CHAPTER II

HISTORICAL

1. Botanical Aspects of *Gloriosa superba* L.

Gloriosa superba L. (Fig.2) or commonly known as Climbing Lily, Superb Lily or Glory Lily is a perennial herbaceous climbing plant in the family of Liliaceae. The name "Gloriosa" is from the Latin "Gloriosus" which means full of glory. It refers to the beauty of the flowers (Chopra *et al.*, 1965). It has a number of Thai local names,including a general name : Dong dueng (ดองดึง); in Chai Nat province : Kaam puu (ก้ามปู) ; in Chon Buri : Khom khwaan (คมขวาน), Bong khwaan (บ้องขวาน), Hua khwaan (หัวขวาน) ; in Central : Daao dueng (ดาวดึงส์), Waan kaam puu (ว่านก้ามปู) ; in Nakhon Ratchasima : Phan mahaa (พันมหา) ; in Northern : Ma khaa kong (มะขาโก้ง) [เต็ม สมิตินันทน์, 2523].

G. superba is native to Africa, and is now cultivated in all the tropical areas,including Madagascar, India, Ceylon, China, Indochina and on the adjacent isles (Thakur *et al.*, 1975). The plant is monocotyledon , tall weak-stemmed plants , supporting themselves by means of tendril-like prolongations of the leaves. Stem 1.5-6 m high, given off from the angle of the young tubers. The

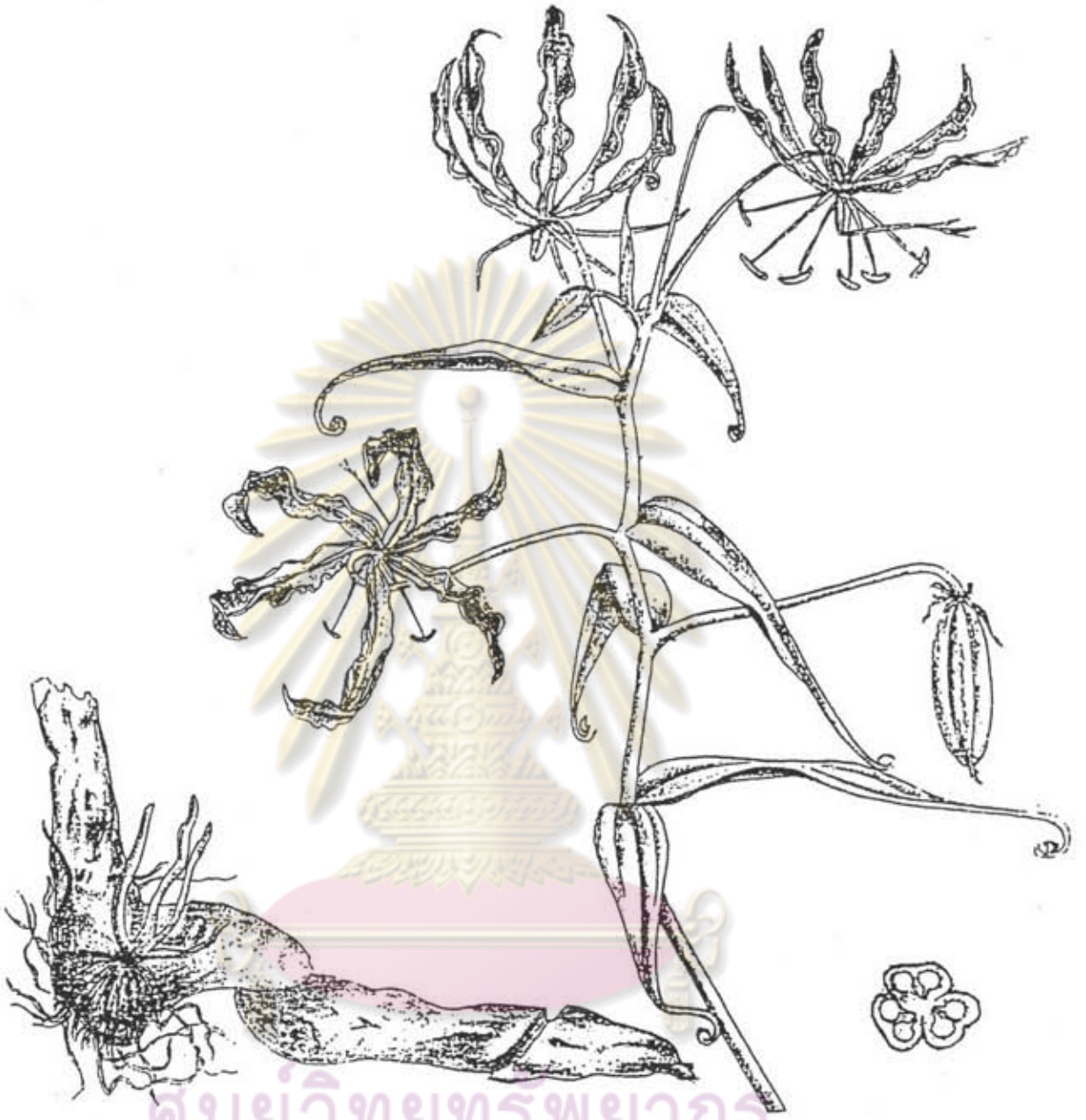


Fig.2 *Gloriosa superba* L. (Liliaceae).

leaves are ovate-lanceolate, 7.5-20 cm long and 2.4-5 cm wide. The flowers occur many and showy, long-stalked, borne singly in the axils of the upper leaves. Their perianth are composed of six distinct long petals which are somewhat undulate. Petals are 5-8 cm long and less than an inch wide , opening yellow , but changing to yellow-red and deep scarlet to crimson from base to apex. Six stamens are long and spreading, with versatile anthers. The ovary is 3-loculed, style long, and bent upward near the base. The fruits are capsule with three lobes and 4-6 cm long. When the capsules are ripe, the top of them will be opened and give a number of seeds which are orange to red-brown color (Bailey, 1963). The tuber is fleshy, cylindrical, bifurcated, usually V-shaped with the two limbs equal or unequal in length, often 15-20 cm long, and 2-4 cm in diameter, budding from both of the ends (Quisumbing, 1951 ; Sastri, 1956 ; Hooker, 1973 ; Trimen, 1974).

G. superba is not difficult to grow. It can be grown either from seeds or tubers. These tubers should be rested in early winter, and started in pots in January to March. When potting the old tubers, offsets may be removed (when they occur) and grown separately for the production of new plants. The tubers may be cut in two for purposes of propagation. Let the plants stand near a pillar or other support. Give freely of water when the plants are

growing and they prefer sandy soil and sunlight (Bailey, 1963). The plants flower during the months of July and August and the fruits with ripe seeds are ready for harvest towards the end of September and October. The plant being a perennial , then the fruits can be harvested for a number of years from the same planting (Sarin *et al.*, 1974).

2. The uses of *G. superba*

The individual parts of *G.superba* are highly toxic and have since ancient times been used in popular medicine (Thakur *et al.*, 1975). The tuber of this plant is believed by Hindu and Mohammedan physicians to have valuable medicinal properties. According to Dutt, it constitutes one of the seven minor poisons of Sanskrit writers and had one of its synonyms "garbhaghatini" or "the drug that causes abortion". The tubers of this plant are, indeed, popularly believed in India to be highly poisonous and are used to some extent at least , to commit suicide and to procure abortion. The United States Dispensatory records the tubers , stalks and leaves to be an acrid narcotic poison. The Kols believed that the tubers yielded a violent poison , then they used to tip theirs arrows (Chopra *et al.*, 1965).

G. superba plant has also been used in indigenous

medicines in many countries (Chopra *et al.*, 1965).

In India , the tuber is used for blood diseases, swellings , wounds , abscesses and pain , as a tonic, stomachic, cholagogue , anthelmintic , abortifacient, used to remove the placenta from the uterus, colic, laxative , itching , thirst , antiperiodic , alterative , purgative , leprosy , piles , chronic ulcers , and as a remedy for snake and scorpion bite. In case of the treatment of gonorrhoea , the white powder obtained by repeated washing and grinding is given internally up to 12 grains, mixed with honey. It is used also in the form of paste as an external application in parasitic skin diseases and as a cataplasm in neuralgic pains (Quisumbing, 1951). Powdered tuber and made into a paste is applied to the navel , suprapubic region and the vagina for promoting labour (Chopra *et al.*, 1965). *G. superba* plant has been reported to possess antifertile activities (Malhi and Trivedi, 1972) and insecticidal or insect-repellent properties (Chopra *et al.*, 1965). Furthermore, the tuber is given to cattle for the expulsion of worms (Chopra *et al.*, 1969).

