CHAPTER II

HISTORICAL

1. Botanical Aspect of *Curcuma longa* L.

Curcuma longa L. (Fig.1) is in the family of Zingiberaceae. Its synonym is *Curcuma domestica* Val. Its local names in various countries are Turmeric, Indian Saffron, Curcuma, Yellow Root (English) (Reynold, 1989); Kunyit (Indonesia); Luyang-Dilow (Philippines); Hsanwen (Burma); Chiang Huang, Yu Chin (Chinese); Wat Kam (Malaya); Manjal (Tamil); Ukon (Japanese) and Khaminchan (Thai) (Public Health, Ministry, 1990).

Curcuma longa is mostly cultivated in India,China, Indonesia and other tropical countries including Thailand. This plant is a perennial herb with freshy rhizomes and tuberous roots. The rhizomes contain a main bright orange-yellow compound called "curcumin" (Burkill, 1935; Claus, 1956; Parry, 1969; Sastri, 1950; Trease and Evans, 1983; Youngken, 1950). The height of this plant is up to one metre. Its leaves are lanceolate up to 40 cm long and 7-8 cm wide. Petiole is thin, with the small ligule and ciliate sheath at the edge. Inflorescence apical is on the leafy shoot, 10-15 cm long and 5-7 cm

Fig 1. *Curcuma longa* L. (Zingiberaceae)

wide. The bracts are white or greenish-white about 5-6 cm long, calyx is tubular, unequally toothed. The corolla is tube funnel-shaped and split down one side, its color is pale yellow with a yellow bar down the lip. Stamen is petaloid, ovary is 3-locule. The fruits are globose capsule with a 3-valved, arillate seed inside (Faculty of Mahidol University, 1986; Public Health, Ministry, 1990; Parry, 1969; Youngken, 1950).

Curcuma longa can grow in most places, ranging from sea level to 1,200 m. This plant prefers sandy and clayey loam containing alluvium or deposit silt with facility for good drianage and adequate water (Public Health, Ministry, 1990; Sastri, 1950). This crop is usually ready for harvest in 7-9 months after planting.

2. Microscopy of *Curcuma longa* Rhizome

2.1 Intact Rhizome

Figure2 (Faculty of Pharmacy Mahidol University, 1986) shows transverse sections of turmeric rhizome which depict the following characteristic features :

1. Cork, composed of many layers of thin-walled, rectangular, brownish cork cells.

2. Cortex, consisting of a broad zone of reserve parenchyma with intercellular spaces, these cells being filled with starch granules, yellowish volatile oil or yellow coloring matter. A layer of thin-walled, rectangular pseudoendodermal cells divides the cortical zones into the outer and inner parts, the latter about twice as broad. The fibrovascular bundles are found scattered throughout this zone and occur more numerously just beneath the pseudoendodermis.

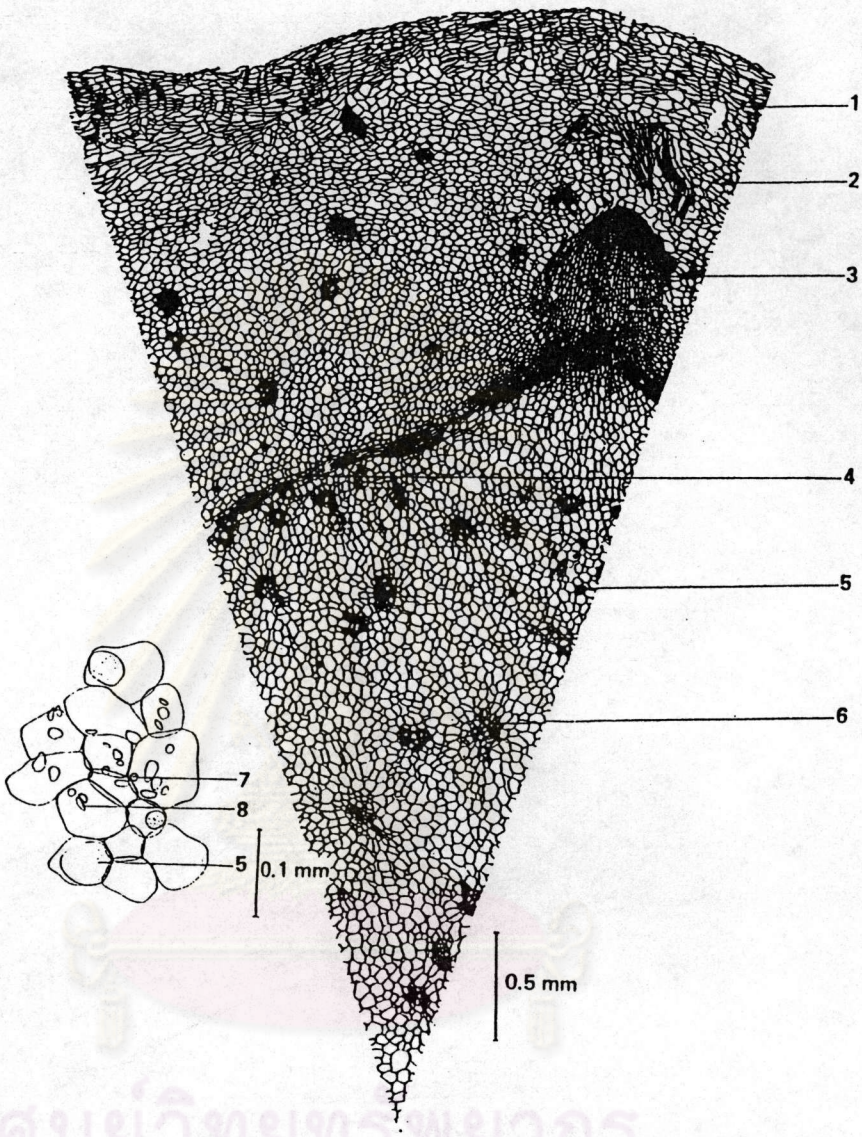


Fig 2. Transverse section of *Curcuma longa* L. rhizome

1. cork
2. parenchyma
3. root primordia
4. pseudoendodermis
5. parenchyma with yellow coloring matter
6. vascular bundle
7. oil droplets
8. starch granules

2.2 Powdered Turmeric

Powdered turmeric is bright orange-yellow with an aromatic odor and a pungent taste. The diagnostic characters (Fig 3.)(Faculty of Pharmacy Mahidol University, 1986) are as follows :

1. The abundant groups of thin-walled, round to oval parenchymal cells, filled with gelatinised starch, oil droplets and bright yellow coloring matter "curcumin" which is soluble in aqueous mount. Some isolated parenchymal cell filled with gelatinised starch are also present.

2. The fairly abundant fragments of brownish cork cells which appear polygonal in surface view.

3. The fairly abundant fragments of vessels with reticulate and scalariform thickening.

4. Few fragments of epidermis, in surface view, composing of straight-walled, polygonal to elongated cells with some cicatrices.

5. The occasional starch granules which are simple, flattened, oblong to oval or irregular in outline, some with a small point hilum situated at the narrow end, 15-30 μm long and 10-17 μm wide.

