DERIVATIVES OF 2-BENZYLIDENECYCLOHEXANONE AS POTENTIAL ANTINEOPLASTIC AGENTS

A Thesis

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T O S A N D R A

ABSTRACT

The aim of this research was to prepare some substituted 2-benzylidenecyclohexyl isothiocyanates, which would be screened against L-1210 lymphoid leukemia and the KB tumor. The first step in the synthesis involved the preparation of substituted (E)-2-benzylidenecyclohexanones, which are α,β-unsaturated ketones, a class of compounds known to have alkylating properties. Only the substituted (E,E)-2,6-bis-(dichlorobenzylidene)cyclohexanones were isolated in the syntheses designed to yield (E)-2-(dichlorobenzylidene)cyclohexanones. All benzylidene derivatives were submitted for screening. The unsubstituted compound, (E)-2-benzylidenecyclohexanone, has a high level of activity against the KB tumor in vitro, and a low level of toxicity in mice.

Ultraviolet irradiation resulted in isomerization of the substituted (E)-2-benzylidenecyclohexanones to the Z isomers. (Z)-2-Benzylidenecyclohexanone was isolated from the ultraviolet irradiation of the E isomer.

Ultraviolet spectroscopy and the Braude equation were employed to study the degree of coplanarity of the conjugated system for some of the benzylidene derivatives.

The mass spectra of the substituted (E)-2-benzylidenecyclohexanones and the substituted (E,E)-2,6-bis-benzylidenecyclohexanones were examined in detail. One of the major fragmentation pathways involved an intramolecular attack by the carbonyl oxygen on the ortho position

of the aromatic ring, resulting in the formation of a benzopyrylium ion.

The second step in the synthesis involved the preparation of the substituted (E)-2-benzylidenecyclohexanone oximes from the corresponding ketones. The mass spectra of the oximes were also examined. A major fragmentation pathway involved an intramolecular attack of the nitrogen of the oxime function on the ortho position of the aromatic ring, analogous to a major fragmentation pathway of the ketones.

Lithium aluminum hydride reduction of the substituted (E)-2-benzylidenecyclohexanone oximes was expected to yield the substituted (E)-2-benzylidenecyclohexylamines. However, high yields of substituted 1-benzyl-1,2-epiminocyclohexanes were obtained. Aziridines have a wide range of biological activity, including anticancer activity. The substituted 1-benzyl-1,2-epiminocyclohexanes were submitted for screening against L-1210 lymphoid leukemia and the KB tumor.

Treatment of 1-benzyl-1,2-epiminocyclohexane with acetic anhydride under reflux resulted in pyrolytic cis-elimination, with the formation of 3-acetamido-2-benzyl-1-cyclohexene.

The Leuckart Reaction was employed in an attempt to convert (E)-2-benzylidenecyclohexanone directly to the corresponding primary amine, (E)-2-benzylidenecyclohexylamine. Several reaction systems were investigated, each yielding the desired amine in only trace amounts.

A third synthesis was attempted, which was to involve the lithium aluminum hydride reduction of the substituted (E)-2-benzylidene-

cyclohexanones to the corresponding unsaturated alcohols, conversion of the alcohols to the tosylate derivatives, formation of the azides from the tosylates, and lithium aluminum hydride reduction of the azides to give the (E)-2-benzylidenecyclohexylamines. The amines could then be used in the Hofmann mustard oil reaction to form the (E)-2-benzylidenecyclohexyl isothiocyanates.

Reduction of (E)-2-benzylidenecyclohexanone with lithium aluminum hydride gave (E)-2-benzylidenecyclohexanol. p-Toluenesulfonyl chloride did not react with this alcohol at room temperature or under ether reflux. Treatment of the lithium salt of (E)-2-benzylidene-cyclohexanol with p-toluenesulfonyl chloride gave the elimination product, 3-benzylidene-1-cyclohexene, in high yield.

