

CHAPTER II

HISTORICAL



1. Botanical aspects

Menispermaceae

Plants of the family Menispermaceae are dioecious climbers, rarely erect plants, and stipules absent. Leaves spiral, often palmatinerved at base; petiole often swollen at base. Flowers small, usually 3-merous; sepals, petals and stamens free or united; carpels free usually 1-6. Fruits consisting of usually 1-6 drupes with style-scar sometimes close to base or lateral; endocarp usually bony and often ornamented; sometimes horseshoe-shaped, usually with a condyle, i.e. a ventral intrusion into the seed-cavity. Seed with endosperm present or absent (Smitinand and Larsen, 1991).

According to Forman (1978, 1982 and 1985) the members of Menispermaceae were divided into five tribes, there are Coscinieae, Menispermeae, Tiliacoreae, Tinosporeae and Fibraureae. But two of these, Tinosporeae and Fibraureae should probably be combined. The following combinations of characters characterize the tribes in Asia.

Coscinieae: Sepals imbricates. Petals 0. Stamens either all or only the inner 3 connate. Carpels 3-6. Drupe with style-scar sublateral towards base on lateral. Endocarp smooth or fibrio-pilose subglobose with condyle obsolete, or subhemispherical with condyle deeply intrusive and 2-chambered. Endosperm present, sometimes ruminant. Seed broadly ellipsoidal or cup-shaped. Embryo with thin foliaceous divaricate cotyledons, which are sometimes much, folded.

Menispermeae: sepals usually free in 1-few whorls or sometimes connate when in 1 whorl, the innermost whorl sometimes valvate, or spiral. Petals (0-)3-6(-9) sometimes connate. Female flowers with perianth sometimes reduced to 1-2 parts. Stamens free or partly connate or united into a peltate synandrium. Carpels 1-6. Drupe strongly curved with style near base. Endocarp with \pm horseshoe-shaped dorsal region

usually ornamented with projections or transverse ridges; condyle, deeply intrusive, either lamelliform and \pm ovate with the seed-cavity curved around its margin or hollow with 1-2 chambers, sometimes perforate. Endosperm usually presents, but absent in *Pachygone*. Seed elongate, strongly curved. Embryo elongates and curved with contiguous cotyledons.

Tillacoreae: sepals imbricate or inner whorl valvate and sometimes connate. Petals rarely absent. Stamens free or connate. Carpels 3-10. Drupe with style-scar near base or lateral. Endocarp smooth, wrinkled, rugose or coarsely reticulate; straight and condyle absent or curved with condyle intrusive and septiform. Endosperm usually absent, but present and ruminant in *Tiliacora*. Seed ellipsoidal, straight. Embryo with thick accumbent cotyledons or elongate and strongly curved with elongate contiguous cotyledons.

Tinosporeae (include *Fibraureae*): sepals imbricate, rarely connate at the base. Petals 6 or 0. Stamens free or united into a peltate synandrium. Carpels 3(-4). Drupe with style-scar terminal. Endocarp spiny, verrucose, rugose or smooth, condyle a ventral hollow or longitudinal groove or deeply intrusive and clavate endosperm present, sometimes ventrally ruminant. Seed usually straight and ventrally hollowed or grooved sometimes cup-shaped. Embryo with foliaceous divergiculate or imbricate cotyledons.

The family contains 73 genera, and about 350 species; which are almost entirely tropical. There are 22 genera and 51 species in Thailand; of which 9 species are endemic (shown by asterisk) (Smitinand and Larsen, 1991). All of them are as follows:

1. *Albertisia*

Albertisia papuana Becc.

A. puberula Forman

2. *Anamirta*

Anamirta cocculus (L.) Wight & Arn.

[Mae nam nong (แม่น้ำนอง) (Northern); thaowan thong

(เถาวัลย์ทอง) (Southwestern); wai din (หวายดิน), Kho khlan (โคกลาน) (Central); thao kha-nom (เถาชะโนม), lumpri (ลุมพรี) (Southeastern)].

3. *Arcangelisia*

Arcangelisia flava (L.) Merr.

[Khamin khrua (ขมิ้นเครือ) (Southeastern); khamin ruesi (ขมิ้นฤๅษี), hap (ฮับ) (Peninsular)].

4. *Aspidocarya*

Aspidocarya uvifera Hook.f. & Thorns.

