CHAPTER II

HISTORICAL

1. Taxa and Description

Alangium salviifolium Wang. has a number of synonyms, including *A. salviifolium* Wang subsp. *hexapetalum* Wang, *A. hexapetalum* Lamk. and *A. lamarckii* Thw.; known in Thai as ปรู๋ Pruu, ผลู Phuu (central), ปรู๋ Pruu (Northern, Northeastern), มะเกลือกา Ma-kluea-kaa (Prachinburi), มะตาปู๋ Ma-taa-puu (Chiang Mai).

The family Alangiaceae is a small family with only one genus (*Alangium*) and about 17 species (Mabberley, 1987). It generally occurs in tropical and subtropical regions of the old world (Keng, 1987). The characteristic of this family are as follows : Tree or shurbs, sometimes spiny. Leave alternate, simple, stipules 0. Flowers hermaphrodite, in a axillary cymes : pedicles articulated. Calyx truncate or with 4-10 teeth. Petals 4-10 mostly linear valvate, at length recurved sometime connate at base. Stamen the same number as an alternate with petals or 2-4 times as many, free or slightly connate at the base, more or less villous inside; anther 2 celled, linear, opening length wise. Disk cushion-like. Ovary inferior, 1-2 celled; style simple, calvate or 2-3 lobed; ovule solitary, pendulous, with 2 integuments. Fruit a drupe crowned by the sepals and disk 1-seed stone. Seed with embryo about equal to endosperm.

A. salviifolium is a small tree with more or less spinescent branches; bark light colored; young parts pubescent. Leaves variable 7.5-12.5 by 2.5-5.7 cm, narrowly ablong or ovate-lanceolate, more or less accuminate, subobtuse, entine, glabrous above, pubescent on the nerve and prominently reticulately veined beneath, base round or acute; petioles 6-13 mm long, densely pubescent flower few, in axillary

fascicles; pedicle 3-6 mm long, densely pubescent, jointed at the top. Calyx tusbinate 3 mm long, densely silky pubescent; teeth triangular, 0.85 mm long petals 5-10 (usually 6), densely pubescent outside, 1.3-2 cm long and about 5 mm wide, narrow linear, reflexed. Stamens numerous (usually more than 20) nearly as long as the petal; filaments hairy at base. Style as long as the stamens; stigma very large. Fruit when young ovoid or ellipsoid, becoming nearly globular when ripe 1.3-2.0 cm diam., crowned by the persistant calyx-limb, finely pubescent, not or obscurely ribbed, pusplish red; endocarp bony; albumen fleshy outside friable inside, not at all ruminate; cotyledons foliaceous, flat, not crumpled (Kirtikar and Basu, 1993).