6. The covering trichomes, which are unicellular, elongated with slightly thick-walled and faintly striated.

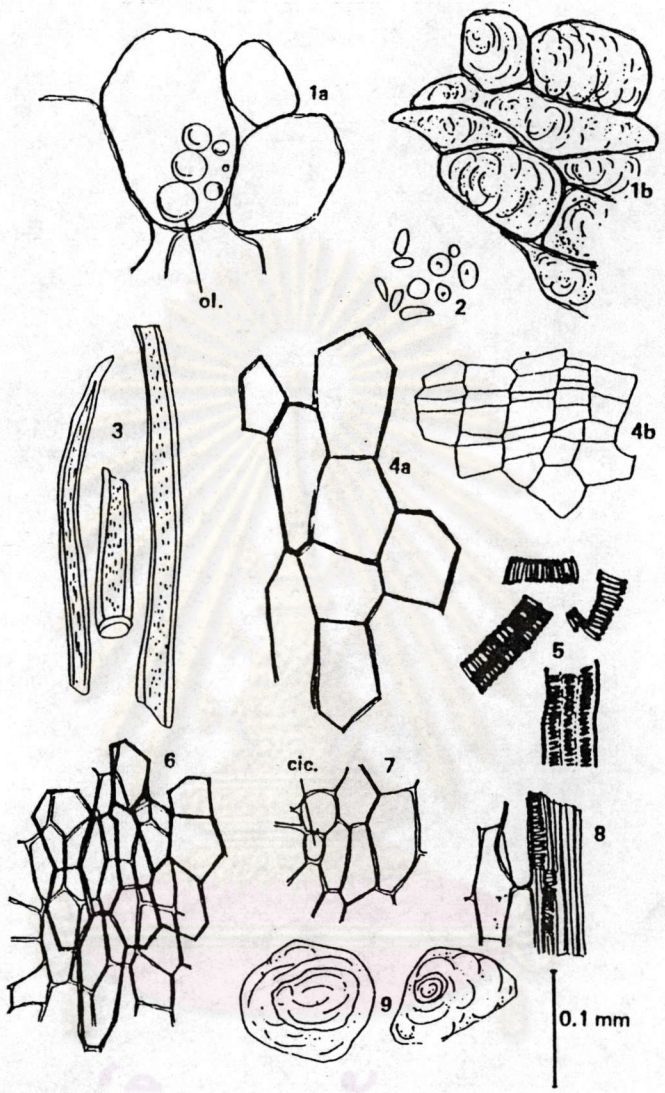


Fig.3 Powder of *C. longa* rhizome

1. parenchymatous cells containing a. oil globules (ol.), b. gelatinized starch
2. starch granules
3. fragments of covering trichomes
4. cork: a. in surface view, b. in sectional view
5. fragments of vessels; spirally, reticulately and scalariformly thickened
6. cork in surface view with underlying epidermis
7. epidermis in surface view showing a cicatrix (cic)
8. parenchymatous cells in longitudinal view, associated with vessels and fibers
9. isolated parenchymatous cells filled with gelatinized starch

3. Specification of High Quality Turmeric Powder

Commercially, turmeric powder should comply with requirements given in the table 1.

Table 1. Specification of high quality turmeric powder

Characteristic	Requirement	Method of test
Moisture content (%w/w)	$\leq$ 10	-TP* appendix 4.12
		-ISO** 939
Foreign matter (%w/w)	$\leq$ 2	-BP*** 1988
		Appendix XID
Total ash (%w/w)	$\leq$ 9	-ISO 928
		-BP 1988 App. XII
Acid insoluble ash (%v/w)	$\geq$ 1.5	-ISO 930
		-BP 1988 App. XIK
Volatile oil content (%w/w)	$\geq$ 7	-BP 1988 App.XIE
Curcuminoid content (%w/w)	$\geq$ 2	-ISO 5566
	$\geq$ 4	-ASTA**** method 18
	$\geq$ 7	-Public Health of
		Thailand,Ministry,
		1990

* = Thai Pharmacopoeia (Public Health of Thailand,Ministry, 1987)

** = International Organization for Standardization (International Organization for Standardization, 1983)

*** = British Pharmacopoeia (The medicine commission, 1973)

**** = American Spice Trade Association (American Spice Trade Association, 1958)

4. Chemical Constituents of *Curcuma longa* L.

There are a number of reports on the chemical constituents in turmeric rhizome. The group of compounds commonly found in turmeric are curcuminoids, volatile oil, oleoresins, carbohydrates, proteins and minerals. List of the compounds found in *C. longa* rhizome is shown in Table 2.

Table 2. Chemical constituents of *C. longa* rhizome

Chemical group	Chemical Constituent	Reference
Curcuminoids	curcumin	Roughley and Whiting, 1973
	demethoxycurcumin	Roughley and Whiting, 1973
	bisdemethoxycurcumin	Roughley and Whiting, 1973
	dihydrocurcumin	Ravindranath and Satyanarayana, 1980
Volatile oil		
-monoterpenes	p-cymene	Guenther, 1952; Parry, 1969
	p-cymenene	Guenther, 1952; Parry, 1969
	α-pinene	Guenther, 1952; Parry, 1969
	α-phellandrene	Guenther, 1952; Parry, 1969
	1,8-cineol	Guenther, 1952; Parry, 1969
	terpinolene	Guenther, 1952; Parry, 1969
	terpineol	Guenther, 1952; Parry, 1969
	camphene	Guenther, 1952; Parry, 1969
	sabinene	Guenther, 1952; Parry, 1969

Table 2. (Continued)

Chemical group	Chemical Constituent	Reference
-sesquiterpenes	β-caryophyllene	Guenther, 1952; Parry, 1969
	α-zingiberene	Guenther, 1952; Parry, 1969
	α-curcumene	Guenther, 1952; Parry, 1969
	bisabolene	Guenther, 1952; Parry, 1969
	β-sesquiphellandrene	Guenther, 1952; Parry, 1969
	α-Atlantone	Guenther, 1952; Parry, 1969
	β-Atlantone	Guenther, 1952; Parry, 1969
-sesquiterpene	ar-turmerone	Trease and Evans,1983; Parry,1969
ketones	β-turmerone	Trease and Evans,1983; Parry,1969
	α-turmerone	Trease and Evans,1983; Parry,1969
	curlone	Tang and Eisenbrand, 1992
Carbohydrates	arabinose	Trease and Evans, 1992
	fructose	Trease and Evans, 1992
	glucose	Trease and Evans, 1992
	starch	Parry, 1969
Oleoresin	-	Parry, 1969; Youngken, 1950
Mineral	-	Parry, 1969; Youngken, 1950

5. The Uses of *C. longa*

C. longa rhizome has long been used for thousand years as a spice, coloring agent in food, household medicine, insect repellent and cosmetics. It is not a true spice, but rather a condiment that has been used in the preparation of curries, pickles and many spicy foods. It is also one of the chief ingredient of curry powder. Turmeric paper is also an official reagent in the British Pharmacopoeia for testing boron, boric acid and alkalinity.

