Structure Elucidation & Isolation:

Terpenoids, Carotenoids, Vitamins and Quassinoids

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Extraction, isolation and structure elucidation of

- Terpenoids:
  - Camphor
  - Menthol
  - Citral.
- Carotenoids:
  - β- Carotene.
- Vitamins:
  - α -Tocopherol.
- Quassinoids:
  - Quassin.

Terpenoids

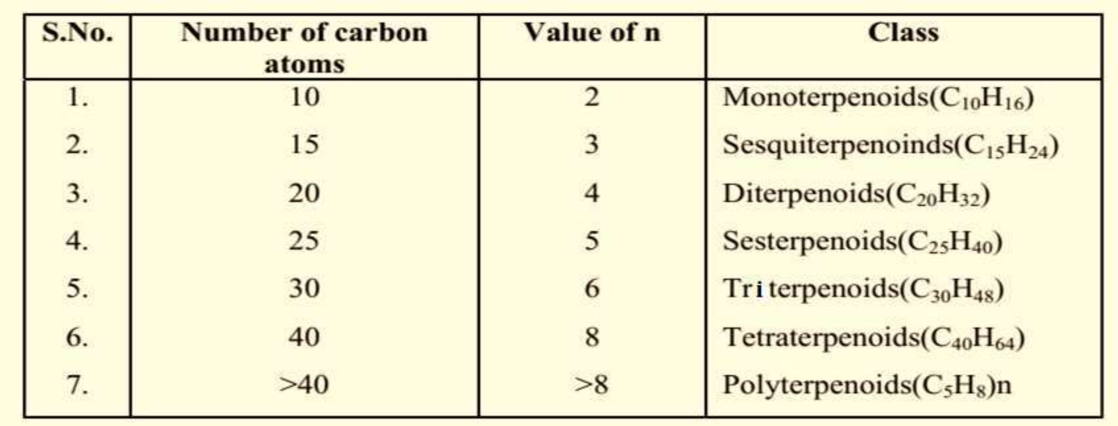
Terpenoids are defined as the hydrocarbons of plant origin having the general formula -$(C_5H_8)n$ *where n is the* number of linked isoprene (2-methyl-1,3-butadiene) units (*isoprenoids).*

Terpenoids form a group of compounds, the majority of which occurs in plant kingdom.

- Mono and sesquiterpenoids are the essential oils obtained from sap and tissues of plants and trees.
- Di and Tri Terpenoids are obtained from plant and tree gums and resins.
- Tetra terpenoids form a group of compounds known as carotenoids.
- Polyterpenoid : Rubber .

CLASSIFICATION OF TERPENOIDS

They can be classified on the basis of value of‘n’ i.e., isoprene unit or number of carbon atoms present in the structure.



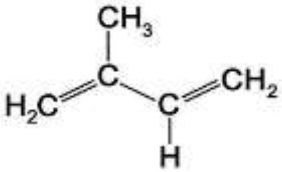
S.No.	Number of carbon atoms	Value of n	Class
1.	10	2	Monoterpenoids($C_{10}H_{16}$)
2.	15	3	Sesquiterpenoinds($C_{15}H_{24}$)
3.	20	4	Diterpenoids($C_{20}H_{32}$)
4.	25	5	Sesterpenoids($C_{25}H_{40}$)
5.	30	6	Tri terpenoids($C_{30}H_{48}$)
6.	40	8	Tetraterpenoids($C_{40}H_{64}$)
7.	>40	>8	Polyterpenoids(C_5H_8)n

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

- i) Acyclic Terpenoids: They contain open structure e.g., citral
- ii) Monocyclic Terpenoids: They contain one ring in the structure e.g., menthol
- iii) Bicyclic Terpenoids: They contain two rings in the structure. E.g., camphor.
- iv) Tricyclic Terpenoids: They contain three rings in the structure. E.g., abietic acid
- v) Tetracyclic Terpenoids: contain four rings in the structure. E.g., gibberellic acid

ISOPRENE RULE

In 1887, Wallach pointed out the isoprene rule from the following facts:

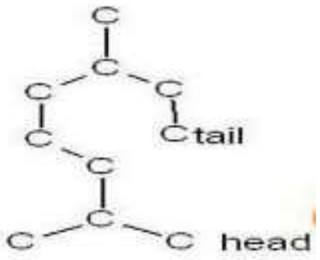


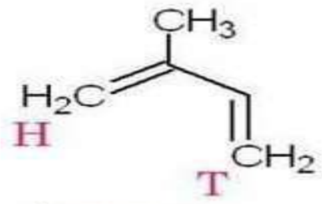
The empirical formula of almost all naturally occurring terpenoids is -(C_5H_8)*n*

Thermal decomposition of almost all terpenoids gives isoprene as one of the products.

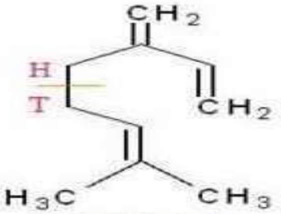
Special isoprene rule:

- Proposed by Ingold in 1925
- Isoprene units in terpenoids are linked in " head to tail" fashion.
- The branched end (head) is isopropyl and other end being the (tail)

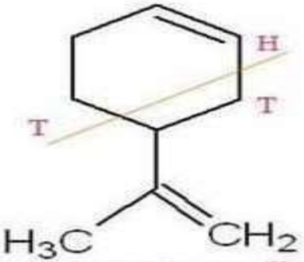




ISOPRENE (1)



MYRCENE (2)



LIMONENE (2)

MONOTERPENOIDS

The monoterpenoids are composed of two isoprene units. These are the simplest natural occurring terpenoids which are isolated from essential oils obtains from the leaves, roots and barks of various plant.

