SYNTHESIS, STRUCTURAL CHARACTERIZATION AND IDENTIFICATION OF METABOLITES OF NITROARENES

by

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ABSTRACT

Nitroarenes are a class of fused ring aromatic hydrocarbons substituted with nitro groups. They are released into the environment by combustion processes. Nitropyrenes and nitrofluoranthenes are of particular importance because of the mutagenic and carcinogenic properties that they display. Efficient and convenient routes of preparation of the individual nitropyrenol, acetamidopyrenol, nitrofluoranthenol and acetamidofluoranthenol isomers in high purity and good yields are described here. The approach to synthesis was acetylation in the case of the pyrene metabolites and benzoylation in the case of the fluoranthene metabolites, followed by conversion to the corresponding ester and nitration. Purification was carried out before or after hydrolysis of the ester, based on the ease of separation of the esters compared to the phenols. The metabolites synthesized were then characterized by H^1-NMR and mass spectrometry.

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CONTENTS

FIGURES

I. INTRODUCTION

Nitrated polycyclic aromatic hydrocarbons (Nitro- PAHs) or nitroarenes are a class of fused ring aromatic hydrocarbons, substituted with one or more nitro groups. Incomplete combustion processes are the predominent source of most of the nitroarenes detected in the environment (Zielinska et al., 1986). Nitroarenes display a broad spectrum of mutagenic, genotoxic and carcinogenic properties. Some members of this group are direct acting mutagens that depend upon reduction of the nitro function by bacterial nitroreductases, while others exhibit only low levels of potency, or require metabolic activation.

Of this class of compounds, nitropyrenes and nitrofluoranthenes are ubiquitous environmental contaminants, and are of particular concern because of the mutagenic and carcinogenic properties that they display (Ball et al., 1986; Bauer and Howard, 1991; El-Bayoumy et al., 1988; Sugimura and Takayama, 1983) .

Studies have shown that chemical species that react with DNA to produce tumors are not necessarily the parent compounds , but could also be metabolites of the parent compounds, that are formed as intermediates of chemical reactions that the compounds undergo. For example, some metabolites of nitropyrene, the nitropyrenols and acetamidopyrenols have tested positive in mutagenicity assays. By analogy with nitropyrene, it was hypothesized that nitrofluoranthene metabolites - nitrofluoranthenols and acetamidofluoranthenols would also be active. This was the reason for the necessity to obtain them in sufficient quantities in order to be able to work with them.

The particular objectives of this research were to chemically synthesize by an efficient method, the metabolites (principally, the oxidation products) of 1-nitropyrene, 3-nitrofluoranthene and of the oxidized derivatives of 1-aminopyrene and 3-aminofluoranthene. The structures of the metabolites synthesized were then be characterized using analytical chemistry techniques. The metabolites were then be analyzed for mutagenicity by the Ames assay (Ames et al., 1975).

II. LITERATURE REVIEW

II.A. Nitro PAHs in the environment

Nitrated polycyclic aromatic hydrocarbons are environmental contaminants widely present in airborne particulate matter, as detected from environmental sources including diesel and gasoline engine emissions, cigarette smoke condensates, fly ash particles, used motor oil, and emissions originating from incinerators, residential home heaters and wood burning stoves (Rosenkranz and Mermelstein, 1985).

1-Nitropyrene has been detected in airborne particulate organic matter (Pitts et al., 1978), diesel exhaust particulate matter (Schuetzle, 1983), coal fly ash (Mumford and Lewtas, 1982), wood stove emissions (Gibson, 1982), service station effluent (Manabe et al., 1984), photocopier toners (Rosenkranz et al., 1980) and in Japanese grilled chicken (Kinouchi et al., 1986).

2-Nitrofluoranthene has been detected in extracts from ambient air particulate matter from urban and rural areas (Nielsen et al., 1984; Pitts et al., 1985). 3-Nitrofluoranthene has been detected mainly in diesel emissions (Paputa-Peck et al., 1983; Schuetzle, 1983).

II. B. Formation of Nitro- PAHs

Nitro- PAHs were formed by the nitration of PAHs present in combustion gases and on particulate matter either during combustion or while circulating in ambient air (Nielsen et al., 1984; Zielinska et al., 1986).

1-Nitropyrene, a direct by-product of incomplete combustion processes, was identified as the single most abundant nitro-PAH in diesel engine exhaust (Paputa-Peck et al., 1983). It was formed synthetically on filters by the reaction of pyrene with gaseous N_2O_5 (Pitts et al., 1985). 3-Nitrofluoranthene, also identified as a combustion by-product in diesel engine exhaust, was readily produced by the direct nitration of the fluoranthene molecule by N_2O_5 or NO_x (Schuetzle et al., 1982; Zielinska et al., 1986). It has been

reported that the reaction of fluoranthene with N_2O_5 on filters formed the 1-, 3-, 7- and 8- isomers of nitrofluoranthene (Sweetman et al., 1986).

2-Nitrofluoranthene, formed predominantly in ambient air, was formed by the reaction of volatile organic fluoranthenes with N_2O_5 after free radical formation at the C-3 position (Pitts et al., 1985; Zielinska et al., 1986; Zielinska et al., 1987).

II. C. Structure Activity Relationships (SAR)

The genotoxicity of nitro-PAHs in the Salmonella typhimurium plate incorporation assay is largely due to the interaction of an arylnitrenium ion formed through reduction of the nitro group. Protonation of the hydroxylamine intermediate results in the loss of a water molecule from this compound and formation of the nitrenium ion, the ultimate reactive species that binds to DNA. Delocalization of the positive charge from the nitrenium ion into the aromatic ring produces carbonium ions at the ortho and para positions. The lifetime of the charged electrophile would be increased by stabilization of the aromatic carbonium ion, leading to an increase in its chances of binding to DNA. Increased formation of DNA adducts would increase mutagenic potency.

Vance and Levin (1984) have determined that four factors favor nitro- reduction of the nitro- PAHs by Salmonella typhimurium and hence enhance the formation of the electrophilic metabolites that react with DNA. The structure activity relationships defined are:

(1) Physical dimension and degree of aromaticity.

(2) Isomeric position of the nitro group.

(3) Conformation of the nitro group with respect to the plane of the aromatic rings.

(4) Ability to resonance- stabilize the ultimate electrophile.

According to the requirements set forth by Vance and Levin (1984), 3-nitrofluoranthene was predicted and found to be a highly mutagenic compound. It has the optimum length of three aromatic rings. By adding an additional benzene ring, 3-

nitrobenzo[k]fluoranthene was found to have reduced mutagenicity. It was observed that nitro- PAHs with nitro groups sterically forced out-of-plane of the aromatic ring structure were weakly mutagenic or nonmutagenic (Vance and Levin, 1984). The cause for the nitro group to be out of a coplanar position was the location of the nitro substituent between two peri positions or in a bay region.

The genotoxicity of nitro- PAHs was enhanced by structural features that contributed to the resonance stabilization of the reactive nitrenium ion (Vance and Levin, 1984).

II. D. Metabolism of Nitro- PAHs

Under anaerobic incubation conditions, bacterial and mammalian enzymatic systems showed the capacity to participate in the reductive metabolism of nitro- PAHs (Rosenkranz and Mermelstein, 1983).

II. D.1. Bacterial metabolism

Biological activation of most nitro- PAHs is thought to occur through the enzymatic action of nitroreductase enzyme present in the Salmonella typhimurium strains. The nitroreductases convert 1-nitropyrene to 1-nitrosopyrene and then to the corresponding N-hydroxylaminoPAH. Three pathways have been suggested for further metabolism of the N-hydroxylaminoPAH intermediates. One pathway involved acid catalyzed decomposition of the intermediate to produce highly electrophilic arylnitrenium ions that could bind to DNA (Karpinsky et al., 1982; Howard et al., 1983b; Patton et al., 1986). A second pathway involved enzymatic reduction of the intermediate to form amino- PAHs that were capable of undergoing acetylation to form acetamido-PAHs. The third pathway involved direct enzymatic O-acetylation of the hydroxylamine intermediate by the O-acetyltransferase enzyme to form N-acetoxy PAHs which were capable of yielding arylnitrenium ions after acid- catalyzed decomposition (Djuric et al., 1986c; Heflich et al., 1985a; McCoy et al., 1983; Rosenkranz and Mermelstein, 1983). Nitropyrene was reduced by Salmonella typhimurium to yield 1-aminopyrene which can

undergo acetylation to form N-acetyl-1-aminopyrene by acetyl CoA dependent acetyl transferase activity (Orr et al., 1985; Messier et al., 1981).

The proposed oxidation and reduction pathways in the metabolism of 3-nitrofluoranthene were thought to follow the pathways of metabolic activation of 1-nitropyrene (Consolo et al., 1989; Bauer and Howard, 1991; Mitchell et al., 1993). 3-Nitrofluoranthene is reduced to 3-nitrosofluoranthene which is further reduced to N-hydroxy-3-aminofluoranthene with a subsequent reduction producing 3-aminofluoranthene, the endproduct of nitroreduction.

The metabolism of 2-nitrofluoranthene is similar to the pathway for 3-nitrofluoranthene with the exception that the nitro group is positioned on C-2 rather than C-3 (Mitchell et al., 1993).

3-Aminofluoranthene and 2-aminofluoranthene are susceptible to acetylation to form N-acetyl-3-aminofluoranthene and N-acetyl-2-aminofluoranthene, respectively.

II. D.2 In vivo metabolism of nitro- PAHs

The metabolism of 1-nitropyrene administered by various methods to male rats produced metabolites that were eliminated in the feces and the urine. Both unchanged 1-nitropyrene and its metabolites- 1-aminopyrene, 6- and 8-hydroxy-1-aminopyrene have been detected in feces. Metabolites in the bile included 1-aminopyrene, 6- and 8-hydroxy-1-acetamidopyrene and 4,5-dihydroxy-4,5-dihydro-1-nitropyrene. Urinary metabolites included 3- and 8-hydroxy-1-nitropyrene, 1-acetamidopyrene, 6- and 8-hydroxy-1-aminopyrene,6- and 8-hydroxy-1-acetamidopyrene, and their conjugates (Ball et al., 1984b; Ball et al., 1985; El-Bayoumy and Hecht, 1984; Kinouchi et al., 1986).

Studies carried out with rat and mouse liver microsomes with 3-nitrofluoranthene showed that 1-, 6-, 7-, 8-, 9-, and 10-hydroxy-3- nitrofluoranthene were the primary metabolites (Ball et al., 1985; Ball et al., 1986b; Howard et al., 1988; Moller et al., 1988).

Metabolism of 2-nitrofluoranthene from the livers of rats resulted in 8-hydroxy-2-nitrofluoranthene and 9-hydroxy-2-nitrofluoranthene (Zielinska et al., 1987).

II. E. Formation of DNA adducts by nitro- PAHs.

The mutagenicity of nitro- PAHs has been attributed to the covalent binding of the ultimate electrophile to DNA (Dietrich, 1987; Singer and Greenberger, 1983).

When nitro- PAHs reacted with DNA, the major adduct formed was N-(deoxyguanosin-8-yl)-amino PAH, by the covalent bonding of the nitrogen atom from the nitro-PAHs to the C-8 atom of 2'-deoxyguanosine (Andrews et al., 1986; Dietrich, 1987; Howard et al., 1983b).

When metabolites of 1-nitropyrene were reduced with xanthine oxidase in the presence of hypoxanthine, N-(deoxyguanosin-8-yl)-1-aminopyrene was produced, along with two minor adducts which were not characterized (Howard et al., 1983b).

The major DNA adduct formed when 3-nitrofluoranthene was chemically reduced to N-hydroxy-3-aminofluoranthene and subsequently reacted with calf thymus DNA was identified as N-(deoxyguanosin-8-yl)-3-aminofluoranthene (Dietrich et al., 1988).

DNA adducts of 2-nitrofluoranthene have been reported but not definitively characterized in the literature (Herreno-Saenz et al., 1992)

The "aminofluorene insertion" model was developed primarily to explain the damage that the N-(deoxyguanosin-8-yl) adducts of nitro- PAHs causes to DNA (van Houte et al., 1987). The adducts could produce distortions in the DNA which could result in a mutation or a transformed cell. The model suggested that the adducts caused deformities in the structure of DNA by insertion of the aminofluorene moiety between the normal DNA bases. Altering the DNA helix could result in misrepair of the deformed site by the DNA repair enzymes (van Houte et al., 1987).

II. F. Carcinogenicity of nitro- PAHs

Cancer induction by chemicals is believed to be a multi- step process that involves interactions between environmental and endogenous factors. The model for

carcinogenesis includes several stages which ultimately form a tumor or malignant neoplasm, three of which are recognized: initiation, promotion and progression (Hodgson and Levi, 1987).

Initiation is the stage which is rapid and irreversible, where the ultimate carcinogen interacts with DNA. The chemical can either act directly as a carcinogen or require metabolic activation to a procarcinogen to convert it to a reactive electrophile that makes covalent binding to DNA possible. This stage results in the formation of adducts, which, if left unrepaired or are misrepaired, would lead to a mutation in the DNA sequence (Hodgson and Levi, 1987; Williams and Weisburger, 1993).

The second stage in tumor formation is promotion. Promoters can act either by increasing the number of initiated cells or decreasing the latency period. Giving a promoting agent after a dose of an initiating agent enhances carcinogenesis. Promoting agents are not in themselves carcinogens (Hodgson and Levi, 1987; Williams and Weisburger, 1993).

Tumor progression is the final stage in carcinogenesis, which leads to increased growth rates, invasion of healthy tissues and metastases (Hodgson and Levi, 1987; Williams and Weisburger, 1993).

1-Nitropyrene showed carcinogenic activity in both rats and mice. Hirose et al., (1984) showed that highly purified 1-nitropyrene induced malignant fibrous histiocytomas at the site of subcutaneous injection in female Sprague- Dawley rats. They also reported that the mammary glands of these rats developed adenocarcinomas. Other recent studies revealed that 1-nitropyrene, 1-nitrosopyrene and 1-aminopyrene induced mammary adenocarcinomas after p.o. administration in female Sprague- Dawley rats, 1-nitropyrene being the most carcinogenic and 1-aminopyrene being the least (El-Bayoumy et al., 1988). Intraperitoneal or subcutaneous injection of 1-nitropyrene induced adenocarcinomas and fibroadenomas in the mammary glands of female CD rats (Imaida

et al., 1991). 1-Nitropyrene produced lung and liver tumors in A/J mice and newborn CD-1 mice on i.p. injection (El-Bayoumy et al., 1984a; Wislocki et al., 1986).

In other studies, 1-nitropyrene has been shown to be nontumorigenic. 1-Nitropyrene was negative on mouse skin on s.c. administration (El- Bayoumy et al., 1982; Tokiwa et al., 1984). The reduced metabolites of 1-nitropyrene, 1-acetamidopyrene and N-hydroxy-1-acetamidopyrene did not produce mammary tumors when administered i.p. or s.c. to newborn female CD rats (Imaida et al., 1991).

The ambiguity of the carcinogenicity data for 1-nitropyrene makes it difficult to predict its effect on humans.

3-Nitrofluoranthene gave rise to malignant fibrous histiocytomas on subcutaneous administration to male F344 rats (Ohgaki et al., 1982; Sugimura and Takayama, 1983).

At present, there is very little data on the carcinogenicity of 2-nitrofluoranthene.

II. G. Mutagenicity of nitro- PAHs

Most of the nitro- PAHs are direct acting mutagens. This is because they do not require exogenous metabolic activation (provided by the addition of S9 liver fractions) for mutagenicity in the Ames Salmonella mutagenicity assay (Rosenkranz and Mermelstein, 1985). However, they do require nitroreduction by bacterial enzymes. Indirect acting nitro- PAHs require exogenous metabolic activation for their mutagenicity.

II. G.1 Mutagenicity of 1-nitropyrene

1-Nitropyrene accounted for nearly half of the direct acting mutagenicity of diesel particulates in Salmonella typhimurium strain TA98 in the absence of metabolic activation. 1-Nitropyrene was shown to be a potent direct acting mutagen in Salmonella typhimurium strains TA98 and TA98/1,8-DNP_6. The mutagenicity was greatly reduced upon addition of an exogenous metabolic activation system. Nitroreduction and acetylation played more important roles than oxidation, in mutagenicity for 1-nitropyrene. 1-Nitropyrene was shown to be weakly mutagenic without S9 activation in

Chinese hamster ovary cells and Chinese hamster V79 cells (Heflich et al., 1990; Berry et al., 1985).

1-Aminopyrene, the end product of the reduction reaction of 1-nitropyrene (Messier et al., 1981) was found to be less mutagenic with and without S9 than the parent compound (Ball et al., 1984a). 1-Acetamidopyrene, the product of acetylation of 1-aminopyrene (Ball et al., 1984a) was found to be nonmutagenic in the absence of S9 and a potent mutagen after addition of S9 (Ball et al., 1984a)

II. G.2 Mutagenicity of 3-nitrofluoranthene

3-Nitrofluoranthene was shown to be a direct acting highly mutagenic compound in Salmonella typhimurium strains TA98 and TA98/1,8-DNP_6, and extremely mutagenic in strain YG1024 (Ball et al., 1986; Consolo et al., 1989; Vance and Levin, 1984; Greibrokk et al., 1985; Shane et al., 1991; Zielinska et al., 1988; Nakagawa et al., 1987). Reduced mutagenicity was observed in the strains with addition of metabolic activation (Ball et al., 1986; Shane et al., 1991). 3-Nitrofluoranthene was also found to be mutagenic with and without S9 in Chinese hamster V79 cells.

3-Aminofluoranthene, the reduction product of 3-nitrofluoranthene (Bauer and Howard, 1991; Belisario et al., 1990), was found to be less mutagenic than its parent compound (Ball et al., 1986). Mutagenicity was increased by the addition of metabolic activation.

3-Acetamidofluoranthene, produced by the acetylation of 3-aminofluoranthene, was found to be nonmutagenic in strain TA98 and TA98/1,8-DNP_6 in the absence of S9, and mutagenic upon addition of S9 (Ball et al., 1986).

II. G.3 Mutagenicity of 2-nitrofluoranthene

2-nitrofluoranthene was a stronger direct acting mutagen in Salmonella typhimurium strain TA98 than in strain TA98/1,8-DNP_6. Mutagenicity was reduced in both strains upon addition of S9.

II. H. Analytical Techniques

Mass spectrometry with exact mass measurements was employed to confirm the elemental composition and major structural features as demonstrated by fragmentation patterns.

Nuclear Magnetic Resonance (NMR) spectral analysis was carried out on the synthesized compounds to characterize the positions of the substitutions.

III. MATERIALS AND METHODS

III.A.1 Materials

All chemicals were purchased from Aldrich Chemical Company (Milwaukke, WI), unless otherwise specified.

1-Acetoxypyrene, used to synthesize the pyrene metabolites, was synthesized by the acetylation of 1-hydroxypyrene, purchased from Aldrich Chemical Company, and also by oxidation of 1-acetylpyrene (synthesized from pyrene by Dr. Sangaiah) by metachloroperoxybenzoic acid (mCPBA) .

8-Benzoylfluoranthene, obtained from the Friedel Crafts reaction on fluoranthene (Campbell and Easton, 1949), was used to synthesize the fluoranthene metabolites.

For the oxidation reactions, dimethyl dioxirane (DMDO) was synthesized from oxone (Adam et al., 1987).

All dry reagents were obtained by distillation from calcium hydride.

For column chromatography, grade 60 silica gel was used. For the preparative plate thin layer chromatography (TLC), 500 μm silica plates were purchased from The Anspec Company (Ann Arbor, MI).

III.A.2 Instrumental Analysis

Electron impact 70 eV mass spectrometer VG-70-250 SEQ by direct probe sample insertion was used for characterization by mass spectrometry.

Nuclear Magnetic Resonance spectral analysis was carried out using the NMR model Bruker AMX 500 spectrometer, the solvent used being acetone-d_6, unless otherwise specified.

For the normal phase HPLC, an ISCO model 2360 gradient programmer with ISCO model 2350 HPLC pump and Alltech Sil 10x250 mm column was used. For the reverse phase HPLC, an ISCO model 2360 gradient programmer with an ISCO model 2300 HPLC pump and Zorbax ODS 9.4x250 mm column was used, and stopped flow UV spectra were acquired with a Perkin-Elmer LC-85B spectrophotometric detector.

Melting points of the compounds synthesized were measurd by Fisher-Johns melting point apparatus.

III.B. Synthesis of the metabolites of pyrene

III.B.1 Nitration of 1-acetoxypyrene

1-Acetoxypyrene (240 mg) was dissolved in 6 ml of acetic anhydride and 2 ml of the nitration mixture (prepared by dissolving 0.5 ml HNO_3 in 11 ml acetic anhydride, in ice) was added with cooling in ice water and stirring. The reaction mixture was cooled in ice for about 5 minutes and allowed to come to room temperature. After 2.5 hours, 20 ml of d.I.water was added to the reaction mixture and stirring was continued for 10 minutes. The yellow solid was filtered on a Whatman 1 filter paper and vacuum dried. The yield of the mixture of 1-acetoxynitropyrenes was 231 mg (82%).

III.B.2 Hydrolysis of the mixture of 1-acetoxynitropyrenes

The mixture of the 1-acetoxynitropyrenes (231 mg) was dissolved in 25 ml of methanol and 2 ml of 3N NaOH wase added. A water condenser was fitted and the mixture was allowed to reflux over a oil bath heated to 80°C. After 3 hours, 0.5 ml of concentrated hydrochloric acid was added to the contents. The hydrolyzed product was extracted with 50 ml of methylene chloride, water was removed using sodium sulfate and the methylene chloride was rotary-evaporated.

A small amount of the mixture of 1-hydroxynitropyrenes was analyzed by the normal phase HPLC with 100% methylene chloride as eluent. The chromatogram showed the presence of three isomers- the 1-hydroxy-6-nitropyrene, 1-hydroxy-8-nitropyrene and 1-hydroxy-3-nitropyrene, in that order of elution. Crude separation was carried out on 3cmx30cm silica gel column eluted with methylene chloride and final purification was carried out by normal phase HPLC with methylene chloride as eluent. The yield of 1-hydroxy-6-nitropyrene was 71.5 mg (36%; red color), the yield of 1-hydroxy-8-nitropyrene was 54.1 mg (27%; red-brown color) and the yield of 1-hydroxy-3-nitropyrene was 23.6 mg (12%; red-orange color).

III.B.3 Hydrogenation and acetylation of 1-hydroxy-6-nitropyrene

1-Hydroxy-6-nitropyrene (10 mg) was dissolved in ethyl acetate in a two-necked round bottomed flask. Platinum oxide (10 mg) was added and the reaction mixture was stirred under hydrogen at room temperature. After 45 min, 10 drops each of acetic anhydride and dry pyridine were added and stirring was continued under argon gas for about 10 hours. The solution was filtered through a sintered glass frit of pore size 4-5.5μ m and the solvent was evaporated under argon gas. The yield of 1-acetoxy-6-acetamidopyrene was 13 mg.

III.B.4 Hydrolysis of 1-acetoxy-6-acetamidopyrene

1-Acetoxy-6-acetamidopyrene (13 mg) was dissolved in 5 ml of methanol. About 5 ml of methanolic ammonia (obtained by bubbling ammonia gas into methanol, in ice) was added and stirred at room temperature for 1 hour. The formation of acetamido hydroxypyrene and disappearance of acetamidoacetoxypyrene was followed with reverse phase HPLC, with 1:1 methanol:water as eluent, gradient 50 to 100% methanol in water over 25 minutes. Methanol was evaporated using argon gas to give 1-hydroxy-6-acetamidopyrene (11.7 mg).

