

Screening and Evaluation of Bioactive Components of Methanol Root Extract of *Anthocleista nobilis* G. Don. by GCMS Analysis

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Research

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Abstract

Background: Medicinal plants have great importance in African medicine and are also used as precursors in drug discovery. The medicinal value of plants lies in their bioactive constituents which usually allow them to fight against several diseases. Plant-based natural constituents can be derived from any part of the plant like roots, bark, leaves, flowers, fruits, seeds. The present study was designed to determine the bioactive/biochemical compounds present in the methanol extract of *Anthocleista nobilis* root.

Materials and Methods: GC-MS analysis of the methanol extract of *A. nobilis* root was performed using a Perkin Elmer GC Clarus 500 system comprising an Agilent technologies 5975 MSD model detector and a gas chromatograph interfaced to a mass spectrometer with the aid of the Turbo mass 5.0 software.

Results: The study results of the GC-MS analysis provided different phytochemical compounds possessing several biological activities such as antimicrobial, antifungal, anticancer, and anti-inflammatory activities. This study therefore, showed that root of *Anthocleista nobilis* is a source of biologically active metabolites. Furthermore, root extract revealed the presence of diverse chemical constituents.

Conclusion: The results of the present study suggest a recommendation of *A. nobilis* root as a plant of phytopharmaceutical importance.

Background

The use of plants for medicinal purposes dates back to earlier recorded human history. Traditional medicines chiefly containing medicinal plants have always played a vital role as important alternatives to conventional medicines in developing countries. The use of medicinal plants or their products is more popular especially among the poor communities that inhabit rural areas and lack access to health. Alternatively, there has been an enormous increase in the demand of medicinal plants across the globe for their chemical diversity and for the production of newer therapeutic moieties to control various diseases. In spite of tremendous advancement made in the discovery of new synthetic drugs, medicinal plants have still retained their therapy in the literature. Therefore, research on medicinal plants always remained a potential area of investigation (Mahmad et al., 2017). Plant-based natural compounds can be derived from any part of the plant like roots, bark, leaves, flowers, fruits and seeds. Screening for active compounds from plants has led to the invention of new medicinal drugs which have different protection and treatment roles against various diseases (Vijisarah and Subramaman, 2014). The modern method describing the identification and quantification of active constituents in plants material may be useful for proper standardization of herbal and its formulation. Use of plants as a source of medicine has been inherited and is an important component of the health care, and as well, becomes the source of many potent and powerful drugs (Kanthal et al., 2014). Many years ago, people around the globe have healed the sick with herbal derived remedies, and handed down through generation among the indigenous populations (Thomas et al., 2013).

The medicinal values of plants, *Anthocleista nobilis* for instance, lies in the bioactive phytochemical constituents that are non-nutritive chemicals that produce definite physiological effects on human body and protect them from various diseases. Plants defend themselves from pathogens and other herbivore enemies by elaborating a variety of bioactive secondary metabolites that may have multiple molecular sites of action. Accordingly, exploitation of these useful plants has spread rapidly to safeguard increasing population from various pathogens and ailments (Arora et al., 2017). Phytochemicals are protective and disease preventing particularly for some forms of cancer and heart disease (Lakshmi et al., 2011). For thousands of years, people all over the world have used medicinal plants as base in making traditional medicines and had given great advantages to mankind to come up with new remedies (Krishnaiah et al., 2011). Medicinal plants contain bioactive compounds, for instance, saponins, tannins, flavonoids, essential oils etc. These phytochemicals are produced as a result of normal metabolic activities of plants and are also known as secondary metabolites (Meskin et al., 2002). These are the originator of medicinal properties within plants, as exemplification, antimicrobial (Sachidananda et al., 2015), antioxidant (Wu et al., 2004) and most importantly antidiabetic (Firdous, 2014). It is generally believed that the medicinal value of plants is due to some plant secondary metabolites that produce certain physiological action in human body. The plant secondary metabolites are important for the human consumption as food and used in the pharmaceutical industry for required special attention (Hill, 1952). Many plants are good sources of antioxidants and other bioactive compounds containing phenolics, alkaloids, amino acids, ascorbic acid etc. Due to increasing demand, seeking therapeutic drugs from plants has grown tremendously. Such preparations contain various bioactive compounds of high therapeutic value and becoming popular in the area of medicine for their less expensive and less side effects etc., compared to modern allopathic drugs.

The traditional medicines in the last few decades emerged to have immense acknowledgements and it is estimated that 80% of community depend on traditional medicine for their primary healthcare (Arora et al., 2017). Traditional medicines are not only contributing to primary health care, but also in the development of modern drugs (Vedarathy, 2003). Various types of traditional medicine and other medical practices referred to as complementary or alternative medicine are increasingly used in both developing and developed countries. Countries like India and China are popularly known when it comes to traditional medicines because they believe them to be safer, more effective and inexpensive (Katewa et al., 2004). Ayurveda stresses the use of plant-based medicine and treatments. But when compared, the Chinese medicine is more established than Ayurvedic medicine. This is due to even after Chinese people migrating to other countries they still follow their own culture. And also the Chinese people wherever in the world are actively participating in export and import of their medical system (Aneesh et al., 2009). It is a sad fact that nowadays we are moving away from nature and due to our undisciplined life style, new diseases are being identified. But the fact is that our rich nature contains remedy for all diseases. Potentially valuable treasures in medicinal plants remain unexplored. By considering the scope of these medicinal plants we have to use more amounts of time and resources into developing medicines by medicinal plants. If we can come back to our nature, culture and tradition on use of medicinal plants, it can therefore, bring up a bright and healthy new generation (Aneesh et al., 2009). In Africa too, there is an abundance of natural

resources such as plants and therefore, the indigenous society are inseparable from the natural environment. The ethnic groups are utilizing their traditional knowledge and experience in inheriting them to their younger generations in order to treat ailments; and their daily lives are depended on nature and this has influenced and helped them in forming traditional knowledge (Halim et al., 2013). Extraction is the main step for the recovery and isolation of bioactive components from plant parts. The analysis and extraction of plant matrices play an important role in the development, modernization and quality control of herbal formulation. Therefore, the extraction of bioactive compounds from plants for therapeutic targets also needs active principle to be identified (Arora et al., 2017).

Gas chromatography-mass spectrometry (GC-MS) is used as a technique that serves a broad range of applications aimed at sample identification, quantitative determination or both. The sample identification (qualitative analysis) needs a high degree of selectivity whereas quantitative analysis requires high accuracy (the precision and trueness) (Fialkov et al., 2006). GC-MS is one of the valuable tool for the identification of phytochemical compounds. It combines two analytical techniques to a single method of analyzing mixtures of chemical compounds. Gas chromatography separates the components of the mixture and mass spectroscopy analyses each of the components separately. It is the best technique to identify the bioactive compounds/constituents of long chain hydrocarbon, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc (Azhagumurugan and Rajan, 2014; Baskaran et al., 2016). The first step in investigating the presence of metabolites in any medicinal plants is by phytochemical screening that gives a broad idea on the nature of chemical constituents (Konig and Hochmuth, 2004). To identify the compound, processing data from GC-MS must fulfil two criteria which includes; correct determination of mass spectrum of individual compounds; and accurate calculation of the abundance of chromatographic peaks corresponding to those compounds in each sample. Moreover, for sample introduction into GC-MS, there are three considerations. Firstly, the constituent of the sample must be volatile and secondly the analytes must be present at concentration which is appropriate to it. Thirdly, while injecting the sample, the sample must not degrade the separation (Fialkov et al., 2006).

Anthocleista nobilis (G. Don.) belonging to the family *Loganiaceae*, is a small to medium-sized tree growing to 30-m tall. It is commonly found in tropical African habitats such as the Mascarene Islands and Madagascar as well as Southern, Western, and Eastern part of Nigeria. Also, it is a ubiquitous flora found densely grown everywhere in the forest, farmland, swampy area of land, roadsides among others in tropical rain forest. The bark is smooth and pale grey. The inner bark is cream-yellow and granular, whereas the twig has two spines above the leaf axis. The leaves are simple, broad and opposite, crowded at the end of branches, and petiole is 1–6 cm long. It is a photoautotroph. It exhibits tap root system, and the root can be erect, bend or curve. It is commonly called Candelabrum, Cabbage tree, Cabbage palm, or Palma christi in English language. It is also locally known as Uko nkirisi in Igbo language, Apa-Ora in Yoruba language, Kwari in Hausa language, Ogugu in Ilaje/Ikale language, and Duwa kuchi in Nupe language (Dokosi, 1998; Ayodele et al., 2013). Conventionally, *A. nobilis* is used in the treatment of fever, stomach ache, diarrhea, and gonorrhoea. It is also used as strong purgative, diuretic, and as poultice for treating sores in parts of West Africa (Madubunyi and Asuzu, 1998). It is used as vapour bath for the treatment of leprosy, venereal diseases, and dysmenorrhoea. Its root decoction is usually taken to regulate

menstruation and also as an abortifacient. In Mbanjo community in Imo State, Nigeria, the root bark decoctions are mostly used in the treatment of diabetes mellitus, gastrointestinal worms, malaria, and jaundice (Madubunyi and Asuzu, 1998), while in Ilaje and Ikale communities in Ondo State, Nigeria, the root tinctures are mostly used as antioxidant, and in the treatment rheumatism and arthritis. Furthermore, it is used in local medicine in parts of West Africa for curing fever, arthritis, stomach ache, diarrhea, and gonorrhoea, and are also as poultice for sores (Irvine, 1961). The source material, *Anthocleista nobilis*, is a plant of strong medicinal properties but so far there is no enough research performed on determination of the chemical composition of its root in literature. Hence, in continuation to the on-going project on the survey of medicinal plants, I herein report, the GC-MS based chemical profiling of the crude extract from the root of *A. nobilis* with the aim of confirming the ethnomedicinal use of the plant.

