



Chemistry of Terpenoids

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Accepted on: 25-05-2014; Finalized on: 30-06-2014.

ABSTRACT

Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastics, and other pharmaceutical functions. Plants do not only accumulate terpenes for herbivore defense, but also emit volatile blends in response to herbivory and many other biotic and abiotic stresses. These terpene-containing volatiles attract natural enemies of the attacking herbivores but due to the complexity of these volatile blends, it is difficult to attribute a specific function to a particular terpene. The importance of terpenes in both nature and human application is difficult to overstate. Basic knowledge of terpene and isoprene biosynthesis and chemistry has accelerated the pace at which scientists have come to understand many plant biochemical and metabolic processes. The abundance and diversity of terpene compounds in nature can have ecosystem-wide influences. Although terpenes have permeated human civilization since the Egyptians, terpene synthesis pathways are only now being understood in great detail. The use of bioinformatics and molecular databases has largely contributed to analyzing exactly how and when terpenes are synthesized. The steroids and sterols in animals are biologically produced from terpenoid precursors. Sometimes terpenoids are added to proteins, e.g., to enhance their attachment to the cell membrane; this is known as isoprenylation.

Keywords: Isoprene, Isolation, Secondary metabolites, Spectroscopic methods and structure elucidation, Terpenes.

INTRODUCTION

There are many different classes of naturally occurring compounds. Terpenoids also form a group of naturally occurring compounds majority of which occur in plants, a few of them have also been obtained from other sources. Terpenoids are volatile substances which give plants and flowers their fragrance. They occur widely in the leaves and fruits of higher plants, conifers, citrus and eucalyptus.¹

The term 'terpene' was given to the compounds isolated from terpenite, a volatile liquid isolated from pine trees. The simpler mono and sesquiterpene is chief constituent of the essential oils obtained from sap and tissues of certain plant and trees. The di and tri terpenoids are not steam volatile. They are obtained from plant and tree gums and resins. Tertraterpenoids form a separate group of compounds called 'Carotenoids'.

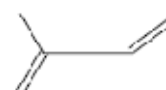
The term 'terpene' was originally employed to describe a mixture of isomeric hydrocarbons of the molecular formula $C_{10}H_{16}$ occurring in the essential oils obtained from sap and tissue of plants, and trees. But there is a tendency to use more general term 'terpenoids' which include hydrocarbons and their oxygenated derivatives. However the term terpene is being used these days by some authors to represent terpenoids.²⁻⁶

By the modern definition: "Terpenoids are the hydrocarbons of plant origin of the general formula $(C_5H_8)_n$ as well as their oxygenated, hydrogenated and dehydrogenated derivatives."

Isoprene rule

Thermal decomposition of terpenoids give isoprene as one of the product. Otto Wallach pointed out that terpenoids can be built up of isoprene unit.⁷

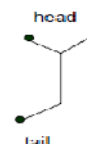
Isoprene rule states that the terpenoid molecules are constructed from two or more isoprene unit.



Isoprene unit

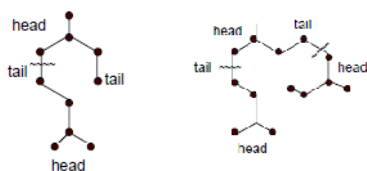
Further Ingold suggested that isoprene units are joined in the terpenoid via 'head to tail' fashion.

Special isoprene rule states that the terpenoid molecule is constructed of two or more isoprene units joined in a 'head to tail' fashion.⁸

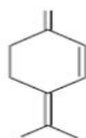


But this rule can only be used as guiding principle and not as a fixed rule. For example carotenoids are joined tail to tail at their central and there are also some terpenoids whose carbon content is not a multiple of five.

