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THE MECHANISM OF THE REACTION OF OZONE
WITH PYRENE AND BENZ[a]ANTHRACENE
IN ACETONITRILE/WATER MIXTURE

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Major professor

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THE MECHANISM OF THE REACTION OF OZONE WITH PYRENE AND BENZ[a]ANTHRACENE IN ACETONITRILE/WATER MIXTURE

By

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ABSTRACT

THE MECHANISM OF THE REACTION OF OZONE WITH PYRENE AND BENZ[a]ANTHRACENE IN ACETONITRILE/WATER MIXTURE

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The ozonation of pyrene and benz[a]anthracene was studied. The toxicity of ozonation products was assessed by monitoring gap junction intercellular communication (GJIC). The intermediate ozonation pyrene products were more inhibitory to GJIC than pyrene and the final products. Therefore, the identification of the ozonation products and an understanding of the reaction pathways that occur in aqueous solutions is important in minimizing the formation of toxic products during ozone treatment.

The ozonation products were identified and quantified using gas chromatography/mass spectrometry (GC/MS). For the ozonation of pyrene, 14 products including aldehyde and carboxylic acid substituted phenanthrene- and biphenyl-type oxidation products were identified. Initial ring cleavage of pyrene at the 4,5 position produced phenanthrene-type products. Prior to the complete disappearance of pyrene, secondary ring cleavage at the 9,10 position occurred. After the disappearance of pyrene, the concentration of the phenanthrene-type products decreased dramatically and the secondary ring cleavage biphenyl-type products predominated. A stoichiometric ratio of 1.68 moles ozone/mole pyrene was required to completely destroy pyrene. For the ozonation of benz[a]anthracene, 15 products including quinone type and phenyl-naphthyl type products were identified. Ozone attacked either on the carbon at the 7 and/or 12 position producing the quinone or hydroxyl functional groups, or at the 5,6 position resulting in ring cleavage and the formation of phenyl-naphthyl type products. These products were formed simultaneously.

Initial attack by ozone on either the bond or atom having the lowest localization energy occurred preferentially. For pyrene, ring cleavage occurred at the bond having the lowest energy, and resulted in a stepwise ring cleavage pathway. For the ozonation of benz[a]anthracene, both atom attack and bond attack occurred, but higher preference was for bond attack.

In aqueous systems, ·OH radicals have greatest effect on the initial bond attack type of ozonation reactions and have least effect on initial atom attack type of reactions. Free radicals also reacted with ozonation products. Conducting the ozonation experiments in aqueous solutions containing a non-participating solvent were more reliable in predicting aqueous products than were organic solvent. Using the results of these studies we were able to predict ozonation products for compounds that have not been previously studied.

In honor of the giant in my life - my courageous and intelligent father.

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A	GC area response of product
AI	GC area response of internal standard
A_{py}	GC area response of pyrene
BaA	benz[a]anthracene
[BaA]	concentration of benz[a]anthracene, mM
C_i	ozone influent concentration, mM
C_e	ozone effluent concentration, mM
4-CPA	4-carboxy-5-phenanthrenecarboxyaldehyde
FL	fluorene
GC	gas chromatography
GJIC	gap junction intercellular communication
L_a	the lowest atom localization energy, eV
L_b	the lowest bond localization energy, eV
M	reactant
[M]	reactant concentration, M
$[M]_o$	initial reactant concentration, M
MS	mass spectrometry
NA	naphthalene
O_3	ozone
$[O_3]$	ozone concentration, M or mM
$[\overline{O_3}]$	average ozone concentration, M
$[O_3]_o$	initial ozone concentration, M
$d[O_3]$	ozone consumption, mM
PAHs	polycyclic aromatic hydrocarbon
PH	phenanthrene
PY	pyrene
[PY]	pyrene concentration, mM
Q	gas flow rate, mL/min
RP-HPLC	reverse phase-high performance liquid chromatography
S_w	aqueous solubility, ppb

k	rate constant, $M^{-1} s^{-1}$ or $mM^{-1} s^{-1}$
t	reaction time, sec
$t_{1/2}$	reaction half-life, sec
v/v	volume to volume ratio
w/v	weight to volume ratio
Δ	concentration difference
ξ	stoichiometric coefficient

Roman Numeral assigned for ozonation products ID

CHAPTER 1

INTRODUCTION

1.1 Environmental Significance

Polycyclic aromatic hydrocarbons (PAHs) are found in many petroleum products including crude oils, motor oils, gasoline and heating fuels. The predominant routes by which PAHs enter the environment are petroleum spillage and the release of PAHs during the combustion of petroleum products with subsequent wet or dry deposition. It is estimated that nearly 230,000 tons/year of total PAHs enter the aquatic environment from various sources (Neff, 1979 p.227). As a result of the release of PAHs to the environment, these compounds find their way into drinking waters, soils, plants and wastewater. In a review article by Harrison et al. (1975), it was noted that concentrations of benz[a]pyrene of 40 - 1300 mg/L have been found in dried soil taken from presumably uncontaminated forests and fields. In plants and vegetables, concentrations of individual PAHs have been found to range from 5 to 110 mg/kg of dry material. Raw sewage from domestic sources can contain significant levels of PAHs (Bourcart et al., 1965; Harrison et al., 1975). Rainfall may increase the levels of PAHs in sewage by more than 100-fold over that which occurs during dry weather periods. Because of their low water solubilities (Futoma et al., 1981), PAHs are rapidly adsorbed to organic and inorganic particulate materials or are accumulated in the tissues of aquatic organisms. PAHs released to the environment tend to remain close to the

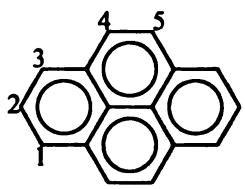
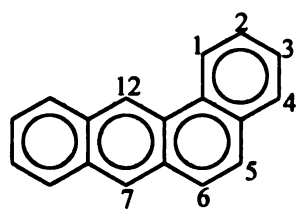
sites of deposition. Therefore, lakes, rivers, estuaries, and coastal marine environments near centers of human population are the primary repositories of aquatic PAHs. In Great Lakes water, high concentrations of pyrene and fluoranthene (11.2 and 10.6 ng/liter, respectively) have been found (Eadie, 1983). The concentration of individual PAHs in sediments ranged from 30 to 750 mg/kg dry wt.

PAHs are of significant environmental importance because they are not readily biodegradable (Sims and Overcash, 1983). They are persistent in soils (Stevens et al., 1989), bioaccumulated (Smith et al., 1984; Solbakken et al. 1983; Hallett et al., 1983), and either carcinogenic or potentially carcinogenic (Griest et al., 1987; Cavalieri et al. 1980; Thorton et al., 1981). Sixteen PAHs are listed among the 129 U.S. EPA Priority Pollutants (EPA, 1984).

1.2 Choice of Model Compounds

Two PAHs will be used as model compounds (listed in Table 1.1); they are pyrene and benz[a]anthracene. The target compounds are chosen because 1) they belong to different classes of PAHs and should have different ozonation mechanisms, 2) they are reactive with both ozone and the ·OH radical, 3) they are recalcitrant (Howard et al., 1991), and 4) they have a range of mutagenic activities but are not strongly carcinogenic to reduce healths risks during laboratory studies.

Table 1.1 Target compounds studied

Compound	Pyrene	Benz[a]anthracene
Abbreviation	PY	BaA
Relative Carcinogenicity[b]	-[b]	+[b]
Most Reactive Bond[a]	4,5	5,6
Lowest Bond-Localization Energy[a]	1.06	1.03
Most Reactive Atom[a]	3	7
Lowest Atom-Localization Energy[a]	1.51	1.35
Structure		

a. Bailey, 1982. In: *Ozonation In Organic Chemistry*. 39(2): 43-76.
b. Harrison, 1975. *Wat. Res.*, 9:331-346.
Relative activity on mouse epidermis: +++, active; ++, moderate; +, weak; -, negative

1.3 Background

The most important processes that govern the fate of PAHs in the environment are photooxidation, chemical oxidation and biodegradation. In atmospheric systems chemical oxidation by ozone or the ·OH radical along with photooxidation are the predominant mechanisms responsible for the degradation of PAHs (Neff, 1979). In soil systems, only low molecular weight hydrocarbons, such as substituted benzenes, can be biodegraded. The higher molecular weight PAHs, however, are highly recalcitrant with half-lives on the order of 210 days to 5.2 years (Howard et al., 1991). Both chemical oxidation and biodegradation can be exploited in developing systems to treat PAHs. If the PAHs are first oxidized using ozone or hydroxyl radicals resulting in ring cleavage, then the byproducts may be much more easily biodegradable than the parent compounds. This process would be applicable for wastewater treatment in which ozonation could be used as a pretreatment followed by some form of biological treatment (WPCF, 1986; Holzer et al., 1991; Gilbert, 1988).

Ozone is a very strong oxidant, more powerful than other oxidants commonly used in water and wastewater treatment. Numerous applications exist for ozonation in the area of wastewater treatment, including the processing of shale oil (Masten and Davies, 1994). While in the United States, ozone is primarily used in water treatment for disinfection and DPB control, it is also used to control odor, improve suspended solids removal, improve performance of granular activated carbon units and improve the biodegradability of wastewater (Rice, 1985; Gasi et al., 1990; Robson and Rice, 1991). Ozone would be

highly useful for the remediation of wastewaters and industrial effluents containing PAHs. For example, ozonation coupled with biodegradation could be used for the ultimate destruction of PAHs in the environment. Ozonation could be used to break the ring structure, followed by biological degradation for the subsequent oxidation of the ozonation byproducts. Therefore, an investigation, especially in terms of the determination of aqueous phase rate constants, reaction byproducts and the effect of competing substrates, is highly pertinent to the treatment of wastewaters.

Preliminary work on the use of gaseous ozone for the decomposition of PAHs in soils has revealed very promising results (Yao and Masten, 1992). It has been shown that ozone is capable of degrading several PAHs (including pyrene, chrysene and phenanthrene) in the soil; however, it is still unclear what degradation products are produced.

Ozone reacts with olefinic compounds by a 1,3-dipolar cyclic addition across the double bond to produce an trioxalane (also called a molozonide), which rapidly decomposes to form aldehydes and organic acids (as shown in Figure 1.1). When ozone reacts with aromatic compounds, three types of ozone attack can occur, resulting in either substitution or ring cleavage. These are: 1) 1,3-dipolar cycloaddition (ozonolysis) at the bond or bonds having the most double bond character (the bond with the lowest bond-localization energy); 2) electrophilic ozone attack on the atom with the lowest atom-localization energy; 3) conjugate addition where there is a reactive diene system (a system of lowest para-

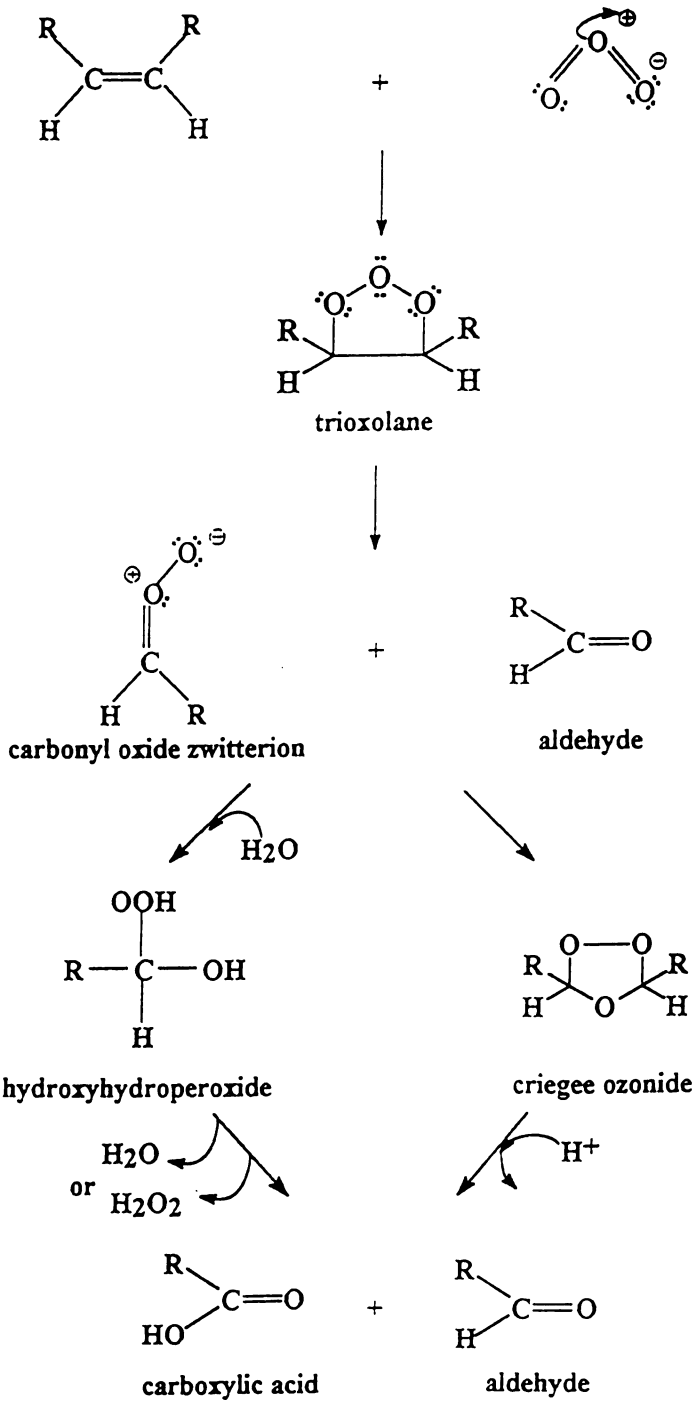
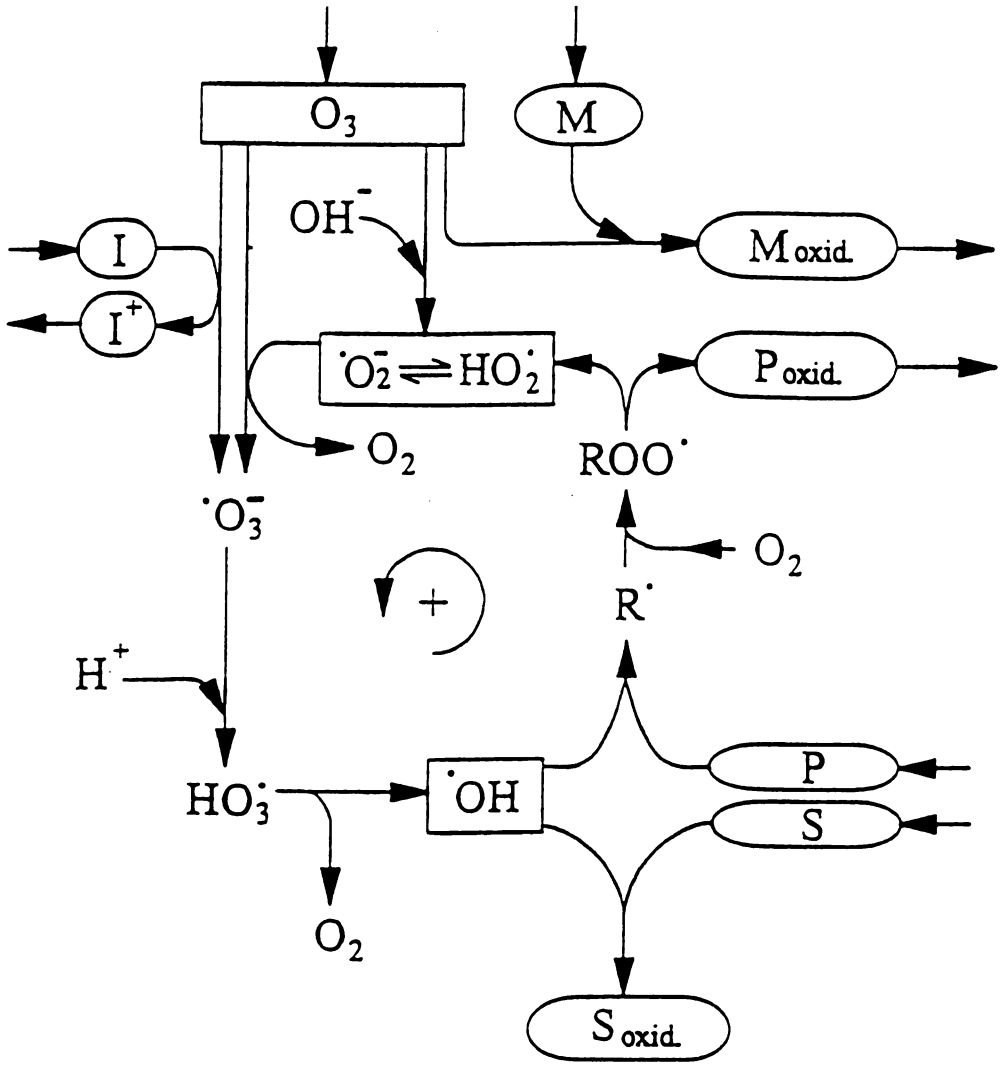


Figure 1.1 Mechanism of the Ozonation Reaction
(Adapted from Bailey, 1972)

localization energy).

When ozone reacts with PAHs, which type of attack ozone will occur depends on where the PAHs has the lowest-localization energy. For example, ring cleavage occurred when phenanthrene reacted with ozone (Bailey, 1982; Meineke and Klamberg, 1978). Gas phase ozonolysis of aromatic compounds resulted in the formation of CH_3COCHO as the major product with small amounts of carbon monoxide, carbon dioxide and formic acid (Atkinson, 1984). The mechanism for the formation of these products is not known. The gas phase reaction of benz[a]pyrene and ozone has also been studied (van Cauwenberghe et al., 1979; Finlayson-Pitts and Pitts, 1988). Such substitution reactions of benz[a]pyrene resulted in the formation of quinones and epoxides, whereas ring cleavage reactions resulted in the formation of dialdehydes, benzanthronedicarboxylic acid and other dicarboxylic acid compounds. The mechanisms of these reactions is not fully understood.

In water, ozone degrades to form hydroxyl radicals (as shown in Figure 1.2). The rate of degradation depends on pH, and on the concentrations, types of chemicals and ions present. Hydroxyl radicals (·OH), being less selective and a stronger oxidant than ozone, will oxidize many compounds that do not or only very slowly react with ozone. The mechanisms of these reactions are complex and the oxidations may occur via three pathways: hydrogen abstraction from a $-CH_2$ group, radical addition, and electron transfer. The secondary radicals formed during these reactions can react with additional ozone, with



LEGEND

M - Micropollutant (can also act as an initiator, scavenger, or promoter)
$M_{oxid.}$ - Oxidized form of micropollutant
P - Promoter
$P_{oxid.}$ - Oxidized form of promoter
I - Initiator
I^+ - Protonated form of initiator formed by electron transfer reaction
S - Scavenger
$S_{oxid.}$ - Oxidized form of scavenger

Figure 1.2 Reactions of Aqueous Ozone in the Presence of Micropollutant M (Adapted from Staehelin and Hoigné, 1985)

one another, or with the target compound(s). Organoperoxides can be formed by the reaction of the carbonyl (C·) radical with any oxygen present. These compounds can enter into a chain reaction involving ozone and the hydroxyl radicals resulting in the formation of new radicals or superoxide. Other compounds can react with the free radicals produced to terminate the chain reaction. (Staehelin and Hoigné, 1985).

The ozonation of PAHs in aqueous solution has been studied by a number of researchers. Ozone reacts with PAHs with rate constants on the order of $>10^3$ M^{-1} s^{-1}. The rate constants for the reaction of selected PAHs with ozone and half-lives are given in Table 1.2. Radding et al. (1976) determined the rate constants for the reaction of ozone and several PAHs in water at 25 °C. Hoigné and Bader (1983) determined the rate constant for the ozonation of naphthalene in water. Their experiments were conducted at 20 °C, pH 2 and in water containing 1 mM *tert*-butyl alcohol. They assumed that the reaction order with respect to ozone concentration is one, and found that the reaction is first order with respect to organic compounds. Hoigné and Bader state that the stoichiometric coefficients usually range from 1 to 5 and calculated the rate constant by assuming a second order reaction and a stoichiometric coefficient equal 2.5. Contrastingly, Legube et al. (1986b) determined the rate constant for the reaction of ozone with naphthalene in organic solvent at 1 °C, pH 5.6. They found that the stoichiometric coefficient for the initial reaction step is 2, and that the reaction is second order. Other researchers assumed that the reaction order and stoichiometry were identical to that assumed by Hoigné and Bader (Butković et al., 1983; Corless et

Table 1.2 Rate constants for the reaction of ozone and various PAHs in water

Compound	Rate constant, k[a] ($M^{-1}s^{-1}$)	Calculated $t_{1/2}$[b] (sec)	Ref.
Naphthalene	3,000	2.3	1
	550	12.5	2
Pyrene	170	41	3
	39,000	0.18	4
Benz[a]pyrene	110	63	3
	6,200	1.1	4
Benz[a]anthracene	260	27	3
Dimethylbenzanthracene	> 680	< 10	3
Dibenzanthracene	280	25	3
Fluorene	fast	completely destroyed	5
Phenanthrene	rapid oxidation of center ring, then very slow oxidation		5
	15,000	0.46	4
Anthracene	fast		5
Acenaphthene	fast		5
Acenaphthylene	fast		5
3,4-Benzofluoranthene	intermediate		5
11,12-Benzofluoranthene	intermediate		5

[a] Tested at pH = 1~7.

[b] Calculated from $t_{1/2} = 0.69/(k\,[\overline{O_3}])$, where $[\overline{O_3}] = 10^{-4}$ M.

Ref.: 1. Hoigné and Bader, 1983; 2. Legube et al., 1986; 3. Radding et al., 1976; 4. Butković et al., 1983; 5. WPCF, 1986.

al., 1990; Kuo, 1985). However, those researchers did not confirm the reaction order and stoichiometry.

Pryor et al. (1983) found that in solutions of dichloromethane (a non-participating solvent), linear free-energy relationships between the rate constants and the calculated orbital parameters of the nine unsubstituted PAHs studied could be obtained. They also found that electrophilic attack by ozone to yield a sigma-complex was the presumed rate-limiting step in the oxidation of these compounds. Although Lee and Hunter (1985) did not determine reaction rate constants, they did find that greater than 99% decomposition of naphthalene, fluoranthene, benz[b]fluoranthene, benz[k]pyrene, and benz[a]fluoranthene could be achieved when aqueous solutions of these compounds were oxidized using an ozone dosage of 21.3 mg/L and one hour contact time. The most recalcitrant of the compounds studied was benz[a]anthracene; 54% of this compound was degraded at the conditions given above. In the WPCF Manual of Practice No. FD-11 (1986) relative rate constants are listed for a number of PAHs. Though no numerical values for the rate constants are presented, qualitative indication of the reaction rates are given.

Several experiments have been conducted to identify ozonation products of a number of PAHs. The products identified are listed in Table 1.3. Dreher and Klamberg (1988) studied the degradation of PAHs in dilute aqueous systems and identified the

Table 1.3 Byproducts formed during the ozonation of various PAHs

Compound oxidized	Products	Ref.
Naphthalene	orthophthaldialdehyde; phthalaldehydic, phthalic, oxalic, formic, oxomalonic acids, hydrogen peroxide, a cyclic peroxide	1,6
	cis and trans-2-formylcinnamaldehyde, 1,4-naphthoquinone, phthalic acid	2
Phenanthrene	diphenic acid from center ring oxidation, then carbon dioxide and oxalic acid; biphenyl carboxylic acid, phthalic acid	3;6
Fluorene (FL)	oxalic acid, FL-oxide, mono- and de-hydroxyl-FL, 7-hydroxy-FL and 7-hydroxy-FL oxide	3;7
Anthracene	anthraquinone, phthalic acid	3;6
Naphthacene	Naphthaquinone, anthraquinone, phthalic acid	6
Acenaphthene	7-formyl-1-indanone, 7-hydroxy-1-indanone, 1-indanone, 1-indanone-7-carboxylic acid, indan-1,7-dicarboxylic acid	4;7
Acenaphthylene	1,8-naphthalenedialdehyde, 1-naphthoic acid, 1,2-epoxy-acenaphthalene, naphthaldehydic acid	4
Fluoranthene	FL-quinone, FL-acid	7
Pyrene	short chain aliphatic compounds	5
Chrysene	biphenyl carboxylic acid, phthalic acid	6

Ref.: 1. Legube et al., 1986; 2. Marley et al., 1987; 3. WPCF, 1986; 4. Kuo, 1985; 5. Corless, 1990; 6. Meineke and Klamberg, 1978; 7. Dreher and Klamberg, 1988.

major byproducts formed from the ozonation of these compounds. The ozonation of fluorene, acenaphthene and fluoranthene resulted in ring cleavage and a number of carboxylated benzenes, quinones and aliphatic compounds. Meineke and Klamberg (1978) studied the ozonation of other PAHs in dilute aqueous systems. They found that the main products of compounds, such as anthracene and naphthacene, are quinone or quinone substituted with carboxylic acids. For the other compounds investigated, e.g., phenanthrene and chrysene, the final products were biphenyl carboxylic acid and phthalic acid. Other researchers (NAS, 1972; Neff, 1979) found that the reaction of PAHs with ozone could result in the cleavage of phenanthrene-like bonds producing various diacids, quinones (by the oxidation of anthracene at the 9,10-like position), diones, and the oxidation of alkyl side-chains.

The products formed from the reaction of ozone with low molecular weight hydrocarbons (e.g., from the ozonation of naphthalene and substituted benzenes) have been successfully identified (Legube et al., 1983, 1986). Legube et al. (1986a) extensively studied the ozonation of naphthalene in aqueous solution and proposed three reaction pathways for the initial attack of ozone on naphthalene. These pathways are: (1) one electrophilic substitution of ozone on carbon 1 (or 2) of the naphthalene molecule, (2) two simultaneous 1,3-dipolar cyclic additions on both the 1,2 and 3,4 bonds of the naphthalene molecule, and (3) one 1,3-dipolar cyclic addition of ozone on the 1,2 bond of the naphthalene molecule.

Several mechanisms for the reactions of ozone with PAHs have been proposed, but have not been confirmed. The major focus of the research that has been conducted on the ozonation of PAHs has been to determine the kinetics of the reaction of ozone with these compounds in aqueous solutions (e.g., Hoigné and Bader, 1983; Staehelin and Hoigné, 1985). While some work has focused on investigating the products formed from the higher molecular weight compounds, because of the low aqueous solubility of PAHs much of this work has also been accomplished in organic solvents. However, as water acts as a participating solvent, different reaction products would be expected in water as compared to organic solvents. As a result, our knowledge of the mechanisms by which ozone reacts with PAHs in either natural waters or soils is severely lacking.

Although PAHs ozonation products may be more easily biodegradable than the parent compounds, little research has been done to evaluate the toxicity of the ozonation products. Some researchers (Pearson, 1982; Pelkonen and Nebert, 1982; Aryton et al., 1990) have shown that oxidation products of PAHs, such as hydroxylated and epoxidized forms of benz[a]pyrene which are produced biologically, are more carcinogenic form than their parent compounds. Il'nitskii et al. (1968) monitor the carcinogenicity of PAHs during ozonation. They used 60 times the ozone : PAH ratio and a treatment time of 2.5 min; they found the carcinogenic hydrocarbons were inactivated. However, Marley et al. (1987) found some of the ozonation products were shown to have significant toxicity. They found naphthalene ozonation products, such as o-phthalaldehyde, were shown to inhibit yeast

growth or were mutagenic in the Ames test. Upham et al. (1994) also found that the ozonation of PAHs resulted in the formation of initial byproducts that were more toxic than the parent compound. Therefore, an investigation of toxicity of the ozonation products needs to be addressed.

1.4 Research Objective

The major focus of this research is to investigate the pathways by which ozone reacts with PAHs in an aqueous system. This is central for understanding and predicting the role of ozonation in degrading PAHs in waters and other environment systems. To achieve such goal the following questions need to be answered:

1. Are the ozonation products more toxic?
2. Are the mechanisms by which the three different classes of PAHs (phenanthrene-, pyrene- and anthracene-types) react with ozone different? If so, are the byproducts resulting from each of these types of reactions different?
3. Can a better understanding of the reaction pathways be used to predict which products will be formed from PAHs based upon their classification?
4. How does the presence of other reactive species affect the rates and pathways of the reaction between the targeted PAH and ozone?

The following specific aims are designed in an attempt to answer the above questions:

1. To determine the kinetics of the reaction of ozone with pyrene, chrysene and benz[a]anthracene in the aqueous phase.

2. To assess the toxicity of ozonation products.
3. To identify the byproducts formed from the reaction of ozone with each of the target compounds.
4. To propose and verify a reaction pathway for the primary reaction of ozone with the target PAHs.
5. To investigate the effect of other reactive species on the pathway of the reaction of ozone with the target compounds.

1.5 Organization of Thesis

The objective of Chapter 2 was to investigate the toxicity of the ozonation products formed from pyrene. A non-genotoxic bioassay that monitors gap junction intercellular communication (GJIC) was used to assess the toxicity after the ozonation of pyrene. The results show the importance of using a biological assay to assess the amount of ozone required to reduce toxicity. The results also suggested that identifying the ozonation products is necessary. By identifying these ozonation products and the reaction pathways in aqueous solutions, treatment alternatives that would minimize the formation of toxic products can be developed. Observations described in this chapter show the feasibility of using ozone to remove pyrene and its toxic byproducts, as long as the ozone dosage was greater than 4.5 mol of ozone/mol of pyrene.

In Chapters 3 and 4, the ozonation pathway of pyrene and benz[a]anthracene are

investigated. The ozone consumption data obtained for the initial reaction with the target compound along with the identification of byproducts from the initial attack on the PAH molecule by ozone was used to propose the ozonation pathway. The ozone dosages were varied to result in different extents of reaction. The concentrations of target compounds and their ozonation products were quantified upon different extents of reaction. The ozonation products which were generated were identified. The effect of free radicals on the oxidation of the target compounds were also studied. The oxidation products were identified to determine if the presence of other reactive species affected the reaction pathway. In Chapter 3, the ozonation of pyrene was observed to occur by a stepwise ring cleavage, and in the oxidation of aldehydic moiety or functional group. Fourteen products including aldehyde and carboxylic acid substituted phenanthrene- and biphenyl- type oxidation products were identified. Two pairs of isomers were identified. In Chapter 4, the results of studies investigating the reaction of ozone with benz[a]anthracene are reported. Fifteen products including seven pairs of isomers were identified. Ozonation by bond attack (resulted in formation of phenyl-naphthyl type products) and atom attack (resulted in formation of quinone type products) occurred simultaneously.

In conclusion, a detailed discussion of ozonation pathways and an evaluation of the predictability of ozonation for the degradation of PAHs in aqueous systems is provided in Chapter 5. Bond- and atom-localization energies were used to predict the pathways by which PAHs would react with ozone. In comparing our results with previously published

researches, most of which were performed in organic solvents, the importance of the ·OH radical in aqueous reactions is emphasized. Based on these conclusions, the pathways by which other similar type of PAHs would react with ozone have been predicted.

In Appendix A, a kinetic study for the reaction of ozone with pyrene is reported. The reaction order of ozone with pyrene and the stoichiometric coefficient was investigated. Confirming these parameters would allow for a higher degree of confidence in predicting the rates of reaction of PAHs with ozone in other complex systems. An assumption of ozone direct reaction with target compound was made. Therefore, a second-order reaction was observed. However, the results observed show that, due to the extremely rapid reaction of ozone with PAHs, other reactive species were involved in the reaction. As such, the assumption that this reaction is overall second order reaction could not applied. In order to obtain the kinetic data for the reaction of ozone with only the parent compound, a stopped flow system should be used.

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CHAPTER 2

DETERMINATION OF THE EFFICACY OF OZONE TREATMENT SYSTEMS USING A GAP JUNCTION INTERCELLULAR COMMUNICATION BIOASSAY

ABSTRACT

A non-genotoxic bioassay that monitors gap junction intercellular communication (GJIC) was used to assess the presence of potential tumor promoters after the ozonation of pyrene, a polycyclic aromatic hydrocarbon (PAH). Water and ozone were not rate-limiting reactants in a solution containing 10 % water/90 % acetonitrile (v/v) and 5 mM pyrene during ozonation. Pyrene (5 mM) was oxidized at various ozone dosages, and the resultant mixtures were assayed for their effect on GJIC. Approximately 4.5 mol of ozone/mol of pyrene was required to remove all the intermediate byproducts found to be inhibitory to GJIC. At 3.6 mol of ozone/mol of pyrene, the mixture, which was slightly inhibitory to GJIC, was fractionated by RP-HPLC into 13 components, of which only one component was inhibitory to GJIC. Just 1.6 mol of ozone/mol of pyrene was needed to remove > 90 % of the pyrene, which showed that monitoring only the removal of the parent compound was insufficient for the assessment of the treatment efficacy.

2.1 Introduction

Environmental pollutants are a major concern from an ecological and human health perspective. This concern has led to a massive effort to clean up toxic waste dump sites and contaminated aquifers along with the development of numerous treatment technologies. The efficacy of a treatment process is most often assessed by monitoring the contaminant of interest. However, when a treatment process involves chemical transformations, the detection of the parent compound provides no toxicological information about the reaction products or the parent compound. Upham et al.(*1*) has shown that the ozonation of PAHs resulted in the formation of products more toxic than the parent compound. Also, numerous researchers (*2-4*) have shown that oxidation of simple aromatic compounds such as benzene or 1,3,5-trichlorobenzene can result in the formation of a very large number of byproducts. The identification and quantification of a large number of byproducts, such as those formed during chemical or biological processes is prohibitively expensive and time consuming. An evaluation of chemical and biological treatment technologies needs to be made using toxicological screening methods to assess the effects of the byproducts on biological systems. The treatment process can then be engineered to minimize the toxicological effects of the byproducts.

Cancer is a primary health concern resulting from human exposure to environmental pollutants. In the past, *in vitro* assays for mutagenicity (genotoxicity) of environmental contaminants have been used for the detection of potential carcinogens.

Many chemicals appear not to be genotoxic (*5-7*) but rather to modify gene expression by their ability to alter the homeostatic control of multicellular organisms (*8*). Intercellular communication is a crucial biological process that maintains the homeostatic health of multicellular organisms (*9, 10*). A membrane structure called the gap junction permits the transfer of low molecular mass ions and molecules ($\leq$ 1200 Da) between contiguous cells (*11*). Amino acids, cAMP, calcium, inositol, and triphosphates are regulatory molecules known to diffuse through gap junctions (*12-14*). The gap junction has been linked to many regulatory roles such as growth control, developmental and differentiation processes, synchronization, and metabolic regulation (*15*). Chronic exposure to epigenetic toxicants that have been implicated in tumor promotion during carcinogenesis (*15-17*), in teratogenesis (*18*), in reproductive dysfunction (*19-21*), and in the alteration of muscle contractions in the heart and uterus (*22, 23*) are also known to effect gap junction intercellular communication (GJIC). Trosko et al. (*24*) also noted that neurotoxicological effects could result from the inhibition of intercellular communication in the central nervous system. A mutation in the connexin 32 gene that encodes for gap junction proteins has been implicated in the cross-linked Charcot-Marie-Tooth syndrome, which is a peripheral neuropathy (*25*).

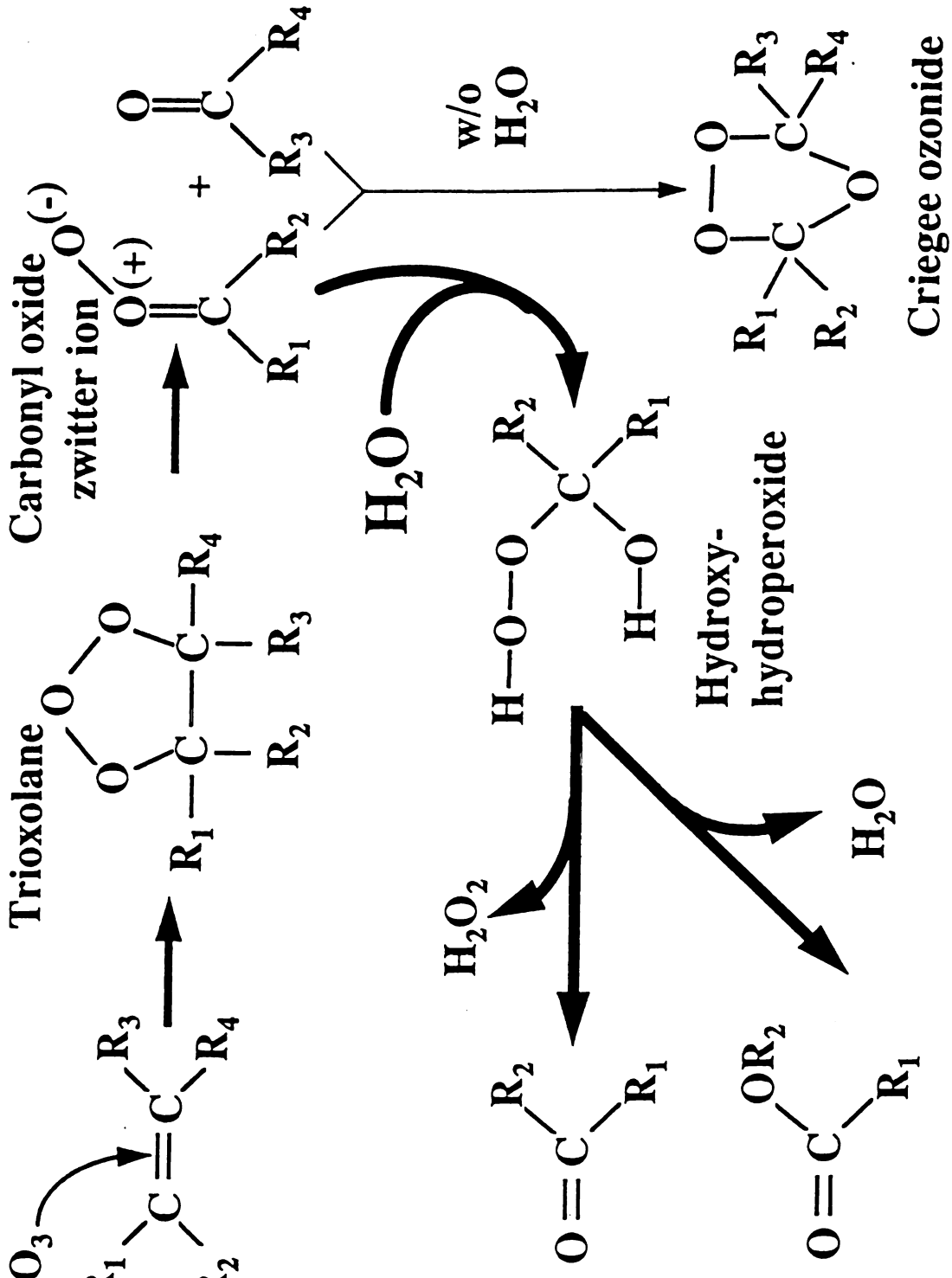
It is clear that the inhibition of GJIC can result in pathological consequences including carcinogenesis (*16, 26*). Thus, the effects of pollutants on the mammalian gap junction can give invaluable toxicological information. Previous toxicological evaluation

of ozone byproducts has focused primarily on genotoxic events (e.g., see *27-29*). The objective of this research was to use a bench-scale, completely-mixed, semi-continuous reactor to assess the efficacy of the ozonation of aqueous organic compounds to remove products that are inhibitory to GJIC. Pyrene was used as the target compound.

As PAHs (e.g., pyrene) usually have low water solubilities, one must either ozonate the chemicals in dilute aqueous solutions and concentrate the products for bioassays or ozonate the compound in more concentrated organic solutions that can be then directly used for biological assays. The latter was chosen in this study to avoid potential losses or contamination of the products during the concentration step.

As water is a participating solvent in the reaction of ozone with unsaturated or aromatic hydrocarbons (*30* and Figure 2.1), the addition of water to an ozone reactor containing a high concentration of an organic solvent is necessary. The primary products of ozonating in the presence of water are H_2O_2 and compounds that contain carboxylic acid and aldehyde functional groups (*30*, Figure 2.1). In the absence of water the primary product is the Criegee ozonide. It was essential to choose a solvent that was nonreactive with ozone and miscible with water. Therefore, it was also necessary to establish conditions wherein water not a rate-limiting reactant to ensure that the ozonation pathway favored the reaction of the carbonyloxide zwitter ions with water.

Figure 2.1 Reaction of ozone with an unsaturated carbon in the presence and absence of water. The primary products in the presence of water are H_2O_2, an aldehyde, and a carboxylic acid if R_2= H.


Trioxolane
Carbonyl oxide
zwitter ion
w/o
H_2O
H_2O
Hydroxy-
hydroperoxide
H_2O_2
H_2O
Criegee ozonide

2.2 Methods

2.2.1 Cell Culture

WB-F344 rat epithelial cell lines were obtained from Drs. J. W. Grisham and M. S. Tsao of the University of North Carolina (Chapel Hill, NC). Cells were cultured in 2 mL of D medium (Formula No. 78-5470EG, GIBCO Laboratories, Grand Island, NY), supplemented with 5% fetal bovine serum (GIBCO Laboratories, Grand Island, NY) and 50 mg/mL gentamicin (Quality Biological, Inc., Gaithersburg, MD). The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air. The cells were grown in 35 mm^2 plastic petri dishes and the culture medium was changed every other day. Bioassays were conducted with confluent cultures that were obtained after 2-3 days of growth.

2.2.2 Bioassay of GJIC

The scrape loading/dye transfer (SL/DT) technique was adapted after the method of El-Fouly et al. (*31*). The cells were incubated with 20 μL of the target compounds for 60 min unless indicated otherwise. A primary product of the reaction of ozone with aromatic hydrocarbons and alkenes is hydrogen peroxide (*30, 32*); therefore, hydrogen peroxide was removed from the growth medium by the addition of catalase (1000 Units/mL; Sigma Chemical Co., St. Louis, MO) to cell cultures immediately preceding the addition of the ozonated solutions. Also, GJIC assays were conducted at noncytotoxic levels of the samples as determined by the neutral red uptake assay kit (Sigma Chemical Co., St Louis, MO). Following incubation, the cells were washed with 3 mL of phosphate

buffered saline (PBS). Lucifer yellow was added to the washed cells and three scrapes were made with a surgical steel-blade scalpel under low light intensities. The purpose of the three scrapes was to ensure that the scrape traversed a large group of confluent cells. After a 3-minute incubation period the cells were washed with 10 mL of PBS and then fixed with 2 mL of a 5 % formalin solution. The distance the dye traveled from the scrape was measured from photographs of microscopic images (magnification 200×) made of the fixed cells using a Nikkon Diaphot-TMD epifluorescence phase-contrast microscope illuminated with an Osram HBO 200W lamp and equipped with a 35-mm FA camera (Nikkon, Japan).

The distance the dye migrated was measured from the cell layer at the scrape to the edge of the dye front that was visually detectable. Due to the slight irregularity of the dye front's edge, the distance along one scrape per dish was measured every 1 cm for a total of 8 cm (at 200×, 1 cm = 50 mm). The average cell length was 25 mm; therefore, the 1-cm measurement on the photograph was equivalent to two cells. Each scrape chosen for photographic analysis was from a group of cells that were homogeneous in cell morphology and confluency. The nine measurements of distance were averaged and reported as the distance the dye traveled for the chosen group of cells representing the cell population of one plate. The photographs were taken and developed at the same time. Three plates per treatment were measured as described, and the values were reported as an average ± 1SD. An analysis of variance (ANOVA) was used for a test of significance

for the results. A Dunnett's multimean range t-Test was used for a test of significance between each treatment and the control (*33*).

2.2.3 Ozonation

Ozone was generated in dried oxygen by electric discharge using a Polymetrics Model T-408 ozone generator (San Jose, CA). Ozone was bubbled into a 100 mL solution of 5 mM pyrene (99% purity, Sigma Chemical Co., St. Louis, MO), dissolved in a acetonitrile/water solvent mixture. Acetonitrile was chosen as the organic solvent because it is miscible with water, has little effect on GJIC (*1*), is immediately amenable to RP-HPLC analysis, and has a low reactivity with ozone with a half life of 18 years (*34*). The acetonitrile (99.8%, purity) was purchased from EM Science (Gibbstown, NJ). The pH of the reactor solution was approximately 2. The above reactions were carried out in a 125 mL gas washing bottle. The ozonated solutions were continually mixed with a magnetic stirrer and stir bar. Effluent gaseous ozone was trapped in 2% (w/v) KI in water. The flow of ozone was regulated at 100 mL/min with a Sidetrack flow controller (Sierra Instruments Inc, Monteray, CA). The i.d. of the tubing was 1/8". All the tubing, connectors and valves were constructed of Teflon®. The concentration of ozone in the influent and effluent gas stream was measured spectrophotometrically at 258 nm using a UV-vis spectrophotometer (Model 1201, Shimadzu Scientific Instruments, Japan). The molar absorptivity coefficient for ozone is 3000 M^{-1} cm^{-1} (*35*). The flow cells were quartz cuvettes with a path length of 0.2 cm. Reactions were terminated by flushing the reactor

with helium for 3-4 min to remove detectable levels of ozone. Quenching agents were not used to remove ozone so as to avoid the formation of radical species and the overloading of the RP-HPLC columns and to simplify the evaluation of the biological effects of the ozonated mixtures.

2.2.4 HPLC Analysis

A Gilson HPLC unit (Worthington, OH) equipped with two pumps (Model 303), manometric module (Model 802b), dynamic mixer (Model 811), UV detector (Model 116), sample injector (Model 231) and diluter (Model 401), and a 5mm 4.6 × 250 mm C-18 Partisphere RP column (Whatman, Clifton, NJ) was used for the chromatographic separations. The eluent was monitored at two wavelengths (210 and 240 nm). The parent compound was separated from the oxidized products by two different linear gradient systems. Linear gradient system A consisted of 50%:50% (V:V) acetonitrile/water at the time of injection and increased to 95%:5% acetonitrile/water 15 min after injection. The mobile phase was held at this composition for an additional 4 min and then decreased back to 50%:50% acetonitrile/water mixture giving a total run time of 20 min. Linear gradient system B consisted of 15%:85% (v:v) acetonitrile/water at the time of injection and increased to 25%:75% acetonitrile/water 15 min after injection. The mobile phase was held at this composition for an additional 4 min and then decreased back to a 15%:85% acetonitrile/water mixture giving a total run time of 20 min. Linear gradient system A separated the less polar components found at the lower ozone dosages while

system B separated the polar components found at the higher ozone dosages. Fraction collection was conducted manually. Each fraction was freeze-dried and reconstituted in 90% acetonitrile at a volume equivalent to the injection volume.

2.3 Results

Pyrene (500 mM) was ozonated in an acetonitrile solvent containing various concentrations of water ranging from 5.6 (10%) to 33 M (60%). The ozonation byproducts of these reactions were collected after the reactor reached reaction-limited conditions and separated using RP-HPLC (Figure 2.2A). The chromatographic profiles obtained for each concentration of water had essentially the same fingerprint. Various concentrations of pyrene ranging from 0.5 to 5.0 mM were ozonated in solutions containing 90 % acetonitrile/10% water (v/v). The ozonated mixtures were diluted by a factor determined from the initial concentration of pyrene in the solution relative to the lowest initial concentration of pyrene (0.5 mM); e.g., the solution containing an initial concentration of 5.0 mM was diluted 10-fold and separated by RP-HPLC (Figure 2.2B). These chromatographic profiles were also the same relative to retention times.

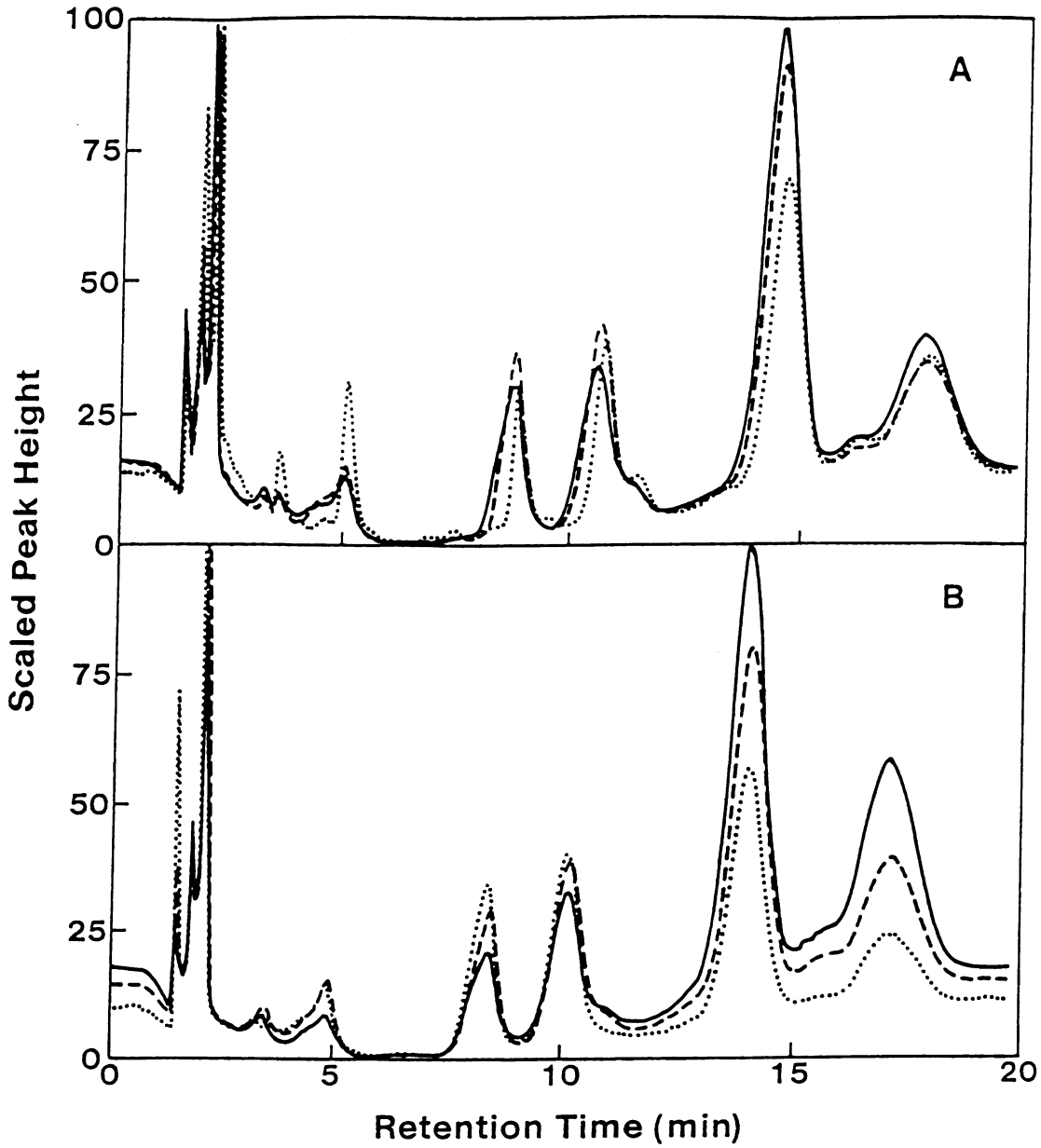


Figure 2.2 (A) Chromatographic separation by RP-HPLC of ozonated pyrene that was dissolved in various concentrations of acetonitrile/water mixtures. The solid, dashed, and dotted lines were mixtures containing 10%, 25%, and 60% water, respectively. This separation used linear gradient system B.
(B) Chromatographic separation by RP-HPLC of various concentrations of pyrene ozonated in a 90%/10%, acetonitrile/water mixtures. The solid dashed, and dotted lines were mixtures containing initial concentrations of 5.0, 2.5, and 1.0 mM pyrene, respectively. This separation used linear gradient system B.

The ozone demand, which includes the actual chemical demand and the dissolution of ozone into solution, exerted during the reaction of ozone with pyrene is shown in Figure 2.3A. Figure 2.3B is an example of the raw data obtained during the reaction of ozone with 0.1 mM pyrene. Ozone demand was calculated as the difference between influent and effluent gaseous ozone concentrations as a function of time. Using gas flow rates, the change in concentration of ozone was converted to a change in mass. The ozone demand of the reactor in the absence of pyrene is represented by the curve with the closed squares which showed that the ozone demand rapidly approached zero. The addition of pyrene increased the ozone demand of the system. The amount of ozone consumed remained constant and at a high level for a longer period at higher concentrations of pyrene.

After the reaction involving pyrene with ozone was terminated, samples were collected for HPLC analysis. Ozonation reactions were terminated by flushing the system with helium, which resulted in the rapid removal of ozone from the system (Figure 2.3B). A three dimensional representation of chromatographic profiles with increasing contact time of ozone with pyrene is shown in Figure 2.4. As the contact time with ozone was increased the products tended to elute from the column at shorter retention times (i.e., they are more polar).

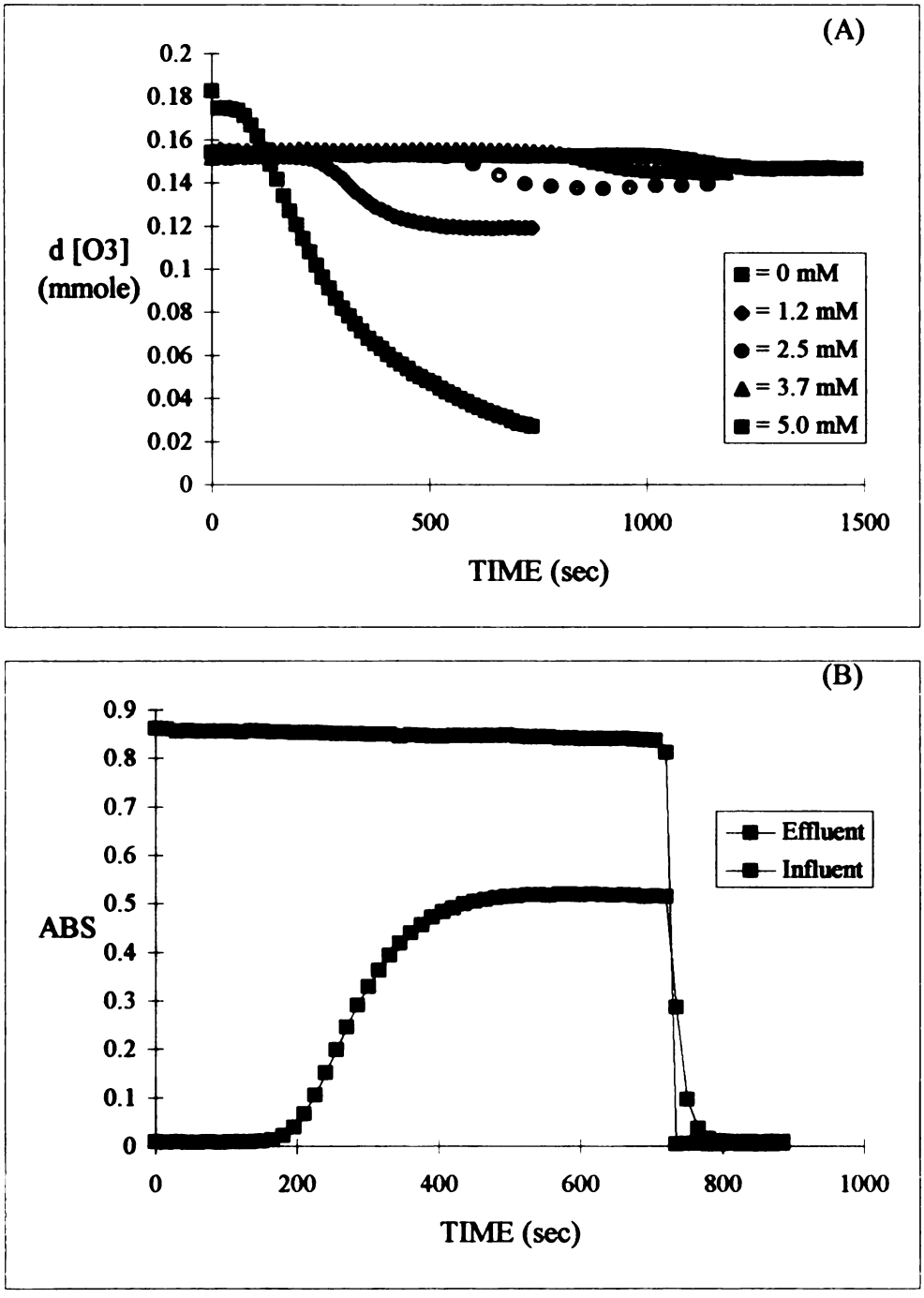
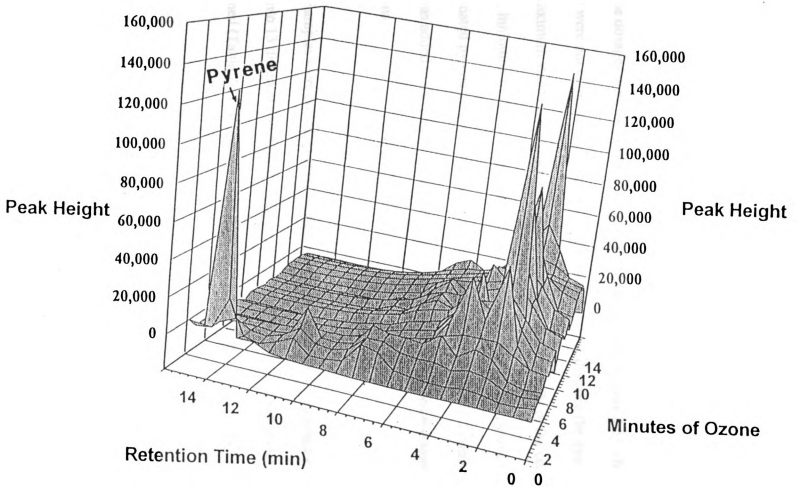


Figure 2.3 (A)Ozone demand of the reactor for various concentrations of pyrene
(B)An example of the raw data collected during the reaction of ozone with 0.1 mM pyrene.

