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HYDROCARBONS FROM PLANTS:  
BIOSYNTHESIS AND UTILIZATION

S.E. Taylor and M. Calvin

September 1986

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# **HYDROCARBONS FROM PLANTS: BIOSYNTHESIS AND UTILIZATION**

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## **ABSTRACT**

Plant hydrocarbons are a large and diverse group of compounds, with hundreds of different structures known, all generated from one of two biosynthetic processes. This paper will discuss the current understanding of these two pathways, isoprenoid biosynthesis and alkane biosynthesis, and also illustrate possible commercial uses for these compounds.

## **Introduction**

Plant hydrocarbons are a well studied and well reviewed topic, with interest dating back to historical times. Despite all of this attention, the biosynthesis of these compounds and their biological function is still not well characterized. To discuss the entire topic of plant hydrocarbons in this paper is impossible, as entire books have been published on the subject<sup>1,2,3</sup>. Instead we will limit our treatment of this topic to a general overview of the current understanding of hydrocarbon biosynthesis in higher plants, followed by specific examples of the various hydrocarbons to illustrate the wide range of current and potential uses for these phytochemicals. The goal of this paper is to generate more interest in this field, specifically concerning: A) the mechanism and control of hydrocarbon biosynthesis; B) the identification of biologically active or chemically useful compounds; and C) the mode of action of the biologically active molecules.

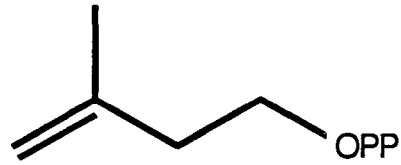
The exact definition of a "plant hydrocarbon" depends upon who is doing the defining. For purposes of this paper we will limit our coverage to compounds that are generated through one of two pathways: either isoprenoid biosynthesis or alkane biosynthesis via fatty acid synthesis.

## **GENERAL BIOSYNTHETIC PATHWAYS**

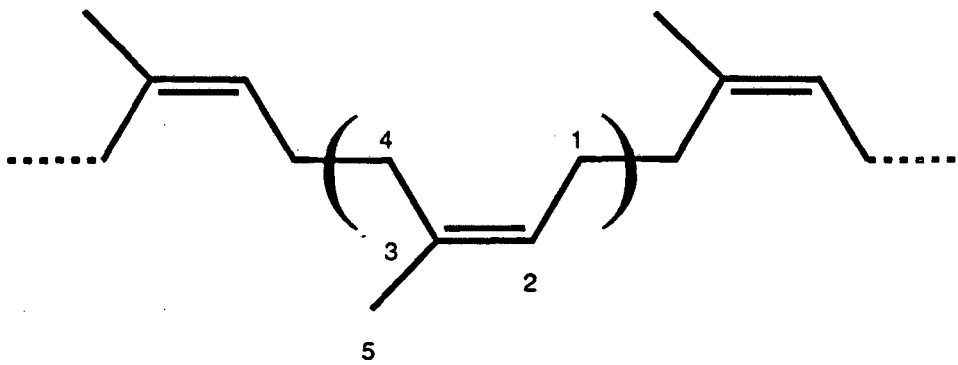
### **Isoprenoid Biosynthesis**

One major class of plant hydrocarbons, the isoprenoids, is synthesized from isopentenyl pyrophosphate (IPP)(figure 1) . These branched, 5 carbon molecules (or "isoprene units") are combined to make elongated carbon skeletons that are the backbone of plant terpenes, sterols, gibberilins, abscisic acid, carotenoids, phytol, quinone side chains, polyisoprenoids (i.e. rubber) and isoprenoid alkaloids.

The IPP molecules are synthesized from acetyl-CoA: first acetoacetyl-CoA is produced by condensation of two acetyl-CoA molecules, followed by a further condensation reaction with an additional acetyl-CoA



**Isopentenyl pyrophosphate**



**isoprene unit**

Figure 1

Structures of isopentenyl pyrophosphate and  
the "isoprene" unit

to form  $\beta$ -hydroxy- $\beta$ -methylglutaryl-CoA (HMG-CoA) (figure 2). HMG-CoA is reduced to mevalonic acid (MVA), which is phosphorylated and then decarboxylated to generate IPP.

HMG-CoA is a substrate for two competing reactions: 1) the conversion to MVA, catalyzed by the enzyme HMG-CoA reductase; and 2) the breakdown to acetyl-CoA and acetoacetate, catalyzed by HMG-CoA lyase. Until recently, the conversion of HMG-CoA to MVA was thought of as the committed step in isoprenoid biosynthesis, but a MVA shunt which recycles MVA back to acetyl-CoA may exist in plants as it does in animals<sup>4</sup>. Still, work out of our laboratory<sup>5</sup> strongly suggests that the conversion of HMG-CoA to MVA is the rate limiting reaction between acetate and the isoprenoids, and thus plays a key role in the control of hydrocarbon biosynthesis.

The IPP molecule acts as a building block for the manufacture of the long chain carbon skeletons. First a molecule of IPP is isomerized to 3,3-dimethyl-allyl pyrophosphate (DMAPP), which is the terminal isoprene unit in the construction of the terpenes. Additional IPP molecules are joined to the DMAPP by a tail to head condensation, generating carbon chains increasing in 5 carbon increments (figure 3). Ring closures, phosphorylations, methylations and oxidations are performed on these carbon skeletons to generate the plant terpenoids.

### **Alkane Biosynthesis**

The second major class of plant hydrocarbons are generated from the removal or the reduction of the carboxylic acid head group of fatty acids. These compounds also arise from acetyl-CoA, utilizing the fatty acid synthetase complex to make the C16:0 palmitic acid, which can be further elongated by a separate system to generate the very long chain fatty acids (eg C30, C32) that are the precursors for the most common plant hydrocarbons, the C29 and C31 straight chain alkanes<sup>6,7</sup>. These alkanes are probably generated by the reduction of the acid moiety to an aldehyde, followed by a decarbonylation (liberating CO) to form the free alkane<sup>8</sup> (figure 4). These hydrocarbons can be converted to secondary alcohols or