In Ceylon, the tuber is used in the treatment of bruises and sprains (Quisumbing, 1951). The flower is used in religious ceremonies (Watt and Breyer-Brandwijk, 1962). In Persia, the tuber is used in the treatment of

haemorrhage from the nose , nocturnal seminal emissions and impotence (Watt and Breyer-Brandwijk,1962). In Guinea, the juice of the ground leaves is used to destroy lice in the hair (Chopra *et al*, 1965). The tubers are used in cataplasm for neuralgia (Kirtikar and Basu, 1935). In Yunan , the tuber is useful in bowel complaints , as an astringent, expectorant,used in bleeding piles and thirst; the flower for fever and thirst (Kirtikar and Basu, 1935).

In Madras, the tuber is applied around windows and doors to ward off snakes (Watt and Breyer-Brandwijk,1962). It is also used as an external application in parasitical affections of the skin (Kirtikar and Basu, 1935). The Hindus used the flowers in the worship of Siva (Clewer, Green, and Tutin, 1915). In South Africa, the tuber is used as an antiparasitic and as a remedy for ascites (Watt and Breyer-Brandwijk, 1962). In Java, the plant is recorded as being used homocidally and the grated fruit has been used for poisoning dogs by mixing it with their food (Watt and Breyer-Brandwijk, 1962).

It has been reported that fresh extracts of *G. superba* tubers are employed successfully for inducing polyploidy in maize (Sastri, 1956) and the tuber extract also shows antibiotic activity against *Staphylococcus aureus* (Sastri, 1956 ; Chopra *et al.*, 1969).

In Thailand , *G. superba* has long been used as folkloric medicine by boiling its tuber and the extract is consumed for the treatment of flatulence , high gas, expectorant, rheumatism, gout, leprosy, wounds and some types of cancer in human (เสงี่ยม พงษ์บุญรอด, 2522). Dried tubers, made into a powder, are used for the treatment of gonorrhoea whereas fresh tuber is grated and prepared as a remedy against the bites of snakes, centipedes, scorpions and also used in parasitic affection of the skin by prepared as external application (เสงี่ยม พงษ์บุญรอด, 2522). Furthermore, the tuber of *G. superba* has also been used as anthelmintic in cattle and as insecticide (ชมรมธรรมชาติศึกษาไทย, 2521).

It has also been reported that tuber and seed coat of *G. superba* are composed of methylcolchicine which can double chromosome in plants. The compound was, therefore, suggested to use for plant breeding work for the purpose of development of new strains of garden flowers or economic crops (ลัดดาวัลย์ บุญรัตนกรกิจ และ ถนอมจิต สุภาวิดา, 2522; บรีดี เอกะวิภาต, 2523).

3. Chemical Constituents of *G. superba*

Since 1880 , when Warden isolated a neutral bitter principle, superbine, which is extremely poisonous from the tuber of *G. superba* (Warden, 1880), the investigation

for other constituents in *G. superba* tubers has continued. Until 1915, Clewer *et al.* reported that colchicine was present in the tuber of *G. superba* with the content of 0.3 % , and the compound had actions and effects identical to colchicine obtained from *Colchicum autumnale* (Clewer *et al.*, 1915 ; Burkill, 1935). Subsequent investigation on other constituents in various plant parts of *G. superba* has found that the groups of compounds commonly found in the plant are alkaloids , phytosterols , organic acids , fatty acids , carbohydrates and resins. The list of these compounds is shown in Table 1.

4. Colchicine

4.1 History

Colchicine in impure form (obtained from *Colchicum* spp.) has been known to man for thousands of years (Eigsti and Dustin, 1955). It is the active ingredient of one of eighteen plants still in use of the approximately 700 listed in the Ebers Papyrus of ancient Egypt (1550 B.C.). Dioscorides, Nero's personal physician, provided the earliest remaining complete botanical description of *Colchicum autumnale* "the autumn crocus or meadow saffron , whose seeds , powdered corm , and dried flowers contain sufficient colchicine to effect relief of pain" (Dalton, 1979).

Table 1 Chemical constituents of various parts of *G. superba*

Chemical group	Chemical substance	Plant parts			Reference
		Tubers	Seeds	Leaves and flowers	
alkaloid	superbine	+			Clewer *et al*, 1915
	colchicine	+	+	+	Clewer *et al*, 1915
	gloriosine	+			Subbaratnam., 1952
	N-formyldeacetylcolchicine	+	+	+	Thakur *et al*, 1975
	2-demethylcolchicine	+			Thakur *et al*, 1975
	3-demethylcolchicine	+	+	+	Thakur *et al*, 1975
	β-lumicolchicine	+		+	Thakur *et al*, 1975
	γ-lumicolchicine	+		+	Thakur *et al*, 1975
	N-formyl-β-lumicolchicine	+			Thakur *et al*, 1975
	N-formyl-γ-lumidesacetyl-colchicine	+	+	+	Thakur *et al*, 1975
	cornigerine	+		+	Thakur *et al*, 1975;
				(lf*)	Dvorackova *et al*, 1984
	3-demethyl-β-lumicolchicine	+		+	Thakur *et al*, 1975
	3-demethyl-N-formyl-N-desacetyl-β-lumicolchicine	+			Thakur *et al*, 1975
	3-demethyl-γ-lumicolchicine	+			Thakur *et al*, 1975
	2-demethyl-β-lumicolchicine	+			Thakur *et al*, 1975
	2-demethyl-N-formyl-N-des-acetyl-β-lumicolchicine	+			Thakur *et al*, 1975
	3-demethyl-N-formyl-N-des-acetylcolchicine	+			Thakur *et al*, 1975
	lumiderivative x	+	+		Thakur *et al*, 1975
	2,3-demethyl-N-desacetyl-colchicine	+	+	+	Thakur *et al*, 1975
	dimethylcolchicine			+ (lf*)	Chopra *et al*, 1969

Table 1 (continued)

Chemical group	Chemical substance	Plant parts			Reference
		Tubers	Seeds	Leaves and flowers	
alkaloid (continue)	2,3-demethylcolchicine	+			Thakur *et al*, 1975
	2-demethyl-N-formyl-N-desacetylcolchicine	+			Thakur *et al*, 1975
	3-demethylcolchiceine	+			Thakur *et al*, 1975
	2-demethylcolchiceine	+			Thakur *et al*, 1975
	N-formyl-N-deacetyl-γ-lumicolchicine	+			Dvorackova *et al*, 1984
	colchifoline	+			Dvorackova *et al*, 1984
	10,11-oxy-10,12a-cyclo-10,11-secocolchicine			+ (lf*)	Dvorackova *et al*, 1984
	(s)-(+)-floramultine (bechuanine)	+	+		Dvorackova *et al*, 1984
	1,12-dihydroxy-2,10,11-tri-methoxyhomoaporphine	+	+		Dvorackova *et al*, 1984
	colchicamide		+		Dvorackova *et al*, 1984
	2-demethylcolchifoline		+		Dvorackova *et al*, 1984
	3-demethylcolchifoline		+		Dvorackova *et al*, 1984
	colchicoside		+		Dvorackova *et al*, 1984
	isoperlolyrine		+		Dvorackova *et al*, 1984

Table 1 (continued)

Chemical group	Chemical substance	Plant parts			Reference
		Tubers	Seeds	Leaves and flowers	
phytosterol	stigmasterol	+			Clewer *et al*, 1915
	β-sitosterol	+			Merchant and Joshi, 1976
phytosterolin	stigmasterol glucoside	+			Clewer *et al*, 1915
	β-sitosterol glucoside	+			Merchant and Joshi, 1976
organic acid	salicylic acid	+			Clewer *et al*, 1915
	benzoic acid	+			Clewer *et al*, 1915
	2-hydroxy-6-methoxybenzoic acid	+			Clewer *et al*, 1915
	tartaric acid	+			Watt and Breyer-Brandwijk, 1962
	chelidonic acid			+ (young lf*)	Chopra *et al*, 1969
saturated fatty acid	palmitic acid	+			Clewer *et al*, 1915
unsaturated fatty acid	linoleic acid	+			Clewer *et al*, 1915
	oleic acid	+			Clewer *et al*, 1915
fatty alcohol		+			Clewer *et al*, 1915
amine	choline	+			Clewer *et al*, 1915
phenolic compound	monomethyl-γ-resorcylate	+ (young root)			Watt and Breyer-Brandwijk, 1962
enzyme	enzyme which hydrolyses amygdalin	+			Clewer *et al*, 1915
essential oil	furfuraldehyde	+			Clewer *et al*, 1915

Table 1 (continued)

Chemical group	Chemical substance	Plant parts			Reference
		Tubers	Seeds	Leaves and flowers	
carbohydrate	dextrose	+			Clewer *et al*, 1915
	starch	+			Mehra and Khoshoo,1915
resin	phenolic compound	+			Clewer *et al*, 1915
	resinous material	+			Clewer *et al*, 1915

