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

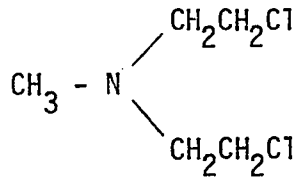
1.1 Chemotherapy of cancer

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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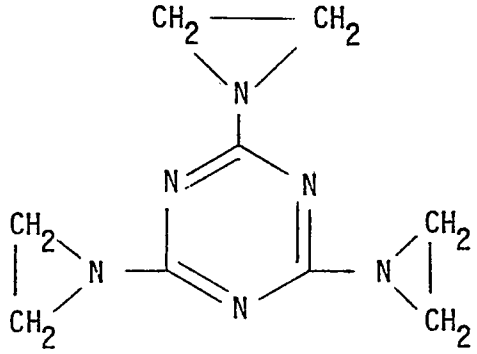

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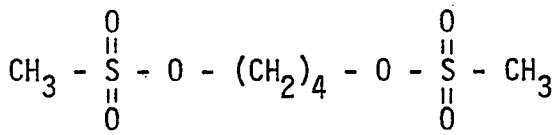


(3)

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)

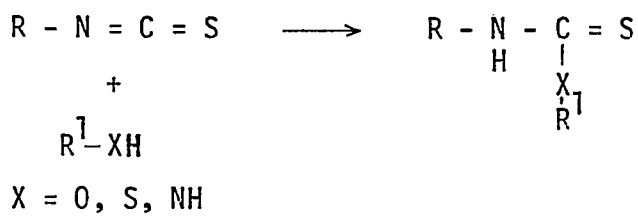
$$(HS\text{-}\overset{\overset{S}{\|}}{C}\text{-}NH\text{-}CH_2\text{-})_2 \longrightarrow (S{=}C{=}N\text{-}CH_2\text{-})_2$$

(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).

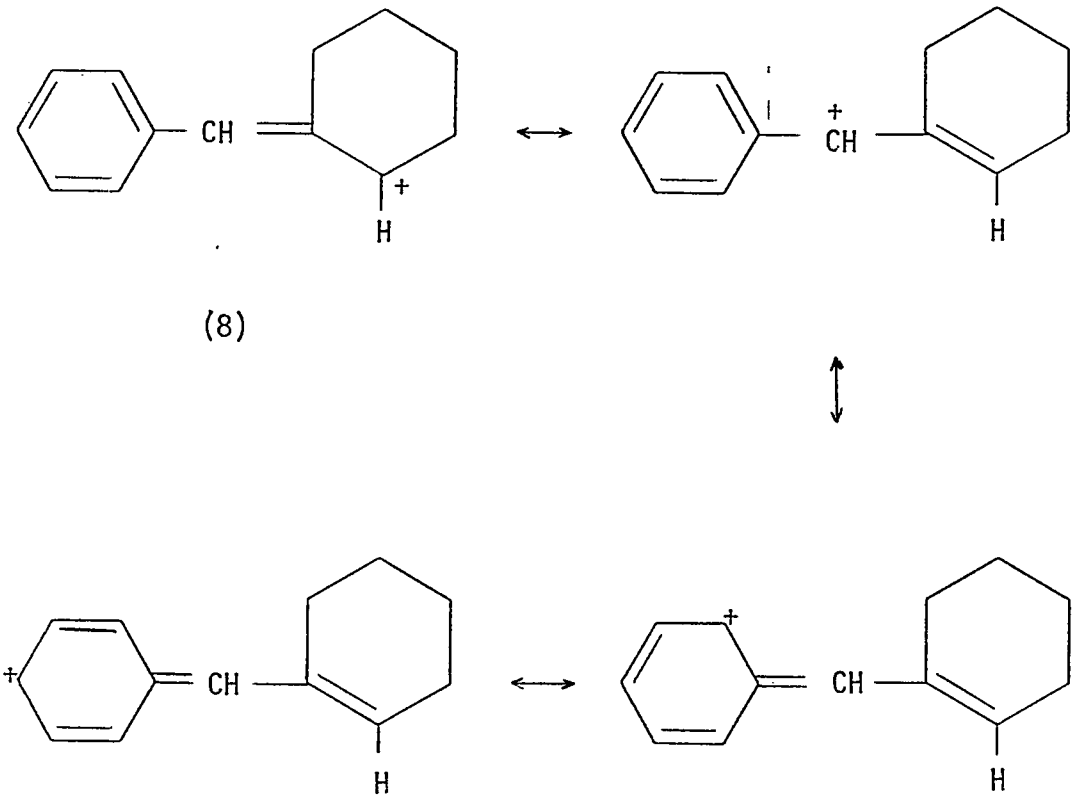
$$CH_2 = CH - CH_2^{(+)} \longleftrightarrow {}^{(+)}CH_2 - CH = CH_2$$

(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

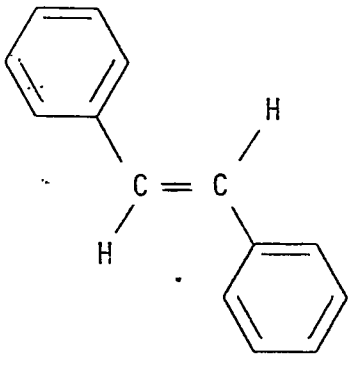


(8)

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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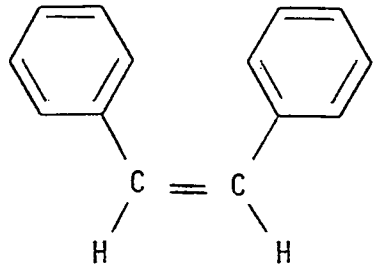
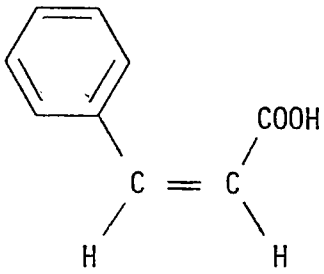


(10)

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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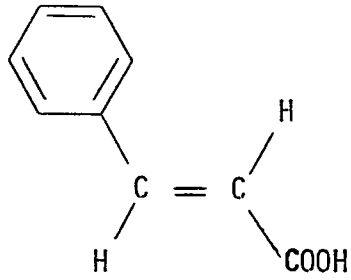
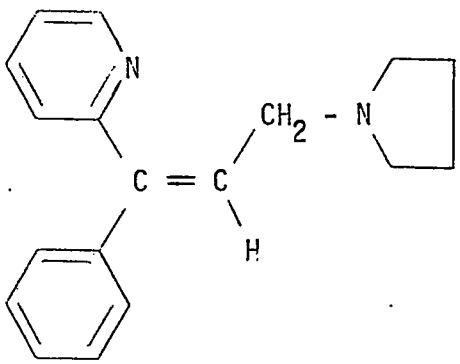


(12)

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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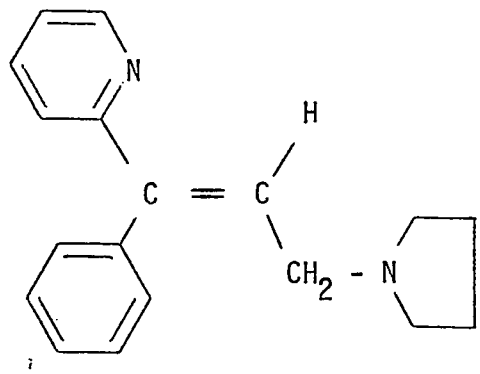
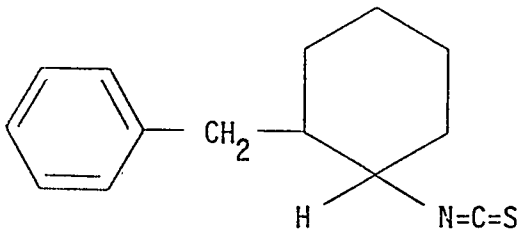


(14)

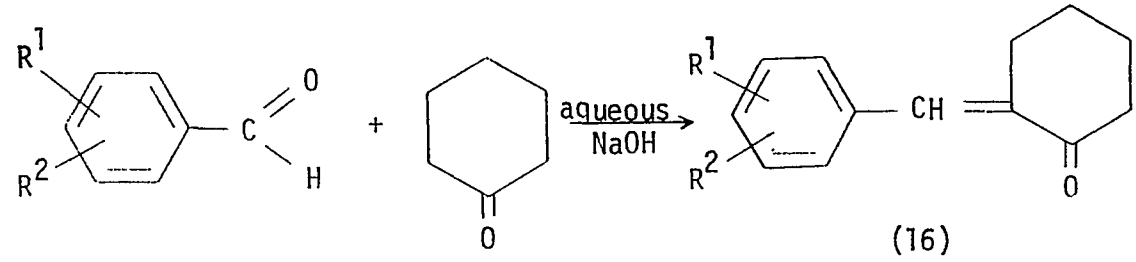
Comparison of the biological activity of 2-benzylidenecyclohexyl isothiocyanate and its substituted derivatives should indicate whether planarity of the molecule enhances reactivity. A comparison of the biological activity of the corresponding substituted and unsubstituted ketones and ketoximes may also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

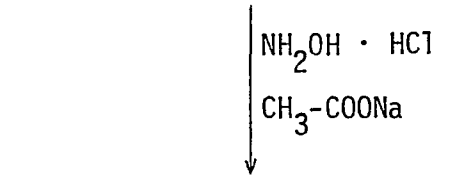
R^1 = 2-Cl; R^2 = 6-Cl

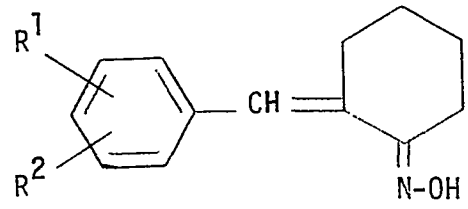
R^1 = 2-Cl; R^2 = 4-Cl

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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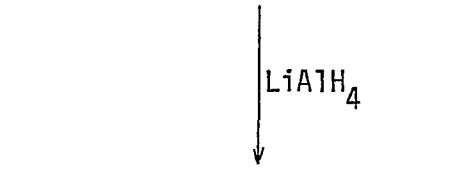
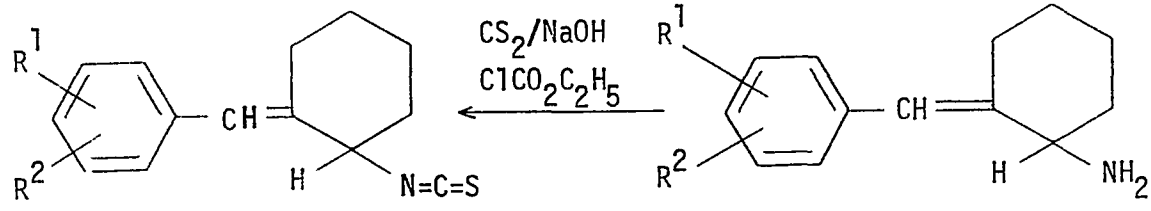
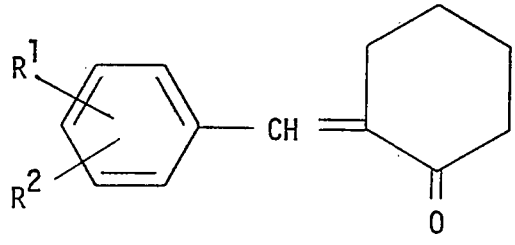




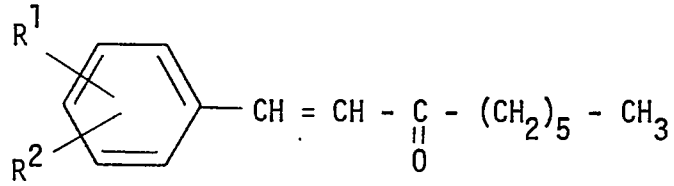
(19) (18)

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)

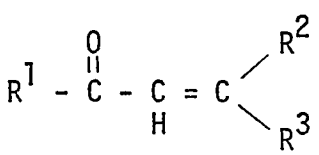


(20)

[1]Taylor (1972).

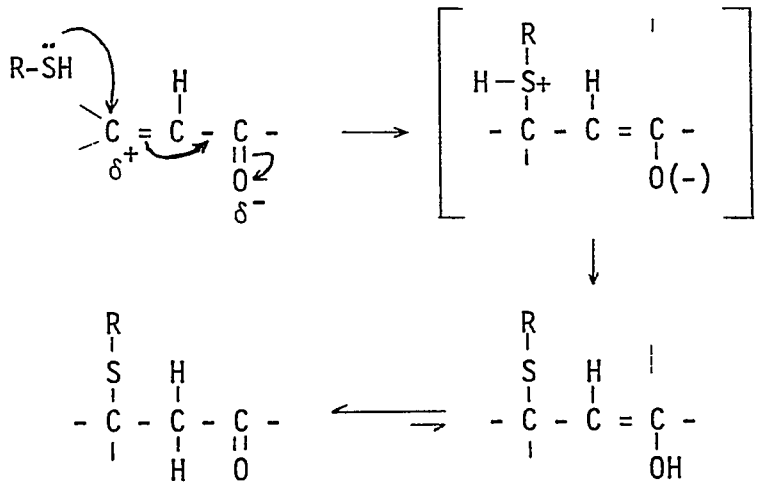
It is generally considered that drug molecules become associated at receptor surfaces by the alignment of certain functions on the molecule with complementary sites on the receptor. The receptor site is the component of the cell with which the drug molecule interacts. Most effective drug-receptor interaction 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)

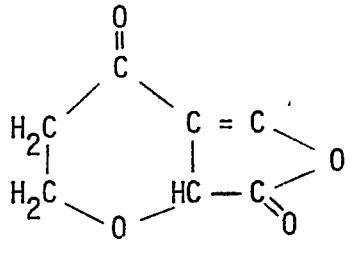
A Michael-type conjugate addition reaction may be involved, which is essentially a 1:4 addition mechanism, giving the saturated ketone (22).



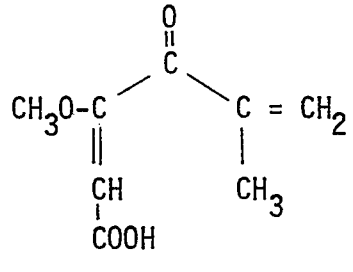
(22)

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



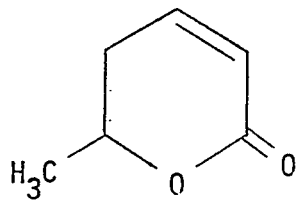
(23)



(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).

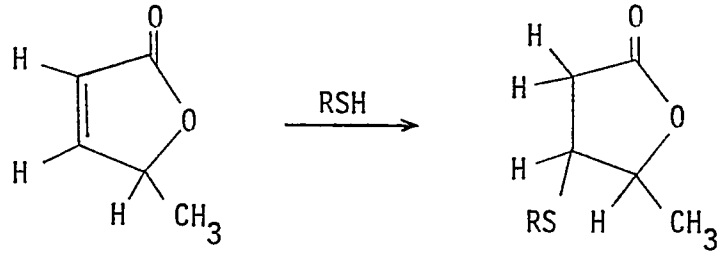


(25)

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).

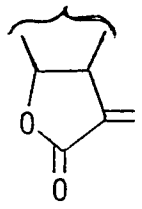
Kupchan and coworkers (1970b) investigated the reactions of endocyclic α,β-unsaturated γ-lactones such as (26) with various sulfhydryl compounds. Cysteine appeared to be the most reactive of

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.

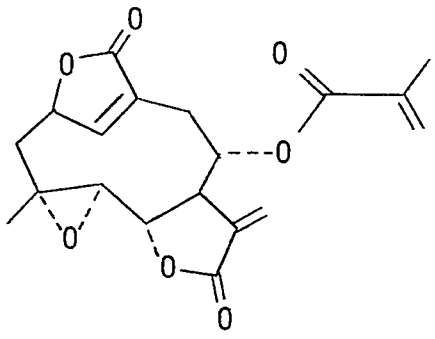


(26)

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



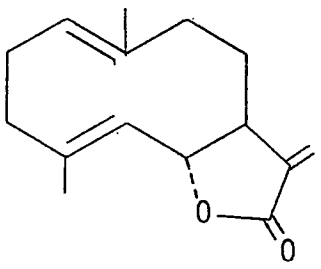
(27)



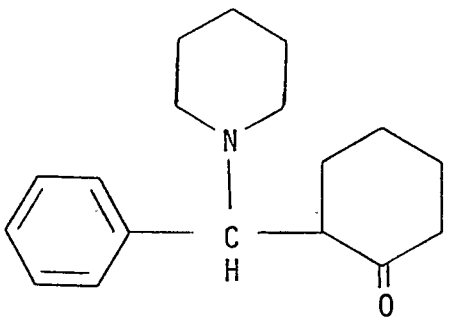
(28)

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).



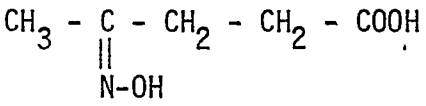
(29)



(30)

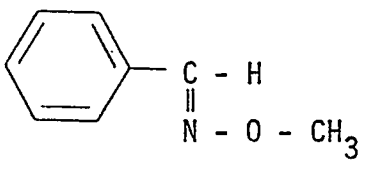
In a study of the relative nucleophilic reactivities of amino groups and mercaptide anions in addition reactions with α,β-unsaturated compounds, Friedman et al. (1965) found that, at comparable pK_a values and steric environments, mercapto anions are about 280 times more reactive than amino groups.

The second stage in the synthesis is the preparation of the substituted 2-benzylidenecyclohexanone oximes (17). Takamiya (1960) examined the antitumor activities of several oximes against Ehrlich ascites carcinoma and Crocker sarcoma 180. Levulinic acid oxime (31) showed a clear antitumor activity *in vitro* and a slight inhibitory effect *in vivo*. The bacteriostatic action of several oximes has been

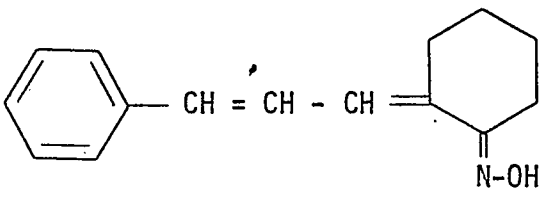


(31)

investigated by Gray and Lambert (1948). Among those oximes tested was O-methylbenzaldoxime (32), which inhibited the growth of Staphylococcus aureus at 1:800, β-hemolytic streptococcus at 1:2000, Salmonella typhi at 1:1200, Bacterium coli at 1:600, Proteus vulgaris at 1:800, and Bacillus subtilus at 1:1200. An α,β-unsaturated oxime (33) has been found active as an anti-obesity agent (Smith Kline and French, 1972). This oxime has structural similarities to the substituted 2-benzylidene-cyclohexanone oximes (17) to be prepared in the present investigation.



(32)



(33)

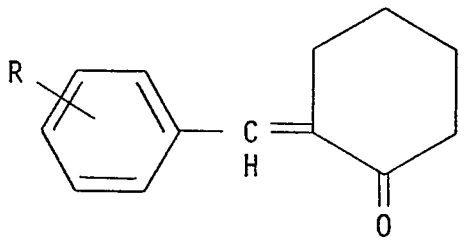
The latter oximes (17) will be submitted for anticancer screening and also for selected pharmacological screenings by Smith Kline and French Laboratories.

The third stage in the synthesis is the preparation of substituted 2-benzylidenecyclohexylamines (18) which will be used in the Hofmann mustard oil reaction to form the substituted 2-benzylidenecyclohexyl isothiocyanates (19), by a method similar to that of Moore and Crossley (1955).

2. DISCUSSION OF THE EXPERIMENTAL WORK

2.1 Preparation of substituted 2-benzylidenecyclohexanones and substituted 2,6-bis-benzylidenecyclohexanones

2.1.1 Preparation of substituted 2-benzylidenecyclohexanones



(34) R = H

(35) R = 2-Cl

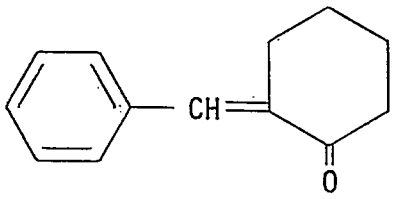
(36) R = 3-Cl

(37) R = 4-Cl

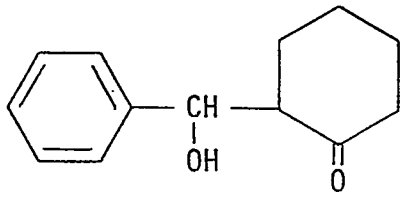
(38) R = 4-$N(CH_3)_2$

The condensation of an aromatic aldehyde with an aliphatic aldehyde or ketone in the presence of a relatively strong base (hydroxide or alkoxide ion) to form an α,β-unsaturated aldehyde or ketone is known as the Claisen-Schmidt reaction. This reaction was utilized to prepare the substituted 2-benzylidenecyclohexanones required in the present investigation.

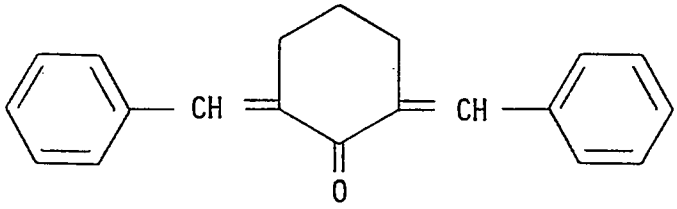
The reaction conditions need to be chosen carefully for this reaction as five products (34), (39-42) are possible when benzaldehyde is condensed with cyclohexanone in the presence of base (Vorlander and Kunze, 1926). In addition to the intermediate aldols (39), (41), and (42) and 2,6-bis-benzylidenecyclohexanone (40) being formed, it is possible for two molecules of 2-benzylidenecyclohexanone (34) to condense together to give the diketone (43)(Tilichenko and Kharchenko, 1959). In the present investigation, the reaction conditions of Walton (1957) were followed to give the desired ketones (34-38) in yields ranging from 12% to 62%.



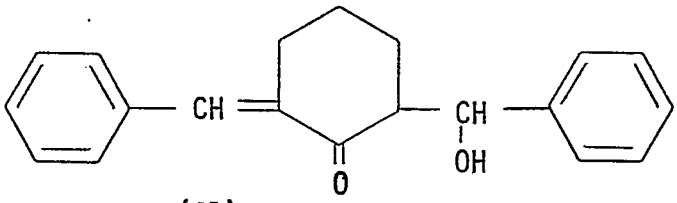
(34)



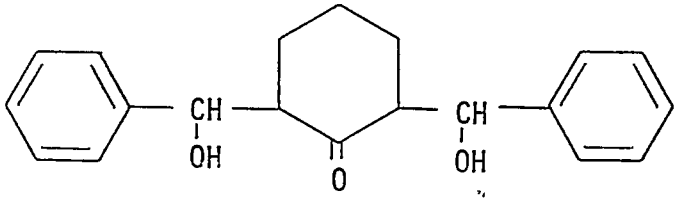
(39)



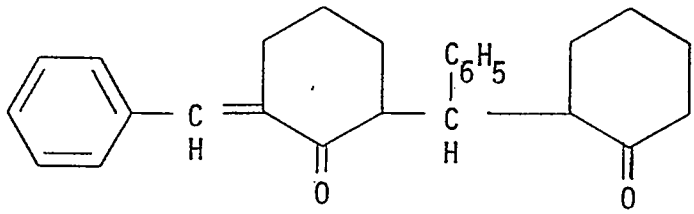
(40)



(41)

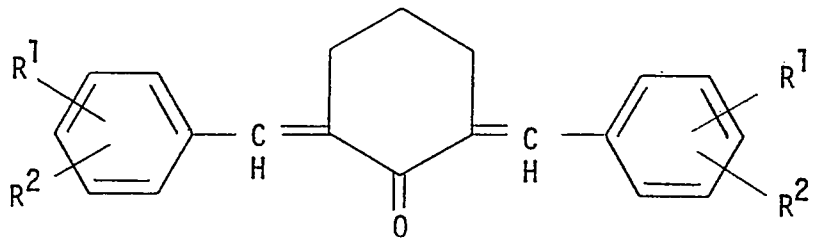


(42)



(43)

2.1.2 Preparation of substituted 2,6-bis-benzylidenecyclohexanones



(44) R^1 = 2-Cl; R^2 = 6-Cl

(45) R^1 = 2-Cl; R^2 = 4-Cl

(46) R^1 = 3-Cl; R^2 = 4-Cl

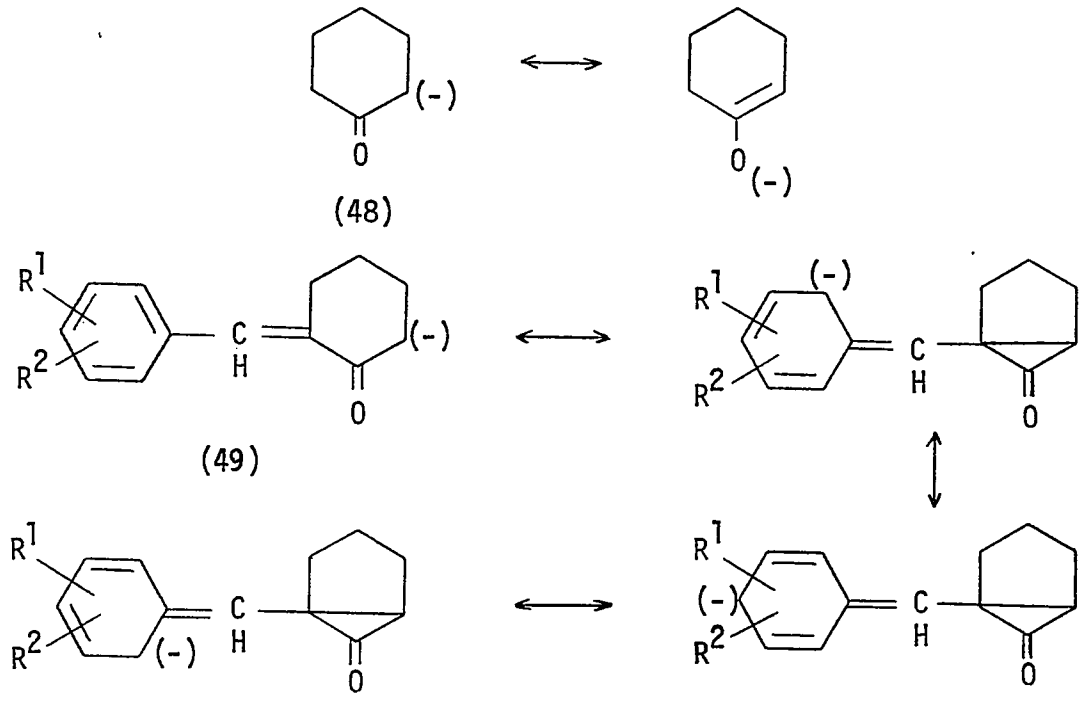
(47) R^1 = 4-$N(CH_3)_2$; R^2 = H

2.1.2.1 Proposed mechanism involved in the formation of the bis-compounds

When the aromatic aldehyde employed in the Claisen-Schmidt reaction was a dichlorobenzaldehyde, such as 2,4-dichloro-, 3,4-dichloro-, or 2,6-dichlorobenzaldehyde, the only product isolated was the substituted 2,6-bis-benzylidenecyclohexanone (44-46). Employing

a large excess of cyclohexanone, a low reaction temperature, and a catalytic amount of base still gave the 2,6-bis-product.

The rate of formation of the product from the addition of the enolate anion of cyclohexanone (48) to the substituted benzaldehyde will depend on the stability of the anion. However, when the substituted 2-benzylidenecyclohexanone is formed it can proceed to an anion (49) in the presence of base. Thus there are two competing anions for the substituted benzaldehyde, one derived from cyclohexanone (48) and the other derived from the substituted 2-benzylidenecyclohexanone (49). In the case of the anions derived from the dichlorinated 2-benzylidenecyclohexanones (49, $R^1 = R^2 = Cl$) extensive resonance stabilization may be possible by a Favorski-type intermediate (Sutherland, 1972), resulting in more stable anions than those derived from cyclohexanone. The higher concentration of these anions (49) would lead to formation of the bis-product (44-46).

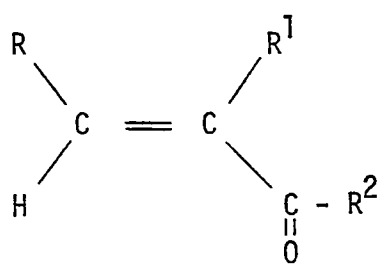


(48)

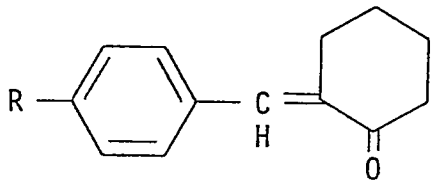
(49)

2.1.3 Stereochemistry of the substituted 2-benzylidenecyclohexanones and substituted 2,6-bis-benzylidenecyclohexanones

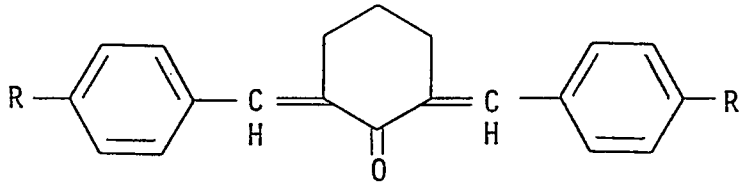
The stereochemistry of the Claisen-Schmidt reaction is of interest in that the α,β-unsaturated ketone (50) normally has an E configuration of the carbonyl group with respect to the larger group (R) at the beta carbon atom (House, 1965, Nielsen and Houlihan, 1968). In the case



(50)



(51) R= Cl, Br, I



(52) R= Cl, Br, I

(53) R= H

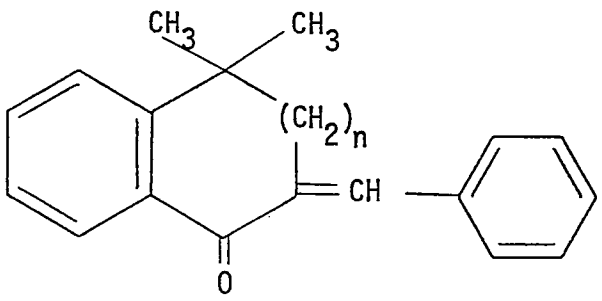
of some 2-(4-halobenzylidene)cyclohexanones (51) and 2,6-bis-(4-halobenzylidene)cyclohexanones (52) it has been shown from dipole moment measurements that both groups of compounds have structures

with the halophenyl group in an E configuration with respect to the carbonyl group (Huitric and Kumler, 1956). Arbuzov and coworkers (1969) calculated the dipole moments for 2-benzylidenecyclohexanone (34) and 2,6-bis-benzylidenecyclohexanone (53). Their calculations also favored the isomer with the phenyl group and the carbonyl group in an E configuration.

Hassner and Mead (1964) have shown that condensation of benzaldehyde with 2-methylcyclohexanone and 2,2-diphenylcyclohexanone gave the E isomers (ε=16,000 and 17,000 respectively) which were converted to the Z isomers (ε=6700 and 7810 respectively) by ultraviolet irradiation. The molar absorptivities of the α,β-unsaturated ketones prepared in the present study by the Claisen-Schmidt reaction ranged from ε10,940 to ε23,700 for the substituted 2-benzylidenecyclohexanones (34-38) and from ε14,920 to ε45,170 for the substituted 2,6-bis-benzylidenecyclohexanones (44-47). The molar absorptivities of the substituted 2-benzylidenecyclohexanones (56-59), prepared by ultraviolet irradiation, ranged from ε5,575 to ε11,080. In each case the isomer from ultraviolet irradiation experienced a hypsochromic shift of the uv max and a decrease in absorption intensity compared to the corresponding isomer from the Claisen-Schmidt reaction. Thus it was concluded that the E isomers were formed in the Claisen-Schmidt reaction and conversion to the Z isomers occurred by the ultraviolet irradiation.

2-Benzylidene-4,4-dimethyl-1-tetralone (54) and 2-benzylidene-3,3-dimethyl-1-indanone (55) have been shown to have the E configuration

by nmr spectroscopy (Kevill et al., 1964). The vinylic proton in (54)



(54) n = 1

(55) n = 0

and (55) gave a signal at δ 7.71 and δ 7.65 respectively for the E isomers, and on ultraviolet irradiation the Z isomers were formed in which the vinylic proton had absorptions at δ 6.63 and δ 6.80 respectively.

The nmr spectra of the substituted 2-benzylidenecyclohexanones (34-38) prepared in the present study showed a signal for the vinylic proton ranging from δ 7.51 to δ 7.30 and the nmr spectra of the substituted 2,6-bis-benzylidenecyclohexanones (44-47) showed a signal for the vinylic proton ranging from δ 7.85 to δ 7.52. These δ values are consistent with the E configuration for these compounds. The nmr spectra of the photoisomers (56-59), showed a signal for the vinylic proton ranging from δ 6.25 to δ 6.42, shielded in comparison to the vinylic proton of the E isomers in which the diamagnetic anisotropy

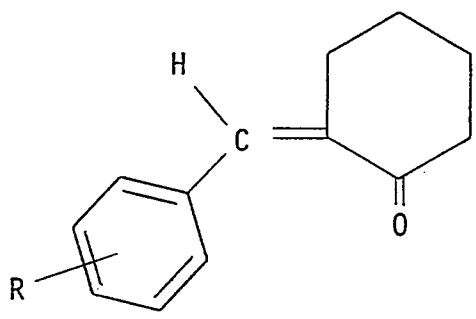
of the carbonyl group deshields the vinylic proton.

Kevill and coworkers (1964) observed a long-range coupling between the vinylic proton and the methylene protons in the E and Z isomers of (54) and (55). The methylene protons appeared as a doublet (J = 2 Hz) and the vinylic proton appeared as a somewhat ill-defined triplet. In the substituted 2-benzylidenecyclohexanones (34-38, 56-59) and 2,6-bis-benzylidenecyclohexanones (44-47) prepared in the present study, the vinylic proton appeared as a triplet (J = 2 Hz) which also indicated a long-range coupling between the vinylic proton and the methylene protons at C-3 or C-3 and C-5 of the cyclohexanone ring.

Braude and Timmons (1955) found that the frequency of the carbonyl bands of hindered α,β-unsaturated ketones,as seen in their ir spectra, approaches that of saturated ketones. This is a consequence of the reduced extent of effective conjugation in the hindered, non-planar compounds. Hassner and Mead (1964) found that the values of Δ, which represents frequency differences, in cm^{-1}, between the C=O and the first C=C absorption band, were in the range of 75-90 cm^{-1} for several substituted (E)-2-benzylidenecyclohexanones and cyclopentanones and 60-65 cm^{-1} for substituted (Z)-2-benzylidenecyclohexanones. In the present investigation the substituted (E)-2-benzylidenecyclohexanones (34-37) gave Δ values of 72-80 cm^{-1} whereas the substituted (Z)-2-benzylidenecyclohexanones (56-59) gave Δ values of 51-64 cm^{-1}.