1-Hydroxy-8-nitro pyrene (4 mg) and 1-hydroxy-3-nitropyrene (9.6 mg) were converted to their respective acetamido forms by the same procedure in yields of 4.8 mg and 10.3 mg, respectively.

NMR and mass spectra data were obtained on all the nitropyrenols and acetamidopyrenols for structural characterization.

III.C. SYNTHESIS OF METABOLITES OF FLUORANTHENE

III.C.1 Oxidation of 8-benzoylfluoranthene

8-benzoylfluoranthene (459 mg) was dissolved in 15 ml methylene chloride and the solution was treated with mCPBA (675 mg). The reaction mixture was cooled to 0° C and trifluoroacetic acid (171 mg) in 15 ml methylene chloride was added dropwise. The reaction mixture was stirred at room temperature (protected from light) for five days.

The methylene chloride was evaporated to dryness. The residue was dissolved in approximately 10 ml of benzene- hexane (3:1) and chromatographed over a 3cmx30 cm silica gel column, using the same solvent system as eluent. Five blue fluorescent bands were obtained, the second band being the 8-benzoyloxy fluoranthene (289.1 mg; 60%) and the fifth band being the starting material (68.9 mg; 15%).

III.C.2 Nitration of the 8-benzoyloxyfluoranthene

To a solution of 8-benzolyoxyfluoranthene (130mg) in acetic acid (2 ml) and methylene chloride (4 ml), was added 0.86 ml of the nitration mixture (prepared by dissolving 0.5 ml of 70% HNO_3 in 11 ml acetic anhydride, on ice) dropwise with cooling. Cooling was removed after 5 minutes. Stirring was continued at room temperature for 7.5 hours. To the reaction mixture cooled in ice, 50 ml of distilled water was added and stirred for 10 minutes. The resultant solid was extracted into methylene chloride and filtered through sodium sulfate to remove traces of waterand then applied to a 3cmx30 cm silica gel column with methylene chloride: hexane (3:2) as eluent. The starting material (77.2 mg; 60%) was obtained along with a mixture of three yellow colored isomers- the 8-nitro-9-benzoyloxyfluoranthene, 3-nitro-9-benzoyloxyfluoranthene and 3-nitro-8-benzoyloxyfluoranthene, in that order of elution. The three isomers were separated by normal phase HPLC with 70:30 methylene chloride : hexane as eluent, in the same elution order as that from the column.

III.C.3 Hydrolysis of the nitrated benzoyloxyfluoranthenes

3-Nitro-8-benzoyloxyfluoranthene (25.5 mg) was dissolved in 20 ml of methanol and 5 ml of 0.5N sodium hydroxide solution was added. The yellow solution was stirred and refluxed in an oil bath at 80° C for 2 hours. The solution turned purple. The methanol was rotary evaporated and the aqueous solution was cooled and acidified with 0.5 ml of concentrated HCl. Solid sodium bicarbonate (0.5 gm) was added to the solution when it became alkaline. The solution was extracted with methylene chloride (50 ml), dried using sodium sulfate and rotary evaporated to give 3-nitro-8-hydroxyfluoranthene (15.4 mg;

53%). The same procedure was carried out with the 3-nitro-9-benzoyloxyfluoranthene and the 8-nitro-9-benzoyloxyfluoranthene.

III.C.4 Hydrogenation and acetylation of 3-nitro-8-hydroxyfluoranthene

3-Nitro-8-hydroxyfluoranthene (4 mg) was dissolved in 8 ml of ethyl acetate and stirred with 4 mg of platinum oxide under hydrogen gas for 45 min. Then 4 drops each of acetic anhydride and dry pyridine were added and stirring was continued for about 10 hours under argon gas. The solution was filtered through a sintered glass frit of pore size 4-5.5 μm and and evaporated under argon gas to give 3-acetamido-8-acetoxyfluoranthene.

III.C.5 Hydrolysis of 3-acetamido-8-acetoxyfluoranthene

3-Acetamido-8-acetoxyfluoranthene was dissolved in 5 ml of methanol. 5 ml of methanolic ammonia was added and stirred at room temperature for about 3 hours. Monitoring of the reaction was carried out as for the analogous pyrene derivative.

The same procedure was followed for the hydrogenation, acetylation and hydrolysis of 3-nitro-9-hydroxyfluoranthene and 8-nitro-9-hydroxyfluoranthene. NMR and mass spectral analyses were carried out on the nitrofluoranthenols and acetamidofluoranthenols.

III.D. ATTEMPTED SYNTHESIS OF 3-NITRO-9-HYDROXYFLUORANTHENE

The above procedure for the synthesis of 3-nitro-9-hydroxyfluoranthene (section III.C.3) gave low yields of the compound since it was the minor isomer. Also, the hydrogenation, acetylation and hydrolysis of 3-nitro-9-hydroxyfluoranthene into 3-acetamido-9-hydroxyfluoranthene (sections III.C.4 and III.C.5) produced incomplete reaction products. Hence, other methods were attempted to synthesize 3-nitro-9-hydroxyfluoranthene in larger quantities.

III.D.1.(i) Bromination of 3-nitrofluoranthene (Campbell and Keir, 1955)

3-Nitrofluoranthene (400 mg) was dissolved completely in 10 ml of nitrobenzene. 500 μl of bromine was added dropwise at room temperature. Stirring was continued at

room temperature for 2 hours. The reaction was stopped, neutralized with aqueous sodium bicarbonate (5%), extracted with chlorobenzene (50 ml), concentrated on the rotary evaporator and the solid was crystallized from chlorobenzene. Yield of 9-bromo-3-nitrofluoranthene : 266.6 mg (41%).

III.D.1.(ii) Hydrolysis of 3-nitro-9-bromofluoranthene (Rice et al., 1983)

n-Butyl Lithium (0.53 ml; 1.4 M) was added to a solution of 3-nitro-9-bromo fluoranthene (120 mg) in dry tetrahydrofuran (THF;20 ml) at room temperature under argon . After 1.5 hours of stirring, t-butyl hydroperoxide (43 µl, 90%) was added and stirring was continued for 1 hour. The reaction mixture was poured into 50 ml water. The compound was extracted with methylene chloride, dried over sodium sulfate and rotary evaporated. Analytical TLC with silica plate and methylene chloride as eluent showed the presence of only starting material.

III.D.2. Hydrogenation, acetylation and hydrolysis of 3-nitro-9-bromofluoranthene

3-Nitro-9-bromofluoranthene (100 mg) was dissolved in ethyl acetate (50 ml). 100 mg of platinum oxide was added and stirred in a hydrogen atmosphere. After 45 minutes, 20 drops each of acetic anhydride and dry pyridine were added. The acetylation was continued under argon for about 10 hours. The 3-acetamido-9-bromofluoranthene was filtered through a sintered glass frit of pore size 4-5.5 µl and dried under argon gas.The compound was characterized by NMR. n-Butyl lithium (0.106 ml) was added to a solution of 3-acetamido-9-bromo fluoranthene (25 mg) in dry THF (10 ml) at -80° C under argon. After 1 hour of stirring at room temperature, t-butyl hydroperoxide (8.6 µl) and stirring was continued for 1 hour. The reaction mixture was treated with 50 ml water and extracted with ethyl acetate (50 ml), dried with sodium sulfate and dried under argon. NMR analysis of the product showed that hydrolysis of the bromo substituent on the 3-acetamido-9-bromofluoranthene did not take place.

III.E. OXIDATION WITH mCPBA

III.E.1 Oxidation of 3-aminofluoranthene with 1 equivalent of mCPBA

mCPBA (50% pure; 0.3 gm) dissolved in 50 ml of methylene chloride was added to 3-aminofluoranthene (0.162 gm) dissolved in 10 ml of methylene chloride. Stirring was continued at room temperature for 2 hours. The reaction mixture was washed with sodium bicarbonate, dried with sodium sulfate and evaporated under argon gas. The compound was redissolved in 5 ml of methylene chloride and was purified using a preparative TLC plate with 1:1 methylene chloride- hexane as eluent. The three bands were scraped off the TLC plate, the silica gel was powdered, dissolved in 1% methanol-chloroform solution and filtered through a sintered glass frit of pore size 40-60 μm. NMR analysis showed that the three bands were starting material (R_f=0.9), 3-nitrofluoranthene (R_f=0.6) and 2-hydroxy-3-nitrofluoranthene (R_f=0.5), in that order.

III.E.2 Oxidation of 3-aminofluoranthene with 3 equivalents of mCPBA

mCPBA (0.8 gm) dissolved in 50 ml methylene chloride was added to 3-aminofluoranthene (0.162 gm) dissolved in 10 ml of methylene chloride. Stirring was continued for 16 hours at room temperature. The solution was washed with sodium bicarbonate, dried with sodium sulfate and rotary evaporated. It was then chromatographed on silica gel with 1:1 methylene chloride-hexane as eluent. NMR analysis of the three bands collected showed 3-nitro-2-hydroxyfluoranthene as the major product and 3-nitrofluoranthene as the minor product, and some starting material.

III.F OXIDATION WITH DMDO

III.F.1 Oxidation of 1-aminopyrene (Murray et al., 1989)

DMDO (7.5 ml) was added to 1-aminopyrene (32.5 mg) dissolved in 5 ml of acetone with cooling. Cooling was removed after 5 minutes and stirring was continued for 30 min. The acetone was evaporated under argon, the solid was dissolved in methylene chloride (5 ml) and chromatographed on a preparative TLC plate with 1:1

methylene chloride-hexane as eluent. 1-nitropyrene (R_f=0.5) was the only compound formed. Yield of 1-nitropyrene: 30 mg (81%).

III.F.2 Oxidation of 3-aminofluoranthene (Murray et al., 1989)

DMDO (7.5 ml) was added to a solution of 3-aminofluoranthene (32.5 mg) in acetone (5 ml) with cooling. Cooling was removed after 5 minutes and stirring was continued for 30 min. The acetone was evaporated under argon, the solid was dissolved in methylene chloride (5 ml) and chromatographed on a preparative TLC plate with 1:1 methylene chloride-hexane as eluent. The products were worked up as before and were identified as 3-nitrofluoranthene (R_f=0.7) and fluoranthene-2,3-quinone (R_f=0.4), in that order of elution.

Yield of 3-nitrofluoranthene : 4 mg (11%)

Yield of fluoranthene-2,3-quinone: 13.5 mg (39%)

III.F.3 Oxidation of 2-nitrofluoranthene

2-Nitrofluoranthene (10mg) was dissolved in 4 ml of acetone. After dissolution, 4 ml of DMDO was added and stirring was continued at room temperature protected from light for six weeks. The progress of the reaction was followed by the normal phase HPLC, using 100 % methylene chloride as eluent. At the end of six weeks, the HPLC profile did not suggest product formation.

III.F.4 Oxidation of 3-nitrofluoranthene

3-Nitrofluoranthene (10 mg) was dissolved in 4 ml of acetone. Then, 4 ml of DMDO was added and stirring was continued at room temperature protected from light for six weeks. The progress of the reaction was followed by normal phase HPLC, using 100 % methylene chloride as eluent. At the end of six weeks, the HPLC profile did not suggest product formation.

III.G. SYNTHESIS OF 3-ACETAMIDO-2-HYDROXYFLUORANTHENE

3-Nitro-2-hydroxyfluoranthene (4 mg; obtained from the mCPBA oxidation of 3-aminofluoranthene) was dissolved in 5 ml of ethyl acetate. 4 mg of platinum oxide was

added and stirred in a hydrogen atmosphere for 1 hour. Then, 4 drops each of acetic anhydride and dry pyridine were added and stirring was continued under argon gas for about 10 hours. The reaction mixture was filtered through a sintered glass frit of pore size 4-5.5 μm and the solvent was evaporated under argon gas. The solid was dissolved in 5 ml of methanol and 5 ml of methanolic ammonia was added. Stirring was continued at room temperature for 3 hours. The progress of the reaction was followed by reverse phase HPLC as described for 1-hydroxy-6-acetamidopyrene. The methanol was evaporated to dryness under argon gas.

NMR and mass spectral analyses confirmed the formation of 3-acetamido-2-hydroxyfluoranthene.

IV. RESULTS

The compounds 6-nitro-1-hydroxypyrene and 8-nitro-1-hydroxypyrene were synthesized first in the 1960's but were available then in minute quantities (Abe and Saito, 1964). Acetamidopyrenols were known from rat urine (Ball et al., 1984) . 3-Nitro-8-hydroxyfluoranthene and 3-nitro-9-hydroxyfluoranthene were isolated previously from a biological incubation mixture (Ball et al., 1985; Howard et al., 1988), but their synthesis has not been described.

Several synthetic methods were employed in the synthesis of the metabolites. These were:

(1) Direct oxidation with two oxidizing agents- DMDO (oxidation of 2-nitrofluoranthene, 3-nitrofluoranthene, 1-aminopyrene and 3-aminofluoranthene) and mCPBA (oxidation of 3-aminofluoranthene)

(2) Hydrolysis of the acetoxy (as in the case of nitropyrenols) or the benzoyloxy (as in the case of nitrofluoranthenols) nitroarenes.

(3) Conversion of other groups such as 3-nitro-8-bromofluoranthene, which has so far been unsuccessful.

(4) Hydrolysis of acetoxyacetamidoarenes.

IV. A Mass Spectral analyses

Mass spectrometry with exact mass measurements was carried out to confirm the identity of the compounds synthesized. The identity of the compounds was obtained by examination of fragmentation patterns that are characteristic for the major functional groups are. The molecular ion suggested the molecular formula of the compound, while exact mass measurements confirmed the elemental composition.

The presence of nitrogen in a compound is indicated by an ion with an odd mass-to-charge ratio. The typical fragmentation pattern for a compound having a nitro group is the loss of 30 mass units corresponding to a -NO group and loss of 46, denoting loss of a nitro group. In acetamido compounds, the acetyl group is cleaved, resulting in a loss of

43 mass units and the nucleus sometimes regains a proton, resulting in a loss of 42 mass units, confirming the presence of an acetyl group.

IV. B. NMR spectral analyses

Nuclear Magnetic Resonance (NMR) spectral analysis was carried out to characterize the substitution patterns of the compounds synthesized, and to distinguish between positional isomers.

IV. B.1 NMR spectra of the nitropyrene derivatives

The NMR spectra for all the nitropyrenols had eight signals in the aromatic region, which is consistent with the presence of eight protons on the di-substituted pyrene compounds. The general features used for assigning identifications were that protons ortho and peri to the nitro group shift downfield and protons ortho to the hydroxy group shift upfield and protons peri to the hydroxy group shift downfield.

In the 6- and 8-nitro-1-hydroxy pyrenes, the proton at position C2 appears at 7.6 ppm, due to the shielding effect of the hydroxy group adjacent to position C2. The same proton appears as a singlet more downfield at 8.3 ppm in the 3-nitro-1-hydroxypyrene, due to the deshielding influence of the nitro group.

IV.B.1(c)1-Hydroxy-3-nitropyrene

The proton at C4 is peri to the nitro group, and has shifted most downfield. Its signal is located at 8.65 ppm. The proton at C10 is shifted downfield to 8.55 ppm since it is peri to the hydroxy group. At 8.4 ppm and 8.35 ppm are located the peaks for protons at C8 and C6. A doublet at 8.35 ppm is identified as a signal for the proton at C9. The singlet at 8.3 ppm corresponds to the proton at position C2, indicating that its two adjacent protons have been substituted. The peak at 8.25 ppm corresponds to the proton at position C5 and the triplet at 8.15 ppm corresponds to the proton at C7.

IV.B.1(a) 1-Hydroxy-6-nitropyrene

The NMR of this compound was carried out in $CDCl_3$. The nitro group at position C6 will make the proton at position C7 appear downfield, due to the electron

withdrawing nature of the nitro group. The three signals in the downfield region between 8.6 and 8.8 ppm signify protons at positions C5, C7 and C10, in that order. The signal for the proton at C8 appears at 8.24 ppm. Protons at C4 and C9, being in the K-region, appear in the region between 8.0 and 8.1 ppm.

IV.B.1(b) 1-Hydroxy-8-nitropyrene

The NMR spectrum indicates that all the signals are doublets, suggesting that each proton has only one proton in its immediate neighbourhood. The protons at positions C9 and C10 appear most downfield, since they are peri to the nitro and hydroxy groups, respectively. One of the signals at 8.7 ppm corresponds to the proton at C7. The peaks at 8.0 and 8.15 ppm correspond to the protons at positions C5 and C4. Irradiating at 8.95 ppm collapsed the signal at 8.7 ppm, therefore the signal at 8.95 ppm is the proton at C10 and the signal at 8.7 ppm is the proton at C9. Irradiating at 8.7 ppm collapsed the doublets at 8.95 and 8.1 ppm, therefore the signal at 8.7 ppm is the protons at C9 and C7, and the signal at 8.1 ppm is the proton at C6.

IV. B.2 NMR spectra of the acetamidopyrenols

The NMR spectra of the acetamidopyrenols have eight peaks in the downfield region, justifying the presence of eight protons on the compounds.

As in the nitropyrenols, the protons peri and ortho to the acetamido group appear downfield, the protons peri to the hydroxy group appear downfield, whereas the proton ortho to the hydroxy group appears upfield. The proton at position C2 appears at 7.6 ppm in the 6- and 8-acetamido-1-hydroxypyrenes, and at 8.3 ppm in the 3-acetamido-1-hydroxypyrene. In all the acetamidopyrenes, the methyl protons on the acetamido group appear at 2.33 ppm and they have a three-proton intensity. The signal for the -NH on the acetamido group appears at 9.6 ppm in all the isomers.

The NMR spectra of the individual acetamidohydroxypyrenes are not discussed in detail since structural identity is ensured by synthesis from the corresponding nitrohydroxypyrenes.

IV. B.3 NMR spectra of the Nitrobenzoyloxyfluoranthenes

In fluoranthene, protons at positions C8 and C9 are most upfield.

The spectra of the nitrobenzoyloxyfluoranthenes have thirteen signals, accounting for eight protons on the di-substituted fluoranthene molecule and five protons on the phenyl ring of the benzoyloxy substituent. The meta protons on the phenyl ring of the benzoyloxy substituent appear at around 7.6 ppm, the para protons at 7.8 ppm and the ortho protons appear in the region between 8.2 and 8.4 ppm.

IV.B.3(a)3-Nitro-8-benzoyloxyfluoranthene

The metacoupled doublet of doublet at 7.4 ppm corresponds to the proton at position C9. The metacoupled doublet for the proton at C7 appears at 8.0 ppm. The triplet at 7.9 ppm corresponds to the proton at C5. The two doublets at 8.6 ppm and 8.7 ppm correspond to the protons at positions C2 and C4, which appear downfield because of their proximity to the nitro group. The rest of the peaks in the region between 8.2 and 8.3 ppm denote the protons at positions C6, C1 and C10 of the fluoranthene molecule.

IV.B.3(b)3-Nitro-9-benzoyloxyfluoranthene

The metacoupled doublet of doublets at 7.4 ppm corresponds to the proton at C8, it being the most upfield proton in fluoranthene. The triplet at 7.9 ppm denotes the proton at position C5. The metacoupled doublet at 8.1 ppm corresponds to the proton at C10, which is metacoupled to the proton at C8. The proton at position C6 appears upfield at 8.1 ppm due to the benzoyloxy group. The doublet at 8.2 ppm corresponds to the proton at C7. The doublet at 8.5 ppm denotes the proton at C2. In the region between 8.2 ppm and 8.3 ppm are present the ortho protons on the phenyl ring of the benzoyloxy substituent (the doublet on the right side of the bunch), and the protons on C1 and C4 of the fluoranthene molecule.

IV.B.3(c)8-Benzoyloxy-9-nitrofluoranthene

The triplet at 7.6 ppm corresponds to the protons at positions C2 and C5. The two doublets at 8.1 and 8.2 ppm correspond to the protons at C3 and C4 due to resonance

with the benzoyloxy and nitro groups, respectively. The peak at 8.3 ppm corresponds to the proton at C7. The peaks for the protons at C1 and C6 appear downfield, at 8.4 and 8.45 ppm, due to their presence in the bay region. The proton at C10 appears at 8.85 ppm, since it is next to the nitro group.

The NMR spectra of the individual nitrohydroxyfluoranthenes and acetamidohydroxyfluoranthenes follow those of the corresponding benzoyloxynitrofluoranthenes, and so they are not discussed in detail.

V. Discussion

The metabolites of 1-nitropyrene and the 3-nitrofluoranthene have been synthesized and their structures characterized by NMR and Mass Spectrometry. These compounds have been synthesized in good yields and with minimal purification required of the final products.

1-Aminopyrene and 3-aminofluoranthene were oxidized by mCPBA and DMDO were synthesized and the products were characterized by NMR and mass spectrometry. mCPBA oxidation of 3-aminofluoranthene gave 3-nitro-2-hydroxyfluoranthene and 3-nitrofluoranthene as products, whereas oxidation of 3-aminofluoranthene with DMDO produced 3-nitrofluoranthene and fluoranthene-2,3-quinone as products. Both mCPBA and DMDO oxidize amino groups to nitro groups. In polycyclic aromatic systems, both the oxidizing agents first epoxidize the C1-C2 bond connected with the amino group, and the epoxide subsequently rearranges to a phenol. The difference in the oxidizing agents arises due to different reaction mechanisms of the hydroxy-amino group. The amino group is oxidized to a nitro group with mCPBA. DMDO however, oxidizes to a quinone faster than to a nitro group. The oxidation with DMDO of 2-nitrofluoranthene and 3-nitrofluoranthene did not give any product, because of the deactivating effect of the nitro group. The various methods employed for the synthesis of 3-nitro-9-hydroxyfluoranthene and 3-acetamido-9-hydroxyfluoranthene were unsuccessful. In 8-benzoyloxyfluoranthene (from which the fluoranthene metabolites were synthesized), the most activated positions are C3 and C9. Nitration preferentially yields 3-nitro-8-benzoyloxyfluoranthene and 8-nitro-9-benzoyloxyfluoranthene. Since position C4 is not activated for nitration, very small amounts of the 3-nitro-9-benzoyloxyfluoranthene isomer are obtained.