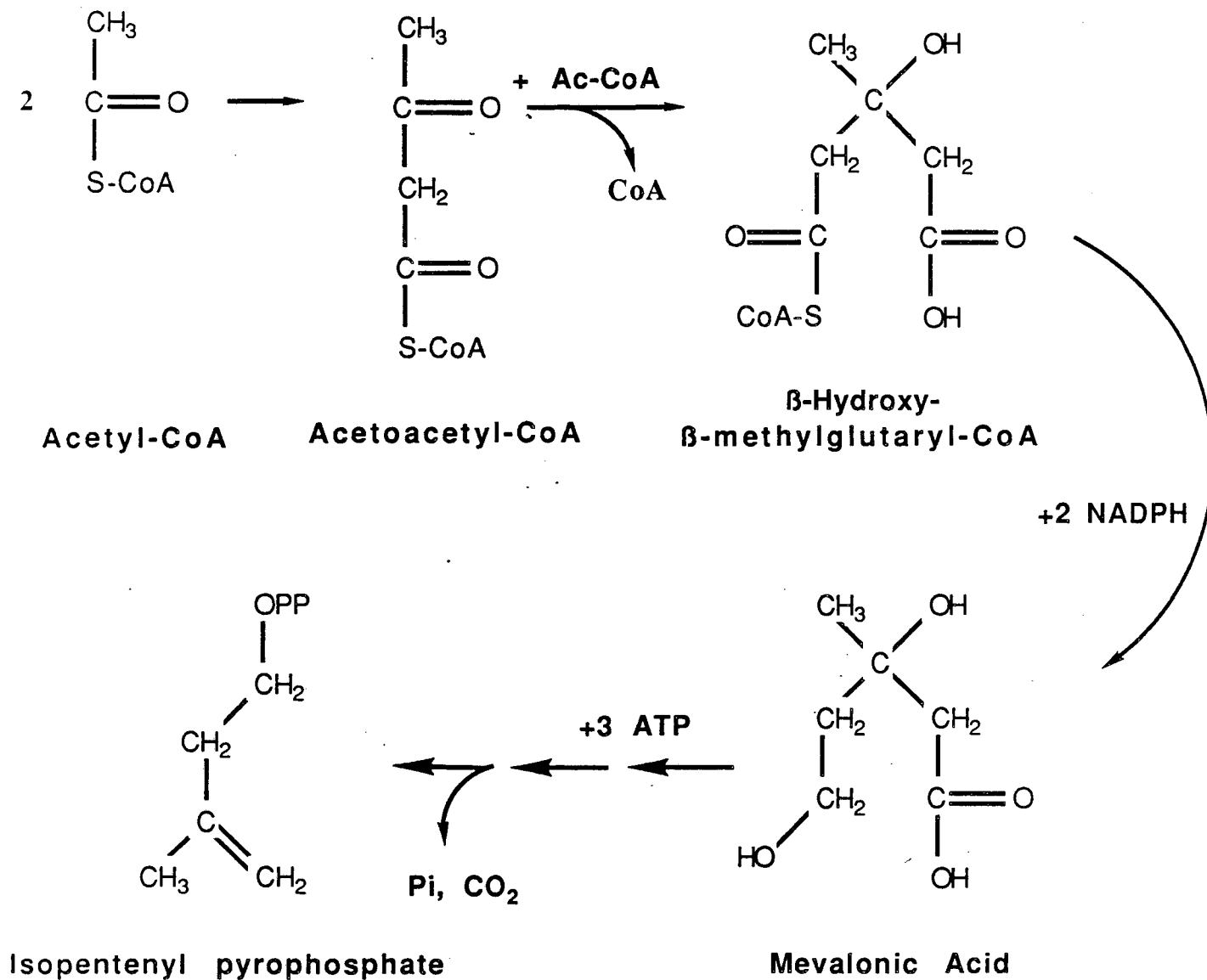


Figure 2. Synthesis of isopentenyl pyrophosphate from acetyl-CoA



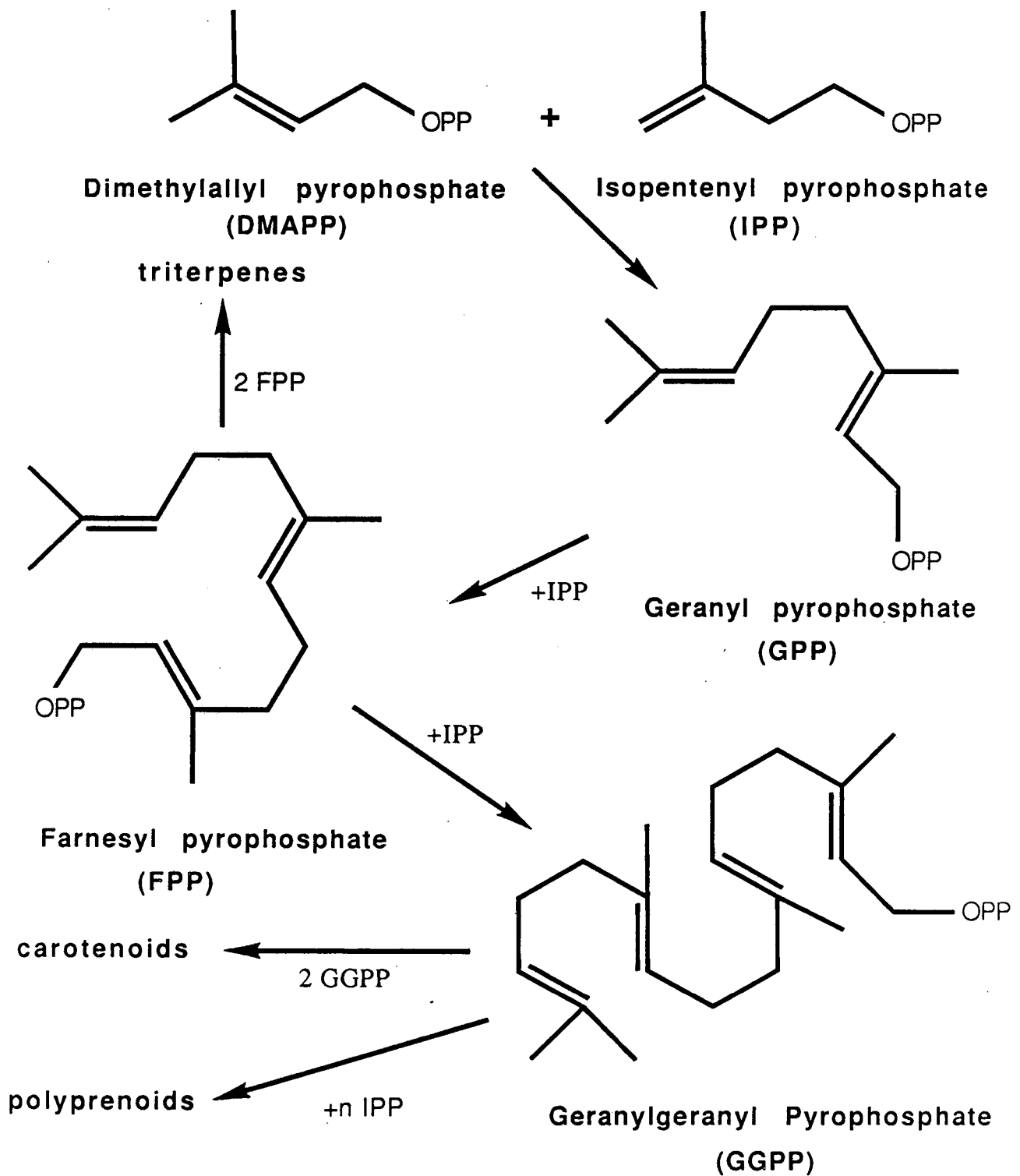


Figure 3. Synthesis of the terpenes from isopentenyl pyrophosphate (IPP)

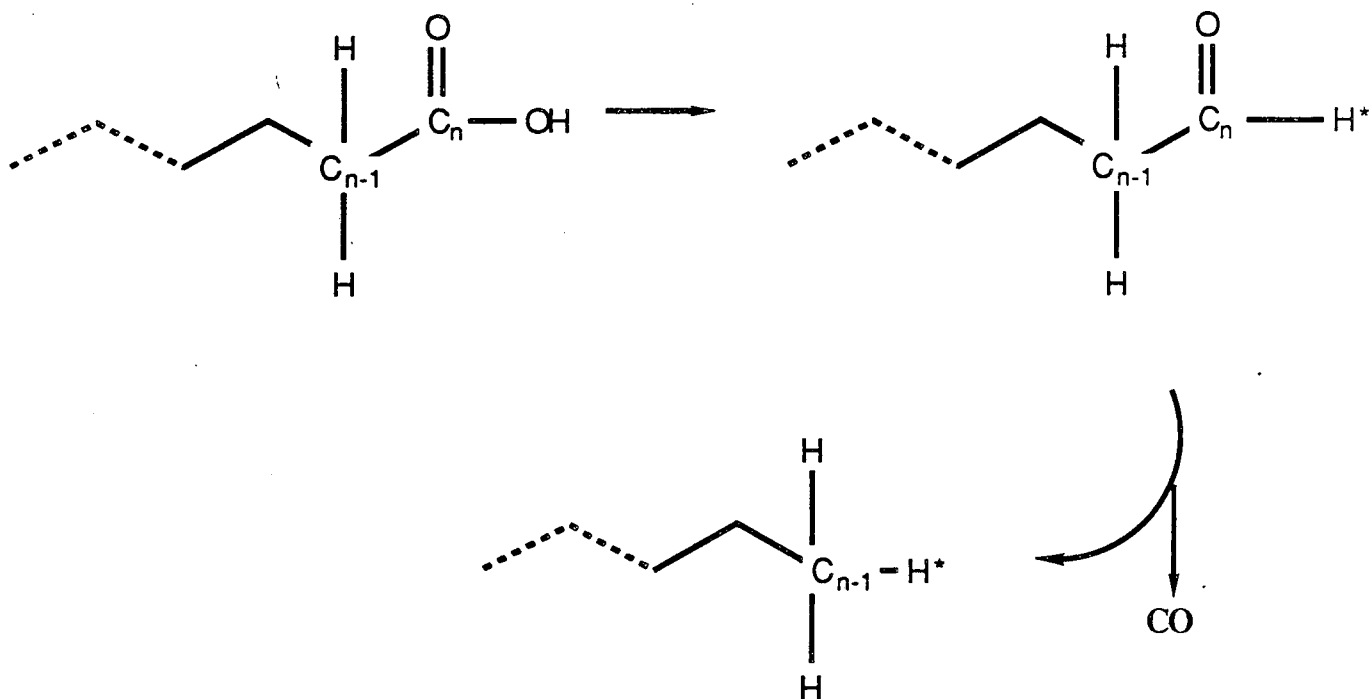


Figure 4 Production of a free alkane from a fatty acid via an aldehyde intermediate. Note that the proton on the terminal carbon of the aldehyde is conserved.

ketones<sup>9</sup>.

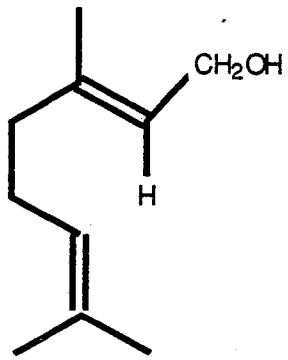
## CLASSES OF HYDROCARBONS

### Monoterpenes and Sesquiterpenes

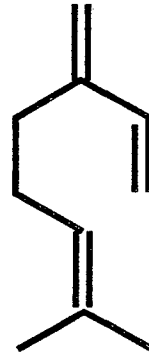
The C<sub>10</sub> monoterpenes and the C<sub>15</sub> sesquiterpenes, of which there are several hundred structural varieties, are found throughout the plant kingdom. They make up the economically important steam extractable "essential oils". Devon and Scott<sup>10</sup> have reported on the existence of fifteen major and fifteen minor structural classes of monoterpenes, but a more simple classification can be done, dividing these compounds into one of four types: acyclic, cyclohexanoic, cyclopentanoic, and irregular (figure 5). For the sesquiterpenes, Devon and Scott have identified 30 main skeletons and 70 minor ones; both acyclic and cyclic sesquiterpene structures have been identified. Some sesquiterpene glycosides, esters, and alkaloids and polyoxygenated sesquiterpenes are found in the nonvolatile fraction of plant extracts<sup>11</sup>.

The monoterpenes are derived from the joining of two isoprene units. Three of the structural classes, the acyclic, cyclohexanoic, and cyclopentanoic compounds, are derived from a tail to head condensation of IPP and DMAPP molecules. The fourth group consists of molecules formed from reactions joining an IPP molecule to the middle of the DMAPP, or by rearrangement after the tail to head condensation has occurred.