2.1.4 Isomerization of substituted 2-benzylidenecyclohexanones

A 1% w/v solution of (E)-2-benzylidenecyclohexanone (34) in chloroform, originally prepared for glc analysis, was analyzed after standing for three months in diffuse room light. The solution now contained two compounds, 45% of the original E isomer and 55% of a compound which was suspected to be the Z isomer formed by photo-isomerization. It was decided to attempt the photoisomerization of (E)-2-benzylidenecyclohexanone (34) to (Z)-2-benzylidenecyclohexanone (56) by irradiation with a UV lamp to confirm the identity of the compound formed in diffuse room light. (Z)-2-benzylidenecyclohexanone was isolated from the irradiated product and had a retention time identical to that of the compound suspected to be the Z isomer. The melting point of the Z isomer (37-38^{o}) was lower than that of the E isomer (55^{o}) which is consistent with an E to Z isomerization.



(56) R = H

(57) R = 2-Cl

(58) R = 3-Cl

(59) R = 4-Cl

The interconversion of geometrical isomers can often be accomplished by heating. Hassner and Mead (1964) converted (Z)-2-benzylidene-6-methylcyclohexanone to the E isomer by heating at 225° for two hours. Similarly, in the present work, a sample of (Z)-2-benzylidenecyclohexanone (56) was converted into the E isomer by heating at 120° for 18 hours.

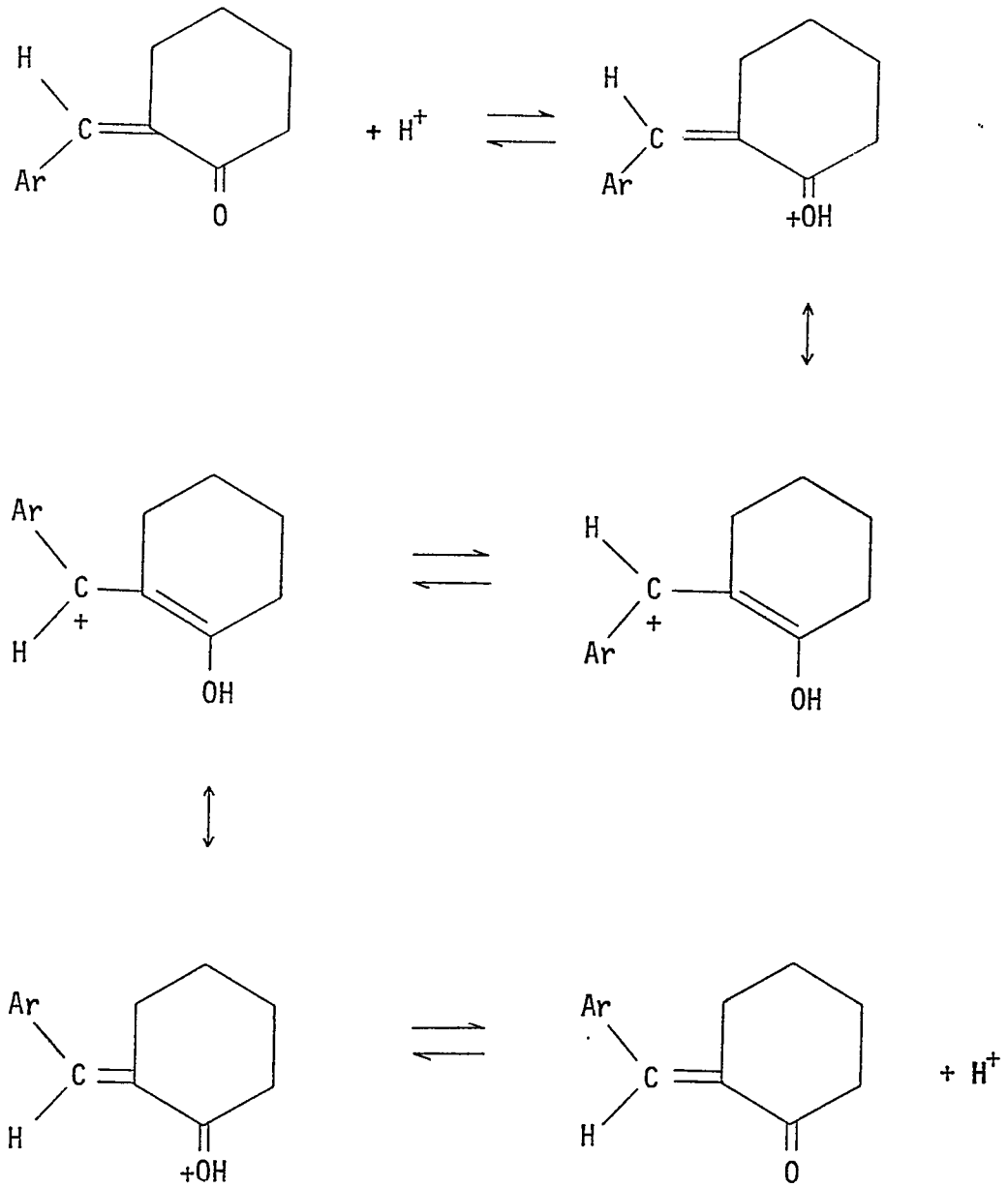
The ultraviolet irradiation of (E)-2-(2-chlorobenzylidene)-cyclohexanone (35) gave a mixture of two main compounds, one of which was unchanged E ketone, plus small amounts of other constituents. Isolation of the other major compound, thought to be the Z isomer (57), was not possible as rapid and almost complete reversion back to the E isomer (35) occurred when isolation was attempted.

The nmr spectrum of the crude irradiation product showed signals at δ 7.43 and δ 6.47 integrating for approximately one proton; they were assigned to the vinylic proton of the E and Z isomer respectively. Approximately 75% of the signal was at δ 7.43 and 25% of the signal was at δ 6.47. The glc analysis of the same sample indicated 65% of the E isomer and 22% of the Z isomer. Thus it was evident that the Z isomer was indeed being formed but was reverting back to the E isomer.

Hassner and Mead (1964) were unsuccessful in the attempted isomerization of (E)-2-(4-chlorobenzylidene)-6-methylcyclohexanone by ultraviolet irradiation. They suggested that the photoequilibrium lies in favor of the E isomer, or possibly a minor impurity was acting

as a quencher. However, a 1% w/v solution of (E)-2-(2-chlorobenzylidene)-cyclohexanone (35) in chloroform which had been exposed to diffuse room light for three months was examined by glc analysis and was found to contain two compounds, 40% of the original E isomer and 60% of a new compound with the same retention time as the Z isomer (57) produced by ultraviolet irradiation. Thus the Z isomer was stable in a solution exposed to diffuse room light but a 1% w/v solution of the irradiated product had the Z isomer quickly revert back almost entirely to the E isomer. In another experiment a solution of (E)-2-(2-chlorobenzylidene)-cyclohexanone in 95% ethanol was irradiated for one hour. Glc analysis showed the presence of three compounds, present in concentrations of 77% (presumably the Z isomer), 21% (unchanged E isomer), and 2%. The ultraviolet spectrum of the irradiated product showed a uv max at 267 mμ compared with a uv max at 278 mμ for the E isomer. An estimate of the molar absorptivity, based on 77% of the Z isomer, gave ε6,765 compared with ε10,940 for the E isomer.

The methanolic solution of (35) exposed to prolonged ultraviolet irradiation was strongly acidic (pH 1-1.5). The presence of halogen ions was established by chemical means (silver nitrate-nitric acid). Thus the presence of hydrochloric acid was indicated in the irradiation solution, produced from a side reaction during the irradiation. The reaction conditions appeared to be too severe in this irradiation. A shorter irradiation period from a greater physical distance, with the use of appropriate filters, could possibly allow the isolation of



Ar = 2-chlorophenyl

Scheme 2 Isomerization of 2-(2-chlorobenzylidene)cyclohexanone by acid.

the desired Z isomer. Acids are particularly effective in bringing about the isomerization of α,β-unsaturated carbonyl compounds, perhaps via the mechanism shown in Scheme 2 (Eliel, 1962). Halogen acids have been employed to convert Z isomers to the E isomers (Kevill et al., 1964).

1% w/v solutions of (E)-2-(3-chlorobenzylidene)cyclohexanone (36) and (E)-2-(4-chlorobenzylidene)cyclohexanone (37) contained the Z isomer, (58) and (59) respectively, after being exposed to diffuse room light for 3 months or to ultraviolet irradiation for one hour. Ultraviolet irradiation of a solution of (E)-2-(4-dimethylaminobenzylidene)-cyclohexanone (38) for one hour did not appear to cause photoisomerization of the E isomer to the Z isomer. Ultraviolet spectroscopy of the irradiated solution indicated unchanged E isomer.

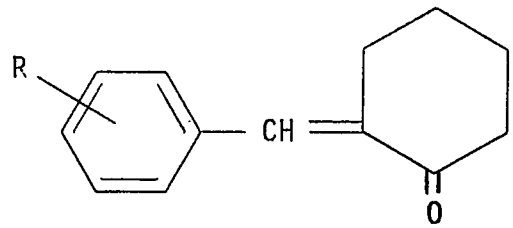
2.1.5 Calculation of the interplanar angle (θ) for substituted 2-benzylidenecyclohexanones and substituted 2,6-bis-benzylidenecyclohexanones

The angle of twist in sterically hindered conjugated systems may be estimated by the method of Braude and coworkers (1954, 1955) based on ultraviolet spectroscopy. The value of the angle of twist between groups is designated θ and is derived from the following equation, in which ε_0 is the molar absorptivity for the reference compound of the series and ε is the molar absorptivity for the substituted derivatives. The degree of coplanarity between functional groups in a series of

$$\cos^2 \theta = \varepsilon / \varepsilon_0$$

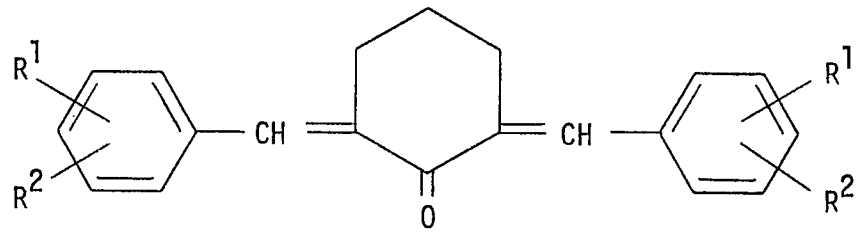
aroylhydrazones was recently calculated by Dimmock and coworkers (1972) using Braude's equation.

Table I Ultraviolet spectra of substituted 2-benzylidenecyclohexanones



Compound	R	Configuration	uv max (mμ)	ε	$\varepsilon/\varepsilon_0$	θ
(34)	H	E	290	14,725	1.00	0
(35)	2-Cl	E	278	10,940	0.6312	37
(36)	3-Cl	E	284	12,975	0.7694	29
(37)	4-Cl	E	293	16,370	1.00	0
(56)	H	Z	272	5,575	0.3787	52

Table II Ultraviolet spectra of substituted 2,6-bis-benzylidenecyclohexanones



Compound	R^1	R^2	Configuration	uv max (mμ)	ε	$\varepsilon/\varepsilon_o$	θ
(40)	H	H	E	330	39,880	1.00	0
(45)	2-Cl	4-Cl	E	318	21,925	0.3847	52
(46)	3-Cl	4-Cl	E	329	34,725	0.7057	33
(44)	2-Cl	6-Cl	E	289	14,920	0.2091	63

The ratios of $\varepsilon/\varepsilon_0$ listed in Table I are based on the assumption that in the absence of steric effects, ortho substituents increase the absorption maxima by a similar amount as when the substituent is in the para position (Braude et al., 1954). Thus the molar absorptivities for the 2-chloro and 3-chloro derivatives, (35) and (36), are reduced by 1,645 which represents the difference in molar absorptivity between the unsubstituted compound (34) and the 4-chloro derivative (37). There are four chlorine atoms in the substituted compounds listed in Table 2. The molar absorptivities for the bis-compounds (44-46) are reduced by 6,580 which is four times the difference in molar absorptivity between the unsubstituted compound (34) and the 4-chloro derivative (37) in Table I.

The value of θ for the ortho-chloro ketone (35) indicates that inhibition of coplanarity of the aromatic ring with the ethylenic linkage is caused by an ortho chloro substituent. In addition, a chlorine atom in the meta position of the aromatic ring, as found in the meta-chloro ketone (36), exerts a buttressing effect causing the aromatic ring to be out of plane with the remainder of the molecule. In the Z isomer (56) a severe steric interaction between the ortho hydrogen of the benzene ring and the carbonyl oxygen causes the benzene ring to be forced out of coplanarity with the rest of the absorbing system, giving a large value for θ. Indeed an examination of Drieding models of the two isomers (34) and (56) reveals that in the E isomer the entire chromophore can be nearly coplanar whereas in the Z isomer steric

interaction between the ortho hydrogen of the benzene ring and the carbonyl oxygen is unavoidable in a planar conformation. This condition if reflected both in a decreased intensity of absorption and a hypsochromic shift of the uv max in the ultraviolet spectrum of the Z isomer compared to the E isomer.

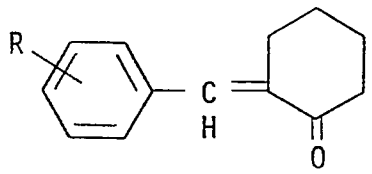
In the bis compounds (Table 2), the greatest inhibition of coplanarity is observed for the bis 2,6-dichloro compound (44) which has chlorine atoms in all four ortho positions of the aromatic rings. In the bis 2,4-dichloro compound (45) there is only one ortho substituent on each aromatic ring and the angle of twist is reduced. A buttressing effect is again observed for a meta chlorine substituent as found in the bis 3,4-dichloro compound (46).

2.1.6 Mass spectra of substituted 2-benzylidenecyclohexanones and substituted 2,6-bis-benzylidenecyclohexanones

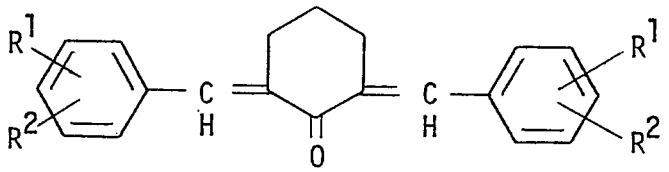
2.1.6.1 Introduction

Since metabolic studies of the compounds in Series 1 and Series 2 are to be determined *in vivo* it was considered of interest to study their mass spectral fragmentations in detail. A comparison of the mass spectra of these compounds with the mass spectra of their metabolites isolated from body tissues and fluids in test animals (i.e. rats) should give some indication of the *in vivo* biotransformations. In addition, metabolic studies of Series 1 and 2 will be compared to metabolic studies on Series 3 so a comparison of the mass spectral fragmentations of Series 1 and 2 with Series 3 is required.

In the mass spectra of a series of substituted styryl ketones (Series 3) Smith *et al*. (1972) showed that an intramolecular aromatic substitution reaction occurred in the molecular ion to give a benzopyrylium ion, after preliminary *E* to *Z* isomerization. This fragmentation pattern was pronounced when the aromatic ring contained an *ortho* chlorine atom and was only of minor significance when the *ortho* positions contained solely hydrogen atoms.The styryl ketones in Series 3 are capable of existing in the s-*cis* and s-*trans* configuration (Dimmock *et al*., 1968) but the exocyclic α,β-unsaturated cycloalkanones (Series 1 and 2) have a fixed s-*cis* configuration of the olefinic group with respect to the carbonyl function. In Series 1 and 2, after the prior isomerization of the *E* isomer to the *Z* isomer, the carbonyl



Series 1



Series 2

(34) R = H

(35) R = 2-Cl

(36) R = 3-Cl

(37) R = 4-Cl

(38) R = 4-$N(CH_3)_2$

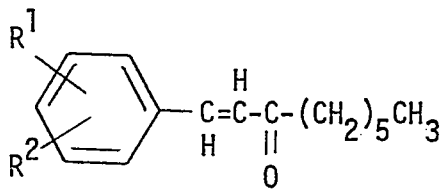
(53)[1] $R^1 = R^2 = H$

(44) R^1 = 2-Cl; R^2 = 6-Cl

(45) R^1 = 2-Cl; R^2 = 4-Cl

(46) R^1 = 3-Cl; R^2 = 4-Cl

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



Series 3

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

[1]Prepared by Mr. G. B. Baker, College of Pharmacy, University of Saskatchewan, Saskatoon.

oxygen atom is in close proximity to the ortho substituent of the aromatic ring which should allow the intramolecular aromatic substitution reaction to be a major fragmentation pathway. It was also considered of interest to examine the effect of substituents on the aromatic ring on the formation of various benzopyrylium ions in the light of the relative stabilization of these ions compared with the corresponding parent ion.

The mass spectral data for the major ions of the compounds in Series 1 and 2 are recorded in Tables III and IV. Figures 1 to 4 are bar graphs of compounds (34), (35), (53), and (44) respectively.

2.1.6.2 Mass spectrum of (E)-2-benzylidenecyclohexanone

Figure 1 indicates the mass spectrum of (E)-2-benzylidenecyclohexanone (34). This spectrum exhibits an intense M-1 peak at m/e 185 (a) such that the ratio M-1/M=1. This ion presumably has the benzopyrylium structure and is formed by the loss of a hydrogen atom after E to Z isomerization has occurred. Indeed the mass spectrum of the Z isomer (56) is virtually identical to that of the E isomer (34). At 15 eV the M-1/M ratio of (34) is 0.36, whereas in the case of the unsubstituted styryl ketone in Series 3, there is absence of the M-1 peak at this ionization voltage. In both compounds further fragmentation at 15 eV is negligible.Thus the geometry of the rigid structure in (34) allows a hydrogen to be abstracted more readily than the corresponding flexible acyclic analog in Series 3.

Table III Relative intensity and m/e values of the principal ions observed in the 70eV mass spectra of compounds in Series 1

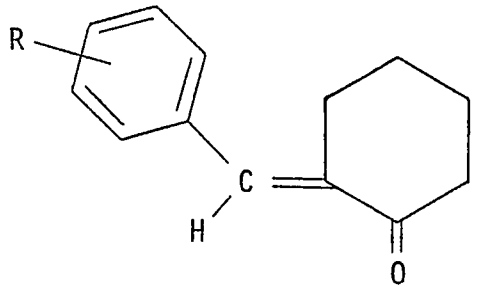
(34)	m/e	186(M)	185	158	157	143	130	129	128	127	117	115	102	91	77	67
	R.I.	100	100	18	9	11	32	46	27	10	36	60	17	24	12	39
(35)	m/e	220(M)	185	129	128	127	115	67								
	R.I.	3	100	17	15	9	15	11								
(36)	m/e	220(M)	219	192	185	164	157	151	129	128	127	116	115	67		
	R.I.	59	35	12	57	4	13	24	47	25	15	12	37	100		
(37)	m/e	220(M)	219	192	185	164	157	151	129	128	127	116	115	67		
	R.I.	100	52	18	44	9	15	22	44	22	15	10	22	42		
(38)	m/e	229(M)	228	201	173	172	160	158	129	128	115					
	R.I.	100	32	12	23	19	9	13	11	7	11					

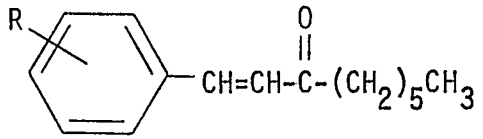
Table IV Relative intensity and m/e values of the principal ions observed in the 70eV mass spectra of compounds in Series 2.[1]

(53)	m/e	274(M)	273	246	218	217	129	128	115							
	R.I.	100	95	9	10	14	9	11	20							
(44)	m/e	410(M)	375	152	151	149										
	R.I.	1	100	11	9	10										
(45)	m/e	410(M)	375	152	151	149										
	R.I.	1	96	8	7	13										
(46)	m/e	410(M)	409	375	347	312	202	188	185	183	170	160	149	128	127	115
	R.I.	76	36	78	30	40	12	12	12	15	14	30	67	32	31	21
(47)	m/e	360(M)	359	332	331	212	166	134								
	R.I.	100	35	16	17	19	31	14								

[1]The base peaks for compound (45) was at m/e 377 and for compound (46) was at m/e 412 due to the presence of more than one chlorine atom in the ions. The ions used for discussion will be those with m/e values corresponding to the presence of only the Cl^{35} isotope.

Table V M-1/M and M-Cl/M ratios at 70 eV for the substituted (E)-2-benzylidenecyclohexanones and a series of mono-substituted 1-phenyl-1-nonen-3-ones[1]

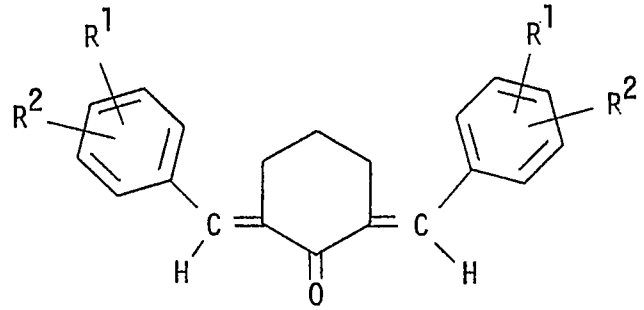


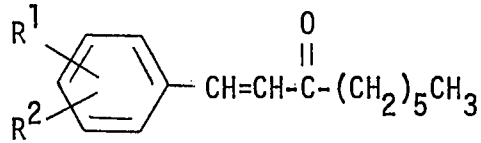


Substituent (R)	Series 1 M-1/M	Series 1 M-Cl/M	Series 3 M-1/M	Series 3 M-Cl/M
2-Cl	0.3	33.3	0.1	4.5
3-Cl	0.6	1.0	---	---
4-Cl	0.5	0.4	0.1	0.0
4-H	1.0	---	0.1	---
4-$N(CH_3)_2$	0.3	---	0.1	---

[1]Data taken from Smith et al. (1972).

Table VI M-1/M and M-Cl/M ratios[2] at 70 eV for the substituted (E,E)-2,6-bis-benzylidenecyclohexanones and a series of substituted 1-phenyl-1-nonen-3-ones[1]





Substituents (R^1 and R^2)	Series 2 M-1/M	Series 2 M-Cl/M	Series 3 M-1/M	Series 3 M-Cl/M
$R^1 = R^2 = H$	1.0	---	0.1	---
R^1 = 4-$N(CH_3)_2$; $R^2 = H$	0.4	---	0.1	---
R^1 = 3-Cl; R^2 = 4-Cl	0.5	1.0	0.1	0.0
R^1 = 2-Cl; R^2 = 4-Cl	0.3	96.0	0.1	2.5
R^1 = 2-Cl; R^2 = 6-Cl	0.0	100.0	0.0	16.7

[1]Data taken from Smith et al. (1972).

[2]Ratios were calculated using the peaks corresponding to the parent and the fragment ions containing only the Cl^{35} isotope. The ion corresponding to the loss of a chlorine atom can also arise from the M+2 peak with loss of Cl^{37}.

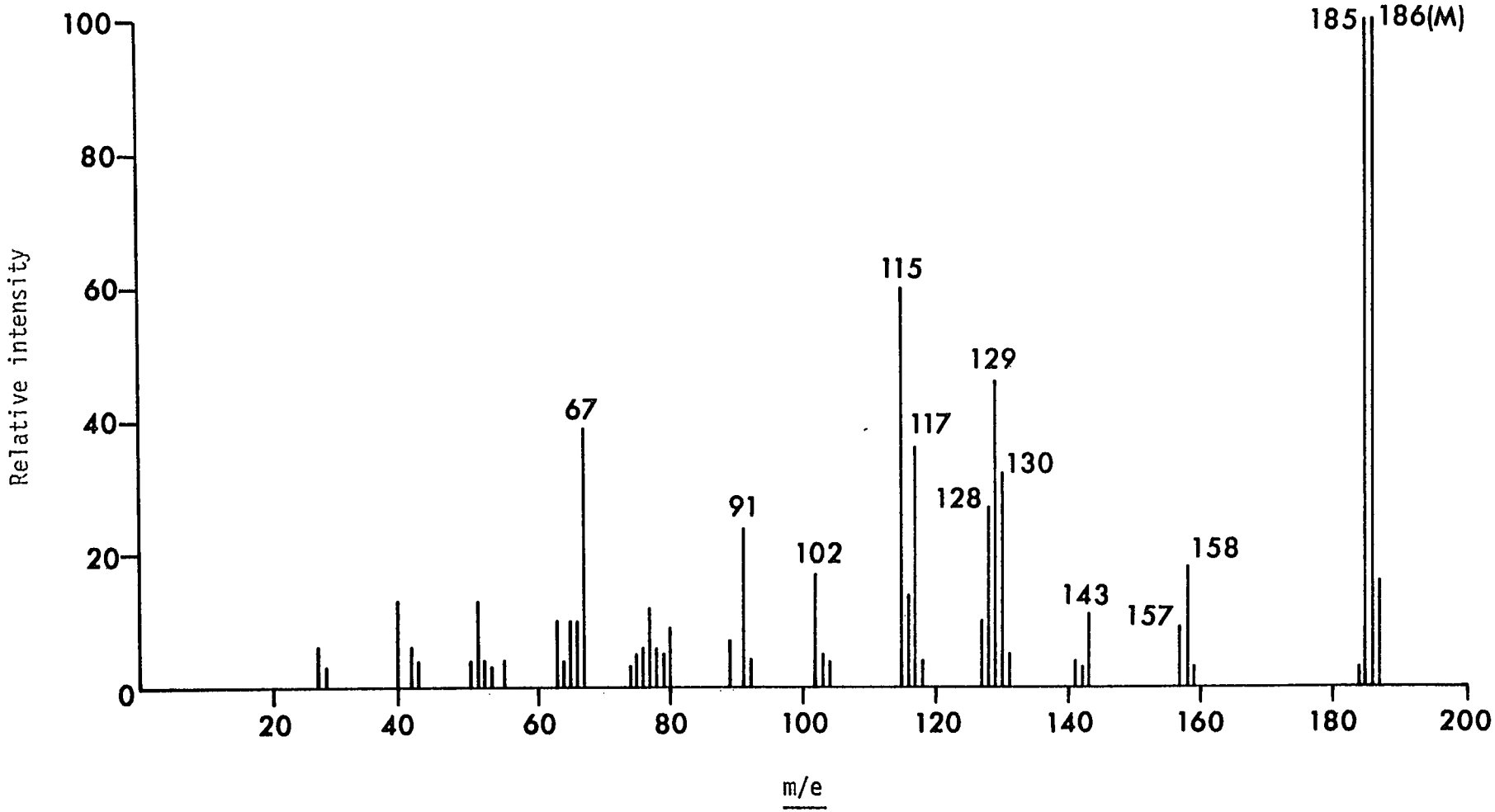


Figure 1. Mass spectrum of (E)-2-benzylidenecyclohexanone

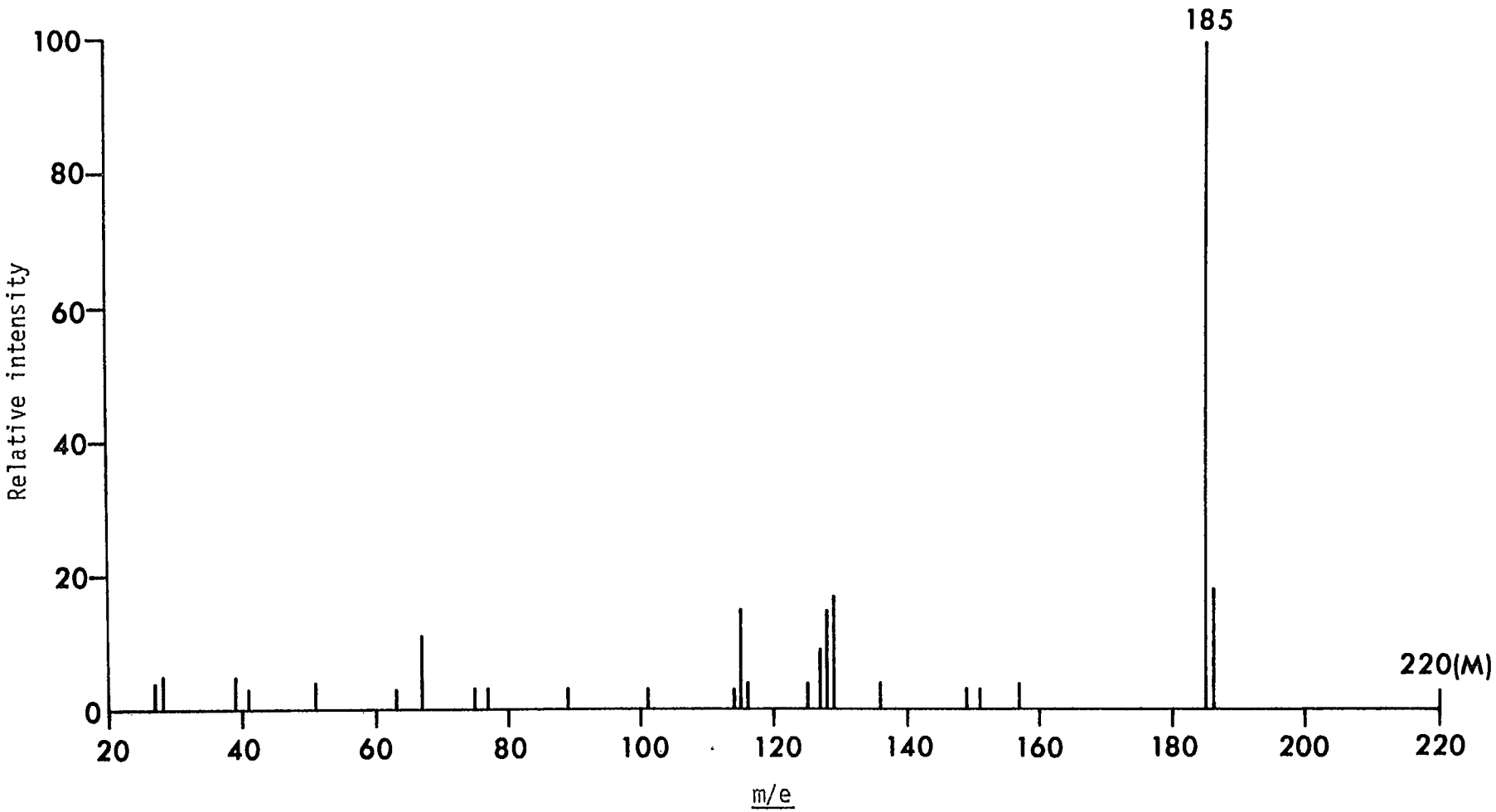


Figure 2. Mass spectrum of (E)-2-(2-chlorobenzylidene)cyclohexanone

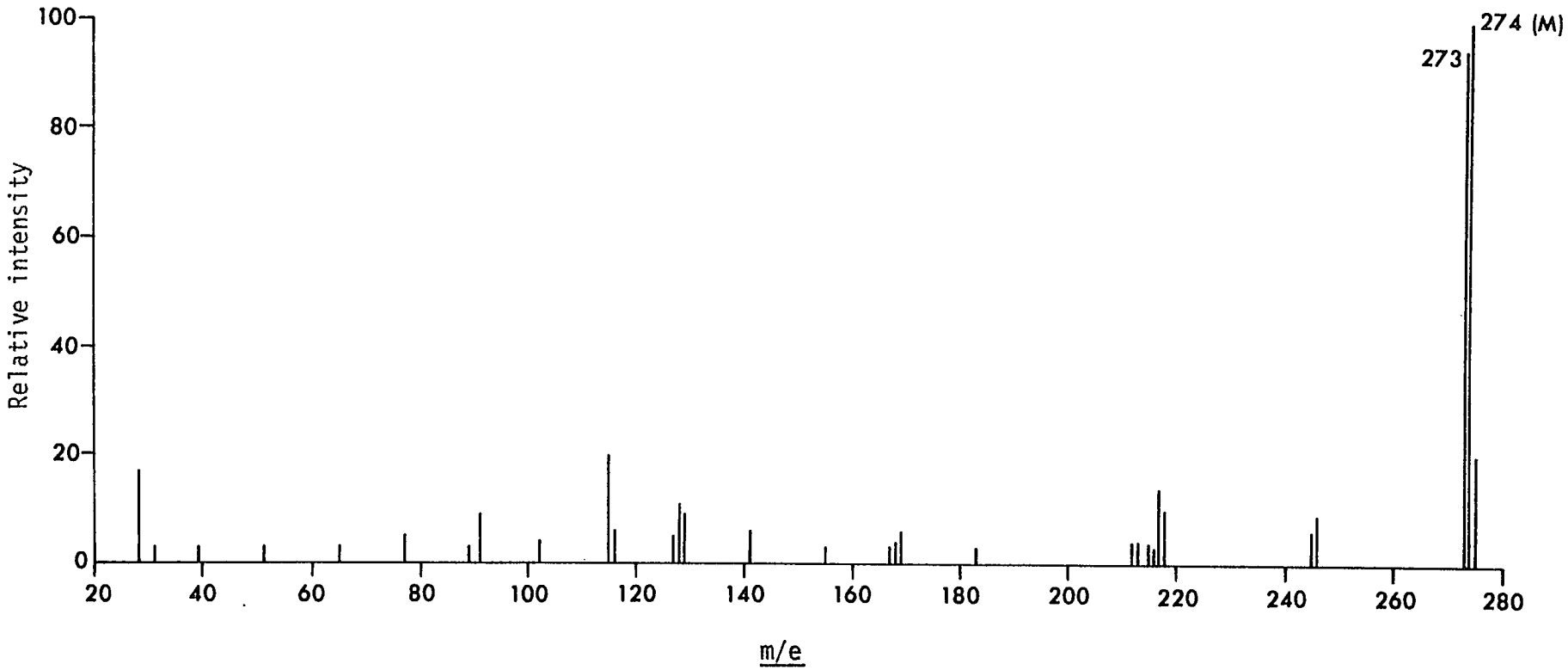


Figure 3. Mass spectrum of (E,E)-2,6-bis-benzylidenecyclohexanone

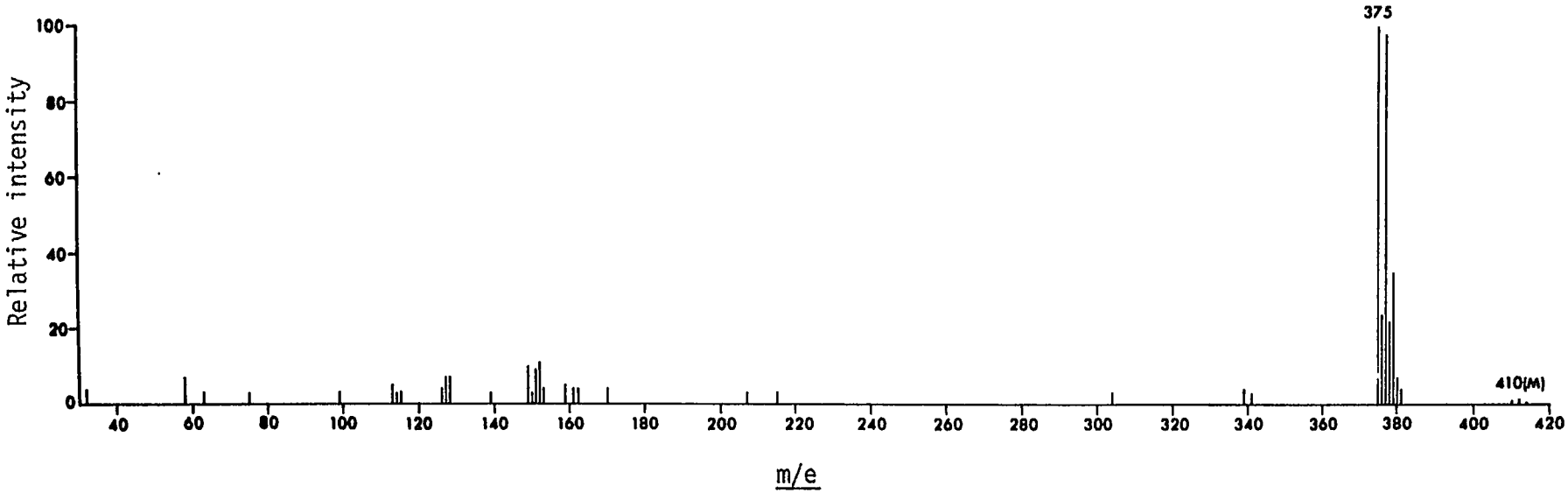
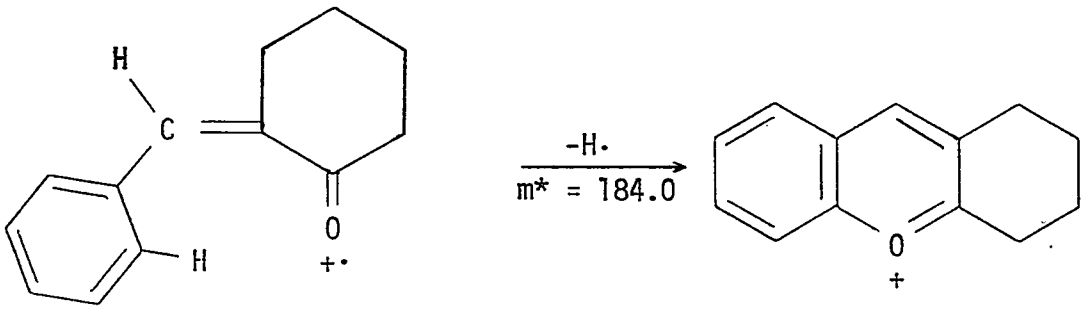


