DESIGN OF NOVEL NUCLEAR SUBSTITUTED STYRYL KETONES.
EVALUATION FOR ANTITUMOR, CYTOTOXIC
AND ANTIMICROBIAL ACTIVITIES

A Thesis
Submitted to the Faculty of Graduate Studies and Research
in Partial Fulfilment of the Requirements
For the Degree of
Doctor of Philosophy
in the Department of Chemistry and Chemical Engineering

by

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Saskatoon, Saskatchewan
May, 1978

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TO MY PARENTS
ABSTRACT

The preparation, mass spectral properties, antineoplastic, antimicrobial and other pharmacological properties of 1-(hydroxyphenyl)-1-nonen-3-ones and related \( O \)-benzoyl esters, \( O \)-ethers and Mannich bases are presented. The effect of a Mannich base on the incorporation of labelled precursors into certain biopolymers is outlined.

Mass spectral data, spectra and fragmentation patterns of the compounds prepared are given. Of particular interest are the fragmentation patterns of 1-(orthosubstituted phenyl)-1-nonen-3-ones which showed the dependence of the formation of the benzopyrylium ion on the leaving group abilities of the ortho-substituent as well as the stabilities of the resultant benzopyrylium ions.

The alkaline hydrolysis of the \( p \)-substituted benzoyl esters of 1-(hydroxyphenyl)-1-nonen-3-ones was undertaken in order to seek a correlation between the rate of hydrolysis of the esters and the \textit{in vivo} P388 lymphocytic leukemia screening data. All the esters were inactive and hence a clearcut correlation was not possible.

The esters showed the expected increase of the second order rate constant as the acyl substituents became more electron withdrawing. This increase in the second order rate constant was more pronounced in the \( p \)-substituted benzoyl esters of 1-(2-hydroxyphenyl)-1-nonen-3-one probably because of the
more effective destabilisation of the crowded transition state. This series of esters had a rho value of 2.43 and the esters of 1-(4-hydroxyphenyl)-1-nonen-3-one had a rho value of 1.84. The alkaline hydrolysis of p-substituted phenyl benzoates was undertaken in order to determine the p value of

\[
0
\]

\[-\text{CH}==\text{CHCC}_6\text{H}_{13},\]

which was found to be +0.25. The rho value of this series of esters was 1.65.

The 1-(alkoxyphenyl)-1-nonen-3-ones showed significantly higher activities against P388 lymphocytic leukemia cells in vivo. Since the ethers do not hydrolyse in basic media, the ethers may be able to reach the target site. The claim has been made that the fluid around some cancer cell systems is more acidic than the fluid surrounding normal tissue cells, and thus the ethers may preferentially hydrolyse to the corresponding active phenol close to the cancer cells. The water soluble Mannich bases of the 1-(hydroxyphenyl)-1-nonen-3-ones showed slightly higher activities compared to the parent compounds. With the exception of the Mannich bases, all the compounds screened were non-toxic at the highest dose used (200 mg/kg).

1-(Hydroxyphenyl)-1-nonen-3-ones and the related Mannich bases showed significant activities. In addition,
the Mannich bases showed marked antihistaminic, analgesic and anti-inflammatory effects.

The effects of (E)-4-dimethylaminomethyl-1-(3,4-dichlorophenyl)-1-nonen-3-one (NC97) on L1210 lymphoid leukemia cells in vitro is described. The incorporation of deoxythymidine and deoxyuridine into DNA was inhibited to the extent of 84-90% and 20-29% respectively at the 1-5/μg/ml dose. This effect is opposite to that exhibited by the clinically used alkylating agents chlorambucil and cyclophosphamide. The incorporation of uridine into RNA was inhibited by 49-66%, while the incorporation of leucine into protein was inhibited 71-79% at the 1-5/μg/ml dose.
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1.0.0.0 INTRODUCTION

1.1.0.0 Introduction to the Disease of Cancer

Cancer has the second highest mortality rate in North America and other industrialised countries, being surpassed only by cardiovascular diseases. There are many cases of cancer originating from viruses, radiation and chemicals, and a large majority of the causes are directly or indirectly related with industrialisation. Since the African and other third world nations are rapidly industrialising, the likelihood of cancer being a major killer in these countries appears rational. Cancer is, therefore, a world wide concern and many researchers in both industrialised and less industrialised countries are involved in research in an effort to combat this plague.

The fundamental problem in cancer is the cancer cell. The cancer cell is not subject to homeostatic control mechanisms of the host, i.e. it undergoes unchecked proliferation. Since the daughter cells of a cancerous cell are cancerous, the problem is believed to be genetic (Busch, 1974).

All cells contain cancer genes; that is, all cells are potentially cancerous. In cancer cells, the genes are derepressed and in non-cancer cells, the genes are repressed which means that cancer can form in any part of the body.

Cancer cells show a number of differences from normal tissue cells. For example, cancer cells have darker
staining nuclei and more chromatin than normal cells and undergo multiple mitoses. Some of the pathological characteristics of cancerous cells are those of invading normal tissue, undergoing metastasis and the loss of contact inhibition (Mercer and Easty, 1961; Abercrombie and Ambrose, 1962) as well as prolonged survival under adverse conditions (Warburg, 1926). Cancer cells have been shown to differ morphologically from normal cells. Thus the microvilli (Mercer and Easty, 1961) found in some cancer cells are non-existent in normal cells, and mitochondria in certain cancers are markedly different from the corresponding normal tissue cells (Smetana, 1970).

Of prime importance to the medicinal chemist are some biochemical differences between normal and cancerous cells. These include elevated t-RNA methyltransferase activity (Lee et al., 1977), abnormal homocysteine metabolism (McCully and Kilmer, 1976), elevated levels of enzymes which operate in the nucleic acid pathway (Bresnick, 1974) and reduction in activity of some mitochondria-specific enzymes (White and Nandi, 1976).

1.2.0.0 Treatment of Cancer

Despite differences claimed between certain cancerous cells and the corresponding normal cells, in the majority of cases, exploitable variants are non-existent since the cancer cell is almost invariably identical to the
normal cell (Bodansky, 1975). The conventional methods of cancer treatment are surgery, radiotherapy and chemotherapy, but all three of these methods have inherent limitations. While tumours which are detected early in their development may respond well to treatment, the situation is different in cases where the disease is already disseminated widely, and where the tumour is inoperable. In these cases, surgery is either palliative or impossible and radiotherapy has to be limited in order to avoid radiation damage to noninfected tissue.

For these reasons, and the fact that patients in less developed nations do not have facile access to surgical and radiotherapeutic treatments, the development of new anticancer drugs is of critical importance, especially as serious disadvantages with currently available antineoplastic drugs are extant. The ultimate goal of cancer chemotherapy is the discovery of a drug that will check the growth of cancer cells or completely destroy them without damaging normal tissues of the host, i.e. a drug of high therapeutic index against neoplastic conditions. The difficulty in producing such a drug is compounded by the several varieties of cancers, lack of specificity of the drug for neoplastic tissues, toxic effects, coupled with the mounting expenses of research.

1.2.1.0 Cancer Chemotherapy

At the present time, the major classes of cytotoxic drugs fall into five groups; namely, antimetabolites, plant
alkaloids, antibiotics, hormones and alkylating agents.

Antimetabolites are compounds that structurally resemble natural body substrates. They act irreversibly in place of the natural body substrates, thus interfering with the normal cell function (Werkheiser, 1961) leading to cell death. Classic examples are the folic acid (Ia) antagonists, for example methotrexate (Ib); antipurines, for example 6-mercaptopurine (IIb) and antipyrimidines, for example 5-fluorouracil (IIIb).

The folic acid antagonists function by affecting the mechanism of folic acid activation. The activation pathway involves the reduction of folic acid (F) to dihydrofolic acid \((\text{FH}_2)\) which is subsequently reduced to tetrahydrofolic acid \((\text{FH}_4)\). The activated \(\text{FH}_4\) is required for the 1-carbon transference occurring in purine and pyrimidine synthesis. The formation of \(\text{FH}_4\) shown below is markedly elevated in rapidly proliferating tissue, such as neoplastic growths.

\[
\begin{align*}
  \text{F} & \xrightarrow{\text{FH}_2-R} \text{FH}_2 \\
  \text{FH}_2 & \xrightarrow{\text{FH}_4} \text{FH}_4 \\
  \text{NADPH} & \xrightarrow{\text{NADP}} \text{NADP} \\
  \text{FAA} & \xrightarrow{\text{FAA}} \text{FAA} \\
  \text{FH}_2 & \xrightarrow{\text{FH}_4} \text{FH}_4 \\
  \text{NADPH} & \xrightarrow{\text{NADP}} \text{NADP}
\end{align*}
\]

FAA = Folic acid antagonist

\(\text{FH}_2-R\) = dihydrofolate reductase

Scheme 1. Blockage of Folic Acid Activation by Folic Acid Antagonists
Two plant alkaloids used extensively in the treatment of cancer are vincaleukoblastine and leukocristine, which are extracted from the periwinkle, Catharanthus roseus (Cutts et al., 1960; Johnson et al., 1963; Whitelaw and Kimm, 1964). In addition, colchicine and demecolcine derived from the autumn crocus, Colchicum autumnale, have been used for the treatment of leukemia (Sokal and Krauss, 1964). A number of investigators are engaged currently in
examining alkaloids for possible anticancer activities (Kupchan et al., (a) 1971; (b) 1976; (c) 1977; Kupchan and Streelman, 1976; Dominguez et al., 1976; Miles et al., 1977).

A number of antibiotics have been screened for antineoplastic activities and several have reached clinical trials. These drugs act at various points in the sequence of DNA to RNA to protein. As of January, 1975, five antibiotic anticancer drugs had been released for clinical use in U.S.A. Bleomycin and mitomycin were shown to interfere with DNA synthesis while dactinomycin and doxorubicin were shown to inhibit both DNA and RNA synthesis, but their effects on RNA synthesis and, therefore, on the synthesis of protein, appeared to be more important. Ribosomal-RNA synthesis is inhibited by dactinomycin at doses that have little or no effect on the synthesis of t-RNA, m-RNA and DNA. Minthramycin was shown to have little effect on DNA synthesis (Martin, 1977).

Hormones are chemicals that regulate metabolic processes in the body. They are responsible for stimulating or inhibiting cell growth. The steroid hormones are specific for cancers arising from tissues responsive to these hormones. Estrogens have been used successfully in the treatment of carcinoma of the prostate gland (Huggins and Hodges, 1941) and in metastatic mammary cancer in postmenopausal patients (Escher and Kaufman, 1961). A number of progestins have been
noted to cause remission of the metastatic carcinoma of the endometrium (Kelly and Baker, 1961) and adrenocortical hormones produce temporary remissions in acute lymphoblastic leukemia (Ellison, 1956) and to a smaller extent in lymphomas. Androgens are mainly associated with the treatment of breast cancer in premenopausal patients (Cole, 1970).

The final major group of chemotherapeutic agents available for treating cancer is the alkylating agents and it is studies with this group of compounds that form the basis of this thesis. Biological alkylating agents may be defined as compounds that transfer alkyl groups to cellular nucleophiles such as amino or thiol groups.

Research into alkylating agents as potential anti-cancer agents was derived from studies of the physiological action of two war gases; namely, sulphur mustard gas during World War I and nitrogen mustard gas during World War II. A number of nitrogen mustards having the general structure \( R-N(CH_2CH_2Cl)_2 \) have clinical utilities and are used today. The nature of the substituent on the nitrogen atom \((R)\) has been varied, for example, phenyl and substituted phenyl groups as well as amino acid and sugar residues.

Mustine hydrochloride (IV) was the first difunctional alkylating agent to be used clinically. This compound has many disadvantages, including powerful vesicant properties, high chemical reactivity and non-selectivity for tumourous
cells. Since the advent of mustine hydrochloride, many nitrogen mustards have been synthesised for clinical use. The most widely used of these are melphalan (V) and cyclophosphamide (VI).

\[
\begin{align*}
\text{IV} & : \quad H - \begin{array}{c}
\text{CH}_2\text{CH}_2\text{Cl} \\
\text{CH}_2\text{CH}_2\text{Cl} \\
\end{array} \cdot \text{HCl} \\
\text{V} & : \quad \text{HOOC-CH-CH-CH-Cl}_2 \\
\text{NH}_2 & \\
\text{VI} & : \quad \text{N(CH}_2\text{CH}_2\text{Cl})_2 \\
\end{align*}
\]

Other groups of alkylating agents have been studied and in some cases, namely, polyaziridines, dimethane sulfonate esters and diepoxides, clinically acceptable compounds have resulted. A polyaziridine, triethylenemelamine (TEM) (IX), was known as a cross linking agent in the textile industry (Boesen and Davis, 1969a). Since it was believed that alkylating agents owe their activity to cross-linking with the near opposite N-7 guanine residues in DNA (Stock, 1971), triethylenemelamine and other polyfunctional ethyleneimine compounds were evaluated for anticancer activities. Some of the most active compounds in this group are organophosphorous derivatives and where the ethyleneimine ring has
been attached to quinone and heterocyclic nuclei, e.g. Thio TEPA (VII), Trenimon (VIII) and TEM (IX).

The dimethanesulphonate esters have the general structure (X) and the most widely used derivative is busulfan (X, n = 4). A number of diepoxides have been prepared as biological alkylating agents for use in the treatment of cancer and diepoxybutane (XI) and eponate (XII) showed promise in preliminary animal experiments. However, the clinical results have been disappointing (Boeson and Davis, 1969b).
The need for additional biological alkylating agents in the treatment of cancer has been stressed recently (Connors, 1969; Heidelberger, 1969). A group of compounds that merit consideration in this regard are α,β-unsaturated ketones. While cyclic α,β-unsaturated ketones have been the subject of considerable interest and pharmacological evaluations, details of the screening results of acyclic α,β-unsaturated ketones is of far sparser occurrence. In addition, detailed, systematic investigations in this latter series of compounds regarding chemical constitution and biological activity is woefully neglected and this thesis is a contribution to partially rectifying this situation. A brief review of α,β-unsaturated ketones is presented, along with other data pertinent to the scope of this investigation.

α,β-Unsaturated ketones are capable of alkylating cellular nucleophiles, via the Michael reaction (Scheme 2). Of the many nucleophiles studied, it has been found that thiols are the most active towards activated olefinic linkages (Friedman et al, 1965; Kupchan, 1970).

\[
\begin{align*}
&\text{R}^1\text{H} \quad \text{H} \quad \text{H} \quad 0 \\
&\text{N} \quad \text{R}^1 \quad / \quad / \\
&\text{C} = \text{C} \\
&\text{H} \quad \text{C} = \text{O} \\
&+ \quad \text{RSH} \\
&\text{R}^2 \\
&\text{C} = \text{O} \\
&\text{R} \\
&\text{C} = \text{O} \\
&\text{SR} \\
&\text{R}^2 \\
&\text{R}^2
\end{align*}
\]

Scheme 2. Alkylation of Thiols by α,β-Unsaturated Ketones.
The importance of the olefinic bond for cytotoxicity in carbonylenes has been shown by the reduction (Kupchan, 1971; Lee et al., 1972) and cysteine addition (Kupchan et al., 1970) of the olefinic bond adjacent to a carbonyl group in the sesquiterpenoid lactones. In addition, a marked decrease in cytotoxic activity was observed when the olefinic bond adjacent to the carbonyl function in glaucatubulone ester quassinoid (XIII) was reduced (Kupchan, 1976).

\[
\begin{align*}
\text{XIII} & \quad \text{R} = (a) \text{ H, (b) acyl} \\
\end{align*}
\]

\[
\begin{align*}
\text{XIV} & \quad \text{R}^1, \text{R}^2 \quad \text{H, O, C=C, CH}-(\text{CH}_2)_4\text{CH}_3, \text{CH}_2\text{N(CH}_3)_2\cdot\text{HCl}
\end{align*}
\]
As Scheme 2 indicates, the reactivity of the β carbon atom of α,β-unsaturated ketones will be enhanced by electron withdrawing groups and diminished by electron donating groups. When \( R^1 \) is a substituted phenyl group, the nature of ring substituents which have different \( \sigma \) values, will mean a corresponding variation in the charge of the β carbon atom. While optimal antineoplastic and cytotoxic activities depend on a plethora of variables, a correlation between the charge of the β carbon atom and pharmacological activity may emerge. In the case of a series of Mannich bases (XIV), such a correlation appeared to exist (Dimmock and Taylor, 1975), where increasing the fractional positive charge of carbon atom 1 was accompanied by an increase in activity against P388 lymphocytic leukemia.

The cytotoxicity of a number of drugs containing hydroxy groups has been abolished on removal of the hydroxy function indicating the importance of this group in conferring biological activities. A study of some substituted benzylidene acetophenones (chalones) against Ehrlich's ascites sarcoma in mice showed that chalcones in which there were hydroxy groups on the benzoyl ring retarded the increase in the volume of the ascitic fluid and the presence of hydroxy groups on the benzylidene ring checked the increase in the number of cells in the ascitic fluid (Kabiev and Veremenichev, 1971). Moreover, it was shown that ortho hydroxy chalcones
were more effective in controlling tumour growth than the para and meta isomers. It is of interest to note that in a series of substituted acrylophenones, the presence of an ortho hydroxy group was important in conferring significant antimicrobial activity (Geiger, 1948). Acetylation of the 16-hydroxy group of fabacein (XV) led to reduced cytotoxic activity (Kupchan, 1973). This observation was interpreted as a possible destruction of the activation of $\alpha,\beta$-unsaturated keto group to nucleophilic attack as a result of the loss of the 16-hydroxy function.

In a cytotoxicity test of five flavonoids obtained from Lychnophora affinis (XVI) in cell cultures of human carcinoma of the nasopharynx (KB screen), it was found that only the compounds with hydroxy substituents showed activity (Le Quesne et al., 1976).
It has been suggested that the hydroxy group at position 3 of hymenoxon (XVII), a sesquiterpenoid lactone from *Hymenoxys Odorata* DC (Bitterweed), may play a significant role in the toxicity of this compound (Pettersen and Kim, 1976).
1.2.2.0. Latentiation of Phenols

The hydroxy group of phenolic compounds is susceptible to rapid metabolic inactivation in vivo. The two major routes of metabolism of phenolic compounds are conjugation of the hydroxy group to form the aryl glucosiduronic acids and secondly, conjugation to form the mono-esters of sulphuric acid (Williams, 1959).

\[
\text{ArOH} \xrightarrow{\text{conjugation}} \text{ArOSO}_3\text{H} + \text{ArO} \begin{array}{c} \text{COOH} \\ \text{OH} \\ \text{OH} \end{array}
\]

The hydrophilic nature of the metabolites hinders their transportation through cell membranes and are they generally readily excreted via the kidneys. Furthermore, the hydroxy group may, by hydrogen bonding, form intermolecular complexes of sufficient size to hinder interaction with receptor sites (Rice et al, 1977).

To overcome some of the problems of administering phenolic derivatives in vivo, the concept of drug latentiation can be employed. This process is defined as the chemical modification of a biologically active compound which generates the parent molecule in vivo (Harper, 1962).

It has been noted that the C-15 ester function of (XIII) is required for antileukemic activity and it is possible that the ester group is required for transportation purposes since variations in the ester function have little effect upon in vivo activity of (XIII) (Kupchan, 1976).
The ester prodrugs may undergo alkaline hydrolysis before reaching the target areas. This situation can be remedied by the use of ethers as protecting groups. Ethers are stable under basic conditions. This concept has been used in preparing (XVIIIb) which is a latentiated form of acetaminophen (XVIIIa), the prodrug hydrolysing rapidly and completely releasing (XVIIIa) under conditions similar to those encountered in the gastrointestinal tract (Repta and Hack, 1973).

\[
\begin{align*}
\text{XVIII} & \quad \text{XIX} \\
\text{(a) } R = H & \quad \text{(b) } R = \quad \text{O}
\end{align*}
\]

The cell membrane is a sandwich of a lipid centre between two layers of proteins. It is apparent, therefore, that those molecules with a high lipid/water partition coefficient will diffuse fastest into the cell. A study of the effect of chain length of alkylated resorcinols XIX on antibacterial activity showed that activity increases to maximum at \( n = 6 \), then falls off to zero when \( n = 8 \) (Wilson et al, 1977). The
explanation for this is that as the value of n increases, the partition coefficient increases, thus allowing more drug to penetrate the cell and thus kill that cell. However, as the value of "n" increases beyond six, very little of the drug is present in the aqueous phase, so that a correspondingly small amount of drug is able to reach the cell.

