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

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by

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TO MY PARENTS

ABSTRACT

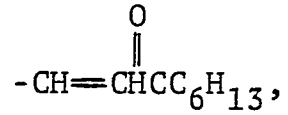
The preparation, mass spectral properties, antineoplastic, antimicrobial and other pharmacological properties of 1-(hydroxyphenyl)-1-nonen-3-ones and related *O*-benzoyl esters, *O*-ethers and Mannich bases are presented. The effect of a Mannich base on the incorporation of labelled precursors into certain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



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 homeostastic 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.

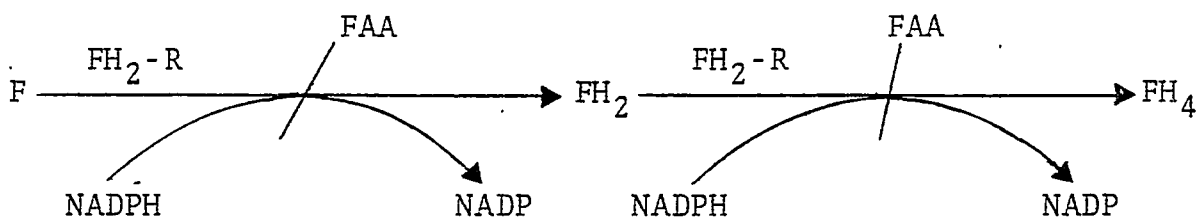
1.2.1.0 Cancer Chemotherapy

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).

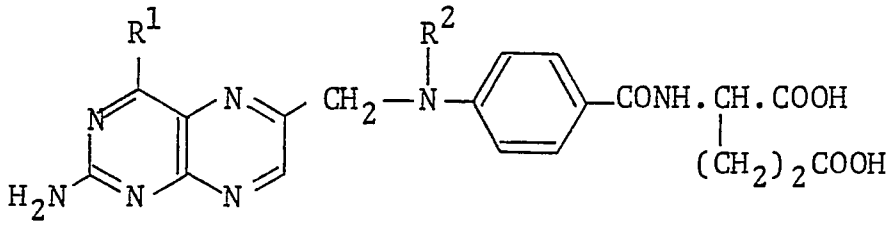
The folic acid antagonists function by affecting the mechanism of folic acid activation. The activation pathway involves the reduction of folic acid (F) to dihydrofolic acid (FH_2) which is subsequently reduced to tetrahydrofolic acid (FH_4). The activated FH_4 is required for the 1-carbon transference occurring in purine and pyrimidine synthesis. The formation of FH_4 shown below is markedly elevated in rapidly proliferating tissue, such as neoplastic growths.



FAA = Folic acid antagonist

FH_2-R = dihydrofolate reductase

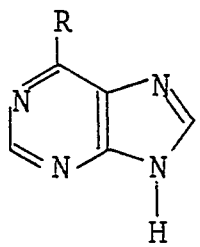
Scheme 1. Blockage of Folic Acid Activation by Folic Acid Antagonists



I

(a) R^1 = OH, R^2 = H

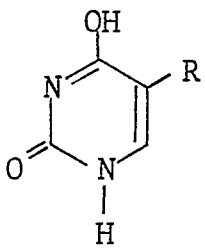
(b) R^1 = NH_2, R^2 = CH_3



II

(a) = H

(b) = SH



III

(a) R = H

(b) R = F

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

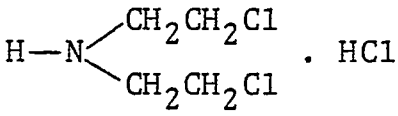
noted to cause remission of the metastatic carcinoma of the endometrium (Kelly and Baker, 1961) and adrenocortical hormones produce temporary remissions in acute lymphoblastic leukemia (Ellison, 1956) and to a smaller extent in lymphomas. Androgens are mainly associated with the treatment of breast cancer 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.

