CHAPTER 2

CHEMICAL CONSTITUENTS OF ZINGIBERACEOUS PLANTS

Zingiberaceae is known to contain numerous amount of chemical substances which altogether account for the various valuable medical usage served by this family. More and more scientists have become inquisitive of the chemical identities of this family. It is also interesting to note that not only the phytochemists are investigating Zingiberaceae, but scientists from other fields such as the pharmacologists and the botanists are also involved as they are also keen to identify the bioactive constituents of this family.

2.1 AROMATIC OR BENZENOID COMPOUNDS

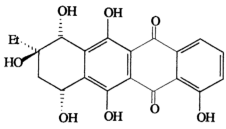
The aromatic or benzenoid compounds are actually the phenolics and some may display neutral or acidic character due to the absence of a free phenolic group or the presence of an acidic group[21]. Phenolic compounds are widely distributed from micro-organisms to higher plants and animals. Biological activities of this group of compounds are not as outstanding as compared with those of alkaloids and steroids. Although initially phenolics are assumed to be biological 'waste products', their biological activities are much more diverse than previously expected. There are some well-known examples of these compounds possessing medicinal uses such as antibiotics, e.g. α-rhodomycinone **5**, purgatives, e.g. sennoside A and B **6** and components showing allelopathic effects, e.g. juglone **7**. Table 2.1 illustrates the classification of the phenolic constituents in plants as provided by Harborne and Simmons[22].

TABLE 2.1 : THE CLASSIFICATION OF THE PHENOLIC CONSTITUENTS IN PLANTS[22]

Number of carbon atoms	Families of Phenols
C_6	[O] Simple phenols
$C_6 - C_1$	[#] Phenolic acids and related compounds
$C_6 - C_2$	[O] Acetophenones and phenylacetic acids
$C_6 - C_3$	[#] Cinnamic acids and related compounds
$C_6 - C_3$	[#] Coumarins, isocoumarins and chromones
C_{15}	[#] Flavones
C_{15}	[#] Isoflvones and isoflavonoids
C_{15}	[#] Flavonols, dihydroflavonols and related compounds
C_{15}	[#] Anthocyanidins
C_{15}	[#] Chalcones, aurones and dihydrochalcones
C_{30}	[O] Biflavonyls
$C_6 - C_1 - C_6$, $C_6 - C_2 - C_6$	[O] Benzophenones,xanthones and stilbenes
C_6, C_{10}, C_{14}	[O] Quinones
C_{18}	[O] Betacyanins

[#] The families of widey distributed phenolic constituents[23].

[O] The families of less widely distributed phenolic constituents.



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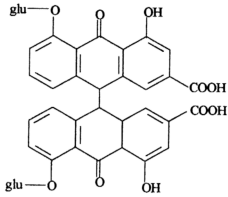

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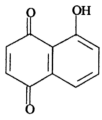

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2.2 TERPENES

Terpenes are generally divided into three groups[24] :

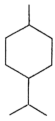
a) mono- and sesquiterpenes - C_{10} and C_{15} substances which are derived from geranyl pyrophosphate and farnesyl pyrophosphate, respectively;

b) diterpenes - C_{20} compounds biogenetically derived from geranylgeranyl pyrophosphate;

c) sesterterpenes - C_{25} compounds genetically derived from geranylfarnesol,
triterpenes - C_{30} substances derived from squalene and
higher terpenes - terpenes with more number of carbon atoms.

For years the monoterpenes have been known as components of essential oils of higher plants. Typical skeletons include acyclic, monocyclic and bicyclic

systems, e.g. menthane **8** and pinane **9**. There are monoterpenes possessing four- or five-membered rings and geminal dimethyl cyclohexane rings, and others do not obey the isoprene rule (refer section 2.3.2), as found in *Compositeae* plant. The sesquiterpenes and diterpenes are mainly of plants and fungi origin. The former has an outstanding diversity of carbon skeletons compared with other classes of terpenoids and may be acyclic or cyclic hydrocarbons, alcohols, ketones or lactones[25]. The latter include resin acids and gibberellins. The sesterterpenes are the newest and the smallest class in the terpenoid family and are found as fungal metabolites in ferns, marine sponges, lichens and insect secretions. The triterpenes form the largest group and are widely distributed in the plant kingdom either in the free states or as esters or glycosides.



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2.3 AROMATIC (OR BENZENOID) COMPOUNDS AND TERPENES OF ZINGIBERACEAE

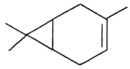
The constituents of Zingiberaceae vary from simple essential oils or volatile components such as 3-carene **10**[26] and 1:8-cineol **11**[27], from *Kaempferia galanga* L.[28,29], to higher terpenes such as (E)-labda-8(17),12-diene-15-ol-16-al **12**, from

Alpinia formosana[30], or more complex aromatics such as 2-methoxy-8-(2,4,5-trimethoxyphenyl)-naphto-1,4-quinone **13**, from *Zingiber cassumunar* Roxb.[31].

Extensive work on the identification of essential oils from Zingiberaceous plants have been performed by many scientists as these plants are known for their aromatic characteristic.

