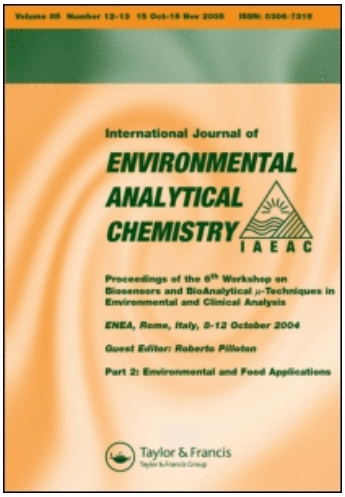

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Characterization of a Nitrogen-Containing Octylphenol Ethoxylate Metabolite by Chemical Derivatization and Degradation in Combination with Mass Spectrometry

Yoshiko Fujita[ab]; James A. Campbell[c]; Gary M. Mong[c]; Martin Reinhard[a]
[a] Department of Civil and Environmental Engineering, Stanford University, Stanford, CA, USA [b] Biotechnologies Department, INEEL, Idaho Falls, ID, USA [c] Pacific Northwest National Laboratory, Richland, W A, USA

To cite this Article Fujita, Yoshiko , Campbell, James A. , Mong, Gary M. and Reinhard, Martin(2001) 'Characterization of a Nitrogen-Containing Octylphenol Ethoxylate Metabolite by Chemical Derivatization and Degradation in Combination with Mass Spectrometry', International Journal of Environmental Analytical Chemistry, 81: 1, 41 — 54
To link to this Article: DOI: 10.1080/03067310108044357
URL: http://dx.doi.org/10.1080/03067310108044357

Intern. J. Environ. Anal. Chem., Vol. 81, pp. 41–54
Reprints available directly from the publisher
Photocopying permitted by license only

CHARACTERIZATION OF A NITROGEN-CONTAINING OCTYLPHENOL ETHOXYLATE METABOLITE BY CHEMICAL DERIVATIZATION AND DEGRADATION IN COMBINATION WITH MASS SPECTROMETRY

YOSHIKO FUJITA[a,†], JAMES A. CAMPBELL[b], GARY M. MONG[b] and MARTIN REINHARD[a,*]

[a]*Department of Civil and Environmental Engineering, Stanford University, Stanford, CA 94305, USA;* [b]*Pacific Northwest National Laboratory, Richland, WA 99352, USA*

(Received 21 November 2000; In final form 26 March 2001)

An analytical approach combining chemical derivatization and degradation techniques with mass spectrometry (MS) was applied for the characterization of a bacterial metabolite of 2-bromooctylphenoxy acetic acid. The metabolite contains nitrogen and its proposed structure is 2-aminomethoxy-3-bromo-5-(1,1,3,3-tetrabutyl)phenol. Chemical ionization MS and high resolution MS provided the molecular weight and elemental composition, respectively, of the molecule, and chemical modifications provided evidence for the presence or absence of specific functional groups. In particular, methylation with diazomethane and acylation with acetic anhydride indicated the presence of a phenolic hydroxyl group, while the results of an attempt to propylate the compound with boron trifluoride/propanol indicated the absence of a carboxylic acid group. Treatment with hydrobromic acid suggested the presence of an ether functionality. Hydrogenation and dehalogenation with palladium catalyst provided little new structural information for this compound, but the results were consistent with those from the other analyses. MS/MS data supported the proposed structure as well. Approaches such as the one described here may be useful for the identification of other trace organic unknowns detected in the environment or in laboratory studies.

Keywords: Octylphenol ethoxylate; Biotransformation; Derivatization; GC/MS

*Corresponding author. Fax: +1-650-723-0308. E-mail: reinhard@ce.stanford.edu
†Current address: Biotechnologies Department, INEEL, Idaho Falls, ID 83415, USA.

INTRODUCTION

In both the field and the laboratory, researchers in the fields of environmental aquatic chemistry and microbiology often encounter novel organic compounds at μg/L concentrations with structures that are not immediately apparent using routine chemical analyses. These "environmental unknowns" are generally not included in commercial chemical inventories and difficult to synthesize, and thus standard reference compounds with which to compare the unknown are often unavailable. This factor, along with the typically low concentrations of these compounds and their presence in complex mixtures, hinders the application of nuclear magnetic resonance (NMR) spectroscopy, which is commonly applied in other fields of chemistry for structural elucidation. Gas chromatography–mass spectrometry (GC/MS) has been widely used and is a powerful tool for identification for trace organic compounds in environmental matrices, but by itself GC/MS often cannot be relied upon to unambiguously identify unknowns, if reference compounds cannot be analyzed for comparison.