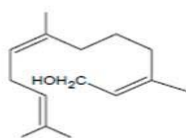
In applying isoprene rule we look only for the skeletal unit of carbon. The carbon skeletons of open chain monoterpenoids and sesquiterpenoids are,



Examples



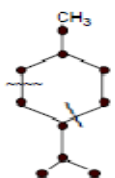
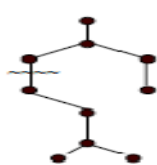
Myrcene (monoterpene)



Farnesol (Sesquiterpene)

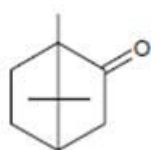
Ingold (1921) pointed that a gem alkyl group affects the stability of terpenoids. He summarized these results in the form of a rule called 'gem dialkyl rule' which may be stated as Gem dialkyl group tends to render the cyclohexane ring unstable where as it stabilizes the three, four and five member rings."

This rule limits the number of possible structure in closing the open chain to ring structure. Thus the monoterpene open chain give rise to only one possibility for a monocyclic monoterpene i.e the p-cymene structure.



P-cymene structure

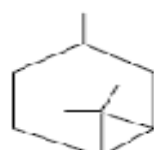
Bicyclic monoterpene contain a six member and a three member ring. Thus closure of the ten carbon open chain monoterpene gives three possible bicyclic structures.



Camphor(6+5) system



Pinane(6+4) system



Carane(6+3) System

CLASSIFICATION OF TERPENOIDS

Most natural terpenoids hydrocarbon have the general formula $(C_5H_8)_n$. They can be classified on the basis of value of n or number of carbon atoms present in the structure.⁹⁻¹¹ (Table-1)

Each class can be further subdivided into subclasses according to the number of rings present in the structure.

Acyclic Terpenoids: They contain open structure.

Monocyclic Terpenoids: They contain one ring in the structure.

Bicyclic Terpenoids: They contain two rings in the structure.

Tricyclic Terpenoids: They contain three rings in the structure.

Tetracyclic Terpenoids: They contain four rings in the structure.

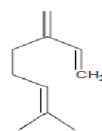
Table 1: Classification of Terpenoids

Number of carbon atoms	Value of n	Class
10	2	Monoterpenoids ($C_{10}H_{16}$)
15	3	Sesquiterpenoids ($C_{15}H_{24}$)
20	4	Diterpenoids ($C_{20}H_{32}$)
25	5	Sesterpenoids ($C_{25}H_{40}$)
30	6	Troterpenoids ($C_{30}H_{48}$)
40	8	Tetraterpenoids ($C_{40}H_{64}$)
>40	>8	Polyterpenoids (C_5H_8) _n

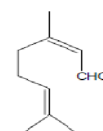
Some examples of mono, sesqui and di Terpenoids:

Mono Terpenoids

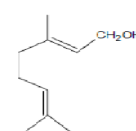
i) Acyclic Monoterpenoids



Myrcene

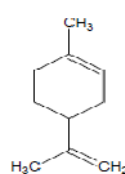


Citral

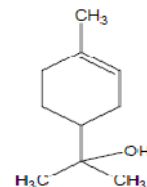


Geraniol

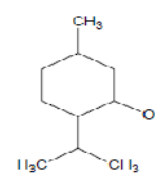
ii) Monocyclic monoterpene



Limonene



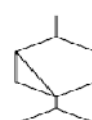
α -Terpineol



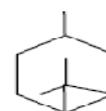
Menthol

iii) Bicyclic monoterpene: These are further divided into three classes.

a) Containing -6+3-membered rings



Thujane



Carane

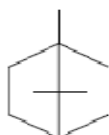
b) Containing -6+4- membered rings



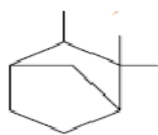
Pinane



c) Containing -6+5-membered rings

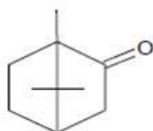


Bornane (Camphane)

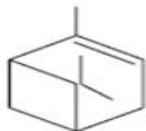


non bornane (iso camphane)

Some bicyclic monoterpenes are



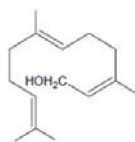
Camphor



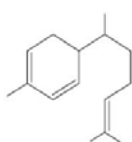
α -pinene

Sesquiterpenoids

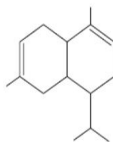
i) Acyclic sesquiterpenoids ii) Monocyclic sesquiterpenoids iii) Bicyclic sesquiterpenoids