Materials And Methods

Chemicals, reagents and instruments

Hexane, chloroform, ethyl acetate, methanol, syringe, Whatman's syringe filter and rotary evaporator were obtained from Sigma-Aldrich. All chemicals were of analytical grade and used without any further purification. The GC-MS analysis was conducted on a Perkin Elmer Clarus 500, GC-MS spectrometer equipped with vF-5 MS fused silica capillary column of 30 m x 0.25 i.d and 0.25 µm film thickness.

Plant material and sample collection

The roots of *Anthocleista nobilis* were collected in November, 2019 within the premises of Ondo State University of Science and Technology (OSUSTECH), Okitipupa, Nigeria. They were identified by the herbarium Curator, Botany Programme of the Department of Biological Sciences, Faculty of Science, OSUSTECH.

Preparation of plant material

The fresh roots of *Anthocleista nobilis* were detached from the whole uprooted plant, rinsed in water and spread on laboratory tables where they were dried under room temperature. The plant material were then transferred to an oven set at 40 °C for 5-10 minutes before been reduced to fine powder with the aid of a mechanical grinder.

Preparation of extract

200 g of the powdered plant material was macerated in 1 litre of methanol for 48 hours. The mixture was sieved using porcelain cloth and was further filtered using No.1 Whatman filter paper. The filtrate was concentrated using rotary evaporator and the crude concentrate was then stored at 4 °C until required for further experiment.

GC-MS analysis of crude extract

The sample of *Anthocleista nobilis* roots was prepared and diluted using methanol. 2 mL of crude sample was suctioned using syringe and filtered by using Whatman's syringe filter (0.2 µm) and transferred into glass vials. Then, 1 µL of distilled sample was analysed by injecting into GC-MS with a split injector at 300 °C. The vF – 5 MS fused silica capillary column (30 m x 0.25 mm x 0.25 µm) was employed. The temperature programme was 50 °C, held for 10 minutes, increased at 3 °C/minutes for 250 °C and finally hold for 10 minutes. Inert helium gas was employed as a carrier gas at a constant flow rate of 1.0 mL/minutes.

Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standards and Technology (NIST) having more than 62,000 patterns of the spectrum of the known components stored in the NIST library. The compound were identified by comparison of their retention indices (RI) with those provided in NIST library. Identification was assumed when a good match of RI was achieved (Khan *et al.*, 2016).

Results And Discussion

The total ion chromatogram (TIC) of methanol root extract of *Anthocleista nobilis* showing the GC-MS profile of the compounds identified is given in Fig. 1. The chromatogram showed twenty three (23) peaks revealing twenty seven (27) different compounds of which eleven (11) compounds were unknown and sixteen (16) compounds were known for its medicinal properties. The compounds were identified based on the mass fragmentation pattern and comparing the peak area and retention time of the NIST database. The chemical composition of the crude methanol extract is summarized in Table 1, with the most abundant compound being 5-Hydroxymethylfurfural (11.11%) used as Antioxidant agent (Shapla *et al.*, 2018), followed by 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (7.41%) used as Antimicrobial, anti-inflammatory, antioxidant agent (Padmashree *et al.*, 2018), and Heptanoic acid (7.41%) of unknown medicinal property (Table 1). The detailed mass spectra of the twenty three compounds identified by GC-MS analysis of *A. nobilis* root are shown in Figs. 2–24. On comparison of the mass spectra of constituents with the main library, all these compounds were characterized and identified. Also, the comparison of the compound's mass spectra with the data base gave more than 100% match as well as confirmatory compound structure match.

Mass spectrometry becomes a vital tool in the hands of the organic chemists and biochemists because of its potential to supply the definitive, qualitative and quantitative information on molecules based on their structural compositions. Gas chromatography is attached to a Mass Spectrometer (GC-MS) enables mixture of small molecules mainly organic compounds of low molecular weight (< 600) which can be analysed. Plants produce diverse phytochemicals known as secondary metabolites. It is well known that plants produce these metabolites to protect themselves from pathogenic attacks. The structures of the various phytochemicals which contribute to the medicinal activity of the plant methanol extract of *Anthocleita nobilis* are shown in Fig. 2–24.

The available literature supports that the identified compounds (secondary metabolites) of *Anthocleista nobilis* root possess several biological activities such as antimicrobial, antifungal, anticancer, and anti-inflammatory activities (Salido et al., 2016; Thepa *et al.*, 2017). Owing to the biological activities of the plant-derived metabolites, as observed in the methanol extract of *Anthocleista nobilis* root, many source material of plant origin have become a field of great scientific interest, and further study of these phytoconstituents may prove the medicinal importance in future.

Six compounds were used as flavouring agent such as 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one; 2-propenoic acid, 2-methyl-,2[[2[(1,3 dioxobutyl)amino] ethoxy]carbonyl]amino]ethyl ester; 9,12-Octadecadienoic acid, methyl ester; 9-Octadecenoic acid; Octadecanoic acid; Carbonic acid, hexadecyl prop-1-en-2-yl ester.

Four compounds were used as antibacterial, antimicrobial and antibiotic agent such as 2-propenoic acid, 2-methyl-,2[[2[(1,3 dioxobutyl)amino] ethoxy]carbonyl]amino]ethyl ester; 4H-Pyran-4-one,2,3-dihydro-3,5 dihydroxy-6-methyl-; 2,4-Hexadiyne-1,6-diol; 9,12-Octadecadienoic acid (Z,Z)-.

Four compounds have anti-inflammatory properties such as 4H-Pyran-4-one, 2,3-dihydro-3,5 dihydroxy-6-methyl-; n-Hexadecanoic acid; 9,12-Octadecadienoic acid, methyl ester; 9-Octadecenoic acid.

Also, four compounds were found to have antioxidant properties, in that compounds such as 4H-Pyran-4-one, 2,3-dihydro-3,5 dihydroxy-6-methyl-; 5-Hydroxymethylfurfural; n-Hexadecanoic acid; Tetradecane, 1-bromo-.

Two compounds were used as antiacne, cosmetic and perfumery agent such as 9,12-Octadecadienoic acid (Z,Z)-; Octadecanoic acid.

One compound was used as preventive against cardiovascular disease such as 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-. (Table 1; Figs. 2–24).

From the information provided so far; it can be inferred that natural bioactive phytocompounds have been suggested as alternative sources for antibacterial, antimicrobial, anti-inflammatory, antioxidant, antiacne, antioxidant, antibiotics etc. The chemical features of these constituents differ considerably among different species. This approach is alluring, in part, because they constitute a potential source of bioactive compounds that have been professed by the general public as comparatively safe and often act at multiple and novel target sites, thereby increasing the potential for resistance.

Table 1: Gas chromatography-mass spectrometric profile of methanol extract of *A. nobilis* root

Sr.no.	Chemical compounds	Molecular formula	Molecular weight (g/mol)	Retention time (min.)	Bioactivities
1	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C ₆ H ₈ O ₄	144.12	4.034	Food-grade flavour ingredient (Padmashree <i>et al.</i> , 2018)
2	2-propenoic acid, 2-methyl-2[[2[(1,3 dioxobutyl)amino] ethoxy]carbonyl]amino]ethyl ester	C ₁₃ H ₂₀ N ₂ O ₆	300.31	5.236	Flavouring agent, antibacterial (Padmashree <i>et al.</i> , 2018)
3	Furazanamine,4-azido-.delta	C ₂ H ₃ N ₆ O	127.09	5.491	Unknown
4	4H-Pyran-4-one,2,3-dihydro-3,5 dihydroxy-6-methyl-	C ₆ H ₈ O ₄	144.12	6.227	Antimicrobial, anti-inflammatory and antioxidant capacity (Padmashree <i>et al.</i> , 2018)
5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C ₆ H ₈ O ₄	144.12	6.339	Same as sr./no. 4
6	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126.11	7.485	Antioxidant (Shapla <i>et al.</i> , 2018)
7	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126.11	7.523	Same as sr./no. 6
8	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126.11	7.552	Same as sr./no. 6
9	Ethanone,1-(2-hydroxy-4 methoxyphenyl)-	C ₉ H ₁₀ O ₃	166.17	9.529	Unknown
10	Heptanoic acid	C ₇ H ₁₄ O ₂	130.18	11.118	Unknown
11	Heptanoic acid	C ₇ H ₁₄ O ₂	130.18	11.171	Unknown
12	Preg-4-en-3-one,17.alpha.-hydroxy -17.beta.-cyano-	C ₂₁ H ₃₂ O ₂	316.5	11.893	Unknown
13	2,4-Hexadiyne-1,6-diol	C ₆ H ₆ O ₂	110.11	12.248	Antibiotic (Padmashree <i>et al.</i> , 2018)
14	Benzenemethanol,3 methoxy-4-nitro	C ₈ H ₉ NO ₄	183.16	12.754	Unknown
15	Adenosine,4'-de(hydroxymethyl)-4'-[N-ethylaminoformyl]-	C ₂₀ H ₂₂ N ₆ O ₆	442.4	13.100	Unknown
16	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	14.899	Antioxidant, anti-inflammatory (Rai <i>et al.</i> , 2016)
17	9,12-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294.5	15.916	Anti-inflammatory (Baskaran <i>et al.</i> , 2016)

Table 1: Continued

Sr.no.	Chemical compounds	Molecular formula	Molecular weight (g/mol)	Retention time (min.)	Bioactivities
18	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280.4	16.308	Antimicrobial, antiacne (Padmashree <i>et al.</i> , 2018)
19	9-Octadecadienoic acid	C ₁₈ H ₃₄ O ₂	282.5	16.362	Anti-inflammatory (Rai <i>et al.</i> , 2016)
20	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.5	16.528	Cosmetic, flavouring agent, perfumery (Padmashree <i>et al.</i> , 2018)
21	9,12,15-Octadecatrienoic acid, (Z, Z,Z)-	C ₁₈ H ₃₀ O ₂	278.4	17.269	Preventive against cardiovascular disease (Arora <i>et al.</i> , 2017)
22	cis-3-Butyl-4-vinyl-cyclopentene	C ₁₁ H ₁₈	150.26	17.607	Unknown
23	Carbonic acid, hexadecyl prop-1-en-2-yl ester	C ₂₀ H ₃₈ O ₃	326.5	19.289	Flavouring agent (Rai <i>et al.</i> , 2016)
24	Ethanol, 2-(octadecyloxy)-	C ₂₀ H ₄₂ O ₂	314.5	19.578	Unknown
25	2-Chloropropionic acid, hexadecyl ester	C ₁₉ H ₃₇ ClO ₂	332.9	19.628	Unknown
26	Cyclopropanecarboxylic acid, 3-(2, 2-dichloroethenyl)-2,2-dimethyl,(3phenoxyphenyl)methyl ester,trans-	C ₂₁ H ₂₀ Cl ₂ O ₃	391.3	19.701	Unknown
27	Tetradecane, 1-bromo-	C ₁₄ H ₂₉ Br	277.28	20.110	Antioxidant (Rai <i>et al.</i> , 2016)

