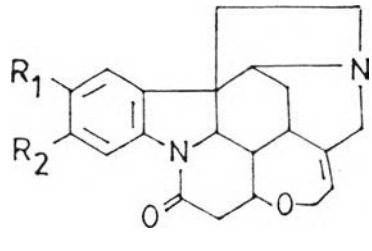
CHAPTER I

GENERAL INTRODUCTION

Introduction

The genus *Strychnos* is the largest genus of the tribe Strychneae family Loganiaceae. It is organized into 12 sections (table 1) base on combination of such botanical features as the length of the corolla tube, the nature of the indumentum on the inner surface of the corolla, the arrangement of the tendrils, the shape and indumentum of the seeds, and the insertion of the stamens and indumentum of the stamens and pistil (Leeuwenberg, 1969, quoted in Bisset and Phillipson, 1971). The genus *Strychnos* comprises about 200 species ranging from forest lianes to shrubs and trees, all of which are pantropical in distribution. They can be subdivided into three geographically separated groups of species, one in South and Central America with 70 species and 2 varieties (Krukoff, 1972), one in Africa with 75 species (Bisset, and Phillipson, 1971; Ohiri, Verpoorte, and Baerheim Svendsen, 1983) and one in Asia and Australia with 44 species (Anet, Hughes, and Ritchie, 1952; Bisset, 1974). All of the species, except *Strychnos potatorum* Linn. which is found in both Africa and Asia (Bisset, and Phillipson, 1971; Bisset et al., 1973), are clearly separated among these three continents.

Strychnos species are the wealthy sources of indole alkaloids (Leeuwenberg, 1980; Kisakürek, Leeuwenberg, and Hesse, 1983). The very first alkaloids to be isolated were strychnine (1) and brucine (2), both of which obtained from the seeds of *Strychnos ignatii* Berg. and *Strychnos nux-vomica* L. in 1818-1819. It took over 120 years for their complete structures elucidation in 1946. Then several phytochemical and pharmacological studies have been taken in order to correlate chemical structures with activities and usages which mostly for the preparation of arrow and dart poisons. Up till now, there were several phytochemical investigations of these *Strychnos* plants and large amount of informations were published and reviewed according to their geographically separated groups, America (Marini-Bettòlo, and Bisset, 1972; Bisset, 1972a), Africa (Bisset, and Phillipson, 1971; Bisset, 1972a; Ohiri et al., 1983) and Asia (Bisset, 1972a; Bisset, and Phillipson, 1976).

