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1-NITROSPYRENE AND 1-NITROSO-8-NITROPYRENE

THE SYNTHESIS AND MUTAGENICITY
OF
1-NITROSO-PYRENE AND 1-NITROSO-8-NITRO-PYRENE

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TITLE: **THE SYNTHESIS AND MUTAGENICITY OF
1-NITROSO-PYRENE AND 1-NITROSO-8-NITROPYRENE**

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ABSTRACT

1-nitropyrene and 1,8-dinitropyrene are environmental pollutants and direct-acting mutagens in bacteria. Studies have shown that reduction of the nitro group is essential for the expression of the mutagenicity of these compounds and the formation of covalent DNA adducts in Salmonella typhimurium. It has also been shown that the corresponding amino compounds are only slightly mutagenic. Since the reduction of nitro compounds to amino compounds must proceed via the nitroso and hydroxylamino intermediates, it has been proposed that the hydroxylamines derived from 1-nitropyrene and 1,8-dinitropyrene are the ultimate mutagens.

1-nitrosopyrene and 1-nitroso-8-nitropyrene were synthesized and reduced to their corresponding hydroxylamines with ascorbic acid. While 1-nitrosopyrene (50,226 rev/nmole) was 50X more potent than 1-nitropyrene (985 rev/nmole) in the Ames Test, 1-nitroso-8-nitropyrene (8,000 rev/nmole) was 10X less potent than 1,8-dinitropyrene (85,830 rev/nmole). The hydroxylamines derived from these compounds proved to be very labile species and could not be characterized.

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic Acid
HPLC	High Pressure Liquid Chromatography
LPLC	Low Pressure Liquid Chromatography
M ⁺	Molecular Ion
Nitro-PAH	Nitrated Derivatives of Polycyclic Aromatic Hydrocarbons
PAH	Polycyclic Aromatic Hydrocarbons
TLC	Thin Layer Chromatography
X.O.	Xanthine Oxidase

1. INTRODUCTION

1.1 Polycyclic Aromatic Hydrocarbons and Their Nitrated Derivatives

Polycyclic aromatic hydrocarbons (PAH) are a class of aromatic compounds consisting of two or more condensed rings (Figure 1). Many of these compounds are known to be by-products of incomplete combustion processes from a wide range of sources, such as gasoline and diesel engines, coal-fired power plants and forest fires(1).

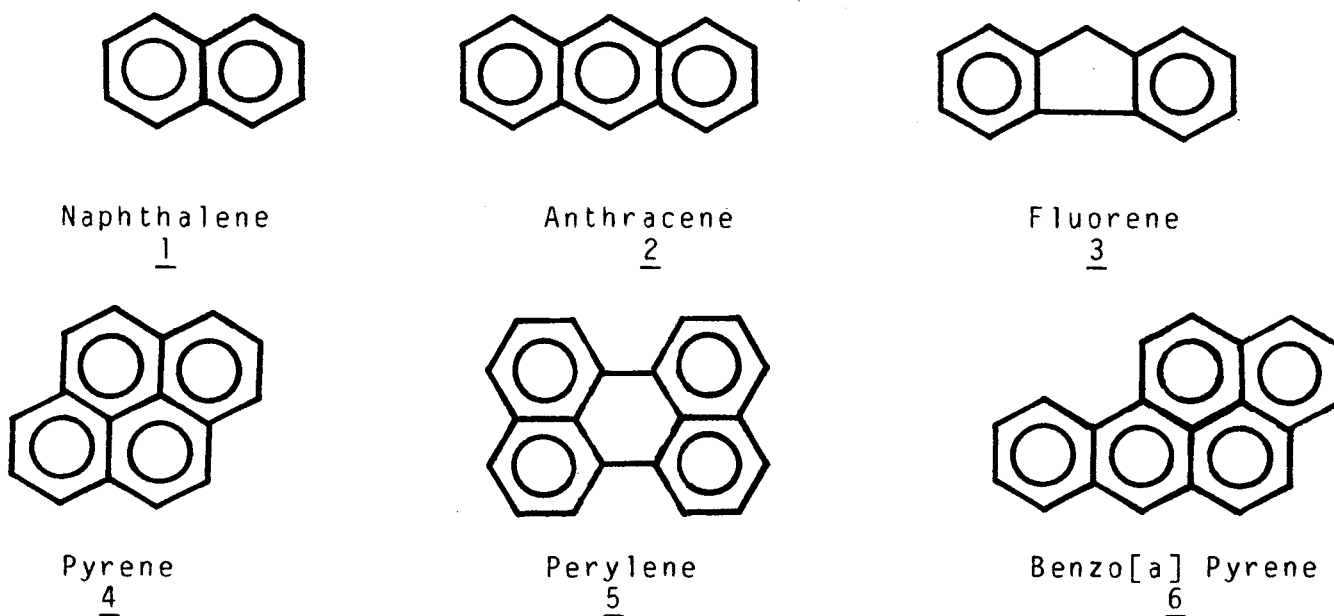


Fig. 1

Because of the demonstrated health risks posed by these compounds, a large scientific effort has been mounted over the past two decades in order to understand the chemical, biological and environmental effects of these compounds. Much of this

work has involved the development of analytical procedures to monitor the levels of these compounds in air and water samples(2). Other research efforts have been directed at studying the mechanisms whereby these compounds exert their effects(3).

Recently, nitrated(4) and oxygenated PAH(5) have been identified in organic extracts of diesel exhaust and urban air particulate; a few examples of these nitrated aromatics are shown in Figure 2. Some of these compounds, in particular 1-nitropyrene 9 and 1,8-dinitropyrene 10 have been shown to be powerful mutagens in bacteria(4). In mammals, these two compounds are reported to be potent carcinogens in preliminary studies(6).

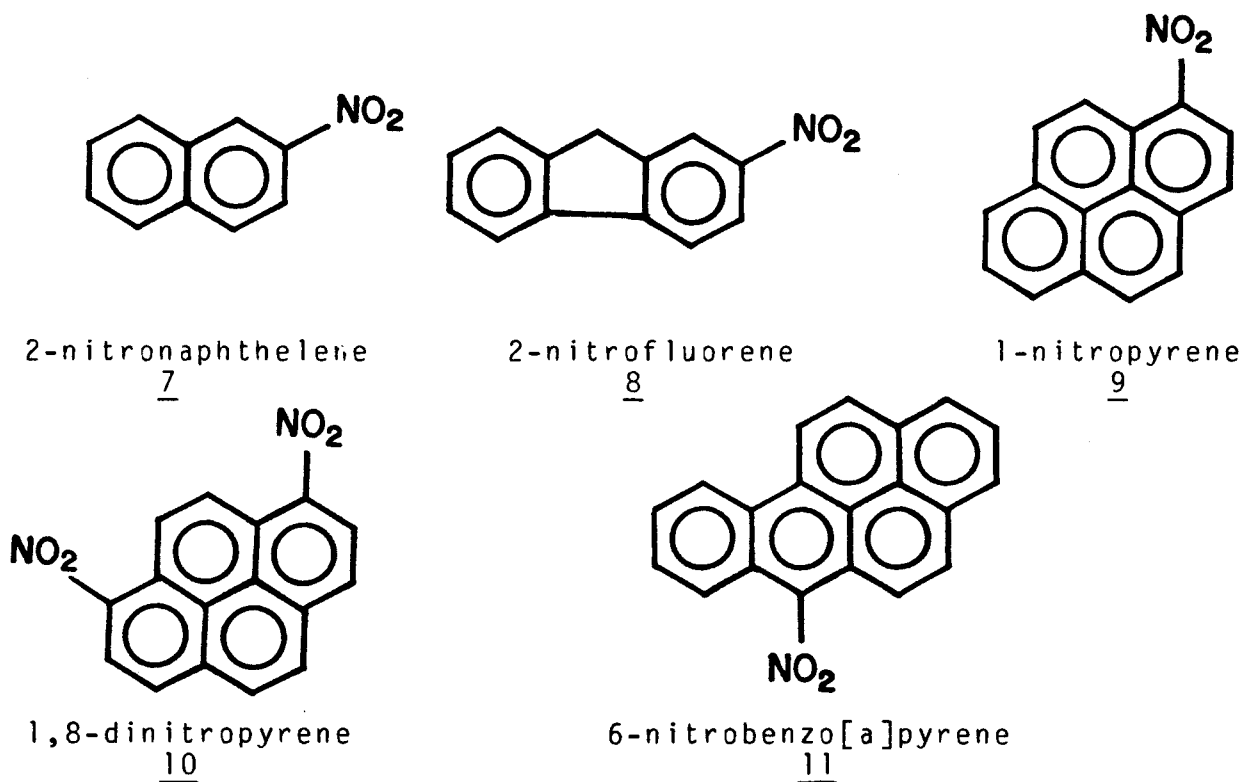


Fig. 2

1.2 The Ames Assay

Mammalian testing of suspected carcinogenic substances involves the use of animals as a model for man. In most of these experiments, the test animal is treated repeatedly with the chemical compound, either by injection or ingestion over a period of time. Tumor initiation is then monitored as a function of dose and time. These experiments are very expensive and time consuming, requiring many animals over the course of 1-2 years. However, Ames and coworkers(7) developed a bacterial mutagenicity test that is now widely used as a rapid screening technique for compounds suspected of having carcinogenic activity. While there is no necessary correlation between a compound's mutagenicity in bacteria and its carcinogenicity in animals, of 300 compounds which gave positive responses in the Ames test, 87% were found to be carcinogenic in animals(8).

The test employs a variety of mutant strains of the bacterium Salmonella typhimurium, one of which lacks the ability to synthesize histidine, an essential amino acid. When placed on a standard agar plate (which lacks histidine), these organisms do not grow. However, on exposure to chemical mutagens or to ultraviolet light, a given bacterium may suffer a mutation in the histidine gene such that it can now synthesize histidine. From this "reverted" cell which can now grow on this medium, a colony of cells develops; after a period of 48 hours, the number of colonies on the plate is determined. Since there will always be some spontaneous

reversions, all data is reported as the number of revertants per nmole of substance above this background level. Even though methods and procedures have been carefully standardized, most compounds exhibit some degree of variability in their responses upon repeated testing under the same conditions. Qualitatively, the test is a good indicator of relative mutagenic potencies (Table I).

Chemical compounds can be divided into two classes, depending on whether the addition of oxidative enzymes to the test plate is required to elicit a mutagenic response. Chemicals that cause mutations in the absence of oxidative enzymes are called "direct-acting", whereas those that require this addition are called "indirect-acting" mutagens. For example, alkylating agents, such as dimethyl sulfate $(\text{CH}_3)_2\text{SO}_4$ (which reacts directly with DNA), behave as "direct-acting" mutagens, while PAH such as anthracene 2 and benzo[a]pyrene 6 are of the "indirect-acting" type. As a class, nitro-PAH can exhibit both direct and indirect mutagenicities. However, the observation that some potent direct-acting nitro-PAH are present in diesel exhaust extracts(9), prompted a great deal of research interest directed towards examining their mutagenicity in bacteria, their carcinogenicity in animals, metabolism and their mode of action.

TABLE I

a) **Mutagenicity of Different Chemicals in Salmonella typhimurium strain TA98**

Chemical	Revertants per nmole(6)
Benzo[a]pyrene <u>6</u>	376*
6-nitrobenzo[a]pyrene <u>11</u>	1
proflavin <u>12</u>	1
1,9-diaminoacridine <u>13</u>	10
2-nitronaphthalene <u>7</u>	1
2-nitrofluorene <u>8</u>	18
1-nitropyrene <u>9</u>	1,000
1,8-dinitropyrene <u>10</u>	73,000

b) **Correlation of Animal Carcinogenicity and Bacterial Mutagenicity (1)**

Class of Compounds	Known mutagens detected as carcinogens (carcinogen/mutagen)
Aromatic amines	23/25
Alkyl halides	17/20
PAH	26/27
nitroaromatics	28/28
Nitrosamines	20/21
Azo dyes and diazo compounds	11/11

* requires activation by oxidative enzymes

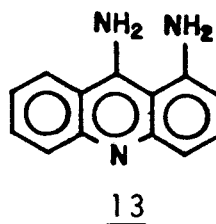
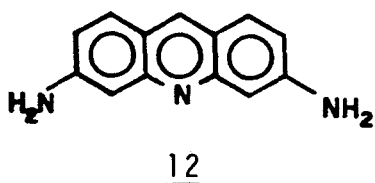
1.3 Mammalian Testing of Nitroaromatics

Thus far, studies on cultured mammalian cells have shown a pattern similar to that of the bacterial assays(10). In animals, it has been shown that some nitro-PAH, upon repeated application, induce tumors in the test animals(11). In some tests, the order of carcinogenicities of those compounds in a given animal did not correlate with their order of mutagenicities in the Ames test. However, there is insufficient data as yet to make a good correlation between the incidence of tumor induction in animals and mutagenicity in bacteria.

1.4 Basis for the Mutagenicity of Nitro-PAH

While specific mechanisms leading to mutations in bacteria and tumor induction in mammals are a matter of conjecture, it is generally felt that the mutagenicity and carcinogenicity of chemical compounds results from the interaction of these compounds (or their metabolites) with DNA. These interactions can be classified into one of two general types:

- 1) Non-covalent interactions such as the intercalation of planar aromatic compounds (e.g. proflavin 12 and 1,9-diaminoacridine 13) between the bases of the DNA helix.



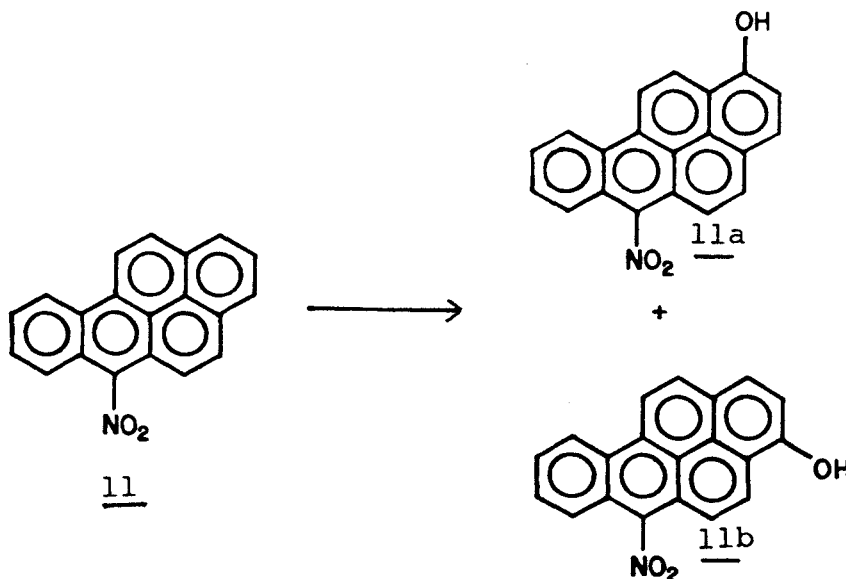
While some nitroaromatics are capable of intercalation with DNA, it has been demonstrated that nitroarenes do not react directly with purified DNA to give covalent adducts (13). However, covalent DNA adducts have been observed when these compounds are incubated with Salmonella typhimurium (14). These findings indicate that nitroarenes are transformed by the bacteria into reactive intermediates that interact covalently with DNA.

1.5 Metabolism of Nitro-PAH

Two pathways have been recognized for the metabolism of nitro-PAH: oxidative and reductive pathways.

i) Oxidative metabolism:

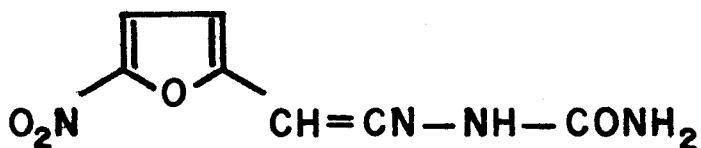
The in vitro metabolism of 6-nitrobenzo[a]pyrene 11 was shown to produce hydroxylated derivatives, mainly the 1-hydroxy-6-nitro- 11a and the 3-hydroxy-6-nitro- 11b isomers (15).



It has been suggested that, as in the case of benzo[a]pyrene 6, the nitro-PAH 11 is converted to an epoxide by the oxidative enzymes. The epoxide then rearranges in water to give the phenolic products 11a and 11b.

ii) Reductive metabolism:

Reduction of the nitro group has been shown to be essential for the expression of the mutagenicity of several nitro-substituted mutagens (16), such as 5-nitrofurans (e.g. nitrofurazone 5-NF). In bacteria, this reduction is done by enzymes called nitroreductases. Bacterial strains deficient in these enzymes are mutagenized to a much lesser extent by the same compounds (17).



5-NF

The metabolism of 1-nitropyrene 9 by *Salmonella typhimurium* has been shown to proceed by this pathway. Following the incubation of 1-nitropyrene 9 with the bacteria, 1-aminopyrene 20 and 1-N-acetylamino pyrene 21 were identified as metabolites (18). Both of these compounds are formed as a result of reduction of the nitro group, and both are weak, indirect-acting mutagens. The step-wise reduction of the nitro-group to the amino group proceeds via the nitroso and N-hydroxylamino intermediates 18 and 19 (Figure 4).