Drugs may also cross the cell membrane by "mediated transport" (Albert, 1968). Special receptor substances called "translocators" catalyse the transfer of particular molecules into the cells. This "carrier hypothesis" involves very specific reactions by means of which metabolic energy is used to either form or open special covalent links in cell membranes.

Whereas it is essential that cytotoxic drugs be able to cross the cell membranes, it has been observed that some degree of water solubility is essential for biological activity (Lappas, 1976). Mannich bases have been shown to have antitumor properties (Schoenenberger et al., 1970; Taylor, 1973). Formation of the hydrochloride or other salts of the Mannich bases would impart water solubility to the parent compounds. In the body, the Mannich bases could undergo either elimination (Tramontini, 1973) giving α,β-, α',β'-unsaturated ketones which are difunctional alkylating agents, or reverse Mannich reaction giving the starting ketone (Riviere, 1960).
1.3.0.0. **α,β-Unsaturated Ketones as Potential Antimicrobial Drugs**

It was noted in 1945 that the antibiotics Clavicin XX and penicillic acid XXI have olefinic bonds adjacent to the carbonyl function and that the activity of these compounds was reduced by the presence of sulphhydryl compounds such as cysteine and thioglycollate (Geiger and Conn, 1945).

Many compounds containing α,β-unsaturated keto functions have since been shown to exhibit antimicrobial properties (Morgan et al., 1969; McGowan et al., 1948; Misra, 1976; Geiger, 1948; Ishida et al., 1960 and Taylor, 1973).

1.3.1.0 **Miscellaneous Biological Activities**

The high saluretic and diuretic activity of some diacylvinyllaryloxy acetic acids XXII has been attributed to the presence of the olefinic bond in conjunction with the carbonyl group (Bicking et al., (a) 1976; (b) 1976).
It has been proposed that these compounds act by binding the sulphhydryl-containing enzymes (Schultz et al., 1962; Komorn and Cafruny, 1965). Chalcones have been shown to have anthelmintic (Laliberte et al., 1967), spasmolytic (Formanek et al., 1958), choleretic (Formanek and Hoeller, 1961) and many other properties which have been reviewed recently (Dimmock and Wong, 1976).

1.4.0.0 The Synthesis of \( \alpha,\beta \)-Unsaturated Ketones and Related Compounds

1.4.1.0 The Aldol and Related Reactions

The aldol and related reactions involve removal of an \( \alpha \)-hydrogen by a base to give a carbanion (equation 1). The carbanion then adds onto the carbonyl carbon of an aldehyde or ketone, resulting in an alcohol (equations 2 and 3). If the alcohol contains an \( \alpha \)-hydrogen, dehydration leading to an \( \alpha,\beta \)-unsaturated ketone or aldehyde may occur (equation 4).
Proton abstraction and addition are fast equilibria, so that equation 2, the addition step, is rate determining.

The aldol and related reactions vary in the nature of the substrate containing the active hydrogen and that containing the carbonyl components. Some of these reactions are the Knoevenagel reaction, the Perkin reaction, Darzens
reaction, the Wittig reaction, the Thorpe reaction and the Claisen-Schmidt reaction. In this study, only the aldol, Claisen-Schmidt and the Knoevenagel reactions will be considered.

1.4.1.1 The Aldol Condensation

In the aldol condensation, the \(\alpha\)-carbon of an aldehyde or ketone adds to the carbonyl carbon of another aldehyde or ketone. This condensation can be either acid or base catalysed. However, the reaction is usually carried out under basic conditions. The product of the aldol condensation is a \(\beta\)-hydroxy aldehyde or ketone and is called the aldol or ketol, respectively (equation 3). The aldol condensation is reversible.

Five aldehyde/ketone combinations are possible in aldol condensations. These combinations are (i) two molecules of the same aldehyde, (ii) two molecules of the same ketone, (iii) two different aldehydes, (iv) two different ketones and (v) aldehyde/ketone combination.

In the cases of both (i) and (ii), each gives one self condensation product while the combinations summarised in (iii), (iv) and (v) above, on the other hand, give two different condensation products as well as two different cross-condensation products if both substrates have \(\alpha\)-hydrogens. This situation is undesirable where a large yield of one of the products is required.
In the condensation of an aromatic aldehyde with a ketone, the aromatic aldehyde does not undergo self condensation, but gives a cross condensation product. The condensation of an aromatic aldehyde with a ketone is called the Claisen-Schmidt reaction.

1.4.1.2 The Claisen-Schmidt Reaction

When the Claisen-Schmidt condensation is carried out between an aromatic aldehyde and a methylalkyl ketone, the nature of the product is dependent on whether a basic or acidic catalyst is used. When a basic catalyst is used, the aldehyde condenses with the methyl group, whereas when an acidic catalyst is used, the aldehyde condenses with the \( \alpha \)-methylene group of a methyl alkyl ketone.

\[
\text{ArCHO} + H_2\text{CCCH}_2\text{R} \underset{\text{base}}{\xrightleftharpoons{\text{acid}}} \text{ArCH}=-\text{CCH}_2\text{R} \\
\text{ArCHO} + H_2\text{CCCH}_2\text{R} \underset{\text{base}}{\xrightleftharpoons{\text{acid}}} \text{ArCH}=\text{CCH}_3
\]

The intermediate product of a Claisen-Schmidt reaction is a ketol, equation 3. In the presence of strong acidic and basic catalysts and relatively high temperatures, the intermediate ketol may dehydrate to form an \( \alpha,\beta \)-unsaturated ketone. The base catalysed dehydration is believed to proceed via the enolate anion and then elimination of the hydroxide
anion (equation 5).

\[
\begin{align*}
\text{Ar}-\text{CH}-\text{CH}-\text{C} & \rightleftharpoons \text{Ar}-\text{CH}-\text{C}=\text{C} & \rightleftharpoons \text{ArCH}=\text{CH}-\text{C} \\
\text{OH} & \uparrow & \text{OH} \\
\end{align*}
\]

Equation 5

The product of the Claisen-Schmidt reaction has the carbonyl group stereoselectively **trans** to the larger group at the \( \beta \) carbon atom, i.e. the product is the (E) geometrical isomer (Hassner and Mead, 1964; Brink, 1969; House and Ro, 1958; Zimmerman, 1963; Taylor, 1973). Scheme 3 shows the mechanism for the preferred formation of the (E) isomer. The carb anon (A) from equation 5 undergoes free rotation along the \( \text{C}_\alpha - \text{C}_\beta \) bond to give the conformation (B) which in turn loses OH to give the olefinic product via the transition state (C). In the transition state (C), there is partial bond breakage of \( \text{C} - \text{OH} \) and partial bond formation between \( \text{C}_\alpha \) and \( \text{C}_\beta \) with a concomitant depletion of the electron density of the anion electron pair. The accompanying Newman projections are viewed along the \( \text{C}_\alpha - \text{C}_\beta \) bond.
Scheme 3. Mechanisms of Preferred Formation of (E) Isomer in Claisen-Schmidt Condensations
House (1972) has given a possible explanation for the preferred formation of the (E) isomer; namely, that the conformation (B) of the carbanion is energetically more favoured than the conformation (A) because there is less steric interaction between the planar enolate system and the substituent Ar at the β carbon atom. House further notes that a more important reason may be that the transition state (C) leading to the trans product has the two large groups, Ar and COR\textsuperscript{1} anti.

The reactivity of the carbonyl group of the aromatic aldehyde depends on the nuclear substituents. Electron withdrawing substituents decrease the electron density at the carbonyl carbon making it more susceptible to nucleophilic attack. Electron donors have a reverse effect. Since the rate determining step involves the addition of the enolate anion to the carbonyl carbon of the aldehyde, the rate of the Claisen-Schmidt reaction might be expected to be related to the Hammett σ values of the nuclear substituents. The Hammett equation is discussed on page 47 of this thesis.

It is, however, interesting to note that the Claisen-Schmidt reaction often fails to produce the desired products if the nuclear substituent is strongly electron withdrawing, for example o-, m- and p-nitro, p-cyano and p-carbamoyl.

(Zimmer and Rothe, 1959; Taylor, 1973; Misra, 1977). Different reaction conditions are, therefore, required for the synthesis of styryl ketones of such nuclear substituents.
One such reaction is the Knoevenagel reaction.

1.4.1.3 The Knoevenagel Reaction

The Knoevenagel reaction is closely related to the Claisen-Schmidt reaction. This reaction involves the treatment of an aldehyde or ketone with an active methylene compound in the presence of catalytic amounts of secondary amines and at least a catalytic amount of an acid. The active methylene compounds used in classical Knoevenagel reactions include esters of malonic, acetoacetic and cyanoacetic acids, as well as phenylacetonitrile, benzylketones and aliphatic nitro compounds. The reactions are usually carried out in refluxing anhydrous benzene with a provision for a continuous removal of water.

A proposed mechanism of the Knoevenagel reaction is given in equations 6 and 7. The nuclear substituted aryl-aldehyde reacts with a secondary amine, then the acid, followed by dehydration to give an iminium cation (equation 6). The iminium cation then reacts with the enol form of the ketone to give an aldol-like intermediate (equation 7). The aldol-like intermediate breaks down to give the desired styryl ketone and the secondary amine, equation 8.

\[
R\text{-CHO} + HNR_2 \xrightleftharpoons{H^+} \underset{R}{\text{CH=NR}_2}^+ 
\]

Equation 6
1.4.2.0 The Mannich Reaction

The Mannich reaction is an important synthetic route for introducing a basic function, which, when transformed into the ammonium salt renders the molecule water soluble. The Mannich reaction is also an important biosynthetic route in natural products.

The Mannich reaction involves the condensation of formaldehyde and primary or secondary ammonium salt with a compound containing an active hydrogen, for example the enolisable
α-hydrogen of ketones and aldehydes and nitroalkanes. The reaction is carried out under acidic conditions in protic solvents like ethanol, methanol, isopropanol, water and acetic acid. Although ammonia and primary amines may be used for the condensation, the resultant Mannich base may condense with one or two additional molecules of formaldehyde and active hydrogen compound. Secondary amines, particularly dimethylamine, piperidine, pyrrolidine and morpholine, are the most commonly used bases in Mannich reactions.

The mechanism of the Mannich reaction is shown in equations 9 to 11. Equation 9 is the same as equation 6. The iminium cation then reacts with the ketone to give a protonated β-aminoketone, equation 10, which loses the proton to give β-aminoketone, equation 11.
Equation 11

If there is more than one active hydrogen, the Mannich base may condense with one additional molecule of aldehyde and the amine to form the bis product, XXIII.

\[
R^1\overset{\text{O}}{\longrightarrow}C\overset{\text{CH(CH}_2\text{NR}_2)_2}{\longrightarrow}
\]

XXIII

The position of aminomethylation of nuclear substituted arylalkyl ketones, XXIV, is pH dependent.

XXIV
It has been observed that if the substituent, R, is electron donating, for example 4-hydroxy and 4-methoxy, aminomethylation in acidic media takes place at the α-methylene of the alkyl group. In basic media, however, it was observed that aminomethylation takes place at the position ortho to the nuclear substituent (Gautier et al., 1964; Brandes and Roth, 1967). A proposed mechanism is outlined in equations 12 - 14.

\[
\begin{align*}
\text{R-O-C-CH}_2\text{R}^1 & \rightarrow \text{R-O=}
\end{align*}
\]

Equation 12

\[
\begin{align*}
\text{R-O-C-CH}_2\text{R}^1 + \text{CH}_2\text{N(CH}_3\text{)}_2 & \rightarrow \text{R-O=}
\end{align*}
\]

Equation 13

\[
\begin{align*}
\text{D} & \rightarrow \text{R-O-C-CH}_2\text{R}^1 + \text{HCl}
\end{align*}
\]

Equation 14
A possible explanation for the pH dependence is that under acidic conditions, the hydroxy and methoxy groups may be protonated and, therefore, electron withdrawing, thus, preventing electrophilic aromatic substitution as proposed in equations 12 to 14.

14.3.0 Mass Spectrometry of Styryl Ketones

In the biochemical sciences, mass spectrometry is becoming an invaluable tool in the identification of drug metabolites. It is particularly suited for this purpose because the metabolites, usually obtainable in nanogram quantities, differ from their precursors by a single chemical group only.

Mass spectrometry also has a wide applicability in natural products. It has been used for the determination of the location of functional groups in long chain fatty acids, peptide sequencing and confirmation of structures of proteins sequenced by classical methods as well as the sequencing of oligosaccharides. Other uses of mass spectrometry are determination of molecular weights and empirical formulae, structure elucidation and isomeric differentiation.

The mass spectral fragmentation pattern of ketones is, in general, uncomplicated, giving abundant molecular ions which break down by well known pathways, namely, the α-, β- and γ-fissions.

α-Cleavage is the rupture of the bond between the carbonyl carbon and either of the α-carbon atoms. The charge is usually retained on the carbonyl fragment ion (the acylium
In dialkylketones, the more intense peak at 70 eV is that resulting from the loss of the larger alkyl group. Alkyl aryl ketones behave similarly; undergoing α-fission to give the alkyl carbonyl as well as the phenyl carbonyl fragment ions. If, however, the aryl alkyl ketone has nuclear substituents, the extent of the phenyl-carbonyl carbon bond fission is affected by the nature of the substituent, being favoured by electron withdrawing substituents. In such cases, the charge is retained on the arylcarbonyl component.

In the case of unbranched α,β-unsaturated ketones, α-cleavage occurs such that the resultant fragment ion contains the olefinic bond.

\[
\begin{align*}
RCH\equiv CH - C - R^1 & \rightarrow RCH\equiv CH - C\equiv O + \\
\end{align*}
\]

The acylium ion resulting from α-scission subsequently loses carbon monoxide, usually with a concomitant appearance of a metastable peak.

\[
\begin{align*}
R\equiv C\equiv O & \rightarrow R^+ \\
\end{align*}
\]

When the alkyl chain attached to the carbonyl group has three or more carbon atoms, β-cleavage, with an exclusive
transfer of a γ-hydrogen atom to the carbonyl oxygen atom occurs through a six-membered cyclic transition state (Willhalm, B. and Thomas, 1965; Beard et al., 1965). This cleavage is called the McLafferty rearrangement. The McLafferty rearrangement may be concerted, as illustrated below, or stepwise (McLafferty, 1966). The products of the McLafferty rearrangement are an olefin and an enol ion radical. The enolic radical does not tautomerise to the keto form (Budzikiewicz et al., 1967).

![Equation 15](image)

Equation 15

If R in equation 15 has three or more carbon atoms, a second McLafferty rearrangement is possible. The enolic ion radical fragments through a six-membered cyclic transition state to give a secondary McLafferty product, equation 16 or 17.

![Equation 16](image)

Equation 16
In branched alkyl ketones, the McLafferty rearrangement preferentially involves the larger alkyl group which gives a more stable alkene. \(\gamma\)-Hydrogen atom migration preference is primary < secondary < tertiary, resulting, respectively, in progressively more highly substituted and hence more stable neutral alkenes. If, on the other hand, the McLafferty rearrangement is thought of as a stepwise process, preference for radical formation is primary < secondary < tertiary radical.

Simple \(\gamma\)-cleavage gives less intense ions than the \(\alpha\)- and \(\beta\)-cleavage ions cited above, but is preferred over simple \(\beta\)- or \(\delta\)-cleavage. The enol form of the ketone may fragment at the \(\gamma\)-C-C bond giving a stable allylic radical. Alternatively, the keto form may fragment to give a four-membered cyclic oxonium ion.
Styryl alkyl ketones and alkylcinnamates exhibit the normal α-, β- and γ-fission fragment ions. In addition, it has been noted that they also exhibit M-1 fragmentations. These ions have been shown by deuterium labelling experiments to arise through loss of an aromatic hydrogen atom (Beynon et al., 1959; Itagaki et al., 1966; Ronayne et al., 1966). It has been suggested (Clausen et al., 1966; Ronayne et al., 1966) that the M-1 ion arises from intramolecular substitution in the molecular ion, thus forming a relatively stable benzopyrilium ion, XXVI.

In a recent study of some nuclear substituted styryl ketones, Smith and co-workers (Smith et al., 1972) observed intense [M-substituent] peaks. These peaks were, however, observed only when the substituent was ortho to the styryl double-bond, suggesting that the attack of the carbonyl oxygen occurs only at the position ortho to the styryl double-bond (Scheme 4).
Scheme 4. Mechanism for the Formation of Benzopyrillum Ions

The loss of the ortho substituent leading to a benzopyrillum ion has also recently been observed in a study of arylidene cycloalkanones (Aizenshtat et al., 1977), iso-flavine and 2'-substituted isoflavines (Eguchi et al., 1977), and 2,2'-disubstituted stilbenes (Mintas et al., 1977).

It might be expected that, for steric reasons, the formation of the intermediate XXV (R > H) would be retarded when the attack occurs at the carbon bearing the substituent, thus resulting in greater peak intensity of the \([M - H]\) fragment ion. Smith and co-workers (Smith et al., 1972), however, found that when \(R = \text{Ortho} - \text{Cl}\), the ratio of the peaks is \([M - \text{Cl}]/[M - H] \approx 36\), suggesting that the steric
factors are relatively unimportant compared to the leaving group ability of the ortho-substituent. It was, therefore, of interest to study the effect of ortho-substituents of varying leaving group abilities.

1.5.0.0 Ester Formation and Hydrolysis

Ester formation in acid, equation 18, is formally the reverse of ester hydrolysis.

\[
\text{R'}\text{COOH} + \text{ROH} \rightleftharpoons \text{R'}\text{COOR} + \text{H}_2\text{O}
\]

Equation 18

The forward and reverse reactions pass through the same transition state. In practice, however, ester formation and hydrolysis are carried out under different reaction conditions. In particular, ester formation between carboxylic acids and alcohols under alkaline conditions is, in general, not feasible because of the formation of a carboxylate anion.

Esterification of ionised carboxyl groups can be achieved by reaction of the carboxylic acid in the presence of p-toluenesulfonyl chloride in pyridine, equations 19 and 20.

\[
\text{R'}\text{COO}^- + \text{ArSO}_2\text{Cl} \xrightarrow{\text{pyridine}} \text{R'}\text{COOSO}_2\text{Ar} + \text{Cl}^-
\]

Equation 19
Esters may be formed by reaction of the carboxylic acid with a carbonium ion, equations 21 and 22.

\[
\begin{align*}
(CH_3)_3CCl & \rightarrow (CH_3)_3C^+ + Cl^- \\
R'COOH + (CH_3)_3C^+ & \rightarrow R'COOC(CH_3)_3 + H^+
\end{align*}
\]
Equation 21

Aryl alkanoates may be prepared from phenol and the corresponding alkanoyl anhydride; for example, substituted phenyl acetates may be prepared from substituted phenols and acetic anhydride, equations 23 and 24.

\[
\begin{align*}
\text{Equation 23}
\end{align*}
\]
Arylbenzoate esters may be prepared from benzoyl chlorides and the corresponding phenols, equation 25.
Ester hydrolysis may proceed by a number of pathways, involving either acyl-oxygen or alkyl-oxygen bond cleavage. Since the bond fissions involve nucleophilic substitution, they may proceed either by an unimolecular or bimolecular mechanism. Both acid and base catalysed ester hydrolyses are possible; thus making eight possible pathways of ester hydrolysis. Ingold proposed a shorthand notation for the eight mechanisms, namely $B_{AC1}$, $B_{AC2}$, $B_{AL1}$, $B_{AL2}$, $A_{AC1}$, $A_{AC2}$, $A_{AL1}$ and $A_{AL2}$ (Ingold, 1953). "B" and "A" refer to base and acid catalysed hydrolyses, respectively, "AC" and "AL" refer to acyl-oxygen and alkyl-oxygen cleavage, respectively. "1" and "2" refer to the molecularity of the rate determining step.