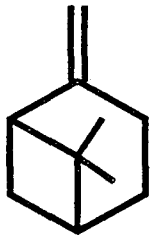
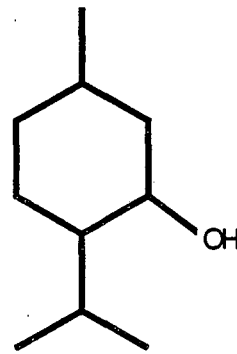
The acyclic monoterpenes are probably derived from geranyl pyrophosphate (GPP) (figure 6). The exact route for synthesis of the cyclic monoterpenes is unclear. While GPP is on the direct route of isoprenoid biosynthesis, its trans configuration at the  $\Delta^2$  double bond makes it hard for some to envision its cyclization, and neryl pyrophosphate (cis at the  $\Delta^2$  double bond) has been proposed as an alternative precursor. There is contradictory evidence supporting each of these compounds as the

Acyclic

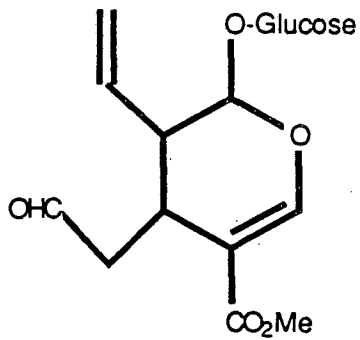
Geraniol



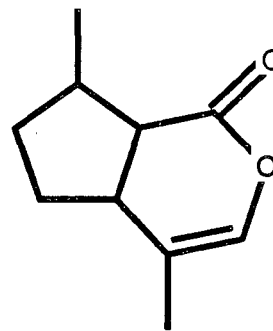
Myrcene

Cyclohexanoid $\beta$ -Pinene

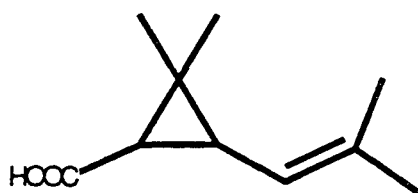
Menthol

Cyclopentanoid

Secolganin



Nepetalactone

Irregular

Chrysanthemic acid

Figure 5 Examples of various monoterpene structural classes

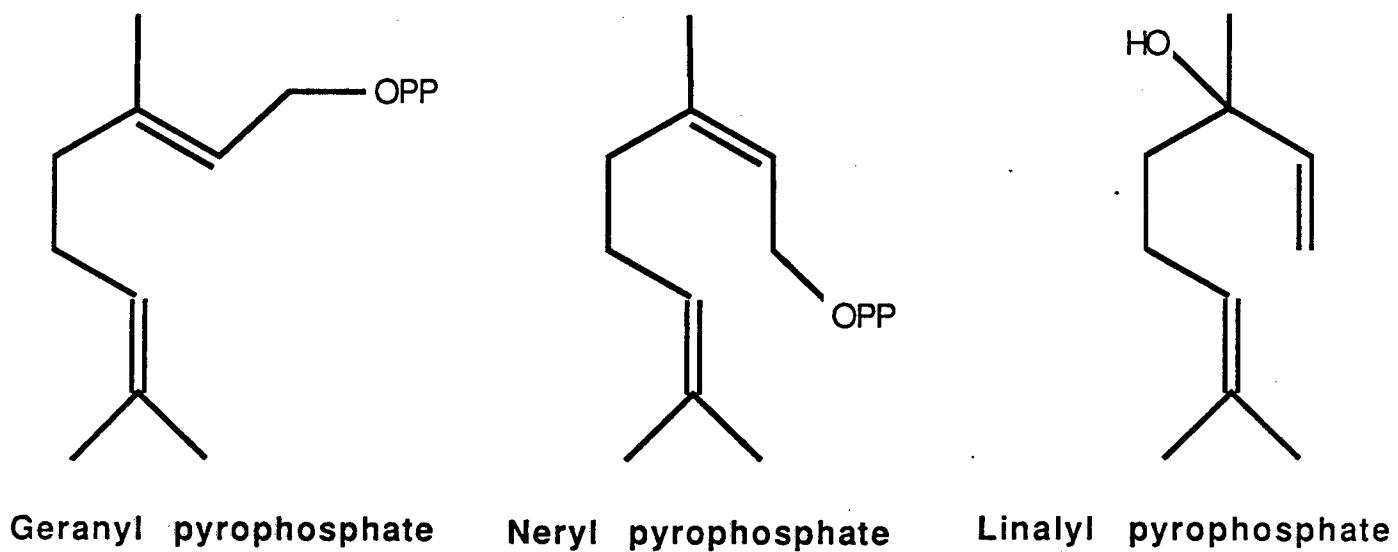
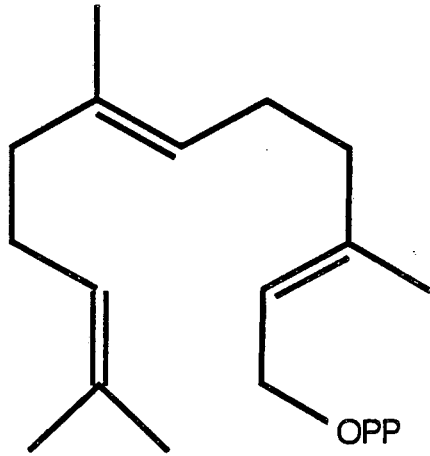


Figure 6 Proposed substrates for the cyclization reaction involved in the formation of the cyclic monoterpenes

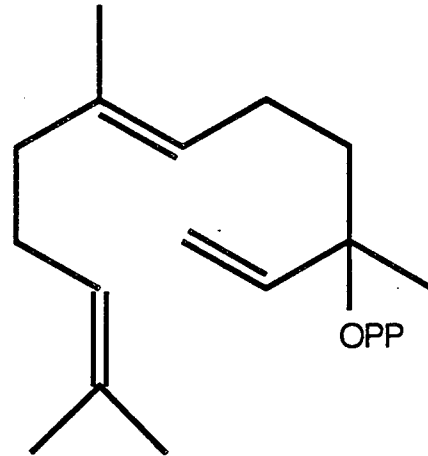
precursor for the cyclic monoterpenes, though recently Croteau et al<sup>12</sup> have reported on the isolation of enzyme systems that catalyze the cyclization of GPP in plants. This apparently occurs via linalyl pyrophosphate<sup>13</sup>, and the same enzyme is presumably responsible for both the isomerization and the cyclization reactions. The wide variety of monoterpenes (and sesquiterpenes) is due to one of two separate mechanisms: there have been reports of multiple products arising from one enzyme, and also multiple cyclases each generating a different product. A more thorough review on the enzymology of monoterpene biosynthesis can be found in Cori M.<sup>14</sup> and in Croteau and Cane<sup>15</sup>.

The sesquiterpenes arise from farnesyl pyrophosphate (FPP), a C15 compound composed of three isoprene units (figure 7). Like GPP, FPP is trans at the  $\Delta^2$  double bond, and for some configurations requires a trans to cis isomerization before any cyclization can occur. This probably occurs via nerolidyl pyrophosphate, at least for the initial cyclization products containing 6 membered rings<sup>16</sup>. Isomerization before cyclization is not required when the initial product is a 10 or 11 carbon member macrocycle, though it still may occur<sup>15</sup>. After the initial cyclizations occur, these structures undergo further rearrangements and/or oxidations to generate the multiple structures composing the sesquiterpenes (figure 8). The wide diversity of the sesquiterpenes is in part due to the initial cyclization of FPP to the 10 or 11 carbon member macrocycles (e.g. germacrene C, figure 8), which allow a wider range of rearrangements and ring closures than the 6 member rings of monoterpenes.

The economic uses of the monoterpenes and sesquiterpenes are wide and varied. Since ancient times there has been a large demand for these compounds as flavorings and in the manufacture of perfumes. One of the most popular flavorings is mint oil. Isolated from either Mentha piperita or M. arvensis, the major constituent of this oil is menthol (figure 5) (or the corresponding ketone, menthone). Demand for this oil has recently reached 22,000 tons annually<sup>17</sup>.



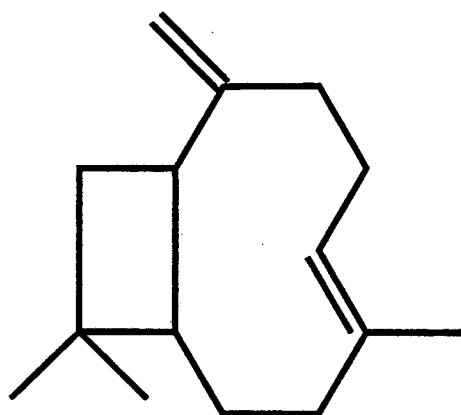
Farnesyl pyrophosphate



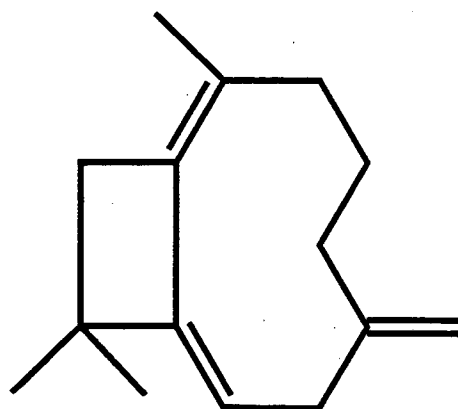
Nerolidyl pyrophosphate

Figure 7

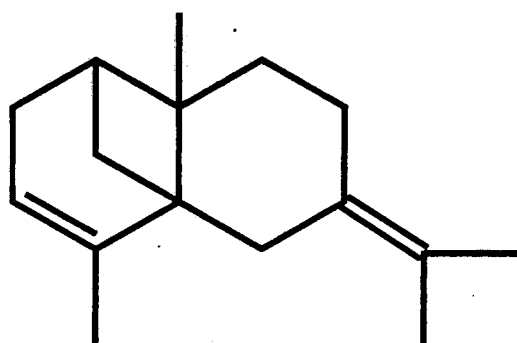
Proposed substrates for the cyclization reaction involved in the formation of the sesquiterpenes



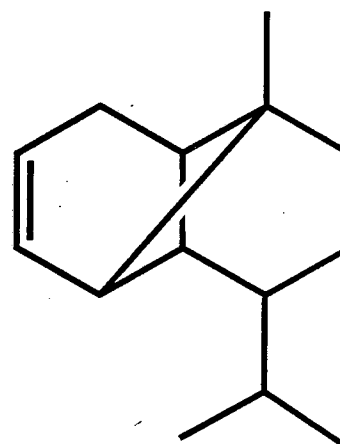
**Caryophyllene**



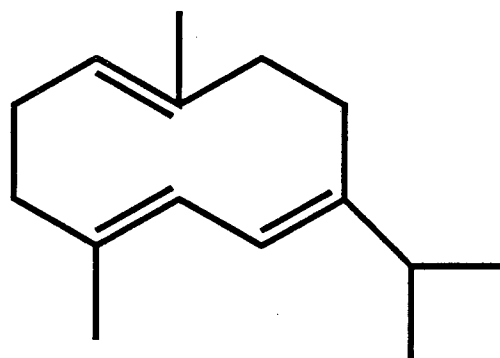
**Humulene**



**Bergamotene**



**Copaene**



**Germacrene C**

Figure 8. Examples of cyclic sesquiterpenes



Grasses from the genus Cymbopogon are also used for commercial production of monoterpenes. The composition of the essential oil will vary from species to species, though citral (figure 9), geraniol, citronellal (figure 9), pipertone and geranyl acetate are the most common major constituents. The two most common cultivated species are C. flexuosus and C. citratus (also called lemongrasses); both are high in citral. Citral is commercially important as it is a starting material for the preparation of the ionones, which are used in cosmetics, flavorings, perfumes, and the production of synthetic Vitamin A<sup>18</sup>.