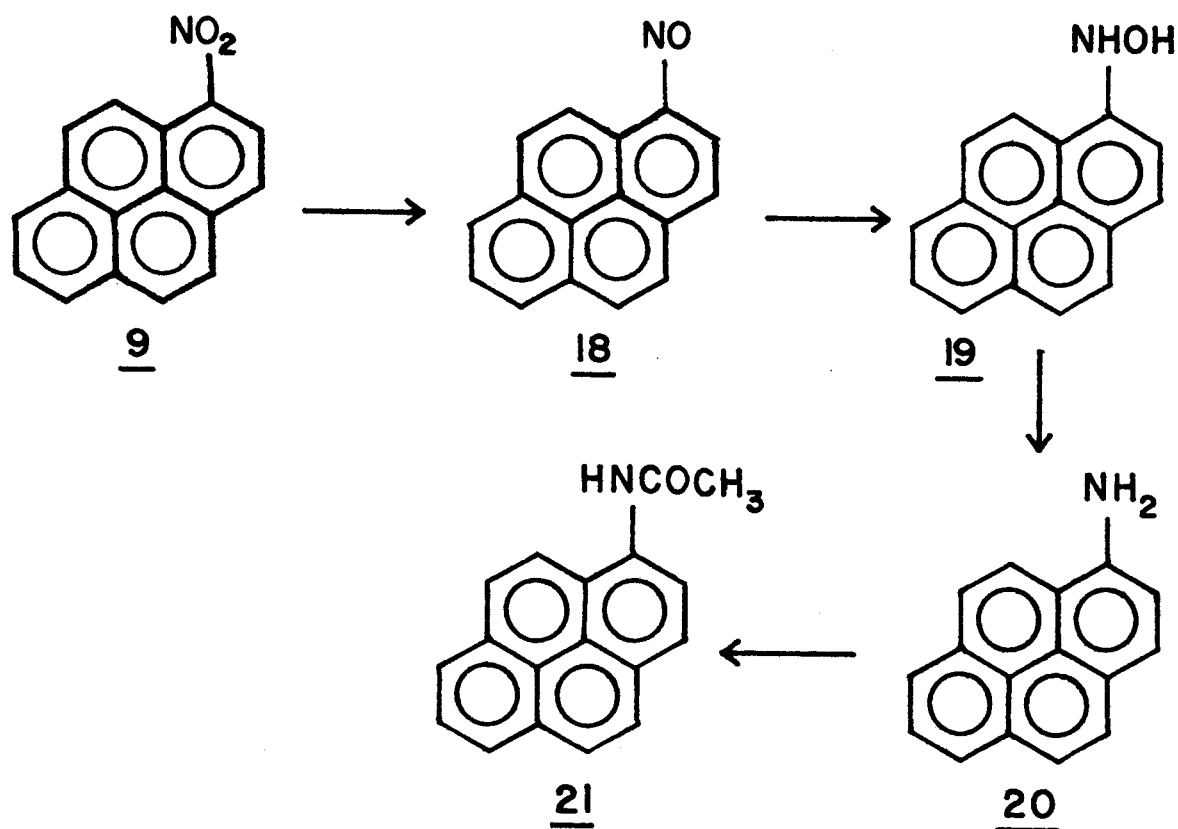
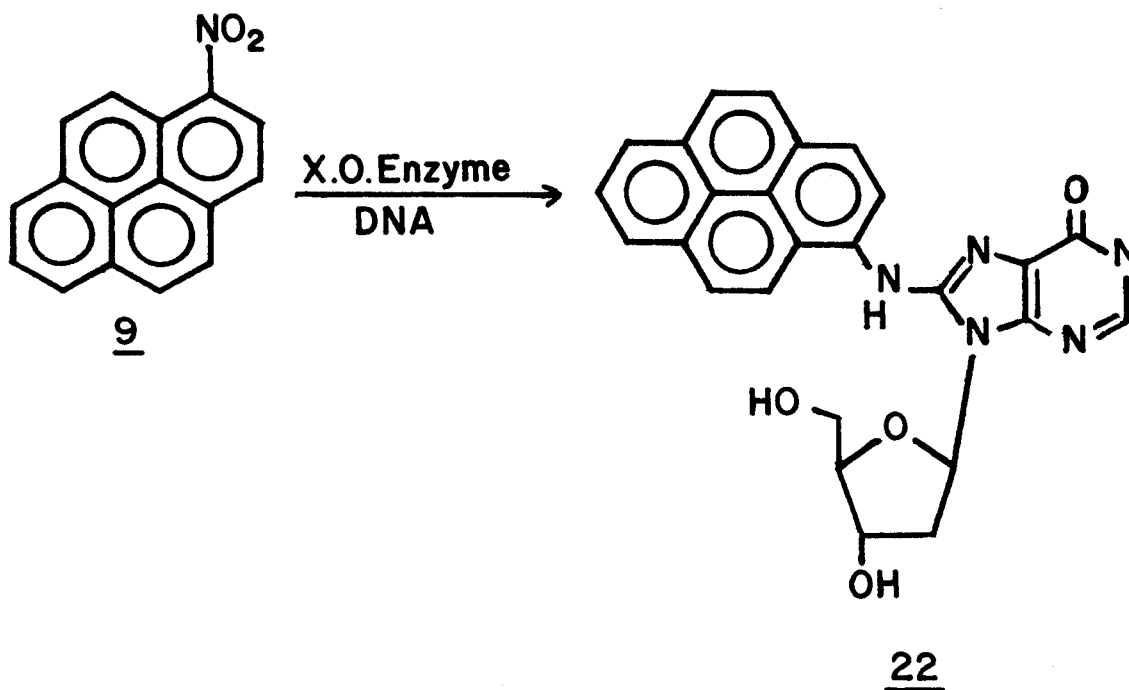


FIG. 4

As yet, no one has reported the presence of either reduction intermediates 18 and 19 in a bacterial system treated with 1-nitropyrene. Howard et al(19) used the enzyme xanthine oxidase to catalyze the in vitro reduction of 1-nitropyrene 9 in the presence of DNA. After hydrolysis of the DNA, they isolated a guanosine adduct identified by mass spectrometry and $^1\text{H-nmr}$ as N-(deoxyguanosin-8-yl)-1-aminopyrene 22.



In this adduct, the pyrene nitrogen is at the hydroxylamine oxidation level, indicating that the adduct may have been formed directly from the hydroxylamine 19 or indirectly by reaction with 1-nitrosopyrene 18 followed by reduction. It has been proposed(19) that the N-hydroxylamine 19 is the proximate mutagen involved in the covalent binding with DNA.

Metabolism studies with 1,8-dinitropyrene 10, one of the most potent direct-acting mutagens in Salmonella typhimurium, have revealed a reductive pattern similar to that observed for 1-nitropyrene 9, (Figure 5). The 1-amino-8-nitro 25, 1,8-diamino 27 and 1-N-acetylamino-8-amino 28 derivatives have been isolated and identified(18). All of these derivatives show substantially lower mutagenic activities compared to 1,8-dinitropyrene 10. Neither the 1-nitroso-8-nitro 23 nor the 1-N-hydroxylamino-8-nitro 24 derivatives have been detected in extracts of Salmonella typhimurium incubations with 1,8-dinitropyrene. In a similar fashion to the 1-nitropyrene case, the expression of the mutagenicity of 1,8-dinitropyrene 10 has been proposed to involve the N-hydroxylamine intermediate 24 as the reactive species in the covalent binding to DNA.

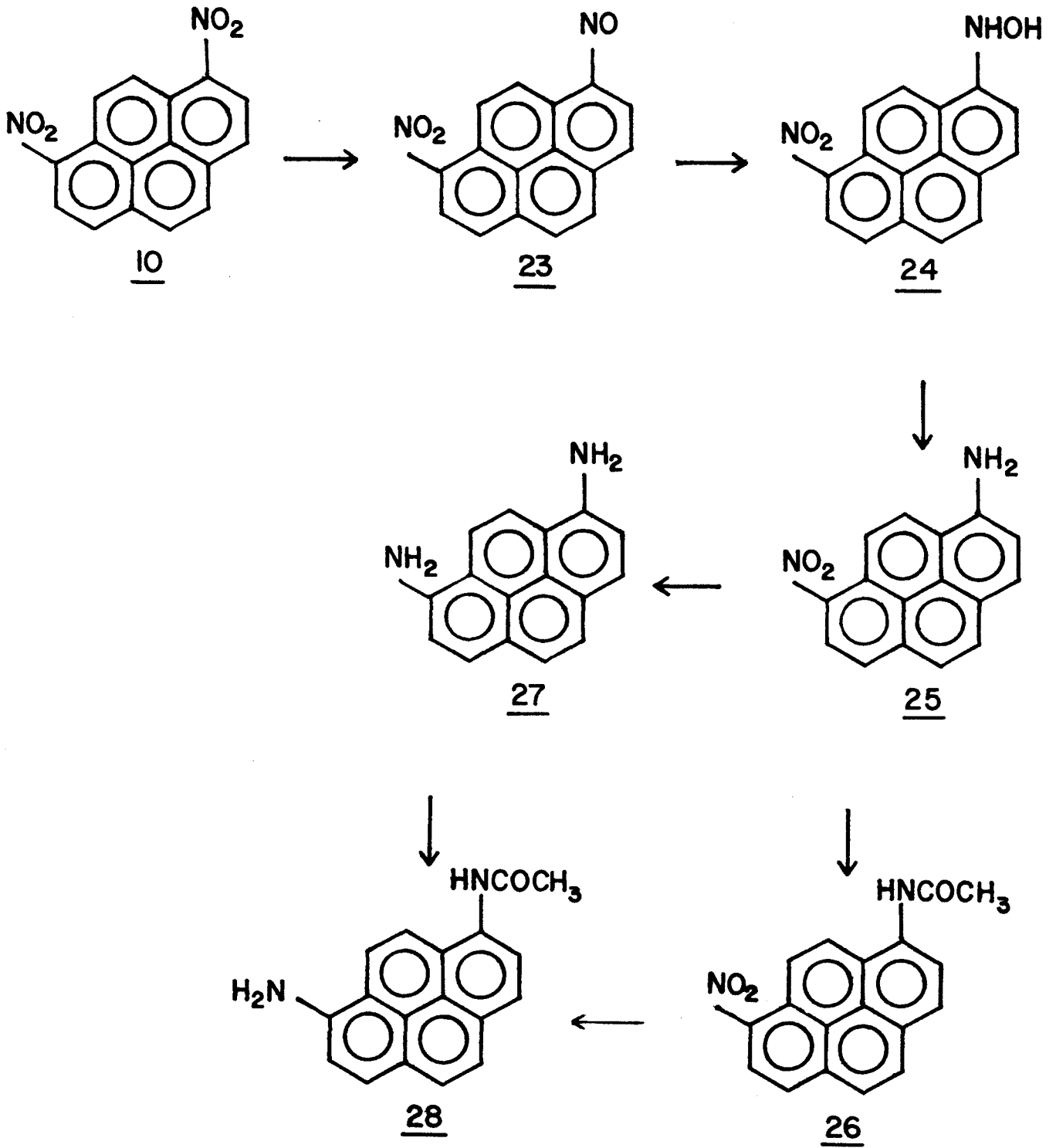


FIG. 5

1.6 The Chemistry of Nitroso and N-hydroxylamino Aromatics

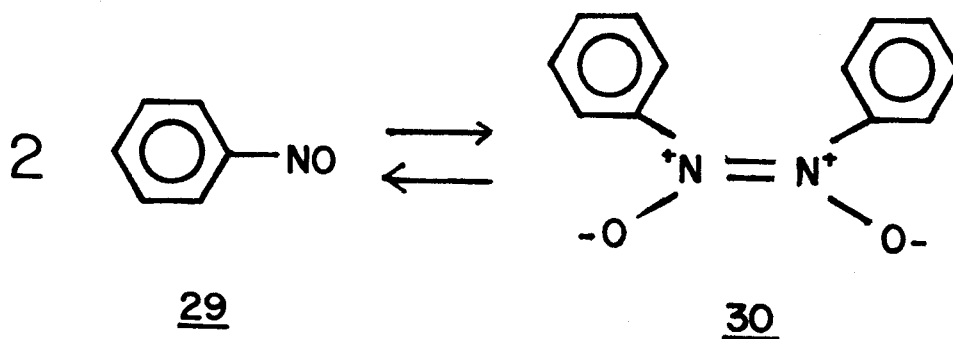
Most of the chemistry of nitroso and N-hydroxylamino substituted aromatics is based upon experience with derivatives of benzene, naphthalene 1 and fluorene 3; little work has been reported on polynuclear aromatic derivatives. Standard preparative methods give poorer yields of these compounds as the number of aromatic rings increases; presumably, the reactivity of these nitroso and hydroxylamino groups is greater than that observed in the smaller ring derivatives(20).

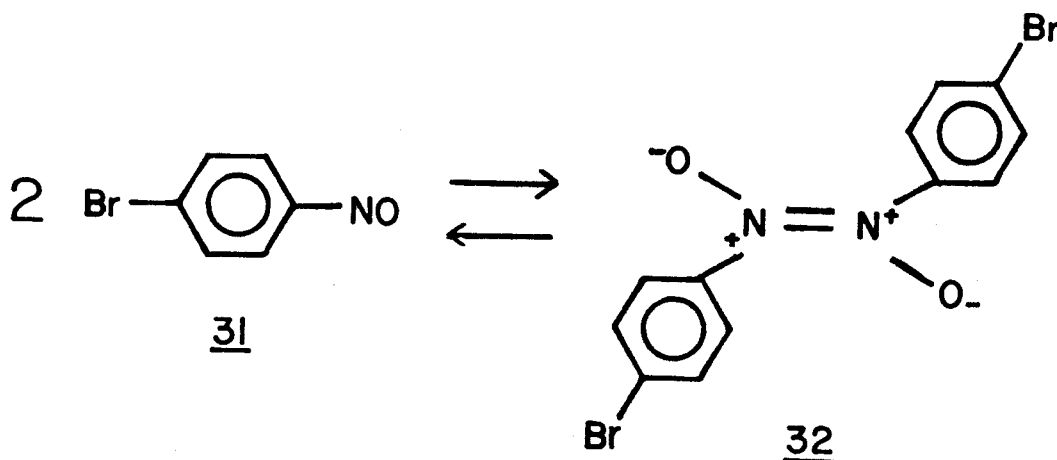
1.6.1. The Nitroso Group

The nitroso group in aromatic systems exhibits a wide range of reactions including dimerization, oxidation, reduction, condensation with amines and hydroxylamines, and photochemical reactions.

1.6.1.1. Dimerization

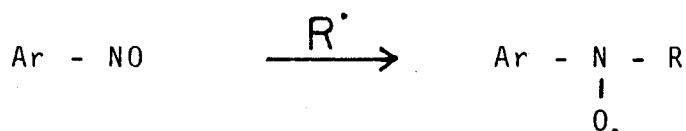
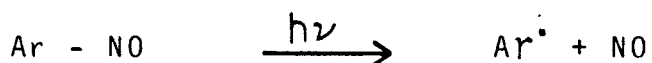
Nitroso compounds dimerize readily in solution. Nitrosobenzene 29, for example, dimerizes predominantly in a cis form 30, while the p-bromo substituted derivative 31 dimerizes in the trans form 32 (21).





1.6.1.2. Photolysis and Radical Formation

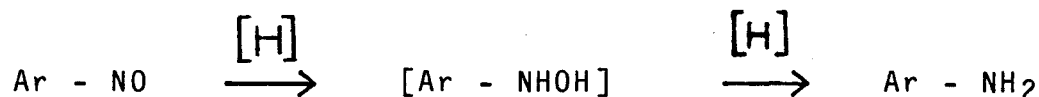
Nitroso compounds readily undergo photolysis and reaction with radical species to generate nitrogen oxide and nitroxide radicals (22).



Ar = Aromatic

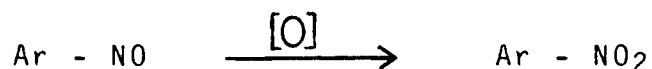
1.6.1.3. Reduction

Aromatic nitroso compounds are easily reduced to the corresponding amines by catalytic hydrogenation (23), and by metal/acid reductions such as tin or zinc in hydrochloric acid (24). These reductions proceed via the hydroxylamine which may be isolated in some cases.



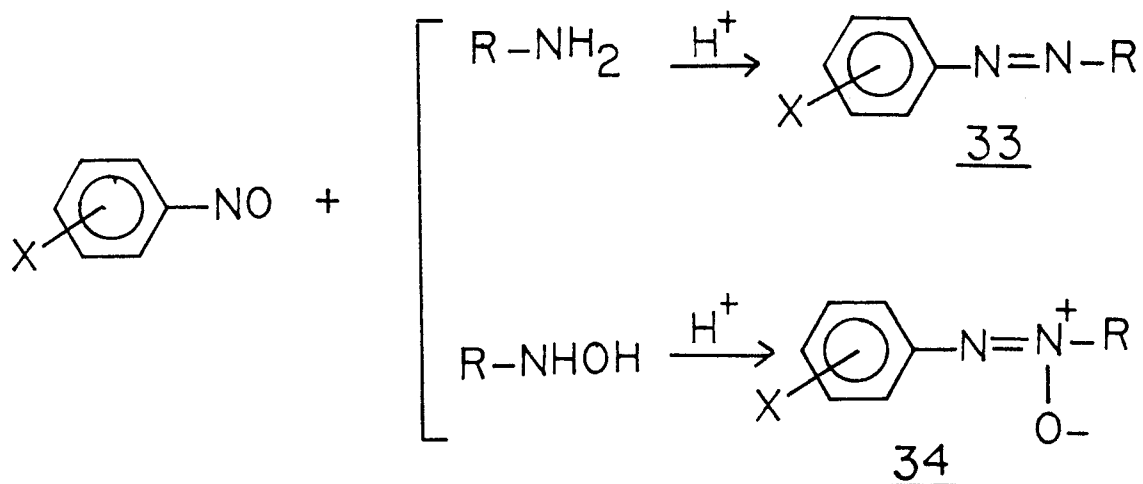
1.6.1.4. Oxidation

Many oxidizing reagents such as peracids, peroxides and chromium (VI) reagents convert the nitroso group to the nitro group (25).



1.6.1.5. Reaction with Amines and N-hydroxylamines

As electrophiles, nitroso compounds react under acid catalysis with primary amines to give azo compounds 33 and with N-hydroxylamines to give azoxy products 34 (21).

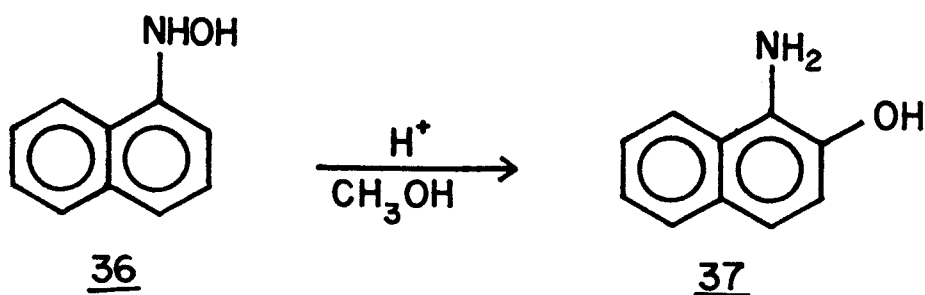


1.6.2 The N-Hydroxylamino Group

The N-hydroxylamine group, on the other hand, is generally more reactive than the nitroso group, readily undergoing rearrangements, oxidations, reductions and disproportionation reactions.

1.6.2.1. Reactions in Protic Solvents

N-Hydroxylamino aromatics undergo the Bamberger rearrangement in aqueous and protic solvents, or solvents where traces of acid are present to give the corresponding hydroxy-aromatic amines (26). For example, 1-N-hydroxylamino-naphthalene 36 rearranges in methanol under acid catalysis to 1-amino-2-hydroxy-naphthalene 37.

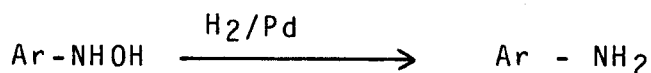


1.6.2.2. Reactivity Towards Oxygen

In solution in the presence of oxygen, N-hydroxylamino arenes undergo oxidation very easily to give the corresponding nitroso and nitro derivatives (27).

