CHAPTER II

HISTORICAL

Distribution

1. Alkaloids and Their Occurrence

Alkaloids should be defined simply as naturally occurring nitrogenous compounds. But, for practical considerations the definition must be more restrictive with some exceptions. In place of this, numerous attemps have been made to provide a system of classification into which most alkaloids can be placed. The most widely accepted classification system, due to Hegnauer (Cordell, 1981), which classified alkaloids into 3 groups, True alkaloids, Protoalkaloids (Biological amines), and Pseudoalkaloids. The major sources of alkaloids in the past has been the flowering plants (Angiosperms). However, there are numerous group of alkaloids isolated from the lower plants, animals, microorganisms, and marine natural products. In the higher plant system of Engler, there are 60 orders, and 34 of these contain alkaloid-bearing species. However, of the over 10000 genera, alkaloids are reported only in 8.7 %, and this distribution is very uneven. The most important alkaloid-containing families are the Liliaceae, Amaryllidaceae, Compositae, Lauraceae,

Ranunculaceae, Menispermaceae, Papaveraceae, Leguminosae, Rutaceae, Loganiaceae, Apocynaceae, Solanaceae, and Rubiaceae (Cordell, 1981). Among those alkaloids, indole alkaloids have played an important part in the development of the chemical and biological sciences.

2. Chemical and Botanical Aspects of Indole Alkaloids

Indole alkaloids are defined as the natural organic products containing either the indole nucleus or an oxidized, reduced, or substituted equivalent of it, e.g. oxindole, pseudoindoxyl, dihydroindole (indoline), indolenine, N-acyl-indole (Hesse, 1978; Leeuwenberg, 1980; Kisakurek, Leeuwenberg and Hesse, 1983). Figure 1 illustrates the foundamental structure of indole alkaloids.

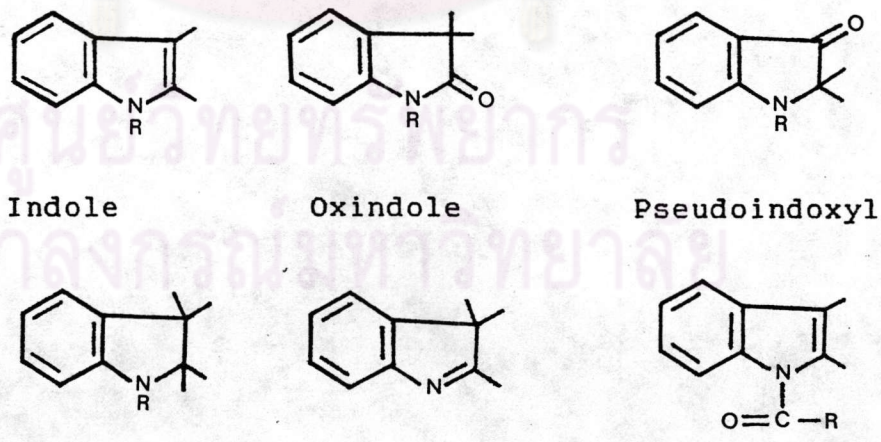
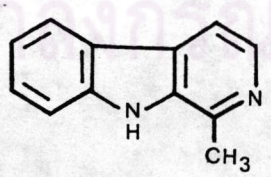
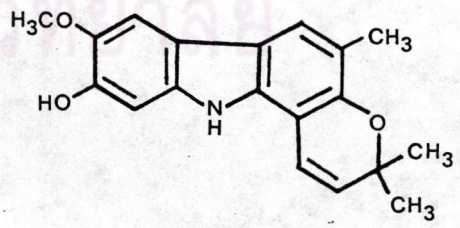


Figure 1 The foundamental structures of indole alkaloids

The number of indole alkaloids of known structure today amounts to approximately 1400. The majority of indole alkaloids have been isolated from the three plant families of the order Gentianales (Contortae), Rubiaceae, Apocynaceae, and Loganiaceae. But they are also known to occur in other plant families, e.g. Alangiaceae, Rutaceae, Elaeocarpaceae, Simaroubaceae, Zygophyllaceae, and Leguminosae. With respect to their structural features, the indole alkaloids can be divided into two main classes. The first class is that of the simple indole alkaloids. They do not present a structural uniformity, having only the indole nucleus or a direct derivative of it as a common feature. Depending upon the constitution of the rest of the molecule, their occurrence is either distributed in many plant families (e.g. harman) or restricted to very few or only one family (e.g. koenigine obtained only from Rutaceae). Because relatively only a small number of these compounds are known, a chemotaxonomic examination of this heterogeneous class of plant bases seems to be irrelevant.



Harman



Koenigine

The second class is the monoterpenoid-derived indole alkaloids. They contain two structure-elements :

tryptamine with the indole nucleus and a C_9- or C_{10}-monoterpene moiety, derived from the iridoid, secologanin. Very probably, because of both of the common components and the biogenetic relationships, the occurrence of the second class of indole alkaloids is more specific and thereby suitable for comparative chemotaxonomic considerations. This class of indole alkaloids occur most frequently in the families Apocynaceae, Loganiaceae, and Rubiaceae (Kisakurek, Leeuwenberg and Hesse, 1983).

3. Botanical Aspects of the Rubiaceae

The Rubiaceae is a large family containing approximately 500 accepted genera and about 6000-7000 species. In 1958, Verdcourt divided members of the Rubiaceae into the following 5 subfamilies (Hemingway and Phillipson, 1980) :-

(1) Subfamily Cinchonoideae

Tribe : Naucleeae, Cinchoneae, Cephalantheae, Virectarieae, Rondeletieae, Catesbaeeae, Mussaendeae (= Isertieae), Heinsieae, Gardenieae, Coffeeae, Retiniphylleae, Altberteae, Vanguerieae, Chiococceae

(2) Subfamily Rubioideae

Tribe : Psychotrieae (= Psathureae), Coussareae, Morindeae, Triainolopideae, Schradereae, Urophylleae, Craterispermeae, Knoxieae, Paederieae, Coccocypseleae, Hamelieae,

Argostemmateae, Ophiorrhizeae, Rubieae, Cruckshanksieae, Anthospermeae, Hedyotideae, Spermacoceae

(3) Subfamily Guettardoideae

Tribe : Guettardeae

(4) Subfamily Hillioideae

Tribe : Hillieae

(5) Subfamily Henriquezieae

Tribe : Henriquezieae

In the view of chemotaxonomy, only 10 out of these 35 tribes contain monoterpenoid-derived indole alkaloids, namely, Naucleeae, Cinchoneae, Cephalantheae, Urophylleae, Rondeletieae, Mussaendeae, Psychotrieae, Ophiorrhizeae, Hamelieae, and Guettardeae. In more restricted view, only 36 out of the 500 genera have been reported to contain monoterpenoid-derived indole alkaloids and about 50 genera have non-monoterpenoid or unidentified alkaloids. The monoterpenoid-derived alkaloidal genera are listed as follows (Ridsdale, 1978a,b; Hemingway and Phillipson, 1980) :-

(1) Tribe Naucleeae

Genus : *Adina, Anthocephalus, Breonadia, Breonia, Gyrostipula, Haldina, Ludekia, Metadina, Khasiaclunea, Myrmeconauclea, Neobreonia, Nauclea, Neonauclea, Pertusadina, Sarcocephalus, Sinoadina*

(2) Tribe Cinchoneae

Genus : *Cinchona, Corynanthe (= Pseudocinchona), Coutarea, Ladenbergia, Pausinystalia, Remijia, Mitragyna, Uncaria*

(3) Tribe Cephalanthus

Genus : *Cephalanthus*

(4) Tribe Rondeletieae

Genus : *Pogonopus*

(5) Tribe Mussaendeae

Genus : *Isertia*

(6) Tribe Psychotrieae

Genus : *Cephaelis, Palicourea*

(7) Tribe Urophylleae

Genus : *Pauridiantha*

(8) Tribe Ophiorrhizeae

Genus : *Ophiorrhiza*

(9) Tribe Hamelieae

Genus : *Hamelia*

(10) Tribe Guettardeae

Genus : *Antirhea, Guettarda, Timonius*

Chemistry of the Alkaloids

1. Alkaloids Isolated from Species of *Mitragyna*

Over the past 30 years *Mitragyna* alkaloids have received considerable attention. The more recent work has not resulted in any unexpected novel structure of alkaloids but rather in the isolation of new isomers together with observation of alkaloidal pattern variations. The alkaloids which have been reported in 10 species of *Mitragyna* are shown as follows :-

1.1 The Asian Species

Mitragyna diversifolia (Wall.ex G.Don) Havil.

leaf : mitraphylline, isomitraphylline, ajmalicine, javaphylline, mitrajavine, angustine (Shellard *et al.*, 1966,1967; Phillipson *et al.*, 1974).

Mitragyna hirsuta Havil.

leaf : mitraphylline, isomitraphylline, mitrajavine, hirsutine, hirsuteine, rhynchophylline, isorhynchophylline (Shellard *et al.*, 1966; Shellard, Tantivatana and Beckett, 1967; Phillipson *et al.*, 1973).

Mitragyna parvifolia (Roxb.) Korth.

leaf : rhynchophylline, isorhynchophylline, rotundifoline, isorotundifoline, akuammigine and its N-oxide, speciophylline (uncarine D) and its N-oxide, pteropodine (uncarine C),

isopteropodine (uncarine E), uncarine F and its N-oxide, ajmalicine, 3-isoajmalicine, mitraphylline, isomitraphylline, hirsutine, dihydrocorynantheine, tetrahydroalstonine, dihydrocorynantheol and its N-oxide, corynantheidol, corynoxeine, rotundifoleine, isorotundifoleine, angustine, rhynchociline, ciliaphylline (Shellard and Phillipson, 1964b; Shellard, Phillipson and Gupta, 1968a,b,1969a; Shellard and Houghton, 1971,1972a,b,c,d,1973a, b,1974a,b; Phillipson *et al.*, 1974; Hemingway *et al.*, 1975; Shellard and Lala, 1977).

stem bark : hirsutine, tetrahydroalstonine, pteropodine, isopteropodine, ciliaphylline, rhynchociline, rhynchophylline, isomitraphylline, uncarine F, speciophylline, ajmalicine, mitraphylline, isorhynchophylline, akuammigine (Shellard, Phillipson and Gupta, 1969b; Shellard and Houghton, 1971,1972b,c,d,1974a; Shellard and Lala, 1977).

root bark : pteropodine, isopteropodine, rhynchophylline, isorhynchophylline, uncarine F, hirsutine, hirsuteine, speciophylline, mitraphylline, isomitraphylline, corynoxeine, corynantheine, dihydrocorynantheine, rhynchociline, ciliaphylline, akuammigine (Shellard and

Houghton, 1971,1972b,c,d,1974a; Shellard and Lala, 1977).

stipule : speciophylline, uncarine F, mitraphylline, isomitraphylline, pteropodine, isopteropodine (Shellard and Houghton, 1972c,d).

growing point :

corynoxeine, pteropodine, isopteropodine, speciophylline, uncarine F, mitraphylline, isomitraphylline (Shellard and Houghton, 1972c,d).

stem xylem :

corynoxeine, mitraphylline, isomitraphylline, rhynchophylline, isorhynchophylline (Shellard and Houghton, 1972c,d).

root xylem :

hirsutine, hirsuteine, rhynchophylline, isorhynchophylline, dihydrocorynantheine, mitraphylline, isomitraphylline, corynoxeine (Shellard and Houghton, 1972c,d).

Mitragyna rotundifolia (Roxb.) O.Ktz.

leaf : rotundifoline, isorotundifoline (mitragynol), corynoxeine, rhynchophylline and its N-oxide, isorhynchophylline and its N-oxide, isocorynoxeine, 3-isoajmalicine, ciliaphylline, specionoxeine, mitraphylline, isomitraphylline (Barger, Dyer and Sargent, 1939; Badger, Cook and Ongley, 1950;

Shellard and Phillipson, 1964a; Houghton and Shellard, 1974; Soontranont, 1979).

stem bark : mitraphylline, isomitraphylline, corynoxeine, isocorynoxeine, rhynchociline, rhynchophylline, isorhynchophylline (Houghton and Shellard, 1974).

root : mitraphylline, isomitraphylline, corynoxeine, isocorynoxeine, isorhynchophylline, rhynchophylline, rhynchociline, ciliaphylline (Houghton and Shellard, 1974).

Mitragyna speciosa (Korth.) Havil.

leaf : mitragynine, speciofoline, rhynchophylline, isorhynchophylline, isocorynantheidine, corynantheidine, rotundifoline (stipulatine), isorotundifoline, isomitraphylline, mitraphylline, speciogynine, speciophylline, paynantheine, isopaynantheine, ajmalicine, 3-isoajmalicine, speciociliatine, mitrafoline, isomitrafoline, isospeciofoline, corynoxine, corynoxine B, corynoxeine, isospecionoxeine, specionoxeine, mitraciliatine, mitrajavine, 3-dehydromitragynine, javaphylline, akuammigine (Field, 1921; Ing and Raison,1939; Beckett, Lee and Tackie, 1963; Hendrickson and Sims, 1963; Beckett *et al.*, 1965,1966a,b,c,d; Beckett, Shellard and Tackie, 1965; Trager *et al.*, 1968; Hemingway *et al.*, 1975;

Shellard, Houghton and Resha, 1978b,c; Houghton and Said, 1986).

stem bark : rotundifoline, ciliaphylline, rhynchophylline, isorhynchophylline, isospecionoxeine, rhynchociline, specionoxeine, mitraphylline, isomitraphylline, mitragynine oxindole A, mitragynine oxindole B, mitraciliatine, speciogynine, javaphylline, speciociliatine (Hendrickson and Sims, 1963; Shellard, Houghton and Resha, 1978b,c).

root bark : rhynchociline, ciliaphylline, rhynchophylline, isorhynchophylline, isospecionoxeine, specionoxeine, speciogynine, mitraciliatine, speciociliatine, mitrajavine (Shellard, Houghton and Resha, 1978c).

Mitragyna tubulosa (Arn.) Havil.

leaf : mitraciliatine, ciliaphylline and its N-oxide, rhynchociline, hirsutine, isorhynchophylline, rhynchophylline, isorotundifoline, rotundifoline, mitraphylline, isomitraphylline (Rungsiyakul, 1973; Shellard and Rungsiyakul, 1973).

stem bark, stem xylem : mitraciliatine, hirsutine, isorhynchophylline, rhynchophylline, rhynchociline, ciliaphylline (Rungsiyakul, 1973).

root bark : rhynchociline, hirsutine, rhynchophylline, isorhynchophylline, ciliaphylline (Rungsiyakul, 1973).

root xylem : isorhynchophylline (Rungsiyakul, 1973).

1.2 The West African Species

Mitragyna inermis (Willd.) O.Ktz.

leaf : uncarine F, rhynchophylline and its N-oxide, isorhynchophylline and its N-oxide, rhynchociline, ciliaphylline, rotundifoline, isorotundifoline, speciophylline, speciogynine, mitraciliatine (Badger, Cook and Ongley, 1950; Beckett and Tackie, 1963a; Shellard and Sarpong, 1969,1970,1971a; Shellard, Phillipson and Sarpong, 1971).

stem bark, root bark : ciliaphylline, uncarine F, isorhynchophylline, rhynchophylline, isorotundifoline, rotundifoline, rhynchociline, speciophylline (Shellard and Sarpong, 1970).

Mitragyna ledermannii (K.Krause) Ridsd., comb. nov.

leaf : rhynchociline, ciliaphylline, rhynchophylline, isorhynchophylline, isorotundifoline, rotundifoline, mitraciliatine (Badger, Cook and Ongley, 1950; Beckett, Shellard and Tackie, 1963b; Beckett and Tackie, 1963a;

Shellard and Sarpong, 1970).

stem bark : rhynchophylline, isorhynchophylline, rotundifoline, isorotundifoline, rhynchociline, ciliaphylline (Badger, Cook and Ongley, 1950; Shellard and Sarpong, 1970).

root bark : rhynchophylline, isorhynchophylline, rotundifoline, isorotundifoline, rhynchociline, ciliaphylline (Shellard and Sarpong, 1970).

Mitragyna stipulosa (DC.) O.Ktz.

leaf : rotundifoline, isorotundifoline, hirsutine, mitraphylline, corynoxeine, rhynchophylline, isorhynchophylline (Beckett, Shellard and Tackie, 1963a; Beckett and Tackie, 1963a,b; Shellard and Sarpong, 1970; Houghton *et al.*, 1976).

stipule, flower : rotundifoline, isorotundifoline, rhynchophylline, isorhynchophylline (Beckett, Shellard and Tackie, 1963a).

stem bark : rhynchophylline, isorhynchophylline, rotundifoline,isorotundifoline, mitraphylline (Beckett, Shellard and Tackie, 1963a; Shellard and Sarpong, 1970).

root bark : rhynchophylline, isorhynchophylline, rotundifoline, isorotundifoline (Shellard and Sarpong, 1970).

1.3 The East African Species

Mitragyna rubrostipulata (K.Schum.) Havil.

leaf : mitraphylline, isomitraphylline, rotundifoline and its N-oxide, isorotundifoline, rhynchophylline and its N-oxide, isorhynchophylline (Badger, Cook and Ongley, 1950; Shellard and Lala, 1978).

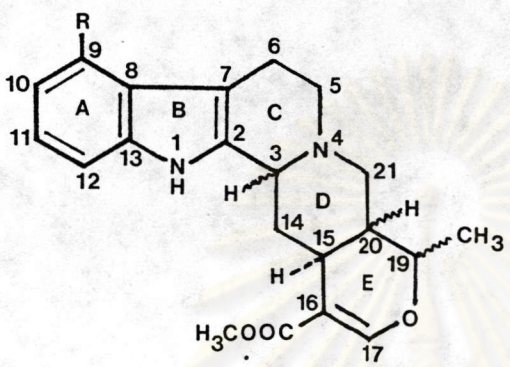
stem bark : mitraphylline, isomitraphylline, rotundifoline and its N-oxide, isorotundifoline, rhynchophylline and its N-oxide, Isorhynchophylline (Badger, Cook and Ongley, 1950; Seaton *et al.*, 1960; Hendrickson and Sims, 1963; Shellard and Lala, 1978).

root bark : mitraphylline, isomitraphylline, hirsutine, hirsuteine, rhynchophylline and its N-oxide, isorhynchophylline, isorotundifoline, rotundifoline and its N-oxide (Shellard and Lala, 1978).

