THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF 2-SUBSTITUTED MALONDIALDEHYDES

by

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We accept this thesis as conforming
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ABSTRACT

The synthesis of 2-aryl- and 2-methylmalondialdehydes is described, commencing with commercially available substrates. This class of compounds represents the C-conjugation of the electrophilic side chain with a substituent polycyclic aromatic hydrocarbon, both putative mutagens. Although O-conjugates of polycyclic aromatic hydrocarbons are well documented, and known to be formed in nature, the properties of polar C-conjugates are relatively unexplored.

The 2-arylmalondialdehydes, (which exist as β-hydroxy acroleins) and two classes of derivatives, the β-N,N-dimethylamino-acroleins, and the β-ethoxyacroleins were tested for mutagenicity using the Ames system. A new strain of Salmonella typhimurium, TR2705 (TA94), was used which is more sensitive to cross linking agents such as malondialdehyde. The aromatic substituents investigated were phenyl, 1- and 2-naphthyl, 1-pyrenyl, 6-chrysenyl, and 1-benzo(a)pyrenyl. None of these proved positive in TR2705, but three benzo(a)pyrene derivatives, the acetamide, the N,N-dimethylaminoacrolein, and the malondialdehyde, were found to be positive in TA98, a strain sensitive to PAH. β-Methoxyacrolein itself was found to be positive in TR2705, while malondialdehyde was less so. Interestingly, even 2-methyl-3-ethoxyacrolein was statistically inactive, showing the deactivating effect of a 2-substituent.

The utility of colorimetric tests for 2-substituted malondialdehydes was also investigated. The commonly used reagent, thiobarbituric

acid, which gives a distinct red colour at 532nm with unsubstituted malondialdehyde, does not give a colour with the 2-substituted compounds. 2-Methylindole, however, does give a colorimetric test (λ max $\sim$ 560nm). The intensity of the colour was dependent on the point of substitution on the aromatic ring.

EXAMINERS:

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LIST OF ABBREVIATIONS

BaP	benzo(a)pyrene
Chry	chrysenyl
CI	chemical ionisation
D	deuterium
DMF	dimethylformamide
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
E	entgegen
EI	electron impact
ESR	electron spin resonance
Et	ethyl
IR	infra red
Me	methyl
MDA	malondialdehyde
1-,2-nap	1- or 2-naphthyl
NMR	nuclear magnetic resonance
PAH	polycyclic aromatic hydrocarbons
Ph	phenyl
ppb	parts per billion
ppm	parts per million
PUFA	polyunsaturated fatty acid
Py	pyrenyl
TBA	thiobarbituric acid
TLC	thin layer chromatography

TTN	thallium trinitrate
UV	ultraviolet
Z	zusammen

ACKNOWLEDGEMENTS

Special thanks are due to Dr. P.R. West and Dr. R.H. Mitchell for their guidance throughout the course of this work. I am also grateful to the University of Victoria for the award of a Graduate Supplement, and to the Department of Chemistry for the use of its research facilities.

There are many people without whose help the project would have been impossible. These include among others; the mechanical and glass shops for the design and construction of special equipment; Miss Margaret Liu and the laboratory of Dr. M.J. Ashwood-Smith, for the performance of the Ames tests; Dr. A.G. Briggs for his skills with a glass torch; and to the members of Chemistry Stores for their help in procuring the basic chemicals.

The companionship of my fellow graduate students, post-doctoral fellows and sundry others has played a great role in making my stay in Victoria an unforgettable one.

Finally, I would like to thank Mrs. J. Kinnis, for her skill and patience in the typing of this thesis.

Dedication

To my parents and to my sister

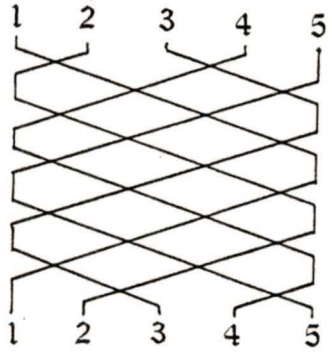
To the
Abbotts, Royalls
Barkers and Bakers

Past, Future and Present

Ringers in an oil-lit belfry—Bitton? Kelston? who shall say?
Smoothly practising a plain course, caverned out the dying day
As their melancholy music flooded up and ebbed away.

And an undersong to branches dripping into pools and wells
Out of multitudes of elm trees over leagues of hills and dells
Was the mathematic pattern of a plain course on the bells.

Bristol – 1945
John Betjeman



CHAPTER I

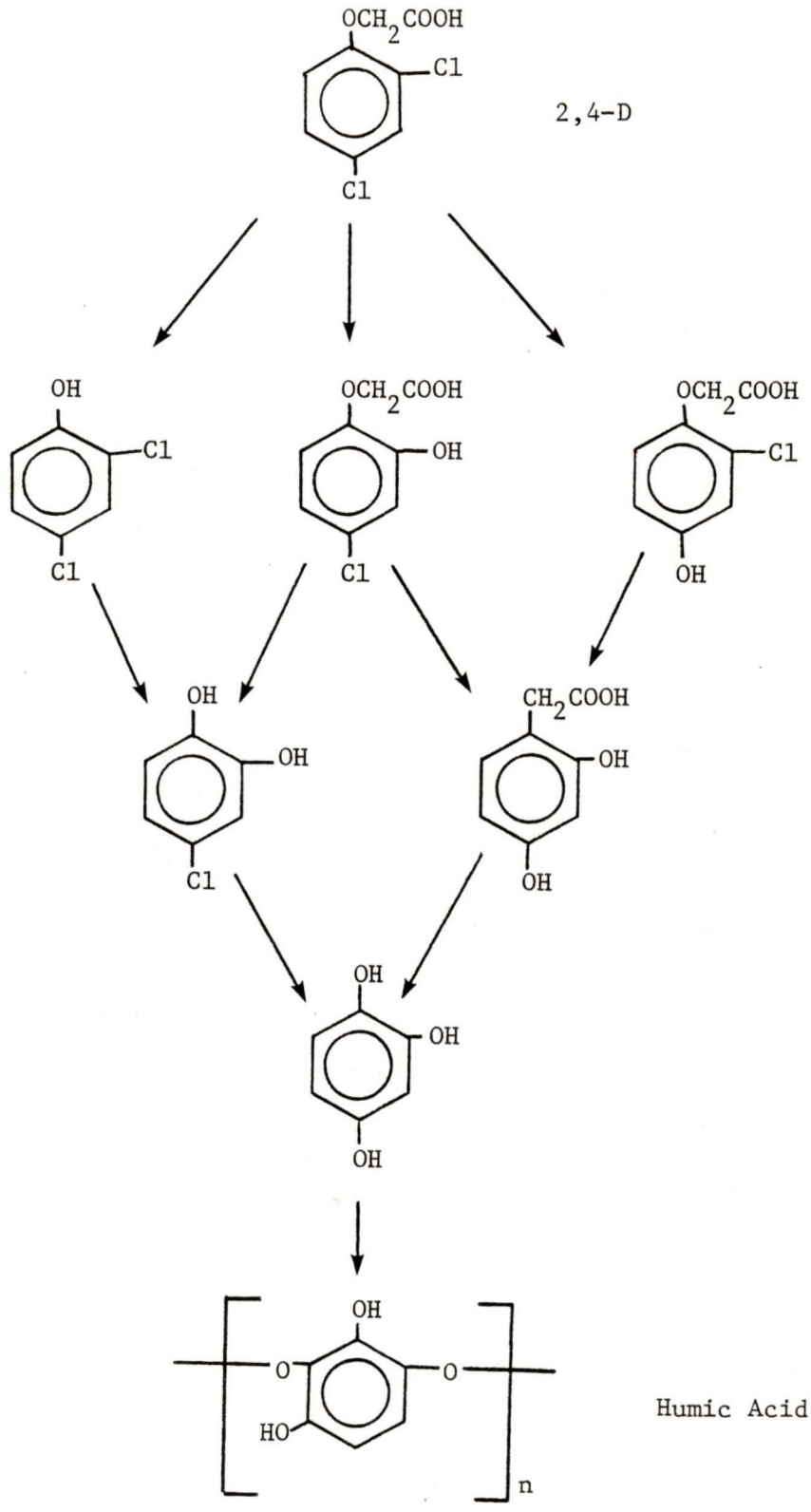
1. An Introduction

In the past quarter century, modern society has become increasingly aware of the impact of foreign organic compounds or pollutants on the natural environment. These compounds have a wide range of chemical structures, and an equally wide range of sources. Two main pathways are prominent in the introduction of these compounds into the environment. Fungicides, herbicides, insecticides, and similar chemicals are deliberately put into the environment to help man provide food for an expanding population. Other toxic pollutants are released through ignorance, or carelessness, through the combustion of fossil fuels, and additives to these fuels.

At present, the principal role of the chemist in environmental toxicology is the investigation of the persistence of manmade chemicals in the biosphere. Pollutant residency time depends on the available degradation routes, and their relative rates. The removal processes include both chemical and microbial pathways. From a chemist's perspective, the presence of oxygen, sunlight, heat and water allow reactions to occur in the largely aqueous environment. Hence, chemical degradation processes include oxidation, photolysis, and hydrolysis which can transform the pollutant into either a less hazardous form (detoxification), or into a form with enhanced toxic activity.

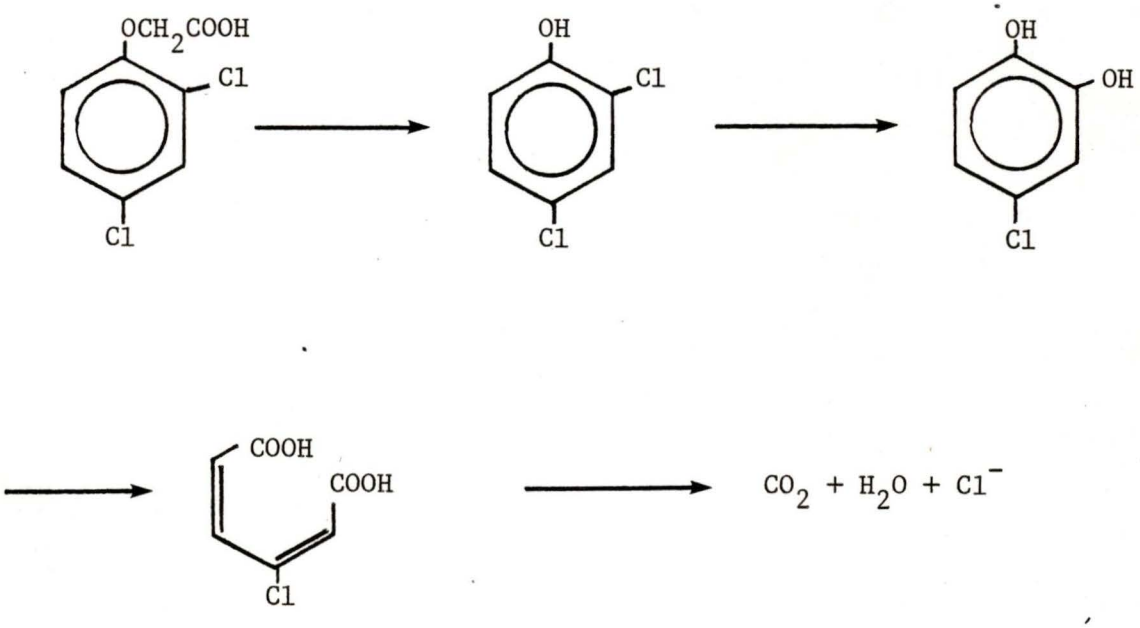
For example, a combination of photolysis and hydrolysis converts the organic herbicide, 2,4-D into a harmless component of soil (Figure 1).

Figure 1. The Photodecomposition of 2,4-D



Microbes also play an important part in the degradation of non-natural products. They initiate a variety of basic chemical transformations to achieve detoxification, including dehalogenation, stepwise dealkylation, hydrolyses of esters and amides, oxidation, cleavage of rings, and formation of water soluble conjugates. Indeed, some microbes use the very pollutant they are attacking as a source of energy. As an example, 2,4-D is also detoxified by an Achromobacter species of bacteria[2] (Figure 2).

Figure 2. The Microbial Decomposition of 2,4-D



In the present work, one of the principal unexplored areas of chemical degradation has been of interest, namely the synergistic or combined effects of two or more pollutants in the complex environmental medium. By combination, chemical conjugates can be formed that involve

fragments derived from more than one pollutant. This can be represented schematically as follows:

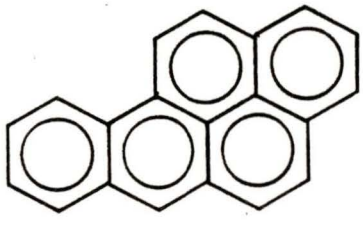
Pollutant	A → B	Decomposition product
Pollutant	C → D	Decomposition product
	B + D → E	Conjugate

The conjugate E would be formed in the decomposition of pollutants A and C. A closely related phenomenon is the possibility that a poly-functional, high molecular weight pollutant may be degraded to more than one potentially active form, or to a form that has more than one potentially active site for harmful biological interaction.

In this work, we are interested in the mutagenic activity of simple oxygenated compounds with electrophilic centres, such as malondialdehyde (MDA) (1) and glyoxal (2) and the ubiquitous polycyclic

$OHC-CH_2-CHO$ (1) $\qquad$ OHC-CHO (2)

aromatic hydrocarbons (PAH), some of which are among the most potent carcinogens known; an example is benzo(a)pyrene (BaP) (3).



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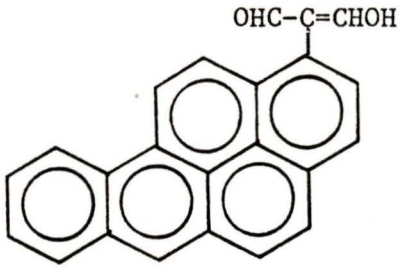
Conjugate E could represent here a structure incorporating both of these elements (4).



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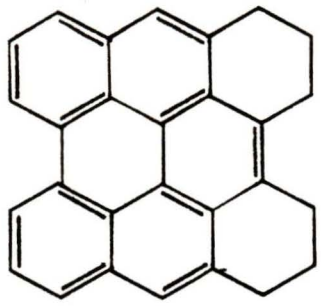
The choice of malondialdehyde as the conjugated group arises from the widespread occurrence of this degradation product in the oxidation and radiolysis of lipids and carbohydrates.[3,4,5] Hence the potential exists for PAH coupling with MDA derived free radicals.

As an introduction to the properties of such complex conjugates, a brief survey of the pertinent chemical reactions of PAH will be outlined, (i.e. photo-oxidation and metabolism), together with an overview of lipid peroxidation emphasizing the origin of MDA and related substances.

2. Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAH) have been found to be common in the environment, though in very low concentrations.[6] Before the advent of human civilization, PAH were formed by the action of forest fires, or in deep sediments, as products of geological transformations. Some PAH, such as hexahydro-meso-naphthodianthrene (5) may also have been biosynthesized. The hydrocarbon (5) was identified in



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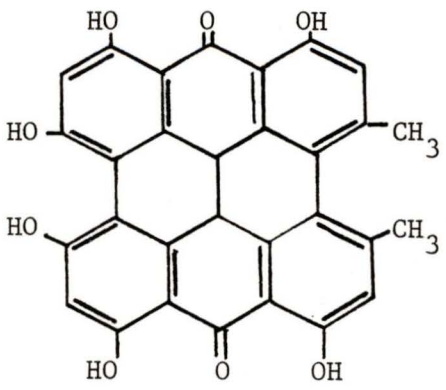
the fossil remains of a sea lily, Millericrinus, and has a similar structure to fossil pigments. In recent years the biosynthesis of PAH has been controversial.

There are numerous reports, especially in older literature, of the isolation of PAH from plant material. However, these findings are questionable on the grounds that the isolation techniques could lead to pyrolytic formation of PAH from suitable precursors. There are, however, many known natural products with a PAH like structure, e.g. hypercin (6) found in St. John's Wart (Hypercium perforatum).[7]



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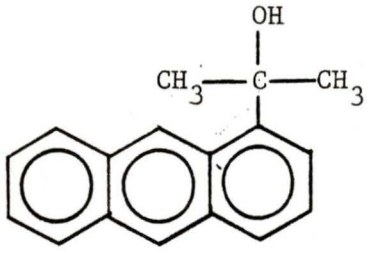
PAH are also released into the environment by the combustion of fossil fuels such as oil and coal. Due to the complexity of the reactions involved, it is hard to relate the products to the original structure.[8]

All sources considered, the input of PAH into the environment is vast, and is summarized in Table 1, below. BaP is listed apart to indicate the relative amounts of this toxic material.[9]

Table 1. Sources of Polynuclear Aromatic Hydrocarbons Released Into the Environment. (1979)

Source	(input in 1000's of kg per year)	
	BaP	PAH (total)
Biosynthesis	25	2700
Petroleum spillage	25-30	170000
Domestic/Industrial wastes	29	4400
Surface runoff from land	118	2940
Fallout from air	500	50000
Total input	697	230040

Once in the environment, PAH can then be acted upon by the various degradation routes available. On exposure to the air, in sunlight, PAH are photooxidized by ozone or hydroxyl radical ($^{\cdot}OH$).[10] In aquatic environments, photooxidation is mediated by singlet oxygen. In the presence of ozone and light, PAH have half lives of several minutes to a few hours, alkylated PAH tending to be more rapid in degrading. Alkylated PAH closely related to the conjugate (e.g. 7), can be electrophilically attacked at the side chain, or the ring system, the two modes competing.



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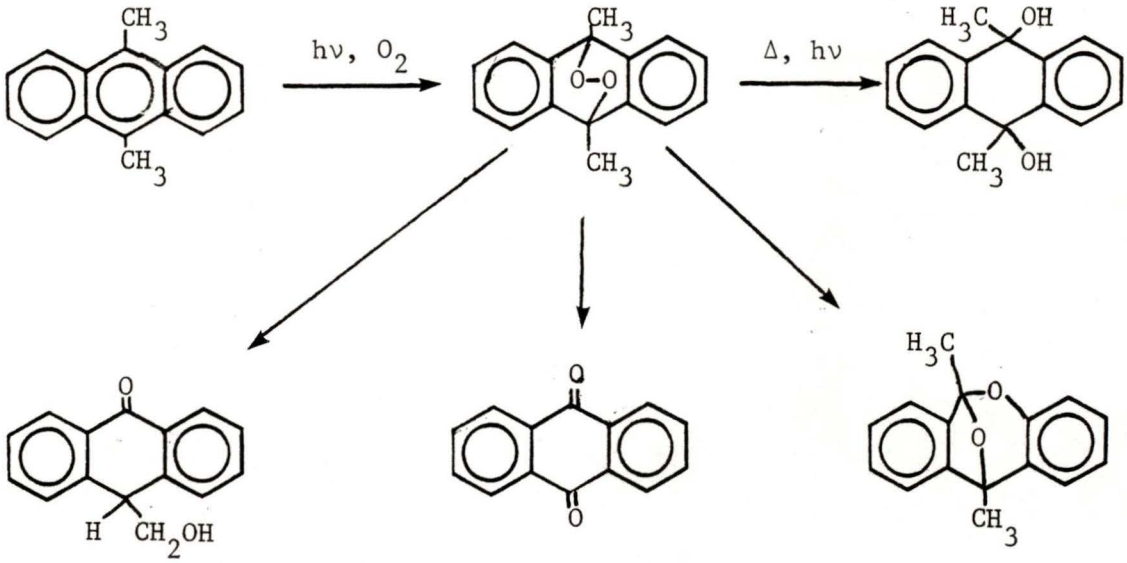
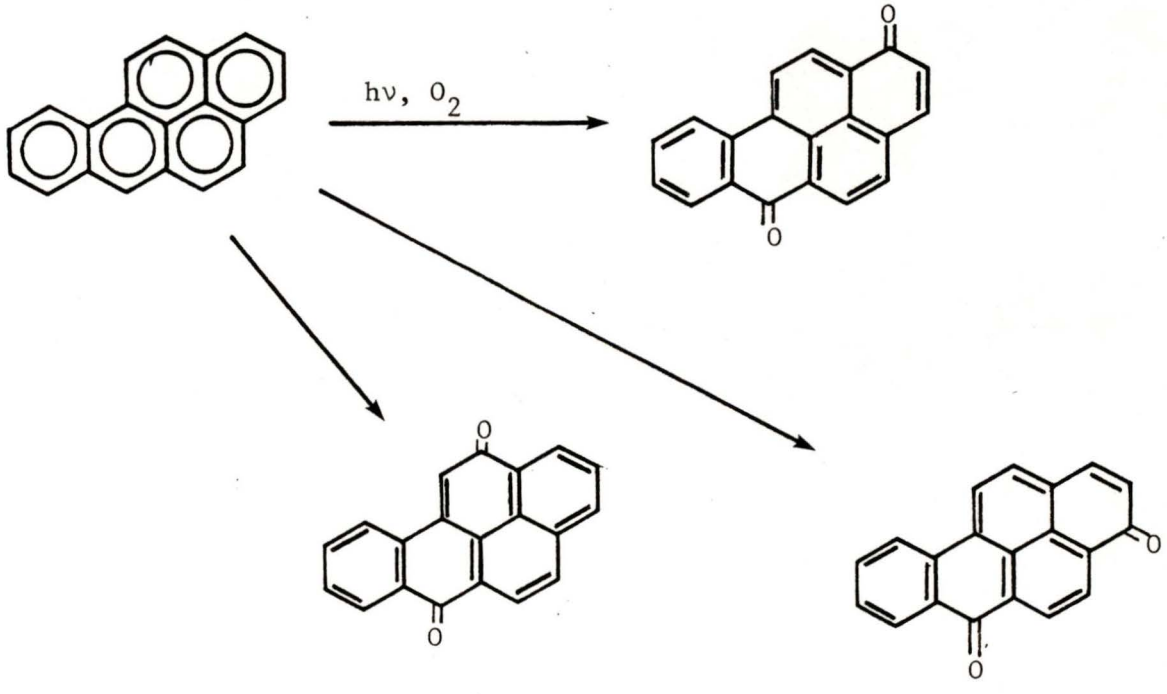
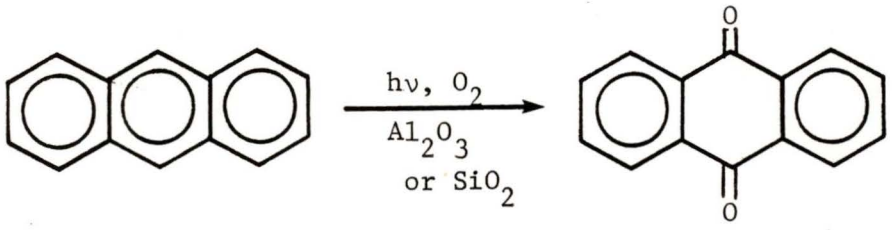
The principal products of unsaturated PAH photooxidation are endoperoxides. Hydrolysis or pyrolysis of the initial intermediates initiates ring cleavage and dealkylation, leading to a variety of compounds. If, for steric reasons, no endoperoxide can be formed, quinones are produced (Figure 3).

Figure 3. Photooxidation of Typical Polynuclear Aromatic Hydrocarbons.





