

Phytochemical and Gas Chromatography-Mass Spectrometric (GC-MS) Analyses of *Whitfieldia lateritia* Leaf

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Abstract: *Whitfieldia lateritia* is a medicinal plant widely used in folkloric medicine of Africa and Asia for the treatment of ailments such as inflammation, anemia and liver damage and boosting of blood. The phytochemical and GC-MS analyses of *Whitfieldia lateritia* leaf were carried out using standard methods. Qualitative phytochemical analysis revealed presence of flavonoids, alkaloids, saponins, cardiac glycosides and tannins from the sample. Nineteen (19) chemical constituents were identified from GC-MS analysis of the sample which include 5-(1-methyl ethylidene)-1,3-cyclopentadiene (1.12%), hept-2-ene (12.2%), octa-1,3,5,7-tetraene (4.5%), (1-mthylethyl) benzene (cumene) (0.7%), 3,5-dimethylhepta-3,5 dien-1-yne (0.6%), hexane (0.3%), 5-(1-methylethylidene)-1,3-cyclopentadiene (0.8%), butanoic acid (1.8%), oct-2-ene (1.1%), hexadecanoic acid (22.8%), nona-1,3-diene (9.4%), non-1-ene (26.9%) and octadecanoic acid (9.2%). Result obtained showed that the leaf extract of *Whitfieldia lateritia* has non-1-ene (26.9%) as the highest and hexane (0.3%) as the least chemical compound. These relative diverse chemical constituents may be responsible for the medicinal properties of *Whitfieldia lateritia* leaf.

Key words: GC-MS analysis • Chemical constituents • Phytochemicals • *Whitfieldia lateritia*

INTRODUCTION

Throughout the history plants have been used by human beings for medicinal purpose and even in modern time's plant have formed the basis of many pharmaceutical in use [1]. Plants provide a vast array of secondary metabolites against environmental stress or other factors like pest attacks, wounds and injuries. The secondary metabolites produced by plants have been found to have various therapeutic uses from time immemorial [2]. Many of the modern medicine in the early history contain phytochemicals that have beneficial effects on long-term health when consumed by humans and can be used to effectively treat human diseases [3].

Herbal medicines do not differ in terms of how they work [5]. Medicinal plants are becoming more of main stream as improvement in analysis and quality control along with advantages in clinical research have shown the treatment and prevention of diseases [6]. Phytochemical are natural bioactive compound found in various parts of

plants, such as a root, stem bark, leaves, seed and fruits. Secondary metabolisms are non-nutritive plant chemicals that have protective properties. Many higher plants produce economically important organic compounds such as oils, resins, tannins, natural rubber, gums, waxes, dyes, flavours and fragrances, pharmaceuticals and pesticides [7].

Whitfieldia lateritia commonly called blood plant is a flowering plant belonging to the family of *acanthaceae* [8]. *Whitfieldia lateritia* is native to Sierra Leone but recently has been observed in several parts of the world like Nigeria. In Nigeria per say, it can be found in large number in places like Ivo, Ikwo and Izzi Local Government Areas of Ebonyi State, Nigeria. It is usually called by different names in several regions where they are found as "Ogwu obara" in Igbo, "Ogu'n eje" in Yoruba and "Magani jinni" in Hausa language [8]. The use of herbs for the treatment of disease is almost universal among industrialized and non-industrialized societies and often more affordable than purchasing synthetic drugs. Despite

the use of *Whitfieldia lateritia* leaves for the treatment of various diseases, there is paucity of documented data available regarding Gas chromatography–mass spectrometric (GC/MS) analysis of the chemical constituents. This study therefore evaluates the Gas chromatography–mass spectrometric (GC/MS) analysis of the chemical constituents of *Whitfieldia lateritia* leaves.

MATERIALS AND METHODS

Materials

Plant Collection: The fresh leaves of *Whitfieldia lateritia* were collected from Ikwo area of Ebonyi State. The plant was identified by a taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. Some parts of the plant were also deposited in the herbarium for reference purpose.

Preparation of Plant Sample: The leaves were destalked, washed and shade dried at ambient temperature with constant turning to averts fungal growth. The dried leaves were later milled to obtained the vegetable leaf meals (VLMs) using an electric blender and was stored in 4°C temperature in refrigerator in well labeled air-tight containers for analysis.

Preparation of *Whitfieldia lateritia* Ethanol Leaf-Extract: Exactly 40grams of dried powdered leaves of *Whitfieldia lateritia* were extracted successively with 300ml of ethanol in an orbital shaker for 24hours at room temperature. The extract was filtered using what-man N0.1 filter paper to remove extractable substances at every 3hrs interval. The combined extracts were then evaporated with rotary evaporator and the dried extracts were stored at 4°C in air-tight sterile container in refrigerator.

Methods

Preliminary Phytochemical Analysis: The preliminary phytochemical screening for the presence of tannins, sapanins, alkaloids, cardiac glycosides, flavonoids and others were carried out on the ethanol leaf-extract of *Whitfieldia lateritia*.

Test for the Presence of Tannins: This was carried out by the method of Harborne (1973) [9].

Principle: Tannins are secondary metabolites of plant species and consist of sugar and non-sugar parts. They are capable of undergoing hydrolysis when inserted into dilute acids or boiling water to give rise to products such as polyhydroxyl phenolic compounds. They are reactive

following the possession of functional groups called hydroxyl group (OH). They participate in redox reaction to give characteristics colour change on the reagent applied.

Procedure: One milliliter (1ml) of crude extract of the sample was collected using syringe and dispensed into test tube. Then, one milliliter (1ml) of ferric chloride (FeCl_3) was added to the test tube. A dirty green precipitate was observed which showed the presence of tannins.

Test for the Presence of Saponins: This was carried out by the method of Harborne (1973) [9].

Principle: Saponins are glycosides with distinctive foaming characteristics. They consist of a polycyclic aglycone that is either a choline steroid or triterpenoid attached through C_3 and an ether bond to a sugar side chain. The aglycone is referred to as the sapogenin and steroid saponins are called saraponins. The ability of saponins to foam is caused by the combination of the non-polar sapogenin and the water soluble side chain (hydrophilic part), which have hydroxyl groups (OH) as functional group.

Procedures

Frothing Test: Two milliliters (2mls) of the extract were diluted with 5ml of distilled water in a test tube. The mixture was stirred vigorously for about 5mins and was allowed to stand for 30minutes. Frothing which persisted for this duration indicated the presence of saponins.

Emulsion Test: An emulsion is any thick liquid in which tiny drops of oil or fat are evenly distributed. Two to Five (2-5) drops of olive oil were added to 3mls of the sample in a test tube, stirred vigorously and allowed to stand for 30mins. Emulsification that was observed for this duration indicated the presence of saponins.

Test for Presence of Alkaloids: This was carried out by the method of Trease and Evans (1989) [10].

Principle: Alkaloid can be detected as loose complexes following their ability to react with some reagents by producing characteristics colour changes depending on the type of reagent used. Alkaloids have an amino group (NH_2) as their functional group as in nicotine.

Procedure: Two milliliters (2mls) of the extract was collected using syringe and was dispensed into a test tube, the test tube was heated for 2mins and 5mls of hydrogen (Hcl) was added and heated again and allowed

to cool. The mixture was divided into A and B. To A, 2 drops of Meyer's reagent was added and white precipitate was observed which showed the presence of Alkaloids. To B, 2 drops of Dragendroff's reagent was added and the formation of red precipitate was observed which confirmed the presence of alkaloids.

Test for the Presence of Flavonoids: This was carried out by the method of Harborne (1973) [9].

Principle: Flavonoids are colourless or pale yellow glycosides that are not soluble in non-polar solvents. They are compound that are oxidize by ethyl-acetate. They react with polar solvent to produce colour changes in accordance with the level of redox reactions that are likely to take place. Flavonoids also reacts with sodium hydroxyl group (NaOH) to form a yellow colour following the reaction of the hydroxyl group (OH) with the ketone functional group.

Procedure: Five milliliters (5ml) of the extract was collected using syringe and was dispensed into a test tube. Exactly 10mls of distilled water, 5mls of dilute ammonium hydroxide (NH₄OH) and few drops of tetraoxosulphate (VI) acid (H₂SO₄) were added in the test tube. A yellow colouration was observed which showed the presence of flavonoids.

