SYNTHESIS, ANTINEOPLASTIC EVALUATION AND KINETIC STUDIES OF SOME MANNICH BASES

A Thesis

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To Winnie, Karthik and Ramya

ABSTRACT

Mannich bases have been found to display a wide range of biological activities. However, there is a paucity of information in the literature regarding the anticancer properties of these compounds. Therefore three different types of Mannich bases, viz., those derived from (a) acetophenones, (b) benzalacetones and (c) 1-phenyl-1-nonen-3-one were prepared and were evaluated against P388 lymphocytic leukemia in mice.

The Mannich bases derived from acetophenones could be broadly divided into three groups: (i) 3-amino-1-aryl-1-propanone hydrobromides, (ii) 3-amino-1-aryl-1-propanone methobromides and (iii) 3-amino-2-aminomethyl-1-aryl-1-propanones. Of these compounds, while (i) and (ii) were uniformly inactive against P388 lymphocytic leukemia, (iii) showed variable and sometimes, appreciable activity. Representative compounds from (i) and (iii) were also screened for respiration-inhibitory activity in rat liver mitochondria. It was found that (iii) were approximately one hundred times more active than (i). A Topliss analysis of the results obtained in the case of (iii) revealed a $-\sigma$ dependency for activity in the P388 screen and a $+\sigma$ dependency for respiration-inhibitory activity in rat liver mitochondria. An attempt was then made to explain the anticancer activities found in the cases of (i), (ii) and (iii) in terms of the

susceptibility of each series of compounds to β-elimination whereby acrylophenones were generated which could act as alkylating agents. Stability studies and kinetic experiments were carried out to achieve this objective and revealed that under the experimental conditions utilised,while no sign of β-elimination was discerned in the case of (i), (ii) underwent extremely rapid elimination to generate the corresponding acrylophenones at pH 7.4. Since both (i) and (ii) were inactive against P388 lymphocytic leukemia, the appreciable activity displayed by some members of (iii) was explained in terms of optimal rates of breakdown of the parent compounds to generate the alkylating species. One compound belonging to this series, viz., 3-dimethylamino-2-dimethylaminomethyl-1-(4-methoxyphenyl)-1-propanone dihydrochloride was designated a selected agent compound by the National Cancer Institute, U.S.A. and was screened against a number of other tumours.

The Mannich bases derived from benzalacetones could be divided into two groups: (1) 5-amino-1-phenyl-1-penten-3-ones and (2) 1-aryl-5-dimethylamino-4-dimethylaminomethyl-1-penten-3-one dihydrochlorides. Of the former, 5-diethylamino-1-phenyl-1-penten-3-one hydrobromide was found to show appreciable activity against P388 lymphocytic leukemia. It is not clear whether the activity of this compound is due to the decomposition product, viz., 1-phenyl-1,4-pentadien-3-one or the α,β-unsaturated ketone moiety in the parent Mannich base. The screening data of the complete series of 1-aryl-5-

dimethylamino-4-dimethylaminomethyl-1-penten-3-one dihydrochlorides have not been received in their final form.

The Mannich bases derived from 1-phenyl-1-nonen-3-one were inactive against P388 lymphocytic leukemia.

Finally two anils derived from benzalacetone were synthesized. These compounds were conceived as prodrugs of benzalacetone, a compound active against P388 lymphocytic leukemia. Of these, N-(1-methyl-3-phenyl-2-propenylidene)-benzenamine, designated a selected agent compound by the National Cancer Institute, U.S.A. was screened against several tumours. It was active against P388 lymphocytic leukemia and the colon 38 tumour.

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1.0.0.0 INTRODUCTION

1.1.0.0 The disease of cancer

Cancer occupies second place as the causa mortis, next to cardiovascular diseases in the United States. Although cancerous lesions have been found even in dinosaur bones, only in the twentieth century has there been concern over the disease. Progress in the cure of the former major causes of death has inevitably led to a rise in the incidence of cancer (Kauffman and Foye, 1981).

Cancer refers not to a single disease but to a group of diseases which may be initiated by the following: carcinogenic chemicals present in cigarette smoke, industrial pollutants, and the diet; radiation; oncogenic viruses; chronic mechanical or thermal trauma; parasitic infection; genetic predisposition; and the aging process (Pratt and Ruddon, 1979a). While "tumour" is a general term indicating any abnormal mass or growth of tissue that is not necessarily life-threatening, a "cancerous" tumour is a malignant neoplasm of considerable danger (Kauffman and Foye, 1981). The principal characteristics of benign and malignant neoplasms are as follows:

	Benign	Malignant
1.	Encapsulated	Nonencapsulated
2.	Noninvasive	Invasive
3.	Highly differentiated	Poorly differentiated
4.	Rare mitoses	Mitoses relatively common
5.	Slow growth	Rapid growth
6.	Little or no anaplasia	Anaplastic to varying degrees

7. No metastasis Metastasis

It should be emphasized that most of the above differences are relative and not invariable. The critical difference appears to be point (7), in that benign tumours do not have the capacity to metastasize, whereas malignant tumours do (Pitot, 1981a). A metastasis is a secondary growth originating from the primary tumour and growing elsewhere in the body (Kauffman and Foye, 1981). However, the fact that a number of benign neoplasms have been found to take on the behaviour of malignant neoplasms during their natural history would seem to point to the artificiality of such a distinction (Pitot, 1981b).

Furthermore, the malignant cell may show changes in cell membrane structure and function, alteration in levels of certain enzymes and the appearance of inappropriate gene products such as "oncofetal" antigens. Malignant cells also display alteration in growth characteristics. Thus they grow without the restraints that regulate normal tissue growth (e.g. differentiation, organ size limitation, hormonal regulation). The question may be posed as to what causes such alterations in the malignant cell. The possible answers include (a) genetic mutation or damage brought about by chemicals or irradiation or both, (b) expression of abnormal genetic information introduced into cells by oncogenic viruses, (c) gain or loss of chromosomal material by neoplastic cells, (d) derepression of "oncofetal" genes that

are present but normally repressed in adult cells, and (e) alterations in the post-transcriptional processing of critical cellular macromolecules. Whatever the cause of these phenotypic alterations, the end result is that the transformed cell has some selective advantage for growth over normal cells. The statement that cancer cells grow without the restraints that regulate normal tissue growth does not necessarily imply that their division rate is higher than that of normal cells. The critical change in the cancer cell is that it does not differentiate normally. The genes coding for differentiation appear to be shut off or inadequately expressed, while the genes coding for cell proliferation are expressed when, in fact, they should not be (Pratt and Ruddon, 1979b).

1.2.0.0 The treatment of cancer

The three most common approaches to the treatment of cancer are surgery, radiotherapy and chemotherapy. Immunotherapy has also been tried but with very little success. While surgery and radiotherapy can often eradicate localized tumours they can fail when the cancer has metastasized to other areas of the body. In contrast, chemotherapy is not so much limited by metastasis as it is by the total mass of the tumour(s). Chemotherapy combined with surgery or radiotherapy or both has increased survival rates for a number of solid tumours that were treated with very little success by only one therapeutic modality (Pratt and Ruddon,

1979c). But the fact remains that the total problem of cancer has not yet been solved. Given below are some of the principal reasons for this lack of success (Korolkovas and Burckhalter, 1976; Pratt and Ruddon, 1979d).

(a) The majority of biochemical differences between normal and neoplastic cells are quantitative rather than qualitative. Few qualitative differences between normal and malignant cells are known (TABLE I) and even those that are known have not been effectively exploited except in the case of leukemia cells that lack the enzyme L-asparagine synthetase.

(b) Malignant cells very rapidly develop resistance to anticancer agents. Combination therapy, although effective in some cases, does not always solve this problem.

(c) Since some tumours are poorly irrigated by blood, easy access of drugs to the cancer cells is hampered.

(d) An ideal way of assessing the therapeutic usefulness of a potential antineoplastic agent is not available. The findings with experimental transplanted animal tumours are not necessarily extrapolated to human tumours.

(e) Most antineoplastic agents produce toxic side effects including immunosuppression.

(f) Under certain conditions most antineoplastic agents are also carcinogenic.

Antineoplastic agents can be divided into the following classes: alkylating agents, antimetabolites,

antibiotics, plant alkaloids, enzymes and miscellaneous agents, hormones and radioactive isotopes.

TABLE I Some "qualitative" differences between normal and malignant cells> (Pratt and Ruddon, 1979e)

Cell constituents or process	*Observed alteration*	*Cell type affected*
Cell Membrane	Presence of tumor-associated antigens	Numerous animal and human cancer cells *in vivo* and *in vitro*
	Cellular agglutination by lectins	Transformed human and animal cells in culture Human leukemia and lymphoma cells
	Loss of epidermal growth factor binding	Mouse cells transformed with viruses or chemicals
	Absence of LETS protein*	Transformed animal cells in culture
	Presence of plasminogen-activators	Transformed animal cells in culture; indirect evidence for presence in human cancers
Placental gene products	Synthesis of placental hormones	Various types of human malignant tumor cells *in vivo* and in cell culture
	Presence of placental isoenzymes (e.g., placental alkaline phosphatase)	Various types of human malignant cells *in vivo* and in cell culture
Enzymes	Lack of L-asparagine synthetase	Certain human and animal lymphoma and leukemia cells *in vivo* and *in vitro*
	Presence of reverse transcriptase	RNA tumor virus-transformed cells; certain human leukemia and mammary tumor cells
Cyclic nucleotide metabolism	Increased cGMP content in proliferating tumor cells	Animal tumor cells in culture and *in vivo*
	Altered cAMP binding proteins	Cultured animal cells transformed with oncogenic viruses; animal tumors *in vivo*
Chromatin-associated proteins	Presence of specific nonhistone proteins	Various rat tumors *in vivo* and *in vitro*; human leukemic cells
Angiogenesis	Presence of tumor angiogenesis factor	Animal tumor cells *in vivo* and *in vitro*
Differentiation	Inability to complete normal differentiation process	Mouse tumor cells *in vivo* and in culture, e g., teratocarcinoma, Friend erythroleukemia, neuroblastoma; human neuroblastoma
Blockade of cells in G_1 or at G_1/S boundary	Inability of malignant cells to enter quiescent phase in response to nutritional deficiency or cell-synchronizing agents	Virus-transformed hamster cells; human melanoma and colon carcinoma cells

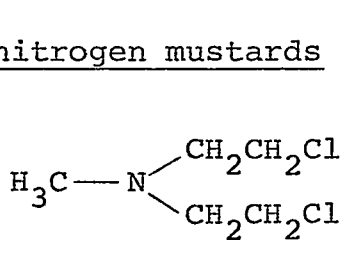
*LETS protein, Large external transformation sensitive protein.

1.2.1.0 Alkylating agents in cancer treatment

The area of interest in this investigation is the alkylating agents. Hence the discussion of chemotherapeutic agents used in the treatment of cancer will be confined to this class of agents.

Since the first trial of nitrogen mustard in a patient with lymphosarcoma in 1942 (Gilman and Philips, 1946), a large number of alkylating agents have been synthesized and screened for antitumour activity. All these compounds react in such a way that an alkyl group or a substituted alkyl group becomes covalently attached to cellular constituents. The alkylating agents used in cancer chemotherapy may be divided into five classes, viz., nitrogen mustards, nitrosoureas, triazenes, methane sulphonic acid esters, and ethylenimines.

1.2.1.1 The nitrogen mustards



(1)

The nitrogen mustards of which mechlorethamine(1) is a representative are highly reactive compounds that form covalent bonds with nucleophilic groups in proteins and nucleic acids. The nucleophilic groups in question include amino, carboxyl, sulfhydryl and imidazolyl moieties (Pratt and Ruddon, 1979f). The proposed mechanism of action for

mechlorethamine is given in fig. 1. The reaction of major importance in the cytotoxic effect of nitrogen mustards is thought to be the formation of a covalent bond between the drug and the 7-nitrogen of guanine (Geiduschek, 1961). As shown in fig.1, after forming a covalent bond with one molecule of guanine, the second chloroethyl group can alkylate another nucleophilic group which in some cases is the 7-nitrogen of another guanine molecule (Pratt and Ruddon, 1979f). If the two guanine moieties are in adjacent strands, the two strands are cross-linked. Consequently, the DNA strands cannot separate and replication is prevented (Crossland, 1980). It has been shown that mechlorethamine in low doses inhibits DNA synthesis in cultured mammalian cells more rapidly and to a greater degree than it inhibits RNA or protein synthesis (Brewer et al., 1961).

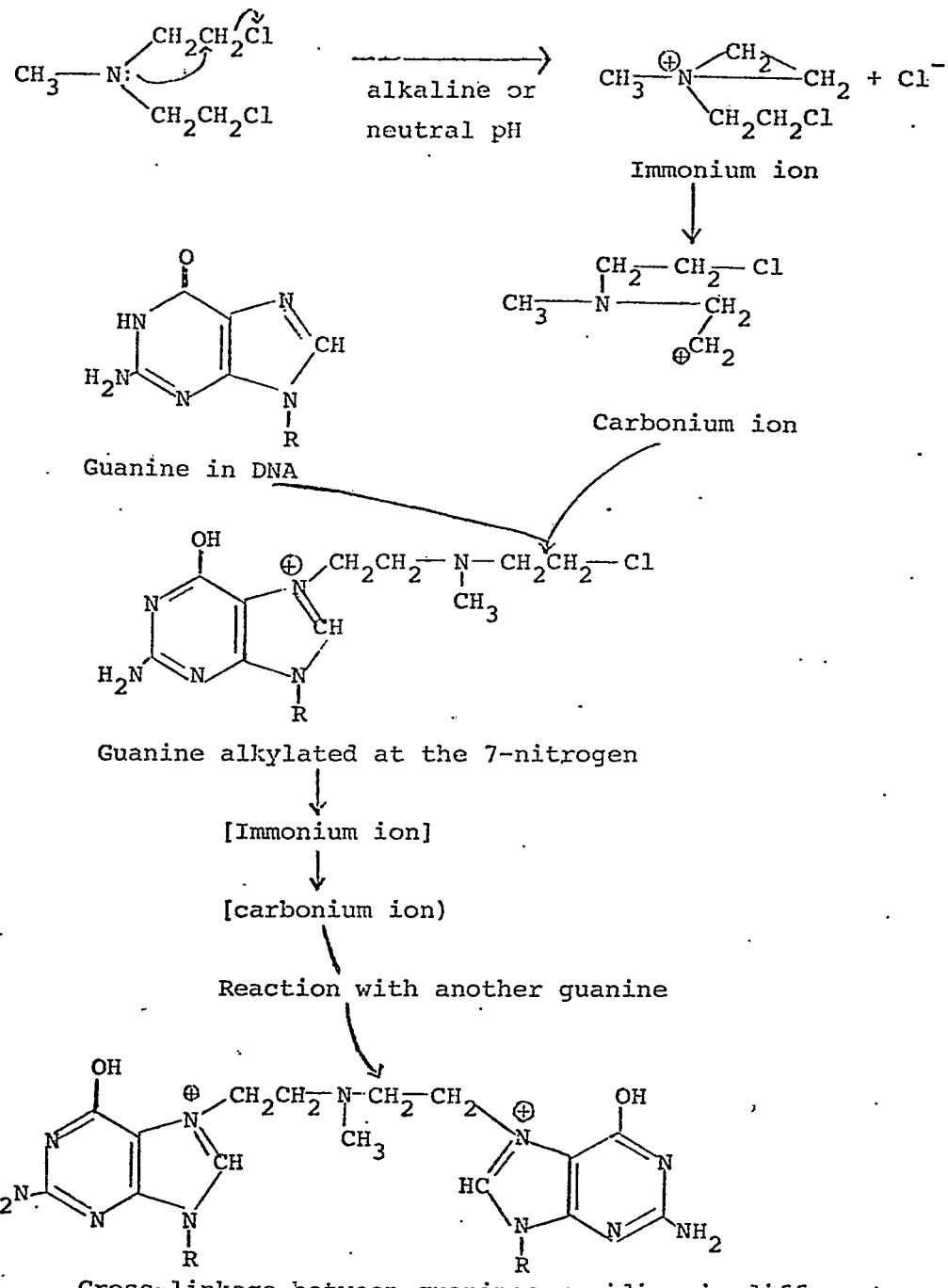
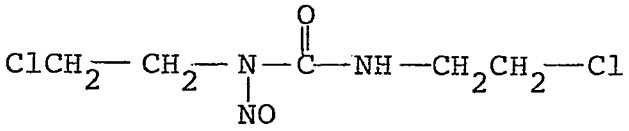


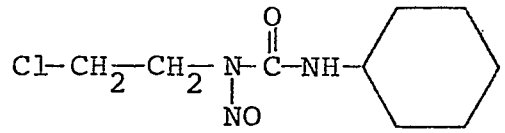
fig. 1[a]. The mechanism by which nitrogen mustard becomes covalently bonded to the 7-nitrogens of two guanine residues.

[a] Taken from Pratt and Ruddon (1979g).

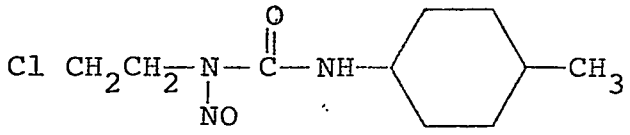
1.2.1.2 The nitrosoureas



1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU; 2)



1-(2-chloroethyl-3-cyclohexyl-1-nitrosourea (CCNU; 3)



1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (methyl-CCNU; 4)

The mechanism of action of the nitrosourea compounds (BCNU, CCNU, methyl-CCNU) is much more complex and less well understood than that of the nitrogen mustards. These compounds decompose in aqueous media to alkylating and carbamoylating intermediates. Reed et al. (1975) have proposed the mechanism of decomposition presented in fig. 2. The nitrosoureas are capable of alkylating several cellular constituents, including nucleic acids and proteins. Interestingly, even those nitrosoureas bearing a single 2-chloroethyl group (e.g. CCNU and methyl-CCNU) can cause interstrand cross-links in DNA (Kohn, 1977). Hence inhibition of DNA synthesis is a possible effect.

The carbamoylation reaction mentioned earlier predominantly involves proteins and the reaction occurs between the isocyanate moiety and the ε - amino groups of lysine (Schmall et al., 1973). The contribution of this reaction to the overall cytotoxic efficacy of the nitrosoureas is not well understood.

The mechanism of action of this series of compounds is complicated by the formation not only of the chloroethyl carbonium ion (see fig. 2) but also of numerous other alkylating moieties such as vinyl carbonium ions (Montgomery et al., 1967), 2-chloroethylamine, and probably 2-chloroacetaldehyde which is capable of alkylating thiol groups and could be formed by the action of aldehyde dehydrogenase on 2-chloroethanol (Pratt and Ruddon, 1979i).

BCNU and CCNU are highly effective against Hodgkin's disease, non-Hodgkin's lymphoma, certain tumours of the brain and malignant melanoma (Carter, 1976a).

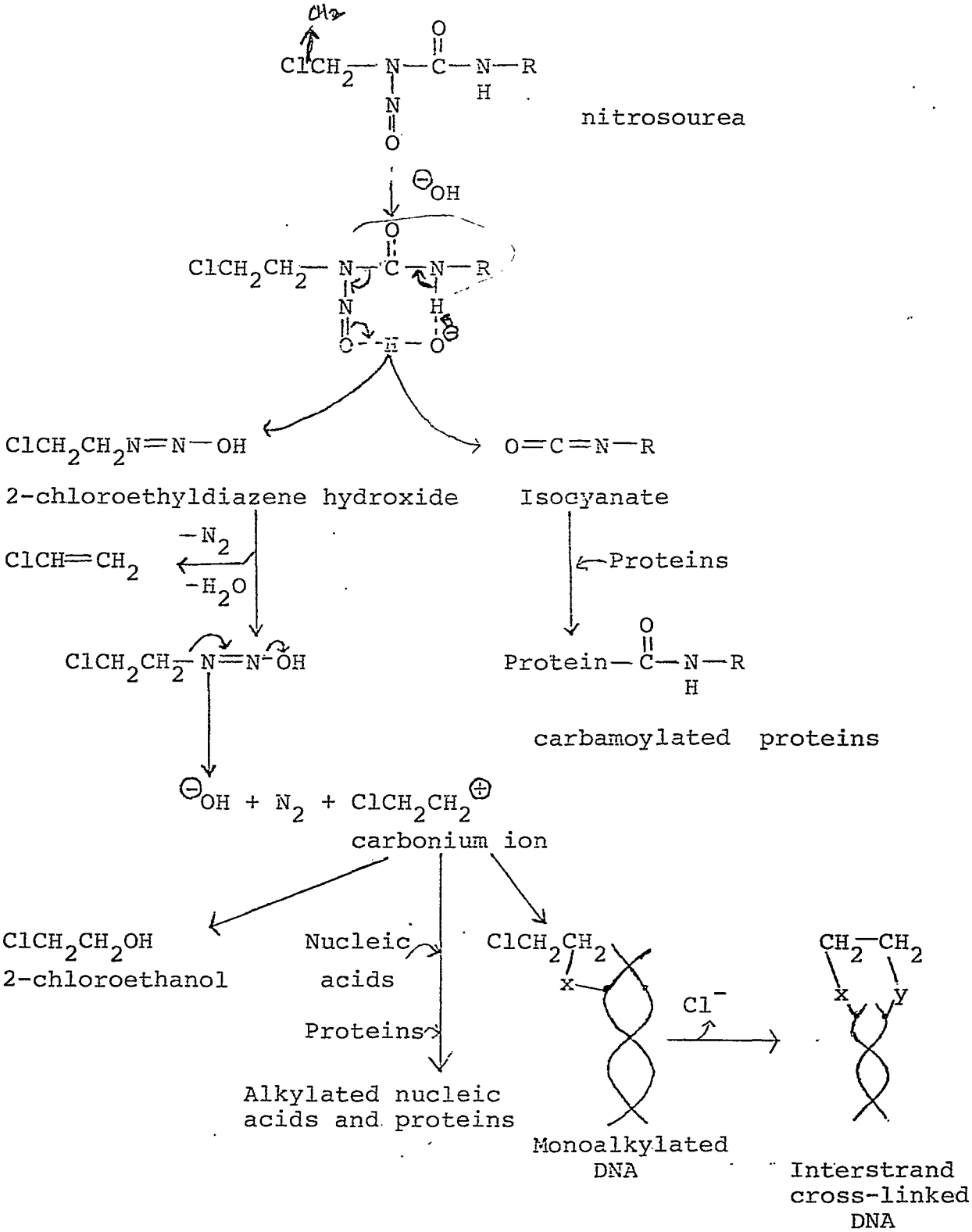
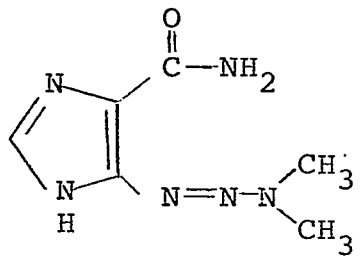


fig. 2[a]. Mechanisms of alkylation and carbamoylation by nitrosoureas.

[a] Taken from Pratt and Ruddon (1979h)

1.2.1.3 The triazenes

Dacarbazine (5), a representative of the triazene class of antineoplastic agents is a structural analogue of 5-aminoimidazole-4-carboxamide. The cytotoxicity of this compound, however, is due to its alkylating ability, not to an antimetabolite action. As shown in fig. 3, this compound can



(5)

decompose to produce the methyl carbonium ion (Loo, 1975) which can attack nucleophilic groups in DNA and other cellular constituents (Pratt and Ruddon, 1979j). Skibba and Bryan (1971) have noted that an important site of in vivo alkylation is the 7-position of guanine.

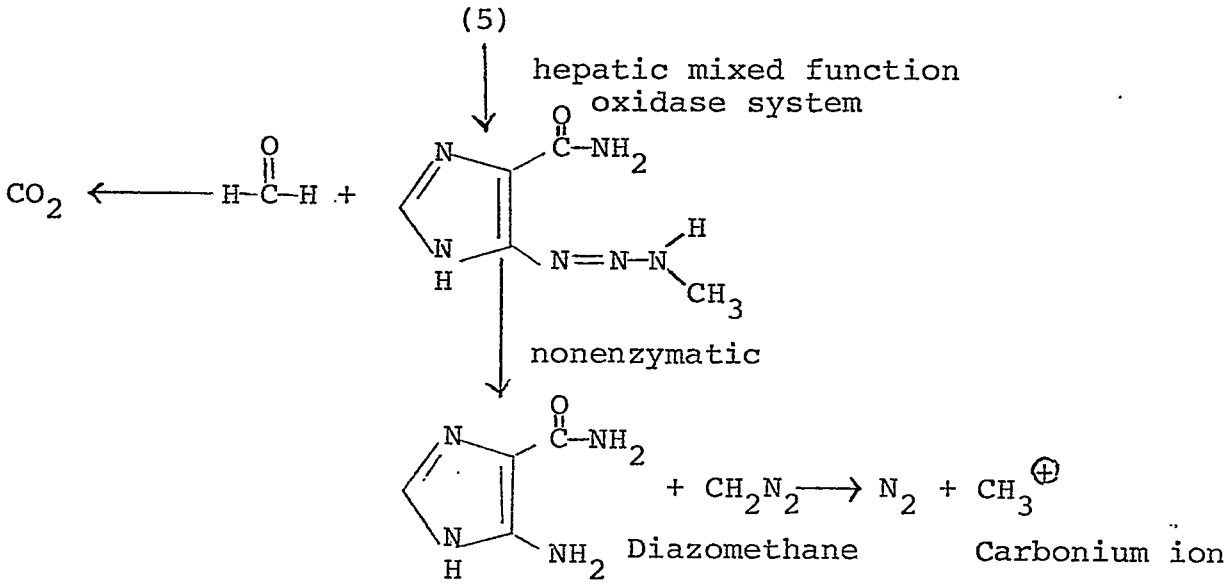
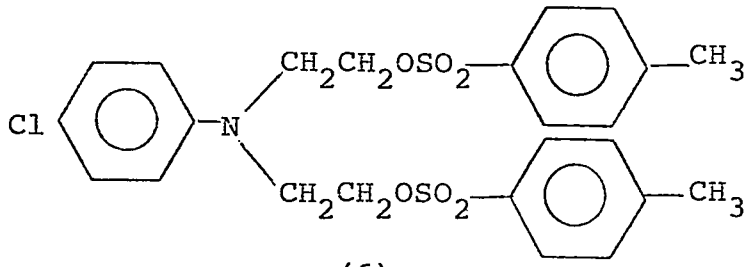


fig. 3[a]. Scheme for the metabolism of dacarbazine

[a] Taken from Pratt and Ruddon (1979j)

Dacarbazine is considered to be the most active single agent against malignant melanoma and is also active against soft tissue sarcomas, particularly in combination with other agents (Carter, 1976b).

1.2.1.4 The methane sulphonates



(6)

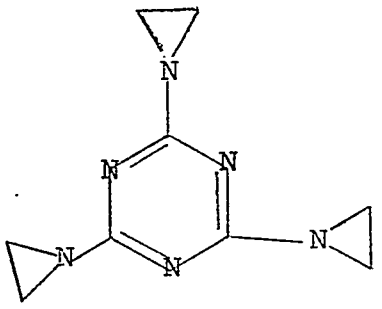
$CH_3SO_2OCH_2CH_2CH_2CH_2OSO_2CH_3$

(7)

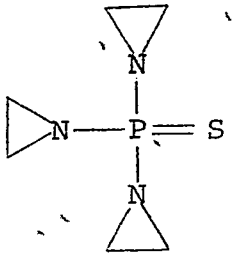
In an effort to enhance the therapeutic efficacy of the nitrogen mustards, Haddow and Timmis (1951) prepared a series of sulphonic acid esters out of which (6) was found to have striking activity against various tumours and reduced marrow toxicity. This observation and the work that followed resulted in the development of busulfan (7). This compound is active against chronic granulocytic leukemia (Pratt and Ruddon, 1979i) and has a chemical reactivity that is considerably less than that of nitrogen mustard. It can react with a variety of cellular constituents (Fox, 1975), one of the more important reactions from the point of view of anticancer activity being the alkylation of the 7-nitrogen of guanine (Brooks and Lawley, 1961).

Although diguanyl derivatives are formed during DNA-drug reaction, with busulfan these appear to represent only intrastrand linkages (Kohn et al., 1966).

1.2.1.5 Ethylenimines



(8)



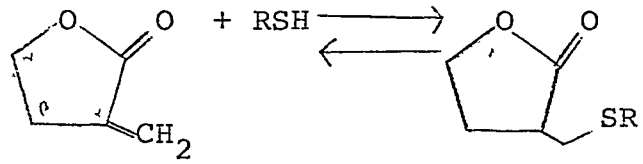
(9)

Triethylenemelamine (TEM,8) and triethylenethiophosphoramide (Thio - TEPA, 9) are representatives of the ethylenimine class of alkylating agents. Their mechanism of action is similar to that of the nitrogen mustards, although (8) and (9) are more reactive under acidic conditions, whereas the mustards are more reactive at alkaline pH (Pratt and Ruddon, 1979k).

1.3.0.0 α,β - Unsaturated carbonyl compounds

Although the classes of alkylating agents discussed above are useful in the treatment of various tumours, the high degree of chemical reactivity of these compounds leads to indiscriminate reactions with many cell constituents and, consequently, narrow therapeutic indices. Hence a need for more selective alkylating agents is indicated(Kupchan, 1976). An increasing number of antitumour terpenoids possess structures and chemical reactivity which suggest that these compounds act by selective alkylation of growth-

regulatory macromolecules (Cassady and Suffness, 1980a; Kupchan, 1976). The reactive groups are α,β-unsaturated ketones, α,β-unsaturated lactones and α,β-unsaturated esters or epoxides. One of the most common moieties found in these antitumour natural products is the α-methylene-γ- lactone (10) which is capable of undergoing Michael addition with



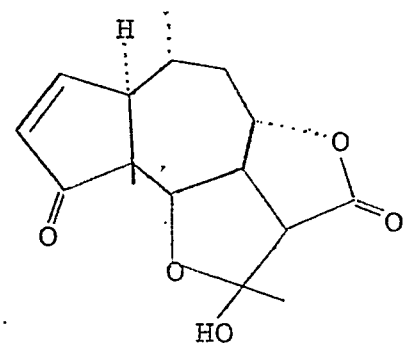
(10) (11)

fig. 4. Reaction of an α-methylene-γ-lactone with a thiol.

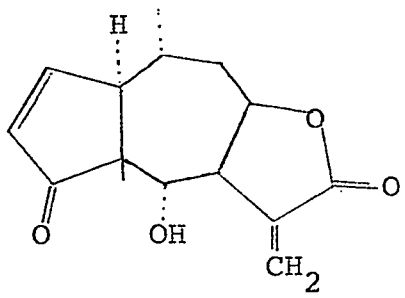
nucleophiles such as thiols (Cassady and Suffness, 1980b; fig. 4). It is noteworthy that in such compounds the olefinic bond is exocyclic, a characteristic which has been shown to be important for cytotoxic activity. The corresponding endocyclic analogues (butenolides) were much less active. A comparison of the reactivities of the two structural types showed that in general the endocyclic compounds reacted slowly and reversibly with cysteine, while the α-methylene-γ-lactones reacted rapidly to form stable products (Kupchan et al. 1971). One would expect the α-methylene-γ-lactone moiety to react with amines in a manner analogous to thiol addition but when such a comparison was attempted it was found that the reaction with amines was much slower than that with thiols (Kawanata and Inayama, 1971; Lanz et al., 1976).

In order to test the hypothesis that the tumour-inhibitory activity of these terpenoid lactones resulted from selective alkylation of sulfhydryl groups in key enzymes controlling cell division (Kupchan, 1974), a series of compounds, viz., vernolepin, elephantopin, eupacunin, and euparotin were tested and found to inhibit phosphofructokinase as a result of reaction with sulfhydryl groups (Hanson et al., 1970). In addition, vernolepin was found to inhibit glycogen synthetase (Smith et al., 1972a).

While the α-methylene-γ-lactone moiety is important for the antitumour activity of the compounds mentioned above, tenulin(12) which lacks an α-methylene-γ-lactone moiety, but possesses a cyclopentenone moiety, also shows appreciable antitumour activity (Cassady and Suffness, 1980c). Furthermore, reduction of the olefinic bond in the α,β-unsaturated ketone moiety of the sesquiterpenoid lactone, helenalin (13) gave a dihydro derivative with sharply reduced cytotoxic activity (Lee et al., 1972).



