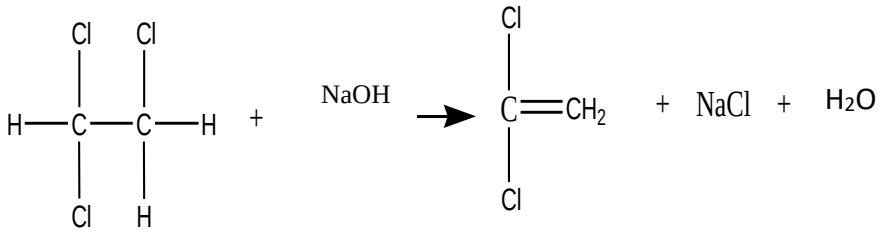
CHAPTER ONE

INTRODUCTION

1.1 Background of study

Vinylidine chloride, also referred to as 1,1-dichloroethylene (1,1-DCE) is a colourless liquid (b.p. 32.2°C), produced by dehydrochlorination of 1,1,2 – trichloroethane [$Cl_2CH.CH_2Cl$], a relatively unwanted by-product formed in the production of 1,1,1–trichloroethane and 1,2–dichloroethane. Conversion to 1,1–DCE involves a base – catalyzed reaction



1, 1, 2-trichloroethane 1, 1-dichloroethylene (Rossberg *et al*., 2006)

1,1–DCE is mainly used as a comonomer in the polymerization of vinyl chloride, acrylonitrile, and acrylates. Inhibitors such as the monomethyl ether of hydroquinone are usually added to prevent the polymerization of 1,1–DCE on storage. 1,1 –DCE is also used in semi conductor device fabrication for growing high purity silicon dioxide (SiO_2) films. 1,1–DCE is thus an important chemical substance which serves as a solvent as well as a monomer (Rossberg *et al*., 2006).

1,1–DCE is considered a potential occupational carcinogen by the US National Institute for Occupational Safety and Health.

As with several other alkenes, 1,1–DCE can be polymerized to form the homopolymer poly(vinylidene chloride), PVDC, a form that is not commercially important because experimental data have been generated which demonstrated unequivocally that it undergoes what has been described as catastrophic decomposition at its melt temperature (above 125°C),

producing HCl (Piringer and Baner, 2008; Marianne, G., 2017). 1,1 – DCE as monomer forms copolymers with other monomers such as vinyl chloride and these copolymers are commercially viable (Paisley, 2007).

Polyvinylidene chloride resins and coatings have reportedly been a part of flexible packaging for sometime. They are presented as possessing a unique combination of functional characteristics which have made them find numerous applications. According to Paisley, (2007) PVDC products are available in a variety of forms, such as aqueous dispersions or latex, for coating on a number of different film and paper substrates, extrudable powders for production of multilayer films and sheets, and soluble powders for solvent – based coatings. Unique properties possessed by PVDC copolymers enable their use for protection from moisture loss or gain, protection against oxidation of ingredients, prevention of oil and grease permeation, and excellent printing characteristics(Michel, 2013). Paisley, (2007) is also on record as asserting that PVDC – coated biaxially-oriented polypropylene (BOPP) films held, as at 2005, a 53% share of barrier film use in the USA. Such a figure does indicate the extensive use of PVDC products and therefore of their importance.

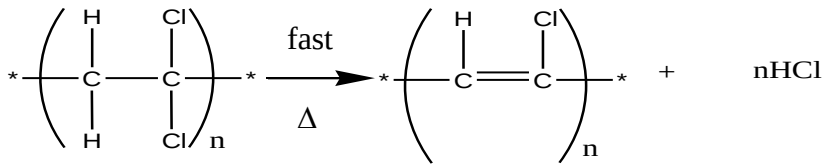
Again according to Paisley, (2007), during production of PVDC, other comonomers/additives are usually introduced, depending on the quality of copolymer desired. Thus, one comonomer/additive may be introduced to improve heat processability by decreasing melt temperature or to enable suitability of the polymer for film production, while another is introduced to provide some desired properties that would enhance polymer aesthetics, such as printability, adhesion, and / or thermoforming shrink flexibility. Thus several of these comonomers/additives may be added depending on desired effects. Additives are common in general polymer use. For instance, plasticizers are used to make PVC more flexible and therefore

more easily processed. On the other hand, terpolymers are increasingly in use, and include the acrylonitrile - butadiene - styrene (ABS) system which is generally formulated as an unbranched, head - to - tail terpolymer in which the individual comonomers are statistically distributed (Lewis, 1993).

Details of the microstructure of PVDC copolymers on the other hand are not yet available but it is reasonable to speculate that the two bulky chlorine atoms on one carbon atom joined to neighbouring carbon by a double bond in the monomer 1,1- DCE would provide sufficient steric hindrance to free rotation such that the relative stereochemistry in the resulting PVDC copolymer would not be random but would be predominantly syndiotactic leading to crystallinity to a greater degree. Thus PVDC copolymers are generally high density and high crystallinity with relatively few defect sites. High vinylidene chloride (VDC) content copolymers do however undergo thermally-induced degradative dehydrochlorination at process temperatures, and these degradations have been of interest ever since such copolymers came into use (Matheson and Boyer, 1952).

The dehydrochlorination process has more recently come under close scrutiny, and has been studied using largely thermogravimetric techniques (Howell *et al*., 2000).

Data generated in these thermogravimetric studies have been used to propose that only HCl is lost during degradation, and that weight loss of a PVDC copolymer sample directly indicates the extent of degradation which is usually represented as follows:



This is the primary degradation process which accompanies the processing of the polymer. The early stage of dehydrohalogenation is uncomplicated by interfering processes, and the only product observed by evolved gas analysis is hydrogen chloride (Howell and Rajaram, 1993; Yue and Economy, 2017).

1.2 Statement of Problem

Thermally-induced degradation of vinylidene chloride copolymers has resulted in considerable losses of polymer material during the production process. The homolysis of the C – Cl bond set up a conjugated polyene sequence, which resulted in the coloration of the copolymers, thereby making them unsuitable for use as films or inhibiting their use as barrier films in packaging applications for food and pharmaceuticals (The Dow Chemical Company, 2005).

A second and more serious consequence of the degradation is the evolution of hydrogen chloride which reacts with the walls of process equipment, commonly stainless steel, at process temperatures, to form iron (III) chloride, a strong Lewis acid catalyst which can enhance the dehydrohalogenation process.

Thus these two problems, namely, thermal dehydrochlorination of PVDC copolymers at process temperatures (a production problem) and the degradation of production equipment by the resulting product of dehydrochlorination would need to be solved, using suitable comonomers and / or appropriate additives.

1.3 Aim and objectives of study:

The aim of this study is to determine the effect of extracted oxygen heterocycle on the dehydrochlorination vinylidene chloride copolymer.

The Specific objectives are to:

i. Extract suitable chemical additive, precocene I which would reduce its rate of dehydrochlorination and loss of polymer material.

ii. Characterize the proposed additive using gas chromatography mass spectrometry (GC-MS), Fourier transform infrared (FTIR), Beynon's Table, atomic absorption spectrometer (AAS) and National Institute of Standards and Technology (NIST) Chemical webbook.

iii. Incorporate the oxygen heterocyele as additive into the copolymer and subject the product to thermogravimeetric analysis (TGA).

iv. Determine the rate constant (ki and kp) data in terms of derived thermodynamic parameters such as entropy, activation energy, free energy.

v. Relate the thermodynamic parameters to the dehydrochlorination process.

1.4 Scope of Study

This study covered:

Extensive literature search for a commercial source of the chemical additive proposed for use in these experiments, namely, simple oxygen heterocycle, or alternatively a convenient and efficient method of preparation (synthesis) of a simple chromene. Ultimately, extraction of precocene I from a plant source was adopted as the popular practice in the various literature surveyed;

Collection and identification of the plant source of the proposed oxygen heterocycle, precocene I;
Extraction and separation of precocene I from other extractives using gas chromatography (GC);

Isolation of separated precocene I by trapping in liquid nitrogen;

Structural elucidation of separated heterocycle using MS, AAS and confirmation using appropriate chemical webbook database;
Incorporation of the additive, 7- methoxy - 2, 2-dimethyl -1-benzopyran (precocene 1), into a vinylidene chloride–methyl acrylate copolymer;

Scanning of the resulting PVDC copolymer – plus – additive product using a Thermogravimetric Analyzer (TGA) for data on thermal dehydrochlorination following incorporation;

Analyzing TGA data for relevant thermodynamic parameters and inferring possible action of the additive on the dehydrochlorination process.

1.5 Significance of Study

The result of this work will hopefully help: to solve PVDC production problems arising from degradation of PVDC copolymers under production temperatures, namely, eliminate the corrosion effect of evolved HCl by preventing such degradation and / or eliminating the degradation altogether;

Provide an avenue for improved preservation of materials coated with PVDC copolymers whether food or pharmaceuticals, and this includes materials of construction.

CHAPTER TWO

LITERATURE REVIEW

2.1 Early Studies of Vinylidene Chloride (VDC) Copolymers

Vinylidene chloride (1,1–DCE) does not appear to have received much mention in the chemical literature before about the 1950's. For instance, a book on polymers, written by Meares, (1965), a prominent author, and published in 1965, discussed the 1,1–disubstituted ethylenes of general formula CH_2**:**CXY (where X and Y are two dissimilar substituents), and merely cited the similarity in the behaviours of these ethylenes as monomers on polymerization with those of other vinyl monomers without going into any details or mentioning cases in which the substituents are the same.

1,1–DCE also does not feature in the more recent book by Parshall and Ittal, (1992) probably because its polymerization procedure does not appear to require sophisticated catalysts, such as those treated in the book, to produce its several copolymers.

Much of the recent research on 1,1–DCE has been carried out in private (company) research laboratories, the most prominent of which is The Dow Chemical Company (2005). Only towards the end of the twentieth century have publications begun to appear in the open chemical literature on 1,1–DCE polymers, much of it published by Howell and his group.

2.2 Characteristics and Uses of Polyvinylidene Chloride (PVDC) Copolymers

The diverse properties and uses of PVDC copolymers make their study rather compelling as well as rewarding both commercially and academically. Dow researchers have reportedly made polyvinylidene chloride into a dark green film which acquired the name *Saran*^{TM} (Cole *et al*., 2003).

Cole *et al.*, (2003) give other uses of *Saran*^{TM} to include habitually and successfully spraying it on U.S. military fighter planes to guard against salty sea spray, thereby preventing degradation due to the rusting process. Car makers have also been reported to habitually use some formulation of *Saran*^{TM} for upholstery. Yet another formulation was devised by Dow that was free of unpleasant odour and green colour, both characteristics arising from the thermally–induced degradation of PVDC. A very important specialty use of *Saran*^{TM} is as *Saran Wrap*, a plastic transparent food wrap introduced in the nineteen fifties, and regarded as the most well known household use of polyvinylidene chloride (Paisley, 2007).

Desirable properties of *Saran Wrap* include good thermal stability in use under ambient conditions, a superior chemical resistance to attack by alkalis and acids, insolubility in oil and most organic solvents, very low moisture regain, and imperviousness to mould, bacteria and insects. It is also used to produce a variety of fibre, both monofilament and multifilament (Cole *et al*., 2003). *Saran*^{TM} is however soluble in polar solvents such as methanol, acetone and ethylacetate. *Saran Wrap* has continued to be in use as food wrap, and such other persistent use as well as variety of use of PVDC has aroused interest in both the kinetics and mechanism of degradation of PVDC polymers to enable amelioration of loss at production temperatures (Strandburg *et al.*, 1991).

Some formulation of polyvinylidene chloride is currently being applied as water – based coating to other plastic films such as biaxially-oriented polypropylene (BOPP) and polyethylene terephthalate (PET) to increase the barrier properties of these films (Paisley, 2007) in their various applications.

Such varieties of use of vinylidene chloride / methyl acrylate (VDC/MA) copolymer formulations have been rationalized on the basis of the chemical structure of the copolymers.

2.3 Chemical structure in the vinylidene chloride / methyl acrylate copolymer.

Copolymerization in the vinylidene chloride / methyl acrylate (VDC/MA) system results in regular structure of the resulting copolymer. This signifies the predominance of the head - to - tail arrangement which has been confirmed for most vinyl polymers examined. Such copolymers have been generally described as unbranched, noncross-linked and therefore highly crystalline (http://www.bing.com). These characteristics are linked to the chemical structures of the reacting monomers as well as to the manner of the attachment of the pendant group along the polymer chains. The two chlorine atoms on the vinylidene chloride unit as well as the methyl carboxylate group on the methyl acrylate group are polar groups and therefore contribute to the polarity of the resulting copolymer. Such polar groups bring about relatively high dipolar forces both intermolecularly and intramolecularly thereby raising the glass temperature. The raised glass temperature is desirable characteristic in copolymers such as PVDC. It is also reasonable to speculate that the two bulky chlorine atoms on one carbon atom joined to neighbouring carbon by a double bond in the monomer 1,1-DCE would provide sufficient steric hindrance to free rotation such that the relative stereochemistry in the resulting PVDC copolymer would not be random but would be predominantly syndiotactic leading to crystallinity to a greater degree and elevated glass temperature. Thus PVDC copolymers are generally high density and high crystallinity with relatively few defect sites. High vinylidene chloride (VDC) content copolymers do however undergo thermally-induced degradative dehydrochlorination at process temperatures, and these degradations have been of interest ever since such copolymers came into use (Matheson and Boyer, 1952). The situation regarding the polarity and therefore the crystallinity of poly (vinyl chloride) (PVC), with which some authors have sought to compare polyvinylidene chloride / methyl acrylate, is different. Poly (vinyl chloride) is a homogeneous polymer. It has

only one polar group, chloride, attached to its chain length. The relative stereochemistry of the chloride centres in PVC is random. PVC is thus largely atactic with only limited crystallinity associated with it (Handbook of Plastic, 2002).

2.4 PVDC Decomposition Studies

Research has enabled the proposal that PVDC decomposition is thermally induced, and occurs in general above 125°C, producing HCl as the sole product (Piringer and Baner 2008). The degradation process is presented in Figure 2.1.

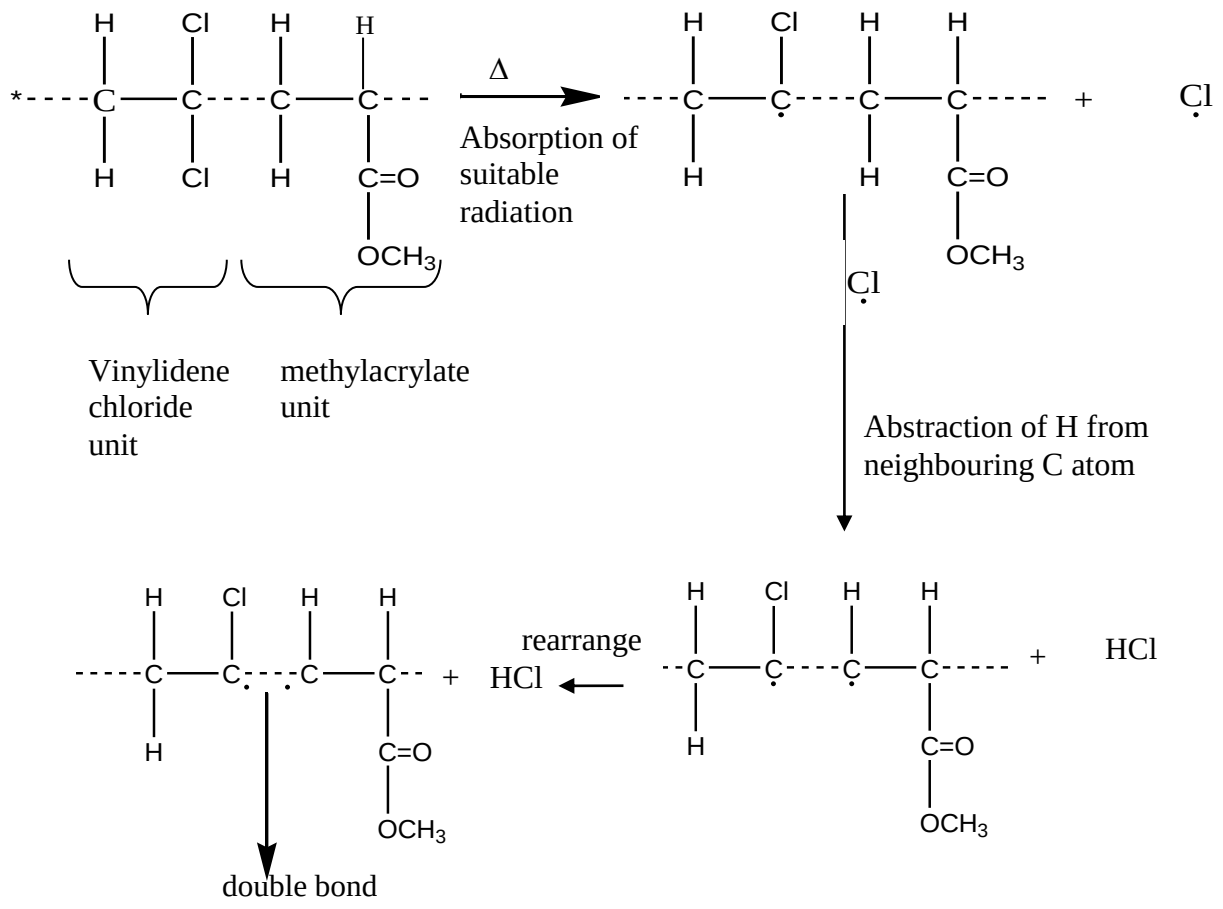


Figure 2.1 Degradation of vinylidene chloride / methyl acrylate polymers (Howell and Liu, 1994).

Homolysis of the C – Cl bond set up a conjugated polyene sequence, which resulted in the coloration of the copolymers, thereby making them unsuitable for use as films or inhibiting their use as barrier films in packaging applications for food and pharmaceuticals (The Dow Chemical Company, 2005).

A second and more serious consequence of the degradation is the evolution of hydrogen chloride which reacts with the walls of process equipment, commonly stainless steel, at process temperatures, to form iron (III) chloride, a strong Lewis acid catalyst which can enhance the dehydrohalogenation process. A major research effort has thus been directed towards obviating this corrosion effect of evolved HCl (Howell and Liu, 1992), as well as avoidance of the regeneration of allylic dichloromethylene (= CCl_2) units in the copolymer main-chain which serve as initiation sites for the degradation and subsequent propagation of the dehydrochlorination reaction (Howell, 1987).

Open literature on this research effort indicates that additives have continued to be introduced into the production mix which would remove hydrogen chloride as it is formed, to prevent the reaction with the walls of process equipment. Such additives have included basic substances, whether passive (for example, magnesium oxide) (Howell and Sastry, 1993) which negatively impact clarity of finished items, particularly for film production, or organic (pyridine derivatives) (Howell and Liu, 1992), which are reportedly too basic to absorb evolved hydrogen chloride. Some of these introduced additives have however reportedly also raised compatibility issues (passive bases), or have proved too basic (hindered amines) to function satisfactorily as stabilizing additives in the copolymers (Howell and Uhl, 2000; Howell and Rajaram, 1993). The search for a solution to the dehydrohalogenation process would appear to be a continuing one.

Thus these two problems, namely, thermal dehydrochlorination of PVDC copolymers at process

temperatures (a production problem) and the degradation of production equipment by the resulting product of dehydrochlorination would need to be solved, using appropriate additives / comonomers without necessarily compromising the compositions of the various formulations on which their various applications depend. The polymer degradation easily propagates, leaving polyene sequences in use long enough to absorb light, and therefore change the colour of the material; worse still, it leads to a significant loss of product during the production process.

A fundamental aim of further research in PVDC production is therefore to minimize this loss by formulating a product in such a way as to reduce to its barest minimum or eliminate completely this loss of material by dehydrochlorination.

Recourse has been made in this direction to the formulation of copolymers with other monomers, such as acrylates and simple vinyl monomers, thus progressively reducing the amount of vinylidene chloride monomer and consequently making the resulting copolymers commercially viable (Wessling, 1977).

2.5 Thermal Stability Studies: Effects of Use of various additives.

Matheson and Boyer, (1952) are among notable researchers who first studied the thermal stability of PVDC and demonstrated that the dehydrochlorination of VDC and its copolymers occurs noticeably at temperatures above 150°C; the dehydrochlorination followed a first order process. The authors also showed that the process fits into a typical chain kinetics involving the well-known initiation, propagation, and termination steps which characterize the polymerization process in general. Figure 2.2 illustrates a typical chain kinetic scheme for vinylidene chloride polymer degradation under a programmed temperature regime (Collins *et al*., 1999).

This chain kinetic scheme has since been applied in studies aimed at elucidating the effects of comonomers and or additives on the rate of degradation of resultant PVDC copolymers.

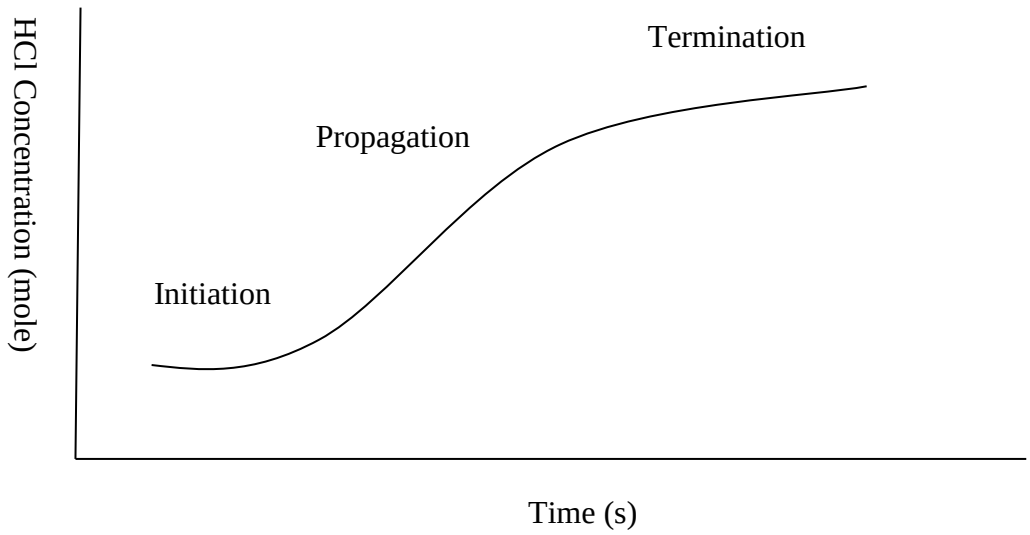


Figure 2.2 Illustration of the Thermal Degradation of a typical Polyvinylidene Chloride Polymer under programmed temperature (Collins *et al*., 1999)

In line with Figure 2.1, the only product observed by evolved gas analysis during such degradation is hydrogen chloride. Monitoring of decomposition is done using thermogravimetric analysis (Howell and Rajaram, 1993; Howell and Liu, 1994)

A major research area in the dehydrochlorination process has been concerned with the effective scavenging of evolved HCl. Howell and Smith, (1988) have explained that these degradations stem from defect structures arising from internal unsaturation (allylic dichloromethylene groups) which serve as initiation sites, and efforts have been made to proffer solutions by seeking to prevent such polyene sequences from arising or building up. While passive bases such as magnesium oxide and tetrasodium pyrophosphate have been suggested and introduced into the polymer melt during processing to absorb evolved HCl and partially overcome this problem, (Howell and Rajaram, 1993), the suggestion has been made that the presence of inorganic bases would negatively impact clarity of finished items, particularly for film applications.

Subsequently, organic bases have been sought which would be compatible with the polymer and also be capable of absorbing evolved HCl. Recent reports indicate that present practice involves the use of some hindered organic bases some of which are still considered too basic for the purpose and may aid in the production of vinylidene chloride – like entities (Howell and Uhl, 2000).

The challenge thus, still remains how best to obviate the degradation of PVDC copolymers without collateral damage to production equipment.

All the papers reviewed in the foregoing adopted the same strategy with regard to finding a remedy for the deterioration of PVDC copolymers through dehydrochlorination. The strategy was to adopt the number and composition of monomer components as in the vinylidene chloride / methyl acrylate copolymer and to study the effect of addition of various chemical entities generally referred to as diluents. Recently some Chinese researchers have adopted a different strategy. Zhao *et al*., (2017), synthesized what they described as a novel copolymer in which they used the usual vinylidene chloride / methyl acrylate system onto which they grafted a third monomer, glycidyl methylacrylate (GMA). The resulting terpolymer was designated as VDC / MA / GMA or PVMG. The chemical structure of Glycidyl methylacrylate is shown in figure 2.3.

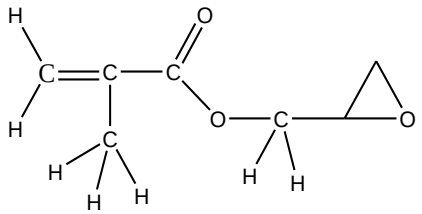


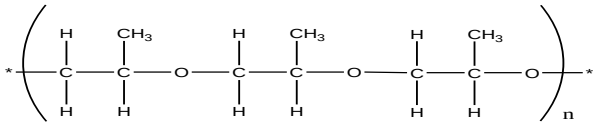
Fig. 2.3 Chemical structure of Glycidyl methylacrylate

The compound is a derivative of methyl methacrylate. It has two component chemical substances, namely, methyl acrylic acid, and propylene oxide. In combination, the propylene oxide blocks the carboxylic group and prevents homopolymerization of glycidyl methylacrylate.

Thus glycidyl methylacrylate is a complex monomer.

Under (appropriate) conditions, the ring-strained propylene oxide polymerizes to chains whose enantiomeric forms are not equivalent and which form branches deliberately introduced in the preparation of graft polymers. This can be seen from the following structures in Fig. 2.4:

(a)



and

(b)

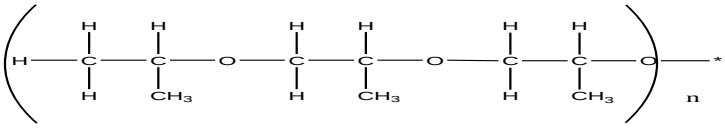


Fig.2.4 enantiomeric forms of polymerized propylene oxide

Such branches are chemically distinct from the main chain but serve to establish cross-links between polymer main chains (Handbook of Plastics, 2002).

The brackets are used to indicate those atoms in the zig-zag chains which lie below the plane of the paper. These zig-zag chains bind to various sites along the copolymer main chain thereby forming cross-links, in addition to stronger cross-links formed by the carboxyl pendant group. Overall result is that the terpolymer PVMG departs from the characteristics of PVDC which make for the varied use of the latter. This was acknowledged by the authors who stated clearly that the existence of GMA caused cross-linking. The novel terpolymer has only recently been described and patented. Future studies will however enable the establishment of its characteristics side-by-side with PVDC. Only a non cross-linked polymer can exist in the viscous state in which it can exist for processing (Handbook of Plastics, 2002).

2.6 Chemical basis for choice of additive

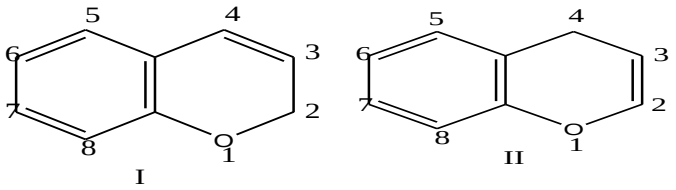
A fact that has been established since the beginning of the systematic study of polymers is that to form part of a chain, an atom must clearly have a minimum valency of two. The oxygen atom in an oxygen heterocyclic organic substance satisfies this condition. The ability of an oxygen atom bound in this way to take part in interparticle interactions (H–bond formation is one of such interaction) also stands it in good stead in providing an extra point of attachment for possible weak interaction with adventitious chemical groups occurring along the chain when conditions for interaction exist and collision probabilities are appropriate. The possibility of incorporating the heterocycle into a copolymer chain, such as would be afforded by the presence of a double bond in the heterocycle, would also make polyene sequences that would occur from dehydrochlorination highly unlikely, as it would tend to separate the units that would favour such conjugation along the polymer chain. There is also a third possibility: a compound with easily detachable H-containing group(s) from which H-abstraction can occur.

The chromenes offer a typical example of the oxygen heterocycle proposed for study in this project. Simple chromenes are generally stable. The double bond in the 3 : 4 position in these chromenes is stabilized by conjugation with the benzene nucleus but offers an extra point of attachment for any adventitious group or even for attachment to the main polymer chain. Also, H-abstraction from a chromene molecule would be quite competitive from any of its several H-atoms compared with H-atoms from the copolymer chain. Furthermore, chromene derivatives, which include their chromene – 2 – one counter parts, have been associated with hypothermal characteristics (Soine, 1991) and may well play roles in mixtures or in combination that would affect heat absorption by components of the combination or mixtures. A fifth possibility is therefore that the effect of adding a low molecular weight substance to a polymer is to lower

some aspect of the heat requirement of the polymer, such as the latent heat of melting, or energy required to sustain the glassy state.

2.7 Chromenes

Bicyclic oxygen heterocycles that result from the fusion of benzene ring with 5, 6 – positions of either 2H– or 4H– pyran ring systems are designated as 2H - chromenes (2H - 1 - benzopyrans), and 4H – chromenes (4H – 1 – benzopyrans) (Ellis, 1977). The structures of these isomeric substances are shown in Figure 2.5.



2H – Chromene
(2H – 1 – benzopyran)

4H – Chromene
(4H – 1 – benzopyran)

Figure 2.5 Chromene Isomers

Their Chromanone derivatives are similarly named.

Several studies have been carried out on this class of natural products along their biological property lines by Isman *et al*.,(1986),Okunade (2002), and by Ribeiro *et al*., (2010) to mention but a few, and reviews are available (Kamboj and Saluja, 2008); Pratap and Ram, (2014). The pyran moiety in a 2H - benzopyran molecule has an active double bond in the 3 : 4 position which is retained in several of its derivatives, but which has been shown in recent microbial studies of the biotransformation of precocene II to lead to identifiable metabolites through the opening up of this 3:4 double bond (Sariaslani *et al*., 2014). Pratap and Ram, (2014) have reviewed the natural and synthetic chromenes with emphasis on their versatility in Organic Synthesis. The 3 : 4 double bond of several chromenes, both synthetic and natural, tend to be

stable with respect to chemical reaction. The two rings present in the molecule tend to reinforce each other.

2.7.1 Occurrence of Chromenes.

2H-Chromene derivatives have been described as natural products known to be widely distributed in nature and specifically present in the plant genus *Ageratum*, a precocious flowering plant (Burkill, 1985). Isolations of chromenes from this plant genus have been done from the species *Ageratum houstonianum* (Dike *et al*.,1991), from *Ageratum fastigiatum* (Del-Vechio *et al*., 2008), as well as from *Ageratum conyzoides* (Gonzalez *et al*., 1991). One of the most studied of the genus *Ageratum*, namely, *Ageratum conyzoides* (AC), has been known to contain several chromene derivatives (figure 2.6) in its phytochemistry and these derivatives include coumarins, as well as precocenes I, II and III. Both coumarins and precocenes are chromene derivatives with the difference that precocenes have dimethyl groups in the C – 2 position in place of a carbonyl group (ChEBI, 2016).

Kamboj and Saluja (2008) reported that the essential oil of AC was a complex mixture of 213 compounds of which about 51 have been identified, while Ekundayo *et al*., (1988) had stated that among the major constituents of the essential oil of AC, the oxygenated sesquiterpene hydrocarbon comprised ageratochromene (32.90%).

According to Rana and Blazquez, (2003), the volatile oil obtained from the aerial parts of AC amounted to 30%.

Figure 2.6: Structure of chromenederivatives

No further clarification was given regarding the substance ageratochromene, which would suggest that this name was grouped among other members of a family of similar substances present in the essential oil or reflected the origin of the substance, as it turned out to be. The most common components of the essential oil of AC were identified in a paper by Katsuri *et al*., (1973) as 7-methoxy-2,2-dimethylchromene, (precocene I); 6,7-dimethyl derivative of ageratochromene (precocene II); and ageratochromene dimer (Katsuri *et al*., 1973).

The chemical structure of ageratochromene dimer was subsequently described by Fraga *et al*., (1999) in attempts to generate data on insect antijuvenile hormones (AJH), as well as to study whether precocenes having more than a 2,2-dimethylpyranyl ring are able to induce strong activity as anti juvenile hormones (Fraga *et al*.,1999).

A generalized structure of some functionalized 2H-chromenes is presented in Figure 2.7 (Pratap and Ram, 2014).

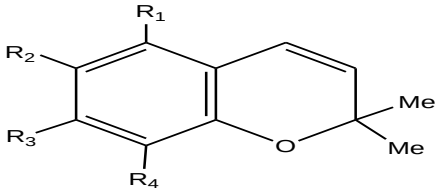


Figure 2.7: Structure of functionalized 2H-Chromenes

$R_1 = R_2 = R_4 = H$; $R_3 = OMe$,	Precocene I	or	6 - Demethyoxychromene
$R_1 = R_4 = H$; $R_2 = R_3 = OMe$,	Precocene II	or	Ageratochromene
$R_1 = R_3 = R_4 = H$; $R_2 = AcO$,	Precocene III	or	6 – Acetylchromene
$R_1 = R_4 = H$; $R_2 = AcO$; $R_3 = OH$,			Eupatoriochromene

2.7.2 Availability of Chromenes.

Various substituted 2H-chromenes have been reported to be widely distributed in nature and are isolated from their natural sources for use in traditional medicine practice (Dean, 1963). These natural sources include plants within the tribe, *Eupatorieae*. Family *Ageratum* is a member of

this tribe. It also includes the plant *Eupatorium odoratum* L., an invasion plant (Owolabi *et al*., 2010). Thus, one method for sourcing chromenes is by extraction from their natural sources. Considered as terpenoids, the chromenes would be classed among the phenylpropanoids and would most likely be extracted from the mainly terpenoid essential oil of a plant such as AC. Such an essential oil, according to Rana and Blazquez, (2003), is a complex mixture of compounds. Chemically, however, terpenoids are generally lipids-soluble compounds. According to Harbonne (1998), they are normally extracted from plant tissues with light petroleum, ether or chloroform and can be separated by chromatography on silica gel or alumina using the same solvents. They are generally not soluble in hydrophilic solvents such as water or ethyl alcohol (ethanol)

Separations using these procedures have their associated problems which Harbonne has enumerated (Harbonne, 1998), and include the diverse chemical entities present in essential oils as well as the varying quantities in which they appear. Both of these associated conditions make extraction and separation into separate constituents a particularly challenging task. Only the recent availability of the gas chromatograph – mass spectrometry (GC-MS) instrumentation with its associated Agilent programming system has it been presently possible to attempt separations as well as isolations (GC) and subsequent identifications of constituent peaks (MS) of components with relative ease (Owolabi *et al*., 2010). Percentage yields, particularly of high boiling substances in such essential oils are however abysmally minimal. Owolabi *et al*., (2010) identified each of the individual fifty – five (55) components in the essential oil of *C. odorata* based both on their retention indices, RI, on the GC., as well as by comparison of their mass spectral fragmentation patterns with those reported in the literature. The highest concentration of 42.2% was obtained for α-pinene component, followed by β-pinene (40.6%). Both α- and β-

pinene are C_{10} monoterpene isomers of relatively low boiling point (Finar, 2004). The solvent extraction with ethanol (Adebayo *et al*., 2011) gave higher concentrations of mostly sugar adducts (Figure 2.8). No free precocenes were reportedly isolated.