5. *Cissampelos*

Cissampelos hispida Forman

C. pareira L. var. *hirsuta* (Buch. ex DC.) Forman

[Khong khamao (ขงเขมา) (Northern); khrua ma noi (เครือหมาน้อย) (Eastern); kon pit (ก้นปัด) (Southwestern); krung khamoa (กรุงเขมา), sifan (สีฟัน) (Peninsular)].

6. *Cocculus*

Cocculus hirsutus (L.) Theob.

C. laurifolius DC.

[Yang nan ton (ย่านางต้น) (North-eastern, Central); sakae dong (สะแกดง) (North-eastern); suramarit (สุรามฤต) (Eastern)].

C. orbiculatus (L.) DC.

7. *Cosciniium*

Cosciniium blumeianum Miers

C. fenestratum (Gaertn.) Colebr.

[Khrua hen (เครือเหิน) (Northeastern), kramin khrua (ขมิ้นเครือ) (Southeastern)].

8. *Cyclea*

Cyclea atjehensis Forman

C. barbata Miers

[Krung badan (กรุงบาดาล) (South-eastern); krung khamao (กรุงเขมา) (Peninsular)].

C. laxiflora Miers

C. polypetala Dunn

C. varians Craib

9. *Diploclisia*

D. glaucesces (Blurne) Diels

[Ma nim dam (มะหนิมดำ), duk khrua (ดูกเครือ) (Northern);
khrua sai kai (เครือใส่ไก่) (Shan/Northern); tap tao (ตับเต่า)
(Peninsular)].

10. *Fibraurea*

Fibraurea tinctoria Lour.

[Khamin ruesi (ขมิ้นฤๅษี), khamin khrua (ขมิ้นเครือ), man miat (มัน
เมียด) (Peninsular); thaowan thong (เถาว์ถ้อง)(Southwestern);
kamphaeng chet chan (กำแพงเจ็ดชั้น) (Central)].

11. *Haematocarpus*

Haematocarpus validus (Miers) Bakh.f. ex Forman

12. *Hypserpa*

Hypserpa nitida Miers

[Haen kuern (แฮนกี๋ม) (Northeastern)].

13. *Limacia*

Limacia blumei (Boerl.) Diels

L. oblonga Hook.f. & Thorns.

L. scanderis Lour.

14. *Pachygone*

Pachygone dasycarpa Kurz

[Nam phrom (น้ำพรม) (Northern); ya nang chang (หญ้านางช้าง)
(Eastern)].

P. odorifera Miers

15. *Parabaena*

Parabaena sagittata Miers

[Phak nang (ผักหนั่ง) (Shan/Northern)].

16. *Pericampylus*

Pericampylus glaucus (Lamk.) Merr.

[Salit hom kha (สลิดหม่อมกา) (Northern); yan tap tao (ย่างต๊ับเต๋า)
(Peninsular)].

17. *Pycnarrhena*

Pycnarrhena lucida (Teijsm. & Binn.) Miq.

[Ya nang ton (ย่านางตัน) (Southwestern)].

P. poilanei (Gagnep) Forman

18. *Sinomenium*

Sinomenium acutum (Thunb.) Rehder & Wilson

19. *Stephania*

Stephania brevipes Craib

[Bua khrua (บัวเครือ) (Northern)].

S. capitata (Blume) Spreng.

S. crebra Forman

S. elegans Hook.f. & Thoms.

[Se-khi-pho (เสกขีพอ) (Karen/ Northern)].

S. glabra (Roxb.) Miers

[Phanang nang (พ่านังนัง) (Northern)].

S. glandulifera Miers

S. japonica (Thunb.) Miers

[Kon pit (ก้นปัด), bai kon pit (ใบก้นปัด) (Central); pang pon (ปัง
ปอน) (Northern); tap tao (ต๊ับเต๋า), yan pot (ย่านปด) (Peninsular)].

S. oblata Craib

S. papillosa Craib

S. pierrei Diels

[Bua khrua (บัวเครือ) (North-eastern); bua bok (บัวบก)
(Southwestern, Eastern and Central); kot hua bua (โกฐหัวบัว),
sabu lueat (สบู่เลือด) (Central)].

S. reticulata Forman

[Tap tao (ต๊ับเต๋า) (Peninsular)].