Figure 2.4 3-D plot of RP-HPLC chromatograms of 5 mM pyrene reacted with ozone for different ozone contact time intervals. The average flow rate was 1.25 mmol of ozone/min. This separation used linear gradient system A.


Pyrene
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
Peak Height
14
12
10
8
6
4
2
0
Retention Time (min)
Minutes of Ozone
Peak Height

Pyrene was ozonated at various dosages ranging from 0 to 4.4 mmol of ozone/mmol of pyrene. The reactor reached reaction-limited conditions at 4.4 mmol of ozone/mmol of pyrene. Reaction-limited conditions were determined by observing the time it took for the effluent ozone gas to reach concentrations that remained fairly constant over time (Figure 2.3B). Ozonated samples (20 mL aliquots) obtained at each of the ozone dosages were assayed for biological effects on GJIC (Figure 2.5). These samples were not cytotoxic as compared to the control and the distance the dye traveled had a maximum standard deviation for toxicity was equivalent to 10% of the control. Initially, inhibition of GJIC did not begin to decreased until 50% of the pyrene was gone. The ozonated pyrene mixture remained inhibitory until approximately 2.2 mmoles of ozone was added to the reactor. This was also when the reactor reached reaction-limited conditions.

Samples were collected at an ozone dosage of 1.8 mmol and separated on RP-HPLC into 13 different components (Figure 2.6). The effect of each fraction on GJIC was determined (Table 2.1). It appeared that only peak 13 was inhibitory to GJIC.

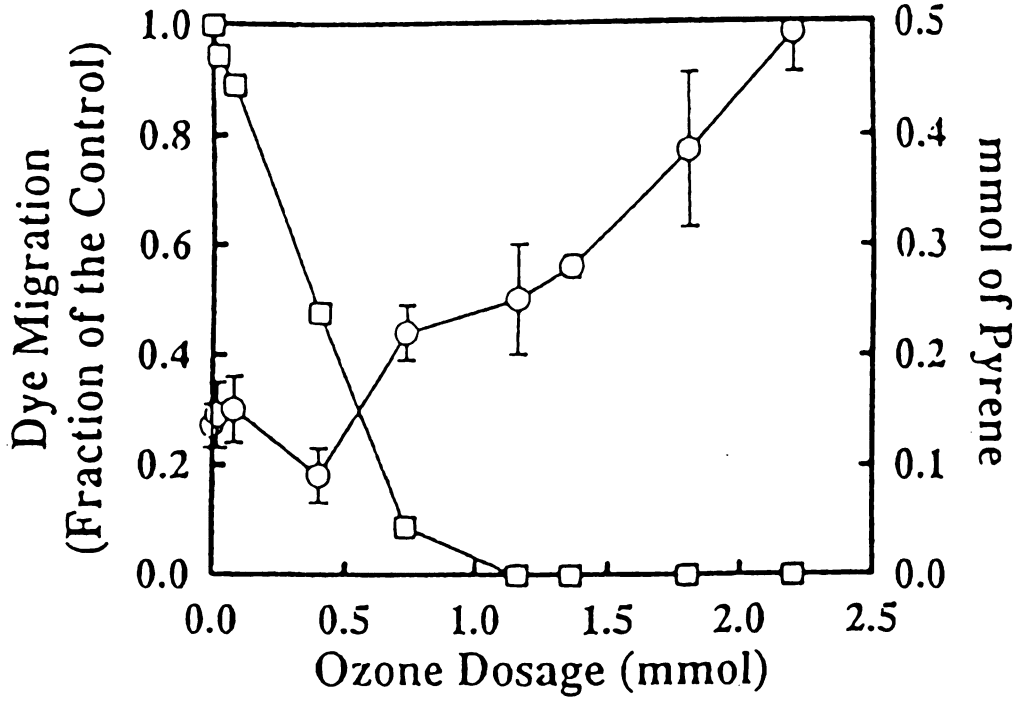


Figure 2.5 Determination of the ozone dosage required for the removal of pyrene and the oxidation byproducts from the solution that were inhibitory to GJIC.
□ = the concentration of pyrene, which was an average of two replicates, with a detection limit of 500 nM as determined using RP-HPLC.
○ = the inhibition of GJIC, which was an average ± 1 SD of the distance the dye traveled from three different plates per experiment.

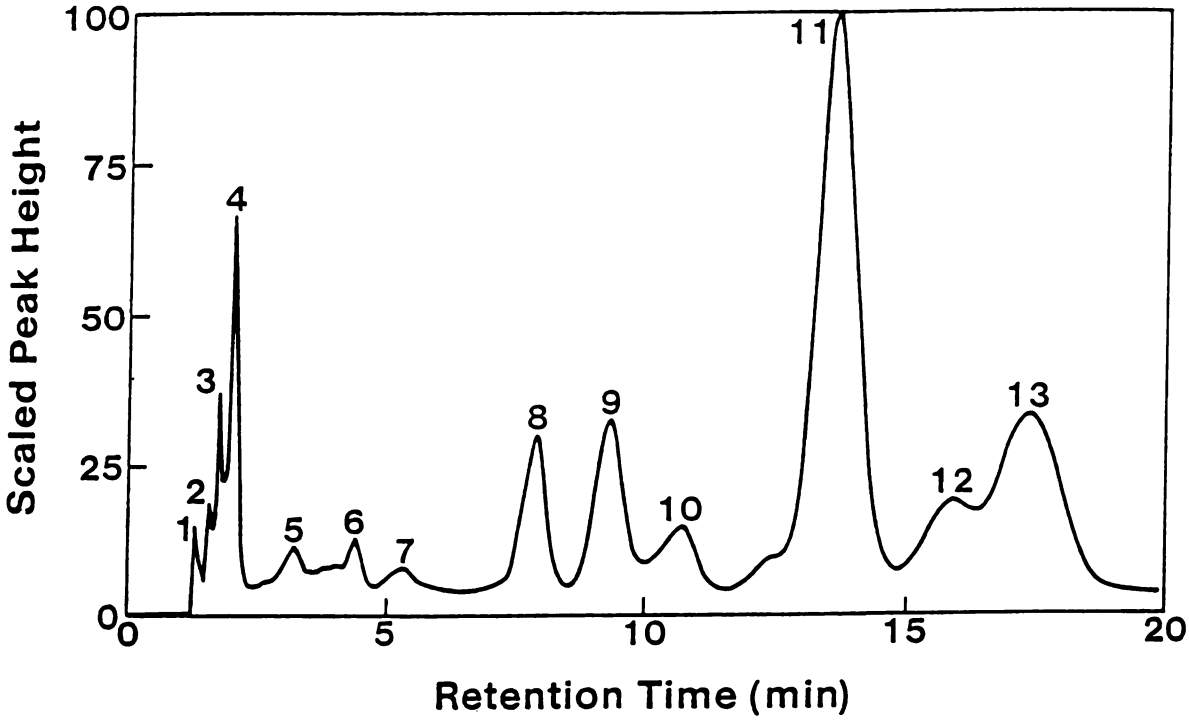


Figure 2.6 Representative chromatogram of the by-product peaks collected for analysis of GJIC activity shown in Table 2.1. This separation used linear gradient system B.

Table 2.1 Effect of ozonated byproducts of pyrene on GJIC

Compound added to cells	Fraction of control[a]
Control (acetonitrile)	1.00 ± 0.05
Pyrene (unozonated)	0.31 ± 0.10[b]
Ozonated pyrene, HPLC peak 1	0.94 ± 0.06
Ozonated pyrene, HPLC peak 2	0.99 ± 0.06
Ozonated pyrene, HPLC peak 3	1.16 ± 0.10
Ozonated pyrene, HPLC peak 4	0.75 ± 0.22
Ozonated pyrene, HPLC peak 5	0.98 ± 0.05
Ozonated pyrene, HPLC peak 6	1.05 ± 0.17
Ozonated pyrene, HPLC peak 7	1.09 ± 0.07
Ozonated pyrene, HPLC peak 8	1.11 ± 0.04
Ozonated pyrene, HPLC peak 9	1.22 ± 0.11
Ozonated pyrene, HPLC peak 10	1.14 ± 0.05
Ozonated pyrene, HPLC peak 11	0.99 ± 0.01
Ozonated pyrene, HPLC peak 12	0.93 ± 0.13
Ozonated pyrene, HPLC peak 13	0.52 ± 0.25[b]

[a] Each datum represents an average ± 1 SD of the distance the dye traveled in three different plates per experiment. All plates were from the same batch of cells. The average distance the dye traveled in the control was 140 μm. The ozone dose was 1.8 mmol ozone/0.5 mmol of pyrene. The above results were significant at $p \leq 0.01$ as determined by ANOVA.

[b] These averages were found to differ significantly from the control at $p \leq 0.01$ as determined by a Dunnett's *t*-Test.

2.4 Discussion

One of our objectives was to determine the optimum conditions for ozonating a contaminant in an organic solvent system in which the reaction was not rate limiting in either ozone or water. Water is a participating solvent in the reaction of ozone with unsaturated and aromatic hydrocarbons (*30*), and if water is not present in the system in sufficient quantity, the primary product is the Criegee ozonide, which is not the primary product formed in an aqueous environment (Figure 2.1). High concentrations of many organic molecules can be attained in a 90% acetonitrile/10% water mixture; therefore, we wanted to determine if ozonation in 10% water will result in the same kind of products as ozonations at very high water concentrations (e.g., at 60%).

The lack of major differences between the RP-HPLC chromatograms at low and high concentrations of water shows that water was not a rate-limiting reactant (Figure 2.2A). Similarly, there were no changes in the RP-HPLC chromatograms of the byproducts at various initial concentrations of pyrene (Figure 2.2B), indicating that ozone was not rate-limiting in this system. This shows that a pure aqueous system can be mimicked by ozonating high concentrations of organic compounds such as pyrene in a relatively nonpolar solvent/water mixture. The mixture of byproducts formed in such a system can be directly tested for biological effects without the need to concentrate the products.

The samples described above were ozonated at reaction-limited conditions in which the effluent ozone concentrations remained constant with time. These conditions followed a period of a high ozone demand where the effluent concentrations were zero (Figure 2.3B). This period of maximal ozone demand increased with increasing concentrations of pyrene (Figure 2.3A). As apparent from the HPLC chromatograms (see Figure 2.4), during this period of maximum ozone demand, pyrene disappears and intermediate products form and also subsequently disappear or diminish in concentration. Only the most polar compounds (shorter retention times) continued to increase in concentration with ozonation time. This is expected since the ozonation of organic compounds usually results in the formation of products containing aldehyde, carboxylic acid and hydroxyl functional groups (refs *30, 38* and Figure 2.1). Increasing the water solubility of an organic compound would probably increase the mobility of the compound(s) in an aqueous environment. This is significant since the more water soluble compounds would be more easily mobilized into aquifers, streams and lakes.

At steady state, the concentration of effluent ozone was not equivalent to the influent ozone concentration (Figure 2.3B), which was probably the result of autocatalytic reactions of ozone in aqueous solutions (*36*) and pressure differences from diffusion processes across the liquid/air interface. Autocatalytic processes were minimized by ozonating the solution at an approximate pH of 2 where the decomposition of ozone to hydroxyl radicals is slow (*37*); this allowed us to assess the toxicity of the

mixture that was primarily the result of ozonation reactions.

The reaction of pyrene, benz[a]pyrene and fluoranthene at low ozone dosages results in the formation of products that are more inhibitory than the parent compound (*1*). Therefore, our ultimate goal was to determine if higher ozone dosages can remove all inhibitory byproducts formed from the ozonation of a polycyclic aromatic hydrocarbon such as pyrene. The removal of all the inhibitory components was achieved at an ozone dosage of approximately 4.5 mol of ozone/mol of pyrene (Figure 2.5). The ozonated pyrene solution was most inhibitory at the point where 50 % of the pyrene was removed, showing that the intermediate products enhanced the toxic effect of pyrene. Although, only 1.6 mol of ozone/mol of pyrene was needed to remove most of the pyrene, the solutions ozonated at this level were still inhibitory to GJIC. This showed that monitoring only the removal of the parent compound could result in the production of intermediate products that are also biologically unsafe. Hydrogen peroxide, which is formed during the ozonation of unsaturated and aromatic hydrocarbons (Figure 2.1), may also play an important role in the inhibition of GJIC because ozonated mixtures of pyrene were more inhibitory when hydrogen peroxide was not removed from the mixture (*1*).

The ozonation byproducts formed during the reaction of pyrene at low ozone dosages were separated by RP-HPLC into several components. All fractions were shown to be inhibitory to GJIC (*1*). In this paper, the byproducts formed were

chromatographically separated (see Figure 2.2) at high ozone dosages (approximately 3.6 mol ozone/mol pyrene). At this ozone dosage the mixture of byproducts was still slightly inhibitory to GJIC (Figure 2.5). Only one out of the 13 components was found to be inhibitory to GJIC (Table 2.1) as determined by the Dunnet's test. This component (peak 13) was one of the less polar compound(s) as indicated by the longer HPLC-retention time. Due to the lack of power in the Dunnett's test, another peak such as peak 4 might have been significant if more than three replicates were obtained but the overall conclusion remains the same, i.e., the intermediate byproducts of ozonated pyrene, which were very inhibitory to GJIC, readily reacted with ozone resulting in the formation of stable compounds, most of which are not inhibitory to GJIC.

Observations described in this paper show the feasibility of using ozone to remove pyrene and many of its byproducts and the importance of using a biological assay to assess the amount of ozone required to reduce toxicity. In particular, the use of a non-genotoxic assay is unique compared to previous work which employed genotoxic and cytotoxic assays. The use of a non-genotoxic bioassay should make a significant contribution in the development of risk assessment models for water treatment technologies that are concerned with removing carcinogenic compounds. This is especially true since most carcinogens appear not to be genotoxic (*5-7*) but rather demonstrate tumor promoting activity, which has been linked to the down regulation of GJIC (*16*). The most common genotoxic assay employed has been the Ames test which is

based on a prokaryotic bacterial system that makes extrapolation to mammalian systems difficult. Furthermore, Ames test results are often conflicting. For example, the treatment of water by ozone has shown both an increase (*28*) and a decrease (*27*) in mutagenicity. Tumor initiating properties determined in vivo also showed no effect (*27*). Also, mutagenicity is no longer equated with carcinogenicity. For example, water treated with either chlorine, chlorine dioxide or monochloroamine was concentrated 4000× by XAD resin adsorption and tested for mutagenicity and lung adenomas (*27*). The samples were all mutagenic but did not cause the formation of any tumors.

2.5 Conclusion

In conclusion, the simple monitoring of the disappearance of pyrene during chemical treatment is insufficient to protect human health and the environment. Bioassays, such as GJIC, must be employed during the design, testing, and monitoring of the engineered processes. In our system, we specifically showed that 4.5 mol of ozone/mol of pyrene were required to remove all components inhibitory to GJIC, whereas only 1.6 mol of ozone/mol of pyrene was required to remove most of the pyrene.

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CHAPTER 3

THE OZONATION OF PYRENE IN ACETONITRILE/WATER MIXTURE: PATHWAY AND PRODUCT IDENTIFICATION

ABSTRACT

The pathway by which ozone reacts with pyrene, a polycyclic aromatic hydrocarbon (PAH), is described in this paper. Pyrene was dissolved in a 90 % acetonitrile : water (v/v) mixture to achieve an initial concentration of 5 mM pyrene. The ozone dosages were varied to obtain different extents of reaction. A stoichiometric ratio of 1.68 moles ozone/mole pyrene was required to complete destroy pyrene. The ozonation products were identified by gas chromatography / mass spectrometry (GC/MS). Fourteen products including aldehyde and carboxylic acid substituted phenanthrene- and biphenyl- type oxidation products were identified. Several ring-cleavage reactions occurred sequentially: i) one ring cleavage of pyrene occurred at the 4,5 position, with phenanthrene-type products predominating; and ii) once secondary ring cleavage occurred, the concentration of pyrene and the phenanthrene-type products decreased dramatically, while the concentration of biphenyl-type products increased. The oxidation of pyrene by hydroxyl free radicals was found to be involved in the reaction, even at pH 3.7.

3.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are derived from the incomplete combustion of organic matter. Mineral fuels, coal derived oils, tobacco smoke and vehicle exhaust (*1*) contain PAHs. The release of PAHs to the environment has resulted in the presence of detectable levels of PAHs in air, water and soil (*2*). As PAHs are sparingly soluble or insoluble in water and recalcitrant, they will tend to accumulate on solid phases, including on air-borne particulate matter and soil organic matter. The accumulation of PAHs in the environment has resulted in the need for remediation processes that will reduce the risk of human exposure to these chemicals, many of which are carcinogenic or potentially carcinogenic (*2*).

Ozone, which is used both in water and wastewater treatment, is a very powerful oxidant that can react with PAHs with rate constants on the order of $>10^3$ $M^{-1} s^{-1}$ (*3,4*). The products formed from the reaction of ozone with PAHs have been identified by several investigators (*5-10*). In most studies (*5-8*), the ozonation products were generated in pure organic solvents. Neff (*8*) found that, in organic solvents, the reaction of PAHs with ozone produces various diacids, quinones, and diones, and results in the oxidation of alkyl side chains. However, limited data describing the oxidation of PAHs in aqueous solutions are available (*11*). Additionally, when ozonation reactions were performed in aqueous solutions excess ozone was used and only the final products were identified. Dreher and Klamberg (*9*) found that in dilute aqueous solutions, the ozonation of fluorene, acenaphthene and fluoranthene in the presence of excess ozone resulted in ring cleavage with the formation of a number of carboxylated benzenes, quinones and

aliphatic compounds.

Pyrene, the target PAH used in this study, can be completely destroyed using ozone treatment (*11,12*). While the toxicity of pyrene has been investigated (*13*), to our knowledge, no studies other than those of Upham et al. (*12,14*) have investigated the toxicity of the products formed from the ozonation of pyrene. These studies have shown that the ozonation of pyrene results in the formation of several intermediates that are more inhibitory to gap junction intercellular communication (GJIC) than is pyrene (*14*). It was also shown that an ozone dosage of 1.6 moles ozone/mole pyrene was required to oxidize pyrene entirely, whereas a dosage of 4.5 moles ozone/mole pyrene was required to destroy all products inhibitory to GJIC (*12*). Based upon this work, it became evident that research is needed to identify the products formed from the reaction of ozone with pyrene. By identifying these products and the reaction pathway, it is our aim to develop treatment alternatives that would minimize the formation of toxic products. The pathway by which ozone reacts with PAHs has been studied in a non-participating solvent (*15*), and Bailey (*16*) proposed a reaction pathway based upon the results. However, the reaction pathway has not been investigated in water, which is a participating solvent.

The objective of this study is to identify the ozonation products and to propose a pathway for the reaction of pyrene with ozone in aqueous solutions. As the intermediates formed from the reaction of ozone and pyrene were found to inhibit GJIC more than pyrene, pyrene was used as the model compound. The ozone dosages were varied to result in different extents of reaction. The ozonation products obtained after pyrene had

reacted to different extents were identified. Also, the relative concentrations of pyrene and its ozonation products were quantified at different extents of reaction.

3.2 Methods

Ozonation experiments

Ozone was generated from dried oxygen by electric discharge using a Polymetrics Model T-408 ozone generator (San Jose, CA). The system is illustrated in Figure 3.1. Pyrene solution was added to a 125 mL gas washing bottle containing 100 mL mixture of acetonitrile /water (90:10 v/v). The tubing (1/8" i.d.), connectors and valves were constructed of Teflon® or stainless steel. Gaseous ozone was bubbled into the pyrene solution which was continuously mixed using a magnetic stirrer and stir bar. Prior to commencing ozonation, helium was passed through the system to clean it. After purging with helium, ozone was allowed to flow into the system but by-passed the reactor to a trap containing 2% (w/v) KI in water. When the influent ozone reached steady state, the influent ozone was then passed through the reactor to commence the reaction. The flow of ozone into the reactor was regulated at 100 mL/min using a Sidetrack flow controller (Sierra Instruments Inc., Monteray, CA). The concentrations of ozone in the influent and effluent gas streams were measured spectrophotometrically at 258 nm using a UV-vis spectrophotometer (Model 1201, Shimadzu Scientific Instruments, Japan). The absorbance values were converted to concentration units using a molar absorptivity coefficient for ozone of 3000 M^{-1} cm^{-1} (*17*). Quartz flow cells with a path length of 0.2 cm were used. To avoid the formation of radical species, ozone was not quenched using

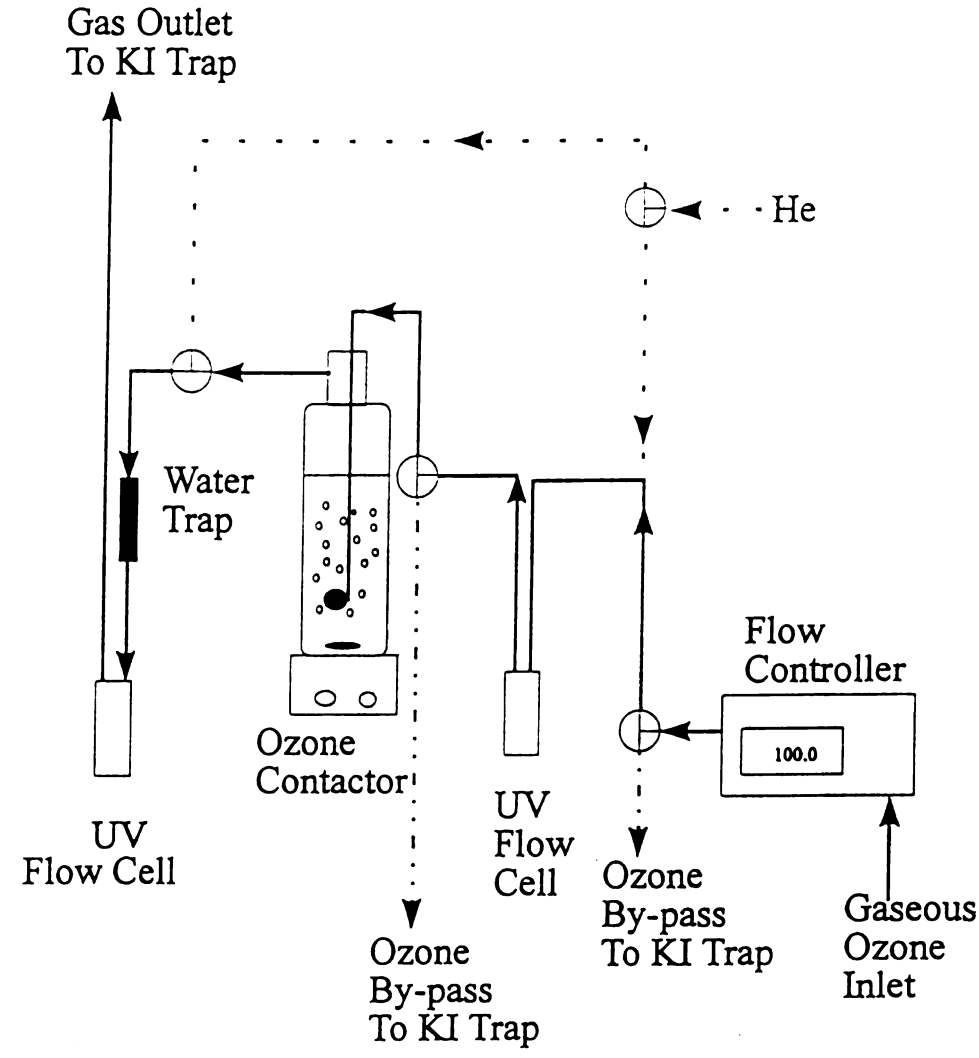


Figure 3.1 Configuration of ozonation system

chemical reagents but rather, reactions were terminated by purging the ozone from the reactor with helium. An average of 15 to 30 sec were required to purge the ozone to below the detection limit (ABS < 0.001).

When the concentration of ozone in the effluent gas was constant, i.e., when the byproducts did not react further with ozone (as illustrated in Figure 3.2), the reaction can be considered to be complete.

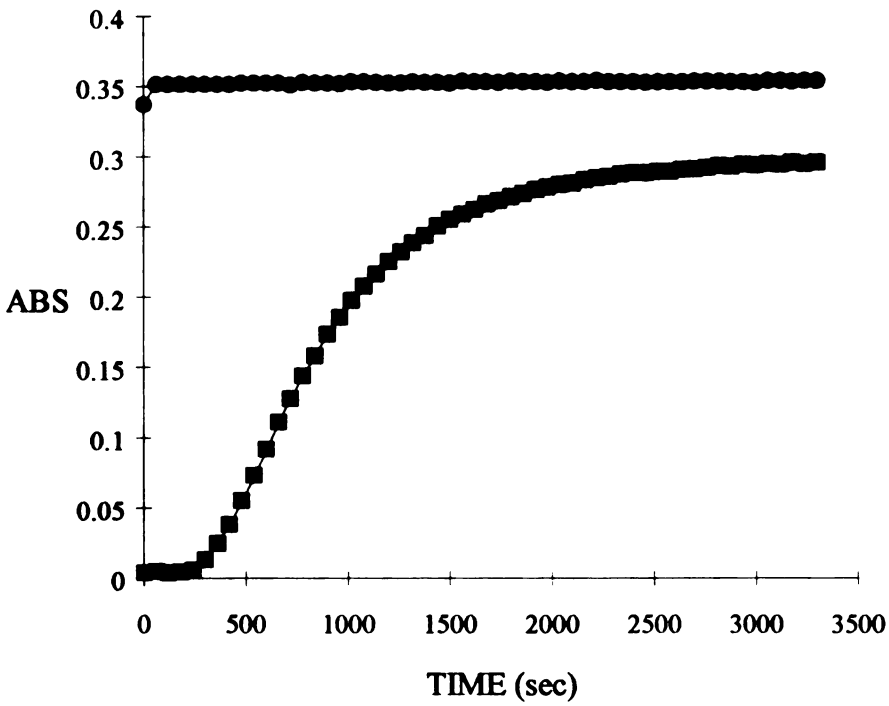


Figure 3.2 Ozone breakthrough curve for ozonation of 5 mmoles/L pyrene dissolved in 90% CH_3CN/water solvent mixture. • Influent ozone absorbance; ■ Effluent ozone absorbance.

In order to study the reaction pathway for the primary reaction of ozone with each of the target PAHs, the byproducts from the initial attack of ozone on the PAHs need to be identified. The initial reaction byproducts were generated in the same manner as that described above except the reaction was stopped before ozone was detected in the off-gas.

Since pyrene has very low aqueous solubility (S_w = 32 ppb at 24 °C), pyrene was dissolved in acetonitrile so as to achieve sufficiently high concentrations of the ozonation products to allow for their direct identification by gas chromatography/mass spectrometry (GC/MS). Concentration procedures such as solvent extraction were avoided to minimize losses of ozonation products. Acetonitrile (99.8% purity, EM Science, Gibbstown, NJ) was chosen as the organic solvent because it is miscible in water and it has a low reactivity with ozone ($t_{1/2} \geq 18$ years at pH 7 and $[\overline{O_3}] = 20.8$ mM) (18). Water was added to the acetonitrile at a sufficiently high concentration for the water to act as a participating solvent. As evidenced in a previous study (*12*), the HPLC chromatographic profiles for various concentrations of water ranging from 10% to 60% (volume ratio) were identical. Therefore, a 90% acetonitrile/water (v/v) mixture was chosen for all the experiments. In aqueous solutions, the reaction of ozone with OH^- will form ·OH radicals. To minimize side reactions resulting from the oxidation of pyrene by ·OH radicals, acidified distilled water was used in preparing the solvent mixture. Distilled water was acidified to pH 2 using phosphoric acid (85% purity) and reduced the pH of the solvent mixture to 3.7. Pyrene (99% purity, Sigma Chemical Co., St. Louis, MO) was dissolved in 100 mL of the acetonitrile/water mixture (90:10) to achieve a pyrene

concentration of 5 mM.

Determination of hydrogen peroxide

The presence of hydrogen peroxide was determined by modifying the N,N-diethyl-p-phenylenediamine (DPD) method (*19*). The ozonated solution (5.4 mL) was mixed with 0.6 mL of sodium phosphate buffer solution to maintain a pH > 6. After 10 sec, the solution was transferred into a UV cell of 1 cm pathlength. The color was measured at 554 nm after 45 sec.

Analysis of ozonation products

Ozonated samples were evaporated under helium and the residue was dried over P_2O_5 in a vacuum desicator for 8 hours. The completely dried sample was derivatized by silylation using bis-trimethylsilyl / trifluoroacetamide (BSTFA) + 1% of trimethylchlorosilane (TMCS) (Regis Technologies Inc., Morton Grove, IL) at 100°C for 20 min to convert all free -OH and -COOH groups into their volatile TMS-ether (-$OSiMe_3$) and TMS-ester (-CO_2SiMe_3) derivatives, respectively.

The ozonation products obtained were identified by GC/MS. GC/MS was performed using a JEOL AX-505H double-focusing mass spectrometer coupled with a Hewlett-Packard 5890J GC (Norwalk, CT). A DB5MS (30 m length × 0.32 mm i.d. × 0.25 μm film thickness) fused silica capillary column (J&W Scientific, Rancho Cordova, CA) was employed for GC separation. A splitless injector was used with a column head

pressure of 10 psi using helium as the carrier gas, producing a flow rate of ca. 1 mL/min. The initial column temperature was held for 2 min at 100°C, ramped at 20°C/min to 220°C, then ramped at 5°C/min to 260°C, and finally ramped at 20°C/min to 300°C. The mass spectrometer was operated in electron impact mode. Mass calibration of the spectrometer was performed using perfluorokerosine. The molecular ions of several derivatized products (those whose molecular ions have a low intensity in the electron impact mode) were confirmed by gas chromatography - chemical ionization (methane) - mass spectrometry (GC-CI(CH_4)-MS).

Quantitation

The relative amounts of pyrene and the ozonation products were assessed using GC/MS by determining the area response of each compound relative to an internal standard. The internal standard, 1,8-naphthalaldehydic acid (97% purity, Aldrich Chemical Co., St. Louis, MO), was dissolved in pure acetonitrile to obtain a stock solution concentration of 5 mM. The sample solutions were prepared by adding 25 μL of the stock solution containing the internal standard into 50 μL of each of the ozonated solutions prior to the drying step. The samples were then derivatized as described above.

The presence of the analytes was detected using GC/MS. A HP 5970 mass spectrometer ChemStation (Norwalk, CT) was used. A SPB-1 (30 m length × 0.25 mm i.d. × 0.25 μm film thickness) fused silica capillary column (Supelco Inc., Supelco Park, Bellefonte, PA) was employed for GC separation. A splitless injector was used with a

column head pressure of 7.5 psi using helium as the carrier gas, producing a gas flow rate of ca. 1 mL/min. The initial column temperature was held for 5 min at 200 °C, ramped at 5 °C/min to 260 °C, then ramped at 20 °C/min to 300 °C. The mass spectrometer was operated in electron impact mode. Mass calibration of the spectrometer was performed using perfluorotributylamine.

3.3 Results

Pyrene was oxidized using ozone at dosages ranging from 0.02 to 2.35 mmoles of ozone. In all experiments, the initial pyrene concentration was 5 mmoles/L. The effect of ozone demand on the residual pyrene concentration, relative to the internal standard, is shown in Figure 3.3. The residual pyrene concentration was determined using the GC area response for pyrene (A_{py}) relative to that for 1,8-naphthalaldehydic acid (25 µL of 5 mM) which was used as an internal standard (AI). The ozone demand was calculated for each specific reaction time:

$$Ozone\,Demand = (C_i - C_e) \times Q \times t$$

where C_i and C_e are the influent and effluent gaseous ozone concentrations, respectively, Q is the gas flow rate and t is the reaction time. Based on the regression line obtained from the plot of the first 6 data points (see Figure 3.3), at an ozone/pyrene ratio of 1.68 ± 0.05 moles ozone/mole pyrene, pyrene had completely reacted. This compares to 1.6 moles ozone/mole pyrene that was determined in an earlier study (*12*).

By varying the extents of reaction, 14 products were identified (see Figure 3.4).

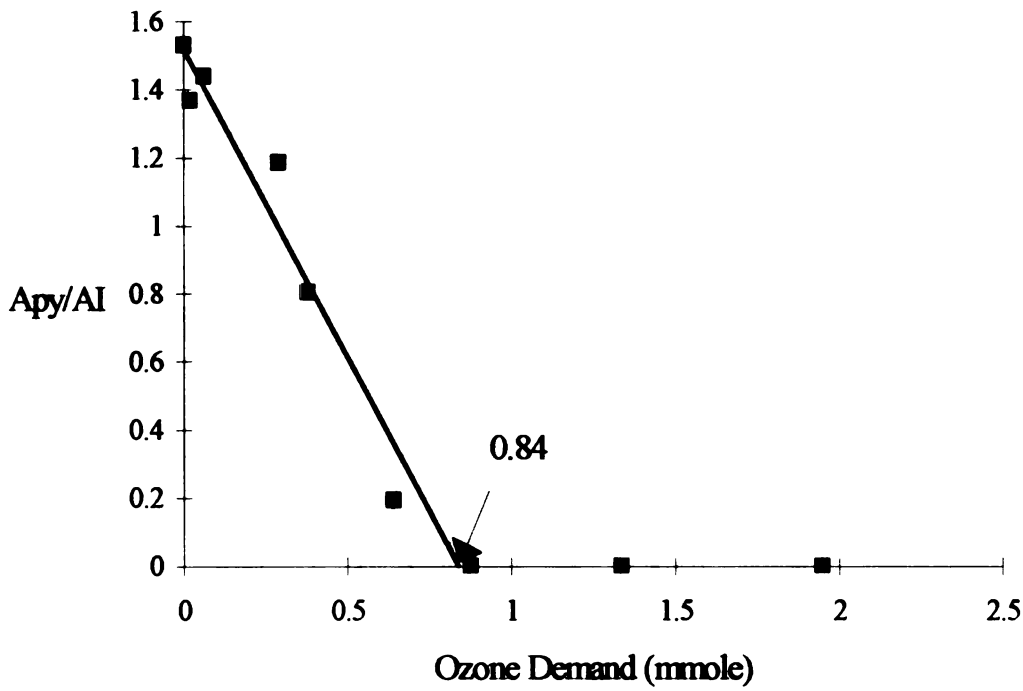


Figure 3.3 The effect of ozone dosage on the residual pyrene concentration. The residual pyrene concentration is determined by the GC area response for pyrene (A_{py}) relative to the response for 1,8-naphthalaldehydic acid (25 μL of 5 mM) used as an internal standard (AI). The solid line represents the linear regression based on the first six data points. The arrow corresponds to the point where 0.5 mmole of pyrene had completely reacted by a total ozone demand of 0.84 mmole, i.e., a ratio of 1.68 moles ozone/mole pyrene.

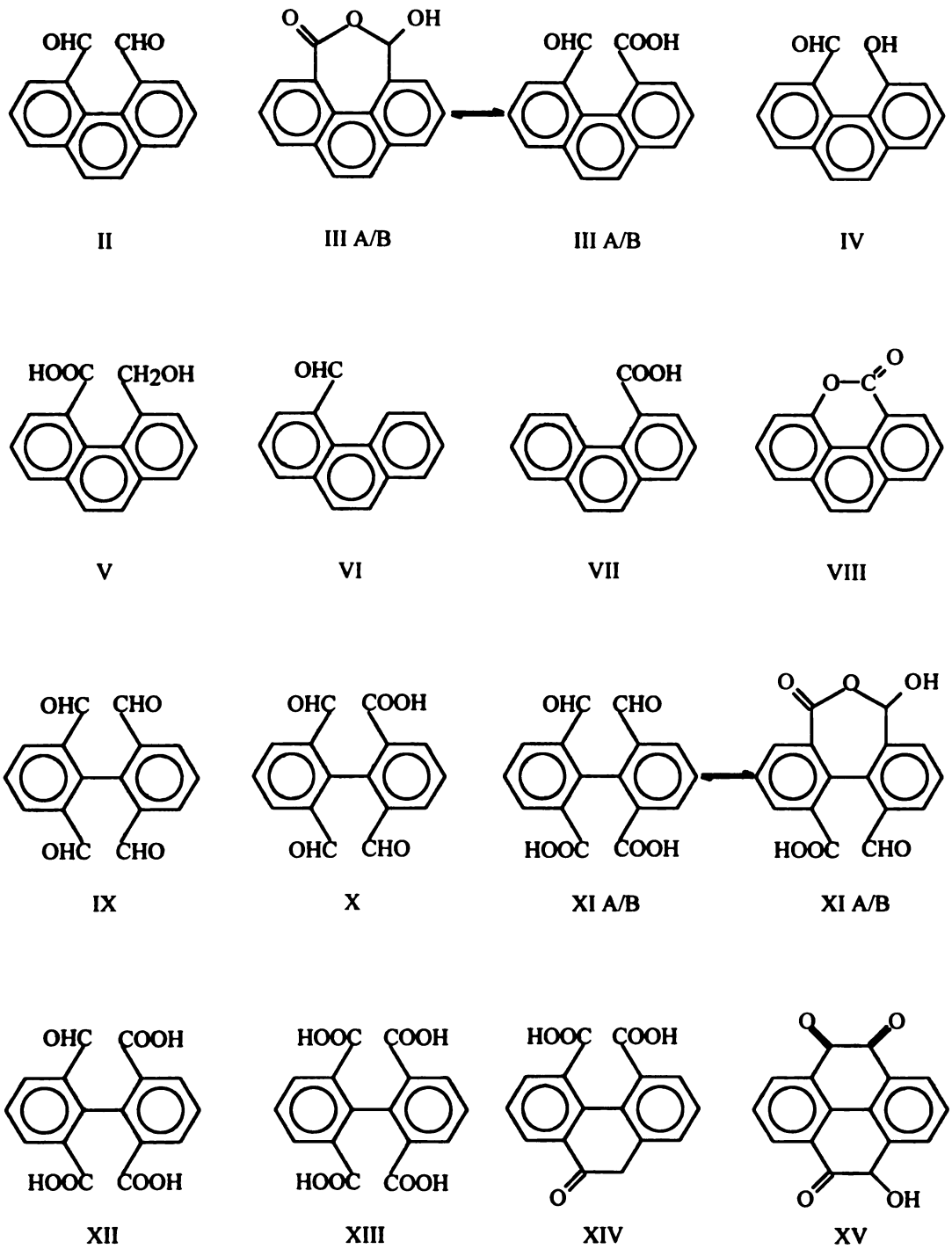


Figure 3.4 Products formed from the ozonolysis of pyrene.

The GC/MS characteristics of each product are given in Table 3.1. Two typical GC chromatograms (for ozone dosages of 0.38 and 1.34 mmoles) are provided as Figure 3.5a and 3.5b, respectively.

Table 3.1 GC/MS characteristics of pyrene ozonation products

product ID	GC retention time	molecular weight of parent compd.	molecular weight of TMS deriv.[a]	Important Ion peaks, m/z				
I	8'25"	202	202	202 (M+.)	101			
II	9'38"	234	234	234 (M+.)	205	176	151	102
III A/B	10'26"	250	322	322 (M+.)	293	205	189	176
III A/B	11'05"	250	322	322 (M+.)	307	294	205	189
IV	8'35"	222	294	294 (M+.)	293	205	176	151
V	9'04"	252	396	396 (M+.)	307	293	279	189
VI	8'05"	206	206	206 (M+.)	205	176	151	103
VII	8'50"	222	294	294 (M+.)	279	205	177	151
VIII	9'10"	220	220	220 (M+.)	193	163	110	
IX	9'00"	266	266	266 (M+.)	237	209	181	152
X	9'23"	282	354	354 (M+.)[b]	325	295	237	221
XI A/B	9'45"	298	442	442 (M+.)[b]	427	325	309	269
XI A/B	9'53"	298	442	442 (M+.)[b]	427	398	325	235
XII	9'58"	314	530	530 (M+.)[b]	515	471	385	323
XIII	10'19"	330	618	618 (M+.)[b]	603	558	323	221
XIV	7'50"	282	426	426 (M+.)	411	337	249	175
XV	10'13"	264	336	336 (M+.)[b]	295	246	237	221

[a] theoretical value based on molecular weight of proposed TMS derivative

[b] derivatived molecular ion confirmed using CI

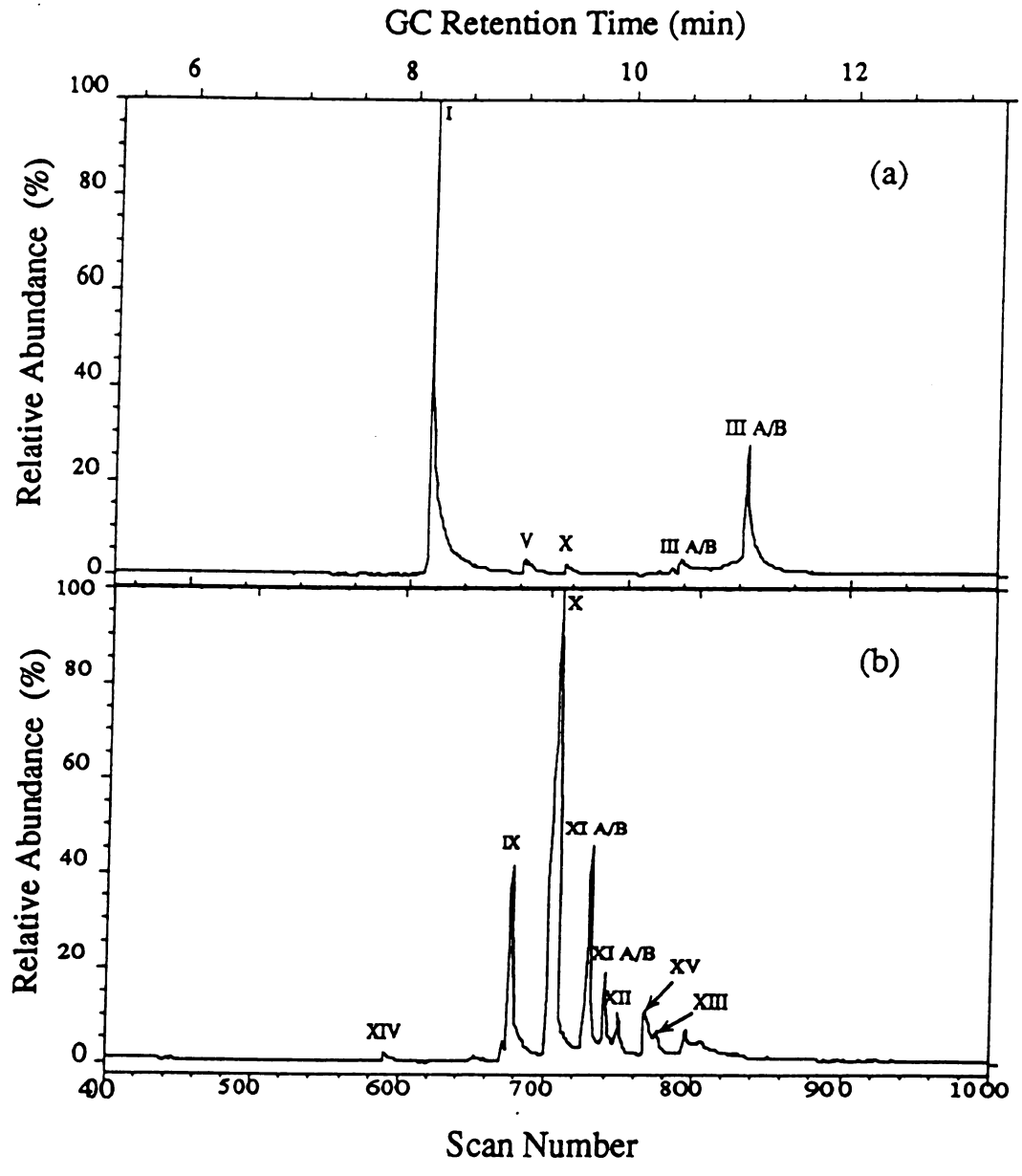
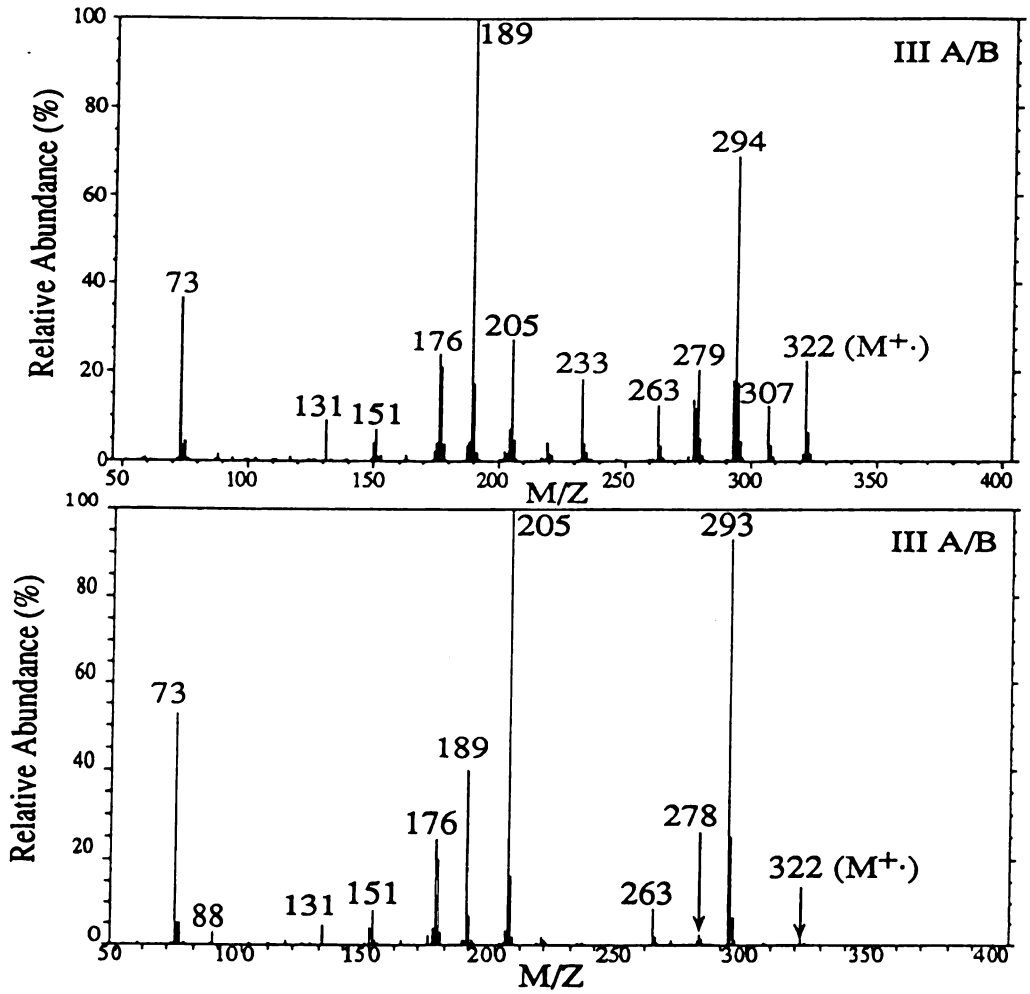


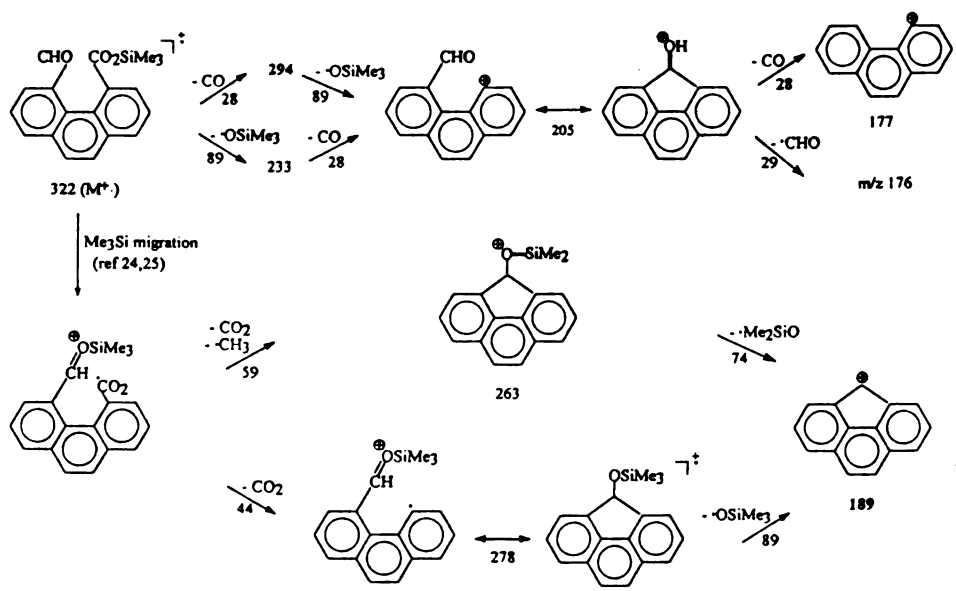
Figure 3.5 (a)The reaction of pyrene (0.5 mmole) with 0.38 mmole of ozone.
(b) The reaction of pyrene (0.5 mmole) with 1.34 mmoles of ozone.
The Roman numerals corresponds to the products listed in Figure 3.4.

Although library searches of standard spectra in NIST MS Search (ver 1.5, 1996) and BenchTop/BPM Search (ver 3.10d, Wiley & Son, 6th ed., 1994) were conducted, due to the paucity of information in these data base, no matches were found. The tentatively assigned identifications of the ozonation products were based on interpretations of the mass spectra (see Appendix B). Prior to the disappearance of pyrene, one ring cleavage phenanthrene-type products predominated. The major product of this type was 4-carboxy-5-phenanthrenecarboxyaldehyde (4-CPA, IIIA/B); its mass spectra and the interpretation of the mass spectra are shown in Figure 3.6. Once pyrene had completely reacted, secondary ring cleavage occurred and biphenyl-type products predominated. The major product of this type was 2',6,6'-biphenyltrialdehyde-2-carboxylic acid (X); its mass spectrum and the interpretation of the mass spectrum are provided in Figure 3.7.

Two pairs of structural isomers have been tentatively identified. The first pair, III A/B (mass spectra of TMS derivatives are shown in Figure 3.6), contains aldehyde and acid functional groups. The second pair, XI A/B (mass spectra of TMS derivatives are shown in Figure 3.8), contains dialdehyde and diacid functional groups. When an aldehyde and a carboxylic acid functional group are adjacent to one another, a cyclization may occur. For example, an equilibrium between the original and the cyclized structure of 2-carboxybenzaldehyde  exists. After derivatization of this compound with TMS, two peaks were observed using GC/MS. Only one commercial compound, 4-CPA (Sigma-Aldrich Library of Rare Chemicals, Milwaukee, WI), could be

Figure 3.6 Mass spectra and structure interpretation of fragmentation patterns for 4-carboxy-5-phenanthrenecarboxyaldehyde (III A/B) derivatized by BSTFA + TMCS. The migration of Me_3Si within a molecule is described in References 24,25. The bold numbers correspond to the important peaks on the mass spectra.