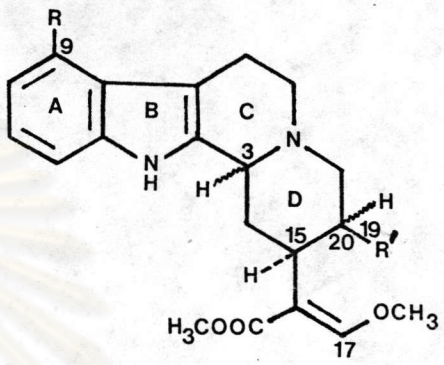
2. Basic Structure of Heteroyohimbine and Oxindole Alkaloids

Nearly all of alkaloids reported to be present in the genus *Mitragyna* are of heteroyohimbine-type (Corynanthe-type) and the corresponding oxindoles. The alkaloids represent variants of these structures, differing in their stereochemistry and/or aromatic substitution. There are 2 types of both heteroyohimbines

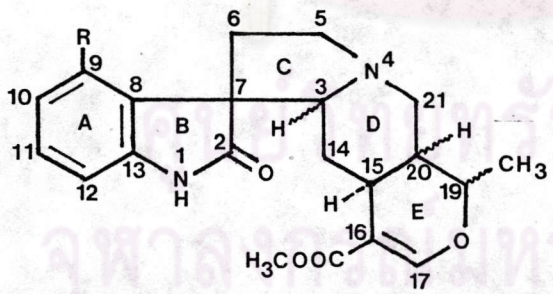
and oxindoles depending upon the nature of ring E, i.e. closed E ring (pentacyclic alkaloids) and open E ring (E *seco*, or tetracyclic alkaloids) as shown in Figure 2.



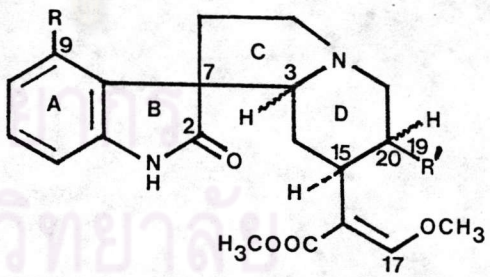
Pentacyclic heteroyohimbines



Tetracyclic heteroyohimbines



Pentacyclic oxindoles



Tetracyclic oxindoles

Figure 2 Basic structures of heteroyohimbine and oxindole alkaloids

3. Configurations of Heteroyohimbine and Oxindole Alkaloids

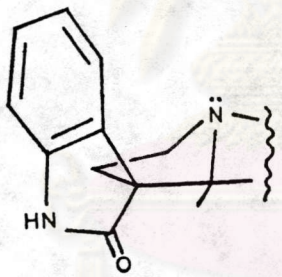
Heteroyohimbine and the corresponding oxindole alkaloids have asymmetric centers at C(3), C(15), and C(20), though all those isolated so far have C(15)-Hα. Thus four possible diastereomers can exist which are designated as *normal*, *pseudo*, *allo*, and *epiallo*. The closed E ring alkaloids also have an asymmetric center at C(19). In all known *Mitragyna* alkaloids the C(19)-CH_3 is α. The open E ring alkaloids may show geometric isomerization because of the double bond between C(16) and C(17), though in all known alkaloids the C(17)-H is *cis* to the C(16) carbomethoxy group.

In addition, the oxindole alkaloids have an asymmetric center at C(7), i.e. ring C attached to ring B at the spiro carbon, C(7), in two different ways. One of which the lactam carbonyl lies below the plane of C/D rings being termed the A series and those of which the lactam carbonyl lies above the plane of C/D rings being termed the B series. Thus eight isomers of oxindoles are possible.

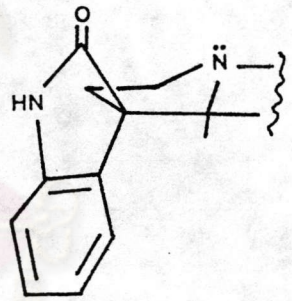
The four isomers of heteroyohimbines and eight of oxindoles are summarized with their configurations in Table 1.

Table 1 Configuration terminology for heteroyohimbine and oxindole alkaloids

Configuration	C(3)-H	C(15)-H	C(20)-H	C(7) series of oxindole
Normal	α	α	β	A or B
Pseudo	β	α	β	A or B
Allo	α	α	α	A or B
Epiallo	β	α	α	A or B

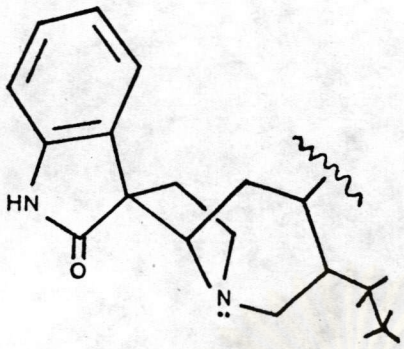


A series oxindoles

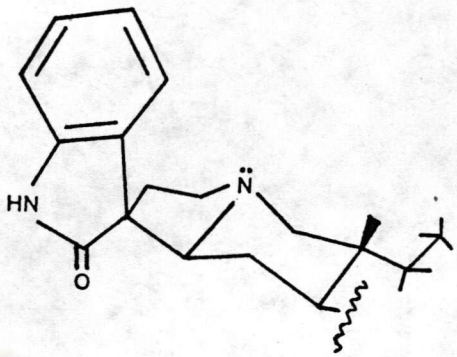


B series oxindoles

Furthermore, in both types of oxindole alkaloids, the lone pair of electrons on N(4) may either be on the same side of C(7) as the lactam carbonyl group or on the opposite side, the former are known as *syn* and the latter as *anti* alkaloids (Shamma *et al.*, 1967).



syn-oxindoles



anti-oxindoles

In *Mitragyna* alkaloids, substitution (R) in the aromatic ring have been found, but only at C(9), and is either a hydroxy or methoxy group. In addition, in the open E ring alkaloids R'may be either an ethyl or a vinyl group.

The configurations of known heteroyohimbine and oxindole alkaloids isolated from the genus *Mitragyna* are summarized as follows :-

3.1 Pentacyclic heteroyohimbine alkaloids

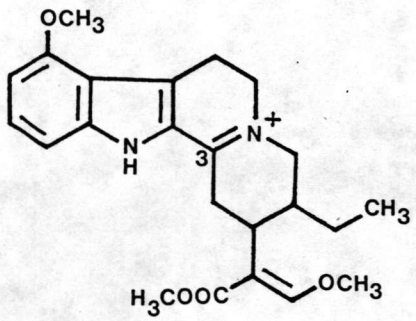
Alkaloid	C(9)-R	Configuration
ajmalicine	H	*normal*
3-isoajmalicine	H	*pseudo*
akuammigine (*)	H	*epiallo*
mitrajavine	OCH_3	*pseudo*
tetrahydroalstonine	H	*allo*

Note : (*) = with its N-oxide

3.2 Tetracyclic heteroyohimbine alkaloids

Alkaloids	C(9)-R	R'	Configuration
corynantheidine	H	CH_2-CH_3	*allo*
corynantheidol	H	CH_2-CH_3	*allo*
corynantheine	H	CH=CH_2	*normal*
dihydrocorynantheine	H	CH_2-CH_3	*normal*
dihydrocorynantheol (*)	H	CH_2-CH_3	*normal*
hirsuteine	H	CH=CH_2	*pseudo*
hirsutine	H	CH_2-CH_3	*pseudo*
isocorynantheidine	H	CH_2-CH_3	*epiallo*
isopaynantheine	OCH_3	CH=CH_2	*pseudo*
mitraciliatine	OCH_3	CH_2-CH_3	*pseudo*
mitragynine	OCH_3	CH_2-CH_3	*allo*
paynantheine	OCH_3	CH=CH_2	*normal*
speciociliatine	OCH_3	CH_2-CH_3	*epiallo*
speciogynine	OCH_3	CH_2-CH_3	*normal*

Recently, a totally new tetracyclic heteroyohimbine alkaloid, 3-dehydromitragynine, has been isolated from the fresh leaves of *Mitragyna speciosa* (Korth.) Havil. This is the first alkaloid of this type to be isolated from natural sources (Houghton and Said, 1986). Presumably 3-dehydromitragynine is unstable and does not withstand the drying process which is why it had not previously been isolated.



3-Dehydromitragynine

3.3 Pentacyclic Oxindole Alkaloids

Alkaloid	C(9)-R	Configuration	C(7) series
isomitraphylline	H	*normal*	A
isopteropodine	H	*allo*	A
javaphylline	OCH_3	*normal*	A
mitraphylline	H	*normal*	B
pteropodine	H	*allo*	B
speciophylline (*)	H	*epiallo*	A
uncarine F (*)	H	*epiallo*	B

3.4 Tetracyclic Oxindole Alkaloids

Alkaloid	C(9)-R	R'	Configuration	C(7) series
ciliaphylline (*)	OCH_3	CH_2-CH_3	*normal*	B
corynoxeine	H	CH=CH_2	*normal*	B
corynoxine	H	CH_2-CH_3	*allo*	A
corynoxine B	H	CH_2-CH_3	*allo*	B
isocorynoxeine	H	CH=CH_2	*normal*	A
isomitrafoline	OH	CH_2-CH_3	*allo*	B
isorhynchophylline (*)	H	CH_2-CH_3	*normal*	A
isorotundifoleine	OH	CH=CH_2	*normal*	B
isorotundifoline	OH	CH_2-CH_3	*normal*	B
isospeciofoline	OH	CH_2-CH_3	*epiallo*	A
isospecionoxeine	OCH_3	CH=CH_2	*normal*	A
mitrafoline	OH	CH_2-CH_3	*allo*	A
mitragynine oxindole A	OCH_3	CH_2-CH_3	*allo*	A
mitragynine oxindole B	OCH_3	CH_2-CH_3	*allo*	B
rhynchociline	OCH_3	CH_2-CH_3	*normal*	A
rhynchophylline (*)	H	CH_2-CH_3	*normal*	B
rotundifoleine	OH	CH=CH_2	*normal*	A
rotundifoline (*)	OH	CH_2-CH_3	*normal*	A
speciofoline	OH	CH_2-CH_3	*epiallo*	B
specionoxeine	OCH_3	CH=CH_2	*normal*	B

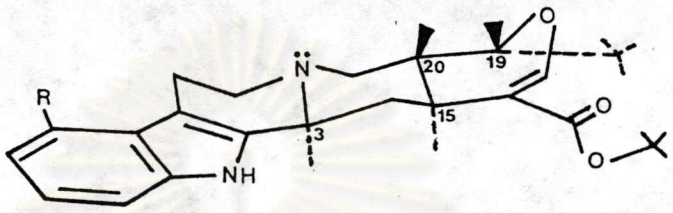
4. Preferred Conformations

The preferred conformation of heteroyohimbine and oxindole alkaloids were established by Trager, Lee and Beckett (1967) and Trager et al. (1968). The conformational analysis is based on the assumption that : (i) an estimation of the nonbonded interaction in piperidine ring (ring D) can be approximated by analogy with the nonbonded interaction arising in the correspondingly substituted cyclohexane, (ii) in all the conformations examined, ring C is assumed to be in the half chair conformation by analogy with cyclohexene and only the chair forms of ring D are considered by analogy with cyclohexane, and (iii) an estimation of the magnitude of the nonbonded interactions of each conformation (ignoring any possible solvation effects) was made with the aid of Dreiding models.

There is only one stable conformation from different ring D chair conformations (three for each of the four configurations of the heteroyohimbines and two for each of the eight configurations of the oxindoles). The preferred conformations of alkaloids isolated from the genus *Mitragyna* are shown as follows :-

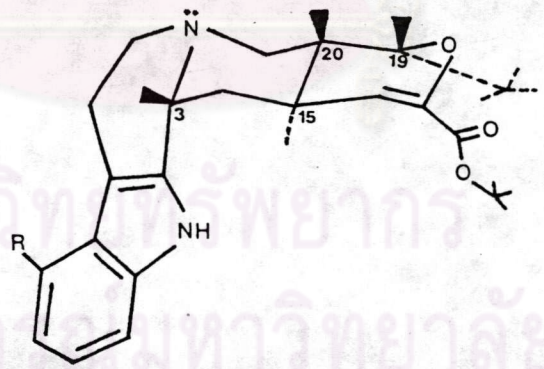
4.1 Pentacyclic Heteroyohimbine Alkaloids

4.1.1 *Normal* configuration



R = H : Ajmalicine

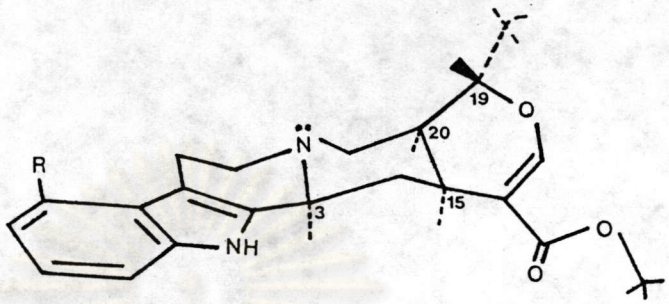
4.1.2 *Pseudo* configuration



R = H : 3-Isoajmalicine

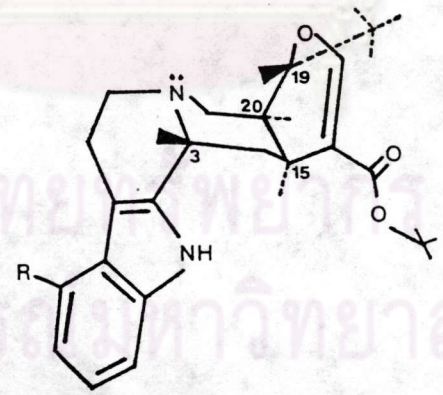
R = OCH_3 : Mitrajavine

4.1.3 *Allo* configuration



R = H : Tetrahydroalstonine

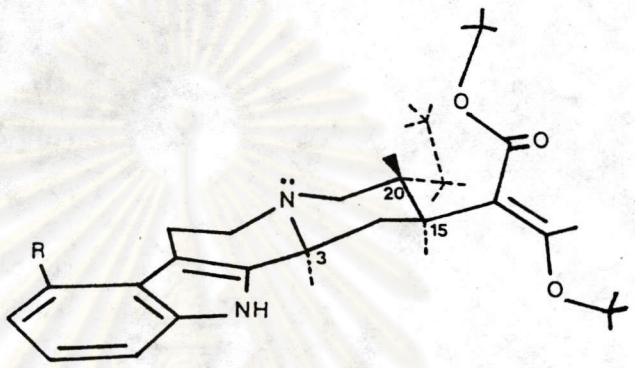
4.1.4 *Epiallo* configuration



R = H : Akuammigine

4.2 Tetracyclic Heteroyohimbine Alkaloids

4.2.1 *Normal* configuration

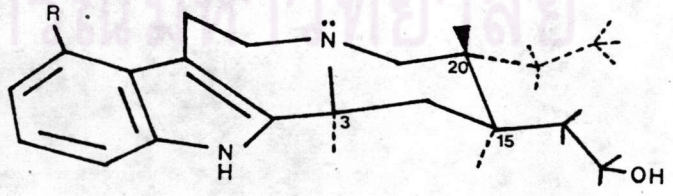


R = H : Dihydrocorynantheine

R = OCH_3 : Speciogynine

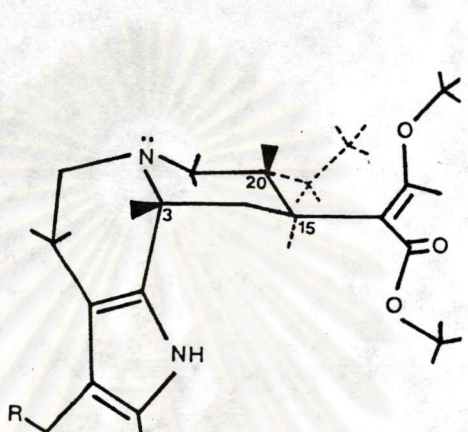
R = OCH_3, C(20)-Et = vinyl : Paynantheine