The metabolites of the pyrenols and fluoranthenols synthesized were tested in the Ames assay for mutagenicity by Zhang and Renninger (unpublished data). It was noted that the 1-nitro-6-hydroxypyrene produced 6 rev/nmole in TA98 strain without S9

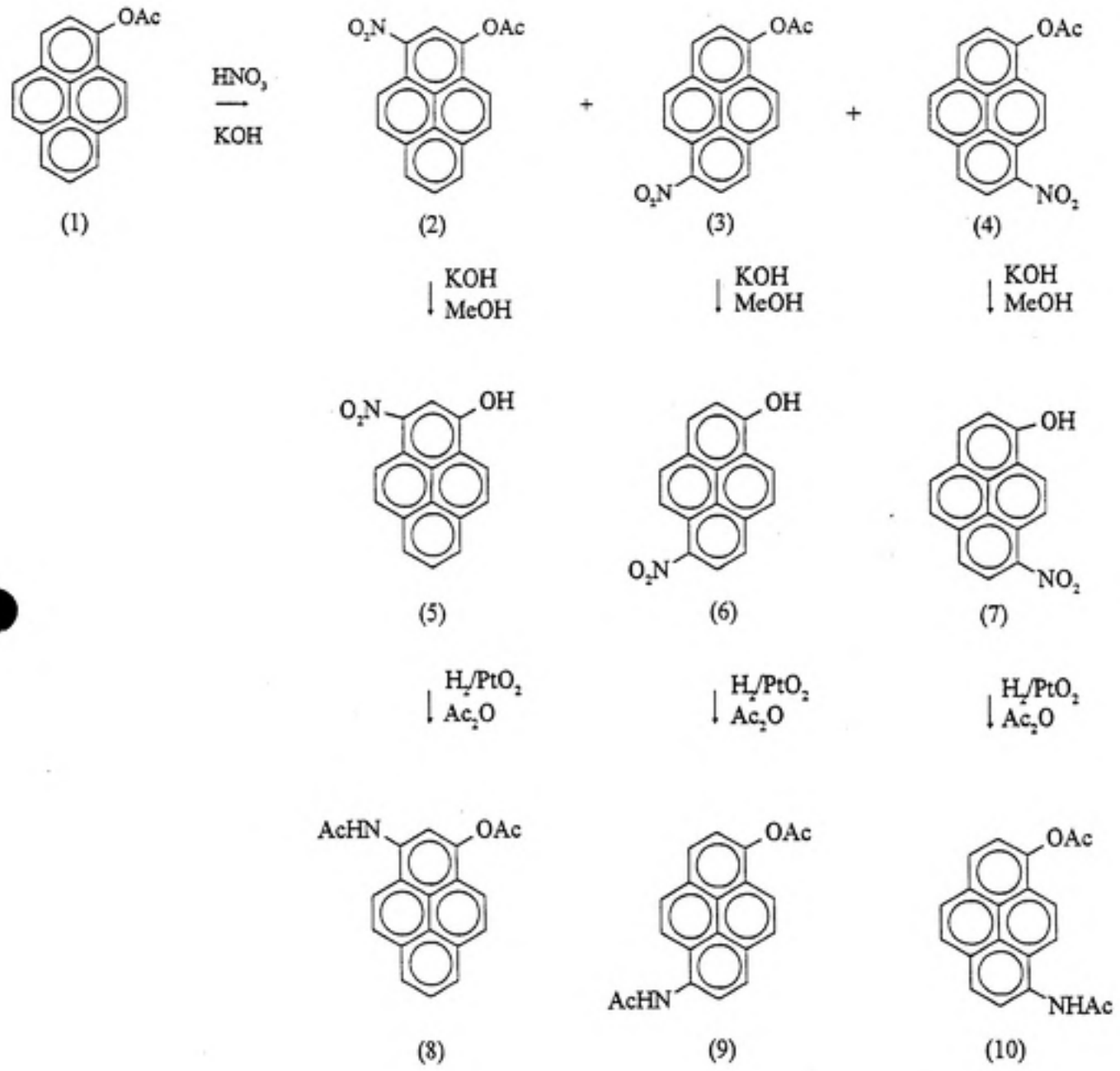
enzyme, and 121 rev/nmole with S9 (Renninger, unpublished data). The same compound produced a low count without S9 and around 350 rev/nmole with S9 when the experiment was repeated by Zhang at the optimum S9 concentration (unpublished data). When 1-acetamido-6-hydroxypyrene was tested by Renninger, it produced no rev/nmole in TA98 strain without S9 and 17 rev/nmole with S9 enzyme. When tested by Zhang it produced no rev/nmole without S9 and around 800 rev/nmole in TA98 with S9 at the optimum concentration. The 1-nitro-8-hydroxypyrene and 1-nitro-3-hydroxypyrene were seen to be moderately active without S9, producing 2.9 rev/nmole and 8.8 rev/nmole, and active with S9, producing 8.8 rev/nmole and 11.2 rev/nmole respectively. Their respective acetamidos were inactive, the 1-acetamido-8-hydroxypyrene producing 0.6 rev/nmole in TA98 without S9 and 0.4 rev/nmole with S9, and the 1-acetamido-3-hydroxypyrene producing 0.69 rev/nmole without S9 and 0.01 rev/nmole with S9 (Zhang, unpublished data).

Among the nitrofluorathenes, only the 3-nitro-8-hydroxyfluoranthene was active, with 43 rev/nmole in TA 98 without S9 and 55.4 rev/nmole with S9. The 3-nitro-9-hydroxyfluoranthene showed 7.2 rev/nmole without S9 and 6.1 rev/mole with S9, the 8-nitro-9-hydroxyfluoranthene produced no rev/nmole both without and with S9. The 3-nitro-2-hydroxyfluoranthene was moderately active with 11.9 rev/nmole without S9, and with 5.1 rev/nmole with S9. The 3-nitro-8-benzoyloxyfluoranthene was very active with 28.5 rev/nmole in TA 98 without S9 and 93.2 rev/nmole with S9. The 3-acetamido-8-hydroxyfluoranthene and 3-acetamido-2-hydroxyfluoranthene were inactive, the former producing 1.24 rev/nmole without S9 and 2.0 rev/nmole with S9, and the latter producing 1.1 rev/nmole without S9 and 1.2 rev/nmole with S9 (Zhang, unpublished data). The 3-acetamido-9-hydroxyfluoranthene has not yet been tested for mutagenicity. 2-Nitrofluoranthene produced about 200 rev/nmole in the TA98 strain both without and with S9. 3-Nitrofluoranthene was highly active without S9, producing 2420 rev/nmole

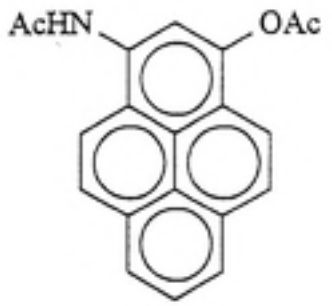
and 222 rev/nmole with S9. 3-Aminofluoranthene was also active, producing 43 rev/nmole without S9 and 113 rev/nmole with S9 enzyme.

The above mutagenicity data from the Ames assay provides the tools to carry out a direct evaluation of some of the genotoxic properties of the metabolites. It also provides a comparison between the mutagenicity of the metabolites and the parent compound, which leads to conclusions as to whether the pathway in which a metabolite is formed is an activation or a detoxification pathway.

From the Ames assay data, it follows that in pyrene, the 1,6- isomers were more active than the rest of the metabolites. 1-Acetamido-6-hydroxypyrene was considerably more active than 1-nitropyrene. In the case of fluoranthene, the activity of all derivatives was less than that of the parent fluoranthene. Hence it can be concluded that the 1,6-isomers of pyrene follow an activation pathway, while the fluoranthene derivatives seem to be detoxification products.

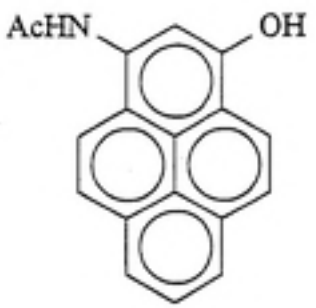


SYNTHESIS OF THE NITROPYRENE METABOLITES



(1)

NH_3 / MeOH



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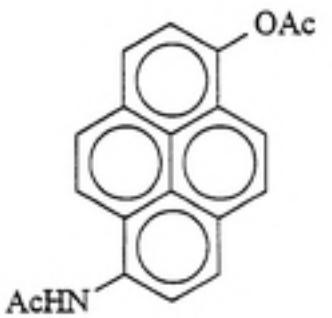

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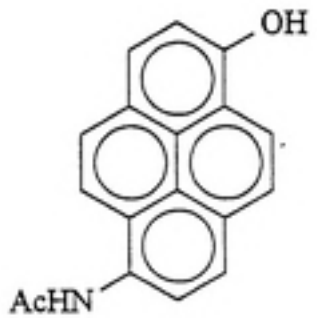
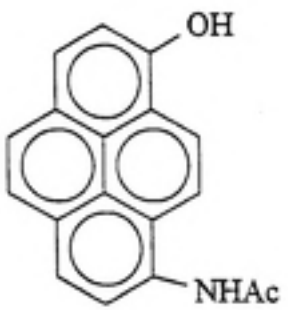


(4)

(5)

NH_3 / MeOH



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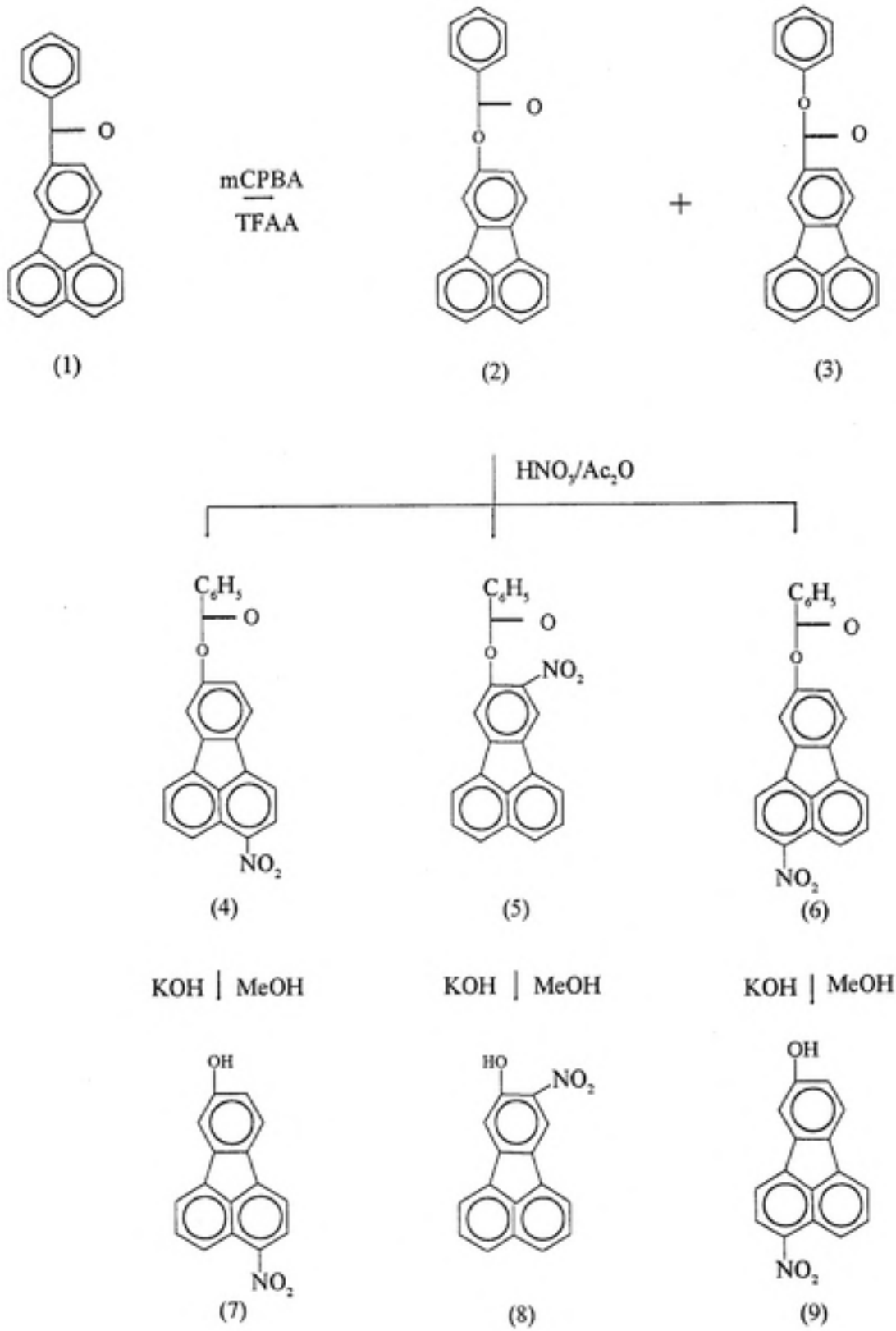
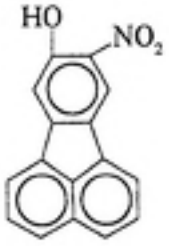
SYNTHESIS OF THE NITROPYRENE METABOLITES (CONTD)



SYNTHESIS OF THE NITROFLUORANTHENE METABOLITES

(1)

H_2/PtO_2 | Ac_2O



(2)

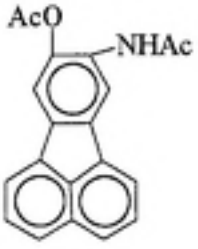
H_2/PtO_2 | Ac_2O

(3)

H_2/PtO_2 | Ac_2O

(4)

NH_3 | MeOH



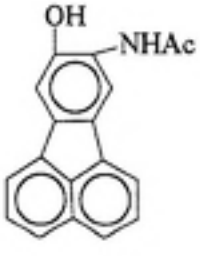
(5)

NH_3 | MeOH

(6)

NH_3 | MeOH

(7)



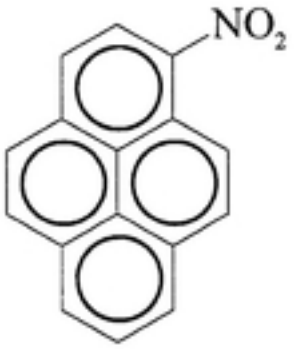
(8)

(9)

SYNTHESIS OF THE NITROFLUORANTHENE METABOLITES (CONTD)

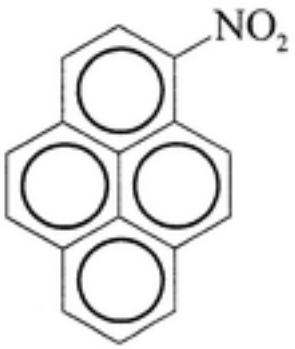
(1)

mCPBA



(2)

DMDO



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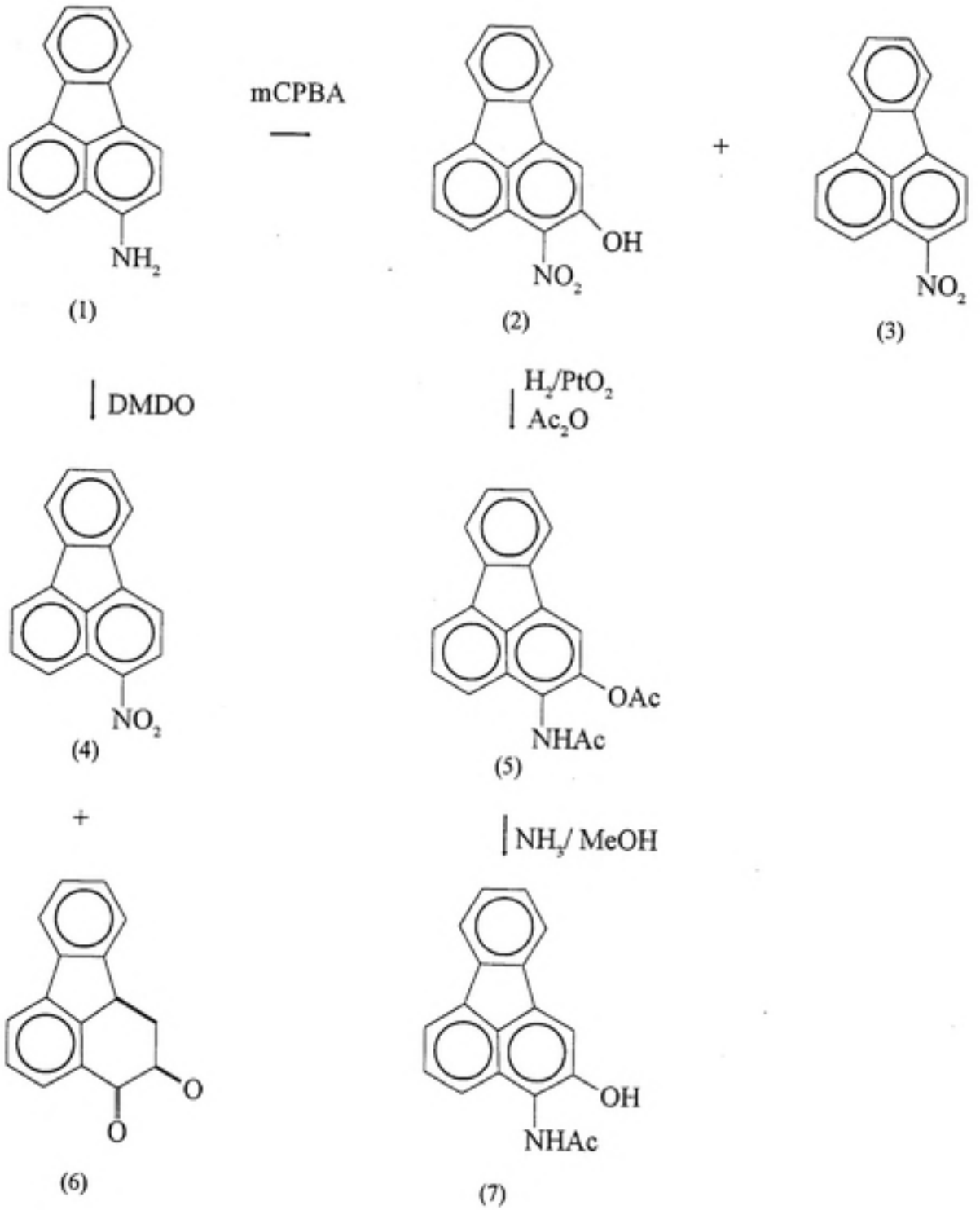
OXIDATION OF 1-AMINOPYRENE



OXIDATION OF 3-AMINOFLUORANTHENE

Synthesis of the nitropyrene metabolites

1 1-acetoxypyrene
2 1-acetoxy-3-nitropyrene
3 1-acetoxy-6-nitropyrene
4 1-acetoxy-8-nitropyrene
5 1-hydroxy-3-nitropyrene
6 1-hydroxy-6-nitropyrene
7 1-hydroxy-8-nitropyrene
8 1-acetoxy-3-acetamidopyrene
9 1-acetoxy-6-acetamidopyrene
10 1-acetoxy-8-acetamidopyrene

Synthesis of the nitropyrene metabolites (contd)

1 1-acetoxy-3-acetamidopyrene
2 1-hydroxy-3-acetamidopyrene
3 1-acetoxy-6-acetamidopyrene
4 1-hydroxy-6-acetamidopyrene
5 1-acetoxy-8-acetamidopyrene
6 1-hydroxy-8-acetamidopyrene

Synthesis of the nitrofluoranthene metabolites

1 8-benzoylfluoranthene
2 8-benzoyloxyfluoranthene
3 8-carboxy fluoranthene phenyl ether
4 3-nitro-8-benzoyloxyfluoranthene
5 9-nitro-8-benzoyloxyfluoranthene
6 3-nitro-9-benzoyloxyfluoranthene
7 3-nitro-8-hydroxyfluoranthene
8 9-nitro-8-hydroxyfluoranthene
9 3-nitro-9-hydroxyfluoranthene

Synthesis of the nitrofluoranthene metabolites (contd)

1 3-nitro-8-hydroxyfluoranthene
2 9-nitro-8-hydroxyfluoranthene
3 3-nitro-9-hydroxyfluoranthene
4 3-acetamido-8-acetoxyfluoranthene
5 9-acetamido-8-acetoxyfluoranthene
6 3-acetamido-9-acetoxyfluoranthene
7 3-acetamido-8-hydroxyfluoranthene
8 9-acetamido-8-hydroxyfluoranthene
9 3-acetamido-9-hydroxyfluoranthene

