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By

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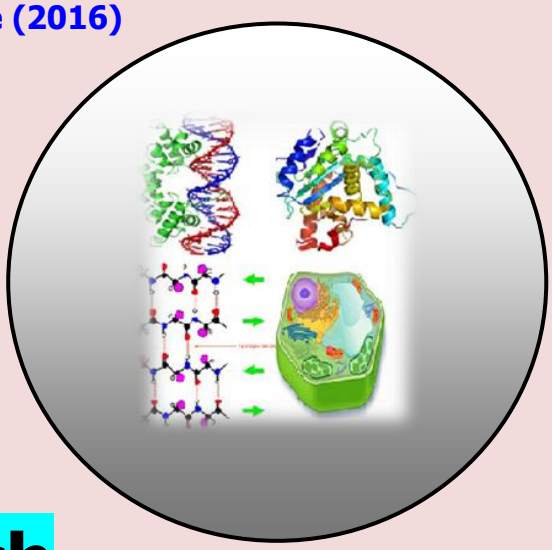
ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

UGC Approved Journal No. 62923
MCI Validated Journal
Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 35 (2) 2018 Pages No. 928-995



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited: Index Copernicus International (Europe), Validated Medical Council of India, World Science Index, Polish Ministry of Science and Higher Education (Poland, Europe) Research Bible (Japan), Scientific Journals Impact Factor Master List, Directory of Research Journals Indexing (DRJI),Indian Science. In, Database Electronic Journals Library (Germany), Open J-Gate, J Gate e-Journal Portal, Info Base Index, International Impact Factor Services (IIFS) (Singapore), Scientific Indexing Services (USA), International Institute of Organized Research (I2OR), Cosmos Science Foundation (Germany), Science Library Index (UAE), Eye Source, Swedish Scientific Publication, World Cat, International Innovative Journal Impact Factor, Einstein Institute for Scientific Information {EISI} and Impact Factor.pl - Kompendiumwiedzy o czasopismachnaukowych, Philadelphia citefactor.org journals indexing Directory Indexing of International Research Journals

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J. Biol. Chem. Research. Vol. 35, No. 2: 928-995, 2018
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 35/02/2011/2018

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

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RESEARCH PAPER

Received: 09/08/2018 **Revised: 29/10/2018** **Accepted: 13/12/2018**

Mass Spectroscopy Techniques and Library of Monosaccharide Derivatives, Oligosaccharide and Glycosides: A Review

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ABSTRACT

In earlier days the structural studies of glycocompounds depended upon paper chromatography, sequential exoglucosidase digestion and quantitative methylation analysis. In recent years HPLC, ^{1}H, ^{13}C and 2D NMR and Mass spectroscopy, FAB, MALDI and ES-MS are being used for structure elucidation of these compounds. Of all the modern techniques used for oligosaccharides/oligoglycosides structure elucidation, NMR (1D and 2D techniques) in combination with Mass spectrometry (FAB, MALDI and ES) yields the complete stereoscopic structure of oligosaccharides/oligoglycosides. The Mass spectrometry is the most basic technique which is used in the structural elucidation of glyco-compounds i.e. glycosides, glycoprotein and oligosaccharide etc. It not only provides the molecular formula but also gives the information regarding the glycosidic linkage and sequences. It is also helpful in pin pointing the appended groups in oligosaccharide and oligoglycosides. In the present review we have made a mass library of oligosaccharide, oligoglycosides and synthetically prepared glycocompounds. Various mass fragmentation patterns have been discussed in detail, providing information regarding glycosidic linkages, appended groups and structure. The mass spectrometry data of synthetic and naturally occurring oligosaccharide and oligoglycosides has been compiled in review article.

Keywords: Mass spectroscopy, mass fragmentation pattern, glycocompounds, glycosides and oligosaccharide.

INTRODUCTION

Since the time emmemorial the natural products were the basis of medicinal chemistry which is providing lead for newer medicines. The compounds isolated from natural sources were so meager in quantity that they could not fulfill the requirements of the mankind therefore it was necessary to synthesize them for getting the required quantity. So, for the purpose of their synthesis their stereoscopic structure was required which was done by chemical degradation and chemical transformation in the present scenario and with the advent of science it is being done by physiochemical techniques like NMR and Mass Spectrometry. Which play decisive role in structure elucidation of natural products. Simultaneously it is also been seen that the compounds containing carbohydrates in them or carbohydrate itself are more effective and less toxic so in the present paper we have selected glycocompounds for their structural analysis by the physiochemical technique with special emphasis on mass spectroscopy. The increased understanding of the ubiquitous role of carbohydrates

in biology forms the basis for a new scientific discipline, glycobiology. Carbohydrates play vital roles in numerous biological processes. Separation and structural elucidation of carbohydrate oligomers is crucial for meaningful structure-activity studies. The structural elucidation of constituent glycans by techniques similar to those used in proteomics is still at a nascent stage. The analysis of biologically important glycocompounds was made complicated by structural complexities, i.e. stereochemistry, linkage, anomericity, poor resolution ability in chromatography and meager quantity (Harvey, D.J 1996).Currently, the lack of chromophores in native carbohydrates is typically addressed by introducing fluorescent or chromophoric tags to improve detect ability during chromatographic separation. The labeling is most commonly done by reductive amination of the aldehyde in the presence of sodium cyanoborohydride (Hase S. 1996). Whereas low molecular mass carbohydrates can be labeled very efficiently in this manner, high-molecular mass oligosaccharides often suffer from reduced labeling efficiency presumably because of steric reasons. It has created new demands for analytical tools for structure elucidation of complex oligosaccharides comprising composition, sequence, branching, and linkage analysis, including anomericity and finally also rings size and absolute configuration. The structural analysis of the carbohydrate is usually carried out by a combination of chemical and enzymatic methods .The structure determination of oligosaccharides is a difficult task because of their presence in meager quantity and low resolution on chromatography. In earlier days the structural studies of oligosaccharides depend upon paper chromatography, sequential exoglucosidase digestion and quantitative methylation analysis etc. Some frequently used techniques is periodate oxidation, Smith degradation, permethylation analysis, acetolysis, alkaline degradation (β-elimination), and sequential degradation with glycosidases. In recent years with the advent of modern chromatographic techniques (HPLC) and recent physicochemical techniques like NMR ^{1}H, ^{13}C and 2D NMR and Mass spectroscopy FAB, MALDI and ES-MS, most of the problems that are unattended previously seem to be resolved. Despite the high degree of sophistication reached by these methods, it is evident that still some uncertainty remains even with regard to the monosaccharide composition. Of all the modern structural methods for oligosaccharides/oligoglycosides, NMR (l D and 2D techniques) in combination with Mass spectrometry (FAB, MALDI and ES) yields the complete stereoscopic structure of oligosaccharides/oligoglycosides, with or without prior structural knowledge. Besides the NMR, the Mass spectrometry is the most basic and a developed technique which is used in the structural elucidation of glyco compounds i.e. glycosides, glycoprotein and oligosaccharide. In the present study we have used the Mass spectrometry as a bench tool in the structural elucidation of various glycocompounds.

History of Mass spectrometry

The technique of mass spectrometry had its beginnings in J.J. Thomson's vacuum tube where in the 18th century in 1886 the existence of electrons and "positive rays" was demonstrated. Thomson, the physicist, observed in his book "Rays of Positive Electricity and Their Application to Chemical Analysis" that the new technique could be used profitably by chemists to analyze chemicals. The primary application of mass spectrometry remains in the realm of physics for nearly thirty years. It was used to discover a number of isotopes, to determine the relative abundance of the isotopes, and to measure their "exact masses", i.e., atomic masses to within a precision of 1 part in 10^6 or better. These important fundamental measurements laid the foundation for later developments in diverse fields ranging from geochronology to biochemical research. The history of MS (Borman et. al 2003) with Sir Joseph John Thomson's "theoretical and experimental investigations on the conduction of electricity by gases for which Thomson was awarded the 1906 Nobel Prize in Physics (Thomson J.J 1899). In the first decade of the 20th century, Thomson went on to construct the first mass spectrometer (then called a parabola spectrograph), in which ions were separated by their different parabolic trajectories in electromagnetic fields and detection occurred by the ions striking a fluorescent screen or photographic plate (Thomson J.J 1911). In the year 1922 Thomson's, Francis W. Aston Nobel Prize in Chemistry designed a mass spectrometer that improved the resolving power by an order of magnitude, allowing Aston to study isotopes. During the same period, A. J. Dempster also improved on resolution with a magnetic analyzer and developed the first electron impact source, which ionizes volatilized molecules with a beam of electrons. Thomson, Aston, and Dempster built a strong foundation of MS theory and instrument design, making it possible for those who followed to develop instruments capable of meeting the demands of chemists and biologists. Wien demonstrated that canal rays could be deflected by passing them through superimposed parallel electric and magnetic fields. Sir Joseph J. Thomson (1856–1940) was credited with the birth of mass spectrometry through his work on analysis of negatively charged cathode ray [6] particles and of positive rays with a parabola mass spectrograph (Thomson J.J 1913).

The ability to separate molecules based on different size and charge was first described in 1912 by J. J. Thomson and expressed as the mass/charge ratio with the unit Thompson (Th). Despite years of intense MS development, the goal of analysing large macromolecules remained elusive for over 70 years. The drive to equip bioscience with novel tools to study the identity and structure, as well as the functional characteristics, of large biomolecules strongly permitted the development of mass spectrometric techniques and methods. The history of mass spectrometry dates back more than one hundred years and has its roots in physical and chemical studies regarding the nature of matter. The chemists were creating an instrument with enough accuracy for the analysis of both the elements and small organic molecules. The answer would come in four different forms: magnetic sector double focusing, time-of-flight (TOF), quadrupole, and Fourier transform ion cyclotron resonance (FT-ICR) and mass analyzers. Alfred O. C. Nier developed the high-mass-resolution double-focusing instrument. New technique played a profound role in the field of chemical analysis. In the next two decades, however, the developments of mass spectrometry continued in the hands of renowned physicists like Aston, Dempster, Bainbridge, and Nier (Grayson M.A. 2002). During this time, mass spectrometry played a pivotal role in the discovery of new isotopes and in determining their relative abundances and accurate masses. In the 1940s, chemists ultimately recognized the potential of mass spectrometry as an analytical tool and applied it to monitor a petroleum refinery stream. Mass spectrometry played a major role in the Manhattan Project, during World War II to perform isotopic analysis and separate 235U from 238U (Nier A.O 1953). The first commercial mass spectrometer became available in 1943. William E. Stephens proposed the concept of TOF MS in 1946 (Stephefls W.E. 1946). In a TOF analyzer, ions were separated on the basis of differences in their velocities as they move in a straight path toward a collector. TOF MS is fast, capable of high resolving power and high accuracy, and applicable to chromatographic detection, and it is used for the mass determination of large biomolecules because of its virtually limitless mass range. The principles of ion cyclotronresonance (ICR) mass spectrometry were introduced in 1948 (Sommer et. al 1951) initially described by J. A. Hipple (Hipple J.A. 1949). Applications to organic chemistry began in the 1950s and exploded in the 1960s and 1970s. From the early 1950s, high resolution experiments and accurate mass measurement for the determination of elemental formulae were developed and demonstrated using double focusing magnetic sector field mass spectrometers. It paved the way for accurate mass measurements of a variety of compounds. The concept of quadrupole mass analyzer and ion traipses mass detectors was First reported in the mid-1950s by Wolfgang Paul the quadrupole mass filter (Pauland W. et. al 1953, Paul W. et al 1958) has proved to be ideal for coupling to GC and, more recently, LC. In such a device, a quadrupolar electrical field (comprising radiofrequency and direct-current components) was used to separate ions. Paul later shared the Nobel Prize in Physics for his work on ion trapping. The double-focusing instruments, offered excellent dynamic range, were quite stable, and were also readily applied to tandem MS experiments features that make them popular for quantitative analysis and drug discovery applications (Yost R.A et.al 1978). In 1959 John Beynon published a report demonstrating that the *m/z* value of an ion can be measured with sufficient accuracy so as to determine its elemental composition (Beynon J.H. et.al 1959). A variety of techniques were established to achieve high accuracy using double focusing magnetic sector field mass spectrometers. Klaus Bieman described the use of photographic plates and a Mattauch-Herzog geometry sector field instrument for precise measurement of line position (Bieman K 1964). The development of gas chromatography (GC)/MS in the 1960s marked the beginning of the analysis of seemingly complex mixtures by mass spectrometry (Ryhage. R. 1964 & Watson J.T etal 1965) The 1960s also witnessed the development of tandem mass spectrometry (MS/MS) (Jennings K.R 1968); the emergence of this technique is a high point in the field of structure analysis and unambiguous quantification by mass spectrometry. By the 1960s, mass spectrometry had become a standard analytical tool in the analysis of organic compounds. Two important developments were presented on Mass Spectrometry and Allied Topics in 1965. Campbell and Halliday provided a theoretical evaluation by relating statistical variation in peak position to ion abundance (Campbell A.J 1965). Green et al. demonstrated the digitization of spectra recorded electronically on magnetic tape for accurate mass measurement, using an MS9 mass spectrometer (Green B.N et.al 1965). Accurate mass measurements are no longer restricted to double focusing magnetic sector field instruments and are now carried out using a variety of mass spectrometers. Fourier transform mass spectrometry (FTMS) offers the highest mass resolution and mass accuracy of any mass spectrometer (Marshall A.G 2002). Chemical ionization, a “soft” mode of ionization, described by M.S.B. Munson and F.H. Field in 1966 (Munson M.S.B et.al 1966), was the use of chemical ionisation (CI), which for the first time made it possible to ionise thermo-labile biomolecules. In CI, abundant reagent gas ions were first formed by electric discharge of a reagent gas, and the reagent ions then in turn ionize volatilised molecules of interest.

The current decade has seen the introduction of two new types of ion traps, the quadrupole linear ion trap (LIT) and the orbitrap, for mass spectrometric analysis (Hager J.W 2002 & Makarov A. 2000). A variety of hybrid tandem mass spectrometry systems were available for enhanced performance in tandem mass spectrometry. The coupling of high-performance liquid chromatography (HPLC) with mass spectrometry, first demonstrated in the 1970s (Baldwin M.A et.al 1973 & McFadden W.H et.al 1976) and later optimized with an ESI(Covey T.R et.al 1991) interface is another high point that has provided chemists and biochemists with one of their most useful instruments. Improvements in detection devices and the introduction of fast data systems have also paralleled these developments. Currently, mass spectrometry has found a niche in the biomedical field and life sciences and is at the fore front of proteomics techniques. In 1974, Melvin B. Comisarow and Alan G. Marshall revolutionized ICR by developing FT-ICR MS (Comisarow M.B et.al 1974). The major advantage of FT-ICR MS was that it allowed many different ions to be measured at once, and sub-part-per-million-accuracy is now routinely possible with commercial instruments. All of these mass analyzer designs and even combinations of different techniques for tandem MS are still used today and are continually being developed for new applications. Plasma desorption (PD), introduced in 1976 (Macfariane R.D et. al 1976), uses high-energy ions to desorb and ionise molecules. The challenge in the 1980s was how to find a way to analyse high-molecular-weight compounds by mass spectrometry and to make mass spectrometry into a powerful detector for liquid separation techniques. The application of "soft ionization" electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) allowed MS to evolve into the realm of biology. A milestone was passed when M. Barber et. al. described the successful use of a non-volatile chemical protection environment next to the molecules to enable polar and thermally labile compounds to survive the ionisation process. The accelerated atoms (and later also ions) of e.g. argon, caesium or xenon was used for mass determination of small biomolecules (i.e. mol. wt. <10 kDa). This technique was termed as fast atom bombardment (FAB) in 1981 (Barber M et.al 1981), and the closely related method liquid matrix secondary ion mass spectrometry (LSIMS) did not solve the problem of reaching higher masses but had a major impact on expectations of future success. The introduction of the electrospray (ES) and soft laser desorption (SLD) methods could meet both needs. The resulting explosion of applications can be examplified by the demonstration that the ES technique is so mild that viral material can remain viable after an electrospray ionisation (ESI) process (Bothner B. et.al 1998). The SLD method allowed easy access to singly charged ionisation of intact biomolecules in complex matrices. Several unique developments in gentler modes of ionization have allowed the production of ions from compounds of large molecular mass and compounds of biological relevance. These methods include electrospray ionization (ESI) (in 1984–1988) (Fenn J.B. et.al 1989) and matrix-assisted laser desorption/ionization (MALDI) in 1988 (Karas M. et.al 1988 and Tanaka K et.al 1988). Malcolm Dole first conceived the technique in the 1960s, for their work on developing soft ionization techniques suitable for large-biomolecule analysis; Fenn and Tanaka shared the 2002 Nobel Prize in Chemistry. ESI and MALDI have made MS increasingly useful for sophisticated biological experiments, such as the sequencing and analysis of peptides and proteins using techniques pioneered by Klaus Biemann studies of noncovalent complexes and immunological molecules; DNA sequencing; and the analysis of intact viruses. Brian Chait to develop MS methods to study noncovalent interactions have clearly demonstrated the method's utility in studying protein–protein complexes or even in examining subcellular components (Katta V et.al 1991).

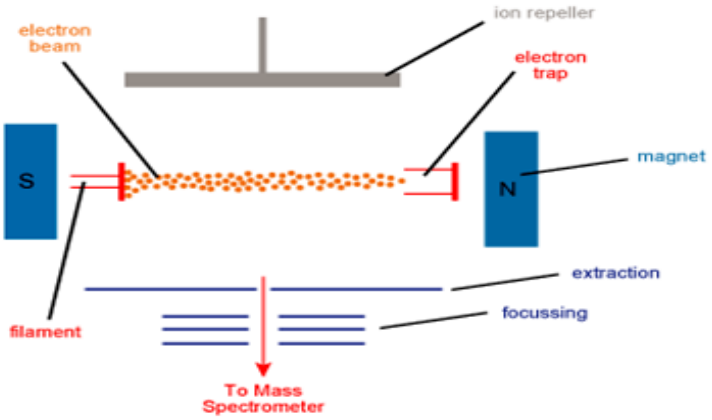
Now, a variety of desorption and ionization methods have been developed to separate matter according to their mass/charge (m/z) values. Via these techniques, mass spectrometry can be applied to measure the characterization of proteins and nucleic acids. Mass spectrometry has unique capabilities; it provides unsurpassed molecular specificity because of its unique ability to measure accurate molecular mass and to provide information on structurally diagnostic fragment ions of an analyte. It provides ultra high detection sensitivity. Mass spectrometry has the ability to detect a single molecule; the detection of molecules in attomole and zeptomole amounts has been demonstrated. It has unparalleled versatility to determine the structures of most classes of compounds. It is applicable to all elements. It is applicable to all types of samples: volatile or nonvolatile; polar or nonpolar; and solid, liquid, or gaseous materials. In combination with high-resolution separation devices. Massspectrometry (Siuzdak G 1999) (MS) is an analytical technique that measures the molecular masses of individual compounds and atoms precisely by converting them into charged ions. Quite often, the structure of a molecule can also be deduced. Mass spectrometry is also uniquely qualified to provide quantitative information of an analyte at levels of structure specificity and sensitivity that are beyond imagination.

Mass spectrometry allows one to study reaction dynamics and chemistry of ions, to provide data on physical properties such as ionization energy, appearance energy, enthalpy of a reaction, proton and ion affinities, and so on, and to verify molecular orbital calculations-based theoretical predictions. Thus, mass spectrometry probably is the most versatile and comprehensive analytical technique currently at the disposal of chemists and biochemists. Accurate mass measurement of small molecules is used to determine elemental formulae.

INSTRUMENTATION TECHNIQUES

MODES OF IONIZATION

A variety of ionization techniques are used for mass spectrometry. Most ionization techniques excite the neutral analyte molecule which then ejects an electron to form a radical cation (M^+). Other ionization techniques involve ion molecule reactions that produce adduct ions (MH+). The most important considerations are the physical state of the analyte and the ionization energy. Electron ionization and chemical ionization are only suitable for gas phase ionization. Fast atom bombardment, secondary ion mass spectrometry, electrospray, and matrix assisted laser desorption are used to ionize condensed phase samples. The ionization energy is significant because it controls the amount of fragmentation observed in the mass spectrum. Although this fragmentation complicates the mass spectrum, it provides structural information for the identification of unknown compounds. Some ionization techniques are very soft and only produce molecular ions; other techniques are very energetic and cause ions to undergo extensive fragmentation. Various ionization techniques are given as under:



$AB + e^{-*} \rightarrow A^+ + B^- + e^-$

$AB + e^{-*} \rightarrow A^+ + B^* + 2e^-$

} fragment ion formation

$AB + e^{-*} \rightarrow [AB^{+\bullet *}] + 2e^-$

radical molecular ion formation

$[AB^{+\bullet *}] \rightarrow AB^{+\bullet}$

$\rightarrow$ secondary fragmentation

Gas-Phase Ionization Techniques

Electron Ionisation and Chemical Ionisation are generally considered to be the 'classical' methods of analyte ionisation. Both techniques are still routinely used for the analysis of low-mass, volatile; thermally stable organic compounds, especially when coupled with gas chromatography (GC-MS). Both techniques follow the same basic setup and source design.

Electron Ionisation (EI) (Fales HM et.al 1972)

In Electron ionization technique the analyte must be vaporized; this is usually achieved by heating the probe tip containing a droplet of the analyte in solution. If the sample is thermally unstable, this will often be the first cause of sample fragmentation. Once in the gas-phase, the analyte passes into an EI chamber.

Where it interacts with a homogeneous beam of electrons typically at 70 electron volts energy. The electron beam is produced by a filament (rhenium or tungsten wire) and steered across the source chamber to the electron trap. A fixed magnet is placed, with opposite poles slightly off-axis, across the chamber to create a spiral in the electron beam. This is to increase the chance of interactions between the beam and the analyte gas. There are no actual collisions between analyte molecules and electrons ionisation is caused by electron ejection from the analyte or by analyte decomposition.

A side-view of an EI source

In the analyte molecule AB, the first two processes that might occur are the direct result of energy transfer from the electron beam to the analyte, causing primary fragmentation and the second main cause of fragment ions in the spectrum. The third process is electron ejection from the analyte to create the energised radical ion. This can then either lose energy through 'ion cooling' and stabilise (accounting for the radical molecular ion in the spectrum) or lose energy through secondary fragmenting the third cause of fragment ions in the mass spectrum. These high levels of fragmentation in EI spectra often result in the the technique being termed a 'hard' method of ionisation. The harsh conditions required volatilizing some types of analyte and the high levels of residual energy possessed by the ions after ionisation because the high levels of fragment ions observed in the mass spectrum.

(a) $CH_4 + e^{-*} \longrightarrow CH_4^{+\bullet} + 2e^-$ methane molecular ion formation

(b) $CH_4^{+\bullet} + CH_4 \longrightarrow CH_5^+ + CH_3^\bullet$ carbocation formation

(c) $CH_5^+ + AB \longrightarrow CH_4 + ABH^+$ protonated analyte formation

(d) $CH_5^+ + CH_4 \longrightarrow C_2H_9^+$ side reaction carbocation formation

(e) $C_2H_9^+ + AB \longrightarrow [AB+C_2H_9]^+$ analyte adduct ion formation

Condensed-Phase Ionization Techniques: Ionization of Solid-State Samples

DESORPTION IONIZATION METHOS	
Technique	**Means of Ionization**
Fast Atom Bombardment (FAB)	Impact of high velocity atoms on a sample dissolved in a liquid matrix.
Secondary ion MS (SIMS)	Impact of high velocity ions on a thin film of sample on a metal substrate (or dissolved in a liquid matrix - liquid SIMS).
Plasma Desorption	Impact of nuclear fission fragments, e.g. from ^{232}Cf, sample embedded in a solid organic matrix.
Matrix Assisted Laser Desorption / Ionozation (MALDI)	Impact of high energy photons on a sample embedded in a solid organic matrix.
Field Desorption	Imposition of high electric field gradient on a sample deposited on a special solid support.
Electrospray	Formation of charged liquid droplets from which ions are desolvated or desorbed.

The ion formation reactions in EI

Holgerrson et, al. has used electron ionization mass spectrometry (Olgersson J. H.) for structure characterization of glycosphingolipids containing eleven sugars moieties.

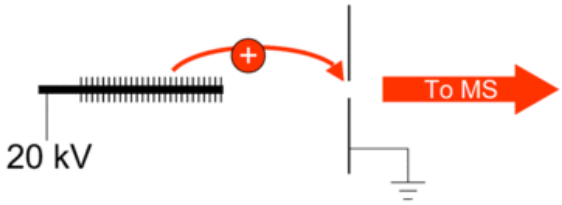
Chemical Ionisation (CI)

Chemical ionisation is a lower energy alternative to EI for volatile analytes. In CI, there is a reagent gas (ammonia or methane) in the ion chamber.Inside the ion source, the reagent gases present in large excess compared to the analyte. Electrons entering the source preferentially ionize the reagent gas. The resultant collisions with other reagent gas molecules createan ionization plasma.

Positive and negative ions of the analyte are formed by reactions with this plasma (DeHoffhin et.al 2003).Ion formation in CI using methane as the reagent gas. In equation (a), methane is ionised by an electron beam in the same way as with EI. Equation (b) shows the ionised reagent gas reacting with un-ionised reagent gas to form the carbocation (protonated methane). This step requires the CI reagent gas to be at a critical pressure too low a pressure and no ionisation of the analyte can take place. Equation (c) shows proton transfer from the carbocation to the analyte (AB) to form the protonated analyte molecule (ABH^+). If the pressure of the reagent gas is too high, then the side reactions (d) and (e) can also occur, leading to formation of the analyte adduct ion.**Ion formation reactions in CI-MS**

In CI, ionisation is due to proton transfer and is therefore a much lower energy process. This results in less residual energy being possessed by the protonated molecules so that fragmentation is greatly reduced. CI still requires volatilisation of the analyte, so thermal degradation of the analyte can still lead to fragment ions being observed. CI is generally considered a much 'softer' ionisation method than EI. Determination of the structure of oligosaccharides by mass spectrometry (ChithovS et.al 1976) is directly analogous of sequencing polypeptides by the same technique. It provides information about the molecular weight, subunit structure, and position sequence. Conventional techniques will give reliable information concerning subunit structure in unknown oligosaccharides. Chizav et. al. has compared the sequencing of polysaccharide by doing the CI spectra of permethylated glycosylalditols.

Field desorption (FD)/field ionization (FI)

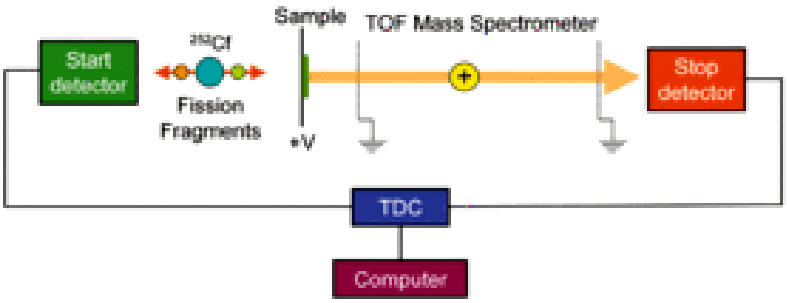


Field desorption ionization with emitter at left and mass spectrometer at right

Field desorption (FD)/field ionization (FI) was first reported by Beckey in 1969(Beckey H.D 1969).In field ionization, a high-potential electric field is applied to an emitter with a sharp surface, such as a razor blade, or more commonly, a filament from which tiny "whiskers" have formed. This results in a very high electric field which can result in ionization of gaseous molecules of the analyte. Mass spectra produced by FI have little or no fragmentation. They are dominated by molecular radical cations $M^{+\cdot}$ and less often, protonated molecules $M+H^+$. In FD, the analyte is applied as a thin film directly to the emitter, or small crystals of solid materials are placed onto the emitter. Slow heating of the emitter then begins, by passing a high current through the emitter, which is maintained at a high potential (e.g. 5 kilovolts). As heating of the emitter continues low-vapor-pressure materials get desorbed and ionized by alkali metal cation attachment. Many earlier applications of FD/FI to analysis of polar and nonvolatile analytes such as polymers and biological molecules have largely been supplanted by newer ionization techniques. FD/FI remains one of the only ionization techniques that can produce simple mass spectra with molecular information from hydrocarbons and other particular analytes. The most commonly encountered application of FD/FI at the present time is the analysis of complex mixtures of hydrocarbons such as that found in petroleum fractions. The recently developed liquid injection FD ionization (LIFDI)(Linden H.B 2004) technique "presents a major breakthrough for FD-MS of reactive analytes" (Gross JH 2004)Transition metal complexes are neutral and due to their reactivity, do not undergo protonation or ion attachment. They benefit from both: the soft FD ionization and the safe and simple LIFDI transfer of air/moisture sensitive analyte solution. This transfer occurs from the Schlenk flask to the FD emitter in the ion source through a fused silica capillary without breaking the vacuum. Field desorption mass spectrometry (Linscheid M et.al 1981) has been used to analyze carbohydrate polymers with 5 to 14 hexose units without derivatization. The molecular weight of the oligosaccharide was determined by means of the abundant quasimolecular ions of the type MNa+, MH+, MNa2 2+, and MNa3 3+. Fragmentation of glycosidic linkages was observed in varying extents.

Plasma desorption ionization mass spectrometry

Plasma desorption ionization mass spectrometry (Macfarlane R.D et.al 1976 & Hilf E.R 1993) (PDMS; also called fission fragment ionization) is a mass spectrometry technique in which ionization of material in a solid sample by bombarding it with ionic or neutral atoms formed as a result of the nuclear fission of a suitable nuclide, typically the Californium isotope ^{252}Cf.

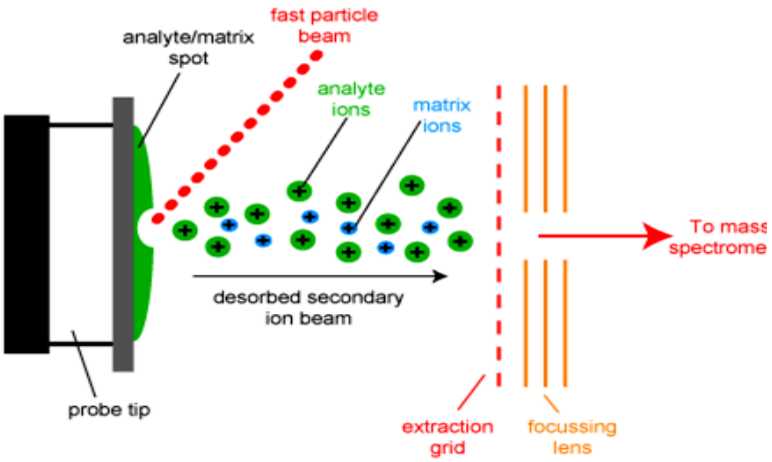


A representation of a plasama desorption time-of-flight mass spectrometer.

^{252}Cf plasma desorption mass spectrometry on a "reflect" desorption instrument (MSBX) was used to study oligosaccharides from bacterial O-specific antigenic chains. This method was suggested for determining the composition of oligosaccharides of O-specific polysaccharide chains from bacteria.

Fast Atom Bombardment (FAB)

The development of fast particle desorption culminate with the development of FAB by Michael Barber in the early 1980's (Barber M et.al 1981). The techniques of FAB and LSIMS are very similar in concept and design as they both involve the bombardment of a solid spot of the analyte/matrix mixture on the end of a sample probe by a fast particle beam. The matrix (a small organic species like glycerol or 3-nitro benzyl alcohol) is used to keep a homogenous sample surface. The particle beam is incident onto the surface of the analyte/matrix spot, where it transfers its energy bringing about localized collisions and disruptions. Some species are ejected from the surface as secondary ions by this process. These ions are then extracted and focused before passing to the mass analyser. The polarity of ions produced depends on the source potentials. In FAB, the particle beam is a neutral inert gas (Ar or Xe) at 4-10 keV and in LSIMS; the particle beam is ions (usually Cs^+) at 2-30 keV. Both methods are comparatively 'soft' ionisation methods very little residual energy is possessed by the ions after desorption making them particularly suited to the analysis of low volatility analytes. FAB-MS (Fereza A.M et.al 2006, Viverge D et.al 1997) was used for elucidating the structure of lactose derived oligosaccharide from Goat's milk.



The mechanism of fast particle beam ionisation mass spectrometry (FAB)

Laser desorption (LD)/ ionisation

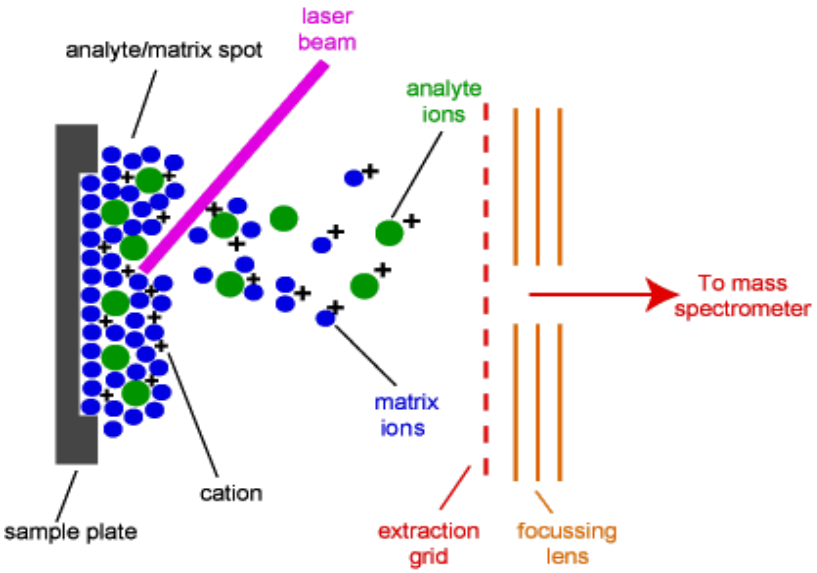
In the early 1960's, it was demonstrated that the irradiation of low-mass organic molecules with a high-intensity laser pulse lead to the formation of ions that could be successfully mass analyzed. This was the origins of laser desorption (LD) ionisation.

Other the next few decades, the technique underwent substantial development, culminating in the extension of the technique to the volatilisation of non-volatile biopolymers and organic macromolecules. There was a sharp cut off in mass at about 5-10 kDa, limiting the technique's application. The other main limitation was that ions were created in bursts which prevented the technique from being coupled to scanning mass analysers. In fact LD was only really successful when coupled to TOF mass analysers. Matrix-assisted laser desorption (Zhao .Y et.al 1997) ionization MS has been used to determine molecular masses of oligosaccharides and to obtain sequence, branching, and linkage information. The application of MS to structure elucidation is attractive because the technique is potentially rapid and sensitive

Matrix-assisted Laser Desorption/Ionisation (MALDI)

In 1987, Michael Karas and Franz Hillenkamp (Karas M. et.al 1987) successfully demonstrated the use of a matrix (a small organic molecule) in LD to circumvent the mass limitation. The matrix had a strong absorbance at the laser wavelength and was highly sublimable (Beavis R.C et.al 1993). A low concentration of the analyte was mixed with this matrix onto a probe or metal plate and introduced into a pulsed laser beam. A substantial burst of ions was produced with each laser pulse. An unexpected side effect of the matrix was that it allowed for the laser incidence spot to be refreshed between each pulse, thus greatly enhancing shot-to-shot reproducibility. This was the foundation of matrix-assisted laser desorption/ionisation (MALDI). Later developments by Koichi Tanaka demonstrated the application of MALDI to a whole range of biological macromolecules. This lead him to receive a part share with John Fenn on the 2002 Noble prize for chemistry (Tanaka K. 2003), making him the 5th mass spectrometry pioneer to receive such an honor.