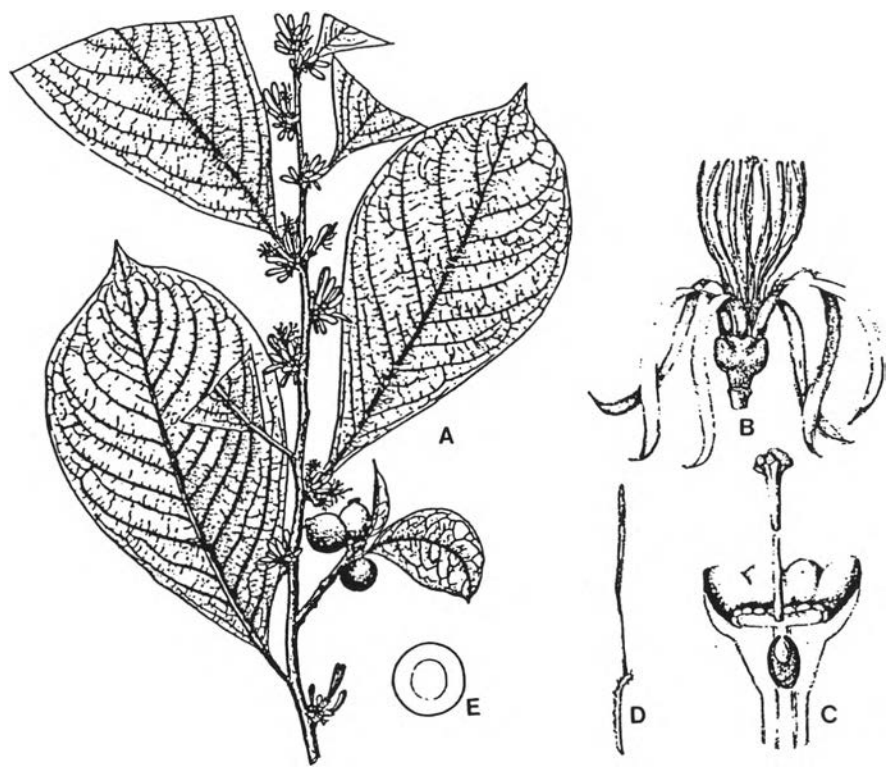

Figure 1 *Alangium salviifolium* Wang. (A) leafy shoot, axillary flowers and fruiting shoot (B) flower with short calyx tube, long recurved petals, numerous stamens and single stlye with a lobed stigma (C) half section of gynoecium showing ovary with pendulous ovule (D) stamen with hairy filament and basifixed anther (E) cross section of fruit

2. Chemical Constituents of *Alangium salviifolium* Wang

The compounds that have been found in this plant are listed in Table 1 and source of their structures are shown in Appendix.

Table 1 Chemical constituents reported to be present in *Alangium salviifolium* Wang

Compounds	Category	Plant Part	References
alamaridine	alkaloid	seed	Bhattacharjya, Mukhopudhyay and Pakrashi, 1986
alamarine (20)	alkaloid	seed	Pakrashi *et al.*, 1980
dihydroalamarine (22)	alkaloid	seed	Pakrashi *et al.*, 1985
dihydroisoalamarine (23)	alkaloid	seed	Pakrashi *et al.*, 1985
isoalamarine (21)	alkaloid	seed	Pakrashi *et al.*, 1980
alancine (12)	alkaloid	stem bark	Chattopadhyay *et al.*, 1984
alangamide (5)	alkaloid	seed	Pakrashi and Ali, 1969
alangicine (9)	alkaloid	root bark	Pakrashi and Ali, 1967
alangidiol	triterpene	leaf	Achari, Pal and Pakrashi, 1975
alangimarckine (28)	alkaloid	leaf, root bark	Willaman and Li, 1920
alangimaridine (16)	alkaloid	seed	Pakrashi *et al.*, 1980
alangimarine (17)	alkaloid	seed	Pakrashi *et al.*, 1980
isoalangimarine (18)	alkaloid	seed	Pakrashi *et al.*, 1980
alangimarinone (19)	alkaloid	seed	Pakrashi *et al.*, 1980
alangine A	alkaloid	root bark	Bhakuni, Dhar and Dhar, 1960
alangine B	alkaloid mixture	root bark	Bhakuni *et al.*, 1960

Table 1 (Continued)

Compounds	Category	Plant Part	References
alangiside (30)	glucoside	root, leaf, fruit	Shoeb *et al.*, 1975
3-O-demethyl-2-O-methyl alangiside (31)	glucoside	fruit	Itoh *et al.*, 1994
3-O-demethyl-2-O-methylisoalangiside (34)	glucoside	fruit	Itoh, Tanahashi and Nagakura, 1995
methylalangiside	glucoside	fruit	Itoh *et al.*, 1994
methylisoalangiside (33)	glucoside	fruit	Itoh *et al.*, 1995
isoalangiside (32)	glucoside	fruit	Itoh *et al.*, 1995
demethylneoalangiside (35)	glucoside	fruit	Itoh *et al.*, 1995
neoalangiside (36)	glucoside	fruit	Itoh *et al.*, 1995
ankorine (11)	alkaloid	leaf	Dasqupta *et al.*, 1965
betulin	triterpene	kernel	Pakrashi *et al.*, 1968
betulinaldehyde	triterpene	kernel	Pakrashi *et al.*, 1968
betulinic acid	triterpene	kernel	Pakrashi *et al.*, 1968
hydroxylactoneA betulinic acid	triterpene	kernel	Pakrashi *et al.*, 1968
bharatamine (29)	alkaloid	seed	Paknashi *et al.*, 1983
cephaeline (2)	alkaloid	root bark	Albright, Van Meter and Goldman, 1965
		root bark	Willaman and Li, 1970
		stem bark	
		seed	Achari *et al.*, 1980
demethylcephaeline (4)	alkaloid	stem bark	Pakrashi and Achari, 1970
isocephaeline (3)	alkaloid	seed	Achari *et al.*, 1980
emetine (1)	alkaloid	stem bark	Budzikiewicz, Pakrashi and Vorbruggen, 1964
protodehydroemetine (10)	alkaloid	leaf	Willaman and Li, 1970

Table 1 (Continued)