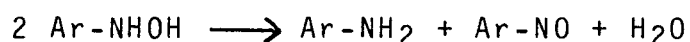
1.6.2.3. Reactivity Towards Reducing Agents

The reduction of N-hydroxylamino aromatics to amino aromatics is very facile and can be readily effected by reducing agents such as Zn or H₂/Pd (28).



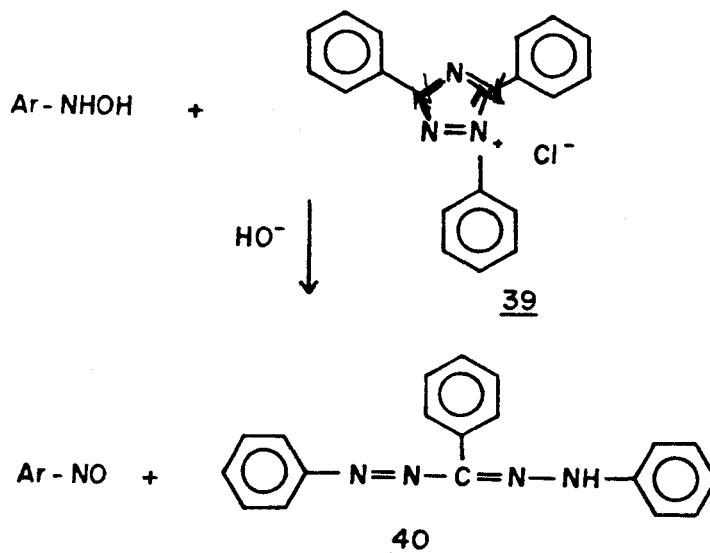
1.6.2.4. Disproportionation Reactions

In addition to being easily reduced and oxidized, these compounds undergo disproportionation reactions to give the amino and nitroso derivatives which, in some cases, condense to the azoaromatic derivative(29).



1.6.2.5. Reaction with Tetrazolium Salts

N-Hydroxylamines reacts instantaneously with 2,3,4-triphenyl-tetrazolium chloride 39 in the presence of base to afford a deep red precipitate, the formazan 40. Since this reaction is very selective towards the N-hydroxyl group even in the presence of the nitro, nitroso, amino, azo and azoxy groups(20), it is commonly used to detect the presence of hydroxylamines in reaction mixtures.



1.7 The "Hydroxylamine" Hypothesis

In case of 1-nitropyrene and 1,8-dinitropyrene it has been proposed(6) that the hydroxylamino intermediates formed by reduction of the nitro group are the proximate mutagens involved in covalent binding to DNA. In support of this hypothesis are the following:

- i) Reduction of the nitro group is essential for the expression of the mutagenicity of these nitro-PAH.
- ii) The corresponding amino and N-acetylamino compounds are only slightly mutagenic and cannot account for the high degree of potency of the parent nitro-PAH.
- iii) The hydroxylamino group is more reactive than the nitroso group, and therefore the hydroxylamino intermediates are expected to be more reactive towards DNA as compared to the nitroso intermediates.

If this hypothesis is correct, it should follow that 1-nitroso-pyrene 18 and 1-N-hydroxylaminopyrene 19 are equally, if not more, mutagenic than 1-nitropyrene 9 in the Ames test. Similarly, the 1-nitroso-9-nitropyrene 23 and 1-N-hydroxylamino-8-nitropyrene 24 should exhibit the same degree of potency as 1,8-dinitropyrene 10.

The main objective of this work is to synthetically prepare the nitroso and N-hydroxylamino derivatives of both 1-nitropyrene and 1,8-dinitropyrene and then evaluate their mutagenicities.

1.8 Synthetic Routes to Nitroso and N-hydroxy Aromatics

The reduction of the nitro group to the amino group is one of the most facile reactions in organic chemistry and can be accomplished by a variety of reagents(30). Metals such as zinc, tin and iron are commonly used as the reducing agents. Hydrogenation over palladium and platinum catalysts is also useful and produces the same results. Usually, the reduction proceeds rapidly to give the amino adduct and only under special conditions is it possible to obtain good yields of the nitroso and hydroxylamino intermediates.

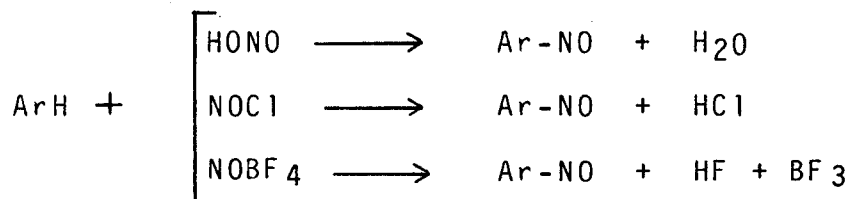
In terms of oxidation, the amino group can be oxidized to the nitro group using different oxidizing reagents. Again, depending on the conditions employed, it is possible to stop the oxidation at an intermediate stage. This has been investigated in benzene and its substituted derivatives; little work has been done on polynuclear aromatics. For both the oxidation of amino-PAH and reduction of nitro-PAH, it is believed that as the number of rings in a given aromatic system increases, the more difficult it becomes to stop the oxidation or the reduction at an intermediate stage(20).

In brief, the methods that can be used to prepare nitrosoarenes include:

1. Nitrosation

One of the older methods of preparing nitrosoaromatics

is the nitrosation of aromatics with nitrous acid(31). Recently, nitrosyl chloride (NOCl) and nitrosonium tetrafluoroborate (NOBF₄) have been employed as nitrosating agents(32).



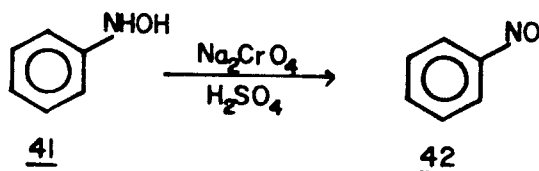
Ar = Aromatic

2. Reduction of Nitroaromatics

It has been reported (33) that the reduction of dinitroaromatics to the corresponding nitro-nitroso derivatives can be done by stannous oxide in alkaline alcoholic solvents. It has been also noted that the best yields of the nitroso adduct were obtained in neutral media.

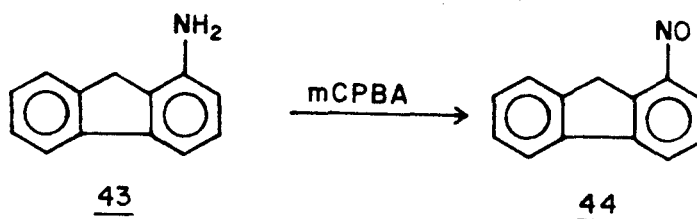
3. Oxidation of Aromatic N-hydroxylamines

Sodium dichromate in sulfuric acid oxidizes phenylhydroxylamine 41 to nitrosobenzene 42 in 70-80% yield(34). Other reagents that are known to effect the same oxidation are mercuric oxide, potassium ferricyanide and periodic acid.



4. Oxidation of Aromatic Amines

Potassium persulfate in sulfuric acid, known as Caro's acid, have been used successfully to oxidize aromatic amines to the corresponding nitroso compound(35). Peroxy acids have been also used to do the same oxidation; such as the oxidation of 1-aminofluorene 43 with m-chloroperoxybenzoic acid gives 1-nitrosofluorene 44 (36).



For the preparation of N-hydroxylamine aromatics, it is expected that the same oxidation and reduction methods would be useful provided that the reagents and reaction conditions are modified to enhance the stability of the hydroxylamine sought.

There are no literature reports of reagents that specifically oxidize aromatic amines to their hydroxylamines. One of the most common methods used for preparing aromatic N-hydroxylamines is the reduction of the corresponding nitro-aromatic with zinc in ammonium chloride. The reduction of nitrobenzene by this method gives a 65% yield of hydroxylaminobenzene. However, this reagent has been reported as ineffective in reducing nitro-naphthalene or any other aromatic ring system bigger than benzene-(30). Ammonium sulfide or sodium sulfide, in the form of polysulfides are believed to be most useful in this case.

1.9 Objectives

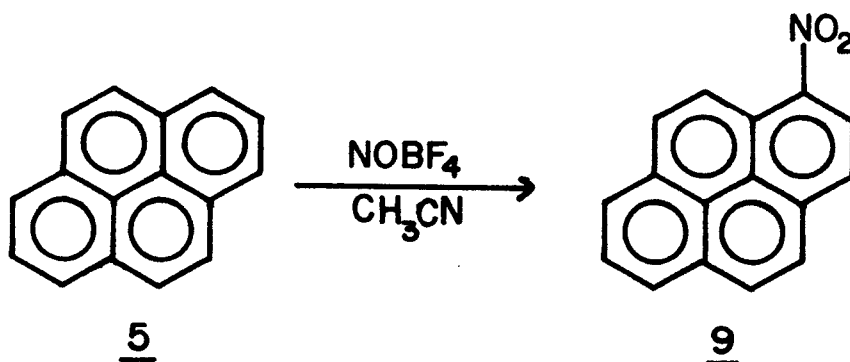
The objectives of this work are:

- 1) Prepare and characterize 1-nitrosopyrene and N-hydroxy-1-aminopyrene
- 2) Prepare and and characterize 1-nitroso-8-nitropyrene and 1-N-hydroxylamino-8-nitropyrene
- 3) Evaluate the mutagenicities of these compounds by the Ames assay as compared to 1-nitropyrene and 1,8-dinitropyrene

2. RESULTS AND DISCUSSION

2.1 Synthesis of 1-nitrosopyrene

Nitrosating agents, such as nitrous acid and nitrosyl chloride, have been used to prepare nitroso aromatics. Therefore, it seemed that a simple method to prepare 1-nitrosopyrene 18 would be the nitrosation of pyrene 5 with an appropriate nitrosating reagent. However, the reaction between pyrene 5 and nitrosonium tetrafluoroborate [NOBF_4] yielded only 1-nitropyrene 9 instead of the desired nitroso product 18.



Yost and Gutmann (36) prepared 2-nitrosobiphenyl and 2-nitroso fluorene from their corresponding amines by oxidation with *m*-chloro-peroxybenzoic acid (mCPBA). Thus, it was necessary to prepare 1-aminopyrene 20 and investigate its oxidation with mCPBA. Amino groups are usually introduced on a given aromatic system as nitro groups and then reduced by catalytic hydrogenation or reducing agents. The nitro group can be easily introduced on pyrene 5 by direct nitration and then purification of the mononitrated product according to the procedure of Dewar et al.(37).

Pyrene 5 was nitrated with nitric acid in acetic anhydride to give 1-nitropyrene 9 and other dinitrated isomers. 1-Nitropyrene 9 was separated by crystallization in 74% yield and reduced quantitatively by hydrogenation over Adam's catalyst (PtO_2) to 1-aminopyrene 20; oxidation of the amine 20 with 2 equivalents of mCPBA in methylene chloride at 0°C was very rapid and within seconds most of the amine had been consumed (Figure 6).

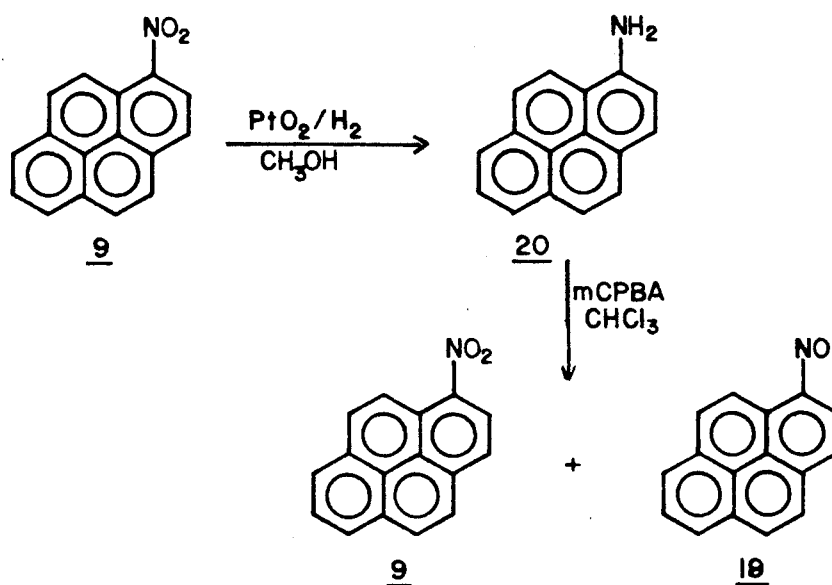
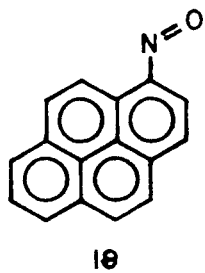


Fig. 6

Analysis of the crude oxidation product by reversed-phase HPLC showed two major products, 1-nitropyrene (less than 10%) and another less polar product. There was no evidence of any aminopyrene. This new product was purified on a prepacked silica gel column to give a bright orange compound with a melting point of $151\text{-}152^\circ\text{C}$.



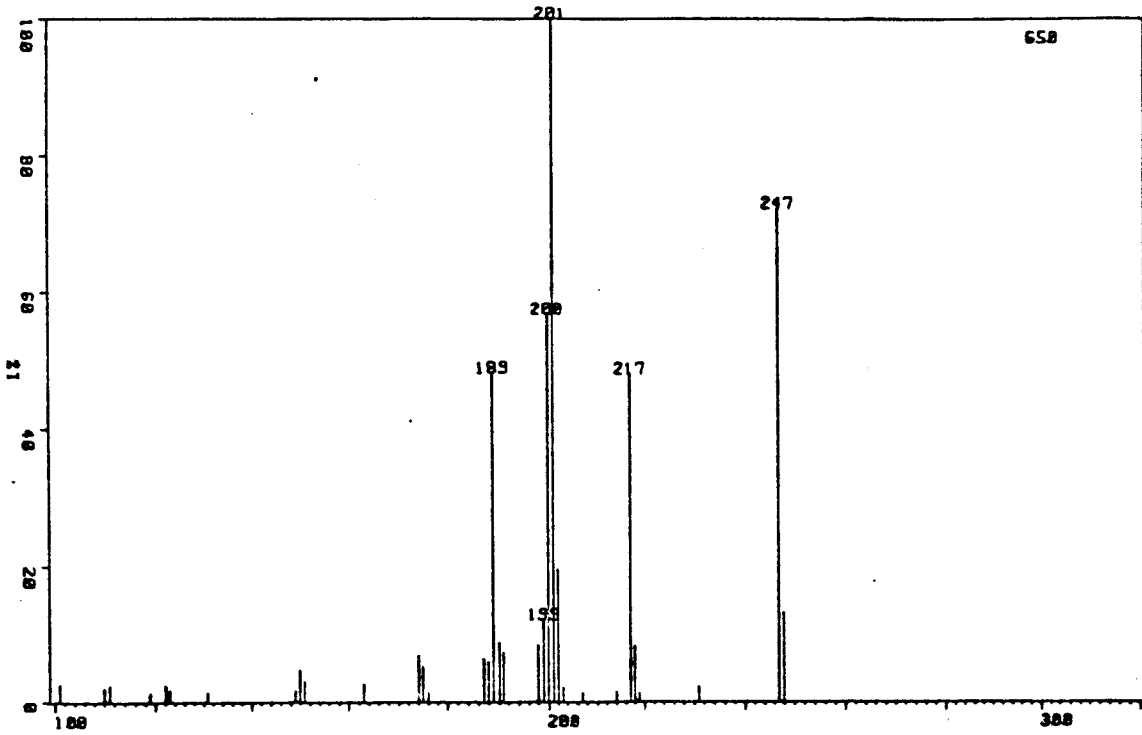
<u>Peak</u>	<u>Description</u>	<u>Intensity</u>
231	M ⁺	50%
217		30%
201	M ⁺ - [NO]	100%

The mass spectrum of 1-nitrosopyrene 18 showed major peaks at m/z 231, 217 and 201. The m/z 231 and 201 peaks correspond to the molecular ion and the M⁺-[NO] peaks respectively. The m/z 217 peak is unusual in that it represents a formal loss of 14 mass units from the molecular ion. Such a loss would be attributed to either the loss of a [CH₂] fragment or the loss of a nitrogen atom. Loss of a [CH₂] fragment has not been observed before in the mass spectra of pyrene derivatives and it is highly unlikely that such a loss would occur before loss of the nitroso group. Alternatively, the loss of a nitrogen atom following a rearrangement of the nitroso group such that the oxygen atom is bonded to the ring system is equally unlikely. However, the mass spectrum of nitropyrene (M⁺ = 247) also shows a peak at m/z 217. Previous work had shown that the intensity of this peak can vary dramatically depending on probe temperature and type of ionization (38). It was suspected and later confirmed by analysis of 1-[¹⁵N]-nitropyrene (M⁺ = 248) that some nitropyrene was being reduced to amino-pyrene within the mass spectrometer ion source and that some m/z 217 intensity was due to the molecular ion, M⁺, of this aminopyrene (Figure 7).