The mechanism of MALDI is believed to consist of three basic steps,
(i) **Formation of a 'Solid Solution':** It is essential for the matrix to be in access thus leading to the analyte molecules being completely isolated from each other. This eases the formation of the homogenous 'solid solution' required to produce a stable desorption of the analyte.

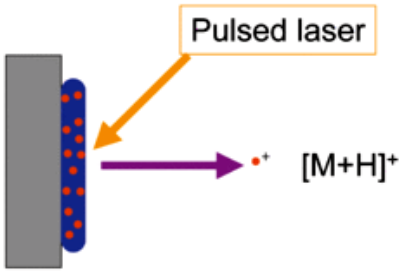


The mechanism of MALDI

(ii) **Matrix Excitation:** The laser beam is focused onto the surface of the matrix-analyte solid solution. The chromophore of the matrix couples with the laser frequency causing rapid vibrational excitation, bringing about localised disintegration of the solid solution. The clusters ejected from the surface consist of analyte molecules surrounded by matrix and salt ions. The matrix molecules evaporate away from the clusters to leave the free analyte in the gas-phase.

(iii) **Analyte Ionisation:** The photo-excited matrix molecules are stabilized through proton transfer to the analyte. Cation attachment to the analyte is also encouraged during this process. It is in this way that the characteristic $[M+X]^+$ (X= H, Na, K etc.) analyte ions are formed. These ionisation reactions take place in the desorbed matrix analyte cloud just above the surface.

The ions were then extracted into the mass spectrometer for analysis. Matrix-assisted laser desorption/ionization (MALDI) is a soft ionization technique used in mass spectrometry, allowing the analysis of biomolecules (biopolymers such as proteins, peptides and sugars) and large organicmolecules (such as polymers, dendrimers and other macromolecules), which tend to be fragile and fragment when ionized by more conventional ionization methods. It is most similar in character to electrospray ionization both in relative softness and the ions produced (although it causes much fewer multiply charged ions). The ionization is triggered by a laser beam (normally a nitrogen laser). The matrix was used to protect the biomolecule from being destroyed by direct laser beam and to facilitate vaporization and ionization. Matrix-assisted (Broberg et.al 2000) laser desorption/ionization mass spectrometry (MALDI-MS) is a powerful technique that characterizes underivatised oligosaccharides. It was used in quantitation of human milk oligosaccharides in bacterial fermentation.



MALDI depicted with matrix in light black and analyte in black

Lasers Used for MALDI		
Laser	**Wavelength (nm)**	**Reference**
Nitrogen laser	337	(Tanaka 1988)
Nd:YAG	355, 266	(Karas et.al1985)
Er:YAG	2940	(Overberg et.al 1990)
CO_2	10,600	(Overberg et.al 1991)

Atmospheric pressure AP-MALDI

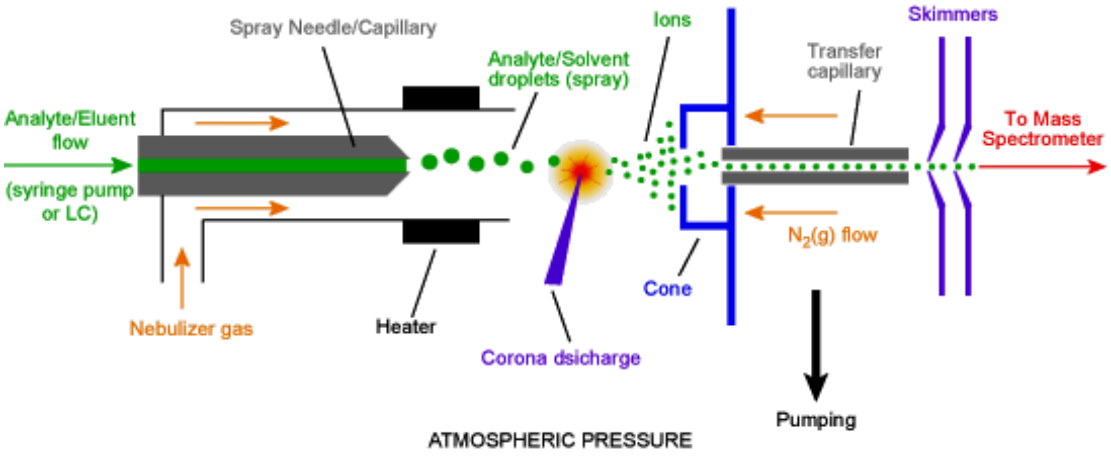
Atmospheric pressure (AP) matrix-assisted laser desorption/ionization (MALDI) is an ionization technique (ion source) that in contrast to vacuum MALDI operates at normal atmospheric environment.(Laiko VV et.al 2000)The main difference between vacuum MALDI and AP-MALDI is the pressure in which the ions are created. In vacuum MALDI, ions are typically produced at 10 m Torr or less while in AP-MALDI ions are formed in atmospheric pressure. Disadvantage of the AP MALDI source is the limited sensitivity observed and the limited mass range. AP-MALDI is used in mass spectrometry (MS) in a variety of applications ranging from proteomics to drug discovery fields. AP-MALDI mass spectrometry include: proteomics, DNA/RNA/PNA, lipids, oligosaccharides, phosphopeptides, bacteria, small molecules and synthetic polymers, similar applications as available also for vacuum MALDI instruments. The AP-MALDI ion source is easily coupled to an ion trap mass spectrometer or any other MS system equipped with ESI (electrospray ionization) or nanoESI source. An atmospheric pressure matrix-assisted laser desorption/ionization (AP MALDI)(Zhang .J et.al 2005) source coupled to Fourier transform ion cyclotron resonance mass spectrometry (FT ICR MS) under UV laser and solid matrix conditions has been demonstrated to analyze a variety of labile oligosaccharides including O-linked and N-linked complex glycans released from glycoproteins.

Condensed-Phase Ionization Techniques: Ionization of Liquid-State Samples

Atmospheric Pressure Chemical Ionisation (APCI)

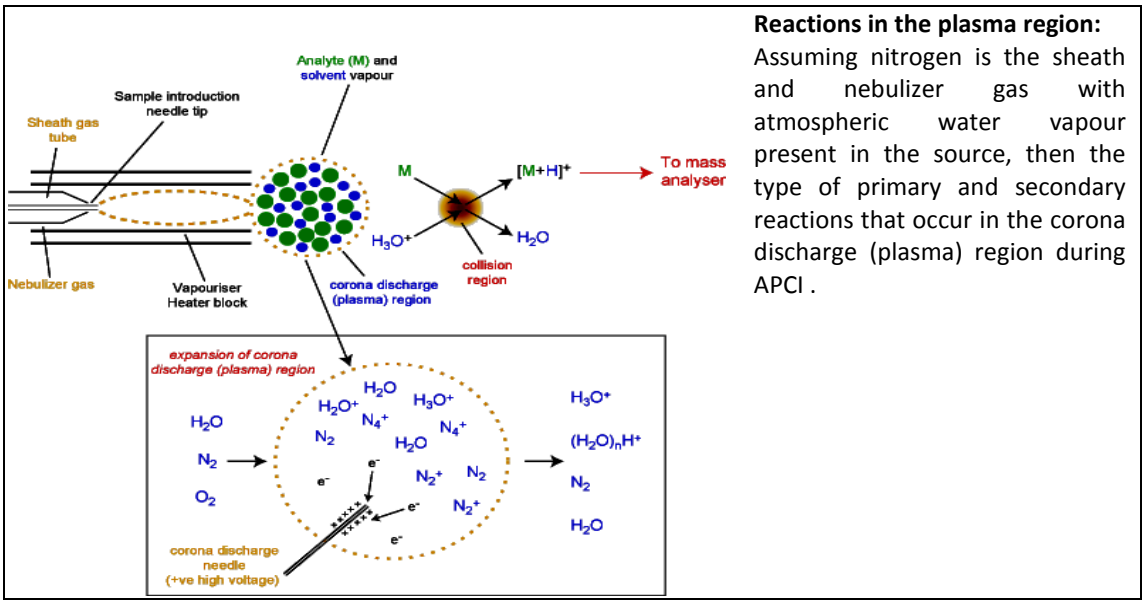
Atmospheric pressure chemical ionisation (APCI) is an analogous ionisation method to chemical ionisation(CI). The significant difference is that APCI occurs at atmospheric pressure and has its primary applications in the areas of ionisation of low mass pharmaceutical compounds (APCI is not suitable for the analysis of thermally labile compounds). The general source set-up shares a strong resemblance to electrospray ionisation(ESI) and as such is most commonly used in conjunction with HPLC or other flow separation techniques. Where APCI differs to ESI, is in the way ionisation occurs.

In ESI, ionisation is bought about through the potential difference between the spray needle and the cone along with rapid but gentle desolvation. In APCI, the analyte solution is introduced into a pneumatic nebulizer and desolved in a heated quartz tube before interacting with the corona discharge creating ions.



The components of an APCI source

The corona discharge replaces the electron filament in CI the atmospheric pressure would quickly "burn out" any filaments and produces primary N2°+ and N4°+ by electron ionisation. These primary ions collide with the vaporized solvent molecules to form secondary reactant gas ions - e.g. H_3O+ and (H_2O) nH+. These reactant gas ions then undergo repeated collisions with the analyte resulting in the formation of analyte ions. The high frequency of collisions results in a high ionisation efficiency and thermalisation of the analyte ions. This results in spectra of predominantly molecular species and adducts ions with very little fragmentation. Once the ions are formed, they enter the pumping and focusing stage in much the same as the other atmospheric pressure ionisation sources.



Reactions in the plasma region:

Assuming nitrogen is the sheath and nebulizer gas with atmospheric water vapour present in the source, then the type of primary and secondary reactions that occur in the corona discharge (plasma) region during APCI .

The mechanism of APCI

Electrospray Ionisation (ESI)

The pioneering experiments by Malcom Dole *et al* demonstrated the use of electrospray to ionise intact chemical species and the technique of electrospray ionisation (ESI) (Chapman S 1937 & Dole M et.al 1968) by John Fenn demonstrated for the first time the use of ESI for the ionisation of biologically important compounds and their subsequent analysis by mass spectrometry (Fenn J.B et.al 1984).

This gave him the Nobel Prize for chemistry for the 4th time (Fenn J.B 2003). In late 1980's, Fenn successfully demonstrated the basic experimental principles and methodologies of the ESI technique, including soft ionisation of involatile and thermally labile compounds, multiple charging of proteins and intact ionisation of complexes (Pramanik B.N et.al 2002). The analyte is introduced to the source in solution either from a syringe pump or as the eluent flow from liquid chromatography. Flow rates are typically of the order of 1µl min^{-1}. The analyte solution flow passes through the electrospray needle that has a high potential difference (with respect to the counter electrode) applied to it (typically in the range from 2.5 to 4 kV). This forces the spraying of charged droplets from the needle with a surface charge of the same polarity to the charge on the needle. The droplets are repelled from the needle towards the source sampling cone on the counter electrode. As the droplets traverse the space between the needle tip and the cone and solvent evaporation occurs.

As the solvent evaporation occurs, the droplet shrinks until it reaches the point that the surface tension can no longer sustain the charge (the Rayleigh limit) at this point a "Coulombic explosion" occurs and the droplet is ripped apart.

$$N_2 + e \longrightarrow N_2^+ + 2e$$

$$N_2^+ + 2N_2 \longrightarrow N_4^+ + N_2$$

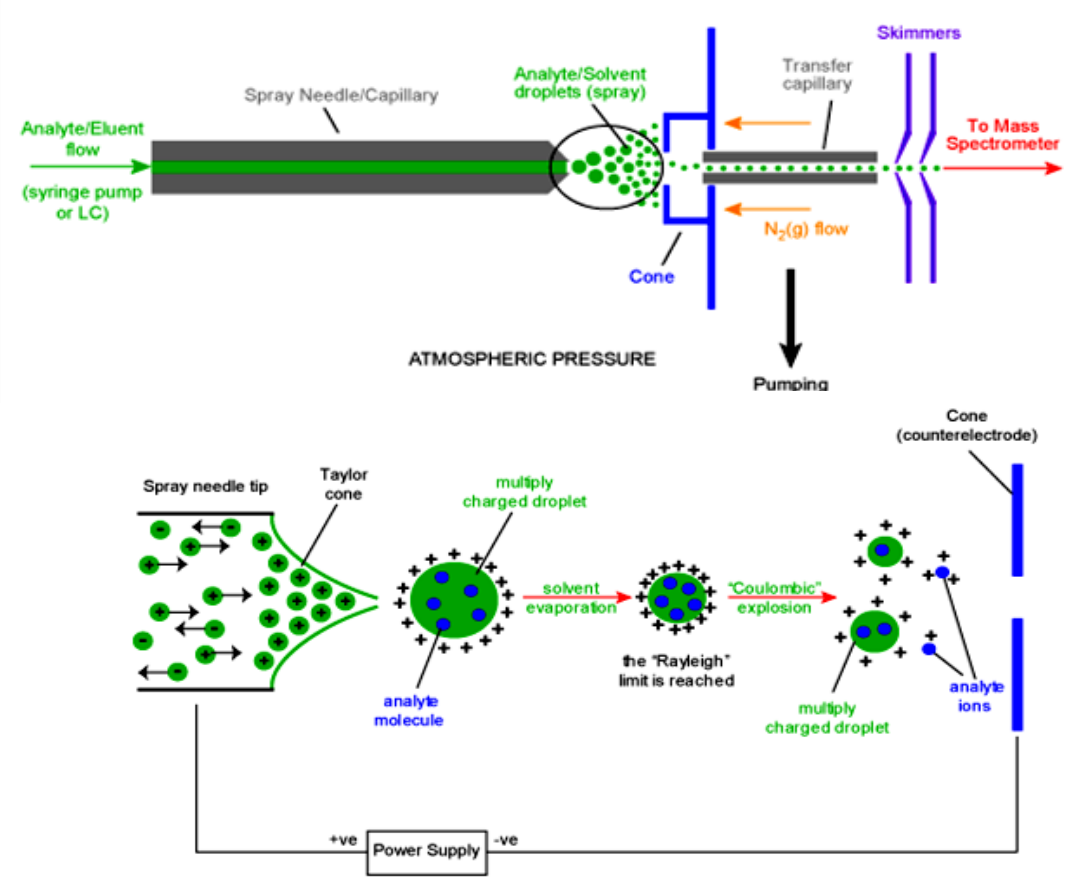
$$N_4^+ + H_2O \longrightarrow H_2O^+ + 2N_2$$

$$H_2O^+ + H_2O \longrightarrow H_3O^+ + OH^\cdot$$

$$H_3O^+ + H_2O + N_2 \longrightarrow H^+(H_2O)_2 + N_2$$

$$H^+(H_2O)_{n-1} + H_2O + N_2 \longrightarrow H^+(H_2O)_n + N_2$$

The most abundant secondary cluster ion is (H2O) 2H + along with significant amounts (H2O) 3H+ and H3O +. The reactions listed above are ways to account for the formation of these ions during the plasma stage. The protonated analyte ions are then formed by gasphase ion-molecule reactions of these charger cluster ions with the analyte molecules. This results in the abundant formation of [M+H] + ions.



A schematic of an ESI source

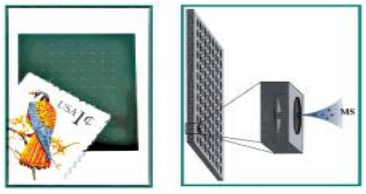
This produces smaller droplets that can repeat the process as well as naked charged analyte molecules. These charged analyte molecules (they are not strictly ions) can be singly or multiply charged. This is a very soft method of ionisation as very little residual energy is retained by the analyte upon ionisation. This is why ESI-MS is such an important technique in biological studies where the analyst often requires that non-covalent molecule-protein or protein-protein interactions are representatively transferred into the gas-phase.

The mechanism of ion formation in ESI

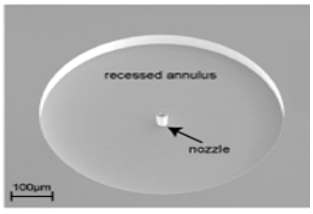
Electrospray (Kogelberg H. et.al 2004 and Viseux N et.al 1997) tandem mass spectrometry used in conjunction with reversed-phase liquid chromatography was applied to characterize permethylated oligosaccharides. N-Acetylhexosamine-containing carbohydrates yielded under these conditions protonated molecular ions which underwent extensive fragmentation, even under lowenergy collision-induced dissociation. MS/MS spectra of $[M + H]^+$ ions are characterized by simple fragmentation patterns which result from cleavage of the glycosidic bonds and thus allow a straight forward interpretation. A systematic study of various oligosaccharides showed that information on sugar sequence and branching could be obtained. The nature of the substituent linked in position 3 of HexNAc-containing fragments could easily be assigned as the result of a specific secondary fragmentation process. MS/MS experiments carried out on fragment ions were proven to be useful for the structural characterization of oligosaccharide subunits. Thus, this approach constitutes a powerful tool for the structural assignment of moieties derived from larger glycans.

NanosprayIonisation

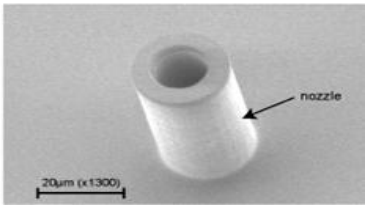
Nanospray-ESI is a development of ESI for spraying very low amounts of very low concentration samples (nmol/mL). The technique has an increased tolerance to high aqueous solvents and salt contamination. Spectra can be obtained from pg of material with very little clean up being required. This increased performance is the result of lowering the inner diameter of the spray needle and reducing potentials normally used in ESI. Standard nanospray uses disposable tips, but has problems with signal reproducibility between tips. Nanospray ionization (Vilm M. 1996) is a low flow rate version of electrospray ionisation. A small volume (1-4 microL) of the sample dissolved in a suitable volatile solvent, at a concentration of ca. 1 - 10 pmol/microL, is transferred into a miniature sample vial. A reasonably high voltage (ca. 700 - 2000 V) is applied to the specially manufactured gold-plated vial resulting in sample ionisation and spraying. The flow rate of solute and solvent using this procedure is very low, 30 - 1000 nL/min, and so not only is far less sample consumed than with the standard electrospray ionisation technique, but also a small volume of sample lasts for several minutes, thus enabling multiple experiments to be performed. A common application of this technique is for a protein digest mixture to be analysed to generate a list of molecular masses for the components present, and then each component to be analysed further by tandem mass spectrometric (MS-MS) amino acid sequencing techniques. This technique (Pfenninger A. et.al 2002) could be demonstrated that MSn experiments of deprotonated neutral oligosaccharides can be applied for structural analysis of oligosaccharides out of complex isomeric mixtures. Linear, fucosylated, and branched structures can be differentiated by making use of specific fragmentation channels which result in a separation into subgroups of the present isomeric mixture. The presence of both linear and branched structures of neutral oligosaccharides, out of complex isomeric mixtures, has been revealed by mass spectrometry.



(a) Size comparision of the nanomate chip



(b) A Scanning Electron Micrograph image showing the annulus and spray nozzle



(c) Scanning Electron Micrograph (1300) showing a single spray nozzle

Mass Analyzers

Magnetic Sector: The first mass spectrometer, built by J.J. Thompson in 1897, used a magnet to measure the *m/z* value of an electron. Sector instruments have higher resolution and greater mass range than quadrupole instruments, but they require larger vacuum pumps and often scan more slowly.

Magnetic sector instruments are often used in series with an electric sector for high resolution and tandem mass spectrometry experiments.Magnetic sector instruments separate ions in a magnetic field according to the momentum and charge of the ion. Ions are accelerated from the source region into the magnetic sector by a 1 to 10 kV electric field. This acceleration is significantly greater than the 100 V acceleration typical for a quadrupole instrument. Since the ions are charged, as they move through the magnetic sector, the magnetic field bends the ion beam in an arc. The radius of this arc (r) depends upon the momentum of the ion (μ), the charge of the ion (C) and the magnetic field strength (B).

$r = \mu/C*B$

Ions with greater momentum will follow an arc with a larger radius. This separates ions according to their momentum, so magnetic sectors are often called momentum analyzers. The momentum of the ion is the product of the mass (*m*) and the velocity (*v*). The charge of the ion is the product of the charge number of the ion (*z*) and the charge of an electron (e).

$r = m/z*v/B*e$

The velocity of an ion is determined by the acceleration voltage in the source region (V) and the mass to charge ratio (*m/z*) of the ion rearranges to give the *m/z* ion transmitted for a given radius, magnetic field, and acceleration voltage as:

$M/z = r^2B^2e/2V$

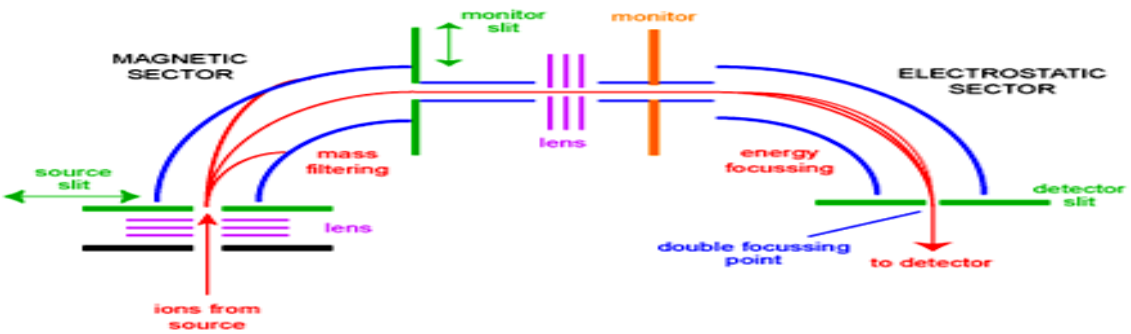
Only one *m/z* value will satisfy for a given radius, magnetic field and acceleration voltage. Other *m/z* ions will travel a different radius in the magnetic sector. Older magnetic sector instruments use a photographic plate to simultaneously detect ions at different radii. Since each *m/z* has a different radius, they strike the photographic plate at a different location. Modern instruments have a set of slits at a fixed radius to transmit a single *m/z* to the detector. The mass spectrum is scanned by changing the magnetic field or the acceleration voltage to transmit different *m/z* ions. Some new instruments use multichannel diode array detectors to simultaneously detect ions over a range of *m/z* values.

Electric Sector/Double Focusing Mass Spectrometers

In the 1950's, the first commercial mass spectrometers were sector instruments. An electric sector consists of two concentric curved plates. A voltage is applied across these plates to bend the ion beam as it travels through the analyzer. The voltage is set so that the beam follows the curve of the analyzer. The radius of the ion trajectory (r) depends upon the kinetic energy of the ion (V) and the potential field (E) applied across the plates.

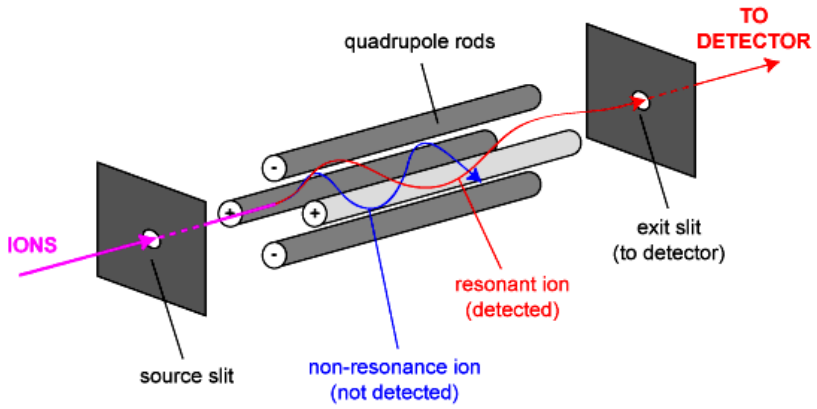
$r = 2V/E$

An electric sector will not separate ions accelerated to a uniform kinetic energy. The radius of the ion beam is independent of the ion's mass to charge ratio so the electric sector is not useful as a standalone mass analyzer. An electric sector is useful in series with a magnetic sector. The mass resolution of a magnetic sector is limited by the kinetic energy distribution (V) of the ion beam. This kinetic energy distribution results from variations in the acceleration of ions produced at different locations in the source and from the initial kinetic energy distribution of the molecules. An electric sector significantly improves the resolution of the magnetic sector by reducing the kinetic energy distribution of the ions. The effect of the electric sector is for a reverse geometry (BE) instrument with the magnetic sector (B) located before the electric sector (E). They consist of some combination of a large electromagnetic ('B' sector), and some kind of electrostatic focusing device ('E' sector). A standard 'BE' geometry double focusing instrument that is, a dual sector instrument consisting of magnetic sector followed by an electrostatic sector.



A sector mass spectrometer

Ions enter the instrument from the source (bottom left) where they are initially focused. They enter the magnetic sector through the source slit where they are deflected according to the left-hand rule. Higher-mass ions are deflected less than lower-mass ions. Scanning the magnet enables ions of different masses to be focused on the monitor slit. The ions have been separated only by their masses. To obtain a spectrum of good resolution - i.e. where all ions with the same *m/z* appear coincident as one peak in the spectrum, ions have to be filtered by their kinetic energies. After another stage of focusing the ions enter the electrostatic sector where ions of the same *m/z* have their energy distributions corrected for and are focused at the double focusing point on the detector slit. Single sector instruments are also used in the specialist area of isotope ratio mass spectrometry (IRMS).



Magnetic Sector Mass Spectrometers

Quadruple and Triple Quadrupole (QQQ) Mass Analysis

The quadrupole mass analyser was developed in parallel with the quadrupole ion trap by the third Nobel Prize winning mass spectrometry pioneer, Wolfgang Paul (Paul W. 1990). A quadrupole mass analyser consists of four parallel rods that have fixed DC and alternating RF potentials applied to them. Ions produced in the source of the instrument are then focused and passed along the middle of the quadrupoles. Their motion will depend on the electric fields so that only ions of a particular *m/z* will be in resonance and thus pass through to the detector. The RF is varied to bring ions of different *m/z* into focus on the detector and thus build up a mass spectrum. The trajectory of the ions through the quadrupole is actually very complex.

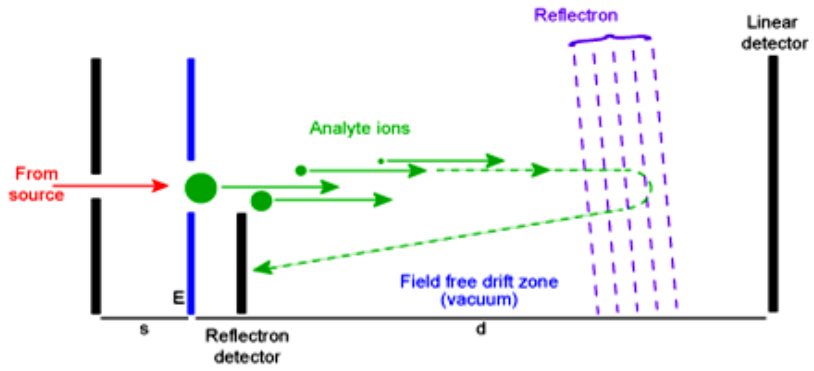
Quadrupole mass analyser

The two opposite rods in the quadrupole have a potential of + (U+Vcost)) (labeled '+' on) and the other two - (U+Vcost)) where 'U' is the fixed potential and Vcost) is the applied RF of amplitude 'V' and frequency '□ '. The applied potentials on the opposed pairs of rods varies sinusoidally as cost) cycles with time 't'. This results in ions being able to traverse the field free region along the central axis of the rods but with oscillations amongst the poles themselves. These oscillations result in complex ion trajectories dependent on the *m/z* of the ions. Specific combinations of the potentials 'U' and 'V' and frequency '□ ' will result in specific ions being in resonance creating a stable trajectory through the quadrupole to the detector. All other *m/z* values will be non-resonant and will hit the quadrupoles and not be detected. The mass range and resolution of the instrument is determined by the length and diameter of the rods. They are very commonly used in conjunction with either gas-chromatography or liquid-chromatography as a simple high throughput screening system. Quadrupoles can also be placed in tandem to enable them to perform fragmentation studies the most common set-up is the triple quadrupole (QQQ) (Yost R.A et.al 1978) mass spectrometer which enables basic ion fragmentation studies tandem Mass spectrometry MS/MS to be performed. This technique (Douglas M. et.al 1998) was used for structural characterization of carbohydrate sequence, linkage and branching in a Neutral Oligosaccharide and N-Linked Glycans.

Time-of-Flight (TOF) Mass Analysis

The time of flight (TOF) describes the method used to measures the time that it takes for a particle, object or stream to reach a detector while traveling over a known distance. In time-of-flight mass spectrometry, ions are accelerated by an electrical field to the same kinetic energy with the velocity of the ion depending on the mass-to-charge ratio.

Thus the time-of-flight can be used to determine the mass-to-charge ratio (Cotter et.al 1994). The time-of-flight of electrons is used to measure their kinetic energy. Innear infrared spectroscopy, the time-of-flight method is used to estimate the wavelength dependent optical path length. With an ultrasonic flow meter measurement, the principle is used to speed of signal propagation upstream and downstream of flow, in order to estimate total flow velocity. Optical time-of-flight sensors also exist, but depend on timing individual particles following the flow rather than using Doppler changes in the flow itself (as this would require generally high flow velocities and extremely narrow-band optical filters time-of-flight mass spectrometry (TOF-MS) is the simplest method of mass measurement to conceptualize, although there are hidden complexities when it comes to higher resolution applications. Their design was published in 1955(Wiley W.C et.al 1955). TOF-MS has really come into its own in recent years as being an essential instrument for biological analysis applications this is especially the case with the coupling of TOF-MS to MALDI and ESI ionisation methods and the development of high-resolution and hybrid instruments (for example Q-TOF and TOF-TOF configurations). The inherent characteristics of TOF-MS are extreme sensitivity (all ions are detected), almost unlimited mass range and speed of analysis. This makes TOF-MS one of the most desirable methods of mass analysis.



A Time-of-Flight mass spectrometer operating in Reflection Mode

The ions were introduced either directly from the source of the instrument or from a previous analyser (in the case of Q-TOF) as a pulse. This results in all the ions receiving the same initial kinetic energy. As they then pass along the field free drift zone, they are separated by their masses, lighter ions travel faster. This enables the instrument to record all ions as they arrive at the detector and so accounts for the techniques high sensitivity. The equation of TOF separation is:

$$\frac{m}{z} = 2e\mathbf{E}s\left(\frac{t}{d}\right)^2$$

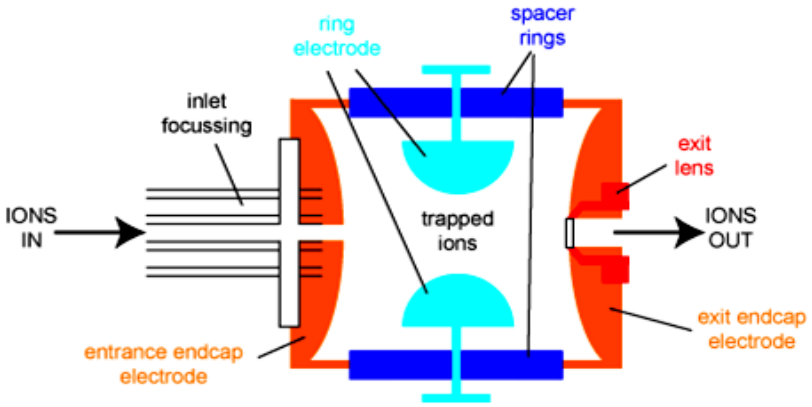
m/z is mass-to-charge ratio of the ion
E is the extraction pulse potential
s is the length of flight tube over which E is applied
d is the length of field free drift zone
t is the measured time-of-flight of the ion

All the ions are given the same initial kinetic energy by the extraction pulse and then drift along the field free drift zone where they will be separated so that all ions of the same *m/z* arrive at the detector at the same time. The pulse is not felt by all ions to the same intensity and so a kinetic energy distribution for each discrete *m/z* exists. This lowers the resolution by creating a time-of-flight distribution for each *m/z*(Cotter R.J 1992). This is relatively easily corrected for by the application of a reflectron at the end of the drift zone(Mamyrin et.al 1973). This consists of a series of electric fields which repulse the ions back along the flight tube usually at a slightly displaced angle resulting in a refocusing of ions with the same *m/z* value on the reflectron detector. This technique was used for structural characterization of oligosaccharides, The 1→3 and 1→6 linkage was confirmed by different fragmentation patterns in oligosaccharides.

Quadruple Ion Trap (QIT) Mass Analysis

The quadrupole ion trap (QIT) mass analyser was developed in parallel with the quadrupole mass analyser by the third Nobel Prize winning mass spectrometry pioneer, Wolfgang Paul (Paul W 1990 & Stafford G.C et.al 1984) in the 1980's. Commercial QIT instruments are very common in Chemical, Biochemical and Forensics laboratories and they are very amenable to being couple with ESI and MALDI ionisation as well as being coupled with liquid chromatography.

The ions, produced in the source of the instrument, enter into the trap through the inlet and are trapped through action of the three hyperbolic electrodes: the ring electrode and the entrance and exit end cap electrodes. Various voltages are applied to these electrodes which results in the formation of a cavity in which ions are trapped. The ring electrode RF potential, an a.c. potential of constant frequency but variable amplitude, produces a 3D quadrupolar potential field within the trap. This traps the ions in a stable oscillating trajectory. The exact motion of the ions is dependent on the voltages applied and their individual mass-to-charge (*m/z*) ratios. For detection of the ions, the potentials are altered to destabilize the ion motions resulting in ejection of the ions through the exit end cap. The ions were usually ejected in order of increasing *m/z* by a gradual change in the potentials. This 'stream' of ions is focused onto the detector of the instrument to produce the mass spectrum.



A Quadrupole Ion Trap Mass Analyser

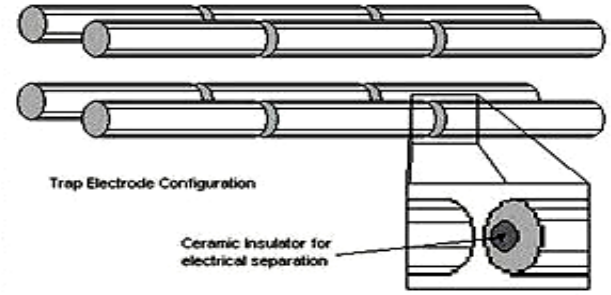
The nature of trapping and ejection makes a quadrupolar ion trap especially suited to performing MSn experiments in structural elucidation studies. It is possible to selectively isolate a particular *m/z* in the trap by ejecting all the other ions from the trap. Fragmentation of this isolated precursor ion can then is induced by CID experiments. The isolation and fragmentation steps can be repeated a number of times and is only limited by the trapping efficiency of the instrument. MS5 experiments are fairly routine with this set-up as is the coupling of liquid chromatography to perform LC-MSn studies. This technique was used for structural characterization of carbohydrate sequence, linkage and branching in a Neutral Oligosaccharide and N-Linked Glycans (Douglas M et.al 1998).