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I. INTRODUCTION

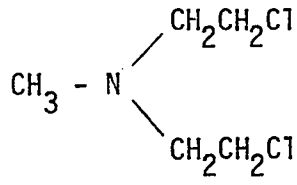
1.1 Chemotherapy of cancer

Cancer is the malignant unrestrained proliferation of somatic cells. Leukemia is an uncontrolled multiplication of the leukoblastic tissues, or ammature leukocytes, which generally leads to an increase in the white cells of the blood. The two major types of leukemia are myelogenous leukemia and lymphatic leukemia. In myelogenous leukemia there is an increase in the number of neutrophils, eosinophils, or basophils whereas lymphatic leukemia involves a proliferation of lymphoid cells only (Boyd, 1967). Chemotherapy has been used quite extensively in the treatment of leukemic patients since often the disease is too widespread for surgical treatment.

The development of chemotherapeutic agents has provided an additional choice of therapy for the treatment of cancer, supplementing surgery and radiation in the control of the disease. The principal classes of drugs that have been found useful in the treatment of cancer are the alkylating agents, antimetabolites, natural products such as the vinca alkaloids, antibiotics, and hormones.

The three major types of alkylating agents used in the chemotherapy of neoplastic diseases are the nitrogen mustards, the ethyleneimines, and the alkyl sulfonates, represented respectively by mechloroethamine (1),

triethylenemelamine (2), and busulfan (3).



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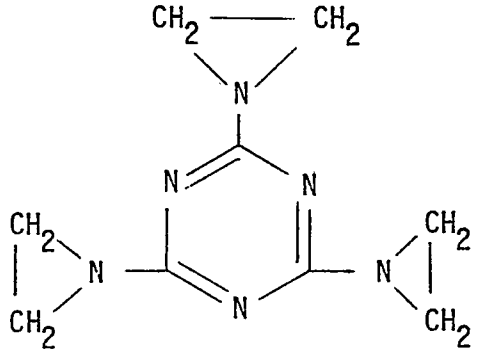

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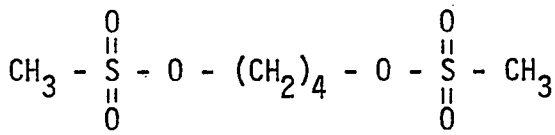


(3)

These alkylating agents can readily form covalent linkage with various nucleophilic substances, including such biologically important moieties as the sulfhydryl and carboxyl groups of proteins, and the phosphate and imidazole groups of nucleic acids. Studies on the interaction of alkylating agents with DNA have shown that the

point of attack on the DNA molecule is predominantly at the 7-position of the purine base, guanine (Brookes and Lawley, 1961, 1964a, 1964b).

1.2 The aims of the present investigation

A group of alkylating agents which have received little attention as antineoplastic drugs are the isothiocyanates, and the aim of the present investigation was to prepare some novel isothiocyanates which would be screened against various cancers, including L-1210 lymphoid leukemia in mice and the KB tumor *in vitro*.

Isothiocyanates have a wide range of biological activity. Very dilute solutions of allyl isothiocyanate have been found to have anticancer activity, especially against lymphoma in chickens (Baranger, 1968). Cyclohexyl isothiocyanate has been patented as a fungicide and nematocide (Kuehle, 1962). Ethylene bisdithiocarbamic acid (4) and its salts (Dithane) are much used as agricultural fungicides, the action resulting from degradation to the corresponding isothiocyanate (5) (Sijpesteijn and van der Kerk, 1954). Garmaise *et al.* (1971)

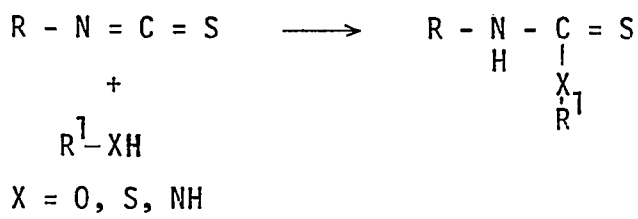
$$(HS\text{-}\overset{\overset{S}{\|}}{C}\text{-}NH\text{-}CH_2\text{-})_2 \longrightarrow (S{=}C{=}N\text{-}CH_2\text{-})_2$$

(4) (5)

prepared a series of ω-bromoalkyl isothiocyanates which showed the anthelmintic activity characteristic of many isothiocyanates. Zsolnai

(1966) found that phenyl isothiocyanate and benzyl isothiocyanate have significant fungistatic activity and some tuberculostatic and ascaricidal activity. He also found tuberculostatic and ascaricidal activity in allyl isothiocyanate and cyclohexyl isothiocyanate. In addition, benzyl isothiocyanate was shown to have bacteriostatic activity.