* lf - leaf

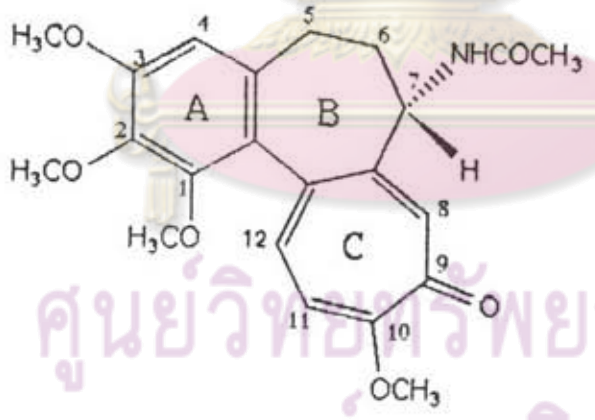
The use of *Colchicum* for the treatment of gout was documented in approximately 560 A.D. and its use appeared to be widespread until the eleventh century. Although the relief of pain was obtained quickly, its high toxicity led to disuse. British formularies (London Pharmacopoeia and Complete English Dispensatory) did list and then discard *Colchicum* in the early 1600's but it was not until the early 1800's that the use of colchicine became widely established (Wyatt, Grady and Sy-rong Sun, 1981).

Presently, colchicine is a highly studied and widely applicable compound for medicinal use and for biochemical and biomedical research. This is because the

compound has been found to have other biological characteristics. For example, it has been shown to be highly specific association with microtubule proteins and to have effects on basic cell functions such as mitosis, secretion , cell morphology , motility , intracellular transport of macromolecules , microtubular assembly , and mitogenic activation (Clark and Garland, 1978).

4.2 Structure and Some Physicochemical Properties

Colchicine is structurally N-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo [a] heptalen-7-yl)-,(S)-acetamide. It has a formular of $C_{22}H_{25}NO_6$ and molecular weight of 399.44. Its structure is shown below :



$C_{22}H_{25}NO_6$

MW. 399.44

The structure of colchicine

Colchicine is pale yellow crystals , amorphous scales, or powder. It is odorless or nearly odorless, and darkens on exposure to light.

For physicochemical properties, colchicine shows its infrared spectrum with the principal bands of 1028, 1248 and 1495 cm^{-1} as shown in Fig.3 (BP, 1988) , its UV-absorption spectrum (in 95 % EtOH) with the λ_{max} values at 243 and 350.5 nm (log ϵ 4.47, 4.22) (Fig.4) (Merck Index, 1989). For proton NMR (^{1}H-NMR) spectrum 360 MHz in $CDCl_3$ (TMS = internal standard) (Fig.5), colchicine shows a singlet aromatic proton at 6.55 ppm for H-4, a broad singlet at 7.69 ppm for H-8, two doublets at 7.39 (H-12) and 6.93 ppm (H-11) with their coupling constant(J) of 11 Hz, a broadened doublet at 8.64 ppm for a proton attached to acetamido nitrogen (NH), a singlet at 4.03 ppm for methoxy proton of 10-OCH_3, 3.67(1-OCH_3), 3.95(2-OCH_3) and 3.92 ppm for 3-OCH_3, a doublet of triplets at 4.66 ppm for H-7 which coupling with both the H-6 protons (J = 11.8 , 6 and 5.8 Hz) and with the NH proton (J = 6 Hz) (Meksuriyen, Lin, and Cordell, 1988). For Carbon-13 NMR (^{13}C-NMR) spectrum (Fig.6), colchicine shows signals at 178.4 ppm (C=O), 168.9 (NHCO), 163.8 (C-10), 134.7 (C-12), 112.3 (C-11), 108.0 (C-4), 60.7 and 60.9 for C-14 and C-13, respectively, 55.9 and 56.0 for C-18 and C-15, respectively, 51.7 (C-7), 36.0 (C-6), 29.4 (C-5) and 22.4 ppm for C-17 (Hufford, Capraro, and Brossi,1980). Finally, for mass spectrum (Fig.7), colchicine shows the molecular ion at m/e 399 (molecular peak) and also other main peaks at m/e 371, 312, 297 and 281 as shown in Table 2 and Fig.7 (Wilson *et al.*, 1963 ; Schonharting *et al.*, 1973).

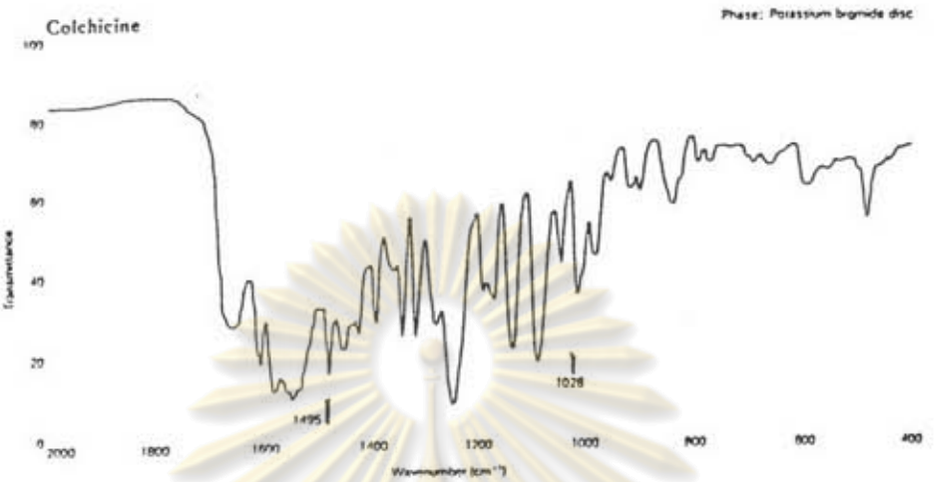


Fig.3 Infrared spectrum of colchicine.

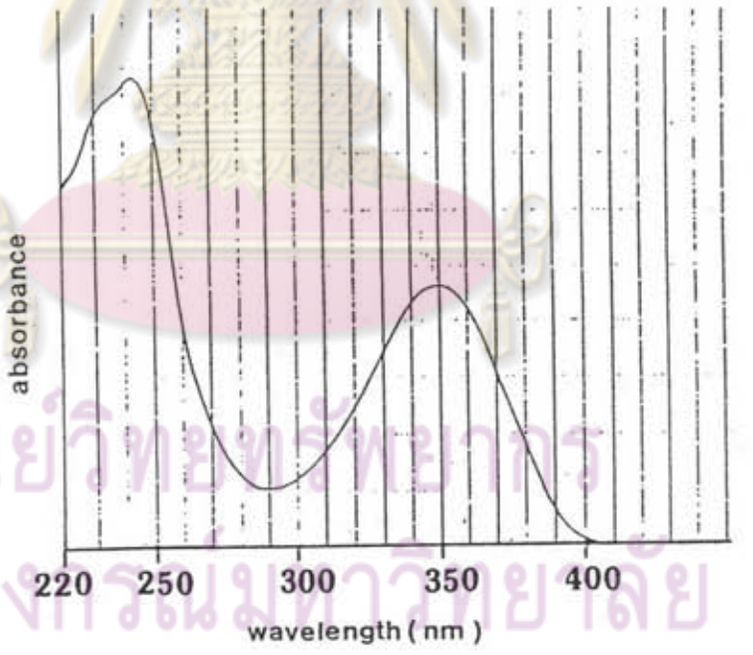


Fig.4 Ultraviolet spectrum of colchicine (in 95% EtOH).

