CHAPTER II

HISTORICAL

1. Botanical aspect of *Piper nigrum* Linn.

Piper nigrum Linn. (Fig. 1) is in the family of Piperaceae. It local names in various countries are pepper (English); pepe (Italian); pimenta (Spanish); poiure (French); prik-thai (Thailand). The name "Pepper" is from the Sansakrit "Pippali". It means to fruit of *Piper nigrum* which has characteristic pungency and aroma (Stanford, 1934).

Piper nigrum is native of southern India but is now cultivated extensively in the tropical areas including Thailand. The plant is a perennial, climbing shrub or vine with a smooth and woody. The plant clings to a tree or other support by means of numerous short rootlets produced at the joints of the stem. In its natural stalk, the plant may reach a height of 20 to 25 feet, but under cultivation it is usually kept down to 15 feet. The leaves are alternate, simple, ovate, dark green, entire with nearly rounded base and a some what acute tip. The flowers are very small, whitish, sessile, perfect and borne in pendulous, dense in spikes. The fruit is small, containing a single seed, sessile, nearly globular; at first green then yellowish and

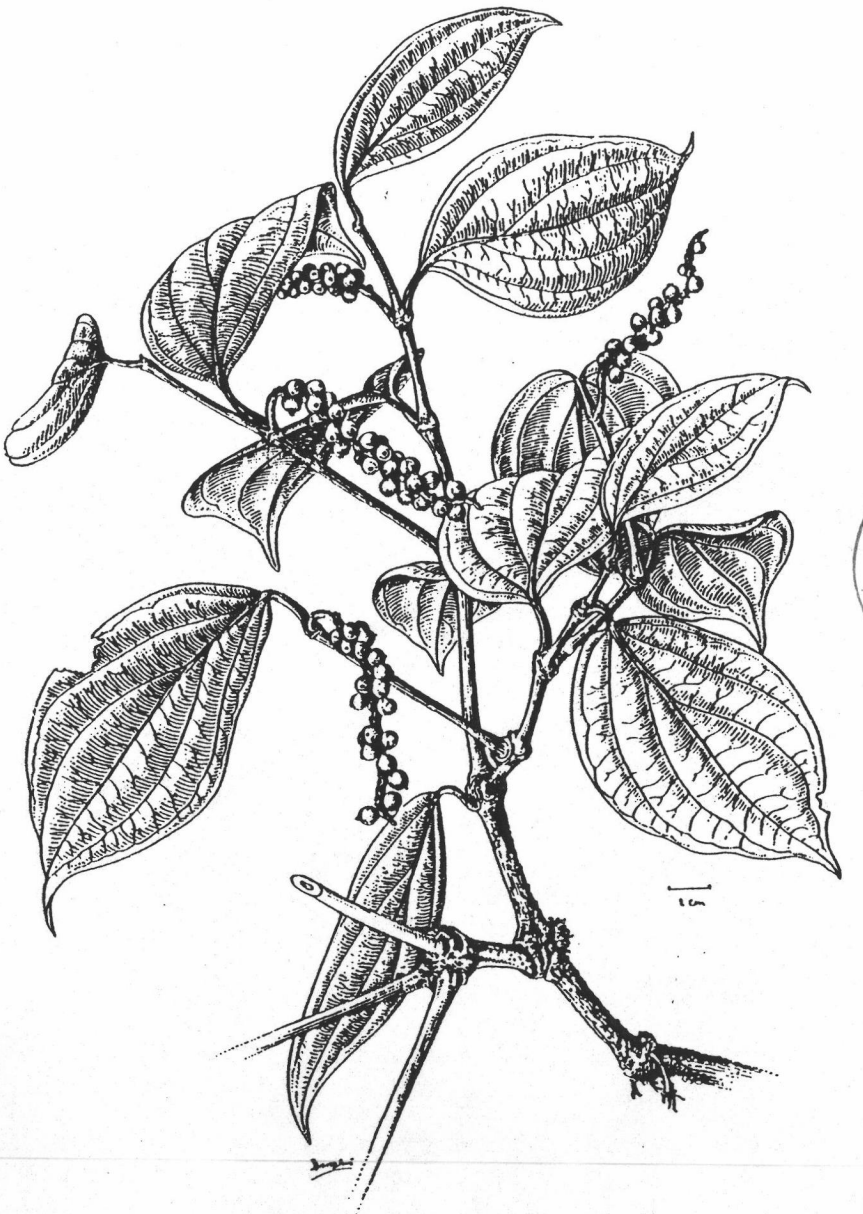


Figure 1 *Piper nigrum* L (Piperaceae)

finally red when ripe. The fruit is borne on spikes 4 to 5 inch long, each spike carrying from 50 to 60 berries.

Piper nigrum requires a warm, humid, tropical climate, with heavy rainfall and intermittant spells of dry weather and some shade. The most suitable soil a well-drained vegetable loam. The land should be flat; hillsides, if used for the growing of pepper, should be terraced (Guenther, 1952).

2. Microscopy of *Piper nigrum*

A cross section of the black pepper berry consists of the following (Fig. 2). Pericarp consists of : a) an pericarp of polygonal cells with containing dark brown contents; b) hypoderm of polygonal cells and groups of radially elongated stone cells with dark contents; c) a mesocarp containing isolated oil cell, fibrovascular bundles and on its inner side definite band of oil cells; d) an endocarp containing a single layer of beaker cells with strongly thickened inner tangential wall; and e) a spermoderm containing a double row of brown pigment cells, the inner ones darker than the outer (Claus, 1956).

Within the pericarp, lies the seed consists of endosperm, a small embryo, embedded in endosperm and a large amount of perisperm in which there is frequently a central cavity. Perisperm consists of hyaline layer, aleurone cells and reserve parenchyma, most of the cells

containing starch masses a scattered number, oil globules, resin masses and piperine (Claus, 1956; Perry, 1969; Trease and Evans, 1978; Youngken, 1950).