The identification of unknown trace organic compounds in the environment can therefore pose a formidable challenge, and alternate approaches to characterization are desirable. Selective derivatization and/or chemical degradations have not been widely used as diagnostic tools for structural elucidation by environmental researchers. In this article we describe the characterization of a novel bacterial metabolite using an approach that relies on a combination of chemical modifications and MS techniques, as well as familiarity with biochemical pathways that have been characterized previously. The precise structure of the compound is not confirmed, but evidence for the presence of specific functional groups is presented.

In a previous publication, we described the cometabolism of brominated octylphenoxyacetic acid (BrOP1EC; Fig. 1) in laboratory cultures of groundwater organisms using octylphenoxyacetic acid (OP1EC) as the primary substrate, and reported the detection of two persistent brominated metabolites [1]. OP1EC is an example of an alkylphenol ethoxycarboxylate (APEC), formed during biological sewage treatment of alkylphenol polyethoxylate (APE) nonionic surfactants, and it can be brominated during chlorine disinfection in the presence of bromide [2,3]. APECs and other APE residues reach the environment in discharged sewage effluents, and are of concern because they can be toxic to aquatic organisms [4,5], and recently they have also been implicated as estrogen mimics [6]. In the transformation study, one of the BrOP1EC metabolites was recognized readily as brominated octylphenol. The mass spectrum of the other indicated the

presence of nitrogen and bromine but its structure could not be discerned immediately. Because of the novelty of the incorporation of nitrogen during transformation, an extensive effort was made to elucidate the structure of the unknown.

Mass spectral library searches provided no matches, nor near matches, with the spectrum of the unknown. Because the compound was at trace concentrations (μg/L), and available only in small (< 250 mL) batches of culture medium, NMR was not practical for structural analysis. Therefore, an approach which relied on a combination of various mass spectrometric and chemical derivatization/degradation techniques was developed. GC with chemical ionization MS (CI-MS) and high resolution MS (HRMS) provided the molecular mass and elemental composition, respectively, and the results of the chemical treatments, analyzed by GC/MS, provided information on the specific functional groups present in the molecule. In addition, GC with nitrogen-specific detection and GC/MS/MS provided supporting structural information. As a result of the characterization effort, a structure was proposed for the metabolite, shown in Fig. 1 along with the parent compound BrOP1EC. The name of the structure is 2-aminomethoxy-3-bromo-5-(1,1,3,3-tetramethylbutyl)phenol, and the compound is hereafter referred to as AMeBrOP. This paper describes the characterization approach and results leading to the proposed structure of the metabolite. The strategy reported here may be applicable to other similar multifunctional trace organic compounds detected in the environment.

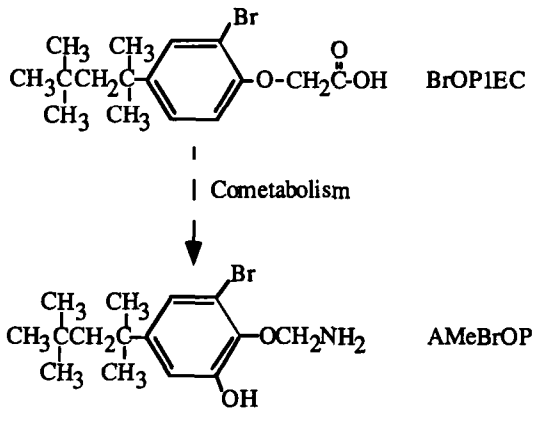

FIGURE 1

EXPERIMENTAL

BrOP1EC–Metabolizing cultures Aerobic groundwater organisms enriched on OP1EC were fed BrOP1EC in combination with OP1EC, and removal of the substrates was monitored routinely by HPLC [1]. When BrOP1EC was removed to non-detectable levels, or at selected time intervals prior to complete removal, samples were taken for detection and characterization of metabolites.

Extraction Samples (10 to 15 mL) of culture medium [1] containing the metabolite were extracted three times with 2–3 mL portions of methylene chloride. The combined extracts were dried with sodium sulfate, and concentrated under a gentle stream of nitrogen to dryness or to a volume appropriate for a subsequent treatment or analysis by GC/MS.