Compounds	Category	Plant Part	References
friedeline	triterpene	leaf	Gupta, Singh and Bhakuni, 1969
lacinilene C	sesquiterpene	seed	Mukhopudhyay *et al.*, 1987
loganic acid	monoterpene	fruit	Kapil *et al.*, 1971
L:N-benzoylphenyl-alaniol	proteid	leaf	Achari, Pal and Pakrashi, 1974
protoemetinol (13)	alkaloid	leaf	Albright, Van Metes and Goldman, 1965
		root bark	Willaman and Li, 1970
10-demethylproto-emetinol (15)	alkaloid	seed	Ali *et al.*, 1982
9-demethylproto-emetinol (14)	alkaloid	seed	Ali *et al.*, 1982
psychotrine (6)	alkaloid	root bark	Budzikeiwicz *et al.*, 1964
		stem bark	Willaman and Li, 1970
		seed	Pakrashi and Ali, 1967
11-hydroxy psychotrine (8)	alkaloid	root bark	Willaman and Li, 1970
demethylpsychotrine (7)	alkaloid	root bark	Pakrashi and Ali, 1967
		stem bark	Pakrashi and Achari, 1970
salsoline	alkaloid	seed	Achari *et al.*, 1980
beta-sitosterol	steroid	kernel	Pakrashi *et al.*, 1968
		leaf	Gupta *et al.*, 1969
stigmasterol	steroid	leaf	Gupta *et al.*, 1969
tubulosine (24)	alkaloid	root bark	Albright *et al.*, 1965
		stem bark	Bhakuni, Jain and Chatuvedi, 1983
10-demethyltubuiosine (27)	alkaloid	root bark	Popelak, Haak and Spingles, 1969
deoxytubulosine (26)	alkaloid	seed, leaf	Battersby *et al.*, 1996

Table 1 (Continued)

Compounds	Category	Plant Part	References
isotubulosine (25)	alkaloid	root bark	Popelak, Haak and Spingler, 1969
venoterpine	monoterpene alkaloid	seed	Achari *et al.*, 1980

3. Biosynthesis of Terahydroisoquinoline Monoterpene Alkaloids and Glucosides

Emetine is a unique of tetrahydroisoquinoline monoterpene alkaloid with emetic and anti-amebic activities and is still an essential drug. In *Cephaelis ipecacuaha* (Rubiaceae) and *Alangium salviifolium* (Alangiaceae), emetine is present together with other ipecac alkaloids such as cephaeline (demethyl emetine), protoemetine. In both plant species, natural alkaloidal glucosides such as ipecoside in *Cephaelis spp.* and alangiside in *Alangium spp.* have also been found (Shoeb *et al.*, 1975).

In a series of isolation and early state of biosynthetic studies, Battersy and Gregory (1968) proposed that the ipecac alkaloid structures arised from the condensation of one monoterpenoid unit and one phenylalanine unit. This initial reaction was conceptual viewed as linkage between primary metabolism and secondary metabolism. The conclusion was based on feeding [2-^{14}C]-tyrosine to *Cephaelis* plant (Figure 2). The radioisotope was detected in two carbon atoms next to the two nitrogen atoms in emetine and cephaeline. In addition, protoemetine was found to have radioactivity in carbon 1 and carbon 3 of the isoquinoline unit. Therefore, protoemetine was suggested not to be the precursor of ipecac alkaloids.

