Chapter I

INTRODUCTION
Though Sin and Sathan have plunged mankind into an ocean of infirmities, yet the mercy of God, which is over all His works, maketh grass to grow upon the mountains, and Herbs for the use of man. And hath not only stampt upon them a distinct forme, but also given them particular signatures, whereby a man may read, even in legible characters, the use of them.

William Coles (1657)

The old-world term for what we call herbal remedies was simples, or, less politely, 'old wives' simples' and these were probably the first remedies known to man when the world was very young. And they are still known to animals, for if we watch our cats and dogs, which are so much nearer the prehistoric ages than ourselves, we shall see the working of their instinct in the care they take in choosing certain grasses and plants as medicines.

Quite early the man realised the curative value of the plants in various ailments. It was, therefore, natural that the science of medicine in the early stage developed around those plants which had curative properties. A continued search for medicinal plants during the last several centuries
has given us a store of innumerable plants which are of great use in the treatment of diseases and promotion of health.

"Whether man was originally destined by nature for a vegetarian or not will probably never be satisfactorily decided but there can be no doubt that in all ages, and under all the varied conditions of his existence upon the earth, he has been dependent, more or less directly, for his support upon the plants growing upon its surface."1

Wrote Pierpoint Johnson and his remark may aptly be applied to the history of medicine.

**Indian medicinal plants:**

The history of medicine and surgery dates back perhaps to the origin of human race. But, as no mode of recording events existed in prehistoric times, there are no data on the methods of treatment practised in that period. In those days, the subject of human suffering and its alleviation was intimately associated with religion, myth and magic. In addition, there must have been certain rational prescriptions. Whenever, the curiosity of the present-day man probes into the past and brings to light even fragmentary information on the ingenious

methods of our ancestors, it makes a fascinating study.

The earliest mention of the medicinal use of plants is found in the "Rigveda", but references to plants in the "Rigveda" are brief. Far more detailed account is available in "Atharva-veda". The period of Rigveda is estimated to be between 3,500 and 1,800 B.C. After the vedas, there is no information on the development of this science in India for a period of about 1,000 years. Then came the two most important works on Indian system of medicine, the works of Charak and Susruta, namely, the Charak-samhita and Susruta-samhita. Susruta-samhita deals with about 700 drugs, some of these were not indigenous to India. With the passing of time, more and more plants found entry into native medicine, taking the number of Indian medicinal herbs to about 1,500. Later during the Buddhist period, considerable progress was made and medicinal plants were cultivated under the direction of highly qualified specialists. Contacts with Greece and Rome, and later with Arabia and Persia, contributed to the enrichment of the Indian Materia Medica and large number of vegetable and other products came into use for the treatment of diseases.

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It has been estimated that out of about 2,000 drugs that have been used in curing human ailments in India, only about 200 are of animal origin and a similar number are of mineral origin. The rest, i.e. about 1,500, are of plant origin. This number is not very large considering the vast area of our country, and the wide variety of plant wealth occurring therein. The great range of temperature (about 49°C to -43°C); rainfall (from 100 mm to over 10,000 mm) and altitude (sea-level to over 6,000 m) in India account for the occurrence of some 20,000 different species of higher plants.

**Aromatic plants of India:**

A large number of aromatic plants containing essential oils are also important from utilitarian point of view. Natural perfume is one of the most remarkable phenomena of plant metabolism and history of aromatic plants has been associated with India since time immemorial. Man has tried to utilise these odoriferous plants for his pleasure from early times. Some have been used as flavouring agents in foods, drinks and in pharmacy. Others have been used as offering to deities, as incense, as principle agents for embalming the dead and for preventing insects from damaging fabrics and food grain. The application of essential oils in day-to-day human activities is very considerable and is rapidly increasing.

Systematic investigation of drugs used in indigenous
medicine in India on modern scientific lines was started more than forty years ago. A number of important medicinal plants prescribed by Kaviraj and Hakims have been investigated.

The study of Indian medicinal plants as a possible source of anticancer drugs has also been in progress for almost 10 years. Laboratory tests, conducted on 800 medicinal plants at C.D.R.I., Lucknow, India has shown anticancer properties in 34 plants.\textsuperscript{4} Illudin-5 Lampterol an anti-tumour substance has been discovered through two independent chemical studies. With the growth of pharmaceutical institutions and chemical laboratories in universities and also in pharmaceutical firms in India, considerable progress has been made in working out the utility of indigenous medicinal plants. This work has brought out the merits and qualities of such drugs as \textit{Paorela corylifolia} in leucoderma, \textit{Plantago ovata} in dysentery, \textit{Adhatoda vasica} in chest diseases, \textit{Holarrhena antidysenterica} in amoebiasis, \textit{Pristimera indica} (antibiotic pristimerin), \textit{Rauwolfia serpentina} as an antidote to insect and snake bites,\textsuperscript{5} and many other as described by Chopra \textit{et al.}\textsuperscript{5}

Besides the above-mentioned plants which have been investigated properly, many plants are used in the practice

\textsuperscript{4} Rajagopalan, T.; \textit{The Indian Express}, 1969, May 18.

of indigenous medicine whose chemical composition and pharmacological properties are not fully worked out. Owing to great extension of indigenous medicine for medical relief in most of the states of the Indian Union during recent years, their use in the treatment of disease has been greatly extended. Examples of these plants have been fully described in Chopra's *Indigenous Drugs of India*. There are large number of plants mentioned in literature which are alleged to have insecticidal or insect-repellent properties. A few examples thus cited above clearly bring out how researches in the field of medicinal plants have played an important role in the cure of human diseases. The importance of work done and utilization of the Indian medicinal plants have been reviewed from time to time by various workers like Chopra et al., Kirtikar and Basu, Nadkarni, Haridas, Zimmer, Dastur and Editors of Wealth of India, Raw materials.

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9 Haridas; *Chikista Chandrodaya,* in 7 Vols, 1955-1964.


12 Wealth of India (Raw materials); Council of Scientific and Industrial Research, New Delhi, 1969.
Any further investigations in this field would, thus be one step ahead to a distant goal of utilising indigenous plant materials for the amelioration of human sufferings on the one hand and provide a profitable source of national wealth on the other.

Chemistry of plant products:

The efficacy of the plant products has most often been shown to be due to an active principle contained therein. These active principles fall under the following group of compounds: essential oils, fixed oils, carbohydrates, tannins, glycosides, protein/amino acids, alkaloids, coumarins, colouring matters, enzymes and others. The description of some of these is briefly reviewed.
ESSENTIAL OILS

Essential oils are the mixture of terpenic hydrocarbons and their oxygenated compounds, (such as alcohols, ethers, aldehydes, lactones, oxides, etc.) a small amount of viscid or semi-solid non-volatile residues (Paraffins, waxes and other products of resinification occurring generally in the plant kingdom. The oils are usually extracted by steam distillation or solvent extraction of either the whole plant or its selected part. Conjugated non-distillable forms, e.g. terpene-β-d-glucosides are also found especially in the floral organs. Progress in the field of essential oils and their constituents has been reviewed by various workers. Remarkable amongst there are Gildemeister and Hoffmann, Simonsen and Barton, Guenther and others. It will be convenient to study essential oils under the following subheadings:

(A) SITES OF SYNTHESIS:

Essential oils mostly occur in plant kingdom as intermediates (perhaps enzyme-bound) in chlorophyta, rhodophyta, gymnosperms and angiosperms.\(^{16}\) It is now known that the oils accumulate in specialized tissues — the oil glands that are usually resin ducts or modified epidermal hairs and the actual sites of synthesis are usually supposed to be secretory cells associated with these glands.\(^{17,18}\) Analysis of the contents of individual oil glands has been feasible especially by application of direct injection techniques to gas-liquid chromatography. The mono- or sesqui terpenes in such glands of several species occurred in similar proportions to those in the oils extracted from whole plants,\(^{19,20}\) but in contrast, the oils extracted from different glands and from different tissues of the same Mentha species were found to be qualitatively different. This contrast may be because of glands being in different stages of development during course of experiment. The oil composition may vary in different

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tissues of the same species. In *Pinus maritima* or *Pinus sylvestris*\(^{21}\) woody parts and needles have varying proportions of monocyclics and bicyclics, although with increase of age the percentage of \(\alpha\) -pinene increases in all tissues, especially in the needles.

(B) ENVIRONMENTAL EFFECTS:

Plants of nominally the same species give different oils when grown in different areas and in different harvests in the same area.\(^{22,23}\) Chromatographic analyses have shown major variations in composition of numerous oils caused by alterations in climate and habitat,\(^{24,25}\) and also by seasonal and diurnal effects.\(^{26,27}\) However, such variations are not found in oils from mature specimens of many species although young plants may be so affected, consequently plant families


\(^{24}\) Von-rudloff, E.; *Phytochemistry*, 1966, 5, 331.


\(^{27}\) Levinson, A.S.; Lemoine, G. and Smart, E.C.; *Phytochemistry*, 1971, 10, 1087.
or individual species must differ in their sensitivity to such factors.

(C) GENETIC CONTROL:

The ability to produce particular monoterpenes, and by inference other terpenoids is generally believed to be under fairly strict genetic control. Comparable investigations have been carried out on the similarly commercially important Pinus species where the monoterpenes composition, and nature of the rearrangements, characteristic of terpene metabolism in these species was shown to be genetically controlled.\textsuperscript{28,29} Genetic analysis\textsuperscript{30} of \textit{Mentha crispa} led to the conclusion that dominant and recessive alleles determined alternative routes of cyclization of a common antecedent ketone to give carvone or menthone. The lemon mints contain predominantly citral and limonene and this simplicity of composition has been considered to imply that they are primitive members of the families.\textsuperscript{31}

\begin{itemize}
  \item Hanover, J.W.; \textit{Heredity}, 1966, \textbf{21}, 723.
  \item Hanover, J.W.; \textit{Forest Sci.}, 1966, \textbf{12}, 447.
\end{itemize}
(D) OCCURRENCE IN ANIMALS:

The great majority of animals do not accumulate monoterpenes although these compounds must be formed as intermediates for steroids, and such intermediates may be enzyme-bound at all times. Nevertheless, certain monoterpenoids, especially iridoids, have been characterised, particularly in Insecta and Arthropoda. Arthropod defensive secretions include citronellal, citral limonene and degraded terpenes. Citral is a trail pheromone for bees.