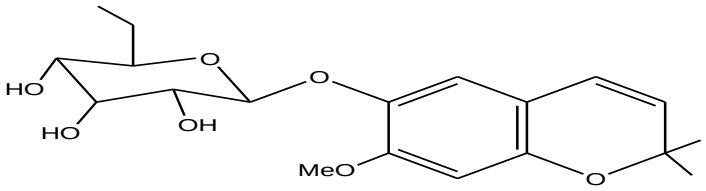
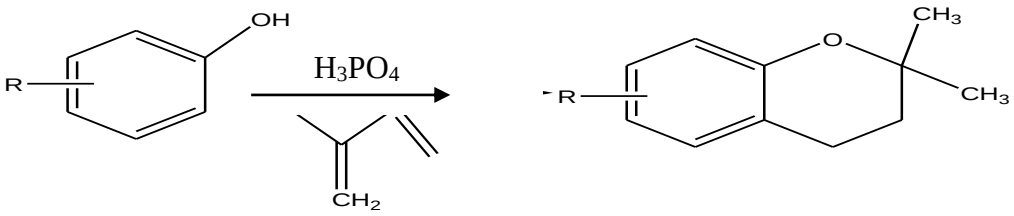


Figure 2.8 2,2 – Dimethylchromene - 7 - methoxy - 6 - O-β-D-glucopyranoside (Adebayo *et al*., 2011)

2.8 Synthesis of Chromenes

Aside from extraction and isolation from their natural source in the tribe *Eupatorieae*, attempts have been made at synthesis of chromenes, as well as their saturated counterparts, the chromans from which they may be derived as shown in Figures 2.9, 2.10, and 2.11. Phosphoric acid (H_3PO_4), was used to attach isoprenes to phenols, leading to the synthesis of 2,2–dimethylchromans (figure 2.9) (Ahluwalia and Arora, 1982):



Phenol Isoprene Chroman
(Saturated benzopyran)

Figure 2.9 Scheme for the Synthesis of 2,2–dimethylchromans (Ahluwalia and Arora, 1982):

The method of Ahluwalia and Arora was in 1997 modified by Kalena *et al*., (1997) who explored the use of a solid catalyst, the macroreticular sulphonic acid exchange resin, Amberlyst 15 for the condensation reaction. This led to a one–step synthesis of 2,2-dimethylchromenes using 3-

hydroxyl-3-methylbut-1-yne as chromenylating agent this is shown in figure 2.10 (Kalena *et al*., 1997):

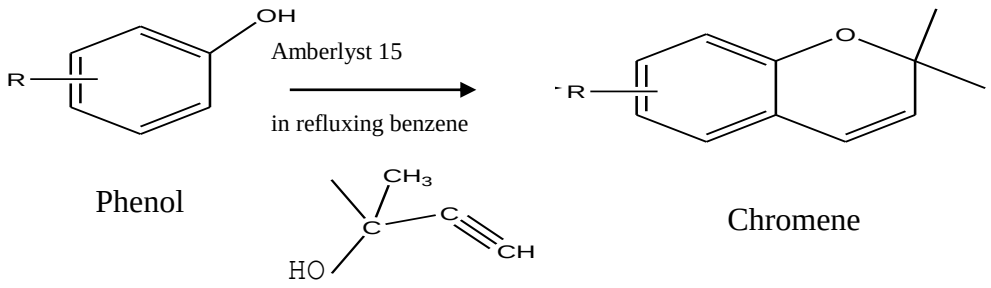


Figure 2.10 Scheme for the Synthesis of 2,2-dimethylchromenes (Kalena *et al*., 1997):

In both of these synthetic routes, yields were reported to be less than 10% in the acid process for chromenes, and 25% in the process with solid Amberlyst 15. Several undesired side-products were obtained in each case, including compounds with more than one pyran ring, as well as some containing the hydroxyl group which were difficult to separate using the separation tools that were available.

The precocenes had earlier been synthesized via 4 – chromanones produced by condensation of appropriately substituted phenols or phenol derivatives with dimethylacrylic acid or its chloride (Bowers *et al*., 1976).

An alternative approach was reaction of appropriate coumarins with methyl magnesium halides and cyclization of the product of this reaction by acid treatment and / or distillation (Hepworth and Livingstone, 1966).

Yields were reported to be 44% for 7 – methoxycoumarin as starting material for the corresponding 2,2 – dimethylchromenes using methyl magnesium iodide. Strunz *et al*., (1983) however reported later that the desired transformation to precocene I could be accomplished with substantially improved yield (73%) by treatment of the resulting product, herniarin with methyllithium (figure 2.11) and, after careful work-up, cyclization of the phenolic alcohol

product under mild conditions on silica gel. The initial step, methylation of the phenolic hydroxy group of umbelliferone, was executed by refluxing the latter with tetramethylortho carbonate in the presence of a catalytic amount of p-toluenesulfonic acid. The methyllitium-silica gel procedure applied to coumarin itself afforded 2,2-dimethylchromene in 83% yield.

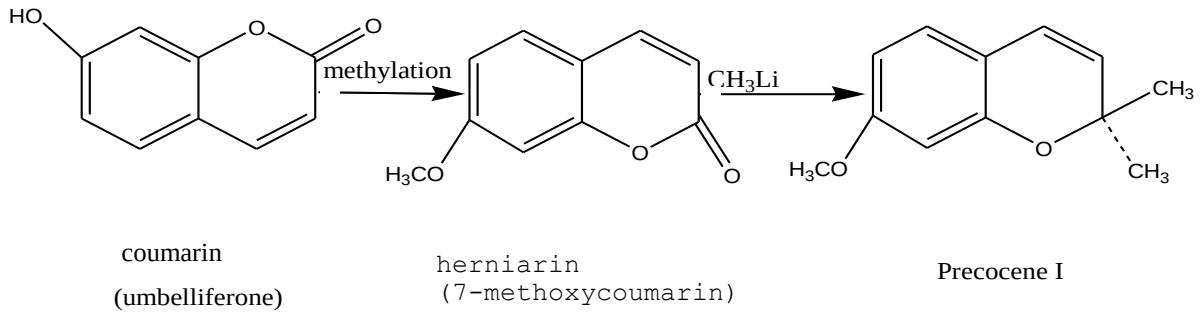


Figure 2.11 Scheme for the transformation of coumarin to Precocene I (Strunz *et al*., 1983)

The alkyllithium step, including the Grignard reagent step required an atmosphere of dry nitrogen to be effective.

There is yet another synthetic method that has been used to produce precocene I. Bissada *et al*., (1994) recorded what they described as a novel and efficient route. This route involved heating four substances in benzene, namely, 3-methoxy phenol, 3-methyl-2-butenal, phenylboronic acid and propanoic acid. By-product water was removed using a Dean – Stark apparatus. The product, precocene I was isolated in 95% yield. The synthetic product was checked for authenticity against the compound isolated by extraction from its natural occurrence, namely, *Ageratum houstinianum*. Details of the solvent used for extraction of the natural product were not given, neither was the procedure for the separation of the extract described. The novel aspect of this method of synthesis was the use of phenylboronic acid as the condensation acid of choice rather than phosphoric acid as used by some earlier workers.

Details of the use of phenylboronic acid in synthesis have subsequently been documented by Chauder *et al*., (1998) and applied by Hiramatsu *et al*., (2013) in their search for antifeedant activity of assorted pyran derivatives against heteropteran insects, grasshoppers, and cockroaches. The synthetic precocene I prepared by the phenylboronic acid procedure and isolated by silica gel column chromatography (CC) was identified by comparison of its physical characteristics with a sample which the authors reportedly extracted from *Ageratum houstinianum*. There were however some irreconcilable differences in some spectroscopic properties of the synthetic products as quoted by Bissada *et al*., (1994) and Hiramatsu *et al*., (2013).

In all these available synthetic methods, the separation of main product Precocene I from associated side-products constituted major problems even in cases where a 95% yield of the required precocene I was reportedly recorded (Hiramatsu *et al*., 2013). These associated side-products included dipyran ring analogues, as well as excess trihydroxyacetophenones used as starting materials for the synthesis.

Thus extraction has remained the option of choice for the availability of simple 2H-chromene derivative such as prococene I. Plants of the family *Asteraceae*, such as *Ageratum conyzoides*, are readily available in Nigeria for extraction purpose.

2.9 *Ageratum conyzoides* Linnaeus

2.9.1 Classification and use

Ageratum conyzoides L. was described by Dalziel, (1937) as a non-food plant. It is used increasingly in ethnomedical practice for the treatment of diverse ailments (Adebayo, 2009).

The following is the scientific classification of *Ageratum conyzoides* L.

Kingdom	*Plantae*
Order	*Asterales*
Family	*Asteraceae*
Tribe	*Eupatorieae*
Genus	*Ageratum*
Species	*A.conyzoides*
Binomial name	*Ageratum conyzoides L.*

2.9.2 Description of the Plant *Ageratum conyzoides*

Kamboj and Saluja, (2008) reviewed the then existing literature on *Ageratum conyzoides* (Billygoat-weed, Whiteweed) and described it as native to Tropical America, especially Brazil, herb is 0.5-1m high, with ovate leaves (2-6cm long) and flowers which are white to mauve. *Ageratum* or white weed is a genus of 40 to 60 tropical American herbs, annuals and perennials from the Sunflower family *Asteraceae*, tribe *Eupatorieae*. The herbs form tussocks or small hills. They grow to average height of about 75cm. The opposite leaves (formed on the stem) are cordate or oval, hairy or tomentose. The margins are slightly toothed or serrate. The fluffy flowers are white to lavender – blue (or mauve) and spread in small compound umbels. They give small dark fruits. The word *Ageratum* is derived from the Greek words *a geras* meaning non-aging and referring to the longevity of the plant; *conyzoides* is derived from *konyz*, the Greek name of *Inula helenium* which the plant is said to resemble. According to the Review the genus *Ageratum* consists of about 30 (thirty) species but only a few species have been

Picture of *Ageratum conyzoides*

Plate 2.1: The plant *Ageratum conyzoides* Linnaeus

phytochemically investigated. The family *Asteraceae* is well marked in their characteristics and cannot be confused with any other; there is however scant reference in the literature to species other than *conyzoides*.

2.9.3 Phytochemistry of*Ageratum conyzoides*

The essential oil obtained from the plant has been reported to have a powerful nauseating odour, and found to be poisonous to rabbits due to the presence of HCN and coumarin(Kamboj and Saluja, 2008). Dalziel, (1937) appeared to have been the first author on record to have stated the non-food use of the plant as well as its use for medicinal purposes. Currently, members of the family *Asteraceae* are largely regarded by Agriculturists and Environmentalists as invasive weeds (Zacchariades *et al*., 2009) and as sources for chemical constituents which are subjects of extensive studies as potential anti juvenile hormone (AJH) agents. These chemical constituents have been named as chromenes, and precocenes I and II in particular. Precocene I has only recently been reported by Mao *et al*., (2010) to have significantly reduced the termite soldier proportion in a termite colony within 40 days.

The phytochemistry of *A. conyzoides* is also quite extensive but low in details. Kamboj and Saluja, (2008) stated that the oil content varies from 0.11 to 0.58% (leaves), and from 0.03 to 0.18% (roots) depending on times of the year and that water distillation of the fresh flowers results in an oil content of 0.2%. These quantities contrast with figures given by Ekundayo *et* al., (1988) and by Rana and Blanquez, (2003). The yield of oil from the petroleum ether extract of the seed was however as high as 26%. Kamboj and Saluja, (2008) also added that the GC-MS analysis of the essential oil of *A. conyzoides* showed that it is a complex mixture of over two hundred compounds consisting of monoterpenes, monoterpenoid hydrocarbons, oxygenated monoterpenoids, sesquiterpenes, sesquiterpenoids, phenylpropanoids and benzenoids. Ekundayo

et al., (1988) gave the exact figure of components as 213. The ethanolic extract of the plant was found to be devoid of tannins. Sujatha *et al*., (1988) had stated earlier that germane to pest control studies was the report that extracts from the plant *Ageratum conyzoides* induced morphogenetic abnormalities in the formation of mosquito larvae, but no active substances were quoted. The subsequent report by Adebayo, (2009) in which various solvents were used to extract active phytochemicals from the leaves of *Ageratum conyzoides* suggested that the petroleum ether and ethylacetate extracts in particular possessed antioxidant, antiviral and anticancer activities and that flavonoids and some chromenes isolated from the plant could be responsible for these activities. Hussien *et al*., (2010) re-investigated the CH_2Cl_2 extract of the air dried aerial parts of *Ageratum conyzoides* obtained from Egypt and isolated a substituted benzopyran which they described as a known compound but did not name. In addition to this, they reported the isolation of a new compound (pyrrolone) which they characterized by MS and by spectroscopy as a yellowish oily material, R_f = 0.40 on TLC using diethylether (Et_2O) / petroleum ether in 2 : 1 ratio; Ir absorption bands at 3350 cm^{-1} (NH) and 1706 cm^{-1} (CO); molecular ion peak at *m/z* 128 (M + H); and molecular formula $C_6H_9O_2N$. This and similar identifications have continued to appear in the chemical literature using GC-MS instrumentation and have been applied to complex mixtures such as the Chinese Herbal Formula *Wu-Zhu-Yu* (Xu *et al*., 2016)

Chromenes were also isolated from the essential oil of the leaves of the plant *Chromolaena odorata* (also known as *Eupatorium odoratum*) in the hydrodistillate obtained by Owolabi *et al*., (2010). Both precocenes I and II were absent in the essential oil obtained and analyzed. The specific chromene, precocene I, was found in trace quantities in the distillate analyzed by GC-MS. *Ageratum* belongs to the same tribe *Eupatorieae* as *Chromolaena odorata*.

2.9.4 *Ageratum conyzoides* the Choice as Source of Precocene I

The choice of *Ageratum conyzoides* (AC) as source for precocene I was based on the demonstrated presence in reasonable quantities of this chemical substance in this plant. The choice was also based on the abundance of the plant in this part of the world, and was supported by the observation that the plant is one on which ants and insects do not feed or under which they do not nestle, hence the research on chromenes as possible AJH agents. This is confirmed in several documentations on AC which lay emphasis on its medicinal properties and on the fact that it is limited to external use only due to its toxicity (Kamboj and Saluja, 2008).

No reliable statement was made in the literature reviewed about the amount of precocene I obtainable from associated plants apart from the trace obtained by Owolabi *et al*., (2010). Therefore various parts of *Ageratum conyzoides* were exploited in the experiments that follow for their yields. Also various solvents were used in order to compare quantities extracted in all cases in which the chemical substances manifested.

A second consideration is that details of experimental conditions for the separation and isolation of the precocenes do not appear to have received much attention in the literature. For instance, where column chromatography or TLC was used, R_f values were not given; in cases where GC – MS instrumentation was used, GC retention times were not listed and no statement was usually made about the method of isolation. It was therefore thought necessary to use a standard mixture obtained from a plant source that would contain no chromene content. The standard mixture for use was considered to be a chloroform extract of the leave of *Moringa oleifera*, a plant the phytochemistry of which is known from the literature not to contain any of the chromenes or chromene derivatives.

2.10 *Moringa oleifera* (MO)

The plant, *Moringa oleifera*, has been described as the best-known species of the family *Moringaceae* which has just one genus, *Moringa* , and fourteen species. These fourteen species make up the *Moringa* genus. (Morton, 1991)

Morton, (1991) also asserts that every part of the *Moringa* plant is used in traditional medicine in Africa, Asia and America.

An abridged review of the medicinal and food uses of *Moringa oleifera* has been prepared by Ozumba, (2008). The nomenclature of this plant as listed by Ozumba include,

Nomenclature

Family	*Moringaceae*
Order	*Brassicales*
Genus	*Moringa*
Species	*Moringa oleifera*
Name	
Horseradish tree	(English)
Ben aile	(French)
Okwe oyibo	(Igbo)
Ewe igbale	(Yoruba)
Zogole	(Hausa)

Cordell, (1981) has stated that the oral history of *Moringa oleifera* is claimed to be copious on the benefits accruable from the treatment or prevention of disease or infection from either dietary or topical administration of *Moringa* preparations in the form of extracts, decoctions, poultices, creams, oils, emollients, salves and powders but that scientific details are not quite well known.

According to Cordell, (1981), major uses of *Moringa oleifera* include the following:

The juice from the leaves is believed to have a stabilizing effect on blood pressure and is administered in the treatment of anxiety and anxiety-related symptoms, malaria, jaundice, as a skin antiseptic, and in malnutrition.

The whole plant shows antibacterial properties.

The poultice prepared from the leaves is claimed to be effective against inflammations and migraine headache; it is thus used in the treatment of rheumatism, as well as for the relief of lower back or kidney pain. Same is also claimed for the root poultice.

The ash from the root is used in the treatment of splenosis. The flowers and the root are claimed to contain the antibiotic pterygospermin which is highly effective on cholera and at high concentration functions as a fungicide.

Information from Fahey, (2006) on *Moringa oleifera* lays considerable emphasis on its commercial and food value, while its use in detoxification as well as in the treatment of digestive disorders is only recently beginning to receive more detailed study to unravel the active materials implicated in such use. For instance the antibiotic activity of *Moringa oleifera* is an area in which extensive anecdotal evidence is available (Eilert *et* al., 1981; Cordell, 2000). Much of the scientific evidence centres on a compound which researchers claim to have isolated from the flower of *Moringa oleifera* and which was named as pterygospermin (Das *et al*., 1957). A molecule of pterygospermin was reported to readily dissociate into two molecules of benzylisothiocyanate. Isothiocyanates are chemical compounds that contain the carbon-sulphur double bond.

The phytochemicals of *Moringa oleifera* are also claimed to afford a variety of fairly unique compounds and in particular those containing the simple sugar rhamnose (Das, *et al*., 1957). The

identity of pterygospermin on which evidence for the antibiotic activity of *Moringa oleifera* is based, was however challenged by some authors who suggested that pterygospermin was not a true molecule but was an artifact of the process of isolation or of structural determination(Eilert *et al*., 1981).

Haristoy *et al*.,(2005) later claimed to have verified and confirmed the identity of 4–(α- L – rhamnopyranosyloxy) benzyl isothiocyanate shown in Figure 2.12as well as its activity against a range of bacteria and fungi, but pterygospermin remains a mystery molecule. The structure for the sugar-benzyl isothiocyanate adduct was given by Haristoy *et al*. as:

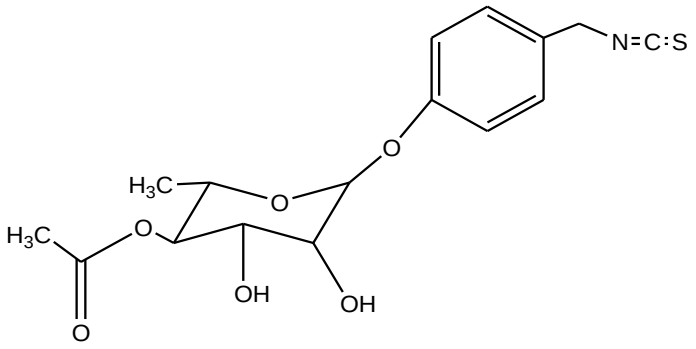


Figure 2.12: Benzyl isothiocyanate – rhamnose sugar adduct (Haristoy *et al*., 2005)

According to Ozumba, (2008), the root bark of *Moringa oleifera* is used in India to prevent enlargement of the spleen and formation of tuberculous glands of the neck, to destroy tumors, and to heal ulcers, but no active substances responsible for this were reported. Mazumder *et al*., (1999) published a paper in which a methanolic extract of the root of *Moringa oleifera* was found to contain some alkaloids but whose chemical structures were not given. This was followed up by Bose, (2007) who considered the anticancer potentials of plants used in folk medicine of Bengal and in which extracts of some of the plants were explored as potential sources of anticancer compounds. According to Bose, the only herb that has been shown to play a role in the treatment of female reproductive disorders is *Moringa oleiferaLam*, whose

effectiveness is derived from a combination of antitumor and hormonal properties. The effectiveness of the *Moringa oleifera* plant in treating ovarian cancer according to him apparently became evident after the publication of studies which demonstrated that benzylisothiocyanate (BITC) and phenethyl isothiocyanate (PEITC) induce apoptosis in ovarian cancer cells *in vitro*.

Thus, *Moringa oleifera* does contain a range of fairly unique compounds. Various reports on the plant and reviews above do not give explicit or reliable information on the details of the plant phytochemicals including their isolation, if any, or structure. What is certain from this review however is that there is no mention of the occurrence of chromenes and coumarins in *Moringa oleifera*. *Moringa oleifera* thus became the referral point, to be used as control in extractions and isolations of chromenes from *Ageratum conyzoides* in which chromenes have been reported to be present. It would hopefully be appreciated from above review of the literature that a major problem in the use of chromene derivatives for further studies of their behaviours in chemical systems is the sparse documentation on the experimental conditions for obtaining them in reasonably pure state and of course identifying them as such.

2.11 Separation, Isolation and Characterization of Mixtures:

2.11.1 Methods of choice for Separation and for Isolation.

There is a great variety of choice in separation methods and the method of choice is generally dependent on what is available to the researcher or what is deemed suitable for the mixture to be separated. Experience shows however that thin layer chromatographic (TLC) method may be used not necessarily to achieve effective separation but to qualitatively test separability. Multi-component mixtures such as extracts are not usually amenable to separation by TLC. The separation and detection of components from a mixture of organic compounds is readily

achievable by gas chromatography. Furthermore limited characterization of unknown components is often possible from retention times appropriate to the particular column used. For instance, Owolabi *et al*., (2010) identified volatile oil components based on their retention indices (RetIndex) determined with reference to a C_9 – C_{21} homologousseriesof normal alkanes before comparison of their mass spectral fragmentation patterns with those reported in the Essential oils literature and stored in the NIST database. On the other hand, mass spectrometry, because of its high sensitivity and fast scan speeds is the technique most suited to provide definite structural information from the small quantities of material eluted from a gas chromatograph, hence its increasing use. RetIndex is however a derived parameter. Its primary data come from the retention time relative to a standard, which is not always available. However, the association of the two techniques in a separation-and-characterization process provides a powerful means of structure identification for the components of natural and synthetic organic mixtures. It is here proposed that the GC-MS procedure is the most ideal for separation, isolation (GC) and characterization (MS) of such extracts as would be expected in this project. It is necessary, nevertheless, to bear in mind that even under optimized elution conditions, matrix – derived signals in the instrumental procedure make finding characteristic compounds quite challenging, sometimes resulting in measurements of only a few peaks or distinguishing them from the background total ion chromatogram (TIC).

2.11.2 GC–MS

The Gas Chromatography-Mass Spectrometry (GC–MS) technique is a combination that is highly compatible and efficient, the gas chromatograph (GC) separating volatile and semi volatile compounds with great resolution, but cannot identify them, whereas mass spectrometry can provide detailed structural information on most compounds such that they can be exactly

identified, but cannot separate them. Ho, (1990) has placed on record the fact that Gas chromatography-mass spectrometry (GC–MS) is acknowledged as a very powerful and ubiquitous analytical technique which has become the analytical method of choice in toxicology, forensics, food science and environmental research. GC–MS instrumentation has virtually replaced the traditional thermal-conductivity detector (TCD) or flame-ionization chromatographic detector (FID) with a very sensitive and information-rich mass spectrometer (MS). This hybrid instrument provides two separate dimensions of information about the components in a complex sample, namely, GC retention times and electron ionization (EI) mass spectra. GC retention time is related to specific chemical properties of the molecules in question (for example, polarity, presence or absence of specific functional groups) while molecular weight (derived from mass spectrum) is indicative of atomic composition. A careful analysis of a fragmentation pattern in the MS can be used to determine the connectivity of the atoms in the original molecule. This is an important step towards structure determination.

Because of its versatility, GC–MS was the major instrument of choice in the analytical procedure reported in this project. Both the qualitative identification and the quantitative measurement of individual components in the complex mixtures studied were affordable by the method and were exploited as much as was possible both to separate (GC) and to isolate and characterize, in order to illustrate the versatility of the method. According to Finar, (2004), Gas chromatography has been particularly useful for isolating terpenoids and for determining their configurational forms from extraction mixtures or from those produced by synthesis.

2.11.3 Information Obtainable from Data from GC-MS

Ideally, the molecular weight of a compound can be directly inferred from its mass spectrum which is the only means by which such an important physical parameter can be obtained from a

physical method of analysis. This information, in combination with the mode of operation of the GC-MS, almost always results in spectral determination of the structures of substances studied. For instance, the SCAN mode affords the continuous and repeated ramping of the monitored *m/z* ratio from a preset lower limit to a preset upper limit, generating a series of complete mass spectra. At the conclusion of each individual scan, the intensities of all the *m/z* ratios within the scan are summed, giving a total ion current. A chromatogram is then constructed by plotting the series of total ion current versus retention time called the total ion chromatogram (TIC). Analyzing a GC-MS chromatogram obtained in the SCAN mode therefore consists of selecting the portion of the TIC that corresponds to a given peak and extracting the mass spectra from that time period.

In the select-ion monitoring (SIM) mode, on the other hand, the Quodrupole remains fixed on a small set of *m/z* ratios, effectively allowing only those predetermined masses to pass through the detector. An analysis in the SIM mode is useful when one is looking for small quantities of (known) compounds under circumstances in which they cannot be separated from other compounds chromatographically, that is, in very complicated mixtures such as in phytochemical situations. In addition, the SIM detection scheme often yields substantially lower detection limits than the SCAN mode as more time is spent monitoring the *m/z* of interest. It also minimizes spectral skew. Automated data collection is also afforded in GC-MS in which upwards of 100-position auto samplers allow the GC-MS to record data without user intervention, thus allowing ample time for data interpretation rather than data collection. Software such as Agilent's Chem Station 1701 DA, are now available as aid to such analysis. Such software enhanced the ability of the instrument to perform effective library searches, mass chromatographic manipulation of data, and produce at least relative quantification of chromatographic peaks. For instance, Hites

and Biemann, (1970) have clearly demonstrated that the data system in mass chromatography sorts through already collected GC-MS data looking for the presence and intensities of certain specified ions which are chosen to characterize specific compounds or compound types expected in the sample. As a further aid to structure elucidation, Response Factors may be used, wherever possible, to provide an alternative to the generation of a calibration curve for each analyte (similar to Beer's law plots in optical spectroscopy) when quantitation is to be attempted. Both the SCAN and SIM modes were used in these studies whenever it was possible or necessary to elucidate the structure determination steps of any desired component. In addition, selection of likely molecular formulae appropriate to mass and isotope abundance measurements was greatly facilitated by data obtainable from Beynon's expanded Table (1960) based on the standard C= 12.000000 up to about mass 250 Da. The Table has become available in abridged form in subsequent volumes on mass spectrometry, such as the book by McLafferty and Turecek, (1993).

2.12 Complementary Identification Techniques

2.12.1 Spectrometry

Infrared (IR) Spectrometry can provide information on aromatic positional isomers that is not available with GC-MS. IR is usually 2 to 4 order of magnitude less sensitive than GC-MS. It may however be used effectively in cases where pure components have been isolated. A second identification spectrometric technique is nuclear magnetic resonance (NMR) spectrometry which can provide detailed information on the exact molecular configuration but again is usually much less sensitive than GC-MS. The technique is useful for detailed attachments of H – species to carbon atoms, again where pure components have been isolated. This is the case for the usually available ^{1}H – NMR equipment as well as ^{13}C- NMR equipment.

2.12.2 Thermogravimetry (TG)

Thermogravimetry (TG) is the branch of thermal analysis which examines the mass change of a sample as a function of temperature (in the scanning mode) or as a function of time (in the isothermal mode) (Hatakeyama and Quinn, 1999).

In this method of analysis, changes in the mass of sample are studied while the sample is subjected to a program in which changes in temperature affect the sample. Thermal changes which bring about changes in mass of a sample include desorption, absorption and decomposition. Thus volatile products or gaseous products lost during the reaction in thermoplastics, thermosets, composites, films and similar materials can be collected and analyzed (Handbook of plastics, 2000).

A thermogravimetric analyzer was used to record data on the kinetics of the dehydrochlorination of the vinylidene chloride – methylacrylate – additive copolymer after incorporation of the additive.

2.13 Observations on the Literature Review

It may be appropriate to record some observations regarding the material reviewed in the literature.

The first observation is that there is to the best of our knowledge, no information in the literature regarding any published studies already carried out on the effect of any oxygen heterocycle on either polyvinylidene chloride (PVDC) copolymers or on any other polymeric material.

The second observation which follows from the first is that the chemistry of the chromenes is yet to be fully explored. Metabolism of precocene II in insects has been studied by Burt *et al*., (1978), and by Haunerland and Boyers, (1985), and parallels drawn between the formation of carcinogenic bay region diol epoxides from polycyclic aromatic hydrocarbons in mammals and

the bioactivation of precocenes by insects and rats (Halpin *et al*., (1982); Pratt *et al*., (1980). Both precocenes I and II are currently widely and increasingly used as tools in experimental arthropod endocrinology. They are consequently considered as prototypes of fourth-generation pesticides. There have also been further studies on insect antijuvenile hormones (AJH) structurally related to 2.2 – dimethyl –7– methoxychromene and 6,7– dimethoxy – 2,2 – dimethylchromene (precocene I and II, respectively) (Brookes *et al*., 1988). Thus although there is evidence of bioactivation of precocenes in animal studies, no reports on the activation of chromenes in general or precocenes in particular either alone or in chemical reactions with other entities, have been encountered in the chemical literature available for review in this project.

Not much of the chemical reactions involving precocene I and II is available in the chemical literature. Pratap and Ram, (2014) suggested that the several chromenes and coumarins extracted from their natural sources and reviewed by them are resources for funtionalization and further exploitation.

Thirdly the establishment and use of standards, controls or reference points serves as a useful aid in qualitative and quantitative science. It is so particularly in chemistry.

Mass chromatographic screening of samples in Forensic Chemistry, for instance, make use of occurrences and or sums of intensities of specific numbers of ions characteristic of different compounds or compound families to determine the presence or absence of such compounds or compound families in given samples (qualitative screening) and to determine amounts present (quantitative screening). Examples of ions so used and the types of compounds screened are tabulated in Table 2.1.

Table 2.1 Ions used in mass - chromatographic analysis of arson accelerants(Ho,1990)

Compound type	*m/z*
Aliphatic	57, 71, 85, 99
Alicyclics and olefinics	55, 69, 83, 97
Alkyl benzenes	91, 105, 119, 133 and also 78, 92,106
Alkyl styrenes	104, 118, 132, 146

No similar data have been located in the chemical literature for the chromenes, although Ho, (1990) has also given two *m/z* values, at 93 and at 136, that serve as aid for the screening of monoterpenes ($C_{10}H_{16}$).

Some of the papers reviewed have however cited the M^+ value for precocene I. Hiramatsu *et al.*, (2013) gave the value as *m/z* 190 for precocene I, and *m/z* 220 for precocene II. It was therefore thought that these two ion masses could be used to qualitatively screen the two plants, namely, *Moringa oleifera* (as blank) and *Ageratum conyzoides* for the presence or absence of precocene I and precocene II, respectively. Thereafter quantitative screening would, in any case(s) of occurrence, be done using a combination of these ion masses and other prominent ion masses detected in the respective mass chromatograms. Xu *et al*.,(2016) adopted this screening procedure with considerable success in their efforts to identify and characterize a total of 168 components in the complex decoction obtained from a traditional Chinese medicine formula used in traditional medicine.

For the identifications in these experiments, samples were compared against control (blank) sample to identify expected or unexpected components. Using such blank procedures resulted in the identification of some 2, 2 – dimethylchromenes. The further characterization of two of these

chromenes as well as a few other compounds was either not attempted or not fully described.

2.14 Analysis of Data obtained in these experiments

Data obtained from the various stages of these experiments have been analyzed using corresponding expressions as follows:

a Data from the Identification and Characterization of Precocene I Additive.

(i) GC data are the characteristic retention times of the various components resulting from the cleanup procedures adopted. These retention times were obtained relative to the non-polar stationary column substance, Carbowax, and were read off the plot of the gas chromatograms recorded in the GC.

(ii) Fragmentation patterns of the various components obtained from the MS were analyzed using the various intensity values of the fragments as well as the relative abundances of named isotope clusters. References were made to Beynon's tables, (1960) as abridged in McLafferty and Turecek, (1993). Molecular structures were determined using the expressions:

$$\text{no of carbon atoms in component compound} = \frac{\text{relative abundance of } M + 1}{1.1 \times \text{relative abundance of } M^+}$$

(McLafferty and Turecek, 1993)

(iii) Index of hydrogen deficiency (IHD) was calculated using the expression

$$IHD = p - q/2 + s/2 + 1$$ (Chapman, 1993)

for a hypothetical chemical formula $C_pH_qO_rN_s$ in which the ratios of the elements are p, q, r, and s, respectively.

(iv) Characterization of precocene I by the determination of density was done by weighing and substituting the masses obtained in the expression

$$\text{density (g mL}^{-1}) = \frac{\text{mass of substance contained in density bottle (g)}}{\text{volume of density bottle (mL)}}$$

b Data from VDC/MA/Precocene I decomposition derived from TGA trace

(i) The concentration versus time data derived from each TGA trace were in general analyzed according to first order kinetics using the expression

$$[A] = [A] \cdot e^{-k_{rate} \cdot t}$$ (Atkins and Paula, 2012)

(ii) The thermodynamic activat ters that govern the decomposition of the VDC/MA/ precocene I entity were rationalized in terms of Transition State Theory. The activated complex according to the theory was analyzed using the Eyring equation that featured in the expressions:

$$\Delta^{\#}G = -RT\ln k^{\#} \quad (2.1)$$

$$\Delta^{\#}G = \Delta^{\#}H - T\Delta^{\#}S \quad (2.2)$$

$$k_{rate} = kT/h \cdot \Delta^{\#}S/R \cdot e^{-\Delta\#H/RT} \quad (2.3)$$

(Isaac, 1977); (Atkins and Paula, 2012)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Materials used in these experiments were;

Deionised water;

Agate mortar and pestle;

Oven;

Furnace;

Beakers,

Analytical balance;

Conical flasks;

Flat bottomed flask (10 Litter);

Methanol, chloroform, n-hexane, ethanol, ethylacetate;

Filter paper (Whatman);

Funnels;

Water bath;

Silica gel (70-230 mesh, Merck);

(TLC) plates;

Iodine tank;

Ruler;

Thermometer;

Abbé refractometer;

Three–necked Quickfit flask;

Microdensity bottle;

Capillary tube;

paraffin bath;

Soxhlet extractor;

Steam distillating equipment;

3.1.1 Reagents

Chemicals used for the preparation of reagents were all of Analar Reagent (AR) quality and included those for the preparation of:

Fehling's solutions;

Meyer's reagent for alkaloids;

$AgNO_3$ for halogens;

All solvents were of Sigma-Aldrich quality;

Concentrated HNO_3 acids;

The comonomer / additive used in the kinetic experiment, 7-methoxy 2,2-dimethyl -1-benzopyran (precocene 1);

VDC/MA copolymer (vinylidene chloride / methylacrylate)

3.1.2 Major Equipment

Vacuum extraction equipment (rotary evaporator with vacuum);

Atomic Absorption Spectrometer (AAS)Pye Unicam 1900;

Thermogravimetric Analyzer (TGA), PE 8000, Temperature Range, room temperature to 1100°C; Heating rate: 0.02 to 250 K / min.

Gas Chromatograph - Mass Spectrometer, GC-MS-QP2010 Plus, Shimadzu, Japan;

The GC-MS consisted of a mass selective detector (EIMS, electron energy = 70 eV), a scan range of 45 – 400 Da, and rate of 4 scans per second. The GC-MS solution software was capable, among other things, of performing a similarity search with linear retention time indices. The library search could be refined to identify actual isomers with the help of the retention time indexing feature.

IR spectra of the isolated and partially characterized liquid precocene I was recorded on JASCO FTIR – 460 Plus spectrophotometer. KBr windows were used for the recording. The Teflon spacer had a thickness of 0.05 mm. Prominent peaks were recorded and are listed in Table 4.8 on page 66. They were used to assign vibration frequencies to the C=C bond stretch in the pyran ring, as well as to the C–O stretch of the aromatic ether ring. Confirmatory information was obtained from the NIST webbook using these data.

3.2 Method

3.2.1 Sample Collection and Identification

Vinylidene chloride copolymer was procured from a commercial source, POLYPRODUCTS Co., Kano. The accompanying literature indicated that the copolymer contained five mole percent of methylacrylate (CH_2=$CHCOOCH_3$).

Other accompanying data include:

Melting range	185 - 200°C
Density	1.86 - 1.88 g mL^{-1}

Samples of *Moringa oleifera* used in these investigations were harvested from the premises of Borromeo Hospital, Onitsha in Anambra State; samples of *Ageratum conyzoides* were collected

from the Government Reservation Area (GRA), Awka also in Anambra State. These samples were identified and confirmed to be authentic samples, by Prof. J. C. Okafor (retd), of Tree Crops and Tropical Ecological Centre, 7 Dona Drive, Independence Layout, Enugu.

3.2.2 Pretreatment of Plant Material

Wherever necessary, the aerial parts and the root system were separately washed in deionised water to thoroughly remove any adhering solid matter. They were separately rinsed in deionised water and any excess water was allowed to drain away. The plant parts were then prepared as follows before use. The leaves or aerial parts were extracted usually after one week of air-drying, while the roots were oven-dried (105^0C) and ground to fine powder using agate mortar and pestle prior to extraction.