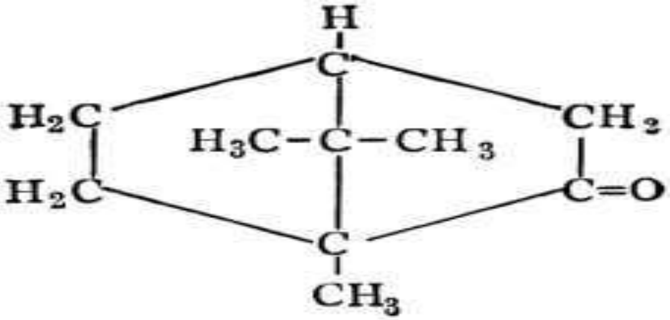
These compounds have pleasant odours and are largely used as perfumery industry.

Monoterpenoids may be divided into three groups:

1. Acyclic monoterpenes. Example- citral.
2. Monocyclic monoterpenes. Example- menthol
3. Bicyclic monoterpenes. Example- camphor

Camphor

- Camphor is derived from the wood of *camphor laurel(Cinnamomum camphora) and other related trees of laurel* family.
- Camphor is a bicyclic mono terpenoid.
- Camphor is a white, crystalline substance with strong odor and pungent taste.
- It is waxy and flammable substance.



STRUCTURAL ELUCIDATION OF CAMPHOR

1) **Molecular formula -** $C_{10}H_{16}O$(bicyclic monoterpenoid)

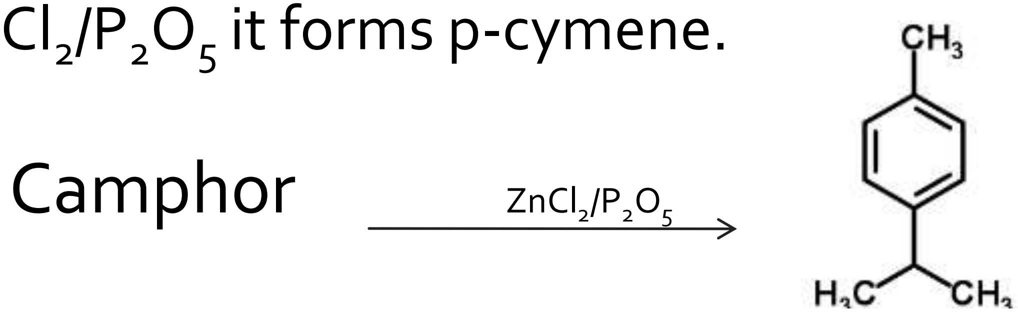
2) **Nature of oxygen atom:** it is ketone as it forms oxime with NH_2OH & phenyl hydrazine with phenylhydrazine.

$C_{10}H_{16}O + H_2NOH \longrightarrow C_{10}H_{16}{=}NOH$

Camphor Hydroxylamine Camphor oxime

3) **Presence of bicyclic ring system:** the parent hydrocarbon of camphor has molecular formula $C_{10}H_{18}$, which corresponds to the molecular formula of bicyclic compounds(CnH2n-2)

4) **Presence of six membered ring:** when distilled with $ZnCl_2/P_2O_5$ it forms p-cymene.



5) Presence of –COCH2- group in camphor is confirmed by its formation to isonitroso derivative with amyl nitrile & HCl.

6)Camphor on oxidation with HNO3 gives camphoric acid & camphoronic acid .

$C_{10}H_{16}O \rightarrow C_{10}H_{16}O_4 \rightarrow C_9H_{14}O_6$

camphor

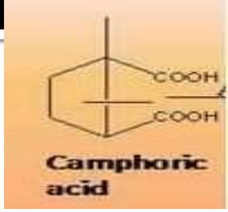
Camphoric acid

Camphoronic acid

- Camphoronic acid is saturated tricarboxylic acid & on distillation at atm pressure gives:

- Bredt suggested that camphoronic acid is 2,3-dimethyl-1,2,3-butanetricarboxylic acid, hence having 3 –CH3 group so camphor must have 3-methyl group.

- On the basis of the formula of camphoric acid "$C_{10}H_{16}O_4$" it was found that camphoric acid is a cyclo pentane derivative and the structure was proposed as:



- Bredt proposed the following reaction to show the relationship between camphor, camphoric acid & camphoronic acid and established the structure of camphor.

Menthol

- It is obtained from the fresh flowering tops of mentha piperita.(labiate) . The active constituent is menthol, menthone, and limonene.
- Menthol is an optically active compound.
- Only its (-) form occurs naturally in peppermint oils.
- Crystalline substance, clear or white in colour, which is solid at room temperature and melts slightly above (43°c).
- Menthol is a 10 carban monocylic terpenes alcohol
- It is used as an antiseptic and anaesthetic. Flavouring agent , carminative. Used in tooth paste. Tooth powder, shaving cream.

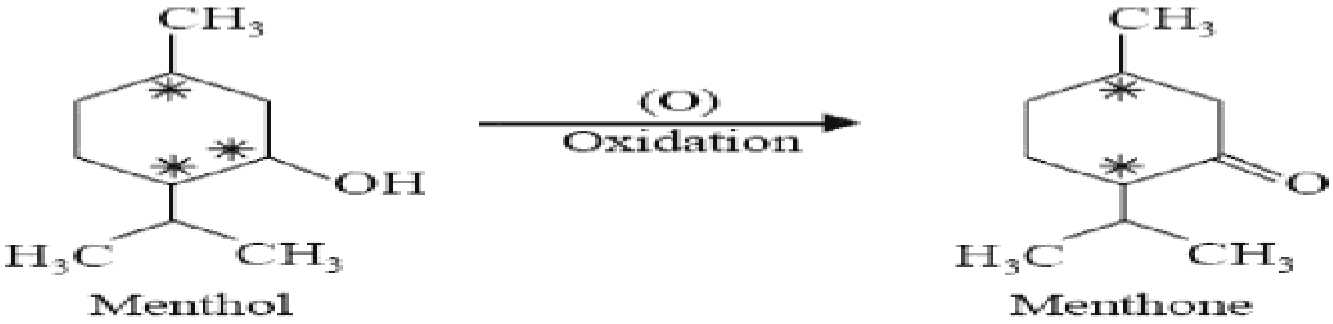
EXTRACTION AND ISOLATION

Extraction and isolation of menthol is done through steam distillation. Steam from a boiler is allowed in the vessel from the bottom, above which the plant material is placed on a grid and the steam carries the vapours of the essential oil to the condenser where they are condensed and collected.