Conclusions

The correlation among the phytochemical constituents with their biological activities is now being the matter of innovative thought. *A. nobilis* is a plant, of which its root is traditionally used for the treatment of rheumatism, arthritis etc. But till date, there are few or no reports on chromatographic analysis of extract of the plant's root, to the best of my knowledge. Here I report the presence of some important compounds in this plant isolated by GC-MS analysis. Thus, this type of study may give information on nature of active principles present in the medicinal plant. These phytoconstituents presumed to be responsible for eliciting the traditional activity of this plant, *Anthocleista nobilis*, and its extract is a good biochemical agent that could be added as a chemical basis in therapeutics.

Abbreviations

Anthocleista nobilis: *A. nobilis*; GC: gas chromatography; MS: mass spectrometry; OSUSTECH: ondo state university of science and technology.

Declarations

Authors' contribution

The design of the research and drafting of manuscript was done by Adekunle O. Ojatula.

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Competing interests

Author declare that he has no competing interest

Availability of data and materials

All data that are relevant to the study are reported within the article.

Consent for publication

The author approved the consent for publishing the manuscript.

Ethics approval and consent to participate

The author has read and agreed the ethics for publishing the manuscript.

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Figures

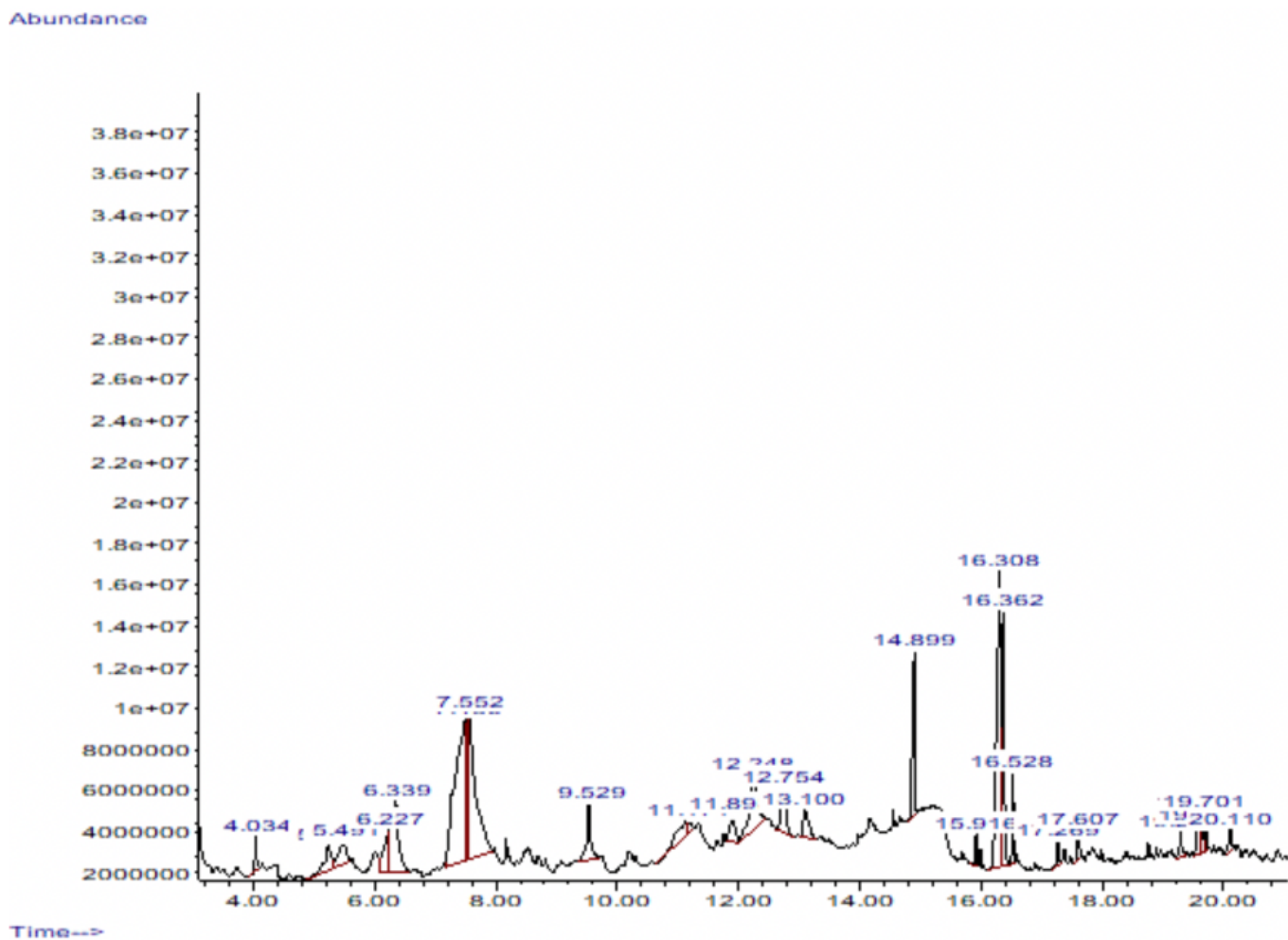


Figure 1

Total Ion Chromatogram (TIC) of methanol extract of *Anthocleista nobilis* root

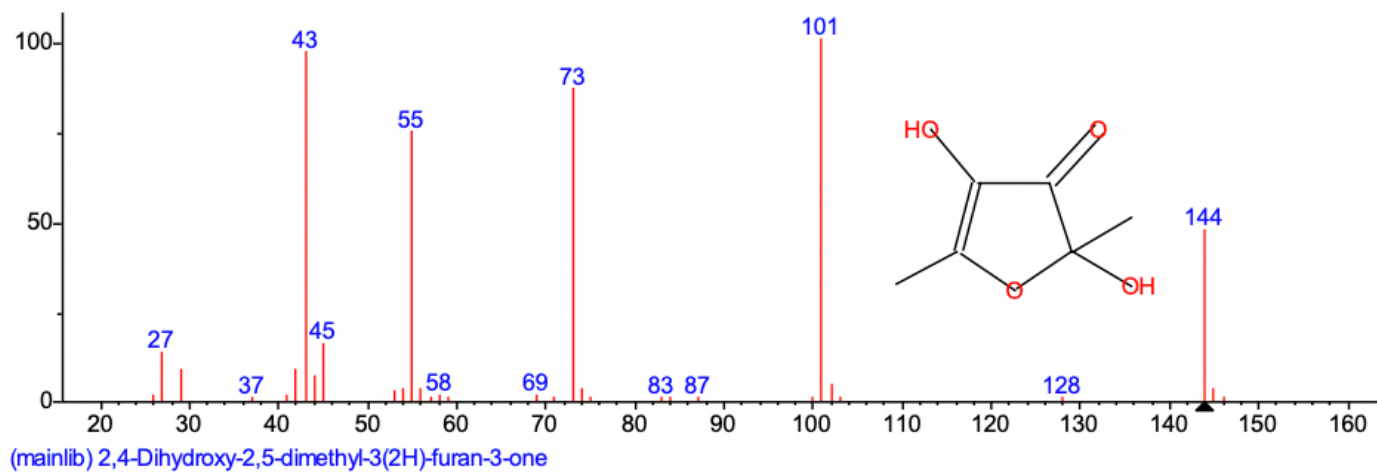


Figure 2

GC-MS spectrum of 2,4-Dihydroxyl-2,5-dimethyl-3(2H)-furan-3-one

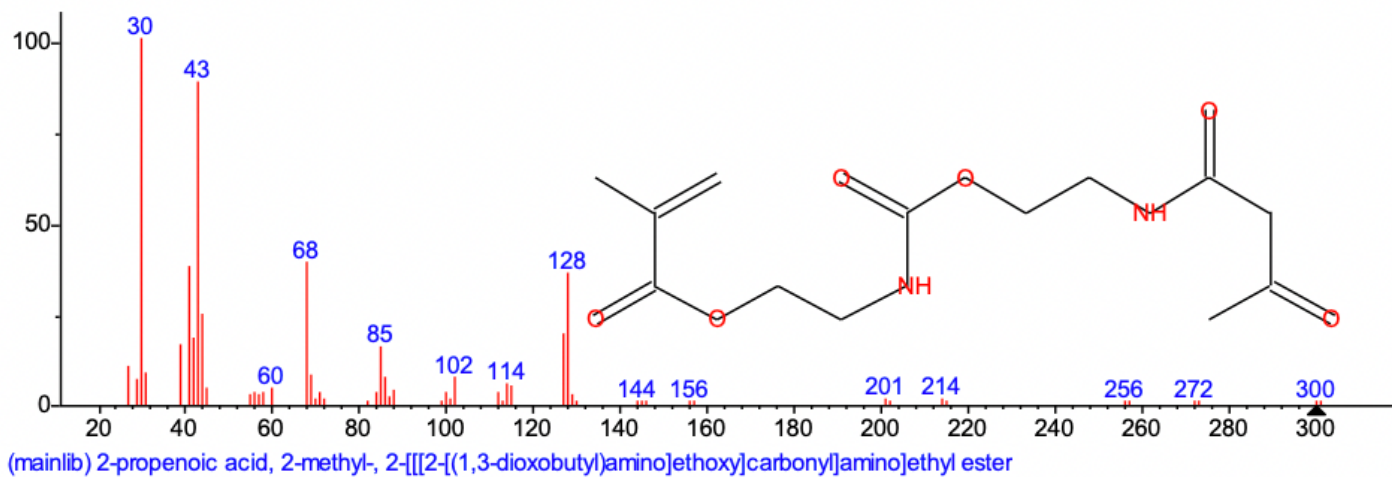


Figure 3

GC-MS spectrum of 2-propenoic add, 2-methyl-, 2[[[2-[(1,3-dioxobutyl)amino]ethoxy]carbonyl]amino]ethyl ester