3.2.3 Variation of Extraction Procedure

Pilot experiment was done using cold maceration procedures and were adopted as follows (Harbonne, 1998), with modification as in Mucuna pruriens (2008); but due to the problem arising from the decay process which was observed on the third day of soaking leaves in deionised water, and which resulted in delayed filtration of extracts, the extraction procedure was varied. 3 L of chloroform and a mixture of 2 L of deionised water and 50 mL of 15 M NH_3 solution were separately introduced into the extraction vessel containing about 2 kg of air-dried leaves or air-dried and finely ground roots before the commencement of extraction. (*Mucuna pruriens*, 2008).

At the end of the extraction procedure adopted, all the concentrated chloroform extracts for the separate plant parts were pooled together, while all the concentrated aqueous extracts were similarly pooled together, for further treatment.

3.3 Further Treatment

3.3.1 Concentration of the extractsand acidification

The pooled concentrated chloroform extract of the leaves of each plant was separately subjected to further solvent removal under reduced pressure, resulting in viscous yellowish-brown slurry (about 250 g) which was cooled and subsequently poured into aqueous acetic acid, (about 100 mL), with stirring. After storing the aqueous acetic acid solution of the slurry at room temperature overnight, the resulting clarified solution was again painstakingly extracted with chloroform, while the aqueous layer left after extraction with chloroform was also left in a partly covered evaporating dish to further reduce in volume.

The pooled concentrated aqueous extract of the leaves was similarly further concentrated by heating in a soxhlet extractor under reduced pressure until a brown viscous slurry resulted. The slurry was cooled and subsequently acidified with dilute acetic acid, mixed in the minimum volume of deionised water (about 10 mL) and stored in a partly covered evaporating dish to further reduce in volume as much as possible.

Both the aqueous and the chloroform extracts of the root systems were similarly separately treated. In no case was any crystal or residue noticed at the bottom of the evaporating dish. These extracts were kept for further analysis as they are mostly acidic.

Attempts were made to obtain essential oil from *Ageratum conyzoides* by steam distillation for assessment of yields of chromenes available and for comparison with yields obtained by extraction. The straight Steam Distillation Method was used. In this method, steam was generated in a separate vessel and contacted with the plant material outside the steam generator through a delivery tube. These attempts failed. Each of the three attempts resulted in a non - resolvable emulsion, partly, because of the disadvantages inherent in hydrodistillation. One of

these disadvantages is that high boiling components, usually with comparatively low volatilities, tend to have incomplete extractions. On the other hand, oxygenated compounds such as phenols have a tendency to dissolve in any condensed water in the still, forming emulsions so that their complete removal becomes impossible (http://agritech.triau.ac.in/horticulture/extraction_ methods_ natural_essential_oil.pdf). There was also the possibility that the hydrophilic substances present in this fragile plant material were quantitatively too low and the lipophilic material quantitatively too high to allow for meaningful extraction through hydrodistillation. The boiling point of the isolated precocene I was 292°C (literature value 292.7°C) under one atmosphere pressure. Volatility would thus be low for hydrodistillation, unless done in specially – constructed stainless steel distillers. Stainless steel is non-reactive and was not available for these experiments.

3.4 AAS Analysis of *Ageratum conyzoides* leaves

2 g of oven-dried *Ageratum conyzoides* was ground to powder using an agate mortar and pestle and ashed in a furnace, the temperature of which was gradually increased from about 105^0C to about 600^0C after which ashing was allowed to proceed for 30 minutes. After cooling to room temperature, the ash sample was very carefully scraped into a 250 mL beaker to which 10 mL of concentrated nitric acid was carefully added. Care was taken to ensure that the ash sample was transferred quantitatively to the beaker by washing the ash container with 5 mL x 3 dil. HNO_3 solution and adding the washings to the 250 mL beaker. The mixture in the beaker was then left overnight in the fume cupboard, gently heated for 2 hours to digest, and the volume of the resulting solution was boiled down to about 2 mL. This procedure was followed by the addition of deionised water to the beaker and further boiling to about 2 mL to further reduce the acidity of

the solution in the beaker. Boiling was continued until no more fumes evolved from this treatment. The resulting clear solution which did not contain any undissolved matter at the bottom of the beaker was quantitatively filtered and further reduced in volume to about 1 mL. It was stored in the refrigerator for use in the determination of its metal content using AAS.

3.5 Separation of Components Obtained from the Extraction Procedure using TLC and Column chromatography.

Extracts were spotted on thin layer chromatographic (TLC) plates coated with silica gel. Elution was carried out using various solvent mixtures which include n-hexane : ethanol (10:1), methanol / aqueous ammonia (200:3), aqueous acetic acid : n-hexane : ethanol (1:10:1). The chromatograms gave spots with R_f values which were recorded.

Preliminary experiments to separate extract mixtures into components either by elution of selected TLC spots on columns using various solvents and/or solvent mixtures which included ethyl acetate, ethanol and hexane or by the use of silica gel on TLC plates and elution with various organic solvents yielded results which were also recorded. Such recorded results did not yield unambiguous statements regarding the number of components in each spot, hence, were subjected to GC-MS treatment for further analysis, and possible comparison of separation data achieved using TLC and column separation methods.

3.6 Separation of Pure Components and Characterization of chromenes using GC-MS

The separation of the various componentsin the various extracts was achieved in the GC using thecharacteristic retention times assigned to each component. The GC characteristic retention times of the identified prcocene I together with the GC column length, film thickness, diameter,

carrier gas velocity and pressure and void time afforded the use of liquid nitrogen for the isolation of the pure Precocene I. Comparison of the various retention times enabled the isolation of a total of three chromenes and two coumarins from *Ageratum conyzoides* leaves. The mass spectrometry (MS) component of the combination with the GC yielded the fragmentation pattern for precocene I. Analysis of the pattern resulted in the assignment of the chromene molecular formula and structure using appropriate Beynon Table, and expression for the hydrogen deficiency index. Conversely, *Moringa oleifera* leaf extracts showed no chromenes / coumarins content. Precocene I which was successfully isolated was further characterized. The mass spectrometry of the pure precocene I was repeated and the spectrum is in appendix 16.

3.7 Further Characterization of Precocene I used as Additive

The Precocene I extracted from *Ageratum conyzoides* was additionally characterised using other procedures as described here.

3.7.1 Boiling point determination: This was carried out using a paraffin bath and a 360°C thermometer attached to a capillary tube using a rubber band. The substance was fed into the capillary tube to a depth of about 2 cm from the top end which was left open, while the bottom was sealed so as not to let in the oil in the paraffin bath. Heating was done using a high-speed flame. The temperature of boiling was noted when tiny bubbles of gas exited from the substance in the capillary, and again noted on cooling when a meniscus began to form. The average value was taken as the boiling point of the substance.

3.7.2 Density: This value was obtained using a 10 mL microdensity bottle which was weighed empty and subsequently reweighed after filling with precocene I to obtain the mass of the substance in the microdensity bottle

The ratio of the mass of precocene I to its volume in the micro density bottle gave the density;

$$\text{Density} = \frac{\text{mass of chromene contained in density bottle}}{\text{Volumn of density bottle}}$$

3.7.3 Refractive index: This value was determined using the Abbé refractometer. The sample was put between two prisms. Light entered the sample from the illuminating prism get refracted at critical angle at the bottom surface of measuring prism and then the telescope was used to measure the position of the border between bright and light areas.

3.7.4 H-deficiency value was calculated from the compound molecular formula obtained from the mass spectrum data.

3.7.5 Ir spectrum, obtained using a JASCO FTIR-460 PLUS spectrophotometer, was used to identify and assign characteristic vibration frequencies to the isolated oxygen heterocycle before incorporation into VDC/MA copolymer.

3.8 Incorporation of extracted precocene I into VDC/MA copolymer

0.1 mole of the identified benzopyran derivative (precocene I) was incorporated as additive / comonomer into the vinylidene chloride copolymer by first heating the copolymer under reflux in methanol (CH_3OH) in a reaction vessel, (a three–necked Quickfit flask), until the previously weighed copolymer dissolved in the solvent. A Quickfit mercury–in–glass thermometer was inserted into the flask through one of the necks. The previously weighed derivative was then added dropwise through the second neck, while the central (third) neck was fitted with a water–cooled twelve – foot condenser for reflux. The addition lasted for about thirty minutes after

which heating was continued for about 60 (sixty) more minutes. The mixture was cooled, the solvent evaporated after the condenser had been removed and the resulting white solid mass thereafter weighed and subjected to thermogravimetric treatment.

3.9 Analysis of the vinylidene chloride / methylacrylate / precocene I product by thermogravimetry

A weighed sample of the vinylidene chloride / methylacrlate / precocene I product was subjected to T G A at three constant temperatures of 170 °C, 180°C, and 190°C. This is because Preliminary studies done from the literature indicated that poly(vinylidene chloride) (PVDC) $(–CH_2–CCl_2–)_n$ has a softening point ranging from 185°C to 200°C (Yue and Economy, 2017). Analysis of the three curves as well as the T G A derived masses was further carried out to derive kinetic as well as thermodynamic data using the equations (2.1) to (2.3) given in chapter two.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results of the sourcing and characterization of precocene I additive

(a) Elemental Analysis of *Ageratum conyzoides* using AAS is presented in Table 4.1

TABLE 4.1 Elemental Composition of *Ageratum conyzoides* from AAS experiment

Element	Concentration (ppm)	mg per 100g	Confidence limit
Ca	3723.17	372.3	0.9925
Fe	2035.57	203.6	0.9917
Zn	124.09	12.4	0.993
Al	9.68	0.9	0.9956
Mg	2.33	0.2	0.9975
Pb	0.30	0.03	0.9962
Cr	0.15	0.015	0.9973
N	0.09	0.009	0.9941
Cu	0.07	0.007	0.9993
Co	0.06	0.006	0.9962
Cd	0.02	0.002	0.9962
K	ND	ND	0.9913
S	ND	ND	0.994
P	ND	ND	0.9961
Na	ND	ND	0.9911

ND = Not Detected

It was necessary, in view of the sourcing of precocene I from its natural origin, to enable statements to be made regarding any effect of matrix materials arising from the environment from which the plant sourced was taken. Such plant source matrix materials include associated chemical elements. These associated chemical elements were separately extracted in acidic media from the whole plant. The extracts were analyzed after using appropriate cleaning process using atomic absorption spectrometry (AAS). The elements found in the extracts, their concentrations, as well as the confidence limit of the data have been presented in Table 4.1.

These data would be of interest in diverse ways. To a nutritionist, they can be linked to WHO standards for the sustenance of health. To an environmentalist, they can be compared to indicators of environmental pollution. For these studies on the deterioration of PVDC by dehydrochlorination, however, none of the elements present in Table 4.1 is known to function in aqueous alkaline media, such as found in *Ageratum conyzoides* as a catalyst, positively or negatively. More importantly, the extraction procedure for precocene I included cleanup procedures which excluded aqueous media that could entrain such extraneous matrix materials. Most importantly, the mass spectrum of the isolated precocene I did not indicate any masses that corresponded to any of the matrix elements.

Additional observations may be made regarding the data in Table 4.1 as well as comparisons related to similar data.

On nutrition, the data in Table 4.1 do indicate that several of the elements usually recommended for good health in humans in micro quantities are present in *Ageratum conyzoides*. These include Fe, Ca, Mg, Zn, Cu, Ni and Cr. Calcium (Ca) and Iron (Fe) in particular each appears in the plant in quantities far greater than the Recommended Daily Allowance (RDA) values of WHO/FAO presented in Table 4.2. Thus, the weed *Ageratum conyzoides* may have been considered as a vegetable for human consumption but for the presence of such heavy metals as lead (Pb) and cadmium (Cd) which are regarded as cumulative environmental pollutants and which are not present in the quoted elemental composition of *Moringa oleifera* reported by Fuglie, (1999) as presented in Table 4.3

TABLE 4.2: Recommended Daily Allowance for a child aged 1 – 3 and a woman during lactation (WHO/FAO) (Fuglie, 1999).

Element	Child (mg per 100g)	Woman (mg per 100g)
Ca	400	1,200
K	800	3,000
P	800	1,200
Mg	150	340
Fe	10	15
Cu	0.8	2

When the data in the three Tables are compared, all the six elements listed in Table 4.2 as recommended by WHO / FAO are present in Table 4.3 for *Moringa oleifera* while some are missing in Table 4.1 which contains data for *Ageratum conyzoides*. For instance, the elements, Ca and Cu are present in the two plants MO and AC, and in quantities that approach those recommended for nutrition but only four are found in AC. Fuglie, (1999) did not include sodium as one of the elements detected in *Moringa oleifera*. The absence in AC of the two recommended microelements, potassium (K) and phosphorus (P), both regarded as playing rather important physiological parts in humans, (Emsley, 1994) is certainly a huge minus for AC.

A comparison may also be made here. As indicated in the literature survey (Chapter Two), Nwankpa, (2015), published a paper in which were included data on the mineral content, among others, of *Ageratum conyzoides*. According to the author who was interested in the nutritional qualities of *Ageratum conyzoides* as compared to its use in traditional medicine, the mineral composition and concentrations of the leaves of the plant were given as follows:

Element	Concentration (mg/100g)
zinc (Zn)	5.75
iron (Fe)	2.52

calcium (Ca)	0.51
phosphorus (P)	0.23
magnesium (Mg)	0.11
sodium (Na)	0.047
potassium (K)	0.057

Compared to data from this work for the same plant presented in Table 4.1, there is agreement in the non-detection of the element sulphur as reported in Table 4.1. Both the diversity of elements in Table 4.1 as well as their respective concentrations, which latter differ from Nwankpa's values by factors ranging from 2 (two) to as much as 100 (one hundred), reflect both areas of interest and the consequent attention paid to details. While nutrition takes account of the leaves (as extracted by Nwankpa), the quest for an additive in a chemical setting (as well perhaps as use in traditional medicine) has set the scene for the exploitation of the whole plant and may account for both variety and concentration of matrix materials.

TABLE 4.3: Elemental composition for *Moringa oleifera* (Fuglie, 1999) compared to AC (see Table 4.1).

Element	Composition(fresh leaves) (mg per 100g)	
	Moringa	*Ageratum*
Ca	440	372.3
K	259	–
S	137	–
P	70	–
Mg	24	0.2
Fe	7	203.6
Cu	1.1	0.007

The two microelements Ca and Cu are recommended in the WHO/FAO RDA (Table 4.2), while Ca, Cu, as well as the element sulphur (S), also present in MO but not in AC, are among the

elements recommended in fertilizer formulations particularly for wheat production. (Emsley. 1994). It must be added, however, that the elemental composition data presented for *Ageratum conyzoides* in Table 4.1 were obtained to complement inferences from preliminary qualitative results which indicated the absence of sulphur and the halogens from *Ageratum conyzoides*, results which were subsequently confirmed by component mass spectra. They should not be regarded as basis for possible recommendation of the plant for or against human consumption or traditional practice.

4.2 Identification Characterization and Confirmation of Precocene I Additive.

4.2.1 Identification of Corresponding Peak in the GC Spectrogram.

The GC peak in the gas chromatogram which corresponded to Precocene I was identified by comparison of the retention times of the various components present in the leaves of *Moringa oleifera* (Sample A, Appendix I) which serve as the control spectrogram, and the retention times of the various components in the chloroform extract of the leaves of *Ageratum conyzoides* (Sample C, Appendix 3). In general, GC retention time as recorded in the gas chromatogram is related to specific chemical properties of a molecule such as volatility, polarity, presence of specific functional group, while molecular mass as derived from the mass spectrum, is indicative of atomic composition (Hites, 1992). Experimental retention times have been compiled and listed in Table 4.4 for the extract from*Moringa oleifera* leaves,

Table 4.4 Retention times and Relative Concentrations of Components under the Gas Chromatogram Peaks of the Chloroform extract of leaves of *Moringa oleifera* (Sample A)

Component Peak no.	Retention time(min)	Relative concentration (%) *
1	4.017	4.7
2	9.033	4.7
3	12.575	4.8
4	24.775	37.3
5	25.942	0.4
6	29.292	12.5
7	31.925	15.6
8	32.608	2.6
9	33.408	7.3

* Concentration data were determined from the area under component peaks.

The extract component under Peak No. 4 in Table 4.4 (retention time 24.775min.) appeared long after peak No. 3 (retention time 12.575 min.) leaving a window of about twelve (12) min. in which no other peak appeared. Such a window did not appear in Table 4.5 in which seven peaks occurred between peak No. 1 (retention time 5.467 min.) and peak No. 9 (retention time 24.767 min.). The retention times for the aerial parts of *Ageratum conyzoides*, are listed in Table 4.5. Compared on the basis of the retention time parameter, the retention time difference between peak No. 4 in Table 4.4 and peak No. 9 in Table 4.5 (10 seconds) is so narrow that it can be reasonably inferred that the two components under each of these two peaks have similar volatility or polarity or similar specific functional groups which are different from the peaks occurring between peak No.1 and peak No. 9 in Table 4.5. These latter seven peaks are therefore due to components that are present in *Ageratum conyzoides* but not present in *Moringa oleifera* leaves.

Table 4.5 Retention times and concentration data of Components under the Gas Chromatogram peaks of the Chloroform extract of aerial parts of *Ageratum conyzoides* (Sample C)

Component Peak no.	Retention time (min.)	Relative concentration (%)*
1	5.467	3.3
2	19.933	9.4
3	21.825	4.4
4	22.483	3.8
5	22.758	3.3
6	23.225	5.2
7	23.642	3.3
8	24.342	6.0
9	24.767	12.8
10	25.542	6.0
11	25.933	11.6
12	27.625	9.4
13	27.875	7.2
14	29.283	6.6
15	31.567	3.3
16	32.617	4.4

* Concentration data were determined from the area under the component peaks

Consideration of the retention time differences among the seven peaks results in the proposition that the retention time difference between peak No. 2 and peak No. 3 in Table 4.5 (about 2 min.) denotes peak No.2 as apart in physical characteristics from peak No.3 and other members of the group of peaks among which the average retention time difference is 30 sec. and no single retention time difference is up to one minute. Thus peak No. 2 in the chloroform extract of the leaves of *Ageratum conyzoides* (Table 4.5) was identified as corresponding to the simplest of the members of the series of components the subsequent fragmentation pattern of which was to be very closely studied.

Similar considerations also led to the identification of three peaks obtained from the chloroform extract of the root system of *Ageratum conyzoides* for closer monitoring and study. The peaks are

numbered 3, 4 and 5 in Table 4.6 and have retention times of 17.183, 17.583 and 17.750 min., respectively. Retention time differences between consecutive members of these three peaks are 24 sec. and 10 sec.

Table 4.6 Retention times and relative Concentration data for components under the Gas Chromatogram Peaks of the $CHCl_3$ extract of root system of *Ageratum conyzoides* (Sample E, Appendix 5).

Component Peak no.	Retention time(min)	Relative concentration (%)*
1	6.375	2.5
2	13.892	2.0
3	17.183	12.9
4	17.583	37.3
5	17.750	19.9
6	23.325	5.0
7	24.583	4.0
8	26.125	9.0
9	29.075	7.5

* Concentration data were determined from the area under the component peak

The extract obtained from the root system of AC using chloroform extractant gave nine (9) peaks indicating nine components. The data are presented in Table 4.6, which also lists the nine components peaks with their respective retention times and relative concentrations.

Comparison similar to those between the respective retention times and retention time differences such as were done for Table 4.4 and 4.5 led to the inference that GC peak No. 3, 4, and 5 in Table 4.6 should be investigated further for the characteristics of the components under those three peaks, to determine the nature of the components under those peaks.

4.2.2 Characterization of Components under the Identified GC Peaks

The first step in the characterization of the components under each of the four identified GC peaks was to study the fragmentation patterns of components under those peaks as provided in their mass spectrograms. Mass spectrometry provides such spectrograms. Among the ways of examining GC-MS data is one that looks at each mass spectrum in turn, in essence stacking up the mass spectra and examining them individually. The advantage in this is completeness of interpretation. This means selecting the portion of the total ion chromatogram (TIC) that corresponds to a given peak and extracting the mass spectra from the specified time period, that is, the retention time (Hites, 1992).

This procedure was adopted for the four selected GC peaks with their retention times as indicated. Structural determination of the components under the identified peaks was both by *a priori* spectral interpretation and by matching their spectra and other data with reference data and / or data from the chemical literature.

The usual characteristic data obtained for each component GC peak in the MS are M^+ (monoisotopic ion mass or parent mass) and corresponds to the molecular mass of the component under the GC peak; M + 1 (used to represent an isotopic cluster peak usually one Dalton from M^+); base peak, which is the highest abundance peak in the fragmentation spectrum and usually designated 100% abundance. The base peak is often considered the most stable ion; M + 2 peak, if present. According to McLafferty and Turecek, (1993), these characteristic data form part of the sensitive fingerprint of a compound subjected to electron ionization (EI) in GC – MS instrumentation

They have been assembled in Table 4.7 for GC peak No. 2 (labeled C2)

Table 4.7 Mass Spectral Characteristic data for GC Peak C2

Species	*m/z*	abundance	Abundance relative to M^+
M+	190	1.9	100
M + 1	191	0.25	13.158
M + 2	–	–	–

4.2.2.1 Procedure for the further Characterization of Component C2

(i) Abundance values were used to obtain the number of carbon atoms in the molecule of the component under the peak, C2, using the relationship

$$\text{no. of C atoms in component under GC peak C2} = \frac{\text{Relative abundance of M + 1}}{1.1 \times \text{relative abundance of } M^+}$$

(McLafferty and Turecek, 1993)

The value is 13.158 / 1.1 = 11.9 ≈ 12.0

(ii) There is no significant peak attributable to M + 2 in the mass spectrum and this is clear indication that the molecule of the component under the peak does not contain the heavy atoms such as Cl, S, Br. (McLafferty and Turecek, 1993)

(iii) There are four major peaks in the mass spectrum of the component under peak C2. These are located at *m/z* values of 190, 175,160 and 132 and are assigned, respectively, to the monoisotopic mass of the molecule under the peak (190), to loss of a CH_3 (methyl) group attached to the molecule (*m/z* 175), to the loss of a second CH_3 (methyl) group also attached to the molecule (*m/z* 160), and to lossof a carbonyl (CO) group (*m/z* 132). The peak at *m/z* 175 is the most prominent of the four major peaks and is the base peak. Both the SCAN mode and the SIM mode were used to obtain these fragmentation data.

These four most intense mass spectral peaks all occur at higher masses, a feature which suggests the presence of an aromatic group in the molecule (McLafferty and Turecek, 1993). In support of this observation, there is a weak peak at *m/z* 77 assignable to a monosubstituted benzene molecule. Stacking up the mass spectra and examining them individually, it was easy to assemble fragments together, that is, find the combinations of elements in the appropriate ranges that added up to the accurate mass (190) being considered. This procedure yielded the result: (Dromey and Foyster, 1979):

Mass	Contribution from
77	benzene ring (monosubstituted)
72	6 ^{12}C atoms out of total of 12
6	6 ^{1}H atoms in two CH_3 group
155	

A benzopyran is under consideration in this case, and there must be at least one oxygen atom (16 Da) in the molecule. This would contribute another mass of 16 Da to the molecular mass, leaving a mass deficit of (190 – 171) to make up the composition of the molecule. The nitrogen rule (Dromey *et al.*, 1979) when used to factor out nitrogen presence in the molecule, thus leaves a mass defect of 19 which can be accommodated by one other oxygen atom as part of the group in the monosubstitution, and leads to the desired molecular formula $C_{12}H_{14}O_2$.

The degree of unsaturation in the molecule which resulted in the number of double bonds has been determined to be 4 (four) using the relationship (Chapman, 1993). The two rings in the molecule complement the 4 (four) double bonds to give a total unsaturation of 6 (six) which results from the total determination of unsaturation using Chapman's relationship formula:

$$\text{H–deficiency} = x - y/2 + z/2 + 1$$

where x, y, z are ratios in which carbon (C), hydrogen (H), and other relevant elements which appear in the molecule.

The structural formula was thus arrived at as shown in figure 4.1:

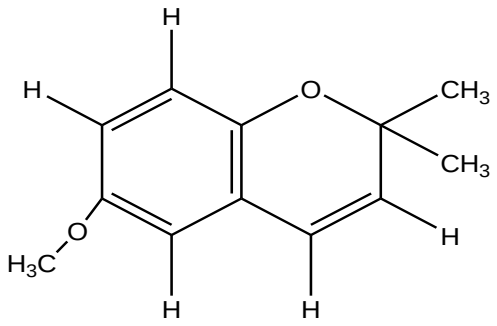


Figure 4.1 Structure of Component C2

3- Methoxyphenol and 3-methyl-2- butenal were used by Canadian researchers as starting materials in the preparation of precocene I (Bissada *et al*., 1994), Hiramatsu *et al*., (2013) also used the same starting materials. The structural formula obtained from these data thus lends support to the basis for the synthetic procedure and the results reportedly obtained by these workers. According to Bissada *et al*., (1994), chromenes are substances obtained from appropriately substituted phenols and suitably funtionalized unsaturated parafinics.

4.2.3 Confirmation of the Component under GC Peak C2

Both the NIST Chemistry WebBook (NIST) and comparison of some additionally generated data with literature values were used in attempts to confirm both the molecular formula and structure of the substance under GC peak C2. For WebBook search, use was made of wild card characters in the molecular formula calculated for the substance by first submitting the formula as $C_{12}H1\%O2\%$, indicating uncertainties in ratio of H and of O as well as supplying the four

prominent MS peak positions obtained from the fragmentation pattern of the component under peak C2. The formula search result was returned as $C_{12}H_{14}O_2$. Other literature results include EIMS: *m/z* 190 for M^+; HREIMS: *m/z*190.0966 also for M^+. NIST also gave the name of the compound as well as the CAS number.

Checks of the literature for characteristic data showed that the most comprehensive tabulation was by Hiramatsu *et al*., (2013). The IR frequencies recorded by these workers were largely in agreement with the experimentally determined peaks obtained from this work, while their ^1H and ^{13}C NMR shifts were in perfect agreement with those received from the NIST Chemistry WebBook following the WebBook search.

The procedure outlined for component peak C2 was also followed for the characterization and conformation of component peak E4 (Table 4.6) resulting in the same conclusion for molecular formula as well as molecular structure for precocene I. The two separate components were thus pooled together after their respective isolation both for characterization and confirmation of the component precocene I. The combined components gave the same fragmentation pattern as the separate components. The combined mass spectrum is presented in Appendix 16.

All the data by which precocene I was characterized are presented in Table 4.8, along with literature values and their sources wherever these are obtained from.

Table 4.8 Physical Characteristics of Precocene I AdditiveUsed in the Kinetic Experiment

Property	value	literature value	formula
$C_{12}H_{14}O_2$		$C_{12}H_{14}O_2$	
	(calculated from mass spectrum)	(source: http://pubchem.ncbirnlon.nih. gov/compound/Precocene_I)	
Molar Mass	190.224 g mol^{-1}	190.0966	
	(calculated from natural isotopic abundance masses)	from (HREIMS) source: (Hiramatsu *et al.*, 2013)	
M^+	m/z 190	m/z 190	
	(from EIMS)	(source: Hiramatsu *etal.*, 2013)	
Base Peak	m/z 175	–	
	(from EIMS)		
Physical State	pale yellow oil	pale yellow oil	
		(source: Hiramatsu *etal*., 2013)	
Boiling Point	292°C (atmosphere);	292.7°C at 760 mm Hg (1 atmos.)	
	redistilled at 68°C / 1 atmos.	(http://www.lookchem.com/6-Demethox yageratochromene/) visited 9/22/2011)	
Density	1.036 g mL^{-1}	1.039 g mL^{-1}	
		(source: http://www.lookchem.com)	
Refractive Index	1.54	1.519 (source: http://www.lookchem.com)	
H-bond donor	none	–	
H-bond acceptor	2		
H-deficiency	6		
	(equivalent to 4 double bonds and 2 rings)		

CAS No.		17596 – 02 – 6
	(source: http://www.lookchem.com)	
IR (film)	(2975, 2890, 1650, 1438, 1275, 738 cm^{-1})	(1617, 1503, 1316, 984, 803) cm^{-1} (source: Hiramatsu, *etal.*, 2013)
^{1}H NMR	–	400 MHz, $CDCl_3$ δ: 1.41 (6H, s, 2Me), 3.74 (3H, s, OMe), 5.44 (1H,d, J=10.1 Hz, H-3), 6.25 (1H,d, J=10.1 Hz, H-4), 6.38 (1H,d, J=8.2 Hz, H-6), 6.39 (1H,s, H-8), 6.86 (1H,d, J=8.2 Hz, H-5),
^{13}C NMR	–	100MHz $CDCl_3$: δ 160.7, 154.3, 127.9, 127.0, 122.0, 114.7, 106.7, 102.1, 76.4 55.3, 28.1

Note: Both ^{1}H NMR and ^{13}C spectroscopic data were provided by NIST Standard Reference Database Number 69 in confirmation of the extracted compound. This was in response to the search in the webbook using derived MS data, namely, molecular ion mass, characteristic ion masses at *m/z* 175, 160 and 132, and molecular formula. Experimental Ir data obtained from this work largely agreed with those of Hiramatsu *et al*., but differed from those reported by Bissada *et al*., who did not record frequencies below about 1450 cm^{-1}

4.3 Preliminary Separation of Components of Extraction mixtures.

Data obtained from TLC Experiment

Table 4.9 presents typical data obtained from initial screening TLC experiment. Spots were identified using iodine vapour in a glass jar.

Table 4.9 shows that two spots were obtained from chloroform extract of the root of *Moringa oleifera* (MO), while three spots resulted from the root extract of *Ageratum conyzoides*. Added base increased the number of spots compared with a neutral eluting medium. According to Harbonne, (1998), spots obtained after acid or alkaline hydrolysis of a plant extract are usually assignable to classes of substances such as alkaloids, cyanogenic glycosides, or even normal amines.

A comparison between the data in Table 4.6 and Table 4.9 for the root system of AC for which the same extracting solvent, chloroform was used, leads to the expectation of at least five (5) TLC spots in Table 4.9, judging by the retention time windows between seemingly dissimilar components as observed in Table 4.6. Additionally, Harbonne, (1998) listed terpenoids in general as extractable in chloroform, among other lipophilic solvents. Attention was therefore subsequently focused on extracts obtained using chloroform ($CHCl_3$) as solvent.

Thus, chloroform solvent extracts were used in obtaining the data presented in Table 4.9, which presents extracts obtained from the root of both plant species, *Moringa oleifera* (MO) and *Ageratum conyzoides* (AC) for their comparative characteristic separation data using TLC separation technique.

The root system of MO, eluted using n-hexane / ethanol (10 : 1) resulted in two spots (R_f 0.36 and R_f 0.53 with added NH_3). This would signify that the two spots contained the same component(s) but in different forms. On the other hand, the extract obtained using the root

system of AC as substrate gave one spot value 0.19 (eluent n-hexane), and two spots, R_f 0.71 and 0.95 (eluent system $MeOH/NH_3$, 200 : 3), indicating qualitative differences in the composition of the two systems.

Recourse was therefore made to column chromatography, where, of course limitations also exist.

Table 4.9 Typical Data from Preliminary TLC Separation Experiments using representative plant extracts obtained in these experiments.

	Plant species / plant part	Extracting solvent	TLC eluent system	observation
1.	*Moringa oleifera* / root (MO)	$CHCl_3$	n – hexane / ethanol (10 : 1)	two spots ; R_f 0.36
			with added NH_3 solution	0.53
2.	*Moringa oleifera* / root (MO)	$CHCl_3$	n – hexane / ethanol (10 : 1)	one spot; R_f 0.36
			no added base / acid	
3.	*Ageratum conyzoides* / root (AC)	$CHCl_3$	MeOH / NH_3 (200 : 3)	two spots; R_f 0.71
				0.95
4.	*Ageratum conyzoides* / root (AC)	$CHCl_3$	n – hexane	one spot; R_f 0.19

❖ Detection of spots was by iodine vapour

For column chromatography, viewing of spots was both visual, and development using iodine vapour, in the absence of a short-wave (254 nm) lamp to enable detection of spots of otherwise coloured components. The data are presented in Table 4.10

Data in Table 4.10 suggest that components in the $CHCl_3$ extract of the root system of *Ageratum conyzoides* number a total of three (3) with R_f values in basic / acidic medium of 0.28 (basic) / 0.35 (acidic), 0.56 / 0.47, 0.77 / 0.65 respectively. The leaves of MO extract resolve visually into two fractions, namely, a red fraction and a yellow fraction. The data for the root system of AC were however useful in focusing attention on the neutral compounds inferred from elution using n-hexane (R_f 0.28). Percolation of dry plant material with hexane is a recognized procedure for isolating natural chromenes from their natural sources such as *E.riparium* Regel, an Australian plant (Anthonsen, 1969). The procedure has also been quoted by Pratap and Ram, (2014).

Red and yellow fractions were also collected from aqueous and $CHCl_3$ extracts respectively of the leaves of *Ageratum conyzoides*, a situation that still left unanswered questions regarding the actual numbers of components in these extracts.

Table 4.6 also contrasts with Table 4.10 in the number of possible component peaks

Thus the task of separation, identification, and / or characterization of individual components in these complex mixtures obtained from the extraction of natural products from natural sources had to shift, rather appropriately, to the combined technique of Gas Chromatography /Mass Spectrometry (GC-MS).

Table 4.10 Typical Column Chromatographic Data of representative plant extracts obtained in these experiments.

	Plant species / plant part	Extracting solvent	eluting solvent	Result	R_f	inference (if any)
1.	AC / root	$CHCl_3$	n-hexane	one spot	0.28	neutral compounds
			Ethanol	one spot	0.56	simple N compounds
			ethyl acetate	one spot	0.77	alkaloids
2.	AC / root	$CHCl_3$	acidification with HOAc / n-hexane / ethanol (50/50)	three spots	0.35 0.47 0.65	
3.	MO / leaves	$CHCl_3$	$CHCl_3$ / MeOH / NH_4OH / H_2O (70:26:2:2)	two spots	red yellow	–
4.	MO / leaves	$CHCl_3$	$CHCl_3$ / NH_4OH	one spot	red	–
5.	AC / leaves	aqueous Extract	$CHCl_3$ / MeOH 17 : 3	one spot	red	–
6.	AC / leaves	aqueous Extract	$CHCl_3$ / MeOH/ NH_4OH/ H_2O (70:26:2:2)	two spots	red yellow	–
7.	AC / leaves	aqueous Extract	H_2O / MeOH 1 : 3	one spot	yellow	–

	Plant species / plant part	Extracting solvent	eluting solvent	Result	R_f	inferences (if any)
8.	AC / leaves	aqueous extract	$CHCl_3$ / NH_4OH	one spot	red	–
9’	AC / leaves	aqueous Extract	NH_4OH / MeOH / acetone (2:1:1)	two spots	red yellow	–

Note (i) Visual observation; no uv lamp was available to aid further spot evaluation.

(ii) Red corresponds to absorption band maximum (λmax) at long wave lengths of visible spectrum (λmax 540 – 600 nm);

(iii) Yellow corresponds to about λmax 350 – 400nm (that is, shorter wavelengths).

4.4 Gas Chromatographic (GC) Data

Gas Chromatographic data for the various plant extracts are presented in the Appendix as follows:

Appendix 1: Gas Chromatogram of the chloroform extract of air-dried leaves of *Moringa oleifera*. This is denoted as Sample A.

Appendix 2: Gas chromatogram of the aqueous extract of air-dried leaves of *Moringa oleifera*, denoted Sample B.

Appendix 3: Gas chromatogram of the chloroform extract of air-dried aerial parts of *Ageratum conyzoides*, Sample C.

Appendix 4: Gas chromatogram of the aqueous extract of air-dried aerial parts of *Ageratum conyzoides*, Sample D.

Appendix 5: Gas chromatogram of chloroform extract of air – dried root system of *Ageratum conyzoides*, Sample E.

Appendix 6: Gas chromatogram of ethanolic extract of air – dried root system of *Ageratum conyzoides*, Sample F.

Appendix 7: Gas chromatogram of ethanolic extract of the flower of *Ageratum conyzoides*, Sample G.

The lipid-soluble components in the extract obtained in sample A (solvent, chloroform, MO, leaves) has nine (9) component peaks compared with four (4) in aqueous medium in Sample B; in Sample C (solvent, chloroform, AC, leaves), there are 16 component peaks compared with Sample D (solvent, aqueous medium, AC leaves.) with five (5) component peaks; in Sample E (solvent, chloroform, AC, root), there are nine (9) component peaks compared with Sample F (solvent, EtOH, AC, root) with six (6) component peaks.