Methylation and diazomethane Extracts were methylated by ethereal diazomethane, prepared in millimolar quantities [7]. Following reaction for approximately 30 min, the reagent was removed by evaporation, and the residue redissolved in methylene chloride for GC/MS analysis. BrOP1EC and OP1EC served as positive controls for the reaction.

Acylation with acetic anhydride Samples were derivatized at room temperature in aqueous solution, based on a method of phenolic amines described by Blau and King [8]. Briefly, 1 mL acetic anhydride was added to 10 mL aqueous sample, and small portions of sodium bicarbonate were added with mixing, until effervescence ceased. The mixture was extracted three times with 2 mL portions of methylene chloride, and the extract was dried and concentrated as usual. Phenylethylamine, *t*-octylphenol, and 4-aminophenol were used as positive controls for the reaction.

Silylation with MSTFA Extracts were trimethylsilylated with 190 μL of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA; Pierce, Rockford, IL, USA), with 10 μL trifluoroacetic acid added as catalyst [9]. The reaction mixtures in sealed borosilicate vials were heated at 70°C for 25 min. The reagents were removed by evaporation, and the residue taken up in methylene chloride for GC/MS analysis. Brominated octylphenol served as a positive control.

Ether cleavage with HBr The ether bond in the metabolite was cleaved by treatment with hydrobromic acid, based on a method described by Landini *et al.* [10] Briefly, 1 mL glacial acetic acid and 0.1 mL hydrobromic acid were added to almost dried extract, and it was refluxed overnight at 120°C. After cooling, the mixture was adjusted to pH 5 with KOH, and extracted with methylene chloride. The solvent was washed with water 4–5 times to remove residual acetic acid, and then the extract was dried

and concentrated as usual for GC/MS analysis. A separate treatment of OP1EC served as a positive control.

Hydrogenation/Hydrodehalogenation with H_2/Pd Ten mL of aqueous sample was placed in a reactor with 1 g/L palladium on alumina catalyst (1% by weight Pd on alumina, 200–400 mesh; Aldrich Chemical Co., Milwaukee, WI, USA), and zero-grade hydrogen gas was bubbled through the solution with constant mixing at room temperature. Half of the reaction mixture was extracted after 1 h, and the remainder was extracted after 6 h. The extracts were dried and concentrated for GC/MS analysis as usual. Brominated octylphenol was a positive control for the reaction.

Propylation using BF3/propanol Extracts were propylated with 14% boron trifluoride in propanol (Alltech Associates, Inc., Deerfield, IL, USA). Approximately 1 mL of BF_3/propanol reagent was added to each dried extract, and the reaction mixtures were heated for 1 h at 100°C in sealed borosilicate vials. After cooling, 1 mL methylene chloride was added, and the mixture was washed three times with 1.5 mL portions of 2% potassium bicarbonate solution. The organic extract was dried with sodium sulfate and concentrated prior to GC/MS analysis. BrOP1EC and OP1EC served as positive controls.

GC with nitrogen-specific detection To confirm the presence of nitrogen in the metabolite, a methylated extract of the compound was injected on an HP 5890 Series II gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) connected with a DB-5 fused silica capillary column (30 m, 0.32 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA) to a chemiluminescent nitrogen-specific detector (Antek 705D; Antek Instruments, Inc., Houston, TX, USA). The detector was operated in the Total Nitrogen mode, whereby organic nitrogen is oxidized at 1000°C, the resulting nitrosyl radical is reacted with ozone to produce excited nitrogen dioxide, and the specific decay is detected by a photomultiplier. The GC temperature program was as follows: 50°C (2 min), heat to 120°C at 10°C/min, heat to 300°C at 5°C/min; total run time 45 min.

MS Techniques Low-resolution electron-impact (EI) MS with high-resolution GC was performed with an HP 5890 GC and a DB-5 fused silica capillary column (30 m, 0.32 mm i.d., 0.25 μm film thickness; J&W Scientific) interfaced to an HP 5970 MSD mass spectrometer. Data were acquired and analyzed with HP Chemstation G1034C software. The GC temperature program was the same as that described above for the GC with nitrogen-specific detection. Scanning was from 20 to 550 mass units, at 1.2 scans/s.

GC with chemical ionization (CI) MS and GC with EI MS/MS analyses of the underivatized metabolite were performed with a Varian 3400 GC

(Varian, Palo Alto, CA, USA) connected to a Finnigan Triple-Stage-Quadrupole Mass Spectrometer (TSQ-70; Finnigan MAT, San Jose, CA, USA). The CI reagent gas was methane; the MS/MS reagent gas was argon. The GC column and temperature program were the same as on the HP EI-MS system, and the MS operating conditions were as described by Ding *et al.* [11].