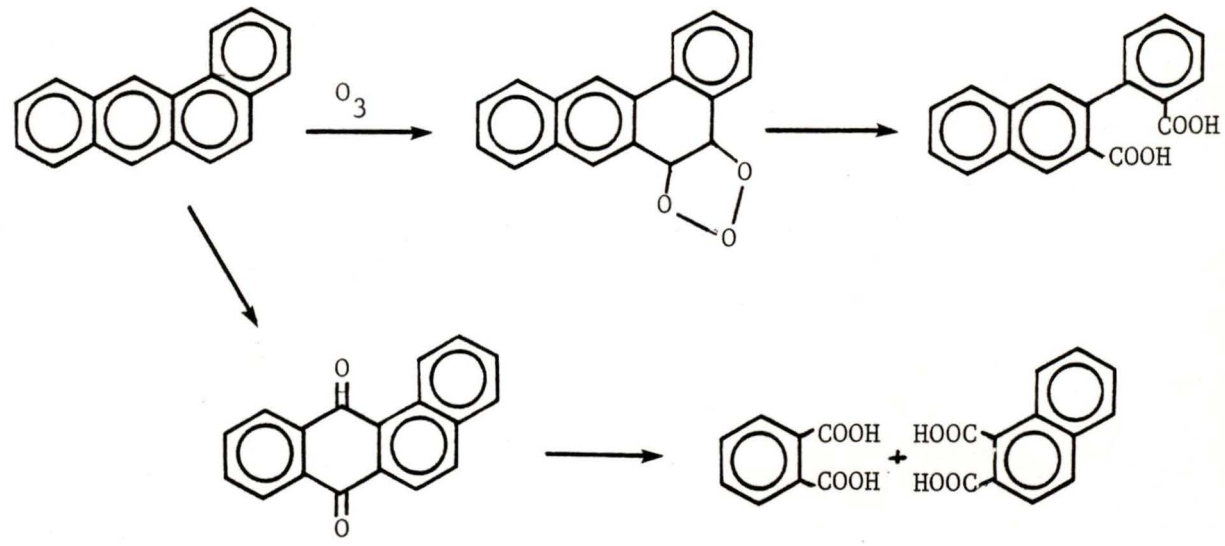
The photoreactivity of PAH absorbed onto particulate matter appears to be more rapid than in solution.[11] A direct pathway that does not involve endoperoxides as intermediates is proposed. For example, anthracene is oxidized to anthraquinone as follows:

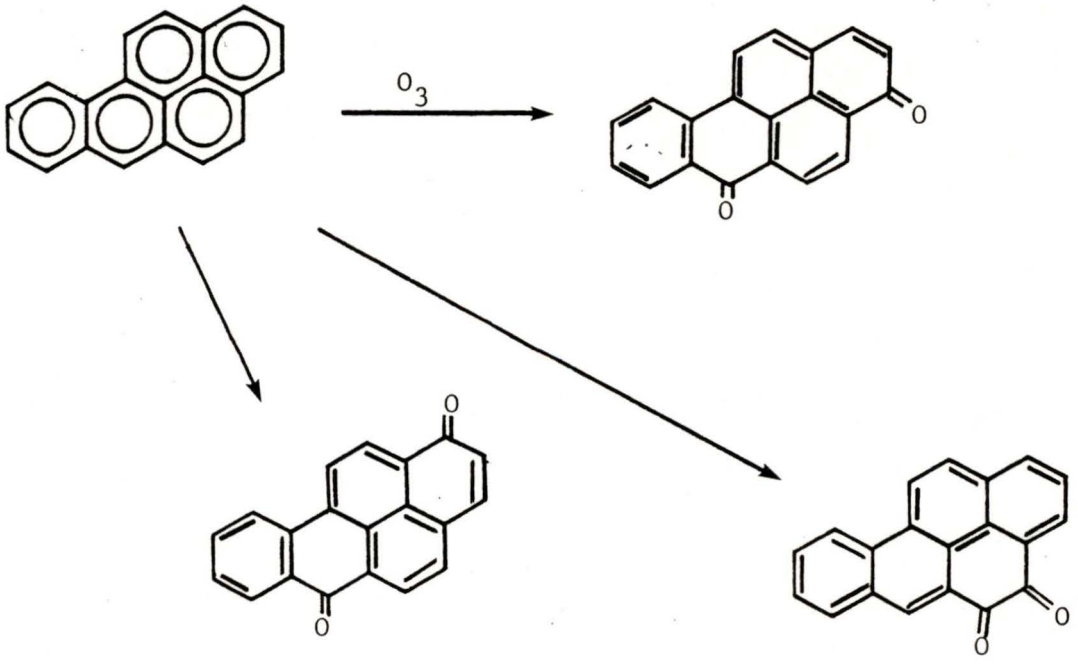


PAH are also chemically oxidized. The chlorination that is used for the removal of pathogens from drinking water, and for the treatment of industrial wastes, is an oxidizing process. The action of sodium hypochlorite at concentrations of 0-13.2 mg/l of "free chlorine" on different PAH has been reported.[12] In combination with a sedimentation stage, chlorination can appreciably reduce the PAH content of river waters[12] (e.g. 100ppb of pyrene reduced to 18ppb after treatment).

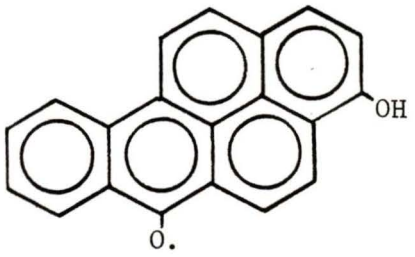
Ozone is also used for water treatment in Europe. Chlorination may produce toxic byproducts (i.e. CCl_4 and $CHCl_3$). Ozonolysis is being adopted in North America as well. Ozone reacts readily with PAH, such as benzanthracene, the reactions including cleavage of phenanthrenoid bonds giving diacids, oxidation of anthracene 5,10 like positions, to give quinones, and oxidation of alkyl side chains, (Figure 4).

Figure 4. Ozonolysis of Representative Polynuclear Aromatic Hydrocarbons.





The BaP quinones shown easily undergo reduction with borohydride to give the semiquinone anion radicals or with ascorbate to produce neutral radicals (e.g. 8).



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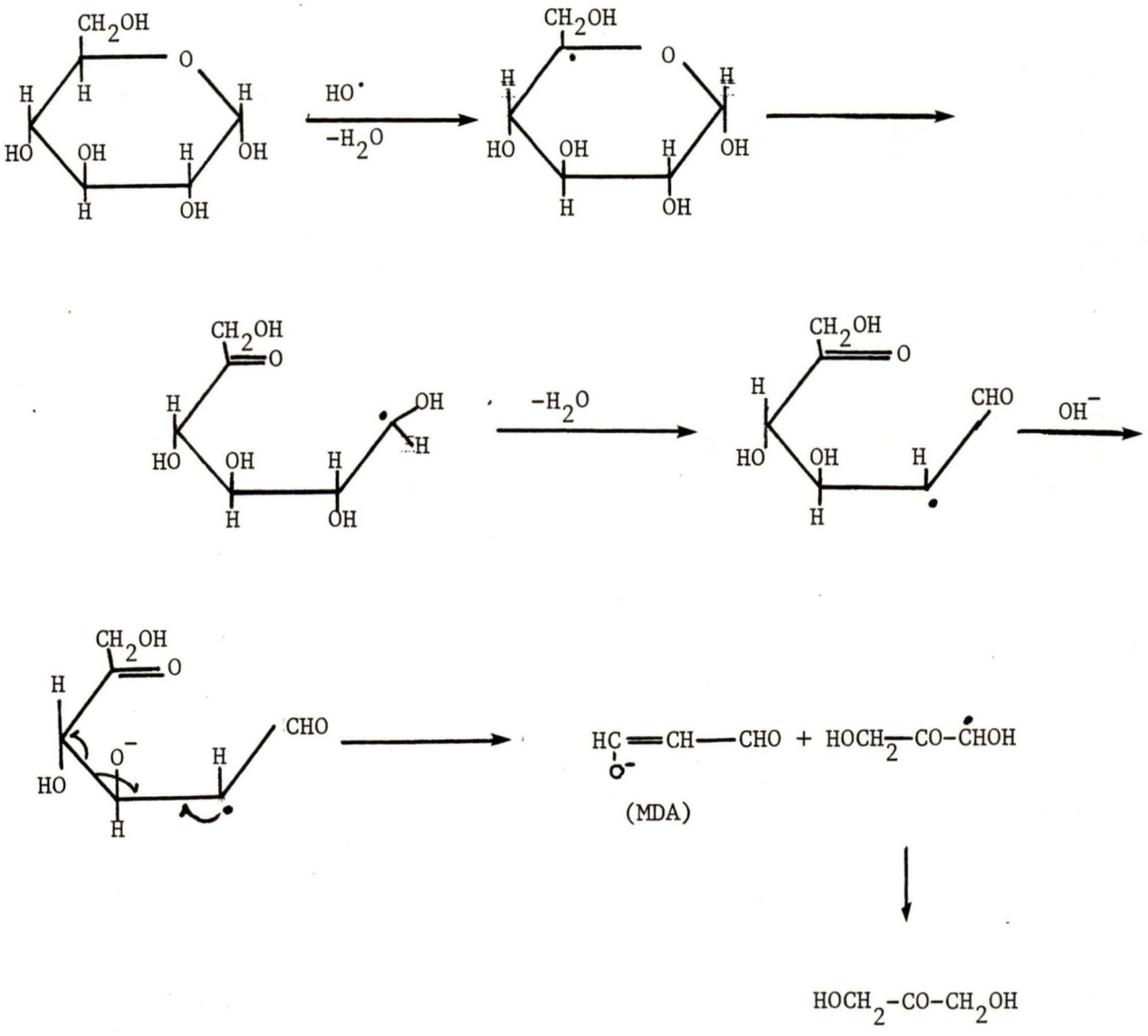
BaP can form four different radicals under different conditions,[15] the 6-oxy BaP radical, BaP anion and cation radicals, and an uncharacterised species derived from thermalised BaP. The 6-oxy radical is the most interesting, and has been identified (by ESR spectroscopy) in mixtures of BaP incubated with rat liver homogenates, and also in solutions of BaP irradiated with light in the presence of oxygen.[16] Autoxidation, a chemical oxidation of 6-hydroxy BaP with ceric sulphate also produces the 6-oxy radical, which was found to bind covalently with DNA.[14] Clearly, such species will also interact with readily oxidized lipids or carbohydrates to initiate cooxidation and potential conjugate formation.

In addition, carbohydrates can form free radicals that can conjugate with PAH. Irradiation of sugars produces a series of radicals that can attack PAH, and then decompose. One of the principal

products in the radiolysis of D-glucose is malondialdehyde. The suggested pathway leading to its formation is as follows:



Attack at differing positions on the ring by the hydroxyl radical can lead to differing amounts of MDA, and in other sugars, also to compounds such as glyoxal (2).

PAH are now known to exhibit mutagenic and carcinogenic behaviour.[23] Of the unsubstituted compounds, the lower members, benzene (9), naphthalene (10) and anthracene (11) are all relatively inactive, even in the presence of enzymes. However, the observed

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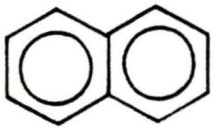

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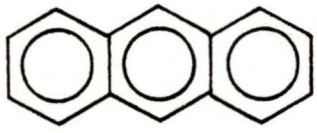

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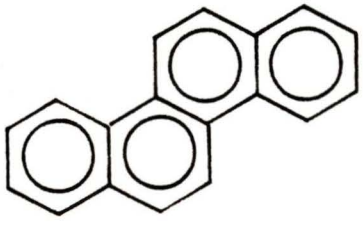
activity increases with the fusion of four rings, as in chrysene (12) and benzanthracene (13).



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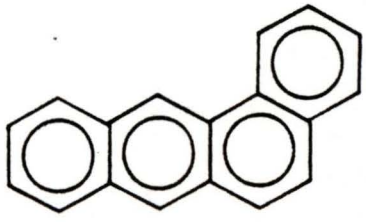

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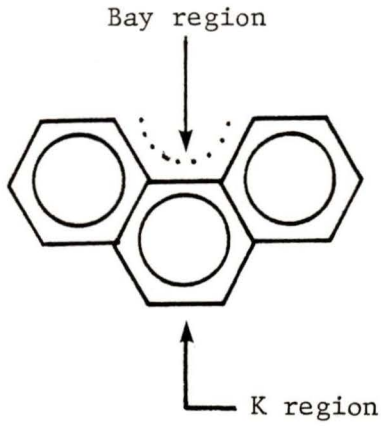
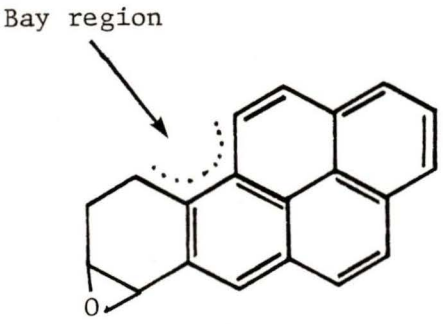
This mutagenic activity has been associated, first with the presence of a "K region", and latterly with a "bay region" in the molecules. Definitions of these terms are in order.



14

Phenanthrene (14) illustrates both a K region and a bay region. The K region may be viewed as an exposed bond,[18] which is readily attacked and that the π electron density is somewhat localized on the bond. Phenanthrene undergoes osmium tetroxide oxidation at this position. Alkyl sustituents elsewhere on the rings increase the π electron density, making the bond more reactive. It may be this enhanced reactivity that makes alkylated PAH more potent carcinogens. For example, the 8-methyl- and 12-methylbenzanthracenes both show an enhanced activity over unsubstituted benzanthracene. Alternatively, oxidation may occur at the side chains.

The bay region can be typified by the sterically crowded region between the 1 and 10 positions of phenanthrene (14). The metabolic formation of a peroxide proximate to such a region creates the potential for a highly reactive carbocation to be formed at the position indicated.



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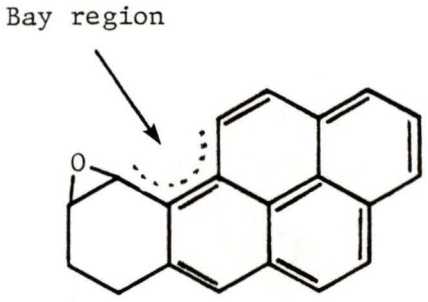

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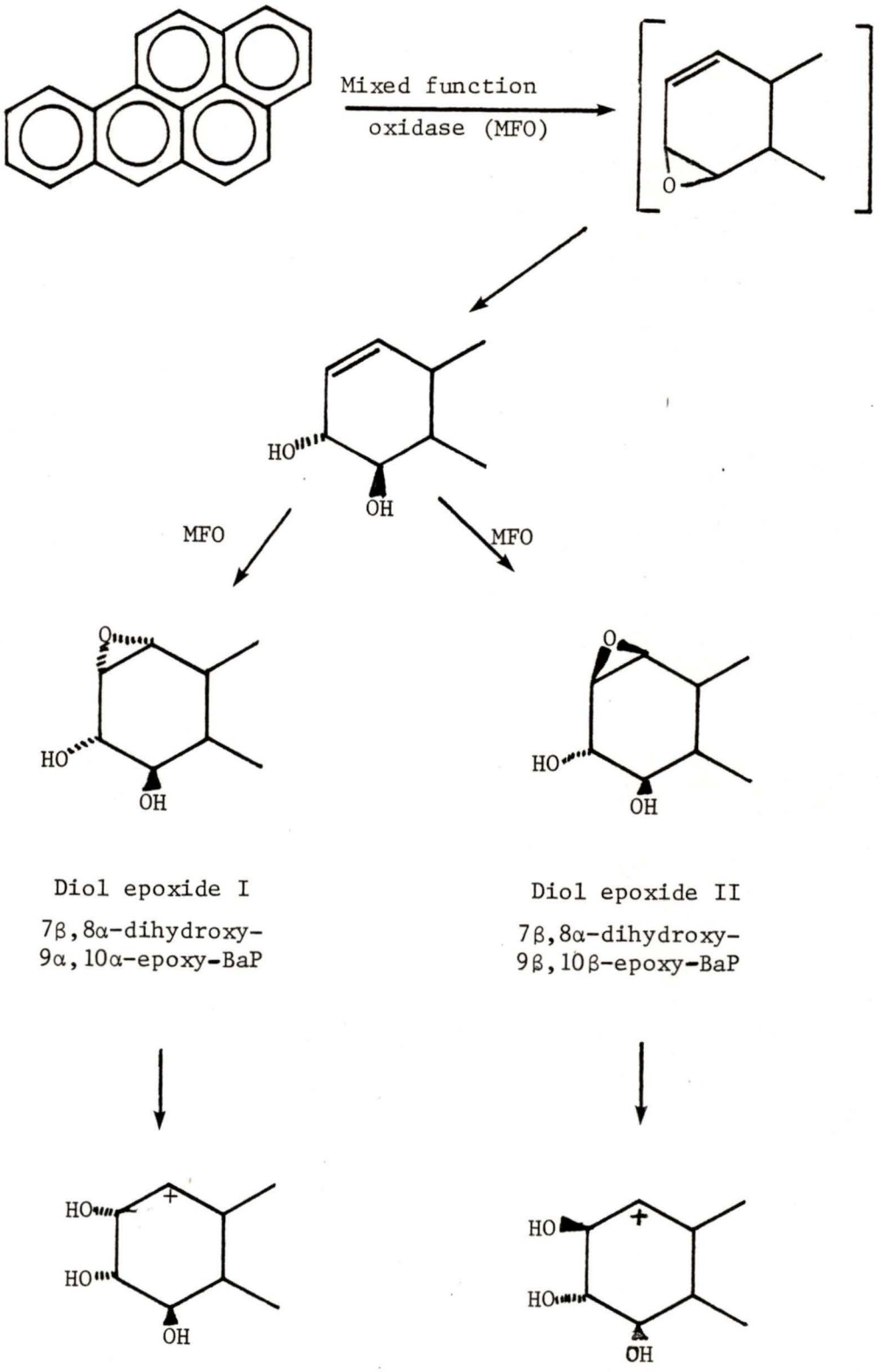
An example of the difference in reactivity is shown in the BaP epoxides, (15) and (16). The 9,10-epoxide (16) is 10 times as reactive as the 7,8-epoxide (15).[14]

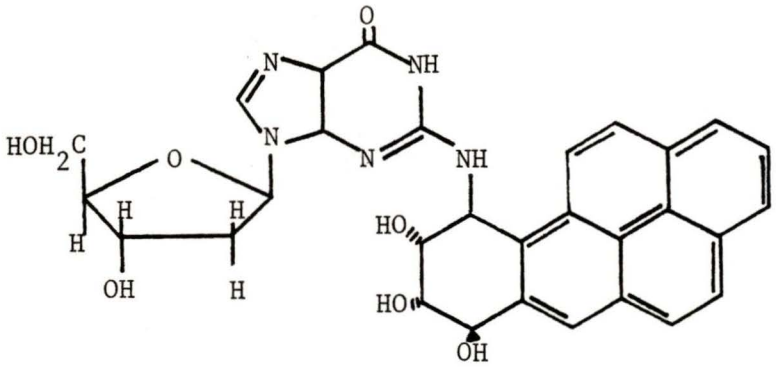
In contrast, attempts to link K region epoxides with carcinogenic metabolites have been unsuccessful. The K region oxides of benzanthracene and BaP have low activities when compared to the parent hydrocarbon. As many PAH with a K region also have a bay area, simultaneous metabolic activation of both sites may well have led to the former's early correlation with cancer.

Enzymic activation of aromatic compounds plays an important role in their mutagenicity. The metabolism of BaP produces metabolites that are more mutagenic than BaP itself.[21] The resulting metabolites, the BaP-7,8-diol-9,10-epoxides are postulated to form as follows[22] (Figure 5).

Figure 5. Mammalian Metabolism of Benzo(a)pyrene.



Note that the ultimate carcinogen is a bay region epoxide. The inclusion of the epoxide ring makes the 10-position available for electrophilic attack on macromolecules such as DNA. The major product is postulated to have the structure shown below.[23]



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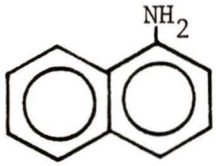
This major DNA conjugate is derived from the guanine moeity, although some adenine reacts with the activated 10 position.

Additionally, substituted aromatic compounds can be metabolically active at a side chain position. An example of such metabolic activation, and the effect of different ring substitution is found in the naphthylamines (18) and (19).



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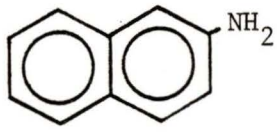

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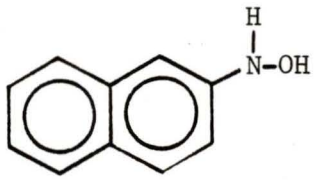
2-Naphthylamine (19) is a human carcinogen, identified as being responsible for bladder cancer,[24] whereas 1-naphthylamine is inactive. The evidence suggests that unmetabolised, the amines are weakly, if at all active, but that metabolism through an N-hydroxyl intermediate, produces a potently active derivative of 2-naphthylamine alone. Supporting this, 2-naphthylhydroxylamine (20) has been found to display powerful carcinogenicity.[25]



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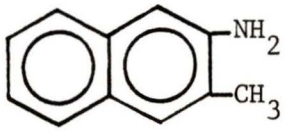
2-Naphthylamine does not induce tumors at sites of injection of test animals, it needs a metabolic activation step. Notably, the addition of a 3-methyl group (21) transforms the molecule into a topical



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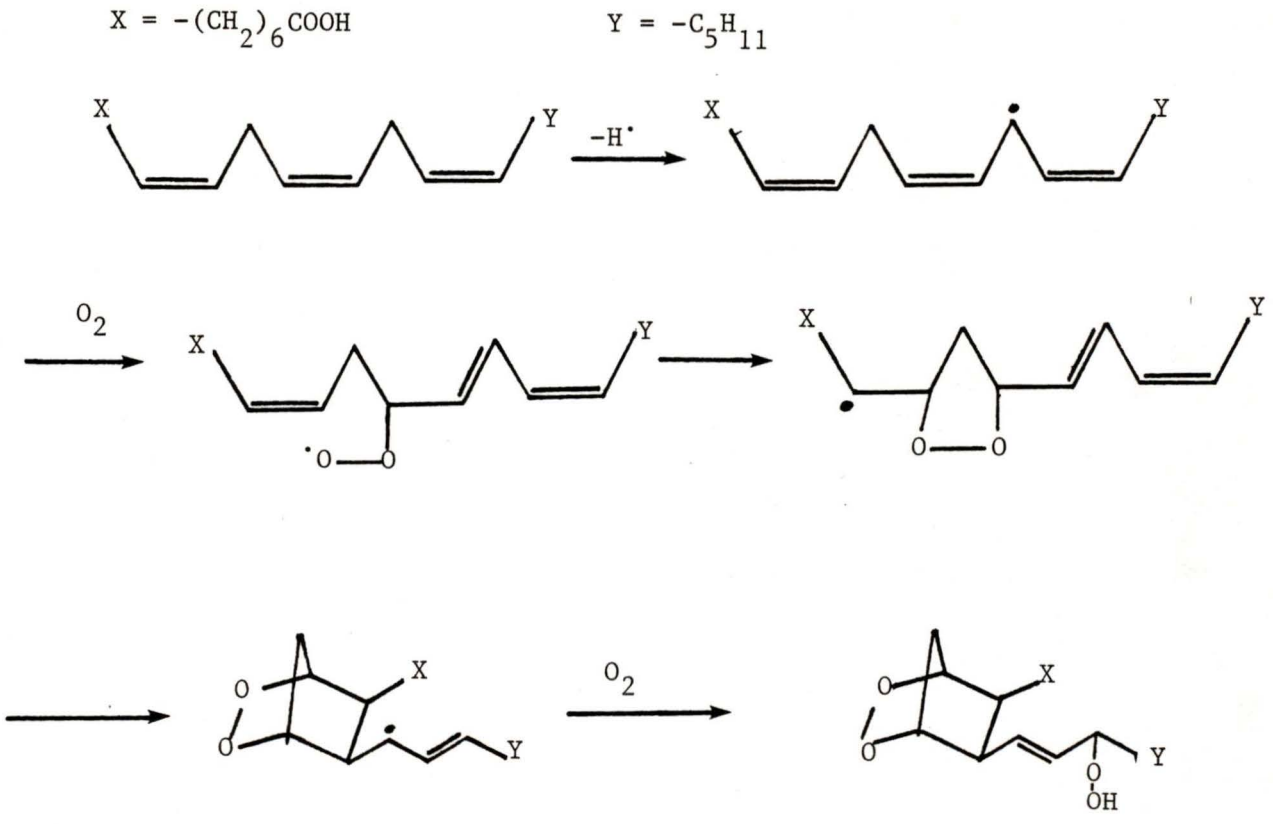
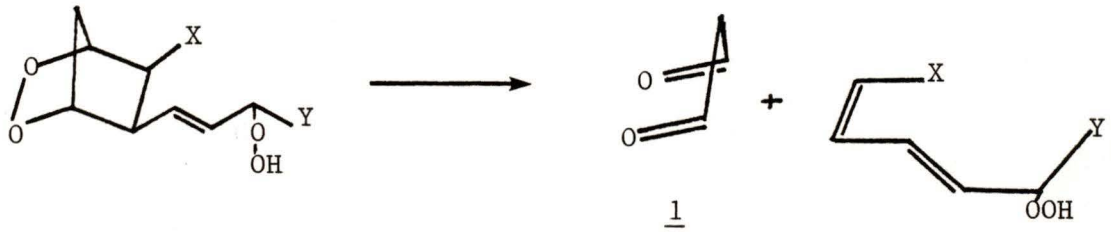
carcinogen,[26] which does not need metabolic activation to produce local tumors.