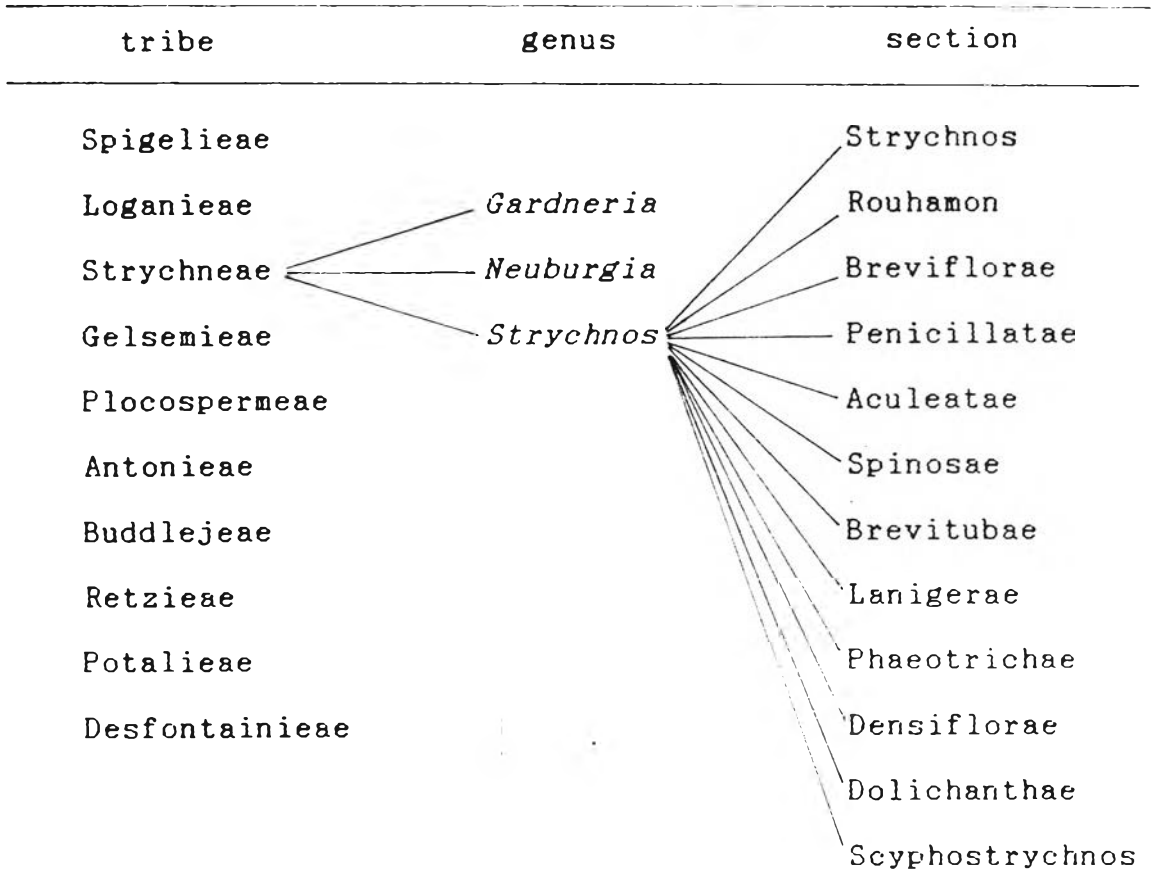


1 Strychnine ; $R_1 = R_2 = H$

2 Brucine ; $R_1 = R_2 = OCH_3$

Table 1

Position of the genus *Strychnos* within the family Loganiaceae



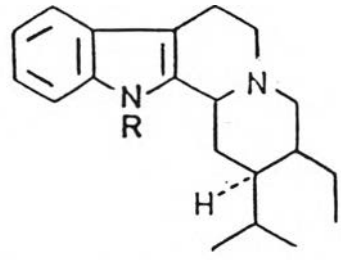
Classification of *Strychnos* alkaloids

The major constituents of *Strychnos* species are the group of terpenoid indole alkaloids, of which more than 350 alkaloids have been isolated (Kisakürek, Leeuwenberg, and Hesse, 1983). A basis for the classification of these indole alkaloids is proposed by Kompis, Hesse and Schmid (1971) and Kisakürek and Hesse (1980). They devided terpenoid indole alkaloids into 8 types according to their characteristic skeleton (figure 1) : Corynanthean (C-type), Vincosan (D-type), Vallesiachotaman (V-type), Strychnan (S-type), Aspidospermatan (A-type), Plumeran (P-type), Eburnan (E-type) and Ibogan (J-type). But at present, only 5 types of terpenoid indole alkaloids belonging to the *Strychnos* species are found, they are the C-, D-, V-, S- and A-types. The most abundant alkaloids are of the S-type and the lesser ones are of the C-type (Kisakürek, Leeuwenberg, and Hesse, 1983).

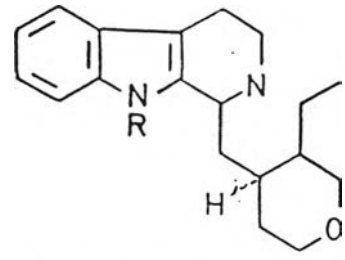
Another classification (figure 3) outlined by Coune. These alkaloids were organized into groups base on the sites of bond migrations and the sites of ring formation of the metabolic products during the processes of biosynthesis which leading to the individual skeletons (Coune, 1980, quoted in Ohiri et al., 1983).

According to these two classifications, Charoendee Pingsuthiwong (1986) rearranged the *Strychnos* alkaloids into 2 main classes : monomeric indole alkaloids and bisindole alkaloids. Monomeric alkaloids were subdivided

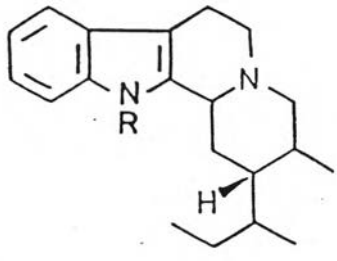
Figure 1 : The indole alkaloid skeletons.