III A/B
Relative Abundance (%)
M/Z
189
294
73
205
176
233
279
322 (M+·)
263
307
131
151
III A/B
Relative Abundance (%)
M/Z
205
293
73
189
278
176
322 (M+·)
263
88
131
151


CHO
CO2SiMe3
322 (M+·)
- CO
28
294
- ·OSiMe3
89
- ·OSiMe3
89
233
- CO
28
CHO
205
OH
- CO
28
177
- ·CHO
29
m/z 176
Me3Si migration
(ref 24,25)
OSiMe3
CH
CO2
- CO2
- ·CH3
59
SiMe2
263
- ·Me2SiO
74
189
- CO2
44
OSiMe3
CH
278
OSiMe3
- ·OSiMe3
89

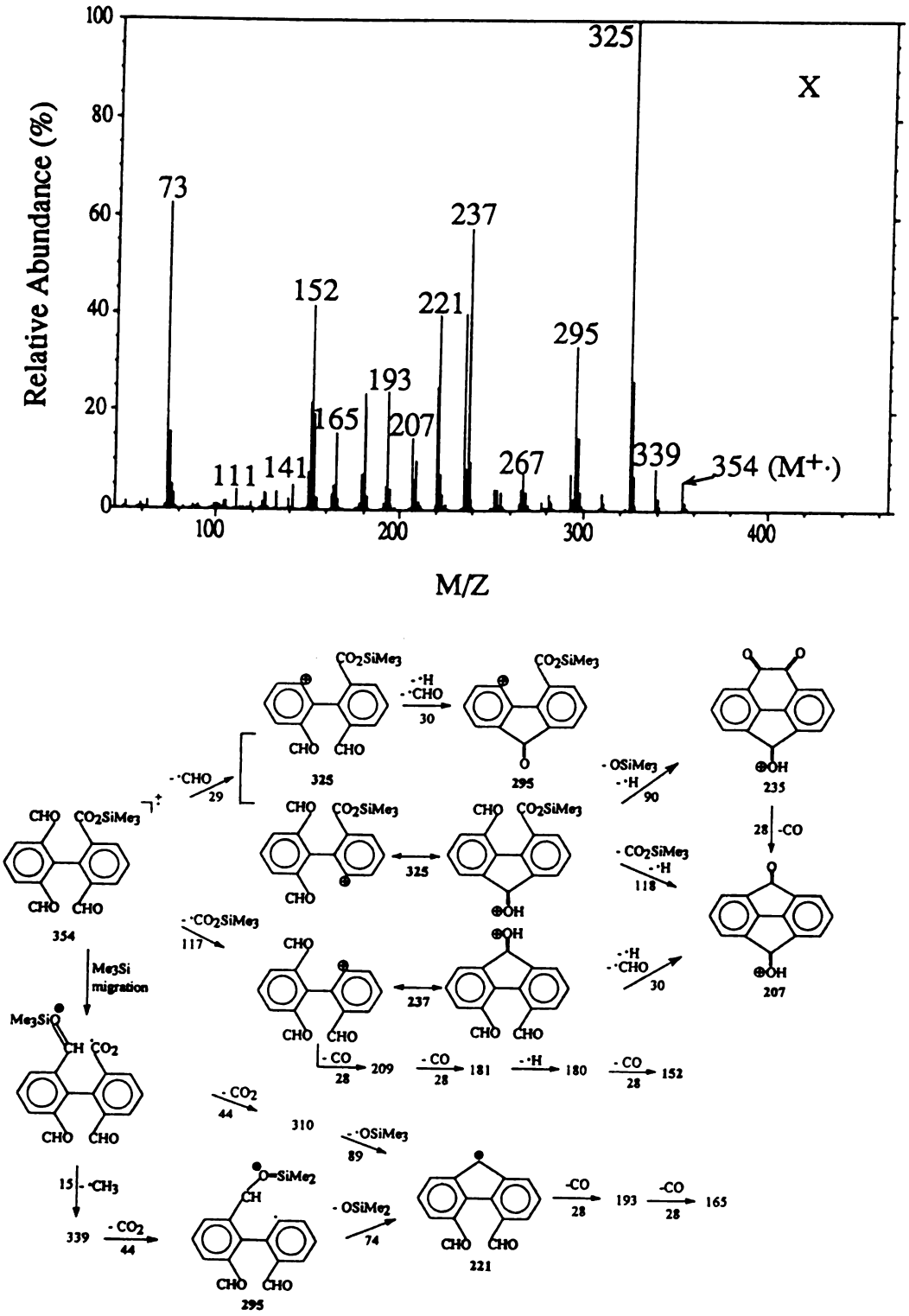


Figure 3.7 Mass spectrum and structural interpretation of fragmentation patterns for 2',6,6'-biphenyltrialdehydo-2-carboxylic acid (X) derivatized by BSTFA + TMCS

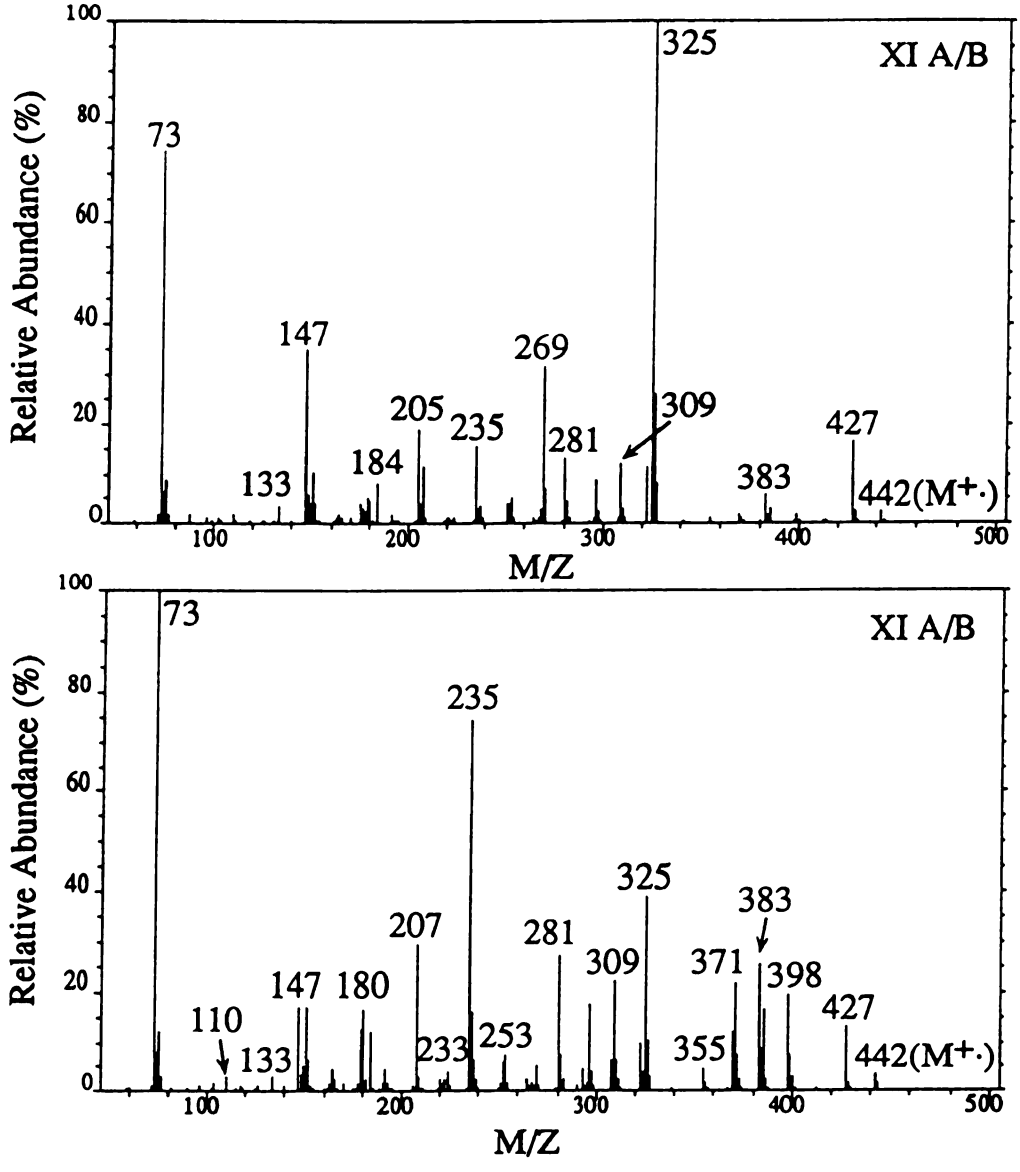


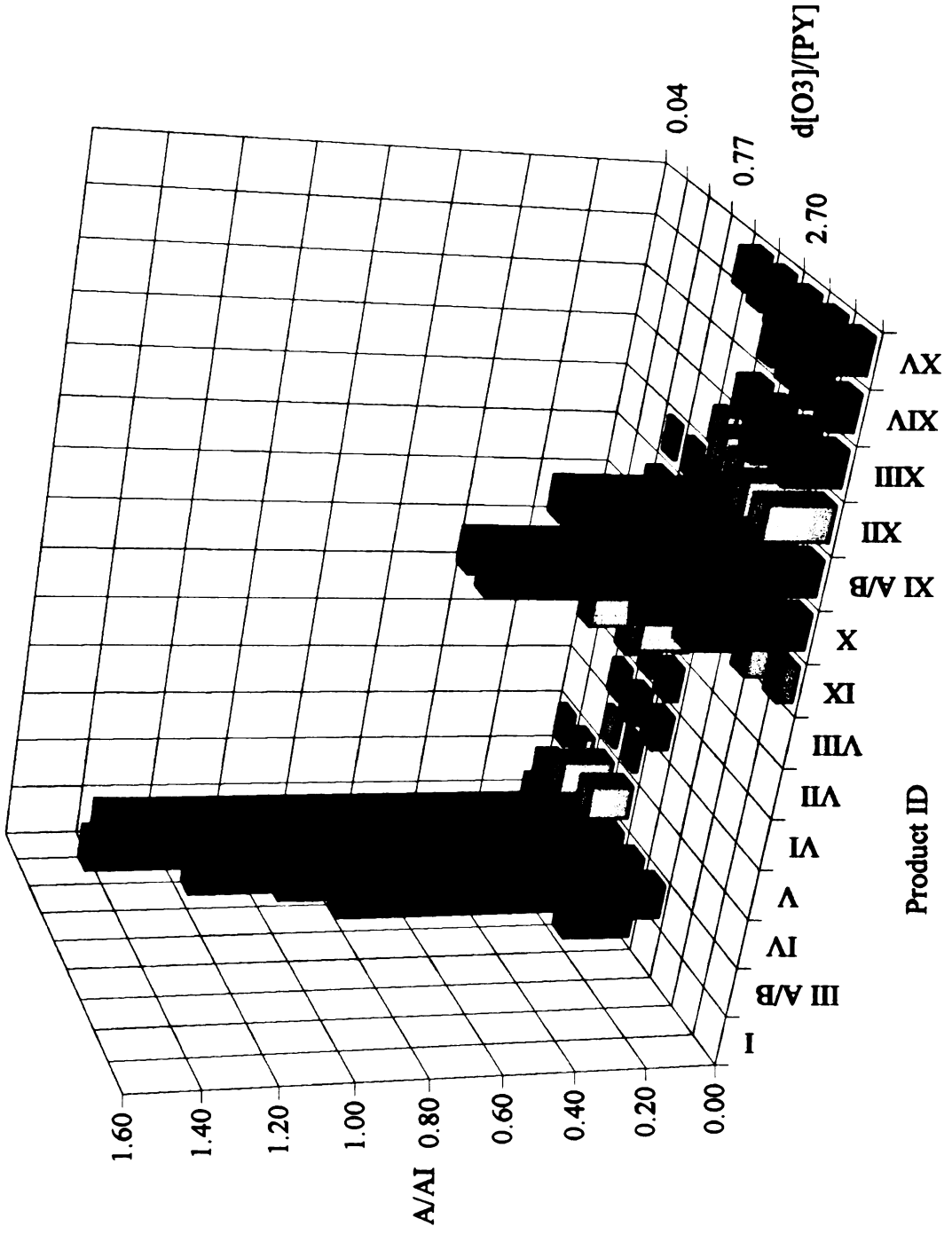
Figure 3.8 Mass spectra of the isomeric forms of XI A/B.

obtained but it contained impurities, including compounds VI, X, and XI A/B. This formulation was prepared so as to compare its GC/MS spectra with that of compound III A/B. Using GC/MS, the TMS derivative of 4-CPA was found to exist in two isomeric forms which were identical to those shown in Figure 3.6.

Several of the products identified appeared to have resulted from the oxidation of pyrene by free radicals, most likely by ·OH. These include one major product, IV, and minor products, II, V, IX, XIV and XV. To test this hypothesis, two experiments were performed either in pure acetonitrile or in an acetonitrile/water solvent mixture that contained t-butanol (1 M) as an ·OH radical scavenger. In solutions containing t-butanol, the sample solutions contained qualitatively higher concentrations of hydrogen peroxide than in the solvent mixture without t-butanol. Products V, XIV and XV were not produced in either pure acetonitrile or in the presence of t-butanol; however, the relative concentration of product IV increased. Products II and IX were formed in the acetonitrile /water solvent mixture both in the presence and absence of t-butanol, but were not found in pure acetonitrile.

The effect of the ozone : pyrene ratio (reported as moles ozone/mole pyrene) on the distribution of the products and their relative concentrations is shown in Figure 3.9. The product concentrations are given as the ratio of the GC area response of the analyte relative to that of the internal standard (A/AI). Product II and phenanthrene-4,5-dicarboxylic acid were found only during preliminary experiments in which the ozonated solutions were concentrated by freeze-drying and the concentrate was reconstitutied in

Figure 3.9 The relative concentrations (A/AI) of pyrene and its ozonation products obtained under different ozone : pyrene ratios. The product concentrations were determined relative to 1,8-naphthalaldehydic acid (25 μL of 5 mM) used as an internal standard.


A/AI
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Product ID
I
III A/B
IV
V
VI
VII
VIII
IX
X
XI A/B
XII
XIII
XIV
XV
d[O3]/[PY]
0.04
0.77
2.70

acetonitrile. As such, no quantitative data were obtained. At an ozone : pyrene ratio of 0.04 mole ozone/mole pyrene, only products III and IV were observed. Initially, concentrations of these products increased as the ozone : pyrene ratio increased. When the ozone : pyrene ratio was increased to 0.58 mole ozone/mole pyrene, several intermediates, including compounds V, VI, VII and VIII, could be detected. Additionally, the secondary-ring-cleavage products X and XI were detected. At an ozone : pyrene ratio $\leq$ 1.30 moles ozone/mole pyrene, reaction product III predominated; several of the secondary-ring-cleavage products (e.g., IX~XII and XV) appeared. When the ozone : pyrene ratio was increased to the point where pyrene had completely reacted, the concentration of product III decreased dramatically. The concentrations of the other phenanthrene-type products (e.g., IV, V, VI, VII and VIII) also decreased to below detection limits. At this time, compound X became the predominant product. Upon further increases in the ozone : pyrene ratio (to > 1.77 moles ozone/mole pyrene), the concentration of product III decreased to below its detection limit and the concentrations of the biphenyl-type products gradually increased. When the ozone : pyrene ratio reached 2.70 moles ozone/mole pyrene, the concentration of compound X reached a maximum value. Upon further increases in the ozone : pyrene ratio, the concentration of compound X decreased and the concentration of other biphenyl-type products increased. At ozone : pyrene ratios between 2.70 and 5.00 moles ozone/mole pyrene, compound XI A/B became the predominant product .

To confirm the proposed ozonation pathway, the ozonation of commercial 4-CPA was investigated. This mixture was prepared and ozonated in the same manner as that used with pyrene. The effect of the ozone : 4-CPA ratio (reported as moles ozone/mole 4-CPA) on the distribution of the products and their relative concentrations is shown in Figure 3.10.

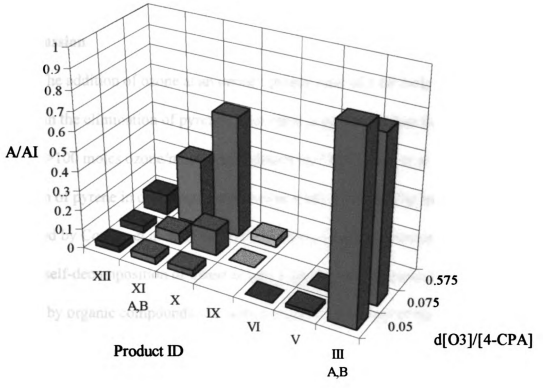


Figure 3.10 The relative concentration (A/AI) of 4-CPA and its ozonation products obtained under different ozone : 4-CPA ratio. The product concentrations were determined relative to 1,8-naphthalaldehydic acid (25 μL of 5 mM) which was used as an internal standard (AI).

Secondary ring cleavage products, such as products X ~ XII, appeared when the ozone dosage was only 0.05 mole ozone/mole 4-CPA. Products IV, VII and VIII were not found. Product V and VI were present, but below detection limits. Upon increases in the ozone dosage, the concentration of products X ~ XII increased, but products V and VI disappeared. Also, product IX was observed upon increasing the ozone dosage. At an ozone dosage of 0.56 mole ozone/mole 4-CPA, 4-CPA had completely reacted, and only products IX ~ XII were detected. Product X predominated.

3.4 Discussion

The addition of ozone at an ozone : pyrene ratio of 1.68 moles ozone/mole pyrene resulted in the elimination of pyrene*. This value is much less than the stoichiometric ratios of >100 moles ozone/mole pyrene determined by Corless et al. (*11*) for the ozonation of pyrene in dilute aqueous solution at pH 4.5 - 6.0. The large stoichiometric ratios used by Corless et al. may be necessary since in dilute aqueous solutions, the aqueous self-decomposition of ozone is very important as compared to the consumption of ozone by organic compounds. As such, a smaller percentage of the applied ozone would go to the oxidation of the target chemicals than we would observe in our more concentrated solutions. Based upon this study, at stoichiometric ratios < 5 moles ozone/mole pyrene, rapid destruction of the ozonation intermediate products also occurred. Based upon results from a previous study (*12*), at these ozone dosages, all the

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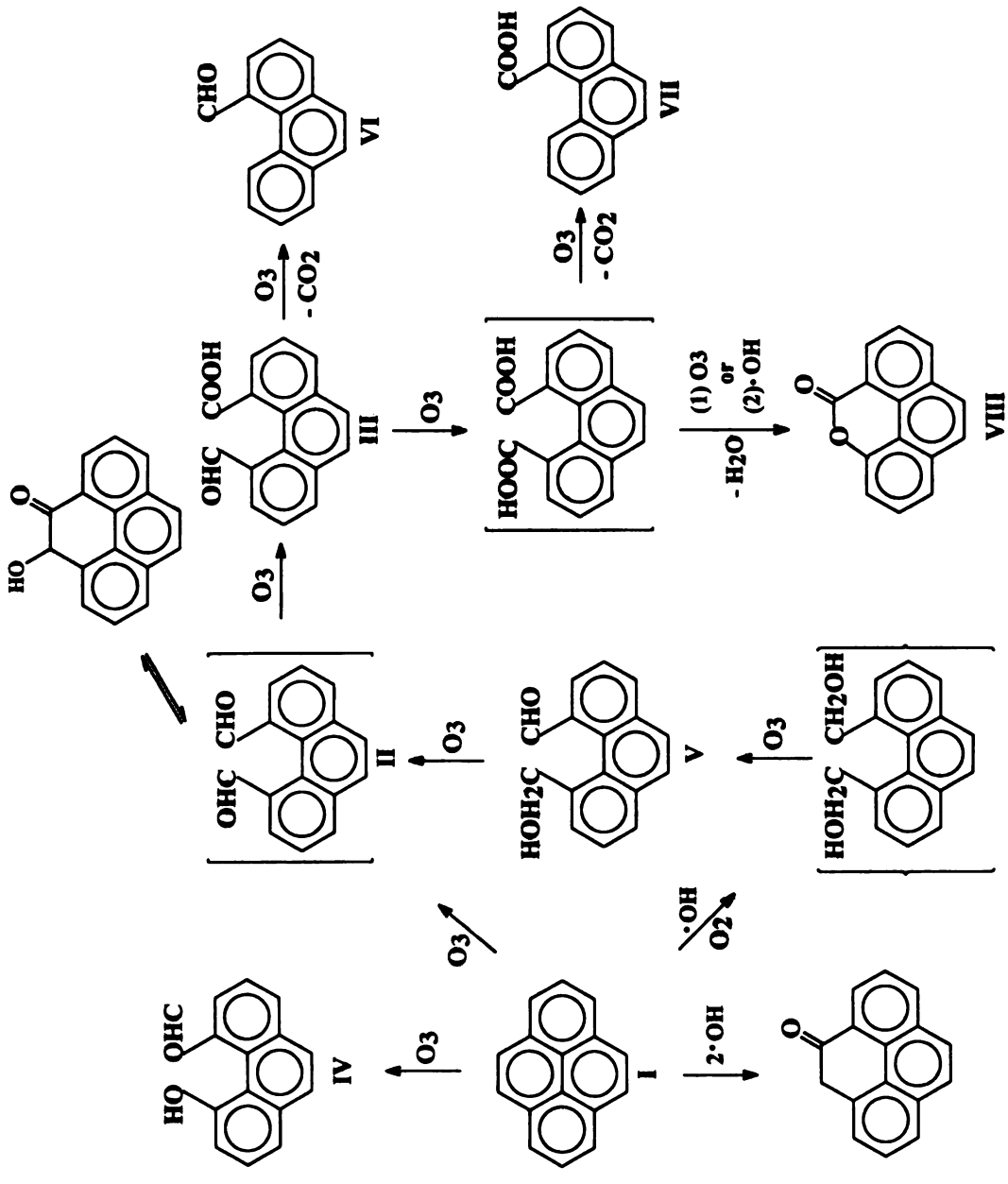
* Note: The reaction of ozone with ozonation products occurs prior to the elimination of pyrene; therefore, ozone would be consumed simultaneously by pyrene and the initial by-products. This would be expected to result in a stoichiometric ratios greater than one which is the value that would be expected if ozone reacted only with pyrene.

intermediate products which were found to have the potential to promote tumor growth are removed. Thus, it is likely that at the ozone dosages used in water and wastewater treatment for the oxidation of pyrene, ozonation would result in a reduction in epigenetic toxicity.

Based upon the products identified along with the sequence of their appearance, a pathway for the ozonolysis of pyrene is proposed (see Figure 3.11). Only the pathway for the formation of one ring cleavage products is shown in the figure. The pathway by which second ring cleavage occurs is similar. Ozone directly attacked the double bond causing ring cleavage of pyrene at the 4, 5 (or 9, 10) position. At the early stages of the reaction, ozone reacted with pyrene to form six one-ring cleavage phenanthrene-type products. Pyrene reacted to form a dialdehyde , e.g., product II, at a concentration below its detection limit. An internal rearrangement of II involving the dialdehyde groups could result in ring closure with a ketone and a hydroxyl functional groups substituted on the cyclic ring. The major ozonolysis product from pyrene was a phenanthrene substituted with an aldehyde and carboxylic acid (III). This is consistent with the pathway proposed by Bailey (*16*). Upon further ozonation, either the aldehydic functional group was oxidized to form a carboxylic acid or the hydrogen on the carboxylic acid was released and decarboxylation resulted in the formation of minor products VI, VII and VIII. The decarboxylation product VIII is consistent with that shown by Davies and Waring (as cited in Sheldon and Kochi (*20*)). When 4-CPA, the commercial compound of product III, was ozonated, products VII and VIII were absent from the oxidized mixture. This suggests that ring cleavage occurs preferentially over decarboxylation.

Figure 3.11 Proposed pathway for the first ring cleavage of pyrene during ozonolysis. Compounds shown in brackets [ ] were detected only in ozonated solutions which were concentrated by freeze-drying and reconstituted in acetonitrile. The compound shown in { } braces is hypothesized, and would be formed by a sequence of reactions involving ·OH in the presence of O_2.


HO
O
OHC
HO
IV
O3
I
2·OH
O
O3
·OH
O2
OHC
CHO
II
O3
HOH2C
CHO
V
O3
HOH2C
CH2OH
O3
OHC
COOH
III
O3
CHO
VI
O3
-CO2
HOOC
COOH
(1) O3
or
(2)·OH
-H2O
O
O
VIII
COOH
VII
O3
-CO2

The direct reaction of ozone with pyrene could also result in the formation of product IV. The ozonolysis of pyrene in solutions containing t-butanol as an ·OH radical scavenger or in pure acetonitrile, resulted in an increase in the concentration of product IV (as compared to ozonolysis in the solvent mixture without radical scavenger). This result is surprising since product IV would not be formed by the classical Criegee pathway. While it is unclear exactly how product IV is formed, one possibility is that a trioxalane forms, followed by a rearrangement to form an aldehyde group and a hydroxyhydroperoxide which undergoes decarboxylation to form an alcohol. This hypothesis was substantiated by the results of an ozonation experiment in which 4-CPA (III) was used as the parent compound. Product IV was absent in the oxidized mixture.

In aqueous solutions (pH > 4), ozone autocatalytically decomposes to form hydroxyl radicals. The ·OH radicals, which enter into a cyclic reaction, enhance the rate of ozone self-decomposition (*21*). They may also react with PAHs to form phenyl-type compounds that can be further oxidized to form ketones. Although at pH 3.7 the decomposition of ozone to form ·OH radicals would not be expected to be significant, work by Masten et al. (*22*) has shown that, even at low pH, ·OH radicals can still responsible for degradation of TCB. Work by Xiong et al. (*23*) has shown that the formation of ozonation products such as glyoxalic acid or other ketoacids can participate in the initiation step for the decomposition of ozone, resulting in the formation of ·OH radicals. Based upon our results, it appears that even at pH 3.7, ·OH radicals were involved in the reaction of pyrene and the production of product V and the ketone type products XIV and XV. When using t-butanol (1M) as an ·OH radical scavenger or when

conducting the ozonolysis reactions in pure acetonitrile, the products V, XIV and XV were not observed. Hydroxyl radical attack, with the addition of oxygen on pyrene, would result in the formation of the diol intermediate. The reaction of this diol intermediate with ozone and the subsequent dehydrogenation would form product V. Upon further ozonation and dehydrogenation of product V, a dialdehyde would form, e.g., compound II. This could explain why products II were found during the ozonolysis of pyrene in the solvent mixture but not found in the pure acetonitrile since the diol would not be formed in a acetonitrile. The ketone type products, XIV and XV, likely resulted from the direct attack of ·OH radicals on pyrene followed by dehydration. Ozonation products will also react with ·OH radicals. For example, product III reacts with ozone to form a dicarboxylic acid intermediate. This intermediate can further react with ozone, followed by the dehydrogenation of the carboxylic acid functional group. Alternatively, it can react with ·OH. Again dehydration of the carboxylic acid functional group would follow. In either case, the formation of a phenantherene with one carboxylic acid group and a $\cdot CO_2$ radical would result. This species can undergo electron transfer/rearrangement of $\cdot CO_2$ and subsequent the decarboxylation of one of the carboxylic acids, resulting in the formation of product VIII.

Upon further ozonation, ozone reacted with the phenanthrene-type compounds at the 9,10 position resulting secondary ring cleavage and the formation of biphenyl-type products (IX ~ XV). This pathway is not shown in Figure 3.11, however the pathway for the second ring cleavage is expected to be similar to that observed for cleavage of the first ring. As the concentrations of the biphenyl-type products increased gradually, the

concentrations of the phenanthrene-type products decreased dramatically. After the major phenanthrene-type product (III) had disappeared, the ring cleavage biphenyl-type products X and XI predominated.

3.5 Conclusions

A pathway for the ozonation of pyrene in acetonitrile/water mixtures has been described. As sufficient water was present for it to participate in the ozonation reaction involving pyrene, this work is relevant to aqueous systems. Ozonation resulted in stepwise ring cleavage and in the oxidation of aldehydic functional groups. Even at pH 3.7, the ·OH radicals appeared to be involved in the reaction of the primary ozonation products.

The identification of the reaction products was accomplished by GC/MS. Future work will include preparatory TLC and NMR to confirm the product identification. Once pure fractions of the products are obtained, these individual products will be ozonated to further investigate the mechanistic pathway.

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CHAPTER 4

THE OZONATION OF BENZ[a]ANTHRACENE IN ACETONITRILE/WATER MIXTURE: PATHWAY AND PRODUCT IDENTIFICATION

ABSTRACT

The pathway for the ozonolysis of benz[a]anthracene (BaA), a polycyclic aromatic hydrocarbon (PAH), has been described in this paper. Benz[a]anthracene was dissolved in a 90 % acetonitrile : water (v/v) mixture to achieve an initial concentration of 1 mM. The ozonolysis of benz[a]anthracene was evaluated using different ozone dosages. Gas chromatography followed by mass spectrometry (GC/MS) was used to separate and identify ozonation products. Fifteen products including seven pairs of isomers were identified. Ozone reacted simultaneously by bond and atom attack with benz[a]anthracene. Ozone attack on the carbon at the 7 and/or 12 position produced the quinone or hydroxyl functional groups. The bond attack type of reaction occurred at the 5,6 position and caused ring cleavage resulting in phenyl-naphthyl type products. Free radicals appeared be involved in the bond attack type of reaction of benz[a]anthracene. Ozonolysis of benz[a]anthracene occurred preferentially by bond attack rather than by the atom attack type of reaction.

4.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are found in many petroleum products including crude oils, motor oils, gasoline and heating fuels. The predominant routes by which PAHs enter the environment are petroleum spillage and the release of PAHs during the combustion of petroleum products with subsequent wet or dry deposition during rainfall or fallout (*1*). As a result of the release of PAHs to the environment, these compounds find their way into drinking waters, soils, plants and wastewater. Preliminary work on the use of gaseous ozone for the decomposition of PAHs in soils have revealed very promising results (*2*). It has been shown that ozone is capable of degrading several PAHs (including pyrene, chrysene and phenanthrene) in soils. Numerous applications exist for ozonation in the area of wastewater treatment, including the processing of shale oil (*3*). However, it is still unclear what products are produced from the ozonation of PAHs either in soils or water.

Ozone is highly reactive with the PAHs. The reaction rate constants of ozone with PAHs range from 5×10^2 to 1×10^5 $M^{-1}s^{-1}$. The products formed from the reaction of ozone with many PAHs have been identified prior to when gas chromatography/mass spectrometry (GC/MS) was extensively used (*4-9*). Products were identified by mixture melting points or by comparison with published infrared spectra. In most of the published literature, the ozonation products were generated either in organic solvents, such as methylene chloride, methanol, and chloroform, or in acetic acid. Nevertheless, only a limited number of products have been identified using these systems. While several mechanisms for the reaction of ozone with PAHs have been proposed, these mechanisms

are questionable since they are based upon the inconclusive identification of products and results obtained in organic solvents.

In this study, benz[a]anthracene was chosen as the model PAH compound. Benz[a]anthracene, a known carcinogen, is found in petroleum in concentrations of 1.2 to 90 ppm. The objective of this study is to identify the ozonation products of benz[a]anthracene by GC/MS and to propose a pathway for the reaction of benz[a]anthracene with ozone in aqueous solutions, and to verified results with the previously published results identified by melting points or infrared spectroscopy (*7-13*). The ozonation products obtained after benz[a]anthracene had reacted to varying extents were identified. Also, the relative concentrations of benz[a]anthracene and its ozonation products were quantified.

4.2 Methods

Ozonation experiments

Ozone was generated in dried oxygen by electric discharge using a Polymetrics Model T-408 ozone generator (San Jose, CA). Gaseous ozone was continually bubbled into a 125 mL gas washing bottle containing 100 mL of 1 mM benz[a]anthracene (99% purity, Sigma Chemical Co., St. Louis, MO) in a acetonitrile/water mixture (90/10 volume ratio). As evidenced in a previous study (*14*), 10% water (volume ratio) was sufficient to act as a participating solvent. Therefore, a 90% acetonitrile/water (v/v) mixture was chosen for all the experiments. Acetonitrile was chosen as the organic

solvent because it is miscible with water and it has a low reactivity with ozone ($t_{1/2} \geq 18$ years at pH 7 and $[\overline{O_3}] = 20.8$ mM) (*15*). Phosphoric acid used to lower the pH. The pH of the reactor solution was approximately 3.9.

Influent gaseous ozone was continually bubbled into the gas washing bottle containing the benz[a]anthracene solution. The solutions were mixed with a magnetic stirrer and stir bar. Effluent gaseous ozone was trapped in an aqueous 2% (w/v) KI solution. The flow of ozone was regulated at 120 mL/min with a Sidetrack flow controller (Sierra Instruments Inc, Monteray, CA). All the tubing (i.d. 1/8"), connectors and valves were constructed of Teflon®. The concentration of ozone in the influent and effluent gas stream was measured spectrophotometrically at 258 nm using a UV-vis spectrophotometer (Model 1201, Shimadzu Scientific Instruments, Japan). The molar absorptivity coefficient for ozone is 3000 M^{-1} cm^{-1} (*16*). The flow cells were quartz cuvettes with a path length of 0.2 cm. An influent gaseous ozone concentration of 0.5 mM was used in all experiments. Reactions were terminated by flushing the reactor with helium; 2 min was required to purge ozone to non-detectable levels. After ozonation, 2 g of Na_2SO_3 was added into the solution to remove the residual organic radicals.

Identification

Separation and purification of the ozonation products were attempted using reverse phase medium pressure column followed by thin layer chromatography (TLC). However, this procedure failed because the silica on the TLC surface catalyzed the further

oxidation of PAHs by any free radicals present (see Appendix C). Ozonated samples were then prepared for direct separation and identification using GC/MS. Ozonated samples were dried under helium. The residue was further dried over P_2O_5 in a vacuum desicator. The completely dried sample was derivatized by silylation using bis-trimethylsilyl / trifluoroacetamide (BSTFA) + 1% of trimethylchlorosilane (TMCS) (Regis Technologies Inc., Morton Grove, IL) at 100 °C for one hour to convert all free -OH and -COOH groups into their volatile TMS-ether ($-OSiMe_3$) and TMS-ester ($-CO_2SiMe_3$) derivatives, respectively.

GC/MS was performed using a JEOL AX-505H double-focusing mass spectrometer coupled with a Hewlett-Packard 5890J GC (Norwalk, CT). A DB5MS (30 m length × 0.32 mm i.d. × 0.25 μm film thickness) fused silica capillary column (J&W Scientific, Rancho Cordova, CA) was employed for GC separation. A splitless injector was used with a column head pressure of 10 psi using helium as the carrier gas, producing a flow rate of ca. 1 mL/min. The initial column temperature was held for 2 min at 100 °C, ramped at 20 °C/min to 220 °C, then ramped at 5 °C/min to 280 °C, and finally ramped at 20 °C/min to 300 °C. The mass spectrometer was operated in electron impact mode. Mass calibration of the spectrometer was performed using perfluorokerosine.

Quantitation

The relative concentrations of benz[a]anthracene and the ozonation products were quantified by determining the area response of each compound relative to an internal

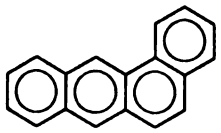
standard. The same GC/MS as previously described was used. The internal standard, 1,8-naphthalaldehydic acid (97% purity, Aldrich Chemical Co., St. Louis, MO), was dissolved in pure acetonitrile to obtain a stock solution having a concentration of 1 mM. The sample solutions were prepared by adding 50 μL of the stock solution containing the internal standard into 100 μL of each of the ozonated solutions prior to the drying step. The samples were, then, derivatized as described above.

4.3 Results

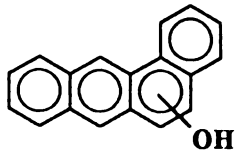
The ozonation products that have been identified are illustrated in Figure 4.1. Fifteen products including 8 pairs of structural isomers were identified. Typical GC chromatograms for ozone dosages of 0.05, 0.11, 0.20 and 0.42 mmole are provided (Figure 4.2a, 4.2b, 4.2c and 4.2d, respectively). The GC/MS characteristics of each product are given in Table 4.1. Library searches of standard spectra in NIST MS Search (ver 1.5, 1996) and BenchTop/BPM Search (ver 3.10d, Wiley & Son, 6th ed., 1994) were conducted. Matches of TMS-derivatized mass spectra of compounds V, X, XIII, XV, and XVI were obtained. The CAS registered numbers for these compounds are listed in Table 4.1.

The spectra of compounds XIV and XVI were compared to that of the commercially available preparations of 2-carboxybenzaldehyde and 2-phthalic acid (97% and 99.5%, respectively. Aldrich, Milwaukee, WI). The tentatively assigned identifications of the other ozonation products were based on interpretations of the mass spectra and comparison with other known spectra from library searches.

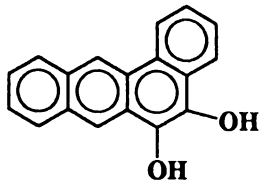
Figure 4.1 Products formed from the ozonolysis of benz[a]anthracene



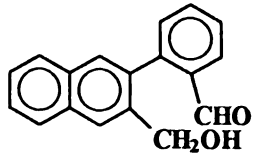
I



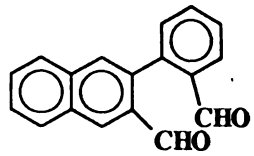
II A



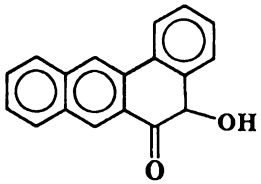
III A



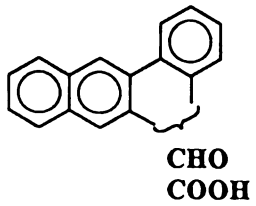
IV



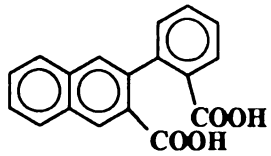
V



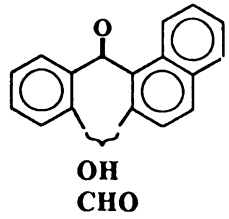
VI



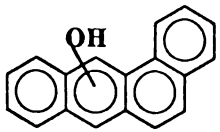
VII A,B



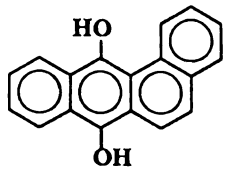
VIII



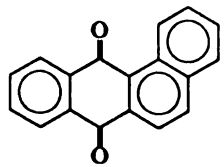
IX A,B



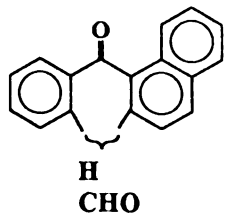
II B



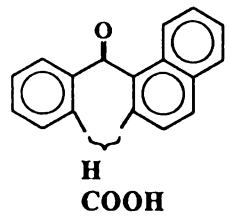
III B



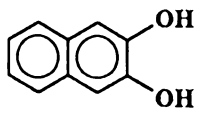
X



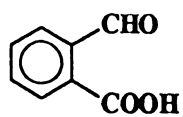
XI A,B



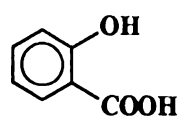
XII A,B



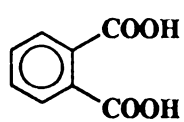
XIII



XIV A,B



XV



XVI

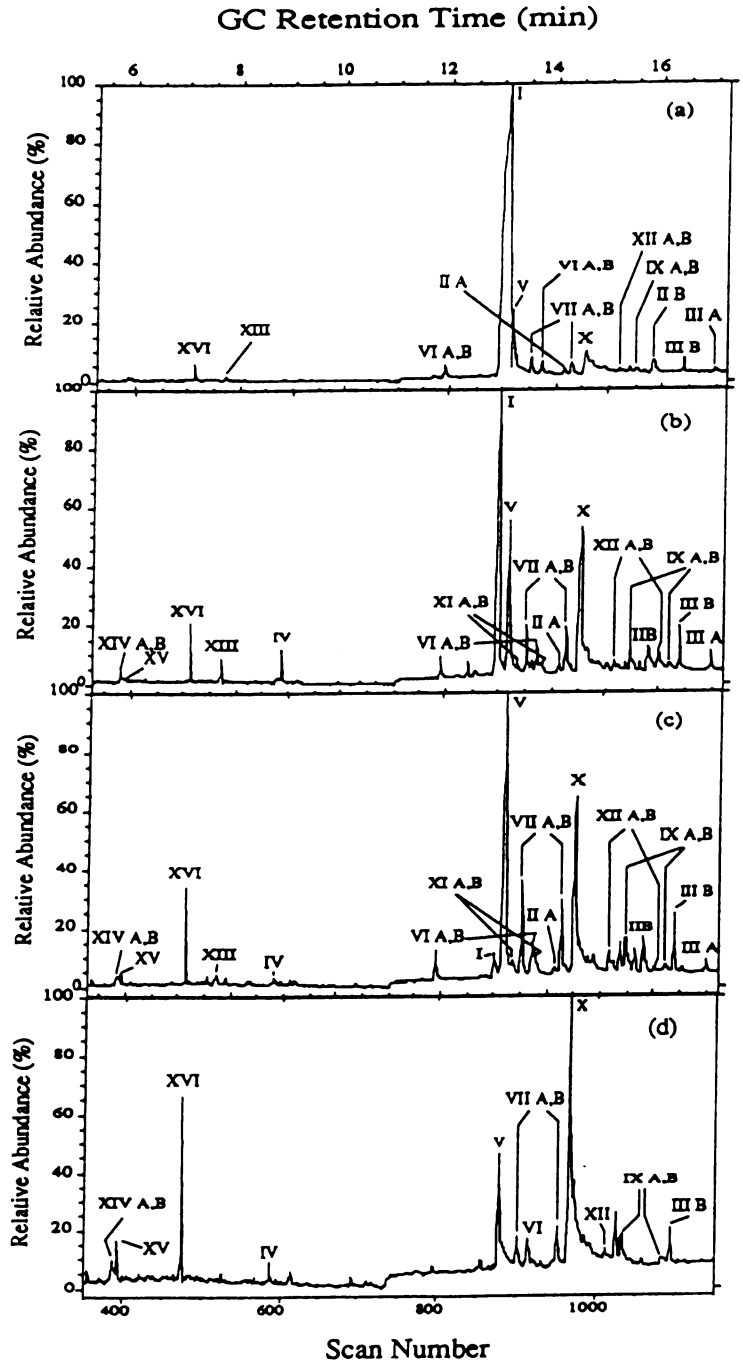


Figure 4.2 Typical GC chromatogram. Figures (a), (b), (c), (d) correspond to the reaction of benz[a]anthracene (0.1 mmole) with 0.05, 0.11, 0.20 and 0.42 mmole of ozone, respectively. The Roman numerals corresponds to the products listed in Figure 4.1.

Table 4.1 GC/MS characteristics of benz[a]anthracene ozonation products

product ID	GC retention time	molecular weight of parent compound	molecular weight of TMS derivative						PBM Search CAS Reg. #
				Important Ion peaks, m/z					
XIV A,B	5'48"	150	222	222 (M+.)	207	179	133	105	
XV	5'54"	138	282	282 (M+.)	267	209	193	135	3789-85-3
XIV A,B	6'01"	150	222	222 (M+.)	207	177	163	133	
XVI	7'07"	166	310	310 (M+.)	295	265	221	193	2078-22-0
XIII	7'42"	160	304	304 (M+.)	289	273	216	186	1032-28-6
IV	8'52"	262	334	334 (M+.)	319	304	291	274	
VI A,B	11'53"	260	332	332 (M+.)	317	303	243	215	
I	13'00"	228	228	228 (M+.)	202	114	101		56-55-3
V	13'11"	260	260	260 (M+.)	231	202	115	101	132335-14-9
XI A,B	13'20"	260	260	260 (M+.)	231	215	202	176	
VII A,B	13'31"	276	348	348 (M+.)	319	231	215	202	77573-41-2**
VI A,B	13'43"	260	332	332 (M+.)	317	303	243	215	
XI A,B	13'45"	260	260	260 (M+.)	231	218	202	176	
II	14'08"	244	316	316 (M+.)	301	285	270	226	
VIII	14'13"	292	436	436 (M+.)	421	319	202		
VII A,B	14'16"	276	348	348 (M+.)	319	231	215	202	
X	14'32"	258	258	258 (M+.)	230	202	129	101	2498-66-0
XII A,B	15'09"	276	348	348 (M+.)	331	316	231	215	
IX A,B	15'28"	276	348	348 (M+.)	333	316	231	193	
II	15'48"	244	316	316 (M+.)	301	285	270	226	
XII A,B	16'00"	276	348	348 (M+.)	333	303	231	215	
IX A,B	16'12"	276	348	348 (M+.)	333	316	231	193	
III	16'24"	260	404	404 (M+.)	389	315	301	285	
III	17'00"	260	404	404 (M+.)	389	316	286	241	

* theoretical value based on the molecular weight of the proposed TMS derivative

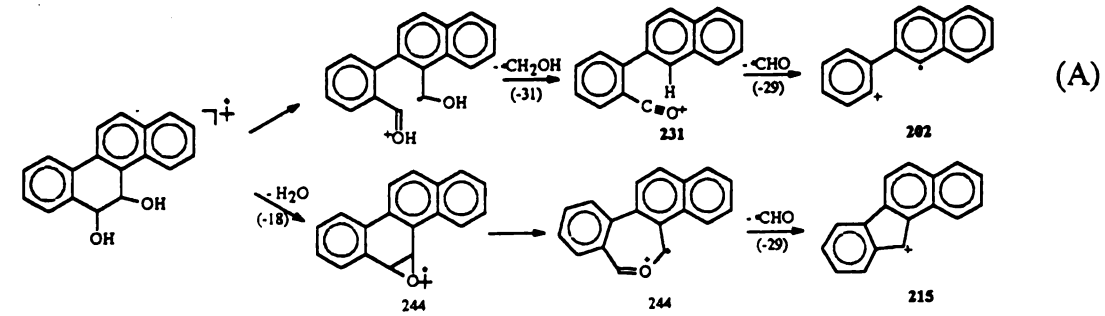
** a comparison mass spectrum on m/z 202, 215, 231

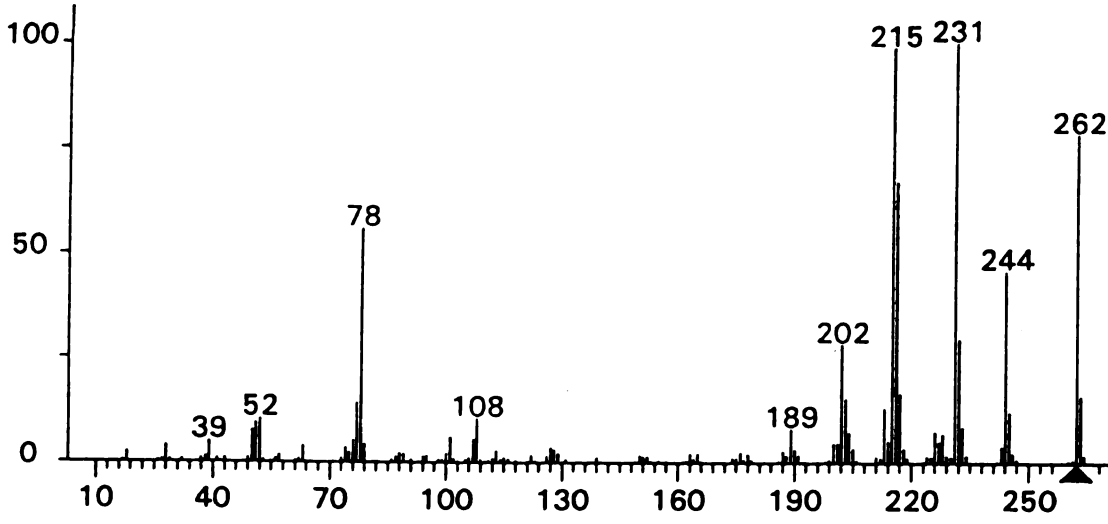
For example, the identical ions characteristic of 5,6-dihydrochrysenediol (see Figure 4.3A) listed in NIST library were compared to the mass spectrum of the TMS derivative of product VII A,B (see Figure 4.3B). The mass spectra and the interpretations of these spectra of all other products are provided in Appendix D.

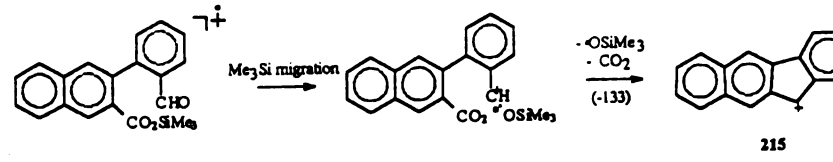
Two types of ozonation products were found. Compounds IIA, IIIA, and IV to VIII are phenyl-naphthyl type ozonation products. The major product of this type was 2-(2'-formyl)phenyl-3-naphthaldehyde (V). Compounds IIB, IIIB, and IX to XII are quinone type ozonation products. The major product of this type was benz[a]anthraquinone (X). Lower molecular weight products, such as dihydroxy-naphthalene (XIII), 2-carboxybenzaldehyde (XIV), 2-hydroxybenzoic acid (XV) and 2-phthalic acid (XVI), were also found.

The effect of the ozone : benz[a]anthracene (BaA) ratio (reported as moles ozone/mole BaA) on the distribution of the products and their relative concentrations is shown in Figure 4.4. Benz[a]anthracene was oxidized using ozone at stoichiometric ratios ranging from 0.29 to 9.88 moles ozone/mole BaA. In all experiments, the initial BaA concentration was 1 mM. The concentrations of the products are given as the ratio of the GC area response of the analyte (A) relative to that for 1,8-naphthalaldehydic acid (50 μL of 1 mM), which was used as an internal standard (AI). Due to their low concentrations, products VIII and XI could not be quantified throughout all experiments. At an ozone : BaA ratio of 0.29 mole ozone/mole BaA, products IIA, V, VI and VII, which are phenyl-naphthyl type products, along with quinone type products IIB, IIIB, X and XII were

Figure 4.3 (A) Mass spectrum of 5,6-dihydrochrysenediol obtained from NIST library search. The abundant ions m/z 202, 215 and 231 are characteristic of *o,o'*-disubstituted phenyl-naphthyls.
(B) Mass spectrum of an ozonation product, 2-(2'-formyl)phenyl-3-naphthoic acid or 2-(2'-carboxy)phenyl-3-naphthaldehyde (VII A,B), derivatized as the trimethylsilyl ester using BSTFA + 1 % TMCS. The phenyl-naphthyl backbone was evidenced by the presence of triplet ions m/z 202, 215 and 231, where m/z 215 was formed through a Me_3Si- migration mechanism (cf:Ref 18,19) followed by loss of CO_2 and Me_3SiO (= 44 +89 = 133) from the molecular ion m/z 348.


(A)
-CH2OH
(-31)
-CHO
(-29)
231
202
-H2O
(-18)
244
244
-CHO
(-29)
215


100
50
0
215
231
262
78
244
202
52
39
108
189
10
40
70
100
130
160
190
220
250


Me3Si migration
-OSiMe3
- CO2
(-133)
215

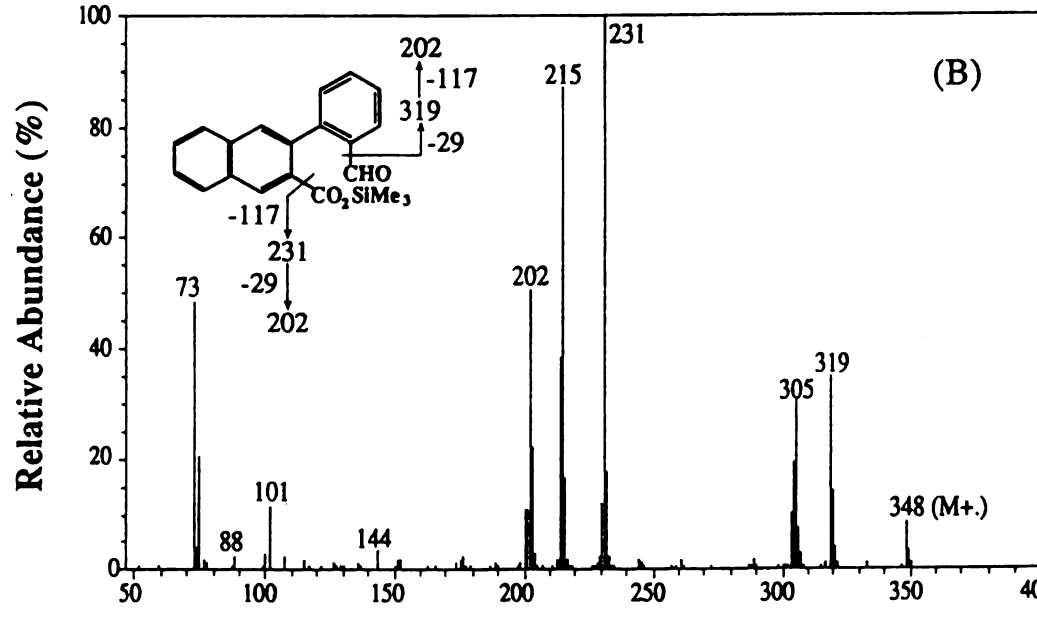
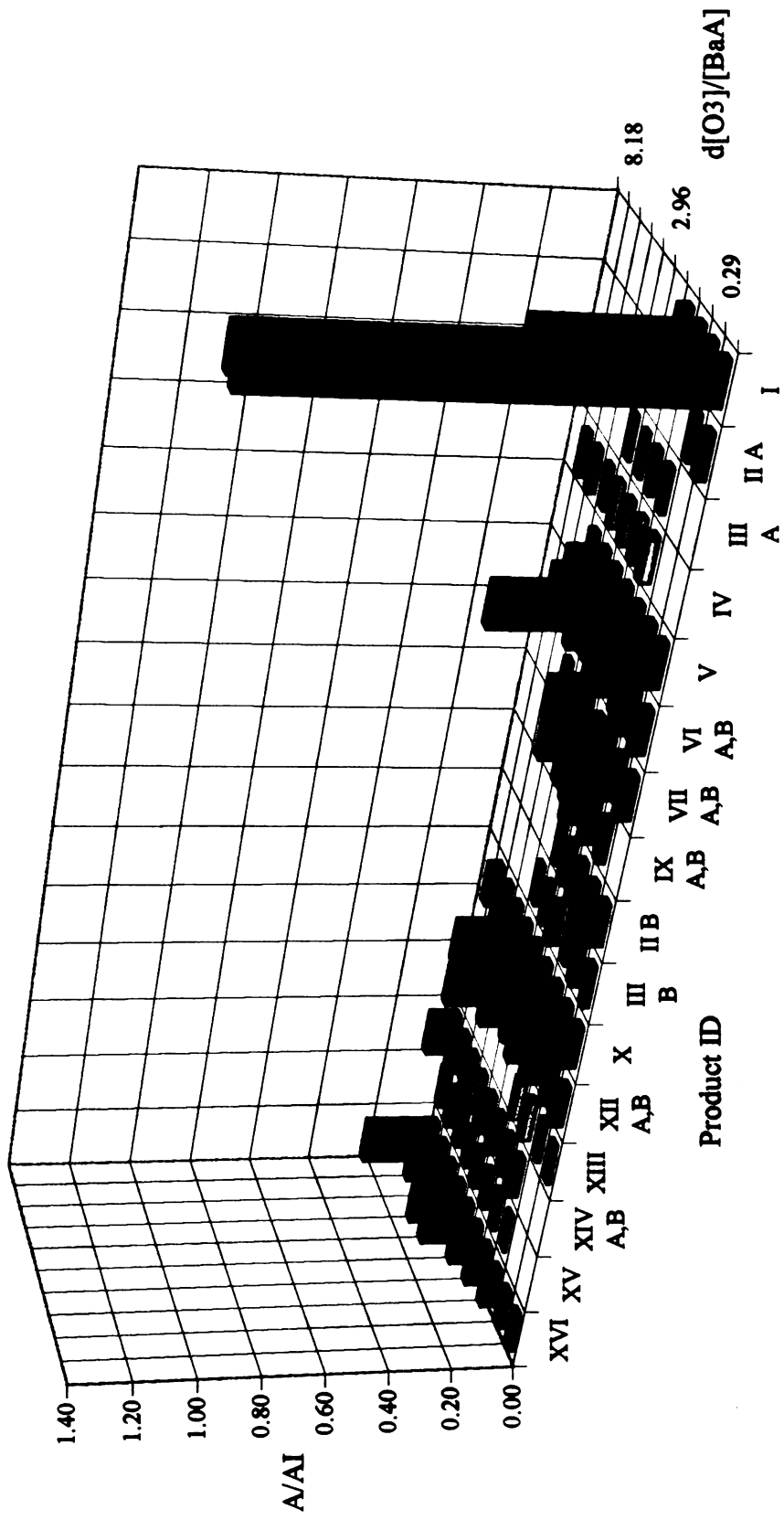

Relative Abundance (%)
(B)
202
-117
319
-29
CHO
CO2SiMe3
-117
231
-29
202
73
231
215
202
319
305
348 (M+.)
101
88
144
M/Z

Figure 4.4 The relative concentrations of benz[a]anthracene and its ozonation products obtained under different ozone : BaA ratios. The product concentrations were determined by the GC area response for compound (A) relative to the response for 1,8-naphthalaldehydic acid (50 μL of 1 mM) used as an internal standard (AI).


A/AI
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Product ID
I
II A
III A
IV
V
VI A,B
VII A,B
IX A,B
II B
III B
X
XII A,B
XIII
XIV A,B
XV
XVI
d[O3]/[BaA]
0.29
2.96
8.18

detected. Also, at this ozone dosage, products XIII and XVI had already formed. As the ozone : BaA ratio increased, the concentration of all products present increased. When the ozone : BaA ratio was 1.08 moles ozone/mole BaA, the concentration of BaA decreased dramatically. Additionally, product IIA disappeared, and products IIIA, IV, XIV and XV were found. At an ozone : BaA ratio of 1.99 moles ozone/mole BaA, the concentration of compounds V and X reached their maximum values, and the relative concentration of BaA decreased to 0.03. At this time, compound V predominated. Upon further increases in the ozone : BaA ratio, the concentrations of all compounds present (except products XIV, XV and XVI) began to decrease. The concentration of product V decreased faster than that of product X, and product X became predominant. At an ozone : BaA ratio of 2.96 moles ozone/mole BaA, the parent compound and product XIII disappeared. Upon further increases in the ozone : BaA ratio (to > 5.50 moles ozone/mole BaA), the concentration of product V decreased to below its detection limit. At this ratio, compound XVI predominated. At an ozone : BaA ratio of greater than 8.20 moles ozone/mole BaA, only products XIV, XV and XVI were detected.