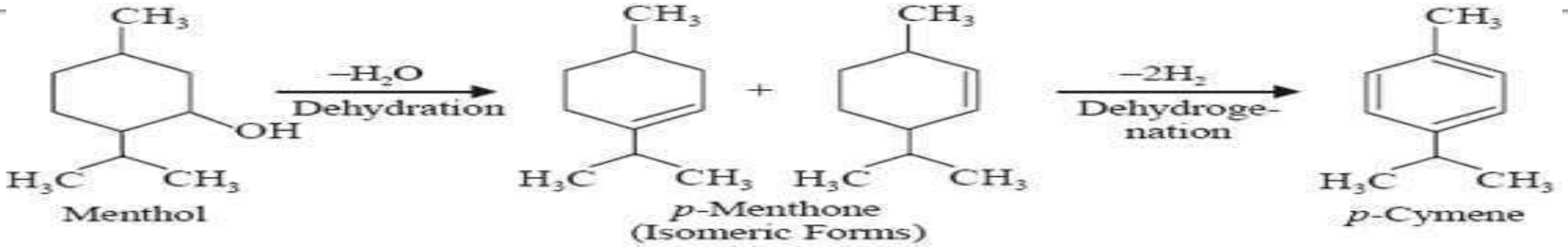
ELUCIDATION OF MENTHOL

- **Molecular formula-** $C_{10}H_{20}O$
- **Presence of alcoholic group -** as menthol forms esters readily with acids, this means that it must posses an alcoholic group.
- **Presence of secondary alcohol**

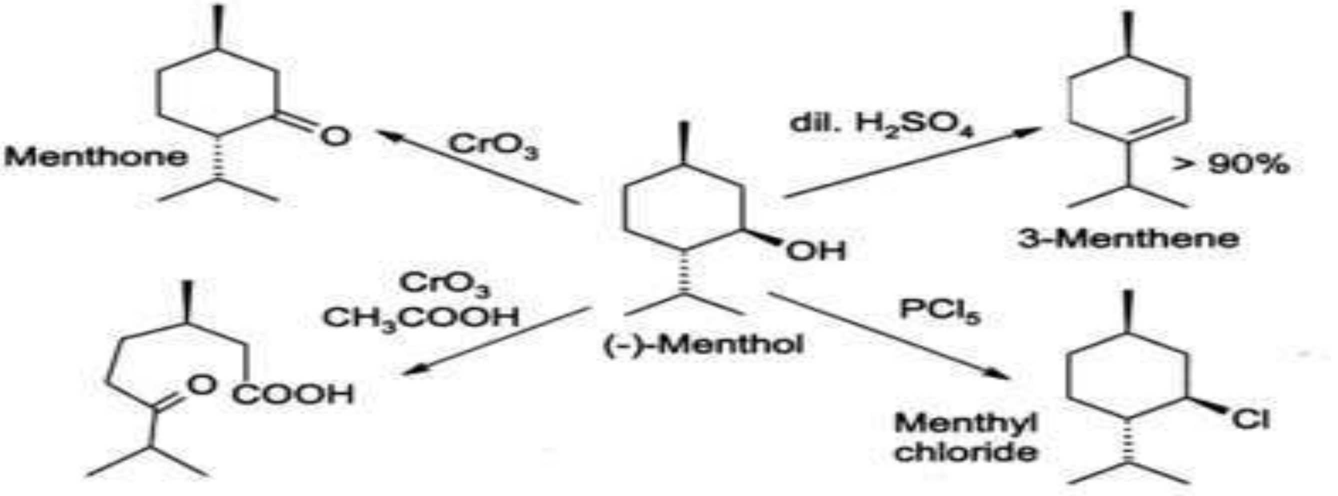
The oxidation of menthol yields ketone, indicating that the hydroxyl group in menthol is secondary in nature.



- **Presence of p-menthane skeleton** menthol on dehydration and dehydro halogention form p-cymene.



- **Explaining the structure of menthol**



Citral

- It is considered to be the most important member of the acyclic monoterpenes because the structure of remaining terpenoids in this group are based on the structure of citral.
- It is obtained from the steam distillation of Cymbopogan flexuosus (Gramineae) commonly knowns as lemon grass.
- Citral is acyclic monoterpenoids, it is a major constituent of lemon grass oil in which it occurs to an extent of 60-80%. It is a pale yellow liquid having strong lemon like odor and can be obtained by fractional distillation under reduced pressure from lemon grass oil.

EXTRACTION AND ISOLATION

Transfer 10ml of lemon grass oil, 100ml of water to a 250 ml round bottom flask. Place a boiling chip into the flask. Connect the flask to a simple distillation apparatus. Heat the mixture to boiling, and collect the distillate at graduated cylinder. Isolate the citral obtained from mixture of water and citral by extracting it with diethyl ether. Evaporate the ether layer to get citral.

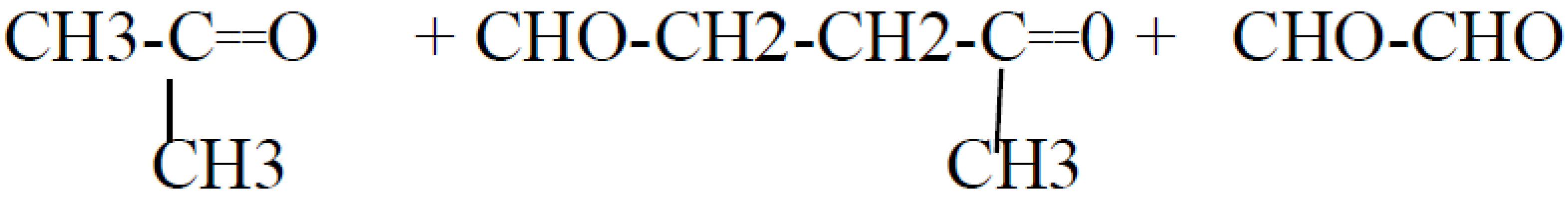
ELUCIDATION OF CITRAL

- **Molecular formula-** $C_{10}H_{16}O$
- **Presence of 2 double bond -**As citral adds on two moles of bromine or hydrogen, this shows that it contain 2 double bond

$$C_{10}H_{20}O \longleftarrow C_{10}H_{16}O \longrightarrow C_{10}H_{16}OBr_4$$

tertrahydrocitral citral citral tetrabromide

- Citral on ozonolysis yields acetone, laevuladehyde and glyoxal. This shows that citral is an acyclic compounds containing 2 double bond.