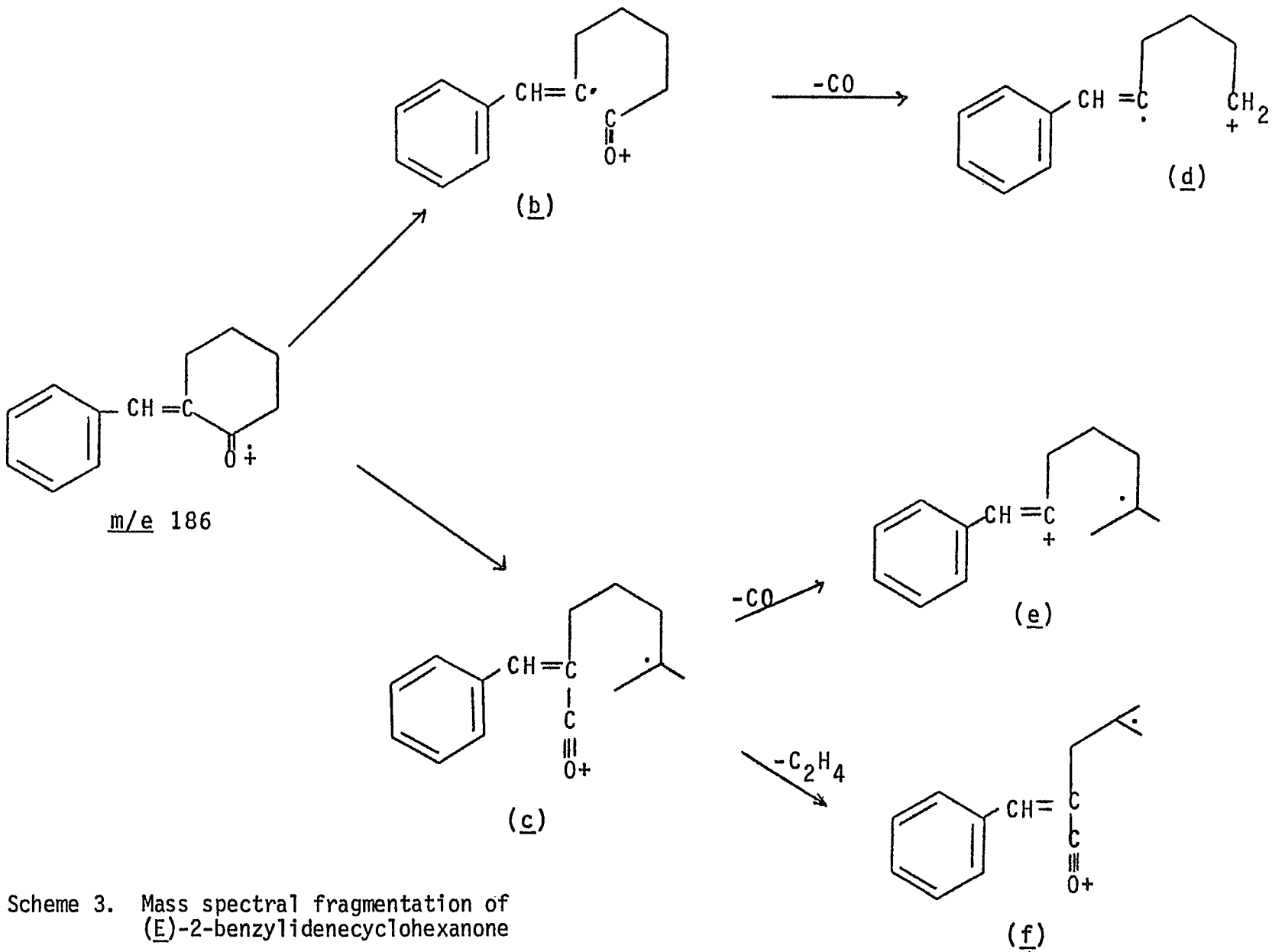
Figure 4. Mass spectrum of (E,E)-2,6-bis-(2,6-dichlorobenzylidene)cyclohexanone



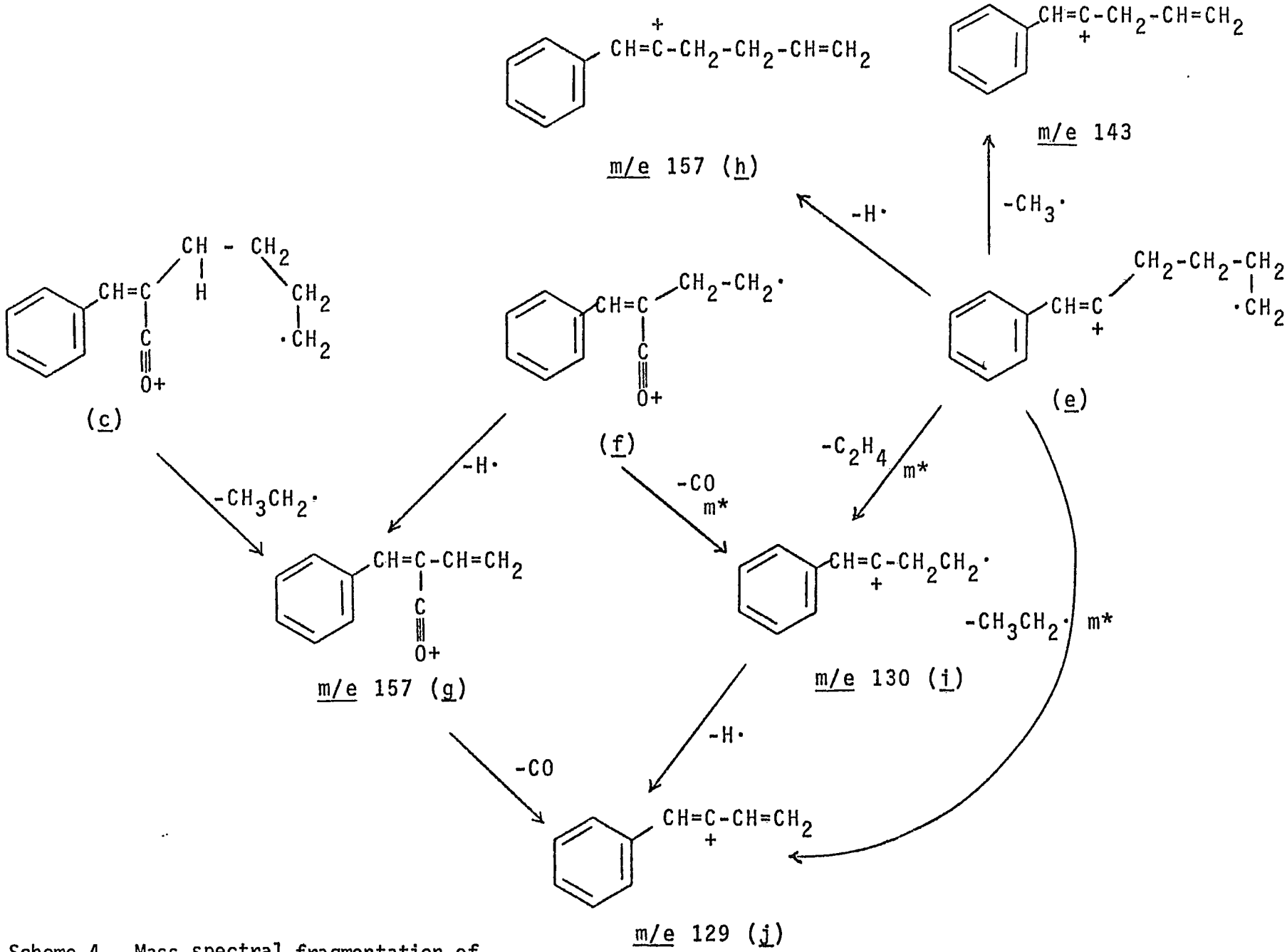
(34) (Z) m/e 186 (a) m/e 185

The ion at m/e 158 (M-28) can arise from either of two possible α-cleavages of the parent ion to form structures (b) and (c) with subsequent loss of carbon monoxide or ethylene to give (d), (e) and (f) , m/e 158 (Scheme 3). It is expected that (c) would be more favored than (b) for the α-cleavage product due to resonance stabilization. Consequently, the loss of 28 mass units from the parent ion would emanate principally from (c) to give (e) and (f). Indeed, when the ion at m/e 158 was examined using high resolution mass spectrometry it was found that ions (e) and (f) were formed in the approximate ratio of 2:1.

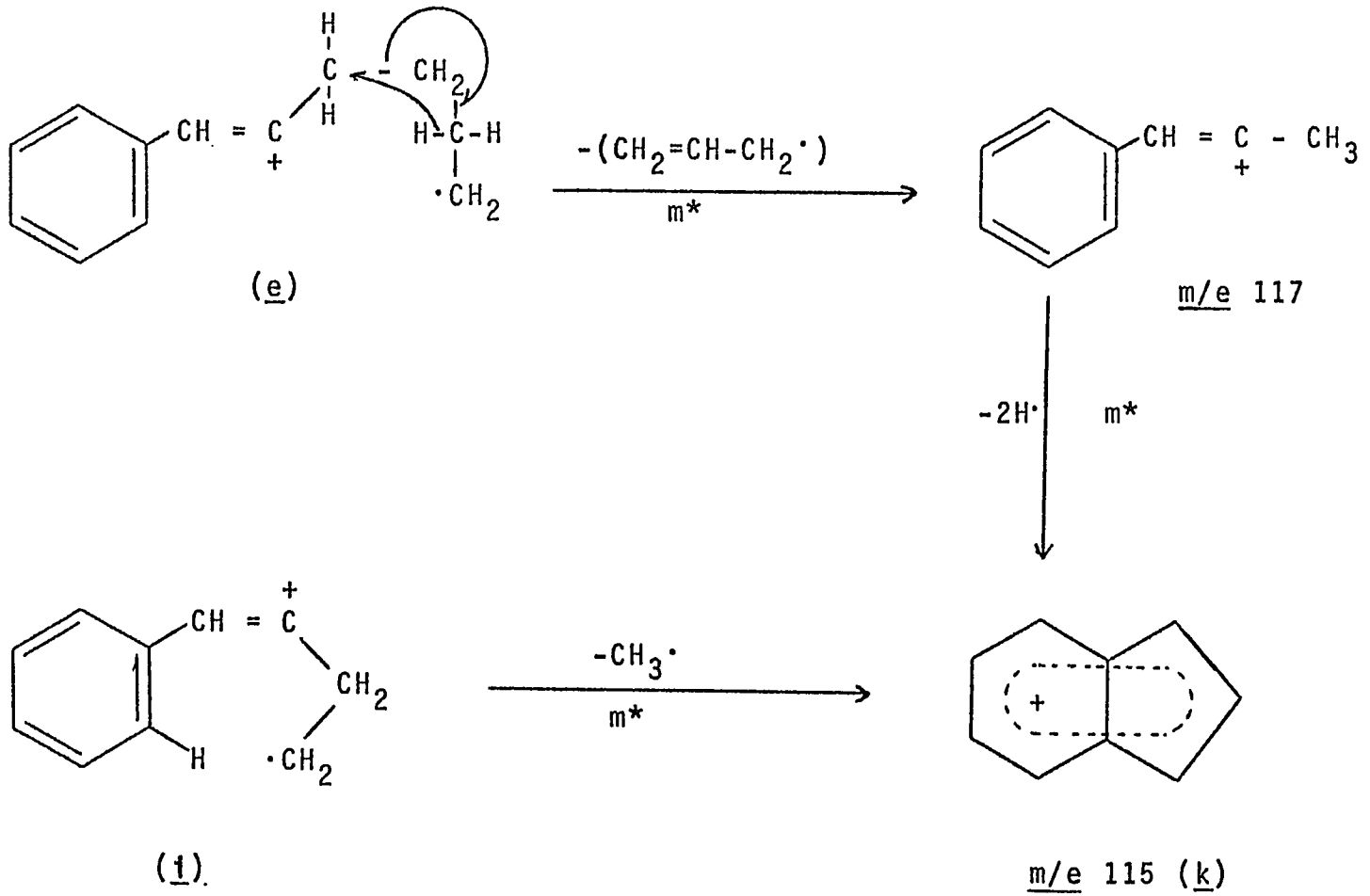
Further fragmentation is shown in Scheme 4. The peak observed in the mass spectrum at m/e 157 could be formed from (c) by loss of an ethyl radical or from (f) by loss of a hydrogen atom to give an ion in both cases with structure (g). As well, (e) could lose a hydrogen atom to give (h). Structure (e), m/e 158, can lose a methyl radical to give m/e 143 or ethylene (m* = 107.0) to produce m/e 130 (i). Structure (i) can also arise from (f), m/e 158, by loss



Scheme 3. Mass spectral fragmentation of (E)-2-benzylidenecyclohexanone



Scheme 4. Mass spectral fragmentation of (E)-2-benzylidenecyclohexanone



(e)

(1)

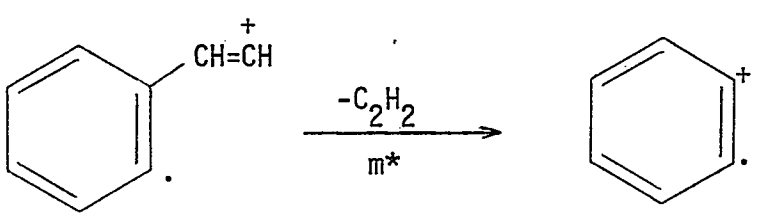
m/e 115 (k)

Scheme 5. Mass spectral fragmentation of (E)-2-benzylidenecyclohexanone

of carbon monoxide. The ion at m/e 129 can arise from several pathways, one of which is confirmed by the observation of the appropriate metastable ion. This ion, with a suggested structure (j), can arise from (i) by loss of a hydrogen atom or from m/e 158 (e) by loss of an ethyl radical (m* = 105.3). A third possibility is that the ion at m/e 157 (g) loses carbon monoxide. The peak at m/e 128 presumably corresponds to an ion with a "naphthalenelike" structure and is derived from (j).

Scheme 5 shows that the precursor of the ion at m/e 117 is m/e 158 (e) as evidenced by a metastable ion at m/e 86.6. Identical processes involving loss of the allyl radical were observed for both the 3-chloro and 4-chloro compounds, (36) and (37), respectively, as indicated by the appropriate metastable peaks. The ion at m/e 117 loses two hydrogen atoms to give m/e 115 (m* = 113.0). An indenyl structure (k) has been proposed for m/e 115 (Willhalm and Thomas, 1968) and this ion was observed to lose acetylene (m* = 68.9). The indenyl cation is also confirmed to be formed from m/e 130 (i) by loss of a methyl radical (m* = 101.7).

The ion at m/e 102 could have the structure as illustrated below since it loses acetylene (m* = 56.6) to give m/e 76. The composition of the ion at m/e 91 is presumably $C_7H_7^+$ with a



m/e 102 m/e 76 (ionized benzyne)

tropylium structure since this ion also loses acetylene (m* = 46.4) to give m/e 65.

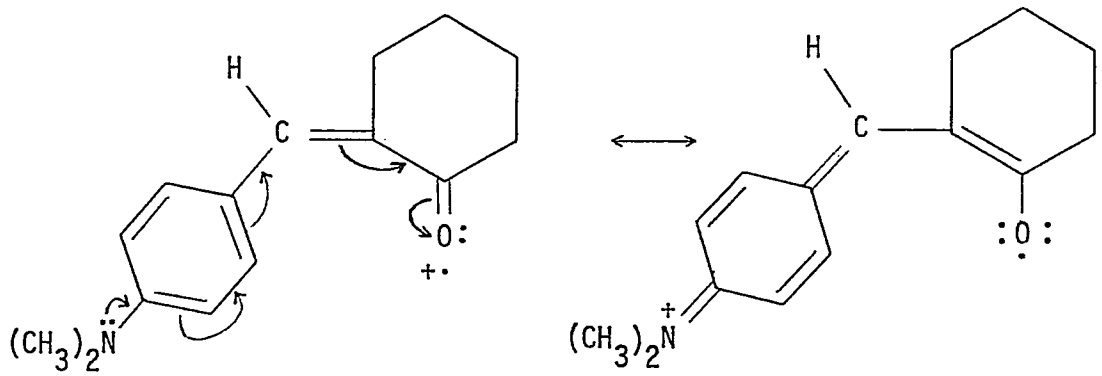
The origin and structure of the ion at m/e 67 is of particular interest since this ion appears in the spectra of all the substituted (E)-2-benzylidenecyclohexanones except the 4-dimethylamino derivative (38). In fact, the most intense ion in the spectrum of the 3-chloro substrate (36) is at m/e 67. High resolution mass spectrometry has shown this ion to have the elemental composition, $C_5H_7^+$. It is noteworthy that m/e 67 does not appear in any of the spectra obtained for the bis substrates (53, 44-47) which suggests that the origin of the ion is from the cyclohexanone ring. It has been proposed that m/e 67 has the structure of the pentadienyl cation, CH_2=CH-CH=CH-CH_2^+, and a mechanism has been proposed for its formation from cyclohexene (Budzikiewicz et al., 1967a). As well, 1-pentyne loses a hydrogen atom to give m/e 67, the most intense ion in the spectrum (Dolejšek et al., 1966).

2.1.6.3 Mass spectra of the substituted (E)-2-benzylidenecyclohexanones

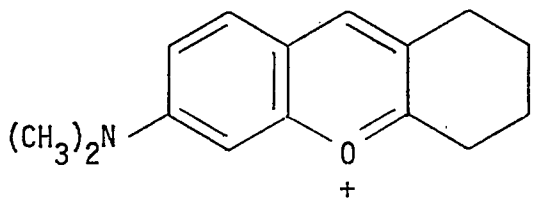
The effect of substituents on the intramolecular aromatic substitution reaction was investigated for both the loss of a hydrogen atom and a chlorine atom from the parent ion. The results are shown in Table V.

The loss of a hydrogen atom from the parent ion to give the M-1 ion was confirmed for all the substituted (E)-2-benzylidene-cyclohexanones by the observation of the appropriate metastable peak except for the 2-chloro compound (35) where the intensity of the parent ion and M-1 ion was extremely weak. It is seen from Table V that the ratio of M-1/M varies from 1.0 for the unsubstituted compound (34) to 0.3 for the 4-dimethylamino substrate (38). A comparison with the styryl ketones in Series 3 shows that the M-1/M ratio is considerably less for Series 3 since facile α-cleavage and McLafferty rearrangement compete.

The correlation of substituents with log Z/Z_0 values, where Z is defined as M-fragment/M for the substituted compound and Z_0 refers to the unsubstituted compound has received considerable attention in recent years (Bursey and McLafferty, 1966; Bursey, 1968; Howe et al., 1969). A comparison of the M-1/M ratio for (34) and (38) is the most meaningful since these compounds do not have the competing fragmentation of the loss of chlorine from the parent ion and, indeed, (38) has as the major fragmentation process the loss of a hydrogen atom. The decrease in the ratio M-1/M from 1.0 to 0.3 for compounds (34) and (38), respectively, may be explained by considerable stabilization of the parent ion from the electron-donating dimethylamino function in (38), making attack of the carbonyl oxygen on the aromatic ring less favored. It is assumed that ease of isomerization of the E substrates to the Z isomers is independent of substituents on the benzene ring. Alternatively, the presence of two hetero atoms in (38) allows



the possibility of charge localization on either atom which might lead to fewer fragment ions as observed. In fact, the ionization potentials of N,N-dimethylaniline and cyclohexanone are 7.14 and 9.14 eV, respectively (Kiser, 1962), which suggests that the positive charge probably resides mainly on nitrogen. It should be noted that the stabilization of the M-1 ion is not the prime factor since (1) would be more stable than the corresponding unsubstituted benzopyrylium ion.



(1)

A comparison of the M-1/M ratio for the chloro compounds (36) and (37) with the unsubstituted compound (34) is difficult due to the competing M-Cl reaction. Table V indicates that both the 3-chloro

and 4-chloro substrates give M-1/M ratios considerably less than for the unsubstituted compound. This is reasonable for the 4-chloro compound (37) since the chlorine can stabilize the parent ion by the mesomeric effect as discussed for the dimethylamino substrate. The 3-chloro substituent (36) cannot directly conjugate with the carbonyl function and presumably makes the substitution reaction less favorable than for the unsubstituted compound by withdrawing electron density from the benzene ring. It should be noted that the 3-chloro substituent is lost approximately twice as readily as the 4-chloro substituent from the parent ion in a competing reaction with the M-1 process. Thus a higher M-1/M ratio might exist for the 3-chloro than for the 4-chloro substrate in the absence of the M-Cl reaction.

The parent ion is not stabalized for the 3-chloro derivative relative to the 4-chloro substrate as is shown from the data in Table III where it is seen that the parent ion for the 3-chloro substrate (36) has a relative intensity of 59 whereas the parent ion for the 4-chloro compound (37) is the most intense peak. The base peak for the 3-chloro compound is at m/e 67, $C_5H_7^+$, which further suggests that the chlorine in the 3-position on the ring does not stabalize the parent ion and fragment ions bearing the substituent, which leads to increased intensity of ions derived from the cyclohexanone ring ($C_5H_7^+$). This is borne out by the data for the 4-dimethylamino compound (38) where the parent ion and subsequent fragment ions bearing the dimethylamino group are stabilized by the electron-donating substituent; no ion at m/e 67 was observed (Table III).

The loss of chlorine from the parent ion was confirmed for (35), (36), and (37) by the metastable ion at m/e 155.6 (220→185). The loss of the chlorine from the 2-chloro compound (35), as expected, was considerably more favored than loss from either the 3-chloro (36) or 4-chloro (37) substrates; Table V, M-Cl/M = 33.3, 1.0, and 0.4, respectively. This indicates the ease of formation of the benzopyrylium structure, ion (a), by the intramolecular displacement of the 2-chloro substituent. Compounds (36) and (37) presumably lose a halogen atom by simple carbon-chlorine bond cleavage as does chlorobenzene (McLafferty, 1962). The loss of the ortho chlorine from (35) is considerably more favored than loss of the ortho chloro from 1-(2-chlorophenyl)-1-nonen-3-one (Series 3); M-Cl/M = 33.3 and 4.5, respectively, Table V. This trend was also observed for the loss of hydrogen from the unsubstituted substrates of Series 1 and 3. The M-Cl peaks for the 3-chloro (36) and 4-chloro (37) substrates are considerably more significant than for 1- (4-dichlorophenyl)-1-nonen-3-one (Series 3), Table V, M-Cl/M = 1.0, 0.4, 0.0, respectively. This is probably due to the fact that α-cleavage and McLafferty rearrangement processes are prominent in Series 3, making the M-Cl reaction less significant.

The loss of 28 mass units from the parent ion was discussed for the unsubstituted compound (34). A similar loss was observed in the cases of (36), (37), and (38) but the 2-chloro compound (35) did not exhibit an ion resulting from loss of either carbon monoxide or ethylene from the parent ion due to the much greater propensity for loss of the ortho chlorine. The M-CO ion (i) can be

stabilized by an electron-releasing substituent such as the dimethylamino group in the 4-position of the aromatic ring.

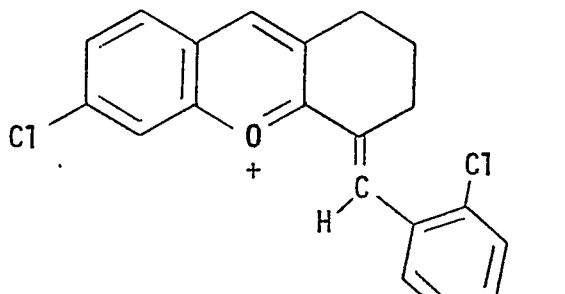
The mass spectral data in Table III shows that the substituted (E)-2-benzylidenecyclohexanones generally follow identical fragmentation pathways to the unsubstituted compound although there is a marked variation in the relative intensity of the fragment ions. With the exception of the 2-chloro substrate (35), the loss of 28 mass units was observed and the respective M-28 ions further lost 28 and 41 mass units (CH_2= CH - CH_2.) and as well peaks at m/e 129, 128, 115, and 67 were observed with the exception of the dimethylamino compound (38) which did not exhibit a m/e 67 peak in its spectrum.

2.1.6.4 Mass spectra of the substituted (E,E)-2,6-bis-benzylidenecyclohexanones

The mass spectra of the substituted (E,E)-2,6-bis-benzylidenecyclohexanones (53, 44-47) were determined and the major ions with their relative intensities recorded in Table IV. The ratio of M-1/M and M-Cl/M for these compounds is shown in Table VI along with ratios obtained in Series 3.

It is noted from the data in Table IV that there is generally considerably less fragmentation at 70 eV for the bis compounds in Series 2 than for the compounds in Series 1. The only major ions

for (E,E)-2,6-bis-benzylidenecyclohexanone (53) and (E,E)-2,6-bis-(4-dimethylaminobenzylidene)cyclohexanone (47) are the M and M-1 ions. The M-Cl ion is the only major ion found in the spectra of both (E,E)-2,6-bis-(2,6-dichlorobenzylidene)cyclohexanone (44) and (E,E)-2,6-bis-(2,4-dichlorobenzylidene)cyclohexanone (45) with the parent ion for each of these compounds having a relative intensity of approximately 1.0. The M-Cl/M ratio in both cases is approximately 100 in comparison with a ratio of 33 observed for (E)-2-(2-chlorobenzylidene)cyclohexanone (35). This difference presumably arises from the increased stability of the fragment ion when it retains an electron-donating ortho or para substituent. Isomerization at only one double bond of the bis

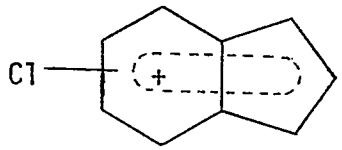


M-Cl ion from (45)

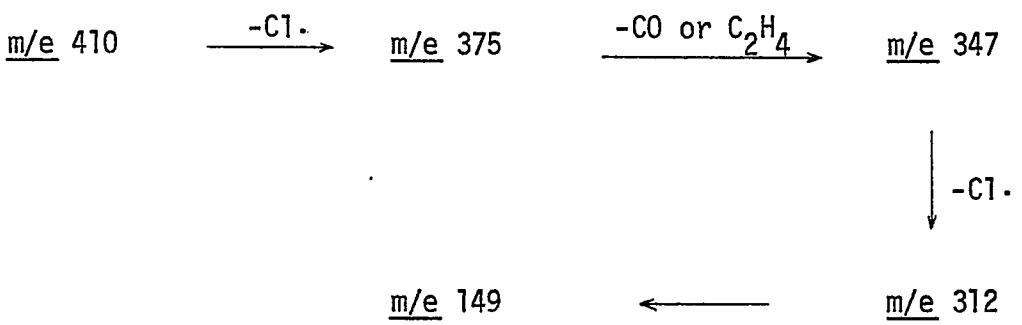
compounds is necessary for the intramolecular substitution reaction. The second aromatic ring, where the two chlorines are retained, is able to stabilize the benzopyrylium ion, as shown above.

The only substrate in the bis series (Series 2) which exhibited significant fragmentation was (E,E)-2,6-bis-(3,4-dichlorobenzylidene)cyclohexanone (46) and the fragmentation is illustrated in Scheme 6. The formation of the ions down to m/e 312 presumably proceeds

as described for the unsubstituted compound (34) in Series 1. It is suggested that the ion at m/e 149 has the structure (m), an analogous structure to m/e 115 (k) discussed earlier. The loss of a chlorine atom from the bis-3,4-dichloro compound (46) occurs with approximately the same ease as from (E)-2-(3-chlorobenzylidene)cyclohexanone (36) since both processes involve cleavage of the meta chlorine atom from the aromatic ring. The M-Cl/M ratio for 1-(3,4-dichlorophenyl)-1-nonen-3-one (Series 3) as shown in Table VI is zero since this substrate can fragment by pathways not available in the cyclohexanone series.

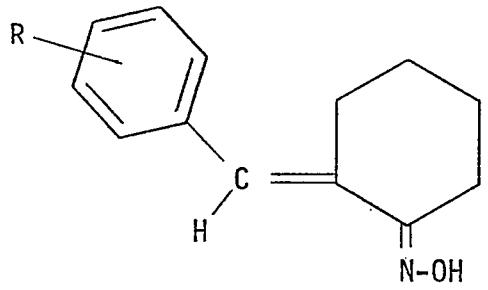


(m)



Scheme 6 Mass spectral fragmentation of (E,E)-2,6-bis-(3,4-dichlorobenzylidene)cyclohexanone

2.2 Preparation of substituted (E)-2-benzylidenecyclohexanone oximes



(60) R = H

(61) R = 2-Cl

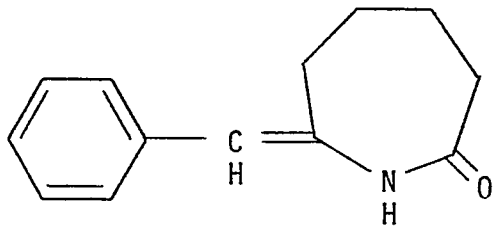
(62) R = 4-Cl

(63) R = 4-$N(CH_3)_2$

2.2.1 Configuration of the oximes

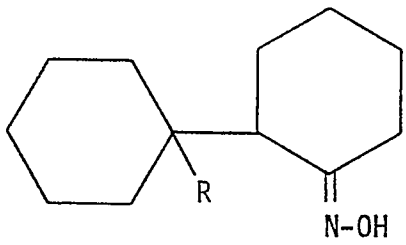
The substituted (E)-2-benzylidenecyclohexanone oximes (60-63) were prepared from the corresponding E ketones by treatment with hydroxylamine, released from hydroxylamine hydrochloride by the action of sodium acetate. Oximes of unsymmetrical ketones are capable of existing in the E and Z forms so two oximes are possible upon the oximation of each of the substituted (E)-2-benzylidenecyclohexanones. Of the oximes prepared, only 2-benzylidenecyclohexanone oxime has been reported in the literature. Vavon and Conia (1952) assumed the presence of only one isomer, as evidenced by a sharp melting point for the oxime and the semicarbazone of 2-benzylidenecyclohexanone. The unsubstituted oxime has also been prepared by Sato et al. (1970). They observed only one signal in the olefinic region of the nmr spectrum

which indicated the presence of one isomer, and thin-layer chromatography (tlc) showed only one spot. In addition, the Beckmann rearrangement of 2-benzylidenecyclohexanone gave one lactam (64) solely, which indicated that the oxime had the E configuration of the oxime hydroxyl group and the benzylidene group.

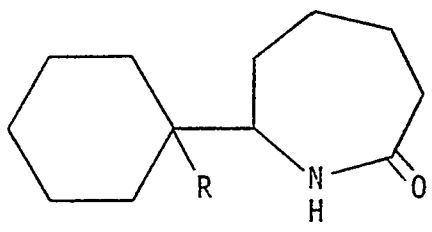


(64)

The E configuration has also been assigned as the most probable structure in several 2-substituted cyclohexanone oximes (Kelly and Matthews, 1970), based on the high resolution mass spectra of the lactams (66) resulting from the Beckmann rearrangement of oximes such as (65).



(65)



(66)

R = H, Cl

The nmr spectrum of each of the substituted (E)-2-benzylidene-cyclohexanone oximes (60-63) prepared in the present investigation showed only one signal for the vinylic proton. Thus it was assumed that the oximes have the E configuration of the oxime hydroxyl group with respect to the benzylidene group.

2.2.2 Gas-liquid chromatography (glc) and thin-layer chromatography (tlc) of the oximes

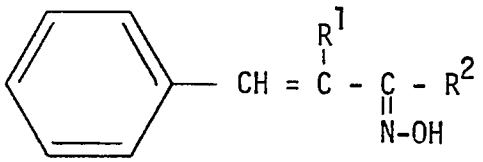
The glc analysis of each of the substituted (E)-2-benzylidene-cyclohexanone oximes (60-63) showed several peaks. Since the column temperatures had to be fairly high (ca. 200°) thermal decomposition of the oximes was suspected and attempts to detect any geometrical isomers were then confined to tlc.

Suwinski et. al. (1967) separated the isomers of oximes prepared from derivatives of benzylideneacetone by crystallization from ethanol or by preparative tlc on silica in a 5:1 benzene-ethyl acetate solvent system. Repeated recrystallization of the substituted (E)-2-benzylidenecyclohexanone oximes prepared in the present study from ethanol or acetone did not change the glc results. No resolution of the unsubstituted oxime (60) or the 2-chloro oxime (61) was observed using tlc with a 5:1 benzene-ethyl acetate solvent system.

Buzlanova and Stepanovskaya (1965) used tlc on silica gel for various oximes. The solvent systems used were 8:12:4:1.5 petroleum

ether-carbon tetrachloride-ethyl ether-ethanol and 15:3:1.5 petroleum ether-ethyl ether-ethanol. The spots were developed by exposure to chlorine or by spraying with a saturated solution of copper acetate in ethanol. These solvent systems were used to check the composition of the oximes (60) and (61) by tlc. Again resolution into more than one spot was not observed.