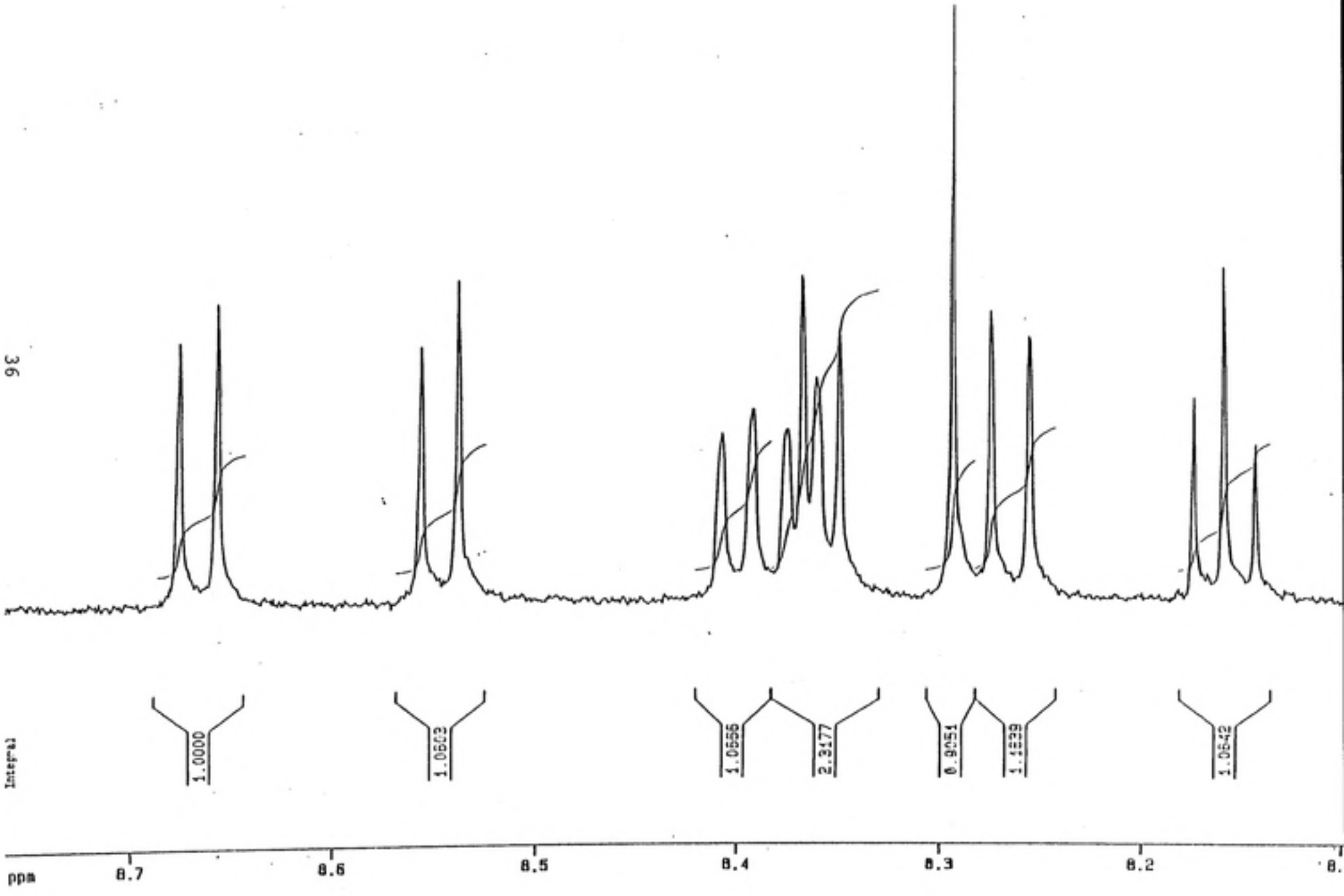
Oxidation of 1-aminopyrene

1 1-aminopyrene
2 1-nitropyrene
3 1-nitropyrene

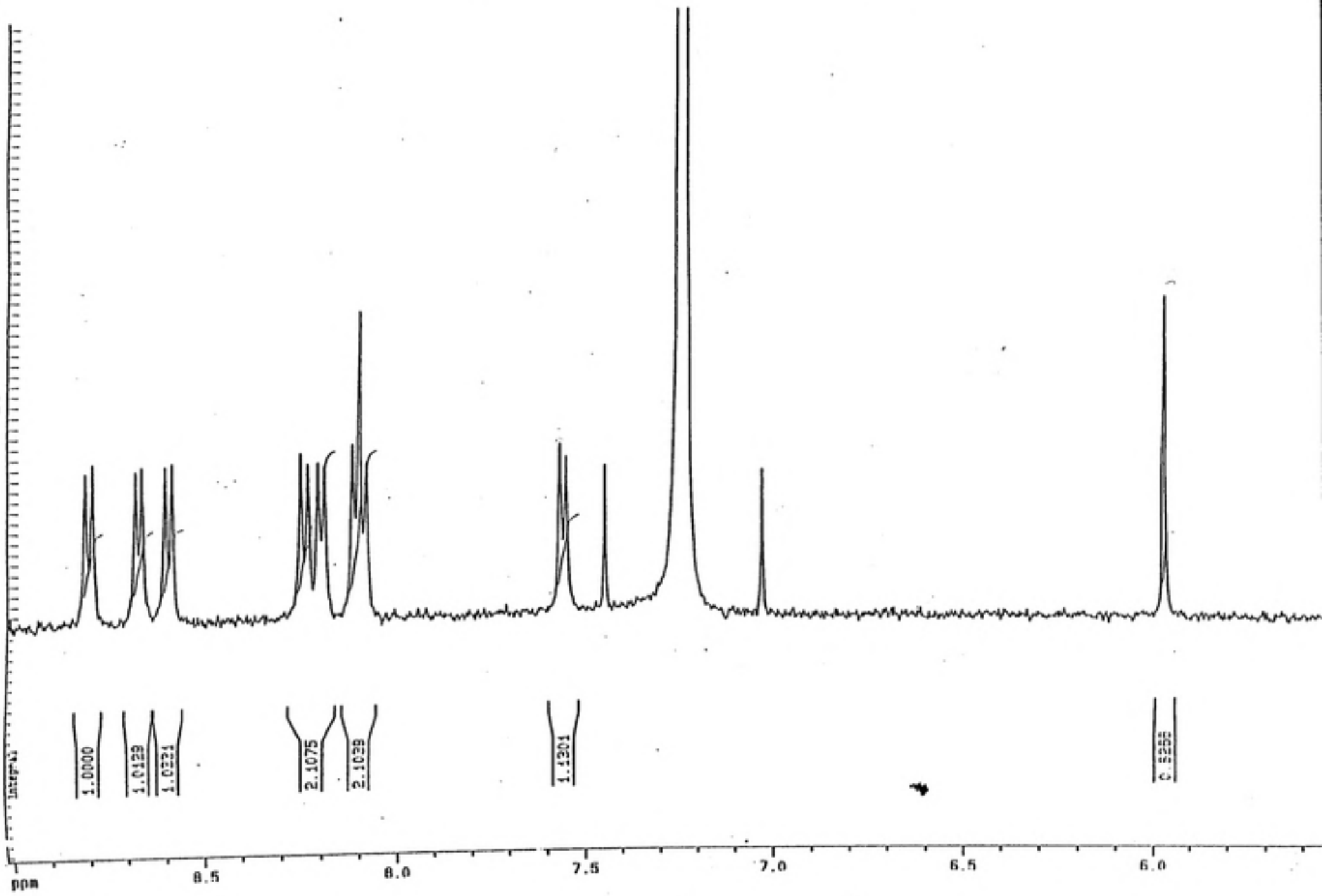
Oxidation of 3-aminofluoranthene

1 3-aminofluoranthene
2 2-hydroxy-3-nitrofluoranthene
3 3-nitrofluoranthene
4 3-nitrofluoranthene
5 2-acetoxy-3-acetamidofluoranthene
6 Fluoranthene-2,3-quinone
7 2-hydroxy-3-acetamidofluoranthene

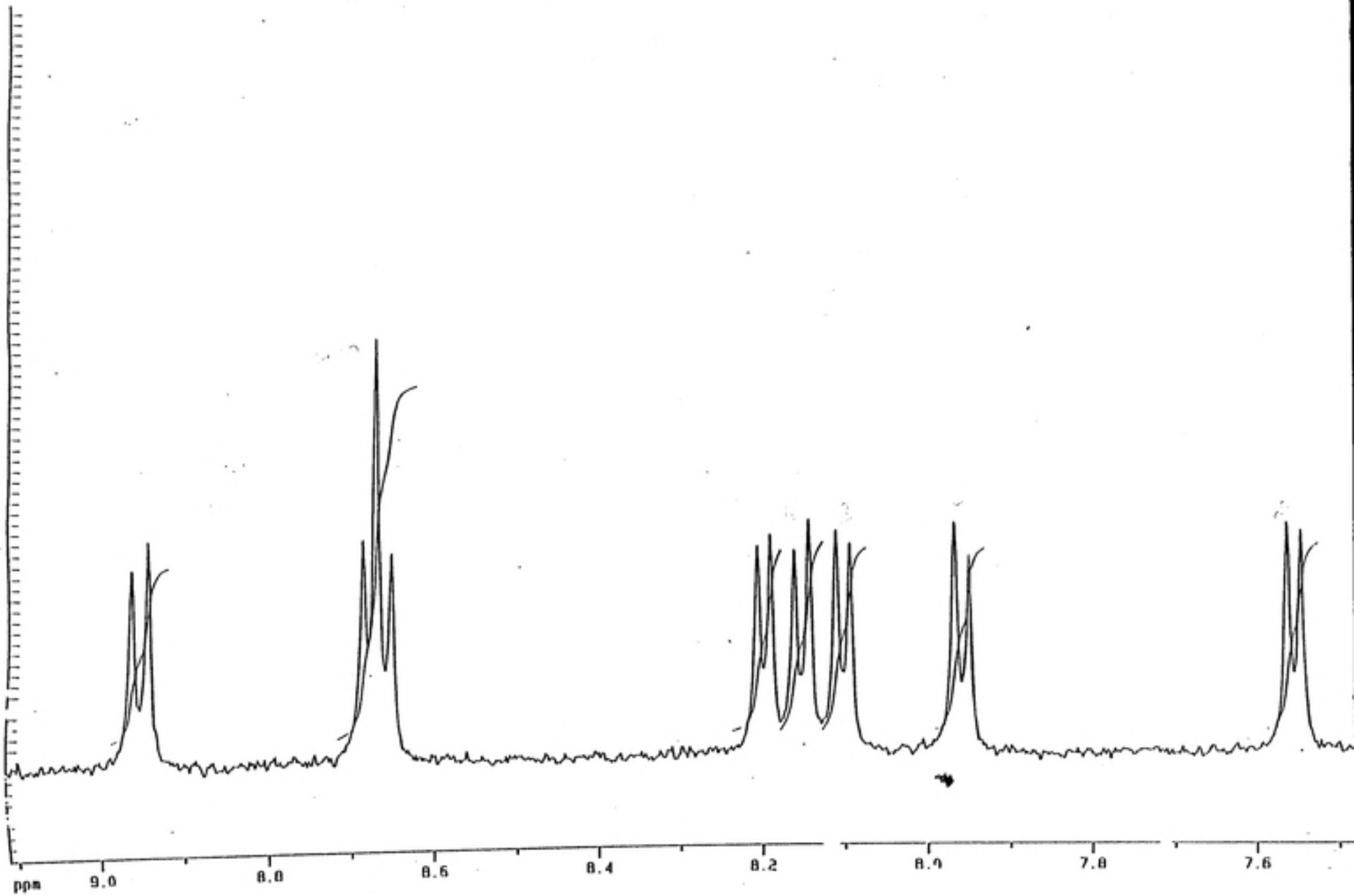
NMR SPECTRUM OF 1-NITRO-3-HYDROXYPYRENE



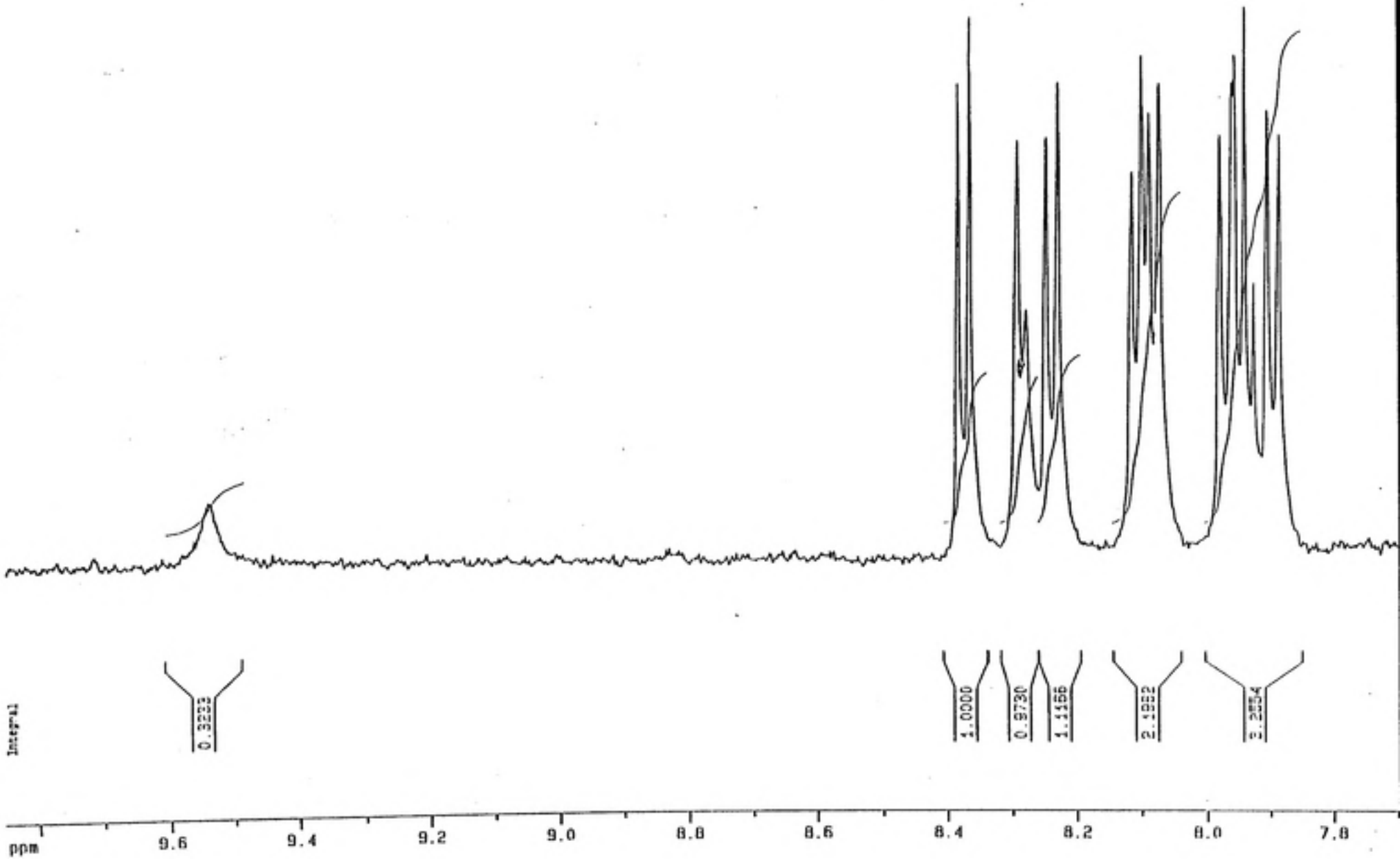
NMR SPECTRUM OF 1-NITRO-6-HYDROXYPYRENE



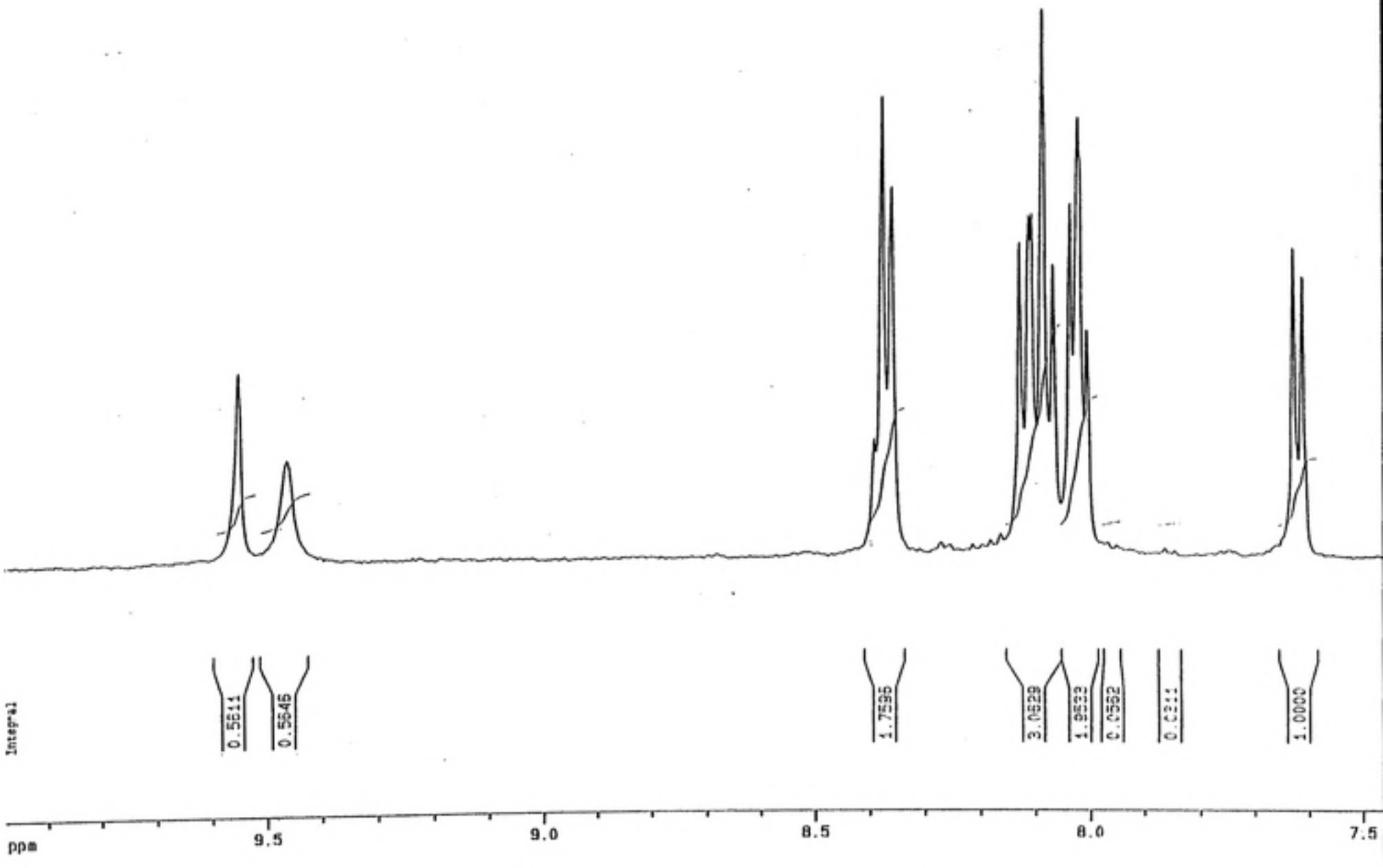
NMR SPECTRUM OF 1-NITRO-8-HYDROXYPYRENE



NMR SPECTRUM OF 1-ACETAMIDO-3-HYDROXYPYRENE



NMR SPECTRUM OF 1-ACETAMIDO-6-HYDROXYPYRENE



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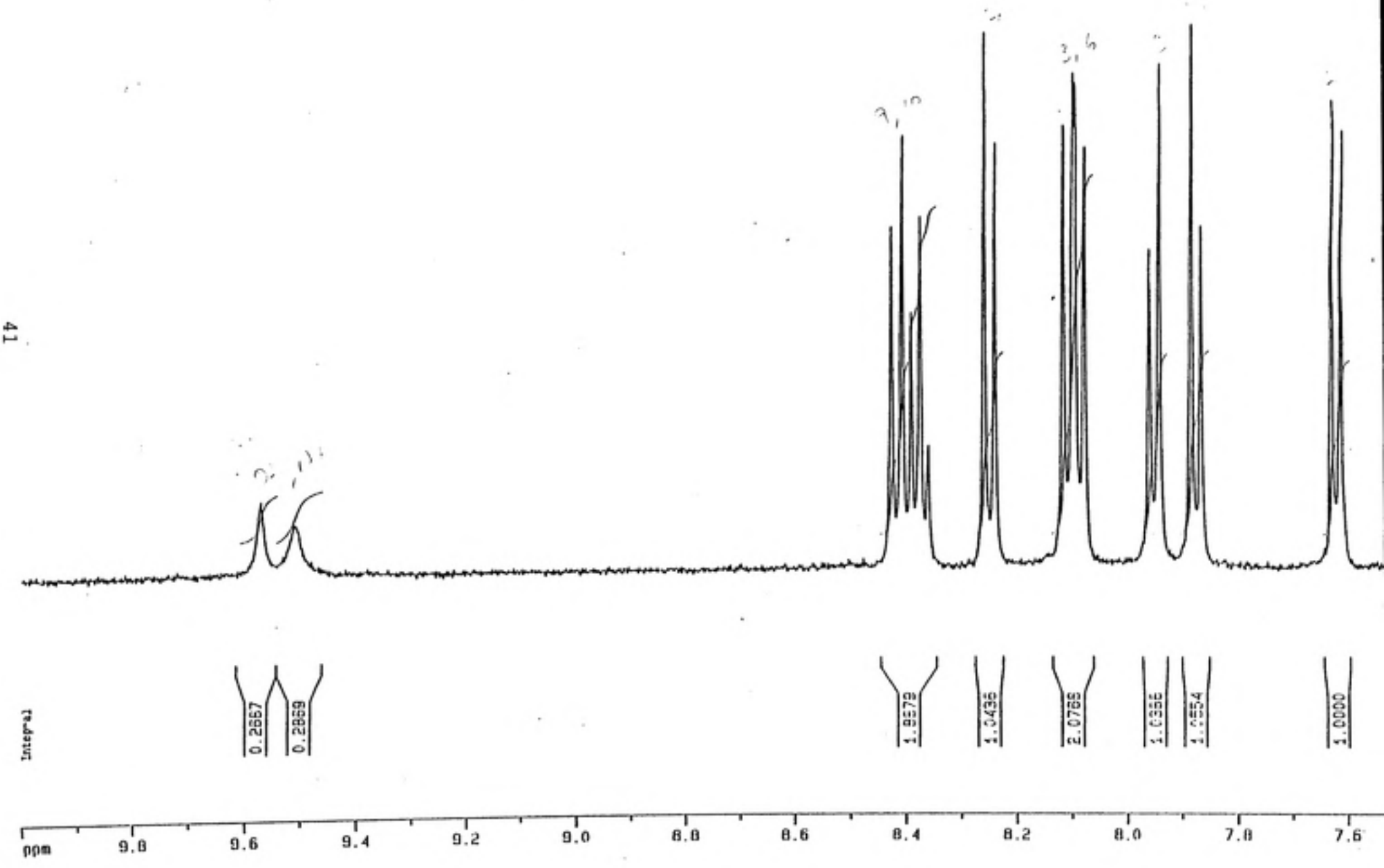
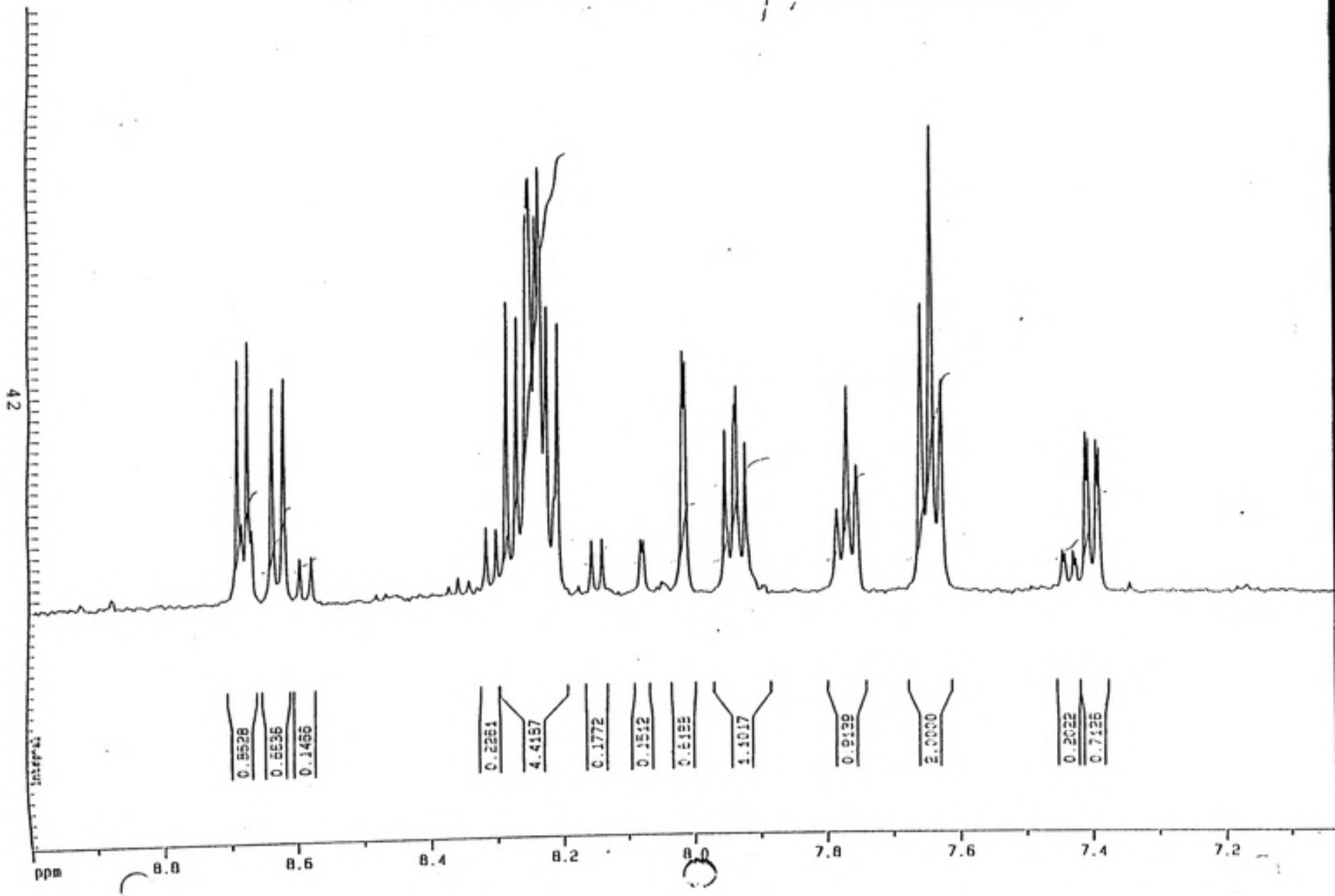