Ion trap

An **ion trap** is a combination of electric or magnetic fields that captures ions in a region of a vacuum system or tube. Two notable types of ion traps are the Penning trap and the Paul trap. For example, in an electron gun (a device emitting high-speed electrons, such as those in CRTs) an ion trap may be implemented above the cathode (using an extra, positively-charged electrode between the cathode and the extraction electrode) to prevent its degradation by positive ions accelerated backward by the fields intended to pull electrons away from the cathode. Ion trap mass spectrometry (ITMS) (Lobvi E. 2007) and the application of this technique to the structural analysis of carbohydrates. The basic principles of operation of the electrostatic ion traps are applicability of the technique to the structural characterization of carbohydrates is illustrated with the analysis of arabinoxylan oligosaccharides by ion trap mass spectrometry.

Linear ion trap

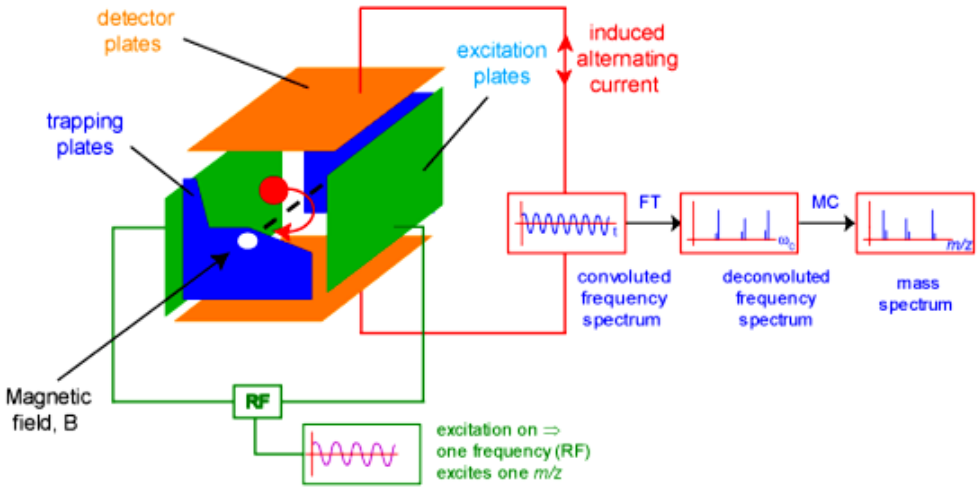
Linear ion trap uses a set of quadrupole rods to confine ions radially and a static electrical potential on end electrodes to confine the ions axially.(Douglas DJ et.al 2005)The linear form of the trap can be used as a selective mass filter or as an actual trap by creating a potential well for the ions along the axis of the electrodes(Raymond E. 2000). Advantages of the linear trap design are increased ion storage capacity, faster scan times, and simplicity of construction (although quadrupole rod alignment is critical, adding a quality control constraint to their production. This constraint is additionally present in the machining requirements of the 3D trap) (schwartz Jae C et.al 2002).



Linear ion trap

Fourier-transform Ion Cyclotron Resonance (FT-ICR)

Alan Marshall and Melvin Comisarow in the 1970's develop the technique (Hipple J.A et.al 1951). The first paper appeared in Chemical Physics Letters in 1974 (Cooley J.W et.al ……). It is a type of mass analyzer (or mass spectrometer) for determining the mass-to-charge ratio (m/z) of ions based on the cyclotron frequency of the ions in a fixed magnetic field (Marshall A.G et.al ……). The ions are trapped in a Penning trap (a magnetic field with electric trapping plates) where they are excited to a larger cyclotron radius by an oscillating electric field perpendicular to the magnetic field. The excitation also results in the ions moving in phase (in a packet). The signal is detected as an image current on a pair of plates which the packet of ions passes close to as they cyclotron. The resulting signal is called a free induction decay (FID), transient or interferogram that consists of a superposition of sine waves. The useful signal is extracted from this data by performing a Fourier transform to give a mass spectrum. Fourier transform ion cyclotron resonance (FTICR) mass spectrometry is a very high resolution technique in that masses can be determined with very high accuracy. Many applications of FTICR-MS use this mass accuracy to help determine the composition of molecules based on accurate mass. This is possible due to the mass defect of the elements. Another place that FTICR-MS is useful is in dealing with complex mixtures since the resolution (narrow peak width) allows the signals of two ions of similar mass to charge (m/z) to be detected as distinct ions. This high resolution is also useful in studying large macromolecules such as proteins with multiple charges which can be produced by electrospray ionization. These large molecules contain a distribution of isotopes that produce a series of isotopic peaks. Because the isotopic peaks are close to each other on the *m/z* axis, due to the multiple charges, the high resolving power of the FTICR is extremely useful. FTICR-MS differs significantly from other mass spectrometry techniques in that the ions are not detected by hitting a detector such as an electron multiplier but only by passing near detection plates. Additionally the masses are not resolved in space or time as with other techniques but only in frequency. Thus, the different ions are not detected in different places as with sector instruments or at different times as with time-of-flight instruments but all ions are detected simultaneously over some given period of time.



FT-ICR-MS showing the ion trapping, detection and signal generation

Due to the ion-trap nature of FT-MS, it is possible to measure the ions without destroying them; this enables further experiments to perform on the ions. The most common of these would be some kind of fragmentation study (MS/MS or MSn) for structural elucidation experiments, but also other gas-phase reactions and studies can be performed - e.g. gas-phase basicity calculations, gas phase kinetics, ion dissociation studies as well as the study of ion-molecule or ion-ion interactions. Alan Marshall has a published a number of reviews of FT-ICR(Marshall A.G et.al 1985) and its applications.

Orbitrap

An orbitrap is a mass spectrometer invented by Alexander Makarov. It consists of an outer barrel-like electrode and a coaxial inner spindle-like electrode that form an electrostatic field with quadro-logarithmic potential distribution(Hu Q et.al 2005).The orbitrap is a modification of the ion trap developed by Kingdon in the early 1920s (Kingdon KH et.al 1923). The Kingdon trap consists of a thin central wire and an outer cylindrical electrode. A static applied voltage results in a radial logorithmic potential between the electrodes. In 1981, Knight introduced a modified outer electrode that included an axial quadrupole term that confines the ions on the trap axis (Knight R.D 1981). Neither the Kingdon nor the Knight configurations were reported to produce mass spectra. Scigelova and Makarov show the resolving power of Orbitraps have a high mass accuracy, a high resolving power and a high dynamic range. Like FTICR-MS. The orbitrap resolving power is proportional to the number of harmonic oscillations of the ions; as a result the resolving power is inversely proportional to the square root of *m/z* and proportional to acquisition time.

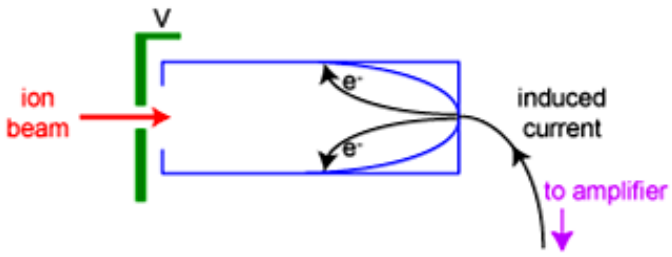
Methods of Ion Detection

Detectors

Mass analysis i.e. the separation of bunches or streams of ions according to their individual mass-to-charge (*m/z*) ratio, is only part of the job of a mass spectrometer. Without some form of accurate and reliable ion detection. All mass spectrometers, apart from FT-ICR- which by its very definition is a combined mass analyser and detector, require an ion detector. The choice of detector depends on the design of the instrument and the type of experiment it was designed to perform. The detector generates a signal from incident ions by either generating secondary electrons, which are further amplified or by inducing a current generated by a moving charge (similar to FT-ICR). The earliest ion detectors (back in the days of Thomson and Aston) consisted of photographic plates located at the end of the mass analyser. All ions of a given *m/z* would impact at the same place on the photographic plate making a spot. The darkness of the spot was indicative of the intensity of that particular *m/z*. The most common types of ion detector are as under,

1. The Faraday Cup or Cylinder

The Faraday cup or cylinder electrode detector is very simple. The basic principle is that the incident ion strikes the dynode surface which emits electrons and induces a current which is amplified and recorded. The dynode electrode is made of a secondary emitting material like CsSb, GaP or BeO. The Faraday cup is a relatively insensitive detector but is very robust. It is ideally suited to isotope analysis and IRMS.

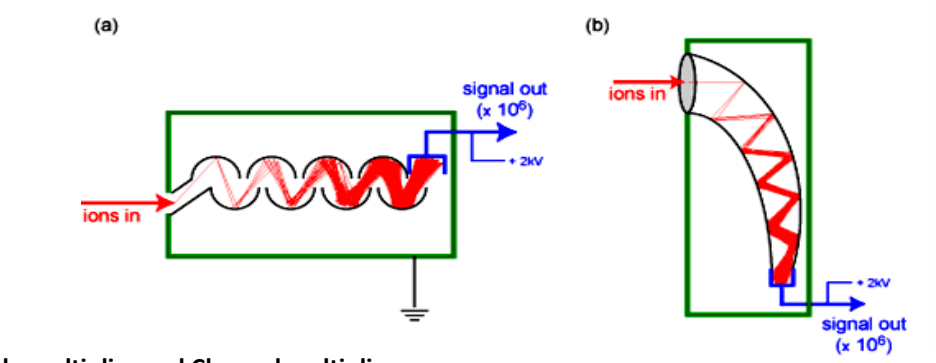


Faraday Cup or Cylinder Electrode

2. The Electron Multiplier

Electron multipliers are probably the most common means of detecting ions, especially when positive and negative ions need to be detected on the same instrument. There are two types of electron multiplier, but they both work essentially by extending the principles of the Faraday cup. A Faraday cup uses one dynode and as a result produces one level of signal amplification. One type of electron multiplier has series of dynodes maintained at increasing potentials resulting in a series of amplifications.

The other type (the channel multiplier) has a curved ('horn' shaped) continuous dynode where amplifications occur through repeated collisions with the dynode surface. In both cases, ions pass the conversion dynode (depending on their charge) and strike the initial amplification dynode surface producing an emission of secondary electrons which are then attracted either to the second dynode, or into the continuous dynode where more secondary electrons are generated in a repeating process ultimately resulting in a cascade of electrons.



Dynode multiplier and Channel multiplier

3. The Photomultiplier or Scintillation Counter

In the photomultiplier (or scintillation counter) the ions initially strike a dynode which results in electron emission. These electrons then strike a phosphorous screen which in turn releases a burst of photons. The photons then pass into the multiplier where amplification occurs in a cascade fashion much like with the electron multiplier. The main advantage of using photons is that the multiplier can be kept sealed in a vacuum preventing contamination and greatly extending the lifetime of the detector. Photomultipliers are now probably the most common detectors in modern mass spectrometers. A photomultiplier, showing the conversion of the ion/electron signal into photon(s) which are then amplified and detected by the photomultiplier.

The Photomultiplier or Scintillation Counter

TANDEM MASS SPECTROMETRY

Tandem mass spectrometry (MS-MS) is used to produce structural information about a compound by fragmenting specific sample ions inside the mass spectrometer and identifying the resulting fragment ions. This information can then be pieced together to generate structural information regarding the intact molecule. Tandem mass spectrometry also enables specific compounds to be detected in complex mixtures on account of their specific and characteristic fragmentation patterns. A tandem mass spectrometer is a mass spectrometer that has more than one analyser, in usually two. The two analysers are separated by a collision cell into which an inert gas (e.g. argon, xenon) is admitted to collide with the selected sample ions and bring about their fragmentation. The analysers can be of the same or of different types, the most common combinations being:

Quadrupole - quadrupole

Magnetic sector - quadrupole

Magnetic sector - magnetic sector

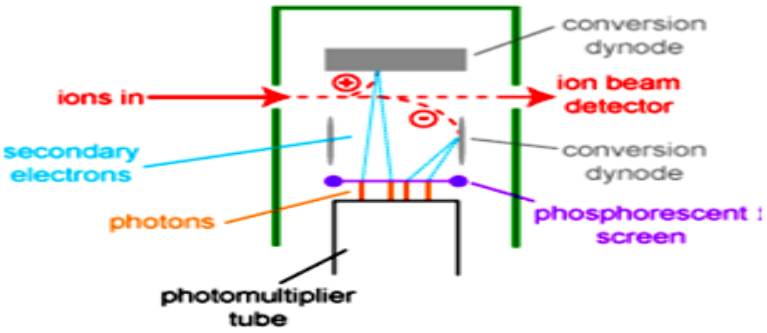
Quadrupole - time-of-flight

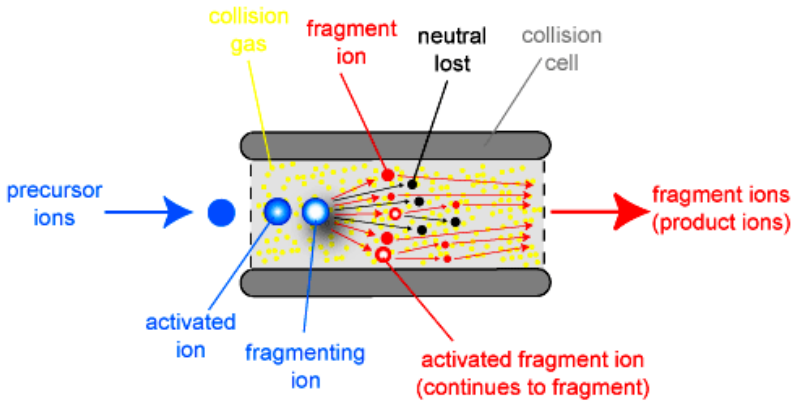
The purpose of this technique is to develop tandem mass spectrometric (Zhongrui Zhou et.al 1990 and Yoo E et. al 2005). Methods to determine detailed carbohydrate structures on permethylated or partially methylated oligosaccharides for future applications on biologically active glycoconjugates and to exploit a faster method of synthesizing a series of structural isomeric oligosaccharides to be used for further mass spectrometry and instrumental analysis. This technique can be applied to larger oligosaccharides for assigning the linkages for correct determination of position and type of linkage by the tandem mass spectroscopy.

Collision Induced Dissociation (CID)

In MS/MS and MSnstudies, the precursor is fragmented in a collision cell or chamber before the mass spectrum is acquired. This produces a product ion scan for the particular precursor ion. This type of data is used for sequencing (peptides and sugars), structural elucidation and analyte identification through fragment fingerprinting.

The most common method of fragmentation is collision induced dissociation (CID), it is also called collisionally activated decomposition (CAD). The precursor ion enters the collision cell containing a high pressure of an energized, chemically inert collision gas - Ar, He, N_2, CO_2 etc.





A CID fragmentation

The precursor ion undergoes repeated collisions with the collision gas, building up potential energy in the molecule, until eventually the fragmentation threshold is reached and the product ions are formed. The types of fragmentation that occur vary considerably with the type of product ion and the amount of energy involved. At lower energies (close to the threshold), fragmentation reactions are often limited to neutral losses (H_2O, MeOH, CO, CO_2, MeCN etc.) depending on the nature of the precursor ion. These neutral losses are often not considered structurally significant, although they can be used to obtain information about functional groups. At higher energies, retro-synthetic type reactions are often observed. These are much more structurally significant, and often result in cleavage of the molecule at characteristic positions. If the energy is too high, C-C bong cleavage can occur leading to uncontrolled fragmentation. Usually it is best to work at around the fragmentation threshold, or just above, to maintain most control over the fragmentation processes. Ion-trap and FT-MSinstruments allow for the most control over CID, but also tend to produce less energetic reactions. Triple quadrupole and QTof instruments tend to produce more energetic CID with more fragmentation, but less operator control. Ion-trap and FT-MS allow multistage fragmentation experiments to be conducted though, which is essential for structural elucidation studies. More on CID fragmentation and structural elucidation is in the application. Structural information can be obtained from fragmentation-induced collision induced by decomposition (CID)(Kovacik V et.al 1995, Reinhold VN et.al, Harvey D. et.al 1997, Morris HR et.al 1996 and Tseng K et.al 1999). The fragmentation is governed primarily by glycosidic cleavages between the monosaccharide rings and by cross-ring cleavages. High energy CID spectra of underivatised oligosaccharides ionised by MALDI to give the sodium adduct Na+ (MNa+), may be detected by a combination of a magnetic sector mass spectrometer with an orthogonal acceleration tandem TOF analyser. CID spectra have also been recorded using tandem MS on an ES quadropole/orthogonal acceleration TOF mass spectrometer.

Uses of Mass spectrometry with GC, LC and other separation techniques

Mass spectrometry is a particularly powerful detector for separation techniques like gas chromatography (GC), liquid chromatography (LC), capillary electrophoresis and supercritical fluid chromatography because of its great sensitivity and ability to identify chemical compounds positively.

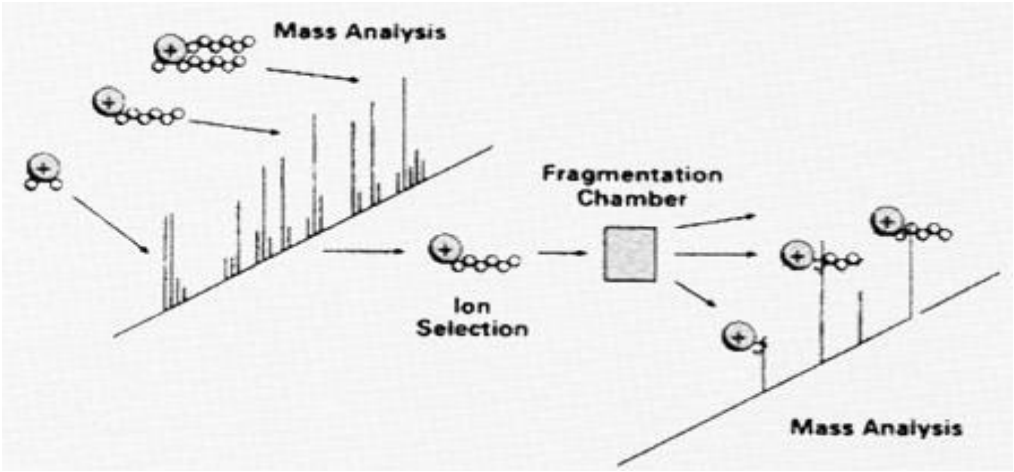
The challenge in interfacing a mass spectrometer to a separation system like a gas or liquid chromatography in maintaining the required vacuum in the mass spectrometer while introducing flow from the chromatograph. Interfaces that restrict or reduce the gas flow into the mass spectrometer (e.g. flow splitters or devices that differentially remove carrier gas from the GC effluent) have made the combination of gas chromatography and mass spectrometry (GC/MS) a widely used technique.

Mass spectrometry in quantitative analysis

Researchers are not always interested in obtaining full mass spectra in cases where the compounds are already known. Rather, they may want to confirm the presence of specific substances or measure how much is present. This is commonly done in environmental pollutant work and in pharmacokinetic studies where the goal is quantization at very low concentrations in complex mixtures. The mass spectrometer is set to monitor only m/z values of ions representative of the molecules of interest so that valuable detection time is not wasted.

Mass spectrometry/mass spectrometry (MS/MS)

Coupling two stages of mass analysis (MS/MS) can be very useful in identifying compounds in complex mixtures and in determining structures of unknown substances. In product ion scanning, the most frequently used MS/MS mode, product ion spectra of ions of any chosen m/z value represented in the conventional mass spectrum are generated. From a mixture of ions in the source region or collected in an ion trap, ions of a particular m/z value are selected in the first stage of mass analysis. These "parent" or "precursor" ions are fragmented and then the product ions resulting from the fragmentation are analyzed in a second stage of mass analysis. If the sample is a pure compound and fragment-forming ionization is used, the product spectra obtained from the fragment ions in the normal mass spectrum can provide much additional information for structural analysis.



In MS/MS, mass analyzer

Elemental mass spectrometry

In elemental mass spectrometry, a technique used mostly for inorganic materials, the elemental composition of a sample is determined rather than the structural identities of its chemical constituents. Elemental mass spectrometry provides quantitative information about the concentrations of those elements. The ion source used in elemental MS is ordinarily an atmospheric-pressure discharge such as the inductively coupled plasma (ICP) or a moderate-power device such as the glow-discharge source. In either case, the decomposition of the sample into its constituent atoms and ionization of those atoms occurs in a specially designed source. The resulting atomic-ion beam is then separated or sorted by a mass spectrometer and the signal as a function of m/z used to determine the sample composition.

MASS SPECTROSCOPY: (Alving K et.al 1999)

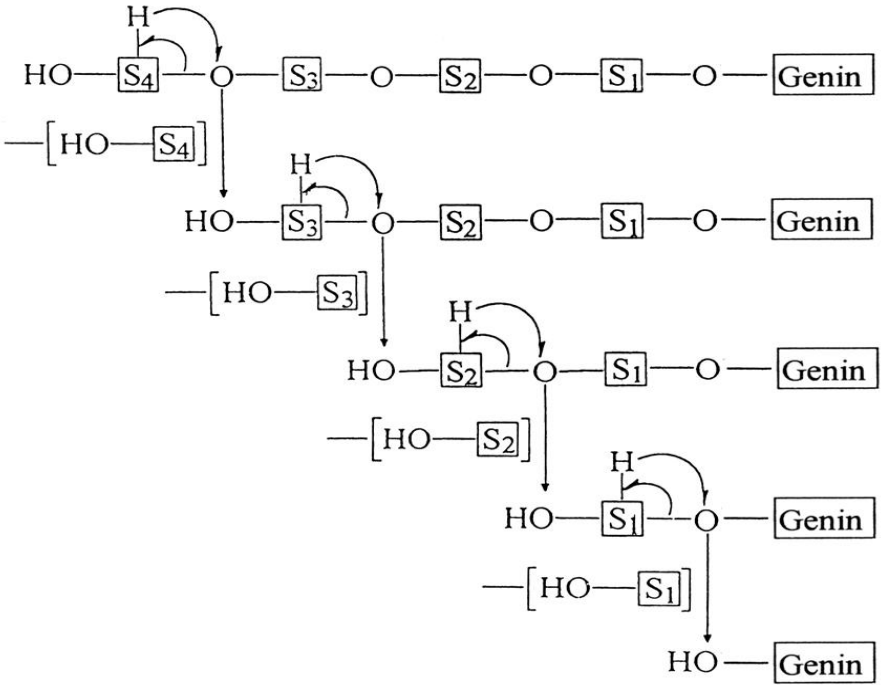
The characterization and analysis of glycoconjugates glycosides and oligosaccharides by mass spectrometry (McCloskey J.A 1990) has undergone a number of improvements in the last 10 years, especially with the development of methods capable of ionizing and analyzing these compounds in their native states. Mass spectrometry is one of the oldest and broadly applicable analytical tools in the chemical sciences in biological research. Mass spectroscopy is an important analytical tool for structure elucidation of complex oligosaccharides/oligoglycosides (Khare M.P et.al 1987) comprising composition, sequence, branching, and linkage analysis, including anomericity and finally also the rings sizes and absolute configuration.

Mass Spectroscopy of Steroid and Steroidal Glycosides

Mass Spectroscopy plays a decisive role in structure elucidation of natural products particularly in steroids and steroidal glycosides.

Structural Features of Steroidal Glycosides (Pregnane, Nor-Pregnane and Cardiac)

Glycosides are compounds in which the sugar is linked to an aglycon moiety through its anomeric carbon by an acetal linkage when the aglycon is a C-21 steroid, (i.e. pregnane) or C-23 steroid, and then it is a pregnane glycoside or cardiac glycoside respectively. They are found in nature either in free form or as O-glycosides containing sugar moiety linked to an alcoholic hydroxyl group mostly present at C-3 (Prakash K. et.al 1992), C-20 (Srivastava O.P et.al 1982) or both(Mu Q.Z et.al 1986) (bisdesmosidic glycosides) through acetal linkage. Pregnane glycosides, containing one (Chen Z.S et.al 1991) to six (Itokawa H et.al 1988 and Chen J et.al 1990) sugar units have been isolated from the extracts of different parts of plants, i.e., roots, stem, seeds etc and their structures has been elucidated by mass spectrometry.



H - Transfer in Oligoglycoside and Elimination of Terminal Sugar Residues

Mass fragmentation pattern of pregnaneoligoglycoside

The difficulties in the application of mass spectrometry (MS) to the field of glycosides (mono to oligo) which was initially limited due to relatively low volatility of these compounds have been overcome, partially, due to new inlet techniques and also by pioneering studies on specific volatile derivatives. MS gave adequate information to understand the characteristic fragmentation and rearrangements of the sugar moiety. Tschesche et al. (1965) have studied stereochemical differences in the molecule by mass spectral fragments. From the study of the mass spectral analysis of underivatised glycosides, it was apparent that the technique of field desorption (FD) in conjunction with the usual electron impact (El) method offers appreciable advantage. In particular higher mass ion peaks such as molecular ions were more evident in FD mass spectra but the ionization does not give extensive fragment ions as do the El mass spectra which often provide more valuable structural information. Of late, Fast atom bombardment (FAB) is increasingly gaining acceptance as one of the most important ionization methods in mass spectrometry. FAB mass spectra of pregnane glycosides invariably give a protonated or an alkali metal cationized molecular ion (when alkali metal salt is added to the liquid matrix) alongwith the fragments arising out of the fragmentation of the molecule thus helping in determining the molecular weight and structures of these biologically important compounds. In pregnaneoligo glycosides the individual monosaccharide units break according to (Brown at al. 1971) along with the hydroxyl group displacement everytime it gets detached from the glycosidic bond.

The stepwise elimination of monosaccharide units leads to mass fragment corresponding to the genin. Sometimes, it is also seen that (M^+-genin) i.e. tetra, tri and disaccharide fragment is obtained (Srivastava et al. 1994b) which further fragments by repeated H- transfers in the oligosaccharide accompanied the elimination of terminal sugars less water, until only the monosaccharide remains.

Basic Structure of Pregnanes and their Mass Fragmentation

Pregnanes are C-21 steroidal compounds having the usual perhydro-1, 2-cyclopentanophenanthrene ring system with β-oriented angular methyl groups at C-21 and C-13 and a two carbon side chain at C-17. Usually pregnane derivatives (Kennard O et.al 1968) possess a β-oriented hydroxyl group at C-14. The configuaration at C-5 has commonly been found to be α-excepting molecules containing a Δ^5. Like all other naturally occurring steroidal compounds, pregnanes also have a hydroxyl group at C-3, which is, however, always β-oriented. Characteristic features of pregnanegenins reported so far may be summarised as follows:

a) Double bond at C-5 (Δ^5) (Trigg P.I et.al 1989)

b) Fusion of ring B and C is always trans.

c) Fusion of ring C and D is cis if hydroxy group at C-14 and trans if hydrogen at C-14.

d) Two carbon chains at C-17, either hydroxy ethyl or acetyl group, in either α or β-configuration.

e) Additional hydroxyl groups at 5α, 6β, 7α, 8β, 11α, 12α or 12β, 14β, 15α, 16α, 17β, 20 and 21 which may be partially esterified.

f) Carbonyl functions at C-1, C-12, C-15 and C-20.

Variations in the common steroidal skeleton of pregnane derivatives are also known where cyclic ethers are also present introducing new rings with C-20. Hirundigenin (Zhang Z.X et.al 1988) gave an unusual modified skeleton for pregnane. Some 14, 15 seco (Srivastava S. et.al 1991)and 13, 14; 14, 15 disecopregnanes 23 have also been isolated. The nor pregnanes are also steroidal compounds having the same basic perhydrocyclopentano phenanthrene nucleus along with a substituent at the C-17 position as in the pregnane glycosides. The only difference being the absence of one or more carbon atoms from the usual C-21 skeleton. When the angular methyl group at C-10 or C-13 is absent then the pregnanegenin termed as a 19-nor or 18-nor pregnane respectively. The MS is greatly useful in assigning the substituent groups in the aglycon part of the pregnane glycoside. The location of hydroxyl group strongly influences the fragmentation of pregnane derivatives. The positions and number of acyl groups present in the aglycon could well be interpreted by the information obtained from the mass spectral fragments. The nature of acid group of acyl derivative could be ascertained by the spectral fragments obtained in the lower mass region of methanolysis product of the glycosides. From the recent study of MS of polyhydroxy pregnanes (Tschesche R et.al 1965 and Hayashi K et.al 1975) a correlation between the structures and the pattern of fragmentation have been deduced. The characteristic fragmentation patterns are schematically presented in following pages.

a) Pregnanes having 3-OR-Δ^5 (R=H or acyl group) undergo retro-Diels-Alder fission followed by elimination of –ROH molecule and Me radical to give prominent peaks. Similarly, retro-Diels-Alder cleavage is observed in 11-OR pregnanes (R=H or acyl group) after elimination of oxygen function as –ROH involving C-9H.

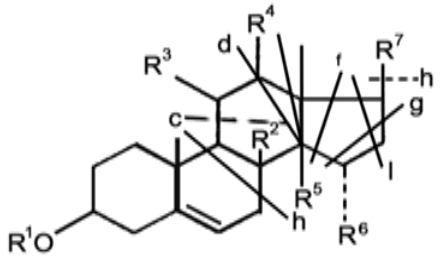
b) 14β-hydroxy pregnanes having 20-keto, 17β side chain undrego D-ring cleavage with the loss of ethylene molecule, the fragments thus reported are M^+-28; M^+-26, M^+-74; M+-85 and M^+-89. This fragmentation is highly stereoselective and is independent of presence or absence of other functional groups in the molecule and compounds with 17α - side chain do not show this fragmentation instead M^+-51, M^+-69, M^+-88, M^+-18 and M^+-n. H_2O-$COCH_3$ is obtained.

The only difference between the fragmentation of pregnanes and C-21 nor pregnanes is the loss of the side chain. In the case of the pregnanes loss of ions of m/z 45 or m/z 43 from M^+ is seen which is caused by the fragmentation of CH (OH) CH3 or $COCH_3$ side chains respectively while in the case of C-21 nor pregnanes the loss is of m/z 15 only.

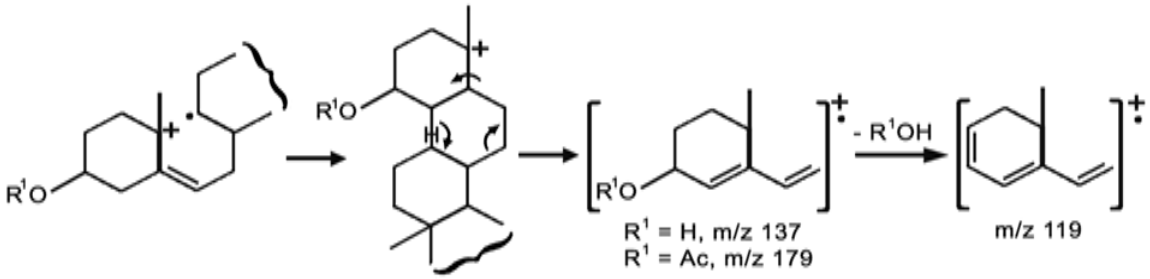
Basic Skeleton of Cardenolides (Srivastava S 1990) and Bufadienolides (Brown P et.al 1972)

About 5 dozen cardiac genins have been isolated from plants till date which are defined a steroids having the perhydrocyclopentanon phenanthrene nucleus and an α, β-unsaturated lactone ring at C-17. These genins can again be classified in to different groups i.e. Cardenolides and Bufadienolides. The cardenolides are C-23 steroidal compound having 5-membered unsaturated lactone rings at C-17 whereas bufadienolides are C-24 steroids with six membered lactone ring at C-17. The most common member of Digitoxigenin, a cardenolide genin having 5βH, it fulfills most of the fundamental requirements necessary for cardenolide.