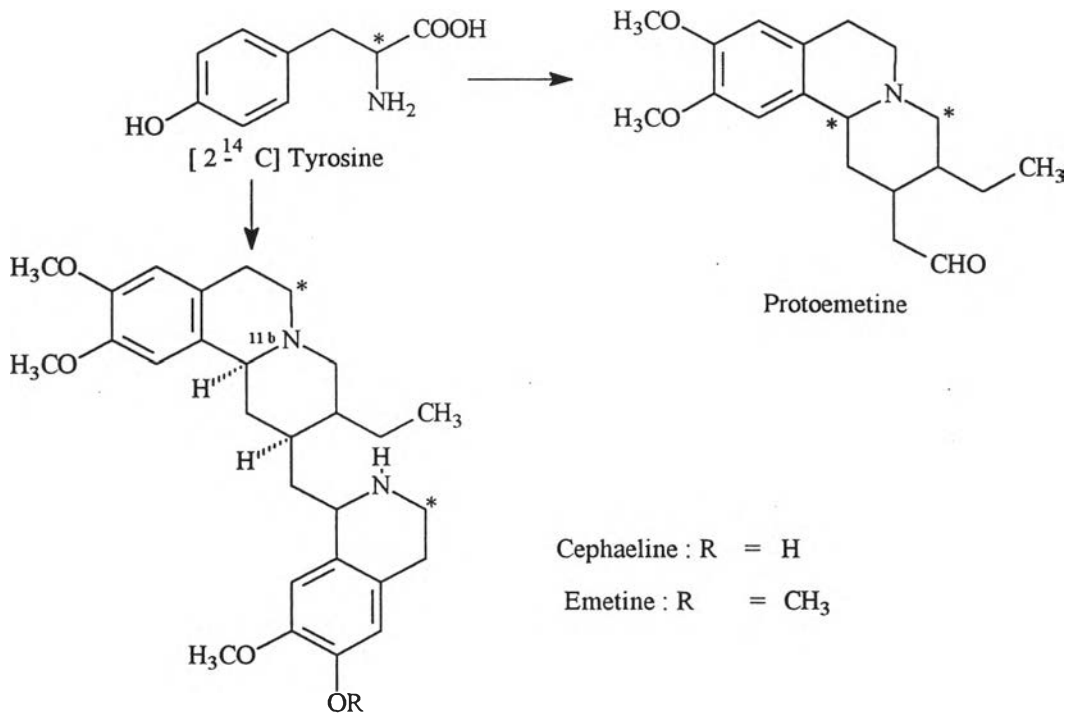

Figure 2 [2-^{14}C]-tyrosine as precursor of both protoemetine and emetine

Similar to the study of phenylpropanoid unit, the monoterpenoid unit has been elucidated based on the principle of feeding experiments. Battersby and Gregory (1968) proved the biosynthetic hypothesis by feeding [2-^{3}H] geraniol in *Cephaelis ipecacuaha*. The labelled geraniol was converted into loganin, a precursor for both ipecoside and cephaeline. The C9 unit of the ipecac alkaloids and C10 of ipecoside are therefore of monoterpenoid origin (Figure 3).