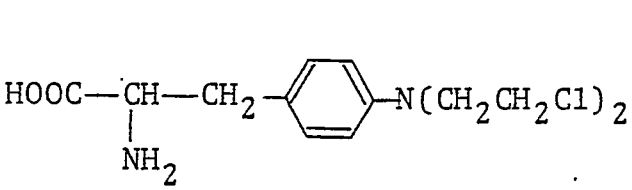
Research into alkylating agents as potential anti-cancer agents was derived from studies of the physiological action of two war gases; namely, sulphur mustard gas during World War I and nitrogen mustard gas during World War II. A number of nitrogen mustards having the general structure $R\text{-}N(CH_2CH_2Cl)_2$ 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

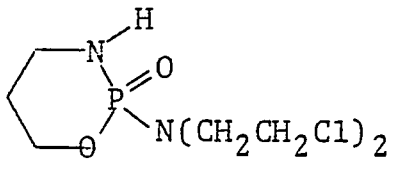
cells. Since the advent of mustine hydrochloride, many nitrogen mustards have been synthesised for clinical use. The most widely used of these are melphalan (V) and cyclophosphamide (VI).



IV



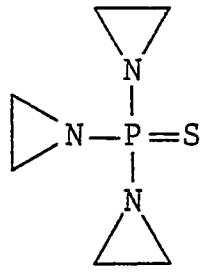
V



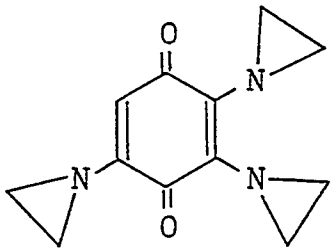
VI

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

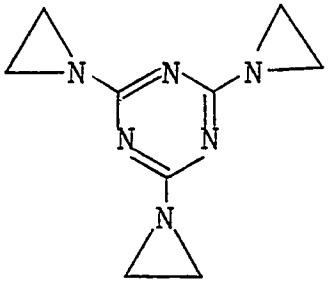
been attached to quinone and heterocyclic nuclei, e.g. Thio TEPA (VII), Trenimon (VIII) and TEM (IX).



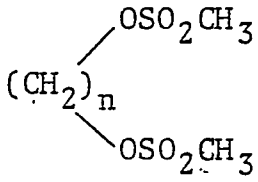
VII



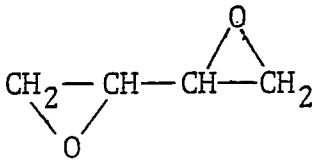
VIII



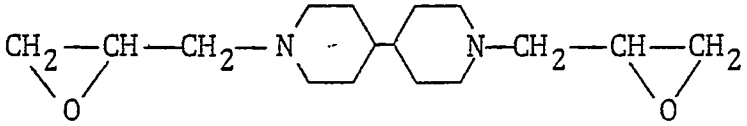
IX



X



XI

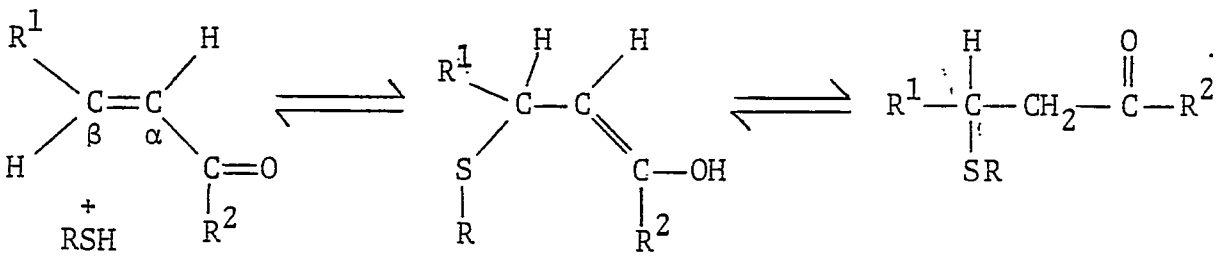


XII

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).