(E) BIOLOGICAL SIGNIFICANCE:

Three theories have been proposed to account for the biological significance of terpenoids as a class. The first is that the terpene pool maintains the respiratory coenzymes in a reduced form by acting as a substrate for metabolism to provide ATP when other sources have been depleted. Linked to this is a suggestion that the monoterpenes provide a pool


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of material for the synthesis physiologically important pigments.

The second proposal is that the majority of terpenoids have no function but are side products of an evolving network from which the essential terpenoids — plant hormones, phytosterols and carotenoids — are being selected.

According to the third theory plants and microorganisms are considered to produce terpenes during periods of dormancy of either the whole organism or of localized tissues, in order to maintain the appropriate enzyme systems in an active state. Specific monoterpenes are claimed to act as growth, heat and transpiration regulators and as participants in photosynthesis. Few essential oils have been shown to possess antimicrobial properties.

Chemistry of Essential Oils:

The investigations on the chemistry of essential oils was started by Dumas followed by many other workers. Berthelot

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studied mainly the hydrocarbons with the formula $\text{C}_{10}\text{H}_{16}$ and Kekulé coined the name "Terpene" for these hydrocarbons in 1866. Semmler then isolated many terpenes and terpenic derivatives from essential oils. Essential oils consist of a mixture of compounds acyclic, alicyclic, aromatic and heterocyclic in character, containing carbon hydrogen and oxygen. Sometimes sulphur and Nitrogen containing compounds are also encountered. The earliest attempt to rationalize the pattern of structure of the monoterpenes was the rule proposed by Wallach in 1887 who supposed such compounds to be constructed from isoprene units (I). Robinson later extended this "Isoprene Rule" that in terpenes (known at that time), the isoprene units are almost invariably linked in a head to tail fashion (limonene (II) and campher (III)).

![Chemical Structures]

(I)  
(II)  
(III)

Contd.

41 Dayal, B. and Purohit, R.M.; Flavour Industry, 1971, 2, 484.

42 Dumas, J.B.; Liebigs Annalen Der Pharmacie, 1833, 6, 1245.
Ruzicka and co-workers\textsuperscript{43,44} amended and proposed a "Biogenetic isoprene rule" which played an important role in placing the chemistry of terpenes, on a rational footing. The terpene analogues - Terpenoids - are compounds having a more distant connection with terpenes and are broadly classified\textsuperscript{45} on the basis of their carbon skeleton.

The mono (C\textsubscript{10}H\textsubscript{16}) and sesqui (C\textsubscript{15}H\textsubscript{24}) terpenes are frequent constituent of essential oils. The diterpenes C\textsubscript{20}H\textsubscript{32} are of less common occurrence, the triterpenes (C\textsubscript{30}H\textsubscript{48}) are mostly obtained from plant or tree gums and resins, and are non-volatile in steam.

**Monoterpenes:** In the monoterpenes series all the four possible types, viz. acyclic, monocyclic bicyclic and tricyclic terpenes are observed as illustrated by representative compounds (IV, V, VI, VII).

\[\text{myrcene (IV)} \quad \text{limonene (V)} \quad \alpha \text{-pinene (VI)} \quad \text{tricyclene (VII)}\]

\textsuperscript{43} Ruzicka, L.; Eschenmoser, A. and Heusser, H.; \textit{Experientia}, 1953, 9, 357.

Contd.
Sesquiterpenes:

Semmler\textsuperscript{46} and Sehreiner and Kremer\textsuperscript{47} independently classified sesquiterpenes into four main groups, i.e. acyclic, monocyclic, bicyclic and tricyclic but the sesquiterpenes can best be classified\textsuperscript{48} into the groups of related compounds according to their carbon skeleton as follows:

\textbf{Sesquiterpenoid lacking carbon rings:}

Farnesol and Nerolidol are the most important members of this small group.

\begin{center}
\begin{tabular}{c c}
\includegraphics[width=0.4\textwidth]{farnesol.png} & \includegraphics[width=0.4\textwidth]{nerolidol.png} \\
Farnesol (VIII) & Nerolidol (IX)
\end{tabular}
\end{center}

\textsuperscript{Contd.}

45 De Mayo; \textit{Mono and Sesquiterpenoids}, 1959, 2, 2.
46 Semmler; \textit{Ber}, 1903, 36, 1037.
48 Barton and Mayo; \textit{Quart. Rev.}, 1957, 11(3), 189-211.
Cadalene type sesquiterpenoids:

Ruzicka and Meyer\textsuperscript{49} showed that cadinene (x) on dehydrogenation with sulphur yields cadalene (xi). Other compounds included in this group are a number of monocyclic sesquiterpenoids which afford cadalene type skeleton on cyclisation, or, are structurally closely related to it, e.g. zingiberene (xii) and $\gamma$-curcumene (xiii).

\textbf{cadinene (X)} \hspace{1cm} \textbf{cadalene (XI)}

\textbf{zingiberene (XII)} \hspace{1cm} \textbf{$\gamma$-curcumene (VIII)}

\textsuperscript{49} Ruzicka and Meyer; \textit{Helv. Chem. Acta.}, 1921, 4, 505, 508.
**Eudalene type sesquiterpenoids:**

Terpenes belonging to this group are eudesmol (xiv) and others which yield eudalene (xv) on dehydrogenation.

![Eudesmol (XIV) and Eudalene (XV)]

**Azulenic type sesquiterpenoids:**

The azulenic type terpenoids can be subdivided into guaiazulene type (xvi) and vetibazulene type (xvii) and zierazulene type. Generally the azulenes themselves never occur in nature, although this is open to some question.

![Guaiazulene (XVI) and Vetivazulene (XVII)]
The detailed and exhaustive chemistry of terpenes is nicely dealt in standard works and, therefore, requires no elaboration here.

Biosynthetic pathways of terpenes:

The isoprene rule proposed by Wallach and extended by Robinson was further developed into "Biogenetic isoprene rule" by Ruzicka and his collaborators. This generalization, which is now universally accepted, states that naturally occurring terpenoids are derived either directly or by way of predictable sterospecific cyclizations, rearrangements, and dimerizations from acyclic C-10, C-15, C-20 and C-30 precursors - geraniol, farnesol, geranylgeraniol, and squalene,

50 Stahl, E.; Ber., 1954, 87, 202, 205, 1626.
respectively. Although isoprene is not found in plants it has been isolated on pyrolytic decomposition of some of monoterpenes and other higher terpenoids. This five carbon fragment postulated in the biosynthesis of terpenoids probably has its origin in a two carbon unit acetic acid and this view was backed by using acetic acid labelled both at the methyl and the carbonyl group. The C-5 unit was also postulated to arise from degradation of carbohydrates, proteins, amino acids and other classes of plant metabolites. These views have been well-summarised. Many C-10 compounds have been implicated as progenitors of monoterpenes, including citral, geraniol, limonene, ocimene and others.

Modern knowledge:

Modern knowledge of biochemical construction of C\textsubscript{5} units, its condensation and subsequent modification of the product has been gathered as a result of radioisotope studies of the synthesis of steriods in both cell-free system and tissues slices from liver, and in cell-free systems from Yeast as well as a few investigations using intact higher plants. A common pattern of synthesis for steriods and terpenes emerged from this work. Carbondioxide is the only radio active precursor that can be fed in the plants under physiological conditions but its use as a precursor has limited applications and compound further along the biogenetic pathways are normally fed. The most common method of introducing such presumed precursors is via the stems of cutshoots, to cut petioles\textsuperscript{61} or intact plants by means of a cotton wick,\textsuperscript{62} but other methods used are by injection into bulbs,\textsuperscript{63} by spraying on to leaves\textsuperscript{64} or by incorporating into roots followed by repotting of the plant.\textsuperscript{62}

Formation of geranyl pyrophosphate (GPP):

The biosynthesis of geranyl pyrophosphate from acetate

\textsuperscript{61} Cromwell, B.T. and Roberts, M.F.; \textit{Phytochemistry}, 1964, 3, 369.

\textsuperscript{62} Austin, D.J. and Meyers, M.B.; \textit{Ibid.}, 1965, 4, 245.


\textsuperscript{64} Johnson, D.F.; Heftmann, E. and Houghland, G.V.C.; \textit{Arch. Biochem. Biophys.}, 1964, 104, 102.
in liver and Yeast systems has been elucidated, and the scheme (I) summarizes the situation.65

(Where P and PP represent phosphate and pyrophosphate groups, respectively.)

Scheme I

\[
\begin{align*}
\text{HOOC} & \text{OH} & \text{HOOC} & \text{OP} & \text{HOOC} & \text{OP} \\
\text{\text{XVIII}} & \rightarrow & \text{\text{XIX}} & \rightarrow & \text{\text{XXI}} & \rightarrow \\
\text{\text{XXI}} & \rightarrow & \left[ \text{\text{XXI}} \right] & \rightarrow & \text{\text{XXII}} & \rightarrow \\
\text{\text{XXII}} & \rightarrow & \text{\text{XXIII}} & \rightarrow & \text{\text{(XXII) + (XXIII)}} & \rightarrow \\
\text{\text{(XXIV)}} & \rightarrow & \text{\text{(XXV)}} & \\
\end{align*}
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Acetoacetyl co-enzyme A, formed by self condensation of acetyl co-enzyme A in the presence of β-keto acyl thiolase, condenses with another molecule of acetyl co-enzyme A to form β-hydroxy-β-methyl glutaryl co-enzyme A (HMG-Co A) that is irreversibly converted by a specific reductase (possibly with the intermediate formation of a hemithioacetal adduct formed from HMG and the thiol group of a enzyme) into (R) - (+) - mevalonic acid (MVA, xviii). This product is sequentially phosphorylated to 5-phosphomevalonic acid (MVAP, xix) and 5-pyrophosphomevalonic acid (MVAPP, xx) and the latter is converted into isopentenyl pyrophosphate (IPP, xxii), probably with the intermediate formation of the triphosphoester (xxi). IPP and 3,3-dimethylallyl pyrophosphate (DMAPP, xxiii) are joint substrates for prenyl transferase that catalyzes the formation of GPP and possibly neryl pyrophosphate (NPP, xxv) in what can be formally regarded as a coupled SN^2 - E2 process. These products are the parents of the acyclic and alicyclic monoterpenes respectively. It is generally accepted that this biogenetic route is also followed in higher plants, although alternative pathways have been proposed that involve the formation of HMG from malonyl co-enzyme A^66 and from leucine.\(^7\) IPP and DMAPP may be regarded jointly as "active isoprene" the biochemical equivalent

of the isoprene unit. Chain extension of GPP or NPP by sequential addition of IPP can lead to the whole family of terpenoids. The enzymes involved in the formation of GPP from IPP and DMAPP have been investigated\(^\text{68}\) and stereochemical details most elegantly worked out.\(^\text{69}\)

**Acyclic compounds and cyclohexane derivatives:**

Possible biogenetic routes to monoterpenes may readily be drawn up. The proposals of Ruzicka and his co-workers\(^\text{43}\) for the pattern of monoterpenes biogenesis are outlined in scheme II.