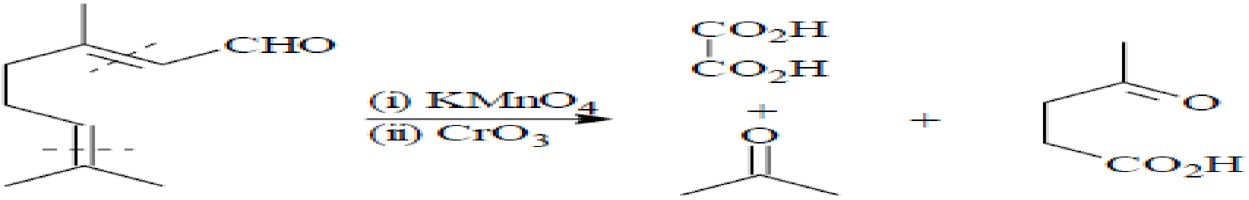


$$CH3\text{-}\underset{\underset{CH3}{|}}{C}{=}O \quad + CHO\text{-}CH2\text{-}CH2\text{-}\underset{\underset{CH3}{|}}{C}{=}0 + \quad CHO\text{-}CHO$$

- C-skeleton of citral: when citral is heated with potassium hydrogen sulfate, it cyclises to p-cymene, indicate that citral is a acylic compound.

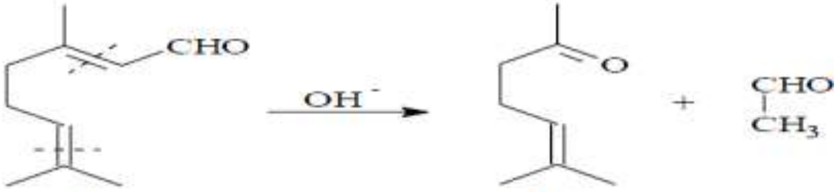
$$C_{10}H_{16}O \xrightarrow{KHSO_4} C_{10}H_{14} \text{ (p-cymene)}$$

- Oxidation of citral with alkaline permanganate, followed by chromic acid, gives acetone, oxalic and laevulic acids.



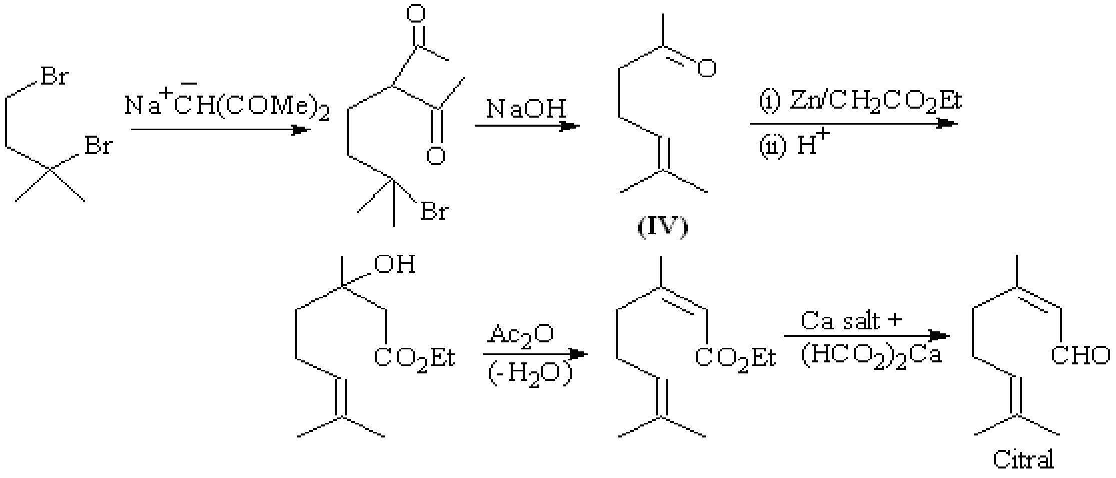
(III)

- Citral on boiling with aqueous potassium carbonate converted into 6-methylhept-5-en-2-one (IV) and acetaldehyde. The formation of these products is readily explained that citral is product of aldol condensation of these two.



(III) (IV)

- Citral is treated with sodium bisulfate to forms mono as well as bisulfite addition product, which indicates that one of the double bond is conjugated with carbonyl group.
- The structure of citral was confirmed by the synthesis of methylheptenone, the conversion of this into geranic ester, which was then converted into citral by heating a mixture of the calcium salts of geranic and formic acids.



Carotenoids

Carotenoid, are the group of non-nitrogenous, yellow, orange, or red pigments (biochromes) that are almost universally distributed in living things.

Carotenoids also called tetraterpenoids, are organic pigments that are produced by plants and algae, as well as several bacteria and fungi.

Carotenoids are composed of eight isoprene units(C_{40}).

they are various colors usually yellow to red pigments

Carotenoids are the precursor of Vitamin A & are powerful antioxidants that helps in preventing some form of cancer and heart disease.

CLASSIFICATION

There are **two general classes of carotenoids:** 1.**carotenes and 2.xanthophylls.**

- Carotenes consist only of carbon and hydrogen atoms; beta-carotene is the most common carotene.
- Xanthophylls have one or more oxygen atoms; lutein is one of the most common xanthophylls.

β- Carotene


Image credit: istockphoto.com/RapidEye

- Beta-carotene is a compound that converts into vitamin A when taken in diet and acts as antioxidant which helps to protect cells from damage.
- It decreases the risk of breast cancers, Prevent osteoarthritis, Prevents asthma attacks,etc
- The sources are carrots, sweet potatoes, dark leafy greens, such as kale and spinach ,romaine lettuce, squash, cantaloupe ,red and yellow peppers ,apricots.