Fragmentation 'b'



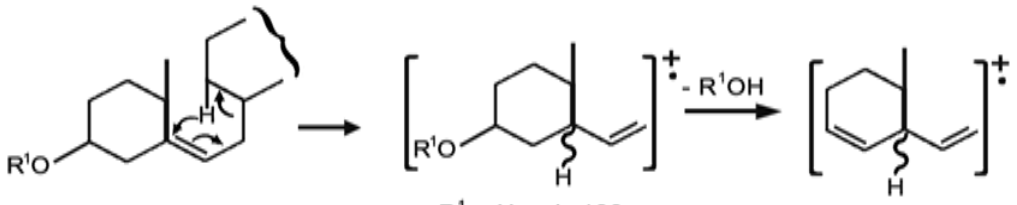
(i)



R^1 = H, m/z 137
R^1 = Ac, m/z 179

m/z 119

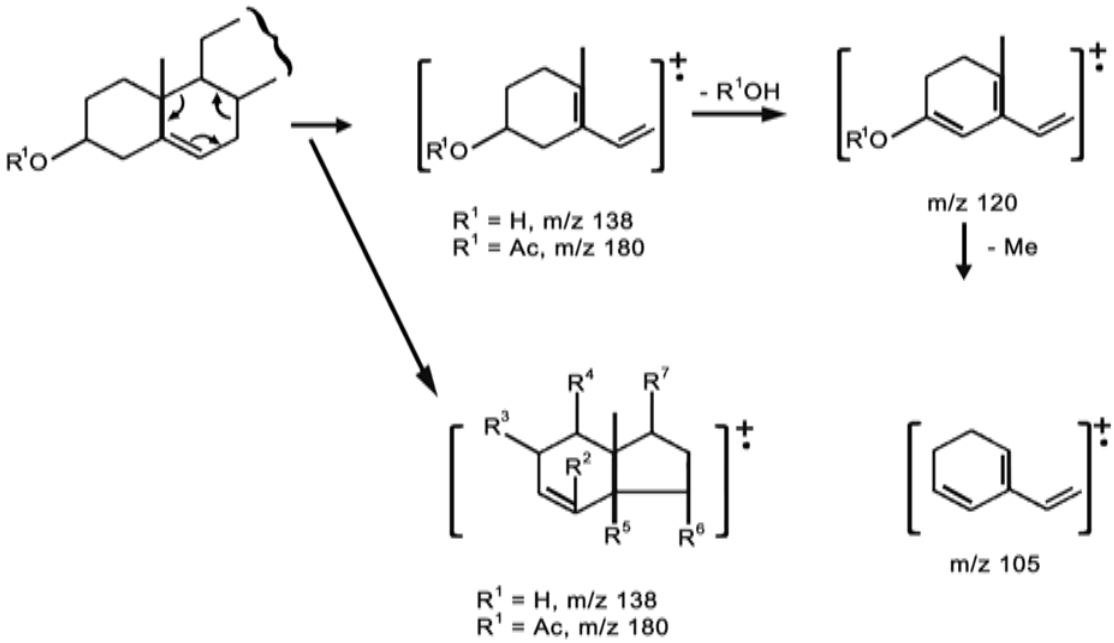
(g)



R^1 = H, m/z 139
R^1 = Ac, m/z 181

m/z 121

(iii) RDA at Δ^8



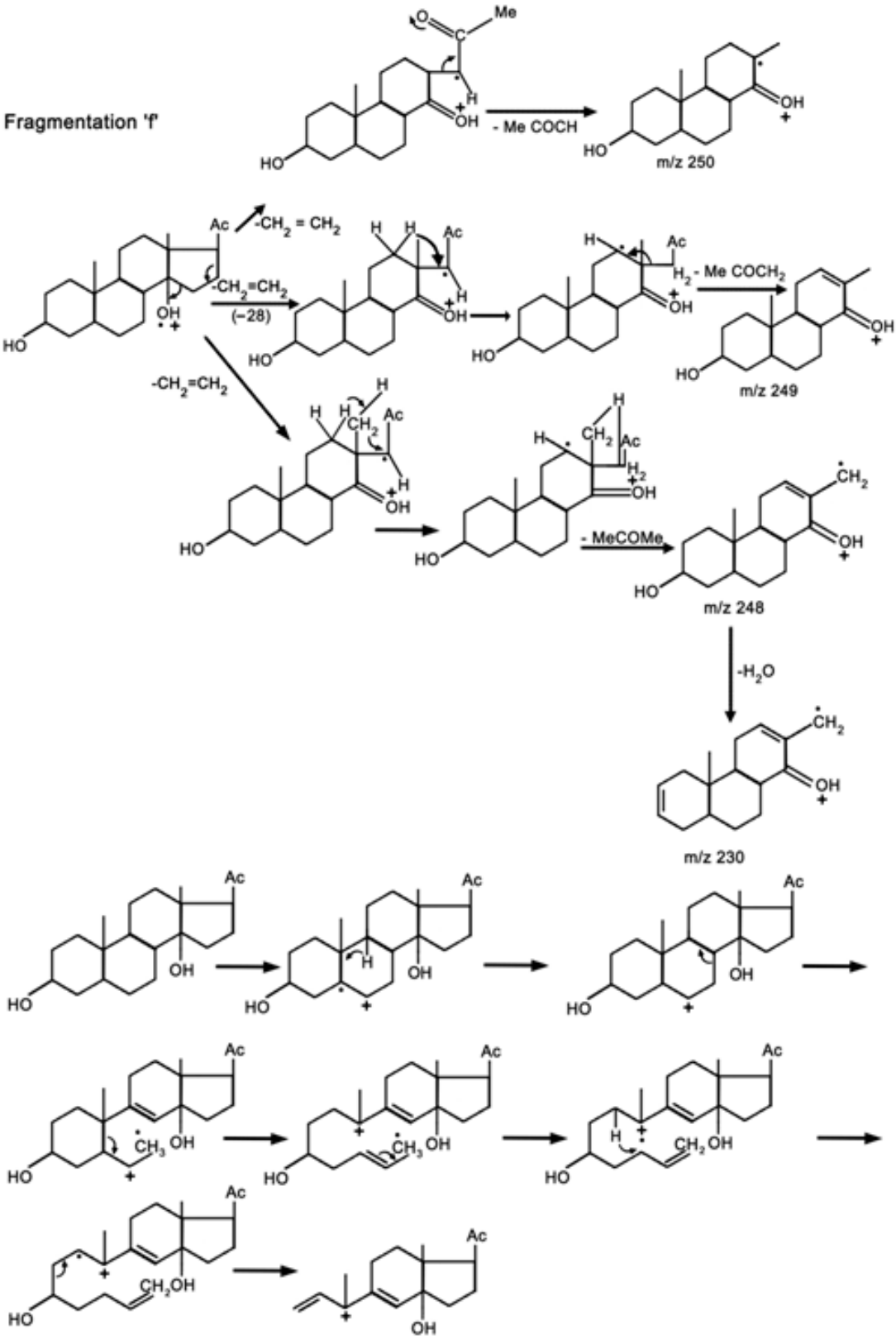
R^1 = H, m/z 138
R^1 = Ac, m/z 180

m/z 120

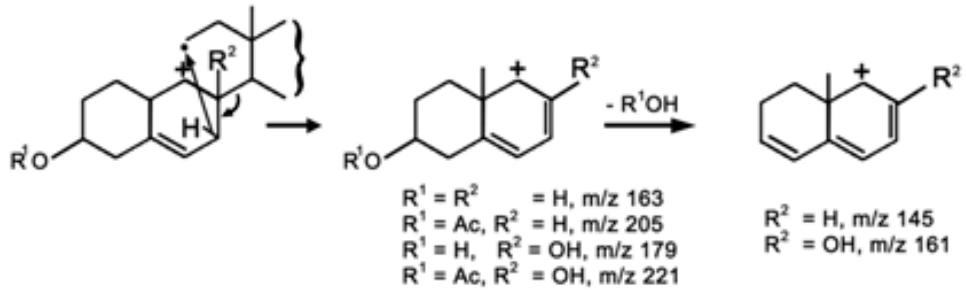
- Me

m/z 105

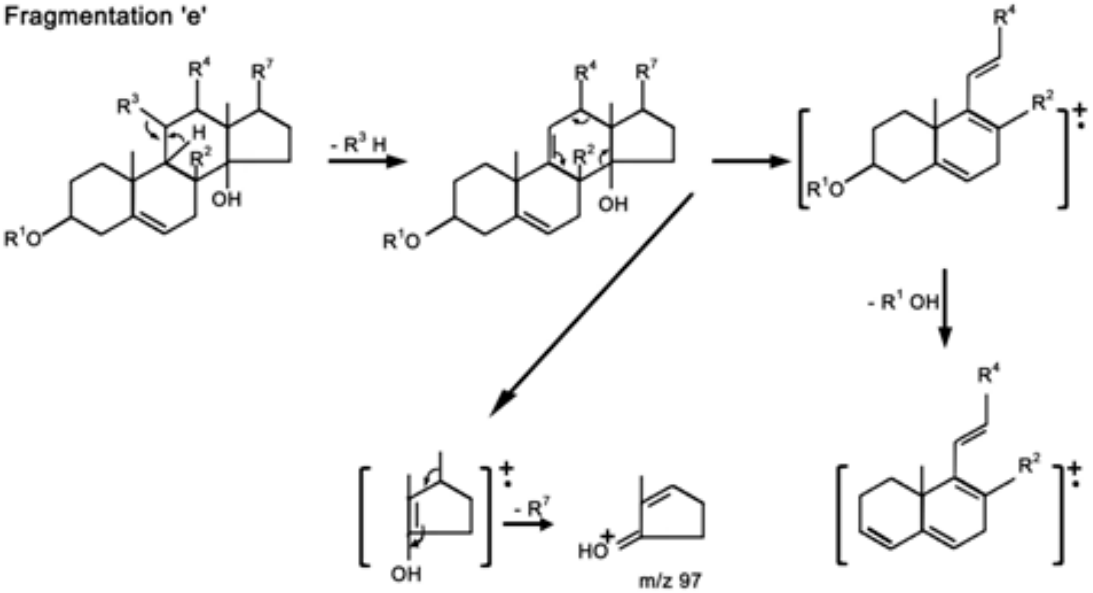
R^1 = H, m/z 138
R^1 = Ac, m/z 180


Fragmentation 'f'
- Me COCH
m/z 250
-CH2 = CH2
-CH2=CH2
(–28)
- Me COCH2
m/z 249
-CH2=CH2
- MeCOMe
m/z 248
-H2O
m/z 230

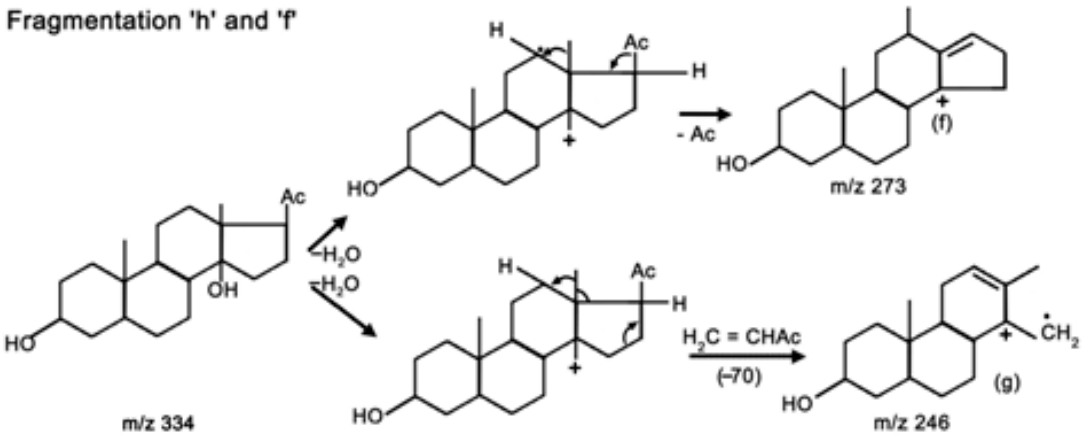
Fragmentation 'c'



Fragmentation 'e'



Fragmentation 'h' and 'f'



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The cardenolides may be assumed as a derivative of this parent cardenolide. Some other unique characteristic features of cardenolides genins are as follows,

1. The presence of isomeric groups at C-3, C-5 and C-17.
2. Double bonds at C-4, C-5 and C-16.
3. Hydroxy group substitutions at 1β, 2α, 3β, 5β, 11α, 11β, (12β) α, 15β, 16β and 19. In few cases ester functions are also reported.
4. Carbonyl functions at C-11, C-12 and C-19.
5. Epoxy groups at three sites, namely 7-8β, 8-14β and 11-12β.

Angular methyl groups at C-10 and C-13 are also found in this aglycon, as in the sterols, however in some of the cardenolides angular aldehydic, hydroxyl-methyl or carboxylic group at C-10 are also encountered. The C-11 hydroxy group when present is mostly of α-configuration unlike those obtained from animal's sources. In analogy with known process in animal organism as well as in microorganism it may be assumed that in plants hydroxylation occurs after the formation of the steroidal skeleton which has also been experimentally in the case of 12-βhydroxy groups. Structural variations around the typical structural pattern are numerous but they usually involve epimerisation at C-3, C-5, C-17 and sometimes the addition of oxygen function at other position.

Mass fragmentation pattern of cardiac glycosides

The mass spectroscopy has proved to be most useful tool for assigning the no. and position of substituents in Cardenolides. It was demonstrated that the location of hydroxyl group strongly influence the fragmentation of cardenolides. From a recent study of mass spectra of large number of cardenolides Pettit et.al (Brown p et.al 1972). Deduced a correlation between their structure and fragmentation pattern which is very useful in the elucidation of their structures. Some representative fragments and fragmentation routes are presented as under-

a) Cardenolides having a hydroxyl group at position-3 undergo Retro-Diels Alder fission after the elimination of a molecule of water.

b) It has been proposed that the fragment ion peaks at m/z 111, m/z 124, m/z 163 and m/z 164 in the MS of cardeno-lides arise out of butenolide and C ring of cardenolides having an unsubstituted C ring.

c) Mass spectra of cardenolide having one hydroxyl group in ring A, B and C in addition to C-14 hydroxyl group contain strong ion peaks at m/z 221, m/z 203, m/z 213, mhz 231, m/z 249, m/z 259 and m/z 241. However, when 2 hydroxyl groups are present in ring A, B or C in addition to C -14 hydroxyl group, the ion peaks present in their mass spectra are some what different.

d) The prominent ion peak at m/z 251 in the MS of genins with a carbonyl and a hydroxyl group in ring C, is analogous to the ion peak at m/z 221 in the MS of genins having one hydroxyl group in ring A, B or C in addition to that at C-14. Other characteristic fragment ion peaks of this group of genin are m/z 233, m/z 251, m/z 294, m/z 276, m/z 258.

e) Characteristic mass fragments for cardenolides and bufadienolides shows the most important fragments at m/z 111 and m/z 123 respectively which arise by the fragmentation of the five membered and six membered lactone ring respectively.

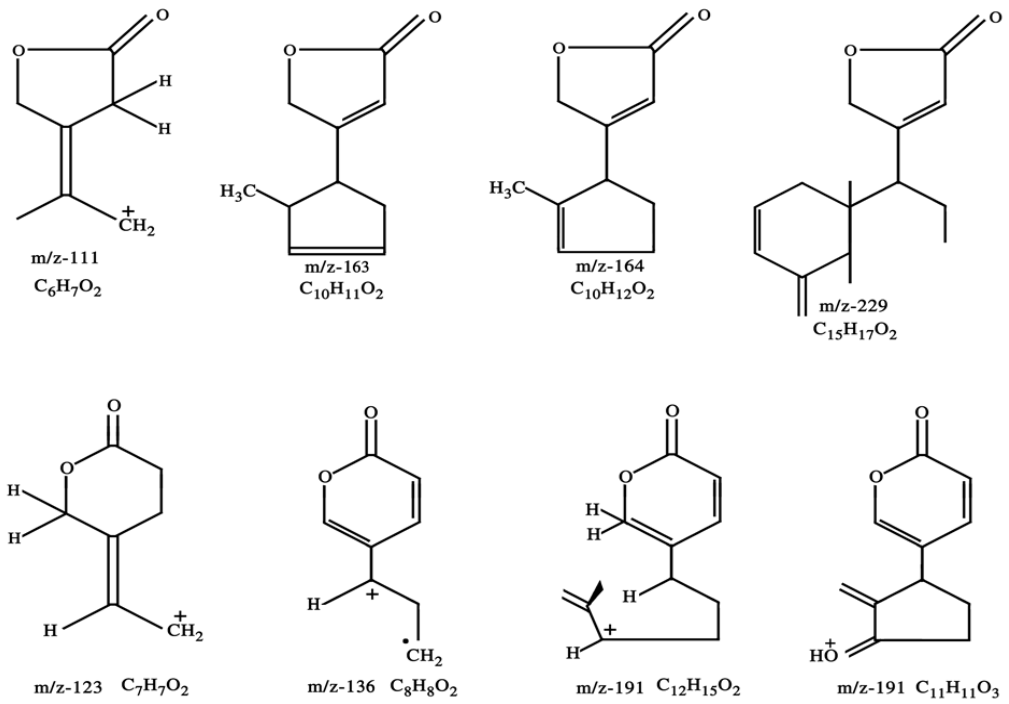
f) The other important fragments for cardiac genins are those involving the D ring with five and six membered lactone ring and giving fragments at m/z 163/164 and 191 respectively.

g) The cardenolides also show another characteristic fragment at 229 which encorporates the ring C and D along with the five membered lactone rings.

All the other fragmentations in the cardenolides, bufadienolides and the pregnanes resemble very much with each other because of the fact that they arise from the perhydrocyclopentanophenantherene nucleus. The stereochemistry (a or ß orientation) of the C-17 side chain can also be determined by Mass spectrometry.

Mass Fragmentation Pattern of Oligosaccharides

Mass spectrometry also provides useful information regarding the sequence of monosaccharides in the oligosaccharide chain linked to the genin moiety in the cardiac as well as the pregnane glycosides. The individual monosaccharide units get detached from the molecular ion peak at the point of linkage along with hydroxyl group displacement. The stepwise elimination of the monosaccharide units starting from the terminal end leads to the formation of the genin fragment.



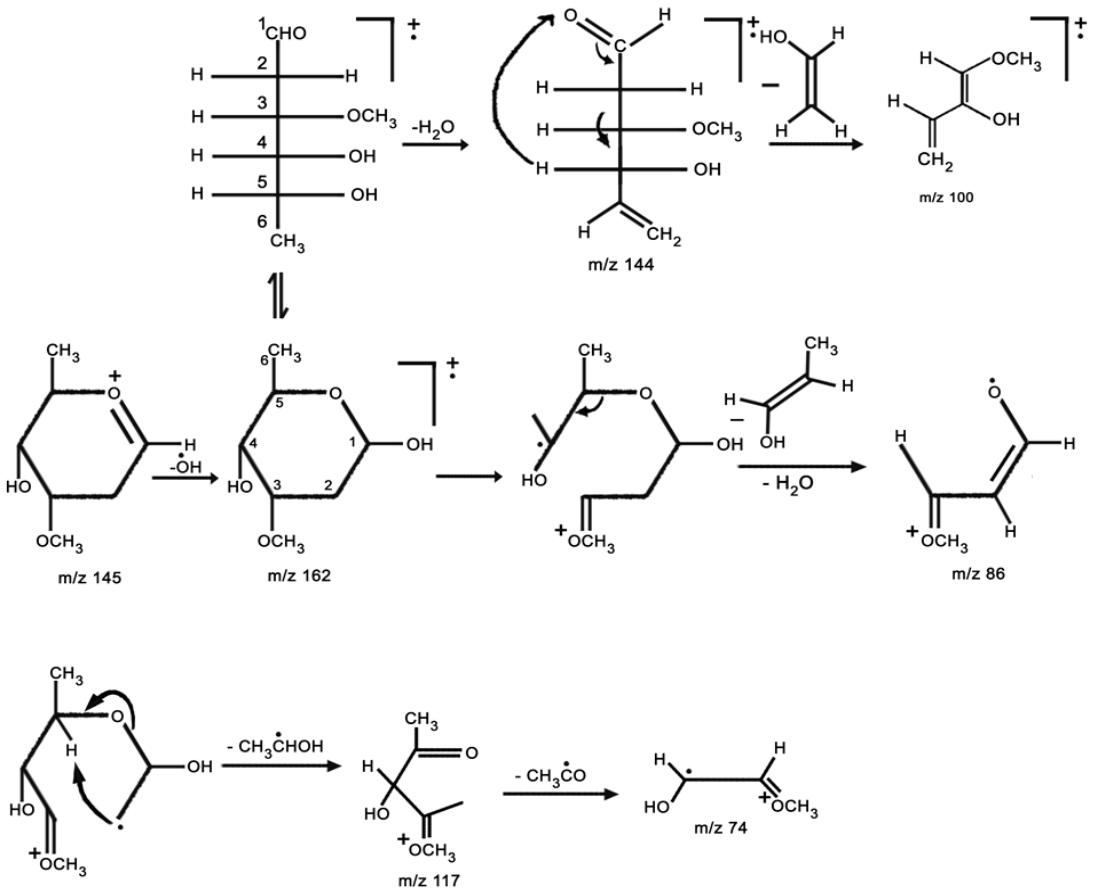
1-Mass fragmentation pattern of 2-de-oxy sugars

With the aim of using mass spectrometry in the structural elucidation of oligosaccharides, a high resolution LI mass spectrum of a model 2-deoxyhexose disaccharide, β methyl pachybioside, was taken to establish the principles which might govern fragmentation pathways for these compounds. The high-resolution mass spectral results, in addition to giving information about the elemental composition of the compound, also furnish equally well information on the composition of fragment ions and thus greatly assist in the correct interpretation of a mass spectrum. The verification of the elemental composition of an ion 'is quite important, whenever its genesis is discussed, for the purpose of determining a fragmentation mechanism for the given structure.

Mass fragmentation pattern of 2- de-oxy sugar

The high-resolution mass spectrum of methyl pachybioside shows its highest-mass ion peak at m/z 318.1678 (1, M-H_2O, $C_{15}H_{26}O_7^+$).The first decomposition pathway (Scheme 1) shows the formation of those fragments which are formed by the usual loss of the elements of water, methanol, and CH_3CHO in different sequences. It also includes the retro Diels Alder fragmentation initiated by the double bond created by the loss of water or methanol, yielding species having the following m/z values:

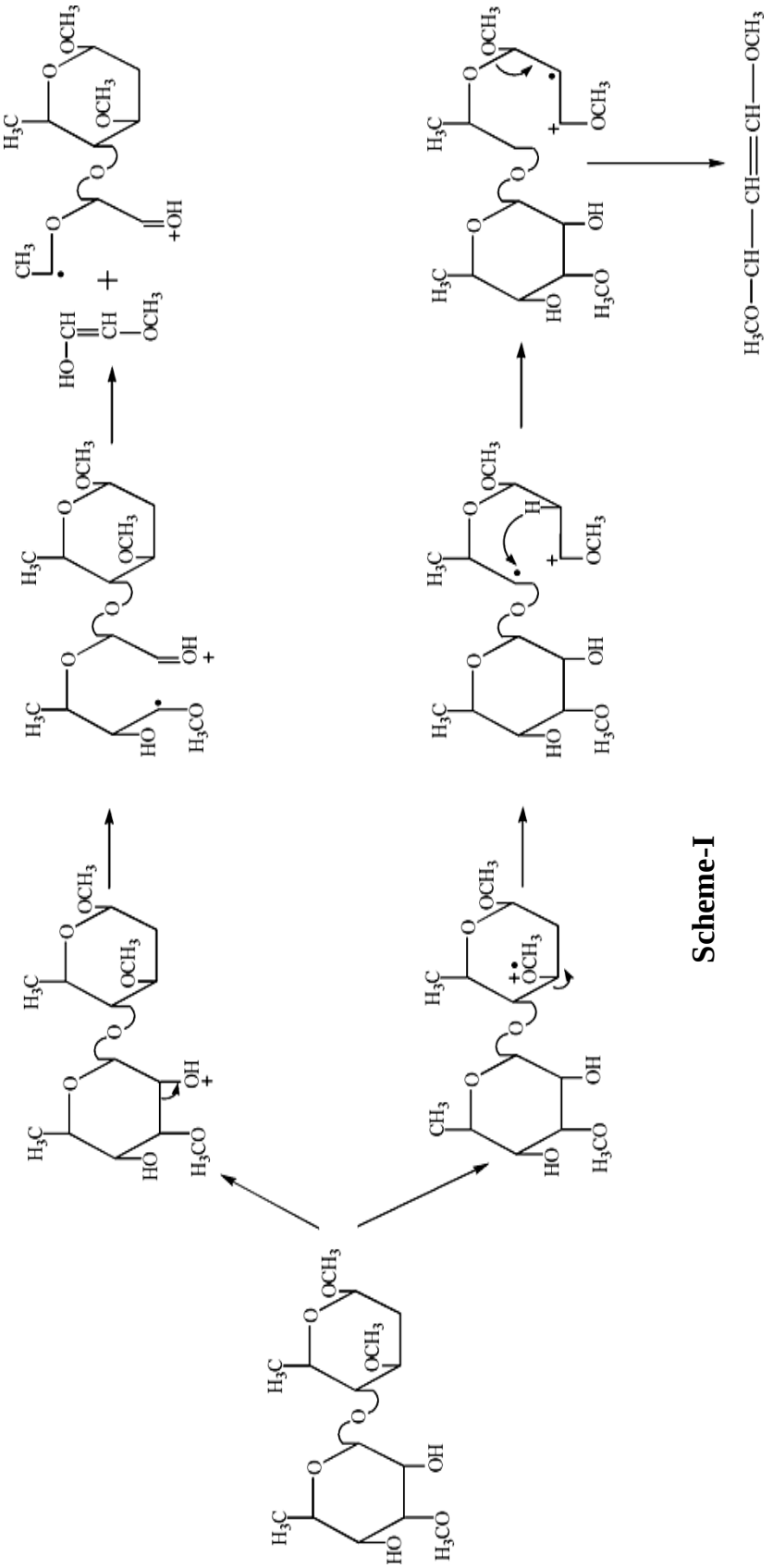
318.1678 (1, M-H_2O, $C_{15}H_{26}O_7$)
260.1243 (2, 318-CH_2CHOCH_3, $C_{12}H_{20}O_6$)
228.1000 (3, 260-CH_3OH, $C_{12}H_{16}O_5$)
305.1602 (4, M-OCH_3, $C_{14}H_{25}O_7$)
273.1326 (5, 305-CH_3OH, $C_{13}H_{21}O_6$)
229.1071 (6, 273-CH_3CH, $C_{11}H_{17}O_5$
197.0816 (7, 229-CH_3OH, $C_{10}H_{13}O_4$)
272.1257 (8, M-CH_3OH, $C_{13}H_{20}O_6$)
260.1243 (9, M-CH_3OH -CH_3OH, $C_{13}H_{20}O_6$)



Besides these, one can also visualize fragmentation pathway II (Scheme 2) wherein the formation of the radical ion at the oxygen atom of the 2-OH group of the normal sugar leads to a 2-3 bond cleavage and loss of a small fragment 10 ($C_3H_6O_2$) of m/z 74.0373. The same type of fragmentation can be anticipated in the 2-deoxy sugar unit by radical-ion formation at the C-3 oxygen atom resulting in the cleavage of the C3-C4 bond which again loses a small fragment 11 ($C_5H_9O_2$) of mass m/z 101.0603.

Fragmentation route III is presented in Scheme 3 in which H-transfers break the disaccharide into monosaccharide units which undergo further fragmentation yielding species having the following m/z values:

145.8815 (12, 176-OCH_3, $C_7H_{13}O_3$)
113.0601 (13, 145-CH_3OH, $C_6H_9O_2$)
95.0498 (14, 113-H_2O, C_6H_7O)
144.0753 (15, 176-CH_3OH, $C_7H_{12}O_3$)
100.0519 (16, 144-CH_3CHO, $C_5H_8O_2$)
118.0616 (17, 176-$CH_3CH{=}CHOH$, $C_5H_{10}O_3$)
87.0450 (18, 118-OCH_3, $C_4H_7O_2$)
117.0551 (19, 118-H, $C_5H_9O_3$)
161.0814 (20, $C_7H_{17}O_4$)
129.0549 (21, 161-CH_3OH, $C_6H_9O_3$)
111.0443 (22, 129-H_2O, $C_6H_7O_2$)



Scheme-I

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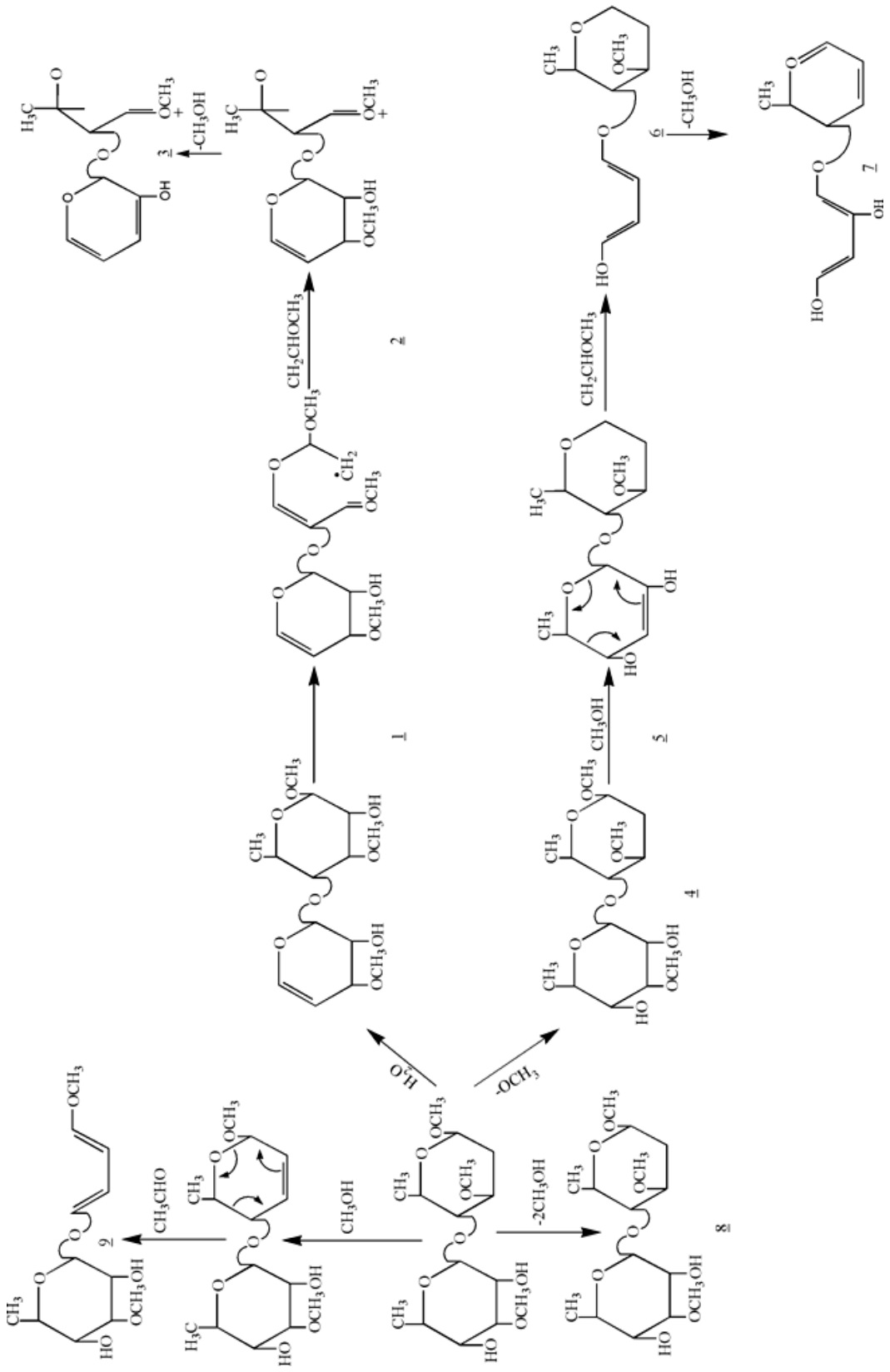
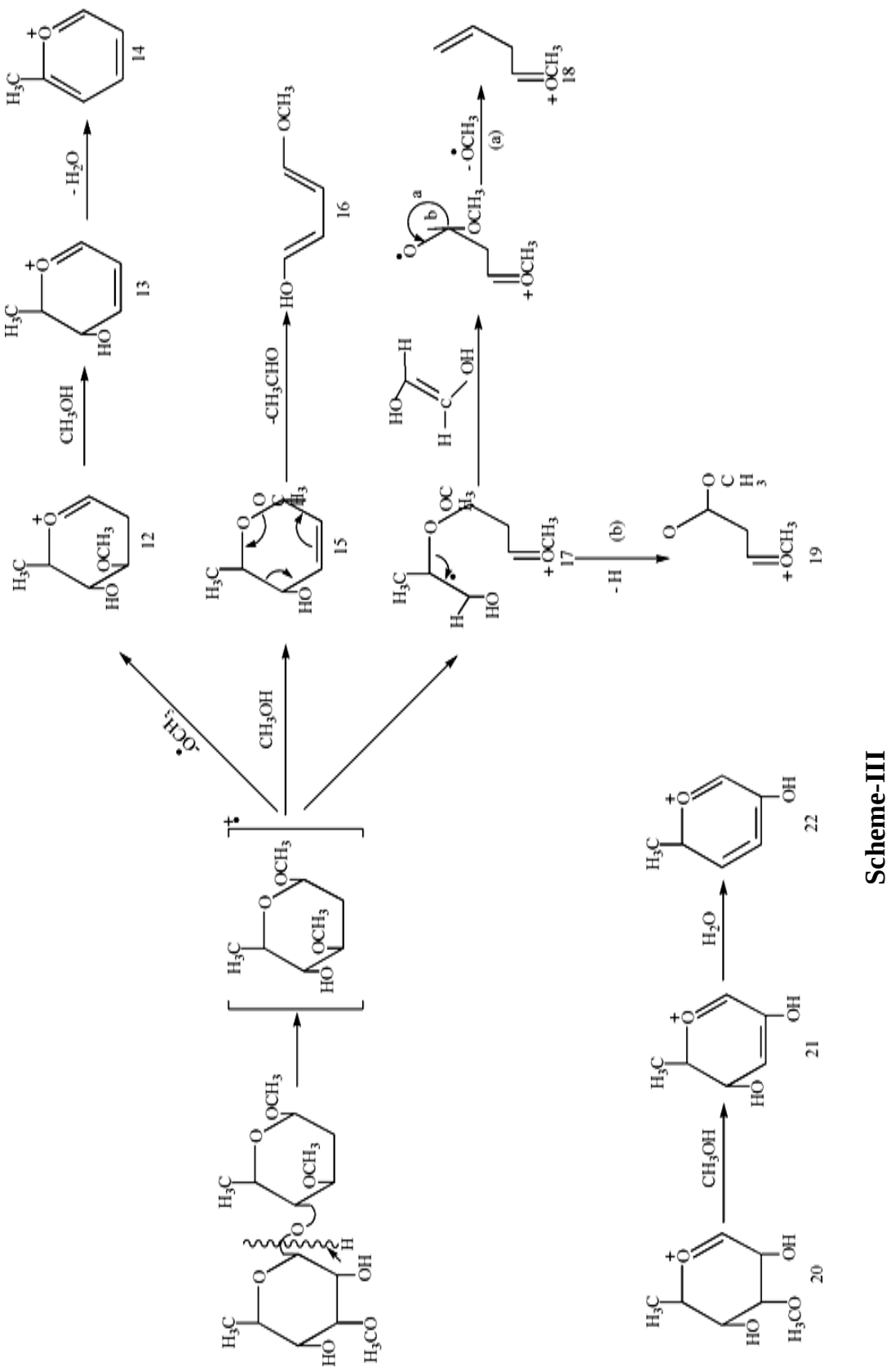
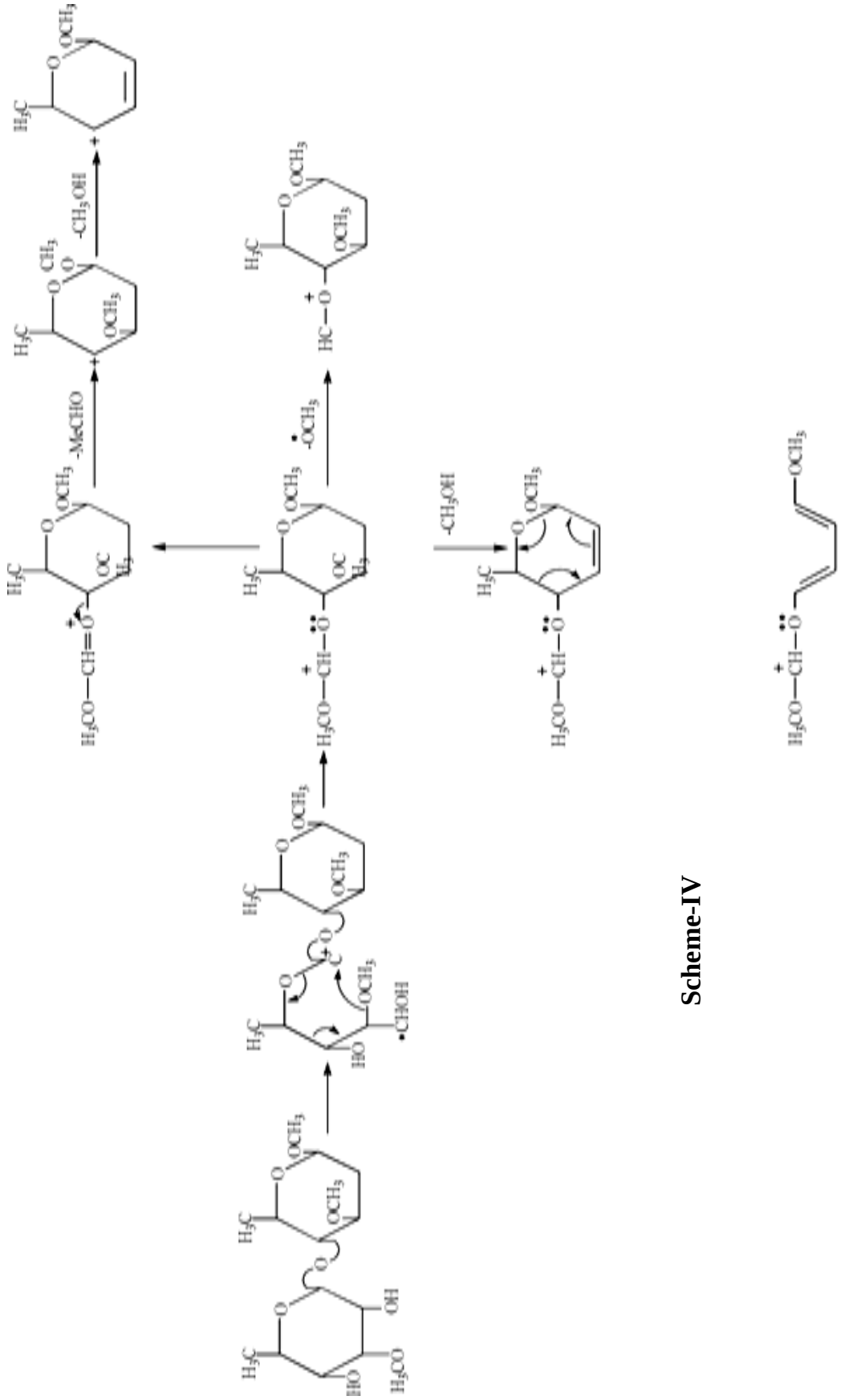
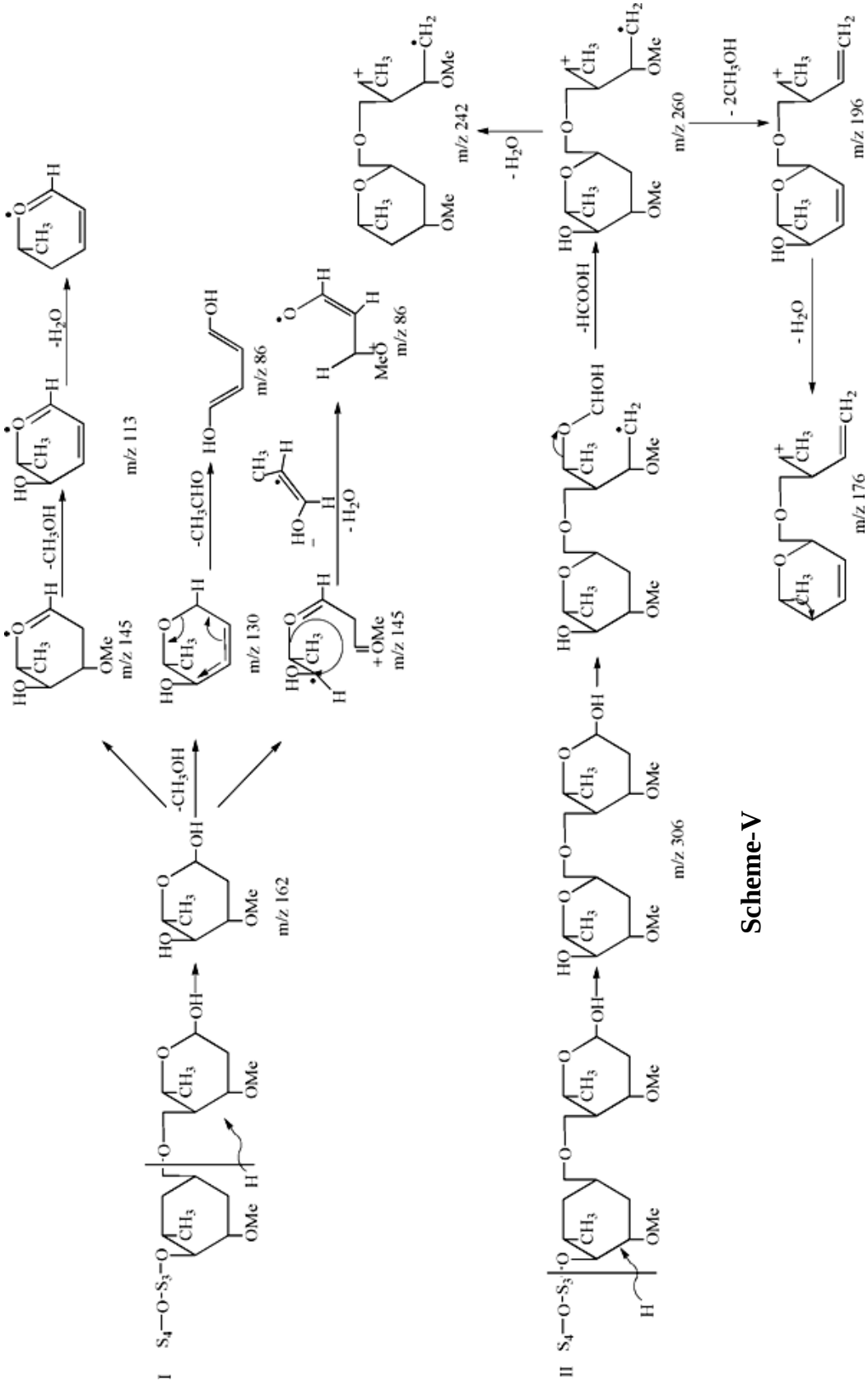




