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PHYTOCHEMICAL AND GAS CHROMATOGRAPHY-MASS SPECTROMETRIC (GC-MS) ANALYSES OF *WHITFIELDIALATERITIA* LEAF

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Abstract

Whitfieldialateritia 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 boasting of blood. The phytochemical and GC-MS analyses of *Whitfieldialateritia* 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 *Whitfieldialateritia* 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 *Whitfieldialateritia* leaf.
Key Words: GC-MS analysis, Chemical constituents, phytochemicals, *Whitfieldialateritia*

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, sterm bark, leaves, seed and fruits. Secondary metabolisms are non-nutritive plant chemicals that have protective properties. Many higher plants produce economically

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important organic compounds such as oils, resins, tannins, natural rubber, gums, waxes, dyes, flavours and fragrances, pharmaceuticals and pesticides [7].

*Whitfieldialateritia*commonly called blood plant is a flowering plant belonging to the family of *acanthaceae*[8]. *Whitfieldialateritia*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 "Ogwuobara" in Igbo, "Ogu`neje" 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 *Whitfieldialateritia*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 *Whitfieldialateritia*leaves.

Figure 1: *Whitfieldialateritia* Leaves with Flower

Materials and Methods

Materials

Plant Collection: The fresh leaves of *Whitfieldialateritia*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.

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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 *Whitfieldialateritia*Ethanol Leaf-Extract

Exactly 40grams of dried powdered leaves of *Whitfieldialateritia*were extracted successively with 300ml of ethanol in an orbital shaker for 24hours at room temperature. The extract was filtered using what-man No.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 *Whitfieldialateritia.*

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 triterpeniod 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 (NH_4OH) and few drops of tetraoxosulphate (VI) acid (H_2SO_4) 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 $FeCl_3$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 *Whitfieldialateritia*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^{o}C held at 80^{o}C for 1minute, rate 5^{o}C/min and at 200^{o}C for 20min. Field ionization detector (FID) Temperature of 300^{o}C, injection temperature of 220^{o}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 *Whitfiedialateritia* leaves.

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

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

Table 1: Phytochemical Screening of *Whitfieldialateritia* Ethanol leaf-Extract

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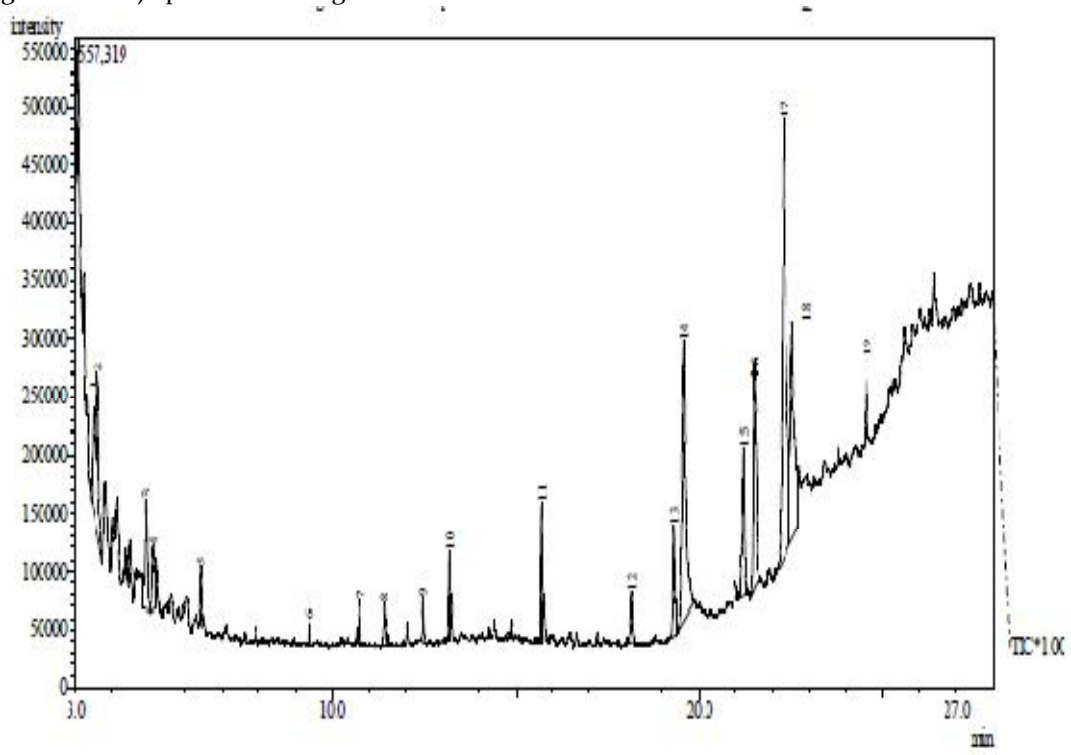


Figure 2: Chromatogram of Ethanol Extract of *Whitfiedialateritia* Leaf.

Result of GC-MS Analysis of *Whitfiedialateritia*

The ethanol extract of the leaf of *Whitieldialateritia* showed nineteen peaks from the GC-MC chromatogram. These peaks indicate the presence of nineteen compounds (1-19) in the extract (Figure 2). 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.

Table 2: GC-MS Analysis and Mass Spectral Data of Ethanol Fraction from the leaf of *Whitfieldialateritia* 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-mthl thylidene) – 1, 3-cyclopentadiene	C_8H_{10}	106	1.12%	27	3.500
2	Hept-2-ene	C_7H_{14}	98	12.2%	19	3.616
3	Octa-1,3,5,7-tetraene	C_9H_{13}	106	4.5%	10	4.912
4	(1-mthylethyl) benzene	C_9H_{13}	121	0.7%	16	3.145
5	3, 5 dimethylhepta -3,5dien 1-yne	C_9H_{12}	120	0.6%	16	6.403
6	Hexane	C_6H_{14}	86	0.3%	10	9.373
7	Hexane	C_6H_{14}	86	0.5%	11	10.794
8	5-(1-mthylethylidene)-1,3-Cyclopentadiene	C_8H_{14}	106	0.8%	10	11.413
9	Butanoic Acid	$C_4H_8O_2$	88	1.8%	15	12.482
10	Hept-2-ene	C_7H_{14}	98	0.7%	22	13.208
11	Hept-2-ene	C_7H_{14}	98	1.3%	23	15.692
12	Butanoic Acid	$C_4H_8O_2$	88	1.0%	16	19.173
13	Oct-2-ene	C_8H_{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_9H_{14}	98	3.0%	34	21.215
16	Nona-1,3-diene	C_9H_{16}	124	9.4%	31	21.498
17	Non-1-ene	C_9H_{18}	126	26.9%	49	22.307
18	Octadecanoic Acid	$C_{18}H_{36}O_2$	284	9.2%	38	22.535
19	Non-1-ene	C_9H_{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

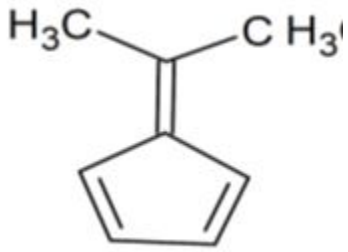
metabolites are present in higher plants. They include alkaloids, steroids, flavonoids, saponnins, glycosides, phenol, tannins, and many others. The active principles of many drugs found in plants are secondary metabolites.