$A_{AL2}$ and $B_{AC1}$ have not been observed; $B_{AL2}$ and $A_{AC1}$ have been observed in strongly alkaline and acid media respectively, and even then, only rarely. $B_{AL1}$ and $A_{AL1}$ are generally observed during the hydrolysis of esters of tertiary alcohols and secondary alcohols that give stable carbonium ions. $A_{AC2}$ is the most common mechanism for acid hydrolysis and $B_{AC2}$ is the most common mechanism for alkaline hydrolysis of esters.

1.5.1.0 Mechanism of the $A_{AC2}$ Reaction

It is well-established that the $A_{AC2}$ reaction is first order in both ester and acid concentrations. Equations 26 to 29 outline the mechanism of the $A_{AC2}$ reaction. Protons transfer to the starting ester, equation 26, and from the
product acid, equation 29, are fast equilibria. The rate constant $k_4$ of equation 27 and $k_5$ of equation 28, are large, whereas $k_3$ and $k_6$ are small.

\[
R'\text{--C--OR} + H^+ \overset{k_1}{\underset{k_2}{\rightleftharpoons}} R'\text{--C--OR}^+ + \text{H}_2\text{O}
\]

Equation 26

\[
R'\text{--C--OR}^+ + \text{H}_2\text{O} \overset{k_3}{\underset{k_4}{\rightleftharpoons}} \text{HO--C--OR} + H^+
\]

Equation 27

\[
\text{HO--C--OR}^+ + H^+ \overset{k_5}{\underset{k_6}{\rightleftharpoons}} R'\text{--C--OR}^+ + \text{ROH}
\]

Equation 28

\[
R'\text{--C--OH}^+ \overset{k_7}{\underset{k_8}{\rightleftharpoons}} R'\text{--C--OH} + H^+
\]

Equation 29
Applying the steady state approximation to the above equations leads to the rate expression (equation 30).

\[
\text{Rate} = \frac{k_1 k_3}{k_2} [\text{Ester}][H^+] - \frac{k_6 k_7}{k_8} [\text{ROH}][\text{Acid}][H^+]
\]

Equation 30

If \( \frac{k_1 k_3}{k_2} \gg \frac{k_6 k_7}{k_8} \), then

\[
\text{Rate} = \frac{k_1 k_3}{k_2} [\text{Ester}][H^+]
\]

Equation 31

Equation 31 is the relationship observed for the \( A_{AC2} \) reaction.

1.5.2.0 Base Catalysed Ester Hydrolysis

The hydroxide anion, is one of the most commonly used bases in the alkaline hydrolysis of esters (Kirsch et al., 1968; Chaw et al., 1971; Humffray and Ryan, 1967; Capon and Ghosh, 1966; Cooper and Williams, 1962). However, nitrogen containing bases, particularly imidazole, have been extensively studied (Bender and Turnquest, 1956; Bruice and Schmir, 1956; Bruice and Benkovic, 1966; Wallerberg et al., 1971). Imidazole is of particular interest because the imidazole ring of the histidine residue of the hydrolytic enzymes has been shown to be responsible for the catalytic activity of these enzymes (Doherty and Vaslow, 1952; Wagner-Jauregg and Hackley, 1953; Hammond and Gutfreud, 1955).
The most common base catalysed ester hydrolysis is the $B_{AC2}$ pathway. It can proceed by two possible routes; namely, (i) direct attack of the carbonyl carbon of the ester by a nucleophile, equations 32 to 33,

\[ \text{Nu} \rightarrow \text{Nu} \quad \text{Equation 32} \]

or (ii) indirect attack by nucleophile, equations 34 to 36.

\[ \text{Equation 33} \]

\[ \text{Equation 34} \]

\[ \text{Equation 35} \]
Equations 34 to 36 represent a general catalysis mechanism (Bamford and Tipper, 1972). Both direct and indirect mechanisms are overall second order, being first order in both the ester and the nucleophile and are, therefore, kinetically indistinguishable. The general base catalysis involves an entrophy lowering termolecular process, making it less favourable than the bimolecular direct attack.

If, in the direct attack process, the nucleophile "Nu" is a much better leaving group, i.e. a much weaker base, than the leaving group RO:, the intermediate breaks down to give the starting materials, i.e. \( k_2 \gg k_3 \). In this case, nucleophilic or direct attack catalysis will not occur. Since, however, the hydroxide anion is one of the poorest leaving groups, alkaline hydrolysis catalysed by the hydroxide anion will invariably proceed by nucleophilic or direct attack. General base catalysis is mainly observed for esters with strongly electron-withdrawing acyl substituents and poor leaving groups (Bamford and Tipper, 1972).

The hydroxide catalysed ester hydrolysis is well known to be first order in both ester and hydroxide anion concentrations and involves acyl-oxygen cleavage (Bunton et al.,
1956a, 1957b, 1957c, 1961d; Bender et al., 1956a, 1957b, 1958c. The reaction proceeds by nucleophilic attack since the product of partial removal of a proton from water gives a species less reactive than the hydroxide anion. A proposed mechanism of hydroxide catalysed ester hydrolysis is given in equations 37 to 39.

\[
R' - \text{C} - \text{O} \quad + \quad \text{OH} \quad \xrightarrow{k_1 \quad k_2} \quad R' - \text{C} - \text{OH} \\
\]

Equation 37

\[
R' - \text{C} - \text{OH} \quad \xrightarrow{k_3 \quad k_4} \quad R' - \text{C} - \text{OH} \quad + \quad \text{RO}^+ \\
\]

Equation 38

\[
R' - \text{C} - \text{OH} \quad + \quad \text{RO}^+ \quad \rightarrow \quad R' - \text{C} - \text{O} \quad + \quad \text{ROH} \\
\]

Equation 39

The overall reaction is formally reversible, but in basic media, the carboxylic acid is readily ionised to the carboxylate anion, equation 39, which is not susceptible to nucleophilic attack, i.e. the equilibrium constant for the
proton transfer is very high and, therefore, the reaction is practically irreversible. Esterification in basic media does not, therefore, occur.

Applying the steady state approximation to equations 37 to 39 gives equation 40.

\[
\text{Rate} = \frac{k_1 k_3 \text{[Ester][OH]} + k_3 k_4 \text{[RCOOH][RO]}}{k_2 + k_3}
\]

Equation 40

Since the last step is practically irreversible, [RCOOH] and [RO\textsuperscript{-}] are very small, and, therefore, the new approximation is equation 41.

\[
\text{Rate} = \frac{k_1 k_3 \text{[Ester][OH]}}{k_2 + k_3}
\]

Equation 41

If \(k_3 \gg k_2\), the rate is given by equation 42.

\[
\text{Rate} = k_1 \text{[Ester][OH]}
\]

Equation 42

Equation 42 is the relationship observed for B\textsubscript{AC}2 reactions.
1.5.3.0 The Hammett Equation

Since the rate of alkaline hydrolysis of esters is largely determined by the rate of addition of hydroxide anion to the carbonyl group of the esters, any substituent which withdraws electrons from the carbonyl group will increase the reactivity of the ester. Electron-donating substituents will decrease the reactivity of the ester. The most successful quantitative measure of the polar effect is the Hammett reaction constant, rho, obtained from the Hammett equation, 43.

The Hammett equation is a linear free energy relationship first postulated by Hammett (Hammett, 1940). The Hammett equation was derived from the dissociation constants of substituted benzoic acids. If $K_{R-C_6H_4COOH}/K_{C_6H_5COOH}$ represents the relative dissociation constants of meta- and para-substituted benzoic acids relative to the unsubstituted benzoic acid and $k_R/k$ are the relative rates of reactions of a series of meta- and para-benzenoid compounds, then the Hammett equation may be represented by equation 43.

$$\log \frac{k_R}{k} = \sigma \rho$$

Equation 43

Where $\log \frac{K_{R-C_6H_4COOH}}{K_{C_6H_5COOH}} = \sigma$ since Hammett arbitrarily assigned rho = 1.0 for the ionisation of the benzoic acids in water at 25°.
The parameter $\sigma$, is characteristic of the substituent and represents polar effects, both inductive and resonance. The parameter $\rho$, is a characteristic of the reaction series and represents the overall sensitivity of the reaction to polar effects. Electron-withdrawing substituents have positive sigma ($\sigma$) values, whereas electron donors have negative $\sigma$ values. As a result, if a rate constant is increased by electron-withdrawing substituents, the $\rho$ value will be positive. If, on the other hand the rate is increased by electron-donating substituents, $\rho$ value will be negative. Kirsch et al. (1968) have determined the $\rho$ values for the alkaline hydrolysis of acyl-substituted phenyl benzoates ($\rho = +2.021$) and aryl-substituted phenyl benzoates ($\rho = +1.238$).

Because of steric effects, the Hammett equation generally fails in case of aliphatic and ortho-substituted benzene derivatives. One of the pioneering works in the study of steric effects in alkaline hydrolysis of esters is that of Taft (Taft, 1952). Taft proposed a linear free energy relationship, equation 44, similar to the Hammett equation.

$$\log \frac{k}{k^0} = fA$$

Equation 44

where $f$ is the reaction constant, a proportionality factor giving the susceptibility of the series to structural effects; and $A$ is a substituent constant obtained as $A = \log \frac{k}{k^0}$ when $f = 1.0$. 
A more recent study (Nishioka et al., 1975) has shown a good correlation between the logarithmic value of the second order rate constant of the alkaline hydrolysis of ortho-substituted phenyl esters and steric as well as field effects (equation 45).

\[ \log k = \rho \sigma + \delta E_s + \beta F + C \]

Equation 45

where \( E_s \) is the Taft-Kutter-Hansch steric effect constant and \( F \) is the Swain-Lupton-Hansch field effect constant.

1.6.0.0 Sites of Action of Alkylating Agents

Drugs act at different sites in living organisms and the principal target sites of xenobiotics are (i) DNA, RNA and processes associated with the biosynthesis and function of these compounds, (ii) mitochondria, (iii) enzymes, (iv) cell membranes and (v) nonspecific cellular constituents. The primary site of action for a number of alkylating agents involve (i) above, particularly DNA, while recent studies, however, indicate that in addition, alkylating agents interfere with respiratory processes in the mitochondria.

1.6.1.0 Effect of Alkylating Agents on DNA

It has been claimed that a number of biological alkylating agents cause alterations of the structure of nucleic acids (Kishimoto and Lieberman, 1964; Crathorn and
The suggestion has been made that DNA is the primary target site and that the cytotoxic effects of bifunctional alkylating agents are due to alkylation and cross-linking at N-7 of the guanine residues in DNA (Harrap and Gascoigne, 1976; Brookes and Lawley, 1961; Lawley and Brookes, 1967).

A number of observations, however, indicate that the site of action of biological alkylating agents is more complex than being simply due to cross-linking of DNA. For example, experiments with radiolabelled alkylating agents in vivo have failed to correlate the sensitivity of the tumour to a drug with the amount of alkylating agent bound to cellular DNA (Wheeler and Alexander, 1964; Trams et al., 1961). In other experiments, no evidence of cross-linking, chain scission, exhaustive deletion of guanine or changes in base composition of DNA was observed in DNA isolated from Lettre-Ehrlich tumour cells following treatment with nitrogen mustard (Golder et al., 1964). Thirdly, no evidence of cross-linking was found in DNA isolated from cultured mouse fibroblast cells treated with nitrogen mustard which inhibited the growth of the cells (Brewer and Aronov, 1963). Finally, although, in general, mainly bifunctional alkylating agents have exhibited cytotoxicity, some monofunctional alkylating agents, notably 5-aziridinyl-2,4-dinitrobenzamide (CB 1954) show good antitumour activity in certain experimental tumours (Ross and Mitchley, 1964). Since these monofunctional compounds cannot cross-link,
they must act at a different site. It has further been shown that the CB 1954-resistant Walker tumour is also resistant to the bifunctional alkylating agent, melphalan, suggesting that CB 1954 and melphalan have a similar mechanism of action (Connors and Melzack, 1971).

The inhibition of DNA synthesis by alkylating agents may be caused by action of the compounds on one or more enzymes involved in DNA synthesis. Certain bifunctional alkylating agents inhibit DNA synthesis with little or no effect on RNA and protein synthesis (Crathorn and Roberts, 1966; Brewer et al., 1961; Levis et al., 1963). However, a recent study has shown monofunctional alkylating sesquiterpenoid lactones to inhibit RNA and protein synthesis and virtually no inhibition of DNA synthesis (Hladon et al., 1977).

The biosynthetic pathways of DNA, RNA and protein are summarised in Figure 1.
Fig. 1. The biosynthesis of the informational biopolymers DNA, RNA and protein.
1.6.2.0 Effect of Alkylating Agents on Mitochondria

Several anticancer agents have been shown to interfere with mitochondrial function (Gosalvez et al., 1976). In a study of the effect of α,β-unsaturated ketones and related Mannich bases on mitochondrial function in the yeast Saccharomyces cerevisiae, a correlation was shown between cytotoxic activities and interference with mitochondrial function. In addition, a correlation between murine toxicity of these compounds and inhibition of mitochondrial function was found (Dimmock et al., 1976a). In a related study, (E)-2-Benzylidenecyclohexanone, which has a high level of cytotoxicity in the KB screen (0.25 μg./ml.) was found to inhibit mitochondrial function in the same yeast (Dimmock et al., 1976b). Furthermore, the effect of alkylating agents on mitochondrial function has been demonstrated by Rutman and co-workers who found significant inhibitions of mitochondrial RNA and protein synthesis as well as specific interference in respiration when Ehrlich ascites tumour cells were treated with mechlorethamine (HN2) (Rutman et al., 1976).

1.7.0.0 The Aims of the Present Investigation

Both cyclic and acyclic α,β-unsaturated ketones have a wide variety of biological properties some of which have been summarised recently (Dimmock and Wong, 1976). Whereas there are many literature references to the biological activities of cyclic α,β-unsaturated ketones, very few systematic studies have been carried out with acyclic
systems.

It has also been found that the presence of hydroxy, ester and ether functions enhance bioactivity in some biological systems. In this study, therefore, the synthesis of nuclear hydroxy styryl ketones, their corresponding O-benzoyl esters and alkyl ethers will be carried out. Since some degree of water solubility is required for bioactivity, Mannich bases of the corresponding nuclear hydroxy styryl ketones will be synthesised.

The second phase of the study involves the screening of the compounds against P388 lymphocytic leukemia in vivo. In vitro dose-response studies will also be carried out using lymphoid leukemia L1210.

The compounds will also be screened against various strains of bacteria and fungi as well as some mammalian disorders.

The rates of alkaline hydrolysis of the esters will be determined and an attempt will be made to correlate the kinetic data with the P388 in vivo data.

In the final phase of the project, an attempt will be made to determine the mechanism involved in anticancer activity. It is believed that alkylating agents exert their activity by reacting with cellular nucleophiles, primarily those found in the DNA to RNA to protein pathway. Labelling experiments will be carried out to determine if the synthesis
of any of the macromolecules is inhibited in the presence of these compounds.
2.0.0.0 RESULTS AND DISCUSSION OF THE EXPERIMENTAL WORK

2.1.0.0 Synthesis of the Alkyl Styryl Ketones and Related Compounds

The compounds were synthesised as shown in Scheme 5, except compounds XXXIIIe, XXXIIIf, and XXXIV which were synthesised by the Knoevenagel reaction.

2.1.1.0 Attempted Preparation of (E)-1-(hydroxyphenyl)-1-nonen-3-ones from Hydroxybenzaldehydes and 2-octanone

The condensation of hydroxybenzaldehydes with 2-octanone in aqueous sodium hydroxide solution yielded brown tars and starting materials. In the presence of sodium hydroxide, the hydroxybenzaldehydes ionise to give electron donating phenoxide anions. Electron donating substituents on the phenyl ring increase the electron density at the carbonyl carbon, thus making it less susceptible to nucleophilic attack. This behaviour has been observed by Zimmer and Rothe (1959) in an attempted base catalysed condensation of 4-hydroxy-3-methoxybenzaldehyde (vanillin) with γ-butyrolactone. In sodium hydroxide, vanillin ionises to form a phenoxide anion.

\[
\begin{align*}
\text{HO} & \quad \text{CHO} \quad \overset{\ominus}{\text{OH}} \quad \overset{\ominus}{:O} \quad \text{CHO} \\
\text{H}_3\text{CO} & \quad \text{H}_3\text{CO}
\end{align*}
\]

\[\sigma_\rho (O^-) = 1.00\]
In the same study (Zimmer and Rothe, 1959) it was noted that condensations with alkoxybenzaldehydes under the same conditions worked well. It was, therefore, decided to protect the hydroxy group of the hydroxybenzaldehydes. Several groups, notably ethers, esters and acetals, may be used for protecting the hydroxy group of phenolic compounds. For this study it was decided to react the phenolic benzaldehydes with either chloromethyl methyl ether or chloromethyl ethyl ether to form the corresponding alkoxy methyl ethers, which are known to be susceptible to facile cleavage in the presence of acid. These syntheses were carried out successfully.

\[
\begin{align*}
\text{OCH}_2\text{OR} & \xrightarrow{\text{H}^+} \text{OH} \\
\text{CHO} + \text{CH}_2\text{OR} & \text{R} = \text{CH}_3, \text{CH}_2\text{CH}_3
\end{align*}
\]

2.1.2.0 Preparation of (E)-1-(alkoxyphenyl)-1-nonen-3-ones

The condensation of the alkoxybenzaldehydes with 2-octanone in the presence of aqueous sodium hydroxide (3% w/v) gave 21 to 67% of the desired products, and, in some cases, resinous dimeric products were formed (mass spectral evidence). The formation of the dimeric products was observed earlier by Heilbron and Irving [1928; 1929] when they carried out base catalysed condensations of alkyl
(n-propyl to n-nonyl) methyl ketones with substituted benzaldehydes. The dimers, usually obtained in low yields (2 - 18%), were originally thought to be cyclobutane derivatives, but were later shown by Nielsen and Dubin (1963) to be cyclohexyl derivatives. The mechanism of cyclisation is shown in equation 46.

\[
\begin{align*}
\text{Equation 46}
\end{align*}
\]

2.1.3.0 **Preparation of (E)-1-(hydroxyphenyl)-1-nonen-3-ones**

Phenylalkyl ethers undergo alkyl carbon oxygen cleavage under acidic conditions. Attempted cleavage of the alkoxymethoxy protecting group using a combination of sulfuric acid and glacial acetic acid gave unidentified resinous products. Attempted cleavage with either glacial acetic acid, trifluoracetic acid, hydrochloric, hydrobromic and hydriodic acids gave the same results, namely, brown tars and resinous products. The desired products were obtained
Scheme 5. Synthetic Routes for Some Derivatives of 1-phenyl-1-nonen-3-one
XXXIII

\[
\begin{array}{ccc}
R^1 & R^2 & R^3 \\
a: & 2-\text{OCH}_3 & 5-\text{OCH}_3 & \text{H} \\
b: & 3-\text{OCH}_3 & 4-\text{OCH}_3 & \text{H} \\
c: & 3,4\text{-methyleneedioxy} & \text{H} \\
d: & 3,4\text{-methyleneedioxy} & \text{CH}_2\text{N(CH}_3)_2\text{.HCl} \\
e: & 3-\text{NO}_2 & \text{H} & \text{H} \\
f: & 4-\text{NO}_2 & \text{H} & \text{H} \\
\end{array}
\]

XXXIV

XXXV

a: \( R = \text{OCH}_2\text{OCH}_3 \)

b: \( R = \text{OH} \)
in good yields (67 to 71%) by heating a mixture of the ether (XXVIIa, d or e) with aqueous formic acid (85%, V/V) under reflux for 15 - 20 minutes.