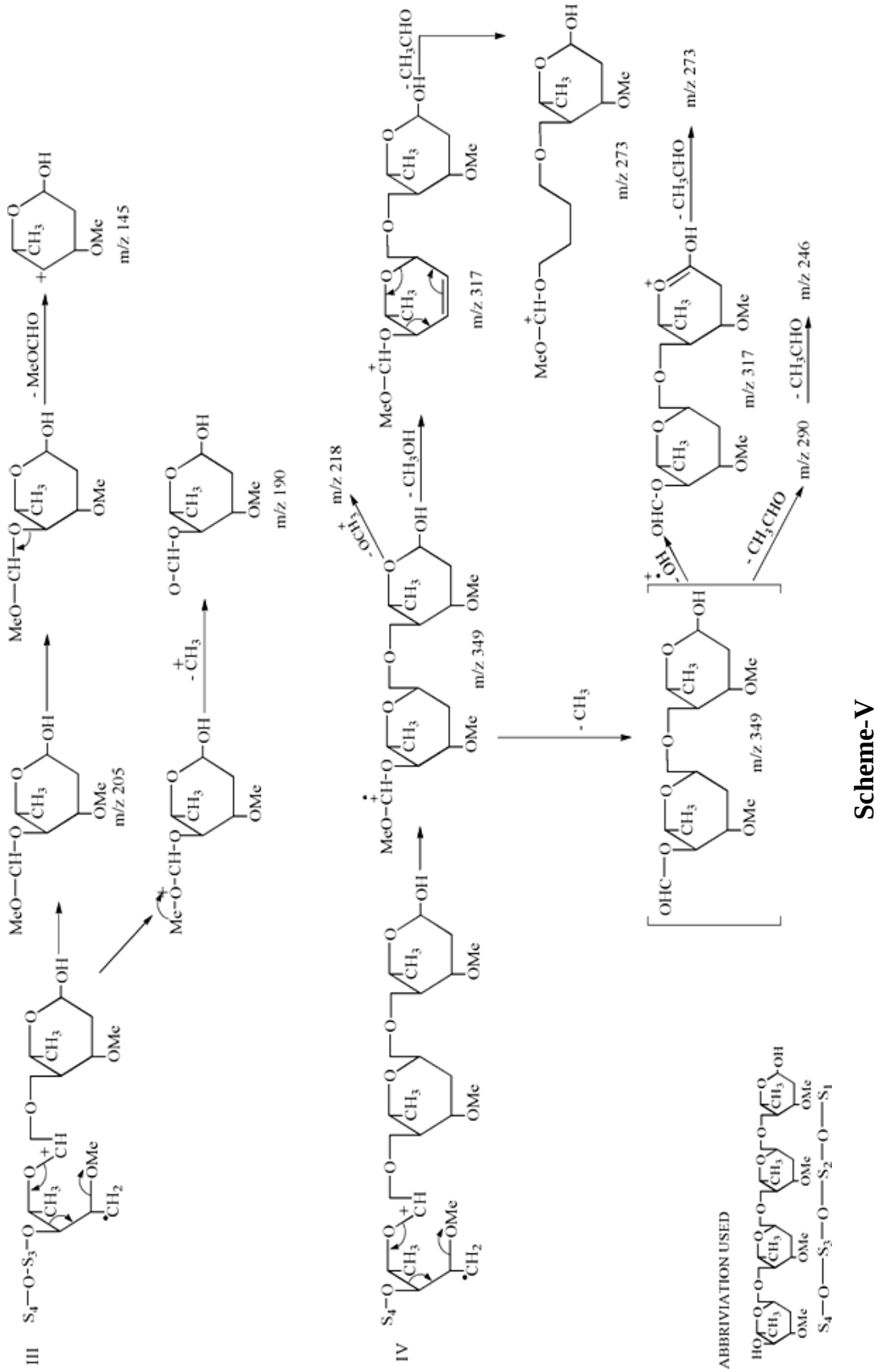
Scheme-III



Scheme-IV



Scheme-V


III
IV
m/z 145
m/z 205
m/z 190
m/z 218
m/z 349
m/z 317
m/z 273
m/z 290
m/z 246
- MeOCHO
- CH3OH
- CH3CHO
- CH3
ABBRIVIATION USED

Scheme-V

Fragmentation route IV (Scheme 4) defines the genesis of ions to be found in the rearrangement involving migration of the methoxyl group after radical-ion cleavage of the C_1-C_2 bond, followed by the migration of the C-3 methoxyl group to C-1, resulting in the cleavage of a normal sugar unit. Further fragmentation of the monosaccharide unit is presumed to be arisingfrom the characteristic fragmentation pattern of 2, 6-dideoxyhexoses reported by Brown et al 1972.This route gives species with the following m/z values:
219.1231 (23, $C_{10}H_{19}O_5$)
159.1021 (24, 219-MeOCHO, $C_8H_{15}O_3$)
127.0758 (25, 159CH_3OH, $C_7H_{11}O_2$)
188.1059 (25, 219-OCH_3, $C_9H_{16}O_4$)
187.0951 (27, 219-CH_3OH, $C_9H_{15}O_4$)
143.0708 (28, 187-CH_3CHO, $C_7H_{11}O_3$)
Based on the major fragmentation pathways that are operative in the mass spectra of underivatised disaccharides studied so far, almost all the prominent fragment ions of the tetrasaccharide orthenthose (Deepak D et.al 1999 and Tiwari K.N et.al 1983) could be interpreted in the context of the proposed structure. The mass spectrum of orthenthose fails to display its molecular ion, as it contains mass peaks of only smaller fragments comprised of monosaccharide and disaccharide units. A structurally significant ion peak is recorded at m/z 306 (6%), which corresponds to a disaccharide fragment. The relatively intense peak in the higher mass region at m/z 290 (100%) corresponds to a fragment formed from the disaccharide fragment resulting from the rearrangement-cleavage of 1. Fragmentation routes and II (Scheme 5) represent repeated H-transfers in the oligosaccharide accompanied by the elimination of terminal sugars 1ess water, giving rise to an ions of the same minimal mass as the molecular ion of the corresponding oligosaccharide with one less monosaccharide residue, and so on until only the monosaccharide remains.

Fragmentation routes III and IV (Scheme 5) show the genesis of ion formed in the rearrangement involving migration of the methoxyl group after radical-ion cleavage of the C1-C2 bond, followed by the migration of the C-3 methoxyl group to C-i, resulting in the cleavage of the oligosaccharide. Further fragmentation of the smaller monosaccharide units is likely via processes characteristic of the fragmentation pattern of 2, 6-dideoxyhexoses reported by Brown et al. These account for most of the major peaks in the spectrum that fully support the structure for oleandrotetrose.

2-Mass fragmentation pattern of normal sugars (Chai W et.al 2001, Reis A et.al 2003 & Kogelberg H. et.al 2004)

ESI give molecular weight information and can be used to obtain branching information. ESI mass spectra can be used to obtain branching information. ESI spectra can be obtained on native or derivatized Oligosaccharides. ESI mass spectra of Oligosaccharides are normally obtained in positive- ion mode where they readily form pseudomolecular ions with H^+, Na^+ and NH_4^+. Larger glycans and N-linked glycans generally form doubly charged ions (e.g. $[M+2H^+]^{2+}$). ESI analysis in negative-ion mode is less sensitive, unless the oligosaccharide is phosphorylated or contains sialic acids. The negative ion spectra are usually composed of $[M-H]^-$ ions.

Fragmentations obtained from oligosaccharides can be summarized as follows:

(1) All fragment ions produced from single cleavage are from the nonreducing terminal.

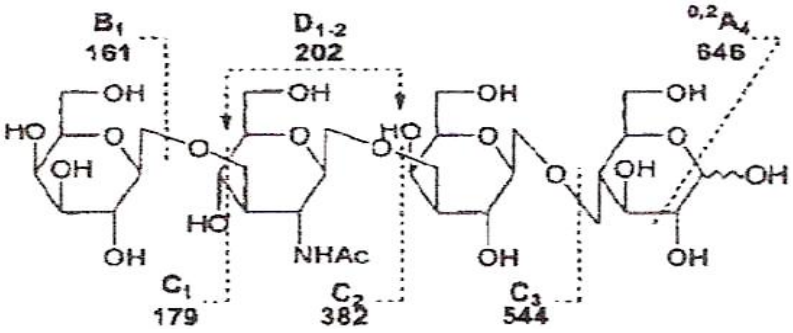
(2) Oligosaccharide sequence information can be deduced unambiguously from a complete set of C-type fragments.

(3) Partial linkage information can be deduced from D- and ^{0,2} A-type fragmentations. A 3-linked GlcNAc or Glc produces a unique D-type ion by double C-Z cleavages. If the 3-linked GlcNAc is not substituted, a D-ion is produced at m/*z* 202. If the 3-linked GlcNAc is substituted by a 4-linked Fuc, the D-ion shifts to m/*z* 348 (202 + 146). Similarly, if there is a Gal at the 4-position of -3GlcNAc, the D-ion is at m/*z* 364 (202 + 162). A mono-4-substituted GlcNAc or Glc gives 0,2 A-type cleavage. Further substitution at the 3-position of GlcNAc or Glc prevents this fragmentation.

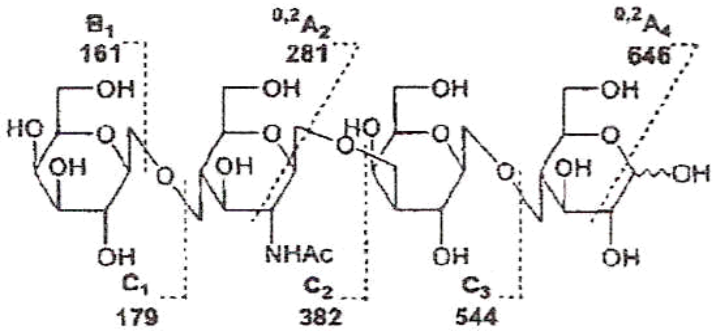
The structural difference between tetrasaccharides LNT and LNnT is the linkage of the non reducing terminal Gal to GlcNAc, with a β1-3 linkage in LNT and a β1-4 linkage in LNnT. Their CID-MS/MS spectra clearly identify this linkage difference. In both spectra, the sequence can be derived from a complete set of C-type fragment ions (C1, m/z 179; C2, m/z 382; C3, m/z 544) while only a single B-type ion is present from the non reducing terminal (B1 at m/z 161).

The A-type ring fragmentation occurs at 4-linked GlcNAc or 4-linked Glcresidues. As indicated in Figure, both LNT and LNnT give $^{0,2}A_4$ ions at m/z 646 from cleavage of the reducing terminal -4Glc. More usefully, a $^{0,2}A_2$ ion (m/z 281, together with its dehydrated ion m/z 263) is produced from the -4GlcNAc in LNnT but not from the -3GlcNAc in the spectrum of LNT. However, this 3-linked GlcNAc residue in LNT gives a unique ion at m/z 202. This is assigned as a C2-Z2 double cleavage, designated as D^{1-2}, due to favorable fragmentation at the reducing side of the glycosidic oxygen. Thus the -3GlcNAc type 1 linkage in LNT and a 4GlcNAc type 2 linkage in LNnT can be readily differentiated by the $^{0,2}A_2$ ion (m/z 281) and D1-2 ion (m/z 202), respectively.

(a)LNT fragmentation



(b) LNnT fragmentation



Thus, the distinctive D- and 0,2 A-type fragmentations are important for differentiating oligosaccharide type 1(3-linked GlcNAc) and type 2 (4-linked GlcNAc) chains and to define the blood group H, Le a /Le x and Le b /Le y determinants: fragment ions at m/*z* 348/528 define a Le a antigen while m/*z* 364/528 indicate a Le x ;a Le b shows fragment ions at m/*z* 348 and 674, and a Le y give an unique fragment ion at m/*z* 510.

All the primary structure of natural productsare characterized by their sequence, linkage, and stereochemistry. Additionally, the large diversity in the monosaccharides due to chemical modification and isomerism, the labile nature of the glycosidic bonds and the poor intrinsic basicity all combine to make the structural elucidation of natural products significantly more difficult than that of other biopolymers. There is currently no analogous method for determining natural products structures with the sensitivity, reliability, and accuracy.

The lack of a rapid method for the complete structural elucidation of natural products remains a major barrier for understanding structure-function relationships of this important class of compounds. The structural elucidation of natural products is a difficult task even under the best circumstances. There is a wide array of tools ranging from chemical to spectroscopic that provide structural determination, but often these methods require long and tedious separation procedures while still limited in sensitivity. The complete structural elucidation of complex natural products requires minimally micromoles of material when often only picomoles or less is available. This is further complicated by the heterogeneity of the compounds as their release from a single glycoprotein produces a collection of compounds. We will call this collection of compounds a library since the collection is composed of combinations involving a small finite set of residues.

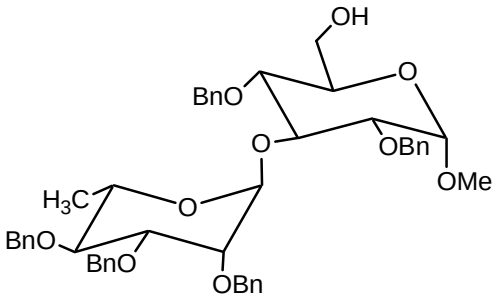
Fragmentation behavior, Characteristic mass increments and their meaning for the analysis of milk oligosaccharides (Pfenninger A et.al 2002)

Analysis	Mass increments(Da)	Interpretation	Assignment
Sequence	162	Cleavage of hexaose(Hex-H_2O)	1st Loss=glucose further Loss=galactose
	203	Cleavage of HexNAc (GlcNAc- H_2O)	HexNAc= GlcNAc
	308(146+162)	fucose residue on Hex (Hex+Fuc- H_2O)	(1→3) Linked Fucose on Glc (1→2)Linked Fucose on Gal In case of (1→2) Linked Fucose in combination with loss of 164 Da
	349(146+203)	fucose residue on HexNAc (HexNAc+Fuc)	(1→3) Linkage(lacto-neo) or (lacto) In case of (1→2) Linked Fucose in combination with loss of 164 Da
	185	clevage of "anhydroHexNAc (GlcNAc- $2H_2O$)	This GlcNAc was linked to a fucose in combination with loss of 164Da
	144	clevage of "anhydro Hex (Hex-$2H_2O$)	Branching site Often the combination with the ring fragment-72Da
Linkage	No ring fragments	(1→3) linkage of the monosaccharide subunit"above"	Observed either between a) LacNAc subunits or b) Gal and GlcNAc in the lacto-neo-series.
	60/78	(1→4) linked hexose	Exclusive observed at reducing end: (1→4) linked glucose in lactose
	101/119	(1→4) linked GlcNAc	Lac- neo-LacNAc
	164	Elimination of (1→3) linked Fuc	At Glc or GlcNAc, i.e. GlcNAc is (1→4) linked (lacto-neo-series)
	72	Gal-2 H_2O at branching site.	Characterstic ring fragment of a (1→6)linked"anhydro" Gal-2 H_2O, which lost a (1→3) branch. Often in combination with loss of 18 Da and 144 Da. Deprotonated reducing end is a "anhydro"Gal-2 H_2O
Branching	{n(365)+a(146)+18} n=1,2,3…, A=0,1,2…	Branching site: n and a determine compositin of the branch.	In combination with chacterstic ring fragments(-18Da and 72Da)s

Often, it is possible to determine the structures of the more abundant components but the structure of the minor components remain somewhat inaccessible. We propose an approach to the structural elucidation of minor components in natural products libraries based on the construction of a catalog of specific sub-structural components that can be used to rebuild the total structure of the unknown components. The method begins with the release (or synthesis) of the library. The structures of the most abundant components are then determined by a combination of methods including chemical degradation, nuclear magnetic resonance, and mass spectrometry. This represents the most difficult and time-consuming step. Structural similarities exist between different natural products, as specific sub structural components are preserved among different compounds. We propose that a catalog of sub-structural components can be identified and characterized by different mass spectrometry techniques. The catalog is constructed from a set of known compounds that have been fully structurally elucidated by, for example, nuclear magnetic resonance and mass spectrometry. The catalog consists of the characteristic fragmentation patterns belonging to a set of specific sub-structural components.

COMPOUND 1

Compound Name : Methyl 2,3,4-tri-O-benzyl-O-α-L-rhamnopyranosyl-(1-3)-2,4-di-O-benzyl-O-β-D-glucopyranoside



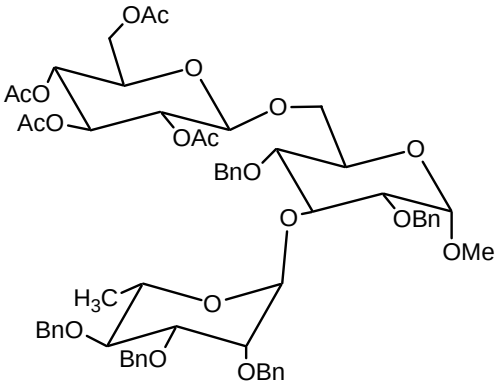
Mol. Formula: $C_{48}H_{54}O_{10}$

Mol. Wt. : 790

ES-MS: m/z 791$[M+H]^+$, 759$[M -OMe]^+$, 741$[759 -H_2O]^+$, 664$[M^+-BnOH + H_2O]^+$, 621$[741-BnCHO]^+$, 604$[621-OH]^+$, 578$[621 -CH_3CO]^+$, 552$[604 -2OH + H_2O]^+$, 522$[M -2BnO]^+$, 496$[604 -BnOH]^+$, 467$[M^+-3C_6H_5CH_2OH]^+$, 383$[S_2-H_2O]^+$, 339$[S_1-H_2O]^+$, 313$[M^+-3BnO -BnCHO]^+$, 311$[M^+-3BnCHO + BnO]^+$, 229$[467-2C_6H_5CH_2CO]^+$, 205$[313 -C_6H_5CH_2OH]^+$, 181$[229 -OCH_3-OH]^+$, 123$[181-CH_3CHCHOH]^+$.

COMPOUND 2

Compound Name : Methyl 2,3,4,6-tetra-O-acetyl-O-β-D-glocopyranosyl-(1-6)-2,3,4-tri-O-benzyl-O-α-L-rhamannopyranosyl-(1-3)-2,4-di-O-benzyl-O-β-D-glucopyranoside



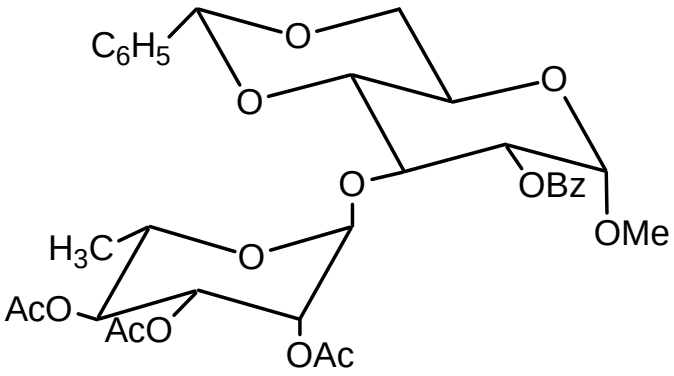
Mol. Formula : $C_{62}H_{72}O_{19}$

Mol. Wt. : 1120

ES-MS: m/z 1121$[M+H]^+$, 1090$[M -2CH_3]^+$, 1029$[M -OBn]^+$, 957$[M-BnOH-COCH_3]^+$, 938$[M -2OBn]^+$, 751$[M -S_2]^+$, 703$[M -S_3]^+$, 608[disaccharide $-AcOH-H_2O -OH]^+$, 559$[703 -BnOH-2H_2O]^+$, 527$[559 -MeOH]^+$, 472$[608 -OBn-OH]^+$, 459$[703 -BnOH - 2OAc -H_2O]^+$, 417$[M -S_1-S_2]^+$, 363$[M -S_1-S_2-3H_2O]^+$, 331$[363 -MeOH]^+$, 299$[331-MeOH]^+$, 271$[331 -AcOH]^+$, 241$[299-CH_3CH_2CHO]^+$, 197$[241 -CH_2CHOH]^+$, 91$[331-4AcOH]^+$.

COMPOUND 3

Compound Name : Methyl 2,3,4-tri-O- acetyl-O-α-L-rhamnopyranosyl-(1-3)-2-O-benzoyl-4,6-O-benzylidene-O-β-D-glucopyranoside

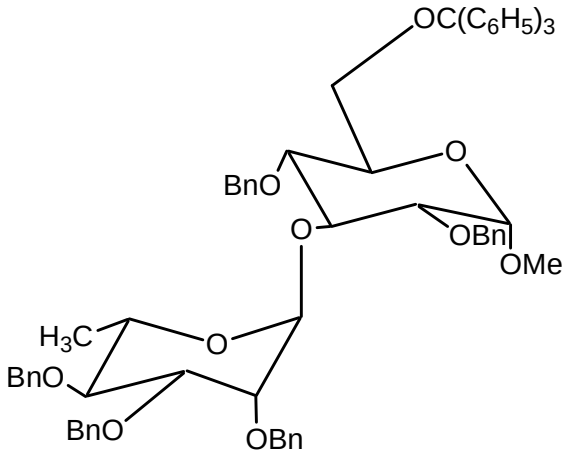


Mol. Formula: $C_{33}H_{38}O_{14}$
Mol. Wt. : 658

ES-MS: m/z 658[M^+], 626[M^+–CH_3OH]$^+$, 598 [M^+–CH_2OHCHO-OAc]$^+$, 596[626–2CH_3]$^+$, 552[596–CH_3CHO]$^+$, 550[M^+–$C_6H_5CH_2OH$]$^+$, 508[552–CH_3CHO]$^+$, 369[M^+–S_2]$^+$, 351[369–H_2O]$^+$, 339[369–CH_3OH]$^+$, 289[M^+–S_1]$^+$, 273[S_1–OH]$^+$, 231[S_1–OBz–OH]$^+$, 213[231–H_2O]$^+$, 179[213–2OH]$^+$, 171[S_2–2OAc]$^+$, 136[179–CH_3CO]$^+$.

COMPOUND 4

Compound Name : Methyl 2,3,4-tri-O-benzyl-O-α-L-rhamnopyranosyl-(1-3)-2,4-di-O-benzoyl-6-O-trityl-O-β-D-glucopyranoside

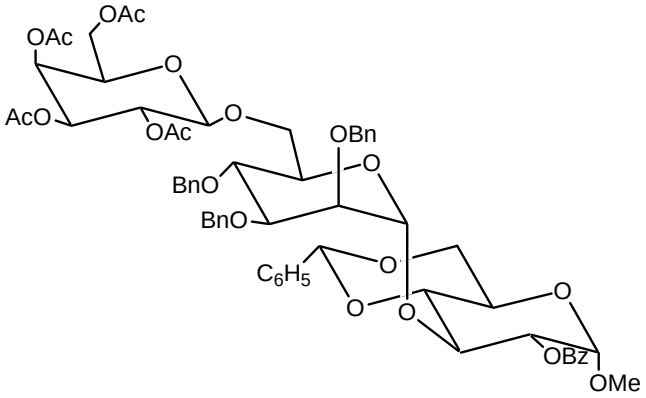


Mol. Formula : $C_{67}H_{68}O_{10}$
Mol. Wt. : 1032

ES-MS: m/z 1001[M –OCH_3]$^+$, 925[M–OBn]$^+$, 790[M –Tr]$^+$, 773[M – (C_6H_5)$_3$CO]$^+$, 759[M^+–$C_{20}H_{17}O$]$^+$, 665[M^+–3BnOH –CH_3CO]$^+$, 663[790 –CH_2OHCHO–CH_3OH –H_2O–OH]$^+$, 603[665–CH_3CHO–H_2O]$^+$, 551[665 –MeOCHO–CH_3CHO]$^+$, 549[665–2$CH_3CHCHOH$]$^+$, 495[603–BnOH]$^+$, 459[495–H_2O]$^+$, 391[459–CH_3OH–2H_2O]$^+$, 367[459–CH_2OHCHO–CH_3OH]$^+$, 333[391–CH_2CHOCH_3]$^+$.

COMPOUND 5

Compound Name : Methyl 2,3,4,6-tetra-O-acetyl-O-β-D-galactopyranosyl-(1-6)-2,3,4-tri-O-benzyl-O-α-D-mannopyranosyl-(1-3)-2-O-benzoyl-4,6-O-benzylidene-O-β-D-glucopyranoside



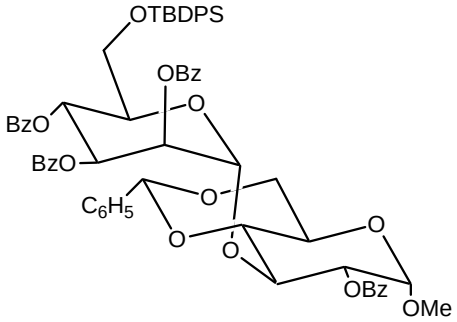
Mol. Formula : $C_{62}H_{68}O_{21}$

Mol. Wt. : 1148

ES-MS: m/z 1148$[M^+]^+$, 1118$[M^+–2CH_3]^+$, 1042[1118–OAc–OH]$^+$, 1058[1148–CH_3OH–$CH_3CHCHOH]^+$, 1026$[M^+$–OBz$]^+$, 950[1058–$C_6H_5CH_2OH]^+$, 933[950–OH]$^+$, 901[933–$CH_3OH]^+$, 888[950–$C_6H_5CH_2CHO–H_2O]^+$, 764$[M^+–S_1–3H_2O]^+$, 664$[S_1+S_3–2H_2O]^+$, 648[764–$2CH_3CHCHOH]^+$, 531$[S_2–C_9H_{90}]^+$, 369$[M^+–762–OH]^+$, 331$[M^+–800–OH]^+$, 229$[S_1–C_6H_5COOH–H_2O]^+$, 211$[S_3–2AcOH]^+$, 169[229–$CH_3COOH]^+$, 91$[S_3–4AcOH]^+$.

COMPOUND 6

Compound Name : Methyl 2,3,4-tri-O-benzoyl-6-O-trt-butyl diphenyl silyl-O-α- D-mannopyranosyl-(1-3)-2-O-benzoyl-4,6-O-benzylidene-O-β-D-glucopyranoside



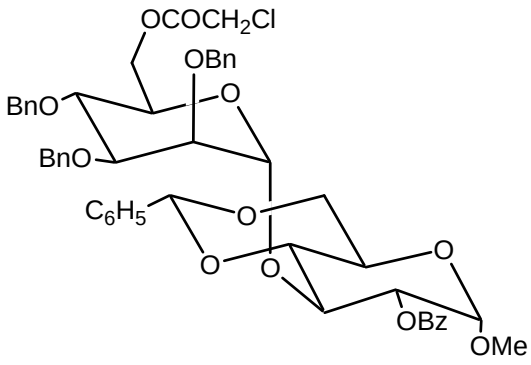
Mol. Formula : $C_{65}H_{49}O_{16}Si$

Mol. Wt. : 1064

ES-MS: m/z 1098$[M^+]$, 1066$[M^+$–MeOH$]^+$, 976$[M^+–C_6H_5COOH]^+$, 714$[M^+–S_2]^+$, 578[714–$C_6H_5CH_2COOH]^+$, 547[578–OMe$]^+$, 469[547–MeOCHO–$H_2O]^+$, 453[714–2HOBz–OH$]^+$, 303[547–2OBz$]^+$, 197[714–2HOBz–OTBDPS–$H_2O]^+$.

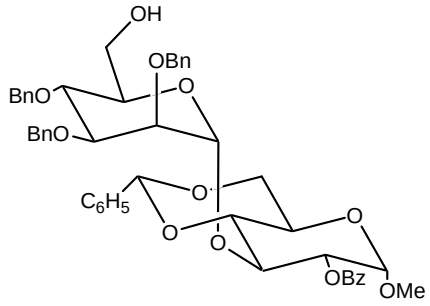
COMPOUND 7

Compound Name : Methyl 2,3,4-tri-O-benzoyl-6-O-chloroacetyl-O-α-D-mannopyranosyl-(1-3)-2-O-benzoyl-4,6-O-benzylidene-O-β-D-glucopyranoside



Mol. Formula: $C_{50}H_{51}ClO_{13}$
Mol. Wt. : 894
ES-MS: m/z 894[M^+], 862[M^+–CH_3OH]$^+$, 802[M^+–92]$^+$, 786[M^+–BnOH]$^+$, 772[M^+–C_6H_5COOH]$^+$, 678[M^+–2BnOH]$^+$, 666[M^+–HOBz–HOBn]$^+$, 634[678–H_2CCHOH]$^+$, 532[M^+–2BnOH–HOBz]$^+$, 508[M^+–S_2]$^+$, 416[508–OAc]$^+$, 369[M^+–S_1–OH]$^+$, 311[369–$H_3CCHCHOH$]$^+$, 240[M^+–654]$^+$, 149[S_1–2BnCHO+BnO]$^+$,91[S_1–3BnOH–OAc]$^+$.

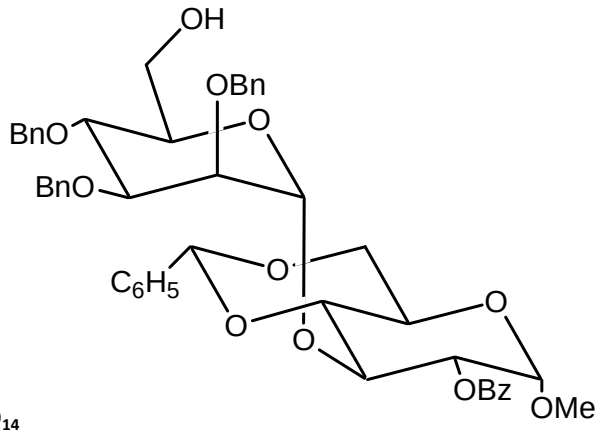
COMPOUND 8
Compound Name : Methyl 2,3,4-tri-O-benzoyl-O-⍰-D-mannopyranosyl-(1-3)-2-O-benzoyl-4,6-O-benzylidene-O- ⍰-D-glucopyranoside



Mol. formula : $C_{48}H_{50}O_{12}$
Mol. Wt. : 818

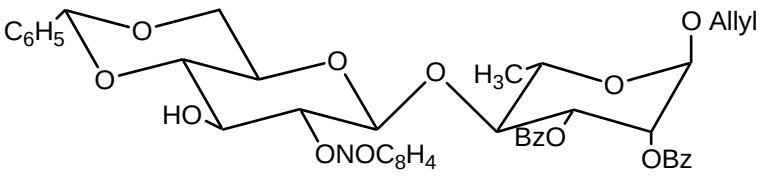
ES-MS: m/z 818[M^+], 800[818–H_2O]$^+$, 787[M^+–OCH_3]$^+$, 753[787–2OH]$^+$, 663[M^+–BnCHO–H_2O–OH]$^+$, 577[M^+–HOBz–BnO]$^+$, 575[M^+–$C_6H_5CH_2COOH$–OBn]$^+$, 433[M^+–385]$^+$, 313[S_1–BnCHO]$^+$, 219[S_1–2BnO]$^+$.