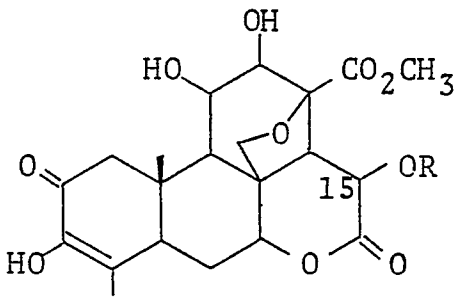
The need for additional biological alkylating agents in the treatment of cancer has been stressed recently (Connors, 1969; Heidelberger, 1969). A group of compounds that merit consideration in this regard are α,β-unsaturated ketones. While cyclic α,β-unsaturated ketones have been the subject of considerable 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).



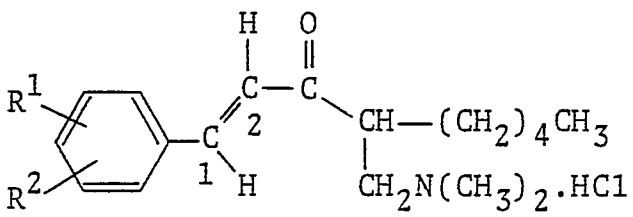
Scheme 2. Alkylation of Thiols by α,β-Unsaturated Ketones.

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).



R = (a) H, (b) acyl

XIII

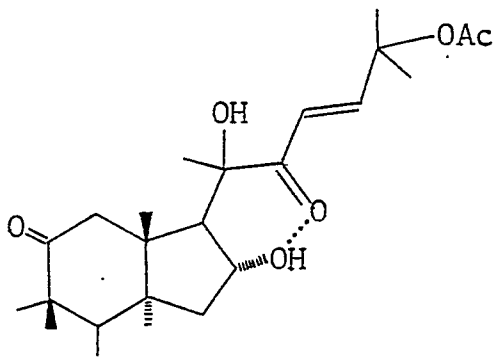


XIV

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 σ 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.

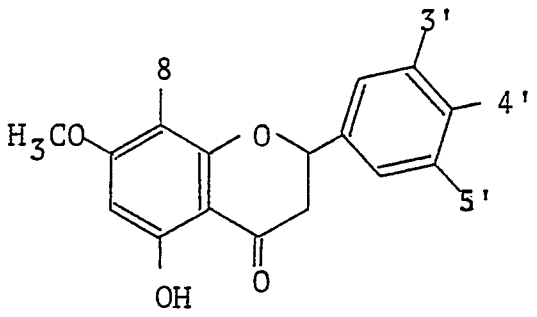
The cytotoxicity of a number of drugs containing hydroxy groups has been abolished on removal of the hydroxy function indicating the importance of this group in conferring biological activities. A study of some substituted benzylidene acetophenones (chalcones) against Ehrlich's ascites sarcoma in mice 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 Vermenichev, 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 α,β-unsaturated keto group to nucleophilic attack as a result of the loss of the 16-hydroxy function.



XV

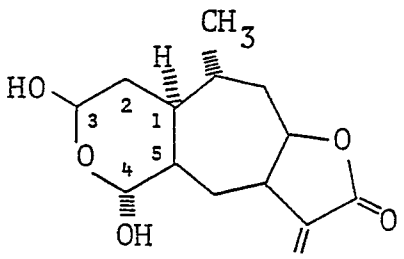
In a cytotoxicity test of five flavonoids obtained from *Lychnophora affinis* (XVI) in cell cultures of human carcinoma of the nasopharynx (KB screen), it was found that only the compounds with hydroxy substituents showed activity (Le Quesne *et al.*, 1976).



XVI

3'	4'	5'	8	KB
OCH_3	OCH_3	H	OCH_3	-
OCH_3	OCH_3	H	H	-
OCH_3	OH	H	OCH_3	+
OH	OCH_3	OCH_3	OCH_3	+
OH	OCH_3	H	H	+

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).



XVII