PRODRUGS AND DERIVATIVES
OF
ALPHA, BETA-UNSATURATED KETONES
DESIGNED AS ANTICANCER AGENTS

A Thesis

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in Partial Fulfilment of the Requirements

For the Degree of

Doctor of Philosophy

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by

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To

MY PARENTS

who encouraged the work

and

SUREKHA

who endured it

ABSTRACT

In the chemotherapy of cancer, a number of different classes of drugs are used. Of these, alkylating agents constitute about 30% and while a few of the less common cancers can be effectively treated by chemotherapy or adjuvant therapy, the drugs are marked by lack of specificity and high toxicity. Moreover, the vast majority of cancers cannot be treated satisfactorily by any therapy at all. Therefore, there is a need for better and more selective anticancer drugs. The present project may be considered to consist of the following two areas.

I) Design, synthesis and antineoplastic evaluation[1] of novel candidate antineoplastics of the type-

a) Mannich bases and related compounds.

b) α,β-Unsaturated ketones and their derivatives.

II) Physicochemical, stability and *in vitro* studies of selected compounds.

The compounds were designed as alkylating agents so that they would alkylate important biomacromolecules in the rapidly proliferating cancer cells. They were, therefore, either strong alkylators *per se* or were designed to generate such a species *in vivo*.

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1 The *in vivo* evaluation for anticancer activity was undertaken by the National Cancer Institute (NCI), U.S.A.

Ia) 3-Dimethylamino-2-dimethylaminomethyl-1-(4-methoxyphenyl)-1-propanone dihydrochloride (28), submitted previously by this laboratory has been designated a Selected Agent Compound by the NCI. It was also shown, at that time, that a slow release of the potential alkylating acrylophenone species from such Mannich bases was favourable for good anti-cancer activity.

In keeping with this observation, a number of dibasic and monobasic Mannich bases derived from acetophenones and other aryl methyl and alkyl methyl ketones and their corresponding acrylophenones, were prepared. While two of the acetophenone bis-Mannich bases showed presumptive antileukemic activity, one compound, viz. a bis-Mannich base derived from 4-(4-methoxyphenoxy)acetophenone demonstrated confirmed antileukemic activity.

Ib) Under Ib were synthesized the following types of compounds.

(i) 1-Aryl-1-nonen-3-ones. A number of α,β-unsaturated ketones have been synthesized previously in this laboratory, a few of which demonstrated, inter alia, appreciable anticancer activity. Hence, a series of 1-aryl-1-nonen-3-ones was synthesized.

(ii) Thiol adducts derived from (i). At the time this project was undertaken, the ethanethiol adduct of 1-(2-

chlorophenyl)-1-nonen-3-one (XVIa) had been designated a Selected Agent Compound by the NCI. Therefore, ethanethiol adducts from series (i) were prepared and tested. Also prepared were adducts of (XVIa) using thiol-containing amino acids and other polar compounds.

(iii) Also prepared were some thiosemicarbazones and semicarbazones derived from (ii).

The antileukemic screening data for the majority of compounds (Ib) is not available at the present time.

High resolution NMR spectroscopy of the thiosemicarbazones showed them to exist as a mixture of syn and anti isomers. Also, possible intramolecular hydrogen bonding in the syn isomers, between the secondary amino (NH) function and the ortho substituent on the aryl ring or the thioethyl grouping was demonstrated.

II) Physicochemical, stability and in vitro studies of selected compounds.

The stability of (28) was studied at 37°C in phosphate buffer of pH 7.4 when, after 5 minutes of incubation, the corresponding acrylophenone (XIIf) was isolated. However, when incubated for 96 hours, both (28) as well as the acrylophenone derivative, (XIIf) underwent a retro-Mannich reaction to form the corresponding mono-Mannich base.

The kinetics of the elimination reaction of some of the acetophenone bis-Mannich bases was studied at 37°C in formate buffer of pH 3.5. However, no correlation between the rate of deamination and antileukemic activity could be found.

In vitro studies with 2-dimethylaminomethyl-1-(4-methoxyphenyl)-2-propen-1-one hydrochloride (XIIf) indicated it to be a potent inhibitor of the growth of P388 cells. Laser flow cytometry showed that it arrests cell cycle transit in a cycle-phase non-specific manner.

When the unsubstituted acrylophenone derivative was examined for its effect in vitro on mouse mitochondrial respiration, it was found to inhibit respiration with an ID_{50} of 1.1 μmoles.

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1.0.0.0 INTRODUCTION

1.1.0.0 The nature of cancer

Cancer has been defined as a relatively autonomous growth of tissue (Pitot, 1981). It is basically a disease of cells characterized by impairment or ineffectiveness of the normal cellular control and maturation mechanisms that regulate multiplications and other functions required for homeostasis in a complex multicellular organism (Salmon, 1978). In other words, the state of dynamic equilibrium involving the whole process of cell division and replacement that exists in rapidly proliferating normal tissues, is disrupted in the process of cancerous growth (Creasey, 1981a). The six major features of cancer are as follows (Salmon, 1978).

1) Excessive cell growth which is usually in the form of a tumor.

2) Undifferentiated cells and tissues which are similar to embryonic tissues.

3) Invasiveness, namely the ability to grow into adjacent tissue.

4) The ability to metastasize i.e. spread to new sites and establish new growths.

5) A type of "acquired heredity" in which the progeny of the cancer cells retain the same cancerous properties.

6) A shift of metabolism towards increased building of macromolecules from nucleosides and amino acids, and an increased catabolism of carbohydrates for cellular energy.

Next to heart disease, cancer is the principal cause of deaths in the U.S.A., causing over 350,000 fatalities a year. With present methods of treatment, one-third of patients are cured with initial surgery or radiation therapy (Salmon, 1978).

Cancer can be caused by a variety of agents which may be grouped into two categories. In the first category there are those that are basically endogenous to the host, such as genetic predisposition, psychological makeup, immune competence, and endocrine or metabolic factors. To these innate factors a second category may be added namely assaults of external origin, such as chemical carcinogens, radiation, sunlight and viruses or other infectious agents (Creasey, 1981b).

The fact that a cancer cell is able to pass on its characteristic features to a large progeny as the tumor grows, suggests that the initial events underlying malignant transformation include inheritable genetic changes. Three theories have evolved to explain the type of inherited change involved. 1) Genetic mutation in the germ cells such that the initial change is inherited in a Mendelian pattern.

2) Somatic mutation in which the primary events originate in tissue cells during the lifespan of the individual who gets cancer. 3) Epigenetic changes that permanently alter gene expression (Creasey, 1981c).

1.2.0.0 Treatment modalities in cancer

There are three major treatment modalities for cancer. These are surgery, radiotherapy and chemotherapy. Surgery and radiotherapy can often eradicate primary or localized disease but may ultimately fail because the cancer has metastasized to other areas of the body. In such instances, chemotherapy, if used properly, may control or eliminate metastatic disease and reduce mortality. Immunotherapy is currently an experimental method ; its future role is unclear. Today an interdisciplinary approach, coordinating the three major treatment modalities (so-called adjuvant therapy), is used in the management of cancer. Although, some of the less common cancers can be cured by chemotherapy alone (e.g. Hodgkin's disease, Burkitt's lymphoma) or, by adjuvant therapy (e.g. Wilm's tumor, Ewing's sarcoma), most of the more common types of cancer (viz. solid tumors) cannot be treated effectively by any therapy at all.

In the chemotherapy of cancer the following classes of compounds are used.

1) Alkylating agents e.g. nitrogen mustard, cyclophosphamide, vide infra.

2) Antimetabolites e.g. methotrexate, fluorouracil, cytarabine.

3) Antibiotics e.g. mitomycin C, doxorubicin, bleomycin.

4) Vinca alkaloids e.g. vincristine, vinblastine.

5) Hormones e.g. glucocorticoids, estrogens.

6) Miscellaneous drugs e.g. L-asparaginase, hydroxyurea.

Most of the anticancer drugs employed clinically exert their antitumor effect by inhibiting nucleic acid (DNA or RNA) or protein synthesis. This inhibition can occur through cross-linking of bases in DNA (e.g. the bifunctional alkylating agents) or binding to and inactivation of enzymes necessary for the synthetic processes. It can also occur by substitution of bases in nucleic acids with inactive analogs or through breakage of DNA by antitumor drugs such as bleomycin. Thus DNA, RNA and protein molecules and/or the processes involved in their syntheses appear to be important cellular targets for anticancer agents (Prestayko, 1980). The principal mechanisms of action of some major anticancer drugs are summarized in table I.

Table I. Principal mechanisms of action of some major anticancer drugs[a]

Site of inhibition	*Drug*	*Mechanism of action*
Purine synthesis	Methotrexate	Inhibits one-carbon transfer required for purine ring synthesis
	Mercaptopurine and thioguanine	Inhibit purine ring synthesis and interconversion of purines
	Hydroxyurea	Inhibits conversion of ribonucleotides to deoxyribonucleotides
Pyrimidine synthesis	Methotrexate	Inhibits one-carbon transfer required for synthesis of dTMP from dUMP
	Fluorouracil	Inhibits dTMP formation by blocking thymidylate synthetase
	Hydroxyurea	Inhibits conversion of ribonucleotides to deoxyribonucleotides
	Azaribine	Inhibits UMP formation
DNA polymerase	Cytarabine	Competitively inhibits incorporation of dCTP into DNA
DNA (direct interaction)	Nitrogen mustards Nitrosoureas Busulfan Dacarbazine Thiotepa Mitomycin C	React covalently with DNA, often cross-linking the strands
	Bleomycin	Causes DNA breakage
	Dactinomycin Daunorubicin Adriamycin	Intercalate between base pairs and inhibit nucleic acid synthesis
	Mithramycin	Nonintercalative binding to DNA to inhibit nucleic acid synthesis
Protein synthesis	L-Asparaginase	Deaminates asparagine, starving the cell for this amino acid
Protein function	Vincristine Vinblastine	Disrupt microtubles, producing metaphase arrest

[a] Taken from Pratt and Ruddon(1977c).

Alkylating agents (which is the area of interest in this investigation) are compounds which are capable of reacting in a manner such that an alkyl group or a substituted alkyl group becomes covalently linked to cellular constituents. The following five major classes of alkylating agents are employed in cancer chemotherapy.

1) Nitrogen mustards e.g. mechlorethamine, cyclophosphamide.
2) Nitrosoureas e.g. carmustine (BCNU), lomustine (CCNU).
3) Triazenes e.g. dacarbazine.
4) Methanesulfonic acid esters e.g. busulfan
5) Ethylenimines e.g. triethylenemelamine (MEM).

The mechanism of action of mechlorethamine (nitrogen mustard) is shown in figure 1, and serves to exemplify the alkylation reaction and its biological consequences. The strained three-membered ring of the immonium ion intermediate is highly reactive and can attack such nucleophilic groups as amino, carboxyl, sulfhydryl, or imidazole moieties in proteins and nucleic acids. One favoured reaction of major importance is the formation of a covalent bond between the drug and the 7-nitrogen group of guanine. The process may be repeated with the other chloroethyl side chain and the immonium ion so formed may react with another guanine base resulting in cross-linking between DNA strands or linking between bases within the same strand of DNA. Alternatively, the second side chain may react with water to produce

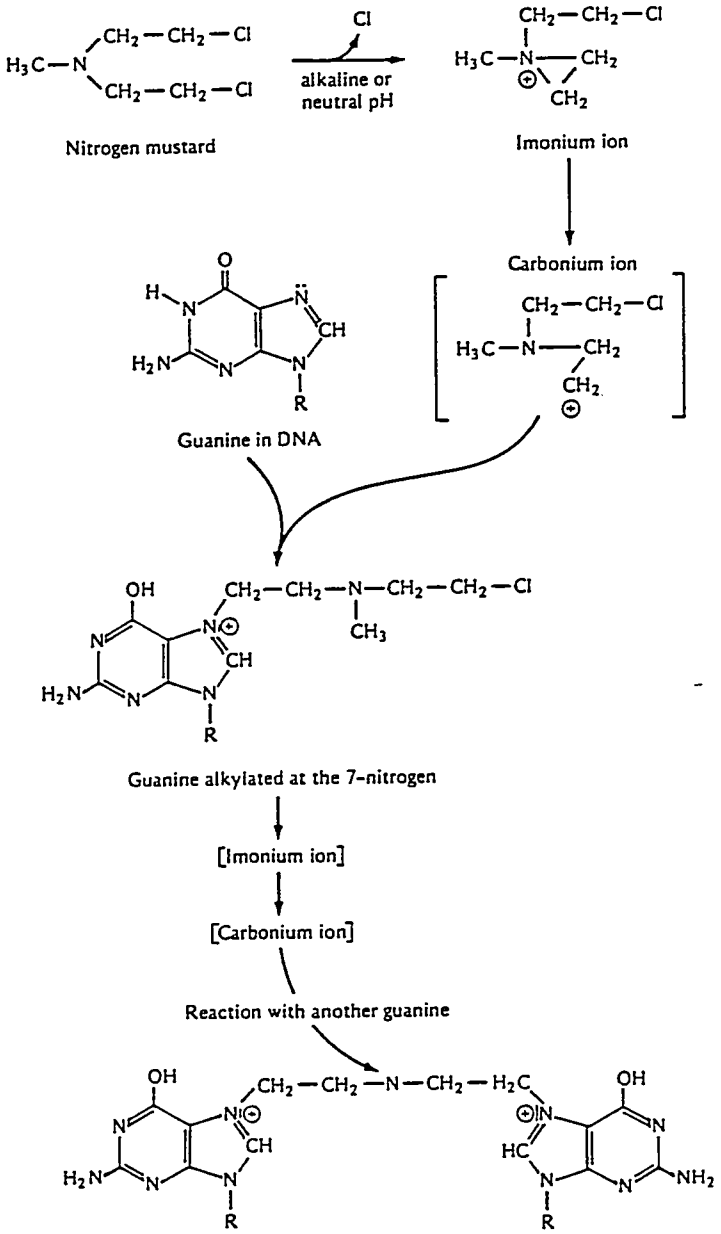


Figure 1. The mechanism by which nitrogen mustard becomes covalently bonded to the 7-nitrogens of two guanine residues (Pratt and Ruddon, 1977d).

monoalkylated DNA guanine units. Although DNA alkylation may have several different biological effects, an important one is the inhibition of DNA replication (Pratt and Ruddon, 1979e).

1.3.0.0 Approaches to the development of new anticancer drugs

The ultimate clinical effectiveness of any anticancer drug requires that it kill malignant tumor cells in vivo at doses that allow enough cells in the patient's critical tissues (e.g. bone marrow) to survive so that recovery can occur. That this is extremely difficult to accomplish is attested to by the fact that out of the nearly 300,000 compounds that have been evaluated as anticancer agents, 40 or so are today considered useful, in varying degrees, in the treatment of cancer. The difficulty of finding new agents lies in the inability to identify and define, by chance or design, an exploitable biochemical difference between normal mammalian host cells and invading cancer cells, such as exists between mammalian cells and bacterial cells and on which the selective toxicity of antibacterial agents such as the sulfa drugs, penicillins and tetracyclines is based (Montgomery, 1979). Added to this is the problem of low predictability of animal tumor models to the neoplasms found in the clinic (especially the solid tumors). Despite this lack of any well-defined metabolic basis for design, however, useful new agents continue to be produced. Most of the

effective new antineoplastic agents derive from random screens for tumor-inhibitory activity combined with limited application of rational drug design. Once a "lead" compound is discovered it is then subjected to systematic structural modification or analog development.

1.3.1.0 Analog Development

Upon the discovery of a "lead" compound, structural modification is carried out 1) in an effort to improve its anticancer therapeutic potential 2) to aid in the elucidation of the biochemical and pharmacological mechanisms of action of the compound. Molecular modifications may also be designed to achieve specific goals such as the following.

1) Development of more potent analogs with respect to anticancer activity.

2) Elimination or minimization of the side effects responsible for rate-limiting host toxicity.

3) Separation of components of the spectrum of action, such as host toxicity and antineoplastic activity into separate molecular entities.

4) Development of analogs with differences in tissue specificity.

5) Modulation of the pharmacokinetic properties of the compound that alter either dose-effect or time-concentration relationships (Kozarich et al., 1979a).

Some of the chemical approaches to accomplish these goals are as follows.

1) The preparation of a series of homologous compounds by progressive changes in structure.

2) The application of the isosteric principle, which involves the insertion of substituents with equal steric properties and equal lipid solubility and/or charge distribution.

3) The employment of certain physicochemical parameters related to partition coefficients and charge distribution.

4) Resolution of isomeric mixtures.

For most efficient analog development, modifications of these kinds should be accompanied by tests for antineoplastic activity in experimental test systems and for appropriate biochemical and pharmacological assays in an effort to effectively direct synthetic efforts (Kozarich et al., 1979a).

1.3.2.0 Quantitative structure-activity relationships

In the absence of information that would permit directed modification to achieve a specific goal, approaches to quantitative structure-activity relationships (QSAR) can be employed (Hansch and Fujita, 1964). These techniques are used to derive quantitative correlation between biological activity and such physicochemical parameters as hydrophobicity, electronic effects, and steric effects of the drug series. The most widely employed QSAR is the linear

free-energy related method of Hansch. The Hansch model expresses biological response as a function of hydrophobic, electronic and steric factors, and may be written in the form of the equation given below.

$$\log(1/C) = -k_1\pi^2 + k_2\pi + k_3\sigma + k_4E_s + k_5$$

C is the concentration of drug required to produce a standard measurable biological effect; π is the hydrophobic parameter; σ is the sum of the Hammett substituent constants for the compound indicating the electronic contribution; E_s is the sum of the Taft steric constants for each substituent on the ring. QSAR has been used extensively in drug development (Kozarich et al., 1979b).

The lipophilic properties of each member of a series of compounds is frequently the single most important factor in determining biological activity. Log P (partition coefficient) factors in QSAR are believed to determine the rate of drug migration to the site of action. A compound with the optimum Log P reaches the desired site most rapidly and in the highest concentration, while compounds with lower and higher Log P values tend to reside longer in aqueous and lipid phases, respectively. The importance of Log P to the development of cancer chemotherapeutic agents has been discussed by Cain (Kozarich et al., 1979c).

An obvious limitation to the Hansch approach is the necessary requirement for synthesis and screening of a number of compounds before any meaningful correlation can be

obtained and the next step towards optimization can be taken. As a result other approaches to analog design in QSAR have been developed. Topliss (1972) described the more direct decision-tree approach to the modification of aromatic rings and side chains which involves the stepwise synthesis and evaluation of a series of compounds. Recently, Topliss (1977) reported another noncomputerized, compound-set, method for applying Hansch approach to aromatic substituent selection. This approach will be discussed in more detail in section 4.7.1.0, vide infra.

Another nonquantitative approach which has been developed is called the "sequential simplex method" (Kozarich et al., 1979d). This procedure resembles the "Topliss tree" approach in that once an initial set of three compounds is synthesized, the biological activity of each successive compound is used to determine the next analog to be prepared. This is continued in a stepwise manner until maximum activity is reached.

The isolipophilic approach takes advantage of the predominant influence of lipophilicity on the activity of many anticancer agents. Once a sufficient number of compounds have been prepared by any of the above approaches, multiple regression analysis may be performed to verify the assumed parameter dependencies (Kozarich et al., 1979e).

1.3.3.0 Target-directed enzyme inhibitors

While the vast majority of drugs used clinically have been obtained empirically by screening procedures, a new rational approach to drug design has become more prevalent in recent years. This approach utilizes the increased knowledge of the mechanism of many enzyme catalyzed reactions and deals with the design of inhibitors. Two such general classes of inhibitors are called transition-state analogs and suicide inhibitors (Kozarich et al., 1979f).

Transition-state analogs are stable compounds which resemble in structure the substrate portion of the transition state for a particular reaction. These analogs should be potent and specific inhibitors of a given enzymatic reaction since the absolute rate theory predicts that enzymes will bind an "altered substrate" (i.e. the transition-state) much more tightly than they bind normal substrates. For example, adenosine deaminase catalyzes the conversion of adenosine to inosine as shown in figure 2. The rate-determining step probably involves nucleophilic attack by water bound to the enzyme on the substrate, forming the tetrahedral intermediate (1). Based on this information, it is easy to understand why coformycin (2) is an exceptionally potent inhibitor of adenosine deaminase.

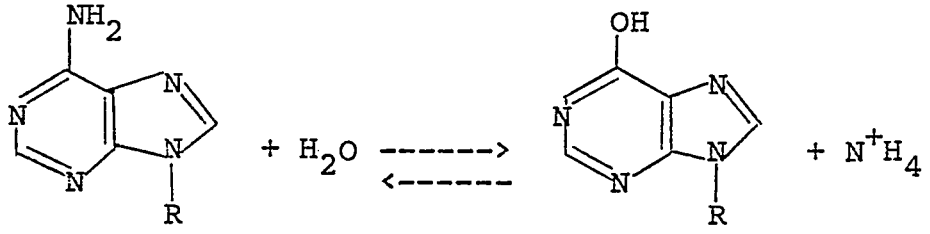
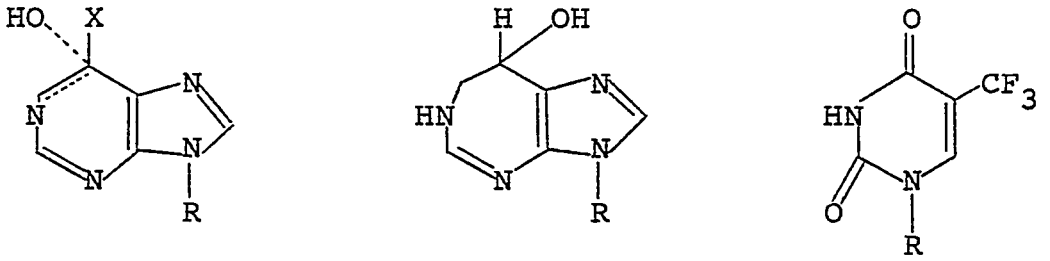


Figure 2. The conversion of adenosine to inosine by adenosine deaminase (R = ribose).



(1) (2) (3)
R=deoxyribose monophosphate

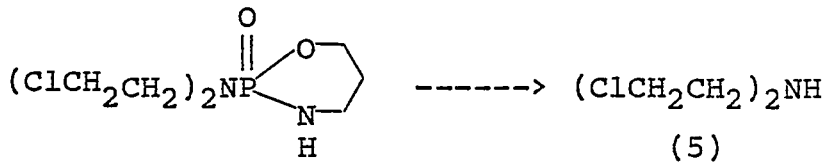
The suicide inhibitors are also irreversible and specific inhibitors and act by binding to the active site of the target enzyme which catalyzes their conversion to a reactive species. This reactive species then inactivates the enzyme by interacting with an amino acid residue at the active site of the enzyme. An example of a suicide inhibitor used in chemotherapy is 5-trifluoromethyluridine monophosphate (3).

1.4.0.0 The prodrug approach

The term prodrug is used to describe compounds which undergo biotransformations prior to eliciting a pharmacological effect (Stella et al., 1980). This

approach can be used to increase the usefulness of certain drugs, in regard to potencies and/or to decrease toxicities. The term prodrug has been used synonymously with other terms such as drug latentiation, bioreversible derivatives, congeners, etc. Drug latentiation has been defined as the chemical modification of a biologically active compound to form a new derivative which will liberate the parent compound in vivo (Kupchan et al., 1965).

The prodrug approach has been applied in a very successful manner in the design of some alkylating agents in cancer chemotherapy (Connors, 1976). For example, the clinically used drug, cyclophosphamide (4) was designed as a latent nitrogen mustard which would be transformed to the active form (5), nor-nitrogen mustard ,by the enzyme phosphamidase whose levels had been reported to be higher in many tumors. However, the clinical activity of cyclophosphamide was later shown to be due to a series of complicated metabolic transformations (Connors, 1976).



(4) (5)

1.5.0.0 Antineoplastic evaluation of the compounds

The antineoplastic evaluation of the compounds described in this thesis was carried out by the National Cancer Institute (NCI) U.S.A. According to their present three-step strategy (Vendetti, 1983), new agents are initially tested in the mouse P388 lymphocytic leukemia prescreen. Leukemia P388 has been selected as a prescreen because 1) its response to clinically active compounds of various classes of drugs is qualitatively similar to that of the L1210 system and 2) it is quantitatively more sensitive than the leukemia L1210 screen (Goldin et al., 1979). Agents showing a percent increase in lifespan $\geq$ 27% over control on two occasions are tested in a modified panel of four transplanted tumors. In the third stage, compounds that are positive in any of the screens at the second stage are subject to secondary screening against a number of tumors (table II). The characteristics of various tumors comprising the tumor panels, are summarized in table III.

Table II. Flow of drugs through NCI preclinical *in vivo* screens[a]

Step	Tumor(s)	Tumor site	Treatment site	Time of treatment initiation
1- Prescreen	P388	Ip	Ip	Day 1
2- Modified panel	L1210	Ip	Ip	Day 1
	B16	Ip	Ip	Day 1
	Mx-1	Src[b]	Ip	Day 1
	M5076	Ip	Ip	Day 1
3- Secondary screening[c]	L1210	Sc	Ip	Day 1
	B16	Sc	Ip	Day 1
	B16	Ip	Sc	Day 1
	M5076	Sc	Ip	Day 1
	MX-1	Sc	Ip	When sc tumor is established
	CD	Sc	Ip	When sc tumor is established
	C8	Sc	Ip	Day 1

a Taken from Venditti (1983).

b Tumor implanted under kidney capsule (subrenal capsule).

c Specific assays for each drug selected on basis of prior responsiveness.

Table III. Characteristics of tumors comprising the prescreen and the subsequent screens in the NCI Tumor Panel[a]

Tumor	Code	Origin of tumor	Host of origin	Histologic description	Test host
			Mouse tumors		
Leukemia (prescreen)	P388	Chemically induced with methyl-cholanthrene	DBA/2 mouse	Lymphocytic leukemia	BDF1 or CDF1
Melanoma	B16	Spontaneous at base of ear	C_{57}B1/6 mouse	Melanoma	BDF1 or B6C3
Breast	CD	Spontaneous	$CD8F_1$ mouse	Mammary adeno-carcinoma	CD8F1
Colon	C8	Induced by chemical carcinogen, 1,2-dimethyl-hydrazine	C_{57}B1/6 mouse	Colon adeno-carcinoma	BDF1
Leukemia	L1210	Chemically induced with methyl-cholanthrene	DBA/2 mouse	Lymphocytic leukemia	BDF1 or CDF1
			Human tumor xenografts		
Breast	MX-1	Human breast	Isolated in nude mice	Infiltrating duct cell carcinoma	Nu/Nu Swiss

a Taken from Staquet et al. (1983).

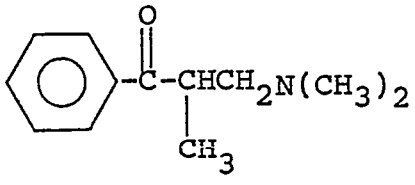
1.6.0.0 The present project

The present research project, which is actually part of an ongoing research program, can be considered to consist of two main areas.

1) Mannich bases and related compounds.

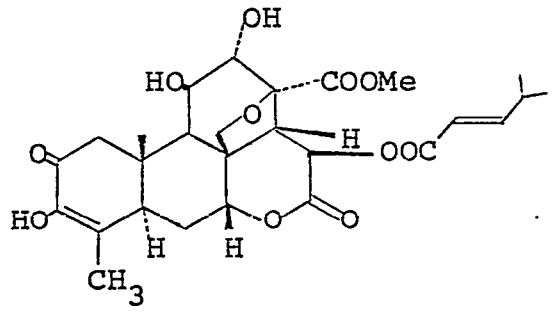
2) α,β-Unsaturated ketones and their derivatives.

Examination of the literature indicates that while a few Mannich bases derived from substituted acetophenones possess anticancer properties [e.g. (6)], there is, in general, a paucity of information on the anticancer properties of such compounds. On the other hand, a number of naturally occurring terpenes have been shown to possess potent anticancer activity [e.g. bruceantin, (7)]. Also many synthetic α,β- unsaturated carbonyl compounds possess antineoplastic properties [e.g. (8)]. The literature relating to the above two areas as well as the circumstances leading to the synthesis of such compounds will be reviewed in more detail in section 2.0.0.0, vide infra.



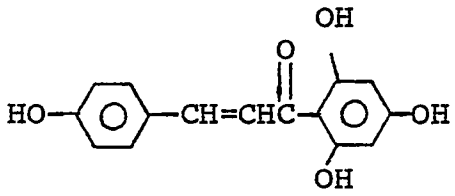
(6)

(Schoenenberger et al., 1969)



(7)

Bruceantin
(Suffness and Douros, 1979)



(8)

(Kabiev and Vermenichev, 1971)

2.0.0.0 Rationale of the study

2.1.0.0 Introduction

The obtention of anticancer drugs on a rational basis appears to be very difficult indeed (Montgomery, 1979; Paull et al., 1984). This is due to the fact that the majority of the known biochemical differences between normal and cancer cells are quantitative rather than qualitative (Pratt and Ruddon, 1979a) and hence, are not exploitable. Nevertheless, active research in the past few decades has resulted in the development of a number of clinically useful anticancer drugs (Montgomery, 1979). The majority of anticancer drugs have DNA as the target and are more effective against those cancers that are characterized by rapid cellular proliferation (Montgomery, 1979; Kohn, 1979). However, normal tissues with a high growth fraction such as the bone marrow are affected as well. Consequently, most anticancer drugs are marked by high toxicity (Pratt and Ruddon, 1979b). Also, because of their involvement with DNA, they have the potential of being mutagenic and carcinogenic (Sieber and Adamson, 1975). Therefore, there is an urgent need for more selective anticancer drugs with an entirely new target and/or a mechanism of action (McVie and Tomlinson, 1983).

The present project, which is actually part of an ongoing research program, represents an effort in this direction. It may be divided into two main areas viz.

1) Mannich bases and related compounds, and 2) α,β-unsaturated ketones and their derivatives. The Mannich bases can be expected to breakdown, under certain conditions, into α,β-unsaturated ketones (Tramontini, 1973; Dimmock et al., 1983a). Therefore, kinetic measurements of the rates of deamination of the bis-Mannich bases were also planned.

Many compounds containing an α,β-unsaturated carbonyl moiety have been shown to possess antitumor activity (Kupchan et al., 1971; Kabiev and Vermenichev, 1971; Hall et al., 1977). α,β-Unsaturated ketones, per se (Posner, 1902; 1904), and those derived from Mannich bases (e.g. Dimmock et al., 1983a) have been shown to react with nucleophiles such as thiols to give β-ketothioethers. This property has been invoked to explain the antitumor (Hall et al., 1977), antibacterial (Geiger and Conn, 1945), and antifungal (Baluja et al., 1964) activities of a number of carbonylenes. This susceptibility of α,β-unsaturated ketones to attack by nucleophiles suggests that these compounds may behave as alkylating agents which is a class of compounds with useful anticancer properties. Also, the conjugated unsaturated ketones have been found to exhibit a greater avidity for S nucleophiles rather than O and N nucleophiles (Baluja et al., 1964; Waddell et al., 1983; Dimmock et al., 1983b). This selectivity is not surprising in view of the classification of sulfur and enone as soft Lewis base and Lewis acid, respectively, whereas

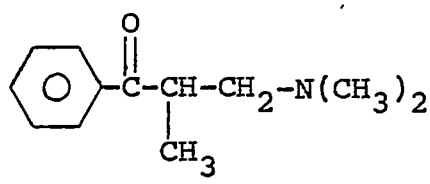
nitrogen and oxygen nucleophiles are considered to be hard Lewis bases (Ho, 1975). This unique Michael selectivity of the conjugated enones suggests that these compounds may have an advantage over other clinically used alkylating agents where carcinogenic (Farmer, 1982) and mutagenic (Cairns, 1980) properties have been noted, presumably because of their interaction with functional groups of the nucleic acids. Indeed, a series of Mannich bases containing an α,β-unsaturated carbonyl moiety did not show mutagenicity when examined in the Ames test (Dimmock et al., 1980a; Torigoe et al., 1983) and some chalcones even exhibited antimutagenic activity (Torigoe et al., 1983). Also, some selective toxicity to those tumors which are more rapidly growing than the corresponding normal tissues is conceivable since thiol levels may be more abundant just prior to and during mitosis (Emmelot, 1964) and hence more susceptible to alkylation. Moreover, P388, EL-H, and L1210 leukemia cells have been shown to require sulfhydryl compounds for tissue cell proliferation (Toohey, 1975).

In the following sections, the rationale for the design and synthesis of the individual classes of compounds is discussed in some detail.

2.2.0.0 1-Aryl-3-dimethylamino-2-dimethylaminomethyl -1-propanone dihydrochlorides (II)

Mannich bases derived from acetophenones have been found to display a wide variety of bioactivities. These properties include antibacterial (Agarwal and Tayal, 1967; Lemin et al., 1969), antimicrobial (Schoenenberger et al., 1969), analgesic (Atwal et al., 1969), anaesthetic (Tronche et al., 1962; Kurihara et al., 1964), antifungal (Plastino et al., 1962), cholinolytic (Mndzhoyan et al., 1969), and other miscellaneous (Kudrin et al., 1962a; Kudrin et al., 1962b; Bastide et al., 1962) activities.

A few reports have been published on the anticancer activities of the aromatic β-aminoketones. Schoenenberger et al. (1969), reported that the compound (6) decreased the weight of sarcoma 180 by 40%.



(6)

Mannich bases in which the amino group is the bis-(2-chloroethyl)amino moiety have also been synthesized (Muhlstadt et al., 1963) and tested (Werner et al., 1970). The latter workers tested 63 Mannich bases, many of them derived from acetophenones, as potential antitumor agents against Ehrlich ascites carcinoma in mice. Most of the 41 N

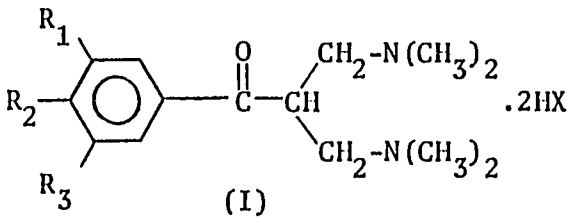
mustard C-Mannich bases tested were moderately to strongly active, while the 9 N mustard N-Mannich bases and the 13 Mannich bases without a N mustard group had slight or no activity, thereby suggesting the activity to be associated with the N mustard function.

Recently, Dimmock et al. (1983a; 1984) prepared a number of bis-Mannich bases derived from acetophenone for evaluation against P388 lymphocytic leukemia (table IV, Ia-l). Some of their observations were as follows.

1) Compounds with electron donating substituents, particularly methyl and methoxy, were quite active (Ib,c,d,l).

2) Compound (Ic), when examined under simulated physiological conditions, was found to deaminate more slowly than compound (If) which was biologically inactive. Thus, elimination seemed to be favoured by electron withdrawing substituents on the aromatic ring. Also, an optimal rate of breakdown seemed to be associated with anticancer activity, i.e., if the decomposition was too rapid (as in the case of If), then the α,β-unsaturated ketone generated was sequestered prior to interaction with leukemic cells, whereas if it was too slow or even refractory to deamination, there would be insufficient α,β-unsaturated ketone present for presumed nucleophilic attack.

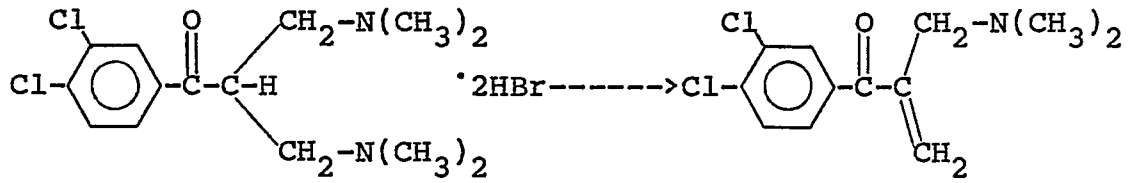
Table IV. Antineoplastic activity of 1-aryl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrohalides, prepared previously in this laboratory, against P388 lymphocytic leukemia in mice.



(I)

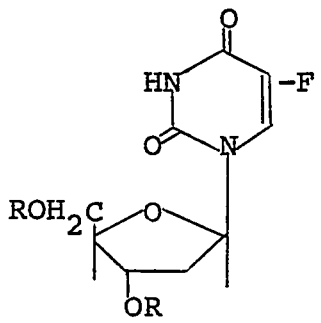
Compound	R_1	R_2	R_3	X	T/C % (mg/Kg)[a]
Ia	H	H	H	Br	117 (25)
Ib	H	OCH_3	H	Cl	128 (25)
Ic	H	OCH_3	H	Br	136 (25)
Id	H	CH_3	H	Br	138 (25)
Ie	H	Cl	H	Br	116 (25)
If	Cl	Cl	H	Br	108 (12.5)
Ig	OCH_3	H	H	Cl	114 (12.5)
Ih	OCH_3	OCH_3	H	Br	124 (12.5)
Ii	OCH_3	OCH_3	OCH_3	Br	111 (50)
Ij	H	OH	H	Br	118 (6.25)
Ik	CH_3	H	H	Br	114 (25)
Il	CH_3	CH_3	H	Br	128 (12.5)

a The figures are the ratios of the survival time of the treated(T) animals to control(C) animals expressed as a percentage. A value of 127 or more indicates activity. Data taken from Dimmock et al.(1983a; 1984).



(If)

In support of the above observation is the work done by Kawaguchi et al. (1985) who prepared a number of 3',5'-diester prodrugs of 5-fluoro-2'-deoxyuridine [FUdR, (9)] and evaluated them for antitumor activity against L1210 leukemia.



(9)

They observed that the longer alkyl chain diesters of FUdR possess higher antitumor activity than the corresponding short chain diesters or FUdR itself. This, in turn, was shown to be due to a slower rate of FUdR regeneration with esterases from the former.

3) They also reported that the Mannich base (If) reacted easily with a biomimetic thiol, 2-mercaptoethanol, under simulated physiological conditions, to give (10). The following scheme was proposed to explain the formation of (10).

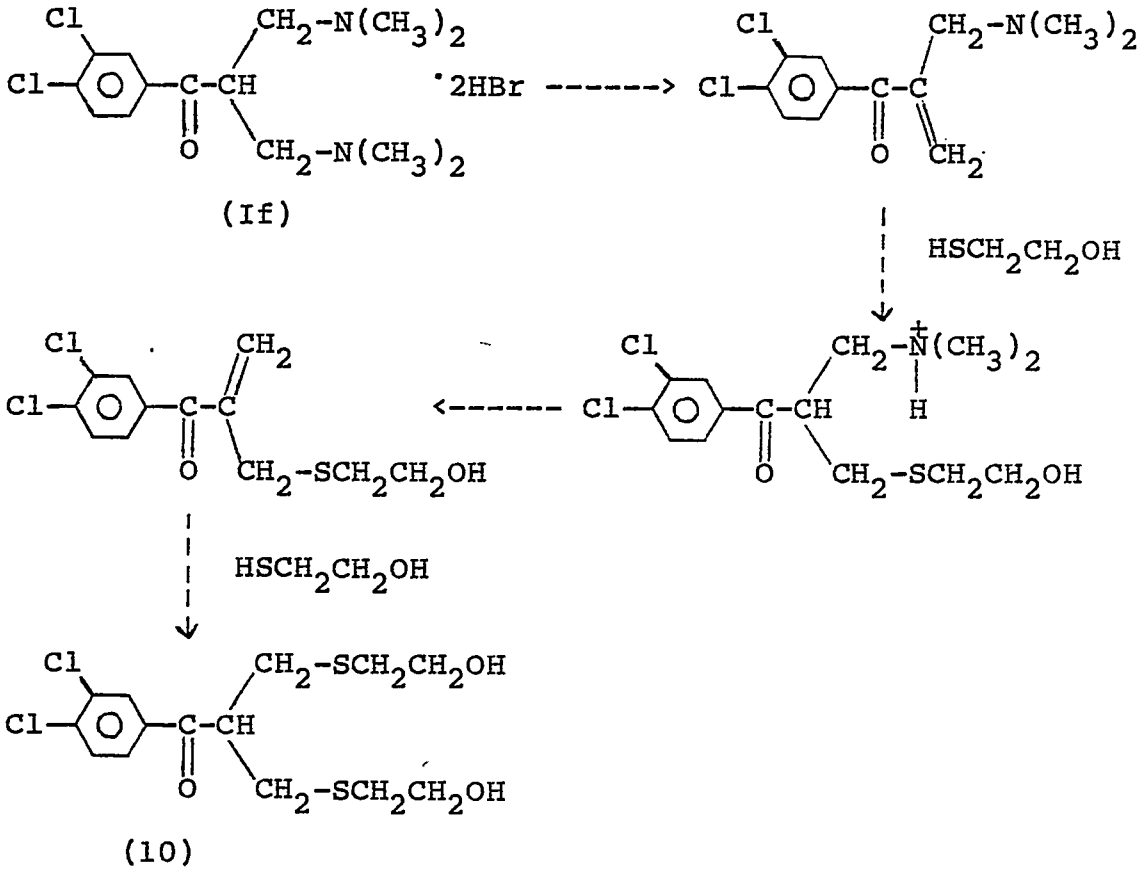


Figure 3. Proposed scheme for the formation of compound (10) from compound (If).