In the course of isolating the compounds from Zingiberaceae, either out of mere curiosity of their contents or due to the need to identify components that possess medicinal properties, many aromatic compounds have been identified. For example, Itokawa *et. al.* reported the isolation of carboaromatic compounds, i.e. 1'-acetoxychavicol acetate **1** and 1'-acetoxyeugenol acetate **2** as antitumour principles from *Alpinia galanga*[17]. Kuroyanagi *et. al.* reported thirteen aromatic components from the rhizomes of *Zingiber cassumunar* Roxb., which included the following compounds: cis-4-[(*E*)-3,4-dimethoxystyryl]-3-(2,4,5-trimethoxyphenyl)-cyclohex-1-ene **14**, *trans*-3-(3,4-dimethoxyphenyl)-4[(*E*)-3,4-dimethoxystyryl]-cyclohex-1-ene **15** and *trans*-3-(3,4-dimethoxyphenyl)-4-[(*E*)-2,4,5-trimethoxystyryl]cyclohex-1-ene **16**[31]. Jurgens *et. al.* isolated five compounds from *Curcuma comosa*; 1,7-diphenyl-4(*E*),6(*E*)-heptadien-3-ol **17**, 1,7-diphenyl-6(*E*)-hepten-3-one **18** and 1,7-diphenyl-6(*E*)-hepten-3-ol **19**[32].

There are only a few reported cases of diterpenes from Zingiberaceae. Itokawa *et. al.* isolated two diterpenes from the rhizomes of *Alpinia speciosa* K. Schum.[33] The compounds are labda-8(17),12-diene-15,16-dial **20** and 15,16-bisnorlabda-8(17),11-dien-13-one **21** which belong to the labdane[34] and



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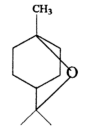

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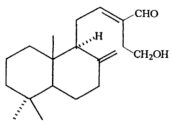

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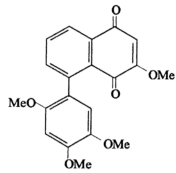

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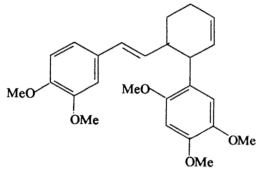

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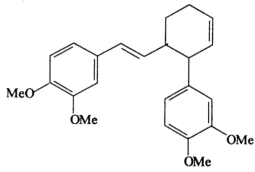

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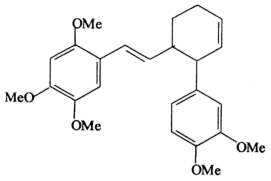

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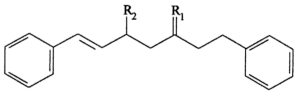


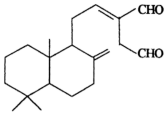
17 R_1 = H, OH; R_2 = H, $\Delta^{4,5}$
18 R_1 = O; R_2 = H
19 R_1 = H, OH; R_2 = H

bisnorlabdane types, respectively. A labdane dialdehyde identified to be (*E*)-8β,17-epoxylabd-12-ene-15,16-dial **22**, isolated from the seeds of Afromomum daniellii, was reported by Kimbu *et. al.*[35]. Tuchinda *et. al.* isolated two pimarane diterpenes from the hexane extract of the rhizomes of *Kaempferia pulchra*, i.e. 2α-acetoxysandaracopimaradien-1α-ol **23** and sandaracopimaradien-1α,2α-diol **24**[36].

In their work, Apisariyakul *et. al.* reported the isolation of turmeric oil and curcumin **25**, a sesquiterpene, from *Curcuma longa*[20,37]. Fasihuddin and Hasmah reported the presence of curcumin and essential oils (monoterpenes), which included carvone **26** and camphor **27**, in the local kunyit (*Curcuma domestica*); and cineol **11** and eugenol **28** from *Alpinia galanga* (*Languas galanga*), locally known as "lengkuas" [37]. Humulene oxide **29** (sesquiterpene) was isolated from *Zingiber zerumbet* (Damodaran *et. al.*)[38].

2.3.1 Biogenesis / biosynthesis of Zingiberaceae aromatic or benzenoid compounds

The mode of synthesis of compounds in plants is an interesting field to study. The terms "biogenesis" and "biosynthesis" are sometimes used without distinction. However, it is customary that the former term is used for a hypothesis, and the latter for an experimentally proven route[39]. Glucose is photosyntesized in green plants and usually serves as the main source of carbon in the synthesis of aromatic compounds. The biosyntheses of many of these products have been extensively examined by means of isotopically labelled precursors.



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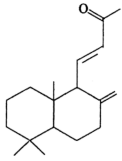

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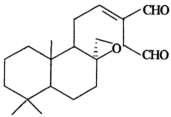

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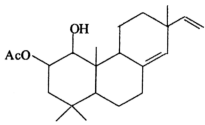

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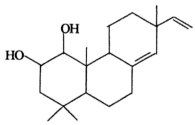

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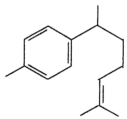

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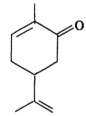

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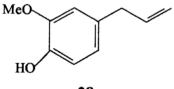

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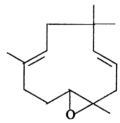

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There are several biosynthetic pathways leading to the formation of aromatic or benzenoid compounds. They are :

i. The shikimic acid pathway[40].