This research work showed that *WhitfieldiaLateritia* 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.* (2010) [12] which revealed the presence of alkaloids and absence of glycosidesin *Talinumtriangulare*leaf in both dry and wet samples. Offor*et al.* (2015) [13] and Aja*et al.* (2015) [14] also reported the presence of all the phytochemicals in various concentrations in *Terminaliacatappa*leaf, *Cajanuscajan*leaf and seed respectively.

The ethanol extract of the leaf of *Whiftieldialateritia* 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 *Whiftieldialateritia* leaf. Aja*et al.*(2014) [5] identified the presence of sixteen GC-MS constituents in *Moringaoleifera* 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 *Vitexdoniana* 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-(methylethylcidene)-1,3-cycopentadienne is active in the treatment of prostate cancer, acne, seborrhea and hirsutism (Swain, 1985) [4]. Hept-2-ene beta-lactamase inhibition efficiency (Swain, 1985) [4] .Butanoic acid is currently considered therapeutic in the treatment of colorectal cancer and hemoglobinopathies (Tabacco, 2006) [6].

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.



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

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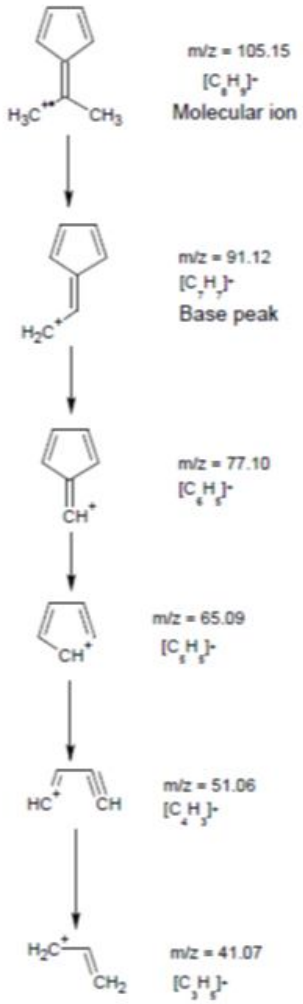

Scheme.1 Fragmentation pattern of 5-(1-methylethylidene)-1, 3-cyclopentadiene

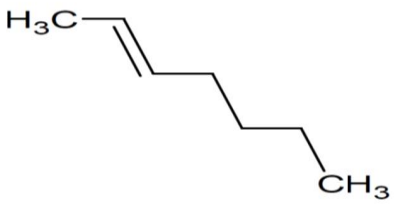

Figure.2: Hept-2-ene

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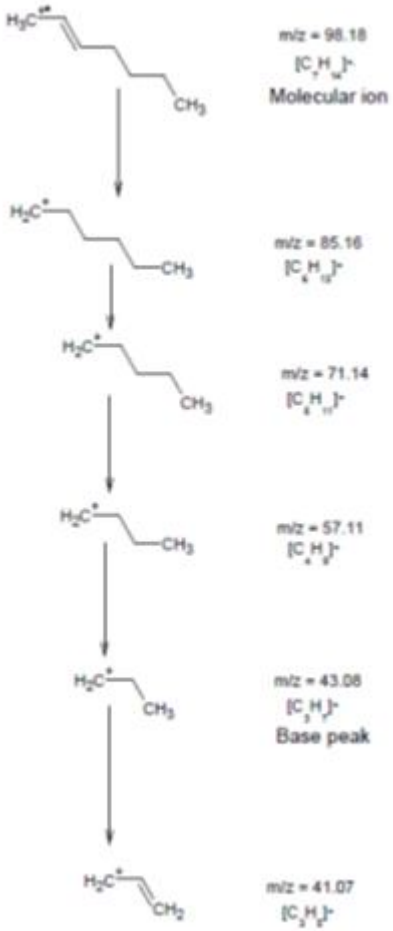

Scheme:2 Fragmentation pattern for Hept-2-ene

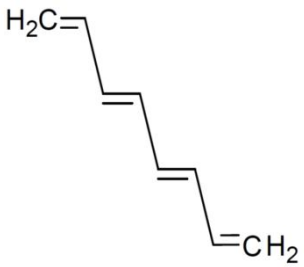

Figure.3:Octa-1,3,5,7-tetaene

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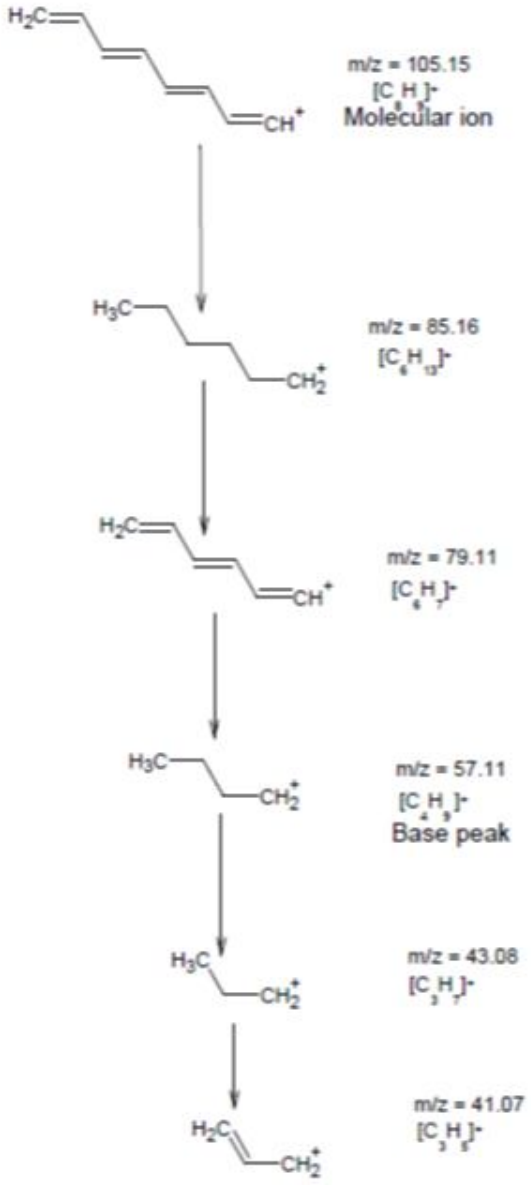
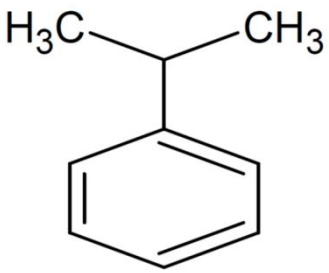