These data are summarized in Table 4.11. and are expectedly fewer than the numbers reportedly obtained from the steam - distilled essential oil from the plant AC reported by other researchers such as Kamboj and Saluja, (2008). The ethanolic extract of AC leaves obtained by Adebayo, (2009) contained a total of eighteen (18) components, including several hydroxyl compounds which have been excluded in these experiments by the use of chloroform $CHCl_3$ as extracting solvent. His figure for ethanol is however comparable to the sixteen obtained here. Handling of these fewer numbers of component peaks obtained by solvent extraction has of course been easier, an added reason for the choice of the procedure, which the GC-MS experiment has resolved much better than either TLC or column chromatography. Besides, extractions by hydrodistillation contain many more varieties of components of varying volatilities suitable for the fragrance industry for which they are intended (Burnett, 2014).

Table 4.11 Summary of GC peaks obtained from Extractions.

Plant species / Part extracted	Extracting solvent	no. of GC component peaks
MO / leaves	$CHCl_3$	9
	Aqueous medium	4
AC / leaves	$CHCl_3$	16
	Aqueous medium	5
AC / root	$CHCl_3$	9
	Aqueous medium	6

4.5 Mass Spectrometric (MS) Data.

The primary mass spectrogram of each component peak appearing in each of the samples A to G is presented in Appendixes 8 - 14. This is the so-called fragmentation pattern of the component under the peak

4.6 Combined Secondary Data from GC – MS

More relevant to the analysis of the fragmentation patterns are the secondary data derivable from both the gas chromatographic peaks and those from the mass spectrograms. These are fully presented in Tables 4.11 to 4.17.

4.6.1 General Inferences drawn from the Various Tables of Combined Secondary Data

Secondary data are data extracted from the primary data, for the purpose of further analysis. The primary data are in the form obtainable from Gas Chromatographic data, while secondary mass spectrometric data are obtainable from the fragmentation patterns.

Secondary Gas chromatographic data include the retention time as well as the relative concentration of the component under each GC peak, while the secondary mass spectrometric data include values for M^+ and such other accompanying fragmentation masses as M + 1, M + 2 and base peak. These values are used in the considerations that lead to the molecular formula and structure of the component under the GC peak.

For further analysis, the following symbols are used (McLafferty and Turecek, 1993):

M^+ stands for MS peak corresponding to the *m/z* value of the monoisotopic ion mass, also referred to as molecular ion mass or parent ion mass. Ideally, the molecular mass of the original component molecule can be inferred from this parameter which is considered as the mass peak containing atoms of the most abundant isotope for each element (^{12}C, ^{16}O, ^{1}H, etc) present in the molecule. The value is often one of the highest and most characteristic *m/z* ratio ions in the E.I. mass spectrum.

M+1 is used to represent an isotope cluster peak, usually found one Dalton upward from M^+ in the E.I. mass spectrum.

M+2 is also an isotope envelope peak two Daltons upward from M^+ in the E.I. mass spectrum. This is usually found in fragmentation patterns of compounds that contain isotopes of +2 mass heavier than the usual natural isotope, such as ^{37}Cl.

The base peak is the highest abundance peak (usually designated 100%) in the system of MS peaks for each component. It is almost if not always given by the most stable ion.

Assembled in Tables 4.12 - 4.17 are the peaks in the total ion chromatogram obtained for each component present in each of the various extracts of the two plants used in these experiments.

It is necessary to observe at this point that the fragmentation patterns of the components under most of the GC peaks are in general quite extensive, almost always resulting in rather low concentrations of the ion mass assigned to each monoisotopic ion (M^+) peak. This phenomenon is suggestive of a largely aliphatic, rather than aromatic, compound composition. The base peak is however easily identifiable as being usually a stable fragment of the original molecule in a few cases. The monoisotopic ion peak is however not always easily identifiable, and even in cases where it tends to occur and thus be identified, identification is with considerable uncertainty. Several attempts to ameliorate this situation in cases where they occurred by seeking to run increasing quantities of component, or even to run the instrument in the select-ion monitoring (SIM) mode to provide much greater sensitivity than the scan mode, or even to apply the fast automated scan/SIM type (FASST) data acquisition technique which the SHIMADZU PLUS accommodates did not succeed for lack of technical expertise. These further discussions have consequently been focused on instances in which considerable certainty can be associated with the measured concentration-dependent identification of the monoisotopic ion, M^+, and its associated M+1 ion peak. Beynon Tables, available in abridged version in McLafferty and Turecek, (1993) have also been consulted in a limited number of cases. These identifications are

presented in the form of such other information as molecular formula, and associated molecular structure, wherever possible.

The combined GC-MS secondary data for the chloroform extract of the leaves of the plant *Moringa oleifera* are listed in Table 4.12 for each of the nine (9) component peaks. There is no component peak in the retention time interval (generally referred to as retention time window) between thirteen (13) minutes and twenty-four (24) minutes, which corresponds to the retention time window between component peak No. 3 (retention time 12.575 min.) and component peak No. 4 (retention time 24.775 min.). There is also no component in the MS that is associated with *m/z* 190 (the monoisotopic ion mass expected for precocene I) or with the base peak at *m/z* 175 (the most stable ion peak). The inference from these data is that there is no GC peak and no corresponding MS fragmentation data that are attributable to the presence of precocene I in the phytochemical profile of the leaves of *Moringa oleifera*. This same inference applies to precocene II. These two inferences justify the use of *Moringa oleifera* as blank (control) in sourcing chromenes in these experiments.

A second observation is that only one of the components in Table 4.12 shows a measurable M + 1 peak, an indication that only for this component may a reliable molecular formula be determined. It is further inferred, from the position of the major peaks at lower levels of fragmentation masses of the components in the chloroform extract of the leaves of *Moringa oleifera* that the majority of components are largely paraffinic, as distinct from aromatic. The inversion of masses after GC peak 7 was noted, and is often attributed to hydrolysis or adsorption effects (Hites, 1992).

Table 4.12 GC-MS Combined data for Chloroform extract of leaves of *Moringa oleifera* (Sample A)

GC component peak no.	GC Retention time(min)	M^+	M+1	M+2	Base peak
1	4.017	114	-	-	43
2	9.033	94	95	-	94
3	12.575	133	-	-	91
4	24.775	223	-	-	57
5	25.942(unresolved)	256	-	-	43
6	29.292(unresolved)	269	-	-	57
7	31.925(unresolved)	336	-	-	67
8	32.608(unresolved)	129	-	-	57
9	33.408(unresolved)	279	-	-	57

Table 4.13 details the characteristic secondary data for all four (4) component peaks present in the aqueous extract of the leaves of *Moringa oleifera*. The retention time windows have resolved into three, and there is no GC peak that corresponds to the retention time expected for precocene I or II. There are also no fragmentation masses suggestive of having their origins in the expected fragmentation pattern of precocene I or II. All the components in the extract are of paraffinic origin, as indicated by the positions of the major fragment masses, while only two of the components exhibit readily measurable M + 1 MS peaks from which their respective molecular formulae may be calculated from the respective fragmentation patterns.

Table 4.13 GC-MS Combined data from Aqueous extract of *Moringa oleifera* (Sample B)

GC. component peak no.	GC Retention time(min)	M^+	M+1	M+2	Base peak
1	3.267	59	-	-	44
2	25.967	256	257	-	43
3	27.667	282	-	-	55
4	27.892	284	285	-	43

All masses are in the correct ascending order in terms of both GC retention time and monoisotopic ion masses.

Secondary data for the $CHCl_3$ extract of the leaves of *Ageratum conyzoides* have been assembled in Table 4.14. Of the sixteen (16) GC component peaks, three exhibited M + 1 peaks. Seven showed base peaks at high mass values and these include the three peaks that have mass values for the M + 1 peak. Thus the majority of the components are inferred to be paraffinic.

GC component peak No. 2 (retention time 19.933 min.) has the following characteristic values: *m/z* 190 (M^+), *m/z* 191 (M + 1), base peak *m/z* 175. It is located in the GC window where there are no interferences from extraneous peaks, unlike several other succeeding peaks that occur either as unresolved peaks or where the base line has departed from running parallel to the time axis. Measurement in this part of the retention window and of the fragmentation ion masses are consequently expected to be relatively error-free.

Table 4.14 GC-MS Combined data for Chloroform extract of Aerial parts of *Ageratum conyzoides* (Sample C)

GC component no.	GC Retention time (min)	M^+	M+1	M+2	Base peak
1	5.467	106	-	-	91
2	19.933	190	191	-	175
3	21.825	189(est)	-	-	43
4	22.483	136	-	-	41
5	22.758	216	-	-	201
6	23.225	248	-	-	233
7	23.642	262	-	-	247
8	24.342	224	225	-	153
9	24.767	223	-	-	57
10	25.542	270	-	-	74
11	25.933	256	257	-	43
12	27.625	264	-	-	55
13	27.875	284	-	-	59
14	29.283	269	-	-	57
15	31.567	149	-	-	105
16	32.617	239	-	-	43

The occurrence of GC peak No. C2 is immediately followed by the occurrence of two peaks, namely, Nos. C3 and C4 which have lower values of M^+ for assigned monoisotopic masses, but with increasingly retention times. This is also the case with a few other GC peaks, namely, No. 8 (retention time 24.342; M^+ 224) after peak C7 (retention time 23.642; M^+ 262), and No. C9 (retention time 24.767; M^+ 223). Such inversions have been explained in terms of hydrolysis of adducts (Hites, 1992) or rearrangement due to H – migrations (McLafferty and Turecek, 1993). In support of the hydrolysis proposition, Margolin and Long, (1973), who studied the hydrolysis of chloroform ($CHCl_3$), proposed a mechanism which is generally believed to accommodate a rate-determining decomposition of the conjugate base of deprotonated $CHCl_3$ to give dichloro carbene. The latter is produced free of energy requirements and not as solvate. The volume of

activation ($\Delta V^{\#}$) is +16 cm^3 mol^{-1}, according to data published by LeNoble, (1978). The positive value of the volume parameter for this hydrolysis reaction is indicative of charge delocalization onto d-orbitals in chlorine after the loss of hydrogen ion (H^+) by $CHCl_3$, a factor long held to account for the acidity of chloroform in basic medium (Margolin and Long, 1973).

The hydrolysis scheme was presented (LeNoble, 1978) as:

$$CHCl_3 + OH^- \rightleftharpoons CCl_3^- + H_2O \xrightarrow{slow} :CCl_2 + Cl^-$$

$$:CCl_2 + :CCl_2 \rightarrow C_2Cl_4$$

Some similarity between this scheme and the dehydrochlorination of vinylidene chloride copolymer seems obvious.

Table 4.15 lists secondary GC-MS data for the five component peaks obtained in the GC from the aqueous extract of the leaves of *Ageratum conyzoides* from which some observations can be made.

These include the total absence of any retention and fragmentation mass data that could be linked to precocenes I and II . It can therefore be validly inferred that precocene I, although present in the $CHCl_3$ extract of the aerial parts of *Ageratum conyzoides*, is not ordinarily soluble in aqueous media.

Three of the five component peaks in Table 4.15 have prominent fragmentation lines to high masses, indicating either aromatic characteristics or reasonably heavy atom presence in the molecules, while only two have measurable associated M + 1 values.

Table 4.15 GC-MS Combined data for Sample D (Aqueous extract of aerial parts of *Ageratum conyzoides* (AC))

GC component no.	GC Retention time (min)	M^+	M+1	M+2	Base peak
1	4.06	164	166	168	94
2	5.467	106	107	-	91
3	20.775	206	-	-	191
4	25.925	256	-	-	43
5	27.608	264	-	-	55

In the $CHCl_3$ extract of the root system of *Ageratum conyzoides* (Table 4.16), peak No. 4 (retention time 17.583 min.) has *m/z* 190 (M^+), *m/z* 191(M + 1), and base peak *m/z* 175. These data indicate the presence of precocene I in the root system of *Ageratum conyzoides*. Secondly, while two component peaks, at retention times 17.583min. (peak No. 4) and 23.325 min (peak No. 6), respectively, have measurable associated M + 1 mass values, the other seven have no prominent M + 1 intensity.

Some inversions in the EI fragmentation patterns are also noticeable in Table 4.16. For instance, component peak No. 1 (retention time 6.375min.) has associated *m/z* 136 as M^+ with base peak at *m/z* 93, while component peak No. 2 (retention time13.892 min.) also has associated *m/z* 136 as M^+ but with base peak at *m/z* 95, a difference of two (2) Da. While this may suggest isomeric structures possibly occasioned by processes associated with hydrolysis and consequent desorption from GC column (Hites, 1992), some other changes in the ascending order of elution further complicate the characterization process. Examples in Table 4.16 include:

Peak No. 3 has retention time 17.183 min., M^+ at *m/z* 204, base peak at *m/z* 41;

Peak No. 4 has retention time 17.583 min., M^+ at *m/z* 190, base peak at *m/z* 175;

Peak No. 5 has retention time 17.750 min., M^+ at *m/z* 204, base peak at *m/z* 69;

Thus while the retention times for these three peaks are in the expected increasing order, the M^+ value increases from peak No.3 to peak No.4, and then decreases at peak No.5 increasing again through peak Nos. 6, 7, and 8 before falling to below 316 at peak No. 9.

Table 4.16 GC-MS Combined data for $CHCl_3$ extract of root system of *Ageratum conyzoides* (AC) (Sample E)

GC component Peak no.	GC Retention time (min)	M^+	M+1	M+2	Base peak
1	6.375	136	-	-	93
2	13.892	136	-	-	95
3	17.183	204	-	-	41
4	17.583	190	191	-	175
5	17.750	204	-	-	69
6	23.325	223	-	-	57
7	24.583	256	-	-	73
8	26.125	316	-	-	301
9	29.075	295	-	-	55

Table 4.17 shows the GC-MS secondary data for the EtOH extract of the root system of *Ageratum conyzoides*.

Comparison with data in Table 4.16 in which data for the $CHCl_3$ extract have been presented, shows clearly that component peaks Nos. 1 – 6 in Table 4.16 are completely absent from Table 4.17, indicating the differences in solubilities of components in the two solvents used. Component peak No.7 in Table 4.16 has exactly the same value of retention time (24.583 min.) as component peak No. 1 in Table 4.17, the same value of *m/z* 256 (M^+) but different base peak values. This would indicate same molecular ion mass values but different stable ions and hence

different molecular form and solubilities. The same inference can be made for component peak No.9 in Table 4.16 and component peak No. 5 in Table 4.17. Both peaks recorded the same retention time of 29.075 min., and the same *m/z* 295 for M^+ and the same base peak value of *m/z* 55. Whereas however Table 4.17 records all data in the formal ascending order in both retention time and molecular ion mass, two inversions of the order are noticeable in the data in Table 4.16 including that involving peak Nos. 7, 8 and 9. As already remarked, such reverses of elution order are traceable to a number of factors such as migration of atoms (for example H) or group of atoms (example methyl) to form more entities, or interaction with the material of the separating column resulting in bleeding of adsorbents, or the splitting of adducts of, say, sugars with other interacting substances (McLafferty and Turecek, 1993). Hites, (1992) has detailed matters that can go wrong in GC-MS experiments and offered suggestions for amelioration some of which have been observed in these Tables.

Table 4.17 GC-MS Combined data for EtOH extract of root system of *Ageratum conyzoides* (AC)

GC component no.	GC retention time (min)	M^+	M+1	M+2	Base peak
1	24.583	256	257	-	43
2	26.308	282	-	-	55
3	26.533	284	285	-	43
4	27.600	269	-	-	43
5	29.075	295	-	-	55
6	29.283	297	-	-	43

Data in Table 4.18 have been presented to enable confirmation of chloroform ($CHCl_3$) as the only solvent among others used in these experiments that has been able to extract chromenes.

Table 4.18 GC-MS Combined data for EtOH extract of fresh flower of *Ageratum conyzoides* (AC)

GC component no.	GC Retention time(min)	M^+	M+1	M+2	Base peak
1	14.275	150	151	-	77
2	24.592	256	-	-	43
3	26.308	282	-	-	55
4	26.533	284	-	-	43
5	27.600	269	-	-	57
6	29.075	295	-	-	55
7	29.283	297	-	-	57
8	30.783	265	-	-	55

Aside from two GC peaks, namely, that at peak No. 1 (retention time 14.275 min; M^+ 150) and at peak No. 8 (retention time 30.783 min, M^+ 265), the other six (6) GC peaks in Table 4.18 occur as a replica of those in Table 4.17.

4.6.2 Other Components in the Extracts obtained from the Two Plants

Combined GC-MS data were scanned and analysed for other GC component peaks and their corresponding fragmentation patterns in the MS to enable identification of other chromenes that may be present or for record purposes. To effect valuable results, attention was focused on chloroform extracts of AC which have been shown in the foregoing to contain extracted precocene I, and comparison have been made with other extracts whenever it is necessary to illustrate inferences made or conclusion drawn. Components were therefore divided into two classes, namely, lipophilic components, that is, those soluble in, and extractable using chloroform as distinct from hydrophilic compounds, that is, those soluble and extractable in aqueous and water-like solvents such as ethanol. Some Tables have thus been repeated for emphasis and or clarity.

4.6.3 Lipophilic Components Extracted from the Plant *Moringa oleifera* (MO)

The components are listed in Table 4.12 and are for those extracted from the leaves of *Moringa oleifera*. Each component is tabulated along with its GC retention time and some MS characteristic lines from its fragmentation pattern. These lines are each presented under M^+ (the monoisotopic ion mass), M+1 (isotopic cluster), M+2 (isotopic envelope peak two Da upward from M^+), and base peak (the highest abundance peak usually designated as 100%).

Table 4.12 GC-MS Combined data for Chloroform extract of leaves of *Moringa oleifera* (Sample A) (Repeat)

GC component no.	GC Retention time(min)	M^+	M+1	M+2	Base peak
1	4.017	114	-	-	43
2	9.033	94	95	-	94
3	12.575	133	-	-	91
4	24.775	223	-	-	57
5	25.942(unresolved)	256	-	-	43
6	29.292(unresolved)	269	-	-	57
7	31.925(unresolved)	336	-	-	67
8	32.608(unresolved)	129	-	-	57
9	33.408(unresolved)	279	-	-	57

a Component No. 1 (A1) has a GC retention time of 4.017 min. The identified monoisotopic ion mass at *m/z* 114 is an even number which is expected to accommodate an even number of nitrogen (N) atoms in the molecule if nitrogen is present.

The concentration of this ion mass is low, suggesting an aliphatic substance origin. There is no characteristic isotope pattern, M+1 or M+2, and no molecular ion mass at both *m/z* 30 and *m/z* 44. These absences allow the inferences that the component molecular formula cannot be

determined by calculation from the (M+1) /M^+ relationship; that the halogens and sulphur are not present in the molecular composition, hence the absence of M+2, and that elemental composition has zero nitrogen content (absence of the two ion masses at *m/z* 30 and at *m/z* 44). Three additional lines are present in the fragmentation pattern at *m/z* 85, 71, and 57 which, according to Ho, (1990) are ion masses used in mass-chromatographic identification of aliphatics. Although the fourth characteristic ion mass at *m/*z 99 for aliphatics is not present in the MS of component A1, the two lines at *m/z* 43 (base peak) and 57 together add up to about 99 (with hydrogen abstraction) and would result in the two ions at 43 and 57 which show up in the fragmentation pattern.

The concentration of base peak at *m/z* 43 (the strongest line) suggests that the mass ion producing this fragmentation mass is more stable than the one producing the line at *m/z* 57. The line at *m/*z 41 must also result from the loss of an oxygen atom from a fragment mass in going from *m/z* 57 to *m/z* 41 (higher concentration than both at *m/z* 71 and *m/z* 57).

These data suggest that the molecular formula $C_7H_{14}O$ is the most appropriate for the identified monoisotopic ion mass of 114, and that the suggested molecular structure is that of a ketone, namely, n-butylethyl ketone shown in figure 4.2.

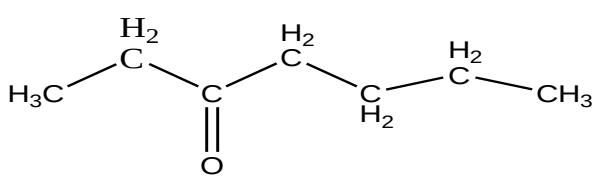


Figure 4.2 Structure of component A1

This is most likely to be a hydrolysis product. The component peak A1 is the weakest in the GC of the nine component peaks. The retention time of four (4) minutes puts it among the class of substances expected to be present in essential oils if present as such in such oils.

b Component peak No. A2 (retention time 9.033min.)

The identified monoisotopic ion mass at *m/z* 94 is also the base peak. The relative concentrations of the M^+ and M+1 peak ions allow a determination of the number of carbon atoms in the component molecule as six (6) and a molecular formula C_6H_6O.

This allows a choice between a phenol molecule (C_6H_5OH, an aromatic acidic substance) and a substituted furan (aldehyde or vinyl). The third alternative, with N in the molecular formula, is readily ruled out because the monoisotopic ion mass at *m/z* 94 is even and cannot contain less than two nitrogen atoms in the formula of a C_6 compound.

The absence of even a weak ion peak in the region *m/z* 70 – 77, rules out the phenolic substance alternative. For the furan alternative, loss of the ethylenic ($CH=CH_2$) group with hydrogen abstraction will result in a fragment ion mass at *m/z* 66 (this is the case). The aldehyde alternative would first lose CO (28) to give *m/z* at 66 as well. Neither alternative can be selected without further inquiry using appropriate complementary identification techniques.

c Component A3. (retention time 12.575 min.)

The monoisotopic ion mass value of 133 (odd) Implies that an odd number (1,3,5,…) of nitrogen atoms would be pesent in the molecule. The loss of *m/z* 29 from the molecular ion (133 – 104) is indicative of a CN group in the molecule, while a strong peak at *m/z* 91 (base peak) indicates that a tropylium ion ($C_7H_7^+$) is present also in the molecule and results from the loss of the CNO group. Also the prominent mass ion at *m/z* 77 is indicative of the presence in the molecule of a monosubstituted benzene ring which would add mass 14 Da (CH_2) to give the ion mass at 91. These and other concentration features give the component the following identity:

Compound formula : C_8H_7NO

Compound name : benzyl isocyanate.

The structure of the identified compound is shown in figure 4.3

Compound structure :

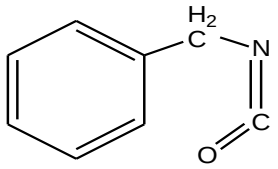


Figure 4.3 Structure of component A3

CAS number : 3173-56-6

RetIndex : O

The RetIndex of zero suggests that the actual number has not been assigned to the molecule in the chromatographic scheme. All the ions at *m/z* 91, 105, 119, and 133 used for the identification of alkylbenzenes in GC-MS analysis are present in the MS spectrum of this compound. The HCN reportedly extracted from the flowers of *Moringa oleifera* by Das, (1957), would thus be obtained from this component, and not from the benzylisothiocyanate presumed to be a hydrolysis product of pterogospermin (Das, 1957).

d Component peak A4 (retention time 24.775 min)

Peak A4 is the most abundant GC peak in the chloroform extract of MO leaves and has associated retention time of 24.775 min. This retention time is outside the retention time window for C_{10} molecular compounds, according to Hites and Biemann, (1970), and Ho, (1990).

The monoisotopic ion mass assigned to *m/z* 223 is an odd number and suggests an odd number of nitrogen (N) element in the molecular formula. The identified monoisotopic ion mass in the MS is weak, an indication that the component is a largely paraffinic substance. This is supported by the base peak at *m/z* 57. The next fragmentation ion downfield from the monoisotopic ion mass is at *m/z* 205. This is much weaker than the monoisotopic ion peak and is suggestive of the loss of 18 Da (H_2O) from the side-chain of the monoisotopic ion mass. The peak at *m/z* 57 (base

peak) suggests that the side-chain attached to the component molecule is the acetamide group. This would leave a molecular ion fragment at about *m/z* 165 which is present in the spectrum. Breakdown of this ion would result in other ion peaks at *m/z* 65, assigned to a C_5 unsaturated group, and at *m/z* 77, usually assigned to substituted benzene ring. The near absence of an isotope cluster peak in the MS makes the determination of the number of carbon atoms in the molecule of the component rather unreliable. The differences in the consecutive ion masses at *m/z* 77 and 65, 57 and 43, 41 and 29, as well as 39 and 27 however suggest considerable unsaturation in the molecule which combines characteristic ions of several classes in its mass-chromatographic ion composition. Thus the mass ions at *m/z* 104 and 112 are indicative of an alkyl styrene, the line at *m/z* 65 suggests that a C_5 ring system (unsaturated) is a component fragment, while the line at *m/z* 165 is suggestive of a fused system of two C_6 and one C_5 rings.

Even the unreliable concentration measurements obtained from the fragmentation pattern of the component under GC peak A4 yielded two figures for the number of carbon atoms per molecule, namely, fifteen (15) and sixteen (16). The C_{16} formula was not consistent with the monomolecular ion mass of 223, while the C_{15} formula yielded a molecular formula of $C_{15}H_{13}ON$. The structural formula which is consistent with the data and which accommodates a five-member ring structure is shown figure 4.4.

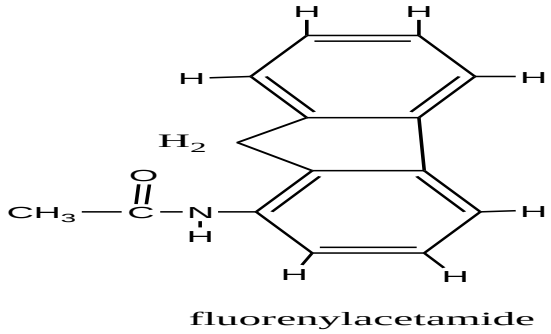


Figure 4.4 **Structure of component A4**

e Component peak A5 (retention time 25.942 min.)

The monoisotopic ion mass located at *m/z* 256 is of low concentration and there is no isotope cluster. The base peak is at *m/z* 43. The fragmentation pattern is extensive, and suggestive of a paraffinic component. The successive lines at *m/z* 227, 213, 199, 185, 171, and 157 are indicative of a homologue (Hites, 1992). There are no characteristic ions in the fragmentation pattern, and this coupled with the skewed nature of the GC peak makes further inferences about this component most uncertain. There are no characteristic ion masses to enable further screening.

f Component peak A6 (retention time 29.292 min.)

The monoisotopic ion mass for this component is at *m/z* 269, 13 Da heavier than the component under component peak A5. The most that can be inferred about this component is that it is largely paraffinic and that the GC peak appears in a retention window in which unresolved peaks occur.

g Component peaks A7 (retention time 31.925 min.), A8 (retention time 32.608 min.) and A9 (retention time 33.408 min)

These three components have appeared in the MS in a reverse order of magnitude of the respective lines assignable to their monoisotopic ion mass.

While the retention times increase from A7 to A8 and then to A9, the identified monoisotopic masses first increase from A6 (269) to A7 (336), then decrease to A8 (129) and increase to 279 at A9. This would occur when fragmentation is preceded by dissociation on the column before or during fragmentation (Hites. 1992). It could also occur if the spectrum is contaminated by a substance that was not originally in the sample being fragmented (Hites, 1992). There is accordingly a possibility that the component under peak A8 is the product of hydrolysis of the

adduct under peak A7 with monoisotopic ion mass at *m/z* 336 which is the largest mass in the spectrum. That the mass ion at *m/z* 129 under GC peak A8 is not a true molecular ion mass is discernible from the fact that the next lower MS fragment ion appears at *m/z* 125 only 4 Da short (Hites, 1992).

For peak A7, the loss of mass 201 Da in the MS in going from *m/z* 336 to the next lower ion peak at *m/z* 135 is indicative of dissociation during fragmentation. The subsequent fragmentation pattern is complex.

The GC component peak A9 has a fragmentation pattern three lines of which are usually assigned to three important ions of the common plasticizer di-(2-ethylhexylphthalate). The lines are at *m/z* 149 (s), *m/z* 167(m), and *m/z* 279 (w). Hytes (1990) remarks that these three lines are particularly abundant (5 to 20%) in polyvinylchloride-based plastic products such as Tygon tubing which may be used in GC-MS septa and which are subject to bleeding at certain prolonged temperatures. The spectrum in peak A9 is thus suspicious.

4.6.4.1 Lipophilic Components Extracted into chloroform from the Aerial parts of *Ageratum conyzoides*.

All the lipophilic components extracted into chloroform ($CHCl_3$) solvent from the aerial parts of the plant *Ageratum conyzoides* are listed in Table 4.14 which includes sixteen (16) component peaks in the GC. Component peak No. 2 (C2) was identified as the peak under which the additive 2,2 – dimethyl – 7 – methoxy chromene was the component which was used in the kinetic experiment. Other component peaks are examined using the same procedure.

Table 4.14 GC-MS Combined data from Chloroform extract of *Ageratum conyzoides* (AC) (Repeat)

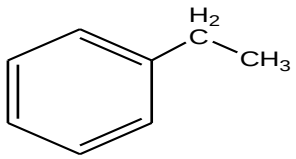
GC component no.	GC Retention time (min)	M^+	M+1	M+2	Base peak
1	5.467	106	-	-	91
2	19.933	190	191	-	175
3	21.825	189	-	-	43
4	22.483	136	-	-	41
5	22.758	216	-	-	201
6	23.225	248	249	-	233
7	23.642	262	-	-	247
8	24.342	224	225	-	153
9	24.767	223	-	-	57
10	25.542	270	-	-	74
11	25.933	256	257	-	43
12	27.625	264	-	-	55
13	27.875	284	-	-	59
14	29.283	269	-	-	57
15	31.567	149	-	-	105
16	32.617	239	-	-	43

a GC Component peak No. C1 (retention time 5.467 min.)

The mass spectrum of this C1 peak with GC retention time 5.467 min. enables the assignment of the ion mass at *m/z* 106 to the monomolecular ion. This is an even number and so the component molecule may contain an even number of nitrogen (N) atoms in its formula or none at all. There is a loss of 15 Da from this mass to give the ion at *m/z* 91, followed by another loss of 14 Da to give the ion mass at *m/z* 77. The base peak at *m/z* 91 is indicative of an alkyl substituted benzene ring (McLafferty and Turecek, 1993). The benzyl radical ion which is assigned to this base peak ion may be present as such in the molecule or may result from molecular rearrangements. The presence in the fragmentation pattern of an (M – 1) peak at *m/z* 105 does however suggest that the component molecular formula does indeed contain the benzylic group which would rearrange

to form the tropylium ($C_7H_7^{+}$.) ion radical to give an ion peak at *m/z* 91, and fragment further to give mass ions at *m/z* 77 ($C_6H_5^{+}$) and at *m/z* 65 (C_5H_5).

The molecular formula has been calculated using the (M + 1) concentration of 8.81% to be C_8H_{10}. Given the retention time of 5.467 min. which falls within the time frame for the monoethylbenzenes (Ho, 1990), the molecular structure arrived at is shown in figure 4.5:



Structural formula of C_8H_{10}

Figure 4.5 Structure of component C1

The alternative structure, C_6H_5 $(CH_3)_2$ is ruled out by the (M – 1) ion mass at *m/z* 105.

b GC Component peak C2 (retention time 19. 933 min.)

The component under this peak was isolated using liquid nitrogen, characterized by several physical methods and used as additive in the kinetic experiment. Detailed data from the characterization experiments have been presented in Table 4.8

Other GC component peaks in Extract C (Plant species, *Ageratum conyzoides*; plant part, aerial parts; solvent, chloroform).

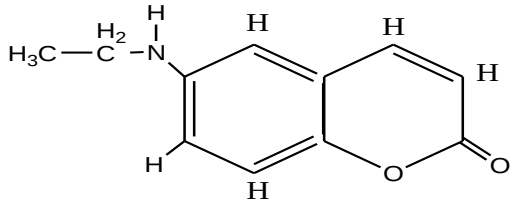
These other GC peaks, from peak C3 to peak C16, appear as a cluster of separate peaks at individual retention times that are very close to each other (peaks C4 to C7) or as unresolved doublets (C8 to C16), the latter within a retention time window where interferences do occur. Considerations of the peaks follow, but assignments would be tentative until supporting evidence is available in all cases so indicated.

c GC Component peak No. C3 (retention time 21.825 min.)

This GC peak is weak.

Concentration considerations indicate that the component is a C11 or C12 compound, with one of three possible molecular formulae, namely, $C_{11}H_{11}NO_2$, $C_{11}H_9O_3$, and $C_{12}H_{11}O_2$. The monomolecular ion mass corresponding to each of these three formulae and assigned to *m/z* 189 is odd and, according to the nitrogen rule, would include at least one N atom. This rules out the other two molecular formulae, leaving one formula, $C_{11}H_{11}NO_2$ for consideration.

The loss of 70 Da from the monomolecular ion mass to produce the next lower ion mass at *m/z* 121 which rearranges to *m/z* 119 indicates the loss of mass C_2H_2N (43Da) and another mass C_2H_3 (27 Da). Both these ion mass peaks appear in the mass spectrum. Characteristic ion peaks at *m/z* 55 and *m/z* 109 indicate that the component is an alicylic / olefinic molecule, while peaks at *m/z* 91 and *m/z* 119 place it among monoalkylbenzenes. Thus the component is complex, consisting of the two. The loss of oxygen indicates the presence of a keto group. Since in alkyl-substituted aromatic compounds, cleavage is very probable at the bond beta to the ring, resulting in a stabilized ion, a base peak is more likely to result from such cleavage and noting that the component is unsaturated, the molecular structure can properly be represented as a substituted coumarin as shown in Fig. 4.6:



(Structure of N-ethylaminocoumarin (189)

Figure 4.6 Structure of component C3

The 6-amino and 6-acetamido derivatives of chromenes have been reported to have anti-depressant and antipyretic properties (Kamboj and Saluja.2008; Miller, and Wood, US Patent 1968).

d GC Component peak C4 (retention time 22.483 min.)

This weak GC peak appeared as an unresolved doublet with a longer retention time of 22.483 min. compared with 21.825 min. for GC peak C3, but with a lower value of ion mass at *m/z* 136 assignable to the monoisotopic ion mass. The ion mass peak at *m/z* 136 is indicative of an alicyclic compound with molecular formula $C_{10}H_{16}$. All the characteristic ion mass peaks for acyclics are present in this mass spectrum. These appear at *m/z* 55, 69, 83 and the cluster of ion masses around *m/z* 91. This cluster represents rearrangements for stable ion formation in the fragmentation of this highly unsaturated component. The ion masses at *m/z* 93 and 136 are characteristic of acyclic monoterpenes. The mass at *m/z* 136 is weak, while those at *m/z* 69 (strong) and *m/z* 67 (weak) are both characteristic of ions of monoterpeniods. The base peak is at *m/z* 41, assignable to $C_3H_5^+$ ion. The two pairs of peaks at *m/z* 105 and 91 as well as those at *m/z* 69 and 55 indicate the loss of 14 Da each, signifying the presence of two CH_2 groups in the molecule. Literature search indicated that these characteristics fit the monoterpene myrcene, a substance that occurs in some natural oils and can form adducts with other compounds such as oxygenated substances (Finar, 2004). The structural formula is shown in figure 4.7:

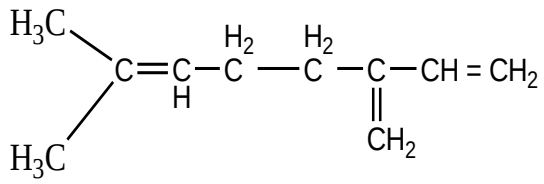


Figure 4.7 Sketch of terpenoid

This is represented in terpenoid practice as in figure 4.8:

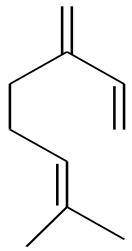


Figure 4.8 Systematic name: 7-methyl-3-methyleneocta-1,6-diene

The component is most likely a monoterpene aglycon of an adduct.

e GC Component peak No. C5 (retention time 22.758 min.)