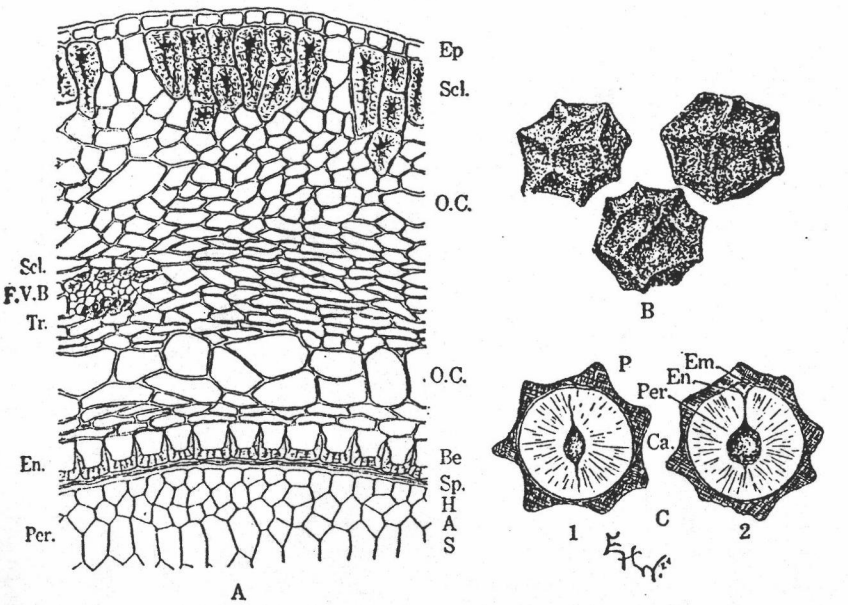


Figure 2 Cross section-Black Pepper. *B*, commercial whole fruit 3.5 to 6 mm . in diameter, blackish brown and coarsely reticulate. *C*, lens view of sections of whold fruit; *(1)* transverse and *(2)* lontigitudinal: *P*, pericarp; *Per*, perisperm; *En*, endosperm; *Em*, embryo. *A*, transverse section of the pericarp: *Ep*, epidermis; *Scl*, sclerenchyma; *OC*, oil cells; *FVB*, fibrovas cular bundle; *Tr*, tracheae; *En*, endodermis of beaker cells; *Sp*, spemo erm; *Per*, perisperm hyaline layer, *A*, aleurone layer, *S*, starch layer. (Drawings by E.H.Wirth.)

Powdered black pepper (Fig. 3) is grayish brown; aromatic in odour consists of numerous stone cell, beaker cells, compact masses of minute starch grains, parenchyma fragments containing oil cells with brownish suberied cells, yellowish oil globules and needle shaped piperine crystals (Claus, 1956; Youngken, 1950).

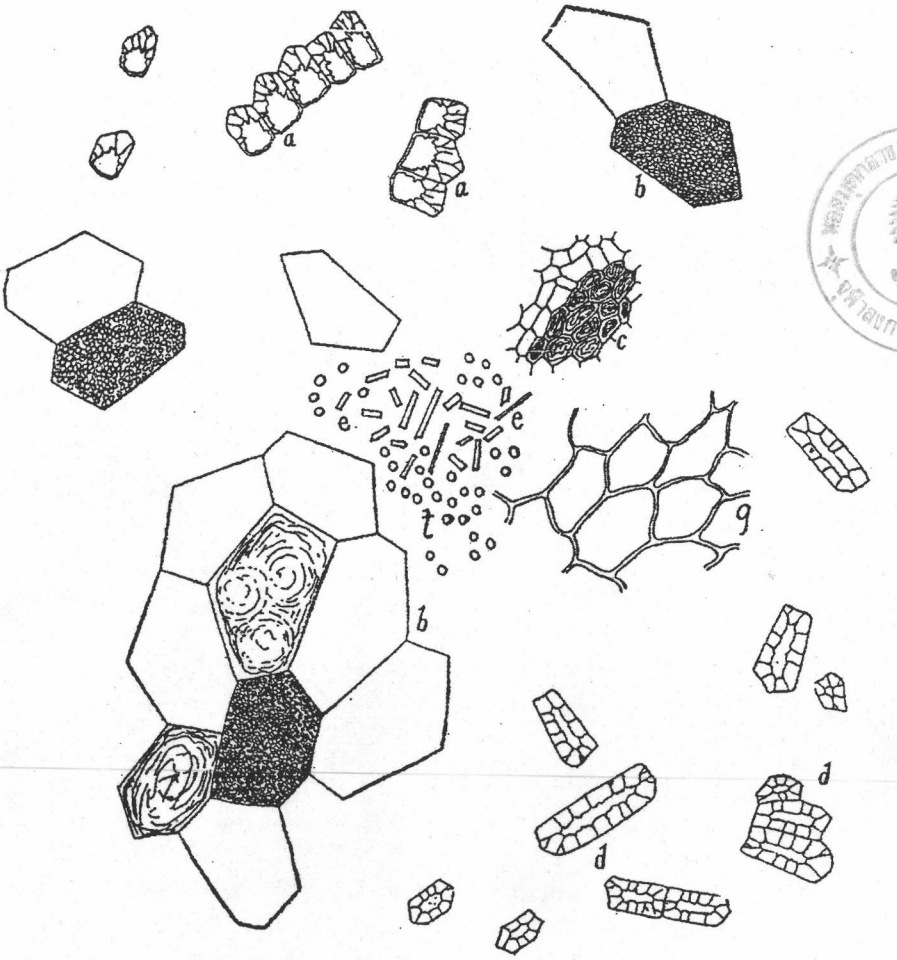


Figure 3 Powdered Black Pepper. *(a)* Stone cells with unequally thickened walls *(b)* perisperm tissue with starch and resin; *(c)* epidermal cells; *(d)* typical stone cells; *(e)* crystals of piperine; *(f)* starch granules; *(g)* parenchyma. *(After Schneider.)*

3. The uses of *Piper nigrum*

Piper nigrum, both black and white pepper are available whole, cracked, coarsely ground or finely ground. Black pepper is prepared from the whole unripe but fully developed berry. Native producers spread the freshly picked spikes on reed mats, exposing them to the sun for about a week. To remove the berries from the stalks the heaped-up material is beaten with sticks, or native, the latter procedure entailing a minimum of waste. On drying in the sun, the colour of the berries changes from green (or red) to dark brown or almost black. During the drying process the material must be turned over frequently to prevent formation of mildew. After completion of the drying, the berries can be separated from the stalks, leaflets and other impurities. Drying process is carried out as rapidly as possible, to prevent formation of mold in the final product. For white pepper, the older and more frequently practiced procedure is the following; freshly picked spikes bearing ripe (red) berries are piled on mats or on a concrete floor and beaten with sticks, or treaded upon, until the berries drop off the stalks. The berries are the placed into concrete tanks filled with slowly running water warmed by the sun. After eight to ten days of soaking, the skins decay and come off the berries. The removal is hastened by trampling upon the thick mass in the water. After cleaning with running water, the white berries are spread in the sun to dry, frequently turned over, and finally winnowed in the usual way

(Guenther, 1952). Pepper has numerous culinary uses, including seasoning and flavouring of soups, meats, poultry, fish, eggs, vegetables, salads, sauces and gravies (Duke, 1985). It is employed commercially in dehydrated and canned soups, poultry dressings, pickles, condiments, pickling spice mixes, baked goods, confections, curry powder blends, nonalcoholic beverages and practically all meat seasonings (Farrell, 1985).

