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Short Communication

Decomposition of two methylbenzothiophene sulphoxides in a commercial gas chromatography injection port liner

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ABSTRACT

2-Methylbenzothiophene sulphoxide and 3-methylbenzothiophene sulphoxide were produced as metabolites by an aromatic hydrocarbon-degrading bacterial culture. Because dibenzothiophene sulphoxide is know to decompose in gas chromatography injectors, the two bacterial products were tested for their stability in a gas chromatography injection port containing a standard Jennings/cup mixing chamber. The two methylbenzothiophene sulphoxides were purified by silica gel chromatography and reversed-phase high-performance liquid chromatography. These sulphoxides decomposed in the injection port giving the corresponding methylbenzothiophene as the major product and the corresponding sulphone as a minor product. The proportions of decomposition products were quite variable and the amount of decomposition was greatest in an injection port liner that had been soiled by the routine analyses of dozens of petroleum samples.

INTRODUCTION

Benzothiophene and alkylbenzothiophenes are found in crude oils [1], shale oils [2,3] and coal tar [4]. Some of these compounds have been shown to be "biodegradable" in laboratory studies [5–7] and petroleum reservoir investigations [8]. However, little is known about the identities of the metabolites of these biotransformations or about the fates of the metabolites in laboratory cultures and in the environment [9]. Recent investigations showed that bacterial cometabolism of 3-methylbenzothiophene (3-MBT) [10] and 2-methylbenzothiophene (2-MBT) [11] yielded the sulphoxides and sulphones of these two compounds.

Dibenzothiophene (DBT) has been used extensively as a model compound to study the metabolism of organosulphur compounds found in petroleum [9]. DBT sulphoxide has been observed as a metabolite in bacterial [12,13] and mammalian [14] systems. Upon gas chromatographic (GC) analysis, DBT-sulphoxide has been shown to decompose in hot injection ports to DBT [15,16] and DBT sulphone [15]. The stabilities of benzothiophene sulphoxides to analysis by GC have not been addressed.

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Many chemical reactions that oxidize the sulphur atom in benzothiophenes do not stop at the sulphoxide, but they continue and produce the sulphone. Although the bacterial culture that we are studying produces trace amounts of sulphones from 3-MBT and 2-MBT, the more abundant oxidation products are the corresponding sulphoxides [10,11]. Surprisingly, this culture will not form the sulphoxides or sulphones of benzothiophene or some other isomers of methylbenzothiophene. This bacterial culture was used to produce the sulphoxides of 3-MBT and 2-MBT and these were isolated by high-performance liquid chromatography (HPLC) to determine whether they would decompose in a commercial GC injection port.

EXPERIMENTAL

Microbial production of the sulphoxides and sulphones of 3-MBT and 2-MBT

Pseudomonas strain BT1 was grown under shake-flask conditions at 28°C in 200 ml of mineral medium supplemented with trace metals [10]. To provide sufficient amounts of metabolites, seven replicate cultures were incubated simultaneously. For metabolite production from 3-MBT, each culture contained 50 μl of 1-methylnaphthalene, which served as the growth substrate, and approximately 10 μl of 3-MBT. To produce the metabolites from 2-MBT, each culture contained 250 mg of glucose/l, which served as the growth substrate, and approximately 10 mg of 2-MBT. After 14 days of incubation, the cultures were acidified to below pH 2 with HCl and extracted with methylene chloride (5 times 15 ml). The extracts were dried over anhydrous sodium sulphate and replicate extracts were combined and concentrated prior to column chromatography. The sulphoxides were identified by GC–mass spectrometry (MS) and GC–Fourier transform infrared spectroscopy (FTIR) [10,11] whereas the sulphones were identified by comparisons of the GC–FTIR spectra from the culture extracts with those of authentic standards.

Isolation of the sulphoxides and sulphones of 3-MBT and 2-MBT

The polar metabolites were isolated by silica gel column chromatography using a 30 cm × 1.1 cm I.D. glass column. A slurry of 7 g of silica gel (Fisher Scientific, 100–200 mesh, Type 150A, Grade 644, activated at 125°C for 24 h) in methylene chloride was poured into the column and the solvent was displaced with 50 ml of *n*-pentane. The methylene chloride extract from the culture was adsorbed to 0.2 g of silica gel, the solvent was removed under a stream of nitrogen gas, and the sample was loaded onto the column which was then developed with 5 ml of *n*-pentane, 5 ml methylene chloride–*n*-pentane (20:80), 25 ml of methylene chloride–*n*-pentane (50:50) and 30 ml methanol–benzene (50:50). The first 50 ml of the column effluent were discarded and the last 12 ml from the column contained the polar metabolites.