R = H, C(20)-Et = vinyl : Corynantheine



R = H : Dihydrocorynantheol

4.2.2 *Pseudo* configuration



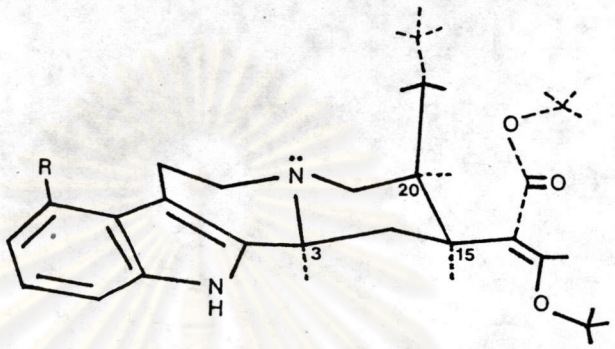
R = OCH_3 : Mitraciliatine

R = OCH_3, C(20)-Et = vinyl : Isopaynantheine

R = H : Hirsutine

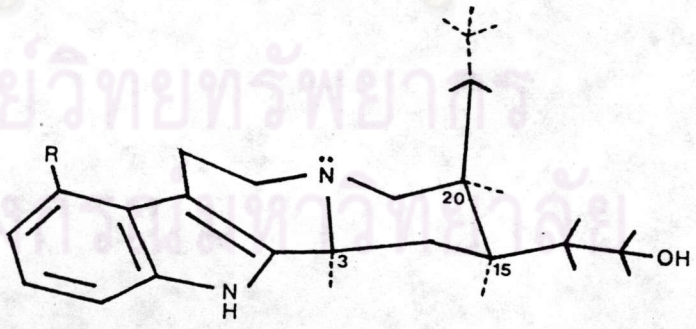
R = H, C(20)-Et = vinyl : Hirsuteine

4.2.3 *Allo* configuration



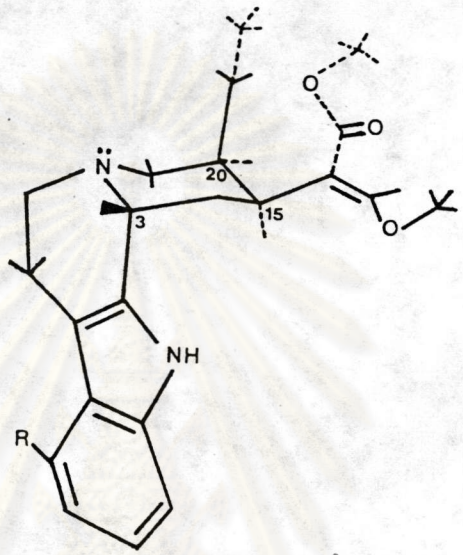
R = OCH_3 : Mitragynine

R = H : Corynantheidine



R = H : Corynantheidol

4.2.4 *Epiallo* configuration

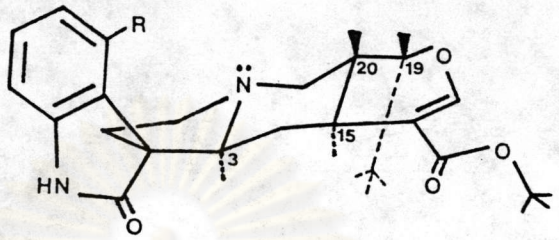


R = H : Isocorynantheidine

R = OCH_3 : Speciociliatine

4.3 Pentacyclic Oxindole Alkaloids

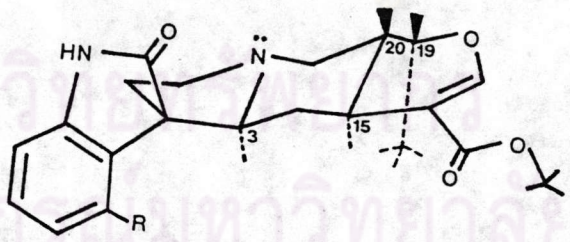
4.3.1 *Normal* configuration : A series



R = H : Isomitraphylline

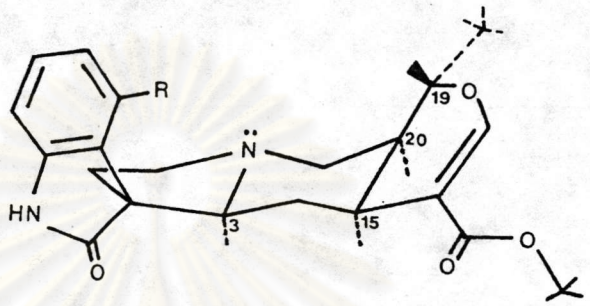
R = OCH_3 : Javaphylline

4.3.2 *Normal* configuration : B series



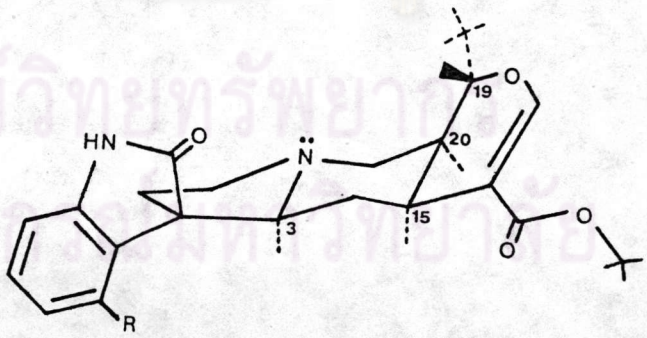
R = H : Mitraphylline

4.3.3 Allo configuration : A series



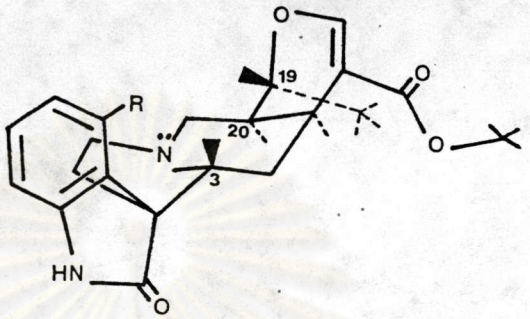
R = H : Isopteropodine

4.3.4 Allo configuration : B series



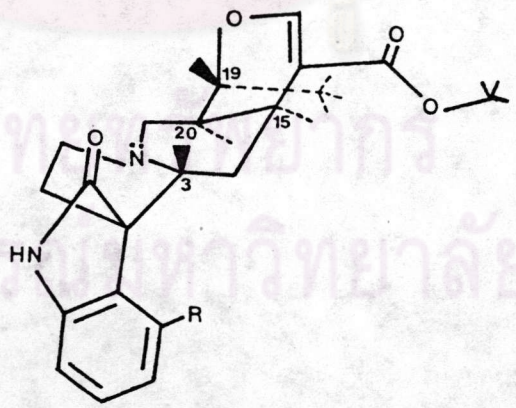
R = H : Pteropodine

4.3.5 *Epiallo* configuration : A series



R = H : Speciophylline

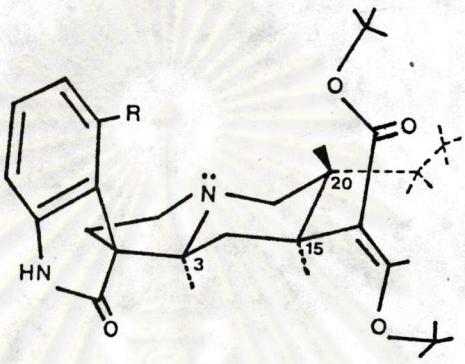
4.3.6 *Epiallo* configuration : B series



R = H : Uncarine F

4.4 Tetracyclic Oxindole Alkaloids

4.4.1 *Normal* configuration : A series



R = OH : Rotundifoline

R = OH, C(20)-Et = vinyl : Rotundifoleine

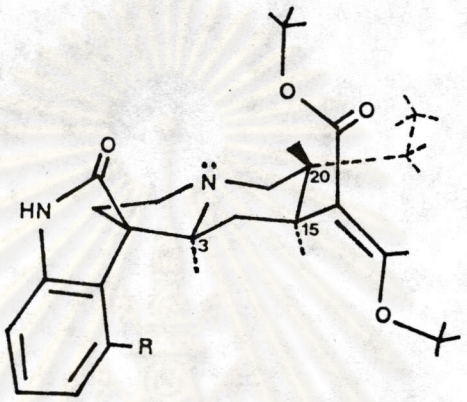
R = H, C(20)-Et = vinyl : Isocorynoxeine

R = OCH_3, C(20)-Et = vinyl : Isospecionoxeine

R = OCH_3 : Rhynchociline

R = H : Isorhynchophylline

4.4.2 *Normal* configuration : B series



R = OH : Isorotundifoline

R = OH, C(20)-Et = vinyl : Isorotundifoleine

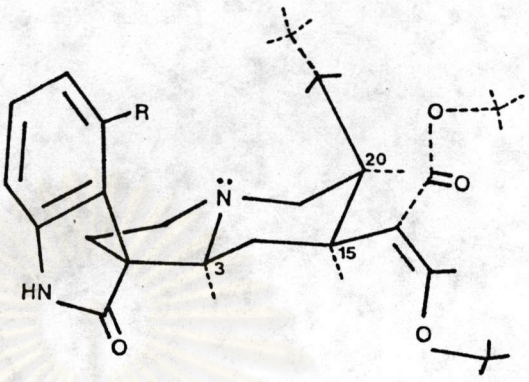
R = H, C(20)-Et = vinyl : Corynoxeine

R = OCH_3, C(20)-Et = vinyl : Specionoxeine

R = OCH_3 : Ciliaphylline

R = H : Rhynchophylline

4.4.3 *Allo* configuration : A series

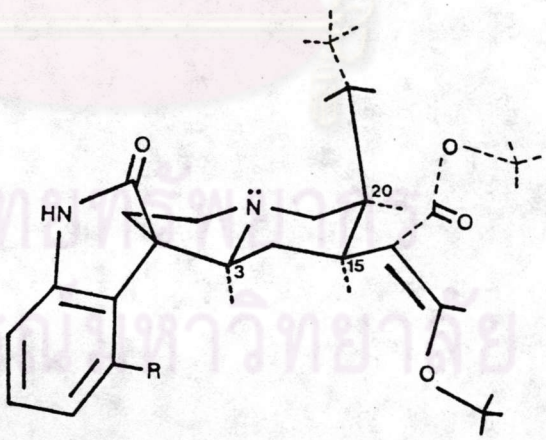


R = OH : Mitrafoline

R = OCH_3 : Mitragynine oxindole A

R = H : Corynoxine

4.4.4 *Allo* configuration : B series

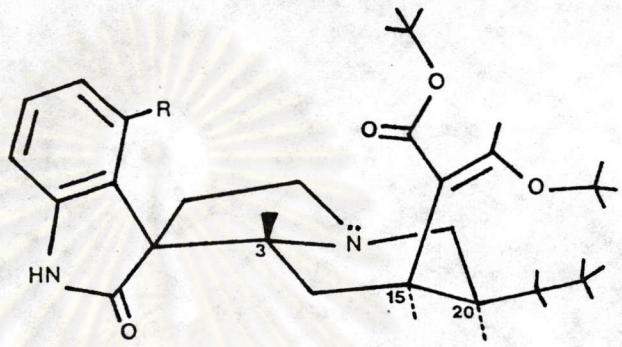


R = OH : Isomitrafoline

R = OCH_3 : Mitragynine oxindole B

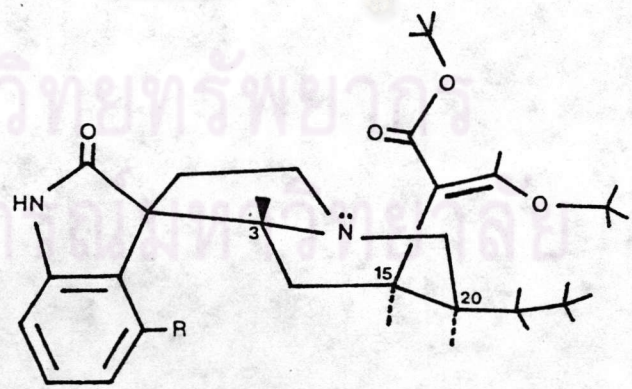
R = H : Corynoxine B

4.4.5 *Epiallo* configuration : A series



R = OH : Isospeciofoline

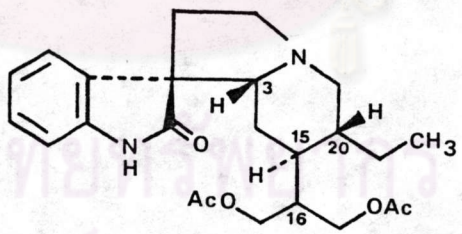
4.4.6 *Epiallo* configuration : B series



R = OH : Speciofoline

4.5 *Pseudo* Configuration of Oxindoles

The *pseudo* oxindole alkaloids were formerly, by conformational studies, concluded to be unstable to exist because of the steric interference between the oxindole unit and the underside of ring D (Trager *et al.*, 1968). Not until 1976 that Brown and Platt has clearly shown by synthesis of the diacetates $C_{24}H_{32}N_2O_5$ from condensation of dihydrosecologanin aglycone and 2-oxytryptamine that alkaloids of this conformation can exist and are reasonably stable (Brown and Platt, 1976). However, no *pseudo* oxindole alkaloid has yet been isolated from natural sources.



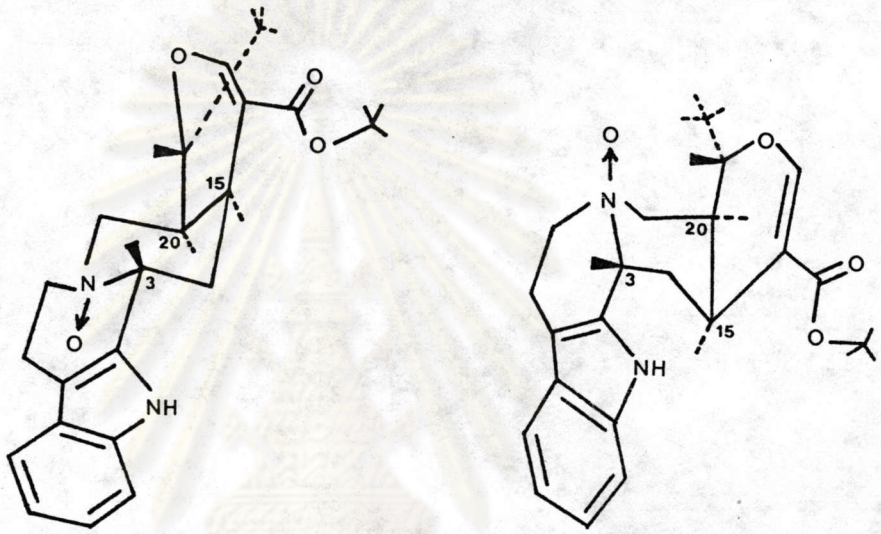
The diacetates $C_{24}H_{32}N_2O_5$

5. Alkaloid N-oxides from *Mitragyna* Species

The earlier examinations of the leaves of *Mitragyna rotundifolia* (Roxb.) O.Ktz. by Shellard and Phillipson (1964a) indicated the presence of a 'base-line' alkaloid but were unable to determine its structure. The isolation of a similar substance from the leaves of *Mitragyna inermis* (Willd.) O.Ktz. enabled Shellard, Phillipson and Sarpong (1971) to characterize it as isorhynchophylline N-oxide. Further study shown it to be anti-isorhynchophylline N-oxide (Phillipson, Rungsiyakul and Shellard, 1973). They also isolated and characterized rhynchophylline N-oxide from the same plant. Shellard and Rungsiyakul (1973) isolated and characterized ciliaphylline N-oxide from the leaves of *Mitragyna tubulosa* Havil. The precence of N-oxides of four other alkaloids, viz. akuammigine, speciophylline, uncarine F, and dihydrocorynantheol were reported from the leaves of *Mitragyna parvifolia* (Roxb.) Korth. (Shellard and Houghton, 1974a). In 1978, Shellard and Lala reported the presence of rhynchophylline N-oxide and *anti*-rotundifoline N-oxide from the leaves, stem bark, and root bark of *Mitragyna rubrostipulata* (K.Schum.) Havil.

Most of the isolated N-oxides from several species of *Mitragyna* are of tetracyclic oxindole alkaloids. They are of rhynchophylline, isorhynchophylline, rotundifoline, and ciliaphylline. The other N-oxides isolated are of

akuammigine, dihydrocorynantheol, speciophylline, and uncarine F.



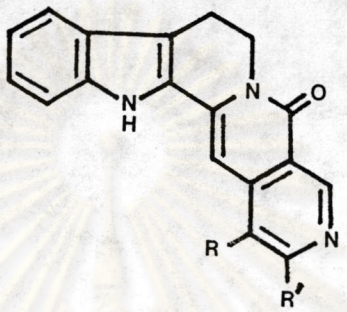
cis *trans*

Akuammigine N-oxides

6. Other Indole Alkaloid from *Mitragyna* Species

The only other indole alkaloid reported to be present in species of *Mitragyna* is angustine, and its occurrence in this genus appears to be restricted. It has been detected in only *Mitragyna javanica* Koord et Val. and *M. parvifolia* (Roxb.) Korth. Angustine belongs to the pyridino-indolo-quinolizidinone group. Other alkaloids in this group, angustoline and angustidine, which have not, as yet, been found in *Mitragyna* species were reported to be present in *Uncaria* and *Strychnos* species together with angustine (Phillipson *et al.*, 1974).

Angustine and angustoline are possibly derived from a combination of tryptamine unit and a secologanin unit closely related to gentianine. Alternatively angustine might possibly be an artefact which formed by reaction of vincoside- or isovincoside-lactam with ammonia. Angustidine might be formed by the loss of C(21) from the secologanin portion of a corynanthe precursor (Au, Cheung and Sternhell, 1973). Recently, Phillipson, Hemingway and Ridsdale (1982) proposed that pyridino-indolo-quinolizidinone alkaloids might arise possibly by ring elaboration of strictosidine (isovincoside), a nitrogenous glycoside intermediate obtained from condensation of tryptamine and secologanin.



Angustine	:	R =	$CH=CH_2$	R' =	H
(Angustoline	:	R =	$CH(OH)CH_3$	R' =	H)
(Angustidine	:	R =	H	R' =	CH_3)

7. The *Mitragyna* and *Uncaria* Alkaloids

The two genera of the subtribe Mitragyninae, *Mitragyna* and *Uncaria*, considered to bear close relationship in both botanical characters and alkaloidal contents. They are similar in yielding heteroyohimbines and oxindoles along with less significant amounts of pyridino-indolo-quinolizidinones (Saxton, 1965,1968,1973; Bindra, 1973; Szantay et al., 1986). Recently, the same type of the triterpenoid, two new ursolic acids and two new quinovic acid glycosides, were isolated from the root of *Uncaria florida* Vidal. and the leaves of *Mitragyna hirsuta* Havil., respectively (Likhitwitayawuid, 1986). This is the further evidence which indicates the homogenity of the plants in the subtribe Mitragyninae, supporting the taxonomic revision of Ridsdale in 1978. The genus *uncaria* now has 34 recognized species, contrasting with the 120 species names appearing in the Index Kewensis (Phillipson, Hemingway and Ridsdale, 1978; Ridsdale, 1978a). However, there are some differences in alkaloidal content between the two genera as listed in Table 2, e.g. no C(9)-methoxy substituted alkaloids were found in *Uncaria* while it was common in *Mitragyna*, and there were fewer species with C(9)-hydroxy alkaloids than in *Mitragyna*. *Uncaria* yielded pentacyclic heteroyohimbines and the corresponding oxindoles with C(19)-methyl either α or β configuration while C(19)-methyl β configuration has not as yet, been found in *Mitragyna* species. Some *Uncaria*

species exhibit considerable infraspecific variation in alkaloidal content and there is a wide variety of structural types than in *Mitragyna* (Phillipson, Hemingway and Ridsdale, 1978,1982).