The presence of an M – 15 peak (loss of CH_3) in the fragmentation pattern of this component comfirms the assignment of the molecular ion peak to the peak at *m/z* 216, while concentration values indicate that the component is a C_{14} compound. The base peak is at *m/z* 201 which loses 16 Da to give the next lower ion peak at *m/z* 185, signifying the presence of an oxygen atom in the base peak. Further fragmentation of the ion mass at *m/z* 185 indicates a loss of 57 Da to give an ion peak at *m/z* 128, an ion mass that is characteristic of alkyl-substituted naphthalene or naphthalene-like moieties (Ho, 1990). This ion mass peak at *m/z* 128 can be assigned to the chromene skeleton that has lost its attachments (three), namely, CH_3, O, mass of 57 ($(CH_3)_3C$), and thus has the form shown in figure 4.9:

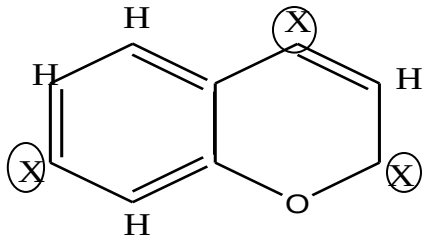


Figure 4.9 chromene skeleton

The circled x's indicate the three points from which attachments were lost.

The rest of the fragmentation pattern justifies the molecular formula $C_{14}H_{16}O_2$, and the most likely molecular structure is that shown in figure 4.10:

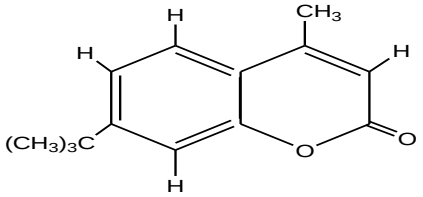


Figure 4.10 Structural formula of 4-methyl-7-trimethylbutyl coumarin (216)

f GC Component peak No. C6 (retention time 23.225 min.)

The ion mass at *m/z* 248 selected as the monoisotopic ion mass is accompanied by an (M -15) mass loss to give a peak ion at *m/z* 233. This peak at *m/z* 233, is the base peak which has also lost additional 16 Da to give the peak at *m/z* 217. This would also be interpreted to mean that the molecular ion mass lost a total mass of 31 Da to give the ion peak at *m/z* 271. Concentration measurements and considerations suggest that the component has a C_{14} molecular formula which, on treatment using the method of Dromey and Foyster, (1979), yielded the molecular formula $C_{14}H_{16}O_4$. Ion peaks at *m/z* 248, 233,128 and 91 which were selected for monitoring in the retention time window between 22.8 min. and 23.4 min. enabled the assignment of the fragmentation ion which appeared at peaks *m/z* 128, 91 and 77 as those associated with the fragmentation of chromenes, The two peaks at *m/z* 59 (CH_3COO–) and 43 (CH_3CO) denote as attached acetate (CH_3COO) group. The structural formula of the chromene ($C_{14}H_{16}O_4$) is in figure 4.11:

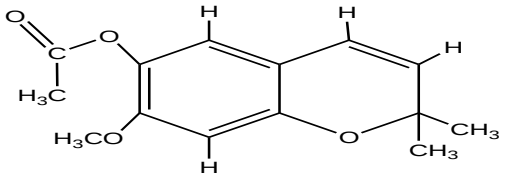


Figure 4.11 Structure of 6-acetoxy-7-methoxy-2, 2-dimethyl-2H-chromene (248)

Some 6-acetyl chromene derivatives were listed by Kamboj and Saluja, (2008) as components of the essential oils obtained from *Ageratum conyzoides* in various parts of the world including Vietnamese oil, Congo oil and Brazil oil but none was or has been reported for Nigerian oil.

g GC Component peak No. C7 (retention time, 23.642 min.)

The ion mass peak at *m/z* 262 (monoisotopic ion mass) has lost 15 Da (CH_3) to give the next lower ion peak at *m/z* 247 (base peak). Further fragmentations result in ion mass peaks at *m/z* 115 and 77 characteristic of chromenes. The loss of 14 Da (CH_2) from the ion peak at *m/z* 217 to give a peak at *m/z* 203 indicates that the component under the peak is a homologue of component C6, with the molecular formula $C_{15}H_{18}O_4$, and structural formula shown in figure 4.12:

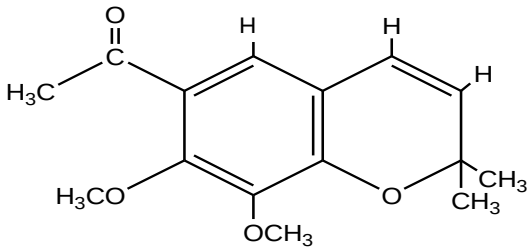


Figure 4.12 Structural formula of methylripariochromene (262)

Pratap and Ram, (2014) have quoted Anthonsen's work (Anthonsen, 1969) in which a component methylripariochromene was extracted and characterized as a chromene from an Australian plant, *E. riparium* Regel, a member of the Tribe Eupatorieae (same Tribe as *Ageratum conyzoides*). The same chromene component was also extracted and identified by Taylor and Wright, (1971) from the Jamaican weed *Eupatorium riparium* Regel. Both groups of researchers used chloroform ($CHCl_3$) as the extract solvent. Methylripariochromene is a derivative of precocene III which has not been identified as such in any of the chloroform extracts obtained in these experiments.

h GC Component Peak No. 8 (retention time 24.342 min.) to GC peak C16 (retention time 32,617 min.).

Component peak No. C8 is an unresolved doublet, and is the first of the series of peaks C8 to C16 recorded within a retention time window additionally complicated by background noise. The fragmentation pattern of the component under peak C8 is complex, with several ion peaks accompanied by clusters of weak peaks which may be regarded as H-rearrangements but also as fragmentation resulting from the unresolved GC peak. These clusters of peaks which have been notably absent from the GC peaks earlier considered have been a feature in all the GC peaks from C8 to C16, with the sole exception of GC peak No. C15 which is considered as arising from an impurity peak. For instance, the mass spectrum of the component under GC peak C15 includes an ion mass peak at *m/z* 149 which ordinarily may be assigned to the monomolecular mass of the component. The appearance of this monomolecular mass at a retention time of 31.567 min. is one of the reversals in the mass spectrum which has been noticeable in these spectra. It has occurred long after component peak No. 8 (retention time 24.342 min.; M^+ 224); peak No. 9 (retention time 24.767 min.; M^+ 223) ; peak No. 10 (retention time 25.542 min.; M^+ 270) ; peak No. 11 (retention time 25.993 min.; M^+ 256) ; peak No. 12 (retention time 27.625 min.; M^+ 264) ; peak No. 13 (retention time 27.875 min.; M^+ 284) ; peak No. 14 (retention time 29.283 min.; M^+ 269).

The loss of 44 Da (COO) from this ion mass at m/z 149 to give a fragment ion at *m/z* 105 (base peak) indicates that the component under this peak is a phthalate. There is however no reason to expect a phthalate (synthetic product) in a chloroform extract obtained from a natural source. Thus, the appearance of this GC peak No. C8 is implausible unless it is considered as a contaminant peak. More importantly, Hites, (1992) has documented three important ions at *m/z*

149, 167 and 279 and warned that they individually or collectively indicate a very common contaminant in MS, namely, di (2-ethylhexyl) phthalate, and which is present in several polyvinylchloride – based plastic products such as Tygon tubing and or septa. The appearance therefore of these three mass ion peaks at *m/z* 149, at *m/z* 167and at *m/z* 279, in the fragmentation pattern of component peak No. A9, where *m/z* 57 is the base peak, the presence of the two mass peaks at *m/z* 149 and *m/z* 167 in the fragmentation pattern of the GC component peak C9 where the base peak is at *m/z* 149, as well the mass ion peak at *m/z* 149 in the fragmentation pattern of GC peak C15, make assignments of the GC peaks C8 to C16 ambiguous. Further assignments will therefore not be pursued any further in these studies until complementary instrumentation is available for relevant information.

4.6.4.2 Lipophilic Components Extracted from the root system of *Ageratum conyzoides*

The nine (9) peaks obtained in the GC from the chloroform ($CHCl_3$) extract of the root system of *Ageratum conyzoides* are listed in Table 4.16 along with their respective GC retention times, and secondary data derived from their individual mass fragmentation patterns.

Table 4.16 GC-MS Combined data for $CHCl_3$ extract of root system of *Ageratum conyzoides* (AC) (Sample E) (Repeat)

GC component no.	GC Retention time (min)	M^+	M+1	M+2	Base peak
1	6.375	136	-	-	93
2	13.892	136	-	-	95
3	17.183	204	-	-	41
4	17.583	190	191	-	175
5	17.750	204	-	-	69
6	23.325	223	-	-	57
7	24.583	256	-	-	73
8	26.125	316	-	-	301
9	29.075	295	-	-	55

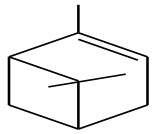
Table 4.16 shows that two GC peaks have two different retention times but exhibit the same value of monoisotopic ion mass. The GC peaks are Nos. 1 (E1), and 2 (E2). The GC retention times are 6.375 min., and 13.892 min., respectively. Additionally, the base peaks have different values. While peak E1 has a base peak value of 93, peak E2 has a base peak value of 95, a difference of 2 Da. Thus although the monoisptopic ion mass at *m/z* 136 has the same value for the two peaks, the degree of unsaturation in the two components is different; the component under peak E1 is more unsaturated than the component under peak E2.

These two peaks are the weakest peaks in the GC. The fragmentation patterns of both components under the two peaks exhibit ion mass peak at *m/z* 93 and at *m/z* 136, both of which are ions characteristic of monoterpenoid hydrocarbons.

That GC peak E1 exhibits a molecular ion M^+ 136, has a base peak at *m/z* 93, as well as a peak at *m/z* 91 makes the assignment of the component under it as α-pinene most probable. Thus α-pinene would be the major component under GC peak E1.

GC peak E2, with its longer retention time of 13.892 min., but the same molecular ion peak M^+ at 136, appears to be a monoterpenoid (isoprene) adduct from a prenylated chromene.

The pinene isomer (figure 4.13) most likely to be involved in the proposed adduct formation would be the beta isomer with the unsaturation point at the 1, 7 position through which it would form the adduct.



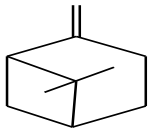


Figure 4.13 **α- pinene** **β- pinene**

both isomers occur naturally

GC component peak No. E3 (retention time 17.183 min.) and peak No. E5 (retention time 17.750 min.)

The components under the two peaks exhibit two different retention times in between which GC peak No. 4 (retention time 17.583 min.) appeared. Both component E3 and component E5 exhibit the same monomolecular ion mass of 204. These two components have two different fragmentation patterns. The ion mass for the two components assigned to *m/z* 204 as the monoisotopic mass loses 15 Da (CH_3) in both cases to give the next ion mass peak at 189. However, the base peak has different values. The component under GC peak No.3 has a base peak at *m/z* 41 which shows that the unsaturation group C_3H_5 is the most stable ion. GC peak component 5 has a base peak at *m/z* 69.

By the nitrogen rule, there is no possibility in either case that the formula (molecular) has any nitrogen (N) content. Additionally, one component, with *m/z* 41 as the base peak, has no oxygen content, while the other, at *m/z* 69, contains at least one oxygen (O) atom in its molecular formula. Concentration determinations give the component of the peak at E3 as a sesquiterpenoid (figure 4.14), C_{15} hydrocarbon compound, while the E5 (figure 4.15) component has C_{13} composition.

Appropriate molecular formulae were obtained as:

E3: $C_{15}H_{24}$

E5: $C_{13}H_{16}O_2$

Corresponding structures are:

E3:

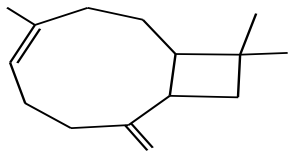


Figure 4.14 **4, 11, 11-trimethyl-8-methylenebicycloundec-4-ene**
(α- and β- caryophyllene)

E5:

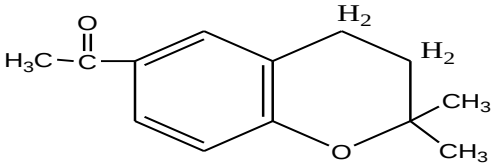


Figure 4.15 **6- acetyl- 2, 2- dimethyl- 3, 4- dihydrochroman**

The difference in mass of the base peak in each of the two cases may be rationalized on the basis that component E3 would have an unsaturated moiety (CH_2=$CHCH_2$. = 41) as base peak while component E5 would have a branched unit (CH_3 – $CH(CH_3)$ – CH = CH_2. = 69) as base peak following their structural differences.

Kamboj and Saluja (2008) reported that caryophyllene, a macrocyclic sesquiterpenoid, is a major component of the essential oil of *Ageratum conyzoides* found in Cameroun oil and in Pakistani oil. Ekundayo *et al*., (1988) have also reported that 6- acetyl -2, 2-dimethyl-3, 4-dihydrochromene was among the component chromene derivatives isolated from the hexane extract of the aerial parts of *Ageratum conyzoides*.

a GC Component Peak No. E4.

The component under GC Peak No. E4 was identified and characterized as precocene I and used in the kinetic experiment. The inversion in the order of increasing molecular mass with increasing retention time between GC peak No. E3 and GC peak No. E4 may thus be interpreted as arising from a difference in functional groups present in the components under each of the two peaks. GC peak No. E4 is the highest concentration in the chloroform ($CHCl_3$) extract of the root system of *Ageratum conyzoides*.

b GC Components E6 – E9

These four GC peaks have occurred within a retention time window in which interferences have been pointed out as occurring. Identification within this window are therefore unreliable and will not be presented.

4.6.5 Hydrophilic Components Extracted from both plant Sources.

With very few expectations, all the components of extract that appeared in GC peaks with retention times longer than twenty four (24) minutes in general exhibited unresolved doublets and had fragmentation patterns which featured interfering impurity peaks. In addition no water-like solvent extract indicated chromene content, using the four MS lines at *m/z* 175, 160, 132 and 77. Separations were therefore not attempted.

4.7 Kinetic Data obtained from the TGA Result.

4.7.1 Acquisition of primary Data, from TGA curve.

The TGA trace obtained for the decomposition of the vinylidene chloride / methylacrylate (5 mole %) copolymer in the isothermal mode at a constant temperature of 170°C after incorporation of precocene additive is reproduced in Figure 4.16. The trace is a plot of mass loss (%) against time (t) in minutes. It thus enables the derivation of kinetic data with which the decomposition process can be studied and characterized.

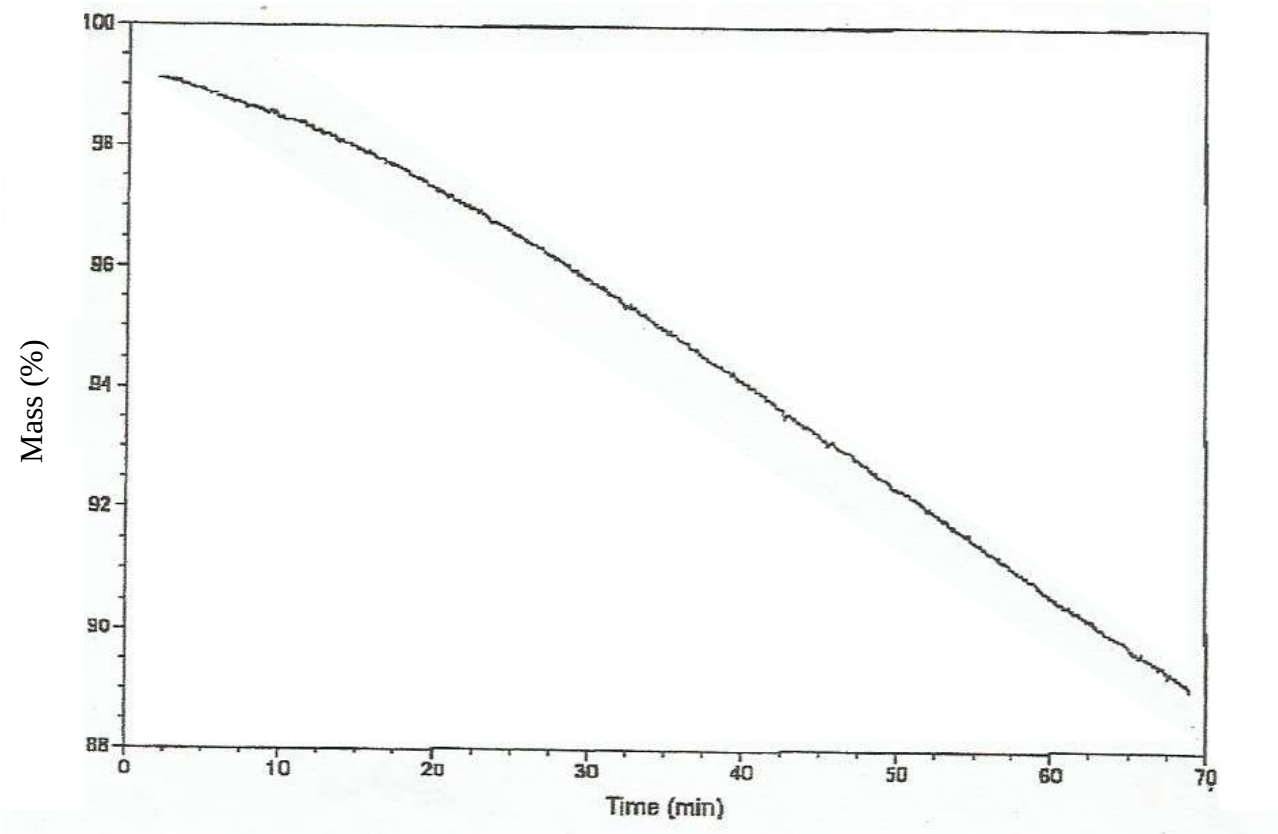


Figure 4.16 Thermogravimetric trace of the isothermal Degradation of Vinylidene Chloride / Methylacrylate (five mole per cent) / precocene I (0.1 mole %) additive at 170°C

Accordingly, two segments are clearly discernible in the TGA trace. The first segment extends from zero time to about ten (10) minutes and is characterized by a more gentle slope. It is recognizable as the initiation segment of the decomposition process during which energy is absorbed to start the process. This first segment gradually progresses into the second segment which ranges from about twenty (20) minutes to about forty (40) minutes and constitutes the propagation stage of the decomposition process. This is the segment in which the energy absorbed in the first segment, during the initiation stage serve to overcome the energy barrier to the decomposition process.

4.7.2 Derivation of Secondary Data from TGA Trace

Data derived from the TGA trace have been assembled in Table 4.19. They have been obtained as follow:

The mass of copolymer / additive remaining undecomposed after time (t) is (Wt)

Table 4.19 TGA Kinetic Data Extracted from the trace in Figure 4.16

W_o (mass of copolymer at time zero) = 0.99 W_∞ (mass of copolymer at infinite time) = 0.466

Time (min)	Time (s)	W_t	$(W_\infty - W_o / W_\infty - W_t)$	$\ln (W_\infty - W_o / W_\infty - W_t)$
2.2	132	0.98	1.0194	0.0192
3.3	198	0.96	1.0607	0.0589
5.5	330	0.949	1.0849	0.0815
6.6	396	0.04	1.1055	0.1003
9.8	588	0.931	1.1269	0.1195
13	784	0.84	1.4010	0.3372
16.4	984	0.768	1.6795	0.5185
19.7	1182	0.706	2.1833	0.7808
23	1380	0.654	3.7872	1.0250
26	1560	0.609	3.6643	1.2986
29.5	1770	0.572	4.9434	1.5980
32.8	1968	0.538	7.2778	1.9848
36	2160	0.51	11.9091	2.4773
39	2340	0.486	26.2	3.2657
43.6	2616	0.466	∞	∞

was determined from the TGA trace. These values of Wt and their corresponding times were therefore tabulated in Table 4.19 and used to obtain the plot in Figure 4.17

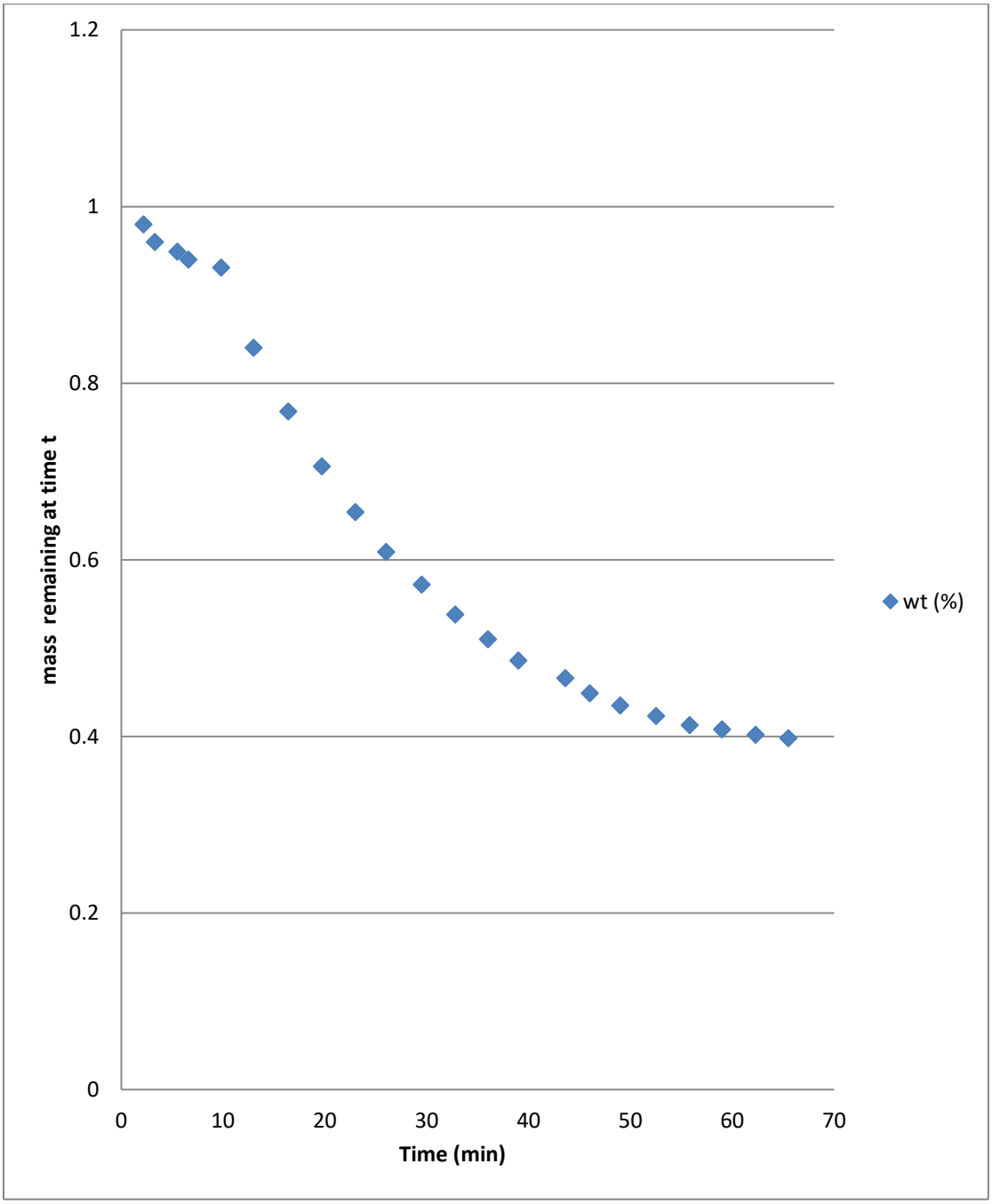


Figure 4.17 Plot of mass of Terpolymer remaining at time t (min) during degradation at 170°C

In the determination of these Wt values, cognizance was taken of the fact that both initiation and propagation phases of degradation can be deduced from the plot of mass remaining versus time shown in Figure 4.17. The initiation phase is however more easily recognized in the plot of ln $[(W_\infty - W_o) / (W_\infty - W_t)]$ versus time as shown in Figure 4.18, where:

W_∞ is mass of sample at infinite time (t_∞), taken as that mass of sample which remained after about one – third of the initial mass of vinylidene chloride component had been lost; this would correspond to the loss of about one mole of hydrogen chloride from each vinylidene chloride repeat unit in the polymer.

W_t is the sample mass remaining at any time, t, during the experiment.

W_o is the sample mass at time zero, t_o, taken as the time at which the first point on the graph was obtained.

The linearity of the plot in Figure 4.18 confirms the first order kinetic of the decomposition process, and results in the derivation of the rate constant for the initiation process. Figure 4.19 shows the corresponding plot of data obtained at long degradation times for the propagation process and gives the rate constant for the propagation of degradation. The linearity of both plots is in accord with results obtained from earlier studies of this degradation process such as those by Matheson and Boyer, (1952). Plots obtained in Figure 4.18 and Figure 4.19 were carried out using SCATTER in Microsoft Excel 2007.

The value for the rate constant for the initiation of decomposition of vinylidene chloride / methyl acrylate / precocene I obtained from Figure 4.18 was 1.16×10^{-5} mol s^{-1}, while that for propagation obtained from Figure 4.19 was 2.32×10^{-5} mol s^{-1}.

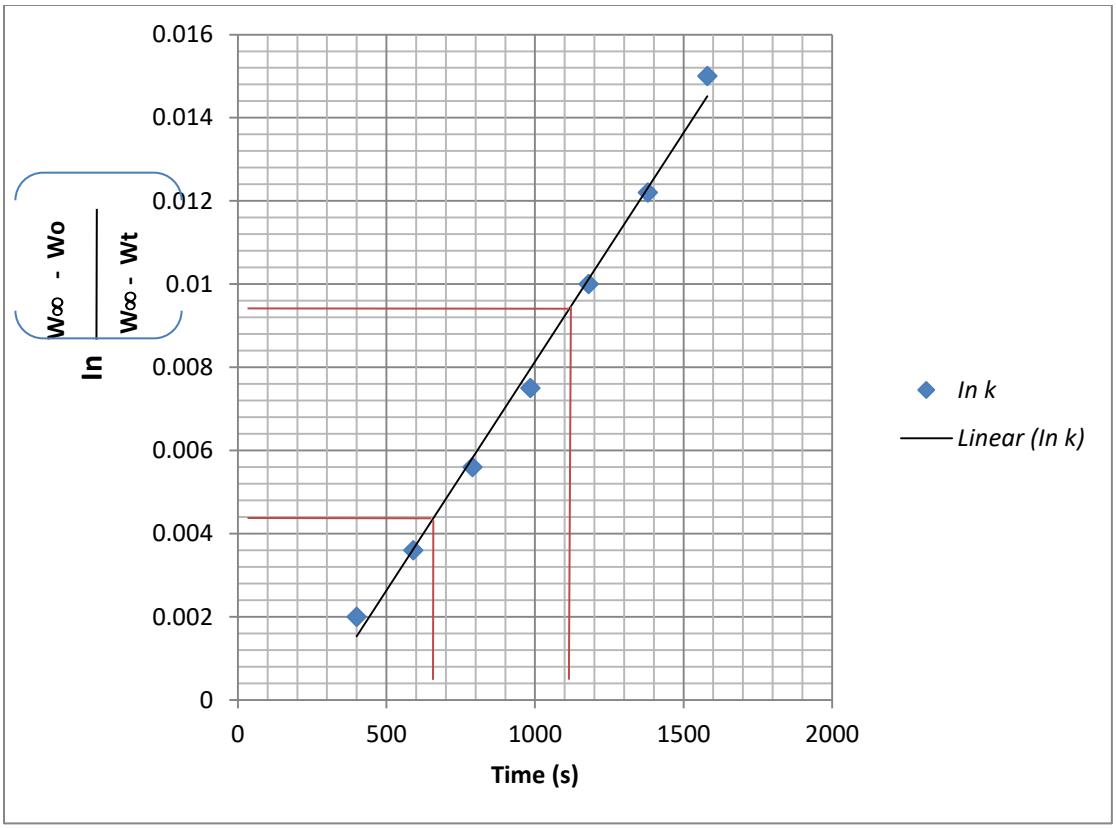


Figure 4.18 Plot of Data for the initiation of Degradation of PVDC / methyl acrylate copolymer / precocene I (0.1%) at 170°C

Slope = $\Delta y / \Delta x$

= (0.0092 - 0.004) / (1100 – 650)

= 0.0052 / 450

= 1.16×10^{-5}

y = mx + c = $1.16 \times 10^{-5} \times 1580 + c = 0.018328 + c = 0.015$

c = 0.015 - 0.018328 = - 0.003328

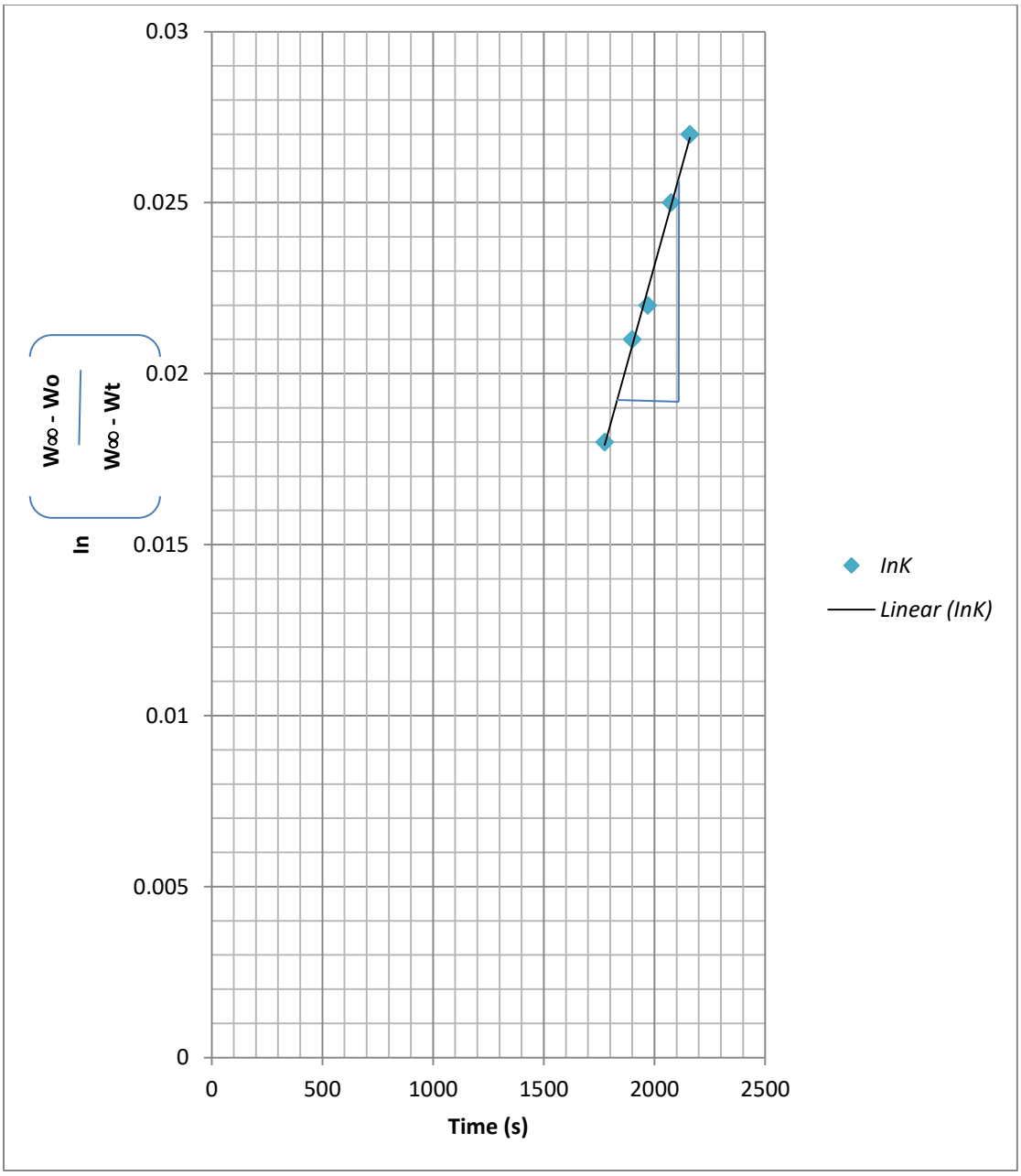


Figure 4.19 Plot of Data for the propagation of Degradation of PVDC / methyl acrylate copolymer / precocene I (0.1%) at 170°C

Slope = $\Delta y / \Delta x$ = (0.0255 - 0.019) / (2100 – 1820)

= 0.0065 / 280

= 2.32×10^{-5}

y = mx + c = $2.32 \times 10^{-5} \times 2075 + c$ = 0.04814 + c = 0.025

c = 0.025 - 0.04814 = -0.02314

4.7.3 Temperature Variation Studies of the Dehydrochlorination

Rate Data were additionally obtained at two other temperatures, namely, 180°C and 190°C. These additional temperatures were chosen to include part of the melting range of the VDC / MA copolymer used for these experiments. The temperature variation was to enable the derivation of the thermodynamic data for the decomposition process. TGA traces obtained at each of three different temperatures, namely 170°C, 180°C and 190°C, were generally similar in form, showing areas of initiation and of propagation at each temperature. Whereas, however, the initiation regime took relatively longer times with increase in temperature, the propagation sequence showed correspondingly shorter times.

Similar treatment of the mass – loss – versus – time data for the three temperatures resulted in corresponding values of rate constants for both initiation and propagation sequences. These values have been assembled in Table 4.20. Table 4.20 also includes literature data from Howell *et al*., (2007) for corresponding data using vinylpyridine as additive, for the purpose of comparison.

The data from Howell *et al*. (2007) also presented in Table 4.20 are for vinylidene chloride / methyl acrylate (five percent) without additive and with 4-vinylpyridine (0.1 mole %) additive. Both sets of data result in higher values for the rate constant both for initiation of degradation and for propagation sequence, while data from the incorporation of precocene I additive give comparatively lower rate values for the initiation sequence, but almost equal values for the propagation sequence at two temperatures, namely, 170°C and 180°C. At 190°C, however, the change of value from 8.97 x 10^{-5} s^{-1} (without additive) and 9.28 x 10^{-5} $s^{-1}$with 0.1% 4 - vinylpyridine, to 7.6 x 10^{-5} s^{-1} (with precocene I additive) becomes quite considerable. These changes indicate a lowering of the tendency of this natural phenomenon to occur. This means in

effect that the spontaneity of the system suffered a reversal at about 190°C. This observation prompted further efforts to obtain thermodynamic parameters of activation for the degradation reaction from these data.

Table 4.20 Values of Rate constant (k_i) for initiation and rate constant (k_p) for Propagation in the Thermal Degradation of the PVDC / methyl acrylate (5%) copolymer without and with additive (0.1%):

(a)	[additive] (mole %)	k_i x 10^5 (s^{-1})	k_p x 10^5 (s^{-1})	temperature (°C)
	0	1.54	2.07	170*
	0	3.31	4.27	180*
	0	6.27	8.97	190*
(b)	[4 – vinylpyridine]			
	0.1	1.86	2.26	170*
	0.1	3.65	4.76	180*
	0.1	6.62	9.28	190*
(c)	[precocene I]			
	0.1	1.16	2.32	170
	0.1	2.50	4.41	180
	0.1	5.58	7.60	190

* (a), (b) Data taken from Howell *et al*., (2007)
(c) Data obtained from these experiments.

4.7.4 Derivation of thermodynamic parameters for the Degradation reaction

Data from the temperature variation of the degradation reaction tabulated in Table 4.20 have been used to determine various possible parameters for the assessment of the tendency of a reaction such as the degradation reaction under consideration to move in a particular direction.

Data obtained for 0.1 mole of additive (precocene I), to the copolymer have been used to prepare appropriate plots to further characterize the nature of the degradation.

Accordingly, plots have been obtained of ln k versus [reciprocal of T (K)] for both the initiation, ln k_i, and the propagation, ln k_p, steps.

Figure 4.20 yields the value of the Arrhenius energy E_a of activation for the initiation process. The slope of this plot is given by -16 x 10^3 and is equal to $-E_a/R$, where R is gas constant; E_a, activation energy, has the value 133 kJ mol^{-1} from this plot. The plot of ln k_p/T versus 1/T in Figure 4.21 yielded a value of 98 kJ mol^{-1} for the propagation process. The plot yielded the value of enthalpy of activation for propagation of dehydrochlorination. The slope gives the value of $\Delta H^{\#}$ from the relationship slope = $-\Delta H^{\#}/R$.