M07056 25 CE-830705.2
CAL: CALML STA:

1.51

29



M0630C 19 ¹⁵NO₂-pyrene
CAL: CALML STA:

30-JUN-1966
1.26

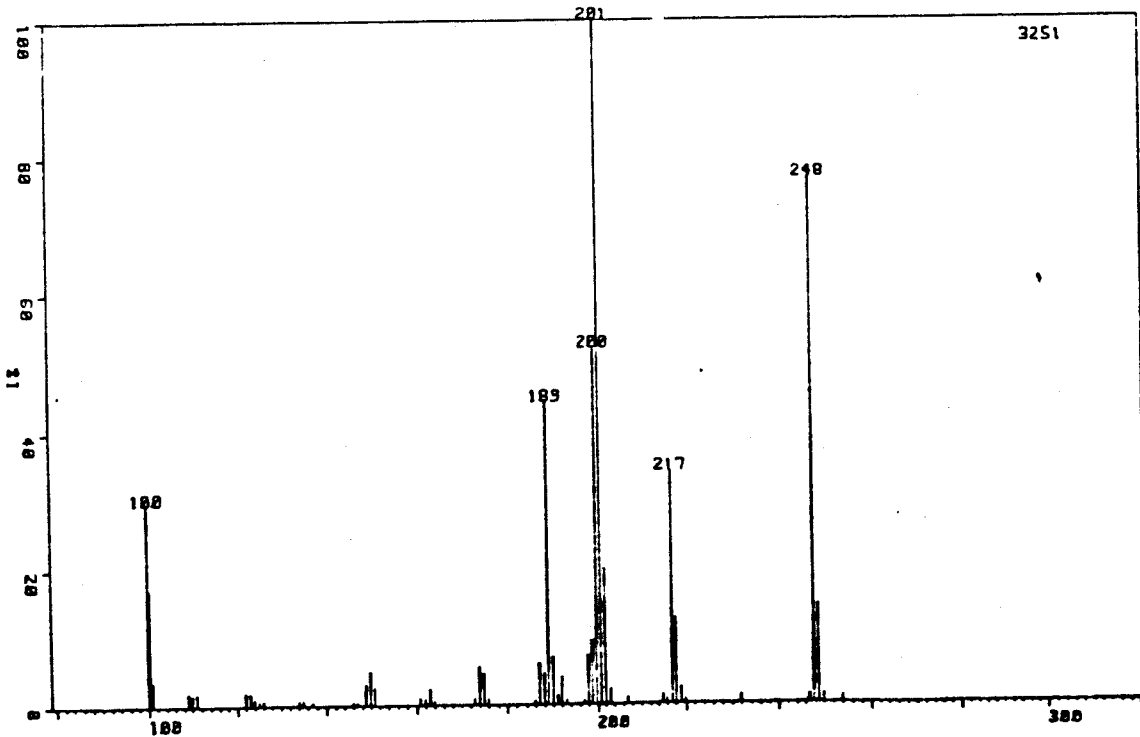
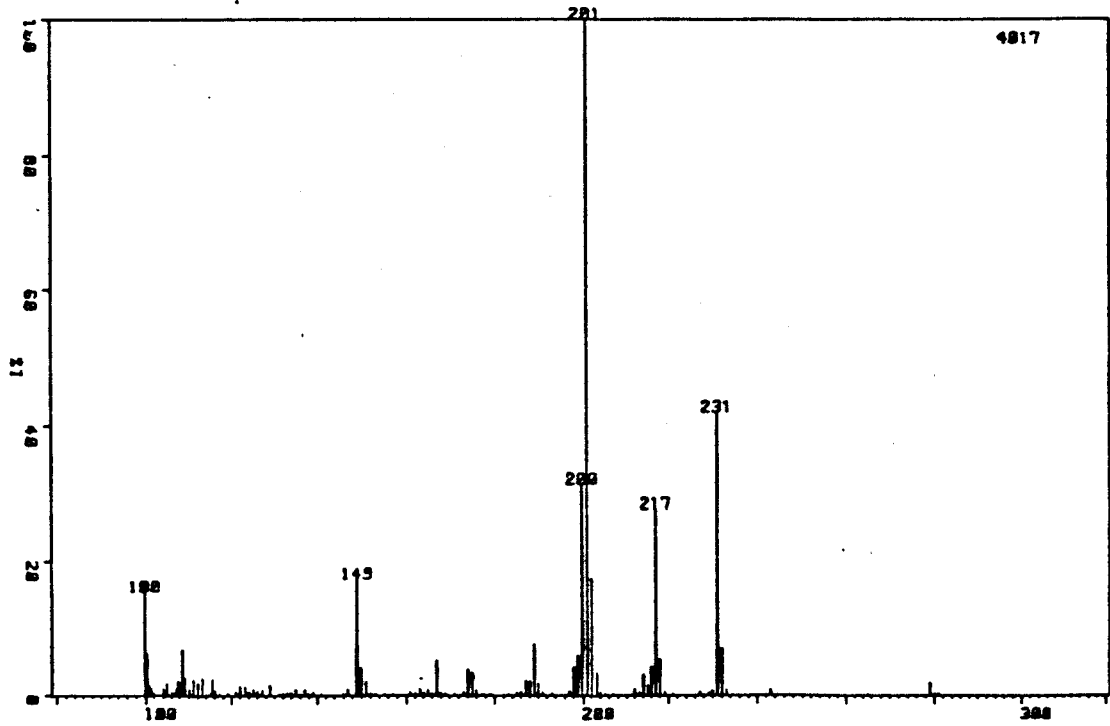


FIG.7

M0419C 18 CE-030419 NITROSD-PYRENE
CAL: CALFA STA:

1:24

30



M0630K 21 CE-030613 (15)NITROSD-PYRENE
CAL: CALML STA:

30-JUN-
1:34

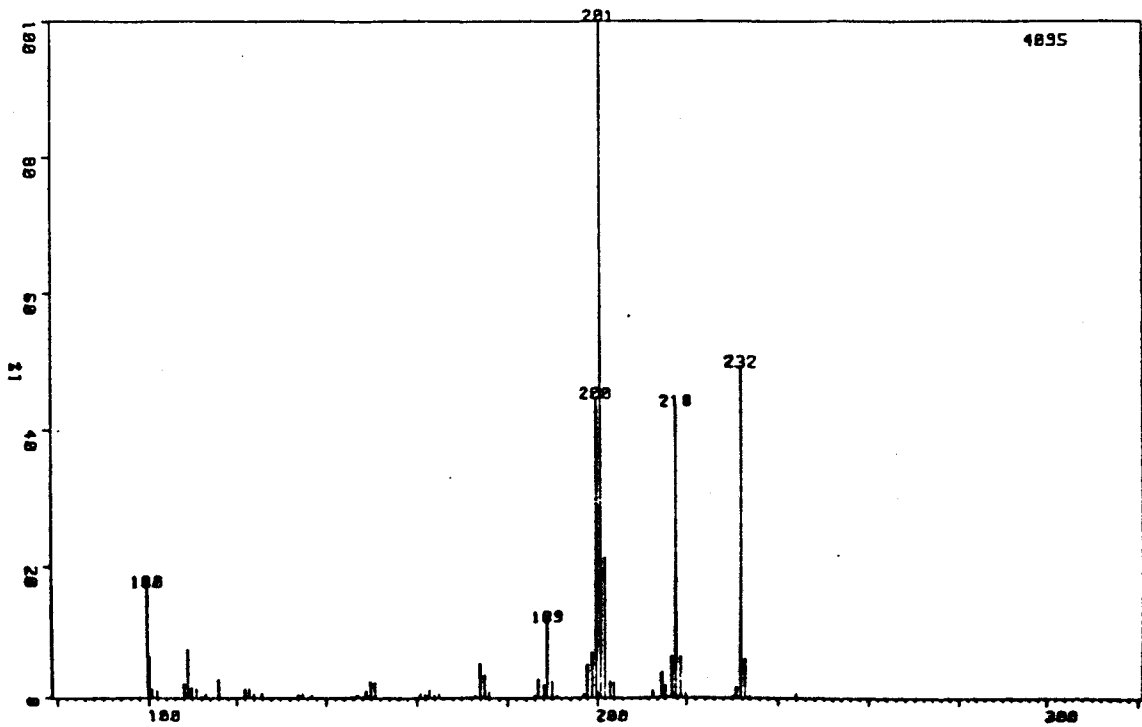
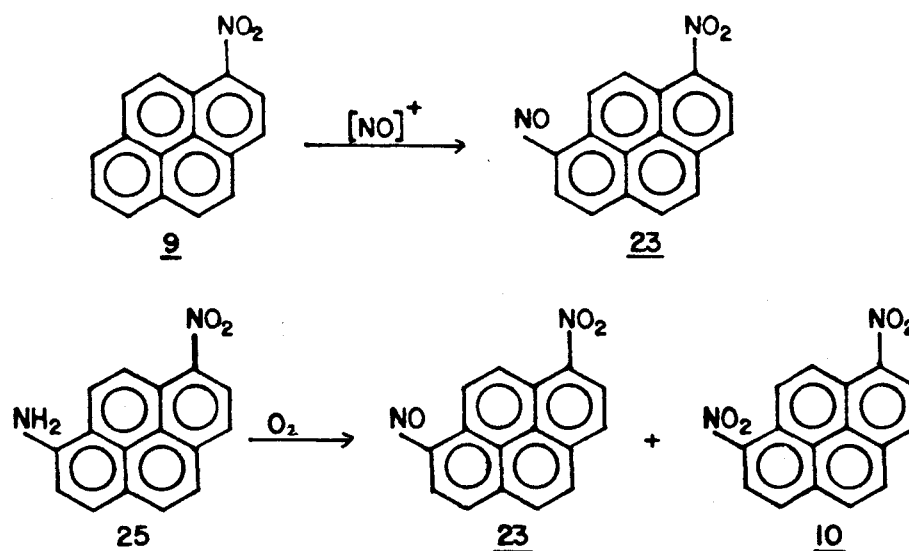


FIG. 8

Thus, 1-¹⁵[N]-nitrosopyrene was prepared for mass spectral analysis by nitration of pyrene with [¹⁵N]-HNO₃ (99 atom % ¹⁵N) in acetic anhydride, reduction to the amine and oxidation as described above. The loss of a nitrogen atom from M⁺(m/z 232) would give a peak at 217 corresponding to a M⁺-[¹⁵N]. While reduction of the nitroso group would show a peak at m/z 218 corresponding to the molecular ion of 1-¹⁵[N]-aminopyrene. The mass spectrum of 1-¹⁵[N]-nitrosopyrene showed a strong ion at m/z 218 and no intensity at 217, clearly indicating the facile reduction of the nitroso group to the amino group within the ion source (Figure 8).

2.2 Synthesis of 1-nitroso-8-nitropyrene

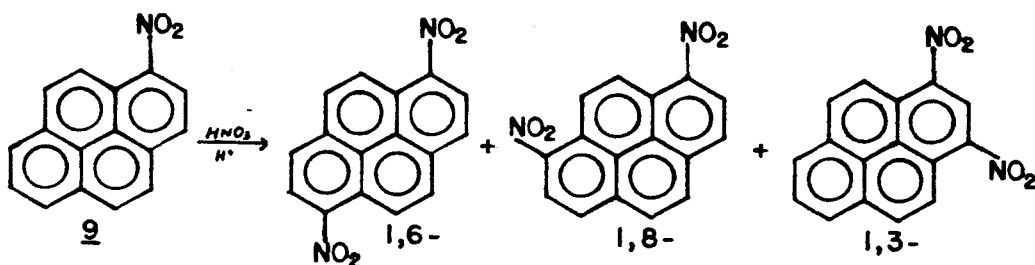
For the synthesis of 1-nitroso-8-nitropyrene 23, two approaches can be considered: first, the nitrosation of 1-nitropyrene 9 and second, oxidation of 1-amino-8-nitropyrene 25.



Since the direct nitrosation of pyrene with NOBF_4 had failed to yield the desired nitroso derivative in our hands, there was little enthusiasm for this approach. Even if the direct nitrosation were successful, the product should contain a mixture of three nitroso-nitropyrenes: the 1,3-, the 1,6- and the 1,8-isomers. However the peracid oxidation of 1-amino-8-nitropyrene 25 is expected to yield only two products: 1-nitroso-8-nitropyrene 23 and 1,8-dinitropyrene 10. Those two compounds, 23 and 10, are most likely easier to separate than the mixture of three nitroso-nitro isomers.

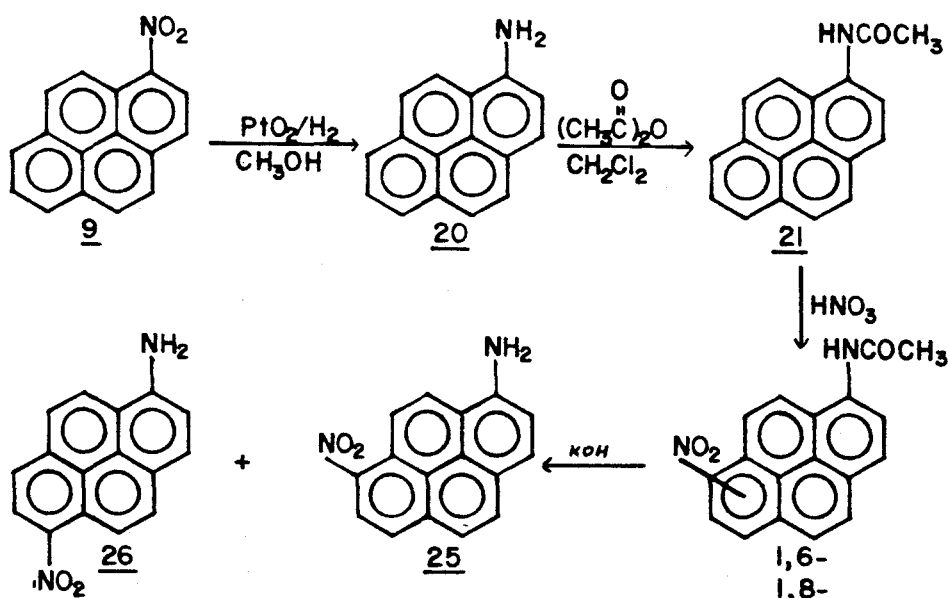
To prepare 1-amino-8-nitropyrene 25, partial hydrogenation of 1,8-dinitropyrene gives only poor yields. The major product obtained in these preparations is 1,8-diaminopyrene 27 (38)

In addition, the preparation of 1,8-dinitropyrene 10 requires the nitration of 1-nitropyrene 9 and then separation of the three dinitrated isomers: the 1,8-, the 1,6-, and the 1,3-dinitropyrenes. The dinitropyrenes are insoluble in most organic solvents except for dimethyl sulfoxide and dioxane; this makes their separation and purification difficult and time-consuming.

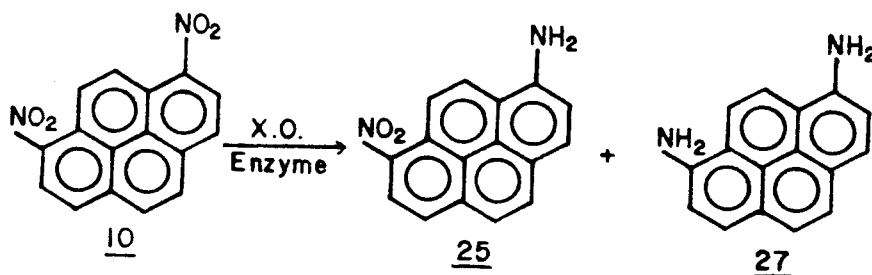


An alternative approach for the preparation of 1-amino-8-nitropyrene 25 is to introduce the amino group and the nitro group on pyrene separately. This can be accomplished by introducing the amino group first and then nitration to give the amino-nitro compound. However, the amino group should be protected before nitration and one way to do that is by acetylation to make the amide. Then, after nitration, the acetyl group can be easily cleaved by hydrolysis to regenerate the amine.

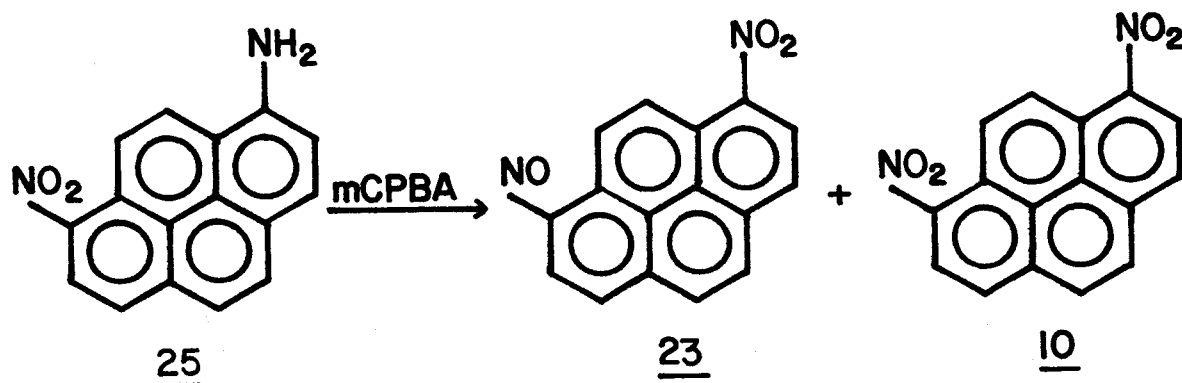
1-nitropyrene 9 was reduced, as before, by catalytic hydrogenation to give 1-aminopyrene 20. Acetylation of the amine 20 followed by nitration gave a mixture of two isomers identified as the N-acetylamino-nitropyrenes by mass spectrometry ($M^+ = 304$). The two isomers (1,6- and 1,8-) were separated by low pressure chromatography and then hydrolyzed in base to give 1-amino-8-nitropyrene 25 and 1-amino-6-nitropyrene 26.

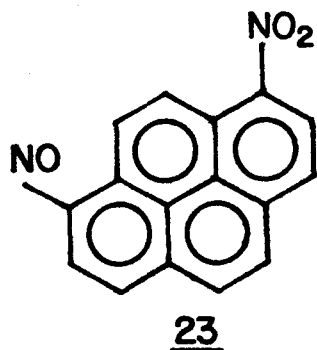


The isomers were identified by comparison with uv absorption spectra and analytical chromatographic data of authentic samples of 1-amino-8-nitropyrene 25 and 1-amino-6-nitropyrene 26, prepared by enzymatic reduction of 1,8- and 1,6- dinitropyrene respectively with xanthine oxidase.



1-amino-8-nitropyrene 25 was then reacted with mCPBA to give some 1,8-dinitropyrene and a new substance as the major product which was identified as 1-nitroso-8-nitropyrene 23. Purification of this product by HPLC gave an orange product (mp=219-220°C) which was shown to satisfy the formula $C_{16}H_8N_2O_3$ by high resolution mass spectrometry. The low resolution mass spectrum was also consistent with this formulation.





<u>Peak</u>	<u>Description</u>	<u>Intensity.%</u>
276	M ⁺	80
262	NO → NH ₂	20
246	M ⁺ - [NO]	60
232	262 - [NO]	10
216	246 - [NO]	50
200	246 - [NO ₂]	100

The oxidation of the amino-nitro derivative 25 with mCPBA is much slower than with 1-aminopyrene 20 under the same conditions. This difference is likely due to the electron withdrawing effect of the nitro group on the aromatic system which decreases the nucleophilicity of the amino group. However, even in the presence of only 2 mole equivalents of peracid, the amount of dinitro product increased with longer reaction times.

When the reaction was allowed to go to completion, more 1,8-dinitropyrene 10 was obtained than the desired nitro-nitroso product 23. This suggests that the nitroso group in nitroso-nitropyrene 23 is oxidized to the nitro level about as readily as the amine of amino-nitropyrene 25 is oxidized to the nitroso level. In the case of aminopyrene oxidations, the amino to nitroso oxidation is much more facile.

2.3 Synthesis of 1-N-hydroxylaminopyrene

Regarding the synthesis of hydroxylamino aromatics, very little work has been done on the preparation of these compounds from their corresponding amines. Most of the literature reports that describe the preparation of such compounds, involve the reduction of nitroaromatics with different reducing agents. As mentioned earlier, N-hydroxylaminobenzene and 1-N-hydroxylaminonaphthalene were prepared from the corresponding nitro compound by reduction. Usually, the hydroxylamine as well as the amine are formed under these conditions. Also, the pH of the reaction and the solvent used affect considerably the yield of the hydroxylamine(20).