In China and Japan, turmeric rhizome has been used as stomachic, stimulant, carminative, hematic or styptic in all kinds of hemorrhages and a remedy for a certain type of jaundice and other liver trouble. Externally, it is applied to minor wounds and certain skin eruptions as a maturative. A decoction affords relief for a burning sensation in eye diseases. It is considered to be very good for irregular menstruation. It promotes blood circulation, dissolves blood clots and also prescribed as remedy for abdominal, chest and back pains (Perry, 1980).

In Indonesia, *C. longa* is a part of numerous native compound medicines. It dispels itching and is gargled as a mouthwash for inflammed gums. A decoction used as antidysentery and anticholeretic. It applied topically for relief pain of insect bites (Perry, 1980).

In Philippines, the tincture or the juice of turmeric may be given to treat bronchial disease and used as antiseptic by applied crushed rhizomes to the affected area. A decoction as tea used as carminative or antiflatulent (Perry, 1980).

In India, turmeric is used as stomachic, tonic and blood purifier. It is also prescribed as an antiperiodic alterative. Mixed with warm milk is beneficial in common cold. The juice of the fresh rhizome is used as an antiparasitic for many skin diseases. Externally, it is applied to indolent ulcers and a paste made from the powdered rhizome along with lime forms a remedy for inflammed joints. A decoction of the rhizome is said to relieve the pain of purulent opthalmia. Turmeric oil distilled from the dried rhizome has antiseptic properties. It is an antacid and in small doses acts as carminative, stomachic, appetizer and tonic. In large dose, however, it appears to act as antispasmodic inhibiting excessive peristaltic movements of the intestine. Turmeric oil is also used as flavoring of spicy food products and to a smaller extent in perfumes of heavy oriental character. In India and Pakistan, rice and wheat are stored by mixing with 2% of powdered turmeric (Jilani and Su, 1983; Sastri *et al.*, 1950). Turmeric is also used for dyeing wool, silk and unmordanted cotton to which it imparts a yellow shade in an acid bath.

Sometimes, it is used in combination with other natural dyes like indigo and safflower to impart different shades (Sastri *et al.*, 1950).

In Thailand, turmeric rhizome has long been used in household remedy since ancient times. For example, it was rubbed or applied to the affected area for relief iching, swelling or insect bites etc. For dyspepsia, powdered turmeric has been manufactured in form of capsule (Public Health, Ministry, 1990).

6. Chemistry,Distribution and Detection of Diarylheptanoids

6.1 Chemistry and Distribution of Diarylheptanoids

A large number of diarylheptanoids (Fig 4.) are mostly found in nature as plant pigments. Most of them are crystalline materials ranging from yellow to red color. They are soluble easily in methanol and acetic acid. The plants containing these diarylheptanoids have been used as drugs, food and coloring agents. These diarylheptanoids are found in the rhizomes of zingiberaceous plants and some other plants.

Diarylheptanoids are two cinnamate units condensed with one malonate unit. They are derived from phenylpropanoid pathway which leads to the formation of compounds with C_6-C_3-$(C_2)_n$ unit. These include bisferuloyl-

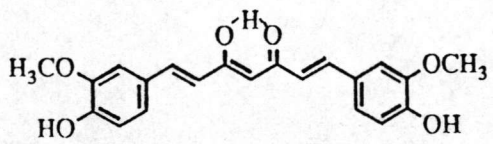
methane (1), 4-hydroxycinnamoylferuloylmethane (2) and bis-4(hydroxycinnamoyl)methane(3) which are commonly known as curcumin, demethoxycurcumin and bisdemethoxycurcumin respectively. These curcuminoids were isolated from *Curcuma longa* in 1815 by Vogel and Pelletier (Vogel and Pelletier, 1815). Dihydrocurcumin (4) which is the minor component was also isolated from the rhizome of *C. longa* (Ravindranath and Satyanarayana 1980).

Renewed interest has been evoked by the recent discovery of compounds containing the 1,9-diarylskeleton which are C_9-chain homologs of curcumin derivatives, and assigned as curcumin- 1(5), curcumin-2(6) and curcumin-3(7) (Tang and Eisenbrand, 1992).

In other *Curcuma* species, curcuminoids have also been found in the rhizomes. For example, two new curcuminoids have been isolated from the rhizome of *C.xanthorrhiza* Roxb. Both compounds are octahydrocurcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-heptane-3,5-diol) (8) and 1-hydroxy-1,7 bis (4-hydroxy-3-methoxyphenyl-1-heptene -3,5-dione)(9) (Shinichi *et al.*, 1987). One new similar diarylheptanoid,1-(4-hydroxyphenyl)-7 phenyl heptane 3,5-diol) has been isolated from the rhizomes of *Alpinia officinarum* Hance. (Shinichi *et al.*, 1987). Some of zingiberaceous plants have also been reported to yield curcuminoids in their rhizomes, for example, the species

of *C. zedoaria*, Roscoe., *Zingiber cassumunar* Roxb. and *Z.zerumbet* Smith. (นันทวัน บุณยะประภัศร, 2530; Youngken, 1950).

For other families,a group of diarylheptanoids has been found in the extracts of male flower (catkins) of *Alnus* species from Betulaceae (Birch family). These diarylheptanoids have been called "Yashabushi-ketol compounds" (10, 11, 12, 13) (Asakawa *et al*, 1969). In addition, centrolobol (14), centrolobin (15) and de-O-methylcentrolobin (16) have been isolated from *Centrolobium* species of Leguminosae (Craveiro and Prado, 1970) and the m,m-bridge biphenyls myricanol (17) and myricanone (18) from *Myrica nagi* Thunb. of Myricaceae (Roughley and Whiting, 1973). Asadinin (19) and its derivatives from *Ostrya japonica* are also closely related in structure as the 9-phenylphenalen-1-ones haemocorin(20),lachnanthoxide (21), lachnanthoflurone and lachnanthocarpone (Weiss and Edwards, 1969).



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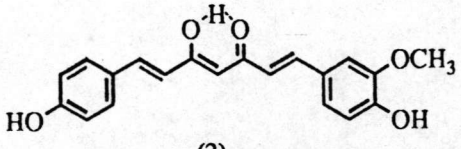

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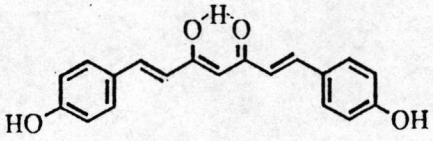

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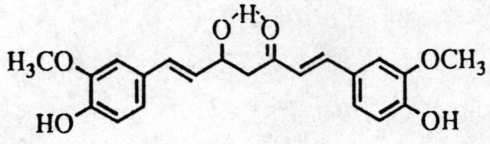

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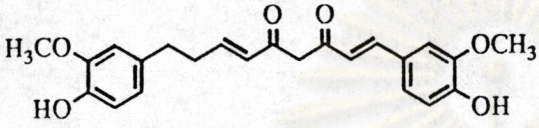

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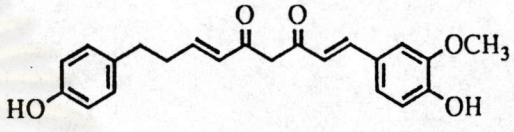