3. Peroxidation of Lipids

The very evident development of rancidity in fats, such as butter and tallow, on exposure to air and light is the most visible sign of oxidation of lipids. It is now well established that it is the polyunsaturated fatty acids (PUFA) that undergo autoxidation to form bicyclic hydroperoxides, analogous to prostaglandin endoperoxides[3,27,28] as follows:

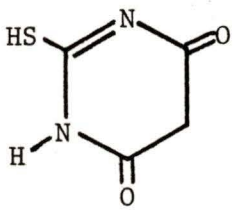


The action of heat or hydrolysis on this endoperoxide effects a retro-cyclo addition reaction, and liberates malondialdehyde (MDA), (1)



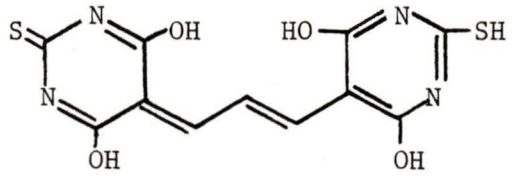
1

Historically, MDA was first detected in such peroxidized PUFA colorimetrically through its red condensation product with thiobarbituric acid (TBA), (22). This complex has an absorance maximum at 532nm,



22

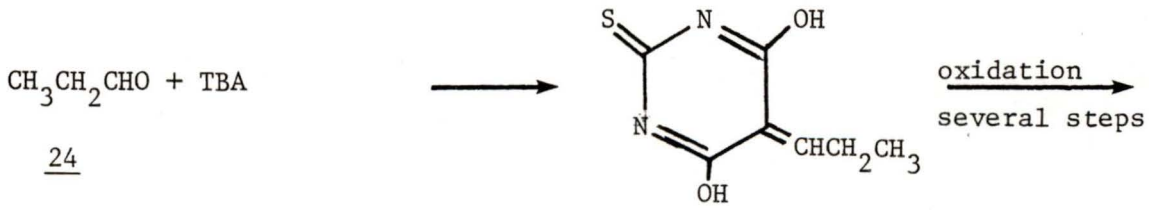
with a sufficiently high extinction coefficient to be extremely sensitive. The pigment produced is believed to have the following structure[29] (23).

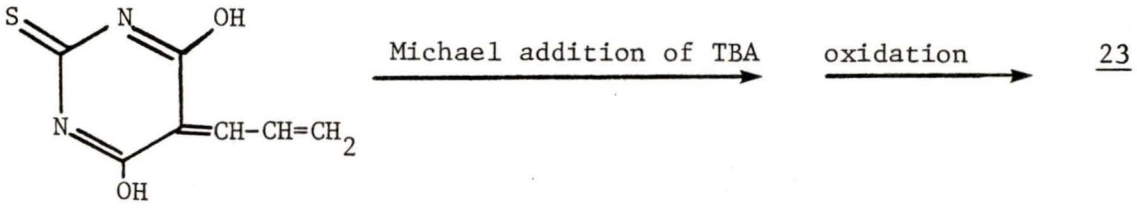


23

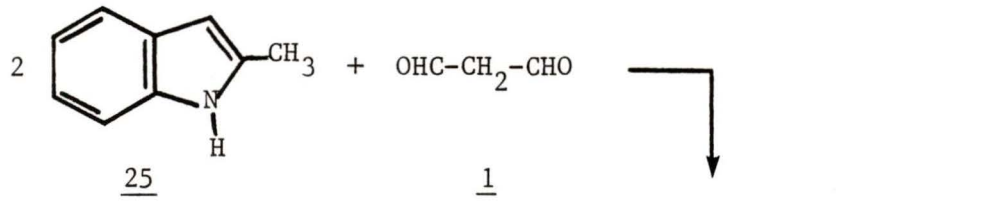
This so called TBA test has found wide use in the detection of the rancidity of fats[30] and the general freshness of foods.[31,32]

Certain factors, however, reduce the efficiency of the TBA test in the quantification of the level of PUFA oxidation in complex biological samples. Firstly, fatty acids give different yields of MDA on oxidation, and highly saturated fats give little or no MDA. Secondly, many other compounds, such as sugars, aldehydes and cyclic peroxides give a positive TBA test. Propanal (24) gives a red TBA colour if left to stand in air. It is thought to undergo oxidation and condense in the following manner:[27]

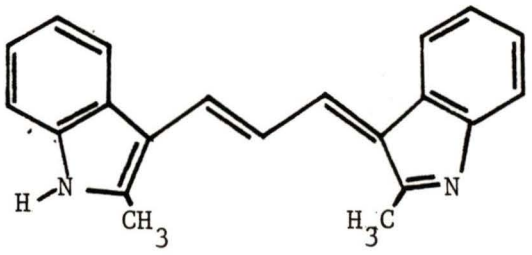




Another colorimetric test for MDA is the analogous condensation reaction with 2-methylindole (25).[33] The colored adduct absorbs at 555nm, and has the following postulated structure.



25 1



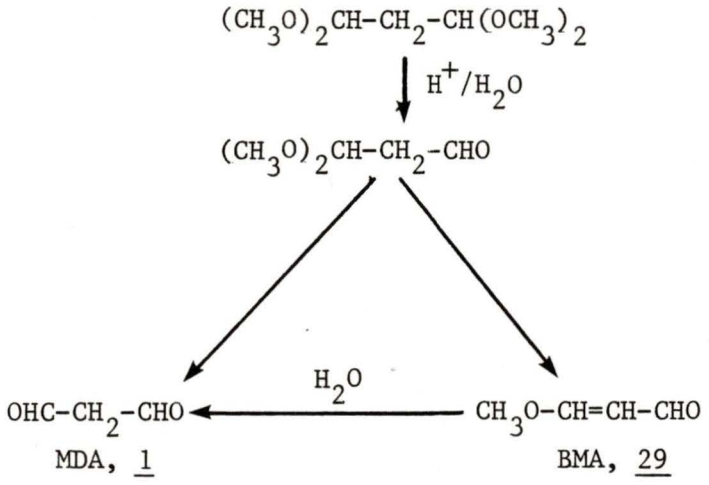
26

While this test has found some use in the detection of MDA in irradiated glucose solutions,[34] it has not been widely exploited to date. We have found 2-methylindole to be much more effective in the detection of substituted malondialdehydes.

In view of the presence of MDA in meats and foodstuffs, an estimate was needed of its potential mutagenicity. Initial studies[4,35,36] using the Ames test, a bioassay technique using the reversion characteristics of histidine-deficient Salmonella typhimurium, showed that MDA was mutagenic, but that antioxidants could reduce the observed activity. However, since pure MDA is an unstable compound,[37] fresh solutions of MDA must be prepared just prior to use. The standard method of preparation involves the acidic hydrolysis in aqueous

solution of 1,1,3,3-tetramethoxypropane (TMP), (27) with Dowex 50 (x2) resin. The supposed MDA that was formed was assayed by the TBA test. Recent research[38] though has cast doubts on the validity of this early work.

The acidic hydrolysis of TMP (or its ethoxy analogue) not only forms MDA, but also the partially hydrolysed derivatives, dimethoxypropanal (DMP), (28) and β-methoxyacrolein (BMA), (29). These are thought to be formed as follows.[38]



DMP and BMA were both assayed separately in the Ames test, and both were found to be mutagenic. BMA was 25-32 times, and DMP 22-27 times as active as a carefully prepared pure sample of the stable sodium salt of MDA. The latter proved to be only a weak mutagen.

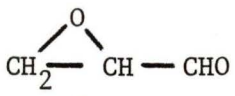
The mutagenicity of MDA and related substances have been associated with their well-established binding to DNA and proteins.[39,40] Both MDA and its homologue, glyoxal (2), have been shown to crosslink purines. In the case of MDA, the linkage has an amino-imino-propene

structure.

$$R - N = CH - CH = CH - NH - R$$

The products obtained are fluorescent, and loss of ribonuclease enzymatic activity has been correlated with the formation of these fluorescent adducts. On reaction of MDA with purines, fluorescent products with similar spectral characteristics to those of the MDA crosslinked DNA were formed.

Other compounds related to MDA in both structure and electrophilicity have also been investigated for mutagenicity. Both glycidaldehyde (30) and β-propiolactone (31) have been studied.[41]



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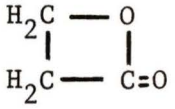


31

These compounds are structural isomers of MDA, and both have been shown to be mutagenic. 1,2-dicarbonyl compounds, such as glyoxal (2) and biacetyl (32), found in butter and beer, have also been shown to

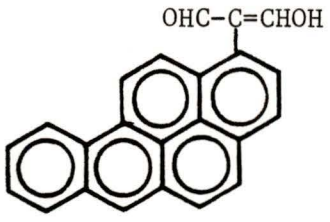
$CH_3CO.CO\ CH_3$ 32

be mutagenic. Glyoxal is quite mutagenic, biacetyl, less so. Tentative structure correlations have been attempted in these cases, suggesting that monocarbonyl compounds are inactive, α-dicarbonyls are active (to some degree), and a related dicarbonyl compound, dimethyl oxalate (33) is inactive.

$$\begin{matrix} COOCH_3 \\ COOCH_3 \end{matrix}$$ 33

4. Summary

In the present study, a series of model conjugates incorporating both an electrophilic malondialdehyde side chain, and a potentially mutagenic polynuclear aromatic ring system has been synthesised. A typical example is:



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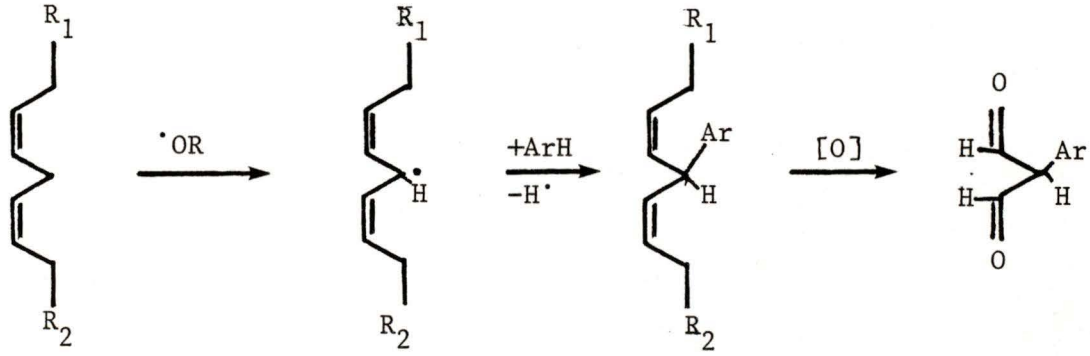
Autoxidation of lipophilic materials in the presence of PAH may give rise to conjugates of the same general structure as the model series: a potential reaction scheme is shown below.



In choosing the PAH framework, aromatic substrates ranging from the simple benzene and naphthalene to the more complex pyrene, chrysene, and benzo(a)pyrene were used. Note that the series incorporates both inactive (benzene) and highly mutagenic (benzo(a)pyrene) aromatic substrates. In view of the difference in biological activity of 1- and 2-naphthyl-derivatives (e.g. the amino compounds), 2-(1-naphthyl)-and 2-(2-naphthyl)-malondialdehydes were synthesised.

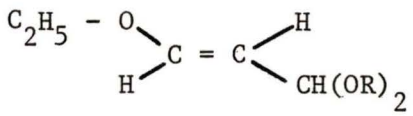
CHAPTER II

CHEMICAL RESULTS AND DISCUSSION

1. Introduction

(i) Properties of Malondialdehyde

Malondialdehyde (1), for such a simple molecule, is deceptively hard to prepare in a pure form. Hüttel,[37] described it as a hygroscopic crystalline solid that melts to form a red liquid between 72 and 74°C. On standing for two days at room temperature it gave evidence of instability by turning yellow. An aqueous solution gave red colours with Schiff's reagent and iron (III) chloride, and immediately reduced ammoniacal silver nitrate - all indicative of its readily oxidizable reactive aldehyde groups. Hüttel prepared MDA from β-ethoxyacrolein acetal (34), by hydrolysing it, neutralizing the resulting solution

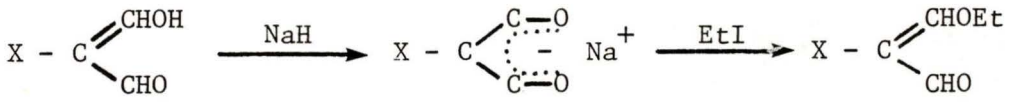


34

with sodium hydroxide (Note that MDA is a reasonable strong Brønsted acid, pKa = 4·46[42]) and subsequently isolating the sodium salt of MDA. The salt was dissolved in ether, and treated with ethereal hydrogen chloride. The ether was removed to leave MDA, which was then purified by sublimation.

The acidity of MDA allows facile conversion to the β-alkoxy-acrolein by simple treatment with base and alkyl iodide, (Scheme 1).

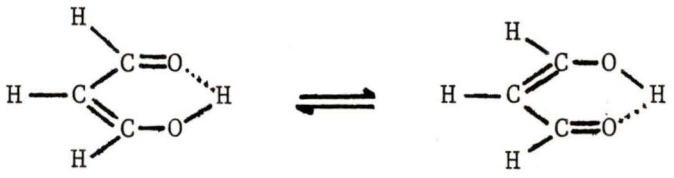
Scheme 1



35 36 37

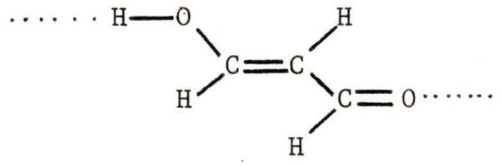
Studies on the structure of MDA are incomplete and sometimes contradictory, undoubtably due to the deterioration of the compound, possibly by polymerisation.

In the gas phase, MDA has been shown to have the following conformation.[43,44] In solution, both this intramolecular hydrogen



1a 1b

bonded form, and an intermolecular hydrogen bond have been detected by IR and NMR; the relative amounts and stabilities of the conformers depend on the polarity of the solvent.[45]

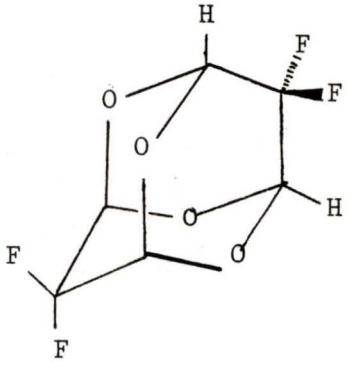


1c

Solid MDA has been studied in the temperature range 150-230 K;[46] and by analysis of the IR spectrum the open chain structure (1c) has been assigned.

(ii) 2-Substituted Malondialdehydes

The syntheses of simple 2-substituted malondialdehydes have been reported previously, including the 2-halo-, 2-cyano-, 2-methyl- and 2-hydroxy-derivatives, among others. An attempt to prepare a 2,2-difluoromalondialdehyde resulted in the production of a dimer[47] (38),

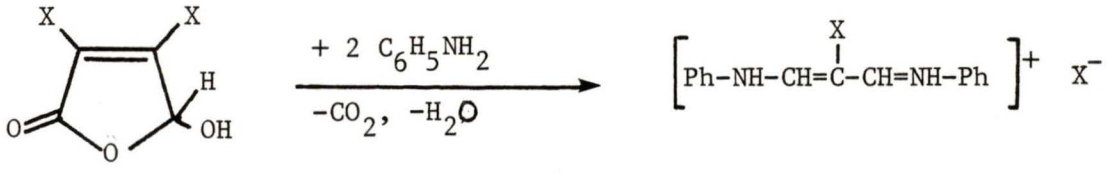


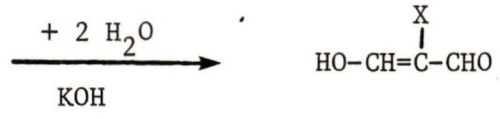
38

(Cf the stable hydrate of chloral, CCl_3-$CH(OH)_2$).

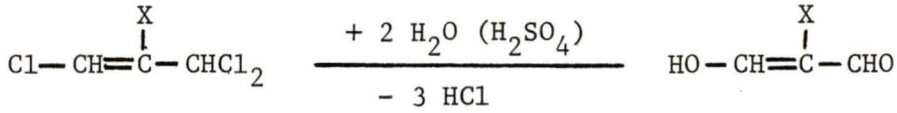
2-Chloro- and 2-bromo-MDA have been prepared from mucochloric or mucobromic acids, as shown below.[48]

X = Cl, Br





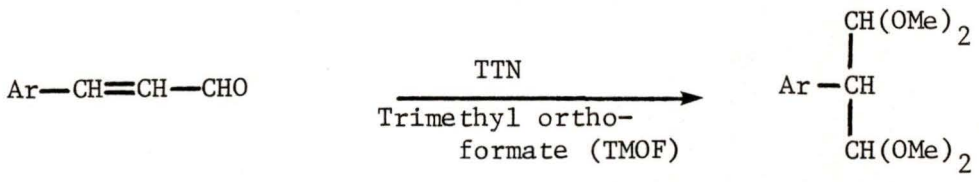
The acids react with aniline to give, after decarboxylation, the halomalondialdehyde dianil hydrogen halides. Alkaline hydrolysis of these dianils leads to the free malondialdehydes. The 2-chloro and 2-bromo MDAs are also accessible from 1,2,3,3-tetrahalo-1-propenes. Acid hydrolysis of these halides gave 2-chloro or 2-bromo MDAs.[48]



X = Cl, Br

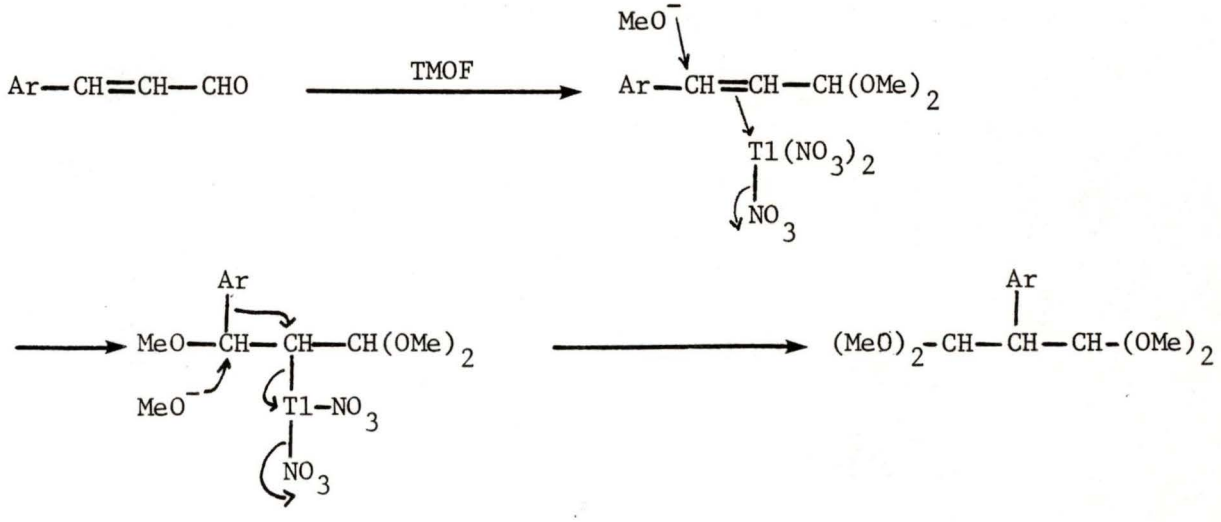
(iii) The Thallium Trinitrate Route to MDA

A relatively recently reported route to 2-substituted malondialdehydes, which we decided to investigate at first was the oxidative rearrangement of cinnamaldehydes (39) with clay supported thallium trinitrate (TTN),[49] to give 2-arylmalondialdehyde bis(dimethyl)acetal (40) directly.



39 40

The reaction proceeds (Scheme 2) by conversion of the aldehyde to the acetal, rendering the double bond more nucleophilic.[50]



An oxidative rearrangement of the aryl group proceeds rapidly to yield the 2-aryl malondialdehyde bis(dimethyl)acetal. The clay

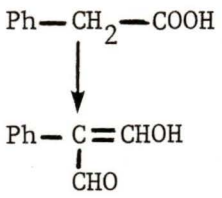
support enhances the rate of reaction by providing greater surface area for the reaction, and the presence of pores in the clay is thought to organise the reactants and lower the entropy of activation.

Unfortunately, however, our attempts to repeat the work of Taylor et al[49] did not produce the desired product (see Experimental). The source of the problem was later found to be in the TTN reagent. The original work was carried out with freshly prepared TTN (unmentioned in the literature account). Use of a commercially available product, stabilised with nitric acid, had the effect of reducing the oxidative activity of the TTN. This was only discovered on correspondence with Prof. Taylor, at which time we had already begun investigation of the route described below.

(iv) Vilsmeier Approach to MDAs

In the light of the above experimental difficulties, we turned to the Vilsmeier reaction. Basically, the Vilsmeier route[51] to 2-substituted MDA consists of two steps: preparation of the N,N-dimethylaminoacrolein from the corresponding substituted acetic acid, and alkaline hydrolysis of these acroleins to the free MDAs. 2-Cyano- and 2-fluoro- MDAs have both been prepared, beginning with cyano- or fluoroacetic acid. The cyano compound may also be prepared from acetonitrile.

Of interest in the present work is the application of the Vilsmeier reagent to arylacetic acids or diethoxyalkanes to prepare aryl or alkylmalondialdehydes respectively.



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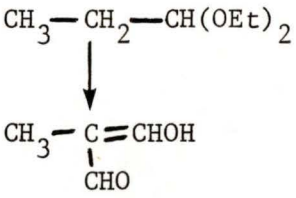
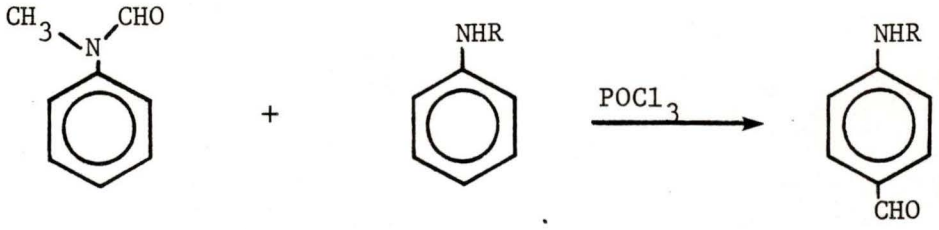
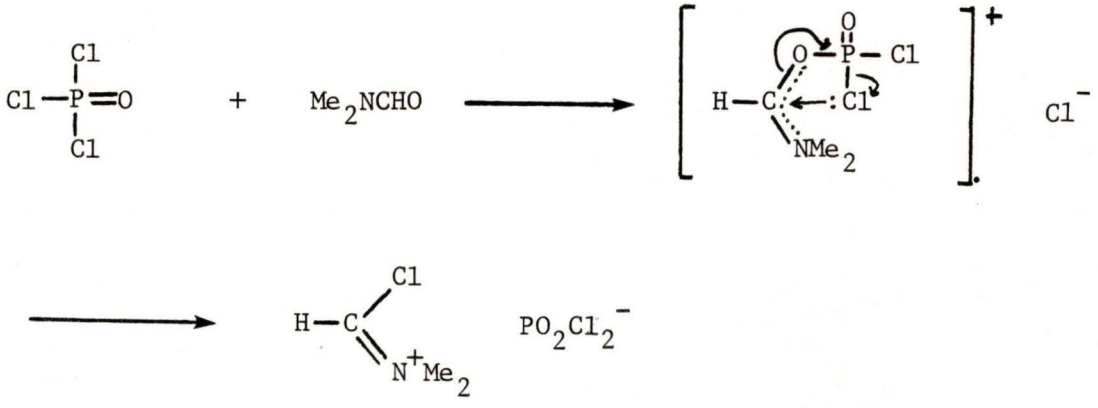


42

Historically, the Vilsmeier reaction was a special type of Friedel-Crafts reaction[52] using an amide in conjunction with acid chlorides, especially phosphorous oxychloride, to prepare aromatic aldehydes.[52]

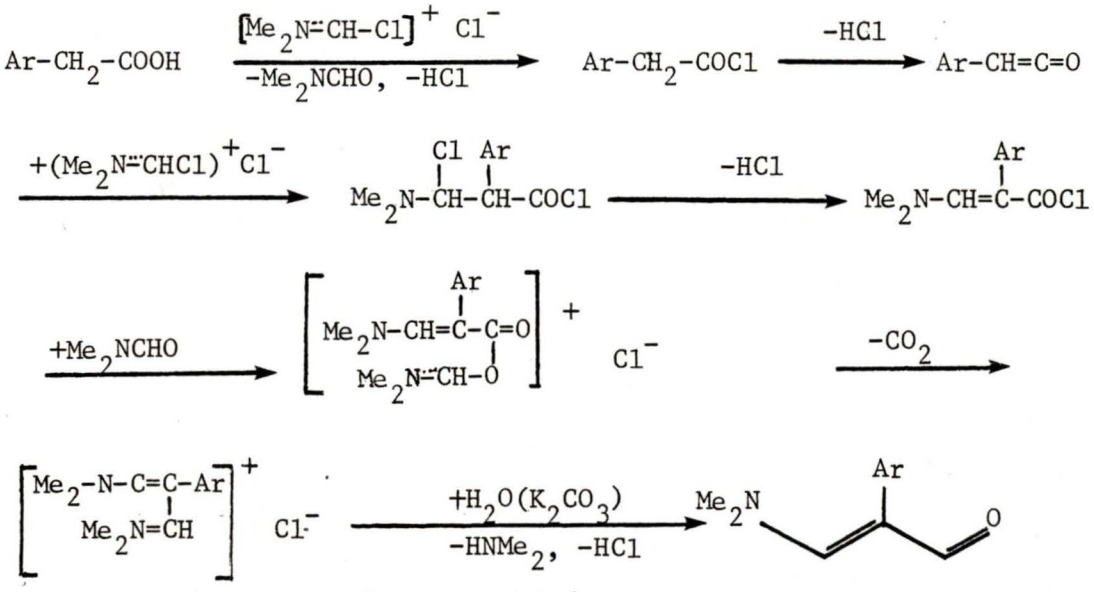


More generally phosphorus oxychloride reacts with amides such as dimethyl formamide (DMF) to give salt like adducts.