Test for the Presence of Cardiac Glycoside This was carried out by the method of Harborne (1973) [9].

Principles: Cardiac glycosides are organic compounds that are capable of undergoing hydrolysis in the presence of dilute acids, alkali or enzymes.

Procedure: Two milliliters (2mls) of the extract was collected into a test tube and 5ml of glacial acetic acid was added and then 2mls of FeCl₃ and 2mls of concentrated ferric acid were added too. A brown ring formation at inter phase of the mixture indicated the presence of deoxy sugar characteristics of cardiac glycosides.

GC-MS Analysis

Procedures: GC-MS analysis of the ethanol extract of *Whitfieldia lateritia* leaf was performed using Shimadzu Japan gas chromatography QP2010 plus with a fused gas chromatography (GC) column (2010) coated with poly-methyl silicon (0.25nm x 50m) and the conditions were as follows: Temperature programming from 80-200°C held at 80°C for 1minute, rate 5°C/min and at 200°C for 20min. Field ionization detector (FID) Temperature of 300°C, injection temperature of 220°C, carrier gas nitrogen at a

flow rate of 1ml/min, split ratio of 1:75. Gas chromatography mass spectrum was conducted using GCMS-QP 2010 plus Shimadzu Japan with injector temperature of 220°C and carrier gas presence of 116.9kpa. The column length is 30m with a diameter of 0.25mm and flow rate of 50ml/min. Elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5 Kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra bank. German Hermlez 233M-Z centrifuge was used.

Component Identification: Chemical constituent of the extract was identified by matching the peak with computer Wiley Ms libraries and confirmed by those comparing mass spectra of the peaks and those from literature

RESULTS

Result of Phytochemical Analysis of *Whitfieldia lateritia*

Leaves: The result of the phytochemical analysis of methanol extract of *Whitfieldia lateritia* leaf revealed the presence of cardiac glycosides, flavonoids, saponins and tannins as shown in Table 1.

Result of GC-MS Analysis of *Whitfieldia lateritia*:

The ethanol extract of the leaf of *Whitfieldia lateritia* showed nineteen peaks from the GC-MC chromatogram. These peaks indicate the presence of nineteen compounds (1-19) in the extract (Figure 1). The composition of the extract contain non-1-ene (26.9%), hexadecanoic acid (22.8%), nona-1,3-diene (9.4%) and octadecanoic acid (9.2%) as the major chemical compounds as shown in Table 2.



Fig. 1: *Whitfieldia lateritia* Leaves with Flower

Table 1: Phytochemical Screening of *Whitfieldia lateritia* Ethanol leaf-Extract

Phytochemicals	Remarks
Alkaloid	Negative
Cardiac glycosides	Positive
Flavonoids	Positive
Saponins	Positive
Tannins	Positive

Table 2: GC-MS Analysis and Mass Spectral Data of Ethanol Fraction from the leaf of *Whitfieldia lateritia* Showing Molecular Formula, Molecular Weight, Percentage Content, Mass Peak and Retention Time

Peak	Compound	Molecular Formula	Molecular Weight	Percentage Content	Mass Peak	Retention Time
1	5-(1-mthyl thylidene) – 1,3-cyclopentadiene	C ₈ H ₁₀	106	1.12%	27	3.500
2	Hept-2-ene	C ₇ H ₁₄	98	12.2%	19	3.616
3	Octa-1,3,5,7-tetraene	C ₉ H ₁₃	106	4.5%	10	4.912
4	(1-mthylethyl) benzene	C ₉ H ₁₃	121	0.7%	16	3.145
5	3, 5 dimethylhepta -3,5 dien 1-yne	C ₉ H ₁₂	120	0.6%	16	6.403
6	Hexane	C ₆ H ₁₄	86	0.3%	10	9.373
7	Hexane	C ₆ H ₁₄	86	0.5%	11	10.794
8	5-(1-mthylethylidene)-1,3-Cyclopentadiene	C ₈ H ₁₄	106	0.8%	10	11.413
9	Butanoic Acid	C ₄ H ₈ O ₂	88	1.8%	15	12.482
10	Hept-2-ene	C ₇ H ₁₄	98	0.7%	22	13.208
11	Hept-2-ene	C ₇ H ₁₄	98	1.3%	23	15.692
12	Butanoic Acid	C ₄ H ₈ O ₂	88	1.0%	16	19.173
13	Oct-2-ene	C ₈ H ₁₆	112	1.1%	25	19.299
14	Hexadecanoic acid	C ₁₆ H ₃₂	224	22.8%	37	19.600
15	Hept-2-ene	C ₇ H ₁₄	98	3.0%	34	21.215
16	Nona-1,3-diene	C ₉ H ₁₆	124	9.4%	31	21.498
17	Non-1-ene	C ₉ H ₁₈	126	26.9%	49	22.307
18	Octadecanoic Acid	C ₁₈ H ₃₆ O ₂	84	9.2%	38	22.535
19	Non-1-ene	C ₉ H ₁₈	126	1.8%	38	24.552

DISCUSSION

The plant kingdom represents an enormous reservoir of biologically active compound with various chemical structures and protective/disease preventive properties. These phytochemicals often known as secondary metabolites are present in higher plants. They include alkaloids, steroids, flavonoids, saponins, glycosides, phenol, tannins and many others. The active principles of many drugs found in plants are secondary metabolites.

This research work showed that *Whitfieldia lateritia* ethanol leaf- extract is rich in some phytochemical like flavonoids, tannins, cardiac glycoside and saponin. These compounds have been known to possess medicinal activities particularly antibacterial activity [11]. The result of this study was not in correlation with the report of Aja *et al.*, [12] which revealed the presence of alkaloids and absence of glycosides in *Talinum triangulare* leaf in both dry and wet samples. Offor *et al.*, [13] and Aja *et al.*, [14] also reported the presence of all the phytochemicals in various concentrations in *Terminalia catappa* leaf, *Cajanus cajan* leaf and seed respectively.

The ethanol extract of the leaf of *Whitfieldia lateritia* showed nineteen peaks from the GC-MC chromatogram (Figure 2). The result showed that non-1-ene (26.9%), hexadecanoic acid (22.8%), nona-1,3-diene (9.4%), octadecanoic acid (9.2%) and hept-2-ene (17.2%) as the major GC-MS constituents of *Whitfieldia lateritia* leaf. Aja *et al.*, (2014) [5] identified the presence of sixteen GC-MS constituents in *Moringa oleifera* leaf with 9-octadecenoic acid (20.89%), L-(+)-ascorbic acid- 2,6-dihexadecanoate(19.66%), 14-methyl-8-hexadecenal (8.11%), 4- hydroxyl-4-methyl-2-pentanone (7.01%), 3-ethyl-2, 4-dimethylpentane(6.14%) and phytol (4.24%) as the major constituents. Nweke *et al.*, (2015) [11] also identified the presence of ten (10) GC-MS constituents of methanol leaf extract of *Vitex doniana* with 6-octadecenoic acid (24.19%) as the major and 1-tridecyne (2.4%) as the least constituents respectively. Non -1-ene (26.9%) is an antidepressants use for the treatment of antipsychotic disorder (Tabacco, 2006). 5-(mthylethylidene)-1,3-cyclopentadienne is active in the treatment of prostate cancer, acne, seborrhea and hirsutism [4]. Hept-2-ene beta-lactamase inhibition efficiency [4]. Butanoic acid is currently considered therapeutic in the treatment of colorectal cancer and hemoglobinopathies [6].