Additionally, a totally new pentacyclic heteroyohimbine alkaloid with C(14)-hydroxy substitution has been isolated from the leaves of *Uncaria attenuata* Korth. and characterized as 14-β-hydroxy-3-isorauniticine which is the first alkaloid being reported as having the substitution at the position other than in the aromatic ring (Ponglux *et al.*, 1980). However, this compound was later revised from 14-β-hydroxy-3-isorauniticine to 14-α-hydroxyrauniticine by NMR spectral analysis and partial synthesis (Yamanaka *et al.*, 1986).

Table 2 Comparison of alkaloids isolated from the *Mitragyna* and *Uncaria*

	Mitragyna 10 species	*Uncaria* 34 species
(1) Number of species containing alkaloids	10	21
(2) Heteroyohimbines and corresponding oxindoles	common	common
(3) C(9)-OCH_3 substitution	common	not found
(4) C(9)-OH substitution	common	infrequent
(5) C(14)-OH substitution	not found	rare
(6) C(19)-CH_3 β-configuration	not found	infrequent
(7) Gambirtannines (benzenoid E ring pentacyclics)	not found	rare
(8) Roxburghines (2 tryptamine units + secologanin)	not found	rare
(9) Yohimbines and the corresponding oxindoles	not found	rare

8. Chemical Transformation of *Mitragyna* Alkaloids

8.1 *In Vitro* Studies

8.1.1 Isomerization of heteroyohimbine alkaloids

Weisenborn and Diassi (1956) and Wenkert and Roychaudhuri (1956) demonstrated that the heteroyohimbine alkaloids may be isomerized at C(3) by an oxidation-reduction reaction using mercuric acetate as an oxidising agent, and zinc and hydrochloric acid as a reducing agent. The reaction is shown in Figure 3.

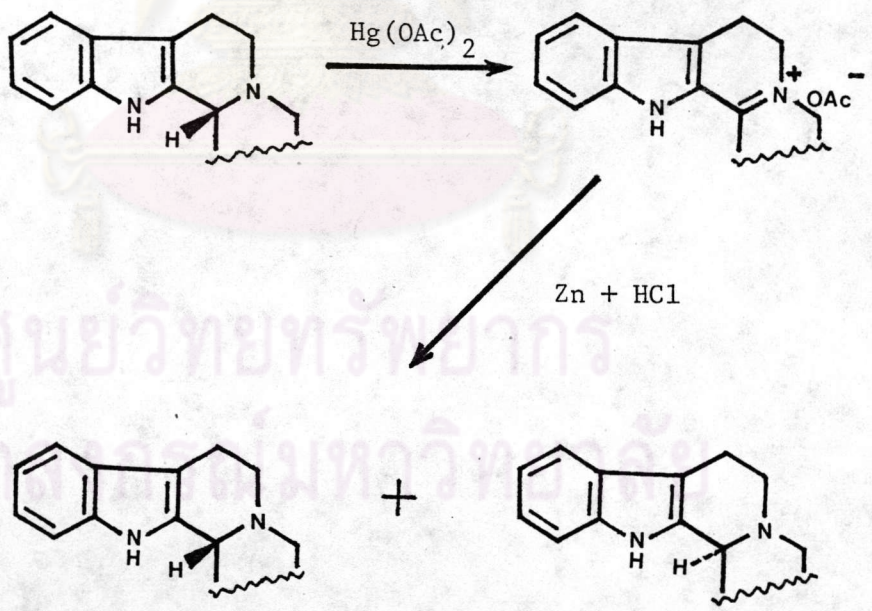


Figure 3 Oxidation-reduction reaction of heteroyohimbine alkaloids

Selected examples of the isomerization of heteroyohimbine alkaloids using this method are given as follows :-

(1) ajmalicine (*normal*) ⟶ 3-isoajmalicine (*pseudo*)

(Wenkert and Roychaudhuri, 1956)

(2) mitrajavine (*pseudo*) ⟶ isomitrajavine (*normal*)

(Shellard and Sarpong, 1971c)

(3) mitragynine (*allo*) ⟶ speciociliatine (*epiallo*)

(Trager, Phillipson and Beckett, 1968)

(4) paynantheine (*normal*) ⟶ isopaynantheine (*pseudo*)

(Beckett, Dwuma-Badu and Haddock, 1969; Shellard, Houghton and Resha, 1978c)

(5) corynantheidine (*allo*) ⟶ isocorynantheidine (*epiallo*)

(Trager, Phillipson and Beckett, 1968; Beckett, Dwuma-Badu and Haddock, 1969; Shellard, Houghton and Resha, 1978c)

(6) speciogynine (*normal*) ⟶ mitraciliatine (*pseudo*)

(Trager, Phillipson and Beckett, 1968)

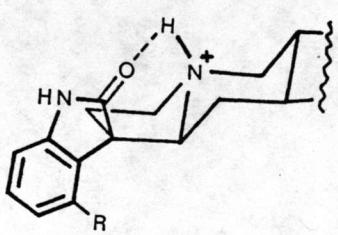
(7) hirsutine (*pseudo*) ⟶ dihydrocorynantheine (*normal*)

(Trager, Phillipson and Beckett, 1968)

8.1.2 Isomerization of oxidole alkaloids

Oxindole alkaloids may be isomerized at C(3) and/or C(7) centers by refluxing in pyridine (basic isomerization) or acetic acid (acidic isomerization). The isomerization involves scission and reformation at the C(3)-C(7) bond and hence possible inversion of one or both of the centers (Seaton *et al.*, 1960; Trager *et al.*, 1968). Starting with a given oxindole isomer, four stereomeric compounds should result upon isomerization, i.e. two (A and B) with C(3)-Hα and two (A and B) with C(3)-Hβ. The isomerization of a *normal* oxindole results in only two products, which are the *normal* A and B isomers, hence supported the former conclusion by Trager *et al.* (1968) that *pseudo* oxindole alkaloids are too unstable to exist.

The medium of isomerization determines the predominant isomer in the final mixture (at equilibrium). In acidic isomerization, the B oxindoles predominant due to the stabilization of the conjugated base by formation of an intramolecular hydrogen bond between the protonated lone pair electron of N(4) and the lactam carbonyl group.



(Hydrogen-bond formation of the B oxindoles)

This stabilization is not possible with the A oxindoles as the lactam carbonyl is below the plane of the C/D rings.

In basic isomerization, the A oxindoles predominate and this is thought to be destabilization due to the electrostatic repulsion between the lone pair electron of N(4) and lactam carbonyl group in the free base form of the B isomers (Finch and Taylor, 1962a,b; Trager *et al.*, 1968).

Selected examples of the isomerization of oxindole alkaloids from several equilibration studies are shown as follows :-

(1) mitraphylline or isomitraphylline —Py→ 80 % isomitraphylline + 20 % mitraphylline

(Seaton *et al.*, 1960)

mitraphylline or isomitraphylline —Ac→ 20 % isomitraphylline + 80 % mitraphylline

(Seaton *et al.*, 1960; Beckett *et al.*, 1966a)

(2) rhynchophylline
or
isorhynchophylline

$\xrightarrow{Py}$ 80 % isorhynchophylline + 20 % rhynchophylline

$\xrightarrow{Ac}$ 30 % isorhynchophylline + 70 % rhynchophylline

(Trager *et al.*, 1968)

(3) specionoxeine
or
isospecionoxeine

$\xrightarrow{Py}$ 35 % isospecionoxeine + 65 % specionoxeine

$\xrightarrow{Ac}$ 50 % isospecionoxeine + 50 % specionoxeine

(Trager *et al.*, 1968)

(4) rotundifoline
or
isorotundifoline

$\xrightarrow{Py}$ 10 % isorotundifoline + 90 % rotundifoline

$\xrightarrow{Ac}$ 40 % isorotundifoline + 60 % rotundifoline

(Trager *et al.*, 1968)

(5) corynoxine
or
corynoxine B

$\xrightarrow{Py}$ 80 % corynoxine + 20 % corynoxine B

$\xrightarrow{Ac}$ 20 % corynoxine + 80 % corynoxine B

(Trager *et al.*, 1968)

(6) mitrafoline
or
speciofoline

$\xrightarrow{Py}$ 40 % mitrafoline + 40 % speciofoline + 10 % isomitrafoline + 10 % isospeciofoline

$\xrightarrow{Ac}$ 50 % mitrafoline + 25 % speciofoline + 15 % isomitrafoline + 10 % isospeciofoline

(Hemingway *et al.*, 1975)

(7) pteropodine $\xrightarrow{Py}$ 60 % pteropodine
+ 40 % isopteropodine
+ traces of speciophylline and uncarine F

$\xrightarrow{Ac}$ 40 % pteropodine
+ 10 % isopteropodine
+ 40 % speciophylline
+ 10 % uncarine F

(Beecham *et al.*, 1968)

(8) isopteropodine $\xrightarrow{Py}$ 95 % isopteropodine
+ 5% pteropodine

$\xrightarrow{Ac}$ 10 % isopteropodine
+ 40 % pteropodine
+ 40 % speciophylline
+ 10 % uncarine F

(Beecham, *et al.*, 1968).

(9) speciophylline $\xrightarrow{Py}$ 10 % speciophylline
+ 30 % pteropodine
+ 30 % isopteropodine
+ 30 % uncarine F

$\xrightarrow{Ac}$ 40 % speciophylline
+ 40 % pteropodine
+ 10 % isopteropodine
+ 10 % uncarine F

(Beecham *et al.*, 1968)

(10) uncarine F $\xrightarrow{Py}$ 30 % uncarine F
+ 30 % pteropodine
+ 30 % isopteropodine
+ 10 % speciophylline

$\xrightarrow{Ac}$ 10 % uncarine F
+ 40 % pteropodine
+ 10 % isopteropodine
+ 40 % speciophylline

(Beecham *et al.*, 1968)

Note : Py = basic isomerization by refluxing with pyridine

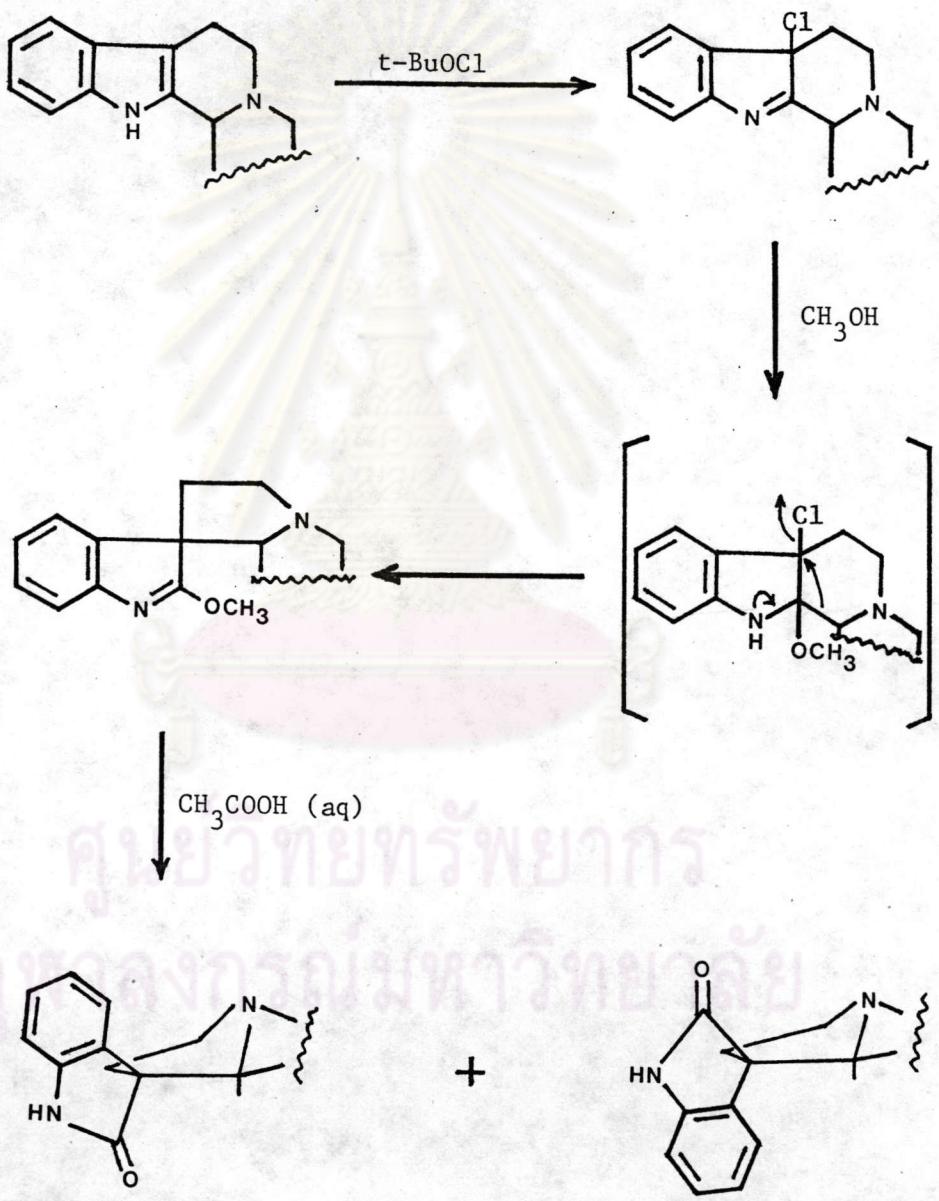
Ac = acidic isomerization by refluxing with acetic acid, mercuric acetate, or hydrochloric acid

8.1.3 Conversion of heteroyohimbine to oxindole alkaloids

On the basis of biogenetic considerations, attemps were made to convert indole alkaloids to the corresponding oxindoles. The conversion involves the oxidation of the indole to 3-substituted indolenine, followed by rearrangement to corresponding oxindoles.

Finch and Taylor (1962a,b) and Shavel Jr. and Zinnes (1962) demonstrated that yohimbine and heteroyohimbine alkaloids are transformed into an epimeric mixture of C(7) chloroindolenines by action of tertiary butyl hypochlorite. Methanolysis of the chloroindolenines yield the imido esters which on hydrolysis in refluxing aqueous acetic acid give two isomeric oxindoles, A and B, as shown in Figure 4.

Figure 4 Conversion of heteroyohimbine to the corresponding oxindole alkaloids through the chloroindolenine



Oxindole A

Oxindole B

The selected examples of the conversion of heteroyohimbine alkaloids to their corresponding oxindoles using this method are summarized as follows :-

(1) ajmalicine (*normal*) ⟶ mitraphylline (*normal* B) + isomitraphylline (*normal* A)
(Finch and Taylor, 1962a; Shavel Jr. and Zinnes, 1962)

(2) corynantheidine (*allo*) ⟶ corynoxine (*allo* A) + corynoxine B (*allo* B)
(Beckett, Dwuma-Badu and Haddock, 1969)

(3) mitraciliatine (*pseudo*) ⟶ rhynchociline (*normal* A) + ciliaphylline (*normal* B)
(Shellard and Sarpong, 1971b)

(4) mitragynine (*allo*) ⟶ mitragynine oxindole A (*allo* A) + mitragynine oxindole B (*allo* B)
(Beckett, Dwuma-Badu and Haddock, 1969; Shellard, Houghton and Resha, 1978a)

(5) mitrajavine (*pseudo*) ⟶ javaphylline (*normal* A) + isojavaphylline (*normal* B)
(Shellard and Sarpong, 1971b)

(6) speciociliatine (*epiallo*) ⟶ speciociliatine oxindole A (*epiallo* A) + speciociliatine oxindole B (*epiallo* B)

(Shellard, Houghton and Resha, 1978a)

Furthermore, Zinnes and Shavel Jr. (1966) converted the carbocyclic E ring indole alkaloid, *pseudo*-yohimbine into *normal* oxindoles. Therefore there is possibility that the *pseudo* heteroyohimbine alkaloids in some *Mitragyna* species could also be transformed to the *normal* oxindoles (Shellard and Sarpong, 1971b).