The formation of acyclics such as myrcene, citronellol or cis-ocimene from GPP has many *in vitro* analogies, and mono-cyclization of the ion (a) formed from NPP to give α-terpineol or terpinen-4-ol, is also chemically reasonable, although the biochemical obtains are open to conjecture. For the latter process, either epoxides (which have been isolated from several essential oils), 70 or sulfonium compounds formed with a thiol group of an enzyme 71 may be involved as indicated below:

**Epoxide path:**

![Epoxide Path Diagram]

**Sulfonium compound path:**

![Sulfonium Compound Path Diagram]

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71 Birch, A.J. and Smith, H.; in ref. 65, p. 245.
According to a recent study\textsuperscript{72} the bicyclic skeletons of pinane and borane series could be directly formed from acyclic precursors by decomposition of certain unsaturated epoxides:

(i) \[ \text{EnzSH} \rightarrow \text{SEnz} \rightarrow \text{Enz} \]

(ii) \[ \text{Enz} \rightarrow \text{NA DPH} \rightarrow \text{Enz} \]

The generation of intermediate carbenes may be feasible at the enzyme surface where water and other potential scavengers may be locally excluded.

Other scheme for bicyclisation \textit{in vivo} have been proposed. Radical induced cyclisation (probably photo-chemically promoted) of cis-ocimene or myrcene to form $\alpha$-pinene or $\beta$-pinene have been suggested as alternatives to the formally ionic routes.\textsuperscript{43,73}


\textsuperscript{73} Burwell, R.L.; \textit{J. Amer. Chem. Soc.}, 1951, \textbf{73}, 4461.
The abundance of linalool and the decrease in its concentration relative to that of limonene during the maturation of citrus fruit was held\textsuperscript{74} to indicate that LPP (linaloyl-pyrophosphate) rather than NPP was the precursor of cyclic monoterpenes.

**Tracer studies:**

Detailed knowledge of the biosynthetic processes and how they come only with the advent of radioisotopes as a mechanistic tool. Reasonable analogs were quickly drawn between the well understood mechanisms of formation of steroids in animals or microorganisms and those of monoterpenes in plants the latter

\textsuperscript{74} Atgaway, J.A.; Pieringer, A.P. and Barabas, L.J.; *Phytochemistry*, 1967, 6, 25.
pathway is often considered to be an offshoot of the former, and although the direct evidence for this is actually very slender, this hypothesis is probably very close to the truth. The paucity of evidence arises from the oft-demonstrated finding that uptake of MVA into the monoterpenes of whole plants or intact plant tissues is generally extremely low. Incorporations are usually in the range 0.01 - 0.1% of the applied tracer and sometimes no significant incorporations can be demonstrated over periods of several days after feeding the labelled precursor. These low incorporations would undoubtedly lead to the conclusion that MVA was not a precursor of terpenoids. If there was any evidence whatsoever form an alternative path.

Few explanations that have been advanced for low incorporation of MAV are:

(i) MVA may not readily penetrate to the intracellular sites of terpenoid synthesis.

(ii) Such $^{14}C$ MVA as can thus penetrate may not be able to intervene in the biosynthetic pathways. MVA never occurs in vivo as the free acid or lactone in significant quantities but is probably enzyme bonded.

(iii) MVA that does enter the "conveyor belt" system may be shunted into physiologically important steroids and carotenoids rather than into the monoterpenes pool.

(iv) If MVA is indeed an obligatory intermediate, its intracellular concentration must be very low, and so its addition in large quantities, including the unphysiological $S$ isomer, during the feeding experiments, may well lead to inhibition by feedback mechanisms of enzymes involved in terpenoid synthesis.

(v) Lastly the unavoidable introduction of large quantities of MVA results in degradation of the additive to products that may also act as enzyme inhibitors.

In support of these explanations there is excellent evidence for utilisation of the MVA pathway in the biosynthesis of monoterpenes in petals. Within 1 hr. of feeding the flower head of a hybrid tea rose with $2\cdot^{14}C$ MVA, up to 11% of the applied tracer had been incorporated into geraniol, nerol and citronellol and their glucosides, the sugar moity was unlabelled.76,77

77 Francis, M.J.O. and Allcock, C.; Phytochemistry, 1969, 8, 1339.
Specific labelling:

More accurate information may be obtained by the use of precursors specifically labelled with tracer at particular position (followed by degradation of purified products of biosynthesis to locate the site of tracers), to determine the fundamental question of possible independence of routes to GPP and NPP, the parents of acyclic and higher terpenoids and of cyclic monoterpenes respectively. NPP could be formed either by direct coupling of IPP with DMAPP or by the isomerisation of the preformed GPP or LPP. Bantherpe et al.\textsuperscript{78} have ruled out the former pathway by using (4R) - [4-3H\textsubscript{1}] MVA (xxvi) and its (4S) isomer (xxvii).

![Diagram of MVA (xxvi) and (xxvii)]

Incorporation of each isomer in turn in admixture with 2 - \textsuperscript{14}C MVA (to act as a marker) into a hybrid tea rose and isolation of geraniol and nerol both free and bonded as \(\beta\)-glucosides showed that the 4S hydrogen was stereospecifically lost in all cases; thus when the 4R and 4S isomers of MVA were
fed together with $^2\text{H}^4\text{C}$ MVA such that the hydrogen
-3/carbon -14 ratios were 1.99 and 1.25, respectively, geraniol and nerol were recorded from their $\beta$-glucosides in which the ratios were 1.97 and 0.05 and 2.01 and 0.09 respectively. A similar pattern was obtained from $\alpha$-pinene produced by Pinus attenuata. Direct condensation of the C-5 compound to form NPP would have resulted in the loss of the 4R hydrogen.

The incorporation of $\left[2\text{H}^4\text{C}\right]$ acetate into citronellal and $\left[2\text{H}^4\text{C}\right]$ MVA into 1,8 cineole in branches of Eucalyptus citriodora and Eucalyptus globulus respectively has been investigated.

Irregular structure:

Under this heading two classes of compounds can be grouped as follows:

(i) Degraded monoterpenes that contain less than ten carbon atoms.

(ii) Compounds that apparently break in isoprene rule, in its simpler statements, in containing C-5 units that are not linked head to tail.

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79 In ref. 63, p. 124.
81 In ref. 63, p. 137.
The first class presents no biogenetic problem. An early example was cryptone (xxviii) which is almost certainly formed in vivo from β-phellandrene (xxix), with which it co-occurs. Others are the arthropod defensive substances (xxx), (xxxii), (xxxiii) the origin of which can be reasonably deduced, although no tracer studies have been carried out. Santene (xxxiii) is believed to be formed by the following pathway and all the presumed intermediates have been identified as co-occurring in sandalwood oils.

84 Berry, P.A.; Macbeth, A.K. and Swanson, T.B.; Ibid., 1937, 1448.
The oils of *Pinus jeffreyi* and *Pinus sabiniana* consist predominantly of n-heptane, but as \[ 2 - ^{14}C \] HMG was not incorporated into this compound, it was concluded\(^8^7\) to be of polyketide rather than of mevalonoid origin. Such conclusions are questionable in view of the negligible incorporations of MVA and biogenetically related compounds into many products that are of undoubted mevalonoid origin. In this context, it is interesting that leucine was incorporated in over 80% yield into amyl alcohol and its acetate in disks of banana fruit and in Yeast,\(^8^8,^8^9\) and this amino acid may be a precursor of certain unusual "terpenoids".

In the case of second class some of the irregularly linked C-10 compounds are very probably formed by well-established rearrangements of precursors biosynthesized, with conventional head-to-tail linking of the C-5 units, and thus come within the province of the operation of the biogenetic isoprene rule. Examples are fenchane derivatives such as fenchol (xxxiv) derived from the ion (xxxv) (Scheme III) and isocamphane derivatives such as camphene (xxxvi) derived from (xxxvii)

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87 Sandermann, W.; Schweers, W. and Beinhoff, O.; Chem. Ber., 1960, 93, 2266.


by a similar Wagner-Meerwein shift.  

Scheme III

A more unusual type of rearrangement gives carquejol (xxxvii) which occur in the oil of the same name\textsuperscript{91} (Scheme IV),

Scheme IV

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and is the only known naturally occurring 6-menthane derivative. Another speculative proposal is the derivation of (xxxix) from thujone (Scheme V).

Scheme V

(XXXIX)

One of the most discussed compounds of this class is artemisia kelone°² (xxxx). A novel route for its biosynthesis was implied by the discovery that $2 - ^{14}C$ MVA was not detectably incorporated into the compound formed by Santolina chamaecyparissus under conditions where the regularly constructed and co-occurring monoterpenes were significantly labelled.°³ These observations have been confirmed, but the same precursor was found to be normally incorporated into artemisia ketone produced by Artemisia annua such that the position of label allowed

92 Hanson, J.R.; Perfum. Essent. Oil Rec., 1967, 58, 787.
delineation of the route of synthesis.\textsuperscript{94} On degradation about 92% of the incorporated tracer was deduced to be at C\textsubscript{9} and C\textsubscript{10} and only about 8% was located at C\textsubscript{7} and C\textsubscript{8}, thus asymmetric labelling occurred, although not to such an extreme as in the monoterpenes.