This pathway is responsible for the biosynthesis of several building blocks essential for all life such as lignin. It leads from carbohydrate through a common series of alicyclic stages (one of them is shikimic acid) to about six or seven primary aromatic compounds, to the three aromatic acids (phenylalanine, tyrosine and tryptophan), to several vitamins and to an immense variety of the so called "secondary metabolites" which include the majority of known alkaloids, pigments, etc.

ii. The acetate or polyketide pathway[41].

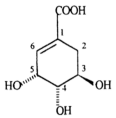
This pathway is furnished by the head-to-tail combination of -CO-CH_2- units derived from acetate and malonate. Most of the compounds often termed "polyketides" (which arise from this pathway) or so-called "secondary metabolites" (substances that occur only in a limited number of organisms and the function of which frequently appears not to be that of an intermediate in metabolism).

iii. The mevalonic acid pathway[40].

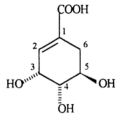
This pathway leads to the formation of aromatic terpenoids and steroids containing aromatic rings.

In the present study, the first pathway, i.e. the "shikimic acid pathway", will be discussed briefly since this pathway is involved in the biosynthesis of the compounds isolated from *Kaempferia galanga* Linn. The biosynthesis of aromatic

or benzenoid compounds involving this pathway is established from studies carried out by Davis[42,43]. This pathway is the major metabolic route leading to the formation of aromatic compounds in living systems[44]. It operates in microorganisms and in higher plants, but not in animals, which is why the latter are dependent on a dietary supply of the aromatic amino acids phenylalanine, tyrosine and tryptophan. The structure for shikimic acid **30** is as shown below, with two systems of numbering; one being the older while the other being the modern numbering. The latter numbering system will be used in the following discussions, in which the numbers for carbons 3 and 5, and 2 and 6, are interchanged[40,45].



older numbering



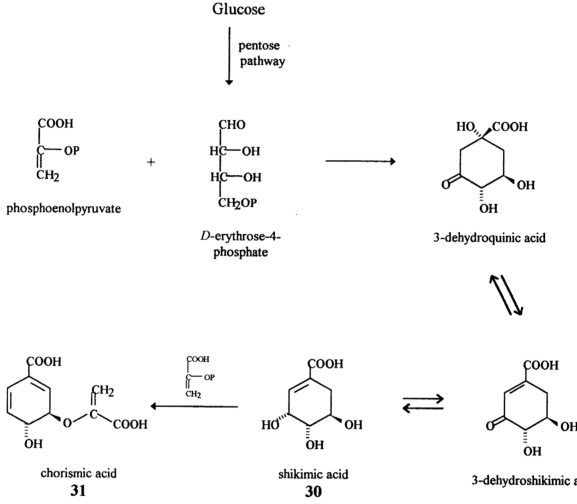
modern numbering

shikimic acid

30

Scheme 2.1 outlines the biosynthesis of phenolic compounds via the "shikimic acid pathway". The first part of the shikimate pathway is the sequence of reactions leading to the formation of chorismic acid **31**, which is common to all the branches of the pathway. The building blocks of shikimic acid are phosphoenolpyruvate and *D*-erythrose-4-phosphate. Both substrates combined in a reaction catalyzed by the enzyme DAHP synthetase to give the 7-carbon sugar 3-deoxy-D-arabino-heptulosonic acid-7-phosphate (DAHP), which is subsequently cyclized to the first carbocyclic compound, 3-dehydroquinate. Hydrolysis of the latter followed by reduction of the carbonyl function then gives shikimic acid which

SCHEME 2.1 BIOSYNTHESIS OF PHENOLIC COMPOUNDS VIA SHIKIMIC ACID PATHWAY[44,46]



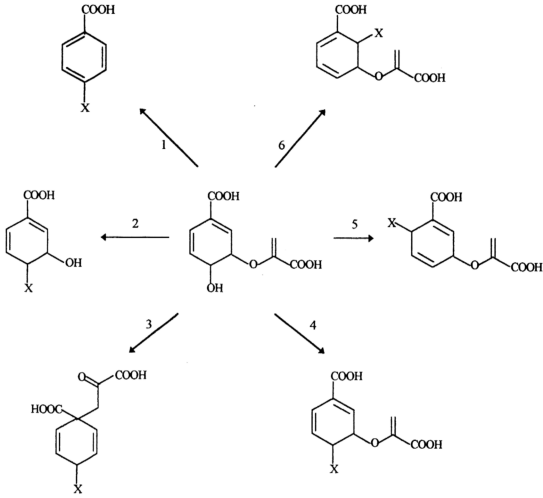
is subsequently phosphorylated in the 3-position. Attachment of a 3-carbon side chain, provided by another molecule of phosphoenolpyruvate, followed by a 1,4-elimination of the elements of phophoric acid then leads to the formation of chorismic acid. In his book, Floss expressed his opinion in that 'the name "shikimate pathway" is a misnomer, and the pathway should more appropriately be called the "chorismate pathway" ', since chorismic acid is the product of this common branch of pathway and is the key compound which, via its many reaction possibilities, opens up routes to the formation of a wide spectrum of aromatic compounds[44].