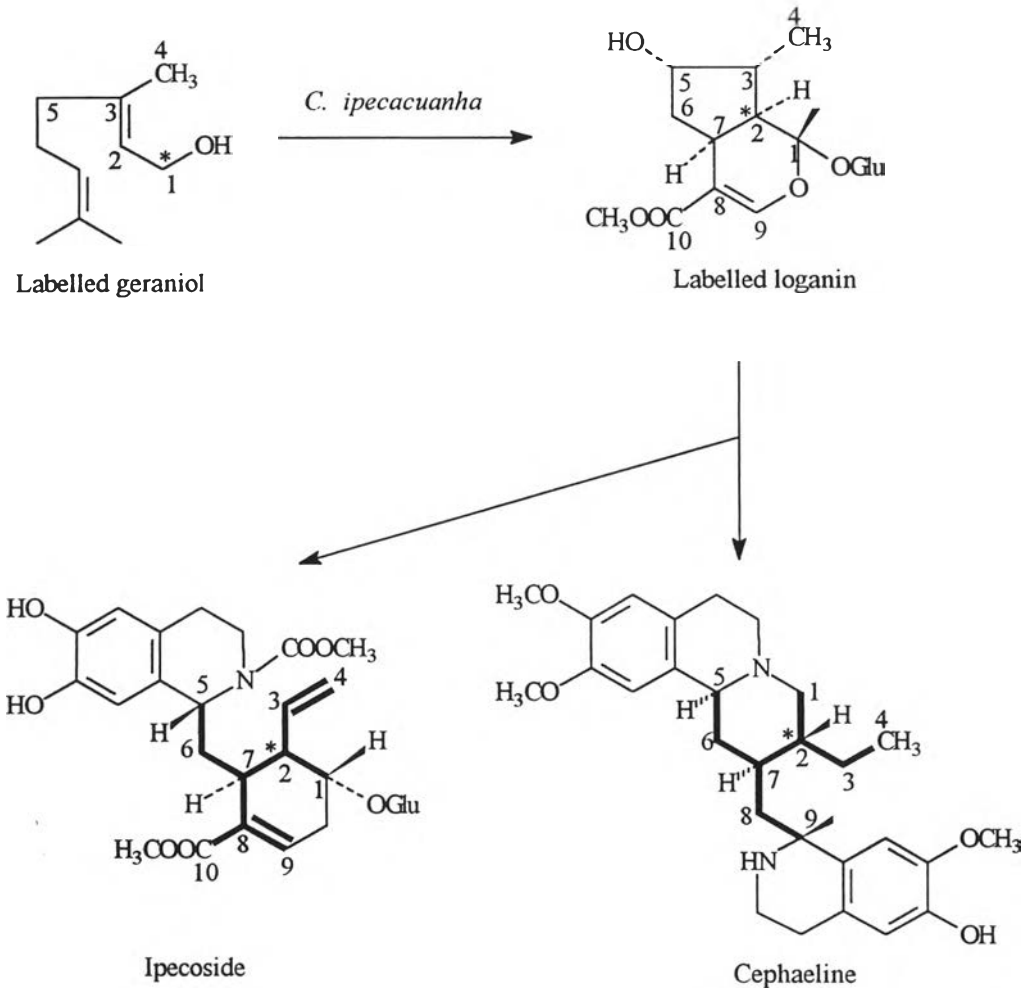

Figure 3 [2-^{14}C]-geraniol (monoterpenoid unit) as the precursor of ipecoside and cephaeline

Later, Battersby, *et al.* (1969) and Parry (1971) demonstrated that loganin was cleaved into secologanin followed by condensation either with 3,4 dihydroxyphenylamine (dopamine) to form deacetylipecoside as the major product together with its isomer. Only deacetylipecoside, not deacetylisoipecoside which can be biologically converted into ipecoside, emetine and cephaeline (Figure 4). In the same way secologanin was also reacted with tryptamine to form the indole alkaloids.

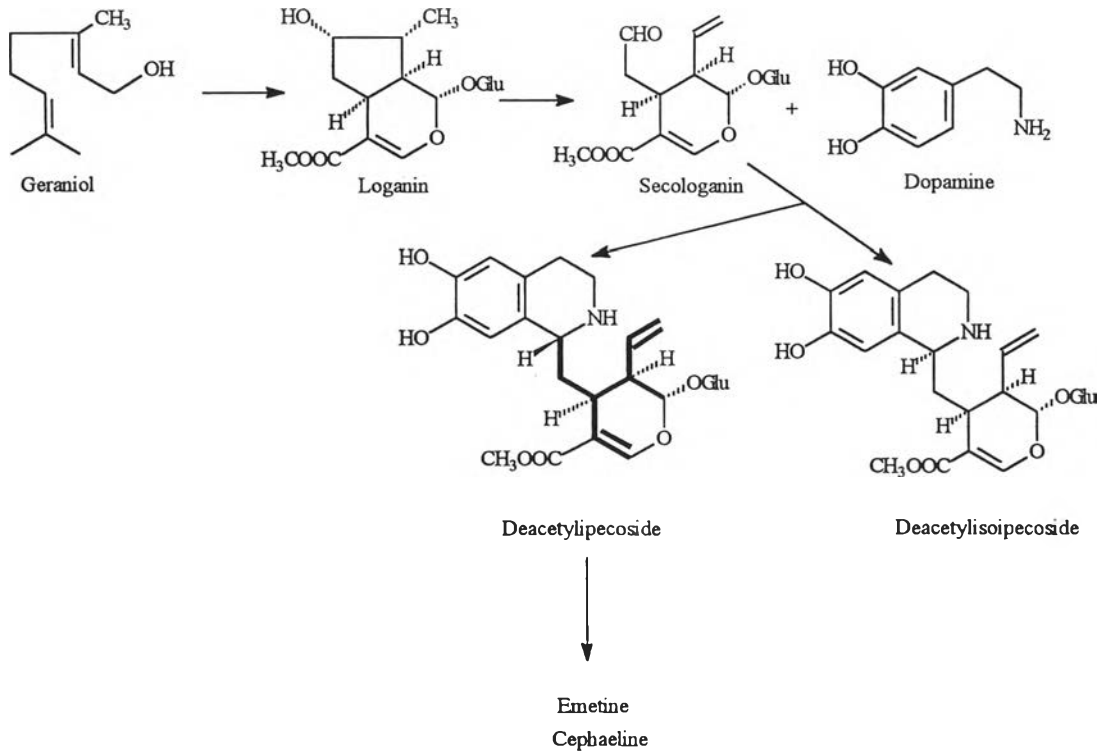

Figure 4 The biosynthetic pathway which proposed by Battersby and Parry (1971)

The sterochemistry at C1 of natural ipecoside determined by x-ray analysis was found to have *R* or β configuration. It has been proved that the alkaloidal glucosides have its C-1 hydrogen atom in the β-configuration rather than the α-configuration (Kennard *et al.*, 1971). In addition to the feeding experiment using [^{14}C] -deacetylipecoside and [^{14}C]-deacetylisoipecoside which prepared from secologanin and [2-^{14}C] dopamine under mild acidic condition, the [^{14}C]-deacetylipecoside with β-configuration appeared to be the sole precursor of ipecac alkaloids, cephaeline and emetine, while deacetylisoipecoside was not found to be incorporated (Battersby 1971). That was surprising when compared the C-1 configuration of β-deacetylipecoside and α-deacetylisoipecoside with C-11β of cephaeline (emetine) showing at α configuration rather to β configuration.

