CHARPTER II

HISTORICAL

1. Taxa and Description

The Alangiaceae is a small, woody family which comprises both a single Old World genus, *Alangium*, with about 20 species and a single New World genus, *Matteniusa*, with three species (Heywood ed.,1978).

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

The plants of *Alangium* spp. are characterized by bisexual and borne on bracteolate, jointed pedicels in axillary cymes. They are regular, with equal numbers (4-10) of sepals and petals, the sepals eventually bending backwards and downwards, often more or less twisted, The petals are sometimes united at the base, and are more or less hairy on the inner surface. The number of stamens varies from four to forty, and they are free or united with the petals, with often hairy filaments and usually basifixed introrse anthers. A nectary disk is generally present at the base of the single style which surmounts the usually unilocular, inferior or superior ovary. The stigma is club-shaped, sometimes two- or three-lobed. One pendulous, anatropous ovule develops in the locule, and the drupaceous fruit is single seeded with a hard endocarp, and usually crowned with the

persistent calyx, The seed has a fleshy endosperm. They are trees or shrubs with alternate, usually simple leaves without stipulates.

The plants of *A. salviifolium* is characterized by its stamen(10-18) more than petals; filaments in or below the middle with a long hairy appendix, upwards glabrous; connective glabrous; cymes very shortly peduncled, densely appressed- hairy; flowers 5-8 (usually 5)-merous; petals yellowish white or white, on the outside shortly tomentose, 14-21 mm; ovary 1-celled; style glabrous; stigma capitate, lobed; fruit broadly ellipsoid, velutinous, 1 1/4- 1 3/4 cm long, red. Leaves elliptic or oblong- obovate, from a rounded or obtuse base, acuminate, entire, pinnate nerved with a 3-nerved base, glabrous or sparingly hairy on the nerves beneath, 7 1/2-18 cm by 3-9 cm; petiole 1/2-11/2 cm. Mostly climbing.

Figure 2 *Alangium salviifolium* (a) leafy shoot, axillary flowers and fruiting shoot (x 2/3); (b) flower with short calyx tube, long recurved petals, numerous stamens and single style with a lobed stigma (x2); (c) half section of gynoecium showing ovary with pendulous ovule (x6); (d) stamen with hairy filament and basifixed anther (x3); (e) cross section of fruit (x2)

2. Chemical Constituents of *A. salviifolium*

The compounds that have been found in *A. salviifolium* are listed in Table 1.

Table 1 List of compounds in *A. salviifolium*

Compounds	Plant parts	Reference
Alamarine	seeds	Pakrashi *et al.*,1985
Alancine	stem bark	Chattopadhyay *et al.*,1984
Alangamide	seeds	Pakrashi *et al.*,1969
Alangicine	root bark	Pakrashi *et al.*,1967
Alangimarckine	leaves	Battersby *et al.*,1966
Alangimaridine	seeds	Pakrashi *et al.*,1985
Alangimrinone	seeds	Pakrashi *et al.*,1985
Alangine A	root bark	Singh *et al.*,1948
Alangine B	root bark	Singh *et al.*,1948
Alangiside	roots,leaves,fruits	Kapil *et al.*,1971
Alangol	seeds	Bhargava and Dutt
Ankorine	leaves,branches	Dasgupta,1965
Benzoylphenylalaninol	leaves	Schenk *et al.*,1961
Bharatamine	seeds	Pakrashi *et al.*,1983
Cephaeline	root bark	Albright *et al.*,1965
Demethylcephaeline	stem bark	Pakrashi *et al.*,1970
9-Demethylprotoemetinol	seeds	Battersby *et al.*,1959
10-Demethylprotoemetinol	seeds	Battersby *et al.*,1959
9-Demethylpsychotrine	root bark,stem bark	Pakrashi *et al.*,1967
10-Demethyltubulosine	root bark,stem bark	Popelak *et al.*,1966
Deoxytubulosine	fruits, seeds	Battersby *et al.*,1965
Dihydroalamarine	seeds	Schof *et al.*,1957
Dihydroisoalamarine	seeds	Tschesche *et al.*,1961
Emetine	root bark	Battersby *et al.*,1959
Isoalamarine	seeds	Pakrashi *et al.*,1985
Isoalangimarine	seeds	Pakrashi *et al.*,1955
Isocephaeline	seeds	Battersby *et al.*,1959
Isotubulosine	root bark	Pakrashi *et al.*,1970
3-O-demethyl-2-O-methylalangiside	dried fruits	Atsuko *et al.*,1994
Protoemetinol	-	Battersby *et al.*,1959
Psychotrine	seeds,root bark,stem bark	Battersby *et al.*,1959
1,2,3,4-Tetrahydro-6-hydroxy-7-methoxy-1-methylisoquinoline	-	Spath *et al.*1934
Tubulosine	root bark,stem bark	Pakrashi *et al.*,1970