S. rotunda Lour.

S. suberosa Forman

[Bua bok (บัวบก) (Central); boraphet phung chang (บอระเพ็ดพุง
ข้าง) (South-western)].

S. subpetata H.S. Lo

S. tomentella Forman

S. venosa (Blume) Spreng.

[Plao lueat khrua (เปล้าเลือดเครือ) (Northern); cho koe tho
(ชอเกาะเทอ) (Karen/Northern); krathom lueat (กระท่อมเลือด)
(Northeastern); kling klang dong (กึ่งกลางดง) (South western);
boraphet yang daeng (บอระเพ็ดยางแดง) (Peninsular)].

20. *Tiliacora*

Tiliacora triandra (Colebr.) Diels

[Choi nang (จ้อยนาง) (Northern); Thao ya nang (เถาย่านาง)
(Eastern, Central); thaowan khieo (เถาวัลย์เขียว) (Southeastern)].

21. *Tinomiscium*

Tinomiscium petiolare Hook.f. & Thoms.

[Pharai hothong (ฝ้ายร้อยห่อทอง) (Peninsular)].

22. *Tinospora*

Tinospora baenzigeri Forman

[Chung chang ling (จุงจางลิง), Chingcha chali (ชิงช้าชาติ)
(General)]

T. crispa (L.) Hook.f. & Thoms.

[Boraphet (บอระเพ็ด) (Central)]

T. siamensis Forman

T. sinensis (Lour.) Merr.

[Ping kaling (ปิงกะลิง) (Northern); sali thao chali (สลีเถาชาติ)
(Central)].

Cyclea (Smitinand and Larsen, 1991)

Slender woody climbers. **Leaves** often peltate, palmately nerved. **Inflorescences** axillary, terminal or cauliflorous, pseudoracemose or thyrsoid. **Male flowers:** sepals 4(-5), free or connate into a 4(-5)-lobed calyx. **Petals** 4, free or connate into a ± cup-shaped corolla or rarely 0. **Stamens** connate into a peltate synandrium; anthers 4-5, dehiscent transversely. **Female flowers:** sepals and petals 1-3, rarely petals 0. **Staminodes** 0. **Carpel** 1, stigma 3-5-fid. **Drupes** curved with style-scar near base, obovate to rotund in outline, sometimes pubescent; endocarp bony with the condyle a central cavity, around which the seed is curved, perforate ventrally between style-scar a base; often perforate laterally, dorsally ornamented with 3-6 rows of tubercles. **Seeds** horseshoe-shaped; embryo narrow, terete, embedded in endosperm.

Key to the species of *Cyclea*

1. Leaves not peltate. Male sepals joined only at base 1. *C. atjehensis*
1. Leaves peltate or if subpeltate then male calyx subglobose
 2. Leaves slightly peltate or subpeltate with petiole attached usually, up to ca 2 mm; from the base, upper surface with fine reticulation, young stems hispid. Inflorescences born on old leafless stems. Male calyx subglobose 2. *C. polypetalala*
 2. Leaves clearly peltate with petiole attached usually, 4-25 mm, from the base, upper surface with lax reticulation. Inflorescences often axillary. Male calyx campanulate or sepals joined only at base
 3. Stems, petioles and lamina pubescent. Male sepals joined only at base 3. *C. varians*
 3. Stems, petioles and lamina hispid (apart from any puberulence). Male calyx campanulate
 4. Male flowers with calyx pilose and corolla copular 4. *C. barbata**
 4. Male flowers with calyx glabrous and petals free 5. *C. laxiflora*

**Cyclea barbata* Miers (Smitinand and Larsen, 1991)

- Vernacular: Krung badan (กรุงบาดาล) (South-eastern); krung khamao (กรุงเขมา) (Peninsular)], Lun huan teng (China), Cincao (Malayu), Camcao (Jawa Tengah), Patha/Patakkilannu (India)
- Distribution: Widespread in Northern, Southeastern, Southwestern and Peninsular regions. Assam, Burma (type), S. Vietnam, Sumatra and Java.
- Ecology: In evergreen, bamboo and deciduous forests, also in scrub, sometimes on limestone; up to 1100 m alt. Flowering February-November; fruiting November-February (-April).