Scheme.3: Fragmentation Pattern for Octa-1,3,5,7-tetraene



(1-Methylethyetyl) Benzene Cumene

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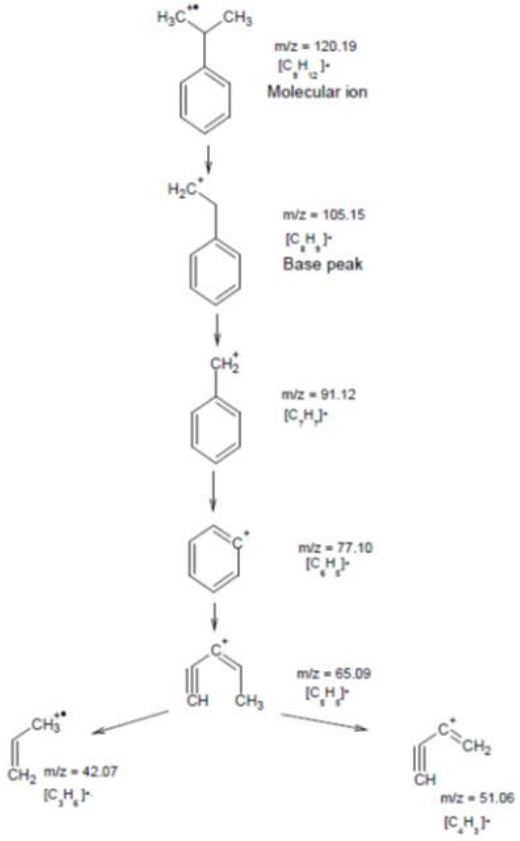

Scheme.4: Fragmentation Pattern for (1-Methylethyl) benzene (Cumene)

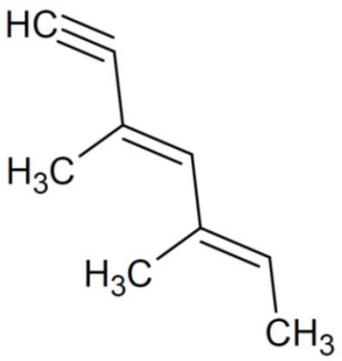

Figure.5: 3,5-dimethylhepta-3,5-dien-1-yne

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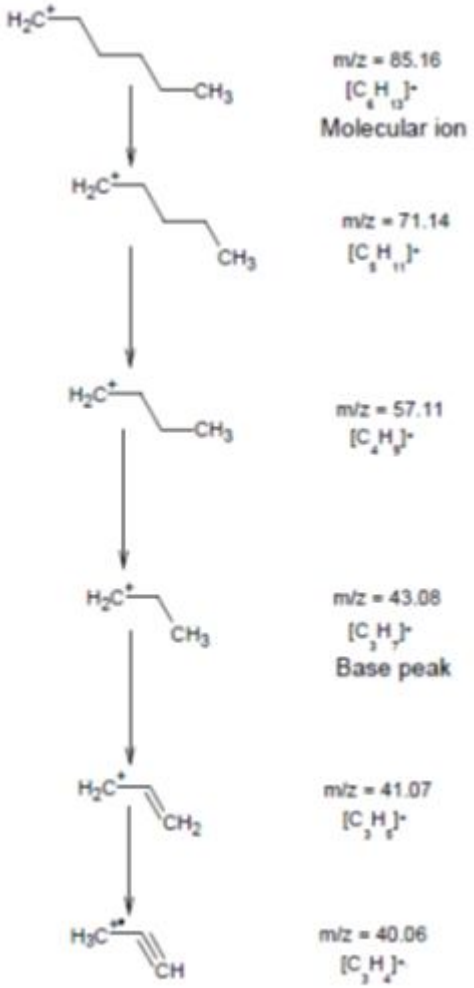
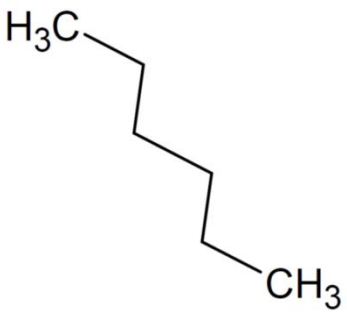


Scheme.6: Fragmentation pattern for Hexane



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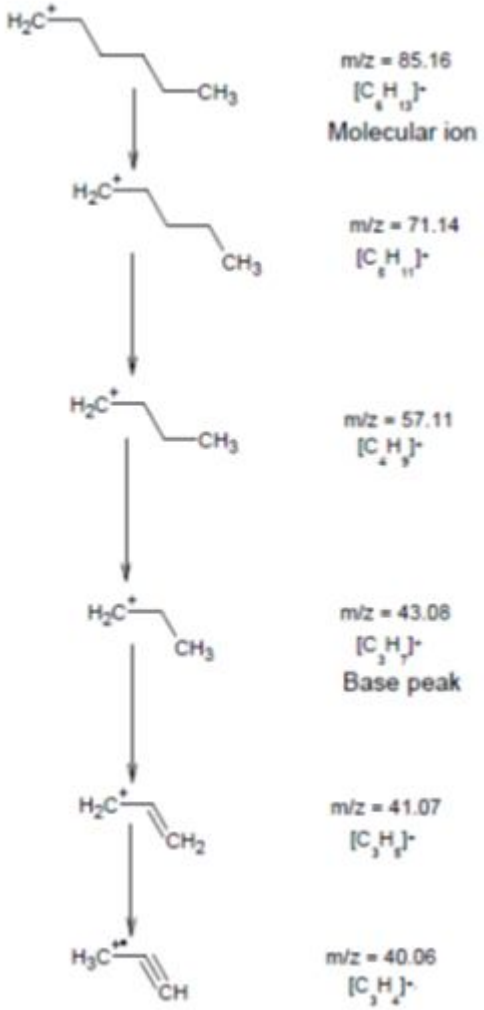

