CHAPTER II

HISTORICAL

1.Botanical aspects

1.1 Botanical aspects of *Stephania* species

The Menispermaceae is a large family containing 73 genera and about 350 species. Most of them are tropical, except a few species of *Coculus* which extend into North America and temperate Asia, and 2 species of *Menispermum* which are found in North America and North Asia (Forman, 1986). In Thailand, there are 22 genera and 51 species, nine of which are endemic (Forman, 1991).

The *Stephania* is one of 73 genera of the Menispermaceae. It is characterized by climbers, rarely erect herbs, stems woody or herbaceous, tuberous rootstock sometimes present, often above ground.

Leaves peltate usually ovate to suborbicular, palmately nerved. Inflorescences axillary or arising from old, leafless stems usually composed of peduncled umbelliform cymes, which are solitary or racemosely arranged, at least the first (-second) order(s) of branching umbellate (in Thai *spp.*), the ultimate branching sometimes irregular, or sometimes the cymes condensed to disciform capitula. Male flowers symmetrical, sepals free, imbricate, 6 or 8 in two equal or unequal whorls, or only 2-3 in *S. capitata*, usually ± obovate. Petals free, 3 or 4, rarely 0-2, usually ± broadly obovate with lateral margins often involute. Stamens connate into a peltate synandrium ; anther-cells 4-8, dehiscing transversely. Female flowers symmetrical or asymmetrical. Sepals 1-8. Petals 2-4, both similar to male. Carpel 1, style very short or absent; stigma shortly lobed or divaricaltely laciniate. Drupes obovoid with style-scar near base, glabrous; endocarp bony, dorsally bearing a horseshoe-shaped band of 2 or 4 longitudinal rows of process, or transverse ridges; condyle often perforate. Seeds horseshoe-shaped; embryo with cotyledons ± equalling the radicle, surrounded by endosperm (Forman, 1991).

There are about 45 species in the Old World tropices. Among these, there are 15 species in Thailand (Forman, 1991). These include

1. *Stephania brevipes* Craib
   [Bua Khruea (บัวเครือ) (Northern)]
2. *Stephania capitata* (Bl.) Spreng.
3. *Stephania creba* Forman
4. *Stephania elegans* Hook. f. & Thoms.
   [Se-khi-pho (เส่ผิพอ) (Karen/Northern)]
5. *Stephania glabra* (Roxb.) Miers
   [Phanang nang (ผนังนั่ง) (Northern)]
6. *Stephania glandulifera* Miers
7. *Stephania japonica* (Thunb.) Miers
   [Kon pit (ก้นปิด),bai kon pit (ใบก้นปิด) (Central); pang pon (ปังปอน)(Northern);tap tao (ตับเต่า), yan pot (ย่านปด) (Peninsular)]
8. *Stephania oblata* Craib
9. *Stephania papillosa* Craib
10. *Stephania pierrei* Diels
    [Bua khruea (บัวเครือ)(North-Eastern); bua bok (บัวบก)(South-Western,Eastern and Central); kot hua bua (โกฐหัวบัว), sabu lueat (สบู่เลือด)(Central)]
11. *Stephania reticulata* Forman
    [Tap tao (ตับเต่า)(Peninsular)]
12. *Stephania suberosa* Forman
    [Bua bok (บัวบก) (Central); boraphet phung chang (บอระเพ็ดพุงช้าง)(South-Western)]
13. *Stephania subpeltata* H.S.Lo
14. *Stephania tomentella* Forman
15. *Stephania venosa* (Bl.) Spreng.
    [Plao lueat khruea (เปล้าเลือดเครือ) (Northern); cho kor tho(ชอเกอทอ) (Karen/Northern); krathom lueat (กระท่อมเลือด)(North-Eastern); kling klang dong (กลิ้งกลางดง)(South-Western); boraphet yang daeng (บอระเพ็ดยางแดง)(Peninsular)].

1.2 Botanical aspect of *Stepania pierrei* Diels

Stephania pierrei Diels (Figure 1) is in the family of Menispermaceae (เต็ม สมิตินันท์, 2523; Forman, 1991). This plant could be found distributed in Thailand and Cambodia. It was described by Diels as being synonymous with *S. rotunda* Lour. (Diels, 1910). The following morphological descriptions were given:

Stephania pierrei Diels n. sp. - *S. rotunda* Lour. Gagnepain in Fl. gen Indochine I (1980) 148 cum var. lappacea Gagnepain (partim?). Planta e tubere magno orta, herbacea, scandens. Rami sulcato-striati glabri; ramuli breves nonnunquan flexuosi folia et inflorescentias gignentes. Foliorum petiolus 2.5 - 4 cm longus; lamina papyracea subtus pallidior fere orbicularis apice plerumque rotunda orbicularis raro obsolete obtusoacuminata, 2.5 - 5.5 cm diamet., nervi tennes subtus vix prominuli. Inflorescentiae hand umbellatae, paniculatae, graciles glabrae, 5 - 8 cm longae, rami longe nudi apice cymulam gignentes, pedicelli 1-2 mm longi. Flores subrotato - expansi 2.5 mm diamet, sepala 6 subcoriacea, spathulato - obovata apice incurva 1.2 - 1.5 mm longa, 0.8 -1 mm lata, petala nulla, synandrium amplum, brevissime filamentatum, subsessile, 1.5 mm diamet. Drupa late oliqua obovata compressa 8 mm longa etlata, endocarpium utrinque costulis transversis ad angulos nodoso incrassatis atque praeterea sevie tuberculorum minutorum ornatum in facie laterali planum sublaeve.

The morphological characters of *S pierrei* Diels are similar to those of *S. erecta* which was described by Craib (Craib, 1922) as follows :

S. erecta Craib. (Menispermaceae -Coculeae), ab affini *S pierrei* Diels, caulibus erectis, foliis crassioribus distinguenda. *Caules* annui, sub anthesin erecti, saepissime simplices, 7 -30 cm alti, superne glauci,inferne pallidiores,annotini straminei, striati, glabri. *Folia* inventute sicca glauca, ovata vel rotunda,apice obtusa, mucronulata, 3 cm longa, 2.5 cm lata, rigidiuscula, glabra, nervis circa 11 radiantibus, nervulis vix conspicuis, subtus pallidiora, marginata, petiolo ad 4 cm longo suffulta. *Pseudumbellae* axcillares vel inferiores ex axillis foliorum squamiformium ortae, 5 - fere 10 mm diametro, pedunculo communi 8 -20 mm longo suffultae, glabrae; pedunculi partiales breves; pedicelli 1.5 -2 mm longi, apice articulati. *Flores* expansi 2.5 mm diametro. *Sepala* saepe varie et irregulariter connata, lanceolata vel ovato-

lanceolata, 1.3 mm longa, 0.8 mm lata, saepe tridentata. *Petala* haud evoluta. Synandrium vix 1.25 mm diametro, brevissime stipitatum.

Because of their similarity in morphology, these two plants have been taxonomically recognized as a single species, and the two scientific names considered synonyms (Craib, 1931; Forman, 1962).

Based on the above botanical descriptions, it should be noted, however,that there is a slight difference in the habitat of the two plants. *S. erecta* is clearly a herb with erect stem, while *S. pierrei* appears to be scandent (Likhitwitayawuid, *et al.*,1993b). In the Flora of Thailand, *S. pierrei* Diels was described by Forman (Forman, 1991) as follows:

S. pierrei Diels (*S. rotunda* Lour or *S.erecta* Craib) is erect herb, entirely glabrous, arising from a tuber, up to 8 (or more?) cm diam.; stems up to 30 cm high. *Leaves* mostly suborbicular, (2-) 3-6 cm diam., sometimes mucronulate at the apex, reticulation rather lax on both surfaces, stiffly papyraceous; petiole 2-3.5 cm long. *Male inflorescences* : cymes axillary, about 1 cm long, with slender peduncles, 7-8 mm long; or arising from the axils of reduced leaves along an axillary shoot to 5 cm long. *Male flowers* on pedicels 1-2 mm. *Sepals* 4 or 5, yellow, freshly usually unequal some joined up to half their length obovate, 1-2 mm long. *Petals* 0.Synandrium broad, sessils or on stalk to 0.5 mm. *Female flowers* unknown. *Drupes* suborbicular to subobvate in outline, 7-8 mm diam.; endocarp with a small central perforation dorsally bearing 4 rows of 16-19 curved, flattened, and ridged projections.

It shoud be emphasized that the chemical evidence suggested that *S. erecta* and *S. pierrei* are not identical, and shoud be separated as separate species.

2. Chemistry of isoquinoline alkoloids

2.1 Chemical aspects of isoquinoline alkaloids

Among plant alkaloids, isoquinoline alkaloids have played an important part in the development of the chemical and biological sciences.

Alkaloids with the isoquinoline ring system, or those derived from a phenylalanine unit and therefore related structurally and biogenetically to the isoquinolines, comprise at least 25 different types as follows (Govindachari and Viswanathan, 1972):

1. Simple isoquinolines
2. 1-phenylisoquinolines
3. 1-benzylisoquinolines
4. Cularine group
5. Phthalideisoquinolines
6. Protoberberines
7. Protopine group
8. Benzophenanthridines
9. Aporphines
10. Proaporphines
11. Dibenzo pyrrocolines
12. Morphine group
13. Protostephanine
14. Hasubanans
15. Pavine group
16. Ochotensine group
17. Rhoeadine group
18. Bisbenzylisoquinolines
19. Emetine group
20. Erythrinans
21. Amaryllidaceae alkaloid
22. Indole isoquinolines
23. 1-Phenethylisoquinolines
24. Isopovine group
25. Terpene alkoloids

The number of isoquinoline alkaloids of known structures approximately 1,800 which are in the forms of both tetrahydroisoquinolines and quaternary isoquinoline salts. The majority of isoquinoline alkaloids have been isolated from the following nine plant familes: Annonaceae, Berberidaceae, Fumariaceae, Hernandiaceae, Lauraceae, Menispermaceae, Papaveraceae, Ranunculaceae and Rutaceae. However, some have been found in other plant families, such as Alangiaceae, Amaryllidaceae,

Cactaceae, Combretaceae, Convolvulaceae, Euphorbiaceae, Leguminosae, Magnoliaceae, Monimiaceae, Nymphaeaceae and Rubiaceae. With respect to their structural features, the isoquinoline alkaloids can be devided into two main classes (Menachery *et al*, 1986). The first class is the simple isoquinoline alkaloids. (Figure 2)

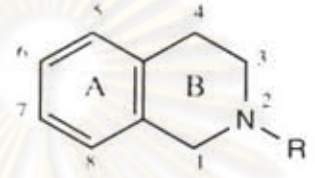


Figure 2 Structure of simple isoquinoline alkaloid

The simple isoquinolines are structurally the simplest of the isoquinoline alkaloids. They are usually bicyclic, although tricyclic species, such as peyoglutan and mescalotam are also included among them. The nitrogen function in ring B is often tertiary and N-methylated, but it may also be secondary, N-formylated, N-acetylated, N-ethylated or oxidized to the imine stage. Quaternary simple isoquinoline such as lophotine and 2-methyl-6,7-dimethoxy isoquinolium salt, have also been isolated. Of more than passing interest is pilicarpine, the only trimeric isoquinoline alkaloid fully characterized. Simple isoquinolines display great varieties in their substitution patterns, depending upon their biogenetic origin. Most simple isoquinolines have been obtained from the Cactaceae, but they also occur among the Alangiaceae, Annonaceae, Berberidaceae, Euphobiaceae, Leguminosae, Menispermaceae, Papaveraceae and Ranunculaceae (Menachery *et al*, 1986).

