

Saftić S, Fedorak PM & Andersson JT (1993) Transformations of methyldibenzothiophenes by three *Pseudomonas* isolates. Environ. Sci. Technol. 27: 2577–2584

Shriner RL, Fuson RC, Curtin DY & Morrill TC (1980) The Systematic Identification of Organic Compounds, 6th edn. John Wiley & Sons, New York

Silverstein RM, Bassler GC & Morrill TC (1991) Spectrometric Identification of Organic Compounds, 5th edn. John Wiley & Sons, New York

Sokol PE (1973) Organic Syntheses Coll. Vol. 5 John Wiley & Sons, Inc., New York. (pp. 706–708)

Speight JG (1980) The Chemistry and Technology of Petroleum. Marcel Dekker Inc., New York

Stridsberg R & Allenmark S (1974) Studies of the benzo[*b*]-thiophene-3(2H)-one system. Chemica Scripta 6: 184–192

Westlake DWS (1983) Microbial activities and changes in the chemical and physical properties of oil, pp. 102–111. In: Donaldson EC & Clark JB (Eds) Proc. 1982 International Conference on Microbial Enhancement of Oil Recovery, Shangri-La, Afton, OK. Bartlesville Energy Technology Centre, Bartlesville, OK

Willey C, Iwao M, Castle RN & Lee M (1981) Determination of sulfur heterocycles in coal liquids and shale oils. Anal. Chem. 53: 400–407

Williams JA, Bjorøy M, Dolcater DL & Winters JC (1986) Biodegradation of South Texas Eocene oils - effects on aromatics and biomarkers. Org. Geochem. 10: 451–461