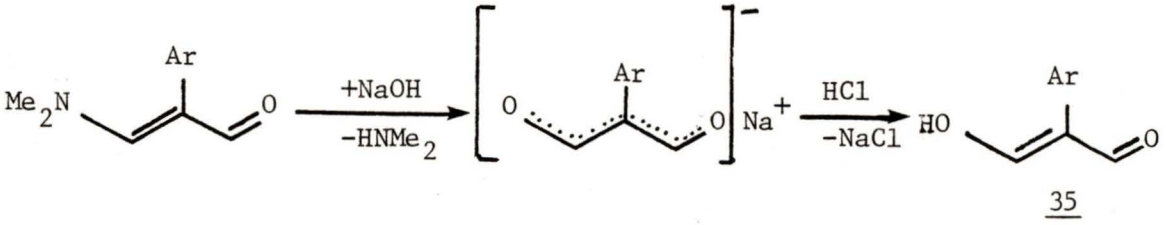


These adducts are then reacted with the substituted acetic acids. To prepare the simple alkyl MDAs, e.g. 2-methylmalondialdehyde, the 1,1 -diacetals are used.

The mechanism of the reaction is poorly understood, but believed to follow the Scheme below.[53]



The initial product, a 2-substituted dimethylaminoacrolein (43) may be isolated, and then hydrolysed under basic conditions to the 2-substituted MDA.



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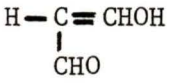
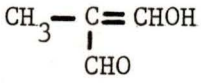
The malondialdehydes prepared by this route in the current work are shown in Figure 6.

Figure 6. The Structures of 2-Substituted Malondialdehydes: Goals of the Current Study.



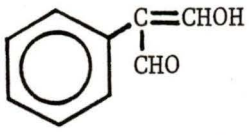
Malondialdehyde

1



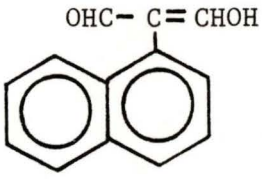
2-Methylmalondialdehyde

42



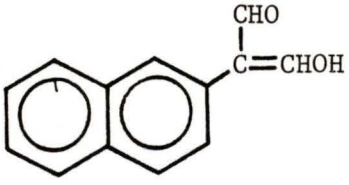
2-Phenylmalondialdehyde

41



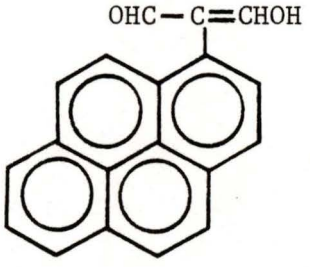
2-(1-Naphthyl)malondialdehyde

44



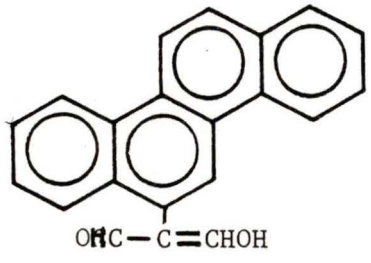
2-(2-Naphthyl)malondialdehyde

45



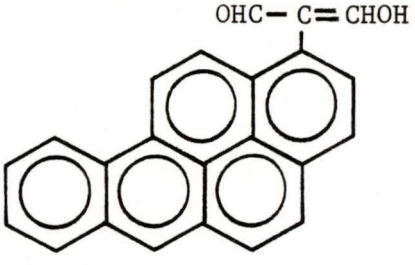
2-(1-Pyrenyl)malondialdehyde

46



2-(6-Chrysenyl)malondialdehyde

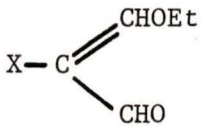
47



2-(1-Benzo(a)pyrenyl)-malondialdehyde

4

Significantly, sodium malondialdehyde may be prepared by this route, starting from 1,1-diethoxyethane, and this method does not incur the hydrolysis by-products formed when MDA is produced by acidic hydrolysis of TMP. Such hydrolysis leads to DMP, (28) and BMA, (29). Unfortunately, these two compounds are the actual mutagens, detected by the Ames test on crude MDA, and great confusion has arisen in the literature from the use of raw hydrolysates. The Vilsmeier method presents a clear improvement in obtaining pure MDA for biological studies. Indeed, since ultimately the BMA has proved to be more mutagenic than MDA, we have pursued the synthesis and assay of this latter class of derivatives as well, (e.g. (37)) to give a clear picture of the



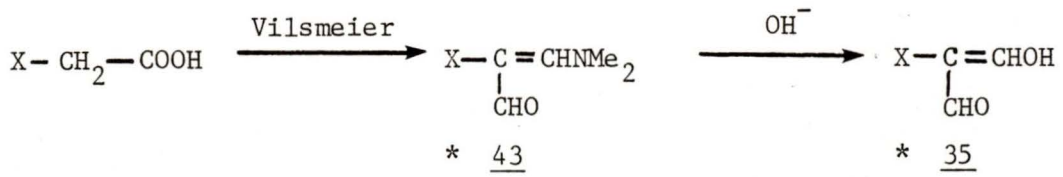
37

effect of 2-substituents on biological activity.

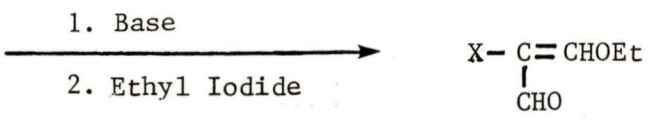
(v) Present Work

Ultimately, the Vilsmeier route has been used to prepare the derivatives shown in Figure 6 via the corresponding dimethylamino-acroleins. The synthetic relationships of the MDAs and related materials that have been synthesised and tested are shown below. The three highly electrophilic and potentially mutagenic derivatives are

indicated by an asterisk:



* 43 * 35

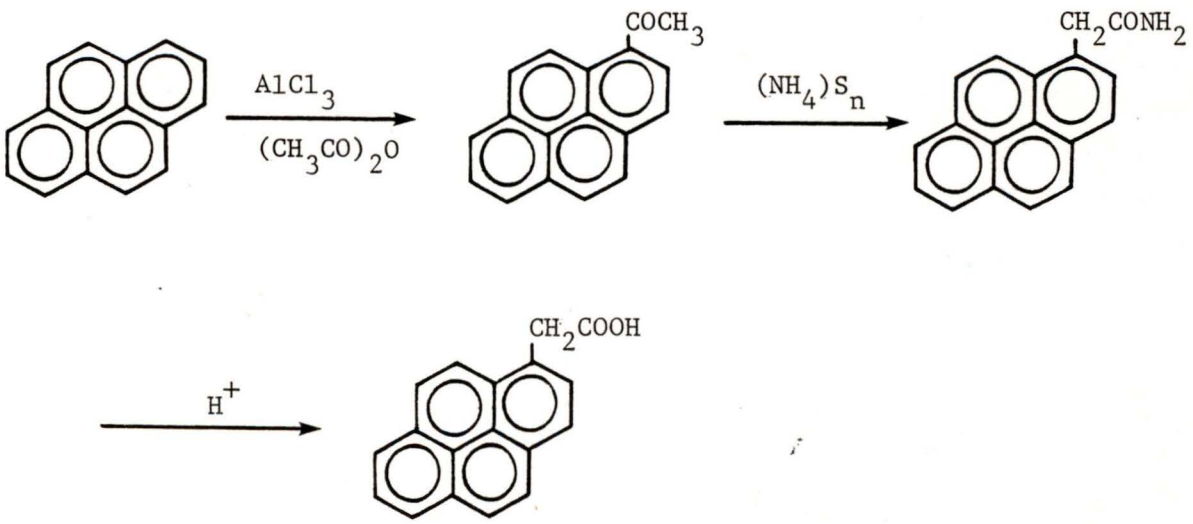


* 37

The compounds (41), (44) and (45) were synthesised from the commercially available acetic acid derivatives.

However, for pyrene, chrysene, and BaP, the arylacetic acids must be prepared from the parent hydrocarbon. An answer to this synthetic challenge was found in the literature[54] as a synthetic route to 1-pyrenylacetic acid. Once the suitability of this route had been proven for pyrene, it was extended to chrysene and BaP.

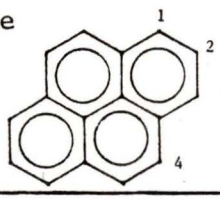
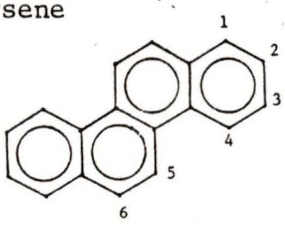
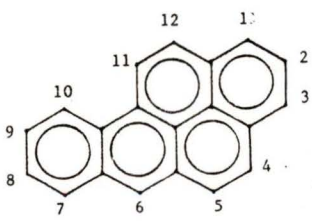
Basically, the route was a three step synthesis of the acetic acid, as shown below.



The initial step is a Friedel-Crafts acetylation of the pyrene nucleus. This is an electrophilic substitution at the position of the nucleus which has the greatest free valence.[55] Conceptually, a numerical form is given to the abstraction of residual π bonding.

The greater the π bonding of one position of the ring to adjacent positions, the less π bonding capacity remains to accomodate an attacking regent. Some sample values of this free valence value (Fr) are shown below (Table 2).

Table 2. Calculated Values of Reactivity Index (Fr) For Selected Hydrocarbons.

Hydrocarbon	Position	Fr
Benzene	1	0.399
Naphthalene	1 2	0.453 0.404
Pyrene 	1 2 4	0.468 0.393 0.452
Chrysene 	1 2 3 4 5 6	0.451 0.403 0.408 0.442 0.440 0.457
Benzo(a)pyrene 	1 2 3 4 5 6 7 8 9 10 11 12	0.472 0.394 0.470 0.455 0.455 0.530 0.459 0.403 0.413 0.442 0.440 0.458

From Table 2, it can be seen that pyrene has the greatest Fr value at the 1-position, consistent with acetylation at this position experimentally. Theory predicts substitution at the 6-position for both chrysene and BaP. Experimentally, 6-acetylchrysene is formed. However, in BaP, the 6-position is a hindered 5-anthracene type position, hence acetylation occurs at the next most reactive site, the relatively less hindered 1-position.

The Friedel-Crafts step, which goes in high yield is followed by the conversion of the ketone to the corresponding acetamide by ammonium polysulphide.

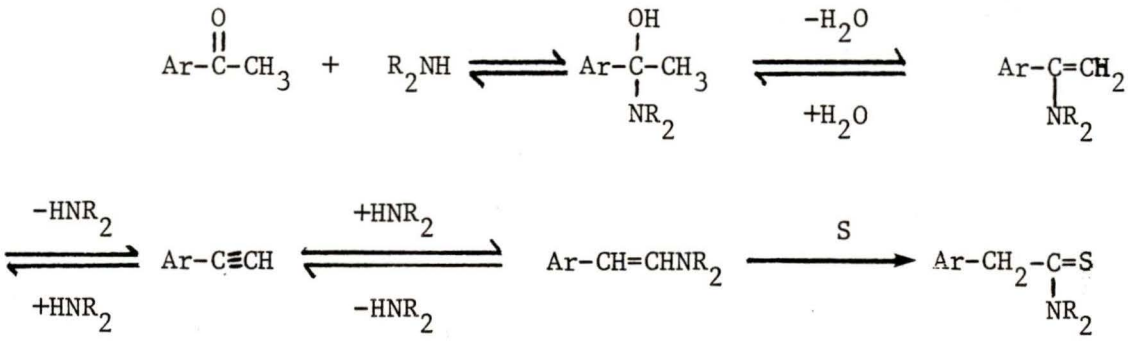
$$X - CO - CH_3 \xrightarrow{(NH_4)S_n} X - CH_2 - CONH_2$$

This process, the Willgerodt reaction, was first reported for acetophenone in 1887.[57] The procedure has been applied to other aryl ketones with varying degrees of success.

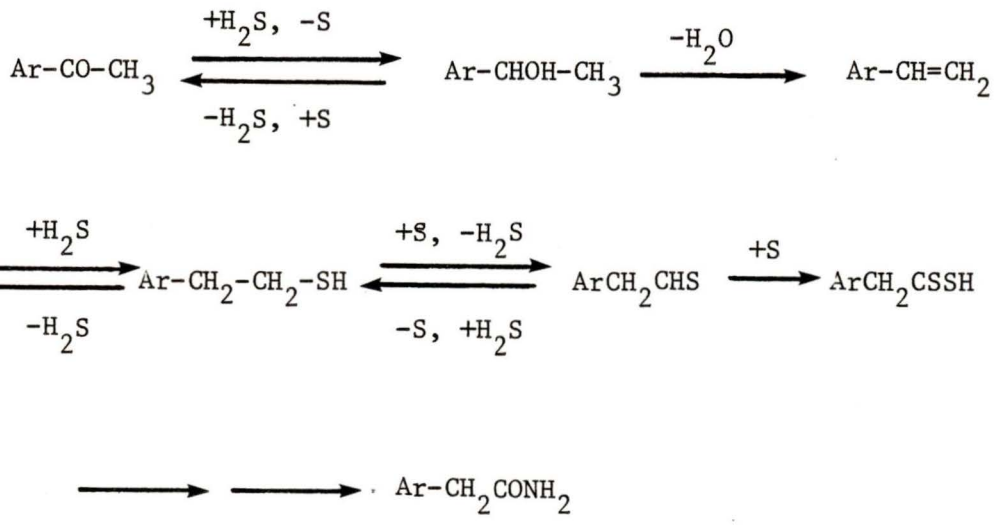
Methyl ketones provided the highest conversion in the Willgerodt reaction, with decreasing yield for each additional methylene (CH_2) group added, i.e. for ethyl, propyl, and higher homologues. In all cases the amide is produced at the terminal carbon atom.

$$Ar\text{-}CO\text{-}CH_2CH_2CH_3 \xrightarrow{(NH_4)S_n} Ar\text{-}CH_2CH_2CH_2CONH_2$$

The above result might imply a rearrangement of the side chain, but studies with branched side chains and radiolabelled acetophenone[57] show no skeletal rearrangement. In consequence several mechanisms have been postulated. One of the first was that of Carmack (Scheme 5).



Another mechanism has been proposed by King and McMillan (Scheme 6).

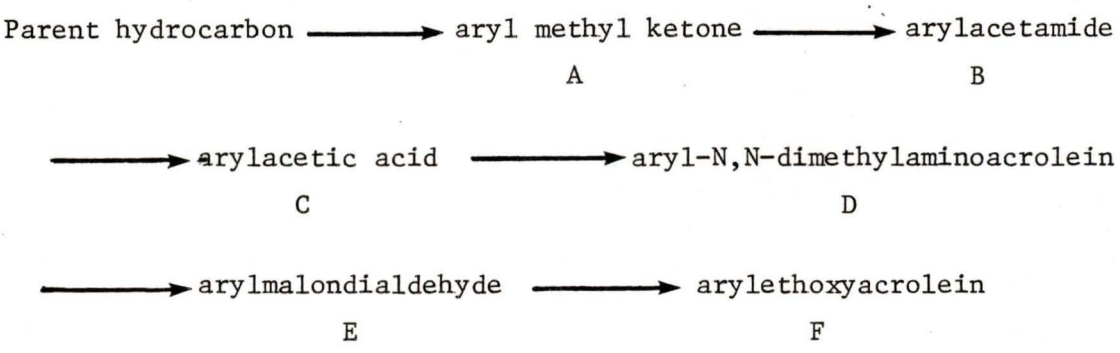


Once the amide was obtained, a simple acidic hydrolysis provided the aryl acetic acid in high yield. Thus the starting materials for the Vilsmeier reaction were then in hand.

2. RESULTS

The overall sequence incorporating both arylacetic acid synthesis and conversion to subsequent products can be summarized (Scheme 7) with the main compounds designated A - F. Characterization and modification of existing methods are outlined in the following sections for each of these intermediates.

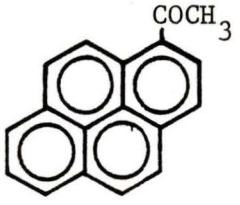
Scheme 7



A Aryl methyl ketones

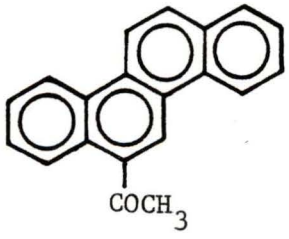
Acetylation of aromatics is a standard reaction, well documented in the literature. However, considerable variations in experimental difficulty are encountered, depending on choice of solvent.

In the present work, the well known compound 1-acetylpyrene (48) was first synthesised by the literature method.[54] This employed



48

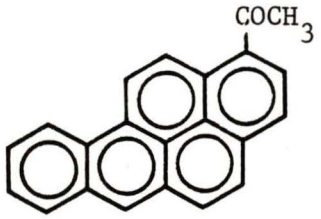
aluminium trichloride as the Friedel-Crafts catalyst, and acetic anhydride as the acetylating agent. Subsequently, chrysene was acetylated with acetyl chloride and aluminium trichloride in dichloromethane (according to literature)[59] to give 6-acetylchrysene (49).



49

Use of dichloromethane as a solvent gave, in our hands, advantages over the use of nitrobenzene. The ease of removal of dichloromethane offered a reduced preparation time for 1-acetylpyrene. Finally, the

literature method for BaP[60] (50) used the alternative easily removed but more hazardous solvent, carbon disulphide.



50

Assignment of the position of substitution in polynuclear aromatics was made historically by laborious derivativization and comparison. Currently, use of high field NMR, with knowledge of chemical shifts and splitting constants affords much easier identification.

In their ^{1}H NMR spectra, all three compounds show a sharp singlet at 2.85 ppm* for the methyl ketone. 1-Acetylpyrene (48) shows a 6H multiplet in the aromatic region (7.91-8.18 ppm) (see Figure 7), a doublet at 8.26 ppm from H-2 (J=8.1 Hz), and a doublet at 9.02 ppm for the phenanthrenoid H-10 (J=9.5 Hz), which falls within the carbonyl deshielding cone.

6-Acetylchrysene (49) has 5 distinct groups in the aromatic region of the ^{1}H NMR spectrum (Figure 7). H-2,3,8, and 9 show as a multiplet at 7.63-7.71 ppm. H-1 and -12 correspond to a multiplet between 7.81 and 7.95 ppm. H-10, a phenanthrenoid 1,10 type position, is a doublet at 8.49 ppm, the other two phenanthrenoid hydrogens, H-4 and -10 and the deshielded H-7 occur as a multiplet, at 8.59-8.78 ppm.

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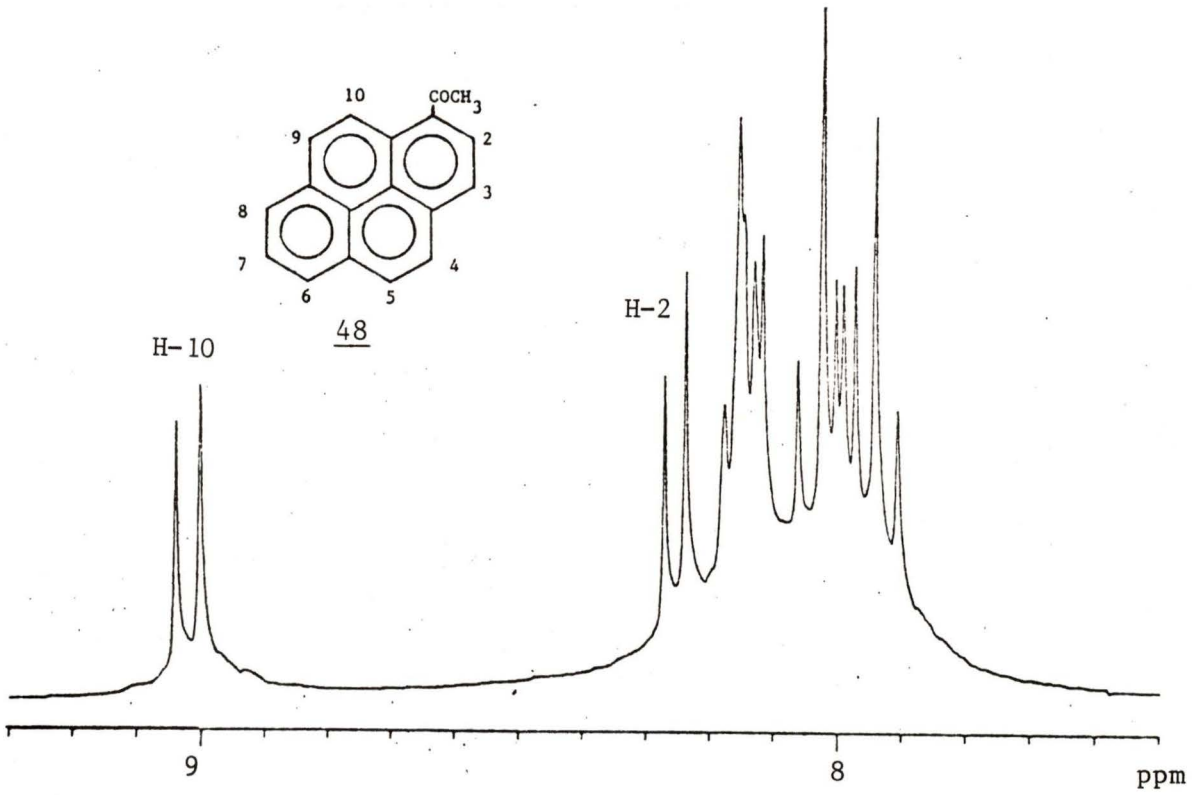
* All chemical shifts are given in ppm downfield from tetramethylsilane as standard.

Figure 7. The Aromatic Region of the ^{1}H NMR Spectra of the Aromatic Substituent in Compounds 48, 49, and 50.