The second class is the benzyl-derived isoquinoline alkaloids. They do not present a structural uniformity having benzylisoquinolines act as precursors to so many other naturally occuring isoquinoline types such as pavines, isopavines, bisbenzylisoquinolines, cularines, protoberberines, erythrima base and others.

Because relatively a large number of isoquinoline alkaloids in this class are known, their occurrence are distributed in many plant families.

2.2 Aporphine alkaloids

Among the isoquinoline alkaloids, the aporphines are considered the largest group. Up till now, at least 684 aporphinoid alkaloids have been recodnized. Their structures can generally be represented as follows:

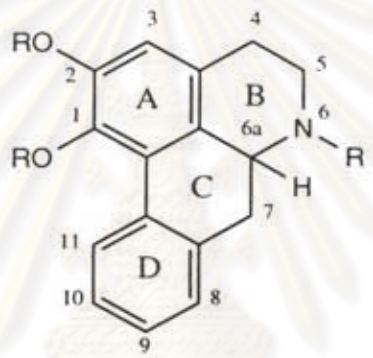


Figure 3 Main structure of aporphine alkaloid

The alkaloids of this group are distributed in at least 18 plant families (Cordell, 1981), of which the most important are Annonaceae, Berberidaceae, Fumariaceae, Hernandiaceae, Lauraceae, Menispermaceae, Monimiaceae, Papaveraceae and Ranunculaceae (Guinandeu *et al.*, 1975; 1979; 1983; 1988; 1994)

The nitrogen atom is usually substitued. Tertiary aporphines can be also attached with methyl, formyl and acetyl groups, and rare to find N-carbamyl and N-β-D-glucosidic aporphines. Several quarternary aporphine salts with two methyl groups or N-oxides attached to the nitrogen are also known. Aprphines are known with C-6a steriochemistry either (*R*) or (*S*)-configurations.

The most diverse structural feature of the aporphines is the oxygenated pattern. Positions 1 and 2 are always oxygenated, either by hydroxy, methoxy or methylene dioxy groups. It is common to find further oxygen substituents at C-4, C-5, C-7, C-9 and C-11, and occasionally at C-3 and C-8 positions. It is rare to fine -O-β-D-glucosidic substituent at C-2. The methylene dioxy bridge is always found between C-1 and C-2 or C-9 and C-10 and rare to find at C-2 and C-3, C-8 and C-9, and C_4 and C_5

C-4, C-5 and C-7 are common to find oxygen substituents in the form alcoholic aporphines, oxoaporphines or/ and dioxyaporphines.between C-6a and C-7, after loss of hydrogen atom can be formed dehydroaporphines. The nitrogen -C-6 bond can be to form phenanthrenes (Guinandeu, *et al.*, 1975; 1979; 1983; 1994).

Guinandeu, *et al.* (1994) divided aporphinoids alkaloids in 18 groups as follows:

1. Aporphines
2. Oxoaporphines
3. 5-Oxo / or 4,5-Dioxoaporphines
4. 4- or/ and 7-Oxygenated aporphines
5. Dehydroaporphines
6. 7-Methyl / or 7-Formyldehydroaporphines
7. 7-Hydroxy-7-methylaporphines
8. 7,7-Dimethyl aporphines
9. Azaanthracenes
10. Azafluorenes
11. 1-Azaoxoaporphines
12. Diazafluoranthrene
13. 4,5-Dioxo-1-azaaporphinoids
14. Azaphenanthrenes
15. Tropoloisoquinolines
16. Azafluoranthrenes
17. Phenanthrenes
18. Miscellaneous

2.3 Isoquinoline alkaloids isolated from the *Stephania spp.*

Since 1790, the *Stephania* were definited by Lour. in the Flora of Cochinchina and after then the alkaloids of the *Stephania* have received considerable attention for a long time. The *Stephania* are distributed in the Old World tropics. The vast majority of alkaloids found in the *Stephania* are the benzylisoquinoline type. The alkaloids and their structures which have been reported for *Stephania* species are shown in Tables 1 and 2.

Table 1 Isoquinoline alkaloids isolated from the *Stephania* species

Plant species	Alkaloids	Alkaloid type	Structure	Reference
Stephania bancroftii	(-)-Tetrahydropalmatine	Protoberberine	P14	Bartley, Baker and Carvalho, 1994.
	(-)-Stephanine	Aporphine	A26	
	(-)-Crebanine	Aporphine	A8	
	Ayuthianine	Aporphine	A5	
	(+)-Sebiferine	Morphinan	M5	
	(+)-Stepharine	Proaporphine	Pa3	
Stephania capitata	Crebanine	Aporphine	A8	Thornber, 1970.
	Cycleanine	Bisbenzylisoquinoline	B4	
	d-Dicentrine	Aporphine	A11	
	Epistephanine	Bisbenzylisoquinoline	B7	
	Phanostenine	Aporphine	A23	
	Stephanine	Aporphine	A26	
Stephania cepharantha	Aromoline	Bisbenzylisoquinoline	B1	Sugimoto, Sugimura and Yamada,1988., Thornber, 1970.
	Berbamine	Bisbenzylisoquinoline	B2	
	Cepharamine	Hasubanan	H1	
	Cepharanthine	Bisbenzylisoquinoline	B3	
	Cycleanine	Bisbenzylisoquinoline	B4	
	Homoaromoline	Bisbenzylisoquinolne	B9	
	Isotetrandrine	Bisbenzylisoquinoline	B13	
Staphania drinklagei	(+)-Corydine	Aporphine	A7	Thornber, 1970.
	Dicentrine	Aporphine	A11	
	(+)-Isocorydine	Aporphine	A14	
	(-)-Roemerine	Aporphine	A24	
Stephania elegans	Aknadinine	Morphinan	M1	Singh, Kumar and Bhaduni, 1981.
	Cyclanoline	Protoberberine	P4	
	Cycleanine	Bisbenzylisoquinoline	B4	
	Epihernandolinol	Morphinan	M3	
	Hasubanonine	Hasubanan	H6	
	Isochondodendrine	Bisbenzylisoquinoline	B12	
	Isotetrandrine	Bisbenzylisoquinoline	B13	
	Magnoflorine	Aporphine	A18	
	Methylcorydalmine	Protoberberine	P7	

Table 1 (continue)

Stephania erecta	(+)-*N*-Methytelobine	Bisbenzylisoquinoline	B15	Prawat, *et al.*, 1982., Tantisewie, *et al.*, 1989., Likhitwitayawuid, *et al*, 1993a.
	(+)-1,2-Dehydrotelobine	Bisbenzylisoquinoline	B6	
	(+)-2-Norisotetrandrine	Bisbenzylisoquinoline	B17	
	(+)-Isotetrandrine	Bisbenzylisoquinoline	B13	
	(+)-2-Northalrugosine	Bisbenzylisoquinoline	B20	
	(+)-Thalrugosine	Bisbenzylisoquinoline	B27	
	(+)-Homoaromoline	Bisbenzylisoquinoline	B9	
	(+)-Stephibaberine	Bisbenzylisoquinoline	B25	
	(+)-Daphnandrine	Bisbenzylisoquinoline	B5	
	(+)-2-Norcepharanthine	Bisbenzylisoquinoline	B16	
	(+)-Cepharanthine	Bisbenzylisoquinoline	B3	
	(+)-2-Norobaberine	Bisbenzylisoquinoline	B18	
	(+)-Obaberine	Bisbenzylisoquinoline	B21	
Stephania glabra	Columbamine	Protoberberine	P2	Bhakuni and Gupta,1982., Patra, Ghosh and Metra,1980
	(-)-Corydalmine	Protoberberine	P3	
	Cycleanine	Bisbenzylisoquinoline	B4	
	Dehydrocorydalmine	Protoberberine	P5	
	Tetrahydropalmatine	Protoberberine	P14	
	Palmatine	Protoberberine	P9	
	Jatrorhizine	Protoberberine	P6	
	Stepharanine	Protoberberine	P10	
	Stepharotine	Protoberberine	P11	
	(-)-Stepholidine	Protoberberine	P12	
Stephania hernandifolia	4-Demethylhasubanonine	Hasubanan	H3	Thornber, 1970.
	4-Demethylnorhasubanonine	Hasubanan	H4	
	Isochondodendrine	Bisbenzylisoquinoline	B12	
	Fanchinoline	Bisbenzylisoquinoline	B8	
	Isotelobine	Bisbenzylisoquinoline	B14	
Stephania japonica	Cyclanoline	Protoberberine	P4	Matsui, *et al.*, 1978,1982,1984. Yamamura and Matsui, 1985.
	Epistephamiersine	Hasubanan	H5	
	Hasubanonine	Hasubanan	H6	
	Homostephanoline	Hasubanan	H7	
	Hypoepistephanine	Bisbenzylisoquinoline	B10	
	Insularine	Bisbenzylisoquinoline	B11	
	Lanuginosine	Aporphine	A16	
	Magnoflorine	Aporphine	A18	
	Metaphanine	Hasubanan	H10	
	Miersine	Hasubanan	H11	
	Oxoepistephamiresine	Hasubanan	H12	
	16-Oxoprometaphanine	Hasubanan	H13	
	Oxostephabinine	Hasubanan	H14	
	Oxostephamiersine	Hasubanan	H15	
	Oxostephanine	Aporphine	A21	
	Oxostephasunoline	Hasubanan	H16	
	Protostephanine	Miscellaneous Base	Mis 1	
	Prometaphanine	Hasubanan	H17	

Table 1 (continue)

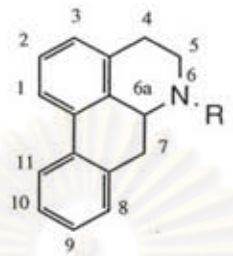
Stephania japonica	Stebisimine	Bisbenzylisoquinoline	B22	
	Stephamiersine	Hasubanan	H18	
	Stephanine	Aporphine	A26	
	Stephasunoline	Hasubanan	H19	
	Stepinonine	Miscellanous Base	Mis.2	
	Steponine	Protoberberine	P13	
	Thalrugosine	Bisbenzylisoquinoline	B26	
Stephania kwansiensis	Tetrahydropalmatine	Protoberberine	P18	Thornber, 1970.
Stephania longa	Longanone	Hasubanan	H8	Lao, Tang and
	Longetherine	Hasubanan	H9	Zu,1982.,
	Stephabyssine	Hasubanan	-	Deng and
	Stephaboline	Hasubanan	-	Zhao1993.
Stephania pierrei	(-)-Anonaine	Aporphine	A1	Likhitwitayawuid
	(-)-Asimilobine	Aporphine	A3	*et al*,1993b.
	(-)-Asimilobine-2-*O*-β-D-glucoside	Aporphine	A4	
	(-)-Capaurine	Protoberberine	P1	
	(+)-Codamine	Tetrahydrobenzyliso quinoline	T1	
	(-)-Corydalmine	Protoberberine	P3	
	Cassythicine	Aporphine	A6	
	(-)-Delavaine	Hasubanan	H2	
	(-)-Dicentrine	Aporphine	A11	
	(-)-Isolaureline	Aporphine	A15	
	(-)-Nordicentrine	Aporphine	A19	
	Magnoflorine	Apophine	A18	
	(-)-*N*-Methyltetra hydropalmatine	Protoberberine	P11	
	(±)-Oblongine	Tetrahydrobenzyliso quinoline	T3	
	(-)-Phanostenine	Aporphine	A23	
	(-)-Roemeroline	Aporphine	A25	
	(+)-Reticuline	Tetrahydrobenzyliso quinoline	T4	
	(-)-Salutaridine	Morphinan	M4	
	(-)-Tetrahydropalma tine	Protoberberine	P14	
	(-)-Tetrahydrostepha bine	Protoberberine	P15	
	(-)-Thaicanine	Protoberberine	P16	
	(-)-Xylopine	Aporphine	A33	
	(-)-Xylopinine	Protoberberine	P17	
Stephenia sasakii	Berbamine	Bisbenzylisoquinoline	B2	Kunitomo, *et al.*,
	Bisakanadinine	Morphinan	M2	1980.,
	Cepharanthine	Bisbenzylisoquinoline	B3	Kunitomo, 1981.
	Crebanine	Aporphine	A8	
	Dehydrocrebanine	Aporphine	A9	
	Dehydrostesakine	Aporphine	A10	
	4,5-Dioxydehydro-crebanine	Aporphine	A12	
	4-Hydroxycrebanine	Aporphine	A13	
	d-Isocorydine	Aporphine	A14	
	Lanuginosine	Aporphine	A16	