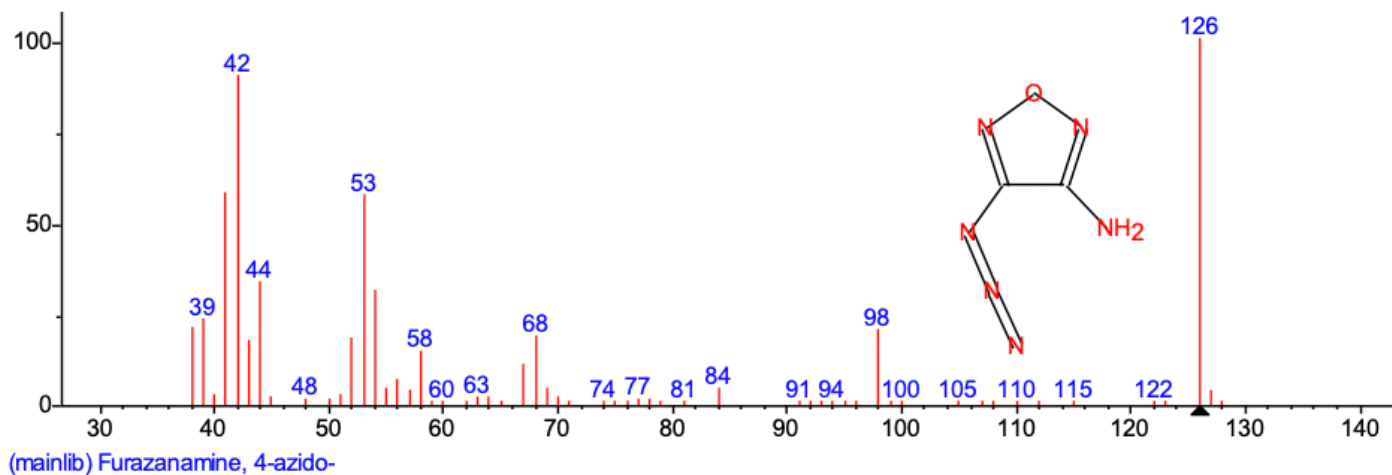


Figure 4

GC-MS spectrum of Furazanamine, 4-azido-

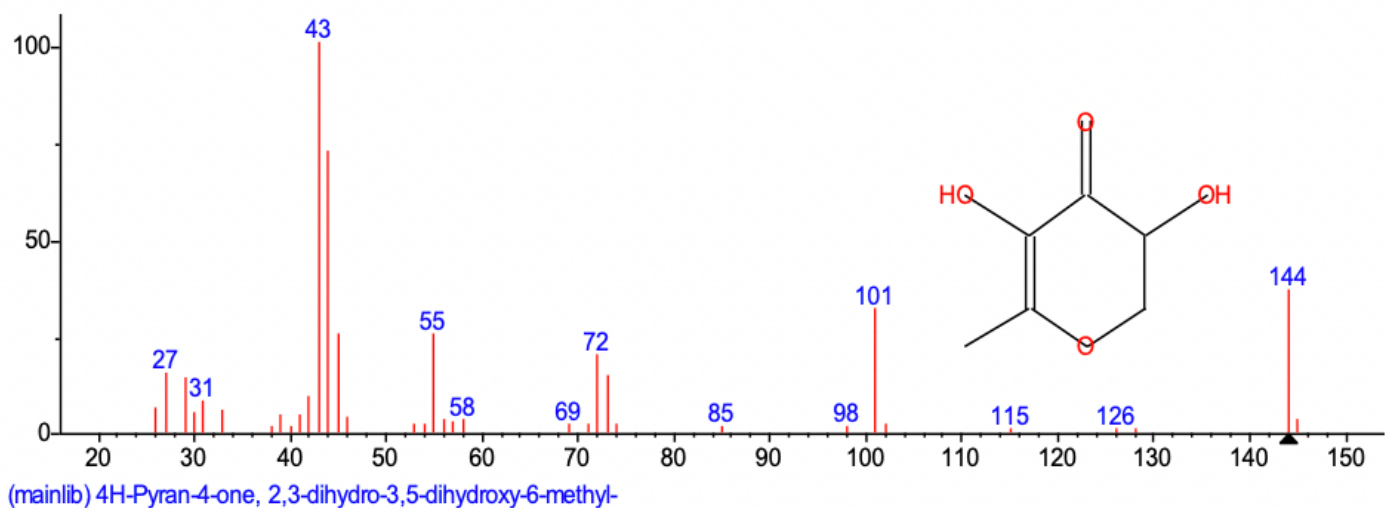


Figure 5

GC-MS spectrum of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl

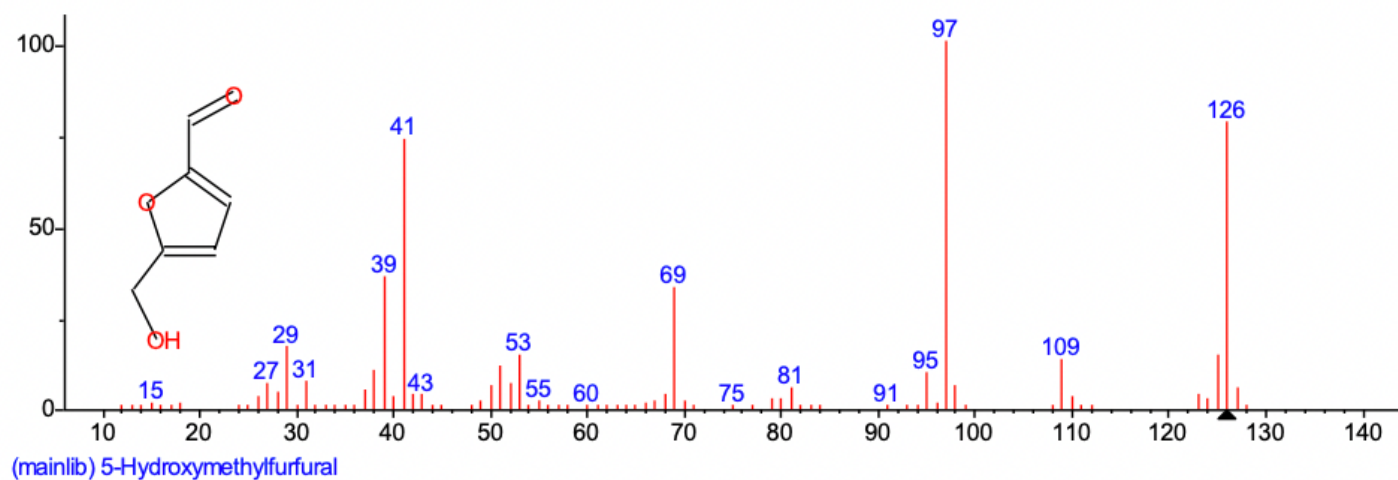


Figure 6

GC-MS spectrum of 5-Hydroxymethylfurfural

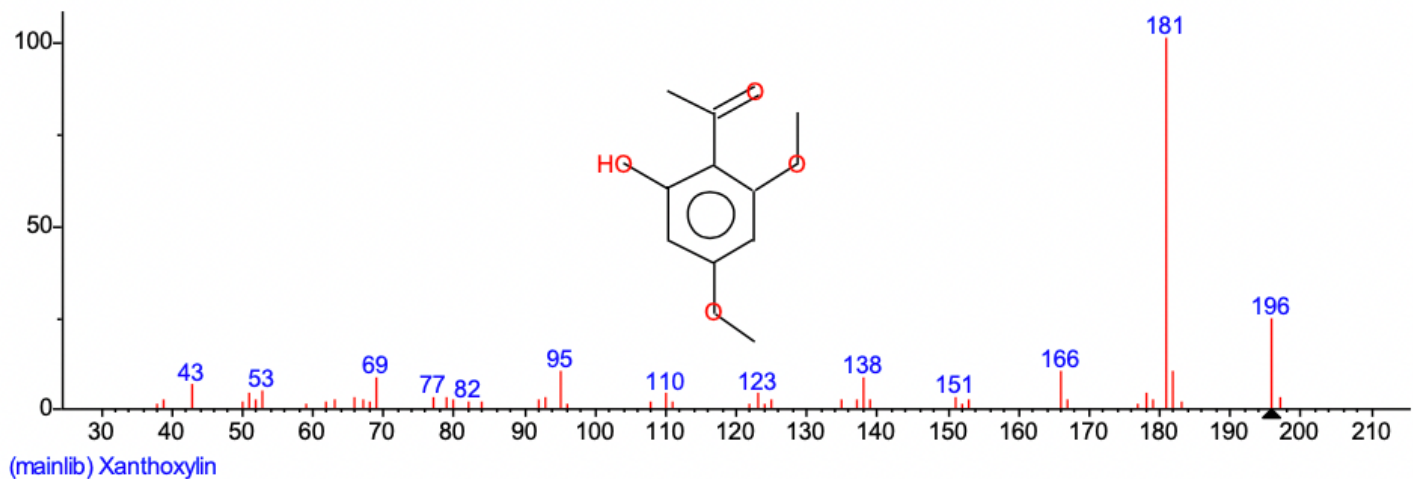


Figure 7

GC-MS spectrum of Xanthoxylin

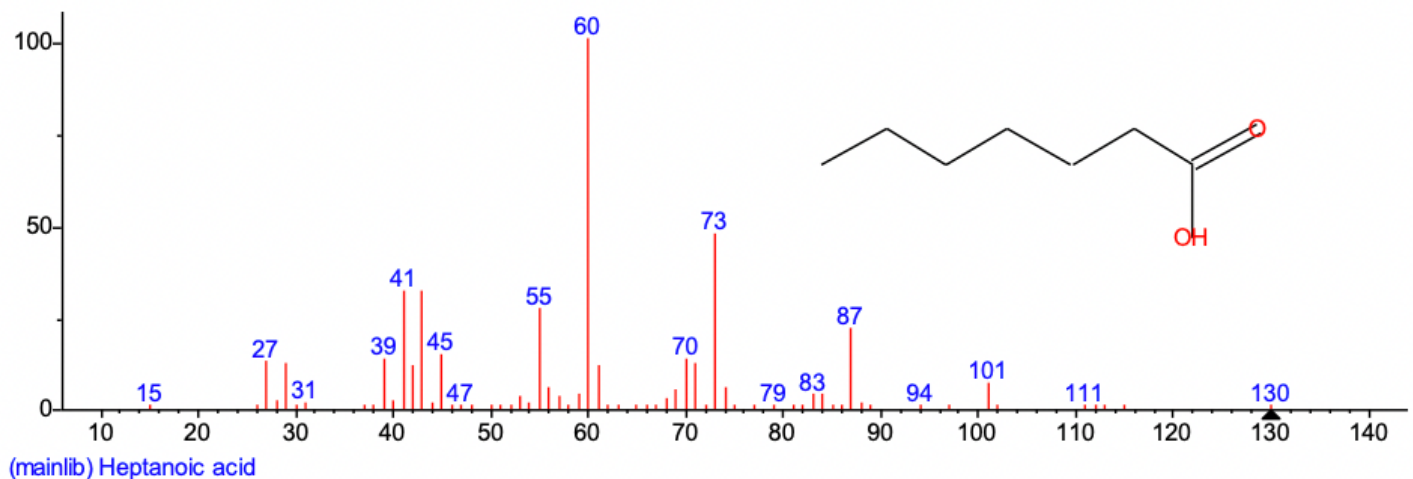


Figure 8