The main constituent of the essential oil of citrus peels is limonene (figure 9). While often considered as just a by-product of the juice industry, this compound is used in the chemical industry as a feedstock. Wang and Huffman<sup>19</sup> project an increased demand for limonene as a starting compound in the synthesis of commercially important organic compounds.

A second historical use for monoterpenes and sesquiterpenes are as medicinal compounds. These terpenes have been used as analeptics, analgesics, anesthetics, anthelmintics, antiarrhythmics, antibiotics, antiepileptics, antihistaminics, antiinflammatories, antirheumatics, antitumor agents, chloretics, diuretics, expectorants, hypotensive agents, pugnatives, sedatives, and spasmolytics<sup>20</sup>. Though usually it is the total essential oil that has been credited with these effects, some studies have identified individual monoterpenes as the active constituents. In most cases the mechanism of biological activity has yet to be determined.

For example, thymol (figure 9) is an excellent antiseptic, exhibiting twenty times the activity of phenol. Effective against bacteria, molds and yeast, thymol is used in the preservation of medical specimens, and can be found in mouthwash. Terpinen-4-ol, a major component of juniper oil, has been used effectively as a diuretic, and cineol from eucalyptus oil is used as an expectorant. Camphor (figure 9), an irritant which induces localized heating, is used in linaments and chest rub preparations for colds. Wagner

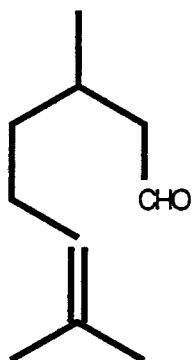
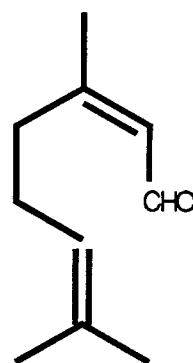
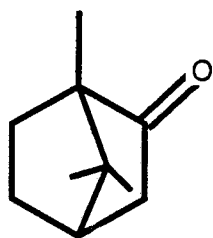
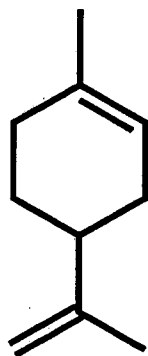
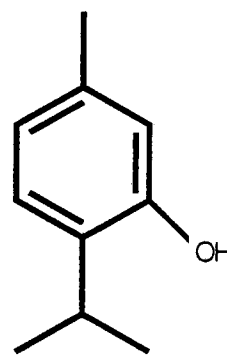
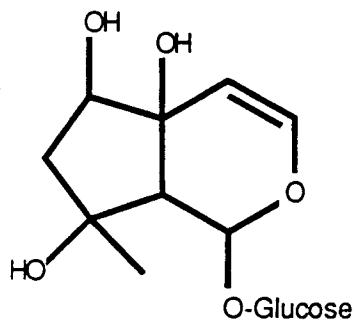
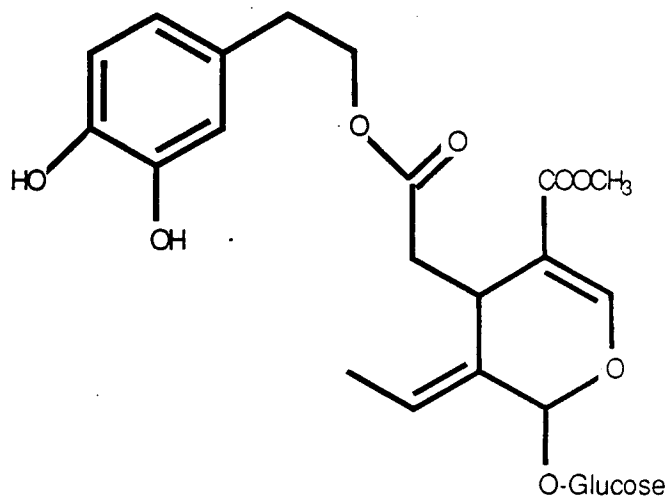
**Citronellal****Citral****Camphor****Limonene****Thymol****Harpagide****Oleuropein**

Figure 9. Commercially important and/or biologically active monoterpenes

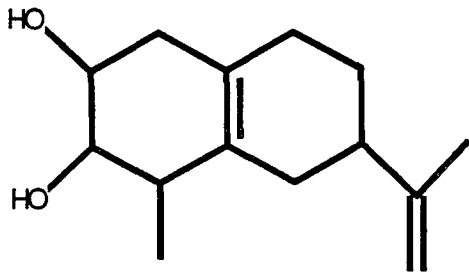
and Sprinkmeyer<sup>21</sup> have reported that citral, citronellal, and limonene were excellent sedatives.

Another medically important group of monoterpenes are the iridoids (e.g., harpagide, figure 9). These compounds are cyclopentanoic monoterpenes, and are commonly found as glucosides. Harpagide, an iridoid glucoside from the root of Harpagophytum procumbens, has been found to have analgesic effects. In some instances the cyclopentane ring has been opened; these compounds are known as secoiridoids. The secoiridoid oleuropein (figure 9), found in olives, has been shown to exhibit hypotensive action. Some of the iridoids have also been found to contain covalently bound chlorine<sup>22</sup>.

Sesquiterpenes also have medicinal uses. They are utilized in the pharmacological industry as "bitter principals". Sesquiterpene lactones have been reported to exhibit strong antitumor/ cytotoxic activity, apparently via an interaction with enzymatic sulfhydryl groups<sup>20</sup>.

A significant proportion of the plant phytoalexins are monoterpenes and sesquiterpenes, especially those from the Solanaceae. These compounds are produced by a plant in response to an infection by bacteria, fungus or nematode. They are thought to impart some protection to the plant, though an *in vivo* function has not been determined for most of the phytoalexins. A possible commercial use might be found for these as pesticides. For example rishitin (figure 10), a compound derived from a sesquiterpene and found in potato exposed to fungus<sup>23</sup>, has been reported to inhibit fungal growth<sup>24</sup>. Another candidate for commercial exploitation is oleoresin, the sap that exudes from pine trees when they are attacked by predators. This exudate, which has strong antimicrobial activity, is made up of a mixture of fatty acids, volatile terpenes (turpentines) and non-volatile resins. The active compound(s) still must be identified.

The monoterpenes and sesquiterpenes may also have a use as pesticides



**Rishitin**

Figure 10 A sesquiterpene phytoalexin

or herbicides (growth regulators). Some are already used commercially: extracts from the pyrethrum flower, called pyrethrins, have been used as insecticides. The active ingredient in these preparations is derived from the irregular monoterpene chrysanthemic acid (figure 5). Iridoids have been found to be successful in the control of insects as they repel predators. For example Neptalactone (figure 5), found in catnip, is a good insect repellent in addition to being a feline attractant. Some sesquiterpenes are known to cause stomatal closure<sup>25</sup>; this response may allow the use of these compounds as herbicides.

The use of plant hydrocarbons as an alternative to petroleum has also been proposed. The best candidates are the monoterpenes and sesquiterpenes, which can be used directly as fuel and chemical feedstock without any further modifications. Turpentine, the volatile portion of pine resin, is in large part made up of the monoterpenes  $\alpha$ -pinene,  $\beta$ -pinene (figure 5) and camphene. Turpentine is used as a solvent and a raw material for chemical synthesis, and could possibly function as an alternative to petroleum as a fuel. Lipinsky<sup>26</sup> reported that turpentine has an energy content equivalent to that of gasoline (both with 40 gigajoules/metric ton), and Wang and Huffman<sup>19</sup> suggest that it may be used in combination with gasoline to make a fuel substitute (gasotine?).

The fruit of the Pittosporum resiniferum tree are also high in  $\alpha$ -pinene and  $\beta$ -pinene, and are currently under study in the Phillipines as a possible alternative energy source. The oil, isolated from the fruits by steam distillation, is comparable to gasoline in terms of utilization as a fuel<sup>27</sup>. A related species with a similar fruit hydrocarbon profile, P. undulatum (commonly found in California), has also been proposed as a source of fuel<sup>27</sup>.

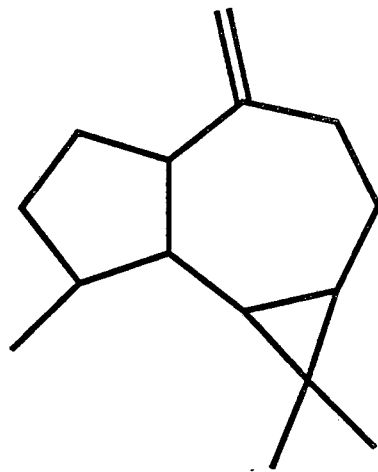
Oil extracted from Croton sonderinians, commonly called marmeleiro oil, is another candidate as a possible source of fuel. This species, a native of Brazil, is enriched in the monoterpene  $\alpha$ -pinene and in the sesquiterpenes  $\beta$ -caryophyllene (figure 8), humulene (figure 8) and elemene. Its handling

and combustion properties are very similar to that of diesel oil<sup>28</sup>.

Trees from the genera Copaifera and Dipterocarpus are also being studied for the commercial production of photosynthetically derived fuels. Oil extracted from the heartwood of the Brazilian C. multijuga tree has been found to contain mostly cyclic sesquiterpenes (carophyllene, bergatomenone and copaene, figure 8). One tree will produce about 50 liters of oil per year. This oil can be used directly in a diesel engine, or may be cracked into gasoline-type compounds by passage over a Mobil zeolite shape specific catalyst<sup>27</sup>.

