# CHAPTER ONE INTRODUCTION

* 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 [Cl2CHCH2Cl], 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

Cl Cl Cl

H2O

NaOH

H C C

H + C CH2

+ NaCl +

H2O

Cl H Cl

NaOH

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 (SiO2) 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](https://www.sciencedirect.com/science/article/pii/B9780323358248000153)., 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:

n

H

Cl

C

Cl n

fast

H Cl

\* C \*



H

\* C C \*

+ nHCl

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).

# 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.

# 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:

* + 1. Extract suitable chemical additive, precocene I which would reduce its rate of dehydrochlorination and loss of polymer material.
		2. Characterize the proposed additive using gas chromatography mass spectrometry (GC-

MS), Fourier transform infrared (FTIR), BeynoQ¶V 7DEOH DWRPLF DEVR (AAS) and National Institute of Standards and Technology (NIST) Chemical webbook.

* + 1. Incorporate the oxygen heterocyele as additive into the copolymer and subject the product to thermogravimeetric analysis (TGA).
		2. Determine the rate constant (ki and kp) data in terms of derived thermodynamic parameters such as entropy, activation energy, free energy.
		3. Relate the thermodynamic parameters to the dehydrochlorination process.

# 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.

# 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

* 1. **Early Studies of Vinylidene Chloride (VDC) Copolymers**

Vinylidene chloride (1,1±DCE) does not appear to have received much mention in the chemical OLWHUDWXUH EHIRUH DERXW WKH ¶V )RU LQVW

a prominent author, and published in 1965, discussed the 1,1±disubstituted ethylenes of general formula CH2**:**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.

# 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 *SaranTM* (Cole *et al*., 2003).

Cole *et al.,* (2003) give other uses of *SaranTM* 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 *SaranTM* 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 *SaranTM* 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). *SaranTM* 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.

# 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](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).

# 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.

H Cl H H Cl H H

H

C

\*

H

\* C C C



C C C C

Absorption of suitable radiation

+ Cl

H Cl H

C=O

H H C=O

OCH3

Vinylidene methylacrylate chloride unit

absorption of H from

neighbouring C atom

Cl

OCH3

Abstraction of H from neighbouring C atom

unit

H Cl H H

H Cl H H

C C C C

rearrange

+ HCl C C C C

+ HCl

H C=O

H C=O

double bond

OCH3

OCH3

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

Fig. 1.2 Representationof theDegradation of Vinyldene chloride 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 (= CCl2) 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).

# 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.

Termination

Propagation

Initiation

Time (s)

HCl Concentration (mole)

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

c

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.

H O

C C C

O C

O

H C

H H H

H H

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:

H CH3

H CH3

H CH3

(a)

n

\* C C

O C C O

C C O \*

H H H H H H

(b)

and

H H H H H H

H

C C O C C O C C O \*

H CH3

H CH3

H CH3 n

# 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).

# 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.

# 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.

5 4 5 4

6 3 6 3

7 2 7 2

O 8 O

8 1 II 1

I

|  |  |  |
| --- | --- | --- |
|  | 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.

# 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%.

O O

CH3

O

CH3

coumarin

chromene

**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).

R1

e

R2

M

R3

O

Me

R4

**Figure 2.7: Structure of functionalized 2H-Chromenes**

|  |  |  |  |
| --- | --- | --- | --- |
| R1 = R2 = R4 = H; R3 = OMe, | Precocene I | or | 6 - Demethyoxychromene |
| R1 = R4 = H; R2 = R3 = OMe, | Precocene II | or | Ageratochromene |
| R1 = R3 = R4 = H; R2 = AcO, | Precocene III | or | 6 ±Acetylchromene |
| R1 = R4 = H; R2 = AcO; R3 = OH, |  |  | Eupatoriochromene |

# 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 -pinene component, followed by -pinene (40.6%). Both - and -

pinene are C10 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.