3. Biosynthetic Studies of Monoterpenoid Isoquinoline Alkaloids

The emetine alkaloids belong to the unique ipecac group of the extremely large isoquinoline alkaloids. These alkaloids have been proposed to be derived from one monoterpenoid unit (*via* secologanin) and one phenylalanine/tyrosine unit. The condensation of both units gives rise to alkaloids exemplified by ipecoside and protoemetine. Protoemetine by combination with a second phenylpropanoid amino acid unit gives rise to the emetine group. Alternatively, further combination of protoemetine with a tryptamine unit gives rise to the typical alkaloids of *A. salviifolium* eg alangimarckine (Figure 3). This assumption was speculated on the precursor-product relationship and based mainly on the visual dissection of a molecule into recognizable precursor fragments (Battersby, Burnett and Parsons,1969). In 1968, Battersby and Gregory proved the hypothesis by feeding [2-^{14}C]-tyrosine to the intact plant. After a suitable peroid of time, the plant was worked up for the purified products and the isotope content was measured for the preferable location in the molecule. It was found that the label is located in the two carbon atoms next to the two nitrogen atoms in emetine and cephaeline(Figure 4). On the other hand protoemetine, a related alkaloid, had radioactivity in isoquinoline carbons 1 and 3. Therefore, protoemetine was concluded not to be the precursor of emetine.

Battersby and Parry, in 1971, also used labelled [O-methyl-^{3}H, 6,6-$^{3}H_2$]-secologanin in their experiments. They found that labelled secologanin indeed efficiently incorporated into deacetylipecoside , cephaeline and emetine. They concluded that one C6-C2 unit from tyrosine or DOPA seemed to react with secologanin, to give deacetylipecoside in the same way as the condensation of tryptamine and secologanin to give strictosidine in monoterpenoid indole alkaloid biosynthesis. Then the structural rearrangement of deacetylipecoside followed by condensation with a second molecule of dopamine and methylation gives rise to emetine has been purposed (Figure 5).