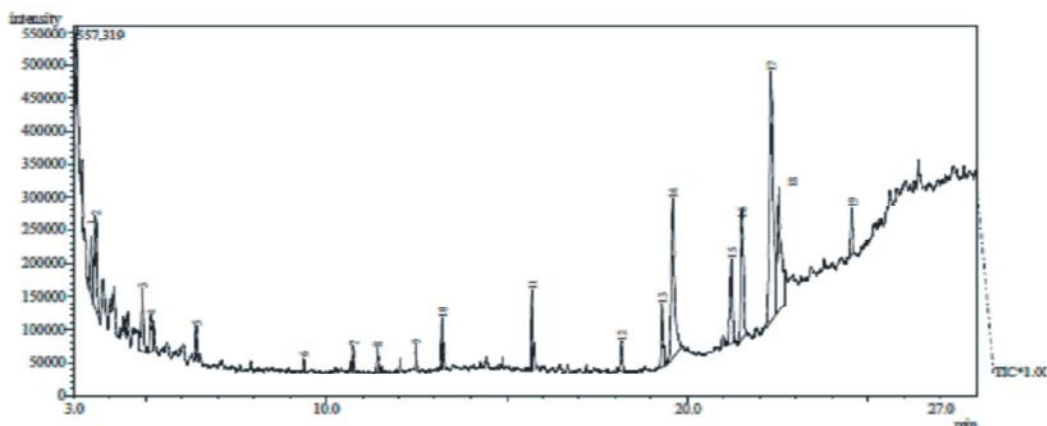


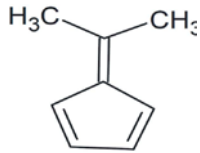
Fig. 2: Chromatogram of Ethanol Extract of *Whitfieldia lateritia* Leaf.

CONCLUSION

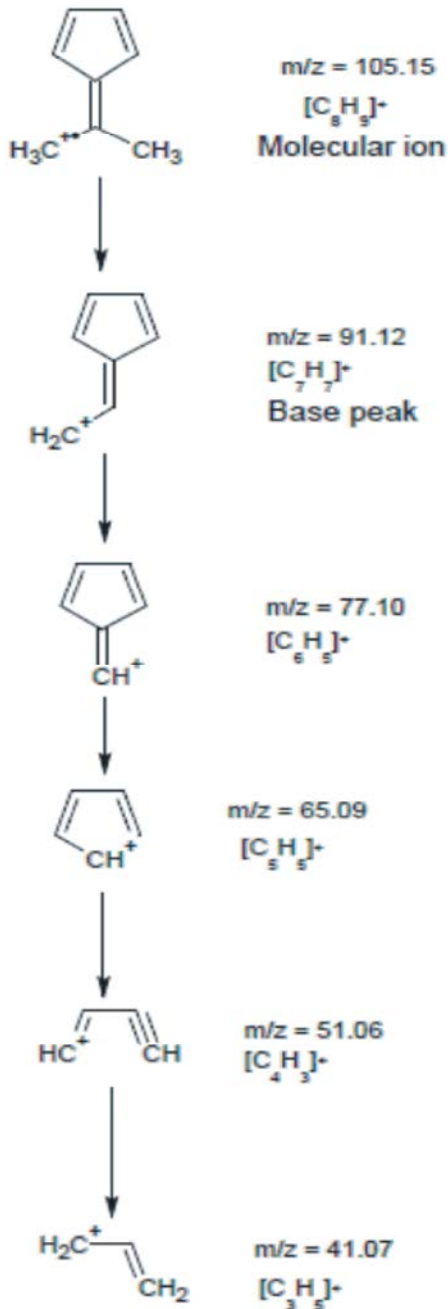
The results showed that the extract of this plant is rich in bioactive compounds which could explain the rationale behind the use of this plant in traditional medicine. The GC-MS constituents showed that the plant contain nineteen chemical compounds with non-1-ene (26.9%) as the highest constituents.

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5-(1-methylethylidene)-1,3-cyclopentadiene



Scheme 1: Fragmentation pattern for 5-(1-methylethylidene)-1,3-Cyclopentadiene

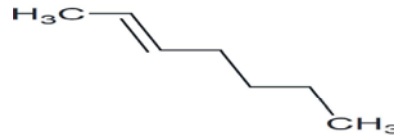
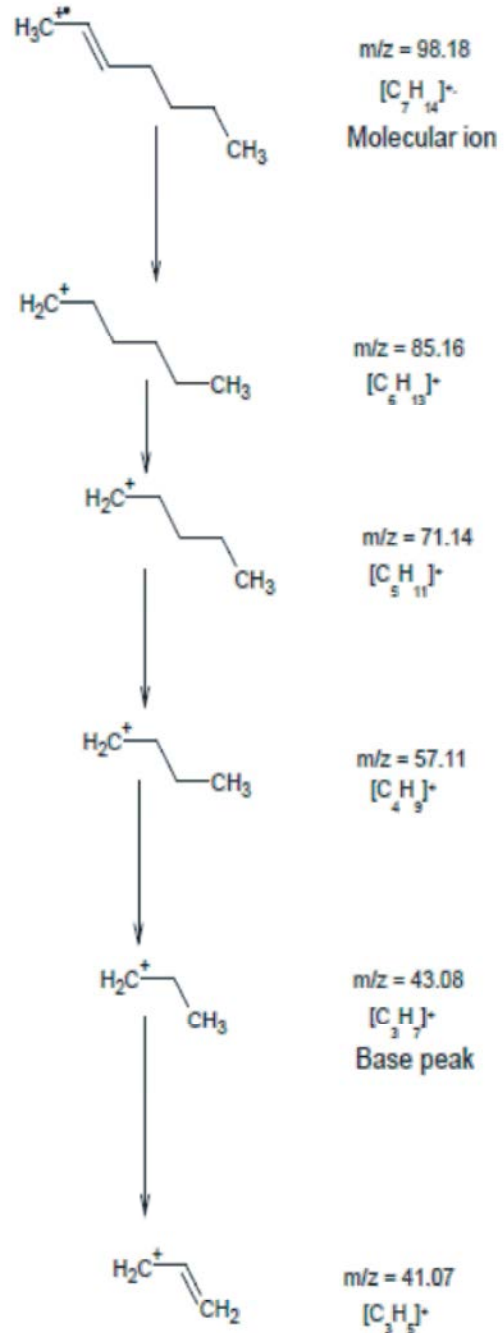
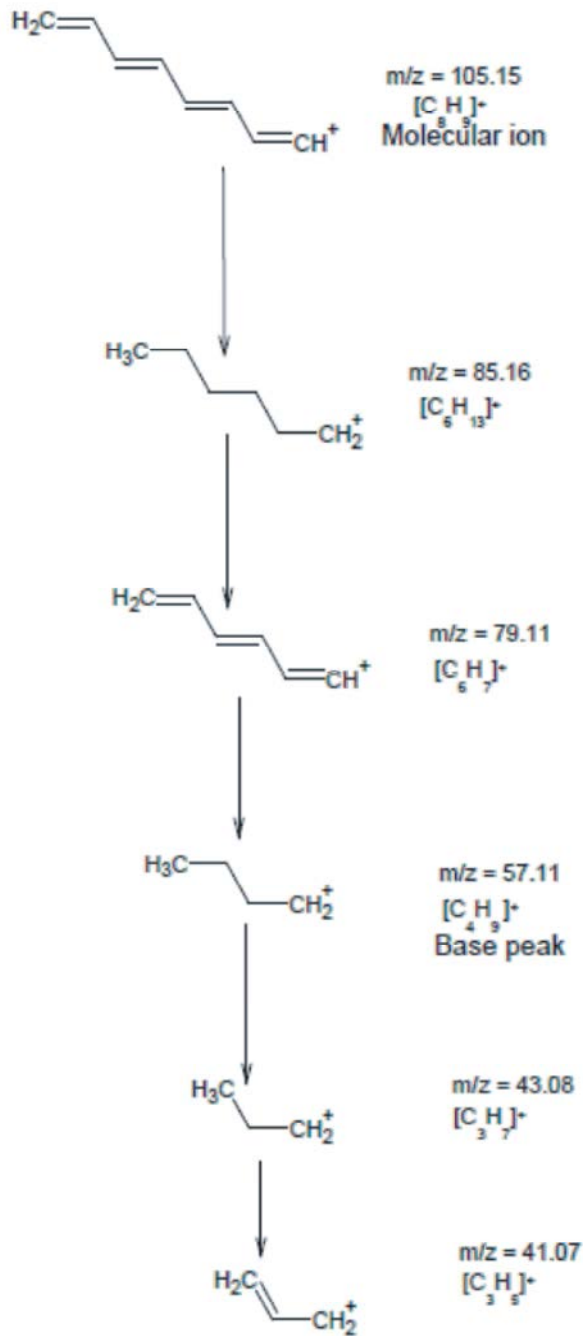
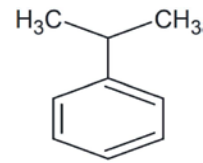
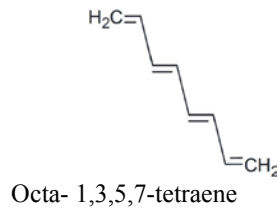