Isolation and extraction of β-CAROTENE

1-carrots are dried and powdered it
2- Powder is extracted with petroleum ether repeatedly at room temperature .the extract are combined and then concentrated at 30-40 c under reduced pressure.
3- In concentrated extract carbon disulfide is added, then small amount of ethanol is added to this solution to remove the colorless impurities.
4- In mother liquor ethanol is added from which crude carotenes are precipitated and filtered off.
5- Finally carotenes are recrystallized from petroleum ether

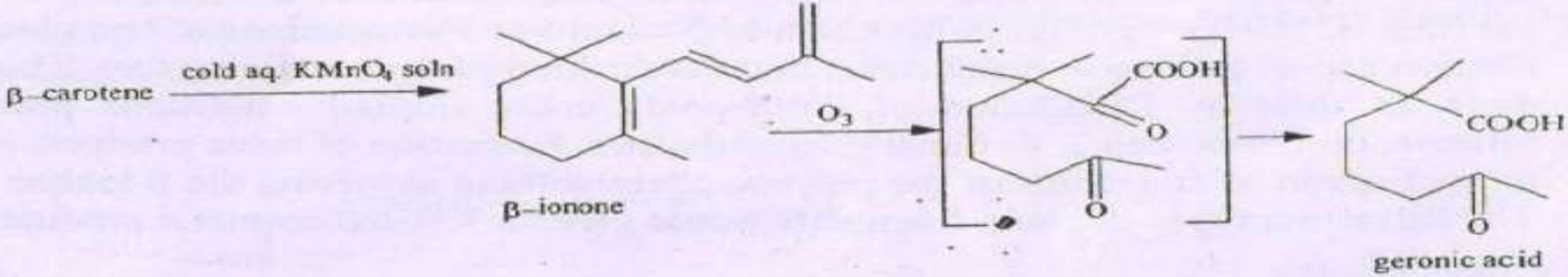
Structure Elucidation

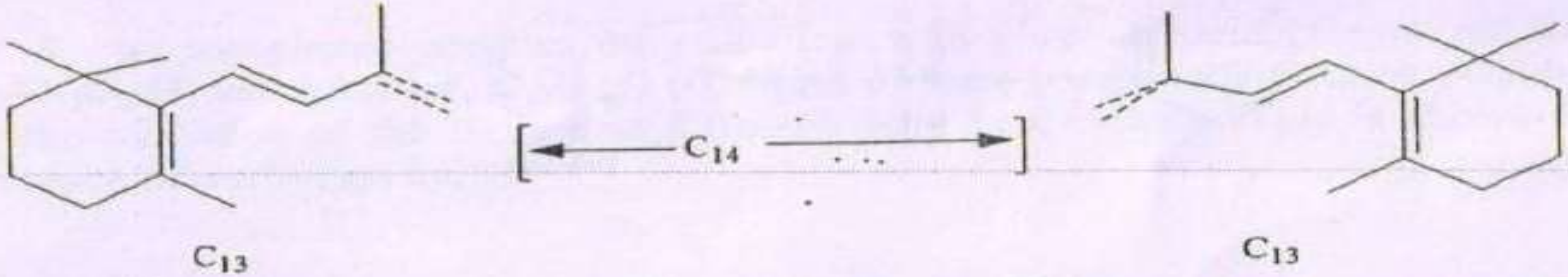
1. Molecular formula of β- carotene is $C_{40}H_{56}$.

2. β- Carotene is catalytically hydrogenated with eleven molecules of hydrogen to forms perhydro- β- carotene. This shows presence of 11 double bond in β- carotene.

3. β- Carotene is treated with five molecules of maleic anhydride to form a crystalline product. This shows that it contains fie conjugated double bonds.

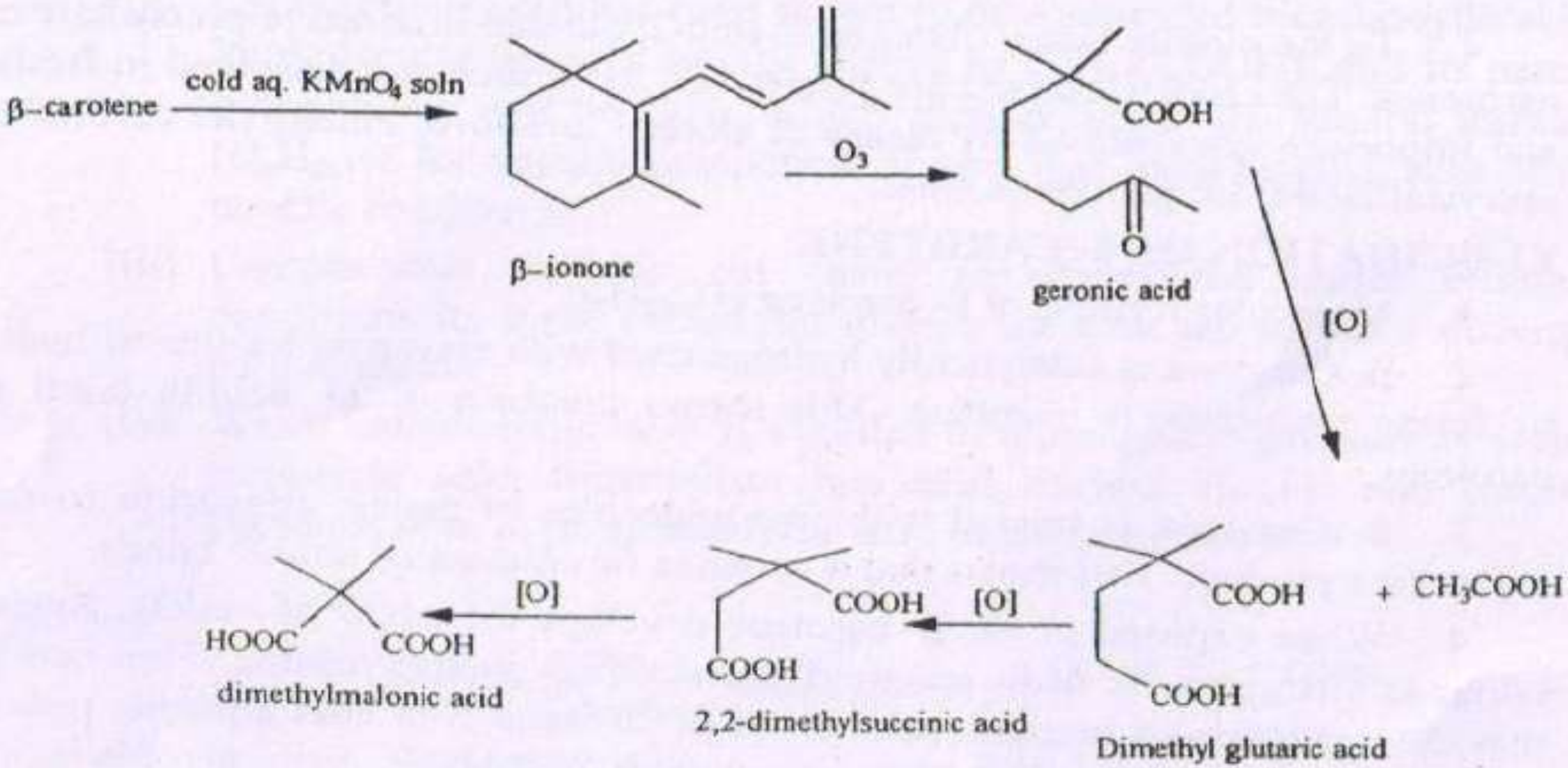
4. When exposed to air, β- carotene develops the odour of violets. Since this odour is characteristic of β- ionone. Presence of β- ionone residue. This confirmed that the oxidation of benzene solution of β- carotene with cold aqueous potassium permagnate gives β- ionone. Now β- iononeis ozonolysis gives geronic acid this shows that presence of two β- ionone residue in β- carotene.