About six species of Dipterocarpus are utilized for the production of gurjun balsam oil. Commonly used in varnishes and lacquers, the oil from these species is now being considered as a fuel source since it is high in sesquiterpenes (allo-aromandendrene [figure 11],  $\beta$ -carophyllene,  $\gamma$ -gurjunene,  $\alpha$ -humulene). One species, D. grandiflorus, has been reported to produce 1 kg. of oleoresin per day, of which about 40% is the high sesquiterpene-containing volatile oil. This oil has the same energy content as crude oil (petroleum), and a study is currently underway in the Philippines to determine the feasibility of the commercial exploitation of this species as an energy crop<sup>29</sup>.

Recently, Nishimura and his coworkers<sup>30</sup> have proposed an integrated approach to the exploitation of plant hydrocarbons. Their aim is to identify all of the possible commercial products that can be obtained from a species (or genus) in order to enhance the profitability of its farming. They have chosen trees from the Eucalyptus genus, in order to combine the growing of these trees as a biomass source with the production of specific hydrocarbon products. Eucalyptus leaves are high in monoterpenes and sesquiterpenes (up to 3.5% of the fresh weight in some species), and Nishimura has begun to identify some of these compounds as being biologically active. In addition to isolating these terpenes for use as fuels,<sup>31</sup> they have also found components that act as germination and growth inhibitors, as insect repellants, and as flavorings and chemical



**Allo-Aromadendrene**

Figure 11 The major hydrocarbon component of the oil of the D grandiflorus tree. This sesquiterpene could be used as an alternative to petroleum

feedstocks.

### **Diterpenes**

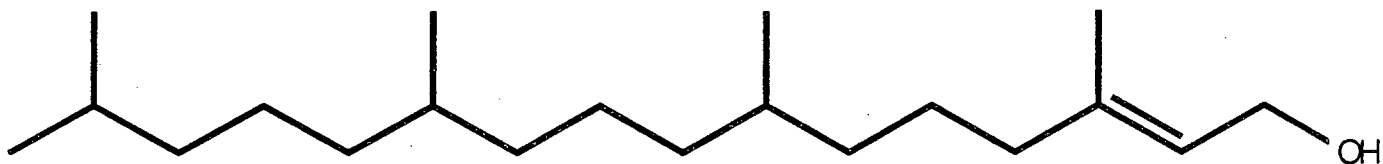
Diterpenes are derived from geranylgeranyl pyrophosphate (GGPP), a C<sub>20</sub> compound made from 4 isoprene units. There are four basic structural classes of diterpenes: acyclic, bicyclic, polycyclic, and macrocyclic (figure 12).

The acyclic diterpenes arise from the dephosphorylation, rearrangement, and/or reduction of GGPP. The most common of the acyclic diterpenes are phytol and its derivatives, which are found as esterified side chains in chlorophyll and prenylquinones. Phytol (figure 12) is generated by a three step reduction of the  $\Delta^{6,10,14}$  double bonds of GGPP. At least in the case of chlorophyll synthesis, this apparently occurs after the GGPP molecule has been esterified to chlorophyllide<sup>32</sup>.

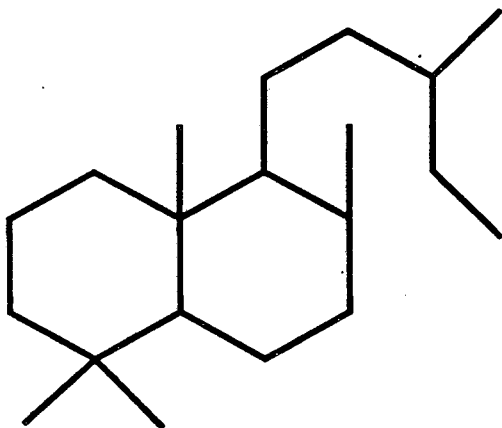
For most of the cyclic diterpenes, the initial product of the cyclase catalyzed reaction is a bicyclic molecule. This does not require trans-cis isomerization of the  $\Delta^2$  double bond (as required in monoterpene and sesquiterpene biosynthesis) as the cyclization occurs far enough down the carbon skeleton to be unaffected by the stereochemistry. The synthesis of the polycyclic diterpenes does not arise by a concerted cyclization of GGPP, but instead requires a bicyclic molecule as a starting point. The macrocycles probably arise from an interaction between a carbonium ion at the C1 carbon (derived by the removal of the pyrophosphate) and the terminal double bond at the C14 carbon, giving rise to the 14 or 15 membered macrocycles. It also has been proposed that many polycyclic diterpenes arise from rearrangements of the macrocycles and not from further cyclization of bicyclic molecules<sup>32</sup>.

Though many pharmacological properties have been attributed to diterpenes, including functioning as antibiotics, expectorants, hypotensive agents, and purgatives, the major medical interest is in regard to their

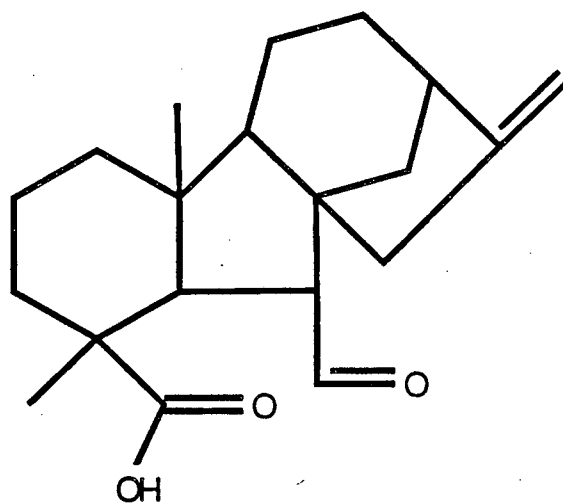
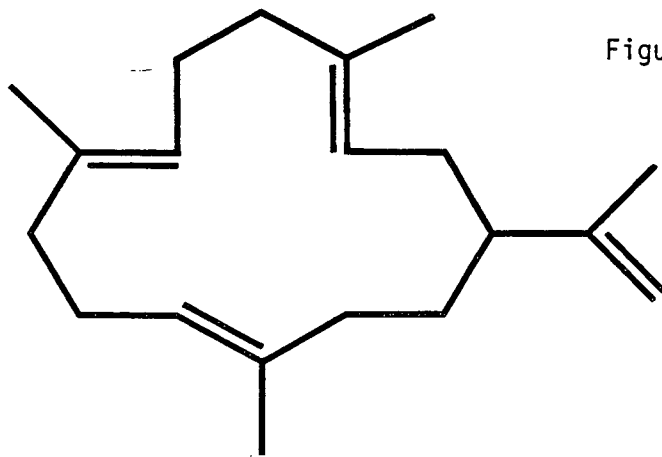


Acyclic

Phytol

Bicyclic

Labdane

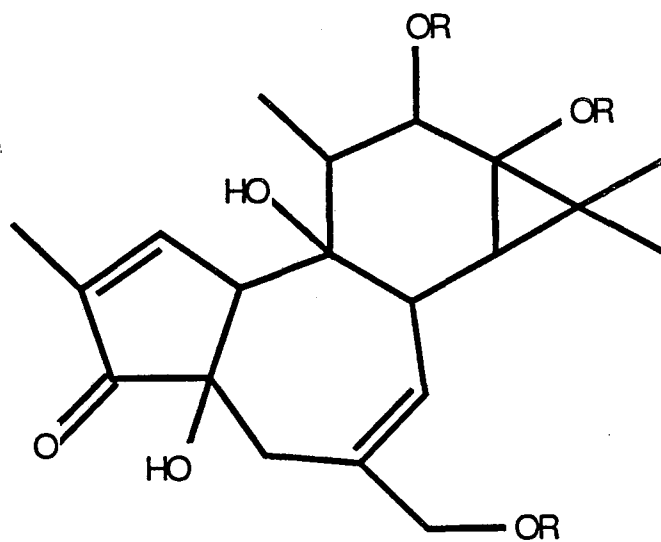
PolycyclicGibberellin A<sub>12</sub>-aldehydeMacrocyclic

Cembrane

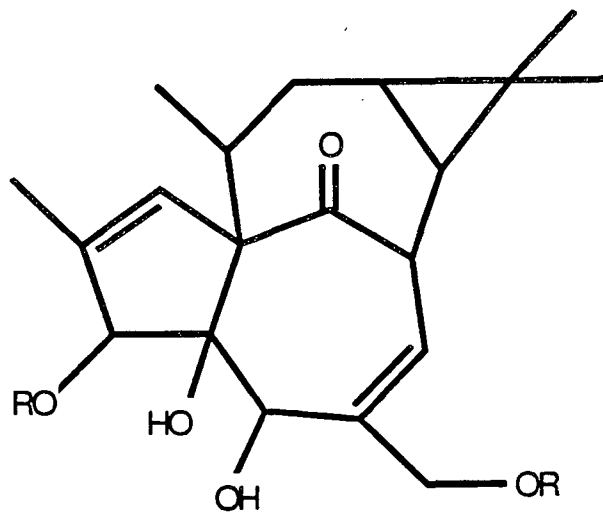
Figure 12 Examples of the various diterpene structural classes

antitumor/ tumor promotion activities. At least 15 different diterpenes have been identified as having antitumor activity<sup>20</sup>, many of them derived from macrocyclic molecules (figure 13). Kupchan and his co-workers<sup>33-35</sup> have suggested that these inhibitors function by alkylation of biologically active sulfhydryl groups. Another mode of action is suggested by the tumor promoting activity of the esters of the diterpene phorbol. It has been reported that phorbol esters such as 12-O-tetradecanoylphorbol-13-acetate (TPA) will bind to and activate protein kinase C, an enzyme credited with a role in the regulation of cell division. The enzyme normally requires diacylglycerol as an activator, but TPA will substitute for this compound<sup>36</sup>. Kupchan has found that with different side chains on a related diterpene (ingenol di-benzoate, figure 13), the diterpene becomes an antitumor agent<sup>34</sup>, suggesting that it is the ester components (fatty acids) of the diterpene esters that impart the tumor promotion activity, and antitumor agents can be developed by substitution of these side chains. What is not clear is if the side chains are important for the binding of the diterpene to an enzyme, or instead are active via some surfactant-type activity<sup>37</sup>. Whatever the mode of action, such antitumor activity raises the possibility of the commercial utilization of these compounds as antitumor agents. These compound may alter normal cell growth by changing protein kinase C activity, so their use as antitumor agents may be limited.