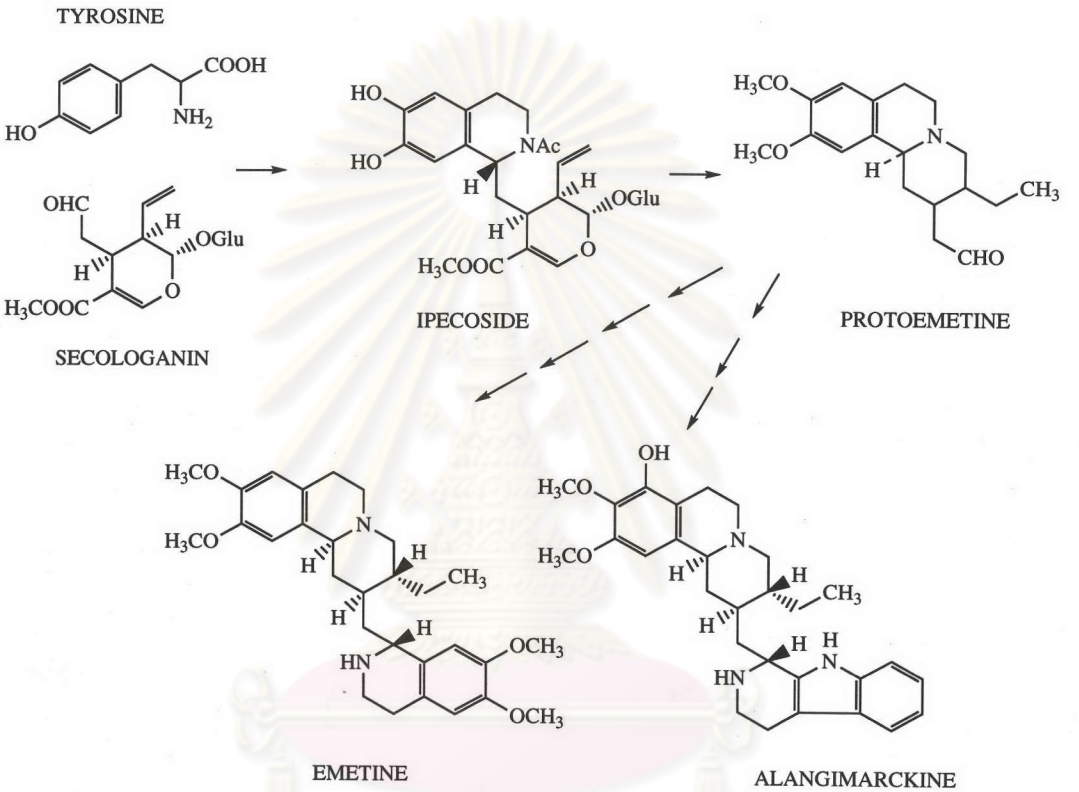

Figure 3 Biogenesis pathway of the emetine group of alkaloids from Dictionary of Alkaloids 1989.

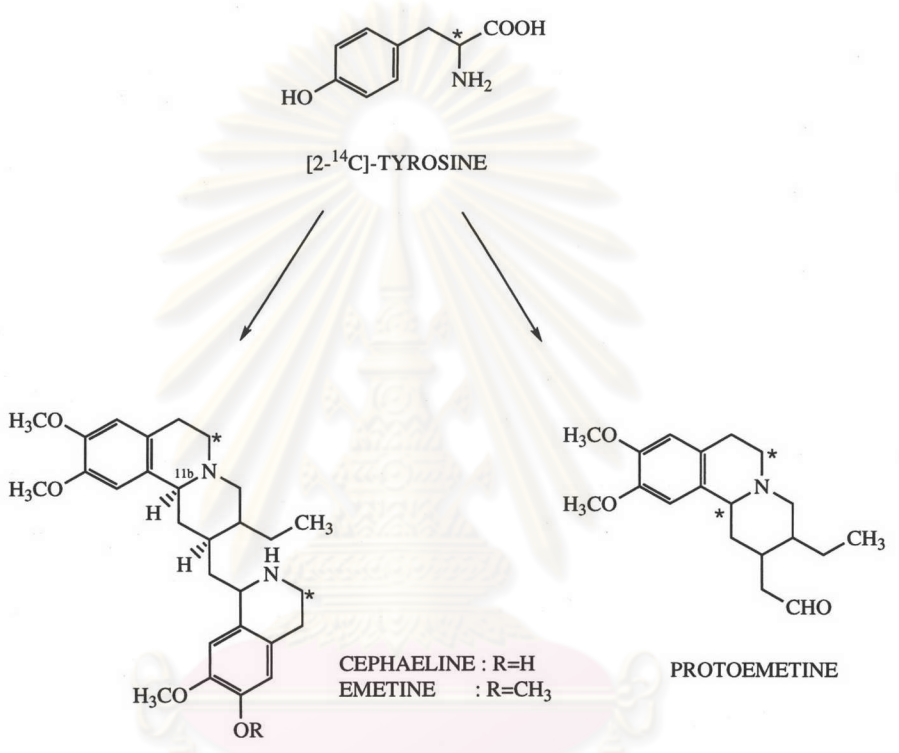

Figure 4 [2-^{14}C]-Tyrosine acts as precursor of both protoemetine and emetine.