Slender climber, up to ca 5 m; **roots** thick and long; **stems** herbaceous or woody, hispid when young, later glabrescent. **Leaves** ovate, deltoid-ovate broadly ovate, 6-17.5 by 4-12.5 cm, base slightly emarginate, truncate or rounded, apex acutely acuminate obtuse with a finely mucronate acumen, margin often hispid, hispid to subglabrous above, puberulous to tomentose below, occasionally hispid along nerves, papery; petioles hispid, 3-6.5 cm, attached 6-25 mm above the base of lamina. **Male inflorescences** axillary or cauliflorous, 7-12(-30) by 0.5-4(-12) cm, puberulous; flowers in spaced, dense, subcapitate, hairy clusters, born on short lateral branches, 1-4(-7) cm. **Male flowers** with pedicels, 1-2 mm. **Calyx** greenish, campanulate, pilose, 1.5-2 mm long; lobes 4 (or 5), triangular, about half the length of tube. **Corolla** gamopetalous, cupular, margin truncate or obtusely lobed, 0.75 mm long, glabrous. **Synandrium** 1.5-2 mm long, usually exserted. **Female inflorescences**, similar to male but usually broader, 11-19 by 3-7 cm. **Female flowers** sessile in dense heads. **Sepals** 2, rhomboid to obovate, 0.5 mm long and broad, pilose on outer surface. **Petals** 2, ± reniform opposite to and much , broader than sepals, 0.5 by 0.75-1 mm, glabrous. **Carpels** 1 mm long, densely pilose; stigma 3-lobed. **Drupes** white, oblique obovate to rotund in outline, 5-7 by 4-5.5 mm, puberulous; endocarp bearing 4 dorsal rows of papilliform tubercles, and a lateral subentire curved ridge bordering the condyle on each side.

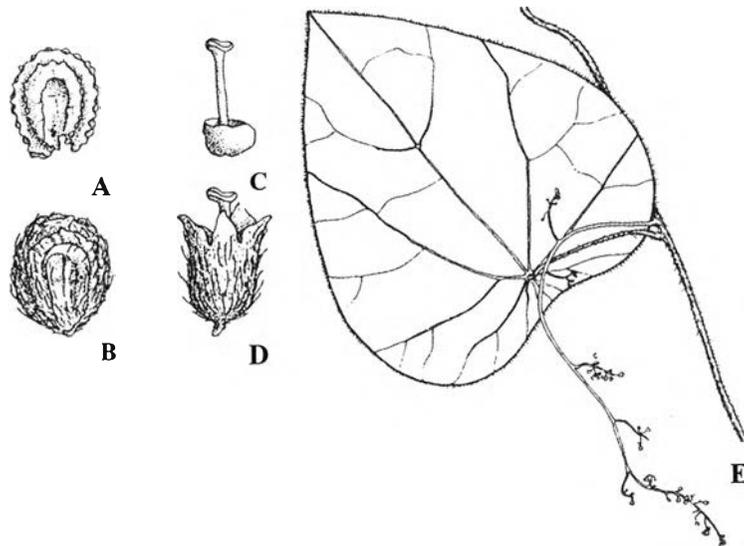


Figure 1. *Cyclea barbata* (Wall.) Miers : A. endocarp, x4; B. drup, x4; C. corolla with synandrium, x8; D. male flower; E. leaf with male inflorescence, x 2/3 (Smitinand and Larsen, 1991)

2. Chemical constituents

Cyclea barbata contain many kinds of bisbenzylisoquinoline (BBI) alkaloids, which are derived biogenetically via pheno-oxidative coupling of two units of benzyltetrahydroisoquinoline alkaloids (Guha *et al.*, 1979). Bisbenzylisoquinoline alkaloids exert hypotensive, antimicrobial, analgesic, muscle relaxant, antitumor, antiarrhythmic, platelet-aggregation inhibitor, histamine antagonist, anti-flagellated protozoa.

The chemical constituents of *Cyclea barbata* (Wall.) Miers are summarized in Table 1.

Table 1. The chemical constituents of *Cyclea barbata* (Wall.) Miers.