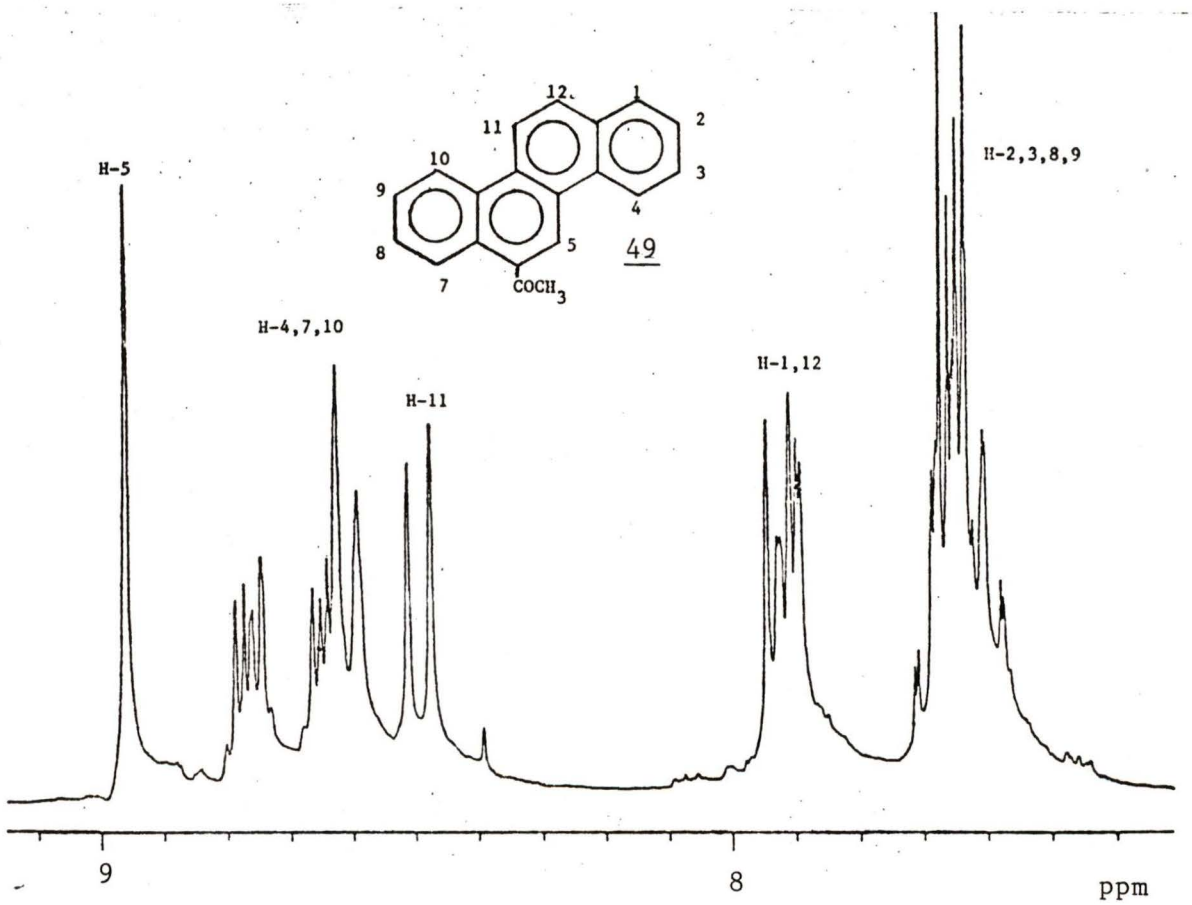
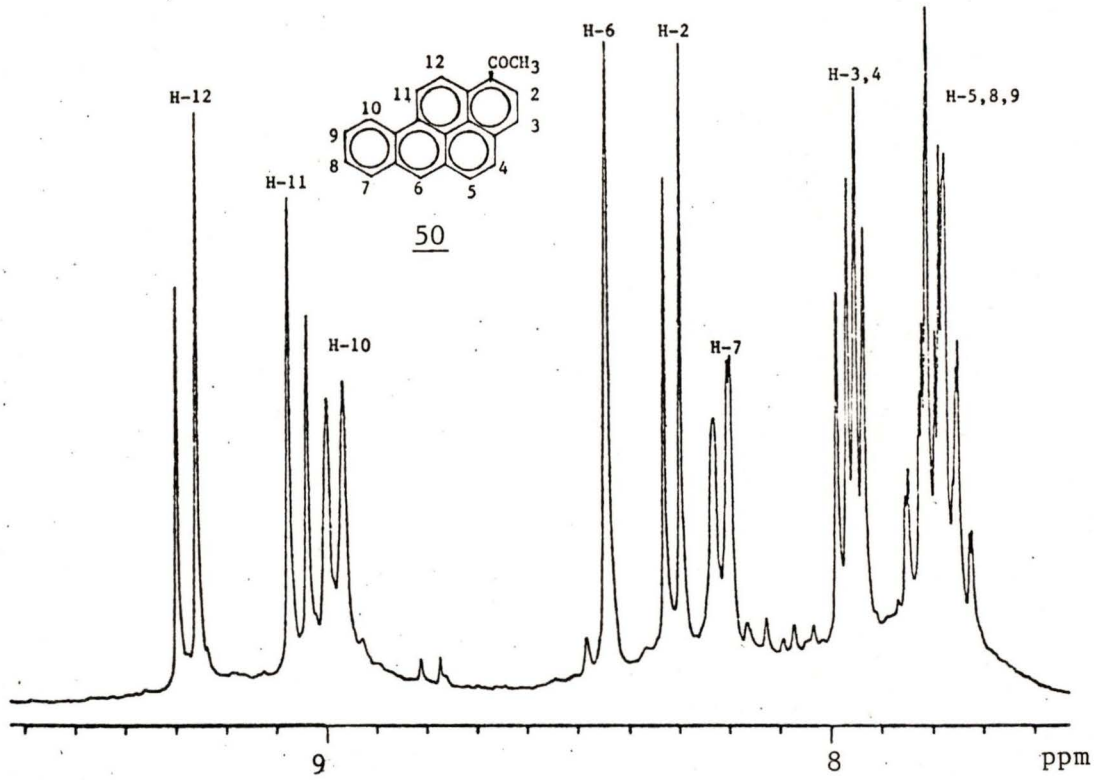


Figure 7 continued.



50

The strongly deshielded H-5 is a singlet at 8.96 ppm.

1-Acetylbenzo(a)pyrene (50) has 8 aromatic groupings (Figure 7). The 3H grouping of H-8,9 and H-4 or 5 is between 7.72 and 7.87 ppm, with H-5 or 4 and H-3 as a multiplet between 7.93 and 7.98 ppm. H-7 occurs as a multiplet between 8.19 and 8.22 ppm, H-2 as a doublet at 8.31 ppm. H-6 is a singlet at 8.44 ppm. The phenanthrenoid H-10 is a doublet at 8.98 ppm. H-11 and -12 are assigned to doublets at 9.05 and 9.28 ppm, which may be interchangeable.

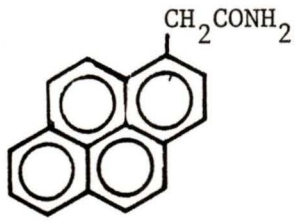
In each case ^{13}C NMR obtained show a methyl carbon at ca. 30 ppm ((48), 30.27; (49), 30.11; (50), 30.17 ppm) and the correct numbers of aryl CH and quaternary aryl carbon centres. In addition, each show a peak from the carbonyl group ((48), 202; (49), 201.91; (50) 201.50).

B Arylacetamides

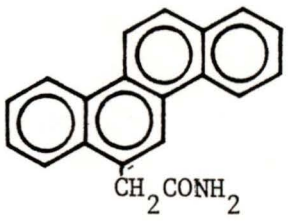
Experimentally, the Willgerodt reaction of the aryl ketones with ammonium polysulphide is carried out at 160°C in dioxane,[54] generating a significant internal pressure in the sealed system. While the initial experiments with 1-acetylpyrene employing Carius tubes were made without incident, later runs with both 1-acetylpyrene and 6-acetylchrysene repeatedly exploded in the muffle furnace. Accordingly, a procedure was developed where the Carius tube was placed in the cylinder of a high pressure hydrogenator which contained dioxane. No further explosions occurred when the hydrogenator was raised to 160°C. It is recommended that this pressure compensation procedure approach be used when volatile and corrosive reagents and solvents are to be heated at elevated temperatures.

With this revised technique in hand, the successful synthesis of 6-chrysenylacetamide and 1-benzo(a)pyrenylacetamide in 87 and 77% yields respectively were achieved. Both of these compounds are previously unreported.

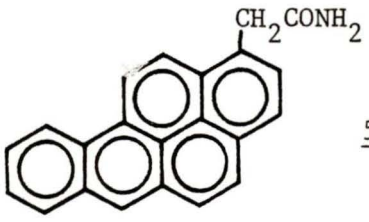
The ^{1}H NMR spectra of these acetamides show a 2H singlet



51



52



53

at ca. 4.2 ppm from the CH_2 group ((51), 4.21 ppm; (52), 4.10 ppm; (53), 4.20 ppm). Due to restricted rotation, the NH_2 protons in the amide group are observed as a pair of broad singlets. Both the pyrene and benzo(a)pyrene showed the NH protons clearly separated at 7.1 and 7.7 ppm, while the chrysene acetamide had an overlap of the NH_2 and aromatic protons. However, the NH_2 protons were exchanged on addition of D_2O to the solution in d_6-DMSO, though reduced solubility caused some precipitation. As expected, each acetamide showed aromatic proton regions similiar to the corresponding acetyl compounds.

As further confirmation of the amide functionality, the ^{13}C NMR showed the CH_2 carbon at 54.71 ppm in 6-chrysenylacetamide, and at 40.05 ppm in 1-benzo(a)pyrenylacetamide. The corresponding carbonyl carbon peaks are at 172.06 and 172.07 ppm respectively. Both compounds

showed appropriate aryl CH carbons, the chrysene compound clear quaternary carbons, while the presence of an impurity made assignment of quaternary carbons in the benzo(a)pyreneacetamide difficult.

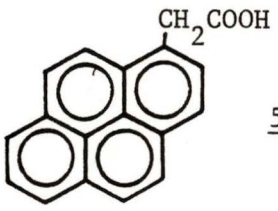
The IR specta all show a broad band at ca. 3400 cm^{-1}, and a sharp band at ca. 1600 cm^{-1}, from the NH_2 and CO groups respectively.

The CI mass spectral data obtained were consistent with the expected molecular weights for the acetamides.

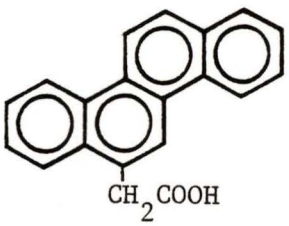
C Arylacetic Acids

Either basic or acidic hydrolysis of amides may be used to prepare arylacetic acids.

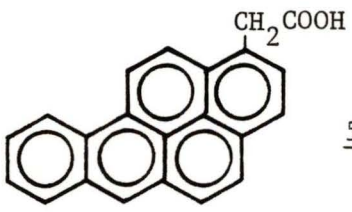
In this work, the acidic hydrolysis of the acetamides proceeded smoothly, following the procedure used to prepare 1-pyrenylacetic acid (54) by Bachmann and Carmack.[54] The only minor difficulty encountered was in the hydrolysis of 1-benzo(a)pyrenylacetamide which is less soluble than the pyrene and chrysene analogues, necessitating a four-fold increase in the glacial acetic acid solvent. While 1-pyrenylacetic acid has been previously reported, both 6-chrysenyl (55) and 1-benzo(a)-pyrenylacetic acids (56) are new compounds.



54



55



56

In all cases the ^{1}H NMR data obtained show a sharp singlet from the CH_2 group ((54), 4.20 ppm; (55), 4.40 ppm: (56), 4.55 ppm) and the expected aromatic proton groupings. The acidic hydrogen was detected at room temperature in 6-chrysenylacetic acid (13.6 ppm) as a broad singlet. In the other compounds, the OH could not be seen and the d_6-DMSO solvent used precluded a variable temperature search. The ^{13}C NMR spectrum was obtained for 6-chrysenylacetic acid, which showed the CH_2 carbon at 40.50 ppm, the carbonyl carbon at 172.10 ppm, and the correct numbers of aryl CH and quaternary carbons. The sparing solubility of the benzo(a)pyrene analogue, even in DMSO, precluded a satisfactory ^{13}C NMR spectrum.

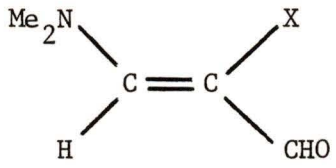
The IR spectra of (55) and (56) were consistent with acidic functions, having a broad peak in each case above 3000 cm^{-1} and a sharp carbonyl peak at about 1690 cm^{-1}.

The mass spectrum data obtained agreed with the calculated molecular weights.

D Derivatives of Malondialdehyde: β-N,N-Dimethylamino-and β-Ethoxy-acroleins

i) β-N,N-Dimethylaminoacroleins

The initial products isolated from the reaction of the Vilsmeier reagent with arylacetic acids or 1,1-diethoxyethanes are the N,N-dimethyl-aminoacroleins (43):



43

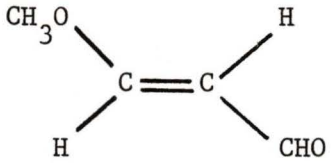
Experimentally, the elementary members of the series (the unsubstituted and methyl compounds, (57), X=H, (58) X=CH_3) are prepared straightforwardly in modest yields of ca. 30% after 1 hour reaction time.[61] The procedure recommended when X=aryl was a reaction time of 18 hrs[51] and the yields obtained were about 80%.

However, in our hands, lower yields were obtained in the synthesis of the higher members of the series (pyrene, chrysene, and benzo(a)pyrene) and thus a modified work-up procedure was developed. Since sodium hydroxide solution at 50°C will cause some hydrolysis of the N,N-dimethylaminoacrolein to the malondialdehyde, cold, more dilute sodium hydroxide solution was tried, and indeed this raised the yield from 50 to 70%, especially in the case of the chrysene and benzo(a)pyrene derivatives.

The unsubstituted, the 2-methyl-, and the 2-phenyl- (59) dimethylaminoacroleins are all oils, but give single spots on TLC using silica gel. Hence in routine preparation of the subsequent malondialdehydes, they were not further purified.

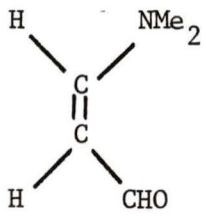
In view of the interest in the unsubstituted compound as an analogue of the demonstrated mutagen, β-methoxyacrolein (29), the

unsubstituted dimethylaminoacrolein (57) was purified by distillation and subjected to Ames testing.

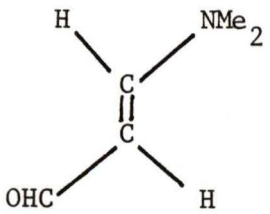


29

The naphthyl analogues (60) and (61) are both sharp melting after recrystallisation from cyclohexane, and have melting points of 94-96°C and 130-132°C respectively. The pyrene (62), chrysene (63) and benzo(a)pyrene (64) derivatives were more difficult to crystallise and the first two showed melting points of 119-122 and 128-136°C respectively. Structurally, it may be noted that the dimethylamino-acroleins may exist as diastereomers, eg:



57a (Z)



57b (E)

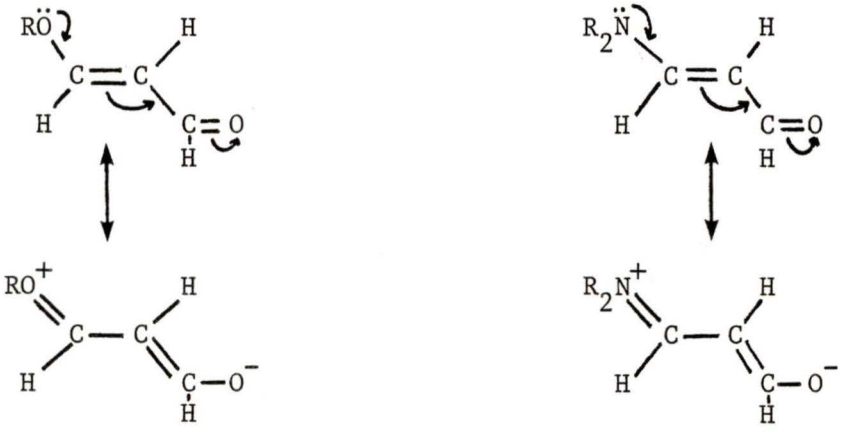
(see section D iii, pg. 53). Although the aryl compounds that we synthesised all gave single spots on TLC, and single peaks on HPLC under the conditions we tried, this does not prevent these compounds existing as a mixture of isomers.

The N,N-dimethylaminoacroleins all had appropriate MH^+ peaks in the methane CI mass spectrum.

ii) β-Ethoxyacroleins

Since the unsubstituted β-methoxyacrolein (29) is known to be a more significant mutagen than free MDA, it was possible that only the alkoxy compounds would give positive Ames response over the entire series. Therefore an attempt was made in each example to convert the malondialdehyde to the O-alkyl derivative. However, lack of sufficient malondialdehyde starting material after mutagen testing requirements were met did not allow synthesis of the ethoxy compound in the chrysene and pyrene series.

The N,N-dimethylamino and alkoxy groups are comparable electronically, and it was of interest to see if their mutagenic potential would be similar.

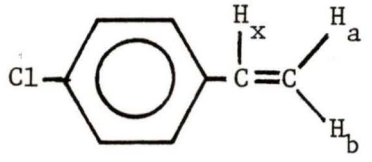


The methoxy derivative of malondialdehyde (BMA), (29) was prepared by hydrolysis of tetramethoxypropane (TMP), (27), (see pg. 24).[38] Separation of BMA from starting material was difficult, though greater than 90% purity was obtained by column chromatography over silica gel twice.

The remaining compounds were prepared by a standard Williamson synthesis using ethyl iodide and the alkoxide generated by sodium hydride treatment of the malondialdehyde.[51] In our hands, BMA, the 2-methyl (65), 2-phenyl (66), and 2(2-naphthyl) (67) compounds were obtained as oils, while the 1-naphthyl (68) derivative was a solid. However, both mass spectra and analyses were again satisfactory.

iii) Structural Assignment of β-Ethoxyacroleins and β-N,N-Dimethyl-aminoacroleins.

It is well established that in compounds such as the styrene (69) the trans coupling constants for protons Hx, Hb is large compared to the cis and gem values. In p-chlorostyrene (69) the coupling constants are as follows:



69

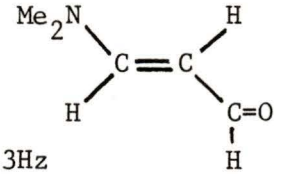
J_{bx} = 18 Hz (trans)
J_{ax} = 11 Hz (cis)
J_{ab} = 2 Hz (gem)

In addition, a hydrogen syn to an aryl group experiences some of the deshielding associated with the ring current. In the above example, Hb has a chemical shift of 5.7ppm, while Ha being trans, and therefore further from the ring, has a value of δ = 5.3ppm.

This information is of value in interpreting the data obtained for the β-dimethylaminoacroleins and ethoxy acroleins.

In the proton NMR, the β-N,N-dimethylamino compounds show as a 6H broad singlet at ca. 3 ppm from the dimethylamino group. Clearly, restricted rotation is expected[62] and in the case of the pyrene derivative (62), the slow limit spectrum with two distinct methyl resonances is obtained. Each compound also shows an aldehydic and vinylic proton.

In the unsubstituted β-N,N-dimethylaminoacrolein (57) the E configuration has been assigned[63,64] on the basis of coupling constant data.



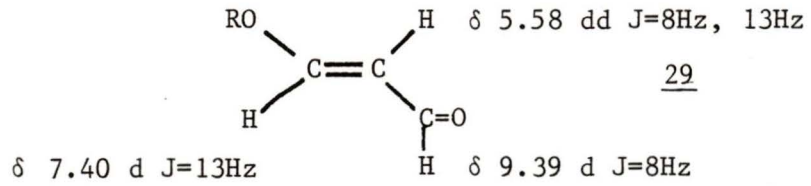
δ 5.02 dd J=13Hz,8Hz

57

δ 7.14 d J=13Hz

δ 9.03 d J=8Hz

Our data obtained for BMA is also consistent with an (E) configuration confirming an early assignment made on an acetal hydrolysis mixture.[65]



29

A summary of chemical shift data for the vinylic and aldehydic protons in substituted β-N,N-dimethylamino- and β-alkoxyacroleins appears below.

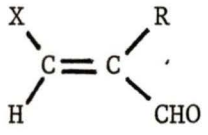
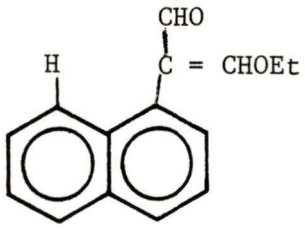


Table 3. Chemical Shifts of the Aldehydic and Vinylic Protons in the Substituted β-Dimethylamino- and β-Ethoxyacroleins.

X	R	Number	δ H-Cx=	δ CHO
NMe_2	H	57	7.14	9.03
	Ph	59	6.84	
	1Nap	60	7.02	9.24
	2Nap	61	6.84	9.11
	Py	62	7.31	9.01
	Chry	63	7.22	9.34
MeO	H	29	7.40	9.30
EtO	Me	65	6.90	9.26
	Ph	66	6.97	9.26
	1Nap	68	7.43	9.59
	2Nap	67	6.93	9.19

The gross chemical shift of the vinyl proton at ca. 7 ppm suggests the aryl group is trans to it, and thus (E) isomers are formed. This vinylic proton appears to become more deshielded as the aryl system becomes larger. This may be due to different conformations or rotamers about the aryl-ethylene bond. We note that 1-naphthyl type substituents have larger chemical shifts for the vinylic proton than do the 2-naphthyl or phenyl cases. This is consistent with the larger chemical shift for 1-naphthylethoxyacrolein (68).



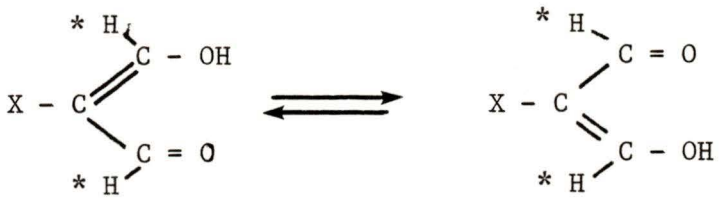
68

Clearly, peri-interactions[66] in the 1-naphthyl cases may create different steric demands.

E Malondialdehydes

Synthesis of the series of malondialdehydes by hydrolysis of the N,N-dimethylaminoacrolein precursors showed no apparent differences in the course of the reaction, however the yields varied widely in each case. The initially isolated sodium salt of each malondialdehyde was immediately converted to the free dialdehyde. The unsubstituted MDA was used as the salt.

The ^1H NMR spectra of all the malondialdehydes show two characteristic resonances.



35a 35b

A peak observed at ca. 8.6 ppm arises from the two starred protons, which are equivalent as a result of the rapid interconversion of the two tautomers. The remaining enolic hydrogen is observed at ca. 12 ppm, and is exchanged in D_2O as expected.

As anticipated for a dynamic effect, lowering the temperature allowed observation of the approach of the slow limit spectrum. In the case of the phenyl (41) and naphthylmalondialdehydes (44) and (45), greater resolution was obtained in the observance of the OH proton, as exchange with bulk solvent slows down. On the other hand, at $-60^{\circ}C$, as the slow limit is approached, the starred protons start to broaden, and begin to appear as two discrete resonances. In the case of the pyrene (46) and chrysene (47) derivatives, however, reducing the temperature also reduced the solubility, with a corresponding loss of signal strength.

The two equivalent protons appear at a position in accordance with the averaging of an aldehyde and vinylic proton. The data for these protons is presented below.

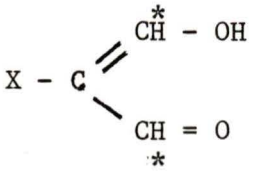


Table 4. Chemical Shifts of the Enolic Protons in 2-Subsituted Malondialdehydes.

X	Number	*H ppm
H	1	8.38
Me	42	8.37
Ph	41	8.61
1Nap	44	8.51
2Nap	45	8.78
Py	46	8.63
Chry	47	8.61

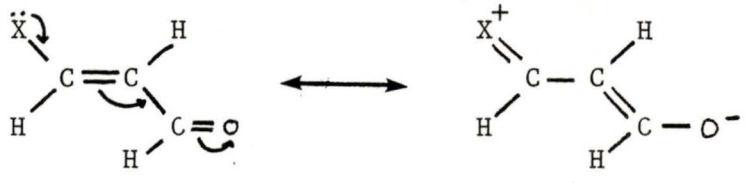
Note the enhanced deshielding of the averaged protons in the aromatic substituted compounds. The rest of the ^{1}H NMR spectra were in accord with the substitution pattern of the individual aromatic rings.