Scheme.7: Fragmentation pattern for Hexane

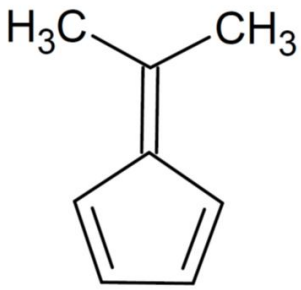

Figure.13:5-(1-Methylethylidene)-1,3-Cyclopentadiene

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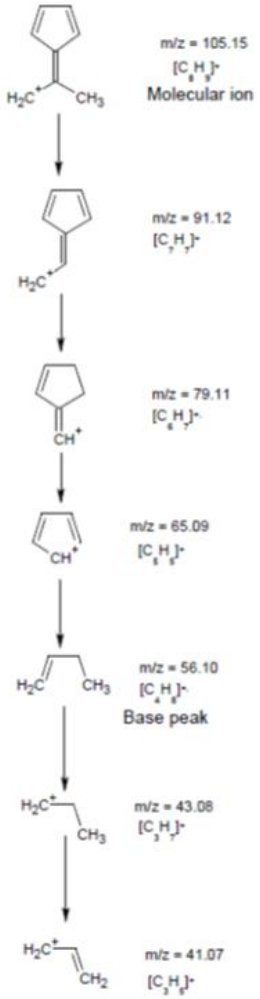
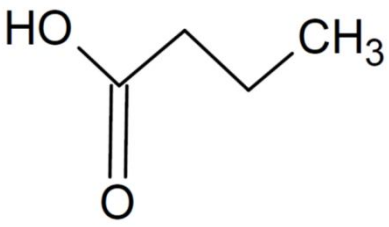


Scheme.8: Fragmentation pattern for 5-(1-methylethylidene)-1,3-Cyclopentadiene



Butanoic Acid

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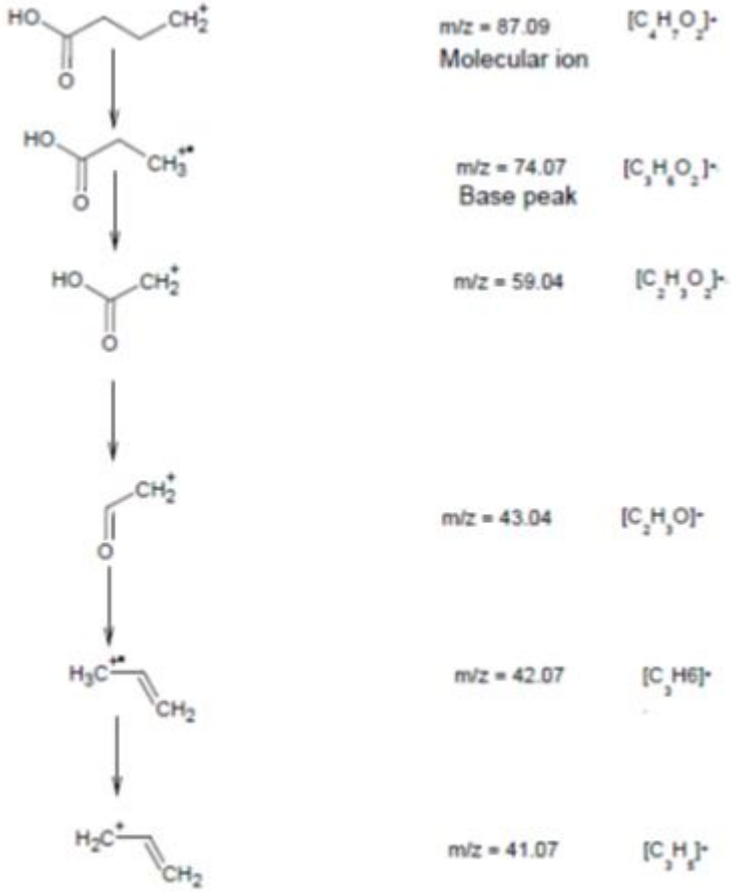
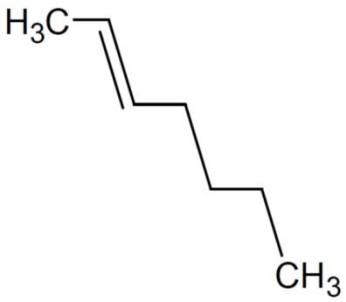


Scheme.9: Fragmentation pattern for Butanoic Acid



Hept-2-ene

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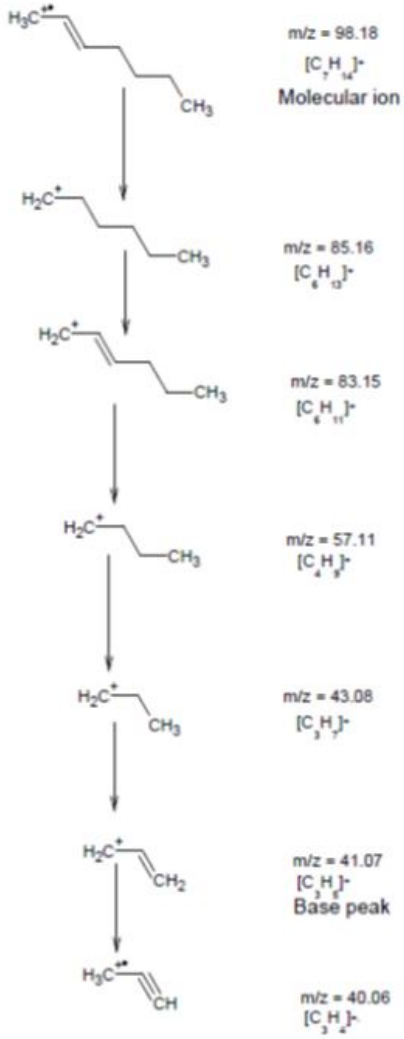

Scheme.10: Fragmentation pattern for Hept-2-ene

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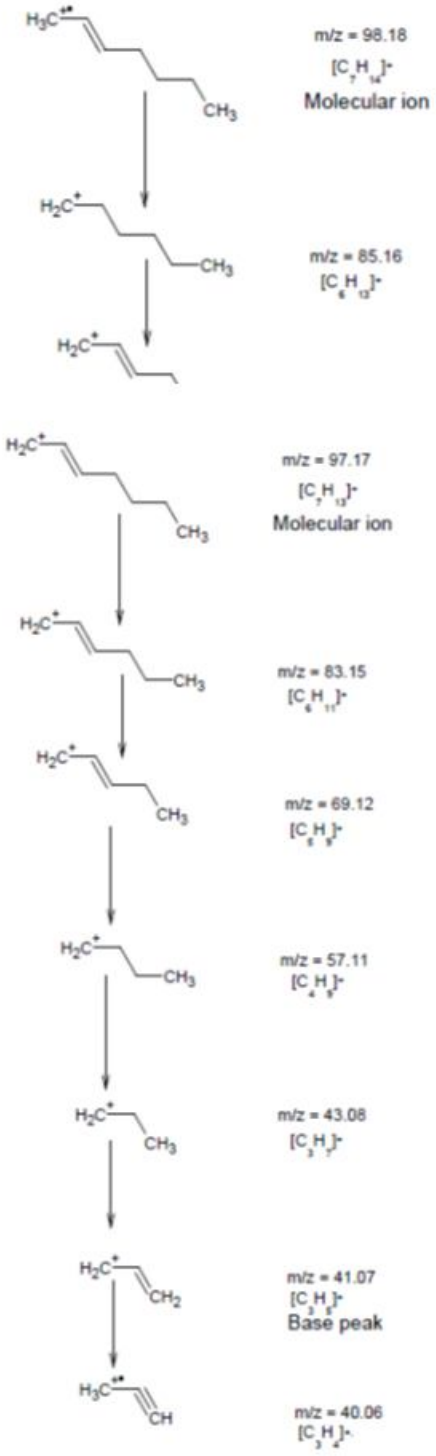