Therefore, based on the known chemistry of hydroxylaminoaromatics and the literature reports, a number of considerations were important in our approach to the synthesis of hydroxylaminopyrene 19:

- 1) It is expected that 1-N-hydroxylaminopyrene 19 is more reactive than 1-N-hydroxylaminobenzene or 1-N-hydroxylaminonaphthalene. Thus it would be very labile to reduction as well as oxidation and other reactions such as disproportionation and rearrangement.
- 2) In the event that 1-N-hydroxylaminopyrene 19 were produced, it is conceivable that it could undergo condensation reactions with 1-nitrosopyrene 18, 1-N-hydroxylaminopyrene 19 or 1-aminopyrene 20.

- 3) It is expected that the yield and stability of the hydroxylaminopyrene compound can be enhanced by avoiding acidic media and protic solvents used during the preparation and purification steps.

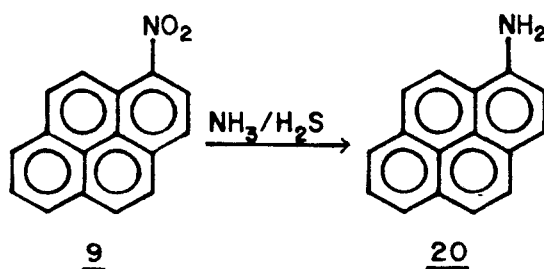
For the preparation of 1-N-hydroxylaminopyrene 19, four methods were attempted: a) reduction of 1-nitropyrene with zinc, b) photolysis of 1-azidopyrene in water, c) reduction of 1-nitrosopyrene with triethylsilane, d) reduction of 1-nitroso-pyrene with ascorbic acid.

2.3.1 Reduction of 1-nitropyrene with Zinc

It has been reported that the reduction of nitrobenzene to hydroxylaminobenzene can be accomplished by using zinc metal in ammonium chloride(39). A similar reduction of nitropyrene 9 was reported by Lund and Berg (40) to produce 1-N-hydroxylamino-pyrene 19. The product was identified by elemental analysis.

Using the same procedure that was reported by Lund and Berg, the reduction of 1-nitropyrene 9 with zinc in an ammonium chloride/ammonium hydroxide solution was carried at room temperature and under an atmosphere of nitrogen gas. The addition of ammonium hydroxide to the reaction mixture is believed to increase the stability of the N-hydroxy product 19(40). However, analysis of the reaction mixture revealed no reduction of the starting material.

It has been reported by T. Patrick et al (20) that some nitropolynuclear aromatic compounds are not reduced by Zn/NH₄Cl. Such compounds include nitro-naphthalene 7 and nitrofluorene 8, which can be reduced instead by ammonium sulfide (NH₄)₂S_x. The nitro aromatic is dissolved in ethanol and then NH₃ gas is bubbled through the solution first followed by H₂S gas. 1-Nitropyrene 9 was reduced by this method but the only product that was observed was 1-aminopyrene 20.



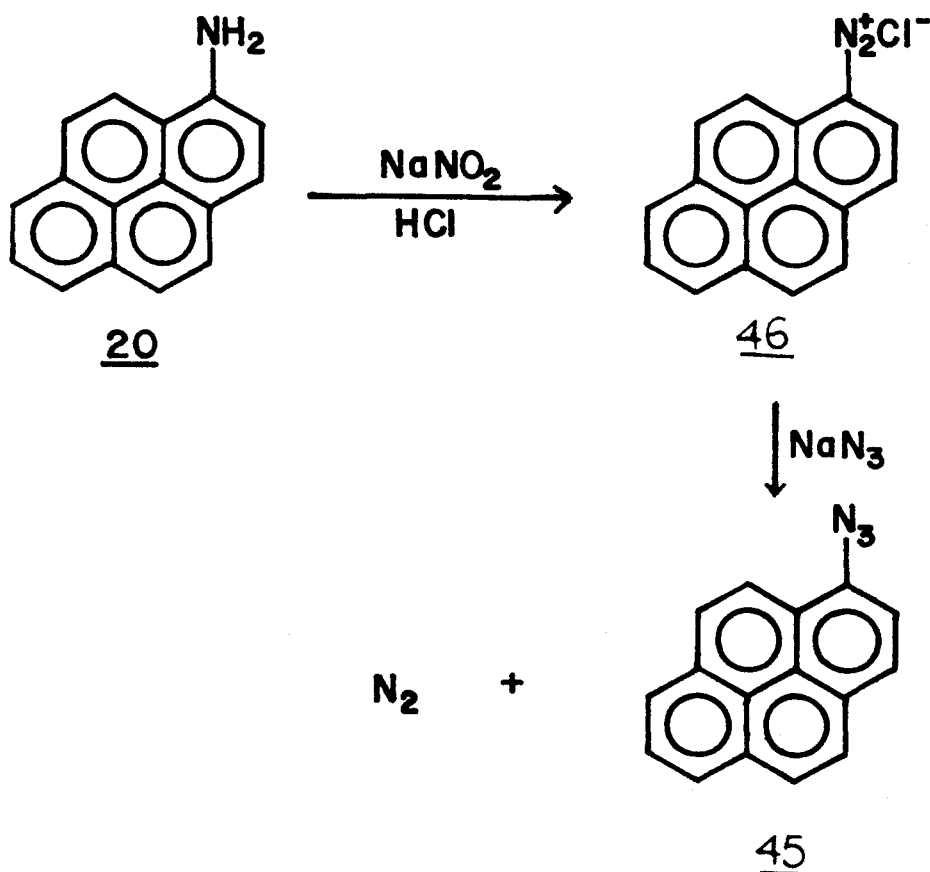
It appears that the reduction potential difference between hydroxylamino and aminopyrene is very small as compared to that of nitro and aminopyrene. As the hydroxylamino compound 19 is formed, further rapid reduction occurs giving aminopyrene 20.

2.3.2 Photolysis of 1-Azidopyrene in Water

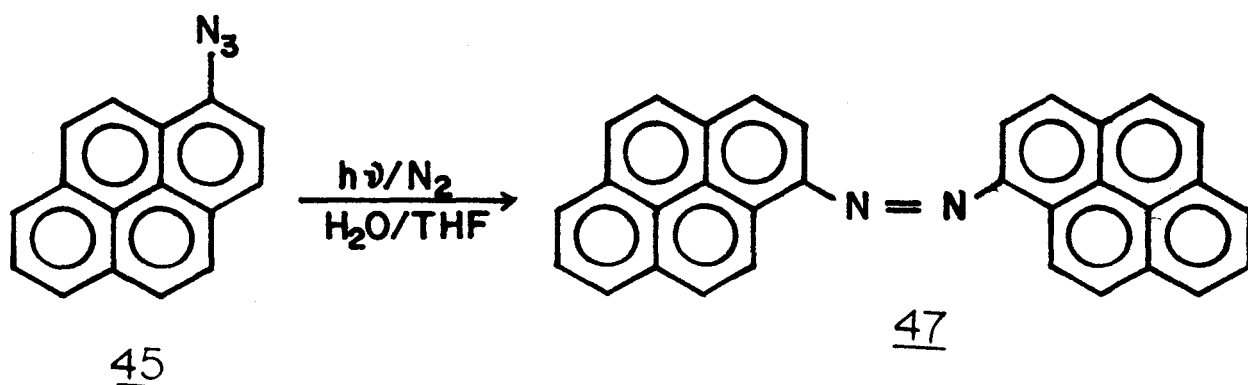
It is well known that azido compounds, upon photolysis, produce the corresponding nitrene with elimination of nitrogen. Nitrenes are very reactive and undergo insertion reactions very efficiently. In hydroxylic solvents, such as H₂O, nitrenes insert into the O-H bond to give the corresponding hydroxylamine.

Given that, it was believed that the photolysis of 1-azidopyrene 45 in H₂O would generate the corresponding nitrene which then inserts into the O-H bond to give hydroxylaminopyrene.

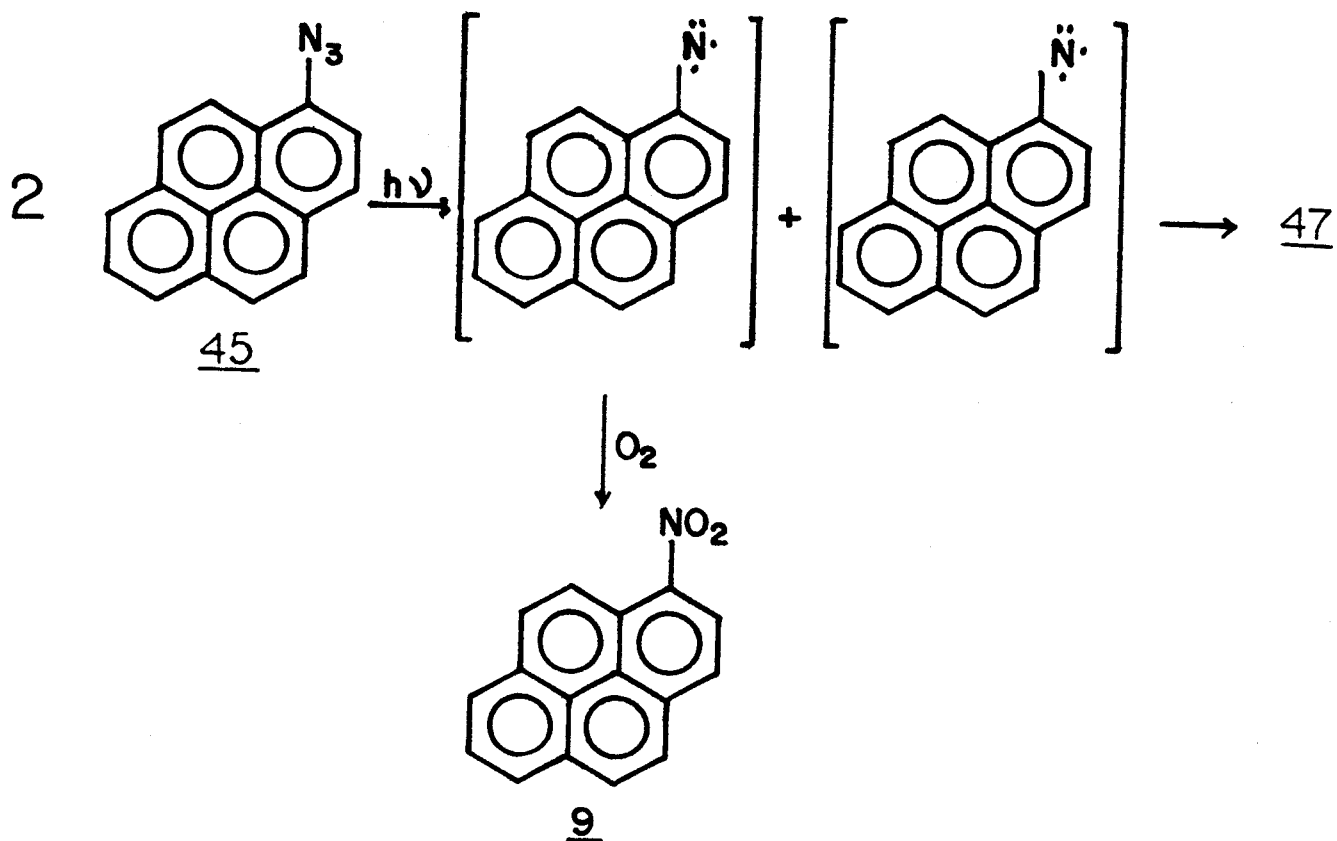
1-azidopyrene 45 was prepared according to the procedure by D.P. Smith et al (41). 1-Aminopyrene 20 was converted to the diazonium salt 46 by nitrosation with sodium nitrite in an acidic acetone-water solvent. The diazo salt 46 was then reacted with sodium azide to give 1-azidopyrene 45.



Since 1-azidopyrene 45 is insoluble in water, the solvent used for photolysis was a mixture of water and tetrahydrofuran (THF). The photolysis is very fast and within 2-3 minutes over 90% of the azido compound is photolyzed. HPLC analysis of the photolyzed solutions along with mass spectral data showed that azopyrene 47 was the only compound formed ($M^+ = 430$).



When the solvents were not deoxygenated prior to photolysis, 1-nitropyrene 9 was formed in addition to azopyrene, presumably by insertion of the nitrene into the oxygen double bond:

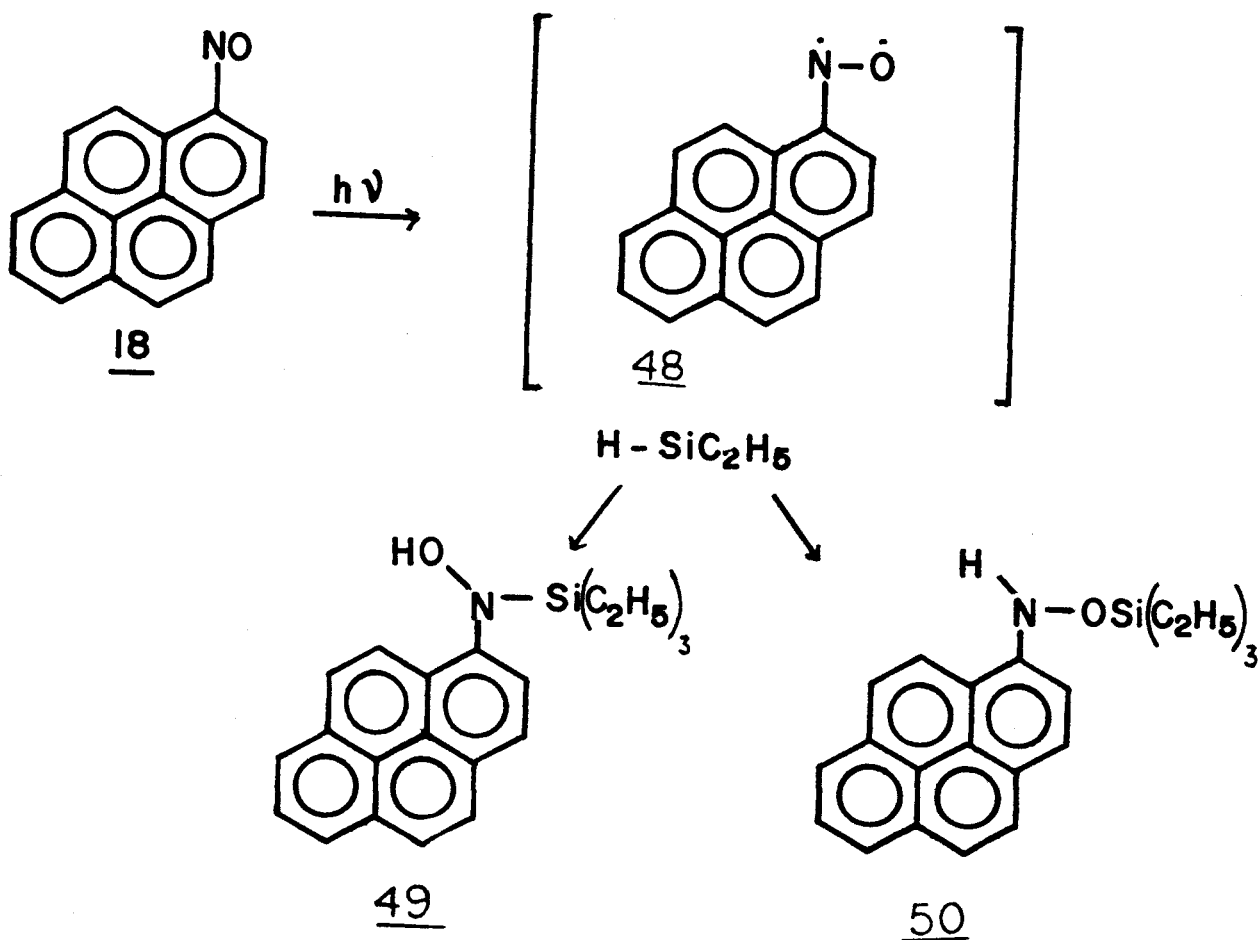


2.3.3 Reduction of 1-nitrosopyrene with Triethylsilane

The use of silyl reagents as protective groups and for preparing derivatives of certain compounds is well documented in the literature(42). The advantage of preparing the silyl derivative of N-hydroxylaminopyrene is that the silylated form would be more stable and less susceptible to oxidation or reduction.

It has been reported that nitrosoalkanes, upon exposure to visible light in the presence of different silanes, react to give the corresponding N-silyl and O-silyl derivatives(43).

Therefore, we thought that the photolysis of 1-nitrosopyrene 18 in triethylsilane should give a mixture of 1-N,N-hydroxytriethylsilylaminopyrene 49 and 1-N-hydroxy-0-triethylsilylaminopyrene 50. Upon photolysis, a diradical intermediate 48 should form which is then trapped by the silane to give the N- and O- silyl derivatives:



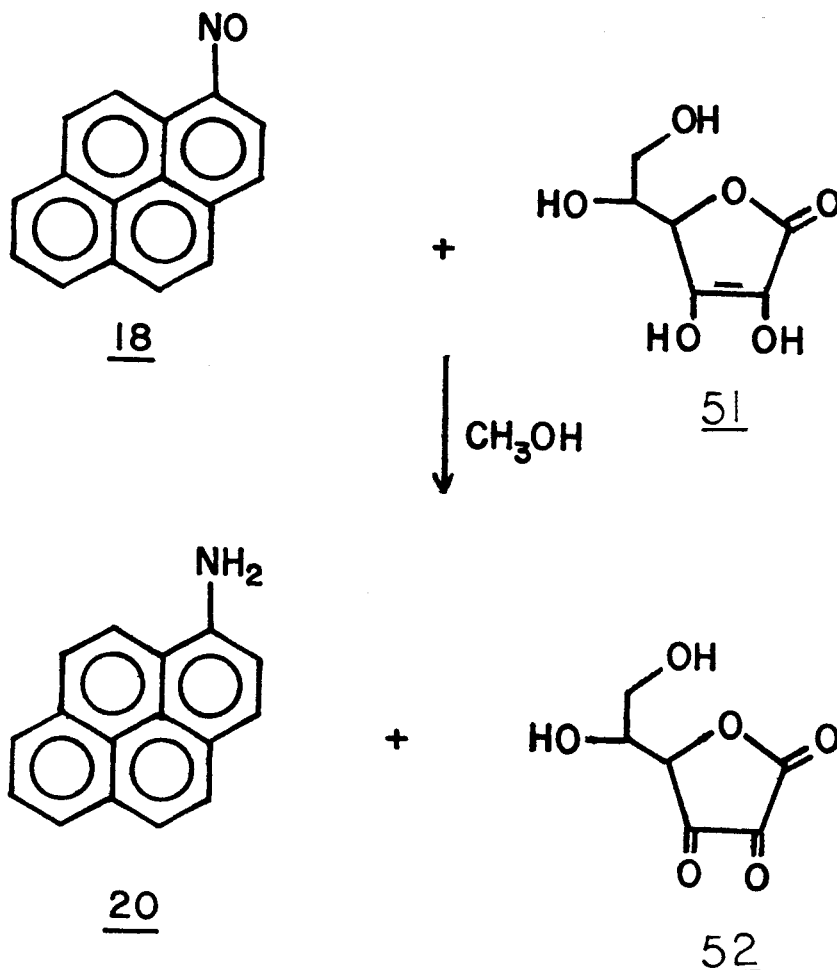
Contrary to our expectations, there was no reaction between 1-nitrosopyrene and triethylsilane upon photolysis in THF. However, in the presence of palladium, as a catalyst, 1-aminopyrene 20 was produced; the silane is just acting as a hydrogenating agent in this reaction.