Scheme 2.2 illustrates the various reaction possibilities of chorismic acid. These include :

1. aromatization by elimination of the side chain,
2. hydrolytic removal of the side chain to 3,4-disubstituted 3,4-dihydrobenzoic acids,
3. Claisen rearrangement,
4. direct displacement reactions of the substituent in the 4-position,
5. allylic displacement reactions of the substituent in the 4-position and
6. bis-allylic displacement reactions of the substituent in the 4-position.

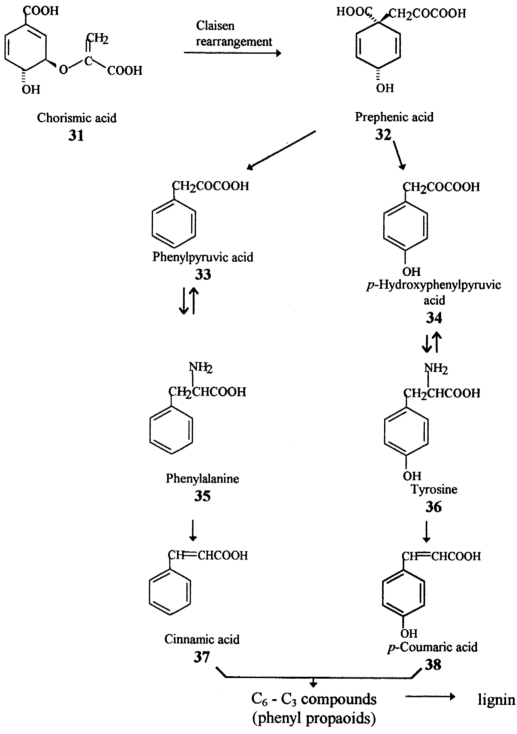
Scheme 2.3 outlines the formation of phenylalanine and tyrosine, which then lead on to the formation of phenylpropanoids. Claisen rearrangement of chorismic acid **31** forms prephenic acid **32**, a common non-aromatic intermediate, which in many organisms, represents a secondary branch point. The following major reactions involve the rearrangement and subsequent aromatization of prephenic acid

SCHEME 2.2 REACTIONS OF CHORISMIC ACID



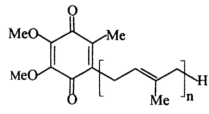
X = substituent or H

SCHEME 2.3 OUTLINE ON THE FORMATION OF PHENYLALANINE AND TYROSINE, AND SUBSEQUENT FORMATION OF PHENYLPROPANOIDS FROM CHORISMIC ACID



32 to form phenylpyruvic acid **33** or *p*-hydroxyphenylpyruvic acid **34** which then undergo transamination to form phenylalanine **35** and tyrosine **36**, respectively.

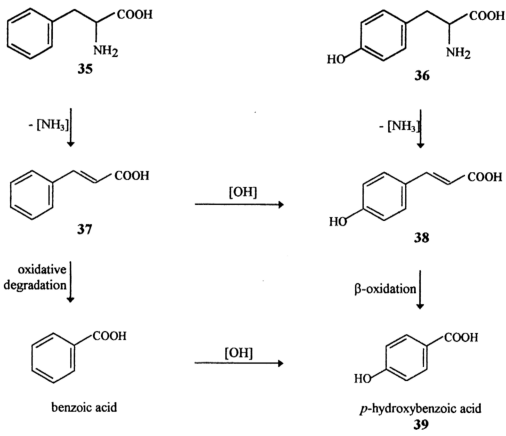
They are many additional branches of the shikimate pathway, some of which are unique to only certain organisms, while others are essential pathways but are of less quantitative significance than the main branches leading to the formation of the three aromatic amino acids. An example which can be considered involving the latter category is the route leading from chorismic acid to *p*-hydroxybenzoic acid **39**, the precursor of ubiquinones **40** (Coenzyme Q)[21]. Ubiquinones **40** exist very widely in bacteria, fungi, algae, higher plants, vertebrates and invertebrates, and play a major role in respiratory electron transport.