Various α,β-unsaturated aldoximes and ketoximes have been detected by tlc on silica gel, using 3:1 benzene-ethyl acetate as solvent (Unterhalt, 1966). Only one isomer was found for each of the following ketoximes (67-69). No resolution into more than one spot was observed for the unsubstituted oxime (60) and the 2-chloro oxime



(67) R^1 = H; R^2 = C_6H_5-CH=CH

(68) R^1 = CH_3; R^2 = CH_3

(69) R^1 = H; R^2 = C_6H_5

(61) when tlc on silica gel was carried out with a 3:1 benzene-ethyl acetate solvent system. Other solvent systems employed for the tlc

of (60) and (61) included 4:1:2 butanol-glacial acetic acid-water and 10:1:1 benzene-ethanol-glacial acetic acid. Again no resolution into more than one spot was observed.

The nmr spectrum of each of the substituted (E)-2-benzylidene-cyclohexanone oximes (60-63) was readily assignable to the desired structure and the carbon-hydrogen analyses were acceptable for the desired oximes. The product from the lithium aluminum hydride reduction of the oximes (60), (61), and (62) contained greater than 99% of one compound (glc analysis) which indicates that the oximes were not a mixture of several compounds as shown by their glc analysis.

2.2.3 Mass spectra of substituted (E)-2-benzylidenecyclohexanone oximes

2.2.3.1 Introduction

One of the major fragmentation pathways in the mass spectra of the substituted (E)-2-benzylidenecyclohexanones (34-38) and substituted (E, E)-2,6-bis-benzylidenecyclohexanones (44-47) is an intramolecular aromatic substitution with loss of an ortho substituent to give a benzopyrylium ion (Section 2.1.6). In order to further investigate this aromatic substitution reaction, it was decided to examine the mass spectra of the substituted (E)-2-benzylidenecyclohexanone oximes (60-63). Since oximes have two hetero atoms the possibility of charge localization on either atom exists which might lead to fewer fragment ions emanating from the cyclohexanone ring, favoring

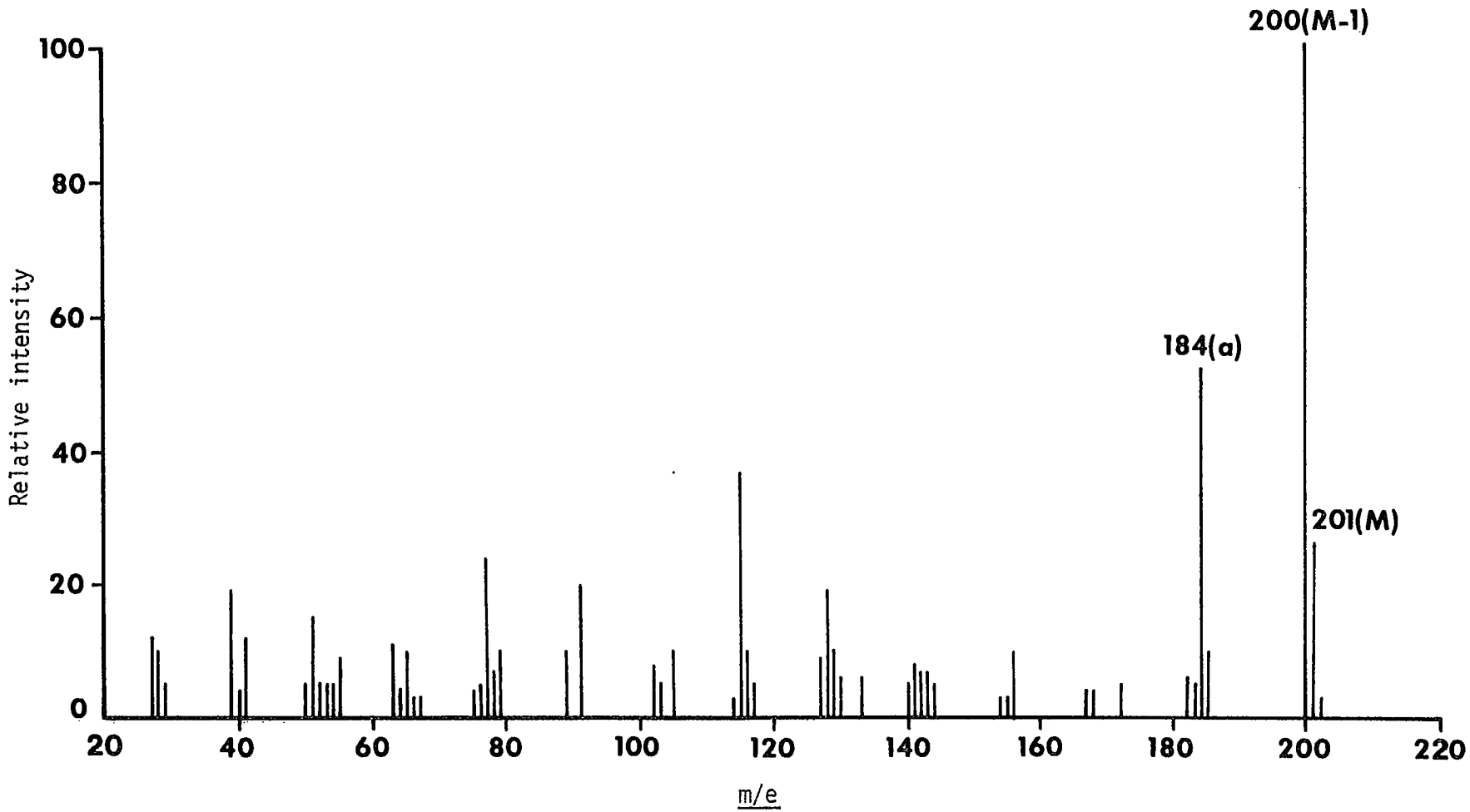


Figure 5. Mass spectrum of (E)-2-benzylidenecyclohexanone oxime

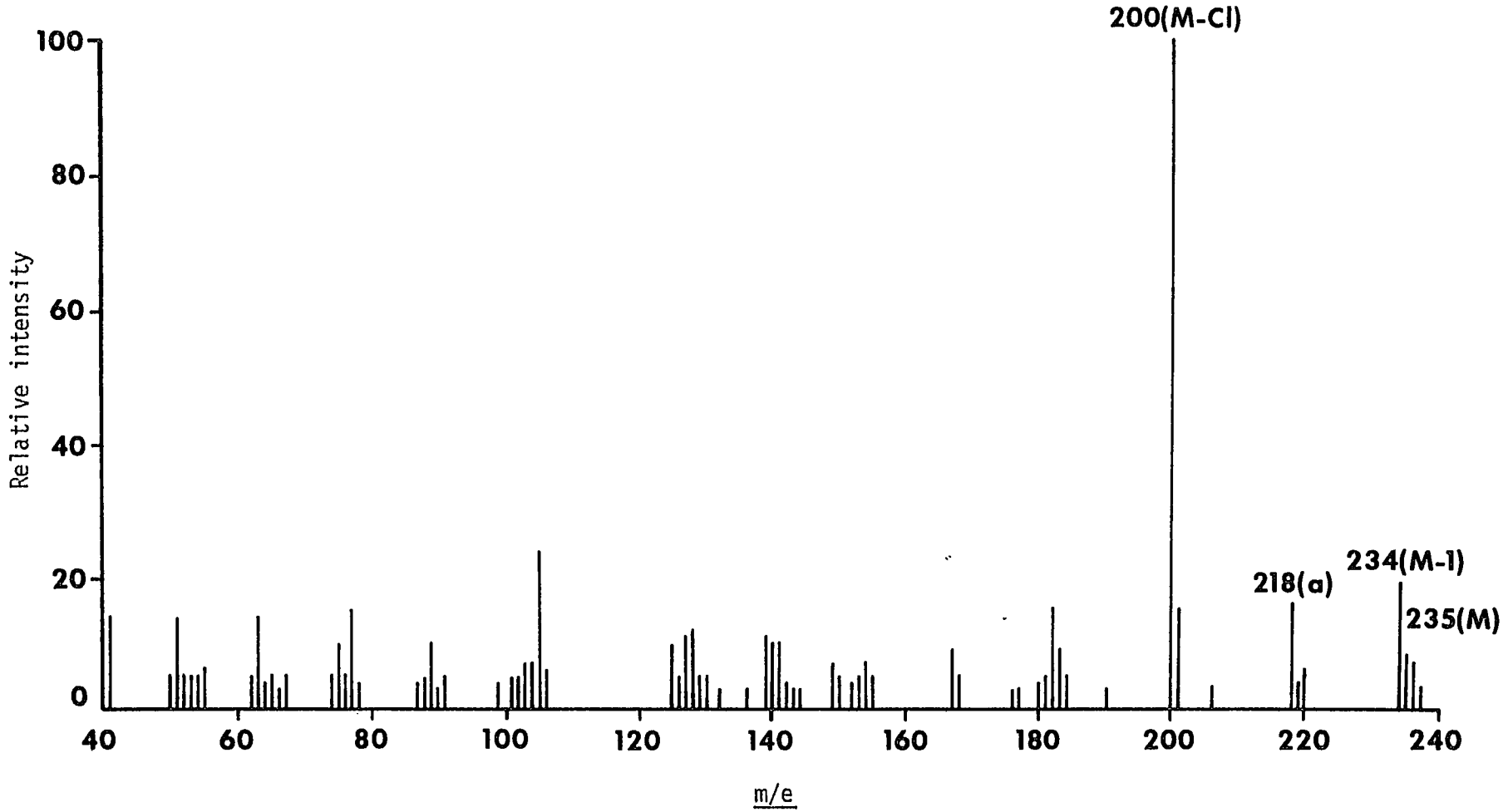


Figure 6. Mass spectrum of (E)-2-(2-chlorobenzylidene)cyclohexanone

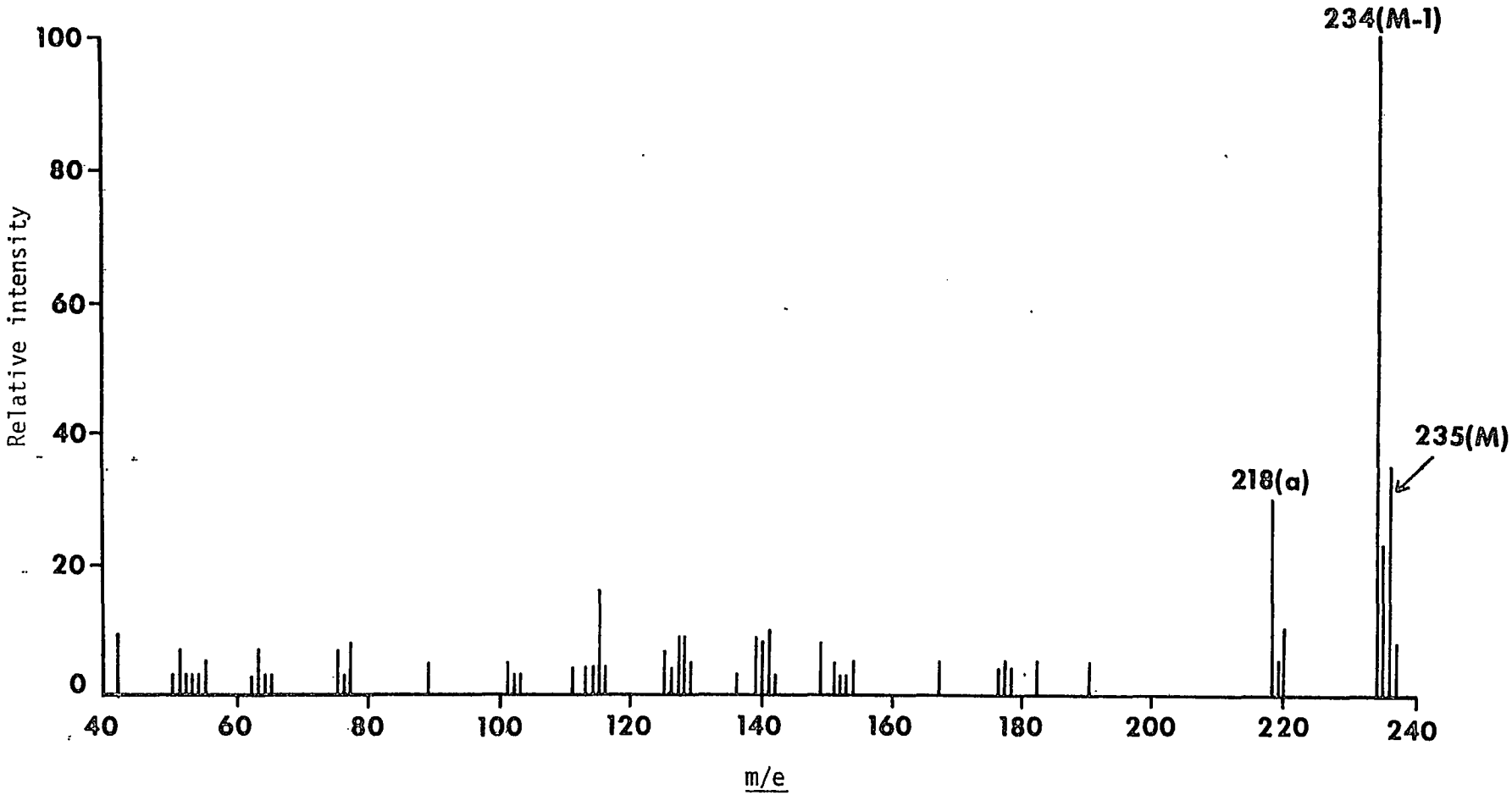


Figure 7. Mass spectrum of (E)-2-(4-chlorobenzylidene)cyclohexanone oxime

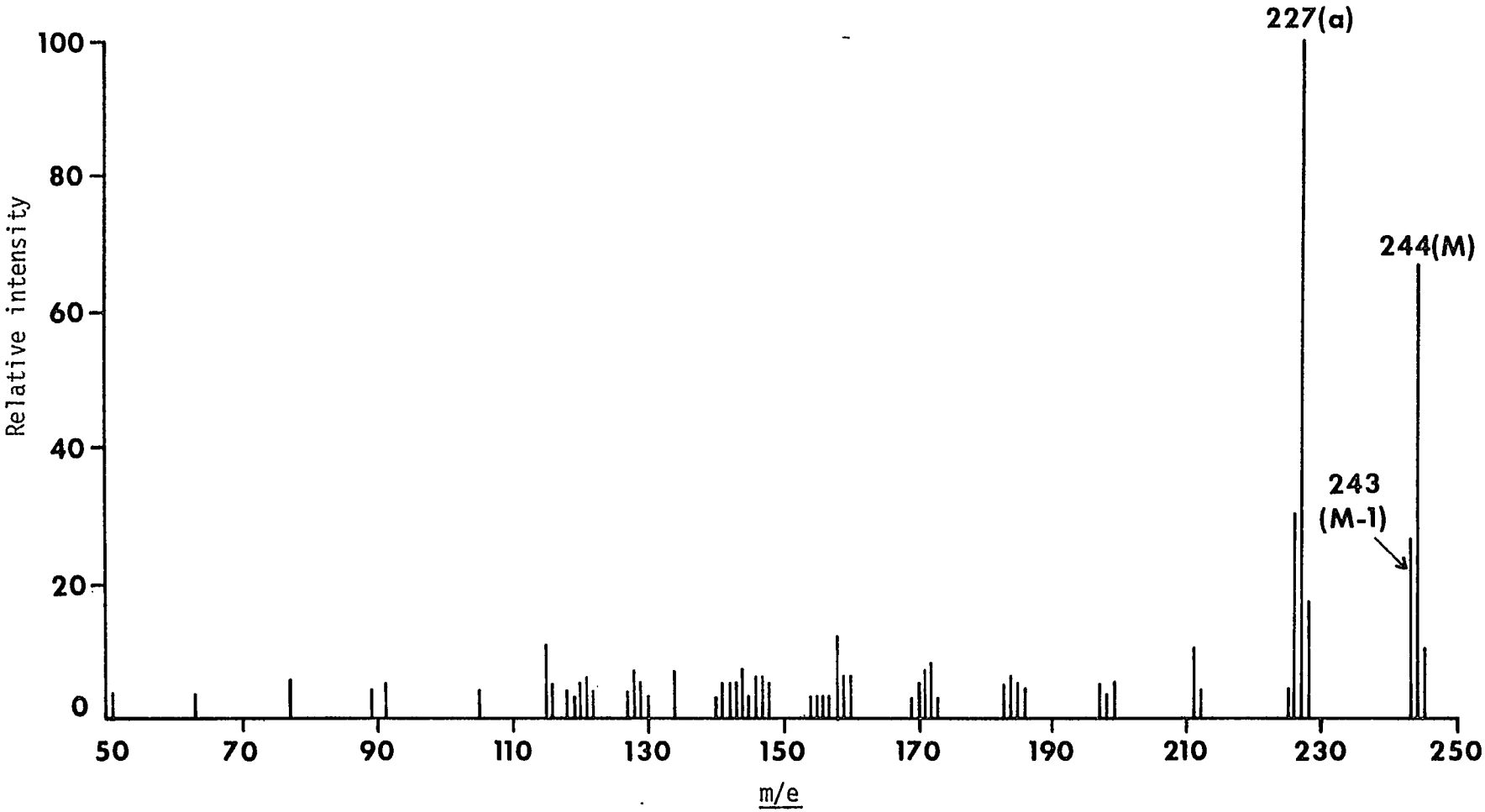


Figure 8. Mass spectrum of (E)-2-(4-dimethylaminobenzylidene)cyclohexanone

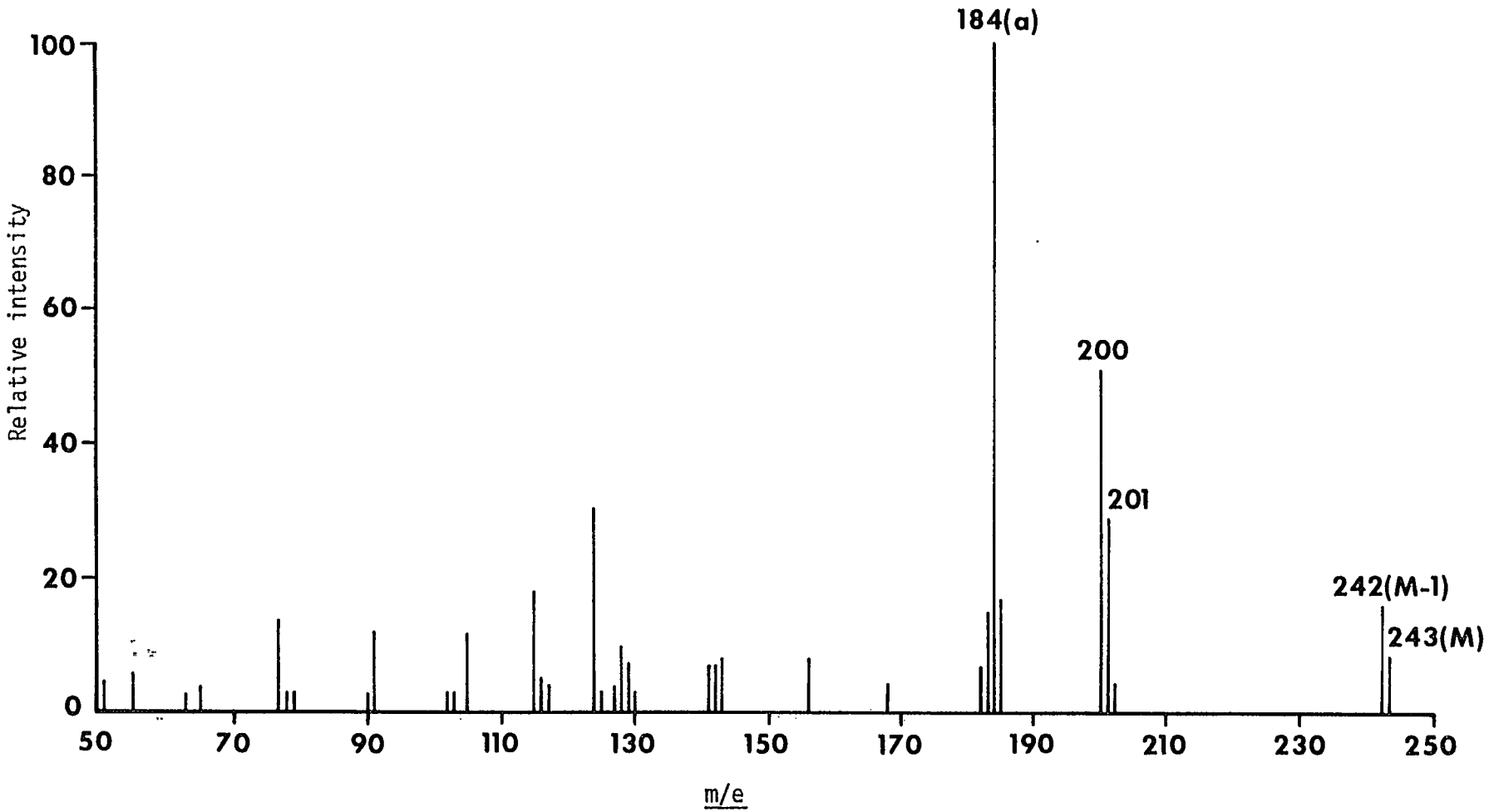
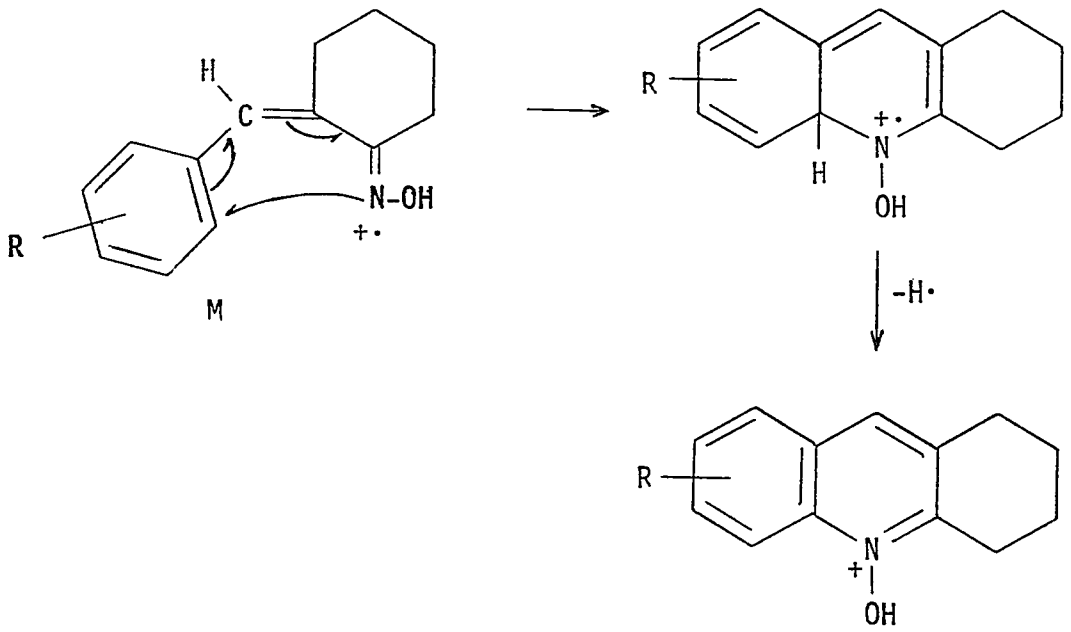


Figure 9. Mass spectrum of (E)-2-benzylidenecyclohexanone oxime acetate

the formation of the substitution product. Furthermore, ring closure with nitrogen in the ring rather than oxygen might be expected to be more favorable since an electron in the C=N bond is more available than in the C=O bond due to the difference in electronegativities of the two hetero atoms.

2.2.3.2 Results and discussion

A striking feature of the mass spectra of the four oximes (44-47) is a large peak at M-1. In fact this peak is approximately three times more intense than the parent ion peak for all the substrates except the 4-dimethylamino derivative (47) where it is only one-third as intense as the parent ion. The spectrum of the acetate of the unsubstituted oxime (98), Fig. 9, shows an appreciable M-1 peak which indicates that the M-1 peak in the oximes is not due to loss of the hydrogen atom from the hydroxyl group. Since the intramolecular aromatic substitution reaction of the substituted (E)-2-benzylidenecyclohexanones, after prior E to Z isomerization, leads to the formation of a stable benzopyrylium ion, it is reasonable to assume that such a mechanism is operative in the case of the oximes with nitrogen bonding to the aromatic ring. In all cases a metastable peak was observed for this process.

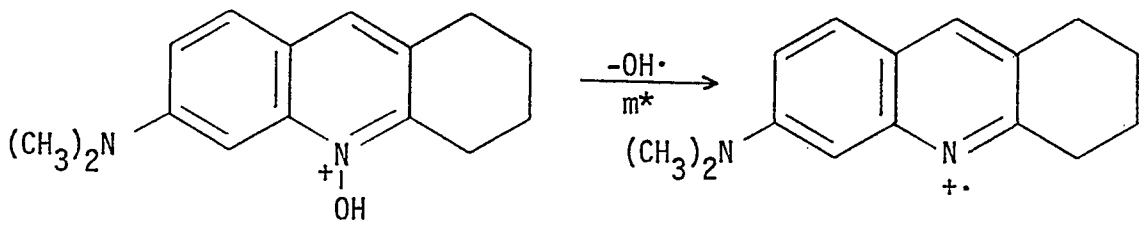


M

M-1

The formation of the M-1 ion in the oxime series appears to be more favorable than for the substituted (E)-2-benzylidene-cyclohexanones as the ratio M-1/M is greater for the oximes with the exception of the 4-dimethylamino oxime (47). Indeed, the M-1 ion represents the base peak in the mass spectra of the unsubstituted oxime (60) and the 4-chloro oxime (62), Figs. 5 and 7 respectively. This high ratio for M-1/M is surprising since it is expected that the oxime parent ions would be relatively more stable than the ketone parent ions due to the presence of the two hetero atoms, resulting in less fragmentation. However, the stability of the product ion from the intramolecular aromatic substitution appears to be the dominant

driving force for the loss of a hydrogen atom from the parent ion. For the 4-dimethylamino substrate (47) the strongly electron-donating para substituent makes the parent ion sufficiently stable due to greater charge delocalization, so that further fragmentation is retarded. The electron-donating substituent influences markedly the mode and ease of fragmentation as shown by the observation of a fairly intense ion resulting from the loss of a hydroxyl radical from the M-1 ion. Such a process is very minor or non-existent for the other substrates. Presumably, the electron-donating substituent allows the formation of this odd-electron ion while for the other substrates the loss of a hydroxyl radical from the M-1 ion would lead to a relatively more unstable ion.

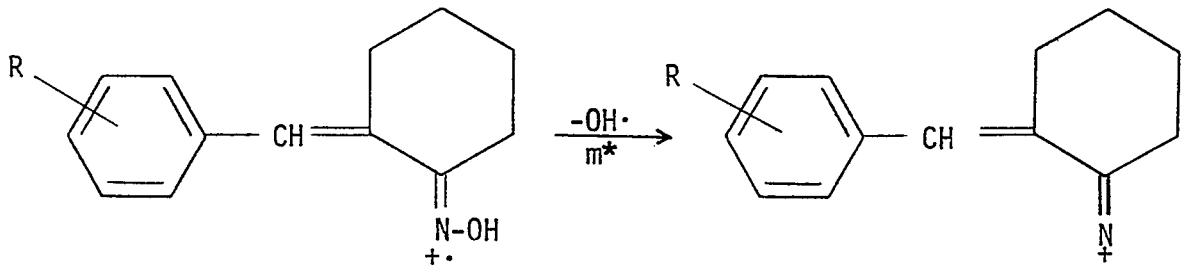


M-1

In the ortho-chloro oxime (61), as in the corresponding ketone, the loss of the ortho chlorine from the parent ion is extremely facile leading to the most intense peak in the mass spectrum, Fig. 6. This process is confirmed by the appropriate metastable peak.

The loss of the para chlorine from the parent ion of the para-chloro oxime (62), however, is not observed, Fig. 7, in contrast to the corresponding ketone where such a loss of chlorine was observed. This is consistent with a more facile cyclization process for the oxime and consequently the competing process, loss of chlorine, is not observed.

A major fragmentation pathway observed for oximes (60) to (63) is the loss of a hydroxyl radical from the parent ion to form the even-electron M-OH species, ion (a), Figs. 5-8. In fact,



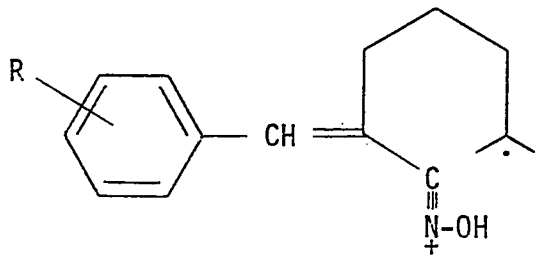
(a)

M M-OH

ion (a) is the most intense ion in the spectrum of the 4-dimethylamino oxime (63). Such a process has been observed for aliphatic aldoximes and ketoximes as well as alicyclic ketoximes (Budzikiewicz et al., 1967) but is of minor importance due to the competing McLafferty rearrangement. However, benzophenone oxime has the M-OH ion as the base peak (Budzikiewicz et al., 1967) which suggests that its formation is made

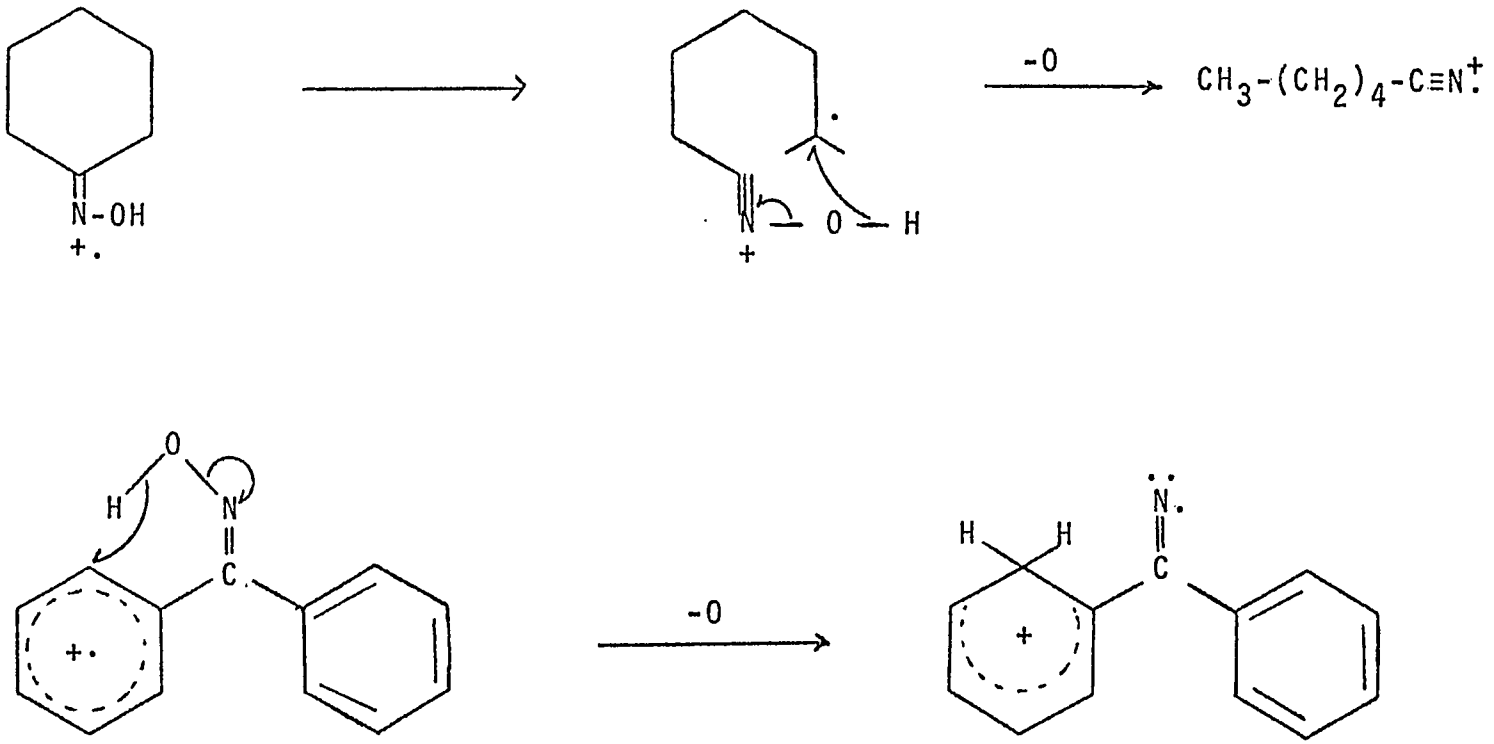
favorable by conjugation.

An interesting feature of the mass spectra of aliphatic ketoximes and aromatic ketoximes (Budzikiewicz et al., 1967) is the loss of an oxygen atom from the parent ion which is a very rare process. This fragmentation pathway may be represented as shown in Scheme 7. Loss of an oxygen atom from the oximes studied in the present investigation was not observed, as confirmed by a high resolution study of the M-16 ions for compounds (60) and (61). The cyclic mechanism leading to the loss of an oxygen atom, as suggested for the aromatic ketoximes, is not favorable in the present series and the product of α-cleavage (b) is stabilized by conjugation.



(b)

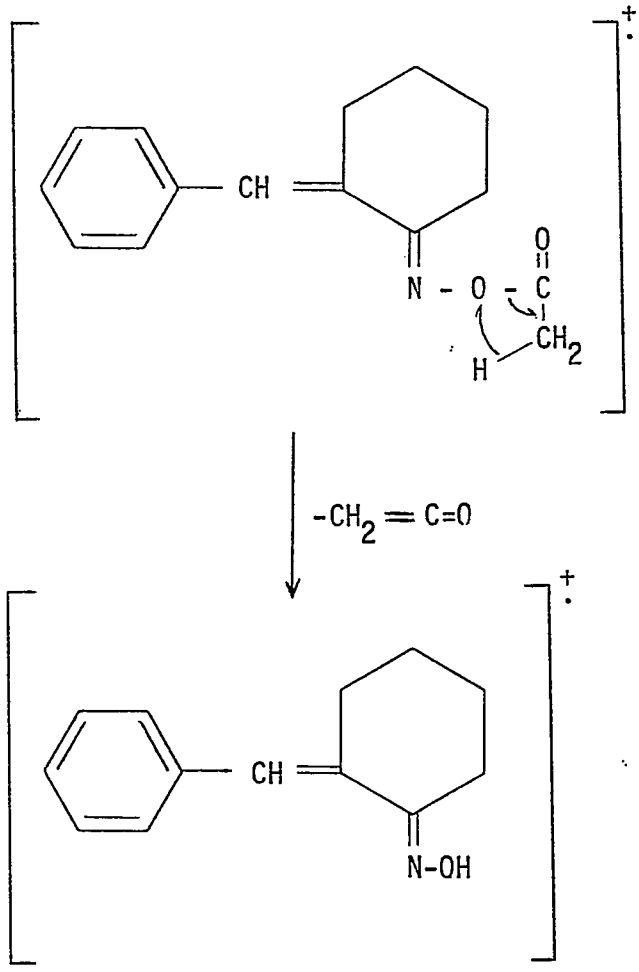
In the study of the fragmentation patterns of the substituted (E)-2-benzylidenecyclohexanones (34-38) α-cleavage with loss of ethylene was observed. Such a process was not operative in the present study and this is consistent with the observation that benzophenone oxime undergoes α-fission with charge retention on the



Scheme 7. Mass spectral fragmentation of an oxygen atom from aliphatic and aromatic ketoximes

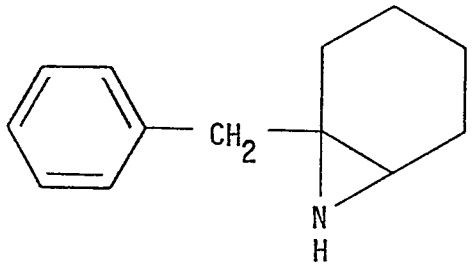
phenyl ring but not on the nitrogenous moiety (Budzikiewicz et al., 1967).