4) A Topliss analysis (Topliss, 1977) of the data indicated a greater importance of the Hammett σ value over the Hansch π parameter (viz. $\pi - 3\sigma$ or $-\sigma$).

Therefore, as a logical extension of the above work, and to test further the validity of the hypothesis that an optimal rate of breakdown was associated with anticancer activity, the preparation of a number of different Mannich bases was contemplated.

It is conceivable that the rate of deamination of the bis-Mannich bases will be determined by the acidity of the α-methine hydrogen and by the nature of the leaving group in the amine function. Therefore, with reference to figure 4, the rate of deamination may be influenced by the following ways.

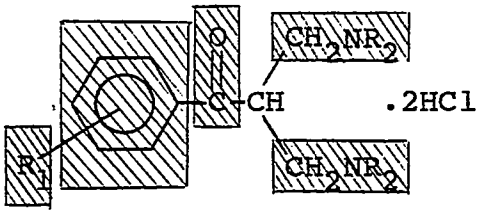


Figure 4. General structure of the bis-Mannich bases

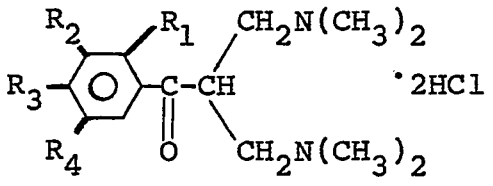
1) by varying the electronic nature of the substituents R_1 on the benzene ring,

2) by replacement of the benzene ring with other aryl, alkyl or cycloalkyl groups,

3) by reduction of the carbonyl group,

4) by varying the basicity of the amine function NR_2.

As a first step, therefore, preparation of series (II) was proposed. Here, the aim would be to retard deamination by aryl substitution with methyl and methoxy groups with special reference to ortho substitution.

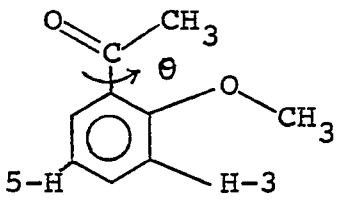


(II)

	R_1	R_2	R_3	R_4
a)	CH_3	H	H	H
b)	CH_3	H	CH_3	H
c)	CH_3	H	H	CH_3
d)	CH_3	H	CH_3	CH_3
e)	OCH_3	H	H	H
f)	OCH_3	H	OCH_3	H
g)	OCH_3	H	H	OCH_3
h)	OCH_3	OCH_3	OCH_3	H
i)	OCH_3	H	OCH_3	OCH_3
j)	H	$--OCH_2O--$		H
k)	Cl	H	H	H

2-Methoxyacetophenone has been shown to exist in the conformation (11) in which the methoxy group lies in the benzene plane and cis to H-3, while the acetyl group

predominantly prefers an arrangement in which the carbonyl group lies trans to the methoxy group. The angle θ is not very large i.e. $<5^{o}$ (Schaefer et al., 1984).



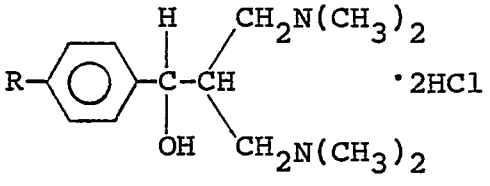
(11)

A recent ^{13}C NMR investigation of substituent effects in 2-methyl-, 2-methoxy-, and 2,6-dimethyl-acetophenone shows clearly the very different effects in the 2,6-dimethyl compound, indicating essentially no conjugation in the last case, but variable effects in the former (Goethals et al., 1982). Similarly, a lanthanide-induced shift NMR investigation of steric effects in 2,4,6-trimethyl-acetophenone indicated the acetyl group to have a dihedral angle of $60\text{-}90^{o}$ with the aromatic ring with the methyl C-C-C angles being unaffected (Abraham et al., 1983). This study also illustrated the lack of conjugation of the carbonyl group with the aromatic ring.

Compounds with ortho substitution were therefore emphasized in the proposed series of compounds, to see what effect, if any, such substitution would have on the stability of the molecule through steric impedence of planarity between the carbonyl bond and the benzene ring.

2.3.0.0 1-Aryl-3-dimethylamino-2-dimethylaminomethyl -1-propanol dihydrochlorides (III)

As shown in figure 4, another way of lowering the rate of deamination is to reduce the carbonyl function to the corresponding alcohol. It is conceivable, on electronic grounds, that the alcohol group should be considerably less electron withdrawing than the carbonyl group and hence this modification should decrease the acidity of the α-methine hydrogen and, in turn, the rate of deamination.

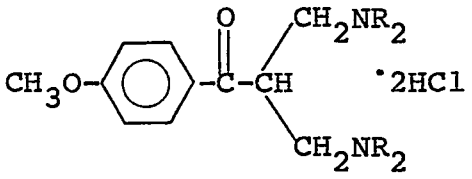


(III)
R=H or OCH_3

2.4.0.0 3-Amino-2-aminomethyl-1-(4-methoxyphenyl) -1-propanone dihydrochlorides (IV)

As discussed in section 2.2.0.0 compound (Ib) showed good activity against P388 lymphocytic leukemia (T/C%=128) and was designated a Selected Agent Compound by the National

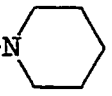
Cancer Institute, U.S.A. (Dimmock et al., 1984). This compound, along with the corresponding para-methyl derivative, has the optimum aryl substitution pattern. As discussed earlier, the rate of formation of the corresponding acrylophenone should be influenced by the basicity of the amine function and hence, it was proposed to prepare series IV.

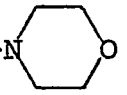


(IV)

(I): b) NR_2= $-N(CH_3)_2$

(IV): a) NR_2= $-N(C_2H_5)_2$

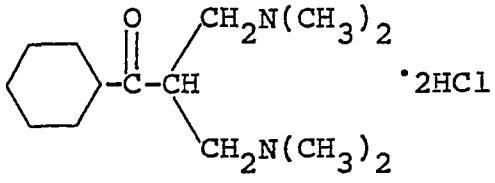
b) NR_2= 

c) NR_2= 

d) NR_2= 

The pKa values of dimethylamine, diethylamine, piperidine, morpholine and pyrrolidine are 10.73, 10.84, 11.12, 8.50 and 11.31, respectively (Albert and Serjeant, 1984). Thus, the rate of decomposition predicted would be IVc > Ib > IVa > IVb > IVd.

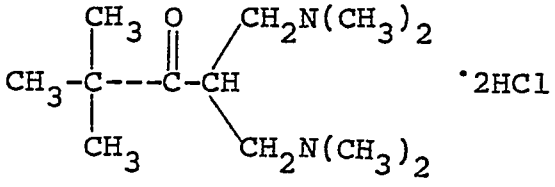
2.5.0.0 1-Cyclohexyl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrochloride (V)



(V)

Another way of lowering the rate of deamination would be to replace the phenyl ring by a cyclohexyl group, as the latter would be expected to be more electron donating than the former. Another advantage of such a modification would be that it would permit some variation in the partition coefficient of the molecule. Correlations between lipophilic character, which is a measure of the fraction of the dose administered reaching its receptor compartment as measured by the partition coefficient in 1-octanol-water, and various biological activities have proven very meaningful (Kupchan et al., 1971).

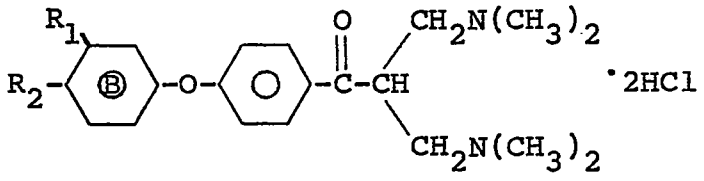
2.6.0.0 1-Dimethylamino-2-dimethylaminomethyl-4,4-dimethyl-3-pentanone dihydrochloride (VI)



(VI)

The preparation of this compound was contemplated for reasons similar to those invoked for the preparation of (V), in section 2.5.0.0.

2.7.0.0 <u>1-(4-Aryloxyphenyl)-3-dimethylamino -2-dimethylaminomethyl-1-propanone dihydrochlorides (VII)</u>



(VII)

	R_1	R_2
a)	H	H
b)	H	Cl
c)	Cl	Cl
d)	H	OCH_3
e)	H	CH_3

Preparation of series (VII) was contemplated for the following reasons.

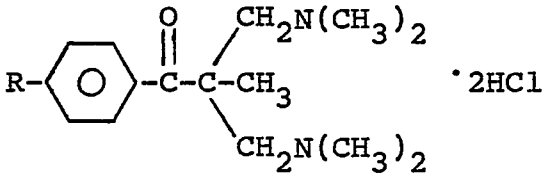
1) Although the Hammett σ value of the phenoxy group (σp= -0.32) is close to that of the methoxy group [(σp= -0.28), Exner, 1978], it was hoped that the placement of an additional electron donating group on the aryl ring B would help in reducing the rate of deamination.

2) The introduction of an aryloxy group would also cause changes in the partition coefficient of the molecule [π (phenoxy)= 1.34, π (methoxy)= -0.03; Taylor and Kennewell, 1981]. This, in turn, could influence anticancer activity.

3) Detection of anticancer activity in the above series of compounds would permit a Topliss analysis of the data

[(Topliss, 1977), section 4.7.1.0]. This, in turn, would aid in the selection of new substituents for the synthesis of potentially more potent analogs.

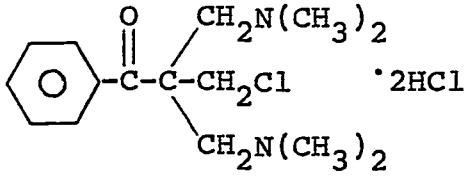
2.8.0.0 1-Aryl-3-dimethylamino-2-dimethylaminomethyl-2-methyl-1-propanone dihydrochlorides (VIII)



(VIII)
R= H, CH_3

The preparation of these two compounds was contemplated in order to aid in verification of the theory that the activity of the bis-Mannich bases is due to their deamination to the corresponding α,β-unsaturated ketones and that an optimal rate of breakdown is associated with good anticancer activity. This is because compounds (VIII) would not be expected to undergo deamination to the corresponding acrylophenone and would, therefore, be predicted to be inactive against P388 lymphocytic leukemia.

2.9.0.0 2-Chloromethyl-3-dimethylamino-2-dimethylamino-methyl-1-phenyl-1-propanone dihydrochloride (IX)



(IX)

This compound was designed for reasons similar to those invoked for the preparation of (VIII), in section 2.8.0.0. In addition, it was hoped that the presence of the β-chlorine atom would increase the acidity of the α-methine hydrogens in β-chloropropiophenone, and thus, aid in the Mannich reaction.

2.10.0.0 1-Aryl-3-dimethylamino-1-propanone hydrohalides (X)

Mono-Mannich bases derived from acetophenone have been known to breakdown under certain conditions, e.g. heat, aqueous alkali, etc., to α,β-unsaturated ketones (Blicke, 1942).

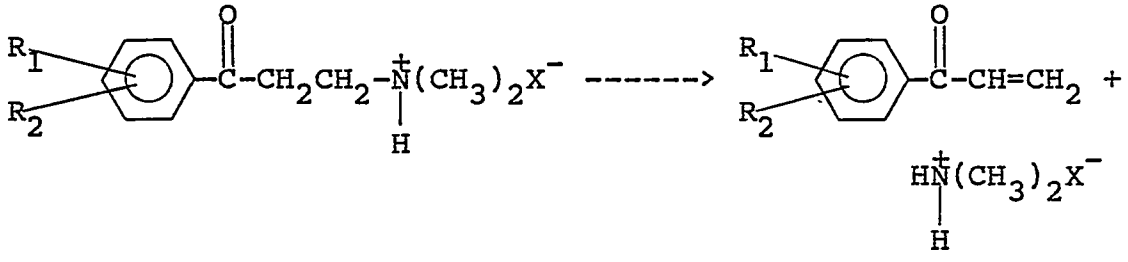


Figure 5. Decomposition of a Mannich salt.

As noted earlier (section 2.1.0.0), several α,β-unsaturated ketones and lactones exhibit potent anticancer properties which could derive from their interaction with cellular thiols. A similar mechanism of action is also conceivable in the present case. In support of this postulate is the work done by Andrisano et al. (1967) who reacted a number of β-dialkylaminopropiophenones with

thiophenols and obtained the corresponding sulfides. An elimination-addition mechanism was proposed for this reaction.

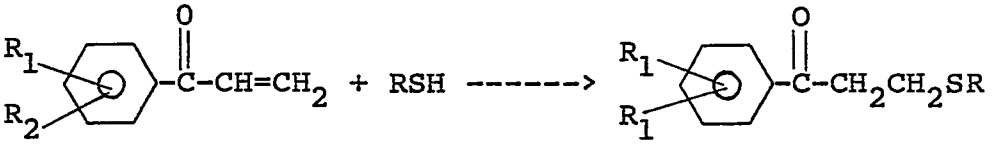
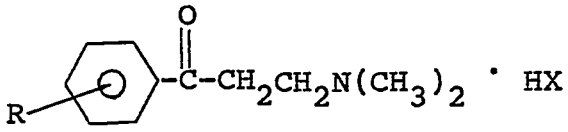


Figure 6. Addition reaction of a thiol with an acrylophenone.

Schoenenberger and Bastug (1971) obtained a correlation between the rate of breakdown of acetophenone mono-Mannich bases and their antimicrobial activity. It was thought possible that a similar correlation might exist between the anticancer activity of the proposed compounds and their stability.

Recently, Dimmock et al. (1983a; 1984) prepared several of these mono-Mannich bases for evaluation against P388 lymphocytic leukemia in mice. They observed that these compounds were uniformly inactive and less toxic than their bis-counterparts. The inactivity was thought to be due to their greater stability in vivo based on experiments using simulated physiological conditions.

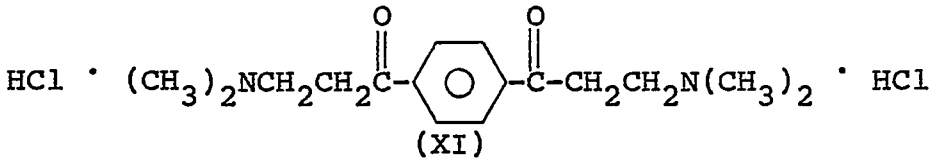
A proposal was therefore made to prepare series (X) to determine if any useful structure-activity relationships accrue or whether the trend observed earlier, viz. that of the mono-Mannich bases being uniformly inactive, was continued.



(X)

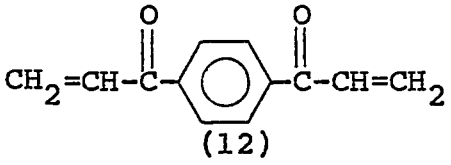
R: as in series (II), section 2.2.0.0
X= Cl, Br

2.10.1.0 1,4-bis(3-Dimethylaminopropionyl)benzene dihydrochloride (XI)



(XI)

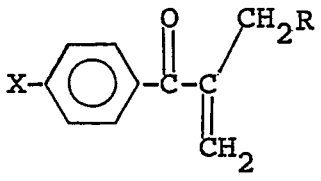
The preparation of this compound was undertaken for reasons similar to those invoked for the preparation of series (X), in section 2.10.0.0. In addition, the potential breakdown product of (XI) would be (12), which would be a bis-alkylating agent.



(12)

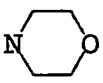
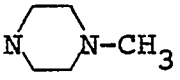
2.11.0.0 1-Aryl-2-dimethylaminomethyl-2-propen-1-one hydrochlorides (XII)

2-Aminomethylacrylophenones appear to exhibit a variety of biological properties. For example, Lesieur et al. (1986) recently prepared a series of nine substituted acrylophenones (XIII). Some of their observations were as follows.



(XIII)

X= H, Cl or OCH_3

R= $N(CH_3)_2$,  or 

1) All of the nine compounds were potent antimicrotubular agents acting in the same concentration range as colchicine, which is a potent antimicrotubular and anticancer agent.

2) These authors also suggested that the antineoplastic activity reported for some of the β-aminoketones in the literature, is probably related to their antimicrotubular effect.

3) The compounds reduced markedly the adenosine diphosphate (ADP)-induced platelet aggregation.

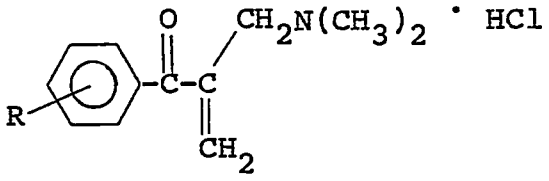
4) The compounds caused a marked diminution in the serum triglyceride, cholesterol and phospholipid level.

5) The nine compounds were also found to be potent inhibitors of the growth of three different fungi.

Mallevais et al. (1984) showed that the diuretic, ethacrynic acid, which is an α,β-unsaturated ketone, is also an antimicrotubular agent. They concluded that it inhibits tubulin polymerization by covalent binding with a cysteine residue near the binding site of colchicine.

Recently, Gupta et al. (1981) reported that some 2-aminomethylacrylophenones of the general structure similar to that shown above, possess potent spermicidal activity.

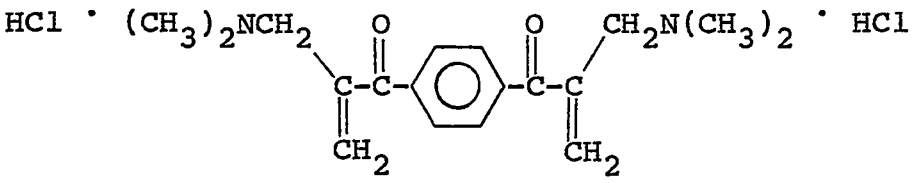
It was contemplated that if the activity of the bis-Mannich bases was indeed due to the acrylophenone moiety, and also in view of the above facts, it might be of interest to prepare a corresponding series of substituted acrylophenones (XII). Comparison of the bioactivity of the acrylophenones with that of the bis-Mannich bases and the mono-Mannich bases could permit useful structure-activity relationships to emerge.



(XII)

R= H, CH_3, OCH_3, OCH_2O, OC_6H_5 or Cl.

2.11.1.0 1,4-bis(2-Dimethylaminomethyl-1-oxo-2-propenyl)benzene dihydrochloride (XIV)

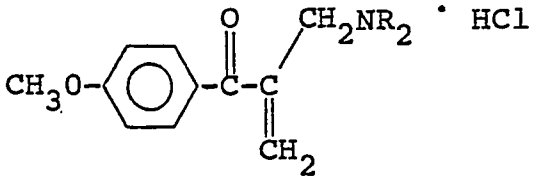


(XIV)

Preparation of this compound was proposed for reasons similar to those mentioned above in section 2.11.0.0. Also, this compound would have the potential of being a bis-alkylating agent.

2.12.0.0 2-Aminomethyl-1-(4-methoxyphenyl)-2-propen-1-one hydrochlorides (XV)

This series of compounds was also prepared for reasons similar to those mentioned for the synthesis of (XII) in section 2.11.0.0. In particular, the activity of these compounds could be compared with that of the 3-amino-2-aminomethyl-1-(4-methoxyphenyl)-1-propanone dihydrochlorides (IV) proposed in section 2.4.0.0.

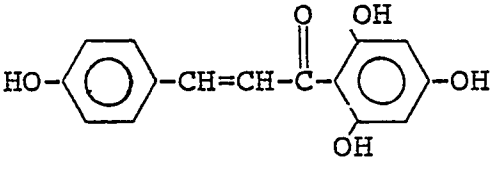


(XV)

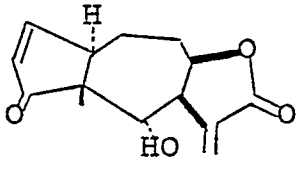
NR_2: as in (IV) section 2.4.0.0

2.13.0.0 1-Aryl-1-nonen-3-ones (XVI)

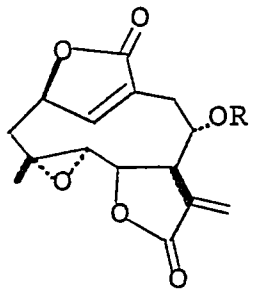
Much of the rationale for the synthesis of these compounds has been discussed in detail in the preceding sections. Below are given the structures of a few of the compounds containing one or more α,β-unsaturated carbonyl moieties which have been reported in the literature to possess good anticancer activity.



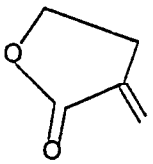
(8)
substituted chalcone
(Kabiev and Vermenichev, 1971)



(13)
helenalin
(Hall et al., 1977)



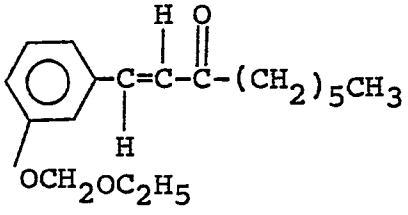
(14)
elephantopin
(Kupchan et al., 1971)



(15)

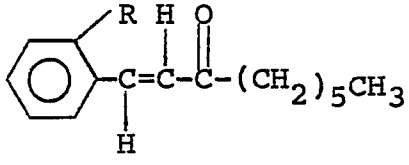
In the case of the naturally occurring sesquiterpene lactones, it has been shown that the anticancer activity is critically dependent on the presence of the α-methylene-γ-lactone grouping [(15), Kupchan et al., 1971] or the cyclopentenone ring system (Hall et al., 1977). The latter group of workers also showed that helenalin alkylated the thiol group of reduced glutathione and L-cysteine in vitro, and that it did not alkylate nucleophiles of purine bases. They suggested that the inhibition of tumor growth by helenalin was due to the CH_2=C-C=O system alkylating by rapid Michael addition the thiol groups of key regulatory enzymes of nucleic acid and chromatin metabolism.

Several conjugated styryl ketones have been prepared and evaluated for anticancer activity in this laboratory (Dimmock and Taylor, 1975; Dimmock and Smith, 1980c; Dimmock et al., 1982). The ether (16) increased the median survival time by 25 and 30% in mice bearing P388 lymphocytic leukemia and B16 melanocarcinoma, respectively (Dimmock et al., 1979a).



(16)

Therefore, in keeping with the above facts and also as a logical extension of the ongoing research program in this laboratory, the preparation of series (XVI) was contemplated.

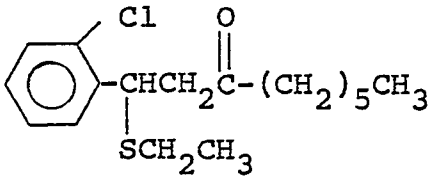


(XVI)

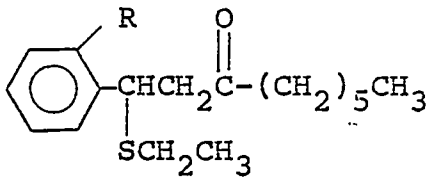
R= H, Cl, Br, F, CH_3 or OCH_3

2.14.0.0 <u>1-Aryl-1-ethylthio-3-nonanones (XVII)</u>

At the time when synthesis of these compounds was undertaken, 1-(2-chlorophenyl)-1-ethylthio-3-nonanone (XVIIa) prepared previously in this laboratory, had been designated a Selected Agent Compound by the National Cancer Institute, U.S.A. It prolonged the survival of mice bearing P388 lymphocytic leukemia by 21% and was relatively non-toxic. Therefore, molecular modification of this compound was proposed with a view to obtaining compounds with increased biological activity and allowing useful structure-activity relationships to emerge.

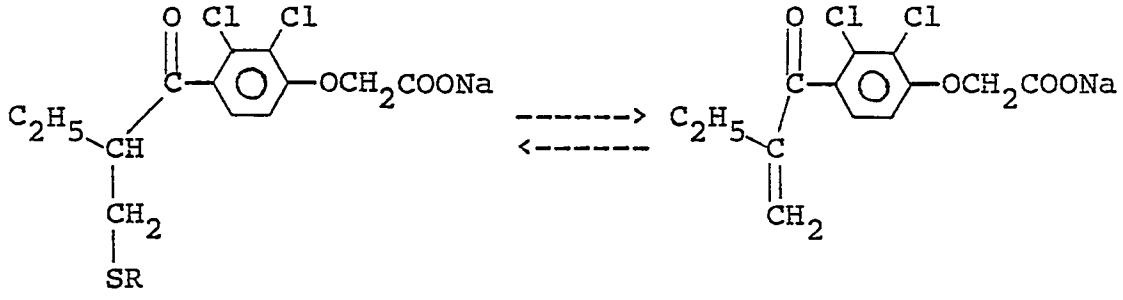


(XVIIa)



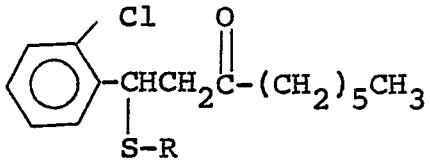
(XVII)
R= H, Cl, Br, F, CH_3 or OCH_3

As a first step, the preparation of series (XVII) was undertaken. The rationale behind the preparation of these compounds was that they would possibly undergo a retro-Michael reaction in vivo, preferably in neoplastic tissue, to generate the parent alkylating α,β-unsaturated ketone and ethanethiol. Indeed, Michael adducts have been found to undergo a retro-Michael reaction under a variety of conditions including base catalysis and heat (Bergmann et al., 1959). Thiol adducts (17) of ethacrynic acid (18) were found to yield the parent α,β-unsaturated ketone (18) under certain in vitro conditions (Koechel and Cafruny, 1973; 1975).



(17) (18)

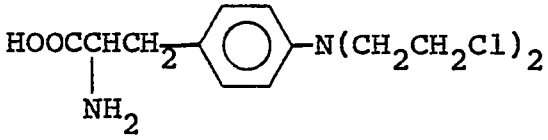
2.15.0.0 1-Alkylthio-1-(2-chlorophenyl)-3-nonanones (XVIII)



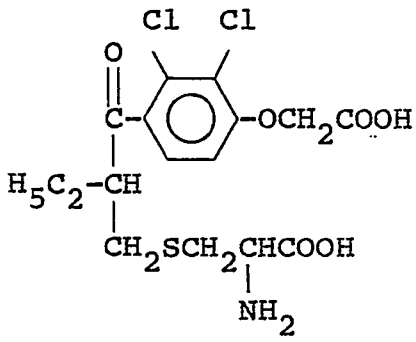
(XVIII)

The preparation of this series of compounds was envisaged for reasons similar to those mentioned above in section 2.14.0.0. In addition, the nature of the R groups possessed various characteristic properties which could augment the anticancer activity of the unsaturated ketone. Also, many of these thiols possess tumor growth-inhibiting properties, some of which are discussed below.

1) As alkylating agents need to penetrate cellular and nuclear membranes to reach the presumed target sites, it seemed logical to researchers to attach the cytotoxic group to carrying structures that are related to substances normally involved in cell growth, such as amino acids, carbohydrates, nucleic acid bases, and steroids (Ross, 1974). An example of a clinically used compound designed by such a concept is melphalan (19) in which the amino acid, phenylalanine, is used as the carrier group.



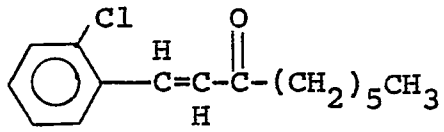
(19)



(20)

Similarly, it has been suggested that the cysteine moiety of the ethacrynic acid-cysteine adduct (20) serves as a carrier for the diuretic, ethacrynic acid (Koechel and Rankin, 1982).

In this regard, the preparation of adducts of 1-(2-chlorophenyl)-1-nonen-3-one (XVIa) with amino acids such as L-cysteine, N-acetyl-L-cysteine and L-cysteine ethyl ester hydrochloride was contemplated. It may be noted that compound (XVIa) prepared previously in this laboratory, showed marginal activity in the P388 lymphocytic leukemia screen (T/c%= 118, Dimmock et al., 1980a).



(XVIa)

2) A number of interesting biological properties have been reported for L-cysteine and its derivatives. For example, L-cysteine hydrochloride prolongs the survival of DBA/2 mice bearing L1210 leukemia by 10% (Knight et al., 1983). L-

Cysteine has also been shown to stereospecifically inhibit aerobic glycolysis exclusively in Ehrlich ascites tumor cells without affecting the same in normal tissues (Kedryna et al., 1983). Similarly, S-trityl-L-cysteine was found to have antileukemic activity with T/C% of 217 (Zee-Cheng and Cheng, 1970; 1972). Preparation of the adducts of (XVIa) with derivatives of L-cysteine was therefore contemplated so that an in vivo retro-Michael reaction of the adducts could provide a two-pronged attack by generating the conjugated enone and L-cysteine.

3) Thiols such as L-cysteine, L-cysteine methyl ester hydrochloride, and glutathione have been shown to form chelate complexes with ferric iron (Hamed et al., 1983) and other metal ions (Li and Manning, 1955). Many compounds have been shown to owe their anticancer activity to chelation of metal ion enzyme cofactors (DiStefano et al., 1983). This aspect of chelation and anticancer activity will be discussed in greater detail in section 2.16.0.0.

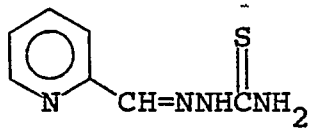
4) Mice fed a diet supplemented with 2-mercaptoethanol (21) have been shown to have an increased mean and maximum life span and a postponed onset and decreased incidence of tumors. Also, the immune responses of these animals were higher compared to the controls (Heidrick et al., 1984).

$HSCH_2CH_2OH$ (21) $HSCH_2CH_2NH_2$ (22)

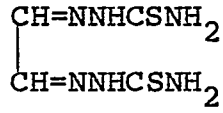
5) It has been suggested that the radioprotector, cysteamine (22), affords DNA protection by binding its two terminal functional groups to two consecutive phosphate groups along the same DNA strand by electrostatic interaction (Liquier et al., 1983).

2.16.0.0 1-Aryl-1-ethylthio-3-nonanone thiosemicarbazones (XIX) and semicarbazones (XX)

A thiosemicarbazone possessing antitumor activity against L1210 leukemia in mice was first reported in 1956. The compound, 2-formylpyridine thiosemicarbazone (23), was later used as the model for further studies by other workers who synthesized and evaluated numerous α,N-heterocylic carboxaldehyde thiosemicarbazones as antineoplastic agents (Klayman et al., 1983). Since then, a number of other thiosemicarbazones with anticancer activity have been reported. For example, glyoxal bis-thiosemicarbazone (24) and related derivatives were shown to be active against sarcoma 180; the mechanism of action was ascribed to metal binding (French and Freedlander, 1958).



(23)



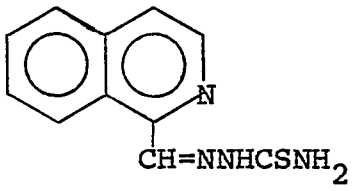
(24)

Metal chelation has also been invoked to explain the anticancer activity of 1-formylisoquinoline thiosemicarbazone [(25), Moore et al., 1970]. The enzyme, ribonucleoside

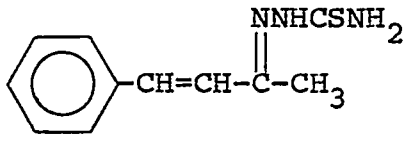
diphosphate reductase, which reduces ribonucleotides to deoxyribonucleotides, is inhibited by (25), by iron binding. This results in interference of DNA biosynthesis, which in turn suppresses tumor growth.

Recently, Mishra et al. (1983) showed that benzalacetone semicarbazone and dibenzalacetone semicarbazone form complexes with a number of divalent metal ions including Cu, Zn, Ni, Co, Mn and Cd.

1-Phenyl-1-buten-3-one thiosemicarbazone (26), prepared previously in this laboratory (Dimmock et al., 1981), was shown to have confirmed activity in the P388 screen (T/C%= 137) and to form a 1:1 chelate with cupric ions. This chelation was thought to be responsible, at least partially, for its mode of action.



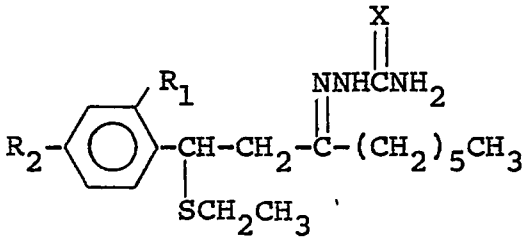
(25)



(26)

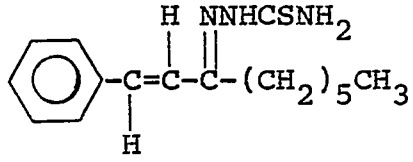
Thus, data exist supporting the idea that metal chelation by thiosemicarbazones and semicarbazones can result in the inhibition of neoplastic growth. It was therefore contemplated that the anticancer potential of 1-aryl-1-ethylthio-3-nonanones (XVII) could be further enhanced by their conversion into the corresponding thiosemicarbazones (XIX) and semicarbazones (XX). Preparation of the compound

(XXI) was proposed with a view to elucidate the relative importance of the conjugated olefinic bond in the expression of anticancer activity.



(XIX): X= S

(XX): X= O
R_1= H, Cl, Br, F, CH_3 or OCH_3
R_2= H, Cl or CH_3

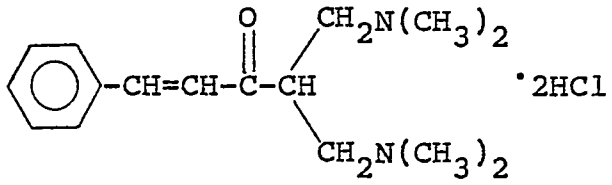


(XXI)

2.17.0.0 Stability studies of compounds under simulated physiological conditions

2.17.1.0 5-Dimethylamino-4-dimethylaminomethyl-1-phenyl -1-penten-3-one dihydrochloride (27)

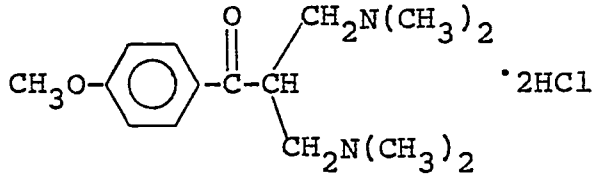
A number of monobasic and dibasic Mannich salts derived from conjugated styryl ketones have been prepared in this laboratory and evaluated for antileukemic activity. While some of the former were active, the latter were uniformly inactive (Dimmock and Patil, 1986). In order to explore the possibility that the absence of antineoplastic activity of the dibasic Mannich salts was due to a breakdown to one or more inactive compounds, it was decided to examine the stability of (27) in phosphate buffer (pH 7.4), at 37°C.



(27)

2.17.2.0 3-Dimethylamino-2-dimethylaminomethyl -1-(4-methoxyphenyl)-1-propanone dihydrochloride (28)

It was pointed out in section 2.2.0.0 that the antileukemic activity of some of the acetophenone bis-Mannich bases was presumably due to their breakdown in vivo to the corresponding acrylophenones. In this context, the bis-Mannich bases could be considered as prodrugs of the alkylating acrylophenones. Therefore, it was deemed important to demonstrate that the bis-Mannich bases do indeed yield the corresponding acrylophenones under simulated physiological conditions. It was therefore proposed to make a thorough investigation of the behavior of a representative bis-Mannich base (28) and also of the corresponding acrylophenone formed therefrom in phosphate buffer (pH 7.4), at 37°C.



(28)

2.18.0.0 Kinetic studies

It was also indicated in section 2.2.0.0 that an optimal rate of breakdown of the bis-Mannich bases was probably necessary for good anticancer activity. In keeping with this hypothesis, Mannich bases were designed which would deaminate relatively slowly. Therefore, in order to test the validity of this hypothesis, a kinetic study of the elimination reaction of the bis-Mannich bases, under simulated physiological conditions, was proposed so that the rates of deamination could be correlated with any observed anticancer activity. Ultraviolet spectroscopy (UV) was the method of choice for studying the kinetics because of the obvious advantages of simplicity, sensitivity, accuracy and reproducibility. However, it was also decided to measure the rate of deamination of a representative bis-Mannich base (28) by a NMR method because in addition to providing comparative data, the NMR method would also aid in establishing the structure of the breakdown product(s).

3.0.0.0 MATERIALS AND METHODS

Melting points

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

Nuclear magnetic resonance (NMR) spectroscopy

NMR spectra were recorded on a Varian T-60 spectrometer. High resolution NMR spectra were determined on a Brucker AM-360-WB spectrometer by Mr. M. Mazurek, National Research Council, Saskatoon, and on a Brucker AM 300 FT NMR spectrometer equipped with a variable temperature unit (BVT-1000) and an Aspect 3000 computer by Mr. D. Leek, Department of Chemistry. Tetramethylsilane was used as the internal standard for the spectra recorded in chloroform-d or carbon tetrachloride. When the solvent used was deuterium oxide, sodium 2,2-dimethyl-2-silapentane-5-sulfonate was used as the internal standard. The abbreviations used are s (singlet), d (doublet), t (triplet), and m (multiplet). Chemical shifts are described as δ(ppm) values downfield from the internal standard.

Mass spectroscopy (MS)

The mass spectra were recorded at 12 and 70 eV on a VG Micromass MM16 F mass spectrometer equipped with a 2025 data system by Dr. G. McKay, College of Pharmacy.

Infra red (IR) spectroscopy

Infra red spectra were recorded on a Beckman Aculab 4 IR spectrophotometer.

Ultra violet (UV) spectroscopy

UV spectra were recorded on a Gilford Response UV-VIS spectrophotometer equipped with a temperature controlling device.

Chromatography

Thin-layer chromatography (TLC) was performed using Eastman chromatogram sheets, type 13254 (silica gel with fluorescent indicator). Spots were observed under short-wave ultraviolet light. All the compounds reported in this thesis were found to be homogeneous by TLC unless otherwise indicated. Column chromatography was performed using Baker analyzed silica gel (60-200 mesh), Fisher Scientific neutral alumina (Brockman activity I, 80-200 mesh) and microcrystalline cellulose, avicel® from Merck. All of the oils purified by chromatography were found to be homogeneous by TLC, unless otherwise indicated.

Elemental analyses

Elemental analyses were performed by Mr. R. E. Teed, Department of Chemistry, using a Hewlett-Packard Model 185 C-H-N Analyser. All samples were dried over phosphorus pentoxide at 60°C under reduced pressure before being analyzed.

Incubation at 37°C

This was carried out using a Dubnoff metabolic shaking incubator.

Freeze-drying

Compounds were freeze-dried on a Labconco® freeze dryer 18.

Drying and purification of solvents and reagents was carried out according to the literature procedures (Vogel, 1978).

Antineoplastic evaluation

The anticancer screening was carried out by the Drug Research and Development Division of the National Cancer Institute (NCI), Bethesda, Maryland, U. S. A., using their protocols (Geran et al., 1972). Unless otherwise indicated there were no mortalities at doses indicating maximum anti-neoplastic activity.

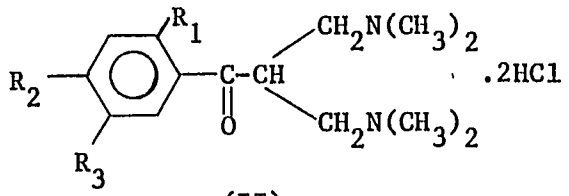
3.1.0.0 Synthesis of the Mannich reagent, N,N-dimethyl(methylene)ammonium chloride (XXII)

This compound was prepared in analogy to a literature procedure (Bohme and Hartke, 1960). A solution of acetyl chloride (19.63g, 0.25 mol) in dry methylene chloride (200ml) was added slowly with stirring to a solution of N,N,N',N'-tetramethyldiaminomethane (25.5g, 0.25 mol) in dry methylene chloride (200 ml). The solid that precipitated was filtered immmediately, washed with dry methylene chloride and dried in vacuo to obtain the title compound, N,N-dimethyl-(methylene)ammonium chloride. This compound was stored in vacuo over phosphorous pentoxide and used as and when required without any further purification. NMR (DMSO-d_6): δ 3.52 [s, 6H, $=\overset{+}{N}(CH_3)_2$], 8.05 (s, 2H, $CH_2=\overset{+}{N}$).