Diterpenes have an established role in the chemical industry. A large part of the nonvolatile fraction (rosin) of the pine oleoresin is made up of diterpene acids (resin acids), and these compounds are used in rubber production, paper sizing, and in synthetic resins and ester gums. From 1960 to 1980, the production of rosin in the United States has dropped 32%, and the domestic production of the high quality gum rosin has dropped to zero<sup>38</sup>. In contrast, demand for these products in the United States remains high so that importation of rosin is necessary. This has led Hoffmann and McLaughlin<sup>38</sup> to propose the herbaceous perennial Grindelia camporum as an economic substitute for the normal pine generated rosins. The major constituent of this species is grindelic acid, similar to the



**Phorbol**



**Ingenol**

Figure 13 Diterpenes used in cancer research. Esters of phorbol have been found to be cocarcinogens; some ingenol esters exhibit antitumor activity (R = benzoate)

major constituent of pine, abietic acid (figure 14). They are currently selecting for improved resin yield to attain a level that would make cultivation of this species economically possible.

Diterpenes may have other commercial uses. There are important diterpene growth regulators: the gibberilins are derived from GGPP. In addition casbene (figure 15), a macrocyclic-derived diterpene, has been found to exhibit growth regulatory activity, inhibiting growth in dwarf maize plants after endogenous applications of gibberilins<sup>39</sup>. Casbene has also exhibited antifungal activity, and since the enzyme responsible for its synthesis has been found to increase in activity in response to fungal infection in *Ricinus communis*, it has been identified as a phytoalexin. Casbene apparently operates as a phytoalexin by inhibiting diterpene production in the fungi<sup>39</sup>.

### **Triterpenes**

The triterpenes are composed of six isoprene units. They are not on the direct isoprenoid pathway but instead represent a branch point off of FPP; two FPP molecules are joined to make a C<sub>30</sub> molecule (squalene), and this is the starting point of the triterpenes. Further reactions, including rearrangements, substitutions, alkylations and dealkylations are carried out on these triterpene skeletons to generate the final products. There are three major structural classes of triterpenes: acyclic, tetracyclic, and pentacyclic (figure 16), with a few tricyclic compounds also known. Often a distinction is made between the tetracyclic and pentacyclic compounds: the tetracyclic are referred to as plant sterols, while the pentacyclic are called triterpenes. This has arisen because the synthesis of the plant sterols is similar to the pathway for cholesterol synthesis in mammals, while the pentacyclic compounds are not found in animals. Both classes of compounds have a common precursor, and in this paper we will treat them all as triterpenes.

The one major acyclic triterpene is squalene. As mentioned above, it is synthesized by a two step head to head condensation of two FPP molecules.

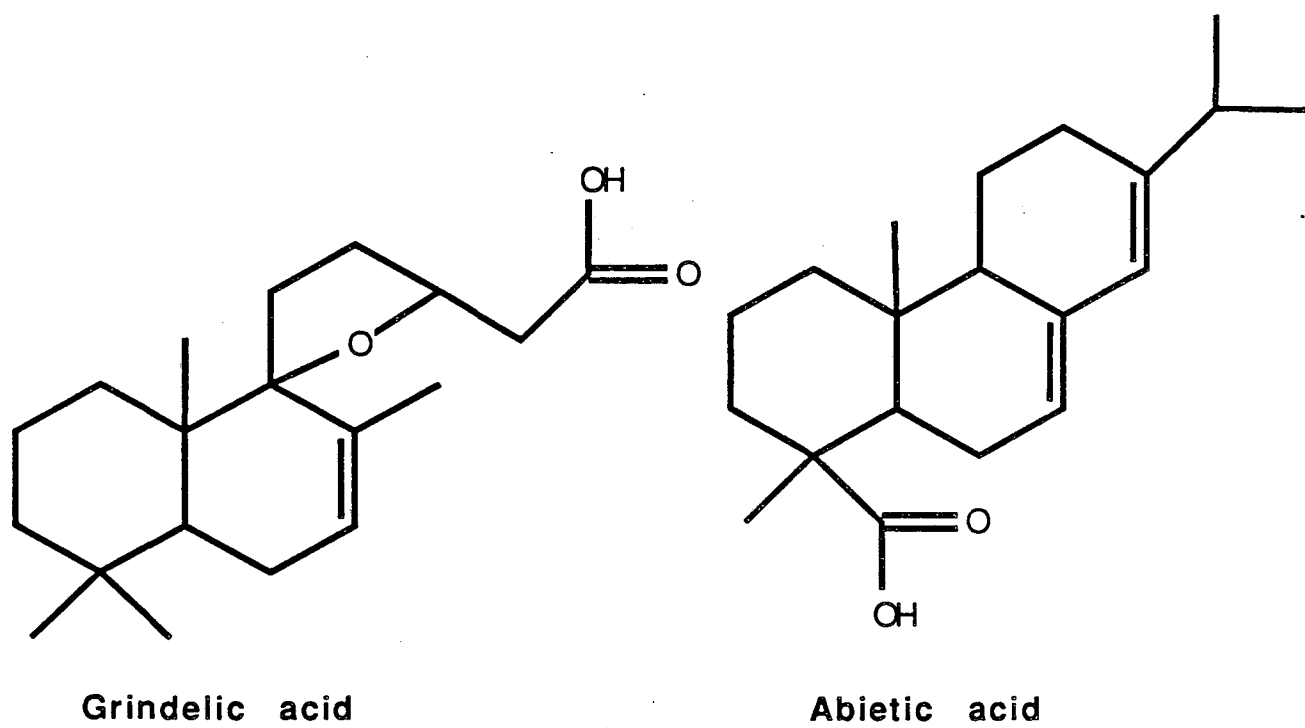
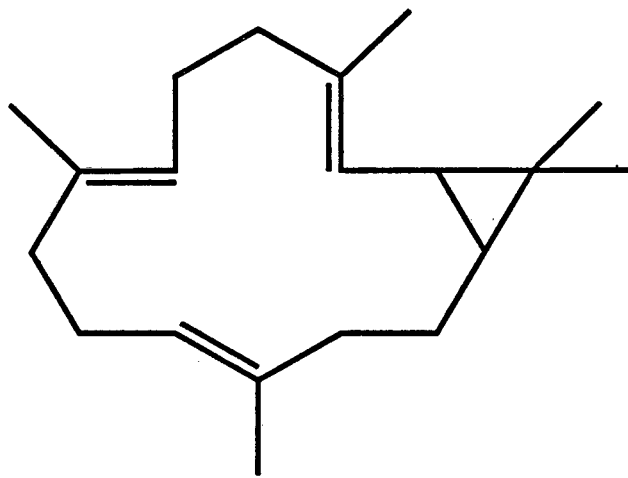
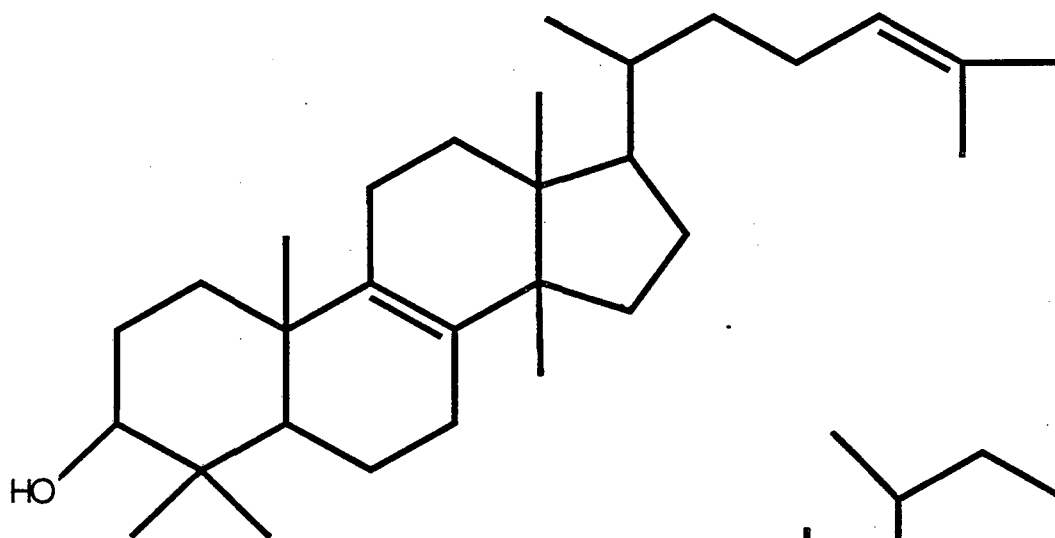


Figure 14 Two diterpene resin acids; grindelic acid is the major component of the rosin of G. camporum, and abietic acid is the major component of the rosin of Pinus sp.