GC-MS spectrum of Heptanoic acid

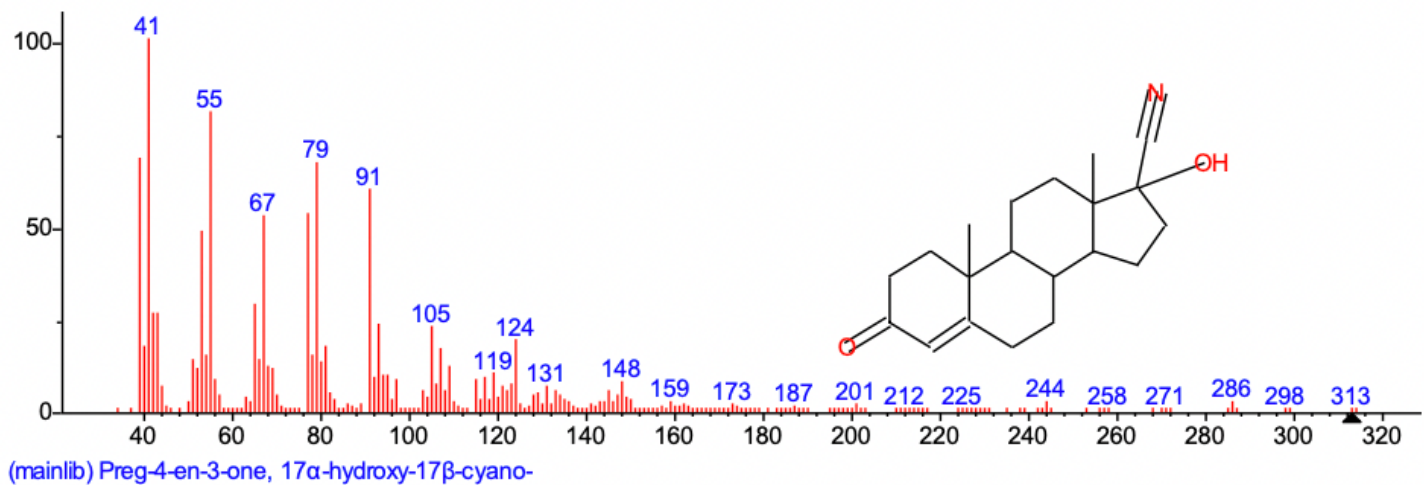


Figure 9

GC-MS spectrum of Preg-4-en-3-one, 17 α -hydroxy-17 β -cyano-

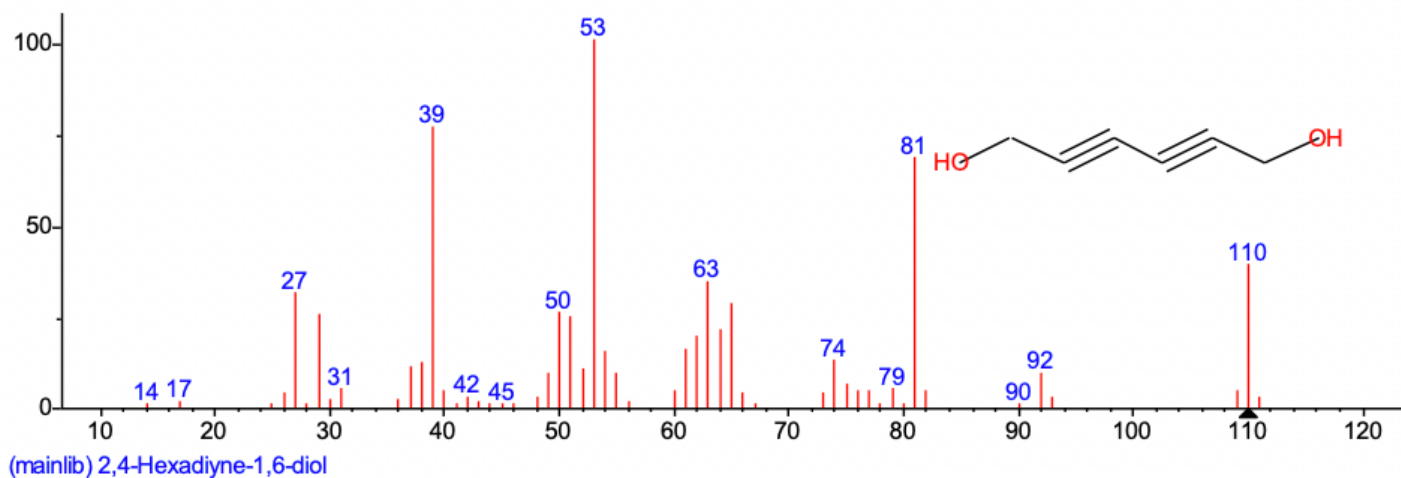


Figure 10

GC-MS spectrum of 2,4-Hyxadiyne-1,6-diol

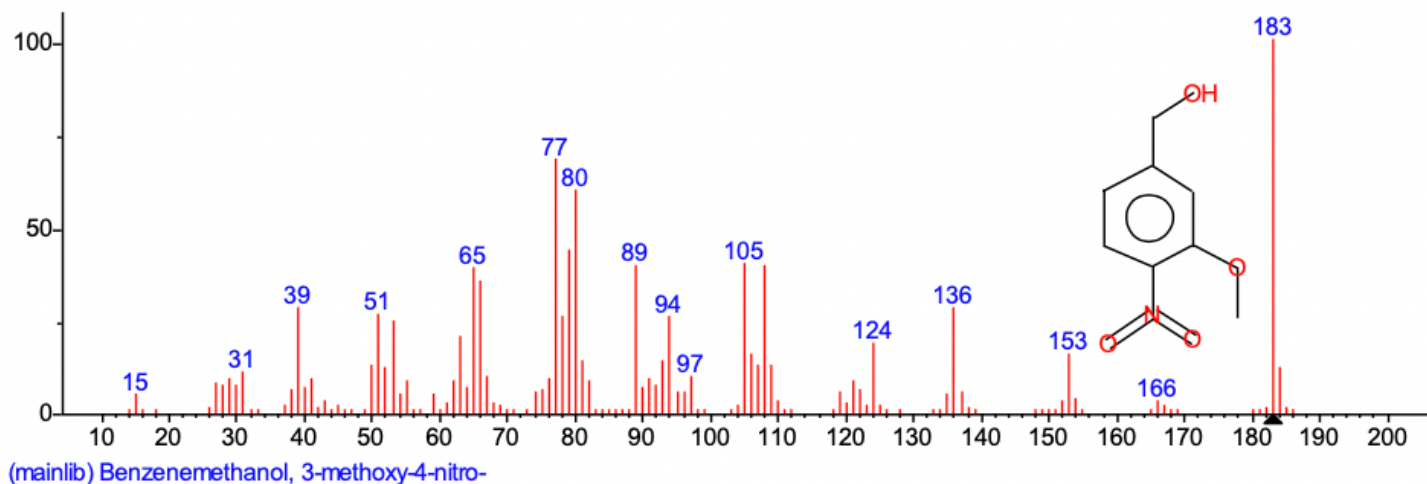


Figure 11

GC-MS spectrum of Benzenemethanol, 3-methoxy-4-nitro-

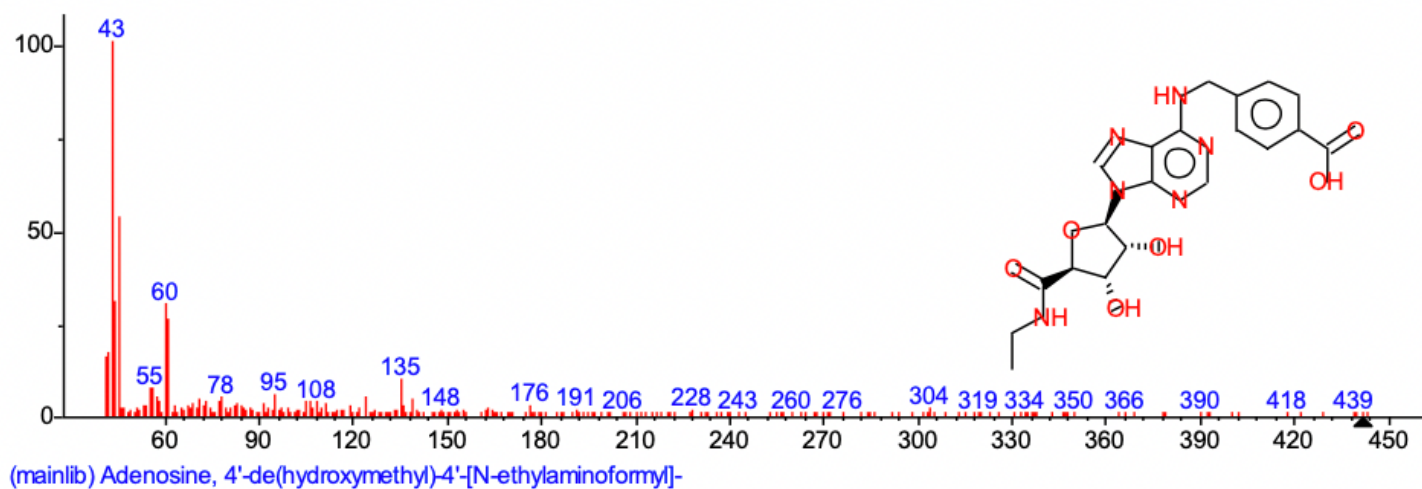


Figure 12

GC-MS spectrum of Adenosine, 4'-de(hydroxymethyl)-4'-[N-ethylaminofomyl]-