As found in the monoterpenoid indole alkaloids, the immediate synthetic precursors had the β-rather than the α-configuration. This was concluded despite the

fact that the cleared configurations would have represented the correct stereochemical relationship between the precursor and bearing mean while, leading to suggestion products of an inversion from β to α configuration during in biosynthetic reaction sequences, a reaction of much controversy and inversion. However, the biosynthesis of the indole alkaloids was proved by both tracer feeding experiment and enzymatic studies, the strictosidine (isovincoside) with 3α-configuration rather than vincoside (3β-configuration) was the common precursor for the biosynthesis of the 3α as well as the 3β-monoterpenoid indole alkaloids (Stockigt and Zenk, 1977). These results showed the correct stereochemical relationship of the corynanthe type alkaloids being clarified via identified with 3 α-configuration. This finding made it nescessary to reinvestigate the biosynthesis of the ipecac alkaloids and glucosides.

In 1978 Nagakura, Hofle and Zenk synthesized the pure epimers of highly labelled [^{3}H, ^{14}C]-deacetylipecoside and deacetylisoipecoside to reinvestigate the biosynthesis of ipecoside and the ipecac alkaloids, cephaeline and emetine. By precursor feeding in leaves or seeding *C. ipecacuanha* and apical cutting of *A.salviifolium,* deacetylipecoside is exculsively and specifically incorporated into nitrogenous glucosides, ipecoside in *C. ipecacuanha* and alangiside in *A. salviifolium*, both of which chemical structures have the β-configuration. No incorporation in the ipecac alkaloids have been observed. In contrast, the epimer with the α-configuration of deacetylisoipecoside is the true precursor of the ipecac alkaloids such as cephaeline and emetine. In both of plants, the radioactive labellings were not found in the glucoside fraction with β-configuration. It is important to note that the 1α-deacetylisoipecoside and 1β-deacetylipecoside are precursors of the ipecac alkaloids and its glucosides, whereas in the indole alkaloids series the formation of the 3β-alkaloids from the 3α-precursor strictosidine proceed with loss of the corresponding hydrogen atom. These results, contrasted to the previous assumption by Battersby1971, no epimerization of the precursor in the biosynthesis of ipecac alkaloids was involved. The α-epimer deacetylisoipecoside is the key intermediate in the formation of the multitude of ipecac alkaloids presented by cephaeline and emetine.

The C-1 β epimer, deacetylipecoside is either acetylated in the plant, to give ipecoside, or its methyl ester group is hydrolysed and subsequently transformed to alangiside. The biosynthetic pathway was suggested that the condensation of dopamine and secologanin built up both of deacetylipecoside and deacetylisoipecoside. While the epimer with β-configuration is metabolically inactivated by acetylation (ipecoside) or lactamization (alangiside), the α epimer is further transformed, most likely involving protoemetine as intermediate, to several monoterpenoid isoquinoline alkaloids including cephaeline and emetine, all processing α-configuration (Figure 5). There is a good reason to assume that the condensation of secologanin and dopamine occured enzymatically within the plants.

The first of enzymatic study in the process of dopamine and secologanin condensation was obtained by crude cell-free system from the leaves of *A. salviifolium*. The enzyme activity was strictly observed based on the presence of the product and the absence of both substrates, dopamine and secologanin. A couple of reaction products were formed. The study suggested that two enzymes are involved in the condensation of dopamine and secologanin to form two opposite epimers of (*R*)-deacetylipecoside and (*S*)-deacetylisoipecoside. Since the secondary products with tetrahydro-isoquinoline monoterpene skeleton in *A. salviifolium* have been found to have both the (*R*)-form of nitrogenous glucosides and (*S*)-form of emetine type alkaloid. They pointed that (*R*) and (*S*) epimer are the first intermediates of the biosynthetic pathways of these two alkaloid groups. This finding appeared to be consistent with the result of feeding experiment reported previously.