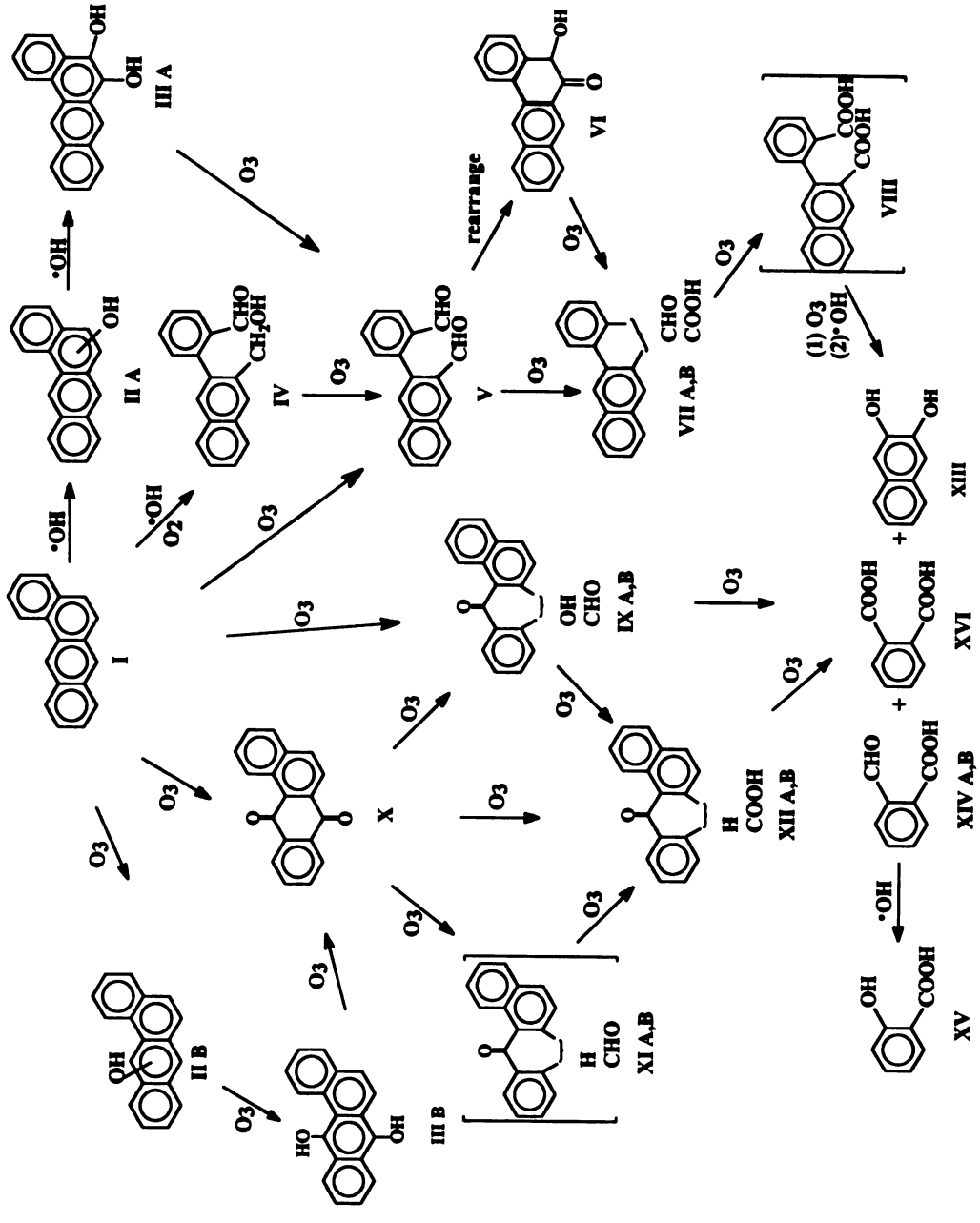
Several of the products identified appeared to have resulted from the oxidation of benz[a]anthracene by free radicals, most likely by ·OH. These include minor products, II A and B, IIIA and B, IV and XIII. To prove this hypothesis, experiments were performed in pure acetonitrile with the ozone dosage varying from 0.05 to 0.50 mmole. Product IIA was not produced, and product IV was not detected until the ozone dosage had increased to 0.50 mmole. Although product IIIA was detected, it was found in low concentrations.

As observed in the acetonitrile/water mixture, products IIB, IIIB and XIII were also detected in pure acetonitrile when a low ozone dosage were used.

4.4 Discussion

Ozone is known to react with aromatic compounds resulting in either substitution by atom attack or ring cleavage by bond attack. The location of the attack of ozone can be correlated with the lowest atom- and bond- localization energies of the target compound. Based upon the products identified, along with the sequence of their appearance, a pathway for the ozonation of benz[a]anthracene is proposed (see Figure 4.5). Both atom and bond attack occurred during ozonation. Atom attack by ozone on the carbon at the 7 or/and 12 position produced compounds with hydroxyl functional groups, e.g., IIB and IIIB. Upon further ozonation of these compounds, benz[a]anthraquinone (X) was produced. Atom attack by ozone at the 7 and 12 position resulted in a trioxalane ring structure; product IX and benz[a]anthraquinone (X) were produced. This pathway was consistent with that proposed by Bailey (*8*). Upon further ozonation, the hydroxyl and/or aldehydic functional group was oxidized to form a carboxylic acid, resulting in the formation of minor products XI and XII. Additionally, the ketone bond can be cleaved resulting in formation of low molecular weight compounds, e.g., XIII, XIV, XV and XVI. Products IIB, IIIB, IX, X and XVI are consistent with products presented by previous researchers (*9-13*).

Figure 4.5 Proposed pathway for the ozonation of benz[a]anthracene. Compounds shown in brackets [ ] were detected at concentrations which were below detection limit.


I
II A
II B
III A
III B
IV
V
VI
VII A,B
VIII
IX A,B
X
XI A,B
XII A,B
XIII
XIV A,B
XV
XVI
•OH
O2•OH
O3
rearrange
(1) O3
(2)•OH
OH
CHO
CH2OH
COOH
HO
H

The bond attack type of reaction occurred on the bond having the lowest energy, i.e., at the 5,6 position and resulted in ring cleavage. Benz[a]anthracene reacted to form a dialdehyde, e.g., product V (2-(2'-formyl)phenyl-3-naphthaldehyde). An internal rearrangement involving the dialdehyde groups on V could result in ring closure with ketone and hydroxyl functional groups substituted on the cyclic ring (product VI). Upon further ozonation, the aldehydic functional group was oxidized to form a carboxylic acid (product VII and VIII). However, further ozonation could also result in cleavage of phenyl-naphthyl type products at the bond between the phenyl and naphthyl groups to produce dihydroxy-naphthalene (XIII) and other low molecular weight compounds. Since the ozonolysis of benz[a]anthracene would be expected to result in the direct formation of products such as V, IX and X, the appearance of low molecular weight compounds, such as XIII, XIV, XV, XVI, at low ozone dosages substantiated the hypothesis that these compounds can be produced by the direct ozonolysis of products V, IX and X.

Free radicals may also be involved in these reactions to form phenyl-type compounds. When benz[a]anthracene was ozonated in pure acetonitrile, the product IIA did not form and the concentration of product IIIA and IV decreased (as compared to that observed in 90% acetonitrile/water solution). The hydroxyl radical could attack at the 5 or 6 position, resulting in the formation of products IIA and IIIA. Also, hydroxyl radical attack, with the addition of oxygen on benz[a]anthracene, followed by dehydrogenation would result in the formation of product IV (*11*). Upon further ozonation and dehydrogenation of $-CH_2OH$ on product IV, a dialdehyde would form, i.e., product V. In pure acetonitrile, product IIIA was produced at lower concentrations (as compared to that

observed in 90% acetonitrile/water solution) and product IV was not produced until the stoichiometric ratios were greater than 3.77 moles ozone/mole BaA. These products may result from the reaction of organic peroxides, generated during the ozonation auto-decomposition chain reaction (*17*). Free radicals can also react with 2-phthalic acid (product XVI), resulting in the formation of 2-hydroxybenzoic acid (product XV) (*11-13*).

A comparison of the effect of ozone demand on the formation of products V (the major product from bond attack pathway) and X (the major product from atom attack pathway) is shown in Figure 4.6 (determination was done as shown on page 62). Although bond and atom attack occur simultaneously from the beginning of the reaction, the bond attack reaction occurs preferentially. Before benz[a]anthracene completely disappeared, products V and X reached their maximum concentrations. However, the maximum concentration of product V was 25% higher than product X. Upon further increasing the ozonation dosage, product V appeared to decrease more rapidly than did product X. Prior to the disappearance of product X, all of the phenyl-naphthyl type products formed from ozone attack had disappeared.

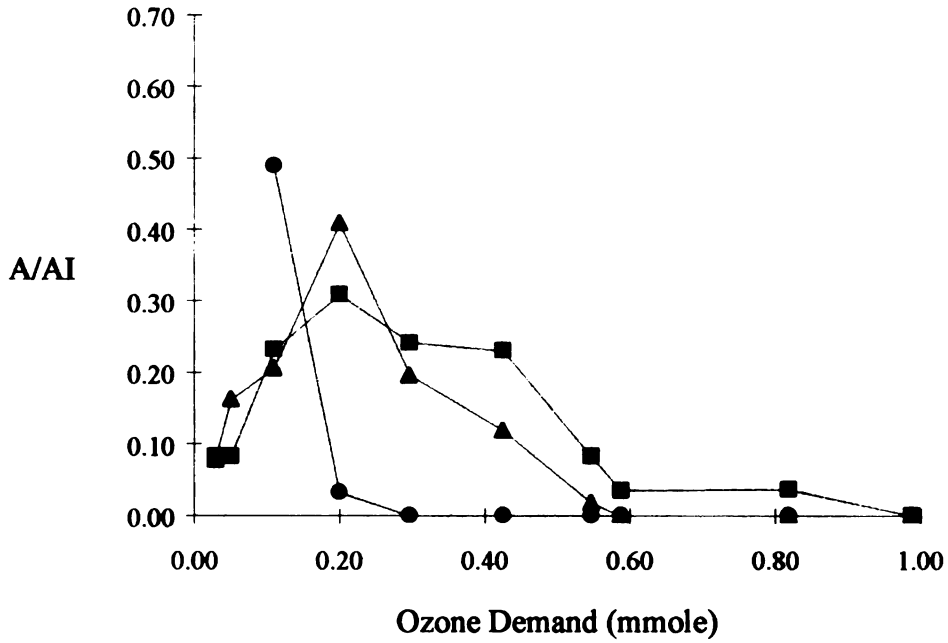


Figure 4.6 The effect of ozone dosage on the concentrations of two major products, 2-(2'-formyl)phenyl-3-naphthaldehyde (V) and benz[a]anthraquinone (X). The residual concentration is determined by the GC area response for compound (A) relative to the response for 1,8-naphthalaldehydic acid (50 μL of 1 mM) used as an internal standard (AI). ● BaA; ▲ Product V; ■ Product X.

4.5 Conclusions

A pathway for the ozonation of benz[a]anthracene in acetonitrile/water mixtures has been described. Ozonation resulted in parallel reactions by both atom and bond attack. The atom attack at the 7 and/or 12 position produced the quinone or hydroxyl functional groups. The bond attack type of reaction occurred at the 5,6 position and caused ring cleavage resulting in phenyl-naphthyl type products. The bond attack reaction occurs preferentially.

The identification of the reaction products was accomplished by mass spectrometry. Future work will include HPLC purification, and NMR to further confirm the product identification. Once pure fractions of the products are obtained, these individual products will be further investigated to assess their toxicity.

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CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The overall objective of this thesis was to gain a better understanding of the reactions involved in the ozonation of PAHs in order to predict the products formed from the ozonation of PAHs in waters and other environmental systems. The major focus of this study was to investigate the pathways of the reactions of ozone with pyrene and benz[a]anthracene in a aqueous system. The target compounds were chosen from two different classes of PAHs to compare different ozonation pathways. An review of the literature related to general ozone chemistry has been presented in Chapter 1. The subsequent three chapters contain descriptions of experimental investigations of two PAHs. Each of these chapters can stand alone and is published or currently in the process of being published. A conclusive discussion based on these research results is provided in this chapter.

The aqueous solubilities of PAHs are very low. Therefore, the reaction of ozone with PAHs has been previously conducted either in dilute aqueous solution (*1-7*) or in organic solvent (*8-17*). However, in dilute aqueous solution, excess ozone was usually used. When excess ozone is involved in reaction, only final products are observed and the

reaction pathway is not predictable (*4,8*). To obtain more concentrated solutions of PAHs, most research was conducted using organic solvents. Some organic solvents, such as methanol or methylene chloride, will react with ozone and produce organic peroxides (*1,18*). This poses an additional difference in reaction products as compared to using water. In this study, acetonitrile was chosen as the organic solvent because it has a low reactivity with ozone, it is miscible with water, it is immediately amenable to RP-HPLC analysis, and it has little effect gap junction intercellular communication (GJIC) which was used as a bioassay. In the water that was present (and dissolved in acetonitrile), ·OH radicals will form from the decomposition of ozone. Additionally, in our studies, sufficient water was present such that water can participate in the direct ozone reactions, through hydrolysis type reactions. As such, reaction pathways involving both the direct reaction with ozone and the direct reaction involving ·OH radical reaction were proposed.

The toxicity of ozonation pyrene products was assessed by monitoring GJIC, a tumor promoter bioassay. The observations indicated that the ozonation of pyrene resulted in the initial formation of products which were more inhibitory to GJIC than was pyrene, itself. Upon further ozonation, the toxicity of the reaction products was reduced. Therefore, identification of the ozonation products became important. By identifying these ozonation products and understanding the reaction pathways that occur in aqueous solutions, treatment alternatives that would minimize the formation of toxic products can be developed. Gas chromatography/mass spectrometry (GC/MS) used with library searches of standard spectra and the GC/MS analysis of commercially available

preparations provided more convincing product identification than other studies using melting point or IR determination.

A reaction pathway involving ozone bond attack which results in the stepwise ring cleavage of pyrene and where ozonation byproducts compete with pyrene for ozone was hypothesized. A stoichiometric ratio of 1.68 moles ozone/mole pyrene was required to completely destroy pyrene, indicating that the reaction of ozone with ozonation products occurs prior to the elimination of pyrene; therefore, ozone would be consumed simultaneously by pyrene and the initial byproducts. This would be expected to result in a stoichiometric ratios greater than one which is the value that would be expected if ozone reacted only with pyrene.

The initial bond attack occurred during the reaction of ozone with pyrene. Fourteen products[1] including aldehyde and carboxylic acid substituted phenanthrene- and biphenyl-type oxidation products formed from the reaction of ozone with pyrene were identified. Ozonation resulted in stepwise ring cleavage and in the oxidation of aldehydic functional groups. First, ring cleavage of pyrene occurred at the 4,5 position, with phenanthrene-type products predominating. Similar results were found by researchers who used organic solvents (*10-12,15-16*); except that they observed that the main product formed was phenanthrene-4,5-dicarboxylic acid. In our studies, 4-carboxy-5-

[1] These products were numbered from II to XV as shown in Figure 3.4.

phenanthrenecarboxyaldehyde was the main product. Upon further ozonation, we found that secondary ring cleavage at the 9,10 position occurred.

While the reaction of ozone with pyrene resulted initially in the formation of phenanthrene-type products, further ozonolysis reaction resulted in the formation of biphenyl-type products. Until pyrene had completely disappeared, the phenanthrene-type products predominated (over the biphenyl-type products). Once the phenanthrene-type products had reacted, the biphenyl-type products predominated. This sequence of reactions further supports the hypothesis that pyrene competes with its ozonation byproducts for ozone.

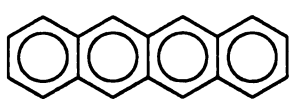
Even at pH 3.7, ·OH radicals appeared to be involved in the reaction of the primary ozonation products. This is thought to be true, because: (i) compound VIII is likely to have been formed from the decarboxylation of an adjacent dicarboxylic acid by ·OH radicals (*19*); (ii) the reaction of ·OH radicals with pyrene followed by ozonation would result in formation of compound V(*20*); (iii) the presence of ketone type products, XIV and XV, would result from ·OH radical attack on pyrene followed by dehydration.

For the ozonolysis of benz[a]anthracene, the ozonation pathway differed from that observed with pyrene. The initial bond and atom attack appeared to occur simultaneously in the reaction of ozone with benz[a]anthracene. Fifteen products[2], which resulted from

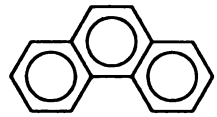
[2] These products were numbered from II to XVI as shown in Figure 4.1

these two pathways, were identified. The bond attack type of reaction occurred at the 5,6 position of benz[a]anthracene and caused ring cleavage resulting in phenyl-naphthyl type products. Bond attack (e.g., ring cleavage) resulted in the formation of product V but not VIII, and only a trace amount of VIII. Atom attack on the carbon at the 7 and/or 12 position produced the quinone or hydroxyl functional groups. Similar products were observed in organic solvent (*8,21-25*). The Ozonolysis of benz[a]anthracene appeared to occur preferentially by the bond attack type of reaction. Although the oxidation of benz[a]anthracene by hydroxyl free radicals did not result in high concentrations of products, ·OH radical was found to be involved in the reaction. Hydroxyl free radicals appeared to result in the formation of initial products (IIA and IV), and subsequent oxidation of product IIA to form a diol product (IIIA). Also, ·OH radicals can be involved in the further oxidation of ozonation products as described in the previous paragraph. However, our work confirms the observations made by Bailey et al. (*25*) that products IIB and IIIB could be formed in the absence of ·OH radicals.

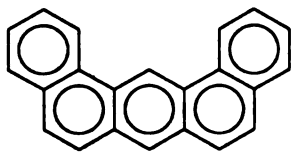
In aqueous systems, ·OH radicals significantly affect the ozonolysis reaction of compounds where ozone reacts by initial bond attack. For those compounds which react with ozone by both attack on the bond and on an atom, the influence of ·OH radicals is less important. However, in any initial atom attack type of reactions, only ozone is involved. For example, Bailey (*26*) showed that, even in the presence of hydrogen peroxide, only initial atom attack product was observed in the reaction of ozone with naphthacene (shown in Figure 5.1).



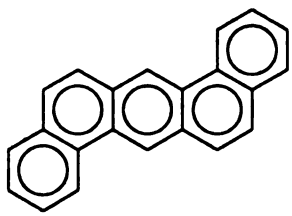
Naphthacene



Phenanthrene



Benz[a,j]anthracene



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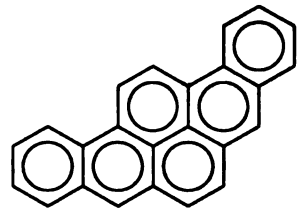
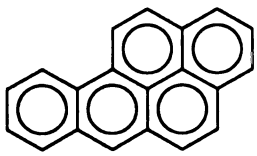
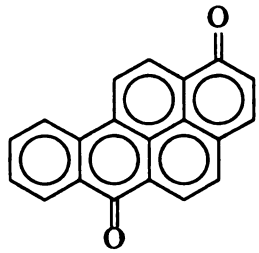


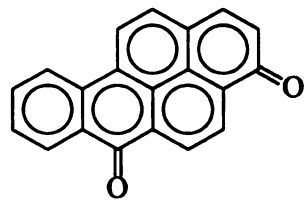
Benz[r,s,t]pentaphene



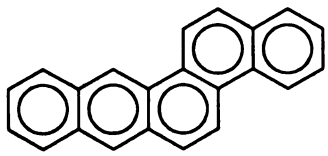
Benz[a]pyrene



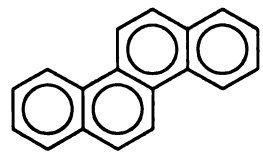
1,6-quinone benz[a]pyrene



3,6-quinone benz[a]pyrene



Benz[b]chrysene



Chrysene

Figure 5.1 Structure of the example PAHs

Where ozonolysis occurs by initial bond attack and ·OH radicals is involved in the reaction of ozonation products, ozonolysis needs to be studied in the presence of water. The results of studies conducted in non-participating solvents can only predict the products (or pathway) formed from initial ozone attack but can not precisely assess the importance of ·OH radicals. Although some researchers (*12,15,16,21,22,25*) have used participating organic solvents, any further reaction to produce non-ozonation products need to be proven (Appendix C). The use of a mixture containing water and a non-participating solvent, as done in this study, is advantageous over using participating solvents such as methanol. This is because in the acetonitrile/water mixture the importance of the free radical reaction can be investigated, yet side reactions involving the participating solvent (e.g., methylation reaction) can be avoided.

In comparing of the reactions of ozone with different PAHs, it is apparent that the lowest localization energy of the target compound is important. Ozone attack either on the bond or atom depends on which of them has the lowest localization energy. For pyrene, the lowest bond localization energy (L_b) is much smaller than the lowest atom localization energy (L_a), i.e., $L_a - L_b = 0.45$; therefore, a bond attack occurs. Other compounds which have $L_b << L_a$ also show the initial ozone bond attack reaction only. For example, with phenanthrene, which has $L_a - L_b = 0.72$, ozonation resulted in ring cleavage by ozone attack on the 9,10 position (*2,26,27*). Moniconi et al. (*28,29*) found that bond attack occurred during the ozonation of dibenz[a,j]anthracene ($L_a - L_b = 0.40$) and dibenz[a,h]anthracene ($L_a - L_b = 0.46$). However, for benz[a]anthracene, the localization energy between bond and atom is smaller, i.e., $L_a - L_b = 0.32$, resulting in competition between bond attack and atom

attack. Moniconi and Salce (*30,31*) also found that bond and atom attack occurred when ozone reacted with benz[r,s,t]pentaphene. Unfortunately the L_b of benz[r,s,t]pentaphene is not available. When the localization energy for the lowest bond was closer or even larger than the localization energy for the lowest atom, initial atom attack by ozone occurred. For example, the ozonation of benz[a]pyrene ($L_a - L_b = 0.13$) resulted in the formation of benz[a]pyrene-3,6-dione and benz[a]pyrene-1,6-dione (*32,33*) by initial atom attack.

Based on our research, some unknown reaction pathways of ozone with previously unstudied PAHs can be predicted. For example, the ozonation of benz[b]chrysene, which has $L_a - L_b = 0.21$, is expected occur by both bond and atom attack. However, because the difference between L_a and L_b is smaller than benz[a]anthracene, products formed from atom attack may have a higher yield than from bond attack, e.g., quinone type of products are expected as the major products. The ozonation of chrysene, of which our study has not been completed due to the problems separating the products, may also be predicted. For chrysene, although the bond and atom having the lowest energy happen to be at the same location, and $L_a - L_b = 0.55$ (*34*), ozone is expected to attack on the lowest energy bond and produce phenyl-naphthyl type of products. This precise prediction of ozone bond attack reaction is consistent with the work done by Copeland et al. (*23,24*). The phenyl-naphthyl product is similar to the benz[a]anthracene products formed by ozone initial bond attack. Therefore, the ozonation of chrysene in aqueous solution can be expected to occur by similar pathway involving ring cleavage as observed for the ozonation of benz[a]anthracene.

5.2 Recommendations

This study identified the products formed from the ozonation of selected PAHs using GC/MS directly. It also opened many directions for future research. We recommend that:

1. the separation procedure be improved for purifying ozonation products. Preparatory TLC and reverse phase medium pressure LC resulted in further oxidation of the products;
2. the ozonation pathway for chrysene be investigated using a improved separation procedure followed by GC/MS identification;
3. the identified reaction products be confirmed using NMR;
4. the mechanistic pathway for the reaction of ozone with the purified products be further investigated to verify the proposed pathways;
5. the toxicity of ozonated products be investigated;
6. the effect of presence of other reactive substrates on the pathway of the reaction of ozone with the target compounds be investigated.

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APPENDICES

APPENDIX A

APPENDIX A

KINETICS STUDY

A.1 Introduction

In recent years, the polycyclic aromatic hydrocarbons (PAHs) have caused much concerned among environmental scientists and engineers. The PAHs are generated and released to the environment by human activities. Low concentrations of these chemicals have been shown to be carcinogenic to mammals (*1,2*).

Ozone is a more powerful oxidant than the other oxidants used in water and wastewater treatment. The increasing interest in using ozone for treatment is based on the successful operations, mainly in Europe, as well as in Canada, Japan and United States (*3*). Therefore, the ozonation kinetics study for the degradation of PAHs is important for Evaluating treatment efficiency.

Only a few studies related to the kinetics of the ozonation of non-dissociating PAHs have been published (*4-8*). The rate constants and stoichiometric coefficients for the reaction of ozone with four PAHs are presented in Table A.1. Kuo (*4*) determined the rate constants for the reaction of ozone and several PAHs in water at 25 °C. Kuo (*4*) and Hoigné and Bader (*5*) determined the reaction rate constants of naphthalene by monitoring ozone consumption. On the contrary, Butković et al. (*6*) and Kuo (*4*) measured the change in the PAH (except

Table A.1 Rate constants and stoichiometric coefficients for the ozonation of PAHs

Compound	Stoichiometry Coeff.	Rate Law	k M^{-1} s^{-1}	Ref
Naphthalene	Use 2 mole O_3/mole NA	$-\frac{\partial[O_3]}{\partial t} = k_{O_3}[O_3][M]$	k = 843 (25°C, pH=3)	4
			k = 9845 (35°C, pH=3)	4
			k = 3202 (25°C, pH=5~6)	4
	1~5 mole O_3 /mole M Use 2.5 in this paper	$-\frac{\partial[O_3]}{\partial t} = k_{O_3}[O_3][M]$	kO_3 = 3000±600 (20°C, pH=2)	5
	2 mole O_3/mole NA	$-\frac{\partial N}{\partial t} = k_N[O_3][N]$	k_N = 550±80 (1°C, pH=5.6)	8
Phenanthrene	Use 2 mole O_3/mole PH	$-\frac{\partial[M]}{\partial t} = k'[M]$	k = 1.9~4.7×10⁴ (25°C, pH=2.21~7)	4
	-	$-\frac{\partial[M]}{\partial t} = k'[M]$	k = 15000±120 (25°C, pH=1~7)	6
	calculate $\Delta[O_3]/\Delta[M]$	$-\frac{\partial[M]}{\partial t} = k_M[O_3][M]$	k_N = 2400±200 (20°C, pH=7)	7
Pyrene	-	-	(18~21°C, pH =?)	1
	-	$-\frac{\partial[M]}{\partial t} = k'[M]$	k = 40000±5000 (25°C, pH=1~7)	6
Benz[a]pyrene	-	$-\frac{\partial[M]}{\partial t} = k'[M]$	k = 6200±1000 (25°C, pH=7)	6
		$k' = k[O_3]$		

for naphthalene) concentrations in solutions where ozone was in excess; pseudo-first order reaction conditions were used to determine the reaction rate constants. Beltrán et al. (*7*) and Legube et al. (*8*) measured both ozone and PAH consumption at different reaction times. They also determined the stoichiometry coefficient of the initial reaction. This method provides a better understanding of the initial reaction of ozone with a target compound.

In order to study the initial ozonation reaction, the stoichiometry coefficient of the reaction of ozone with PAH was determined. Hoigné and Bader (*5*) state that the stoichiometric coefficients usually range from 1 to 5. They calculated the reaction order for aromatic compounds by assuming that the stoichiometric coefficients were equal to 2.5. In most subsequent papers, researchers assumed that the reaction order and stoichiometry were identical to that assumed by Hoigné and Bader (*5*). However, those researchers did not confirm the reaction order and stoichiometry. Contrastingly, Legube et al. (*8*) determined the rate constant for the reaction of ozone with naphthalene in organic solvent at 1 °C, pH 5.6. They found that the stoichiometric coefficient for the initial reaction step is 2, and that a second order reaction is verified. Beltrán et al. (*7*) found the stoichiometric coefficient for phenanthrene to be 1. This is the evidence for ozone direct reaction with phenanthrene. In this study, the kinetics of the reaction of ozone with pyrene were investigated. Both ozone and pyrene concentration were monitored.

A.2 Kinetics Model

If the decomposition of the target compounds is due solely to the direct reaction of the compounds with ozone (and not due to reaction with secondary oxidants), then the direct reaction of ozone with solute M can be expressed as:

$$M + \xi\, O_3 \rightarrow M_{oxid} \qquad [1]$$

where ξ is the stoichiometric coefficient (i.e., the number of moles of ozone consumed per mole of M transformed to M_{oxide}). The change in concentrations of reactions with time can be written as:

$$-\frac{dM}{dt} = k[M]^m [O_3]^n \qquad [2]$$

where m, n are the reaction order for solute and ozone. Work done by Bader and Hoigné (*5*) shows a second order reaction for some non-dissociating organic compounds; therefore, an assumption of a second order reaction was made, i.e., m = n = 1. The solution to equation [2] can be derived as:

$$\ln \frac{[O_3]\cdot[M]_o}{[O_3]_o\cdot[M]} = ([O_3]_o - \xi\,[M]_o)\,kt \qquad [3]$$

This expression can be written as:

$$\ln A = k\,T$$

where $A = \frac{[O_3]\cdot[M]_o}{[O_3]_o\cdot[M]}$, and $T = ([O_3]_o - \xi[M]_o)t$. Values for ξ can be calculated as:

$$\xi = \frac{[O_3]_o - [O_3]}{[M]_o - [M]} = \frac{\Delta[O_3]}{\Delta[M]} \qquad [4]$$

If $\ln\frac{[O_3]\cdot[M]_o}{[O_3]_o\cdot[M]}$ vs $([O_3]_o-\xi[M]_o)*t$ is linear, the second order reaction is confirmed and k can be determined.

A.3 Method

Kinetics Measurements

This study was conducted at room temperature; and, since ozone has lower degradation rate at lower pH (*9*), the reaction was controlled at pH 2. All aqueous ozone solutions were prepared in acidic deionized (DI) water. Phosphoric acid (85% purity) was added to acidify the DI water to a pH of 2. Ozone was produced from dried oxygen using a ozone generator (Polymetrics, Inc., San Jose, CA, Model T-408). Aqueous ozone solutions were prepared by bubbling gaseous ozone through a one liter vessel containing the acidified water. The aqueous ozone concentrations ranged from 0.05 to 0.3 mM.

Because of the low aqueous solubility of pyrene (*2*), a stock solution was prepared in pure acetonitrile. Since a detection limit for pyrene (using HPLC/ UV detection) of 0.1 mg/l was obtained, and the reaction of ozone and pyrene in aqueous solutions can be very fast, the kinetics experiments were conducted in solutions containing an initial concentration of at least 10 mg/L pyrene.

To terminate the reaction, any residual ozone in the reaction solution was quenched using indigo. Work done by Bader and Hoigné (*10*) has shown that indigo reacts immediately with ozone and can be employed in this manner. The indigo stock solution

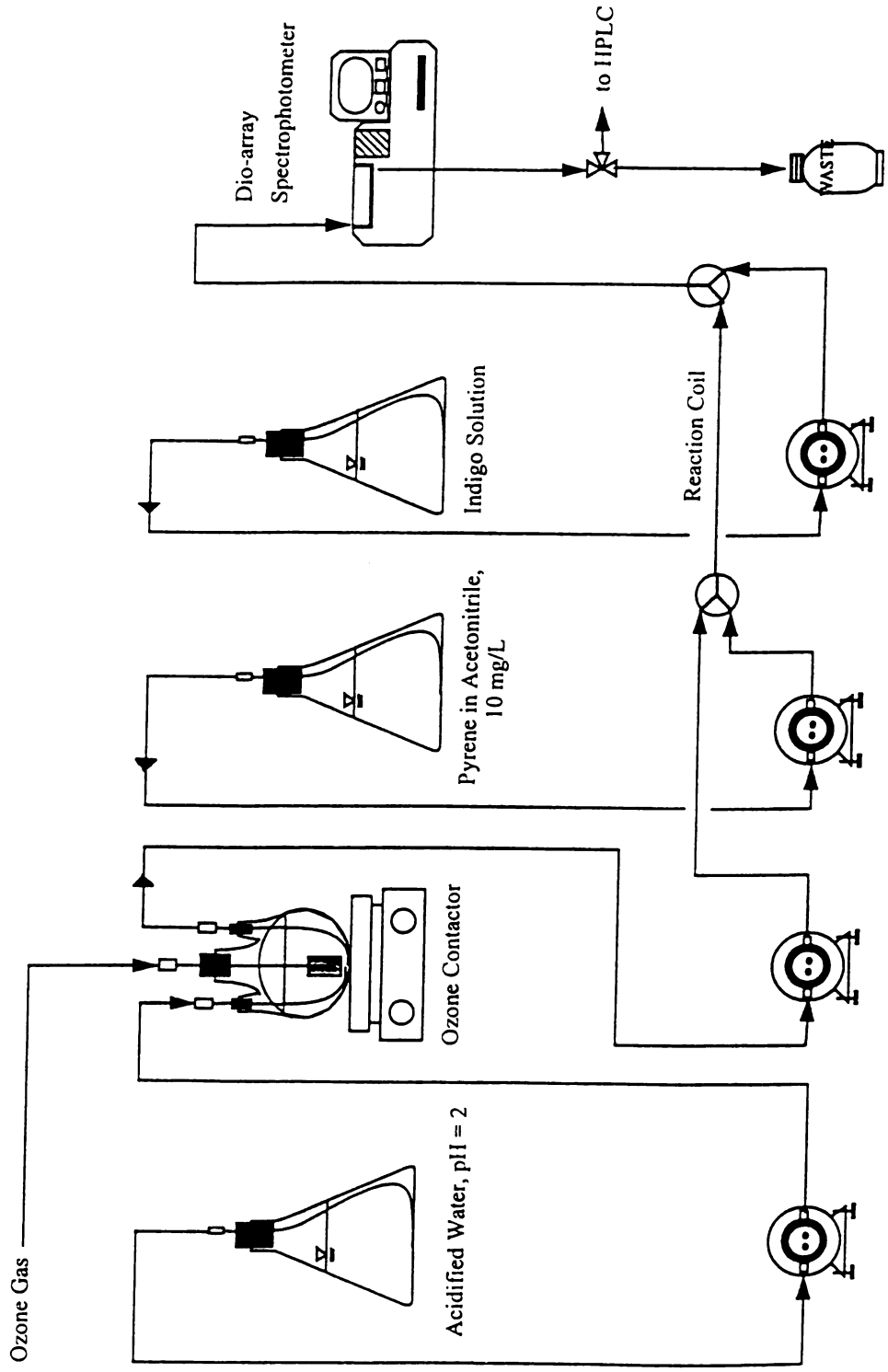
contained 1 mM of potassium indigotrisulfonic acid (~95 % purity. Aldrich, WI) in pH 2 water. As the percentage of solvent was found to affect the solubility of pyrene, the reagent solutions were prepared so as to maintain a constant acetonitrile/water mixture during mixing. Thus, the stock indigo solution was diluted to 0.25-0.5 mM (depending on the initial ozone concentration). This maintained the ratio of 50% acetonitrile to pH 2 water in all reaction solutions.

The ozonation reaction were studied in a continuously flowing apparatus (illustrated in Figure A.1). All solutions were pumped using a FMI RP pump (Model RH/QG, Fluid Metering, Inc., Oyster Bay, NY). Acidified water was continuously pumped into the ozone contactor to maintain a constant concentration of ozone in the stock solution. Reactants were continuously pumped through a Y-shaped mixer. The reaction retention times were controlled by the volume contained in the reaction coil. The reaction was quenched by mixing the solution in the reaction coil with the indigo stream in the second Y-shaped mixer. The residual ozone concentration was determined by the indigo method (*10*). At the end of the system, the reaction solution was collected, and residual pyrene concentration was determined using HPLC.

Analytical Techniques

Aqueous Ozone. Aqueous ozone was withdrawn from the ozone contactor using a gas-tight glass syringe. In solutions that did not contain pyrene or the reaction products, the ozone concentration was determined directly using UV spectrophotometer at 258 nm. A dio-array spectrophotometer (Model # 8452A, Hewlett Packard, Palo Alto, CA) was used for this

Figure A.1 Configuration of kinetics apparatus


Ozone Gas
Acidified Water, pH = 2
Ozone Contactor
Pyrene in Acetonitrile, 10 mg/L
Indigo Solution
Reaction Coil
Dio-array Spectrophotometer
to HPLC
WASTE

purpose. An extinction coefficient of 3000 M^{-1} cm^{-1} was used to convert the absorbance units to concentration. In solutions containing pyrene or the reaction products, ozone was measured using the indigo method (*10*). The final ozone concentration was determined by the decrease in the absorbance at 600 nm as compared to the blank which contained no ozone.

Pyrene Concentration. Aliquots of the sample solutions were collected before and after ozone reacted with pyrene. HPLC was carried out using a Gilson system equipped with a 5μm 4.6 x 250 mm Partisphere RP C^{18} column (Whatman, Clifton, NJ). An UV detector (Model No. 116) was used to measure the absorbance at 240 nm. The mobile phase contained in 85% acetonitrile and 15% water mixture flowing at a flow rate of 1.5 ml/min.

A.4 Results and Discussion

The stoichiometric coefficient for ozonation of pyrene in aqueous solution can be calculated according to Equation [4]. The experimental results for ξ are shown in Figure A.2. Using a linear regression of all the data points, the stoichiometric coefficient, ξ, was determined to be 2.0 ± 0.02, $r^2 = 0.92$. Based upon these experimental results, one pyrene reacted with two ozone. However, based upon previous research (*11*), except when ozone was present in excess (*12*), only one bond attack ozonation products are produced. As such, ξ would be expected to be one. The fact that we obtained $\xi = 2.0$ suggests that ozone was consumed either by self-decomposition of ozone or by the reaction of ozone with products formed by the initial one bond attack on pyrene.

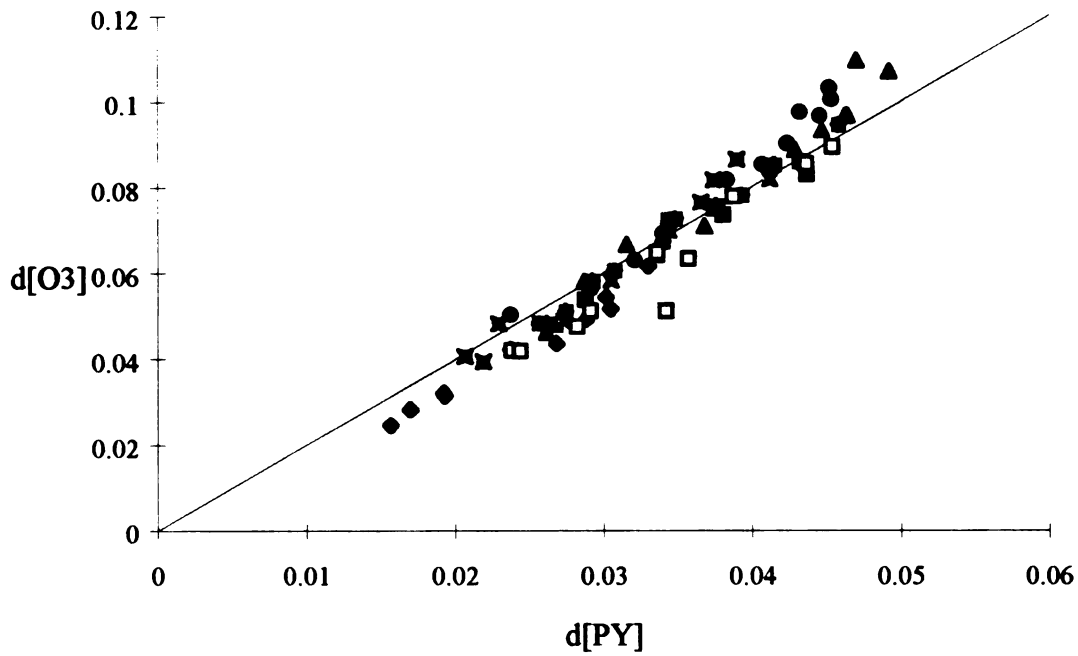


Figure A.2 The stoichiometric coefficient for the reaction of ozone with pyrene. Different symbols indicated different set of data; solid line is linear regression result with r^2 = 0.92.

When equation [3] was applied, the reaction rate constant, k, could be obtained by plotting lnA vs T (shown as Figure A.3). A reaction rate constant, k of 3.718 ± 0.066 $mM^{-1} s^{-1}$, $r^2 = 0.96$ was observed. On the contrary, Butković et al. (*6*) obtaine a rate constant of 40000 ± 5000 $M^{-1} s^{-1}$ for the reaction of ozone with pyrene. The large difference in values of k may because in their studies ozone was in excess and they only measured the change in the concentration of pyrene. However, any organo peroxide or ·OH free radicals generated during the reaction may react with pyrene, itself. As a result, an over estimate of the rate constant may have occurred. In this study, both ozone and pyrene concentrations were monitored. However, since the reaction between ozone and pyrene is very fast, the ozone consumption measured during the experiment may not only be due to the reaction of ozone with pyrene but by any intermediates formed along with ozone self-decomposition. Therefore, the assumption that the decomposition of pyrene is due solely to the direct reaction of ozone can not be applied in this case.

In order to get more accurate results for ozone direct reaction, a shorter reaction time is required for this fast reaction. As the equipment required to do these experiments, such as stop-flow reaction, was not available, we were not able to obtain the desired kinetic data.

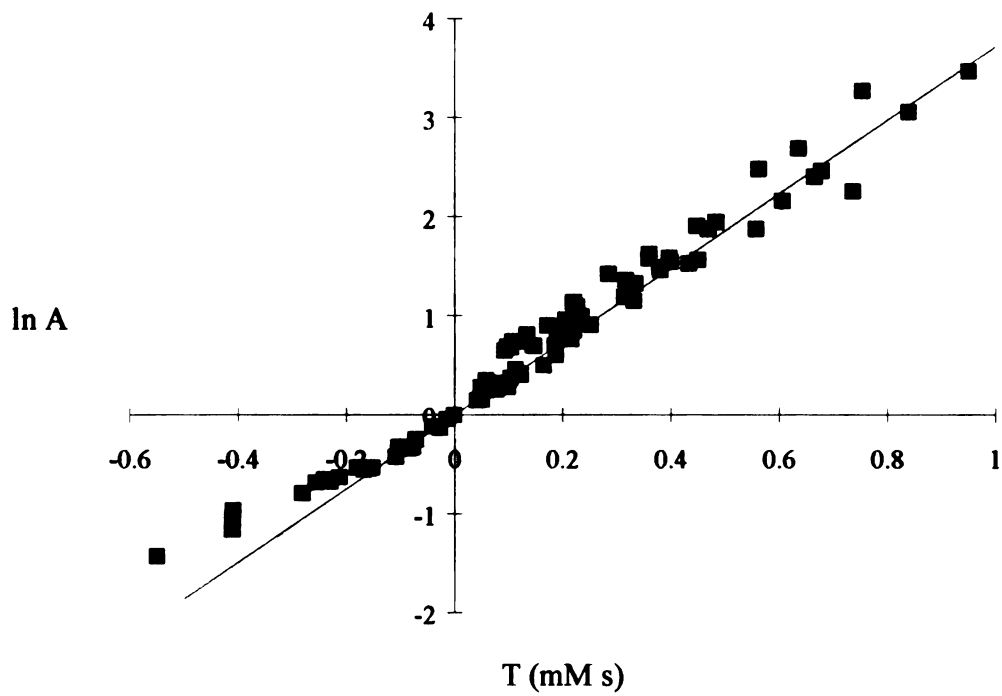


Figure A.3 The rate constant for the reaction of ozone with pyrene in aqueous solution. A plot of lnA vs T. Where $A = \frac{[O_3]\cdot[M]_o}{[O_3]_o\cdot[M]}$, and $T = ([O_3]_o - \xi[M]_o)t$.
■ = calculated k from each data point; solid line is the linear regression result ($r^2 = 0.96$).

A.5 References

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APPENDIX B

APPENDIX B

MASS SPECTRA AND INTERPRETATION OF SILYLATED PYRENE OZONATION PRODUCTS

In this appendix, the raw spectra obtained from GC/MS analysis is followed by the interpretation of these spectra for pyrene ozonation products. Products were silylated by BSTFA + 1% TMCs and heated at 100°C for 20 min prior to GC/MS analysis. The silylation reagent converts -OH or -COOH functional groups to $-OSiMe_3$ or $-COOSiMe_3$.

Pyrene mass spectrum from library search in NIST MS Search (ver 1.5, 1996) is illustrated to allow the comparison of the raw mass spectrum obtained from GC/MS analysis with published spectra. The commercial compound, 4-carboxy-5-phenanthrenecarboxyaldehyde (4-CPA, Sigma-Aldrich Library of Rare Chemicals, Milwaukee, WI), was prepared so as to compare its GC/MS spectra with that of compound III A/B. From the GC chromatographs and mass spectra of 4-CPA before and after TMS derivative was found to exist in two isomeric forms which were identical to those shown in compound III A/B.

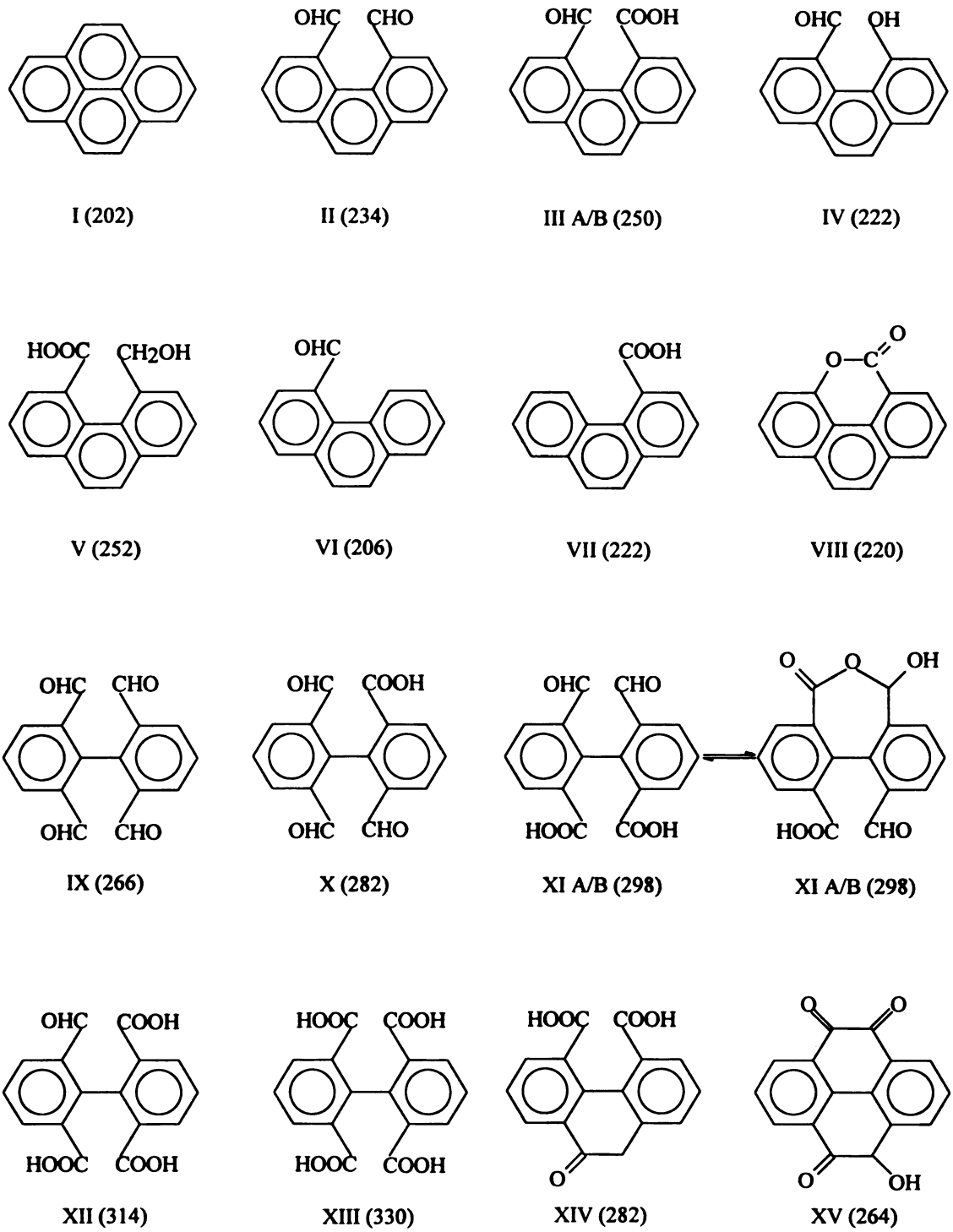


Figure B.1 Products formed from the ozonolysis of pyrene. Roman numbers correspond to the ID assigned to each product. Molecular weights are given in parentheses.

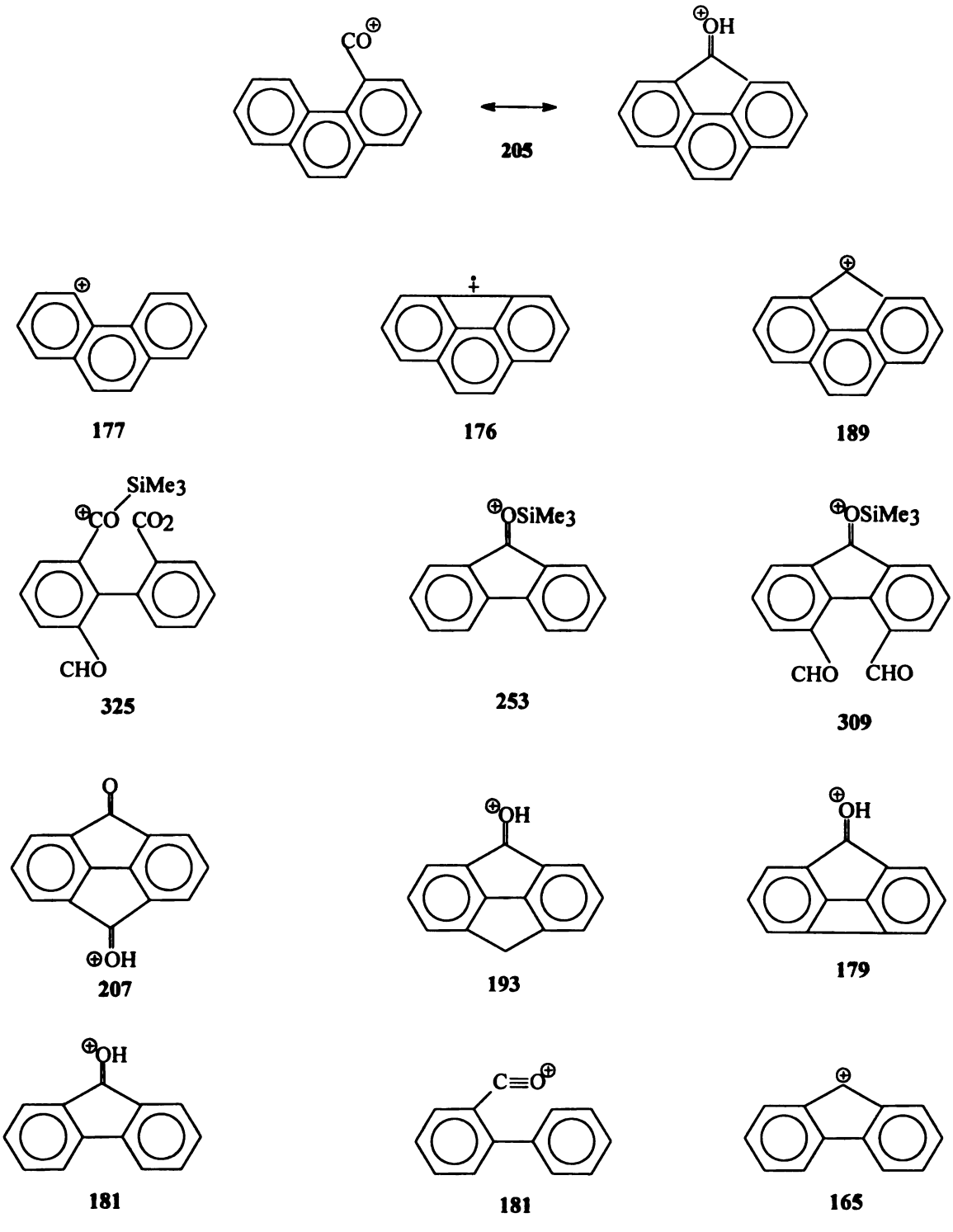
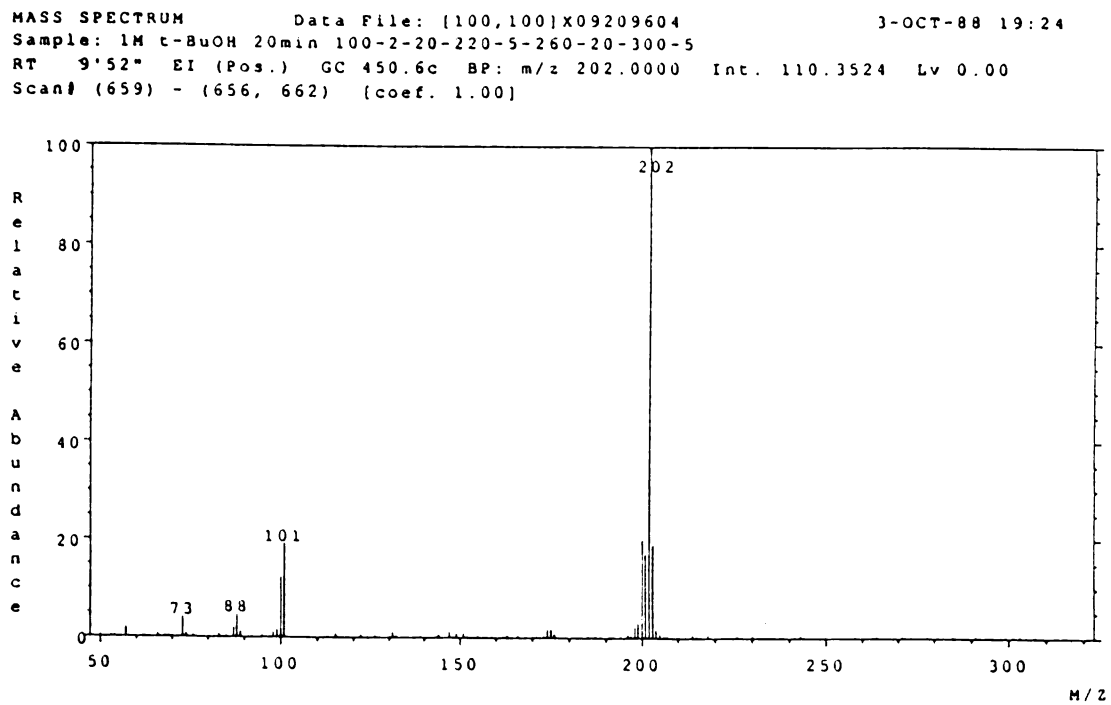
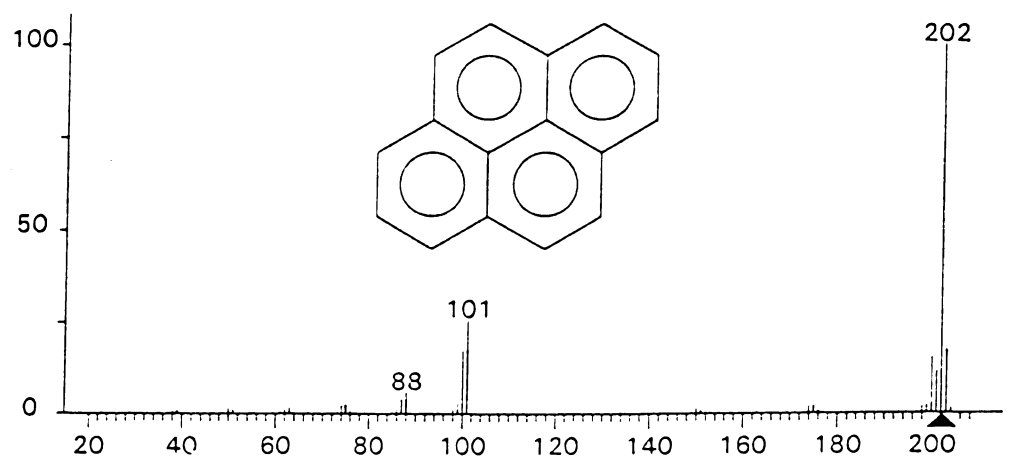


Figure B.2 Common ions of mass spectra of ozonation products formed from pyrene.