3.2.0.0 Synthesis of 1-aryl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrochlorides (IIa-d, Table V)

Compounds (IIa-d) were prepared by the following general method. A mixture of the appropriate acetophenone (0.019 mol), Mannich reagent (7.47g, 0.08 mol) and dry acetonitrile (50 ml) was stirred at 45-50°C for 24-90 hours. In case of compound (IId) the mixture was also refluxed for 8 hours at the end of the stirring period. In each case, the precipitated solid was filtered, washed with hot acetonitrile and dried. Pure (IIa) was obtained by recrystallizing this solid. Compounds (IIb-d) were obtained by dissolving the crude solid in a little water, followed by lyophilization of the solution. The solids so obtained were purified by recrystallization from suitable solvents. In the case of compound (IId), a number of recrystallizations were required in order to obtain a sufficiently pure compound. The structures of all the compounds were confirmed by NMR and IR spectroscopy and elemental analysis. The spectroscopic data generated for a representative compound (IIb) are as follows. NMR (D_2O): δ 2.97 [s, 12H, $N(CH_3)_2$], 3.17-3.87 [m, 5H, $C^2H(CH_2)_2$], 3.93 (s, 3H, aromatic 4-OCH_3), 4.07 (s, 3H, aromatic 2-OCH_3), 6.60-6.87 (m, 2H, aromatic H at C-3 and C-5), 7.82 (d, 1H, J=9Hz, aromatic H at C-6); IR (KBr): ν 2800-2340 ($\overset{+}{N}H$), 1660 (C=O) cm^{-1}.

Table V. Physical data of 1-aryl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrochlorides(IIa-d)



(II)

Compound	R_1	R_2	R_3	Time of stirring (hours)	Recrystallization solvent	Yield (%)	Melting point (°C)	Molecular formula	Analysis(%) Calculated C	H	N	Found C	H	N
IIa	OCH_3	H	H	24	Methanol/Ether	22	155.0	$C_{15}H_{26}Cl_2N_2O_2$	53.41	7.77	8.31	53.70	7.84	8.31
IIb	OCH_3	OCH_3	H	90	Methanol/Ether	68	143.7	$C_{16}H_{28}Cl_2N_2O_3$	52.32	7.68	7.63	52.65	7.78	7.67
IIc	OCH_3	OCH_3	OCH_3	48	Methanol/Ether	79	175.2	$C_{17}H_{30}Cl_2N_2O_4$	51.38	7.61	7.05	51.24	7.99	6.81
IId	H	OCH_2O		24	Methanol	13	184.2	$C_{15}H_{24}Cl_2N_2O_3$	51.29	6.89	7.98	50.55	6.64	7.57

3.2.1.0 Reaction of 2,4-dimethylacetophenone with the Mannich reagent

A mixture containing 2,4-dimethylacetophenone (2.96g, 0.02 mol), the Mannich reagent (7.47g, 0.08 mol) and dry acetonitrile (50 ml) was stirred at 45-50°C for 32 hours. The excess of undissolved Mannich reagent was removed by filtration and washed with hot acetonitrile. The filtrate was evaporated in vacuo to yield a dry solid, which was dissolved in water and lyophilized. The solid thus obtained when subjected to fractional crystallization from methanol-ether, gave 3-dimethylamino-2-dimethylaminomethyl-1-(2,4-dimethylphenyl)-1-propanone dihydrochloride [0.01g, (29)], m.p. 145-147°C. NMR (D_2O): δ 2.27-2.57 (br s, 6H, aromatic CH_3 at C-2 and C-4), 3.00 [s, 12H, $N(CH_3)_2$], 3.23-4.00 [m, 5H, $C^2H(CH_2)_2$], 7.20-7.53 (m, 2H, aromatic H at C-3 and C-5), 7.90 (d, 1H, J=9Hz, aromatic H at C-6).

Anal. Calc. for $C_{16}H_{28}Cl_2N_2O$: C 57.31 H 8.42 N 8.36

Found : C 56.63 H 8.45 N 8.17

The mother liquor obtained above was evaporated in vacuo to yield a solid which was crystallized repeatedly from acetone to give 2-dimethylaminomethyl-1-(2,4-dimethylphenyl)-2-propen-1-one hydrochloride [0.40g, 8%, (XIIc)], m.p. 156-159°C. The structure of this compound was elucidated by NMR and IR spectroscopy and elemental analysis, and confirmed by comparison of its TLC (benzene: methanol, 9:1), melting point and the spectral character-

istics with those of an authentic sample of the compound prepared by an unambiguous route, vide infra. NMR (D_2O): δ 2.30 (s, 3H, aromatic CH_3 at C-4), 2.37 (s, 3H, aromatic CH_3 at C-2), 3.03 [s, 6H, $N(CH_3)_2$], 4.17 (s, 2H, CH_2N), 6.20 (s, 1H, $C=CH_2$), 6.67 (s, 1H, $C=CH_2$); IR (KBr): ν 1655 (C=O), 1620 ($C=CH_2$) cm^{-1}.

Anal. Calc. for $C_{14}H_{20}ClNO$: C 66.26 H 7.94 N 5.52

Found : C 66.66 H 7.61 N 5.45

3.2.2.0 Attempted synthesis of 3-dimethylamino -2-dimethylaminomethyl-1-(2,5-dimethylphenyl) -1-propanone dihydrochloride

A mixture containing 2,5-dimethylacetophenone (2.96g, 0.02 mol), the Mannich reagent (7.47g, 0.08 mol) and dry acetonitrile (50 ml) was stirred at 45-50°C for 20 hours. The excess of undissolved Mannich reagent was removed by filtration and washed with hot acetonitrile. The filtrate was evaporated in vacuo to yield a dry solid which was washed with anhydrous ether and dried. The solid was then dissolved in water and the aqueous solution was lyophilized. NMR spectroscopy (300 MHz) of the solid thus obtained indicated it to be almost exclusively a mixture of the corresponding acrylophenone and dimethylamine hydrochloride. Fractional crystallization of this mixture, first from acetone, and then from methanol-ether gave 2-dimethylaminomethyl-1-(2,5-dimethylphenyl)-2-propen-1-one hydrochloride [1.38g, 27%

(XIId)], m.p. 142-146°C. The structure of this compound was elucidated by NMR and IR spectroscopy and elemental analysis and confirmed by comparison of its TLC (benzene: methanol, 9:1), melting point and the spectral characteristics with those of an authentic sample of the compound prepared by an unambiguous route vide infra. NMR (D_2O): δ 2.20 (s, 3H, aromatic CH_3 at C-5), 2.30 (s, 3H, aromatic CH_3 at C-2), 2.93 [s, 6H, $N(CH_3)_2$], 4.07 (s, 2H, CH_2N), 6.13 (s, 1H, $C=CH_2$), 6.57 (s, 1H, $C=CH_2$), 6.87-7.27 (m, 3H, C_6H_3).

Anal. Calc. for $C_{14}H_{20}ClNO$: C 66.26 H 7.94 N 5.52

Found : C 67.01 H 7.78 N 5.43

3.2.3.0 Attempted synthesis of 3-dimethylamino-2-dimethylaminomethyl-1-(2-methylphenyl)-1-propanone dihydrochloride

A mixture containing 2-methylacetophenone (1.74g, 0.013 mol), the Mannich reagent (4.74g, 0.051 mol), and dry acetonitrile (25 ml) was stirred at 45-50°C for 24 hours. The reaction mixture was worked up as in section 3.2.2.0. The solid obtained after lyophilization was crystallized once from acetone. NMR spectroscopy (360 MHz) indicated it to be a mixture of the bis-Mannich base, the corresponding acrylophenone, and dimethylamine hydrochloride. No attempts at further purification of the mixture were undertaken.

3.2.4.0 Attempted synthesis of 3-dimethylamino -2-dimethylaminomethyl-1-(2,4,5-trimethylphenyl) -1-propanone dihydrochloride

A mixture containing 2,4,5-trimethylacetophenone (0.50g, 0.003 mol), the Mannich reagent (1.15g, 0.012 mol) and dry acetonitrile (8 ml) was stirred at 45-50°C for 20 hours and worked up as in section 3.2.2.0. The solid obtained after lyophilization was subjected to fractional crystallization from acetone when two unequal fractions were obtained. The smaller portion was identified by NMR spectroscopy to consist mainly of dimethylamine hydrochloride. NMR spectroscopy of the larger fraction indicated it to be a mixture of two compounds namely 2-dimethylaminomethyl-1-(2,4,5-trimethylphenyl)-2-propen-1-one hydrochloride and dimethylamine hydrochloride in the ratio of 60:40. The NMR data for this mixture is as follows. NMR (D_2O): δ 2.27 (s, 9H, aromatic CH_3), 2.80 [s, 6H, $N(CH_3)_2$ of dimethylamine hydrochloride], 3.00 [s, 6H, $N(CH_3)_2$], 4.13 (s, 2H,CH_2N), 6.23 (s, 1H,C=CH_2), 6.70 (s, 1H, C=CH_2), 7.10 (s, 1H, aromatic H at C-3), 7.17 (s, 1H, aromatic H at C-6). No further attempt at purification of the mixture was undertaken.

3.2.5.0 Attempted synthesis of 1-(2,5-dimethoxyphenyl) -3-dimethylamino-2-dimethylaminomethyl -1-propanone dihydrochloride

A mixture containing 2,5-dimethoxyacetophenone (3.00g, 0.017 mol), the Mannich reagent (6.22g, 0.07 mol) and dry acetonitrile (45 ml) was stirred at 45-50°C for 20 hours and

worked up as in section 3.2.2.0. NMR spectroscopy of the crude solid obtained after lyophilization indicated it to consist mainly of the corresponding acrylophenone and dimethylamine hydrochloride with small amounts of the desired bis-Mannich base. Therefore, fractionation of this mixture was not undertaken.

3.2.6.0 Attempted synthesis of 3-dimethylamino-2-dimethylaminomethyl-1-(2,3,4-trimethoxyphenyl)-1-propanone dihydrochloride

A mixture containing 2,3,4-trimethoxyacetophenone (0.50g, 0.0024 mol), the Mannich reagent (0.89g, 0.0096 mol) and dry acetonitrile (10 ml) was stirred at 45-50°C for 8 days and worked up as in section 3.2.2.0. The solid obtained after lyophilization was crystallized once from methanol-ether. NMR spectroscopy indicated it to be a mixture of 2-dimethylaminomethyl-1-(2,3,4-trimethoxyphenyl)-2-propen-1-one hydrochloride and dimethylamine hydrochloride. NMR (D_2O): δ 2.73 [s, 6H, $N(CH_3)_2$ of dimethylamine hydrochloride], 2.97 [s, 6H, $N(CH_3)_2$], 3.73-4.00 (m, 9H, aromatic OCH_3), 4.13 (s, 2H, CH_2N), 6.27 (s, 1H, $C=CH_2$), 6.60 (s, 1H, $C=CH_2$), 6.87 (d, 1H, J=9Hz, aromatic H at C-5), 7.17 (d, 1H, J=9Hz, aromatic H at C-6).

3.2.7.0 Attempted synthesis of 1-(2-chlorophenyl) -3-dimethylamino-2-dimethylaminomethyl -1-propanone dihydrochloride

A mixture containing 2-chloroacetophenone (0.50g, 0.003 mol), the Mannich reagent (1.21g, 0.013 mol) and dry acetonitrile (7 ml) was stirred at 45-50°C for 24 hours. The reaction mixture was cooled overnight in the refrigerator and the precipitated excess of the Mannich reagent was filtered. The filtrate was worked up as in section 3.2.0.0. The solid obtained after lyophilization was subjected to fractional crystallization from acetone whereby dimethylamine hydrochloride and another fraction (0.11g), m.p. 133-140°C, were obtained. NMR spectroscopy of the latter indicated it to be a mixture of 1-(2-chlorophenyl)-2-dimethylaminomethyl-2-propen-1-one hydrochloride (XII k) and dimethylamine hydrochloride in the ratio of 5:1.2. NMR (D_2O): δ 2.77 [s, 6H, $N(CH_3)_2$ of dimethylamine hydrochloride], 3.00 [s, 6H, $N(CH_3)_2$], 4.20 (s, 2H, CH_2N), 6.37 (s, 1H, $C=CH_2$), 6.80 (s, 1H, $C=CH_2$), 7.27-7.67 (m, 4H, C_6H_4). The structure of the acrylophenone (XII k) was confirmed by comparison of the NMR spectrum of the mixture with that of a pure sample of the compound (XII k) prepared by an unambiguous route, vide infra.

3.3.0.0 Synthesis of 1-aryl-3-dimethylamino -2-dimethylaminomethyl-1-propanol dihydrochlorides (III)

These compounds were prepared according to the literature methodology (Albrecht et al., 1962), with some modifications.

3.3.1.0 Synthesis of 3-dimethylamino-2-dimethylaminomethyl -1-phenyl-1-propanol dihydrochloride (IIIa)

3-Dimethylamino-2-dimethylaminomethyl-1-phenyl-1-propanone (3.19g, 0.014 mol) was dissolved in methanol (25 ml) and added to a stirring solution of sodium borohydride (0.52g, 0.014 mol) in aqueous methanol (50% v/v, 65 ml) below 10°C. The addition required 30 minutes. The mixture was stirred for 1 hour while warming to room temperature, and then for 4 hours at 45-50°C. Most of the methanol was removed in vacuo. With cooling, water (22 ml) was added. The base was extracted with 2 portions of ether (35 ml and 25 ml) and the combined ether extracts were dried over anhydrous sodium sulfate. Removal of the ether in vacuo gave 3-dimethylamino-2-dimethylaminomethyl-1-phenyl-1-propanol (2.76g), m.p. 61-63°C. Lit. (Albrecht et al., 1962) m.p. 63-65°C.

The base was dissolved in anhydrous ether and anhydrous hydrogen chloride gas was passed in to precipitate the dihydrochloride. Crystallization from absolute ethanol gave the pure dihydrochloride (2.59g, 62%), m.p. 228-230°(dec). Lit. (Albrecht et al., 1962) m.p. 225-227°C. IR (KBr): ν 3320 (OH) cm^{-1}.

3.3.2.0 Synthesis of 3-dimethylamino -2-dimethylaminomethyl-1-(4-methoxyphenyl) -1-propanol dihydrochloride (IIIb)

Sodium borohydride (0.42g, 0.011 mol) was dissolved in aqueous methanol (6 ml, 67% v/v) below 10°C. 3-Dimethylamino-2-dimethylaminomethyl-1-(4-methoxyphenyl)-1-propanone (2.90g, 0.011 mol) was dissolved in methanol (6 ml) and added to the sodium borohydride solution over a 10 minute period below 10°C. The mixture was stirred while cold for 10 minutes, then 1 hour and 15 minutes at room temperature and 2.5 hours at 45-50°C. The solvent was removed *in vacuo* and water (12 ml) was added to the residue. This was extracted with ether (12 ml x 2) and the combined ether extracts were dried over anhydrous sodium sulfate. The solvent was evaporated *in vacuo* and the residue was dissolved in ethanol (12 ml). This solution was acidified by the addition of ethanolic hydrochloric acid (approximately 3.3N). The solution was diluted with anhydrous ether and cooled in the refrigerator overnight to obtain the pure crystalline dihydrochloride (0.90g, 31%), m. p. 223°(dec). Lit. (Albrecht *et al.*, 1962) m.p. 226.5-227°C.

3.4.0.0 Synthesis of 3-amino-2-aminomethyl -1-(4-methoxyphenyl)-1-propanone dihydrochlorides (Table VI, IVc,d)

The 2-aminomethyl-1-(4-methoxyphenyl)-2-propen-1-one hydrochlorides required for the preparation of the title compounds were obtained as described in section 3.12.0.0.

The free amines were prepared by basification of an aqueous solution of the dihydrochlorides with dilute ammonia solution (10% w/v), followed by extraction with ether. The combined ether extracts were washed once with water and dried over anhydrous sodium sulfate. Removal of the ether *in vacuo* gave the required free base.

The title compounds were prepared according to a literature methodology (Gupta *et al.*, 1981). Thus, a solution of the appropriate 2-aminomethyl-1-(4-methoxyphenyl)-2-propen-1-one (0.0065 mol) and the amine (morpholine or pyrrolidine, 0.0065 mol) in methylene chloride (20 ml) was stirred at room temperature for 24 hours. The solvent was removed *in vacuo* and the residue dissolved in anhydrous ether (IVc) or acetone (IVd). Anhydrous hydrogen chloride gas was passed in to precipitate the crude dihydrochloride which was purified by crystallization from a suitable solvent. The structure of the two compounds was confirmed by NMR and IR spectroscopy and elemental analysis. The NMR data of a representative compound (IVd) is as follows. NMR (D_2O): δ 2.07 (br s, 8H, pyrrolidinyl H at C-3 and C-4), 2.70-4.13 [m, 16H, $C^2H(CH_2)_2$, aromatic OCH_3 and pyrrolidinyl H at C-2 and C-5], 7.20 (d, 2H, *J*=9Hz, aromatic H at C-3 and C-5), 8.20 (d, 2H, *J*=9Hz, aromatic H at C-2 and C-6).

3.4.1.0 Attempted synthesis of 1-(4-methoxyphenyl)-3-(1-piperidinyl)-2-(1-piperidinylmethyl)-1-propanone dihydrochloride (IVb)

A solution of 1-(4-methoxyphenyl)-2-piperidinylmethyl-2-propen-1-one (1.90g, 0.0073 mol) and piperidine (0.62g, 0.0073 mol) in methylene chloride (20 ml) was stirred at room temperature for 24 hours, and worked up as in section 3.4.0.0. The crude dihydrochloride obtained was crystallized from methanol-ether to give the title compound (2.47g), m.p. 151-154°C. Lit. (Albrecht et al., 1962) m. p. 54-57°C (free base). NMR (D_2O): δ 1.73 (br s, 12 H, piperidinyl H at C-3, C-4 and C-5), 2.47-4.00 [m, 16H, $C^2H(CH_2)_2$, aromatic OCH_3 and piperidinyl H at C-2 and C-6], 7.07 (d, 2H, J=9Hz, aromatic H at C-3 and C-5), 8.00 (d, 2H, J=9Hz, aromatic H at C-2 and C-6).

However, satisfactory elemental analysis could not be obtained for this compound and the free base despite crystallizations from methanol-ether and other solvent systems. Also, the compound appeared to undergo decomposition upon storage in a desiccator (calcium chloride), and possibly during recrystallization. This was evidenced by TLC (benzene: methanol, 9:1) and changes in melting point. This compound, therefore, was not evaluated for bioactivity.

Elemental analyses of the dihydrochloride:

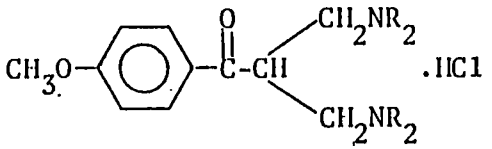
Anal. Calc. for $C_{21}H_{34}Cl_2N_2O_2$: C 60.42 H 8.21 N 6.71

Found : C 57.41 H 7.95 N 6.29
: C 57.77 H 7.98 N 6.35
: C 55.55 H 7.95 N 6.31

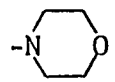
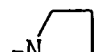
Elemental analyses of the free base:

Anal. Calc. for $C_{21}H_{32}N_2O_2$	: C 73.21 H 9.36 N 6.71
Found	: C 70.25 H 9.28 N 7.92
	: C 69.97 H 9.09 N 7.78

Table VI. Physical data of 3-amino-2-aminomethyl-1-(4-methoxyphenyl)-1-propanone dihydrochlorides(IVc,d)



(IV)

Compound	NR_2	Recrystallization solvent	Yield (%)	Melting point (°C)	Molecular formula	Analysis(%) Calculated C	H	N	Found C	H	N
IVc		Ethanol	60	162-163 (dec)	$C_{19}H_{30}Cl_2N_2O_4$	54.16	7.18	6.65	54.11	7.31	6.46
IVd		Methanol-Ether	41	157-158	$C_{19}H_{30}Cl_2N_2O_2$	58.61	7.77	7.20	58.36	7.80	7.10

3.5.0.0 Synthesis of 1-cyclohexyl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrochloride (V)

A mixture containing cyclohexyl methyl ketone (3.00g, 0.024 mol), the Mannich reagent (8.89g, 0.095 mol) and dry acetonitrile (60 ml) was stirred at 45-50°C for 24 hours. The precipitate was removed, washed with hot acetonitrile and dried. Crystallization from methanol afforded the title compound (2.14g, 29%), m.p. 161-162.5°C.NMR (D_2O): δ 0.97-2.03 [m, 10H, cyclohexyl $(CH_2)_5$], 2.60-4.00 [m, 18H, $N(CH_3)_2$, $C^2H(CH_2)_2$, and cyclohexyl H at C-1].

Anal. Calc. for $C_{14}H_{30}Cl_2N_2O$: C 53.67 H 9.65 N 8.94

Found : C 53.73 H 9.57 N 8.96

3.6.0.0 Synthesis of 1-dimethylamino-2-dimethylaminomethyl-4,4-dimethyl-3-pentanone dihydrochloride (VI)

A mixture containing pinacolone (3.00g, 0.03 mol), the Mannich reagent (11.20g, 0.12 mol) and dry acetonitrile was stirred at 45-50°C for 48 hours. The precipitate was filtered, washed with hot acetonitrile and dried. Crystallization from ethanol afforded the title compound (4.86g, 57%), m.p. 191-193°C(dec). Lit. (Miyano et al., 1982) m.p. 58.5°C (free base). NMR (D_2O, 300 MHz): δ 1.29 [s, 9H, $C(CH_3)_3$], 2.98 [s, 12H, $N(CH_3)_2$], 3.32-3.39 (dd, 2H, CH_2), 3.66-3.73 (dd, 2H, CH_2), 4.13 (quintet, 1H, C^2H).

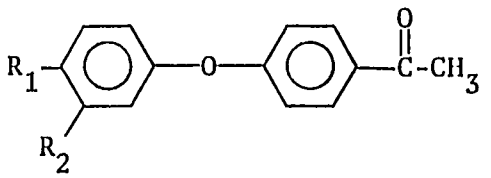
Anal. Calc. for $C_{12}H_{20}Cl_2N_2O$: C 50.17 H 9.82 N 9.75

Found : C 50.24 H 9.37 N 9.66

3.7.0.0 Synthesis of 1-(4-aryloxyphenyl)ethanones (Table VII, XXIIIa-d)

The title compounds with the exception of 1-(4-phenoxyphenyl)ethanone which was obtained commercially (Transworld Chemicals, U.S.A.), were prepared according to a literature methodology (Trust et al., 1979). To a solution of 4-fluoroacetophenone (2.00g, 0.015 mol) and the appropriate phenol (0.02 mol) in N,N-dimethylacetamide (20 ml) was added anhydrous potassium carbonate (2.62g, 0.019 mol). The resulting slurry was heated under nitrogen at 150-155°C for 18 hours. After cooling to room temperature, the mixture was added to water (25 ml) and extracted with benzene. The organic extracts were washed with dilute sodium hydroxide solution and dried over anhydrous magnesium sulfate. Removal of the solvent in vacuo gave a residue which was purified by distillation. The structures of these compounds were confirmed by NMR spectroscopy and/or elemental analysis. The NMR data of a representative compound, 1-(4-phenoxyphenyl)-ethanone, is as follows. NMR ($CDCl_3$): δ 2.50 (s, 3H, CH_3), 6.77-8.03 (m, 9H, aromatic H).

Table VII. Physical data of 1-(4-aryloxyphenyl)ethanones(XXIIIa-d)



(XXIII)

Compound	R_1	R_2	Yield (%)	Boiling point(°C/mm)	Lit. melting point(°C) or boiling point (°C/mm)	Molecular formula	Analysis(%) Calculated C	Calculated H	Found C	Found H
XXIIIa	Cl	H	82	134-135/0.05	66-68[1]	$C_{14}H_{11}ClO_2$	----	----	----	----
XXIIIb	Cl	Cl	82	147-150/0.01	------	$C_{14}H_{10}Cl_2O_2$	59.81	3.59	59.93	3.50
XXIIIc	CH_3	H	80	127-128/0.1	205-210/12[2]	$C_{15}H_{14}O_2$	79.62	6.24	79.55	6.12
XXIIId	OCH_3	H	81	139-141/0.02	234-236/16[3]	$C_{15}H_{14}O_3$	74.36	5.82	74.45	5.86

1 Trust et al., 1979

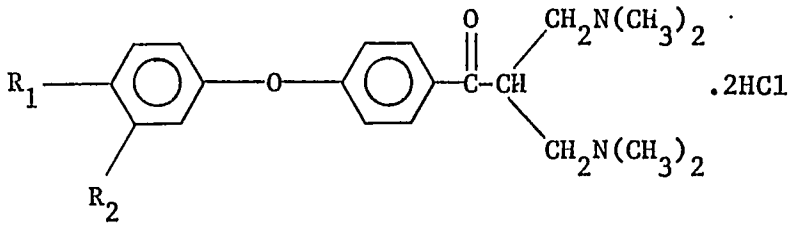
2 Julia and Baillarge, 1953

3 Petit and Buu-hoi, 1961

3.7.1.0 Synthesis of 1-(4-aryloxyphenyl)-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrochlorides (Table VIII, VIIa-e)

Compounds (VIIa-e) were prepared by the following general method. A mixture of the appropriate 4-aryloxy-acetophenone (0.014 mol), the Mannich reagent (5.29g, 0.057 mol) and dry acetonitrile (60 ml) was stirred at 45-50°C for 24 hours. The precipitated solid was filtered, washed with hot acetonitrile and dried. Crystallization from suitable solvents afforded the title compounds. Their structures were confirmed by NMR and IR spectroscopy and elemental analysis. The NMR data of a representative compound, (VIId) is as follows. NMR (D_2O, 300MHz): δ 2.47 (s, 3H, aromatic CH_3), 3.11 [s, 12H, $N(CH_3)_2$], 3.63-3.69 (dd, 2H, CH_2), 3.90-3.97 (dd, 2H, CH_2), 4.74 (quintet, 1H, C^2H), 7.16 (d, 2H, J=8.4 Hz, phenoxy H), 7.25 (d, 2H, J=8.9 Hz, aromatic H at C-3 and C-5), 7.42 (d, 2H, J=8.4 Hz, phenoxy H), 8.25 (d, 2H, J=8.9 Hz, aromatic H at C-2 and C-6).

Table VIII. Physical data of 1-(4-aryloxyphenyl)-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrochlorides(VIIa-e)



(VII)

Compound	R_1	R_2	Recrystallization solvent	Yield (%)	Melting point(°C)	Molecular formula	Analysis(%) Calculated C	H	N	Found C	H	N
VIIa	H	H	Methanol	60	170-172	$C_{20}H_{28}Cl_2N_2O_2$	60.15	7.07	7.02	60.31	6.99	6.91
VIIb	Cl	H	Ethanol	42	162-163	$C_{20}H_{27}Cl_3N_2O_2$	55.37	6.27	6.46	55.34	6.40	6.54
VIIc	Cl	Cl	Absolute Ethanol	42	152-153	$C_{20}H_{26}Cl_4N_2O_2$	51.30	5.60	5.98	51.12	5.54	5.94
VIId	CH_3	H	Absolute Ethanol	40	160-160.5	$C_{21}H_{30}Cl_2N_2O_2$	61.01	7.32	6.78	60.99	7.68	6.91
VIIe	OCH_3	H	Methanol	31	162-163	$C_{21}H_{30}Cl_2N_2O_3$	58.74	7.04	6.53	58.36	7.13	6.62

3.8.0.0 Synthesis of 1-phenyl-1-trimethylsilyloxy -1-propene

This compound was prepared according to literature methodology (Emde et al., 1982). In a three-necked flask provided with a magnetic stirrer, dropping funnel with a calcium chloride guard tube and a reflux condenser connected to a supply of dry nitrogen, was placed a solution of propiophenone (5.00g, 0.037 mol) and triethylamine (4.15g, 0.041 mol) in carbon tetrachloride (30 ml). The solution was stirred at 0-5°C. To this was gradually added a solution of trimethylsilyl trifluromethanesulfonate (9.11g, 0.041 mol) in carbon tetrachloride (10 ml) over a period of about 30 minutes. The cooling bath was removed and the reaction mixture was stirred at room temperature for 2 hours. The lower carbon tetrachloride layer was separated, evaporated in vacuo and the residue was fractionated to obtain the title compound (5.40g, 70%), b.p. 35°C/ 0.06 mm. Lit. (Emde et al., 1981) b.p. 40°C/ 0.1 mm. NMR ($CDCl_3$): δ 0.13 [s, 9H, $Si(CH_3)_3$], 1.70 (d, 3H, J=7 Hz, CH_3), 5.23 (q, 1H, J=7 Hz, C^2H); IR (neat): ν 1670 (C=CH) cm^{-1}.

3.8.1.0 Reaction of 1-phenyl-1-trimethylsilyloxy-1-propene with N,N-dimethyl(methylene)ammonium chloride

This reaction was carried out in a NMR tube. A solution of enol silyl ether (0.07g, 0.00034 mol) in chloroform-d (0.5 ml) was placed in the NMR tube and to this was added the Mannich reagent (0.032g, 0.00034 mol). Upon

mixing, an exothermic reaction occurred, accompanied by disappearance of the Mannich reagent. NMR spectroscopy indicated the complete formation of the trimethyl silyl derivative of the mono-Mannich base in less than 30 minutes. NMR: δ 1.33 (d, 3H, J=7 Hz, C^2HCH_3), 3.03 [s, 6H, $N(CH_3)_2$], 3.13-4.43 (m, 3H, C^2HCH_2), 4.73 [s, 9H, $C=O^+Si(CH_3)_3$], 7.50-8.23 (m, 5H, C_6H_5).

At this point, the second mole of the Mannich reagent was added and the NMR tube incubated at 40°C for several hours. However, the Mannich reagent did not dissolve, and NMR spectroscopy did not indicate any conversion of the mono-Mannich base into the desired bis-Mannich base.

3.8.2.0 Attempted synthesis of 3-dimethylamino-2-dimethylaminomethyl-2-methyl-1-(4-methylphenyl)-1-propanone dihydrochloride (VIII b)

A mixture of 4-methylpropiophenone (0.50g, 0.0034 mol), the Mannich reagent (0.66g, 0.0071 mol) and dry acetonitrile (7 ml) was stirred at 45-50°C for 48 hours. The solvent was removed in vacuo and the residue was triturated with anhydrous ether to obtain a solid. This was filtered, washed with anhydrous ether, and crystallized once from acetone-ether. NMR spectroscopy indicated it to be a mixture of 3-dimethylamino-2-methyl-1-(4-methylphenyl)-1-propanone hydrochloride (the mono-Mannich base) and dimethylamine hydrochloride in a ratio of 5:2.2. NMR (D_2O): δ 1.33(d, 3H, J=7 Hz, C^2HCH_3),

2.43 (s, 3H, aromatic CH_3), 2.80 [s, 6H, $N(CH_3)_2$ of dimethylamine hydrochloride], 2.97 [s, 6H, $N(CH_3)_2$], 3.17-4.33 (m, 3H, C^2HCH_2), 7.30 (d, 2H, J=8 Hz, aromatic H at C-3 and C-5), 7.83 (d, 2H, J=8 Hz, aromatic H at C-2 and C-6).

3.9.0.0 Attempted synthesis of 2-chloromethyl -3-dimethylamino-2-dimethylaminomethyl -1-phenyl-1-propanone dihydrochloride (IX)

A mixture of β-chloropropiophenone (2.00g, 0.012 mol), the Mannich reagent (4.44g, 0.048 mol) and dry acetonitrile was stirred at 45-50°C for 24 hours. The excess of the undissolved Mannich reagent was filtered and washed with hot acetonitrile. The filtrate was evaporated *in vacuo* and the residue was triturated with anhydrous ether to yield a solid which was filtered, washed with cold acetone and dried. NMR spectroscopy of this solid indicated it to consist almost exclusively of 2-dimethylaminomethyl-1-phenyl-2-propen-1-one hydrochloride. This was then dissolved in water and the aqueous solution was extracted once with ether and then basified with dilute ammonia solution (5% w/v). The basic solution was extracted with ether and the combined ether extracts were washed with water and dried over anhydrous magnesium sulfate. The ether was removed *in vacuo* and the residual oil was dissolved in anhydrous ether. The ethereal solution was treated with anhydrous hydrogen chloride gas and the precipitated solid was filtered, washed with ether and dried (0.73g), m.p. 153-154°C. This was again identified

by NMR spectroscopy to be 2-dimethylaminomethyl-1-phenyl-2-propen-1-one hydrochloride (XIIa). Its structure was confirmed by comparison of its TLC (benzene: methanol, 9:1), melting point and spectral characteristics with those of an authentic sample of (XIIa), prepared by an unambiguous route, vide infra. NMR (D_2O): δ 2.93 [s, 6H, $N(CH_3)_2$], 4.10 (s, 2H, CH_2N), 6.37 (s, 1H, $C=CH_2$), 6.63 (s, 1H, $C=CH_2$), 7.27-8.00 (m, 5H, C_6H_5).

3.10.0.0 Synthesis of 1-aryl-3-dimethylamino-1-propanone hydrohalides (Table IX, Xa-g)

Compounds (Xa-f) were prepared by the following general method. A mixture of the appropriate acetophenone (0.055 mol), paraformaldehyde (2.10g, 0.07 mol), dimethylamine hydrochloride (6.00g, 0.074 mol), concentrated hydrochloric acid (0.12 ml) and alcohol (45 ml) was refluxed for 7-18 hours. The ethanol was removed in vacuo. The residue was dissolved in water and extracted with ether to remove the unreacted acetophenone. The aqueous layer was then made basic with dilute ammonia solution (10% w/v) and extracted with ether. The combined ether extracts were washed once with water and dried over anhydrous magnesium sulfate. Removal of the ether in vacuo gave an oil which was dissolved in anhydrous ether and the ethereal solution was treated with the appropriate anhydrous hydrogen halide gas. The precipitated solid was filtered, washed with ether and dried.

Crystallization from suitable solvents gave the pure product. In the case of (Xb&f) the precipitated solid was found to be analytically pure.

Compound (Xg) was prepared as follows. A mixture of 3,4-methylenedioxyacetophenone (2.00g, 0.012 mol), the Mannich reagent (2.28g, 0.024 mol) and dry acetonitrile (25 ml) was stirred at 45-50°C for 20 hours. The precipitated solid was filtered, washed with hot acetonitrile and dried. Crystallization from methanol afforded the pure product. The structure of all the compounds was confirmed by NMR and IR spectroscopy and elemental analysis. The spectroscopic data of a representative compound (Xb) are as follows. NMR (D_2O): δ 2.37 (s, 3H, aromatic CH_3 at C-4), 2.50 (s, 3H, aromatic CH_3 at C-2), 3.03 [s, 6H, $N(CH_3)_2$], 3.57 (s, 4H, CH_2CH_2), 6.97-7.27 (m, 2H, aromatic H at C-3 and C-5), 7.73 (d, 1H, J=8 Hz, aromatic H at C-2); IR (KBr): ν 2400-2800 ($\overset{+}{N}H$), 1680 (C=O) cm^{-1}.

3.10.1.0 Synthesis of 1,4-bis(3-dimethylaminopropionyl) benzene dihydrochloride (XI)

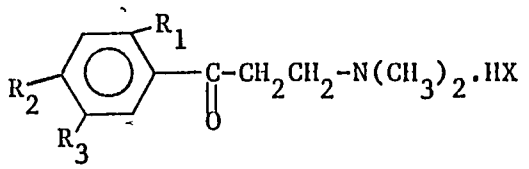
A mixture of 4-diacetylbenzene (3.00g, 0.019 mol), the Mannich reagent (3.63g, 0.039 mol) and dry acetonitrile (100 ml) was stirred at 45-50°C for 24 hours. The precipitated solid was filtered, washed with hot acetonitrile and dried. Crystallization from methanol afforded the pure product (1.82g, 28%), m.p. 210°C(dec). Lit. (Shimojo et al., 1968)

m.p. 224.5°C. NMR (D_2O): δ 3.03 [s, 12H, $N(CH_3)_2$], 3.70 (s, 8H, CH_2CH_2), 8.13 (s, 4H, C_6H_4).

Anal. Calc. for $C_{16}H_{26}Cl_2N_2O_2$: C 55.01 H 7.50 N 8.02

Found : C 55.27 H 8.01 N 7.56

Table IX. Physical data of 1-aryl-3-dimethylamino-1-propanone hydrohalides(Xa-g)



(X)

Compound	R_1	R_2	R_3	X	Time of reflux or stirring (hours)	Recrystallization solvent	Yield (%)	Melting point (°C)	Molecular formula	Analysis(%) Calculated C	Calculated H	Calculated N	Found C	Found H	Found N
Xa	CH_3	H	H	Cl	18	Ethanol/ Ether	27	145-148	$C_{12}H_{18}ClNO$	63.29	7.97	6.16	63.18	8.00	6.10
Xb	CH_3	CH_3	H	Cl	16	------	36	125-126	$C_{13}H_{20}ClNO$	64.58	8.34	5.79	64.36	8.41	6.02
Xc	CH_3	H	CH_3	Cl	16	Ethanol/ Ether	34	141-144	$C_{13}H_{20}ClNO$	64.58	8.34	5.79	64.41	8.26	5.95
Xd	OCH_3	H	H	Br	7	Ethanol/ Ether	33	116-118[1]	$C_{12}H_{18}BrNO_2$	50.01	6.29	4.86	49.86	5.88	4.74
Xe	OCH_3	OCH_3	H	Br	7	Ethanol/ Ether	58	141-143[2]	$C_{13}H_{20}BrNO_3$	49.06	6.34	4.40	49.18	6.20	4.67
Xf	OCH_3	OCH_3	OCH_3	Br	8	-----	36	185-186	$C_{14}H_{22}BrNO_4$	48.28	6.37	4.02	48.22	6.26	3.92
Xg	H	OCH_2O		Cl	20	Methanol	41	189-192[3,4]	$C_{12}H_{16}ClNO_3$	55.92	6.26	5.44	55.47	6.06	5.32

1. Lit.(Carter et al., 1981)m.p. not quoted.
2. Lit.(Carter et al., 1981)b.p. 125-127°C/0.5mm(free base).
3. Lit.(Oswald et al., 1971)m.p. 195-196°C.
4. Lit.(El'tsov, 1964)m.p. 192°C.

3.11.0.0 Synthesis of 1-aryl-2-dimethylaminomethyl -2-propen-1-one hydrochlorides (Table X, XIIa-k)

The title compounds were prepared in analogy to a literature methodology (Gupta et al., 1981). A mixture of the appropriate acetophenone (0.019 mol), dimethylamine hydrochloride (1.58g, 0.019 mol), paraformaldehyde (1.16g, 0.039 mol) and glacial acetic acid (45 ml), was heated under reflux for 1-6 hours. The solvent was removed in vacuo and the residue was triturated with anhydrous ether or acetone (XIIg). The solid thus obtained was filtered and crystallized from the appropriate solvent to yield the pure product.

The structures were confirmed by NMR and IR spectroscopy and elemental analysis. The spectroscopic data of a representative compound (XIIc) is as follows. NMR (D_2O): δ 2.30 (s, 3H, aromatic CH_3 at C-4), 2.37 (s, 3H, aromatic CH_3 at C-2), 3.00 [s, 6H, $N(CH_3)_2$], 4.13 (s, 2H, CH_2N), 6.23 (s, 1H, $C=CH_2$), 6.67 (s, 1H, $C=CH_2$), 6.97-7.40 (m, 3H, C_6H_3); IR (KBr): ν 1655 (C=O), 1620 ($C=CH_2$) cm^{-1}.