Medicinally, pepper is considered aromatic, carminative, stomachic, febrifuge and stimulant. Its action as a stimulant is more obvious especially evident on the mucous membranes of the rectum and urinary organs. For external uses, it is considered rubefacient and irritant. It is regarded as useful remedy in haemorrhoidal affections and in relaxed conditions of the rectum attended with prolapsus. It is likewise given in combination with aperients to facilitate its action and prevent griping (Bose, 1928). It was once employed in treatment of gonorrhoea and chronic bronchitis (Trease and Evans, 1989). When applied locally as a gargle, pepper has been found to be useful in the treatment of relaxed uvula, paralysis of the tongue and in other affections of the mouth or throat (Bose, 1928).

In China, pepper has been suggested for the treatment of cholera, malaria, abdominal fullness, adenitis, cold, colic diarrhea, dysentery, dysmenorrhea, dysuria, furuncles, headache, gravel, urinary calculus, nausea, poisoning due to fish, mushrooms or shellfish (Duke, 1985; Perry, 1980).

A poultice made from the pepper plus salt and vinegar, is used for corns (Duke, 1985).

In Malay Peninsula, pepper is included in tonics, mixtures for indigestion and prescriptions used in and after childbirth. It may be combined with wild bamboo shoots or with honey and ginger as an abortifacient (Ridley, 1897).

In India, pepper was employed in folklore medicine for treatment of asthma, bronchitis, pyrexia, insomnia and abdominal disorders (Atal *et al.*, 1975; Chopra and Chopra, 1955; Kirtikar and Basu, 1944 ; Perry, 1980).

In Thailand, pepper has been used in folklore medicine for carminative, stomachic, febrifuge and expectorant (สาธสนม, 2526).

4. Chemical constituents of *Piper nigrum*

Different parts of *Piper nigrum* have been studied for their chemical constituents, especially the fruit part. Pepper contains 2 to 4% volatile oil and 5 to 9% piperine, piperidine, piperettine and other minor alkaloids (Youngken, 1950). In term of nutrition, the fruit (per 100 g) has been reported to contain 255 calories, 10.5% water, 11.0 g protein, 3.3 g fat, 64.8 g total carbohydrate, 13.1 g fiber, 4.3 g ash, 437 mg Ca, 173 mg P, 28.9 mg Fe, 44 mg Na, 1,259 mg K, 114 μg β-carotene equivalent, 0.11 mg thiamine, 0.24 mg riboflavin and 1.14 mg niacin (Leung, 1980). The groups of compounds found in *Piper nigrum* are isobutylamides, pyrrolidides, piperidides, phenylpropanoids, flavones, benzenoids and terpenoids. The list of the compounds found in various parts of *Piper nigrum* is shown in Table 1 and the list of terpenoids in pepper oil is shown in section 6.1 (Table 3).

Table 1 Chemical constituents found in various parts of *Piper nigrum*.

Plant part	Category	Chemical substance	Reference
Fruits	Isobutylamides	pellitorine (N-isobutyl deca-*trans* -4-dienamide	Ohigashi *et al* ., 1983
		N-isobutyl-*trans* -2-*trans* -4-octadecadienamide	Nakatani and Inatani, 1981
		N-isobutyl-*trans* -2-*trans* -4-eicosadienamide	Raina *et al* ., 1976
		N-isobutyl-*trans* -2-4-*cis* -8-eicosatrienamide	Nakatani and Inatani, 1981
		N-isobutyl-13(3,4-methylene dioxyphenyl) *trans* -2-*trans* -4-*trans* -8-tridecatrienamide	Nakatani and Inatani, 1981
		pipercide	Su and Horvat, 1981 ; Tabuneng *et al* ., 1983
		dihydropipercide	Su and Horvat, 1981
		guineesine	Miyakado *et al* . ,1980
	Pyrrolidides	trichostachine	Nakatani and Inatani, 1981
	Piperidides	piperine	Varzele and Qureshi, 1980
		piperidine	Hewitt *et al*., 1957; Jennings and Wrolstad, 1961
		coumapenine	Nakatani *et al*.,1980
		piperoleines A	Ohigashi *et al*.,1983
		piperoleines B	Ohigashi *et al*.,1983
		piperettine	Spring,1950
		N-5-(4-hydroxy-3-methoxyphenyl)-penta-*trans* -2-*trans* -4-dienoyl-piperidine	Inatani *et al* .,1981

Table 1 (continued)

Plant part	Category	Chemical substance	Reference
	Piperidides	N-*trans*-feroloyl piperidine	Inatani *et al* .,1981
	Miscellaneous alkaloids	N-*trans* -ferulyl tyramine	Nakatani *et al*.,1980
Fruits	Phenylpropanoids	eugenol methyl ether	Russell and Jennings,1969
		myristicin	Russell and Jennings,1969
		safrole	Russell and Jennings,1969
		caffeic acid	Schulz and Herrmann, 1980
	Flavones	kaempferol glycosides	Herrmann, 1979
		quercitrin	Herrmann, 1979
		isoquereitrin	Herrmann, 1979
		rhamnetin glycosides	Herrmann, 1979
		quercetin glycosides	Herrmann, 1979
		rutin	Herrmann, 1979
	Benzenoids	piperonol	Hewitt *et al* .,1957
Stems	Hydrocarbons	n-hentriacontane	Singh *et al* .,1976
		hentriacontan-16-ol	Singh *et al* .,1976
		hentriacontan-16-one	Singh *et al* .,1976
	Piperidides	piperine	Hodorn and Jungkunz,1951
Leaves	Phenylpropanoids	eugenol	Alencar *et al*.,1984
Seeds	Piperidides	piperine	Verzele and Qureshi,1980
		piperanine	Traxler,1971

5. Piperine

5.1 Structure and chemical properties

Piperine (1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine; 1-piperoyl piperidine; $C_{17}H_{19}NO_3$; MW 285.33) (Fig. 4)