O

O

MeO

O

HO

HO OH

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

# 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 (H3PO4), was used to attach isoprenes to phenols, leading to the synthesis of 2,2± dimethylchromans (figure 2.9) (Ahluwalia and Arora, 1982):

OH

H3PO4

CH3

O

R R CH3

CH2

Phenol Isoprene Chroman (Saturated benzopyran)

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):

OH

Amberlyst 15

in refluxing benzene

O

R

Phenol

R

CH3

C

CH

C

HO

Phenol Chromene

Chromene

**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.

HO O O

methylation

H3CO

CH3Li

O

O

H3CO

CH3

O

CH3

coumarin

Coumarim

(umbelliferone)

herniarin

(7-methoxycoumarin)

Herniarin

7-methoxycoumarin

Hemiarin

7-methoxycoumarin

Precocene I

**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.

* 1. ***Ageratum conyzoides* Linnaeus**

# 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.*

* + 1. **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*.

* + 1. **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 CH2Cl2 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, Rf = 0.40 on TLC using diethylether (Et2O) / 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 C6H9O2N. 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*.

* + 1. ***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, Rf 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.

* 1. ***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±(- 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:

N C S

H3C

O

H3C O

O

O

OH OH

**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.

# Separation, Isolation and Characterization of Mixtures:

* + 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 C9 ±C21 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).

# 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.

# 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 ratheU WKDQ GDWD FROOHFWLRQ 6RIW 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

 VLPLODU WR %HHU¶V ODZ SORWV LQ RSWLFDO VSHF

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

IDFLOLWDWHG E\ GDWD REWDLQDEOH IURP %H\QRQ¶V

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).

# Complementary Identification Techniques

* + 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 1H ±NMR equipment as well as 13C- NMR equipment.

# 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.

# 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 (C10H16).

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.

# Analysis of Data obtained in these experiments

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

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

1. 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.
2. 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

QDPHG LVRWRSH FOXVWHUV 5HIHUHQFHV ZHUH PD

McLafferty and Turecek, (1993). Molecular structures were determined using the

|  |  |
| --- | --- |
| expressions: |  |
| no of carbon atomsin component compound | = |  relative abundance of M + 1 1.1 x 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 CpHqOrNs 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

density (g mL-1) = mass of substance contained in density bottle (g)

volume of density bottle (mL)

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

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

[A] = [A] **.** e (Atkins and Paula, 2012)

1. The thermodynamic activat

±krate**.** t

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:

ion parame

|  |  |  |
| --- | --- | --- |
| ¨#G | = - RTInk# | (2.1) |
| ¨#G | = ¨#H - 7¨#S | (2.2) |

krate = kT/h **.** ¨#S/R **.** e ±¨ + 57 (2.3)

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

# CHAPTER THREE MATERIALS AND METHODS

* 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;

# Reagents

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

)HKOLQJ¶V VROXWLRQV

0H\HU¶V UHDJHQW IRU DONDORLGV

AgNO3 for halogens;

All solvents were of Sigma-Aldrich quality; Concentrated HNO3 acids;

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

VDC/MA copolymer (vinylidene chloride / methylacrylate)

# 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.

# Method

* + 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 (CH2=CHCOOCH3).

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.

# 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 (1050C) and ground to fine powder using agate mortar and pestle prior to extraction.

# 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 NH3 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.

# Further Treatment

* + 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\_](http://agritech.triau.ac.in/horticulture/extraction_%20methods_%20natural_essential_oil.pdf)

[methods\_ natural\_essential\_oil.pdf](http://agritech.triau.ac.in/horticulture/extraction_%20methods_%20natural_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.

* 1. **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 1050C to about 6000C 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. HNO3 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.

# 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 Rf 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.

* 1. **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.

# Further Characterization of Precocene I used as Additive

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

* + 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.
		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;

Density = mass of chromene contained in density bottle

Volumn of density bottle

* + 1. **Refractive index:** This value was determined using the Abbé refractometer. The