The "keto-enol" fluxionality is also observed in the ^{13}C NMR as illustrated in Table 5, by the chemical shift observed for the CHO carbons, which are at lower shift than for a normal aldehyde (e.g. CH_3CHO at 201 ppm), and at higher shift than at a CHOH carbon expected to be at ca. 155 ppm). Good correspondence between chemical shifts of similar types of carbon atoms is obtained in each series.

Table 5. ^{13}C NMR Resonances of 2-Substituted Malondialdehydes and Derivatives: Chemical Shift δ of Carbons.

	Malondialdehyde		Dimethylamino acrolein			Ethoxy acrolein		
	CHO	XC(CHO)$_2$	CHO	XC=CNR	CNR$_2$	CHO	XC=COR	=C-OR
Me	179.60	119.61						
Ph	181.08	118.36						
1NAP	181.96	116.68	189.35		158.81	190.29	123.09	167.96
2NAP	181.29	118.30	189.29	114.94	158.3	194.25		168.85
Py	189.29		189.51					
Chry	181.94	117.3	189.57					

A further indication of the bifunctionality of these molecules is seen in the infra red spectra. The characteristic stretch of the carbonyl group in both ethoxyacrolein and dimethylaminoacrolein reflects the loosening of the vibration by extensive interaction with the adjacent π system.

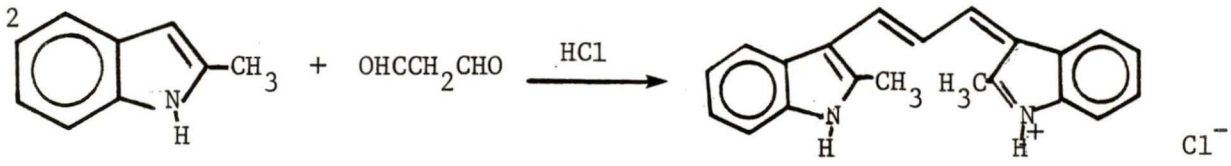


In accordance with this, the internally H bonded carbonyls of the malondialdehyde are loosened even further, and appear at ca. 1570cm^{-1}.

While excellent spectoscopic evidence was obtained to establish the structure of the compounds up to chrysene in complexity, characterization of the BaP series was hampered by insolubility and instability.

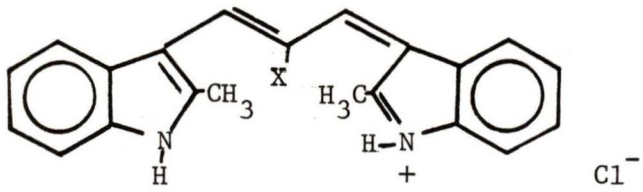
Condensation Reactions of Malondialdehydes

Condensation of 2-methylindole (25) occurs with MDA (1) to give an intensely coloured species (26) with a λ max at 555nm (ε=24000).



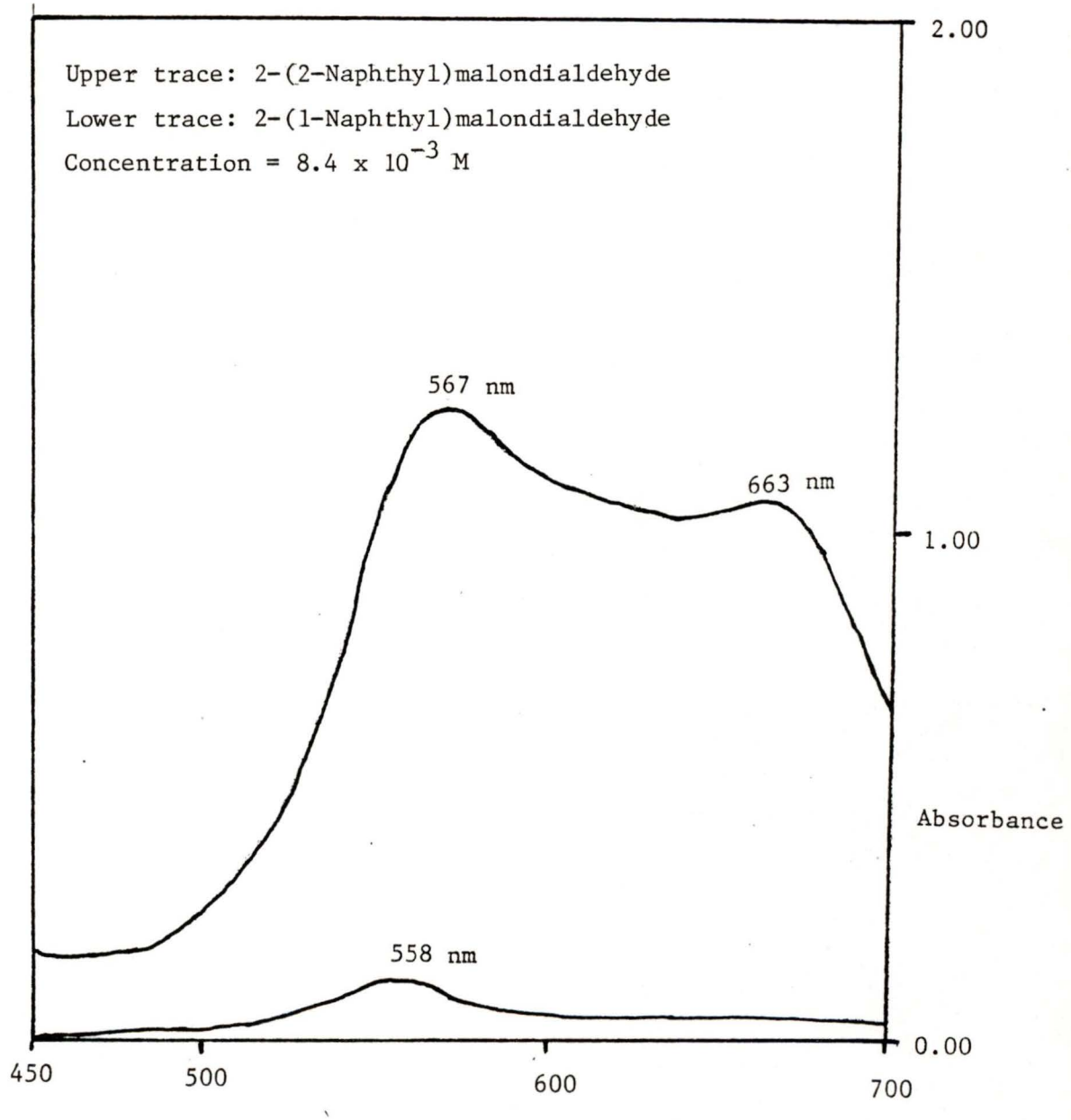
25 1 26

In our hands, use of the reagent also gave distinctive colours with substituted malondialdehydes. The phenyl and 2-naphthyl derivatives give purple solutions with the reagent, with a discrete shoulder in the case of the 2-naphthyl MDA at 663 nm and pronounced tailing to longer wavelengths. This may be due to the further conjugation of the aromatic π system of the substituent X with the basic chromophore.



Interestingly, the 1-naphthyl, pyrene and chrysene malondialdehydes also give an absorbance at ca. 560 nm, but with reduced extinction coefficients. A comparison of the differing intensities is seen in Figure 8. These results are summarized below:

Figure 8. Spectrophotometric Analyses of Malondialdehydes: Reaction of 1- and 2-Naphthyl Derivatives With 2-Methylindole



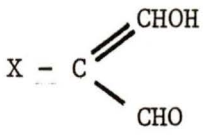


Table 6. Colorimetric Analysis of 2-Substituted Malondialdehydes Using 2-Methylindole: λ max and ε Values.

X	Number	λ nm	ε $L.mol^{-1}.cm^{-1}$.
Ph	41	563.9	766.2
1Nap	44	558.1	123.4
2Nap	45	567.3	797.4
Py	46	569.8	178.8
Chry	47	569.8	274.3
BaP	4	565.6	106.3

Although the lowest value of ε is recorded for BaP, it should be noted that the value may have been depressed by the presence of impurities in the material used. Even though satisfactory identification could not be achieved by standard techniques, the characteristic behaviour shown with 2-methylindole provides valuable evidence that benzo(a)-pyrenylmalondialdehyde has been synthesised.

Although thiobarbituric acid (22) is widely used as a colorimetric indicator for MDA (532nm, ε = 6720), with the substituted malondialdehydes prepared in this work, the characteristic red colour did not form. This does not appear to have been previously reported in the literature. Investigation of the two reagents, TBA and 2-methylindole, with two compounds similar to MDA produced interesting results.

Pentan-2,4-dione (70), and 4,4-dimethoxybutan-2-one (71) were treated with the two reagents. The dione, (70), did not produce a colour with either reagent,[67] while the dimethoxybutan-2-one gave a purple colour with 2-methylindole (λ max = 583 nm, ε = 227.3; 563 nm, ε = 226.1).

$CH_3-CO-CH_2-CO-CH_3$

70

$CH_3-CO-CH_2-CH(OMe)_2$

71

Clearly the utility of 2-methylindole in combination with the TBA test allows detection of substituted malondialdehydes as well as the parent MDA (1) in lipid peroxidation mixtures.

CHAPTER III

BIOLOGICAL RESULTS AND DISCUSSION

The evaluation of the threat to the environment of foreign chemicals, i.e. impact assessment, has been of rapidly expanding interest in the last few decades. In consequence, governmental agencies have been established to monitor the introduction of new materials. Two classes of information are normally required to determine the acceptability of a new chemical. The first is concerned with the level of production use, and disposal of the compound. The second set of information describes the substance's physical, chemical, biological, and environmental properties. This data is generated by a series of tests which cover the following areas: physical/chemical properties, mobility, degradability, accumulation in living organisms, short and long term toxicity, and carcinogeneity (or mutagenicity and teratogenicity). A breakdown of the individual classes is given in Table 7.

Table 7. Typical Characteristics of Environmental Pollutants Considered For Impact Assessment.

PHYSICAL/CHEMICAL PROPERTIES	MOBILITY
Solubility	Absorption onto soils
Partition coefficient	Particles in air, detritus
Dissociation constant	Leaching
Volatility	
TOXICITY	**BIOLOGICAL ACCUMULATION**
Aquatic	Aquatic
Terrestrial	Terrestrial
Human	Airborn
	Human
DEGRADABILITY	**BIOLOGICAL ACTIVITY**
Biodegradation in soil & water	Point mutations, DNA repair
Photodegradation	Chromosome aberrations
Chemical degradation	Cultured cell and *in vitro* mutagenesis, cell transformation
	Chronic tests, teratogenicity

These tests may be arranged in a tiered system of four levels, from the fast, inexpensive tests in the first two levels, to the more complex and time-consuming tests in the upper two levels. (Table 8)[68]

Table 8. Tiered Testing Scheme for Evaluation of Environmental Contaminants.

Level 0	Initial information and basic tests (Routine tests, acute toxicity tests, mutagenicity screen).
Level 1	Simple short term and inexpensive tests (Dosages, test organisms chosen from level 0 data, preliminary hazard assessment).
Level 2	More realistic simulations of expected exposure (Full battery of all mutagenicity/carcinogenetity test categories, used to confirm level 1 results).
Level 3	Most complex and time consuming, including chronic tests and limited area field studies. (Includes teratogenicity testing, of both live-born young, and decrease in fertility, chromosomal abberations. Some test results are hard to interpret in this class with regard to effects on human health.)

Within this tiered system, there exists more than one technique for assaying the biological effect of pollutants. Microbes, insects, and mammalian cells both *in vivo* and *in vitro* are widely used. Mammalian cells are regarded as being more valid than bacterial cells in terms of extrapolation to man. Most of these tests depend on the alteration of the cells exposed to the chemical under investigation, and the degree of activity can be related to the number of organisms showing a characteristic response.

One of the most widely used mutagenicity tests is the Ames test, developed by Professor B. Ames of the University of California.[69] The

test uses specially bred Salmonella typhimurium, the mutant strains developed to revert back to the wild type when acted on by particular mutagens. Most test bacteria have a deleted excision repair system, which enhances the sensitivity of the test. The mutant Salmonella used in the assay are bred for histidine dependence, and revert back to the wild type (i.e. $Hist^- \rightarrow Hist^+$).

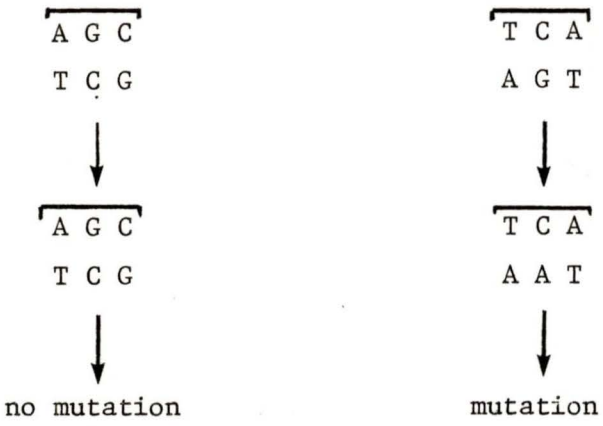
Small amounts of material are needed for the test, typically less than 100 micrograms per plate. The compound under investigation is incorporated into a top layer of agar with the bacteria. Results are measured in numbers of revertant colonies, corrected for background, and good linear dose-respose relationships can be established for low concentrations. At higher concentrations though, the number of revertants falls, due to competing toxicity to the cells.

The addition of a microsomal activation system, S9 (derived from rat liver initiated with Aroclor 1254, a chlorinated biphenyl mixture) increases the scope of the test to include compounds that are metabolized to mutagens. For example, aflatoxin B1, BaP, and 2-acetylaminofluorene have been identified as mutagens in S9 activated systems,[70] as has β-propiolactone.

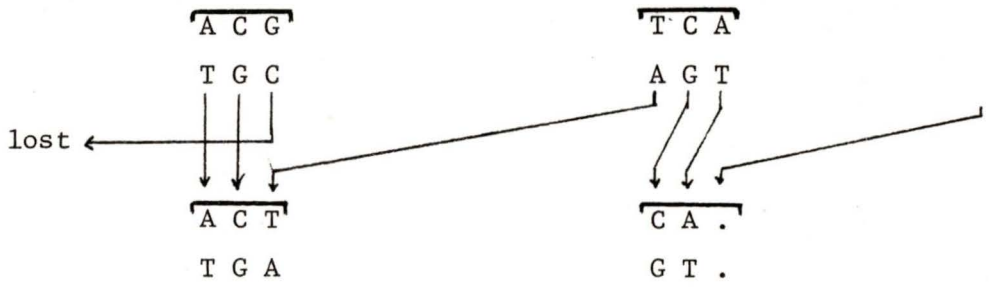
The tester strains themselves can detect different types of mutagenic behaviour. Of the strains used in this study, TA100 detects base pair substitutions, and TA98, frameshift mutations.

The substitution of a base pair is a mutation whereby a wrong base pair is inserted into the DNA sequence. When the strand is replicated, the 'wrong' base pairs up with its natural partner (i.e. adenine with thymine, cytosine with guanine, so that the new DNA

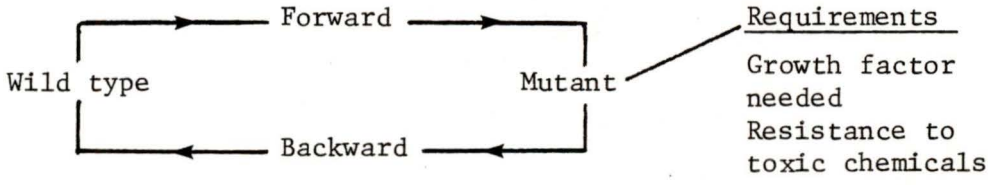
incorporates an incorrect pair of bases.



A frameshift mutation arises from the loss or addition of a base-pair in the DNA sequence. If a base-pair is lost, the DNA, which is read in triplet codons, incorporates the first base-pair from the next codon, and the replicated DNA code becomes scrambled.



Mutations at specific sites such as these can be either forward or backward. A forward mutation transforms the natural (wild) type into a mutant form, whereas a backward type reverts a mutant to the wild type. This latter process is most commonly studied in mutant cells that have known base-pair substitutions or frameshifts. The normal activity (e.g. histidine independence in the case of the Ames test) is restored by a new substitution, or a second deletion and insertion. Backward mutation is highly specific in the type of DNA interaction required, and a compound that does not affect the rate of this type of interaction may still cause other genetic effects (Scheme 8).



Some interferences have been observed in the Ames test. The use of DMSO as a solvent has caused an increased positive response with some chemicals. Another set of interferences are false results, both positive and negative.

False negative results may be observed for known hormonal mutagens (i.e. diethylstilbestrol); or produced by a secondary response to tissue damage; or by an unrepresentative microsomal activation system. False positive results may arise from the incorporation of

the growth factor (e.g. histidine) as an impurity. *In vitro* results may be positive while the *in vivo* results are negative due to the *in vivo* binding of a metabolite to macromolecules other than DNA before the substance under test can react with DNA. An example is dichlorvos, which *in vivo* is rapidly hydrolysed, so that no genetic effects are observed. Interestingly, benzo(a)pyrene diol epoxide I (see Chapter I, pg. 17) is positive in the Ames test system, but is inactive as a tumor initiator of mouse skin.[71]

RESULTS AND DISCUSSION

In this work, the relatively new tester strain, TR2705 (TA94) (kindly provided by Dr. B. Ames after personal visit) was used for the malondialdehyde assays. This strain detects cross-linking agents such as mitomycin and MDA (1), and is several times more sensitive then previously used strains.

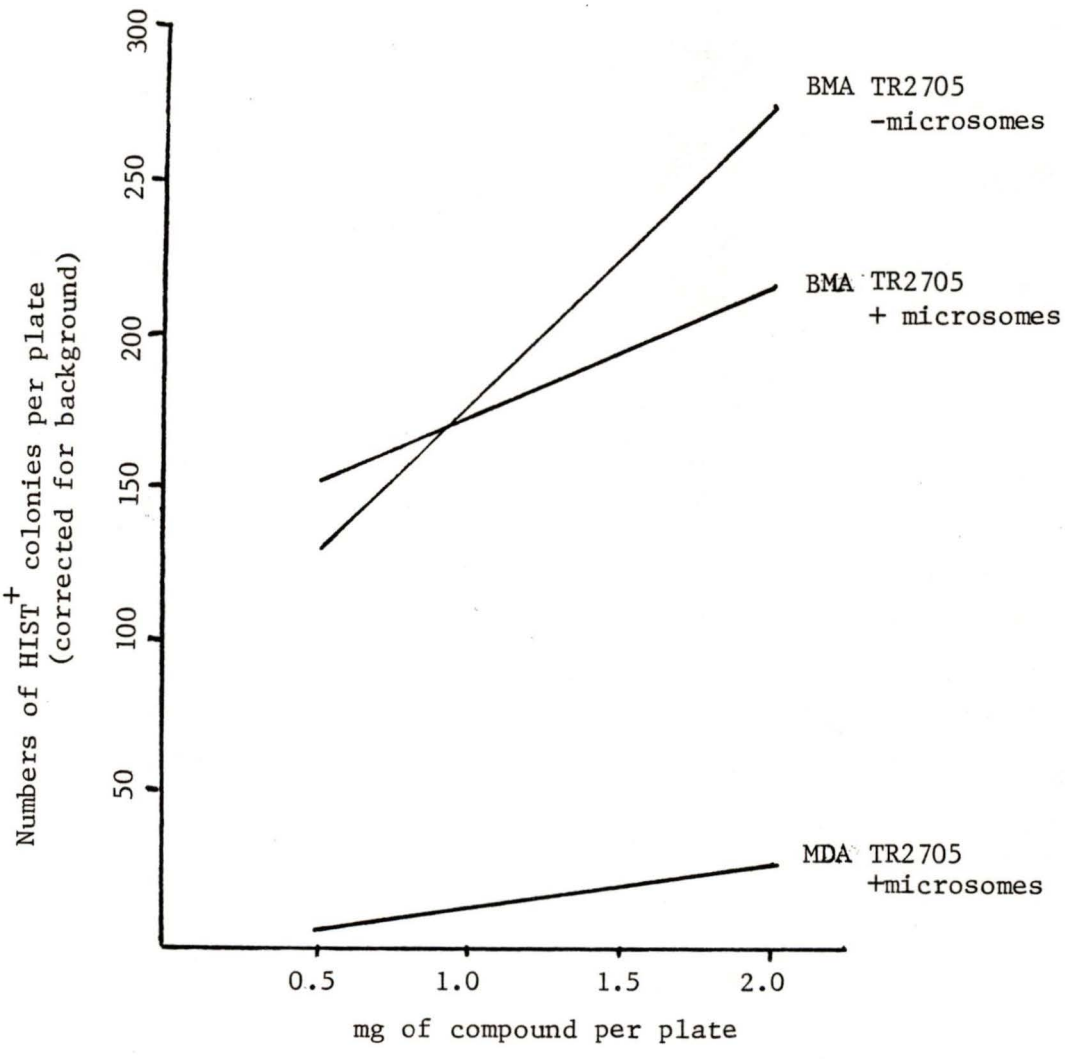
A series of 2-substituted malondialdehydes, derivatives, and precursors were tested in TR2705, and the benzo(a)pyrene derivatives were also tested in TA98, a general screening strain. Table 9 contains a summary of the compounds and the test results.

Table 9. Results of the Ames Test on 2-Substituted Malondialdehydes and Derivatives.

Compound	No.	Strain	Microsomes	Results mg/plate	revertant colonies
Sodium malondialdehyde	1	TR2705	-	-ve	
			+	+ve 2.0	27.7
				1.0	18.0
				0.5	5.0
β-Methoxyacrolein	29	TR2705	-	+ve 2.0	277
				1.0	186
				0.5	123
			+	+ve 2.0	216
				1.0	175
				0.5	151

Compound	No.	Strain	Microsomes	Results
β-N,N-Dimethylaminoacrolein	57	TR2705	+/-	-ve
2-Methylmalondialdehyde	42	TR2705	+/-	-ve
2-Methyl-3-ethoxyacrolein	65	TR2705	+/-	-ve
2-Phenylmalondialdehyde	41	TR2705	+/-	-ve
2-Phenyl-3-ethoxyacrolein	66	TR2705	+/-	-ve
2-(1-Naphthyl)malondialdehyde	44	TR2705	+/-	-ve
2-(2-Naphthyl)malondialdehyde	45	TR2705	+/-	-ve
2-(1-Naphthyl)-3-ethoxyacrolein	68	TR2705	+/-	-ve
2-(2-Naphthyl)-3-ethoxyacrolein	67	TR2705	+/-	-ve
2-(2-Naphthyl)-3-dimethylamino-acrolein	61	TR2705	+/-	-ve
2-(1-Pyrenyl)malondialdehyde	46	TR2705	+/-	-ve

Figure 9. Dependance of the Number of Revertant Colonies For Compounds 29 and 1 With Concentration per Plate.



The following compounds were assayed as spot tests, due to the amounts of compound available. Spot tests are good for quick positive or negative results on small quantities, but are not easily quantified, and a positive result should be followed by a full Ames plate test for confirmation of results. (Table 10)

Table 10. Compounds Assayed as Spot Tests in the Ames System.

Compound	No.	Strain	Microsomes	Result
2-(6-Chrysenyl)malondialdehyde	47	TR2705	+/-	-ve
		TA98	+/-	-ve
1-Benzo(a)pyrenylacetamide	53	TA98	+/-	+ve
2-(1-Benzo(a)pyrenyl)-3-(N,N-dimethylamino)acrolein	64	TA98	+/-	+ve
2-(1-Benzo(a)pyrenyl)malondialdehyde	4	TA98	+/-	+ve
		TR2705	+/-	-ve

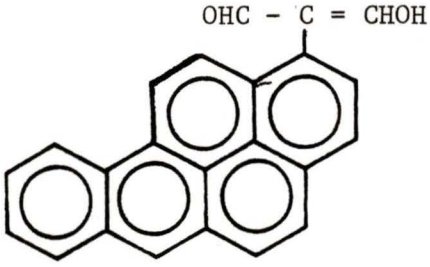
As can be seen from the tables, the results of Marnett and Tuttle[38] have been confirmed. Malondialdehyde sodium salt was found to be a weak mutagen in the presence of the S9 microsomal activation system only. β-Methoxyacrolein (29) was considerably more mutagenic, both with and without S9. The results for MDA (Na) and BMA are presented graphically in Figure 9. This shows a good linearity over the tested concentration range 0.5-2.0 mg/plate. The shape of the graph outside these limits is unknown, but believed to curve down to the origin at the lower end.