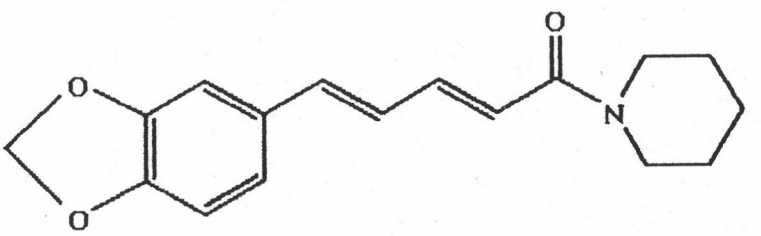
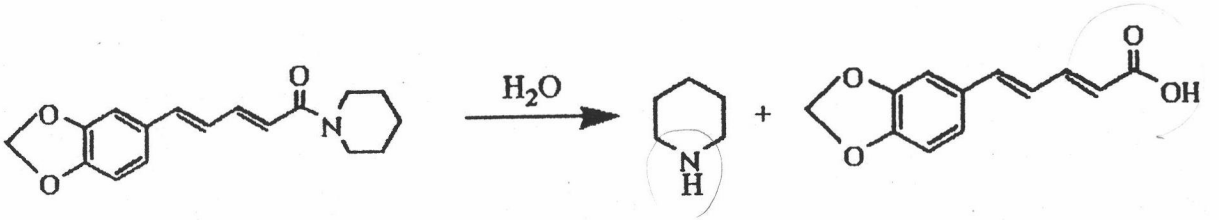


Figure 4 The structure of piperine.

The piperidide alkaloid piperine was first isolated from *Piper nigrum* in 1820 (Purseglove, 1981). It was later isolated from other *Piper* Spp. (Table 2). It has also been found in *Psilocaulon obsimile* (Family Aizoaceae), in fruits of *Xylopia brasiliensis* (Family Annonaceae) (Darnley, 1974) and in leaves of *Rhododendron fauriae* var *rufescens* (Family Ericaceae) (Kawaguchi, 1942). On hydrolysis by acid or alkaline, piperine decomposes into strongly basic piperidine and piperic acid (Fig. 5) (Guenther, 1952). Piperine can be photoisomerized into a mixture other configuration (Grewe *et al.*, 1970).

Table 2 Piperine in *Piper* **Spp.**

Piper	Plant part	Reference
Piper album Vahl.	fruit	Glasl *et al* ., 1976
P. chaba Hunter	stem	Bose, 1935
P. cubeba Linn.	fruit	Hodorn and Jungkun, 1951
P. quineense Schum & Thonn	root	Addae-Mensah *et al* ., 1977;Dwuma-Badu *et al* ., 1976;Okogum and Ekong,1974
	fruit	Addae-Mensah *et al*., 1977
P. longum Linn.	root	Dutta *et al* ., 1977
	fruit	Dhar and Atal, 1967;Govindachari *et al* ., 1969
P. nepalense Miq.	stem	Gupta *et al* ., 1972
P. nigrum Linn.	fruit	Nakatani and Inatani, 1981;Verzele and Qureshi, 1980
	stem	Singh *et al* ., 1976
	seed	Traxler, 1971
P. novae-hollandiae Miq.	stem	Loder *et al* ., 1969
P. peepuloides Roxb.	fruit	Dhar and Raina, 1973
P. retrofractum Vahl.	stem	Mishra and Tewari, 1964
P. sylvaticum Roxb.	root	Barnerji and Dhara, 1974



piperine piperidine piperic acid

Figure. 5 The hydrolysis of piperine.

For the physical properties, piperine has crystalline in monoclinic prism melting at 129-130°C, tastless at first but burning after taste. It is neutral to litmus (pK (18°C) = 12.22; K = $6x10^{-13}$) almost insoluble in water and petroleum ether but readily soluble in alcohol, chloroform, ether, benzene and acetic acid (Budavari, 1989). Piperine has steriochemistry of *trans-trans* form. Its UV spectrum shows λ_{max} at 345 nm (log Σ 4.47). The characteristic spectra (Cordell, 1981) of IR (Fig. 6), NMR (Fig. 7) data on ^{1}H-NMR (Fig. 8) and data on ^{13}C-NMR (Fig. 9) are shown below.

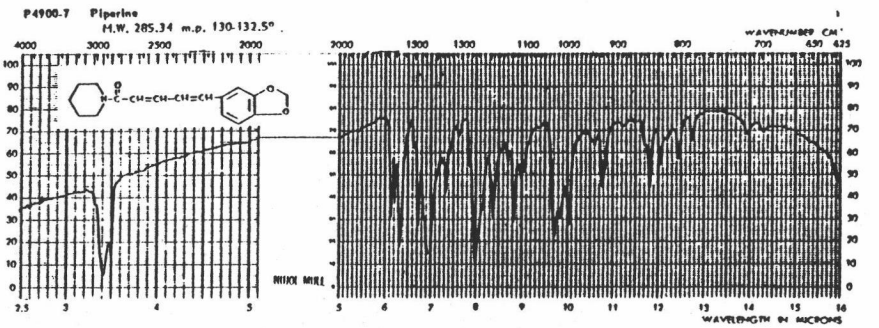


Figure 6 IR spectrum of piperine.

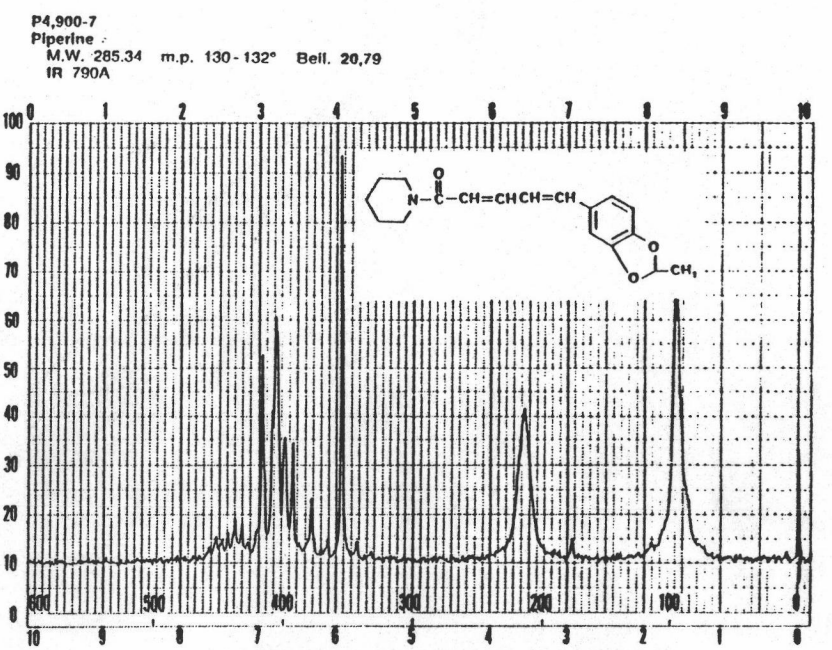


Figure 7 ^{1}H-NMR spectrum of piperine.