Table 1 (continue)

Stephania sasakii	Lioriodenine	Aporphine	A17	
	l-Tetrahydropalmatine	Protoberberine	P14	
	N-Methylpapaverali-nium	Tetrahydrobenzyliso quinoline	T2	
	Phanostenine	Aporphine	A23	
	(R)-Roemeroline	Aporphine	A25	
	Steponine	Protoberberine	P13	
	Stesakine	Aporphine	A27	
Stephania suberosa	Cepharanthrine 2'-*N*-oxide	Protoberberine	-	Patra, *et al*,1986., Amarendra, *et al*., 1987.
	2-Norcepharanthine	Protoberberine	-	
	Norstephasubine	Bisbenzylisoquinoline	B19	
	Stephasubinine	Bisbenzylisoquinoline	B24	
	Stephasubine	Bisbenzylisoquinoline	B23	
Stephania tetrandra	Cyclanoline	Protoberberine	P14	Thornber, 1970.
	Fanchinoline	Bisbenzylisoquinoline	B8	
Stephania venosa	Annonaine	Aporphine	A1	Pharadai,*et al*, 1965,1981., Charls,*et al*,1987, Banerji,*et al*,1994
	Apoglazionine	Aporphine	A2	
	Asimilobine	Aporphine	A3	
	Ayuthianine	Aporphine	A5	
	Corydine	Aporphine	A7	
	Crebanine	Aporphine	A8	
	Kamaline	Aporphine	A34	
	Kikumamanin	Protoberberine	-	
	Mecambroline	Aporphine	-	
	N-Carboxamidoste-pharine	Proaporphine	Pa1	
	Nuciferine	Aporphine	A20	
	O-Methylstephari-nosine	Proaporphine	Pa2	
	Oxostephanosine	Aporphine	A22	
	Reticuline	Tetrahydrobenzyliso quinoline	T4	
	Stepharine	Proaporphine	Pa3	
	Stepharinosine	Proaporphine	Pa4	
	Sukhodianine	Aporphine	A28	
	Tetrahydropalmatine	Protoberberine	P14	
	Thailandine	Aporphine	A29	
	Thalrugosamine	Bisbenzylisoquinoline	-	
	Tuduranine	Aporphine	A30	
	Ushinsunine	Aporphine	A31	
	Uthongine	Aporphine	A32	

Table 2 The structure of formulae in table 1

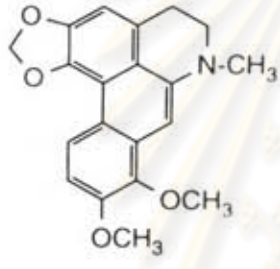
APORPHINES (A)



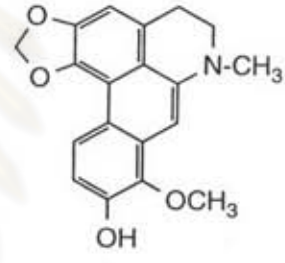
Alkaloid	1	2	4	6	7	8	9	10	11
(-)-Anonaine(A1)	-O-CH_2	-O-	H	H	H	H	H	H	H
Apoglazionine (A2)	OH	OCH_3	H	CH_3	H	H	H	OH	H
(-)-Asimilobine (A3)	OCH_3	OH	H	H	H	H	H	H	H
(-)-Asimilobine-2-*O*-*β*-*N*-glucoside (A4)	OCH_3	glucose	H	H	H	H	H	H	H
(-)-Ayuthianine (A5)	-O-CH_2	-O-	H	CH_3	OH	OCH_3	H	H	H
Cassythicine (A6)	-O-CH_2	-O-	H	CH_3	H	H	OH	OCH_3	H
(-)-Corydine (A7)	OH	OCH_3	H	CH_3	H	H	H	OCH_3	OCH_3
Crebanine (A8)	-O-CH_2	-O-	H	CH_3	H	OCH_3	OCH_3	H	H
(-)-Dicentrine (A11)	-O-CH_2	-O-	H	CH_3	H	H	OCH_3	OCH_3	H
4-Hydroxycrebanine (A13)	-O-CH_2	-O-	OH	CH_3	H	OCH_3	OCH_3	H	H
(+)-Isocorydine (A14)	OCH_3	OCH_3	H	CH_3	H	H	H	OCH_3	OH
(-)-Isolaureline (A15)	-O-CH_2	-O-	H	CH_3	H	H	OCH_3	H	H
Lanuginosine (A16)	-O-CH_2	-O-	H	CH_3	=O	H	OCH_3	H	H
Lioriodenine (A17)	-O-CH_2	-O-	H	CH_3	=O	H	H	H	H
Magnoflorine (A18)	OH	OCH_3	H	$(CH_3)_2$	H	H	H	OCH_3	OH
(-)-Nordicentrine (A19)	-O-CH_2	-O-	H	H	H	H	OCH_3	H	H
Nuciferine (A20)	OCH_3	OCH_3	H	CH_3	H	H	H	H	H
Oxostephanine (A21)	-O-CH_2	-O-	H	CH_3	=O	OCH_3	H	H	H
Oxostephanosine (A22)	-O-CH_2	-O-	H	CH_3	=O	OH	H	H	H
(-)-Phanostenine (A23)	-O-CH_2	-O-	H	CH_3	H	H	OCH_3	OH	H
(-)-Roemerine (A24)	-O-CH_2	-O-	H	CH_3	H	H	H	H	H
(-)-Roemeroline (A25)	-O-CH_2	-O-	H	CH_3	H	H	OH	H	H
(-)-Stephanine (A26)	-O-CH_2	-O-	H	CH_3	H	OCH_3	H	H	H

Table 2 (continue)

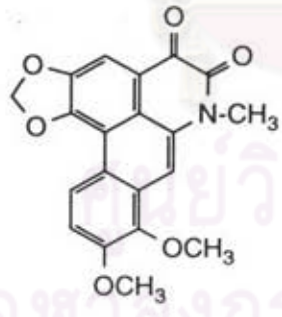
Alkaloids	1	2	4	6	7	8	9	10	11
Stesakine (A27)	-O-CH_2	-O-	H	CH_3	H	OCH_3	OH	H	H
Sukhodianine (A28)	-O-CH_2	-O-	H	CH_3	OH	OCH_3	OCH_3	H	H
Thailandine (A29)	-O-CH_2	-O-	H	CH_3	=O	OCH_3	H	H	H
Tuduranine (A30)	OCH_3	OCH_3	H	H	H	H	H	OH	H
Ushinsunine (A31)	-O-CH_2	-O-	H	CH_3	OH	H	H	H	H
Uthongine (A32)	-O-CH_2	-O-	H	CH_3	=O	OCH_3	OCH_3	H	H
(-)-Xylopine (A33)	-O-CH_2	-O-	H	H	H	H	OCH_3	H	H



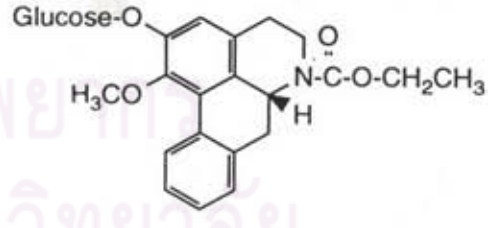
Dehydrocrebanine (A9)



Dehydrostesakine (A10)



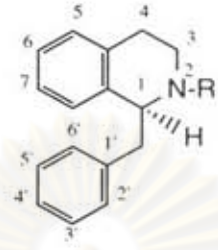
4,5-Dioxydehydrocrebanine (A12)



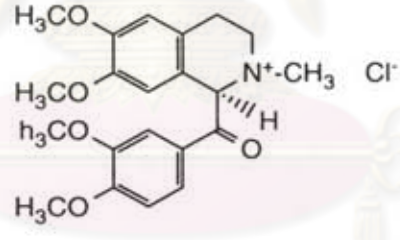
Kamaline (A34)

Table 2 (continue)

TETRAHYDROBENZYLISOQUINOLINE (T)



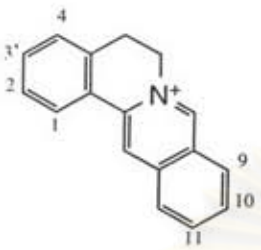
Alkaloids	R	6	7	8	3'	4'
(+)-Codamine (T1)	CH_3	OCH_3	OH	H	OCH_3	OCH_3
(±)-Oblongine (T3)	$(CH_3)_2$	H	OCH_3	OH	OH	H
(+)-Reticuline (T4)	CH_3	OCH_3	OH	H	OH	OCH_3



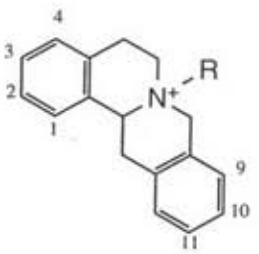
N-methylpapaveraldinium chloride (T2)

Table 2 (continue)

PROTOBERBERINE (P)



Formula A

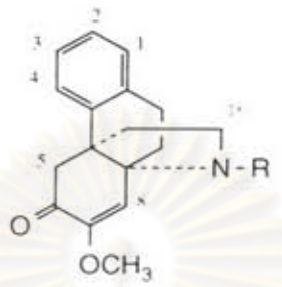


Formula B

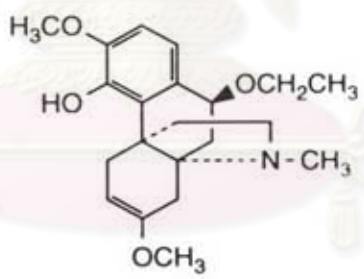
Formula A	Formula B	1	2	3	4	9	10	11	R
	(-)-Capaurine (P1)	OH	OCH_3	OCH_3	H	OCH_3	OCH_3	H	-
Columbamine (P2)		H	OH	OCH_3	H	OCH_3	OCH_3	H	-
Dehydrocory dalmine (P5)	(-)-Corydalmine (P3)	H	OCH_3	OCH_3	H	OCH_3	OH	H	-
	Cyclanoline (P4)	H	OH	OCH_3	H	OH	OCH_3	H	CH_3
Jatrorhizine (P6)		H	OCH_3	OH	H	OCH_3	OCH_3	H	-
	N-Methylcory dalmine (P7)	H	OCH_3	OCH_3	H	OCH_3	OH	H	CH_3
	N-Methyltetrahy dropalmatine (P8)	H	OCH_3	OCH_3	H	OCH_3	OCH_3	H	CH_3
Palmatine (P9)	Tetrahydropalma tine (P14)	H	OCH_3	OCH_3	H	OCH_3	OCH_3	H	-
	Steponine (P13)	H	OCH_3	OH	H	OH	OCH_3	H	CH_3
Stepharanine (P10)	Stepholidine (P12)	H	OH	OCH_3	H	OCH_3	OH	H	-
	Stepharotine (P11)	H	OCH_3	OCH_3	H	OCH_3	OCH_3	OH	-
	Tetrahydrostepha bine (P15)	OH	OCH_3	OCH_3	H	H	OCH_3	OCH_3	-
	Thaicanine (P16)	H	OCH_3	OCH_3	OH	OCH_3	OCH_3	H	-
	Xylopinine (P17)	H	OCH_3	OCH_3	H	H	OCH_3	OCH_3	-