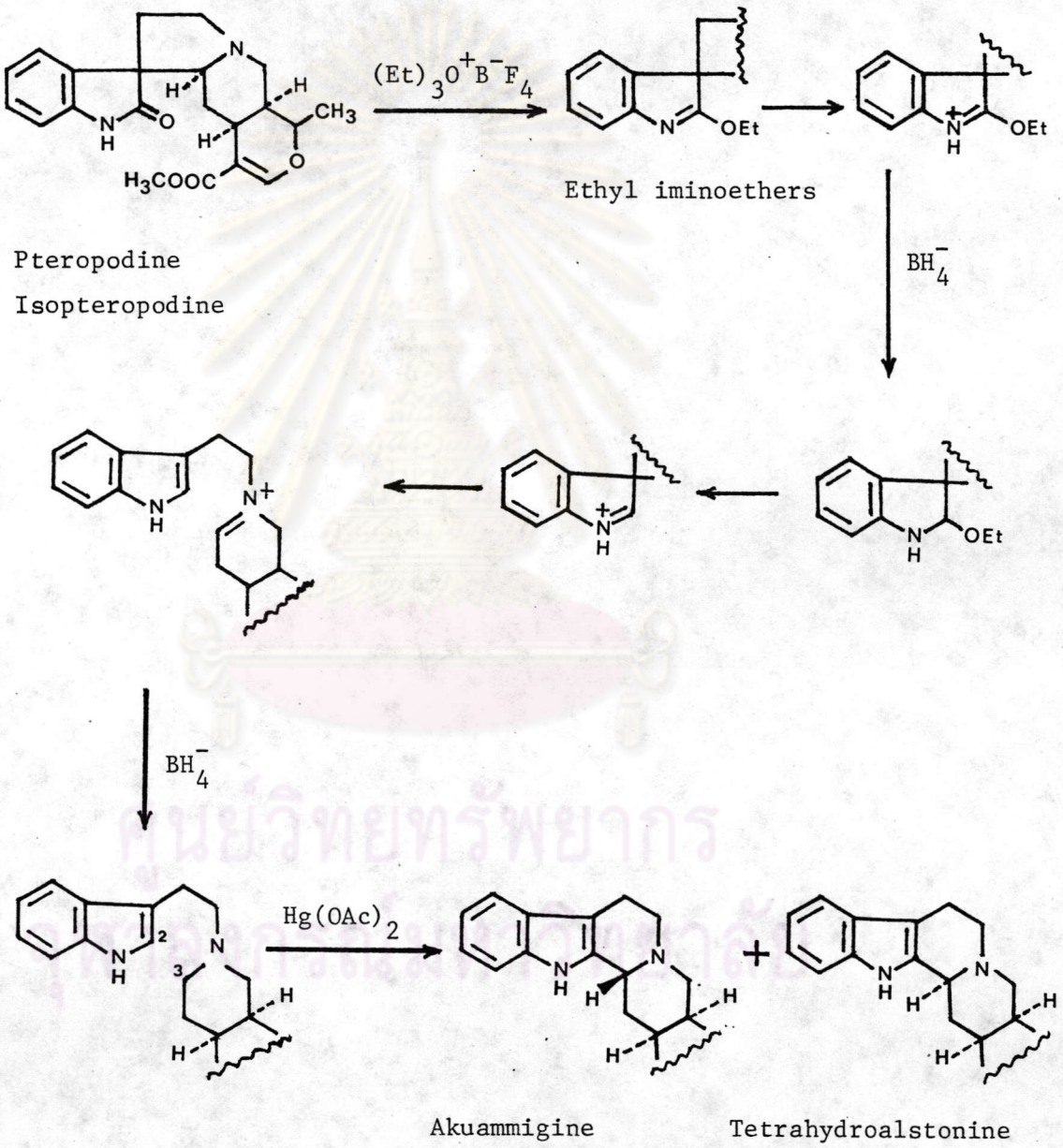
Another method of converting heteroyohimbines to oxindoles is the use of lead tetraacetate to give an acetoxyindolenine which on refluxing with methanol containing acetic acid give the oxindoles. By using this method the indole tetrahydroalstonine has been converted to its corresponding oxindoles, uncarine F, pteropodine, isopteropodine, and speciophylline (Hart, Johns and Lamberton, 1967).

8.1.4 Conversion of oxindole to heteroyohimbine alkaloids

Aimi *et al.* (1972,1973) demonstrated that the chemical conversion of natural oxindoles into the correspoding indoles has been made by way of a sequence of reactions which include formation of iminoethers of

natural oxindoles with Meerwein's reagent, reduction of the iminoethers to 2,3-*seco*-indoles and oxidative cyclization of 2,3-*seco*-indoles by mercuric acetate in diluted acetic acid to the desired natural indoles. Sodium borohydride in acetic acid was found to be a specific reagent for the reduction of oxindole-iminoethers to 2,3-*seco*-indoles which were the key intermediates in these transformation. By using this method pteropodine and isopteropodine have been converted to their corresponding heteroyohimbine alkaloids tetrahydroalstonine and its isomer, akuammigine as shown in Figure 5. They have also similarly converted isorhynchophylline into hirsutine and dihydrocorynantheine.

Figure 5 Conversion of oxindoles to the corresponding heteroyohimbine alkaloids.



8.2 *In Vivo* Studies

By feeding the young plant (less than one year old) of *Mitragyna parvifolia* (Roxb.) Korth. with unlabelled alkaloids, Shellard and Houghton (1972a) observed the presence of isomitraphylline and mitraphylline in the leaves both after feeding ajmalicine and 3-isoajmalicine into the stem xylem. The result showed that conversion of *normal* and *pseudo* heteroyohimbines into *normal* oxindoles occur *in vivo*, which the interconversion of the heteroyohimbines did not seem to take place. Their work also revealed that the specificity of the enzyme systems in this plant might be for the C(9) unsubstituted alkaloids since no oxindole alkaloid was detected after feeding with C(9)- methoxy unsubstituted *normal* and *pseudo* heteroyohimbine alkaloids, isomitrajavine and mitrajavine. The enzyme systems were also specific for the closed E ring alkaloids since there was no evidence for the presence of any oxindole alkaloid correspoding to the open E ring heteroyohimbines when they were fed to the plant.

Shellard and Houghton (1973b) confirmed their work in 1972 by using the ^{14}C-alkaloids in the young plant of *Mitragyna parvifolia* (Roxb.) Korth. They fed ^{14}C-tetrahydroalstonine and ^{14}C-akuammigine separately into the stem xylem of the plant and labelled pteropodine, isopteropodine, speciophylline, and uncarine F were detected in both cases.

Shellard and Houghton (1974a) further examined the distribution of alkaloids in young plants of this species. They fed ^{14}C-alkaloids into the stem bark, stem xylem, and root bark (just below the hypocotylar region). The evidence pointed to the possibility of this plant possessing two biogenetic sites, the leaves and the roots, with mitraphylline being the alkaloid which links the two sites. They fed pteropodine and mitraphylline separately through the stem xylem and pteropodine was shown to be converted to mitraphylline and then mitraphylline was converted via corynoxeine to rhynchophylline. Evidence obtained by feeding ^{14}C-rhynchophylline and ^{14}C-hirsutine separately into the root phloem showed that rhynchophylline, a *normal* oxindole alkaloids, is converted to the *pseudo* indole hirsutine as well as conversion of hirsutine to rhynchophylline. This clearly showed that neither *normal* oxindole alkaloid nor *pseudo* heteroyohimbine alkaloid could be converted to the corresponding *normal* heteroyohimbine alkaloid since no dihydrocorynantheine, the *normal* heteroyohimbine alkaloid corresponding to rhynchophylline could be detected.

Moreover, Shellard and Houghton (1972a) found that when mitraphylline was fed into the stem xylem, rhynchophylline was found in the leaves. The use of ^{14}C-mitraphylline (Shellard and Houghton, 1974a) showed that it was not necessarily the rhynchophylline from the main stem xylem but that the mitraphylline itself was

converted via corynoxeine to rhynchophylline. When ^{14}C-rhynchophylline was used-incorporated in large amounts of rhynchophylline- both mitraphylline and rhynchophylline were detected in the leaves together with ^{14}C-labelled *allo* and *epiallo* closed E ring oxindoles. It would appear that the interconversion involving :

rhynchophylline $\rightleftharpoons$ mitraphylline $\rightleftharpoons$ pteropodine

occurs normally in the leaves but since only small quantities are present in the transportation stream, only the final product-the *allo* and *epiallo* oxindole alkaloids are found.

8.3 N-oxidation of Heteroyohimbine and Oxindole Alkaloids

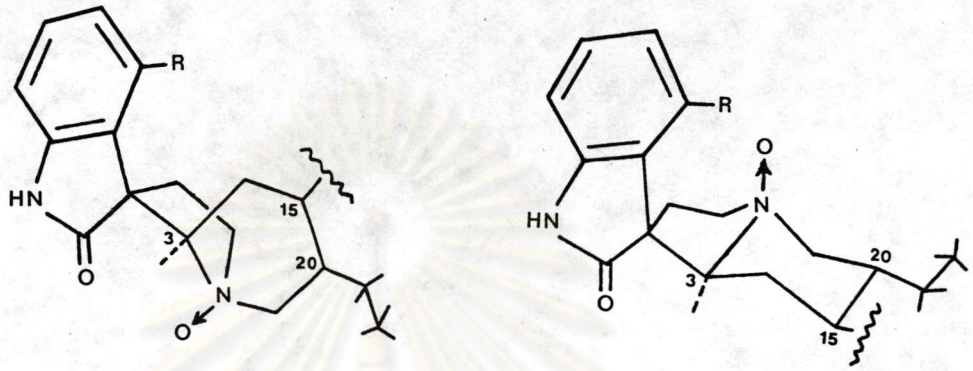
A classical method to prepare tertiary amine N-oxides is the use of hydrogen peroxide. Another more effective method using m-chloroperbenzoic acid with non-aqueous solvents was improved by Craig and Purushothaman (1970). Shellard, Phillipson and Sarpong (1971) prepared the N-oxides of rhynchophylline and isorhynchophylline by treating an ethanolic solution of the alkaloid with hydrogen peroxide solution overnight at room temperature, followed by heating on a boiling water bath for 30 minutes.

Merlini, Nasini and Phillipson (1972) synthesized N-oxides of pentacyclic unsubstituted heteroyohimbine alkaloids by treatment with m-chloroperbenzoic acid.

Those synthesized were 4-*R*-akuammigine, 4-*S*-akuammigine, 4-*R*-tetrahydroalstonine, 4-*R*-3-isoajmalicine, and 4-*R*-ajmalicine N-oxides.

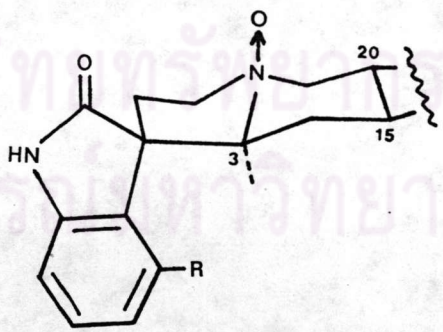
Phillipson, Rungsiyakul and Shellard (1973) have used the both methods in preparing N-oxides of isorhynchophylline (A series), rhynchophylline (B series), rhynchociline (A series), and ciliaphylline (B series) in order to characterize naturally occurring ciliaphylline N-oxide isolated from *Mitragyna tubulosa* Havil. and found that whereas the B series oxindole alkaloids give only one N-oxide, the A series give two-an *anti* and a *syn* N-oxides. Thus isorhynchophylline and rhynchociline appear to form two N-oxides while rhynchophylline and ciliaphylline form one N-oxide. Furthermore Shellard, Houghton and Lala (1977) also used these two methods to prepare N-oxides of rotundifoline (A series) and isorotundifoline (B series) and obtained two rotundifoline N-oxides (*anti*- and *syn*-) and one isorotundifoline N-oxide. The preferred configurations of oxindole alkaloid N-oxides are shown in Figure 6.

Figure 6 The N-oxides of oxindole A and B series



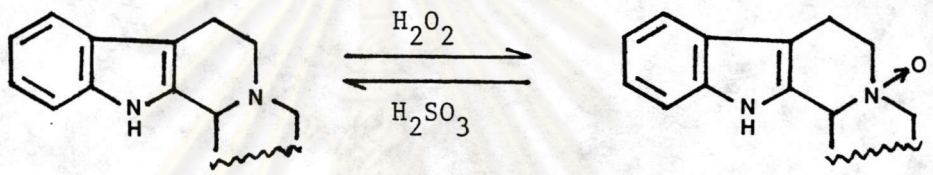
syn- *anti-*

N-oxides of oxindole A

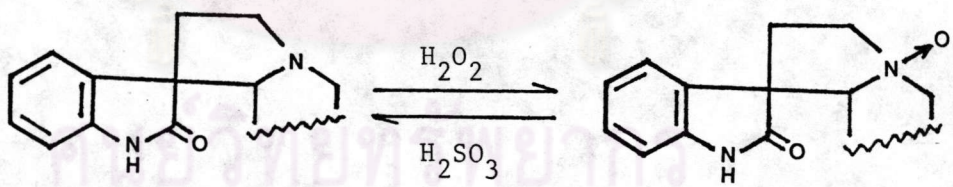


N-oxide of oxindole B

Alkaloid N-oxides are readily reduced to their parent alkaloids without isomerization at C(7) by treated with sulphurous acid and allowed to stand overnight (Shellard, Phillipson and Sarpong, 1971) or treated with concentrated ammonia and excess of ferrous sulphate, and heated on a steam bath for 30 minutes (Merlini, Nasini and Phillipson, 1972). The reaction is summarized in Figure 7.



Heteroyohimbine alkaloids

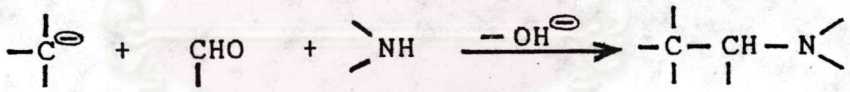


Oxindole alkaloids

Figure 7 Formation and reduction of alkaloid N-oxides

Biogenesis

It has been assumed that complex natural products are assembled from simple molecules which are, in turn, related to common cellular constituents. With the elucidation of the structures of the major groups of alkaloids, and the recognition of common features amongst the structural variants within each group, it becomes apparent that their skeletons could be visually dissected into fragments whose carbon-nitrogen skeleton bore the features of one or more the other of a small number of amino acids. The reassembly of these foundamental substances into the complex product should be carried out by a small number of simple organic reactions. Two of these, the Mannich reaction, i.e. the condensation of an amine, an aldehyde and a carbanion :



and the oxidative coupling of phenols, are of particular importance (Pelletier, 1970). Additionally, it is a function of the range of enzymes at the intermediate stages, rather than a multiplicity of precursors in the initial stages, which accounts for the numerous alkaloids that are known (Cordell, 1981).

There are two terms, biosynthesis and biogenesis, commonly used in discussing the formation of the products of secondary metabolism. Biosynthesis is the experimental

study of the formation of secondary metabolites. Biogenesis is the hypothetical speculation on the precursor-product relationships in a biosynthetic pathway. By means of this definition biogenesis refers to the manner in which the organic substances are synthesized, altered, or degraded by plant or animal organisms. It is based mainly on the visual dissection of a molecule into recognizable precursor fragments (Cordell, 1981).

1. Biogenesis of Indole Alkaloids

Numerous radioactive tracer experiments have demonstrated that the monoterpenoid-derived indole alkaloids are biosynthesized by a general route which begins with the condensation of tryptamine and C_9- or C_{10}-monoterpene moiety, derived from secologanin. This condensation proceeds through a nitrogenous glycoside, in particular strictosidine, the key intermediate in the biosynthetic pathway which gives rise to various types of indole alkaloids.

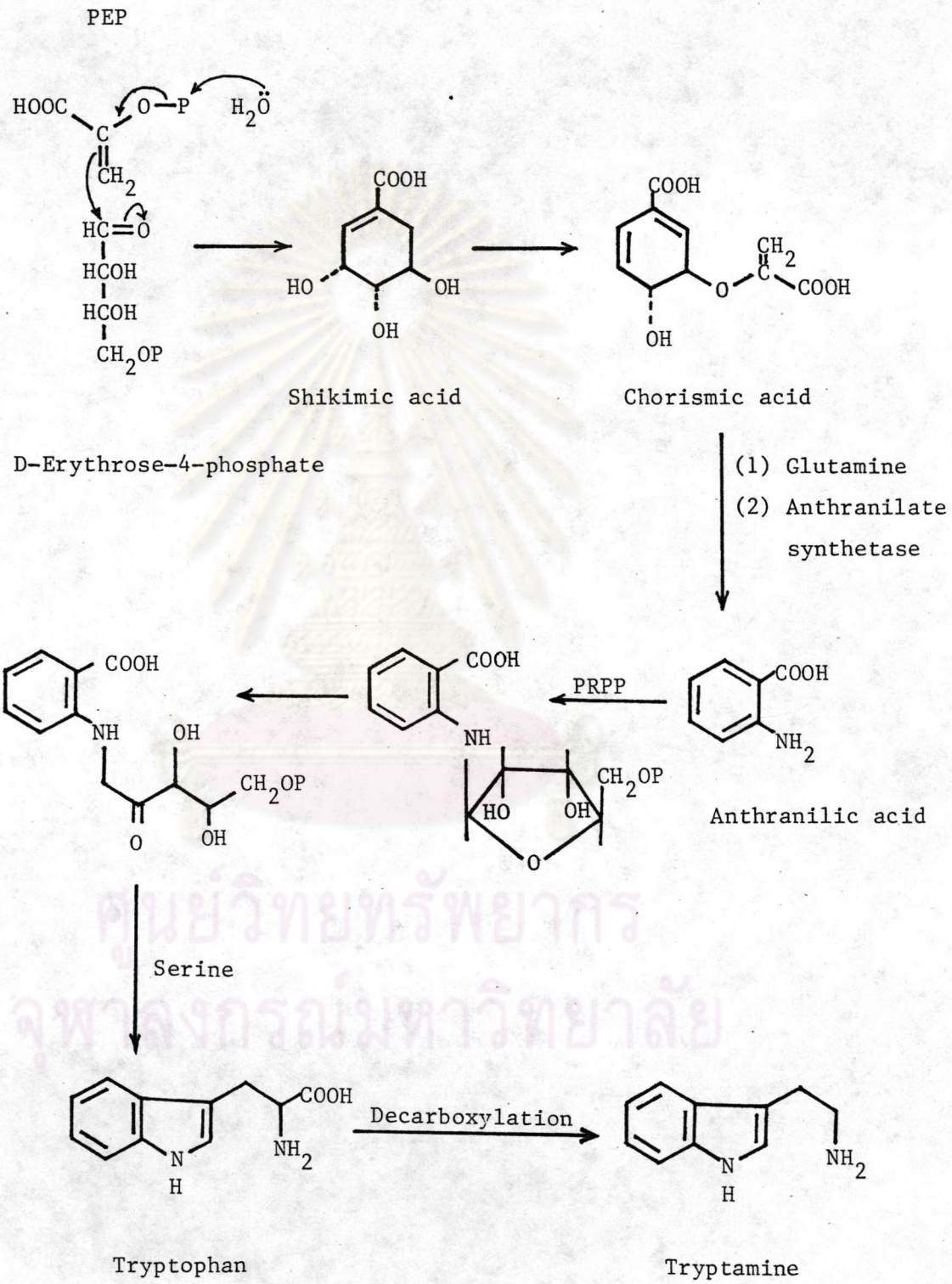
1.1 Biosynthesis of Indole Alkaloid Precursors

1.1.1 Formation of tryptamine

Perkin Jr. and Robinson (1919) had originally suggested that the two nitrogens and the aromatic portion of all the then-known indole alkaloids originate from tryptophan via its decarboxylation product, tryptamine, a fact later experimentally proved (Battersby, Burnett and

Parsons, 1968; Kompis, Hesse and Schmid, 1971). Tryptophan itself is derived from shikimic acid, a key intermediate in shikimic acid pathway. The biosynthesis of shikimic acid pathway comprises of two main stages : the first proceeds from the condensation between a molecule of D-erythrose-4-phosphate, coming from the pentosephosphate cycle, and one of phosphoenolpyruvic acid (PEP), arising from glycolysis to chorismic acid. The second stage involves many ramifications all departing from chorismic acid including anthranilic acid, a precursor of tryptophan. Tryptamine is then formed by decarboxylation of tryptophan via a pyridoxal-bound intermediate (Luckner, 1972; Dalton, 1979; Cordell 1981; Manitto, 1981; Torssell, 1983). The reaction is summarized in Figure 8.