A variety of mechanisms has been proposed, all unbacked by any experimental evidence, for the biogenesis of this compound two of these are:

(a) ring opening of a cyclopropane intermediate derived from linalool\textsuperscript{95} (xxxii).

(b) fission of a carane skeleton\textsuperscript{96} (xxxiii).

\[ (\text{xxxxi}) \quad (\text{xxxxii}) \]

**Antimicrobial properties of Essential Oils:**

Essential oils have been used since antiquity as preservatives and are also used today as germicides and


fungicides. Ancient Egyptians\textsuperscript{97} used essential oils for embalming. The inhibitory and detrimental effects of the essential oils make them bactericidal and fungicidal agents. On account of their bacterial action a number of volatile oils have been employed in the past for the treatment of arogenital infections. A variety of essential oils have been employed therapeutically for their bactericidal actions. A formula\textsuperscript{98} containing essential oils of anise, cajeput and Juniper was devised for the prevention and treatment of cholera. In indigenous medicine essential oils have been used as curatives for intestinal disorders caused by microbes as they tend to minimise putrefaction.

The effect of the essential oils in destroying or in activating microorganisms has received great attention in recent years. Essential oil of ocimum-bacilicum is reported to be active against salmonella typhi,\textsuperscript{99} similarly oil of Piper betle has been successfully used in the treatment of catarrhal disorder and as an antiseptic.\textsuperscript{100} Lard and Husa\textsuperscript{101}

\begin{itemize}
\item \textsuperscript{97} Risler, J.; \textit{Compte rendu de l}, Academic des Sciences, Paris, 1936, \textit{203}, 517.
\item \textsuperscript{99} Khorana, M.L. and Vangikar, M.B.; \textit{Indian J. Pharm.}, 1950, \textit{12}, 134.
\item \textsuperscript{100} Gupta, K.C. and Vishwanathan, R.; \textit{Antibiotics and Chemotherapy}, India, 1956, \textit{6}, 194.
\item \textsuperscript{101} Lard, C.F. and Husa, J.W.; \textit{J. Amer. Pharm. Assoc.}, 1954, \textit{43}, 438.
\end{itemize}
have observed that some essential oils in very low concentrations can inhibit mould growth. Bose et al., Klieve and Hathmacher, Maruzzella et al., Jonver et al., and W. Schweisheimer screened out the antimicrobial effects of a number of essential oils.

Papers on the antibacterial activity of the essential oils have been reported from time to time by various workers.

The reviewed literature shows the successful application of the essential oils which have potent antimicrobial activity in combating certain skin disorders resulting from various bacterial and fungal infections and such studies mark a new approach to cure human sufferings.

FATS AND OILS

General:

Fats and oils are widely distributed both in the animal and vegetable kingdoms. In plants they occur predominantly in seeds and fruits and are also found in varying quantities in roots, branches, stems and leaves. Generally, it is now accepted that fatty oils are formed from carbohydrates. It is noteworthy that the fatty acids found in fats contain an even number of carbon atoms. There is considerable evidence that the fatty acids are produced first and then at a later stage, these are combined with glycerol through the agency of lipase to form the triglycerides. It is believed that glycerol is also formed from carbohydrates. Some relationship generally appears between the composition of fats and the ecological conditions. The fats with a few exceptions from tropical plants are characterised by containing notable percentages of saturated acids, whereas those from plants growing under colder climatic conditions contain large proportions of unsaturated acids. In seeds spores and tubers these function as food reserve to be drawn up during germination and the early life of the plant. In the early stages of germination the fat contents undergo little diminution but following this period it rapidly diminishes. In animals the fat deposit mostly occurs in subcutaneous tissues, liver and intermuscular
contactive tissues. It appears to serve the following functions in animals:

(i) They may be oxidized immediately to carbon dioxide and water. The energy thus liberated is used to produce muscular work and to maintain the body temperature.

(ii) Combine with proteins in formation of cellular protoplasm, cell membranes, etc.

(iii) Some fat deposits may provide padding to protect the internal organs.

(iv) Lastly as a reserve food for future use in case of crisis.

Oils are chiefly obtained from plants or animals by expression or by means of solvent extraction. Plant fixed-oils are chemically triglycerides, i.e. esters of glycerol with saturated or unsaturated fatty acids containing minor portions of sterols (free or as ester), vitamins, pigments, hydrocarbons and other substances. If the fatty acid radical of a fat molecule are alike, the ester is known as simple glyceride and if acid radicals differ in a fat molecule the ester is called a mixed glyceride.
\[
\begin{align*}
\text{CH}_2 - \text{OH} & \quad \text{HOO}\text{CR}_1 \quad \text{CH}_2\text{OO}\text{CR}_1 \\
\text{CH} - \text{OH} + & \quad \text{HOO}\text{CR}_2 \quad \text{CHO}\text{OCR}_2 + 3 \text{H}_2 \text{O} \\
\text{CH}_2 - \text{OH} & \quad \text{HOOCR}_3 \quad \text{CH}_2\text{OO}\text{CR}_3
\end{align*}
\]

Simple and mixed glyceride

**Saponifiable portion:**

When fats are heated with alkalies such as caustic soda they undergo saponification and salts of fatty acids (soap) are formed and glycerine liberated. Fatty acids are mostly aliphatic straight chain acids (except in few cases where ring compounds are found to occur as part of the fatty acid molecule as in epoxy cyclic acids, etc.) containing an even number of carbon atoms ranging from 8 to 24. These fatty acids may be saturated and/or unsaturated.

Depending upon the nature and percentage of these acids, the fixed oils can conveniently divided into three types -

Drying, with iodine value above 130,
Semidrying, with iodine value between 100 and 130, and
Non-drying, with iodine value below 100.

**Unsaponifiable portion:**

It is an important part of the fixed oil which includes all those substances that are not saponified by alkali and are
soluble both in ether and petroleum ether. The unsaponifiable matter chiefly consists of sterols and small quantities of other alcohols like tocopherols and hydrocarbons such as squalene\textsuperscript{108-110} \( \text{C}_{30}\text{H}_{50} \), gadusene \( \text{C}_{18}\text{H}_{32} \),\textsuperscript{111} tricontane \( \text{C}_{30}\text{H}_{62} \) and pentatriconate \( \text{C}_{15}\text{H}_{72} \). These may be separated from sterols and other alcohols by well-known methods. The unsaponifiable matter normally constitutes less than 2% of fixed oils.

**Sterols:**

The sterols\textsuperscript{112} are crystalline polycyclic hydroaromatic, secondary alcohols containing an aliphatic side chain. They are widely distributed in nature occurring both as free and as esters of higher aliphatic acids and may be classified on the basis of occurrence as zoo sterols (in animals), phytosterols (in plants) and mycosterols (in kryptogams particularly in fungi). In plants sterols seems have no

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\textsuperscript{109} Durramond, J.C. and Thorbjarnarson, T.; \textit{Analyst.}, 1935, \textit{60}, 23.


\textsuperscript{111} Nakamiya, J.; \textit{Chem. Abs.}, 1936, \textit{30}, 315.

\textsuperscript{112} Friedman; \textit{"Sterols and Related Compounds,"} Chem. Publishing Company, Brooklighl.
known function, although these have profound importance in
the animal metabolism as hormones, co-enzymes, bile acids
and provitamin D.

**Biosynthesis**

It was considered for many years that the synthesis of
fats and fixed oils by living organisms was effected simply
by a reversal of the reaction responsible for their
degradation. Specifically, these include the hydrolysis of
glycerol fatty acid esters by the enzyme lipase and the
subsequent removal of 2-carbon units as acetyl-CoA from the
fatty acid chain by β-oxidation. Biosynthetic studies
indicate that the formation of these lipids utilizes quite
different chemical pathways. ¹¹⁴

**Biosynthesis of the fatty acid metabolites:**

It has been shown that these are produced by a series
of reactions involving two enzyme complexes plus ATP, TPNH,
Mn++ and carbon dioxide.

¹¹³ Claus, E.P. and Tyler, V.E.; "Pharmacognosy," Vth Edn.,
Acetate first reacts with CoA and the acetyl-CoA thus formed is converted by reaction with carbon dioxide to malonyl-CoA. This in turn reacts with an additional molecule of acetyl-CoA to form a 5-carbon intermediate which undergoes reduction and elimination of carbon dioxide to produce butyryl-CoA. Malonyl-CoA again reacts with this compound to form a 7-carbon intermediate which is reduced to caproyl-CoA. Repetition of the reaction results in a fatty acid containing an even number of carbon atoms in its chain. Thus malonyl-CoA, a 3-carbon compound, is actually the source of the 2-carbon biosynthetic units of the fatty acids.

Pathways of biosynthesis of unsaturated, branched-chain, odd-numbered and otherwise modified fatty acids have not been established in detail. There is evidence that the first step in the production of a mono-unsaturated acid is the formation of the acyl-CoA derivative of its saturated analogue. This is followed by enzymatic desaturation.\textsuperscript{115}

Additional reactions may then ensue. For example, enzymes present in certain fractions of unripe castor seeds are capable of hydroxylating oleic acid (oleyl-CoA) to produce ricinoleic acid.\textsuperscript{116} Thus the probable sequence for the formation of this latter compound may be summarized as follows:

\textsuperscript{115} Korn, E.D.; J. Biol. Chem., 1964, 239, 396-400.
Acetyl-CoA — Stearyl-CoA — Oleyl-CoA — Ricinoleyl-CoA

Stearic acid (Octadecenoic) Oleic acid (9-Octadecenoic) Ricinoleic acid (12-hydroxy-9-Octadecenoic)

The glycerol moiety utilized in lipid biosynthesis derives mainly from the L-isomer of \( \alpha \)-glycerophosphate (L-\( \alpha \)-GP). Reactions involved in the formation of a typical triglyceride are summarized in Fig. I.

L-\( \alpha \)-GP, which may derive either from free glycerol or from the glycolysis intermediate dihydroxyacetone phosphate, reacts successively with two molecules of fatty acyl-CoA to form first L-\( \alpha \)-lysophosphatidic, and then L-\( \alpha \)-phosphatidic acid. The latter compound is converted to an \( \alpha \), \( \beta \)-diglyceride which can either cycle back to the phosphatidic acids or react with another fatty acyl-CoA to form a triglyceride.