(12)



(13)

It was also shown that helenalin and tenulin reacted with cysteine and glutathione by conjugate addition. These compounds also inhibited the glycolytic enzymes phosphofructokinase and hexokinase, the respiratory process, and DNA synthesis (Hall et al., 1977; Lee et al., 1977). Since no evidence of any interaction between helenalin and tenulin and DNA was noted, it was conjectured that the action of these compounds on DNA synthesis was due to an effect on DNA polymerase (Cassady and Suffness, 1980c). These findings are quite interesting in view of the observation that selected thiol inhibitors have shown greater effect against tumours than against normal tissues (Knock et al., 1970). Furthermore, chemicals which react primarily with thiol groups may pose less of a threat to DNA and therefore, are less likely to be carcinogenic than those which are preferentially attracted to oxygen or nitrogen groups (Ashby, 1978).

Although many cyclic α,β-unsaturated ketones have been evaluated against a variety of tumours, relatively little work has been undertaken with acyclic α,β-unsaturated ketones as potential antineoplastic agents. Kabiev and Vernenichev (1971) studied the effect of some substituted chalcones on Ehrlich's ascitic sarcoma in mice. They found that hydroxy groups on the benzoyl ring retarded the increase in the volume of ascitic fluid and hydroxy groups on the benzylidene ring checked the increase in the total number

of tumour cells in this fluid.

A study on the effect of several acyclic α,β-unsaturated ketones related to furfuralacetone on the Ehrlich ascites tumour in mice has also been undertaken (Furst et al., 1954). Furfuralacetone increased the life span of the mice by approximately 17 per cent. In the series of compounds evaluated, an α,β-unsaturated ketone moiety was found to be necessary for antitumour activity.

1.4.0.0 The Hammett equation

Hammett (1935; 1937) found that the reactions of m- and p- substituted benzene derivatives could often be correlated by a relationship now known as the Hammett equation (equation 1).

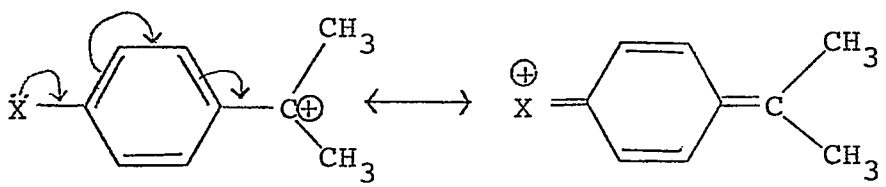
$$\log k/k_o = \sigma\rho \text{ - - - - - - - - - (1)}$$

The symbol k represents the rate or equilibrium constant for the substituted, and k_o, the rate or equilibrium constant for the unsubstituted compound. The substituent constant σ depends only on the substituent and represents the ability of the substituent to donate or withdraw electrons. It is defined by

$$\sigma = \log \frac{K_{X\text{-}C_6H_4COOH}}{K_{C_6H_5COOH}} \text{ - - - - - - - (2)}$$

where $K_{X\text{-}C_6H_4COOH}$ is the ionization constant for the substituted benzoic acid and $K_{C_6H_5COOH}$ that for benzoic acid. The reaction constant, ρ, measures the sensitivity of the reaction to electron donation or withdrawal. From equations (1) and (2) it follows that ρ is unity for the ionization of benzoic acids. Since σ is positive for electron-withdrawing substituents and negative for electron-releasing substituents, a positive ρ indicates that the reaction is accelerated or the equilibrium favoured by electron withdrawal; a negative ρ shows the opposite. The magnitude of ρ shows the sensitivity of the reaction to substituent changes.

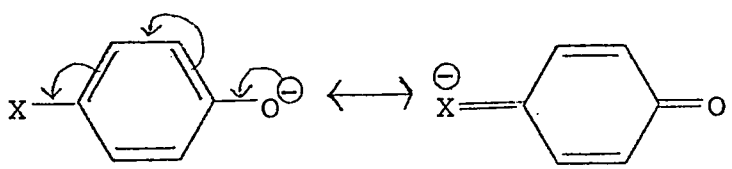
The Hammett equation fails in cases where the substituent can enter into direct resonance interaction with the reaction centre in the transition state. For such cases, two new sets of substituent constants, viz., σ+ and σ-, have been devised. The σ+ values which were devised by Brown and Okamoto (1958) are used in cases where an electron-releasing group enters into a direct mesomeric interaction with a developing positive charge in the transition state (e.g. fig. 5).



X = an electron releasing group

fig. 5. Stabilization of a cumyl carbocation by through-conjugation involving an electron-releasing aromatic substituent.

The σ- values, on the other hand, are used when electron-withdrawing groups interact mesomerically with a developing negative charge in the transition state (Jaffé, 1953; e.g. fig. 6).



X = electron-withdrawing substituent

fig. 6. Stabilization of a phenoxide anion by through-conjugation involving an electron-withdrawing substituent.

1.5.0.0 Isotope effects

The use of isotope effects has proved extremely valuable in the study of reaction mechanisms. For example, when a hydrogen atom in a reactant molecule is replaced by deuterium, there is often a change in the rate of a reaction. Such effects are known as deuterium isotope effects and are expressed by the ratio k^H/k^D. Studies of isotope effects owe their usefulness to the fact that isotopic substitution has little effect on the qualitative chemical reactivity of the substrate but often has a significant effect on the rate at which reaction occurs (Carey and Sundberg, 1977a). The first part of this discussion will be concerned with primary kinetic isotope effects, i.e., reactions in which a bond to the isotopically substituted atom is broken at the transition state of the rate-determining step.

The zero-point energy of a vibration is given by $E^\circ = \frac{1}{2} h\nu$ where h is Planck's constant, 6.6238×10^{-27} erg-sec. and ν, the frequency of vibration. The frequency, in turn, is related to the properties of the bond by Hooke's law, i.e. $\nu \propto (k/\mu)$ where k is the force constant and μ, the reduced mass. The force constant k is not appreciably affected by changing C-X to C-X^1 where X and X^1 are the lower and higher mass isotopes of the same element. On the other hand, the reduced mass, μ, which is defined by equation 3, is increased by such an alteration (Gilliom, 1970; Jones, 1979a).

$$\mu_{xy} = \frac{m_x m_y}{m_x + m_y} \quad - - - - - - - - \quad (3)$$

μ_{xy} = Reduced mass for a bond between x and y

m_x = mass of atom x

m_y = mass of atom y

Since the zero-point vibrational energy of a bond is inversely proportional to the reduced mass, D-C, D-O, D-N, etc. have lower energies in the ground state than the corresponding H-C, H-O, H-N, etc. Therefore, complete dissociation of a D-C bond, for example, requires more energy than that for an H-C bond (fig. 7).

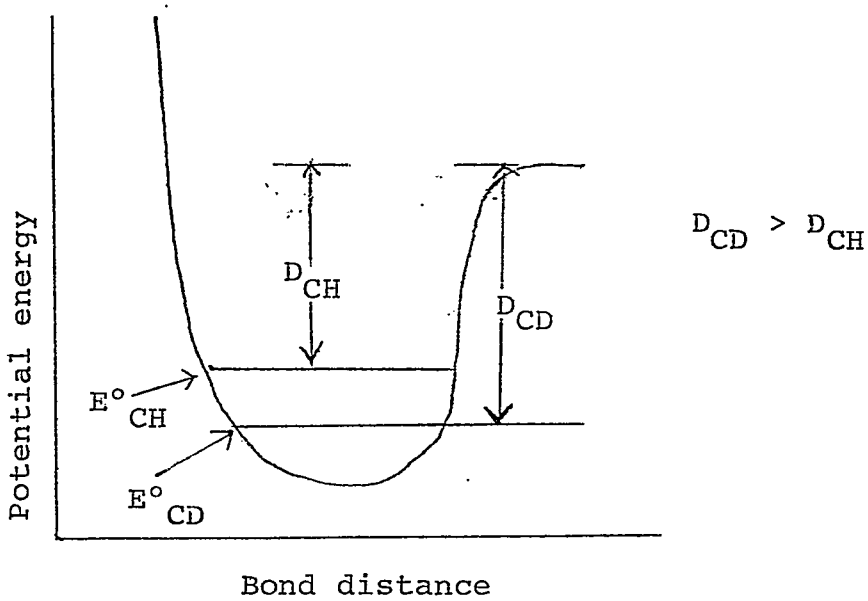


fig. 7. Differences in zero-point vibrational energies and bond dissociation energies of C-H and C-D bonds (Jones, 1977a).

It follows that if an H-C bond is broken in the rate-determining step, the rate would be lowered by the substitution (with deuterium). On the other hand, if the H-C bond is not broken at all or is broken in a non-rate-determining step, substitution of deuterium for hydrogen would be expected to cause little or no change in rate (March, 1977a).

The magnitude of the rate difference depends on the nature of the transition state. The maximum effect occurs when the hydrogen being transferred is bound about equally to the atoms between which it is being transferred. The calculated maximum for the isotope effect k^H/k^D involving C-H(D) bonds is about 7 at room temperature and becomes lower at higher temperatures. When bond-breaking is more or less than half complete, the value is lower (Westheimer, 1961). Two useful pieces of information regarding a reaction mechanism may be obtained from primary isotope effects: Firstly, if the k^H/k^D value is 2 or more it is evidence that the bond to the substituted hydrogen atom is being broken in the transition state. Secondly, from the magnitude of the isotope effect it becomes possible to obtain a qualitative indication as to where the transition state lies with regard to product and reactant. Thus a relatively low isotope effect indicates that the bond to hydrogen is very little or very completely broken in the transition state, i.e. the structure of the transition state should resemble either the reactant or the product. On the other

hand, if the isotope effect is close to 7, the hydrogen atom is about equally bonded in the transition state to the two atoms between which the transfer occurs (Carey and Sundberg, 1977b).

Deuterium isotope effects have been found to occur even where it is certain that the C-H bond is not broken at all in the reaction. Such effects are called secondary isotope effects and are usually in the range, k^H/k^D = 0.7-1.5. Secondary isotope effects may be divided into α and β effects. In a β secondary isotope effect, substitution of deuterium for hydrogen beta to the position of bond breaking slows the reaction. For example, the relative rates for the solvolyses of the two isopropyl bromides, $(CH_3)_2CHBr$ and $(CD_3)_2CHBr$, are 1.00 and 0.75, respectively (Leffek et al., 1960). The cause of β isotope effects is thought to be hyperconjugation in the transition state (fig. 8).

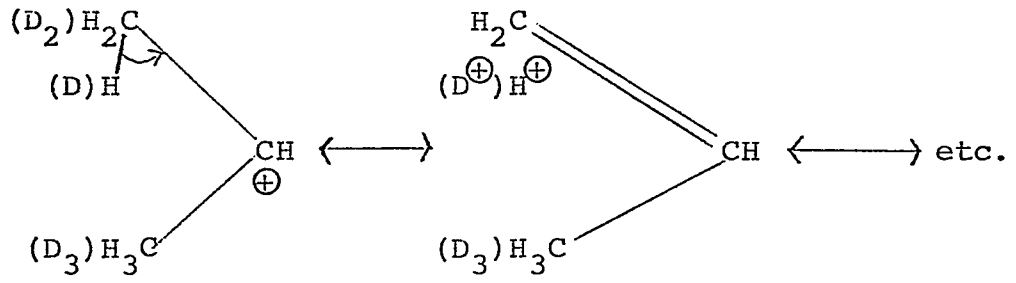


fig. 8. Hyperconjugative forms of isopropyl carbocation.

The greater the carbocation character of the transition state the greater are the β isotope effects (Bender and Feng, 1960; Jones and Bender, 1960). The α secondary isotope effects result from a replacement of hydrogen by deuterium at the carbon containing the leaving group. If an sp^3-hybridized carbon in the ground state is converted to an sp^2-hybridized carbon in the transition state, a hydrogen bonded to that carbon will experience a decreased resistance to C-H bending. The freeing of the bending mode is greater for a C-H bond than for a C-D bond since the amplitude of the former is greater, and the result will be a normal secondary isotope effect (i.e. $k^H/k^D > 1$). Examples of such reactions include the S_N1 solvolysis reactions of alkyl halides. However, if coordination increases on going from the ground state to the transition state, an inverse isotope effect (i.e., $k^H/k^D < 1$) will be obtained [e.g. cyanohydrin formation (March, 1977b; Carey and Sundberg, 1977b).]

1.6.0.0 Elimination reactions

Elimination reactions can be classified according to the relative placement of the carbon atoms from which elimination occurs (Carey and Sundberg, 1977c):

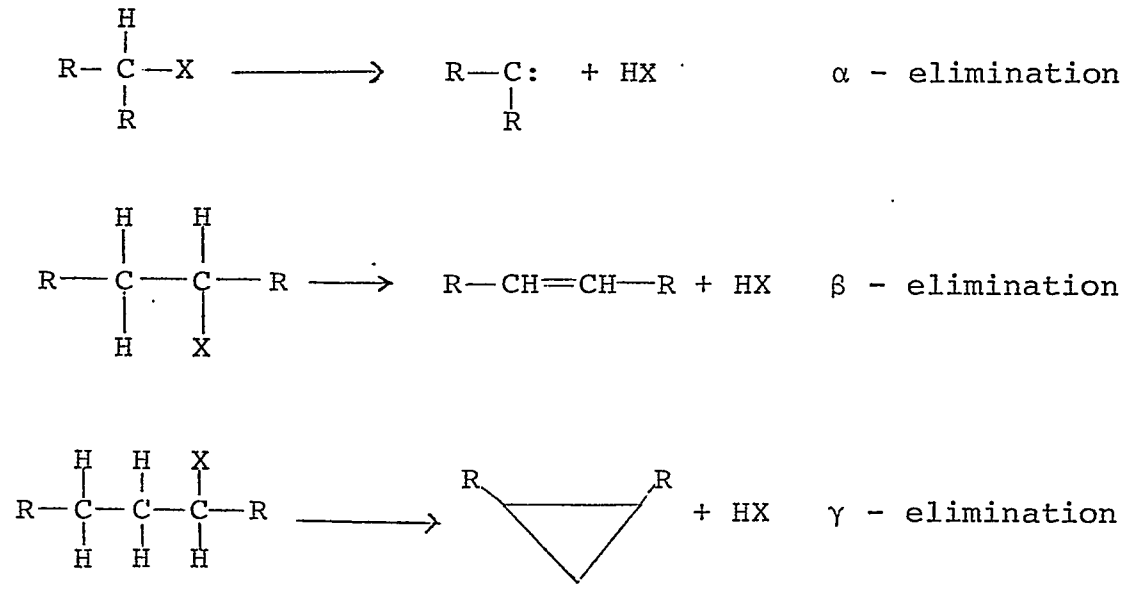


fig. 9. Classification of elimination reactions.

The β-eliminations can be further divided into two types, with one taking place largely in solution and the other mostly in the gas phase (March, 1977c). Discussion in this section will be confined to β-eliminations which occur in solution.

1.6.1.0 Mechanisms of β-eliminations

1.6.1.1 The E1 mechanism

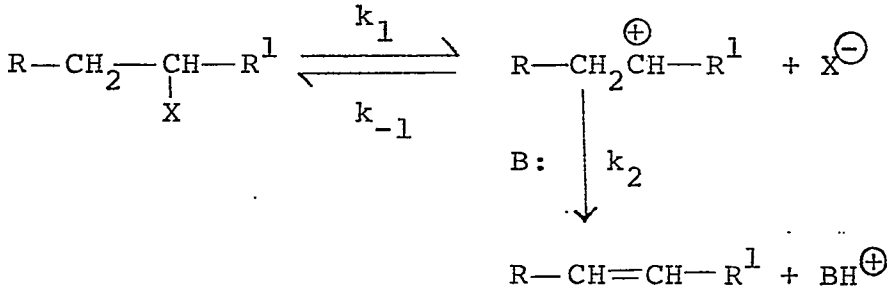


fig. 10. The E1 mechanism

The E1 mechanism is a two-step process in which the rate-determining step is usually the unimolecular ionization of the substrate to give a carbocation which loses a β-proton in a fast step to a base, usually the solvent (fig. 10). The kinetics is usually first order in substrate (March, 1977d). In an E1 reaction there is only a small secondary deuterium isotope effect, but a significant leaving group isotope effect (Jones, 1979b).

1.6.1.2 The E2 mechanism

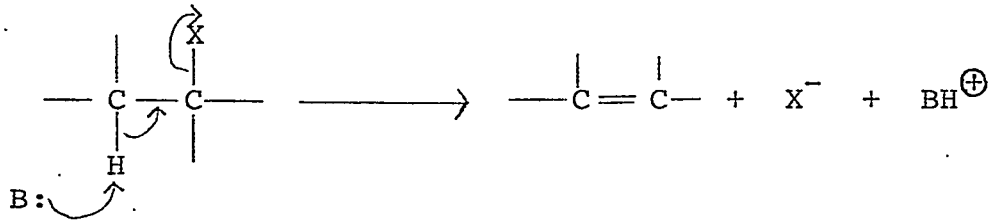


fig. 11. The E2 mechanism

As depicted in fig. 11, the E2 mechanism is a bimolecular process in which abstraction of a proton beta to the leaving group is concerted with departure of the leaving group. It is a one-step mechanism which is kinetically second order: first order in substrate and first order in base. Since the C-H and C-X bonds are broken in the rate-determining step, two types of primary isotope effects are observed for E2 reactions, viz., a hydrogen-deuterium isotope effect in the range 3 to 8 and a leaving group isotope effect (March, 1977e).

1.6.1.3 The ElcB mechanism

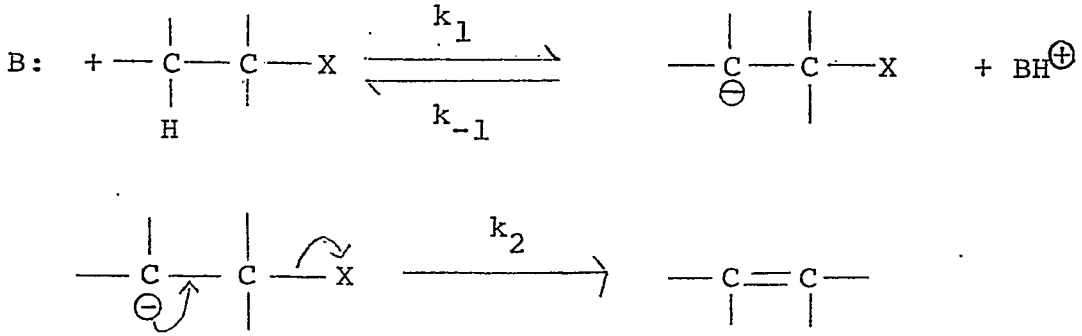


fig. 12. The ElcB mechanism

The ElcB mechanism occurs in the case of reactants having substituent groups that can effectively stabilize the intermediate carbanion. There are three kinetic possibilities in the case of ElcB reactions:

(1) The carbanion returns to starting material faster than it forms product ($k_{-1} >> k_2$); the first step is reversible (pre-equilibrium ElcB).

(2) The formation of product is faster than return of the carbanion to starting material ($k_2 >> k_{-1}$). In this case the first step is essentially irreversible.

(3) A very acidic substrate can be completely ionized to the anion in an excess of base. This is followed by a first order decomposition in the second step, unaffected by changes in the concentration of base (March, 1977f; Jones, 1979b).

With the exception of the third limiting case, it is often difficult to distinguish between E2 and ElcB

by kinetic analysis. So several non-kinetic methods have been employed to make the distinction. The classic method is isotope exchange (Saunders and Cockerill, 1973a). If pre-equilibrium ElcB is operative, one can detect the incorporation of deuterium into the substrate from a labelled solvent if the reaction is stopped partway. This reaction, however, does not show any deuterium isotope effect, though there can be an isotope effect from the leaving group (Jones, 1979c). A distinction between ElcB (irreversible) and E2 may be made by studying the leaving group isotope effect. In the case of ElcB (irreversible), unlike in the case of E2, the C-X bond is broken in the fast step and hence a leaving group isotope effect will not be observed in this case (Saunders and Cockerill, 1973b).

1.6.1.4 The ylid mechanism

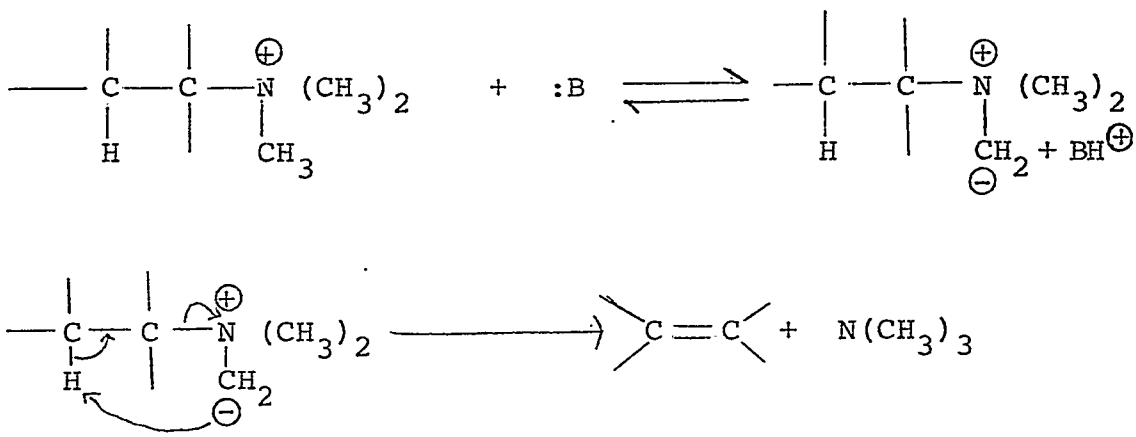


fig. 13. The ylid mechanism

The ylid mechanism has been shown to operate in certain cases (e.g., ammonium and sulphonium salts) where the normal E2 mechanism is not sterically favoured. In this the base, instead of removing the β-hydrogen, removes one of the methyl hydrogens (fig. 13). The most obvious way to distinguish between this mechanism and the ordinary E2 mechanism is by deuterium labelling. For example, if the reaction is carried out on a quaternary ammonium hydroxide deuterated on the β-carbon, then the amine that is eliminated should contain deuterium if the ylid mechanism is operative. But if the E2 mechanism is in operation, the amine will contain no deuterium (Jones, 1979d; March, 1977g).

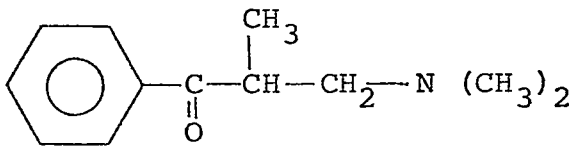
2.0.0.0 AIMS OF THE PRESENT INVESTIGATION

2.1.0.0 Introduction

Mannich bases have been found to display a wide range of biological activities, including antimicrobial effects (Florestano et al., 1957; Dimmock et al., 1975; Dimmock et al., 1976a; Varma and Nobles, 1968), analgesic (Janssen, 1962; Viterbo et al., 1974) and anticonvulsant (Daruwala et al., 1974) properties, local anaesthetic activity (Blicke and Blake, 1930; Wilson and Kyi, 1952) and psychotropic properties (Shut et al., 1972). Several Mannich bases screened by Varma et al. (1970) were found to inhibit pyruvic acid oxidation.

2.2.0.0 1-Aryl-3-dimethylamino-1-propanone hydrobromides(I)

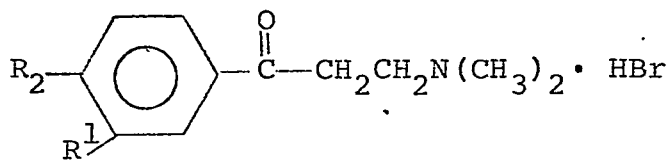
A few reports have been published on the anticancer properties of β-aminoketones derived from substituted acetophenones. Schoenenberger et al. (1969) have reported the inhibition of Sarcoma 180 tumour by (14).



(14)

Mannich bases in which the amino group is the bis-(2-chloroethyl)amino moiety have also been synthesized (Pettit and Settepani, 1962) and tested (Werner et al., 1970). The latter workers have reported the effects of sixty-eight Mannich bases with and without the nitrogen mustard moiety and sixteen comparison compounds on transplanted tumours such as Ehrlich ascites carcinoma, L1210 leukemia, myeloid leukemia, Crocker sarcoma and Walker carcinosarcoma. They found that activity against the Ehrlich ascites carcinoma was closely associated with the nitrogen mustard function since Mannich bases without this group were essentially inactive.

Apart from reports such as those mentioned above, there is a paucity of information in the literature on the anticancer properties of Mannich bases derived from substituted acetophenones. Therefore, the synthesis of compounds Ia-k with a view to studying their potencies versus P388 lymphocytic leukemia was contemplated.



I

a. $R^1 = R^2 = H$

b. $R^1 = CH_3$; $R^2 = H$

c. $R^1 = H$; $R^2 = CH_3$

d. $R^1 = OCH_3$; $R^2 = H$

e. $R^1 = H$; $R^2 = OCH_3$

f. $R^1 = OH$; $R^2 = H$

g. $R^1 = H$; $R^2 = OH$

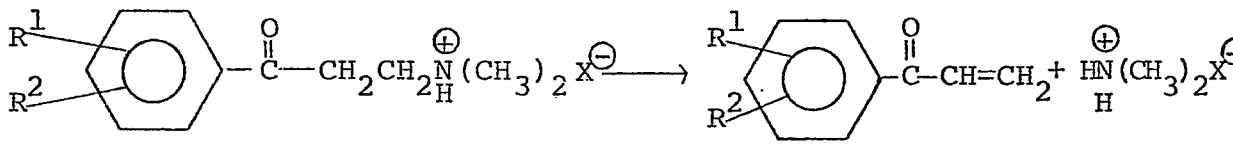
h. $R^1 = NO_2$; $R^2 = H$

i. $R^1 = H$; $R^2 = NO_2$

j. $R^1 = H$; $R^2 = Cl$

k. $R^1 = R^2 = Cl$

Furthermore, under certain conditions, e.g., heat, aqueous alkali, etc., Mannich bases are known to undergo β-elimination to generate α,β-unsaturated ketones (Blicke, 1942; fig. 14).

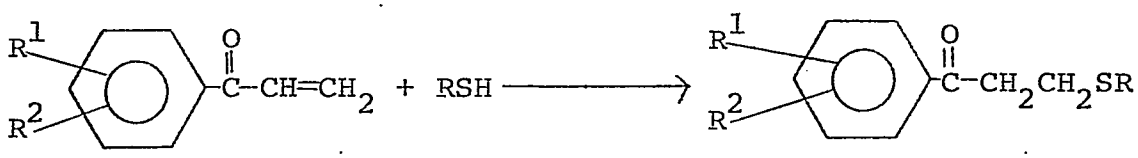


(15)

fig. 14. Decomposition of a Mannich salt

As noted earlier (Section 1.3.0.0), several α,β-unsaturated ketones and lactones display potent antineoplastic and cytotoxic activities. Since the bioactivity of these compounds seems to derive from the fact that these compounds are capable of undergoing conjugate addition with biological nucleophiles such as thiols resulting in cell death, a similar mechanism of action is conceivable in the case of

the acrylophenones generated upon decomposition of compounds belonging to series I (fig. 15).



(16)

fig. 15. Addition reaction of a thiol with an acrylophenone

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 sulphides. An elimination-addition mechanism was proposed for this reaction. To obtain corroborative evidence for what was proposed, it was decided to react a representative compound, Ie, with a biomimetic thiol, viz., 2-mercaptoethanol, under simulated physiological conditions (37°C and an aqueous buffer of pH 7.4).

Schoenenberger et al. (1969) have claimed a correlation between the rate of breakdown of Mannich bases and fungistatic activity. It was thought that a similar correlation between anticancer activity of I and rate of breakdown might emerge. Therefore, aromatic substituents with diverse σ values were chosen (TABLE II) with a view to altering the acidity of the hydrogens beta to the amine leaving group in the parent compound, Ia. Since the rate-determining step in such elimination reactions often involves

abstraction of the β-hydrogen by base, the different analogues would be expected to decompose at different rates. With these points in mind, a study of the kinetics of elimination of these compounds under simulated physiological conditions was contemplated. Since it was also decided to study the kinetics of elimination of the corresponding methobromides, the hydrobromide salts were prepared instead of the more conventional hydrochlorides with a view to keeping the anion constant so as to permit a meaningful comparison of the kinetic behaviours of the two series.

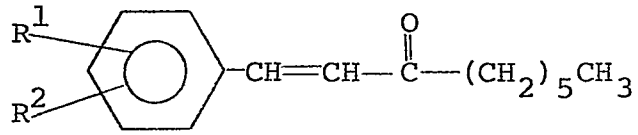
TABLE II. Hammett sigma values of the aromatic substituents[a] employed in series I.

Substituent(s)	σ
H	0.00
3-CH_3	-0.07
4-CH_3	-0.17
3-OCH_3	+0.12
4-OCH_3	-0.27
3-OH	+0.12
4-OH	-0.37
3-NO_2	+0.71
4-NO_2	+0.78
4-Cl	+0.23
3,4-Cl_2	+0.60[b]

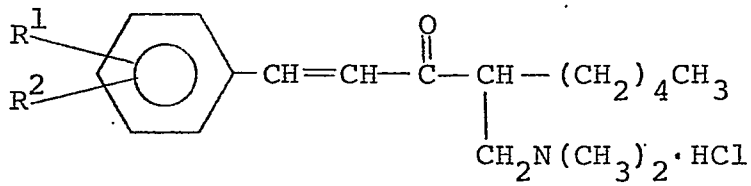
[a] Taken from Carey and Sundberg (1977d)

[b] The Σσ value was compounded from the σ_m and σ_p values for chloro group.

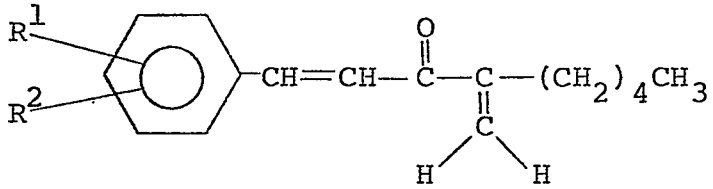
Earlier workers in this laboratory (Dimmock et al., 1976b) found a positive correlation between inhibition of mitochondrial function in yeast and activity in the KB screen in a series of α,β-unsaturated ketones (17), the corresponding Mannich bases (18) and related derivatives including (19).



(17)



(18)



(19)

a. R^1 = 4-Cl; R^2 = H

b. R^1 = 3-Cl; R^2 = 4-Cl

Thus 83 percent of the compounds tested either had mitochondrial respiration-inhibitory properties and significant activity in in the KB screen or were inactive in both tests. Similarly, 78 percent of the compounds tested showed murine toxicity at

the dose level employed, i.e. 400 mg/kg, and inhibition of mitochondrial function or had no effect on either biological parameter. Therefore, it was decided to screen some representative compounds from series I for respiration-inhibitory properties in rat liver mitochondria.

2.3.0.0 1-Aryl-3-dimethylamino-1-propanone methobromides (II)

Quaternary ammonium compounds have a wide variety of biological properties (Cavallito, 1960; Cavallito, 1980). Examples of some of the bioactivities associated with these compounds are given in TABLE III.

TABLE III. Examples of some biological activities of quaternary ammonium compounds (Burger, 1970; Warner and Warner, 1981)

Drug Prototype	Biological Activity
Acetylcholine	Cholinergic
Decamethonium	Curareform
Hexamethonium	Hypotensive
Neostigmine	Cholinesterase-inhibitory
Methantheline	Anticholinergic
Benzalkonium chloride	Antiseptic

While the quaternary ammonium group itself is very important for the activity of each of the prototypes given in TABLE III, in the 1-aryl-3-dimethylamino-1-propanone methobromides proposed to be synthesized for the purpose of this investigation, the trimethylammonium function was

conceived as a leaving group so that the alkylating species, namely, the acrylophenone, could be released <u>in vivo</u> upon deamination of the parent compound (fig. 16).