Farnesol



Zinziberene



Cadinene

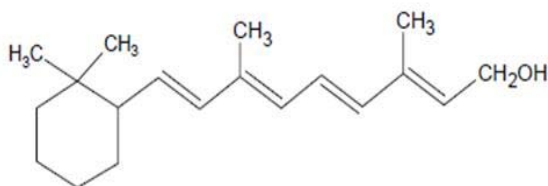
Diterpenoids

i) Acyclic diterpenoids



Phytol

ii) Mono cyclic diterpenoids



Vitamin A

Isolation of Mono And Sesquiterpenoids¹²⁻¹³

Both mono and sesquiterpenoids have common source i.e essential oils. Their isolation is carried out in two steps:

- i) Isolation of essential oils from plant parts
- ii) Separation of Terpenoids from essential oils.

i) Isolation of essential oils from plant parts

The plants having essential oils generally have the highest concentration at some particular time. Therefore better yield of essential oil plant material have to be collected at

this particular time. e.g. From jasmine at sunset. There are four methods of extractions of oils.

- a) Expression method
- b) Steam distillation method
- c) Extraction by means of volatile solvents
- d) Adsorption in purified fats

Steam distillation is most widely used method. In this method macerated plant material is steam distilled to get essential oils into the distillate form these are extracted by using pure organic volatile solvents. If compound decomposes during steam distillation, it may be extracted with ether at 50°C. After extraction solvent is removed under reduced pressure.

ii) Separation of Terpenoids from essential oil

A number of terpenoids are present in essential oil obtained from the extraction. Definite physical and chemical methods can be used for the separation of terpenoids. They are separated by fractional distillation. The terpenoid hydrocarbons distill over first followed by the oxygenated derivatives.

More recently different chromatographic techniques have been used both for isolation and separation of terpenoids.

General Properties of Terpenoids¹⁴⁻¹⁶

1. Most of the terpenoids are colorless, fragrant liquids which are lighter than water and volatile with steam. A few of them are solids e.g. camphor. All are soluble in organic solvent and usually insoluble in water. Most of them are optically active.
2. They are open chain or cyclic unsaturated compounds having one or more double bonds. Consequently they undergo addition reaction with hydrogen, halogen, acids, etc. A number of addition products have antiseptic properties.
3. They undergo polymerization and dehydrogenation
4. They are easily oxidized nearly by all the oxidizing agents. On thermal decomposition, most of the terpenoids yields isoprene as one of the product.

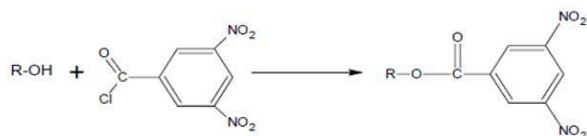
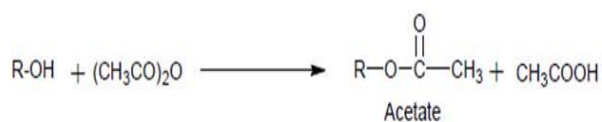
General Methods of Structure Elucidation of Terpenoids¹⁷⁻¹⁹

i) **Molecular formula:** molecular formula is determined by usual quantitative analysis and mol.wt determination methods and by means of mass spectrometry. If terpenoid is optically active, its specific rotation can be measured.

ii) **Nature of oxygen atom present:** If oxygen is present in terpenoids its functional nature is generally as alcohol aldehyde, ketone or carboxylic groups.

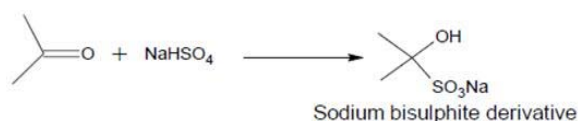
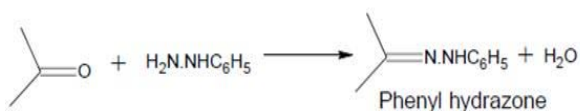
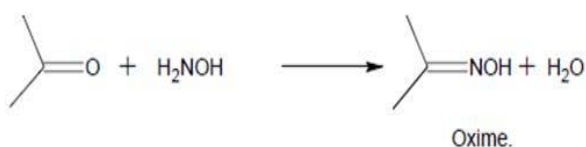
a) **Presence of oxygen atom present:** presence of –OH group can be determined by the formation of

acetates with acetic anhydride and benzoate with 3,5-dinitrobenzoyl chloride.