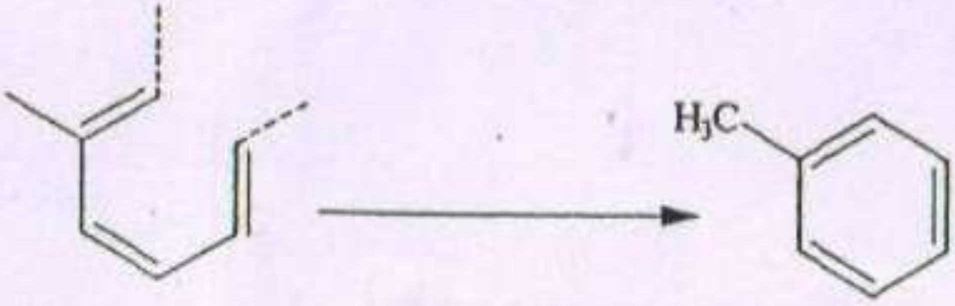


The colour of β- carotene is due to extended conjugation, the C_{14} portion of the molecule will be conjugated. The presence of conjugation in this central portion confirmed that β- carotene forms an adduct with five molecules of maleic anhydride.

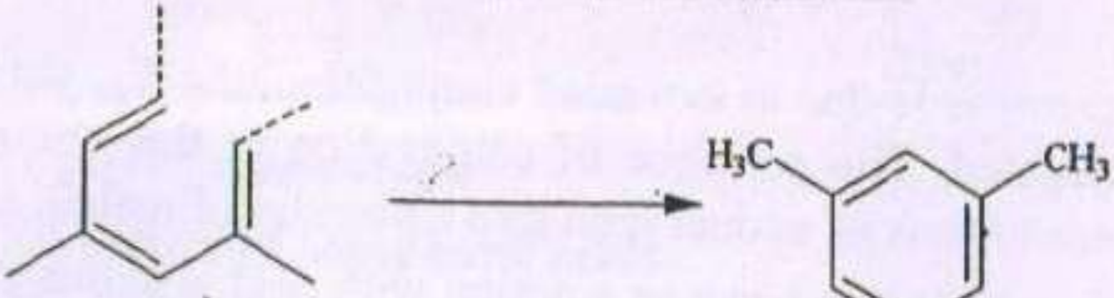
5. Oxidation of β- carotene in benzene solution with cold aqueous permagnate gives a mixture of β- ionone, dimethyl glutaric acid, 2, 2-dimethylsuccinic acid dimethyl malonic acid and acetic acid. Presence of two β- ionone residues. Some methyl side chains in the central C_{14} portion of the molecule.



6. Number and position of side chain: Kuhn-Roth side chain determination is applied to β- carotene, it yield ~ 5.4 molecules of acetic acid. This indicates that there are four –C (CH_3) = groups in the chain of β- carotene. The positions of two placed in the two end of β- ionone residue and find the position of the remaining two. This was done as follows. Distillation of carotenoids under normal conditions produces toluene, m-xylene and 2, 6- dimethylnaphthalene. Formation of these products is due to cyclisation of fragments of the polyene chain without involving the β-ionone ring. The following types of chain fragments would give the desired aromatic products:



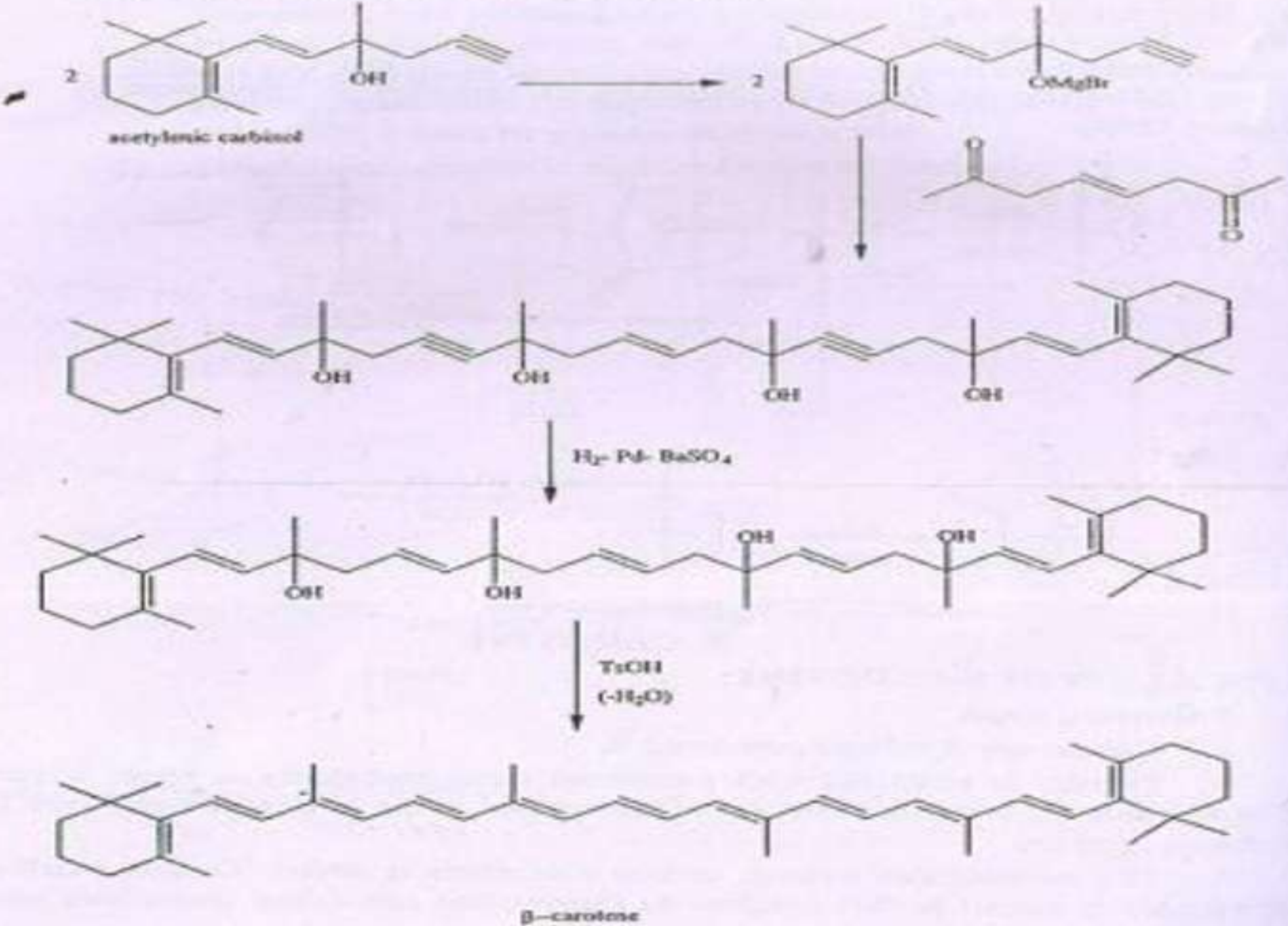
Toluene



1, 3

m-xylene

8. Synthesis: the structure of β- carotene is confirmed by synthesis
(a) (Karrer et al; 1950): (Yield was poor)



Vitamins: α -Tocopherol

- The term 'vitamin E' refers to a group of closely related compounds which occur naturally and which are, to different degrees, anti-sterility factors.
- The most biologically active one is α –tocopherol
- The main source of α and β-tocopherol is wheat germ oil; the compound is obtained from cotton seed oil. Wheat germ oil was first subjected to chromatographic analysis to remove sterols, etc., and then the α and β-tocopherol were purified by conversion into their crystalline allophones or 3,5-dinitrobenzoates.
- Hydrolysis of these derivatives gave the tocopherols as pale yellow oils.

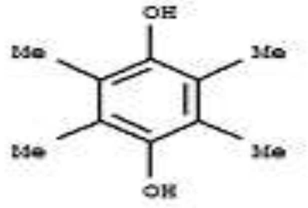
EXTRACTION AND ISOLATION

Wheat germs are dried and pressed to collect the oil. The oil is then treated with 20% alcoholic KOH in absence of oxygen. The unsaponified portion contain sterol and vitamin E, the former are removed by precipitation with digitonin. Now the distillation of the remaining oil gives vitamin E fraction at 200-250°C under reduced pressure, this process gives less yield of vitamin.

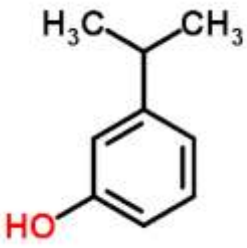
ELUCIDATION OF TOCOPHEROL

1. Molecular formula is $C_{29}H_{50}O_2$
2. Tocopherol forms monoacetate. Monoester and monoether. Indicate that one of the oxygen atom is present as hydroxyl group.
3. The second oxygen atom was found to be present as a cyclic ether.
4. Tocopherol is heated with with 350°C to yield duroquinol indicating presence of benzenoid nucleus.

$C_{29}H_{50}O_2 \xrightarrow[350^\circ C]{}$

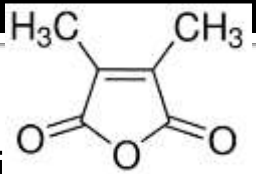


5. Tocopherol is heated with HI gives Ψ-cumenol. This is shown that presence of one free hydroxyl group



6.Oxidation of tocopherol with chromic acid under mild condition yields dimethyl maleic anhydride and an optically active saturated lactone A.