3.11.1.0 Synthesis of 1,4-bis(2-dimethylaminomethyl -1-oxo-2-propenyl)benzene dihydrochloride (XIV)

A mixture of 4-diacetylbenzene (2.00g, 0.012 mol), dimethylamine hydrochloride (2.00g, 0.025 mol), paraformaldehyde (1.48g, 0.049 mol) and glacial acetic acid (35 ml) was heated under reflux for 2 hours. The residue obtained after removal of the solvent in vacuo was triturated with acetone and the resulting solid was crystallized from water-

acetone to yield the pure product (1.01g, 28%), m.p. 250-253°C(dec). NMR (D_2O): δ 2.97 [s, 12H, $N(CH_3)_2$], 4.13 (s, 4H, CH_2N), 6.40 (s, 2H, $C=CH_2$), 6.67 (s, 2H, $C=CH_2$), 7.77 (s, 4H, C_6H_4).

Anal. Calc. for $C_{18}H_{26}Cl_2N_2O_2$: C 57.91 H 7.02 N 7.51

Found : C 57.44 H 7.15 N 7.40

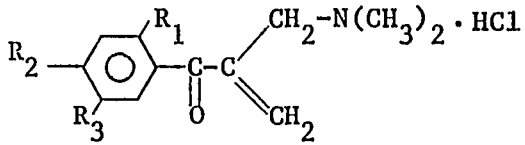
3.11.2.0 Conversion of 3-dimethylamino-1-(2,4-dimethylphenyl)-1-propanone hydrochloride into 2-dimethylaminomethyl-1-(2,4-dimethylphenyl)-2-propen-1-one hydrochloride (XIIc)

A mixture of 3-dimethylamino-1-(2,4-dimethylphenyl)-1-propanone hydrochloride [(table IX, Xb), 0.44g, 0.0018 mol], paraformaldehyde (0.16g, 0.0055 mol) and glacial acetic acid (7 ml) was refluxed for 2 hours. The reaction mixture was cooled and worked up as for (XIIa-k) to give the corresponding acrylophenone (0.11g, 23%), m.p. 151.5-153°C.

Anal. Calc. for $C_{14}H_{20}ClNO$: C 66.26 H 7.94 N 5.52

Found : C 65.99 H 7.45 N 5.65

Table X. Physical data of 1-aryl-2-dimethylaminomethyl-2-propen-1-one hydrochlorides(XIIa-k)



(XII)

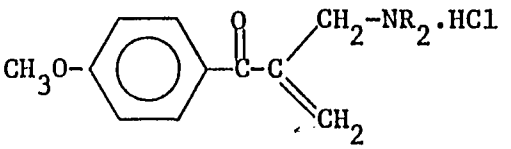
Compound	R_1	R_2	R_3	Time of reflux (hours)	Recrystallization solvent	Yield	Melting point (°C)	Molecular formula	Analysis(%) Calculated C	H	N	Found C	H	N
XIIa	H	H	H	3	Ethanol/Ether	45	156-158[1]	$C_{12}H_{16}ClNO$	63.85	7.15	6.21	64.00	7.51	6.30
XIIb	CH_3	H	H	6	Acetone	44	145-148	$C_{13}H_{18}ClNO$	65.13	7.57	5.84	65.24	7.83	5.85
XIIc	CH_3	CH_3	H	6	Acetone	37	151-153	$C_{14}H_{20}ClNO$	66.26	7.94	5.52	66.30	8.17	5.48
XIId	CH_3	H	CH_3	6	Acetone	47	148-149.5	$C_{14}H_{20}ClNO$	66.26	7.94	5.52	66.70	7.82	5.66
XIIe	OCH_3	H	H	6	Acetone	60	148-150	$C_{13}H_{18}ClNO_2$	61.05	7.09	5.48	61.02	7.08	5.39
XIIf	H	OCH_3	H	3	Ethanol/Ether	28	151-152[2]	$C_{13}H_{18}ClNO_2$	61.05	7.09	5.48	61.19	7.36	5.39
XIIg	OCH_3	OCH_3	H	1	Ethanol/Ether	18	159-162	$C_{14}H_{20}ClNO_3$	58.84	7.05	4.90	58.45	7.03	4.97
XIIh	OCH_3	OCH_3	OCH_3	2	Ethanol/Ether	69	154-160	$C_{15}H_{22}ClNO_4$	57.05	7.02	4.44	57.07	7.12	4.52
XIIi	H	OCH_2O		6	Ethanol/Ether	36	167-169	$C_{13}H_{16}ClNO_3$	57.88	5.98	5.19	57.47	5.56	4.78
XIIj	H	OC_6H_5	H	3	Ethanol/Ether	50	167-168	$C_{18}H_{20}ClNO_2$	68.02	6.34	4.41	68.33	6.59	4.20
XIIk	Cl	H	H	6	Acetone	62	146-149	$C_{12}H_{15}Cl_2NO$	55.40	5.81	5.38	55.49	5.91	5.48

1. Lit.(Bard et al., 1985) m.p.158.16 °C.
2. Lit.(Lesieur et al., 1986) m.p.149-153 °C.

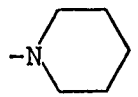
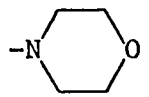
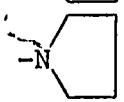
3.12.0.0 Synthesis of 2-aminomethyl-1-(4-methoxyphenyl) -2-propen-1-one hydrochlorides (Table XI, XVb-d)

The title compounds were prepared according to the literature methodology (Gupta et al., 1981). A mixture of 4-methoxyacetophenone (4.00g, 0.027 mol), the appropriate amine hydrochloride (0.027 mol), paraformaldehyde (1.76g, 0.059 mol) and glacial acetic acid (30 ml) was heated under reflux for 12-24 hours. The solvent was removed in vacuo and the residue was triturated with anhydrous ether or cold acetone (XVd). The solid thus obtained was purified by crystallization from appropriate solvents. The structures were confirmed by NMR spectroscopy and elemental analysis. The NMR data of a representative compound (XVd) is as follows. NMR (D_2O): δ 2.13 (br s, 4H, pyrrolidinyl H at C-3 and C-4), 2.87-3.87 (m, 4H, pyrrolidinyl H at C-2 and C-5), 3.97 (s, 3H, aromatic OCH_3), 4.23 (s, 2H, CH_2N), 6.30 (s, 1H, $C=CH_2$), 6.60 (s, 1H, $C=CH_2$), 7.13 (d, 2H, J=9 Hz, aromatic H at C-3 and C-5), 7.87 (d, 2H, J=9 Hz, aromatic H at C-2 and C-6).

Table XI. Physical data of 2-aminomethyl-1-(4-methoxyphenyl)-2-propen-1-one hydrochlorides(XV)



(XV)

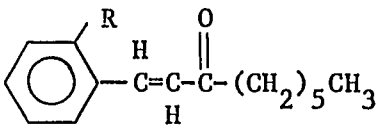
Compound	NR_2	Time of reflux (hours)	Recrystallization solvent	Yield (%)	Melting point (°C)	Molecular formula	Analysis(%) Calculated C	H	N	Found C	H	N
XVb		17	Ethanol/Ether	32	172-173[1]	$C_{16}H_{22}ClNO_2$	64.96	7.50	4.74	65.21	7.42	5.02
XVc		24	Ethanol/Ether	20	171-173[2] (dec)	$C_{15}H_{20}ClNO_3$	60.50	6.77	4.70	60.82	7.48	4.93
XVd		12	Acetone	27	142-144.5	$C_{15}H_{20}ClNO_2$	63.93	7.15	4.97	64.11	7.30	4.56

1. Lit.(Gupta et al., 1981) m.p.185 °C.
2. Lit.(Lesieur et al., 1986) m.p.180-181 °C.

3.13.0.0 Synthesis of 1-aryl-1-nonen-3-ones (Table XII, XVIa-f)

The title compounds were prepared according to a literature methodology (Smith et al., 1972). A mixture containing 2-octanone (0.60 mol), the appropriate aromatic aldehyde (0.50 mol), sodium hydroxide (0.25 mol), and water (300 ml) was heated under reflux with vigorous mechanical stirring for 24 hours. After cooling to room temperature, the mixture was extracted repeatedly with benzene, the combined organic extracts were washed with water and were dried over anhydrous magnesium sulfate. The benzene was evaporated in vacuo and the unreacted aldehyde and 2-octanone were removed by distillation. The residue was purified by distillation or recrystallization. The structures of these compounds were confirmed by NMR spectroscopy and elemental analysis. The spectroscopic data generated for a representative compound, (XVIa), are as follows. NMR ($CDCl_3$): δ 0.77-2.00 [m, 11H, $(CH_2)_4CH_3$], 2.67 (t, 2H, J=7 Hz, C^4H_2), 6.60 (d, 1H, J=16 Hz, C^2H), 7.07-8.10 (m, 5H, C^1H and C_6H_4); IR (neat): ν 1700 (s-cis C=O), 1680 (s-trans C=O), 1620 (C=C), 985 [(E)-CH=CH] cm^{-1}.

Table XII. Physical data of 1-aryl-1-nonen-3-ones(XVI)



(XVI)

Compound	R	Yield (%)	Boiling point(°C/mm)	Lit. boiling point(°C/mm)	Molecular formula	Analysis(%) Calculated C	Calculated H	Found C	Found H
XVIa	Cl	57	110-112/0.08	122/0.15[1]	$C_{15}H_{19}ClO$	71.84	7.63	71.65	7.56
XVIb	Br	50	134-136/0.1	----	$C_{15}H_{19}BrO$	61.02	6.48	61.08	6.46
XVIc	F	60	129-131/0.6	----	$C_{15}H_{19}FO$	76.88	8.17	76.98	8.18
XVId	H	65	148-150/0.06	133-134/0.8[1]	$C_{15}H_{20}O$	83.28	9.32	83.52	9.34
XVIe	CH_3	43	124-125/0.1	----	$C_{16}H_{22}O$	83.42	9.62	83.77	9.35
XVIf	OCH_3	42	138-140/0.08	145-146/0.45[2]	$C_{16}H_{22}O_2$	78.00	9.00	78.05	9.00

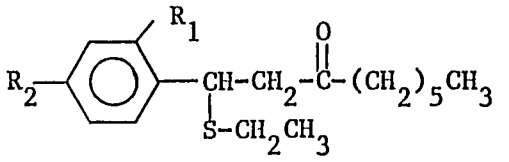
1 Smith et al., 1972

2 Dimmock et al., 1982

3.14.0.0 Synthesis of 1-aryl-1-ethylthio-3-nonanones (Table XIII, XVIIa-h)

A mixture of the appropriate 1-aryl-1-nonen-3-one (0.12 mol), ethanethiol (0.13 mol), piperidine (0.02 mol) and benzene (100 ml) was stirred at room temperature for 24 hours. The solution was then washed successively with aqueous hydrochloric acid (5% w/v), followed by aqueous sodium hydrogen carbonate solution (5% w/v) and finally with water. The organic layer was dried over anhydrous magnesium sulfate and removal of the benzene, in vacuo, gave colorless oils which were purified by distillation (XVIIa-f). Compound (XVIIg) decomposed on attempted distillation. Compounds (XVIIg & h) were therefore purified by column chromatography using silica gel and eluting the product with a mixture of equal amounts of petroleum ether (b.p. 60-80 °C) and benzene. The spectroscopic data of a representative compound, (XVIIa), are as follows. NMR ($CDCl_3$): δ 0.80-1.90 [m, 14H, $(CH_2)_4CH_3$ and $SCH_2C\underline{H}_3$], 2.40 (m, 4H, C^4H_2 and $SC\underline{H}_2CH_3$), 2.93 (d, 2H, $\underline{J}$=8 Hz, C^2H_2), 4.93 (t, 1H, $\underline{J}$=8 Hz, C^1H), 7.00-7.67 (m, 4H, C_6H_4); IR (neat): ν 1725 (C=O) cm^{-1}.

Table XIII. Physical data of 1-aryl-1-ethylthio-3-nonanones(XVII)



(XVII)

Compound	R_1	R_2	Yield (%)	Boiling point(°C/mm)	Molecular formula	Analysis(%)			
						Calculated		Found	
						C	H	C	H
XVIIa	Cl	H	65	134-136/0.1[1]	$C_{17}H_{25}ClOS$	65.25	8.05	65.25	7.99
XVIIb	Br	H	85	146-148/0.2	$C_{17}H_{25}BrOS$	57.13	7.05	57.52	7.09
XVIIc	F	H	66	121-125/0.03	$C_{17}H_{25}FOS$	68.87	8.50	69.18	8.43
XVIId	H	H	76	121-123/0.1[1]	$C_{17}H_{26}OS$	73.33	9.41	73.64	9.40
XVIIe	CH_3	H	68	136-138/0.35	$C_{18}H_{28}OS$	73.92	9.65	73.93	9.67
XVIIf	OCH_3	H	67	138-142/0.03	$C_{18}H_{28}O_2S$	70.08	9.15	70.14	9.15
XVIIg	H	Cl	61	----------	$C_{17}H_{25}ClOS$	65.25	8.05	64.94	8.01
XVIIh	H	CH_3	79	----------	$C_{18}H_{28}OS$	73.92	9.65	73.83	9.68

1. Lit.(Dimmock et al., 1980b), no data quoted.

3.15.0.0 Synthesis of 1-alkylthio-1-(2-chlorophenyl) -3-nonanones (XVIII)

3.15.1.0 Synthesis of 1-(2-aminoethylthio) -1-(2-chlorophenyl)-3-nonanone hydrochloride (XVIIIa)

A mixture of 1-(2-chlorophenyl)-1-nonen-3-one [(XVIa), 6.62g, 0.026 mol], cysteamine hydrochloride (2.00g, 0.018 mol) and ethanol (40 ml) was heated under gentle reflux for 24 hours in an atmosphere of nitrogen. After removal of the solvent in vacuo, the residue was placed on a dry column of cellulose. The unreacted ketone was eluted with petroleum ether (b.p. 60-80°C), and use of a mixture of equal amounts of benzene and petroleum ether (b.p. 60-80°C) gave the title compound as an oil (4.62g, 72%). NMR ($CDCl_3$): δ 0.60-1.77 [m, 11H, $(CH_2)_4CH_3$], 2.13-2.57 (m, 2H, C^4H_2), 2.60-3.50 (m, 6H, C^2H_2 and SCH_2CH_2N), 4.80 (t, 1H, C^1H), 7.00-7.77 (m, 4H, C_6H_4), 8.33 (br s, 3H, $\overset{+}{N}H_3$); IR (neat): ν 3670-2330 ($\overset{+}{N}H_3$ and CH), 1720 (C=O), 1615 ($\overset{+}{N}H_3$) cm^{-1}.

Anal. Calc. for $C_{17}H_{27}Cl_2NOS$: C 56.03 H 7.47 N 3.84

Found : C 56.28 H 7.42 N 3.72

3.15.2.0 Synthesis of 1-(2-chlorophenyl) -1-(2-hydroxyethylthio)-3-nonanone (XVIIIb)

A mixture of 1-(2-chlorophenyl)-1-nonen-3-one [(XVIa), 5.00g, 0.02 mol], 2-mercaptoethanol (2.28g, 0.029 mol), piperidine (0.57g, 0.0067 mol) and benzene (50 ml) was stirred at room temperature for 24 hours. The reaction mixture was

then washed successively with aqueous hydrochloric acid (5% w/v), aqueous sodium hydrogen carbonate solution (5% w/v) and water. The organic layer was dried over anhydrous magnesium sulfate. After removal of the solvent *in vacuo*, the residue was purified on a silica gel column and the product eluted with benzene-methanol (95:5) to give the title compound (4.82g, 75%).

An attempt to purify the residue by distillation resulted in substantial decomposition to the starting materials as evidenced by TLC and NMR of the distilled product. NMR ($CDCl_3$): δ 0.87-1.50 [m, 11H, $(CH_2)_4CH_3$], 2.30-2.80 [m, 5H, C^4H_2 and SCH_2CH_2OH (1H exchanged with D_2O)], 3.00 (d, 2H, J=7 Hz, C^2H_2), 3.75 (t, 2H, J=6 Hz, SCH_2CH_2OH), 5.00 (t, 1H, J=7 Hz, C^1H), 7.07-7.67 (m, 4H, C_6H_4); IR (neat): ν 3720-3120 (OH), 1710 (C=O) cm^{-1}.

Anal. Calc. for $C_{17}H_{25}ClOS$: C 62.08 H 7.66

Found : C 62.13 H 7.69

3.15.3.0 Synthesis of 1-(2-chlorophenyl)-1-[(2,3-dihydroxy-4-mercaptobutyl)thio]-3-nonanone (XVIIIc)

1-(2-Chlorophenyl)-1-nonen-3-one [(XVIa), 3.00g, 0.012 mol] was added dropwise to a solution of dithioerythritol (7.40g, 0.048 mol) and piperidine (0.20g, 0.0023 mol) in benzene-ethanol (3:1, 80 ml) with vigorous stirring. After stirring the solution at room temperature for 1 hour, the ethanol was removed *in vacuo* and after dilution with a

little benzene, the residue was worked up as for (XVIIIb) to give the title compound as a clear, colorless oil (4.10g, 85%). This compound was found to be unstable at room temperature and hence was not evaluated for bioactivity. NMR ($CDCl_3$): δ 0.63-1.93 [m, 11H, $(CH_2)_4CH_3$], 2.10-4.27 (m, 13H), 4.67-5.13 (m, 1H, C^1H), 6.97-7.80 (m, 4H, C_6H_4); IR (neat): ν 3140-3720 (OH), 1720 (C=O) cm^{-1}.

Anal. Calc. for $C_{19}H_{19}ClO_3S_2$: C 56.34 H 7.21

Found : C 56.57 H 7.25

3.15.4.0 Synthesis of 1,4-bis-[1-(2-chlorophenyl) -3-oxo-nonylthio]-2,3-dihydroxybutane (XVIIId)

1-(2-Chlorophenyl)-1-nonen-3-one [(XVIa), 3.25g, 0.013 mol], dithiothreitol (0.50g, 0.0032 mol), and piperidine (0.55g, 0.0064 mol) were dissolved in benzene (25 ml). If necessary, a few drops of ethanol were added to dissolve any insoluble dithiothreitol. The solution was warmed on a steam bath for 5 minutes, cooled and worked up as for (XVIIIb) to give the title compound as a clear, colorless oil (1.81g, 85%). This compound was found to be unstable at room temperature and hence was not evaluated for bioactivity. NMR ($CDCl_3$): δ 0.77-2.00 [m, 22H, $(CH_2)_4CH_3$], 2.17-2.73 (m, 8H), 2.80-3.20 (m, 6H), 3.27-3.83 (m, 2H),

4.70-5.17 (m, 2H, $C\underline{H}CH_2$), 7.00-7.73 (m, 8H, aromatic H); IR (neat): ν 3720-3180 (OH), 1730 (C=O) cm^{-1}.

Anal. Calc. for $C_{34}H_{48}Cl_2O_4S_2$: C 62.20 H 7.37

Found : C 62.39 H 7.29

3.15.5.0 Synthesis of ethyl 2-amino-3-[1-(2-chlorophenyl) -3-oxo-nonylthio]propionate hydrochloride (XVIIIe)

1-(2-Chlorophenyl)-1-nonen-3-one [(XVIa), 3.25g, 0.013 mol] and L-cysteine ethyl ester hydrochloride (2.00g, 0.011 mol) were dissolved in alcohol (40 ml) and the solution stirred at room temperature for 50 hours. The solvent was removed in vacuo and the residue was suspended with vigorous stirring in a little volume of petroleum ether (b.p. 60-80°C) and purified as for (XVIIIa) to give the title compound (3.45g, 73%). NMR ($CDCl_3$): δ 0.63-1.90 [m, 14H $(CH_2)_4CH_3$ and $COOCH_2C\underline{H}_3$], 2.03-2.60 (m, 2H), 2.93-4.97 (m, 8H), 6.97-8.03 (m, 7H, C_6H_4 and $\overset{+}{N}H_3$); IR ($CHCl_3$ soln): ν 2320-3360 ($\overset{+}{N}H_3$ and CH stretch superimposed), 1760 (C=O) cm^{-1}.

Anal. Calc. for $C_{20}H_{31}Cl_2NO_3S$: C 55.04 H 7.16 N 3.21

Found : C 55.61 H 7.02 N 3.29

3.15.6.0 Synthesis of sodium 2-(N-acetylamino)-3 -[1-(2-chlorophenyl)-3-oxo-nonylthio]propionate (XVIIIf)

A mixture of 1-(2-chlorophenyl)-1-nonen-3-one [(XVIa), 4.00g, 0.016 mol], N-acetyl-L-cysteine (2.17g, 0.013 mol) and ethanol (80% v/v, 40 ml) was adjusted to pH 8.5 with a

solution of sodium hydroxide (5% w/v) in ethanol (80% v/v). After stirring the reaction mixture at room temperature for 50 hours, the solvent was removed *in vacuo* and the residue was purified in the same manner as (XVIIIa) to give the title compound (4.96g, 86%), m.p. 98-100°C (softens at 60°C). NMR ($CDCl_3$): δ 0.77-1.63 [m, 11H, $(CH_2)_4CH_3$], 1.70-2.07 (d, 3H, $NHCOCH_3$), 2.10-2.57 (m, 2H), 2.87 (m, 4H), 4.20 (m, 1H), 4.77 (m, 1H), 6.83-7.67 (m, 5H, C_6H_4 and $NHCOCH_3$); IR ($CHCl_3$ soln): ν 3200-3520 (NH), 2820-3140 (CH), 1725, 1670, 1655, 1630, 1540, 1420.

Anal. Calc. for $C_{20}H_{27}ClNNaO_4S$: C 55.10 H 6.24 N 3.21

Found : C 55.14 H 6.19 N 3.24

3.15.7.0 Attempted synthesis of sodium 2-amino-3-[1-(2-chlorophenyl)-3-oxo-nonylthio]propionate (XVIIIg)

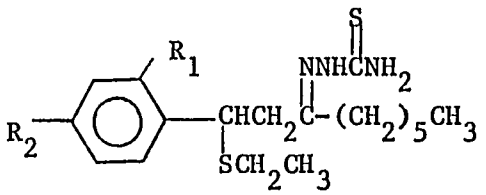
A mixture of 1-(2-chlorophenyl)-1-nonen-3-one [(XVIa), 1.00g, 0.004 mol], L-cysteine (0.40g, 0.0033 mol), and ethanol (80% v/v, 15 ml) was adjusted to pH 8.5 with a solution of sodium hydroxide (1N) in ethanol (80% v/v). The air in the flask containing the solution was evacuated and the solution stirred at room temperature for 65 hours. TLC (benzene) indicated very little or no reaction. The solvent was removed *in vacuo*, the residue diluted with water and the mixture extracted repeatedly with ether. The combined ether extracts were dried over anhydrous magnesium sulfate. Removal of the ether *in vacuo* gave a yellowish oil (0.80g)

which was identified to be the unreacted ketone by comparison of its TLC and NMR characteristics with those of an authentic sample of the ketone (XVIa).

3.16.0.0 Synthesis of 1-aryl-1-ethylthio-3-nonanone thiosemicarbazones (Table XIV, XIXa-h)

A mixture of the appropriate 1-aryl-1-ethylthio-3-nonanone [(XVIIa-h), 0.10 mol], thiosemicarbazide (9.11g, 0.10 mol), acetic acid (5.61g, 0.094 mol) and ethanol (300 ml) was heated under reflux for 24 hours. Refrigeration of the reaction mixture containing (XIXa) led to a solid being deposited which was recrystallized from ethanol. In the case of compounds (XIXb-h), the solvent was removed *in vacuo* and the resultant oil was dissolved in benzene. Any solid that deposited was removed by filtration and the solution applied to a chromatography column of neutral alumina. The product was eluted with benzene-methanol (95:5). The spectroscopic data generated for a representative compound (XIXa) are as follows. NMR ($CDCl_3$): δ 0.63-1.70 [m, 14H, $(CH_2)_4CH_3$ and $SCH_2\underline{C}H_3$], 2.03-2.70 (m, 4H, C^4H_2 and $SC\underline{H}_2CH_3$), 2.83 (d, 2H, $\underline{J}$=8 Hz, C^2H_2), 4.53-5.00 (m, 1H, $\underline{J}$=8 Hz, C^1H), 5.90-7.80 (m, 6H, C_6H_4 and $CSNH_2$), 8.53 and 8.90 (s, 1H, *anti* and *syn* NH, respectively); IR ($CHCl_3$ soln): υ 3560, 3420, 1590, 1510 cm^{-1}.

Table XIV. Physical data of 1-aryl-1-ethylthio-3-nonanone thiosemicarbazones(XIX)



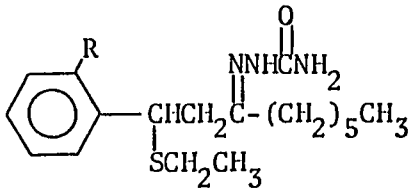
(XIX)

Compound	R_1	R_2	Yield (%)	Melting point (°C)	Molecular formula	Analysis(%)					
						Calculated			Found		
						C	H	N	C	H	N
XIXa	Cl	H	59	82.5	$C_{18}H_{28}ClN_3S_2$	56.01	7.31	10.88	55.96	7.12	10.72
XIXb	Br	H	90	90.3	$C_{18}H_{28}BrN_3S_2$	50.22	6.55	9.76	50.30	6.51	9.65
XIXc	F	H	94	oil	$C_{18}H_{28}FN_3S_2$	58.50	7.64	11.37	58.57	7.58	10.91
XIXd	H	H	91	71.0	$C_{18}H_{29}N_3S_2$	61.49	8.31	11.95	61.57	8.37	12.03
XIXe	CH_3	H	95	oil	$C_{19}H_{31}N_3S_2$	62.42	8.55	11.49	62.20	8.47	11.26
XIXf	OCH_3	H	90	90.7	$C_{19}H_{31}N_3OS_2$	59.80	8.19	11.01	59.78	8.15	10.63
XIXg	H	Cl	77	oil	$C_{18}H_{28}ClN_3S_2$	56.01	7.31	10.89	56.06	7.26	10.23
XIXh	H	CH_3	76	oil	$C_{19}H_{31}N_3S_2$	62.42	8.55	11.49	62.59	8.77	11.00

3.16.1.0 Synthesis of 1-aryl-1-ethylthio-3-nonanone semicarbazones (Table XV, XXa,b)

A solution of semicarbazide hydrochloride (0.20g, 0.0018 mol), and sodium acetate trihydrate (0.25g, 0.0018 mol) in methanol (5 ml) was added to a vigorously stirred solution of the appropriate 1-aryl-1-ethylthio-3-nonanone [(XVIIa or d), 0.0018 mol] in methanol (5 ml). The resultant mixture was stirred at room temperature for 24 hours. The solvent was removed *in vacuo*, the residue treated with benzene and any insoluble material was removed by filtration. The organic solution was concentrated and purified as for (XVIIIb) to give the title compounds as oils. The spectroscopic data generated for a representative compound (XXa) are as follows. NMR ($CDCl_3$): δ 0.63-1.63 [m, 14H, $(CH_2)_4CH_3$ and $SCH_2C\underline{H}_3$], 1.93-2.60 (m, 4H, $SC\underline{H}_2CH_3$ and C^4H_2), 2.80 (d, 2H, $\underline{J}$=8 Hz, C^2H_2), 4.80 (t, 1H, $\underline{J}$=8 Hz, C^1H), 5.60 (br s, 2H, $CSNH_2$), 6.90-7.60 (m, 4H, C_6H_4), 8.33 (s, 1H, NH); IR ($CHCl_3$ soln): ν 3560, 3440, 1700, 1570 cm^{-1}.

Table XV. Physical data of 1-aryl-1-ethylthio-3-nonanone semicarbazones(XX)



(XX)

Compound	R	Yield (%)	Melting point (°C)	Molecular formula	Analysis(%)					
					Calculated			Found		
					C	H	N	C	H	N
XXa	Cl	86	oil	$C_{18}H_{28}ClN_3OS$	57.96	7.56	11.26	58.10	7.47	10.95
XXb	H	88	oil	$C_{18}H_{29}N_3OS$	64.44	8.71	12.52	64.30	8.40	12.20

3.16.2.0 Synthesis of 1-phenyl-1-nonen-3-one thiosemicarbazone (XXI)

A mixture of 1-phenyl-1-nonen-3-one [(XVId), 1.00g, 0.0046 mol], thiosemicarbazide (0.42g, 0.0046 mol), acetic acid (0.26g, 0.0043 mol) and ethanol (10 ml) was heated under reflux for 24 hours. The reaction mixture was worked up as for (XIXb-h) to give the title compound (1.24g, 93%) as an oil. NMR ($CDCl_3$): δ 0.63-1.93 [m, 11H, $(CH_2)_4CH_3$], 2.03-2.80 (m, 2H, C^4H_2), 6.57-7.80 (m, 9H, C_6H_5, C^1H, C^2H, and $CSNH_2$), 8.83 and 9.40 (s, 1H, anti and syn NH, respectively).

Anal. Calc. for $C_{16}H_{23}N_3S$: C 66.39 H 8.01 N 14.52

Found : C 66.31 H 7.94 N 14.19

3.17.0.0 Stability studies

3.17.1.0 Preparation of 5-dimethylamino-1-phenyl-1-penten-3-one hydrochloride (30) from 5-dimethylamino-4-dimethylaminomethyl-1-phenyl-1-penten-3-one dihydrochloride (27)

A solution of 5-dimethylamino-4-dimethylaminomethyl-1-phenyl-1-penten-3-one dihydrochloride [0.50g, 0.0015 mol, (27)] in tromethamine hydrochloride buffer (0.01 mol, pH 7.40, 500 ml) was placed in an incubator at 37°C. After 1 hour, TLC (benzene-methanol, 9:1) revealed the absence of (27) (R_f=0.35) and the presence of a major spot (R_f=0.19) along with a trace of one or more compounds at the point of application of the mixture. The solution was lyophilized and the residue was dissolved in a small quantity of water. This was extracted with chloroform and the organic extracts were dried over anhydrous magnesium sulfate. Removal of the solvent *in vacuo* gave an oil (0.10g, R_f=0.19) which was dissolved in chloroform and the solution was treated with anhydrous hydrogen chloride gas. Addition of anhydrous ether gave a solid which was filtered, washed with ether and then with ice cold acetone to give a colorless solid (0.07g, 19%, R_f=0.19). Recrystallization from acetone afforded the *retro*-Mannich product, m.p. 151-152.5°C, which had identical NMR, IR, TLC and MS characteristics as an authentic sample of 5-dimethylamino-1-phenyl-1-penten-3-one hydrochloride (30) prepared by an unambiguous literature

methodology (Dimmock et al., 1976). NMR (D_2O, 300MHz): δ 2.94 [s, 6H, $N(CH_3)_2$], 3.48 (s, 4H, CH_2CH_2), 6.88 (d, 1H, J=16.3 Hz, C^2H), 7.46-7.83 (m, 6H, C_6H_5 and C^1H); IR (KBr): ν 1650 (C=O), 1615 (C=C), 970 [(E)-CH=CH] cm^{-1}; EIMS: m/e (relative intensity) 203 (67, M-HCl), 158 (38), 157 (46), 131 (61), 103 (59), 77 (51), 58 (100).

3.17.2.0 Preparation of 2-dimethylaminomethyl-1-(4-methoxyphenyl)-2-propen-1-one hydrochloride (XIIf) from 3-dimethylamino-2-dimethylaminomethyl-1-(4-methoxyphenyl)-1-propanone dihydrochloride (28)

A solution of 3-dimethylamino-2-dimethylaminomethyl-1-(4-methoxyphenyl)-1-propanone dihydrochloride [(28), 1.00g, 0.003 mol] in phosphate buffer (0.25 mol, pH 7.40, 40 ml) was placed in an incubator at 37°C. After 5 minutes, the reaction mixture was cooled to room temperature and extracted with chloroform (2 x 20 ml) and the combined chloroform extracts were dried over anhydrous magnesium sulfate. Removal of the solvent in vacuo gave an oil (0.65g) which was identified by NMR spectroscopy to be 2-dimethylaminomethyl-1-(4-methoxyphenyl)-2-propen-1-one (56). NMR ($CDCl_3$): δ 2.33 [s, 6H, $N(CH_3)_2$], 3.33 (s, 2H, CH_2N), 3.83 (s, 3H, aromatic OCH_3), 5.59 (s, 1H, $C=CH_2$), 5.83 (s, 1H, $C=CH_2$), 6.86 (d, 2H, J=8.5 Hz, aromatic H at C-3 and C-5), 7.77 (d, 2H, J=8.5 Hz, aromatic H at C-2 and C-6). The oil was dissolved in anhydrous ether and the solution was treated with anhydrous hydrogen chloride gas. The precipitated solid

was filtered, washed with ether and dried. Crystallization from ethanol-ether gave the corresponding hydrochloride [(XIIf), 0.55g, 73%], m.p. 149-150°C. The structure of this compound was confirmed by comparison of its melting point and NMR characteristics with those of an authentic sample of the compound, prepared by an unambiguous route, vide supra.

3.17.3.0 Preparation of 3-dimethylamino-1-(4-methoxyphenyl) -1-propanone hydrochloride(31) from 3-dimethylamino -2-dimethylaminomethyl-1-(4-methoxyphenyl)-1 -propanone-dihydrochloride (28)

A solution of 3-dimethylamino-2-dimethylaminomethyl-1-(4-methoxyphenyl)-1-propanone dihydrochloride [(28), 0.50g, 0.0015 mol] in phosphate buffer (0.25 mol, pH 7.4, 400 ml) was incubated at 37°C for 72 hours. At the end of this period, another 100 ml of the buffer was added to the reaction mixture and the incubation was continued for a further 24 hours. TLC (benzene-methanol, 9:1) indicated almost complete absence of [(28), R_f=0.39] and the appearance of a new spot (R_f=0.18). The reaction mixture was cooled to room temperature and extracted with chloroform (2 x 100 ml). The combined chloroform extracts were washed once with water and dried over anhydrous magnesium sulfate. Removal of the solvent in vacuo gave an oil (0.25g) which was dissolved in anhydrous ether and treated with anhydrous hydrogen chloride gas. The precipitated solid was filtered,

washed with ether and dried. Crystallization from acetone gave the retro-Mannich product, 3-dimethylamino-1-(4-methoxyphenyl)-1-propanone hydrochloride [(31), 0.062g, 17%], m.p. 167-170°C. This compound had similar NMR characteristics and melting point as an authentic sample of (31) prepared by an unambiguous route, vide infra. NMR (D_2O, 300MHz): δ 2.96 [s, 6H, $N(CH_3)_2$], 3.54-3.64 [(m, on expansion shows two very close triplets), 4H, CH_2CH_2], 3.92 (s, 3H, aromatic OCH_3), 7.11 (d, 2H, J=8.93 Hz, aromatic H at C-3 and C-5), 8.03 (d, 2H, J=8.93 Hz, aromatic H at C-2 and C-6).

3.17.4.0 Preparation of 3-dimethylamino-1-(4-methoxyphenyl)-1-propanone hydrochloride(31) from 2-dimethylaminomethyl-1-(4-methoxyphenyl)-2-propen-1-one hydrochloride (XIIf)

This preparation was carried out using the methodology for converting (28) to (31), section 3.17.3.0, using 2-dimethylaminomethyl-1-(4-methoxyphenyl)-2-propen-1-one hydrochloride [(XIIf), 0.50g, 0.002 mol]. The oil (0.32g), obtained after removal of the chloroform in vacuo was dissolved in anhydrous ether and treated with anhydrous hydrogen chloride gas. The precipitated solid was purified by crystallization from acetone to give 3-dimethylamino-1-(4-methoxyphenyl)-1-propanone hydrochloride [(31), 0.032g, 7%], m.p. 164-167°C. Its structure was confirmed by

comparison of its TLC, melting point and NMR characteristics with those of an authentic sample of (31) prepared by an unambiguous route, vide infra.

3.17.5.0 Synthesis of 3-dimethylamino-1-(4-methoxyphenyl) -1-propanone hydrochloride (31)

A mixture of 4-methoxyacetophenone (0.50g, 0.0033 mol), the Mannich reagent (0.66g, 0.0071 mol) and dry acetonitrile (7 ml) was stirred at 45-50°C for 2 hours. The excess of the Mannich reagent was filtered and washed with hot acetonitrile. The filtrate was kept in the refrigerator overnight. The deposited crystals were filtered, washed with cold acetonitrile and dried. Crystallization from ethanol gave 3-dimethylamino-1-(4-methoxyphenyl)-1-propanone hydrochloride [(31), 0.11g, 14%], m.p. 165-167°C. Lit. (Albrecht et al., 1962) m.p. 182-184°C. NMR (D_2O): δ 3.03 [s, 6H, $N(CH_3)_2$], 3.63 (s, 4H, CH_2CH_2), 3.97 (s, 3H, aromatic OCH_3), 7.13 (d, 2H, J=9 Hz, aromatic H at C-3 and C-5), 8.07 (d, 2H, J=9 Hz, aromatic H at C-2 and C-6).

3.18.0.0 Kinetic studies

3.18.1.0 Method I: UV spectroscopy

The kinetics of the elimination reaction of the acetophenone bis-Mannich bases were measured under pseudo first order conditions at a pH of 3.50 and 37°C employing the Guggenheim method (Bunnett, 1975).

A Gilford Response UV-VIS spectrophotometer equipped with a temperature controlling device was used for the measurements. A temperature of 37 ± 0.2°C was used for all measurements unless otherwise indicated. A computerized kinetic program featured with the spectrophotometer was used for the study.

A 0.25 M formate buffer of pH 3.50 was used for the kinetics. Two liters of the buffer solution was prepared by dissolving sodium formate (34.00g) in about 1 liter of double distilled water. To this was added hydrochloric acid (1.40 N, 232 ml). Finally, the volume was adjusted to 2 liters with water, and the pH checked to be 3.50.

3.18.1.1 Determination of the wavelengths of absorption maxima

The approximate wavelengths of the absorption maxima (λ_{max}) and the molar absorptivities (ϵ_{max}) of the bis-Mannich bases and the corresponding acrylophenones were determined using 10^{-4}-10^{-5} mol/liter solutions of the compounds in the

formate buffer at 0°C and also in distilled water at room temperature.

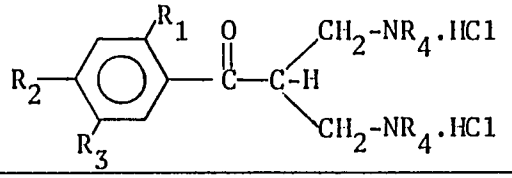
3.18.1.2 General procedure for the measurement of rate constants of β-elimination of the bis-Mannich bases

The Mannich base (Table XVI, a-g, 10^{-5}-10^{-6} mol) was added to approximately 98 ml of the formate buffer, pH 3.5, contained in a 100 ml volumetric flask which was previously incubated at 37°C in a constant temperature circulating water bath. The volume was adjusted to 100 ml with the buffer and the mixture shaken to dissolve the compound as quickly as possible. A stopwatch was started as soon as the compound dissolved. An aliquot of this solution was placed into a thermocuvette which was then transferred immediately to the sample compartment of the spectrophotometer. The stopwatch was stopped as soon as the kinetic program was initiated and the optical density measurements commenced. The delay time (usually between 30-90 seconds) was then included in the calculation of the rate constant. The reference cuvette contained formate buffer. The reaction was followed by measuring the decrease in absorbance at the λ_{max} of the bis-Mannich base at 37°C.

Since the Guggenheim method was employed, optical density readings OD_1, $OD_2 \ldots OD_n$ were recorded at times t_1, $t_2 \ldots t_n$ followed by another set of readings OD'_1, $OD'_2 \ldots OD'_n$ at times $t_1+\Delta t$, $t_2+\Delta t \ldots t_n+\Delta t$. The time interval of Δt

chosen was of the order of 1 to 2 half-lives. A graph of log(OD-OD') as abscissa was plotted against time t, in minutes, as ordinate using a Hewlett Packard HP-37E calculator. The slope, m, was multiplied by -2.303/60 to obtain the pseudo first order rate constant ($K_{\psi}sec^{-1}$). The half-life ($t_{1/2}$) was calculated from the equation $t_{1/2}$= $0.693/K_{\psi}$ and was expressed in minutes. A minimum of 12 different OD and OD' values were used for each compound and all determinations were carried out in triplicate. Measurements were not made beyond 2 to 3 half-lives.