Primary alcoholic group undergo esterification more readily than secondary and tertiary alcohols.

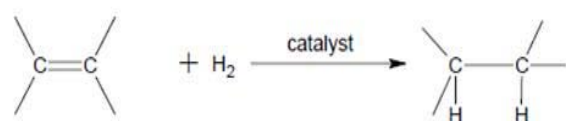
Presence of >C=O group: Terpenoids containing carbonyl function form crystalline addition products like oxime, phenyl hydrazone and bisulphite etc.



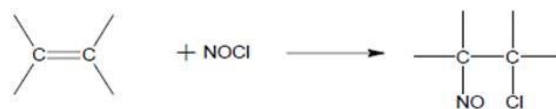
If carbonyl function is in the form of aldehyde it gives carboxylic acid on oxidation without loss of any carbon atom whereas the ketone on oxidation yields a mixture of lesser number of carbon atoms.

iii) **Unsaturation:** The presence of olefinic double bond is confirmed by means of bromine, and number of double bond determination by analysis of the bromide or by quantitative hydrogenation or by titration with monoperphthalic acid.

Presence of double bond also confirmed by means of catalytic hydrogenation or addition of halogen acids. Number of moles of HX absorbed by one molecule is equal to number of double bonds present.



Addition of nitrosyl chloride (NOCl) (**Tilden's reagent**) and epoxide formation with peracid also gives idea about double bonds present in terpenoid molecule.



iv) **Dehydrogenation:** On dehydrogenation with sulphur, selenium, polonium or palladium terpenoids converted to aromatic compounds. Examination of these products the skelton structure and position of side chain in the original terpenoids can be determined.

For example α -terpenol on Se-dehydrogenation yields p-cymene.



Thus the carbon Skelton of terpenol is as follows.



v) **Oxidative degradation:** Oxidative degradation has been the parallel tool for elucidating the structure of terpenoids. Reagents for degradative oxidation are ozone, acid, neutral or alkaline potassium permanganate, chromic acid, sodium hypobromide, osmium tetroxide, nitric acid, lead tetra acetate and peroxy acids. Since oxidizing agents are selective, depending on a particular group to be oxidized, the oxidizing agent is chosen with the help of structure of degradation products.

Table 2: Relation between general formula of compound and type of compounds

General formula of parent saturated Hydrocarbon	Type of structure
$\text{C}_n\text{H}_{2n+2}$	Acyclic
C_nH_{2n}	Monocyclic
$\text{C}_n\text{H}_{2n-2}$	Bicyclic
$\text{C}_n\text{H}_{2n-4}$	Tricyclic
$\text{C}_n\text{H}_{2n-6}$	Tetracyclic

vi) **Number of the rings present:** With the help of general formula of corresponding parent saturated hydrocarbon, number of rings present in that molecule can be determined.

Vii) Relation between general formula of compound and type of compounds: Table 2

For example: limonene (mol. formula. $C_{10}H_{16}$) absorbs 2 moles of hydrogen to give tetrahydro limonene (mol. Formula $C_{10}H_{20}$) corresponding to the general formula. C_nH_{2n} . It means limonene has monocyclic structure.

viii) **Spectroscopic studies:** All the spectroscopic methods are very helpful for the confirmation of structure of natural terpenoids and also structure of degradation products. The various methods for elucidating the structure of terpenoids are;

a) UV Spectroscopy: In terpenes containing conjugated dienes or α,β -unsaturated ketones, UV spectroscopy is very useful tool. The values of λ_{max} for various types of terpenoids have been calculated by applying Woodward's empirical rules. There is generally good agreement between calculation and observed values. Isolated double bonds, α,β -unsaturated esters, acids, lactones also have characteristic maxima.

b) IR Spectroscopy: IR spectroscopy is useful in detecting group such as hydroxyl group ($\sim 3400\text{cm}^{-1}$) or an oxo group (saturated $1750\text{-}1700\text{cm}^{-1}$). Isopropyl group, cis and trans also have characteristic absorption peaks in IR region.

c) NMR Spectroscopy: This technique is useful to detect and identify double bonds, to determine the nature of end group and also the number of rings present, and also to reveal the orientation of methyl group in the relative position of double bonds.

d) Mass Spectroscopy: It is now being widely used as a means of elucidating structure of terpenoids for determining mol. wt., mol. formula, and nature of functional groups present and relative positions of double bonds.