2.3.4 Reduction of 1-nitrosopyrene with Ascorbic Acid

Ascorbic acid 51 is a well-known, naturally occurring, reducing agent in biological systems. It has been successfully used to convert certain nitro and nitrosoaromatics to their corresponding N-hydroxylamines(44). It has also been reported to stabilize these N-hydroxy adducts, such as N-hydroxy-2-aminofluorene formed by reduction of 2-nitrosofluorene.

The study by Howard et al. (19) showed that the formation of the DNA adduct, identified in the incubation of 1-nitropyrene 10 with xanthine oxidase and DNA, was diminished when ascorbic acid 51 was added to the incubation medium. This suggested at first that the N-hydroxylaminopyrene is not stabilized by ascorbic acid but is rapidly reduced to aminopyrene. However, it was anticipated that by avoiding excess ascorbic acid, it might be possible to generate some of the N-hydroxy intermediate when reducing 1-nitrosopyrene.

A solution of 1-nitrosopyrene in methanol was titrated by addition of a methanolic solution of ascorbic acid and the reaction mixture was analyzed by HPLC. Slow disappearance of the nitroso compound was observed with simultaneous build-up of 1-aminopyrene 20; there was no indication of any other material or components being formed.



Recently, Howard et al (44) showed that reduction of 1-nitrosopyrene by ascorbic acid in dimethylformamide yielded a new product different from 1-aminopyrene. Although the attempts to purify and identify this new material were unsuccessful, the current thinking is that this product is the N-hydroxy-1-aminopyrene.19. These findings proved to be very interesting and research efforts are directed right now towards isolation and characterization of this product. If it is in fact the N-hydroxy compound, then mutagenicity and metabolism studies of this compound should provide enough data to establish its implication in mutagenesis.

2.4 Ames Assay Results

2.4.1 The Test Results

Pure samples of 1-nitrosopyrene 16, 1-nitroso-8-nitropyrene 23, 1-nitropyrene 9 and 1,8-dinitropyrene 10 were submitted to Dr. D. McCalla's lab, Department of Biochemistry, McMaster University for testing by the Ames assay. The following results were obtained:

TABLE II
Ames Assay Results

Chemical	Strain (revertants/nmole/plate)		
	TA98	TA98NR	TA98DNP ₆
1-nitropyrene <u>9</u>	985	548	396
1-nitrosopyrene <u>18</u>	50,226	43,420	24,020
1,8-dinitropyrene <u>10</u>	85,830	72,400	1,625
1-nitroso-8-nitropyrene <u>23</u>	8,100	13,700	520

2.4.2 The Strains

The Salmonella typhimurium tester strain TA98 was selected because the mutations induced in this strain are predominantly of the frameshift type (45). Most nitro-PAH induce mutations of this type and are thus most active in this strain. Strain TA98NR was selected for its deficiency in an enzyme which reduces the nitro group of nitrofurans and nitroimidazoles(17). This enzyme has been termed "classical" nitroreductase. Some nitro-PAH, including 1-nitropyrene 9, show considerably decreased activity

in the TA98NR strain. However, mutagenicity of some other nitro-PAH such as 1,8-dinitropyrene 10 is not diminished in this strain. Strains of TA98 which are resistant to the mutagenic activity of 1,8-dinitropyrene have been isolated and TA98DNP₆ is one of them(46).

It was then suggested that more than one nitro reductase might be involved in activating nitro-PAH(47). Further investigations have shown that the N-hydroxy derivatives of fluorene 3 are not significantly mutagenic in the DNP₆ strain as compared to strains TA98 and TA98NR. However, the N-acetoxy derivatives are equally mutagenic in TA98, TA98NR and TA98DNP₆. Thus, it appears that the DNP₆ strain is deficient in esterifying enzyme(s) and that esterification of the N-hydroxy fluorene increases the mutagenic activity of these compounds.

2.4.3. Data Interpretation

The results from Table II show that 1-nitrosopyrene 18 is significantly more mutagenic than 1-nitropyrene 9 and is equally potent in strains TA98 and TA98NR. This implies that 1-nitrosopyrene does not require the "classical" nitroreductase for its activation. Provided that it does not react directly with DNA, then it is metabolized in TA98NR by other reductase(s) similar to the metabolism of 1,8-dinitropyrene. The response

obtained in TA98DNP₆ indicates that esterification of the hydroxylamine 19 derived from 1-nitrosopyrene contributes but is not essential for the expression of the mutagenicity of this compound.

It is also shown in Table II that 1-nitroso-8-nitropyrene 23 is about 10x less mutagenic than 1,8-dinitropyrene 10 in TA98 and TA98NR. This suggests one of the following:

- i) 1-nitroso-8-nitropyrene is not properly metabolized by the bacteria. This could be due, for example, to its inefficient uptake by the bacteria.
- ii) 1-nitroso-8-nitropyrene and, therefore, 1,8-dinitropyrene are not metabolized by the bacteria in the same manner as proposed for 1-nitropyrene. This different bioactivation pathway may or may not involve a "hydroxylamino" intermediate.

2.5 CONCLUSION

The presence of nitro-PAH in the environment and the fact that the most abundant of these, 1-nitropyrene 9 is carcinogenic in male rats presents us with a significant problem. Thus, it would seem that a better understanding of the biological properties of these chemicals is urgently needed. So far, the indication is that the N-hydroxy-1-aminopyrene 17 is extremely labile under the conditions employed and undergoes reactions instantaneously as it is formed. Therefore, it is highly questionable that in a biological system, this N-hydroxy compound would survive long enough to exert its effects on the DNA.

It is also unclear whether 1-nitropyrene and 1,8-dinitropyrene undergo similar metabolic pathways by the bacteria Salmonella. Further investigation into the uptake of 1-nitroso-8-nitropyrene by the bacteria, its metabolism and stability under those conditions is required.

In summary, the inability to prepare the N-hydroxylamino derivatives of 1-nitropyrene and 1,8-dinitropyrene and to evaluate their mutagenicity, leaves the "hydroxylamino" problem unsolved. Certainly, the recent work of Howard et al(44) might provide a better approach for the synthesis of the N-hydroxy derivatives.

It remains to be seen whether isolation of this derivative can be accomplished or further derivatization to a more stable product is required. Another approach, which is currently being investigated, is the electrochemical reduction of 1-nitropyrene and 1-nitrosopyrene. Should this method prove to be successful in preparing the N-hydroxy-1-aminopyrene, then mutagenicity and metabolism studies of this compound should provide greater understanding of the mechanism of mutagenesis of 1-nitropyrene.

3. EXPERIMENTAL

3.1 Materials

For the preparation of 1-aminopyrene, 1-nitropyrene of 97% purity was obtained from Aldrich and platinum dioxide from Alfa chemicals.

For the preparation of 1-nitrosopyrene and 1-nitroso-8-nitropyrene, m-chloroperbenzoic acid was obtained from Sigma (85%) Reagent Grade.

For the synthesis of 1-hydroxylaminopyrene, zinc metal dust (96.2%) was obtained from Mallinckrodt, sodium nitrite from BDH chemicals, sodium oxide (95%) from the Baker Chemical Co., and ascorbic acid (99%) from Sigma.

For the nitration of pyrene and 1-N-acetylamino pyrene, fuming nitric acid (90%) was obtained from the Fisher Scientific Co., and ^{15}N -nitric acid (99% ^{15}N) from Merck, Sharpe and Dohme.

Reagent grades of petroleum ether, diethyl ether, acetone, hexane, ethyl acetate, dichloromethane and acetic anhydride were obtained from the BDH Chemical Co. and Fisher Scientific Co.

3.2 Liquid Chromotography

3.2.1. High Performance Liquid Chromatography (HPLC)

The analysis and purification of the derivatives of pyrene were performed on two HPLC systems.

System 1: Beckman model 110A isocratic liquid chromatograph with a model 110 pump.

System 2: Analab pump model A-60-S with a Valco sample injection valve model CLA-117.

Columns

- A. a Whatman reverse-phase analytical ODS-2 column
(250mm x 4.6mm ID)
- B. an Altex normal-phase 5 μ m ultra-sphere cyano column
(250mm x 4.6mm ID)

Detectors

Beckman model 153 U.V. absorption detector at 254nm.

Solvents

Solvents used were HPLC grade. Hexane, isopropanol, methylene chloride for normal phase and acetonitrile, methanol and water for reverse phase analysis. Deionized water used was purified by an ISDH milliQ purification unit.

3.2.2. Low Pressure Liquid Chromatography (LPLC)

Purification was performed on the same two systems as the HPLC with the exception of using normal-phase reagent grade solvents and a Lobar prepacked column size A (240mm x 10mm) silica gel 60 EM reagent (Merck).

3.2.3. Thin-Layer Chromatography (TLC)

The progress of each reaction was monitored by TLC. Plastic sheets precoated with silica gel 60 F254 (Merck) were developed in (ethyl acetate : hexane) or in (acetone : hexane) solvent systems. The starting materials and products were observed under both shortwave and longwave U.V. light.

3.3 Ultra-violet Spectroscopy (UV)

All UV-visible absorption spectra were acquired on a Unicam SP8-100 ultraviolet spectrophotometer. Samples were dissolved in HPLC grade methanol.

3.4 Mass Spectrometry (MS)

All mass spectra were acquired on a VG micromass 7070F mass spectrometer with an electron energy of 70 ev and a source temperature of 200°C. Samples were prepared for analysis by the following procedure: the compound was dissolved in acetone, transferred into glass capillary tubes and solvent evaporated to dryness under reduced pressure. Samples were introduced into the source via the solid probe inlet.

3.5.1. Synthesis of 1-aminopyrene

To a solution of 1-nitropyrene (1.00g., 4.0 mmoles) dissolved in 50 mls of methanol was added 8.5 mg of platinum oxide. Hydrogenation of this solution at atmospheric pressure resulted in the consumption of 270 mls of hydrogen (12.05 mmoles). The analysis of the reaction mixture showed that all of the starting material had been consumed (Rf of 1-nitropyrene = 0.51, Rf of 1-aminopyrene = 0.28 in 10% ethyl acetate in hexane as a solvent). The reaction mixture was filtered through a 1-inch bed of celite, washed with methanol (2 x 10 mls) and the fluorescent solvent evaporated to dryness to give a pale green solid, 0.8g (92% yield). HPLC analysis of this product on system 1 column A, showed a single peak with a k' of 1.6 in 5% water in Methanol. This product was identified to be 1-aminopyrene. MS, m/z (relative intensity), 217 (M⁺, 100), 189 (20). UV (methanol) λ_{\max} 283(ϵ 23700), 242(ϵ 44100).

3.5.2 Synthesis of 1-nitrosopyrene

1-Aminopyrene (100.0 mg, 0.5 mmoles) was dissolved in 5 ml CH₂Cl₂ and stirred in an ice bath. To this solution, m-chloroperbenzoic acid (166 mg, 1.1 mmole) dissolved in 1 ml cold CH₂Cl₂ was added. The reaction was monitored by TLC (solvent 10% ethyl acetate in hexane, Rf of 1-aminopyrene = 0.28, Rf of 1-nitropyrene = 0.51, Rf of 1-nitrosopyrene = 0.63). After 2 minutes, all the 1-aminopyrene had reacted. The solution was allowed to stir for 5-10 minutes. It was extracted with 5% NaHCO₃ (3 x

10ml). The organic layer was collected and evaporated. The crude brown product was purified on a silica gel column (15cm x 2 cm silica gel, 60-120 mesh).

A yellow colored band was eluted from the column with 10% ethyl acetate in hexane. After the solvent was evaporated, the product was purified by LPLC using 2% acetone in hexane as a solvent to give 1-nitrosopyrene 86% yield. MS, m/z (relative intensity), 231 (M^+ , 50), 217(30), 201(100). UV (methanol) λ max 458(ϵ 15800), 440(ϵ 16000), 396(ϵ 10000), 313(ϵ 16700), 302(ϵ 14000), 259(ϵ 19300), 234(ϵ 48400).

3.6.1. Synthesis of 1- ^{15}N -nitropyrene

Pyrene (200 mg, 1.0mmoles) was dissolved in 10 mls acetic anhydride and the solution was stirred in a 50 ml round bottom flask in an ice bath. ^{15}N -nitric acid (10M, 0.1ml) was dissolved in 1 ml acetic anhydride and then slowly added to the pyrene solution. The reaction was monitored by TLC. (solvent 10% ethyl acetate in hexane, R_f of pyrene = 0.55, R_f of 1- NO_2 pyrene = 0.51). After 3 hours, the TLC showed little starting material left. The reaction was quenched with ice/ H_2O , neutralized with 1M NaOH and extracted with ethyl acetate (2 x 10 ml). The product was then dried and purified on a silica gel column (30cm x 2cm). A yellow non-fluorescent fraction was eluted with 5% ethylacetate in hexane. HPLC analysis of this fraction showed one single

peak identified by mass spectrometry as 1-¹⁵N-nitropyrene (M^+ = 248). Yield 76% (188 mg, 0.76 mmole).

3.6.2. Synthesis of 1-¹⁵N-nitrosopyrene

1-¹⁵N-Aminopyrene and 1-¹⁵N-nitrosopyrene were prepared using the same procedure as described earlier, sections 3.5.1 and 3.5.1.

3.7.1 Synthesis of 1-N-acetylaminopyrene

1-Aminopyrene (400 mg, 1.84 mmoles), prepared as described in section 3.5.1, was dissolved in 15 mls dichloromethane and stirred at room temperature. 5 mls of acetic anhydride was added to the solution. After one hour, a light green precipitate was collected by filtration and washed with (2 x 10 mls) ether. The product, after drying, showed one component by HPLC identified as 1-N-acetylaminopyrene (M^+ = 259). Yield 352 mg, 74%.

3.7.2. Synthesis of 1-N-acetylamino-8-nitropyrene

1-N-Acetylaminopyrene (300 mg, 1.16 mmoles) was dissolved in 9 mls acetic anhydride. To this solution 67 μ l of 90% fuming nitric acid in 1 ml of acetic anhydride was added slowly with stirring. The color of the greyish solution turned to yellow immediately. After 2 hours the TLC showed that all starting material has reacted. (Rf for 1-N-acetylaminopyrene = 0.43, Rf for 1-N-acetylamino-6-nitropyrene = 0.36, Rf for 8-N-acetylamino-8-nitropyrene = 0.46, Solvent : 50% acetone in hexane). The

reaction mixture was quenched with H₂O and neutralized with 2M NaOH. The precipitate was then filtered and dried. (260 mg, 74% yield). The product, a mixture of 1,8- and 1,6-isomers, was purified on a silica prepacked column by LPLC using 60% ethyl acetate in hexane as a solvent. 8-N-acetylamino-1-nitropyrene elutes first and amounts to 40% of the mixture product. MS, m/z (relative intensity), 304(M⁺, 60), 274(40), 200(100). UV (methanol) λ_{\max} 415(6500), 302(5500). R_f for the 1,8-isomer = 0.22, R_f for the 1,6-isomer = 0.31 in 60% ethyl acetate in hexane.

3.7.3. Synthesis of 1-amino-8-nitropyrene

1-N-Acetylamino-8-nitropyrene (24 mg, 0.08 mmoles) was dissolved in 2 mls of 95% C₂H₅OH. To this solution, 1.5 ml of 1M KOH was added at room temperature. An immediate colour change was observed and the original yellow colour of the solution was changing to red. The reaction was warmed and the temperature raised to 50° after which the reaction mixture was refluxed for 2-1/2 hours. It was then cooled to room temperature and extracted with (2 x 5 mls) CH₂Cl₂ and then evaporated to give a deep red coloured product. 1-amino-8-nitropyrene 16.3 mgs (79% yield). MS, m/z (relative intensity), 262(M⁺, 90), 232(60), 216(100), 204(30). UV (methanol) λ_{\max} 482(ϵ 2500), 398(ϵ 1000), 318(ϵ 1800).

3.7.4. Synthesis of 1-nitroso-8-nitropyrene

1-Amino-8-nitropyrene (10 mg, 0.038 mmoles) was dissolved in 5 mls CH_2Cl_2 in a small round bottom flask and stirred at 0°C in an ice bath. 0.6 mls of 200 mg/ml solution of mCPBA in CH_2Cl_2 was added (0.1 mls at 15 minute intervals). The reaction was monitored by TLC using 25% acetone in hexane as solvent, R_f of the starting material 1-amino-8-nitropyrene = 0.25, R_f for the 1-nitroso-8-nitropyrene = 0.45. After 2 hours, the reaction mixture still showed unreacted starting material and was poured onto a silica column (2 cm x 10 cm) and run through with CH_2Cl_2 . A fast running orange band was collected and upon examination by HPLC showed two components corresponding to 1,8-dinitropyrene and 1-nitroso-8-nitropyrene later identified by high resolution mass spectral analysis. MS, m/z (relative intensity, 276(M^+ , 80), 262(20), 246(60), 232(10), 216(50), 200(100). UV (methanol) λ_{max} 436(ϵ 5200), 231(ϵ 10900).

3.8 Synthesis of 1-N-hydroxylaminopyrene

3.8.1 Reduction of 1-Nitropyrene with Zn

1-Nitropyrene (100 mg, 0.4 mmoles) was dissolved in 3 mls ethyl ether and stirred under nitrogen atmosphere while 0.2 mls of a mixture of (NH_4OH : 25% NH_4Cl in H_2O (2:1)) was added followed by 30 mg of zinc dust. The reaction mixture was stirred under nitrogen for 30 minutes and poured over 0.75 mls of petroleum ether. A yellow precipitate was formed and filtered under a nitrogen stream. Mass spectral analysis of the precipitate

as well as HPLC analysis showed the presence of 1-nitropyrene. MS 247(M⁺, 80), 217(50), 201(100). UV.