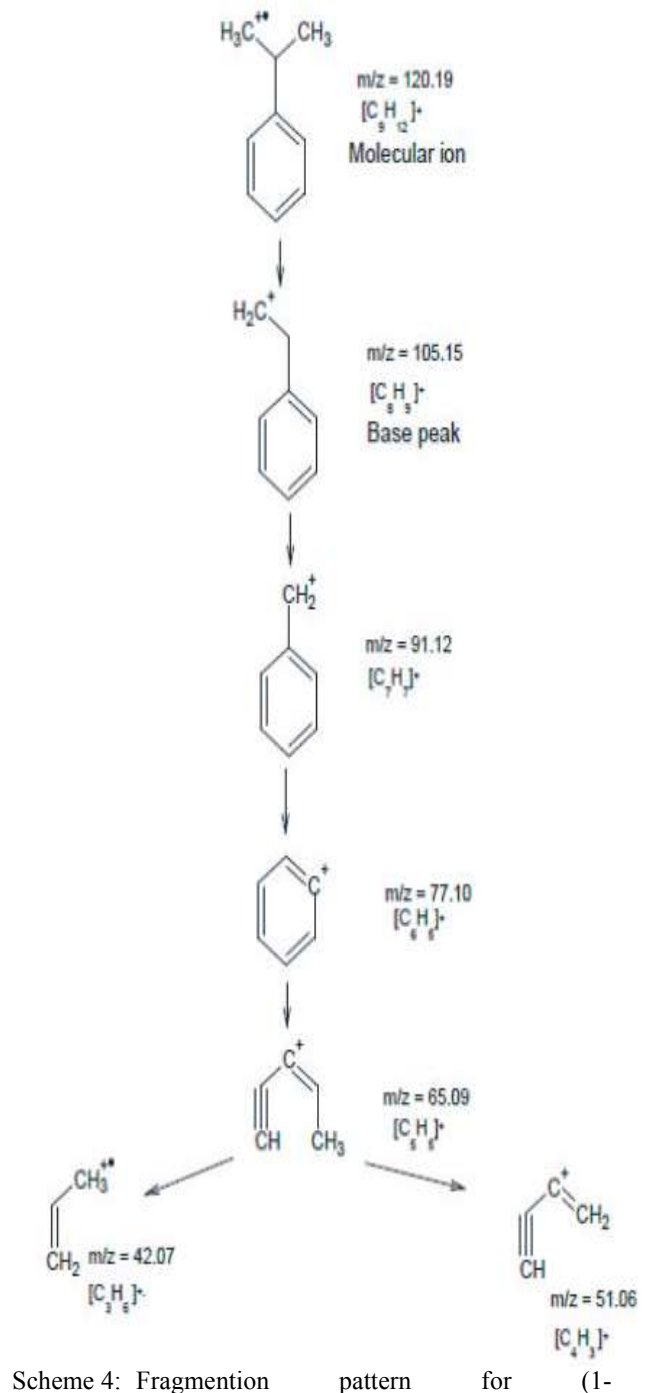
Fig. 2: Hept-2-ene



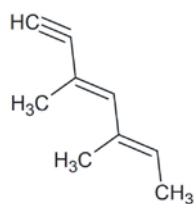
Scheme 2: Fragmentation pattern for Hept-2-ene



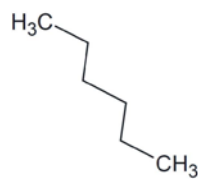
Scheme 3: Fragmentation pattern for Oct-1, 3, 5, 7,-tetraene



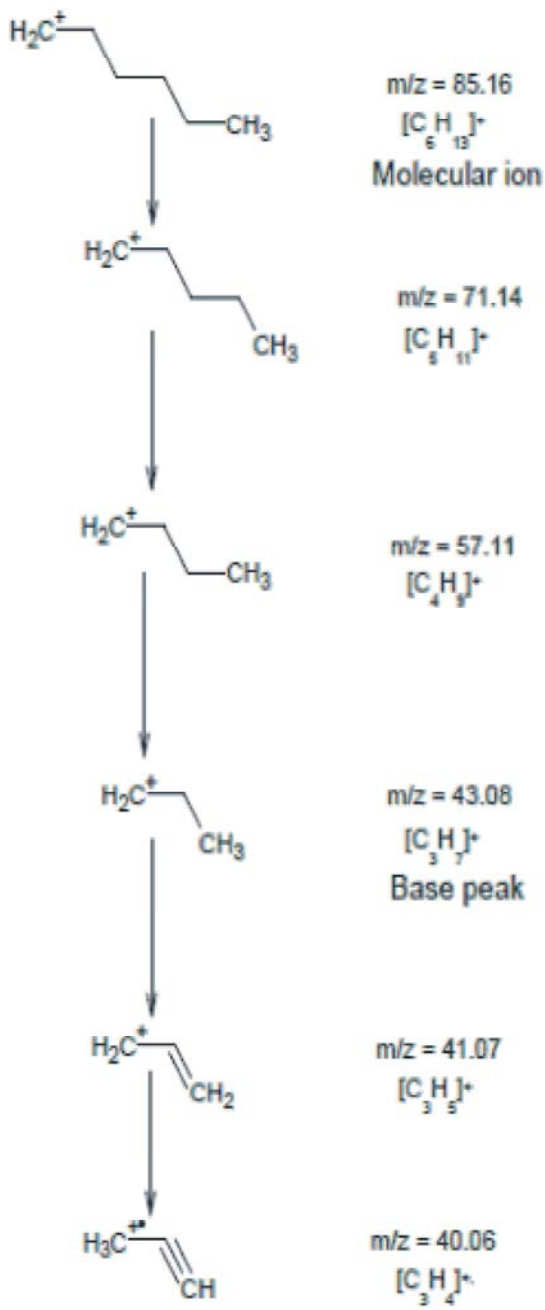
Scheme 4: Fragmentation pattern for (1-Methylethyl)benzene (Cumene)