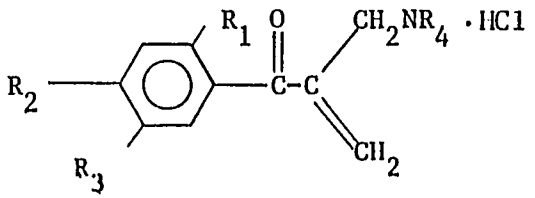
Table XVI. Wavelengths of absorption maxima (λ_{max}) and molar absorptivities (ϵ_{max}) of some acetophenone bis-Mannich bases.



Compound	R_1	R_2	R_3	NR_4	λ_{max}^{Buffer} (nm) [1]	λ_{max}^{Water} (nm) [2]	ϵ_{max}	λ_{max}^{Water} (nm) [2]	ϵ_{max}
a	H	H	H	$-N(CH_3)_2$	255.5	----	----	251.5	8,590
b	OCH_3	H	H	$-N(CH_3)_2$	321.0	259.0	----	320.0	3,370
c	H	OCH_3	H	$-N(CH_3)_2$	295.0	223.0	7,469	287.5	13,047
d	OCH_3	OCH_3	OCH_3	$-N(CH_3)_2$	348.0	282.5	9,056	347.0	8,381
e	H	CH_3O-C$_6$H$_4$-O-	H	$-N(CH_3)_2$	295.0	----	----	289.0	16,142
f	H	Cl-C$_6$H$_4$-O-	H	$-N(CH_3)_2$	293.5	223.5	15,727	288.5	16,711
g	H	OCH_3	H	-N⟨ ⟩	296.5	226.5	8,032	294.5	17,011

1. 0.25 M Formate buffer of pH 3.5 and at 0 °C.
2. At room temperature.

Table XVII. Wavelengths of absorption maxima(λmax) and molar absorptivities (ϵmax) of some substituted acrylophenones in distilled water



Compound	R_1	R_2	R_3	NR_4	λ_{max} (nm)	ϵ_{max}	λ_{max} (nm)	ϵ_{max}
a	OCH_3	H	H	$-N(CH_3)_2$	220.5	-----	315.5	1,741
b	H	OCH_3	H	$-N(CH_3)_2$	224.5	11,856	298.0	11,122
c	OCH_3	OCH_3	OCH_3	$-N(CH_3)_2$	-----	-----	346.0	3,711
d	H	OCH_3	H		225.5	10,441	297.5	10,505

3.18.1.3 A representative kinetic experiment

Data for a typical kinetic experiment in the elimination reaction of 1-[4-(4-chlorophenoxy)]phenyl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrochloride (table XVI, f) are given in table (XVIII). A plot of this data is shown in figure 7. The kinetic data for all of the bis-Mannich bases is presented in table (XIX).

Table XVIII. Data for a typical kinetic experiment in the elimination reaction of 1-[4-(4-chlorophenoxy)]phenyl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrochloride (table XVI,f) in 0.25M formate buffer, pH 3.50, at 37°C, measured by UV spectroscopy (Concentration = 3.74×10^{-5} mole/liter, Δt = 21 minutes)

Time in minutes (t)	Optical density at time t (OD_t)	Optical density at time $t+\Delta t$ ($OD_{t+\Delta t}$)	Δ OD	2+log Δ OD
3.16	0.6258	0.4840	0.1418	1.15168
3.66	0.6197	0.4825	0.1372	1.13735
4.16	0.6135	0.4810	0.1325	1.12222
4.66	0.6080	0.4792	0.1288	1.10992
5.16	0.6023	0.4780	0.1243	1.09447
5.66	0.5970	0.4768	0.1202	1.07990
6.16	0.5918	0.4755	0.1163	1.06558
6.66	0.5868	0.4744	0.1124	1.05077
7.16	0.5817	0.4725	0.1092	1.03822
7.66	0.5771	0.4721	0.1050	1.02119
8.16	0.5725	0.4706	0.1019	1.00817
8.66	0.5682	0.4697	0.0985	0.99344

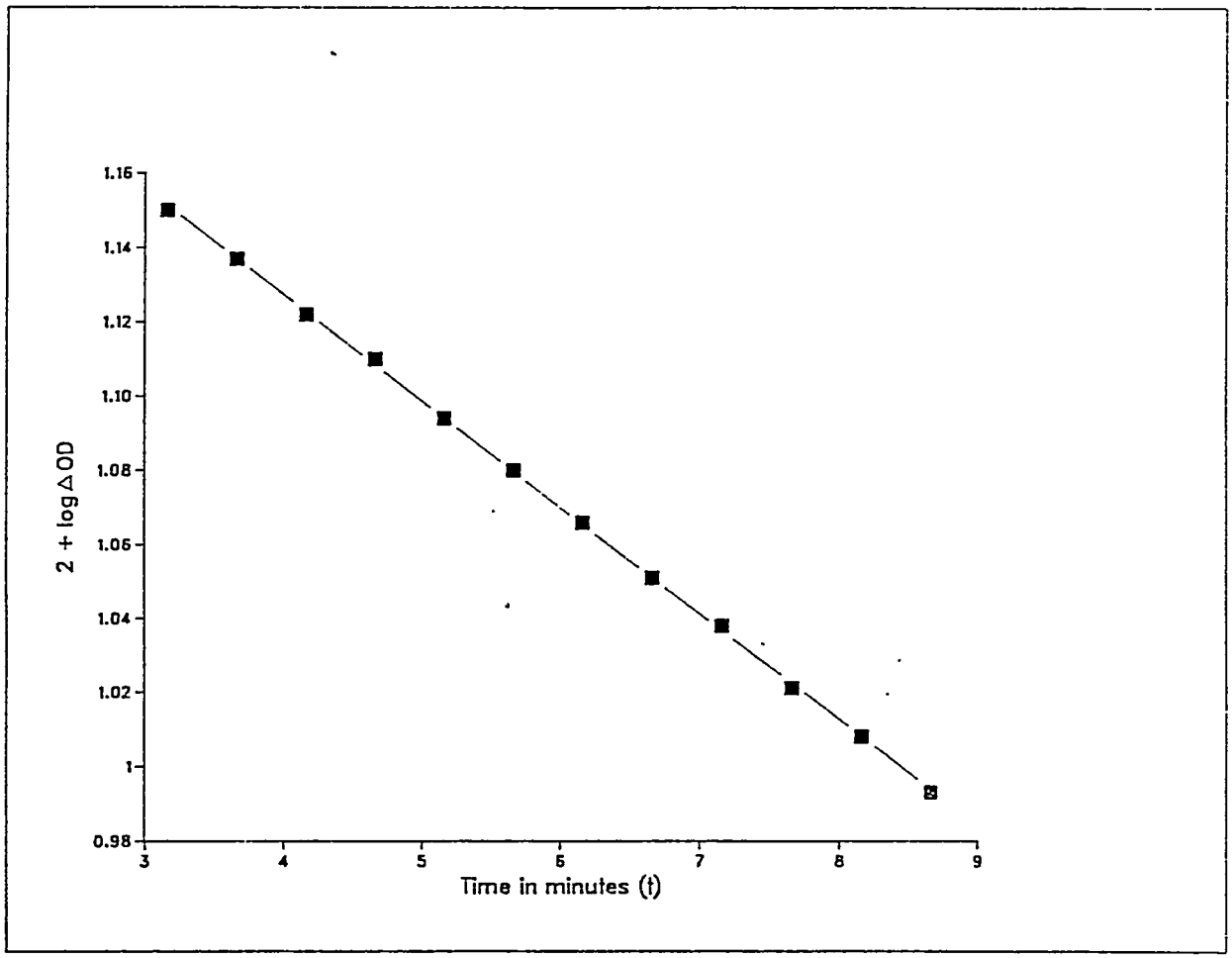
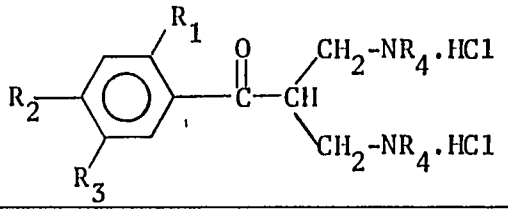


Figure 7. Plot of the data given in table XVIII for a typical kinetic experiment in the elimination reaction of 1-[4-(4-chlorophenoxy)]phenyl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrochloride, in 0.25 M formate buffer, pH 3.50, at 37°C, measured by UV spectroscopy. Slope m = -2.877×10^{-2} min^{-1}. Pseudo first order rate constant, $K_{\psi} = 1.104 \times 10^{-3}$ sec^{-1}. Correlation coefficient r = -0.9999.

Table XIX. Kinetic data for the elimination reaction of some 3-amino-2-aminomethyl-1-aryl-1-propanone dihydrochlorides in 0.25 M formate buffer, pH 3.50, at 37°C, measured by UV spectroscopy



Compound	R_1	R_2	R_3	NR_4	λ (nm)	Pseudo first order rate constant K_ψ [a] sec^{-1}	Standard deviation (SD)	Coeff. of variation (CV,%)	Half-life $t_{1/2}$ (min)	Standard deviation (SD)
a	H	H	H	$-N(CH_3)_2$	255.5	2.462×10^{-3}	$\pm0.0162\times10^{-3}$	0.66	4.69	±0.030
b	OCH_3	H	H	$-N(CH_3)_2$	321.0	8.667×10^{-3}	$\pm0.099\times10^{-3}$	1.15	1.33	±0.015
c	H	OCH_3	H	$-N(CH_3)_2$	295.0	4.707×10^{-4}	$\pm0.043\times10^{-4}$	0.92	24.54	±0.226
d	OCH_3	OCH_3	OCH_3	$-N(CH_3)_2$	348.0	1.157×10^{-3}	$\pm0.014\times10^{-3}$	1.17	9.98	±0.119
e	H	CH_3O-C$_6$H$_4$-O-	H	$-N(CH_3)_2$	295.0	8.736×10^{-4}	$\pm0.212\times10^{-4}$	2.43	13.23	±0.316
f	H	Cl-C$_6$H$_4$-O-	H	$-N(CH_3)_2$	293.5	1.096×10^{-3}	$\pm0.025\times10^{-3}$	2.28	10.54	±0.240
g	H	OCH_3	H	-N(C$_4$H$_8$)	296.5	7.613×10^{-5}	$\pm0.360\times10^{-5}$	4.73	151.95	±7.353

a Mean of 3 determinations.

3.18.2.0 Method II: ^{1}H NMR spectroscopy

The kinetics of the elimination reaction of a representative bis-Mannich base, 3-dimethylamino-2-dimethylaminomethyl-1-(4-methoxyphenyl)-1-propanone dihydrochloride [table XIX, -c, (28)] was also studied by a NMR method under pseudo first order conditions at a pH of 3.50 and 37°C.

A Brucker AM 300 FT NMR spectrometer equipped with a variable temperature unit (BVT-1000) and an Aspect 3000 computer was used for the study. A temperature of 37±0.1°C was used for all the experiments. A computerized kinetic program was used to accumulate spectra at specified time intervals.

A 0.25 M formate buffer of pH 3.50 (pD=3.10) was used for the kinetic study. A quantity of 20 ml of the buffer solution was prepared by dissolving sodium formate (0.34g) in about 10 ml of deuterium oxide (D_2O). To this was added sodium 2,2-dimethyl-2-silapentane-5-sulfonate [(DSS), 0.10g] as an internal standard and hydrochloric acid -d_1 (1.34 N, 3.10 ml). The volume was finally adjusted to 20 ml with D_2O. Preliminary experiments showed that addition of 10 mg of the bis-Mannich base to 0.5 ml of the buffer, does not cause any change in the pH of the medium for at least 3 hours.

3.18.2.1 Kinetic measurements

A NMR tube containing 0.5 ml of the formate buffer was introduced into the spectrometer magnet and allowed to equilibrate for about 10 to 15 minutes at the temperature of the probehead (37±0.1°C). The NMR tube was then ejected and the Mannich base (8.43 mg, 25μM) was introduced into the tube. The contents were shaken to dissolve the compound as quickly as possible. A stopwatch was started as soon as the compound dissolved and the NMR tube reintroduced into the spectrometer magnet. The stopwatch was stopped as soon as the kinetic program was initiated. The delay time (usually between 1 and 2 minutes) was then included in the calculation of the rate constant. Spectra were accumulated every 5 or 10 minutes and the reaction was followed by integration of the olefinic signals (δ ca. 6.37 and 6.60) as they appeared with time. The reaction was terminated after about 2 to 3 half-lives.

A graph of the logarithm of the mean integral value of the olefinic signals, $\log(I_{olefinic})$ as abscissa, was plotted against time (t), in minutes, as ordinate using a Hewlett Packard HP-37E calculator. The slope, m, was multiplied by 2.303/60 to obtain the pseudo first order rate constant ($K_{\psi}\,sec^{-1}$). The half-life, ($t_{1/2}$), was calculated from the equation $t_{1/2} = 0.693/K_{\psi}$ and was expressed in minutes. The experiment was carried out in duplicate.

The mean integral value of the olefinic signals was

corrected each time by relating it to the integral of the DSS signal. Care was taken to see that [NS x (AQ+RD)] did not exceed 10 or 20% of D_1, where

NS= number of scans per spectrum
AQ= acquisition time
RD= relaxation delay
D_1= time interval between spectra

Appropriate corrections were applied to the time scale to take into consideration the values of NS, AQ, and RD.

The NMR spectrum of -c (table XIX) at time t=4.00 minutes, in formate buffer, pH 3.50, at 37°C, is shown in figure 8. Figure 9 shows the NMR spectrum of -c (table XIX) at time t=81.00 minutes (approx. 2.5 half-lives).

The kinetic data for one of the experiments is shown in table (XX), and a plot of those kinetic parameters is shown in figure 10.

The results obtained from the two experiments are as follows.

Experiment	K_ψ (sec^{-1})	$t_{1/2}$ (min)	Correlation (r)
1	3.434×10^{-4}	33.63	0.9577
2	3.325×10^{-4}	34.74	0.9263

Mean $K_\psi = 3.38 \times 10^{-4} \pm 0.077 \times 10^{-4}$ sec^{-1}

Mean $t_{1/2} = 34.19 \pm 0.79$ minutes.

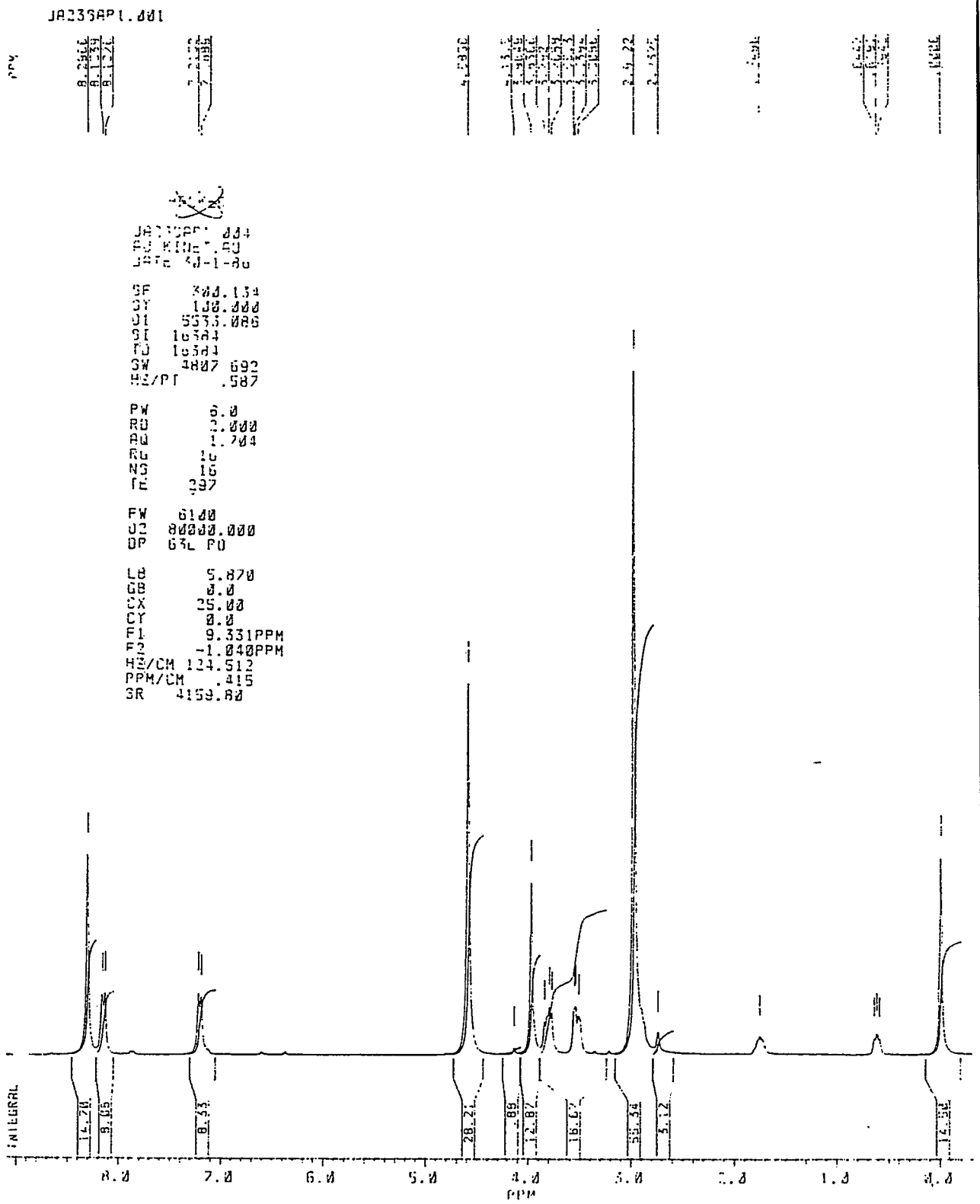


Figure 8. 300 MHz NMR spectrum of 3-dimethylamino-2-dimethylaminomethyl -1-(4-methoxyphenyl) -1- propanone dihydrochloride (table XIX,-c) at time t=4.00 minutes, in formate buffer, pH 3.50, at 37°C.

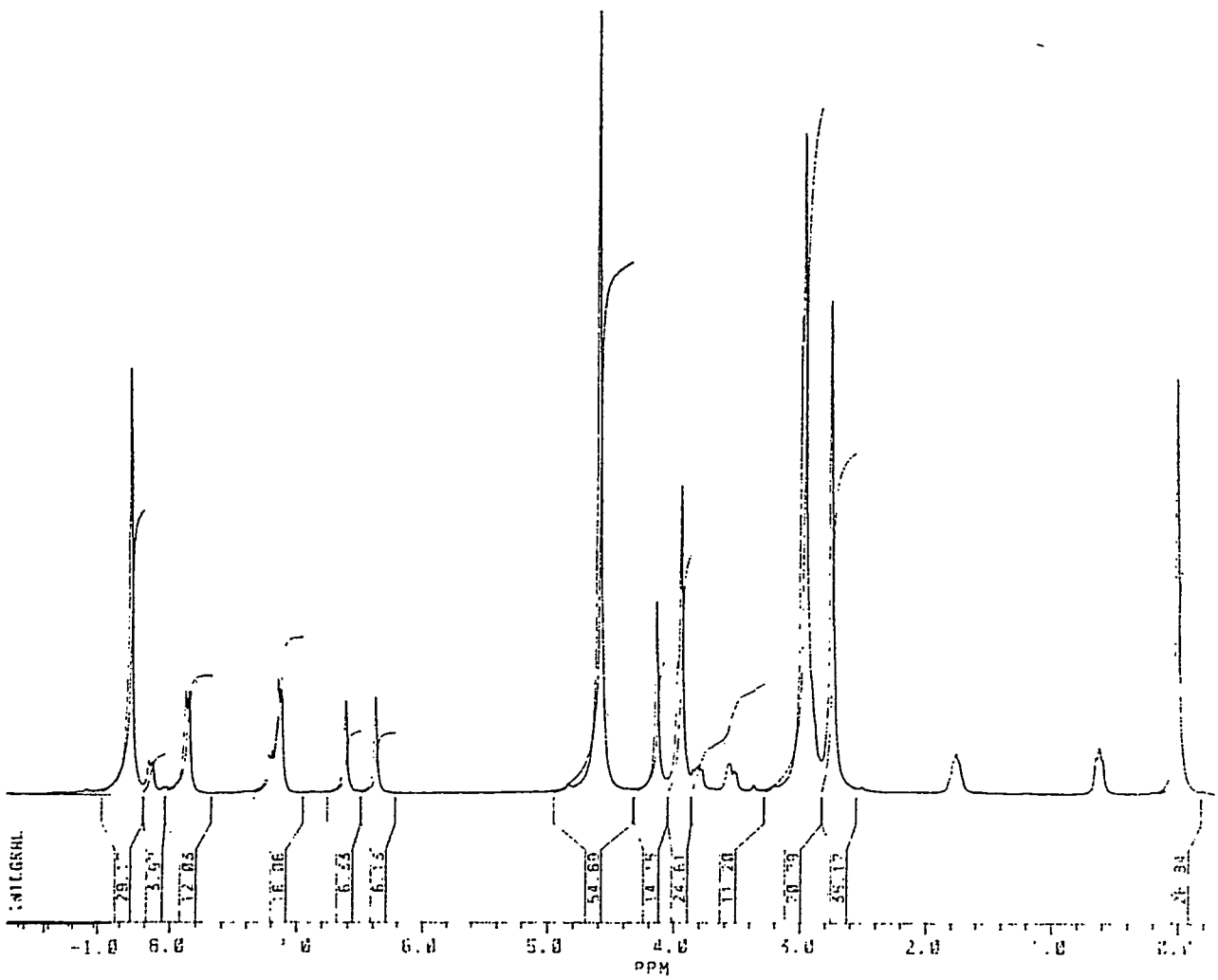


Figure 9. 300 MHz NMR spectrum of 3- dimethylamino-2-dimethylaminomethyl -1-(4-methoxyphenyl)-1-propanone dihydrochloride (table XIX,-c) at time t=59.00 minutes, in formate buffer, pH 3.50, at 37°C.

Table XX. Data for a typical kinetic experiment in the elimination reaction of 3-dimethylamino-2-dimethylaminomethyl-1-(4-methoxyphenyl)-1-phenyl-1-propanone dihydrochloride (table XIX,-c) in 0.25 M formate buffer, pH 3.50, at 37°C, measured by 300 MHz NMR spectroscopy, concentration = 8.43mg (25 μM) in 0.50ml of buffer .

Time in minutes (t)	I^{a}_{DSS}	$I^{b}_{olefinic}$	Corrected $I_{olefinic}$	log $I_{olefinic}$
4	14.50	0.0000	0.0000	0.0000
15	13.23	0.9650	1.0941	0.0391
26	13.13	1.5300	1.7479	0.2425
37	14.22	2.6185	2.7621	0.4412
48	14.39	3.1250	3.2582	0.5130
59	26.84	6.2300	3.4817	0.5418
70	28.69	7.0200	3.6703	0.5647
81	28.86	8.9400	4.6466	0.6671

a Integration of the DSS signal

b Integration of the olefinic signal

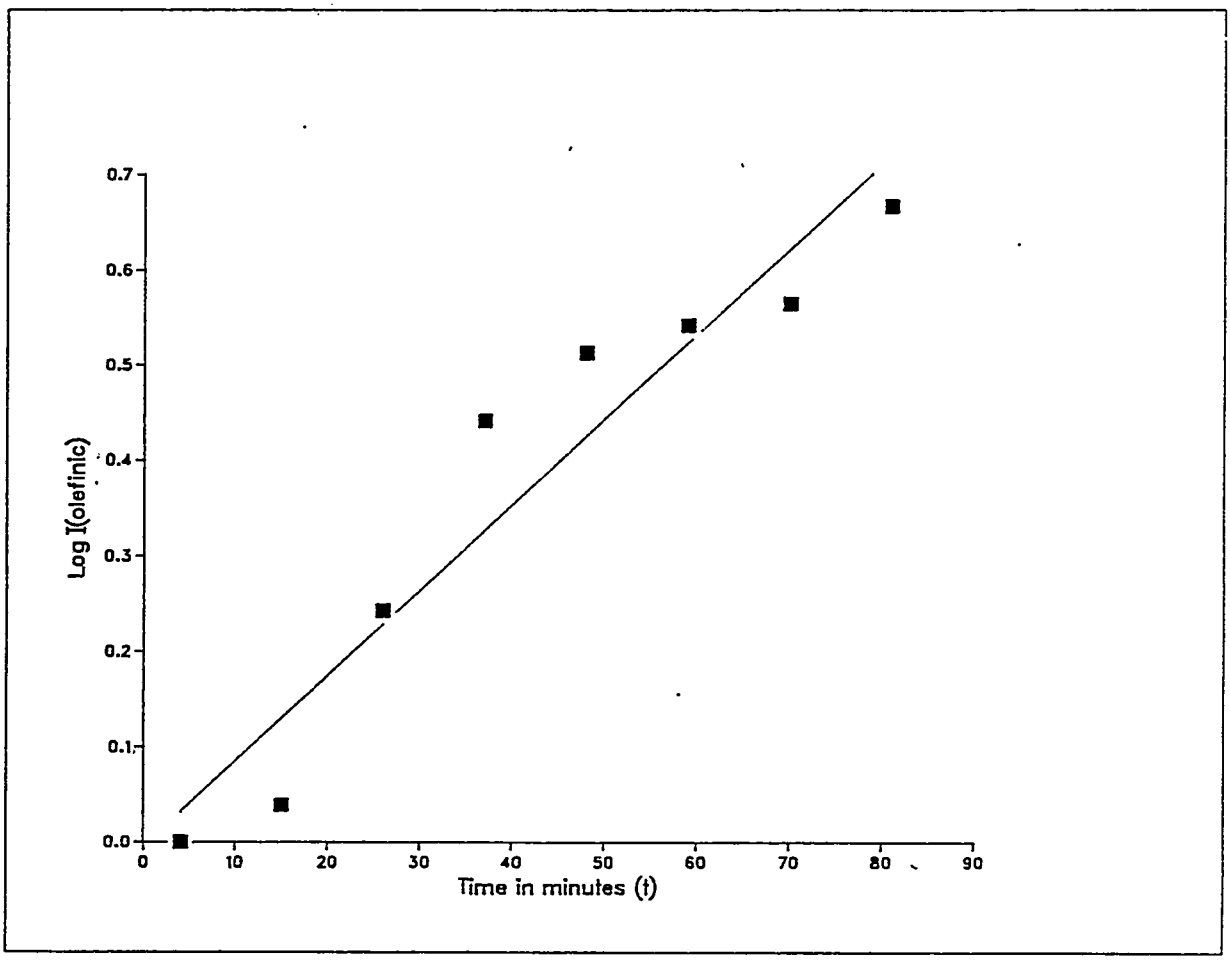
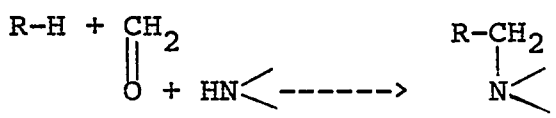


Figure 10. Plot of the data given in table XX, for a typical kinetic experiment in the elimination reaction of 3-dimethylamino-2-dimethylaminomethyl-1-(4-methoxyphenyl)-1-propanone dihydrochloride, in 0.25 M formate buffer, pH 3.50, at 37°C. Slope m = 8.947×10^{-3} min^{-1}. Pseudo first order rate constant, $K_\psi = 3.434 \times 10^{-4}$ sec^{-1}. Correlation coefficient r = 0.9577.

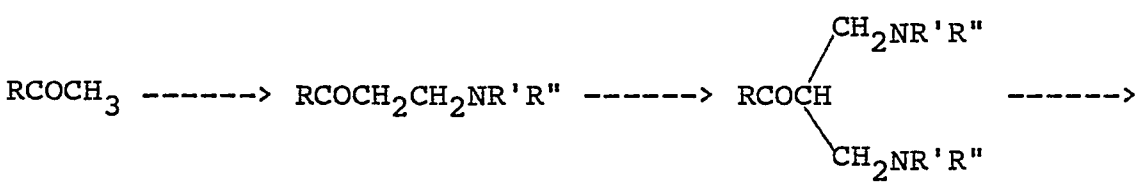
4.0.0.0 Results and discussion

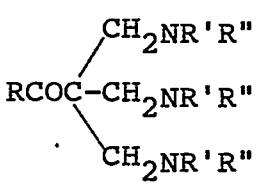
4.1.0.0 Introduction to the Mannich reaction

The Mannich reaction is one of the most important methods available for the introduction of one carbon units into organic molecules. It involves condensation of a substrate (R-H) possessing at least one active hydrogen (alkyl ketones, phenols, NH-heterocycles, etc.) with formaldehyde (or, occassionally other aldehydes) and a primary or a secondary amine (or, occasionally ammonia).

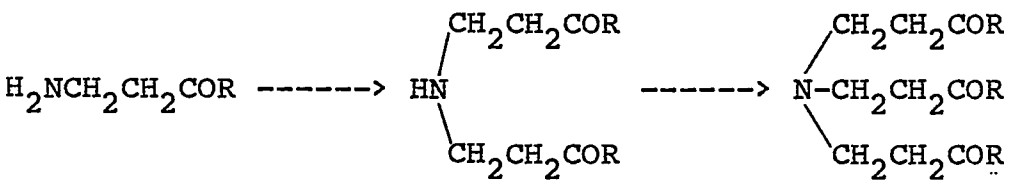


If the compound containing the active hydrogen has more than one replaceable hydrogen atom, multiple aminomethylations can occur.

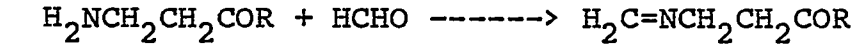




The amine used can also promote the formation of mixed products. Thus, while only single products are possible with secondary amines, two or three products are possible with primary amines and ammonia respectively.



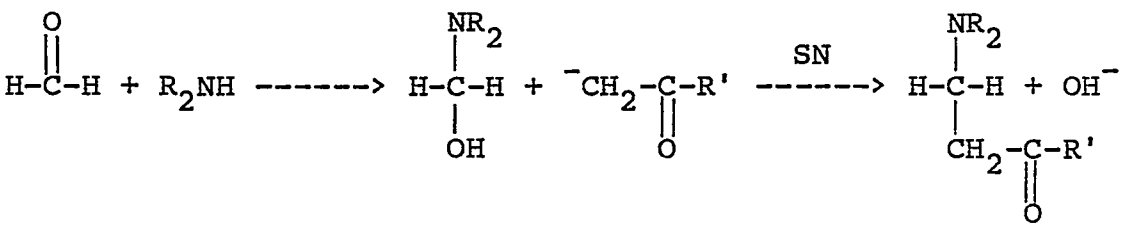
A further reaction is possible whereby condensation of the Mannich base with excess of formaldehyde can occur.



Therefore, a secondary amine (e.g., dimethylamine, diethylamine, piperidine, morpholine or pyrrolidine) is usually used to avoid such side reactions.

The mechanisms of the base-catalyzed and the acid-catalyzed reactions are shown below (March, 1977a).

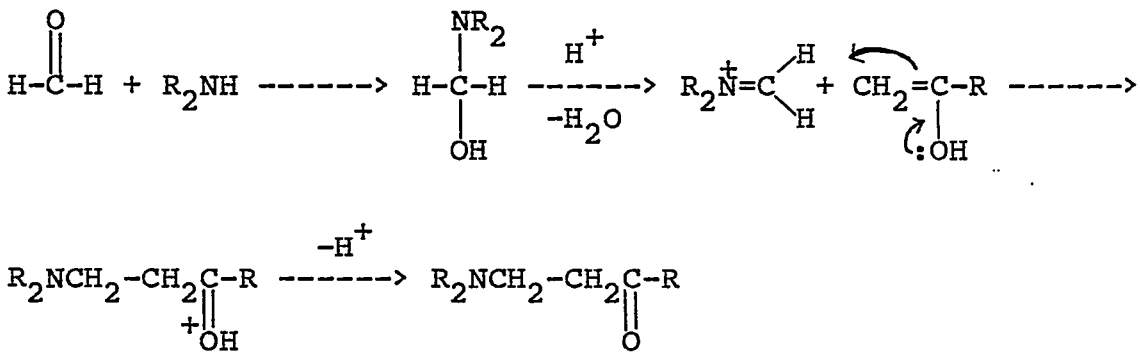
The base-catalyzed reaction proceeds as follows.



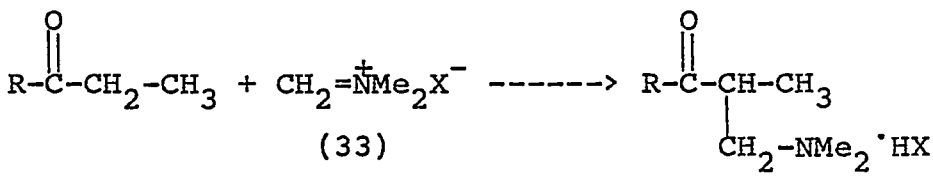
(32)

It is possible that in basic media, the intermediate which undergoes the nucleophilic substitution may be $R_2NCH_2NR_2$ instead of (32).

The acid-catalyzed reaction takes place as illustrated below.



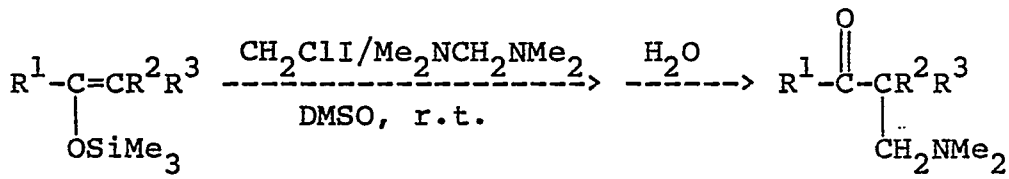
A new approach to the Mannich reaction employs the use of preformed iminium ions (33) directly as the aminomethylating species.

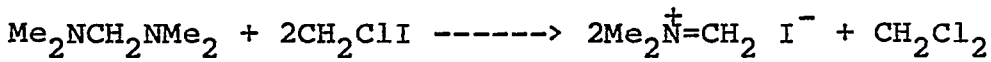


(33)

As noted by Holy et al. (1979) this approach has three basic advantages: 1) reactions are faster since the concentration of iminium ion is higher than that generated via equilibria; 2) lower temperatures are possible; and 3) aprotic conditions may be used. The superiority of this approach over the classical Mannich reaction has been demonstrated in several cases by Kinast and Tietze (1976).

Recently, Miyano et al. (1982) reported a convenient synthesis of Mannich bases from enol silyl ethers by a combination of chloroiodomethane and N,N,N'N'-tetramethyldiaminomethane (TMMD). These workers also showed that the reagent system CH_2ClI/TMMD provides a convenient route to the Eschenmoser's salt (33).





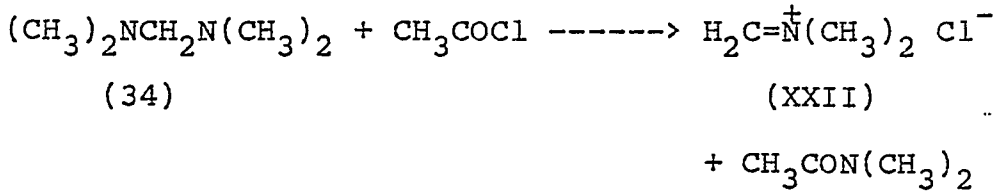
(33)

With unsymmetrical ketones it is possible to obtain a mixture of products using the classical Mannich conditions. Regioselectivity has been obtained by treatment of the ketone with preformed iminium ions (March, 1977a): the use of $Me_2\overset{+}{N}=CH_2$ CF_3COO^- in trifluoroacetic acid gives substitution at the more highly substituted position, while with iso-$Pr_2N^+=CH_2$ ClO^-_4 the reaction takes place at the less highly substituted position.

Regiospecific synthesis of Mannich bases has also been achieved by indirect methods in which specific enols such as silyl enol ethers (Holy and Wong, 1977; Miyano et al., 1982) and enol borinates (Hooz and Bridson, 1973) are generated and allowed to react with dialkyl(methylene)ammonium salts.

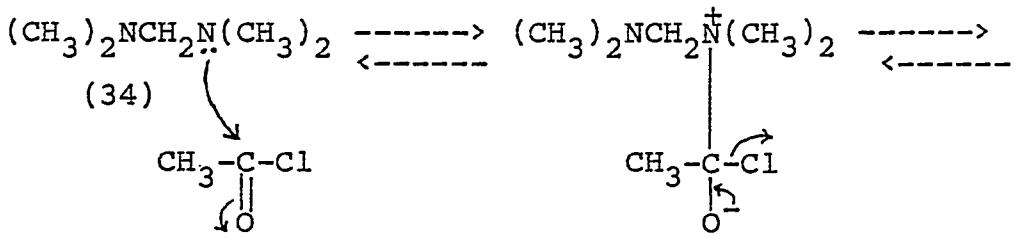
4.1.1.0 Synthesis of the Mannich reagent (XXII)

N,N-Dimethyl(methylene)ammonium chloride (XXII) was prepared in analogy to a literature methodology (Bohme and Hartke, 1960) by the reaction of N,N,N',N'-tetramethyl-diaminomethane with acetyl chloride in methylene chloride.

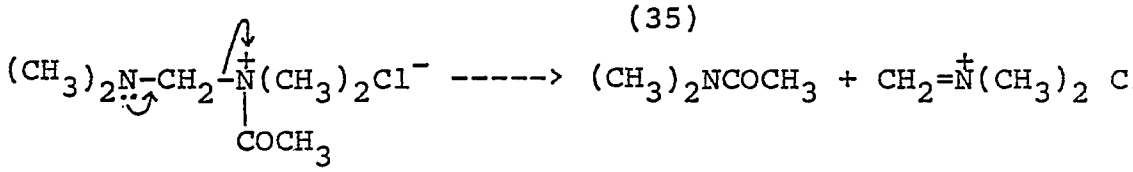


(34) (XXII)

The mechanism of this process can be written as follows.



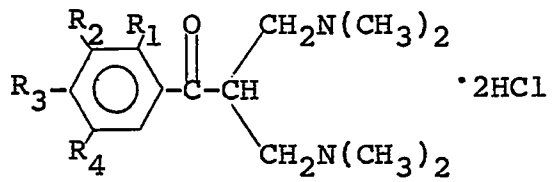
(34) (35)



(36) (37) (XXII)

The mechanism involved here is called the tetrahedral mechanism (March, 1977b). The initial step involves a nucleophilic addition of the diamine (34) to the carbon-oxygen double bond of the acetyl chloride to form a tetrahedral intermediate (35). This intermediate then eliminates the chloride ion to form the unstable intermediate (36) which collapses to give the Mannich reagent (XXII) and N,N-dimethyl acetamide (37).

4.2.0.0 1-Aryl-3-dimethylamino-2-dimethylaminomethyl -1-propanone dihydrochlorides (II)



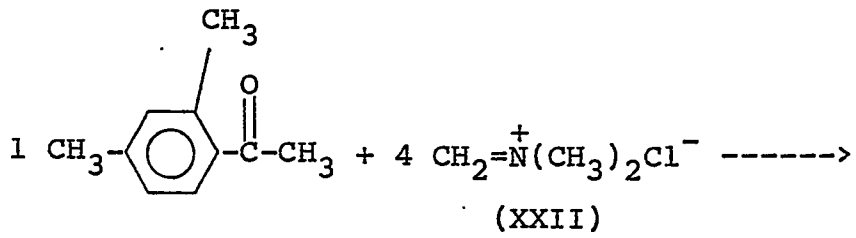
(II)

R_1, R_2, R_3 and R_4: same as section 2.2.0.0

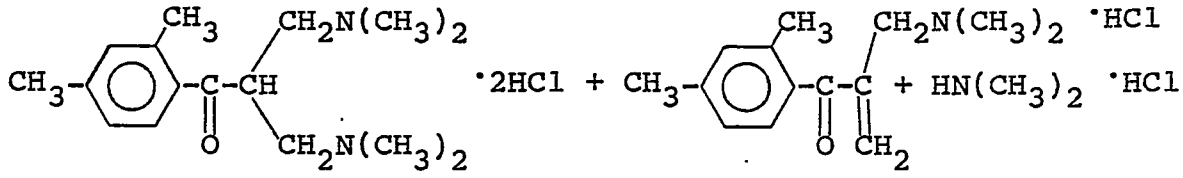
Initial attempts directed towards preparation of this series of compounds were made using two representative ketones, 2-methoxy- and 2,4-dimethoxy-acetophenone, under the classical conditions by reacting the ketones with dimethylamine and formaldehyde. Under these conditions, very little or no reaction occurred. Attention was therefore directed to the use of the preformed Mannich reagent,dimethyl-(methylene)ammonium chloride, because of the advantages mentioned in the preceding section. Considerable experimentation was also necessary, using this methodology, before some of the required Mannich bases could be obtained. Use of the Mannich reagent and the ketone in a ratio of 1:1 or 2:1 always resulted in the formation of either the monobasic compound or a mixture of the monobasic and dibasic compounds. Finally, a ratio of 4:1 was found to be satisfactory. The reaction was carried out by stirring a mixture of the two components in acetonitrile at 45-50°C for the required time periods. Use of higher temperatures lowered the yields of the products. Under these reaction conditions, only 2-methoxy-, 2,4-dimethoxy-, 2,4,5-

trimethoxy- and 3,4-methylenedioxy-acetophenone formed the desired bis-Mannich bases. The physical data of these four compounds is presented in table V, section 3.2.0.0. The yields varied from 13 to 79%.