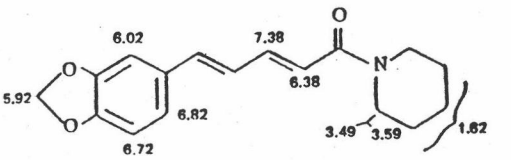


Figure 8 Data on ^{1}H-NMR of piperine.

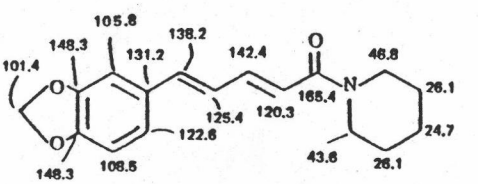


Figure 9 Data on ^{13}C-NMR of piperine.

5.2 Biological activities of piperine

Piperine is considered an active principle of *Piper nigrum* which is commonly used as condiment and employed in folklore medicine for treatment of asthma, bronchitis, pyrexia, insomnia and abdominal disorders (Atal *et al.*, 1975; Chopra and Chopra, 1955; Kirtikar and Busu, 1944; Perry, 1980). There have been a number of reports on its pharmacological activities and toxicity which are described as follows.

5.2.1 Pharmacological activities

Piperine has been found to possess depressant properties on the central nervous system (Pei, 1983; Woo *et al.*, 1979). Piperine and its derivatives have been reported to act as anticonvulsant and can potentiate the sedative effect of some depressing agents such as hexobarbital and pentobarbital (Mujumdar *et al.*, 1990; Pei, 1979, 1983; Pei and Xie, 1980; Shin and Woo, 1979; Woo *et al.*, 1979). Piperine has also been found to decrease blood pressure and respiration rate in cats and dogs (Gabor-Jancso, 1971; Neogi *et al.*, 1971). Recently, it has been shown to decrease blood pressure and heart rate in rats, presumably due to vagus nerve stimulation of the compound (Ukarachata, 1988).

In folklore medicine of many Asian countries including Thailand, pepper has been used in indigenous drug preparations for inducing menstruation and termination of early pregnancy (Chandhoke *et al.*,1978; Piyachaturawat *et al.*, 1982). This is thought to be the

effect of piperine since the compound has been shown to inhibit implantation, produce abortion and delay labor in mice (Chailurkit, 1984; Piyachaturawat *et al.*, 1982).

Piperine has been reported to have non-specific spasmolytic activities in fruit-bat and guinea pig (Cole, 1985), anti-inflammatory activity in rats (Mujumdar, 1990), antipyretic activity in rabbits (Lee, 1984) and analgesic activity in mice (Lee, 1984). It has also been shown to stimulate the movement of male sexual organ of *Daphnia magna* (Viehoever and Conen, 1937) and exert bronchial contraction activity in guinea pig (Szolcsanyi, 1983).

For bactericidal activities, piperine has been reported to have an inhibiting effect on *Lactobacillus plantarum*, *Micrococcus specialis* and two fecal microorganisms, *Escherichia coli* and *Streptococcus faecalis* (James, 1985). The compound has been found to have antifungal activity on *Aspergillus parasiticus* with a 50% inhibition dose of 1,000 ppm and a dose of 10,000 ppm can inhibit aflatoxin production up to 98% (Madhyastha and Bhat, 1984). In addition, piperine has been reported to have bactericidal activity on *Mycobacterium smegmatic* (100 μg/ml) and fungicidal acitivity on *Candida albicans* (100 μg/ml) (Mitscher et al., 1972).

In some experiments, piperine has been found to cause a significant induction of hepatic mixed function

oxidase system (Shin and Woo, 1980, 1985). Recent study has also shown the effect of piperine on the hepatic drug-metabolizing enzymes and demonstrated that piperine is a potent nonspecific inhibitor of drug metabolism (Atal *et al.*, 1985). More recently, it has been found that piperine possesses inhibitory action to liver mitochondrial biogenetics (Reanmongkol *et al.*, 1988).

5.2.2 Toxicity of piperine

It has been reported that piperine markedly increases the efficiency of pyrethrin in killing house flies (Duke, 1985). It improves insecticidal activity like eucalyptus oil. Also, piperine is synergistically insecticidal to rice weevils and cowpea weevils (Harville *et al.*, 1943, 1947; Mutsubara and Tanimura, 1966; Synerholrn *et al.*, 1945). The compound has been used as insect repellent in corn earthworm, *Heliothis obsoleta* (F.) and been weevil, *Acanthoscelides obtectus* (Say) (Freeborn and Wymore, 1929; Lathrop and Keirstead, 1946). In addition, it has been reported that piperine can be used for killing mosquito larvae up to 100% (Choudhury and Das, 1983).

Since piperine and other pepper alkaloids have chemical structures similar to that of the mutagenic urinary safrole metabolite, pungent components of black pepper are sometimes suspected to have mutagenic and carcinogenic activity (Buchanun, 1978). It has been reported that the extract of black pepper can significantly increase the number of tumor-bearing mice

(Shwaireb, 1990). Recent study has shown that black pepper extract induces liver tumors in Egyptian toads (El-Mofly, 1991).

Acute toxicity of piperine has been investigated in various animals including mouse, rat and hamster (Piyachaturawat *et al.*, 1983). It has been reported to have a intravenous LD_{50} value of 15.1 mg/kg body wt. for mice (Piyachaturawat *et al.*, 1983). The daily intake of piperine in adult Indian population as estimated from the curry powder consumption is approximately 17 mg/kg body wt. (Srinavasan and Satyanarayana, 1981). However, there has been no evident report of piperine toxicity in human.

5.3 Identification

Piperine in powdered black pepper can be indentified by microchemical test as follows: 1) mount powdered black pepper in alcohol on a slide, and add a drop of water. Long needles of piperine will be formed readily and separated near the edge of the cover-glass or 2) mount powdered black pepper in hydrochloric acid which has been added a small crystal of cadmium acetate on a slide. After that, shaves of yellow needles consisting of piperine cadmium compound will be separated from powdered black pepper (Claus, 1956).

In addition, piperine from different sources has also been identified by colorimetric, UV-spectrophotometric or thin-layer chromatographic methods (Helrich, 1990;

Rao *et al.*, 1960). Recently, HPLC method has been used widely to identify and analyze piperine because it is more rapid and sensitive than other methods (Geister and Gross,1990; Rathnawatie and Buckle, 1983; Verzele *et al.*, 1979).