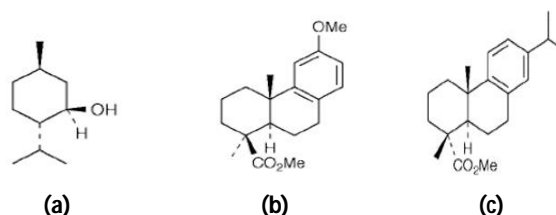
ix) **X-ray analysis:** This is very helpful technique for elucidating structure and stereochemistry of terpenoids.

x) **Synthesis:** Proposed structure is finally confirmed by synthesis. In terpenoid chemistry, many of the synthesis are ambiguous and in such cases analytical evidences are used in conjunction with the synthesis.

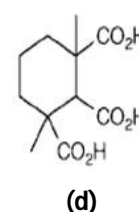
The Determination Of Relative Stereochemistry

The problems associated with establishing the relative stereochemistry of the terpenoids can be divided into those relating to the stereochemistry of the substituent's and those associated with the stereochemistry of the ring junctions. Many of the classical pre-spectroscopic strategies yielded results in the 1940's and 1950's. The assignment of the stereochemistry of the menthols (**a**) utilized the comparative rates of their esterification with *p*-nitrobenzoyl chloride to determine the configuration of the alcohols at C-3. The two neomenthols were esterified more slowly than either menthol or isomenthol. This was attributed to steric hindrance by the isopropyl group and hence this group was placed *cis* to the hydroxy in the

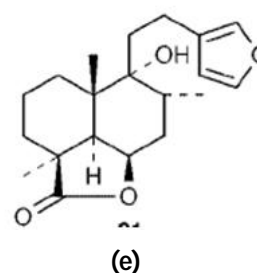
neomenthols. The much slower rate of hydrolysis of methyl podocarpate (**b**) compared to methyl dehydroabietate (**c**) was used to reveal the greater steric hindrance in the former arising from interactions with the methyl group at C-10. The ester is an axial substituent in methylpodocarpate.



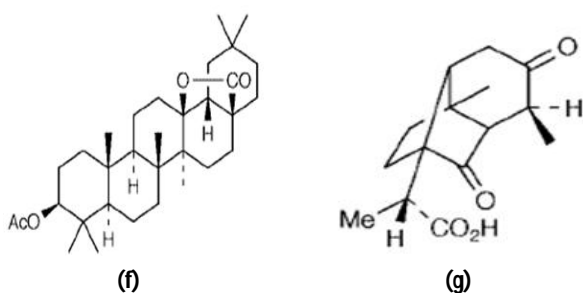
The *trans* A/B stereochemistry of the abietic and pimaric acids was established by examining the *pK* values of the carboxylic acids (**d**) derived by oxidative degradation. These results were then extended to the triterpenes. The results of stereochemical studies in the terpene and steroid series provided many of the examples on which the theories of the conformational analysis of reactions were based.



The conformational stability of *trans* fused α -decalones was used to establish the B/C *trans* configuration in the pentacyclic triterpenes and to establish the *trans* A/B fusion in the diterpenoids such as marrubiin (**e**). An interesting summary of the application of these reactions to the stereochemistry of the eudesmane group of sesquiterpenes appeared in 1960.