As for the putative mutagens, i.e. the substituted malondialdehydes, the β-N,N-dimethylaminoacroleins, and the β-ethoxyacroleins, none showed activity in TR2705. The possible exception is 2-methyl-3-ethoxyacrolein (65) which on first inspection seemed to show a slight activity. Subsequent statistical analysis of the results did not confirm this observed result as significant.

In terms of the results, it is interesting to note that the 2-substitution of a malondialdehyde or ethoxyacrolein renders a potential mutagen totally inactive. The replacement of the methoxy group in β-methoxyacrolein by an N,N-dimethylamino group also has a deactivating effect. Such substitutions could sterically interfere with the spatial requirements for harmful interaction with DNA; the malondialdehydes and ethoxy derivatives being made too bulky to either sit within the double helix and crosslink it, or just by being too large to get to the potential crosslinking site.

The benzo(a)pyrene derivatives tested, while not showing activity in TR2705, did show activity in spot tests in TA98. This is to be expected, given BaP's known mutagenic behaviour. The position of the substitution in our BaP samples, the 1-position, is not able to impede the metabolic activation of the 10 position, as outlined in Chapter I. In these BaP compounds, the substituent plays no part in the formation of the ultimate BaP mutagens, the diol epoxides. It is interesting to speculate what effect a group such as the malondialdehyde moeity would have on observed mutagenicity if attached at the 9- or 10-positions, hampering formation of the diol epoxide.

In this work, three more BaP compounds have been tested, interesting in view of the question of translocation, and metabolism. In the case of the malondialdehyde 4 , although for this material,



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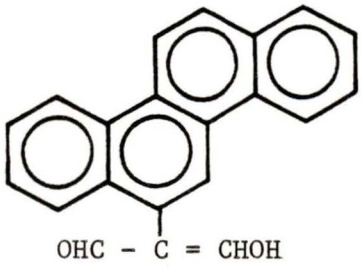
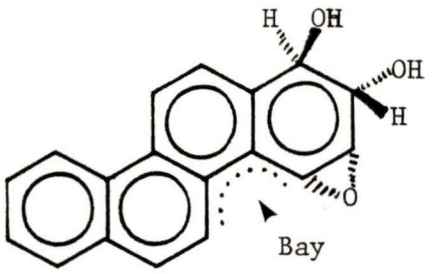
the usual standards of purity were not obtained, all the material tested was water soluble. This compound is a C conjugate of BaP, not an O conjugate, and so not subject to cleavage.

The chrysene malondialdehyde (47) was also tested in TA98 in



47

view of the slight activity of chrysene (12) itself, but showed no activity. There is evidence that the ultimate metabolite of chrysene may be the 1,2-dihydrodiol-3,4-epoxide (72)[72] which has a considerable



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activity over the parent chrysene. In our work, the chrysene is substituted with the malondialdehyde moeity at the 6-position, which is not in a place to interfere with metabolic activation of the bay area. In this case too, it is of interest to speculate on the effect of a bay region substituent on the metabolic activation of chrysene.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Perkin Elmer R12B (60 MHz) or an R32 (90 MHz) spectrometer, the latter was used for variable temperature studies. ^{13}C NMR and some ^{1}H NMR were recorded on a Bruker WM250 MHz multinuclear spectrometer (^{13}C at 62.875 MHz), ^{1}H at 250.132 MHz). Spectra were run in deuterochloroform and at ambient temperature unless otherwise specified. ^{1}H chemical shifts are given in ppm down field from tetramethylsilane as an internal reference, which was also used in the high field spectra. The multiplicity symbols have their usual meaning (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad).

Mass spectra were obtained with a Finnigan 3300 GC/MS (quadrupole) system at 70ev (electron impact, EI) or employing methane chemical ionisation (CI). Where EI spectra are given, good CI spectra were also obtained.

Elemental analyses were performed by Canadian Microanalytical Services Ltd., Vancouver, B.C.

Melting points were obtained with a Reichert hot-stage apparatus equipped with an Omega Engineering digital thermometer.

Dichloromethane, acetone, methanol, ethanol, dimethylformamide, ethylacetate, and N,N-dimethylacetamide were all distilled commerical solvents, stored where necessary over molecular sieves. Dioxane, acetic acid, chlorobenzene and nitrobenzene were all used as received.

Evaporations were carried out under vacuum.

In ^{13}C NMR data an asterisk (*) indicates a line of increased intensity.

Commercial samples of substrates were used as received, and were obtained as follows:-

Substrate	Source
1,1,3,3-tetramethoxypropane	Aldrich
Phosphorus oxychloride	Mallincrodt
p-toluene sulphonic acid	Aldrich
Diethoxpropane	Aldrich
Diethoxyethane	B.D.H.
Phenylacetic acid	Aldrich
1-Naphthylacetic acid	Aldrich
2-Naphthylacetic acid	Aldrich
Acetic anhydride	Fisher
Acetyl chloride	Baker
Pyrene	Aldrich
Chrysene	Aldrich
Benzo(a)pyrene	Aldrich

Sodium Malondialdehyde (1a)[42]

Method (a):- 1,1,3,3-tetramethoxypropane (27) (9.18g, 0.56 mol) was shaken for 2½ hours at room temperature with aqueous sulphuric acid (100 mL, 3%). The resulting solution was brought to pH 8 - 9 with sodium hydroxide solution (1M), and then evaporated to dryness. The sodium salt was extracted with ethanol (100%), the solvent removed,

and the salt purified by dissolution in the minimum amount of water followed by precipitation with acetone to give tan crystals of sodium malondialdehyde (3.18g, 60%).

Method (b):- Phosphorus oxychloride (17.8g, 0.11 mol) was added dropwise to a stirred solution of DMF (14.0g, 0.19 mol) in 1,2-dichloroethane (32 mL). A further amount of dichloroethane (16 mL) was then added, followed by 1,1-diethoxyethane (8.9g, 0.075 mol) dropwise while cooling the reaction to 0°C. The mixture was then heated at 70°C for 15 minutes, and poured onto ice (ca. 30g). Saturated potassium carbonate solution (60 mL) was added to the reaction mixture, and the dichlormethane removed by evaporation. The residue was heated at 90°C for 15 minutes, and extracted with benzene:ethanol (2:1, 4x50mL). The organic extracts were combined, dried, and evaporated to yield 2.25g (30%) of N,N-dimethylaminoacrolein (57) as a dark oil. A small portion was distilled under vacuum, the remainder used directly below:-

bpt 46-49°C (100 torr); NMR (60 MHz) δ 3.06 (6H, bs, N-C$\underline{H}_3$), 5.02 (1H, dd, J_A=13 Hz, J_B=8.5 Hz, $\underline{H}$C=), 7.14, (1H, d, J_A=13 Hz, = C$\underline{H}$N), 9.03 (1H, J_B=8.5 Hz, CHO); IR 3450 (br), 2925, 2820, 2755, 2695, 1612 (br), 1403, 1318, 1197, 1167, 1113, 792, 725, 620 cm^{-1}; MS (CI) $MH^{+\cdot}$ 100 (100).

To the crude oil obtained above (2.15g), sodium hydroxide (2.0 mL, 50%) and water (1 mL) were added, and the mixture warmed to 70°C. The solvent was removed by evaporation, and the residue dissolved in the minimum amount of water. Acetone precipitated the product, 1.56g, 75% (22% based on diethoxyethane) as fine tan needles of sodium malondialde-

hyde. NMR (D_2O, 60 MHz), δ 5.04 (1H, t, J=10 Hz, =CH-), 8.38 (2H, d, J=10 Hz, =CH-O); IR (KBr) 3400 (br), 1580 (br), 1370 (br), 1268, 1172, 1095, 1020, 900, 825 cm^{-1}.

3-Methoxyacrolein (29) (beta-methoxyacrolein, BMA)[38]

A solution of para-toluenesulphonic acid (54.3mg, 0.316 mmol) in water (1.80g, 100 mmol) was added to 1,1,3,3-tetramethoxypropane (27) (16.4g, 100 mmol) and stirred at 80°C for 2 hours. The reaction mixture was distilled at atmospheric pressure to remove methanol (3.20g,100 mmol) and then under reduced pressure, collecting the fraction of boiling point 110/120°C (145 torr). This fraction was chromatographed on silica gel (ca. 750 mL, 60-200 mesh), and the product eluted with dichloromethane:pentane, (3:1). The 29 containing fractions were identified by TLC, analysed by NMR, and those in which the methoxy peak of 27 was less than 29 were pooled and rechromatographed (silica gel, ca. 250mL, dichloromethane:pentane, 3:1). 29 was again identified by TLC and NMR, the 29 containing fractions pooled and evaporated to yield 54mg of 92% (established by NMR) pure oil.
NMR (60 MHz) δ 3.78 (3H, s, OCH_3), 5.58 (1H, dd, J_A=13 Hz, J_B=8 Hz, =C-H), 7.40 (1H, d, J_A=13 Hz), 9.39 (1H, d, J_B=8 Hz, CHO); IR (thin film) 2940, 2835, 1725, 1680, 1620, 1445, 1100 (v.br), 930, 800 cm^{-1}; MS (CI) 87 (MH^+).

2-Methyl-3-(N,N-dimethylamino)acrolein[61] (58)

Phosphorus oxychloride (17.2g, 0.110 mol) was added to a cooled solution of DMF (13.8g, 0.189 mol) in 1,2-dichloroethane (32 mL). A further amount of dichloroethane (16 mL) was added, followed by 1,1-diethoxypropane (10.0g, 0.076 mol). The mixture was heated at 70°C for 15 minutes, and then poured onto ice (ca. 30g). Saturated potassium carbonate solution (60 mL) was added over 10 minutes. The dichloroethane was removed by evaporation, and the residue heated at 90°C for 15 minutes. The residue was extracted with benzene:ethanol (2:1, 4 x 25 mL). The combined organic extracts were dried and evaporated to give 2.26g (26%) of 58, as an orange oil. This was used directly below.

2-Methylmalondialdehyde (42)

Crude (58) from above (2.26g, 0.0228 mol) was added to sodium hydroxide solution (2 mL, 30%) and water (3 mL). The mixture was heated to 70°C, and the solvent evaporated. The residue was dissolved in the minimum amount of water, was filtered, and then acidified with hydrochloric acid (5 M). The solvent was removed, and the residue sublimed under vacuum to give white amorphous crystals of (42) (1.14g, 68%). mp 90-91°C (lit. mp[61] 89-90°C); NMR ^{1}H (250 MHz, Acetone d_6) δ 1.61 (3H, s, $\underline{CH}_3$), 8.37 (2H, s, CHO); ^{13}C δ 5.86 ($\underline{CH}_3$), 119.61 (Me-$\underline{C}$-$(CHO)_2$), 179.60 ($\underline{CHO}$); IR (KBr) 3000 (vbr), 1580 (br), 1412, 1370, 1212, 1008, cm^{-1}; MS (CI, rel%), 87 (100), 85 (60), 58 (70), 56 (60).

2-Phenyl-3-(N,N-dimethylamino)acrolein (59) [51]

Dimethylformamide (20.8g, 0.285 mol) was added with stirring to phosphorusoxychloride (34.5g, 0.223 mol) at 10°C (ice bath) over 10 minutes. The mixture was stirred at 10°C for a further 10 minutes, and then a solution of phenylacetic acid (10.0g, 0.0735 mol) in DMF (40mL) was added over 5 minutes. The mixture was heated at 70°C for 18 hours, then poured onto ice (ca. 300g) and neutralised with anhydrous potassium carbonate. Sodium hydroxide solution (100 mL, 50%) was added, and the mixture held at 60°C until the evolved vapours were no longer basic. The reaction mixture was extracted with dichloromethane (4 x 50 mL) and the organic extract evaporated to give 59 as a dark oil (11.0g, 86%). This was used directly below.

2-Phenylmalondialdehyde (41)

Sodium hydroxide solution (12.5 mL, 25%) was added to a refluxing solution of the crude oil 59 (10.0g, 0.057 mol) obtained above, in ethanol (10mL). The mixture was refluxed for 3 hours, and then evaporated to dryness. The residue was dissolved in water, filtered, and acidified with hydrochloric acid (5 M). The product was separated by filtration, and recrystallised from cyclohexane to give small tan crystals (7.45g, 88%).

mp 94-95°C (lit. mp[51] 92-95°C); NMR (250 MHz) δ 7.23-7.24 (5H, m, ArH), 8.61 (2H, s, CHO/=CH-OH), 14.4 (brs, sharpens at low temperature, -OH); ^{13}C, δ 118.36, 126.56, 127.36, 129.15, (aromatic -CH), 133.56, 181.08, (CHO); IR (KBr), 2600, (vbr), 1580 (br), 1395, 1350 (br), 1230 (br),

1110, 782, 705 cm^{-1}; MS (EI, rel%) 148 (55), 119 (26), 102 (28), 91 (100).

2-(1-Naphthyl)-3-(N,N-dimethylamino)acrolein (60)[51]

This was prepared as above for 2-phenyl-3-(N,N-dimethylamino)-acrolein, except that 1-naphthylacetic acid (10.00g, 0.054 mol) in DMF (26mL) was added to a cooled mixture of DMF (14.14g, 0.19 mol) and phosphorus oxychloride (23.45g, 0.15 mol). The product was isolated as a brown oil (8.73g, 72%) and was recrystallised from cyclohexane to give fine brown crystals.

mp 94-96°C; NMR (90 MHz) ^{1}H δ 2.60 (6H, brs, $C\underline{H}_3$), 7.00-7.90 (m, 8H, ArH+ =C$\underline{H}$N), 9.24 (1H, s, C$\underline{H}$O); ^{13}C δ 125.20, 125.66, 126.05*, 126.43, 127.73, 128.30 (aryl-$\underline{C}$H), 158.81 (=CH N Me_2), 189.35 (-$\underline{C}$HO); IR (KBr), 1595 (br), 1390, 1265, 1175, 1110, 990, 810, 778 cm^{-1}; MS (CI) 226 (100); Anal. (C_{15} H_{15} NO). Calcd. % C, 79.97; H, 6.71; N, 6.22: Found % C, 79.52%; H, 6.68: N, 6.20.

2-(1-Naphthyl)malondialdehyde (44)

Sodium hydroxide solution (17.5 mL, 25%) was added to a refluxing solution of 2-(1-naphthyl)-3-(dimethylamino)acrolein (5.00g, 8.89 mmol) in ethanol (50 mL). The mixture was refluxed for a further three hours, and then evaporated to dryness. The residue was dissolved in the minimum amount of water, filtered and acidified with concentrated hydrochloric acid. The product was collected by filtration, and re-rystallised from cyclohexane to give light off-white crystals of

2-(1-naphthyl)malondialdehyde (44) (4.06g, 81%):
mp 186-187°C; NMR (90 MHz) ^{1}H δ 7.32-8.05 (7H, m, ArH), 8.51 (2H, s, CHO/CHOH), 14.35 (brs, sharpens at -50°C, 1H, OH); ^{13}C 116.68 (=C-CHO) 124.91, 125.56, 126.30, 126.77, 128.12, 128.68, 128.77 (aryl C-H), 130.53, 132.80, 134.06 (quaternary aryl C) 181.96; IR (KBr) 2950 (br), 2640 (br), 1625, 1565 (br), 1418, 1390, 1365, 1262, 1248, 1195, 1140, 790, 772, 720 cm^{-1}; MS (EI), 198 (90), 169 (64), 152 (44), 142 (25), 141 (100).

2-(2-Naphthyl)-3-(N,N-dimethylamino)acrolein (61) [51]

This was prepared exactly as for 2-(1-naphthyl)-3-(N,N-dimethylamino)acrolein (60) except that 2-naphthylacetic acid (10.00g, 0.054 mol) was used. The product was isolated as a dark solid, and recrystallised from cyclohexane to yield 11.54g (91%).
mp 130-132°C (lit mp[51] 131-135°C); NMR (90 MHz) ^{1}H δ 2.64 (6H, bs, NCH_3), 6.84 (1H,s, =CH), 7.20-7.90 (7H, M, ArH), 9.11 (1H, s, CHO); ^{13}C δ 43.6 (b, CH_3), 114.94 (=C -CHO), 125.52, 125.76, 127.08, 127.46, 127.73, 129.43, 129.55 (aryl -CH), 132.11, 132.28, 132.99 (quart. aryl C), 158.3 (b, =CHN), 189.29 (CHO); IR (KBr), 1560 (br), 1385, 1265, 1185, 1112, 1075, 940, 780, 752, cm^{-1}; MS, (CI) 226 (MH^{+}).

2-(2-Naphthyl)malondialdehyde (45)[51]

This was prepared exactly as for 2-(1-naphthyl)malondialdehyde, except that 2-(2-naphthyl)-3-(N,N-dimethylamino)acrolein (5.00g, 22.2 mmol) was used. The product was recrystallised from cyclohexane to yield

3.30g (75%).

mp 141-2°C; NMR ^{1}H (250 MHz) δ 7.36-7.89 (7H, m, ArH), 8.76 (2H, s, CHO/C=CHOH), 14.5 (1H, brs, sharper at -50°C, OH); ^{13}C, δ 118.30 (=CH-CHO), 124,83, 124.94, 126.15, 126.61, 127.77, 127.83, 129.00 (aryl-CH), 130.91, 132.44, 133.71 (quart aryl C), 181.29 (-CHO); IR (KBr) 3000 (br), 2650 (br), 1580 (br), 1400, 1350, 1330, 1250, 1230, 1190, 768, 748, cm^{-1}; MS (EI, rel%) 198 (52), 170 (28), 169 (17), 142 (20), 141 (100); Anal; calcd for $C_{13}H_{10}O_2$ (%) C, 78.77; H, 5.09; Found C, 78.46; H, 5.11.

2-Phenyl-3-ethoxyacrolein (66)[51]

Sodium hydride (143mg, 5.9 mmol) was added to a stirred solution of 2-phenylmalondialdehyde (41) (500mg, 3.4 mmol) in N,N-dimethylacetamide (9 mL). The mixture was stirred until the effervescence had ceased, and then ethyl iodide (1.05g, 6.7 mmol) was added, and the mixture stirred for 24 hours. The solvent was removed by evaporation, and water (ca. 20 mL) added to the residue. The mixture was extracted with ethyl acetate (4 x 25 mL), the organic extracts combined, washed with water and saturated sodium chloride solution, and dried over sodium sulphate. The solvent was removed, and the residue redissolved in dichloromethane and chromatographed by preparative TLC. (20cm x 20cm plates, 0.75mm, silica gel 60 HF 254 + 366). The band travelling at rf 0.45-0.85 was collected, and the solvent removed to yield 2-phenyl-3-ethoxyacrolein (0.46g, 85%) as a pale yellow oil.

NMR (90 MHz) ^{1}H δ 1.28 (3H, t, J = 7 Hz, CH_3), 3.52 (2H, q, J = 7 Hz, CH_2), 6.97 (1H, s, C = H), 7.24 (5H, s, ArH), 9.26 (1H, s, CHO); MS (CI), MH^+

177 (100); IR, 1690, 1624, 1600, 1498, 1451, 1255, (br), 1100 (b), 695 cm^{-1}.

2-(1-Naphthyl)-3-ethoxyacrolein (68)

This was prepared exactly as above for 1-phenyl-3-ethoxyacrolein (66) except that 2-(1-naphthyl)malondialdehyde (45), (0.5g, 2.5 mmol), sodium hydride (106mg, 4.4 mmol), ethyl iodide (0.78g, 5.0 mmol) and N,N-dimethylacetamide (16 mL) were used.

The product was purified by prep. TLC as before to yield light cream crystals of 2-(1-naphthyl)-3-ethoxyacrolein (0.51g, 90%). mp 126-127°C; NMR (250 MHz) ^{1}H δ 1.31 (3H, t, J = 7 Hz, CH_3), 4.18 (2H, q, 7 Hz, CH_2), 7.31 (1H, dd, J_o = 7 Hz, J_m = 1.5 Hz, ArH-2), 7.40-7.53 (3H, m, ArH -3,6,7), 7.61-7.66 (1H, m, ArH -8), 7.82-7.88 (2H, m, ArH -4,5), 9.59 (1H, s, CHO); ^{13}C δ 15.32 (CH_3), 71.78 (OCH_2), 123.09 (=CH-O), 125.36, 125.56, 125.68, 125.83, 128.21, 128.50* (Ar CH), 128.6, 131.50, 133.82 (quat ArC), 167.96 (=CHOEt), 190.29 (CHO); IR 3040, 2980, 2835, 2730, 1655, 1610, 1295, 1238, 1205, 1128, 1090, 1005, 990, 898, 780 cm^{-1}; MS (CI) MH^+ 227 (100); Anal: Calcd for $C_{15}H_{14}O_2$ (%) C: 79.62 , H: 6.24; Found C: 79.64, H: 6.19.

2-(2-Naphthyl)-3-ethoxyacrolein (67)

This was prepared in exactly the same manner as 2-(1-naphthyl)-3-ethoxyacrolein (68), except that 2-(2-naphthyl)malondialdehyde (45), (0.50g, 2.5 mmol) was used. The product was isolated as a clear yellow oil (0.48g, 85%).

bpt 190-200°C (0.75 torr); NMR (90 MHz) ^{1}H δ 1.24 (3H, t, 7 Hz, CH_3), 4.10 (2H, q, 7 Hz, CH_2), 6.93 (1H, s, =CH- Et), 7.24-7.85 (7H, m, ArH), 9.19 (1H, s, CHO); ^{13}C δ 15.26 (CH_3), 72.16 (OCH_2), 125.80, 126.00, 127.12, 127.30, 127.53, 128.27, 128.62 (ArCH), 168.85 (= CHOEt), 194.25 (CHO) (Note: many small peaks were present due to decomposition of the sample); IR 3050, 2980, 2720, 1668, 1622, 1594, 1305, 1258, 1233, 1130, 1082, 1012, 860, 817, 770, 752, 730 cm^{-1}; MS (EI) $M^{+\cdot}$ 226 (49), 172 (16), 170 (27), 156 (28), 155 (100), 141 (67), 127 (92); Anal: Calcd for $C_{15}H_{14}O_2$ (%) C: 79.62, H: 6.24; Found C: 79.06, H: 6.32.

2-Methyl-3-ethoxyacrolein (65)

This was prepared exactly as for 2-(1-naphthyl)-3-ethoxyacrolein, except that 2-methylmalondialdehyde (0.50g, 5.8 mmol), sodium hydride (0.27g, 6.4 mmol), ethyl iodide (0.90g, 5.8 mmol), and N,N-dimethyl-acetamide (16 mL) were used. The product was isolated as a pale yellow oil, (0.52g, 79%).

NMR (90 MHz) ^{1}H δ 1.34 (3H, t, 7 Hz, CH_3), 1.65 (3H, s, CH_3), 4.10 (2H, split q, 7 Hz, CH_2), 6.90 (1H, bs, CH =), 9.26 (1H, s, CHO); IR 2920, 1630, 1215, 1008, 835 cm^{-1}; MS (EI) 114 (45), 85 (100), 68 (30), 57 (50).

1-Acetylpyrene (48)[54]

Method (a):- Acetic anhydride (5.2 mL, 5.6g, 0.055 mol) was added to a suspension of anhydrous aluminium chloride (13.2g, 0.099 mol) in nitrobenzene (50mL) under nitrogen at 0°C with stirring. The mixture was cooled to -5°C, and finely divided pyrene (10.0g, 0.049 mol) was

added over 10 minutes in ca. 1g portions. The mixture was then stirred at 0°C for 7 hours, hydrolysed with hydrochloric acid (1 M, ca. 100 mL) and the nitrobenzene removed by steam distillation. On cooling, the residue was removed by filtration, and recrystallised from methanol to yield yellow plates of 1-acetylpyrene (10.0g, 83%).