5.4 Separation

The separation of piperine is usually performed by organic extraction with chlorinated solvents such as ethylene dichloride and methylene dichloride. For purification, piperine can be separated from the crude extract by using column or thin-layer chromatography. UV light or spraying reagent (vanillin-sulphuric acid) are usually used for detection on the TLC plate (Hornstein, 1966; Wagner *et al.*, 1984).

5.5 The proposed biosynthetic pathway of piperine

Piperine, the pungent principle of pepper is thought to be biosynthesized by the so-called "phenylpropanoid pathway". It originates from the condensation of the N-heterocycle piperidine with the thioester piperoyl-CoA (Fig. 10). According to this pathway, the thioester piperoyl-CoA derived from malonyl-CoA with cinnamyl-CoA, the 3,4-position of methylenedioxy ring is typical of compounds of the phenylpropanoid class. The N-heterocycle piperidine derived from amino acid lysine (Geissmann and Crout, 1969).

Very recently, it has been clearly shown that the enzyme piperidine piperoyltransferase catalyses the

synthesis of piperine in the presence of piperoyl-CoA and piperidine (Geisler and Gross, 1990).

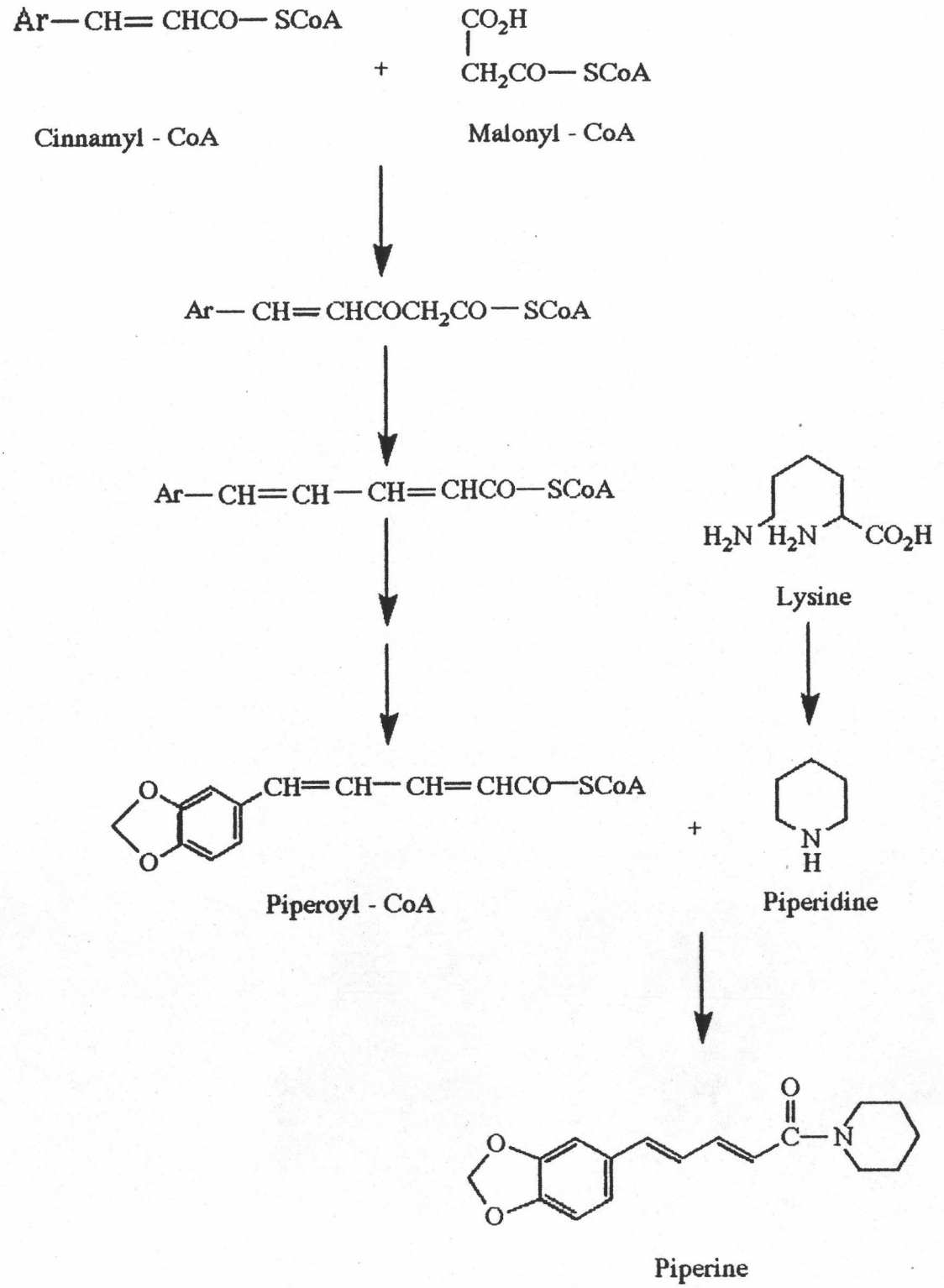


Figure 10 The proposed biosynthetic pathway of piperine.

6. Pepper oil

6.1 Composition of pepper oil

The volatile pepper oil is obtained by steam distillation from dried berries of *Piper nigrum*. It is an almost colorless to bluish-green liquid with a characteristic odour recalling that of whole pepper. The physical and chemical constants of pepper oil are described below (Budavari, 1989).

Specific gravity at 15°C	0.890-0.900
Refractive index at 20°C	1.4935-1.4977
Optical rotation at 25°C	-3° to -5°
Solubility	1:5 in 90% alcohol

A study on the composition in pepper oil was begun during the last century.The pepper oil is primary a complex mixture of hydrocarbon such as monoterpenes (50-80%), sesquiterpenes (20-40%) and small amounts of oxygenated terpene compounds (less than 4%) (Hasselstrom *et al.*, 1957; Ikeda*et al.*, 1962; Jennings and Wrolstad, 1961; Nigam and Handa, 1964; Richard *et al.*, 1971; Wrolstad and Jennings, 1965). The presence of terpenoids in black pepper oil including terpene hydrate, α-phellandrene and β-caryophyllene was reported over 90 years ago (Schreiner and Kremers, 1901). Later, a number of additional substances were identified including those shown in Table 3.