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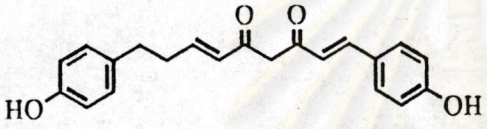

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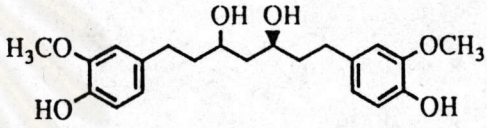

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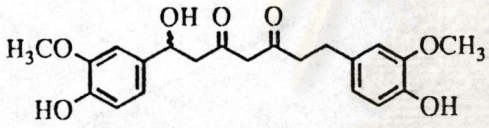

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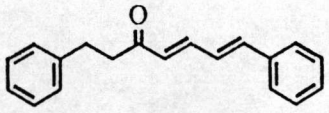

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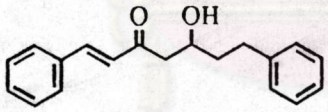

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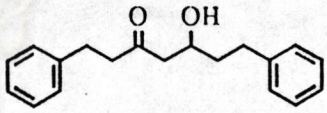

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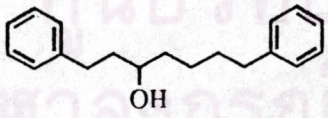

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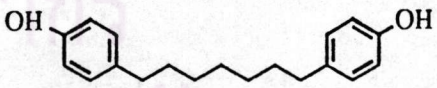

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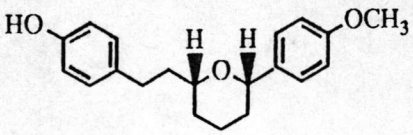

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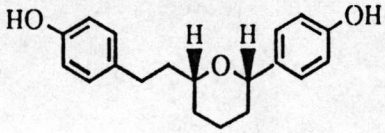

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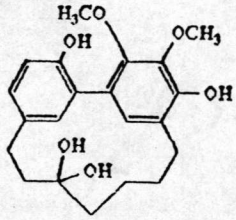
Fig.4 Some diarylheptanoids found in higher plants



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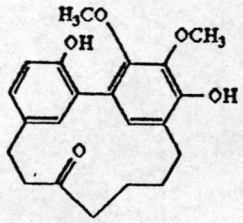

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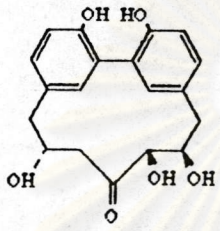

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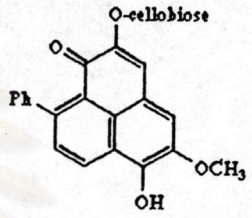

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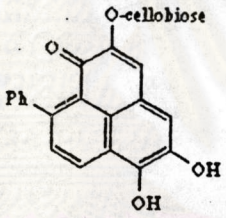

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Fig.4 (continued)

6.2 Detection, Separation and Identification of Diarylheptanoids

6.2.1 Detection

Detection of diarylheptanoids,especially curcuminoids, can be performed by a color reaction method called boric acid test. This test is the official identification for curcumin and turmeric suggested by WHO and FAO (Food and Agriculture Organization, 1971; World Health Organization, 1976). The red coloration when turmeric extract or its active principle curcumin react with boric acid is the basis of the most sensitive spot test for boron (Spicer and Strickland, 1952). Rosocyanin (Fig. 5) is the compound formed when curcumin reacts with boric acid in the presence of a mineral acid. It contains two curcumin molecules co-ordinated around a boron atom associated with one equivalent of an anion.

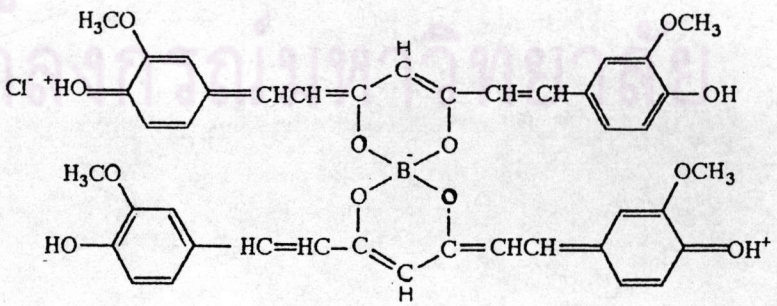


Fig.5 The chemical structure of Rosocyanin

For other diarylheptanoids such as Yashabushi-ketol which was isolated from *Alnus* species, it gives colorless needles when crystallize with n-hexane. The compound also has color reaction with 2,4-dinitrophenyl hydrazine which is the characteristic of ketone and alcohol (Asakawa *et al.*, 1969).

6.2.2 Separation

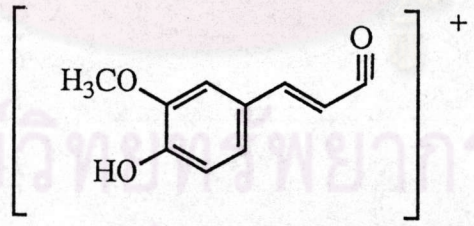
The general procedure for separation of diarylheptanoids is thin layer chromatography on silica gel. There are a number of solvent systems of TLC which have been used for separation of diarylheptanoids. For example, n-propanol : ethyl acetate : water (40:30:30) (Wagner,1984), chloroform: benzene: 95% ethanol (45:45:10) (Stahl,1973) and benzene: methanol (9:1)(Sasri, 1981) have been used for curcuminoids separation; benzene : ethylacetate (19:1) (Asakawa *et al.*, 1969) for Yashabushi-ketol separation; n-propanol : water (8:2) (Thomas, 1971) for haemocorin separation;ethylacetate 100% for lananthoside separation (Weiss and Edwards, 1969); and benzene:acetone (8:2) for centrolobine separation(Craveiro, *et al.*,1970).

6.2.3 Identification

Since most diarylheptanoids,especially curcuminoids are pigments and each type has a characteristic

absorption spectrum, they can simply identified by uv-visible spectrophotometry or fluorometry. Illustrate spectra for some natural diarylheptanoids are given in Table 3.

Curcuminoids can also be identified by their mass spectrum, it was found that MS *m/z*: 368(M^+), 350, 342, 326, 285, 253, 192, 177 are curcumin; MS *m/z* : 338(M^+), 312, 255, 223, 177, 124 are demethoxy-curcumin and MS *m/z* : 308 (M^+), 290, 282, 225 are bisdemethoxycurcumin (Kosuke *et al*, 1985). It has been reported that the fragment ion at *m/z* 177 shows cytotoxic activity (Matthes *et al* , 1980).



m / z 177

Fig.6 Fragment ion of curcumin by mass spectrometry.