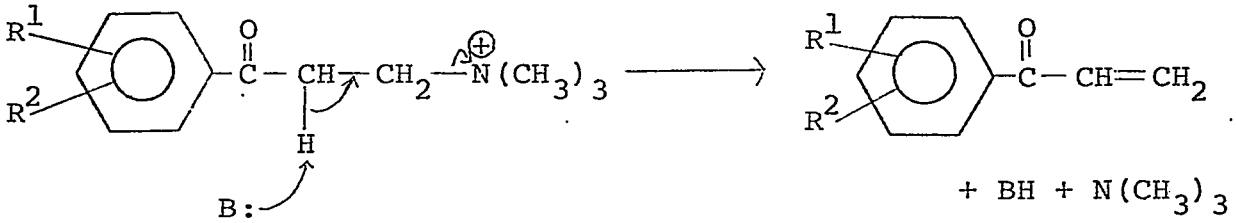
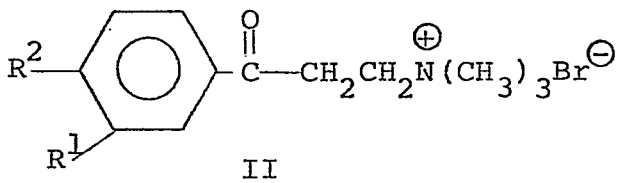


fig. 16. β-Elimination of 1-aryl-3-dimethylamino-1-propanone methobromides



II

a. $R^1 = R^2 = H$

b. $R^1 = CH_3$; $R^2 = H$

c. $R^1 = H$; $R^2 = CH_3$

d. $R^1 = OCH_3$; $R^2 = H$

e. $R^1 = H$; $R^2 = OCH_3$

f. $R^1 = OH$; $R^2 = H$

g. $R^1 = H$; $R^2 = OH$

h. $R^1 = NO_2$; $R^2 = H$

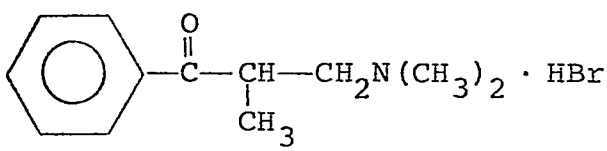
i. $R^1 = H$; $R^2 = NO_2$

j. $R^1 = H$; $R^2 = Cl$

k. $R^1 = R^2 = Cl$

The synthesis of IIa-k was contemplated with the following reasons in view. Firstly, a systematic investigation of the anticancer properties of Mannich base methobromides derived from substituted acetophenones had not been undertaken. Secondly, trimethylamine (pK_a = 9.80) would be expected to be a better leaving group than dimethylamine (pK_a = 10.77)[1]. Therefore, a comparison of the anticancer properties and murine toxicities of series I and II on the basis of the relative leaving group abilities of the two amine functions might be possible. In order to lend some credibility to such a comparison, kinetic studies aimed at quantifying the possible differences in the rates of release of the different acrylophenones were planned. A study of the effects of different aromatic substituents on the rate of elimination was also contemplated.

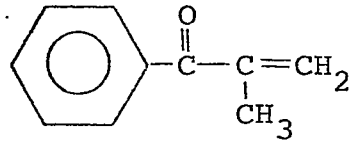
2.4.0.0 3-Dimethylamino-2-methyl-1-phenyl-1-propanone hydrobromide (III)



III

1. Smith, P.J. and Grover, T.S., Unpublished results.

The synthesis of III and its screening versus P388 lymphocytic leukemia were proposed with the following reasons in view. The introduction of a methyl group into carbon-2 of Ia as in III would be expected to decrease the acidity of the hydrogen beta to the amino group and thereby decrease the rate of release of the corresponding acrylophenone (20) in vivo.



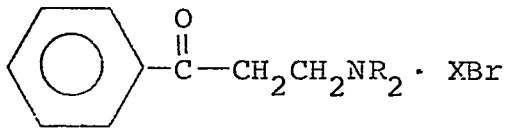
(20)

Furthermore, the methyl group would be expected to enhance the lipophilicity not only of III relative to Ia but also of (20) relative to acrylophenone. Hence it was thought that these factors might bring about an alteration in antineoplastic activity and/or murine toxicity in III relative to Ia. Finally, as noted in Section 2.2.0.0, (14) showed an interesting level of activity in the Sarcoma 180 screen. This coupled with the fact that screening data on the activity of (14) versus P388 lymphocytic leukemia were not available was an additional reason for the synthesis and testing of III.

2.5.0.0 3-Amino-1-phenyl-1-propanones (IV)

The synthesis of IV a, b, c and e was proposed with a view to studying the effect of altering the dimethylamino

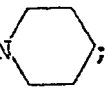
group in Ia on antineoplastic activity and murine toxicity in the P388 screen. The synthesis of the two quaternary ammonium compounds, IV d and f was also contemplated in order that their activities versus P388 lymphocytic leukemia and murine toxicities could be evaluated.



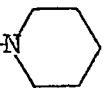
IV

a. $NR_2 = N(C_2H_5)_2$; X = H

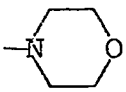
b. NR_2 = ; X = H



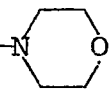
c. NR_2 = ; X = H



d. NR_2 = ; X = CH_3



e. NR_2 = ; X = H

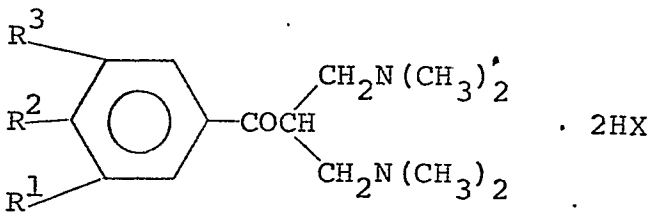


f. NR_2 = ; X = CH_3

This segment of work could be regarded as an extension of what was attempted when the synthesis of series I and II and compound III was proposed. Thus amines with different leaving group abilities might be expected to influence the rate of release of acrylophenone from IV differently. Furthermore, each amino group would be expected to confer unique solubility characteristics on IV. Hence

it was thought that the information obtained from the screening data of series V would complement that obtained from the screening data of I, II and III in arriving at meaningful structure-activity correlations in regard to both antineoplastic activity in the P388 screen and murine toxicity.

2.6.0.0 1-Aryl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrohalides (V)



V

a. $R^1 = R^2 = R^3 = H$; X = Cl

b. $R^1 = R^2 = R^3 = H$; X = Br

c. $R^1 = CH_3$; $R^2 = R^3 = H$; X = Br

d. $R^1 = R^3 = H$; $R^2 = CH_3$; X = Cl

e. $R^1 = R^3 = H$; $R^2 = CH_3$, X = Br

f. $R^1 = R^2 = CH_3$; $R^3 = H$; X = Br

g. $R^1 = OCH_3$; $R^2 = R^3 = H$; X = Cl

h. $R^1 = R^2 = H$; $R^3 = OCH_3$; X = Cl

i. $R^1 = R^3 = H$; $R^2 = OCH_3$; X = Br

j. $R^1 = R^2 = OCH_3$; $R^3 = H$; X = Br

k. $R^1 = R^2 = R^3 = OCH_3$; X = Br

l. $R^1 = R^3 = H$; $R^2 = Cl$; X = Cl

m. $R^1 = R^3 = H$; $R^2 = Cl$; X = Br

n. $R^1 = R^2 = Cl$; $R^3 = H$; $X = Cl$

o. $R^1 = R^2 = Cl$; $R^3 = H$; $X = Br$

p. $R^1 = R^3 = H$; $R^2 = OH$; $X = Br$

Synthesis of Va-p and their screening against P388 lymphocytic leukemia were contemplated for the following reasons. In series I, the methylene protons alpha to the carbonyl function are rendered acidic not only by the benzoyl moiety but also by the electron-withdrawing inductive effect of the dimethylammonium group. This would explain the possible susceptibility of series I to β-elimination. If a second dimethylammonium group is introduced into the molecule as in V, the methine proton alpha to the carbonyl group would be more acidic than the methylene protons adjacent to the carbonyl group in I. Furthermore, the olefin formed after the loss of one dimethylammonium group may react in a facile manner with nucleophiles and expedition of the loss of the remaining onium group could occur generating a further centre for nucleophilic attack (fig. 17).

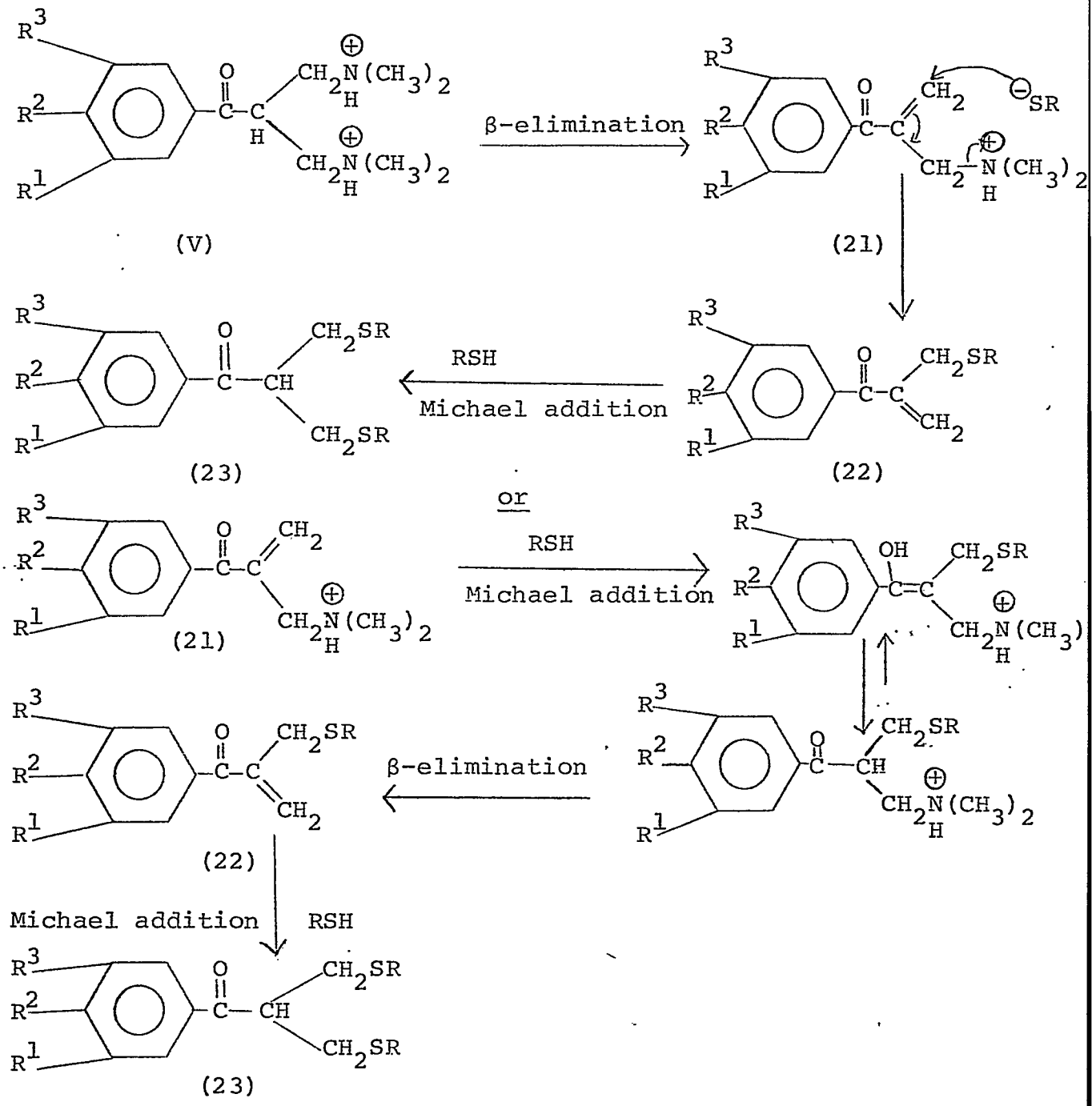


fig. 17. Possible mechanisms of alkylation of cellular nucleophiles such as thiols by 1-aryl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrohalides

For reasons similar to those outlined in Section 2.2.0.0, it was also proposed to screen a few representative compounds from series V for respiration-inhibitory properties in rat liver mitochondria. Furthermore, in order to test the validity of the hypothesis that these compounds could act as bifunctional alkylating agents it was proposed to react a representative compound, Vo, with a biomimetic thiol, 2-mercaptoethanol, under simulated physiological conditions (an aqueous buffer of pH 7.4 and 37°C).

2.7.0.0 3,5-bis-(Dimethylaminomethyl)-4-hydroxyacetophenone dihydrobromide (VI).

Several Mannich base hydrochlorides derived from 1-and 2-naphthols were shown to react with arenethiols in aqueous-alcoholic solutions to give the corresponding sulphides (Andrisano et al., 1970). From the kinetic data obtained, an elimination-addition mechanism was proposed for these reactions (fig. 18).

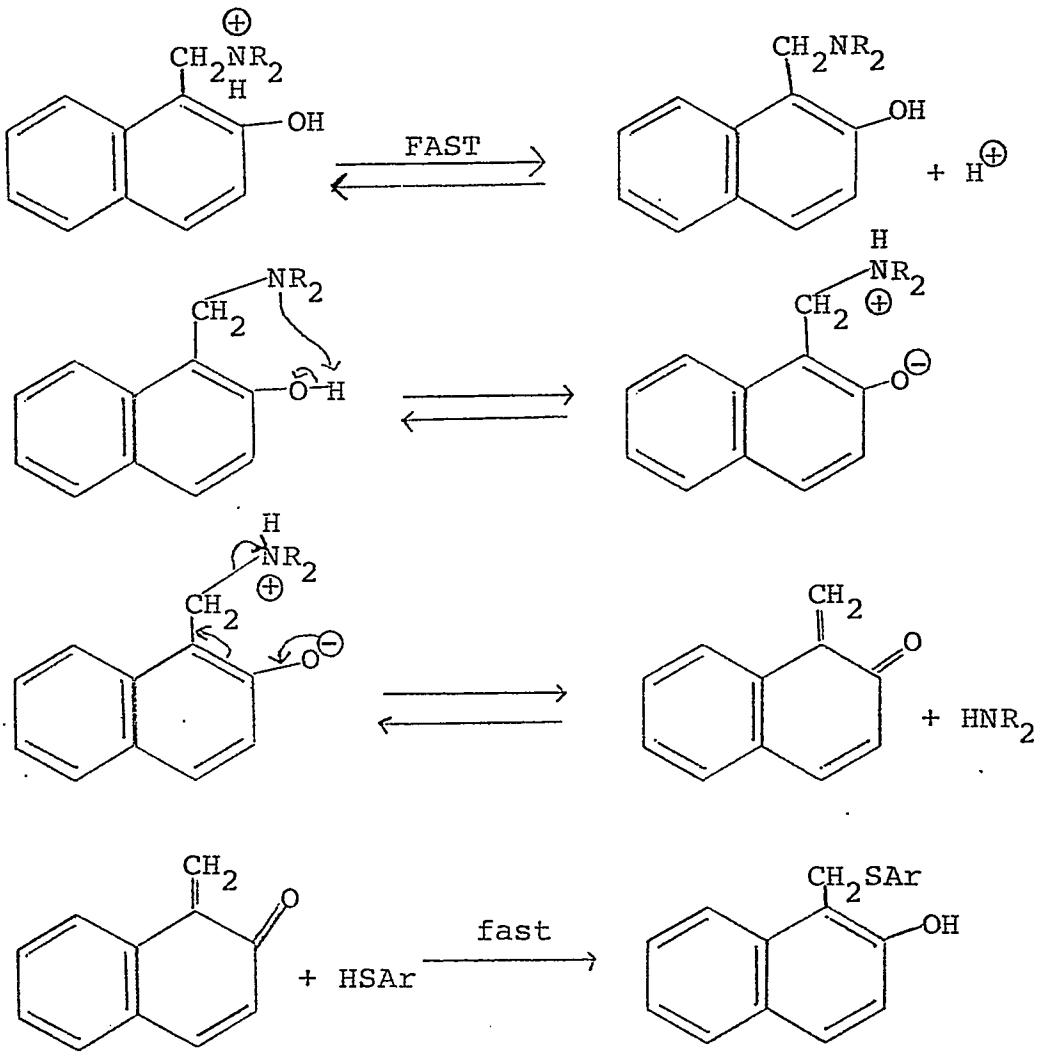
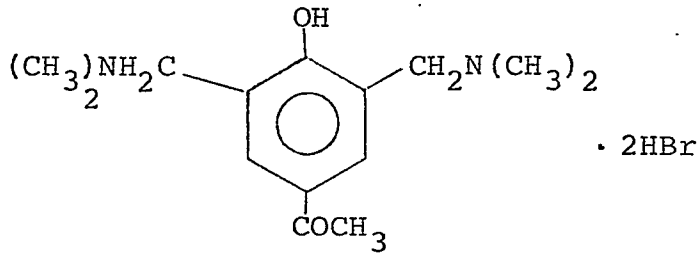


fig. 18. Mechanism of reaction of a Mannich salt of 2-naphthol with an arenethiol.

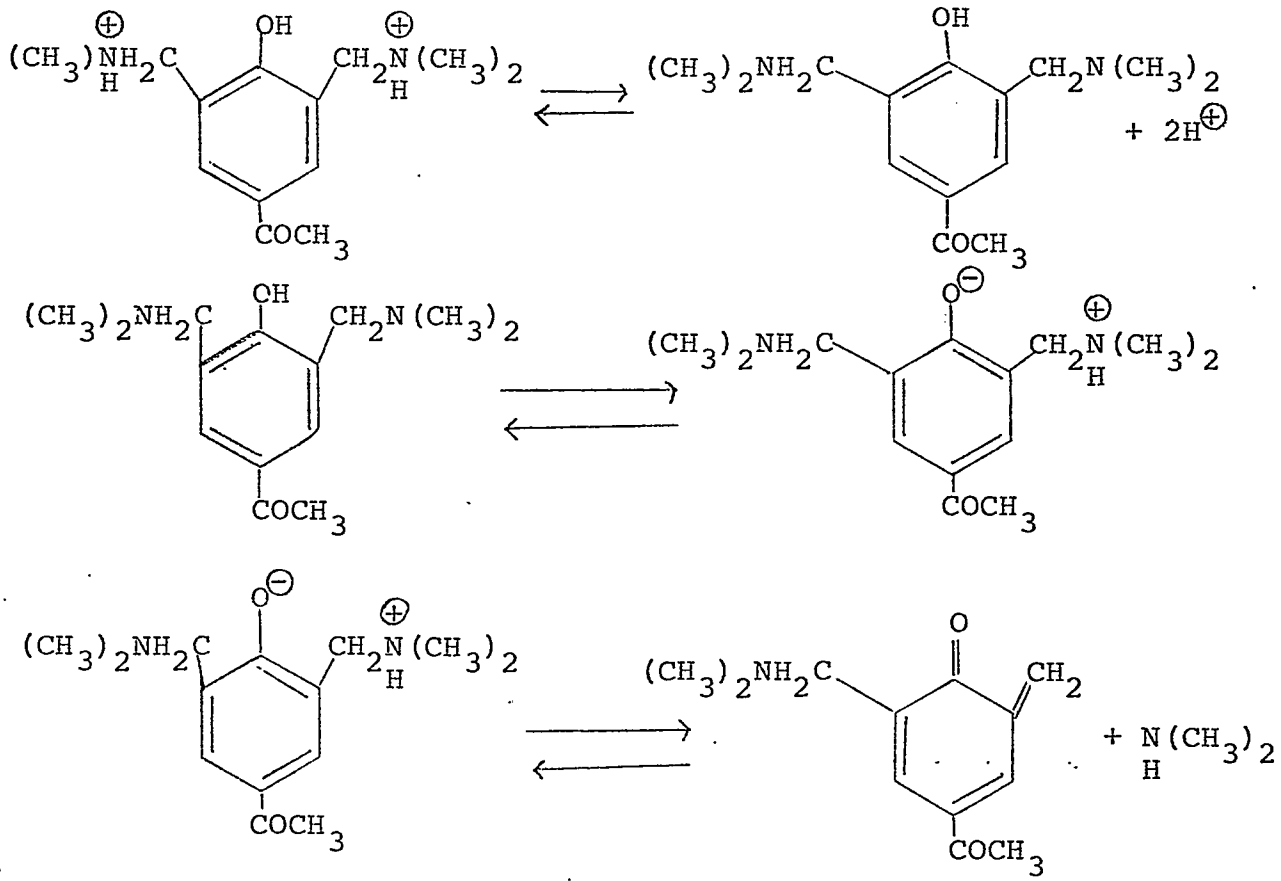
Based on these findings, VI was designed and its synthesis contemplated with a view to evaluating its activity against P388 lymphocytic leukemia.



· 2HBr

VI

It was thought that the presence of two dimethylaminomethyl groups could make VI act as a bifunctional alkylating agent as shown in fig. 19.



(24)

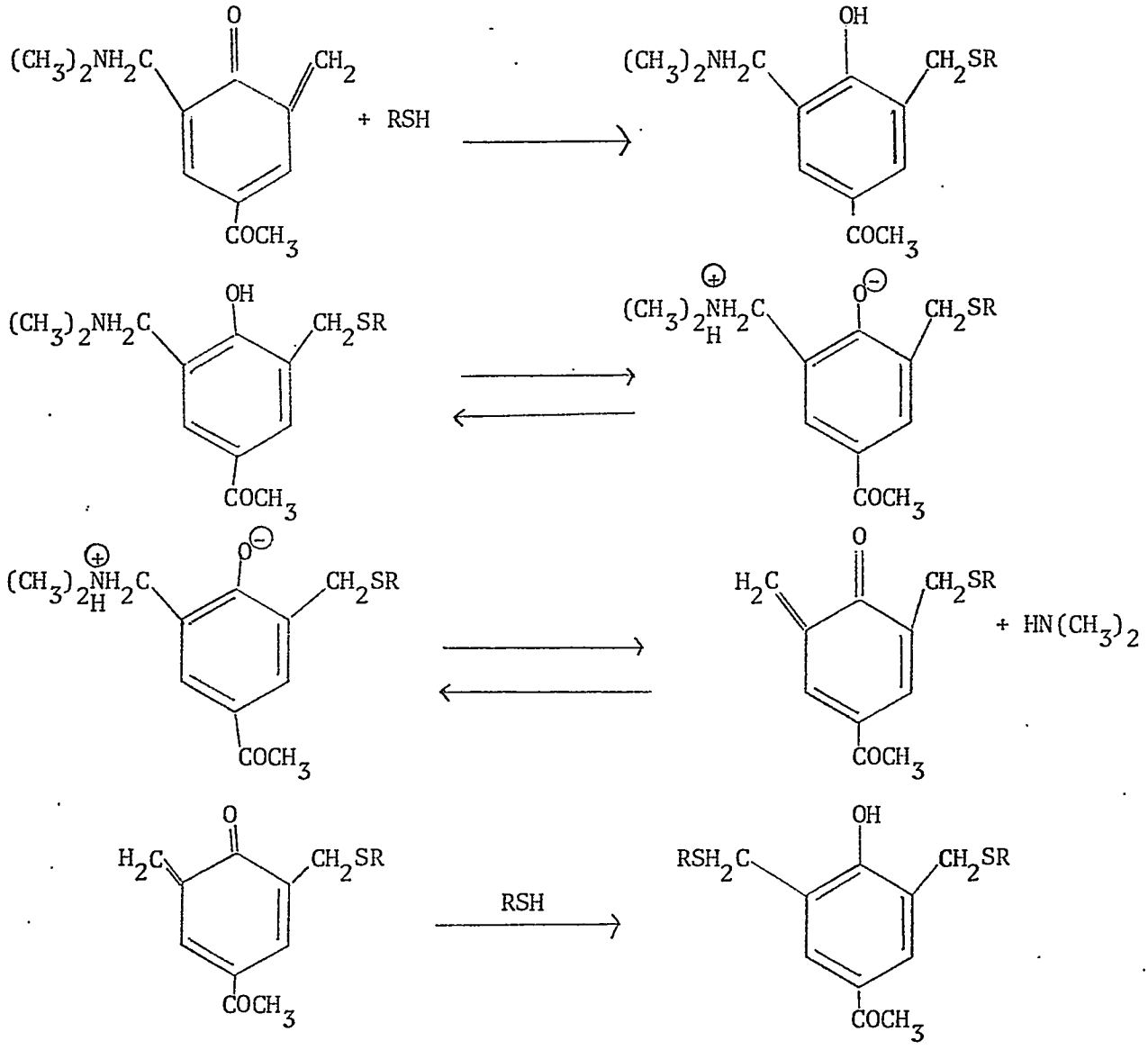
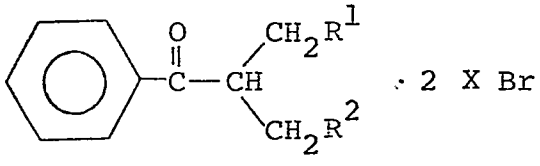


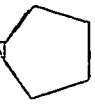
fig. 19. Proposed mechanism of reaction of 3,5-bis-(dimethylaminomethyl)-4-hydroxyacetophenone dihydrobromide with a thiol.

2.8.0.0 3-Amino-2-aminomethyl-1-phenyl-1-propanones (VII)

Several compounds with the general structure VII were proposed to be synthesized for evaluation in the P388 screen.

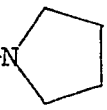


VII

a. $R^1 = R^2 =$  ; X = H

b. $R^1 = R^2 =$  ; X = H

c. $R^1 = R^2 =$  ; X = H

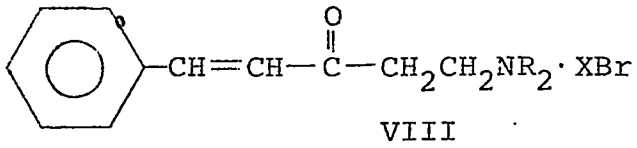
d. $R^1 = N(CH_3)_2$; $R^2 =$  ; X = H

e. $R^1 = R^2 = N(CH_3)_2$; X = CH_3

The structural modification of Va to give VII a-e was dictated by considerations similar to those that led to the molecular modification of Ia to give IIa and IV a-f.

2.9.0.0 5-Amino-1-phenyl-1-penten-3-ones (VIII)

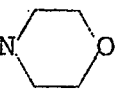
If the Mannich bases derived from acetophenones are reactive with cellular nucleophiles per se, then the insertion of an olefinic linkage between the aromatic ring and the keto function in such compounds to give the general structure VIII should alter the bioactivity dramatically.

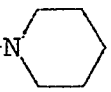


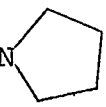
VIII

a. $NR_2 = N(C_2H_5)_2$; X = H

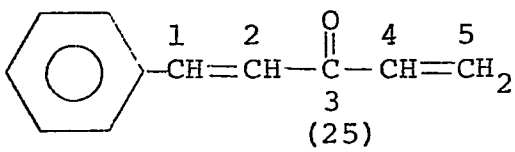
b. $NR_2 = N(C_2H_5)_2$; $X = CH_3$

c. NR_2 =  ; X = H

d. NR_2 =  ; X = H

e. NR_2 =  ; X = H

If deamination occurs <u>in vivo</u>, then an α,β,α',β'-diolefinic ketone (25) will have been formed which may permit cross-linking of DNA strands to occur. It may be added that (25) may be considered similar to a nitrogen mustard in that the strands could be cross-linked by a five-membered arm (fig. 20).



(25)

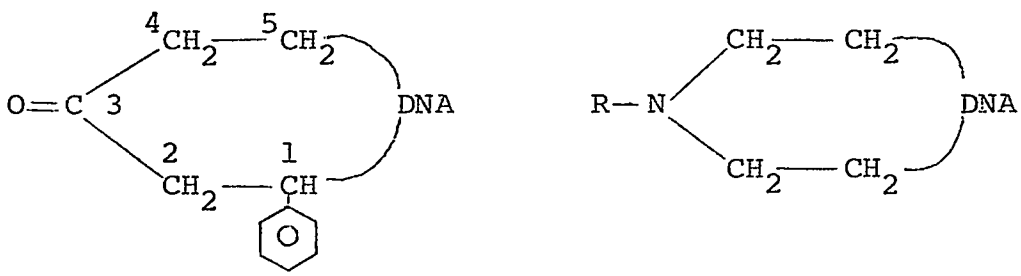
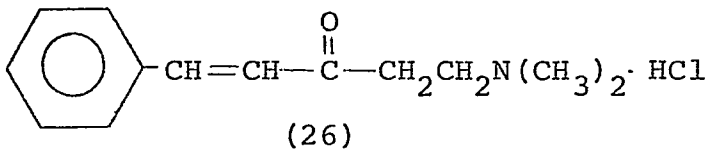


fig. 20. Possible cross-linking of DNA strands by 1-phenyl-1,4-pentadien-3-one and a nitrogen mustard.

A related compound (26) synthesized earlier in this laboratory showed cytotoxic properties in the KB screen but was inactive (T/C % 108) versus P388 lymphocytic leukemia (Dimmock et al., 1979a).

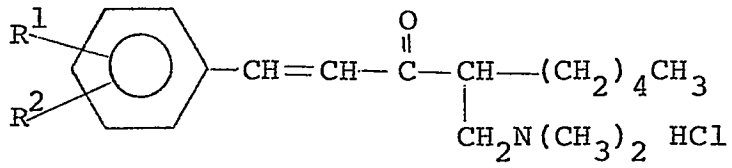


(26)

It was thought that the inactivity of (26) could, at least in part, be due to either of two factors: a) a suboptimal rate of release of the $\alpha,\beta,\alpha',\beta'$-diolefinic ketone (25) and b) suboptimal solubility characteristics of (26) per se or of its free base. Therefore it was thought that these parameters could be varied by introducing amine functions into VIII with different leaving group abilities and different solubility characteristics. Screening of the resultant compounds VIII a-e against P388 lymphocytic leukemia was also contemplated.

2.10.0.0 4-Aminomethyl-1-phenyl-1-nonen-3-ones (IX)

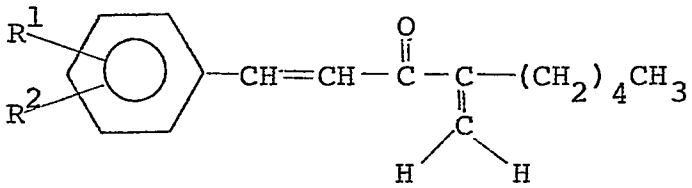
Mannich bases with the general structure (27) have displayed significant antineoplastic and cytotoxic properties (Dimmock and Taylor, 1975).



(27)

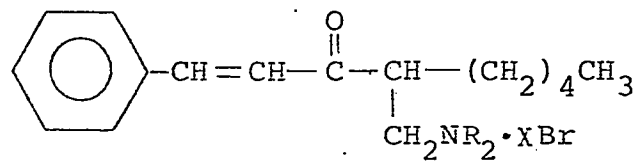
$R^1 = R^2 = H, Cl$

While attempts have been made by earlier workers (Dimmock and Taylor, 1975; Dimmock et al., 1978; Dimmock et al., 1979b) to modify the anticancer activity in this series by using different substituents on the aromatic ring, not many attempts have been made to modify the amine function. If the biological activity of this series of compounds is due to elimination in vivo to release the diolefinic ketone (28), a change in the amino group would alter the rate at which the active species is formed.

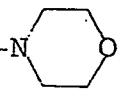


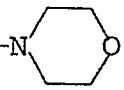
(28)

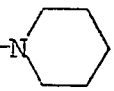
Therefore, the synthesis of IX a-c was proposed with IX a and b incorporating bases weaker than dimethylamine and IX c incorporating a base stronger than dimethylamine.



IX

a. NR_2 =  ; X = H

b. NR_2 =  ; X = CH_3

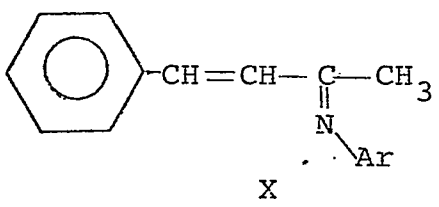
c. NR_2 =  ; X = H

It was considered at the time that these amines would confer different solubility characteristics on IX which might have to be taken into account in the interpretation of the screening data.

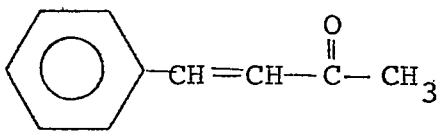
2.11.0.0 Benzalacetone anils (X)

Schiff bases have been known to inhibit the growth of tumours (Hodnett and Willie, 1966; Hodnett and Dunn, 1970). Billman et al. (1969) have tried to exploit the acid liability of Schiff bases to design some potential antineoplastic agents. The rationale used is as follows. Several workers (Eden et al., 1956; Kahler and Robertson, 1943; Meyer et al., 1948) have claimed that the milieu of some tumour cells has a lower pH than that of corresponding normal cells. The increased acidity of tumours has been

ascribed to the greater rate of glycolysis in neoplastic tissue, which leads to increased lactic acid production (Warburg et al., 1924; Cori and Cori, 1925). The average pH value for a number of tumour tissues has been considered to be ∿ 6.5 (Abel et al., 1975). Hence a prodrug such as a Schiff base, permitting the release of a cytotoxic agent (e.g., a ketone, an aldehyde or an amine) under acidic conditions may afford selective toxicity to these tumours with reduced toxicity for normal cells. While the Schiff bases synthesized by Billman et al. (1969) were conceived as prodrugs of an antineoplastic amine, the same principle was employed in the design of Xa and b where the active moiety is an α,β-unsaturated ketone, 4-phenyl-3-buten-2-one or benzalacetone [(29); T/C % 126 (Dimmock and Smith, 1980)].