The remaining seven acetophenones, including the four methyl acetophenones, the two methoxy compounds, and 2-chloroacetophenone, all gave rise to varying mixtures of the desired bis-Mannich base and/or, the corresponding acrylophenone and dimethylamine hydrochloride. In most cases, the identity and composition of the mixtures were established by NMR spectroscopy and by comparison of the NMR spectrum of the mixture with that of authentic samples of the corresponding acrylophenones, where available. In the case of 2,4-dimethyl- and 2,5-dimethyl-acetophenone, the mixture was separated into its components by careful fractional crystallization and the identity of the components established by NMR and IR spectroscopy, elemental analysis and melting point.



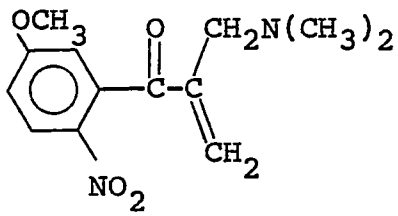
(XXII)



(29, minor) (XIIc) (major)

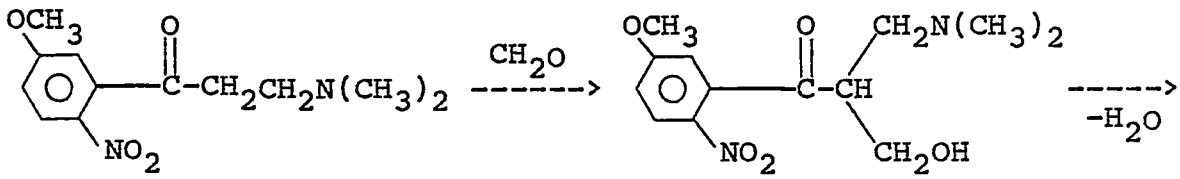
This observation raises two pertinent questions. The first one concerns the formation of the acrylophenones *per se*. The second one deals with the differential behavior of the methyl acetophenones as opposed to the methoxy acetophenones. That is, while at least some of the methoxy-acetophenones formed the desired *bis*-Mannich bases in good yields, none of the methylacetophenones formed the desired compounds in satisfactory yields.

The formation of acrylophenones, *per se*, is perhaps not very surprising. Of some relevance is the report that aminomethylation of 2-nitro- and 2-ethoxycarbonylamino-acetophenones led to the formation of corresponding acrylophenones (e.g. 38) in addition to the expected monobasic propiophenone derivatives (Gevorgyan *et al.*, 1984).

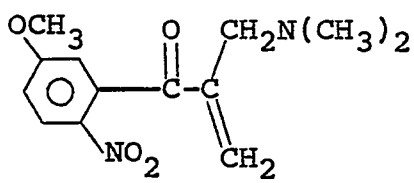


(38)

The formation of (38) was explained by the fact that, under the conditions of the Mannich reaction, the C-hydroxymethyl compound (39) is formed from compound (40) and its dehydration leads to compound (38).



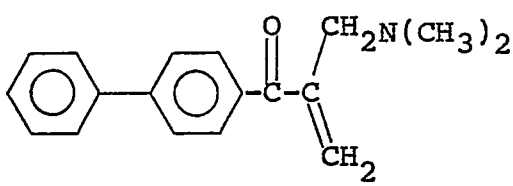
(40) (39)



(38)

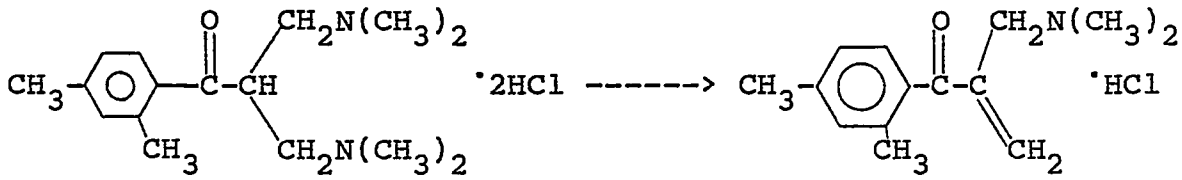
This was confirmed by both physicochemical and analytical methods and by various reactions of compound (38).

Another example is that of formation of compound (41) (Gevorgyan et al., 1984).



(41)

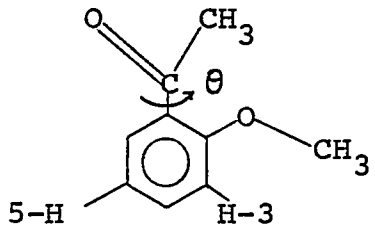
In the above examples, the acrylophenones were formed by the condensation of the monobasic Mannich salt with formaldehyde. However, in the case on hand, such a derivative could possibly arise only by deamination of a bis-Mannich base, as exemplified below.



(29) (XIIc)

There does not appear to be any report in the literature indicating the formation of acrylophenone derivatives under similar conditions.

The answer to both questions posed earlier may be found in some of the literature reports on the conformational preferences of ortho-substituted acetophenones. As discussed in section 2.2.0.0, variable effects are seen in the case of the methyl and the methoxy compounds. 2-Methoxyacetophenone, for example, exists in the conformation (11) in which, to counter the steric hindrance, the methoxy group lies in the plane of the benzene ring and cis to H-3, while the carbonyl group lies trans to the methoxy group. Consequently, the angle θ is not very large (Schaefer et al., 1984).



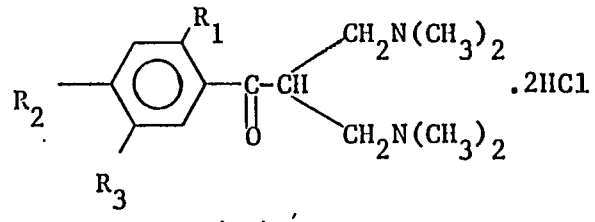
(11)

Such a conformation is probably not possible for the ortho-methylacetophenones because of the relative rigidity of the ortho-methyl group. Consequently, the steric strain would be expected to be more in the latter case. For example, the acetyl group in 2,4,6-trimethylacetophenone was found to have a dihedral angle of 60-90° resulting in a lack of conjugation between the carbonyl group and the aromatic ring (Abraham et al., 1983). This, in turn, would forbid the supply of electrons from the aromatic ring to the electron deficient carbonyl carbon by resonance interaction. It is conceivable that such steric effects should be much more pronounced in the bis-Mannich bases derived from such ortho-substituted acetophenones. It is not clear at this point in time whether deamination is a direct result of an attempt to relieve steric strain in the molecule; the steric strain in the deaminated product would appear, a priori, to be about the same as in the bis-Mannich base. Alternatively, deamination could be a secondary effect produced as a result of the increased acidity of the ∝C-methine hydrogen in the sterically hindered molecule.

The results of the anticancer evaluation of the four Mannich bases against P388 lymphocytic leukemia in mice are shown in table (XXI). As seen from the data, only the 2,4,5-trimethoxy compound shows very good activity (T/C%= 139). However, this activity was not confirmed by the second screener and hence remains presumptive at the moment. The prototype 4-methoxy compound has a T/C% of 128 at a dose of 25 mg/kg. Until a few years ago the criterion for activity was a T/C% value of 120. In this regard, compounds (IIb & IId) may be said to possess marginal activity. A reference compound, 5-fluorouracil, gives a T/C% value of >135 at a dose of 20mg/kg when administered daily by the intraperitoneal route for either five or nine days (Instruction 271F, Developmental Therapeutics Program, Division of Cancer Treatment, NCI, Bethesda, Maryland, November 1983). Although these compounds were designed to deaminate slowly relative to the 4-methoxy compound, kinetic data in table (XIX) indicates that they do not do so. It is therefore quite possible that a major proportion of the acrylophenone generated from these compounds is being inactivated by local reactions before reaching the leukemic cells and/or by breakdown to the corresponding monobasic compounds (section 3.17.3.0) which are devoid of antileukemic activity (vide infra).

The compounds appear to be quite toxic with drug induced mortalities occurring at doses >7.5 mg/kg in case of (IIa & IIb). A related compound (28) prepared previously in this

Table XXI. Antineoplastic activity of 1-aryl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrochlorides against P388 lymphocytic leukemia in mice (IIa-d)



(II)

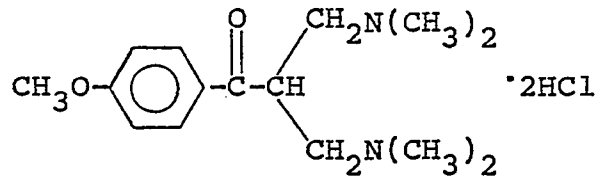
Compound	R_1	R_2	R_3	Maximum T/C%[a] (dose in mg/kg)	Toxicity in mice[b] (dose in mg/kg)
IIa	OCH_3	H	H	112(7.5)	0/6(30), 5/6(15)
IIb	OCH_3	OCH_3	H	121(7.5)	0/6(30), 4/6(15)
IIc	OCH_3	OCH_3	OCH_3	139(60)[c], 124(120)[c]	0/6(240), 5/6(120), 6/6(60)
IId	H	OCH_2O		122(15)	0/6(120), 5/6(60), 6/6(30)

a Anticancer activity is expressed as the ratio of the survival time of the treated(T) animals to control(C) animals given as a percentage. All of the compounds were initially screened at 240, 120 and 60 mg/kg and if mortalities occurred at these doses, they were reduced to non-lethal levels. A compound should increase the median survival time by at least 27% to be considered active.

b These figures are the numbers of survivors on the fifth day after commencement of the dosage schedule of nine daily doses given intraperitoneally.

c Presumptive activity not confirmed by the second screener, awaiting further evaluation.

laboratory, has been subjected to a thorough toxicological assessment in rats (Dimmock et al., 1984).



(28)

It was concluded that this compound, at a single 25mg/kg dose or a series of 12.5mg/kg doses, is very irritating and severly damages any tissue with which it comes into contact. This observation probably accounts for the severe murine toxicity of the present series of compounds as well as related derivatives.

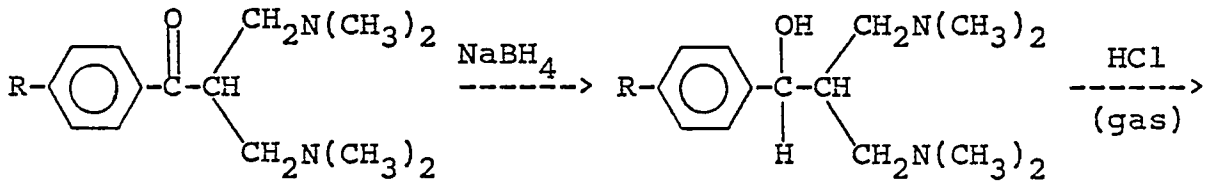
The following conclusions may be drawn from this limited study.

1) The validity of the hypothesis that an optimal rate of breakdown is essential for anticancer activity cannot be substantiated to any great extent. This is mainly because of the small number of compounds that could actually be prepared. In the present series, if the 2,4,5-trimethoxy compound is eventually deemed to be inactive, it would lend support to the above hypothesis in that the ortho-substituted compounds have a shorter half-life ($t_{1/2}$) than the prototype 4-methoxy compound, vide supra, and hence are all inactive. If, on the other hand, the activity of (IIc) were to be confirmed then it would militate against the hypothesis.

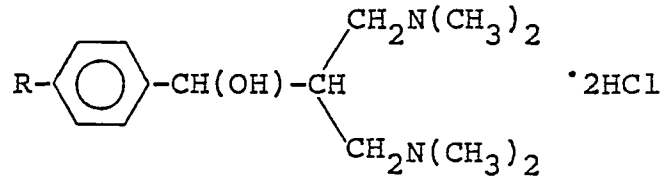
2) Ortho-substitution appears to be detrimental to the stability of the bis-Mannich bases. This is evident in the small number of compounds that could be prepared satisfactorily and in their shorter half-lives (Table XIX, section 3.18.1.2).

4.3.0.0 1-Aryl-3-dimethylamino-2-dimethylaminomethyl -1-propanol dihydrochlorides (III)

The synthesis of the title compounds was accomplished by a literature procedure (Albrecht et al., 1962). This involved sodium borohydride reduction of the free bases of the corresponding aminoketones followed by the conversion into the dihydrochloride salts. The reduction was facile and the yields were good.



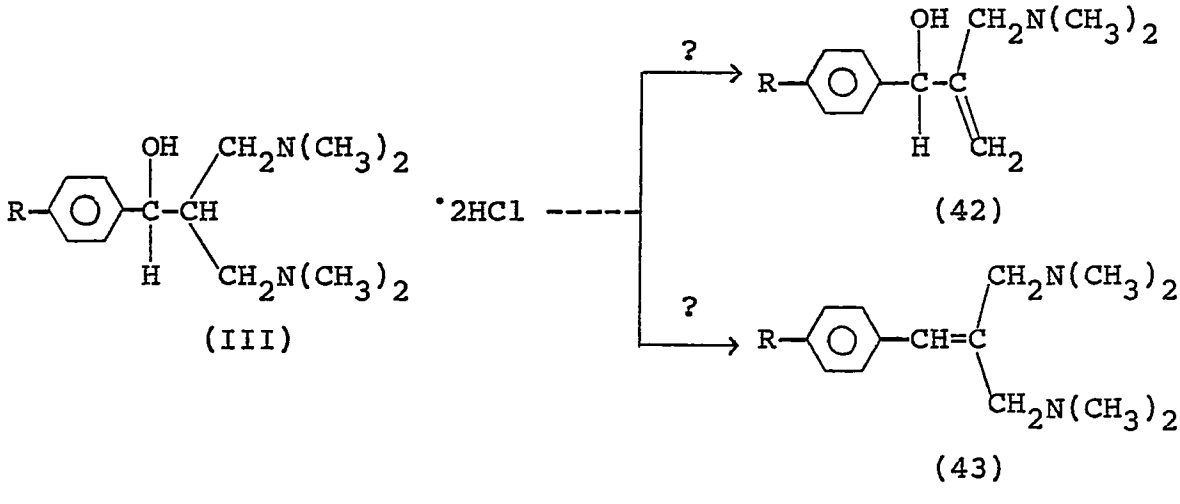
R= H, OCH_3



(III)

The alcohols (III) would be expected to breakdown under physiological conditions either by a deamination process or by dehydration to form (42) and (43) respectively. However, a comparison of the properties of the leaving groups in the

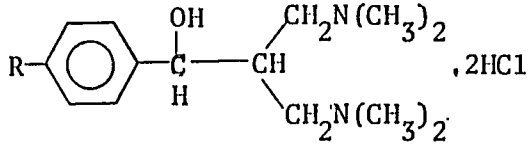
two mechanisms would suggest that the dehydration process, in which the conjugated olefin (43) would be formed, would be favoured.



The antineoplastic evaluation of the alcohols is presented in table (XXII). It can be seen from the data that 1) both of the alcohols are bereft of antileukemic activity and 2) in comparison with the bis-Mannich bases (table XXI), they appear relatively non-toxic since there were no mortalities even at a dose of 240 mg/kg.

The absence of antileukemic activity and murine toxicity may be due to the following reasons 1) Chemically, the alcohols may be too stable, in vivo, to generate any alkylating species. To test this possibility, the stability of the alcohol (IIIa) was examined in phosphate buffer, under simulated physiological conditions of pH 7.46 and 37°C, by NMR spectroscopy. The compound was found to be stable for at

Table XXII. Antineoplastic activity of 1-aryl-3-dimethylamino-2-dimethylaminomethyl-1-propanol dihydrochlorides (III) against P388 lymphocytic leukemia in mice



(III)

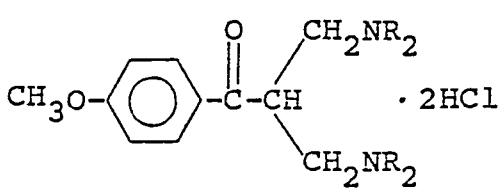
Compound	R	Maximum T/C%[a] (dose in mg/Kg)	Toxicity in mice[b] (dose in mg/Kg)
IIIa	H	109(240)	6/6(240)
IIIb	OCH_3	101(240), 102(60)	6/6(240)

a,b Same as footnotes a and b, Table XXI.

least 48 hours. 2) Even if the alcohols were to breakdown, *in vivo*, into (42) or (43), it is possible that these breakdown products may not possess the desired alkylating ability and/or antineoplastic properties. 3) The alcohols may be rapidly eliminated from the body by facile metabolism such as O-glucuronidation.

From the point of view of SAR it would thus appear that the carbonyl group is essential for antileukemic activity (and probably affects murine toxicity). One may compare, for example, the antileukemic activity of the 4-methoxy *bis*-Mannich base (T/C%= 128, table IV) with that of the corresponding alcohol (T/C%= 102).

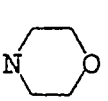
4.4.0.0 3-Amino-2-aminomethyl-1-(4-methoxyphenyl) -1-propanone dihydrochlorides (IV)



(IV)

a) NR_2= $N(C_2H_5)_2$

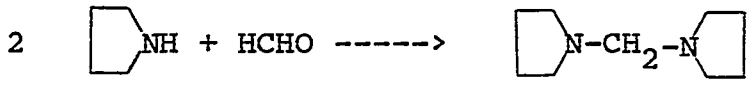
b) NR_2= 

c) NR_2= 

d) NR_2= 

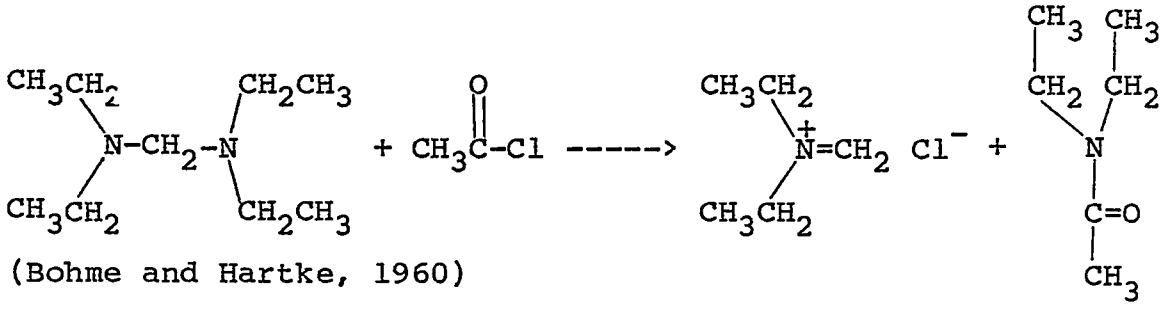
Initial attempts at the preparation of the above series of compounds were made by the traditional approach by heating under reflux a mixture containing 4-methoxyacetophenone, formaldehyde, the appropriate amine and ethanol. Under these conditions, the reaction was usually incomplete and considerable difficulty was experienced in the work-up of the reaction mixture.

It was then decided to use the preformed immonium salts where possible. The diaminomethanes necessary for the preparation of these salts were either obtained commercially (N,N,N',N'-tetraethyldiaminomethane, dimorpholinomethane and dipiperidinomethane) or were prepared as per the literature methodology [dipyrrolidinomethane (44); Korb and Fernandez, 1971].



(44)

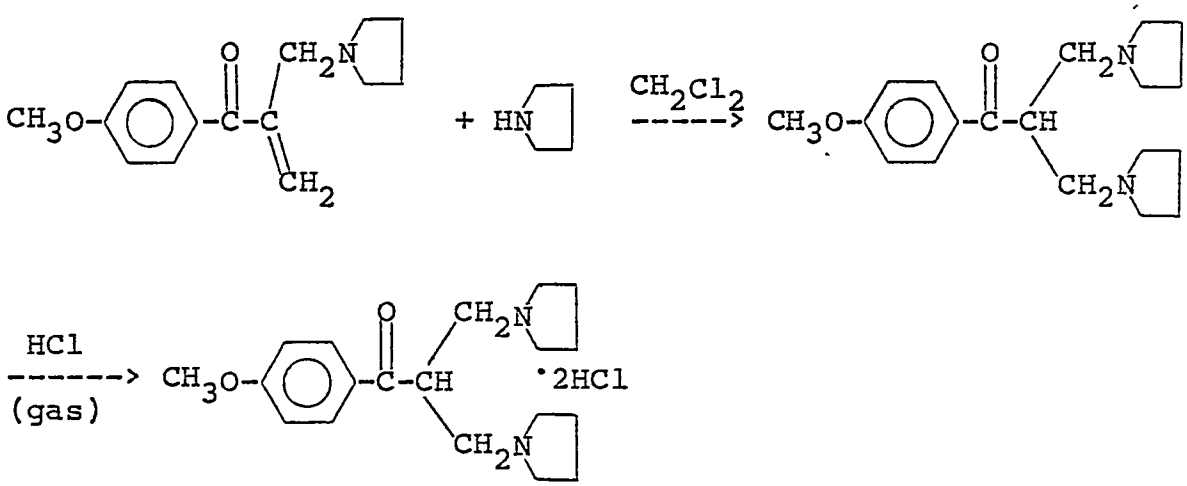
These diaminomethanes were then converted into the corresponding immonium salts in analogy to literature procedures by reacting them with acetyl chloride as exemplified below.



(Bohme and Hartke, 1960)

However, attempted reaction of these immonium salts with 4-methoxyacetophenone, in different molar proportions, proved unsatisfactory. In some cases, only the monobasic compound was formed while in others, a mixture of the monobasic and dibasic compounds resulted which was difficult to work-up.

Finally, the synthesis of some of the bis-Mannich bases was accomplished by the Michael addition of the appropriate amine (e.g. pyrrolidine) to the corresponding acrylophenone derivative, in analogy to a literature methodology (Gupta et al., 1981).

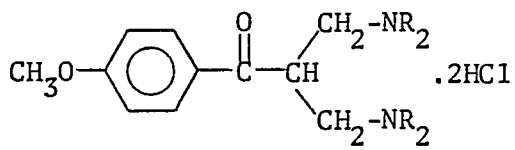


Using this procedure, the pyrrolidino (IVd) and the morpholino (IVc) compounds could be prepared satisfactorily in an average yield of about 50%. Although the piperidino compound (IVb) was prepared and characterized by NMR spectroscopy, it failed to give satisfactory combustion analyses. Also, it was found to undergo decomposition upon storage and

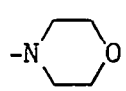
possibly during recrystallization. Therefore, this compound was not submitted for anticancer evaluation. Despite numerous attempts using all of the methodologies described above, the diethylamino compound (IVa) could not be obtained satisfactorily because of difficulties in the work-up.

The activity of the two Mannich bases against P388 lymphocytic leukemia, is shown in table (XXIII). As seen from the data the pyrrolidino bis-Mannich base (IVd) meets the criterion for activity with a T/C% of 128. However, this acitivity is presumptive at the moment and has not been confirmed by the second screener and is therefore, awaiting further evaluation. It was predicted on the basis of leaving group abilities in section 2.4.0.0, that this compound would deaminate slowly relative to (28) and therefore, had the potential of being biologically active. Examination of the kinetic data in table (XIX) indicates that the compound does indeed deaminate slowly relative to (28); perhaps too slowly. However, it is possible that the compound (IVd) may have a higher partition coefficient than (28) and this factor might alter its cellular uptake and/or aid in the expression of antineoplastic activity. The importance of partition coefficient in the determination of various biological activities, is well established (Kupchan et al., 1971).

Table XXIII. Antineoplastic evaluation of 3-amino-2-aminomethyl-1-(4-methoxyphenyl)-1-propanone dihydrochlorides (IVc,d) against P388 lymphocytic leukemia in mice

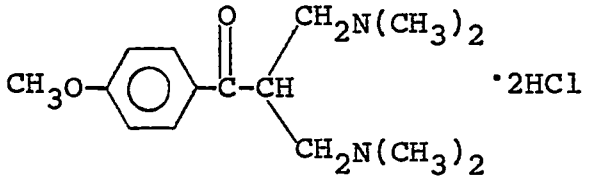


(IV)

Compound	NR_2	Maximum T/C%[a] (dose in mg/kg)	Toxicity in mice[b] (dose in mg/kg)
IVc		111(120)	0/6(240), 5/6(120), 6/6(60)
IVd		128[c](30),121[c](15)	0/6(120), 3/6(60)

a,b Same as footnotes a and b, table XXI.

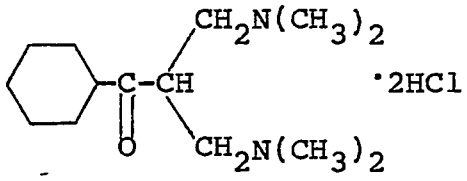
c Presumptive activity not confirmed by second screener, awaiting further evaluation.



(28)

The inactivity of the morpholino compound (IVc) is probably due to its rapid deamination, in vivo [pKa of dimethylamine and morpholine are 10.73 and 8.50, respectively (Albert and Serjeant, 1984)].

4.5.0.0 1-Cyclohexyl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrochloride (V)



(V)

The synthesis of this compound was accomplished very conveniently by the reaction of cyclohexyl methyl ketone with the Mannich reagent in a yield of 29%. Unfortunately, this compound was inactive in the P388 screen.

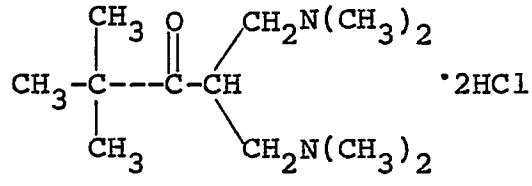
Activity against P388 lymphocytic leukemia in mice:

Maximum T/C%* (dose in mg/kg) : 117(7.5)

Murine toxicity* (dose in mg/kg): 0/6(30), 6/6(15)

[* For an explanation of the terminology, please see footnotes a and b, table XXI]

4.6.0.0 1-Dimethylamino-2-dimethylaminomethyl-4,4-dimethyl-3-pentanone dihydrochloride (VI)



(VI)

This compound was also prepared in a very facile manner by the reaction of pinacolone with dimethyl(methylene)-ammonium chloride in 57% yield. However, it failed to meet the criterion for antileukemic activity.

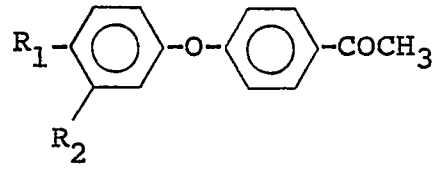
Activity against P388 lymphocytic leukemia in mice:

Maximum T/C%* (dose in mg/kg): 110(120)

Murine toxicity* (dose in mg/kg): 0/6(240), 6/6(120)

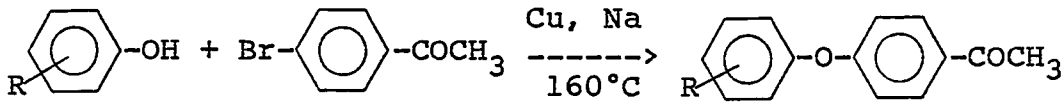
[* For an explanation of the terminology, please see footnotes a and b, table XXI]

4.7.0.0 1-(4-Aryloxyphenyl)ethanones (XXIII)

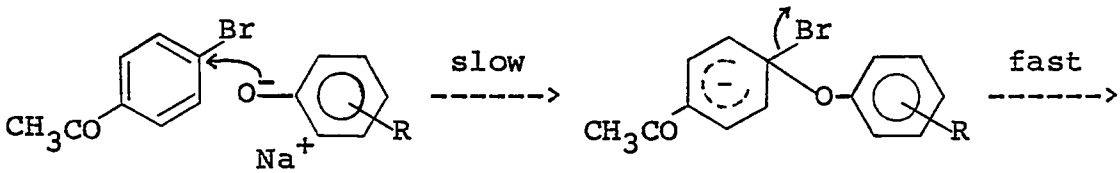


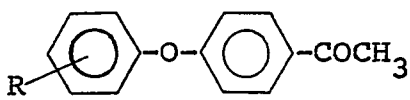
(XXIII)

4-Aryloxyacetophenones have been previously prepared by the Ullmann ether synthesis by reacting phenols with 4-bromo-acetophenone using copper catalysts (Litvinenko et al., 1983; Julia and Baillarge, 1953) as exemplified below.



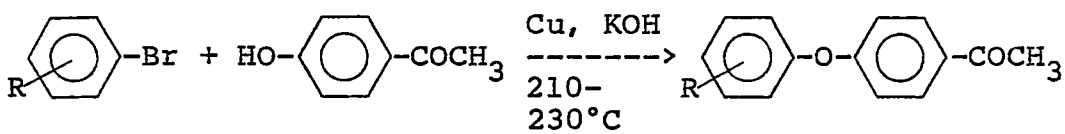
The reaction probably proceeds by the SNAr mechanism (March, 1977c).



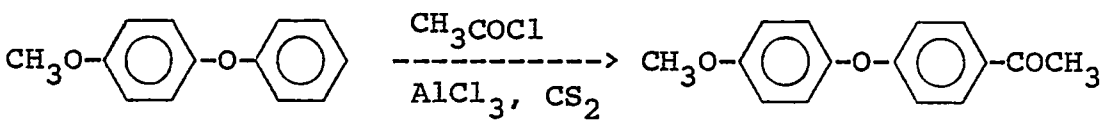


Copper probably functions by forming aryloxycopper(I) intermediates which then react in a facile manner with the arylhalide (March, 1977c).

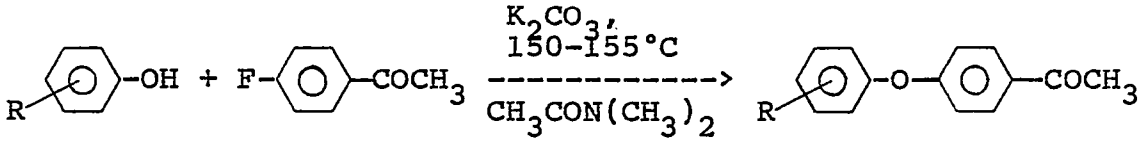
In addition, the reaction was carried out by treating 4-hydroxyacetophenone with an aryl bromide (Kimoto et al., 1953).



Aryloxyacetophenones have also been prepared by Friedel-Crafts acetylation of diphenyl ethers (Petit and Buu-hoi, 1961; Kimoto et al., 1953; Adams and Noller, 1941) as illustrated below (Petit and Buu-hoi, 1961).

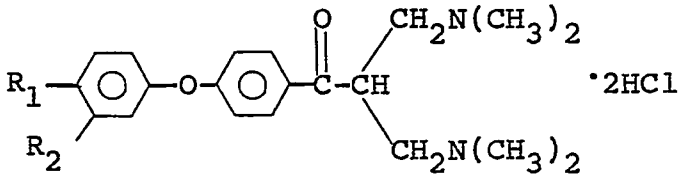


Trust et al. (1979) reported a minor variation of the Ullmann ether synthesis in which aryloxyacetophenones were prepared by the condensation of 4-fluoroacetophenone with phenols under the following conditions.



This method was claimed to offer marked improvements over the traditional method in respect of both yield and ease of preparation. This method was therefore employed for the synthesis of the required aryloxyacetophenones which were obtained in excellent yields (>80%).

4.7.1.0 1-(4-Aryloxyphenyl)-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrochlorides (VII)



(VII)

This series of compounds was prepared very conveniently by reacting the appropriate aryloxyacetophenone with four moles of the Mannich reagent. The yields ranged from 30 to 60%. The aryl substitution pattern in this series was chosen so as to permit a Topliss analysis of the data (Topliss, 1977).

According to this approach, an initial small group of compounds consisting of the unsubstituted derivative along with the analogs possessing 4-methoxy, 4-methyl, 4-chloro and 3,4-dichloro substituents is selected, tested and arranged according to potency. The potency order in the group is then compared to the tabulated potency order calculated for various parameter dependencies relating to hydrophobic, electronic and steric effects (table XXIV). From this activity pattern analysis, the probable operative parameters can be deduced and a new substituent selection made for the synthesis of potentially more potent analogs (table XXV).

The anticancer activity of the Mannich bases is shown in table (XXVI). The data indicates that compound (VIIe) has confirmed antileukemic activity (T/C%= 132) while compound (VIIa) has a marginal activity with a T/C% of 123. Mannich bases (VIIb & d) have a T/C% of about 117 followed by compound (VIIc) with a T/C% of 109. Thus, a potency order of $4\text{-}OCH_3 > 4\text{-}H > 4\text{-}CH_3 \geq 4\text{-}Cl > 3,4\text{-}Cl_2$ is thus indicated. Comparing this potency order with those listed in table (XXIV), $-\sigma$ is obtained as a probable operative parameter. Examination of table (XXV), indicates that new substituents such as $4\text{-}N(C_2H_5)_2$, $4\text{-}OCH(CH_3)_2$, $4\text{-}NHC_4H_9$ etc. should be selected to obtain potentially more potent analogs. It is interesting to note that a similar operative parameter (viz. $\pi\text{-}3\sigma$ or $-\sigma$) was also found for the parent acetophenone bis-Mannich bases (section 2.2.0.0).

It is not clear at the present time why only the 4-methoxy compound (VIIe) is uniquely active. Examination of the kinetic data in table [(XIX), section 3.18.1.2] indicates, for example, that compounds with 4-chloro and 4-methoxy substituent on the phenoxy ring have approximately the same chemical half-life.

The series of aryloxy bis-Mannich bases also appears to be quite toxic with maximum tolerated doses of approximately 5-30 mg/kg.

Table XXIV. Potency order for various parameter dependencies[a]

Substituents	Parameters									
	π	$2\pi-\pi^2$	σ	$-\sigma$	$\pi+\sigma$	$2\pi-\sigma$	$\pi-\sigma$	$\pi-2\sigma$	$\pi-3\sigma$	E_4^b
3,4-Cl_2	1	1-2	1	5	1	1	1-2	3-4	5	2-5
4-Cl	2	1-2	2	4	2	2-3	3	3-4	3-4	2-5
4-CH_3	3	3	4	2	3	2-3	1-2	1	1	2-5
4-OCH_3	4-5	4-5	5	1	5	4	4	2	2	2-5
H	4-5	4-5	3	3	4	5	5	5	3-4	1

[a]Taken from Topliss (1977)

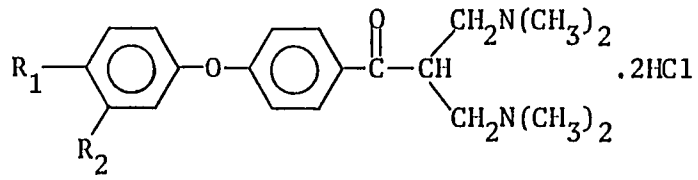
[b]Unfavourable steric effect from 4-substitution

Table XXV. New substituent selection[a]

Probable operative parameters	New substituent selection
π, $\pi+\sigma$, σ	3-CF_3, 4-Cl; 3-CF_3, 4-NO_2; 4-CF_3; 2,4-Cl_2; 4-c-C_5H_9; 4-c-C_6H_{11}
π, $2\pi-\sigma$, $\pi-\sigma$	4-$CH(CH_3)_2$; 4-$C(CH_3)_3$; 3,4-$(CH_3)_2$; 4-$O(CH_2)_3CH_3$; 4-OCH_2Ph; 4-$N(C_2H_5)_2$
$\pi-2\sigma$, $\pi-3\sigma$, $-\sigma$	4-$N(C_2H_5)_2$; 4-$N(CH_3)_2$; 4-NH_2; 4-NHC_4H_9; 4-OH; 4-$OCH(CH_3)_2$; 3-CH_3, 4-OCH_3
$2\pi-\pi^2$	4-Br; 3-CF_3; 3,4-$(CH_3)_2$; 4-C_2H_5; 4-$O(CH_2)_2CH_3$; 3-CH_3, 4-Cl; 3-Cl; 3-CH_3; 3-OCH_3; 3-$N(CH_3)_2$; 3-CF_3; 3,5-Cl
<u>Ortho</u> effect	2-Cl; 2-CH_3; 2-OCH_3; 2-F
Other	4-F; 4-$NHCOCH_3$; 4-$NHSO_2CH_3$; 4-NO_2; 4-$COCH_3$; 4-SO_2CH_3; 4-$CONH_2$; 4-SO_2NH_2

[a]Taken from Topliss (1977)

Table XXVI. Antineoplastic evaluation of 1-(4-aryloxyphenyl)-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrochlorides (VII) against P388 lymphocytic leukemia in mice

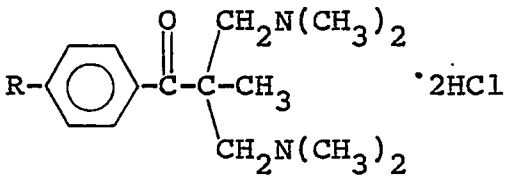


(VII)

Compound	R_1	R_2	Maximum T/C%[a] (dose in mg/kg)	Toxicity in mice[b] (dose in mg/kg)
VIIa	H	H	123(7.5)	0/6(60), 4/6(30), 6/6(15)
VIIb	Cl	H	116(3.75)	0/6(30), 5/6(15), 6/6(7.5)
VIIc	Cl	Cl	109(3.75)	0/6(30), 4/6(7.5), 6/6(3.75)
VIId	CH_3	H	117(7.5)	0/6(60), 6/6(30)
VIIe	OCH_3	H	132(15), 129(7.5) 121(3.75)	0/6(60), 6/6(30)

a,b Same as footnotes a and b, table XXI.

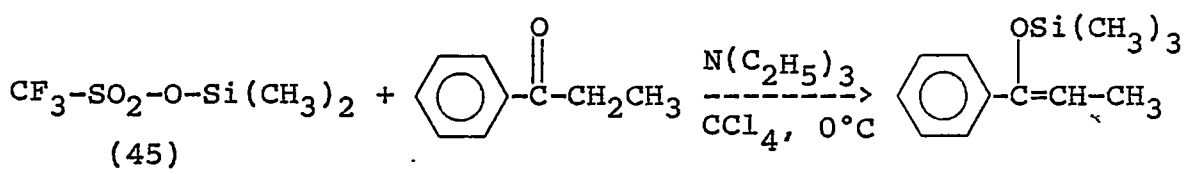
4.8.0.0 Attempted synthesis of 1-aryl-3-dimethylamino -2-dimethylaminomethyl-2-methyl-1-propanone dihydrochlorides (VIII)



(VIII)
R= H, CH_3

The preparation of these two compounds was first attempted by reacting the appropriate propiophenone with the Mannich reagent in different molar ratios. Only the monobasic compound could be formed under these conditions.

Enol silyl ethers have been reacted with preformed iminium salts to obtain the desired products (Holy et al., 1979; Miyano et al., 1982). The reactions were carried out at room temperature and reaction times were short. It was therefore decided to adopt this method. The trimethylsilyl enol ether was prepared by reacting propiophenone with trimethylsilyl trifluoromethanesulfonate (45) because of the known exceptionally high reactivity of the triflate (Emde et al., 1982).

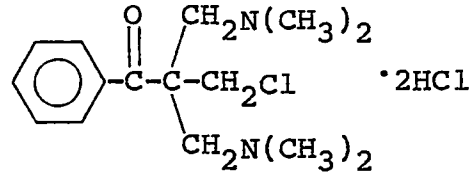


(45)

When the enol silyl ether was reacted with an equimolar quantity of dimethyl(methylene)ammonium chloride in a NMR tube, a brisk, exothermic reaction occurred, accompanied by

disappearance of the Mannich reagent. NMR spectroscopy indicated the complete formation of the trimethyl silyl derivative of the monobasic compound in less than 30 minutes. However, no further reaction could be detected upon addition of the second mol of the Mannich reagent and incubation of the NMR tube at 40°C for several hours. The inability to form the bis-Mannich base is probably due to the steric strain that would ensue upon the introduction of the second dimethylaminomethyl group.