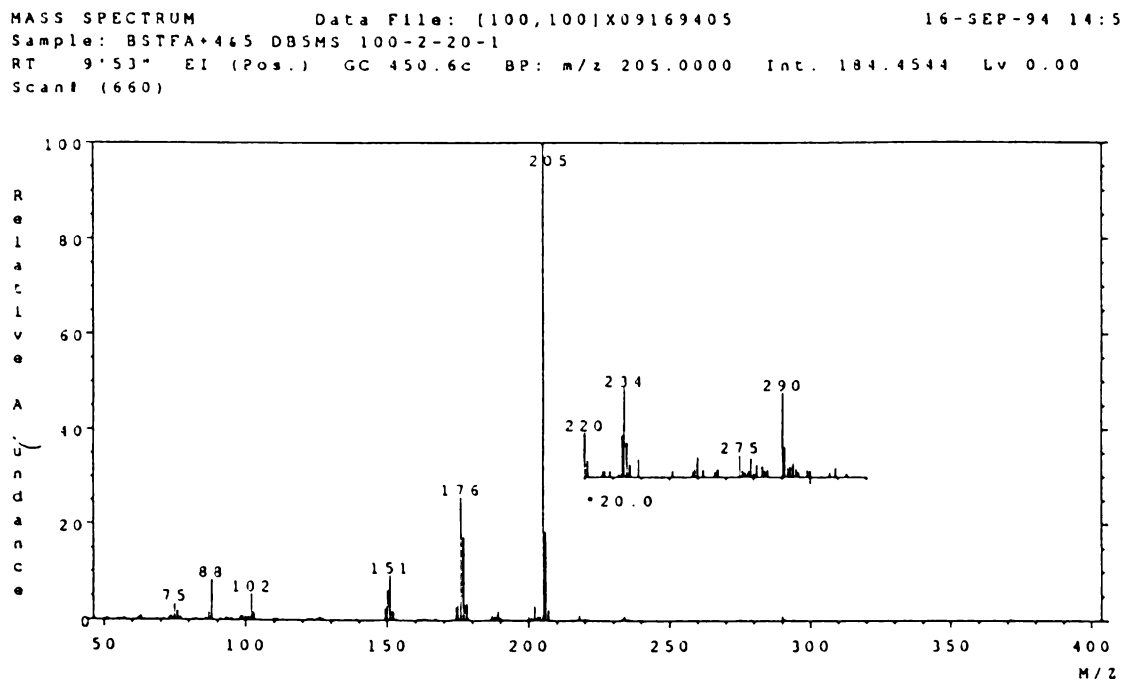
Mass Spectrum of pyrene (I)



Mass spectrum of pyrene obtain from NIST database (NIST#: 9063, CAS#: 129-00-0)



Mass Spectrum of the silylation product II



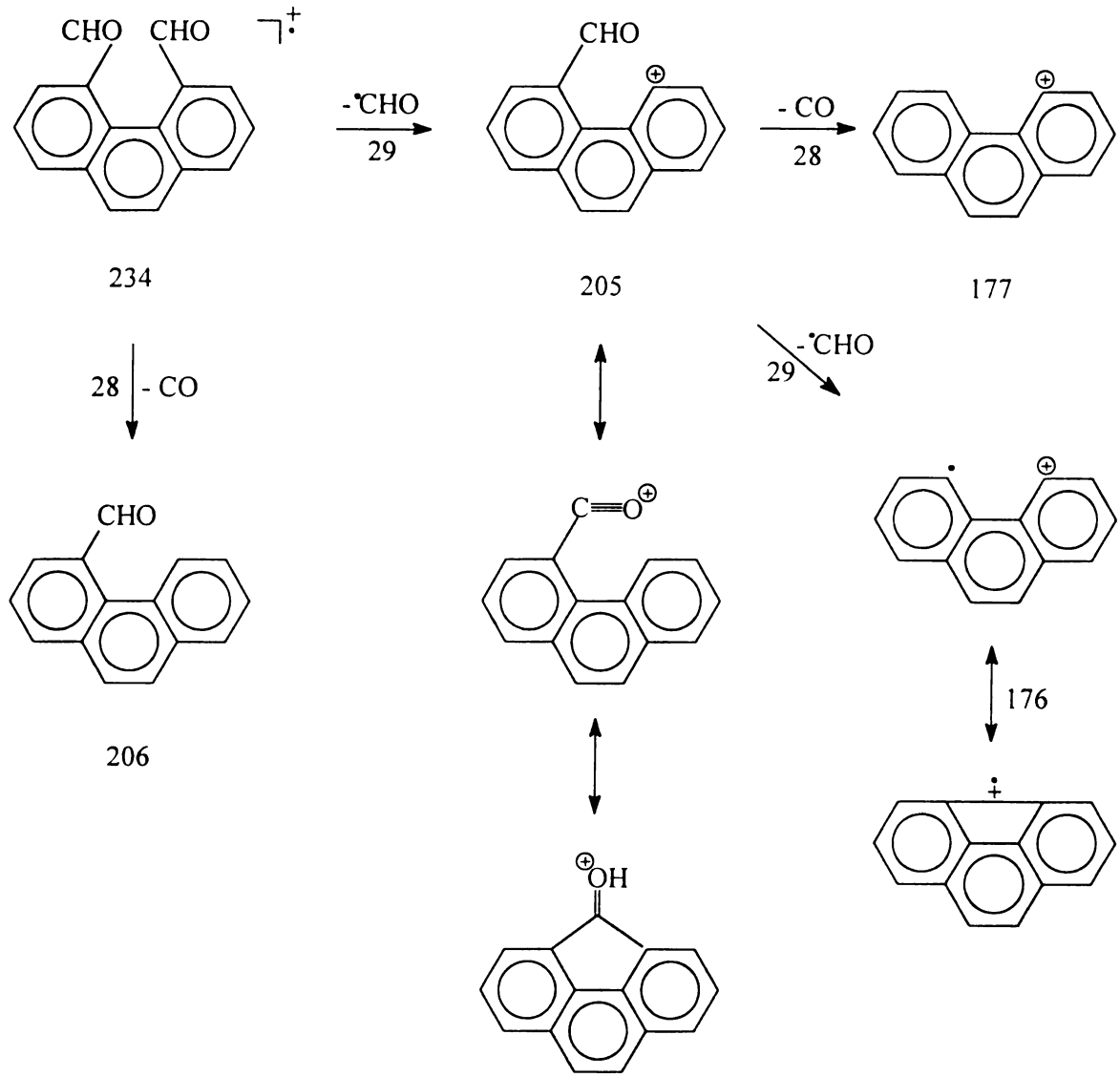
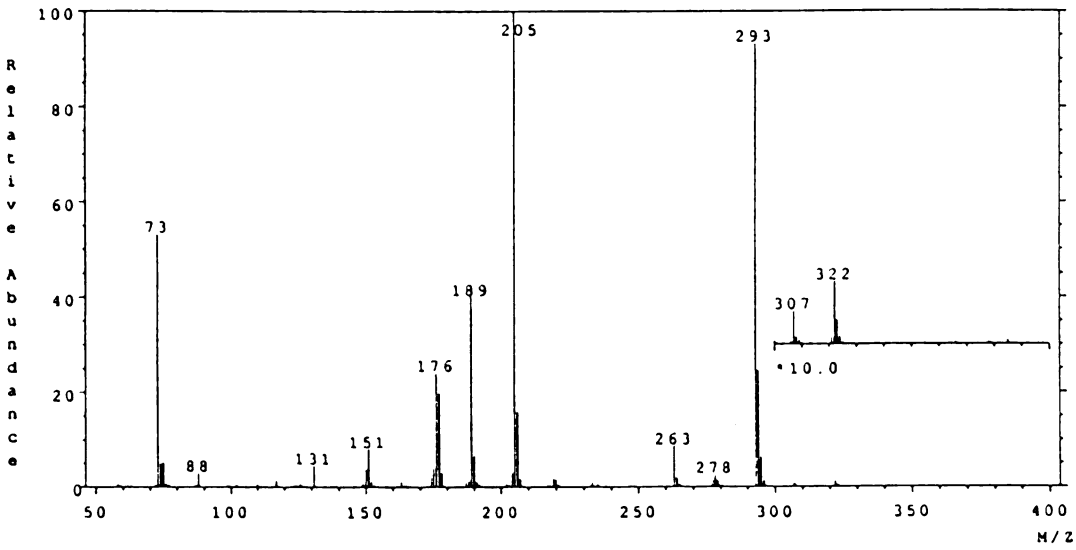


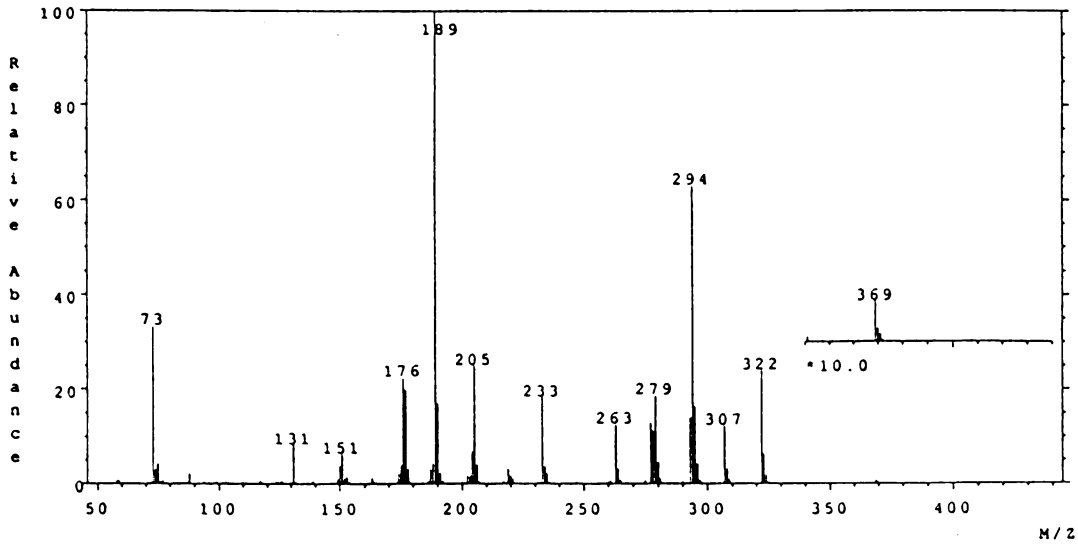
Figure B.3 Interpretation of the mass spectrum for the silylation product II. Molecular ion weight = 234.

Mass Spectra of the silylation product III A,B

MASS SPECTRUM Data File: [100,100]X09209612 3-OCT-88 22:27
Sample: in CH3CN 2min 100-2-20-220-5-260-20-300-5
RT 13'19" EI (Pos.) GC 450.6c BP: m/z 205.0000 Int. 1200.2550 Lv 0.00
Scan# (890) - (880, 892) [coef. 1.00]



MASS SPECTRUM Data File: [100,100]X09209612 3-OCT-88 22:27
Sample: in CH3CN 2min 100-2-20-220-5-260-20-300-5
RT 14'12" EI (Pos.) GC 450.6c BP: m/z 189.0000 Int. 1104.9910 Lv 0.00
Scan# (949) - (937, 951) [coef. 1.00]



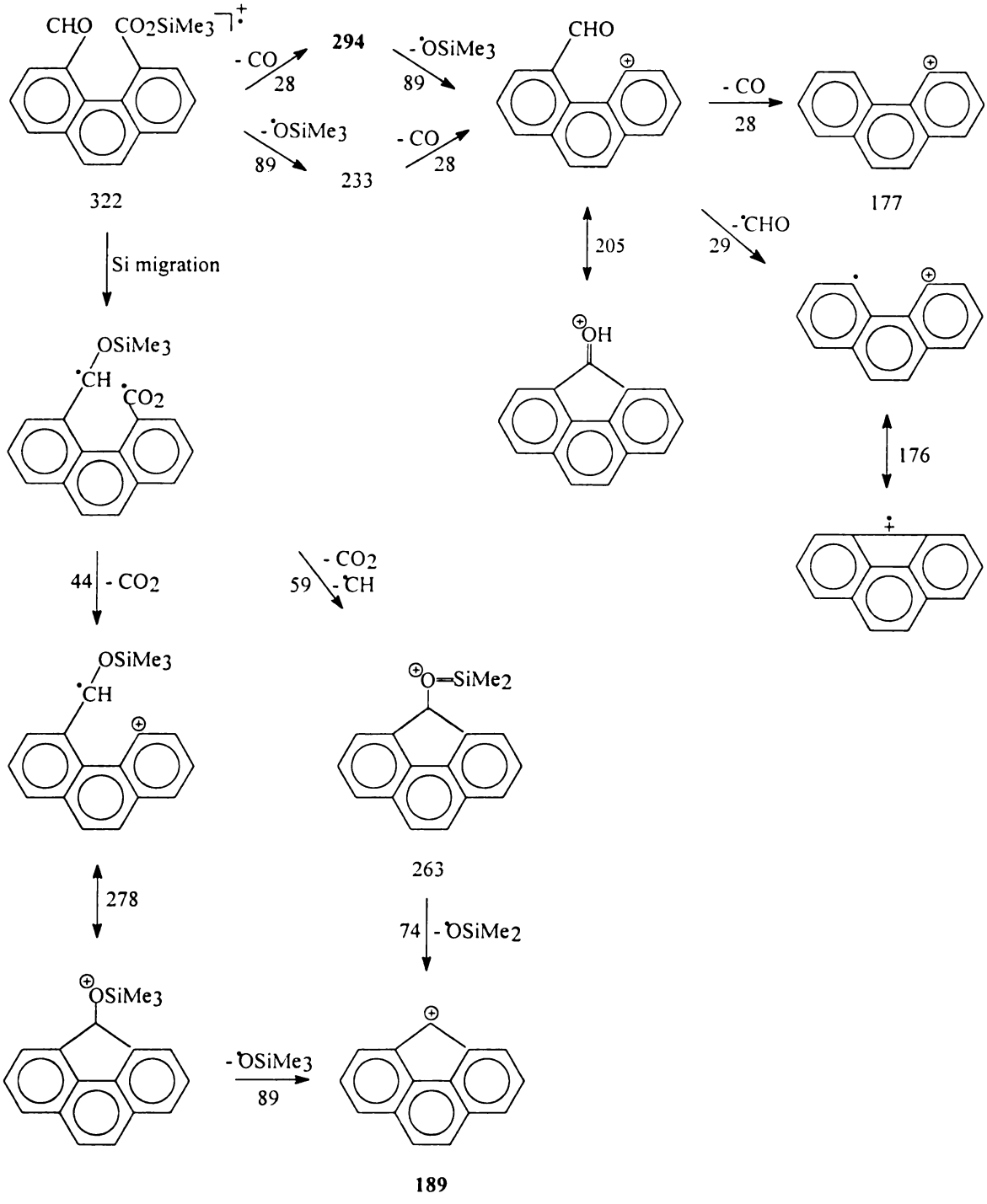
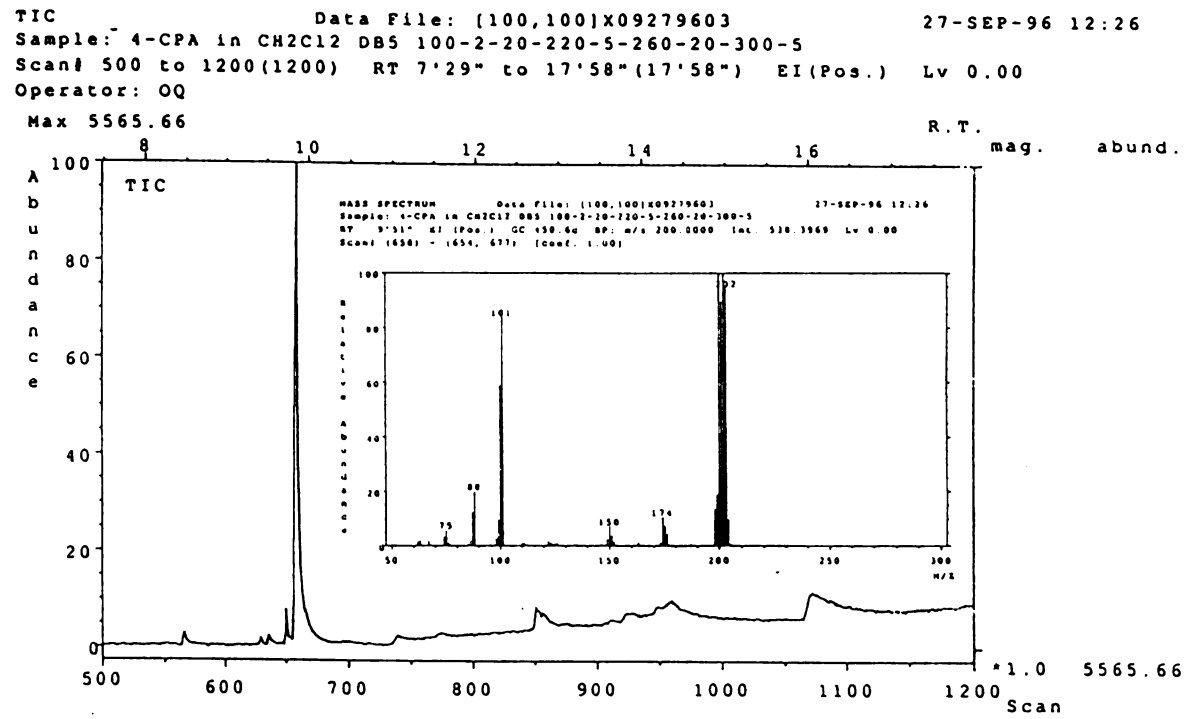
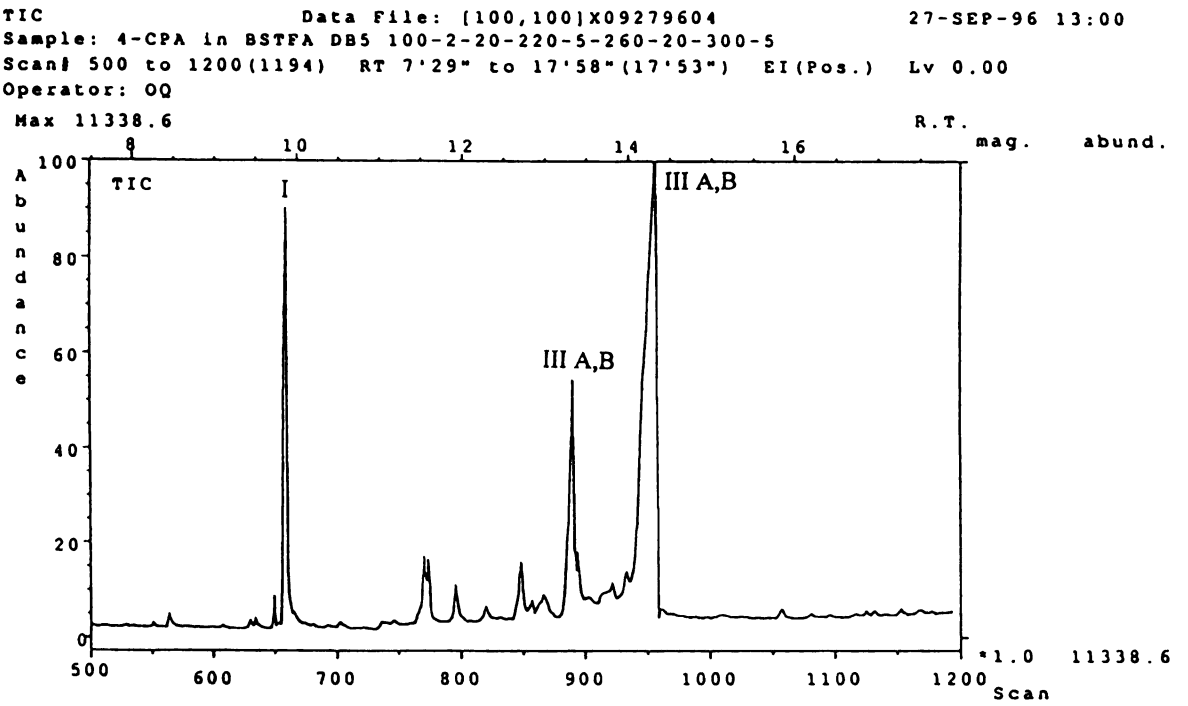


Figure B.4 Interpretation of the mass spectra for the silylation product III A,B. Molecular ion weight = 322.

GC chromatograph of 4-CPA in pure solvent. The insert shows the mass spectrum of the peak.

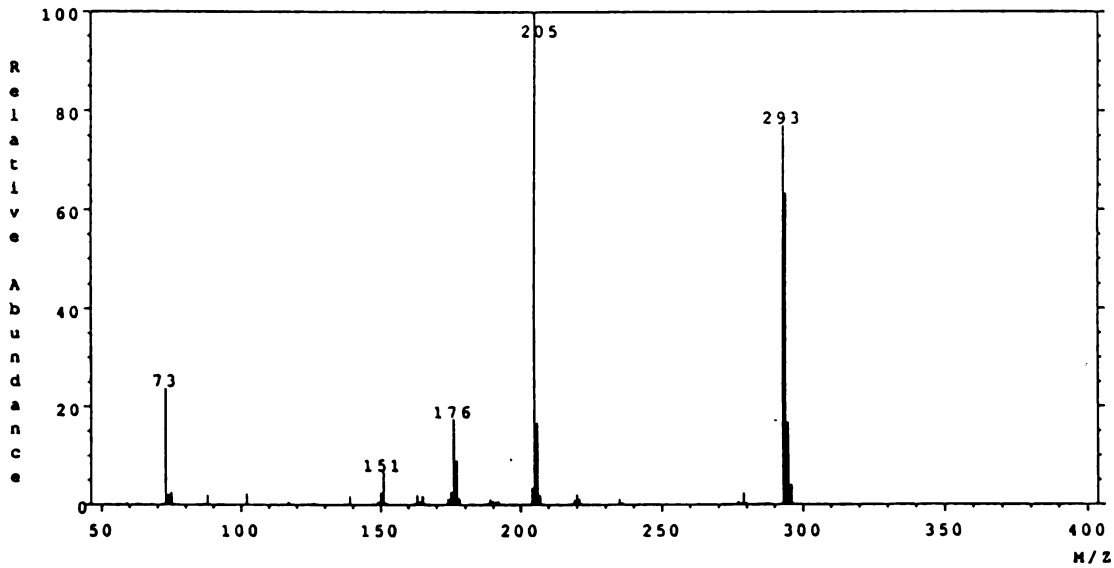


GC chromatograph of 4-CPA in silylation reagent



Mass Spectrum of the silylation product IV

MASS SPECTRUM Data File: [100,100]X09209604 3-OCT-88 19:24
Sample: 1M t-BuOH 20min 100-2-20-220-5-260-20-300-5
RT 10'30" EI (Pos.) GC 450.6c BP: m/z 205.0000 Int. 712.9849 Lv 0.00
Scan# (701) - (695, 703) [coef. 1.00]



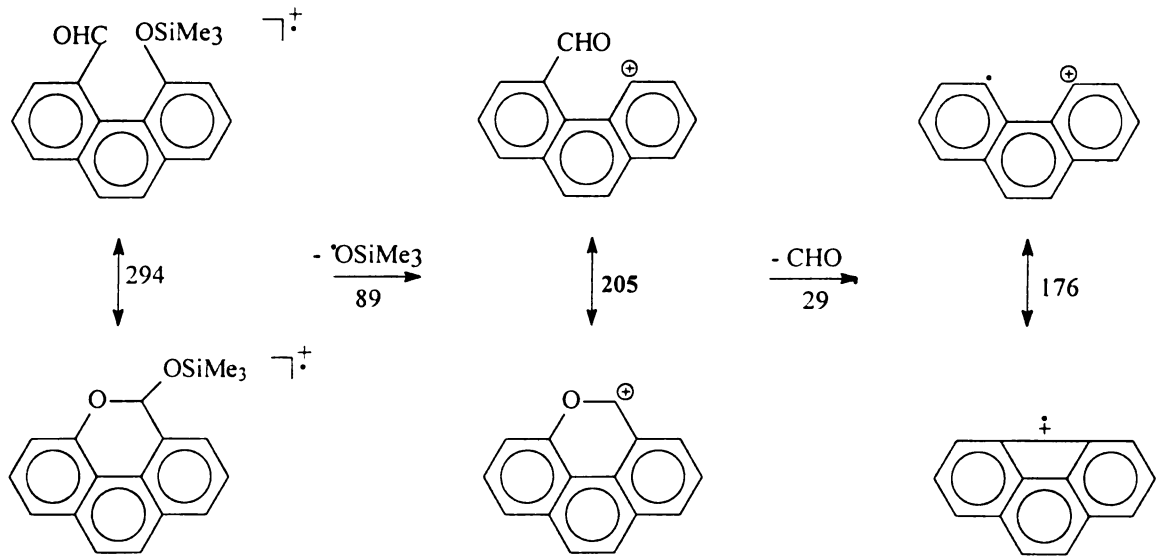
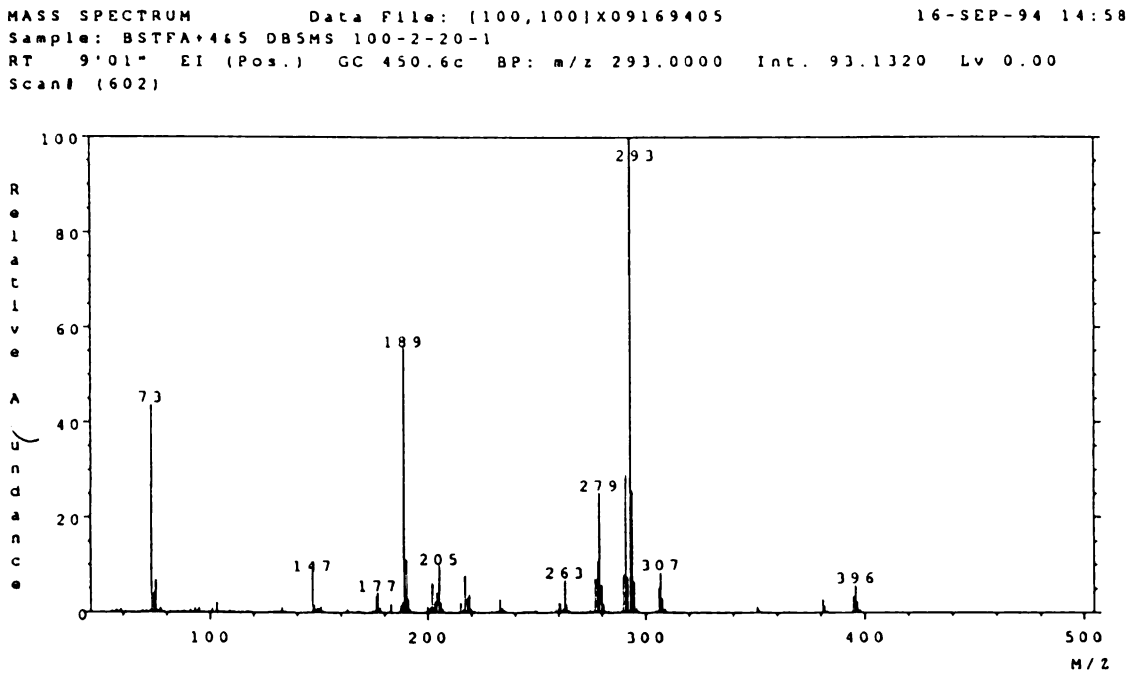


Figure B.5 Interpretation of the mass spectrum for the silylation product IV. Molecular ion weight = 294.

Mass Spectrum of the silylation product V



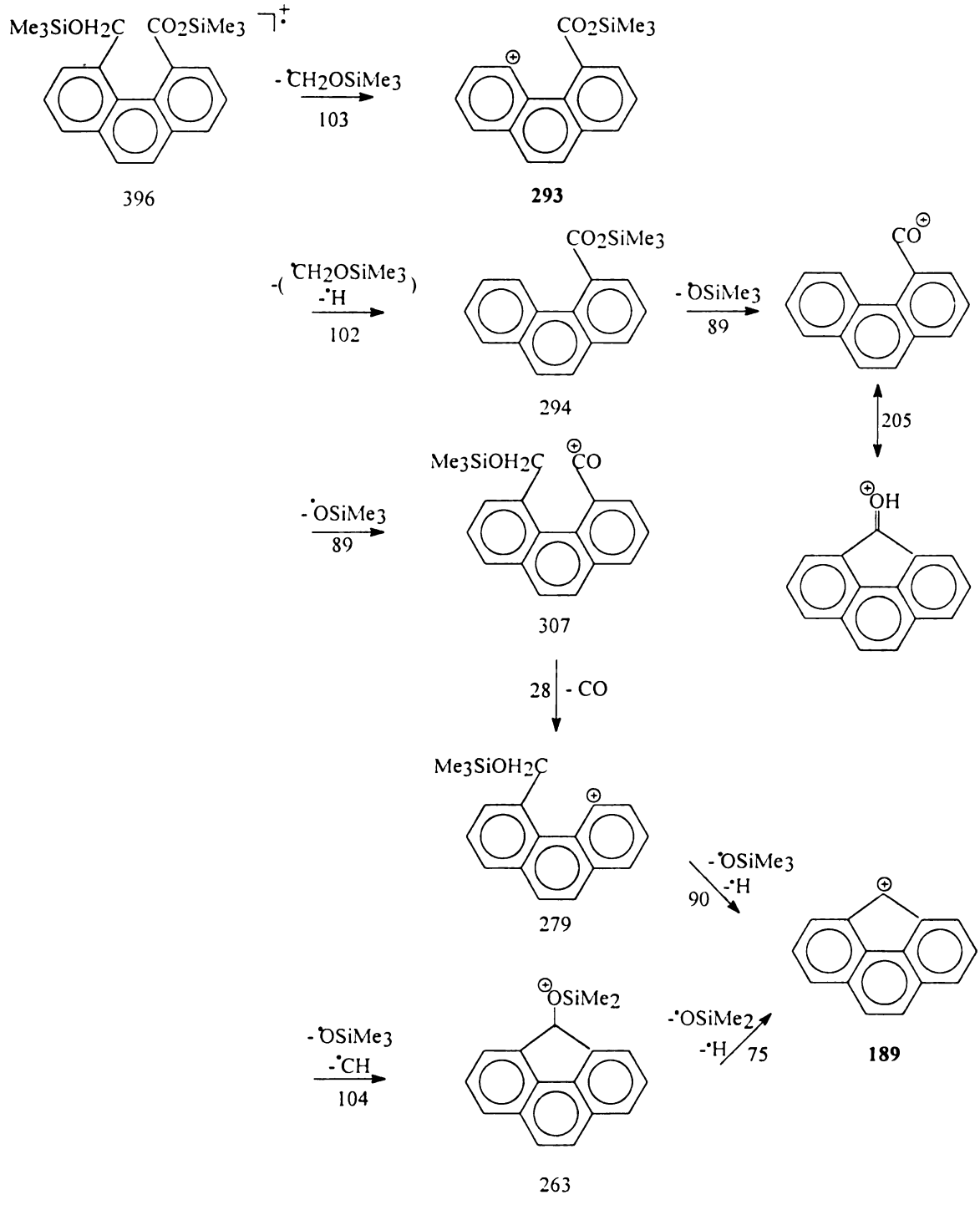
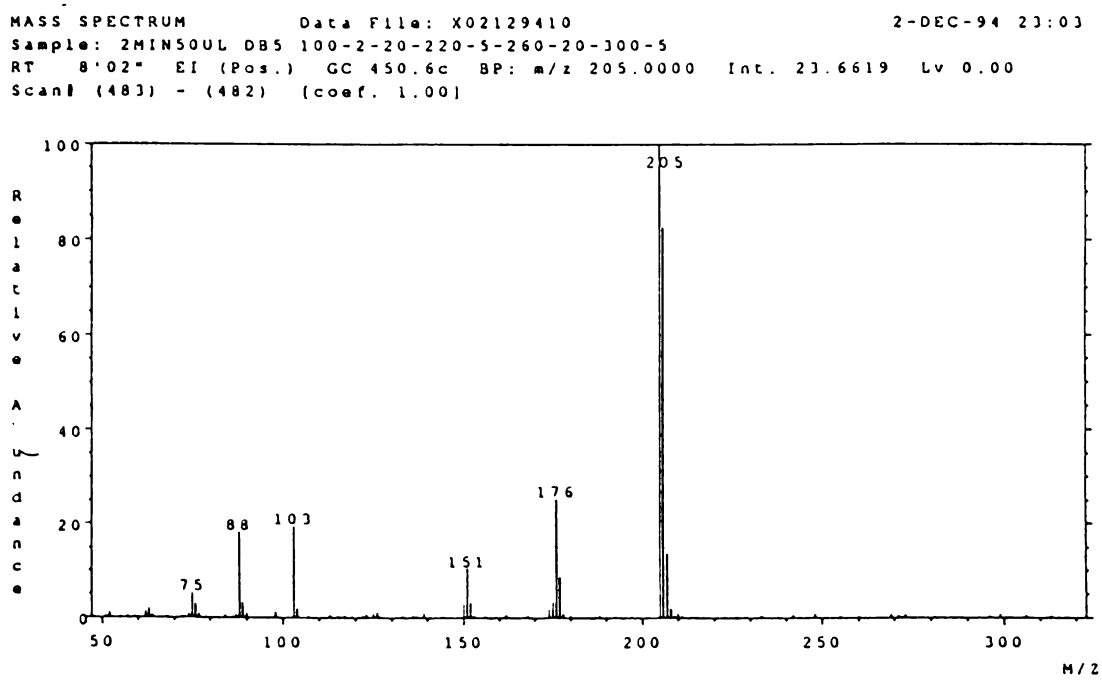


Figure B.6 Interpretation of the mass spectrum for the silylation product V. Molecular ion weight = 396.

Mass Spectrum of the silylation product VI



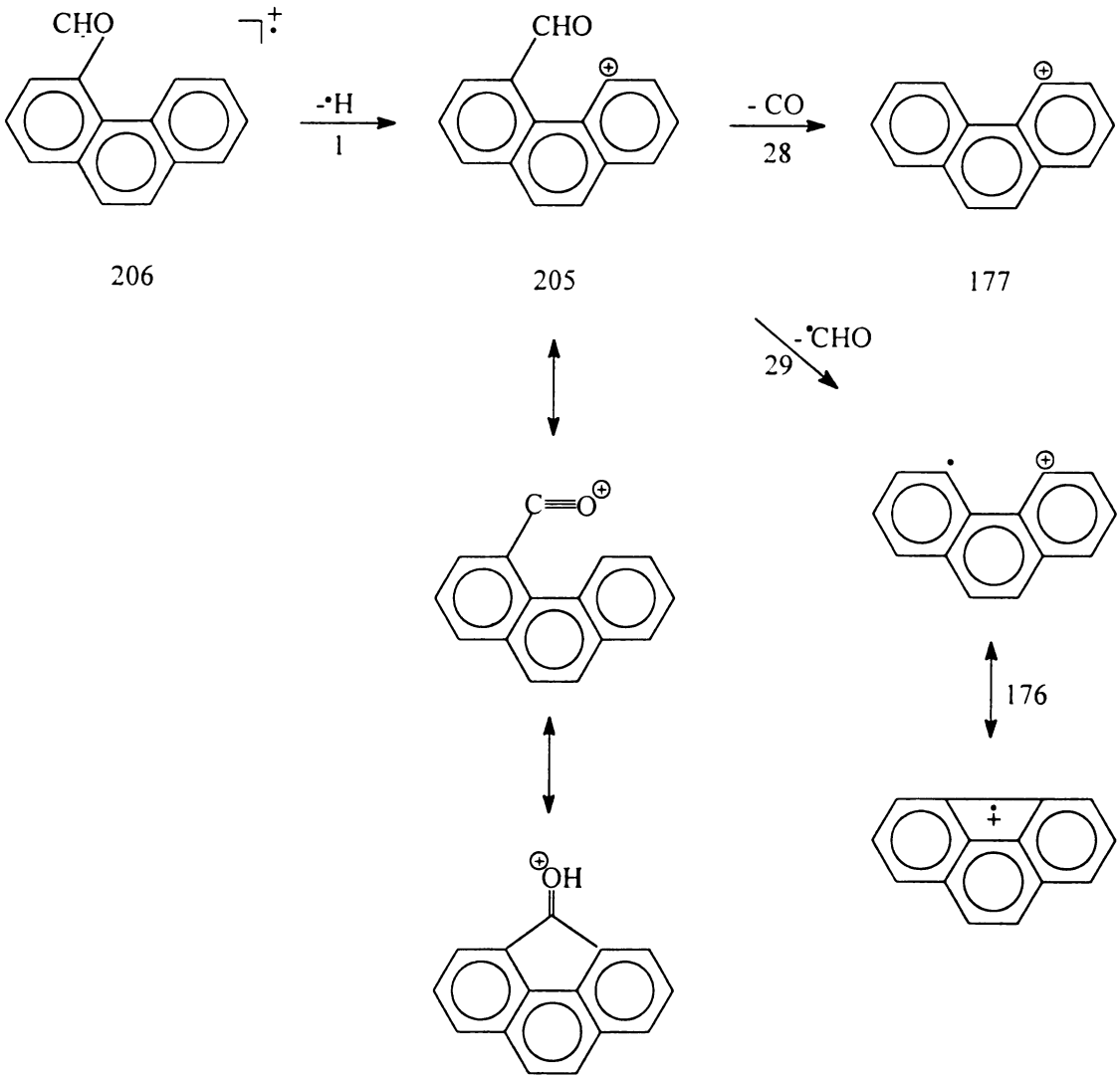
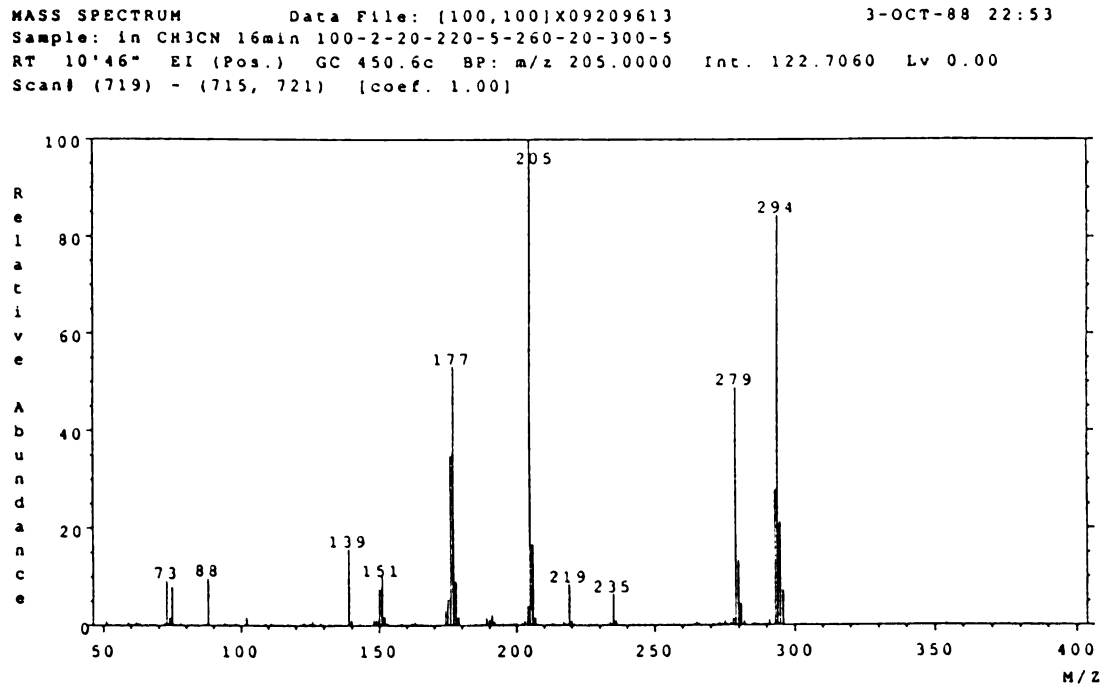


Figure B.7 Interpretation of the mass spectrum for the silylation product VI. Molecular ion weight = 206.

Mass Spectrum of the silylation product VII



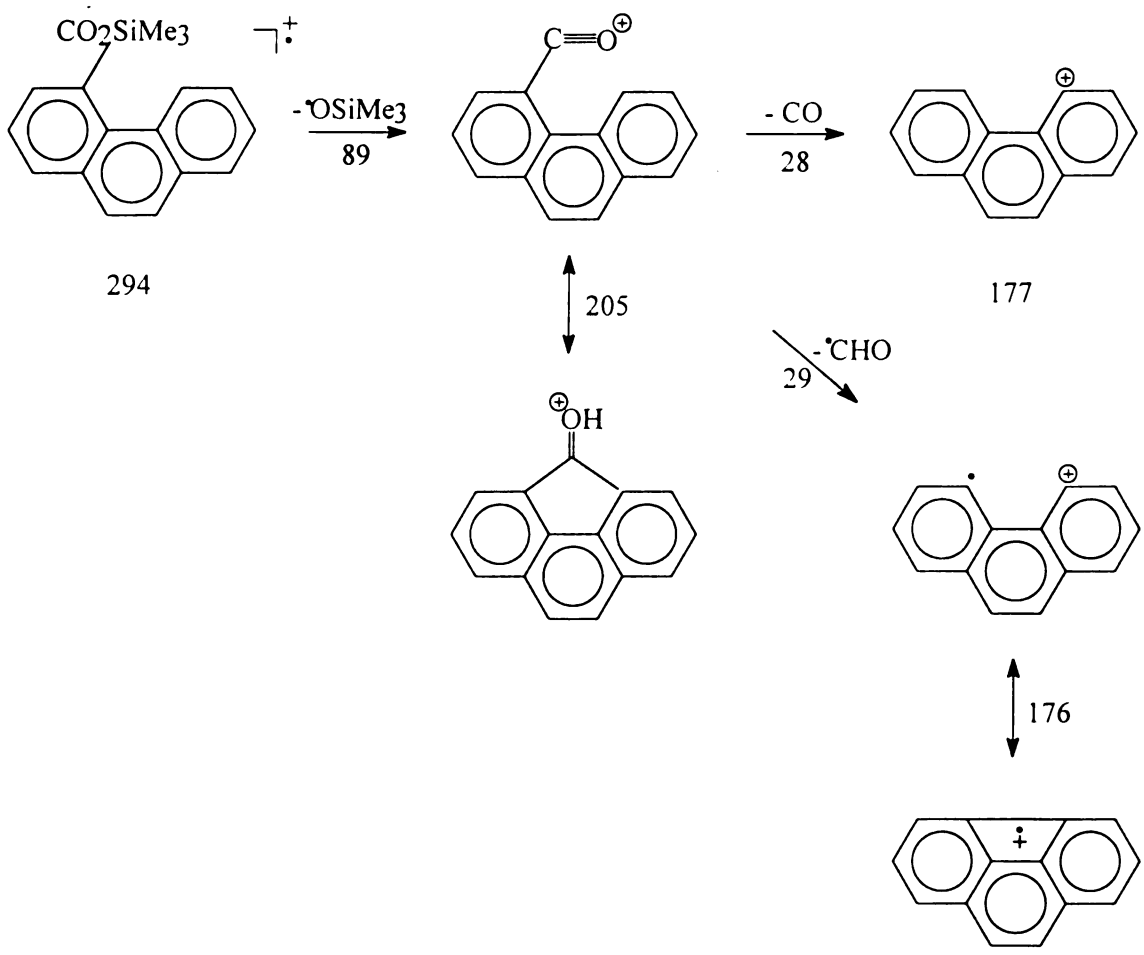
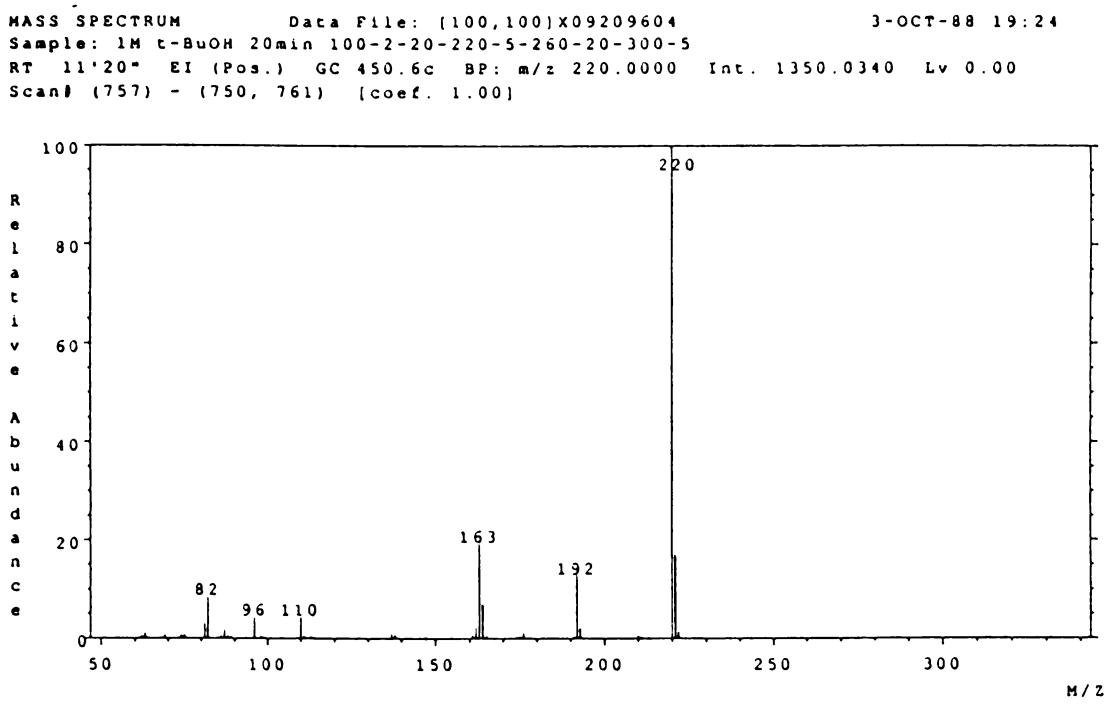


Figure B.8 Interpretation of the mass spectrum for the silylation product VII. Molecular ion weight = 294.

Mass Spectrum of the silylation product VIII



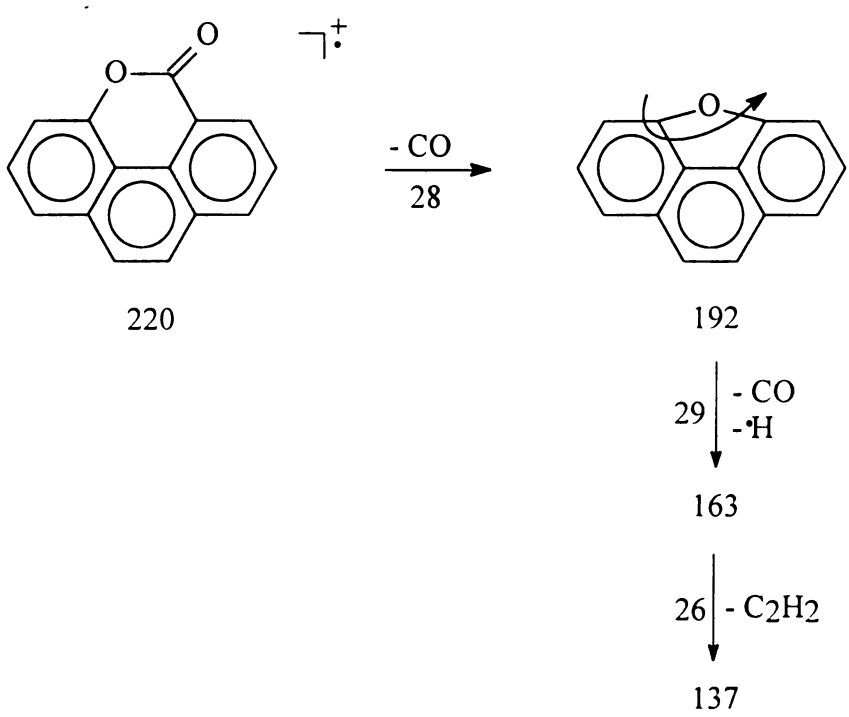
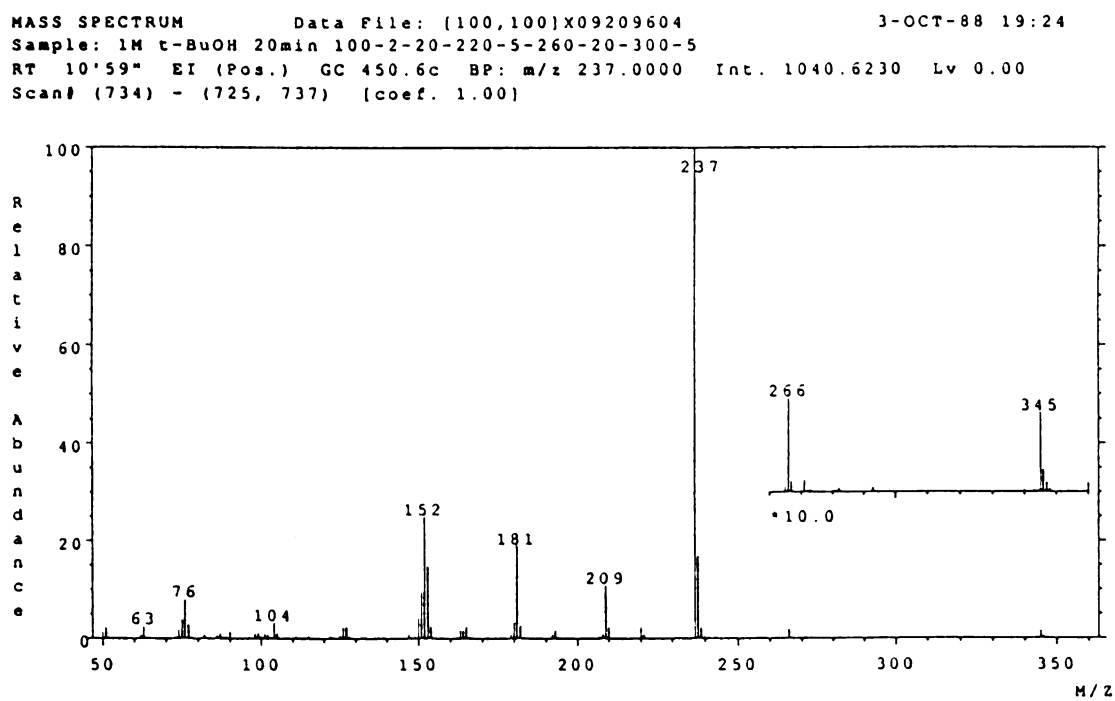


Figure B.9 Interpretation of the mass spectrum for the silylation product VIII. Molecular ion weight = 220.

Mass Spectrum of the silylation product IX



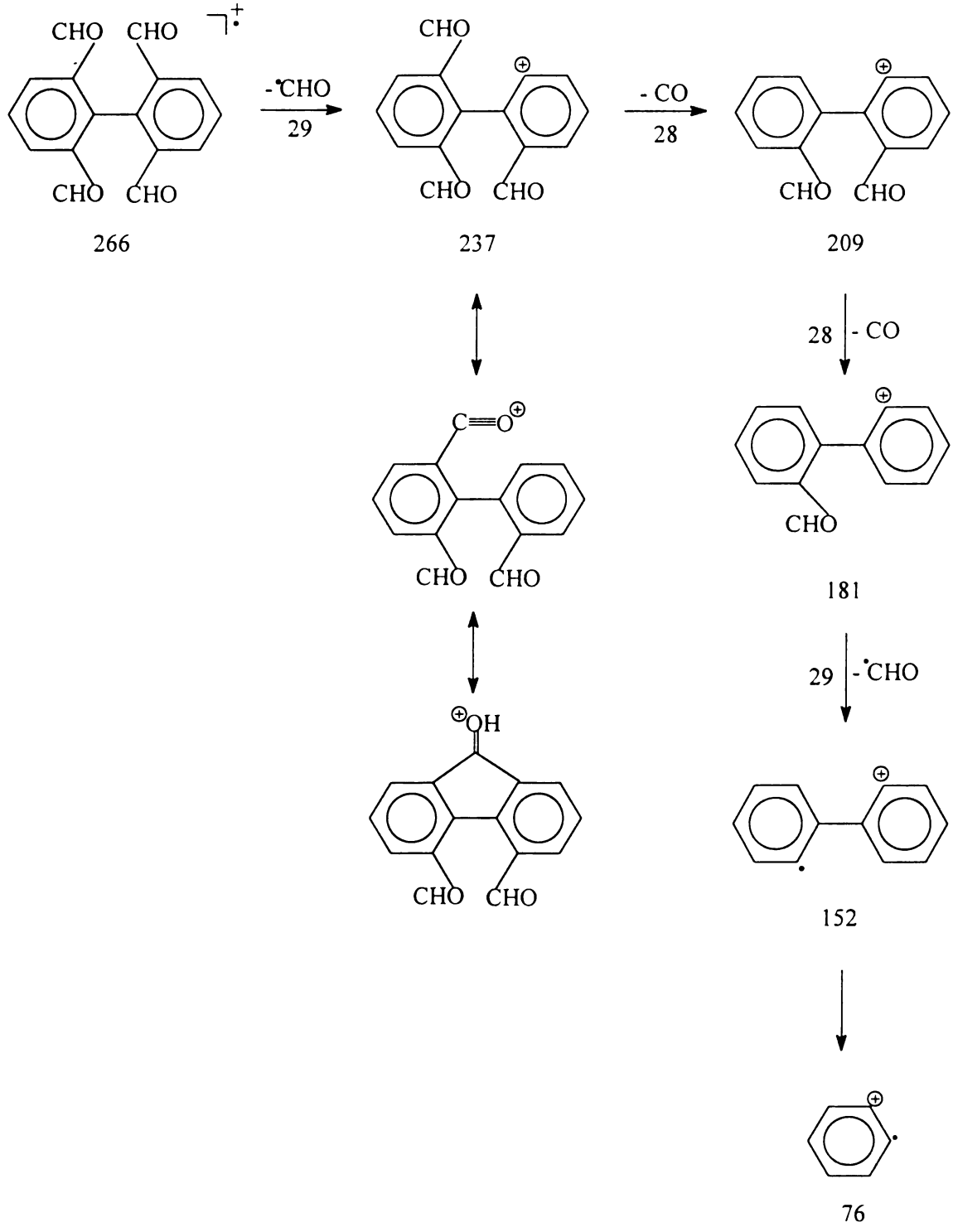
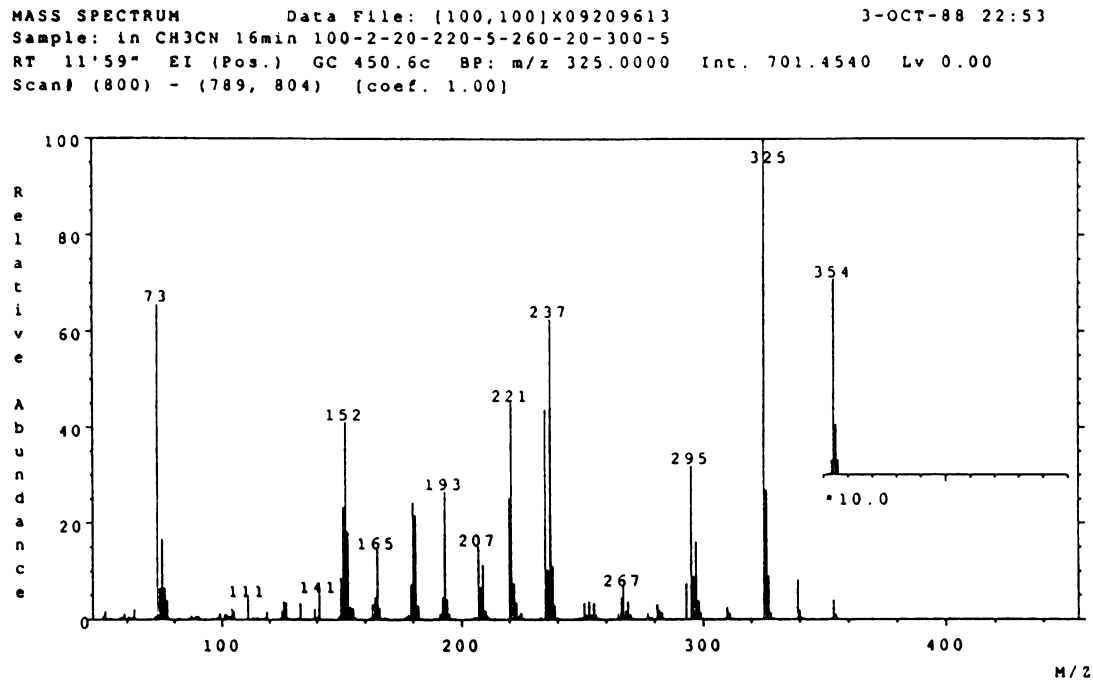


Figure B.10 Interpretation of the mass spectrum for the silylation product IX. Molecular ion weight = 266.