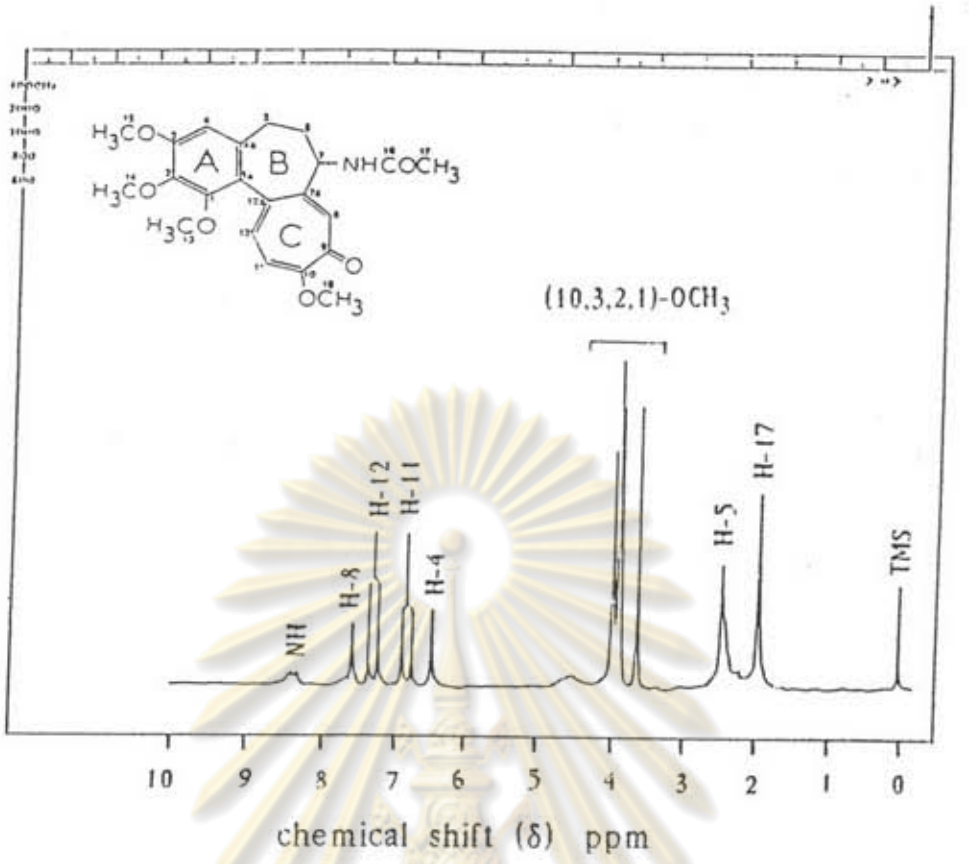


Fig.5 Proton-NMR spectrum of colchicine.

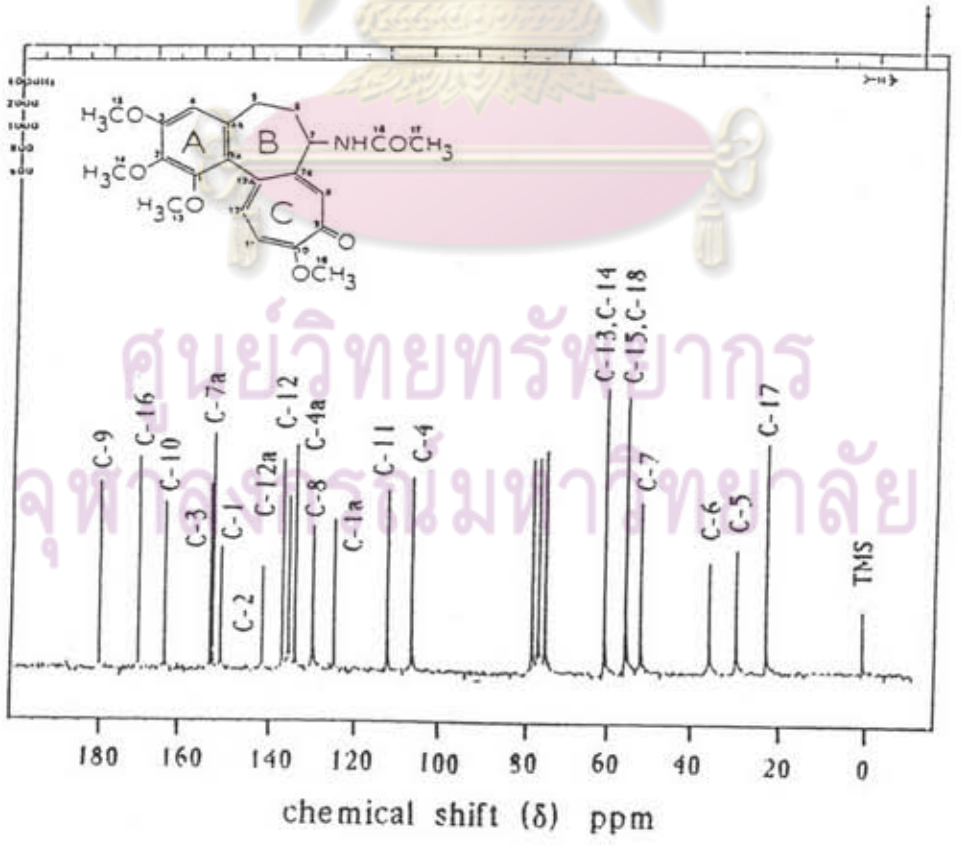


Fig.6 Carbon-13 NMR spectrum of colchicine.

Table 2 Mass spectrum fragmentation pattern of colchicine.

m/e	Species
399	M^+
371	M^+-CO
312	$M^+(371)-CH_3-\underset{\displaystyle OH}{\overset{\displaystyle |}{C}}-NH$
297	$M^+(312)-CH_3$
281	$M^+(312)-OCH_3$

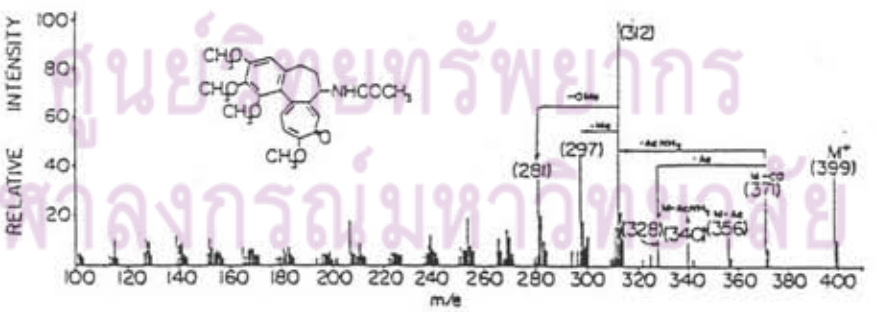


Fig.7 Mass spectrum of colchicine.

For other properties, colchicine shows its melting point range of 142-150 °C, pKa 12.35 at 20 °C (in aqueous solution) and its 0.5 % solution gives a pH of 5.9 . In term of solubility, it is freely soluble in alcohol or chloroform, sparingly soluble in water (1g/22 ml), benzene (1g/100 ml), ether (1g/220 ml) and practically insoluble in petroleum ether (Merck Index, 1989). The specific rotation given in the British Pharmacopoeia (BP, 1988) for a 1% aqueous solution is -425 to -450 ° (at 19.5-20.5 °C).

4.3 Chemical Reaction and Degradation of Colchicine

Colchicine is converted into a mixture of three photoisomers in the presence of ultraviolet light (Pelletier, 1970 ; Dalton, 1979). These are β-lumicolchicine , γ-lumicolchicine and α-lumicolchicine (Fig.8). These tetracyclic structures are formed with loss of the tropolone ring.

Upon acid hydrolysis, a sequence of reaction was purposed which led to the formation of colchicinic acid (Fig.9) (Eigsti and Dustin, 1955 ; Dalton, 1979). However, the conversion of colchicine to colchiceine and other products also occurs during alkaline hydrolysis with pH>13 (Wilczok *et al.*, 1979). There is no appreciable hydrolysis to colchicine occuring in neutral or slightly alkaline (pH 8.1) solutions even after 2 months storage (BP, 1980).

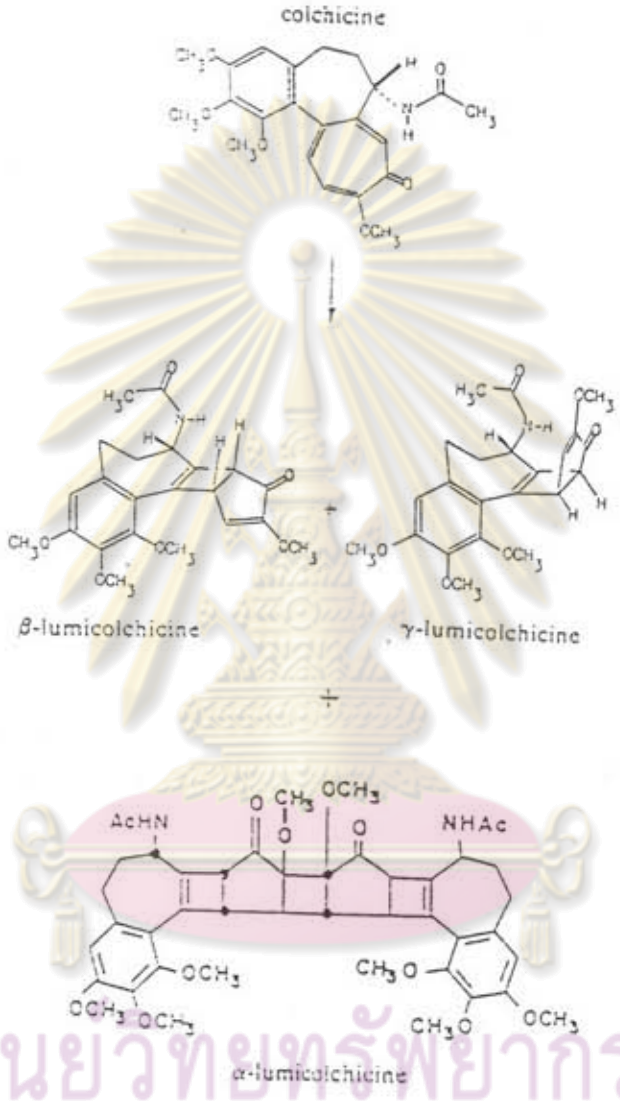


Fig.8 Reaction of colchicine with ultraviolet light.