3.8.2. Synthesis and photolysis of 1-azidopyrene

1-Aminopyrene (50 mg, 0.23 mmoles) was dissolved in 4 mls of a mixture of acetone:water (1:1). The solution was then stirred in an ice bath followed by the addition of 0.025 ml of concentrated Hydrochloric acid. A solution of sodium nitrite (25 mg, 0.36 mmoles) in 1 ml of water was added dropwise and very slowly. After the TLC showed no starting material left, the round bottom flask containing the reaction mixture was covered with aluminum foil and the subsequent steps were performed in the absence of direct light. Sodium azide (150 mg, 2.3 mmoles) was added to the dark brown solution. The reaction was stirred in the ice bath for 30 min. and then for 1 hour at room temperature. The dark brown mixture was then extracted with (2 x 20 mls) benzene, dried over anhydrous magnesium sulfate and then evaporated to give a greyish product. This product contained 1-azidopyrene (60-65% by HPLC analysis) which was separated by column chromatography (silica gel, 5mm x 100mm) using hexane as a solvent. 1-azidopyrene runs along with the solvent front and when dried gives a white powder with a m.p. (decomposes) 104-105°C. MS, 243(M⁺, 39), 216(19), 215(100), 214(22). UV (methanol) 357(€49000), 340(€38000), 283(€40000), 246(€54000).

Note: Photolysis was done by a UVGL-25 multiband using the

short UV band - 254 nm fixed at a distance of 4cm for 5 minutes. Solutions of $2 \times 10^{-5} \text{M}$ 1-azidopyrene were stirred in borosilicate glass tubes during irradiation. The solvent was then removed by drying under vacuum and the dry solid was then analyzed by HPLC and MS.

3.8.3 Reaction of 1-nitrosopyrene with triethylsilane

Photolysis of 1-nitrosopyrene and triethylsilane reaction mixtures was performed as in sec. 3.8.2. A solution of 2 mg/ml of 1-nitrosopyrene in benzene was stirred with 0.1 ml of triethylsilane in borosilicate glass tubes. Photolysis was done by the UVGL-25 using the short-band - 254 nm fixed at a distance of 4cm. Aliquots were then removed at 10 min. intervals, solvent dried and then analyzed by HPLC and MS.

3.8.4 Reduction of 1-nitrosopyrene with Ascorbic Acid

10ml Solutions of 1-nitrosopyrene ($8.5 \times 10^{-5} \text{M}$) and ascorbic acid ($1 \times 10^{-3} \text{M}$) in methanol were prepared. Four 1ml aliquots of the nitrosopyrene solution were mixed separately with 1, 2, 5 and 10 ml aliquots of the ascorbic acid solution and each reaction mixture was analyzed by HPLC at 10 min intervals from time = 0 min. to time = 60 min. The HPLC analysis was done on system 2 column A using 95% methanol:5% water as a solvent with a flow rate of 2.0 ml/min. For 1-nitrosopyrene: $t_r = 8.5$ min, $k' = 4.25$, and for 1-aminopyrene: $t_r = 4.2$ min, $k' = 1.6$.

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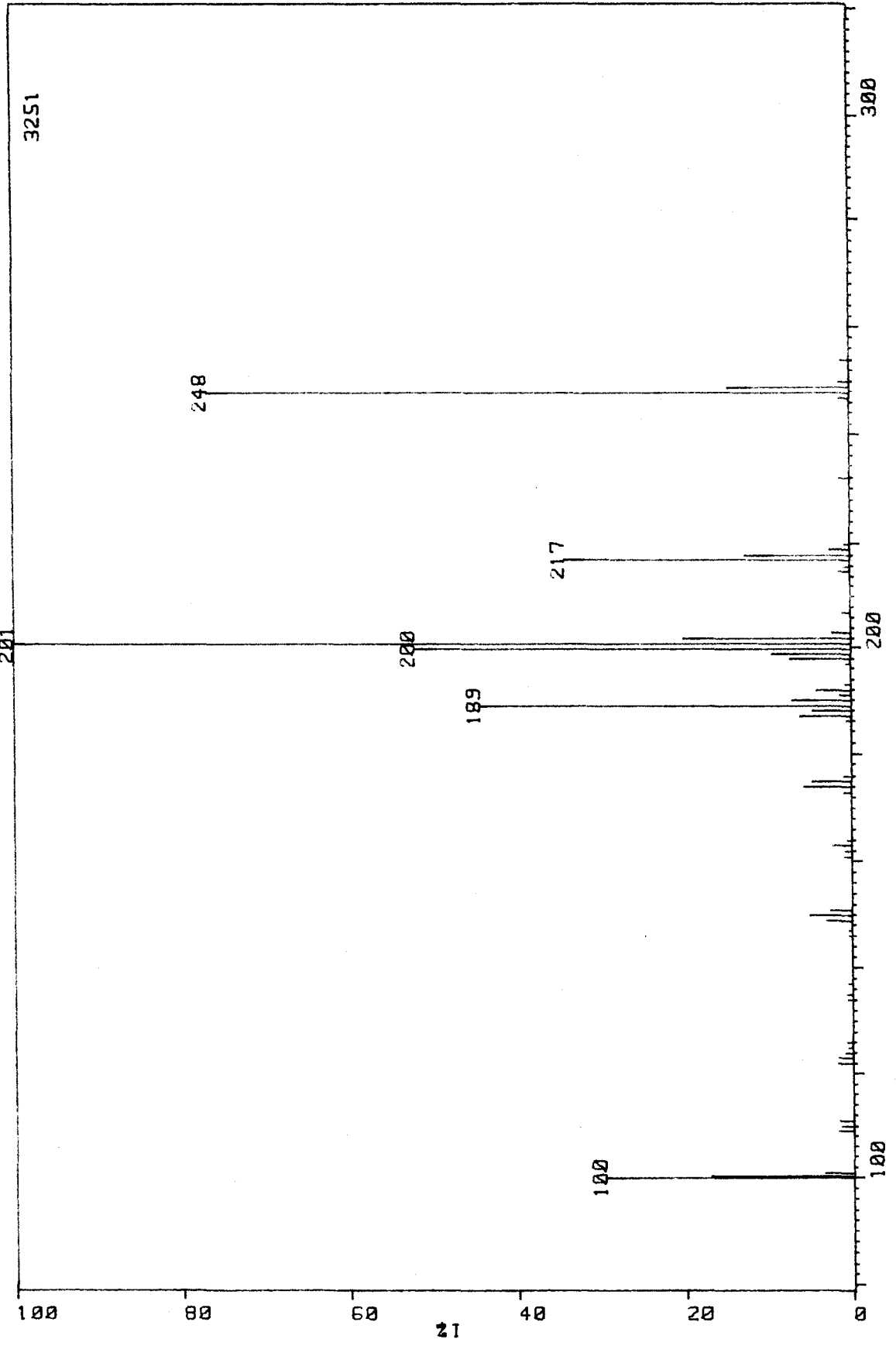
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APPENDIX

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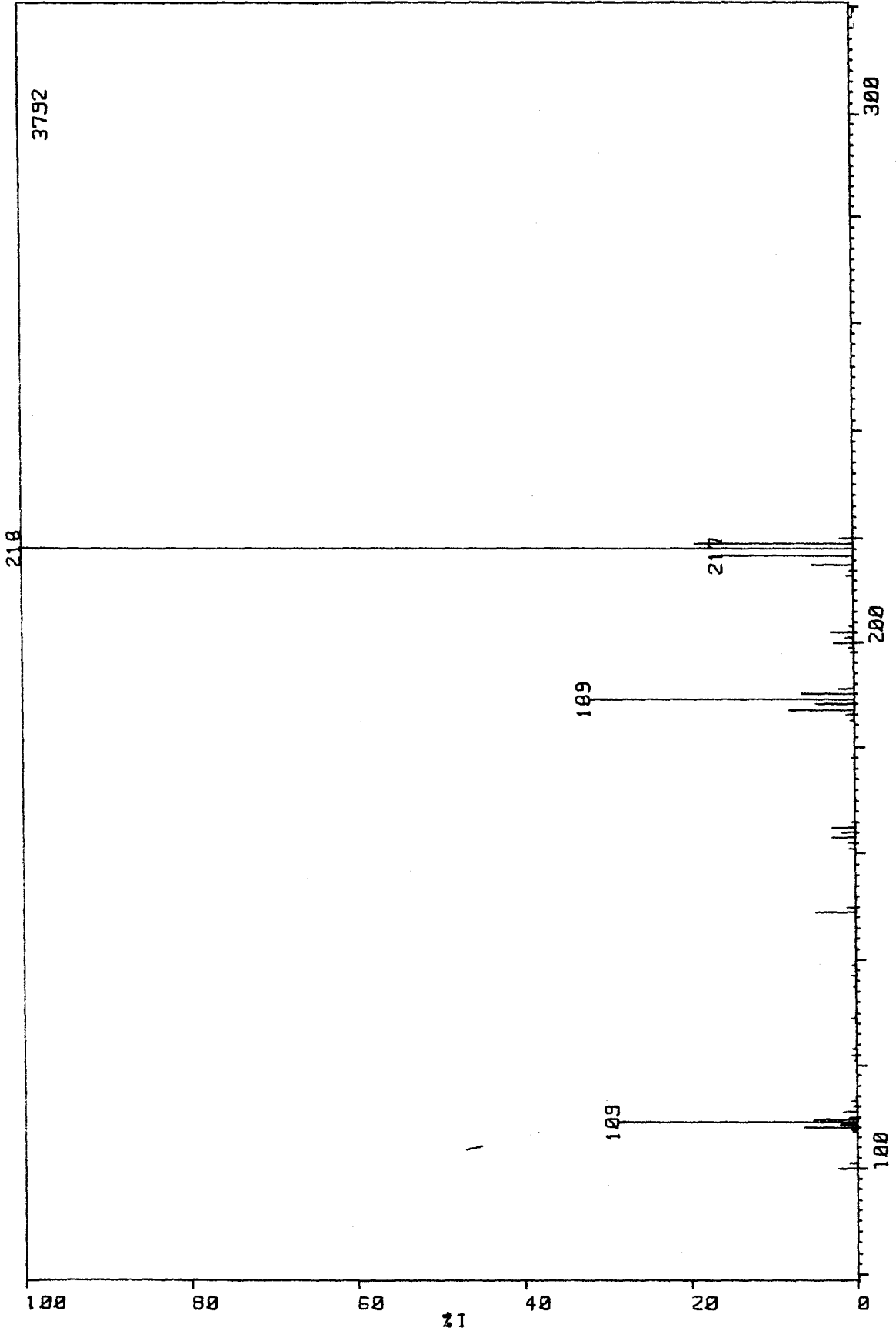
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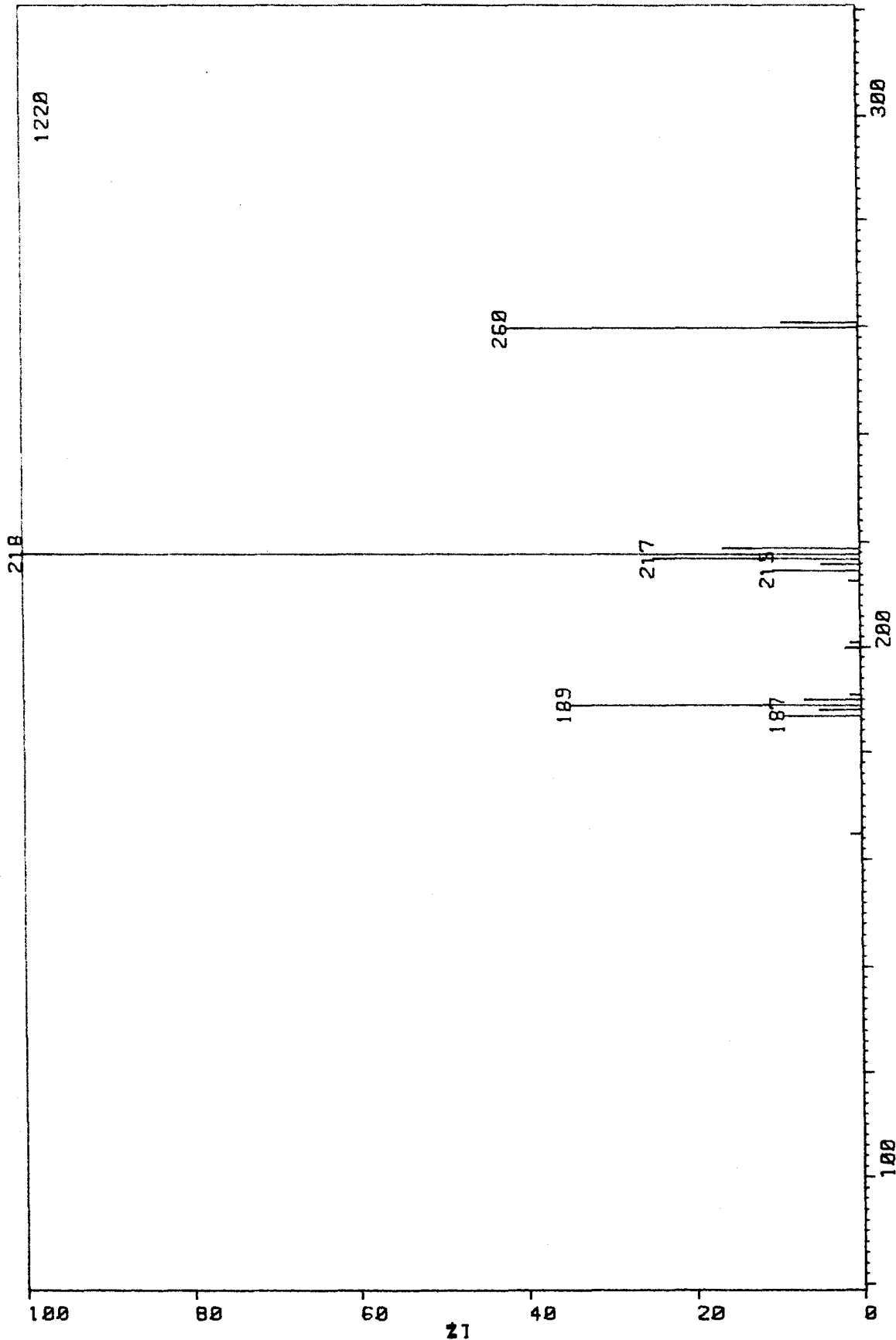
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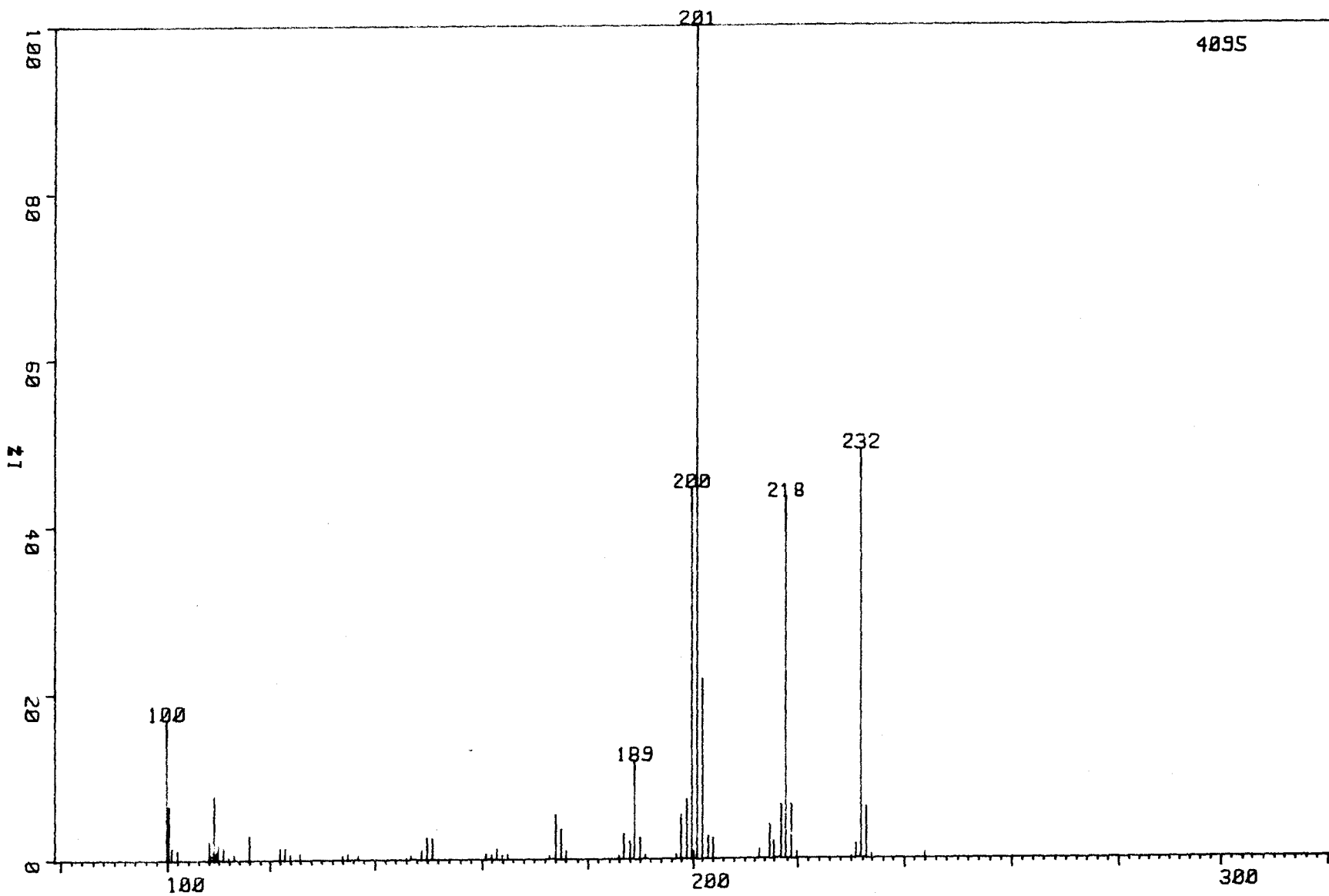