Isothiocyanates combine with nucleophilic groups such as sulfhydryl, amino, and hydroxyl functions as shown below. For example, cyclohexyl



isothiocyanate has been shown to combine with hydroxyl and amino groups *in vitro* (Zahradnik, 1959). Allyl isothiocyanate combines with aromatic amines such as aniline and N-ethylaniline to give the corresponding allylphenylthioureas (Miskidzh'yan and Gladyshevskaya, 1962) and reacts with such aliphatic amines as methylamine, ethylamine, and isopropylamine to form the corresponding allylalkylthioureas (Miskidzh'yan, 1960). The addition of amines to isothiocyanates is generally much faster than the addition of the corresponding alcohols. Rao (1963) investigated the addition of various alkyl alcohols to 4-bromophenyl isothiocyanate to form the corresponding thiourethans (thiocarbamates).

Bacq and Fischer (1946) showed that allyl isothiocyanate reacts with the sulfhydryl groups of ovalbumin. Cattle serum, cysteine, and thioglycolate inhibit the antimicrobial activity of isothiocyanates which suggests that these compounds exert their antimicrobial activity by an intracellular inactivation of sulfhydryl enzymes (Zsolnai, 1966). The fungicidal activity of ethylene bisdithiocarbamic acid (4) is attributed to combination of the isothiocyanate (5), formed upon degradation, with essential sulfhydryl groups (Sijpesteijn and van der Kerk, 1954).

Alkylating agents are thought to react at the cellular level by S_N1 and S_N2 mechanisms. The rate of reaction by the S_N1 mechanism depends on the rate of formation of the carbonium ion which is itself dependent on the stability of the carbonium ion. Allylic derivatives give rise to more stable carbonium ions than alkyl derivatives due to mesomerism. For example, the allyl cation (6) is more stable than the propyl cation (7) which is reflected by greater reactivity of allyl isothiocyanate than propyl isothiocyanate (Zahradnik, 1959).

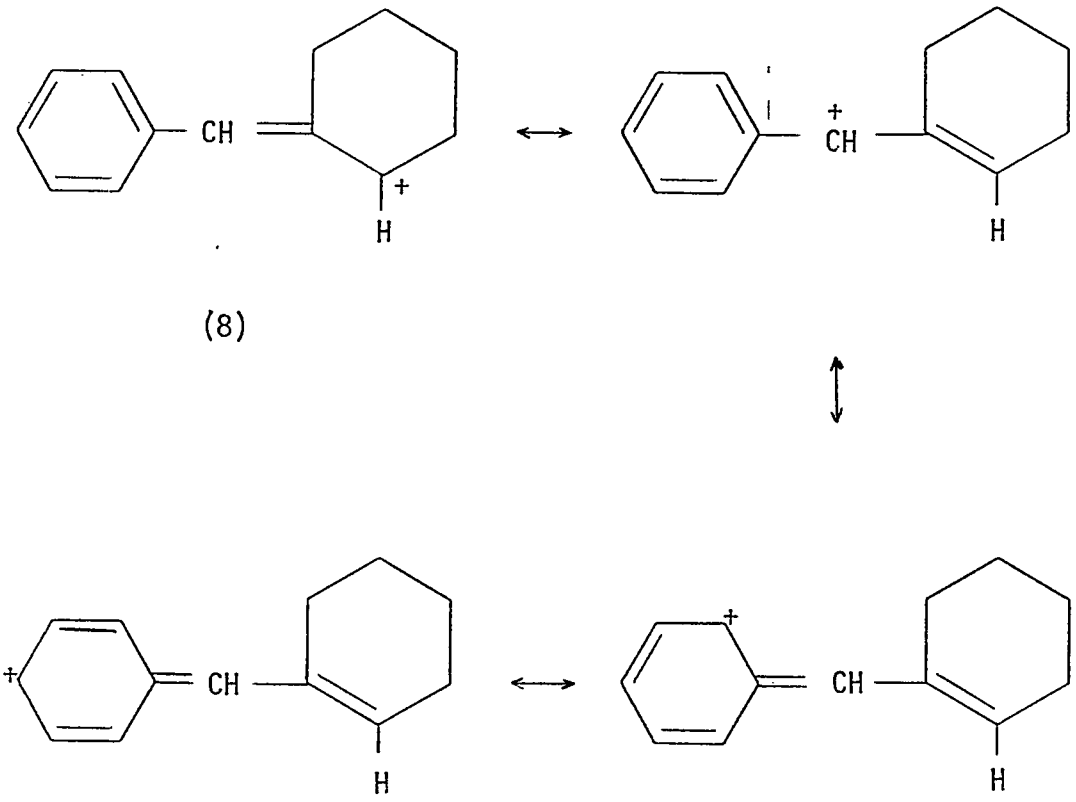
$$CH_2 = CH - CH_2^{(+)} \longleftrightarrow {}^{(+)}CH_2 - CH = CH_2$$