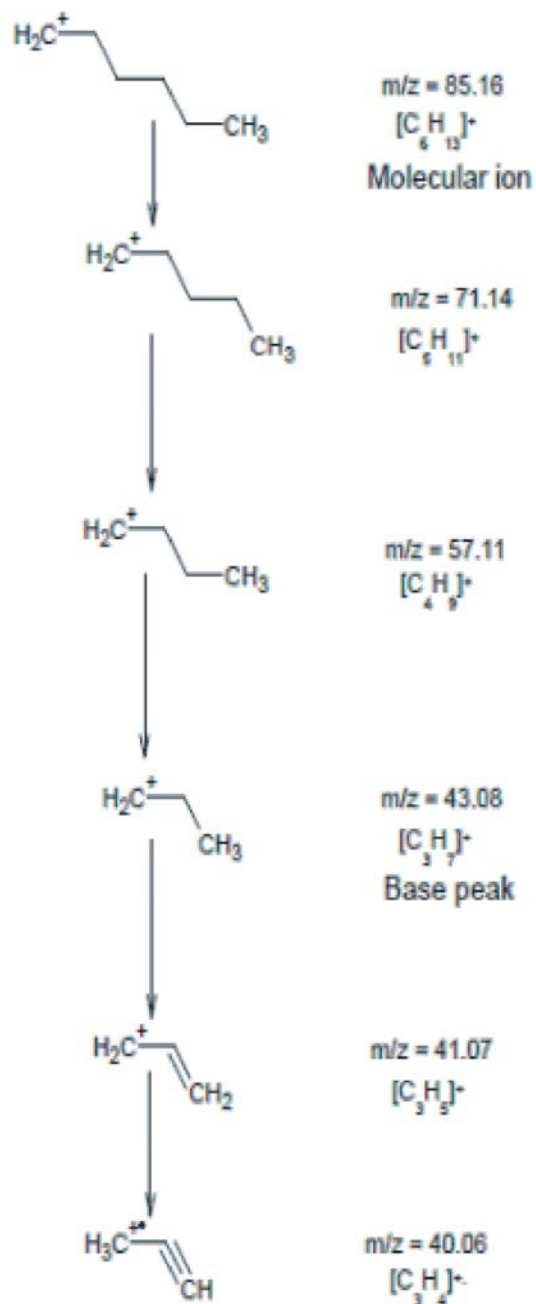
3,5-dimethylhepta-3,5- dien-1-yne



Hexane



Scheme 6: Fragmentation pattern for Hexane



Scheme 7: Fragmentation pattern for Hexane

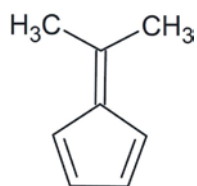
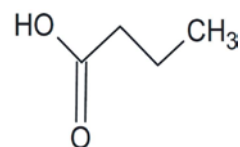
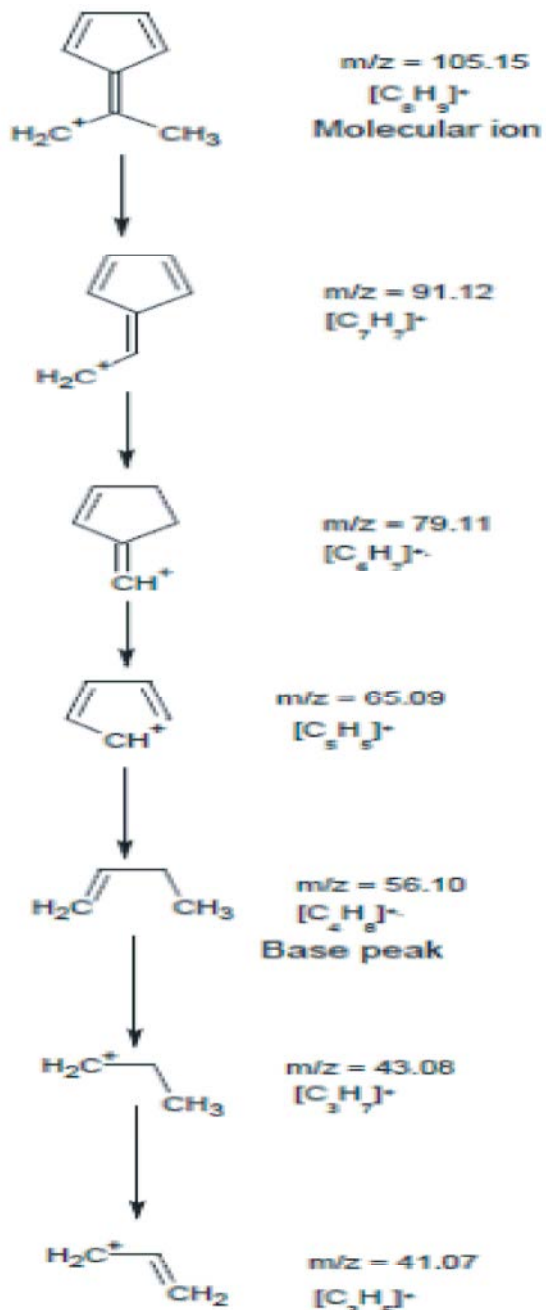


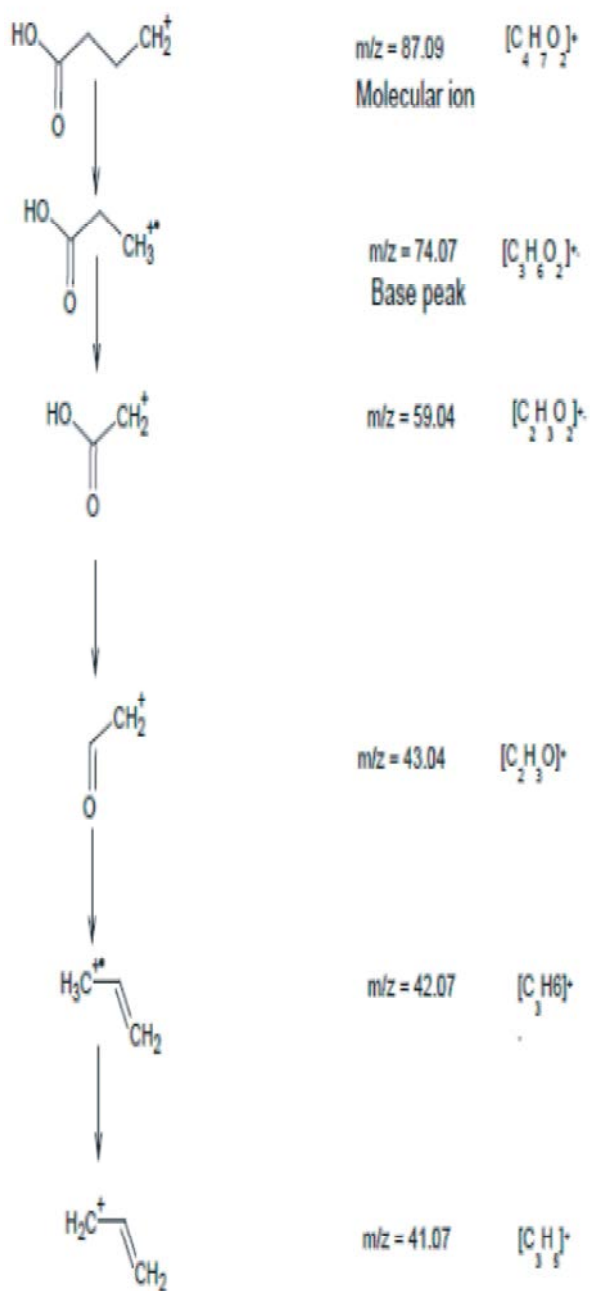
Fig. 13: 5-(1-methylethylidene)-1,3- Cyclopentadiene