COMPOUND 9
Compound Name : Allyl 4,6-O-benzylidene-3-O-chloroacetyl-2-deoxy-2-phthalimido-O-β-D-glucopyranosyl-(1-4)-2,3-di-O-benzoyl-O-⍰-L-rhamnopyranoside



Mol. formula: $C_{46}H_{42}ClNO_{14}$
Mol. Wt. : 867
ES-MS: m/z 867[M^+]$^+$, 809[M^+–AllylOH]$^+$, 719[M^+–148]$^+$, 687[809–BzOH]$^+$, 673[809–$C_6H_5CH_2COOH$]$^+$, 599[M^+ –268]$^+$,581[673 –$C_2H_2ClO_2$] $^+$, 455[M^+–S_1–H_2O]$^+$, 395[M^+–S_2]$^+$, 359[599–2OBz]$^+$, 256[S_1–HOBz–OH]$^+$, 228[S_2–C_6H_5COOH–$C_3H_3ClO_2$]$^+$, 154[S_1–AcOH–NPhth]$^+$, 90[455–365]$^+$.

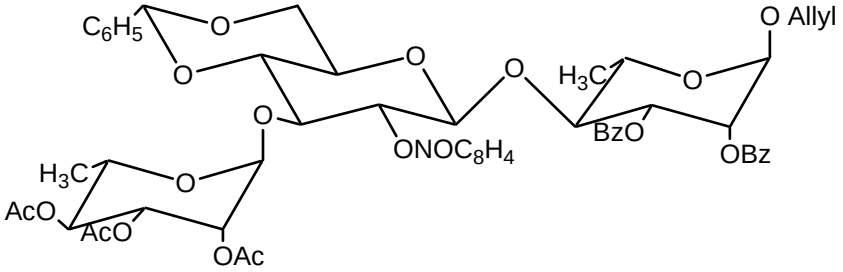
COMPOUND 10
Compound Name : Allyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-O-β-D-glucopyranosyl-(1-4)-2,3-di-O-benzoyl-O-⍰-L-rhamnopyranoside



Mol. formula : $C_{44}H_{41}NO_{13}$
Mol. Wt. : 791
ES-MS: m/z 791[M^+], 792[M^++H]$^+$, 814[M^++Na]$^+$, 734[M^+–OAllyl]$^+$, 669[M^+–BzCOOH]$^+$, 612[M^+–179]$^+$, 680[M^+–S_1–OH]$^+$, 362[S_2–H_2O]$^+$, 292[362 –AllylCHO]$^+$, 274[292–H_2O]$^+$, 256[274–H_2O]$^+$, 226[S_2–$C_6H_5CH_2COOH$]$^+$, 149[17–625–OH]$^+$.

COMPOUND 11

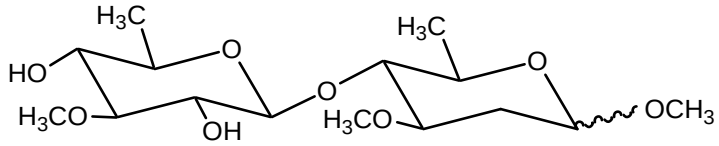
Compound Name : Allyl 2,3,4-tri-O-acetyl-O-⍰-L-rhamnopyranosyl-(1-3)-4,6-O-benzylidene-2-deoxy-2-phthalimido-O-β-D-glucopyranosyl-(1-4)-2,3-di-O-benzoyl-O-⍰-L-amnopyranoside



Mol. formula: $C_{56}H_{57}NO_{20}$
Mol. Wt. : 1063
ES-MS: m/z 1086 [M^++Na]$^+$, 791[M^+–S_3]$^+$, 669[M^+–S_1]$^+$, 355[M^+–708]$^+$, 273[M^+–S_1–S_2]$^+$, 213[S_3–AcOH]$^+$, 153[213–AcOH]$^+$.

COMPOUND 12

Compound : Methyl pachybioside

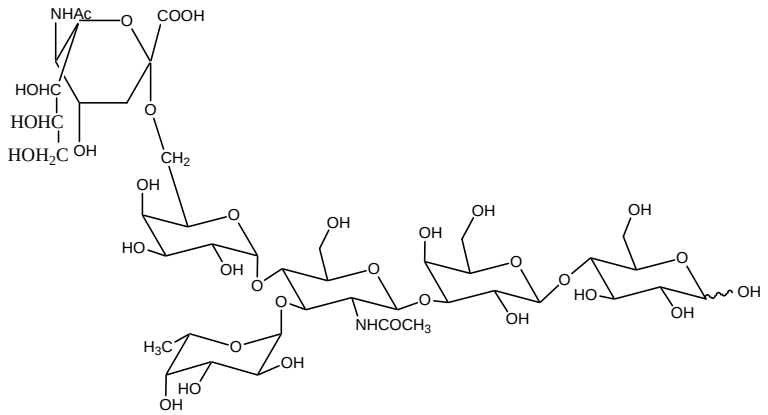


Mol. formula : $C_{15}H_{28}O_8$
Mol. Wt. : 336
ES-MS: 318.1678 [M^+], 305.1602 [M–OCH_3]$^+$, 273.1326[305–CH_3OH]$^+$, 272.1257[M–2CH_3OH]$^+$, 260.1243[318–CH_3CHOCH_3 or M–CH_3OH-CH_3CHO]$^+$, 229.1071[273–CH_3OH]$^+$, 228.1000[260–2CH_3OH]$^+$, 197.0816[229–2CH_3OH]$^+$, 188.1059[219–OCH_3]$^+$, 159.1021[219–OCHO]$^+$, 145.865[176–OCH_3]$^+$, 144.0753[176–CH_3OH]$^+$, 143.0708[187–CH_3CHO]$^+$, 129.0549[161–CH_3OH]$^+$, 127.0758[159–CH_3OH]$^+$, 118.0616[176–CH_3CH=CHOH]$^+$, 117.0551[118–H]$^+$, 113.0601[145–CH_3OH]$^+$, 111.0443[129–H_2O]$^+$, 100.0519[144–CH_3CHO]$^+$, 95.0498[113–H_2O]$^+$, 87.0450 [188–OCH_3]$^+$.

COMPOUND 13 (Ranjan A.K 2016)

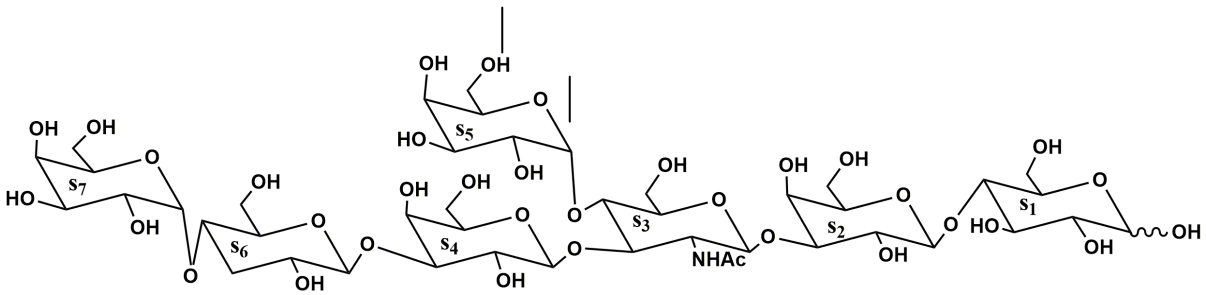
Compound : Asinose

Compound Name: Neu5Acα(2→6)Galα(1→4)GlcNAcβ(1→3)Galβ(1→4)Glc

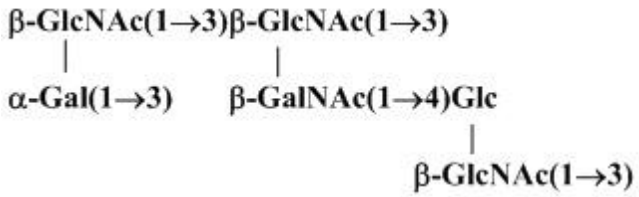


Mol. formula : $C_{43}H_{72}O_{33}N_2$
Mol. Wt. : 1144
ES-MS: 1167 $[M+Na]^+$, 1099 $[M–COOH]^+$, 1081 $[1099–H_2O]^+$, 1041 $[1099–NHCOCH_3]^+$, 1028 $[M–2NHCOCH_3]^+$, 1021 [M+Na-S_4], 1008 [1099 –side chain, HOCH , $HOCH_2$],$^+$ 1003 $[1021–H_2O]^+$, 998 [M–S_6-S_5-S_3-S_2-S_1], 967 [998-CH_2OH], 962 [998–$2H_2O$], 953 [998–COOH], 943 $[1003–CH_2OHCHO]^+$, 940 [998–$NHCOCH_3$], 936 [953 –OH], 907 [998 –Side chain (–CHOH–CHOH–CH_2OH)], 882 [998 -$2NHCOCH_3$], 878 [936 –$NHCOCH_3$], 865 [882 –OH] or [907 –CH_2=C=O], 807 [865 –$NHCOCH_3$], 789 [807 –H_2O], 707 [M – S_6 – S_4], 672 [707 –H_2O, –OH], 665 [707 –CH_2=C=O], 654 [672 –H_2O], 647 [707 –CH_2OHCHO], 629 [647 –H_2O], 612 [672 –CH_2OHCHO], 598 [629 –CH_2OH], 594 [612 –H_2O], 587 [647 – $NHCOCH_3$], 580 [598 –H_2O], 536 [594 –$NHCOCH_3$], 545 [998 –S_6–S_5], 527 [547 –H_2O], 510 [527 –OH], 467 [527–CH_2OHCHO], 449 [467 –H_2O], 391 [449 –$NHCOCH_3$], 383 [529 –S_5]$^+$, 367 [529 –S_2]$^+$, 342 [545 –S_3], 324 [342 –H_2O], 307 [324 –OH], 289 [307 –H_2O]$^+$, 180 [342 –S_2].

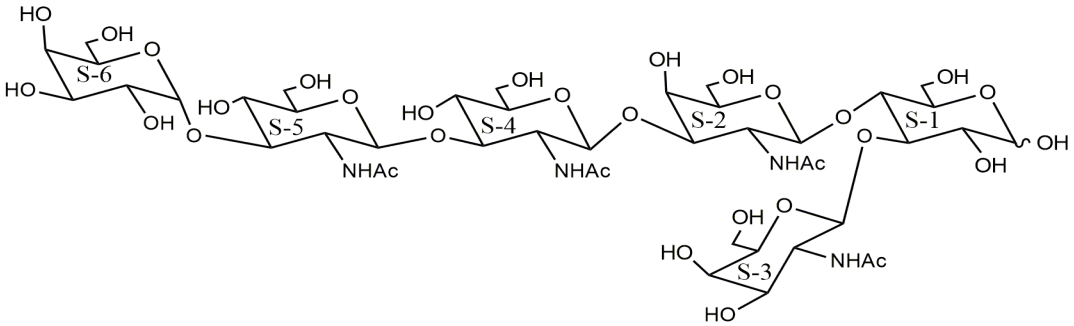
COMPOUND 14(Ranjan A.K 2016)
Compound : Equinose
Compound Name : αGal (1→4)
GlcNAcβ(1→3)Gal β(1→4)Glc
αGal (1→4) βGlc(1→3)βGal (1→3)



Mol. formula: $C_{44}H_{75}O_{36}N$
Mol. Wt. : 1193
ES-MS: m/z1232 $[M + K]^+$, 1135 $[1193–NHCOCH_3]$, 1100 [1135 –H_2O,–OH], 1099 [1135–$2H_2O$], 1093 [1135 –CH_2=C=O], 1057 [1093 –$2H_2O$], 1039 [1099 –CH_2OHCHO], 1022 [1039 –OH], 1031 [M –S_7], 1000 [1031 –CH_2OH], 997 [1039 –CH_2=C=O], 995 [1031–$2H_2O$], 983 [1000 –OH], 978 [995 –OH], 973 [1031 –$NHCOCH_3$], 960 [995–H_2O,–OH], 953 [995 –CH_2=C=O], 935 [995 –CH_2OHCHO], 931 [973 –CH_2=C=O], 900 [931–CH_2OH], 869 [1031–S_5], 851 [859 –H_2O], 834 [869 –H_2O–OH], 820 [851 –CH_2OH], 802 [820 – H_2O], 791 [851 –CH_2OHCHO], 773 [791 –H_2O], 749 [791 –CH_2=C=O], 746 [869 –S_6], 733 [791 –$NHCOCH_3$], 730 [869 –S_6], 694 [730–$2H_2O$], 707 [869 –S_4], 689 [746 –III H_2O], 676 [707–CH_2OH], 671 [746 –III H_2O], 629 [671 –CH_2=C=O], 611 [671 –CH_2OHCHO], 613 [671 –$NHCOCH_3$], 545 [746 –III –S_4], 527 [545 –H_2O], 469 [527 –$NHCOCH_3$], 467 [527 –CH_2OHCHO], 514 [545 –CH_2OH], 472 [514 –CH_2=C=O], 456 [514 –$NHCOCH_3$], 342 [545 –S_3], 325 [342 –OH], 289 [325 –$2H_2O$], 307 [325 –H_2O], 265 [325 –CH_2OHCHO], 223 [265 –CH_2=C=O], 180 [342 –S_2].
COMPOUND 15(Verma P et.al 2017)
Compound : Dicose
Compound Name :

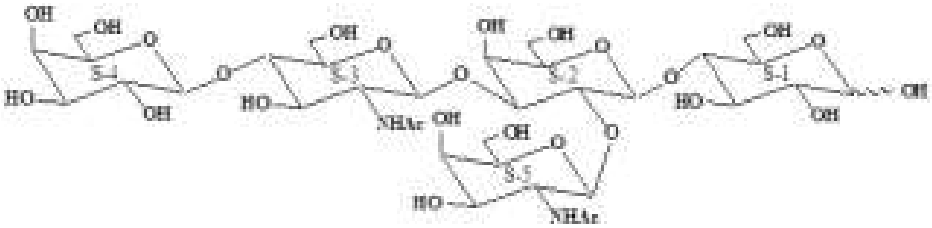


Mol. formula : $C_{44}H_{74}O_{31}N_4$
Mol. Wt. : 1154



ES Mass :1216[M+Na+K], 1193[M+K], 1154 $[M]^+$, 1124[1154–$2CH_3$], 1094[1154–CH_2OH], 1070[1124–$3H_2O$], 992[1154–S_6], 977[992–CH_3], 974[992–H_2O], 926[977–3OH], 916[974–$NHCOCH_3$],908[926–H_2O], 878[908–CH_3], 858[916–$NHCOCH_3$], 789[992–S_5], 731[789–$NHCOCH_3$], 713[731–H_2O], 696[713–OH], 666[696–$2CH_3$], 568[789–S_5], 553[568–CH_3], 539[586–CHO], 536[553–OH], 505[539–2OH], 476[505–CHO], 440[476–$2H_2O$], 383[586–S_3], 365[568–S_4], 331[365–2OH], 316[331–CH_3], 180[383–S_2], 162[365–S_2].

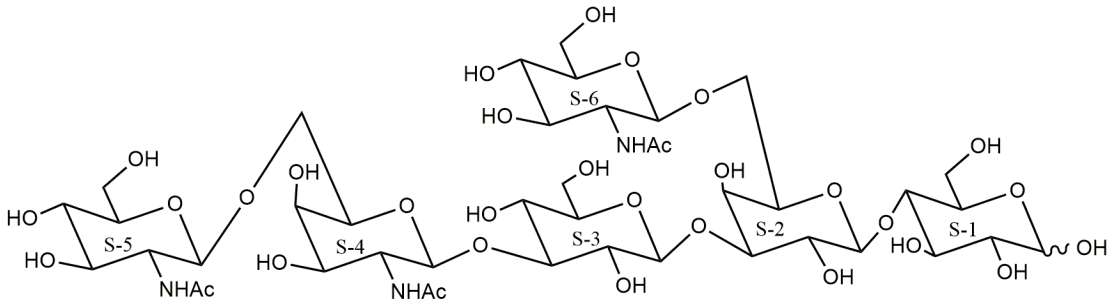
COMPOUND 16 (Gangwar L et.al 2017)
Compound : Bubaliose
Compound Name: β-Gal (1→4) β-GlcNAc(1→3) β-Gal(1→4) β-Glc
β-GalNAc(1→2)



Mol. formula : $C_{34}H_{58}O_{26}N_2$
Mol. Wt. : 910
ES-MS:m/z 972 $[M+Na+K]^+$,949 $[M+K]^+$, 910 $[M]^+$,852 [910 –$NHCOCH_3$], 835[852–OH],817[835–H_2O], 799[835–H_2O], 786[817–CH_2OH], 782[799–OH], 748[910–S_4], 730[748–H_2O], 694[730–$2H_2O$], 688[748–CH_2OHCHO], 676[694–H_2O], 652[688–$2H_2O$], 635[652–OH], 634[652–H_2O], 592[652–CH_2OHCHO], 545[748–S_4], 342[545–S_5], 306[342–$2H_2O$], 289[306–OH], 180[342–S_2].
COMPOUND 17 (Singh P et.al 2017)
Compound : Famiose
Compound Name:

GlcNAc(1→6)-β-GalNAc(1→4)-β-Glc(1→3)-β-Gal(1→4)Glc
↓
GlcNAc(1→6)

Mol. formula: $C_{42}H_{71}N_3O_{31}$
Mol. Wt. : 1113



ES-MS: m/z 1153 $[M+K+H]^+$, 1113 $[M]^{+}$, 1077[M - $2H_2O$], 1060[1077 - OH],, 1042[1060 - H_2O], 1008[1042 - 2OH], 979[1008 - CHO], 936[979 - CH_3CO], 910[1113-S_5], 707[910-S_4], 689[707 - H_2O], 660[689 - CHO], 617[660 - $COCH_3$], 559[617 - $NHCOCH_3$]., 504[707-S_6], 444[504 - CH_2OHCHO], 402[444 - CH_2CO], 384[402 - H_2O], 342[504-S_3], 324[342 - H_2O],288[324 -$2H_2O$], 244[288 -CH_2=CHOH], 180[342-S_2], 145[180 - H_2O -OH],127[145 - H_2O].

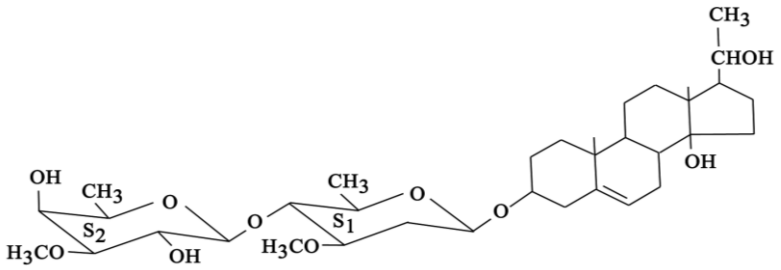
COMPOUND 18

Compound : Denicunine

Compound Name : Calogenin 3-0-3-O-methyl-D-fucopyranosyl-(1→4)-O-β-D-oleandropyranoside

Mol. formula: $C_{35}H_{58}O_{10}$

Mol. Wt. : 638



ES-MS: m/z 677 $[M+K]^-$, 638 $[M^-]$, 623 $[M^-–CH_3]^-$, 605 $[623–H_2O]^-$, 591 $[623–CH_3OH]^-$, 593 $[M–CHOCH_3]^+$, 587 $[605–H_2O]^+$, 576 $[591–CH_3]^+$, 573 $[605–CH_3OH]^+$, 572 $[587–CH_3]^+$, 561 $[593–CH_3OH]^+$, 531 $[576–CHOHCH_3]^+$, 517 $[561–CH_3CHO]^+$, 513 $[531–H_2O]^+$, 478 $[M^+–S_2]^+$, 463 $[478–CH_3]^+$, 460 $[478–H_2O]^+$, 431 $[463–CH_3OH]^+$, 416 $[431–CH_3]^+$, 413 $[431–H_2O]^+$, 373 or (Genin + K)$^-$ $[M–S_2, S_1]^+$, 357 $[460–CHOHCH_3]^+$, 322 $[M^+–Genin]^+$ or $[638–Genin]^+$, 273 $[322–CH_3OH,–OH]^+$, 255 $[273–H_2O]^+$, 241 $[273–CH_3OH]^+$, 229 $[273–CH_3CHO]^+$, 305 $[M^+–Genin–OH]$ or 305 [322–OH], 289 $[Genin–CHOHCH_3]^+$, 253 $[289–2H_2O]^+$, 305 [322–OH], 223 $[253–2CH_3]^+$, 197 $[229–CH_3OH]^+$, 145 [162 –OH]$^+$, 143 [161 –H_2O]$^+$, 129 [161–CH_3OH]$^+$, 127 [145–H_2O]$^+$, 111 [143–CH_3OH] or [129 – H_2O]$^+$, 95 [127–CH_3OH]$^+$.

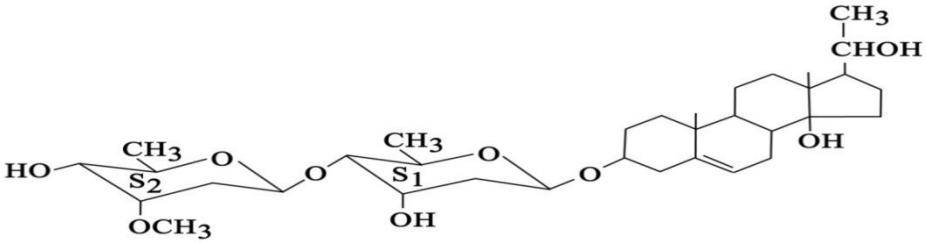
COMPOUND 19

Compound : Heminine

Compound Name : Calogenin-3-O-β-D-cymaropyranosyl-[1-4]-O-β-D-digitoxopyranoside

Mol. formula : $C_{34}H_{56}O_9$

Mol. Wt. : 608

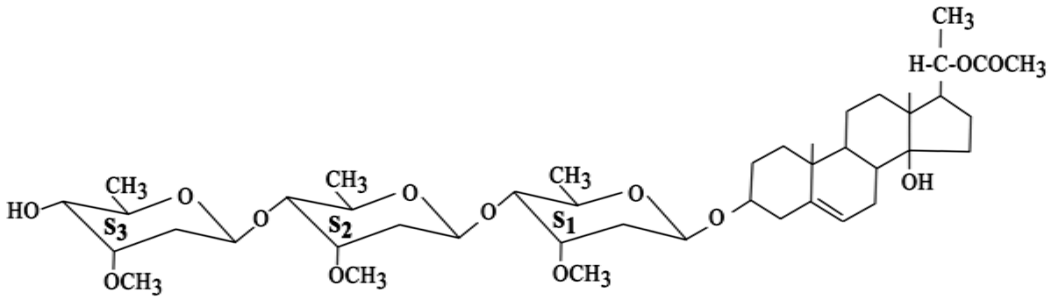


ES-MS:m/z647 $[M^+K]^+$, 609 $[M^+H]$, 578 $[M^+2CH_3]^+$, 575 $[M^+–CH_3, –H_2O]$+, 564 $[M^+–CHOHCH_3]^+$, 548 [578–$2CH_3$]$^+$, 503 [548–$CHOHCH_3$]$^+$, 471 [609–$CHOHCH_3$],$^+$ [503–CH_3OH]$^+$, 464 $[M^+–S_2]^+$, 453 [471–H_2O]$^+$, 449 [464–CH_3]$^+$, 435 [453–H_3O]$^+$, 413 [499–$2H_2O$]$^+$, 369 [413–CH_3CHO]$^+$, 335 [Genin + H] $[M^+–S_1–S_2]^+$, 289 [Genin–$CHOCH_3$]$^+$, 275 $[M^+–Genin]^+$, or [disaccharide–OH]$^+$, 274 [289–CH_3]$^+$, 257 [275–H_2O]$^+$, 253 [289–$2H_2O$]$^+$, 243 [275–CH_3OH]$^+$, 241 [274–CH_3, –H_2O]$^+$, 239 [275–$2H_2O$]$^+$, 225 [257–CH_3OH]$^+$, 223 [253–$2CH_3$]$^+$, 207 [239–CH_3OH]$^+$, 199 [243 –CH_3OH]$^+$, 127 [145 – H_2O]$^+$, 113 [131 – H_2O], 95 [127–CH_3OH]$^+$.

COMPOUND 20

Compound : Ornonine

Compound Name : 20-0-acetyl-calogenin-3-O-β-D-cymaropyranosyl- (1→4)-O-β-D-cymaropyranosyl (1→4)-O-β-D-cymaropyranoside



Mol. formula : $C_{44}H_{72}O_{13}$

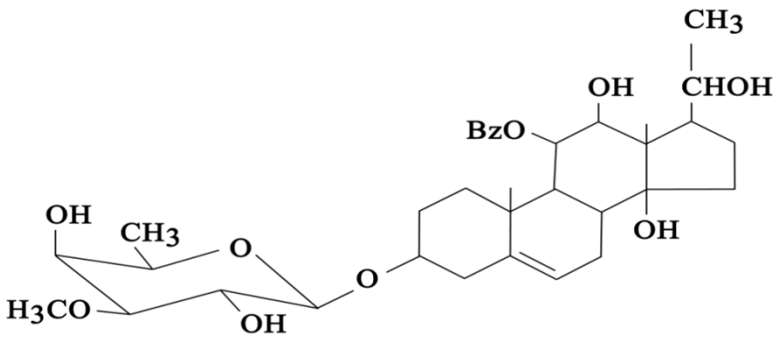
Mol. Wt. : 808

ES-MS: 808 [M^+], 778 [M^+–$2CH_3$], 772 [M^+–$2H_2O$], 760 [778 –H_2O], 749 [M^+–$OCOCH_3$], 740 [772 –CH_3OH], 728 [760 –CH_3OH], 714 [778 –$2CH_3OH$], 708 [740–CH_3OH], 696 [728–CH_3OH], 693 [708–CH_3], 678 [714 –$2H_2O$], 664 [M^+–S_3], 656 [M^+–$CH_3CHOCOCH_3$, CH_3OH, H_2O, CH_3], 649 [664–$\dot{C}H_3$], 634 [664–$2\dot{C}H_3$], 632 [664–CH_3OH], 614 [632–H_2O], 606 [693–$CH_3CHOCOCH_3$], 605 [664–$OCOCH_3$], 600 [632–CH_3OH], 596 [614–H_2O], 581 [596–$\dot{C}H_3$], 577 [664–$CH_3CHOCOCH_3$], 574 [606–CH_3OH] 566 [634–CH_3OH, $2H_2O$], 562 [649–$CH_3CHOCOCH_3$], 534 [566–CH_3OH], 520 [M^+–S_2, S_3], 512 [562 –H_2O, CH_3OH], 494 [512–H_2O], 490 [520–$2\dot{C}H_3$], 488 [520–CH_3OH], 461 [520 – $OCOCH_3$], 458 [520 – $2CH_3$], 488 [520–CH_3OH], 461 [520–$OCOCH_3$], 458 [490–CH_3OH], 450 [M^+–Genin], 444 [488–CH_3OH], 440 [458–H_2O], 433 [520–$\dot{C}H_3CHOCOCH_3$], 408 [440–CH_3OH], 376 [M^+–S_1, S_2, S_3], 329 [450–Na–S_3], 317 [376–$OCOCH_3$], 307 [450+H –S_3], 289 [376 –$CH_3CHOCOCH_3$], 257 [306 –OH, CH_3OH], 239 [257–H_2O], 225 [257–CH_3OH], 207 [239–CH_3OH], 181 [225 – CH_3CHO], 145 [450 –S_2, S_1], 127 [145–H_2O], 113 [145–CH_3OH], 95 [113–H_2O].

COMPOUND 21

Compound : Orgonine

Compound name : 11-O-benozyl-drevogenin-D-3-O-β-D-3-O-Methyl fucopyranoside

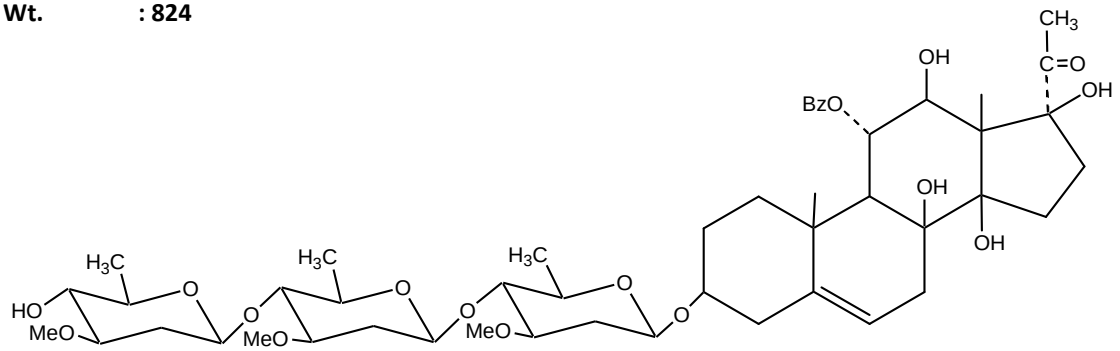


Mol. formula : $C_{35}H_{50}O_{10}$

Mol. Wt. : 630

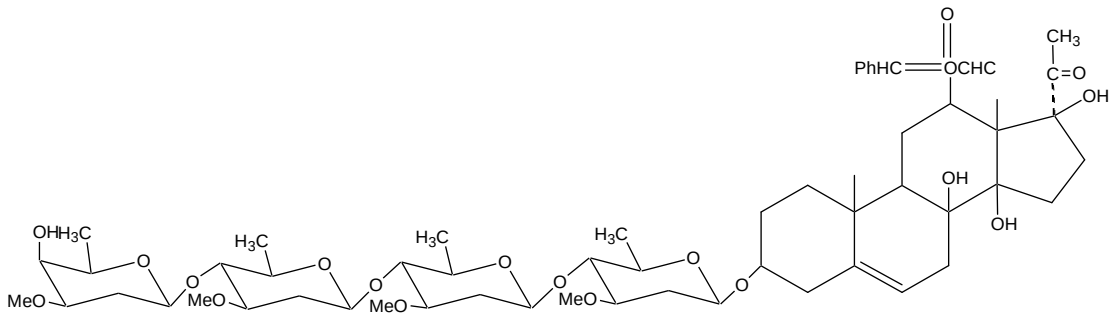
ES-MS: 669 [M^++K^+], 624 [M^++K–$CHOCH_3$]$^+$, 609 [624–$\dot{C}H_3$], 606 [624–H_2O]$^+$, 597 [609–H_2O]$^+$, 592 [624–CH_3OH]$^+$, 588 [606–H_2O]$^+$, 538 [592–$3H_2O$]$^+$, 509 [M^++K–S_1]$^+$ or [Genin +K], 508 [538–$2\dot{C}H_3$], 476 [Genin +K–$\dot{C}H_3$, H_2O]$^+$ [509–$\dot{C}H_3$, H_2O]$^+$ or 431 [476–$CHOHCH_3$]$^+$, 416 [431 –$\dot{C}H_3$]$^+$, 387 [508 –$OCOC_6H_5$]$^+$, 259 [416 –$OCOC_6H_5$, $2H_2O$]$^+$, 217 [Sugar+K]$^+$, [M^+ + K–Genin]$^+$, 167 [217 –$CH_3OH_2H_2O$]$^+$, 164 [217–OH, $2H_2O$]$^+$, 150 [167 – OH]$^+$, 141 [217 –CH_3OH, CH_3CHO].

COMPOUND 22
Compound : Brevine
Compound Name : 11-0-benzoyl-12-β-hydroxy-Lineolon-3-O-methyl-β-D- cymaropyranosyl-(1-4)-O-β-D-cymaropyranosyl-(1-4)-O-β-D-cymaropyranoside
Mol. formula : $C_{40}H_{72}O_{17}$
Mol. Wt. : 824



FD-MS : m/z 932 [M^+], 500 [M^+–trisaccharide], 450 [M^+–sugar]. EIMS m/z (rel.int.): 700(1) [M^+–S_3–$2H_2O$–2OH], 556 (2) [700–S_2]$^+$, 500 (3) [M–trisaccharide]$^+$, 482 (5) [500–H_2O]$^+$, 466 (2) [500–2OH]$^+$, 465 (2) [482–OH]$^+$, 450 (2) [M^+–genin], 447 (1) [465–H_2O]+, 434 (1) [466 –OH –CH_3]$^+$, 430 (1) [447 –OH]$^+$, 412 (3) [430 –H_2O]$^+$, 391 (2) [434 –$COCH_3$]$^+$, 378 (2) [500 –BzOH]$^+$, 361 (1) [378 –OH]$^+$, 354 (450–3MeOH]$^+$, 349 (2) [450 –$C_5H_8O_2$]$^+$, 343 (3) [465 –BzOH]$^+$, 326 (1) [343–OH]$^+$, 308 (2) [326 –H_2O]$^+$, 306 (3) [Disaccharide]$^+$, 300 (3) [343 –$COCH_3$]+, 290 (100) [412–BzOH; 308 –H_2O]$^+$, 196(2) [306 –HCOOH –2MeOH]$^+$, (8) [196 –H_2O]+, 162 (22) [Monosaccharide]$^+$, 161 (10) [$C_{11}H_{13}O$]$^+$, 156 (12) [$C_8H_{12}O_3$]$^+$, 130 (22) [162 –MeOH]$^+$, 113 (15) [156 –$COCH_3$]$^+$, 86 (21) [130 –CH_3CHO; 162–MeCHCHOH -$2H_2O$]$^+$.