Table 3 Ultraviolet-visible and fluorescence absorption of some naturally occuring diarylheptanoids

Plant part	diarylheptanoids	UV-vis λ_{max} (EtOH) (log ϵ) (nm)	Fluorescence		Reference
			λ_{ex} (nm)	λ_{em} (nm)	
rhizome	curcumin	428 (4.76)	433	511	Rouseff, 1988
rhizome	demethoxycurcumin	424 (4.71)	428	505	Rouseff, 1988
rhizome	bisdemethoxycurcumin	418 (4.57)	425	501	Rouseff, 1988
rhizome	dihydrocurcumin	375 (4.39)	-	-	Ravindranath and Satyanarayana, 1980
stem bark	myricanone	217 (4.49)	-	-	Campbell, Tuck and-
stem bark	myricanol	216 (4.51)	-	-	Whiting, 1970
bud	yashabushi-ketol	217 (4.35)	-	-	Asakawa *et al*, 1969
trunk wood	centrolobin	218 (4.40)	-	-	Craveiro *et al*, 1970

7. Structure and Chemical Properties of Curcuminoids

7.1 Curcumin

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl) 1,6 heptadiene 3,5 dione]; $C_{21}H_{20}O_6$; MW 368.37 (Fig 7).

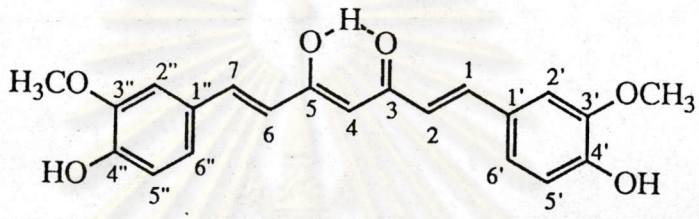


Fig.7 The structure of curcumin

Curcumin was first isolated from *Curcuma longa* Linn (*C.domestica* Val)in 1815 by Vogel and Pelletier (Vogel and Pelletier,1815) and has subsequently been found in other zingiberaceous plants such as *C. zedoaria* Roscoe, *Zingiber zerumbet* Smith. and *Z. cassumunar* Roxb. Curcumin belongs to the chemical group of 1,6 heptadiene 3,5 dione with two (4-hydroxy-3-methoxyphenyl) groups attached to C-1 and C-7 of the molecule. It is relatively nonpolar and soluble in alcohol and glacial acetic acid. It is insoluble in water and ether (Windholz, 1983). It gives a brown color with alkali and a red color with boric acid. It has been reported that curcuminoids are photooxidative, they are more stable in dry powder than

in alcoholic extract. Vanillin, *p*-hydroxybenzaldehyde, ferulic aldehyde, *p*-hydroxybenzoic acid, vanillic acid and ferulic acid were identified as its oxidative products (Khurana, 1988). Curcumin in crystal form has an orange-yellow color with the melting point of 184-186°C (Roughley, 1973). Its uv-vis spectrum shows λ_{max} (EtOH) at 428 nm($\log\epsilon$ =4.76) and its fluorescence spectrum shows λ_{ex}(EtOH) at 433 nm and λ_{em} (EtOH) at 522 nm (Rouseff,1988). For IR spectrum, curcumin shows ν_{max}(KBr) 3550-1760,1615, 1580, 1510, 1420, 1275, 1135, 1110, 1020, 959, 840, 800 (Kosuke *et al*,1985). ^{1}H NMR spectrum(400 MHz, in $CDCl_3$)δ: 7.59 (d,J=15.9 Hz) for H-1, 6.47 (d,J=15.3 Hz) for H-2, 5.80(s) for an enol form, 6.47(d,J=15.3 Hz) for H-6,7.59 (d,J= 15.9 Hz) for H-7, 7.05 (d,J=1.9 Hz) for H-2′ and H-2″,6.93(d,J=8.0)for H-5′, 7.12 (dd,J=1.9 Hz) for H-6′ and H-6″,6.93 (d,J=8.0 Hz) for H-5″, 3.95 (s) for OMe-3′ and 3″,5.87 (br,s) for OH-4′and OH-4″(Musuda *et al*,1992).

7.2 Demethoxycurcumin

Demethoxycurcumin [4-hydroxycinnamoyl (feruloyl) methane]; $C_{20}H_{18}O_5$; MW 338.37 (Fig. 8)

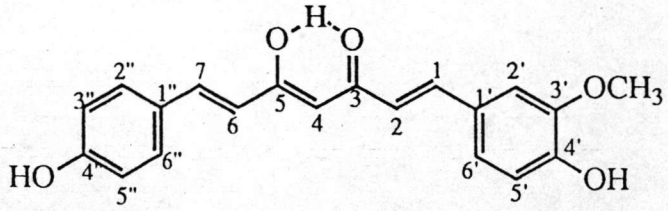


Fig.8 The structure of demethoxycurcumin

Demethoxycurcumin was also first isolated from *C. longa* L. and has been found together with curcumin in the rhizomes of other zingiberaceous plants (Roughley and Whiting, 1973). Demethoxycurcumin belongs to the chemical group of a 4- hydroxy-3 - methoxyphenyl and a 4-hydroxyphenyl attached respectively to C-1 and C-7 of the molecule. It is relatively more polar than curcumin. It is soluble in alcohol and glacial acetic acid like curcumin.

The crystal of demethoxycurcumin has an orange-yellow color with the melting point of 168-169°C (Roughley and Whiting, 1973). Its uv-vis spectrum shows λmax (EtOH) at 424 nm($\log \epsilon = 4.71$) and its fluorescence spectrum shows λ_{ex} (EtOH) at 428 nm and λ_{em} (EtOH) at 505 nm (Rouseff, 1988). For IR spectrum, demethoxycurcumin shows ν_{max} (KBr)at 3550-1740, 1620, 1580, 1560, 1515, 1260, 1135, 970, 960, 815 (Kosuge *et al*, 1985). ^{1}H NMR spectrum (in $CDCl_3$) δ : 7.61 (d,J=15.9 Hz) for H-1 or H-7, 7.59 (d, J=15.9 Hz) for H-1 or H-7, 7.45 (d, J=8.1 Hz) for H-2" and H-6", 7.12 (dd, J=1.8 Hz) for H-6', 7.05 (d, J=1.8 Hz) for H-2', 6.93

(d, J=15.9 Hz) for H-2 or H-6, 6.47 (d, J=15.9 Hz) for H-2 or H-6, 5.86 (br, s) for OH, 5.80 (s) for H-4, 3.95 (s) for OMe-3" (Musuda *et al*, 1992).

7.3 Bisdemethoxycurcumin

Bisdemethoxycurcumin [1,7 bis (4-hydroxycinnamoyl) methane]; $C_{19}H_{16}O_4$; MW 308.37 (Fig.9)

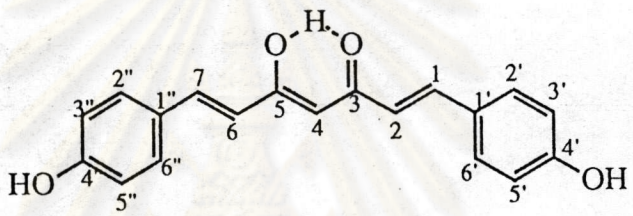


Fig.9 The structure of bisdemethoxycurcumin

Bisdemethoxycurcumin was first isolated together with curcumin and demethoxycurcumin from *C.longa* as well as curcumin and demethoxycurcumin. It belongs to the chemical group of two 4-hydroxyphenyls attached to C-1 and C-7 of the molecule. It is relatively more polar than curcumin and demethoxycurcumin and also soluble in alcohol and glacial acetic acid.