Scheme.11: Fragmentation pattern for Hept-2-ene

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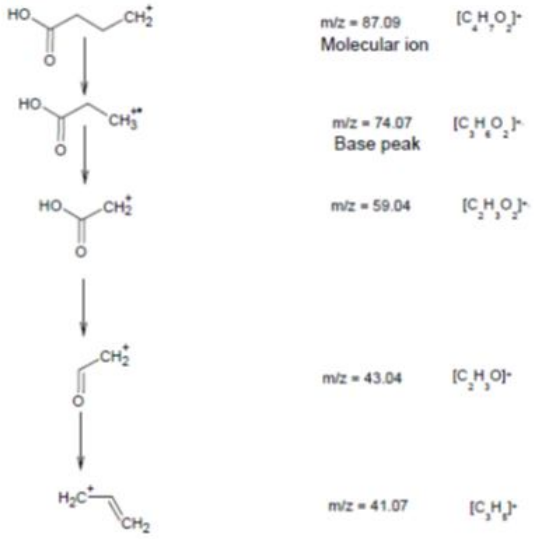
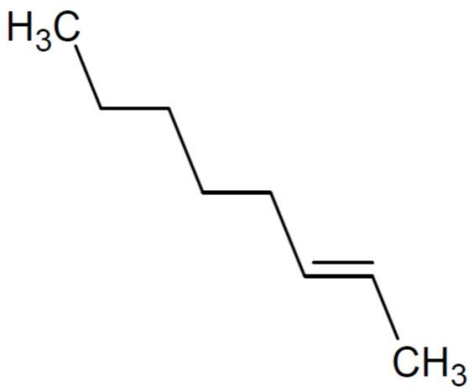


Scheme.12: Fragmentation pattern for Butanoic Acid



Oct-2-ene

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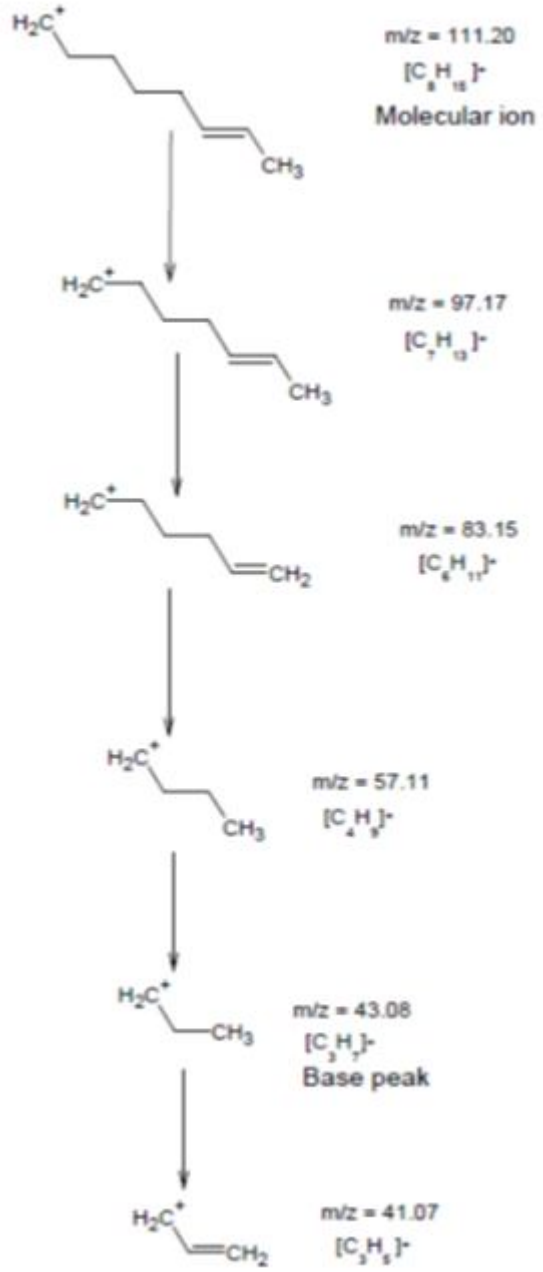