COMPOUND 23
Compound Name: Orthenine
Compound Name : 12-0-β-cinnamoyl-calogenin-3-O-methyl-β-D- cymaropyranosyl-(1-4)-O-β-D-cymaropyranosyl-(1-4)-O-β-D-cymaropyranoside
Mol. formula : $C_{58}H_{88}O_{19}$
Mol. Wt. : 1088



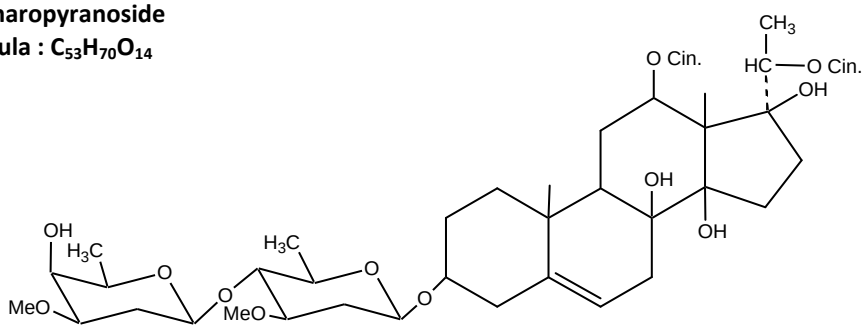
EI-MS: m/z (rel. int.): [M^+] (not observed); 494.2702 (0.42) [M^+–sugars – H_2O] ($C_{30}H_{38}O_6$); 476.2563 (0.92) [494 –H_2O]$^+$ ($C_{30}H_{36}O_5$); 458.2472 (0.87) [476–H_2O]$^+$ ($C_{30}H_{34}O_4$]; 364.2284 (0.47) [M –sugars –PhCHCHCOOH]$^+$ ($C_{21}H_{32}O_5$); 346.2148 [364 –H_2O]$^+$ ($C_{21}H_{30}O_4$); 328.2038 (6.48) [346 –H_2O]$^+$ ($C_{21}H_{28}O_3$); 310.1936 (5.63) [328 –H_2O]$^+$ ($C_{21}H_{26}O_2$); 292.1831 (4.07) [310–H_2O]$^+$ ($C_{21}H_{24}O$); 274.1722 (0.85) [292–H_2O]$^+$ ($C_{21}H_{22}$); 257.1402 (8.02) [tetrasaccharide ion –two sugars –MeOH]$^+$ ($C_{13}H_{21}O_5$); 239.1319 (1.59) [257 –H_2O]$^+$ ($C_{13}H_{19}O_4$); 208.1111 (1.20 [genin –$C_9H_{14}O$ – PhCHCHCOOH –H_2O]$^+$ ($C_{12}H_{16}O_3$); 191.1069 (0.92) [208 –OH]$^+$ ($C_{21}H_{15}O_2$); 181.0895 (1.20) [257 –MeOH –MeCHO]$^+$ ($C_{10}H_{13}O_3$); 145.0873 (100.00) [monosaccharide –OH]$^+$ ($C_7H_{13}O_3$); 120.0940 (5.62) [genin –$C_{21}H_{26}O_6$ –H_2O]$^+$ (C_9H_{12}); 113.0606 (40.903 [145 –MeOH]$^+$ ($C_6H_9O_2$); 105.0707 (6.76) [120 –CH_3]$^+$ (C_9H_9); 95.0500 (9.29) [113–H_2O]$^+$ (C_6H_7O).

COMPOUND 24

Compound Name : Plocinine

Compound Name : 11-0-α, 20-O-β-di-cinnamoyl-ornogenin-3-O-β-D- cymaropyranosyl-(1-4)-O-β-D-cymaropyranoside

Mol. formula : $C_{53}H_{70}O_{14}$



Mol. Wt. : 930

EI-MS: m/z (rel. int.): [M^+] (not observed); 494.2639 (2) [M –sugars –PhCHCHCOOH]$^+$ ($C_{30}H_{38}O_6$); 476.2565 (3) [494 –H_2O]$^+$ ($C_{30}H_{36}O_5$) (3) [476 –H_2O]$^+$ ($C_{30}H_{34}O_4$); 346.2144 (44.6) [494 –PhCHCHCOOH]$^+$ ($C_{21}H_{30}O_4$); 328.2041 (89.1) [346 –H_2O]$^+$ ($C_{21}H_{28}O_3$); 310.1938 (82.8) [328 –H_2O]$^+$ ($C_{21}H_{26}O_2$); 292.1831 (28) [310 –H_2O]$^+$ ($C_{21}H_{24}O$); 274.1720 (22) [292 –H_2O]$^+$ ($C_{21}H_{22}$); 257.2707 (34) [disaccharide ion –MeOH]$^+$ ($C_{13}H_{21}O_5$); 145.0874 (100) [monosaccharide –OH]$^+$ ($C_7H_{13}O_3$); 113.0604 (8) [145 – MeOH]$^+$ ($C_6H_9O_2$); (35) [113 –H_2O]$^+$ (C_6H_7O).

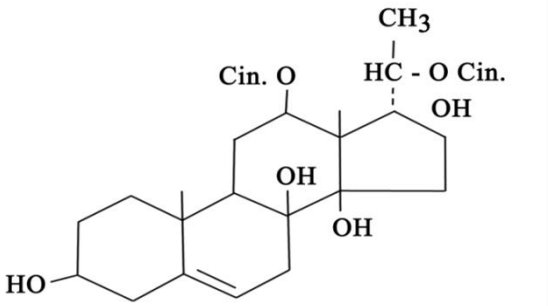
COMPOUND 25

Compound : Ornogenin

Compound Name : 11-0-α-20-O-β-di-cinnamoyl-ornogenin

Mol. formula: $C_{39}H_{46}O_8$

Mol. Wt. : 642



EI-MS: m/z (rel.int.): [M+] (not observed); 494 (8) [M –PhCHCHCOOH]$^+$, 476 (8) [494 –H_2O]$^+$, 468 (6) [–$C_9H_{14}O$–2H_2O]$^+$, 458 (7) [476–H_2O]$^+$, 450 (10) [M –CH_3CHOCOCHCHPh –OH]$^+$, 440 (5) [458 –H_2O]$^+$ 435 (7) [450 –CH_3]$^+$, 432 (11) [450 –H_2O]$^+$, 422 (4.8) [440 –H_2O]$^+$, 417 (4) [435 –H_2O; 432 –CH_3]$^+$, 346 (34) [494 –PhCHCHCOOH]$^+$, 328 (76) [346 –H_2O]$^+$, 310 (39) [328 –H_2O]$^+$, 292 (22) [310 –H_2O]$^+$, 274 (10) [292–H_2O]$^+$, 172 (23) [468–2PhCHCHCOOH]$^+$, 131 (100) [PhCHCHCO]$^+$, 120 (20) [M–$C_{30}H_{32}O_7$ –H_2O]$^+$, 105 (62) [120 –CH_3]$^+$.

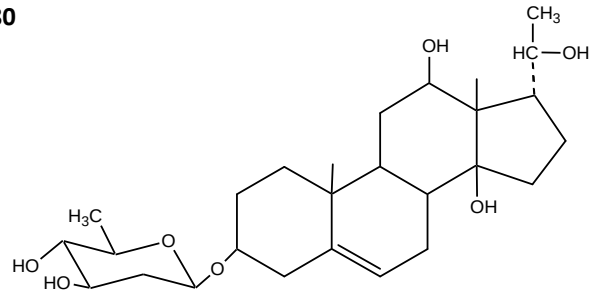
COMPOUND 26

Compound : Locin

Compound Name : 12-β-hydroxy-calogenin-3-O-β-D-fucopyranoside

Mol. formula : $C_{27}H_{44}O_7$

Mol. Wt. : 480



EI-MS m/z (rel.int.): [M^+] (not observed); 332 (2) [M –sugar –H_2O]$^+$, 314 (10) [332–H_2O]$^+$, 269 (12) [314–CH_3CHOH]$^+$, 254 (6) [269–CH_3]$^+$, 241 (3) [269–C_2H_4]$^+$, 236 (17) [254 –H_2O]$^+$, 211 (6) [M–$C_9H_{15}O$]$^+$, 183 (10) [211–CH_2CH_2]$^+$, 168 (15) [183–CH_3]$^+$, 148 (100) [monosaccharide]$^+$, 139 (7) [M–$C_{12}H_{20}O_3$]$^+$, 130 (4) [148–H_2O]$^+$, 123 (14) [168–CH_3CHOH]$^+$, 121 (12) [139 –H_2O]$^+$, 113 (15) [130 –OH]$^+$, 106 (20) [121 –CH_3]$^+$, 105 (27) [123 –H_2O]$^+$, 95 (35) [C_6H_9O]$^+$, 95 (37) [113–H_2O]$^+$.

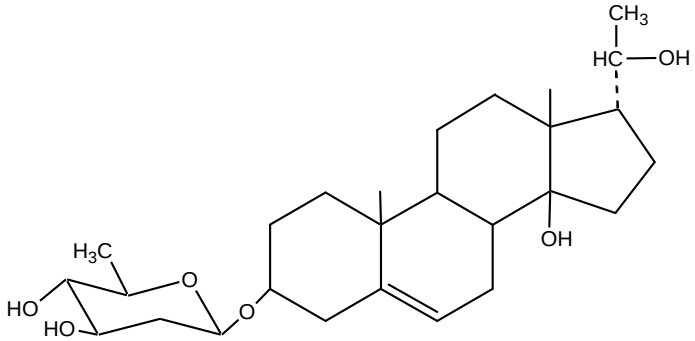
COMPOUND 27

Compound : Calocinin

Compound Name : calogenin-3-O-β-D-fucopyranoside

Mol. formula : $C_{27}H_{44}O_6$

Mol. Wt. : 464



EI-MS m/z (rel.int.): [M^+] (not observed); 419 (11) [M–CH_3CHOH]$^+$, 401 (19) [419 –H_2O]$^+$, 334 (15) [M –sugar]$^+$, 316 (67) [334 –H_2O]$^+$, 289 (35) [334 –CH_3CHOH]$^+$, 271 (30) [316 –CH_3CHOH; 289 –H_2O]$^+$, 253 (25) [271 –H_2O]$^+$, 163 (9) [$C_{11}H_{15}O$]$^+$, 151 (7) [$C_{12}H_{20}O_2$ –CH_3CHOH]$^+$, 145 (9) [163 –H_2O]$^+$, 137 (5) [$C_9H_{13}O$]$^+$, 133 (10) [151 –H_2O]$^+$, 131 (100) [M –genin –OH]$^+$, 119 (8) [137 –H_2O]$^+$, 113 (55) [131 –H_2O]$^+$, 105 (11) [$C_9H_{14}O$ – H_2O –CH_3]$^+$, 95 (15) [113 –H_2O]$^+$.

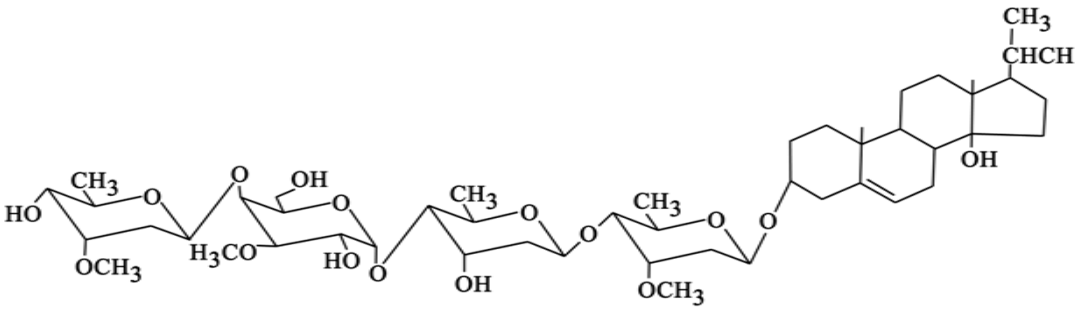
COMPOUND 28

Compound Name : reticulin

Compound Name : calogenin-3-O-β-D-cymaropyranosyl-(1-4)-O-3-O-methyl- α-D-galctopyranosyl-(1-4)-O-β-D-digitoxopyranosyl-(1-4)-O-β-D-cymaropyranoside

Mol. formula : $C_{48}H_{80}O_{17}$

Mol. Wt. : 928



FAB-MS : m/z (rel.int.) 929 (7) [M +1]$^+$, 883 (9) [M – CH_3CHOH]+, 851 (9) [883 –CH_3OH]$^+$, 833 (6) [851 –H_2O]$^+$, 789 (5) [933 –CH_3CHO]$^+$, 784 (5) [M –(S_4-OH)]$^+$, 739 (7) [789 –CH_3OH-H_2O; 784 –CH_3CHOH]$^+$, 721 (4) [739 –H_2O]$^+$, 695 (4) [739 –CH_3OH –CH_2OHCHO]$^+$, 671 (8) [721 –CH_3OH –H_2O]$^+$, 645 (11) [721-CH_3OH-CH_3CHO]$^+$, 639 (9) [671 –CH_3OH]$^+$, 633 (7) [695+H_2O–CH_3CHO]$^+$, 621 (5) [639 –H_2O]$^+$, 613 (10) [tetrasaccharide +H]$^+$, 603 (7) [695 –CH_3OH – CH_2OHCHO]$^+$, 595 (6) [tetrasaccharide –OH]$^+$, 583 (8) [645 –H_2O –CH_3CHO]$^+$, 577 (6) [595 –H_2O]$^+$, 573 (7) [633 –CH_2OHCHO]$^+$, 559 (7) [603 –CH_3CHO]$^+$, 551 (7) [583 –CH_3OH]$^+$, 547 (13) [583 -2H_2O]$^+$, 545 (12) [M – S_4 – (S_3-OH) –CH_3CHOH –H_2O; 577 –CH_3OH]$^+$, 543 (12) [573 -2CH_3]$^+$, 532 (13) [547 –CH_3]$^+$, 527 (8) [545 –H_2O]$^+$, 512 (7) [527 –CH_3]$^+$, 511 (7) [tetrasaccharide –$C_5H_9O_2$]$^+$, 501 (8) [545 –CH_3CHO]$^+$, 497 (15) [512–CH_3]$^+$, 493 (10) [511 –H_2O]$^+$, 491 (10) [551 –CH_2OHCHO]$^+$, 457 [501 –CH_3CHO]$^+$, 451 (8) [595 – (S_4–OH)]$^+$, 443 (11) [493 –CH_3OH–H_2O]$^+$, 439 (15) [457 –H_2O]$^+$, 433 (9) [M-S_4-S_3-(S_2-OH)–CH_3CHOH]$^+$, 429 (17) [493 –2CH_3OH]$^+$, 421 (15) [453 –CH_3OH]$^+$, 419 (15) [451 –CH_3OH]$^+$, 415 (10) [433 –H_2O]$^+$, 383 (10) [415 –CH_3OH; 443–H_3COCHO]$^+$, 379 (8) [439 –CH_2OHCHO]$^+$, 377 (8) [421 –CH_3CHO]$^+$, 369 (20) [429 –CH_2OHCHO]$^+$, 365 (14) [457 –CH_3OH–CH_2OHCHO]$^+$, 359

(15) [419 $-CH_2OHCHO]^+$, 347 (13) [379 $-CH_3OH]^+$, 339 (13) [383 $-CH_3CHO]^+$, 335 (12) $[C_{15}H_{27}O_8]^+$, 333 (12) [365 $-CH_3OH]^+$, 327 (17) [359 $-CH_3OH]^+$, 325 (17) [369 $-CH_3CHO]^+$, 321 (12) [M-(Genin-O-S_1-O-S_2)]$^+$, 317 (18) [335$-H_2O]^+$, 309 (10) [327 $-H_2O]^+$, 303 (12) [335 $-CH_3OH]^+$, 295 (12) [339-$CH_3CHO]^+$, 289 (25) [321$-CH_3OH]^+$, 281 (13) [325 $-CH_3CHO]^+$, 275 (15) [M–Genin-S_4-(S_3-OH)]$^+$, 273 (14) [317$-CH_3CHO]^+$, 265 (36) [309$-CH_3CHO]^+$, 257 (22) [275 $-H_2O$; 289-$CH_3OH]^+$, 243 (17) [303$-H_3COCHO]^+$, 241 (12) [273$-CH_3OH]^+$, 213 (13) [251 $-CH_3CHO]^+$, 199 (15) [243 $-CH_3CHO]^+$, 197 (16) [241 $-CH_3CHO]^+$, 181 (8) [213 $-CH_3OH]^+$, 159 (10) $[C_7H_{11}O_4]^+$, 115 (100) [159$-CH_3CHO]^+$.

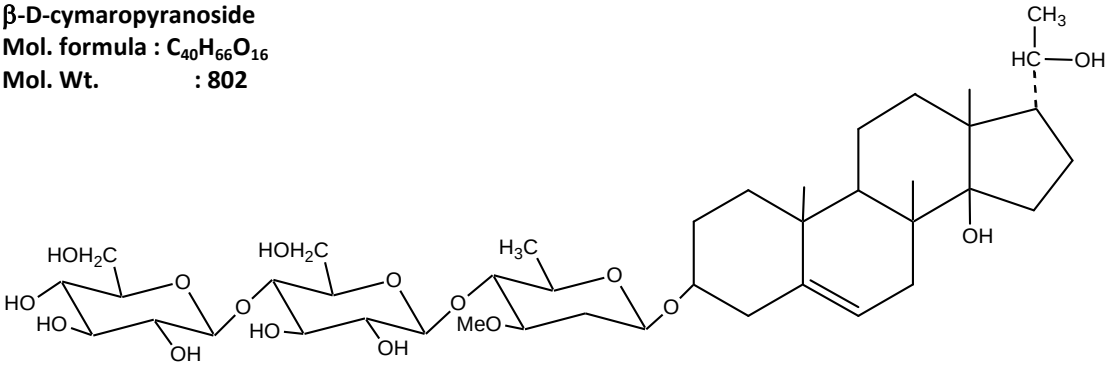
COMPOUND 29

Compound : Leptaculatin

Compound Name : calogenin-3-O-β-D-Glucopyranosyl-(1-4)-O-β-D-Glucoxopyranosyl-(1-4)-O-β-D-cymaropyranoside

Mol. formula : $C_{40}H_{66}O_{16}$

Mol. Wt. : 802



EI-MS: m/z (rel.int.): 460 (2) [M-terminal Glu-(Glu-OH)-$H_2O]^+$, 450 (4) [trisaccharide -$2H_2O]^+$, 436 (3) [trisaccharide $-CH_3OH$ $-H_2O]^+$, 433 (3) [M-terminal Glu-(Glu-OH) $-CH_3CHOH]^+$, 419 (12) [M–terminal Glu-(Glu-OH)]-$CH_3CHCHOH$-H]$^+$, 418 (11) [436-H_2O; 450 $-CH_3OH]^+$, 404 (4) [419 $-CH_3]^+$, 401 (5) [433 $-CH_3OH]^+$, 400 (5) [418 $-H_2O]^+$, 386 (7) [404 $-H_2O]^+$, 383 (5) [401 $-H_2O]^+$, 382 (5) [400 $-H_2O]^+$, 357 (8) [460 $-CH_3CHOH$-$CH_3CHCHOH]^+$, 356 (7) [357 $-H]^+$, 353 (4) [trisaccharide $-C_5H_9O_4]^+$, 342 (22) [trisaccharide –cym]$^+$, 339 (7) [383 $-CH_3CHOH]^+$, 326 (6) [386 $-CH_3CH_3CHOH]^+$, 325 (5) [342–OH]$^+$, 324 (5) [trisaccharide –(terminal Glu –OH]$^+$, 321 (5) [353 $-CH_3OH]^+$, 317 (16) [353–$2H_2O]^+$, 307 (4) [325 $-H_2O$; 324-OH]$^+$, 303 (8) [321 $-H_2O]^+$, 289 (49) [307 $-H_2O]^+$, 285 (15) [303 $-H_2O]^+$, 278 (7) [324 $-OCHOH]^+$, 277 (7) [321 $-CH_3CHO]^+$, 271 (66) [289 $-H_2O$; 317 $-HOCHO]^+$, 264 (10) [342 $-C_2H_4O_2]^+$, 259 (11) [277 $-H_2O]^+$, 257 (13) [289 $-CH_3OH]^+$, 256 (14) [324 $-CH_3OH$-$2H_2O]^+$, 248 (5) [324 $-CH_3OH$ $-CH_3CHO]^+$, 239 (15) [257 $-H_2O$; 285 $-HOCHO]^+$, 231 (5) [277 $-HOCHO]^+$, 229 (8) [289 $-C_2H_4O_2]^+$, 228 (7) [264 $-2H_2O]^+$, 213 (18) [259 $-HOCHO]^+$, 210 (64) [228-$H_2O]^+$, 209 (60) $[C_7H_{13}O_7]^+$, 199 (12) [259 $-C_2H_4O_2]^+$, 197 (10) [229 $-CH_3OH]^+$, 191 (11) $[C_8H_{15}O_5]^+$, 180 (61) [Glu]$^+$, 163 (23) [209–HOCHO; 180 $-OH]^+$, 162 (21) [cym]$^+$, 159 (15) [191 $-CH_3OH]^+$, 145 (29) [cym–OH; 191 –HOCHO; 163 $-H_2O]^+$, 131 (34) [191 $-C_2H_4O_2]^+$, 127 (17) [145 $-H_2O]^+$, 115 (19) [159 $-CH_3CHO]^+$, 113 (23) [145 $-CH_3OH]^+$, $[C_4H_7O_3]$, 95 (58) [127 $-CH_3OH$; 113 $-H_2O$]+, 87 (93) $[C_4H_7O_2]^+$.

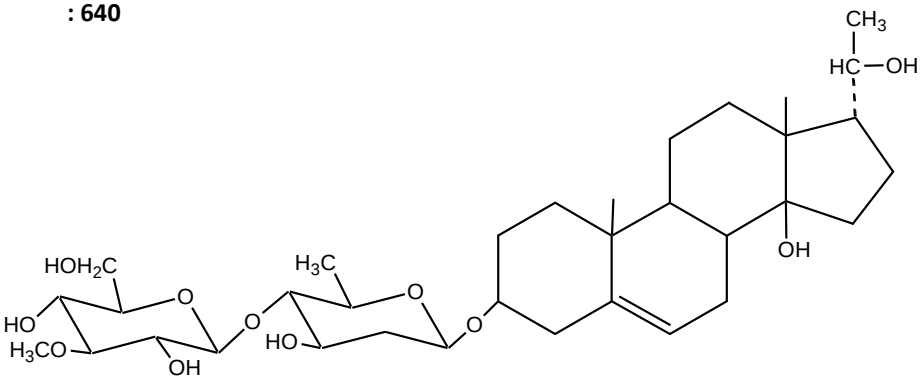
COMPOUND 30

Compound : Deniculatin

Compound Name : calogenin-3-O-β-D-digitoxopyranosyl-(1-4)-O-β-D-Galactopyranoside

Mol. formula : $C_{34}H_{56}O_{11}$

Mol. Wt. : 640



FAB-MS: m/z (rel.int.): 663 (29) $[M+Na]^+$, 622 $[M-H_2O]^+$, 577 (43) $[622-CH_3CHOH]^+$, 559 (22) $[577-H_2O]^+$, 515 (25) $[559-CH_3CHO]^+$, 509 (23) $[559-H_2O-CH_3OH]^+$, 507 (20) $[M-C_5H_9O_4]^+$, 489 (27) $[507-H_2O]^+$, 483 $[515-CH_3OH]^+$, 464 (18) $[M-(Gal-OH)]^+$, 429 (15) $[489-CH_3OCHO]^+$, 419 (13) $[464-CH_3CHOH]^+$, 408 (19) $[483-CH_3-CH_2OHCHO]^+$, 404 (14) $[419-CH_3]^+$, 393 (16) $[408-CH_3]^+$, 386 (13) $[404-H_2O]^+$, 371 (12) $[386-CH_3]^+$, 369 (22) $[429-CH_3-CH_2CHOH]^+$, 351 (10) $[369-H_2O]^+$, 327 (11) $[371-CH_3CHO]^+$, 307 (14) [disaccharide $-OH]^+$, 289 (18) $[307-H_2O]^+$, 211 (10) $[289-H_2O-CH_2OHCHO]^+$.

EI-MS: m/z (rel. int.): $[M]^+$, (not observed), 577 (3) $[M-CH_3CHOH-H_2O]^+$, 419 (3) $[M-(Gal\text{-}OH)-CH_3CHOH]^+$, 401 [577 –(Gal –OH), 419 $-H_2O]^+$, 386 (2) $[401-CH_3]^+$, 371 (2) $[386-CH_3]^+$, 368 (3) $[386-H_2O]^+$, 339 (5) $[401-H_2O-CH_3CHO]^+$, 306 (4) [disaccharide $-H_2O]^+$, 289 (68) [genin $-CH_3CHOH]^+$, 256 (12) $[306-H_2O-CH_3OH$; 289 $-H_2O-CH_3]^+$, 238 (11) $[256-H_2O]^+$, 228 (25) $[306-H_2O-CH_2OHCHO]^+$, 131 (25) [disaccharide $-C_5H_9O_4-H_3COCHO]^+$, 113 (15) $[131-H_2O]^+$.

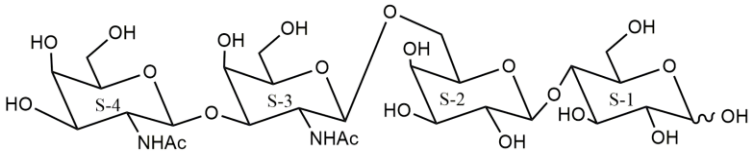
COMPOUND 31 (Singh P et.al 2017)

Compound : Caprose

Compound Name: GalNAc-β-(1→3)-GalNAc-β-(1→ 6)-Gal-β-(1→4)Glc

Mol. formula : $C_{28}H_{48}O_{21}N_2$

Mol. Wt. : 748



ES-MS: m/z 771 $[M+Na]^+$, 748 $[M]^+$, 627$[M-NHCOCH_3]$, 575[748–3H_2O–2OH], 545[748–S_4],545[603–$NHCOCH_3$],495[545–CH_3OH], 487[545–$NHCOCH_3$], 455[487–CH_3OH], 440[545–CH_2OH–CH_3OH–CH_2C=O], 422[440–H_2O],407[455–CH_2OH–OH], 406[455–CH_3OH–H_2O], 391[422–CH_2C=O], 441[545–2CH_2OH–CH_2C=O], 365[440–$NHCOCH_3$–OH], 346[441–CH_2OHCHO–H_2O–OH] 371[406–H_2O–OH], 303[371–4OH], 225[303–CH_2OHCHO–H_2O], 222[303–CH_2OH–CH_3OH–H_2O], 260[324–2CH_3OH], 225[260–H_2O], 169[262–CH_2OHCHO–CH_3OH].

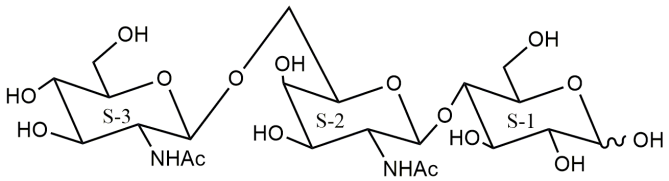
COMPOUND 32 (Singh M. et.al 2016)

Compound : Bosiose

Compound Name: GlcNAc-β-(1→6)-GalNAc-β-(1→4)Glc

Mol. formula : $C_{22}H_{38}O_{16}N_2$

Mol. Wt. : 586



ES-MS: m/z 586 $[M]^+$, 568[M–H_2O], 551[568–OH], 526[586–CH_2OHCHO], 484[526–CH_2C=O], 483[526–CH_3CO], 467[484–OH], 466[484–H_2O], 465[483–H_2O], 449[467–H_2O], 431[449–H_2O], 383[586–S_3], 340[383–$COCH_3$], 325[383–$NHCOCH_3$], 323[383–CH_2OHCHO], 305[323–H_2O], 290[325–H_2O–OH], 259[290–CH_2OH], 242[259–OH].

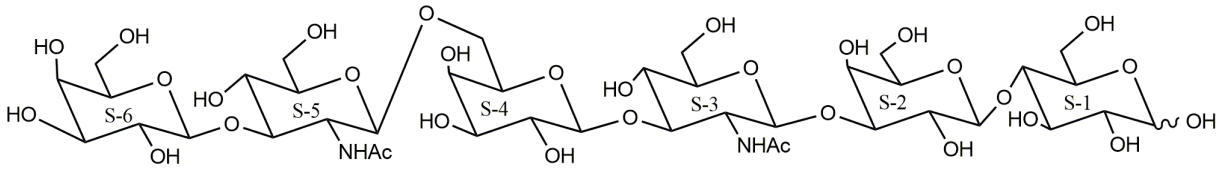
COMPOUND 33 (Singh M. et.al 2016)

Compound : Bovisose

Compound Name:Gal-β-(1→3)-GlcNAc-β-(1→6)Gal-β-(1→3)-GlcNAc-β-(1→3)Gal-β-(1→4) Glc

Mol. formula :$C_{40}H_{68}O_{31}N_2$

Mol. Wt. : 1072



ES-MS: m/z 1134 $[M+Na+K]^+$, 1111$[M+K]^+$, 1073 $[M+H]^+$, 1072$[M]^+$, 1043 [M–CHO], 1025 [1043–H_2O], 1012 [M–CH_2OHCHO], 1008 [M–2CH_3OH], 995 [1012–OH], 994 [1012–H_2O], 966 [1008–CH_2CO], 952 [994–CH_2CO], 923 [958–CHO], 910 [M–S_6], 893 [910–OH], 892 [910–H_2O], 881 [910–CHO], 864 [881–OH], 812 [864–CH_2CO], 806[864–$NHCOCH_3$], 707 [910–S_5], 690 [707–OH], 689 [707–H_2O], 632 [690–$NHCOCH_3$], 627[669–CHO], 591 [627–2H_2O], 545 [707–S_4], 527 [545–H_2O], 503 [545–CH_2CO], 498 [527–CHO], 481[498–OH], 440[498–$NHCOCH_3$], 422[440–H_2O], 406[440–2OH], 384[406–H_2O], 364[406–CH_2CO], 342[545–S_3], 324[342–H_2O], 308[342–2OH], 282[342–CH_2OHCHO], 264 [282–H_2O], 222 [264–CH_2CO], 180[342–S_2], 162[180–H_2O].

COMPOUND 34 (Mani A. et.al 2017)

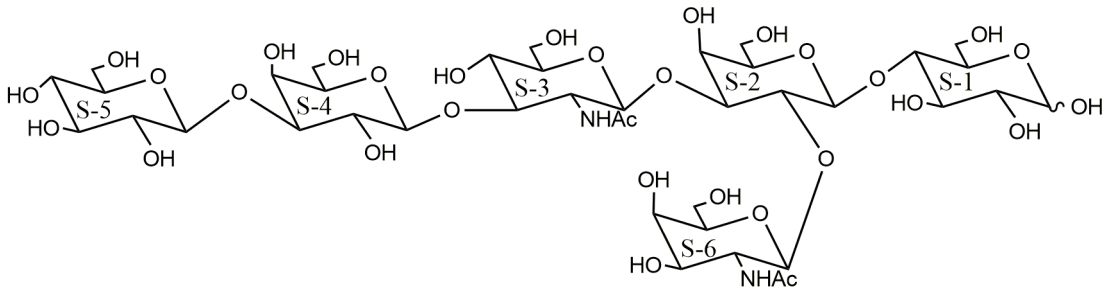
Compound : Dariose

Compound Name:

Glc-β(1→3) Gal-β(1→3) GlcNAc-β(1→3)Gal-β(1→4) Glc
↑
GalNAc-β(1→2)

Mol. formula : $C_{40}H_{68}O_{31}N_2$

Mol. Wt. : 1072



ES Mass: m/z 1111 $[M+K]^+$, 1095 $[M+Na]^+$, 1072 $[M]^+$, 1025 [1072–H_2O,–CHO], 1012 [1072–CH_2OHCHO], 965 [1012–H_2O,–CHO], 919 [965–CH_2OH,–OH], 869 [1072–S_6] 820[838– H_2O], 707[869–S_5] 582 [659–OH, CH_2OHCHO], 565 [582–OH], 545[707–S_4] 496 [527–CH_2OH], 342[545–S_3], 295 [313–H_2O], 180 [342–S_2].

COMPOUND 35 (Singh A.K et.al 2016)

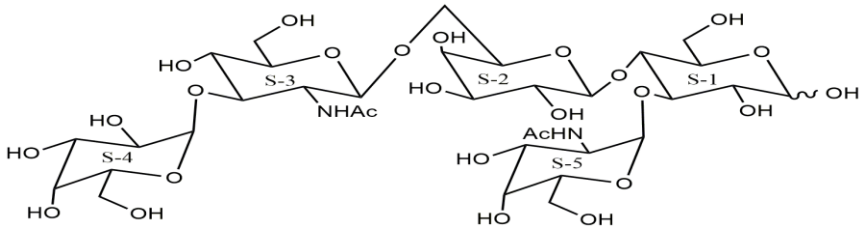
Compound : Grunniose

Compound Name:

Gal-α(1→3) GlcNAc-β(1→6) Gal-β(1→4) Glc
↑
GalNAc-α(1→3)

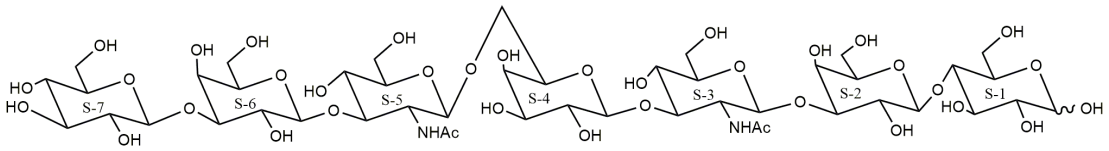
Mol. formula : $C_{34}H_{58}O_{26}N_2$

Mol. Wt. : 910



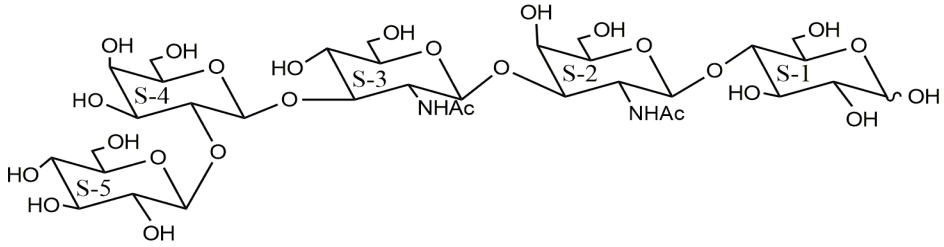
ES-MS:m/z 949 $[M+K]^+$,910$[M]^+$, 893 [910–OH], 892 [910–H_2O], 853 [892–CHOH], 832 [892–CH_2OHCHO], 806 [864–$NHCOCH_3$], 707 [910–S_5], 690 [707–OH], 689 [707–H_2O], 632 [690–$NHCOCH_3$], 630 [690–CH_2OHCHO], 614 [632–H_2O], 596 [614–H_2O], 590 [632–CH_2CO], 545 [707–S_4], 528 [545–OH], 527 [545–H_2O], 499 [528–CHO], 498 [527–CHO], 481[499–H_2O], 465 [499–2OH], 462 [498–2H_2O], 440 [498–$NHCOCH_3$], 422 [440–H_2O], 404 [422–H_2O], 362 [404–CH_2CO], 342 [545–S_3], 324 [342–H_2O], 308 [342–2OH], 307 [324–OH], 277 [308–CH_2OH], 276 [307–CH_2OH], 260 [277–OH], 242 [260–H_2O], 180 [342–S_2], 162 [180–H_2O].