Mass Spectrum of the silylation product X



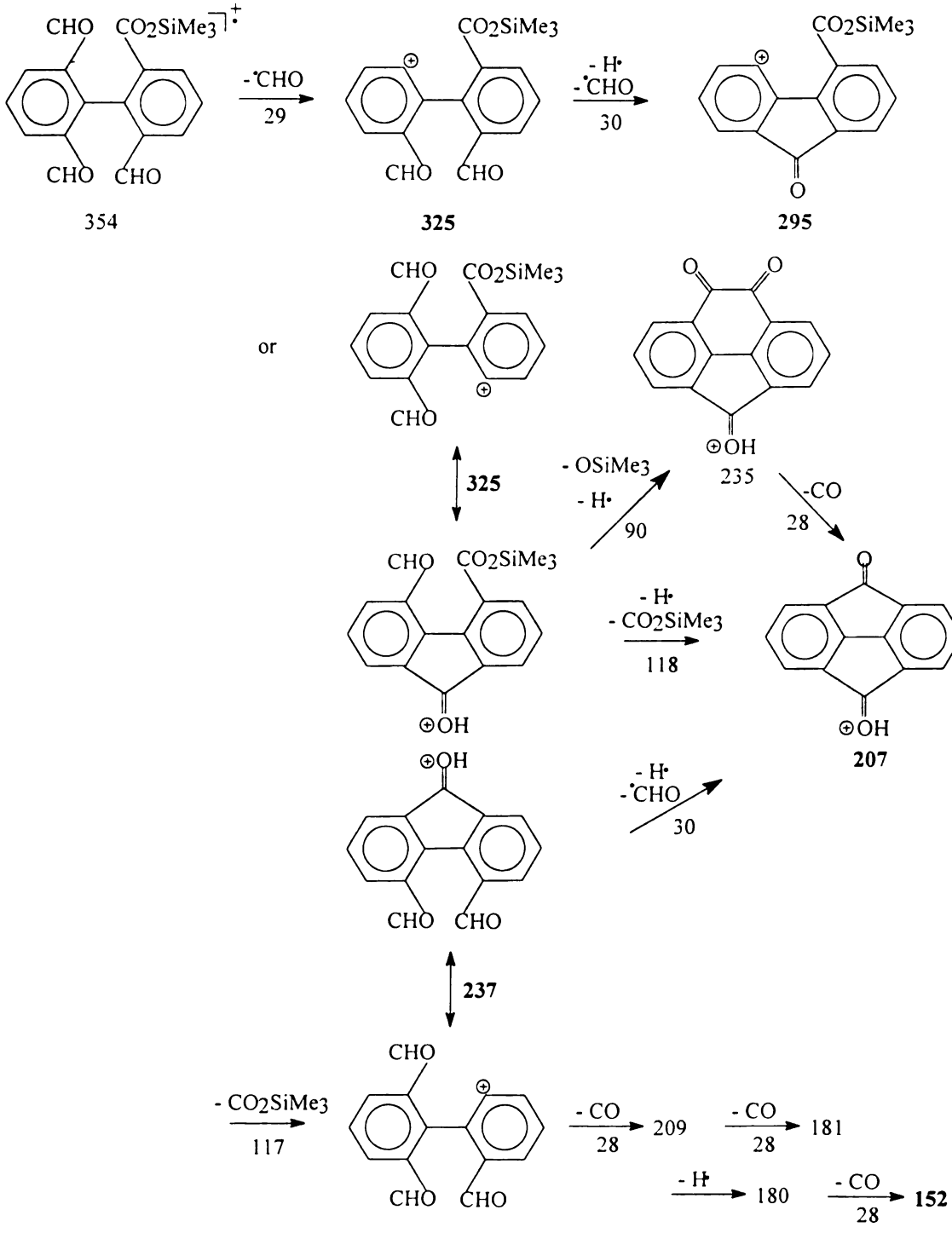


Figure B.11a Interpretation of the mass spectrum for the silylation product X. Molecular ion weight = 354.

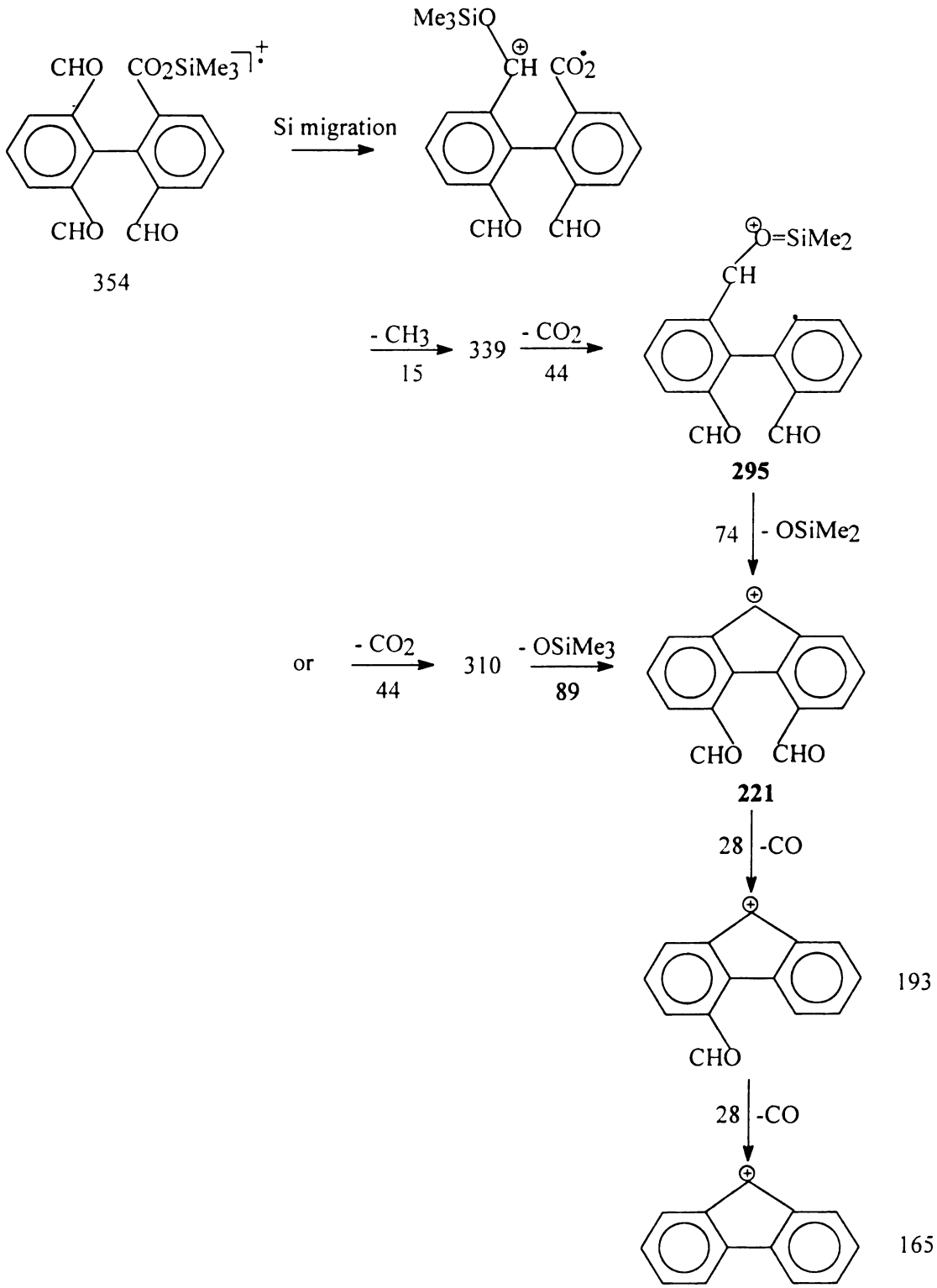
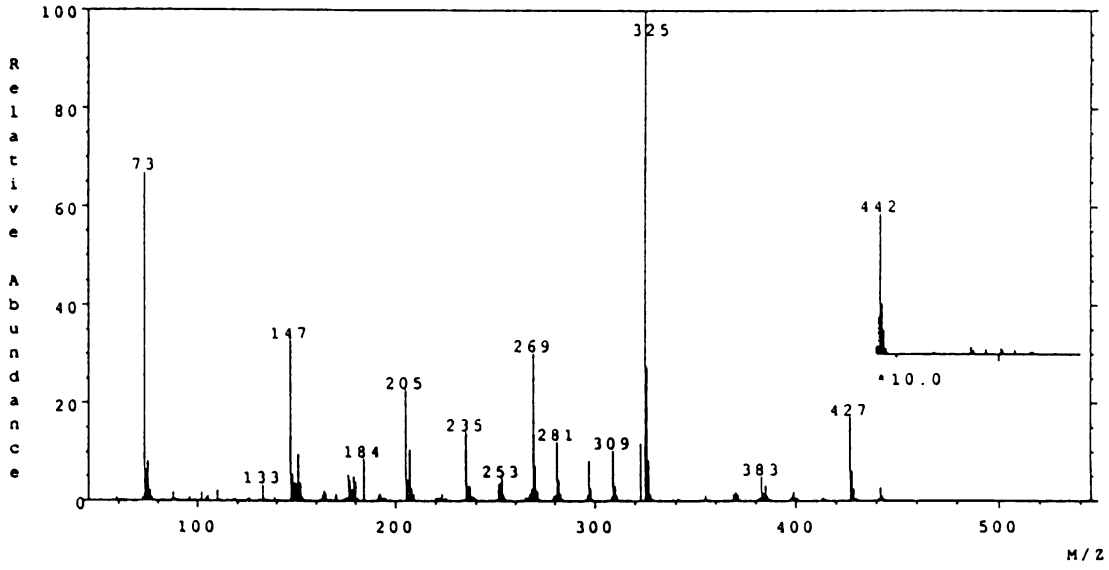


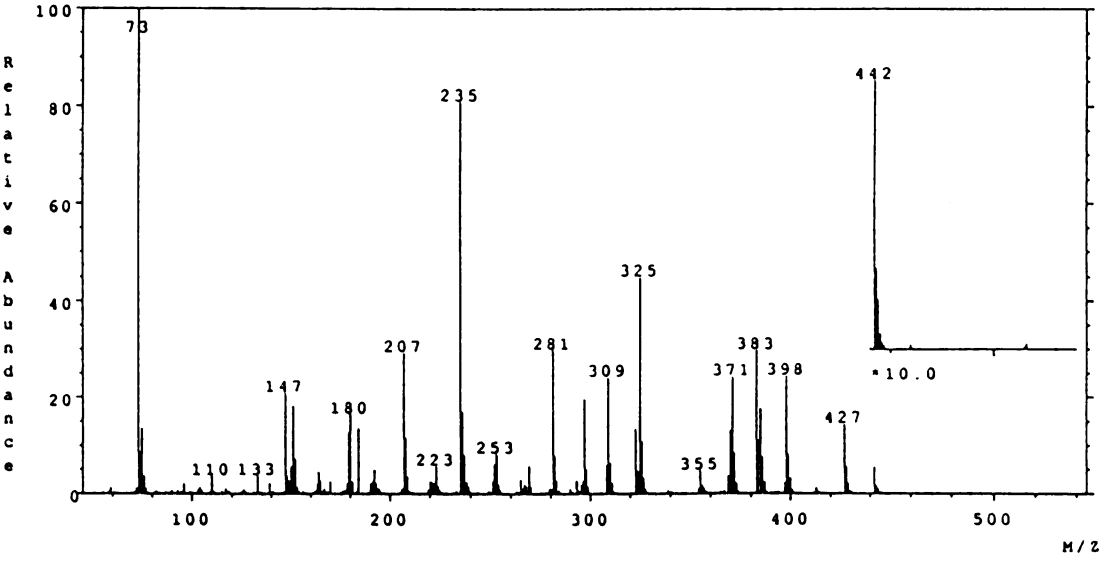
Figure B.11b Interpretation of the mass spectrum for the silylation product X with $SiMe_3$ migration during GC/MS analysis. Molecular ion weight = 354.

Mass Spectra of the silylation product XI A,B

MASS SPECTRUM Data File: [100,100]X09209604 3-OCT-88 19:24
Sample: 1M t-BuOH 20min 100-2-20-220-5-260-20-300-5
RT 12'46" EI (Pos.) GC 450.6c BP: m/z 325.0000 Int. 932.6188 Lv 0.00
Scan# (853) - (843, 857) [coef. 1.00]



MASS SPECTRUM Data File: [100,100]X09209604 3-OCT-88 19:24
Sample: 1M t-BuOH 20min 100-2-20-220-5-260-20-300-5
RT 13'02" EI (Pos.) GC 450.6c BP: m/z 73.0000 Int. 457.0286 Lv 0.00
Scan# (871) - (863, 874) [coef. 1.00]



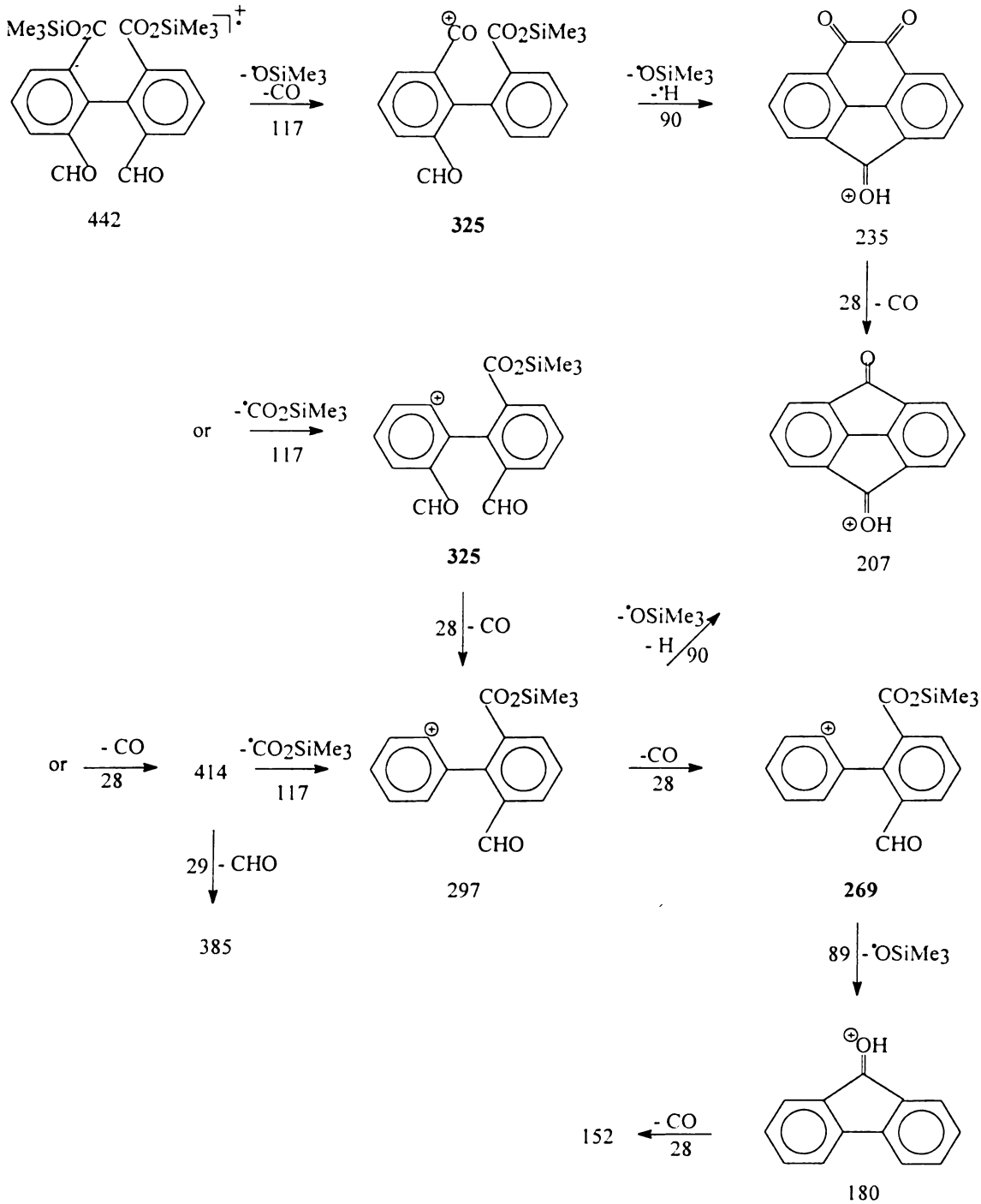


Figure B.12a Interpretation of the mass spectrum for the silylation product XI A,B. Molecular ion weight = 442.

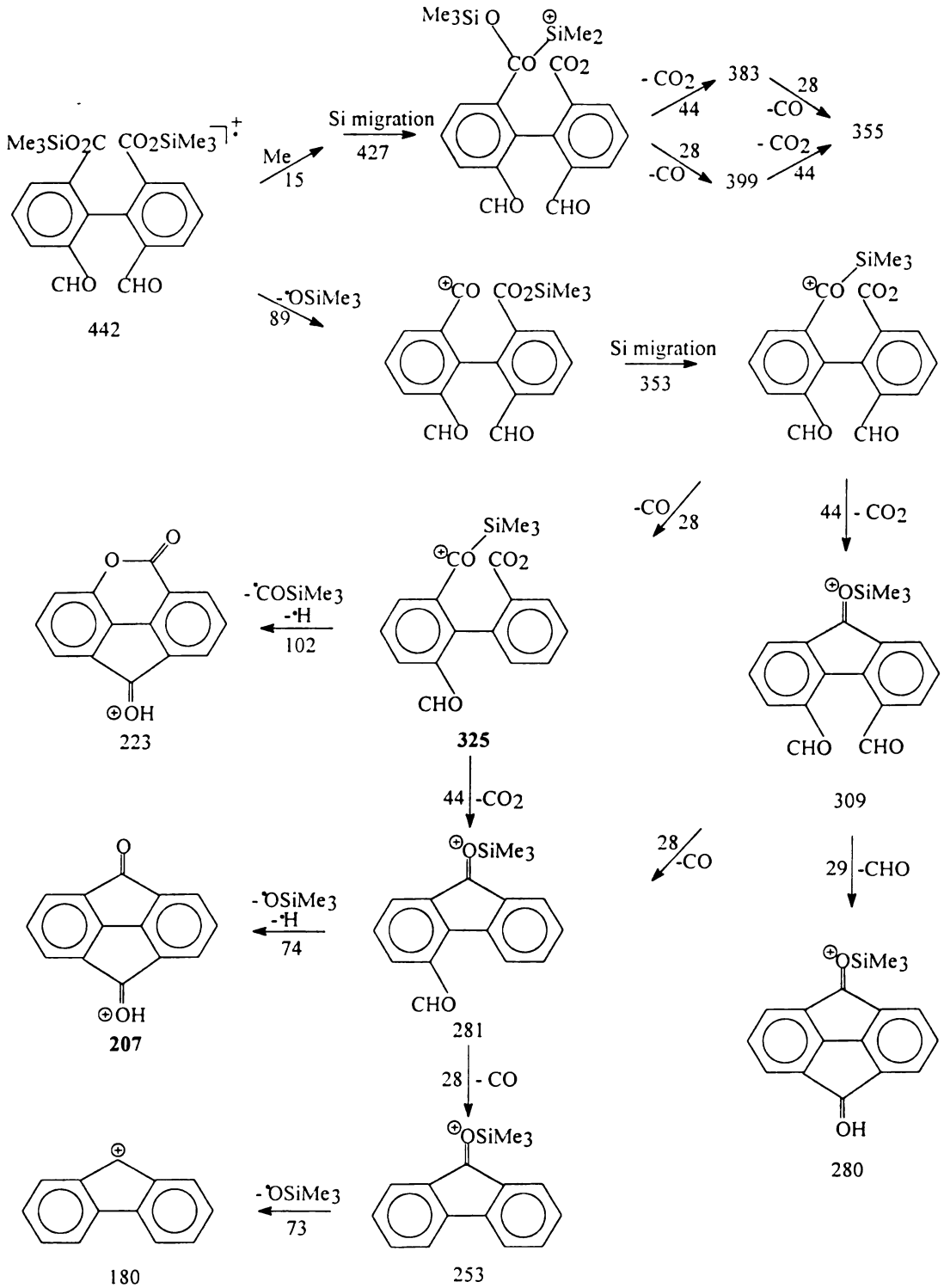


Figure B.12b Interpretation of the mass spectrum for the silylation product XI A,B with $SiMe_3$ migration during GC/MS analysis. Molecular ion weight = 442.

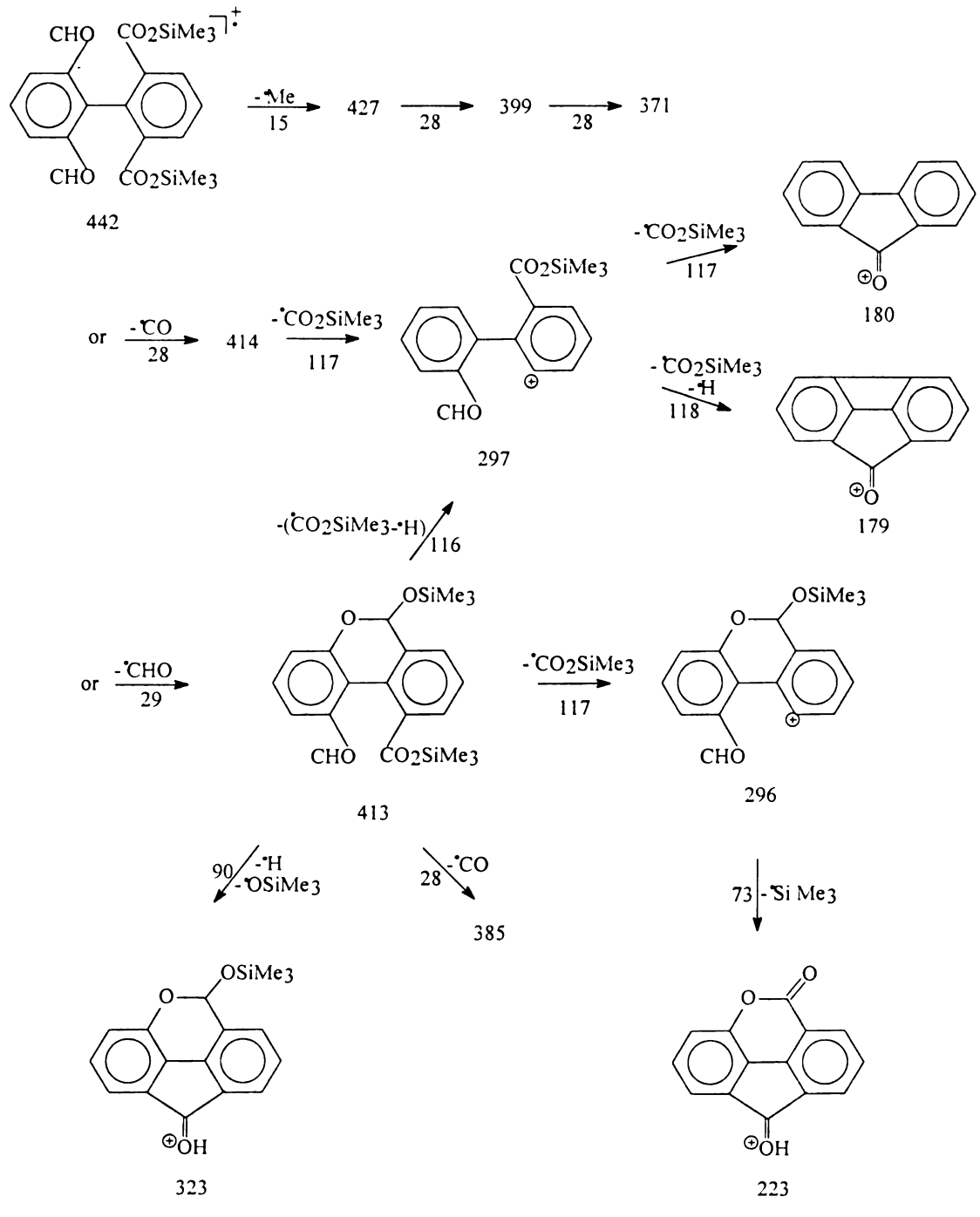


Figure B.12c Interpretation of the mass spectrum for the silylation product XI A,B. Molecular ion weight = 442.

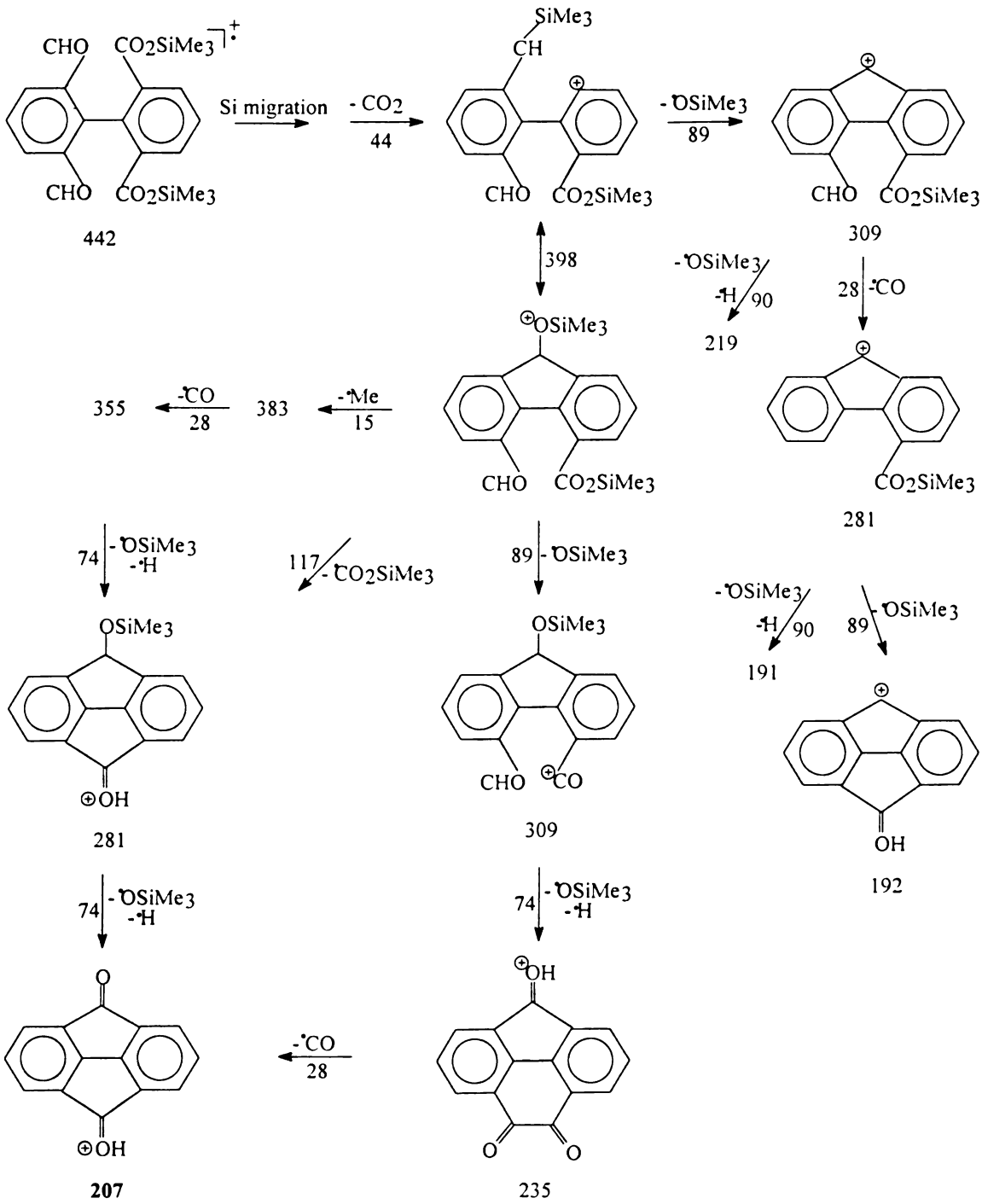
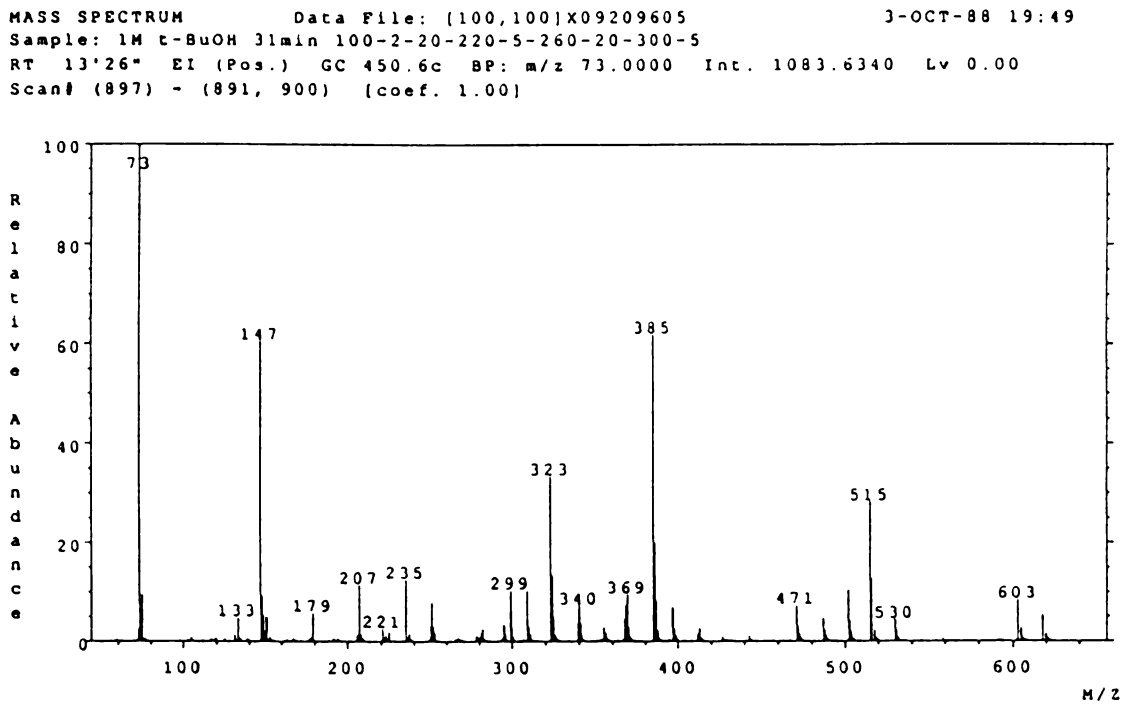


Figure B.12d Interpretation of the mass spectrum for the silylation product XI A,B with $SiMe_3$ migration during GC/MS analysis. Molecular ion weight = 442.

Mass Spectrum of the silylation product XII



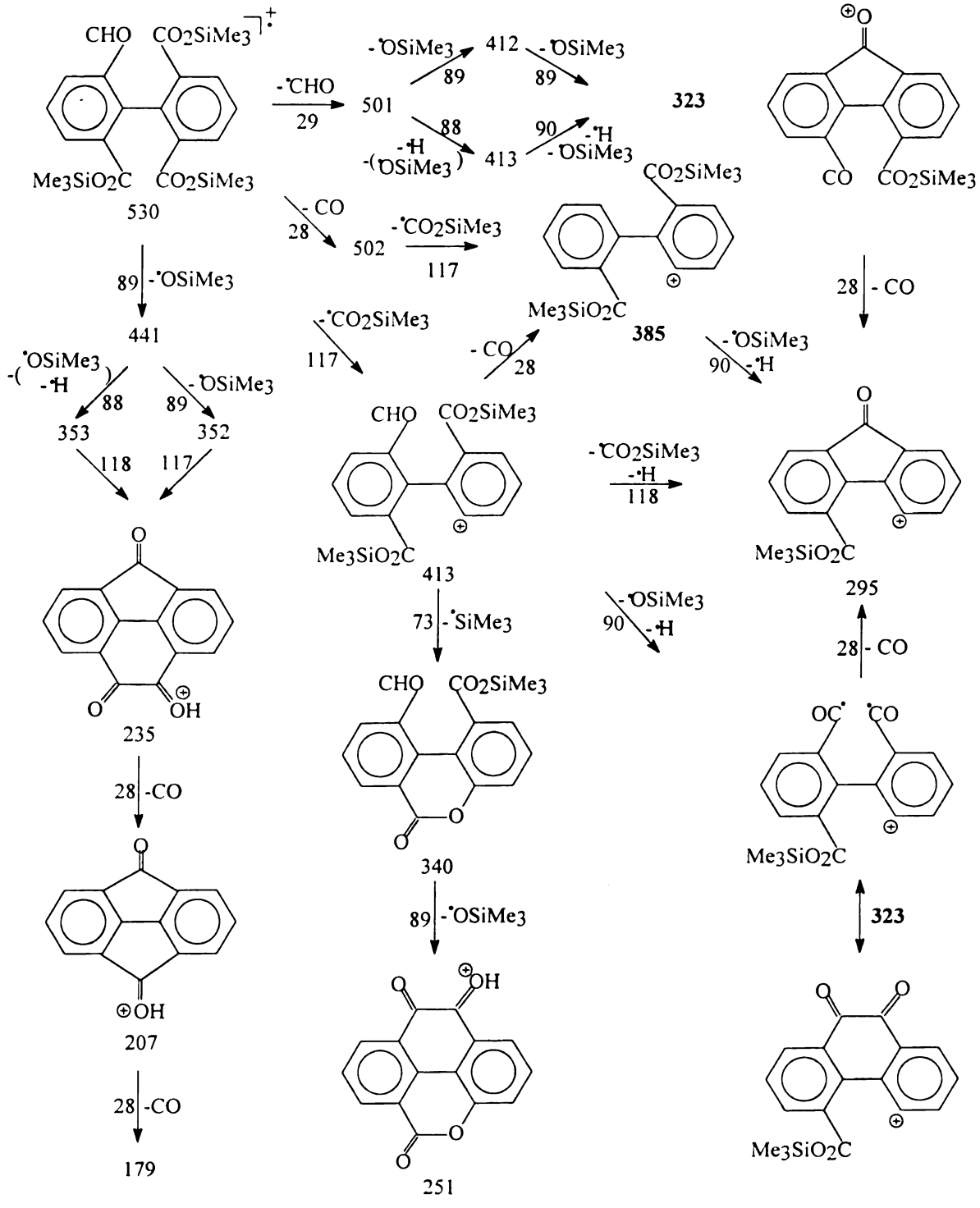


Figure B.13a Interpretation of the mass spectrum for the silylation product XII. Molecular ion weight = 530.

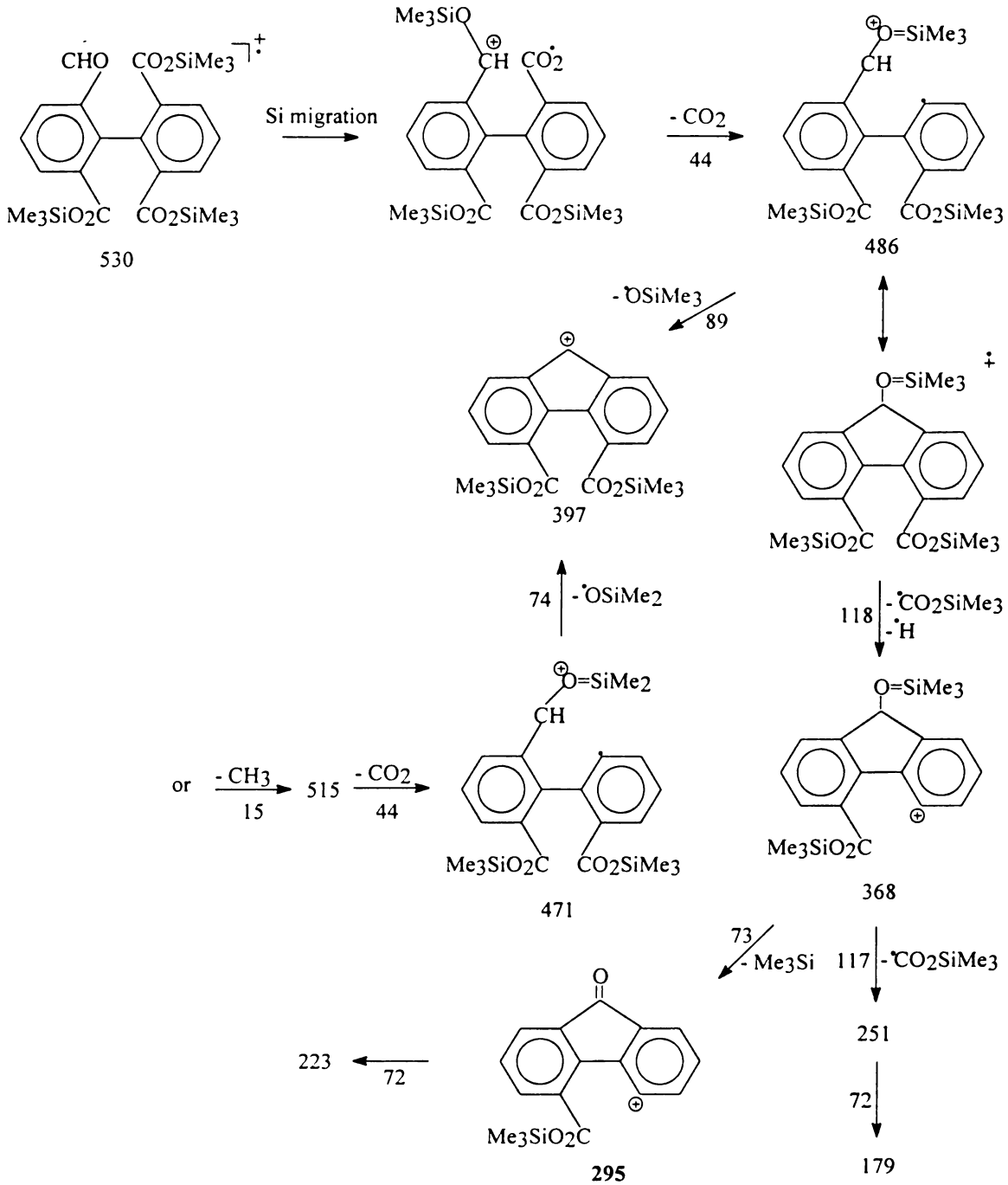
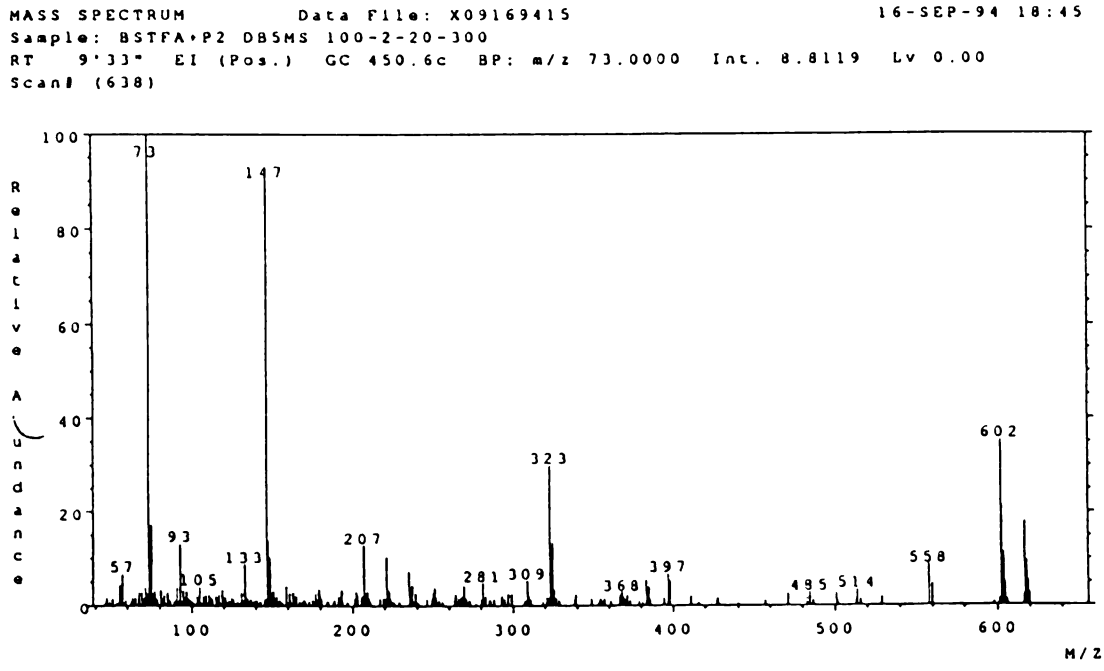


Figure B.13b Interpretation of the mass spectrum for the silylation product XII with $SiMe_3$ migration during GC/MS analysis. Molecular ion weight = 530.

Mass Spectrum of the silylation product XIII



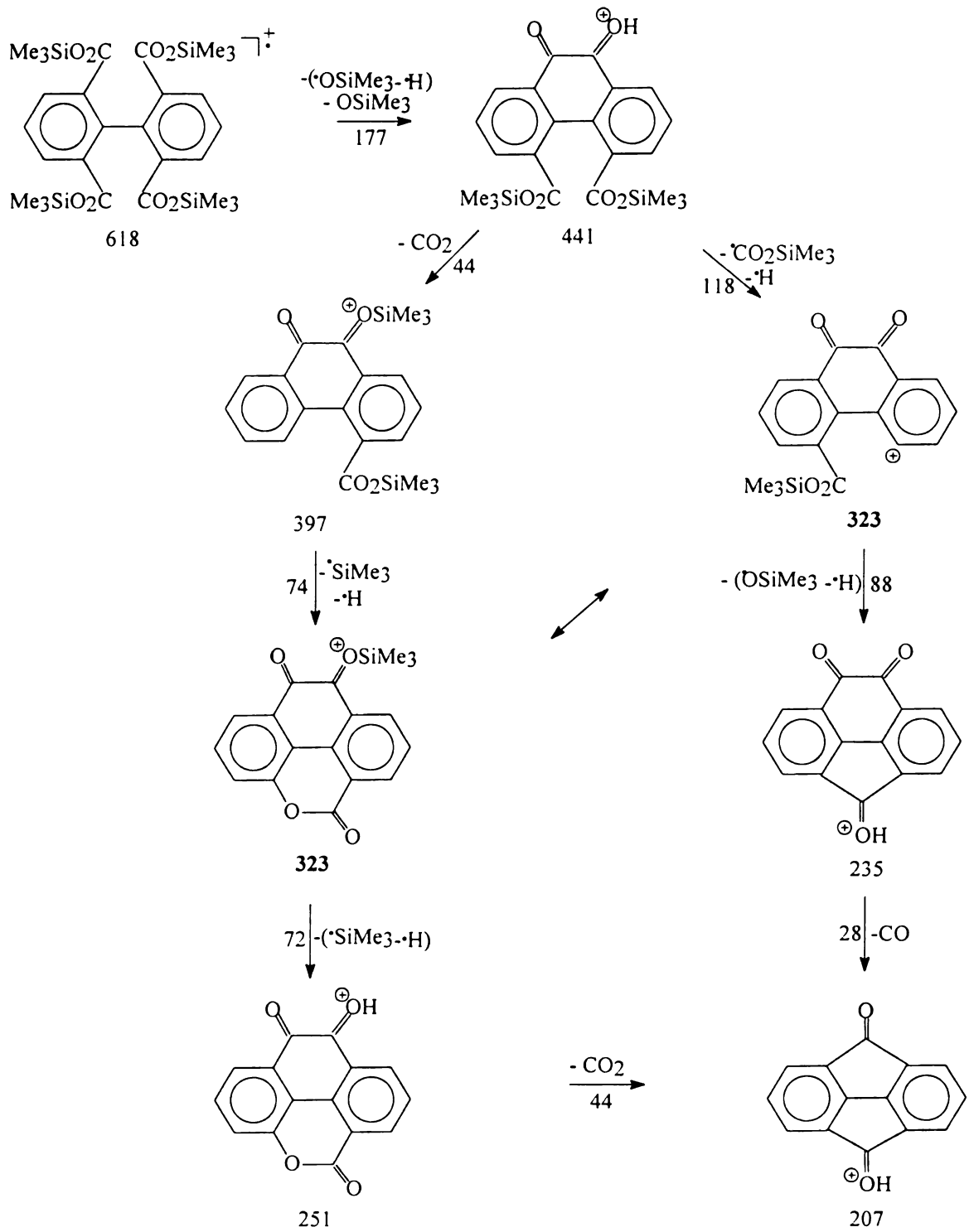
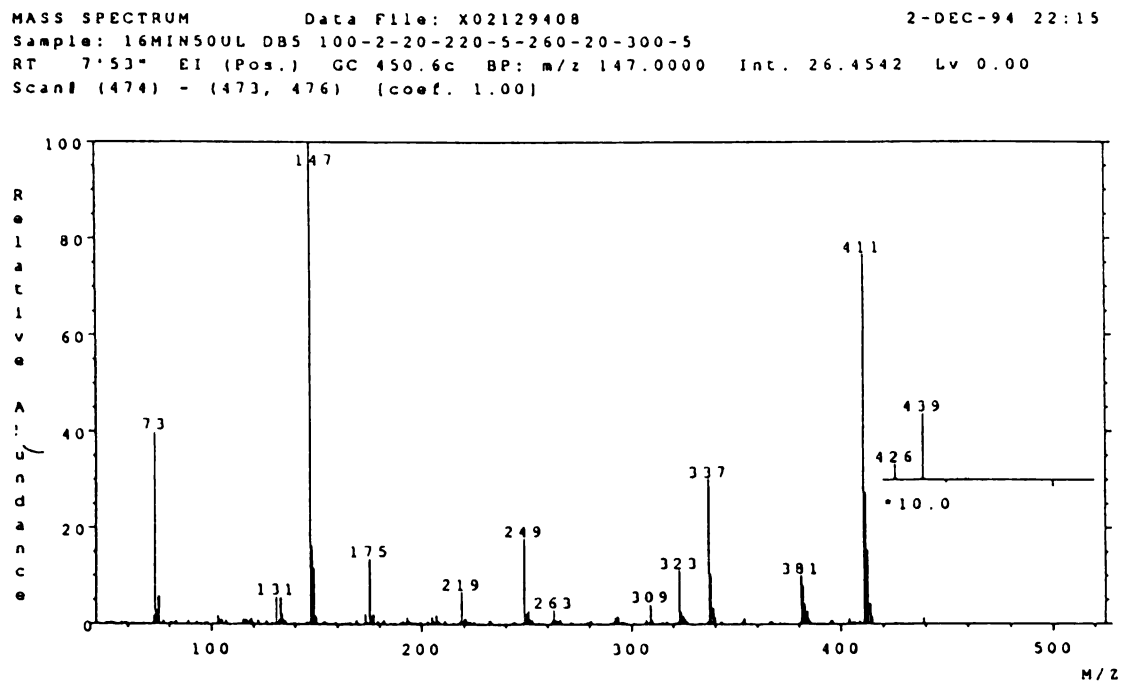


Figure B.14 Interpretation of the mass spectrum for the silylation product XIII. Molecular ion weight = 618.

Mass Spectrum of the silylation product XIV



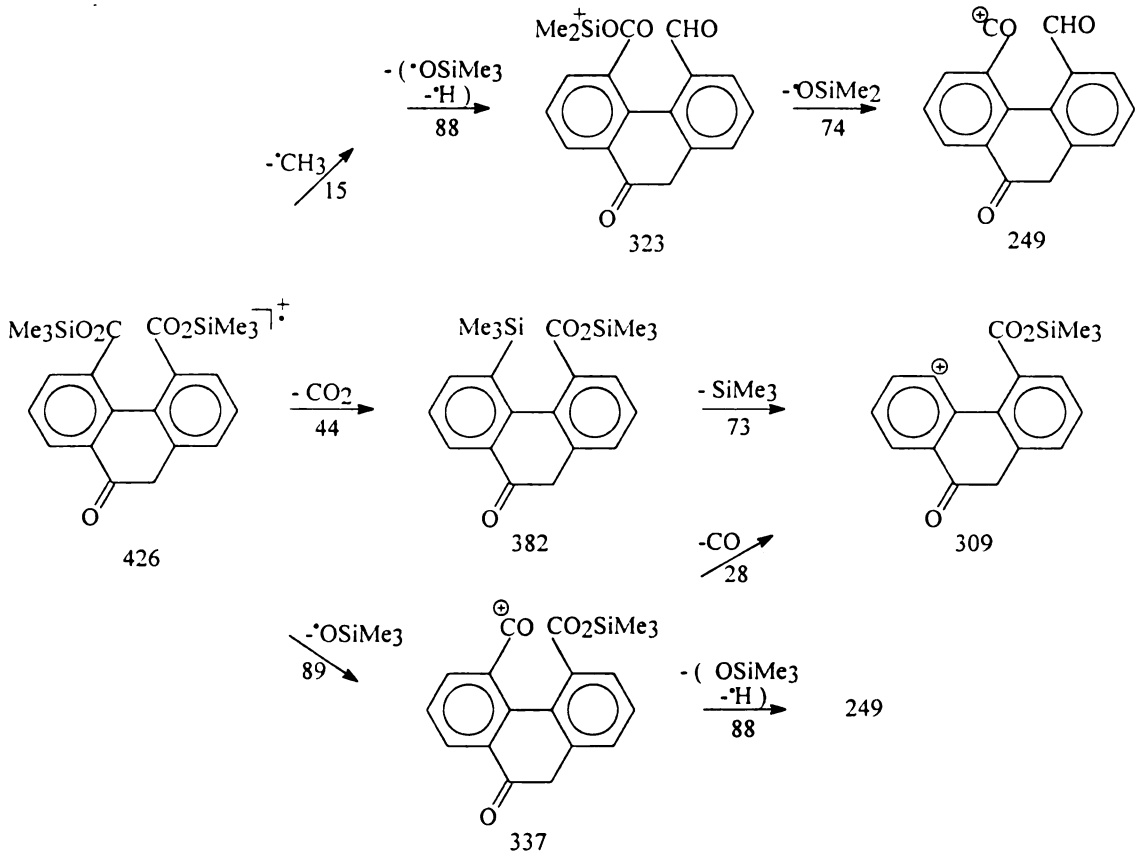
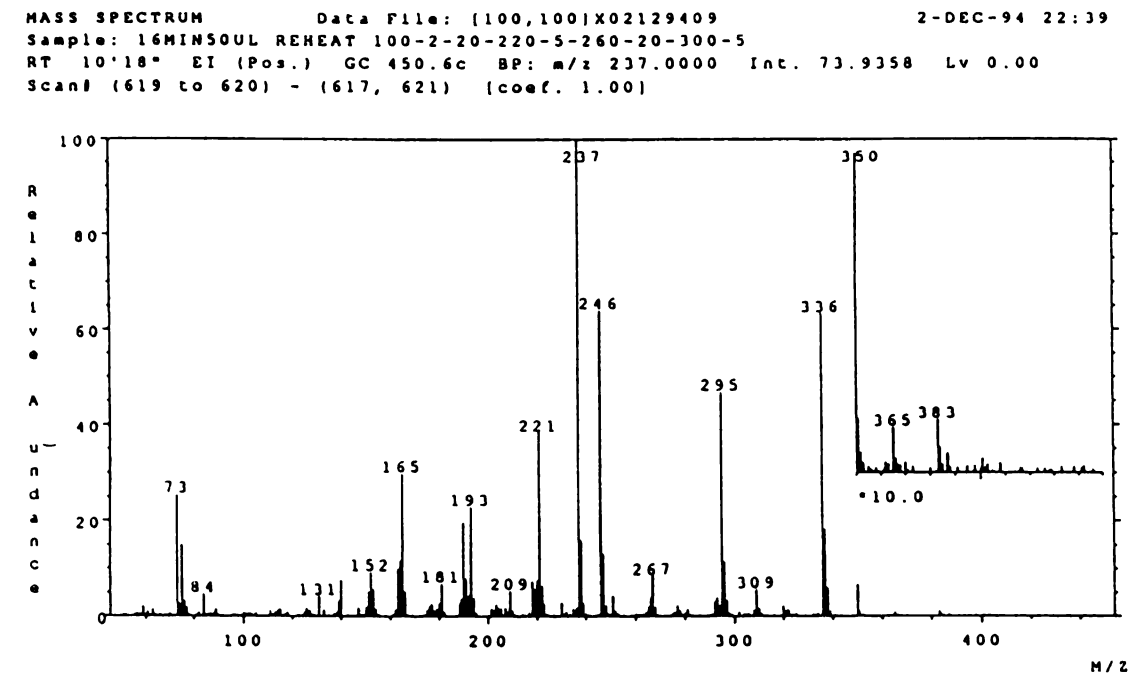
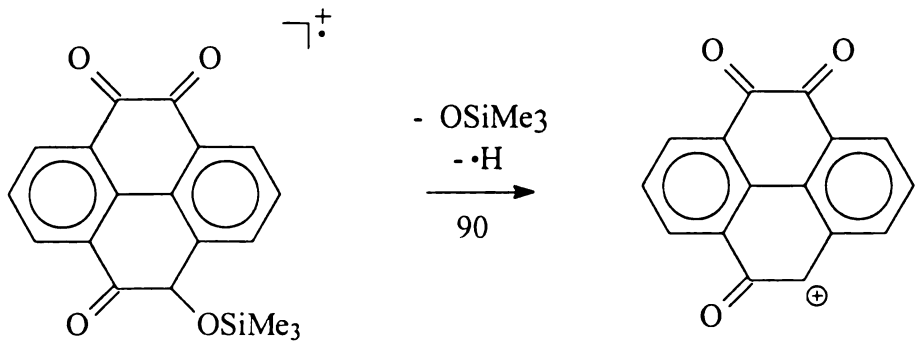


Figure B.15 Interpretation of the mass spectrum for the silylation product XIV. Molecular ion weight = 426.

Mass Spectrum of the silylation product XV





336 246

Figure B.16 Interpretation of the mass spectrum for the silylation product XV. Molecular ion weight = 336.

APPENDIX C

APPENDIX C

OZONATION PRODUCTS PURIFICATION

Benz[a]anthracene ozonated samples were purified using reverse phase medium pressure column (C_{18} column) followed by thin layer chromatography (TLC). Reverse phase medium pressure chromatography (C_{18} silica, 500 x 20 mm glass column, pressure rated 150 psi, DyChrom, Santa Clara, CA) was used to separate the crude products. The mobile phase was controlled by a low pressure pump (Model 81-M-2, Chemco, Sunnyvale, CA) at a flow rate of 4 mL/min. The column was conditioned using the starting solvent mixture. The sample was dissolved in the same solvent mixture and loaded into the system. Two mobile phase gradients were used for the different ozonation times. Gradients of 90/10 and 70/30 methanol/water to pure methanol were applied for 10 min and 20 min ozonation products, respectively. After fractionation of the crude products on the C_{18} column, TLC was employed to further separate some of the fractionated samples. The silica gel plates (20 x 20 cm, 250μm Uniplate, Analtech, Newark, DE) were eluted with hexane/ether mixture or a mixture of benzene/chloroform with a minimal amount of methanol. TLC separation for the fractionated samples from C_{18} column depended on the sample purity and quantity of the sample (minimum loading for each plate < 15 mg).

Flow charts shown in Table C.1 and C.2 give the sequence of separation for 10 min and 20 min ozonation sample; each fraction was analysis by GC/MS.

Table C.1 Separation flow chart for sample reacted with ozone for 10 minutes.