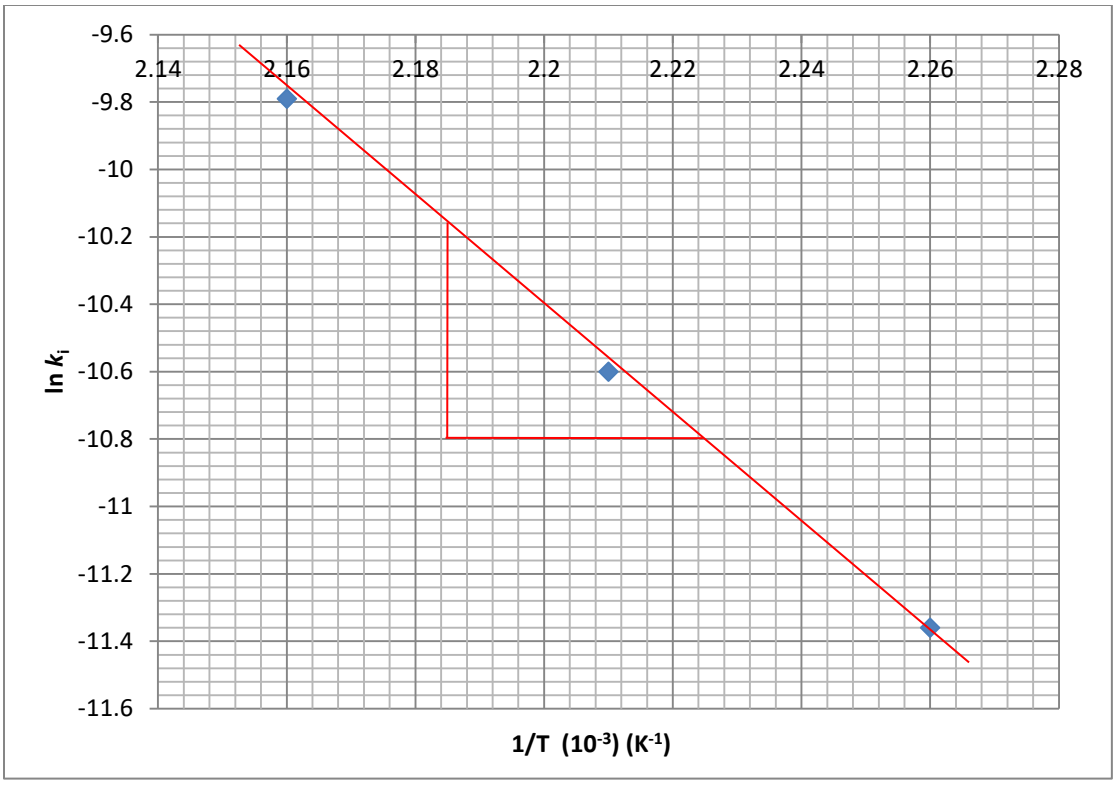


Figure 4.20 Plot of ln k_i versus reciprocal of T for the initiation process.

Slope = $\Delta y / \Delta x$

= -10.16 - (-10.8) / (2.184–2.224) x 10^{-3} = 0.64 / -0.04 x 10^{-3}

= -16 x 10^3 K

The value of the slope (m) is equal to $-E_a$ / R, where R is a constant equal to 8.314 J/mol K^{-1}

-16 x 10^3 = $-E_a$ / 8.314

E_a = 16 x 10^3 x 8.314 = 133024 J mol^{-1}

= 133 kJ mol^{-1}

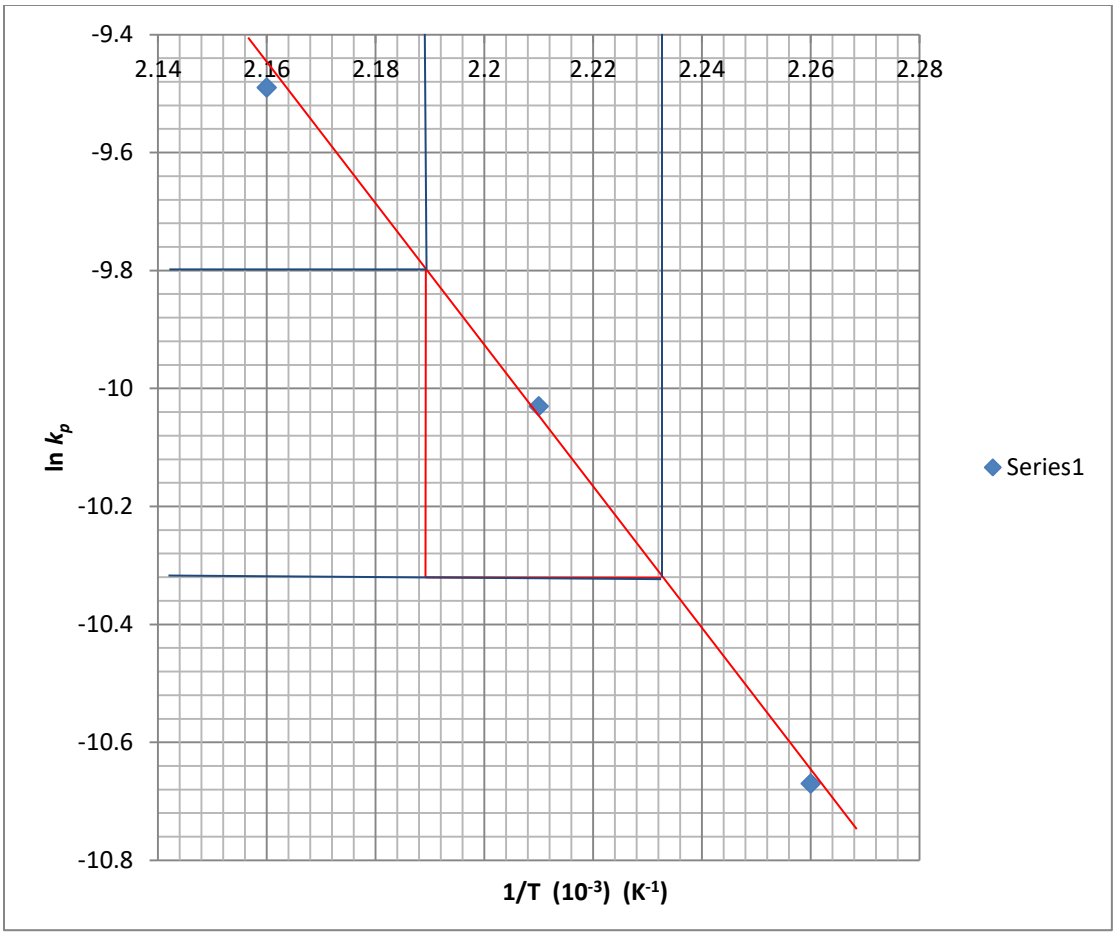


Figure 4.21 Plot of ln k_p versus reciprocal of T for the activation enthalpy for the propagation process.

Slope = -9.8 – (- 10.32) / (2.188 – 2.232) X 10^{-3} = 0.52 / -0.044 x 10^{-3}

= -11818.18 = $-\Delta H^{\#}/R$

$\Delta H^{\#}$ = 11818.18 x 8.314 = 98256.36 J mol^{-1}

$\Delta H^{\#}$ = 98.26 kJ mol^{-1}

The theory of absolute rates, (Fried *et al.*, 1977; Isaacs, 1977) based on transition state concepts, gives an expression for the rate of a chemical reaction for constant pressure conditions, in terms of three fundamental constant, h (plank's constant), k (Boltzman constant) and R (gas constant):

$k_{rate} = kT/h \ . \ e^{\Delta S\# / R} \ . \ e^{-\Delta H\# / RT}$

This is a fundamental equation of activated complex theory, which can be written in the form:

$\Delta S^{\#}/R = \ln k_{rate} + \Delta H^{\#}/R \ . \ 1/T + \text{constant}$

This form enables the evaluation of the activation entropy ($\Delta S^{\#}$) from the experimental rate of propagation. It has been used to determine the value of $\Delta S^{\#}$ by plotting In (k_p/T) versus 1/T in Figure 4.24, and substituting appropriate values for the fundamental constants in the expression:

k, Boltzmann constant, 1.380×10^{-23} J K^{-1}

h, Planck constant, 6.626×10^{-34} J s

$\Delta H^{\#}$ (enthalpy of activation) is assumed to be temperature – invariant in the temperature range 170°C - 190°C.

The plot in Figure 4.24 gives the value of the entropy of activation for the propagation of dehydrochlorination in the presence of precocene I at 190°C as -128 J $K^{-1}mol^{-1}$. Experimental values of Activation parameters for degradation have been assembled in Table 4.20 for comparison.

Table 4.21 Comparison of values of Activation parameters for the propagation step for the Thermal Degradation of Vinylidene Chloride / methyl acrylate copolymer at 190°C with and without additive.

Additive (mol%)	Enthalpy of Activation $\Delta H^{\#}$ (kJ mol^{-1})	Arrhenius Parameter E_a (kJ mol^{-1})	Entropy of Activation $\Delta S^{\#}$ (J K^{-1} mol^{-1})
None	121	125 (Source: Howell *et al*., 2007)	-32
4 – Vinylpyridine (0.1)	116	120 (Source: Howell *et al*., 2007)	-37
Precocene I (0.1)	94 (Source:	133 this work)	-128

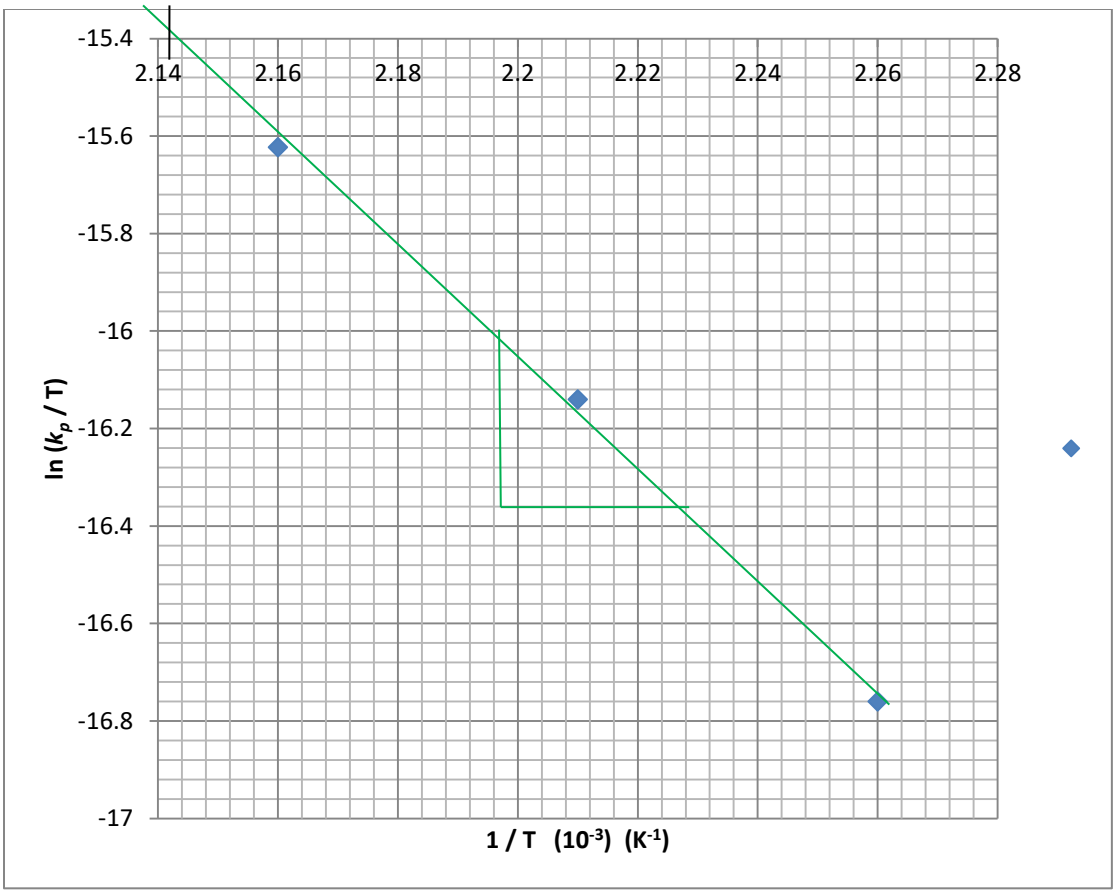


Figure 4.22 Plot of ln k_p / T versus reciprocal of T for the propagation process.

Slope = -16 – (- 16.36) / (2.196 – 2.228) X 10^{-3}

= 0.36 / -0.032 x 10^{-3}

= -11250

Slope = $-\Delta H^{\#}$ / R

$\Delta H^{\#}$ = 8.314 x 11250 = 93532.5 J mol^{-1}

= 93.53 kJ mol^{-1}

$\Delta S^{\#}$ = Intercept x R = -15.38 x 8.314 = -127.87 J mol^{-1} K^{-1}

≈ -128 J mol^{-1} K^{-1}

The thermodynamic activation data in Table 4.21 may be used to analyse and evaluate the molecular configuration in the system. For instance, the value of the enthalpy of activation shows a steady decrease, from 121 kJ mol^{-1} with no additive to the copolymer to the lower value of 116 kJ mol^{-1} in the presence of 0.1 mole % 4 - vinylpyridine additive. The still much lower value of 94 kJ mol^{-1} in the presence of 0.1 mole % precocene I additive is indicative of a considerable change in the sum total of the energy required for breaking old bonds and forming new bonds in the system. This is reflected in the expression:

$$\Delta H = \Delta U + P\Delta V \qquad (4.1)$$

where U represents the internal energy of the system, and V represents the volume which, for a solid substance or even for some liquid systems at moderate pressures, is assumed constant. The $P\Delta V$ term thus vanishes, leaving only changes in internal energy.

In the absence of additive, the bonds to be broken are the C – Cl bond of the vinylidene chloride entity (one bond), as well as the C – H bond of an sp^3 carbon in the copolymer chain. The bond to be formed is the product H – Cl bond. Approximate estimates of the values involved can be made using data from the Tables such as those compiled by Pauling, (1960). The bond energies of the C–Cl bond and of the C – H bond are given therein as 416 kJ mol^{-1}, and 328 kJ mol^{-1}, respectively. It would thus require at least a total of 744 kJ mol^{-1} to break both bonds. Formation of HCl by combination of H· radical and Cl· radical would result in the net introduction of the bond energy of HCl, namely, 432 kJ mol^{-1} to the system, resulting in net energy requirement of 312 kJ mol^{-1}. Thus ΔH is -312 kJ mol^{-1}. Approximately this latter value would also be the value of the enthalpy of activation with added 4 – vinylpyridine. This additive has been characterized

as sufficiently basic to actively strip hydrogen chloride from vinylidene chloride units thereby generating initiation sites for the propagation of thermal degradation (Howell *et al*.,2007).

Precocene I is neither acidic nor basic, and cannot therefore contribute to the promotion of H – abstraction which both acidic and basic substances contribute to. The situation with precocene I additive may thus be rationalized using bond energies and postulating that chromenes, especially precocene I, release the methyl radical ($CH_3\cdot$) with relative ease into the system. The subsequent formation of CH_3Cl from the hot methyl radical ($CH_3\cdot$) released with relative ease from precocene I and the equally hot chloride radical ($Cl\cdot$) available form the vinylidene chloride unit of the copolymer chain would release at least the bond energy of the gaseous methyl chloride product into the system, giving a net value of the entropy of activation, $\Delta S^{\#}$. This value, - 128 J K^{-1} mol^{-1} in the presence of precocene I additive, is the highest of the three values quoted in the Table. The entropy is a parameter that comes into reckoning when stable states of molecular aggregations are considered. Thus the most stable state of any assemblage of molecules is said to be that which has the lowest accessible Gibbs free energy, G, which has the composite nature as in the expression:

$$\Delta G = \Delta U + P\Delta V - T\Delta S$$

The expression $\Delta U + P\Delta V = \Delta H$ has been shown here to result in the estimated value of – 662 kJ mol^{-1}. The value estimated for $T\Delta S$ is about 60kJ mol^{-1} at T = 463 K.

Thus ΔG has the estimated value of –600 kJ mol^{-1}. This negative value predicts a reversal of the direction of reaction, and therefore the nondehydrochlorination direction.

It is therefore postulated that precocene I additive offers a more facile alternative to H – abstraction from the copolymer chain. This postulation is supported by the thermodynamic parameters obtained in these experiments and presented in Table 4.21.

It is important here to observe that all the chromene derivatives identified in the GC–MS experiment had one characteristic in common. Their respective fragmentation patterns in the MS exhibited a relatively strong (M – 15) peak, an indication of the facile breaking of the CH_3 bond at the C-2 position in the chromene molecule. The energy requirement for the breaking of their C–CH_3 bond may be estimated at about 45 kJ mol^{-1} at 190 °C which is relatively low compared with other energy values already considered. The chromene derivatives, in which the carbonyl (C=O) group has replaced the C-2 methyl groups present in the chromenes, do not exhibit the (M – 15) ion peak.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 Summary

A neutral oxygen heterocycle, precocene I, extracted from *Ageratum conyzoides* using chloroform solvent ($CHCl_3$) as the extractant, was separated from other co-components using gas chromatography (GC). Isolation was by trapping in liquid nitrogen while identification was by mass spectrometry (MS) using the fragmentation pattern. The compound was incorporated into VDC/methyl acrylate copolymer. The kinetic data obtained from the TGA analysis of the product of incorporation were analyzed graphically, and derived thermodynamic parameters indicated a more stable arrangement in the product of incorporation with lower degradation spontaneity when compared with known literature values without additives as well as with nitrogen bases.

5.2 Conclusion

5.2.1 The chromatographic separation and isolation (GC) and structural elucidation (MS) procedures have clearly indicated that AC contained terpenoids (chromenes and derivatives). Precocene I, an oxygen heterocyclic compound, was extracted as a pure compound by the method of extraction used.

The incorporated product which was examined visually had no compatibility issue. It is therefore concluded from these statements that a pure compound, precocene I, was proposed and sourced successfully for incorporation into VDC/MA as additive. Also concluded from these statements is that the additive was successfully incorporated into VDC/MA copolymer without the appearance in the product of incorporation of any lumps or boundaries as evidence of different

phases in the resulting copolymer/additive. Precocene I purity can be checked using the MS spectrum (Appendix 16)

5.2.2 There was reduction in the rate of dehydrochlorination of the product of incorporation. At 190°C, however, the change of value from 8.97 x 10^{-5} s^{-1} (without additive) and 9.28 x 10^{-5} s^{-1} with 0.1% 4 -vinylpyridine, to 7.6 x 10^{-5} s^{-1} (with precocene I additive) became quite considerable. These changes indicate a lowering of the tendency of this natural phenomenon to occur. The conclusion from these TGA data is thus that the natural tendency for the copolymer formed by vinylidene chloride / methylacrylate (5 mole per cent) towards dehydrochlorination has been reversed in the presence of 0.1 mole percent precocene I additive.

5.2.3 It is also concluded that the proposed mechanism for the dehydrochlorination, with additive, namely, a transition state mechanism, yielded activation data in support of the lowering of the rate of dehydrochlorination. The negative value of the Gibbs energy for the dehydrochlorination process in the presence of precocene I additive is supportive of this reversal.

5.2.4 It is also concluded from a close look at the matrix elements present in the plant from which precocene I has been extracted that the results of this study have indicated that several of the elements usually recommended for good health in humans in micro quantities are present in *Ageratum conyzoides*. Thus, the weed *Ageratum conyzoides* may have been considered as a vegetable for human consumption but for the presence of such heavy metals as lead (Pb) and cadmium (Cd) which are regarded as cumulative environmental pollutants. Secondly, these elements, referred to as matrix elements, were not co-extracted with precocene I additive incorporated into VDC/MA copolymer. They would therefore not influence the dehydrochlorination of the copolymer, positively or negatively.

5.3 CONTRIBUTION TO KNOWLEDGE

i The solvent used for extraction of precocene I in these experiments has enabled the extraction of reasonable quantity of pure precocene I and not as an adduct. This method of extraction would certainly be of help in future attempt to further study the chemistry of this simple chromene.

ii The retention time parameter has previously not been reported for the chromenes reported in this work in the literature available to us. Precocene I has been characterized in this work in terms of physical parameters such as the retention time and this is a development that will ease the extraction and isolation of this substance whenever it is required for further studies.

iii Most importantly, the work reported here has introduced for the first time the use of a chromene to seek for the solution of the problem of degradation of polyvinylidene chloride copolymers or any other polymer. Data obtained from initial kinetic studies after the incorporation of precocene I into the polyvinylidene chloride / methyl acrylate copolymer have been shown to indicate appreciable reduction of degradation compared with results obtained using other named additives. Both the rate constants of degradation as well as thermodynamic derived data support this view. Such reversal of dehydrochlorination may be intrinsic and not dependent on concentration.

In particular, the thermodynamic activation parameters point to a more ordered system in the presence of precocene I additive

iv The modified extraction procedure adopted in these studies has resulted in the identification of a chloroform-extractable metabolite from the plant specie, *Moringa oleifera* (MO). The metabolite in question is N-2-fluorenylacetamide. This metabolite

has been reported in the literature to be a potential carcinogenic and mutagenic derivative which induces tumors in a number of animal species in the liver, bladder and kidney. The GC peak of this component in the chloroform extract of the leave of MO has the highest concentration. This is the first report to our knowledge that indicates the presence of this chemical compound in *Moringa oleifera*.

5.4 Recommendations for Further Work

It is recommended that the dehydrochlorination process be further studied using the dynamic method in Thermogravimetry.

i. Degradation processes may be studied in two ways, namely, degradation as a function of temperature (dynamic degradation), and degradation at constant temperature as a function of time (isothermal degradation). The latter procedure has been used in this research.

   The dynamic method should hopefully yield complementary data on the stability of vinylidene chloride / methyl acrylate (5 mole percent) / precocene I (variable mole %), including the temperature of maximum dehydrochlorination. This would enable comparison of data obtained therefrom on the relative stabilities of the copolymer with respect to E2 elimination (H – abstraction) in vinylidene chloride / methyl acrylate copolymers as these eliminations concern the generation of initiation sites for the onset of further degradation. Therefore it is necessary to examine this dynamic method using an optimal temperature which, as these data would suggest, is around 190°C.

ii. It is further recommended that experimental data should be generated to determine the nature of the aggregation of the vinylidene chloride / methyl acrylate (5 mole %) copolymer in the presence of precocene I additive. This will enable the assessment of the preferred role played by the additive and therefore the extent of its involvement in bond formation.

iii. A corollary to the second recommendation above is that appropriate data ought to be generated on the relative presence of HCl and or CH_3Cl in mixtures of the copolymer and varied quantities of precocene I additive so as to determine the favoured product of dehydrochlorination.

iv. increased quantities of the component should be run in order to ameliorate the difficulty in the identification of monoisotopic ion peak.

v. The instrument should be run in the select-ion monitoring (SIM) mode to provide much greater sensitivity than the scan mode, or even apply the fast automated scan/SIM type (FASST) data acquisition technique which the SHIMADZU PLUS accommodates in order to ameliorate the difficulty in the identification of monoisotopic ion peak, as this concerns other unresolved peaks.

REFERENCES

Adebayo, A.H (2009). Phytochemical, Biochemical and Toxicological Studies on *Ageratum conyzoides* Linnaeus. Ph.D. thesis, Covenant University, Ota, Nigeria.

Adebayo, A.H., Jia, C. –J., Zhanga, Y,-M., Hea, W. –J., Zenga, G. –Z., Hana, H. –J., Xua, J. –J., Akindahunsi, A. A., and Tana, N. –H. (2011) Toxicological Evaluation of precocene II isolated from *Ageratum* conyzoides L. (Asteraceae) in Sprague Dawley rats. Nat. Prod. Commun. 6, 1263.

Ahluwalia, V. K and Arora, K. K. (1982). Nuclear isoprenylation of phenols using H_3PO_4 J. Chem. Soc., Perkins Trans 1, 335.

Anthonsen, T. (1969). Extraction and Isolation of four new chromenes from the Australian plant *E. riparium Regel*. Acta Chem. Scand. 23, 3605

Atkins, P. and Paula, J. (2012). Atkins' Physical Chemistry. Ninth Edition. Oxford University Press. Chapter 22, pp 791 – 848.

Barnes, C. S., and Occolowitz, J. L. (1964). The Mass Spectrum of some Naturally Occurring Oxygen Heterocycles and Related Compounds. Austr. J. of Chemistry 17, 975.

Beynon, J. H. (1960). Mass Spectrometry and its application to Organic Chemistry. Elsevier, Amsterdam. Several pages, depending on molecular ion, M^+.

Bissada, S., Lau, C. K., Bernstein, M. A., and Dufresne, C. (1994). One-pot Synthesis of precocene I and II and a formal synthesis of robustadial A and B via 2-phenyl – 4H – 1, 3, 2, - benzodioxaborin. Can. J. Chem. 72, 1866.

Bose, C.K. (2007). Possible Role of *Moringa oleifera* Lam Root in Epithelial Cancer Medscape General Medicine 9,26

Bowers, W. S., Ohta, T., Cleere, J. S., and Marsella, P. A. (1976). Discovery of insect Antijuvenile hormones in plants. Science, 193, 542

Brookes, G. T., Ottridge, A. P., and Mace, D. W. (1988). The Effect of some furochromene and benzochromene analogues of 2, 2 - dimethyl - 7 - methoxychromene (precocene I) and benzofuran precursors on *Oncopeltus fasciaticus* (dallas) and *Locusta migratoria migratoriodes* (RαF)Pesticide Science, 22, 41.

Burkill, H.M. (1985). The Useful Plants of West Tropical Africa, Kew: Royal Botanic Gardens. vol. I. p. 51

Burnett, C. (2014). Safety Assessment of Citrus – Derived Peel Oils as Used in Cosmetics. Cosmetic Ingredient Review, Personal Care Products Council. pp 20 – 25.

Burt, M. E., Kuhr, R. J., and Bowers, W. S. (1978). Metabolism of precocene II in the cabbage looper and European corn borer. Pest Biochem. Physiol. 9, 300

Chapman, J. R. (1993). Practical Organic Mass Spectrometry, 2nd ed. New York: Wiley, pp 45 – 51

Chauder, B. S., Lopez, C. C., Lopez, R. S. C., da Silva, A. J. M., and Snieckus, V. (1998). Synthesis of benzopyrans using phenylboronic acid synthesis, p. 279.

ChEBI (Chemical Entities of Biological Interest) @ www. obi.ac.uk/chebi/
last visited Jan. 21, 2016.

Cole, D. J., Browning, E., and Schroeder, F. E. H. (2003). Encyclopedia of Modern Everyday Inventions. Greenwood Publishing Group. pp 129 – 132.

Collins, S. S., Yoda, K., Anazawa, N., and Brikinshaw, C. (1999). Defect Structures in Polymer Mainchain as initiation sites for VDC copolymer Degradation. Polym. Degrad. Stab. 66, 87.

Cordell, G.A. (1981) Introduction to Alkaloids: A Biogenetic Approach. Wiley-Intersicence, New York. pp 10 – 14.

Cordell, G.A. (2000). Biodiversity and Drug Discovery: a symbiotic relationship. Phytochemistry, 55, 470

Dalziel, J.M. (1937) The Useful Plants of West Africa. London:Crown Agents. p. 6.

Das, B.R., Kurup, P.A. and Narasimba, R. P. L. (1957). Antibiotic Principle from *Moringa pterygosperma*. VII. Antibacterial activity and chemical structure of compounds related to pterygospermin. Indian J. Med. Res. 45, 191.

Dean, F. M. (1963). Naturally Occurring Oxygen Ring Compounds. Butterworth, London. Chapter 7, pp. 161 – 168

Del – Vechio – Vierral, G., Orlando, V. S., Celia, H., and Marin, A. C. K. (2008). Antimicrobial activity of Essential oils of *Ageratum fastigiatum* by agar - diffusion method and minimal inhibitory concentration measurements. Rec. Nat. Prod. 3, 52.

Dike, S. Y., Merchant, J. R. and Sapre, N. Y. (1991). Precocene I and II: Isolation from *Ageratum houstonianum* and antihormonal activity in Sensitive insects. Tetrahedron, 47, 4775.

Dromey, R. G. and Foyster, G. T. (1979). Rapid Calculations of Elemental Compositions for high Mass Spectra Data. Dept. of Computing Science, University of Wollongong. Working Paper No. 21, 79 – 83.

Eilert, U., Wolters, B. and Nahrstedt, A. (1981). The antibiotic Principle of Seeds of *Moringa oleifera* and *Moringa stenopetala*. Planta Medica 42, 55.

Ekundayo, O., Laasko, I., and Hiltunen, R. (1988). Essential oils of *Ageratum conyzoide.* Planta Medica 54, 55.

Ellis, G. P. (Editor). (1977). Chemistry of Heterocyclic Compounds. Vol. 31 Chromenes, Chromanones and Chromones. Wiley, New York. pp 10 – 120.

Emblem, A. (2012) Plastics properties for packaging materials in Packaging Technology, ScienceDirect, Woodhead publisher pp 287 - 309.

Emsley, J. (1994). The Consumer's Good Chemical Guide. Transworld Publisher Ltd., London. Chap. 5 pp 24 – 27.

Fahey, J. W. (2006). *Moringa Oleifera:* A Review of the Medical Evidence for its Nutritional, Therapeutic and Prophylactic Properties. Trees for life Journal. pp 25 – 40

Finar, I. L. (2004). Organic Chemistry. Vol 2. Stereochemistry and the Chemistry of Natural Products. 5th Ed. Pearson Education. pp 356 – 359.

Fraga, B. M., Cabiera, I., and Garcia. V. P. (1999). HETEROCYCLES, One – step preparation of the 3, 3′- dimer of precocene II. 51, 2747.

Fried, V., Hameka, H. F., and Blukis, U. (1977). Physical Chemistry. Mac Millan Publishing Co., Inc. London. Pp . 787 – 788.

Fuglie, L. J. (1999). The Miracle Tree- *Moringa Oleifera:* Church World Service, Dakar, Senegal. Pp 15 – 20.

Gonzalez, A. G., Aguiar, Z. E., Grillo, T. A., Luis, J. G., Riviera, A., and Calle, J. (1991). Phytochemistry, Methoxy Flavones from *Ageratum conyzoide*. 30, 1269.

Halpin, R. A., El – Nagger, S. F., Mc Combe , K. M., Vyas, K. P., Boyd, D. R., and Jerina, D. M. (1982). Tetrahedron Lett. Resolution and assignment of absolute configuration to the (+)- and (-)- cis aand trans 3, 4 – diol metabolites of the antijuvenile hormone precocene I. 23, 1655.

Handbook of Plastics, Elastomers and Composites (2002). 4th Ed. McGraw – Hill. Charles A. Harper (Editor - in - Chief) pp 205 – 208.

Harbonne, J. B. (1998) Phytochemical Methods: A guide to Modern Techniques of Plant Analysis. Third Edition. Edmundsbury Press, Bury St. Edmunds Great Britain. p. 52

Haristoy, X., Fahey, J.W., Scholtus, I. and Lozniewski, A. (2005).Evaluation of antimicrobial effect of several isothiocyanates on Helicobacter pylori. Planta Medica 71,326.

Hatakeyama, T. and Quinn, F. X. (1999). Thermal Analysis Fundamentals and Applications to Polymer Science: Thermogravimetry, 2nd Ed. John Wiley & Sons. pp 45 – 71.

Haunerland, N. H. and Bowers, W. S. (1985). Arch. Insect Biochem. And Physiol. Comparative Studies on pharmacokinetics and metabolism of the Antijuvenile hormone precocene II. 2, 55

Hepworth, J. D. and Livingstone, R. (1966). The reaction of coumarins with Grignard reagent to yield methoxycoumarins and subsequent acid treatment to the corresponding dimethylchromenes. J. Chem. Soc. C, 2013.

Hiramatsu, Y., Adfa, M., Ninomiya, M., Yoshimura, Y., and Koketsu, M. (2013). Effect of 2, 2 - dimethylchromenes against the feeding Behavior of *Coptotermes formosanus* Shiraki. Jour. Pest Sc. 38, 228.

Hites, R. A. and Biemann, K. (1970). Mass Chromatographic Screening of chemical compound types in samples using certain specified ions. Anal. Chem. 42, 855.

Hites, R. A. (1992) Handbook of Mass Spectral Environmental Contaminants. 2nd edn. Boca Raton, FL. Lewis Publishers.

Ho, M. H. (1990). Analytical Methods in Forensic Chemistry. Ellis Hovwood Limited Chichester, England. Chap. 2, pp 16 – 20.

Howell, B.A., (1987) Thermally – induced Degradation of Vinylidene Chloride Copolymers: Effect and Defect Structures. J. Polym. Sc., Polym. Chem. Ed., 25, 1681.

Howell, B.A. and Smith, P. B. (1988). Studies of the effects of internal unsaturation and the incidence of Defect Structures on the Dehydrochlorination of Polyvinylidene chloride copolymers. J. Polym. Sc., Polym. Chem. Ed. 26, 1287.

Howell, B.A., and Liu, H. (1992) Degradation of Vinylidene chloride / methyl Acrylate copolymer in the presence of organic nitrogen basesThermochim. Acta, 212, 1

Howell, B.A., and Sastry, B.B.S. (1993). Degradation of Vinylidene Chloride / Methyl Acrylate Copolymers in the presence of phosphine", PROCEEDINGS, 22nd North American Thermal Analysis Society Meeting, pp 122 – 127.

Howell, B.A. and Rajaram, C.V. (1993). Evolved Gas Analysis in the Thermal Degradation of Vinylidene Chloride Copolymer system, J. Therm. Anal., 40, 575.

Howell, B.A., and Liu, M. (1994). Thermogravimetric analysis of the Dehydrochlorination of Vinylidene Chloride Copolymers. Thermochim. Acta, 214, 169.

Howell, B.A., and Uhl, F. M. (2000). Degradation of Vinylidene Chloride / methyl Acrylate Copolymers: Deleterious Effects of Hindered amines. Thermochim. Acta, 357, 113.

Howell, B.A., Ahmed, Z. and Ahmed, S.I. (2000). Thermal Degradation of Vinylidene Chloride Copolymers studied under Thermal analysis. Thermochim. Acta, 357/8,10

Howell, B.A., Odelana, A. O., and Beyer, D. E. (2007). Stability of Vinylidene Chloride Barrier Polymers, in Organic and Physical Chemistry using Chemical Kinetics. Nova Science Publishers Inc., NewYork. pp 257 – 277

http://agritech.triau.ac.in/horticulture/extraction_methods_natural_essential_oil.pdf). (last visited 12/12/2014)

http://pubchem.ncbirnlon.nih. (last visited 10/12/2013)

http://www.bing.com (last visited 18/4/2014)

http://www.lookchem.com/6-Demethoxyageratochromene/ (visited 9/22/2011)

Hussien, T. A., Mohamed, N. S-E., Monstafa, M. F. M., and El-Sayed M. A. (2010). A new Natural pyrrolone from the Egyptian *Ageratum* species. European Journ. Chem., 1, 140.

Isaac, N. S. (1977). Liquid Phase High Pressure Chemistry, John Wiley & Sons. Chap. 4, pp 181 – 198.

Isman, M. B., Yaw, J. V., and Proksch, P. (1986). Naturwissenschaften. Pharmacological Screening of *Ageratum conyzoides* L. 73, 500

Kalena, G. P., Jain, A., and Banerji, A. (1997). Molecules, Amberlyst 15 catalysed Prenylation of phenols: One - step synthesis of Benzopyrans. 2, 100.

Kamboj, A. and Saluja, A.K. (2008) *Ageratum conyzoides* L. A review on its phytochemical and pharmacological profile. Int. J. Green Pharmacy. 2, 59.

Katsuri, T. R., Thomas, M. and E. M. Abraham, E. M. (1973). Essential oil of Ageratum conyzoides. Isolation and structure of two constituents. Indian J. Chem. 11, 9

Kitomura, R. O. S., Romaff, P., Young, M. C. N., Kato, M. J. and Lago, J. H. G. (2006). Phytochemistry, Antifungal activity of two new prenylated chromenes including one with 6- carboxylic acid group isolated from the methylene chloride extract of the leaves of *Piperonia serpens.* 67, 2398.

Lago, J. H. G., Ramos, C. S., Cassanova, D. C. C., Morandim, A. A., Bergamo, D. C. B., Cavalheiro, A. J., Bolzani, V. S., Furlan, M., Guimaraes, E. F., Young, M. C. M., and Kato, M. J. (2004). Some biologically active chromenes isolated from species of the

genus *Piper*, including the prenylated chromene from *Piper gaudichaudianum*. J. Nat. Prod. 67, 1783.