(6)

$$CH_3 - CH_2 - CH_2^{(+)}$$

(7)

In the case of 2-benzylidenecyclohexyl isothiocyanate, the phenyl ring will further stabilize the allylic cation (8) which should enhance the alkylating properties of the isothiocyanate via the S_N1 mechanism. Electron-donating substituents in the ortho

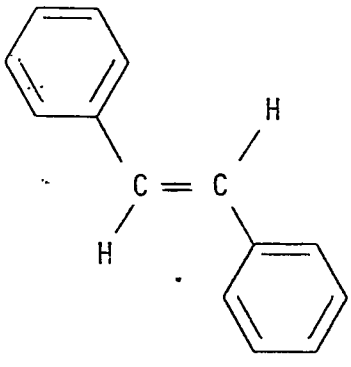


(8)

or para positions of the aromatic ring should increase the stability of the carbonium ion. For example, a dimethylamino group in the para position of the phenyl ring would extend the mesomerism, giving a more stable carbonium ion.

Maximum resonance stabilization of the carbonium ion (8) is expected when the conjugated system is coplanar, which allows for maximum overlap of atomic orbitals. Bulky substituents in the ortho position of the phenyl ring would be expected to force the phenyl ring out of planarity with the rest of the conjugated system, resulting in lower resonance stabilization. The presence of a chlorine atom in the ortho position of the phenyl ring would be expected to exert a steric effect sufficient to twist the phenyl ring out of coplanarity with the ethylenic group. This effect can be observed and the angle of twist determined by ultraviolet spectroscopy and Braude's equation (Braude, 1955).

A further reason for the preparation of substituted 2-benzylidenecyclohexyl isothiocyanates is that transportation to the site of action may be dependent on coplanarity between different groups of the molecule. It has been shown that geometrical isomers with varying degrees of planarity have different adsorption properties on non-biological surfaces. For example, Zechmeister and McNeely (1942) reported that stilbene (9) possessed higher adsorption properties on an alumina column than the corresponding Z isomer, isostilbene (10).



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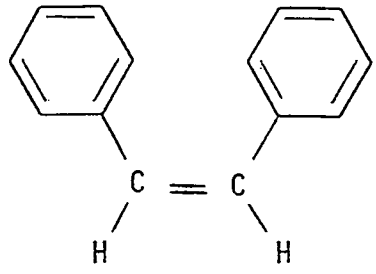
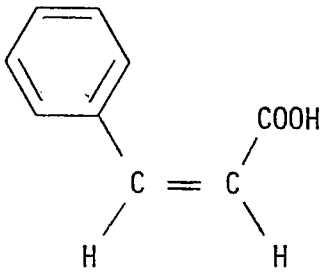


(10)

In certain cases, biological activity resides in the isomer displaying a lack of planarity. (Z)-Cinnamic acid (11) displays activity as a plant growth regulator whereas the corresponding E isomer (12) is inactive (Haagen-Smit and Went, 1935). Alternatively,



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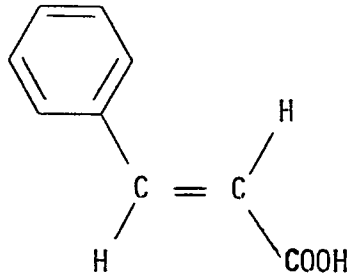
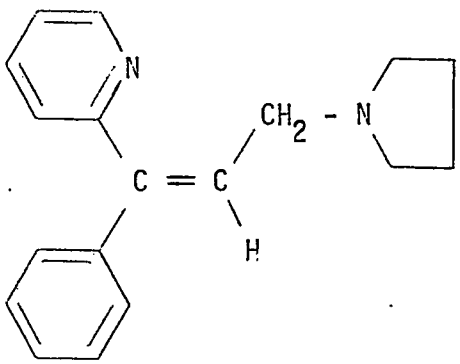


(12)

biological activity may be greater in the planar molecule. Compound (13) has the pyridine ring coplanar with the double bond whereas the other isomer (14) is prevented by steric hindrance from demonstrating this coplanarity. In this case compound (13) has a greater antihistaminic activity than compound (14) (Adamson et al., 1951).