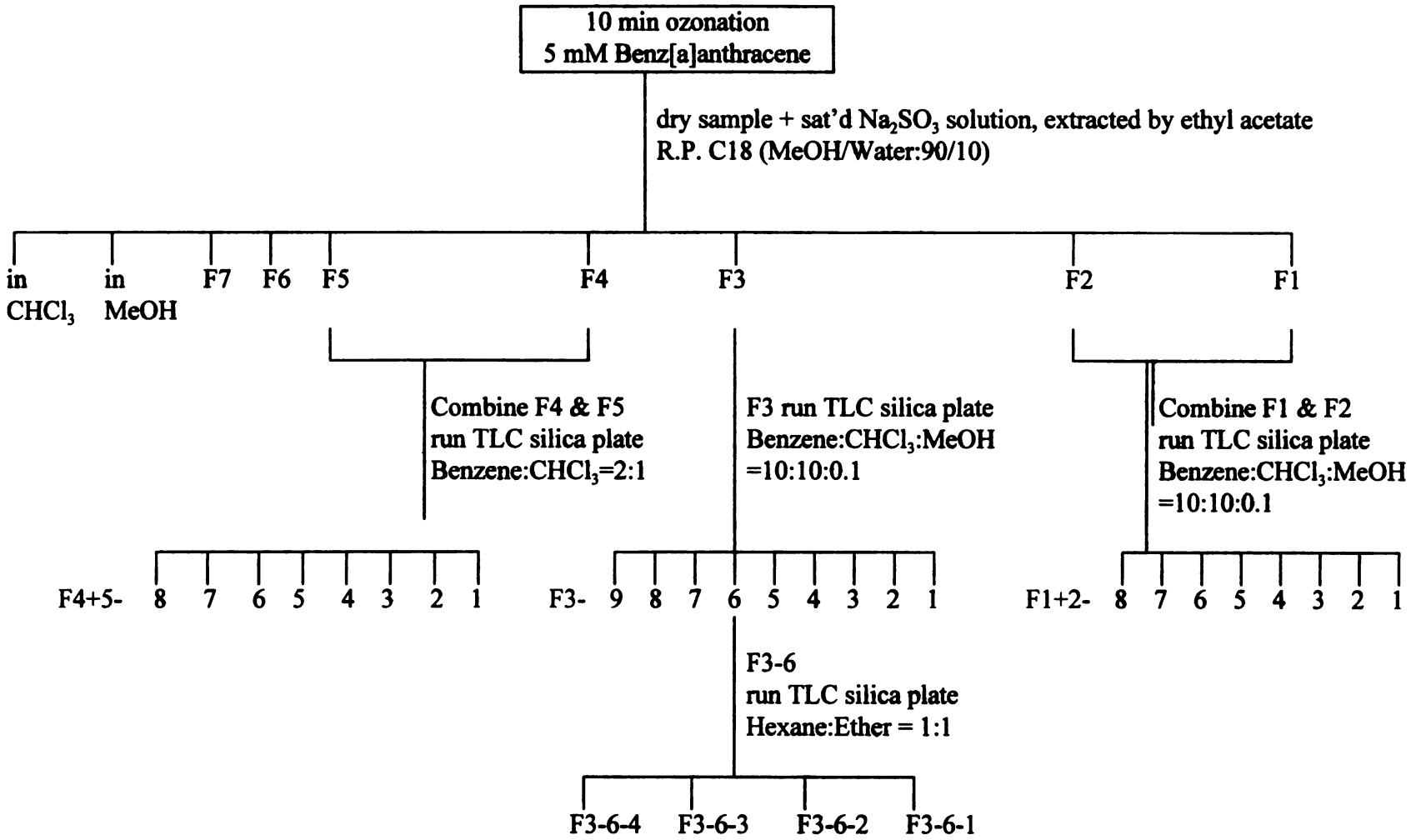
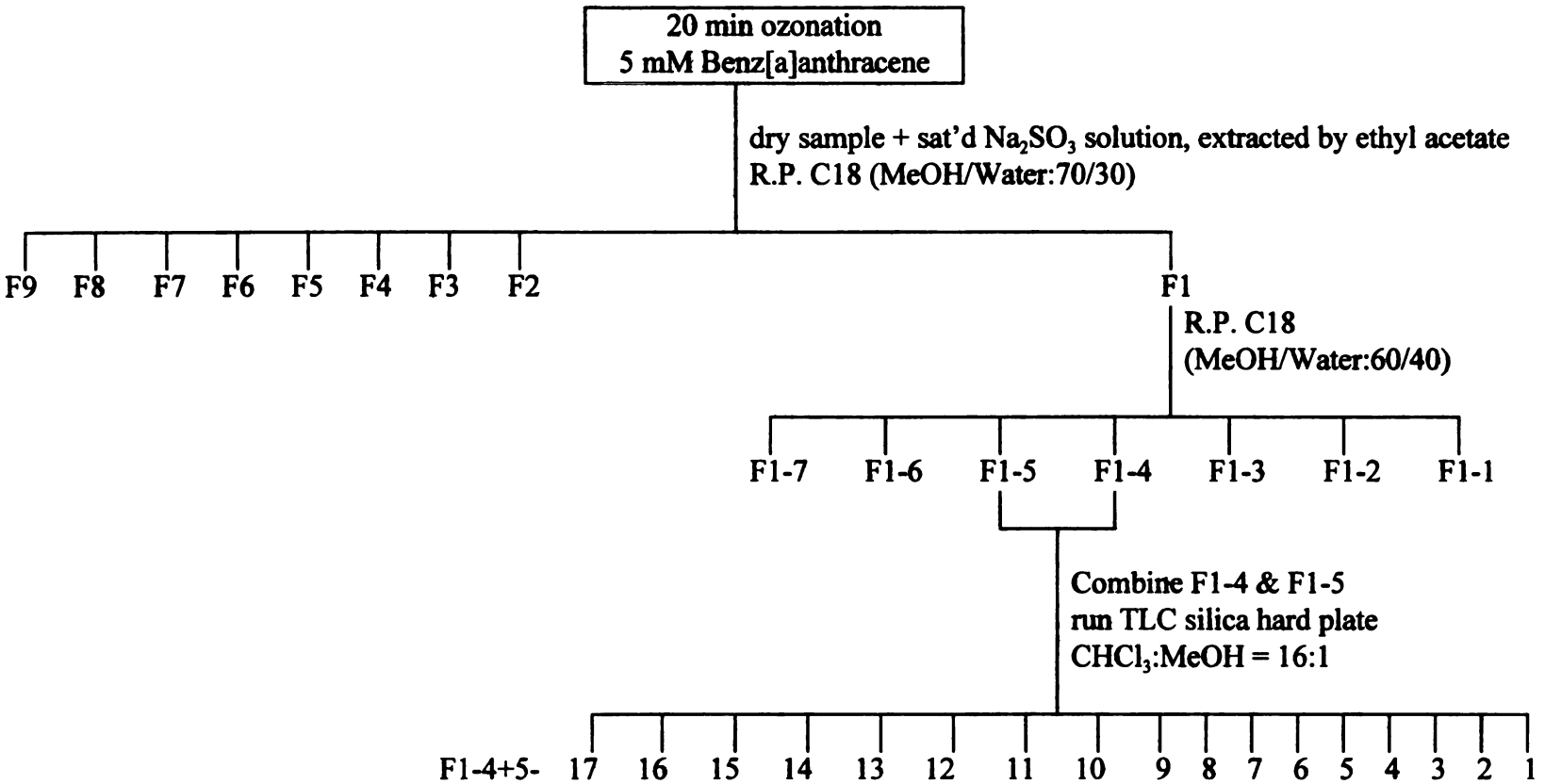


Table C.2 Separation flow chart for sample reacted with ozone for 20 minutes.



Typical GC chromatograms for the samples from the C_{18} column and samples that had undergone additional separation procedures are shown in Figure C.1 and C.2, respectively. After the first separation step, most samples were not pure enough for GC/MS analysis. As shown in Figure C.1, these samples still resulted in shoulders between peaks, causing overlapping of the mass spectra of neighboring compounds. Therefore, the molecular ion for the first separation compounds could not be identified and further purification was required. After a second separation step, some of the fractionated samples, such as F4+5-3 from 10 min ozonation as illustrated in Figure C.2, was pure enough for GC/MS analysis.

Although the separation procedure used could purify (or concentrate) compound V and X, this procedure failed because of several problems. First of all, the silica on the TLC surface catalyzed the further oxidation of PAHs (or ozonation products) by any free radicals present. Therefore, large number of compounds, e.g. ethers or esters, were generated. When these compounds were produced, the similarities in their physical and chemical properties resulted GC peaks that could not be separated, resulting in overlapping mass spectra. Furthermore, these products (ethers or esters), as the major peak shown in Figure C.2, were not produced by the ozone direct reaction pathway.

In conclusion, another separation method should be developed to improve the purity of ozonation products. The purification using reverse phase medium pressure column and TLC as described here can not be used for this purpose.

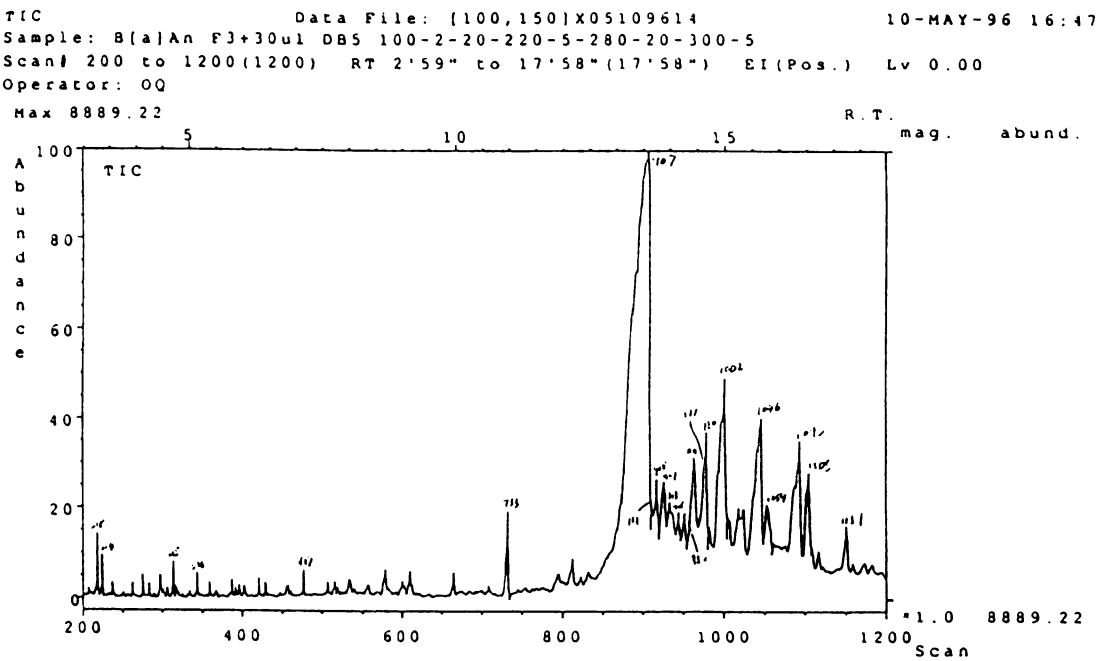


Figure C.1 GC chromatograms of sample F3 from 10 min ozonation sample followed by separation on a C_{18} column.

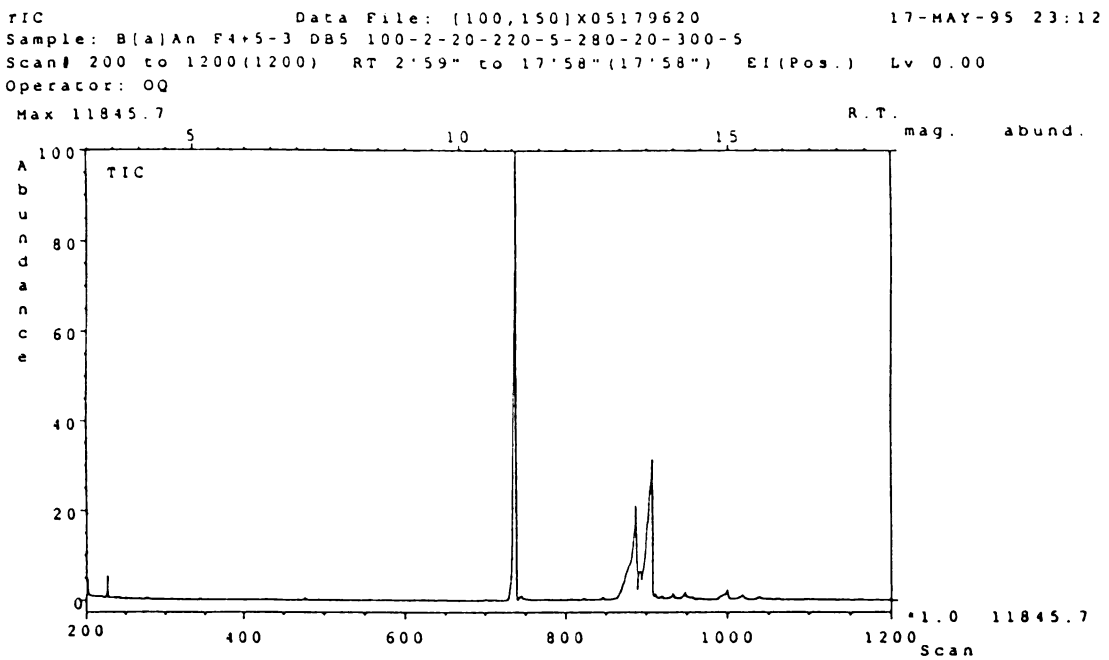


Figure C.2 GC chromatograms of sample F4+5-3 from 10 min ozonation sample followed by a second separation.

APPENDIX D

APPENDIX D

MASS SPECTRA AND INTERPRETATION OF SILYLATED BENZ[a]ANTHRACENE OZONATION PRODUCTS

In this appendix, the raw spectra obtained from GC/MS analysis is followed by the interpretation of these spectra for benz[a]anthracene ozonation products. Products were silylated by BSTFA + 1% TMCs and heated at 100°C for 60 min prior to GC/MS analysis. The silylation reagent converts -OH or -COOH functional groups to $-OSiMe_3$ or $-COOSiMe_3$.

The mass spectra from library search in NIST MS Search (ver 1.5, 1996) or BenchTop/BPM Search (ver 3.10d, Wiley & Son, 6th ed., 1994) which were matched the raw mass spectra from GC/MS analysis are also illustrated. The commercial compound, 2-carboxybenzaldehyde (Sigma-Aldrich Library of Rare Chemicals, Milwaukee, WI), was prepared so as to compare its GC/MS spectra with that of compound XIV A/B. From the GC chromatograph and mass spectra of 2-carboxybenzaldehyde before and after TMS derivatization, the target compound found to exist in two isomeric forms which were identical to those shown in compound XIV A/B.

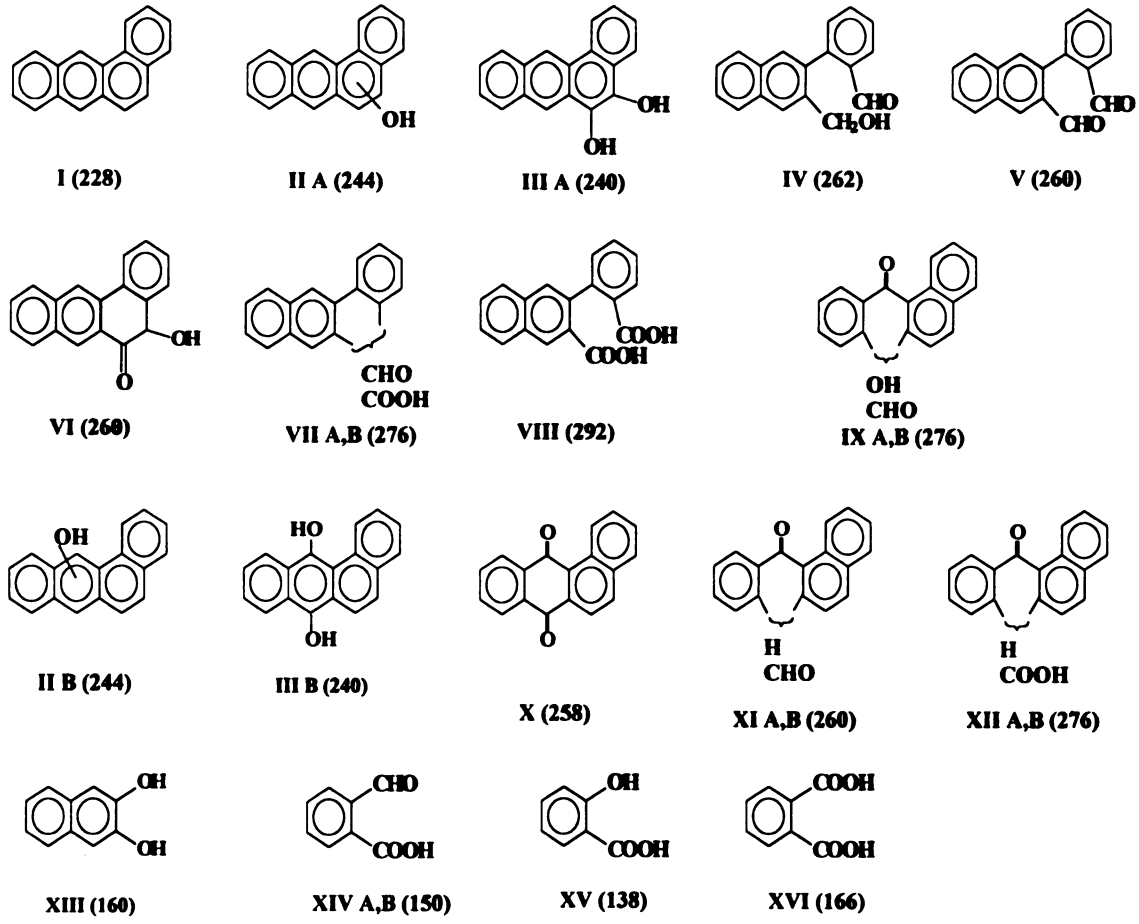
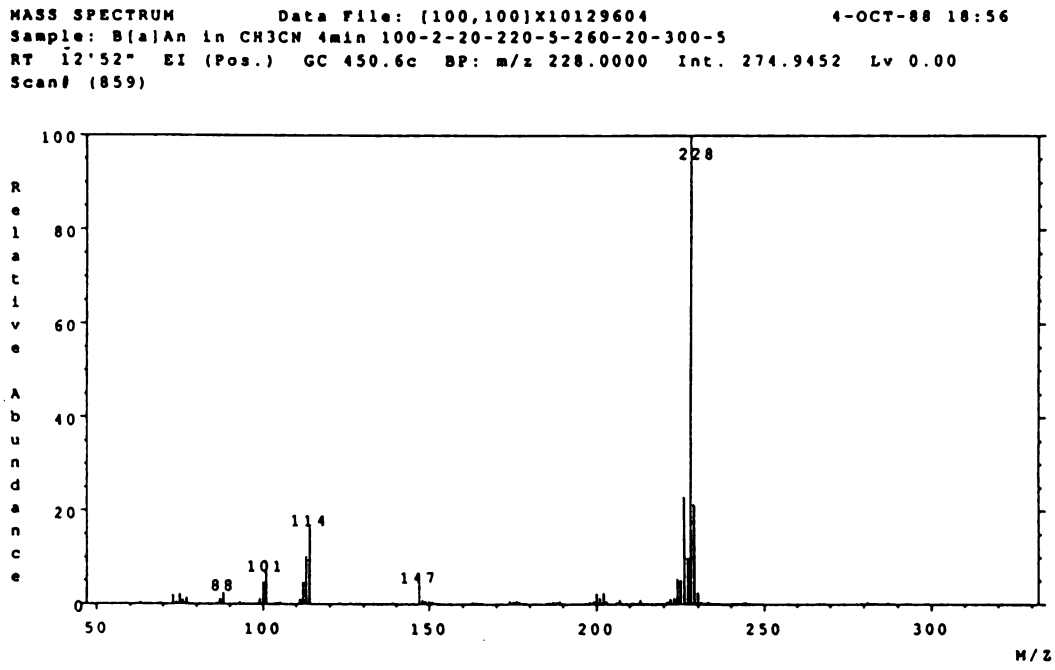
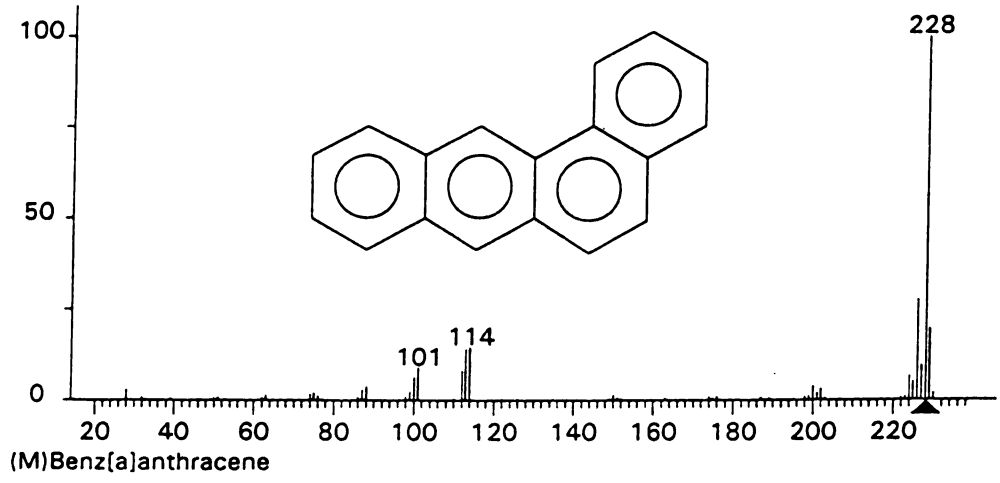


Figure D.1 Products formed from the ozonolysis of benz[a]anthracene. Roman numbers correspond to the ID assigned to each product. Molecular weights are given in parentheses.

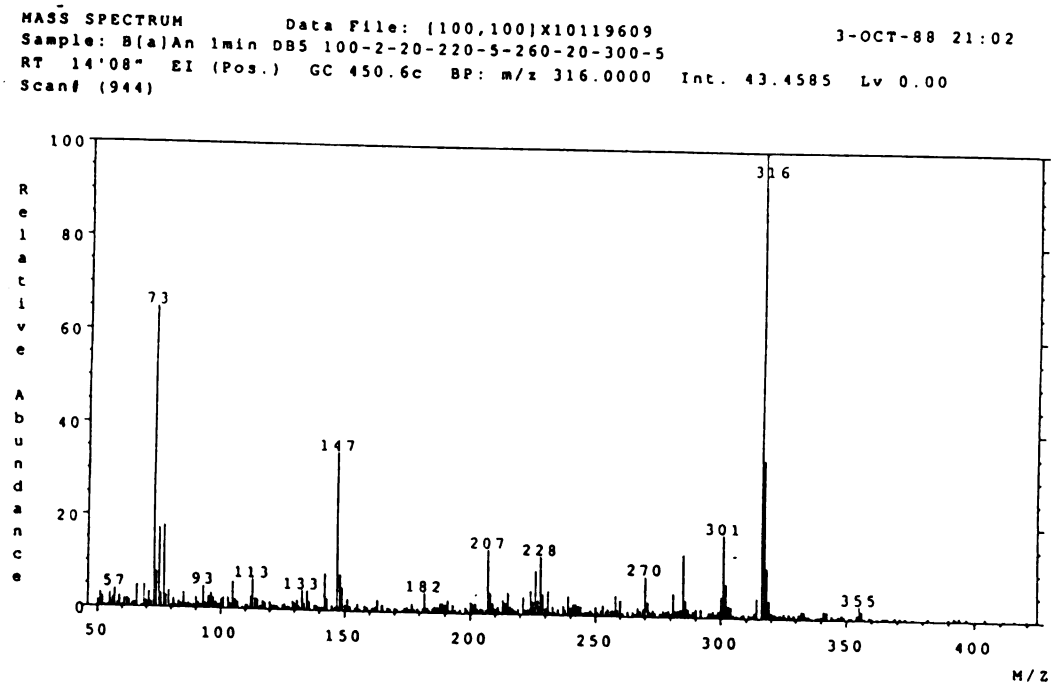
Mass Spectrum of benz[a]anthracene (I)

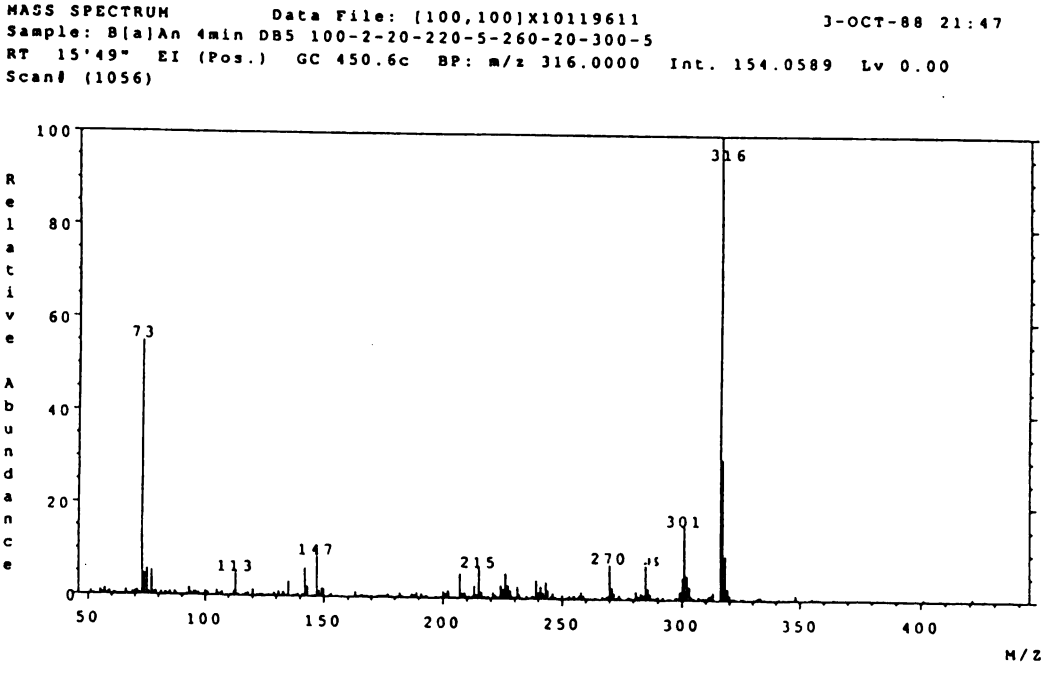


Mass spectrum of benz[a]anthracene obtained from NIST database (NIST#: 114976, CAS#: 56-55-3)



Mass Spectra of the silylation product II A,B





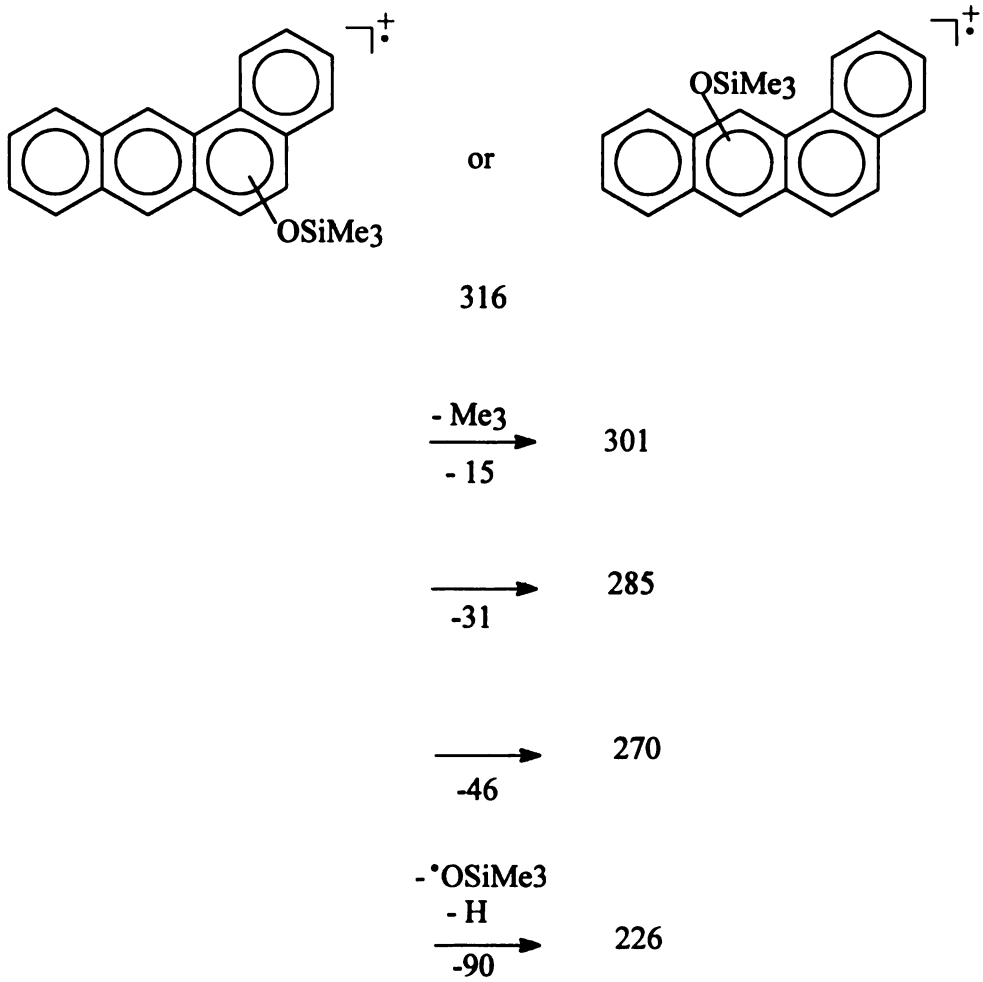
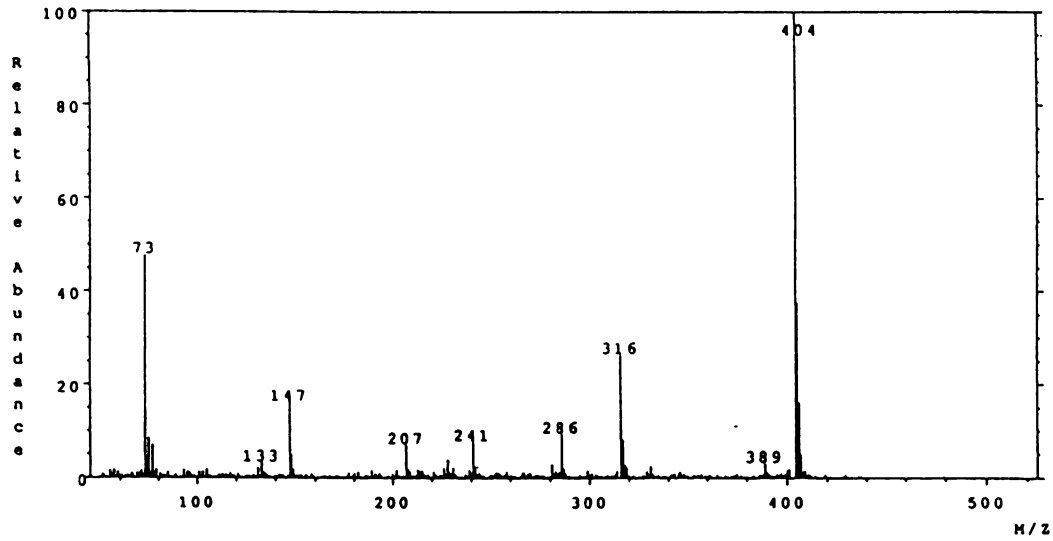


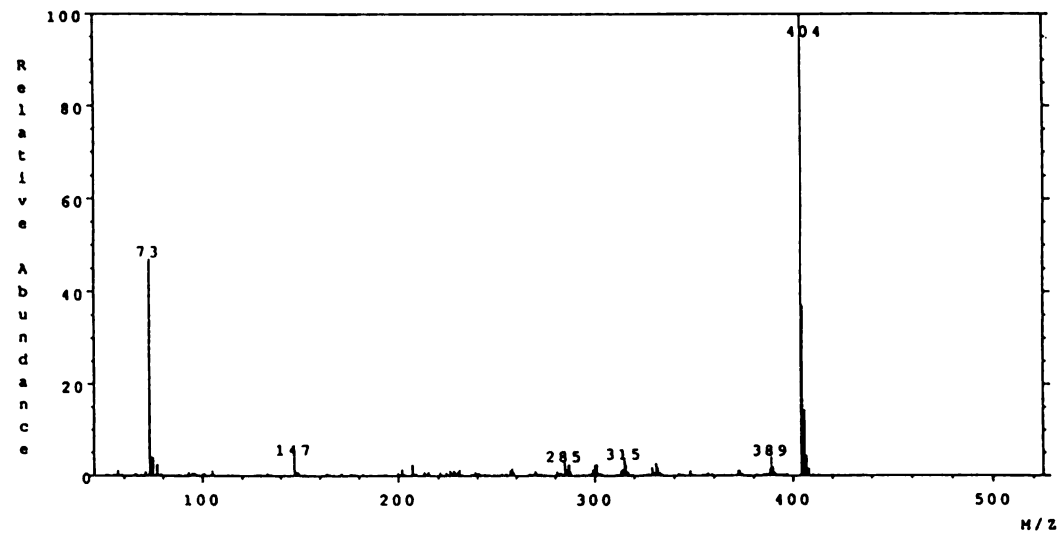
Figure D.2 Interpretation of the mass spectra for the silylation product II A,B. Molecular ion weight = 316.

Mass Spectra of the silylation product III A,B

MASS SPECTRUM Data File: [100,100]X10119610 3-OCT-88 21:24
Sample: B[a]An 2min DB5 100-2-20-220-5-260-20-300-5
RT 17'00" EI (Pos.) GC 450.6c BP: m/z 404.0000 Int. 112.6099 Lv 0.00
Scan# (1135)



MASS SPECTRUM Data File: [100,100]X10119610 3-OCT-88 21:24
Sample: B[a]An 2min DB5 100-2-20-220-5-260-20-300-5
RT 16'24" EI (Pos.) GC 450.6c BP: m/z 404.0000 Int. 316.4309 Lv 0.00
Scan# (1095)



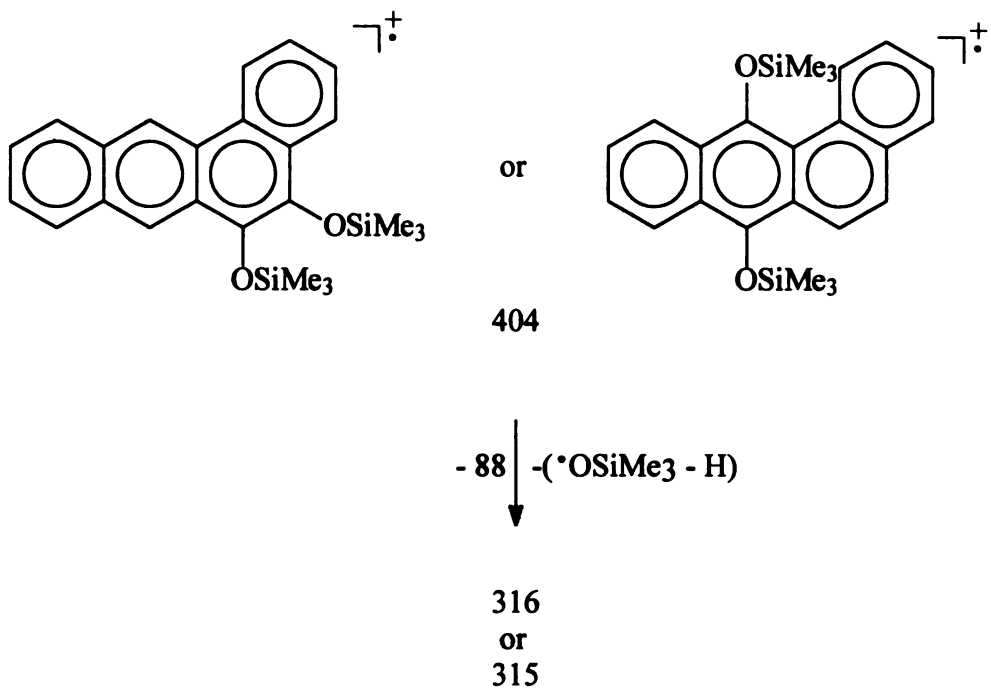
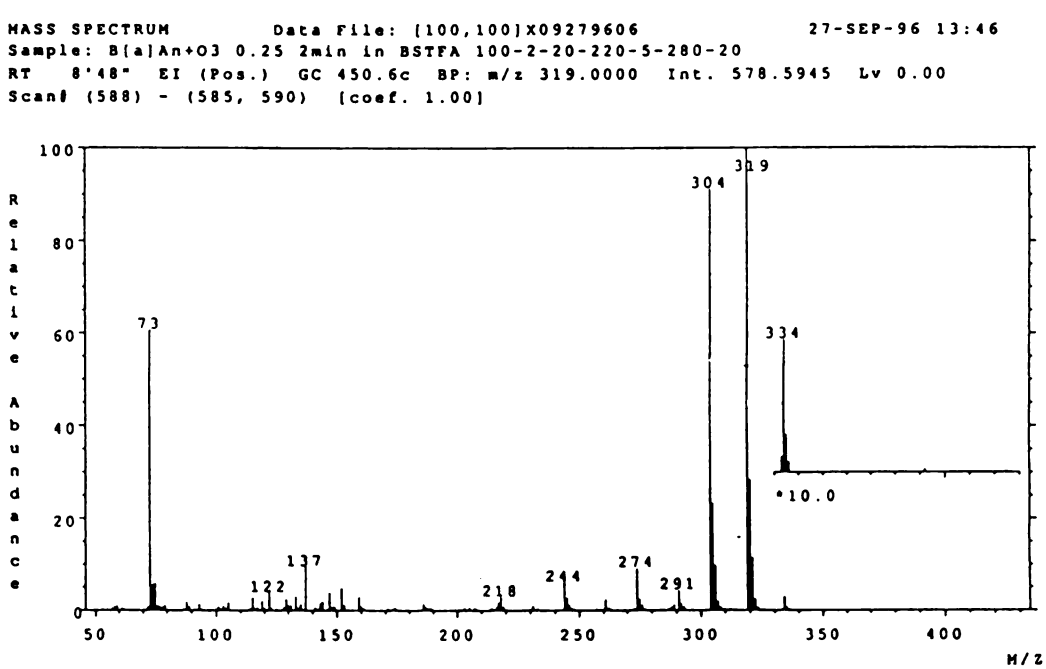


Figure D.3 Interpretation of the mass spectra for the silylation product III A,B. Molecular ion weight = 404.

Mass Spectrum of the silylation product IV



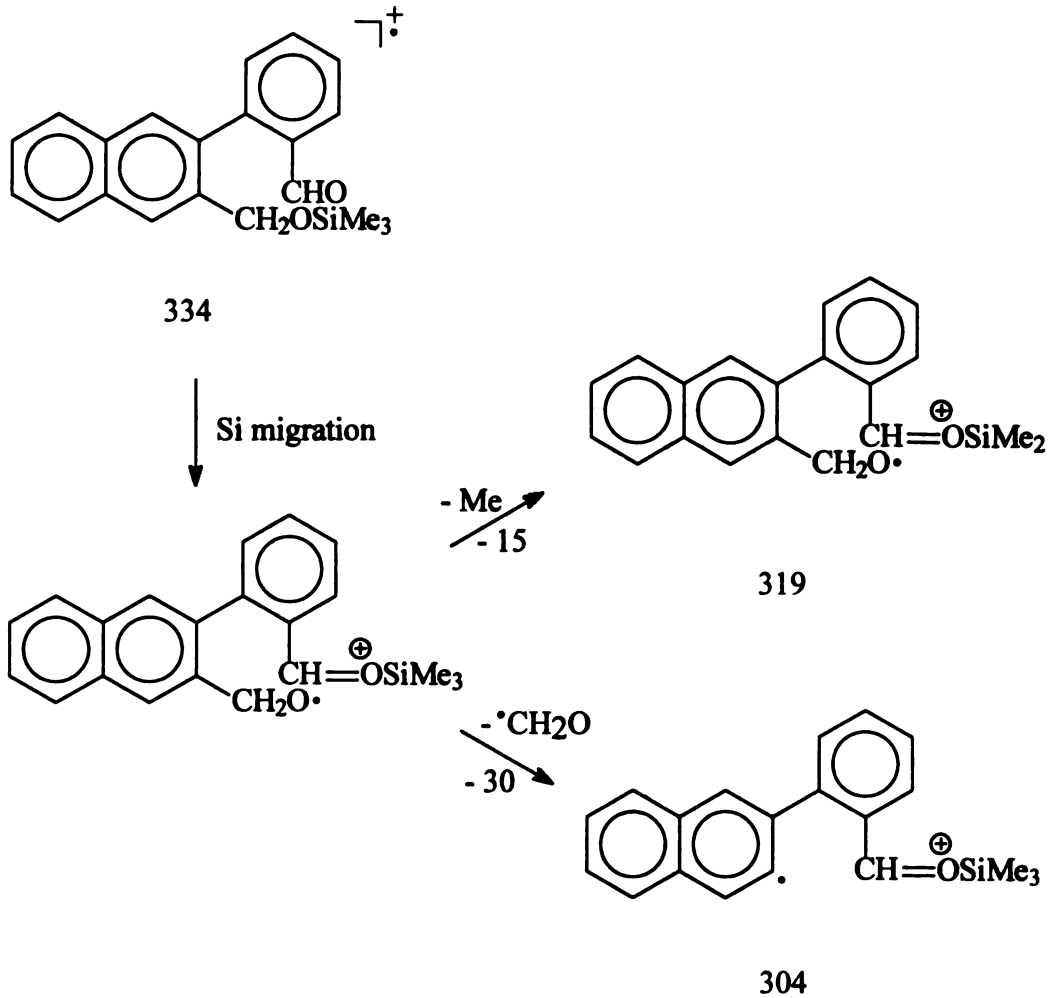
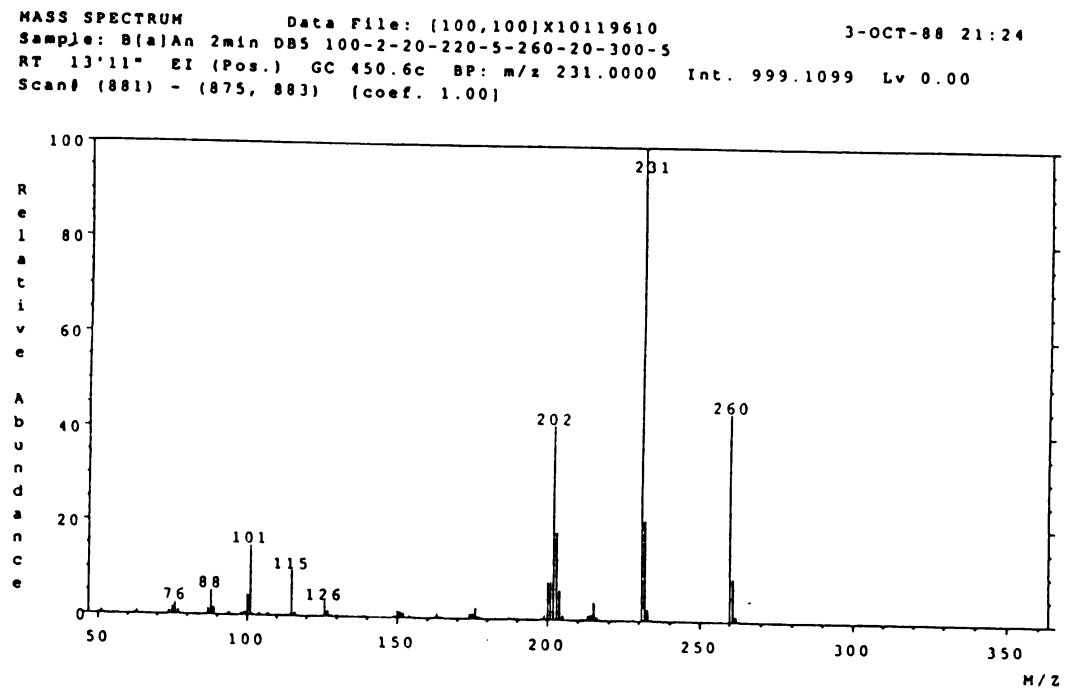
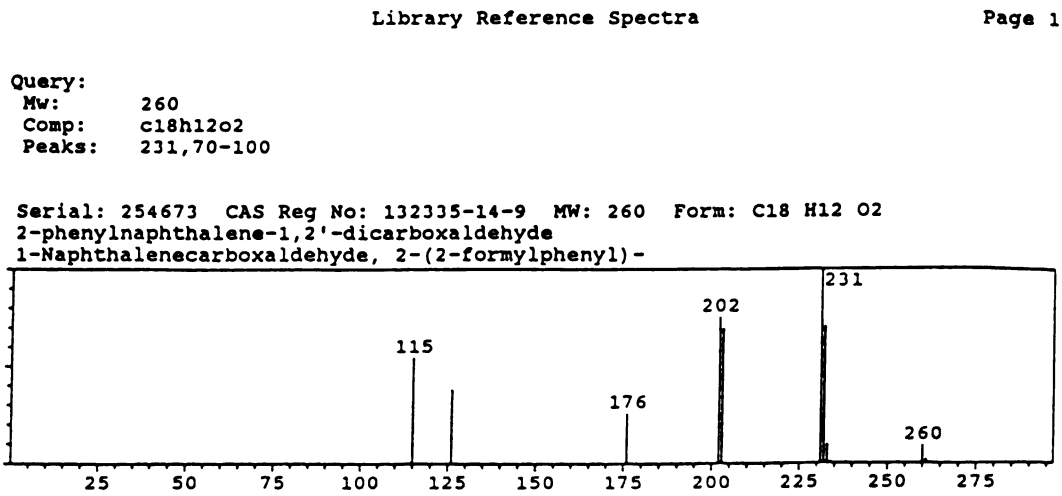


Figure D.4 Interpretation of the mass spectrum for the silylation product IV. Molecular ion weight = 334.

Mass Spectrum of the silylation product V

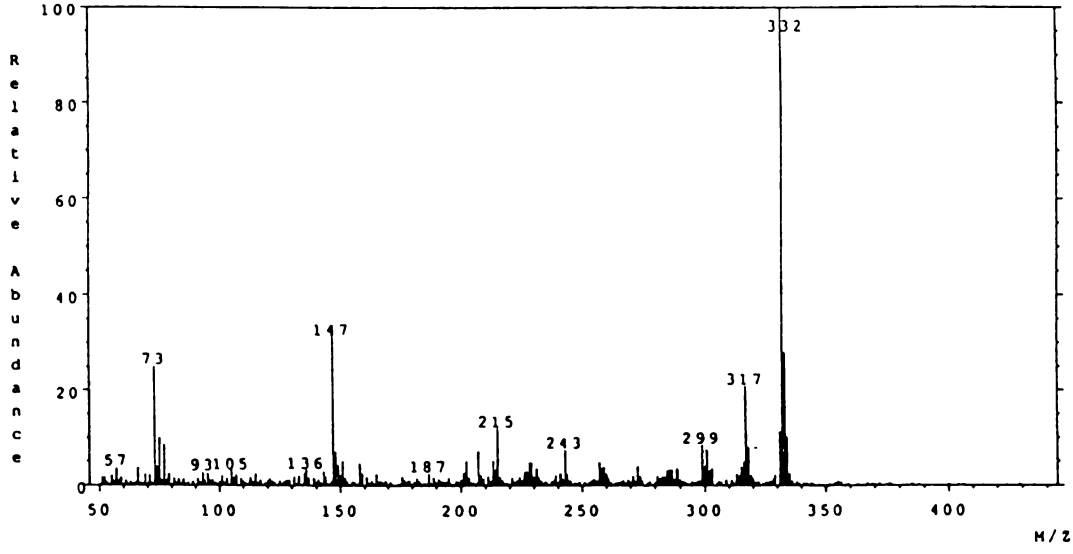


Mass spectrum of 2-(2'-formyl)phenyl-3-naphthaldehyde obtained from BenchTop/BPM database (Serial #: 254673, CAS #: 132335-14-9)

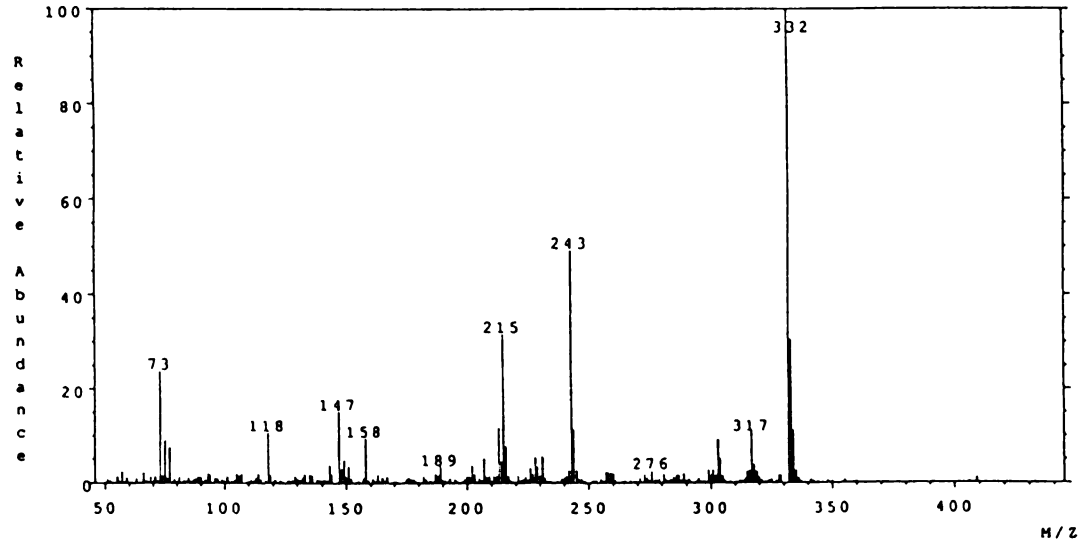


Mass Spectra of the silylation product VI A,B

MASS SPECTRUM Data File: [100,100]X10119610 3-OCT-88 21:24
Sample: B[a]An 2min DB5 100-2-20-220-5-260-20-300-5
RT 11'53" EI (Pos.) GC 450.6c BP: m/z 332.0000 Int. 70.9747 Lv 0.00
Scan# (794)



MASS SPECTRUM Data File: [100,100]X10119610 3-OCT-88 21:24
Sample: B[a]An 2min DB5 100-2-20-220-5-260-20-300-5
RT 13'43" EI (Pos.) GC 450.6c BP: m/z 332.0000 Int. 115.1673 Lv 0.00
Scan# (916)



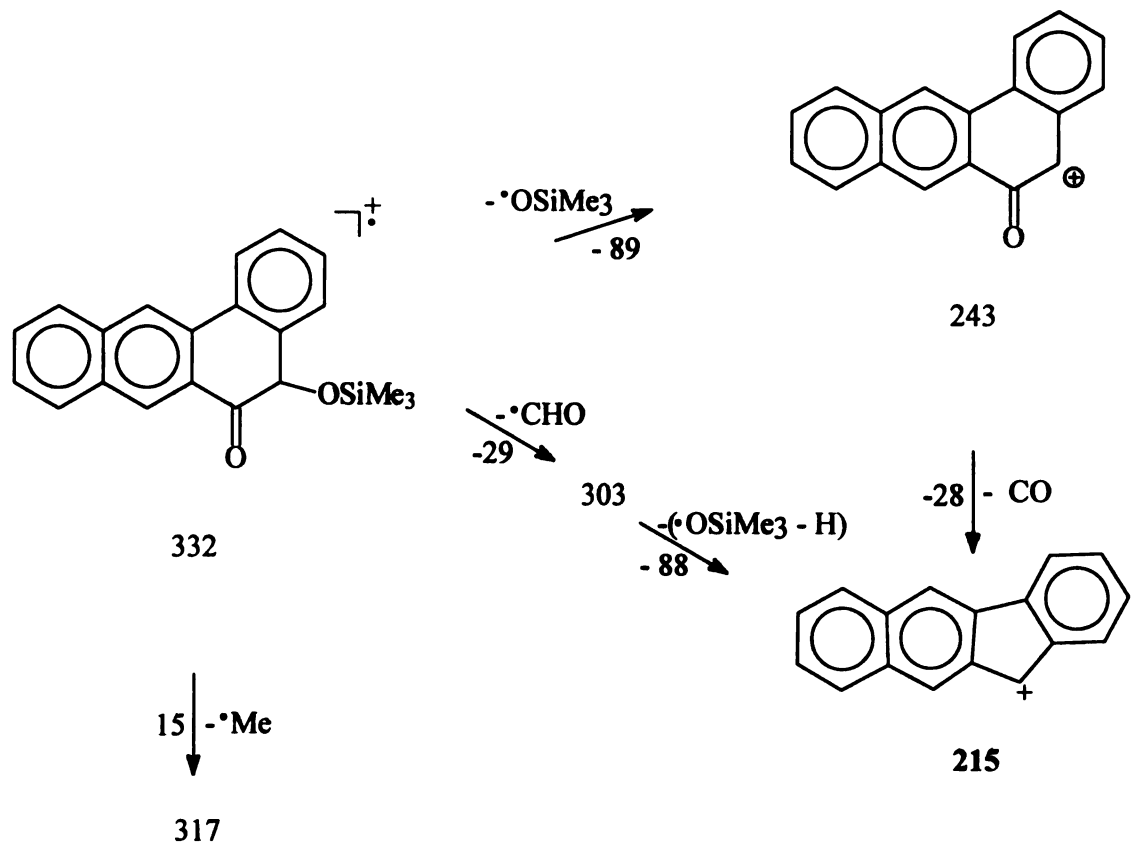
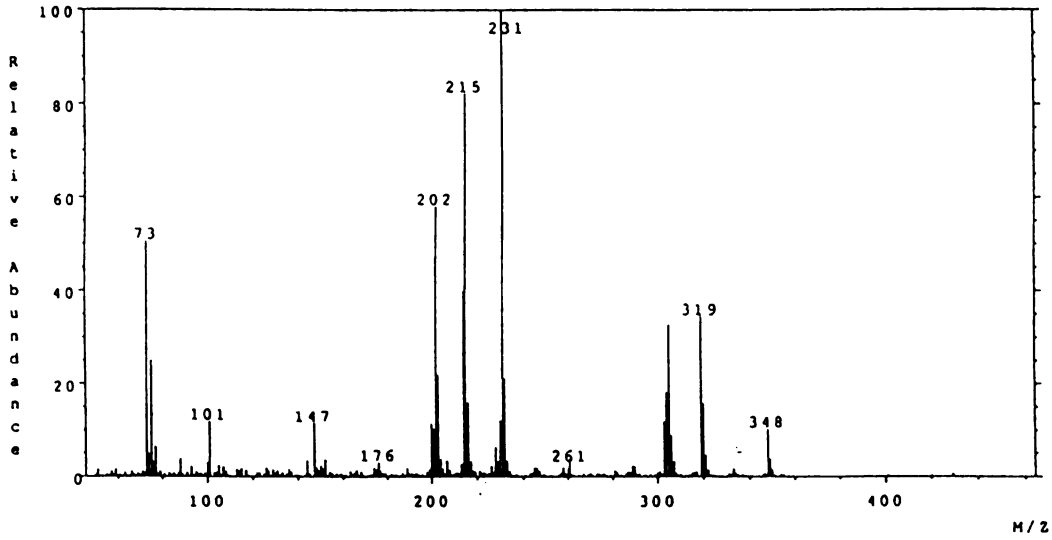


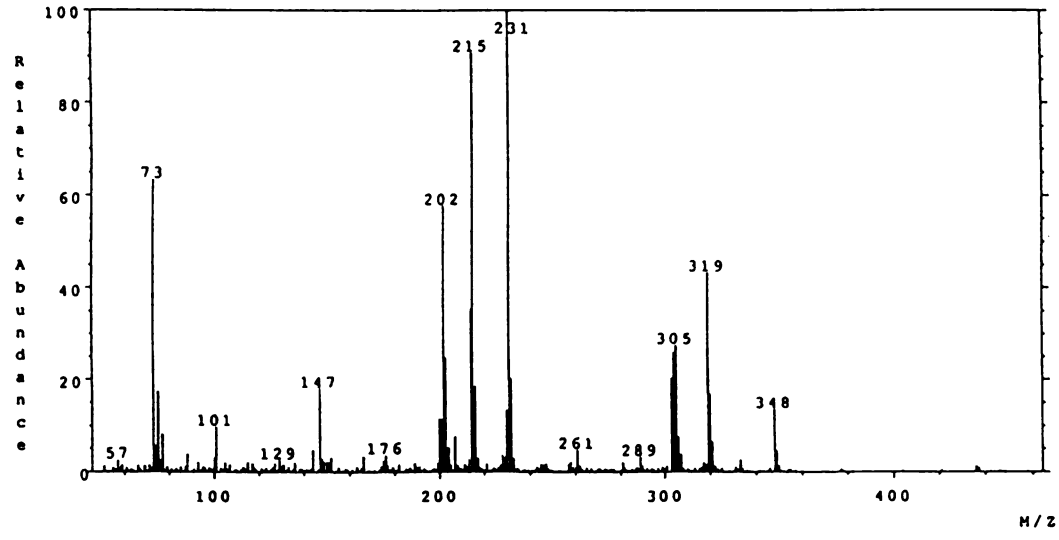
Figure D.5 Interpretation of the mass spectra for the silylation product VI A,B. Molecular ion weight = 332.