Method (b):- Acetic anhydride (5.2 mL, 5.6g. 0.055 mol) was added to a stirred suspension of anhydrous aluminium chloride (13.2g, 0.099 mol) in dichloromethane (100 mL). A solution of pyrene (10.0g, 0.049 mol) in dichloromethane (300 mL), was added over 15 minutes, and the mixture stirred at room temperature for 16 hours, and then hydrolysed with hydrochloric acid (1 M, ca. 200 mL). The organic layer was separated washed, dried, and the solvent removed by evaporation. The product was recrystallised from methanol to yield pale yellow plates of 1-acetylpyrene (11.3g. 93%).

mpt 87-89°C (lit mpt[54] 89-90°C); NMR (250 MHz) ^{1}H δ 2.85 (3H, s, CH_3), 7.91-8.18 (6H, m, ArH), 8.26 (1H, d, J = 8.1 Hz, ArH-2) , 9.02 (1H, d, J = 9.5 Hz, ArH-10); ^{13}C δ 30.27 (CH_3), 123.77, 124.85, 125.90, 126.15, 126.19, 126.89*, 129.40, 129.52 (ArCH), 124.09, 129.33, 130.34, 130.90, 131.73, 133,80 (quat Ar-C), 202, (C=O); IR 1665, 1590, 1500, 1382, 1369, 1350, 1252, 1228, 1198, 1181, 1127, 1095, 945, 900, 836, 732, 710, 703, cm^{-1}; MS (CI) MH^+ 245 (100).

6-Acetylchrysene (49)[59]

Chrysene (5.0g, 0.022 mol) was added in ca. 1g portions to a solution of aluminium trichloride (4.0g, 0.030 mol) and acetyl chloride

(2.0mL, 0.025 mol) in dichloromethane (100 mL) under nitrogen with stirring at 0°C. The mixture was refluxed for 4 hours and then hydroloysed with dilute hydrochloric acid (1M, ca. 50 mL). The organic layer was separated, washed with dilute sodium hydroxide solution (1 M) water, dried, and the solvent removed by evaporation. The residue was recrystallised from acetone to yield yellow plates of 6-acetylchrysene (5.2g, 87%).

mpt 143-4°C (lit mpt[59] 144°C); NMR (250 MHz) ^{1}H δ 2.85 (3H, s, $\underline{CH}_3$), 7.63-7.71 (4H, m, H-2,3,8,9), 7.89-7.95 (2H, m, H-1,12), 8.49 (1H, d, J = 9.1 Hz, H-11), 8.59-8.78 (3H, m, H-2,7,10), 8.96 (1H, s, H-5); ^{13}C 30.11 ($\underline{C}H_3$), 120.83, 122.60, 123.27, 124.13, 126.66, 126.74, 127.04, 127.24, 127.51, 128.74, 129.65(Aryl $\underline{C}$H), 126.11, 128.55, 130.59, 131.01, 132.13, 134.71 (quat Ar$\underline{C}$), 201.91 ($\underline{C}$O); MS (CI) 271 (100).

1-Acetylbenzo(a)pyrene (50)[60]

A solution of benzo(a)pyrene (0.50g, 2.0 mmol) in carbon disulphide (13 mL) was added to a stirred solution of aluminium trichloride (0.50g, 3.7 mmol) in acetylchloride (3 mL, 4.2 mmol) over 10 minutes under nitrogen with cooling. The mixture was stirred for a further 1 hour, and then decomposed with dilute hydrochloric acid and ice (ca. 25 mL). The resulting solution was extracted with chloroform (5 x 50 mL), and the organic extracts combined, washed with sodium bicarbonate, water, dried, and evaporated. The residue was recrystallised from boiling benzene to yield yellow plates of 1-acetylbenzo(a)pyrene (0.53g, 89%)

mpt 252-253°C (lit mp[60] 254°C); NMR (250 MHz) ^{1}H δ 2.85 (3H, s, CH_3), 7.72-7.87 (3H, m, H -5[+],8,9), 7.93-7.98 (2H, m, H -3,4[+]), 8.19-8.22 (1H, m, H -7), 8.31 (1H, d, J = 7.9 Hz, H -2), 8.44 (1H, s, H -6), 8.98 (H, d, J = 8.5 Hz, H -10), 9.05 (1H, d, J = 9.5 Hz, H -11[≠]), 9.28 (1H, d, J = 9.5 Hz, H -12[≠]); ^{13}C δ 30.17 (CH_3), 123.16, 123.54, 124.16, 125.40, 126.46[+], 126.62, 127.27, 128.07, 128.91, 130.45 (aryl CH), 125.80, 127.00, 128.35, 128.54, 129.39, 129.53, 131.39, 132.04, 135.00 (quat Ar$\underline{C}$) 201.50 (CO); MS (CI) MH^+ 295 (100).

1-Pyrenylacetamide (51)[73]

1-acetylpyrene (1.0g, 4.1 mmol) dissolved in dioxane (3.2 mL) was sealed in a thick walled pyrex tube under vacuum with ammonium polysulphide solution (4.0 mL)*. The tube was heated at 160°C for 18 hours, allowed to cool, opened, and the product collected by filtration. Recrystallisation from acetic acid/chlorobenzene (1:1) yielded 1-pyrenylacetamide (0.8g, 76%).

mpt 245-246°C (lit mp[73] 246-247°C); NMR(90 MHz) (DMSO d_6) δ 4.21 (2H, s, $C\underline{H}_2$), 7.1, 7.7 (1H each, bs, $-N\underline{H}_2$), 800-8.60 (9H, m, ArH); IR 3400 (b), 1660, 830, 315, 765, 753, 700 cm^{-1}; Ms (CI) MH^+ 260 (9), 258 (11), 246 (20), 230 (52), 216 (100).

* Ammonium polysulphide was prepared as follows:- sulphur (0.4g) was dissolved in concentrated ammonium hydroxide solution (880) saturated with hydrogen sulphide, (4 mL).

6-Chrysenylacetamide (52)

This was prepared as described above for 1-pyrenylacetamide, except that 6-acetylchrysene (1.0g, 2.4 mol), dioxane (2.9 mL) and ammonium polysulphide solution (3.6 mL) were used. The product was recrystallised from acetic acid/chlorobenzene (1:1) to yield pale yellow needles of 6-chrysenylacetamide (0.92g, 87%).
mpt 292-5°C; NMR (250 MHz)(DMSO d_6) ^{1}H δ 4.10 (2H, s, CH_2), 7.04 (1H, bs, NH_2), 7.42-7.82 (5H, m, NH_2, ArH, -2,3,8,9), 8.06-8.32 (3H, m, ArH, 1,7,12), 8.83-8.89 (4H, m, ArH, -4,5,10,11); ^{13}C δ 4.71 (CH_2), 121.16, 123.18*, 123.66, 124.78, 126.44*, 126.69, 126.88, 128.31 (aryl CH), 127.04, 127.19, 129.63, 130.19, 131.08, 131.72, 132.04 (quat ArC) 172.06 (CO); IR 3420 (b), 1662, 812, 752 cm^{-1}; MS (CI), MH^+ 286 (100), 244 (90); Anal: Calcd for $C_{20}H_{13}NO$; C 84.19, H 5.30, N 5.61%. Found C 83.89, H 5.38, N 4.78%.
N 4.78%.

1-Benzo(a)pyrenylacetamide (53)

This was prepared as described above for 1-pyrenylacetamide, except that 1-acetylbenzo(a)pyrene (0.4g, 1.4 mmol), dioxane (2.1 mL) and ammonium polysulphide (2.1 mL, syringe) were used. The product was recrystallised from acetic acid/chlorobenzene (1:1) to yield orange crystals of 1-benzo(a)pyrenylacetamide (0.31g, 77%).

mpt 297-299°C; NMR (250 MHz) (DMSO d_6) ^{1}H δ 4.20 (2H, s, CH_2), 7.10 (1H, bs, NH_2), 7.70 (1H, bs, NH_2), 7.80-8.41 (7H, m, ArH), 8.60 (1H, d, J = 9.5 Hz, ArH -12), 8.66 (1H, s, ArH-6), 9.18 (1H, d, J =7.7 Hz, ArCH-10), 9.24, (1H, d, J = 9.5 Hz, ArH-11); ^{13}C δ 40.50 ($\underline{C}H_2$), 122.23, 123.21, 124.31, 124.49, 124.58, 126.16, 126.30, 127.32, 127.74, 128.57* (Aryl CH) (Due to impurity peaks, the quaternary carbons could not be assigned). IR 3400 (br), 2920, 1655, 1097, 1015, 930, 900, 745 cm^{-1}; MS (CI) MH^+ 310 (100). Anal: Calcd for C_{22} H_{13} NO C 85.41, H 4.89, N 4.35%. Found C 84.80, H 5.35, N 3.72%.

1-Pyreneacetic acid (54)[54]

Concentrated hydrochloric acid (12M, 7 mL) was added cautiously to a refluxing solution of 1-pyrenylacetamide (0.95g, 3.7 mmol) in glacial acetic acid (14 mL) under nitrogen. The mixture was refluxed for a further 1½ hours, cooled, and a further amount of concentrated hydrochloric acid (7 mL) added. The product was separated by filtration, purified through its water soluble potassium salt, and recrystallised from chlorobenzene to yield cream crystals of 1-pyreneacetic acid (0.89g, 94%).

mpt 220-221°C, (lit mp[54] 222.5-223°C); NMR (90 MHz) (DMSO d_6) δ 4.20 (2H, s, CH_2), 8.27-8.76 (9H, m, ArH); MS (CI) MH^+ 261 (100).

6-Chryseneacetic acid (55)

This was prepared as described above for 1-pyrenylacetic acid (54), except 6-chrysenylacetamide (0.91g, 3.4 mmol), acetic acid (12 mL), and concentrated hydrochloric acid (2 x 6 mL) were used. The product was recrystallised from chlorobenzene to yield 6-chryseneacetic acid (0.89g, 97%).

mpt 284-9°C (with softening); NMR (90 MHz) (DMSO d_6) ^{1}H δ 4.40 (2H, s, CH_2) 7.6-8.0 (4H, m, ArH -2,3,8,9), 8.0-8.4 (3H, m, ArH -1,7,12), 8.8-9.2 (4H, m, ArH, -4,5,10,11) 13.6 (1H, brs, COOH) ^{13}C δ 40.50 (CH_2), 121.19, 123.21*, 123.69, 124.80, 126.47**, 126.72, 126.90, 128.34 (aryl CH), 127.05, 127.22, 129.66, 130.21, 131.10, 131.73, 132.08 (quat ArC), 172.10 (COOH); IR 3050, (1br), 1690, 1210, 900, 815, 750 cm^{-1}; MS (CI) MH^+ 287 (20), 286 (15), 257 (40), 243 (30), 229 (100); Anal: Calcd for $C_{20}H_{14}O_2$ C, 83.90; H, 4.93; Found C, 83.92; H, 4.90.

1-Benzo(a)pyreneacetic acid (56)

This was prepared as described above for 1-pyreneacetic acid except that 1-benzo(a)pyrenylacetamide (0.29g, 0.94 mmol), acetic acid (15 mL) and concentrated hydrochloric acid (2x2.1 mL,syringe) were used The product was recrystallised from chlorobenzene to yield crystals of 1-benzo(a)pyreneacetic acid (0.27g, 93%).

mpt 295-298°C; NMR (250 MHz) (DMSO d_6) ^{1}H, δ 4.55 (2H,s, CH_2), 7.44-8.69 (9H, m, ArH), 9.19-9.29 (2H, m, ArH -10,11); IR 3400 (br), 2910, 1688, 1403, 1310, 772 cm^{-1}; MS (CI), MH^+ 311 (60), 310 (80), 309 (40), 266 (100). Anal: Calcd for $C_{22}H_{14}O_2$ C, 85.14, H, 4.55; Found C, 85.31,

H, 4.98.

2-(1-Pyrenyl)-3-(N,N-dimethylamino)acrolein (62)

Phosphorus oxychloride (0.60g, 3.9 mmol) added to DMF (0.60 mL, 0.56g, 7.7 mmol) over 10 minutes under nitrogen with stirring at 0°C. After stirring for a further 10 minutes, the mixture was allowed to warm to room temperature, and a solution of 1-pyreneacetic acid (0.60g, 2.3 mmol) in DMF (2 mL) added over 10 minutes. The reaction mixture was heated at 70°C for 18 hours, and then poured onto ice (ca. 10g). The resulting solution was neutralised with anhydrous potassium carbonate, and then made strongly alkaline with sodium hydroxide solution (3 mL, 30%). This mixture was warmed at 30°C under a stream of nitrogen until the exhaust gas was no longer basic, allowed to cool, and stirred at ambient temperature for a further 18 hours. The product was collected by filtration, as a dark amorphous solid of 2-(1-pyrenyl)-3-(dimethylamino)acrolein (0.43g, 62%), and used directly in the sythesis of 2-(1-pyrenyl)malondialdehyde (46) below.

A small quantity (ca. 20mg) was chromatographed on an alumina column with dichloromethane to yield light brown crystals. mpt 119-122°C; NMR(90 MHz) ^{1}H δ 2.63 (3H, s, CH_3), 3.33 (3H,s, CH_3), 7.31 (1H, s, HC = R), 7.60-8.30 (9H, m, ArH), 9.01 (1H, s, CHO); ^{13}C δ (methyl, v broad), 124.46, 124.94, 125.03, 125.40, 125.90, 127.28, 127.41, 127.59, 129.81 (Aryl CH), (the quaternary C could not be discerned), 189.51 (-CO); IR, 1595, 1582, 1340, 1265, 1175, 1110, 830, 780, 700 cm^{-1}; MS (EI) $M^{+\cdot}$ 299 (45), 245 (30), 242 (30), 216 (100).

2-(6-Chrysenyl)-3-(N,N-dimethylamino)acrolein (63)

This was prepared in exactly the same manner as described above for 2-(1-pyrenyl)-3-(N,N-dimethylamino)acrolein (62) except that 6-chryseneacetic acid (55) (1.0g, 3.5 mmol dissolved in DMF, 3 mL) phosphorus oxychloride (1.0 mL, 0.61g, 4.0 mol) and DMF, (1.0 mL, 0.94g, 1.3 mmol) were used. The product was obtained as a dark brown amorphous solid (1.1g, 93%) and used directly below in the synthesis of 2-(6-chrysenyl)malondialdehyde (47).

A portion (ca. 50mg) was chromatographed on an alumina column with dichloromethane. 2-(6-Chrysenyl)-3-(N,N-dimethylamino)acrolein (63) was isolated as light brown amorphous crystals.

mp 128-136 (with softening); NMR (90 MHz) δ 2.67 (6H, brs, NMe_2), 7.22 (1H, s, =CH), 7.45-7.80 (4H, m, ArH -2,3,8,9), 7.8-8.2 (3H, m, ArH -1, 7,12) 8.50-8.90 (4H, m, ArH -4,5,10,11) 9.34 (1H, s, CHO); ^{13}C δ 29.69 (CH_3), 121.11, 123.31, 123.49, 124.65, 126.35, 126.58**, 126.90, 127.34, 128.50 (aryl CH) (quaternary C could not be easily discerned) 189.57 (CHO) IR 1625, 1585, 1095, 895, 810, 750 cm^{-1}; MS (CI) MH^+ 326 (55), 258 (100).

2-(1-Benzo(a)pyrenyl)-3-(N,N-dimethylamino)acrolein (64)

This was prepared in the same manner as described above for 2-(1-pyrenyl)-3-(N,N-dimethylamino)acrolein (62) except that 1-benzo(a)-pyrenylacetic acid (200mg, 0.65 mmol) in DMF (5 mL) phosphorus oxychlororide (0.17g, 1.1 mmol) and DMF (0.17 mL, syringe, 0.16g, 2.2 mmol) were used. The crude 2-(1-benzo(a)pyrenyl)-3-(N,N-dimethylamino)acrolein

(64) (151mg, 66%) (MH^+ = 350) so obtained was used directly in the synthesis of 2-(1-benzo(a)pyrenyl)malondialdehyde (4) below.

2-(1-Pyrenyl)malondialdehyde (46)

Sodium hydroxide solution (1.6 mL, 25%) was added to a refluxing solution of crude 2-(1-pyrenyl)-3-(N,N-dimethylamino)acrolein (62) from above (0.40g, 1.3 mmol) in ethanol (2.0 mL, 100%). After refluxing for 3 hours, the reaction mixture evaporated to dryness, the residue dissolved in the minimum amount of water, and acidified with hydrochloric acid (5 M). The product, 2-(1-pyrenyl)malondialdehyde, was collected by filtration, purified through its water soluble potassium salt, and recrystallised from cyclohexane to yield 0.18g (51%). mp 230-231°C; NMR (90 MHz), δ 7.60-8.40 (10H, m, ArH), 8.63 (1H, s, CHO) (in THF: 12.75 (1H, brs, OH)); ^{13}C δ 125.17, 125.69, 126.03*, 127.70, 128.28, 129.34 (aryl CH) 158.90 (= CHOH), 189.29 (CHO) (other quaternary peaks could not be discerned); IR 3020 (br), 1680 (br), 1580 (br), 1250 (br), 840, 700 cm^{-1}; MS (CI) MH^+ 273 (100); Anal: Calcd for $C_{19}H_{12}O_2$; C, 83.81; H, 4.44; Found; C, 83.85 H, 4.87.

2-(6-Chrysenyl)malondialdehyde (47)

This was prepared in exactly the same manner as described above for 2-(1-pyrenyl)malondialdehyde (46) except that 2-(6-chrysenyl)-3-(N,N-dimethylamino)acrolein (63) (0.90g, 2.8 mmol), ethanol (5 mL, 100%), and sodium hydroxide solution (5.4 mL, 25%) were used. 2-(6-chrysenyl)-malondialdehyde (47) was isolated as pale pink crystals (60mg, 7%).

mp 242-245°C ^{1}H NMR (250 MHz) δ 7.61-7.78 (4H, m, ArH,-2,3,-8,9) 7.98-8.15 (3H, m, ArH -1,7,12), 8.59-8.85 (6H, ArH -4,5,10,11, CHO (=CHOH)); ^{13}C δ 117.3 (ArC=), 120.91, 122.97, 123.06, 123.74, 125.74, 126.70, 126.95, 127.05, 127.55, 128.02, 128.67 (aryl - CH)*. 130.33, 131.16, 132.32 (quarternary ArC) 181.94 (CHO); IR 1590 (br), 1245 (br), 813, 784, 751; MH^{+} m/e 299 (100).

* Note considerable variation in intensity was observed, and some smaller peaks. Anal: Calcd for $C_{23}H_{14}O_2$ C, 84.54, H, 4.75% Found C 83.78, H 5.18%.

2-(1-Benzo(a)pyrenyl)malondialdehyde (4)

This was prepared in exactly the same manner as for 2-(1-pyrenyl)-malondialdehyde (46), except that crude (64) obtained above (75mg, 0.21 mmol), ethanol (4 mL) and sodium hydroxide (0.4 mL, 25%) were used. The product (11mg) was isolated, subjected to a 2-methylindole (25) test, and the presence of a weak absorption at ca. 565 nm was taken as an indication that the product was obtained.

Attempted Oxythallation procedure [49]

(1) Preparation of reagent:-

Thallium trinitrate (24.5g, 0.055 mol) was added to a stirred mixture of trimethylorthoformate (62.5 mL) and methanol (50 mL) and the stirring continued until all the thallium salt had dissolved. K-10 Montmorillionite clay (55g) was then added in one portion through a powder funnel; the funnel was rinsed with methanol (12.5 mL) and the

mixture stirred for a further 5 minutes. The solvents were then removed by evaporation to give the thallium trinitrate/K10 reagent as a free flowing powder (ca. 85g).

(2) Use of reagent.

The reagent obtained above (1.20g, ≡ 7.77 mmol TTN) was added to a stirred solution of cinnamaldehyde (1.00g, 7.58 mmol) in dichloromethane (10 mL). The suspension was stirred until a starch iodide test for thallium (III) was negative (ca. 10 minutes). The spent reagent was removed by filtration, the filtrate washed with aqueous sodium bicarbonate, water, then dried over magnesium sulphate. The solvent was evaporated, and the products investigated by NMR.
Only partial conversion to cinnamaldehyde dimethylacetal was indicated by presence of peaks at δ 4.9 (-CH $(OMe)_2$) and δ 3.25 (-CH $(OMe)_2$).

Thiobarbituric acid test

Aqueous 1% thiobarbituric acid solution (1 mL) was added to a solution of the compound to be tested (ca. 10 mmol) in acetone (5 mL) Concentrated hydrochloric acid was added (5 drops) and the mixture warmed at 80°C for 20 minutes. The UV spectrum was recorded immediately using a reference cell containing actone (5 mL), TBA reagent (1 mL), and hydrochloric acid (5 drops) warmed as above, on a Beckmann DU 8 Recording Spectrophotometer.

2-Methylindole test[35]

2-Methylindole (25) reagent (0.126g in 100 mL ethanol) (1 mL) was added to the substance to be tested (ca. 10 mmol) in acetone (5 mL). Glacial acetic acid was added (10 drops) and the mixture heated at 80°C for 1 hour. The UV spectrum was recorded immediately with a reference cell containing reagent (1 mL), acetone (5 mL), and acetic acid (10 drops) warmed as above on a Beckmann DU 8 Recording Spectrophotometer.

Ames Test:-

The method for detecting mutagenicity was the Salmonella/ mammaliam microsome test described by Ames et al.[74] The Salmonella typhimurium strains were TA 110 which detects frameshift mutagens; TR 2705 which detects uvr B repair system; TA 100 which detects base pair substitutions; and TA 98 which detects -2 frameshifts. These were kindly provided by Professor B. N. Ames (Berkeley). The microsomal fractions (S-9) were obtained from rat liver homogenates after induction by a polychlorinated biphenyl mixture (Aroclor 1254). The protein concentration in the S-9 fractions, as determined by the Lowrey method, was consistently around 50 mg/mL. Fresh microsomes were frozen in 2mL aliquots to -79°C, then stored in liquid nitrogen at -196°C until needed. The S-9 mix was prepared as described except that NADP was made up and sterilized by Millipore filtration immediately before use. Test compounds were dissolved in DMSO and stored in the dark at -20°C.

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MeO-CH=CH-CHO

29

β-Methoxyacrolein

EtO-CH=C-CHO

Me

65

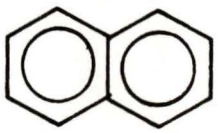
2-Methyl-3-ethoxyacrolein

EtO-CH=C-CHO

66

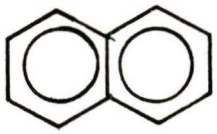
2-Phenyl-3-ethoxyacrolein

EtO-CH=C-CHO



68

2-(1-Naphthyl)-3-ethoxyacrolein



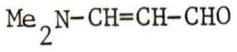
C-OEt

C-CHO

67

2-(2-Naphthyl)-3-ethoxyacrolein

N,N-DIMETHYLAMINOACROLEINS



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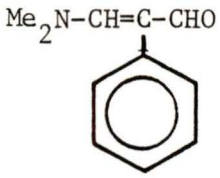
3-N,N-Dimethylaminoacrolein



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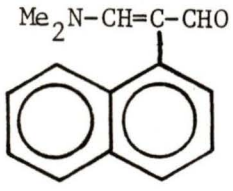
2-Phenyl-3-N,N-dimethylaminoacrolein



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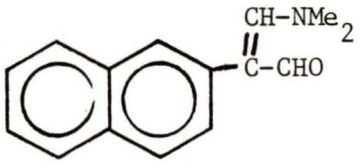

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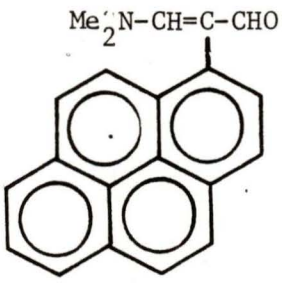

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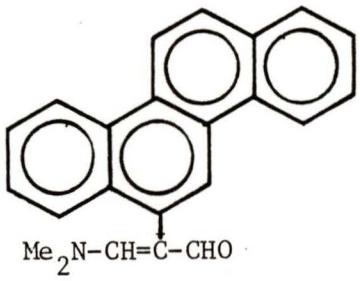
2-(1-Pyrenyl)-3-N,N-dimethylaminoacrolein



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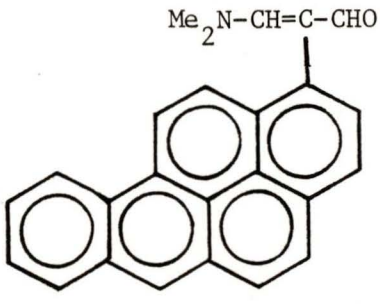
2-(6-Chrysenyl)-3-N,N-dimethylaminoacrolein



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2-(1-Benzo(a)pyrenyl)-3-dimethylaminoacrolein

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