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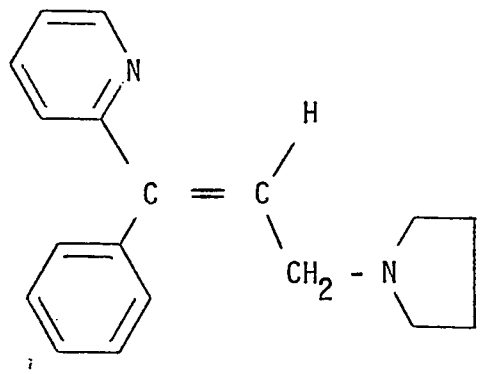
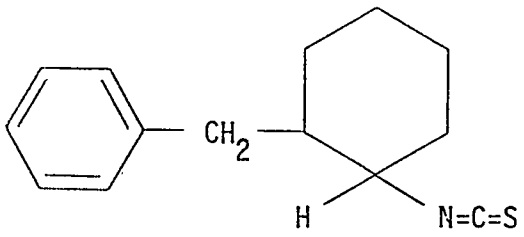


(14)

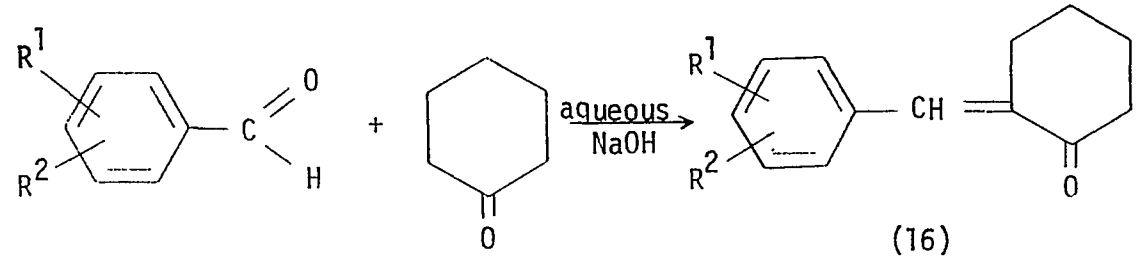
Comparison of the biological activity of 2-benzylidenecyclohexyl isothiocyanate and its substituted derivatives should indicate whether planarity of the molecule enhances reactivity. A comparison of the biological activity of the corresponding substituted and unsubstituted ketones and ketoximes may also indicate whether activity is favored by coplanarity.

In addition it would be interesting to have 2-benzylcyclohexyl isothiocyanate (15) screened to determine if biological activity is decreased by saturation of the olefinic bond of 2-benzylidenecyclohexyl isothiocyanate, analogous to the decreased activity of propyl isothiocyanate as compared to allyl isothiocyanate.



(15)

The proposed route for the synthesis of the isothiocyanates in this program is as shown in Scheme 1. The first stage in this



(16)