In the case of the acetate of the unsubstituted oxime (98) it is observed that the parent ion loses ketene to form (60) which loses a hydrogen atom and also fragments to ion (a) as discussed earlier. The loss of ketene is represented by the following equation.



2.3 Reduction of substituted 2-benzylidenecyclohexanone oximes

2.3.1 Preparation of 1-benzyl-1,2-epiminocyclohexane

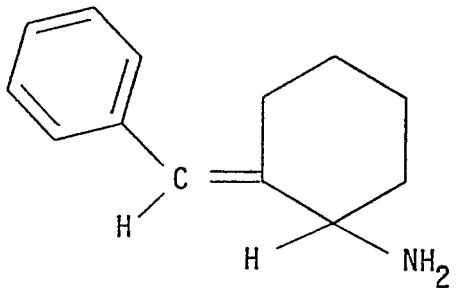


(70)

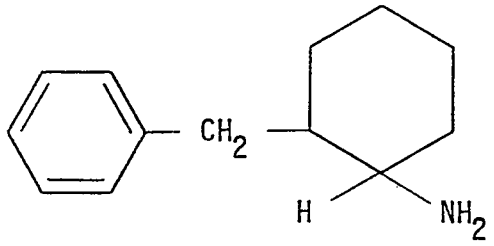
The reduction of oximes with lithium aluminum hydride (LAH) usually gives the corresponding primary amines although the yields vary over a wide range (Gaylord, 1956). It was predicted that LAH reduction of the substituted (E)-2-benzylidenecyclohexanone oximes (17) would yield the corresponding (E)-2-benzylidenecyclohexylamines (18), which would be employed in the Hofmann mustard oil reaction to prepare the substituted (E)-2-benzylidenecyclohexyl isothiocyanates (19).

However, reduction of (E)-2-benzylidenecyclohexanone oxime (60) with LAH gave a pale yellow syrup which contained one compound (glc) in which the olefinic bond was saturated, as evidenced by the loss a signal for the vinylic proton in the nmr spectrum and in addition, loss of the styrenoid absorption was detected by uv spectroscopy. Tests for unsaturation with bromine and potassium permanganate were also negative. Only one replaceable proton on the nitrogen was indicated from the nmr spectrum (D_2O exchange) and chemical tests (i.e. nitrous

acid test, Rimini test) were negative for a primary amine. Thus the reduction product was not (E)-2-benzylidenecyclohexylamine (71) or 2-benzylcyclohexylamine (72).



(71)

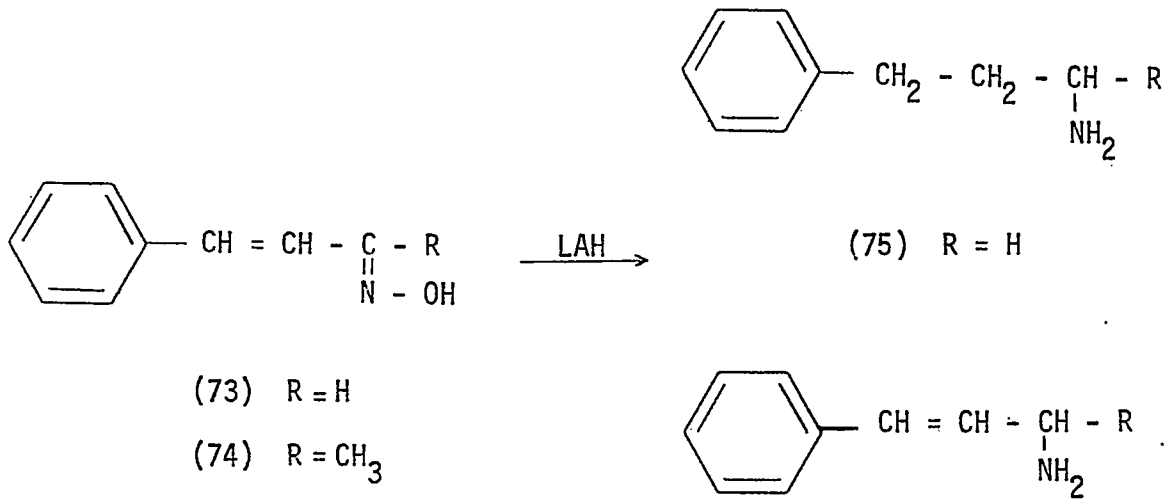


(72)

The mass spectrum of the reduction product gave a parent peak at m/e 187 which also indicated that the saturated primary amine (72), which has a parent peak at m/e 189, was not the product. A prominent peak at m/e 91 was assigned to the tropylium ion, which would come from the benzyl portion of the molecule. A metastable ion for the fragmentation of m/e 91 to m/e 65 was also observed, consistent with the assignment of a tropylium ion for m/e 91. The (M + 1)/(M) ratio of the parent ion indicated that the compound contained 13 carbon atoms, and the odd mass for the parent peak indicated the presence of one nitrogen atom. Thus an empirical formula of $C_{13}H_{17}N$ was indicated.

The reduction of α,β-unsaturated oximes does not always give the α,β-unsaturated amines. Larsson (1950) reported an 84% yield

of 3-phenyl-1-aminopropane (75) from the LAH reduction of cinnamaldoxime (73). Walter (1952), in repeating the reduction, was able to isolate the unsaturated amine (76) in a 53% yield. He also reduced benzylideneacetone oxime (74) to the unsaturated primary amine (77) in a 55% yield. These results are not wholly unexpected, even in view of Larsson's findings, for it is known that in some α,β-unsaturated carbonyl compounds



(73) R = H

(74) R = CH_3

(75) R = H

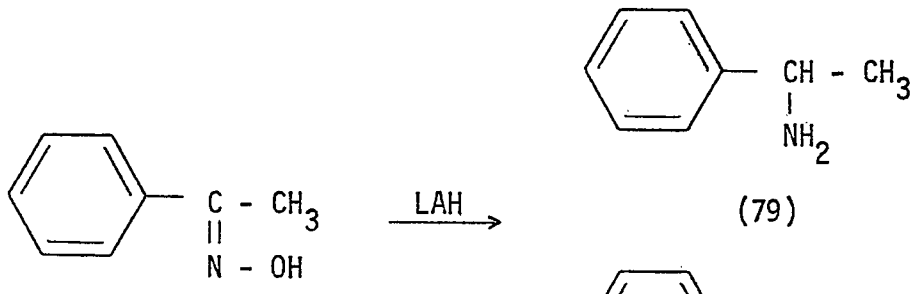
(76) R = H

(77) R = CH_3

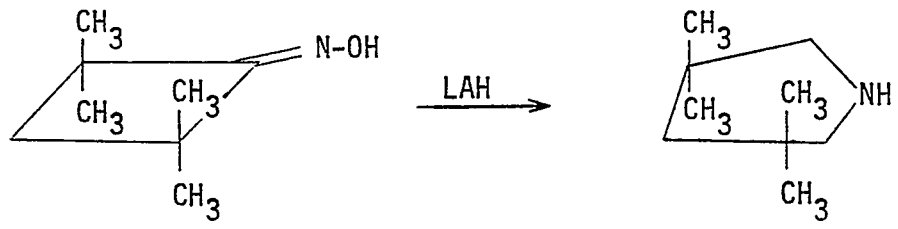
it is possible to direct the reduction with LAH to give either the saturated or unsaturated compound by appropriate choice of conditions (Hochstein and Brown, 1948).

In addition, it is not always primary amines that are formed by reduction of oximes with LAH. The reduction of certain aryl ketoximes

yields the primary amines together with the rearranged secondary amines (Larsson, 1950; Smith et al., 1952; Lyle and Trosianiec, 1955; Petrarca and Emery, 1963). For example, Larsson (1950) found that acetophenone oxime (78) was reduced by LAH to the corresponding amine, 1-phenylethylamine (79) together with 10-15% of the rearranged amine, N-ethylaniline (80). In addition, reductive ring expansion of cyclic ketoximes has also been observed (Blomquist and coworkers, 1959; Harfenist and Magnien, 1958; Lautenschlaeger and Wright, 1963). For example, LAH reduction of 2,2,4,4-tetramethylcyclobutanone oxime (81) gave the secondary amine, 2,2,4,4-tetramethylpyrrolidine (82) (Lautenschlaeger and Wright, 1963).

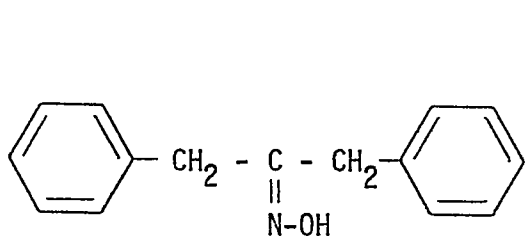


(78) (79) (80)

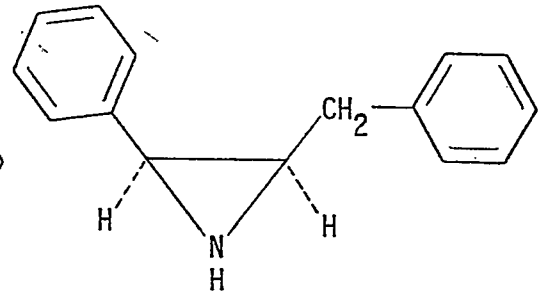


(81) (82)

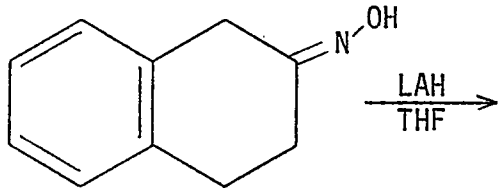
Kitahonoki et al. (1965) reported that the reduction of various ketoximes with LAH in tetrahydrofuran afforded aziridine derivatives. LAH reduction of dibenzylketone oxime (83) and β-tetralone oxime (85) gave the aziridines (84) and (86) respectively. They found that the yields of the aziridines were dependent on the kind of solvent used for the reductions. When ether was used as the solvent instead of tetrahydrofuran the aziridine formation was decreased or totally eliminated.



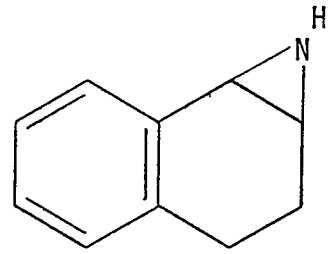
(83)



(84) 73%



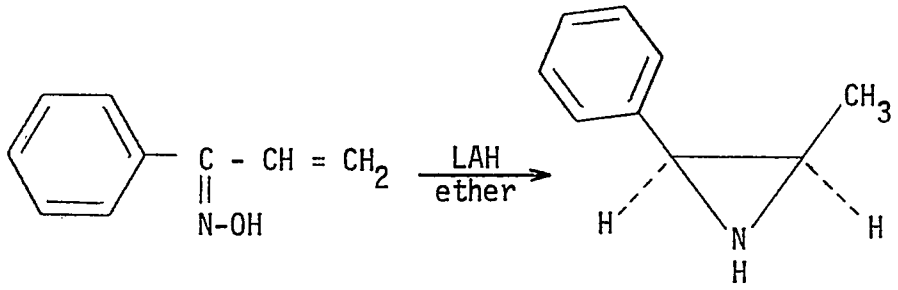
(85)



(86) 40%

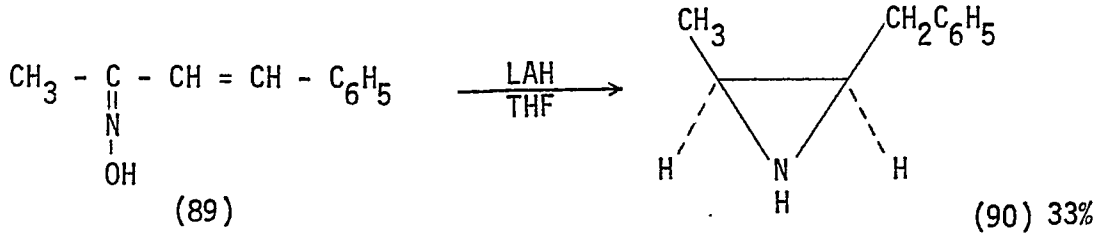
At almost the same time, Shandala et al. (1965) reported one similar example of this type of reaction. Phenyl vinyl ketoxime (87) gave cis-2-phenyl-3-methylaziridine (88) in 50% yield upon reduction with LAH in boiling ether. This was the first example of

aziridine formation from an α,β-unsaturated ketoxime. Since then

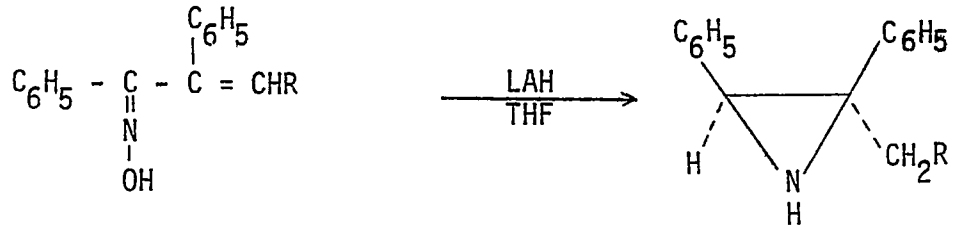


(87) (88)

Kotera and coworkers have investigated the reduction of many ketoximes and aldoximes with LAH, including the reduction of several α,β-unsaturated ketoximes. For example, cis-1-benzyl-2-methylaziridine (90) was obtained from the reduction of benzylideneacetone oxime (89) with LAH. They also obtained aziridines (92) and (94) from the LAH reduction of the α,β-unsaturated ketoximes (91) and (93) respectively (Kotera and Kitahonoki, 1969).



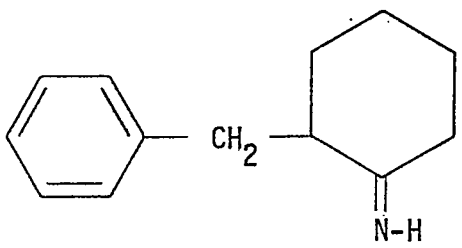
(89) (90) 33%



(91) R = H (92) R = H, 90%

(93) R = C_6H_5 (94) R = C_6H_5, 86%

Thus a survey of the literature indicates that α,β-unsaturated oximes on reduction with LAH afford unsaturated amines, saturated amines, and aziridines. In addition cyclic oximes can undergo ring expansion and aryl ketoximes can give rearranged secondary amines. Apart from one of these types of compounds being formed, another structure considered from the LAH reduction of (60) was an imine (95), which fits the empirical formula. However, the spectral and chemical data indicated that the product obtained by reduction of (E)-2-benzylidene-cyclohexanone oxime with LAH is 1-benzyl-1,2-epiminocyclohexane (70).



(95)

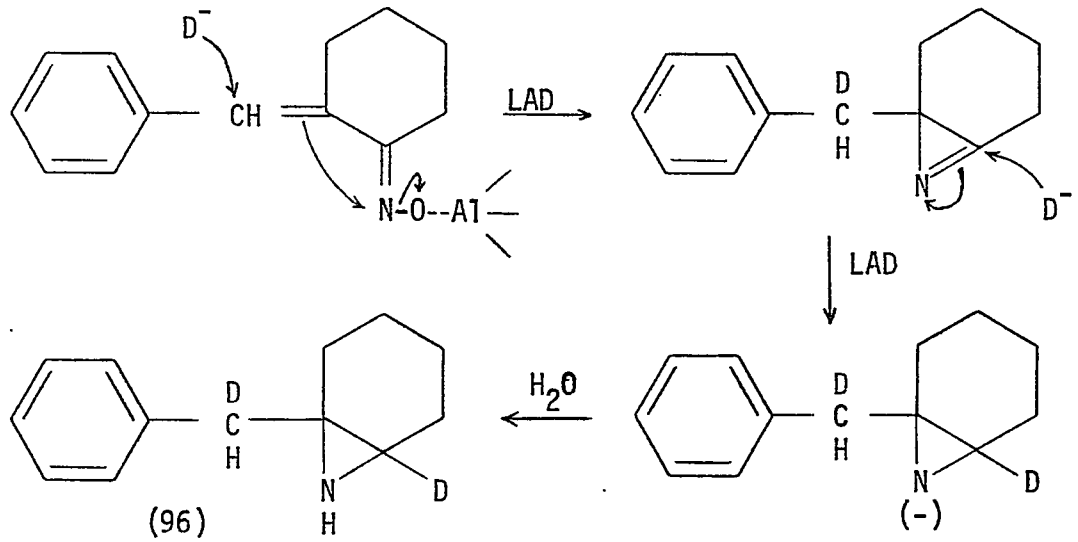
The two hydrogen atoms of the benzylic side chain exhibit an AB quartet in the nmr spectrum of the aziridine (70) since they are not magnetically equivalent because of the adjacent aziridine ring. The magnetic non-equivalence of methylene protons adjacent to an asymmetric center is a common phenomenon. The bridgehead hydrogen at C-2 is slightly deshielded due to the aziridine ring, appearing as a multiplet at δ 2.07-1.83 and the hydrogens at C-3 and C-6 of the cyclo-

hexane ring are also deshielded slightly by the aziridine ring, appearing as a multiplet at δ 1.90-1.50.

In order to inspect the reaction mechanism for the formation of the aziridine (70) the oxime (60) was reduced with lithium aluminum deuteride. The location of deuterium in the deuterated reduction product (96) was determined by comparing its nmr spectrum with that of the non-deuterated reduction product (70). The AB quartet with doublet centers at δ 2.92 and δ 2.63 is absent in the nmr spectrum of the deuterated product and two shaggy multiplets at δ 2.85 and δ 2.68 integrating for one proton now appear which were assigned to the erthro and threo forms due to the CDH group in the C_6H_5-CHD side chain. The bridgehead proton at C-2 (δ 2.07-1.83) in the nmr spectrum of the aziridine (70) is absent in the deuterated analog (96), indicating that introduction of deuterium is at the C-2 position on the cyclohexane ring. A replaceable proton (D_2O exchange) still appears in the nmr of the deuterated compound, with a signal at δ 0.98.

The mass spectrum of the deuterated aziridine (96) had prominent peaks at m/e 189, 97, and 92, which indicated the incorporation of one deuterium in a C_6H_5-CHD side chain and the other deuterium in the epiminocyclohexane portion of the molecule.

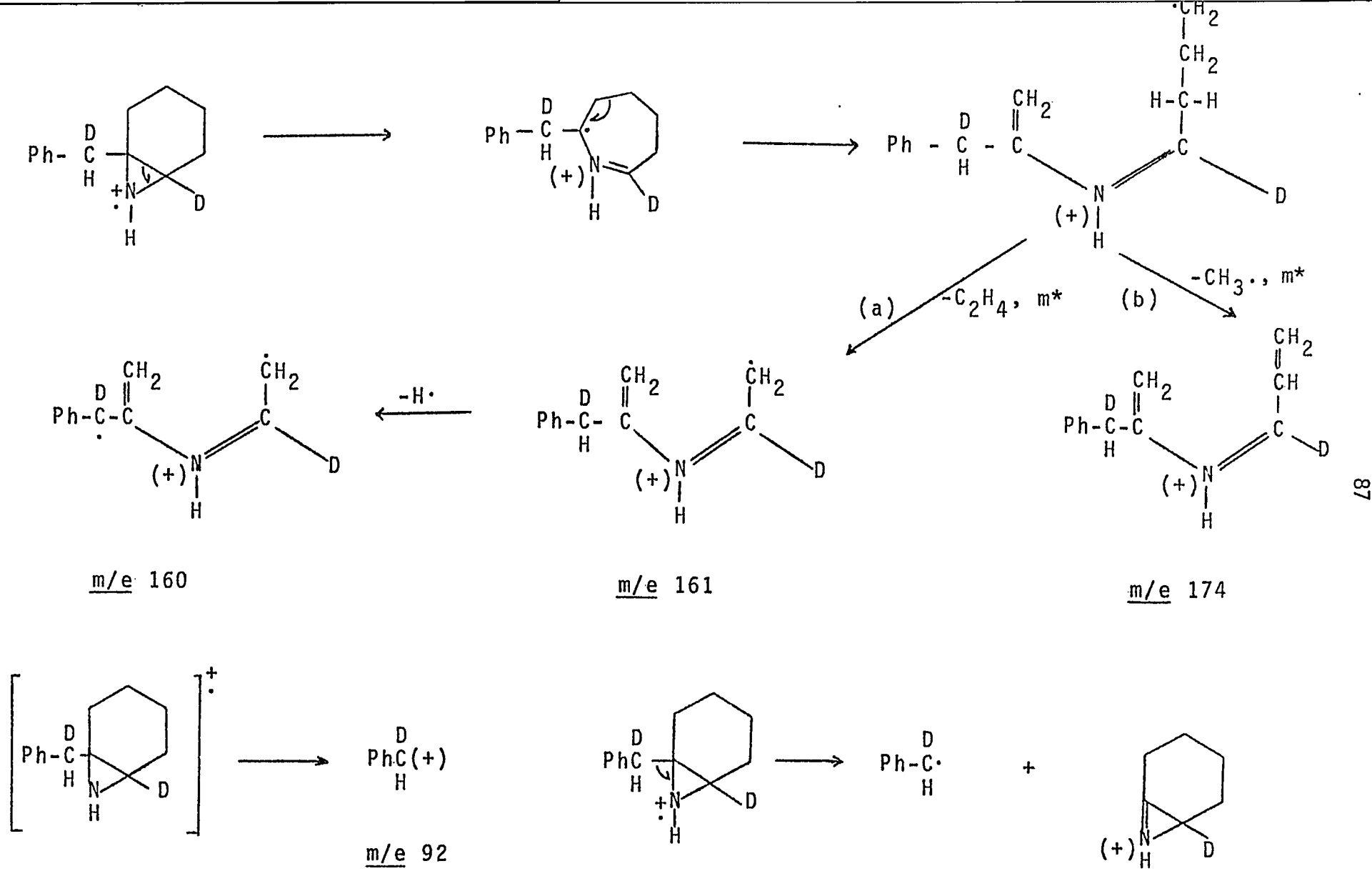
The postulated reaction mechanism for the reduction of the oxime (60) with lithium aluminum deuteride is as follows:



The mass spectrum of the undeuterated aziridine (70) and the deuterated aziridine (96) were compared and the principle peaks are summarized in Table VII, followed by the possible fragmentation pathways for the deuterated aziridine (96), (Scheme 8).

Table VII Major peaks in the mass spectra of aziridines

Undeuterated aziridine (70)		Deuterated aziridine (96)	
m/e	Rel. abundance	m/e	Rel. abundance
187 $(M)^+$	100	189 $(M)^+$	100
186 $(M-1)^+$	56	188 $(M-1)^+$	40
172 $(M-15)^+$	82	174 $(M-15)^+$	36
159 $(M-28)^+$	54	161 $(M-28)^+$	36
158 $(M-29)^+$	74	160 $(M-29)^+$	40
96 $(M-91)^+$	100	97 $(M-92)^+$	60
91 $(M-96)^+$	100	92 $(M-97)^+$	96

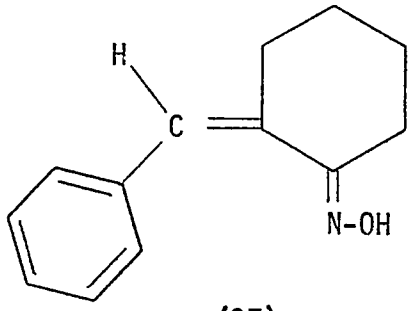


Scheme 8. Mass spectral fragmentation of 1-benzyl-α-d-1,2-epiminocyclohexane-2-d

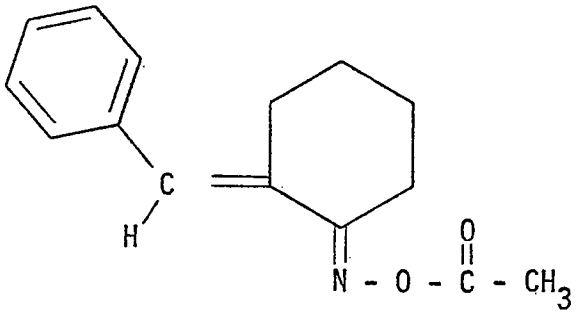
Lithium borohydride reduction of (E)-2-benzylidenecyclohexanone oxime (60) gave a pale yellow syrup which contained 65% of the aziridine (70) and 35% of unreacted oxime (60). Lithium borohydride is intermediate in reducing power between lithium aluminum hydride and sodium borohydride. Sodium borohydride did not reduce the oxime at all. LAH reduction of (Z)-2-benzylidenecyclohexanone oxime (97) gave a reduction product which contained 49% of the aziridine (70), 33% of unreacted oxime (97), and two unidentified compounds, each present in a concentration of approximately 9%.

Lithium borohydride and especially LAH react rapidly with hydroxylic compounds. It was considered that attack at the hydroxyl group of (E)-2-benzylidenecyclohexanone oxime (60) was affecting the course of the reduction by causing steric hindrance of the hydride reducing species to the carbon atom of the C=N bond. It was decided that a reduction should be attempted with an analog of the oxime in which the hydroxyl group was masked. (E)-2-benzylidenecyclohexanone oxime acetate (98) was prepared in the hope that LAH would reduce the oxime function rather than the conjugated double bond. However, the LAH reduction of the oxime acetate (98) gave only the aziridine (70) in a yield of 90%. Kotera and coworkers (1968) found that when O-substituted derivatives, such as the O-methyl and O-acetyl derivative of dibenzylketoxime,(99) and (100) respectively, were used for the LAH reduction the major reduction product was the aziridine (101),

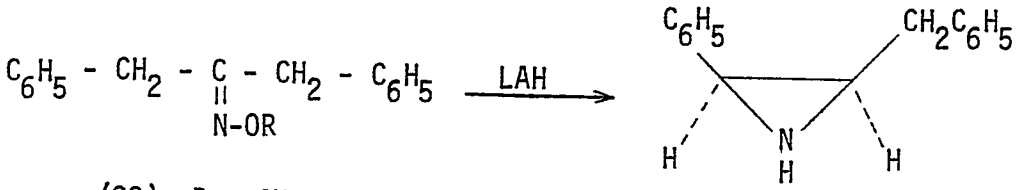
together with a small amount of the primary amine.



(97)



(98)

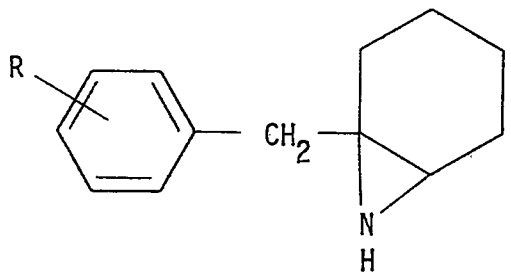


(99) R = CH_3

(100) R = $COCH_3$

(101)

2.3.2 Preparation of related substituted 1-benzyl-1,2-epimino-cyclohexanes



(102) R= 2 - Cl

(103) R= 4 - Cl

(104) R= 4 - $N(CH_3)_2$

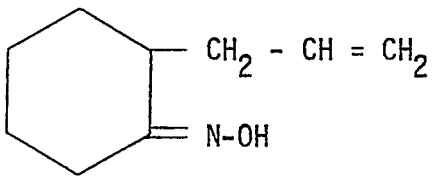
Both (E)-2-(2-chlorobenzylidene)cyclohexanone oxime (61) and (E)-2-(4-chlorobenzylidene)cyclohexanone oxime (62) were reduced by LAH to the arizidines (102) and (103) respectively. The reduction product was essentially one compound in each case (glc) and distillation in vacuo gave the pure aziridine. The product from the LAH reduction of (E)-2-(4-dimethylaminobenzylidene)cyclohexanone oxime (63) contained three compounds, present in concentrations of 59%, 24%, and 17% (glc). Fractional distillation did not change the percentage of each of these compounds in the distillate. The nmr spectrum of the distillate showed signals which were characteristic of the other three aziridines and which were assigned to 1-(4-dimethylaminobenzyl)-1,2-epimino-cyclohexane (104), present as the major compound in the reduction mixture. A small quantity of a white solid formed in the reduction

product over a period of three months which was identified as 2-(4-dimethylaminobenzyl)cyclohexylamine. The retention time of this amine was identical to that of the compound representing 24% of the reduction product (glc). The third compound in the reduction product was not identified.

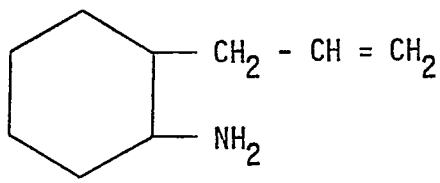
2.3.3 Attempted reduction of (E)-2-benzylidenecyclohexanone oxime with other reducing agents

Other hydride reducing agents were employed in an attempt to get reduction at the ketoxime center of the oxime (60) instead of at the conjugated olefinic bond. However, the attempted reduction of this oxime with sodium borohydride, sodium trimethoxyborohydride, and lithium tri-(t-butoxy)aluminum hydride gave only unreacted oxime.

Booth and King (1958) selectively reduced 2-allycyclohexanone oxime (105) to 2-allylcyclohexylamine (106) using sodium in ethanol.



(105)



(106) 83%

Applying their method to the reduction of the unsubstituted oxime (60) did not result in any acid-soluble product which indicated that no

amine product was formed. The reaction mixture turned black and solidified, probably due to decomposition. The olefinic bond in (60), being part of a conjugated system, would likely be attacked by this reduction system.

Zambito and Howe (1960) reduced the following oxime (107) to the acetamido derivative (108) using zinc in acetic acid and acetic anhydride.

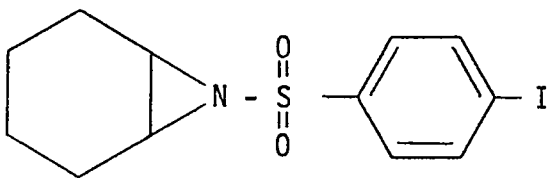
$$HO - N = C(CO_2C_2H_5)_2 \xrightarrow[(CH_3CO)_2O]{Zn \atop CH_3COOH} CH_3CO - NH - CH(CO_2C_2H_5)_2$$

(107) (108)

The attempted reduction of (E)-2-benzylidenecyclohexanone oxime (60) with zinc in glacial acetic acid did not result in any acid-soluble product.

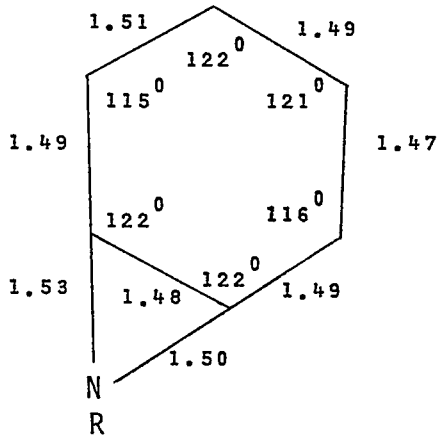
2.3.4 Structure of the aziridines

Some idea of the shape of the epiminocyclohexane portion of the substituted 1-benzyl-1,2-epiminocyclohexanes can be established from the x-ray diffraction studies of Trefonas and Majeste (1965) on 7-(p-iodobenzenesulfonyl)-7-azabicyclo (4.1.0) heptane (109).



(109)

They found that the aziridine ring is fused cis to the cyclohexane ring with fusion angles of 122°. In order to adjust to this widening at the fusion angle, there is a subsequent widening at the back of the cyclohexane ring, resulting in angles of 121° and 121.5°. The cyclohexane ring is flattened appreciably, although still in the chair conformation. The flattening of the cyclohexane ring to accommodate the fusion of the aziridine ring is accompanied by a significant shortening of the bond distances in the cyclohexane ring, resulting in an average value of 1.49 Å for the C - C bond distances. The C - C bond distances in cyclohexane are 1.54 Å and the bond angles are 109° (Hassel and Viervoll, 1947).

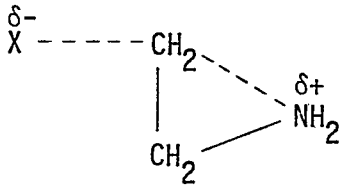


R = p-I-$C_6H_4SO_2$

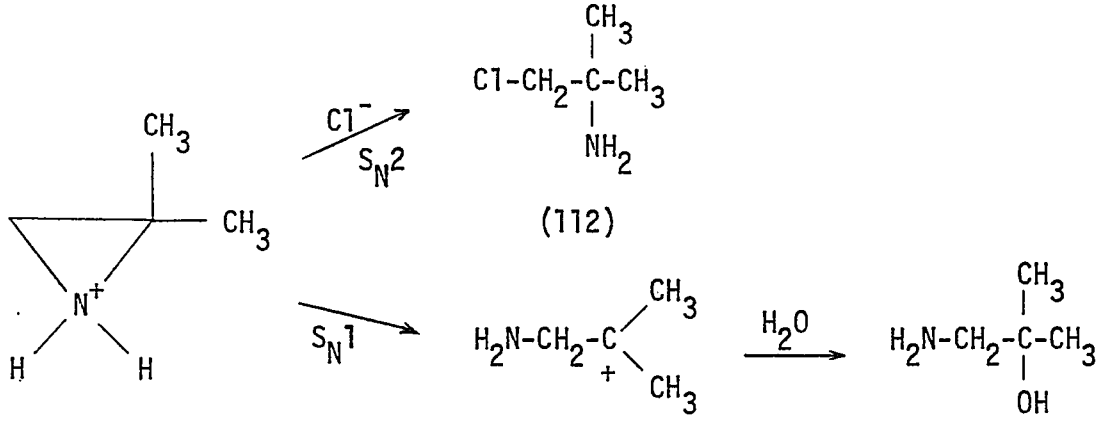
2.3.5 Mechanism of action and biological activity of aziridine derivatives

The mechanism for ring opening of carbon-unsubstituted aziridines by nucleophiles has been established as an S_N2 displacement

at the aziridine carbon atom and may be represented by the reaction of the nucleophile X^- on protonated ethyleneimine (110). For aziridines which possess substituents on carbon, the mechanism for ring-opening reactions is not clearly defined as an S_N1 or an S_N2 type. For example, treatment of 2,2-dimethylaziridine (111) with aqueous HCl leads to 1-methyl-1-(chloromethyl)ethylamine (112) (Fanta and Walsh, 1966) and 1-amino-2-methyl-2-propanol (113) (Schatz and Clapp, 1955). These products can be explained by a mechanism as shown below.