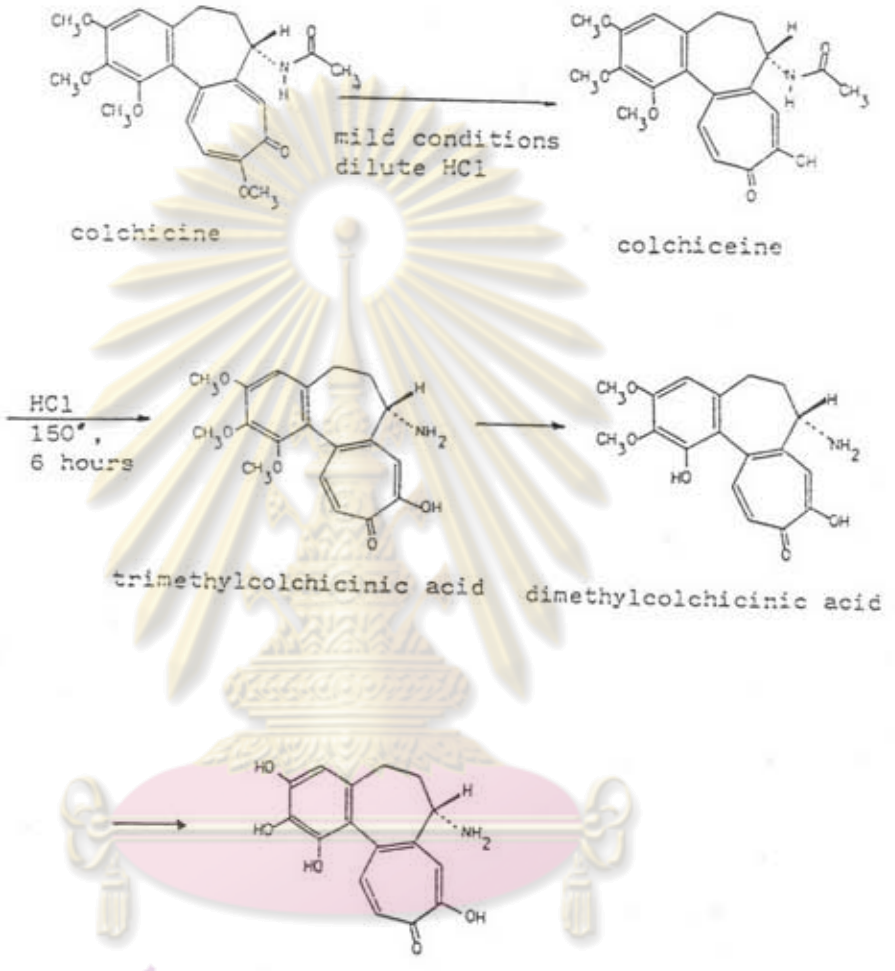
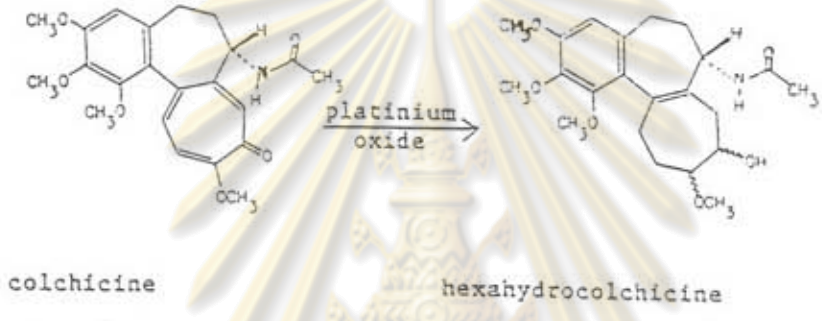


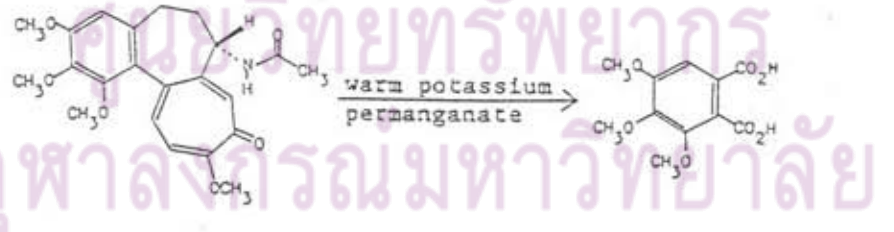
Fig.9 Acid hydrolysis of colchicine.

With hydrogenation reaction, colchicine has been shown to be converted to hexahydrocolchicine in the presence of platinum oxide (Fig.10) (Eigsti and Dustin, 1955 ; Dalton, 1979). Finally, it has been reported that oxidation of colchicine by using warm potassium permanganate leads to the formation of 3,4,5-trimethoxyphthalic acid (Fig.11) (Dalton, 1979).



colchicine hexahydrocolchicine

Fig.10 Hydrogenation of colchicine.



colchicine 3,4,5-trimethoxyphthalic acid

Fig.11 Oxidation of colchicine

4.4 Isolation of Colchicine from Plant Materials

Colchicine is the medicinally active component in *Colchicum autumnale* L. (Liliaceae) and numerous species of *Colchicum*. It has also been found in other Liliaceous species (Table 3). Extraction of colchicine in these plant materials is effected by alcohol (Eigsti and Dustin, 1955). For colchicine isolation, the crude extract is first distilled off the alcohol. The syrupy residue is diluted with water to precipitate the insoluble fats and resins, and filtered. The aqueous solution is then repeatedly extracted with chloroform or digested with lead carbonate. After refiltered, evaporated to a small volume , and extracted with chloroform , the colchicine is recovered as a crystalline addition complex with chloroform. The chloroform is then distilled off in steam or alcohol and evaporation of the residual solution yields amorphous colchicine which may be crystallized from ethyl acetate as pale yellow needles (Eigsti and Dustin, 1955 ; Wyatt *et al.*, 1981).

Moreover, it has been reported on some modifications of colchicine extraction. For example, chromatographic purification of the chloroform solution on alumina (Eigsti and Dustin,1955) ; extraction of the dried powder with petroleum ether to remove fats followed by alcoholic extraction (Eigsti and Dustin, 1955) ; wax and

Table 3 Colchicine-containing plants.

Name	Reference
Androcymbium gramineum L.	Youngken, 1950
A. melanthioides var *stricta* Baker	Malichova *et al.*, 1979
Anthericum ramosum L.	Eigsti and Dustin, 1955
Asphodelus albus Willd.	Eigsti and Dustin, 1955
Bulbocodium ruthenicum Bung.	Eigsti and Dustin, 1955
Colchicum alpinum DC.	Eigsti and Dustin, 1955
C. arenarium Waldst. and K.	Eigsti and Dustin, 1955
C. autumnale L.	Eigsti and Dustin, 1955
C. byzantinum Ten.	Santavy *et al.*, 1981
C. latifolium S.S.	Malichova *et al.*, 1979
C. luteum Baker	Eigsti and Dustin, 1955
C. montanum L.	Eigsti and Dustin, 1955
C. multiflorum Brot.	Eigsti and Dustin, 1955
C. neapolitanum Ten.	Eigsti and Dustin, 1955
Fritillaria montana Hoppe.	Eigsti and Dustin, 1955
Gloriosa rothschildiana O' Brien.	Thakur *et al.*, 1975
G. simplex L. (*G. virescens* Lindl.)	Potesilova *et al.*, 1967; Thakur *et al.*, 1975
G. superba L.	Eigsti and Dustin, 1955
Hemerocallis fulva L.	Eigsti and Dustin, 1955
Iphigenia stellata Kunth.	Sarin *et al.*, 1974
Littonia modesta Hook.	Potesilova *et al.*, 1967
Lloydia serotina Salib.	Eigsti and dustin, 1955
Merendera bulbocodium Ram.	Eigsti and Dustin, 1955
M. caucasica Biel.	Eigsti and Dustin, 1955
M. persica Bois. and Kotsch.	Eigsti and Dustin, 1955
M. sobolifera Fisch	Eigsti and Dustin, 1955
Muscari tenuiflorium Tausch.	Eigsti and Dustin, 1955
Ornithogalum comosum L.	Eigsti and Dustin, 1955
O. umbellatum L.	Eigsti and Dustin, 1955
Sandersonia aurantiaca Hook.	Finnie and Van-Staden, 1991

Table 3 (continued)

Name	Reference
Tofieldia calyculata Whlnd.	Eigsti and Dustin, 1955
T. glacialis Gaud.	Eigsti and Dustin, 1955
Tulipa silpestris L.	Eigsti and Dustin, 1955
Veratrum album L.	Eigsti and Dustin, 1955
V. nigrum L.	Eigsti and Dustin, 1955

paraffin wax for the removal of resin (Smolenski, Crane and Voigt, 1958) ; soxhlet apparatus (Smolenski *et al.*, 1958) were added in colchicine extraction procedures (Wyatt *et al.*, 1981).