Table 2 (continue)

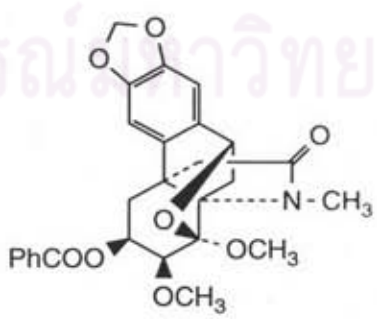
HASUBANAN ALKALOID (H)



Alkaloids	3	4	8	R
Cepharamine (H1)	OCH_3	OH	H	CH_3
Delavaine (H2)	-O-CH_2-O		OCH_3	CH_3
4-Demethylhasubanonine(H3)	OCH_3	OH	OCH_3	CH_3
4-Demethylnorhasubanonine (H4)	OCH_3	OH	OCH_3	H
Hasubanonine (H6)	OCH_3	OCH_3	OCH_3	CH_3
Homostephanoline (H7)	OH	OCH_3	OCH_3	CH_3



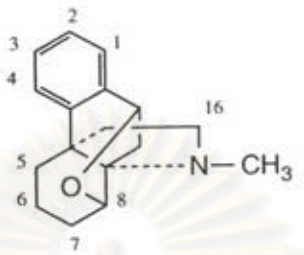
Longetherine (H9)



Oxostephabinine (H14)

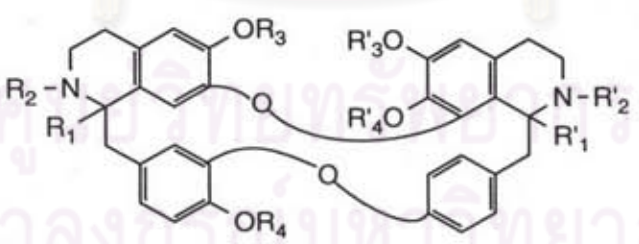
Table 2 (continue)

HASUBANAN ALKALOID (H)



Alkaloids	3	4	6	7	8	16
Epistephamiersine (H5)	OCH_3	OCH_3	=O	OCH_3	OCH_3	H_2
Longanone (H8)	OCH_3	OH	=O	OCH_3	OCH_3	H_2
Metaphanine (H10)	OCH_3	OCH_3	H_2	=O	OH	H_2
Miersine (H11)	OCH_3	OCH_3	OH	OCH_3	OH	H_2
Oxoepistephamiersine (H12)	OCH_3	OCH_3	=O	OCH_3	OCH_3	=O
16-Oxoprometaphanine (H13)	OCH_3	OCH_3	H_2	OCH_3	OH	=O
Oxostephamiersine (H15)	OCH_3	OCH_3	=O	OCH_3	OCH_3	=O
Oxostephasunoline (H16)	OCH_3	OCH_3	OH	OCH_3	OH	=O
Prometaphanine (H17)	OCH_3	OCH_3	H_2	=O	OH	H_2
Stephamiersine (H18)	OCH_3	OCH_3	=O	OCH_3	OCH_3	H_2
Stephasunoline (H19)	OCH_3	OCH_3	OH	OCH_3	OH	H_2

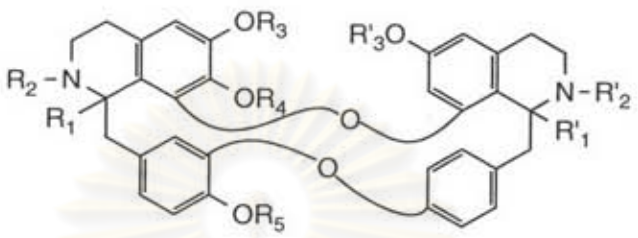
BISBENZYLISOQUINOLINE (B)



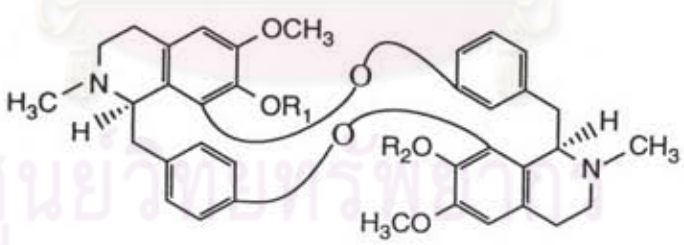
Alkaloid	R_2	R_3	R_4	R_2'	R_3'	R_4'	Configuration
Aromoline (B1)	CH_3	CH_3	H	CH_3	CH_3	H	1-R , 1'-S
Cepharanthine (B3)	CH_3	CH_3	CH_3	CH_3	$-CH_2-$		1-R , 1'-S
Daphnandrine (B5)	H	CH_3	CH_3	CH_3	CH_3	H	1-R , 1'-S
Homoaromoline (B9)	CH_3	CH_3	CH_3	CH_3	CH_3	H	1-R , 1'-S
2-Norcepharanthrine (B16)	H	CH_3	CH_3	CH_3	$-CH_2-$		1-R , 1'-S
2-Norobaberine (B18)	H	CH_3	CH_3	CH_3	CH_3	CH_3	1-R , 1'-S
Obaberine (B21)	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3	1-R , 1'-S
Stephibaberine (B25)	CH_3	CH_3	CH_3	CH_3	H	CH_3	1-R , 1'-S

Table 2 (continue)

BISBENZYLISOQUINOLINE (BERBAMINE TYPE) (B)



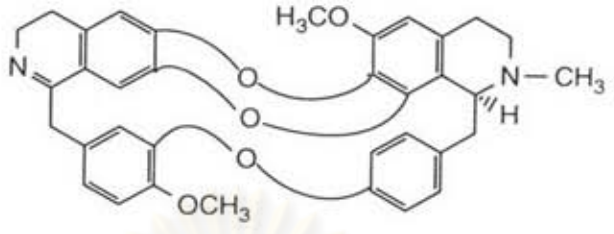
Alkaloid	R_2	R_3	R_4	R_5	R'_2	R'_3	Configuration
Berbamine (B2)	CH_3	CH_3	CH_3	H	CH_3	CH_3	1-R , 1'-S
Fanchinoline (B8)	CH_3	CH_3	H	CH_3	CH_3	CH_3	1-S , 1'-S
Isotetrandrine (B13)	CH_3	CH_3	CH_3	CH_3	H	CH_3	1-S , 1'-S
2-Norisotetrandrine (B17)	CH_3	CH_3	CH_3	CH_3	H	CH_3	1-S , 1'-S
2-Northalrugosine (B20)	H	CH_3	H	CH_3	CH_3	CH_3	1-R , 1'-S
Thalrugosine (B26)	CH_3	CH_3	H	CH_3	CH_3	CH_3	1-R , 1'-S



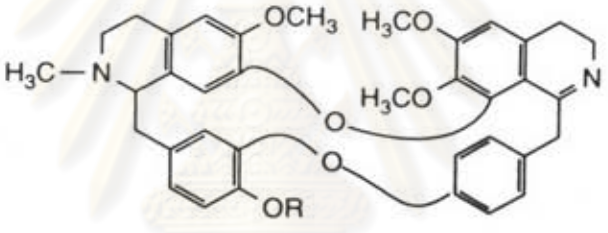
R1, R2 = CH3 : Cycleanine (B4)

R1, R2 = H : Isochondodendrine (B12)

Table 2 (continue)

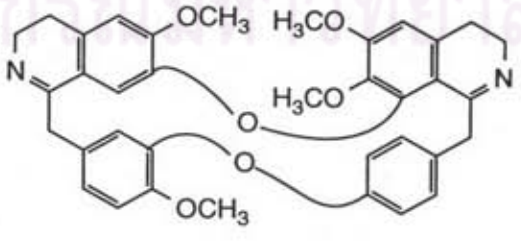


1.2-Dehydrotelobine (B6)



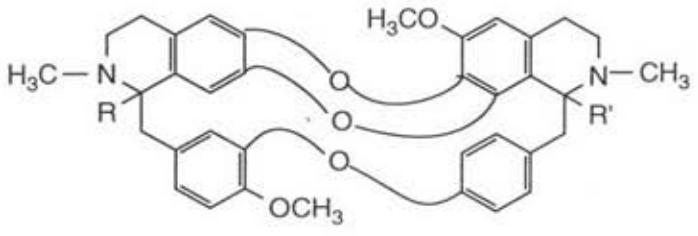
Epistephanine (B7) R= CH_3

Hypoepistephanine (B10) R= H



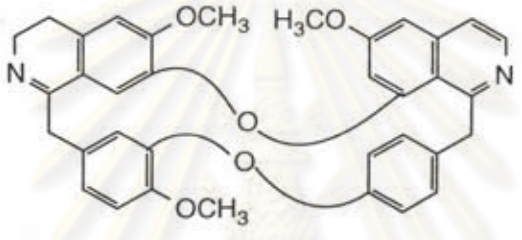
Stebisimine (B22)

Table 2 (continue)

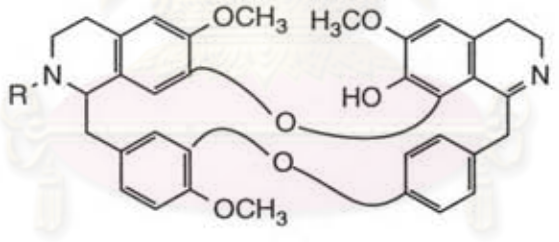


Isotelobine (B14) R,R'= *S*

N-methyltelobine (B15) R,R'= *R*

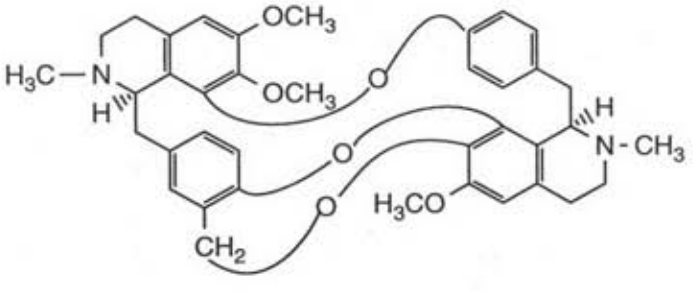


Stephasubinine (B24)



Stephasubine (B23) R= CH_3

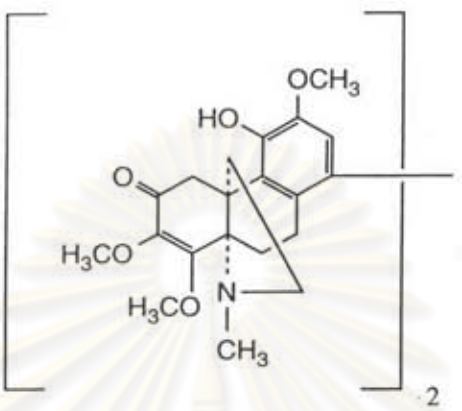
2-Norstephasubine (B19) R= H



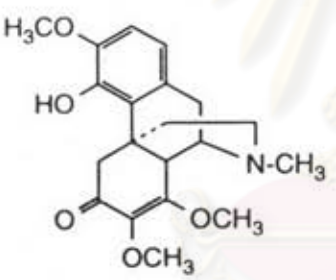
Insularine (B11)