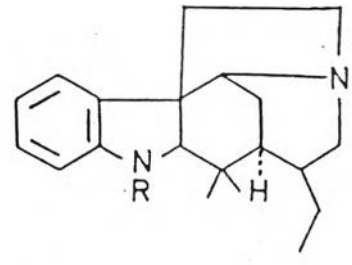
Corynanthean (C-type)



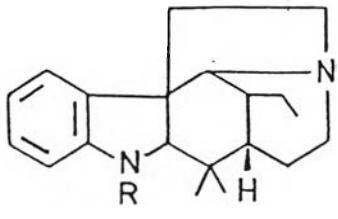
Vincosan (D-type)



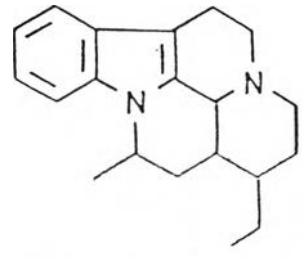
Vallesiachotaman (V-type)



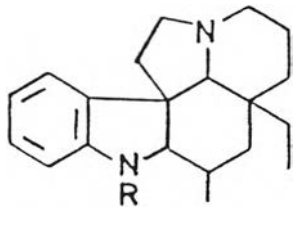
Strychnan (S-type)



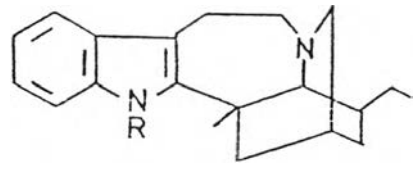
Aspidospermatan (A-type)



Eburnan (E-type)



Plumeran (P-type)



Ibogan (I-type)