GC coupled with high resolution mass spectrometry (HRMS) was performed at Pacific Northwest National Laboratory (PNNL; Richland, WA, USA). Accurate mass measurements were performed using a JEOL SX-102/SX-102 double-focusing, tandem mass spectrometer equipped with a JEOL Unix data system (JEOL USA Inc., Peabody, MA, USA). The instrument was tuned to a resolution of 5000 (10% valley definition). Data were acquired by scanning over the mass range of 50–500 mass units at a rate of one per second. Instrument tuning and real-time mass measurements were performed by leaking a controlled amount of perfluorokerosene (PFK) into the electron-impact ion source from a septum inlet reservoir. Computer-assisted accurate mass assignments and subsequent elemental compositions were made on data obtained from averaging ten consecutive scans over the GC elution profile of the analyte. The instrument was equipped with a Hewlett-Packard 5890 gas chromatograph fitted with a DB-5 capillary column (30 m, 0.25 mm i.d.). The GC oven temperature was held at 50°C for 2 min, then increased at 8°C/min to 300°C. The same equipment and temperature program were used for positive ion CI analyses of the acetylated unknown, using isobutene. The source pressure was approximately 2×10^{-4} torr. The temperature of the source was 200°C, and the mass spectrometer scanned from 70 to 500 mass units at a rate of 1/s.

RESULTS AND DISCUSSION

The brominated nitrogen-containing unknown was found in extracts from cultures metabolizing BrOP1EC, along with another intermediate, brominated octylphenol. In the experiment reported previously, AMeBrOP accounted for an estimated 4% of the metabolized BrOP1EC on a molar basis [1], but in other experiments performed in this laboratory, AMeBrOP has been estimated to account for up to 25% of the metabolized BrOP1EC (data not published). The EI mass spectrum of AMeBrOP is presented in Fig. 2.

Four significant features can be observed in the spectrum shown in Fig. 2: (1) The molecular ion is odd-numbered, at *m/z* 329/331; (2) There are significant even-numbered fragment ions at *m/z* 258/260, *m/z* 230/232,

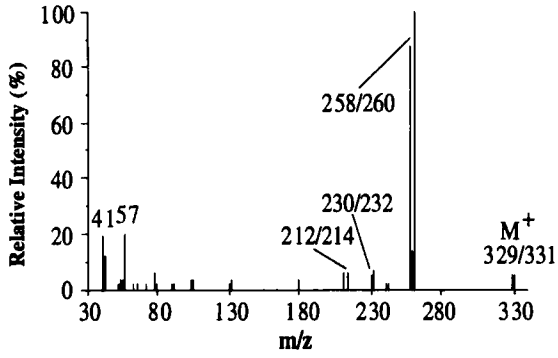

FIGURE 2

and *m/z* 212/214; (3) Several distinctive ions are in pairs separated by 2 mass units in an approximately 1 : 1 ratio of abundance, i.e., *m/z* 329 and 331, *m/z* 258 and 260, *m/z* 230 and 232, and *m/z* 212 and 214; and finally (4) There is a 71 mass unit difference between the molecular ion and the base peak at *m/z* 258/260. The first and second features together suggest the presence of an odd number of nitrogen atoms in the molecule [12] and this was consistent with the strong response of the compound when analyzed by GC with the nitrogen-specific detector. A single peak was detected in the run, eluting at the same retention time as the AMeBrOP on the GC/MS system. The third feature, the pairs of ions separated by 2 mass units in a 1 : 1 ratio, is the characteristic isotopic pattern associated with the presence of a single bromine atom [12]. The fourth feature, the loss of a 71 mass unit fragment form the molecular ion to from the base ion at *m/z* 258/260, is characteristic of benzylic cleavage resulting in loss of the *t*-pentyl group from the branched 1,1,3,3-tetramethylbutyl structure of the side chain [13].