Table 2 (continue)

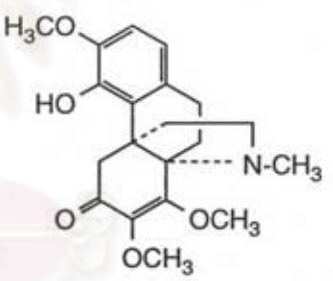
MORPHINAN ALKALOIDS (M)



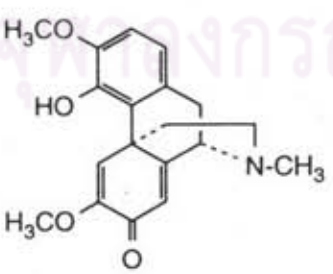
Bisakanadinine (M2)



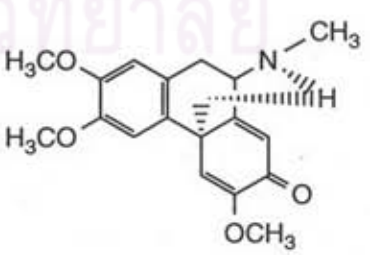
Aknadinine (M1)



Epihernandolinol (M3)



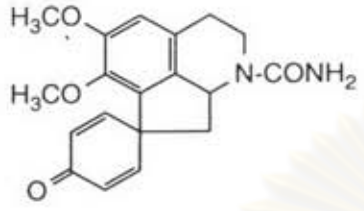
Salutaridine (M4)



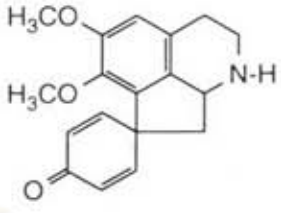
Sebiferine (M5)

Table 2 (continue)

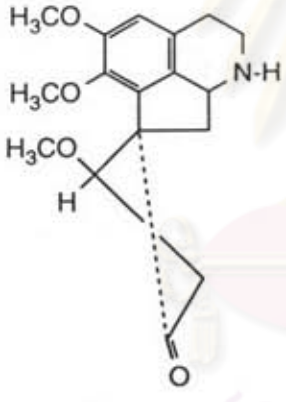
PROAPORPHINE ALKALOIDS (Pa.)



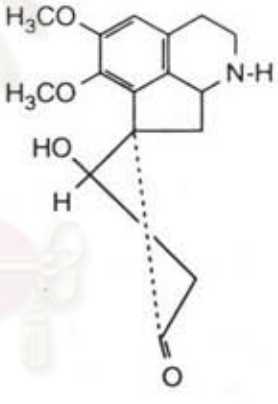
N- carboxamidostepharine (Pa.1)



Stepharine (Pa.3)



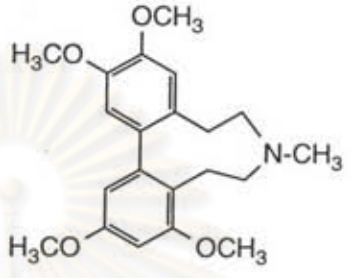
O-methylstepharinosine (Pa.2)



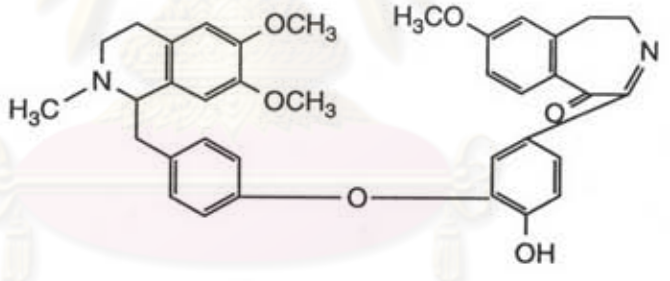
Stepharinosine (Pa.4)

Table 2 (continue)

MISCELLANEOUS BASE (Mis.)



Protostephanine (Mis.1)



Stepinonine (Mis.2)

2.4 Extraction and isolation of isoquinoline alkaloids from *Stephania pierrei*.

Extraction and isolation of isoquinoline alkaloids from *S. pierrei* tuber have been reported very recently (Likhitwitayawuid *et al.*, 1993b). From the tubers, it was exhaustively extracted with 95% ethanol. After evaporation, the syrupy residue was partitioned between chloroform and water (4:1). The chloroform fraction, after removal of the solvent and drying over anhydrous Na_2SO_4, afforded a chloroform extract. The water fraction, after lyophilization, gave an aqueous extract.

The chloroform extract was triturated with 2% acetic acid and filtered. The filtrated was then basified with $NaHCO_3$ and extracted with chloroform to give fraction A. The insoluble material was dried in a vacuum desiccator to afford fraction B.

Fraction A was subjected to column chromatography on silica gel using chloroform and methanol as the solvents in a polarity gradient fashion. Thirty-six 500 ml fractions were collected. Fractions 19 to 22 were pooled, dried in vacuo, and recrystallized from acetone to give (-) tetrahydropalmatine (P14)(0.042 % w/w). Fraction 24 was further purified by preparative TLC eluting with toluene : diethylamine (DEA)(96:4) to afford (-)-xylopinine (P17)(0.000075%w/w). Fractions 25 to 31 were combined and subjected to preparative TLC, using cyclohexane : ethyl acetate (AcOEt) : DEA (5:4:1) as the solvent. The lower band afforded (-)delavaine (H2)(0.00013%). The upper band was removed and further separated by preparative tlc eluting with cyclohexane : AcOEt : DEA (7:2:1) to give (-)-capaurine (P1)(0.000045%w/w) and (-)-thaicanine (P16)(0.00006%w/w). Fractions 32 to 36 were combined, dried and further separated by preparative TLC eluting with toluene : DEA (94:6) to yeild (-)-tetrahydrostephabine (P15)(0.00008%w/w) and (-)-corydalmine (P3)(0.00018%w/w).

Fraction B was chromotographed over silica gel and eluted with a series of chloroform-methanol combinations in a polarity-gradient manner. Seventy-eight fractions (500 ml) were collected. Fractions 6 and 7 gave (-)-tetrahydropalmatine (P14). Fraction 10, after removal of the solvent and drying *in vacuo*, was recrystallized from acetone to afford (-)-dicentrine (A11), (0.006%w/w). Fractions 11 to 36 were combined and dried. The residue was then recrystallized from acetone to give (-)-

dicentrine (A11). The mother liquor was dried and further purified by preparative tlc eluting with toluene : DEA (98:2) to afford (-)-isolaureline (A15)(0.00065%w/w). Preparative TLC of fractions 37 to 39 using AcOEt : methanol (6:1) as the eluent afforded (-)-phanostenine (A23)(0.0002%w/w). Fractions 40 to 41 were pooled, dried and subjected to preparative tlc eluting with cyclohexane : AcOEt : DEA (2:7:1). The more polar band was removed and extracted to give xylopine (A33)(0.000035%w/w). Fraction 42 to 52 were combined, dried, and then chromatographed over a silica gel column, using AcOEt : methanol (5:1) as the eluting solvent. Twenty-50-ml fractions were collected. Fractions 5 to 9 were pooled and evaporated to give a residue which was identified as (-)-cassythicine (A6)(0.000055%w/w). Fractions 11 to 13 were combined and further purified by preparative tlc eluting with cyclohexane : AcOEt : DEA (4:5:1) to afford (-)-salutaridine (M4)(0.00014%w/w) and (+)-codamine (T1)(0.000055%w/w). Fractions 55 to 69 from fraction B were combined and further separated by preparative tlc, using cyclohexane : AcOEt : DEA (1:8:1) as the solvent. Extract of the less polar band gave (+)-reticuline (T4)(0.000055%w/w), whereas that of the more polar one afforded (-)-asimilobine (A3)(0.00026%w/w).

The aqueous extract was redissolved in water and then treated with Mayer's reagent. The precipitates were collected, resuspended in methanol, and the solution was filtered. The filtrate was disignated fraction C, and the precipitate fraction D.

The filtrate (fraction C) was pass through an Amberite IRA-400 (chloride form) column, evaporated, and dried to afford a residue. This residue was then subjected to column chromatography using chloroform and methanol as the solvents with increasing polarity. Twenty 40-ml fractions were collected. Further purification of fraction 4 was carried out by preparative tlc eluting with methanol : NH_4OH (99:1) to give (-)-roemeroline (A25)(0.000025%w/w). Preparative TLC of fraction 7 using methanol : NH_4OH (99:1) as the eluent led to the isolation of (-)-asimilobine (A3). Fraction 15 to 17 were combined, dried and further purified by preparative tlc in chloroform: methanol (9:1) to yeild *N*-methyltetrahydropalmatine (P11)(0.00063%w/w). Separation of fractions 18 to 20 by preparative tlc eluting with methanol : NH_4OH (90:10) afford (±)-oblongine (T3)(0.00003%w/w).

Fraction D was resuspended in acetone : methanol : water (6:2:1) and filtered. The filtrate was then subjected to ion-exchange chromatography (Amberite IRA-400, chloride form), evaporated under reduced pressure and dried. Preparative TLC of the

obtained residue in chloroform : methanol : NH_4OH (14:5:1) afforded magnoflorine (A18)(0.00011%w/w).

2.5 Biosynthesis of aporphine alkaloids

In recent years, by enzyme and feeding experiments of plant cell cultures,it has been shown that (*S*)-reticuline is the central intermediate in the biosynthesis of benzylisoquinolines and many other alkaloids, including aporphines (Figure 4)(Schneider and Zenk, 1985).