2.1.4.0 Preparation of (E)-1-(nitrophenyl)-1-nonen-3-ones

The attempted condensation of o-, m- and p-nitrobenzaldehydes with 2-octanone under the Claisen-Schmidt conditions yielded tars and starting materials. The failure of these reactions to produce the desired products may be attributed to the increased activity of the carbonyl carbon of the nitrobenzaldehydes, leading to multitudinous side reactions. For example, in a study of the condensation of γ-butyrolactones with aromatic aldehydes using sodium methoxide as catalyst, low temperatures and short reaction times, it was observed that when the benzaldehyde substituents were o-, m- and p-nitro, p-cyano and p-acetamido, low yields, tars and unidentified noncrystalline side products were obtained (Zimmer and Rothe, 1959). Forbes and Gregory (1968) and Vinnik and Kisлина (1974) have shown that o- and p-nitrobenzaldehydes in aqueous sodium hydroxide are converted to the corresponding phenols.

In the present study, the condensation of m- and p-nitrobenzaldehydes with 2-octanone was subsequently carried out via the Knoevenagel reaction. Prolonged heating under reflux during the synthesis of XXXIIIif produced compound XXXVI. This is understandably in line with the high reactivity of p-nitrobenzaldehyde.
Attempted condensation of o-nitrobenzaldehyde with 2-octanone by the Knoevenagel reaction failed to give the desired product, but gave 3% of orange crystals which have not been characterised.

2.1.5.0 Preparation of (E)-1-(pentafluorophenyl)-1-non-3-one

An attempted condensation of pentafluorobenzaldehyde with 2-octanone by the Claisen-Schmidt reaction gave starting materials and some resinous products. Ivanova and coworkers (1969) noted that pentafluorobenzaldehyde failed to undergo the Claisen-Schmidt condensation with acetaldehyde and with acetone in alkaline media. Earlier, Barbour and coworkers (1961) found that in the presence of aqueous alkali, pentafluorobenzaldehyde forms pentafluorobenzene and formic acid.
It is also of interest to note that the treatment of pentafluorobenzaldehyde with dimethylamine, sodium methoxide, sodium hydrogen sulfide and sodium and potassium thiophenoxides gave products in which one or more fluorine atoms had been replaced by the nucleophile (Aroskar et al., 1968). In this study, the condensation of pentafluorobenzaldehyde with 2-octanone in piperidinium acetate yielded the desired styryl ketone as well as a piperidinyl-tetrafluorobenzaldehyde (mass spectral evidence).

2.1.6.0 Preparation of (E)-4-dimethylaminomethyl-1-(substituted)phenyl-1-nonen-3-ones

The Mannich bases XXIXa, XXIXb and XXXIIIId were obtained in yields ranging from 32 to 38%. Compounds XXIXa and XXIXb were obtained by acid catalysed aminomethylation of XXVIIc and XXVIIe respectively and XXVIIc and XXVIIe simultaneously underwent acid catalysed cleavage of the ether bond to give the phenolic Mannich bases XXIXa and XXIXb. Direct aminomethylation of XXVIIc gave XXIXb in 16% yield and XXXIIIId was obtained by aminomethylation of XXXIIIc. In this case, however, no cleavage of the ether linkage was observed. This is probably due to the poorer leaving group ability of the methylenedioxy function. In all cases, no ring aminomethylation was observed, and although the substrate styryl ketones had two replaceable hydrogen atoms, no multiple product formation was observed. The low yields of the Mannich bases may be due to the reverse Mannich reaction occurring.
Attempted aminomethylation of XXVIIa, XXXIIIa, XXXIIIb and XXXIIIe gave yellow and yellowish green water soluble oils. Trituration of the oils and storage in vacuo for 17 weeks failed to induce crystallisation.

2.1.7.0 Preparation of \( p \)-substituted benzoates of (E)-1-(hydroxyphenyl)-1-nonen-3-ones

The esters XXXa to XXXg, XXXIb and XXXIc were obtained as solids in yields ranging from 59 to 85%. Compound XXXIa, however, was obtained in crude form as a yellowish/orange viscous oil. Tlc separation of the crude mixture showed the presence of the phenolic starting material XXVIIIa. Attempted column chromatographic separation using the same solvent system did not effect separation. On attempted re-distillation in vacuo, the oil turned brown, probably due to decomposition.

2.1.8.0 Preparation of \( p \)-substituted Phenyl Esters of Benzoic Acid

\[
\text{XXXVII} \quad \begin{array}{l}
\text{a: } R = \text{CH}_3 \\
\text{b: } R = \text{H}^1 \\
\text{c: } R = \text{Cl} \\
\text{d: } R = \text{NO}_2
\end{array}
\]

1. Obtained from British Drug Houses.
The esters XXXVIIa, c and d were obtained as solids in yields ranging from 39 to 64% and were purified by recrystallisation from petroleum ether.
2.2.0.0 Mass Spectroscopy of (E)-1-(substituted phenyl)-1-nonen-3-ones

The data for the mass spectral fragmentation patterns is shown in Table 1 for the para- and meta-substituted compounds and Table 2 for the ortho-substituted derivatives. Representative plots for the para- and meta-compounds are given in Figures 2 to 6; while those of the ortho-compounds are given in Figures 7 to 15.

From Table 1, it is observed that the ethers XXVIIId and XXVIIe have, as their base peaks, the ions \([\text{CH}_2\text{OCH}_2\text{R}]^+\), where \(\text{R} = \text{H}, \text{CH}_3\); whereas the ethers XXXIIIb and XXXIIIc which cannot give the stable ion by ether cleavage a undergoes the usual ketone \(\alpha\)-cleavage b.

The fragmentation patterns of XXXIIIb and XXXIIIc are similar and are represented in Scheme 6 for the fragmentation pattern of XXXIIIc.
Table 1. m/e (Relative intensity) values of the principal ions observed in the 70 eV mass spectra of the meta- and para- substituted styryl ketones

<table>
<thead>
<tr>
<th></th>
<th>m/e (Relative intensity) values of the principal ions observed in the 70 eV mass spectra of the meta- and para- substituted styryl ketones</th>
</tr>
</thead>
<tbody>
<tr>
<td>XXVId</td>
<td>290(M⁺,3), 220(5), 180(5), 161(3), 147(5), 122(3), 121(6), 91(4), 78(4), 65(4), 59(100)</td>
</tr>
<tr>
<td>XXVIIe</td>
<td>276(M⁺,11), 206(4), 191(15), 161(3), 151(11), 45(100)</td>
</tr>
<tr>
<td>XXXIIIc</td>
<td>260(M⁺,22), 203(4), 190(50), 175(100), 160(16), 145(47), 135(24), 117(28), 89(48), 63(23)</td>
</tr>
<tr>
<td>XXXIIIb</td>
<td>276(M⁺,22), 245(2), 219(2), 206(20), 191(100), 175(27), 163(13), 150(23), 78(10), 43(10), 41(12)</td>
</tr>
<tr>
<td>XXXVIIId</td>
<td>232(M⁺,15), 175(4), 162(58), 161(9), 147(100), 119(14), 91(17), 65(11), 43(7), 41(8)</td>
</tr>
<tr>
<td>XXXVIIIf</td>
<td>232(M⁺,12), 175(4), 162(57), 161(13), 147(100), 119(31), 107(14), 91(23), 65(15), 43(12), 41(17)</td>
</tr>
<tr>
<td>XXXa</td>
<td>366(M⁺,1), 135(100), 119(1), 107(4), 92(6), 75(8), 65(2), 43(2), 41(3)</td>
</tr>
<tr>
<td>XXXb</td>
<td>350(M⁺,2), 280(2), 265(1), 119(100), 91(16), 65(4), 43(2), 41(2)</td>
</tr>
<tr>
<td>XXXc</td>
<td>336(M⁺,2), 105(100), 91(6), 78(15)</td>
</tr>
<tr>
<td>XXXd</td>
<td>370(M⁺,2), 300(6), 162(4), 147(8), 144(7), 141(39), 139(100), 103(6), 101(20), 91(4), 75(6), 65(2)</td>
</tr>
<tr>
<td>XXXe</td>
<td>414/416(M⁺,0.6/0.6), 386(3), 384(6), 382(4), 358(1), 356(2), 384(1), 331(1), 329(1), 185(99), 183(100), 155(30), 104(8), 76(41), 75(35), 74(11), 50(35)</td>
</tr>
<tr>
<td>XXXf</td>
<td>361(M⁺,3), 291(10), 276(12), 161(3), 145(22), 144(8), 130(100), 102(30)</td>
</tr>
<tr>
<td>XXXg</td>
<td>381(M⁺,2), 311(13), 295(14), 231(7), 150(100), 145(38), 120(20), 104(36), 92(15), 76(18)</td>
</tr>
<tr>
<td>XXXII</td>
<td>372(M⁺,9), 302(26), 287(45), 247(7), 231(8), 161(55), 160(43), 147(13), 146(24), 145(31), 144(17)</td>
</tr>
<tr>
<td>XXXIIIe</td>
<td>141(39), 119(32), 118(14), 91(15), 78(100), 55(7), 51(20), 43(57).</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th></th>
<th>XXXIIIf 361(M⁺,2), 244(3), 243(2), 219(2), 204(6), 191(91), 176(100), 174(48), 144(21), 130(20), 129(27), 102(61), 76(16), 43(27), 41(28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XXXVI</td>
<td>361(M⁺,15), 218(3), 204(6), 191(100), 176(90), 174(22), 145(15), 144(14), 130(32), 118(8), 100(33), 90(7), 76(11), 43(15), 41(18)</td>
</tr>
<tr>
<td>XXIXa</td>
<td>394(M⁺,25), 377(19), 365(8), 352(19), 351(76), 336(17), 258(7), 216(19), 204(16), 202(19), 176(100), 137(18), 136(22), 130(68), 128(44), 118(21), 116(37), 115(40), 102(72), 90(34), 76(23), 55(18), 44(36), 43(26), 41(50)</td>
</tr>
<tr>
<td>XXIXb</td>
<td>289(M⁺-HCl,3), 244(5), 218(4), 201(10), 148(16), 119(5), 91(7), 58(100), 45(12), 44(19) 289(M⁺-HCl,2), 218(3), 147(6), 119(3), 107(3), 91(4), 58(100)</td>
</tr>
<tr>
<td>XXXIIIId</td>
<td>317(M⁺-HCl,3), 246(3), 175(3), 58(100)</td>
</tr>
</tbody>
</table>
Figure 2. Mass Spectrum of (E)-1-(4-hydroxyphenyl)-1-nonen-3-one, XXVIIIc, at 70 eV.
Figure 3. Mass Spectrum of (E)-1-(3,4-methylenedioxyphenyl)-1-nonen-3-one
Figure 4. Mass Spectrum of (E)-1-(3-nitrophenyl)-1-nonen-3-one, XXXIIIe, at 70 eV.
Figure 5. Mass Spectrum of (E)-1-(benzenesulfonyloxyphenyl)-1-nonen-3-one, XXXII, at 70 eV.
Figure 6. Mass Spectrum of 4-dimethylaminomethyl-(E)-1-(3-hydroxyphenyl)-1-mnen-3-one, XXIXb, at 70 eV.
Scheme 6. The fragmentation pattern of (E)-1-(3,4-methylenedioxyphenyl)-1-nonen-3-one, XXXIIIe, at 70 eV
It is of interest to note that the \( \gamma \)-cleavage, \( M^+ + 203 \), probably by ring closure, in Scheme 6 is observed in a number of other compounds discussed later, in preference to the \( \beta \)-, \( \delta \)- and \( \epsilon \)-simple cleavages.

The compounds of the structure XXXVIII, exhibit parent ions that vary in intensity in the order \( R = H (14\%) > ether (7\%) > ester (2\%) \). This may be attributed to the stability of \( R^+ \) relative to the molecular ion. These compounds fragment in a similar manner, giving the substituent \( R \) as the base peak, except when \( R = H, \) ![structure](XXXVIII) R = H, ester, ether

The phenolic derivatives fragment as shown in Scheme 7, for XXVIIIc.

1. Average value
Scheme 7. The fragmentation pattern of (E)-1-(4-hydroxyphenyl)-1-nonen-3-one, XXVIIc, at 70 eV
As in the ether series, Scheme 7 shows the preference for γ-cleavage over the β-, δ- and ε-simple cleavages. The data from Schemes 6 and 7 suggests that in cases where the substituent R does not form a stable fragment ion, the main fragmentation pathway is via the α-cleavage to the carbonyl.

The main feature of the fragmentation of the esters XXXa to XXXg is the loss of the phenolic radical to give the benzoyl ion. From the data, Table 1, it will be noted that as the substituent on the benzoyl group becomes more electron withdrawing, the α-cleavage to the ketone carbonyl and the McLafferty rearrangements become prominent (compounds XXXf and XXXg) which is presumably due to destabilisation of the benzoyl ion by para-electron withdrawers. This receives support from the observation that the p-OCH₃ ester XXXa gives the stable [H₃CO C₆H₄CO]⁺ ion and ions formed by α-cleavage to the ketone carbonyl and McLafferty rearrangements are not observed.

The fragmentation pattern of the benzoyl ion is as follows:

\[
\begin{align*}
\text{[R-} & \text{C} \equiv \text{O}]^+ & \xrightarrow{-\text{CO}} & \text{[R-} & \text{C} = & \text{]}'^+ \xrightarrow{-R'} & \text{[C} = \text{]}^+ \\
\text{m/e 76}
\end{align*}
\]
When $R = \text{Cl}$ or $\text{Br}$, there is an additional loss of $\text{HCl}$ or $\text{HBr}$ respectively to give the ion at $m/e$ 75. When $R = \text{OCH}_3$, the positive charge on the ion is stabilised and as expected, no significant fragmentation is observed. Because of the destabilising effect of the nitro groups, the $p$-nitrobenzoyl ion fragments to give the ions indicated in Scheme 8.

\[ \begin{align*}
\text{[O}_2\text{N-C\equiv O]}^+ & \quad \text{150(a)} \\
\text{[O}_2\text{N-C\equiv O]}^+ & \quad \text{150(b)} \\
\text{m}^* & \quad \text{NO} \\
\text{120} & \quad \text{-CO} \\
\text{104} & \quad \text{-CO} \\
\text{92} & \quad \text{-CO} \\
\text{76} & \quad \text{-NO}_2 \\
\end{align*} \]

Scheme 8. Fragmentation pattern of the $p$-nitrobenzoyl ion at 70 eV
The fact that there is loss of molecular nitric oxide followed by two losses of carbon monoxide implies that in the mass spectrometer, the ion $150(a)$ is first converted to m/e $150(b)$ before fragmentation (Budzikiewicz et al., (a) 1967).

The fragmentation pattern of the sulfonyloxy ester, XXXII is markedly different from that of the benzoyloxy esters XXXa to XXXg. The ester XXXII exhibits a significant parent ion (9%) as well as the $\alpha$- and McLafferty-cleavage ions, 45 and 26% respectively, Scheme 9.
Scheme 9. The fragmentation pattern of (E)-1-(benzenesulfonyloxyphenyl)-1-nonen-3-one, XXXII, at 70 eV
The nitroderivatives XXXIIIe, XXXIIIf, and XXXVI give complex fragmentation patterns. Scheme 10 showing the fragmentation pattern of XXXIIIe at 70 eV is representative of these compounds.

Scheme 10. The fragmentation pattern of (E)-1-(3-nitrophenyl)-1-nonen-3-one, XXXIIIe, at 70 eV
It is of particular interest to note that compounds XXXIIIe and XXXIIIifloseH₂O and OH from their respective parent ions with the loss of H₂O confirmed by the observation of a metastable ion. It is possible that fragmentation proceeds from the enol form of the ketone, equation 47.

\[
\begin{align*}
\text{CH}=\text{C}-\text{C} & \xrightarrow{\text{OH}} \text{CH}-(\text{CH}_2)_2\text{-CH}_3 \quad \text{+} \\
\text{NO}_2 & \quad \text{261} \\
m^* & \quad \text{H}_2\text{O} \\
\downarrow & \\
\text{CH}=\text{C}-\text{C} & \xrightarrow{} \text{CH}-(\text{CH}_2)_2\text{-CH}_3 \quad \text{+} \\
\text{NO}_2 & \quad \text{243}
\end{align*}
\]
The Mannich bases XXIXa, b and XXXIIIId fragment to give a methylene-dimethylamino cation, \( \text{CH}_2=\text{N} \) , which is the only major fragment ion. The fragmentation pattern of Scheme 11 is representative of these compounds.

Scheme 11. The fragmentation pattern of (E)-4-dimethylaminomethyl-1-(3-hydroxyphenyl)-1-nonen-3-one, XXIXb, at 70 eV
The mass spectra of the ortho-substituted compounds is similar to those of the para- and meta- series, differing only in one important feature, the loss of the ortho-substituent. In a study of some ortho-substituted styryl ketones and related compounds [Smith et al., 1972; 1973] it was shown that these compounds undergo intramolecular cyclisation to give the benzopyrylium ion XXVI. It was, therefore, considered of interest to study the effects of ortho-substituents on the aromatic ring on the formation of various benzopyrylium ions in the light of the relative stabilisation of these ions compared with the parent ion, as well as the leaving group abilities of the substituents. The data corresponding to the ortho-substituted styryl ketones is given in Table 2.
Table 2. m/e (Relative intensity) values of the principal ions observed in the 70 eV mass spectra of the ortho-substituted styryl ketones

<table>
<thead>
<tr>
<th></th>
<th>m/e (Relative intensity) values</th>
<th>Σ [M-ortho]</th>
</tr>
</thead>
<tbody>
<tr>
<td>XXVIIIa</td>
<td>232(M⁺,5), 215(M⁺-OH,4), 162(20), 157(22), 148(10), 147(100), 103(38), 91(26), 65(10)</td>
<td>1.0</td>
</tr>
<tr>
<td>XXXVa</td>
<td>344(M⁺,0.7), 283(M⁺-OCH₂OCH₃,2), 225(7), 214(6), 113(6), 45(100)</td>
<td>0.8</td>
</tr>
<tr>
<td>XXXVb</td>
<td>300(M⁺,8), 283(M⁺-OH,3), 232(16), 230(24), 227(11), 225(17), 219(12), 217(65), 216(14), 215(100), 195(14), 159(20), 123(18)</td>
<td>0.5</td>
</tr>
<tr>
<td>XXVIIa</td>
<td>276(M⁺,2), 215(M⁺-OCH₂OCH₃,3), 161(10), 45(100)</td>
<td>1.1</td>
</tr>
<tr>
<td>XXVIIb</td>
<td>290(M⁺,2), 215(M⁺-OCH₂OCH₂OCH₃,6), 147(20), 59(100)</td>
<td>1.4</td>
</tr>
<tr>
<td>XXXIIIa</td>
<td>276(M⁺,44), 245(M⁺-OCH₃,100), 206(12), 192(9), 191(71), 176(39), 175(30), 161(11), 151(9), 148(11), 133(10), 77(12)</td>
<td>18</td>
</tr>
<tr>
<td>XXXIa</td>
<td>336(M⁺,0.6), 215(M⁺-OCOO,2), 105(100), 77(41)</td>
<td>0.5</td>
</tr>
<tr>
<td>XXXIb</td>
<td>285(M⁺,0.5), 215(M⁺-ClOOO,13), 141(35), 139(100), 111(17)</td>
<td>5.8</td>
</tr>
<tr>
<td>XXXIc</td>
<td>381(M⁺,0.5), 215(M⁺-O₂NOCO,21), 161(11), 151(20), 150(100), 145(14), 120(12), 118(11), 104(43), 92(15), 76(15)</td>
<td>5.1</td>
</tr>
<tr>
<td>XXXIV</td>
<td>306(M⁺,5), 236(75), 221(100), 205(9), 204(19), 193(39), 143(14), 99(7), 55(13), 43(23), 41(32)</td>
<td>0</td>
</tr>
</tbody>
</table>

1. The sum of ³⁵Cl and ³⁷Cl was used in the discussion.
Figure 7. Mass Spectrum of (E)-1-(2-methoxymethoxyphenyl)-1-nonen-3-one, XXVIIa, at 70 eV.
Figure 8. Mass spectrum of (E)-1-(2-ethoxymethoxyphenyl)-1-nonen-3-one, XXVIIb, at 70 eV.
Figure 9. Mass Spectrum of (E)-1-(2-hydroxyphenyl)-1-nonen-3-one, XXVIIIa, at 70 eV.
Figure 10. Mass Spectrum of (E)-1-(3,5-dichloro-2-hydroxyphenyl)-1-nonen-3-one, XXXVb, at 70 eV.
Figure 11. Mass Spectrum of (E)-1-(3,5-dichloro-2-methoxymethoxyphenyl)-1-nonen-3-one, XXXVa, at 70 eV.
Figure 12. Mass Spectrum of (E)-1-(2,5-dimethoxyphenyl)-1-nonen-3-one, XXXIIa, at 70 eV.
Figure 13. Mass Spectrum (E)-1-(2-benzoyloxyphenyl)-1-nonen-3-one, XXXIa, at 70 eV.
Figure 14. Mass Spectrum of (E)-1-(2-(4-chlorobenzoyloxy)phenyl)-1-nonen-3-one, XXXIb, at 70 eV.
Figure 15. Mass Spectrum of (E)-1-{2-(4-nitrobenzoyloxy)phenyl}-1-nonen-3-one, XXXIc, at 70 eV.
The ratio $\% \Sigma [M\text{-}ortho]$ is a measure of the ease of formation of the various benzopyrylium ions, XXXIX.