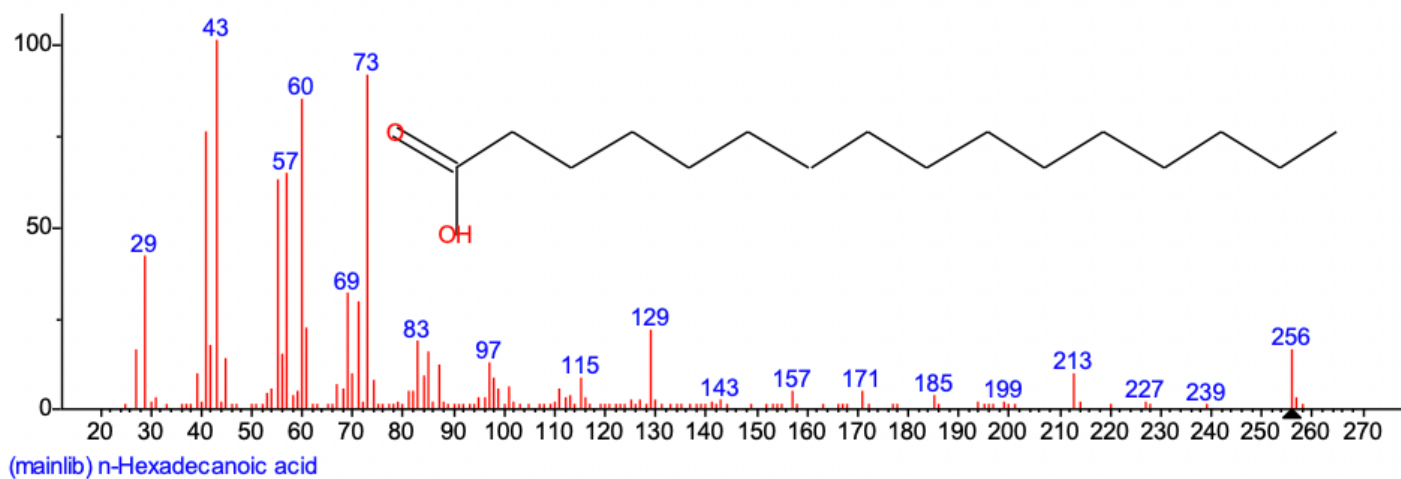


Figure 13

GC-MS spectrum of n-Hexadecanoic acid

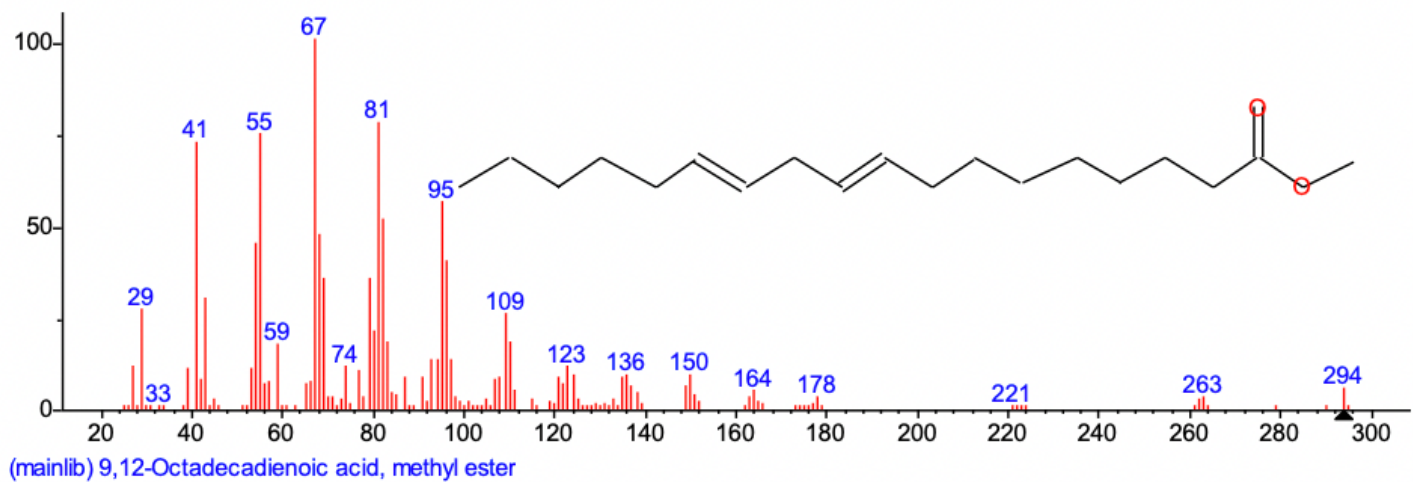


Figure 14

GC-MS spectrum of 9,12-Octadecadienoic, methyl ester

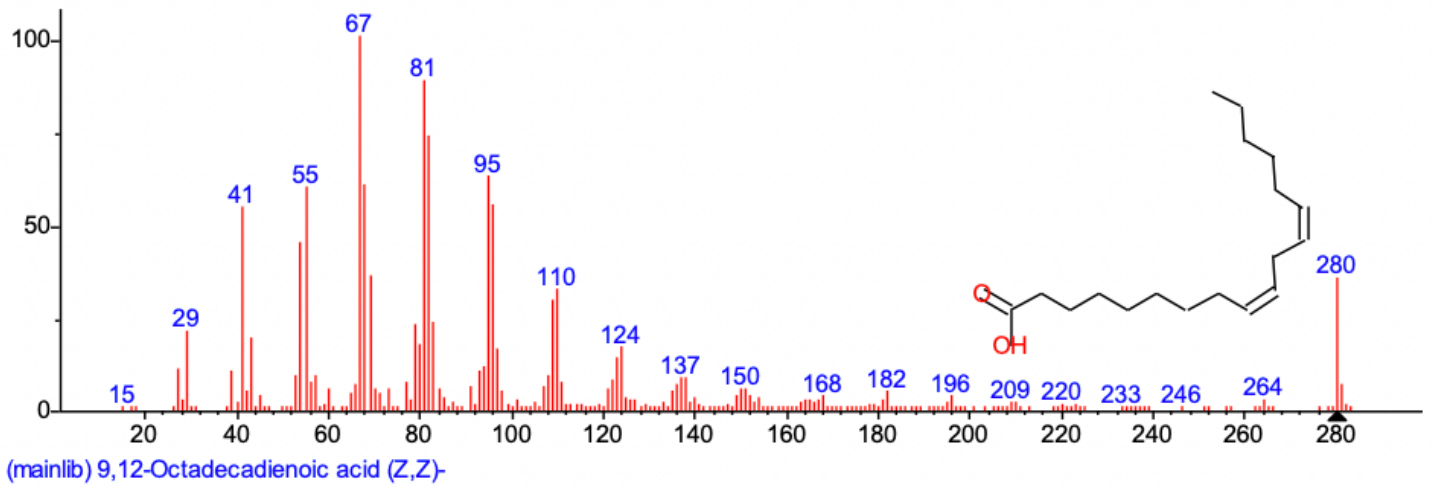


Figure 15

GC-MS spectrum of 9,12-Octadecadienoic acid (Z,Z)-

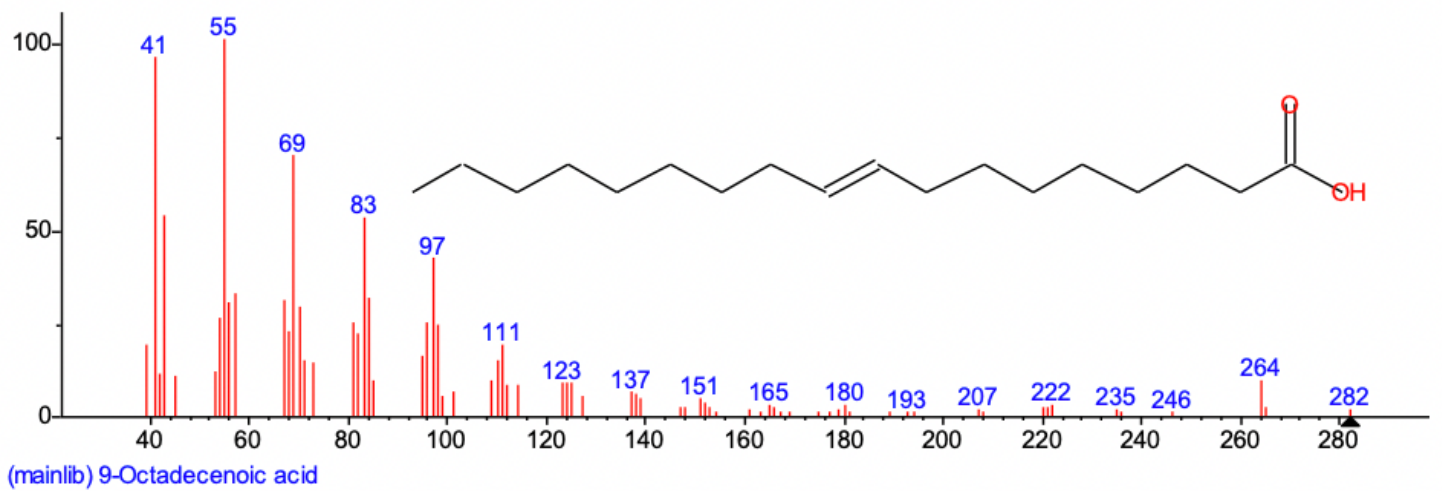


Figure 16

GC-MS spectrum of 9-Octadecenoic acid

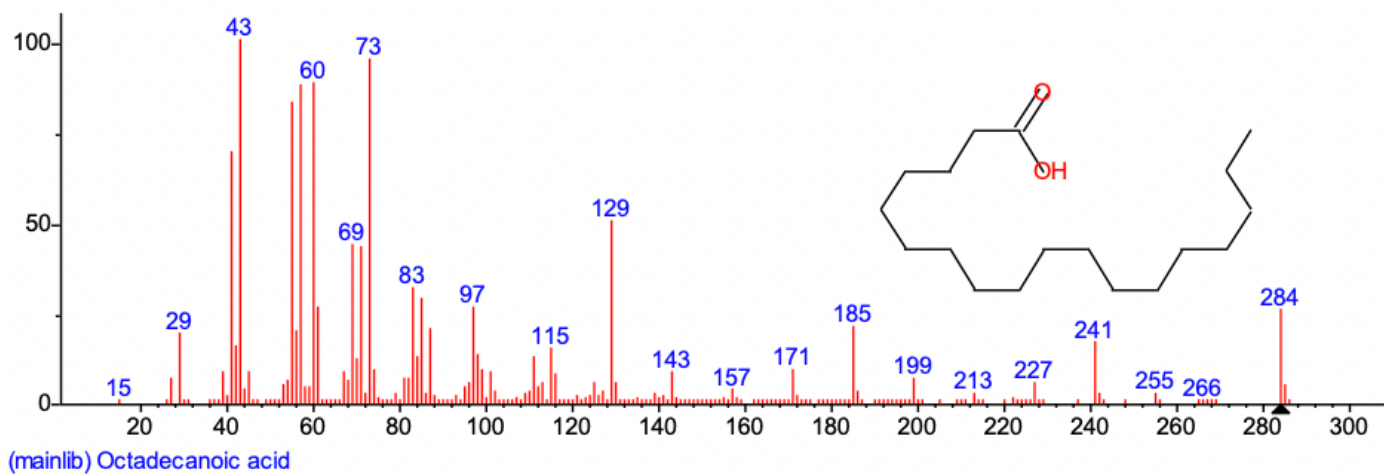


Figure 17