Scheme.13 Fragmentation for Oct-2-ene

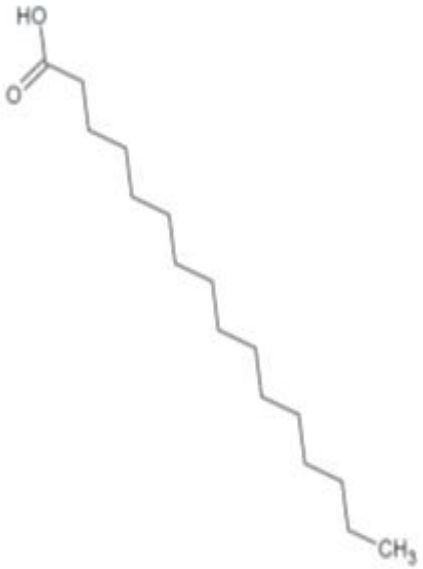

Figure.14: Hexadecanoic acid

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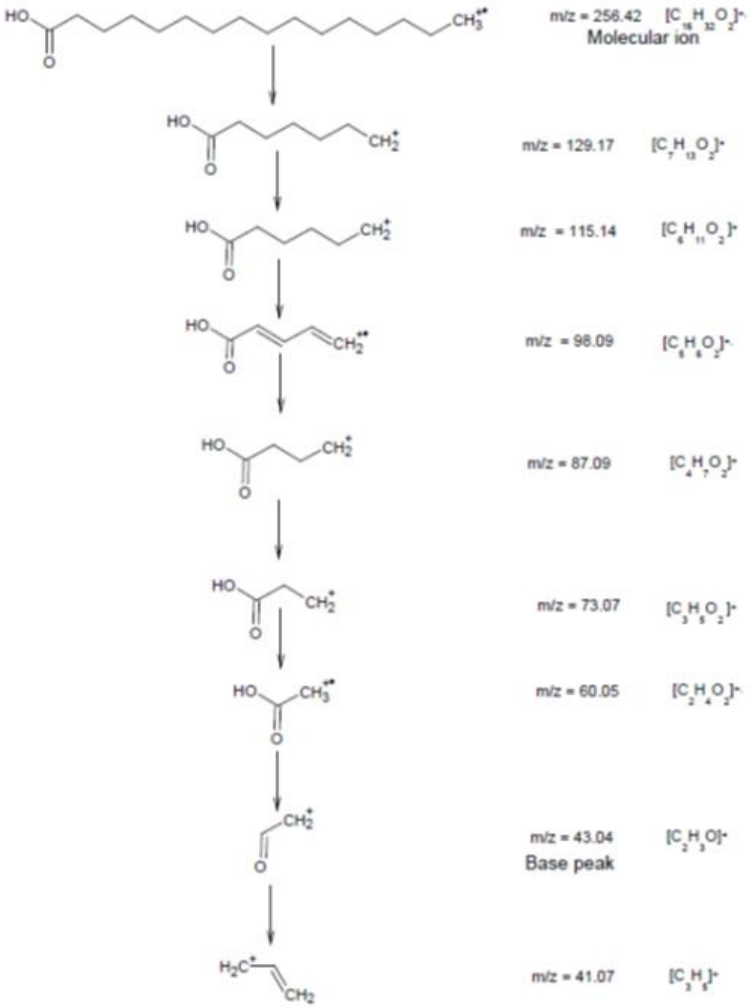

Scheme.14 Fragmentation pattern for Hexadecaonic Acid

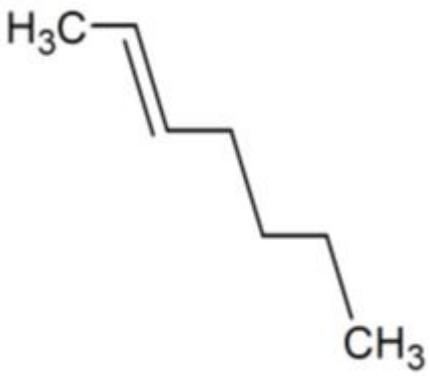

Figure.15 Hept-2-ene

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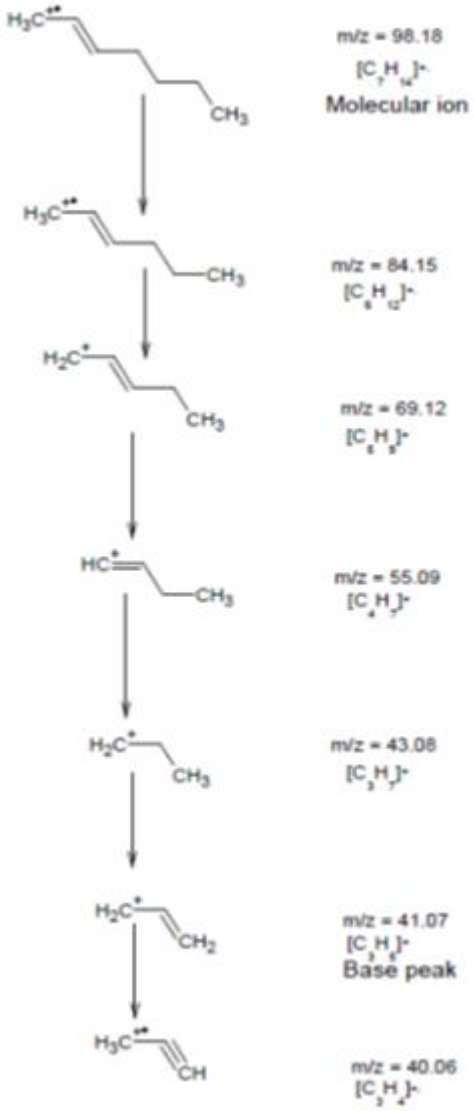
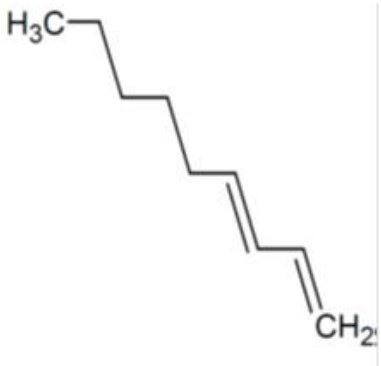


Scheme.15: Fragmentation pattern for Hept-2-ene



Nona-1,3-diene

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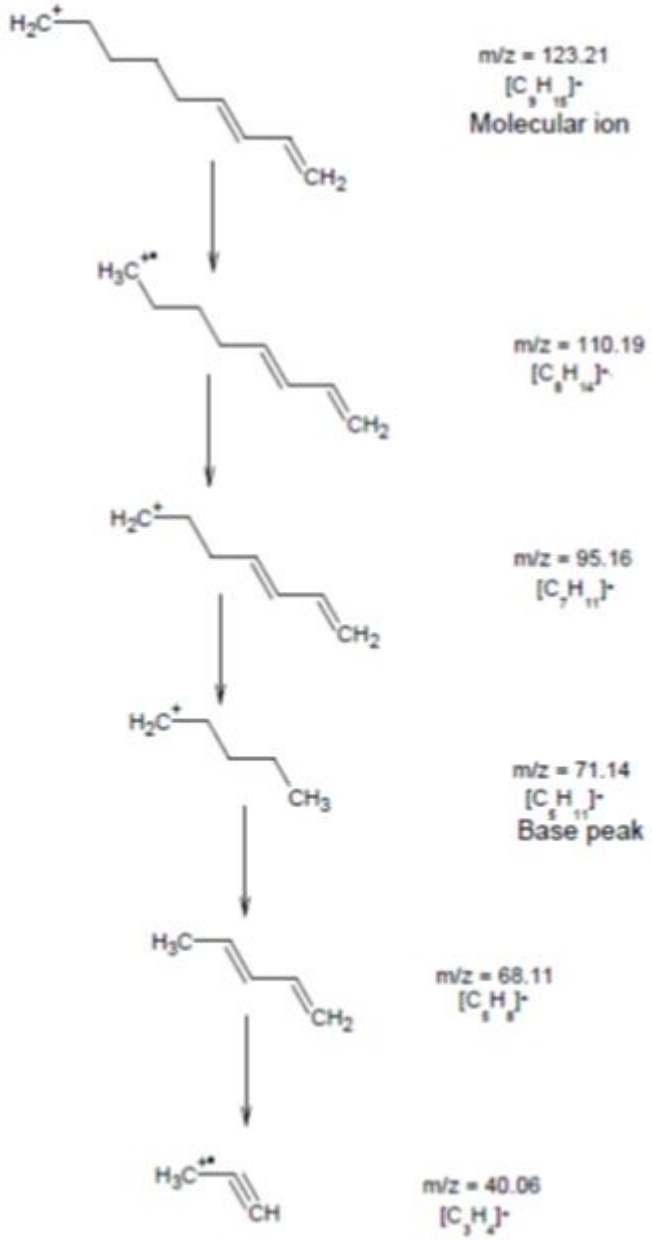