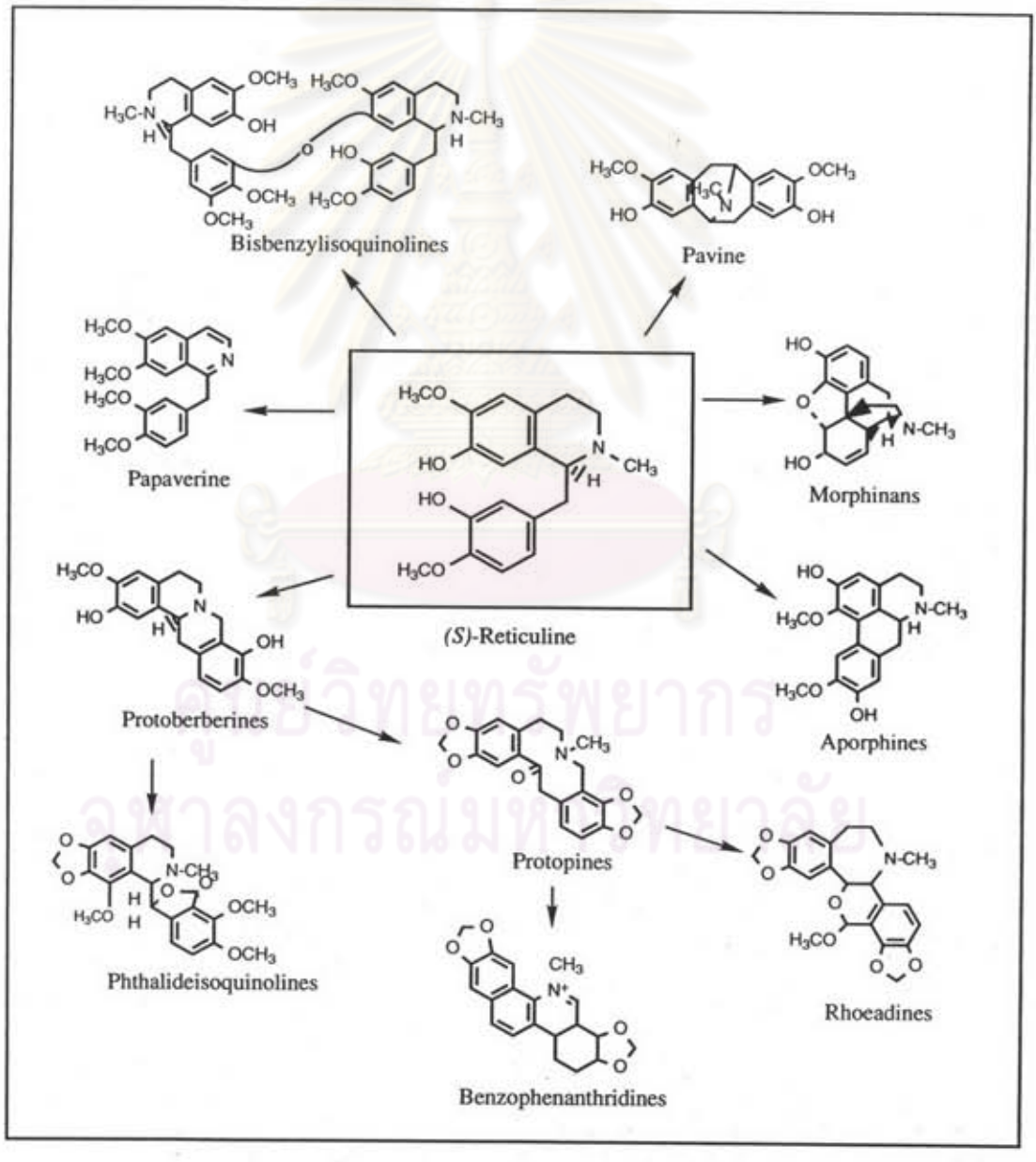


Figure 4 Central role of *(S)*-reticuline in biogenetic pathways of isoquinoline alkaloids

Previously, numerous tracer experiments established that both C_6-C_2 units comprising the benzylisoquinoline skeleton were derived from tyrosine (Spenser,1968) and norlaudanosoline was thought to be the central intermediate for a multitude of isoquinoline alkaloids (Robinson, 1955). This hypothesis was based on tracer experiments using specifically labelled norlaudanosoline (Battersby, 1964). (*S*)-norlaudanosoline was the result of the condensation of dopamine with 3, 4 dihydroxyphenylacetaldehyde, both are derived from a dihydroxylated tyrosine derivative, DOPA (Robinson, 1955). However, the incorporation of labelled DOPA or dopamine into the alkaloids showed that only isoquinoline portion and not the benzylic portion of the alkaloid molecules was labelled (Spenser, 1968). Subsequent experiments also proved that the two C_6-C_2 units derived from tyrosine differ from one another. Feeding experiments with (*S*)-[1-^{13}C]-norcoclaurine have shown that this trihydroxylated precursor is specifically incorporated into protoberberine, aporphine and benzophenanthridine alkaloids in cell suspension cultures as well as in to pavine and benzophenanthridine alkaloids in whole plants (Stadler, 1989). The rates of the incorporation ranged from 2.5 to 36%. This has led to the conclusion that tyrosine is metabolized to dopamine and p-hydroxyphenylacetaldehyde followed by their condensation to form norcoclaurine, thus explaining the lack of incorporation of DOPA or dopamine into the benzylic portion of reticuline derived alkaloids (Stadler,1989) and the norcoclaurine pathway leading to (*S*)-reticuline can be summarized as shown in Figure 5 (Muller and Zenk,1992).

Aporphines have been proposed to be derived from (*S*)-reticuline (Bhakuni, 1977, Brochmann-Hansen, 1971, Barton, 1965, Battersby, 1963) as well as (*R*)-reticuline (Luckner,1990), although the biosynthetic pathway from reticuline to aporphines is still uncompletely known. Until now, there are at least five possible routes for the biosynthesis of aporphines from reticuline. These routes have been proposed on the basis of the orientation of phenolic and methoxy groups (Figure 6)(Cordell,1981).

Among these biogenetic pathways, route V seems to be more available than the others. In this pathway, the aporphines belonging to the (*R*) or (*S*)-series are formed through direct coupling (Figure 7 and 8)(Luckner, 1990).

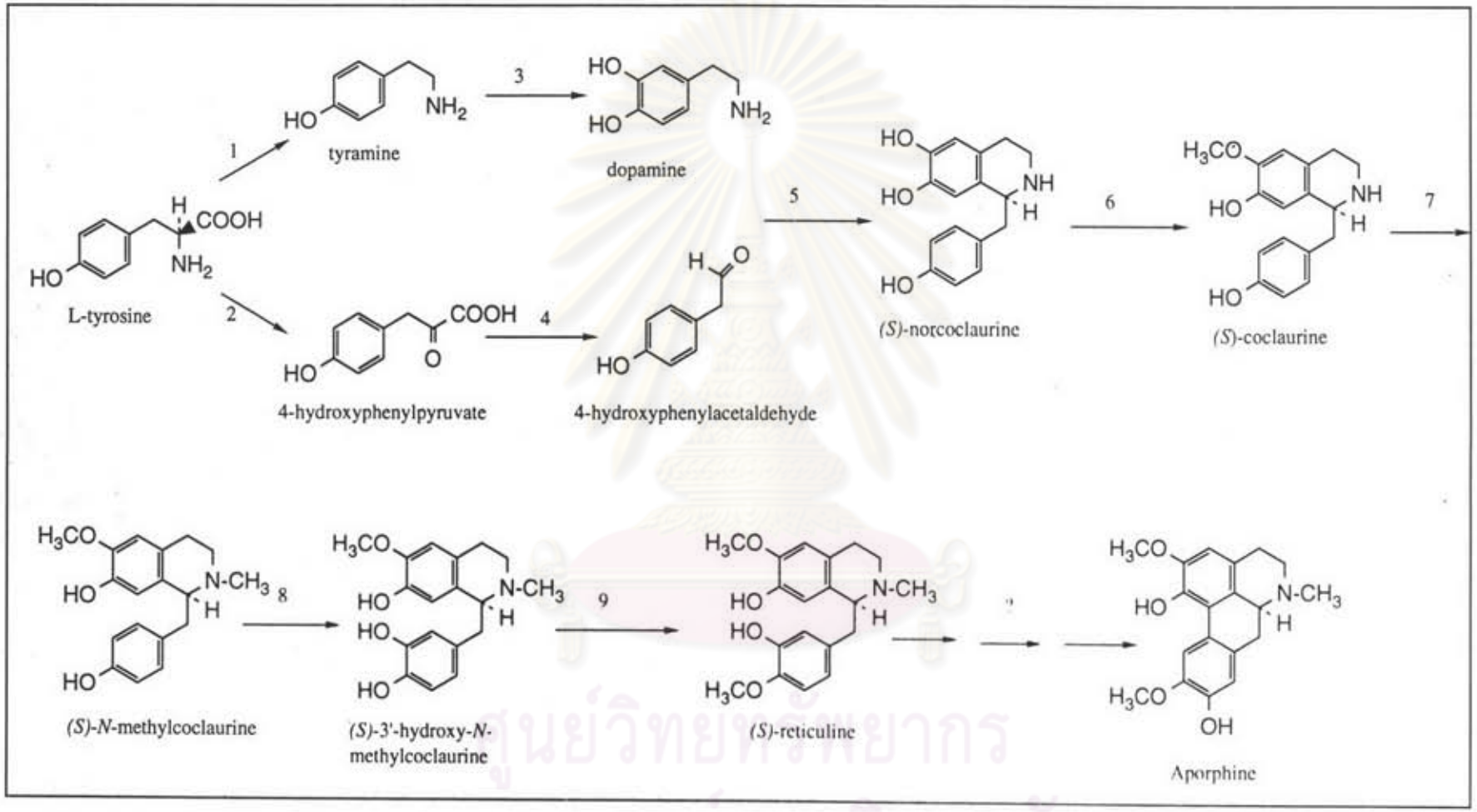


Figure 5 Biosynthetic pathway of *(S)*-reticuline starting from L-tyrosine leading to the biosynthesis of aporphines. The enzyme involed in the biosynthesis: 1=L-tyrosine decarboxlase, 2=L-tyrosine transaminase, 3=phenolase, 4=*p*-hydroxyphenylpyruvate decarboxylase, 5=*(S)*-norcoclaurine synthase, 6= norcoclaurine-6-*o*-methyltransferase, 7=coclaurine -*N*-methyltransferase, 8=phenolase and 9=*(S)*-3'-hydroxy-*N*-methylcoclaurine-4'-*o*-methyltransferase.