HPLC method and fraction collection

The solvent was evaporated from samples of culture extracts and the residues were dissolved in acetonitrile for HPLC analysis using a Hewlett-Packard series 1050 instrument with a reversed-phase LiChrospher 100 RP-18 column (5 μm, 125 mm × 4 mm I.D., Hewlett-Packard). Two mobile phases were used. For routine analysis, acetonitrile–water (50:50) (solvent 1) was used. To obtain baseline separation for the manual collection of the fractions containing the sulphoxides and sulphones, acetonitrile–water (25:75) (solvent 2) was used. In both cases, the flow-rate was 1.0 ml/min and the effluent was monitored at 280 nm.

GC analysis of the sulphoxides and sulphones of 3-MBT and 2-MBT

Culture extracts and collected fractions were analyzed by capillary GC using a 30 m × 0.25 mm O.D. (0.25 μm film thickness) DB-5 column (J & W Scientific) housed in a Hewlett-Packard (Model 5890) GC system equipped with flame ionization detection (FID) and flame photometric detection (FPD). The column effluent was split so that the sample was analyzed simultaneously by the two detection systems [5]. The decomposition products were identified on the basis of their retention times on the GC–FPD chromatogram which matched those of authentic standards. The injector temperature was 250°C and the oven temperature program used for all GC analyses was; 90°C for 2 min, 4°C/min to 250°C which was held for 16 min. All other operating conditions were as previously reported [5]. The injection split ratio was approximately 1:20 and the

injector contained a standard Hewlett-Packard Jennings/cup mixing chamber (part No. 18740-80190) injection port liner packed with 10% OV-1 on 80–100 mesh Chromosorb W HP as suggested by Hewlett-Packard.

Chemicals

3-MBT was purchased from Lancaster Synthesis (Windham, NH, USA) and its sulphone was synthesized by the method for benzothiophene sulphone synthesis [16]. 2-MBT and its sulphone were synthesized as outlined by Andersson [17].

Pesticide-grade solvents were used for extractions and column chromatography and HPLC-grade acetonitrile was used. Water for HPLC work was purified by passage through a Milli-Q apparatus (Millipore).

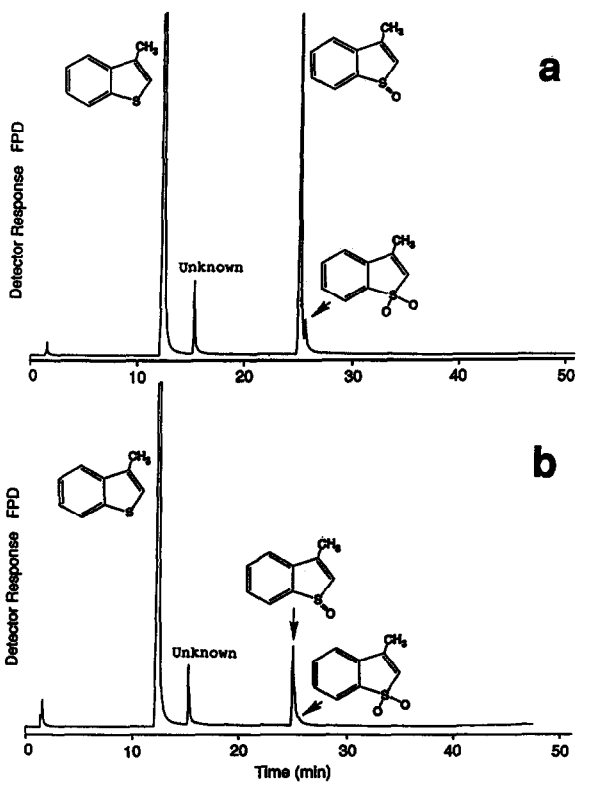

Fig. 1. GC analyses of (a) the polar fraction of extracts from cultures grown in the presence of 3-MBT and (b) the 3-MBT sulphoxide-containing fraction (the shaded portion in Fig. 2a) obtained by HPLC purification.

RESULTS AND DISCUSSION

Fig. 1a shows a GC–FPD chromatogram of the extract of *Pseudomonas* sp. BT1 grown on 1-methylnaphthalene in the presence of 3-MBT. Typically we observed the parent compound 3-MBT in these chromatograms and we assumed that the bacterial cometabolism left some of this compound unoxidized. However, after silica gel column chromatography was used to separate the polar sulphoxide and sulphone from the 3-MBT, GC analysis of the polar fraction gave a chromatogram that was essentially the same as Fig. 1a suggesting that sulphoxide was decomposing to 3-MBT.

This polar fraction was analyzed by HPLC and the resulting chromatogram is shown in Fig. 2a. Decomposition of the sulphoxide and the sulphone would not occur during HPLC analysis [18]. Authentic 3-MBT eluted at 28.18 min under these chromatographic conditions. Although there is a very small peak at 27.82 min, we have been unable to ascertain whether or not this was a trace amount of 3-MBT.