**Importance:**

The most important role of fats, from the quantitative point of view is that of fuel. The energy supplied by fats (9 calories per gram) is more than double of proteins and carbohydrates (4 calories each per gram). Hydrogenation of the liquid oils produces semisolid fats which find extensive use as cooking fats and shortening. Non-edible oils and fats are used for the manufacture of fatty acids, detergents and other fine chemicals. These are also technologically in paints,
Dihydroxyacetone Phosphate (from glycolysis) → Glycerol

\[
\begin{align*}
\text{DHA} & \quad \text{Glycerol} \\
\text{HO-CH-CH_2-O} & \quad \text{HO-CH-CH_2-O} \\
\text{CH_2-O} & \quad \text{CH_2-O} \\
\end{align*}
\]

\[+ \text{CoA-S-C-R} \rightarrow \text{CoA-S-C-R} \rightarrow \text{R-C-O-CH} \]

\[+ \text{CoA} \rightarrow \text{CoA} \rightarrow \text{R-C-O-CH} \]

\[\text{CH_2-O} \quad \text{CH_2-O} \]

L-\(\alpha\)-Glycerophosphate (L-\(\alpha\)-GP) → Fatty acyl-CoA

\[\text{L-\(\alpha\)-GP} \quad \text{Fatty acyl-CoA} \]

\[\text{L-\(\alpha\)-Lysophosphatic acid} \quad \text{Fatty acyl-CoA} \quad \text{L-\(\alpha\)-Phosphatic acid} \]

\[\text{R-C-O-CH} \quad \text{R-C-O-CH} \]

\[\text{CH_2-O} \quad \text{CH_2-O} \]

\[\text{D-\(\alpha\)-\(\beta\)-Diglyceride} \quad \text{Fatty acyl-CoA} \quad \text{Triglyceride} \]

Enzymes and Cofactors Required
1. L-\(\alpha\)-GP dehydrogenase + DPNH + H^+  4. Phosphatic acid phosphatase
2. Glycerokinase + ATP  5. Diglyceride kinase + ATP
3. Acetyl-CoA + ATP

**Fig. 1. Biosynthesis of a triglyceride**
Fig. II BIOSYNTHESES OF FATTY ACIDS
varnishes, polishes, cosmetics, lubricants, etc. and for the manufacture of soaps, candles, explosives, plastics and host of other useful products.

Fixed oils and fats also find use in pharmaceutics. Many drugs contain fixed oils and fats as their principal constituents. Cyclic fatty acids, e.g. chaulmugric acid are used in the treatment of leprosy.

Besides essential oils and fixed oils, the plant products contain various other class of compounds, of which only a few are briefly reviewed.

**CARBOHYDRATE**

Carbohydrates, proteins and fats form the three great classes of food-stuff. Of these carbohydrates form important structural materials for plants and occur in all living cells. Their chief function is to supply energy. The principal sources of useful dietary carbohydrates are cereals, potatoes, sugar cane and sugar beet. Carbohydrates might be employed as the starting material for the biogenesis of other types of compounds in the body, such as fatty acids and certain amino acids.

Besides food-stuff as sugar carbohydrates are used in
many industries or segments of industries, like paper, textile fibres, plastics, drugs, vitamins, etc.

Biosynthesis of carbohydrates\footnote{117}:

Carbohydrates are products of photosynthesis, a biological process that converts electromagnetic energy into chemical energy. In the green plant, photosynthesis consists of two classes of reactions. One comprises the so-called light reactions which actually convert electromagnetic energy into chemical potential. The other class consists of enzymatic reactions which utilise the energy from the light reactions to fix carbon dioxide into sugar. These are referred to as the dark reactions. The results of both of these types of reactions are most simply summarized in the following equation:

\[
2\text{H}_2\text{O} + \text{CO}_2 + \text{light} \xrightarrow{\text{chlorophyll}} (\text{CH}_2\text{O}) + \text{H}_2\text{O} + \text{O}_2
\]

Although this equation summarizes the overall relationships of the reactants and products, it gives no clue as to the nature of the chemical intermediates involved in the process. The elucidation of the reactions by which carbon

\footnote{117 Claus, E.P. and Tyler, V.E.; "Pharmacognosy," 5th Edn., 1965, 51, Lea & Febiger, Philadelphia.}
dioxide is accepted into an organic compound and ultimately into sugars with regeneration of the carbon dioxide acceptor was a major achievement in biosynthetic research. The present concept of this pathway of carbon in photosynthesis, as worked out primarily by Calvin and co-workers is presented in Fig III 118

The essentials of this rather complex cyclic reduction system are as follows. Carbon dioxide from the atmosphere reacts with a five-carbon sugar ribulose diphosphate (RuDP) forming an unstable six-carbon intermediate which breaks down into two molecules of 3-phosphoglyceric acid (PGA). This latter compound is the first stable intermediate in green plant photosynthesis. The carboxyl group of PGA is then reduced to form the corresponding aldehyde, a three-carbon sugar (3P). Five of these three-carbon sugars undergo a series of condensations, dismutations, and rearrangements to produce three five-carbon sugars (ribose 5-phosphate, xylulose 5-phosphate, and ribulose 5-phosphate). These are in turn converted to RuDP which is the carbon dioxide acceptor. Since each of the three molecules of RuDP thus formed can add a molecule of carbon dioxide, there is a net conversion of three molecules of carbon dioxide into one three-carbon organic compound per turn of the cycle.

Energy must be introduced into the carbon cycle in two different places. It is first utilized for the reduction of PGA to Tr.P, a reaction consuming one molecule of adenosine triphosphate (ATP) and one molecule of reduced pyridine nucleotide (PNH). This may be either triphosphopyridine nucleotide (TPNH) or diphosphopyridine nucleotide (DPNH), depending upon the plant species. Since two PGA molecules are reduced for each carbondioxide molecule fixed, the energy requirements for this portion of cycle will be two molecules of ATP and two of PNH. Another molecule of ATP is subsequently utilized in the conversion of ribulose 5-phosphate to RuDP.

The total energy requirement for the fixation of one molecule of carbondioxide into sugar is thus three molecules of ATP and two molecules of PNH. Stated in another way, the synthesis of one mole of hexose phosphate requires 18 moles of ATP and 12 moles of PNH.

The ATP and PNH molecules consumed during carbondioxide fixation are provided by the light and electron transport reactions of photosynthesis. Absorption of light by a photosynthetic unit comprised of 300-500 chlorophyll molecules yields spatially separated oxidized and reduced sites as a result of electron transfer. Subsequently, the oxidized moiety brings about the oxidation of water to produce molecular oxygen; the reduced moiety reduces pyridine nucleotide (PNH) to PNH. In other words, the photosynthetic unit and its associated
electron transport pathways consist of an electron pump mechanism which utilizes light energy to remove electrons from water to produce oxygen. The electrons thus obtained are in turn raised to a sufficiently negative redox potential \( E^1_0 = -0.3V \) to reduce \( PN^+ \) and subsequently carbon dioxide.

The other cofactor necessary for carbon dioxide fixation is ATP. Its synthesis from adenosine diphosphate (ADP) and inorganic phosphate (Pi) utilizes energy available during the electron flow from water to \( PN^+ \). Fig IV illustrates how ATP formation is coupled to the oxidation of water and the reduction of \( PN^+ \) in green plant photosynthesis.

Production of Sucrose:

Sucrose is of considerable metabolic importance in higher plants, since studies have shown that it is not only the first free sugar formed in photosynthesis but also the main transport material. Newly formed sucrose is, therefore, probably the usual precursor for polysaccharide synthesis. Although an alternative pathway consisting of a reaction between glucose 1-phosphate and fructose is responsible for sucrose production in certain microorganisms, the biosynthesis of this important metabolite in higher plants apparently occurs as shown in Fig V.

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Fig. III The path of carbon dioxide fixation in photosynthesis (Modified after R.B. Park)

Fig. IV Generalized concept of light reactions & electron transport pathways of green plant photosynthesis (Modified after R.B. Park)

Fig. V Pathways of sucrose biosynthesis.
Fructose 6-phosphate, derived from the photosynthetic cycle is converted to glucose 1-phosphate which in turn reacts with UTP to form UDP-glucose. This either reacts with fructose 6-phosphate to form first sucrose phosphate and ultimately sucrose or with fructose to sucrose directly. Once formed, the free sucrose may be either remain in situ or be translocated via the sieve tubes to various parts of the plants. A number of reactions, e.g., hydrolysis of invertase or reversal of the synthetic sequence, will convert it to monosaccharides from which other oligosaccharides or polysaccharides may be derived.

**PROTEINS**

Proteins are a class of complex organic compounds which yield amino acids upon hydrolysis. About 19% of the animal body consists of proteins thus skin, muscles, tissues, hair finger nails and blood, all are mostly of proteinous composition. Leather and natural fabrics such as silk and wool are built of proteins. In plants, proteins chiefly occur in seeds, the legumes, cereals and nuts being the richest sources.

Proteins not only form the structural framework of the body also its working machinery as well. These are unquestionably the most important of all known substances in the organic kingdom. The enzymes are known to be proteinic in nature.
Several proteins are used for industrial purposes, Gelatin in photographic films and in making animal glues, casein, in adhesives such as glues, in paints and as basis of certain plastics, soyabean protein in plastics for automobile fittings.