X



(29)

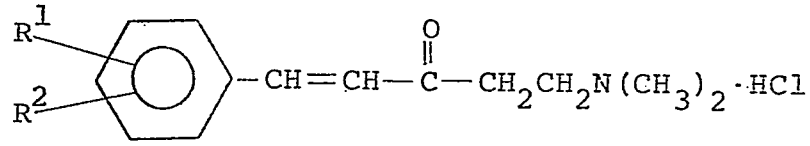
a. Ar = C_6H_5

b. Ar = 4-ClC_6H_4-

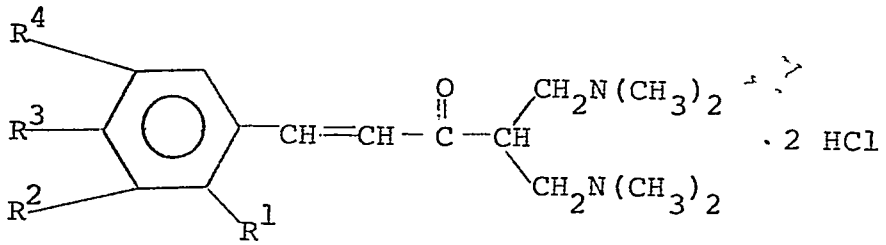
The evaluation of compounds Xa and b in the P388 screen was contemplated so that anticancer activities and murine toxicities of these compounds could be compared with those of (29).

2.12.0.0 1-Aryl-5-dimethylamino-4-dimethylaminomethyl-1-penten-3-one dihydrochlorides (XI)

The structural modification of 1-aryl-5-dimethylamino-1-penten-3-one hydrochloride (30) to give XI a-i was contemplated for reasons similar to those invoked for the proposed molecular modification of I to give V.



(30)



XI

a. $R^1 = R^2 = R^3 = R^4 = H$

b. $R^1 = R^3 = R^4 = H$; $R^2 = CH_3$

c. $R^1 = R^2 = R^4 = H$; $R^3 = CH_3$

d. $R^1 = R^2 = R^4 = H$; $R^3 = OCH_3$

e. $R^1 = R^4 = H$; $R^2 = R^3 = OCH_3$

f. $R^1 = H$; $R^2 = R^3 = R^4 = OCH_3$

g. $R^1 = R^4 = H$; $R^2, R^3 = O-CH_2-O$

h. $R^1 = R^2 = R^4 = H$; $R^3 = OH$

i. $R^1 = R^3 = Cl$; $R^2 = R^4 = H$

As in the case of V, an increase in the rate of elimination would be predicted for XI relative to (30). It was thought that this might be an advantage since the rate constants for the addition of thiols to α,β-unsaturated ketones of the type under consideration are often quite high at physiological pH, i.e., pH 7.4 (Dimmock et al., 1980a) and so if thiol addition occurs with the α,β-unsaturated ketone moiety already present in (30) before the second olefinic moiety is generated, the rate of deamination would be slowed down still further because of two reasons: a) The hydrogens beta to the dimethylammonium group in the thiol adduct (31) would be less acidic than those in (30) and b) the olefin (32) formed from (30) would be more stable than that (33) formed from (31) (fig. 21).

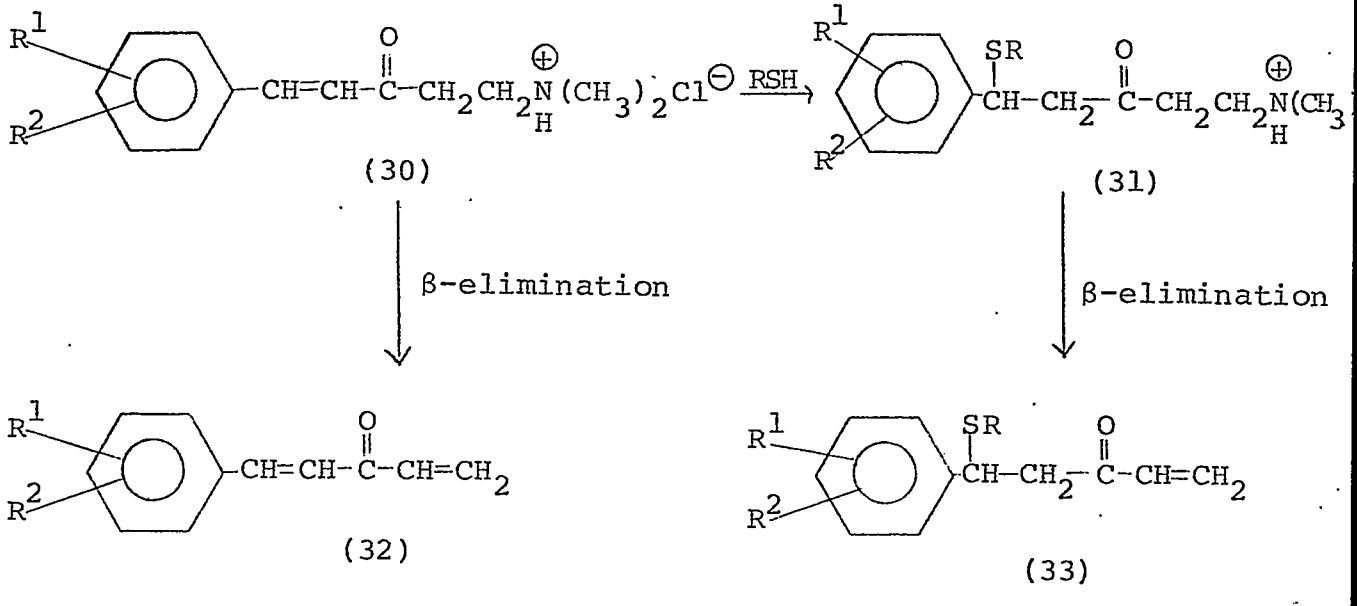


fig. 21. Possible reaction pathways of thiols with Mannich bases derived from conjugated styryl ketones.

From the above argument, it follows that a high probability of formation of the diolefinic ketone (32) is associated with a rate of deamination that is comparable to or more rapid than the rate of thiol addition to (30) to give (31). It was expected that XI a-i would fulfil this requirement. Furthermore, because of their structural similarity not only to VIII but also to V these compounds would be expected to act as trifunctional alkylating agents (fig. 22).

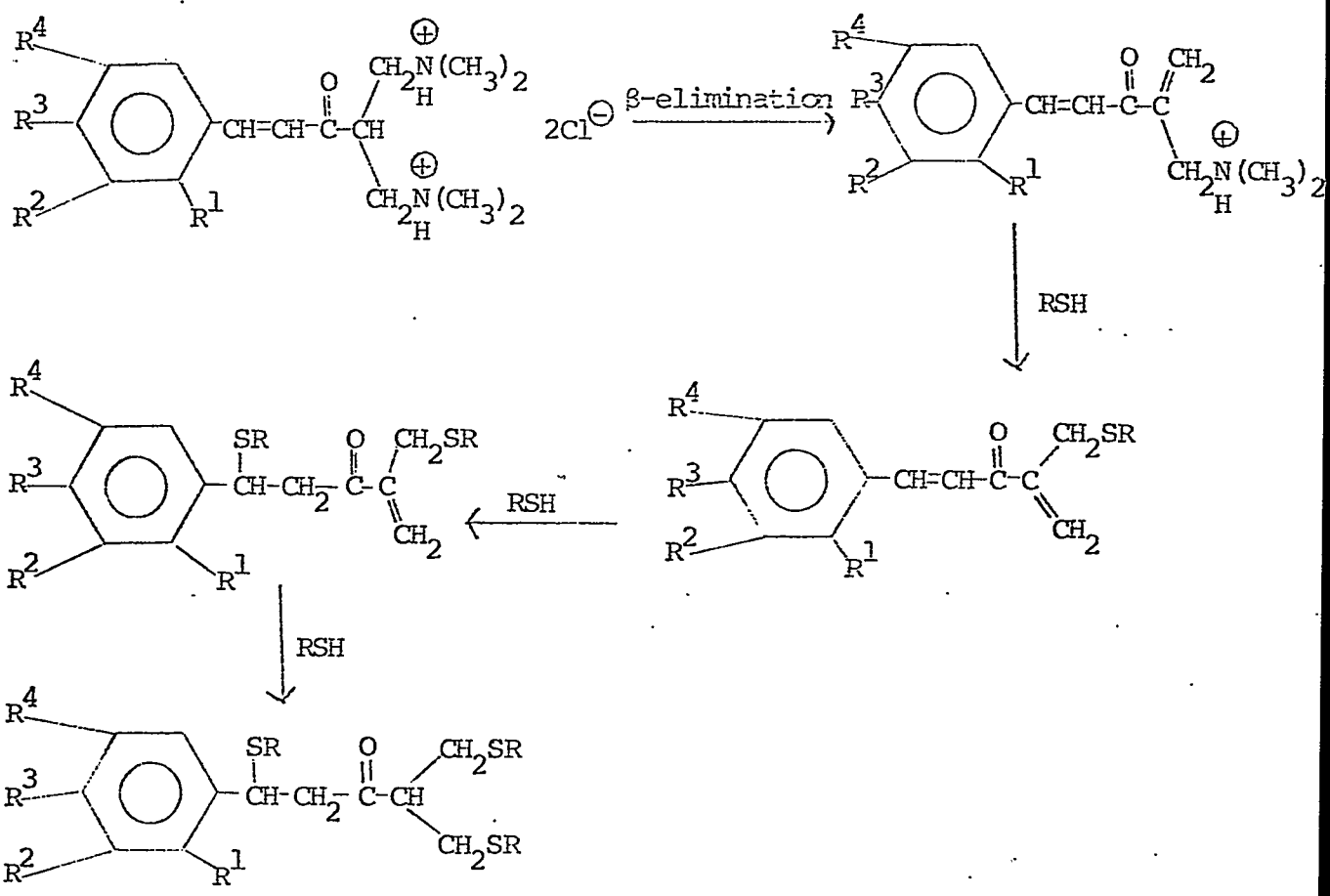


fig. 22. Possible reactions of 1-aryl-5-dimethylamino-4-dimethylaminomethyl-1-penten-3-one dihydrochlorides with thiols.

3.0.0.0 DISCUSSION OF THE EXPERIMENTAL WORK

3.1.0.0 Introduction to the Mannich reaction

The Mannich reaction consists of the condensation of a substrate possessing at least one active hydrogen with an aldehyde (often formaldehyde) and a primary or secondary amine (or, occasionally, ammonia). Examples of Mannich substrates include alkyl ketones, phenols, NH-heterocycles, alkynes, etc. (Tramontini, 1973). The essential consequence of the reaction is the replacement of the active hydrogen by an aminomethyl group (fig. 23).

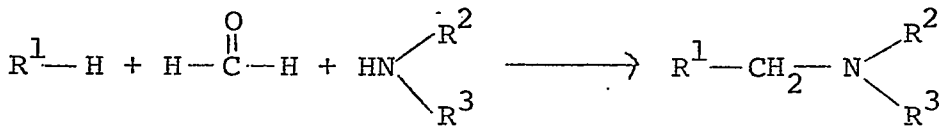


fig. 23. The Mannich reaction.

Since many compounds have more than one replaceable hydrogen, multiple aminomethylations may occur at the position alpha to the carbonyl group in such compounds. Acetophenone, for instance, may give a mono-or disubstituted product depending upon the molar ratio of the reactants used (figs. 24 and 25; Thompson, 1968).

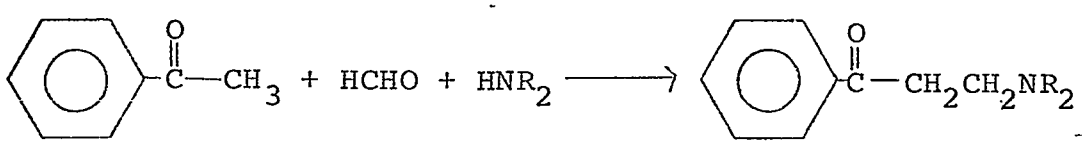


fig. 24. Monoaminomethylation of acetophenone

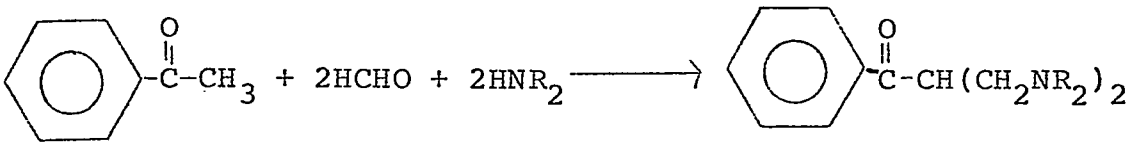


fig. 25. Diaminomethylation of acetophenone

A similar situation prevails in the case of phenols where mono- and disubstituted products including positional isomers are formed as a consequence of multiple aminomethylation (fig. 26; van Marle and Tollens, 1903).

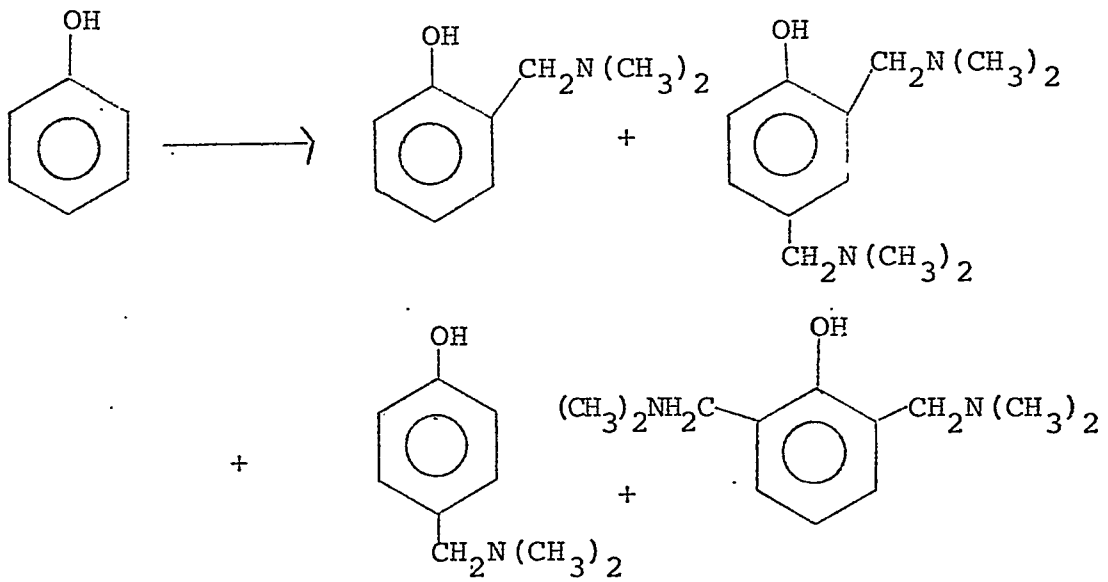
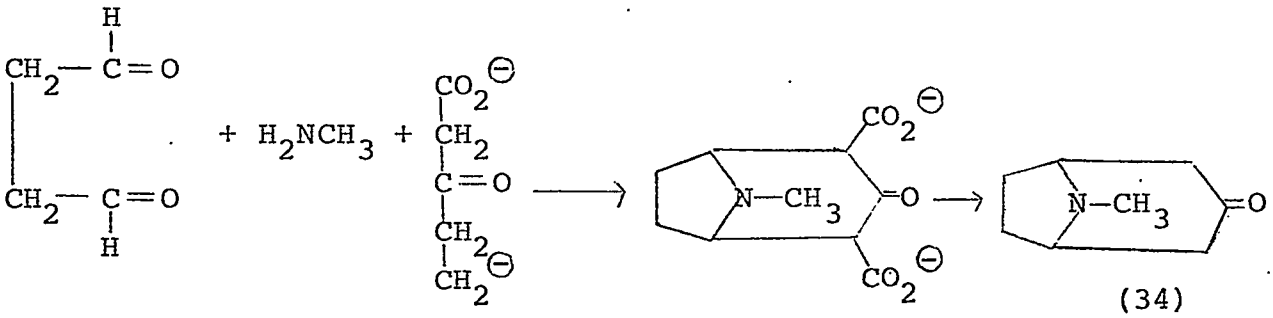


fig. 26. Aminomethylation of phenol

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

Common secondary amines used in Mannich reactions include dimethylamine, diethylamine, pyrrolidine, piperidine and morpholine.

The aldehyde used in the Mannich reaction is generally formaldehyde or its trimer, trioxymethylene. However, in one of the earliest examples of the use of the Mannich reaction in the synthesis of natural products, Robinson (1917) made use of succindialdehyde in his ingenious synthesis of tropinone (34; fig. 27).



(34)

fig. 27. Robinson's synthesis of tropinone

The mechanism of the Mannich reaction under acidic conditions is shown in fig. 28 (Tramontini, 1973; Thompson, 1968; Carey and Sundberg, 1977e).

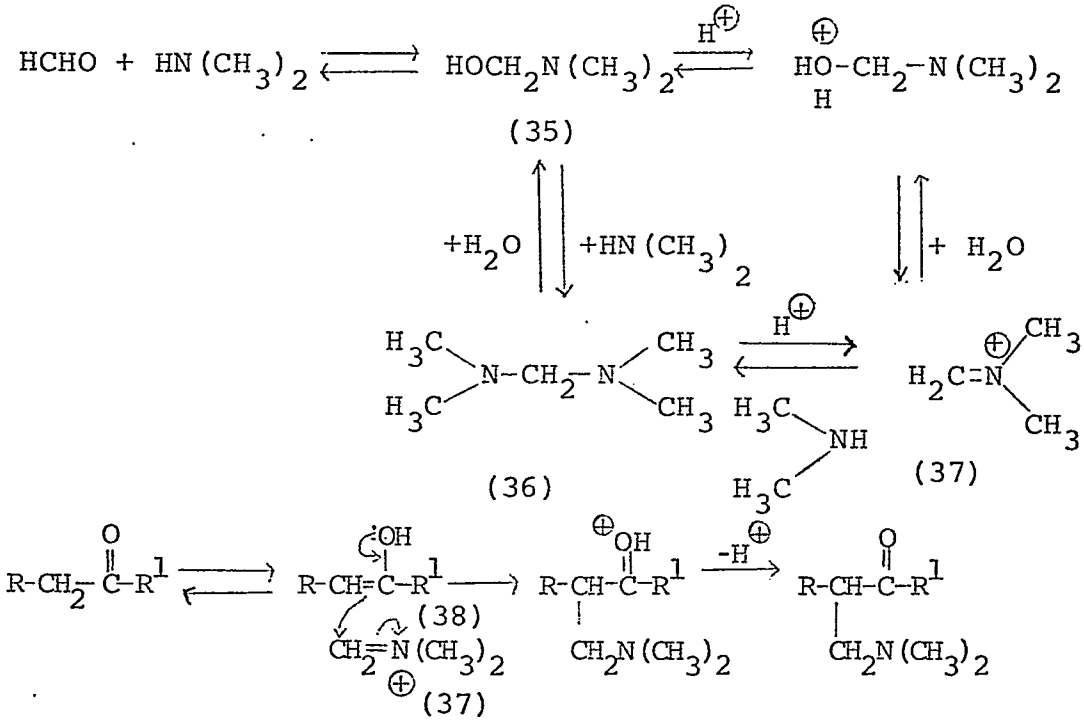


fig. 28. Mechanism of the Mannich reaction under acidic conditions.

Under basic conditions, however, the aminomethylating species has been postulated to be the hydroxymethylamine (35) or the methylene-bis-amine (36). In the case of cyclohexanones, for instance, the mechanism given in fig. 29 is the one postulated by Cummings and Shelton (1960).

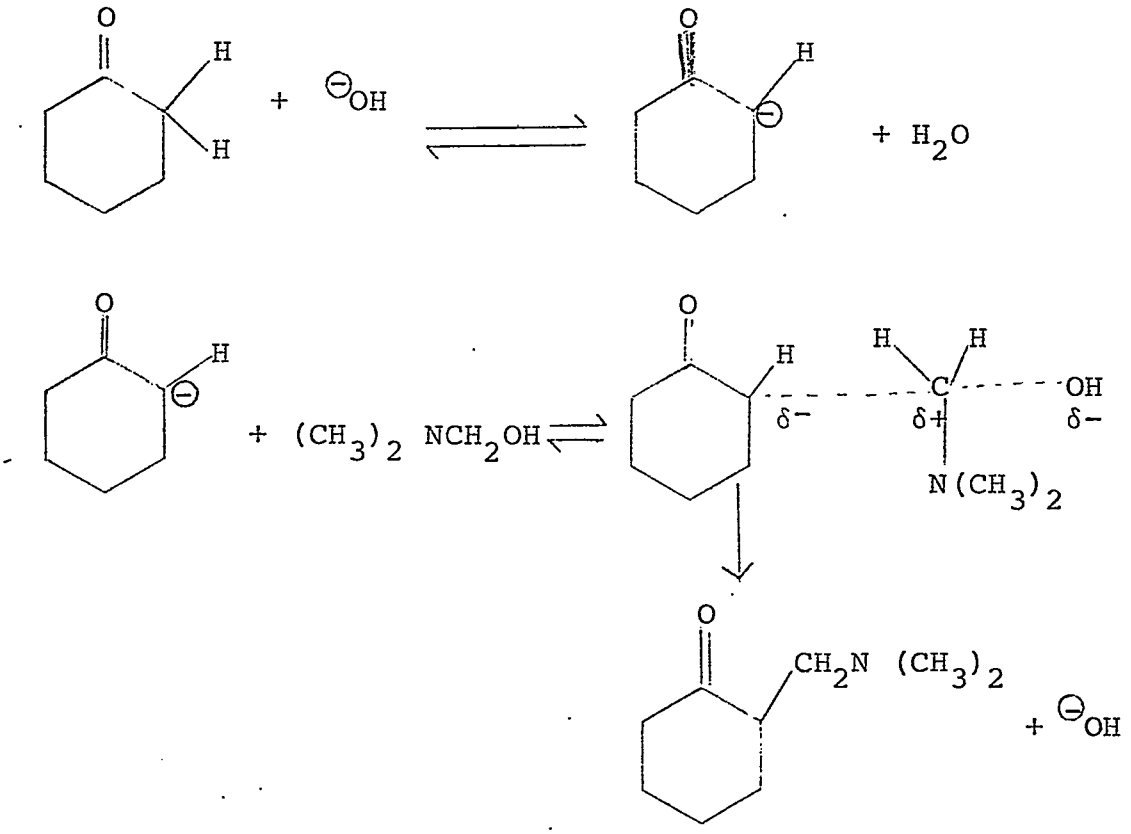


fig. 29. Mechanism of aminomethylation of cyclohexanone due to Cummings and Shelton (1960).

However, a mechanism involving a hydrogen-bonded complex has also been suggested for both carbonyl and phenolic substrates (fig. 30). This mechanism would explain why ortho-substitution predominates in phenolic substrates (Burckhalter et al., 1964; Burckhalter and Lieb, 1961).

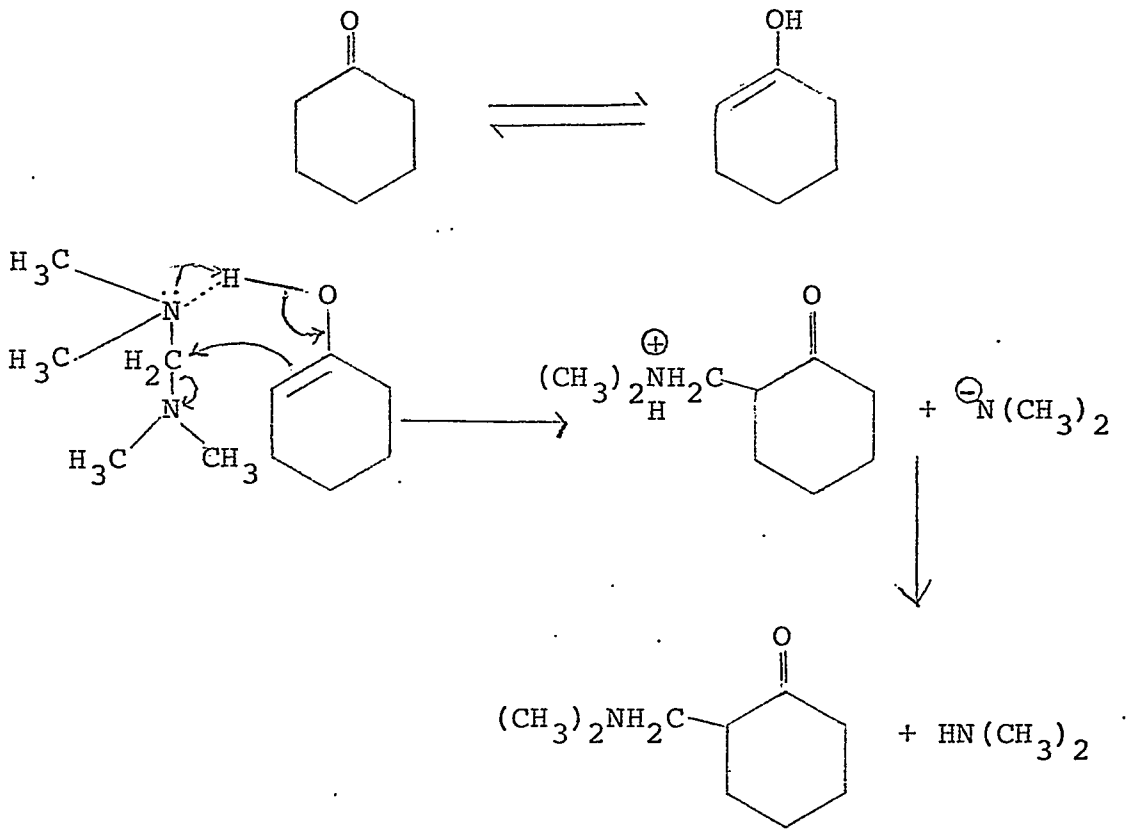
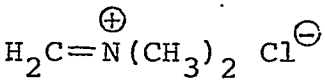


fig. 30. Mechanism of aminomethylation of cyclohexanone involving a hydrogen-bonded complex as intermediate.

As noted earlier, the aminomethylating species in acid-catalyzed Mannich reactions is the iminium ion (37) formed *via* equilibria. Recently, a great deal of success has been achieved by using iminium ions directly as the aminomethylating species. As noted by Holy *et al.* (1979) this approach has several advantages: (1) faster reactions can be expected 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.

Kinast and Tietze (1976) studied the α-aminoalkylation of some aldehydes and ketones using N,N-dimethyl(methylene) ammonium chloride (39) as the alkylating species.



(39)

In several cases they demonstrated the superiority of aminomethylation with the iminium salt (39) over the conditions of the classical Mannich reaction. In the same study it was found that unsymmetrical ketones were almost exclusively aminomethylated at the more highly substituted carbon atom (e.g. fig. 31).

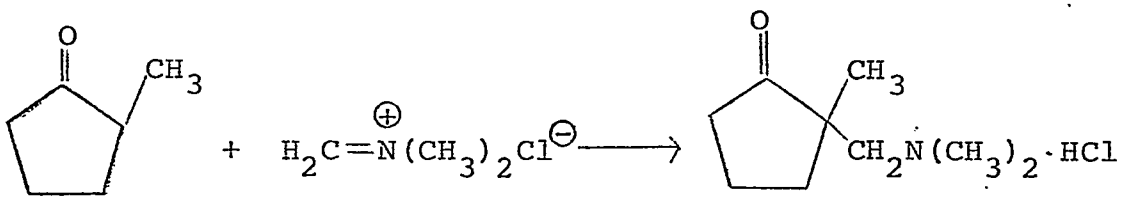


fig. 31. Aminomethylation of 2-methylcyclopentanone with dimethyl(methylene)ammonium chloride.

Recently, a regioselective Mannich reaction of 3-methyl-2-butanone with dimethyl(methylene)ammonium trifluoroacetate(40) has been reported (Gaudry et al., 1979). In this reaction, the ketone is almost exclusively aminomethylated at the less substituted carbon atom (fig. 32).

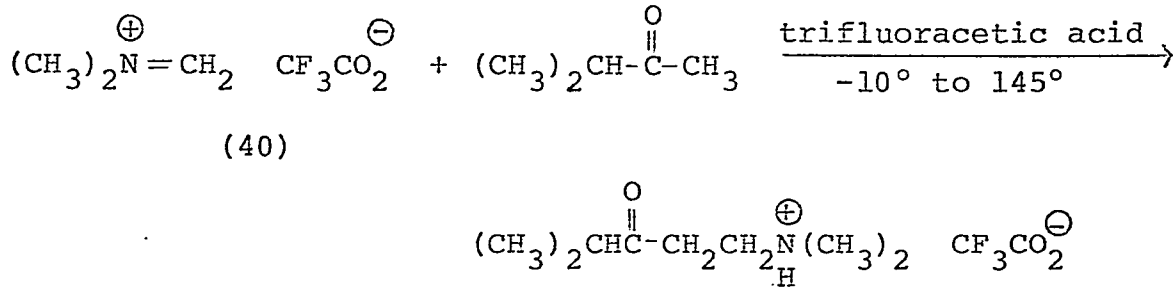


fig. 32. Aminomethylation of 3-methyl-2-butanone with dimethyl(methylene)ammonium trifluoroacetate.

3.2.0.0 Preparation of 3-amino-1-aryl-1-propanone hydrobromides (Ia-k, IVa-c, e)

The method of Maxwell (1943) was adapted for the preparation of Ia-h, IVa-c and IVe. Thus an alcoholic solution of the appropriate acetophenone, paraformaldehyde, the amine hydrochloride and hydrochloric acid was heated under reflux for 2-4 hours to obtain the β-aminoketone in the form of its hydrochloride salt. The free base obtained upon neutralization of this salt was converted to the corresponding hydrobromide using hydrogen bromide gas as shown in fig. 33.

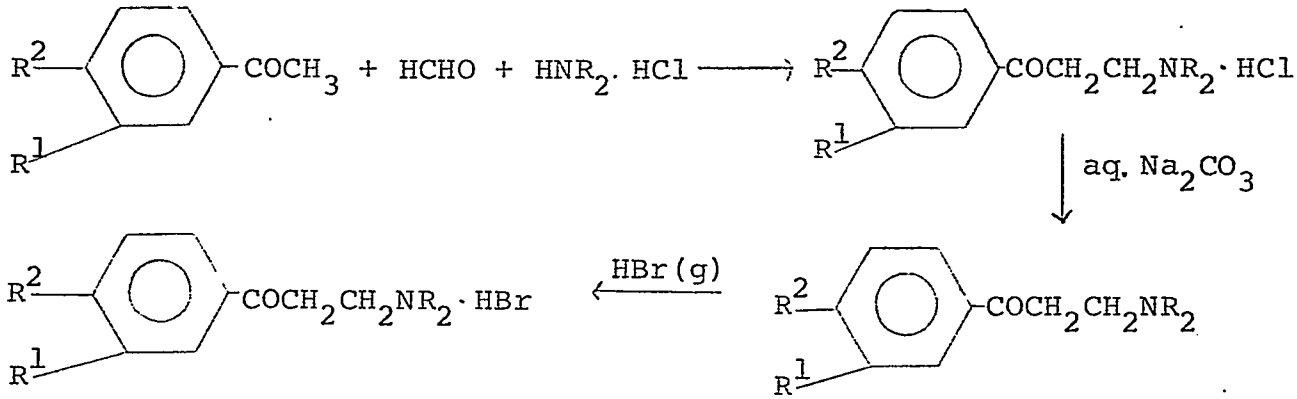


fig. 33. Synthesis of 3-amino-1-aryl-1-propanone hydrobromides using the conditions of the classical Mannich reaction.

In some cases where the method shown in fig. 33 gave poor yields of I (e.g., Ii-k), the use of dimethyl (methylene)ammonium chloride (39) gave significantly higher yields. Compound (39), which was prepared by reacting acetyl chloride with N,N,N′,N′-tetramethyldiaminomethane in methylene chloride, was reacted with the appropriate acetophenone in acetonitrile to obtain the desired product (fig. 34; Kinast and Tietze, 1976).