4.9.0.0 Attempted synthesis of 2-chloromethyl -3-dimethylamino-2-dimethylaminomethyl-1-phenyl -1-propanone dihydrochloride (IX)



(IX)

When the synthesis of this compound was attempted by reacting β-chloropropiophenone and the Mannich reagent in a ratio 1:4, the acrylophenone (XIIa) was obtained which could arise by the following pathway.

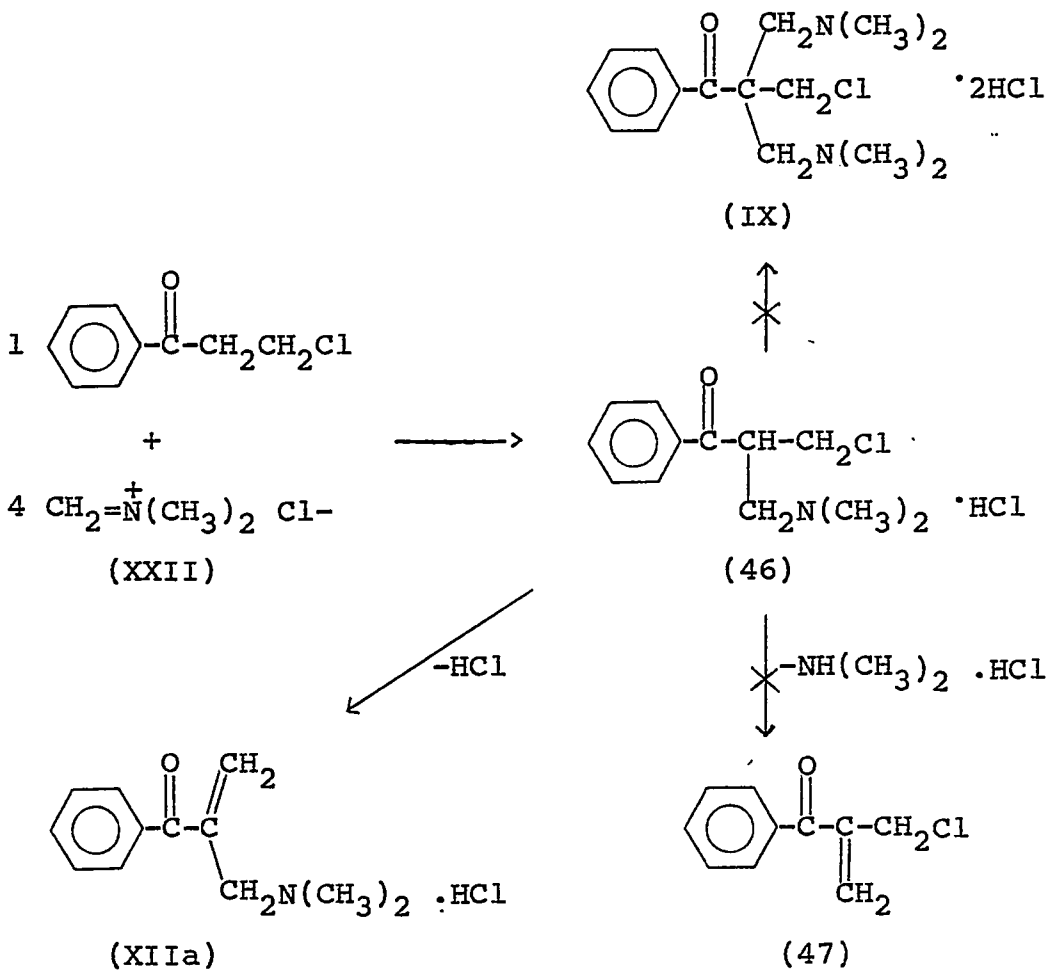
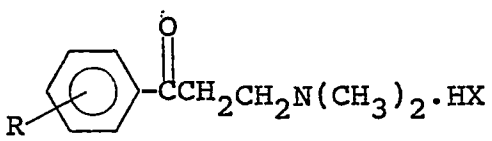


Figure 11. Possible pathway for the formation of 2-dimethylaminomethyl-1-phenyl-2-propen-1-one hydrochloride during the Mannich reaction of β-chloropropiophenone.

The mono-Mannich base (46), once formed, had at least three options. 1) It could react with another mol of the Mannich reagent to form the desired product (IX), which was not formed. Steric factors are probably responsible for this. 2) It could eliminate either hydrogen chloride or dimethylamine hydrochloride forming the acrylophenone (XIIa)

or (47) respectively. Since (XIIa) was obtained exclusively, it would mean that there is preferential elimination of hydrogen chloride. This is contrary to what one might have expected on the basis of leaving group abilities because dimethylamine hydrochloride, with a positively charged nitrogen atom, should have been eliminated preferentially to give the acrylophenone (47).

4.10.0.0 <u>1-Aryl-3-dimethylamino-1-propanone hydrohalides (Xa-g)</u>

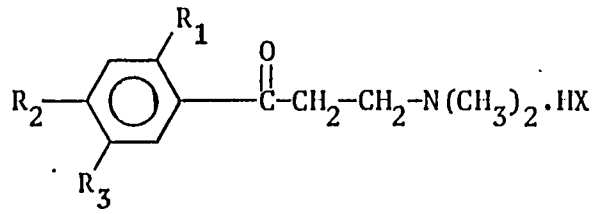


(X)

Compounds (Xa-f) were prepared conveniently by the traditional method by refluxing a mixture of the appropriate acetophenone, dimethylamine hydrochloride, paraformaldehyde, hydrochloric acid and ethanol. The compounds were isolated by basification of the reaction mixture followed by extraction of the free base into ether. The Mannich salts were then precipitated by treatment with the appropriate hydrohalide gas. Compound (Xg) was prepared by reacting 3,4-methylenedioxyacetophenone with the Mannich reagent. The yields of the compounds varied from 27 to 58%. In the case of compounds (Xd-f) it was observed that they could be handled more conveniently in the form of their hydrobromide salts.

The antileukemic activity of compounds (Xb-g) is summarized in table (XXVII). The data indicates that the compounds were uniformly inactive. Of some relevance is the observation that they had lower murine toxicity than the corresponding bis-Mannich bases listed in Table (XXI). In the case of (Xf, Table XXVII), for example, there were no mortalities even at a dose of 240 mg/kg. This observation continues the trend noted earlier in this laboratory viz. that of the monobasic compounds being inactive and less toxic than the corresponding bis-Mannich bases. The inactivity of such compounds has been attributed (Dimmock et al., 1983a) to their greater stability in phosphate buffer (pH 7.4) at 37°C and consequently to the lack of formation of the corresponding alkylating acrylophenone.

Table XXVII. Antineoplastic evaluation of 1-aryl-3-dimethylamino-1-propanone hydrohalides (X) against P388 lymphocytic leukemia in mice

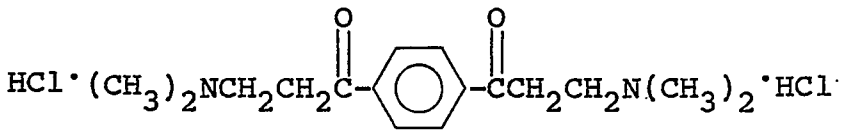


(X)

Compound	R_1	R_2	R_3	X	Maximum T/C%[a] (dose in mg/Kg)	Murine toxicity[b] (dose in mg/Kg)
Xa	CH_3	H	H	Cl	Not available	Not available
Xb	CH_3	CH_3	H	Cl	106(15)	0/6(120), 6/6(60)
Xc	CH_3	H	CH_3	Cl	99(60)	0/6(120), 6/6(60)
Xd	OCH_3	H	H	Br	112(30)	0/6(240), 1/6(120), 6/6(60)
Xe	OCH_3	OCH_3	H	Br	108(60)	0/6(240), 4/6(120), 6/6(60)
Xf	OCH_3	OCH_3	OCH_3	Br	116(120)	6/6(240)
Xg	H	OCH_2O		Cl	100(60)	0/6(240), 6/6(120)

a,b Same as footnotes a and b, table XXI.

4.10.1.0 1,4-bis(3-Dimethylaminopropionyl)benzene dihydrochloride (XI)



(XI)

This compound was prepared by the reaction of 4-diacetylbenzene with the Mannich reagent in 28% yield. However, it failed to meet the criterion for activity.

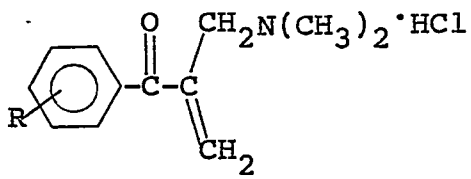
Activity against P388 lymphocytic leukemia in mice:

Maximum T/C%* (dose in mg/kg) : 108(25)

Murine toxicity* (dose in mg/kg): 2/6(200), 6/6(100)

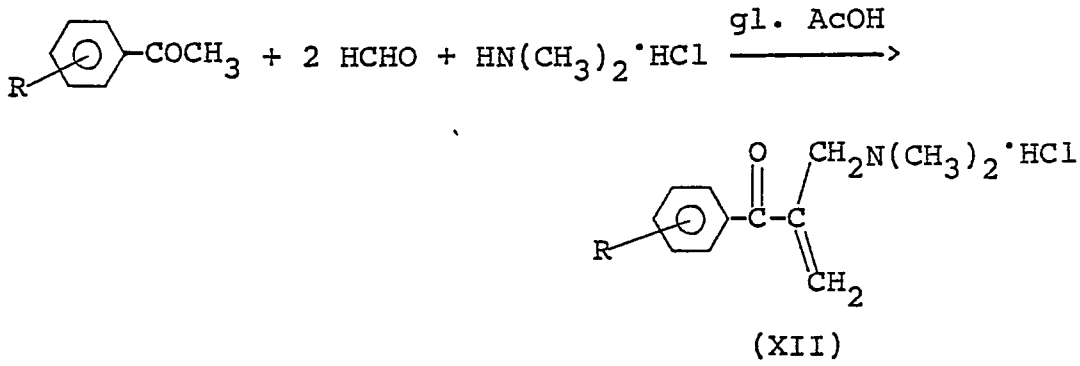
[* For an explanation of the terminology, please see footnotes a and b, table XXI]

4.11.0.0 1-Aryl-2-dimethylaminomethyl-2-propen-1-one hydrochlorides (XIIa-k)



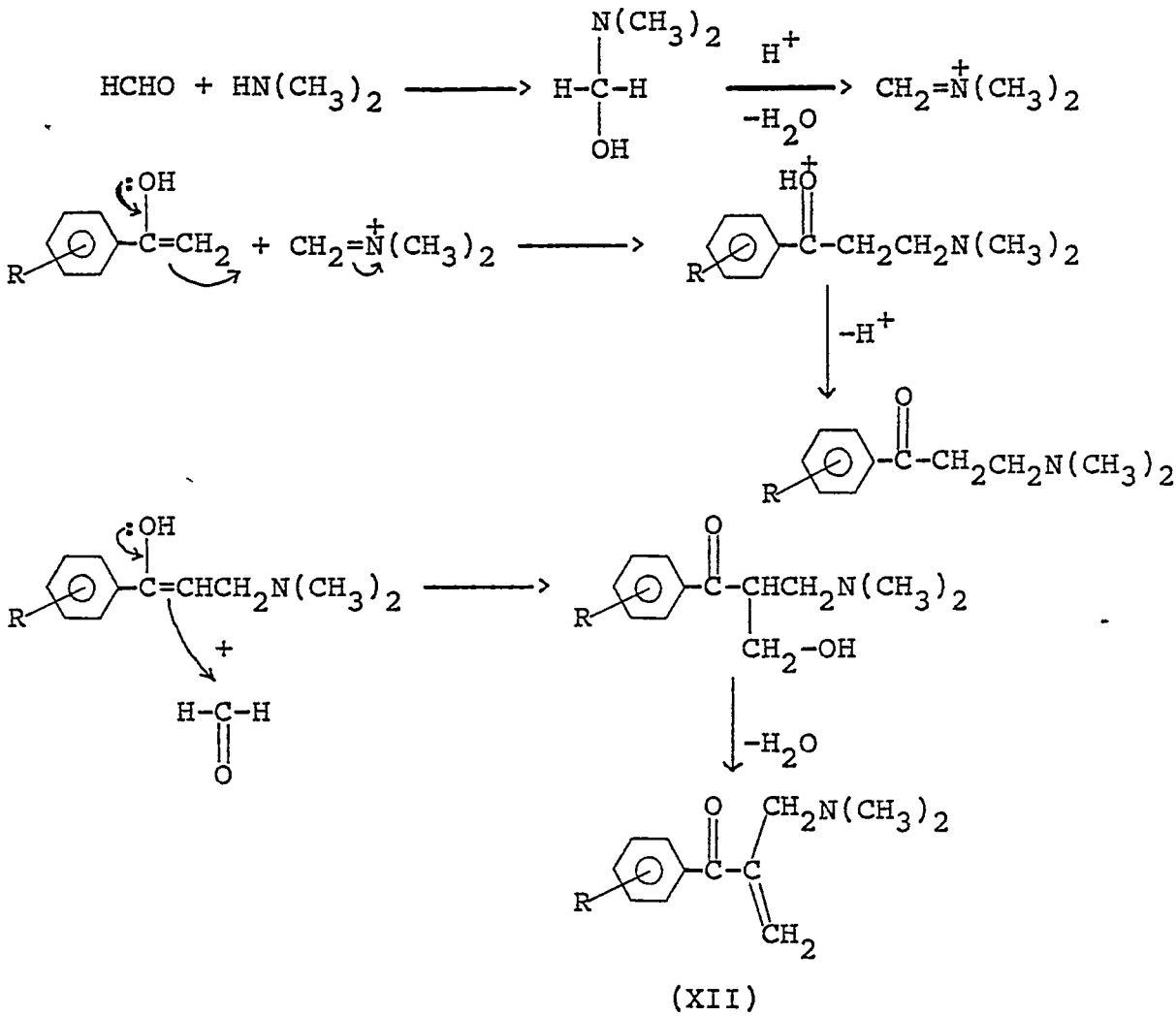
(XII)

The title compounds were prepared in analogy to a literature methodology (Gupta et al., 1981) by refluxing a mixture containing the appropriate acetophenone, dimethylamine hydrochloride, paraformaldehyde and glacial acetic acid in 18-69% yields.



(XII)

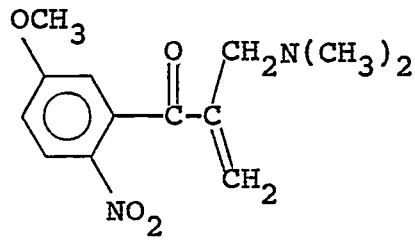
The following mechanism is probably involved.



(XII)

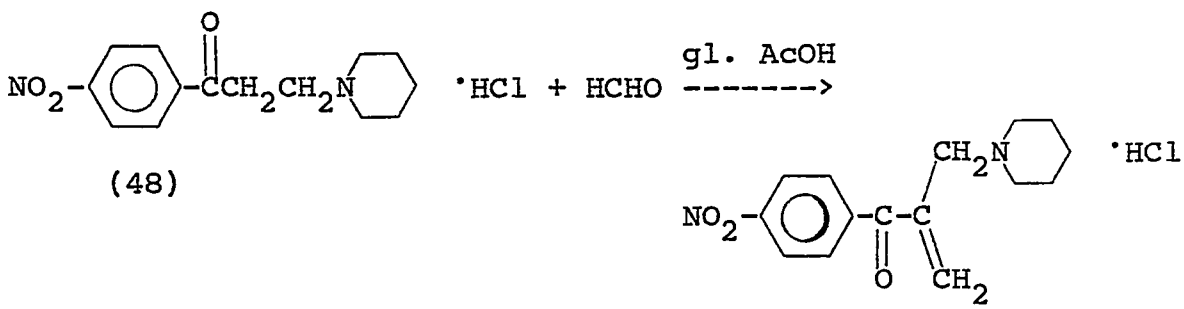
Figure 12. Proposed mechanism for the formation of 1-aryl-2-dimethylaminomethyl-2-propen-1-one hydrochlorides (XII).

The formation and dehydration of a hydroxymethyl intermediate has been proposed to explain the unexpected formation of the acrylophenone (38) in the synthesis of its corresponding monobasic Mannich salt (Gevorgyan et al., 1984, section 4.2.0.0).



(38)

Further evidence for the above mechanism is provided by the fact that Gupta et al. (1981) reacted β-piperidino-4-nitropropiophenone hydrochloride (48) with paraformaldehyde to obtain the corresponding acrylophenone (49).



(48)

(49)

In the preparation of the present series of compounds the above method was also tried out with a representative Mannich base, 3-dimethylamino-1-(2,4-dimethylphenyl)-1-propanone hydrochloride. However, the overall yield obtained by this method was slightly lower (30%) than by the other

"one-pot" reaction (37%).

The anticancer evaluation of these compounds is presented in table (XXVIII). The following conclusions can be drawn from this data.

1) Compounds (XIIa & i) possess marginal antileukemic activity with T/C% of 122 and 123, respectively.

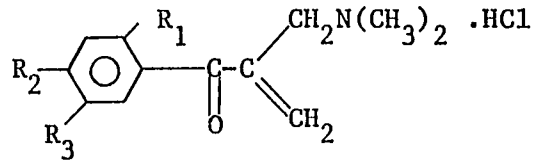
2) All of the remaining compounds are uniformly inactive including compounds (XIIf & h) the corresponding bis-Mannich bases of which had antileukemic activity [T/C%= 128 (table IV) and 139 (table XXI), respectively].

3) All of the compounds possess very high murine toxicity with drug-induced mortalities occurring at less than 15 mg/kg.

4) This data also suggest that the formation of the acrylophenones per se from the corresponding bis-Mannich bases may not be sufficient for the expression of anticancer activity and that other factors such as rate of release of the α,β-unsaturated ketones in vivo are probably important.

A representative compound from this series viz. (XIIf), was examined for its inhibitory activity, in vitro, against the P388 cell line (personal communication from Dr. R.C. Warrington, Dept. of Biochemistry). It was found to be extremely cytotoxic and a number of times more potent than N,N-bis(2-chloroethyl)-N-nitrosourea(BCNU). Laser Flow Cytometry indicated the compound to arrest the cell cycle

Table XXVIII. Antineoplastic evaluation of 1-aryl-2-dimethylaminomethyl-2-propen-1-one hydrochlorides(XII) against P388 lymphocytic leukemia in mice



(XII)

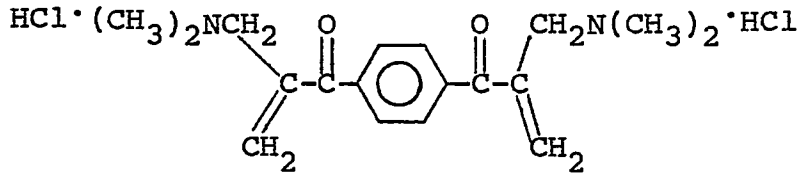
Compound	R_1	R_2	R_3	Maximum T/C%[a] (dose in mg/kg)	Murine toxicity[b] (dose in mg/kg)
XIIa	H	H	H	122(0.94)	0/6(15), 4/6(7.5), 6/6(3.75)
XIIb	CH_3	H	H	108(6.25)	1/6(12.5), 6/6(6.25)
XIIc	CH_3	CH_3	H	112(0.93)	0/6(7.5), 6/6(3.75)
XIId	CH_3	H	CH_3	Not available	Not available
XIIe	OCH_3	H	H	113(1.8)	0/6(15), 6/6(7.5)
XIIf	H	OCH_3	H	113(1.87)	1/6(7.5), 6/6(3.75)
XIIg	OCH_3	OCH_3	H	106(3.80)	1/6(12.5), 6/6(7.5)
XIIh	OCH_3	OCH_3	OCH_3	110(1.8)	0/6(15), 6/6(7.5)
XIIi	H	OCH_2O		123(1.8), 120(3.75)	0/6(15), 3/6(7.5), 6/6(3.75)
XIIj	H	OC_6H_5	H	104(0.93)	1/6(7.5), 6/6(3.75)
XIIk	Cl	H	H	109(1.8)	0/6(15), 6/6(7.5)

a,b Same as footnotes a and b, table XXI

transit in a phase non-specific manner. Thus, it would appear that such compounds are active *in vitro* but not *in vivo*. A possible explanation as to their inactivity *in vivo* is that the compounds may react indiscriminately with cellular constituents as a result of their high chemical reactivity and thus become inactivated prior to reaching the target tissues. Alternatively, they may breakdown, *in vivo*, to the corresponding monobasic compound (section 3.17.4.0) which are devoid of anticancer activity. Similar potent activity has been observed for such acrylophenones against the L1210 cell line *in vitro*, but not *in vivo* (personal communication from Dr. M.L. Mallevais, Unite des Proteines de l'INSERM, Place de Verdun, 59045-Lille, Cedex, France).

Recently, compound (XIIa) was examined for its effect, *in vitro*, on the respiration in isolated mice liver mitochondria (Dimmock *et al.*, 1986). It was found to be a potent inhibitor of respiration with an ID_{50} of 1.14 μmol. This observation may explain, at least in part, the high level of murine toxicity associated with these compounds.

4.11.1.0 1,4-bis(2-Dimethylaminomethyl-1-oxo-2-propenyl) benzene dihydrochloride (XIV)



(XIV)

This compound was prepared by the methodology discussed in the preceding section (4.11.0.0) in 28% yield. It was also found to be inactive in the antileukemic screen.

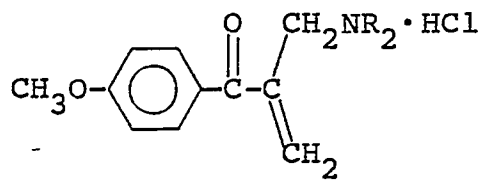
Activity against P388 lymphocytic leukemia in mice:

Maximum T/C%* (dose in mg/kg) : 108(3.12)

Murine toxicity* (dose in mg/kg): 0/6(12.5), 3/6(6.25), 6/6(3.12)

[* For an explanation of the terminology, please see footnotes a and b, table XXI].

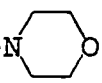
4.12.0.0 2-Aminomethyl-1-(4-methoxyphenyl)-2-propen-1-one hydrochlorides (XV)



(XV)

a) NR_2= $-N(C_2H_5)_2$

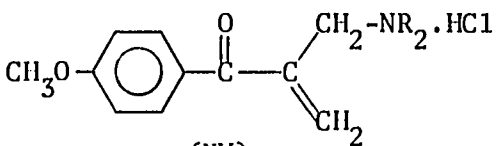
b) NR_2= 

c) NR_2= 

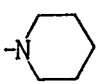
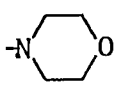
d) NR_2= 

Compounds (XVb-d) were prepared by the methodology indicated in section 4.11.0.0 in yields ranging from 20-32%. Despite numerous attempts, compound (XVa) could not be prepared satisfactorily because of the difficulty in work-up. The antineoplastic activity of (XVd) is summarized in table (XXIX). At the time of writing the thesis, the data for compounds (XVb & c) was not available while compound (XVd) was found to be inactive at the dose levels tested. It is interesting to note that the bis-Mannich base corresponding to compound (XVd) has presumptive antileukemic activity (T/C%= 128, table XXIII, section 4.4.0.0).

Table XXIX. Antineoplastic evaluation of 2-aminomethyl-1-(4-methoxyphenyl)-2-propen-1-one hydrochlorides (XV) against P388 lymphocytic leukemia in mice

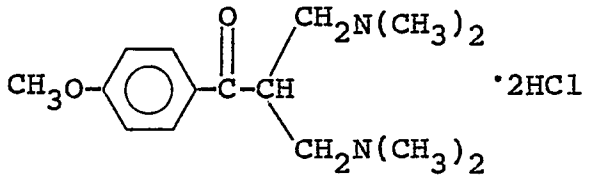


(XV)

Compound	NR_2	Maximum T/C%[a] (dose in mg/Kg)	Murine toxicity[b] (dose in mg/Kg)
XVb		Not available	Not available
XVc		Not available	Not available
XVd		117(1.87)	0/6(30), 3/6(7.5), 5/6(3.75)

a,b Same as footnotes a and b, table XXI.

4.12.1.0 Structure-activity relationships in the acetophenone bis-Mannich bases



(28)

The prototype compound (28) possesses confirmed antileukemic activity in mice (T/C%= 128, T/C% for the dibromide salt= 136, table IV). The limited structure-activity relationships generated thus far may be summarized as follows.

1) Activity is retained when the 4-methoxy group is replaced by a methyl but not by chloro or hydroxyl. Activity is lost when the methoxy group is in the 2 or 3 positions of the aryl ring. When the 4-OCH_3 group was replaced by a series of aryloxy groups, only the 4-(4-methoxy)phenoxy compound was found to be active.

2) Replacement of the aryl ring by a cyclohexyl or a t-butyl group, resulted in a loss of activity.

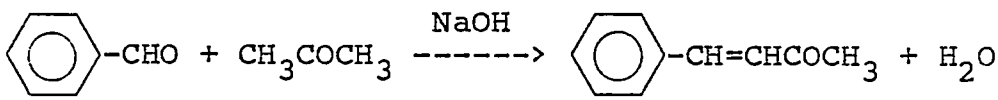
3) Reduction of the carbonyl group to a secondary alcohol also abolishes activity.

4) Activity is retained when the dimethylamino group is replaced by a pyrrolidino group but is lost when replaced by a morpholino group. These effects are probably related to the basicity of the amine function.

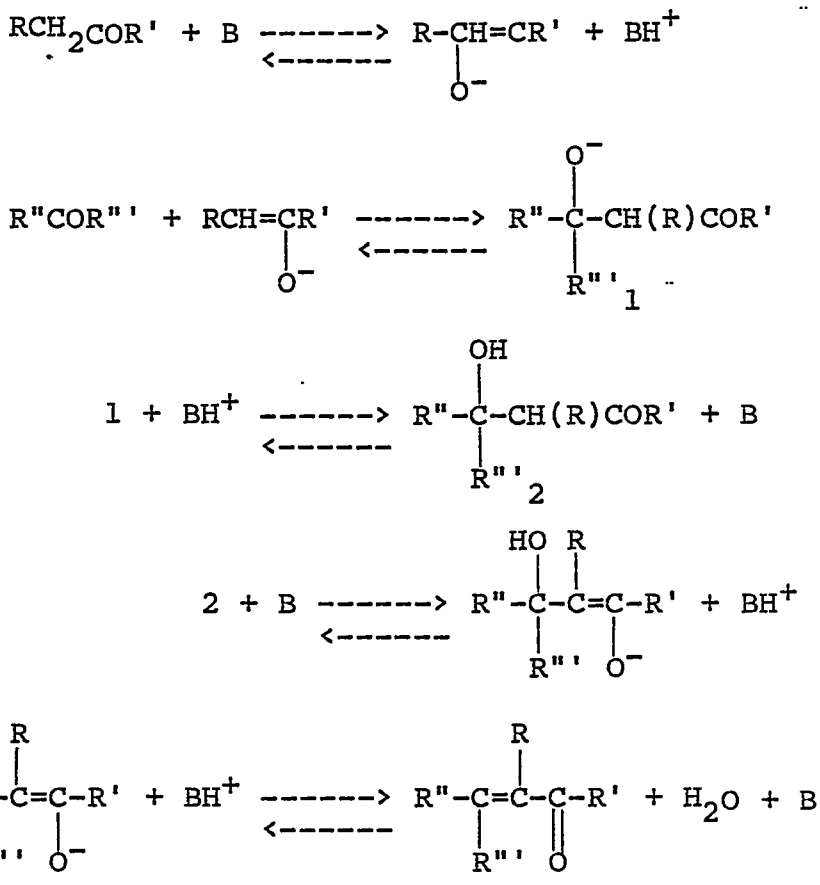
5) All of the acetophenone monobasic compounds and the substituted acrylophenones prepared to this date in this laboratory appear to be virtually devoid of antileukemic activity.

4.13.0.0 Introduction to the Claisen-Schmidt condensation

The aldol condensation has long been recognized to be one of the most versatile synthetic tools in organic chemistry. It includes reactions producing β-hydroxy aldehydes or β-hydroxy ketones by self-condensations or mixed condensations of aldehydes and ketones, as well as reactions leading to α,β-unsaturated aldehydes or ketones, formed by dehydration of the intermediate β-aldols or β-ketols (Nielsen and Houlihan, 1968; House, 1972a). The term aldol condensation has sometimes been applied to many so-called "aldol-type" condensations including the Claisen, Knoevenagel, Doebner, Perkin, Stobbe, and Reformatsky reactions. The condensation of an aromatic aldehyde with an aliphatic aldehyde or ketone in the presence of a relatively strong base (hydroxide or alkoxide ion) to form an α,β-unsaturated aldehyde or ketone is known as the Claisen-Schmidt reaction. However, the term has been extended to include many types of aldehyde-ketone condensations employing either acidic or basic catalysts.

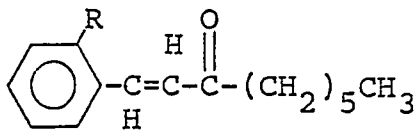


The mechanism of the base-catalyzed condensation involves a series of equilibria, which are shown below.



It is interesting to note that the most highly favoured (and often the most stable) product in the Claisen-Schmidt condensation is the (E)- isomer (trans disposition of the bulkier β-group and the α-carbonyl group).

4.13.1.0 1-Aryl-1-nonen-3-ones (XVI)



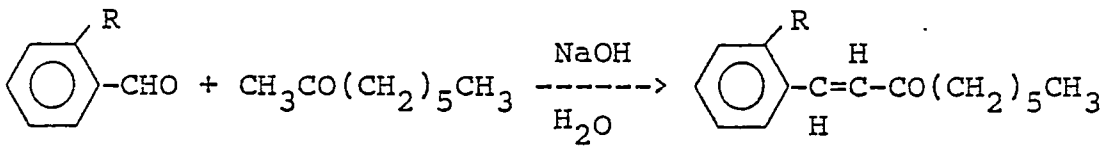
(XVI)

a) R = Cl d) R = H

b) R = Br e) R = CH_3

c) R = F f) R = OCH_3

These compounds were prepared according to a literature methodology (Smith et al., 1972) by heating under reflux a mixture containing 2-octanone, the appropriate aromatic aldehyde, sodium hydroxide and water. Under these conditions, condensation of the aldehyde occurred exclusively at the methyl group of the ketone and the yields ranged from 42 to 65%. The (E)- configuration of the double bond was established by NMR spectroscopy (J = 16 Hz) and by the IR band at 985 cm^{-1}.



(XVI)

When alcohol was used as solvent, only low yields of the desired styryl ketones were obtained. This may have been due to dimer formation (Heilbron and Irving, 1929) or oxidation of the solvent to acetaldehyde leading to aldol polymerization (House, 1972a).

Compounds (XVIa,d & f) have been previously evaluated for antileukemic activity against P388 lymphocytic leukemia in mice, the results of which are summarized below.

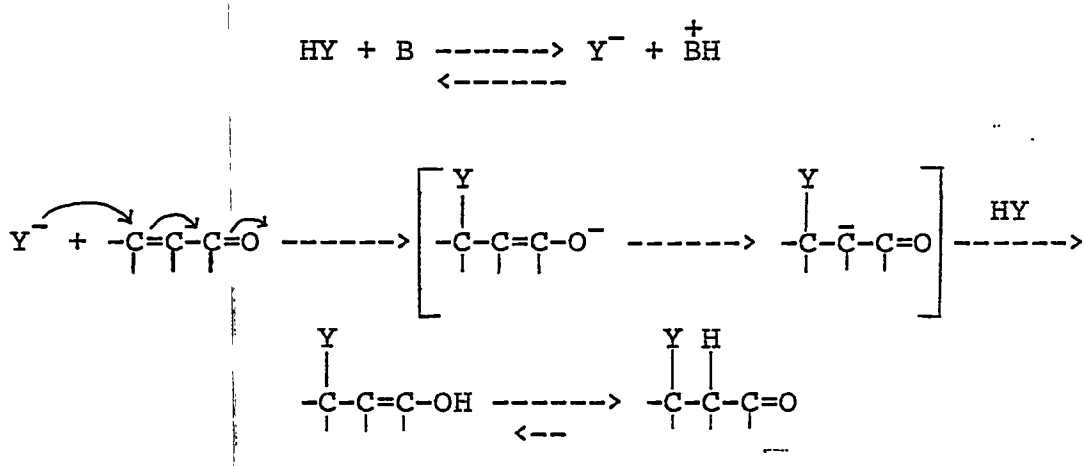
Compound	Maximum T/C% (dose in mg/kg)	Lit. Reference
XVI a	118 (200)	Dimmock et al. (1980a)
XVI d	106 (200)	
XVI f	117 (200)	Dimmock et al. (1982)

The data for the remaining compounds is not available.

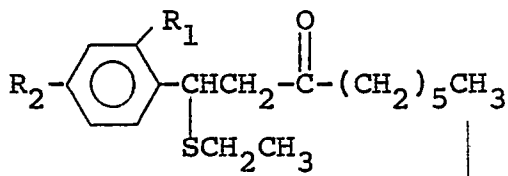
4.14.0.0 Introduction to the Michael Reaction

The nucleophilic addition of enolate (or analogous) anions to the carbon-carbon double bond of α,β-unsaturated ketones, aldehydes, nitriles or carboxylic acid derivatives is known as the Michael reaction (House, 1972b; Bergmann et al., 1959). Nucleophiles other than enolate anions such as alcohols, thiols or amines may also add to the Michael acceptors. Usually weaker bases such as piperidine, pyridine, triethylamine etc. are used to catalyze the condensation.

The mechanism involves a 1,4 nucleophilic addition to the conjugated system of the acceptor (March, 1977d).



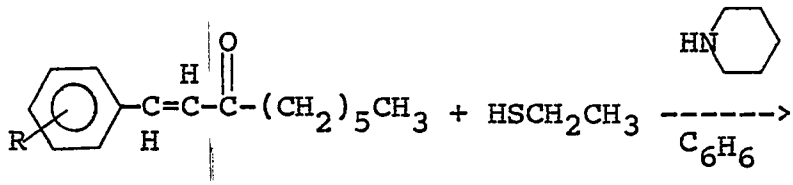
4.14.1.0 1-Aryl-1-ethylthio-3-nonanones (XVII)



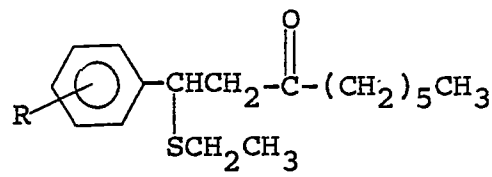
(XVII)

	R_1	R_2		R_1	R_2
a)	Cl	H	e)	CH_3	H
b)	Br	H	f)	OCH_3	H
c)	F	H	g)	H	Cl
d)	H	H	h)	H	CH_3

The compounds (XVIIa-h) were prepared by treating the corresponding unsaturated ketones (XVI) with ethanethiol and piperidine.



(XVI)



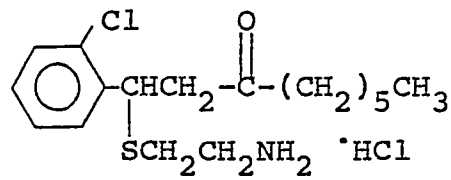
(XVII)

The yields ranged from 61 to 85%. Since compound (XVIIg) decomposed during attempted distillation, compounds (XVIIg&h) were therefore purified by column chromatography on silica gel.

Compounds (XVIIa & d) have been evaluated previously for antileukemic activity (Dimmock et al., 1980a), while the remaining compounds were not screened by the National Cancer Institue, U.S.A. Initial screening of compound (XVIIa) gave a T/C% value of 121 (200mg/kg) but recent data shows that the correct figure is 113 (200 mg/kg). Compounds (XVIId) failed to meet the criterion for activity with a T/C% of 114 (200). There were no mortalities at the highest dose tested in both cases.

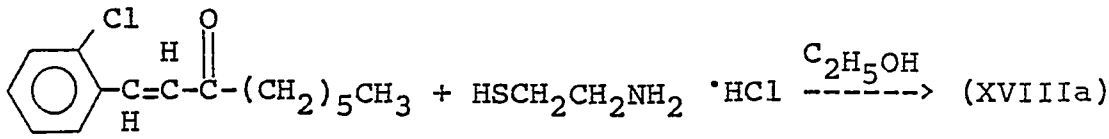
4.15.0.0 1-Alkylthio-1-(2-chlorophenyl)-3-nonanones (XVIII)

4.15.1.0 1-(2-Aminoethylthio)-1-(2-chlorophenyl)-3-nonanone hydrochloride (XVIIIa)



(XVIIIa)

Initial attempts were made to prepare this compound by heating under reflux the unsaturated ketone (XVIa) and cysteamine hydrochloride in the presence of a slight excess of either piperidine or pyridine. Under these conditions either very little reaction occurred or a mixture of products was formed which was not purified. It is possible that under basic conditions the amino group of cysteamine adds to the carbon-carbon double bond and/or condenses at the carbonyl group. This, in turn, could be an intramolecular as well as an intermolecular process. Indeed α,β-unsaturated ketones have been known to form such additions and/or cyclic condensation products with cysteamine (Hankovszky et al., 1974) and also aliphatic diamines and dithiols (Hideg and Lloyd, 1971). It was therefore contemplated that if the reaction was carried out with cysteamine hydrochloride per se (i.e. without any added base) the quaternary amino function would be prevented from participating in the reaction, and when the unsaturated ketone was refluxed with cysteamine hydrochloride in ethanol, the desired product (XVIIIa) was obtained in 72% yield.



(XVIa)

Unfortunately, this compound was inactive as an anti-leukemic agent.

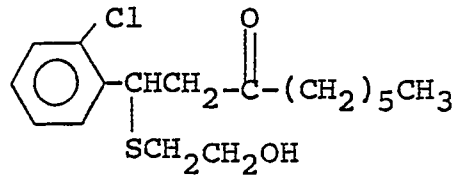
Activity against P388 lymphocytic leukemia in mice:

Maximum T/C%* (dose in mg/kg) : 100(3.75)

Murine toxicity* (dose in mg/kg): 1/6(60), 6/6(30)

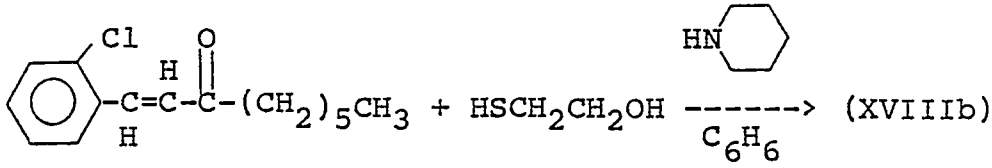
[*For an explanation of the terminology, please see footnotes a and b, Table XXI]

4.15.2.0 1-(2-Chlorophenyl)-1-(2-hydroxyethylthio)-3-nonanone (XVIIIb)



(XVIIIb)

This compound was prepared in 75% yield by the Michael addition of 2-mercaptoethanol to the α,β-unsaturated ketone (XVIa) under catalysis by piperidine.



(XVIa)

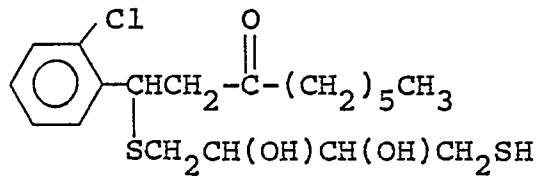
When an attempt was made to purify the compound by vacuum distillation, the distillate was found to consist, by TLC and NMR spectroscopy, mainly of the α,β-unsaturated ketone with

smaller amounts of the desired product. 2-Mercaptoethanol was found condensed in the cooling traps. It would therefore appear that the product undergoes a thermally induced retro-Michael reaction. Michael adducts have been known to undergo a retro-Michael reaction under base catalysis and by heat (Bergmann et al., 1959) and also during gas chromatographic analysis (Onkenhout et al., 1982). The compound (XVIIIb) was therefore purified by column chromatography on silica gel. Activity against P388 lymphocytic leukemia in mice:

Maximum T/C% (dose in mg/kg) : 114(240)

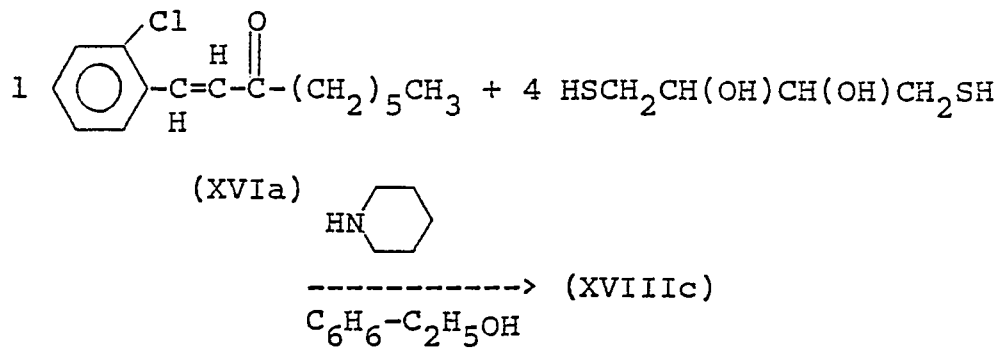
Murine toxicity (dose in mg/kg): 6/6(240).