The above information indicates that in the AMeBrOP, the brominated aromatic ring and octyl(1,1,3,3-tetramethylbutyl) side chain of the parent compound BrOP1EC are intact, but at least one other substituents is present on the ring, including a functional group containing nitrogen. The molecular weight of the compound is 329/331 Da (329 Da for the ^{79}Br isotope, 331 Da for the ^{81}Br isotope), and this was confirmed by CI-MS, which showed the $(M+1)$ ion at *m/z* 330/332 as the base (100% relative intensity) ion. Given the molecular weight, the possibilities for the elemental composition of the compound are limited. Analysis by HRMS determined that the elemental composition of the EI base ions (*m/z* 258/260) was $[C_{10}H_{13}O_2NBr]^+$ (computed error 3 millimass units (mmu)). In addition, the elemental composition of *m/z* 212/214 was determined to be $[C_9H_9OBr]^+$ (computed error 6 mmu)

which represents a difference of 46 (CH_4ON) from *m/z* 258/260. the low abundance, and interference by the mass calibration standard, PFK, prevented accurate mass determination for the molecular ion itself. However, because the loss of the 71 mass unit *t*-pentyl group (C_5H_{11}) is so characteristic of the EI fragmentation of OP1EC and related compounds [13], the elemental composition of AMeBrOP can with virtual certainty be stated as $C_{15}H_{24}O_2NBr$.

Tallying up the masses and compositions of the aromatic ring, bromine, and octyl side chain, of the 329 Da available for the ^{79}Br isotope, only 65 mass units remain to be distributed elsewhere on the ring: one carbon atom, seven hydrogen atoms, two oxygen atoms, and one nitrogen atom. The distribution of these atoms was not immediately apparent from the MS analyses alone, and so various selective derivatization and degradation techniques were applied to the compound, in order to obtain more information about the unknown functional group(s). Table I contains a list of the various chemical treatments applied, and the functional groups tested for. Functional groups other than those included in Table I may be modified by the listed treatments, but their presence was ruled out based on other data; e.g. thiols can be derivatized by diazomethane [14] and acylated [8], but the elemental composition of AMeBrOP as determined by HRMS did not contain sulfur.

Chemical Modifications

Treatment of the AMeBrOP with BF_3/propanol resulted in no change in the AMeBrOP structure. This finding, along with the observations that the AMeBrOP could be successfully chromatographed by GC without

TABLE I Chemical modification techniques

Treatment	*Functional groups tested for:*	*Reference*	*Positive control(s)*
Propylation (BF_3/Propanol)	Carboxylic acids	15	BrOP1EC, OP1EC
Methylation (Diazomethane)	Carboxylic acids, phenols, acidic NH groups	14	BrOP1EC, OP1EC
Acylation (Acetic Anhydride)	Phenols, alcohols, amines	8	*t*-octylphenol, phenylethylamine
Silylation (MSTFA)	Phenols, alcohols, amines	9	Brominated octylphenol
Ether Cleavage (HBr)	Ether	10	OP1EC
Hydrogenation/ Hydrodehalogenation (H_2/Pd)	Aromatic ring, bromine*	16	Brominated octylphenol

*The objective of this procedure was to reduce the ring and alter the EI-MS fragmentation pattern, in order to gain additional information about the compound structure.

derivatization, and also was more efficiently extracted from neutral aqueous solution than from acidified aqueous solution, suggested that the metabolite no longer contained the carboxylic acid group of the BrOP1EC. However, reactions with diazomethane and acetic anhydride did result in modification of the AMeBrOP. Derivatization with diazomethane resulted in the compound whose EI mass spectrum is shown in Fig. 3(a). One methyl group was added, as evident from the 14 mass unit increase, relative to the AMeBrOP spectrum (Fig. 2), in both the base peak and molecular ion. Acylation of the compound yielded the EI mass spectrum shown in Fig. 3(b). Here the spectrum is very similar to that of the underivatized compound shown in Fig. 2 but the increased GC retention time and the molecular ion signal at *m/z* 373 (confirmed by CI-MS) indicated that acylation did occur; the increase of 42 mass units in the molecular ion represents the addition of one acetyl group. Acetic anhydride can also react with amino groups, as evidenced by the positive reactions with phenylethylamine and 4-aminophenol, but no characteristic ions of acetamides, as described by McLafferty and Turecek [12] and seen in the controls, were detected in the acetylated extract containing the unknown. Unfortunately no analogs of the proposed structure, with an amino group attached to a methoxy group, and a bromine atom and a hydroxyl group also on the ring, were available to test as standards.

The reactions with diazomethane and acetic anhydride suggested that a free phenolic group was present in the molecule. The presence of a phenolic hydroxyl group is consistent with published pathways for the aerobic transformation of the chlorinated phenoxyacetic acid herbicides, structurally similar to BrOP1EC; in these compounds, hydroxylation of the aromatic ring is observed prior to ring cleavage [17]. However, in the attempt to silylate the compound with MSTFA, as with the BF_3/propanol, no product was observed for AMeBrOP, although the brominated octylphenol was partially derivatized. This may indicate that the phenolic group on the metabolite is sterically hindered by other constituents on the ring, and therefore the bulky MSTFA reagent was prevented from reacting. The amine, proposed in the structure for AMeBrOP, also appears to be sterically hindered or otherwise unreactive with MSTFA.