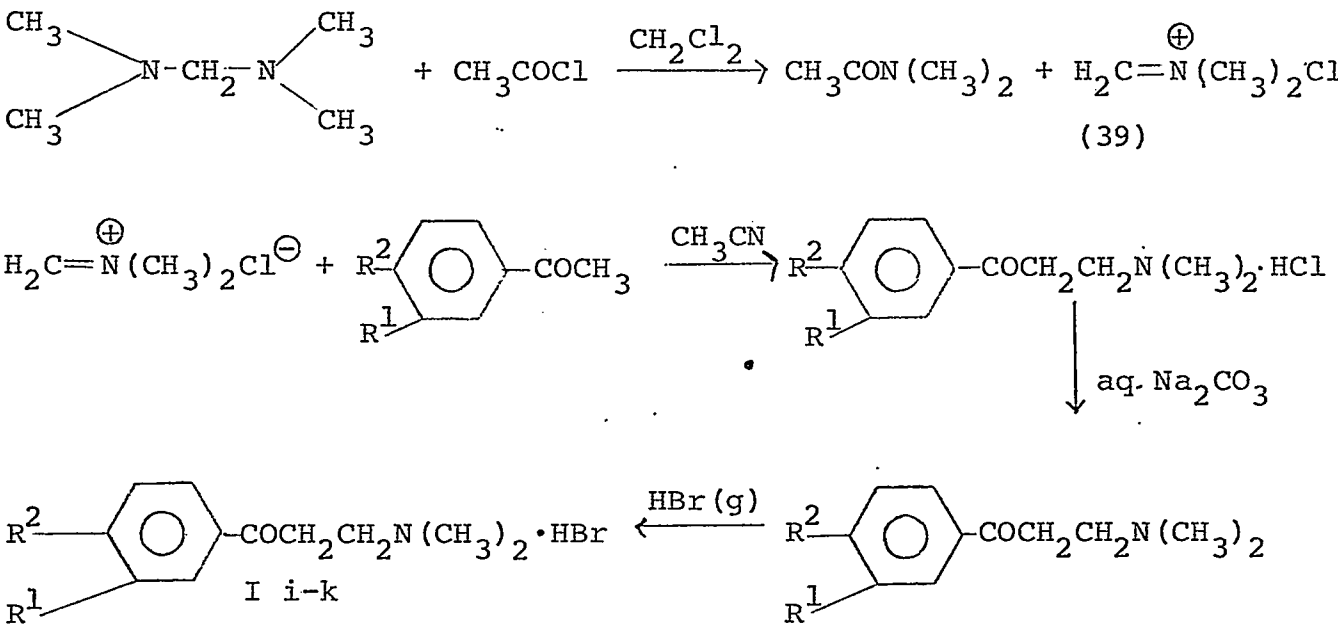


fig. 34. Synthesis of 3-amino-1-aryl-1-propanone hydrobromides using dimethyl(methylene)ammonium chloride as the aminomethylating species.

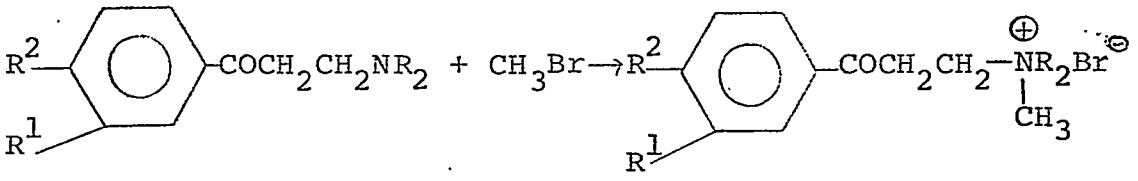
In the case of the 3,4-dichloro compound, Ik, the change in solvent from ethanol to 1,2-dimethoxyethane as suggested by Hirai et al. (1963), while retaining the overall method shown in fig. 33, resulted in a less dramatic increase in yield than

when the method was changed to that shown in fig. 34. Thus, while the yield of the Mannich base hydrochloride increased by 14% compared to Maxwell's method in the first instance, the increase was a more dramatic 56% in the second.

The physical data for compounds Ia-k, IVa-c and IVe are given in TABLES XV (Section 4.1.0.0) and XVII (Section 4.4.0.0).

3.3.0.0 Preparation of 3-amino-1-aryl-1-propanone methobromides (II a-k, IV d and IV f)

Compounds II a-k, IV d and IV f were prepared by quaternizing the corresponding 3-amino-1-aryl-1-propanones with methyl bromide in anhydrous ether or dry acetone as shown in fig. 35.



II a-k, IV d,f

fig. 35. Synthesis of 3-amino-1-aryl-1-propanone methobromides.

In general, the methobromides were obtained in lower yields than the corresponding hydrobromide salts. This could be ascribed to the greater susceptibility of the quaternary ammonium compounds to elimination in the presence of methanol (which was used as a recrystallization solvent) than the corresponding hydrobromide salts.

The physical data for compounds II a-k, IV d and IV f are given in TABLES XVI (Section 4.2.0.0) and XVII (Section 4.4.0.0).

3.4.0.0 Preparation of 3-dimethylamino-2-methyl-1-phenyl-1-propanone hydrobromide (III)

3-Dimethylamino-2-methyl-1-phenyl-1-propanone hydrobromide was synthesized using the method shown in fig. 36.

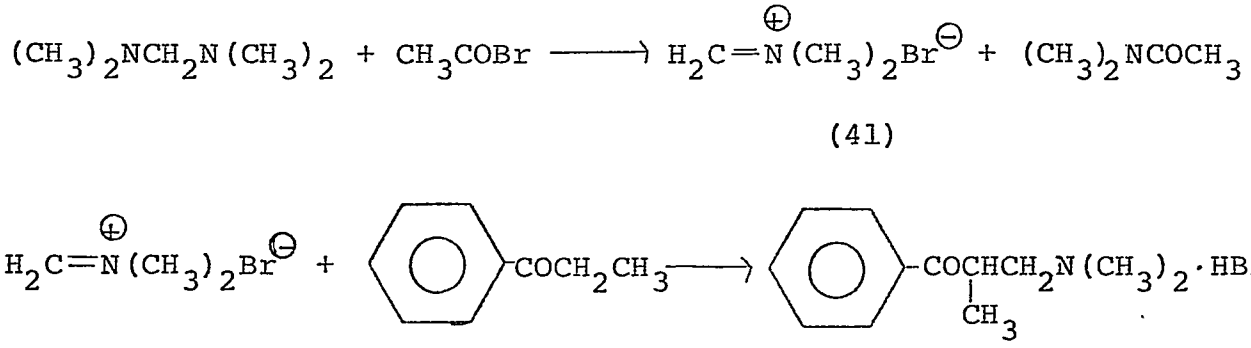
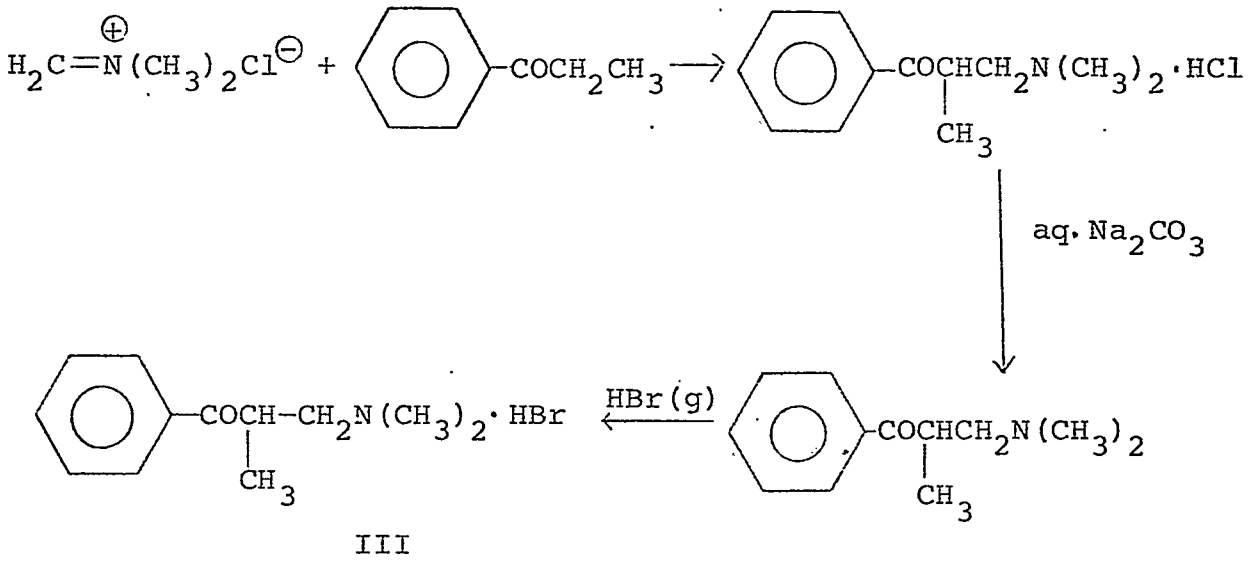


fig. 36. Synthesis of 3-dimethylamino-2-methyl-1-phenyl-1-propanone hydrobromide.

It was thought that the direct use of dimethyl(methylene) ammonium bromide (41) in the Mannich reaction would give a higher yield of the product than a multistep synthetic route such as that shown in fig. 37 since the latter would involve losses due to handling.



III

fig. 37. Multistep synthetic route for 3-dimethylamino-2-methyl-1-phenyl-1-propanone hydrobromide.

The physical data for compound III are given in Section 4.3.0.0.

3.5.0.0 Antineoplastic activity of 3-amino-1-aryl-1-propanone hydrobromides (I a-k, IV a-c, e).

While all β-aminoketone hydrobromides prepared, including I a-k, IV a-c and IV e, are uniformly inactive against P388 lymphocytic leukemia, they display a wide range of murine toxicities (see Sections 4.1.0.0 and 4.4.0.0; TABLES XV and XVII). The inactivity of these compounds is perhaps due to extremely slow rates of release of the corresponding acrylophenones resulting in suboptimal concentrations of the alkylating species at the target site at any given time (see Section 3.23.0.0). The toxicity pattern observed, however, is less easy to explain. While the relatively low

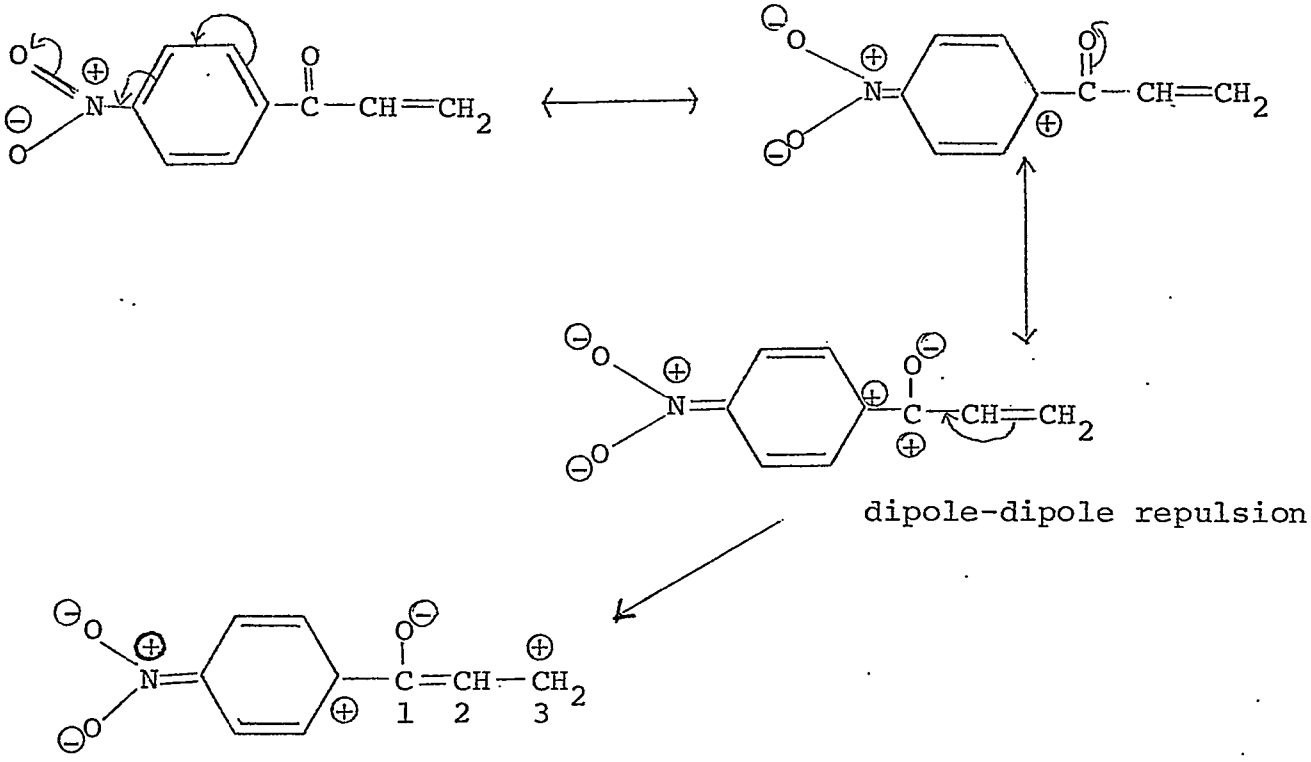
toxicity of the hydroxy compounds, I f and I g may be due to their conversion in vivo to the corresponding O-glucuronides and/or sulphates (Williams, 1959) followed by rapid excretion of the resulting metabolites, the reason for the difference in toxicity between the two nitro compounds, for instance, is less clear. Unlike the case of series V, the variation in toxicity in these series of compounds is not easily explainable in terms of simple differences in the rates of release of the different acrylophenones. Thus on comparing compounds which do not display mortalities at 100 mg/kg (I a-c; f, g, i) with those where deaths are noted at this dose level (I d, e, h, j, k) it is found that both groups contain compounds having aromatic substituents with positive and negative Hammett σ values. In addition, both groups of compounds contain aromatic substituents with hydrophilic (hydroxy, nitro and methoxy groups) and lipophilic (methyl and chloro groups) characteristics (Albert, 1960). Hence toxicity is not explained simply on the basis of the Hammett σ or Hansch π values.

3.6.0.0 Antineoplastic activity of 3-amino-1-aryl-1-propanone methobromides (II a-k, IV d, f)

Like the hydrobromides discussed earlier (Section 3.5.0.0), all β-aminoketone methobromides, including II a-k, IV d and IV f were inactive against P388 lymphocytic leukemia (Sections 4.2.0.0 and 4.4.0.0; TABLES XVI and XVII). While it is difficult to arrive at unequivocal structure-activity

correlations from the data generated, it is conceivable that the inactivity of these compounds against P388 lymphocytic leukemia is due to extremely rapid deamination of the parent compounds. In other words, the data generated suggest that formation of the acrylophenones *per se* is insufficient for antileukemic activity and that other factors such as the rate of release of the α,β-unsaturated ketones *in vivo* are important. If the latter are released rapidly, they may react with nucleophiles present in normal cells and thus may be inactivated prior to reaching the target site. There is some evidence that these compounds undergo rapid decomposition under neutral and basic conditions (Section 3.23.0.0).

On the other hand, the enhanced toxicity of the methobromides relative to the hydrobromides may be due to a more facile generation of the acrylophenones in the case of the former compared to the latter (see Sections 3.10.0.0 and 3.23.0.0). However, the order of toxicity within series II, for example, bears an inverse relationship to ease of elimination. Thus II h and II i, which are the compounds considered to be most susceptible to β-elimination, are the least toxic. The acrylophenones formed from these compounds would be expected to show a greater reactivity towards nucleophiles in general than those formed from II with electron-releasing substituents on the aromatic ring. This would also make II h and II i less selective in their affinity for nucleophiles (e.g., fig. 38).



Relief of dipole-dipole repulsion--contribution of canonical form to resonance hybrid significant--may show a high, non-selective affinity for nucleophiles because of the positive charge on carbon-3.

fig. 38. Possible reason for the high reactivity of 4-nitroacrylophenone towards nucleophiles.

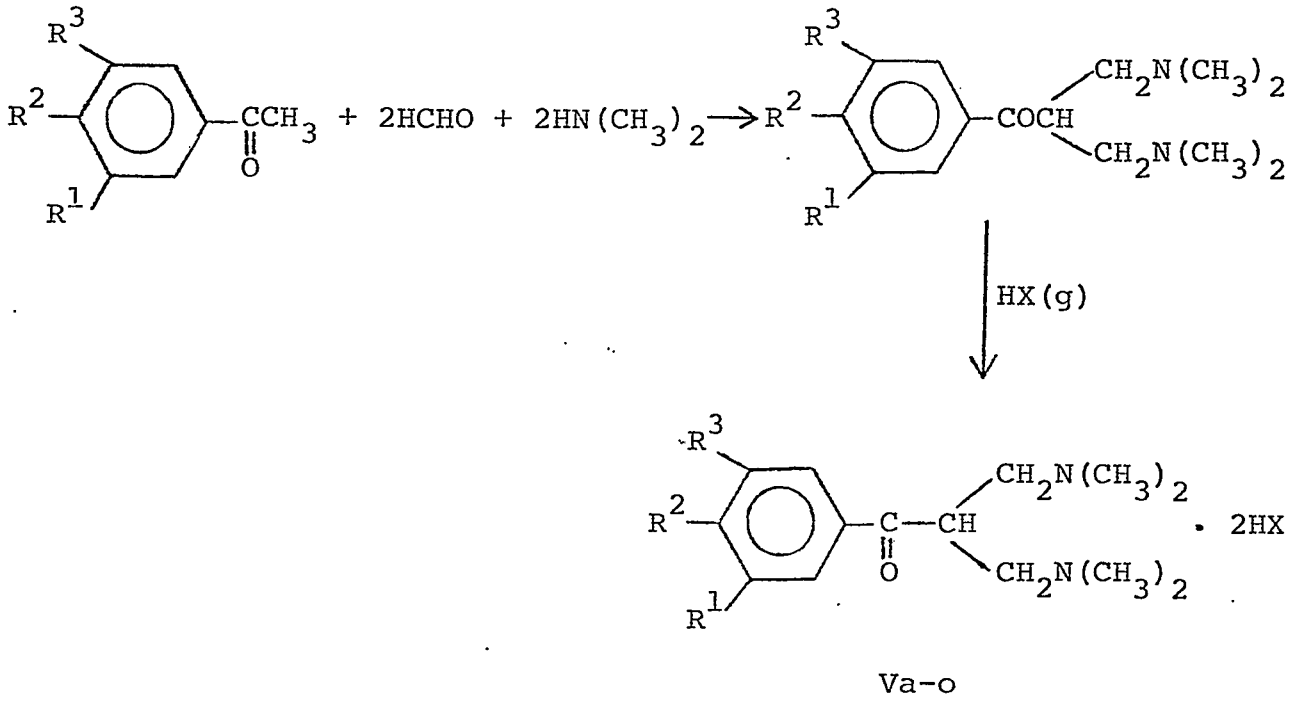
Since low selectivity would mean increased affinity for "weak" nucleophiles such as water, it is conceivable that almost complete detoxification of these compounds occurs before toxic effects become operative. On the other hand, the less reactive acrylophenones, e.g., those formed from II a and II b, may be less prone to detoxification and therefore, more likely to cause biochemical lesions leading to toxicity than their more reactive counterparts.

3.7.0.0 Antineoplastic activity of 3-dimethylamino-2-methyl-1-phenyl-1-propanone hydrobromide (III).

Like the related compounds in series I, II and IV, III is inactive against P388 lymphocytic leukemia. Its murine toxicity is comparable to that displayed by Ia (see Section 4.3.0.0). Since no meaningful structure-activity correlations could be discerned in the case of series I, attempts to relate the activity of III to the activity pattern observed in series I would be injudicious.

3.8.0.0 Preparation of 3-amino-2-aminomethyl-1-aryl-1-propanone dihydrohalides (V, VII) and 3,5-bis-(dimethylaminomethyl)-4-hydroxyacetophenone dihydrobromide (VI)

Compounds Va-o were prepared by a modified method of Lemin et al. (1969). Thus a mixture of the appropriate acetophenone, dimethylamine (25% w/v) formaldehyde (37% w/v) and ethanol was heated under reflux for 3 to 30 hours. The diamine so obtained was converted to the corresponding dihydrohalide salt by treating a solution of the former in acetone with the appropriate hydrogen halide gas (fig. 39).

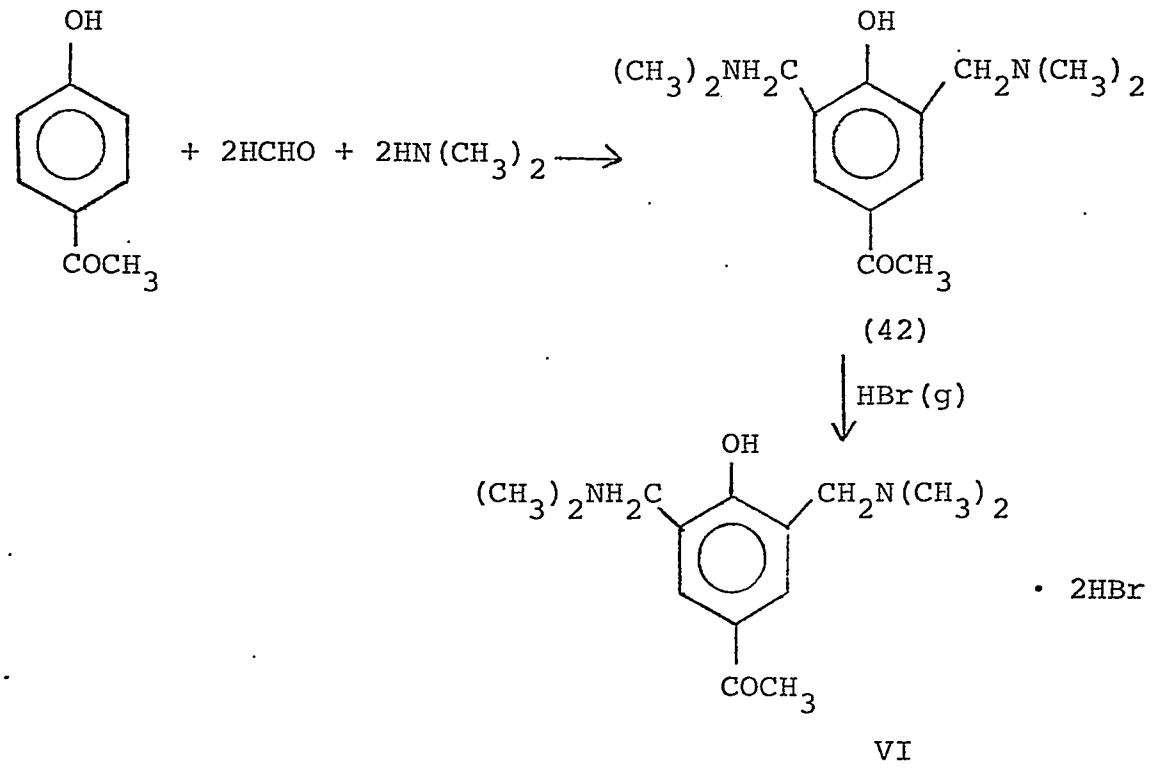


Va-o

fig. 39. Synthesis of 1-aryl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrohalides.

An increase in time of heating under reflux often resulted in an increase in yield up to a certain point. Thus in the case of Vk, when the time of heating under reflux was extended from 3 to 30 hours, the yield increased from 21 to 61% . No further increase in yield was observed with prolonged heating.

As anticipated, when 4-hydroxyacetophenone was reacted as shown in fig. 40, ring aminomethylation occurred to give the diamine (42; Gautier et al., 1964) which was subsequently converted to VI by treatment with hydrogen bromide gas. The physical data for VI are given in Section 4.6.0.0.



VI

fig. 40. Synthesis of 3,5-bis-(dimethylaminomethyl)-4-hydroxyacetophenone dihydrobromide (VI).

Therefore, an alternative route to the synthesis of 3-dimethylamino-2-dimethylaminomethyl-1-(4-hydroxyphenyl)-1-propanone dihydrobromide, Vp, was sought. Since ring aminomethylation is the direct result of the ring activating effect of the hydroxyl function in the starting acetophenone, the latter was converted to 4-(2-tetrahydropyranyloxy)-acetophenone (43) using the method of Bongini et al. (1979) before subjecting it to the Mannich reaction. The tetrahydropyranyl group was later removed by boiling the Mannich base dihydrobromide in methanolic hydrogen bromide (fig. 41).

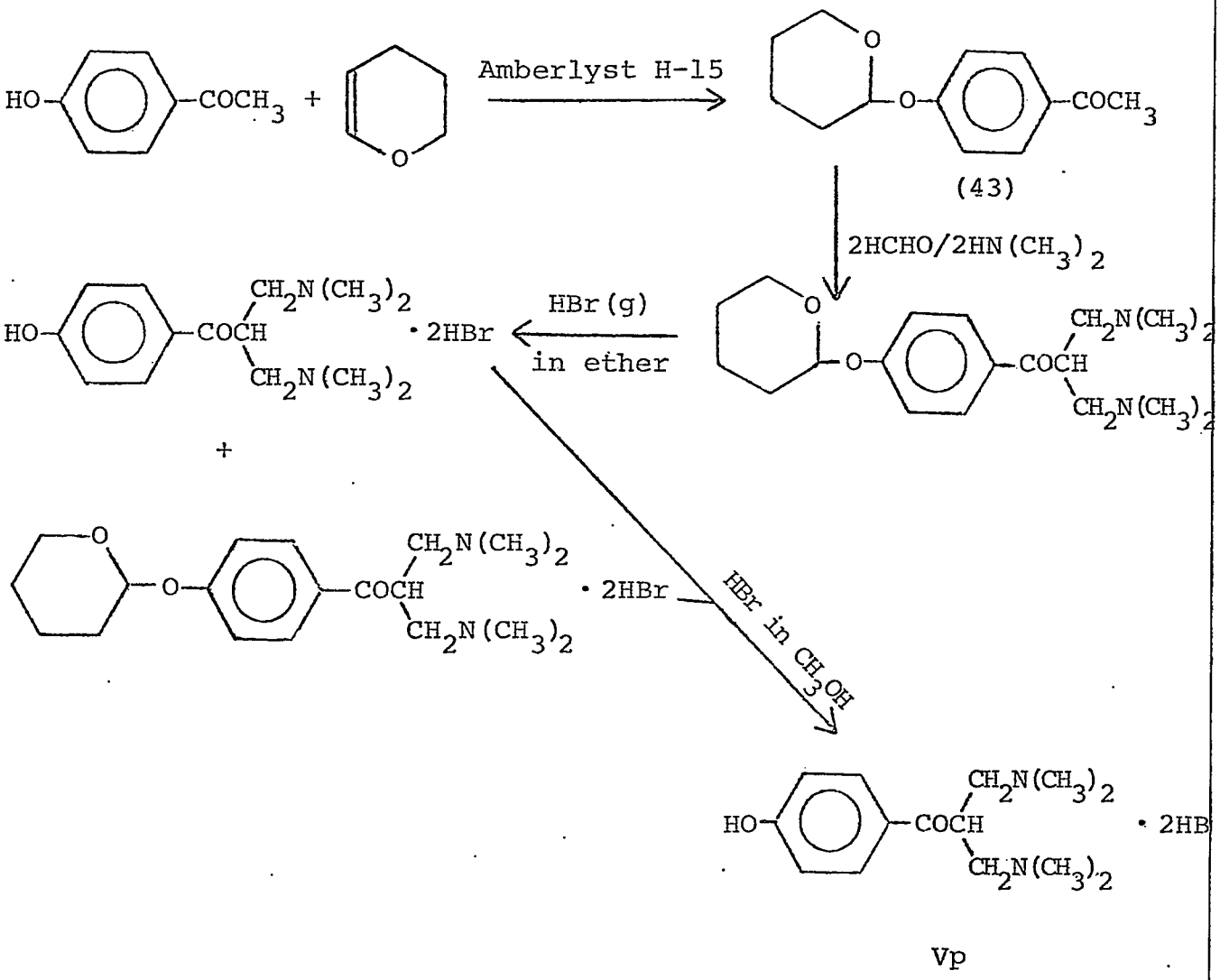


fig. 41. Synthesis of 3-dimethylamino-2-dimethylaminomethyl -1-(4-hydroxyphenyl)-1-propanone dihydrobromide.

The physical data for compounds Va-p are given in TABLE XVIII (Section 4.5.0.0).

Several 3-amino-2-aminomethyl-1-phenyl-1-propanone dihydrobromides, VIIa-c, were also synthesized in the same manner as Va-o using different secondary amines in lieu of dimethylamine (fig. 42).

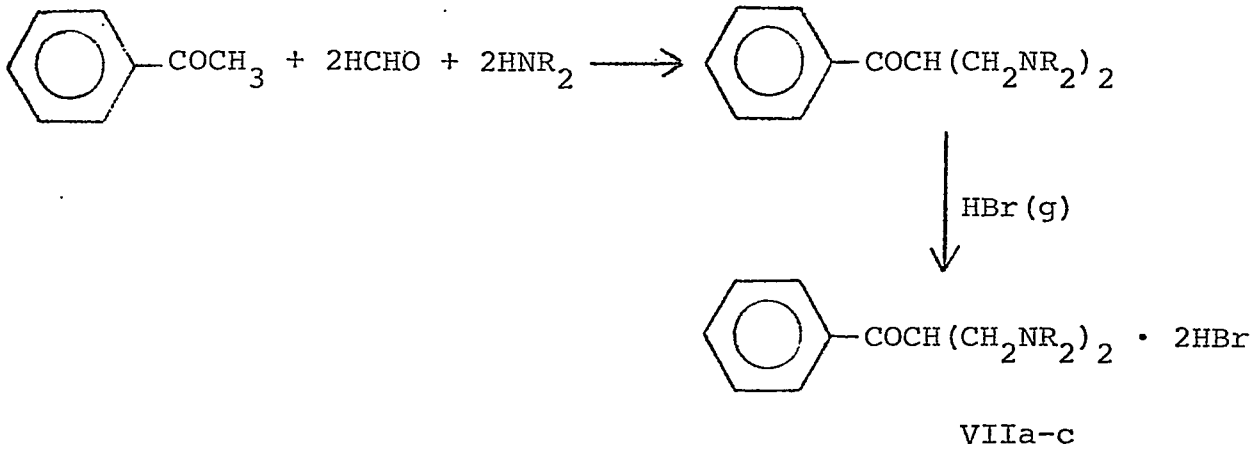
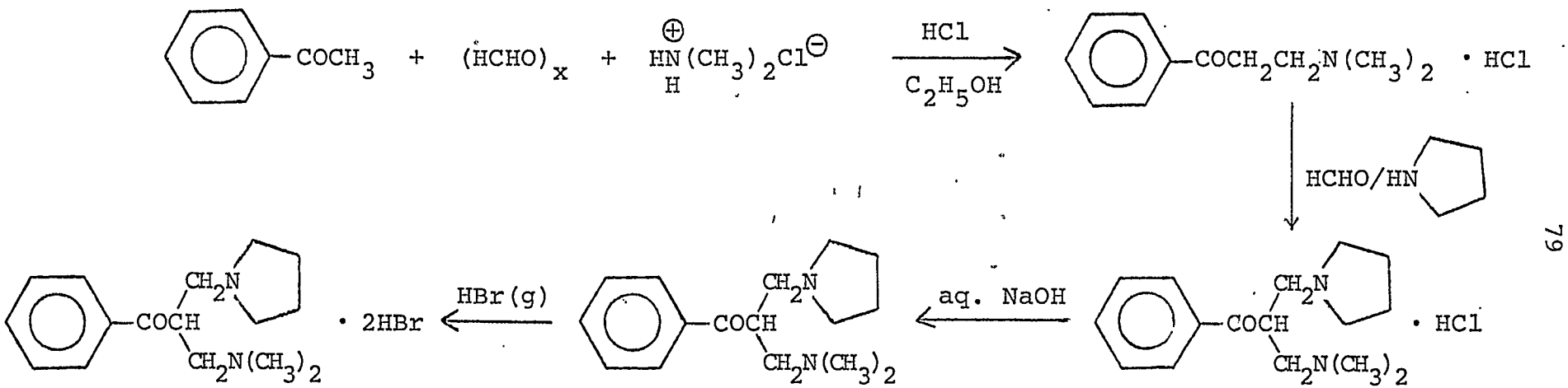


fig. 42. Synthesis of 3-amino-2-aminomethyl-1-phenyl-1-propanone dihydrobromides.