LeNoble, W. G., (1978). High Pressure Chemistry, H. Kelm (Ed.), Reidel, Amsterdam, p. 307

Lewis R. S., (1993) Hawley's Condensed Chemical Dictionary Van Nostrand Reinhold, New York, 10th Ed. pp 70 – 75.

Logothetis, A.L., (1979). The Stability of the Vinylidene Chloride / alkyl acrylate system. J. Polym. Chem., Polym. Chem. Ed. 17, 2541.

Mao, L., Henderson, G., and Dong, S. –L. (2010). Biological activity and the effect of precocene I on the termite soldier population. J Entomol. Sci. 45, 27.

Margolin, Z., and Long, F. A., (1973). Studies of the base hydrolysis of chloroform. J. Amer. Chem. Soc., 95, 2757

Marianne, G., (2017)Miscellaneous Vinyl Thermoplastics in Brydson's Plastics Materials (Eighth Edition) pp. 427–440

Matheson, L.A., and Boyer, B.F. (1952). The Thermal Stability of Vinylidene Chloride Copolymers at modest temperatures (120° - 200°C). Ind. Eng. Chem., 44, 867

Mazumder, U.K, Gupta, M., Chakrabarti, S. and Pal, D. (1999). Evaluation of haematological and hepatorenal functions of methanolic extract of *Moringa Oleifera* Lam. root treated mice. Indian J. Exp. Biol. 37, 612

McLafferty, F. W. and Turecek, F. (1993). Interpretation of Mass Spectra, 4th Ed. Wiley. New York. All Chapters.

Meares, P. (1965). POLYMERS: Structure and Bulk Porperties. D. Van Nostrand Company Ltd. London. pp. 1 – 4.

Michel, B. (2013) 4 – Detailed Accounts of Thermoplastic Resins in Thermoplastics and Thermoplastic Composites (Second Edition), pp 189 – 714.

Miller, J. A., and Wood, H. C. (1968). The Pharmacological activities of some classes of compounds obtained from the plant *Ageratum conyzoides* L.:Anti-depressant, analgesic and antipyretic properties of simple chromenes and chromans. US. Patent 121 : 307.

Morton, J.F. (1991). The Horseradish Tree, *Moringa pterygosperma* (Moringaceae). Economic Botany 45, 318

Mucuna pruriens (2008), available at http://users.lycaeum.org/~desoxy/Mucunapruriens.htm- "Isolation of Alkaloids from the leaves of *Mucunapruriens*"

National Institute for Occupational Safety and Health (NIOSH). Pocket Guide to Chemical Hazards No.0661. Available at http://www.cdc.gov/niosh/npgd0661.html.

NIST Chemistry WebBook, NIST Standard Reference Database Number 69, Linstrom, P. J. and Mallard W. G., (Eds.), available at http://webbook.nist.gov. last accessed (4 / 10 /2013)

Nwankpa, E. M (2015). Vitamin and Mineral content of *Ageratum conyzoides* . Advances in Chemistry and Biochemistry 2,

Okunade, A. L. (2002). Allelochemicals from *Ageratum conyzoides* L. and their effects on related pathogens. Fitoterapia, 73, 1.

Owolabi, M. S., Ogundajo, A., Yusuf, K. O., Lajide, L., Villanneva, H. E., Tuten, J. A., and Setzer, W. N. (2010). Chemical Composition and Bioactivity of the Essential Oil of *Chromolaena odorata* from Nigeria. Rec. Nat. Prod. 4, 72.

Ozumba, N.A. (2008). *Moringa Oleifera*: A Review of its Medicinal and Other Uses. Institute for Development Studies, University of Nigeria, Enugu Campus.

Paisley, K. (2007). PVDC: New Developments, New Opportunities PLACE Conference St. Louis Missouri, USA.

Parshall, G.E. and Ittal, S. D. (1992) Homogeneous Catalysis, 2nd Ed. John Wiley & Sons, Inc., New York

Pauling, L. (1960). Nature of the Chemical bond, 3rd Ed. Cornell University Press. pp 31 - 50.

Piringer, O. G. and Baner, A. L. (2008) Plastic packaging: Interactions with Food and Pharmaceuticals. John Wiley & Sons pp 40 – 45.

Pratap, R. and Ram, V. J.(2014). Natural and Synthetic Chromenes, Fused Chromenes, and Versatility of Chromenes in Organic Synthesis. Chem. Rev. 114, 10476.

Pratt, G. E., Jennings, R. C., Hammeth, A. F., and Brooks, G. T. (1980). Lethal Metabolism of precocene II ascribed to a reactive epoxide. Nature (London), 284

Rana, V. S. and Blazquez, M. A. (2003).Chemical Composition of the volatile oil of *Ageratum Conyzoides* aerial parts. Int . J. Aromather 13, 203

Ribeiro, V. L., dos Santos, J. C., Bordignan, S. A., Apel, M. A., Henriques, A, T., and Poser, V. (2010). Compounds from *Ageratum conyziodes*: Isolation, structural elucidation and insecticidal activity. Bioresour. Technol. 101, 2506.

Rossberg, M., Lendle, W., Pfeiderer, G., Togel, A., Dreher, E–L., Langer, E., Rassaerts, H., Kleinschmidt, P., Strack, H., Cook, R., Beck, U., Lipper, K–A., Torkelson, T. R., Loser, E., Beutel, K. K., and Mann, T. (2006). “Chlorinated Hydrocarbons” in Ullman’s Encyclopedia of Industrial Chemistry, Wiley – VCH, Weinheim, Germany. pp 230 – 272.

Sariaslani, F. S., McGee, L. R., and Ovenall, D. W. (2014). Applied and Environmental Biology Microbial Transformation of Precocene II: Oidation Reaction by *Streptomyces griseus*. 53, 1780.

Soine, T. O. (1991). The Phytochemical and Pharmaceutical profile of 2H-pyran-2-one in isolated and fused ring derivatives. J. Pharm. Sci. 53, 231.

Strandburg, G., Delassus, P. T., and Howell, B. A. (1991) in S. J. Rich and J. H. Hotkiss (Eds.). Food and Packaging Interactions II. ACS Symposium Series, No. 473, Washington DC. Chap. 12, pp 195 – 227.

Strunz, G. M., Brillon, D., and Giguere, P. (1983). An Improved Procedure for the Conversion of Certain Coumarins to 2, 2-dimethyl chromenes: Efficient Transformation of umbelliferone to precocene I. Can. J. Chem. 61, 1963

Sujatha, C. H., Nisar, S., and Jadhi, C. (1988). Evaluation of Plant Extracts for biological activity against mosquitoes, Int. Pest Control, 7, 122.

Taylor, D. R. and Wright, J. A. (1971). Phytochemistry. A natural chromene from the Jamiacan weed *Eupatorim riparium* Regel. 10, 1665.

The Dow Chemical Company. (March, 2005) SARAN^{TM}: PVDC Resins and Films and the Environment. Form No. 190 – 0050 – 0305 SMG.

Wessling , R.A., (1977) “Polyvinylidene Chloride” Gordon and Breach, New York. pp 21 – 27.

Xu, H., Niu, H., He, B., Cui, C., Li, Q., and Bi, K. (2016). Molecules, Comprehensive Qualitative Ingredient Profiling of Chinese Herbal Formula Wu-Zhu-Yu. Decoction via a Mass Defect and Fragment Filtering Approach Using High Resolution Mass Spectrometry.21, 664.

Yue, Z., and Economy, J. (2017)Carbonization and activation for production of activated carbon fibers in Activated Carbon Fiber and Textiles. ScienceDirect,Woodhead Publishing Series in Textiles. Pp 61 – 139.

Zacchariades, C., Day, M., Muniappan, R., and Reddy, G. V. P. (2009). *Chromolaena odorata* L. (Asteraceae). in Biological Control of Tropical Weeds Using Arthropods. Cambridge University Press, UK. pp 130 – 160.

Zhoa, F., Yin, Y., Zhang, D., Ning, P., Fu, M.,Yao, D (2017). Preparation and characterization of novel thermal – stable vinylidene chloride - methyl acrylate – glycidyl methacrylate copolymer. International Journal of Polymer Analysis and Characterization 22, Issue 49 pages. Copyright Patent, 2018.

Appendices

Appendix 1: Gas Chromatogram of the chloroform extract of air-dried leaves of *Moringa oleifera*. (Sample A).

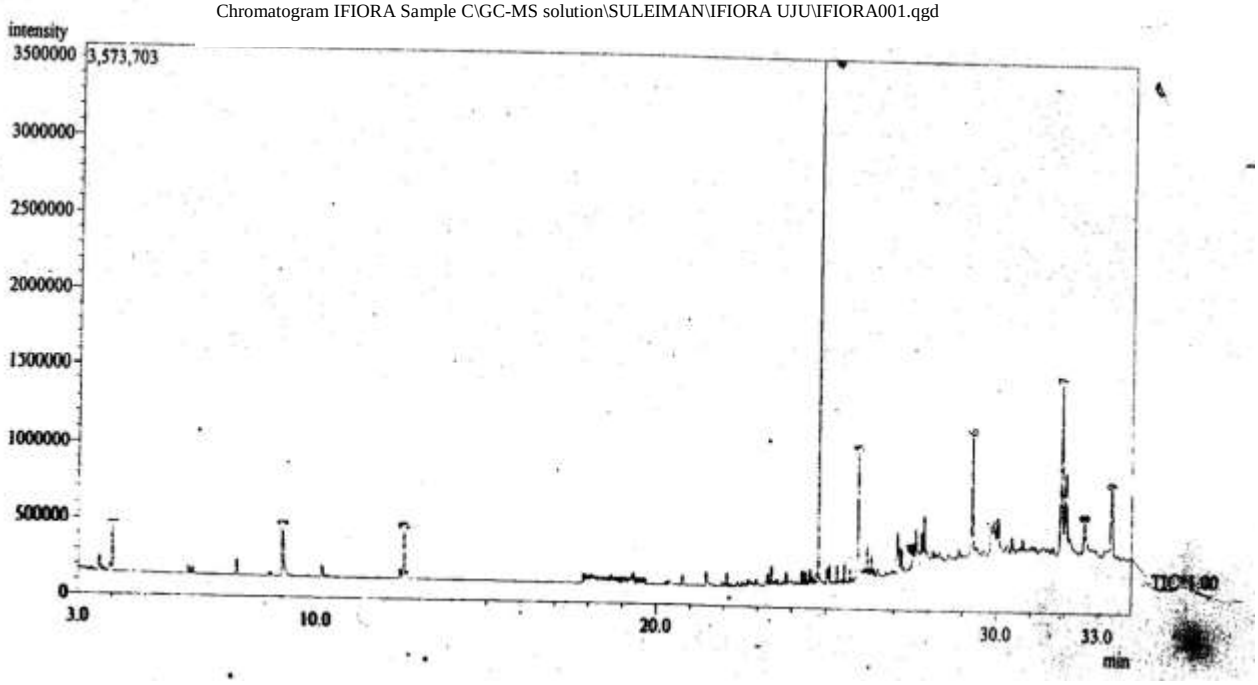
Admin 12/07/2011 11:54:50

GCMS-QP2010 PLUS
SHIMADZU,JAPAN

NARICT, ZARIA
GCMS ANALYSIS

IFIORAUJU (SAMPLE A)

Chromatogram IFIORA Sample C\GC-MS solution\SULEIMAN\IFIORA UJU\IFIORA001.qgd



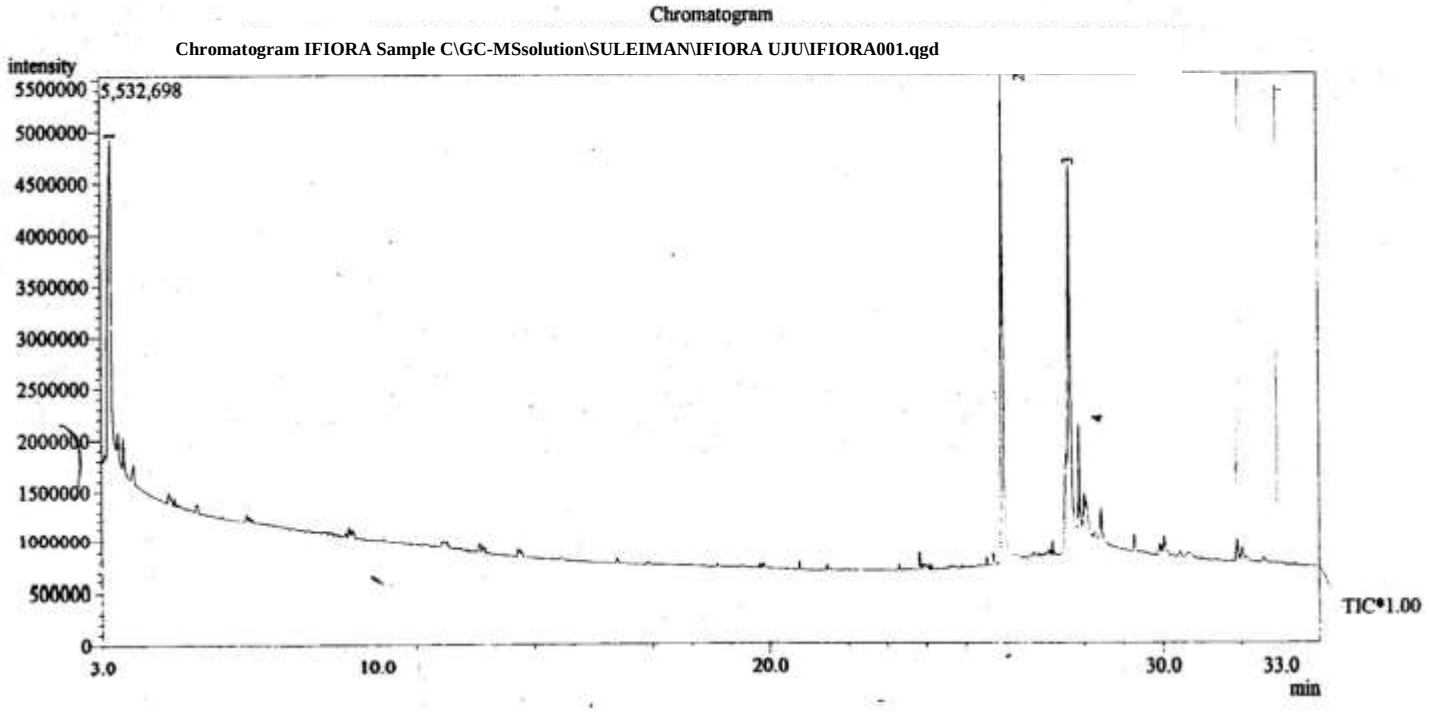
Appendix 2: Gas chromatogram of the aqueous extract ofair-driedleaves of *Moringa oleifera*, (Sample B)

Admin 12/07/2011 12:10:47

GCMS-QP2010 PLUS
SHIMADZU,JAPAN

NARICT, ZARIA
GCMS ANALYSIS

IFIORA UJU (SAMPLE – B)



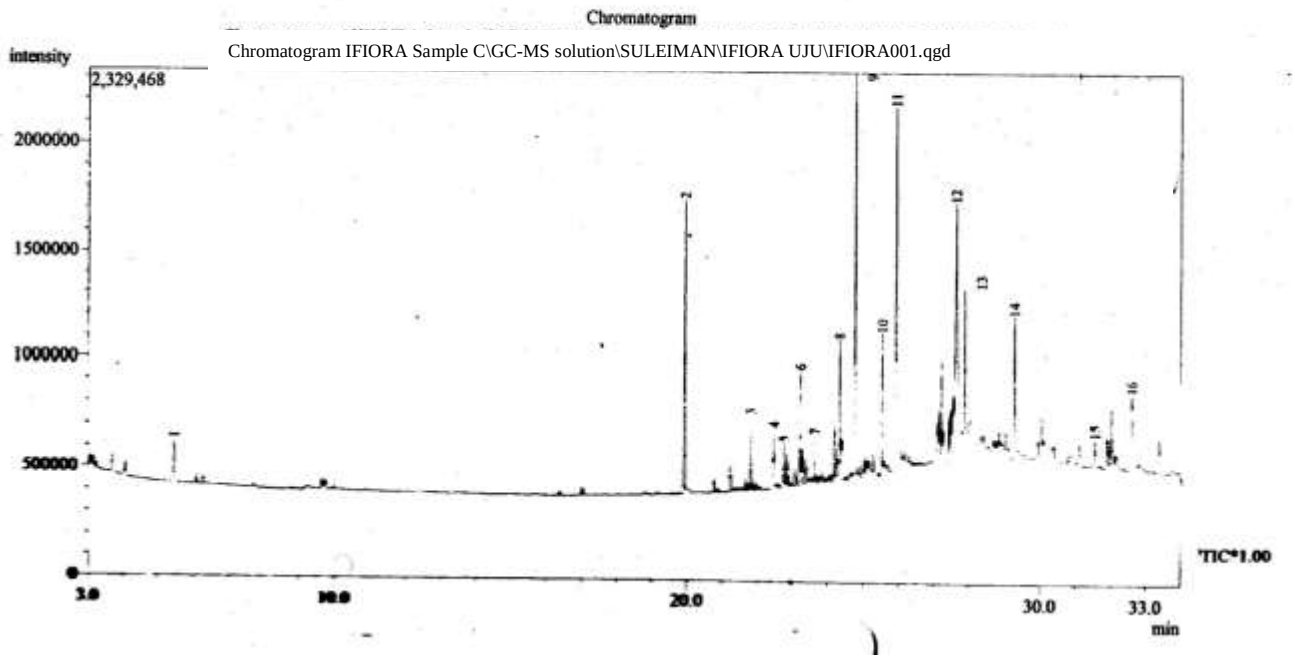
Appendix 3: Gas chromatogram of the chloroform extract of air-dried aerial parts of *Ageratum conyzoides*, (Sample C)

Admin 12/07/2011 12:11:23

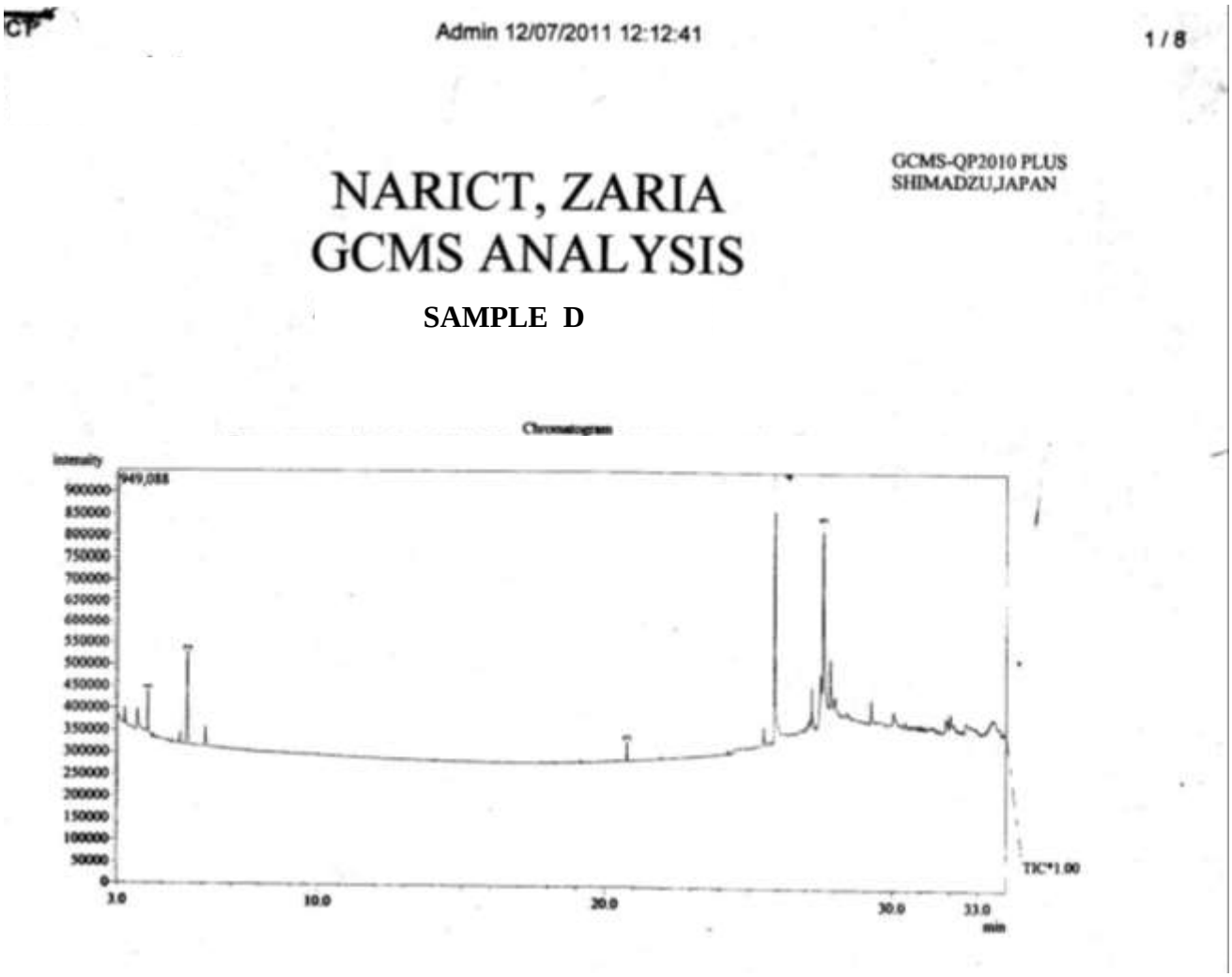
GCMS-QP2010 PLUS
SHIMADZU,JAPAN

NARICT, ZARIA
GCMS ANALYSIS

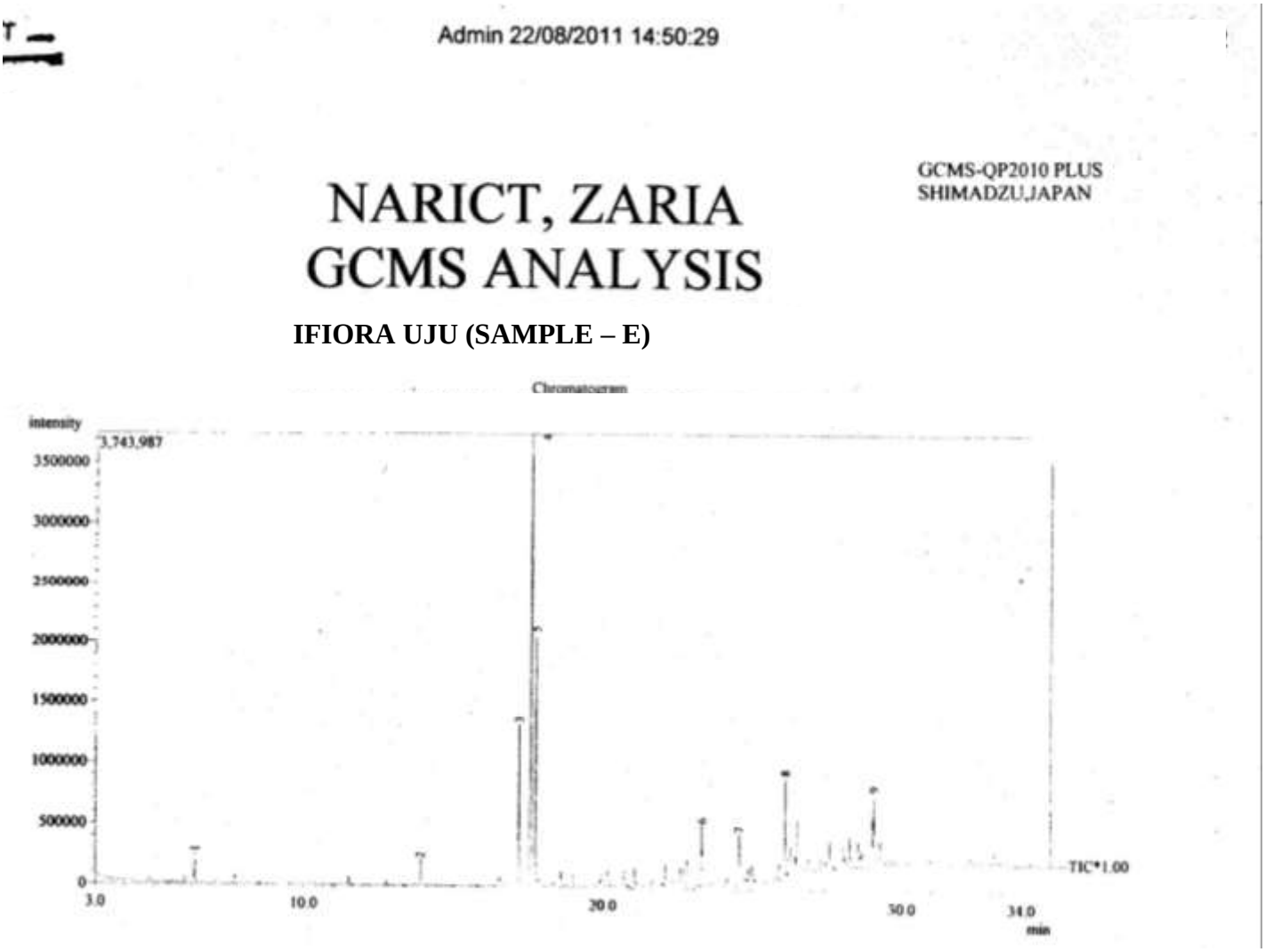
IFIORAA UJU (SAMPLE C)



Appendix 4: Gas chromatogram of the aqueous extract of air-driedaerial parts of *Ageratum conyzoides* (Sample D)



Appendix 5: Gas chromatogram of chloroform extract of air-driedroot system of *Ageratum conyzoides*(Sample E)



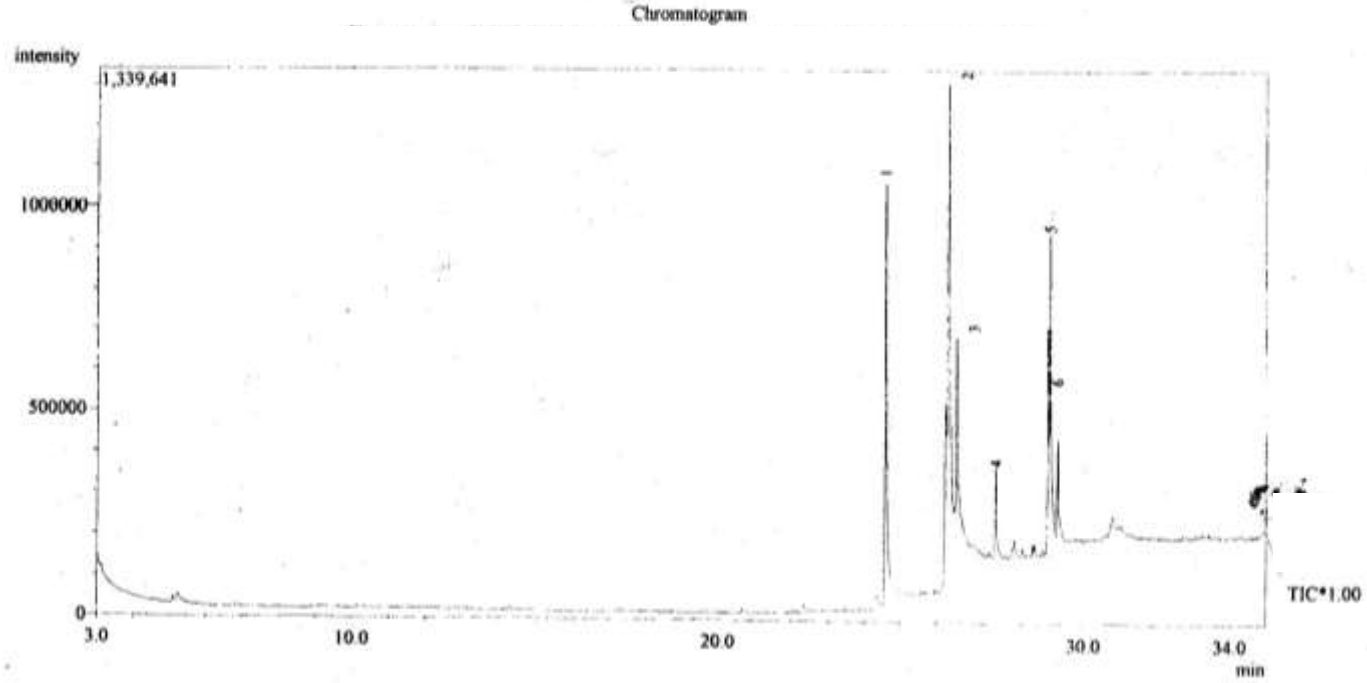
Appendix 6: Gas chromatogram of ethanolic extract of air-driedroot system of *Ageratum conyzoides*, (Sample F)

:T Admin 22/08/2011 14:49:38

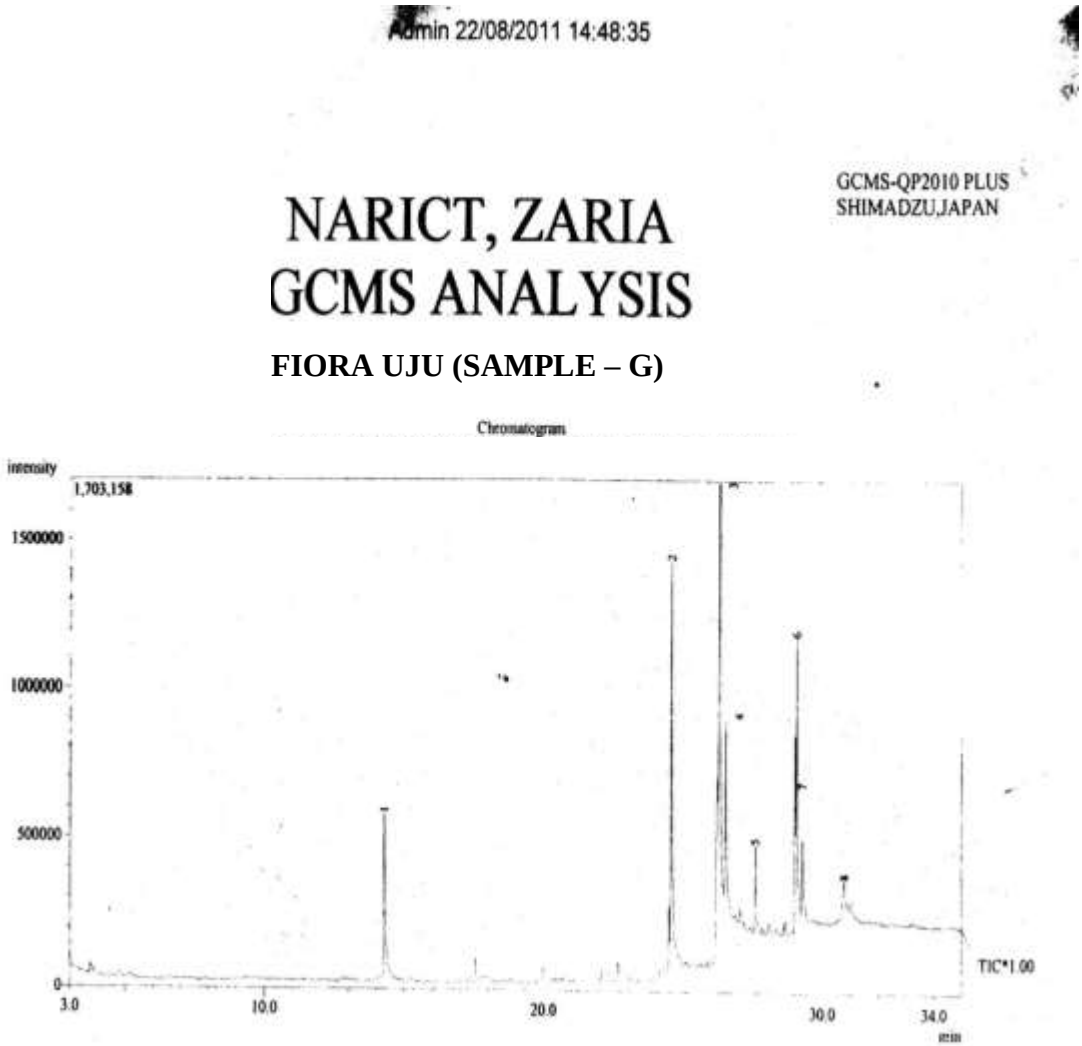
GCMS-QP2010 PLUS
SHIMADZU,JAPAN

NARICT, ZARIA
GCMS ANALYSIS

IFIORA UJU (SAMPLE – F)



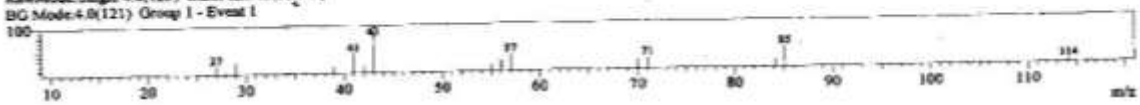
Appendix 7: Gas chromatogram of ethanolic extract of the flower of *Ageratum conyzoides*, Sample G.