4.15.3.0 1-(2-Chlorophenyl)-1-[(2,3-dihydroxy-4-mercaptobutyl)thio]-3-nonanone (XVIIIc)



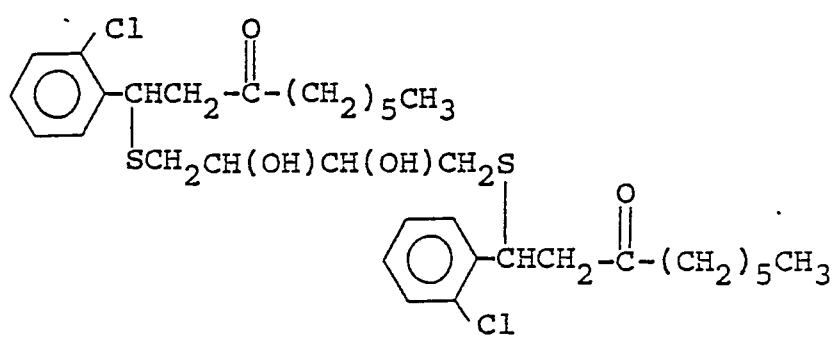
(XVIIIc)

This compound was prepared in 85% yield by the reaction of dithioerythritol with the unsaturated ketone (XVIa) in the presence of piperidine. The ketone and the thiol compound were used in a ratio of 1:4 because use of equimolar quantities always gave a mixture of the mono- and the bis- adducts.



However, this compound was found to be unstable at room temperature and hence was not evaluated for antileukemic activity.

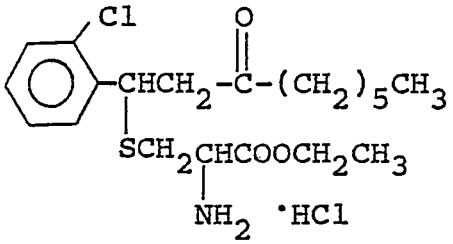
4.15.4.0 1, 4-bis[1-(2-Chlorophenyl)-3-oxo-nonylthio]-2, 3-dihydroxybutane (XVIIId)



(XVIIId)

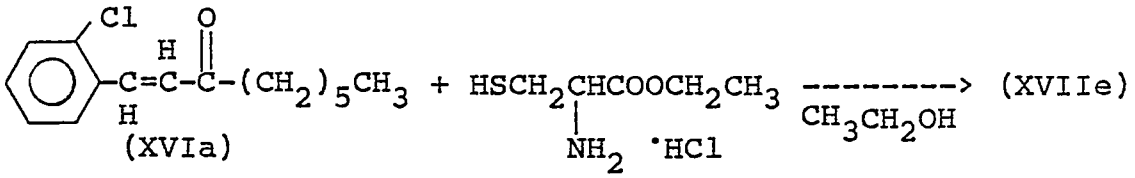
This compound was also prepared in 85% yield by treating dithiothreitol with the unsaturated ketone (XVIa) in a ratio of 1:4, under catalysis by piperidine. However, instability of this compound at room temperature precluded its submission to the National Cancer Institute, U.S.A., for anticancer evaluation.

4.15.5.0 Ethyl 2-amino-3-[1-(2-chlorophenyl)-3-oxo-nonylthio]propionate hydrochloride (XVIIIe)



(XVIIIe)

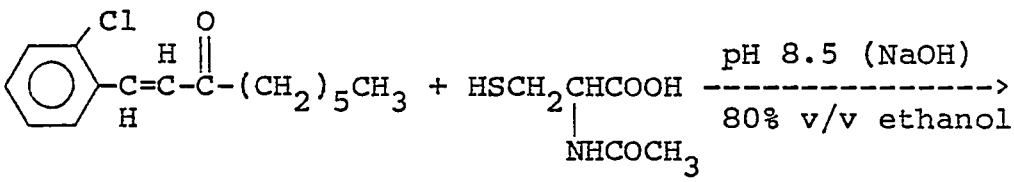
This compound was prepared in 73% yield by the 1,4-addition of L-cysteine ethyl ester hydrochloride to the conjugated enone (XVIa).

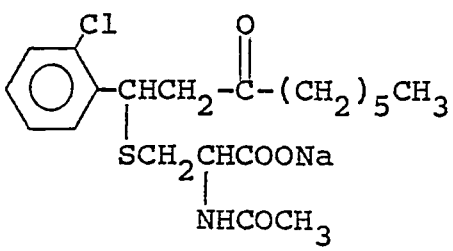


Screening data for this compound is not available.

4.15.6.0 Sodium-2-(N-acetylamino)-3-[1-(2-chlorophenyl)-3-oxo-nonylthio]propionate (XVIIIf)

This compound was prepared by the following synthetic scheme.





(XVIIIf)

Purification by column chromatography on cellulose afforded the pure product in 86% yield. The compound was inactive in the P388 screen and was fairly toxic.

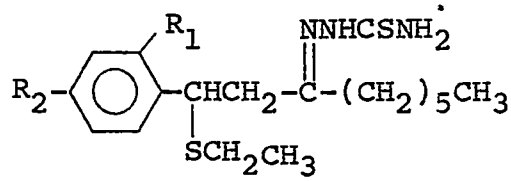
Activity against P388 lymphocytic leukemia in mice:

Maximum T/C%* (dose in mg/kg) : 101(60)

Murine toxicity* (dose in mg/kg): 1/6(240), 6/6(120)

[*For an explanation of terminology, please see footnotes a and b, Table XXI]

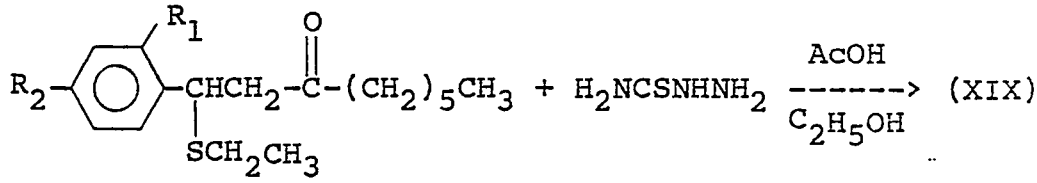
4.16.0.0 1-Aryl-1-ethylthio-3-nonanone thiosemicarbazones (XIXa-h)



(XIX)

R_1, R_2: same as in table (XIV), section 3.16.0.0

These compounds were prepared by heating under reflux a mixture of the appropriate thiol adduct (XVIIa-h), thiosemicarbazide and acetic acid in ethanol for 24 hours. Compound (XIXa) crystallized from the reaction mixture upon cooling while the remaining compounds were purified by column chromatography on neutral alumina. The yields were good and ranged from 59 to 95%. Compound (XIXd) was found to be light sensitive, turning yellow in the presence of light but turned colorless again when kept protected from light.



(XVII)

A representative compound (XIXa) was evaluated for anti-leukemic activity by the National Cancer Institute, U.S.A., and was found to be inactive and relatively non-toxic.

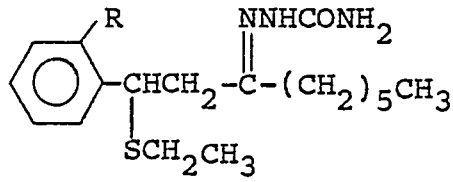
Activity of (XIXa) against P388 lymphocytic leukemia in mice:

Maximum T/C%* (dose in mg/kg) : 96(120)

Murine toxicity* (dose in mg/kg): 5/6(240), 6/6(120)

[*For an explanation of terminology, please see footnotes a and b, table XXI]

4.16.1.0 1-Aryl-1-ethylthio-3-nonanone semicarbazones (XX)



(XX)

a) R = Cl

b) R = H

Compounds (XXa&b) were prepared by condensing semicarbazide hydrochloride with the appropriate thiol adducts (XVIIa&d) in the presence of sodium acetate. The yields of (XXa&b) were 86% and 88% respectively.

A representative compound (XXa) was found to be inactive and non-toxic at the dose levels tested.

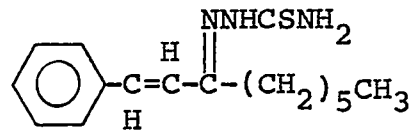
Activity of (XXa) against P388 lymphocytic leukemia in mice:

Maximum T/C%* (dose in mg/kg) : 105(60)

Murine toxicity* (dose in mg/kg): 6/6(240)

[*For an explanation of terminology, please see footnotes a and b, Table XXI]

4.16.2.0 1-Phenyl-1-nonen-3-one thiosemicarbazone (XXI)



(XXI)

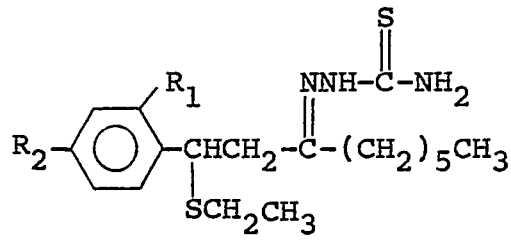
This compound was prepared similarly in 93% yield by condensing thiosemicarbazide with the α,β-unsaturated ketone (XVId) in ethanol, in the presence of acetic acid. The screening data for this compound is not available.

4.16.3.0 NMR Spectroscopy of 1-aryl-1-ethylthio-3-nonanone thiosemicarbazones (XIXa-h)

Thiosemicarbazones can exist in syn and anti configurations (50 & 52) and the ratio of isomers may be determined by NMR spectroscopy. A representative compound (XIXe) revealed that in solution the percentage of the syn form increased with time and the ratio of syn and anti isomers, determined by integration of the methine and secondary amino protons, became constant at the end of two hours. It was assumed that the rate of equilibration of the other compounds in series (XIX) was the same and the NMR spectra were recorded for these derivatives at the end of

three hours. In series (XIX), at equilibrium there was approximately 75% of the anti form except for (XIXe) in which case equal amounts of both isomers existed (table XXX). The assignment of the syn isomers was based on the premise that the thiosemicarbazono group would cause some shielding of the proton at carbon-1 and that hydrogen bonding and steric effects (see below) would enable the secondary amino proton to absorb at a lower field than the corresponding anti isomers.

Table XXX. NMR Spectral data[a] of 1-aryl-1-ethylthio-3-nonanone thiosemicarbazones (XIXa-h) in deuterochloroform at time of equilibria



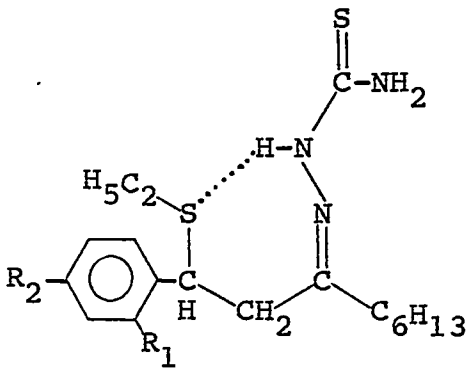
(XIX)

Compound[b]	R_1	R_2	δC^1H		δNH		
			Anti (%)	Syn (%)	Anti (%)	Syn (%)	$\Delta\delta NH$
XIXa	Cl	H	4.81(75)	4.71(25)	8.43(78)	8.81(22)	0.38
XIXb	F	H	4.63(82)	4.43(18)	8.42(80)	8.73(20)	0.31
XIXc	Br	H	4.78(72)	4.71(28)	8.44(77)	8.85(23)	0.41
XIXd	CH_3	H	4.40(74)	4.35(26)	8.42(72)	8.76(28)	0.34
XIXe	OCH_3	H	4.80(50)	4.54(50)	8.40(50)	9.29(50)	0.89
XIXf	H	H	4.12(72)	4.01(28)	8.43(71)	8.60(29)	0.17
XIXg	H	Cl	4.10(73)	3.99(27)	8.46(73)	8.65(27)	0.19
XIXh	H	CH_3	4.09(69)	3.98(31)	8.44(69)	8.56(31)	0.12

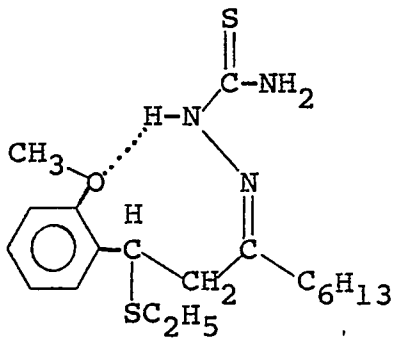
[a] The data generated in table (XXX) was obtained using a Bruker AM-360-WB spectrometer by dissolving 10 μM of the compound in 1 ml of deuterochloroform and recording the spectra after three hours. The spectra of (XIXe, 1 μM) in deuterochloroform and a 10 μM solution of (XIXe) in deuterated dimethylsulfoxide (1ml) were also examined after three hours.
[b] The compounds have been arranged in a different order than in table (XIV) for reasons of clarity.

Table (XXX) indicates that whereas the chemical shifts of the secondary amino proton of the anti isomers is constant at 8.43 (±0.03) ppm, the absorptions of the syn isomers differ from the corresponding anti isomers by 0.36 (±0.05) ppm in (XIXa-d), which contain ortho halo or methyl functions, by 0.89 ppm for (XIXe) and 0.16 (±0.04) ppm for (XIXf-h) in which ortho substituents in the aryl ring are absent. It is conceivable that the increased deshielding of the amino protons in the syn isomers of (XIXa-e) compared to (XIXf-h) is due to steric effects and hydrogen bonding. Thus the presence of an ortho substituent is likely to cause an increase in the rotomers in which the ethylthio group aligns close to the amino function and the resultant hydrogen bonding causes increased deshielding of the proton of the secondary amino (NH) group (50). Also, in the case of (XIXa-c), hydrogen bonding between the NH proton and halo atom could occur. The large increase in the deshielding of the NH

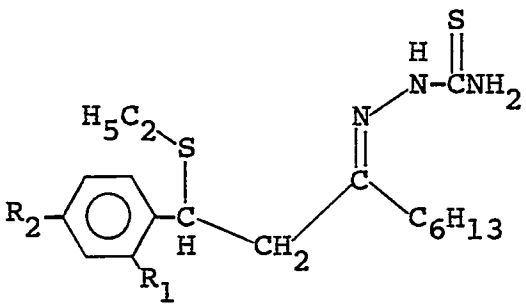
absorption of the syn isomer of (XIXe) compared to the anti isomer is probably due to marked intramolecular hydrogen bonding (51) and this possibility was strengthened by two further experiments. First, comparison of the ratio of syn and anti isomers of (XIXe) and time of equilibration was the same when the concentration of a solution of the compound was reduced tenfold suggesting that hydrogen bonding was intramolecular and not intermolecular. Second, when the spectrum of (XIXe) was determined in deuterated dimethylsulfoxide (which being more polar than deuterochloroform disrupts hydrogen bonding to a greater extent) equilibration of the isomers occurred more rapidly (50 minutes) and the percentage of the syn isomer dropped from 50 to 29%.



[(50), syn isomer]



[(51), syn isomer]



[(52), anti isomer]

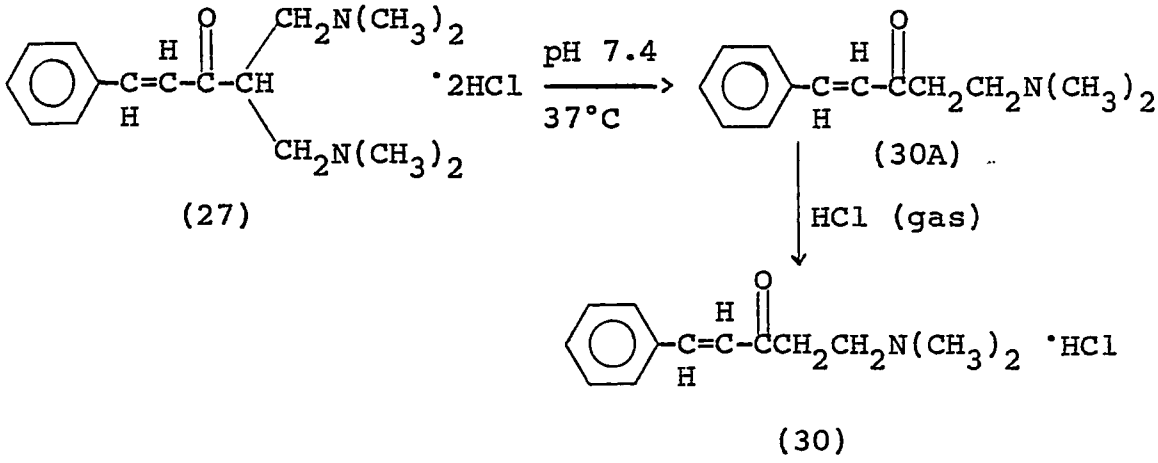
4.16.4.0 Summary of the work on α,β-unsaturated ketones and their derivatives

A number of compounds were prepared under this heading in the present research project. However, not all of the compounds were screened by the National Cancer Institute, U.S.A., for antileukemic activity. A few of the representative compounds that were evaluated were all found to be devoid of activity. Therefore, in the absence of complete biological data, it would be injudicious to attempt any meaningful structure-activity correlations.

4.17.0.0 Stability Studies

4.17.1.0 Stability of 5-dimethylamino-4-dimethylaminomethyl-1-phenyl-1-penten-3-one dihydrochloride (27)

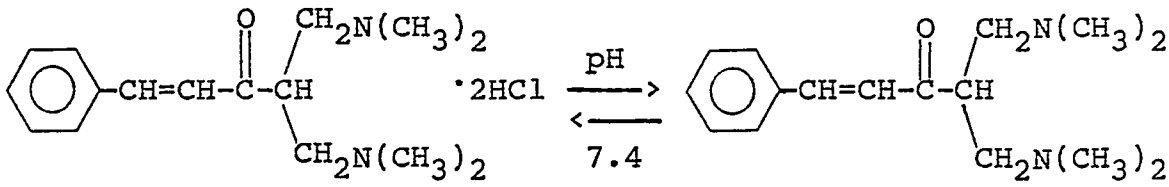
The stability of (27) was examined under simulated physiological conditions employing an aqueous tromethamine hydrochloride buffer of pH 7.4 and a temperature of 37°C. Under these conditions, (27) gave rise to the corresponding monobasic compound (30). Although there are reports in the literature regarding deaminomethylation of a mono-Mannich base to the starting ketone, there does not appear to be any report describing the retro-Mannich reaction of a bis-Mannich base to the corresponding monobasic compound.



(27) (30A) (30)

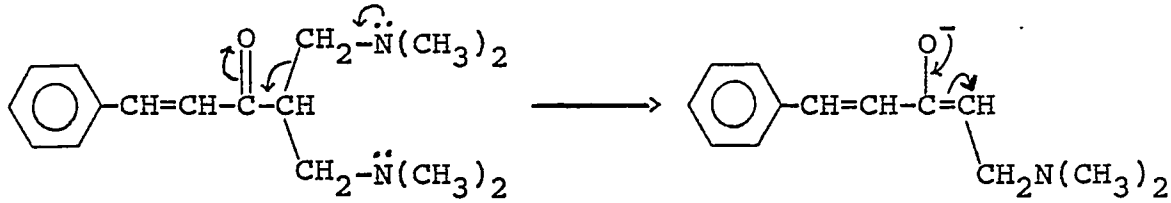
Evidence for the formation and structure of (30) includes its mass spectrum, NMR spectrum and the fact that it had identical NMR, IR, TLC and MS characteristics as an authentic sample of (30) prepared by an unambiguous literature methodology (Dimmock et al., 1976).

Plausible mechanisms for the formation of (30) from (27) are shown in figure 13. Mechanism (a) is the exact reverse of the Mannich reaction involving the intermediate formation of the enolate (53) and the immonium salt (XXII). The latter decomposes readily in the presence of water to form formaldehyde and dimethylamine.

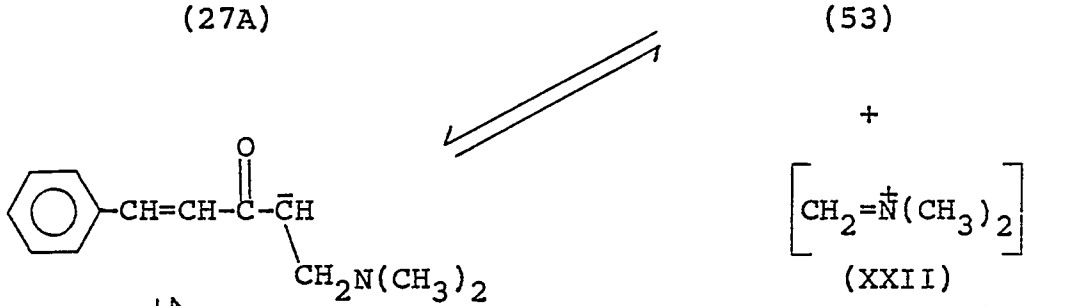


(27) (27A)

Mechanism (a)



(27A) (53)



(30A)

Mechanism continued on page 190 (Figure 13)

Mechanism (b)

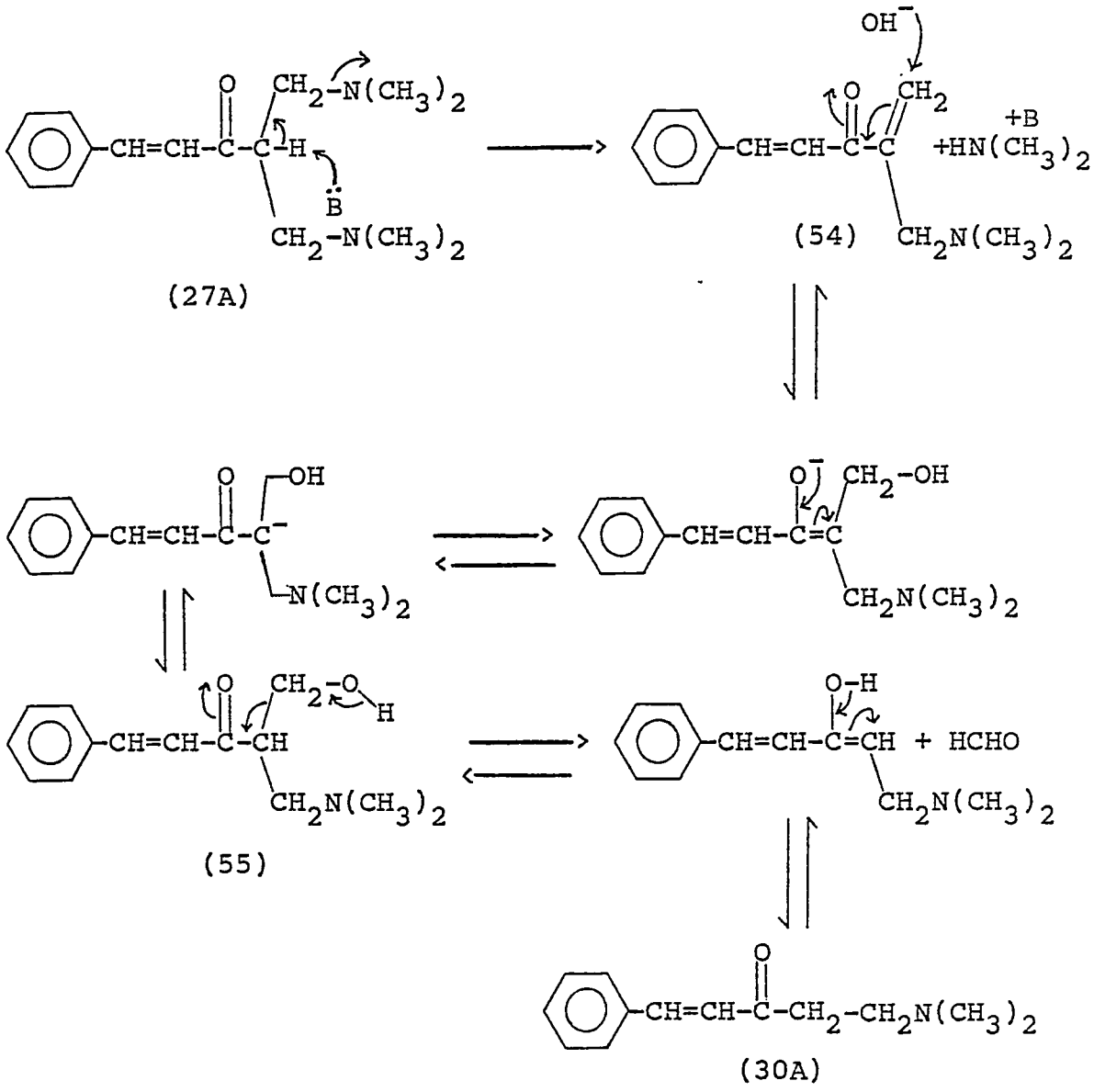


Figure 13. Plausible mechanisms for the formation of (30) from (27)

Mechanism (b), on the other hand, involves the intermediate formation of the enone (54) and the C-hydroxymethyl compound (55). The methine proton in (27A) is quite acidic and therefore, a β-elimination reaction is conceivable forming the doubly conjugated enone (54) and dimethylamine. No attempts were made, however, to isolate (54). Hydration of (54) could then occur to form the hydroxymethyl compound (55). This, in turn, could lose formaldehyde to form the mono-Mannich base (30A).

Evidence in support of mechanism (b) was indirectly obtained during stability studies on an acetophenone bis-Mannich base [(28), section 4.17.2.0, vide infra] during which an intermediate analogous to (54), viz. (XIIf), was actually isolated and characterized.

This study was undertaken to explore the possibility that the absence of antineoplastic properties of compounds of the type (27) could be due to breakdown in vivo to one or more inactive compounds. Since compound (30) is virtually bereft of activity in the P388 screen (Dimmock et al., 1979b), the formation of this compound from (27) under simulated physiological conditions may explain, at least in part, the lack of activity of (27) and related compounds.

4.17.2.0 Stability of 3-dimethylamino-2-dimethylaminomethyl-1-(4-methoxyphenyl)-1-propanone dihydrochloride (28) in phosphate buffer

The stability of (28) was examined under simulated physiological conditions employing a phosphate buffer of pH 7.4 and a temperature of 37°C. Under these conditions, the following observations were made.

1) Incubation of (28) for 5 minutes gave rise to the corresponding acrylophenone, 2-dimethylaminomethyl-1-(4-methoxyphenyl)-2-propen-1-one (56), which upon treatment with HCl gas gave the hydrochloride salt (XIIf) in a yield of 73%. The formation of the acrylophenone (56) from the dihydrobromide salt of (28) has been previously noted in this laboratory (Dimmock et al., 1983a).

2) When the incubation of (28) was continued for 96 hours, the corresponding mono-Mannich base, 3-dimethylamino-1-(4-methoxyphenyl)-1-propanone (31A) was obtained which upon treatment with HCl gas gave the hydrochloride (31) in a yield of 17%.

3) Incubation of the intermediate acrylophenone (XIIf), prepared by an unambiguous route (vide supra), for 96 hours gave rise to the corresponding mono-Mannich base (31A) which upon treatment with HCl gas gave the hydrochloride (31) in a yield of 7%.

The structures of compounds (XIIf & 31) were confirmed by comparison of their TLC, melting point and NMR characteristics with those of the authentic samples prepared by unambiguous routes, vide supra.

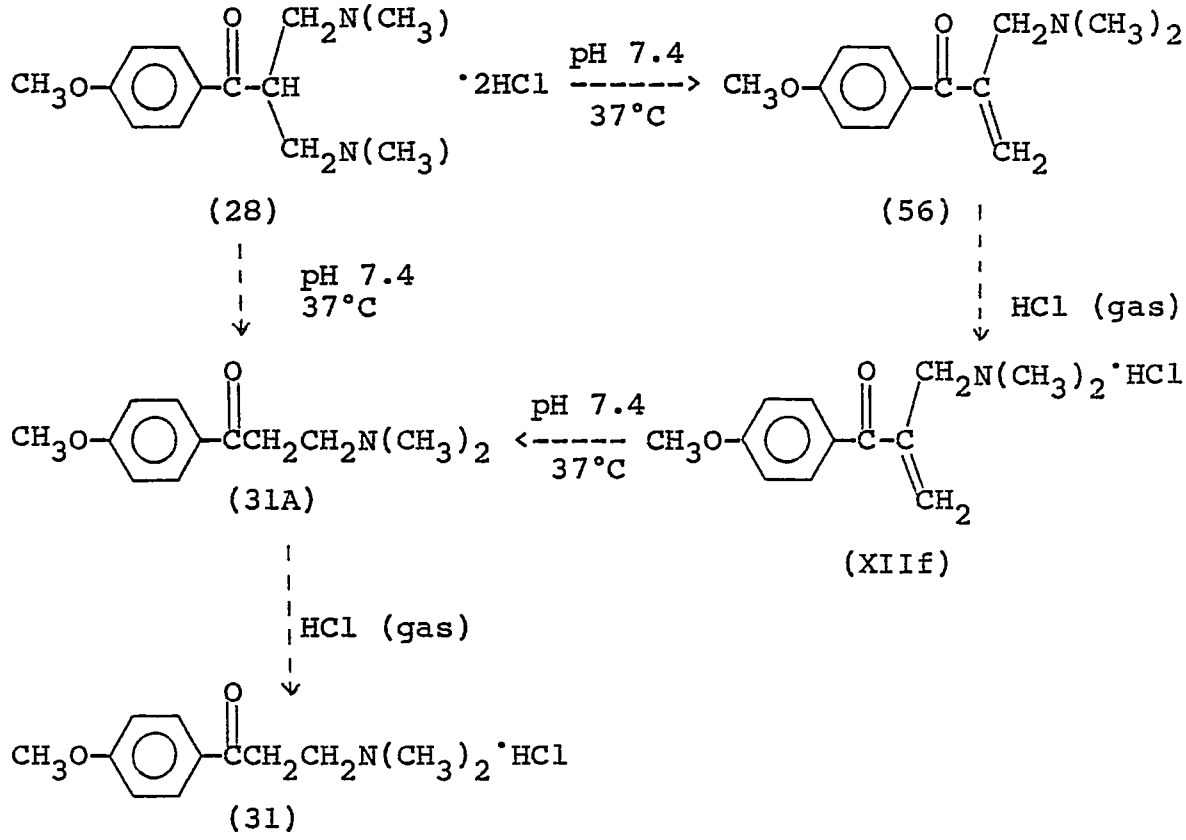


Figure 14. Stability of 3-dimethylamino-2-dimethylamino-methyl-1-(4-methoxyphenyl)-1-propanone dihydrochloride in phosphate buffer.

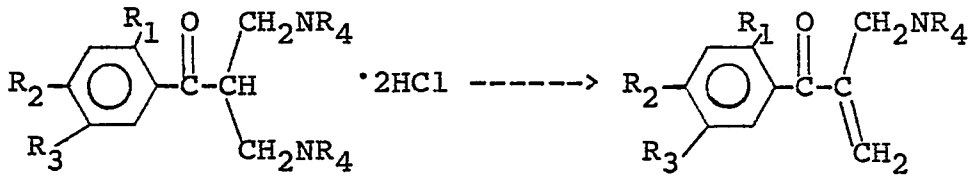
The obtainment of (XIIf) as an intermediate would suggest the operation of a mechanism similar to mechanism (b) discussed in section 4.17.1.0 (vide supra) in the above transformations.

The fact that the acrylophenone (XIIf) is obtained from the bis-Mannich base (28) under simulated physiological conditions, suggests that the latter can serve as a prodrug for the former. It is interesting to note that the bis-Mannich base as well as the acrylophenone can eventually break down to the corresponding mono-Mannich bases. However, these transformations were found to occur relatively slowly. Nevertheless, it is quite possible that the inactivity in the acrylophenone series and of some of the bis-Mannich bases (especially those which deaminate rapidly in vivo) is due, at least in part, to their conversion to the inactive mono-Mannich bases.

4.18.0.0 Kinetic Studies

4.18.1.0 Method I: UV Spectroscopy

The aim of the kinetic studies was twofold. Firstly, to determine the rate of deamination of some of the bis-Mannich bases. Secondly, to determine if there was a correlation between the rate of deamination and the antileukemic activity.



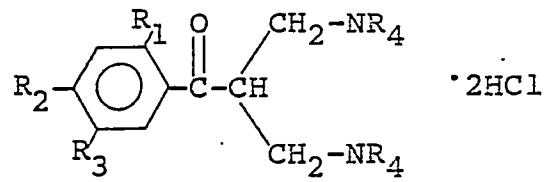
Initially, attempts were made to study the kinetics under simulated physiological conditions employing a phosphate buffer of pH 7.4 and a temperature of 37°C. Under these

conditions some of the bis-Mannich bases, especially those with an ortho-methoxy group, deaminated at a rate too fast to be measurable. After much experimentation, a pH of 3.5 was found to be suitable. Therefore, it was decided to study the kinetics under pseudo first order conditions using a formate buffer of pH 3.5 and a temperature of 37°C.

In the preliminary experiments carried out at this pH and at the λ max of the compound it was observed that the absorbance of the solution decreased steadily for a certain period of time (about 5 to 7 half-lives) after which the behavior was erratic i.e. the absorbance reading either increased or decreased. Furthermore, the absorbance reading did not attain a constant value even after 24 hours. It is possible that the acrylophenones formed by deamination of the bis-Mannich bases were undergoing a further reaction, such as addition of water to the double bond, under the experimental conditions. Therefore, it was decided to use the Guggenheim method (Bunnett, 1975) which does not require a knowledge of the initial or final concentration of the reacting species.

The kinetic data is presented in complete detail in table (XIX). For the purpose of the present discussion, the half-lives of the compounds and their antileukemic activities are tabulated below (table XXXI). The following conclusions may be drawn from the data generated.

Table XXXI. Chemical half-lives ($t_{1/2}$) and antileukemic activities (T/C%) of some 3-amino-2-aminomethyl-1-aryl-1-propanone dihydrochlorides.



Compound	R_1	R_2	R_3	NR_4	Half-life $t_{1/2}$ (min)	Maximum T/C%[a]
a	H	H	H	$-N(CH_3)_2$	4.69	115[1]
b	OCH_3	H	H	$-N(CH_3)_2$	1:33	112
c	H	OCH_3	H	$-N(CH_3)_2$	24.54	128[1]
d	OCH_3	OCH_3	OCH_3	$-N(CH_3)_2$	9.98	139
e	H	CH_3O-C$_6$H$_4$-O-	H	$-N(CH_3)_2$	13.23	132
f	H	Cl-C$_6$H$_4$-O-	H	$-N(CH_3)_2$	10.54	116
g	H	OCH_3	H	-N⟨ ⟩	151.95	128

[a] Anticancer activity is expressed as the ratio of the survival time of the treated animals to control animals expressed as a percentage. A compound should increase the median survival time by at least 27% to be considered active.

[1] Data generated previously in this laboratory.

1) Comparison of the $t_{1/2}$ values of compounds -a, -b and -c indicates the deleterious effect of introducing a methoxy group into the ortho position. Not only does the group fail to act as an electron donor but also it appears to introduce a considerable amount of steric strain into the molecule as suggested by the relatively short half-life of compound -b (1.33 min).

2) Compounds -c and -g differ only in the nature of the leaving group. The pka values of dimethylamine and pyrrolidine are 10.73 and 11.31, respectively (Albert and Serjeant, 1984). It was predicted in section 2.4.0.0 that compound -g would deaminate slower than compound -c which is exactly what is observed. The two compounds have half-lives of approximately 25 and 150 minutes, respectively. The fact that a change in the leaving group causes almost a 6-fold difference in the rate of the reaction is suggestive of cleavage of the C-N bond in the rate determining step of the reaction. However, in the absense of more information and evidence it would be injudicious to speculate upon the mechanism of the reaction. Also, an investigation in that direction was beyond the scope of this work.

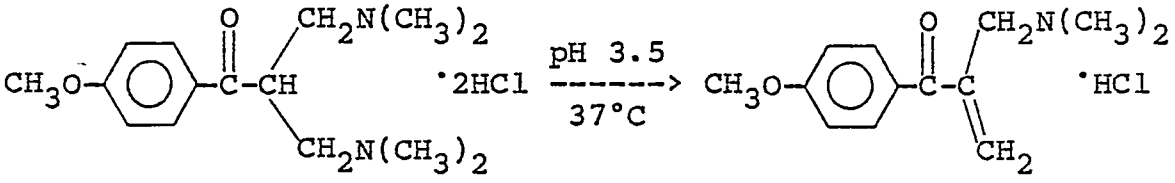
3) Contrary to the prediction, the phenoxy group does not appear to be very useful in retarding deamination in the present series of compounds. This is evident from the half-lives of compounds -c, -e and -f which are 24.54, 13.23 and 10.54 minutes, respectively. Interestingly, compounds -e and -f with a 4-methoxy and a 4-chloro substituent on the phenoxy ring have roughly the same half-lives.

4) Of the compounds listed in table (XXXI) compounds -c and -e have confirmed antileukemic activity while compounds -d and -g have presumptive activity. From the data so far available there does not appear to be any direct correlation between half-life ($t_{1/2}$) and the antileukemic activity (T/C%). Compounds -c and -g for example, have half-lives of about 25 and 150 minutes, respectively and yet have exactly the same level of activity (T/C% = 128). Compounds -e and -f have about the same half-life of decomposition (approximately 13 and 11 minutes, respectively) although compound -e has confirmed antileukemic activity while compound -f is inactive.

4.18.2.0 Method II: NMR Spectroscopy

The kinetics of the elimination reaction of a representative compound, 3-dimethylamino-2-dimethylaminomethyl-1-(4-methoxyphenyl)-1-propanone dihydrochloride (28), was also

studied by a NMR method under conditions similar to those used for the UV spectroscopic method. This involved the use of an excess of 0.25M formate buffer (in D_2O) of pH 3.5 and a temperature of 37°C.



(28) (XIIf)

Under these conditions, the reaction was monitored very easily by integration of the signal for the two olefinic protons of the acrylophenone being formed. These signals appeared at about δ 6.6 and 6.36, in a region which was free from any interfering peaks.

The pseudo first order rate constant obtained for compound (28) by the UV and NMR spectroscopic method were 4.707×10^{-4} and 3.38×10^{-4} sec^{-1}, respectively, and the corresponding half-lives were 24.54 and 34.19 minutes, respectively. The discrepancy between the two values may be due to a number of reasons including the fact that the ionic strength of the solution of the compound used for the two methods would be expected to be different because a much more concentrated solution is required for the NMR spectroscopic method.

4.19.0.0 Summary

In conclusion, the work in this thesis has achieved the following goals. Molecular modification of the 'lead' compound, 3-dimethylamino-2-dimethylaminomethyl-1-(4-methoxyphenyl)-1-propanone dihydrochloride, led to the preparation of a number of derivatives with varying activities towards P388 lymphocytic leukemia in mice. A correlation between the liberation of α,β- unsaturated ketones from the bis-Mannich bases under simulated physiological conditions with antileukemic activity was sought but none was found. However these stability studies revealed that a retro-Mannich reaction of a bis-Mannich base occurred which is the first example of such a phenomenon. Two of the acrylophenones were found to have interesting biochemical properties, on respiration in mitochondria isolated from mouse liver cells and on P388 cells in vivo. Future work should be directed towards investigating the relative lipophilicities of various members of this series of compounds and by molecular modification of one or more of the 'lead' derivatives.

The other phase of the work namely the synthesis and screening of the α,β- unsaturated ketones and related thiol adducts and thiosemicarbazones was less satisfactorily resolved since although the preparation of a number of molecules was accomplished successfully the evaluation of these derivatives by the National Cancer Institute was sporadic and incomplete.

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