Pepper oils from different cultivars have their

Table 3 Constituents identified in black pepper oil

Constituents	Reference
Monoterpene hydrocarbons	
Camphene	Ikeda *et al* , 1962
Δ^3-Carene	Wrolstad and Jennings, 1965
p-Cymene	Wrolstad and Jennings, 1965
Limonene	Hasselstrom *et al* ., 1957
Myrcene	Wrotstad and Jennings, 1965
cis -Ocimene	Ikeda *et al* ., 1962
α-Phellandrene	Hasselstrom *et al* ., 1957
β-Phellandrene	Ikeda *et al* ., 1962
α-Pinene	Hasselsfrom *et al* ., 1957
β-Pinene	Hasselstrom *et al* ., 1957
Sabinene	Ikeda *et al* ., 1962
α-Terpinene	Ikeda *et al* ., 1962
γ-Terpinene	Ikeda *et al* ., 1962
Terpinolene	Wrolstad and Jennings, 1965
α-Thujene	Wrolstad and Jennings, 1965
Sesquiterpene hydrocarbons	
α-*cis* -Bergamotene	Russell *et al* ., 1986
α-*trans* -Bergamotene	Russell *et al* ., 1986
β-Bisabolene	Russell and Else, 1973
δ-Cadinene	Muller *et al* ., 1968
γ-Cadinene	Debrauwere and Verzele, 1976
Calamenene	Debrauwere and Verzele, 1976
β-Caryophyllene	Nigam and Handa, 1964
α-Copaene	Richard *et al* ., 1971
α-Cubebene	Richard *et al* ., 1971
β-Cubebene	Artem'ev and Mistryukov, 1979
ar-Curcumene	Russell and Jennings, 1969
δ-Elernene	Debrauwere and Verzele, 1976

Table 3 (continued)

Constituents	Reference
β-Elemene	Russell and Else, 1973
β-Farnesene	Russell and Else, 1973
α-Guaiene	Debrauwere and Verzele, 1976
α-Humulene	Muller *et al* ., 1968
Isocaryophyllene	Muller *et al* ., 1968
γ-Muurolene	Muller *et al* ., 1968
α-Santalene	Muller *et al* ., 1968
α-Selinene	Lewis *et al* ., 1969a
β-Selinene	Lewis *et al* ., 1969a
α-Patchoulene isomer	Artem'ev and Mistryukou, 1979
Oxygenated monoterpenes	
Borneol	Debrauwere and Verzele, 1975
Camphor	Debrauwere and Verzele, 1975
Cavacrol	Debrauwere and Verzele, 1975
cis -Carveol	Russell and Jennings, 1969
trans -Carveol	Russell and Jennings, 1969
Carvone	Russell and Jennings, 1969
Carvetonacetone	Debrauwere and Verzele, 1975
1,8-Cineole	Debrauwere and Verzele, 1975
Cryptone	Russell and Jenings, 1969
p-Cymene-8-ol	Russell and Jenings, 1969
p-Cymene-8-methyl ether	Debrauwere and Verzele, 1975
Dihydrocarveol	Hasselstrom *et al* ., 1957
Dihydrocarvone	Debrauwere and Verzele, 1975
Linalool	Russell and Jennings, 1969
Myrtenol	Debrauwere and Verzele, 1975
cis -Sabinene hydrate	Russell and Jennings, 1970
trans -Sabinene hydrate	Russell and Jennings, 1970
β-Pinone	Debrauwere and Verzele, 1975
1-Terpinen-4-ol	Richard and Jennings, 1971

Table 3 (continued)

Constituents	Reference
Oxygenated monoterpenes	
1-Terpinen-5-ol	Debrauwere and Verzele, 1975
α-Terpineol	Richard and Jennings, 1971
Phenyl ethers	
Eugenol	Richard and Jennings, 1971
Methyl eugenol	Richard and Jennings, 1971
Myristicin	Richard and Jennings, 1971
Safrole	Richard and Jennings, 1971
Anethole	Artem'ev and Mistryukov, 1979
Oxygenated sesquiterpenes	
β-Caryophyllene alcohol	Debrauwere and Verzele, 1975
Caryophyllene ketone	Richard *et al* ., 1971
Caryophyllene oxide	Debrauwere and Verzele, 1975
Epoxy-dihydrocaryophyllene	Hasselstrom *et al* ., 1957
Nerolidol	Richard and Jennings, 1971
Eudesmol	Artem'ev and Mistryukov, 1979
Miscellaneous	
Butyric acid	Debrauwere and Verzele, 1975
Hexanoic acid	Debrauwere and Verzele, 1975
Benzoic acid	Debrauwere and Verzele, 1975
Cinnamic acid	Debrauwere and Verzele, 1975
Piperonic acid	Debrauwere and Verzele, 1975
Piperonal	Debrauwere and Verzele, 1975
Piperidine	Hasselstrom *et al* ., 1957

Table 4 Aromatic attributes of the components of black pepper oil

Component	Main Character	Subsidiary Attributes
Monoterpene hydrocarbons		
α-Pinene	Pine-like	Warm, Resinous, Refreshing
Camphene	Camphoraceous	Oily, Terpeney
Sabinene	Peppery	Warm, Woody, herbaceous
β-Pinene	Dry woody	Resinous, Pine-like, Terpeney
Myrcene	Sweet balsamic	Resinous, Lemony, Fresh
α-Phellandrene	Peppery	Woody, Fresh, Citrus, Minty
Δ^3-Carene	Penetrating	Sweet, Irritating
α-Terpinene	Lemony	Fresh
β-Phellandrene	Peppery	Minty, Slightly citrus-like
Limonene	Fresh	Light, Orange-like
γ-Terpinene	Herbaceous	Warm, Lemony
Terpinolene	Sweet piny	Slightly anisic
Sesquiterpene hydrocarbons		
β-Caryophyllene	Woody spicy	Dry, Clove-like
β-Farnescene	Sweet	Warm
Humulene	Sweet woody citrus	Penetrating
β-Selinene	Sweet woody	Peppery
α-Selinene	Herbaceous	Warm, Woody, Peppery
β-Bisabolene	Warm spicy	Balsamic, aromatic
Oxygenated compounds		
Linalool	Floral woody	Light, Refreshing, Slightly citrus-like
1-Terpinen-4-ol	Warm pepery	Earthy, Woody
Carvone	Warm herbaceous	Spicy, Slight floral
Caryophyllene ketone	Fruity	Minty

own characteristic odour due to the different proportion of their volatile components. The aromatic characters of the component present in black pepper oil are shown in Table 4 (Lewis *et al.*, 1969b).