COMPOUND 36 (Singh A.K 2016)
Compound : **Vakose**
Compound Name: **Glc-β(1→3)Gal-β(1→3)GlcNAc-β(1→6)Gal-β(1→3)GlcNAc-β(1→3)Galβ(1→4)Glc**
Mol. formula : $C_{46}H_{78}O_{36}N_2$
Mol. Wt. **: 1234**



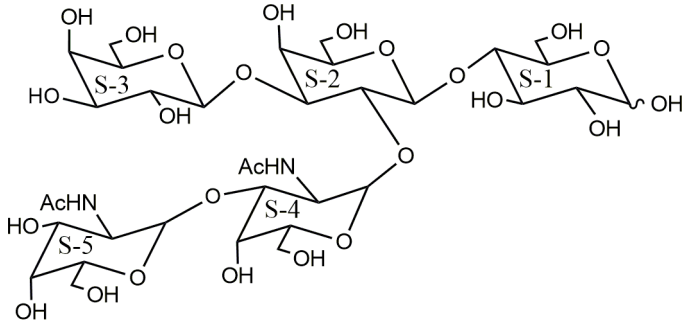
ES-MS:m/z 1296 $[M+Na+K]^+$, 1234 $[M]^+$, 1199 [M–H_2O–OH], 1198 [M–$2H_2O$], 1178 [1196–H_2O], 1139 [1197–$NHCOCH_3$], 1121 [1139–H_2O], 1110 [1139–CHO], 1089 [1121–CH_3OH], 1072 [M–S_7], 1061 [1121–CH_2OHCHO], 1041 [1072–CH_2OH], 1010 [1041–CH_2OH], 993 [1010–OH], 974 [1010–$2H_2O$], 956 [974–H_2O], 933 [993–CH_2OHCHO], 910 [M–S_6], 893 [910–OH], 892 [910–H_2O], 881 [910–CHO], 864 [881–OH], 863 [893–CHOH], 806 [864–$NHCOCH_3$], 805 [863–$NHCOCH_3$], 773 [805–CH_3OH], 763 [805–CH_2CO], 745 [763–H_2O], 727 [745–H_2O], 707 [910–S_5], 690 [707–OH], 689 [707–H_2O], 648 [707–CHOHCHO], 590 [648–$NHCOCH_3$], 558 [590–CH_3OH], 545 [707–S_4], 527 [545–H_2O], 499 [558–CHOHCHO], 481 [499–H_2O], 467 [527–CH_2OHCHO], 465 [499–2OH], 425 [467–CH_2CO], 406 [465–CHOHCHO], 396 [425–CHO], 365 [425–CH_2OHCHO], 364 [406–CH_2CO], 342 [545–S_3], 325 [342–OH], 307 [342–OH–H_2O], 278 [307–CHO], 260 [278–H_2O], 242 [260–H_2O], 224 [242–H_2O], 180 [342–S_2], 162 [180–H_2O].

COMPOUND 37(Singh M. et. al 2015)
Compound : **Meeniose**
CompoundName:β-GalNAc-(1→2)-β-Gal(1→4)-β-GlcNAc-(1→3)-β-GalNAc-(1→4)-Glc
Mol. formula: $C_{36}H_{61}O_{28}N_3$
Mol. Wt. **: 951**



ES-MS:m/z 1013$[M+Na+K]^+$, 990 $[M+K]^+$, 951$[M]^+$, 933 [951–H_2O], 893 [910–$NHCOCH_3$], 862 [893–CH_2OH], 748[951–S_5], 700 [748–OH,–CHO], 657[748–CH_2OHCHO,–CHO], 586[748–S_4], 490[586–$2H_2O$,–CH_2OHCHO],528[586–$NHCOCH_3$], 383[586–S_3], 318[383–CH_2OH,–2OH], 258[318–CH_2OHCHO], 296[383–$NHCOCH_3$,–CHO], 180[383–S_2].

COMPOUND 38(Singh M. et. al 2015)
Compound : **Murtiose**
Compound Name:β-Gal (1→3)-β-Gal(1→4)-Glc
|
α-GalNAc(1→3)-α-GalNAc(1→2)
Mol. formula: $C_{34}H_{58}O_{26}N_2$
Mol. Wt. **: 910**

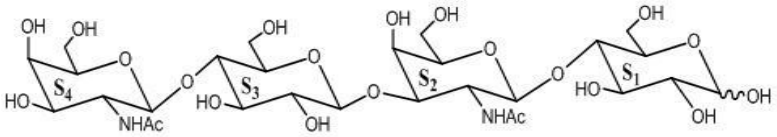


Indexed, Abstracted and Cited in Indexed Copernicus International and 20 other databases of National and International repute

ES-MS: m/z 972 $[M+Na+K]^+$, 949$[M+K]^+$, 910$[M]^+$, 875[910–H_2O,–OH], 851[910–CH_2OCHO], 820[851–CH_2OH], 785[820–OH,–H_2O], 748[910–S_3], 671[748–CH_2OHCHO,–OH], 717 [748–CH_2OH], 545[748–S_5], 507[545–2H_2O, –2H^+], 485[545–CH_2OHCHO], 487[545–$NHCOCH_3$], 342[545–S_3], 284[342–$NHCOCH_3$], 325[342–OH], 281[342–CH_2OHCHO,–H^+].

COMPOUND 39(Rathore R.S et.al 2018)
Compound : Vulgose
Compound Name: GalNAc-β-(1→4)-Glc-β-(1→3)-GalNAc-β-(1→4)-Glc

Mol. formula:$C_{28}H_{48}O_{21}N_2$
Mol. Wt. : 748

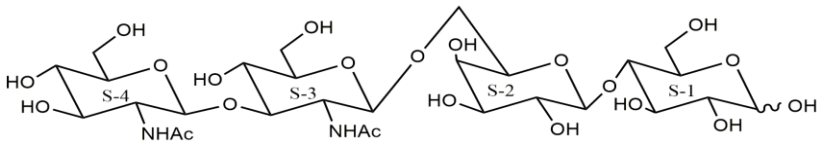


FAB-MS: m/z 787$[M+K]^+$, 749$[M+H]^+$,748$[M]^+$, 751[787–2H_2O], 745[787–CH_2=C=O], 730[M–H_2O], 717[M–CH_2OH], 712[M–2H_2O], 707[749–CH_2=C=O], 706[M–CH_2=C=O], 699[730–CH_2OH], 693[751–$NHCOCH_3$], 688[M–CH_2OHCHO], 672[730–$NHCOCH_3$], 670[712–CH_2=C=O], 653[688–H_2O,–OH], 639[688–CH_2OH,–H_2O], 634[670–2H_2O], 632[M–2$NHCOCH_3$], 596[632–2H_2O], 545[749–S_4] 514[545–CH_2OH], 478[514–H_2O], 472[514–CH_2=C=O], 456[514–$NHCOCH_3$], 443[478–H_2O,–OH], 414[443–CH_2=C=O], 412[443–CH_2OH], [472–CH_2OHCHO],383[545–S_3],365[383–H_2O], 325[383–$NHCOCH_3$], 323[383–CH_2OHCHO], 307[365–$NHCOCH_3$], 305[323–H_2O], 289[307–H_2O], 287[323–2H_2O],180[383–S_2].

COMPOUND 40(Gunjan et.al 2017)
Compound : Bosonose acetate
Compound Name: Glc-α-(1→6)-Gal-β-(1→4)-Glc
Mol. Formula:$C_{18}H_{32}O_{16}$
Mol. Wt. : 966

FAB-MS: m/z 967 $[M+H]^+$, 779[966–CH_3COOH,–CH_3CO,–2CH_2=C=O], 637[967–S_3] 595[637–2CH_2=C=O], 577[619–CH_2=C=O], 559[619–CH_3COOH], 535[577– CH_2=C=O], 517[577–CH_3COOH], 457[577–2CH_3COOH], 391[577–CH_3COOH,–CH_3CO, 2CH_2=C=O], 229[391–2CH_3COOH,–CH_2=C=O], 187[229–CH_2=C=O], 169[229–CH_3COOH], 109[169–CH_3COOH].

COMPOUND 41(Gunjan et.al 2016)
Compound : Indicose
Compound Name:GlcNAc-β-(1→3)-GlcNAc-β-(1→6)-Gal-β-(1→4)-Glc
Mol. formula:$C_{28}H_{48}O_{21}N_2$
Mol. Wt. : 748

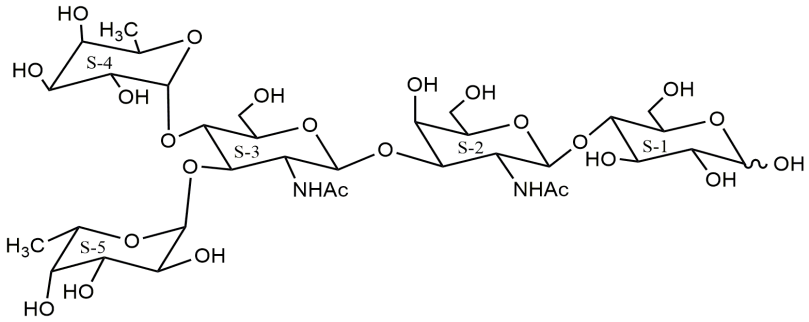


FAB-MS:m/z 810 $[M+Na+K]^+$, 787$[M+K]^+$, 771 $[M+Na]^+$, **:** 748 $[M]^+$,774[810–2H_2O], 742[771–CHO], 714[774–CH_2OHCHO], 686[M–2CH_2OH], 656[714–$NHCOCH_3$], 632 [M–2$NHCOCH_3$], 625[656–CH_2OH], 614[632–H_2O], 583[614–CH_2OH], 583[787–S_4], 583[625–CH_2=C=O], 566[810–GlcNAc–H_2O],523[583–CH_2OHCHO], 465[686–GlcNAc–H_2O], 465[523–$NHCOCH_3$], 430[465–H_2O–OH], 430[465–H_2O–OH], 413[430–OH], 345[406– CH_2OH-CHOH], 329[365–2H_2O], 329[406–CH_2OHCHO–OH], 329[413–2CH_2=C=O],279[345–2CH_3CO], 244[465–GlcNAc–H_2O], 209[244–H_2O–OH],192[329–CH_2OHCHO–CH_2=C=O–H_2O–OH], 167[209–CH_2=C=O], 149[180–CH_2OH], 107[149–CH_2=C=O].

COMPOUND 42(Kumar K et.al 2016)
Compound : **Gaurose**
Compound Name:

Fuc-α-(1→ 4)-GlcNAc-β (1→3) GalNAc-β (1→ 4) Glc
↑
Fuc-α-(1 → 3)

Mol. formula:$C_{34}H_{58}O_{24}N_2$
Mol. Wt. **: 878**

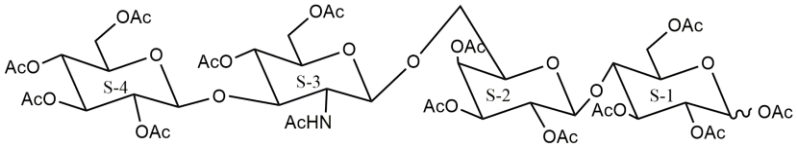


FAB-MS:m/z 940 $[M+Na+K]^+$ m/z 878 $[M^+]$, 775 [878–$2CH_2OH$–CH_3OH–CHO], m/z 732 [878–S_5], 722[878–S_5], 677[775–$3H_2O$–2OH], 688[878–CH_2CO–2OH–$2H_2O$], 687[722–H_2O–OH], 617[687–$2H_2O$–OH], 618[688–CH_2CO–CH_3OH], 586[732–S_4], **556 [],**551[618–H_2O–OH–CH_3OH], 673[722–CH_3OH–OH], 664[722–$NHCOCH_3$], 617[673–CH_2OH–CHO], 538[617–2OH], 497[556–CH_3OH–OH], 465[497–CH_3OH], 466[497–CH_2OH],382[586–S_3], 303[352–CH_3OH–OH], 244[303–CH_2CO–OH], 185[244–CH_2CO–OH], 180[382–S_2].

COMPOUND 43(Khan M. et.al 2017)
Compound : **Indinose acetate**
Compound Name:

Glcβ(1→3)GlcNAcβ(1→6)Galβ(1→4)Glc

Mol. formula:$C_{52}H_{71}O_{34}N$
Mol. Wt. : 1253

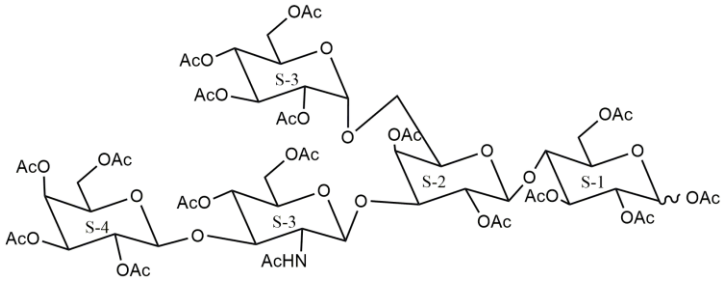


FAB-MS:m/z 1276 $[M+Na]^+$, 1192[1276–$2CH_2$=C=O], 1119[1192–CH_2=C=O, –OCH_3], 1031[1192–CH_3COOH, –CH_3CO, –$NHCOCH_3$], 1014[1031–OH], 970[1120–CH_3COOCH_3COOH–OCH_3], 922 [1253–S_4], 865[1014–$2CH_3COO$–OCH_3] 834[865–OCH_3], 747[865–$2CH_3COO$], 705[747–CH_2=C=O] 659[865–$2CH_3COOH$–$2CH_3CO$], 637[679–CH_2=C=O], 619[922–S_3], 577[619–CH_2=C=O], 517[577–CH_3COOH], 475[517– CH_2=C=O], 426[517–CH_3COOH–OCH_3], 391[475–$2CH_2$=C=O], 373[475–CH_3COOH–CH_2=C=O], 331[619–S_2], 229[331–CH_3COOH,–CH_2=C=O],211[331–$2CH_3COOH$], 169[229–CH_3COOH], 109[169–CH_3COOH].

COMPOUND 44(Gangwar L et.al 2017)
Compound : **Dicusose acetate**
Compound Name:

Galα(1→6)
|
Galβ(1→3)GlcNAcβ(1→3)Galβ(1→4)Glc

Mol. formula:$C_{64}H_{88}O_{41}N_2$
Mol. Wt. **:** 1541

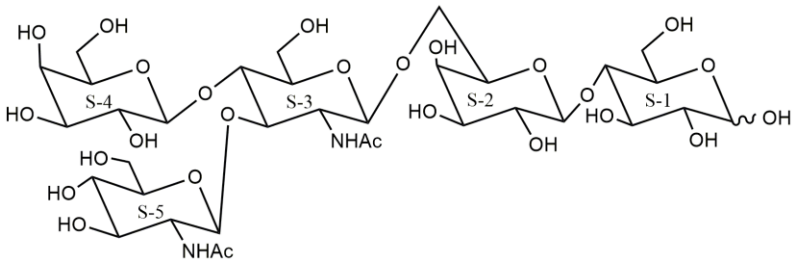


FAB-MS: m/z 1565[M+Na+H]$^+$, 1541[M]$^+$,1507[1565–NHCOCH$_3$], 1434[1507–CH$_2$=C=O,–OCH$_3$], 1347[1507–2CH$_3$COO,–CH$_2$=C=O], 1320[1507–2CH$_2$=C=O,–CH$_3$COOH,–OCH$_3$], 1211[1541–S$_5$], 1275[1434–NHCOCH$_3$,–CH$_2$=C=O,–OCH$_3$],1234 [1565–Gal], 1172[1275–CH$_3$CO(OH)COCH$_3$], 1153[1211–NHCOCH$_3$], 1129[1172–CH$_3$CO], 1069[1129–CH$_3$COOH], 1043[1129–2CH$_3$CO], 981 [1234–3CH$_3$COOH,–OCH$_3$,–CH$_2$=C=O], 924[1541–S$_4$–S$_3$], 903 [1234–Gal], 880[1211–S$_4$], 803 [981– CH$_3$COOH,–2OCH$_3$], 731 [981–3CH$_3$COO,–OCH$_3$,–CH$_2$=C=O], 718 [803–CH$_3$CO,–CH$_2$=C=O], 617[1541–S$_1$,–S$_2$,–S$_5$], 595 [M–S$_5$,–S$_4$,–S$_3$], 577[M–S$_5$,–S$_4$,–S$_3$], 535[577–CH$_2$=C=O], 493[535–CH$_2$=C=O], 391[493–CH$_3$COOH,–CH$_2$=C=O], 247 [M–S$_5$,S$_4$,S$_3$,S$_1$], 229 [331–CH$_3$COOH,–CH$_2$=C=O], 187[229–CH$_2$=C=O], 169[229–CH$_3$COOH], 109[169–CH$_3$COOH].

COMPOUND 45(Khan M et.al 2017)
Compound : **Indose**
Compound Name:

Galβ(1→4)
|
GlcNAcβ(1→6)Galβ(1→4)Glc
|
GlcNAcβ(1→3)

Mol. formula:C$_{64}$H$_{88}$O$_{41}$N$_2$
Mol. Wt. **:** 1540

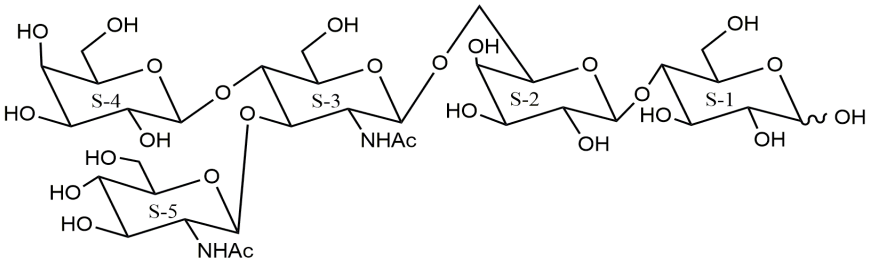


FAB-MS: m/z 1563[M+Na]$^+$, 1540 [M]$^+$, 904 [881–Na], 881[M–S$_4$-S$_5$], 830[904–CH$_3$COOCH$_3$],801[904–CH$_3$COOH-CH$_3$CO], 779[881–CH$_3$COOH–CH$_2$=C=O], 759[801–CH$_2$=Cs=O], 741[801–CH$_3$COOH], 717 [759–CH$_2$=C=O], 701 [759–NHCOCH$_3$], 700[759–CH$_3$COO], 659[701–CH$_2$=C=O], 619[881–S$_3$], 577[619–CH$_2$=C=O], 559[619–CH$_3$COOH], 499[619–2CH$_3$COOH], 457[559–CH$_3$COOH,–CH$_2$=C=O], 390[577–CH$_3$COOH,–3CH$_2$=C=O], 331[M–S$_4$], 289[M–S$_5$] 229[331–CH$_3$COOH–CH$_2$=C=O], 211[331–CH$_3$COOH], 169 [229–CH$_3$COOH], 109[169–CH$_3$COOH].

COMPOUND 46(Gangwar L et.al 2018)
Compound : **Medalose**
Compound Name:

Gal-β-(1→3)GlcNAc-β-(1→6)Gal-β(1→4)Glc
4
↓
1GlcNAcβ

Mol. formula:C$_{34}$H$_{58}$O$_{26}$N$_2$
Mol. Wt. **:** 910



ES mass: m/z933[M+Na]$^+$, 910[M], 748[910–S_4], 545[748–S_5], 342[545–S_3], 180[342–S_2], 527[545–H_2O], 892[910–H_2O], 844[892–CH_2OH], 771[844–CH_2OH], 848[910–2CH_2OH], 789[848–CH_2CO–OH], 758[789–CH_2OH], 861[910–CH_2OH–H_2O], 819[861–CH_2CO], 771[819–CH_2OH–OH],837[910–CH_2OH–CH_2CO], 819[837–H_2O], 730[748–H_2O], 699[730–CH_2OH], 681[699–H_2O], 621[681–CH_2OHCHO], 590[621–CH_2OH], 688[748–CH_2OH–CHO], 652[688– 2H_2O], 621[652–CH_2OH], 579[621–CH_2CO], 659[748–$NHCOCH_3$–CH_2OH], 611[659–CH_2OH–OH],593[611–H_2O], 576[593–OH], 558[576–H_2O], 465[527–2CH_2OH], 406[465–CH_2CO–OH], 375[406–CH_2OH], 357[375–H_2O], 496[545–CH_2OH–H_2O], 479[496–CH_2OH–H_2O], 462[479–OH], 402[462–CH_2OHCHO], 384[402–H_2O], 342[384–CH_2 CO], 293[342–CH_2OH–H_2O], 275[293–H_2O], 257[275–H_2O], 239[257–H_2O], 190[239–CH_2OH–H_2O], 325[342–OH], 266[325–CH_2CO–OH], 218[266–CH_2CO–OH], 200[218–H_2O].

COMPOUND 47(Saksena R et.al 1999)

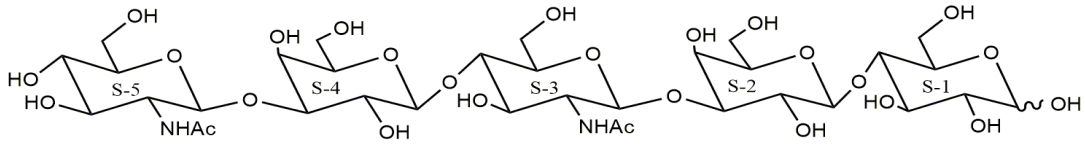
Compound : **Substance P2, $C_{34}H_{58}N_2O_{26}$**

Compound Name:

$$GlcNAc–\beta–(1\rightarrow3)–Gal–\beta–(1\rightarrow4)–GlcNAc–\beta–(1\rightarrow3)–Gal–\beta–(1\rightarrow4)Glc$$

Mol. formula:$C_{34}H_{58}O_{26}N_2$

Mol. Wt. **:** 910

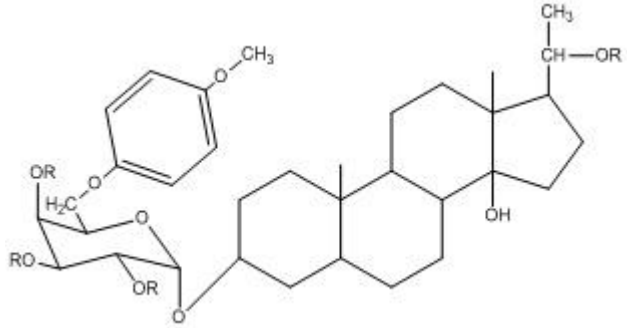


FAB-MS:m/z949[M+K], 933[M+Na], 911[M+H], 893[911–H_2O], 880[911–CH_2OH], 857[893–2H_2O], 853[911–$NHCOCH_3$], 849[880–CH_2OH], 833[893–CH_2OHCHO], 799[857–$NHCOCH_3$], 795[853–CH_2OHCHO], 791[833–CH_2=C=O],775[949– CH_2OHCHO,–CHOH,–$CHNHCOCH_3$], 758[775–OH],749[791–CH_2=C=O], 746[949–S_5], 732[911–S_1], 730[933–S_5], 716[758–CH_2=C=O], 712[730–H_2O], 708[911–S_5], 704[746–CH_2=C=O],698[716– H_2O], 694[712–H_2O],693[746–2H_2O,–OH], 690[708–H_2O],684[746–CH_2OH], 680[698–H_2O], 676[694–H_2O], 672[690–H_2O],657[693–2H_2O], 649[680–CH_2OH], 637[672–H_2O–OH], 632[690–$NHCOCH_3$],586[911–S_2,S_1], 579[637–$NHCOCH_3$], 568[933–S_5–S_4], 567[949-S_5-S_4], 566[657–CH_2=C=O, –H_2O,–CH_2OH], 551[568–OH],546[911–S_5,S_4], 543[579–2H_2O], 529[546–OH], 528[546–H_2O], 515[551–2H_2O], 511[528–OH], 487[529–CH_2=C=O], 468[528–CH_2OHCHO], 409[468–$NHCOCH_3$], 383[911–S_3-S_2-S_1], 365[383–H_2O], 343[911–S_5,S_4,S_3], 342[S_2,S_1], 329[365–2H_2O], 307[383–$NHCOCH_3$, –H_2O], 305[365–CH_2OHCHO], 290[307–OH], 288[342–3H_2O], 257[288–CH_2OH], 247[307–CH_2OHCHO], 226[257–-CH_2OH], 180[S_1].

COMPOUND 48(Neupane P.B et.al 2017)

Compound : Ichnoside

Compound Name:3β, 14β, 20β-trihydroxy-pregnane-3-O-α (6-O-methoxy phenyl) galactopyranoside



Mol. formula: $C_{34}H_{52}O_9$

Mol. Wt. : 604

FAB-MS: m/z627 $[M+Na]^+$, 604 $[M]^+$, 559 $[M - CH_3CHOH]^+$,336 $[M–S_1]^+$, 318 $[genin–H_2O]^+$, 273$[318–CH_3CHOH]^+$, 269$[S1–OH]^+$, 251$[S_1–OH–H_2O]^+$, 249[336– $OHCH_2CH_2CH_2CHCH]^+$,246$[318–CH_3CHOH–CH_2CH]^+$, 233$[251–H_2O]^+$, 166[251– OHCHCHCOHCH]$^+$, 108 $[OHCHCHCOHCH+Na]^+$.

COMPOUND 49(Gunjan et.al 2018)

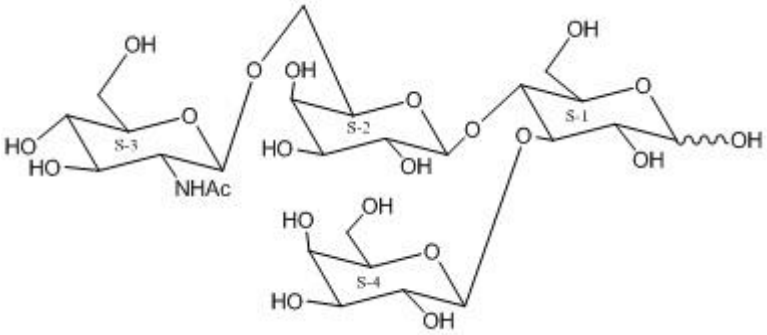
Compound : **Camelose**

Compound Name :

GlcNAc–β–(1→6)Gal–β–(1→4)Glc
|
Gal–β–(1→3)

Mol. formula : $C_{26}H_{45}O_{21}N$

Mol. Wt. : **707**



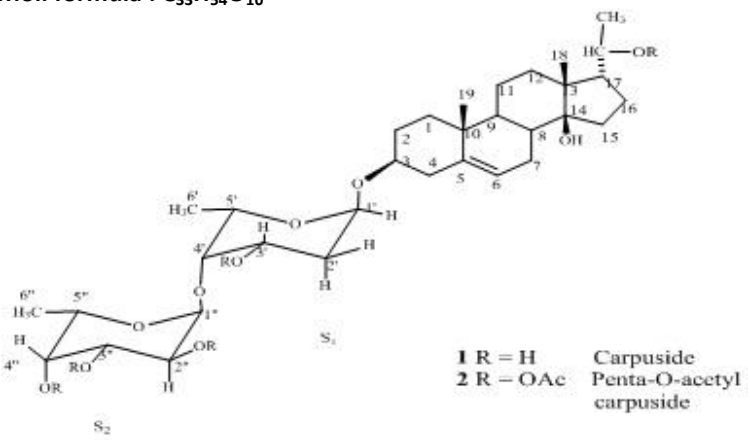
ES-Ms:769$[M+Na+k]^+$, 746$[M+K]^+$, 730$[M+Na]^+$,707$[M]^+$647[707–CH_2OHCHO], 612[647–H_2O–OH], 648[707–CH_2CO–OH], 612[648–$2H_2O$], 552[612–$CH_2CO–H_2O$], 689[707–H_2O], 629[689–$CH_2CO–H_2O$], 612[629–H_2O], 547[612–CH_2OH–2OH], 649[707–$NHCOCH_3$], 618[649–CH_2OH], 601[618–OH], 504 [M–S_3], 456[504–CH_2OH–OH], 421[456–H_2O–OH], 343[421–$CH_2OHCHO–H_2O$], 473[504–CH_2OH], 456[473–OH], 342[504–S_4], 325[342–OH], 294[325–CH_2OH], 232[294–$2CH_2OH$], 242[294–$2H_2O$-OH], 197[232–H_2O–OH], 184[232–CH_2OH–OH], 300[342–CH_2CO], 269[300–CH_2OH], 251[269–H_2O], 233[251–H_2O], 288[342–$3H_2O$], 180[342–S_2], 163[180–OH].

COMPOUND 50(Srivastava A.et.al 2017)

Compound : **Capruside**

Compound Name :calogenin-3-O-α-L-fucopyranosyl-(1→4)-α-L-digitoxopyranoside.

Mol. formula : $C_{33}H_{54}O_{10}$



Mol. Wt. : **610$[M]^+$**

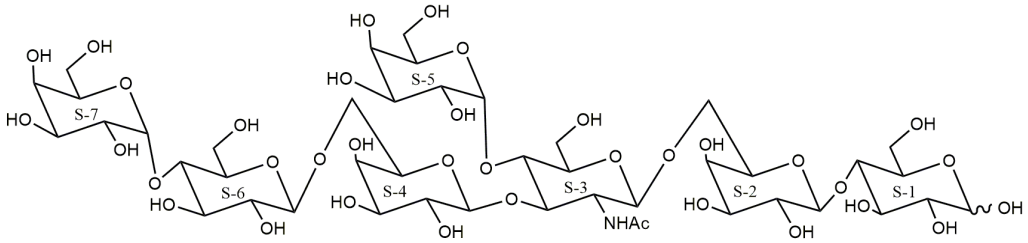
FAB-MS: m/z 633$[M+ Na]^+$, 610$[M]^+$, 565[610–CH_3CHOH], 333[aglycon], 277[disaccharide–OH], 259[277–H_2O], 463[610– S_2], 333[463–S_1], 418[463–CH_3CHOH], 278[418–S_1], 288[genin–CH_3CHOH],592$[M^+–H_2O]$, 547$[M^+–CHOHCH_3–H_2O]$,529[547–H_2O], 485[529–CH_3CHO], 467[485–H_2O], 437[467–$2CH_3$], 547$[M^+–CHOHCH_3–H_2O]$, 403[418–CH_3], 385[403–H_2O], 370[385–CH_3], 326[370–CH_3CHO].

COMPOUND 51(Maurya R.K et.al 2017)
Compound : **Labiose**

Compound Name :

$$\alpha Gal(1\rightarrow4)$$
$$\uparrow$$
$$\alpha Gal(1\rightarrow4)\beta Glc(1\rightarrow6)\beta Gal(1\rightarrow3)\beta GlcNAc(1\rightarrow6)\beta Gal(1\rightarrow4)Glc$$

Mol. formula : $C_{44}H_{75}O_{36}N_1$
Mol. Wt. : 1232$[M+K]^+$

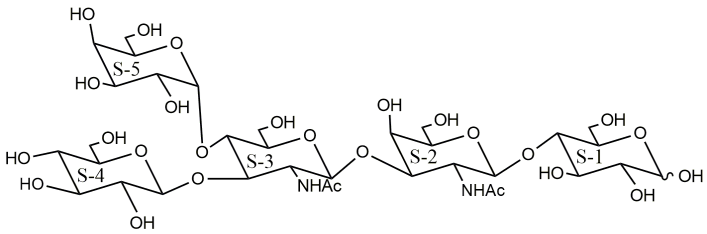


ES-MS: m/z 1232 $[M+K]^+$,1176[M–OH], 1115[M–CH_2OHCHO,–H_2O], 1175[M–H_2O], 1100 [M–H_2O, –OH,–$NHCOCH_3$], 1162 [M–CH_2OH], 1102 [M–CH_2OHCHO, –CH_2OH], 1073[M–H_2O, -CH_2OHCHO,–CH_2=C=O], 1031[1014–S_7],1014 [1031-OH], 954[1031-OH, –CH_3OHCHO], 983[1031–CH_2OH,–OH], 989[1031–CH_2=C=O], 958[1031–CH_2OH,–CH_2=C=O], 922[1031–CH_2OH,–CH_2=C=O,–$2H_2O$], 995 [1031–$2H_2O$],978[1031–$2H_2O$,–OH], 960[1031– $3H_2O$, –OH], 973 [1031–$NHCOCH_3$], 942 [1031–$NHCOCH_3$, –CH_2OH], 1013[1031–H_2O], 940[1031–CH_2=C=O, –CH_2OH, –H_2O], 922[1031–CH_2=C=O,–CH_2OH, –$2H_2O$], 898[1031–CH_2=C=O,–CH_2OH, CH_2OHCHO], 869[1031–S_6], 833[869–$2H_2O$], 791[869–CH_2=C=O,–$2H_2O$], 773[869–$2H_2O$, –CH_2OHCHO], 820[869–H_2O,–CH_2OH], 838[869–CH_2OH], 803[869–CH_2OH, –H_2O, –OH], 796 [869–CH_2OH,–CH_2=C=O]. 707(IV), 697[707–H_2O], 690[707–OH], 689[707–H_2O], 676[707–CH_2OH], 658[707–CH_2OH,–H_2O], 671 [707–H_2O], 647 [707–CH_2OHCHO], 649 [707–$NHCOCH_3$]. 509[545–$2H_2O$], 451[545–$2H_2O$, –$NHCOCH_3$], 467[M–H_2O, –CH_2OHCHO], 472[545–CH_2OH,–CH_2=C=O], 342(VI), 325[342–OH], 289[342–$2H_2O$–OH], 223[342–OH,–CH_2OHCHO], 324[342–H_2O], 293[34–H_2O,–CH_2OH–CH_2=C=O], 300[342–CH_2=C=O], 240[342–CH_2=C=O,–CH_2OHCHO], 869 A [S-5,S-4,S-3,S-2, S-1] C[S-3, S-4, S-5, S-6,S-7], 707 B [S-1,S-2,S-3 S-5], 545 D[S-3,S-4, S-5].

COMPOUND 52(Kumar K et.al 2017)
Compound : **Yakose**
Compound Name:

$$\beta\text{–}Glc(1\rightarrow3)\beta\text{–}GlcNAc(1\rightarrow3)\beta\text{–}GalNAc(1\rightarrow4)Glc$$
$$|$$
$$\alpha\text{–}Gal\ (1\rightarrow4)$$

Mol. formula:$C_{34}H_{58}O_{26}N_2$
Mol. Wt. : 910



ES-MS: m/z 972 $[M+Na+K]^+$, 910 $[M]^+$, 850 [910-CH_2OH-CHO], 807 [850-CH_3CO], 779 [748-$3H_2O$-OH], 780 [850-$2H_2O$-OH], 762 [780-H_2O], 744 [762-H_2O], 748 [910-S_5],712 [748-$2H_2O$], 690[748-$NHCOCH_3$], 655[690-H_2O-OH], 593[712-CH_2OHCHO-CH_2CO-OH], 575[593-H_2O], 573[655-$2CH_3OH$-H_2O], 559[593-2OH], 558[593-H_2O-OH], 500[558-$NHCOCH_3$], 465[500-H_2O-OH], 586 [748-S_4], 526 [586-CH_2OH-CHO], 481 [526-H_2O-OH], 421 [481-CH_2OH-CHO], 386 [421-H_2O-OH], 273 [386-CH_2OHCHO-$2H_2O$-OH], 231 [273-CH_3CO], 223 [273-CH_3OH-H_2O]. 383 [586-S_3] 365 [383-H_2O], 288 [383-CH_3CO-$2H_2O$-OH], 271 [288-OH], 270 [288-H_2O], 236 [271-H_2O-OH], 235 [270-H_2O-OH], 180 [383-S_2], 138 [180-CH_2CO].

ACKNOWLEDGEMENTS

We are thankful to Head, Department of Chemistry, Lucknow University, Lucknow for providing lab facilities. We are also grateful to Prof. Raja Roy, CBMR, SGPGI, Lucknow for providing spectroscopic facilities.

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