**Biosynthesis of Proteins:**

Proteins are synthesized from free amino acids and not by the condensation of preformed peptides or keto acids. The free amino acids are believed to be activated by a reaction with ATP to yield enzyme-bound amino-acyladenylates. In this complex the carboxyl group of the amino acid is linked to the 5'-phosphate of AMP as a mixed anhydride. Each individual amino acids has its own activation enzyme. This type of reaction has been shown to occur in many of the higher plants including spinach, rye, asparagus and tobacco. The next major step forward was the finding that amino acids from the AMP-complexes are transferred to soluble ribonucleic acids (sRNA) yielding amino acyl-sRNA complexes. No separate enzyme is required for the transfer of the amino acid from the AMP complexes to the sRNA. The reaction sequence is given

below. There seems to be at least one sRNA for each amino acid and these substances

\[
\text{ATP + amino acid + enzyme} \quad \text{enzyme - (AMP - amino acid) + PP}
\]

\[
\text{enzyme - (AMP - amino acid) + sRNA} \quad \text{sRNA - amino acid + enzyme}
\]

all have molecular weights of approximately 30,000. Partial separations have been achieved by several procedures but counter current distribution appears to be particularly suitable. In addition to the sRNA at least two other nucleic acid fractions are involved in protein biosynthesis, messenger RNA (mRNA) and ribosomes. mRNA is believed to carry the information necessary to synthesize a given protein. Because the mRNA is only a small portion of the total RNA present in the cell it is difficult to get precise information concerning its composition and properties. However, evidence does exist that it acts as the template for protein synthesis, that it is rapidly synthesized and metabolically unstable and it may mimic the N-base composition of DNA. The hypothesis therefore is that mRNA acts as an information carrier from DNA and plays the role of a template in protein synthesis.

The ribosomes are associated with the particulate fractions of the cell and have been obtained from microsomes, nuclei and mitochondria. They are isolated by ultracentrifugation and their size depends on the magnesium ion concentration of the medium. The major constituents (40 to 60%) of the
ribosomes are proteins that resemble histories and RNA. Experimental evidence indicates that although ribosomes are required for protein synthesis they do not determine the nature of the protein produced. Presumably the ribosomes represent a non-specific part of the total machinery necessary for protein production and only after they have interacted with a specific mRNA will a given protein be synthesized.

Nirenberg and Matthai \(^{123}\) suggest that DNAse treated cell-free preparations will synthesize polypeptides in response to synthetic polynucleotides. Thus the addition of polyuridylic acid led to the formation of polyphenylalanine.

## TANNIN

Tannins are complex organic compounds with molecular weight of the order of 2,000 or more. They are built up from carbon, hydrogen and oxygen. The vegetable tannins are produced during the metabolism of plants and trees. Tannins are usually localized in specific plant parts such as leaves, fruit, barks or stems. Although often found in immature fruits. Since tannins are antiseptic in action, they prevent damage by insects and fungi.

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The largest exporters of tannins are the India, Argentina and South Africa. These countries supply myrobalans, quercracho and wattle respectively. Besides their use in leather tanning and in medicine, tannins find their way into a variety of industries such as ink manufacture, boiler-water treatment, plastics mineral ore floatation, oil-well drilling, ion exchange resins and oxidation-inhibitors.

**Biosynthesis of Tannins:**

Biochemical pathways leading to various individual components of the tannins are known. For example, gallic acid, a typical hydrolysis product of the hydrolyzable tannins, is thought to derive from shikimic acid by two different pathways. In certain microorganisms, 5-dehydroshikimic acid apparently undergoes dehydrogenation to yield gallic acid (Fig. VII).

However, in *Rhus typhina* the biosynthesis takes place from phenyl propanoid precursors, probably as shown in Fig. VII.124

Biosynthesis of catechin, a flavan-3-ol derivative of the type long considered to be the phenolic precursors of condensed tannins, probably results from a combination of the acetate and the shikimic acid (phenyl propanoid) pathways. The biosynthetic route has been found to be characteristic for

Fig. VI Biosynthesis of gallic acid in microorganisms.

Shikimic acid  \[ \xrightarrow{\text{5-Dehydroshikimic acid}} \] Gallic acid

Phenylelamine  \[ \xrightarrow{\text{Cinnamic acid}} \] p-Coumaric acid

Caffeic acid  \[ \xrightarrow{\text{3,4,5-Trihydroxycinnamic acid}} \] Gallic acid

Fig. VII Biosynthesis of gallic acid in Rhus typhina.

3 Acetate  \[ + \] Cinnamic acid (equivalent)  \[ \xrightarrow{\text{Catechin}} \]

Fig. VIII Biosynthesis of Catechin.
all flavonoids of this type which have been investigated. Such simple polyphenol and flavonoid units, together with other closely related compounds and sugars, apparently undergo various condensation and polymerisation reactions to produce the complex tannin molecules.

Although several hypothesis have been advanced to account for their formation, the biosynthesis of a complete tannin has not yet been achieved experimentally.

Available evidence indicates that tannins are probably not translocated in the plant but are formed and accumulate in situ from translocated precursors by local enzyme systems.

MODERN METHODS OF ANALYSIS

Up to past few decades, only conventional chemical methods were used for separation, purification and identification of plant isolates; these methods mostly were fractional distillation under atmospheric and reduced pressure followed by fractional crystallization and preparation of suitable derivatives. For all these comparatively larger quantities of isolate was the prerequisite and when this was separated in the individual constituents the inherent disadvantages being the risk of polymerisation followed by liable change in the structure of the compound(s) originally present therein.
Enormous development in the field of phytochemistry has become possible with the advent of more sophisticated physico-chemical and physical methods and the difficulties previously encountered have been eliminated to a considerable extent. Chromatography has now replaced fractional distillation and the purity of the isolated sample can be determined up to a confident stage by T.L.C. or G.L.C. For characterisation of a compound various physical methods are in vogue these include U.V., I.R., N.M.R., Raman and mass spectra. These techniques are being largely used by Indian workers. Recently Sukh Dev and others have applied I.R. and N.M.R. for identification of Diterpenoid constituents of some indigenous Ayurvedic crude drugs. Some of these techniques which have been utilised by the present author for characterisation of the isolated compound(s) are briefly reviewed below:

CHROMATOGRAPHY:

Chromatography may be defined as the selective adsorption and separation of a mixture of chemical substances on a column or film of adsorbent through which a suitable solvent has been passed. No chemical reaction takes place during the process, and once separated from the mixture, any starting material is recovered chemically unchanged. This technique is used widely

in many of analysis and has resulted in numerous important advances, particularly in biochemistry.

The Russian botanist, Tswett\(^{126}\) usually regarded as the father of chromatography, first described the process in 1906 when he filtered a petroleum ether solution of chlorophyll, extracted from leaves, through a column of calcium carbonate. The possibilities of Tswett's method were fully realised when Kuhn and Lederer\(^{127}\) separated the carotenes and xanthophylls on a preparative scale on columns of alumina and calcium carbonate. In 1938, Reichstein\(^{128}\) introduced the liquid chromatogram and thus extending the applicability of the method to colourless substances also. It has now become a prominent method of isolation, purification and characterisation of constituents that one comes across during the phytochemical investigations.

The chromatographic technique in general has taken the following forms:

(i) Column Chromatography
(ii) Paper Chromatography
(iii) Ion Exchange Chromatography
(iv) Thin Layer Chromatography
(v) Gas-liquid Chromatography

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It would be worthwhile to briefly summarise these, as many of have been used in the present investigations.

**Column Chromatography:**

The method consists in washing the column of developed chromatogram successively with a series of solvents (non-polar to polar). The adsorbents commonly used are alumina, aluminium silicate, magnesia, magnesium silicate, calcium hydroxide, calcium carbonate, silica gel, Fuller's earth, charcoal, powdered sucrose, etc.

Hesse *et al.* \(^{129}\) have established the following order of activity of adsorbents: activated charcoal \(\succ\) silica gel \(\succ\) Franconite \(\succ\) Floridin \(\succ\) acid alumina \(\succ\) basic alumina \(\succ\) \(\text{Cr}_2\text{O}_3\) \(\succ\) Zns \(\succ\) sugar charcoal \(\succ\) \(\text{Al}_2\text{O}_3\) (Merck) \(\succ\) \(\text{CaF}_2\) \(\succ\) CaO.

The elution is carried out by the solvents which are listed below according to their eluting power in increasing order, \(^{130}\) light petroleum ether, cyclohexane, carbon tetrachloride, Trichloroethylene, Toluene, Benzene, Dichloromethane, chloroform, ether, Ethyl acetate, acetone, n-propanol, ethanol, methanol.

Chromatography on alumina column has been employed by

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Rothe to separate oxygenated compounds and hydrocarbons from essential oils. Similarly the analysis of essential oils by column chromatography has been described by Kirchner and Miller, Reitsema, Nigam and Purohit, Dayal and Purohit, and Handa et al. Invert dry column chromatography is yet another addition to the technique of chromatographic separation. Sukh Dev et al. used this technique for the separation of constituents of unsaponifiable matter.

**Paper Chromatography:**

Paper chromatography is similar in principle to column chromatography except that the mixture to be resolved is chromatographed on a sheet of filter paper which acts as the adsorbent. The chemical mixture is applied in a small drop of solution to a filter paper about lin from the end and is allowed to stand in the developing solvent. When the solvent

has run its prescribed length, the paper is removed dried, and the drug or chemical is rendered visible or eluted by a suitable method. The four types of paper chromatography employed are ascending, descending, ascending-descending and circular (radial).

Lower fatty acids C₁ to C₉ were separated by Brown and Hall,¹³⁸ and Kennedy and Barker¹³⁹ using alcohols containing ammonia. The behaviour of high molecular weight fatty acids C₁₀ to C₂₂ was studied by Kaufman and Budwig,¹⁴⁰ who found filter paper a suitable medium for the radiometric determination of fatty acids. Buchman used paper chromatography for the separation of saturated and unsaturated acids. Ashley and Westphal¹⁴¹ have described a micromethod for the separation of 10 to 50 µg. quantities of C₁₂ to C₂₄ acids on filter paper cotted paraffin oil or latex. Quantitative estimation by measurement of the spot areas was achieved by Reid and Lederer.¹⁴²

Various workers have extensively reviewed the paper chromatography of carbohydrates and related compounds notable

amongst them are Hough,\textsuperscript{143} Isherwood,\textsuperscript{144} and Kowcabany.\textsuperscript{145}

Paper chromatography now-a-days mostly used for the separation of amino acids. Boulanger \textit{et al.}, and Thompson and Thompson have nicely reviewed the use of paper chromatography in the field of amino acids.

\textbf{Thin Layer Chromatography:}

Thin layer chromatography (T.L.C.) is a technique, whereby thin layers or films of silica gel are spread on glass plates, and chemical mixtures applied to these films are separated into their respective components by means of suitable solvents. The thin layer films can be developed rapidly and the mixtures resolved with a high degree of sharpness.