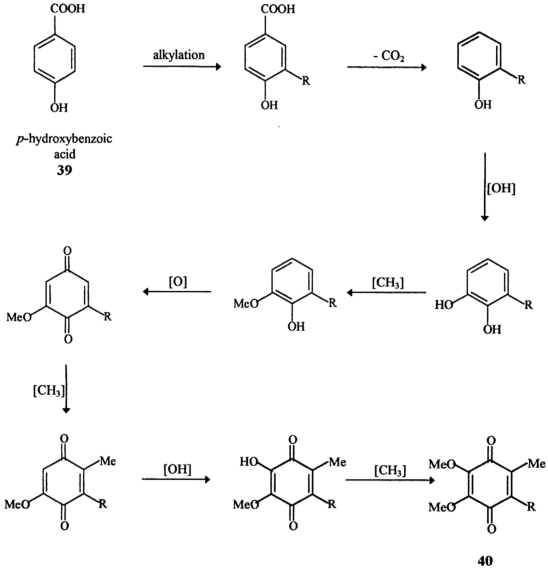
40 n = 6 - 10

The ubiquinones are derived in part from the acetate-mevalonate pathway, and in part from shikimic acid. Experiments with ^{14}C-acetic acid and ^{14}C-malonate proved that the quinone ring is not formed from acetic acid, but the isoprene side-chain does. Scheme 2.5 illustrates a proposed biosynthetic pathway for ubiquinones[47,48]. In higher plants and some fungi, *p*-hydroxybenzoic acid is synthesized by either of two related-transformations of pre-existing aromatic rings : direct *para*-hydroxylation of benzoic acid (derived from cinnamic acid by oxidative

SCHEME 2.4 NORMAL BIOSYNTHESIS OF *PARA*-HYDROXYBENZOIC ACID IN PLANTS



SCHEME 2.5 A PROPOSED BIOSYNTHETIC PATHWAY FOR UBIQUINONES



p-hydroxybenzoic acid
39

40

R = $\left[\text{CH}_2\text{CH}=\text{C}(\text{Me})\text{CH}_2\right]_n\text{H}$

n = 6 - 10

degradation), or β-oxidation of coumaric acid formed either from tyrosine or, more commonly, by hydroxylation of cinnamic acid. (Scheme 2.4).

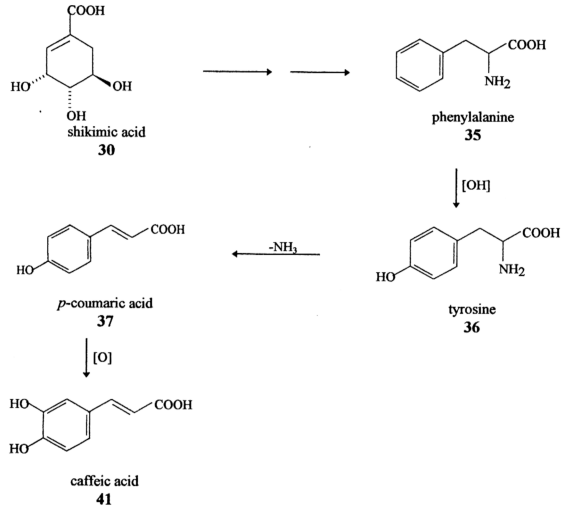
Scheme 2.6 illustrates the biosynthesis of caffeic acid **41**[49]; an example of biologically active plant catechols (*o*-diphenols) and originates from the essential amino acid, phenylalanine, and go through steps of hydroxylation. Plant catechols are mostly acids and have profound hormone-like effects (hormonal or synergistic) on plant growth at extremely low concentrations.

There also exist several nitrogen-containing metabolites derived from shikimic acid, which do not readily fit into any convenient chemical classes. An example of this is the antibiotic, chloramphenicol **43** (chloromycetin)[50]. Its biosynthesis requires *p*-aminophenylalanine **42**, a naturally-occurring amino acid, as a precursor of chloramphenicol. In higher plants, *p*-aminophenylalanine is derived from the shikimate pathway. The pathway is outlined in scheme 2.7.

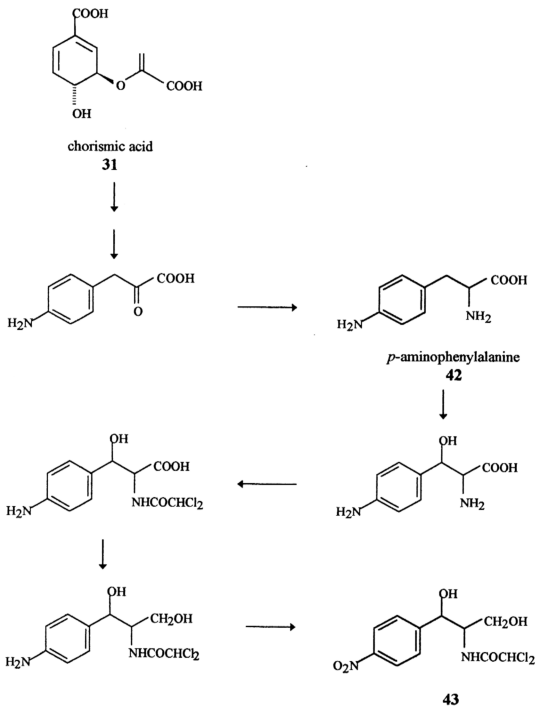
2.3.2 Biogenesis / biosynthesis of Zingiberaceae terpenes