(110)



(111) (112) (113)

Thus it has been suggested that nucleophilic substitutions on aziridines involve competitive S_N1 and S_N2 processes, the latter

being dominant for aziridines with primary carbon atoms, both being important for aziridines with secondary carbon atoms, and the former being the main path for aziridines with tertiary carbon atoms (Buist and Lucas, 1957; Earley et al:, 1958; Schatz and Clapp, 1955).

The broad classification of aziridines into two groups of compounds, activated aziridines and aziridines possessing a basic nitrogen, is based primarily on the reactivity of these compounds toward nucleophilic reagents. Activated aziridines are those derivatives possessing a nitrogen substituent capable of stabilizing the negative charge which is formed on the aziridine nitrogen in the transition state when the compound reacts with a nucleophile. The substituent must be capable of conjugating with the unshared pair of electrons on the aziridine nitrogen, e.g. the carbonyl group of N-acetylaziridine. For a basic aziridine to undergo ring opening with a nucleophile under ordinary conditions, the nitrogen must first be positively charged whereas the activated aziridines readily undergo such ring opening.

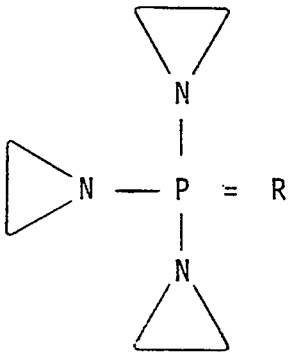
Compounds containing the aziridine ring have profound effects on living cells. Aziridines are very active mutagenic chemicals and are considered to exert their effects by alkylation of some part of parts of the nucleic acid structures involved in cell replication. Ethyleneimine itself has been employed much more than any of its derivatives as a mutagen, and it is one of the most common reagents used by the geneticist to induce mutations. Ethyleneimine and its

simple homologs are too toxic for use in vivo against protists (bacteria, amobae, etc.), but these compounds have been repeatedly suggested for fumigative disinfection.

Aziridine derivatives have a wide range of biological activities which have recently been documented by Dermer and Ham (1969). Aziridines have been patented as germicides and fungicidal activity is described for many aziridine derivatives. A few acylated aziridines have antiviral properties, especially against encephalitis virus. It is established that the antibiotic activity in the mitomycins depends on the presence of the aziridine ring. Another use of aziridines has been as sexual chemosterilants in insects.

The use of aziridines as anticancer drugs has had much more study than other biological effects. Like other alkylating agents, they have greatest palliative value on leukemias and other lymphomas, such as Hodgkin's disease, and are of little value against solid tumors. Considerable similarity is observed between the reactivity of ethyleneimine compounds and the β-halogenoalkylamines (nitrogen mustards). Studies of the kinetics of alkylations by reactive nitrogen mustards strongly support the view that, in aqueous solution, cyclization to an ethyleneimmonium ion precedes the alkylation (Gilman and Philips, 1946). Since the formation of the ethyleneimmonium ion constitutes the initial reaction of the nitrogen mustards, screening programs have been devised to discover useful ethyleneimine derivatives.

These studies have yielded several active compounds, including triethylenemelamine (2), triethylenephosphoramide (114) and triethylenethiophosphoramide (115).

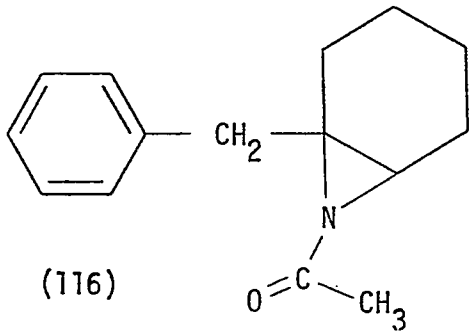


(114) R = O

(115) R = S

The substituted 1-benzyl-1,2-epiminocyclohexanes (70), (102), and (103) prepared in the present study have been dispatched for anticancer screening. It would be of interest to synthesize activated derivatives of these aziridines with N-substituents such as acetyl or benzoyl groups.

2.4 Attempted preparation of the N-acetyl derivative of 1-benzyl-1,2-epiminocyclohexane



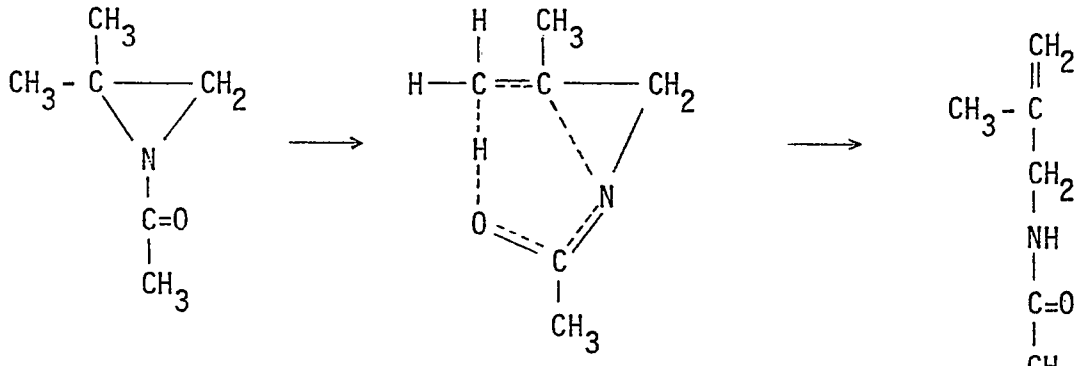
(116)

2.4.1 Pyrolytic cis-elimination of aziridines

Before the structure of the reduction product from (E)-2-benzylidenecyclohexanone oxime (60) had been established as the aziridine (70) the preparation of an N-acetyl derivative of the reduction product was attempted by treatment with acetic anhydride (15 minute reflux). The product isolated from this reaction showed ir absorptions at 3310 cm^{-1} (N-H), 1530 cm^{-1} (Amide II) and 1635 cm^{-1} (Amide I). Nmr spectroscopy indicated the presence of one hydrogen on the nitrogen atom (exchanges with NaOD) and also one olefinic proton was evident. The product did not appear to contain the aziridine ring, and was therefore not the N-acetyl aziridine (116).

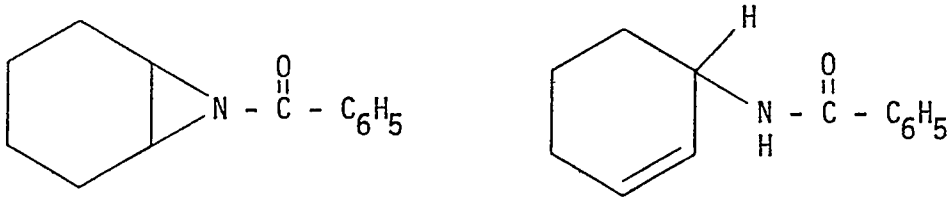
The pyrolytic rearrangement of 1-acetyl-2,2-dimethylethylenimine (117) to give N-(β-methallyl)acetamide (119) was described by Fanta and Deutsch (1958). Evidence was presented that the rearrangement occurred by an intramolecular mechanism similar to the Chugaev

reaction, involving a cyclic transition state (118).



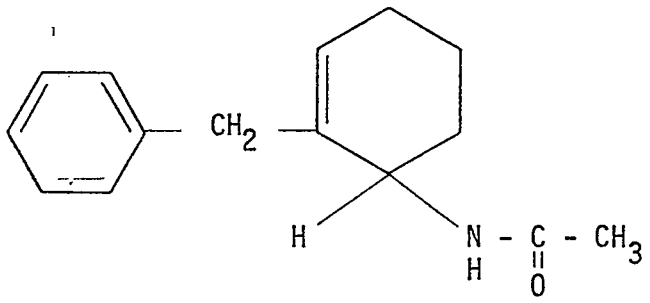
(117) (118) (119)

Since then the thermal isomerization of N-acyl- or N-aroylaziridines to the isomeric N-allylcarboxamides has been the subject of extensive investigation (Kashelikar and Fanta, 1960a, 1960b; Talukdar and Fanta, 1959; Fanta and Walsh, 1965; Fowler and Hassner, 1968). This isomerization has been shown to be stereospecific and has been classified with the well-known group of pyrolytic cis-eliminations (Kashelikar and Fanta, 1960a, 1960b; Fanta and Walsh, 1965). For example Fanta and Walsh (1965) found that N-benzoylcyclohexenimine (120) underwent pyrolysis to the unsaturated amide (121) upon heating in benzene at 200-210°.

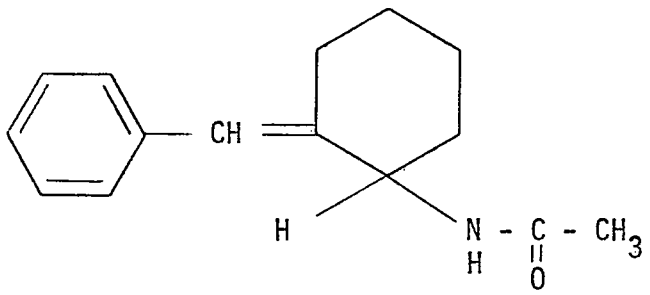


(120) (121)

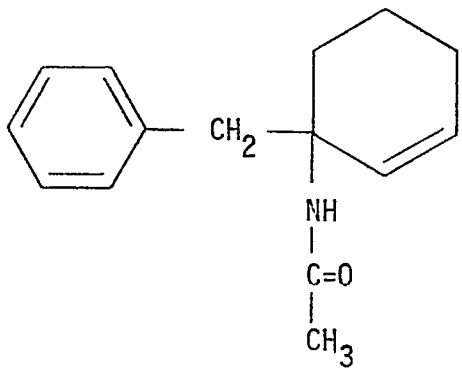
Pyrolytic cis-elimination of the N-acetyl derivative (116) could give the following compounds (122-124). Structure (123) may be eliminated since the nmr spectrum of the product isolated after



(122)



(123)



(124)

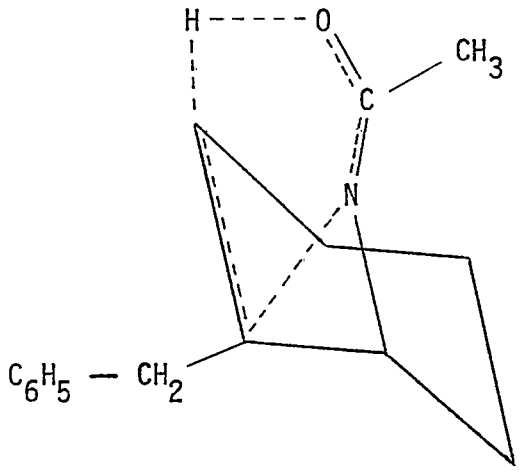
treatment with acetic anhydride showed the presence of the benzyl side chain and uv spectroscopy indicated that styrenoid absorption was absent. The nmr spectrum showed a broad peak at δ 5.77-5.37 integrating for two protons and deuterium exchange with NaOD removed one of these protons, indicating that the amide N-H proton was superimposed on an olefinic proton.

The preparation of the N-acetyl derivative of the deuterated aziridine (96) was also attempted. The nmr spectrum of this N-acetyl derivative was similar to that of the N-acetyl derivative of the non-deuterated aziridine except that the signal at δ 4.40 was absent, which is expected if deuterium is on the bridgehead carbon of the deuterated aziridine. The N-H proton and the olefinic proton were not superimposed in this nmr spectrum; the olefinic proton was present at δ 5.73-5.47 and the N-H proton was present at δ 5.35.

Catalytic hydrogenation of the product from the acetylation of (70) was carried out to saturate any olefinic bonds that were present. The molecular weight of the catalytically-reduced compound was increased by two mass units, indicating the presence of one olefinic bond in the product from the acetylation. The signal at δ 5.77-5.37, present in the nmr spectrum of the acetylated product was reduced to a broad singlet at δ 5.71 in the nmr spectrum of the hydrogenated product. The signal at δ 5.71 disappeared upon the addition of NaOD and was assigned to the amide proton. A comparison of the nmr spectrum

of the acetylated product and the catalytically hydrogenated product indicated that there was only one olefinic proton present in the unsaturated amide formed in the acetylation. This leaves (122) as the correct amide structure.

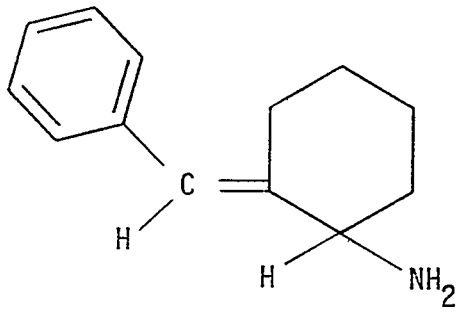
Examination of the Dreiding model of the N-acetyl derivative of the aziridine (116) showed that the carbonyl oxygen could approach the axial hydrogens at C-3 and C-6 of the cyclohexane ring to give cis-elimination. The unsaturated amide (122) isolated follows Saytzeff's rule whereby the most highly substituted olefin is formed. A cyclic transition state (125) for the cis-elimination can be represented as shown:



(125)

2.5 Other attempts to prepare (E)-2-benzylidenecyclohexyl isothiocyanate

2.5.1 Preparation of (E)-2-benzylidenecyclohexylamine by the Leuckart reaction

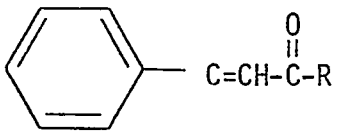


(126)

Since the reduction of the substituted (E)-2-benzylidenecyclohexanone oximes (17) failed to produce the required substituted (E)-2-benzylidenecyclohexylamines (18) it was decided to employ the Leuckart reaction in an attempt to go directly from the substituted (E)-2-benzylidenecyclohexanones (16) to the substituted (E)-2-benzylidenecyclohexylamines (18). The amines (18) could then be converted to the substituted (E)-2-benzylidenecyclohexyl isothiocyanates (19) by the Hofmann mustard oil reaction.

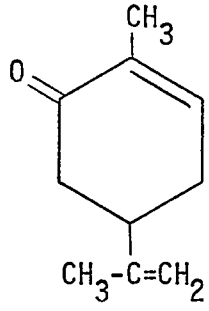
The Leuckart reaction involves the reductive amination of aldehydes or ketones by formamide, ammonium formate, formamide with formic acid, or ammonium formate with formic acid. The reaction is

carried out by heating a mixture of the carbonyl compound and the amide or its derivative, usually in the presence of the reducing agent, formic acid. Primary and secondary amines are obtained as the formyl derivatives whereas tertiary amines are obtained as the formates. However, there are problems associated with undertaking this reaction for α,β-unsaturated ketones. When Ingersoll et al. (1936) applied the Leuckart reaction to benzylideneacetone (127) or to carvone (129) the product obtained was chiefly a neutral resin with only small amounts of the corresponding primary amines. They suggested that the method was unsuitable for application to α,β-unsaturated ketones. Similarly, Walter (1952) did not obtain any primary amine from cinnamaldehyde (128) via the Leuckart reaction.



(127) R = CH_3

(128) R = H



(129)

In the present work, reactions were undertaken with (E)-2-benzylidenecyclohexanone (34), using formamide alone, formamide and

formic acid, concentrated ammonia and formic acid, and ammonium formate with formic acid. In each case a 10% yield of acid-soluble material was isolated from an otherwise resinous reaction mixture. High vacuum distillation gave a colorless syrup in about 1% yield which contained two compounds in concentrations of 85% and 15% as determined by glc analysis. These two compounds are possibly the axial and equatorial amines of (E)-2-benzylidenecyclohexylamine (126). The mass spectrum of the distillate gave a parent peak at m/e 187, assigned to the required amine (126), and the absence of peaks at higher m/e values excluded the presence of secondary amine. The nmr spectrum showed a singlet for the vinylic proton at δ 6.45 and a singlet at δ 1.40 integrating for two protons which was assigned to the primary amine protons (D_2O exchange). The ir spectrum showed absorption peaks at 3296 and 3374 cm^{-1} which were assigned to the N-H stretching vibrations of the primary amine.

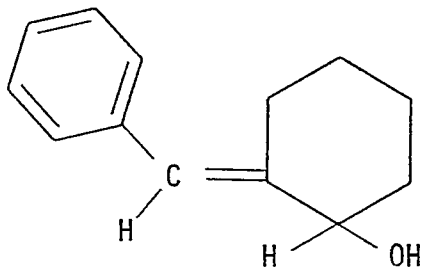
Upon standing the distillate became a colorless water-soluble solid, presumed to be the carbonate salt. It is of interest to note that Booth and King (1958) found that 2-allylcyclohexylamine (106) was rapidly converted into the carbonate on exposure to air.

2.5.2 Attempted preparation of (E)-2-benzylidenecyclohexylamine via an azide

The poor yields of (E)-2-benzylidenecyclohexylamine (126) obtained from the Leuckart reaction were not sufficient to allow the

preparation of (E)-2-benzylidenecyclohexyl isothiocyanate (127) in practical screening quantities. A third route for the synthesis of this isothiocyanate was attempted and is shown in Scheme 9.

2.5.2.1 Preparation of (E)-2-benzylidenecyclohexanol



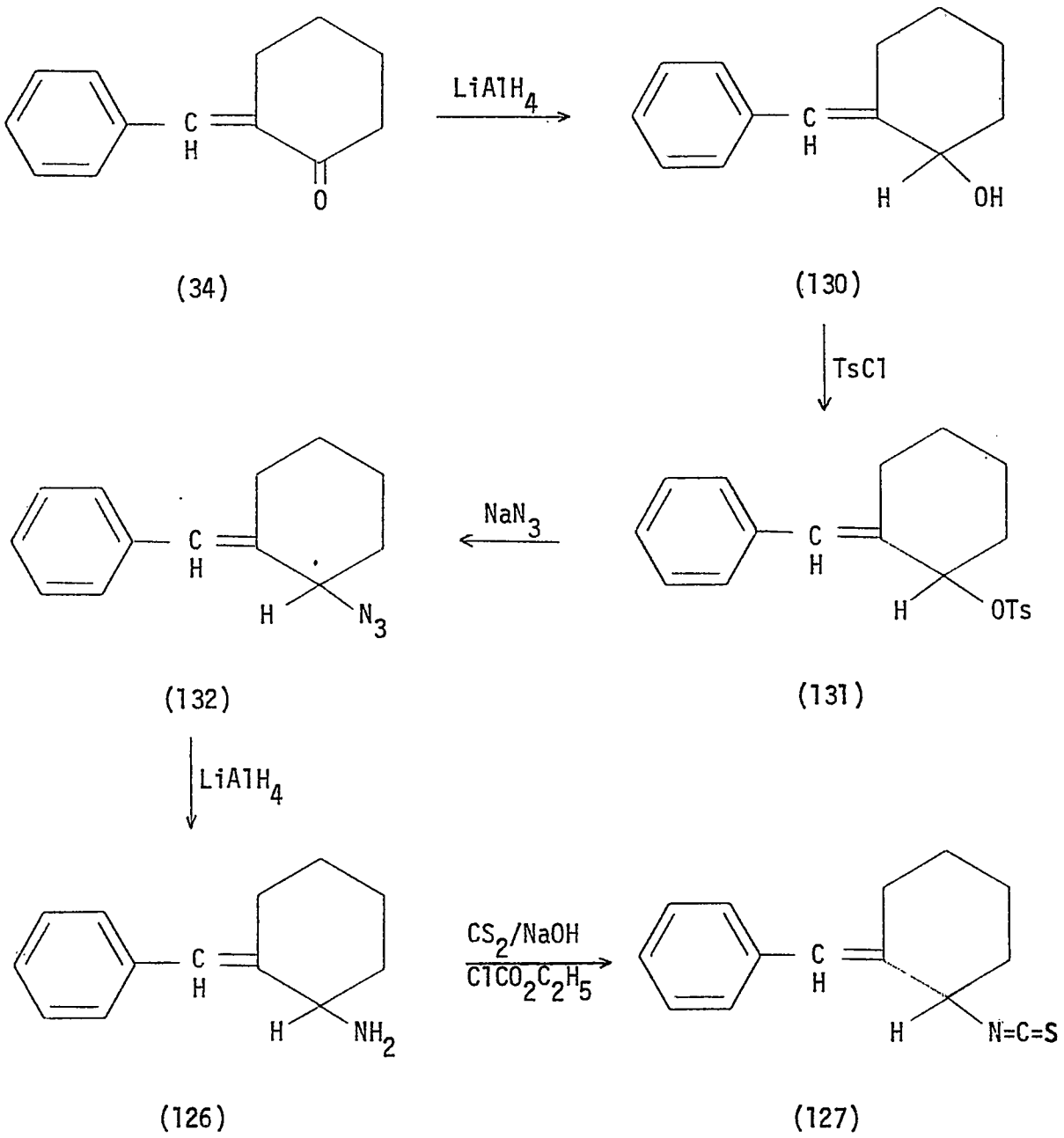
(130)

The first stage in the synthesis depicted in Scheme 9 is the preparation of (E)-2-benzylidenecyclohexanol (130) which is an allylic alcohol, a class of compounds with a wide range of biological activities. Allyl alcohol (133) has a mild bactericidal action against staphylococci, streptococci, proteus, typhoid, and colon bacilli (Morel et al., 1937). Uricchio (1951) found that allyl alcohol was a toxic

$$CH_2 = CH - CH_2OH$$

(133)

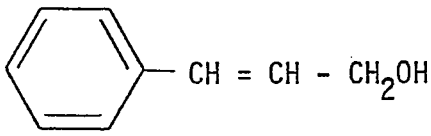
nematocide whereas the saturated analog, propyl alcohol, was relatively ineffective. Corden and Young (1962) found that allyl alcohol was a soil fungicide and it also has been reported to have general soil disinfecting properties (Mikovski and Rosa, 1960) and pesticidal properties for soil-borne pests (Darby et al., 1962).



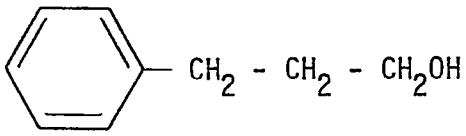
(34) (130) (131) (132) (126) (127)

Scheme 9 Proposed route for the synthesis of (E)-2-benzylidenecyclohexyl isothiocyanate.

Cinnamyl alcohol (134) has also been found to have bactericidal activity, which was much greater than that of the saturated alcohol, hydrocinnamyl alcohol (135) (Bose et al., 1949). Thus, the presence of an α,β double bond is again shown to enhance biological activity. Okazaki and Oshima (1953) reported that cinnamyl alcohol was effective against fungi, and had some activity against tubercle bacteria.



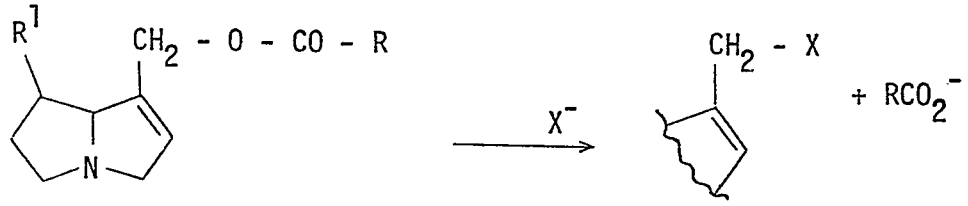
(134)



(135)

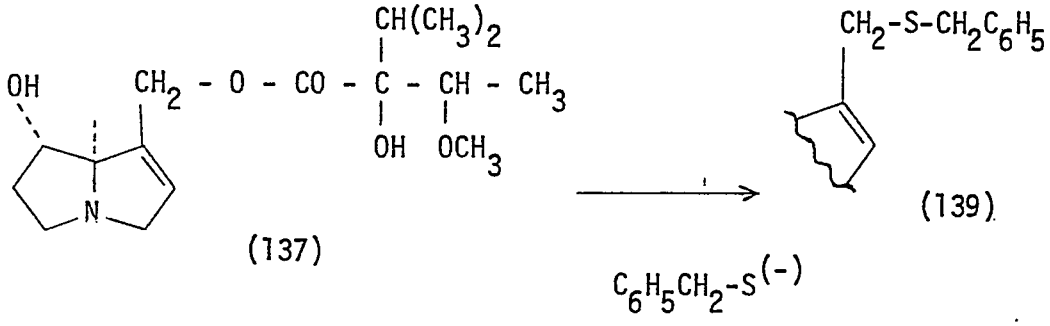
Culvenor and coworkers (1962) suggested that the effects of the pyrrolizidine alkaloids on cell nuclei are due primarily to their ability to act in the cell as alkylating agents. The alkaloids, represented by (136), are allylic esters which may be able to function as alkylating agents by a mechanism involving alkyl-oxygen fission of the ester linkage, which would result in displacement of the anion RCO_2^- by a nucleophilic agent X^-. For example, heliotrine (137) reacts with the benzyl mercaptan anion (138) to give a high yield of the sulphide (139). This reaction also occurs with cysteine. Extension

of this reaction to sulfhydryl groupings in enzymes and other cell constituents is expected.



(136) R^1 = H, OH, or O-acyl

R = alkyl



(137)

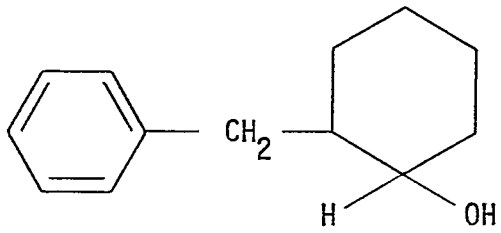
(139)

$C_6H_5CH_2-S^{(-)}$

(138)

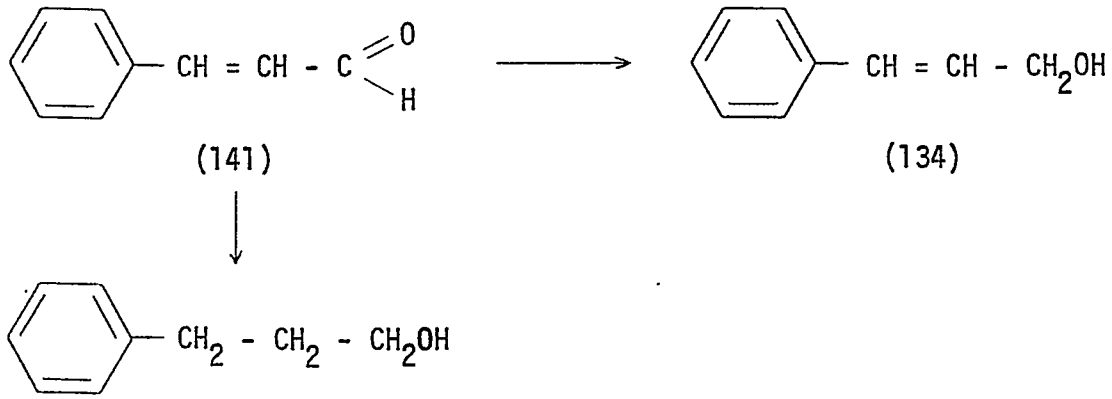
Russell (1954) reported an almost quantitative yield of (E)-2-benzylidenecyclohexanol (130) from the LAH reduction of (E)-2-benzylidenecyclohexanone (34). In the present investigation, the product from the LAH reduction of (E)-2-benzylidenecyclohexanone (Product A) appeared to contain two compounds, in concentrations of 98% and 2%, as determined by glc analysis. Fractional distillation in vacuo and repeated recrystallizations of Product A did not change the percentages of the two compounds in the mixture. The major component of Product A was identified as (E)-2-benzylidenecyclohexanol (130). The nmr spectrum

of Product A showed a signal for the vinylic proton at δ 6.55 and the uv spectrum gave a styrenoid-type absorption at a uv max of 244 mμ (ε14,560). The minor component of the reduction mixture was suspected to be the saturated alcohol, 2-benzylcyclohexanol (140).



(140)

Hochstein and Brown (1948) found that the normal reduction procedure with LAH, as applied to cinnamaldehyde (141), furnished hydrocinnamyl alcohol (135) whereas the reverse mode of addition, carried out by adding the calculated amount of hydride solution to a solution of cinnamaldehyde (141) at a temperature of -10^{o}, resulted in a good yield of cinnamyl alcohol (134).



(141) (134)

(35)

LAH reduction of (E)-2-benzylidenecyclohexanone (34) by the inverse addition procedure used to prepare cinnamyl alcohol (Hochstein and Brown, 1948) gave a product with the same glc analysis as that for Product A obtained from the normal reduction procedure.

LAH is often viewed as a less selective reducing reagent than sodium borohydride. However, Johnson and Rickborn (1970) found that reduction of a conjugated double bond was a substantial competing process in the sodium borohydride reduction of both cyclic and acyclic α,β-unsaturated ketones. Sodium borohydride reduction of the E ketone (34) gave a product with a composition similar to that of Product A obtained from LAH reduction (glc).

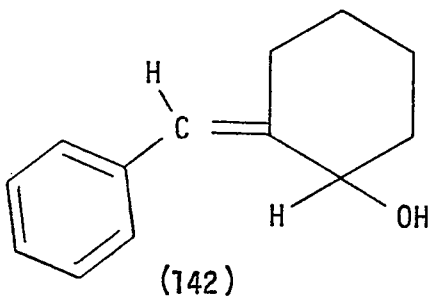
Jackson and Zurquyah (1965) found that attack at the carbonyl carbon atom (1,2-addition) was very much preferred to 1,4-addition when they reduced various α,β-unsaturated ketones with sodium trimethoxyborohydride in tetrahydrofuran solution. Reduction of (E)-2-benzylidenecyclohexanone (34) with sodium trimethoxyborohydride in tetrahydrofuran gave a product with the same composition as that from LAH reduction or sodium borohydride reduction. Thus, in the hydride reduction of the E ketone (34) the olefinic bond is stable, resulting in 1,2-addition of the hydride rather than 1,4-addition.

Catalytic hydrogenation of Product A gave 2-benzylcyclohexanol (140) which did not have a retention time similar to that of the minor component of Product A.

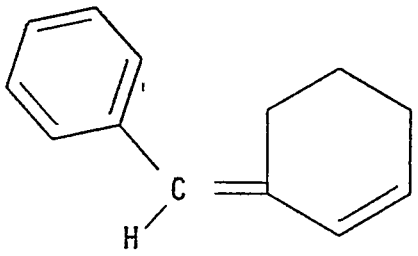
The minor component in Product A was shown not to be the photoisomer, (Z)-2-benzylidenecyclohexanol (142) by comparison

of the retention time of the minor component in Product A with the retention time of an authentic sample of (Z)-2-benzylidenecyclohexanol (142), prepared by the LAH reduction of (Z)-2-benzylidenecyclohexanone (56) (glc analysis).

It was suspected that the minor component detected in Product A was (E)-3-benzylidene-1-cyclohexene (143), formed by the dehydration of (E)-2-benzylidenecyclohexanol on the glc column.



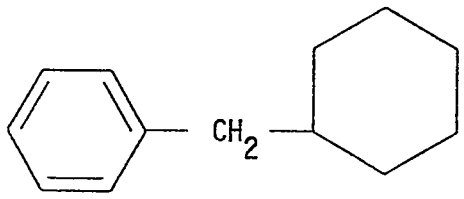
(142)



(143)

The retention time of the minor component in Product A was identical to the retention time of a sample of (E)-3-benzylidene-1-cyclohexene (143), prepared by the acid-catalyzed dehydration of (E)-2-benzylidenecyclohexanol (130). Isothermal temperature determinations ranging from 140° to 210° with the gas-liquid chromatograph did not change the percentage of the minor component in Product A. If dehydration of the E alcohol (130) was occurring on the glc column, it is expected that higher column temperatures would result in more of the elimination product (143). Thus it appeared that (E)-3-benzylidene-1-cyclohexene (143) was actually present as 2% of the product from the LAH reduction

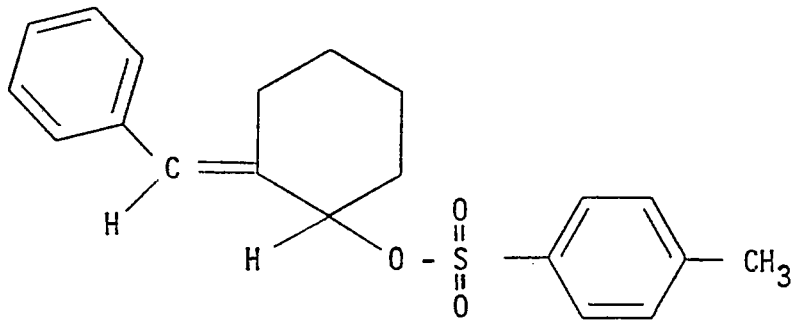
of (E)-2-benzylidenecyclohexanone (34). However, catalytic hydrogenation of this product gave only one compound, 2-benzylcyclohexanol (140). The presence of traces of benzylcyclohexane (144) might be expected.



(144)

(E)-2-benzylidenecyclohexanol (130) and (Z)-2-benzylidenecyclohexanol (142) were both sent for anticancer screening. If the alcohols are found to have activity, then the synthesis of various esters would be of interest, since the esters would represent the latent form of the active alcohol. Enzymatic hydrolysis of the esters *in vivo* would release the active alcohols. Reaction of allylic esters by alkyl-oxygen fission of the ester linkage (Culvenor *et al.*, 1962) is also an interesting concept.

2.5.2.2 Attempted preparation of (E)-2-benzylidenecyclohexyl p-toluenesulfonate

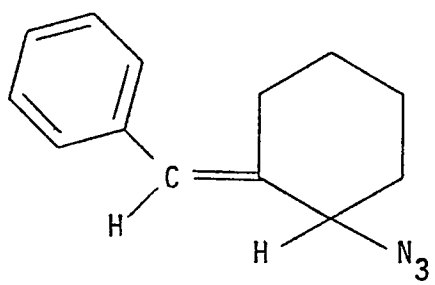


(131)

(E)-2-benzylidenecyclohexanol (130) did not react with p-toluenesulfonyl chloride in the cold (4^{o}), at room temperature, or in a refluxing ether solution. Presumeably the chemical nature of the alcohol is not the reason for the lack of reactivity. Cinnamyl alcohol (134) has been found to undergo almost complete tosylation in two hours when reacted with p-toluenesulfonyl chloride, (Mesnard and coworkers, 1963). The lack of reactivity of (130) is probably due to steric hindrance.

It was decided to prepare the lithium salt of (E)-2-benzylidenecyclohexanol which should be much more reactive with p-toluenesulfonyl chloride but in this case no (E)-2-benzylidenecyclohexyl p-toluenesulfonate (131) was obtained. The major product was identified as (E)-3-benzylidene-1-cyclohexene (143). It would appear that the tosylate (131) did indeed form but was so unstable that it immediately underwent detosylation, giving the elimination product (143). A number of tosylates, particularly unsaturated ones, are reported to decompose in a few hours at 25^{o} (Fieser and Fieser, 1967). Four other unidentified compounds were found in the product, present in concentrations of 12%, 4%, 2%, and 1% (glc analysis).