Plant part	Category	Chemical constituent	Reference
Root	Alkaloid	Tetrandrine, Isotetrandrine, Limacine, Berbamine, Nomoaromoline	Yupraphat <i>et al.</i> , 1974
Root	Alkaloid	Fragchinoline, Isofragchinoline, Thalrugosine, Thaligine	Goepel <i>et al.</i> , 1974
Root	Alkaloid	(+)-Tetrandrine, (-)-Limceine, (+)-Thalrugosine, (+)-Homoaromoline, (-)-Cycleapeltine	Lin <i>et al.</i> , 1993
Root	Alkaloid	(-)-Tetrandine-2'- β -N-oxide, (-)-2'-Norlimacine, (+)-Cycleabarbantine, (+)-Berbamine, (-)-Repandine, (+)-Cycleanorine, (+)-Daphrandrine, (-)-Curine, (+)-Cocclaurine, (-)-N-methylcocclaurine	Guinaudeau <i>et al.</i> , 1993

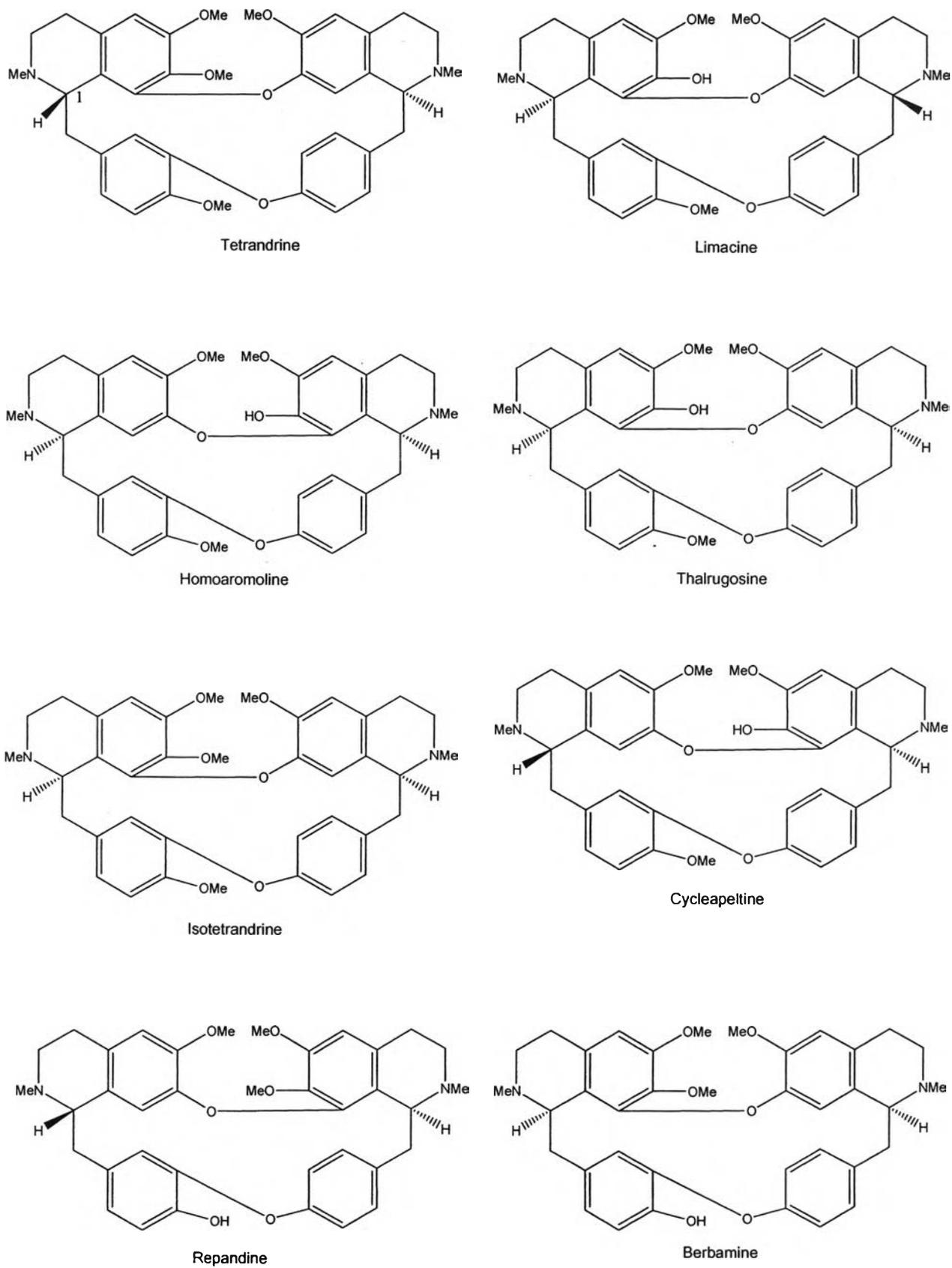


Figure 2. The chemical constituents of *Cyclea barbata* (Wall.) Miers.