NMR SPECTRUM OF 3-NITRO-8-BENZOYLOXYFLUORANTHENE



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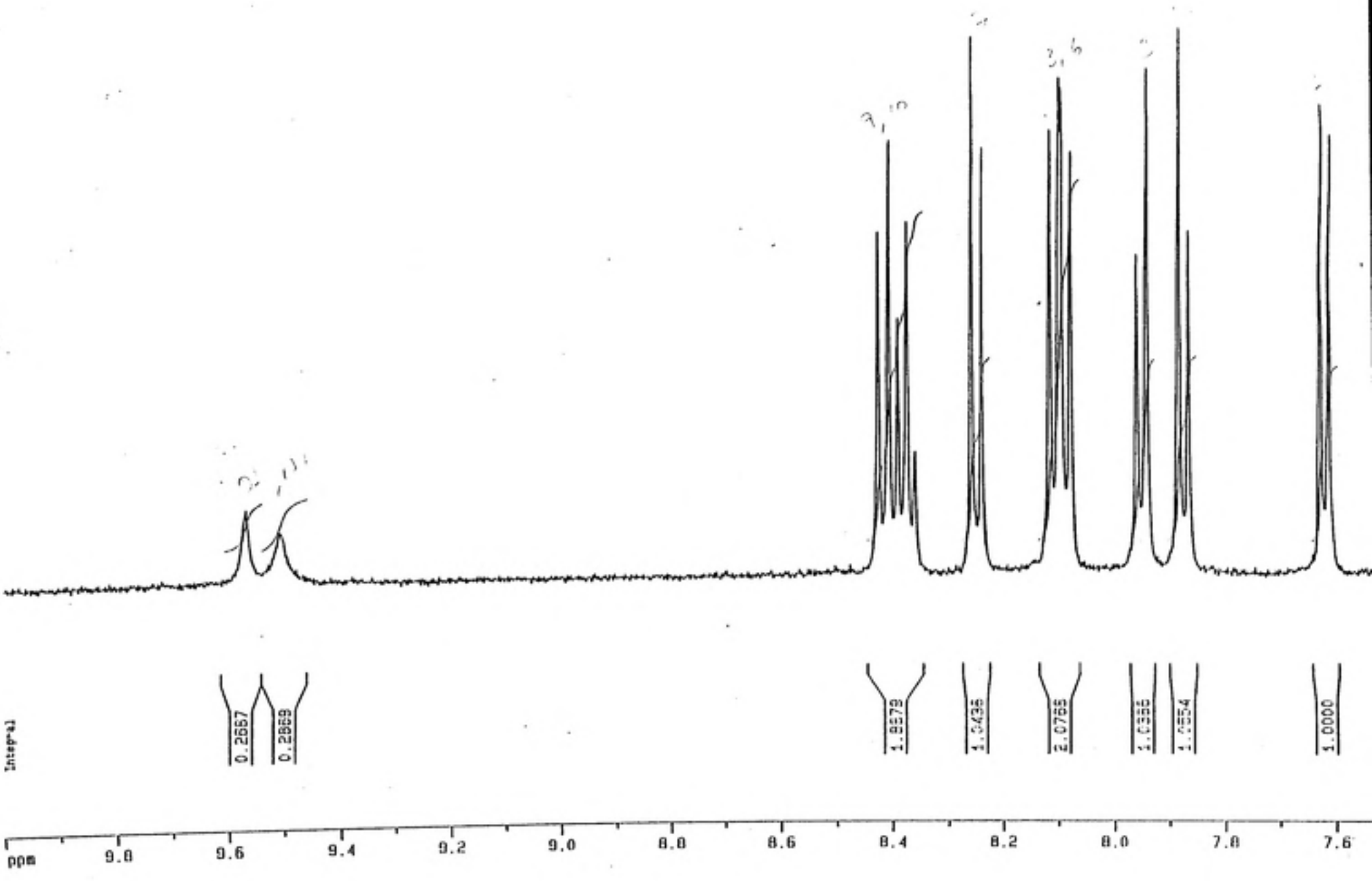
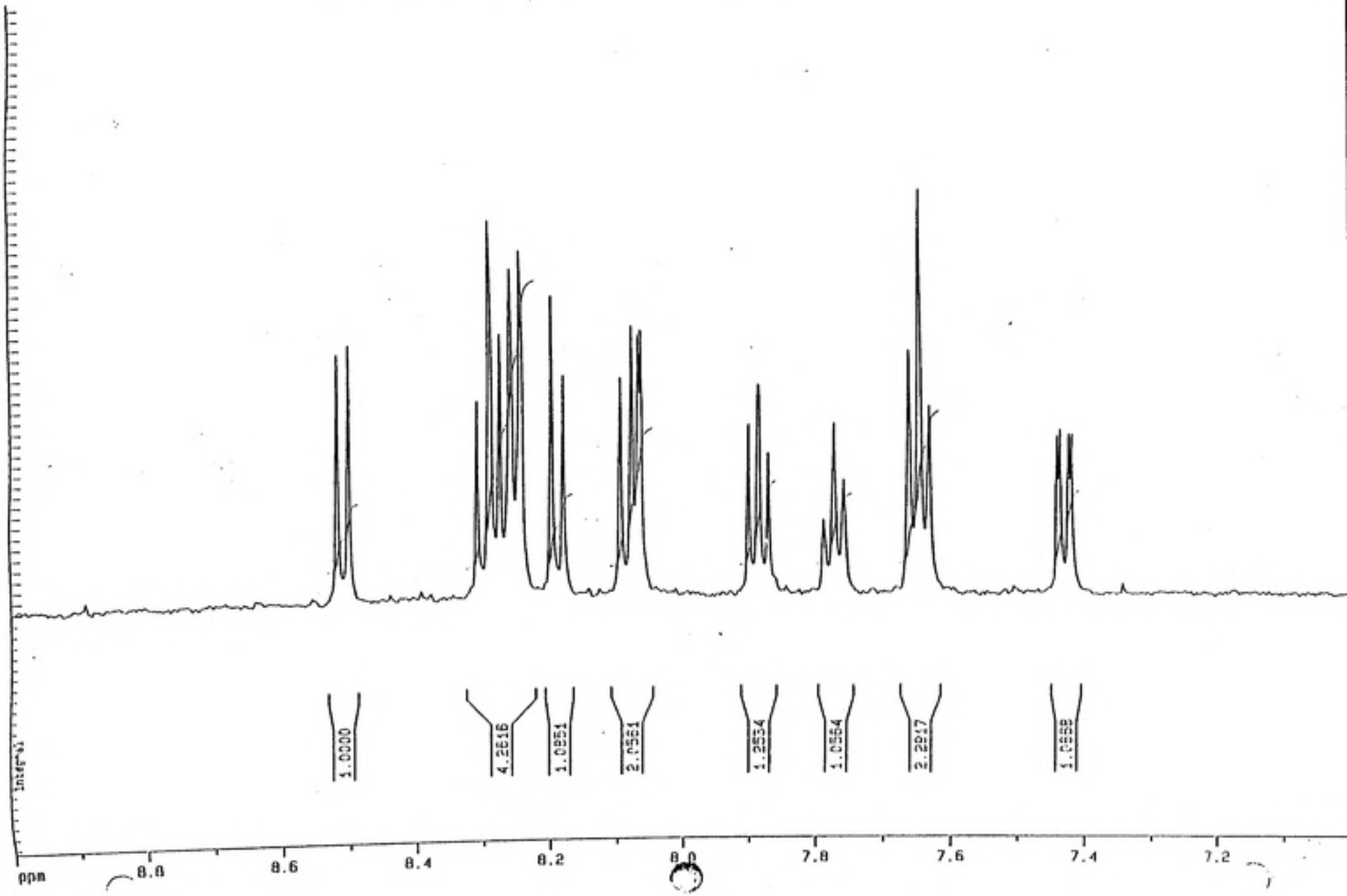


NMR SPECTRUM OF 3-NITRO-9-BENZOYLOXYFLUORANTHENE



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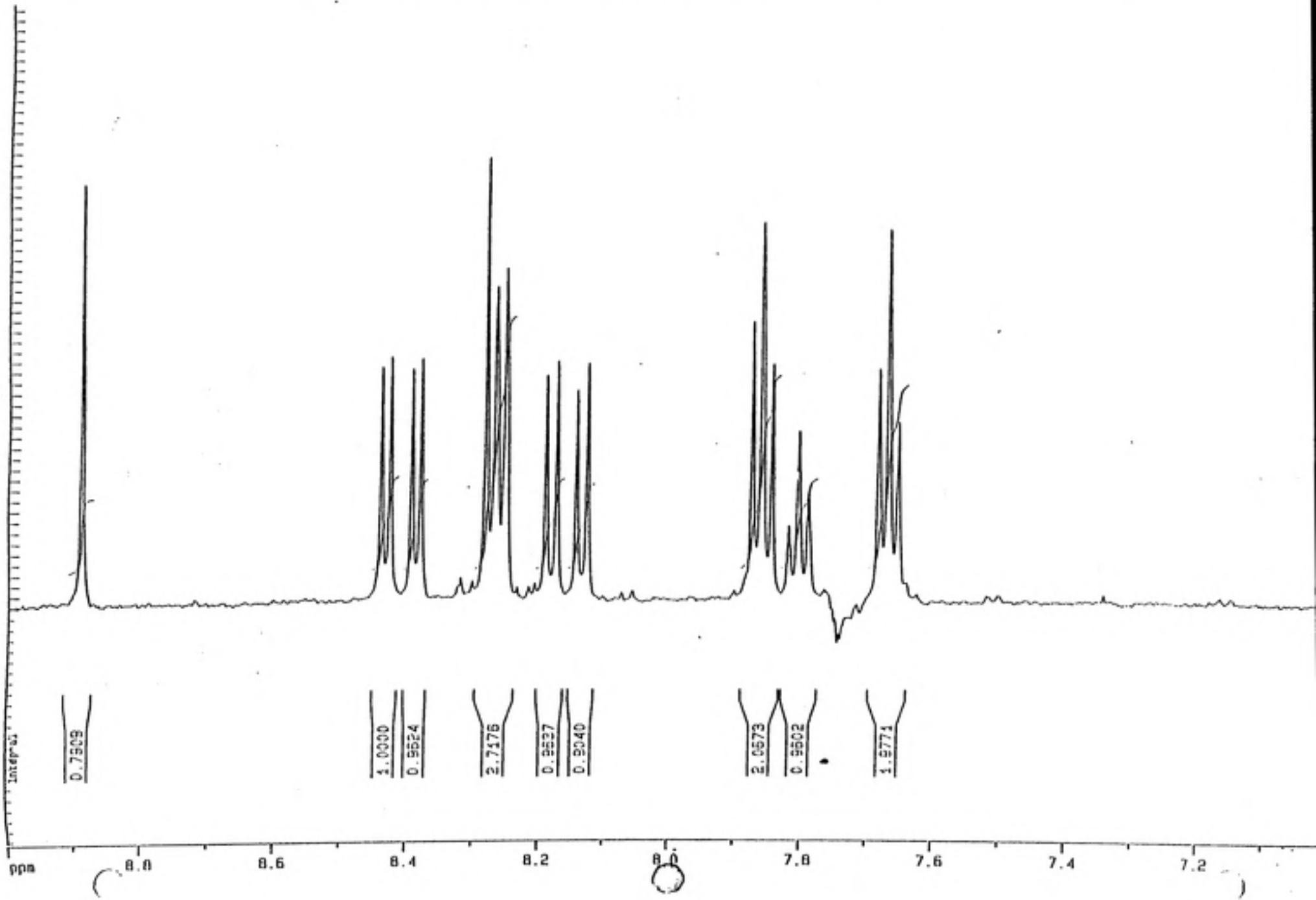

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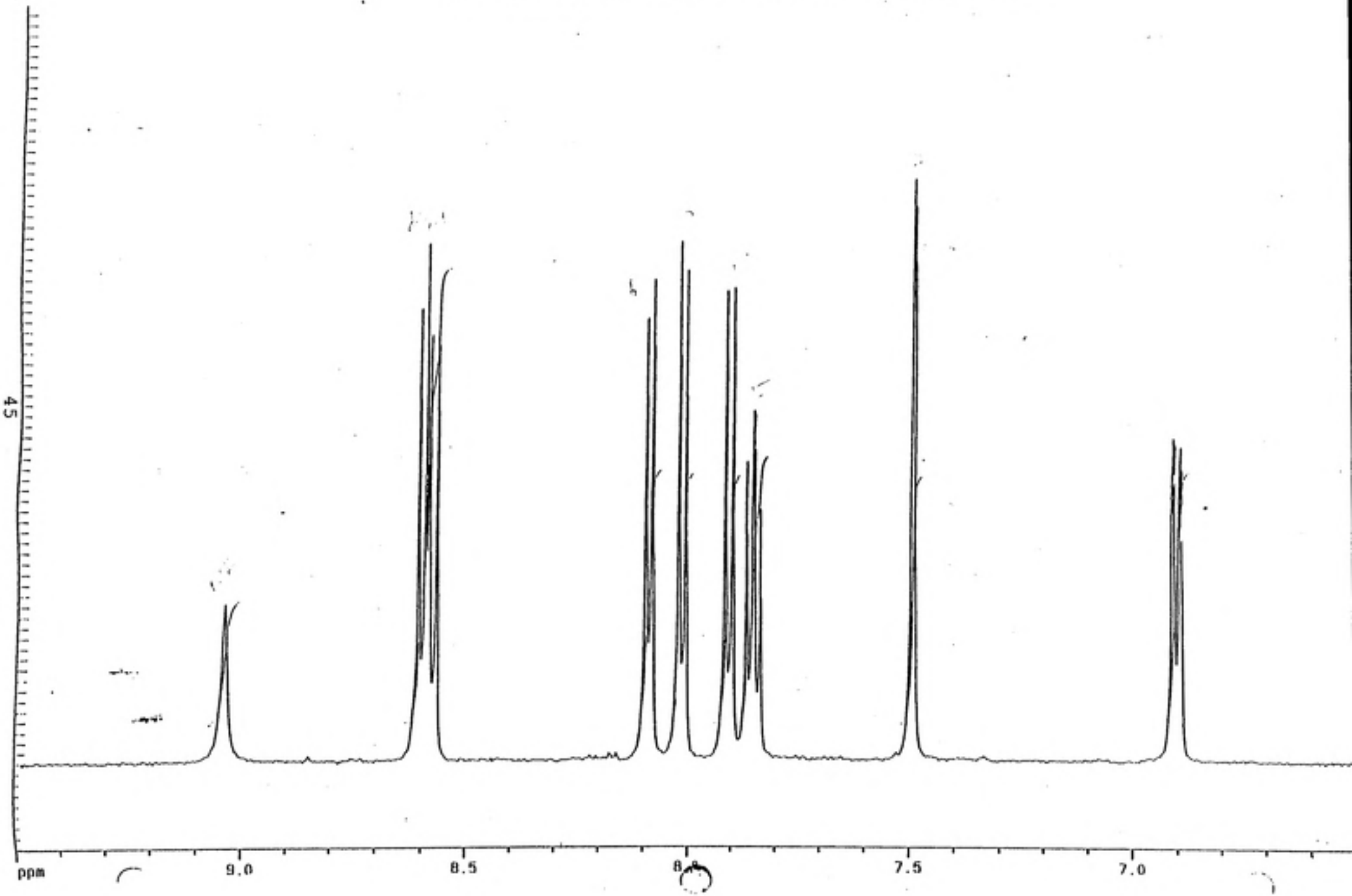

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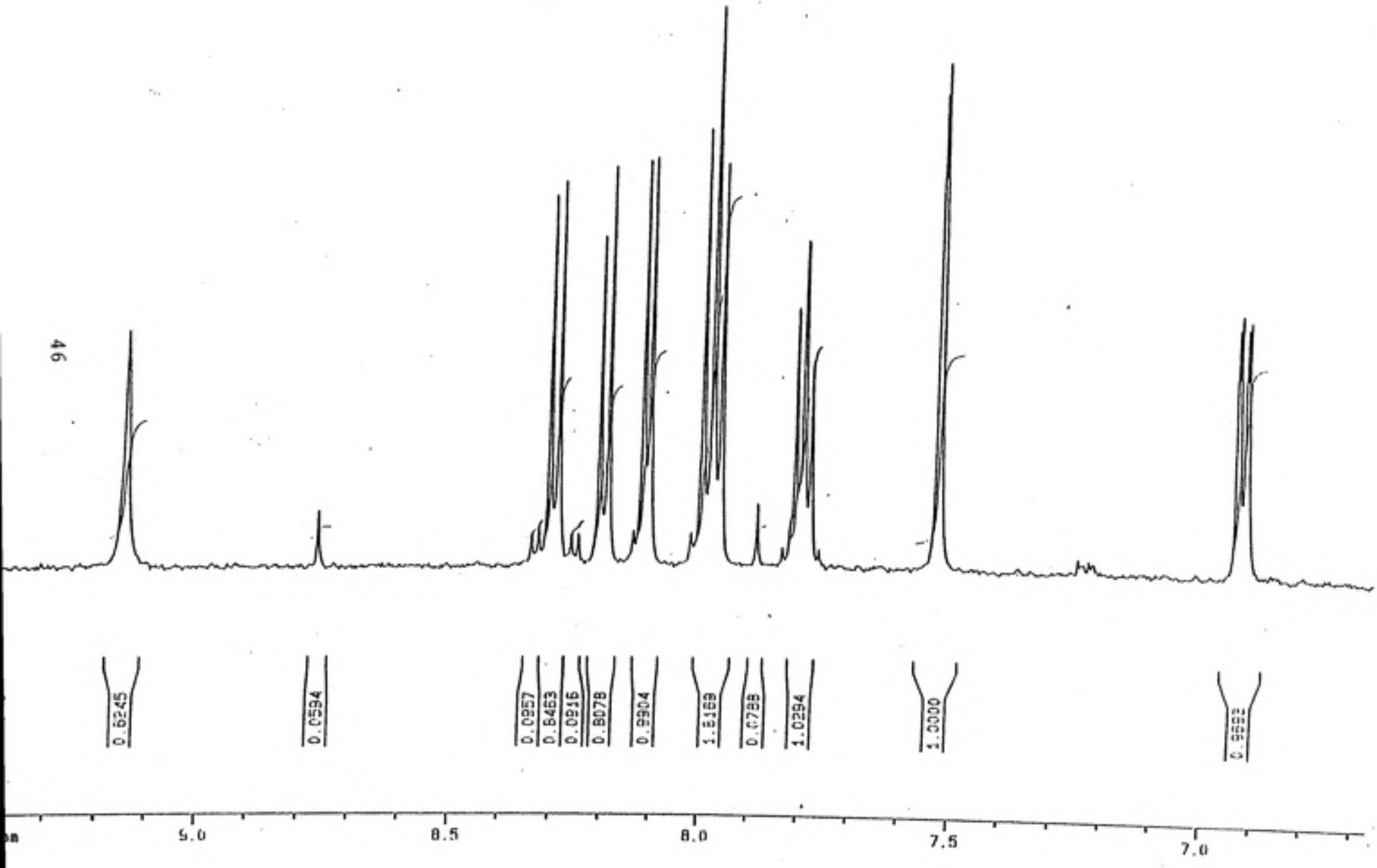
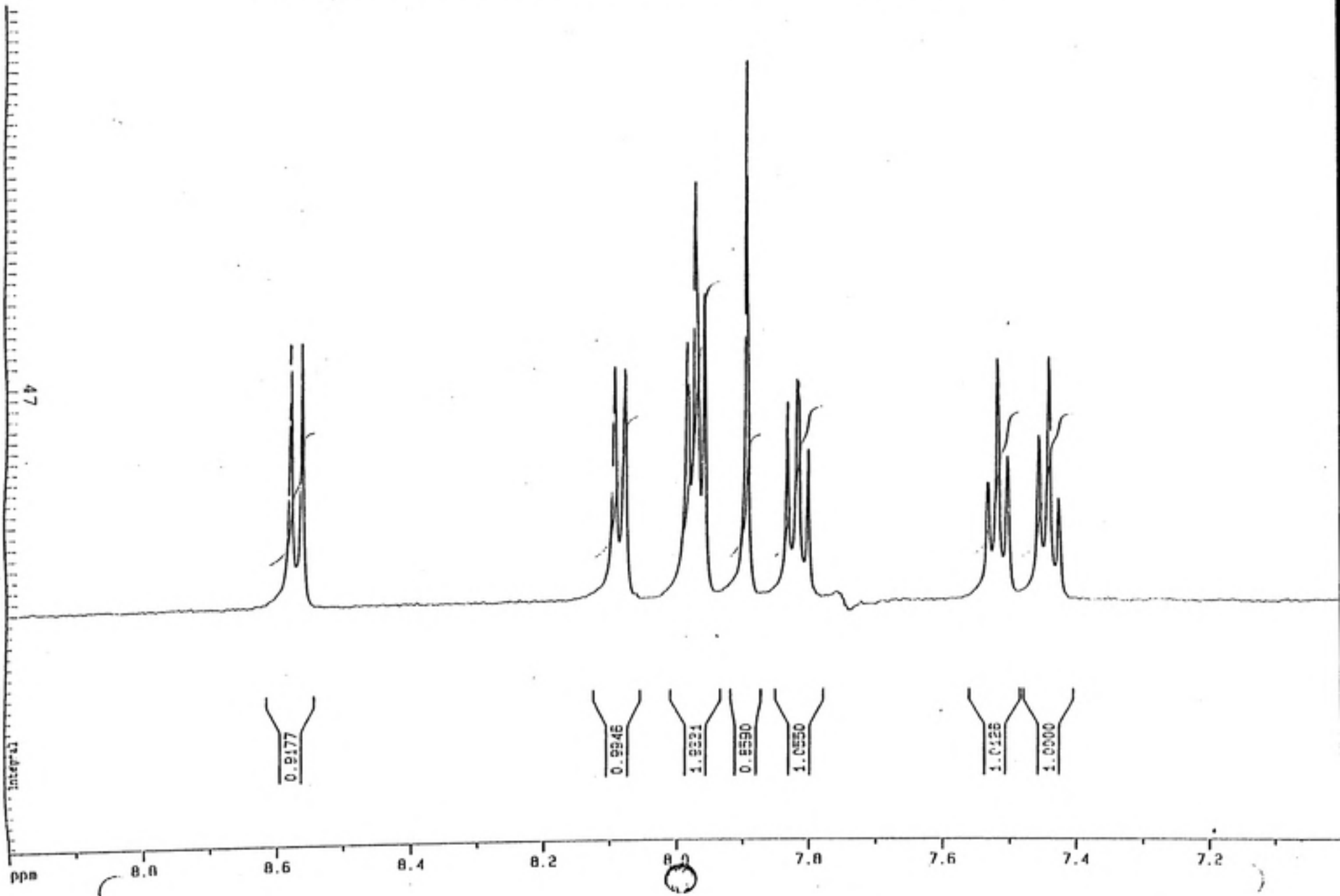
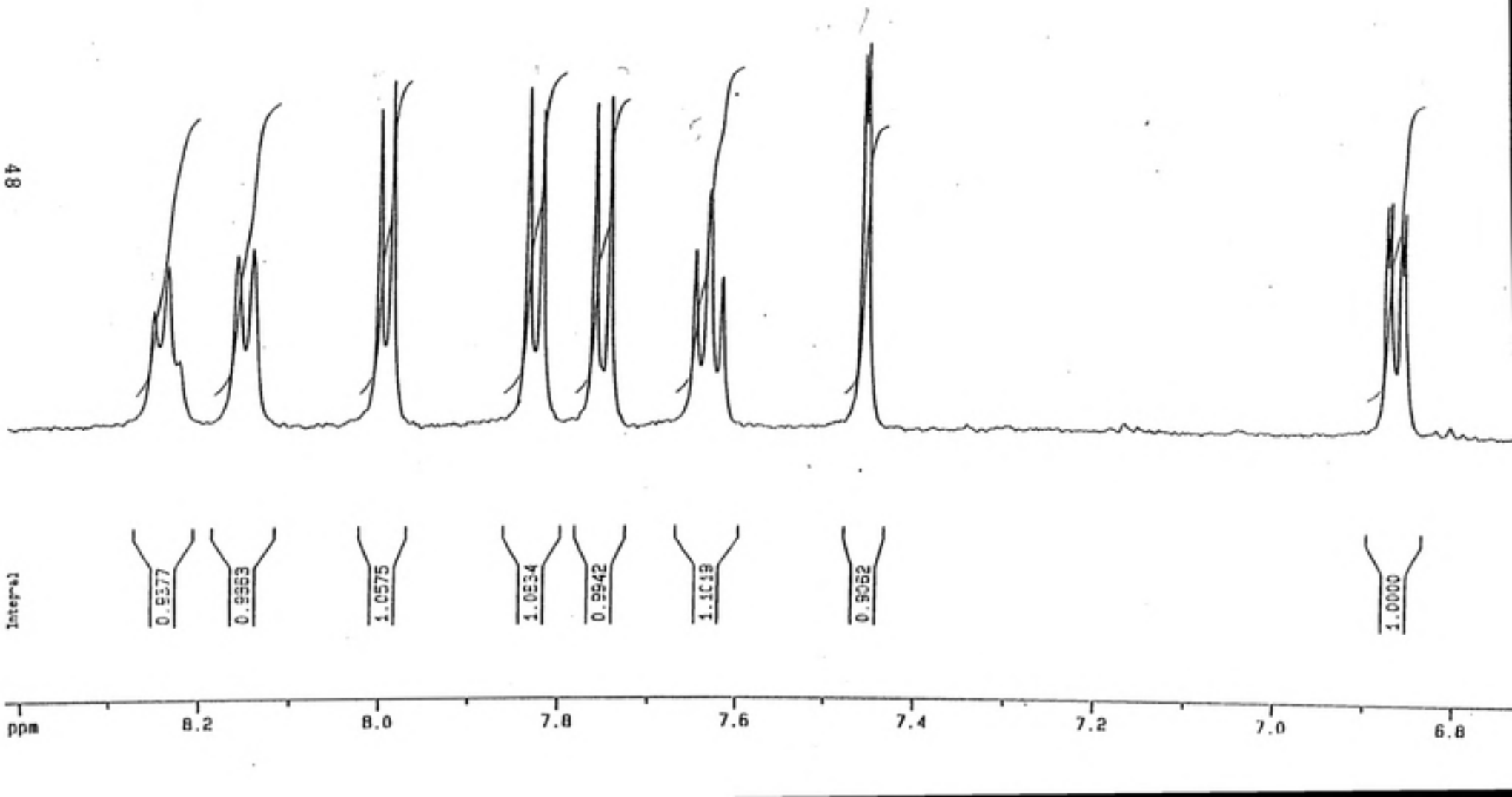