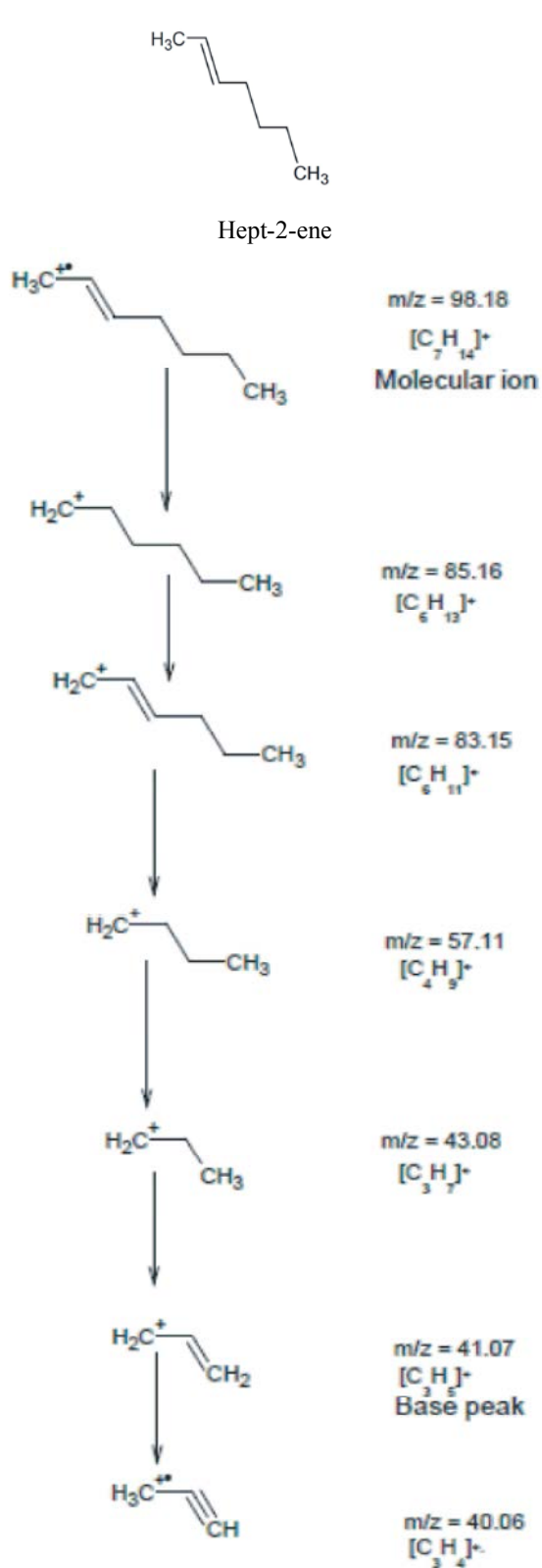
Butanoic acid



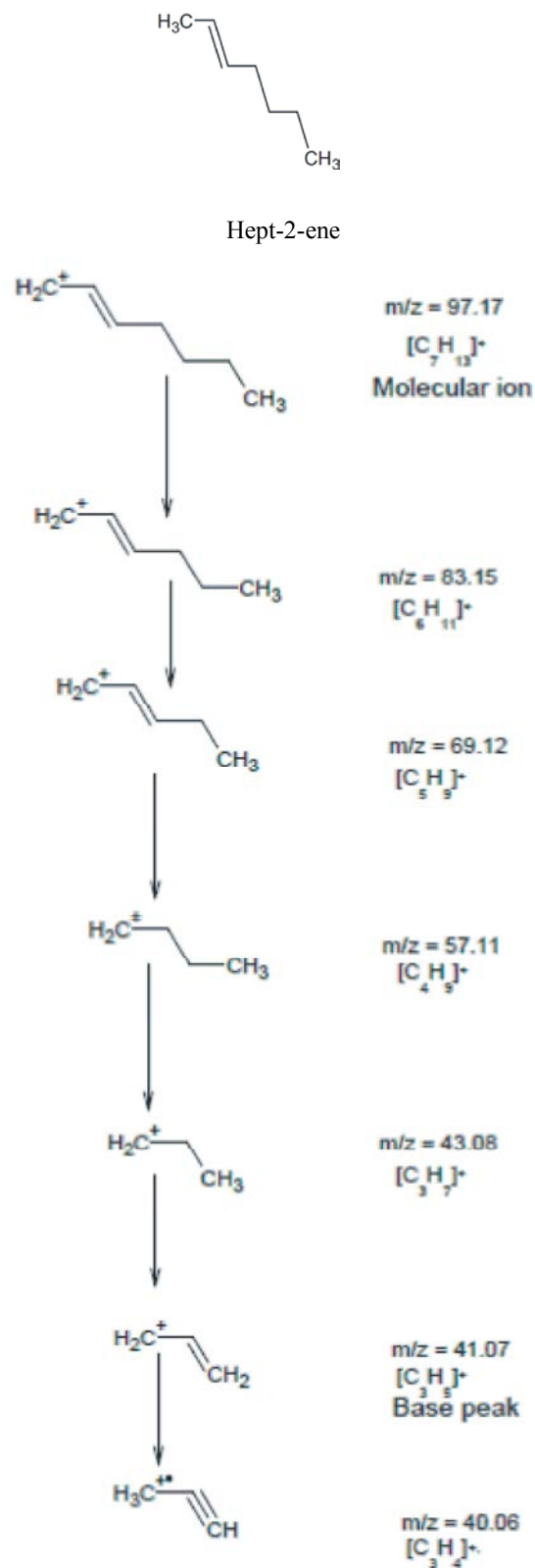
Scheme 8: Fragmentation pattern for 5-(1-methylethylidene)-1,3- Cyclopentadiene



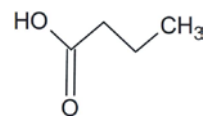
Scheme 9: Fragmentation pattern for Butanoic acid



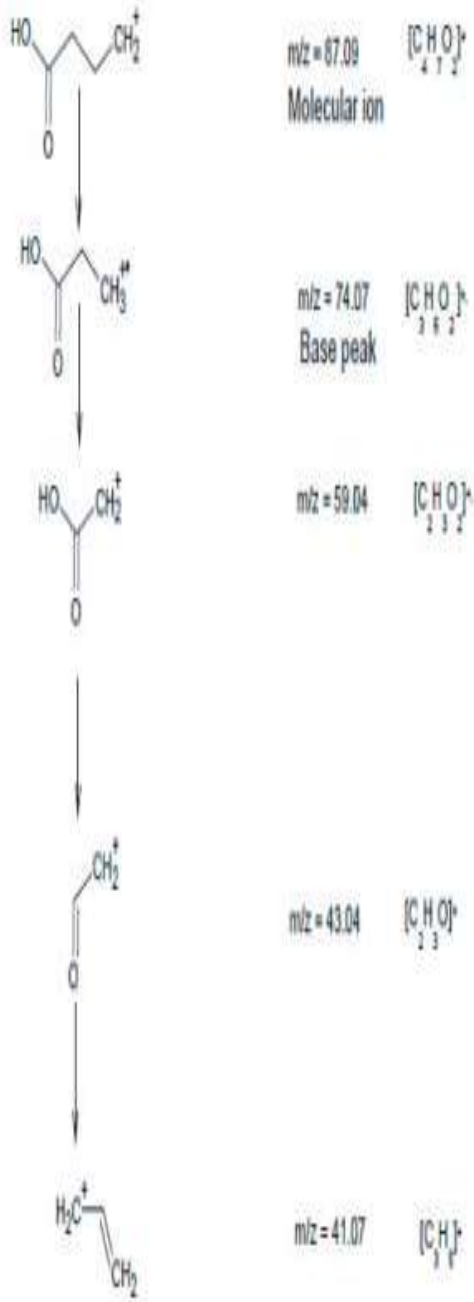
Scheme 10: Fragmentation pattern for Hept-2-ene



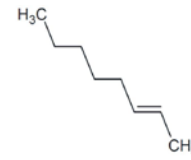
Scheme 11: Fragmentation pattern for Hept-2-ene