The strategy of using cyclization reactions such as lactonization reactions to establish the *cis* stereochemistry of the D/E ring junction in the pentacyclic triterpenes, in which the facile formation of the lactone (**f**) could only be accommodated with *cis* ring junction, is an example. The elucidation of the cyclization reactions of santonin to give the bridged santonic acid (**g**) played an interesting role in establishing the stereochemistry of santonin. However the correct configuration at C-11 was eventually established by X-ray analyses of 2-bromo- α -santonin and 2-bromo- β -santonin.²⁰⁻²¹



THE IMPACT OF INSTRUMENTAL CHROMATOGRAPHIC TECHNIQUES

The development of instrumental chromatographic techniques for the separation of natural products, particularly terpenoids, had widespread ramifications in structure elucidation. The gas chromatographic analysis of essential oils had revealed their complexity and when coupled with mass spectrometry led to the elucidation of the structures of a number of new mono and sesquiterpenoids. The understanding of factors which affect the fragmentation patterns of terpenoids in the mass spectrometer and the use of these as structural tools has become very important. The sensitivity of the methods gave a stimulus to insect chemistry in which a number of volatile mono- and sesquiterpenoids play an important role as pheromones. The conversion of the gibberellin plant hormones to their esters and trimethylsilyl ethers followed by their separation and identification by gas chromatography–mass spectrometry led not only to the elucidation of the structures of over 120 of these compounds but also to their quantitation at various stages of plant development. Confirmatory synthetic and model studies have become a very important part of these instrumental strategies in which very small amounts of the natural product are examined. The development of HPLC as a separation tool and the link with spectroscopic methods has had a similar impact on the isolation and structure elucidation of more polar terpenoid natural products, particularly compounds from marine sources.²²

THE ABSOLUTE STEREOCHEMISTRY OF TERPENOID SECONDARY ALCOHOLS

A number of methods have been developed for establishing the absolute stereochemistry of a secondary alcohol. Studies on the addition of Grignard reagents to the α -keto-esters of optically active terpenoid alcohols showed that asymmetric induction could be used to establish the stereochemistry of a chiral alcohol. The chirality of the atrolactic acid that was formed reflected that of the original secondary alcohol. Horeau's method was based on the kinetic resolution by the alcohol in its esterification by 2-phenylbutyric anhydride. The method is based on using a racemic mixture of the anhydride. One enantiomer will react faster with the chiral secondary alcohol than the other. The optical activity of the remaining 2-phenylbutyric acid will thus reflect the chirality of the secondary alcohol.

An NMR method based on derivatization with (*R*)- and (*S*)- α -methoxy- α -trifluoromethylphenylacetic acid (MTPA) has become a popular strategy for establishing the absolute stereochemistry of terpenoid alcohols. Although it was originally based on an analysis of the ¹⁹F NMR spectrum, a more reliable version uses the high field ¹H NMR spectrum. A correlation was established between the absolute stereochemistry of terpenoid secondary alcohols and the $\Delta\delta$ ($\delta_S - \delta_R$) values of adjacent protons for the (*R*)- and (*S*)-MPTA esters. This correlation was successfully applied in establishing the absolute stereochemistry of a number of marine terpenoids of the cembranolid and xenicane series. In a few instances the absolute configuration of a terpenoid has been determined by X-ray crystallography of a heavy atom derivative, e.g. an ester or an amide containing a bromine atom. The assumption is often made that all of a group of terpenes that occur in a particular plant belong to the same enantiomeric series. However there have been a number of reports where, for example, enantiomeric labdane diterpenes co-occur in the wood of *Oxystigma oxyphyllum* and in the leaves of *Mimosa hostilis*. Hence this assumption in terpenoid structure elucidation is not always valid.²³⁻²⁴

CONCLUSION

Many terpenes remain to be discovered so they will undoubtedly intrigue scientists for years, as their applications are only beginning to be fully realized. Arguably, society has benefited tremendously from terpenes. In addition, understanding the function of genes in terpene production could lead to discovering novel compounds or pathways, which might reveal new important aspects for many human applications. For instance, the ability to up regulate terpene synthesis in *C. langsdorfii* could result in an increase in the diesel-like resin harvested from this tree, might prove beneficial to the global market of biofuels. As we continue into the agro biotechnology age, it is highly likely the applications and potentials of terpenes will be further explored.

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Source of Support: Nil, Conflict of Interest: None.