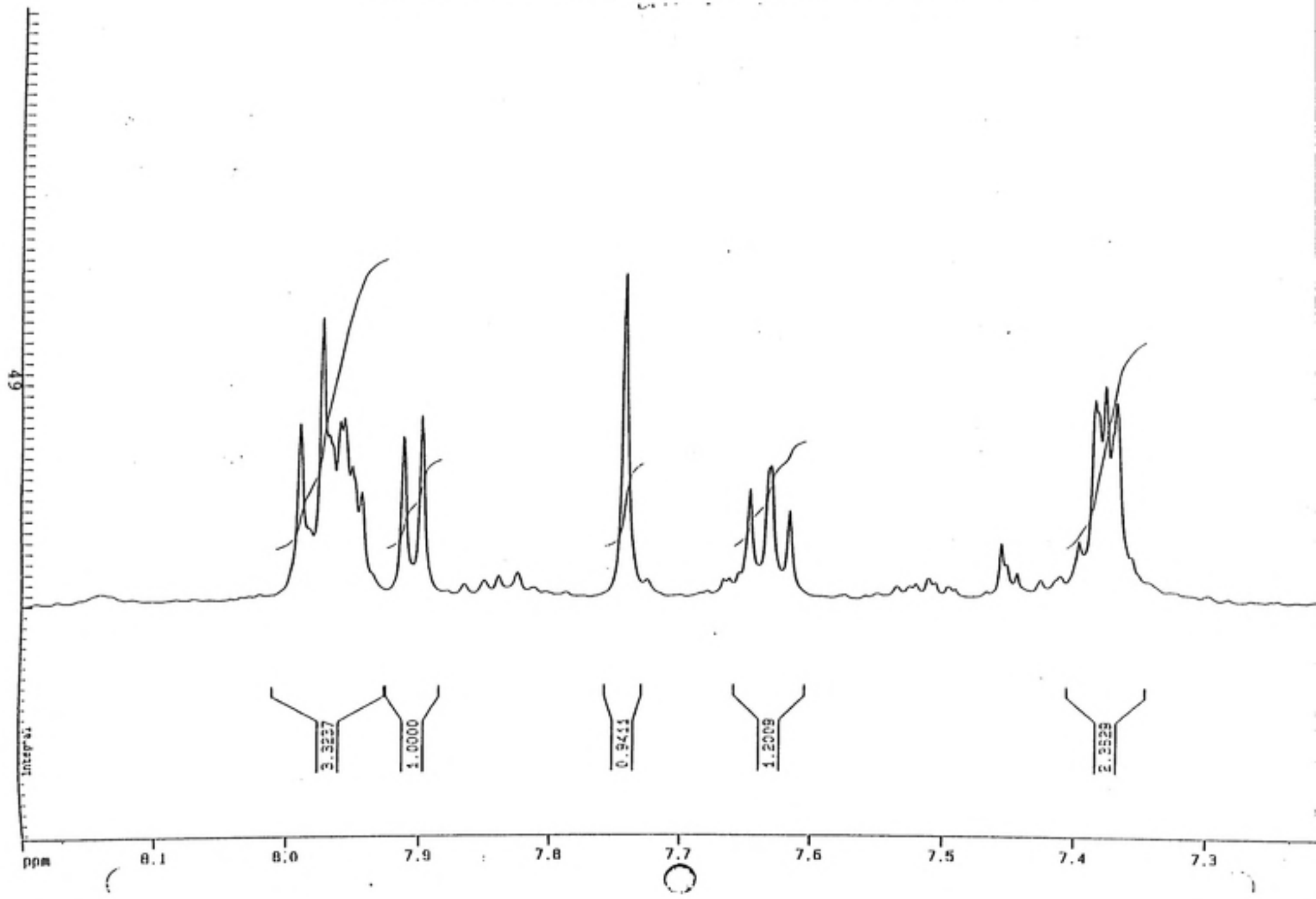
NMR SPECTRUM OF 3-NITRO-2-HYDROXYFLUORANTHENE



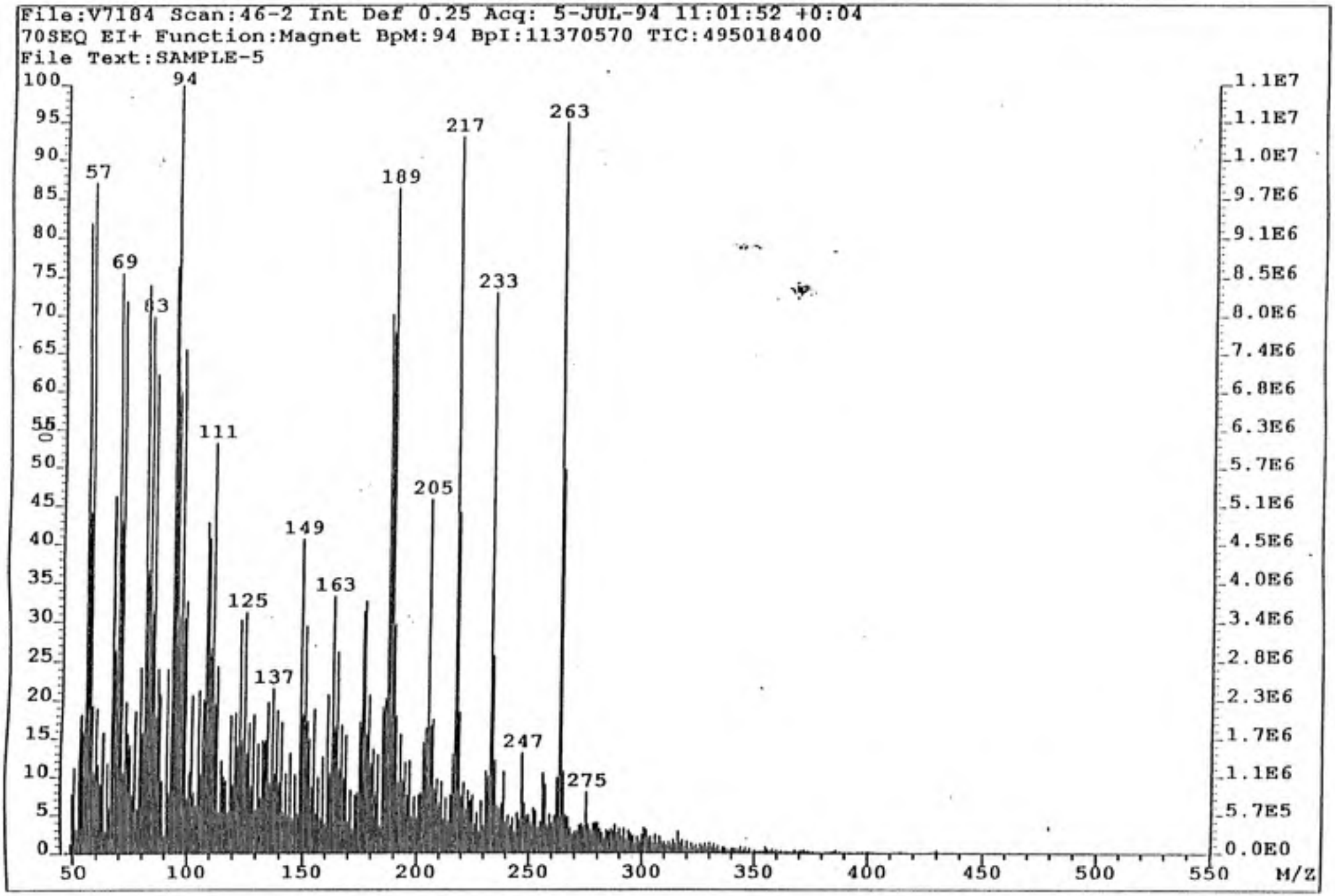
NMR SPECTRUM OF 3-ACETAMIDO-8-HYDROXYFLUORANTHENE



NMR SPECTRUM OF 3-ACETAMIDO-2-HYDROXYFLUORANTHENE



MASS SPECTRUM 1-NITRO-3-HYDROXYPYRENE



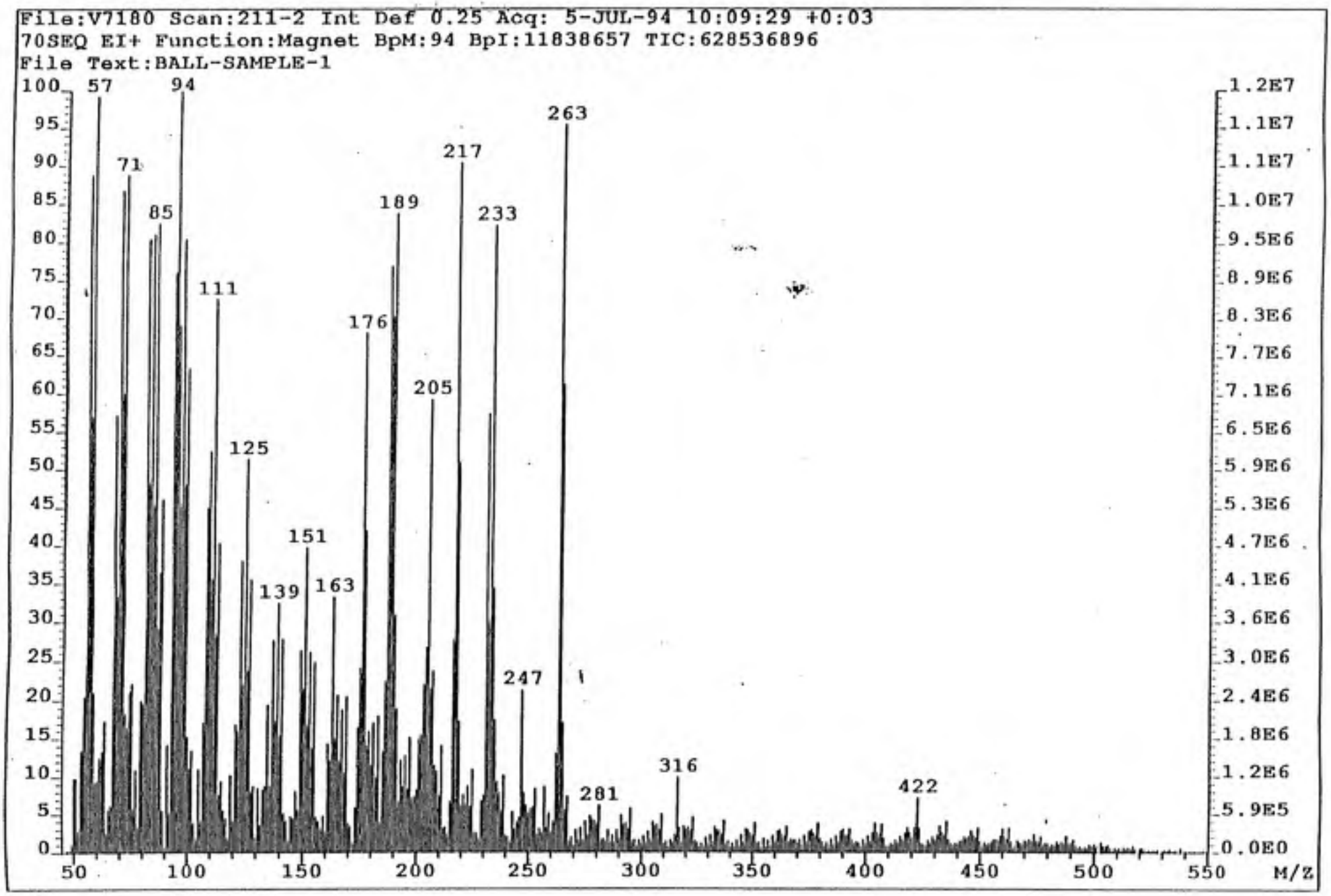
Exact mass : 263.0601

mass-to-charge ratio (m/z)	% intensity	mass; ion lost
263	95	-
247	13	16 ; O-
233	73	30 ; NO-
217	93	46 ; NO_2-
205	46	58 ; C, NO_2-
189	87	74 ; C, OH-, NO_2-

The ion of 100% intensity has an m/z of 94

MASS SPECTRUM OF 1-NITRO-3-HYDROXYPYRENE (CONTD)

MASS SPECTRUM OF 1-NITRO-6-HYDROXYPYRENE



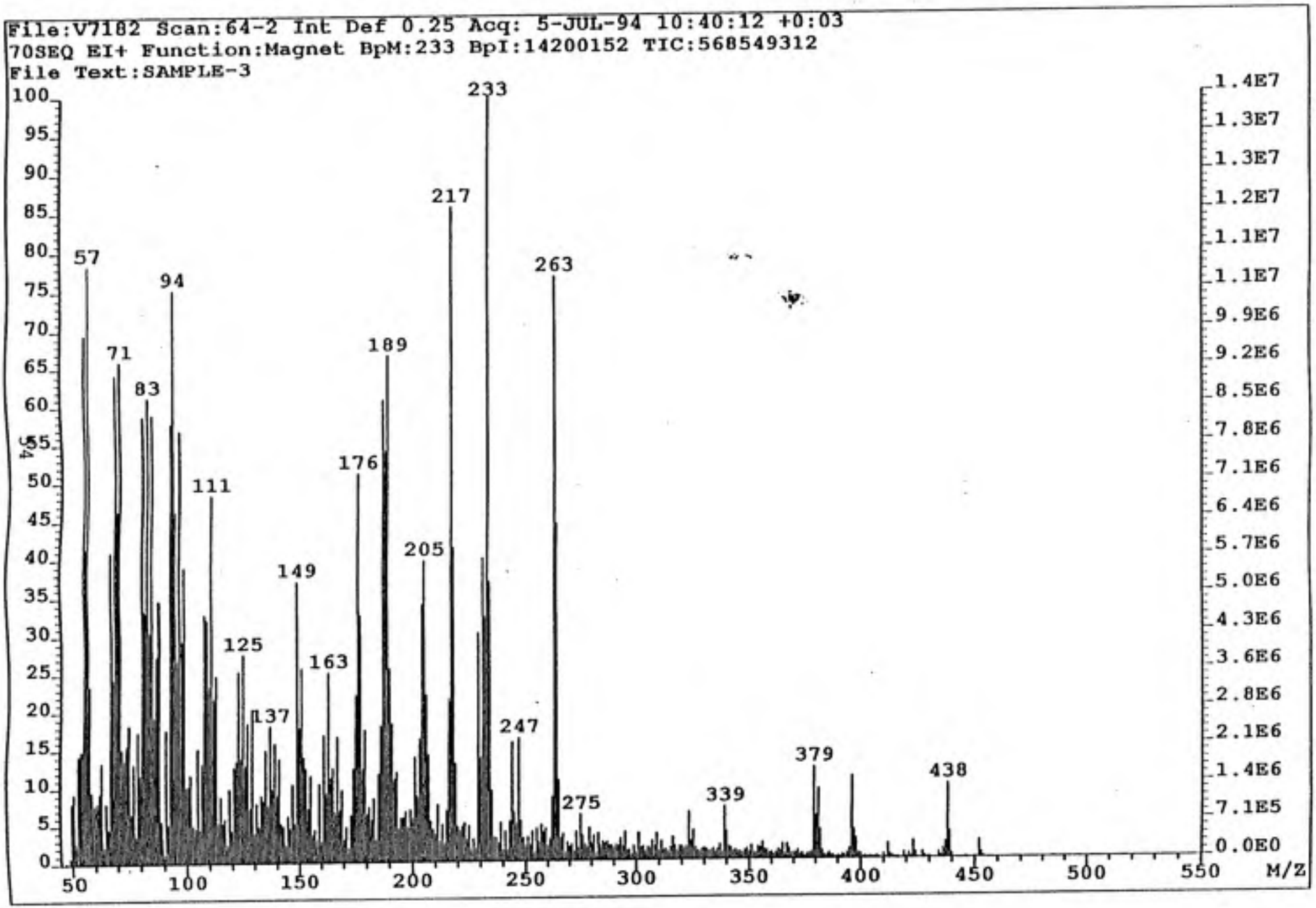
Exact mass : 263.0595

mass-to-charge ratio (m/z)	% intensity	mass ; ion lost
263	95	-
247	21	16 ; O-
233	82	30 ; NO-
217	90	46 ; NO_2 -
205	59	58 ; C, NO_2-
189	84	74 ; C, OH-, NO_2-

The ion of 100% intensity has an m/z of 94

MASS SPECTRUM OF 1-NITRO-6-HYDROXYPYRENE (CONTD)

MASS SPECTRUM OF 1-NITRO-8-HYDROXYPYRENE

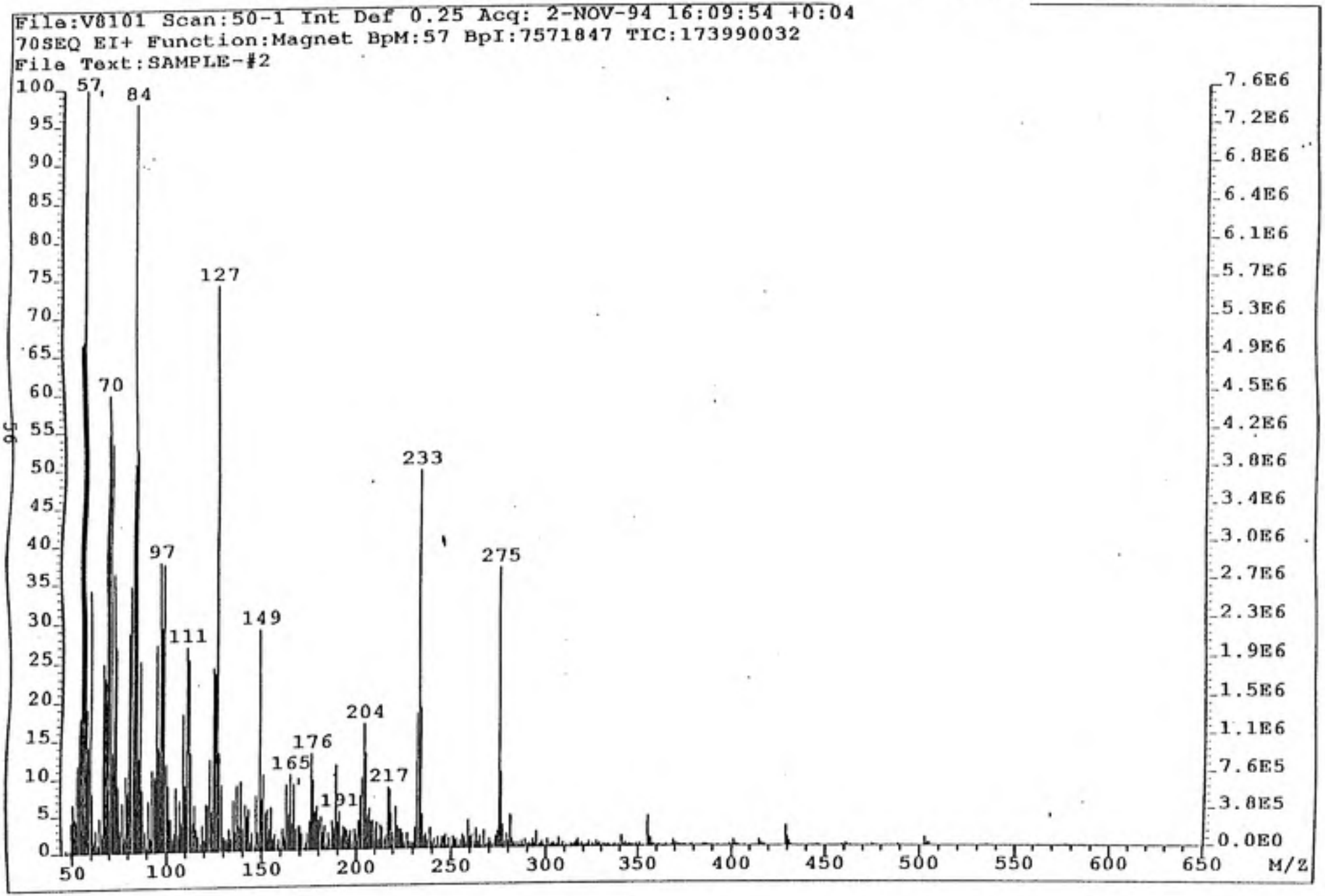


Exact mass : 263.0578

mass-to-charge ratio (m/z)	% intensity	mass ; ion lost
263	77	-
247	16	16 ; O-
233	100	30 ; NO-
217	85	46 ; NO2-
205	40	58 ; C, NO2-
189	67	74 ; C, OH-, NO2-

MASS SPECTRUM OF 1-NITRO-8-HYDROXYPYRENE (CONTD)