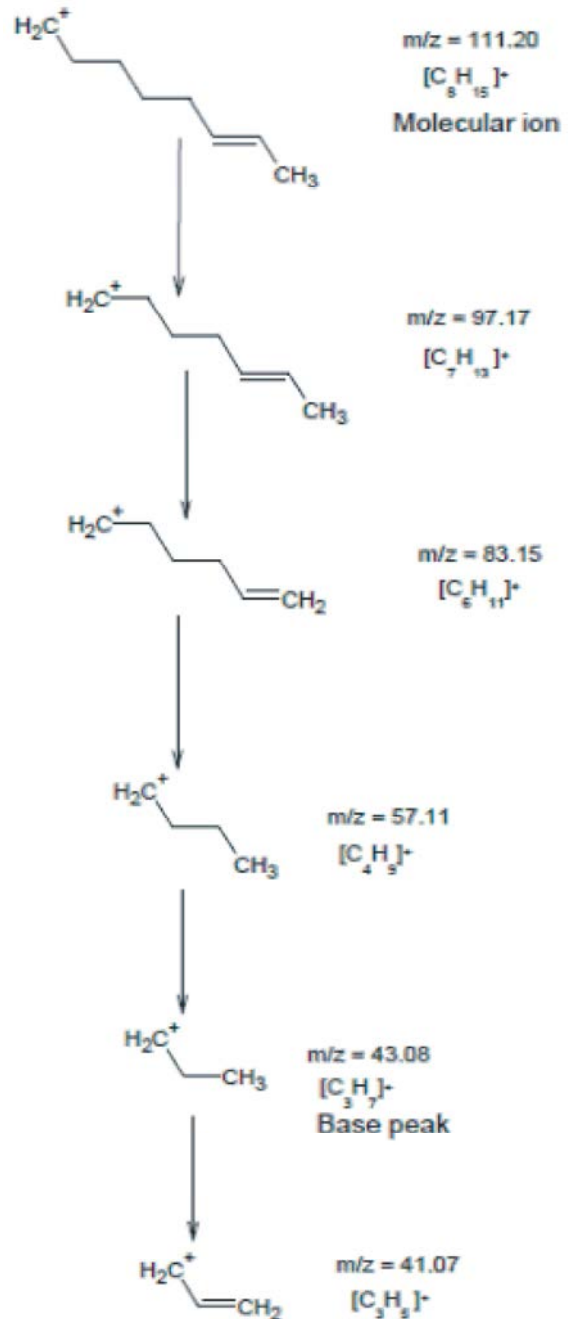
Butanoic acid



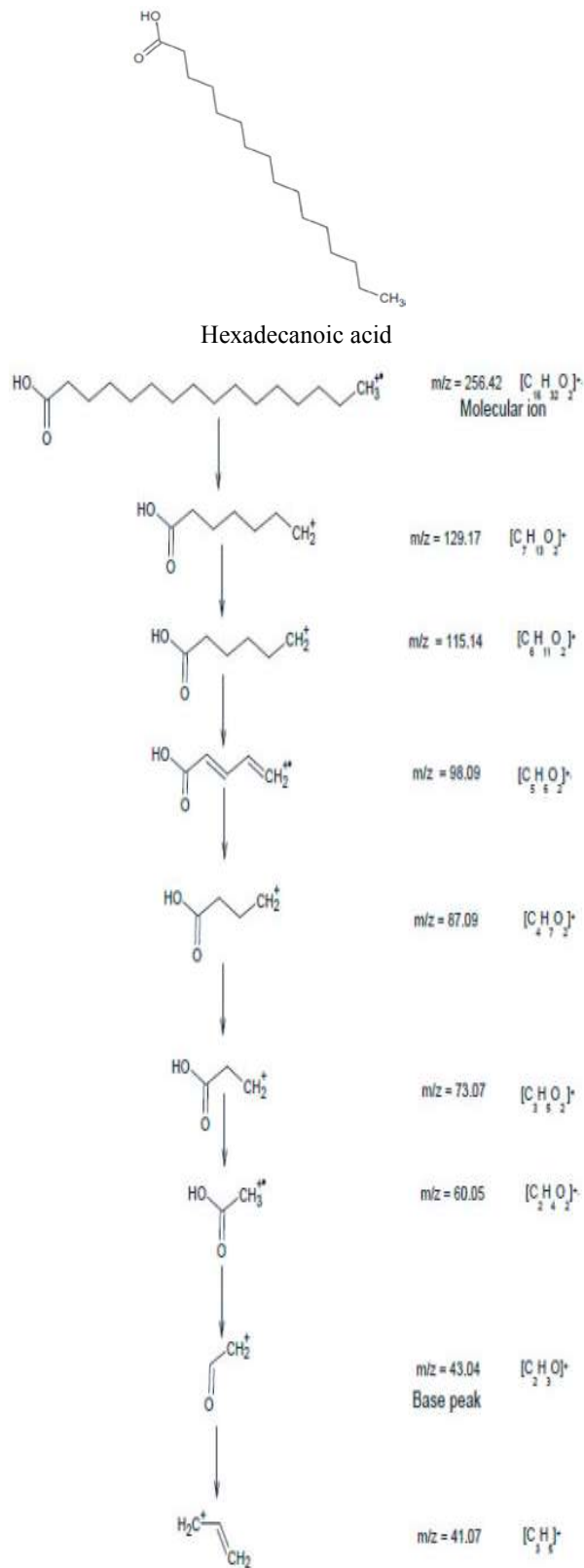
Scheme 12: Fragmentation pattern for Butanoic acid



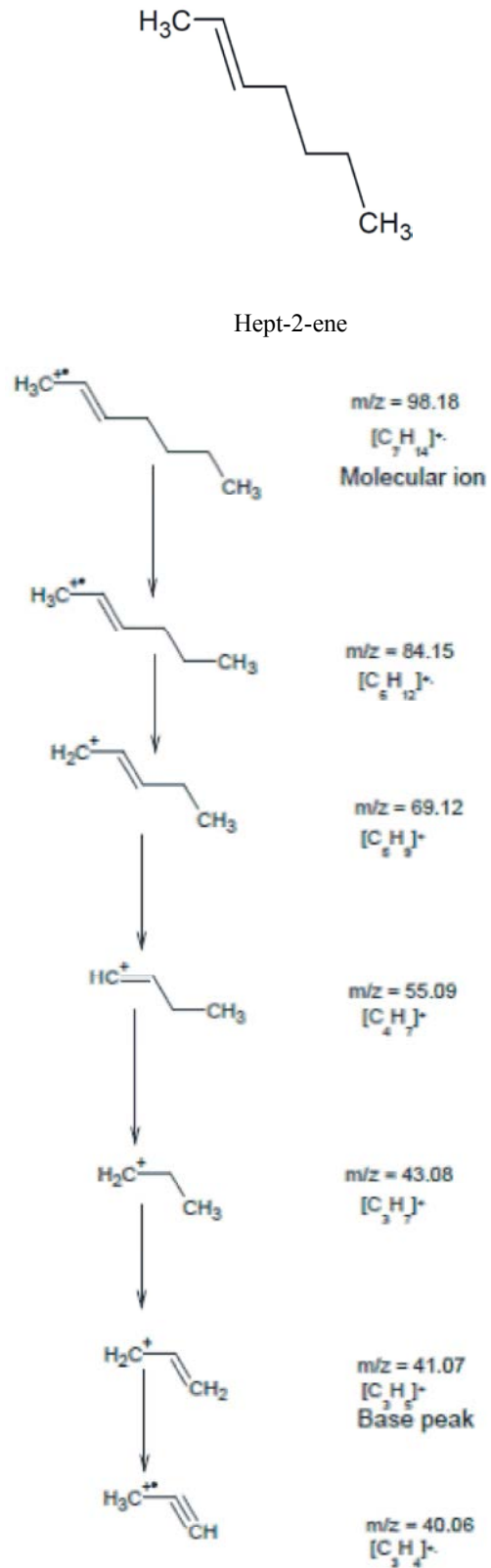
Oct-2-ene



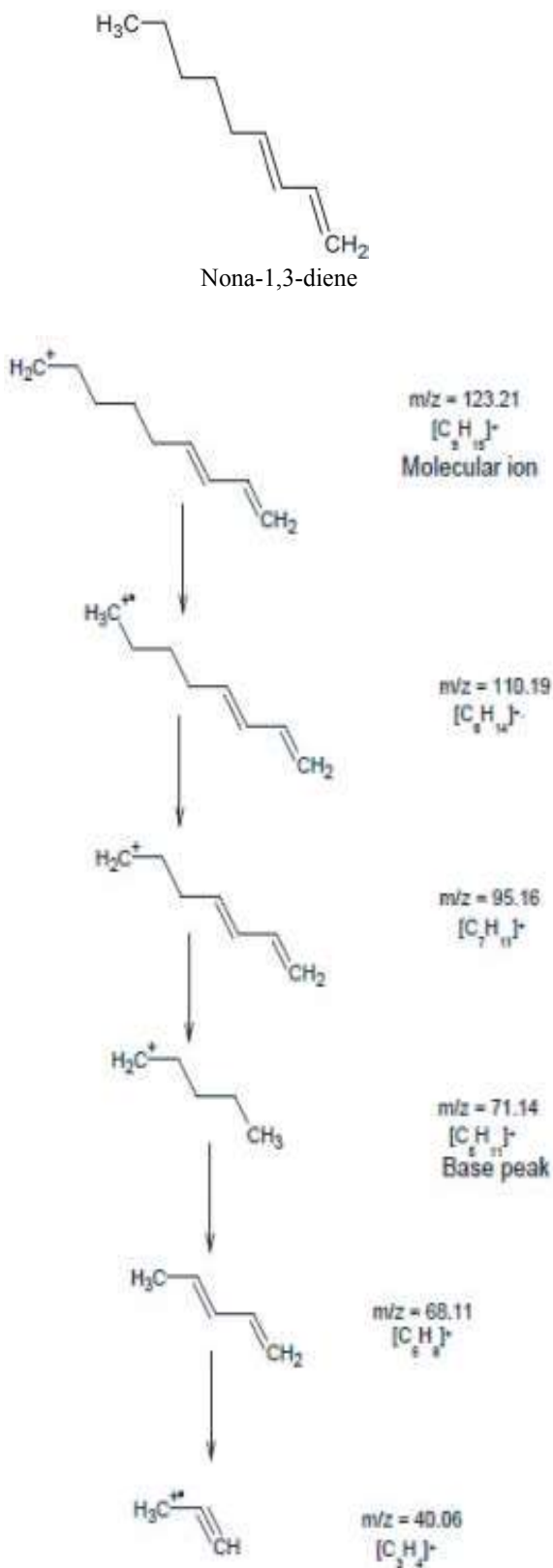
Scheme 13: Fragmentation pattern for Oct-2-ene