![Diagram](image)

XXXIX

The relative stabilities of XXXIX depend on the electronic effects of the substituent(s) R. Electron withdrawing R groups would destabilise this ion, while electron donors would stabilise the positive charge by electron donation. Table 2 shows that when $R = \text{OCH}_3$, the ratio of 18 is about eighteen times that when $R = \text{H}$ and about 30 times that when $R = \text{diCl}$. When $R = \text{H}$, the ratio range is 1.0 - 1.4, depending on the stability of the radical lost, $\text{HO}^- < \text{CH}_2\text{OCH}_3 < \text{CH}_2\text{OCH}_2\text{CH}_3 \leftrightarrow \text{CH}_3\text{OCHCH}_3$. When $R = \text{diCl}$, the ratio range is 0.5 - 0.8. In this case also the loss of $\text{CH}_2\text{OCH}_3$ is preferred over the loss of $\text{HO}^-$. For the pentafluorosubstituted XXXIV, however, no loss of F$^-$ was observed from the parent ion, either indicating the high instability of the M-F ion or the poor leaving qualities of the fluorine atom.

In all the ortho-substituted styryl ketones used in this study, no peaks corresponding to M-1 were found. Smith and coworkers [(a) 1972] observed the preferred loss of an ortho-chlorine atom over the loss of an ortho-
hydrogen which suggests that the steric factors are relatively unimportant compared to the leaving group ability of the ortho-substituent.

2.3.0.0 Hydrolysis of the Esters

The esters were divided into three series: Series I, compounds XXXa to XXXg; Series II, compounds XXXIa to XXXIc; and Series III, compounds XXXVIIa to XXXVIIa.

SERIES I

\[
\begin{align*}
R & = \text{OCH}_3, \text{CH}_3, \text{H}, \text{Cl}, \text{Br}, \text{CN}, \text{NO}_2 \\
\text{Compound } # & = XXX(a) (b) (c) (d) (e) (f) (g)
\end{align*}
\]

SERIES II

\[
\begin{align*}
R & = \text{H, Cl, NO}_2 \\
\text{Compound } # & = XXXI(a)(b) (c)
\end{align*}
\]
SERIES III

\[
\begin{array}{c}
R = \text{CH}_3, \text{H}, \text{Cl}, \text{NO}_2 \\
\text{Compound \#} = \text{XXXVII(a)(b)(c)(d)}
\end{array}
\]

The second order rate constants \( (k_2) \) for the alkaline hydrolysis of the three series of esters are given in Table 3.

Table 3. Second order rate constants \( (k_2 \text{M}^{-1}\text{min}^{-1}) \) for the hydrolysis of benzoyl esters at 37° C.

<table>
<thead>
<tr>
<th>R</th>
<th>SERIES I</th>
<th>SERIES II</th>
<th>SERIES III</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₂</td>
<td>542.23</td>
<td>1645.83</td>
<td>189.67</td>
</tr>
<tr>
<td>CN</td>
<td>402.68</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Br</td>
<td>71.98</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cl</td>
<td>77.11</td>
<td>91.46</td>
<td>22.62</td>
</tr>
<tr>
<td>H</td>
<td>25.34</td>
<td>19.89</td>
<td>8.46</td>
</tr>
<tr>
<td>CH₃</td>
<td>11.09</td>
<td>-</td>
<td>5.49</td>
</tr>
<tr>
<td>OCH₃</td>
<td>6.47</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CH=CHCC₆H₁₃</td>
<td>-</td>
<td>-</td>
<td>25.34</td>
</tr>
</tbody>
</table>

Solvent: 50% (V/V) aqueous 1,4-dioxane

\([\text{NaOH}]: \quad 7.0 \times 10^{-4} - 2.8 \times 10^{-3}\text{M}\)

Ionic Strength \((\mu)\): 2.8 \times 10^{-3}\text{M}
The major steps involved in the hydrolysis of Series I esters are shown in equations 48 to 50.

Equation 48

Equation 49

Equation 50

The rate determining step has been shown to be the addition step, equation 48 (see section 1.5.2.0 of this thesis), and, therefore, the electronic properties of the substituent R would have a significant effect on the rate of attack of the ester carbonyl carbon by the hydroxy anion. Electron withdrawing substituents decrease the electron density at the ester carbonyl carbon, thus making it more
susceptible to attack by nucleophiles. The electron donating substituents, on the other hand, increase the electron density at the ester carbonyl carbon, making it less susceptible to attack by nucleophiles, and, therefore, decreasing the rate of hydrolysis as compared to the unsubstituted ester. This trend was observed during the study of the hydrolysis of Series I, II and III esters.

In order to ensure that no Michael addition occurred in Series I and II esters, the compound XXXIIIf was run through the same procedure carried out for the esters and no addition was observed.

The Hammett plot for Series I, Figure 16, gave a rho (\(\rho\)) value of 1.84, Table 4. This value is consistent with those of 1.98 - 2.04 obtained by Kirsch and coworkers (1968) for the alkaline hydrolysis of phenyl esters of substituted benzoates in aqueous acetonitrile (33%, V/V) at 25\(^\circ\). Representative plots for the alkaline hydrolysis of Series I esters are given in Figures 17 to 23.
Figure 16. The Hammett Plot for the Alkaline Hydrolysis of $p$-substituted Benzoates of (E)-1-(4-hydroxyphenyl)-1-nonen-3-one at 37°
Table 4. The Rho values for the hydrolysis of benzoyl esters at 37°

<table>
<thead>
<tr>
<th>SERIES</th>
<th>RHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.84</td>
</tr>
<tr>
<td>II</td>
<td>2.43</td>
</tr>
<tr>
<td>III</td>
<td>1.65</td>
</tr>
</tbody>
</table>

\[ \sigma_{\rho(CH=CHCC_6H_{13})} = 0.25 \]
Figure 17. Plot for Alkaline Hydrolysis of (E)-1-[4-(4-methoxybenzoyloxy)phenyl]-1-nonen-3-one in Aqueous 1,4-dioxane (50%, V/V) at 37°
Figure 18. Plot for the Alkaline Hydrolysis of (E)-1-[4-(4-methylbenzoyloxy)phenyl]-1-nonen-3-one in Aqueous 1,4-dioxane (50%, V/V) at 37°
Figure 19. Plot for the Alkaline Hydrolysis of (E)-1-(4-benzoyloxyphenyl)-1-nonen-3-one in Aqueous 1,4-dioxane (50%, V/V) at 37°
Figure 20. Plot for the Alkaline Hydrolysis of (E)-1-[4-(4-chlorobenzoyloxy)phenyl]-1-nonen-3-one in Aqueous 1,4-dioxane (50%, V/V) at 37°
Figure 21. Plot for the Alkaline Hydrolysis of (E)-1-[4-(4-bromobenzoyloxy)phenyl]-1-nonen-3-one in Aqueous 1,4-dioxane (50%, V/V) at 37°
Figure 22. Plot for the Alkaline Hydrolysis of \((E) - 1-[4-(4-cyanobenzoyloxy)phenyl]-1\text{-nonen}-3\text{-one}\) in Aqueous 1,4-dioxane (50%, V/V) at 37°.
Figure 23. Plot for the Alkaline Hydrolysis of (E)-1-[4-(4-nitrobenzoyloxy)phenyl]-1-nonen-3-one in Aqueous 1,4-dioxane (50%, V/V) at 37°
The Series II esters showed a similar trend, except that the electron withdrawing substituents seemed to facilitate the hydrolysis more efficiently than in Series I esters, Table 3. The Hammett plot for Series II esters, Figure 24 gave a $\rho$ value of 2.43, which is significantly larger than that for the Series I esters. This may be attributed to a more effective destabilisation of the transition state, XXXVIII thus making the response to electronic effects of substituents more critical. Representative plots for the alkaline hydrolysis of Series II esters are given in Figures 25 to 27.

[Chemical structure image: XXXVIII]
Figure 24. Hammett Plot for the Alkaline Hydrolysis of p-substituted Benzoates of (E)-1-(2-hydroxyphenyl)-1-nonen-3-one at 37°
Figure 25. Plot for the Alkaline Hydrolysis of (E)-1-(2-benzoyloxyphenyl)-1-nonen-3-one in Aqueous 1,4-dioxane (50%, V/V) at 37°
Figure 26. Plot for the Alkaline Hydrolysis of (E)-1-[2-(4-chlorobenzoyloxy)phenyl]-1-nonen-3-one in Aqueous 1,4-dioxane (50%, V/V) at 37°
Figure 27. Plot for the Alkaline Hydrolysis of (E)-1-[2-(4-nitrobenzoyloxy)phenol]-1-nonen-3-one in Aqueous 1,4-dioxane (50%, V/V) at 37°
The hydrolyses of Series III esters were carried out in order to determine the $\sigma_{\text{para}}$ value of -$\text{CH}=\text{CHCC}_6\text{H}_{13}\$. The second order rate constants are given in Table 3. From the Hammett plot, Figure 28 the $\sigma_{\text{para}}$ value of -$\text{CH}=\text{CHCC}_6\text{H}_{13}\$ was found to be +0.25, i.e. similar to that of chlorine or bromine substituents. The $\rho$ value of 1.65, Table 4, is consistent with the fact that aryl substituents of phenylbenzoates have less effect on the reaction than acylsubstituents. Representative plots for the alkaline hydrolysis of Series III esters are given in Figures 29 to 32.
Figure 28. The Hammett Plot for the Alkaline Hydrolysis of p-substituted Phenyl Benzoates at 37°.
Figure 29. Plot for the Alkaline Hydrolysis of 4-methylphenyl Benzoate in Aqueous 1,4-dioxane (50%, V/V) at 37°
Figure 30. Plot for the Alkaline Hydrolysis of Phenyl Benzoate in Aqueous 1,4-dioxane (50%, V/V) at 37°
Figure 31. Plot for the Alkaline Hydrolysis of 4-chlorophenyl Benzoate in Aqueous 1,4-dioxane (50%, V/V) at 37°
Figure 32. Plot for the Alkaline Hydrolysis of 4-nitrophenyl Benzoate in Aqueous 1,4-dioxane (50%, V/V) at 37°
2.4.0.0 Pharmacological Results of some (E)-1-(substituted phenyl)-1-nonen-3-ones and Related Compounds

2.4.1.0 Antineoplastic Activities

The antineoplastic screening results of the compounds against P388 lymphocytic leukemia in mice are summarised in Table 5. The compounds were non-toxic at 200 mg./kg., except for the Mannich bases which were highly toxic at 200 mg./kg., but non-toxic at 100 mg./kg. The toxicities of structurally related Mannich bases have been attributed, in part, to impairment of mitochondrial function (Dimmock et al., 1976).

The phenolic ketones gave marginal increases of 4 - 12% in the mean survival times of the mice. The low activities of the phenolic ketones may be due to rapid O-glucuronide formation and the resultant ethers are readily excreted. The benzoate ester derivatives showed antineoplastic activities similar to those of the corresponding phenols and the rapid hydrolysis of the esters to the phenols may account for this observation. This hydrolysis is facilitated by the presence of the hydrolytic enzymes in vivo.
### Table 5. Antineoplastic activities of some (E)-1-(substituted phenyl)-1-nonen-3-ones and derivatives against P388 lymphocytic leukemia

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<thead>
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<th>Compound Number</th>
<th>(mg./kg.)</th>
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<th>T/C %</th>
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1. 2 out of six survivors at 200 mg./kg.
2. 1 out of six survivors at 200 mg./kg.
3. No survivors at 200 mg./kg.

* The figures are the ratios of the mean survival time of treated animals to control animals expressed as a percentage and indicate the maximum increases in survival time found.
The ester, XXXII, which was not hydrolysed in aqueous sodium hydroxide (1 M) at 37° was also inactive in the P388 lymphocytic leukemia screen. The ether derivatives showed higher activities than the corresponding phenolic ketones. This may be attributed to the fact that ethers are stable in alkaline media, but hydrolyse readily in acidic media. The claim has been made that the interstitial fluid surrounding certain cancerous growths is more acidic than the plasma of blood afferent to the tumors (Kahler and Robertson, 1943). The ethers XXVIId and XXXIIIa showed the highest activities in the P388 pre-screen. Secondary screening of XXVIId against other tumor systems is currently in progress at the NCI Laboratories.

The inactivity of the nitro compounds XXXIIIe and XXXIIIff against the P388 lymphocytic leukemia cells may be due to the indiscriminate reaction of these compounds prior to reaching the tumorous cells, since the carbon-1 of the nitro styryl ketones has a large partial positive charge because of the powerful electron withdrawing effects of the nitro groups.

The generally low activity of the (E)-1-(substituted phenyl)-1-nonen-3-ones may also be a result of two factors; namely, (i) low bioavailability due to insolubility in water, and (ii) the difficulty in the case of the phenolic compounds due to the molecules being ionised, in transportation across membranes. The water soluble Mannich bases
showed antineoplastic activities that were marginally higher than those of the corresponding parent ketones.

2.4.2.0 Cytotoxic Activities of NC 96 and NC 97

The compounds NC 96\(^1\) and NC 97\(^1\) were chosen for preliminary biochemical studies because these compounds have been shown to have significant activities against P388 lymphocytic leukemia in vivo (Taylor, 1975). The T/C % values of 130 and 142% were obtained for NC 96 and NC 97 respectively.

\[
\begin{align*}
  &\text{NC 96}: R^1 = 2\text{-Cl}, R^2 = 4\text{-Cl} \\
  &\text{NC 97}: R^1 = 3\text{-Cl}, R^2 = 4\text{-Cl} \\
\end{align*}
\]

Both compounds inhibited the growth of L1210 lymphoid leukemia cells in tissue culture. The dose of NC 96 that caused 50% inhibition in growth by L1210 cells was 1.57 \(\mu\text{g./ml.}\), and that of NC 97 was 1.31 \(\mu\text{g./ml.}\), Figure 33. The higher cytotoxic activity of NC 97 is consistent with the higher T/C % in the P388 in vivo screen.

Figure 33. Dose Response Curves for NC 96 (□□□□) and NC 97 (○○○○) against L1210 Cells in Tissue Culture.
2.4.2.1 Effect of NC 97 on Incorporation of Labelled Precursors into Biopolymers

In order to gain some information on the mechanism of action of this series of compounds, the effects of NC 97 on the biosynthesis of DNA, RNA and protein were investigated by studying the incorporation of the respective precursor molecules using L1210 cells. Table 6 shows the percentage inhibition of the incorporation of different precursor molecules into the acid insoluble precipitate of L1210 cells.

2.4.2.2 Effect of NC 97 on the Incorporation of Tritiated Deoxythymidine ($^3$H-TdR) and Tritiated Deoxyuridine ($^3$H-UdR)

The nucleosides deoxythymidine, deoxyadenosine, deoxyguanosine and deoxycytidine, after conversion to their corresponding triphosphates, serve as precursor molecules for the biosynthesis of deoxyribonucleic acid (DNA). DNA is synthesised in four main stages; namely, (i) the biosynthesis of purine and pyrimidine ribonucleoside monophosphates; (ii) the conversion of the ribonucleotides to the corresponding deoxyribonucleotides; (iii) the phosphorylation of the deoxyribonucleoside monophosphates to the corresponding triphosphates; and (iv) the polymerisation of the deoxyribonucleoside triphosphates to yield polydeoxyribonucleotides in the presence of an appropriate DNA template. The reaction
The numbers indicate the following enzymes:

1. Thymidylate synthetase
2. Thymidine kinase (salvage pathway)
2'. Thymidylate kinases (main pathway)
3. DNA polymerases

Scheme 12. Reactions Involved in the Synthesis of Thymidine Nucleotides
is catalysed by DNA polymerases, Figure 1. Deoxythymidine and deoxyuridine share common biochemical pathways for incorporation into DNA. The synthesis of thymidine-5'-monophosphate (dTMP) from deoxyuridine-5'-monophosphate de novo is catalysed by thymidilate synthetase. The "salvage pathway" for the synthesis of dTMP utilises pre-formed thymidine. This reaction is catalysed by thymidine kinase. Thymidine-5'-monophosphate is then converted to deoxythymidine-triphosphates by stepwise phosphorylation, Scheme 12. Therefore, by studying the effects of drugs on incorporation of TdR and UdR, it is possible to determine their effect on DNA synthesis.
Table 6. Percent inhibition of the incorporation\(^1\) of tritiated deoxythymidine (\(^3\)H-TdR), tritiated deoxyuridine (\(^3\)H-UdR), tritiated uridine (\(^3\)H-UR) and tritiated leucine (\(^3\)H-leucine).

<table>
<thead>
<tr>
<th>Dose µg./ml.</th>
<th>(^3)H-TdR</th>
<th>(^3)H-UdR</th>
<th>(^3)H-UR</th>
<th>(^3)H-leucine</th>
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<tr>
<td>5</td>
<td>86</td>
<td>27</td>
<td>59</td>
<td>79</td>
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</table>

1. The leukemia L1210 cells were incubated for four hours with different concentrations of NC 97 and pulsed for 20 minutes with appropriate labelled precursors.
The effect of NC 97 on the incorporation of tritiated deoxythymidine ($^3$H-TdR) and tritiated deoxyuridine ($^3$H-UdR) into DNA was, therefore, studied in order to determine whether or not the synthesis of DNA was affected by these drugs.

The results of the inhibition of the incorporation of the precursor molecules are given in Table 6 and Figures 34 to 37. The incorporation of tritiated thymidine was inhibited to the extent of 84 - 90% by NC 97 at doses of 1 - 5 μg./ml. On the other hand, the incorporation of tritiated deoxyuridine was inhibited only 20 - 29% at the same concentrations of NC 97. This data suggests that the de novo pathway of thymidine synthesis was less affected by NC 97 as compared to the salvage pathway. The decreased incorporation of TdR could be due to inactivation of the kinases or DNA polymerases, or due to direct binding with the DNA template, thus destroying its priming ability. With the present data, it is not possible to determine the actual site of inactivation.
Figure 34. Effects of NC 97 on the Incorporation of Deoxythymidine into Acid Insoluble Precipitate of Cultured L1210 Cells

- O-O 1 μg/ml
- ▲▲ 3 μg/ml
- □□△ 5 μg/ml
Figure 35. Effects of NC 97 on the Incorporation of Deoxyuridine into Acid Insoluble Precipitate of Cultured L1210 Cells

- - - 1 μg/ml
- - - 3 μg/ml
- - - 5 μg/ml
It is of interest to note that the effect of NC 97 is opposite to that of the bifunctional alkylating agents cyclophosphamide, VI, and chlorambucil, XXXIX. These drugs have been shown to selectively inactivate dTMP synthetase in vivo (Srinivasan et al., 1977) and inhibit preferentially incorporation of UdR into DNA in cell culture (Srinivasan, 1977).