MASS SPECTRUM OF ACETAMIDO-3-HYDROXYPYRENE



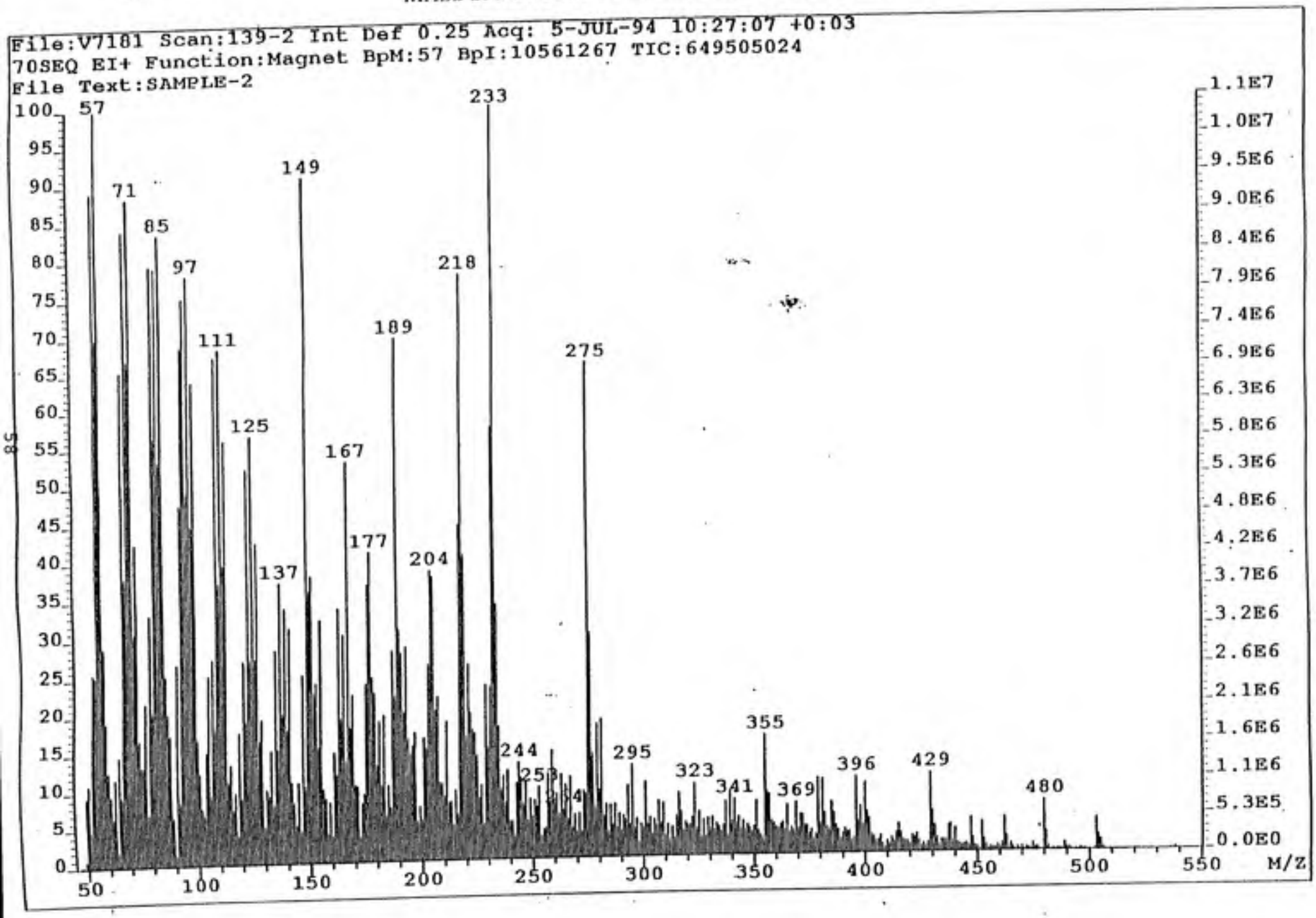
Exact mass : 275.0972

mass-to-charge ratio (m/z)	% intensity	mass ; ion lost
275	37	-
233	50	42 ; $COCH_3$
217	7	58 ; H, $NHCOCH_3$
204	17	71 ; CH, $NHCOCH_3$
189	11	84 ; O, C, $NHCOCH_3$

The ion of 100% intensity has an m/z of 57

MASS SPECTRUM OF 1-ACETAMIDO-3-HYDROXYPYRENE (CONTD)

MASS SPECTRUM OF 1-ACETAMIDO-6-HYDROXYPYRENE



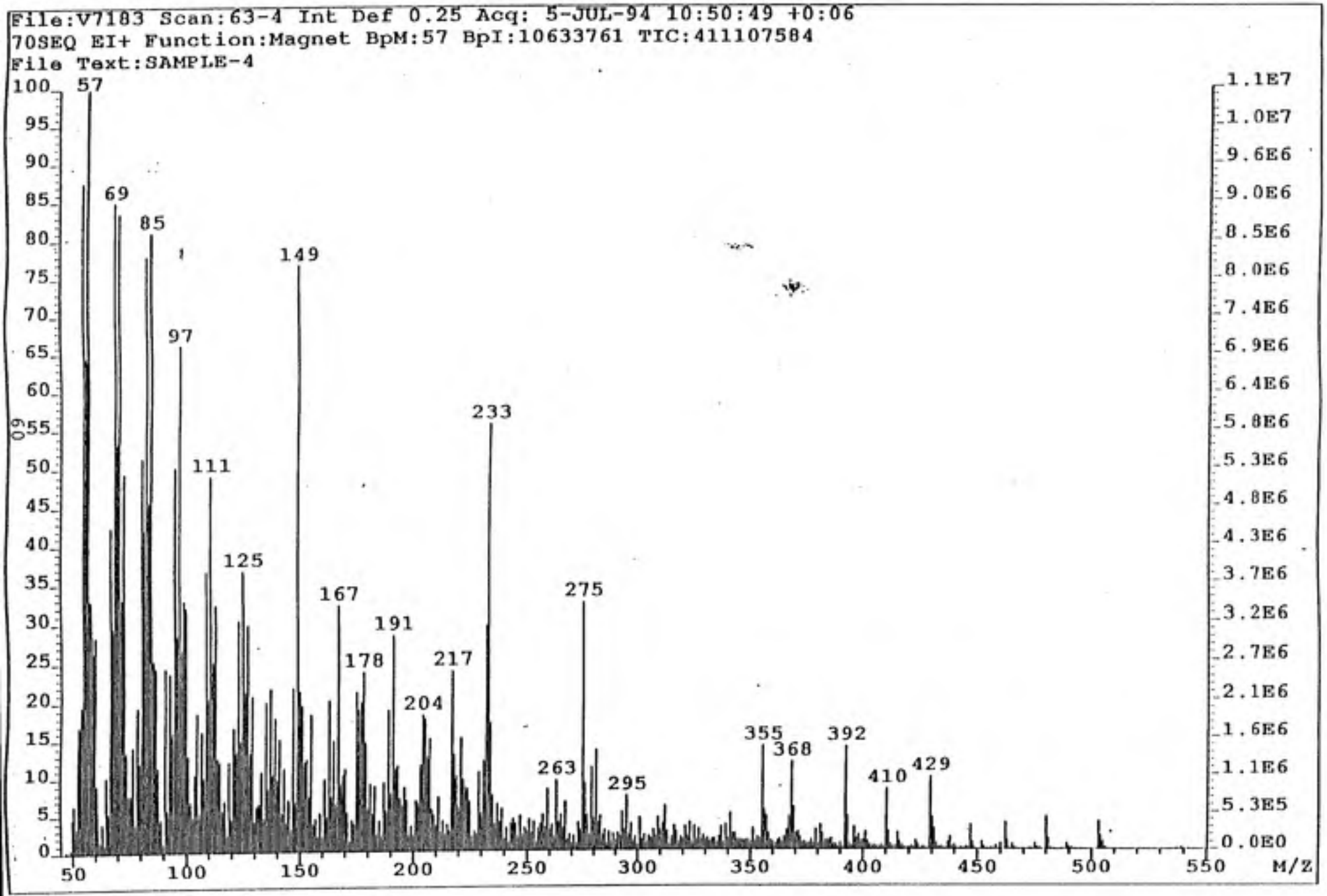
Exact mass : 275.0951

mass-to-charge ratio (m/z)	% intensity	mass; ion lost
275	66	-
233	99	42 ; $COCH_3$-
218	78	57 ; $NHCOCH_3$-
204	38	71 ; CH, $NHCOCH_3$-
189	70	86 ; O-, CH, $NHCOCH_3$-

The ion of 100% intensity has an m/z of 57

MASS SPECTRUM OF 1-ACETAMIDO-6-HYDROXYPYRENE (CONTD)

MASS SPECTRUM OF 1-A TAMIDO-8-HYDROXYPYRENE



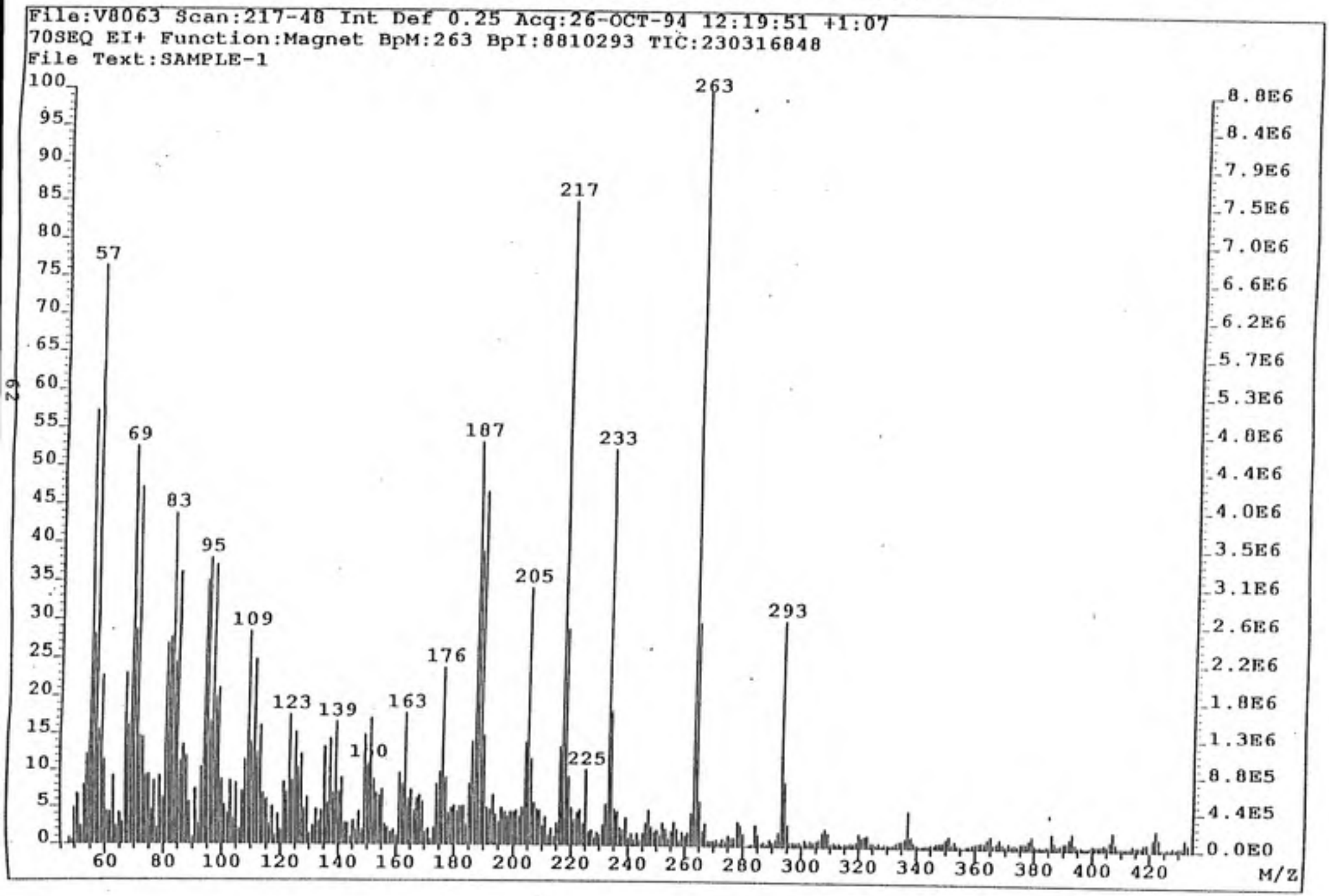
Exact mass : 275.0940

mass-to-charge ratio (m/z)	% intensity	mass ; ion lost
275	33	-
233	56	42 ; $COCH_3$
217	23	58 ; H, $NHCOCH_3$
204	18	71 ; CH, $NHCOCH_3$
191	28	84 ; O, C, $NHCOCH_3$

The ion of 100% intensity has an m/z of 57

MASS SPECTRUM OF 1-ACETAMIDO-8-HYDROXYPYRENE (CONTD)

MASS SPECTRUM OF 3-NITRO-8-HYDROXYFLUORANTHENE

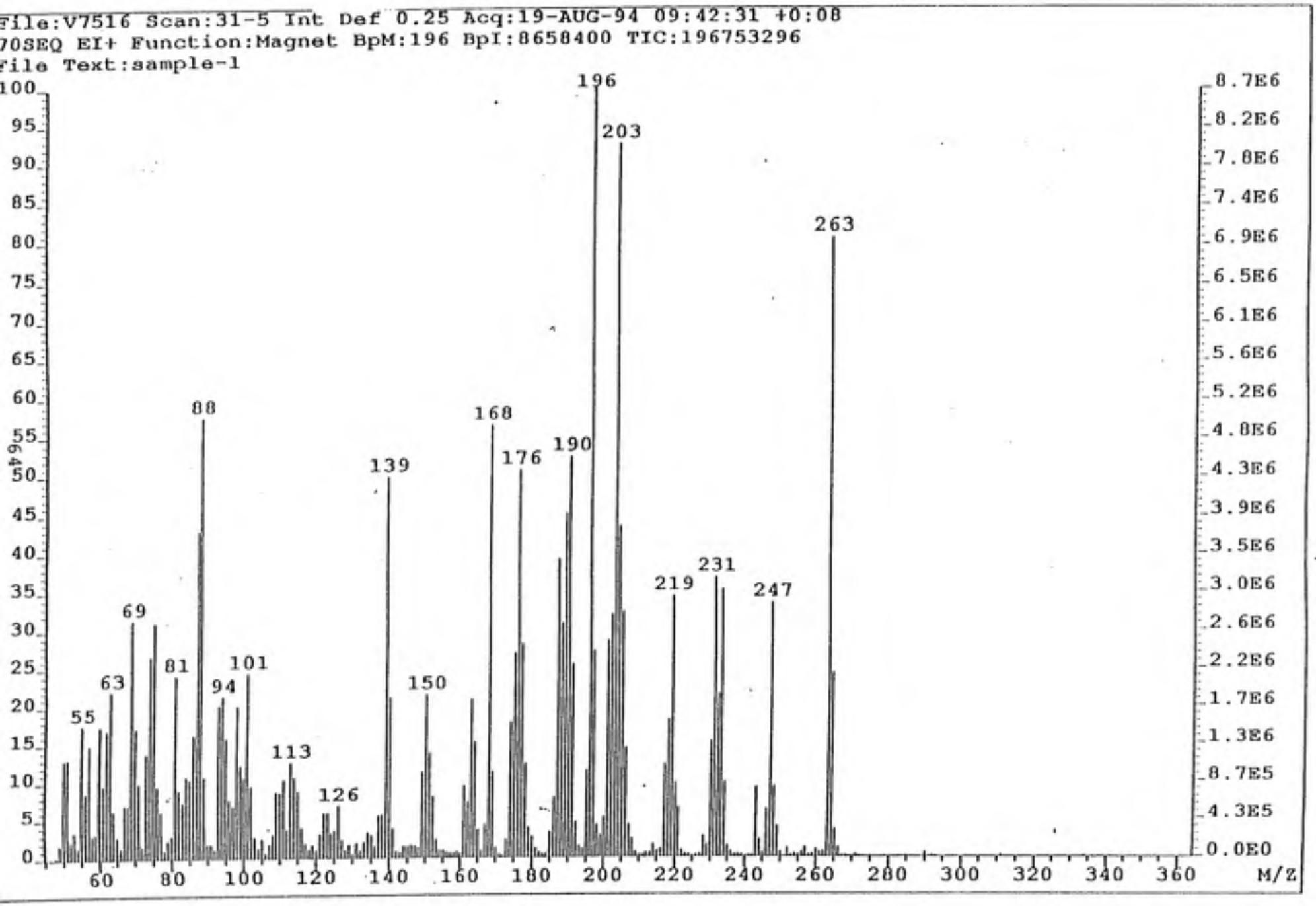


Exact mass : 263.0615

mass-to-charge ratio (m/z)	% intensity	mass ; ion lost
263	100	-
233	52	30 ; NO-
217	85	46 ; NO_2-
205	34	58 ; C, NO_2-
187	53	76 ; C, OH-, NO_2-

MASS SPECTRUM OF 3-NITRO-8-HYDROXYFLUORANTHENE (CONTD)

MASS SPECTRUM OF 3-NITRO-2-HYDROXYFLUORANTHENE

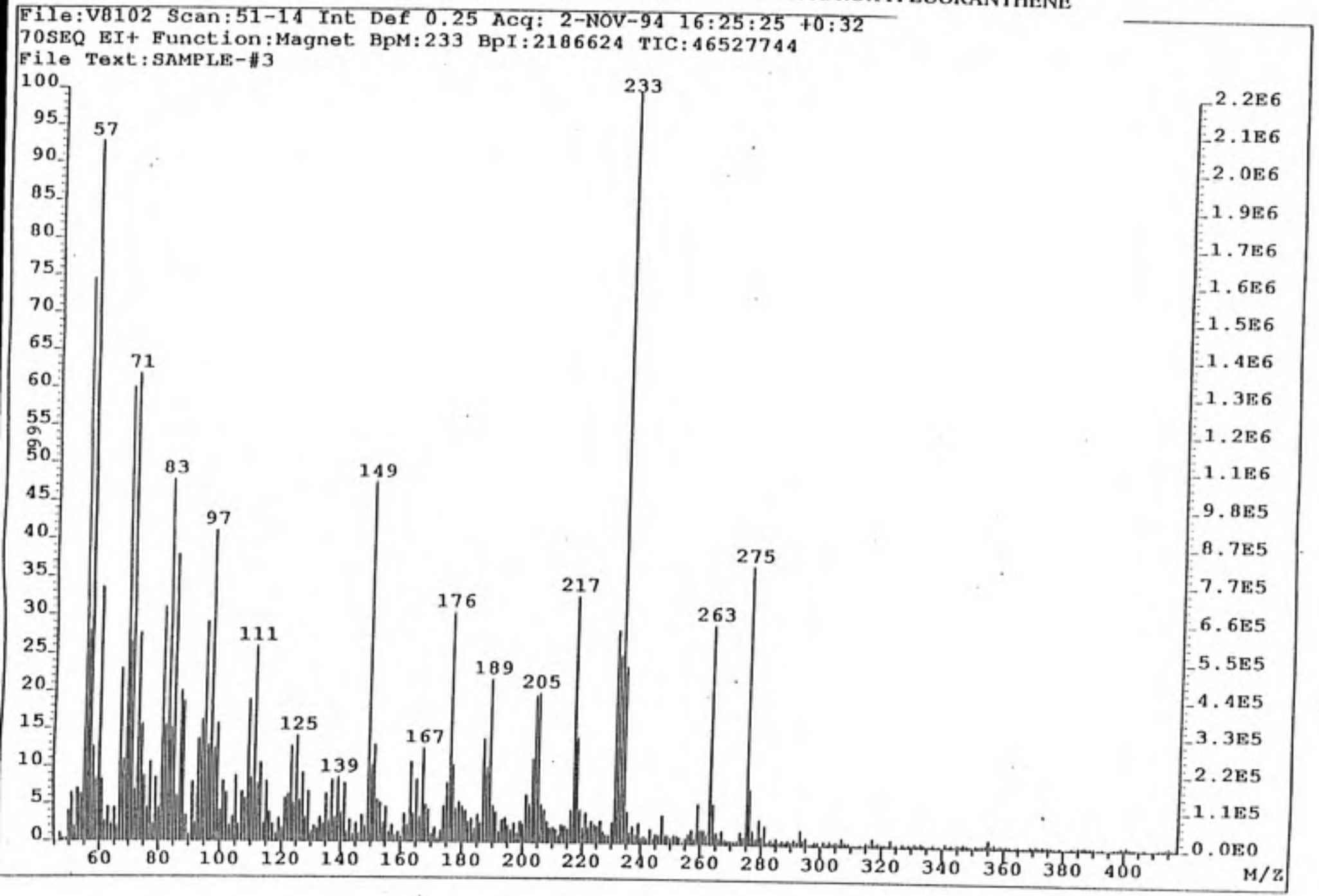


Exact mass : 275.0970

mass-to-charge ratio (m/z)	% intensity	mass ; ion lost
275	37	-
233	100	42 ; $COCH_3$
217	33	58 ; $NHCOCH_3$
205	20	70 ; CH, $NHCOCH_3$
189	22	86 ; O, C, $NHCOCH_3$

MASS SPECTRUM OF 3-NITRO-2-HYDROXYFLUORANTHENE (CONTD)

MASS SPECTRUM OF 3-ACETAMIDO-8-HYDROXYFLUORANTHENE



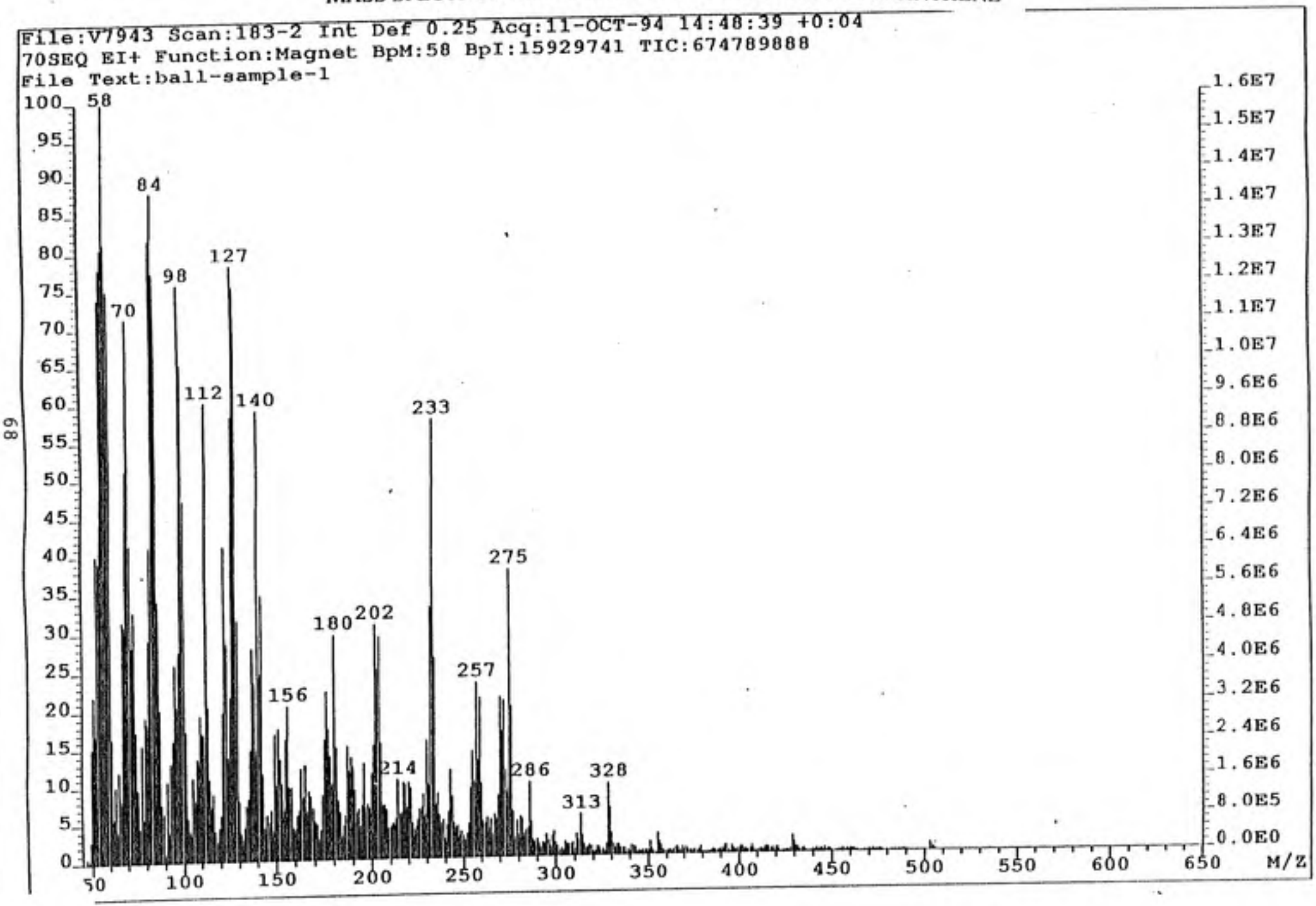
Exact mass : 275.0970

mass-to-charge ratio (m/z)	% intensity	mass ; ion lost
275	37	-
233	100	42 ; $COCH_3$
217	33	58 ; $NHCOCH_3$
205	20	70 ; CH, $NHCOCH_3$
189	22	86 ; O, C, $NHCOCH_3$