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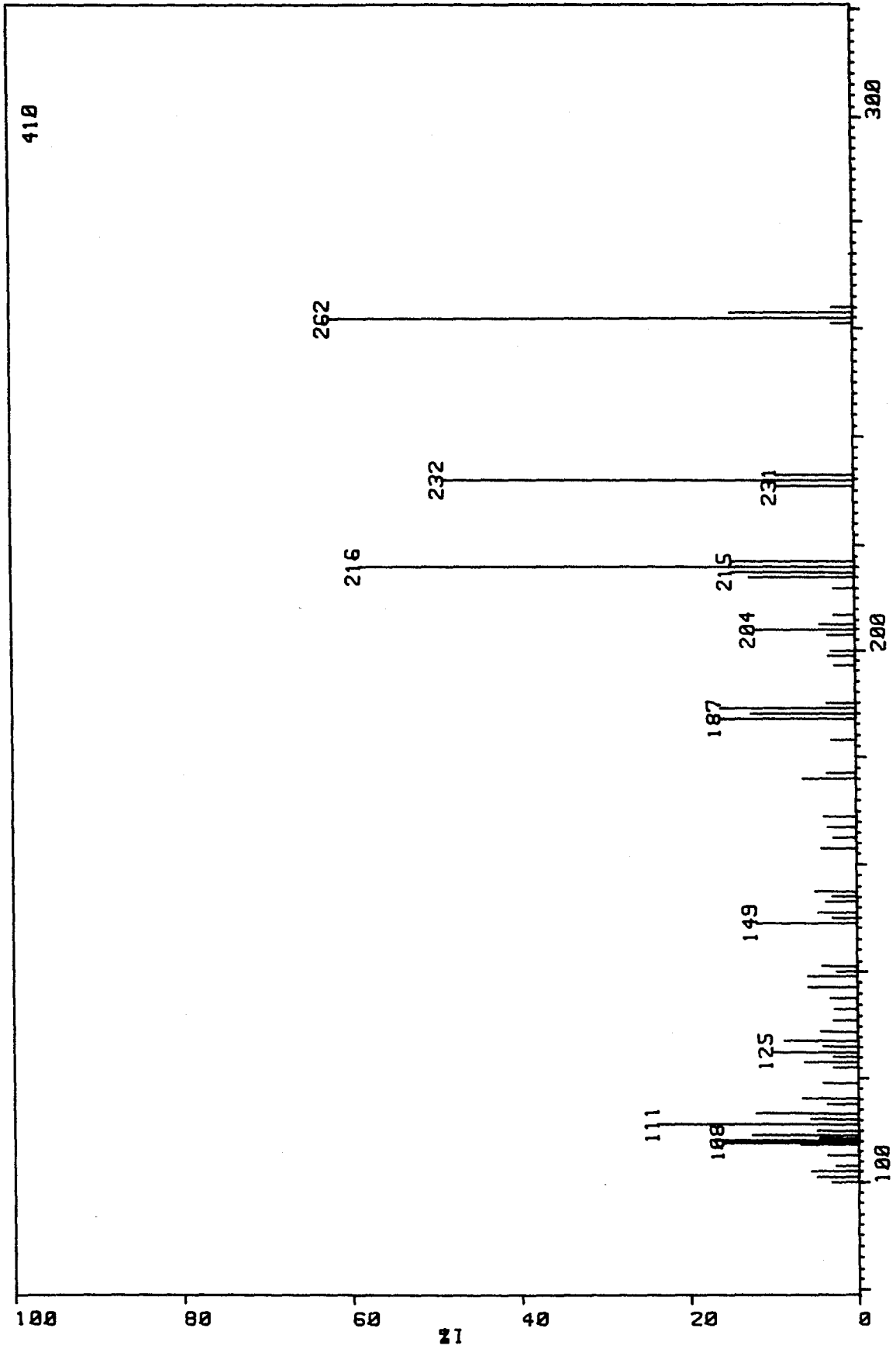
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1.52



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STR:

1,8-ANP

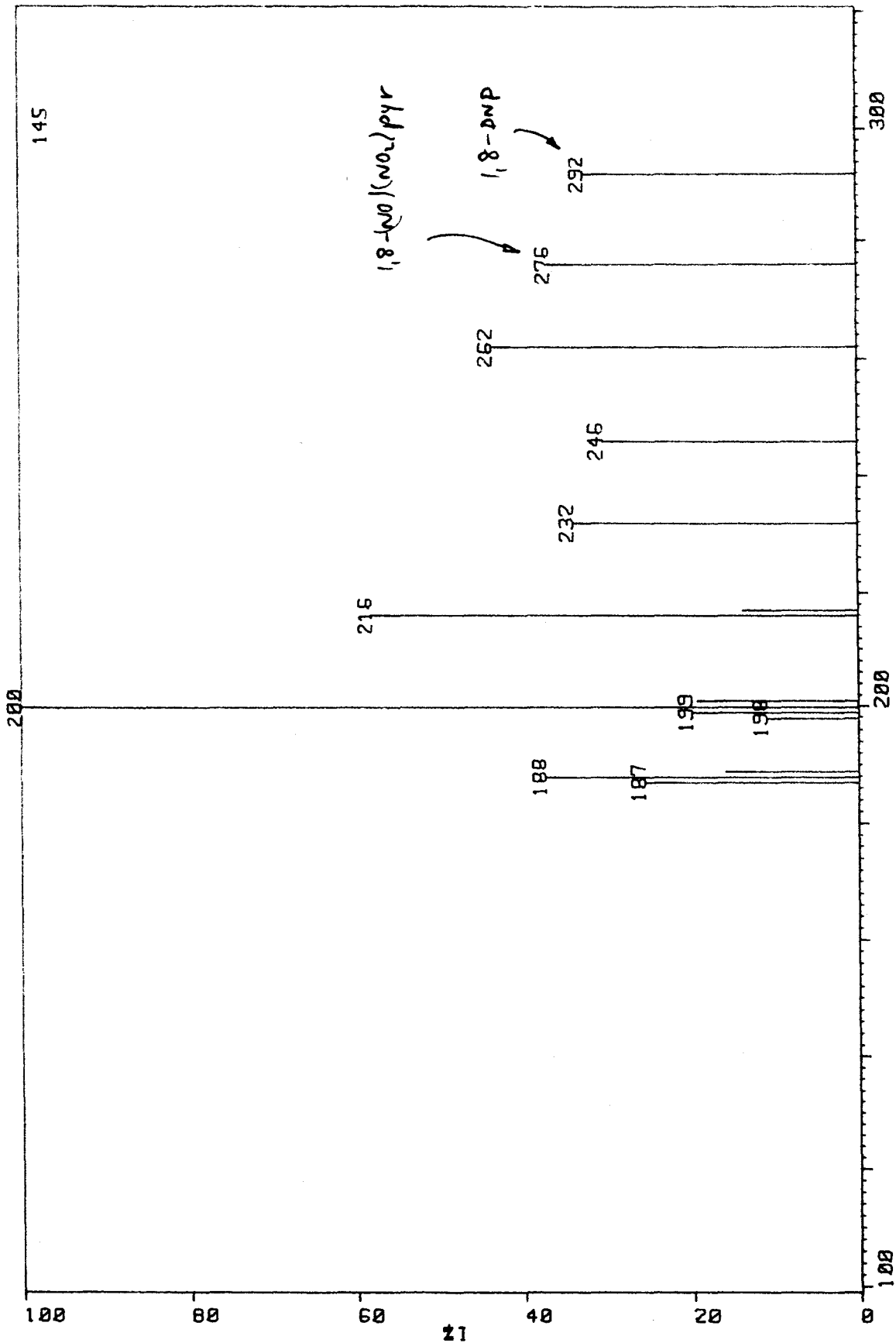
mCPBA
CHCl₃

LOBAR

2 peaks
in 1:2 ratio

30-JUN-75

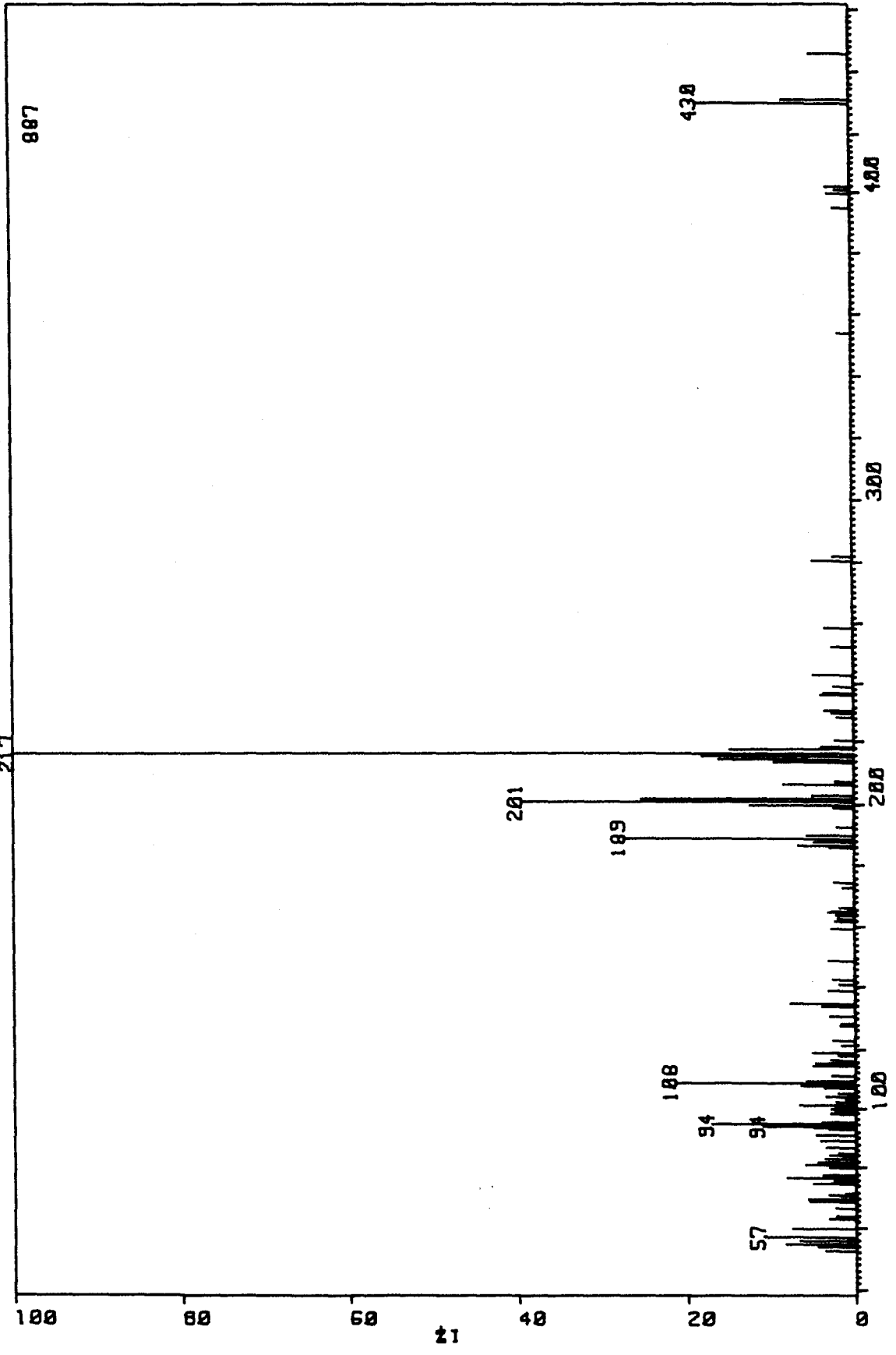
7:3



CE6 53 (830715.1)
CAL: CALFA STR: C.

$\text{pyr-N}_3 \xrightarrow[\phi\text{H}]{h\nu}$ pyr-N=N-pyr (~~isolated~~)
isolated peak

19-AUG-83
3.8

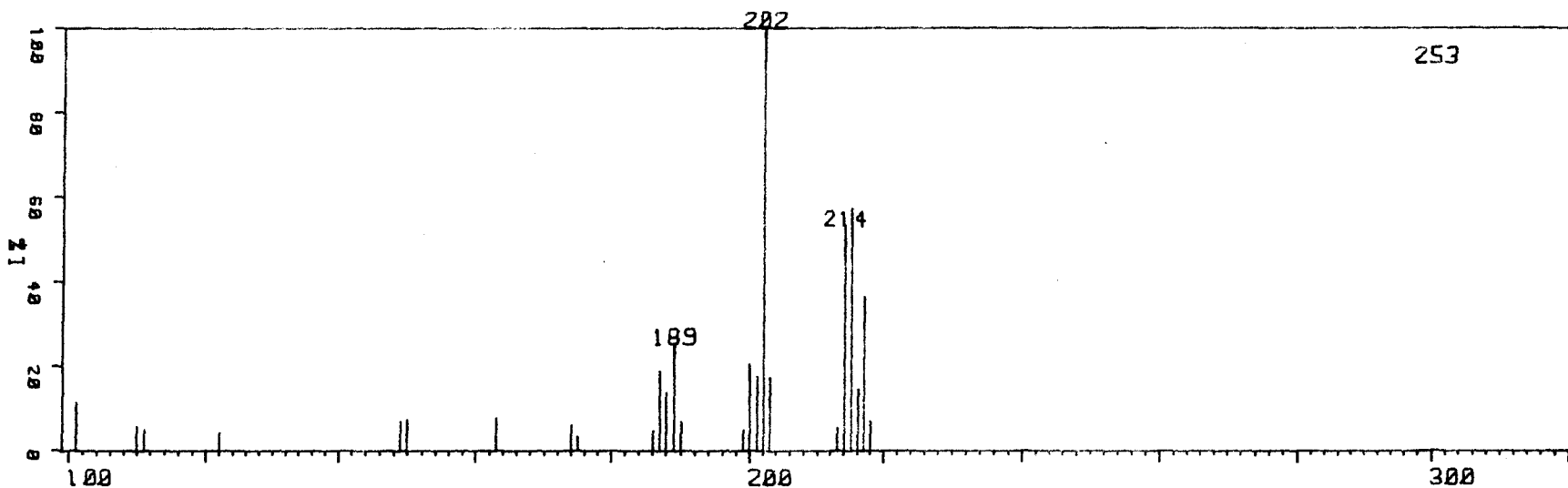


M0713D 9
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CE-830713 AZIDO-PYRENE
STA:

purified by cyano column

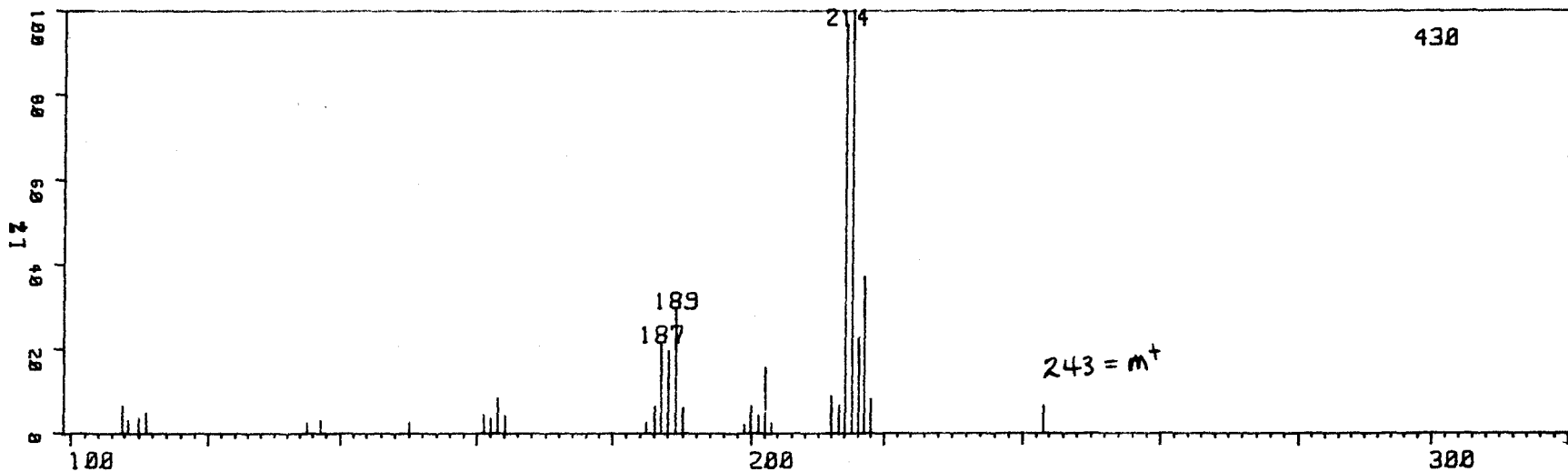
13-JUL-83
0:42



M0713D 18
CAL: CALML

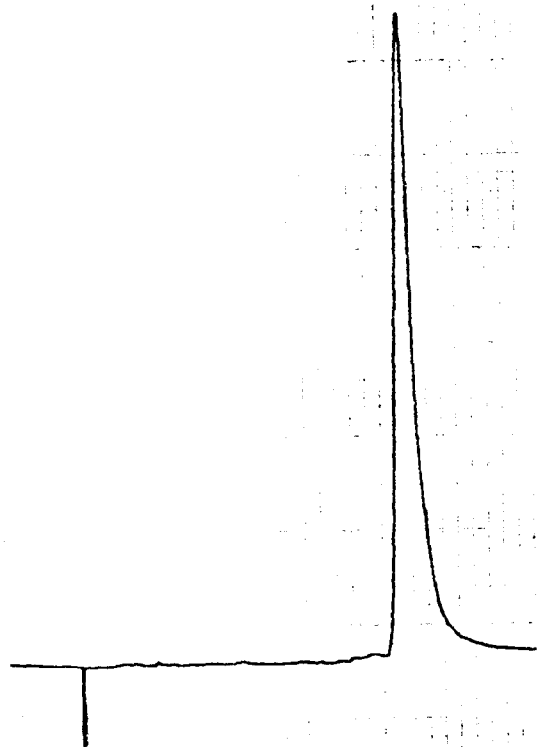
CE-830713 AZIDO-PYRENE
STA:

13-JUL-83
1:21



Conditions

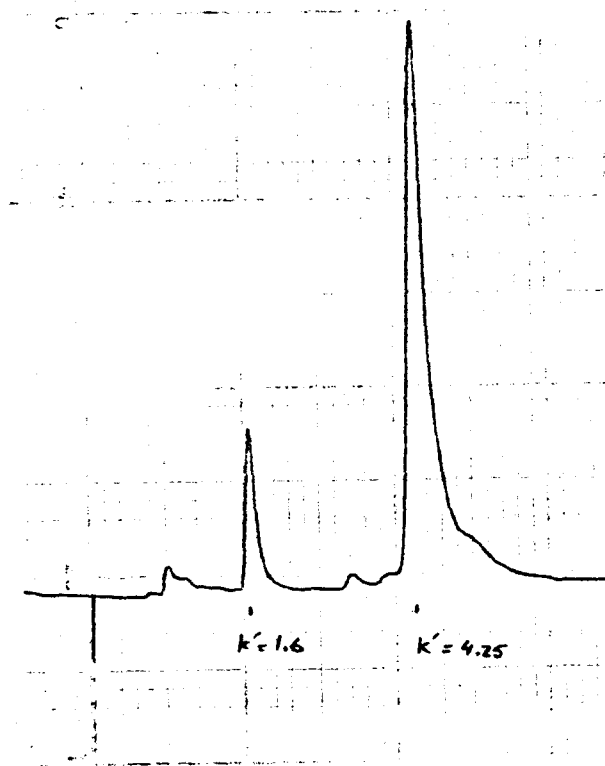
system 2
Column A
Solvent 95% MeOH
5% H₂O



L-NOPyr : Ascorbic Acid

1 : 50

time = 0 min



Same solution

time = 20 min.