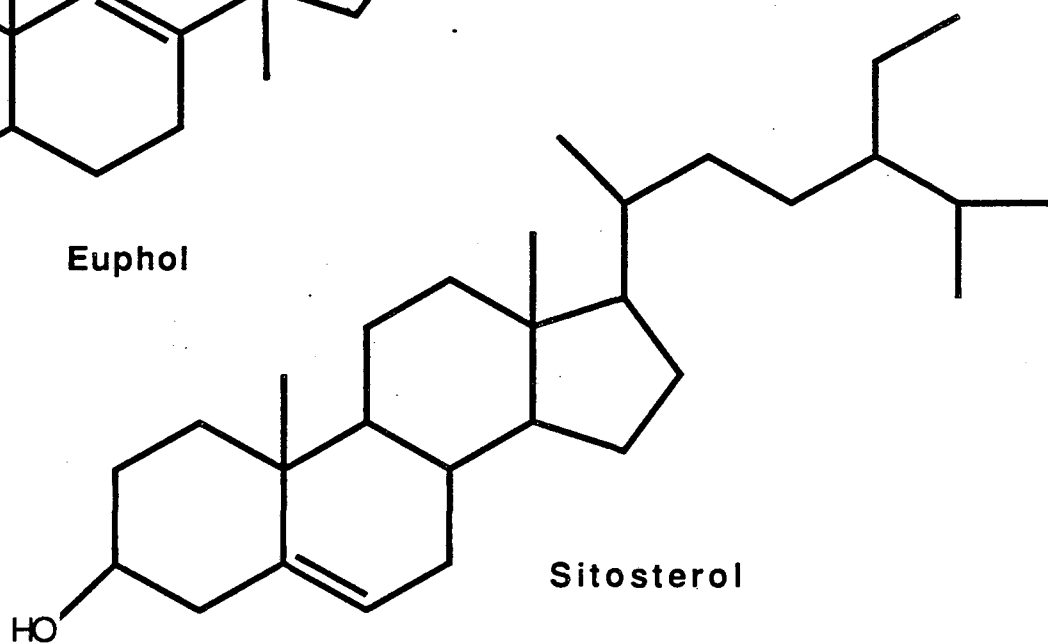


**Casbene**

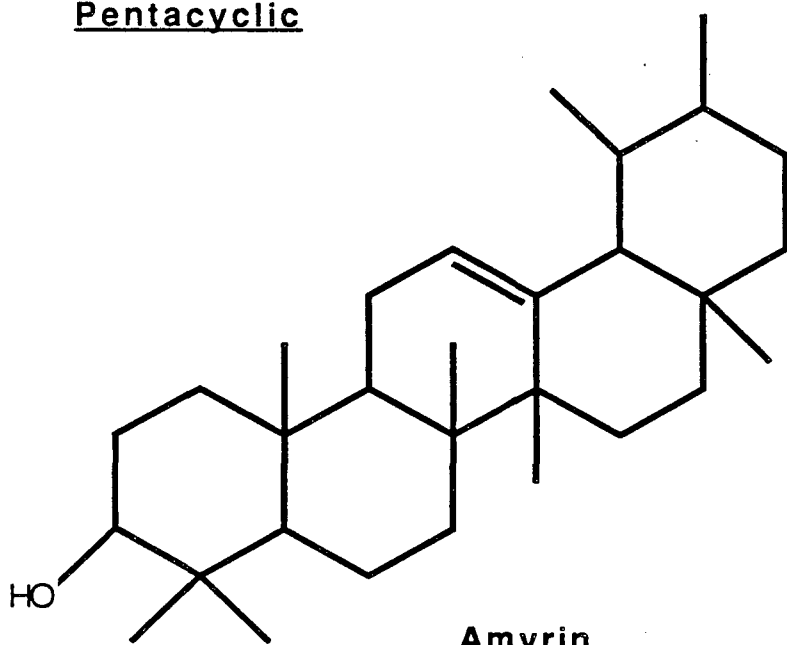
Figure 15. A diterpene phytoalexin

Tetracyclic

Euphol



Sitosterol

Pentacyclic

Amyrin

Figure 16 Examples of the two classes of cyclic triterpenes

The first step results in the synthesis of presqualene pyrophosphate, which subsequently undergoes rearrangement to generate squalene (figure 17).

The cyclic triterpenes are all derived from squalene. This occurs via a two step process in which the squalene is first oxidized to 2,3 squalene epoxide, which then undergoes cyclization to form the cyclic triterpenes. With the use of analogs to postulated high energy intermediates of the cyclization reaction, Taton et al<sup>40</sup> have shown that different cyclase enzymes are used to form the tetracyclic and the pentacyclic triterpenes.

Triterpenes have been reported to have useful pharmaceutical properties, including antifungal, antiinflammatory, antimicrobial and antitumor activity. They have been used in the healing of ulcers, and have been shown to be effective in reducing cholesterol and atherosclerosis in rats<sup>41,42</sup>. The "cardiac glucosides", which are derived from cyclic triterpenes, are important in the treatment of heart ailments. Digitoxin and digoxin (figure 18) are currently used as heart-stimulants.

Commercial exploitation of triterpenes by the chemical industry may also be possible. They may be used as growth regulators: germination is inhibited by certain cucurbitacins (figure 19)<sup>43</sup>. Avenacin, a glycoside of a pentacyclic triterpene, is a fungitoxin<sup>44</sup>. Some alkaloids that are derived from plant sterols, such as solanidine (figure 18), also function as fungitoxins. Plant sterols can also be used as feedstock for the synthesis of important mammalian steroids: diosgenin (figure 18), found in the roots of Dioscorea plants, is used as a chemical feedstock for the synthesis of cortisone and other antiinflammatory agents<sup>45</sup>.

The oxidative breakage of the A-ring, forming seco-triterpenes, generates many biologically active molecules (e.g. roburic acid, figure 20). Many of these compounds have been reported to be phytoalexins, arising in response to microbial, fungal, and insect invasion. They also have been reported to possess alleopathic capabilities<sup>46</sup>. It has been suggested that