2.4.2.3 Effect of NC 97 on the Incorporation of Tritiated Uridine (\(^{3}\)H-UR)

The ribonucleic acids (RNA's) are formed from the precursor nucleosides, adenosine, guanosine, uridine and cytidine. The DNA transcription process dictates the sequence of the nucleotide units in the RNA molecules that are being made under the control of the DNA which acts as a template, Figure 1. The transcription process, mediated by DNA-dependent RNA polymerases, has products that have ribonucleo-
tide sequences complimentary to one of the DNA which was used as a template. Subsequent steps in RNA biosynthesis involve elongation (catalysed by RNA polymerases) and chain termination.

The inhibition of the incorporation of tritiated uridine using L1210 cells was investigated in order to study the effect of NC 97 on RNA synthesis. Other investigators have used this method to study the effects of drugs on RNA synthesis (Connors and Hare, 1975).

The incorporation of uridine was inhibited to the extent of 49-60% at doses of 1 - 5 μg./ml. of NC 97, Table 6 and Figure 36. The inhibition of the incorporation could be a result of one or more effects on the RNA biosynthetic pathway; namely, (i) binding of NC 97 directly onto the DNA template; (ii) inactivation of DNA-dependent RNA polymerase; (iii) inactivation of the RNA polymerases; or (iv) inactivation of the enzymes involved in the conversion of the nucleosides to the corresponding nucleotides.
Figure 35. Effect of NC 97 on the Incorporation of Uridine into Acid Insoluble Precipitate of Cultured L1210 Cells

- 1 µg/ml
- 3 µg/ml
- 5 µg/ml
2.4.2.4 Effect of NC 97 on the Incorporation of Tritiated 1-leucine

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH} - \text{CH}_2 - \text{CH} - \text{COOH} \\
\text{CH}_3 & \quad \text{NH}_2
\end{align*}
\]

Protein synthesis is the final stage in the expression of genetic information. The genetic information is transferred from mRNA to the protein. The initial step in the synthesis of proteins is the activation of the amino acid. This reaction is catalysed by the appropriate amino acid synthetases.

\[
\text{ATP} + \text{amino acid} + \text{Enzyme} \quad \text{(amino acid - AMP)} - \text{Enzyme} + \text{pyrophosphate}
\]

Transfer RNA (tRNA) then recognises a particular aminoacyl-tRNA synthetase so that it can accept the appropriate activated amino acid. The first amino acid in a protein polypeptide chain is methionine with a formyl (CHO) group attached to the free amino group. Formylation takes place after the amino acid has been attached to the tRNA molecule and is catalysed by a transformylase which requires $\text{N}^{10}$-formyl-tetrahydrofolate. The subsequent chain elongation
is catalysed by peptidyl transferase. The final step in protein synthesis is chain termination.

Tritiated $\delta$-leucine was used to study the effect of NC 97 on the synthesis of proteins. The extent of the inhibition of the incorporation of $\delta$-leucine by NC 97 at 1 - 5 $\mu$g./ml. was 71 - 79%, Table 6 and Figure 37. The actual site of the inhibition cannot be determined with the present data as this may take place at any one or more of the protein synthesis steps. Inhibition may also be indirectly due to inhibition of DNA and RNA syntheses.
Figure 37. Effects of NC97 on the Incorporation of Leucine into Acid Insoluble Precipitate of Cultured L1210 Cells

- O-O-O 1 µg/ml
- O-O-O 3 µg/ml
- O-O-O 5 µg/ml

Incubation Time (Hours)
2.4.2.5 Summary

The effect of NC 97 and probably other structurally related styryl ketones seems to be indiscriminate inhibition of the synthesis of the macromolecules. The inhibition occurs in the order DNA > protein > RNA. In DNA synthesis, the dTMP synthetase pathway seems to be affected to a lesser degree than the salvage pathway.

2.4.3.0 Antimicrobial Activities

Table 7 indicates the evaluation of the (E)-1-(substituted phenyl)-1-nonen-3-ones and related compounds for antimicrobial activities. The phenolic ketones showed high potencies against the pathogenic fungi *Trichophyton mentagrophytes* and *Microsporum gypseum*, as well as the yeast *Saccharomyces uvarum*. Further, XXVIIb showed pronounced activity against the fungus *Candida albicans*, whereas XXVIIa showed only marginal activity and XXVIIc was devoid of any activity against this fungus. The ether derivatives showed similar spectra of activities, but the potencies were reduced, except in the case of XXXIIIb that had potencies against the fungi *Trichophyton mentagrophytes* and *Microsporum gypseum* similar to those of the phenolic ketones. The conversion of the phenols to the corresponding esters, however, produced derivatives that showed no activities, except XXXc and XXXg that had marginal activity against the pathogenic fungi *Trichophyton mentagrophytes* and *Microsporum gypseum*. The nitro derivative XXXIIIc also showed high potencies against
Table 7. Antibacterial and antifungal activities of the \((E)-1-(\text{substituted phenyl})-1\text{-nonen-3-ones and derivatives.}\)

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</table>

inactive: minimum inhibitory concentration (MIC) > 500 µg./ml. for both bacteria and fungi

* slightly active: MIC, 100-500 µg./ml. for bacteria
> 10-500 µg./ml. for fungi

** active: MIC, < 100 µg./ml. for bacteria
< 10 µg./ml. for fungi

1. The bacteria designated by numbers 1 to 8 are Escherichia coli (ATCC 8739), Pseudomonas aeruginosa (ATCC 10145), Klebsiella pneumoniae (ATCC 4352), Salmonella typhimurium (G 46), Bordetella bronchiseptica (ATCC 4617), Staphylococcus aureus (ATCC 6538), Streptococcus faecalis (ATCC 8030) and Bacillus subtilis (ATCC 6633).

2. The fungi designated by numbers 1 to 5 are Trichophyton mentagrophytes (ATCC 9533), Microsporum gypseum (ATCC 14683), Aspergillus niger (ATCC 10535), Candida albicans (ATCC 10231) and Saccharomyces uvarum (ATCC 9080).
these fungi. The Mannich bases $\text{XXXIXb}$ and $\text{XXXIIIId}$ showed similar spectra of activities and potencies to the phenols.

In the antibacterial screen, the phenolic derivative $\text{XXIXb}$ showed high potencies against the bacteria Staphylococcus aureus and Bacillus subtilis. In addition, $\text{XXIXb}$ showed high potencies against Klebsiella pneumoniae and Streptococcus faecalis. With the exception of Bacillus subtilis, the ethers, esters, Mannich bases and nitro derivatives were virtually devoid of antibacterial activities.

2.4.4.0 Miscellaneous Activities

The results of the miscellaneous tests are given in Tables 8 and 9. With the exception of the Mannich bases, the compounds generally showed poor activities in the screens listed in Table 8.

Compound $\text{XXVIIIa}$ was devoid of any bioactivity with the exception of the pronounced pressor effect in the cardiovascular screen. Compound $\text{XXVIIIb}$ showed slight analgesic and antidepressant effects, as well as a diminished response to pain. Compound $\text{XXVIIIc}$ showed a very slight inhibition of anaphylactic reaction, as well as a short period of increased motor activity.
<table>
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<th>Anti-inflammatory</th>
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<tr>
<td>XXVIIa</td>
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<tr>
<td>XXVId</td>
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<tr>
<td>XXVIIe</td>
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<tr>
<td>XXXIIIb</td>
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<tr>
<td>XXXIIIc</td>
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<tr>
<td>XXXc</td>
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<td>XXXd</td>
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<tr>
<td>XXXg</td>
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<tr>
<td>XXXII</td>
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<tr>
<td>XXXIIb</td>
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<td>XXXIIc</td>
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<tr>
<td>XXIXb</td>
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<tr>
<td>XXXIIIId</td>
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<td>**</td>
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<td>*</td>
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<td>**</td>
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</tr>
</tbody>
</table>

1 - inactive  
2 * slightly active  
3 ** active
Of the ether derivatives, only XXVIIe showed any significant activity in the analgesic screen. At a dose of 128 mg./kg., a 54% reduction in the number of phenylquinone writhes was found compared to that of acetylsalicylic acid which causes a 50% reduction at a dose of 50 mg./kg. XXVIIe also showed a slight inhibition of the anaphylactic reaction and a slight pressor effect in the cardiovascular screen. The neuropharmacological profile of XXVIIe indicated a reduced sensitivity to pain, as well as intensive grooming and scratching. Compound XXVIIa showed a slight pressor effect and an increase in spontaneous motor activity. Compound XXVIIId showed slight analgesic and anaphylactic activities. It is of interest to note that when XXVIIId was given as a solution in dimethylsulfoxide, it showed a higher activity than when given as a suspension in saline, at the same concentration. Compound XXXIIIc showed a slight pressor response of short duration and a slight inhibition of the anaphylactic reaction. In the antihistaminic screen, XXXIIIc indicated potentiation at low doses and an inhibitory effect at high doses.

The esters showed poor activities, with the exception of XXXg and XXXIb that had significant analgesic activities causing a 59% reduction in phenylquinone writhes at 128 mg./kg. and 50% reduction at 256 mg./kg. respectively. Compound XXXg also indicated a slight inhibition of cutaneous anaphylaxis, and decreased motor activity while XXXIb indicated an increase
in irritability. Both XXXd and XXXIc showed slight analgesic activities, and, in addition, XXXIc showed a slight anti-anaphylactic activity, while XXXd showed a reduced spontaneous motor activity. Compound XXXc showed a slight increase in the mean blood pressure.

The nitro derivatives were completely devoid of any activity.

The Mannich bases XXIXb and XXXIIId had significant activities in all screens except the hypoglycemic tests in which they showed no activity. A detailed breakdown of the more significant activities of the Mannich bases is summarised in Table 6. As indicated in this table, XXIXb and XXXIIId had very significant analgesic and antidepressant activities. A more interesting pharmacological activity, however, was the antihistaminic activities. The dimethylaminoethyl group comprising carbon atom 4 is similar to the general structure found in most antihistaminic drugs. In addition, XXIXb and XXXIIId showed a slight inhibition of histamine-mediated inflammatory and anaphylactic reactions, a stimulant effect on the central nervous system, convulsions and a fall in blood pressure.
Table 9. Details of the miscellaneous activities of the Mannich bases

<table>
<thead>
<tr>
<th>Compound</th>
<th>Analgesic (dose)</th>
<th>Antidepressant (dose)</th>
<th>Cardiovascular (dose)</th>
<th>Antihistaminic (dose)</th>
<th>LD₅₀ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Effect</td>
<td>30 min.</td>
<td>60 min.</td>
<td>BP⁵</td>
<td>HR⁶</td>
</tr>
<tr>
<td>XXIXb</td>
<td>-95.5(64)</td>
<td>-54.5(64)</td>
<td>-34.8(64)</td>
<td>-32.1(20)</td>
<td>-23.2(20)</td>
</tr>
<tr>
<td>XXXIIIId</td>
<td>-99.0(64)</td>
<td>-45.4(64)</td>
<td>-4.34(64)</td>
<td>-39.4(20)</td>
<td>-24.2(20)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>-50.0(50)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>--</td>
<td>-90.0(8)</td>
<td>-49.0(8)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>--</td>
<td>--</td>
<td>--</td>
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<td>--</td>
</tr>
</tbody>
</table>

1. The criterion for activity was as follows: Aspirin, -30 to -50% slightly active, > 50% active; Amitriptyline, (30 minutes) -30% active, (60 minutes) -10% active; Diphenhydramine, -50% active.
2. mg./kg.
3. mg./ml.
4. minimum lethal dose
5. blood pressure
6. heart rate
3.0.0.0 DESCRIPTION OF THE EXPERIMENTAL WORK

3.1.0.0 Preparation of Alkoxymethoxybenzaldehydes

\[ \begin{array}{c}
\text{CHO} \\
\text{OCH}_2\text{OR}
\end{array} \quad R = \text{CH}_3, \text{CH}_2\text{CH}_3 \]

The compounds were prepared by a modified method of Nelson and Wallis (1973). To a solution of the appropriate hydroxybenzaldehyde (61.06 g., 0.50 mole) in 1 l. of diglyme, sodium hydride (24.00 g., 1.00 mole) as a 50% dispersion in oil was added cautiously. Vigorous mechanical stirring was continued during and after the addition of sodium hydride and when the evolution of hydrogen had ceased (after approximately 1.5 hours), 1.00 mole of the appropriate chloromethylalkylether was added, with stirring, in the fume hood. The stirring was continued for another 1.5 hours after which water (300 ml.) was added, and the organic and aqueous layers separated. The aqueous layer was extracted with diethyl ether (3 x 300 ml.) and all the organic fractions combined with the main organic layer. Removal of the solvent afforded the crude product which was purified by distillation to give the desired alkoxymethoxybenzaldehydes as colourless oils, Table 10. The mass spectra of each compound indicated only two prominent peaks; namely, m/e 45 (100%) and m/e 166 (M^+, 7-12%) for methoxymethoxybenzalde-
146

hydes and m/e 59 (H₃CCH₂OCH₂, 100%) and m/e 180 (M⁺, 1 - 3%) for ethoxymethoxybenzaldehydes.
Table 10. Physical Data for Alkoxymethoxybenzaldehydes.

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>R</th>
<th>Yield</th>
<th>B.P.</th>
<th>NMR: Chemical Shift (ppm)</th>
<th>Aromatic</th>
<th>(\text{H} - \text{C} = \text{O})</th>
<th>(\text{OCH}_2\text{O})</th>
<th>(\text{OCH}_3)</th>
<th>(\text{OCH}_2\text{CH}_3)</th>
<th>(\text{OCH}_2\text{CH}_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{O-CH}_3)</td>
<td>68</td>
<td>102(0.55)</td>
<td>6.80-7.87, m, 4H</td>
<td>10.38, s, 1H</td>
<td>5.21, s, 2H</td>
<td>3.47, s, 3H</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{m-CH}_3)</td>
<td>64</td>
<td>95(0.15)</td>
<td>7.05-7.82, m, 4H</td>
<td>9.92, s, 1H</td>
<td>5.32, s, 2H</td>
<td>3.47, s, 3H</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{P-CH}_3)</td>
<td>74</td>
<td>113(0.20)</td>
<td>7.26, d, 2H, J = 8.50</td>
<td>9.89, s, 1H</td>
<td>5.37, s, 2H</td>
<td>3.47, s, 3H</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(\text{O-C}_2\text{H}_5)</td>
<td>63</td>
<td>88(0.14)</td>
<td>6.73-8.32, m, 4H</td>
<td>10.44, s, 1H</td>
<td>5.33, s, 2H</td>
<td>3.78, q, 2H, J = 7.0</td>
<td>1.23, t, 3H, J = 7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{m-C}_2\text{H}_5)</td>
<td>71</td>
<td>82(0.14)</td>
<td>6.87-7.86, m, 4H</td>
<td>, s, 1H</td>
<td>5.25, s, 2H</td>
<td>3.74, q, 2H, J = 7.0</td>
<td>1.20, t, 3H, J = 7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{P-C}_2\text{H}_5)</td>
<td>77</td>
<td>89(0.14)</td>
<td>7.12, d, 2H, J = 9.00</td>
<td>9.58, s, 1H</td>
<td>5.27, s, 2H</td>
<td>3.75, q, 2H, J = 7.0</td>
<td>1.20, t, 3H, J = 7.0</td>
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<td></td>
</tr>
</tbody>
</table>

1. Lit. (Bohnsack and Seibert, 1965) b.p. 100 - 102°/2.5 mm.
2. Lit. (Bohnsack and Seibert, 1965) b.p. 122°/2 mm.
3.1.1.0 Preparation of 3,5-Dichloro-2-methoxymethoxybenzaldehyde

3,5-Dichloro-2-methoxymethoxybenzaldehyde was prepared in 61% yield by the general procedures described previously except that the reaction time was quadrupled. The crude product was recrystallised repeatedly from petroleum ether (b.p. 100 - 120°) to give the required aldehyde as colourless needles, mp. 90 - 92°. NMR (CDCl₃): δ: 7.52 - 7.14 (m, 2, aromatic H), 8.84 (s, 1, CHO), 5.17 (s, 2, OCH₂O) and 3.59 (s, 3, OCH₃). Mass spectrum: m/e, 234/236 (M⁺, 5%) 45 (100%).

3.1.2.0 Preparation of (E)-1-(alkoxymethoxyphenyl)-1-nonen-3-ones

The compounds were prepared by the following general procedure. A mixture of 2-octanone (76.93 g., 0.60 mole), the appropriate alkoxy methoxyl benzaldehyde (0.50 mole) and sodium hydroxide (10 g., 0.25 mole) in water (300 ml.) was heated under reflux with mechanical stirring for 12 - 24 hours. On cooling, the two layers were separated and the aqueous layer washed with benzene (3 x 100 ml.) and combined with the original organic layer. The solvent and excess 2-octanone were
removed using a rotary film evaporator and the remaining crude product was purified by distillation to give the desired ketones as yellow oils (Table 11).

Table 11. Physical Data for (E)-1-(alkoxymethoxyphenyl)-1-nonen-3-ones

<table>
<thead>
<tr>
<th>R</th>
<th>Yield (%)</th>
<th>B.P. (°/mm)</th>
<th>Molecular Formula</th>
<th>Analysis, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>-CH₃</td>
<td>59</td>
<td>178°/0.50</td>
<td>C₁₇H₂₄O₃</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C:73.88</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H:8.75</td>
</tr>
<tr>
<td>m-CH₃</td>
<td>65</td>
<td>177°/0.16</td>
<td>C₁₇H₂₄O₃</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C:73.88</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>H:8.75</td>
</tr>
<tr>
<td>P-CH₃</td>
<td>42</td>
<td>191°/0.70</td>
<td>C₁₇H₂₄O₃</td>
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<tr>
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<td></td>
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<td></td>
<td>C:73.88</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>H:8.75</td>
</tr>
<tr>
<td>O-CH₂CH₃</td>
<td>59</td>
<td>170°/0.10</td>
<td>C₁₈H₂₆O₃</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C:74.45</td>
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<tr>
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<td>H:9.02</td>
</tr>
<tr>
<td>m-CH₂CH₃</td>
<td>67</td>
<td>168°/0.13</td>
<td>C₁₈H₂₆O₃</td>
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<tr>
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<td></td>
<td></td>
<td>C:74.45</td>
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<tr>
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<td></td>
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<td></td>
<td>H:9.02</td>
</tr>
<tr>
<td>P-CH₂CH₃</td>
<td>21</td>
<td>176°/0.10</td>
<td>C₁₈H₂₆O₃</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<td>C:74.45</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>H:9.02</td>
</tr>
</tbody>
</table>

1. On standing, the oil solidified to a very pale yellow waxy solid.
3.1.2.1. Preparation of (E)-1-(3,5-dichloro-2-methoxymethoxy-phenyl)-1-nonen-3-one

The title compound, prepared in 37% yield from 3,5-dichloro-2-methoxymethoxybenzaldehyde and 2-octanone using the previously described procedure was a yellow oil, b.p. 187°/0.25 mm. NMR(CDCl₃): δ: 7.27-7.60 (m, 2, aromatic H), 7.83 (d, 1, C₁H, J₁,₂ = 16.0), 6.76 (d, 1, C₂H, J₂,₁ = 16.0), 2.65 (t, 2, C₄H, J₄,₅ = 7.0), 0.60 - 2.18 [m, 11, (CH₂)₄CH₃], 5.08 (s, 2, OCH₂O) and 3.62 (s, 3, OCH₃). Mass spectrum: m/e 344/346 (M⁺, 0.7/0.4%), 45 (100%).


3.1.2.2 Preparation of (E)-1-(3,4-dimethoxyphenyl)-1-nonen-3-one

Condensation of 3,4-dimethoxybenzaldehyde (30 g., 0.18 mole) with 2-octanone (24.40 g., 0.19 mole) in the presence of sodium hydroxide (4.0 g., 0.10 mole) by the general procedure for the preparation of (E)-1-(alkoxymethoxy-phenyl)-1-nonen-3-ones described earlier, yielded a crude product which was fractionally distilled under reduced pressure to give a yellow oil (29.0 g., 58%) bp. 168°/0.14 mm., that solidified to a waxy solid on standing, as well as an orange viscous oil (4 g.) bp. 183°/0.14 mm. Mass spectrometry indicated that the orange oil had a molecular weight of 552 i.e. twice that of the title compound. Mass spectro-
metry of title compound: m/e 276 (M⁺, 22%), 191 (100%).
Analysis: Calculated for C₁₇H₂₄O₃: C, 73.86; H, 8.76.
Found: C, 73.68; H, 8.73.