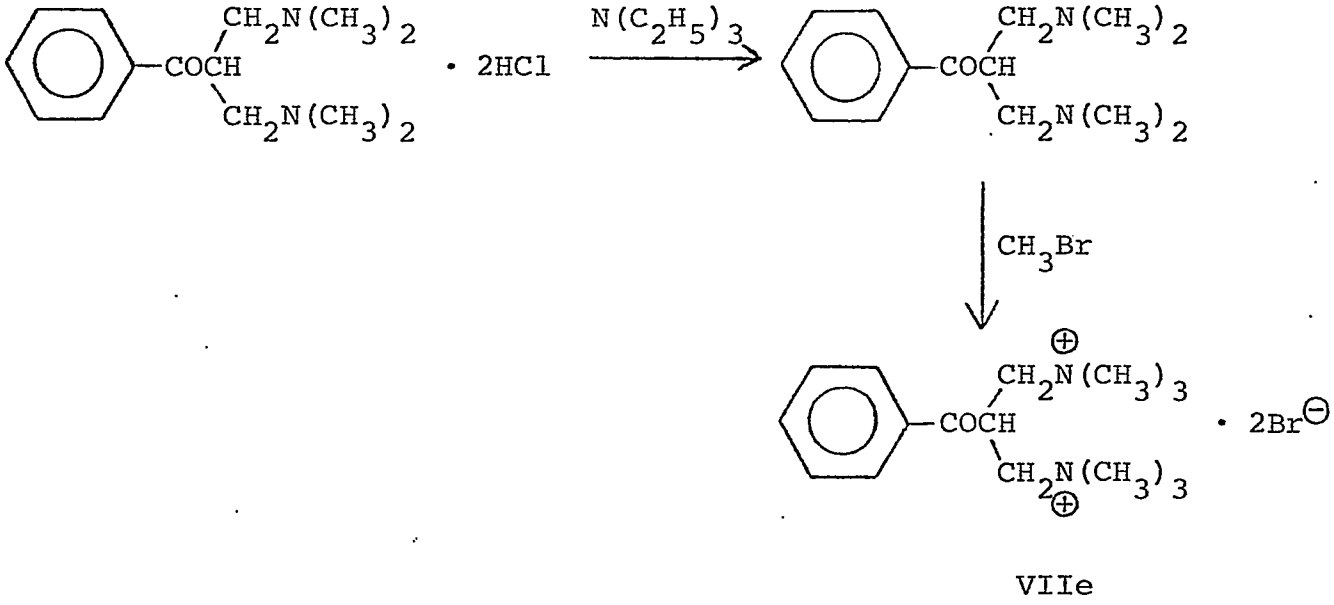
Compound VIId, which incorporates two different amino functions was synthesized using the method of Albrecht et al. (1962; fig. 43).



VIId

fig. 43. Synthesis of 3-dimethylamino-2-(1-pyrrolidinylmethyl)-1-phenyl-1-propanone dihydrobromide.

The corresponding dimethobromide of Vb was prepared as shown in fig. 44.



VIIe

fig. 44 Synthesis of 3-dimethylamino-2-dimethylaminomethyl-1-phenyl-1-propanone dimethobromide

The free base of Va was liberated with triethylamine since it was thought that the latter would be less likely to approach the β-hydrogen and thereby cause elimination than, for example, the hydroxide ion, for steric reasons.

The physical data for VIIa-e are given in Section 4.7.0.0 (TABLE XX).

Of the compounds described in this section, only Va has been reported in the literature (Albrecht et al., 1962). The same patent also reports the synthesis of the

corresponding dihydrochlorides of VIIb and VIIc and the free base of Vh or Vi. The synthesis of the free base of Va has also been reported by Albrecht et al. (1962) and Lemin et al. (1969). There has been no report in the literature on the anticancer activity of these compounds.

3.9.0.0 Mass spectrometry of some 1-aryl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrobromides.

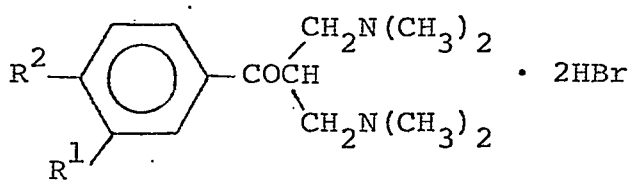
The mass spectral data for some representative compounds from series V are given in TABLE IV. The principal fragment ions formed from V are shown in fig. 45. In this section, an attempt will be made to highlight the main characteristics of the mass spectral fragmentation patterns of the compounds under consideration.

The mass spectra of the compounds selected have some features in common:

Firstly, the radical ion of the free diamine (44), which could be formed as shown in fig. 45 is absent in each case. On the other hand, each spectrum shows peaks of equal intensity at m/z 80 and 82 due to $[HBr]^{+\cdot}$ and also at m/z 79 and 81 due to Br^{+}.

Secondly, the peak due to the radical ion of 1-aryl-2-dimethylaminomethyl-2-propen-1-one (45) which could arise from loss of dimethylamine from (44) is also absent. This pathway, however, cannot be ruled out since

TABLE IV. m/z (Relative intensity) values of the principal ions observed in the 70 eV mass spectra of some 1-aryl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrobromides



Compound	R^1	R^2	m/z (Relative intensity) of principal ions
Vb	H	H	176(23), 172(4), 132(18), 105(44), 84(13), 82(24), 81(8), 80(24), 79(8), 77(33), 58(100), 45(11), 44(21)
Vi	H	OCH_3	206(18), 202(6), 162(35), 135(78), 107(11), 92(18), 84(12), 82(28), 81(11), 80(28), 79(11), 77(24), 58(100), 45(16), 44(32)
Vm	H	Cl	212(3), 210(9), 206(6), 141(18), 139(53), 113(7), 111(22), 84(8), 82(18), 81(6), 80(20), 79(7), 75(15), 58(100), 45(14), 44(27)
Vo	Cl	Cl	246(3), 244(6), 240(3), 202(9), 200(14), 177(4), 175(27), 173(42), 149(2), 147(11), 145(16), 109(10), 84(9), 82(21), 81(8), 80(22), 79(8), 75(6), 74(8), 58(100), 45(15), 44(27).
Vp	H	OH	192(11), 188(4), 148(41), 121(100), 93(22), 84(8), 82(21), 81(8), 80(21), 79(8), 65(23), 58(93), 45(24), 44(44)

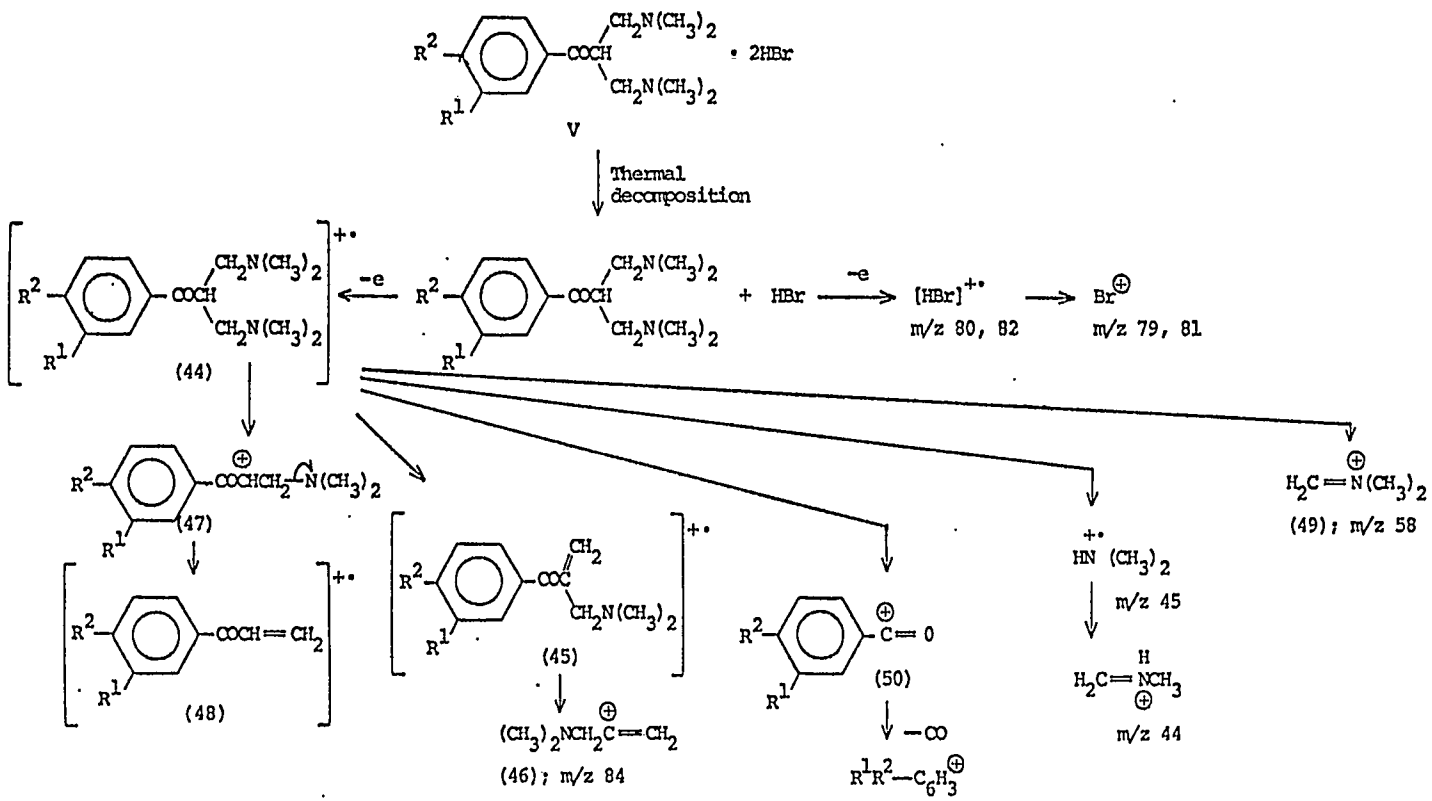


fig. 45. Mass spectral fragmentation pattern for a 1-aryl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrobromide.

the data obtained suggest that this ion, viz., (45), is formed but is of low intensity because of its fragmentation to give (46), m/z 84.

Thirdly, the carbocation (47) may account for the peak having the highest mass to charge ratio in each case. Homolytic fission of the bond gamma to the carbonyl function in (47) gives the radical ion of the acrylophenone, (48).

Finally, the peak due to the dimethyl(methylene) ammonium ion (49; m/z 58) is quite intense in all cases. In fact, it is the base peak in all cases but one, viz., the 4-hydroxy analogue (Vp). In the latter the base peak is due to the acylium ion (50), m/z 121. Stabilization of the positive charge in the acylium ion by the powerful electron-releasing mesomeric effect of the hydroxyl group [σ_p^+ for 4 - OH = -0.85 (Carey and Sundberg, 1977d)] could account for this observation (fig. 46).

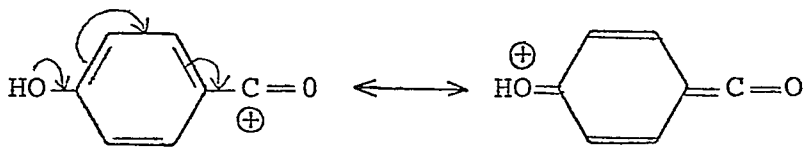


fig. 46. Resonance stabilization of the acylium ion formed in the case of 3-dimethylamino-2-dimethylaminomethyl-1-(4-hydroxyphenyl)-1-propanone dihydrobromide.

The methoxy analogue, Vi, also shows a highly intense peak due to the acylium ion (m/z 135, 78%), an observation

which would seem to point at the stabilization of the acylium ion by the electron-releasing mesomeric effect of the 4-methoxy group [σ_p^+ for 4 - OCH_3 = -0.65; Carey and Sundberg, 1977d]. Interestingly, the peak due to the acylium ion (m/z 105) in the case of the unsubstituted compound has a relative intensity of only 44% .

3.10.0.0 Biological activity of 1-aryl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrohalides (V).

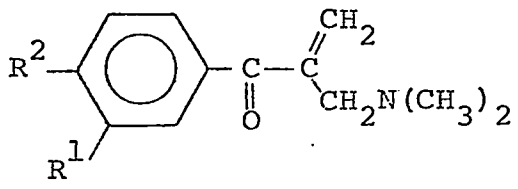
Compounds Va, b, d, e, g-i and l-o were synthesized and with the exception of Vl and Vn were screened by the National Cancer Institute, Bethesda against P388 lymphocytic leukemia (see TABLE XVIII; Section 4.5.0.0). Out of this group, five members containing aromatic substituents with diverse σ values, viz., Vb, e, i, m and o, were chosen and their activity studied in greater detail. Furthermore, an attempt was made to compare the activity of these five compounds with that of its analogues in series I. Thus Ia, c, e, j and k were selected from the latter. In the discussion that follows, series I and V refer only to these representatives unless stated otherwise.

The activity of the two series was first assessed against P388 lymphocytic leukemia [see TABLES XV (Section 4.1.0.0) and XVIII (Section 4.5.0.0)]. The dose scheduling was constant for compounds I and V except fewer doses of Vm and Vo were administered. While the monobasic compounds

(I) were uniformly inactive as noted in Section 3.5.0.0, the dibasic compounds (V) showed varying degrees of activity. The approximate order of potency was Ve, i > Vb, m > Vo. All of the compounds considered showed murine toxicity but whereas no mortalities were found at 50 mg/kg in series I, only the inactive diamine Vo did not cause deaths at this dose level in this series. No animals died when the representative compounds from series V were administered at a dose of 25 mg/kg, and hence, greater antineoplastic activity accompanied by greater murine toxicity was found in V than I.

The reasons for the differences in bioactivities between series I and V were then considered. Lability of a number of Mannich bases to produce the corresponding α,β-unsaturated ketones is well documented (Mollica et al., 1970; Dimmock and Taylor, 1974). On occasions, this property has been invoked to explain the bioactivities of a number of Mannich bases (Gordon et al., 1965; Schoenenberger et al., 1969). As observed in Sections 2.2.0.0 and 2.6.0.0, such deamination processes are possible in the cases of both I and V, leading to the formation of substituted acrylophenones which could undergo attack by cellular nucleophiles such as thiols. Since the rate of deamination in this series of compounds will be greatly influenced by the Hammett σ value of the aromatic substituent, the stabilities of the compounds having groups with the most divergent σ values,

viz., Ik, Vo ($\sigma_{3,4\text{-}Cl_2}$ = +0.60) and Ie, Vi ($\sigma_{4\text{-}OCH_3}$ = -0.27) were examined under simulated physiological conditions (phosphate buffer, pH 7.4, 37°C; see Sections 4.12.2.0. and 4.12.3.0). While only the corresponding free bases could be isolated from the buffer solutions containing Ie and Ik after 1.5 hours, elimination products XIIa and XIIb were isolated from solutions containing Vi and Vo after 5 minutes.



XII

a. R^1 = H; R^2 = OCH_3

b. R^1 = R^2 = Cl

When the temperature was reduced to 5°C, Vo gave rise to a mixture of XIIb and Vo as a free base in a ratio of 10:1 after 5 minutes, while Vi gave rise to the free base and no olefin. After one hour at 5°C, Vi gave a mixture of XIIa and the free base of Vi in a ratio of 7:4 (see Section 4.12.3.0). Hence the comparative rates of deamination of Vi and Vo follow that predicted by a consideration of the electronic effects of the nuclear substituents. The difference between I and V in their ability to undergo elimination to produce the corresponding α,β-unsaturated ketones is probably due to variation in the acidity of the protons

beta to the dimethylamino group(s), since the rate-determining step in such elimination reactions often involves abstraction of the β-hydrogen by base. Since compounds belonging to series I and V were conceived as prodrugs of the corresponding acrylophenones with an affinity for biogenic thiols, a representative compound was selected from each group and its reaction with a model thiol, viz., 2-mercaptoethanol, studied (see Sections 4.12.4.0 and 4.12.5.0). Preliminary experiments involving incubation of Vo and 2-mercaptoethanol in buffer (pH 7.4) at 37°C followed by extraction with chloroform gave a mixture of compounds as revealed by thin-layer chromatography on silica gel (chloroform : methanol 10:1). However, when Vo and the thiol were incubated for a short period of time in a mixture of buffer and chloroform, the product isolated was shown by mass spectrometry to be XIII (fig. 47). This compound could have arisen by either of two mechanisms shown in fig. 17 (Section 2.6.0.0). The mass spectrum also showed the absence of a prominent peak at m/z 58 which is often the base peak in Mannich bases due to the dimethyl(methylene)ammonium ion. It was considered that the lack of formation of the corresponding olefins in the case of I may have led to non-reactivity of the latter with thiols. However, when an experiment similar to that described for Vo was carried out with Ie and 2-mercaptoethanol, the product isolated from the reaction

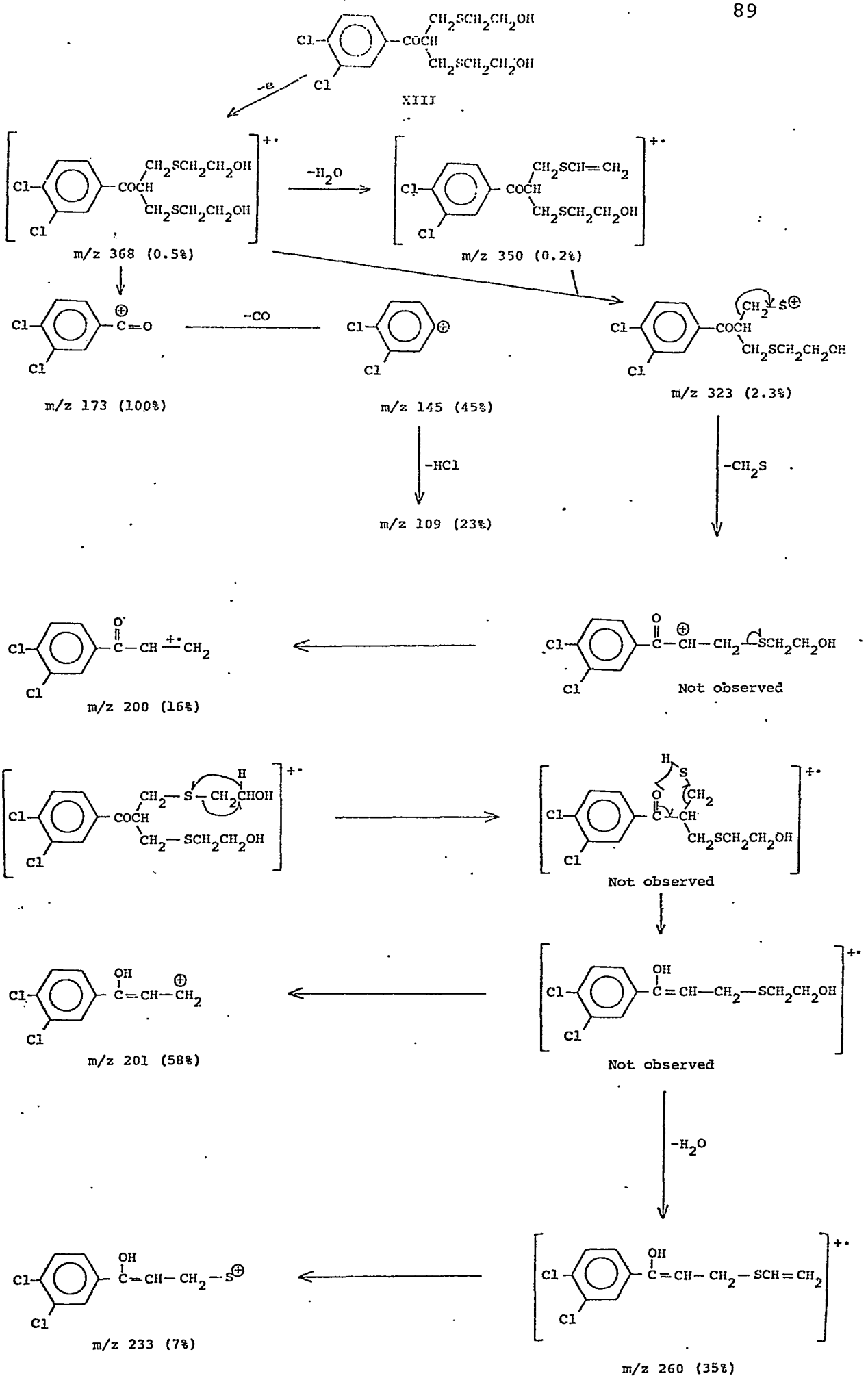


fig. 47. Mass spectral fragmentation pattern for 1-(3, 4-dichlorophenyl)-3-(2-hydroxyethylmercapto)-2-(2-hydroxyethylmercapto)methyl-1-propanone, XIII

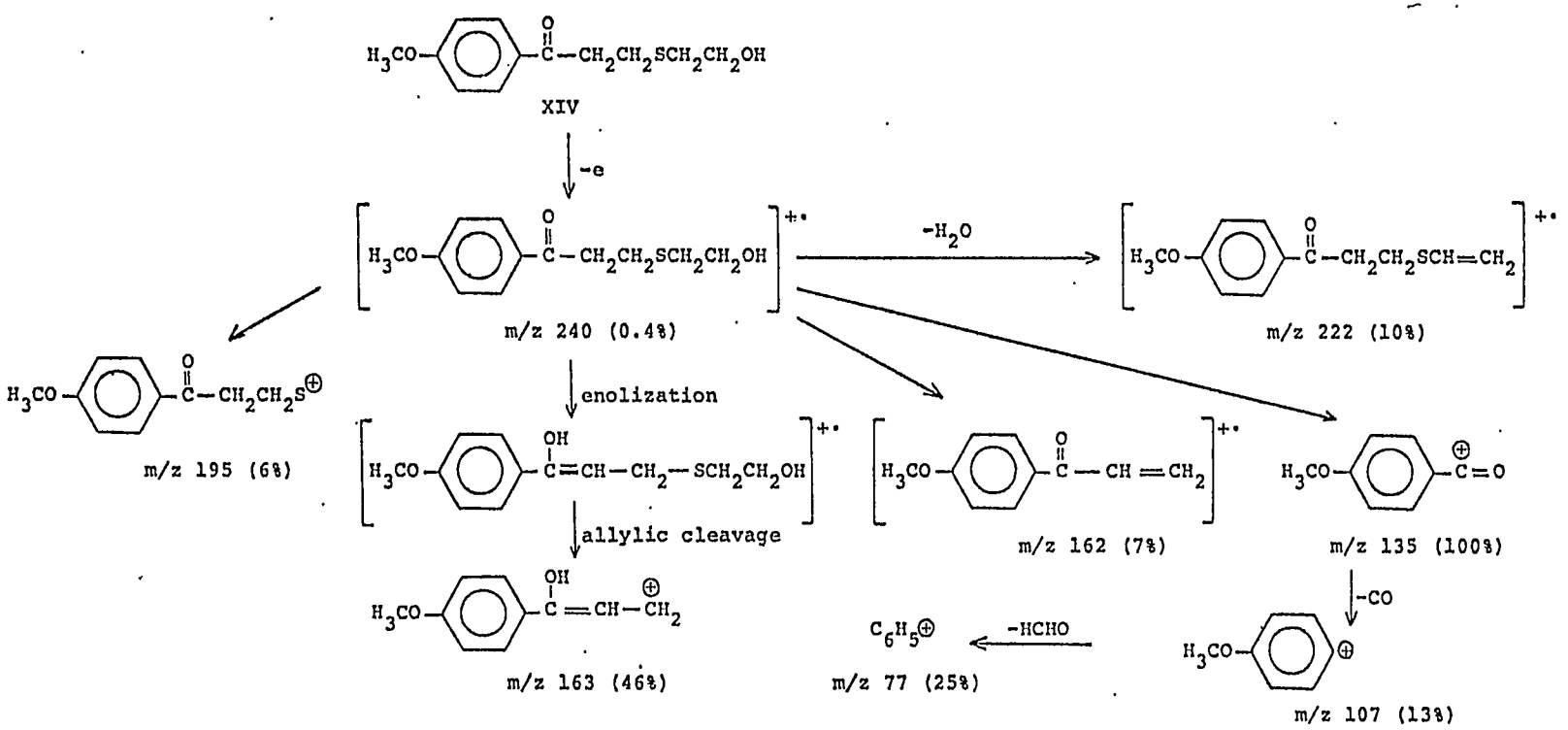


fig. 48. Mass spectral fragmentation pattern for 3-(2-hydroxyethylmercapto)-1-(4-methoxyphenyl)-1-propanone, XIV.

mixture was shown by mass spectrometry (fig. 48) to be 3-(2-hydroxyethylmercapto)-1-(4-methoxyphenyl)-1-propanone (XIV), i.e., the dimethylamino function had been replaced by a 2-hydroxyethylmercapto function. Control experiments showed that the reaction between Ie and 2-mercaptoethanol occurred in the buffer and not necessarily in the organic solvent (see Section 4.12.4.0). Hence elimination in series I may occur in the presence of stronger nucleophiles than hydroxide anion, viz., the 2-hydroxyethylmercapto ion. Although the generalization that a strong nucleophile that is also a weak base should promote substitution rather than elimination is valid in most cases, it is not always true. For example, $PhS^{\ominus}$ is a weaker base than $EtO^{\ominus}$ by a factor of 10^{10}, but in ethanolic solution it induces elimination from t-butyl chloride about ten times faster (Jones, 1979e). The possibility that compounds I and V could react with 2-mercaptoethanol, present presumably in the form of the thiolate anion, by a substitution mechanism was considered. However, this would involve a transition state in which there is a decrease in charge relative to the starting material (fig. 49).

$$RX^{\oplus} + Y^{\ominus} \longrightarrow Y^{\delta-} \ldots R \ldots X^{\delta+}$$

fig. 49. Transition state for an S_N2 reaction involving a positively charged substrate and a negatively charged nucleophile.

Therefore, an increase in solvent polarity in this case would be attended by a large decrease in rate (March, 1977h). So there seems little doubt that these compounds react by an elimination-addition mechanism (see figs. 14 and 15; Section 2.2.0.0).

The effect of I and V on respiration in rat liver mitochondria is given in TABLE V. At low concentrations in both series of compounds, stimulation of respiration occurs and, in general, as the concentration is increased stimulation of respiration increases and then diminishes while inhibition of respiration increases. In addition, the elevation of dose shortens the time period prior to inhibition by I and V. A typical result is illustrated in figures 50 and 51. While from a qualitative view-point, both series of compounds exert similar effects, a quantitative comparison of Ia, c, e, j and k with compounds bearing similar nuclear substituents in series V indicates that each member of I under consideration requires approximately one hundred times the concentration to elicit the same response referable to increases in both stimulation and inhibition as the corresponding member in series V. For example, doses of 25 μmoles of I cause similar percentage inhibitions of respiration in mitochondria as 0.25 μmole doses of V. At comparable dose levels, the olefins XIIa and XIIb have similar respiration-inhibitory properties as their progenitors

TABLE V. Effect of some Mannich bases on respiration in rat liver mitochondria using succinate as the substrate at pH 7.4 and 37°C.

Compound	Concentration, μmoles	Stimulation, %	SE	Time (min.) prior to constant inhibition of respiration	SE	Inhibition, %	SE
Ia	10	37.32	13.22	----	----	0	-----
	25	35.57	6.09	6.72	0.90	54.18	12.21
	50	28.22	5.10	7.76	0.86	57.44	4.87
	100	28.62	2.86	4.43	0.35	72.38	2.19
Ij	5	35.26	7.67	3.68	0.18	48.75	8.92
	10	45.03	12.18	3.36	0.16	50.36	11.79
	25	57.24	4.69	3.08	0.16	63.96	1.88
	35	27.79	5.79	3.38	0.20	86.09	2.11
Ik	5	68.48	15.13	3.89	0.36	84.86	2.73
	10	63.27	6.26	2.22	0.19	63.38	4.73
	25	0	-----	1.00	0.09	87.76	3.91
Ic	10	39.93	7.96	1.50	0.92	4.31	3.28
	25	92.89	6.54	5.60	0.27	62.27	3.95
	50	34.23	8.47	3.58	0.29	46.83	9.68
	100	0	-----	2.95	0.34	78.93	3.67
Ie	10	35.46	1.08	----	----	0	-----
	25	52.18	7.82	----	----	0	-----
	50	64.69	8.45	6.02	0.17	29.75	15.11
	100	49.11	5.61	5.22	0.21	56.58	2.26
Vb	0.01	64.72	7.43	----	----	0	-----
	0.1	38.65	5.29	2.81	1.05	9.81	4.21
	0.25	33.68	4.25	3.64	0.64	53.94	5.22
	1.0	20.02	1.97	5.14	1.10	70.60	4.37
	5.0	34.60	4.29	1.90	0.11	85.27	1.37
	10.0	12.22	3.23	1.28	0.12	87.58	2.79
	25.0	0	-----	1.25	0.06	96.62	1.02
Vm	0.01	32.14	9.19	----	----	0	-----
	0.1	27.41	2.28	4.84	0.23	34.90	4.65
	0.25	37.56	5.44	3.64	0.27	70.97	5.78
	1.0	24.19	3.47	2.32	0.10	83.30	1.97
	10	16.48	1.54	0.84	0.02	89.33	2.46
	25	0	-----	0	----	94.47	2.31
Vo	0.01	38.58	8.65	----	----	0	-----
	0.1	63.90	8.60	4.76	0.21	79.78	1.24
	0.25	79.86	9.44	1.66	0.79	79.08	4.61
	1.0	0	-----	1.17	0.03	89.98	1.09
	10	0	-----	0.20	0.03	97.35	1.16
Ve	0.01	63.32	1.03	----	----	0	-----
	0.1	28.87	6.94	3.32	0.68	23.65	5.67
	0.25	57.85	5.01	6.40	0.38	51.14	1.68
	1.0	56.67	4.47	2.82	0.10	64.88	4.33
	10	0	-----	1.22	0.07	82.82	1.37
	25	0	-----	0.87	0.07	97.36	0.57
Vi	0.01	54.77	14.25	----	----	0	-----
	0.1	18.41	1.99	----	----	0	-----
	0.25	27.75	5.37	4.29	0.37	21.58	4.27
	1.0	34.00	5.99	3.88	0.21	55.60	1.63
	10	33.17	3.32	2.20	0.19	75.62	4.24
	12.5	60.08	7.05	1.88	0.12	78.97	6.61
	15	36.92	3.44	2.32	0.30	80.52	3.12
	25	17.25	7.77	1.64	0.10	90.54	0.99
	50	0	-----	1.57	0.30	97.78	0.78
XIIb	1.0	68.71	7.14	2.18	0.14	84.70	2.50
	10	0	-----	0.65	0.04	90.25	1.76
XIIa	1.0	37.00	8.37	6.94	0.30	36.25	7.10
	10	69.46	4.07	2.52	0.18	74.70	2.89
	25	49.34	4.84	1.42	0.02	81.85	3.06

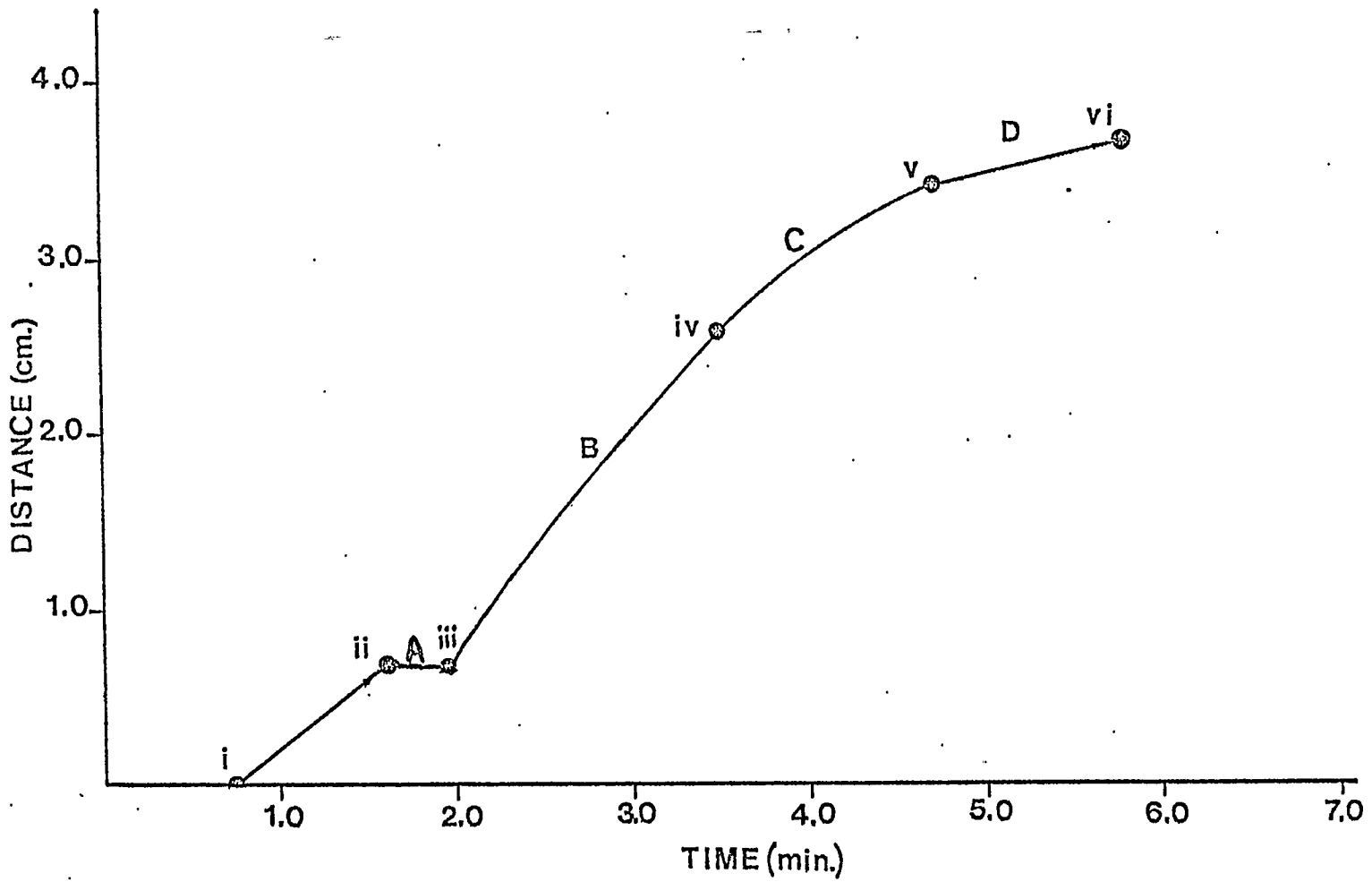


fig. 50. The effect of Ij (25 µmoles) on respiration in rat liver mitochondria at 37°C and pH 7.4. Succinate and Ij were added at (i) and (ii) respectively. The periods A, between (ii) and (iii) and C between (iv) and (v) represent lag periods prior to the obtention of constant maximal stimulation (phase B) and constant maximal inhibition (phase D) of respiration.