GC-MS spectrum of Octadecanoic acid

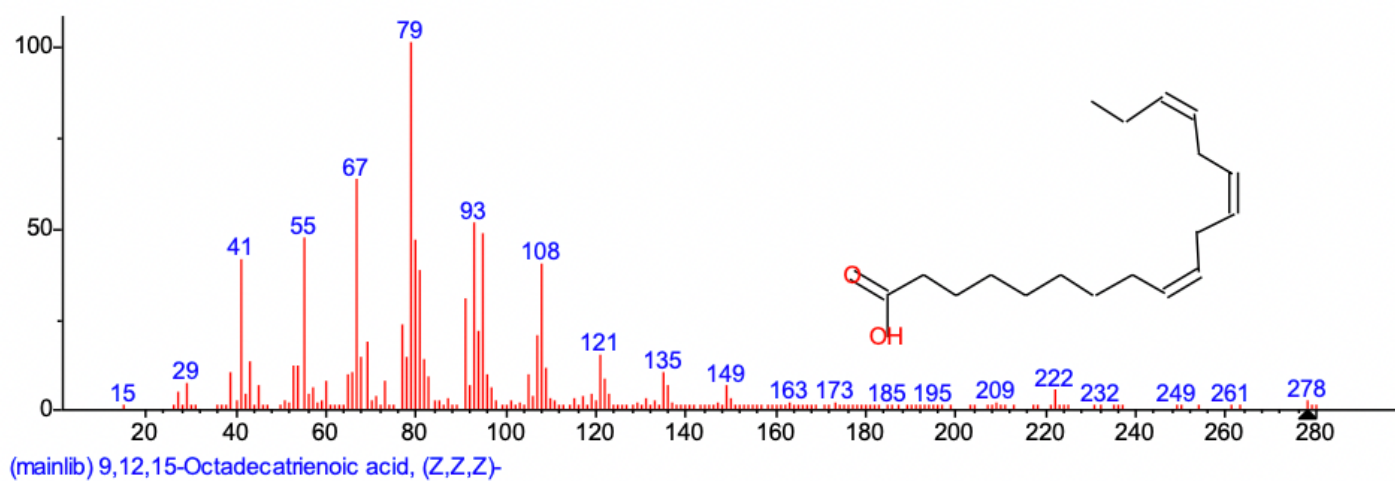


Figure 18

GC-MS spectrum of 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-

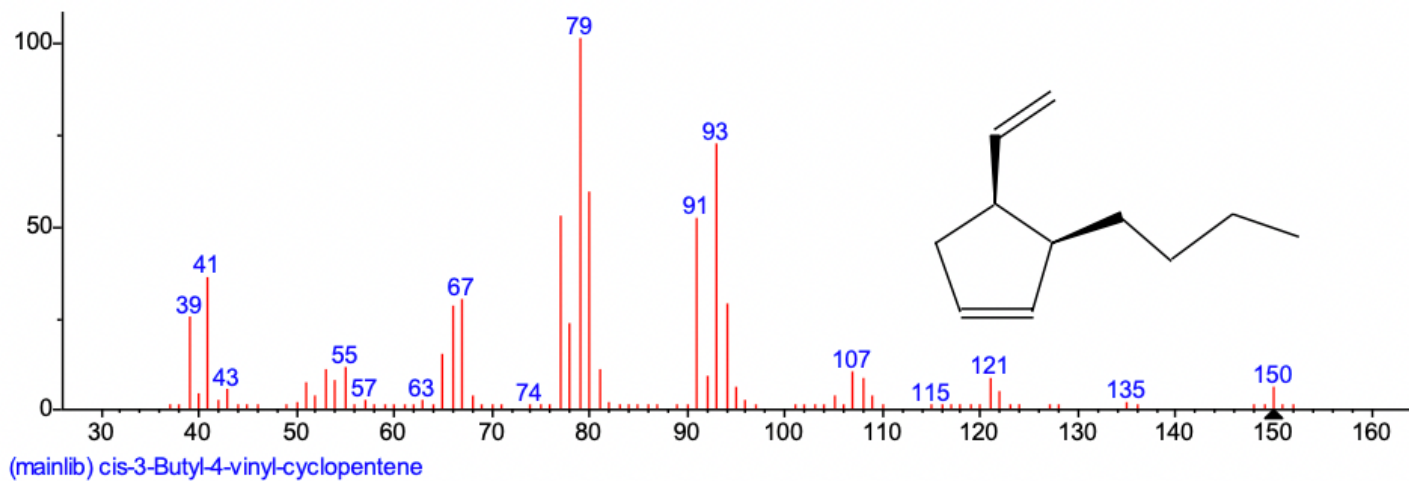


Figure 19

GC-MS spectrum of cis-3-Butyl-4-vinyl-cyclopentene

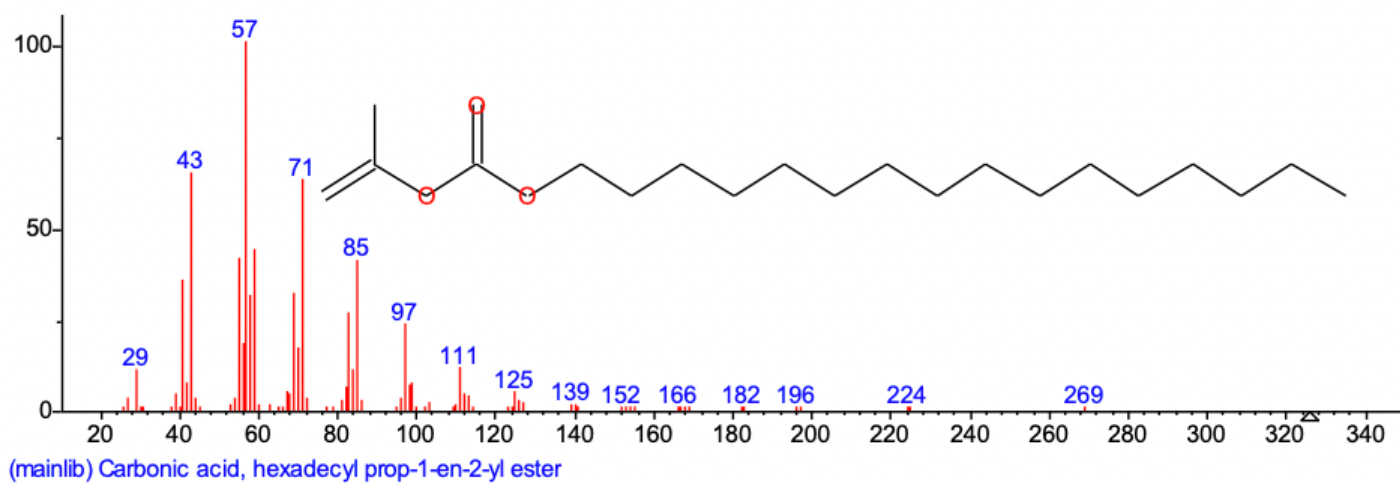


Figure 20

GC-MS spectrum of Carbonic acid, hexadecyl prop-1-en-2-yl ester

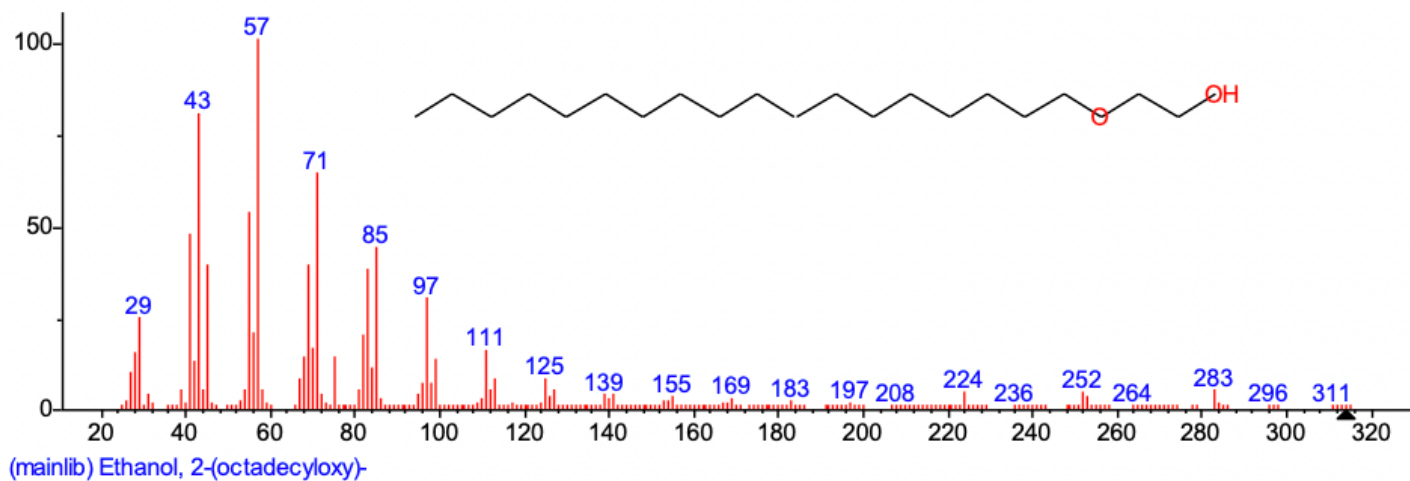


Figure 21

GC-MS spectrum of Ethanol, 2-(octadecyloxy)-