2.5.2.3 Attempted preparation of (E)-2-benzylidenecyclohexyl azide



(132)

The product from the attempted preparation of (E)-2-benzylidenecyclohexyl p-toluenesulfonate contained four unidentified compounds, one of which could have been the desired tosylate (131). However, reaction of this product with sodium azide in an attempt to prepare (E)-2-benzylidenecyclohexyl azide (132) by a method similar to that used by Bose and coworkers (1962) did not change the composition of the resulting product (glc). It appeared that no tosylate (131) was present in the product mixture from the attempted preparation of the tosylate.

3. DISCUSSION OF THE SCREENING RESULTS

3.1 Introduction

Most of the compounds synthesized in this project were submitted to the Drug Research and Development branch of the National Cancer Institute at Bethesda, Maryland, U.S.A. The Cancer Chemotherapy National Service Center changed its name to Drug Research and Development, effective April, 1971. The Cancer Chemotherapy National Service Center was established in 1955 to provide assistance to investigators in cancer chemotherapy in areas such as drug screening, material resources, and information exchange. Over a period of years, the program evolved into a highly integrated program of drug development which comprises a major component of the pre-clinical chemotherapy research of the National Cancer Institute.

Drug Research and Development is composed of three branches, Drug Development, Drug Evaluation, and Program Analysis, all of which are responsible for seeking and developing new and potentially more effective anti-tumor agents. In addition, Drug Research and Development initiates new and better methods for discovering and evaluating novel drugs, and for devising better ways of using active anti-tumor compounds which are currently available. Its activities are carried out through intramural laboratory operations, and through

research and development contracts with pharmaceutical and chemical companies, research institutes, and academic institutions.

The structures of the compounds submitted for screening, with the relevant code numbers, are shown in Table VIII and Table IX, and screening results are shown in Table X and Table XI.

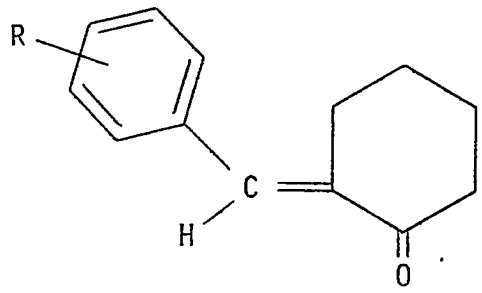
In addition several compounds were sent to Smith Kline and French Laboratories, Philadelphia, U.S.A. for antiviral screening and other selected pharmacological screens.

3.2 Screen against L-1210 lymphoid leukemia

Ascitic fluid (0.1 ml containing 10^5 cells) is injected intraperitoneally in mice (BDF_1 strain) and treatment is begun 24 hours after the injection, with one dose daily intraperitoneally for 30 days. All survivors are sacrificed on day 30. Control animals are treated with the vehicle used for dissolving or suspending the test compounds, the volume being equal to that used for the majority of the test groups.

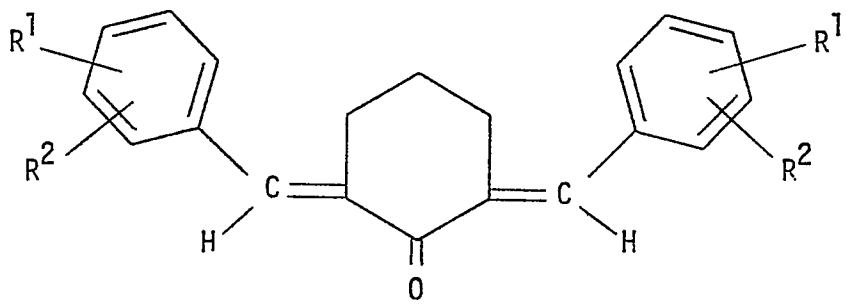
The mean survival time of test animals is compared with that of control animals and the results are expressed as a percentage of control survival time. A T/C value for L-1210 tests is the mean survival time (in days) of the test group divided by the mean survival time of the control group, expressed as a percentage. A T/C $\geqslant$ 125% is the criterion for a synthetic compound to pass stage 1 of the

Table VIII Structures of compounds for which screening results are available (Series 1)



N. C. Number	R
101	H
102	4-$N(CH_3)_2$
104	2-Cl
105	4-Cl
106	4-$\overset{+}{N}(CH_3)_3$ I^-

Table IX Structures of compounds for which screening results are available (Series 2)



N. C. Number	R^1	R^2
103	4-$N(CH_3)_2$	H
107	2-Cl	6-Cl

sequential screening. This value represents an increase of 25% in the mean survival time of test animals over control animals. To pass stage 2 of the sequential screen, a material must produce an effect such that the product of the T/C values of the 2 tests (expressed as a decimal) is $\geqslant$ 1.56.

The confirmation procedure consists of a dose-response experiment at doses both higher and lower than the dose at which the sequential tests were done. Dose-response is determined at four doses: 3/2 X, X, 2/3 X, and 4/9 X, where X is the dose at which the sequential tests were carried out. A dose response with a T/C $\geqslant$ 125% for at least one dose is the criterion for a synthetic compound to pass the confirmation test.

Deaths occurring during the first 5 days of injections for the L-1210 test system are considered toxic deaths. A maximum tolerated dose for a single experiment is defined as the highest dose which produces 2 or fewer deaths in 6 animals or 3 or fewer deaths among 10 animals. When a toxic result (greater than 2/6 or 3/10 deaths) is observed, the test is repeated at an appropriately lower dose until the maximum tolerated dose is reached. If the ratio T/C is less than 85%, the dose is considered toxic and retesting at one-half the dose is carried out.

A reproducible increase in survival of at least 50% is the criterion for acceptance of a material as a potential candidate for clinical evaluation.

Table X *In vivo* screening results against L-1210 lymphoid leukemia

Sample Number	Injection interval (days)	Number of Injections	Dose (mg/kg)	Survivors ( ) of ( )		Mean survival time		Percent (T/C)
						Test	Control	
	4	3	400	6	6	9.3	8.9	104
N.C. 101	4	3	200	6	6	9.3	8.9	104
	4	3	100	6	6	8.7	8.9	97
	1	9	400	6	6	8.7	8.5	102
N.C. 101	1	9	200	6	6	8.7	8.5	102
	1	9	100	6	6	8.7	8.5	102
	4	3	400	6	6	9.2	8.9	103
N.C. 102	4	3	200	6	6	9.2	8.9	103
	4	3	100	6	6	8.3	8.9	93
	1	9	400	6	6	8.2	8.5	96
N.C. 102	1	9	200	6	6	9.3	8.5	109
	1	9	100	5	5	8.4	8.5	98
	4	3	400	6	6	8.7	8.9	97
N.C. 104	4	3	200	6	6	9.3	8.9	104
	4	3	100	6	6	9.3	8.9	104
	1	9	400	6	6	8.5	8.5	100
N.C. 104	1	9	200	5	6	9.0	8.5	105
	1	9	100	6	6	8.3	8.5	97

Table X (contd.)

	4	3	400	6	6	9.2	8.7	105
N.C. 105	4	3	200	6	6	9.3	8.7	106
	4	3	100	6	6	9.0	8.7	103
	1	9	400	6	6	8.7	8.6	101
N.C. 105	1	9	200	6	6	8.5	8.6	98
	1	9	100	6	6	8.2	8.6	95
	4	3	400	0	6	0.0	8.7	
N.C. 106	4	3	200	0	6	0.0	8.7	
	4	3	100	2	6	9.0	8.7	
	1	9	50.0	6	6	8.8	8.5	103
N.C. 106	1	9	25.0	6	6	8.5	8.5	100
	1	9	12.5	6	6	10.0	8.5	117
	4	3	50.0	6	6	9.8	9.2	106
N.C. 106	4	3	25.0	6	6	8.5	9.2	92
	4	3	12.5	6	6	9.0	9.2	97
	4	3	400	5	5	8.8	8.7	101
N.C. 107	4	3	200	6	6	8.8	8.7	101
	4	3	100	6	6	9.7	8.7	111
	1	9	400	6	6	7.8	8.6	90
N.C. 107	1	9	200	6	6	9.3	8.6	108
	1	9	100	6	6	9.0	8.6	104

None of the compounds screened against the L-1210 lymphoid leukemia had activity significant enough for further testing. N.C. 107 had a T/C value of 111% at a dose of 100 mg/kg in one test but lower T/C values were found at this dosage when the test was repeated. N.C. 106 was toxic at doses of 400, 200, and 100 mg/kg. Testing at 50.0, 25.0, and 12.5 mg/kg reduced the toxicity and a T/C of 117% was obtained at a daily dose level of 12.5 mg/kg. This is less than the T/C of 125% required for further testing.

Klucel was used as the vehicle for all compounds except N.C. 106 which was dissolved in saline. Three dose levels per injection were used, namely 400, 200, and 100 mg/kg and two screens for each compound were carried out. In the first screen the interval between injections was 4 days, and there were 3 injections. In the second screen an injection was made daily, with a total of 9 injections. There was no significant difference between these two screens for each compound except at the 12.5 mg/kg dose level for N.C. 106 which gave a much higher T/C value when one injection was made daily for 9 days.

3.3 Screen against the KB (Eagle) cell culture

The KB cell culture is derived from a human carcinoma of the nasopharynx. It was selected as a general *in vitro* screen because of its rapid and reproducible growth as a monolayer culture.

Stock cultures on glass are cultivated on basal medium (Eagle) plus 10% serum derived from calves, humans, or other suitable sources.

The cells are maintained in a state of rapid growth by frequent subculture, generally every 3 or 4 days. The cultures are refed 24 hours before use on a test. A suspension of cells is diluted to a concentration of 10-20 µg of cell protein per ml. Approximately 3.9 ml of the cell suspension is added to each culture to which 0.1 ml of drug solution or suitable control material has already been added. The test is ended after 72 hours.

The determination of cytotoxicity is based on the inhibition of cell protein synthesis. Measurements include the initial protein per tube (C_0), the final protein in control tubes (C), and the final protein in drug-treated tubes (T). The equivalent of a T/C value, in this case $(T - C_0)/(C - C_0)$ is determined at each dose level. Routine testing of compounds is done at 100, 10, and 1 µg/ml. It is assumed that this response varies linearly with the log of the concentration of the candidate drug and is a straight line, within defined limits of response. A computer-calculated ED_{50}, i.e. the dose which inhibits protein synthesis to 50% of the control growth, is the value recorded.

A two-stage testing system was initially chosen. A synthetic material passed the first stage if the ED_{50} was $\leqslant$ 6 µg/ml. In order to pass the second stage, a compound required an arithmetic mean from the first 2 tests of $\leqslant$ 4 µg/ml. Confirmation at a second laboratory with $ED_{50} \leqslant$ 4 µg/ml. was required. These criteria have been tightened to select materials with an $ED_{50} \leqslant$ 1 µg/ml. for retesting at lower concentrations.

Table XI shows the ED_{50} values for the compounds in Series 1 and 2 which were screened against the KB cell culture. N.C. 101 had an ED_{50} of 2.5×10^{-1} µg/ml, which passed stage 2 of the sequential screen. The other compounds are classified as non-toxic and inactive.

Table XI <u>In vitro</u> screening results against the KB cell culture

Sample	Vehicle		ED_{50} (µg/ml)
N.C. 101	D	L	1.0×10
N.C. 101	D		2.5×10^{-1}
N.C. 102	D	M	1.0×10^{2}
N.C. 104	7		2.0×10
N.C. 105	7		2.1×10
N.C. 106	D		4.9×10
N.C. 107	D	M	1.0×10^{2}

<u>Vehicle</u>

D = Alcohol

7 = Not specified

L = less than

M = more than

3.4 Screen against adjuvant-induced arthritis

Adjuvant arthritis is produced by a single intradermal injection of 0.75 mg of M. butyricum suspended in white paraffin oil into a hindpaw (left) footpad of a male Wistar Purina rat. The injected leg becomes inflamed which causes an increase in volume of the leg, with the leg reaching maximal size within 3-5 days (primary lesion). The animal exhibits a decrease in body weight gain during this initial period. The adjuvant arthritis (secondary lesion) occurs after a delay of approximately 10 days and is characterized by inflammation of the non-injected sites (right hind leg), decrease in body weight, and further increases in the volume of the injected hind leg. Test compounds are orally administered daily in 0.5% tragacanth (10 ml/kg body weight), beginning on the day of the adjuvant injection and continuing for 17 days, exclusive of days 4, 5, 11, and 12. Drug activity on the primary lesion (left leg) is determined on day 3 and drug activity on the secondary lesions (both legs) is determined on or after day 10 by comparing leg volumes of the treated group with a control arthritic (vehicle) group. Hind leg volumes are measured by immersing the leg into a mercury reservoir and recording the subsequent mercury displacement.

A compound is considered to have antiarthritic activity if it produces a statistically significant decrease in the inflamed hind leg volumes of the treated groups when compared with arthritic controls. Body weight changes from day 0 are also statistically compared to the arthritic control group. The compounds tested had their activity

compared to that of prednisolone, a steroid of known antiarthritic activity.

Neither NC 107 nor NC 105 had any antiarthritic activity upon the primary lesion or the secondary lesion. No increase or decrease in body weight was observed in the test animals, compared to the arthritic control group.

3.5 Antiobesity screen

Charles River rats (260 - 280 g) are trained over a 2 week period to eat their daily food ration of ground Purina Laboratory Chow in only 5 hours. After training, either vehicle (controls) or drug is administered orally by gastric intubation daily for 5 days. One hour after drug administration, tared food cups containing ground Purina Lab Chow are presented. The cups are weighed after 1 hour of feeding and again after 5 hours of feeding to determine the effect of the drug(s) on food consumption. Water is available ad libitum. Rats are weighed daily before drug administration. Significant activity is defined as a statistically significant reduction in 5 hour food consumption and/or a significant loss in body weight over the 5 day test.

Oral administration of 50.0 mg/kg of NC 102 or NC 103 (in 10 ml/kg of 5% PEG 400 - 95% 0.75% METHOCEL) to Purina-fed male rats daily for 5 days failed to produce a significant reduction in 1 or 5 hour food consumption. There was no significant change in body weight gain compared to controls over the 5 day test period.

3.6 Antiviral screen

Compounds are first checked for lethal toxicity. Three mice are given six 25 mg/kg subcutaneous doses of a test compound (2 doses per day for 3 days). Animals are observed for a 7 day period for death, weight loss, or overt illness. Only compounds passing these toxicity tests are tested against 3 viral infections. Using a group of 10 mice for each infection, all 10 animals in one group are inoculated with an LD_{90} to LD_{95} dose of one of the viruses. The dose of each test compound for each viral infection is 25 mg/kg subcutaneously, given twice daily starting 3 hours before inoculation with the virus and continuing for 3 days (4 days for influenza infection). The viral infections and the duration of the tests for each is as follows:

1. Influenza A-2 (14 days)
2. Herpex Simplex (12 days)
3. Coxsackie B-1 (10 days)

The criterion for activity is a 30% or greater increase in the mean survival time.

To date no antiviral screening results have been received from Smith Kline and French Laboratories.

3.7 Gastric acid secretion

Male Charles River rats (355 - 410 g) are deprived of food with free access to water 18 hours before testing. Under ether anesthesia, a mid-line incision is made and the pyloric portion of the stomach is

exposed and ligated. The incision is closed with wound clips and the animal is permitted to recover. Two hours following pyloric ligation, the animals are sacrificed by a blow on the head, the stomach is removed, and the gastric contents are collected in a graduated centrifuge tube. After centrifugation, the volume and pH of the gastric juice are determined.

A compound is considered active if it produces a statistically significant increase in pH, or a statistically significant decrease in gastric juice volume.

A single oral administration of 50.0 mg/kg of NC 103 (suspended in 1 ml/kg of water) was made 1 hour before the pyloric ligation. No significant change occured in the pH or volume of the gastric juice.

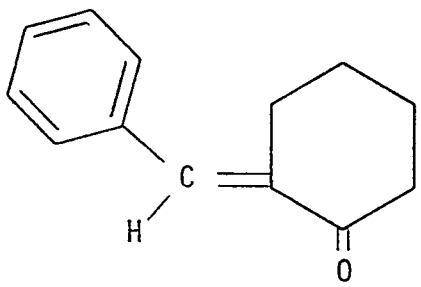
3.8 General test for pharmacological effects

A single oral dose of 50.0 mg/kg of NC 102 or NC 103 (in 5% PEG 400 - 95% 0.75% METHOCEL) was administered to male Charles River rats. All rats survived the test, with no apparent pharmacological effects, the animals appearing normal on the day of administration, 24 hours later, and 7 days later.

4. DESCRIPTION OF THE EXPERIMENTAL WORK

4.1 Preparation of substituted 2-benzylidenecyclohexanones

4.1.1 Preparation of (E)-2-benzylidenecyclohexanone

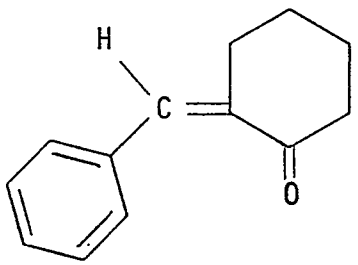


A mixture of cyclohexanone (593 g, 6.04 mol), benzaldehyde (320 g, 3.02 mol), sodium hydroxide (60.5 g, 1.51 mol) and water (1500 ml) was heated at 110° with stirring for 6 hr, and was then stirred at room temperature for 12 hr. The reaction mixture was extracted with ether, the combined ether extracts washed with water, and dried ($MgSO_4$). Removal of the ether under reduced pressure gave a viscous orange syrup which was distilled at 122° and 1.0 mm to give a yellow syrup (414 g). Upon cooling the distillate solidified to a bright yellow solid which contained 2 compounds in concentrations of 0.8% and 99.2% (glc). Recrystallization of this solid from Skelly F or methanol gave pure (E)-2-benzylidenecyclohexanone (348 g, 62%): mp 55° (Durr, 1953, quotes mp 55°, from toluene); uv max 290 mμ (ε14,725) and 224 mμ (ε4,350); ir 1600 (C=C) and 1675 m^{-1} (C=O); nmr δ7.43(t, 1, J =2Hz, C_6H_5-CH=),

7.50 - 7.13 (m, 5, $C_6\underline{H}_5$), 3.00 - 2.67 (m, 2, $C^3\underline{H}_2$), 2.67 - 2.37 (m, 2, $C^6\underline{H}_2$), 2.17 - 1.47 (m, 4, $C^4\underline{H}_2$, $C^5\underline{H}_2$); mass spectrum m/e (rel intensity) 186 (M^+)(100), 185 (100), 158 (18), 157 (9), 143 (11), 130 (32), 129 (46), 128 (27), 127 (10), 117 (36), 115 (60), 102 (17), 91 (24), 77 (12), 67 (39).

Anal. Calcd for $C_{13}H_{14}O$: C, 83.83; H, 7.52. Found: C, 83.77; H, 7.54.

4.1.2 Preparation of (Z)-2-benzylidenecyclohexanone



A solution of (E)-2-benzylidenecyclohexanone (20.0 g) in methanol (300 ml) was irradiated with a Hanovia 450 watt UV lamp (type 679A36) through quartz. The solution was cooled by a water jacket during the irradiation. Aliquot samples were taken at 1 hr intervals and the course of the reaction was followed by glc analysis. After 1 hr irradiation the solution contained 43% of the Z isomer and after 2 hr irradiation the Z isomer represented 69% of the reaction solution. After 3 hr the solution contained 82% of the Z isomer, and irradiation was continued for a further 5 hr, at which time the Z isomer constituted 88% of the reaction mixture, together with 12% of the E isomer. The solvent was removed under reduced pressure and the residual orange liquid was distilled at 122-130° and 2.0 mm to give a yellow syrup (18.3 g)

which contained 94% of the Z isomer and 6% of the E isomer. Crystallization of the distillate from Skelly F gave pure (Z)-2-benzylidenecyclohexanone (12.0 g, 60%) as colorless crystals: mp 37-38°; uv max 272 mμ (ε5,575) and 222 mμ (ε7,900); ir 1631 (C=C) and 1695 cm^{-1} (C=O); nmr δ 7.47-7.07 (m, 5, $C_6\underline{H}_5$), 6.37 (t, 1, J = 2Hz, C_6H_5-C$\underline{H}$ =), 2.87-2.23 (m, 4, $C^3\underline{H}_2$, $C^6\underline{H}_2$), 2.23-1.57 (m, 4, $C^4\underline{H}_2$, $C^5\underline{H}_2$); mass spectrum m/e (rel intensity) 186 (M^+)(98), 185 (100), 158 (15), 157 (8), 143 (8), 130 (31), 129 (40), 128 (22), 127 (8), 117 (37), 115 (44), 102 (17), 91 (28), 77 (13), 67 (61).

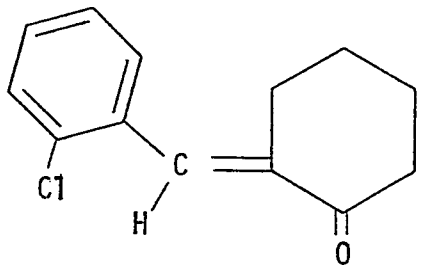
Anal. Calcd for $C_{13}H_{14}O$: C, 83.83; H, 7.52. Found: C, 83.90; H, 7.61.

Glc analysis indicated that (Z)-2-benzylidenecyclohexanone had the same retention time as the compound present in a concentration of 0.8% in the distillate of 4.1.1.

A rapid, short path distillation of the crude (Z)-2-benzylidenecyclohexanone was necessary because it reverts to (E)-2-benzylidenecyclohexanone on prolonged heating. A sample of the Z isomer which was heated at 120° for 18 hr was converted into the crude E isomer (98% E isomer and 2% Z isomer by glc analysis). Crystallization from methanol gave pure (E)-2-benzylidenecyclohexanone.

A 1% w/v solution of (E)-2-benzylidenecyclohexanone in chloroform which was allowed to stand for 3 months in diffuse room light contained 55% of the Z isomer and 45% of the E isomer (glc).

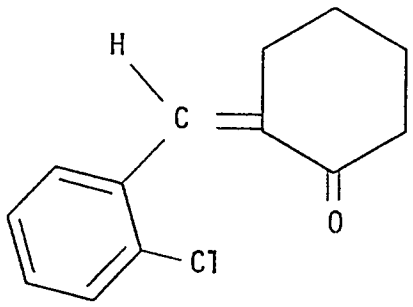
4.1.3 Preparation of (E)-2-(2-chlorobenzylidene)cyclohexanone



A mixture of cyclohexanone (405 g, 4.12 mol), 2-chlorobenzaldehyde (288 g, 2.05 mol), and aqueous sodium hydroxide solution (1 N, 2050 ml) was heated under reflux at 130° with stirring for 20 hr. The cooled reaction mixture was extracted with chloroform. The chloroform extracts were washed with water, dried ($MgSO_4$), and evaporated under reduced pressure to give a viscous dark orange syrup (440 g). Distillation of the crude product at 128° and 1.0 mm gave a bright yellow oil (193 g) which contained 2 compounds (5% and 95%) (glc). The distillate solidified upon cooling. Repeated recrystallization from methanol gave pure (E)-2-(2-chlorobenzylidene)cyclohexanone (120 g, 26%): mp 70.0-70.5° (Campbell and Gilow, 1960, quote mp 69.5-70.5°); uv max 278 mμ (ε10,940) and 228 mμ (ε7,585); ir 1604 (C=C) and 1676 cm^{-1} (C=O); nmr δ 7.50 (t, 1, J = 2Hz, C_6H_4Cl-CH =), 7.47-7.07 (m, 4, C_6H_4Cl), 2.83-2.30 (m, 4, C^3H_2 , C^6H_2), 2.20-1.43 (m, 4, C^4H_2, C^5H_2); mass spectrum m/e (rel intensity) 220 (M^+)(3), 185 (100), 129 (17), 128 (15), 127 (9), 115 (15), 67 (11).

Anal. Calcd for $C_{13}H_{13}ClO$: C, 70.75; H, 5.94. Found: C, 70.63; H, 5.89.

4.1.4 Attempted preparation of (Z)-2-(2-chlorobenzylidene)cyclohexanone



A solution of (E)-2-(2-chlorobenzylidene)cyclohexanone (20.0 g) in methanol (300 ml) was irradiated with ultraviolet light as described previously (4.1.2). After 5.5 hr of irradiation, the reaction mixture had changed color from yellow to green and was shown to contain two main components plus a minor constituent (glc). The original ketone constituted 45% of the reaction mixture and a new compound (presumably the Z ketone), with a shorter retention time, constituted 51%. The reaction solution was irradiated for a further 18.5 hr and was then allowed to stand at room temperature for 20 hr. Removal of the methanol under reduced pressure at 40^{o} gave a brown liquid which solidified upon standing. Glc analysis of this product showed 88% of the E ketone and 1% of the Z ketone plus four other minor constituents. The aliquot after 5.5 hr irradiation showed 92% of the E ketone and 3% of the Z ketone after standing at room temperature for 38 hr.

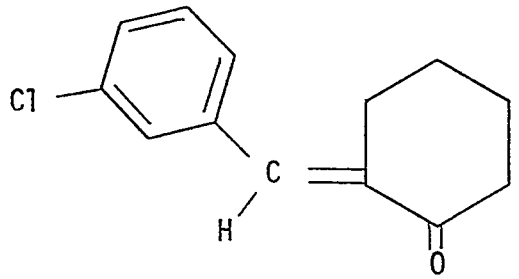
The crude E isomer was irradiated for 19 hr, after which the irradiated solution contained the E isomer (42%) and the Z isomer (43%) plus four other minor constituents (glc). The methanol was immediately removed from an aliquot of the irradiated solution under

reduced pressure at 40° and the residue was again analyzed by glc. The E isomer was now 65% of the mixture and the Z isomer was 22%. The nmr spectrum of this residue showed a signal at δ 7.43 and δ 6.47 integrating for approximately one proton, assigned to the vinylic proton, with approximately 75% of the signal at δ 7.43 and 25% of the signal at δ 6.47. The irradiated solution was found to be highly acidic, with a pH of 1-1.5. A chemical test for the presence of halogen (silver nitrate-nitric acid) was positive, suggesting the presence of chloride ions.

A solution of (E)-2-(2-chlorobenzylidene)cyclohexanone (75 mg) in methanol (100 ml) was irradiated for 1 hr. Glc analysis of the irradiated product indicated three compounds, present in concentrations of 77%, 21% (unchanged E ketone) and 2%. The major compound in the irradiated product was identified as (Z)-2-(2-chlorobenzylidene)cyclohexanone: uv max 267 mμ (ε6,765 based on 77% of the Z ketone); ir 1634 (C=C) and 1696 cm^{-1} (C=O); nmr δ 6.42 (t, 1, J= 2Hz, C_6H_4Cl-CH=).

A 1% w/v solution of (E)-2-(2-chlorobenzylidene)cyclohexanone in chloroform was examined by glc analysis after standing for several months in diffuse room light. Glc analysis indicated the presence of two compounds, whose retention times corresponded to the E ketone (40%) and the Z ketone (60%).

4.1.5 Preparation of (E)-2-(3-chlorobenzylidene)cyclohexanone[1]



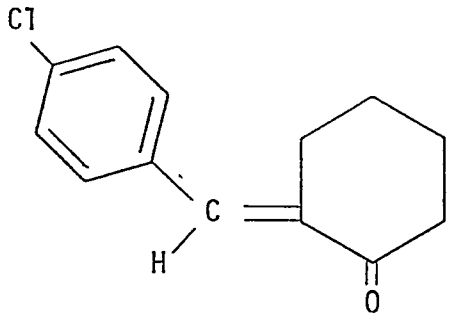
A mixture of cyclohexanone (13.8 g, 0.14 mol), 3-chlorobenzaldehyde (6.9 g, 0.048 mol), sodium hydroxide (0.78 g, 0.019 mol), and water (23.5 ml) was heated under reflux with stirring for 12 hr. The cooled reaction mixture was extracted with benzene, the combined benzene extracts washed with water, and dried ($MgSO_4$). Removal of the benzene under reduced pressure gave a viscous orange syrup which was distilled at 145-150° and 0.5 mm to give a pale yellow oil (1.95 g) which solidified upon cooling. Recrystallization of the solid from methanol gave pure (E)-2-(3-chlorobenzylidene)cyclohexanone (1.32 g, 12%): mp 75-75.5°; uv max 284 mμ (ε12,975) and 227 mμ (ε4,955); ir 1595 (C=C) and 1675 cm^{-1} (C=O); nmr δ 7.30 (t, 1, J = 2Hz, C_6H_4Cl-CH=), 7.47-7.00 (m, 4, C_6H_4Cl), 2.97-2.30 (m, 4, C^3H_2, C^6H_2), 2.17-1.53 (m, 4, C^4H_2, C^5H_2); mass spectrum m/e (rel intensity) 220 (M^+)(59), 219 (35), 192 (12), 185 (57), 157 (13), 151 (24), 129 (47), 128 (25), 127 (15), 116 (12), 115 (37), 67 (100).

Anal. Calcd for $C_{13}H_{13}ClO$: C, 70.75; H, 5.94. Found: C, 70.65; H, 5.85.

[1] Prepared by Mr. M.S. Auyeung, College of Pharmacy, University of Saskatchewan, Saskatoon.

A solution of the pure E ketone (75 mg) in methanol (100 ml) was irradiated for 1 hr. Glc analysis of the irradiated product indicated two compounds, present in concentrations of 95% and 5% (unchanged E ketone). The major compound in the irradiated product was identified as (Z)-2-(3-chlorobenzylidene)cyclohexanone: uv max 268 mμ (ε8,110 based on 95% of the Z ketone); ir 1633 (C=C) and 1691 cm^{-1} (C=O); nmr δ 6.30 (t, 1, J= 2Hz, C_6H_4Cl-CH=).

4.1.6 Preparation of (E)-2-(4-chlorobenzylidene)cyclohexanone



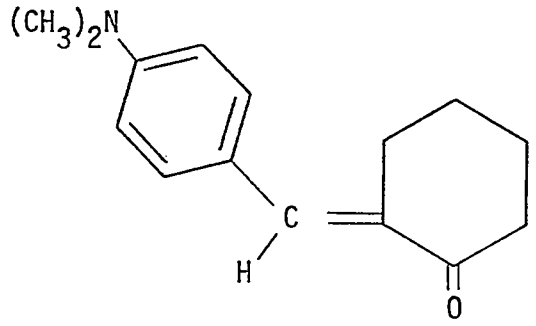
A mixture of cyclohexanone (278 g, 2.83 mol), 4-chlorobenzaldehyde (200 g, 1.42 mol), and aqueous sodium hydroxide solution (1 N, 1420 ml) was heated at 120° with stirring for 18 hr. The reaction mixture was cooled and extracted with chloroform. The chloroform extracts were washed with water, dried ($MgSO_4$), and evaporated under reduced pressure to give a viscous brown syrup. Fractional distillation of the crude product at 140° and 1.0 mm gave a bright yellow syrup (87 g) which solidified upon cooling. Glc analysis showed two compounds (6% and

94%) in the distillate. Recrystallization from methanol gave pure (E)-2-(4-chlorobenzylidene)cyclohexanone (43 g, 14%): mp 56.5-57.0° (Huitric and Kumler, 1956, quote 57-57.5°); uv max 293 mμ (ε16,370) and 225.5 mμ (ε4,790); ir 1599 (C=C) and 1678 cm^{-1} (C=O); nmr δ 7.40 (t, 1, J = 2Hz, C_6H_4Cl-C<u>H</u>=), 7.40-7.20 (m, 4, C_6<u>H_4</u>Cl), 2.77-2.57 (m, 2, C^3<u>H_2</u>), 2.70-2.37 (m, 2, C^6<u>H_2</u>), 2.17-1.47 (m, 4, C^4<u>H_2</u>, C^5<u>H_2</u>); mass spectrum m/e (rel intensity) 220 (M^+) (100), 219 (52), 192 (18), 185 (44), 164 (9), 157 (15), 151 (22), 129 (44), 128 (22), 127 (15), 116 (10), 115 (22), 67 (42).

Anal. Calcd for $C_{13}H_{13}ClO$: C, 70.75; H, 5.94. Found: C, 70.69; H, 5.89.

A solution of (E)-2-(4-chlorobenzylidene)cyclohexanone in methanol (100 ml) was irradiated for 1 hr. Glc analysis showed two compounds in the irradiated product, present in concentrations of 91% and 9% (unchanged E ketone). The major compound in the irradiated product was identified as (Z)-2-(4-chlorobenzylidene)cyclohexanone: uv max 278 mμ (ε11,080 based on 91% of the Z ketone); ir 1632 (C=C) and 1683 cm^{-1} (C=O); nmr δ 6.25 (t, 1, J = 2Hz, C_6H_4Cl-C<u>H</u>=).

4.1.7 Preparation of (E)-2-(4-dimethylaminobenzylidene)cyclohexanone



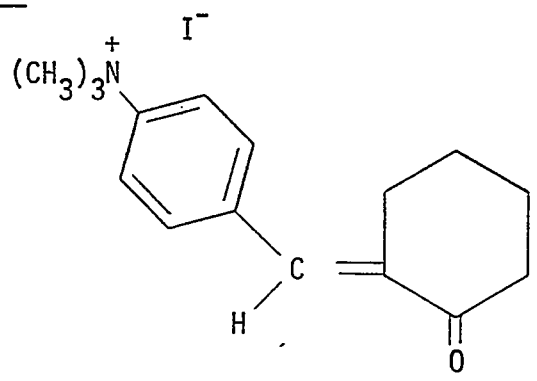
A mixture of cyclohexanone (210 g, 2.14 mol), 4-dimethylaminobenzaldehyde (107 g, 0.72 mol), and aqueous sodium hydroxide solution (2N, 350 ml) was heated under reflux at 120° with stirring for 26 hr. The reaction mixture was cooled and filtered to remove the yellow-orange crystals (128 g) which were heated under reflux with ethanol. The insoluble material (8.5 g) was removed by filtration and yellow crystals (97 g) deposited from the mother liquor. Repeated recrystallization of these crystals from ethanol gave pure (E)-2-(4-dimethylaminobenzylidene)-cyclohexanone as bright yellow crystals (70 g, 43%): mp 127-128° (Shriner and Teeters, 1938, quote mp 127-127.5°); uv max 381 mμ (ε23,700) and 253 mμ (ε6,560); ir 1607 (C=C) and 1664 cm^{-1} (C=O); nmr δ 7.51 (t, 1, J = 2 Hz, $(CH_3)_2N$-C_6H_4-CH=), 7.57-7.23 (m, 2, aromatic C^2H, C^6H), 6.83-6.50 (m, 2, aromatic C^3H, C^5H), 2.98 (s,6, $(CH_3)_2N$),2.97-2.70 (m, 2, C^3H_2), 2.67-2.30 (m, 2, C^6H_2), 2.13-1.47 (m, 4, C^4H_2, C^5H_2); mass spectrum m/e (rel intensity) 229 (M^+) (100), 228 (32), 201 (12), 173 (23), 172 (19), 158 (13), 129 (11).