Vi and Vo although the effect on stimulation of respiration appears somewhat different. Two representative compounds from each series were also examined for their effect on mitochondria at 20°C and in general, stimulation of respiration was increased and inhibition of respiration diminished at this temperature compared to 37°C (TABLE VI). The effect of temperature on the pattern of respiration was considered of interest since recent reports indicate alterations in mitochondrial function on changes in temperature (Psenakova and Kolek, 1981; Goroshinskay et al., 1981). The compounds could serve as biochemical tools. For example, if on reducing the temperature at which respiration was measured to 20°C stimulation of respiration disappeared while inhibition of respiration was retained at the same dose that caused both stimulation and inhibition at 37°C, it would allow a study of the effects of inhibition of respiration alone to be undertaken at the required concentration level.

As noted in Section 2.11.0.0, the pH of some tumours is lower than that of the corresponding normal cells. A number of investigators have shown that the pH of certain tumours is in the range of 7.0 and in addition, the administration of glucose to tumour-bearing animals has lowered the pH of the neoplasm to 6.4, while the pH of normal tissue was unaffected (Eden et al., 1956; Kahler and Robertson, 1943). Furthermore, some Mannich bases were

TABLE VI. Effect of some Mannich bases on respiration in rat liver mitochondria using succinate as the substrate at pH 7.4 and 20°C.

Compound	Concentration, µmoles	Stimulation, %	SE	Time (min.) prior to constant inhibition of respiration	SE	Inhibition, %	SE
Ik	5	27.03	11.26	-----	----	-----	----
	10	70.00	18.45	-----	----	-----	----
	25	68.56	9.50	10.76	1.09	39.70	6.73
Ie	25	42.35	3.89	-----	----	-----	----
	50	60.80	6.18	-----	----	-----	----
	100	66.45	4.58	-----	----	-----	----
Vo	0.1	25.70	4.62	7.62	0.17	43.98	2.98
	0.25	46.54	10.50	3.46	0.16	50.62	11.57
	1.0	29.64	1.92	2.04	0.10	69.16	2.50
	10	-----	-----	0.62	0.13	82.75	2.46
Vi	0.1	5.19	1.80	-----	----	-----	----
	0.25	21.17	3.54	-----	----	-----	----
	10	49.86	5.87	5.78	0.45	48.30	6.69
	15	51.36	7.28	3.62	0.27	61.25	4.76
	25	49.96	8.73	2.98	0.41	68.37	4.45
	50	-----	-----	1.34	0.40	84.37	3.17

found to have increased respiration-inhibitory properties as the pH was lowered from 7.4 to 6.9 and then from 6.9 to 6.4 in mitochondria obtained from both rat liver cells and the Morris 5123 TCH tumour (Dimmock et al., 1980b). Therefore, a comparison of the effect on respiration in mitochondria between a compound displaying significant activity versus P388 lymphocytic leukemia, viz., Vi and an inactive analogue, Vo, at pH values of 7.4, 6.9 and 6.4 was attempted. The results obtained at pH values of 6.9 and 6.4 are summarized in TABLE VII. With the exception of the 1.0 μmole dose for Vo, the largest increase in stimulation of respiration is at the pH of the normal cells (i.e., pH 7.4) in the case of Vo, while maximal increase in stimulation for the active compound, Vi, is at pH 6.4. If antineoplastic activity is influenced by the effect on stimulation of respiration, selective toxicity under the acidic conditions of the cancer cells is conceivable. When inhibition of respiration was examined, an alternate situation was observed, i.e. minimal percentage inhibition of respiration occurs with both Vi and Vo at pH 6.4. Hence the bioactivity of Vi in contrast to Vo is only explicable in terms of the effect on stimulation and not inhibition of respiration if effect on mitochondrial function is associated with anticancer properties and the pH of malignant cells is lower than that of the corresponding normal cells.

TABLE VII - Effect of some Mannich bases on respiration in rat liver mitochondria using succinate as the substrate at pH 6.9 and 6.4 and at 37°C.

Compound	Concentration, moles	Stimulation							Time (min.) prior to constant inhibition of respiration							Inhibition						
		pH 6.9		pH 6.4		Levels of significance (p) between pH values of			pH 6.9		pH 6.4		Levels of significance (p) between pH values of			pH 6.9		pH 6.4		Levels of significance (p) between pH values of		
		%	SE	%	SE	7.4 & 6.9	6.9 & 6.4	7.4 & 6.4	Time	SE	Time	SE	7.4 & 6.9	6.9 & 6.4	7.4 & 6.4	%	SE	%	SE	7.4 & 6.9	6.9 & 6.4	7.4 & 6.4
Vo	0.01	12.23	2.71	30.41	7.15	0.05	0.10	0.50	4.74	0.26	0	0	<0.001	<0.001	>0.50	11.35	2.49	0	----	<0.001	<0.001	>0.50
	0.1	0	----	34.47	7.06	<0.001	0.005	0.01	3.42	0.11	10.22	0.41	0.005	<0.001	<0.001	67.43	3.79	24.13	9.30	0.20	<0.001	<0.001
	0.25	0	----	40.76	6.73	<0.001	<0.001	<0.001	3.50	0.21	6.20	0.25	<0.001	<0.001	<0.001	87.56	2.00	46.09	5.36	0.20	<0.001	<0.001
	1.0	0	----	49.53	7.96	>0.50	<0.001	<0.001	1.90	0.09	3.65	0.13	<0.001	<0.001	<0.001	94.45	1.45	82.29	3.04	0.20	0.005	0.025
Vi	0.25	19.57	4.11	54.22	1.85	0.20	<0.001	0.005	1.78	1.11	0	0	0.10	0.10	0.01	7.33	4.51	0	----	0.05	0.50	0.025
	1.0	17.48	2.98	58.65	6.86	0.10	<0.001	0.01	3.26	0.97	0	0	0.50	0.005	<0.001	12.14	4.57	0	----	<0.001	0.005	<0.001
	10	9.27	4.97	43.33	6.89	0.25	0.005	0.50	3.93	0.31	7.97	0.19	<0.001	<0.001	<0.001	73.01	1.87	49.30	6.72	>0.50	0.005	0.005

In conclusion, the following generalizations regarding chemical structure and bioactivity may be made. Firstly, series V was found to be more active versus P388 lymphocytic leukemia and to display greater murine toxicity than series I which may be associated with the greater instability of V in aqueous media in which decomposition to the corresponding enones occurred. The only exception was Vo which was inactive against the particular tumour under consideration and appeared to be less toxic than Vb, e, i and m. Retrospectively, the five aromatic substituents selected are the ones recommended for a Topliss analysis (Topliss, 1977). According to this approach, an initial small group of compounds consisting of the unsubstituted compound along with the analogues possessing 4-methoxy, 4-methyl, 4-chloro and 3,4-dichloro substituents is selected, tested and arranged in order of potency. A comparison is then made between the potency order in this group and the tabulated potency order calculated for various parameter dependencies relating to hydrophobic, electronic and steric effects. From this analysis, the probable operative parameters can be deduced and a new substituent selection made with a view to enhancing potency (TABLES VIII and IX). Since this method looked promising, an attempt was made to subject the data generated to a Topliss analysis. Rank ordering according to potency of the initial compound group suggested a $-\sigma$ or $\pi - 3\sigma$ dependency for activity

TABLE VIII. 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 IX. 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_2
<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)

of series V in the P388 screen. Secondly, murine toxicity in both I and V may be associated, at least in part, with interaction with nucleophiles. Thirdly, both series of compounds affected respiration in mitochondria isolated from rat liver cells and compounds bearing the same nuclear substituents differed one hundredfold approximately in the doses required to elicit similar effects. Rank ordering according to respiration-inhibitory potency suggests a $+\sigma$ dependency in both series of compounds. This is in contrast to what appears to be required for activity in the P388 screen. Fourthly, maximum antineoplastic activity is found with Ve and Vi although they do not represent the maxima in regard to either rate of decomposition or effect on respiration in mitochondria. Thus if the deamination reaction has a positive Hammett rho value, Vb, m and o would be expected to liberate the corresponding acrylophenones faster than Ve and Vi. I, on the other hand, appears to be much less susceptible to β-elimination than V in aqueous media. In isolated mitochondria, a similar generalization appears valid, i.e. Vb, m and o are more active and I less active than Ve and Vi. It is conceivable, therefore, that an optimal rate of breakdown is required for significant activity versus P388 lymphocytic leukemia. For example, although Vo is rapidly converted to XIIb which can affect mitochondria at very low dose levels, it may be inactivated prior to reaching

the site of action in the case of P388 lymphocytic leukemia. On the other hand, the absence of formation of the acrylophenone or its formation at a very slow rate could lead to insufficient compound for anticancer efficacy.

It was noted earlier that a Topliss analysis of the potency order obtained in the initial compound group selected from series V suggested a $-\sigma$ or $\pi-3\sigma$ dependency for activity in the P388 screen. Vg and a new set of compounds, viz., Vc, f, j, k and p were used to examine the importance of these two parameters and the possible requirement of a methyl or methoxy group at the para position of the aromatic ring to the exclusion of the former. The anticancer screening data are given in TABLE XVIII (Section 4.5.0.0). If activity versus P388 lymphocytic leukemia is $-\sigma$ dependent, the 4-hydroxy analogue, Vp ($\sigma_{4\text{-OH}} = -0.37$) should be more active than either Ve ($\sigma_{4\text{-CH}_3} = -0.17$) or Vi ($\sigma_{p\text{-OCH}_3} = -0.27$). Similar reasoning would predict that the 3,4-dimethyl analogue, Vf [$\sigma_{3,4\text{-(CH}_3)_2} = -0.24$] should have a level of activity comparable to Ve or Vi. While Vf was found to have significant activity [T/C% 130 (12.5)], Vp was inactive [T/C% 118 (6.25)]. The inactivity of Vp is perhaps due to rapid biodegradation, possibly by O-glucuronide and/or sulphate formation followed by rapid excretion of the resulting metabolite(s). Such biotransformation pathways are well-documented for hydroxy compounds

(Williams, 1959). The lowering of activity observed with Vf relative to Ve seems to suggest that a steric factor is operative apart from an electronic factor. A 3-methyl group perhaps hinders proper alignment of the aromatic ring at the receptor site. However, the statistical significance of this difference in bioactivity is not very clear since there is often a considerable difference (i.e. up to ~10 percentage points) in the antineoplastic screening data generated for the same compound at the same dose level on two different occasions. The slight lowering of activity observed in the case of Vf would, however, preclude π-3σ dependency for activity in the P388 screen since the π-3σ value for the 3,4-dimethyl group is 1.89 as compared to 0.79 for the 4-methoxy group (Topliss, 1977). The fact that the 3-methyl analogue, Vc (π-3σ for 3-CH_3 = 0.77; Topliss, 1977) is inactive serves to strengthen this view. The level of activity observed in the case of Vc [T/C% 114 (25)] is more consistent with the view that the σ value of the substituent is an operative parameter. The rate of elimination of Vc ($\sigma_{3\text{-}CH_3}$ = -0.07) may not be very much different from that of Vb [T/C% 120 (12.5)] and this is reflected in their respective potencies. The slight lowering of activity observed in the case of Vc relative to Vb (if indeed it is statistically significant) may again suggest steric hindrance to alignment of the aromatic moiety at the

receptor site. The 3,4-dimethoxy analogue Vj [$\sigma_{3,4-(OCH_3)_2}$ = -0.15] is much less active [T/C% for Vj = 121 (12.5)] than Vi [T/C% 136 (12.5)] a fact that would seem to indicate that the cumulative σ value is more critical for activity than the presence of a methyl or methoxy group at the para position. The 3,4,5-trimethoxy analogue, Vk [$\sigma_{3,4,5-(OCH_3)_3}$ = -0.03] is almost equiactive with Vo ($\sigma_{3,4-Cl_2}$ = +0.60) which would seem to accentuate the detrimental effect of meta substitution on activity.

In conclusion, the following generalizations regarding chemical structure and activity versus P388 lymphocytic leukemia in series V may be made.

(i) By and large, a -σ dependency is indicated for activity.

(ii) π-3σ does not appear to be an operative parameter.

(iii) Meta substitution is detrimental to activity.

3.10.1.0 Activity of 3-dimethylamino-2-dimethylaminomethyl-1-(4-methoxyphenyl)-1-propanone dihydrochloride, Vh, against various tumour systems.

Although a few 1-aryl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrochlorides (Va, d, g and h) were also screened for activity against P388 lymphocytic leukemia, an in-depth study of the structure-activity

relationships was not attempted with this group of compounds. However, a representative compound, Vh, designated a selected agent compound by the National Cancer Institute, U.S.A., has been evaluated in a number of additional tumour systems. As shown in TABLE X this compound was found to be active only against P388 lymphocytic leukemia. It was inactive against all other tumours selected for this study.

TABLE X. Evaluation of 3-dimethylamino-2-dimethylaminomethyl-1-(4-methoxyphenyl)-1-propanone dihydrochloride against various tumours in mice.

No.	Tumour	Treatment Schedule	Maximum activity (dose in mg/kg)[a]	Toxicity[b] (dose in mg/kg)	Comments on the tumour model[c]
1	CX - 1 Colon xenograft	One injection every four days; a total of four injections.	38 (25)	0/6(100), 3/6(50),	First generation transplant human tumour xenografts - considered to be more representative of human tumour response than their animal counterparts.
2	LX - 1 Lung xenograft	One injection every four days; a total of three injections.	64 (50)	0/6(100), 6/6(50)	
3	MX - 1 Breast xenograft	One injection every four days; a total of four injections.	101 (12.5)	0/6(100), 5/6(50), 6/6(25)	
4	$CD8F_1$ Mammary tumour	One injection.	103 (7.81)	1/10(125), 3/10(62.5), 10/10(31.25)	First generation transplant animal tumour.
5	Colon 38	One injection every seven days; a total of two injections.	102 (25)	0/10(100), 1/10(50), 10/10(25)	Solid tumour with a low rate of metastasis.
6	B16 melanocarcinoma	Nine daily injections.	102 (25)	0/10(50), 10/10(25)	Useful for detecting compounds that will be active against slow-growing tumours.
7	Lewis lung carcinoma	Nine daily injections.	105 (12.5)	1/10(50), 10/10(25)	Permits study of the influence of drugs on prevention of dissemination and against metastasis.
8	L-1210 Lymphoid leukemia	Nine daily injections.	105 (1.56)	0.6(50), 6/6(25)	Used as a prescreen for synthetics.
9	P388 lymphocytic leukemia	Nine daily injections.	128(25)	0/6(100), 2/6(50), 6/6(25)	Excellent for detecting low orders of activity - used as a prescreen, especially for natural products.

[a]The figures for tumours 1-5 are the weight differences of the treated animals compared to controls expressed as a percentage. A compound is considered active if the figure is less than 20 for tumours 1-4 and less than 42 for tumour 5. The figures for the remaining tumours 6-9 are the ratios of median survival time of treated animals to controls expressed as a percentage. A compound is considered active if it increases the survival time by more than 25, 25, 40 and 20 per cent when assessed against tumours 6, 7, 8 and 9 respectively.

[b]Toxicity is a measure of the number of survivors on days 15, 11, 11, 34, 20, 5, 5, 5 and 5, respectively when assessed against tumours 1-9.

[c]Based on the article by Goldin et al. (1979).

3.11.0.0 Antineoplastic activity of 3,5-bis-(dimethylaminomethyl)-4-hydroxyacetophenone dihydrobromide (VI).

Compound VI was found to be inactive in the P388 screen [T/C% 101 (50)] and non-toxic at the maximum dose level tried, viz., 200 mg/kg. One possible reason for these observations could be that this compound is less prone to elimination than the 1-naphthol derivatives studied by Andrisano et al. (1970). β-Elimination in this case would result not only in disruption of aromaticity but also in a high energy, ortho-quinoid structure (24). The other possibility is that VI does undergo elimination to give (24) which because of its instability reacts non-selectively with nucleophiles (e.g. water). Hence not enough alkylating species reaches the desired site of action.

3.12.0.0 Antineoplastic activity of 3-amino-2-aminomethyl-1-phenyl-1-propanones (VII).

Just as the substituents on the aromatic ring in compounds belonging to series V can affect the rate of release of the corresponding acrylophenones in aqueous media, a variation in the amino group as in series VII may be expected to have the same effect provided the amino group leaves in a rate-limiting step. While there is very little variation in activity among Vb, VIIc and VIId versus P388 lymphocytic leukemia, there is a wide variation in toxicity [see TABLES XVIII (Section 4.5.0.0) and XX (Section (4.7.0.0)]. Thus Vb is the most toxic of the three, VIId is

of intermediate toxicity and VIIc the least toxic. The replacement of one of the dimethylamino groups in Vb by a 1-pyrrolidinyl group as in VIId brings about a decrease in toxicity. That a pyrrolidinyl group does indeed lower toxicity is confirmed by the observation that the inactive analogue VIIa is less toxic than Vb. Interestingly, the bis-quaternary ammonium compound, VIIe, is even more toxic than Vb. Thus the data generated seem to suggest that the amino group is responsible for at least part of the toxicity associated with series V.

The activity pattern found in this series is less easy to interpret. Of the compounds in this series, VIIc [T/C% 119 (50)] would be expected to undergo decomposition far more rapidly than Vb, VIIa, b and d, the reason being that morpholine (pK_a = 8.7) is the weakest base and therefore, would be expected to be the best leaving group of the amines under consideration.[1] In series V, it was observed that a fast rate of deamination as in Vo resulted in inactivity versus P388 lymphocytic leukemia. Therefore, the fact that VIIc is almost equiactive with Vb calls for an explanation. In this connection it is perhaps relevant that the higher murine toxicity observed in the case of series V made determination of antineoplastic activity at doses higher than 25 mg/kg (except in the case of Vo where the figure was

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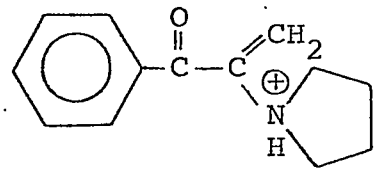
[1] Smith, P.J. and Grover, T.S., Unpublished results.

50 mg/kg) virtually impossible. The inactivity of Vo, the least toxic analogue at 50 mg/kg may be due, not merely to a rapid rate of release of the corresponding acrylophenone, but also to the lack of selectivity of the latter in its affinity for nucleophiles. The strong electron-withdrawing influence of the chloro groups would make this acrylophenone a much better Michael acceptor than the one formed from VIIc (see Section 3.6.0.0). This may cause the alkylating species, viz., the acrylophenone generated from Vo, to be inactivated before it reaches the target site. Thus the possibility of using high doses without fear of causing toxicity may explain why VIIc is almost equiactive with Vb. The bis-quaternary ammonium compound is highly toxic and inactive.

The importance of the leaving group(s) may be seen by comparing the levels of anticancer activity and murine toxicity of the three structurally related compounds, Vb, VIId and VIIa. While VIId [T/C% 124 (12.5)] is almost equiactive with Vb (T/C% 120 (12.5)], its toxicity behaviour [0/6 (100), 5/5 (50)] resembles that of VIIa [0/6 (100), 6/6 (50)]. As noted earlier, the leaving group ability decreases as the basicity of the leaving group increases. Therefore, one would expect Vb to undergo β-elimination much faster than VIIa (pK_a for pyrrolidine = 11.27) provided the amino group leaves in a rate-limiting step. If the

same mechanism is operative in the β-elimination of VIId, one would expect dimethylamine to leave first to give (51).



(51)

Thus the first step in this case, viz., the formation of an acrylophenone is perhaps similar to that one would expect in the case of Vb. On the other hand, the formation of (51) may occur very slowly or not at all in the case of VIIa. This may prevent sufficient concentration of the alkylating species from being present at the desired site of action at any given time. Therefore, VIIa may be inactive for reasons similar to those invoked to explain the inactivity of compounds Ia-k and IVa-c. Compound VIIb was not screened against P388 lymphocytic leukemia since it had already been submitted to the National Cancer Institute, U.S.A. by a different agency in the form of the dihydrochloride salt.

3.13.0.0 Preparation of 5-amino-1-phenyl-1-penten-3-ones (VIII)

The method of Maxwell (1943) was used with some modifications for the preparation of the 5-amino-1-phenyl-1-penten-3-one hydrochlorides which were then converted to VIIIa-e as shown in fig. 52.

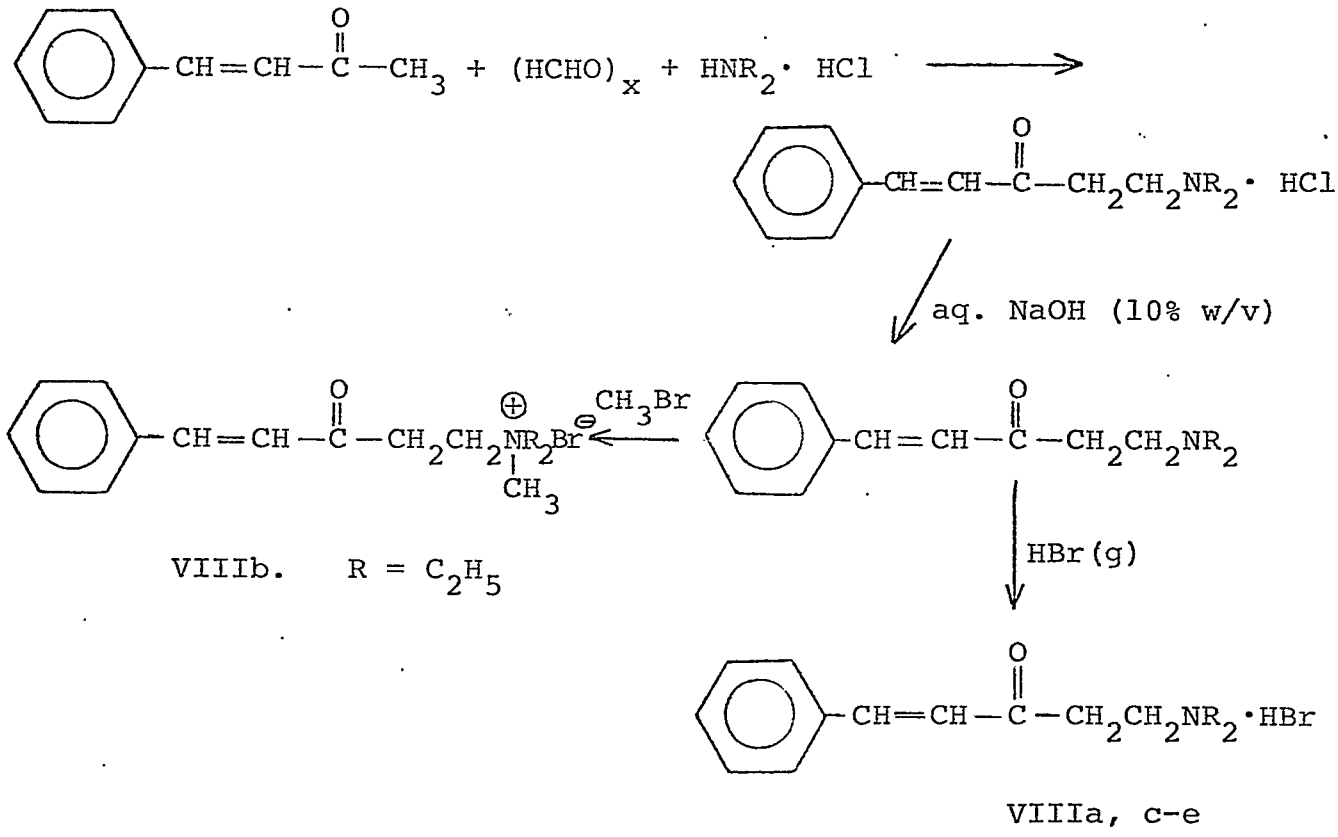


fig. 52. Synthesis of 5-amino-1-phenyl-1-penten-3-ones.

The compounds were obtained in yields ranging from 41-56%. While VIIIb-e were recrystallized from ether-methanol, ethyl acetate was used for the recrystallization of VIIIa (TABLE XXI; Section 4.8.0.0).

3.14.0.0 Antineoplastic activity of 5-amino-1-phenyl-1-penten-3-ones (VIII).

Of the compounds synthesized, only VIIIa and VIIIb were accepted for screening by the National Cancer Institute, U.S.A. since VIIIc-e had already been submitted to them by different agencies in the form of the corresponding hydrochloride

salts. Therefore, the screening data generated for VIIIa and VIIIb (see TABLE XXI; Section 4.8.0.0) were compared with those generated for a related compound, viz., 5-dimethylamino-1-phenyl-1-penten-3-one hydrochloride (26) synthesized in this laboratory (Dimmock et al., 1979a). While (26) was found to be inactive [T/C% 108 (50)], both VIIIa [T/C% 136 (100)] and VIIIb [T/C% 118 (50)] showed interesting levels of activity in the P388 screen. Since the order of basicities in the amines under consideration is $HN(C_2H_5)_2$ > $CH_3N(C_2H_5)_2$ > $HN(CH_3)_2$, activity against P388 lymphocytic leukemia seems to be associated with a slow rate of deamination in this series. (As noted in Section 3.12.0.0, the leaving group ability decreases with an increase in the basicity of the leaving group). However, while the activity pattern observed in this series is similar to that observed in the case of series V, the similarity ends here. Thus in series VIII, the most active compound, VIIIa, also displays the least murine toxicity, a pattern which is in marked contrast to what was observed in the case of series V. However, it would be unwise to hazard a guess as to what moiety is responsible for the biochemical lesion leading to antineoplastic activity in this series. Since 4-phenyl-3-buten-2-one (29) showed significant activity against P388 lymphocytic leukemia (see Section 2.11.0.0), it is possible that the amine moiety does little more than alter the partition

coefficient of the parent molecule (29), which is more favourable in some cases (e.g. VIIIa) from the point of view of activity versus P388 lymphocytic leukemia than in others [e.g. (26)]. Analogue synthesis from the point of view of altering lipophilicity as well as the rate of deamination should throw some light on the parameters that are responsible for activity in the P388 screen in this series.

3.15.0.0 Preparation of 4-aminomethyl-1-phenyl-1-nonen-3-ones (IX).

The scheme used for the synthesis of IXa-c is shown in fig. 53. The physical data for IXa-c are given in TABLE XXII (Section 4.9.0.0).

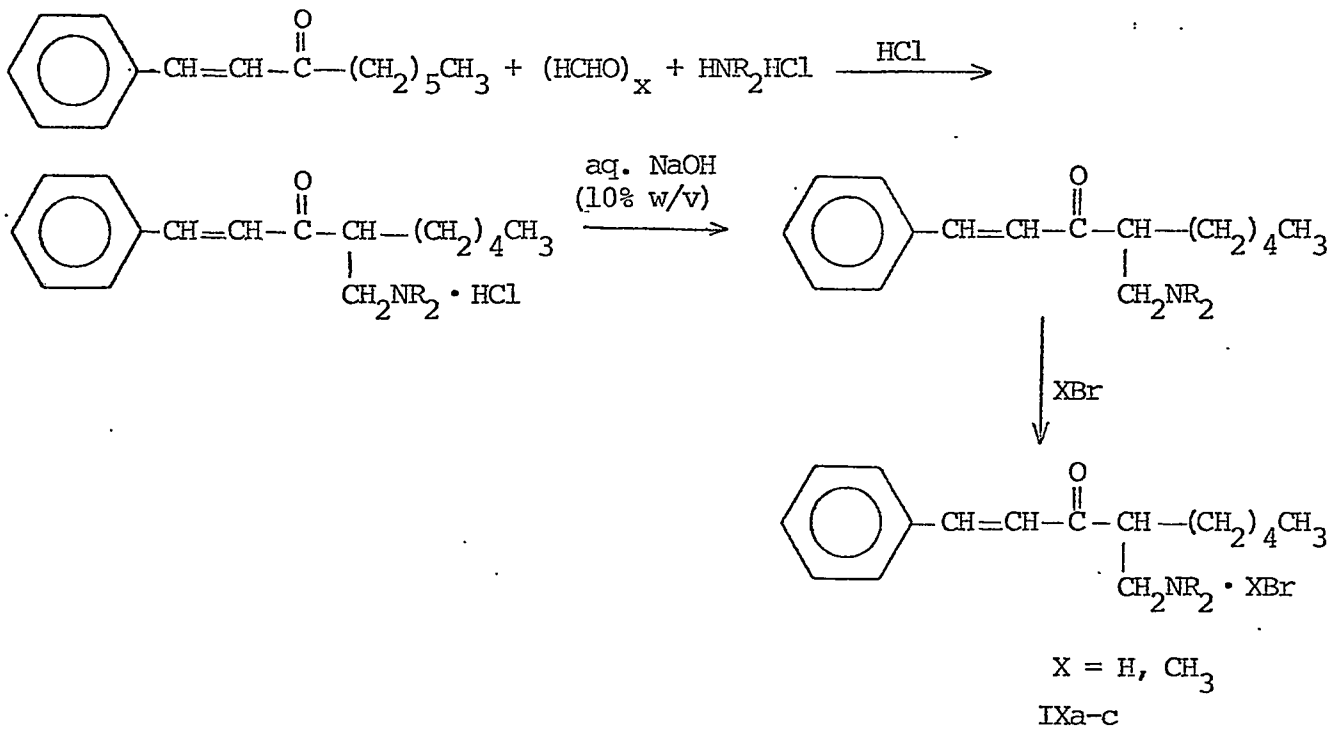


fig. 53. Synthesis of 4-aminomethyl-1-phenyl-1-nonen-3-ones.

The starting ketone, 1-phenyl-1-nonen-3-one (52) was prepared using the method of Smith et al. (1972b; fig. 54).

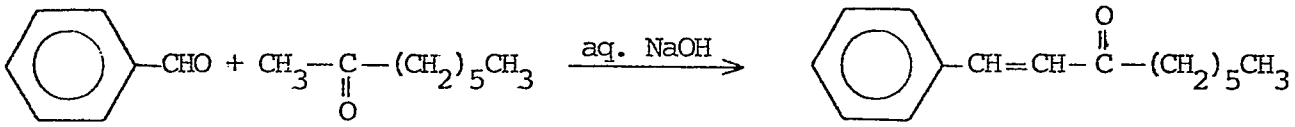


fig. 54. Synthesis of 1-phenyl-1-nonen-3-one.