3.1.2.3 Preparation of (E)-1-(2,5-dimethoxyphenyl)-1-nonen-3-one
A mixture of 2,5-dimethoxybenzaldehyde (83.09 g., 0.50 mole), 2-octanone (76.90 g., 0.60 mole) and sodium hydroxide (10.0 g., 0.25 mole) in distilled water (300 ml.) was heated under reflux for 12 hours. The crude product obtained was fractionally distilled under reduced pressure to give 2,5-dimethoxybenzaldehyde bp. 105/0.3 mm. (16.5 g., 20%) and the title compound (79.0 g., 58%) as a yellow oil, bp. 181°/0.4 mm. that solidified to a wax on standing. The residual tar remaining in the distillation flask was not analysed. Mass spectrometry of the required ketone: m/e 276 (M⁺, 44), 245 (100%).
Analysis: Calculated for C₁₇H₂₄O₃: C, 73.86; H, 8.76.
Found: C, 74.00; H, 8.62.

3.1.2.4 Preparation of (E)-1-(3,4-methylenedioxyphenyl)-1-nonen-3-one
Condensation between 3,4-methylenedioxybenzaldehyde (23.96 g., 0.16 mole) and 2-octanone (23.10 g., 0.18 mole) in the presence of sodium hydroxide (4.0 g., 0.10 mole) in water (100 ml.) by the general method in which the reactants were heated under reflux for 23 hours, afforded the crude product as a yellow solid (19.8 g.). Recrystallisation of the crude
product from petroleum ether (bp. 100 - 120°) gave the title compound (16.4 g., 39%) as colourless needles, mp. 56.5 - 58° [Lit: (Hedenburg and Wachs, 1948) mp. 61°]. Mass spectrum: m/e 260 (M⁺, 22%), 175 (100%).

Analysis: Calculated for: C₁₆H₂₀O₃: C, 73.81; H, 7.75.
Found: C, 73.92; H, 7.95.

3.1.3.0 Preparation of (E)-1-(hydroxyphenyl)-1-nonen-3-ones

A mixture of (E)-1-(2-methoxymethoxyphenyl)-1-nonen-3-one (10.00 g., 0.036 mole), aqueous formic acid (85% v/v, 15 ml., 0.338 mole), and petroleum ether (bp. 100 - 120°, 250 ml.) was heated under reflux for 20 minutes. On cooling, the layers were separated and the petroleum ether layer evaporated to 50 ml. and refrigerated to give 5.8 g. of crude product. A further quantity (0.41 g.) of material was obtained by refrigerating the dark brown aqueous layer. Repeated recrystallisation of the crude ketone from petroleum ether (bp. 100 - 120°) gave (E)-1-(2-hydroxyphenyl)-1-nonen-3-one (5.6 g., 67%) as pale yellow granular crystals, mp. 101 - 102°.

Mass Spectrum: m/e 232 (M⁺, 5%), 147 (100%).
Analysis: Calculated for C₁₅H₂₀O₂: C, 77.54; H, 8.68.
Found: C, 78.03; H, 8.62.

The corresponding meta and para isomers were prepared in an analogous manner, except that petroleum ether was not added to the reaction mixture. The meta isomer was obtained in 73% yield as a colourless powder, mp. 54 - 56°.
Analysis: Calculated for C\textsubscript{15}H\textsubscript{20}O\textsubscript{2}: C, 77.54; H, 8.68.
Found: C, 77.57; H, 8.93.

The para isomer was obtained in 71\% yield as pale yellow granular crystals, mp. 76 - 77\°.

Analysis: Calculated for C\textsubscript{15}H\textsubscript{20}O\textsubscript{2}: C, 77.54; H, 8.68.
Found: C, 77.89; H, 8.42.

3.1.3.1 Preparation of (E)-1-(3,5-Dichloro-2-hydroxyphenyl)-1-nonen-3-one

(E)-1-(3,5-Dichloro-2-methoxymethoxyphenyl)-1-nonen-3-one (10.0 g., 0.028 mole) was converted into the title compound (6.2 g., 74\%) using the previously described methodology for the preparation of (E)-1-(2-hydroxyphenyl)-1-nonen-3-one. The product was recrystallised from petroleum ether (bp. 100 - 120\°) as pale yellow needles, mp. 104 - 105\°. Mass Spectrum: m/e 302 (M\textsuperscript{+}, 6\%), 215 (100\%).

Analysis: Calculated for C\textsubscript{15}H\textsubscript{18}Cl\textsubscript{2}O\textsubscript{2}: C, 59.81; H, 6.02.
Found: C, 59.89, H, 6.23.

3.1.4.0 Preparation of (E)-1-(nitrophenyl)-1-nonen-3-ones

3.1.4.1 Attempted Preparation of (E)-1-(2-nitrophenyl)-1-nonen-3-one

The methodology used for the synthesis of (E)-1-(4-nitrophenyl)-1-nonen-3-one, described later in this thesis, was employed and yielded a black tar that solidified on standing and was not examined further.
Reduction of the time of heating under reflux to one hour gave shiny orange flakes, that were recrystallised from petroleum ether, mp. 213 - 215°. Yield: 1.0 g., 2.9% based on the yield of the expected product. Mass Spectrum: m/e 404 (M⁺, 0.6%), 84 (100%). NMR (CDCl₃-d): complex. Analysis: Calculated for C₁₅H₁₉NO₃: C, 68.97; H, 7.28; N, 5.36. Found: C, 72.16; H, 7.26; N, 13.74.

Further reduction of the time of heating under reflux to 0.5 hour yielded unreacted 2-nitrobenzaldehyde (43% recovery) and a semi-solid black tar which was not examined.

3.1.4.2 (E)-1-(3-nitrophenyl)-1-nonen-3-one

A solution of 3-nitrobenzaldehyde (15 g., 0.10 mole), 2-octanone (14.1 g., 0.11 mole), piperidine (8.1 g., 0.10 mole) and glacial acetic acid (6.6 g., 0.11 mole) in dry benzene (100 ml.) was heated under reflux with mechanical stirring for 24 hours, cooled and extracted with water (3 x 100 ml.). The organic layer was separated, dried and the solvent was removed using a water aspirator to give a residual brown oil which was fractionally distilled under reduced pressure to yield 3-nitrobenzaldehyde, nmr evidence, mp. 55° (1.2 g., 8%) bp. 97°/0.13 mm. and the desired compound (13.5 g., 52%) bp. 215°/0.10 mm. that solidified to give yellow flakes.

1. During one distillation, the residual black tar exploded violently.
Three recrystallisations of the yellow flakes from petroleum ether afforded the title compound as pale, creamish, flaky crystals, mp. 50 - 51°. Mass Spectrum: m/e 261 (M⁺, 2%), 176 (100%).

Analysis: Calculated for C₁₅H₁₉NO₃: C, 68.97; H, 7.28; N, 5.36. Found: C, 69.04; H, 7.31; N, 5.38.

3.1.4.3 (E)-1-(4-nitrophenyl)-1-nonen-3-one

To a vigorously stirred solution of 4-nitrobenzaldehyde (20.0 g., 0.13 mole) and 2-octanone (19.0 g., 0.15 mole) in dry benzene (200 ml.) was added glacial acetic acid (7.8 g., 0.13 mole) and piperidine (11.1 g., 0.13 mole). The mixture was heated under reflux for 4 hours using a condenser attached to a Dean-Stark trap, during which time, water (3 ml.) was collected. The dark brown benzene solution was cooled, extracted with water (3 x 100 ml.) and the benzene was removed in vacuo. To the residual black tar was added petroleum ether (500 ml.) and the mixture heated under reflux for 2 minutes. After cooling at room temperature for 20 minutes, the yellow solution was decanted, evaporated in vacuo to 200 ml. and after storing the solution in a refrigerator overnight, the resultant yellow needles were collected (8.8 g.). The original black tar was extracted again with petroleum ether (500 ml.) and yielded a further quantity of compound (1.7 g.). The combined crude products were recrystallised from petroleum ether to give the title compound (10.5 g., 30%) as yellow needles, mp. 56 - 57.5°. Mass Spectrum: m/e 261
156

(M⁺, 14), 191 (100%).

Analysis: Calculated for C₁₅H₁₉NO₃: C, 68.97; H, 7.28; N, 5.36. Found: C, 69.49; H, 7.45; N, 5.37.

3.1.4.4 (E)-4-(4-nitrobenzylidene)-1-(4-nitrophenyl)-1-nonen-3-one

\[
\begin{align*}
\text{O}_2\text{N} & \hspace{1cm} C = C - C - C - (\text{CH}_2)_4\text{CH}_3 \\
\text{CH} & \hspace{1cm} \text{H} \\
\text{NO}_2 & \hspace{1cm} \text{CH}
\end{align*}
\]

A mixture of 4-nitrobenzaldehyde (20.0 g., 0.13 mole), 2-octanone (19.0 g., 0.15 mole), glacial acetic acid (7.8 g., 0.13 mole) and piperidine (11.1 g., 0.13 mole) was heated under reflux for 36 hours according to the method employed in the synthesis of (E)-1-(4-nitrophenyl)-1-nonen-3-one. The crude product obtained (0.71 g.) was recrystallised from petroleum ether to give the title compound as fine yellow needles (2.5 g., 7%) mp. 168-170°. Mass Spectrum: m/e 394 (M⁺, 25%), 176 (100%).

NMR (CDCl₃): δ: 8.37-7.26 (m, 11, aromatic H and olefinic H), 2.63 - 2.49 (m, 2, C₅H), 1.55 - 0.79 [m, 9, (CH₂)₃CH₃].
Analysis: Calculated for $C_{22}H_{22}N_2O_3$: C, 66.97; H, 5.63; N, 7.11. Found: C, 64.69; H, 5.77; N, 6.70.

3.1.5.0 Preparation of 4-substituted Benzoyl Chlorides

All the 4-substituted benzoyl chlorides, except 4-methoxybenzoyl chloride, were from commercial sources\(^1\). 4-Methoxybenzoyl chloride was prepared as follows. 4-Methoxybenzoic acid (0.16 mole) was cautiously added to thionyl chloride (0.32 mole) and the mixture heated on a steam bath for 1.5 hours, at the end of which time the evolution of sulfur dioxide and hydrogen chloride gases had ceased. Heating was continued for a further 15 minutes and excess of thionyl chloride was evaporated to yield the crude acid chloride (25.5 g.) as a pale yellow oil, which was used in the synthesis of the esters without further purification.

3.1.6.0 Preparation of 4-substituted Benzoates of 1-(hydroxyphenyl)-1-nonen-3-ones

\[\text{Reactions and structures...}\]

1. See appendix for sources.
These compounds were prepared by the following general procedure. To a solution of (E)-1-(hydroxyphenyl)-1-nonen-3-one (5.0 g., 0.022 mole) in dry pyridine (20.0 ml.) was added the appropriate 4-substituted benzoyl chloride (0.025 mole). The solution was heated on a steam bath for 10 minutes, mechanically stirred at room temperature for 24 hours and then poured onto crushed ice (20.0 g.). When all the ice had melted, the solution was extracted with diethyl ether (3 x 100 ml.) and the organic extract was washed with water (3 x 100 ml.). Removal of the ether in vacuo afforded the crude ester which was purified by recrystallisation from diethyl ether or petroleum ether.

In the case of the attempted preparation of (E)-1-(2-benzoyloxyphenyl)-1-nonen-3-one, the crude product was a yellow viscous oil which gave an orange oil (5.4 g.) bp. 198 - 206°C/0.2 mm., on distillation. TLC of the product run on silica gel G plate using chloroform : ethylacetate : diethylamine (92 : 5 : 3) showed two spots with \( R_f \) values of 0.64 and 0.18, and the spot at \( R_f \) 0.64 corresponded to that of (E)-1-(2-hydroxyphenyl)-1-nonen-3-one. Attempted separation of the reaction components using silica gel column chromatography and the solvent mixture used in TLC did not effect separation.

The oil gradually turned brown or attempted redistillation and the distillation was discontinued.
The physical data for the esters is given in Tables 12 and 13.