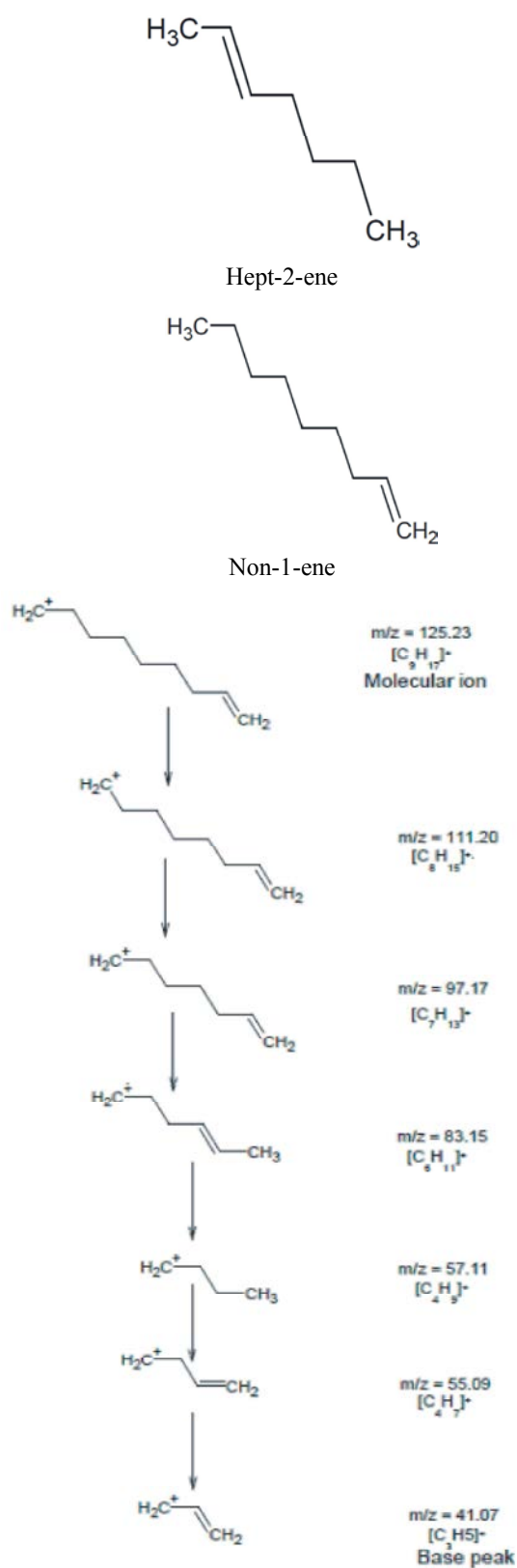
Scheme 14: Fragmentation pattern for Hexadecanoic acid



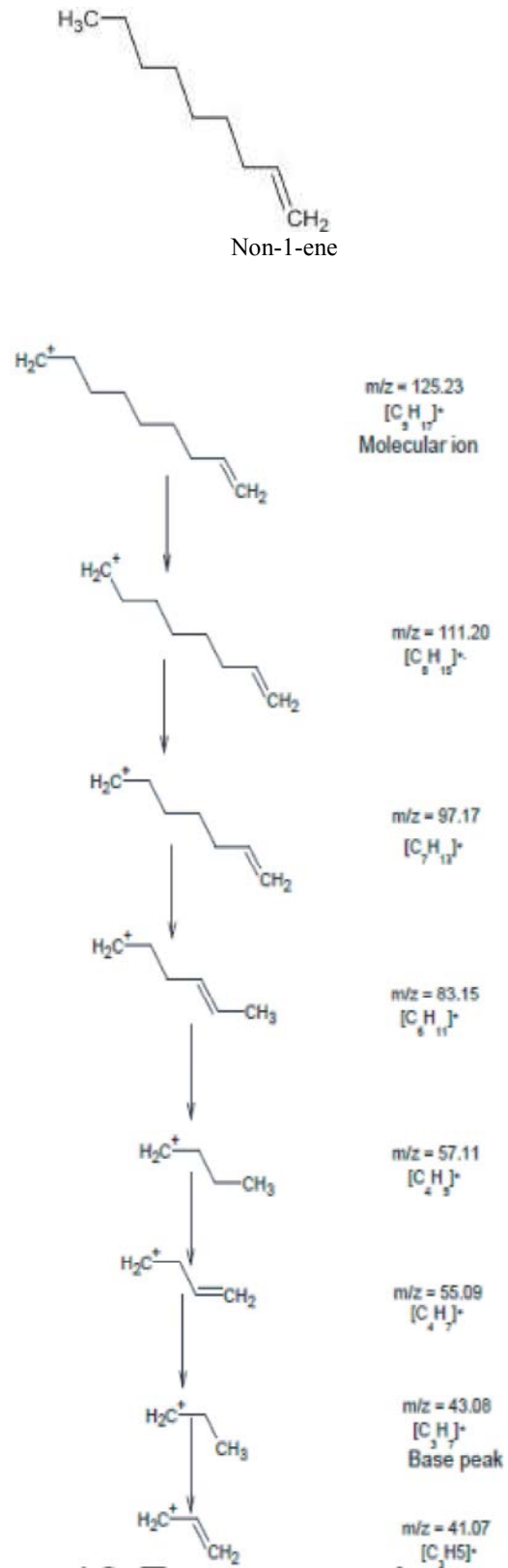
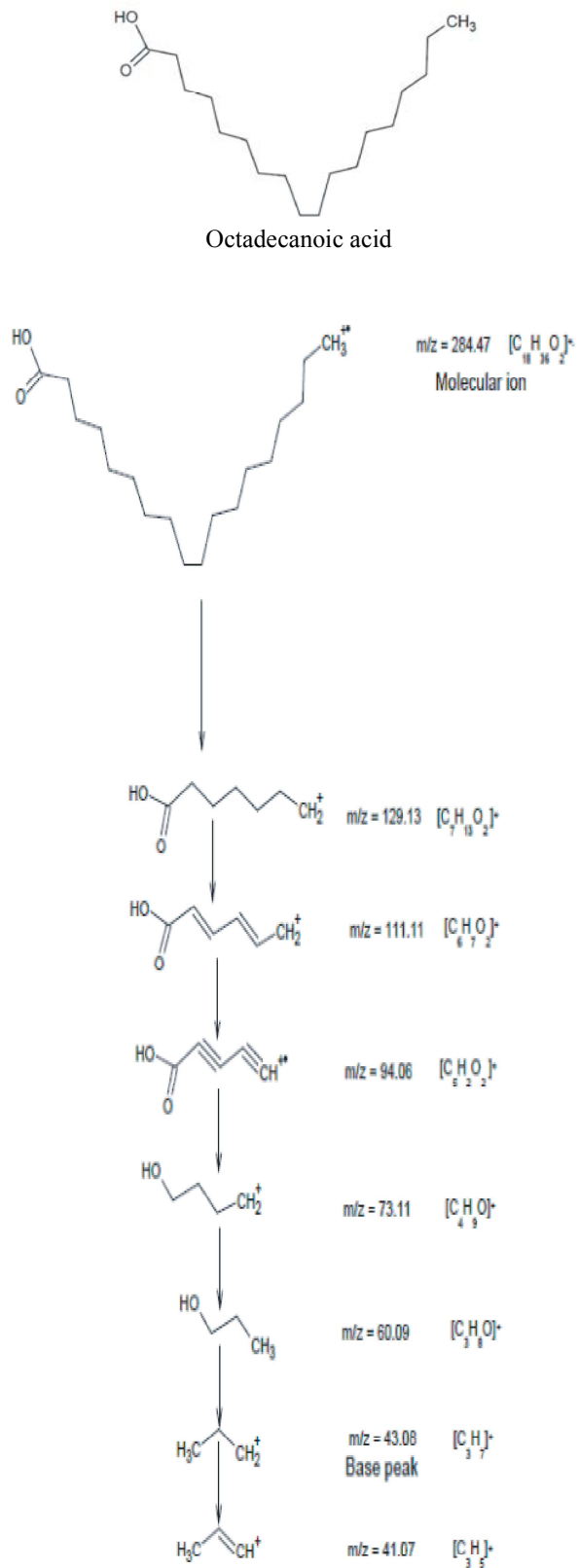
Scheme 15: Fragmentation pattern for Hept-2-ene



Scheme 16: Fragmentation pattern for Nona-1,3-diene



Scheme 17: Fragmentation pattern for Hept-2-ene



Scheme 18: Fragmentation pattern for Octadecanoic acid

Scheme 19: Fragmentation pattern for Non-1-ene