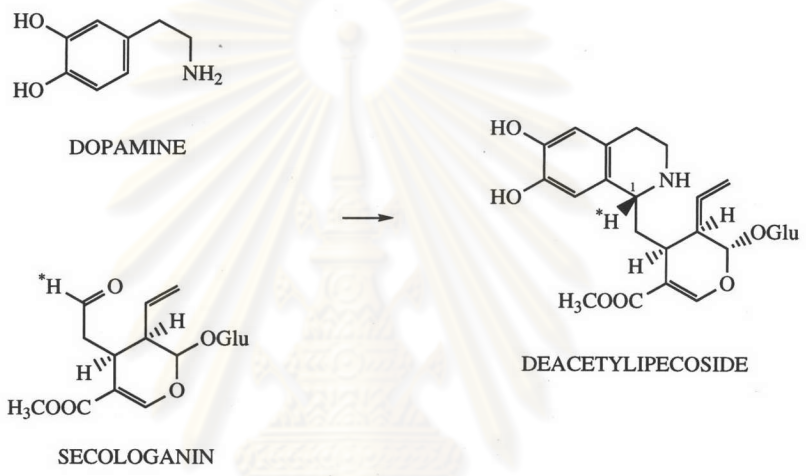

Figure 5 Condensation of dopamine and secologanin gives deacetylipecoside

Although X-ray analysis of the natural ipecoside proved that the glucoside has its C-1 hydrogen atom in the β rather than the α configuration (Kennard *et al.*,1971), feeding experiments using (^{14}C) deacetylipecoside and (^{14}C) deacetylisoipecoside showed that deacetylipecoside (β) was the sole precursor for ipecoside as well as for cephaeline and emetine(Battersby *et al.*,1971). This experiment showed beyond any doubt that cephaeline (and emetine) has its hydrogen atom of the C-11b position in α rather than in the β position. However, according to the biosynthetic study on indole alkaloids, vincoside(3β) was reported to be exclusively incorporated into the monoterpenoid indole alkaloids with 3 α stereochemistry(Stockigt and Zenk,1977). Later, it was able to prove, by both tracer feeding experiments and enzymatic studies, that strictosidine(isovincoside) with 3 α configuration rather than vincoside(3β) was the common precursor for the biosynthesis of the 3 α as well as the 3 β monoterpenoid indole alkaloids. These results showed that the correct stereochemical relationship for the *Corynanthe* type alkaloids was 3 α configuration. This finding made it necessary to reinvestigate the biosynthesis of the ipecac alkaloids and of ipecoside in *Cephaelis ipecacuanha* A.Rich and also the study of the related compounds in *A. salviifolium.*

Nagakura *et al.*(1978) carried out a tracer experiment by feeding (7-^{3}H)-secologanin into the seedlings of *Cephaelis.* The incorporation of secologanin into ipecoside, cephaeline and emetine were about 10%, 0.21% and 0.01% respectively. This clearly established the role of secologanin as precursor for this type of compound and confirms the earlier observation in *Cephaelis*(Battersby and Parry 1969). Furthermore Nagakura *et al.*(1978) synthesized and separated the epimers (deacetylipecoside and deacetyl isoipecoside) from (7-^{3}H) - secologanin and (1-^{14}C) - 3,4 - dihydroxyphen-ethylamine hydrochloride.

The crucial experiment was the independent feeding of the separated epimers to both plant species. (1-^{3}H,3-^{14}C)-Deacetylipecoside(β) was incorporated into ipecoside and alangiside. This clearly established that deacetylipecoside serves as precursor for ipecoside(both compounds having β configuration at C-1) but incorporation of deacetylipecoside into cephaeline and emetine was not observed. Doubly labelled deacetylisoipecoside(α) was administered to both plants, no incorporation was observed in the alkaloidal glucoside fraction with β configuration, but cephaeline and emetine(α configuration) were both unequivocally labelled.

It has been assumed previously that deacetylipecoside, with β configuration, is the sole biosynthesis precursor for the ipecac alkaloids as well as for ipecoside(Battersby and Parry, 1971). Since the ipecac alkaloids possess α configuration. An inversion of configuration was postulated. The results presented later (Nagakura *et al.*,1978) prove that contrary to the previous assumptions, no epimerisation of the precursors in the biosynthesis of ipecac alkaloids is involved.

Deacetylisoipecoside with 1α configuration is incorporated into cephaeline and emetine with retention of configuration. This compound is the key intermediate in the formation of the series of ipecac alkaloids in *Cephaelis* as well as in *Alangium*. Deaectylipecoside with 1β configuration is acetylated to yield ipecoside in *Cephaelis* or enzymatically hydrolyzed and subsequently transformed to alangiside in *Alangium*. In both alkaloidal glucosides, the β configuration is retained. The biosynthetic pathway suggested here as shown in Figure 1.

For us, there is good reason to assume that the condensation of secologanin and dopamine occur enzymatically within the plant. The question of whether one enzyme gives rise to both epimers or two enzymes with stereochemical control are involved should be examined.