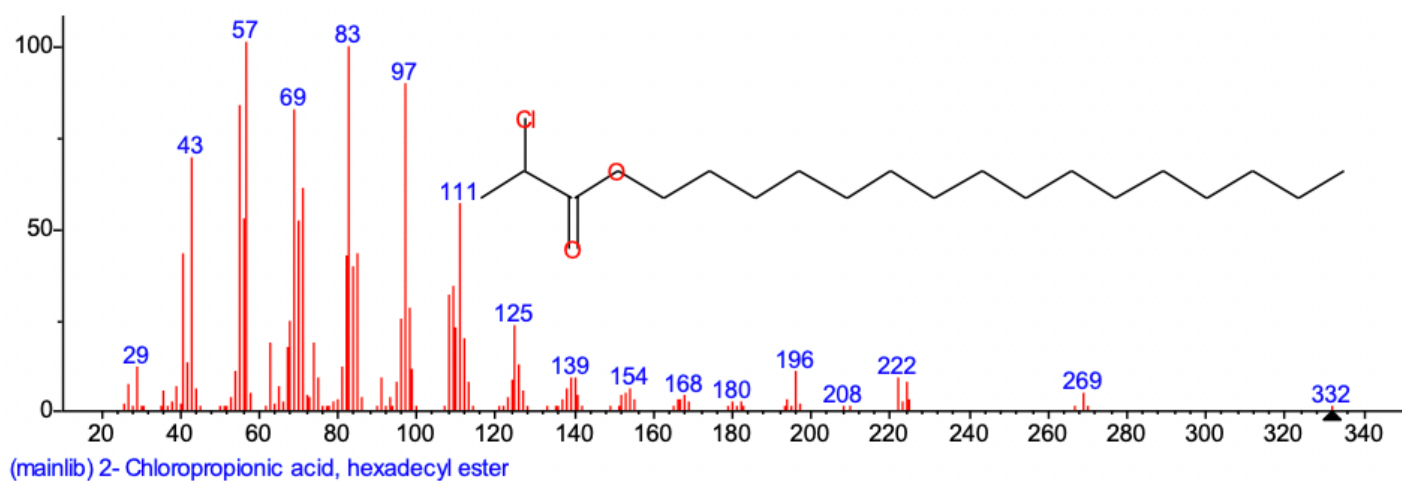


Figure 22

GC-MS spectrum of 2-Chloropropionic acid, hexadecyl ester

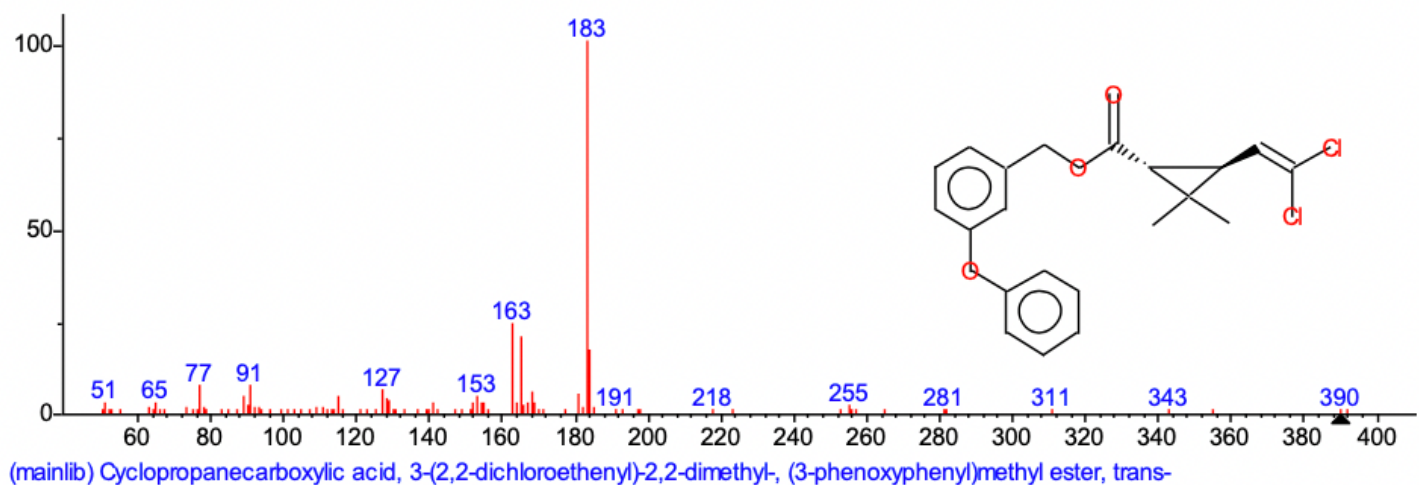


Figure 23

GC-MS spectrum of Cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-, (3-phenoxyphenyl)methyl ester, trans-

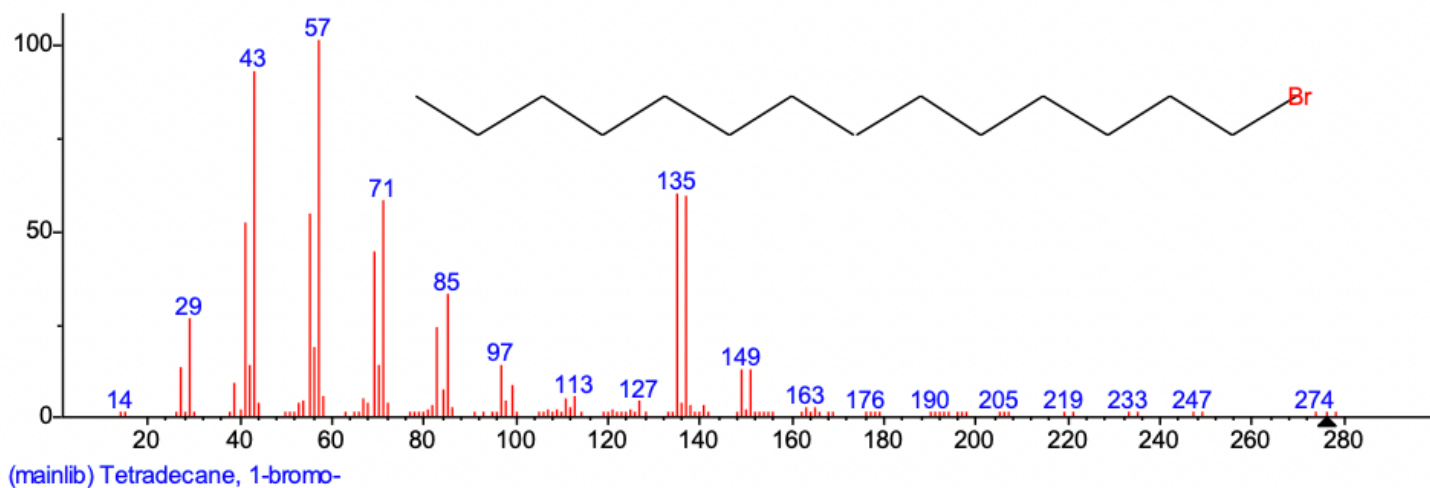


Figure 24

GC-MS spectrum of Tetradecane, 1-bromo-

Supplementary Files

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