Investigations of the molecular structures of terpenes reveal that, in the majority of cases, the carbon skeletons are theoretically built up by the union of two or more isopentane or isoprene **44** units in a head to tail manner[51,52]. This generalization, known as the *isoprene rule* was first suggested by Wallach in 1887 and later elaborated by Robinson. However, with Ruzicka's entrance into the field of higher terpenes in 1938, the possible role of isoprene polymers (such as farnesol

SCHEME 2.6 BIOSYNTHESIS OF CAFFEIC ACID[49]

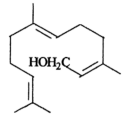


SCHEME 2.7 BIOSYNTHESIS OF CHLORAMPHENICOL



45) in the generation of sesquiterpenes was acknowledged[53]. Ruzicka proposed the *isoprene rule* and pointed out that all terpenoids are built up from isoprene units[39]. For example, limonene **46** is made up of three isoprene units. Nevertheless, there are terpenes which do not obey the *isoprene rule*, either because the number of carbon atoms in the molecule is not a multiple of five or because the skeleton cannot be constructed from isoprene units, e.g. eremophilone **47**.

44



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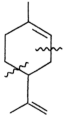

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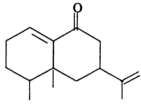
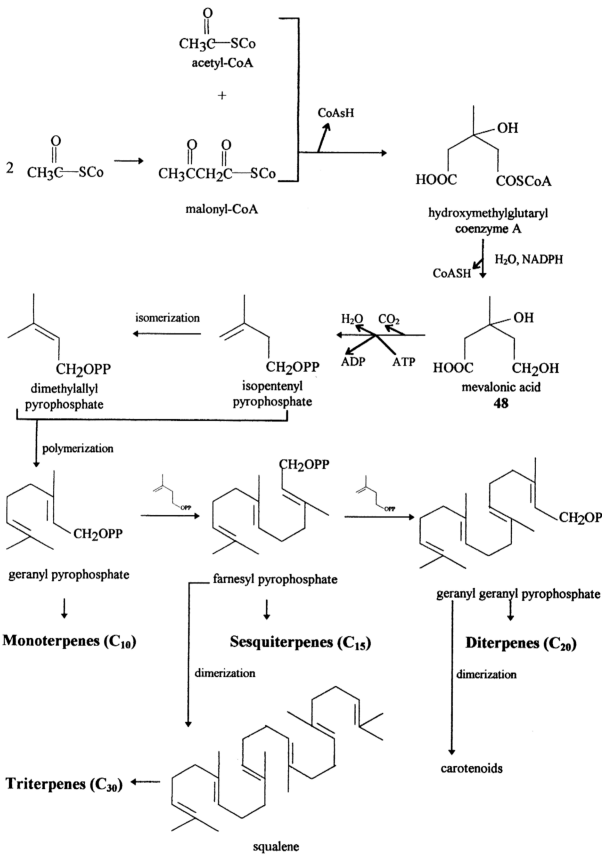


47

The biosynthesis of mevalonic acid **48**, and its subsequent conversion to mono-, sesqui-, di-, sester-, tri- and higher terpenoids is as summarized in scheme 2.8. The first step in the biosyntheses of terpenes from acetate is the formation of a four-carbon unit, malonyl coenzyme A, from two molecules of acetyl coenzyme A. Acetyl coenzyme A and malonyl coenzyme A will react to form hydroxymethylglutaryl coenzyme A, which then undergo irreversible hydrolysis to form free hydroxymethylglutarate and free coenzyme A. Hydroxymethylglutarate then undergo reduction of one of its carboxyl functions to yield mevalonic acid **48**,

SCHEME 2.8 BIOSYNTHESIS OF TERPENES[39,53,54]



followed by phosphorylation to form mevalonic acid-5-pyrophosphate. The latter subsequently undergo decarboxylation to isopentenyl pyrophosphate. Isomerization of this compound forms dimethylallyl pyrophosphate. The polymerization sequences that follow are envisioned as proceeding by an ionization of the carbon-oxygen bond of the dimethylallyl pyrophosphate to create a cationic center which is then attacked by a molecule of isopentenyl pyrophosphate.

2.4 STRUCTURAL ELUCIDATION : GENERAL METHODS AND THEORY.

To elucidate the structures of compounds, natural product chemists apply the spectroscopic technology which include the proton nuclear magnetic resonance (^{1}HNMR), ^{13}CNMR, infrared, ultraviolet and mass. The analytical method that has had the greatest impact on science has been the nuclear magnetic resonance spectroscopy (NMR). Although the first applications in organic chemistry came in the 1950s, the tool was not widely used until the advent of the Varian Associates A-60 spectrometer in the early 1960s. The new experiment provided the final piece of the structural puzzle in many cases; UV and IR gave the functionality, MS gave the molecular weight and formula, and NMR and MS together allowed one to put together the molecular skeleton. The development of fourier transform (FT) method and its application to the NMR experiment in the 1970s was considered to be a revolutionary advancement to organic chemistry and biological chemistry and allowed the elucidation of samples at small quantities (eg. pure compounds with minimum weight of 0.01 mg would be sufficiently detectable for ^{1}HNMR spectra using 270-600 MHz instruments)[55]. With carbon-13 NMR, the chemist could look

directly at the carbon backbone of molecules. The surge of two-dimensional (2D) NMR in the present decade enabled the chemist to obtain the kind of detailed structural 'photograph' that can be obtained in the crystalline state by the costly and time-consuming technique of x-ray crystallography[56].