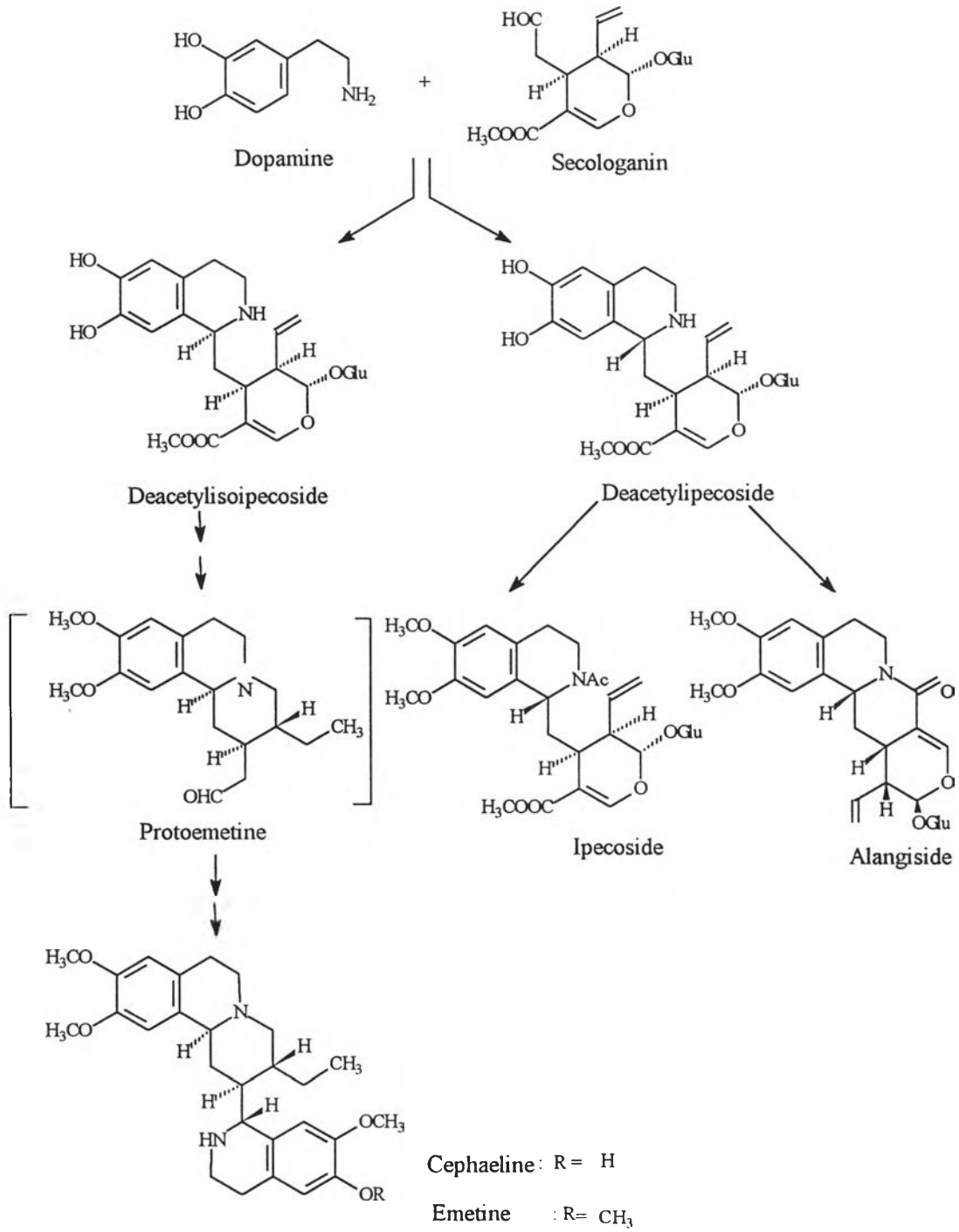

Figure 5 Proposed biosynthetic sequences for the biosynthesis of cephaeline, emetine and the alkaloidal glucosides by Nagakura *et al.*, 1971

In an attempt to understand isoquinoline monoterpene alkaloids and glucosides biosynthetic pathway, the enzymatic condensation of dopamine and

monoterpenoid secologanin is important to the closer investigated. Our research focus on elucidating the first biosynthetic enzyme which involved in this step. The activity of enzymes are investigated. We aim to purify both of enzymes for studies their characteristics and identify the enzymatic products. By these systems will clarify the question of first step of the emetine biosyntheic pathway.

4. Biological Activities of Tetrahydroisoquinoline Monoterpene Alkaloids

Alangium salviifolium Wang is the rich source of tetrahydroisoquinoline monoterpene alkaloids. It have been used as the folkloric medicine to the treatment of leprosy, syphylis, various skin disorder. The root and bark have been used as antiamoebic drugs.