Scheme.16: Fragmentation pattern for Nona-1,3-diene

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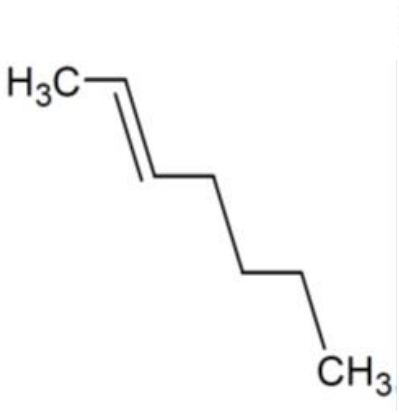
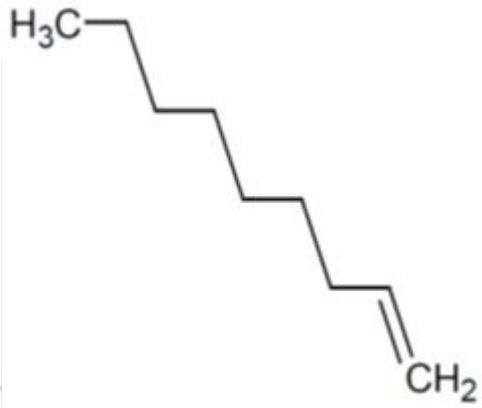
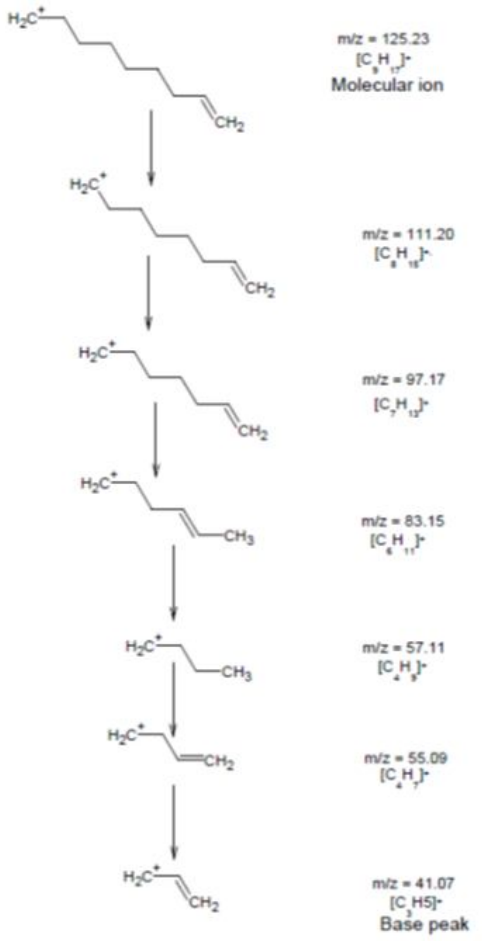


Hept-2-ene



Non-1-ene



Scheme:17 Fragmentation pattern for Non-1-ene

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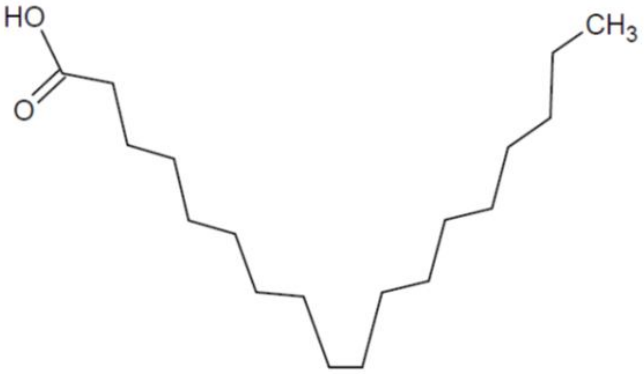
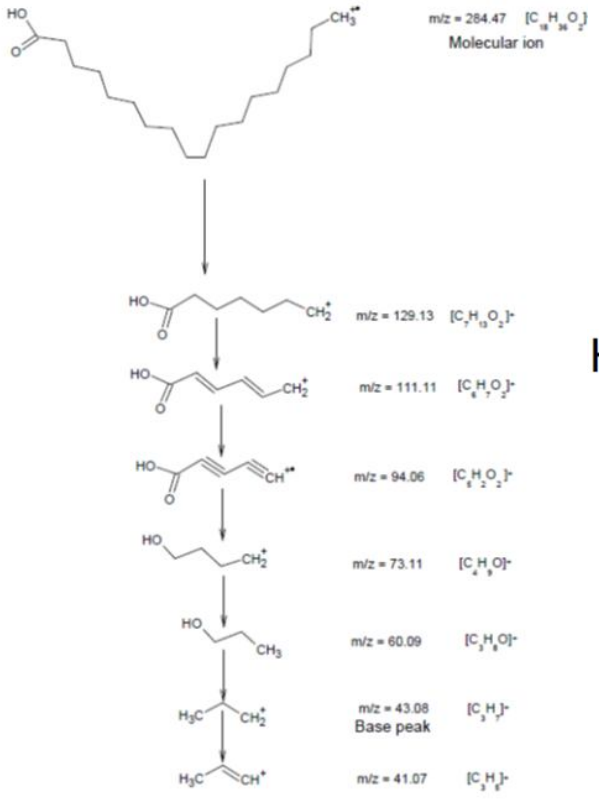
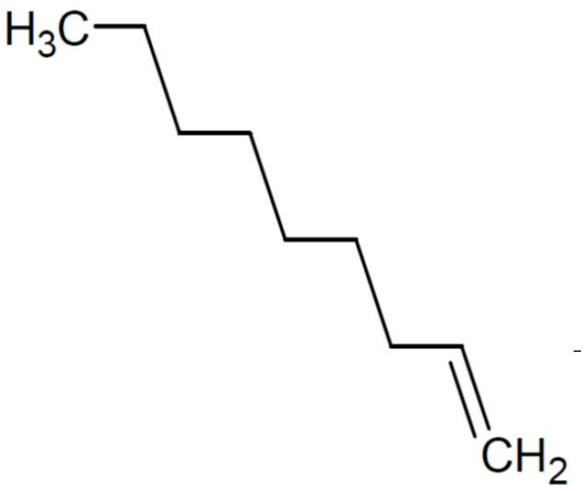