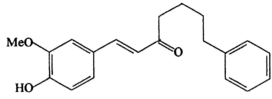
2.4.1 Ultra violet spectra of aromatic or benzenoid compounds[57,58,59]

Benzene displays three absorption bands: 184nm (ε_{max} 60,000) - E_1 band, 204nm (7,900) - E_2 and K bands, and 256nm (200) - B bands.

Benzenoid bands (B bands) are characteristic of the aromatic or heteroaromatic molecules, which are shown as broad bands containing multiple peaks or fine structure in the near-UV region between 230 and 270nm. The characteristic fine structure of these bands may be absent in spectra of substituted aromatics. The fine structure is often destroyed by the use of polar solvents. When a chromophoric group (unsaturated group) such as C=O, C=C or NO_2 is attached to an aromatic ring, the B bands are observed at a longer wavelengths than the more intense K bands.

K bands are due to the $\pi \rightarrow \pi^*$ transitions of conjugated di- or polyene systems and are usually characterized by high molar absorptivity, $\varepsilon_{max} > 10\ 000$. These transitions are unresponsive to solvent polarity since hydrocarbon double bonds are nonpolar. Direct attachment of a chromophore to the benzene ring

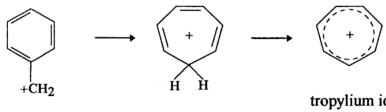
336nm, and on addition of NaOH maximum absorptions shifted to 255 and 404nm, respectively.



49

2.4.2 Mass spectroscopy of aromatic or benzenoid compounds

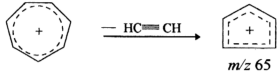
Molecular ions from compounds of the general formula C_6H_5X will fragment by loss of X, or part of X[60]. An aromatic ring in a molecule stabilizes the molecular ion peak , which is usually sufficiently large that accurate intensity measurements can be made on the M+1 and M+2 peaks. A prominent peak (often the base peak) at *m/z* 91 ($C_6H_5CH_2^+$) is indicative of an alkyl substituted benzene ring. Branching at the α carbon leads to masses higher than 91 by increments of 14, the largest substituent being eliminated most readily. It has been proposed that, in most cases, the ion of mass 91 is a tropylium rather than a benzylic cation[61,62].



benzylic cation

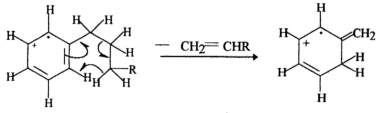
tropylium ion

A peak at *m/z* 65 can be frequently seen, resulting from the elimination of a neutral acetylene molecule from the tropylium ion.



m/z 65

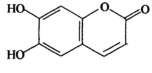
When the alkyl group attached to the benzene ring is longer than C_2, hydrogen migration with elimination of a neutral alkene take place and this account for the peak at *m/z*92.



m/z 92

A characteristic cluster of ions due to α cleavage and hydrogen migration in monoalkylbenzenes appears at *m/z* 77 ($C_6H_5^+$), 78 ($C_6H_6^+$) and 79 ($C_6H_7^+$).

Often identification of aromatic compounds such as phenylpropanoids can be confirmed by UV spectral measurements. For example, caffeic acid and its derivatives have characteristic absorption bands at 243 and 326nm, with a distinctive shoulder at 300nm to the long wave band. Hydroxycoumarins absorb at longer wavelengths than cinnamic acids; aesculetin, the coumarin related to caffeic acid, has absorption bands at 230, 260, 303 and 351nm[63].



aesculetin

2.4.3 ^{1}HNMR spectroscopy of aromatic or benzenoid compounds

When a molecule is placed in a magnetic field, currents are set up in the molecule, and the additional magnetic field at the nucleus due to these currents causes a nuclear magnetic resonance (NMR) shift[64]. Such currents flow around atoms, within bonds and from atom to atom around a benzene ring. Each of these three types of currents can affect the ring hydrogen shifts differently. It is known that the chemical shift of benzene protons is δ 7.27 ppm and the general regions of chemical shifts of aromatic and heteroaromatic protons lie between 6 to 9 ppm, i.e. towards lower field. The so-called "ring-current effect", an example of diamagnetic anisotropy, accounts for the large deshielding of benzene ring protons[65]. Figure 2.1 shows this effect. This also indicates that a proton held directly above or below the ring should be shielded.