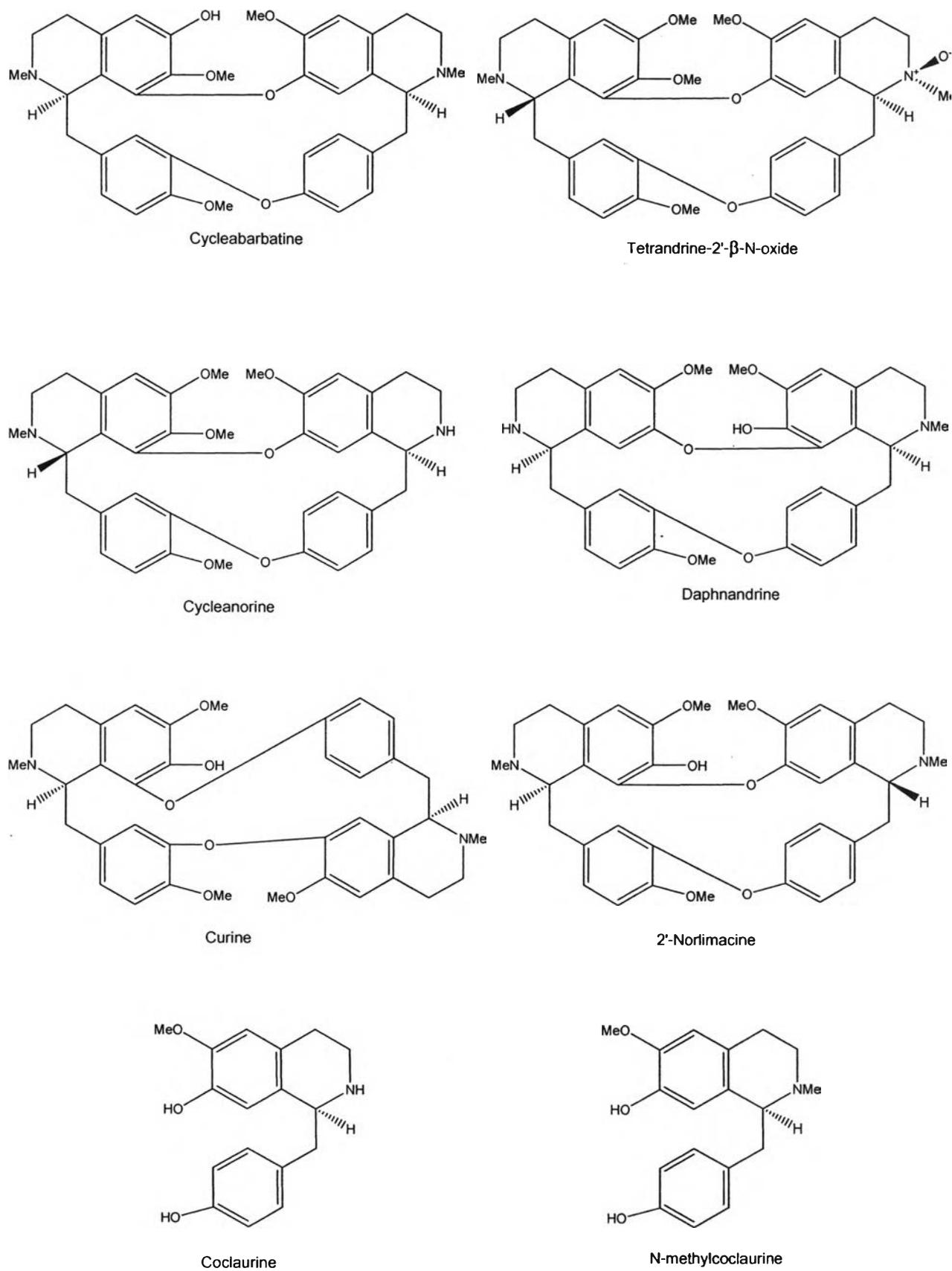


Figure 2. The chemical constituents of *Cyclea barbata* Miers (Continued).