4.5 Detection and Determination of Colchicine

4.5.1 Color Tests

Colchicine is classified as an neutral alkaloid which can form precipitates with many common alkaloidal reagents under suitable conditions (Eigsti and Dustin, 1955). Table 4 summarizes the reagents used for the color tests of colchicine and the resulted color of each reaction (Wyatt *et al.*, 1981).

Table 4 Color tests of colchicine.

Agents	Color
1. Dilute mineral acids and alkalis	intense yellow
2. nitric acid	violet slowly changing to yellow then to green
3. sulfuric acid formaldehyde	yellow
4. ammonium molybdate	yellow
5. ammonium vanadate (Vitali's test)	green → yellow → purple / brown / red-brown
6. ferric chloride T.S.	garnet red
7. sulfuric acid followed by nitric acid	lemon-yellow
	greenish-blue → reddish → yellow or almost colorless
8. excess of sodium hydroxide	red
9. water (color intensified by adding mineral acids)	yellow
10. nitric acid-water-sodium hydroxide	orange-red
11. concentrate nitric acid ; addition of water; followed by sodium hydroxide	violet → brown / red → yellow → orange / red
12. hydroxylamine-sodium hydroxide (warm the solution)	orange

4.5.2 Aqueous Titrimetric Analysis (Residual Titration)

This method is based on the direct titration of the alkaloid colchicine presence in an aqueous solution. Practically, an accurately weighted sample of colchicine is dissolved in excess 0.02 N hydrochloric acid. The excess acid is titrated with 0.02 N sodium hydroxide using methyl orange as indicator (Karawya and Diab, 1975).

4.5.3 Non-aqueous Titrimetric Analysis

The non-aqueous titration procedure is the official method described in the United States Pharmacopoeia XXI (USP XXI, 1985) and the British Pharmacopoeia (BP, 1988). An accurately weight sample of colchicine is dissolved in a mixture of acetic anhydride-toluene (1:2). The end point is determined potentiometrically using 0.02 N perchloric acid as the titrant. An addition non-aqueous titration procedure has also been presented (Karawya and Diab, 1975). In this method, glacial acetic acid containing 3-4 drops of acetic anhydride is used to dissolve colchicine. Titration is accomplished using either crystal violet or potentiometric determination using calomel and glass electrodes, 0.01 N perchloric acid is used as the titrant.

4.5.4 Spectrophotometric Analysis

The official USP XXI (USP XXI, 1985) and BP 1988 (BP, 1988) methods for the analysis of colchicine tablets are spectrophotometric. A portion of powdered tablets is weighted and colchicine is extracted with chloroform from an aqueous solution. The UV spectrum of the chloroform solution is recorded and compared to the USP reference standard (diluted to the same final concentration with chloroform) at the maximum absorbance at about 350 nm. Spectrophotometric analysis is also conducted using nitric acid to dissolve the drug, followed by sodium hydroxide T.S. and diluted with water. The solution is then read at 350 nm, with an additional maximum observed at about 510 nm (Smolenski *et al.*, 1958). In addition to the official methods, spectrophotometric analysis using the hydroxylamine-sodium hydroxide color reaction (orange color) can be accomplished using either readings at 500 nm (Mack and Finn, 1949) or with ferric chloride solutions, after acid hydrolysis (read at 470 nm) (King, 1951 ; Pearce, 1959). Colchicine can also be analyzed after lithium aluminum hydride reduction and extraction from 1 % hydrochloric acid-ammonia-acetic acid solution into carbon disulfide. The organic layer is removed and combined with benzene followed by reading at 445 nm (Karawya and Diab, 1975).

Isonicotinic hydrazide in alkaline media has also been used for reaction with colchicine for a colorimetric determination (Wallace, 1961).

4.5.5 Paper Chromatography

Ascending paper chromatography has been accomplished using Whatman no.1 paper predipped in a 5 % solution of sodium dihydrogen citrate and dried. The solvent system consist of 4.8 g of citric acid in a mixture of 130 ml of water and 870 ml of 1-butanol, Rf x 100 = 83 (Wyatt *et al.*, 1981). Examination is conducted using shortwave ultraviolet light (Clarke,1969). An additional analysis can be performed using formamide/benzene:chloroform:formamide(7:3:1) and longwave ultraviolet detection (Macek, 1972).

4.5.6 Thin-layer Chromatography

Thin-layer chromatography has frequently been used for the analysis of colchicine. Methods of detection and solvent systems are listed in Table 5 (Wyatt *et al.*, 1981).

Table 5 Thin-layer chromatography of colchicine.

Plate	Solvent	Method of detection*	Rf x 100
silica gel F-254	chloroform-acetone-diethylamine (5:4:1)	A,B,C,D,E	47
silica gel F-254	chloroform-methanol-acetic acid (65:15:1)	A,B,C,D,E	75
silica gel F-254	chloroform-methanol (9:1)	A,B,C,D,E	68
silica gel F-254	chloroform-methanol-diethylamine (5:4:1)	A,B,C,D	98
silica gel F-254	totuene-ethanol-aqueous ammonia (170:28:2)	A,B,C,D,F	18
silica gel G	benzene-acetone-ether-10% aqueous ammonia (4:6:1:0.3)	-	15
silica gel G	benzene-acetone-ether-25% aqueous ammonia (4:6:1:0.3)	-	20
silica gel G	chloroform-diethylamine (9:1)	G	41
silica gel G	benzene-ethyl acetate-diethylamine (5:4:1) + 8% methanol	B,G,H	61
silica gel G	methanol-aqueous ammonia (100:1.5)	I	62
silica gel G pretreated with 0.1 N NaOH	methanol	G	57
aluminum oxide F-254	chloroform-acetone-aqueous ammonia (25:20:0.4)	A,B,C,D,E	64
alumina G	chloroform	G	11

*Methods of detection colchicine on TLC plate

A : Shortwave ultraviolet light

B : longwave ultraviolet light

C : 0.5% iodine in chloroform

D : 40% sulfuric acid in methanol followed by heat (105°)

E : 40% sulfuric acid in methanol followed by heat (105°) and longwave ultraviolet light

F : acidified potassium iodoplatinate

G : potassium iodoplatinate

H : antimony (III) chloride

I : p-dimethylaminobenzaldehyde

4.5.7 High-performance Liquid Chromatographic Analysis

High-performance liquid chromatography has been used extensively for the analysis of colchicine (Davis and Klein, 1980 ; Klein and Davis, 1980, 1981) and is the official United States Pharmacopoeia XXII method for the drug substance (USP XXII, 1990). Furthermore, HPLC has also been used for the determination of colchicine and colchicoside in powdered seeds of *C. autumnale* (Forni and Massarani, 1977). The various HPLC systems used for the analysis are given in Table 6 (Wyatt *et al.*, 1981).

Table 6 High-performance liquid chromatographic systems for colchicine.