R^1 = H; R^2 = H

R^1 = 2-Cl; R^2 = H

R^1 = 3-Cl; R^2 = H

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

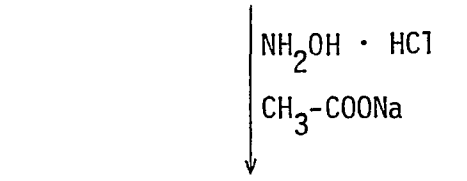
R^1 = 2-Cl; R^2 = 6-Cl

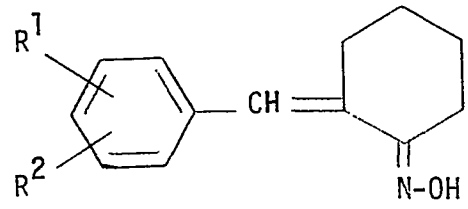
R^1 = 2-Cl; R^2 = 4-Cl

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

R^1 = 4-$N(CH_3)_2$; R^2 = H

R^1 = 4-$\overset{+}{N}(CH_3)_3 I^{(-)}$; R^2 = H





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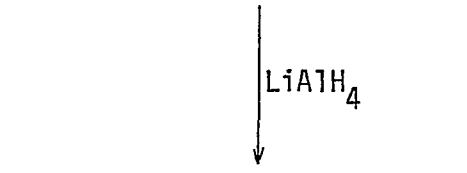
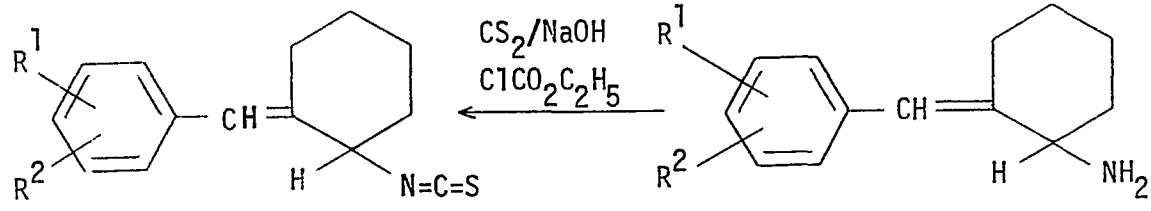
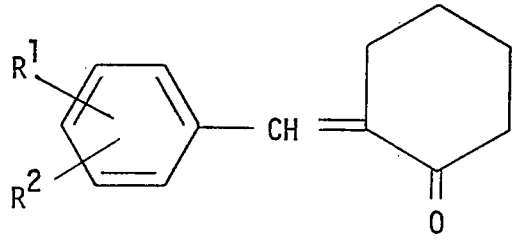




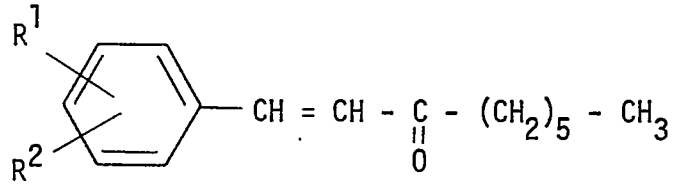
(19) (18)

Scheme 1 Proposed route for the synthesis of substituted 2-benzylidenecyclohexyl isothiocyanates

synthesis is the preparation of the substituted 2-benzylidenecyclohexanones (16), which are α,β-unsaturated ketones, a class of compounds known to be alkylating agents. The substituted 2-benzylidenecyclohexanones (16) represent more rigid cyclic analogs of the flexible open chain derivatives of 1-phenyl-1-nonen-3-one (20). The screening results against L-1210 lymphoid leukemia for derivatives of (16) and (20)[1] will hopefully demonstrate whether rigidity or flexibility is favored for optimum antileukemic activity in these compounds.



(16)

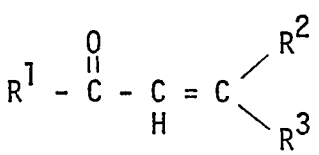


(20)

[1]Taylor (1972).

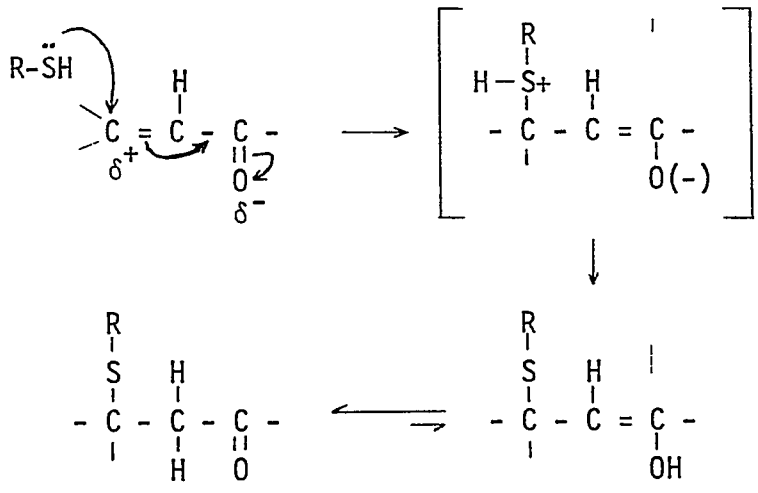
It is generally considered that drug molecules become associated at receptor surfaces by the alignment of certain functions on the molecule with complementary sites on the receptor. The receptor site is the component of the cell with which the drug molecule interacts. Most effective drug-receptor interaction would be expected with rigid molecules in which the distance between functions important for interaction at the receptor is similar to the distance between the complementary sites on the receptor surface. However, a rigid molecule that does not possess the correct molecular dimensions cannot associate at the receptor surface since it has little or no capacity for changing to a more favourable geometry. In contrast, a less rigid molecule is capable of adopting a wide range of conformations, one of which may possess molecular dimensions complementary to the receptor.