T.L.C. not only combines the advantages of paper and column chromatography but in certain cases it is superior to either of them. Now-a-days this technique is almost universally accepted as a modern analytical method in chemical analysis and research. Due to simplicity the technique lends itself to a rapid screening of essential oils for semi-quantitative composition studies. It is also a means of allowing the chemist to analyse for possible adulterants. A number of reviews have

\textsuperscript{143} Hough, L.; \textit{Meth. Biochem. Anal.}, 1954, 1, 205.
\textsuperscript{145} Kowcabany, G.N.; \textit{Adv. Carbohydrate Chem.}, 1954, 9, 304.
appeared in the literature on the use of T.L.C. in essential oil analysis. Of particular interest are the chapters by Stahl\textsuperscript{146} and Kirchner\textsuperscript{147} in their comprehensive texts covering the whole amount of T.L.C. Recently a review on the use of T.L.C. in essential oil analysis has been published by Lawrence.\textsuperscript{148}

Methyl esters of fatty acids have been separated by Malins and Mangold\textsuperscript{149} using the silicone oil impregnated silica gel plates. This method is also used for the separation of amino acid using silica gel G in both two and one dimensional ascending methods. The use of T.L.C. in alkaloids and sterols has been reviewed by Marini-Bettolo.\textsuperscript{150}

\textbf{Gas-liquid Chromatography:}

Gas-liquid chromatography (G.L.C.) is a relatively recent development in the general field of chromatographic techniques but in the last decade, it has become an increasingly popular method among organic chemists for analysis and for the isolation of individual components from complex mixtures. Compared with

\begin{enumerate}
\item Lawrence, B.M.; \textit{Perf. Ess. Oil Rec.}, 1968, 6, 421.
\end{enumerate}
other chromatographic techniques, such as thin layer chromatography and column chromatography, the apparatus required for gas-liquid chromatography is relatively expensive and complicated and it owes its wide popularity primarily to its versatility and efficiency. It has, for example, the advantage of rapid simultaneous quantitative as well as qualitative analysis, and with very little modification of the basic apparatus it is possible to efficiently separate the components of a mixture on a preparative scale. The stationary phase for gas-liquid chromatography is a liquid of low vapour pressure which is absorbed on a porous inert solid support enclosed in a narrow diameter tube.

The rapid expansion in the use of this technique as an analytical tool can be attributed largely to the work of Martin and James who, in 1952, described the separation of a mixture of fatty acids by G.L.C. In the same year they reported the separation of amines. Since the essential oils are mixtures of a wide variety of compounds and are volatile; G.L.C. together with other physico-chemical methods finds extensive application for their analysis. Seher,151 Aratani and Komae152 have reviewed the application of gas chromatography to terpenic compounds and perfume materials. The gas chromatographic examination of some volatile oils have been carried out by

various workers like Nigam et al., Lawrence, and Purohit et al. The application of gas-liquid chromatography has also been extended to aliphatic alcohols, tocopherols, triterpenoid alcohols, unsaponifiable matter, fatty acids and their methyl esters.

ULTRA VIOLET ABSORPTION SPECTROSCOPY:

The absorption spectroscopy using U.V. and visible light was the earliest physical method employed for the examination of molecular structure. Usually the data obtained from ultra violet spectra is used in conjunction with other spectral data for this purpose. With the help of Woodword's rule and the exact position and intensities of the absorption bands, it is possible to get useful information about the degree of substitution in a molecule, and the nature of the double bond.

In the azulene series, visible and to some extent the ultra violet spectra are of great assistance in locating the positions of nuclear alkyl groups. Hantzsch, and

156 Woodword; J. Amer. Chem. Soc., 1942, 64, 72.
158 Hantzsch; Ber., 1912, 45, 553, 903 and 1743.
Auwers first suggested the use of U.V. absorption spectra in the chemistry of terpenes. Ultra violet absorption spectra for the oils of star anise, cinnamon, clove, larender, crispment and peppermint have been recorded by Minutili. The main use of this technique in terpenoids is in the detection of conjugation. The ultra violet spectroscopy of fatty acids has been reviewed by Pitt and Morton, and Kass.

INFRARED SPECTROSCOPY:

The use of infrared spectroscopy has become a standard practice in the structural diagnosis particularly in the field of natural products, where the compounds isolated in the pure form are many a times available in small quantities. The study of infrared spectra leads to a great deal of information, e.g., the presence of various functional groups, hydrogen bonding (intramolecular and intermolecular), the identification of cis- and transisomers, conformational orientations, orientation in aromatic compounds, etc. I.R. spectrophotometry has been employed for qualitative as well as quantitative

161 Pitt and Morton; Prog. Chem. Fats, Other Lipids, 1957, 4, 227.
analysis, but the quantitative applications are of less significance than the qualitative ones.

Bellamy\(^\text{163}\) and Rao\(^\text{164}\) have given good reviews for the interpretation of I.R. absorption of organic compounds. Hansdorff\(^\text{165}\) has illustrated the analytical approach made possible by this technique by means of few examples. Levy\(^\text{166}\) in Canada have collected infrared data of several terpenic compounds. Gunthhard and Plattner\(^\text{167}\) studied the I.R. spectra of azulenes.

**MASS SPECTROSCOPY:**

Mass spectroscopy is carried out by the bombardment of the vapour of the compound at very low pressures with electrons possessing high energies. The molecule is broken down into various positive ions whose relative abundance provides a great deal of information about the structure of the compound. From a mass spectra a wealth of information can be obtained concerning the composition of mixtures of organic compounds.

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166 Levy; Perf. & Ess. Oil Rec., 1958, 42, 715.
and the elemental analysis of solid state sample. A detailed interpretation of the mass spectrum frequently makes it possible to place functional groups into certain areas of the molecule and to see how they are connected with each other. It is an essential adjunct to the use of stable isotopes in investigating reaction mechanisms and in tracer work. By this method molecular weight can be determined directly, even to fraction of a mass unit on more sophisticated spectrometers. Thus it has been successively utilised to distinguish readily between C\textsubscript{30} and C\textsubscript{31} substances in triterpinoids and long chain fatty acids. Using this method even the substances containing isotopic oxygen such as \textsuperscript{16}O and \textsuperscript{18}O or \textsuperscript{35}Cl and \textsuperscript{37}Cl can be separated and identified according to their different mass. Application of this technique to essential oils are also innumerable. The mass spectra of 24 sesquiterpene hydrocarbons and of 12 saturated derivatives of these hydrocarbons produced by catalytic hydrogenation has been studied by Moshonas and Lund\textsuperscript{168}. The details of the applications of mass spectrometry for the characterisation of plant products has been given in standard works.

NUCLEAR MAGNETIC RESONANCE:

N.M.R. is one of the recent and most important tool in the hands of organic chemists to study molecular structure.

\textsuperscript{168} Moshonas, M.G. and Lund, E.D.; Flavour Industry, 1970, 5, 375.
electron distribution, hindered rotation, molecular association, determination of keto-enolic equilibria, and solving many integrate problems of organic chemistry. In fact the discovery and development of N.M.R. spectroscopy is now recognised as one of the most important event in the last fifteen years for the advancement of organic chemistry. The methods of nuclear magnetic resonance was first developed independently by E.M. Purcell\textsuperscript{169} and Felix Bloch\textsuperscript{170} in 1946. A careful study of N.M.R. spectra reveals —

(i) the presence of particular functional group,
(ii) relative number of nuclei present in the group, and
(iii) the relative position of these groups from the multiplicities of the lines.

Use of N.M.R. spectroscopy in the field of natural products were made by several workers.\textsuperscript{171-173,125}

\textsuperscript{170} Bloch, F. et al.; Ibid., 1946, 69, 37.
\textsuperscript{173} Achari et al.; Ibid., 1974, L1(3), 419.
PROBLEM TAKEN AND WORK DONE

In India even now-a-days a large number of indigenous plants and their specified parts are used as household remedies by the people mostly residing in villages out of these some are described in Ayurvedic and Unani books and some are still unpublished. The systematic chemical and biochemical studies of some of the well-known Indian medicinal plants have revealed that their therapeutic potency is due to an active principle mainly present in their essential oils, fixed oils, or other class of compounds. Thus the physiological action of *Mentha piperita* is due to menthol and that of *Ocimum sanctum* to eugenol and so on. Cyclic fatty acids (e.g., chaulmogric acid) are used in the treatment of leprosy.

As such the utilization of plant materials and of the pure compounds isolated from plants has developed in recent years into a systematic search of the indigenous medicinal plants for new drugs.

A knowledge of relationship of biological activity to the chemical constituents of the plants is desirable not only for the discovery of new therapeutic agents but such information may also be of value in disclosing new sources of economic materials. Further a knowledge of chemical constituents of plants would also be useful to those interested in medicine chemotaxonomy and biosynthesis.
Being inspired by the enormous wealth of the plant products of the country and their utility as a source of national wealth and for the amelioration of human sufferings, and with the availability of improved physical methods of analysis in addition to the well-established chemical methods it was thought desirable to work up some essential oils, fixed oils, and mustard oils, proteins, carbohydrates and tannins which have remained hitherto either remained unworked or have been partially investigated at a time when the improved techniques and facilities were not available.

In addition to this antimicrobial efficacy of some essential oils and various extractives of different parts of the medicinal plants against certain pathogenic and non-pathogenic Gram positive and Gram negative microorganisms have also been studied by the present author. The present investigations have, therefore, been carried out as under:

I. **Studies on essential oil of**

   **Zanthoxylum ovalifolium** Wight:

   The essential oil from the seeds of hitherto unworked plant *Zanthoxylum ovalifolium* has been obtained in an yield of 0.8% as a yellowish coloured liquid with a characteristic smell. The oil had the following physico-chemical properties:
Specific gravity (28°C) ... 0.8688
Refractive index (28°C) ... 1.4682
Optical rotation ... + 6.42°
Acid value ... 0.92
Ester value ... 29.70
Ester value after saponification ... 148.0

Oil has been completely analysed and found to contain l-α-phellandrene (16.52%), d-limonene (14.89%), safrole (10.28%), methyl cinnamate (8.52%), citral (5.30%), d-cadinene (5.13%), β-myrcene (2.64%) and α-pinene (1.12%).