Tetrandrine

Structure and Chemical Properties

Tetrandrine is a bisbenzylisoquinoline alkaloid present in *Cyclea barbata*, *Cyclea bermanni*, *Cyclea peltata*, *Cocculus sarmentosus*, *Stephania tetrandrae*, *Stephania hernandifolia*, *Triclisia subcordata* and *Isopyrum thalictroides*. Its chemical name is 6,6',7,12-tetramethoxy-2,2'-dimethyl-berbaman. It has a formula of $C_{38}H_{42}O_6N_2$ and a molecular weight of 622.760 (Southon and Backingham, 1989). The structure of tetrandrine is shown in Figure 3.

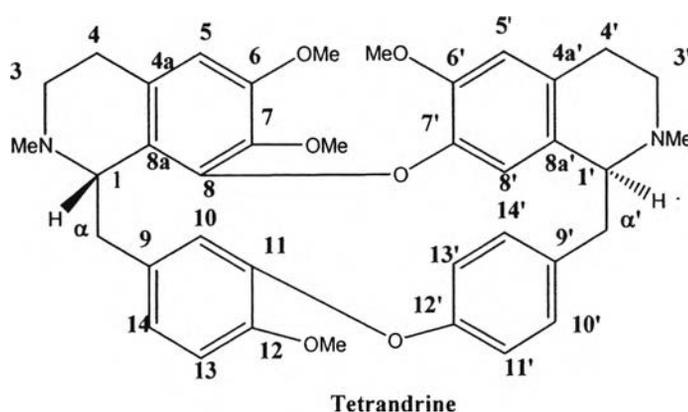


Figure 3. Chemical structure of (+)-tetrandrine.

Tetrandrine is a white crystal, having a bitter taste. It is soluble in methanol, ethanol, chloroform, dichloromethane, and water (Southon and Backingham, 1989).

For physicochemical properties, tetrandrine shows its ultraviolet spectrum (UV) with maximum absorption bands at 214 and 283 nm. For mass spectrum, tetrandrine shows the molecular ion at m/z 622 (M^+ , Calcd. for $C_{38}H_{42}O_6N_2$) and also other main peaks at m/z 607, 591, 485, 431, 395, 311, 198 (base peak), 175, 174, 160. Its proton nuclear magnetic resonance (1H -NMR) spectrum and carbon nuclear magnetic resonance (^{13}C -NMR) spectrum of tetrandrine were shown in Table 2 (Thevand *et al.*, 2004).

Table 2. ^1H -NMR spectral* and ^{13}C -NMR spectral** of (+)-tetrandrine.

Proton	Chemical shift (ppm)	Carbon	Chemical shift (ppm)
H-1	3.75(d, overlapped signal)	C-1	61.55
NMe	2.33(s)	NMe	42.43
H-3 α	2.91(m)	C-3	44.23
H-3 β	3.52(m)	C-4	22.13
H-4 α	2.41(m)	C-4a	128.27
H-4 β	2.93(m)	C-5	105.84
H-5	6.30(s board)	C-6	151.52
6-OMe	3.75(s)	C-7	137.98
7-OMe	3.19(s)	C-8	148.60
H-8	-	C-8a	123.08
H- α (b)	2.52(d, 13.59)	C- α	42.09
H- α (a)	2.70 (t, partly overlapped signal)	C-9	135.11
H-10	6.55(d, 1.64)	C-10	116.32
H-11	-	C-11	149.49
12-OMe	3.93(s)	C-12	147.14
H-13	6.86(d, 8.14)	C-13	111.64
H-14	6.87(dd, 8.26, 1.16)	C-14	122.83
H-1'	3.87(dd, 10.98, 5.6)	C-1'	64.07
N'Me	2.62(s)	N'Me	42.78
H-3' β	2.87(m)	C-3'	45.42
H-3' α	3.43(m)	C-4'	25.38
H-4' β	2.72(m)	C-4a'	128.11
H-4' α	2.95(m)	C-5'	112.83
H-5'	6.51(s board)	C-6'	148.73
6'-OMe	3.37(s)	C-7'	143.88
H-8'	5.99(s board)	C-8'	120.30
H- α '	2.80(t, 11.93, 11.54)	C-8a'	128.21
H- α '	3.25(dd, 12.36, 5.6)	C- α '	38.44
H-10'	6.30(dd, 8.27, 2.23)	C-9'	135.38
H-11'	6.80(dd, 8.27, 2.53)	C-10'	132.78
H-13'	7.14(dd, 8.13, 2.53)	C-11'	122.11
H-14'	7.34(dd, 2.18, 8.18)	C-12'	153.84
		C-13'	122.05
		C-14'	130.27
		6-OMe	55.93
		7-OMe	60.43
		12-OMe	56.27
		6'-OMe	55.98