In order to determine whether an ether bond was present in the molecule, the compound was refluxed with an excess of hydrobromic acid overnight. Extraction of the reaction mixture revealed the presence of the compound exhibiting the EI mass spectrum depicted in Fig. 3(c). Examination of the mass spectrum reveals the approximately 1 : 2 : 1 abundance of ions separated by 2 mass units at *m/z* 362/364/366, *m/z* 291/293/295 and *m/z* 263/265/267; this isotopic pattern is characteristic of compounds containing

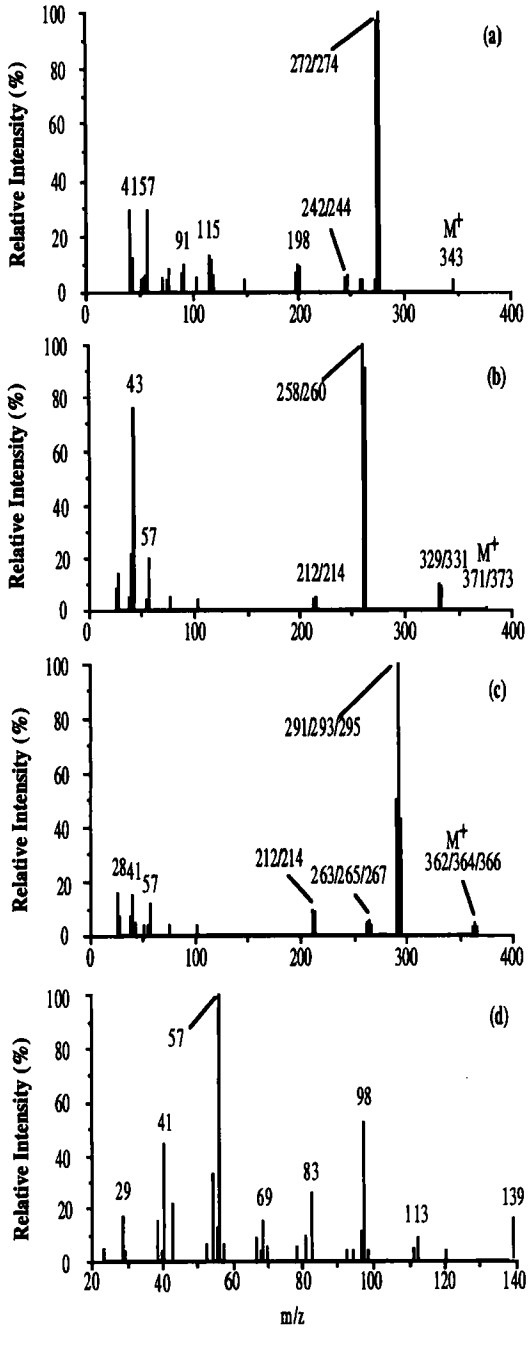

FIGURE 3

two bromine atoms. Also of note is the even-numbered molecular ion at *m/z* 362/364/366, and the odd-numbered base ion at *m/z* 291/293/295, indicating that nitrogen is no longer present in the molecule. This suggests that an ether bond connecting the nitrogen-containing moiety to the aromatic ring was cleaved, and an additional bromine was added to the molecule, most likely at the point of attachment of the ether bond. In general, cleavage of aryl alkyl ethers by HBr results in the phenol and alkyl bromide [18], but perhaps due to the amino group, the aryl bromide product was formed rather than [$BrCH_2NH_2$]. No other brominated compounds resulting from the cleavage were observed by GC/MS. The difference in mass between the ^{79}Br isotope AMebrOP and the product of the ether cleavage (362 Da if both bromines are ^{79}Br) was 33 mass units, meaning that the added bromine replaced a group that accounted for 46 mass units.