Figure 8 Formation of tryptamine



1.1.2 Formation of secologanin

The iridiods are widely distributed in the plant kingdom and often glycosidically bound. An inportant place amongst the iridoids is held by loganin, which is the commonest precursor of the so-called secoiridoids such as secologanin. Loganin itself is derived from mevalonic acid. From many experimental evidences, loganin is a key compound in providing a ten- or (after removed of C(22) by decarboxylation) a nine-carbon unit to non-tryptophan-derived part of the monoterpenoid-derived indole alkaloids (Kompis, Hesse and Schmid, 1971). Figure 9 illustrates the main steps of the biosynthesis of secologanin from mevalonic acid. The early steps involve the condensation of molecules of acetyl CoA with the aid of enzyme β-ketoacylthiolase to give 3-hydroxy-3-methylglutaryl CoA (HMG CoA). The two-step reduction with NADPH then affords mevalonic acid via mevaldic acid. The compound is optically active and in many subsequent studies it has been found that only 3*R*-mevalonic acid is biologically active, whilst the *S*-isomer is metabolically inert (Cordell, 1981; Torssell, 1983). 3*R*-Mevalonic acid is phosphorylated to 5-phosphomevalonic acid, and then to 5-pyrophosphomevalonic acid in the presence of ATP. Followed by *trans* elimination of carbon dioxide and water afford isopentanylpyrophosphate (IPP). Enzyme-mediated stereoselective loss of the *pro*-4*S* hydrogen and stereoselective addition of hydrogen to the *re* side of the

double bond produces dimethylallylpyrophosphate (DMAPP). Stereoselective loss of the *pro*-4*S* proton from IPP in the coupling-elimination reaction with DMAPP produces geranylpyrophosphate, in which the *pro*-4*S* hydrogens of the two mevalonate units are completely lost. The methyl groups of DMAPP are not initially biosynthetically equivalent. Therefore in geranylpyrophosphate, C(10) is specifically derived from C(2) of mevalonic acid and C(8) and C(9) from C(6) of mevalonic acid (Cordell, 1981).

Hydrolysis of geranylpyrophosphate gives rise to geraniol. Experimentally proof establishing geraniol as a specific precursor to members of the Corynanthe-, Iboga-, and Strychnos types were given by Battersby *et al.* (1966) and Loew, Goeggel and Arigoni (1966). No carbon atoms are gained or lost in the interconversion of geraniol to form loganin, but several oxidations are performed at various stages. A *cis-trans* isomerization of the 2,3-double bond of geraniol to give nerol, in which the hydrogen at C(2) of the former is retained in the latter, and hydroxylation of nerol at C(10) to give 10-hydroxynerol. Nerol has been hypothesized as an intermediate, since 10-hydroxynerol is a more effecient precursor than 10-hydroxygeraniol. The route between 10-hydroxynerol to deoxyloganin is not well understood. However, there is evidence to suggest that further oxidation of C(8) and C(10) occurs to give a trialdehyde in which C(8) and C(10) have become equivalent (by tautomerization). Probably ring closure occurs at

this point to give the monocyclic trialdehyde, which exists as the cyclized hemiacetal. Glycosylation, possibly of the hemiacetal gives rise to the known intermediate, deoxyloganin. Hydroxylation at C(7) of deoxyloganin occurs stereospecifically to give loganin, and ring opening of loganin gives secologanin (Dalton, 1979; Cordell, 1981).

Figure 9 Formation of secologanin

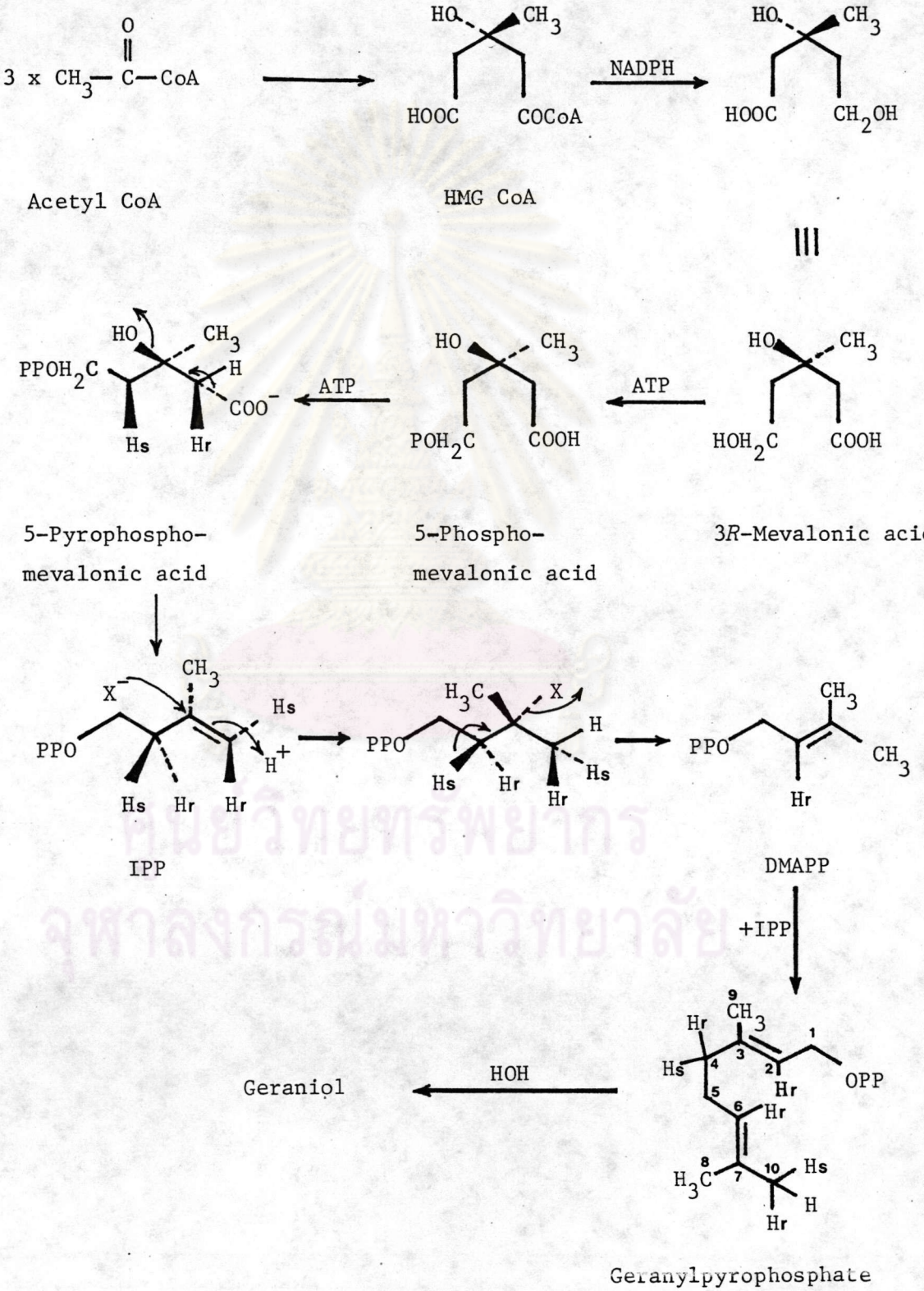
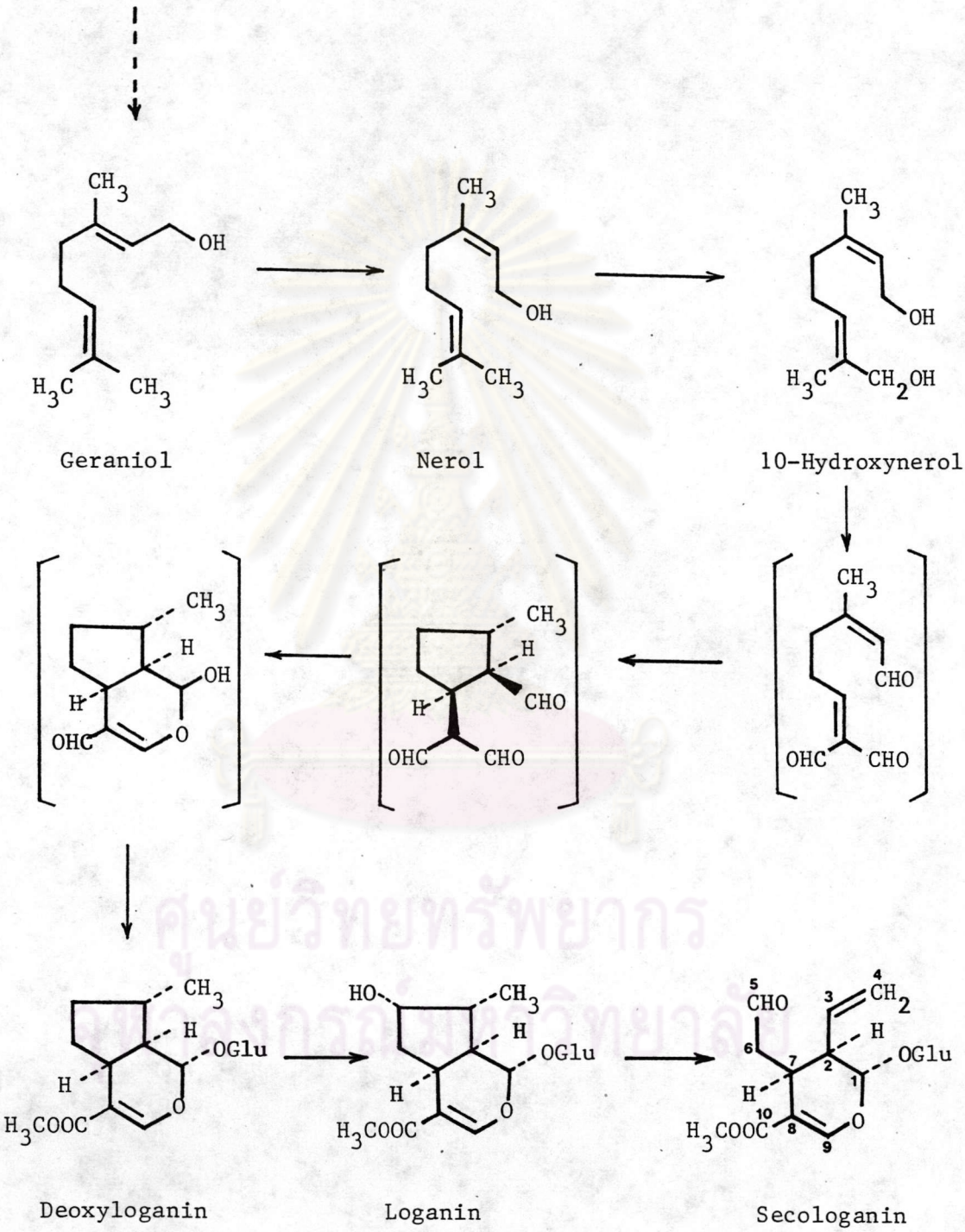


Figure 9 (continued)

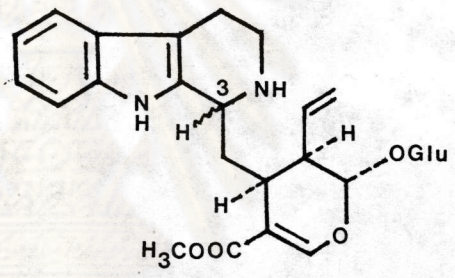


1.2 The Role of Strictosidine (Isovincoside) in the Biosynthesis of Indole Alkaloids

The isolation of strictosidine by Smith (1968) from *Rhazya stricta* and *R. orientalis* as an unstable amorphous pale yellow solid began what continues to be a most exciting period of indole alkaloid chemistry. Shortly after that Battersby and co-workers obtained two nitrogenous glycosides, vincoside and isovincoside, from *Catharanthus roseus* and also succeeded in producing these alkaloids *in vitro* by the condensation of tryptamine and secologanin at pH 6.2. Clearly one of these compounds should be identical with strictosidine which formerly isolated by Smith in 1968, and this was found to be isovincoside. Moreover in original assignment, vincoside and isovincoside are C(3) epimers, viz. vincoside had C(3)-Hα while isovincoside had C(3)-Hβ. From a biosynthetic point of view, it was demonstrated by single- and double-labelling experiments that vincoside was the precursor of representative indole alkaloids from the Corynanthe-, Aspidosperma-, and Iboga types in *C. roseus*. Isovincoside was not involved in these biosyntheses (Battersby, Burnett and Parsons, 1968,1969a,b).

At this point there was a very interesting experiment presented by De Silva, Smith and Warren (1971) which conclusively demonstrated that isovincoside and strictosidine were identical and that the C(3)

stereochemistry of vincoside was incorrect. The complete stereochemistry of strictosidine has been established by a chemical correlation with dihydroantirhine acetate, whose absolute stereochemistry had been deduced. It was demonstrated that no C(3) epimerization had occurred in the correlation. Strictosidine (isovincoside) therefore has C(3)-Hα(*S*), and the C(3)-Hβ(*R*) of vincoside was also confirmed by an X-ray analysis (Mattes *et al.*, 1975).



	Original assignment	Correct assignment
Vincoside	C(3)-Hα	C(3)-Hβ(*R*)
Isovincoside	C(3)-Hβ	C(3)-Hα(*S*)

Several years after obtaining the correct absolute stereochemistry of vincoside and isovincoside, there were many independent experiments determined the fate of strictosidine (isovincoside) in the biosynthetic pathway of indole alkaloids and demonstrated that much of the early work with vincoside as precursor was incorrect.

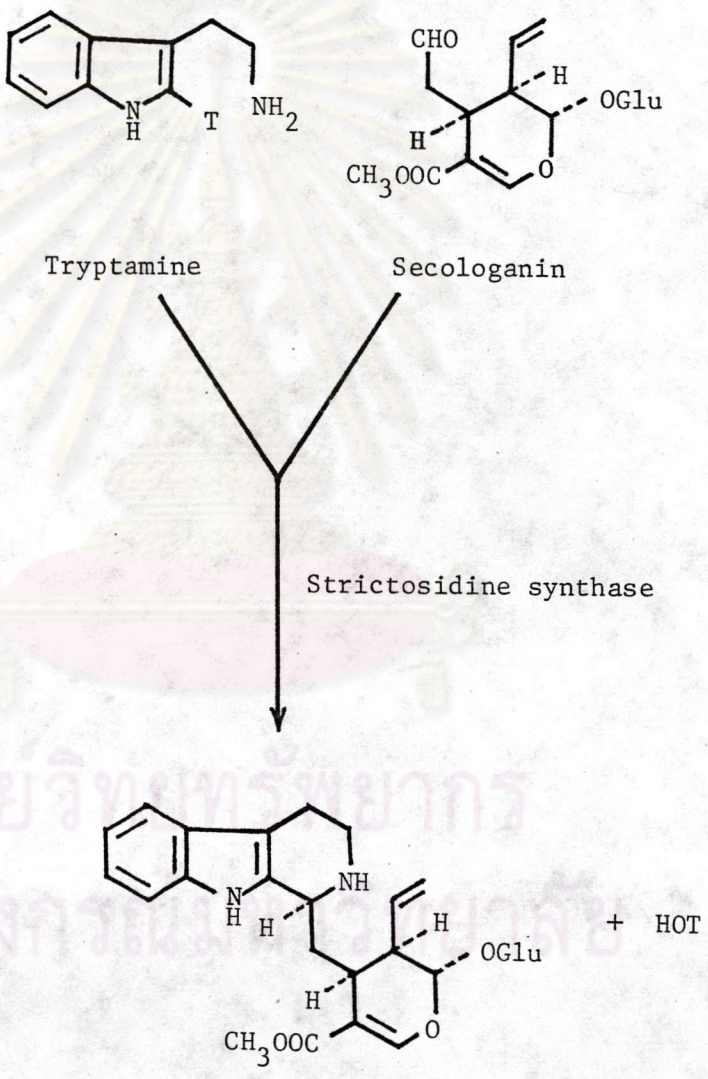
Heckendorf and Hutchinson (1977) studied the biosynthesis of camptothecine which the result showed conclusively that strictosamide, but not vincosamide, was a specific precursor of camptothecine. It was presumed at this point that strictosidine was the precursor of camptothecine. The important evidence was obtained by the incubation of [5-^{14}C, 14-^{3}H] vincoside and strictosidine under identical condensations in a cell-free system from *Catharanthus roseus* and repeated this experiment in aqueous solution feeding to 18-day-old *C. roseus* shoots. The results of these experiments leave no doubt that strictosidine is indeed the precursor of the major alkaloids ajmalicine, vindoline and catharanthine, representing the Corynanthe-, Aspidosperma-, and Iboga types respectively, and that with both cell-free and whole plant systems no radioactive can be detected when vincoside is used as a potential precursor. This means that vincoside is not metabolized to the natural alkaloids of *C. roseus* (Scott *et al.*, 1977). In the same time, the similar results using single labelled vincoside and doubly labelled strictosidine were obtained by Stockigt and Zenk (1977) who have also shown that strictosidine accumulates in cell-free system when alkaloid synthesis is inhibited by a glucosidase inhibitor, δ-D-gluconolactone. And from this experiment the crucial enzyme catalysing the condensation of tryptamine with secologanin to yield exclusively the α-epimer, strictosidine, has been discovered and named

strictosidine synthase. Re-examined the stereochemical point of nitrogenous glycosides by radioactive tracer experiments on *Catharanthus roseus* and *Cephaelis ipecacuanha* (Battersby, Lewis and Tippett, 1978) confirm that the C(3)-Hα isomer is indeed the precursor of indole alkaloids rather than the C(3)-Hβ isomer. Additionally, by using radioactive labelling with cell-free systems and feeding experiments, Rueffer, Nagakura and Zenk (1978) presented the experiment which shows a very interesting biosynthetic significance. The results from this experiment demonstrated that strictosidine is the key intermediate of indole alkaloids not only in *Catharanthus roseus* but also in cell-free systems of *Amsonia*, *Rhazya*, *Cinchona*, *Stemmadenia*, *Uncaria*, and other *Catharanthus* species. More results from this experiment have been extended to other indole alkaloid-producing systems, not only those having the C(3)-Hα stereochemistry but also those with a C(3)-Hβ. In *Rauvolfia canescens* α-yohimbine [C(3)-Hα] and reserpiline [C(3)-Hβ] were studied, and in *Mitragyna speciosa* speciociliatine [C(3)-Hβ] and mitragynine [C(3)-Hα] were examined. In all instances strictosidine was a precursor but vincoside was not. Furthermore strictosidine was incorporated into a series of different alkaloids, including yohimbine-, ajmalicine-, vindoline-, strychnine-, sarpagine types, and gelsemine (Nagakura, Rueffer and Zenk, 1979). This is contrary to the chemical conversion in which strictosidine is

transformed to C(3)-Hα and vincoside to C(3)-Hβ indole alkaloids. This fact shows the limitations of biomimetic experiments with respect to *in vivo* processes. It is important to note that the *in vivo* conversion of strictosidine into C(3)-Hβ alkaloids proceeds with loss of hydrogen at C(3) while it is retained in the formation of the C(3)-Hα series. Recently, the enzyme strictosidine synthase has been partially purified from *Catharanthus roseus* cell suspension cultures and immobilized on CNBr-activated Sepharose. The immobilized enzyme exhibits a thermostability increased 300 fold over that of the soluble enzyme and catalyses exclusively the formation of the C(3)-Hα(*S*)-isomer, strictosidine. The reaction catalysed by this enzyme is depicted in Figure 10 (Pfitzner and Zenk, 1982).