The presence of most of the above constituents have been confirmed by comparing their I.R. spectra.

II. Studies on essential oil of Alpinia khulanian M. Sheriff:

A yellowish coloured oil having camphoraceous odour has been obtained using water and steam distillation method from the rhizomes of Alpinia khulanian in an yield of 0.32%. The oil has been completely analysed and found to have the following physico-chemical constants:
<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity (30°C)</td>
<td>0.9170</td>
</tr>
<tr>
<td>Refractive index (30°C)</td>
<td>1.5122</td>
</tr>
<tr>
<td>Optical rotation</td>
<td>-40°</td>
</tr>
<tr>
<td>Acid value</td>
<td>0.76</td>
</tr>
<tr>
<td>Ester value</td>
<td>110.7</td>
</tr>
<tr>
<td>Ester value after acetylation</td>
<td>132.9</td>
</tr>
</tbody>
</table>

Presence of the following compounds in the oil could be established by fractionation under reduced pressure followed by chromatography and I.R. spectral studies: methylcinnamate (38.82%), cineole 1:8 (20.61%), α-pinene (1.29%), l-borneol (8.80%), Δ³-carene (7.38%), l-camphene (9.15%), methyl chavicol (8.0%), unidentified hydrocarbon (3.14%).

III. Chemical examination of seeds of
Amaranthus blitum var. Oleracea
(Proximate analysis, Fixed Oil, Protein & Carbohydrate)

Proximate analysis of the dried and powdered seeds gave the following values:

- Moisture - 6.12%, Ash content - 7.21%,
- Sulphated ash - 8.45%, Acid insoluble ash - 1.67%

The ash on analysis showed the presence of PO₄³⁻, Cl⁻, NO₃⁻, SO₄²⁻, Fe³⁺⁺, K⁺, Na⁺ and Mg²⁺.
The presence of various class of constituents has been tested in the extracts obtained by the successive solvent extraction. The fixed oil, protein, carbohydrate portions have been analysed in details.

The fixed oil is found to contain the glycerides of palmitic (13.10%), myristic (8.79%), stearic (3.81%), palmitoleic (5.80%), oleic (53.04%) and linoleic (15.46%) acids. Identity of β-amyrin, lupeol, β-sito sterol have been established in the unsaponifiable matter using inverted dry-column Chromatography, and by comparing their I.R. spectra with those of authentic.

Protein has been isolated from the defatted seeds with 10% sodium chloride solution, and amino acids obtained by the acid hydrolysis. The protein of the seeds have thus been found to be condensation-polymer of lysine, aspartic acid, glutamic acid, cystine, arginine, tyrosine, leucine, methionine and proline.

In the carbohydrate part of the seeds presence of fructose, glucose and sucrose has been established. The percentage of the total reducing sugar in the seeds has been estimated 0.7810% w/w as glucose.
IV. Chemical analysis of *Diospyros montana* Roxb.:

(Fixed oil and protein from seeds, Tannins from fruit)

Different parts (fruits, seeds and leaves) of *Diospyros montana* Roxb. were dried, powdered and extracted with solvents from non-polar to polar and the extractives were tested for important chemical constituents. The fixed oil, protein and tannins have been analysed.

**Proximate analysis** of fruits, seeds and leaves have also been carried out.

<table>
<thead>
<tr>
<th></th>
<th>Fruits</th>
<th>Seeds</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture %</td>
<td>38.82</td>
<td>11.76</td>
<td>22.42</td>
</tr>
<tr>
<td>Ash content %</td>
<td>5.90</td>
<td>5.41</td>
<td>14.01</td>
</tr>
<tr>
<td>Sulphated ash %</td>
<td>8.53</td>
<td>12.83</td>
<td>9.36</td>
</tr>
<tr>
<td>Acid insoluble ash %</td>
<td>1.96</td>
<td>1.8</td>
<td>1.05</td>
</tr>
</tbody>
</table>

**Fixed oil** has been obtained from the seeds in an yield of 1.5% and saponification of the oil gave mixed fatty acids 82.5% and unsaponifiable matter 1.04%. The oil has been found to be the glyceride of palmitic (27.91%), stearic (11.09%), oleic (37.08%) and linoleic (23.92%) acids.

The unsaponifiable portion has been found to contain lupeol, β-sito sterol and stigmasterol. Identity of these was also confirmed by their I.R. spectra.
In the protein hydrolysate cystine, arginine, glutamic acid, alanine, proline and leucine could be identified.

Tannins from fruits have been extracted using lead salt method and have been found to contain Gallic and Ellagic acid moieties belonging to the hydrolysable class of tannins.

V. Chemical examination of the fixed oil derived from seeds of *Citrullus vulgaris* var. *fistulosus* (Stocks) Duthie & Futter:

Proximate analysis of the dried and powdered seeds gave the following values:

- Moisture ... 8.2%
- Ash content ... 3.6%
- Alcohol soluble extractive ... 8.42%
- Water soluble extractive 3.17%

The ash on analysis has been found to contain acid insoluble ash 0.72% and sulphated ash 6.85%. Qualitative analysis showed the presence of chloride, sulphate, phosphate, potassium sodium, Iron and calcium. The fibre contents of the seeds have been determined to be 5.2%.

Qualitative chemical examination of various extractives (obtained by successive solvent extraction using different solvent) by established methods indicated that apart from
fixed oil, carbohydrate, amino acids, sterols, saponins and resins were also present.

Yellowish coloured fixed oil was obtained from the seeds in an yield of 18% and has been found to be the glyceride of palmitic 4.20%, stearic 12.20%, oleic 29.95% and linoleic 53.85% acids. Presence of the acids have also been confirmed by comparing I.R. spectra with those of authentic one.

The unsaponifiable portion has been found to contain $\beta$-amyrin and $\beta$-sito sterol. I.R. spectrum also confirmed the presence of these compounds.

VI. Studies on antimicrobial activity of essential oils:

Sixteen essential oils isolated from indigenous aromatic medicinal plants have been studies for their antibacterial and antifungal activity against certain Gram positive, Gram negative pathogenic and non-pathogenic microorganisms.

Bacteria included for the study were—

B. subtilis, P. aeruginosa, S. paratyphi,
E. coli, S. aureus, S. lutea, C. pyogenes,
P. vulgaris, S. enteritidis, P. multocida,
S. pullorum, S. albus.
Fungi included for the study were —

*C. albicans*, *A. niger*, *M. gypsinum*,

*A. fumigatus*, *P. regulosum*, and *C. tropicalis*.

The activity was determined by the filter paper disc diffusion method. The minimum concentration of the essential oils which retain the antimicrobial activity have also been ascertained. The dilutions of the essential oils used were 1:100, 1:250, 1:500 and 1:1000 in ethylene glycol.

It has been observed that most of the essential oils give maximum antimicrobial activity against most of the organisms in their pure form and degree of activity decreases in proportion to their dilution. On comparing the antibacterial and antifungal efficacy of the essential oils, it is concluded that though most of the oils responded satisfactorily for their antimicrobial properties, the oils of *Murraya koenigii*, *Alpinia khulanjan*, *Alpinia calamus*, *Nepeta hindostana* and *Amomum subulatum* in particular gave more promising results as such these can be placed as the potent antimicrobial agents. The most susceptible organisms were found to be those of *B. subtilis*, *C. pyogenes*, *P. multocida*, *S. lutea*, *C. alificans* and *A. fumigatus*. These antibacterial and antifungal studies of the essential oils mark a new approach to cure the human sufferings, specially the common skin diseases and infections which are caused by pathogenic bacteria and fungi.
VII. Studies on antibacterial activity of various extractives of different parts of some medicinal plants:

The in vitro antibacterial activity of various extractives of the different parts of plants like Polyalthia longifolia, Diospyros montana and Putranjiva roxburghii has been determined against Gram positive and Gram negative bacteria using filter paper disc diffusion method.

In general it has been observed that though various extractives of different part of plants have been found to possess potent microbial activity but petroleum ether and carbon tetrachloride extractives in particular are very effective against most of the organisms tested. Petroleum ether extractive of the leaves of P. longifolia has been found to have maximum inhibitory effect and the most susceptible organisms were B. subtilis and C. pyogenes.

VIII. Preliminary chemical examination of leaves and volatile oil of Putranjiva roxburghii:

Dried and powdered leaves of Putranjiva roxburghii were extracted with non-polar to polar solvents. The extractives obtained were tested for important chemical constituents.

By proximate analysis the leaves have been found to contain
moisture (48.72%), ash (8.62%), acid insoluble ash (3.94%),
sulphated ash (6.05%), total calcium (1.62%), water soluble
extractive (18.11%), alcohol soluble extractive (19.63%).

Volatile oil (0.04%) obtained from the leaves gave following
physical constants:

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity (30°C)</td>
<td>0.9374</td>
</tr>
<tr>
<td>Refractive index (30°C)</td>
<td>1.4879</td>
</tr>
<tr>
<td>Boiling point at atmospheric pressure</td>
<td>163°C</td>
</tr>
<tr>
<td>Solubility</td>
<td>Miscible in organic solvents</td>
</tr>
</tbody>
</table>

Supposedly new aromatic isothiocyanate was isolated from the
oil, having the following physico-chemical constants:

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity (30°C)</td>
<td>0.9369</td>
</tr>
<tr>
<td>Refractive index (30°C)</td>
<td>1.4885</td>
</tr>
<tr>
<td>Boiling point at atmospheric pressure</td>
<td>160°C</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>153.00</td>
</tr>
<tr>
<td>Percentage of nitrogen</td>
<td>3.22</td>
</tr>
<tr>
<td>Percentage of sulphur</td>
<td>13.25</td>
</tr>
</tbody>
</table>

I.R. and U.V. spectral analysis of this isothiocyanate has
also been carried out.

The amino acids - glycine, proline, valine and leucine -
were identified in the protein hydrolysate by descending
paper chromatography.

Presence of glucose and lactose was established in the
carbohydrate part of the leaves using ascending paper
chromatography. Total reducing sugar was estimated to be
1.12% w/w as glucose.