Mass Spectra of the silylation product VII A,B

MASS SPECTRUM Data File: [100,100]X10119610 3-OCT-88 21:24
Sample: B[a]An 2min DB5 100-2-20-220-5-260-20-300-5
RT 13'31" EI (Pos.) GC 450.6c BP: m/z 231.0000 Int. 139.3189 Lv 0.00
Scan# (903)



MASS SPECTRUM Data File: [100,100]X10119610 3-OCT-88 21:24
Sample: B[a]An 2min DB5 100-2-20-220-5-260-20-300-5
RT 14'16" EI (Pos.) GC 450.6c BP: m/z 231.0000 Int. 123.2544 Lv 0.00
Scan# (953)



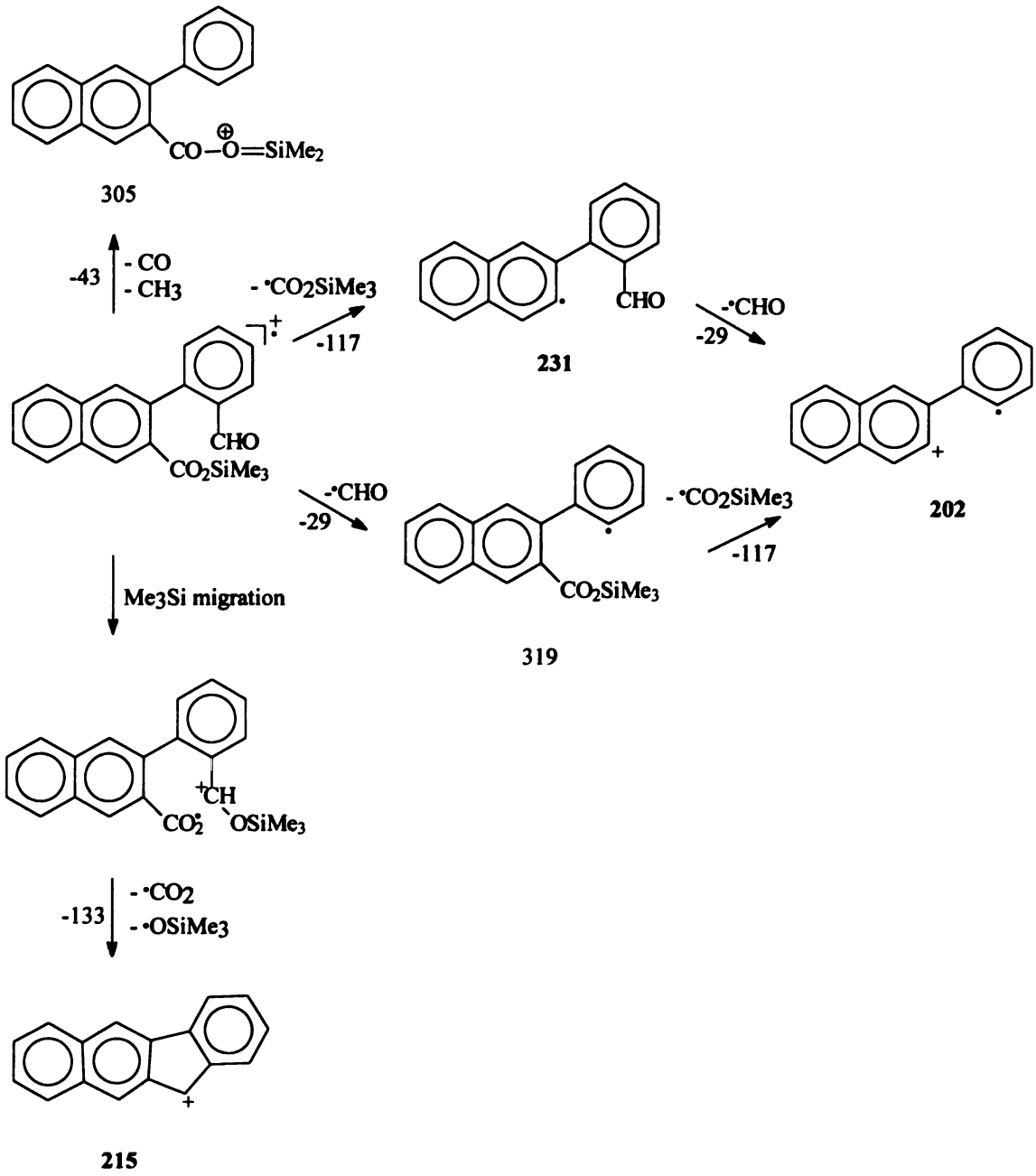
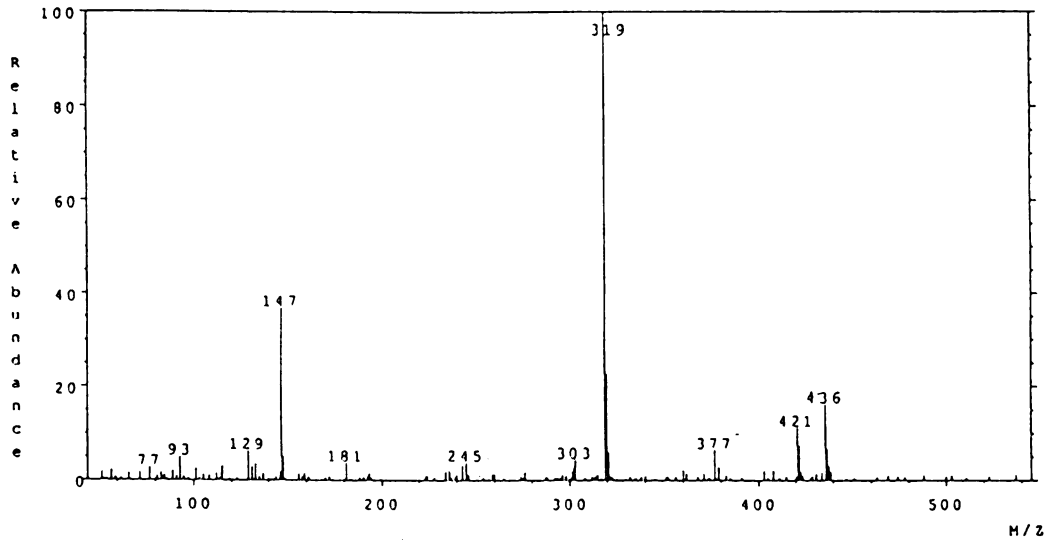


Figure D.6 Interpretation of the mass spectra for the silylation product VII A,B. Molecular ion weight = 348.

Mass Spectrum of the silylation product VIII

MASS SPECTRUM Data File: [100,120]X1U119610 3-OCT-88 21:24
Sample: B[a]An 2min DB5 100-2-20-220-5-260-20-300-5
RT 14'14" EI (Pos.) GC 450.6c BP: m/z 319.0000 Int. 31.2527 Lv 0.00
Scan# (951) - (948, 954) [coef. 1.00]



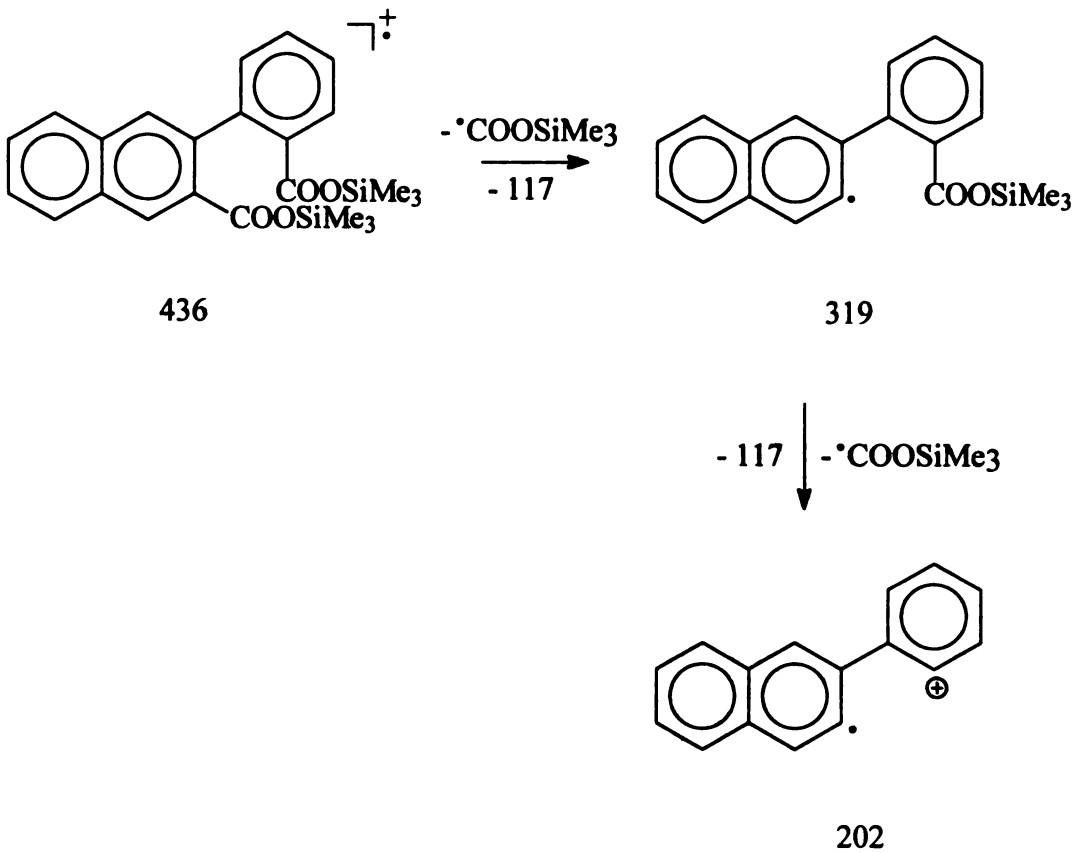
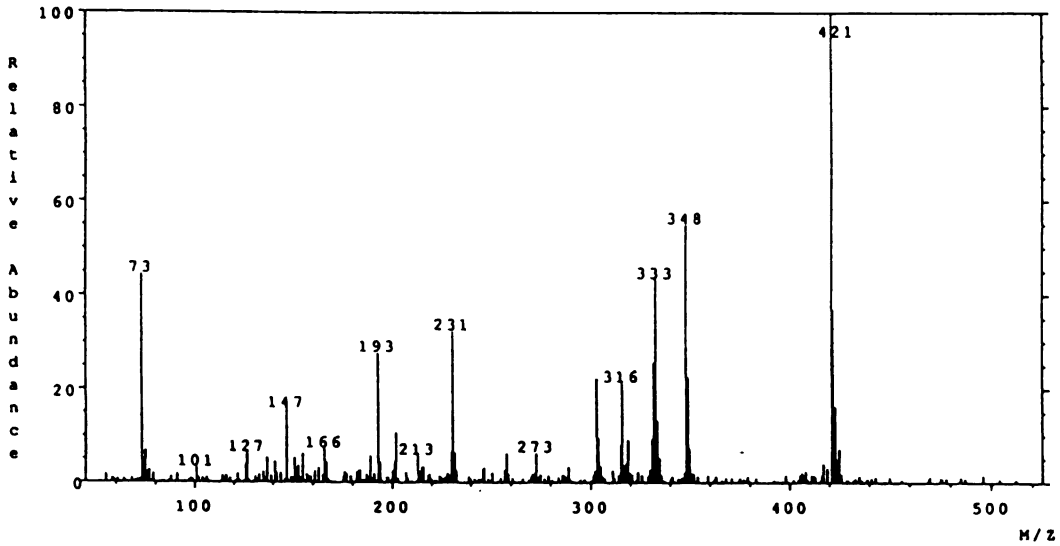


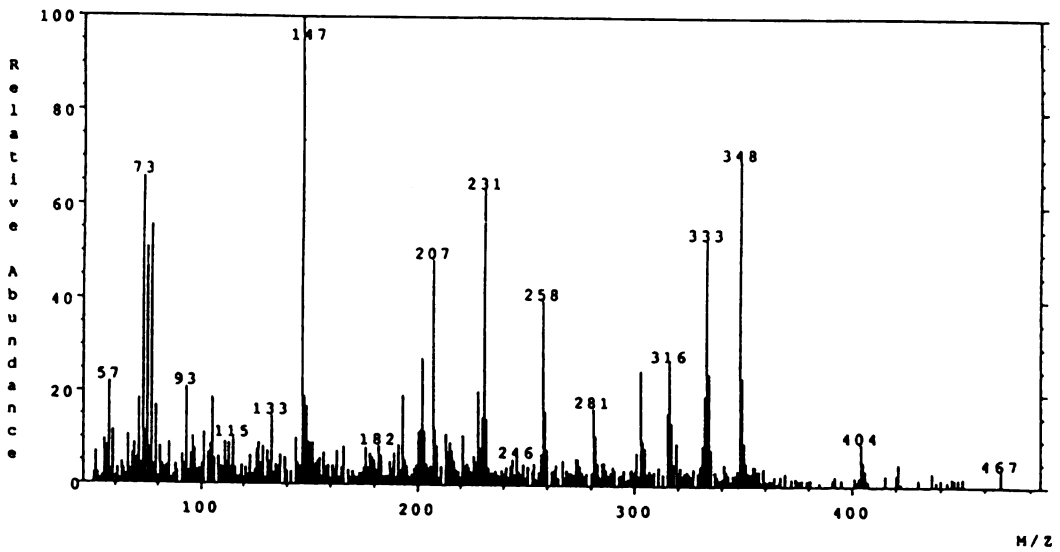
Figure D.7 Interpretation of the mass spectrum for the silylation product VIII. Molecular ion weight = 436.

Mass Spectra of the silylation product IX A,B

MASS SPECTRUM Data File: [100,100]X10119611 3-OCT-88 21:47
Sample: B[a]An 4min DB5 100-2-20-220-5-260-20-300-5
RT 15'28" EI (Pos.) GC 450.6c BP: m/z 421.0000 Int. 35.6202 Lv 0.00
Scan# (1033) - (1029, 1035) [coef. 1.00]



MASS SPECTRUM Data File: [100,100]X10119610 3-OCT-88 21:24
Sample: B[a]An 2min DB5 100-2-20-220-5-260-20-300-5
RT 16'13" EI (Pos.) GC 450.6c BP: m/z 147.0000 Int. 14.9352 Lv 0.00
Scan# (1083)



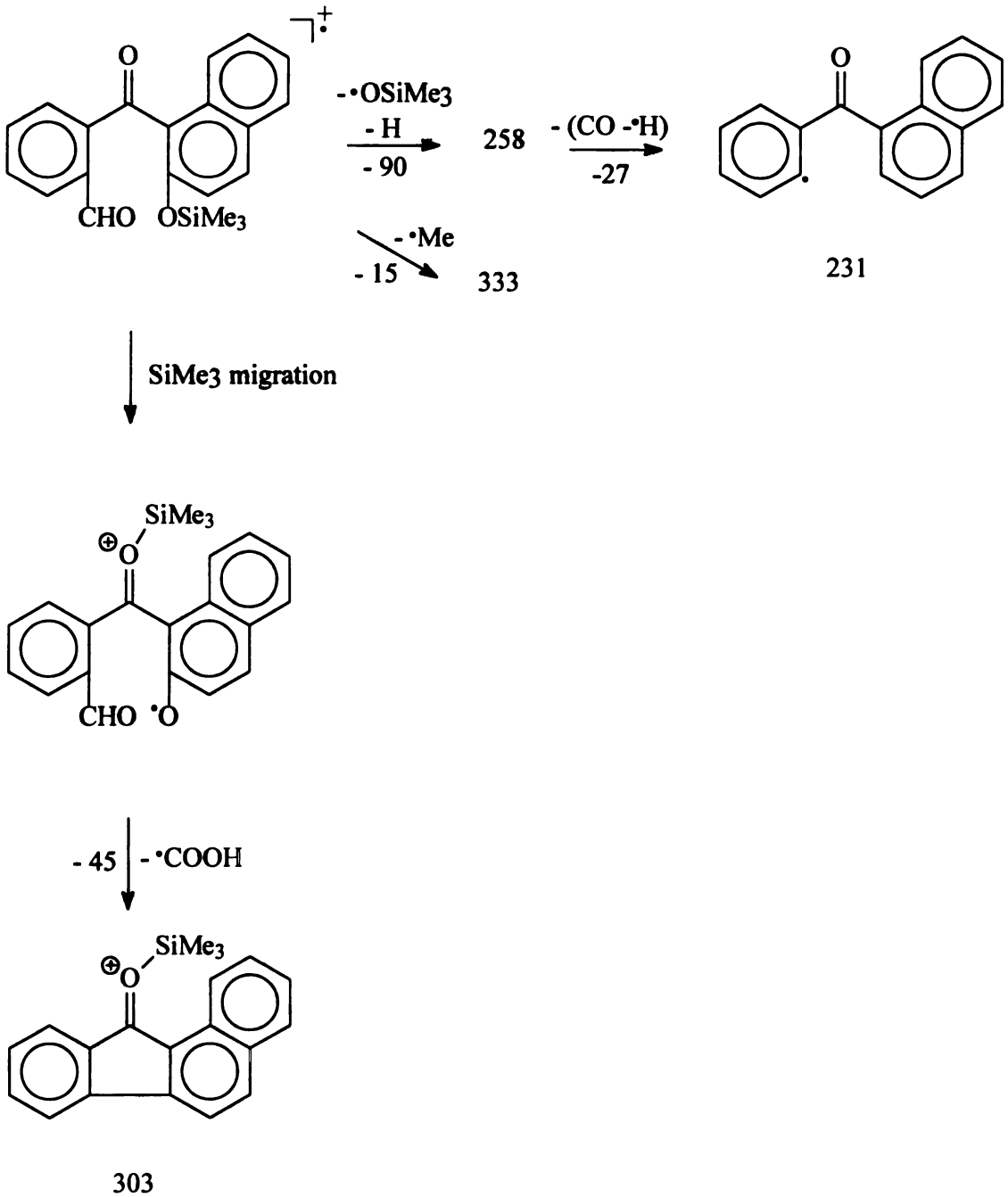
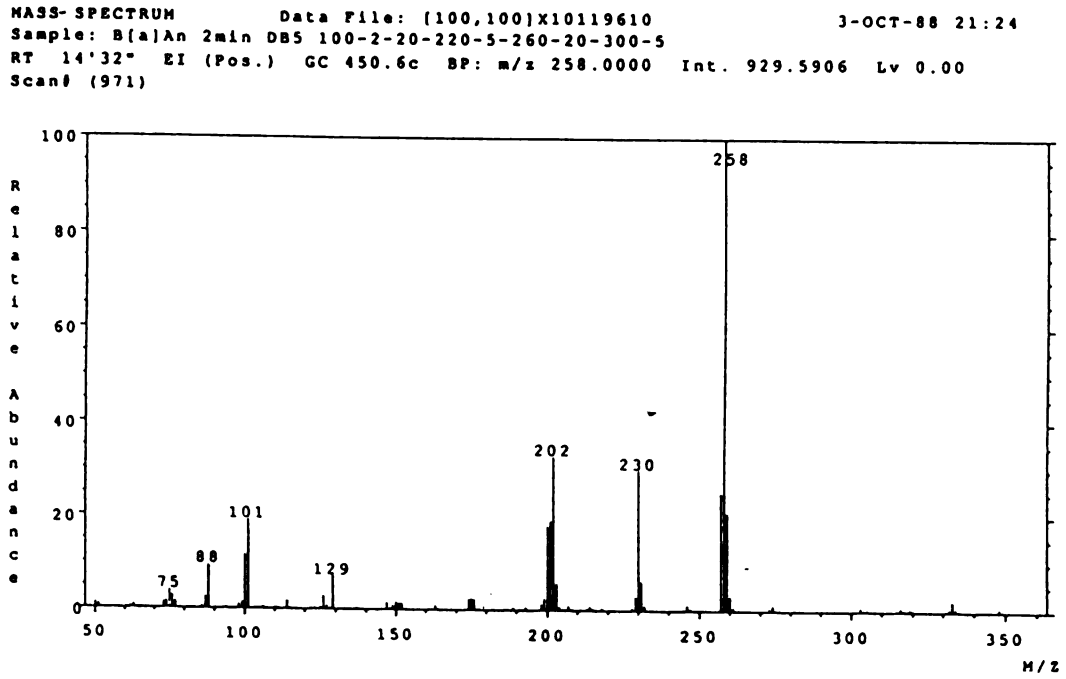
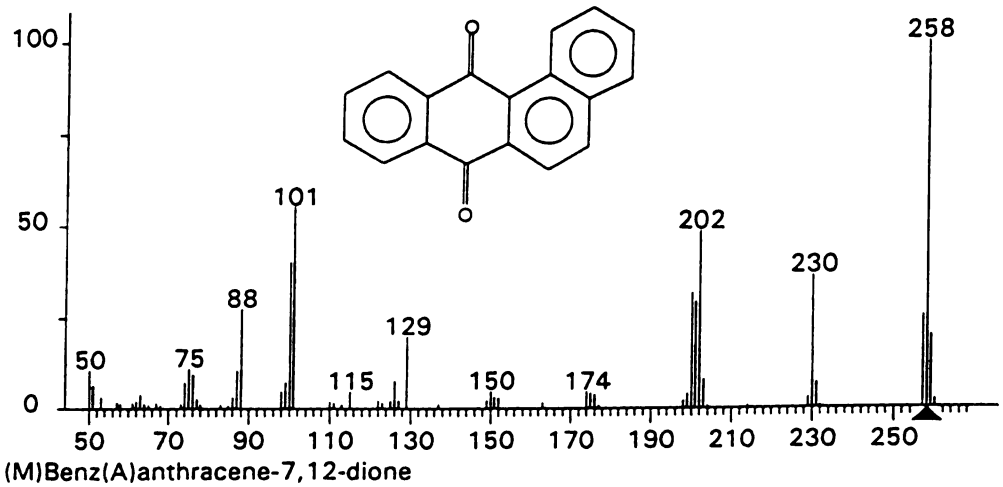


Figure D.8 Interpretation of the mass spectra for the silylation product IX A,B. Molecular ion weight = 348.

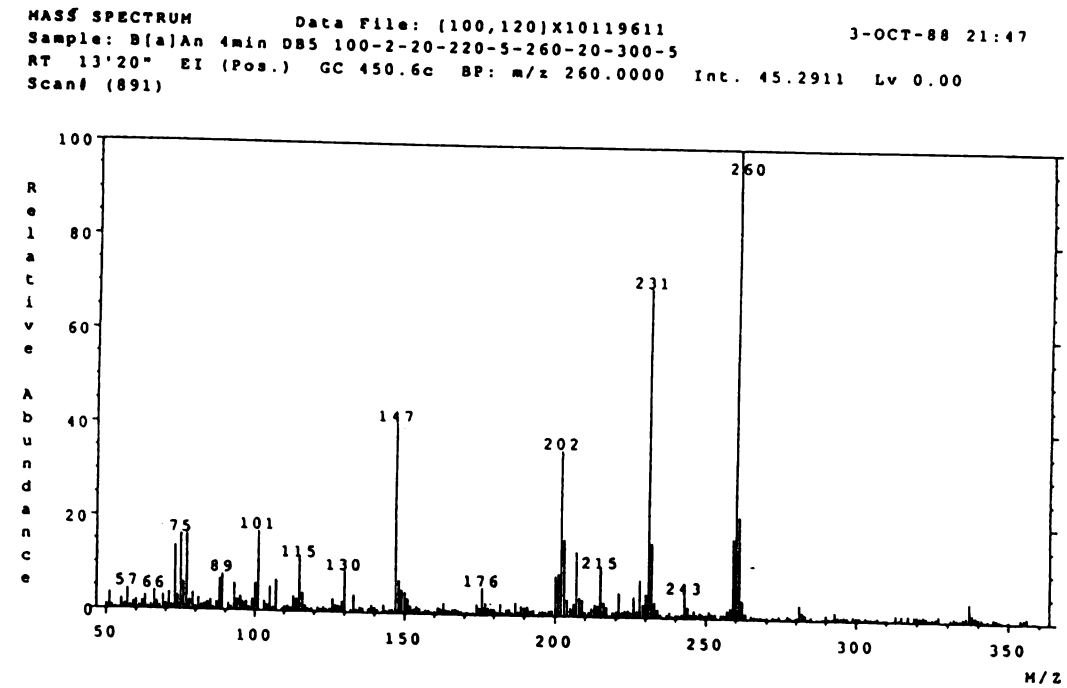
Mass Spectrum of the silylation product X

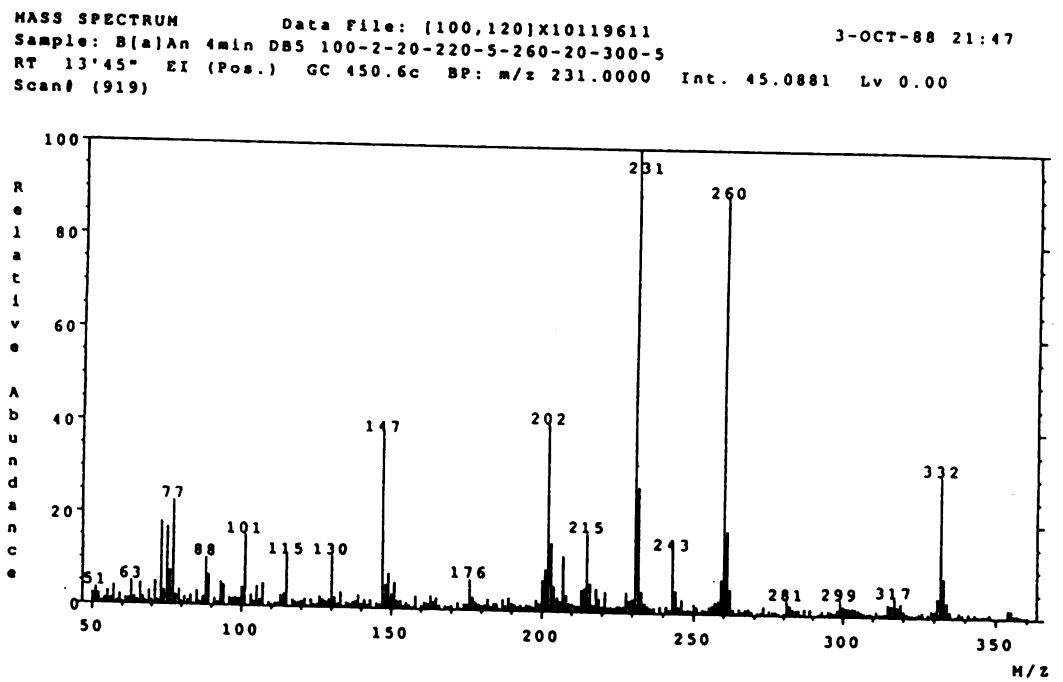


Mass spectrum of benz[a]anthraquinone obtained from NIST database (NIST #: 101274, CAS #: 2498-66-0)



Mass Spectra of the silylation product XI A,B





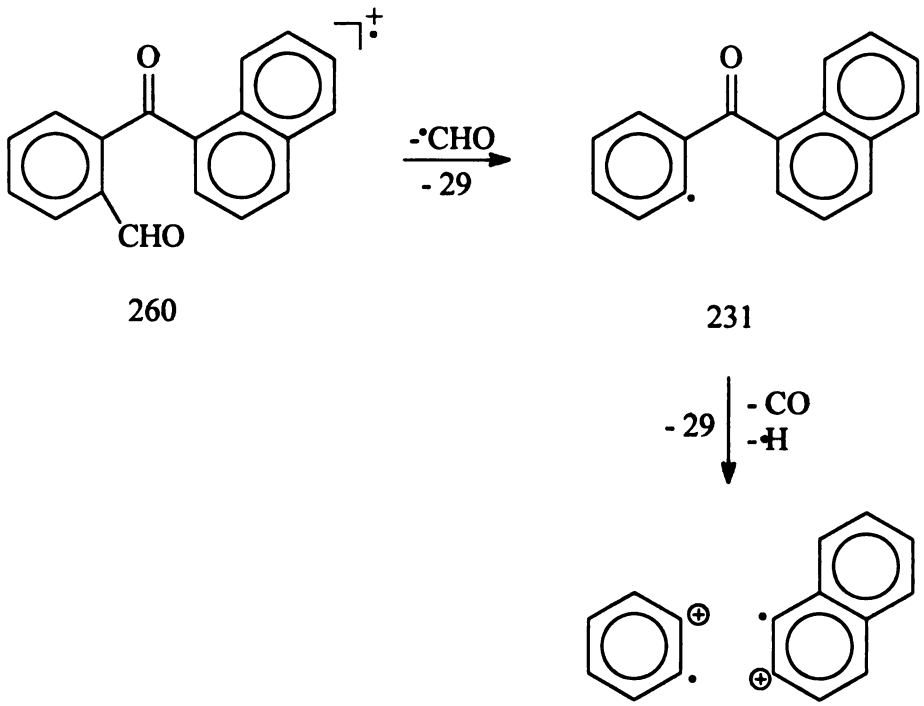
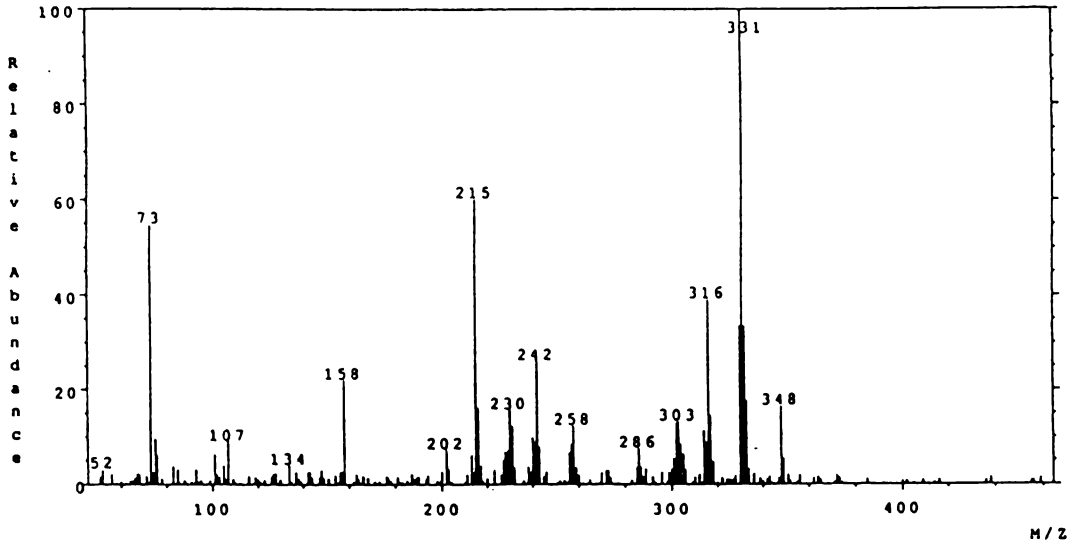


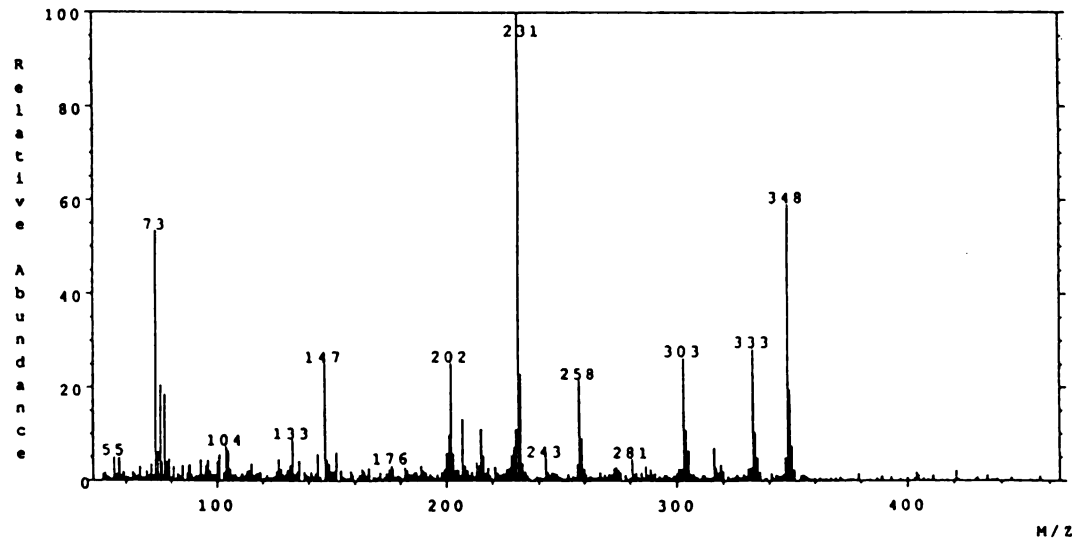
Figure D.9 Interpretation of the mass spectra for the silylation product XI A,B. Molecular ion weight = 260.

Mass Spectra of the silylation product XII A,B

MASS_SPECTRUM Data File: [100,100]X10119611 3-OCT-88 21:47
Sample: B[a]An 4min DB5 100-2-20-220-5-260-20-300-5
RT 15'09" EI (Pos.) GC 450.6c BP: m/z 331.0000 Int. 24.3378 Lv 0.00
Scan# (1012) - (1008, 1015) [coef. 1.00]



MASS SPECTRUM Data File: [100,100]X10119610 3-OCT-88 21:24
Sample: B[a]An 2min DB5 100-2-20-220-5-260-20-300-5
RT 16'00" EI (Pos.) GC 450.6c BP: m/z 231.0000 Int. 53.1997 Lv 0.00
Scan# (1069)



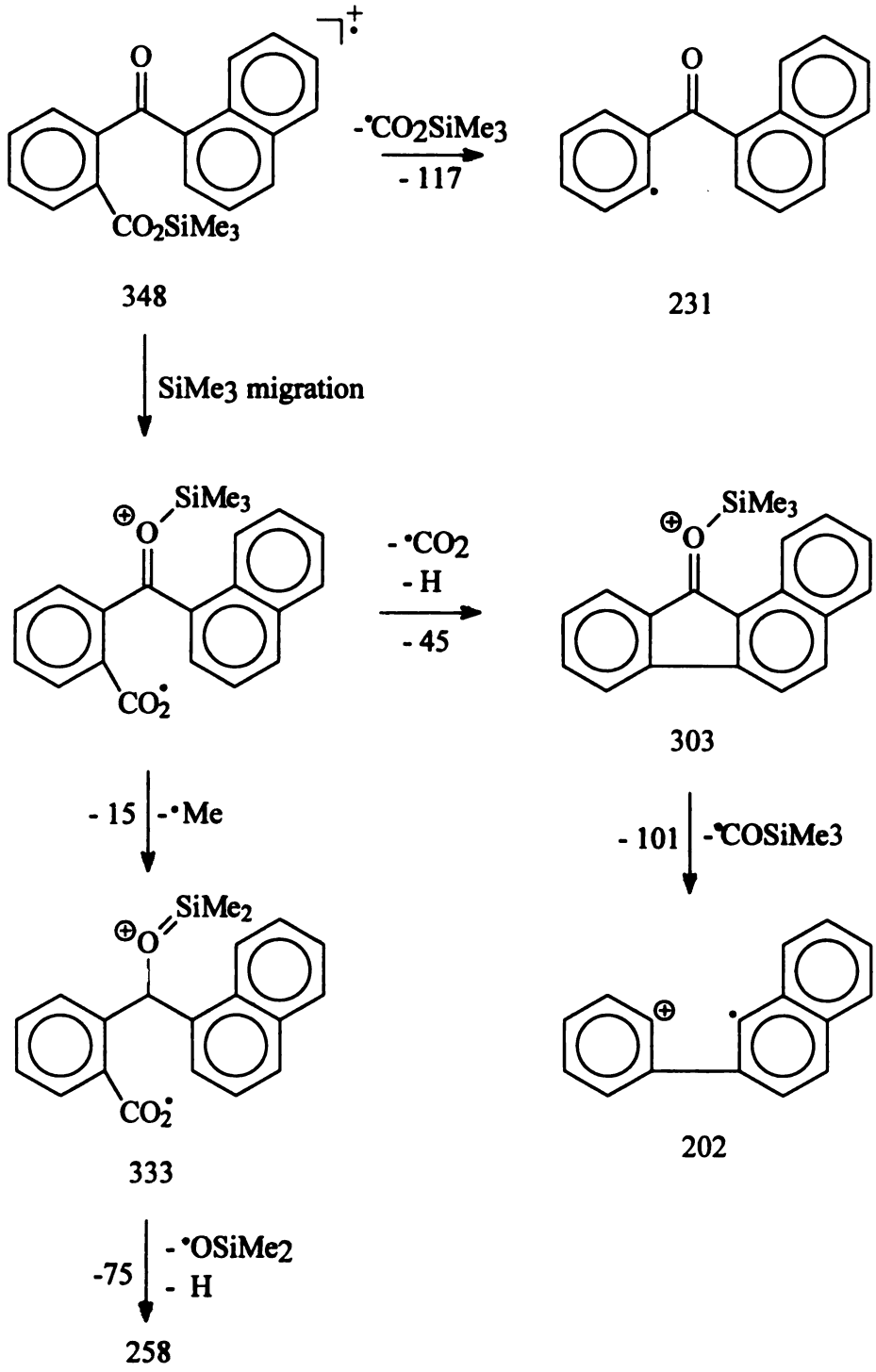
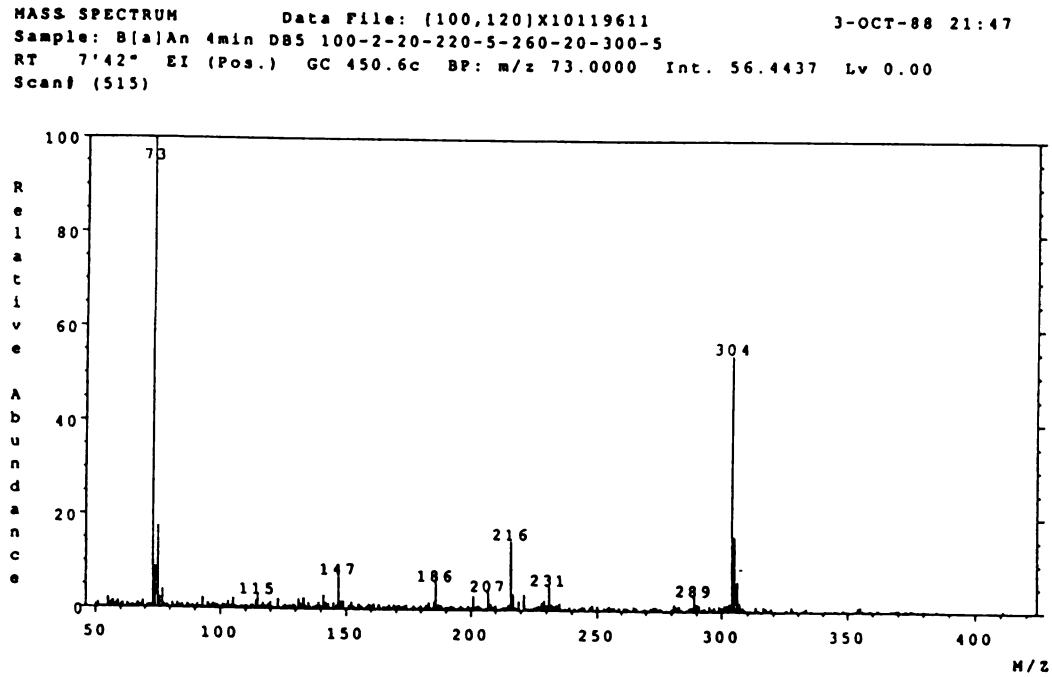
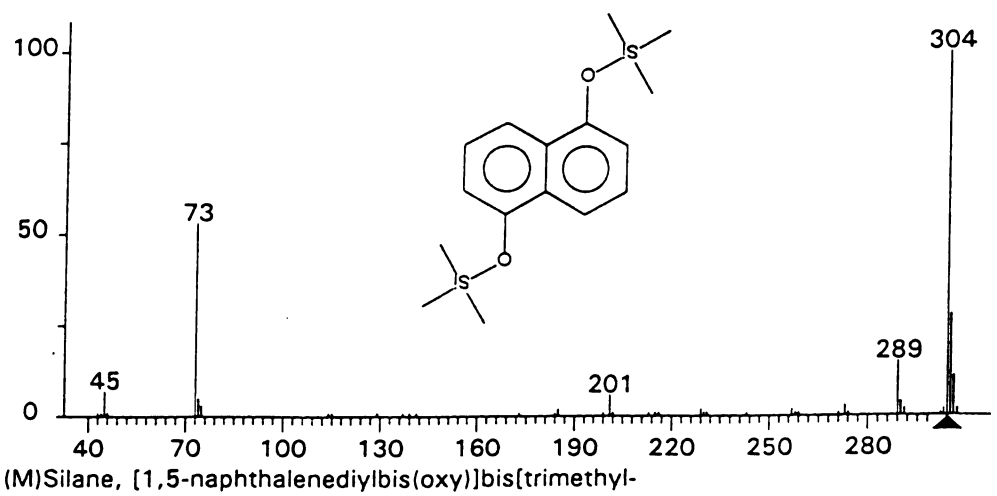


Figure D.10 Interpretation of the mass spectra for the silylation product XII A,B. Molecular ion weight = 348.

Mass Spectrum of the silylation product XIII

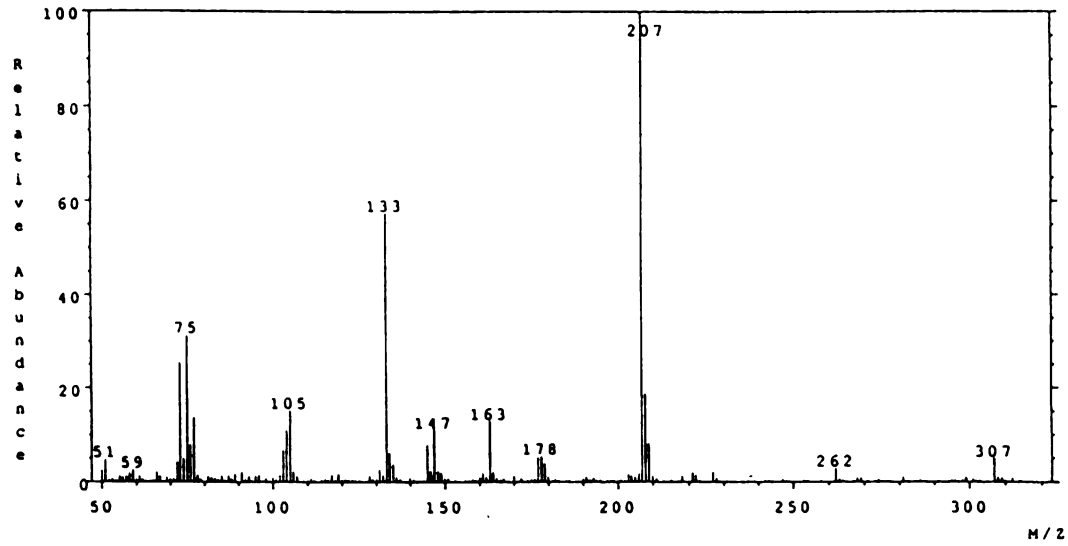


Mass spectrum of silylated dihydroxy-naphthalene obtained from NIST database (NIST #: 40361, CAS #: 1032-28-6)

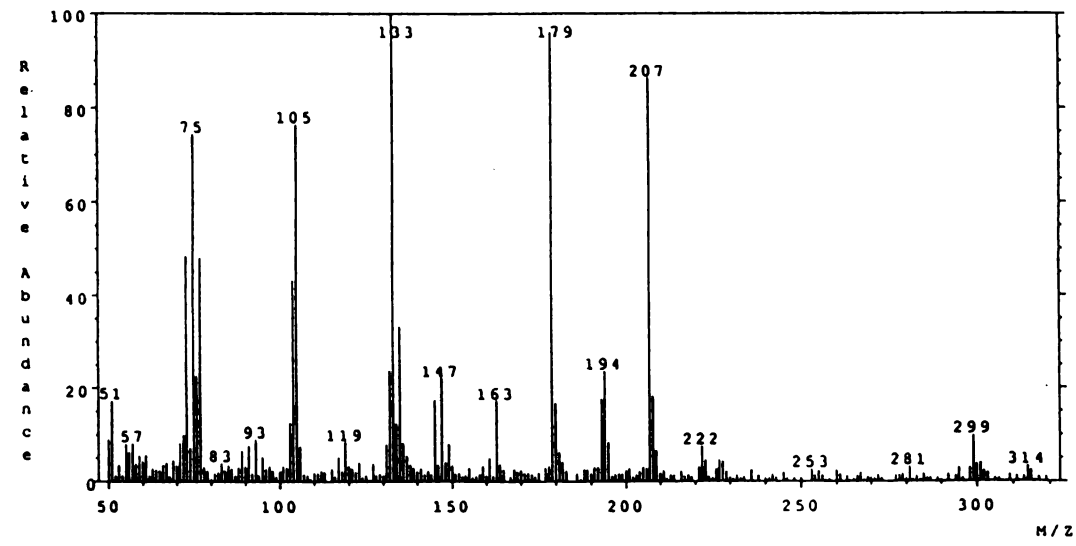


Mass Spectra of the silylation product XIV A,B

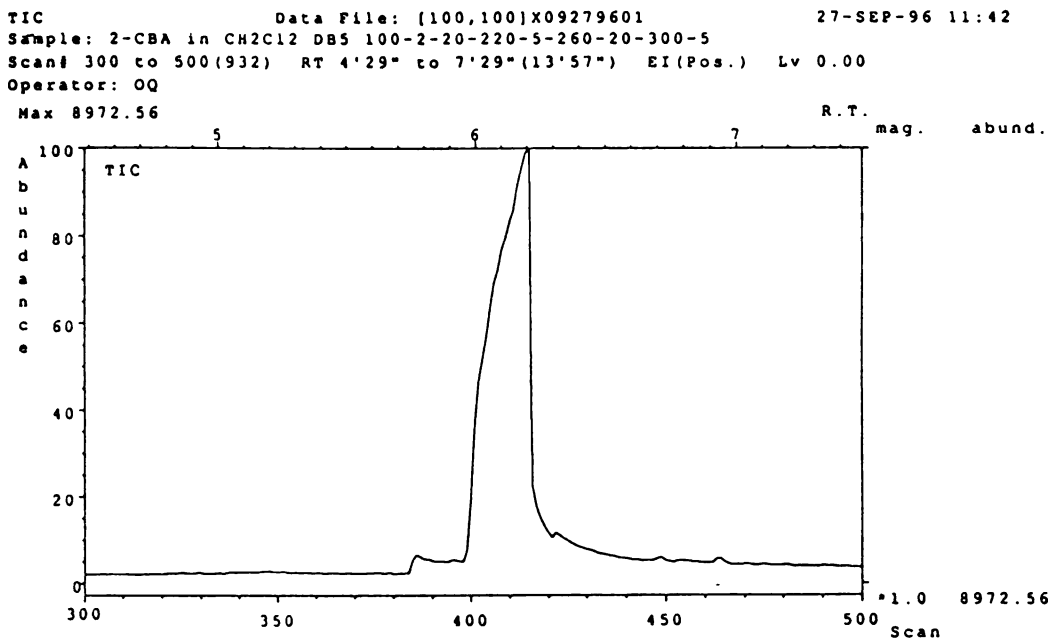
MASS SPECTRUM Data File: [100,120]X10119614 3-OCT-88 22:57
Sample: B[a]An 10min DB5 100-2-20-220-5-260-20-300-5
RT 6'01" EI (Pos.) GC 450.6c BP: m/z 207.0000 Int. 75.2624 Lv 0.00
Scan# (402)



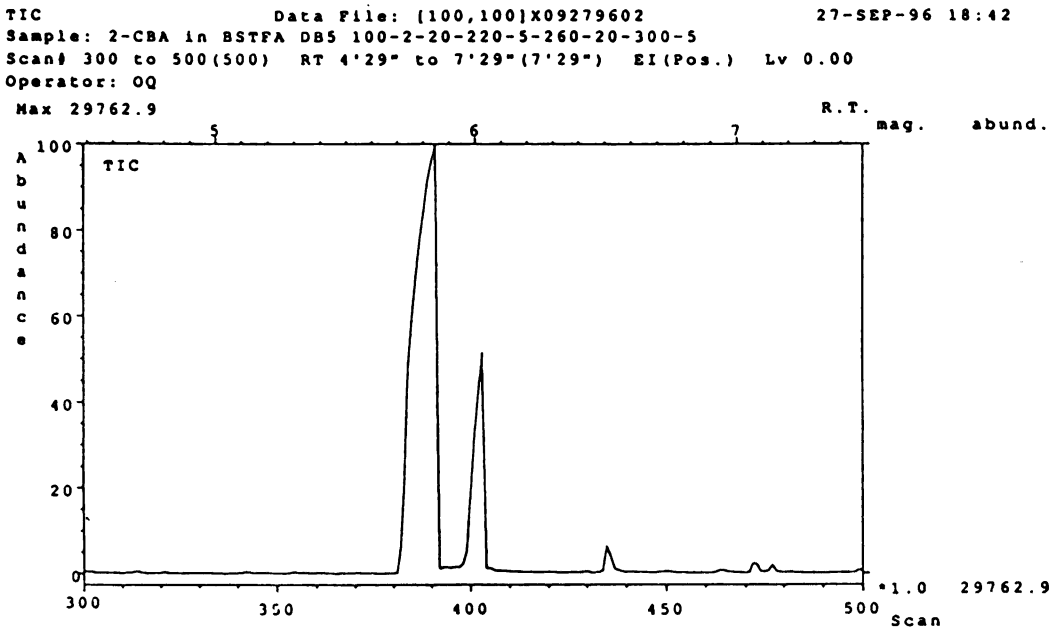
MASS SPECTRUM Data File: [100,100]X10119610 3-OCT-88 21:24
Sample: B[a]An 2min DB5 100-2-20-220-5-260-20-300-5
RT 5'48" EI (Pos.) GC 450.6c BP: m/z 133.0000 Int. 19.5510 Lv 0.00
Scan# (388)



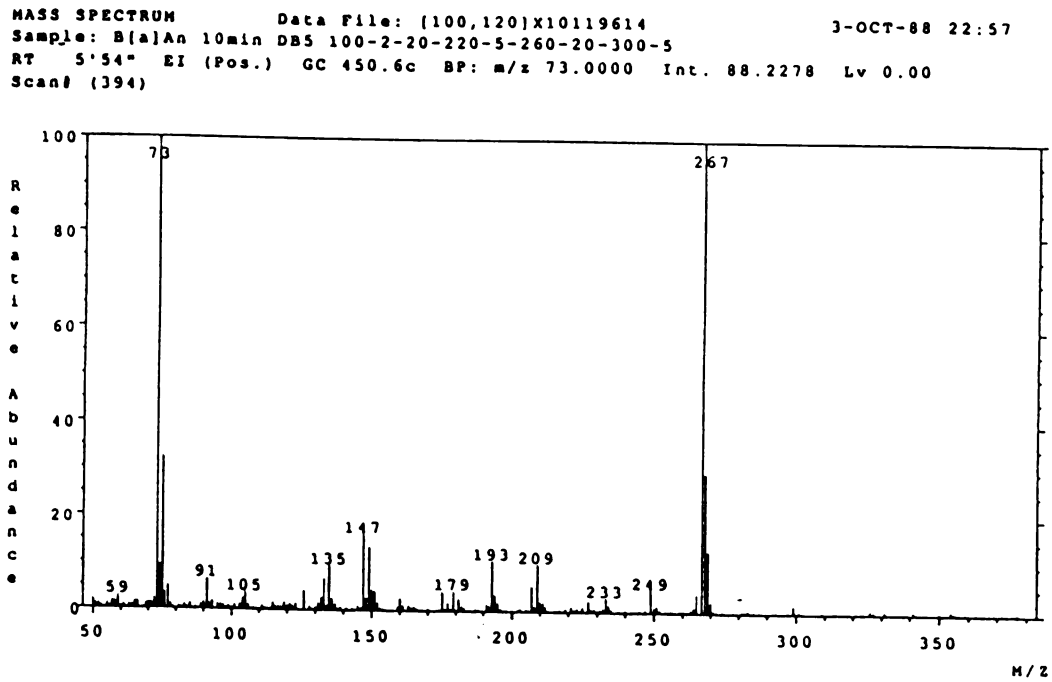
GC chromatograph of 2-carboxybenzaldehyde in pure solvent



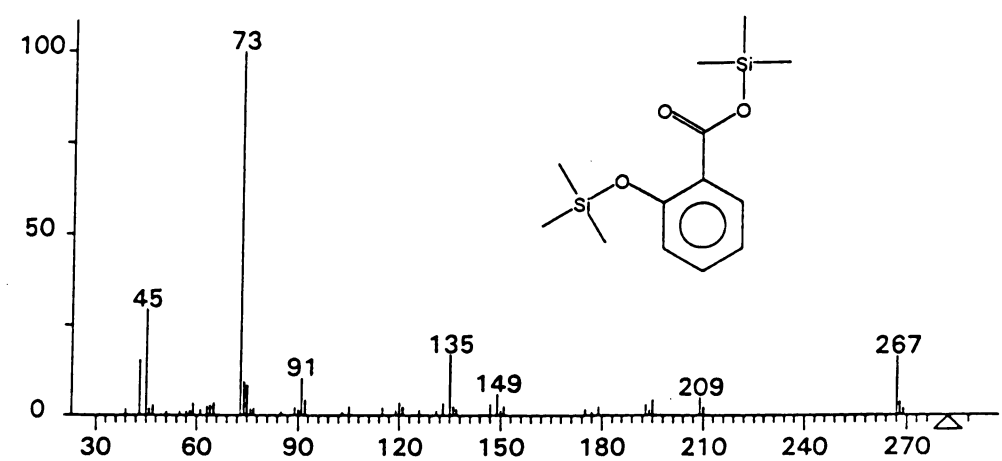
GC chromatograph of 2-carboxybenzaldehyde in silylation reagent



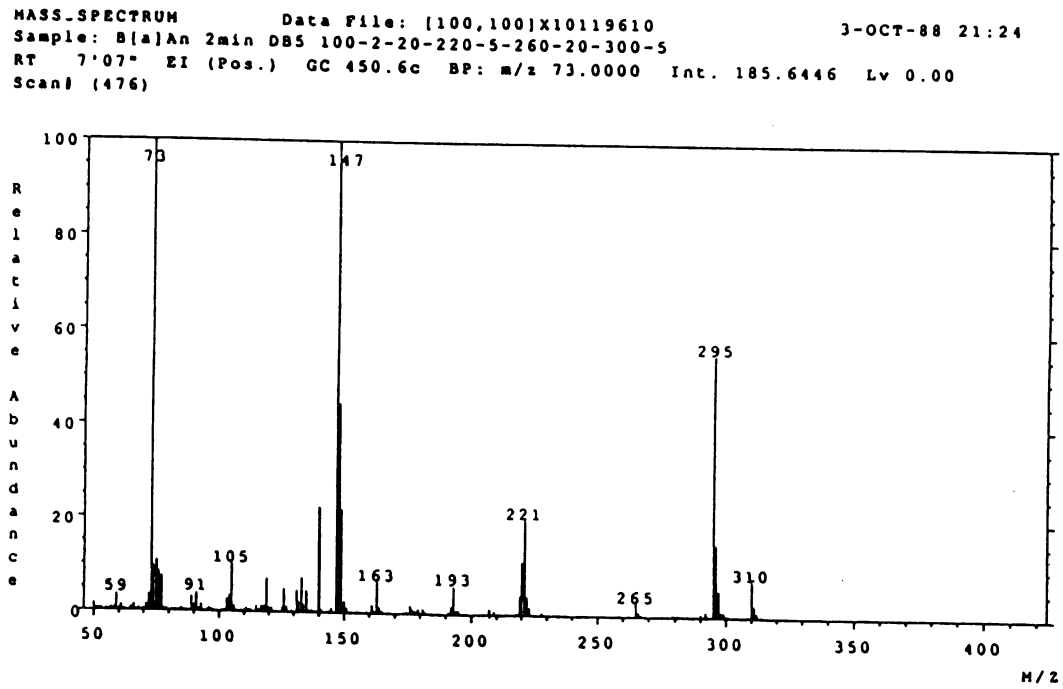
Mass Spectrum of the silylation product XV



Mass spectrum of silylated 2-hydroxybenzoic acid obtained from NIST database (NIST #: 64161, CAS #: 3789-85-3)



Mass Spectrum of the silylation product XVI



Mass spectrum of silylated 2-phthalic acid obtained from NIST database (NIST #: 71607, CAS #: 2078-22-0)