Anal. Calcd for $C_{15}H_{19}NO$: C, 78.56; H, 8.35. Found: C, 78.48; H, 8.35.

The ethanol-insoluble material (8.5 g) removed by filtration was extracted several times with boiling ethanol and recrystallized from acetone to give (E , E)-2,6-bis-(4-dimethylaminobenzylidene)-cyclohexanone as bright orange crystals (7.1 g, 4.3%). The physical and spectral properties are described in 4.2.5.

A solution of (E)-2-(4-dimethylaminobenzylidene)cyclohexanone (20 mg) in 95% ethanol (60 ml) was irradiated with a Hanovia 450 watt UV lamp for 1 hr. The uv spectrum of the irradiated solution was the same as the uv spectrum of the E ketone.

4.1.8 Preparation of (E)-2-(4-dimethylaminobenzylidene)cyclohexanone methiodide



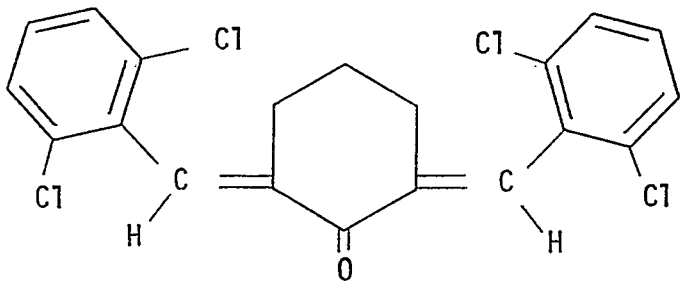
A solution of (E)-2-(4-dimethylaminobenzylidene)cyclohexanone (10.0 g, 0.04 mol) and methyl iodide (12.4 g, 0.08 mol) in anhydrous benzene (100 ml) was heated under reflux at 120° for 16 hr. The hot benzene solution was filtered to remove the yellow precipitate, which was recrystallized from absolute ethanol to give bright yellow crystals

of (E)-2-(4-dimethylaminobenzylidene)cyclohexanone methiodide (6.6 g, 40%): mp 148-150°; uv max 280 mμ (ε13,590) and 219 mμ (ε13,810); ir 1613 (C=C) and 1682 cm^{-1} (C=O); nmr (D_2O) δ 8.13-7.80 (m, 2, aromatic $C^2\underline{H}$, $C^6\underline{H}$), 7.80-7.47 (m, 2, aromatic $C^3\underline{H}$, $C^5\underline{H}$), 7.27 (t, 1, $\underline{J}$ = 2Hz, $(CH_3)_3\overset{+}{N}\text{-}C_6H_4\text{-}C\underline{H}=$) , 3.77 (s, 9, $(C\underline{H}_3)_3N$), 3.07-2.53 (m, 2, $C^3\underline{H}_2$), 2.80-2.33 (m, 2, $C^6\underline{H}_2$), 2.17-1.50 (m, 4, $C^4\underline{H}_2$, $C^5\underline{H}_2$); mass spectrum m/e 229 (M^+).

Anal. Calcd for $C_{16}H_{22}INO$: C, 51,76; H, 5.97. Found: C, 51.37; H, 6.01.

4.2 Preparation of substituted 2,6-bis-benzylidenecyclohexanones

4.2.1 Preparation of (E,E)-2,6-bis-(2,6-dichlorobenzylidene)cyclohexanone



(a) A solution of cyclohexanone (35.4 g, 0.36 mol), 2,6-dichlorobenzaldehyde (20.0 g, 0.11 mol), and aqueous sodium hydroxide solution (1 N, 115 ml) in ethanol (100 ml) was heated at 75° with stirring for 5 hr. The reaction mixture was cooled and filtered to remove the yellow precipitate (10.7 g). The precipitate was washed twice with ethanol and recrystallized from acetone to give bright yellow crystals of (E,E)-2,6-bis-(2,6-dichlorobenzylidene)cyclohexanone (9.0 g, 38%): mp 183°; uv max 289 mμ (ε14,920); ir 1605 (C=C) and 1678 cm^{-1} (C=O); nmr δ 7.52 (t, 2, J = 2Hz, 2 x $C_6H_3Cl_2$-CH=), 7.53-6.93 (m, 6, 2 x $C_6H_3Cl_2$), 2.60-2.30 (m, 4, C^3H_2, C^5H_2), 1.93-1.52 (m, 2, C^4H_2); mass spectrum m/e (rel intensity) 410 (M^+) (1), 375 (100), 152 (11), 151 (9), 149 (10).

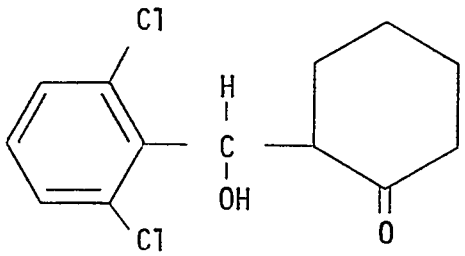
Anal. Calcd for $C_{20}H_{14}Cl_4O$: C, 58.28; H, 3.42. Found: C, 58.17; H, 3.50.

(b) An aqueous sodium hydroxide solution (1 N, 86 ml) was added dropwise to a stirring mixture of cyclohexanone (33.6 g, 0.34 mol) and 2,6-dichlorobenzaldehyde (15.0 g, 0.085 mol). After addition of the

aqueous sodium hydroxide solution was completed, the reaction mixture was heated at 50° with stirring for 20 hr. The water and excess cyclohexanone were removed under reduced pressure and the residue was dissolved in chloroform. The chloroform solution was washed with water, dried ($MgSO_4$), and evaporated under reduced pressure to give a yellow solid which was recrystallized from acetone to give the bis-compound (9.6 g, 54%): mp 180-181°.

(c) A mixture of cyclohexanone (22.8 g, 0.23 mol), 2,6-dichlorobenzaldehyde (10.0 g, 0.057 mol), and aqueous sodium hydroxide solution (0.25 N, 230 ml) was stirred at room temperature for 24 hr. Isolation of the product by the same method as (b) gave the bis-compound (7.2 g, 61%): mp 180-182°.

4.2.2 Preparation of 2-(α-hydroxy-2,6-dichlorobenzyl)cyclohexanone



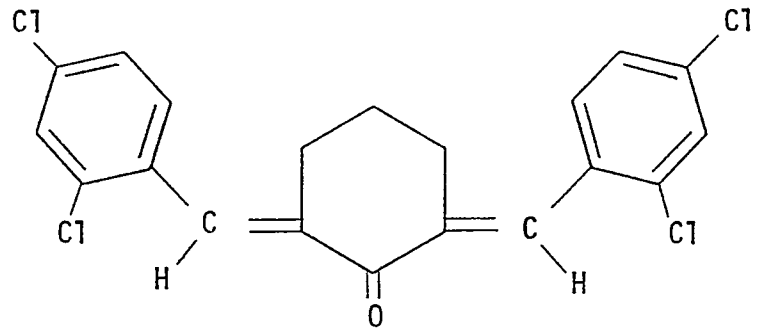
A mixture of cyclohexanone (19.9 g, 0.20 mol), 2,6-dichlorobenzaldehyde (6.04 g, 0.034 mol), and aqueous sodium hydroxide solution (1 N, 18 ml) was stirred at 45° for 2 hr. The reaction mixture was extracted with chloroform, the chloroform extracts washed with

water and dried ($MgSO_4$). Removal of the chloroform and excess cyclohexanone under reduced pressure gave a yellow syrup. Fractional distillation at 160° and 1.0 mm gave a pale yellow syrup (2.96 g) which solidified upon cooling. Recrystallization of this solid from methanol gave colorless crystals of 2-(α-hydroxy-2,6-dichlorobenzyl)-cyclohexanone (2.13 g, 23%): mp 148-149°; ir 1705 (C=O) and 3565 cm^{-1} (OH); nmr δ 7.43-6.93 (m, 3, $C_6\underline{H}_3Cl_2$), 5.83 (d, 1, J = 10Hz, $C_6H_3Cl_2$-C<u>H</u>), 3.77-3.10 (m, 1, $C^2\underline{H}$), 3.63 (broad s, 1, exchanges with D_2O, O<u>H</u>), 2.62-2.28 (m, 2, $C^6\underline{H}_2$), 2.28-1.20 (m, 6, $C^3\underline{H}_2$, $C^4\underline{H}_2$, $C^5\underline{H}_2$); mass spectrum m/e (rel intensity) 273 (M^+)(2), 219 (9), 177 (10), 175 (19), 173 (7), 139 (4), 111 (11), 99 (8), 98 (100), 97 (10), 83 (15), 75 (16), 70 (36), 55 (19), 41 (20), 39 (12).

Anal. Calcd for $C_{13}H_{14}Cl_2O_2$: C, 57.16; H, 5.16. Found: C, 57.08; H, 5.11.

A solution of the hydroxy ketone (0.55 g) in 95% ethanol (10 ml) was acidified with concentrated hydrochloric acid (5 drops) and heated under reflux for 6 hr. The ethanol was removed under reduced pressure and the residue dissolved in ether. The ether solution was washed with water, dried ($MgSO_4$), and evaporated under reduced pressure to give a pale yellow solid (0.49 g), identified as unreacted 2-(α-hydroxy-2,6-dichlorobenzyl)cyclohexanone.

4.2.3 Preparation of (E,E)-2,6-bis-(2,4-dichlorobenzylidene)cyclohexanone[1]



A mixture of cyclohexanone (4.60 g, 0.047 mol), 2,4-dichlorobenzaldehyde (6.86 g, 0.039 mol), and sodium hydroxide (0.78 g, 0.019 mol) in water (24 ml) was heated under reflux at 110° with stirring for 12 hr. The reaction mixture was cooled and extracted with benzene, the benzene extracts washed with water and dried ($MgSO_4$). Removal of the benzene under reduced pressure gave a bright yellow solid which was recrystallized from dimethylformamide to give (E,E)-2,6-bis-(2,4-dichlorobenzylidene)cyclohexanone (1.22 g, 15%): mp 163° (Dibella, 1968, quotes mp 162°); uv max 318 mμ (ε21,925) and 238 mμ (ε15,310); ir 1609 (C=C) and 1670 cm^{-1} (C=O); nmr δ 7.85 (t, 2, J = 2Hz, 2 x $C_6H_3Cl_2$-CH=), 7.48 (s, 2, 2 x aromatic C^3H), 7.28 (s, 4, 2 x aromatic C^5H, C^6H), 2.93-2.57 (m, 4, C^3H_2, C^5H_2), 2.10-1.50 (m, 2, C^4H_2); mass spectrum m/e (rel intensity) 410 (M^+) (1), 375 (96), 152 (8), 151 (7), 149 (13).

Anal. Calcd for $C_{20}H_{14}Cl_4O$: C, 58.28; H, 3.42. Found: C, 58.10; H, 3.40.

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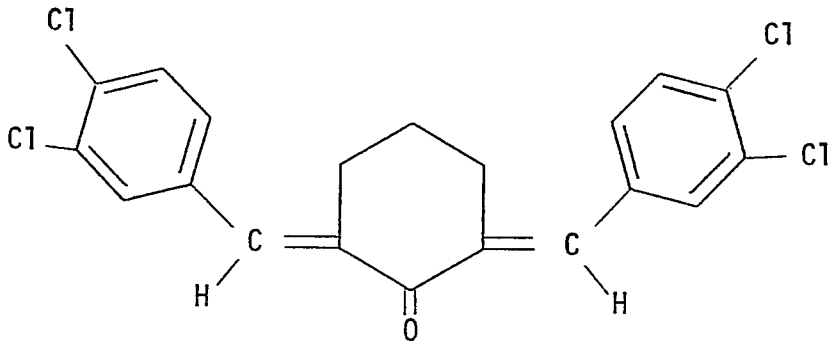
[1] Prepared by Mr. M.S. Auyeung, College of Pharmacy, University of Saskatchewan, Saskatoon.

4.2.4 Preparation of (E,E)-2,6-bis-(3,4-dichlorobenzylidene)cyclohexanone[1]

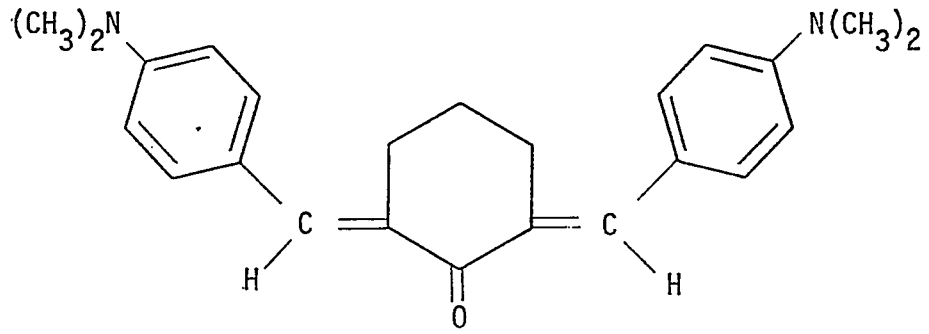


A mixture of cyclohexanone (13.8 g, 0.14 mol), 3,4-dichlorobenzaldehyde (20.6 g, 0.12 mol), and sodium hydroxide (2.35 g, 0.059 mol) in water (71 ml) was heated under reflux at 110° with stirring for 12 hr. The reaction mixture was cooled and extracted with benzene, the organic extracts washed with water and dried ($MgSO_4$). Removal of the benzene under reduced pressure gave an orange-brown residue which was recrystallized from ethanol to give yellow crystals of (E,E)-2,6-bis-(3,4-dichlorobenzylidene)cyclohexanone (3.45 g, 21%): mp 150° (Dibella, 1968, quotes mp 147-148°); uv max 329 mμ (ε34,725) and 241 mμ (ε17,775); ir 1608 (C=C) and 1664 cm^{-1} (C=O); nmr δ 7.65 (t, 2, J = 2Hz, 2 x $C_6H_3Cl_2$-CH=), 7.60-7.13 (m, 6, 2 x $C_6H_3Cl_2$), 3.07-2.70 (m, 4, C^3H_2, C^5H_2), 2.03-1.57 (m, 2, C^4H_2); mass spectrum m/e (rel intensity) 410 (M^+)(76), 409 (36), 375 (78), 347 (30), 312 (40), 202 (12), 188 (12), 185 (12), 183 (15), 170 (14), 160 (30), 149 (67), 128 (32), 127 (31), 115 (21).

Anal. Calcd for $C_{20}H_{14}Cl_4O$: C, 58.28; H, 3.42. Found: C, 58.30; H, 3.41.

[1] Prepared by Mr. M.S. Auyeung, College of Pharmacy, University of Saskatchewan, Saskatoon.

4.2.5 Preparation of (E,E)-2,6-bis-(4-dimethylaminobenzylidene)cyclohexanone

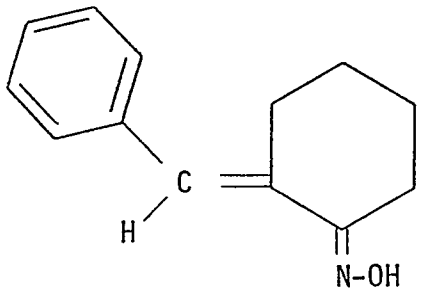


(E,E)-2,6-bis-(4-Dimethylaminobenzylidene)cyclohexanone was isolated from the reaction mixture in the preparation of (E)-2-(4-dimethylaminobenzylidene)cyclohexanone (4.1.7). The ethanol-insoluble residue (8.5 g) from the crude product obtained in 4.1.7 was washed repeatedly with hot ethanol and recrystallized from acetone to give orange crystals of (E,E)-2,6-bis-(4-dimethylaminobenzylidene)-cyclohexanone (7.07 g, 4.3%): mp 250° (Shriner and Teeters, 1938, quote mp 248-249°); uv max (dichloromethane) 432 mμ (ε45,170) and 273 mμ (25,095); ir 1611 (C=C) and 1647 (C=O); nmr δ 7.68 (t, 2, J = 2Hz, 2 X $(CH_3)_2N$-C_6H_4-CH=), 7.57-7.23 (m, 4, aromatic C^2H, C^6H), 6.80-6.50 (m, 4, aromatic C^3H, C^5H), 2.98 (s, 12, 2 X $(CH_3)_2N$), 3.13-2.73 (m, 4, C^3H_2, C^5H_2), 2.10-1.57 (m, 2, C^4H_2); mass spectrum m/e (rel intensity) 360 (M^+) (100), 359 (35), 332 (16), 331 (17), 212 (19), 166 (31), 134 (14).

Anal. Calcd for $C_{24}H_{28}N_2O$: C, 79.96; H, 7.83. Found: C, 79.81; H, 7.72.

4.3 Preparation of substituted 2-benzylidenecyclohexanone oximes

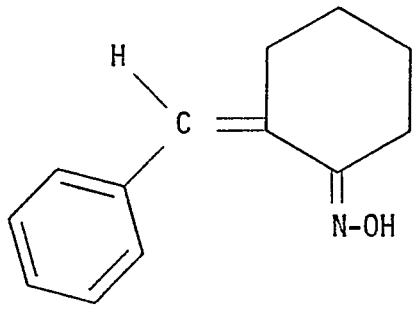
4.3.1 Preparation of (E)-2-benzylidenecyclohexanone oxime



A mixture of (E)-2-benzylidenecyclohexanone (32.2 g, 0.17 mol) in ethanol (100 ml), hydroxylamine hydrochloride (13.2 g, 0.19 mol) in water (20 ml), and sodium acetate (14.8 g, 0.18 mol) in water (20 ml) was heated at 60° with stirring for 1 hr. The volume of the reaction mixture was increased to 250 ml by the addition of water, and the reaction mixture was cooled. The reaction mixture was filtered to remove the colorless crystals (33.9 g) which were washed with water and recrystallized from acetone to give pure (E)-2-benzylidenecyclohexanone oxime (26.1 g, 77%): mp 125-126° (Vavon and Conia, 1952, quote mp 126°); ir 1598 (C=C) and 3216 cm^{-1} (O-H); uv max 273 mμ (ε14,725); nmr δ 9.28 (broad s, 1, exchanges with D_2O, O<u>H</u>), 7.33-7.03 (m, 5, $C_6\underline{H}_5$), 6.83 (t, 1, <u>J</u> = 2Hz, C_6H_5-C<u>H</u>=), 2.80-2.43 (m, 4, $C^3\underline{H}_2$, $C^6\underline{H}_2$), 1.80-1.47 (m, 4, $C^4\underline{H}_2$, $C^5\underline{H}_2$); mass spectrum <u>m/e</u> (rel intensity) 201 (M^+) (26), 200 (100), 185 (10), 184 (52), 156 (10), 129 (10), 128 (19), 116 (10), 115 (37), 105 (10), 91 (20), 89 (10), 79 (10), 77 (24).

<u>Anal</u>. Calcd for $C_{13}H_{15}NO$: C, 77.58; H, 7.51. Found: C, 77.48; H, 7.44.

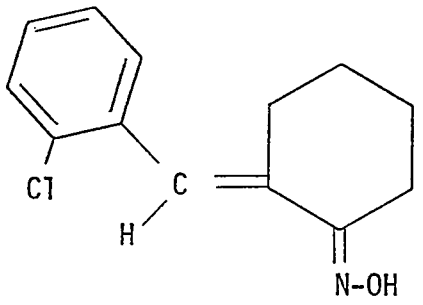
4.3.2 Preparation of (Z)-2-benzylidenecyclohexanone oxime



A mixture of (Z)-2-benzylidenecyclohexanone (4.00 g, 0.0215 mol) in ethanol (35 ml), hydroxylamine hydrochloride (1.64 g, 0.0236 mol) in water (5 ml), and sodium acetate (1.85 g, 0.0225 mol) in water (5 ml) was stirred at room temperature for 10 hr. The ethanol and water were removed under reduced pressure. The residue was dissolved in ether, the ether solution washed with water and dried ($MgSO_4$). Removal of the ether under reduced pressure gave a colorless solid (3.48 g) which was recrystallized from ether-Skelly F to give colorless crystals of (Z)-2-benzylidenecyclohexanone oxime (3.21 g, 74%): mp 90-92°; ir 1597 (C=C) and 3246 cm^{-1} (O-H); uv max 262 mμ (ε 10,550); nmr δ 8.10 (broad s, 1, exchanges with D_2O, O<u>H</u>), 7.40-6.97 (m, 5, $C_6\underline{H}_5$), 6.33 (t, 1, <u>J</u> = 2Hz, C_6H_5-C<u>H</u>=), 2.80-2.20 (m, 4, $C^3\underline{H}_2$, $C^6\underline{H}_2$), 2.03-1.43 (m, 4, $C^4\underline{H}_2$, $C^5\underline{H}_2$); mass spectrum <u>m/e</u> (rel intensity) 201 (M^+) (27), 200 (100), 185 (6), 184 (42), 183 (9), 182 (9), 156 (6), 143 (5), 142 (6), 128 (7), 115 (14), 105 (5), 91 (7), 77 (9).

<u>Anal</u>. Calcd for $C_{13}H_{15}NO$: C, 77.58; H, 7.51. Found: C, 77.57; H, 7.49.

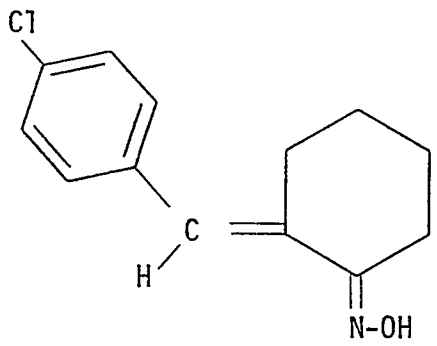
4.3.3 Preparation of (E)-2-(2-chlorobenzylidene)cyclohexanone oxime



A mixture of (E)-2-(2-chlorobenzylidene)cyclohexanone (20.0 g, 0.090 mol) in ethanol (200 ml), hydroxylamine hydrochloride (7.55 g, 0.108 mol) in water (25 ml), and sodium acetate (8.17 g, 0.099 mol) in water (20 ml) was heated at 60° with stirring for 1 hr. The volume of the reaction mixture was increased to 500 ml by the addition of water. The cooled reaction mixture was filtered to remove the colorless precipitate (21.1 g) which was washed with water and recrystallized from acetone to give pure (E)-2-(2-chlorobenzylidene)cyclohexanone oxime (17.3 g, 81%): mp 169°; ir 1589 (C=C) and 3247 cm^{-1} (O-H); uv max 267 mμ (ε12,480); nmr δ 9.31 (broad s, 1, exchanges with D_2O, OH), 7.43-7.00 (m, 4, C_6H_4Cl), 6.87 (t, 1, J= 2Hz, C_6H_4Cl-CH=), 2.83-2.23 (m, 4, C^3H_2, C^6H_2), 1.97-1.40 (m, 4, C^4H_2, C^5H_2); mass spectrum m/e (rel intensity) 235 (M^+)(8), 234 (19), 218 (16), 201 (15), 200 (100), 182 (16), 141 (10), 140 (10), 139 (11), 128 (12), 127 (11), 125 (10), 105 (24), 89 (10), 77 (15), 75 (10), 51 (14).

Anal. Calcd for $C_{13}H_{14}ClNO$: C, 66.24; H, 5.99. Found: C, 66.09; H, 5.93.

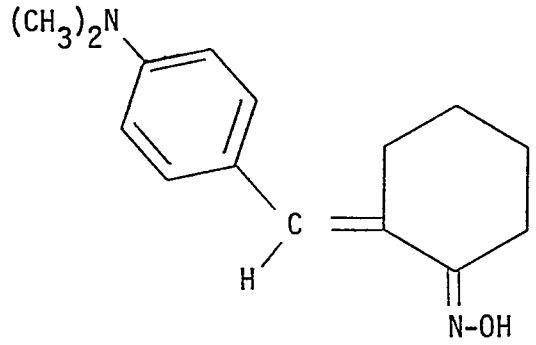
4.3.4 Preparation of (E)-2-(4-chlorobenzylidene)cyclohexanone oxime



A mixture of (E)-2-(4-chlorobenzylidene)cyclohexanone (10.01 g, 0.045 mol) in ethanol (100 ml), hydroxylamine hydrochloride (3.78 g, 0.054 mol) in water (12 ml), and sodium acetate (4.08 g, 0.049 mol) in water (15 ml) was heated at 60^{o} with stirring for 0.5 hr. The volume of the reaction mixture was increased to 250 ml by the addition of water. The cooled reaction mixture was filtered to remove the colorless precipitate (10.05 g) which was washed with water and recrystallized from acetone to give pure (E)-2-(4-chlorobenzylidene)cyclohexanone oxime (9.1 g, 85%): mp 131^{o}; ir 1592 (C=C) and 3247 cm^{-1} (O-H); uv max 277 mμ (ε 17,630); nmr δ 9.36 (broad s, 1, exchanges with D_2O, OH), 7.40-7.00 (m, 4, C_6H_4Cl), 6.77 (t, 1, J = 2Hz , C_6H_4Cl-CH=), 2.77-2.45 (m, 4, C^3H_2, C^6H_2),1.80-1.53 (m, 4, C^4H_2, C^5H_2); mass spectrum m/e (rel intensity) 235 (M^+)(23), 234 (100), 220 (10), 218 (30), 141 (10), 115 (16).

Anal. Calcd for $C_{13}H_{14}ClNO$: C, 66.24; H, 5.99. Found: C, 66.32; H, 6.04.

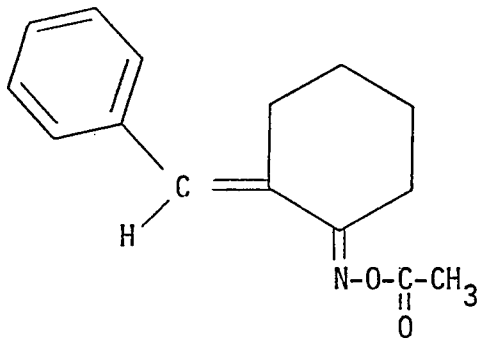
4.3.5 Preparation of (E)-2-(4-dimethylaminobenzylidene)cyclohexanone oxime



A mixture of (E)-2-(4-dimethylaminobenzylidene)cyclohexanone (20.0 g, 0.087 mol) in ethanol (250 ml), hydroxylamine hydrochloride (7.31 g, 0.105 mol) in water (25 ml), and sodium acetate (7.88 g, 0.096 mol) in water (20 ml) was heated at 65° with stirring for 1 hr. Stirring was continued for 2 hr as the oil bath cooled. The volume of the reaction mixture was increased to 500 ml by the addition of water. The cooled reaction mixture was filtered to remove the yellow-orange precipitate (18.45 g) which was washed with water and recrystallized from acetone to give orange crystals of pure (E)-2-(4-dimethylaminobenzylidene)cyclohexanone oxime (15.7 g, 74%): mp 166-168°; ir 1605 (C=C) and 3226 cm^{-1} (O-H); uv max (dichloromethane) 329 mμ (ε24,175); nmr δ 9.21 (broad s, 1, exchanges with D_2O, O<u>H</u>), 7.33-7.03 (m, 2, aromatic $C^2\underline{H}$, $C^6\underline{H}$), 6.80 (t, 1, <u>J</u> = 2Hz, $(CH_3)_2N$-C_6H_4-C<u>H</u>=), 6.77-6.51 (m, 2, aromatic $C^3\underline{H}$, $C^5\underline{H}$), 2.93 (s, 6, $(C\underline{H}_3)_2N$), 2.80-2.47 (m, 4, $C^3\underline{H}_2$, $C^6\underline{H}_2$), 1.80-1.47 (m, 4, $C^4\underline{H}_2$, $C^5\underline{H}_2$); mass spectrum <u>m/e</u> (rel intensity) 244 (M^+) (67), 243 (26), 228 (17), 227 (100), 226 (30), 211 (10), 158 (12), 115 (11).

<u>Anal</u>. Calcd for $C_{15}H_{20}N_2O$: C, 73.73; H, 8.25. Found: C, 73.46; H, 8.19.

4.3.6 Preparation of the acetate of (E)-2-benzylidenecyclohexanone oxime

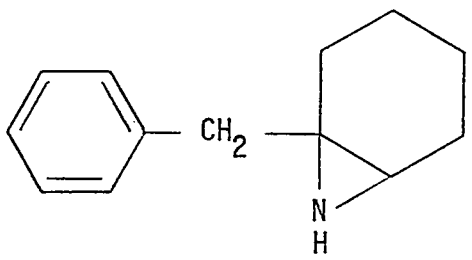


A solution of (E)-2-benzylidenecyclohexanone oxime (3.5 g, 0.0175 mol) in freshly-distilled acetic anhydride (15 ml) was heated under reflux for 0.5 hr. Crushed ice was added to the cooled solution and 10% w/v aqueous sodium carbonate solution was added to decompose the excess acetic anhydride. The gummy yellow precipitate (2.3 g) was removed by filtration and recrystallized from methanol to give colorless needles of pure (E)-2-benzylidenecyclohexanone oxime acetate (1.0 g, 24%): mp 105.5°; ir 1765 cm^{-1} (C=O); nmr δ 7.37-7.10 (m, 5, $C_6\underline{H}_5$), 7.03 (t, 1, $\underline{J}$ = 2Hz, C_6H_5-C$\underline{H}$ =), 2.83-2.50 (m, 4, $C^3\underline{H}_2$, $C^6\underline{H}_2$), 2.18 (s, 3, $C\underline{H}_3$-CO), 1.87-1.47 (m, 4, $C^4\underline{H}_2$, $C^5\underline{H}_2$); mass spectrum m/e (rel intensity) 243 (M^+) (7), 242 (16), 201 (29), 200 (51), 185 (17), 184 (100), 183 (15), 128 (10), 124 (31), 115 (18), 105 (12), 91 (12), 77 (14), 43 (26).

Anal. Calcd. for $C_{15}H_{17}NO_2$: C, 74.05; H, 7.04. Found: C, 74.11; H, 7.06.

4.4 Reduction of substituted 2-benzylidenecyclohexanone oximes

4.4.1 Preparation of 1-benzyl-1,2-epiminocyclohexane



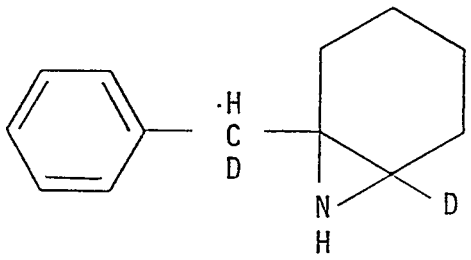
4.4.1.1 Reduction of (E)-2-benzylidenecyclohexanone oxime with lithium aluminum hydride

A solution of (E)-2-benzylidenecyclohexanone oxime (7.55 g, 0.037 mol) in anhydrous ether (180 ml) was added dropwise to a stirring suspension of lithium aluminum hydride (2.16 g, 0.057 mol) in anhydrous ether (40 ml) at such a rate that the ether refluxed gently. The reaction mixture was heated under reflux with stirring for 8 hr, then cooled. The reduction complex was decomposed by adding water dropwise at a rate to gently reflux the ether. The ether layer was separated, washed with water, dried ($MgSO_4$), and evaporated under reduced pressure to give a pale yellow oil (6.48 g) which contained essentially one compound (glc). Fractional distillation of this oil at 78° and 0.2 mm gave pure 1-benzyl-1,2-epiminocyclohexane as a colorless oil (5.19 g, 74%): n_D^{20} 1.5460; ir 3250 cm^{-1} (N-H); nmr δ 7.40-7.00 (m, 5, $C_6\underline{H}_5$), 2.92 and 2.63 (centers of 2 d of AB quartet, 2, J = 14 Hz, C_6H_5-$C\underline{H}_2$), 2.07-1.83 (m, 1, $C^2\underline{H}$), 1.90-1.50 (m, 4, $C^3\underline{H}_2$, $C^6\underline{H}_2$),

1.50-1.07 (m, 4, $C^4\underline{H}_2$, $C^5\underline{H}_2$), 0.73 (broad s, 1, exchanges with D_2O, N<u>H</u>); mass spectrum <u>m/e</u> (rel intensity) 187 (M^+)(100), 186 (56), 172 (82), 159 (54), 158 (74), 96 (100), 91 (100).

<u>Anal</u>. Calcd for $C_{13}H_{17}N$: C, 83.37; H, 9.15. Found: C, 83.20; H, 9.12.

4.4.1.2 Reduction of (<u>E</u>)-2-benzylidenecyclohexanone oxime with lithium aluminum deuteride



A solution of (<u>E</u>)-2-benzylidenecyclohexanone oxime (1.53 g, 0.00759 mol) in anhydrous ether (40 ml) was added dropwise to a stirring suspension of lithium aluminum deuteride (0.477 g, 0.0114 mol) in anhydrous ether (30 ml) at such a rate that the ether refluxed gently. The reaction mixture was heated under reflux for 8 hr, then allowed to stand overnight. Decomposition of the reduction complex and extraction of the reduction product by the procedure used in 4.4.1.1 gave a pale yellow oil (1.29 g) which contained essentially one compound (glc). Fractional distillation of the reduction product at 80° and 0.5 mm gave pure 1-benzyl-<u>α-d</u>-1,2-epiminocyclohexane-<u>2-d</u> (1.08 g, 75%) as a colorless oil: ir 3256 cm^{-1} (N-H); nmr δ 7.47-7.07 (m, 5, $C_6\underline{H}_5$), 2.85 and 2.68 (centers of 2 m, 1, C_6H_5-C<u>H</u>D), 2.00-1.50 (m, 4, $C^3\underline{H}_2$, $C^6\underline{H}_2$), 1.50-1.28 (m, 4, $C^4\underline{H}_2$, $C^5\underline{H}_2$), 0.98 (broad s, 1, exchanges

with D_2O, NH); mass spectrum m/e (rel intensity) 189 (M^+) (100), 188 (40), 174 (36), 161 (36), 160 (40), 97 (60), 92 (96).

4.4.1.3 Reduction of (E)-2-benzylidenecyclohexanone oxime with lithium borohydride

A solution of (E)-2-benzylidenecyclohexanone oxime (4.00 g, 0.0199 mol) in anhydrous ether (100 ml) was added dropwise to a stirring suspension of lithium borohydride (0.652 g, 0.0299 mol) in anhydrous ether (40 ml) at such a rate that the ether refluxed gently.The reaction mixture was heated under reflux with stirring for 6 hr, then cooled. The reduction complex was decomposed by adding water (10 ml) dropwise at a rate whereby the ether refluxed gently. The ether layer was separated, washed with water, and dried ($MgSO_4$). Removal of the ether under reduced pressure gave a pale yellow syrup (3.61 g) which was found to contain 65% 1-benzyl-1,2-epiminocyclohexane and 35% unreacted (E)-2-benzylidenecyclohexanone oxime (glc).

4.4.1.4 Reduction of (Z)-2-benzylidenecyclohexanone oxime with lithium aluminum hydride

A solution of (Z)-2-benzylidenecyclohexanone oxime (2.00 g, 0.00994 mol) in anhydrous ether (25 ml) was added dropwise to a stirring suspension of lithium aluminum hydride (0.75 g, 0.0198 mol) in anhydrous ether (20 ml) at such a rate that the ether refluxed gently. The reaction mixture was stirred at room temperature for 20 hr. The