The crystal of bisdemethoxycurcumin has the yellow color with the melting point of 224-226°C (Roughley

and Whiting, 1973). Its uv-vis spectrum shows λ_{max} (EtOH) at 418 nm ($\log \epsilon$ =4.57) and fluorescence spectrum shows λ_{ex} (EtOH) at 425 nm and λ_{em} (EtOH) at 501 nm (Rouseff, 1988). For IR spectrum, bisdemethoxycurcumin shows ν_{max} (KBr) at 3500-1700, 1625, 1595, 1560, 1510, 1425, 1235, 1135, 835 (Kosuge *et al*,1985). ^{1}H NMR (in d_6-acetone) δ : 7.61 (d, J=15.9 Hz) for H-1 and H-7, 7.55 (d, J=8.6 Hz) for H-2′, H-6′, H-2" and H-6", 6.89 (d,J=8.6 Hz) for H-3′, H-5′, H-3"and H-5, 5.97 (s) for H-4 (Musuda *et al*, 1992).

8. Biological Activities of Curcuminoids

8.1 Biological Activities of Curcumin

Curcumin has been studied for its pharmacological activities both *in vivo* and *in vitro*. It has been shown to have antiinflammatory activity as well as sodium curcuminate and phenylbutazone (Ammon and Wahl, 1990; Ghatak and Basu,1972; Mukhopadhyay *et al*.,1982). Curcumin was found to inhibit hydrogen peroxide free radical (Kunchandy and Rao, 1989; Runchandy and Rao, 1990) and inhibit the pathway of leukotriene B-4 formation in rat peritoneal polymorphonuclear neutrophils which cause inflammation (Ammon *et al*.,1991; and Flynn *et al*., 1986). The compound has also been shown to inhibit gas formation of *Clostridium perfringens* which causes food flatulent (Bhavanishankar and Murthy, 1985). Kiso *et al*. (1983)

has found that curcumin (1 mg/ml) diminishs CCl_4-induced-GOT to 53% and GPT to 20% of the control and suggested that, curcumin posses antihepatotoxic activities (Kiso *et al.*, 1983). For biliary secretion, it has been found that sodium curcuminate has an effect on bile secretion from the cannulated bile duct in anesthetized dogs (Ammon and Wahl, 1990; Ramprasad and Sirsi, 1956, Ramprasad and Sirsi, 1957). It has also been reported that curcumin at doses between 25 and 100 mg/kg i.p. inhibits collagen and adrenaline-induced aggregation of platelets(Srivastava *et al.*, 1985; Srivastava *et al.*, 1986; Kosuge, Ishida and Yamazaki, 1985). Collagen-induced platelets aggregation is known to be associated with an increase in the thromboxane-A_2 (TXA_2) levels. It is therefore conceivable that curcumin may have an anti-TXA_2 activity. For antimicrobial activities, it has been found that alcoholic extract from *C. longa* inhibits growth of most organisms occuring in cholecystis including *Sarcinia, Corynebacterium, Streptococcus* and *Bacillus* strains (Lutomski *et al.*, 1974; Shankar and Murthy,1978; Shankar and Murthy, 1979; Tang and Eisenbrand, 1992). Sodium curcuminate has also been reported to have antibacterial activities, it specifically inhibits *Micrococcus pyogenes* (Ammon and Wahl, 1990; Tang and Eisenbrand, 1992). In addition, the ethanolic extract of *C. longa* has been reported to have anti-amoebic activity against *Entamoeba histolytica* and fungistatic effect against *Aspergillus flavus, Microsporum gypseum,*

Penicillium corymbiforum, P.javanicum, Trichoderma viride and *Trychophytum mentagrophytes* (นันทวัน บุณยะประภัศร, 2530; Ramprasad and Sirsi, 1956; Shankar and Murthy, 1978; Shankar and Murthy, 1979). Recently, ethanol extract of *C. longa* has been reported to have antitumor activity. This was shown by its inhibition of cell growth of chinese hamster ovary cell at a concentration of 0.4 mg/ml and increase survival rate of mice injected Dalton's lymphoma cells (Kuttan *et al.*, 1985; Kuttan *et al.* 1987; Kuttan, 1989). It has also been found that curcumin has an efficacy in reducing chemically-induce tumors in mice and causes the reduction in the expression of papillomas in mouse skin (Soudamini and Kuttan, 1987).

8.2 Toxicity of Curcumin

In case of acute toxicity, it has been found that curcumin has no toxic effects to rats after doses up to 5 g/kg curcumin given orally (Sankaranarayanan and Murthy, 1979; Vijayalaxmi, 1980; Wahlstrom and Blennow, 1978). For whole turmeric or curcumin, it has been shown that after it is fed to the rats at doses normally consumed by humans or at much higher doses (1.25-125 fold), the rats do not show any adverse effects on growth, feeding efficacy ratio, erythrocytes, leucocytes or on the level of blood constituents (Hb, albumin, globulin etc.) (Sambaith *et al.*, 1982). It has also been shown to have no teratogenic effect in bone-marrow cells of

mice in either chromosomal aberrations or the micronucleus test (Vijayalaxmi, 1980).

8.3 Pharmacokinetics of Curcumin

Curcumin administered orally to rats has been found to be excreted in the feces about 75%, while negligible amounts appeared in urine (Ravindranath and Chandrasekhara, 1981; Wahlstrom and Blennow, 1978). Measurements of blood plasma levels and biliary excretion showed that curcumin was poorly absorbed from the gut (Holder, Plummer and Ryan, 1978; Khanna, Signh and Sarin, 1981; Ravindranath and Chandrasekhara, 1982). Curcumin injected intraveneously or added to the perfusate of the isolated liver was actively transported into the bile. It has also been found that the major biliary metabolites of curcumin are glucuronides of tetrahydro-curcumin, hexahydrocurcumin,dihydroferulic acid and traces of ferulic acid (Holder, Plummer and Ryan,1978).

8.4 Biological Activities of Demethoxycurcumin

Demethoxycurcumin has been shown to have antihepatotoxic (Kiso *et al.*, 1983), antiinflammatory (Rao, Basu and Siddiqui, 1982), anticancer (Kuttan *et al.*, 1985; Kuttan, 1987), antibacterial, antifungal (Ammomn and Wahl, 1990, Lutomski *et al.*, 1974) and antiplatelet activities (Kosuge *et al.*, 1985; Takuo and Ishida, 1985). It has been reported that biological activities of

demethoxycurcumin are less than curcumin and its mechanism of actions are similar to that of curcumin which is described in biological activities of curcumin in 8.1.

8.5 Biological Activities of Bisdemethoxycurcumin

Bisdemethoxycurcumin has biological activities similar to that of demethoxycurcumin and it has been found that both biological activities of demethoxycurcumin and bisdemethoxycurcumin are less active than curcumin (Ammon and Wahl, 1990; Tang and Eisenbrand, 1992).