3.16.0.0 Antineoplastic activity of 4-aminomethyl-1-phenyl-1-nonen-3-ones (IX).

As mentioned in Section 2.10.0.0, IXa-c were conceived as structural analogues of compounds (27). While the data received from the National Cancer Institute, U.S.A. up until the commencement of this investigation seemed to attribute maximal antineoplastic activity to the 3,4-dichloro analogue [(27); R^1, R^2 = 3,4-Cl_2; T/C% 142 (6.25)] in the P388 screen, the data received from them subsequently have failed to confirm this finding. Furthermore, since only one set of data is available for the 2,4- and 2,6-dichloro analogues indicating possible activity in the P388 screen, it would be judicious to regard these results with caution. Therefore, the design of IXa-c is perhaps based on a "false" lead, an eventuality that arose out of the fact that the second set of screening data for the 3,4-dichloro compound was received in 1981, ten years after the first results were forwarded to these laboratories.

3.17.0.0 Preparation of benzalacetone anils (X).

Compounds Xa and Xb were prepared by heating under reflux a benzene solution of 4-phenyl-3-buten-2-one or benzalacetone and the appropriate aniline with continuous removal of water formed during the reaction (fig. 55). The physical data for Xa and Xb are given in TABLE XXIII (Section 4.10.0.0).

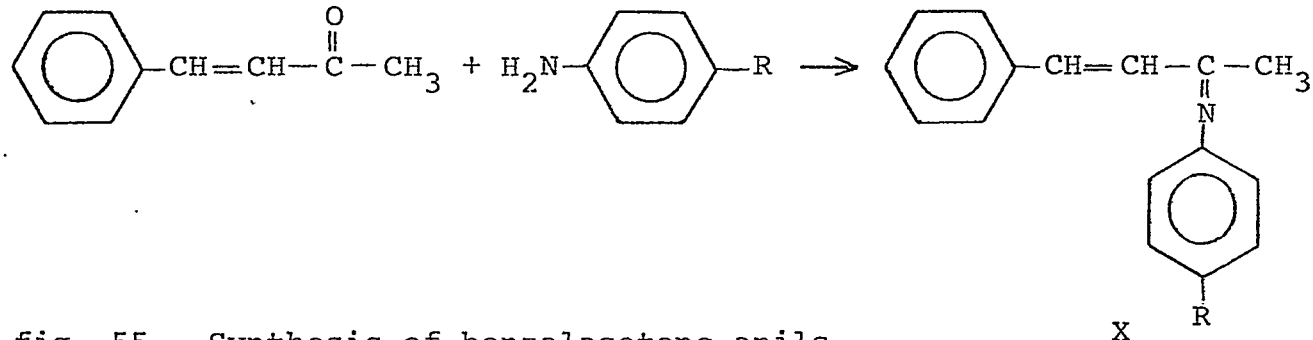
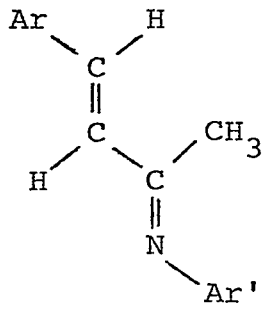


fig. 55. Synthesis of benzalacetone anils.

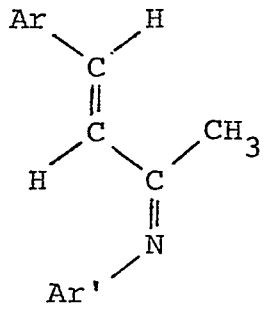
X

3.18.0.0 Geometrical (syn-anti) isomerism in benzalacetone anils (Xa, b).

Anils such as Xa and Xb are potentially capable of existing in two discrete, geometrically isomeric forms represented by structures X' and X".



X' (anti)



X" (syn)

The structure in which the aryl group attached to the nitrogen (Ar') in cis to the methyl is designated the anti isomer (X'); the other configuration (X") being the syn isomer. X' is thermodynamically more stable than X" (Tennant, 1979). The configuration of azomethines in general can be established by NMR spectroscopy (Buchanan and Dawson, 1977). However, no attempt was made to separate the geometrical isomers of the anils under consideration since N-aryl-ketimines exist entirely in the more stable anti form in the solid state, but in solution an equilibrium is established between the syn and anti isomers (Tennant, 1979). The latter was confirmed by NMR spectroscopy in chloroform-d in which the anti and syn isomers of both Xa and Xb were found to exist in a ratio of 2:1, as evidenced by the appearance of two discrete signals in the NMR spectrum due to the methyl group in each compound (see Section 4.10.0.0).

3.19.0.0 Antineoplastic activity of benzalacetone anils (X).

While compound Xa was found to be active in the P388 screen, Xb was found to be inactive (TABLE XXIII; Section 4.10.0.0). However, the sample of Xb which had been synthesized earlier in this laboratory and sent to the National Cancer Institute, U.S.A. for screening was subsequently found to be impure. Hence, the data generated by the National Cancer Institute on this compound should be regarded with suspicion.

TABLE XI. Evaluation of N-(1-methyl-3-phenyl-2-propenylidene)-benzenamine against various tumours in mice.

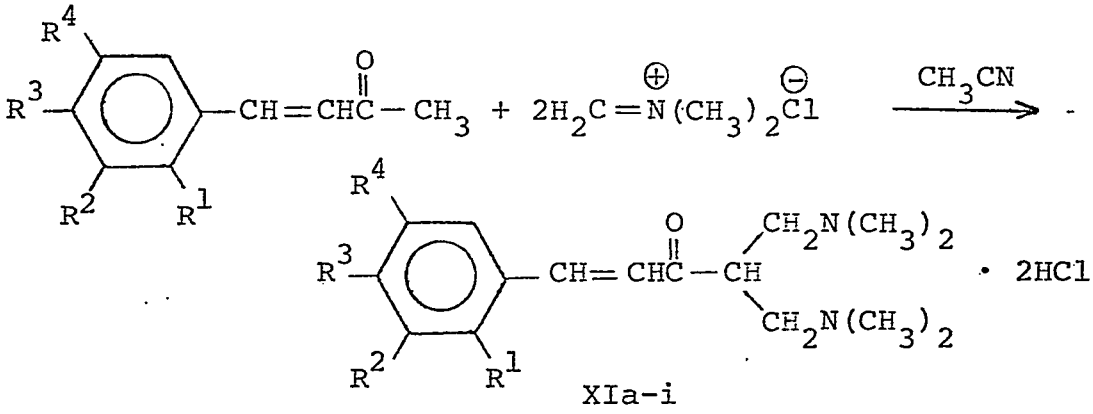
No.	Tumour	Treatment Schedule	Optimum Activity[a] (dose) in mg/kg.)	Toxicity[b] (dose in mg/kg)
1	CX - 1 Colon xenograft	One injection every four days; a total of four injections.	50 (300)	6/6 (600)
2	LX - 1 Lung xenograft	One injection every four days; a total of three injections.	63 (300)	6/6 (2400)
3	MX - 1 Breast xenograft	One injection every four days; a total of three injections.	69 (600)	6/6 (1200)
4	$CD8F_1$ Mammary xenograft	One injection	97 (250)	1/10 (1000), 10/10 (500)
5	Colon 38	One injection every seven days; a total of two injections.	40 (25)	4/10 (400), 9/10 (400), 10/10 (200)
6	B16 Melanocarcinoma	Nine daily injections.	115 (100)	9/10 (400), 10/10 (200)
7	L-1210 Lymphoid leukemia	Nine daily injections.	111 (50)	0/6 (400), 6/6 (200)
8	Lewis lung carcinoma	Nine daily injections.	109 (12.5)	8/10 (400), 10/10 (200)
9	P388 Lymphocytic leukemia	Nine daily injections.	125 (200)	2/6 (400), 6/6 (200)

[a,b]See corresponding footnotes in TABLE XV.

Compound Xa, which was found to display a relatively low level of murine toxicity, was also screened against several other tumour systems. The compound was found to be active only against the colon 38 tumour (TABLE XI).

3.20.0.0 Preparation of 1-aryl-5-dimethylamino-4-dimethylaminomethyl-1-penten-3-one dihydrochlorides (XI).

The title compounds were prepared by reacting a solution of the appropriate 4-aryl-3-buten-2-one in acetonitrile with two molar equivalents of dimethyl(methylene) ammonium chloride (fig. 56).



XIa-i

fig. 56. Synthesis of 1-aryl-5-dimethylamino-4-dimethylaminomethyl-1-penten-3-one dihydrochlorides.

The compounds were recrystallized from methanol, if necessary (see TABLE XXIV, Section 4.11.0.0 for physical data).

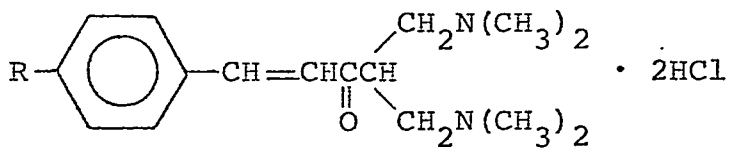
Of the compounds synthesized, the 4-methyl (XIc) and the 4-methoxy (XId) analogues did not give satisfactory microanalytical values. In each case, the carbon content found was at least two per cent lower than the calculated

carbon content, while the hydrogen and nitrogen contents were found to be within acceptable limits (± 0.4% of the calculated values). However, the NMR spectra of these compounds were found to be quite satisfactory (Section 4.11.0.0).

3.21.0.0 Mass spectrometry of some 1-aryl-5-dimethylamino-4-dimethylaminomethyl-1-penten-3-one dihydrochlorides (XI).

Three representative compounds, viz., the unsubstituted (XIa), the 4-methyl (XIc) and the 4-hydroxy (XIh) analogues, were selected for this study. The principal fragment ions formed from XIa, c and h are shown in fig. 57 and the mass spectral data given in TABLE XII.

TABLE XII. m/z (Relative intensity) values of the principal ions in the 70 eV mass spectra of some 1-aryl-5-dimethylamino-4-dimethylaminomethyl-1-penten-3-one dihydrochlorides.



Compound	R	m/z Relative intensity
XIa	H	215(6), 214(3), 202(26), 198(4), 158(28), 157(40), 131(34), 103(41), 84(9), 77(33), 58(100), 45(11), 44(26)
XIc	CH_3	229(4), 228(2), 216(18), 212(2), 172(10), 171(11), 157(28), 145(20), 117(18), 115(13), 102(4), 91(8), 84(5), 58(100), 45(6), 44(13)
XIh	OH	231(6), 230(3), 218(6), 214(4), 188(8), 174(15), 173(9), 147(32), 119(14), 94(8), 91(11), 84(4), 65(9), 58(100), 45(18), 44(37)

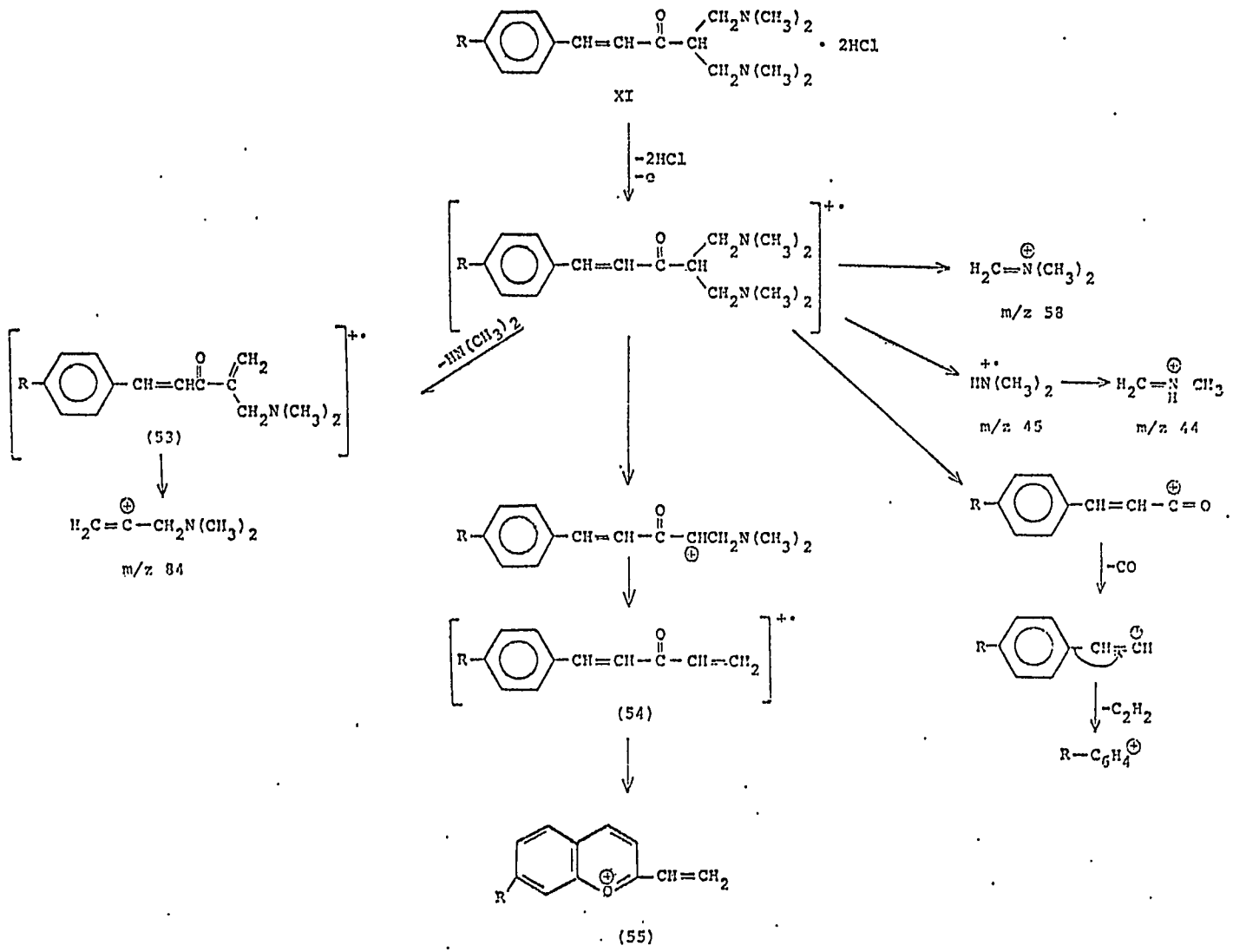


fig. 57. Mass spectral fragmentation pattern for 1-aryl-5-dimethylamino-4-dimethylaminomethyl-1-penten-3-one dihydrochlorides.

Although there are quite a few obvious similarities in the mass spectral fragmentation patterns between 1-aryl-3-dimethylamino-2-dimethylaminomethyl-1-propanone dihydrobromides (V) and XI, there are also certain distinct differences. For example, there is a discernible peak due to the radical ion of 1-aryl-4-dimethylaminomethyl-1,4-pentadien-3-one (53) in the case of XI. As noted in Section 3.9.0.0, the peak due to the corresponding ion is of negligible intensity in the case of V. Furthermore, in series XI, the base peak is due to the dimethyl(methylene)ammonium ion (m/z 58) in each case (including the 4-hydroxy analogue) under consideration.

The olefinic double bonds in XI were shown from the NMR spectra to have the trans arrangement ($J_{1,2}$ = ~16 Hz). However, the mass spectra showed peaks corresponding to the loss of a hydrogen atom from the 1-aryl-1,4-pentadien-3-one radical ion (54) to give the benzopyrylium ion (55). An intramolecular aromatic substitution mechanism has been invoked to account for the formation of such species (fig. 58; Clausen et al., 1966; Ronayne et al., 1966). For this mechanism to become operative, a trans to cis isomerization of the styryl double bond should occur prior to intramolecular aromatic substitution.

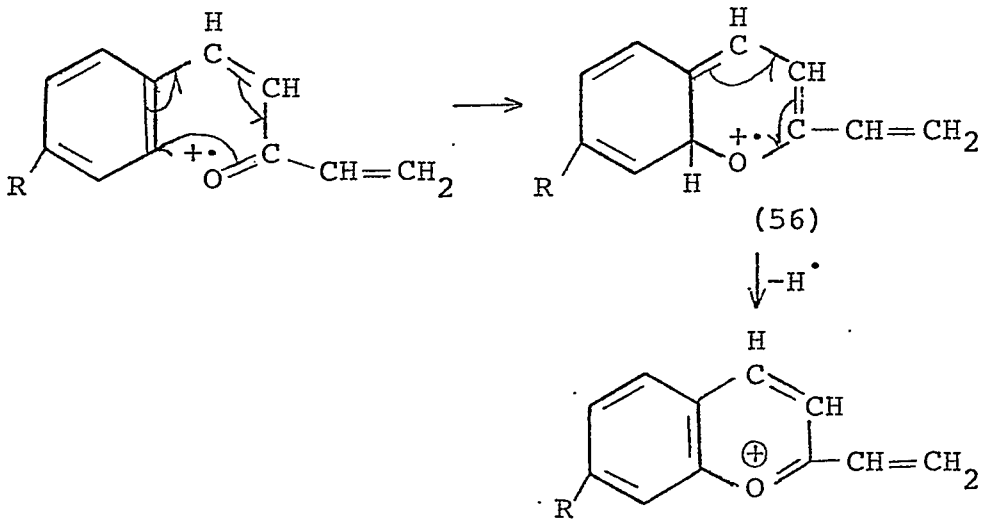


fig. 58. Formation of the benzopyrylium ion from 1-aryl-1,4-pentadien-3-one radical ion.

As suggested by Smith et al. (1972b), the formation of the benzopyrylium ion could proceed by the initial attack of the carbonyl oxygen at the position ortho to the styryl double bond to give the cyclic intermediate (56). This intermediate may lose a hydrogen atom as shown in fig. 58 to give the benzopyrylium ion which is aromatic.

3.22.0.0 Antineoplastic activity of 1-aryl-5-dimethylamino-4-dimethylaminomethyl-1-penten-3-one dihydrochlorides (XI).

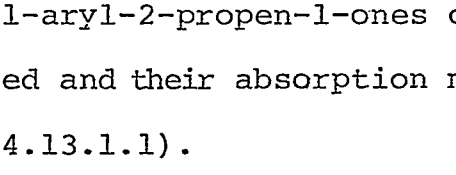
At the time of writing this thesis, the screening data for this series of compounds had not been received in their final form. However, the data received so far suggest that these compounds are highly toxic even at very low dose levels (e.g. 12.5 mg/kg). Screening has been completed for only one compound, viz., XId. This compound did not display significant activity against P388 lymphocytic leukemia [T/C% 106 (6.25)].

3.23.0.0 Kinetic studies

Before an attempt was made to study the elimination reactions of 1-aryl-3-dimethylamino-1-propanone hydrobromides (I) and methobromides (II) quantitatively, some preliminary experiments were carried out with a view to understanding the behaviour of some of the Mannich base hydrobromides and methobromides in solution. Variations in the absorption maxima with time were studied for a number of 1-aryl-3-dimethylamino-1-propanone methobromides in the presence of aqueous solutions of different bases such as sodium hydroxide, sodium carbonate, trimethylamine, etc., at room temperature. When the reaction was carried out under pseudo first-order conditions using a large excess of base, a hypsochromic shift was observed in each case. Since the generation of an α,β-unsaturated ketone moiety should result in a bathochromic shift, the stability of the methobromides was studied first in water, and then in dry methanol (Vogel, 1956) in the absence of base. Although a bathochromic shift was initially observed in water, the final result once again proved to be a hypsochromic shift. A similar behaviour was observed in dry methanol, except that in the latter the shift to a lower wavelength was more gradual. In water and also in methanol (reagent) the reaction rate was so fast as to make recording of optical density readings extremely difficult. It was also found that in aqueous sodium hydroxide

solution, 3-dimethylamino-1-(4-methoxyphenyl)-1-propanone methobromide (IIe) gave at least six chloroform-soluble products as shown by thin-layer chromatography on silica gel (ethyl acetate: petroleum ether, b.p. 30°C-60°C 85:15).

Since kinetic studies proved to be virtually impossible in basic and neutral media, it was decided to study the behaviour of the methobromides in weakly acidic Sørensen's phosphate buffers (Deardoff, 1970). Since the pH of certain cancer cells is considered to be of the order of 6.9, a value which can be decreased to approximately 6.4 by pretreatment with glucose (see Section 3.10.0.0), it was decided to study the kinetics of elimination of II at pH 6.4 (37°C). Accordingly, the relevant 1-aryl-2-propen-1-ones or acrylophenones, XVa-i were synthesized and their absorption maxima determined (TABLE XXV; Section 4.13.1.1).



XV

a. $R^1 = R^2 = H$

b. $R^1 = CH_3$; $R^2 = H$

c. $R^1 = H$; $R^2 = CH_3$

d. $R^1 = OCH_3$; $R^2 = H$

e. $R^1 = H$; $R^2 = OCH_3$

f. $R^1 = OH$; $R^2 = H$

g. $R^1 = H$; $R^2 = OH$

h. $R^1 = H$; $R^2 = Cl$

i. $R^1 = R^2 = Cl$

Optical density readings of a solution of the appropriate methobromide in Sørensen's phosphate buffer, pH 6.4 were recorded at the absorption maximum of the corresponding acrylophenone. The temperature was maintained at 37°C. However, although a decrease in pH was attended by a slower rate of reaction in each case, it was still difficult to work under these conditions because of the high rate of reaction observed in the case of the more reactive members of the series [e.g. IIj (4-Cl) and IIk (3,4-Cl_2)]. By a process of trial and error, the following conditions were arrived at, viz., a temperature of 20°C and a pH value of 5.9.

The Mannich base hydrobromide salts appeared to undergo little more than simple neutralization at pH 7.4 (37°C) over a period of 12 hours and hence, were excluded from further study. Compounds IIh (3-NO_2) and IIi (4-NO_2) were also excluded due to solubility problems.

In the preliminary experiments done at pH 5.9 (20°C) it was observed that the optical density of the solution under consideration increased steadily for a certain period of time (~2 to 3 half-life periods) after which the behaviour was erratic, i.e. the optical density reading

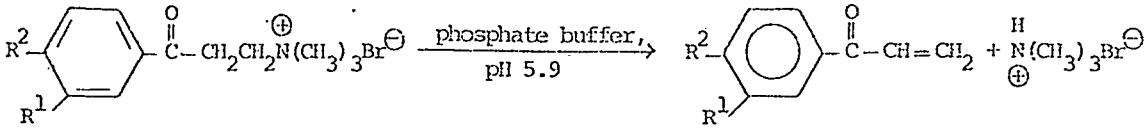
increased or decreased. Furthermore, the optical density reading did not attain a constant value even after 24 hours. Possibly, the acrylophenones formed from β-elimination of IIa-g, j and k were undergoing Michael addition with water under the reaction conditions employed. Therefore, the method of Guggenheim (1926), which does not require a knowledge of the magnitude of the initial or final concentration of the reacting species, was employed for the purpose of determining the pseudo first-order rate constants for the elimination reactions of IIa-g, j and k. The data obtained are given in TABLE XIII. A model kinetic plot is given in Section 5.1.0.0. The Hammett plot for the β-elimination reaction of 1-aryl-3-dimethylamino-1-propanone methobromides at 20°C, pH 5.9 gave a rho (ρ) value of $+1.00 \pm 0.09$ (fig. 59).

An attempt was made to synthesize the 2,2-d_2 analogues of IIa (unsubstituted) and IIe (4-methoxy). Although the compounds were subsequently shown to be only ~70% deuterated, the k^H/k^D values were nevertheless determined for the elimination reaction of these compounds and were found to be 3.29 for IIe and 2.14 for IIa (TABLE XIV).

The following conclusions could be drawn from the data generated.

Of the mechanisms that are possible, E1 is unlikely since the magnitude of the deuterium isotope effects suggests that they are primary. Although deuterium analysis of the 2,2-d_2 analogues of IIa and IIe indicated that the compounds

TABLE XIII. Rates of deamination of some 1-aryl-3-dimethylamino-1-propanone methobromides at 20°C and pH 5.9.



Compound[a]	R^1	R^2	σ[b]	Δt employed (in seconds)	Rate constants $\times 10^4$ sec^{-1}	$\log_{10} \frac{k_R}{k_H}$
IIa	H	H	0.00	3000	(1) 2.13±0.03 (2) 2.01±0.05 (3) 2.15±0.05 Mean: 2.10±0.03[c]	0.00
IIb	CH_3	H	-0.07	3000	(1) 2.19±0.03 (2) 2.28±0.03 (3) 2.25±0.06 Mean: 2.25±0.06[c]	0.0280287
IIc	H	CH_3	-0.17	4000	(1) 1.49±0.02 (2) 1.54±0.03 (3) 1.50±0.03 Mean: 1.51±0.02[c]	-0.1432423
IId	OCH_3	H	+0.12	2400	(1) 3.92±0.07 (2) 4.01±0.05 Mean: 3.97±0.04[c]	0.2765712
IIe	H	OCH_3	-0.27	5000	(1) 1.06±0.03 (2) 1.05±0.01 (3) 1.06±0.02 Mean: 1.06±0.01[c]	-0.2969134
IIf	OH	H	+0.12	3200	(1) 3.43±0.21 (2) 3.23±0.10 Mean: 3.33±0.12[c]	0.2002249
IIg	H	OH	-0.37	10000	(1) 0.847±0.12 (2) 0.886±0.10 (3) 0.872±0.13 Mean: 0.87±0.07[c]	-0.3836996
IIj	H	Cl	+0.23	1800	(1) 3.80±0.06 (2) 4.07±0.08 (3) 3.94±0.06 Mean: 3.94±0.04[c]	0.2732769
IIk	Cl	Cl	+0.60	700	(1) 7.55±0.11 (2) 7.46±0.15 (3) 7.39±0.11 Mean: 7.47±0.07[c]	0.5511013

[a] The concentrations employed were in the range 10^{-4} - 10^{-5} M.

[b] Carey and Sundberg (1977d).

[c] The limits shown are the standard deviation; mean deviation, $r = \pm \frac{\sqrt{r_1^2 + r_2^2 + r_3^2}}{n}$ where r_1, r_2 and r_3 are the standard deviations in k for determinations (1), (2) and (3), respectively, and n is the number of determinations.

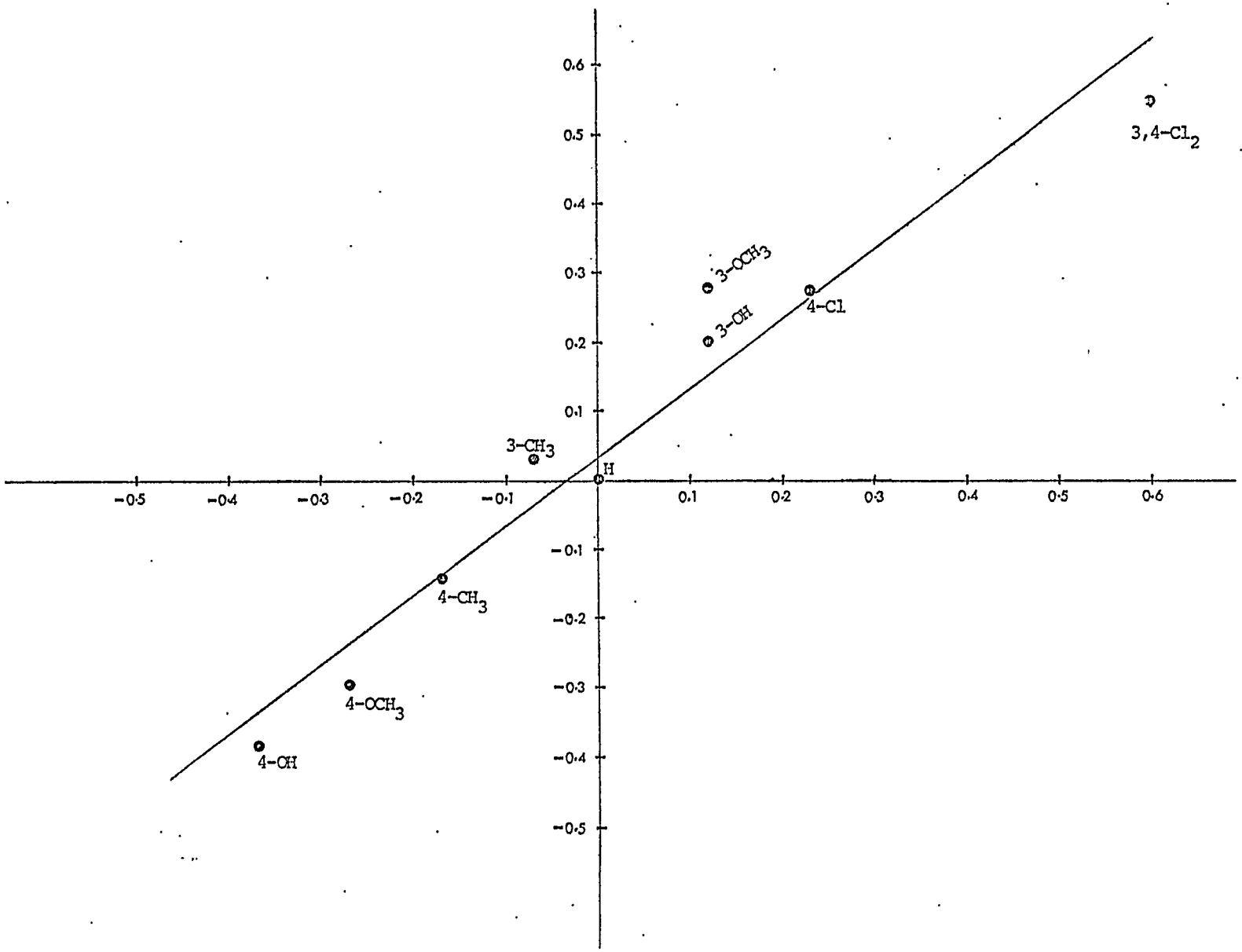
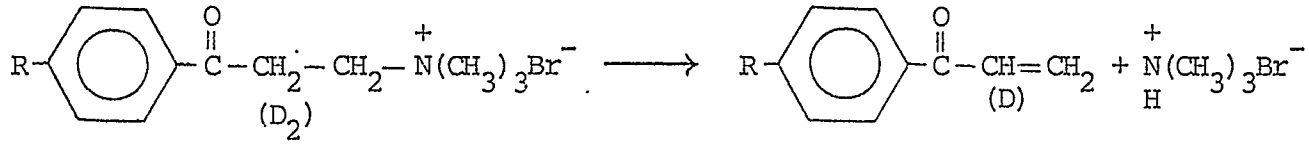


fig. 59. The Hammett plot for the β-elimination of 1-aryl-3-dimethylamino-1-propanone methobromides at 20°C, pH 5.9.

TABLE XIV. Hydrogen-deuterium kinetic isotope effects for the elimination reactions of some representative 1-aryl-3-dimethylamino-1-propanone methobromides at 20°C and pH 5.9.



R	Rate constant for the deuterated analogue × 10^4 sec^{-1}	Rate constant for the undeuterated analogue × 10^4 sec^{-1}	$\frac{k^H}{k^D}$[a]
H	(1) 1.21±0.02 (2) 1.18±0.05 Mean: 1.195±0.027	(1) 2.56±0.01 (2) 2.55±0.01 Mean: 2.555±0.007	2.14±0.05
OCH_3	(1) 0.384±0.01 (2) 0.386±0.01 Mean: 0.385±0.007	(1) 1.25±0.01 (2) 1.28±0.01 Mean: 1.265±0.007	3.29±0.06

[a]Ratio of rates of elimination;

deviation: $\pm(k^H/k^D)[(r_H/k_H)^2 + (r_D/k_D)^2]^{\frac{1}{2}}$

where r is the standard deviation in k.

were only ~70% deuterated, the data should be interpreted with caution. The low deuterium content in each case may be due to any of the following reasons: a) less than reliable deuterium analysis; b) the presence of an impurity such as trimethylammonium bromide which would not be expected to affect the rate of reaction of the 2,2-d_2 analogues significantly; c) contamination with the undeuterated compound which, if present in significant quantities, would be expected to undergo elimination more rapidly than the corresponding deuterated analogue if the C-H(D) bond is broken in a rate-limiting step, thus giving rise to a curved kinetic plot. Since the kinetic plots for both deuterated analogues have correlation coefficients close to unity and also since the rate constants are much lower than those obtained for the corresponding undeuterated analogues, the possibility of these compounds being contaminated with the undeuterated analogues is remote. Furthermore, prior to quaternization with methyl bromide the structures of the two 1-aryl-3-dimethylamino-1-propanone 2,2-d_2 were established by NMR spectroscopy (see Section 4.13.0.0). Since reasons (a) and (b) are less likely to give spurious k^H/k^D values than (c), it is perhaps not unwise to use these values as evidence to exclude E1 as a possible mechanism of elimination in the case of series II. As noted in Section 1.6.1.1, the E1 mechanism usually involves the loss of the β-hydrogen in a fast step. Hence a reaction proceeding