Using solvent 2, fractions of the effluent from the HPLC column containing 3-MBT sulphoxide and 3-MBT sulphone were collected individually and analyzed by GC. The fraction that contained only the 3-MBT sulphoxide gave the GC chromatogram shown in Fig. 1b, in which 3-MBT and, to a lesser extent, its sulphoxide were observed because of decomposition of the sulphoxide in the injection port. Analysis of the sulphoxide-containing fraction by HPLC showed only the presence of 3-MBT sulphoxide (Fig. 2b).

GC analysis of the HPLC fraction containing the 3-MBT sulphone showed only one peak which corresponded to the sulphone. This is consistent with previous observations that the sulphones of alkylbenzothiophenes and DBT do not decompose during GC analysis [15].

Similarly, HPLC was used to obtain the 2-MBT sulphoxide-containing fraction from the polar fraction of the methylene chloride extract of cultures grown in the presence of 2-MBT. HPLC analysis of the 2-MBT sulphoxide-containing fraction showed no other peaks. However, when this fraction was analyzed by GC, 2-MBT and a trace amount of 2-MBT sulphone were observed. These results demonstrated that 2-MBT sulphoxide was also suscepti-

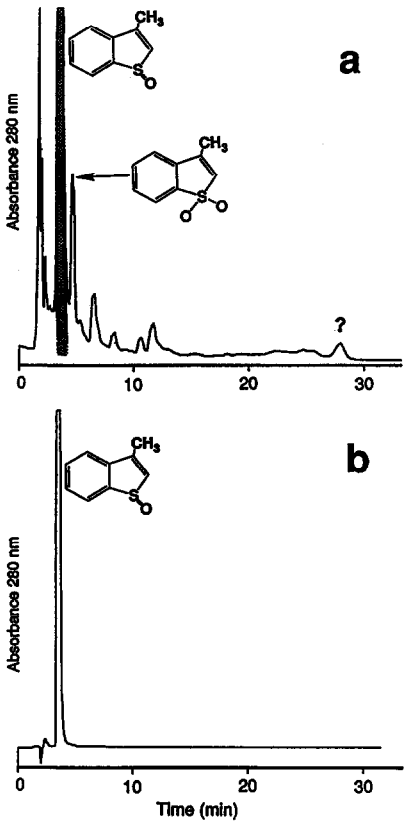

Fig. 2. HPLC analyses of (a) the polar fraction of extracts from cultures grown in the presence of 3-MBT and (b) the 3-MBT sulphoxide-containing fraction (the shaded portion in Fig. 2a) obtained by HPLC purification. Analyses done using solvent 1.

ble to decomposition in the GC injection port.

The GC analyses described above were interspersed among routine analyses of petroleum and petroleum fractions from biodegradation studies. Thus no special consideration was given to the condition of the commercial injection port liner. To assess the effects of the condition of the injection port liner, samples of the polar fraction from a culture grown in the presence of 3-MBT were analyzed and the ratios of 3-MBT/3-MBT sulphoxide and 3-MBT sulphone/3-MBT sulphoxide were calculated based on the FID response (Table I). Injections into a liner that had been used for the analyses of dozens of petroleum samples yielded the largest ratios indicating that the greatest amount of decomposition occurred under these conditions. The ratios were much smaller when a clean liner, with or without packing material was used. The ratios from duplicate injections showed that decomposition of 3-MBT sulphoxide was quite variable, especially when the soiled injection port liner was used. With the cleaned injection port liner, the relative standard deviations of the replicate injections were much smaller, most being $<10\%$.

These investigations demonstrated that GC methods using this commercially available injection port liner led to the decomposition of the two methylbenzothiophene sulphoxides studied. Indeed, the cleanliness of the liner determined the extent and reproducibility of the decomposition. Therefore we suggest that the use of this commercially available injection port liner is not appropriate for the determinations of the amounts of 3-MBT and 2-MBT biotransformed or the amounts of their sulphoxides produced in biodegradation studies. In addition, sulphones detected by the GC analysis of extracts from cultures grown in the presence of these methyl-

TABLE I

RELATIVE PROPORTIONS OF 3-MBT AND ITS OXIDES IN THE POLAR FRACTION OF EXTRACTS FROM CULTURES GROWN IN THE PRESENCE OF 3-MBT

Ratios determined from FID responses of duplicate GC analyses using a commercially available injection port liner under various conditions. R.S.D. = Relative standard deviation.

Liner condition	3-MBT/sulphoxide			Sulphone/sulphoxide		
	Injection 1	Injection 2	R.S.D.	Injection 1	Injection 2	R.S.D.
Packed[a] and soiled by dozens of injections of petroleum samples	22.8	10.8	51%	3.8	1.9	47%
Freshly cleaned and empty	0.10	0.11	8%	0.25	0.27	6%
Freshly cleaned and packed[a]	0.20	0.16	13%	0.28	0.26	4%

[a] With 10% OV-1 on 80–100 mesh Chromosorb W HP.

benzothiophenes may be due to the decomposition of the corresponding sulphoxide in the injection port. Thus HPLC analysis should be used to verify the biologically-mediated formation of these sulphones.

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