MASS SPECTRUM OF 3-ACETAMIDO-8-HYDROXYFLUORANTHENE (CONTD)

Temp. 380 C

MASS SPECTRUM OF 3-ACETAMIDO-2-HYDROXYFLUORANTHENE



Exact mass : 275.0934

mass-to-charge ratio (m/z)	% intensity	mass ; ion lost
275	37	-
233	58	42 ; $COCH_3$
214	10	61 ; H, $NHCOCH_3$
202	29	73 ; CH_2, $NHCOCH_3$

The ion of 100% intensity has an m/z of 58

MASS SPECTRUM OF 3-ACETAMIDO-2-HYDROXYFLUORANTHENE (CONTD)

Ames mutagenicity assay results

(Zhang, Renninger, Rosser, unpublished data; Ball et al., 1986)

Compound	Rev/nmole in TA 98	
	-S9	+S9
6-Nitro-1-hydroxypyrene	6	121
8-Nitro-1-hydroxypyrene	2.9	8.8
3-Nitro-1-hydroxypyrene	8.8	11.2
6-Acetamido-1-hydroxypyrene	0	17
8-Acetamido-1-hydroxypyrene	0.6	0.4
3-Acetamido-1-hydroxypyrene	0.69	0.01
3-Nitro-8-hydroxyfluoranthene	43	55.4
3-Nitro-9-hydroxyfluoranthene	7.2	6.1
9-Nitro-8-hydroxyfluoranthene	0	0
3-Nitro-2-hydroxyfluoranthene	11.9	5.1
3-Acetamido-8-hydroxyfluoranthene	1.24	2
3-Acetamido-9-hydroxyfluoranthene	not yet been tested	
3-Acetamido-2-hydroxyfluoranthene	1.1	1.2
1-Nitropyrene	339	60
1-Aminopyrene	93	33
2-Nitrofluoranthene	200	200
3-Nitrofluoranthene	2420	222
3-Aminofluoranthene	43	113

BIBLIOGRAPHY

Adam, W., R. Curci, and J.O. Edwards, (1989). Dioxiranes: A new class of powerful oxidants. Acc. Chem. Res., 22: 205-211.

Ames, B.N., J. McCann, and E. Yamasaki, (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat. Res., 31: 347-364.

Andrews, P.J., M.A. Quilliam, B.E. McCarry, D.W. Bryant, and D.R. McCalla, (1986). Identification of the DNA adduct formed by metabolism of 1,8-dinitropyrene in *Salmonella typhimurium*. Carcinogenesis, 7: 105-110.

Ball, L.M. and L.C. King, (1985). Metabolism, mutagenicity and activation of 1-nitropyrene in vivo and in vitro. Environ. Internat., 11: 355-362.

Ball, L.M., M.J. Kohan, L.D. Claxton, and J. Lewtas, (1984). Mutagenicity of derivatives and metabolites of 1-nitropyrene: Activation by rat liver S9 and bacterial enzymes. Mutat. Res., 138: 113-125.

Ball, L.M., M.J. Kohan, J.P. Inmon, L.D. Claxton, and J. Lewtas, (1984). Metabolism of 1-nitro[^{14}C]pyrene in vivo in the rat and mutagenicity of urinary metabolites. Carcinogenesis, 5: 1557-1564.

Ball, L.M., M.J. Kohan, K. Williams, M.G. Nishioka, A. Gold, and J. Lewtas, (1985). Metabolism and activation pathways of the environmental mutagen 3-nitrofluoranthene. In Polynuclear Aromatic Hydrocarbons: Tenth International Symposium on A Decade of Progress, ed. M.W. Cooke and A.J. Dennis. 59-70. Columbus, Ohio: Battelle Press.

Ball, L.M. and J. Lewtas, (1985). Rat liver subcellular fractions catalyze aerobic binding of 1-nitro[^{14}C]pyrene to DNA. Environ. Health Perspect., 62: 193-196.

Ball, L.M., M.G. Nishioka, and J. Lewtas, (1986). Hepatic metabolism of the environmental mutagen 2-nitrofluoranthene. Pharmacologist, 28: 136.

Bauer, S.L. and P.C. Howard, (1991). Kinetics and co-factor requirements for the nitroreductive metabolism of 1-nitropyrene and 3-nitrofluoranthene by rabbit liver aldehyde oxidase. Carcinogenesis, 12: 1545-1549.

Beland, F.A., R.H. Heflich, P.C. Howard, and P.P. Fu, (1985). The in vitro metabolic activation of nitro polycyclic aromatic hydrocarbons. In Polycyclic Hydrocarbons and Carcinogenesis, ed. R.G. Harvey. 371-396. Washington, D.C.: American Chemical Society.

Belisario, M.A., R. Pecce, R.D. Morte, A.R. Arena, A. Cecinato, P. Ciccioli, and N. Staiano, (1990). Characterization of oxidative and reductive metabolism in vitro of nitrofluoranthenes by rat liver enzymes. Carcinogenesis, 11: 213-218.

Berry, D.L., G.M. Schoofs, and W.A. Vance, (1985). Mutagenicity of nitrofluoranthenes, 3-aminofluoranthene and 1-nitropyrene in Chinese hamster V79 cells. Carcinogenesis, 6: 1403-1407.

Campbell, N., and Easton, W.W., (1949). J. Chem. Soc., 340-345.

Campbell, N. and N.H. Keir, (1955). The orientation of disubstituted fluoranthene derivatives. J. Chem. Soc., 1233-1237.

Consolo, M.C., M. Anders, and P.C. Howard, (1989). Mutagenicity of the phenolic microsomal metabolites of 3-nitrofluoranthene and 1-nitropyrene in strains of *Salmonella typhimurium*. Mutat. Res., 210: 263-269.

Dietrich, A.M., (1987). "Mass spectrometric characterization of DNA adducts formed from 3-nitrofluoranthene." Ph.D. Dissertation, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Dietrich, A.M., C.R. Guenat, K.B. Tomer, and L.M. Ball, (1988). Identification and characterization of the major DNA adduct formed chemically and in vitro from the environmental genotoxin 3-nitrofluoranthene. Carcinogenesis, 9: 2113-2119.

Dietrich, M.W., W.E. Hopkins, K.J. Ashbury, and W.P. Ridley, (1987). Liquid chromatographic characterization of the deoxyribonucleoside-5'-phosphates and deoxyribonucleoside-3',5'-bisphosphates obtained by ^{32}P-postlabeling. Chromatographia, 24: 545-551.

Djuric, Z., E.K. Fifer, P.C. Howard, and F.A. Beland, (1986). Oxidative microsomal metabolism of 1-nitropyrene and DNA-binding of oxidized metabolites following nitroreduction. Carcinogenesis, 7: 1073-1079.

Djuric, Z., R.H. Heflich, E.K. Fifer, and F.A. Beland, (1986). Metabolic activation of mutagenic and tumorigenic dinitropyrenes. In Biochemical and Molecular Epidemiology of Cancer, ed. C. Harris. 441-447. New York, NY: Alan R. Liss.

Djuric, Z., D.W. Potter, R.H. Heflich, and F.A. Beland, (1986). Aerobic and anaerobic reduction of nitrated pyrenes in vitro. Chem. Biol. Interact., 59: 309-324.

El-Bayoumy, K. and S.S. Hecht, (1984). Identification of trans-1,2-dihydro-1,2-dihydroxy-6-nitrochrysene as a major mutagenic metabolite of 6-nitrochrysene. Cancer Research, 44: 3408-3413.

El-Bayoumy, K. and S.S. Hecht, (1984). Metabolism of 1-Nitro[U-4,5,9,10-^{14}C]pyrene in the F344 Rat. Cancer Res., 44: 4317-4322.

El-Bayoumy, K., S.S. Hecht, T. Sackl, and G.D. Stoner, (1984). Tumorigenicity and metabolism of 1-nitropyrene in A/J mice. Carcinogenesis, 5: 1149-1452.

El-Bayoumy, K., B. Reddy, and S.S. Hecht, (1984). Identification of ring oxidized metabolites of 1-nitropyrene in the feces and urine of germfree F344 rats. Carcinogenesis, 5: 1371-1373.

El-Bayoumy, K., A. Rivenson, B. Johnson, J. DiBello, P. Little, and S.S. Hecht, (1988). Comparative tumorigenicity of 1-nitropyrene, 1-nitrosopyrene, and 1-aminopyrene administered by gavage to Sprague-Dawley rats. Cancer Res., 48: 4256-4260.

El-Bayoumy, K., G-H. Shiue, and S.S. Hecht, (1988). Metabolism and DNA binding of 1-nitropyrene and 1-nitrosopyrene in newborn mice. Chemical Research in Toxicology, 1: 243-247.

Gibson, T.L., (1982). Nitro derivatives of polynuclear aromatic hydrocarbons in airborne and source particulate matter. Atmos. Environ., 16: 2037-2040.

Greibrokk, T., G. Lofroth, L. Nilsson, R. Toftgard, J. Carlstedt-Duke, and J. Gustafsson, (1985). Nitroarenes: Mutagenicity in the Ames *Salmonella*/microsome assay and affinity of the TCDD-receptor protein. In Toxicity of Nitroaromatic Compounds, ed. D.E. Rickert. 167-183. New York: Hemisphere.

Heflich, R.H., E.K. Fifer, Z. Djuric, and F.A. Beland, (1985). DNA adduct formation and mutation induction by nitropyrenes in *Salmonella* and Chinese hamster ovary cells: relationship with nitroreduciton and acetylation. Environ. Health Perspect., 62: 135-143.

Heflich, R.H., P.C. Howard, and F.A. Beland, (1985). 1-Nitrosopyrene: An intermediate in the metabolic activation of 1-nitropyrene to a mutagen in *Salmonella typhimurium* TA1538. Mutat. Res., 149: 25-32.

Heflich, R.H., J.R. Thornton-Manning, T. Kinouchi, and F.A. Beland, (1990). Mutagenicity of oxidized metabolites of 1-nitropyrene in Chinese hamster ovary cells. Mutagenesis, 5: 151-157.

Herreno-Saenz, D., F.E. Evans, T. Heinze, J. Lewtas, and P.P. Fu, (1992). In vitro metabolism and DNA adduct formation from the mutagenic environmental contaminant 2-nitrofluoranthene. Chemical Research in Toxicology, 5: 863-869.

Hirose, M., M.S. Lee, C.Y. Wang, and C.M. King, (1984). Induction of rat mammary gland tumors by 1-nitropyrene, a recently recognized environmental mutagen. Cancer Res., 44: 1158-1162.

Hodgson, E. and P.E. Levi, (1987). A Textbook of Modern Toxicology. New York: Elsevier Science Publishing Co., Inc.

Howard, P.C., F.A. Beland, and C.E. Cerniglia, (1983). Reduction of the carcinogen 1-nitropyrene to 1-aminopyrene by rat intestinal bacteria. Carcinogenesis, 4: 985-990.

Howard, P.C., J.A. Gerrard, G.E. Milo, P.P. Fu, F.A. Beland, and F.A. Kadlubar, (1983). Transformation of normal human skin fibroblasts by 1-nitropyrene and 6-nitrobenzo[a]pyrene. Carcinogenesis, 4: 353-355.

Howard, P.C., R.H. Heflich, F.E. Evans, and F.A. Beland, (1983). Formation of DNA adducts in vitro and in *Salmonella typhimurium* upon metabolic reduction of the environmental mutagen 1-nitropyrene. Cancer Res., 43: 2052-2058.

Howard, P.C., G.D. Mateescu, and M.C. Consolo, (1988). Liver microsomal metabolism of the environmental carcinogen 3-nitrofluoranthene. I. Phenolic metabolites. Carcinogenesis, 9: 911-917.

Howard, P.C., K.A. Reed, and D.R. Koop, (1988). Oxidative metabolism of 1-nitropyrene by rabbit liver microsomes and purified microsomal cytochrome P-450 isozymes. Cancer Res., 48: 4261-4265.

Imaida, K., M. Hirose, L. Tay, M.-S. Lee, C.Y. Wang, and C.M. King, (1991). Comparative carcinogenicities of 1-, 2-, and 4-nitropyrene and structurally-related compounds in the female CD rat. Cancer Res., 51: 2902-2907.

Imaida, K., M.-S. Lee, C.Y. Wang, and C.M. King, (1991). Carcinogenicity of dinitropyrenes in the weanling female CD rat. Carcinogenesis, 12: 1187-1191.

Karpinsky, G.E., E.C. McCoy, and H.S. Rosenkranz, (1982). The chemical activation of non-mutagenic nitrated polycyclic aromatic hydrocarbons to mutagens. Mutat. Res., 92: 29-37.

Kinouchi, T., M. Morotomi, M. Mutai, E.K. Fifer, F.A. Beland, and Y. Ohnishi, (1986). Metabolism of 1-nitropyrene in germ-free and conventional rats. Jpn. Journ. Cancer Res. (Gann), 77: 356-369.

Kinouchi, T. and Y. Ohnishi, (1986). Metabolic activation of 1-nitropyrene and 1,6-dinitropyrene by nitroreductases from *Bacteroides fragilis* and distribution of nitroreductase activity in rats. Microbiol. Immunol., 30: 979-992.

Kinouchi, T., H. Tsutsui, and Y. Ohnishi, (1986). Detection of 1-nitropyrene in yakitori (grilled chicken). Mutat. Res., 171: 105-113.

Manabe, Y., T. Kinouchi, K. Wakisaka, I. Tahara, and Y. Ohnishi, (1984). Mutagenic 1-nitropyrene in wastewater from oil-water separating tanks of gasoline stations and in used crankcase oil. Environ. Mutagen., 6: 669-681.

McCoy, E.C., M. Anders, and H.S. Rosenkranz, (1983). The basis of the insensitivity of *Salmonella typhimurium* strain TA98/1,8-DNP_6 to the mutagenic action of nitroarenes. Mutat. Res., 121: 17-23.

McCoy, E.C., E.J. Rosenkranz, R. Mermelstein, and H.S. Rosenkranz, (1983). Mutagenic specificity and the prediction of mechanisms and bioactivation pathways of genotoxicants: the mutagenicity of 5-nitroacenaphthene as an example. Mutat. Res., 111: 61-68.

Messier, F., C. Lu, P. Andrews, B.E. McCarry, M.A. Quilliam, and D.R. McCalla, (1981). Metabolism of 1-nitropyrene and formation of DNA adducts in *Salmonella typhimurium*. Carcinogenesis, 2: 1007-1011.

Mitchell, C.E., W.E. Bechtold, and S.A. Belinsky, (1993). Metabolism of nitrofluoranthenes by rat lung subcellular fractions. Carcinogenesis, 14: 1161-1166.

Moller, L., M. Corrie, T. Midtvedt, J. Rafter, and J.-A. Gustafsson, (1988). The role of the intestinal microflora in the formation of mutagenic metabolites from the carcinogenic air pollutant 2-nitrofluorene. Carcinogenesis, 9: 823-830.

Mumford, J.L. and J. Lewtas, (1982). Mutagenicity and cytotoxicity of coal fly ash from fluidized-bed and conventional combustion. J. Toxicol. Environ. Health, 10: 565-586.

Murray, R. W., Rajadhyaksha, S. N., Mohan, Lily, (1989). Oxidation of primary amines by dimethyldioxirane. J. Org. Chem., 54: 5783-5788.

Nielsen, T., B. Seitz, and T. Ramdahl, (1984). Occurrence of nitro-PAH in the atmosphere in a rural area. Atmos. Environ., 18: 2159-2165.

Ohgaki, H., N. Matsukura, K. Morino, T. Kawachi, T. Sugimura, K. Morita, H. Tokiwa, and T. Hirota, (1982). Carcinogenicity in rats of the mutagenic compounds 1-nitropyrene and 3-nitrofluoranthene. Cancer Lett., 15: 1-7.

Orr, J.C., D.W. Bryant, D.R. McCalla, and M.A. Quilliam, (1985). Dinitropyrene-resistant *Salmonella typhimurium* are deficient in an Acetyl-CoA Acetyltransferase. Chem.-Biol. Interact., 54: 281-288.

Paputa-Peck, M.C., R.S. Marano, D. Schuetzle, T.L. Riley, C.V. Hampton, T.J. Prater, L.M. Skewes, T.E. Jensen, P.H. Ruehle, L.C. Bosch, and W.P. Duncan, (1983). Determination of nitrated polynuclear aromatic hydrocarbons in particulate extracts by capillary column gas chromatography with nitrogen selective detection. Anal. Chem., 55: 1946-1954.

Patton, J.D., V.M. Maher, and J.J. McCormick, (1986). Cytotoxic and mutagenic effects of 1-nitropyrene and 1-nitrosopyrene in diploid human fibroblasts. Carcinogenesis, 7: 89-93.

Pitts, J.N., Jr., J.A. Sweetman, B. Zielinska, A.M. Winer, and R. Atkinson, (1985). Determination of 2-nitrofluoranthene and 2-nitropyrene in ambient particulate organic matter: evidence for atmospheric reactions. Atmos. Environ., 19: 1601-1608.

Pitts, J.N., Jr., K.A. Van Cauwenberghe, D. Grosjean, J.P. Schmid, D.R. Fitz, W.L. Belser Jr., G.B. Knudson, and P.M. Hynds, (1978). Atmospheric reactions of polycyclic aromatic hydrocarbons: facile formation of mutagenic nitro derivatives. Science, 202: 515-519.

Rice, J.E., E.J. LaVoie, and D. Hoffman, (1983). Synthesis of the isomeric phenols and the trans-2,3-dihydrodiol of fluoranthene. J. Org. Chem., 48: 2360-2363.

Rosenkranz, H.S., E.C. McCoy, D.R. Sanders, M. Butler, D.K. Kiriazides, and R. Mermelstein, (1980). Nitropyrenes: Isolation, identification and reduction of mutagenic impurities in carbon black and toners. Science, 209: 1039-1043.

Rosenkranz, H.S. and R. Mermelstein, (1983). Mutagenicity and genotoxicity of nitroarenes: All nitro-containing chemicals were not created equal. Mutat. Res., 114: 217-267.

Rosenkranz, H.S. and R. Mermelstein, (1985). The genotoxicity, metabolism and carcinogenicity of nitrated polycyclic aromatic hydrocarbons. J. Environ. Sci. Health, C3: 221-272.

Schuetzle, D., (1983). Sampling of vehicle emissions for chemical analysis and biological testing. Environ. Health Perspect., 47: 65-80.

Schuetzle, D., T.L. Riley, T.J. Prater, T.M. Harvey, and D.F. Hunt, (1982). Analysis of nitrated polycyclic aromatic hydrocarbons in diesel particulates. Anal. Chem., 54: 265-271.

Shane, B.S., G.L. Squadrito, D.F. Church, and W.A. Pryor, (1991). Comparative mutagenicity of nitrofluoranthenes in *Salmonella typhimurium* TA98, TA98NR, and TA98/1,8-DNP_6. Environ. Mol. Mutagen., 17: 130-138.

Singer, B. and D. Grunberger, (1983). Molecular Biology of Mutagens and Carcinogens. New York: Plenum Press.

Sugimura, T. and S. Takayama, (1983). Biological actions of nitroarenes in short-term tests on *Salmonella*, cultured mammalian cells and cultured human tracheal tissues: possible basis for regulatory control. Environ. Health Perspect., 47: 171-176.

Sweetman, J.A., B. Zielinska, R. Atkinson, T. Ramdahl, A.M. Winer, and J.N. Pitts Jr., (1986). A possible formation pathway for the 2-nitrofluoranthene observed in ambient particulate organic matter. Atmos. Environ., 20: 797-811.

Tokiwa, H., T. Otofuji, K. Horikawa, S. Kitamori, Otsuka H., Y. Manabe, Kinouchi T., and Y. Ohnishi, (1984). 1,6-Dinitropyrene: mutagenicity in *Salmonella* and carcinogenicity in BALB/c mice. J. Natl. Cancer Inst., 73: 1359-1363.

van Houte, L.P.A., J.T. Bokman, J.T. Lutgering, J.G. Westra, J. Retel, R. van Grondelle, and J. Blok, (1987). An optical study of the conformation of the aminofluorene-DNA complex. Carcinogenesis, 8: 759-766.

Vance, W.A. and D.E. Levin, (1984). Structural features of nitroaromatics that determine mutagenic activity in *Salmonella typhimurium*. Environ. Mutagen., 6: 797-811.

Williams, G.M. and J.H. Weisburger, (1993). Chemical Carcinogenesis. In Casarett and Doull's Toxicology: The basic science of poisons, ed. M.O. Amdur, J. Doull, and C.D. Klaassen. 127-200. 4 ed., Vol. New York: McGraw-Hill, Inc.

Wislocki, P.G., E.S. Bagan, A.Y.H. Lu, K.L. Dooley, P.P. Fu, H. Han-Hsu, F.A. Beland, and F.F. Kadlubar, (1986). Tumorigenicity of nitrated derivatives of pyrene, benz[a]anthracene, chrysene and benzo[a]pyrene in the newborn mouse assay. Carcinogenesis, 7: 1317-1322.

Zielinska, B., J. Arey, R. Atkinson, T. Ramdahl, A.M. Winer, and J.N. Pitts Jr., (1986). Reaction of dinitrogen pentoxide with fluoranthene. J. Amer. Chem. Soc., 108: 4126-4132.

Zielinska, B., W.P. Harger, J. Arey, A.M. Winer, R.A. Haas, and C.V. Hanson, (1987). The mutagenicity of 2-nitrofluoranthene and its in vitro hepatic metabolites. Mutat. Res., 190: 259-266.