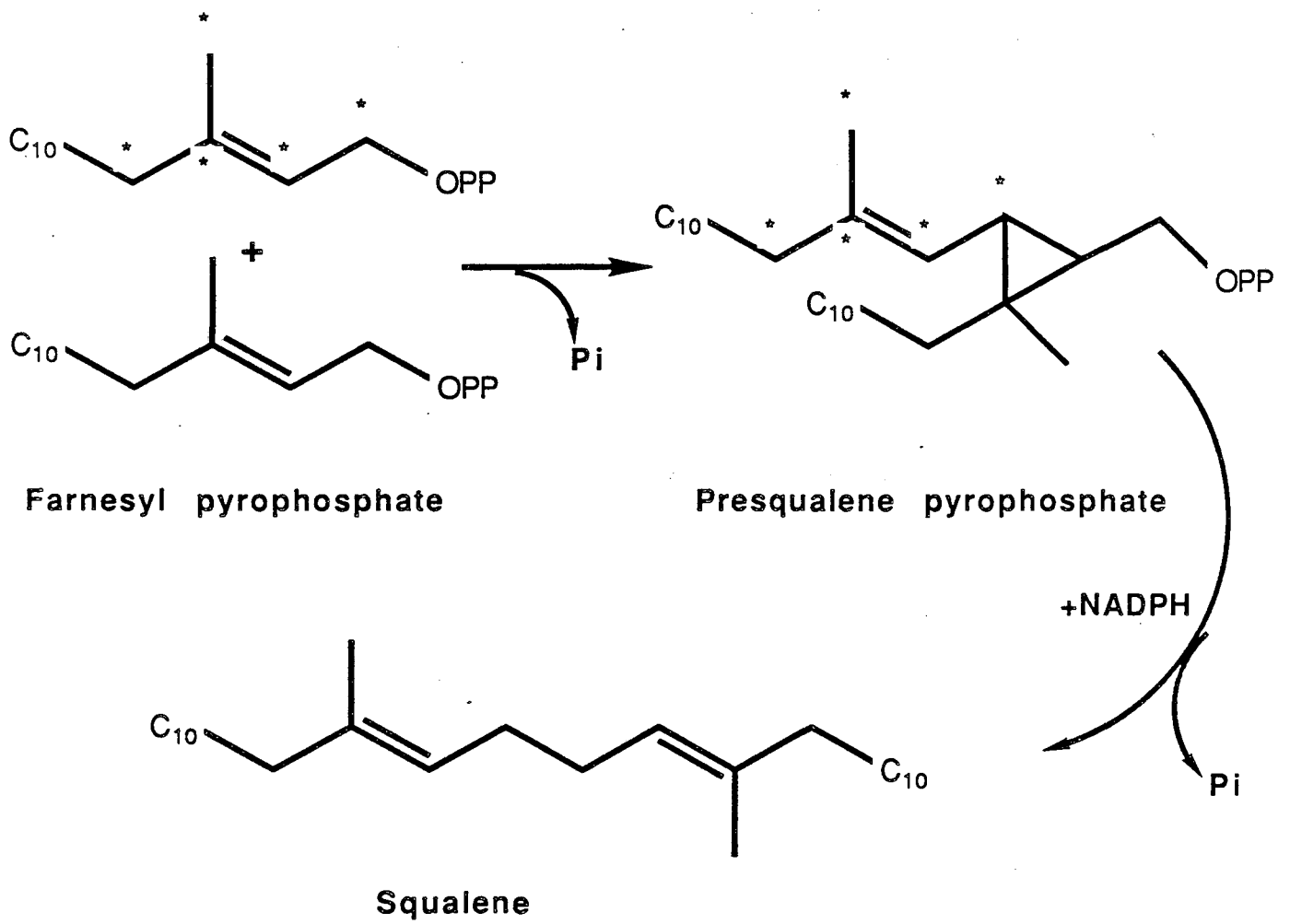


Figure 17 Biosynthesis of squalene from farnesyl pyrophosphate

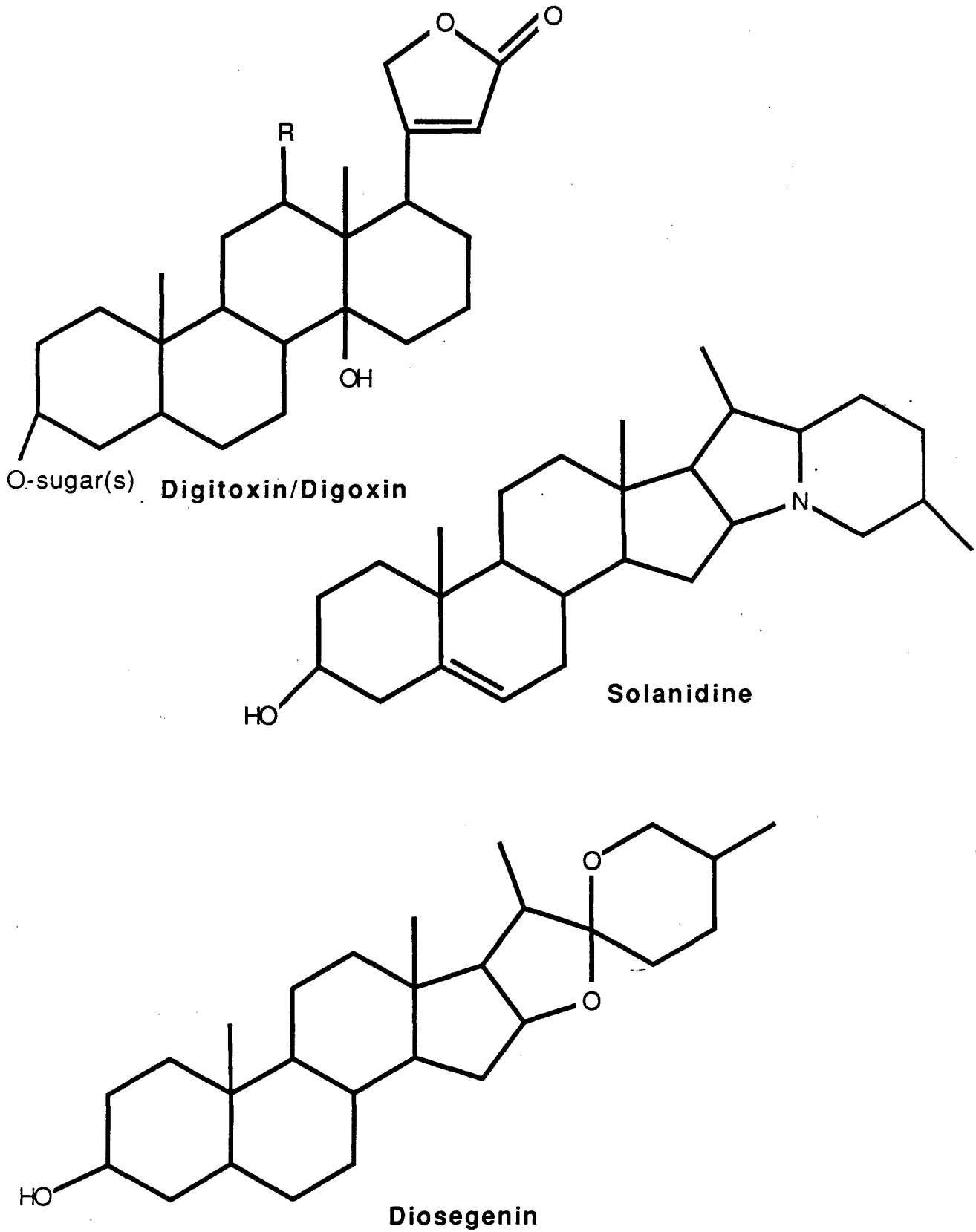
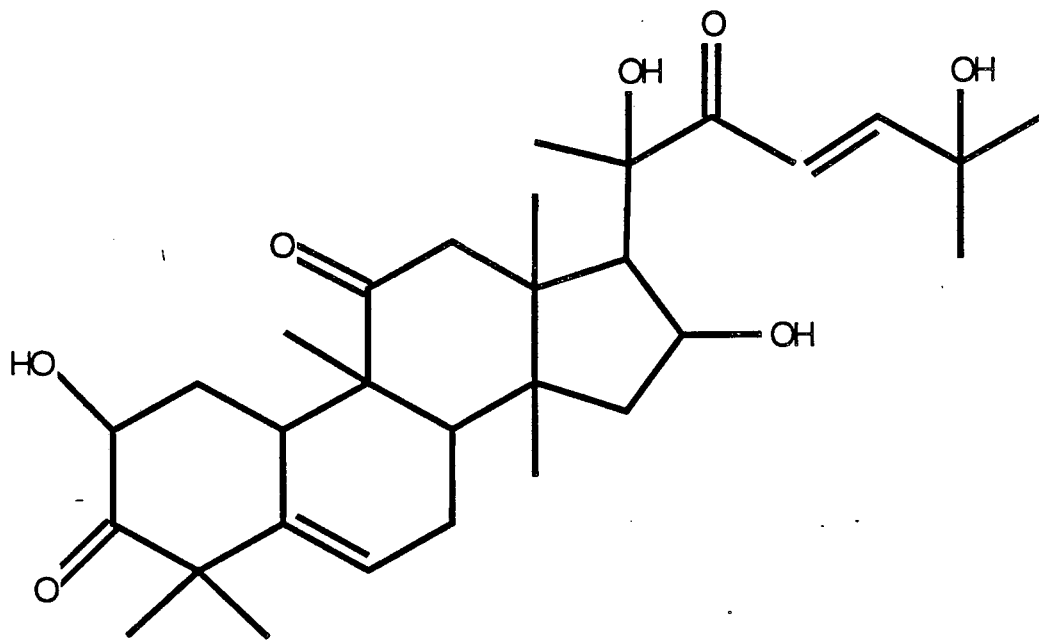


Figure 18. Biologically active triterpenes. (R = H for digitoxin;  
R = OH for digoxin)



**Curcurbitacin D**

Figure 19 An alleopathic triterpene

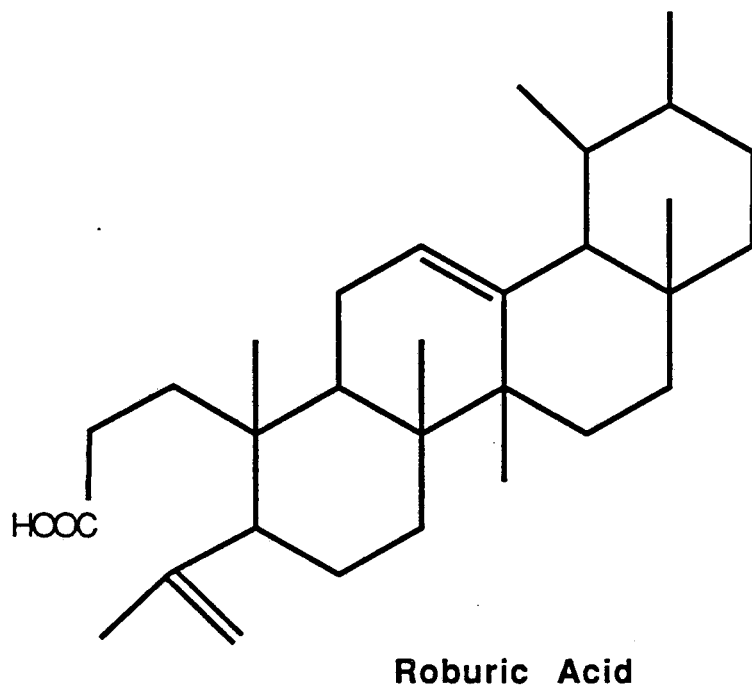


Figure 20 A biologically active seco-triterpene

the activity of the seco-triterpenes arises from an interaction of biological important sites in other organisms with the rigid, oxygenated cyclic triterpene<sup>42</sup>.

Triterpenes can also supplement petroleum as an energy source. Some members of the Asclepiadaeaceae and Euphorbiaeaceae families produce large amounts of triterpenes. These triterpenes, localized in a milky emulsion (or latex), constitute up to 9% of the dry weight of some species. Four species are currently under investigation as possible candidates for "energy farms": Euphorbia lathyris and E. tirucalli from the Euphorbiaeaceae family and Asclepias speciosa (milkweed) and Calotropis procera from the Asclepiadaeaceae family<sup>27</sup>. The triterpenes from E. lathyris have been isolated and identified as cycloartenol, 24-methylene- cycloartenol, lanosterol, an isomer of lanosterol, and butyrospermol<sup>5,47</sup>. If this oil is passed over a Mobil zeolite catalyst it can be cracked to generate high octane gasoline<sup>48</sup>. The major constituent of A. speciosa is the pentacyclic triterpene amyrin (figure 16)<sup>49</sup>; it should also be easily converted to gasoline.

### Other Isoprenoids

There are many additional plant isoprenoid hydrocarbons, some are of commercial interest. Carotenoids, made up of eight isoprene units, are synthesized by a head to head condensation of two GGPP molecules (in a fashion similar to squalene biosynthesis), followed by several desaturation steps. There are acyclic carotenoids (lycopene), bicyclic carotenoids ( $\alpha$ - and  $\beta$ -carotene), and oxygenated bicyclic carotenoids (the xanthophylls).  $\beta$ -carotene is used in nutrition; animals can generate vitamin-A by cleaving  $\beta$ -carotene.

The other important class of isoprenoids are the polyisoprenoids. These are found in two forms in plants: the cis-1,4-polyisoprene structure of rubber and the trans-1,4-polyisoprene structure of gutta. The main source of natural rubber is the Hevea brasiliensis tree, which produces polymers with molecular weights of  $10^5$  to  $10^6$  g/mole. Another potential source is

Parthenium argentatum (guayule), which is rich in rubber that has an average molecular weight that is high enough to be of commercial use. The average molecular weight of gutta molecules is much less than rubber (about 10x), which reduces their useability, though the trans configuration at the double bond imparts thermoplastic properties which may be commercially exploitable<sup>50</sup>.

### Alkanes

The alkanes are derived by removal of the acid moiety from fatty acids. They are most commonly found outside the plant as a component of the cuticular wax. Most alkanes are odd-numbered carbon chains, though even-numbered molecules are also found. The most abundant of the alkanes are the C29 and C31 structures.

The most common uses for these hydrocarbons are in polishes, sizings, emulsions finishes and as a food additive. But there are other potential uses for these products. The jojoba plant contains a significant amount of an alkane-fatty acid ester that can be used as a lubricant. The major alkane constituents are the mono-unsaturated C20 and C22 alkanes<sup>51</sup>. Plant waxes are important in plant-insect interactions<sup>52</sup>, and alkanes might play a role in this: parasitic predators are attracted to crop-damaging insects by the presence of specific alkanes<sup>53</sup>. It is not yet known if plants have a role in the synthesis of these hydrocarbons, but it is possible that they may use this system as a means of protection.

Plants also produce alkanes at levels that may allow their use as an energy crop. Up to 30% of the steam distillate from the fruits of the P. resiniferum tree is made up of alkanes<sup>54</sup>. The major alkane constituents are heptane and nonane, which can be used directly as gasoline substitutes.

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