Column	Mobile phase	UV-detection wavelength
μ Bondapak C-18	20% and 35% acetonitrile-water	254
μ Bondapak C-18	acetonitrile-methanol-phosphate buffer pH 6 (16:5:79)	350
LiChrosorb RP-18	acetonitrile-methanol-phosphate buffer pH 6 (16:5:79)	350
LiChrosorb RP-8	30% acetonitrile-water	254
LiChrosorb Si-60	gradient : acetonitrile-10% acetonitrile in water (0-30%)	254
Zorbax-Sil	87-89% methylene chloride-2-propanol	254
Chromanetics C-8	methanol-water (1:3)	254
Partisil ODS	methanol-water (1:1, 1:2)	254
Hypersil (5 μm)	dichloromethane-2-propanol	240

4.6 The Biosynthesis of Colchicine

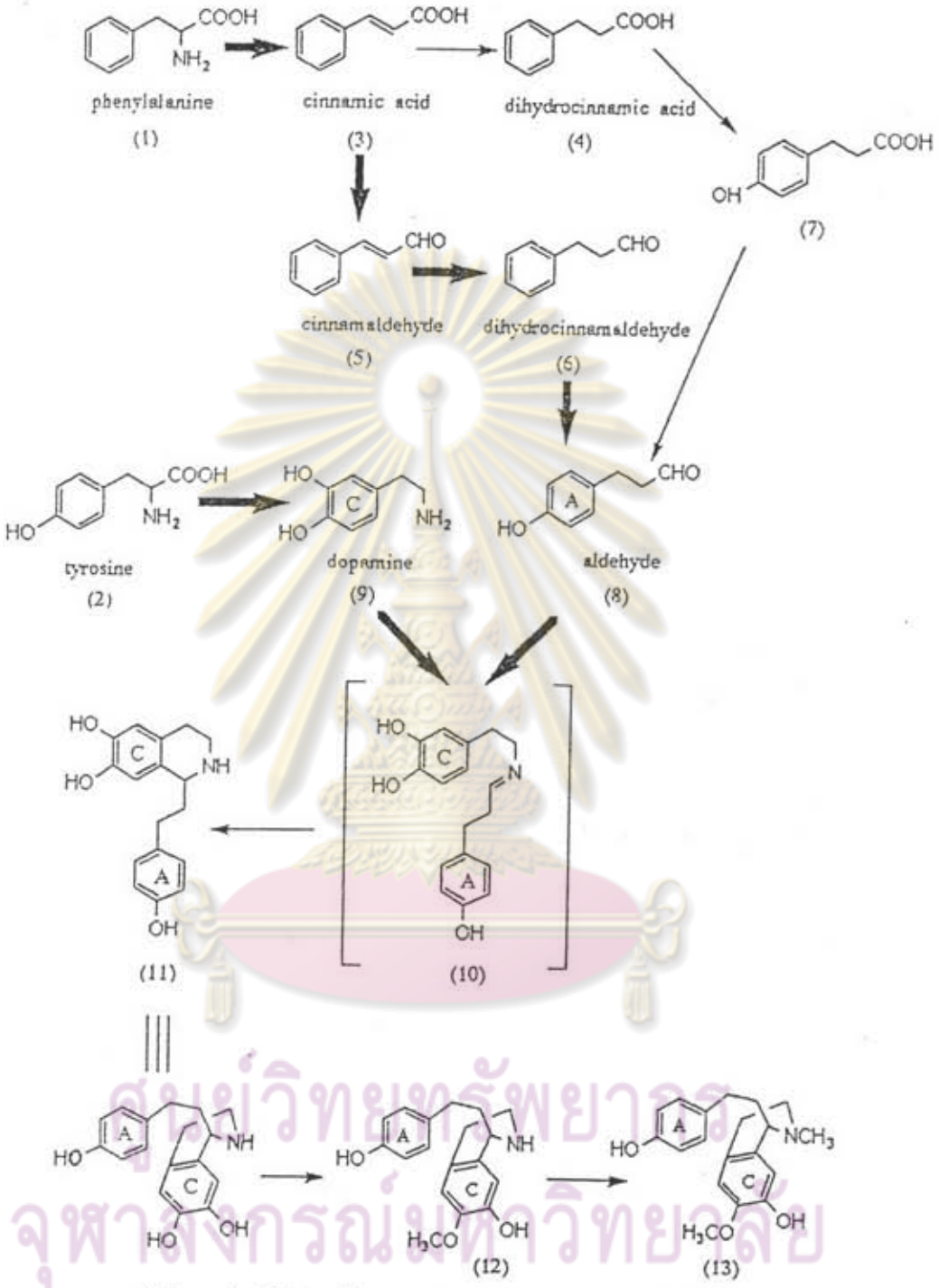
Colchicine is a neutral alkaloid with a unique tropolone ring. The compound consists of an aromatic ring A , with three methoxy groups , a seven-membered ring B , which is substituted with an acetylated amino group , and the tropolone ring C (see(23) in Fig.12).

Tracer feeding experiments have shown that colchicine is originated from one molecule of tyrosine and one molecule of phenylalanine. The tropolone ring C is formed from the aromatic nucleus of tyrosine and C-3 of the side chain *via* dopamine. Ring A of colchicine and C-5, C-6 , and C-7 has been reported to be derived from phenylalanine *via* cinnamic acid (Leete, 1965 ; Battersby *et al.*, 1972 ; Herrick, 1981 ; Battersby , McDonald , and Stachulski, 1983 ; Herbert and Knagg, 1986 ; Herbert, Kattah, and Knagg, 1990).

Early studies on feeding experiments with *Colchicum autumnale* and *C. byzantinum* Ten. established that colchicine (23) is a modified phenethylisoquinoline alkaloid (as autumnaline (16)) which is derived from cinnamic acid (3) *via* cinnamaldehyde (5) , dihydro-cinnamaldehyde (6) and aldehyde (8). The aldehyde is then condensed with dopamine (9) which is derived from tyrosine (2). This route is believed to be a major pathway for the

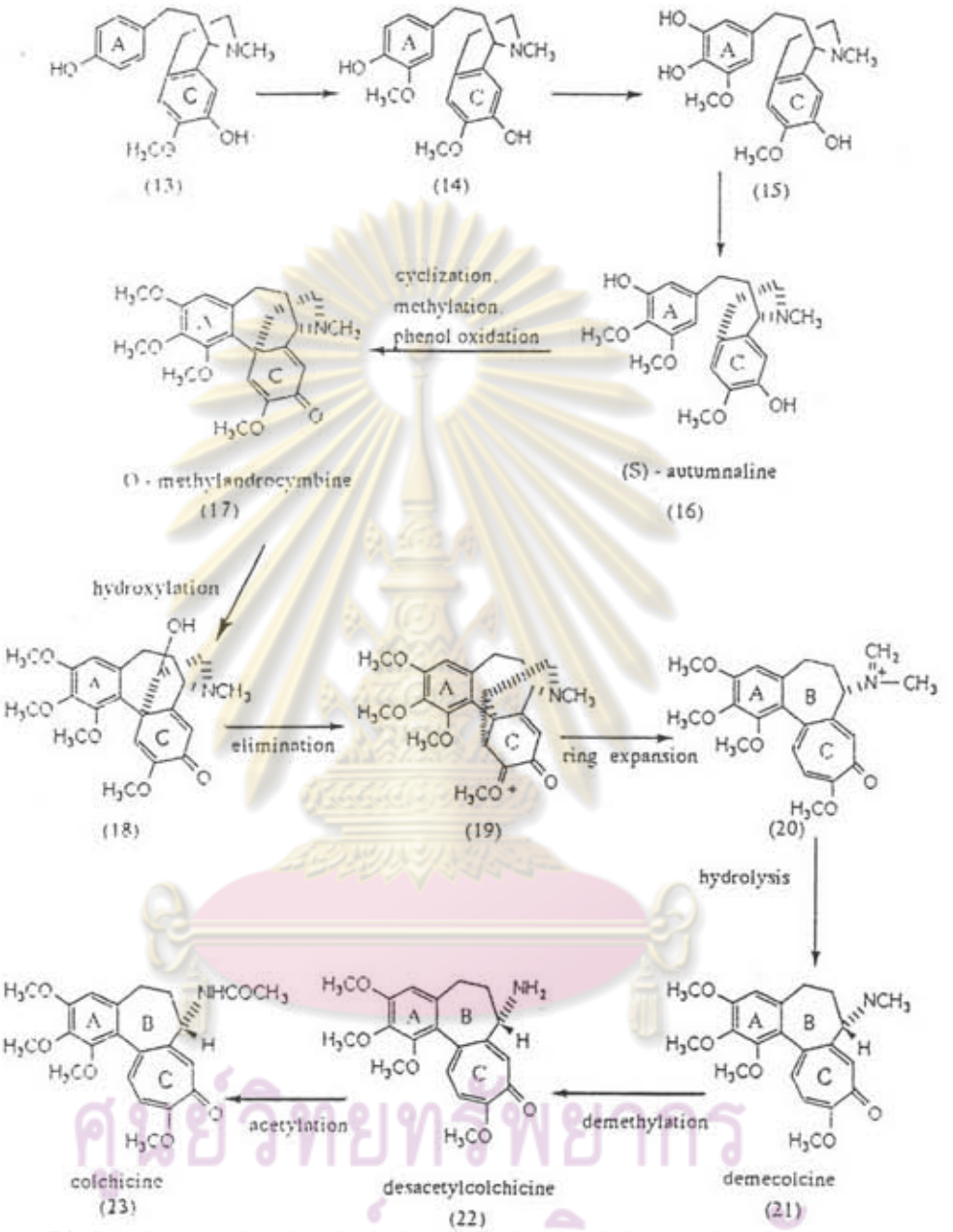
biosynthesis of colchicine (Fig.12, scheme 1, thickened arrows). Alternatively, dihydrocinnamic acid (4) may be utilized by the plants as a minor biosynthetic route (Fig.12, scheme 1, normal arrows). Final resolution of these stages in the biosynthetic pathway must now depend on evidence with isolated enzymes (Herbert and Knagg,1986; Herbert *et al.*, 1990).