Emetine is considered to be one of the most potent amoebicidal agent and is used in the initial treatment of severe cases of acute amoebicidal dysentery (Gilman *et al.*, 1980). The emetine hydrochloride is administered by injection and the complexation with bismuth iodide is used via oral route (Reynolds, 1989). The side effect include abdominal cramp, dizzeness, fainting, vomiting, neuromuscular and cardiovascular effects and pain at the site of injection. The emetine is concentrated in the liver and is still employed to treat amoebic hepatitis either alone or combined with cloroquine. Emetine also has been used as a mild emetic because it has local effect and due to gastrointestinal irritation disentery (Gilman *et al.*, 1980; Reynold, 1989)

Emetine and some derivatives have been tested for their *in vivo* activity against *Entamoeba histolytica* (NIH200) and cytotoxicity to guineas pig ear keratinocytes (GPK) (Keene *et al.*, 1987). Emetine exhibited IC_{50} value of 0.07 μg/ml and 0.02 μg/ml for GPK cytotoxicity. The ratio of GPK cytotoxicity to amoebicidal activity is 0.02. The less than one value of the ratio reflects the highly cytotoxic nature of the compound (Keene *et al.*,1986). The synthetic compound, 3,3-dehydroemetine, has been shown to be 2-3 times less active than emetine in amoebicidal activity at the IC_{50} value of 0.16 μg/ml, but its cytotoxicity (IC_{50} 0.02

μg/ml) was equal to that of emetine (Keene *et al.*, 1987). A synthetic compound 2,3-dehydroemetine, has been shown to be 2-3 times less active than emetine in amoebicidal activity (IC_{50} 0.16 μg/ml) but is cytotoxicity to GPKs (IC_{50} 0.02 μg/ml) is equal to that of emetine (Keene *et al.*, 1987). Removal of the 9,10-dimethoxy substituents from emetine has been found to result in a 52-fold loss of amoebicidal activity (IC_{50} 3.7 μg/ml) and 270-fold loss of cytotoxicity (IC_{50} 5.4 μg/ml) (Keene *et al.*, 1987).

Tubulosine has the same overall stereochemistry as emetine but differs in having a hydroxy substituted tetrahydro-β-carboline moiety in the lower portion of the molecule. This change has been shown to result in a 23-fold loss of amoebicidal activity (IC_{50} 1.6 μg/ml) but retention of cytotoxicity (IC_{50} 0.04 μg/ml) (Keene *et al.*, 1987).

Antitumor activities of emetine, cephaeline, tubulolosine and *O*-methyl-tubulosine in rodent tumor have been reported as active against the L1210 and P388 leukemia cell lines (Brossi, 1985). Typical increases in life span (ILS) values have been found as follows : cephaeline, 30-60% in L12210 and 50% in P388; tubulosine, 30% in L1210 and 80% in P388; and *O*-methyltubulosine, 40-50% in L1210 and 60-80% in P388.

The cardiovascular effects and antiinflammatory activities have also been studied. The alkaloid AL-60 from the stembark has been found to exert a biphasic action on blood pressure of cats injected intravenously with either chloralose-urethane or sodium nembutal anesthesia. The action appeared to be dose-dependent, sustained and prolonged (Dutta and Pakrashi, 1962). The alkaloid AL-60 has later been found to be a mixture of psychotrine, cephaeline, and demethylcephaeline (Pakrashi and Achari, 1970).

The effect of various concentrations of total alkaloid from the seeds has been investigated by intravenous injection of overnight fasted cats anasthetized with

nembutal (Dutta and Pakrashi, 1962). The extract in low doses (0.1-1 mg/kg) showed a transient biphasic action on carotid blood pressure. Marked and prolonged hypotension was observed at a dose of between 1.6-8 mg/kg while with 16 mg/kg pressure was reduced to zero with complete inhibition of the respiration. After a few seconds the pressure again registered a gradual rise and was maintained at 30-40 mm below the normal level and normal respiration was restored.

A total alkaloid fraction isolated from the leaves has been screened for antiinflammatory activity against formalin-induced arthritis in albino rats, using betamethasone (5γ/100g/day) as the reference compound (Prasad, Bhattacharya and Das, 1966). Betamethasone reduced the foot volume, necrosis of the feet, and tenderness following formalin injection, while extracts from *A. salviifolium* leaves increased the inflammatory reaction during the first five days and then significantly reduce foot volume from the 11^{th} day. This is collaborated by the fact that the leaves are used in the indigenous systems of medicine to relieve rheumatic pains when applied in the form of a poultice.