Because the benzylic cleavage of the *t*-pentyl group from the octyl side chain is so dominant during EI fragmentation of the metabolite, an attempt was made to hydrogenate the aromatic ring in order to change the fragmentation pattern and thereby perhaps gain additional information about the carbon skeleton of the molecule and ring substituents. The sample subjected to hydrogenation contained both brominated octylphenol and the AMebrOP, and after 1 h the product whose spectrum is shown in Fig. 3(d) was found in the extract, along with unbrominated octylphenol. After 6 h, the octylphenol was no longer present, and the Fig. 3(d) product was present at greater concentration, suggesting that the AMebrOP and the brominated octylphenol were converted to products with the same spectra (and retention times) under the conditions of the hydrogenation. The spectrum was difficult to interpret, limiting the diagnostic utility of the hydrogenation reaction, but by comparison with published spectra, the spectrum depicted in Fig. 3(d) is suspected to be that of *t*-octyl cyclohexanone. If that is the case, the molecular ion, at *m/z* 210, was not detected. This proposed structure is consistent with the known behavior of halogenated phenols under conditions of hydrogenation with palladium catalyst; bromine is rapidly lost from the ring, and the phenol is readily converted to the cyclohexanone [16]. The loss of nitrogen from the AMebrOP could be explained by cleavage of the *N*-containing alkyloxy group.

GC/MS/MS ANALYSIS

GC/MS/MS provided support for the proposed structure as well. The base ions in the mass spectrum of AMeBrOP, *m/z* 258 and 260, were selected for

daughter ion analyses. The production spectrum for *m/z* 260 in presented in Fig. 4. (Data for the *m/z* 258 ion, containing the ^{79}Br isotope, are analogous, but shifted down by two mass units for the three largest ions.) The first and most abundant fragment ion produced was *m/z* 232, followed by *m/z* 214 and *m/z* 179. These represent neutral losses of 28, 46, and 81 mass units, respectively. The 28 mass unit loss was attributed to a rearrangement loss of ethane from the α,α-dimethyl benzylic group [12]. (Fragmentation of 1,1,3,3-tetramethylbutyl has been investigated by Moldovan and Bayona [19].) The 46 mass unit loss was attributed to the loss of a fragment with the elemental composition of CH_4ON. This was consistent with the elemental compositions determined by HRMS for the *m/z* 258/260 and *m/z* 212/214 ions; the former was $[C_{10}H_{13}O_2NBr]^+$ and the latter $[C_9H_9OBr]^+$, corresponding to a 46 mass unit difference of CH_4ON.

The loss of the same 46 mass unit fragment was seen again when the *m/z* 230 ion, containing the ^{79}Br isotope (and thus analogous to the *m/z* 232 ion) was selected for daughter ion analysis; the most abundant (90%) ion in the product spectrum was *m/z* 184. The 81 mass unit loss in the *m/z* 260 product ion spectrum was due to the loss of the bromine atom; this was confirmed by the reappearance of the *m/z* 179 ion in the product ion spectrum of the *m/z* 258 ion, which contained the ^{79}Br isotope. The loss of bromine was observed again when the *m/z* 184 ion was selected for daughter ion analysis – the most abundant ion was at *m/z* 105, representing the loss ^{79}Br. The presence of the small ion at *m/z* 133 in Fig. 4 may be attributed to simultaneous losses of Br

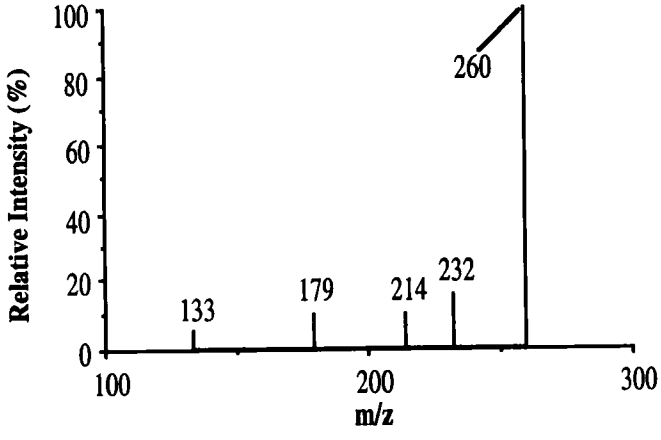

FIGURE 4

TABLE II Proposed neutral losses in EI fragmentation of AMeBrOP

Neutral loss (amu)	*Proposed elemental composition*	*Proposed structure*
71	C_5H_{11}	*t*-pentyl group
28	C_2H_4	Ethene
46	CH_4ON	OCH_2NH_2
79/81	Br	Br
127	CH_4ONBr	$BrOCH_2NH_2$

and CH_4ON as a neutral molecule of $BrOCH_2NH_2$ formed by a rearrangement process. The proposed neutral losses discussed in this section are listed in Table II.