$C_{29}H_{50}O_2 \xrightarrow{CrO_3} C_{21}H_{40}O_2$ (Lactone) +

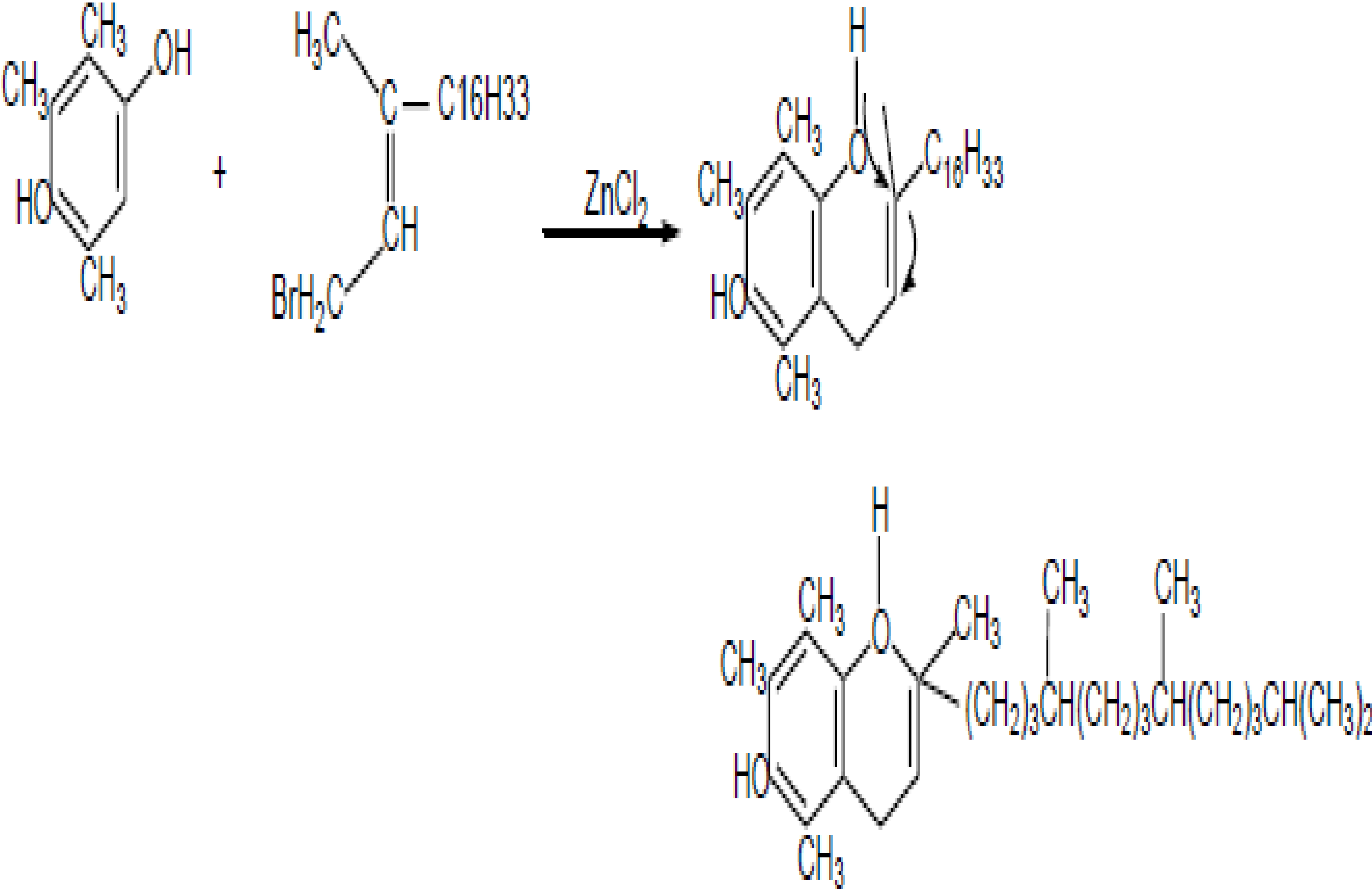


dimethyl maleic anhydride

7. α -tocopherol acetate, on oxidation with chromic acid, forms an acid, $C_{16}H_{32}O_2$(B) and a ketone, $C_{18}H_{36}O$ (C). Both of these compounds must be produced by the oxidation of the lactone at different point in the side chain. This show that presence of methyl group.

8. Structure of α -tocopherol is confirmed by synthesis

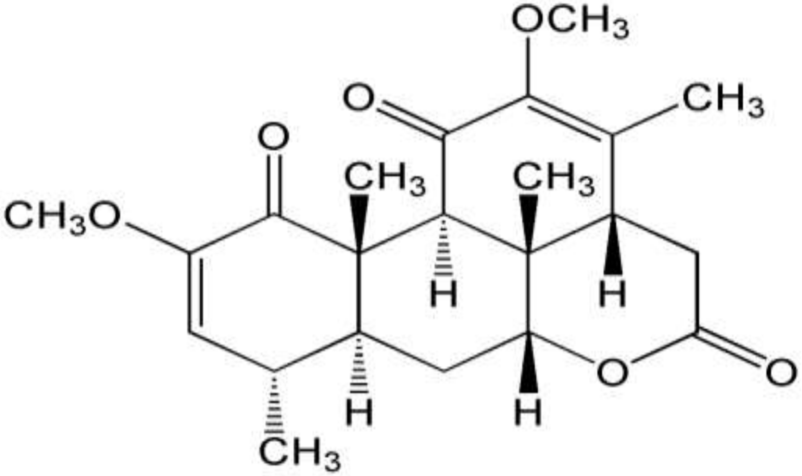
It have synthesized tocopherol by condensing trimethylquinol with phytyl bromide.



(±)-α-tocopherol

Quassinoids: Quassin

- Quassin is an intensely bitter oxygenated triterpenoid characterized by having a lactone structure. Quassin and related compounds constitute the group of quassinoids or



Quassinoids: Quassin

- Quassin is the main constituent of quassia wood, which is the stem wood of *Picrasma excelsa known in commerce as Jamaica quassia or of Quassia amara known in commerce as Surinam Quassia family Simarubaceae .*

Isolation

and neutralized with Na_2CO_3.

- Tannic acid solution is added gradually, until no more precipitate is formed.
- The precipitate is collected, triturated with lead carbonate (to form lead tannate and liberate quassin) then dried on a water bath.
- The produced mass is powdered and repeatedly extracted with alcohol 80 %.
- The combined alcoholic extracts are concentrated and left to allow crystallization of quassin.

Uses

- Quassia wood extract is used as a bitter tonic.
- The drug has anthelmintic properties, and is administered as enema for expulsion of threadworms.
- It also used as insecticide.

THANK YOU