Condensation of the aldehyde (8) with dopamine (9) affords a set of phenethylisoquinolines which are precursors for the alkaloid colchicine (23) and (11) is identified as the first of these followed by (12) and then (13) (Herbert *et al.*, 1990) as shown in Fig.12, scheme 1. The late stages of the biosynthesis of colchicine is known to involve conversion of a phenethyltetrahydro-isoquinoline (S)-autumnaline (16) by cyclization , methylation and phenol oxidation into a dienone (O-methyl-androcymbine)(17). Then hydroxylation of (17) yields the alcohol (18) which undergoes elimination to (19) and ring expansion to generate the tropolone system (20). Subsequently, the immonium salt (20) undergoes hydrolysis to demecolcine (21). Finally , demethylation and acetylation of (21) at nitrogen atom to form desacetylcolchicine (22) and colchicine (23), respectively (Leete, 1965 ; Barker *et al.*, 1967 ; Luckner, 1972 ; Battersby , Sheldrake , and Milner, 1974 ; Dalton, 1979 ; Battersby *et al.*, 1983 ; Herbert *et al.*, 1990).



Scheme 1 : Early and intermediate stages of colchicine biosynthesis.

Fig.12 Biosynthetic pathway of colchicine.



Scheme 2 : The latter stages of colchicine biosynthesis

Fig.12 Biosynthetic pathway of colchicine (continued)

4.7 Biological Activities of Colchicine

Colchicine has been reported to possess anti-inflammatory and anti-tumor activities (Eigsti and Dustin, 1955 ; Woodbury, 1970 ; Creascy, 1975 ; Kastrup, 1988 ; Reynolds, 1989). It has also been reported to be a powerful mitotic poison (Sartorelli and Creascy, 1969 ; Lin *et al.*, 1980 ; Meksuriyen *et al.*, 1988). The compound has been used for a specific treatment of acute attacks of gouty arthritis (Kastrup,1988 ; Gennaro,1990) although the exact mechanism of action is not known. Colchicine apparently exerts its effect by reducing the inflammatory response to the deposited crystals and also by diminishing phagocytosis (Kastrup, 1988). It diminishes lactic acid production by leukocytes directly and by diminishing phagocytosis and thereby interrupts the cycle of urate crystal deposition and inflammatory response that sustains the acute attack (Kastrup, 1988). The oxidation of glucose in phagocytizing as well as in nonphagocytizing leukocytes *in vitro* is suppressed by colchicine (Kastrup, 1988). Although it relieves pain in acute gouty attacks, colchicine is not an analgesic and does not relieve other types of pain or inflammation. Colchicine is not a uricosuric and will not prevent the progression of gout to chronic gouty arthritis. Its prophylactic , suppressive effect helps reduce the incidence of acute attacks and relieve the patient's occasional residual pain and mild

discomfort (Kastrup, 1988 ; Gennaro, 1990).

Colchicine has been found to be an effective inhibitor of mitosis and microtubule assembly (Andreu and Timasheff, 1982). Therefore , colchicine , in proper dilutions , can arrest the plant and animal cell division *in vitro* and *in vivo* (Watt and Breyer-Brandwijk, 1962 ; Chopra *et al.*, 1965). The action is believed to be directly on cell division and resulting in the arrest of mitosis in the metaphase. This is due to the binding of colchicine to the protein tubulin to form a tight complex and induces a conformation change in the protein (Margolis and Wilson,1977 ; Goodwin and Mercer, 1983). This tubulin-colchicine complex inhibits the growth of microtubule and spindle formation in the metaphase. This leads to the arrest of cellular mitosis (Andreu and Timasheff, 1982).

Cytotoxicity of colchicine might be of benefit in cancer therapy (Morton, 1977) as it has been reported that colchicine induces regression of tumours in mice and dogs but its inhibition of cell division appears to be not specific for tumour cells. Furthermore, the effective dose for inhibiting the growth of tumours approaches the lethal dose for the host (Glasby, 1975).

Colchicine has been found to have some degree of antibacterial action on various species of bacteria

including *Staphylococcus aureus* (Sastri, 1956). It also has activities on both normal and cancer cells, especially the cells with high rate of cell division such as bone marrow, tumours, skin and lymphoid structures (Watt and Breyer-Brandwijk, 1962). Colchicine also produces a temporary leucopaenia followed by a leucocytosis, due sometimes to a striking increase in the number of basophils. These haemopoietic effects are apparently due to a direct action on the bone marrow , which may result in agranulocytosis or aplastic anaemia after toxic amounts of colchicine (Watt and Breyer-Brandwijk, 1962).

Colchicine can lower body temperature, increase the sensitivity to central depressants , depress the respiratory centre,enhance the response to sympathomimetic agents , constrict the blood vessels and induces hypertension by central vasomotor stimulation, enhance gastro-intestinal activity by neurogenic stimulation, and alter neuromuscular function (Watt and Breyer-Brandwijk, 1962).

Colchicine is now being widely used in plant breeding work for inducing polyploidy. This action is due to its inhibition of cell separation after the division of chromosomes resulting in polyploidy or doubling of the number of chromosomes. Colchicine is, therefore, used for making hybrids of widely different species or varieties of

garden flowers or economic crops (Chopra *et al.*, 1965), Colchicine has been applied also in animal studies, especially to explore embryonic growth and wound-healing (Morton, 1977).

Besides, colchicine has been used in the treatment of hepatic cirrhosis (Kastrup, 1988) and for familial Mediterranean fever which is prevalent in Egypt (Morton, 1977 ; Kastrup, 1988).

4.8 Pharmacokinetics of Colchicine

Colchicine is well absorbed after oral administration with approximately 31 % of the compound is bound to plasma protein. Large amounts of colchicine and its metabolites enter the intestinal tract in bile and intestinal secretions. The bulk of the absorbed colchicine is excreted within the first 24 hours , especially at high blood levels. High concentrations of colchicine are found in the kidney,liver and spleen. Excretion occurs primarily by biliary and renal routes (Kastrup, 1988).

The dose of colchicine for a treatment of acute gouty arthritis is 0.5 to 1.2 mg initially , follow by 0.5 to 1.2 mg every 1 to 2 hours , until pain is relieved, or nausea , vomiting or diarrhea occurs. The total amount of colchicine needed to control pain and inflammation

during an attack is 4 to 8 mg. Articular pain and swelling typically abate within 12 hours and are usually gone in 24 to 48 hours (Kastrup, 1988 ; Gennaro, 1990).

4.9 Toxicity of Colchicine

Colchicine is classified as an extremely poisonous drug (Thai Pharmacopoeia, 1987 ; USP XXII,1990). It is very toxic when taken in large doses. The most frequent adverse effects of colchicine are those involving the gastrointestinal tract and may be associated with its antimitotic action (Schindler, 1965 ; Reynolds, 1989). Diarrhoea, nausea, vomiting, and abdominal pain are often the first signs of toxicity (Sastri, 1956 ; Watt and Breyer-Brandwijk, 1962 ; Glasby, 1975 ; Kastrup, 1988 ; Reynolds, 1989 ; Gennaro, 1990). The symptoms of toxicity are including burning in the mouth and throat, extreme thirst, difficulty in swallowing, acute gastroenteritis, abdominal discomfort , nausea , violent vomiting and uncontrollable, purging followed by bloody diarrhea and dysenteric, cessation of urine, weak-quick pulse, chills, flatulence , gritting of teeth , vascular damage which result in shock, kidney damage which cause hematuria and oliguria , severe dehydration , hypotension , pain in the extremities, muscular weakness, ascending paralysis of the central nervous system , convulsions and respiratory paralysis. Death usually results from respiratory

depression (Watt and Breyer-Brandwijk, 1962; Morton, 1977; Kastrup, 1988 ; Reynolds, 1989).

Prolonged therapeutic use of colchicine may cause bone marrow depression and result in agranulocytosis, thrombocytopenia and aplastic anemia. Furthermore , it causes peripheral neuritis, myopathy, rashes, azoospermia or oligospermia, and sometimes loss of hair (Morton, 1977; Kastrup, 1988 ; Reynolds, 1989).

The lethal dose of colchicine is estimated to be 65 mg but there have been reported that ingestion of as little as 7 mg of colchicine has caused death (Morton, 1977 ; Kastrup, 1988).