9. The Proposed Biosynthetic Pathway of Curcuminoids

The biosynthesis of curcumin was first studied in 1910 by Lampe and coworkers (Lampe, 1913) using the rhizomes of *C. longa* as plant material. Since then, information obtained from subsequently studies has suggested that curcuminoids in plants are derived from the phenylpropanoid pathway. Roughley and Whiting (1973) proposed that the biosynthesis of curcuminoids would have been related to that of lignans which involve the union of two cinnamate units with a central methylene supplied by malonate (Roughley and Whiting, 1973). As shown in Fig. 10, the biosynthesis of curcumin starts from feruloyl CoA reacting with malonyl CoA to form C_6-C_3-C_2 intermediate (Chi-Kit Wat and Towers, 1979; Roughley and Whiting, 1971; Roughley and Whiting, 1973). This intermediate then

further reacts with the second molecule of feruloyl CoA to form curcumin.

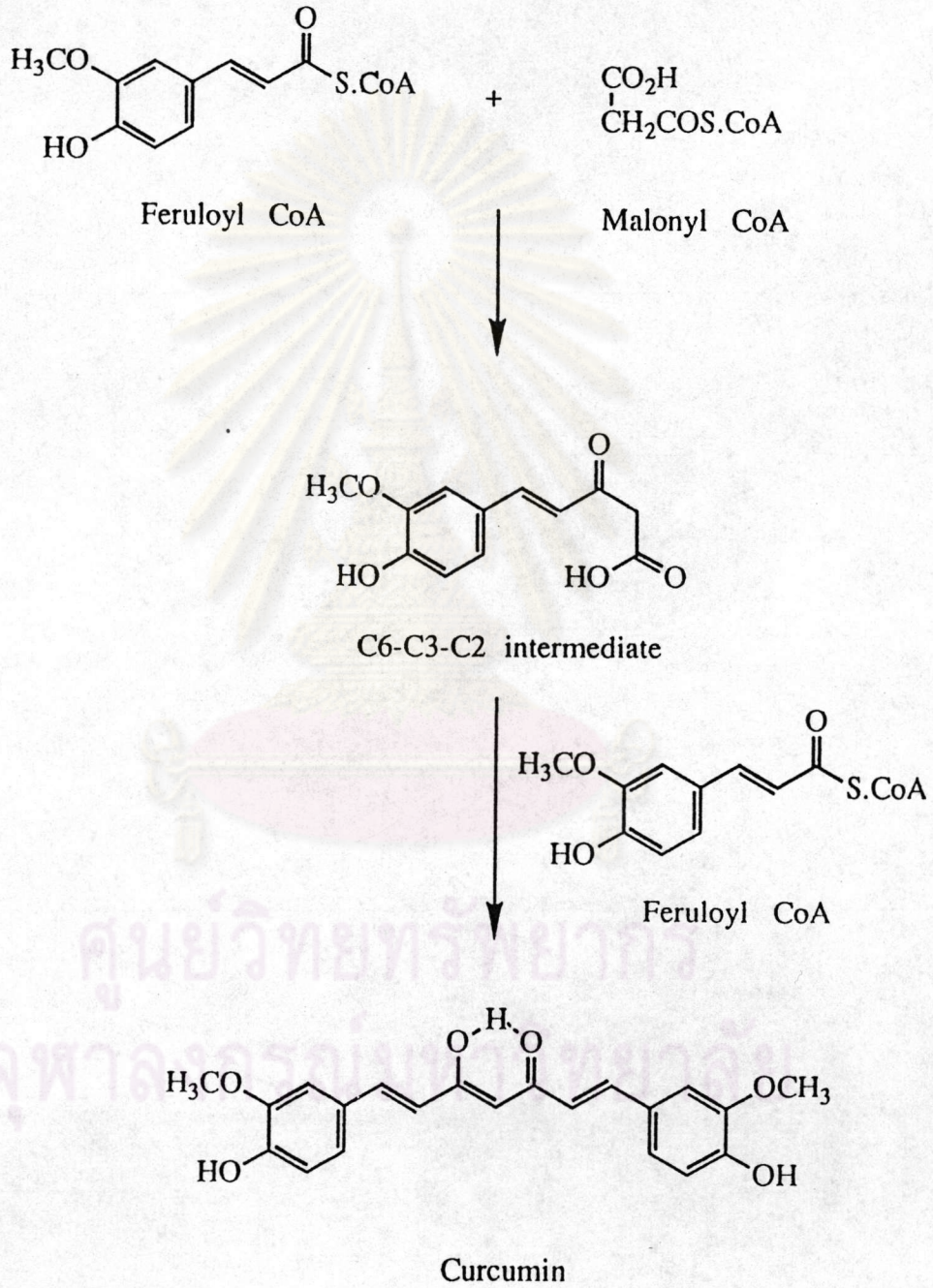


Fig.10 Biosynthetic pathway of curcumin

On the other hand, the biosynthesis of curcumin may be explained by another alternative scheme (Fig 11.) which involves polyketide extension of a cinnamate group with five malonate units. The resulting chain is then reduced and cyclised to give second aromatic ring. The biosynthesis would be completed by hydroxylation and methylation at C-3′ (Roughley and Whiting, 1973).