Octadecanoic Acid



Scheme:18 Fragmentation pattern for Octadecanoic Acid



Non-1-ene

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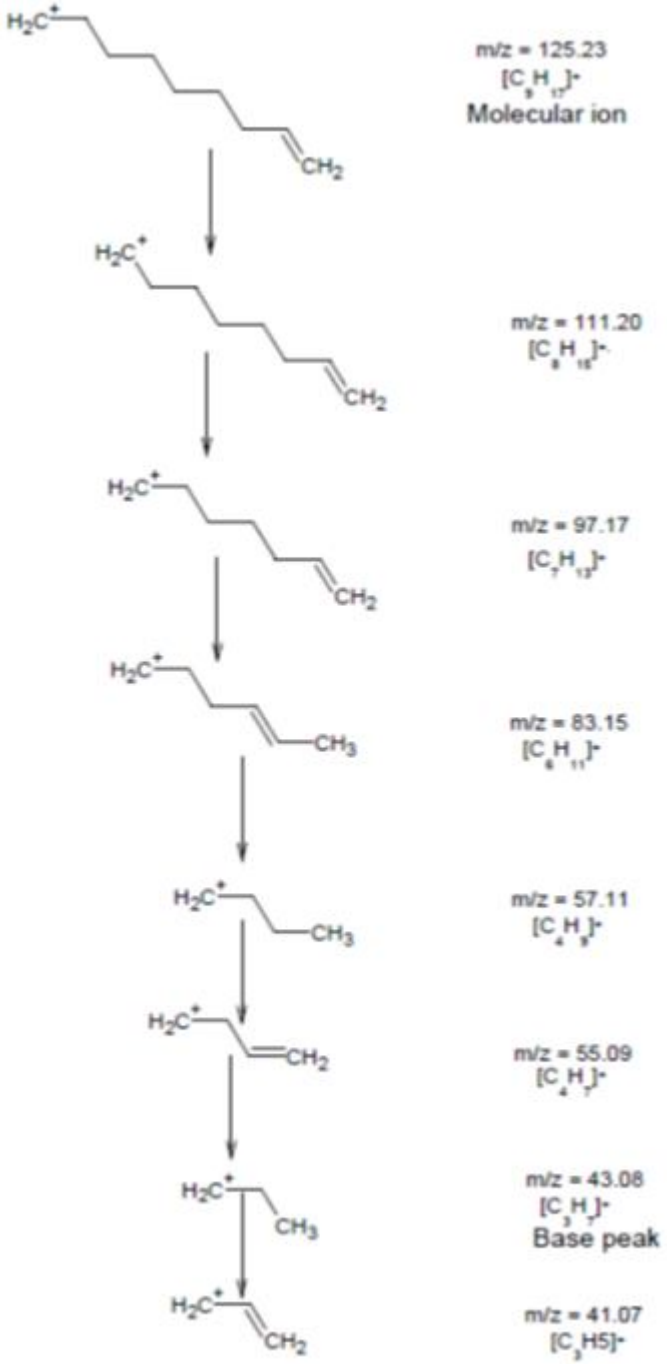

Scheme:19 Fragmentation pattern for Non-1-ene

References

1. Tapsell, L.C., Hemphill, I. and Cobial, L. (2006). Health Benefit of Herbs and Species. The past, the present, the future. *Medical Journal of Austria,* **188**(4):4-24.
2. Gronhaug, T. E., Glaeserud, S., Skogsrud, M., Ballo, N., Bah, S., Diallo, D.and Paulsen, B (2008). Ethnopharmacological survey of six medicinal plants from Mali, West Africa. *Journal of EthnobiologyEthnomedicine,* 4(26): 1-10.
3. Abeloff, A. (2008). Abeloff's Clinical Oncology.4th Edition, Elsevier, Philadelphia, **25**:13-15
4. Swain, E. and Tony, D. (1985). Plants in the development of modem medicine. Harvard University press, UK, 674-680.
5. Aja, P. M., Nwachukwu, N., Ibiam, U. A., Igwenyi, I. O., Offor, C. E and Orji, U. O. (2014). Chemical Constituents of *Moringaoleifera* Leaves and Seeds from Abakaliki, Nigeria, *American Journal of Phytomedicine and Clinical Therapeutics* 2(3):310-321
6. Tabacco , E., Borreani, G. I., Crovetto, G. M., Galassi, G. I., Colombo, D. I., and Cavallarin, I. (2006). Effect of Chestnut Tannin On Fermentation Quality, Proteolysis and Protein Rumen Degradability of Alfalfa Silage. *Journal of Diary Science,***89**(12):4736-4822.
7. Christopher, B. (2003). RHSA-Z Encylopedia of Garden plants, third edition. Dorling Kindersley, London, 738-751.
8. D'Incalci, M., Steward, W.P., and Escher, A.Y. (2005). Use of Cancer Chemo Preventive phytochemical as Anti-neoplastic Agents .*Lancetonocology,***6**(67):899-904.
9. Harborne, J.B. (1973). Text book of Phytochemical Method, 1st Edition, Champmaan and Hall Ltd. London, 110-113.
10. Trease, G. E. and Evans, W. C. (1989). Pharmacognosy, 11th Edition, Macmillian, BrailliaeTiridal Can.
11. Nweke, O. L., Nwachukwu, N., Aja, P. M., Agbafor, K. N., Nwaka, A. C., and R. UchennaEzeilo. (2015). Phytochemical and Gas Chromatograpy-Mass Spectrophotometeric (GC-MS) Analyses of *Vitexdoniana* Leaf from Abakaliki, Ebonyi State, *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS),*10 (5):33-38.
12. Aja, P.M., A.N.C. Okaka, P.N. Onu, U.A. Ibiam and A.J. Uraku, (2010). Phytochemical Composition of *Talinumtriangulare* (Water Leaf) Leaves. Pakistan *Journal of Nutrition*, 9(6): 527-530.
13. Offor, C. E., Ugwu, Okechukwu, P. C., Aja,, P. M. andIgwenyi, I. O. (2015). Proximate and Phytochemical Analyses of *Terminaliacatappa*Leaves, *European Journal of Applied Sciences*, 7 (1): 09-11.
14. Aja, P.M., Alum, E.U., Ezeani, N. N., Nwali, B. U and Edwin, N.(2015). Comparative Phytochemical Composition of *Cajanuscajan*Leaf and Seed,*International Journal of Microbiological Research* 6 (1): 42-46.