The ability of many α,β-unsaturated ketones to react with sulfhydryl compounds was discovered by Posner (1902, 1904). He found that α,β-unsaturated ketones of general structure (21) undergo addition reactions most readily when R^1 is an aromatic radical and R^2 or R^3 is a hydrogen atom.



(21)

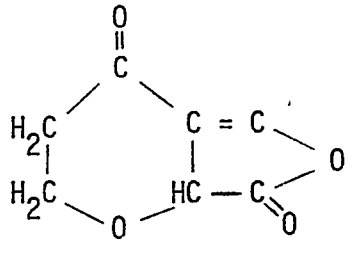
A Michael-type conjugate addition reaction may be involved, which is essentially a 1:4 addition mechanism, giving the saturated ketone (22).



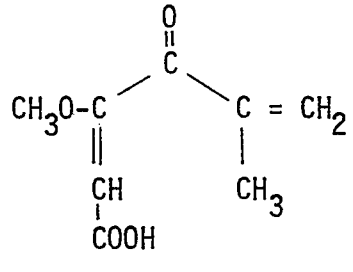
(22)

S-alkylated addition product

Clavacin (23) and penicillic acid (24) are antibiotics which are active against both Gram-positive and Gram-negative bacteria. The α,β-unsaturated ketone group is the only structural feature common to both compounds and was considered likely to be responsible for their antibacterial activity (Conn and Geiger, 1944). The fact that the



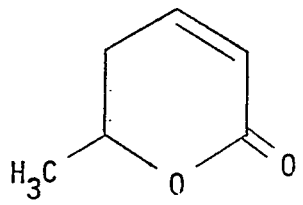
(23)



(24)

antibacterial activities of clavacin and penicillic acid depend on their reaction with sulfhydryl groups was established by Geiger and Conn (1945). They found that both compounds were inactivated by sulfhydryl compounds. Clavacin was inactivated by thioglycolate and by thiosulfate most effectively while penicillic acid was inactivated by cysteine and thioglycolate. Geiger and Conn (1945) synthesized a number of α,β-unsaturated ketones using Posner's model structure (21) as a guide in an attempt to discover α,β-unsaturated ketones with antibacterial activity similar to clavacin and penicillic acid. The most active compound, acrylophenone, fulfilled the structural requirements of (21) perfectly, since here R^1 is a phenyl group, and both R^2 and R^3 are hydrogen atoms. It demonstrated significant bacteriostatic activity and showed fungistatic activity of the same order of magnitude as clavacin. Acrylophenone was also inactivated by both cysteine and thioglycolate. Thus Geiger and Conn concluded that the antibiotic activity of clavacin and penicillic acid, as well as certain synthetic α,β-unsaturated ketones, such as acrylophenone, was due to their reaction with the sulfhydryl groups of bacterial enzyme systems or with sulfhydryl-containing metabolites essential to the bacteria.

The reaction of α,β-unsaturated lactones with thiols has been suggested to play a key role in several biological growth-regulatory phenomena. The selective growth-inhibitory action of δ-hexenolactone (25) on certain animal tissues was shown to be antagonized by cysteine (Hauschka et al.,1945).