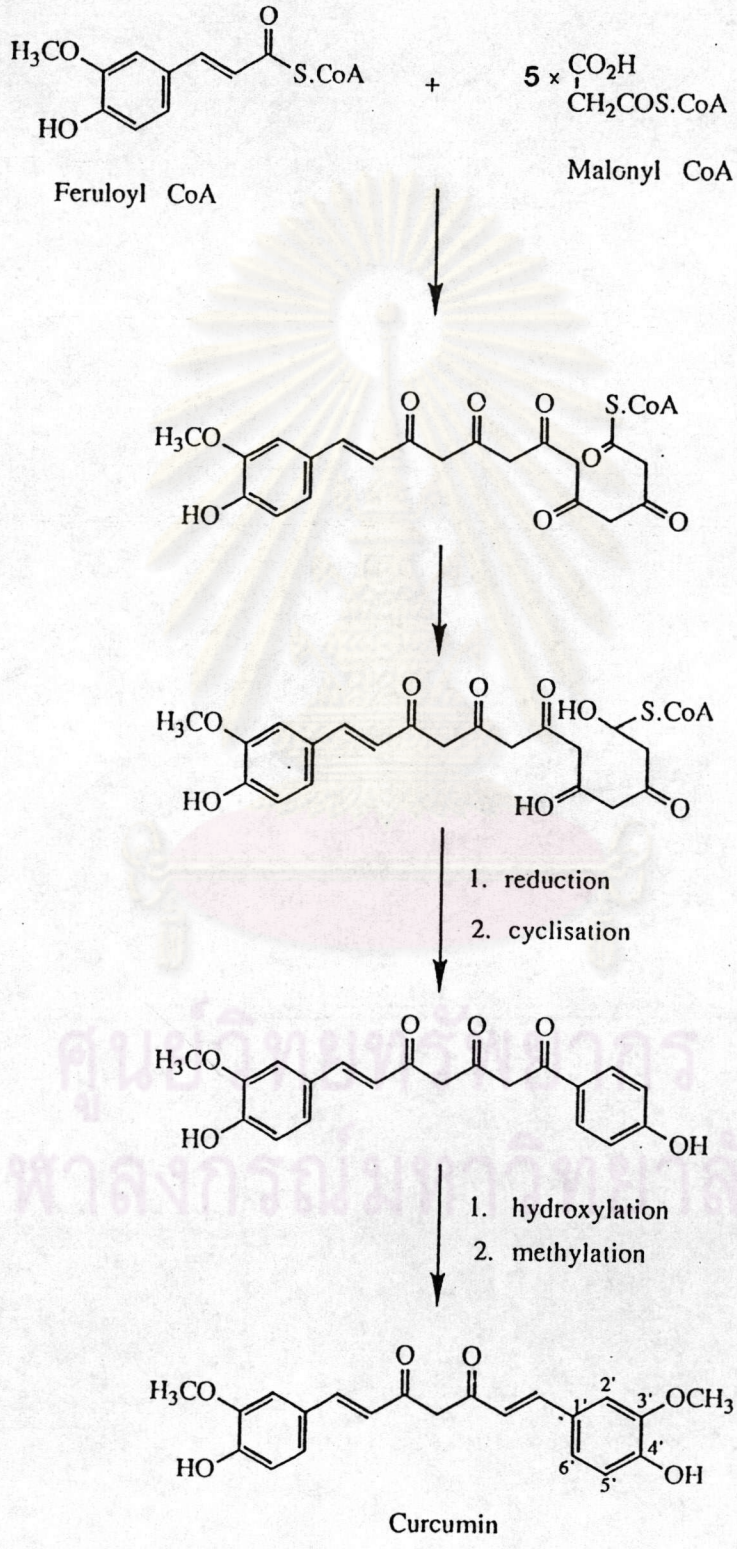


Fig.11 Alternative biosynthetic pathway of curcumin

10. Chemistry and Biological Activities of Turmeric Oil

10.1 Chemical Properties of Turmeric Oil

Turmeric oil which is obtained from the rhizome of *C. longa* is volatile oil with an orange-yellow color and a characteristic odor (Guenther, 1952; Youngken, 1950). It is derived from steam distillation, water distillation or an oil selecting solvent extraction. Its properties can be summarized as follows :

Specific gravity at 15°	: 0.938 to 0.967
Optical rotation	: -13° to -25° or dextrorotatory up to + 28°
Refractive index at 20°	: 1.512 to 1.517
Acid Number	: 0.6 to 3.1
Ester Number	: 6.5 to 16
Ester Number after acetylation	: 28 to 53
Solubility	: Soluble in 0.5 to 1.0 vol. of 90% alcohol

The chemical components of turmeric oil have been subjected to numerous chemical investigations. In general, turmeric oil consists of monoterpenes 10%, sesquiterpenes 25% and sesquiterpene ketones 65% (นิจศิริ เรืองรังษี, 2534). It contains α-pinene, β-pinene, camphene, p-cymene, p-cymenene, terpinolene, α-phellandrene,

caryophyllene, α-zingiberene, α-curcumene, bisabolene, β-sesquiphellandrene, ar-turmerone, α-turmerone, β-turmerone and curlone (Guenther, 1952; Helen, 1982; Tang and Eisenbrand, 1992). Among these components, ar-turmerone ($C_{15}H_{20}O$) are the major one. The structures of some turmeric oil components are shown in Fig.12.

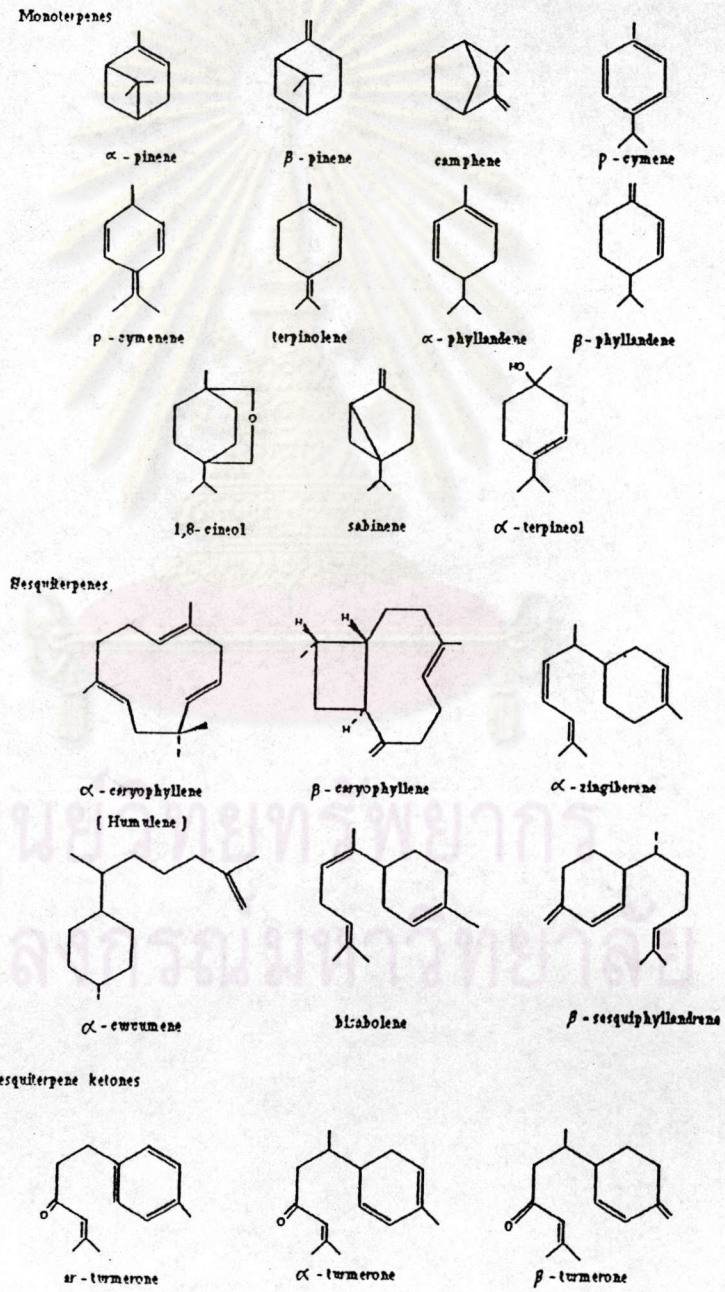


Fig.12 Votatile oil components in turmeric rhizome

10.2 Biological Activities of Turmeric Oil

Turmeric oil has been shown to exhibit many biological activities. For example, it was reported to have antimicrobial activities against *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* (โสภณ เริงสำราญ, 2531). It has been found to suppress acute edema and polyarthritis (Chandra and Gupta, 1972; Chawla and Gupta, 1972). Turmeric oil has also shown to increase bile secretion and bile flow from canulated bile duct in anesthetized dogs (Ozaki and Liang, 1988). Recently, Itokawa *et al.*, (1985) has found that α-curcumene, ar-turmerone and β-atlantone exhibit anticancer activity (Itokawa *et al.*, 1985). For insect repellent activity, it has been reported that turmerone and ar-turmerone give strong repellency to *Tribolium condusum* and *Rhyzopertha dominica* (Helen, 1982).