Therefore it can be stated that strictosidine with C(3)-Hα(*S*) stereochemistry is the central precursor for the elaboration of the monoterpenoid-derived indole alkaloids in the four plant families Apocynaceae, Loganiaceae, Rubiaceae, and Nyssaceae (Nagakura, Rueffer and Zenk, 1979).

Figure 10 Stereospecific condensation of [C(2)-T]-tryptamine and secologanin



C(3)-Hα(*S*)-Strictosidine

1.3 Biogenetic Classification of Indole Alkaloids

The monoterpenoid-derived indole alkaloids are currently classified into 5 base types, Corynanthe-, Aspidosperma-, Iboga-, Strychnos-, and Yohimbe types. This classification arose from the Thomas (1961) and Wenkert(1962) hypotheses of the iridoid, cyclopentanoid monoterpene, intermediate in the biosynthetic pathway to these indole alkaloids which, when cleaved, would give the various skeletal types as shown in Figure 11 (Scott, 1973).

From various experimental proof it was demonstrated that Corynanthe-type alkaloids were formed from strictosidine. The formation of the Corynanthe-type bases from strictosidine requires no skeleton rearrangement. It was thus reasoned that this skeleton type serves as a precursor for the alkaloids with rearranged skeletons (Nakanishi *et al.*, 1983). Table 3 shows the structures of representative alkaloid for each of the 5 base types. Basing on this classification heteroyohimbine alkaloids and the corresponding oxindoles are classified as the member of Corynanthe-type.

Figure 11 The 5 base types of monoterpenoid-derived indole alkaloids

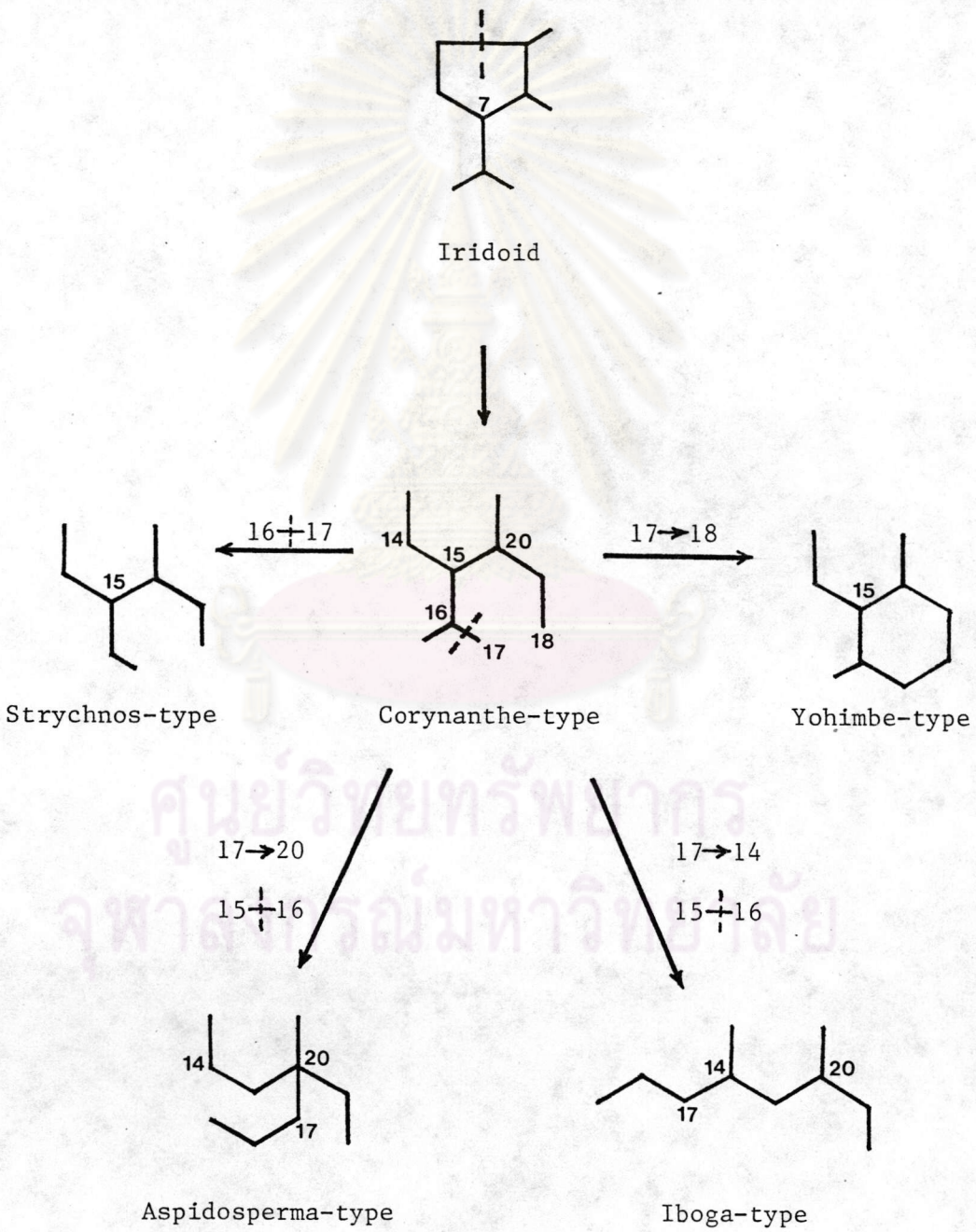
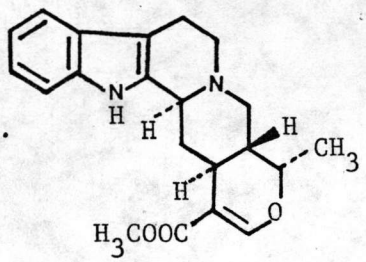
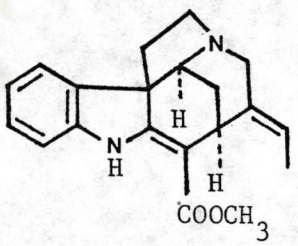
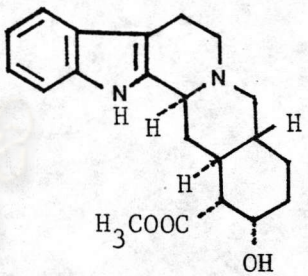
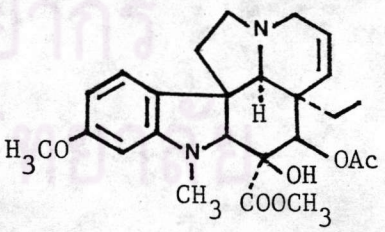
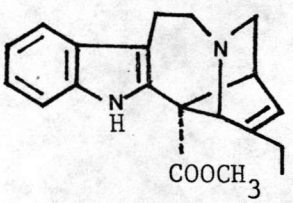


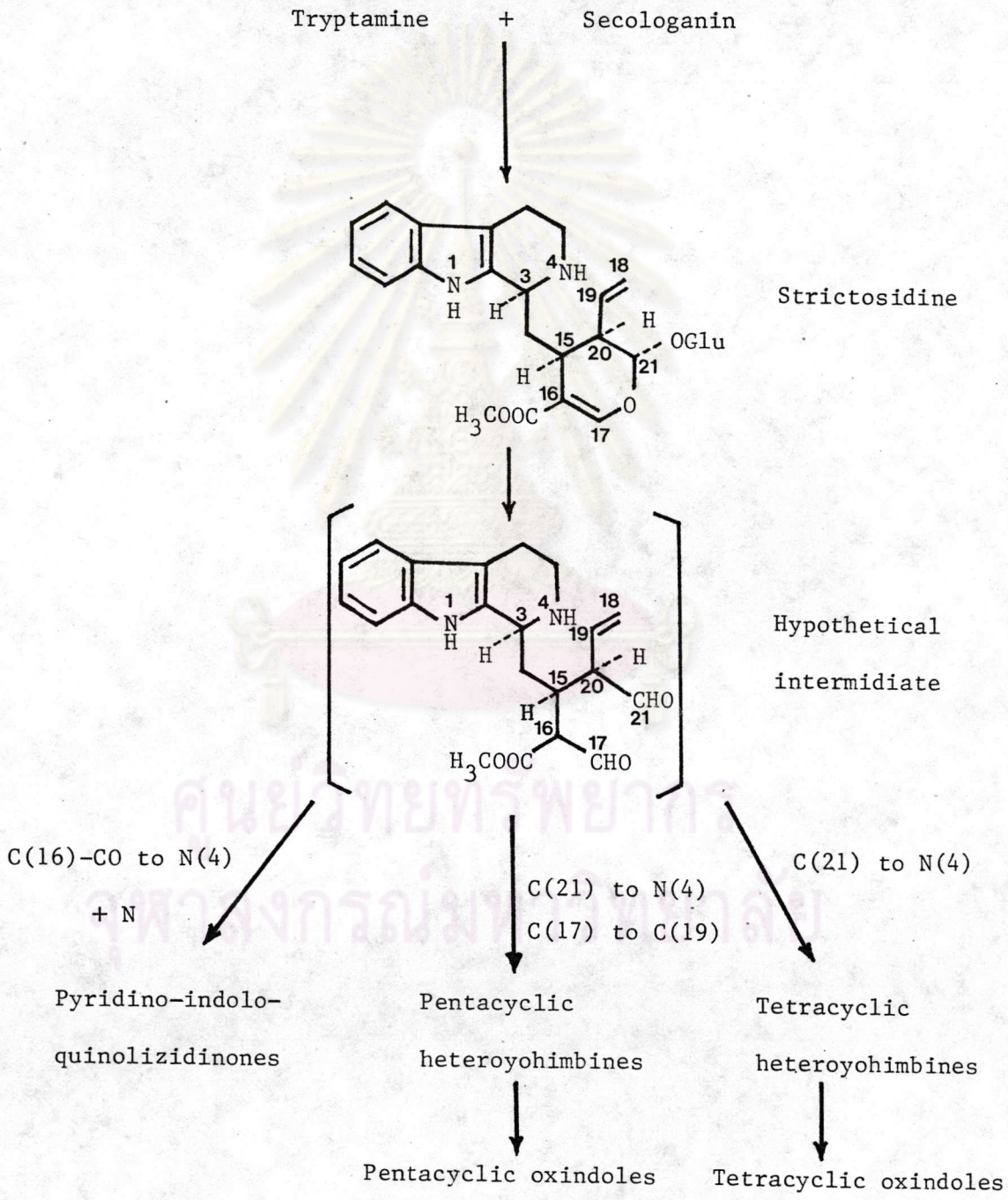
Table 3 The structures of representative alkaloid for each of the 5 base types

Type	Example	Structure
Corynanthe	ajmalicine	
Strychnos	akuammicine	
Yohimbe	α-yohimbine	
Aspidosperma	vindoline	
Iboga	catharanthine	

1.4 Formation of Pentacyclic and Tetracyclic Heteroyohimbines and the Pyridino-indolo-quinolizidinones

Figure 12 illustrates the postulated biosynthetic relationships between the major alkaloids which are specific to the genus *Mitragyna* and *Uncaria*. Structure elaboration by several ring closures of the hypothetical intermediate resulting in formation of various specific skeletal types. A new bond between carbonyl group of C(16) and N(4) together with addition of one nitrogen atom leads to the pyridino-indolo-quinolizidinones. The combination of C(21) with N(4) results in tetracyclic heteroyohimbines which are then transformed to their corresponding oxindoles. The ring closures of C(21) to N(4) and C(17) to C(19) gives pentacyclic heteroyohimbines which can further yield their corresponding oxindoles by specific chemical transformations (Phillipson, Hemingway and Ridsdale, 1982).

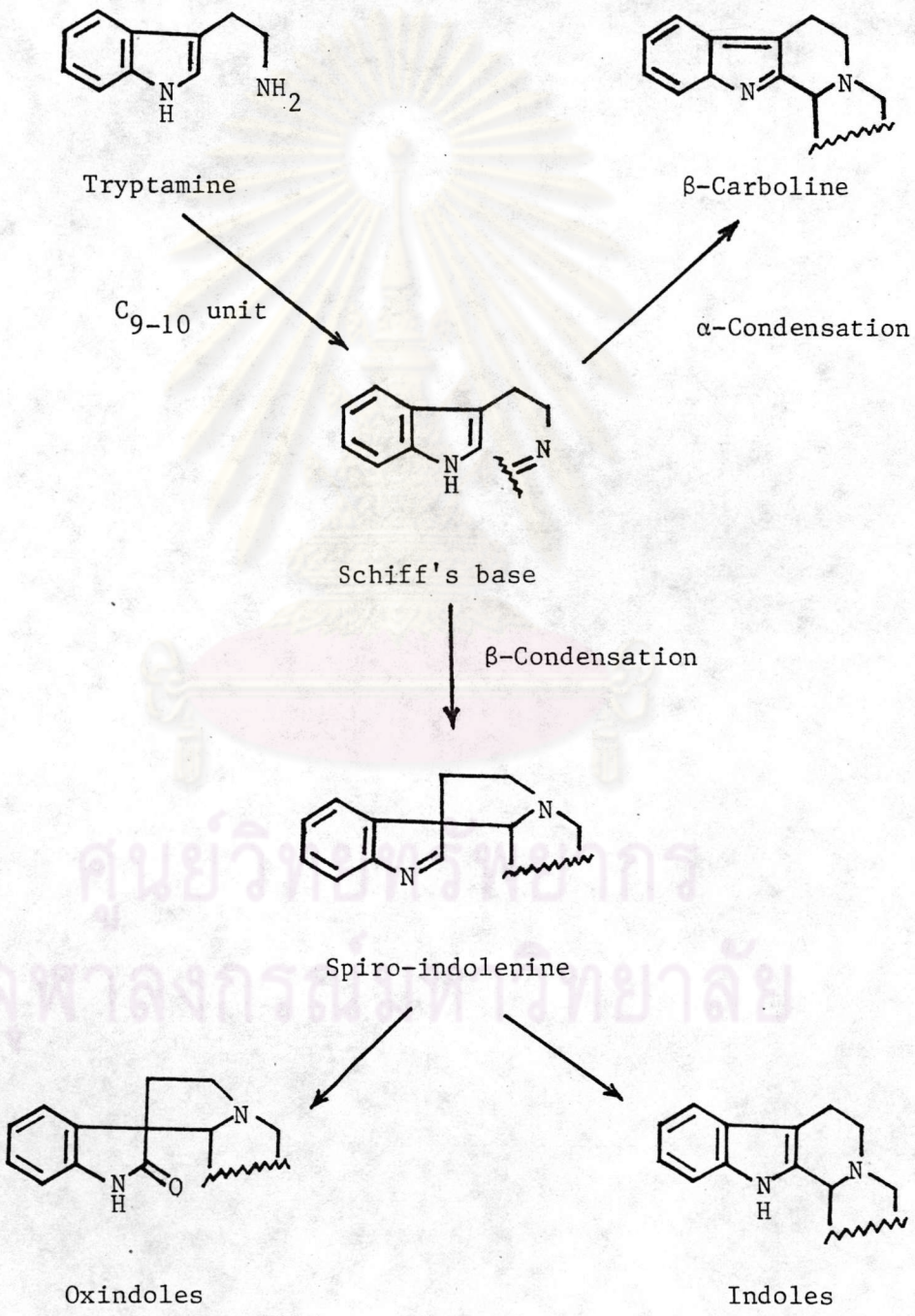
Figure 12 The formation of pentacyclic and tetracyclic heteroyohimbines and the pyridino-indolo-quinolizidinones



2. The Relationship Between Indole and Oxindole Alkaloids

The Woodward proposals regarding the condensation of tryptamine and the C_9- or C_{10}-carbon unit suggest that this may be either an α-condensation to give indoles or a β-condensation to give oxindoles (Shellard, Phillipson and Gupta, 1969b). Furthermore, Jackson and Smith (1968a,b) have proposed that the Schiff's base, which is the initial product obtained from the condensation of tryptamine and secologanin, then undergoes cyclization at either α- or β-position of the indole nucleus to yield the β-carboline or the spiro-indolenine intermediate, respectively (Figure 13). They argued that β-condensation is more favored because the intermediate product, spiro-indolenine, does not necessitate a rearrangement of the π electron system of the benzene ring which would be the case with an α-condensation. The spiro-indolenine can readily isomerize to the indole in acid conditions and can be oxidized to give oxindoles.