Appendix 8: Primary mass spectrum of sample A

Spectrum

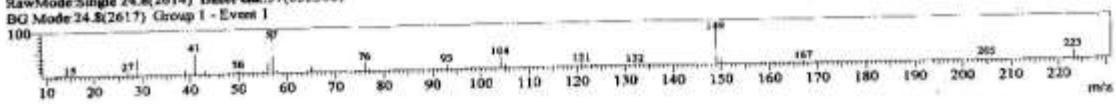
Line#:1 R.Time:4.0(Scan#:123)
MassPeaks:18
RawMode:Single 4.0(123) BasePeak:43(52999)
BG Mode:4.0(121) Group 1 - Event 1



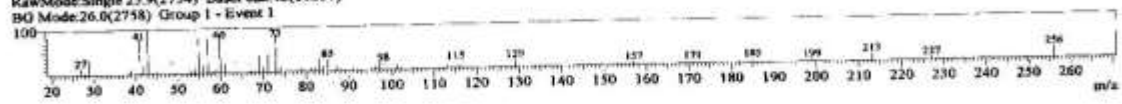
Line#:2 R.Time:9.0(Scan#:725)
MassPeaks:20
RawMode:Single 9.0(725) BasePeak:94(45678)
BG Mode:9.1(728) Group 1 - Event 1

Line#:3 R.Time:12.6(Scan#:1150)
MassPeaks:24
RawMode:Single 12.6(1150) BasePeak:91(40619)
BG Mode:12.6(1155) Group 1 - Event 1

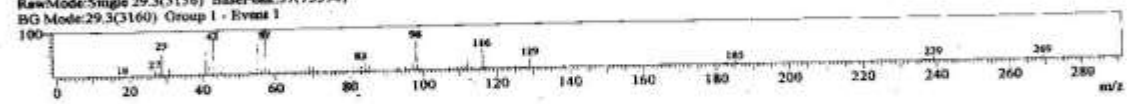
Line#:4 R.Time:24.8(Scan#:2614)
MassPeaks:56
RawMode:Single 24.8(2614) BasePeak:57(632368)
BG Mode:24.8(2617) Group 1 - Event 1



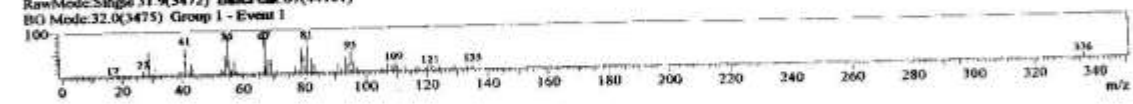
Line#:5 R.Time:25.9(Scan#:2754)
MassPeaks:54
RawMode:Single 25.9(2754) BasePeak:43(61267)
BG Mode:26.0(2758) Group 1 - Event 1



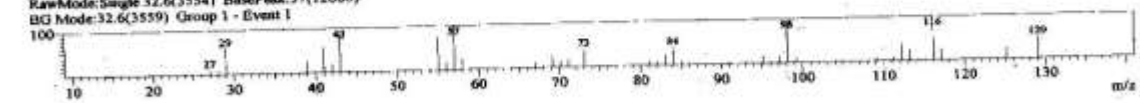
Line#:6 R.Time:29.3(Scan#:3156)
MassPeaks:53
RawMode:Single 29.3(3156) BasePeak:57(73594)
BG Mode:29.3(3160) Group 1 - Event 1



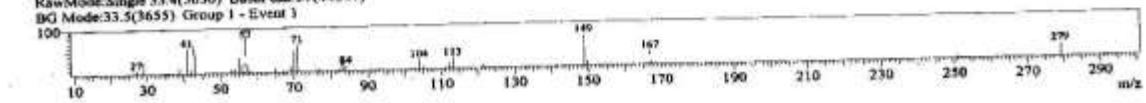
Line#:7 R.Time:31.9(Scan#:3472)
MassPeaks:66
RawMode:Single 31.9(3472) BasePeak:67(44101)
BG Mode:32.0(3475) Group 1 - Event 1



Line#:8 R.Time:32.6(Scan#:3554)
MassPeaks:44
RawMode:Single 32.6(3554) BasePeak:57(12686)
BG Mode:32.6(3559) Group 1 - Event 1



Line#:9 R.Time:33.4(Scan#:3650)
MassPeaks:43
RawMode:Single 33.4(3650) BasePeak:57(44847)
BG Mode:33.5(3655) Group 1 - Event 1

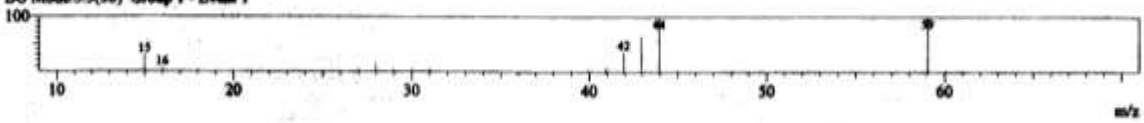


Spectrum Comparison

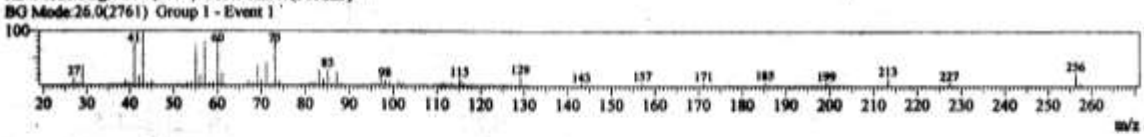
Appendix 9: Primary mass spectrum of sample B

Spectrum

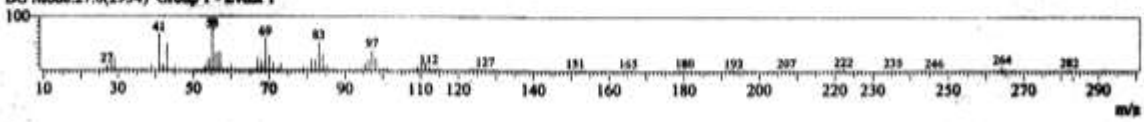
Line#:1 R.Time:3.3(Scan#:33)
MassPeaks:27
RawMode:Single 3.3(33) BasePeak:44(651516)
BG Mode:3.3(36) Group 1 - Event 1



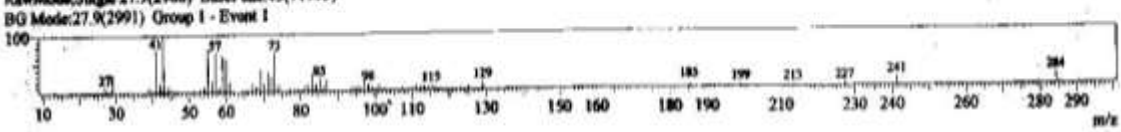
Line#:2 R.Time:26.0(Scan#:2757)
MassPeaks:88
RawMode:Single 26.0(2757) BasePeak:43(383223)
BG Mode:26.0(2761) Group 1 - Event 1



Line#:3 R.Time:27.7(Scan#:2961)
MassPeaks:96
RawMode:Single 27.7(2961) BasePeak:55(268710)
BG Mode:27.6(2954) Group 1 - Event 1

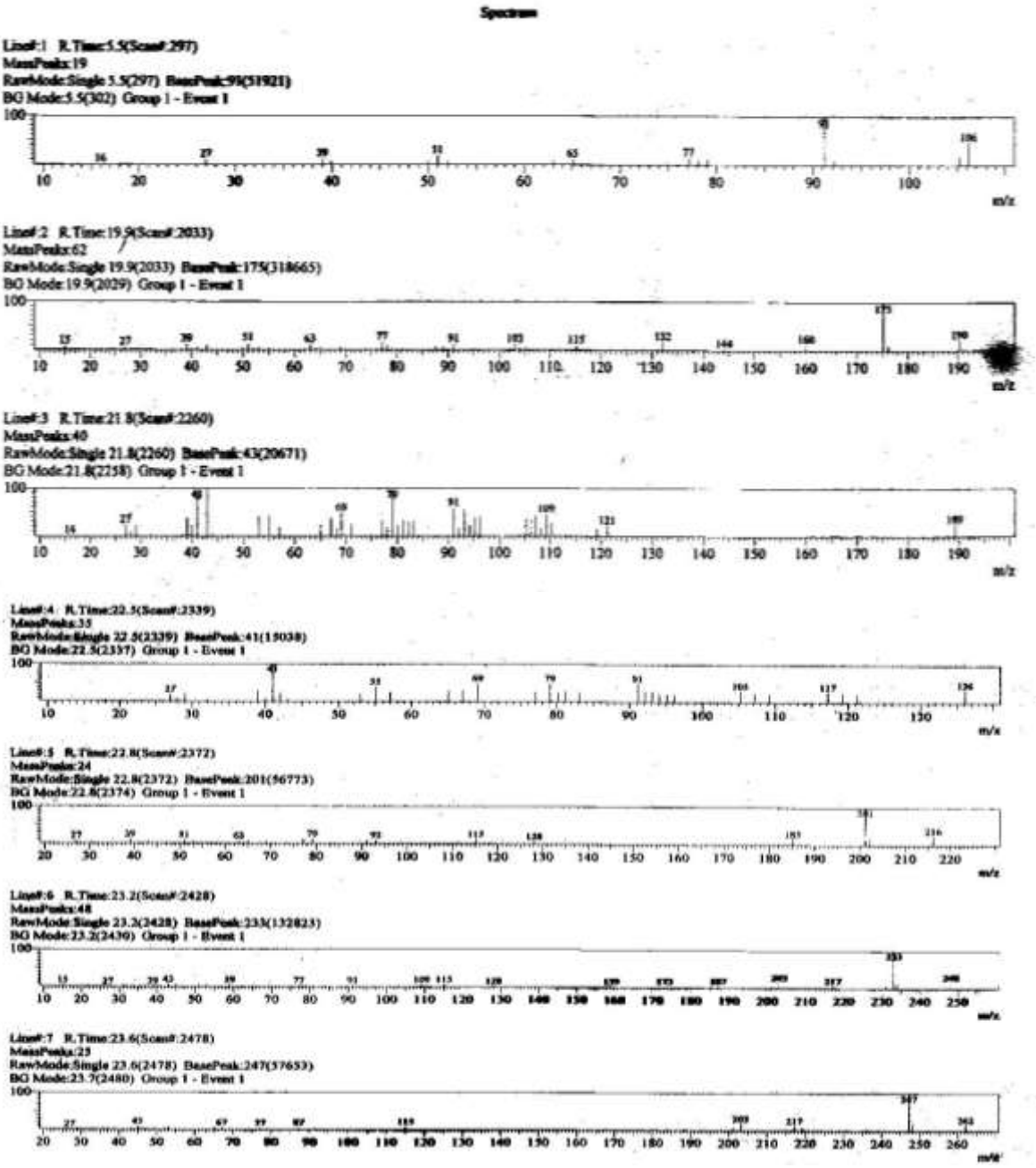


Line#:4 R.Time:27.9(Scan#:2988)
MassPeaks:69
RawMode:Single 27.9(2988) BasePeak:43(71995)
BG Mode:27.9(2991) Group 1 - Event 1

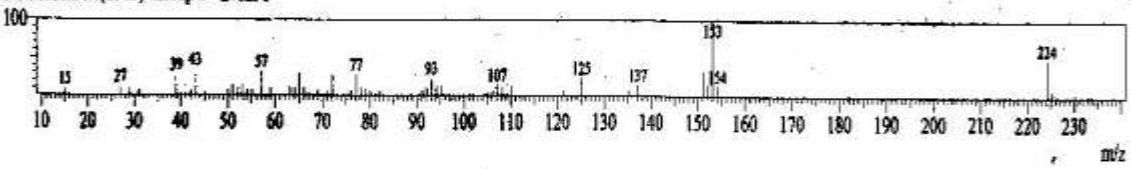


Spectrum Comparison

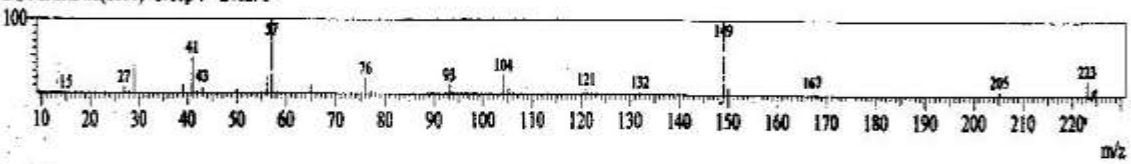
Appendix 10: Primary mass spectrum of sample C



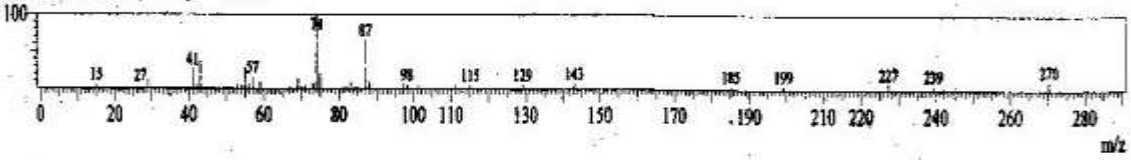
Line#:8 R.Time:24.3(Scan#:2562)
MassPeaks:57
RawMode:Single 24.3(2562) BasePeak:153(57432)
BG Mode:24.4(2565) Group 1 - Event 1



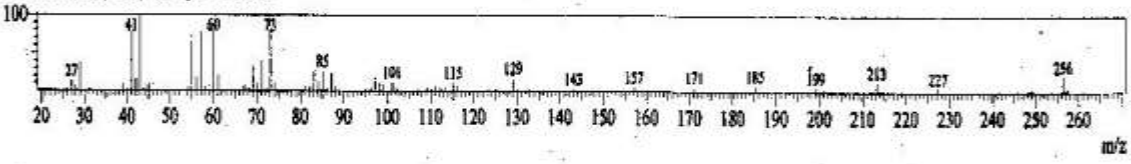
Line#:9 R.Time:24.8(Scan#:2613)
MassPeaks:51
RawMode:Single 24.8(2613) BasePeak:149(294791)
BG Mode:24.8(2616) Group 1 - Event 1



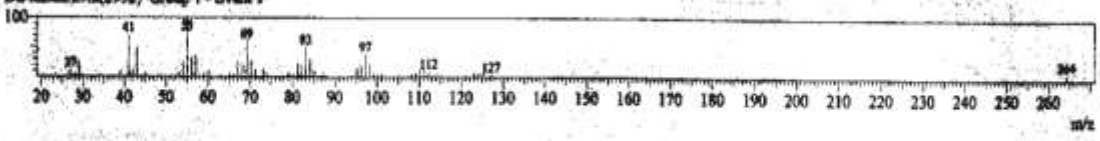
Line#:10 R.Time:25.5(Scan#:2706)
MassPeaks:44
RawMode:Single 25.5(2706) BasePeak:74(75794)
BG Mode:25.6(2708) Group 1 - Event 1



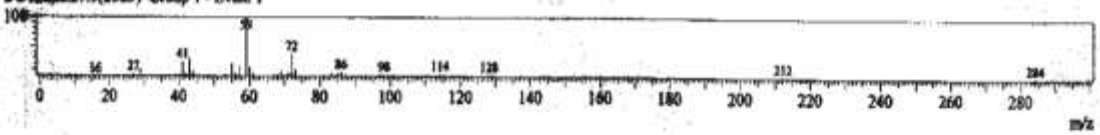
Line#:11 R.Time:25.9(Scan#:2753)
MassPeaks:68
RawMode:Single 25.9(2753) BasePeak:43(122070)
BG Mode:26.0(2757) Group 1 - Event 1



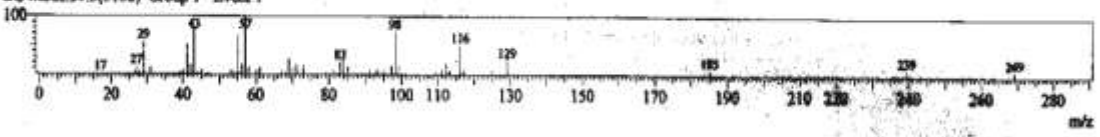
Line#:12 R.Time:27.6(Scan#:2956)
MassPeaks:61
RawMode:Single 27.6(2956) BasePeak:55(86786)
BG Mode:27.6(2952) Group 1 - Event 1



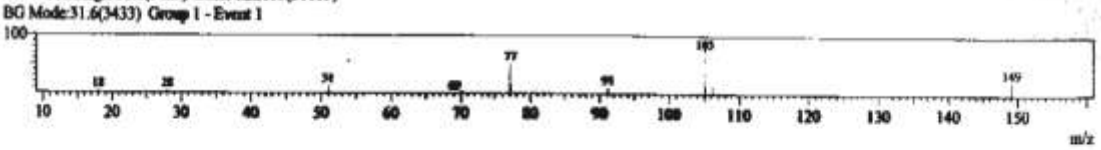
Line#:13 R.Time:27.9(Scan#:2986)
MassPeaks:56
RawMode:Single 27.9(2986) BasePeak:59(130746)
BG Mode:27.9(2989) Group 1 - Event 1



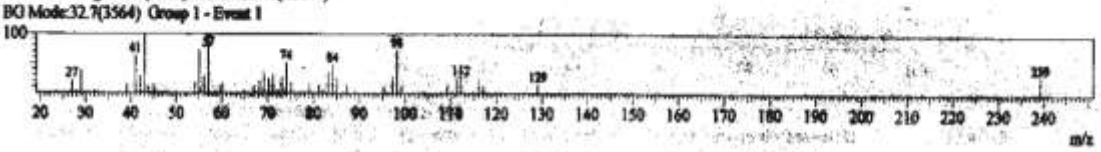
Line#:14 R.Time:29.3(Scan#:3155)
MassPeaks:51
RawMode:Single 29.3(3155) BasePeak:57(41598)
BG Mode:29.3(3158) Group 1 - Event 1



Line#:15 R.Time:31.6(Scan#:3429)
MassPeaks:22
RawMode:Single 31.6(3429) BasePeak:105(31169)
BG Mode:31.6(3433) Group 1 - Event 1



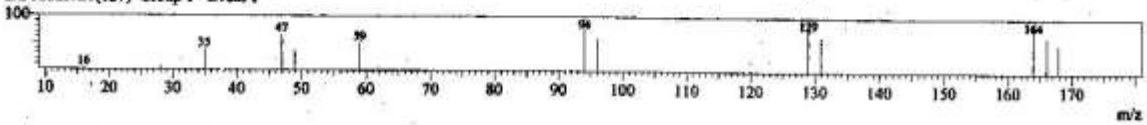
Line#:16 R.Time:32.6(Scan#:3555)
MassPeaks:42
RawMode:Single 32.6(3555) BasePeak:43(22244)
BG Mode:32.7(3564) Group 1 - Event 1



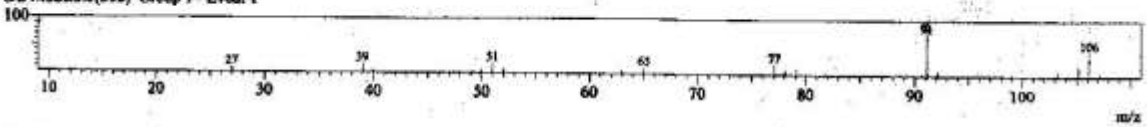
Appendix 11: Primary mass spectrum of sample D

Spectrum

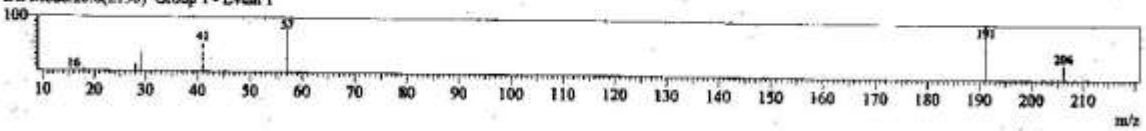
Line#:1 R.Time:4.1(Scan#:129)
MassPeaks:17
RawMode:Single 4.1(129) BasePeak:94(9428)
BG Mode:4.0(127) Group 1 - Event 1



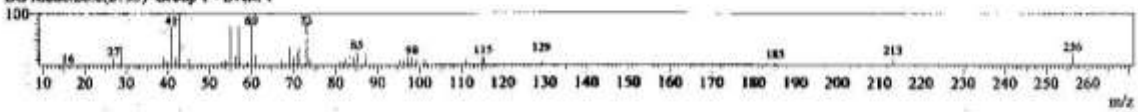
Line#:2 R.Time:5.5(Scan#:297)
MassPeaks:20
RawMode:Single 5.5(297) BasePeak:91(61129)
BG Mode:5.5(302) Group 1 - Event 1



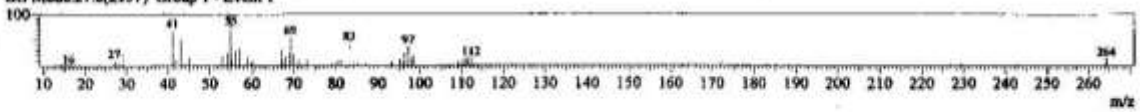
Line#:3 R.Time:20.8(Scan#:2134)
MassPeaks:9
RawMode:Single 20.8(2134) BasePeak:191(12058)
BG Mode:20.8(2136) Group 1 - Event 1



Line#:4 R.Time:25.9(Scan#:2752)
MassPeaks:45
RawMode:Single 25.9(2752) BasePeak:43(45013)
BG Mode:26.0(2755) Group 1 - Event 1



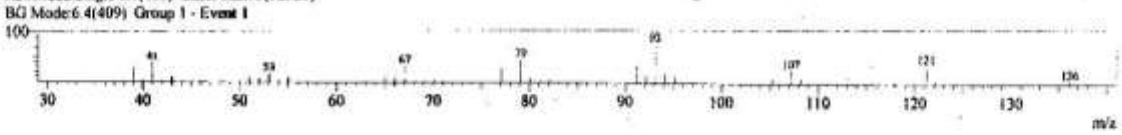
Line#:5 R.Time:27.6(Scan#:2954)
MassPeaks:42
RawMode:Single 27.6(2954) BasePeak:55(24881)
BG Mode:27.6(2957) Group 1 - Event 1



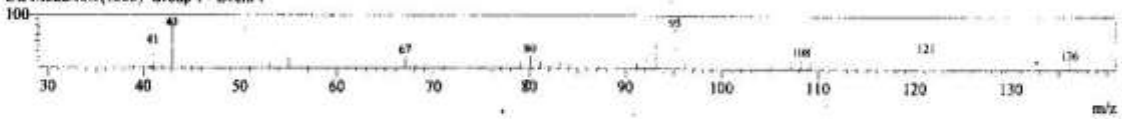
Appendix 12: Primary mass spectrum of sample E

Spectrum

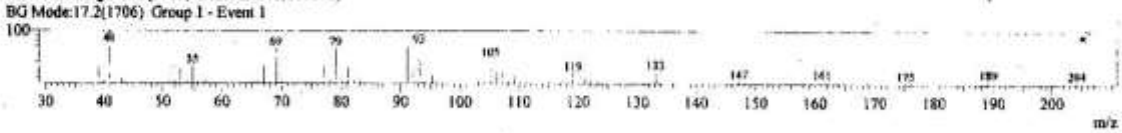
Line#:1 R.Time:6.4(Scan#:406)
MassPeaks:40
RawMode:Single 6.4(406) BasePeak:93(32328)
BG Mode:6.4(409) Group 1 - Event 1



Line#:2 R.Time:13.9(Scan#:1308)
MassPeaks:41
RawMode:Single 13.9(1308) BasePeak:95(30188)
BG Mode:13.9(1305) Group 1 - Event 1

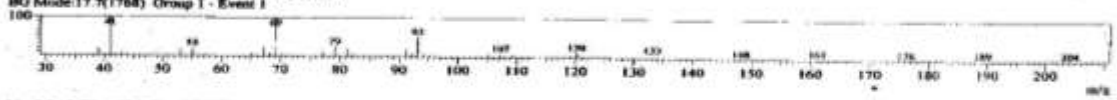


Line#:3 R.Time:17.2(Scan#:1703)
MassPeaks:71
RawMode:Single 17.2(1703) BasePeak:41(110270)
BG Mode:17.2(1706) Group 1 - Event 1

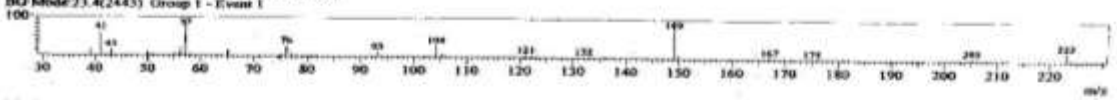


Line#:4 R.Time:17.6(Scan#:1751)
MassPeaks:106
RawMode:Single 17.6(1751) BasePeak:175(1022962)
BG Mode:17.6(1755) Group 1 - Event 1

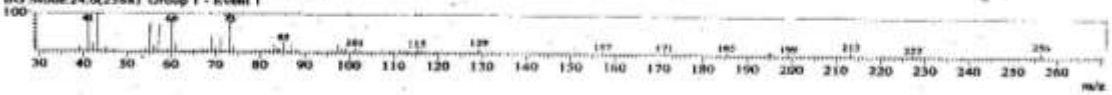
Line#:5 R.Time:17.8(Scan#:1771)
MassPeaks:70
RawMode:Single 17.8(1771) BasePeak:69(249989)
BG Mode:17.7(1768) Group 1 - Event 1



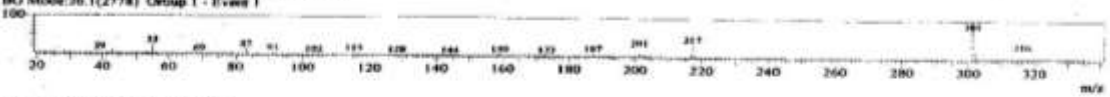
Line#:6 R.Time:23.3(Scan#:2440)
MassPeaks:53
RawMode:Single 23.3(2440) BasePeak:149(748880)
BG Mode:23.4(2443) Group 1 - Event 1



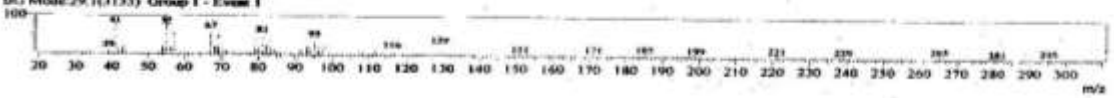
Line#:7 R.Time:24.6(Scan#:2591)
MassPeaks:60
RawMode:Single 24.6(2591) BasePeak:73(31089)
BG Mode:24.6(2588) Group 1 - Event 1



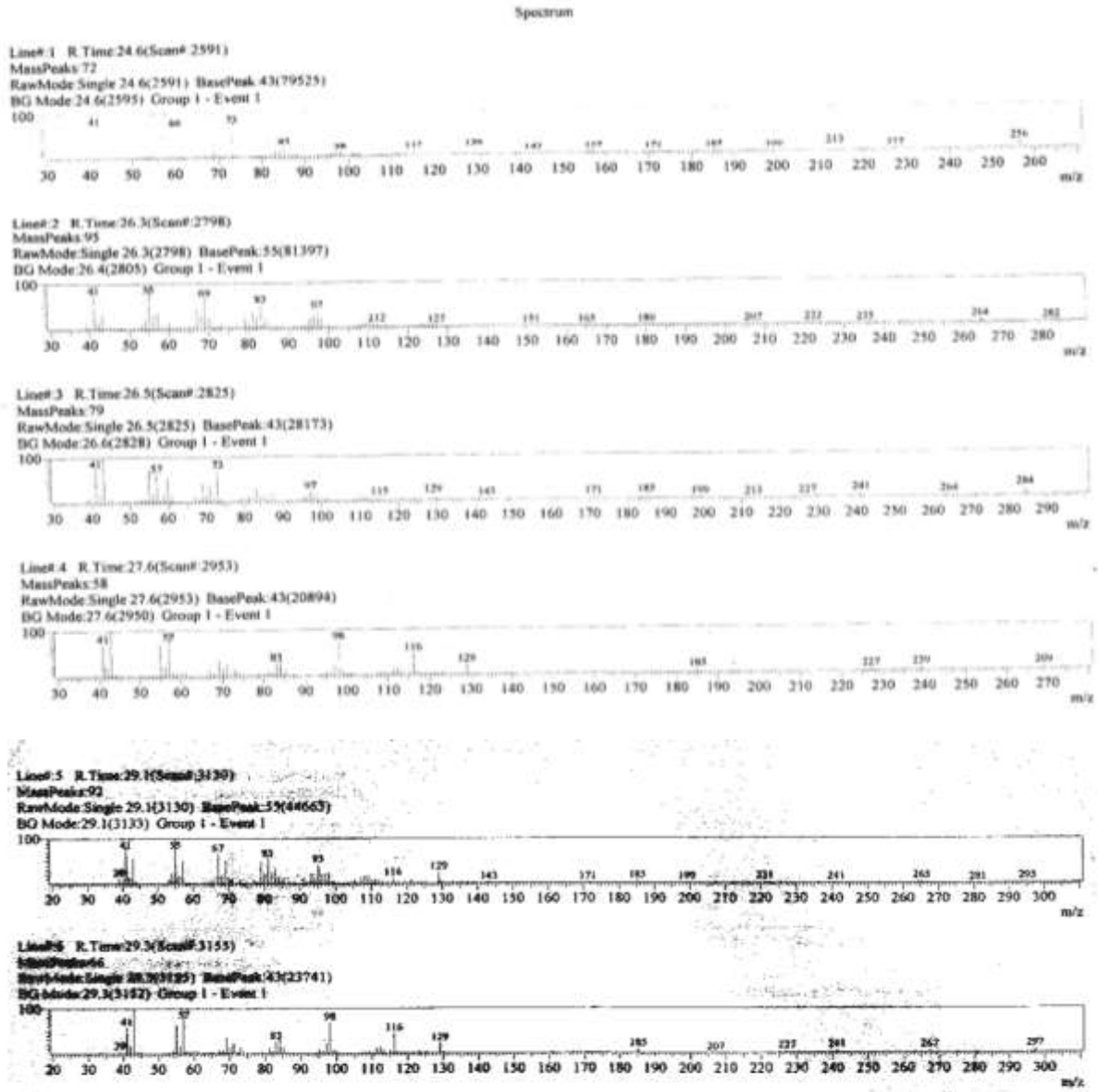
Line#:8 R.Time:26.1(Scan#:2776)
MassPeaks:107
RawMode:Single 26.1(2776) BasePeak:301(149358)
BG Mode:26.1(2778) Group 1 - Event 1



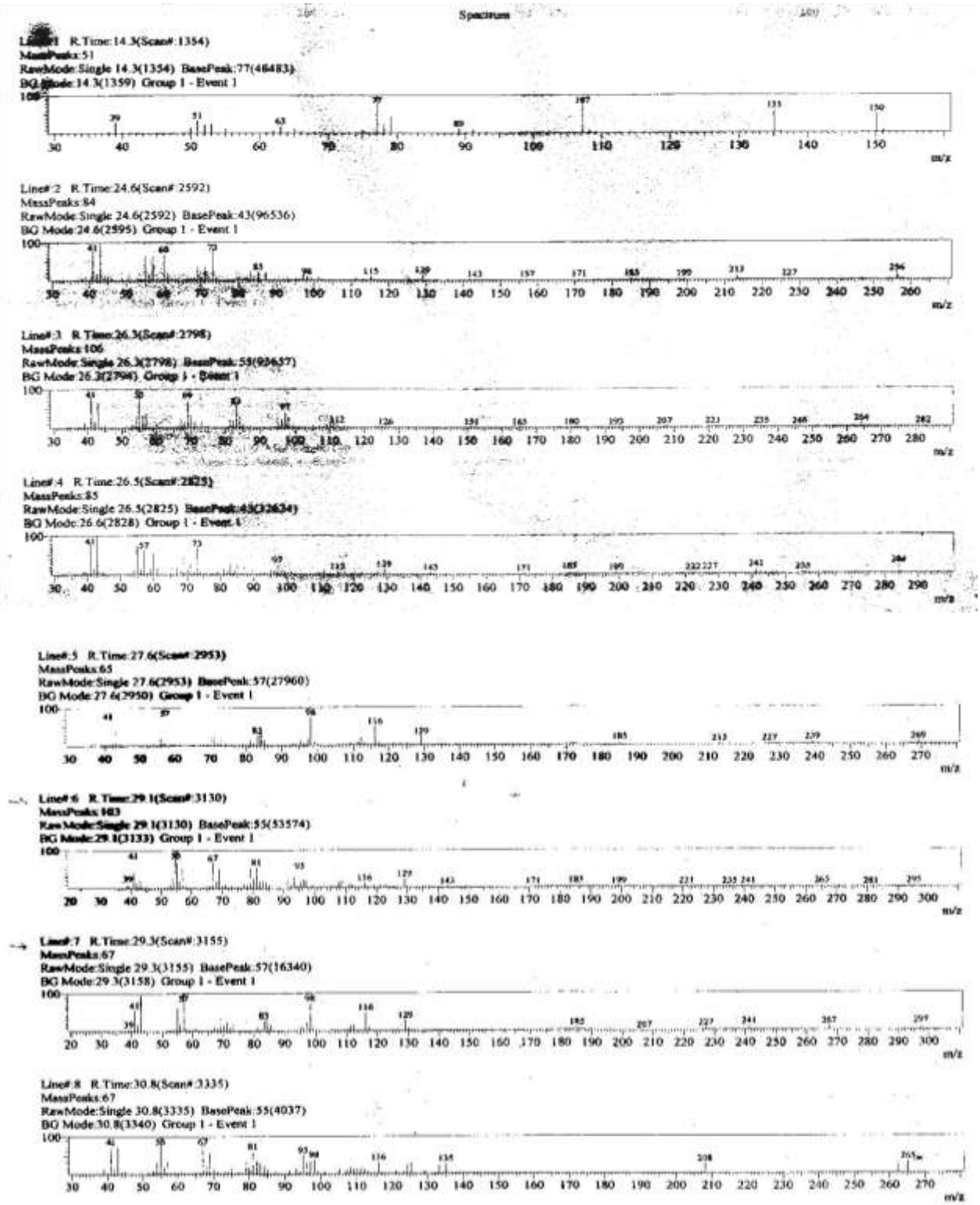
Line#:9 R.Time:29.1(Scan#:3130)
MassPeaks:86
RawMode:Single 29.1(3130) BasePeak:55(35355)
BG Mode:29.1(3133) Group 1 - Event 1



Appendix 13: Mass spectrum of Sample F



Appendix 14: Mass spectrum of Sample G



Appendix 15: Abridged Procedure for the identification of individual components in the Mass spectrum

Procedure for the identification of individual components in the mass spectrum.

1. Recognition of the molecular ion in a mass spectrum.

The apparent loss of 14 Daltons from a supposed molecular ion suggests the presence in the molecule of a homologue since the :CH_2 group has a very high $\triangle$Hf value and does not therefore fragment easily.

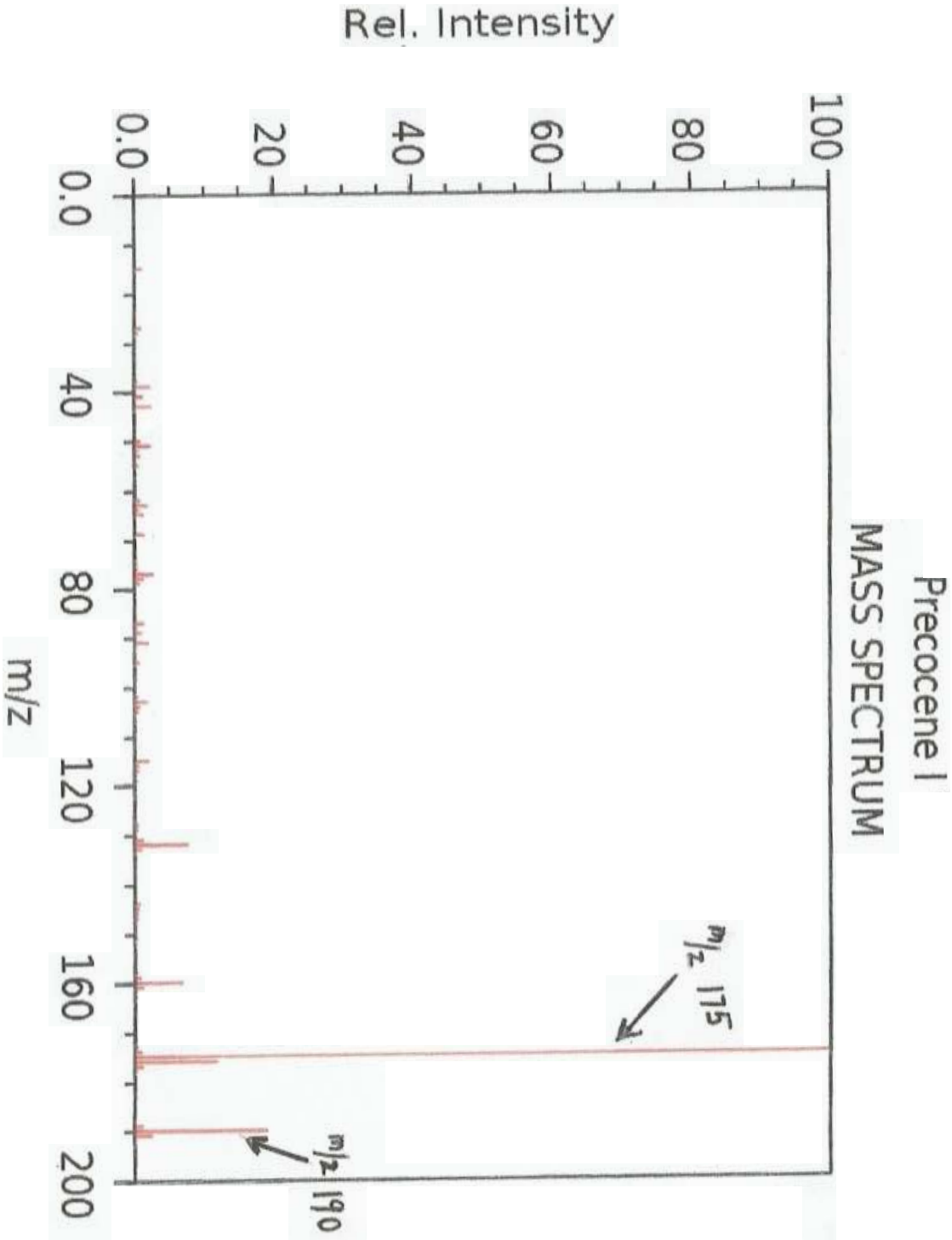
2. If a peak occurs 4 – 13 mass units below a supposed molecular ion, it is possible that either the higher mass peak is not a molecular ion, or the spectrum is of a mixture of compounds.

The molecular weights of compounds containing only C, H, (O) are even, as are those of molecules additionally containing an even number of nitrogen atoms. The molecular weights of compounds containing only C, H, N,(O) are odd when the number of nitrogen atoms are odd, and even when the number of nitrogen atoms are even.

Aliphatic compounds all tend to fragment extensively. The base peak is therefore usually a fragment of the original compound. Electron ionisation (EI) thus provides a sensitive fingerprint of an aliphatic compound, particularly in GC-MS.

3. The intensity of a particular ion is proportional to its concentration in the source at any given time. This is analogous to Beer's law in absorption spectroscopy and enables the use of the same quantification methodologies for GC-MS. Thus a tabulated list of mass-to-charge ratios (m/z) versus abundance values enables an accurate determination of the number of carbon atoms in a molecule, using empirical relationships.

Appendix 16: Mass Spectrum of "Precocene I" isolated and characterized in Table 4.5. Component C2 (leaf extract in chloroform) and E4 (root extract in chloroform) have been combined to obtain this mass spectrum



Appendix 17: Thermogravimetric trace of the isothermal Degradation of Vinylidene Chloride / Methylacrylate (five mole per cent) / precocene I (0.1 mole %) additive at 170°C