*Recorded in CDCl₃. Chemical shift values are reported as δ values (ppm) from TMS at 500 MHz; signal multiplicity and coupling constants (Hz) are shown in parentheses.

** Recorded in CDCl₃. Chemical shift values are reported as δ values (ppm) at 125.8 MHz.

Detection and Determination of Tetrandrine

Tetrandrine has been identified by thin-layer chromatography method (Svendsen and Verpoorte, 1983), and determined for its content by high performance thin-layer chromatography densitometer (Blatter *et al.*, 2004), high performance liquid chromatography (Sun *et al.*, 1998; Chou *et al.*, 2002) and capillary electrophoresis (Li *et al.*, 2004).

Thin layer chromatography (TLC) was performed on silica gel 60 F₂₅₄ plate using ethylacetate: isopropanol: 25% ammonia (5:4:1), chloroform: cyclohexane: diethylamine (5:4:1) or toluene: ethylacetate: diethylamine (7:2:1) as solvent systems. Dragendroff's reagent and iron (III) chloride-perchloric acid reagent are used for detection of the spots (Svendsen and Verpoorte, 1983).

High performance thin layer chromatography (HPTLC) densitometer for determination of tetrandrine was performed on HPTLC silica gel 60 F₂₅₄ plate using toluene: ethyl acetate: methanol: 28% ammonia (10:10:5:0.3) as solvent system. Ultraviolet (UV) light 214 nm is used for detection of the spots (Blatter and Reich, 2004).

High performance liquid chromatography (HPLC) was performed by using C₁₈ column, 250x4.6 mm, 5 μ m. The eluent was acetonitrile-0.05 M dihydrogen phosphate buffer (pH 3.0) (27:73, v/v) containing 0.1% diethylamine and 2 mM sodium heptanesulphonate (Sun *et al.*, 1998) or linear gradient elution with dihydrogenphosphate buffer and acetonitrile (Chou *et al.*, 2002).

Capillary electrophoresis was performed by using uncoated fused silica capillary, 57 cm in length (50 cm to detector) x 75 μm I.D. The running buffer was the mixture of 25 ml 200 mM Ammonium Acetate, 10 ml Acetic acid, and 20 ml Acetonitrile, then diluted to 100 ml with methanol solution in 100 ml volumetric flask (Li *et al.*, 2004).

3. Pharmacological activities of (+)-tetrandrine

The pharmacological activities of (+)-tetrandrine were shown in Table 3.

Table 3. The pharmacological activities of (+)-tetrandrine.

Activity	References
Calcium channel antagonist	Fang and Jiang, 1986 Liu <i>et al.</i> , 1992 Takemura <i>et al.</i> , 1996 Kim <i>et al.</i> , 1997
Vasodilator and Anti-hypertension	Fang and Jiang, 1986 Kim <i>et al.</i> , 1997 Kwan and Achike, 2002
Histamine release inhibitor	Nakamura <i>et al.</i> , 1992
Uterine smooth muscle relaxant	Docon <i>et al.</i> , 1992
Cytotoxic and Anti-malarial	Lin <i>et al.</i> , 1993 Angerhofer <i>et al.</i> , 1999
Anti-arrhythmia	Kim <i>et al.</i> , 1997
Inhibit Ca^{2+} release-activated Ca^{2+} channels in vascular endothelia cells	Kwan <i>et al.</i> , 2001
Immunomodulator	Lai, 2002

Activity	References
Induce cell cycle arrest and apoptosis	Chen, 2002 Lee <i>et al.</i> , 2002
Neuroprotective	Koh <i>et al.</i> , 2003
Inhibitor of tumor proliferation	Wang <i>et al.</i> , 2004
Hypnotic	Zhao <i>et al.</i> , 2004