Figure 13 The relationship between indole and oxindole alkaloids

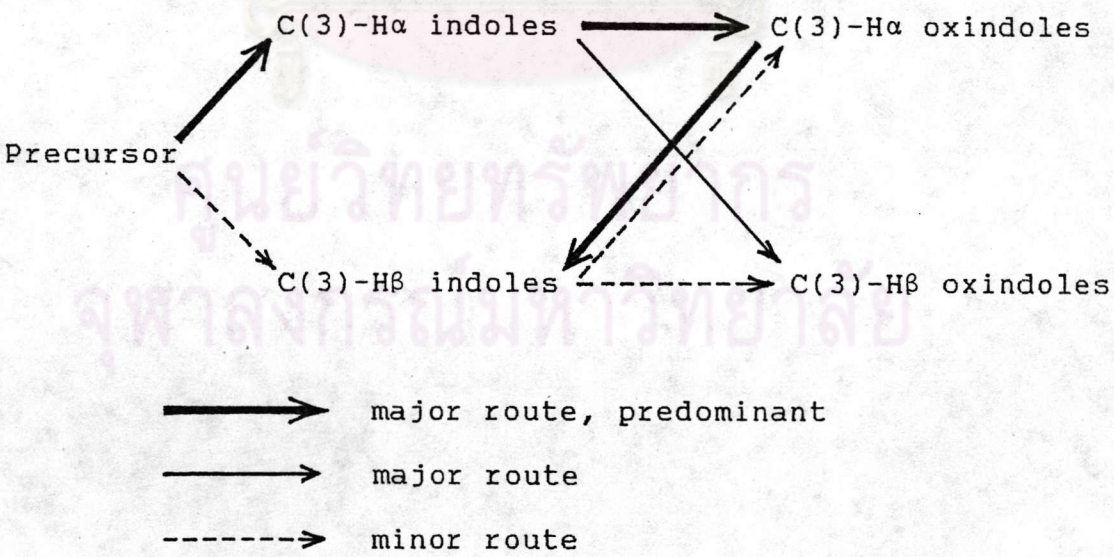


3. Possible Biogenetic Route of *Mitragyna* Alkaloids

Since 1960s to 1970s, basing on the progressive knowledge of chemistry of alkaloids reported to be present in several *Mitragyna* species, their chemical transformations using both *in vivo* and *in vitro* techniques, and the biogenesis of indole and oxindole alkaloids, Shellard and his co-workers have subsequently been postulating hypotheses, and also their modifications, regarding the biogenesis of *Mitragyna* alkaloids (Shellard, Phillipson and Gupta, 1969b; Shellard and Houghton, 1973b, 1974a). These hypotheses and their modifications, which at that time vincoside was believed to be a key intermediate of indole alkaloids, have now been considered invalid in the light of the newly discovered evidence about the key intermediate in the biosynthetic pathway of indole alkaloids carried out by Scott *et al.* (1977), Stockigt and Zenk (1977). These experimental proof demonstrated that strictosidine (isovincoside) not vincoside is the indeed precursor of indole alkaloids. At this point Shellard, Houghton and Resha (1978b) had proposed a modified hypothesis based on strictosidine as a precursor and suggested that the major route of *Mitragyna* alkaloids is via the C(3)-Hβ indoles which are then converted to both C(3)-Hα and C(3)-Hβ oxindoles. Subsequently Rueffer, Nagakura and Zenk (1978) have shown that strictosidine is the common biosynthetic precursor

for alkaloids with C(3)-Hα as well as C(3)-Hβ configuration. They also demonstrated that strictosidine is the precursor for the *allo* (mitragynine) and *epiallo* (speciociliatine) in *Mitragyna speciosa*.

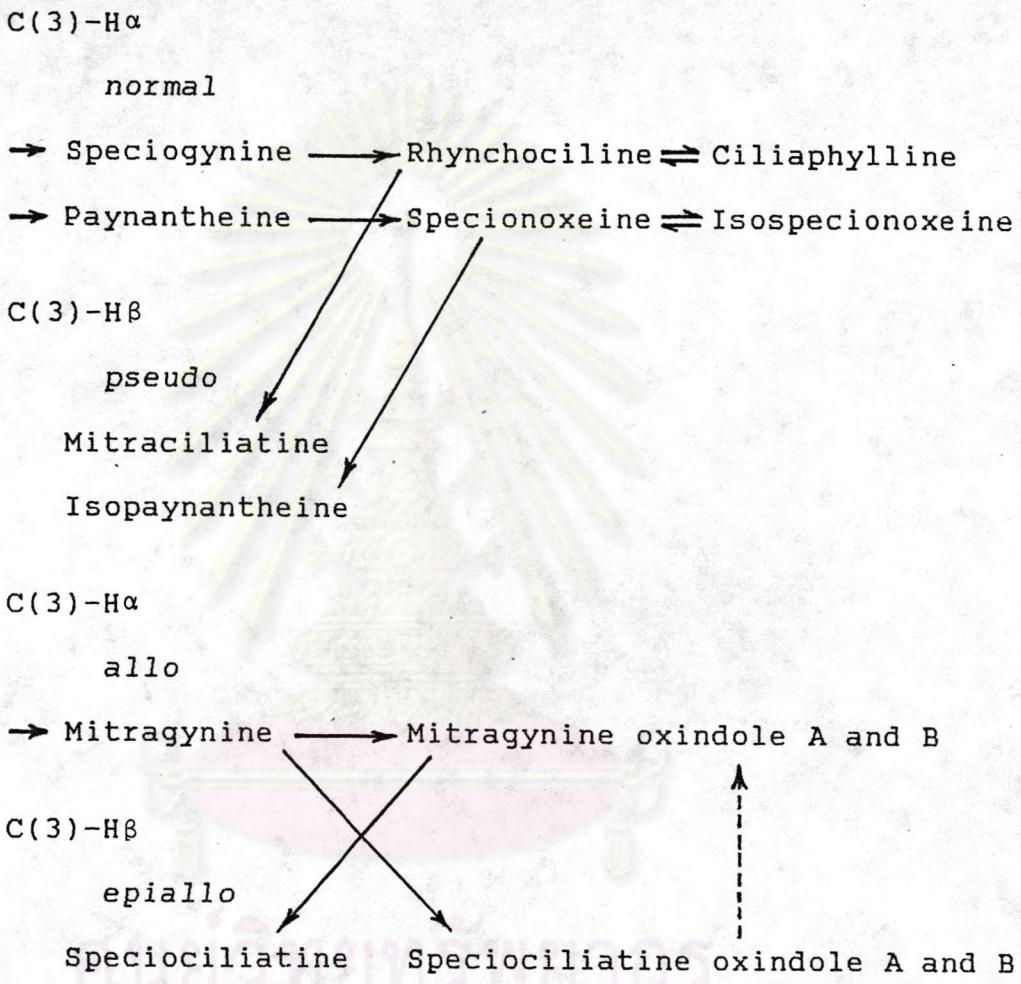
The evidence now available suggests that the major biogenetic route of *Mitragyna* alkaloids is via the C(3)-Hα indole alkaloids which are then converted primarily to the C(3)-Hα oxindole alkaloids and that these oxindoles are then converted to the C(3)-Hβ indole alkaloids. There is probably, in addition, a minor route via the C(3)-Hβ indole alkaloids which are converted to the oxindoles since this has been shown to occur by *in vivo* studies (Shellard and Houghton, 1974a; Shellard and Lala, 1978). The scheme proposed by Shellard, Houghton and Resha (1978c) may be shown diagrammatically as follows :-



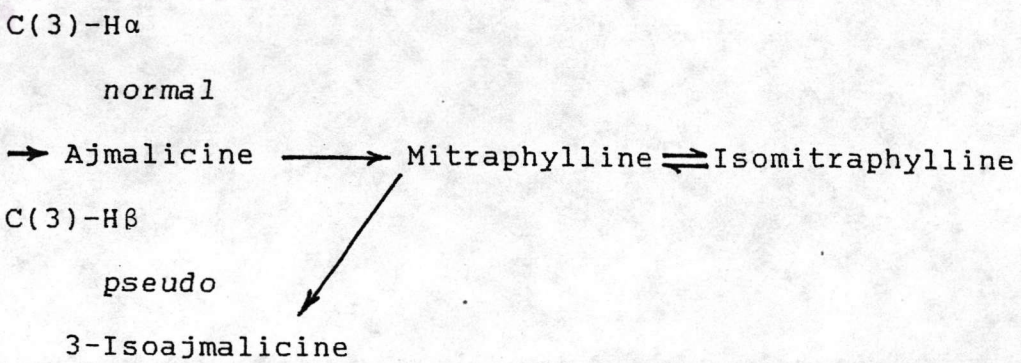
They also proposed a probable biogenetic route of alkaloids in *Mitragyna speciosa* as shown in Figure 14.

Figure 14 The probable biogenetic route of alkaloids in *Mitragyna speciosa*

(1) Open E ring alkaloids [C(9)-OCH_3]



(2) Closed E ring alkaloids [C(9)-H]



Pharmacology of *Mitragyna* Alkaloids

Among naturally occurring compounds the indole alkaloids play important roles as biologically active substances. There are two interesting groups of the monoterpenoid-derived indole alkaloids, namely, the yohimbines and reserpines, which already have found medicinal application in human therapy (Szantay *et al.*, 1986). In *Mitragyna* alkaloids there are some heteroyohimbines and oxindoles which exhibit significant pharmacologic activities.

1. Mitragynine

Since mitragynine was the dominant alkaloid and exclusive to *Mitragyna speciosa* (Korth.) Havil. (Shellard, 1974) it was assumed to be the physiologically active constituent having morphine-like properties. According to Laidlaw, mitragynine is a local anaesthetic (Field, 1921). Grewal (1932) performed a series of experiments on animal tissues and found mitragynine to be a central nervous system stimulant rather than depressant. He indicated that mitragynine has a depressant effect on some isolated tissues, facilitates the passage of impulses in the autonomic nervous system and increases the excitability of the medulla and probably of the motor centers. He also found that mitragynine is a general protozoal poison, but is probably ineffective against bacteria or pathogenic protozoa. Grewal subsequently administered mitragynine to

five men and observed a cocaine-like effect. Moreover, 50 mg of pure mitragynine acetate produced nausea and vomiting in some subjects (Jansen and Prast, 1988).

Mitragynine acetate was found to diminish the excitability of plain muscle, to anaesthetize the cornea, and to be toxic to animals in fairly low doses. In the psychological research 6 human subjects submitted to various laboratory tests after taking doses of 50-100 mg of mitragynine acetate and after doses of 650-1300 mg of the powdered leaves. There were indications that both forms of the drug produced the following effects :- decrease in the time of reaction to stimulus; increased tolerance to that; increased steadiness and capacity for work; flushing of the skin, apparently due to dilatation of superficial blood vessels (Marcan, 1934). Mitragynine-hydrochloride or mitragynine ethanedisulfonate exhibited analgesic and antitussive properties in animals. Unlike the narcotic analgesic, mitragynine had little effect on gastric mobility, failed to produce excitement in cats, was not antagonized by nalorphine, and had only weak respiratory depressant activity in anaesthetized animals at analgesic levels. In the mouse, no evidence of toxicity (tremors and convulsions) was observed after doses as high as 920 mg/kg. Mitragynine was found to be much less active subcutaneously than orally, suggesting that the active analgesic moiety may be a metabolite (Macko, Weisbach and Douglas, 1972).

Zarembo *et al.* (1974) produced two active metabolites from microbial transformation of mitragynine by the fungus *Helminthosporum* sp. (ATCC 20154) were isolated from the biological milieu and their structures were elucidated as mitragynine pseudoindoxyl and hydroxy mitragynine pseudoindoxyl. Mitragynine pseudoindoxyl displayed analgesic activity in the D'Amour-Smith test almost 10-fold (9.4 : 1) stronger than mitragynine when administered by both oral and intraperitoneal routes to animals. Hydroxy mitragynine pseudoindoxyl was 20-30 % more effective than mitragynine.

According to Raffauf, pre-clinical trials in humans of mitragynine which carried out by Smith, Kline and French Laboratories revealed unacceptable side effects (Jansen and Prast, 1988). At this point it appears that mitragynine is positive for use as analgesic, antitussive, and hypothermic agent in animals while there is no evidence of addition properties. But, mitragynine is too toxic for its analgesic activity to have any clinical application, while the corresponding pseudoindoxyl may have potential.

2. Ajmalicine

Ajmalicine is an official pharmacologically active compound for the treatment of circulatory diseases, especially in the relief of obstruction of normal cerebral blood flow and hypertension. It is related chemically to

reserpine but has less antihypertensive activity. Its hypotensive effect is mainly due to a reduction in cardiac out-put and a reduction in peripheral resistance. Like reserpine, ajmalicine causes depletion of noradrenaline stores in peripheral sympathetic nerve terminals and depletion of catecholamine and serotonin stores in the brain, heart and many other organs resulting in a reduction in blood pressure, bradycardia, and central nervous system depression (Reynolds, 1989).

3. Rhynchophylline (Mitrinermine)

Rhynchophylline, injected in doses of 0.1 mg/g, causes in guinea-pig a reduction of temperature of 2-3°C. It is very toxic to paramecium, which is killed within 6 minutes by a 1:1000 solution (Perrot, Raymond-Hamet and Millat, 1936). Small concentrations of rhynchophylline contract the intestine of guinea-pig and rabbit, though large concentration relax them. The uterus of the rabbit is contracted. On the isolated seminal vesicle, rhynchophylline antagonizes the effect of adrenaline and acetyl choline (Raymond-Hamet, 1937,1941). Rhynchophylline is a very potent hypotensor, which is devoid of the effects on the sympathetic system produced by yohimbine (Millat, 1946). The hypotensive effect of the total extracts are, inpart, owing to rhynchophylline (Saxton, 1965).

4. Dihydrocorynantheol

Dihydrocorynantheol exhibits antimicrobial activity against gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus* (Verpoorte, Ruigrok and Svendsen, 1982).

5. Mitraphylline and 3-Isoajmalicine

In the dogs mitraphylline has an hypotensive action but does not counteract the hypertensive and renal vasoconstrictive action of adrenaline (Raymond-Hamet, 1933). Mitraphylline exerts a general depressant effect and in some respects resembles cocaine, but is less active than mitragynine (Saxton, 1965). The low doses intravenous injection of mitraphylline and 3-isoajmalicine separately to anaesthetized rats produced dose-related decrease in blood pressure and heart rate. In higher doses (e.g. 24 mg/kg), the contractile properties of the heart became depressed which was manifested by arrhythmia and heartbeat cessation. The lethal dose in rat of mitraphylline is 96 mg/kg. Studies of mitraphylline and 3-isoajmalicine on isolated rat's and guinea-pig's atria, negative chronotropic, negative inotropic, and negative dromotropic responses had been observed. These effects were resistant to atropine (Archongka, 1983). Mitraphylline and 3-isoajmalicine also reduced spontaneous movements of isolated rabbit jejunum as well as the resting tension of ileum from guinea-pig (Sroysuwan,

1985). These results indicated that mitraphylline and 3-isoajmalicine possessed anticholinergic properties.

Separately preincubation of the rat right atrial strips with mitraphylline and 3-isoajmalicine antagonized positive chronotropic responses of the tissues to 5-hydroxytryptamine (Archongka, 1983). The 5-hydroxytryptamine-induced contraction on isolated aortic strips from rabbit and ileum from guinea-pig were also reduced by 3-isoajmalicine (Sroysuwan, 1985). These results show antiserotonergic properties of 3-isoajmalicine and mitraphylline.

6. Hirsutine and Isorhynchophylline

Hirsutine and isorhynchophylline were examined in the rat superior cervical ganglionic preparation *in situ*. Intraarterial administration at the dose 1 mg of hirsutine exerted ganglion blocking effect lasted longer than that of hexamethonium in 0.5 mg. Inhibitory effect of isorhynchophylline at the dose 1 mg was weak and short-acting (Harada, Ozaki and Sato, 1974). Intraarterial administration of the dose 2 mg of hirsutine to the rat limb preparation (*in situ*) depressed both indirectly and directly elicited contractions of the muscle. Its depressive effect of about 50 % lasted longer than 1 hour. The dose 2 mg of isorhynchophylline showed little depressive effect in both indirectly and directly elicited

contractions, which corresponds to its very weak ganglion-blocking effect (Harada and Ozaki, 1976).

The effects of hirsutine and isorhynchophylline on parasympathetic ganglionic transmission were studied in a preparation of the guinea-pig urinary bladder *in situ*. Hirsutine at the dose 0.5 mg inhibited the contraction of the urinary bladder induced by electrical stimulation of the pelvic nerves in guinea-pig. Their potency was about 50 % of that of hexamethonium. Isorhynchophylline, 0.25 mg, showed a weak inhibitory effect. Both hirsutine and isorhynchophylline at the dose of 0.25 mg depressed the contraction induced by intraarterial dimethylphenyl-piperazinium, with no antagonizing action to the acetylcholine-induced contraction. At the dose 0.003 g/ml, hirsutine and isorhynchophylline elevated the tone of the spontaneous movement of guinea-pig's urinary bladder and augmented its amplitude. The stimulating action of hirsutine was not effected by pretreatment with atropine, hexamethonium, diphenhydramine, or tetrodotoxin. Hirsutine also showed a local anaesthetic action in the isolated frog sciatic nerve preparation. Apparently, hirsutine inhibits bladder contraction by inhibition of the parasympathetic ganglionic transmission and that a blockade of the nicotinic receptors played a role in this effect (Harada, Ozaki and Ohno, 1979).