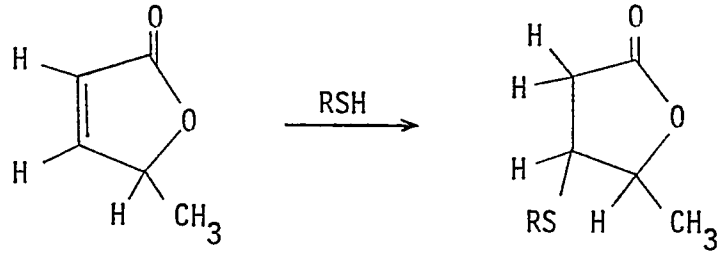


(25)

Since then the search for tumor inhibitors of plant origin has led to the isolation and characterization of a number of sesquiterpene lactones (Kupchan, 1970) that exhibit growth inhibitory activity in vivo against animal tumor systems and in vitro against KB cells. The functional group which is common to these compounds is the α-methylene-γ-lactone grouping. A study of the reactions of tumor-inhibitory conjugated α-methylene lactones with model biological nucleophiles revealed that sulfhydryl compounds were the most reactive of the nucleophiles investigated, which supports the view that a Michael-type addition of sulfhydryl-bearing compounds may play a significant role in the mechanisms by which the lactones exert their biological activities (Kupchan et al., 1970a). The tumor-inhibitory α-methylene lactones have been shown to inhibit the sulfhydryl enzyme, phosphofructokinase, and evidence has been presented to indicate that the inhibition resulted from their reaction with the sulfhydryl groups of the enzyme (Hanson et al., 1970).

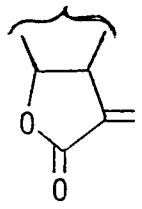
Kupchan and coworkers (1970b) investigated the reactions of endocyclic α,β-unsaturated γ-lactones such as (26) with various sulfhydryl compounds. Cysteine appeared to be the most reactive of

the sulfhydryl compounds investigated. They also found that the exocyclic unsaturated lactone (27) in elephantopin (28) reacted much faster with cysteine than unsaturated endocyclic lactones such as (26). A rate ratio of exocyclic to endocyclic unsaturated lactones of the order of 10^3 was suggested.

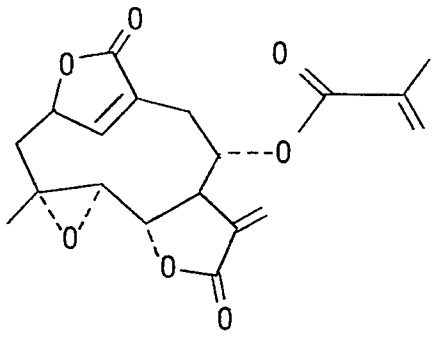


(26)

R = CH_2-CH($^+NH_3$, COO^-) , CH_2-CH(NH-$COCH_3$, $COOCH_3$) , C_3H_7 , CH_2-C_6H_5



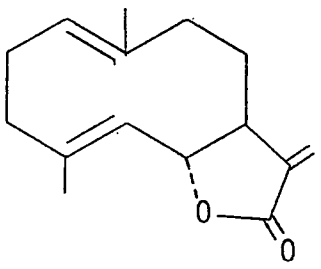
(27)



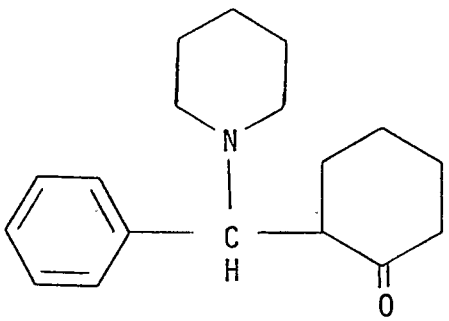
(28)

It has been shown that α,β-unsaturated lactones react with amines in an analogous manner. The addition of dimethylamine to compound (26) has been found to occur in solution (Jones and Young, 1966). The exocyclic unsaturated lactone present in costunolide (29) reacts with dimethylamine to give a stable Michael adduct (Hiremath et al., 1968). Baltzly

and coworkers (1955) studied the addition of a number of secondary amines to cyclic and open-chain analogs of 2-benzylidenecyclohexanone. In these systems the steric requirements of the amine appeared to be quite critical with only cyclic amines and methyl secondary amines adding well. The basicity of the amines appeared to have little relation to the yield of addition product. Such bases as piperidine gave 80-90% of the addition compound (30).



(29)



(30)

In a study of the relative nucleophilic reactivities of amino groups and mercaptide anions in addition reactions with α,β-unsaturated compounds, Friedman et al. (1965) found that, at comparable pK_a values and steric environments, mercapto anions are about 280 times more reactive than amino groups.