6.2 Biological activities of pepper oil

Pepper oil has been reported to have biological activities against microorganisms. It showed antifungal activities againt *Rhizopus nigricans* but has no antifungal activities againt *Fusarium oxysporum, Candida albicans, Phoma betae* and *Saccharomyces cereviseae* (Maruzzella *et al.*, 1960). It has been used to correct intestinal disorders resulting form faulty diets (Mukherji, 1954). Pepper oil is a valuable adjunct in the flavouring of sausages, canned meats, soups, table sauces and certain beverages and liquors. It has been used in perfumery particularly in bouguets of the oriental type (Guenther, 1952).

Some components found in pepper oil such as α-pinene, β-pinene, terpinolene, limonene and α-phellandrene have been shown to inhibit bacterial growth of *Bacillus subtilis, Salmonella enteritidis, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus morganii* and *Escherichia coli* at dilution lower than 20 to 200 times (Katayama and Nagai, 1960).

6.3 Identification

Recently, gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) have been used

to identify the components of pepper oil (Buckle *et al.*, 1985). Identification by GC is usually carried out by the relative retention method which gives information on various components present in volatile oil and characteristic chromatographic pattern of the pepper oil. For mass spectrometry, the components are identified by comparison with the mass spectra of authentic samples analyzed under the same conditions with spectra published in the literature (Masada, 1976; Yamaguchi, 1970) and with spectra in the mass spectrometry library (Linskens and Jackson, 1991).

6.4 Isolation

Commercial preparation of pepper oil is mostly carried out by means of steam distillation. For the laboratory scale, hydrodistillation is more practical. Solvent extraction using ether gives pepper oleoresin which contains both volatile oil and pungent nonvolatile compounds. The basic principle in steam distillation is to boil pepper in distilled water, a process that results in the disruption of gland cell and release of the volatile oil contained therein. The steam containing volatile oil passes through a cold water condenser allowing the volatile oil fraction to float on top of the water. Collection can be made by drawing out the water followed by the volatile oil. Typically, steam distillation for 3 to 5 hours is required (Linskens and Jackson, 1991).

6.5 The proposed biosynthetic pathway of monoterpens and sesquiterpenes in pepper oil

Monoterpenes and sesquiterpenes in pepper oil can be found in high levels. The biosynthetic precursor of terpenes is isoprene unit, the compound arises from acetate *via* mevalonic acid. From Fig. 11, geranyl pyrophosphate is the C-10 precursor of the terpenes and is believed to play a key role in the formation of monoterpenes. It is formed by the condensation of each unit of isopentenyl pyrophosphate and dimethylallyl pyrophosphate. Geranyl pyrophosphate is believed to be the direct precursor of acyclic monoterpenes. However, it must be isomerized to neryl pyrophosphate before the formation of cyclic monoterpenes. Sesquiterpenes are considered to be biosynthesized from farnesyl pyrophosphate by the condensation of geranyl pyrophosphate and isopentenyl pyrophosphate (Porter and Spurgeon,1981; Stumpf and Conn,1980; Tyler *et al.*, 1988).

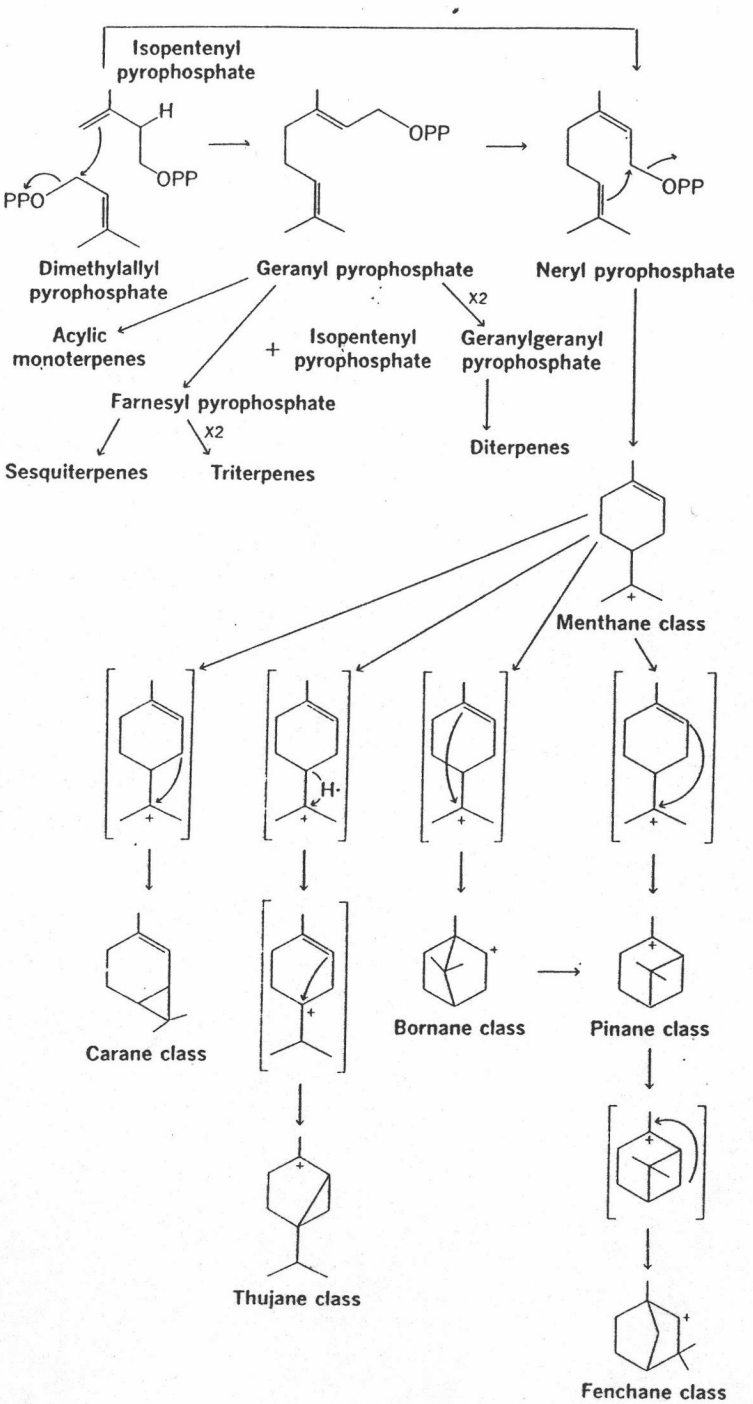


Figure 11 Hypothetic mechanism for biosynthetic of terpenes.