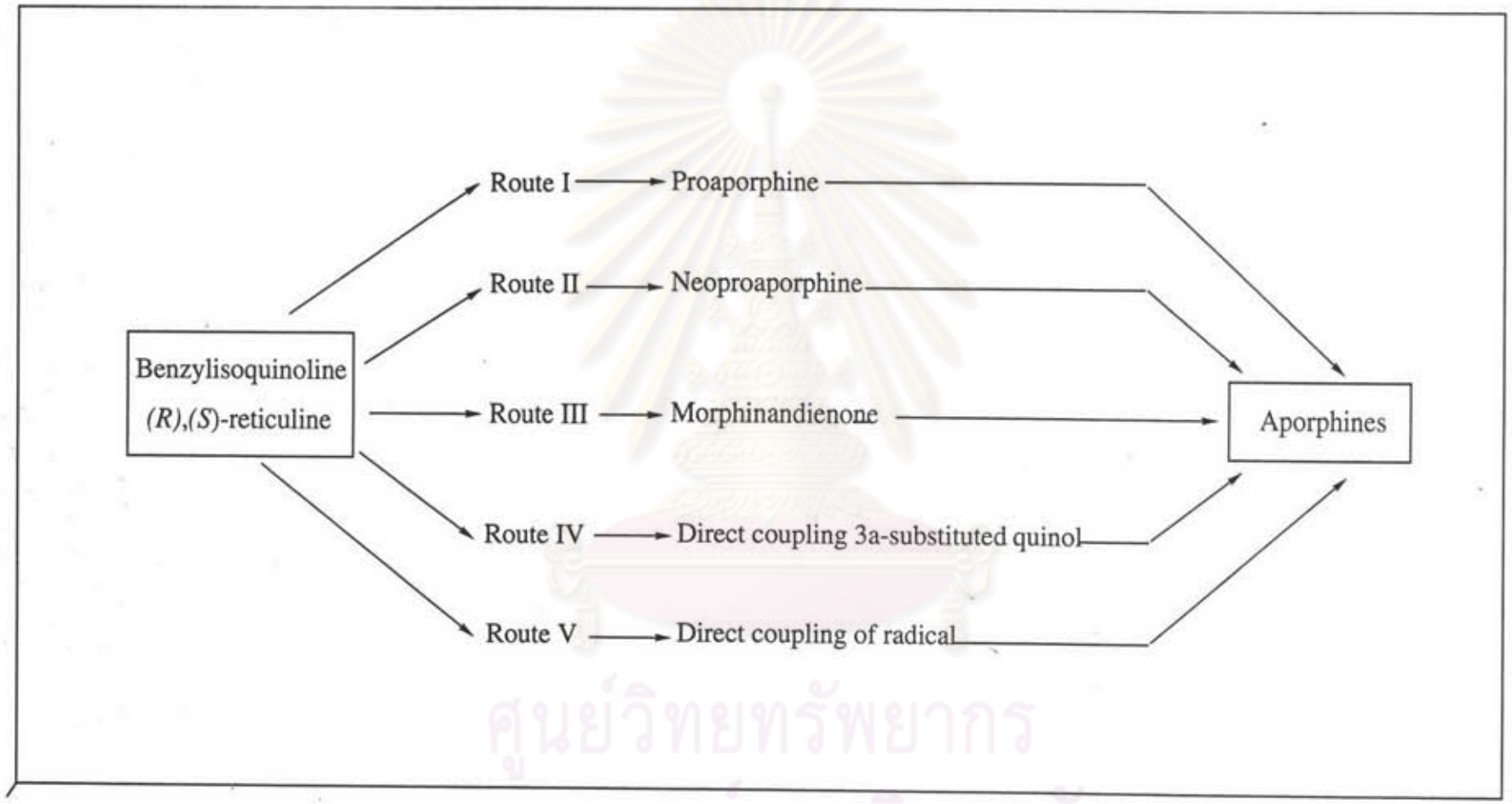


Figure 6 Proposed biogenesis routes of aporphines from benzylisoquinoline

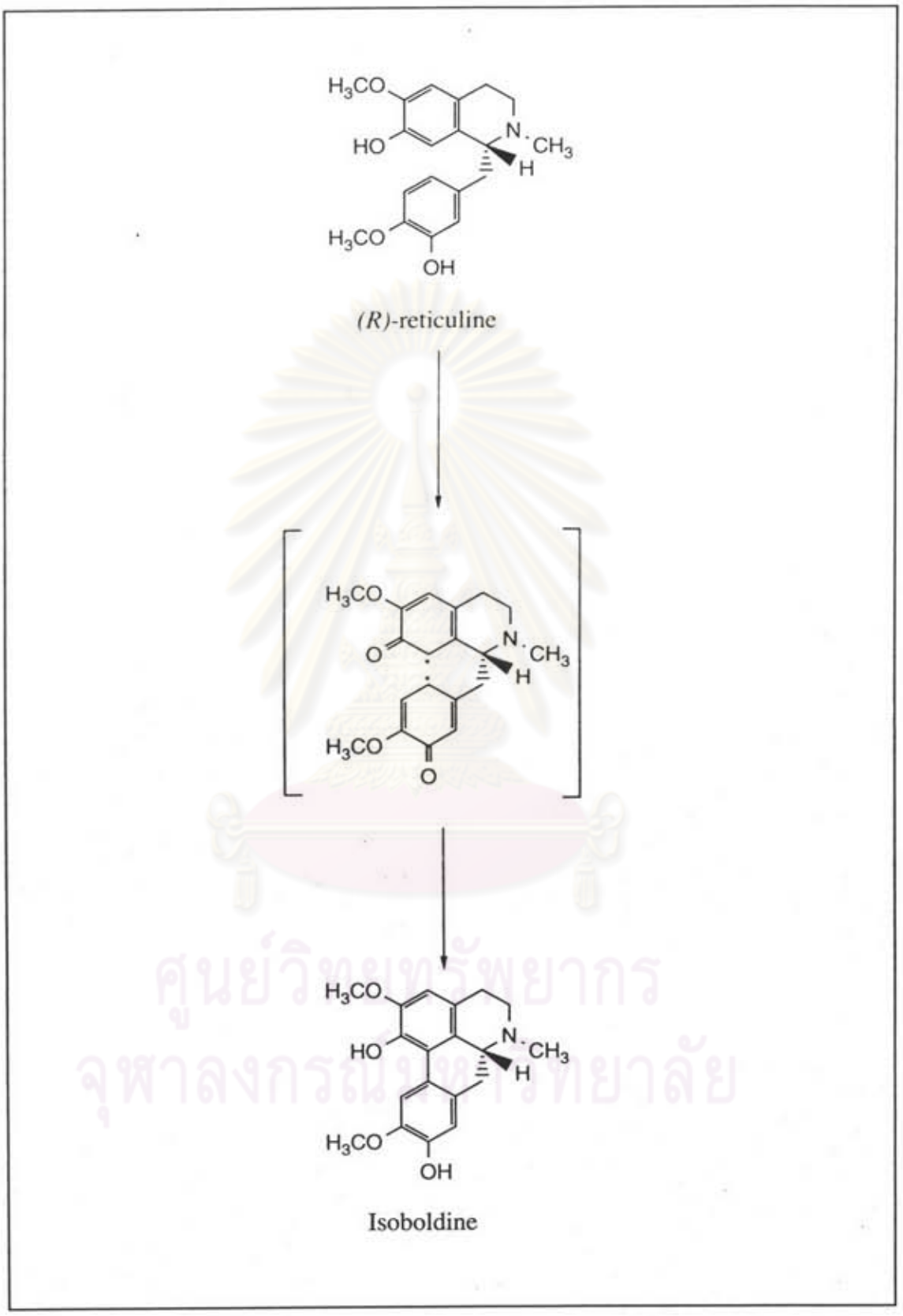


Figure 7 Formation of aporphine alkaloid isoboldine belonging to the *(R)*-reticuline

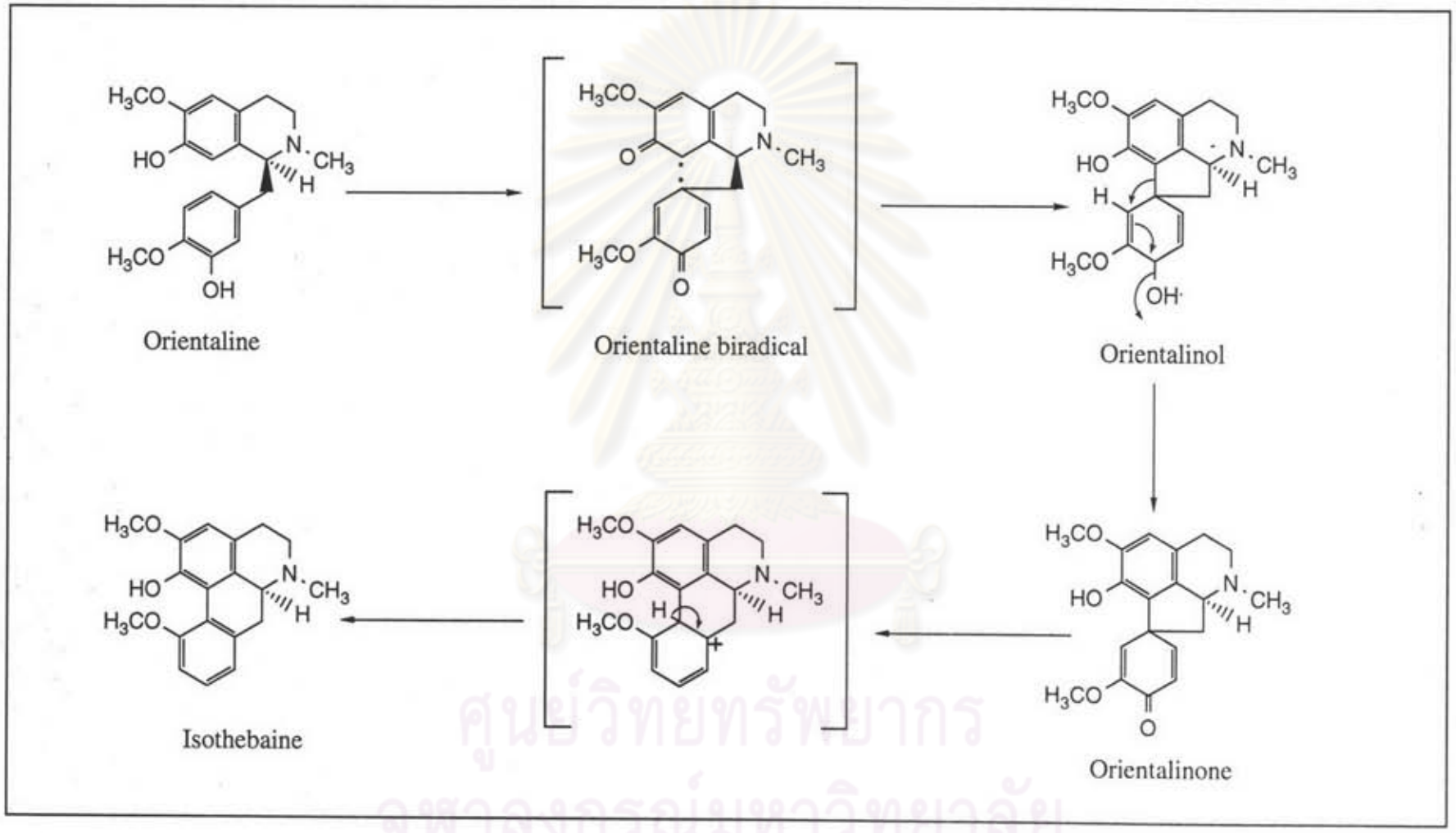


Figure 8 Biosynthesis of isothebaine belonging to the (-)-orientaline

However, the methoxy and hydroxy positions in some aporphine skeletons are not completely corresponded to their position of benzylisoquinoline precursors. This makes this possible route still in question of its major operation in nature.

Nevertheless, theoritically, there are two possible routes of the biosynthetic pathway that occur through direct coupling. These are *ortho - ortho* and *ortho - para* coupling of the precursor, reticuline (Figure 9)(Herbert, 1981).

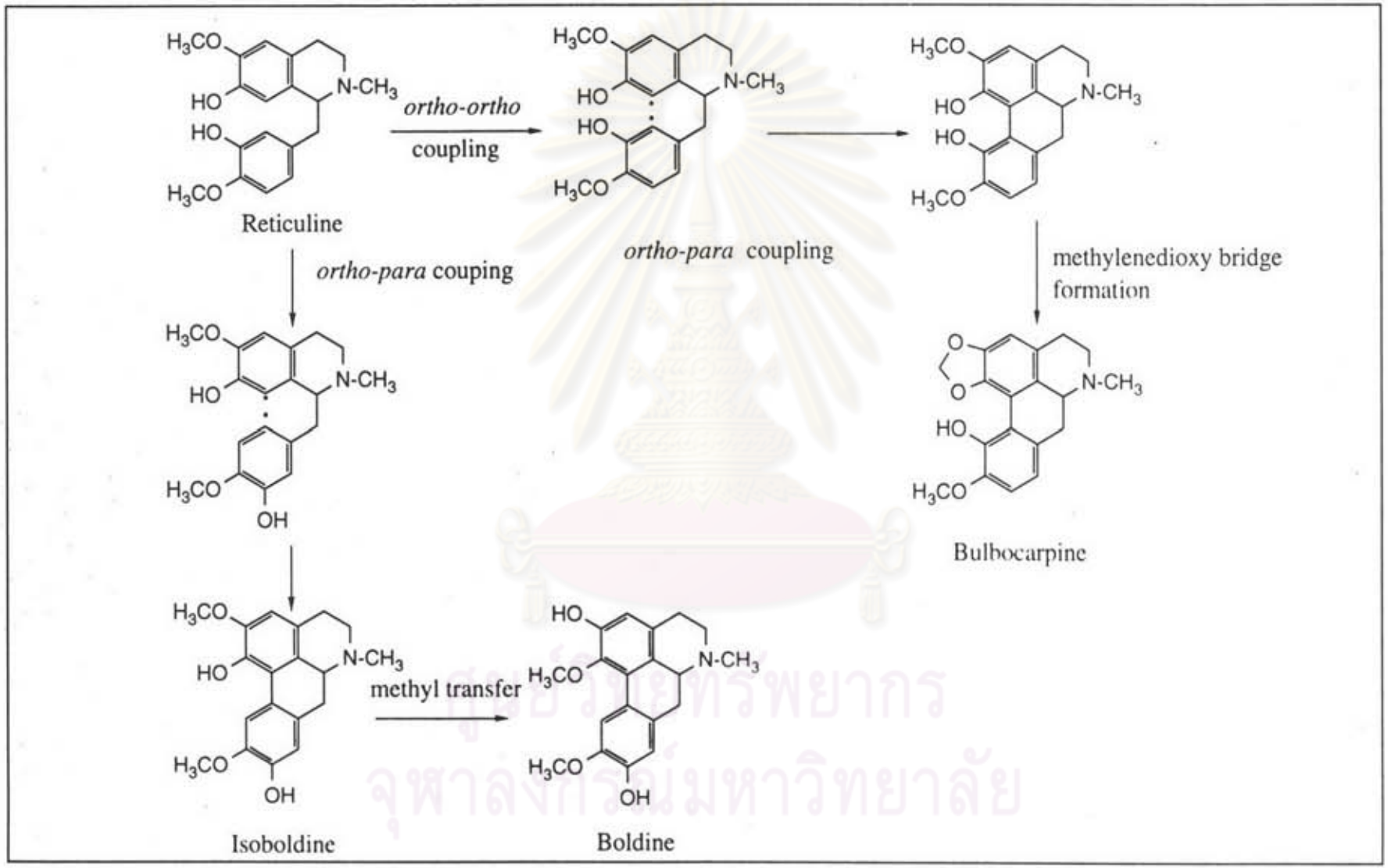


Figure 9 The biosynthetic pathway of aporphines via *ortho-ortho* and *ortho-para* coupling