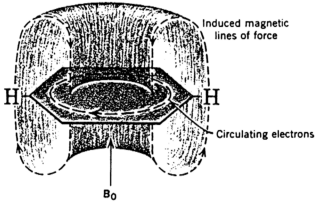
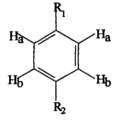


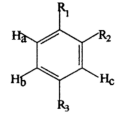
Figure 2.1 Ring current effects in benzene

The position taken by an entering group in a substituted benzene appears to be intimately connected with the value of the electron density at the *ortho, meta* and *para* positions[66]. The fact that the chemical shifts observed in proton resonances reflect differences in the electron distribution about chemically non-equivalent protons arise from the small magnetic fields set up by the electrons which oppose the applied field and are directly proportional to it. This phenomenon is commonly called a diamagnetic shielding of the protons by the electrons. Highet *et. al.*[67] reported that the chemical shifts of protons on aromatic rings reflect the charge density on the carbon atom to which the protons are attached and the observations on substituted benzenes are closely parallel: electron-withdrawing substituents produce downfield shifts in the *ortho* and *para* protons, whereas electron-donating substituents produce an upfield shift; the *ortho* protons are generally more affected than the *para*.

In a 1,4-disubstituted benzene ring, two doublets for AB-type protons can be observed at approximately 7.35 and 6.90 ppm, with *ortho* coupling of 8 Hz[58,68]. In another case, two *meta*-coupled aromatic protons show two doublets at approximately 6.00 ppm with coupling constants of 1.32 Hz. In the case of a 1,2,4-trisubstituted benzene ring, signals for the three aromatic protons may be split into an ABC system with J_{AC} = 0.0 Hz, typical of *ortho-ortho-meta* disposed aromatic protons. A relatively upfield one-proton doublet will appear at 6.70 ppm (J_{AB} = 8.4 Hz), a downfield doublet of doublets and a doublet (one proton each) at 6.80 ppm (dd, J_{BA} = 8.4 and J_{BC} = 2.0 Hz) and 6.90 ppm (J_{CB} = 2.0 Hz)[69].



1,4-disubstituted benzene ring



1,2,4-trisubstituted benzene ring

The chemical shifts of the side-chain hydrogens are determined primarily by their proximity to the aromatic ring[70]. Because of the anisotropy in the magnetic susceptibility of the phenyl ring, protons lying in the plane of the ring experience maximum deshielding. This is predicted to decrease as the protons are located further above or below the plane; protons located directly above the center of the ring should be maximally shielded.

Two doublets at approximately 6.20 ppm and 7.50 ($J_{1,2}$ = 16 Hz) indicate the presence of trans α,β-unsaturated side chain[59,68,71,72]. The presence of a phenolic hydroxyl group can be shown by a singlet at 5.50 ppm which disappears on addition of D_2O[58,69]. A singlet at 3.90 ppm (3H) indicates the presence of a methoxy group attached to the benzene ring[59,68].

2.4.4 ^{13}CNMR spectroscopy of aromatic or benzenoid compounds[110,111]

Direct observation of the carbon skeleton has been available on a practical basis only since the early 1970's due to the availability of FT instrumentation. The ^{12}C nucleus is not magnetically "active" (spin number, *I*, is zero), but the ^{13}C nucleus is like the ^{1}H nucleus which has a spin number of ½. However, the overall

sensitivity of ^{13}C compared with ^{1}H is about 1/5700 since the natural abundance of ^{13}C is only 1.1% that of ^{12}C.

The coupling constant, *J*, values for ^{13}C–H are large (~ 110-320 Hz), hence, proton coupled ^{13}C spectra usually show complex overlapping multiplets that are difficult to interpret. Decoupling of the protons by means of a broadband generator removes these couplings. While the spectrum is being taken, the sample is irradiated with a strong signal encompassing the whole range of frequencies within which the protons in the molecule come into resonance. Eventually, this will cause the geminal protons to be exchanging places rapidly several times during the measurement of the carbon signal. Each carbon atom, therefore, "see" only an average state for the protons near to it, and instead of being coupled, each chemically non-equivalent ^{13}C atom simply gives rise to a single sharp line. The chemical shifts encountered in routine ^{13}C spectra range about 240 ppm downfield from tetramethylsilane (TMS), the internal reference; this is a range of about 20 times that of routine ^{1}H spectra (~ 12 ppm).

Benzene carbon (sp^2) atoms absorb at 128.5 ppm, neat or as solution in $CDCl_3$ (deuterated chloroform) or CCl_4 (carbon tetrachloride). Shifts of the aromatic carbon atom directly attached to a substituent are correlated with substituent electronegativity after correcting for magnetic anisotropy effects. For example, benzene carbon atom attached to an ethylene resonates at 137.6 ppm, while that attached to a methoxy resonates at 159.9 ppm.

Carbons with sp^3 hybridization, ie. of alkanes, show signals in the region of 10 - 58 ppm, while carbons of alkenes (sp^2) produce peaks in the region of 80 - 170 ppm. Furthermore, *sp* carbons of alkynes exhibit signals in the range of 20 - 95 ppm.

^{13}CNMR also permits direct observation of carbon-containing functional groups. The C=O groups (sp^2 carbons) of carboxylic acids and derivatives are in the range of 150 - 185 ppm while sp^3 carbons of ethers (C–O–C) are in the range of 55 - 85 ppm. In addition, alcohols show carbon (sp^3) resonating in the region of 45 - 85 ppm.