We proposed the structure of OCH_2NH_2 for the 46 mass unit fragment assuming the ether structure in the parent compound remained intact. The HRMS data is consistent with this assumption. Mass spectral fragmentation strongly indicates loss of these atoms either as a unit by a fission processes or in combination with Br as neutral molecule $BrOCH_2NH_2$ after rearrangement. With the phenolic hydroxyl group (17 mass units) attached to a different carbon of the ring, the last two hydrogen atoms can be assigned to the two remaining unsubstituted aromatic carbons. The exact locations of the four ring substituents (*t*-octyl group, bromine, hydroxyl, and OCH_2NH_2) relative to each other may be different from the AMebrOP structure depicted in Fig. 1; their relative positions cannot be confirmed from the data available.

Acknowledgements

The authors are grateful to Dr. Wang-Hsien Ding for performing CI-MS and EI-MS/MS analyses, and to Dr. Christoph Schüth for setting up the hydrogenation reaction. Dr. Viorica Lopez-Avila provided assistance in the interpretation of MS data. This research was supported by a grant from the Orange County Water District, Orange County, California, The GC/TSQ-70 was obtained through an equipment grant from the U.S. Department of Energy (DE-FGO5-87ER75377).

References

[1] Y. Fujita and M. Reinhard, *Environ. Sci. Technol.*, **31**, 1518–1524 (1997).
[2] M. Reinhard, N. Goodman and K.E. Mortelmans, *Environ. Sci. Technol.*, **16**, 351–362 (1982).

[3] H.A. Ball and M. Reinhard, in *Water Chlorination. Chemistry, Environmental Impact and Health Effects*; R.L. Jolley, R.J. Bull, W.P. Davis, S. Katz, M.H. Roberts Jr. and V.A. Jacobs (Eds.), Lewis Publishers, Inc., Chelsea MI, 1984, Vol. 5, pp. 1505–1514.
[4] K. Yoshimura, *J. Am. Oil Chem. Soc.*, **63**, 1590–1996 (1986).
[5] M.H.I. Comber, T.D. Williams and K.M. Stewart, *Water Res.*, **27**, 273–276 (1993).
[6] S. Jobling, D. Sheahan, J.A. Osborne, P. Matthiessen and J.P. Sumpter, *Environ. Toxicol. Chem.*, **15**, 194–202 (1996).
[7] H.M. Fales, T.M. Jaouni and J.F. Babashak, *Anal. Chem.*, **45**, 2302–2303 (1973).
[8] K. Blau and G.S. King, in *Handbook of Derivatives for Chromatography*; K. Blau and G.S. King (Eds.), Heyden & Son Ltd., Philadelphia, 1979, pp. 31–47.
[9] R.P. Evershed, in *Handbook of Derivatives for Chromatography*; 2nd Edn., K. Blau and J.M. Halket (Eds.), John Wiley & Sons, New York, 1993, pp. 51–108.
[10] D. Landini, F. Montanari and F. Rolla, *Synthesis*, **10**, 771–773 (1978).
[11] W.-H. Ding, Y. Fujita, R. Aeschimann and M. Reinhard, *Fresenius' J. Anal. Chem.*, **354**, 48–55 (1996).
[12] F.W. McLafferty and F. Turecek, *Interpretation of Mass Spectra*; 4th Edn., University Science Books, Mill Valley, CA, 1993, p. 371.
[13] E. Stephanou, M. Reinhard and H.A. Ball, *Biomed. Environ, Mass Spectrom.* **15**, 275–282 (1988).
[14] T.H. Black, *Aldrichim. Acta*, **16**, 3–10 (1983).
[15] K. Blau and A. Darbre, in *Handboodk of Derivatives for Chromatography*; K. Blau and G.S. King (Eds.), Heyden & Son. Ltd., Philadelphia, 1979, pp. 11–28.
[16] P.N. Rylander, *Hydrogenation Methods*; Academic Press, San Diego, 1985, p. 193
[17] M.L. Rochkind-Dubinsky, G. Sayler and J.W. Blackburn, *Microbiological Decomposition of Chlorinated Aromatic Compounds*; Marcel Dekker, Inc., New York, 1987, Vol. 18.
[18] J. March, *Advanced Organic Chemistry, Reactions, Mechanisms, and Structure*; 4th Edn., John Wiley & Sons, New York, 1992.
[19] Z. Moldovan and J.M. Bayona, *Rapid Comm. Mass Spectrom* **14**, 379–389 (2000).