In the structure of isoboldine (as same as reticuline), there are the methyl group at position C_1 and hydroxyl group at C_2 of ring A, but it has reversed in the same positions in norboldine (as same as boldine) structure. Two possible biosynthetic sequences can be envisaged, either migration of a methyl group *via* a methylenedioxy bridge at ring A (position 1-2 = -O-CH_2-O-) or methylation at the 2-hydroxyl yielding laurotetanine, followed by demethylation of the alternative hydroxyl group (Schneider and Zenk, 1993). In *Litsea glutinosa*, the loss of radioactivity of the methoxy group of ring A from reticuline was shown, indicating rather methylation/demethylation than the methylenedioxy mechanism (Barton *et al.*,1967). To prove these possibilities Schneider and Zenk (1993) administered (*S*)-reticuline triply-labelled with ^{13}C, (*S*)-[1-^{13}C, 6-O$^{13}CH_3$, N-$^{13}CH_3$] reticuline, to cell cultures of *Peunus boldus*, which is source of aporphines, respectively. During incorporation of the labelled precursor into aporphine alkaloids, unexpected transmethylation of the methyl groups were observed by ^{13}C NMR spectroscopy which seem to proceed *via* demethylation, flux of ^{13}C through the C-1 pool, and remethylation. This hypothetical mechanism should start with an oxidative attack on the methyl group yielding a hydroxymethyl moiety which is subsequently split off from as CH_2O by a hydroxymethylase. The CH_2O is transfered to tetrahydrofolate (THF) which, as an intermediate in the biosynthesis of methionine, reacts with homocysteine. Methionine is incorporated into *S*-adenosylmethionine (SAM) from which, in the final step of the flux, the methyl is transfered back to the alkaloid. An argument in support of this hypothesis is the occurence of signal in the ^{13}C NMR spectrum of the crude extract from *Peunus boldus* assignable to methionine (Schneider and Zenk, 1993).

There is considerable need for the intermediates of the C-1 pool, methionine and SAM, in the general metabolism of the plant cell. Methionine is an important building block of proteins and other plant metabolites, while SAM is the general methylating agent in plants. Considering the high flux of the C-1 pool, it is very surprising that ^{13}C, after passing through this sequence, returns to the starting compound. With N-^{14}C labelled reticuline, it was shown that this phenomenon is independent of the concentration of the compound supplied. To account for this shuffing of methyl groups in alkaloid biosynthesis, it must be assumed the demethylation/remethylation proceeds within a subcellular compartment, may be even within a vesicle devoted to the formation of these alkaloids (Amann, Wanner, and Zenk, 1986).

For example, the simplest biosynthetic sequence has been proposed for the formation of bulbocarpine (Herbert, 1981). Oxidative coupling is thought to occur between the sites *ortho* to the *ortho* in reticuline to give intermediate with minor modification affords bulbocarpine. For the other way, because of free rotation of benzylic portions in reticuline structure, oxidative coupling between the sites *ortho* to *para* position is also possible to give isoboldine before methyl transfer to afford boldine.

Schneider and Zenk (1993) have suggested that there are several intermediates of aporphines with different methylation patterns in Ring A as shown in Figure 10.

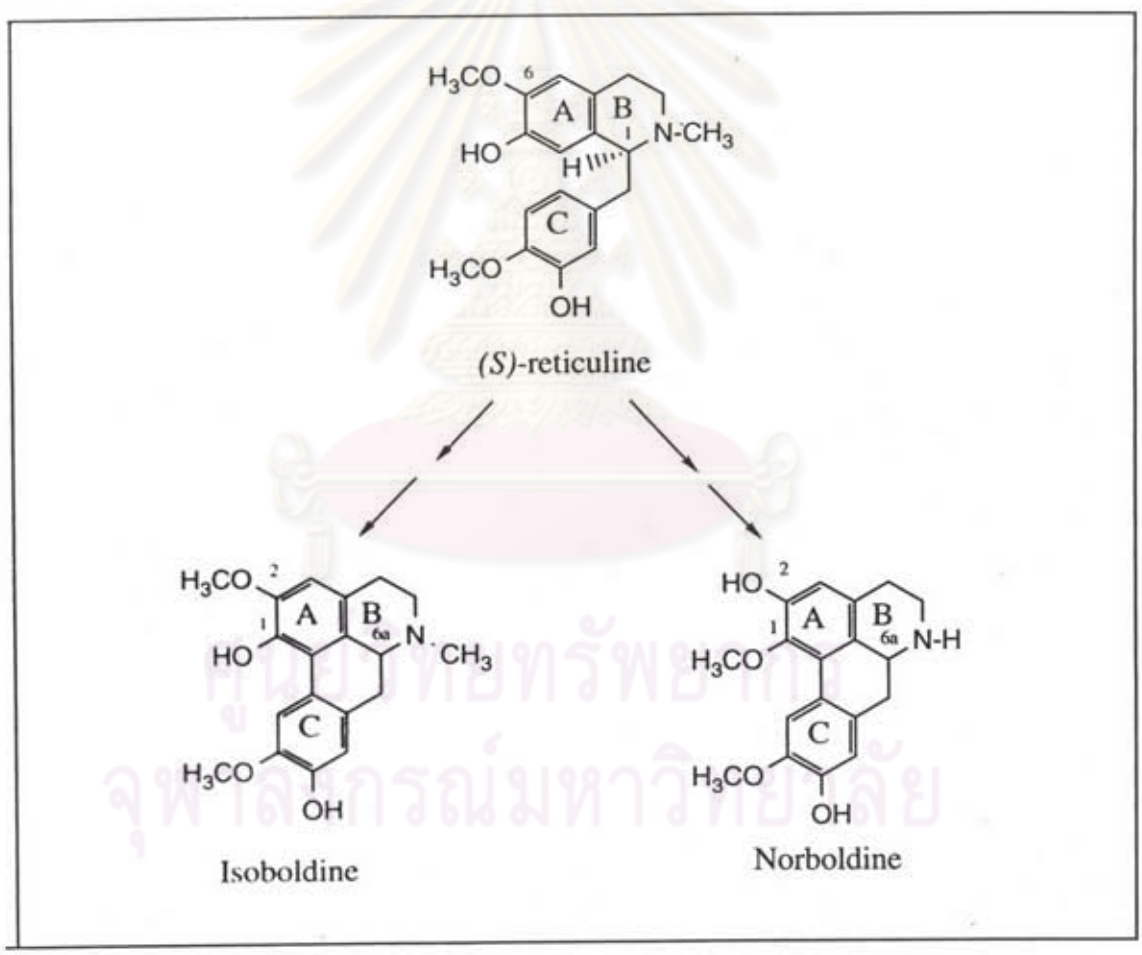


Figure 10 Changes in the methylation patterns in Ring A during the biosynthesis of aporphines starting from *(S)*-reticuline.

Clearly this is an area in need of considerable further study, and this time it is not clear which, or how many, of the possible biosynthetic routes may be operating in order to produce the various aporphine alkaloids.

2.6 The production of aporphine alkaloids by plant cell cultures

Under optimal conditions plant cells are able to grow like microorganisms in media cultures without limitation or aging. Moreover they are totipotent, in that one single cell or protoplast has the whole genetic information for the differentiated plant. Therefore one can expect that under appropriate conditions, plant cell cultures have the ability to produce the whole range of natural products which are isolated from the differentiated plants. This could be of extreme importance for the production of substances especially for pharmaceutical use, since a survey indicated that 23% of all prescriptions contain natural compounds (Farnsworth and Moris, 1976). Plant cell cultures would have several advantages in comparison to differentiated plants. They are independent for instance of geographical and climatical conditions, of plant diseases or animal destruction and therefore the price of the plant drugs could be stabilized. In addition, they could easily be cultivated under special state control.

Since Reinhard (1967) described the production of protoberberine alkaloids by callus cultures of *Berberis vulgaris*, many different structure types of benzylisoquinoline alkaloids have been isolated from callus and suspension cultures. The greatest variety in the substitution patterns on the basic structures have been found in the group of protoberberine alkaloids (Rueffer, 1985). For aporphine alkaloids, only a few natural compounds have been isolated from plant cell cultures and the results of these studies are summarized in Table 3.

Table 3 Aporphine alkaloids isolated from plant cell cultures

Alkaloid name	Source	Reference
Cepharadione A	*Stephania cepharantha*	Akasu *et al.*, 1975
Cepharadione B	*S. cepharantha*	Akasu *et al.*, 1975
Liriodenine	*S. cepharantha*	Akasu *et al.*, 1975
Lysicamine	*S. cepharantha*	Akasu *et al.*, 1975
Norcepharadione	*S. cepharantha*	Akasu *et al.*, 1975
Isoboldine	*Fumaria capreolata*	Rueffer, 1985
Norboldine	*Peumus boldus*	Stadler and Zenk, 1990
Magnoflorine	*Corydalis incisa*	Ikata *et al.*, 1974
	C. pallida	Ikata *et al.*, 1974
	Dicentra peregrina	Ikata *et al.*, 1974, Ikata and Itokawa, 1982
	Eschscholtzia califonica	Ikata *et al.*, 1974
	Papaver somniferum	Ikata *et al.*, 1974
	P. setigerum	Ikata *et al.*, 1974
	P. bracteatum	Ikata *et al.*, 1974
	P. orientale	Ikuta *et al.*, 1974
	P. rhoeas	Ikata *et al.*, 1974
	Thalictrum minus	Ikata and Itokawa, 1982
	Coptis japonica	Ikata and Itokawa, 1982
	Mahonia japonica	Ikata and Itokawa, 1982
	Nandina domestica	Ikata and Itakawa, 1982
	Tinospora caffra	Rueffer *et al.*, 1985
	Chasmanthera dependens	Rueffer *et al.*, 1985
	Stephania japonica	Rurffer *et al.*, 1985
	Dioscoreophyllum cumminisii	Furuya *et al.*, 1983
	Berberis stolonifera	Schneider and Zenk, 1992
	Thalictrum tuberosum	Galneder and Zenk, 1990, Zenk,1991, Schneider and Zenk, 1992
Laurotetanine	*Peunus boldus*	Stadler and Zenk, 1990