Table 12. Physical data for 4-substituted benzoates of 1-(2-hydroxyphenyl)-1-nonen-3-one

```
<table>
<thead>
<tr>
<th>R</th>
<th>Yield %</th>
<th>mp</th>
<th>Molecular Formula</th>
<th>Analysis, %</th>
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<tr>
<td></td>
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<td>Calc.  Found</td>
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<tr>
<td>Cl</td>
<td>68</td>
<td>47-48.5</td>
<td>C_{22}H_{23}ClO_3</td>
<td>C, 71.23</td>
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<td>H, 6.26</td>
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<td>NO_2</td>
<td>59</td>
<td>62-63.5</td>
<td>C_{22}H_{23}NO_5</td>
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<td>H, 6.08</td>
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<td>N, 3.67</td>
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1. Recrystallised from petroleum ether.
Table 13. Physical data for 4-substituted benzoates of 1-(4-hydroxyphenyl)-1-nonen-3-one

![Chemical Structure](R-C-O-O-C==C-C-(CH₂)₅CH₃)

<table>
<thead>
<tr>
<th>R</th>
<th>Yield %</th>
<th>mp</th>
<th>Molecular Formula</th>
<th>Analysis, %</th>
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<tr>
<td>OCH₃</td>
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<td>100-101̊</td>
<td>C₂₃H₂₆O₄</td>
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<tr>
<td>CH₃</td>
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<td>C₂₃H₂₆O₃</td>
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<td>H, 7.48</td>
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<td></td>
</tr>
<tr>
<td>H</td>
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<td>C₂₂H₂₄O₃</td>
<td>C, 78.57</td>
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<td>C, 71.23</td>
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<td>H, 6.26</td>
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<td>129-130.5̊</td>
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<tr>
<td>CN</td>
<td>72</td>
<td>103-105̊</td>
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<tr>
<td>NO₂</td>
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<td>C₂₂H₂₃NO₅</td>
<td>C, 69.26</td>
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<td>H, 6.08</td>
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</tr>
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</table>

1. Recrystallised from petroleum ether
2. Recrystallised from diethyl ether
3.1.7.0 Preparation of (E)-1-[4-(benzenesulfonyloxy)phenyl]-1-nonen-3-one

\[
\text{O} \quad \text{H} \quad \text{O} \\
\text{C=C --C --(CH}_2\text{)}_5\text{CH}_3
\]

A mixture of (E)-1(4-hydroxyphenyl)-1-nonen-3-one (7.5 g., 0.032 mole), benzene sulfonyl chloride (5.71 g., 0.032 mole) in dry pyridine (20.0 ml.) was heated on a steam bath for 1.5 hours and then stirred mechanically at room temperature for a further 1.5 hours. The crude colourless product, isolated by extracting the reaction mixture using the method for the synthesis of (E)-1-[(4-substituted benzoyloxy)phenyl]-1-nonen-3-ones, was recrystallised from methanol to give the title compound (9.1 g., 76%) as colourless flakes, mp. 47 - 48.5 °. Mass Spectrum: m/e 372 (M⁺, 9%), 77 (100%).

Analysis: Calculated for C₂₁H₂₄O₃S: C, 67.70; H, 6.50. Found: C, 68.03; H, 6.51.

3.1.8.0 Preparation of 4-Substitutedphenyl Esters of Benzoic Acid

\[
\text{O} \\
\text{R}
\]
The esters in Table 14 were prepared by the method employed in the synthesis of (E)-1-[(4-substituted benzoyl-oxy)phenyl]-1-nonen-3-ones and the crude products obtained were recrystallised from petroleum ether. Phenyl benzoate was obtained from B.D.H. Chemicals Ltd. and purified by recrystallisation from ethanol, mp. 69.5 - 70° (lit. mp. 71°).

**Table 14. Physical data of p-substituted phenyl esters of benzoic acid**

<table>
<thead>
<tr>
<th>R</th>
<th>Yield %</th>
<th>mp.</th>
<th>Lit. mp.</th>
<th>Molecular Formula</th>
<th>Analysis, %</th>
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<td>C₁₄H₁₂O₂</td>
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<td>H, 5.70</td>
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<td>84-85</td>
<td>C₁₃H₉ClO₂</td>
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<td>N, 3.73</td>
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</table>
3.1.9.0 Preparation of (E)-4-dimethylaminomethy1-1-(hydroxypheny1)-1-nonen-3-one Hydrochlorides

The Mannich bases were prepared by the following general procedure. A mixture of (E)-1-(methoxymethoxyphenyl)-1-nonen-3-one (15.0 g., 0.054 mole), dimethylamine hydrochloride (4.4 g., 0.054 mole) and paraformaldehyde (4.9 g., 0.054 mole) was heated under reflux in absolute ethanol (100 ml.) containing hydrochloric acid (0.5 - 2%) for 24 hour. After removal of the alcohol in vacuo, the residue was suspended in water and extracted with diethyl ether. Removal of the solvent gave unreacted (E)-1-(hydroxyphenyl)-1-nonen-3-one. The aqueous phase was cooled to 0°, basified with aqueous sodium hydroxide (0.1 M) until the pH reached 9.0 and extracted with diethyl ether. The ethereal extract was acidified with ethanolic hydrochloride (10% v/v) and the solvent removed in vacuo to near dryness and the re-aminging solution refrigerated. The crystals deposited were removed and recrystallised from dry acetone.

(E)-4-Dimethylaminomethy1-1-(4-hydroxyphenyl)-1-nonen-3-one hydrochloride was obtained in 37% yield as a colourless powder, mp. 174 - 176°. Mass Spectrum: m/e 289 (M⁺ - HCl, 2%); 58 (100%). NMR (DMSO-d₆) δ: 10.56 (S, 1, NH); 10.22 (S, 1, OH); 7.72 (d, 1, C₁H, J₁₂ = 16.0); 7.64 (d, 2, aromatic H, J₂₃,₂₃ = 8.7); 6.90 (d, 2, aromatic H, J₅₆,₅₆ = 8.7); 6.90 (d, 1, C₂H, J₂₁ = 16.0); 3.80 - 3.00 (m, 3, C₄H₂CH₂); 2.72 [S, 6, N(CH₃)₂] and 1.90 - 0.57 [m, 11, (CH₂)₄CH₃].
Analysis: Calculated for C\textsubscript{18}H\textsubscript{28}ClNO\textsubscript{2}: C, 66.34; H, 8.66; N, 4.30. Found: C, 62.03; H, 8.71; N, 4.08.

(E)-4-Dimethylaminomethyl-1-(3-hydroxyphenyl)-1-nonen-3-one was obtained in 38% yield as a colourless powder, mp. 154-156°. Mass Spectrum: m/e 238 (M\textsuperscript{+}HCl, 3%), 58 (100%). NMR (DMSO-d\textsubscript{6}) δ: 10.83 (S, 1, NH), 9.93 (S, 1, OH), 7.77 (d, 1, C, J\textsubscript{1,2} = 16.0), 7.05 (d, 1, C\textsubscript{2}H, J\textsubscript{2,1} = 16.0), 7.40 - 6.87 (m, 4, aromatic H), 3.88 - 3.00 (m, 3, C\textsubscript{4}HCH\textsubscript{2}), 2.77 [S, 6, N(CH\textsubscript{3})\textsubscript{2}] and 2.00 - 0.57 [m, 11, (CH\textsubscript{2})\textsubscript{4}CH\textsubscript{3}].

Analysis: Calculated for C\textsubscript{18}H\textsubscript{28}ClNO\textsubscript{2}: C, 66.34; H, 8.66; N, 4.30. Found: C, 64.11; H, 8.56; N, 4.06.

An attempt to prepare (E)-4-dimethylaminomethyl-1-(2-hydroxyphenyl)-1-nonen-3-one hydrochloride by the general procedure led to the isolation of a water soluble blue-green oil. Storage of the oil in a vacuum desiccator or trituration with diethylether, petroleum ether (bp. 60-80°) and benzene did not induce crystallisation.

3.1.9.1 Preparation of (E)-4-dimethylaminomethyl-1-(3,4-methylenedioxyphenyl)-1-nonen-3-one hydrochloride

A solution of hydrochloric acid (10 ml.) in ethanol (250 ml.) was added to a mixture of (E)-1-(3,4-methylenedioxyphenyl)-1-nonen-3-one (41.2 g., 0.16 mole), dimethylamine hydrochloride (12.9 g., 0.16 mole) and paraformaldehyde (4.8 g., 0.16 mole) in methanol (12.5 ml.) and the resultant mixture was heated under reflux for 1 hour.
On cooling, a colourless solid deposited, which was recrystallised from acetone to give \((E)-4\text{-dimethylaminomethyl-1-}(3,4\text{-methylenedioxyphenyl})-1\text{-nonen-3-one hydrochloride (18.3 g., 32%) as colourless needles, mp. 149 - 150^\circ.}

**Mass Spectrum:** m/e 317 (M+HCl, 2%), 58 (100%).

**Analysis:** Calculated for C_{19}H_{28}ClNO_{3}: C, 64.47; H, 7.98; N, 3.96. Found: C, 64.78; H, 8.07; N, 3.81.

### 3.1.9.2 Attempted Preparation of \((E)-4\text{-dimethylaminomethyl-1-}(3,4\text{-dimethoxyphenyl})-1\text{-nonen-3-one hydrochloride}

A mixture of \((E)-1\text{-}(3,4\text{-dimethoxyphenyl})-1\text{-nonen-3-one, paraformaldehyde and dimethylamine hydrochloride in ethanol was heated under reflux for 24 hours and a water-soluble yellow oil obtained by the general method for the preparation of \((E)-4\text{-dimethylaminomethyl-1-}(\text{hydroxyphenyl})-1\text{-nonen-3-one hydrochlorides. The oil was dissolved in water, basified with aqueous sodium hydroxide solution (0.1 M) and extracted with diethylether to give a yellow oil corresponding to 21\% yield of the free base of the title compound. **NMR (CDCl}_3\text{-d) } \delta: 7.60 \text{[d, 1, C, H, J}_1,2 = 16.0), 7.30 - 6.88 \text{[m, 3, aromatic H], 6.84 \text{[d, 1, C}_2\text{H, J}_2,1 = 16.0), 3.90 \text{[s, 6, OCH}_3\text{]}, 3.02 - 2.29 \text{[m, 3, C}_4\text{HCH}_2\text{N}, 2.23 \text{[s, 6, N(CH}_3\text{)]}, 1.83 - 0.72 \text{[m, 11, (CH}_2\text{)]}_4\text{CH}_3].

The oil was treated with ethanolic hydrochloric acid (10\% v/v) to give a yellow oil which did not crystallise either on trituration with diethylether, benzene and petroleum ether (60 - 80\°) or by storage in a vacuum desiccator for
17 weeks. Tlc on silica gel G plates using chloroform : ethyl : acetate:: diethylamine (92 : 5 : 3) revealed the presence of at least eight components with \( R_f \) values of 0.03, 0.07, 0.10, 0.44, 0.46, 0.52 (main component), 0.58 and 0.71 when examined under long wave ultraviolet light.

It was found that \((E)-1-(3,4\text{-dimethoxyphenyl})-1\text{-nonen}-3\text{-one}\) had an \( R_f \) value of 0.73 using these chromatographic conditions.

### 3.1.9.3 Attempted Preparation of \((E)-4\text{-dimethylaminomethyl}-1-(3\text{-nitrophenyl})-1\text{-nonen}-3\text{-one Hydrochloride}\)

A mixture of \((E)-1-(3\text{-nitrophenyl})-1\text{-nonen}-3\text{-one}, \) dimethylaminehydrochloride and paraformaldehyde in ethanol was heated under reflux for 24 hours and a water-soluble oil obtained by the general method for the preparation of \((E)-4\text{-dimethylaminomethyl}-1-(\text{hydroxyphenyl})-1\text{-nonen}-3\text{-one hydrochlorides}. The oil was dissolved in water, basified with aqueous sodium hydroxide (0.1 M) and extracted with diethylether to give a yellow oil corresponding to 19% yield of the free base of the title compound. Mass Spectrum: m/e 318 (\( M^+\)-HCl, 0.2%), 247 (0.5), 228 (2), 176 (1), 98 (3), 96 (3), 83 (2), 81 (4), 74 (13), 70 (9), 69 (2), 68 (8), 67 (3), 59 (26), 58 (38), 57 (8), 55 (4), 53 (2), 51 (2), 45 (21), 44 (6), 43 (20), 42 (5), 41 (12), 43 (3), 39 (9), 38 (12), 37 (2), 36 (37), 35 (7), 32 (30), 31 (100), 30 (5), 29 (33), 28 (15), 27 (22), 26 (5).

The oil was treated with ethanolic hydrochloric acid (10% v/v) to give a yellow oil which did not crystallise
either on trituration with diethylether, benzene and petroleum ether (60 - 80°) or by storage in a vacuum desiccator for 17 weeks. Tlc on silica gel G plates using chloroform : ethylacetate : diethylamine (92 : 5 : 3) revealed the presence of at least seven components with Rf values of 0.05, 0.48, 0.54 (main component), 0.61, 0.67, 0.73 and 0.78 when examined under long wave ultraviolet light. It was found that (E)-1-(3-nitrophenyl)-1-nonen-3-one had an Rf value of 0.73 using these chromatographic conditions.

3.1.9.4 Preparation of (E)-1-(pentafluorophenyl)-1-nonene-3-one

The procedure for the synthesis of (E)-1-(4-nitrophenyl)-1-nonen-3-one was followed except that the time of heating under reflux was reduced to 1 hour. The title compound was obtained as a mobile yellow oil (8.5 g., 27%) bp. 114°/1.0 mm. leaving a brown residue in the distillation flask. Mass Spectrum: m/e 306 (M+, 7.0%), 221 (100%). NMR (CDCl3-d) δ: 7.42 (d, 1, C1H, J1,2 = 16.0), 6.92 (d, 1, C2H, J2,1 = 16.0), 2.62 (5, 2, C4H, J4,5 = 7.0), 2.18 - 0.63 [m, 11, (CH2)4CH3].

Analysis: Calculated for C15H15F5O: C, 58.82; H, 4.94. Found: C, 58.37; H, 4.76.

Further distillation of the brown residue gave a yellow viscous oil (0.6 g.).

1. The oil immediately solidified.
3.2.0.0 **Kinetic Studies**

The esters were divided into three groups; namely, (E)-1-[(4-substituted benzoyloxy)phenyl]-1-nonen-3-ones (Series I), (E)-1-[(2-substituted benzoyloxy)phenyl]-1-nonen-3-ones (Series II) and the aryl substituted phenyl benzoates (Series III), Section 2.3.0.0. The rates of the hydrolysis were determined by measuring the optical density (O.D.) of the phenoxide product to 100 percent reaction. The ultra-violet spectra of the individual esters were determined in aqueous 1,4-dioxane (50% v/v) using a Varian Cary 118 spectrophotometer.

In order to check the Beer-Lambert Law for the resultant phenoxide anion, the absorbance of the phenoxide anion in aqueous 1,4-dioxane (50% v/v) was determined at five concentrations. To a known concentration of phenol in aqueous 1,4-dioxane (50% v/v) was added a drop of sodium hydroxide (10 M) solution, shaken and the O.D. noted. A plot of the O.D. versus the phenoxide concentration made. The wavelengths used in the Beer-Lambert Law plots are given in Table 15.
Table 15. Absorption wavelengths of the phenoxides

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<th>( \lambda_{\text{max}} ) nm</th>
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<td>389</td>
</tr>
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<td>405</td>
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<td>( \text{H} )</td>
<td>299</td>
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<tr>
<td>( \text{CH}_3 )</td>
<td>289</td>
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</tbody>
</table>

1. For ortho-phenoxide, \( \lambda_{\text{max}} = 406 \) nm.

For the determination of the rate of formation of the phenoxide anion, a wavelength of the phenoxide least interfering with the ultra-violet spectrum of the corresponding ester was chosen for study. To the main arm of the two-component kinetic flask, figure 38, was added the ester solution (10 ml.). To the side arm of the flask was added sodium hydroxide solution (10 ml.) containing enough sodium chloride to maintain an ionic strength of \( 2.8 \times 10^{-3} \) M after mixing. The side arm was secured in place by means of a rubber band, then the flask was equilibrated at 36.9 ± 0.02°C for 30 minutes. The two fractions were then
quickly mixed by rotating the side arm upwards, shaken to ensure thorough mixing and immediately transferred to the thermostated ultra-violet cells in the spectrophotometer. The reaction was followed by continuous recording of the optical density of the phenoxide anion to infinity, at a previously chosen wavelength. The time lag between mixing and the beginning of recording was approximately 22 seconds and was corrected for in the calculations. Attempted hydrolysis of (E)-1-[(4-benzenesulfonyloxy)phenyl]-1-nonen-3-one under the same conditions was unsuccessful.

The kinetic runs were carried out in duplicate under pseudo first order conditions at two different sodium hydroxide concentrations. The pseudo first-order rate constant, \( k' \), is given by the expression:

\[
k' = 2.303 \log \frac{O.D. - O.D. \cdot 0}{O.D. - O.D. t}
\]

Where \( O.D. \) is the optical density of the solution at \( t = \infty \) and corresponds to 100% reaction (observed \( O.D. \cdot \infty = \text{calculated } O.D. \cdot \infty + 3\% \)), \( O.D. \cdot 0 \) is the initial optical density and \( O.D. t \) is the optical density of the solution at time \( t \). The constant \( k' \) was calculated by plotting \( \log O.D. - O.D. t \) against time. The best fit of the line to the points was obtained by a least squares treatment of the data using an HP 2000 computer.
The second-order rate constants, $k_2$ (Table 3), were calculated from the pseudo first-order constants by dividing $k'$ by the concentration of sodium hydroxide. Representative plots for each compound are given in discussion.

Attempted hydrolysis of Series I esters at pH 7.4 using imidazole as catalyst was unsuccessful.

For each series of esters $\log \frac{k_2R}{k_2H}$ was plotted against $\sigma_p^1$ and the rho value determined off the graph. From the Hammett plot of Series III esters was determined the sigma para value of $-\text{CH} = \text{CHC}_6\text{H}_3$. 

Figure 38. Diagram of the Flask Used in Kinetic Experiments.

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3.3.0.0 Biochemical and Pharmacological Studies

3.3.1.0 Anticancer Screening

The anticancer screening was carried out by the Drug Research and Development Division of the National Cancer Institute, Bethesda, Maryland, U.S.A., using their protocols (Geran et al., 1972). The compounds, dissolved in saline, hydroxypropylcellulose, saline with Tween-80 or saline with alcohol, were administered by the intraperitoneal route to the BDF₁ and CDF₁ strain of mice, implanted with ascitic fluid containing P388 lymphocytic leukemia cells 24 hours prior to treatment. Injections were administered daily for nine consecutive days. For each screen, a three-dose assay was carried out. Six mice were used for each dose. The number of survivors on the fifth day after drug administration was noted. Deaths before the sixth day were considered non-leukemic and formed the basis of the toxicity evaluation. The ratio of the mean survival time of the treated animals (T) to the control animals (C) was calculated as a percentage (T/C %) for each dose. A minimal 25% increase in survival of treated animals over controls is necessary for further screening. A reproducible T/C ≥ 150% is necessary for a compound to be acceptable as a possible candidate for clinical evaluations.

3.3.2.0 Incorporation Studies

Incorporation studies were carried out on L1210 lymphoid leukemia cells in cell culture.
3.3.2.1 **Cell Culture**

The L1210 lymphoid leukemia stock culture was obtained from Dr. J. Manchak, University of Alberta, Edmonton, Alberta, Canada. The cells were cultured in an incubator at 37°C in a 5% carbon dioxide humid atmosphere.

To determine the mean generation time (MGT), the actively growing cells were diluted to $1.84 \times 10^5$ cells/ml. with Fishers Medium containing 10% horse serum, placed in a 35 mm. petri dish and the cells counted at 24, 48 and 72 hours using Model FN Coulter Counter. The mean generation time was determined from the relationship:

$$\text{MGT} = \frac{\text{Elapsed time (hours)}}{\text{Number of doublings}}$$

The elapsed time was 48 hours and the MGT 16 hours. Determinations were carried out in duplicate.

3.3.2.2 **Inhibition of Cell Growth**

The actively growing cells were diluted to $3.68 \times 10^5$ cells/ml. with Fisher's medium containing 20% horse serum. A freshly prepared stock drug solution in water was sterilised by passing through a millipore filter, then serially diluted with Fisher's medium to give twice the desired concentrations. To the cell solution in a 35 mm. petri dish was added 10 ml. of drug solution. The petri dish was then incubated at 37°C in a 5% carbon dioxide humid atmosphere. The cells were counted at 24, 48 and 72 hours. The 50% inhibitory dose (ID$_{50}$) for the drug was calculated using the cell numbers after 48 hours exposure to the drug. For each drug, the cell
count at 48 hours expressed as a percentage of the control cell count was plotted against the drug concentration. The drug concentration giving 50% of control cell count at 48 hours was the ID$_{50}$. Determinations were carried out in duplicate.

3.3.2.3 Leucine, Ribonucleosides and Deoxyribonucleosides Uptake Studies

The methods of Hryniuk and Bertino (1969) and Chabner (1972) as modified by Srinivassan (1977) were employed. A cell suspension (0.8 ml.) containing 2.0 to 2.3 x 10$^6$ cells was placed in loose-cap plastic tubes. To each tube was added a freshly prepared solution of NC 97 in water (0.1 ml.) to give final concentrations of 1, 3 and 5 μg./ml. The cell suspensions were then incubated at 37°C. in a 5% carbon dioxide atmosphere. After 0.5, 1.0, 2.0 and 4.0 hours contact with the drug, 0.1 ml. of $^3$H-TdR (3.0 μCi, 6.52 x 10$^{-6}$M), $^3$H-UdR (3.0 μCi, 8.33 x 10$^{-6}$M), $^3$H-UR (3.0 μCi, 5.17 x 10$^{-6}$M) or $^3$H-leucine (3.0 μCi, 2.83 x 10$^{-6}$M) was added to the tubes that were then re-incubated at 37°C. At intervals of 5, 10, 15 and 20 minutes after addition of the labelled compound, 0.2 ml. of cell suspension was removed and added to 5 ml. cold 10% trichloroacetic acid (TCA) in a test tube. The resulting precipitate was mixed on a Vortex mixer and left overnight at 4°C., then centrifuged for 20 minutes at 4°C. at a speed of 3000 rpm. The supernatant fluid was discarded. The precipitate was washed three times
with 5 ml. cold TCA. The precipitate was then dissolved in 5 ml. NCS solubiliser (strong base in toluene, 0.6 N solution in toluene), diluted with 12 ml. scintillation fluid, and the amount of radioactivity incorporated into DNA, RNA or protein determined with an Isocap liquid scintillation counter. A control cell suspension was run concurrently. All assays were done in duplicate.

The results were plotted as a rate of incorporation of radioactivity per million cells per minute. The percentage ratio of the label incorporated by treated cells after 4 hours pre-incubation with 1, 3 and 5 μg./ml. NC 97 over the control was calculated.

3.3.3.0 Antimicrobial Screening

Antimicrobial screening was carried out by BioResearch Laboratories Limited, Montreal, Quebec, Canada; against the following bacteria: *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 10145), *Klebsiella pneumoniae* (ATCC 4352), *Salmonella typhimurium* (G46), *Bordetella bronchiseptica* (ATCC 4617), *Staphylococcus aureus* (ATCC 6538), *Streptococcus faecalis* (ATCC 8030) and *Bacillus subtilis* (ATCC 6633). The following fungi were also used in the screening: *Trichophyton mentagrophytes* (ATCC 9533), *Microsporum gypseum* (ATCC 14683), *Aspergillus niger* (ATCC 10535), *Candida Albicans* (ATCC 10231) and *Saccharomyces uvarum* (ATCC 9080).
The compounds were dissolved in water or dimethylsulfoxide (DMSO) and diluted serially to various concentrations. The concentrations of the stock solutions were prepared in such a way that when 0.5 ml. of the drug solution was added to 15 ml. of agar media, the desired final concentrations were obtained. Trypticase soy agar media was employed for bacteria and modified Sabouraud agar for the fungi and yeasts. The test organisms were previously grown for 2 days at 35° C. for bacteria and yeasts and one week at 24° C. for fungi. The agar plates were streaked with cell suspension containing approximately $10^5$ organisms/ml. The plates were incubated for 2 to 14 days at 24° C. for fungi and 35° C. for bacteria. The bacteria were assessed at 500 and 100 µg./ml. and the fungi at 500, 250, 100 and 10 µg./ml.

3.3.4.0 Miscellaneous Screens

The tests described below were also carried out by Bio-Research Laboratories Limited in Montreal for analgesic (phenylquinone-induced writhing test) and antidepressant (antagonism of tetrabenzene-induced ptosis) effects, the drugs were administered subcutaneously in mice. Anti-inflammatory (antagonism of carageenan-induced paw edema), hypoglycemic (measurement of blood-glucose concentration) and anti-anaphylactic (inhibition of passive cutaneous anaphylaxis) effects were carried out in rats subcutaneously, orally and intravenously, respectively. Antihistaminic
activity was determined \textit{in vitro} as antagonism of histamine in the isolated guinea-pig ileum preparation. Other tests carried out were the cardiovascular screen, in which the measurement of arterial blood pressure in anesthetised rats was undertaken, as well as the neuropharmacological profile, whereby mice were observed for a period of one hour as well as at the end of five hours after intraperitoneal injection of the compound and the behaviour of the animals noted.
4.0.0.0 APPENDIX

Nmr Spectra

Nmr spectra were run on a Varian A-60 and Bruker WP60 spectrometers, using deuterochloroform as solvent and tetramethylsilane (TMS) as internal standard. Chemical shifts are expressed in δ (ppm) values downfield from TMS. The following abbreviations were used: s(singlet), d(doublet), t(triplet), and m(multiplet).

Ultraviolet Spectra

Ultraviolet spectra were run using a Cary Model 118 Spectrophotometer employing 1 cm. quartz cells.

Mass Spectra

Mass spectra were determined on an AE1 MS-12 single focusing mass spectrometer operated by Mr. D. Bain. Samples were introduced by direct probe method and ionised with a 70 eV electron beam.

Elemental Analysis

Elemental analyses were carried out by Mr. R. Teed using a Hewlett-Packard Model 185 C-H-N Analyser.

Melting Points

Melting points were determined on a Gallenkamp MF-370 apparatus and are uncorrected.

Thin Layer Chromatography

Thin layer chromatograms were run on 20 x 20 cm. glass plates which were pre-coated with 0.5 mm. silica gel G (Silica gel N-HR/UV254 Polygram; Fisher Scientific Company).
Benzoyl Chlorides
The acid chlorides were obtained from Aldrich Chemical Company, Inc., and were used without further purification.

Drying of Organic Extracts
Organic extracts were washed several times with water, dried over anhydrous magnesium sulfate and the solvent removed in vacuo.

Petroleum Ether
The fraction of petroleum ether with a boiling range of 100 - 120° was used unless otherwise stated.

Anhydrous Pyridine
A commercial grade of pyridine was heated under reflux in the presence of potassium hydroxide, distilled and stored over anhydrous barium oxide.

Anhydrous Benzene
Analytical grade benzene was stored over sodium wire in a dry bottle.

1,4-Dioxane
A mixture of analytical grade 1,4-dioxane and sodium hydroxide pellets was heated under reflux for 18 hours, then distilled using a column. The distilled 1,4-dioxane was again heated under reflux in the presence of sodium metal, then distilled at atmospheric pressure and stored without exposing to the air.
5.0.0.0 REFERENCES


Bohnsack, H. and Seibert, W., Reichstoffe, Aromen, Koerperpflegemittel, 15, 321 (1965); Chem. Abstr., 64, 17349g (1966).