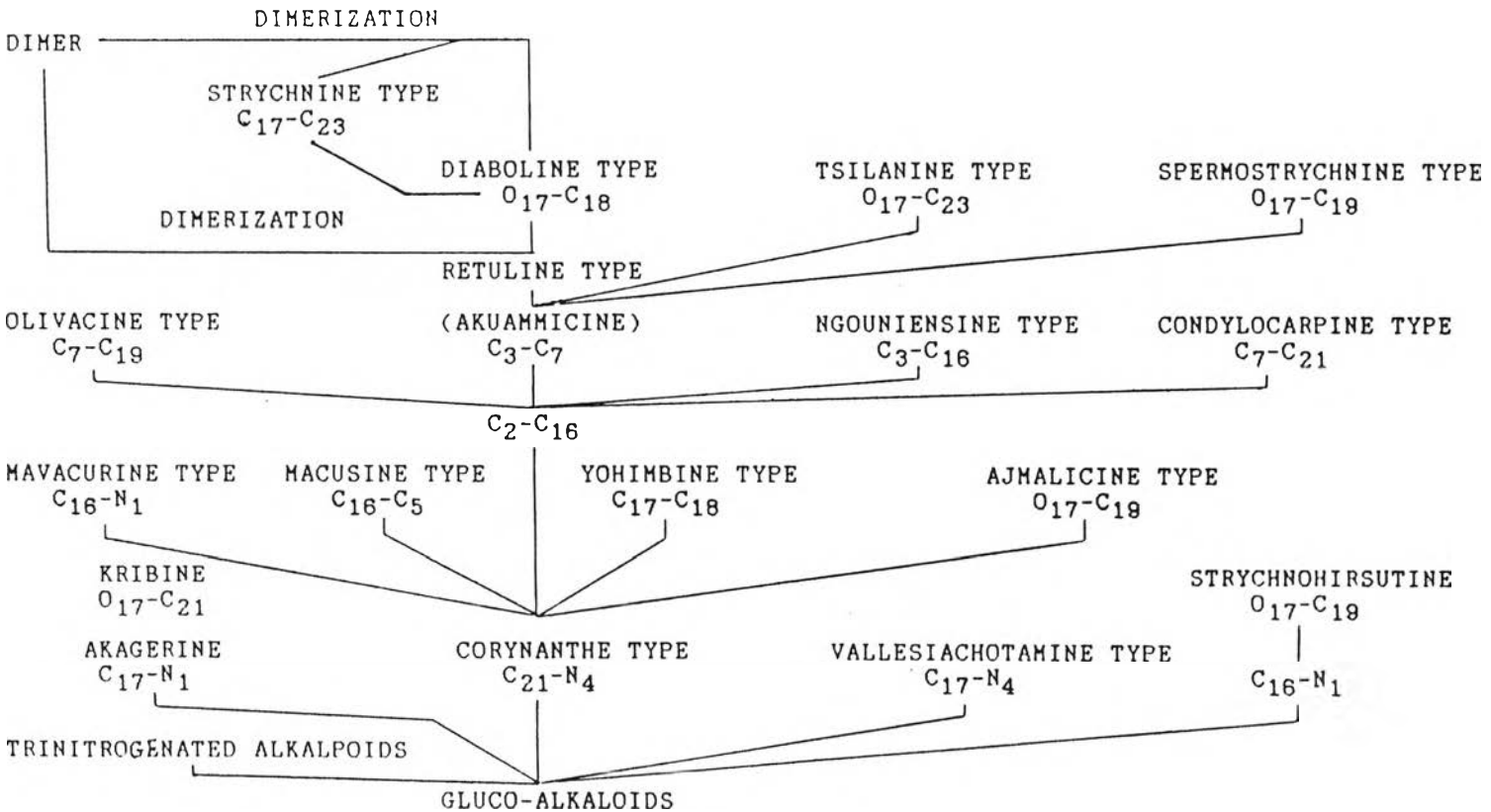


Figure 2 : Biogenetic classification of *Strychnos* alkaloids arranged by Counc

into 6 types, five of which are C-, D-, V-, S- and A-types. The other one was miscellaneous type (M-type) which the alkaloids can not be clearly differentiated. And for bisindole alkaloids, they were subdivided into 2 types : Strychnan-Strychnan type and Strychnan-Corynanthean type. All types of *Strychnos* alkaloids were further subdivided into groups which were designed especially for the alkaloids of this genus (table 2).

Table 2

Subdivision of the main types of *Strychnos* alkaloids

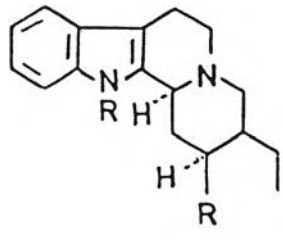
Class	Subdivisions
Class 1	Monomeric indole alkaloids
Type 1	Corynanthean (C-type)
Group C_1	E-*seco*-indole group (3)
C_2	Ajmalicine group (4)
C_3	Yohimbine group (5)
C_4	Akagerine group (6)
C_5	Mavacurine group (7)
C_6	Sarpagine group (8)
C_7	Oxindole group (9)
Type 2	Vincosan (D-type)
Group D_1	Strictosidine group (10)
D_2	Decussine group (11)
Type 3	Vallesiachotaman (V-type)
Group V_1	Antirhine group (12)
V_2	Angustine group (13)

Table 2 (continue)

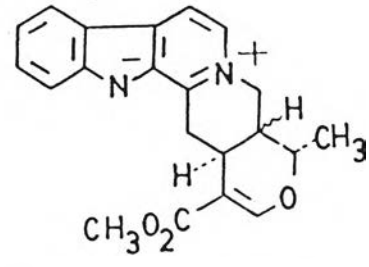
Class	Subdivisions
Type 4	Strychnan (S-type)
	Group S_1 : Retuline group (14)
	S_2 : Diaboline group (15)
	S_3 : Isostrychnine group (16)
	S_4 : Strychnine group (17)
	S_5 : Spermostrychnine group (18)
	S_6 : Tsilanine group (19)
Type 5	Aspidospermatan (A-type)
	Group A_1 : Condylocarpine group (20)
Type 6	Miscellaneous (M-type)
	Group M_1 : Ngouiensine group (21)
	M_2 : Olivacine group (22)

Table 2 (continue)

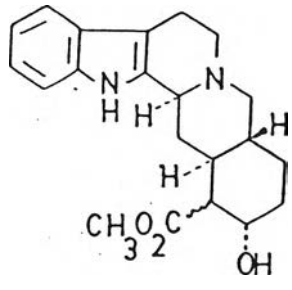
Class	Subdivisions
Class 2	Bisindole alkaloids
	Type 1 Strychnan-Strychnan (S-S type)
	Group B_1 : Retuline-Retuline (S_1-S_1) group
	B_2 : Diaboline-Diaboline (S_2-S_2) group
	B_3 : Retuline-Diaboline (S_1-S_2) group
	B_4 : Isostrychnine-Isostrychnine (S_3-S_3) group
	Type 2 Strychnan-Corynanthean (S-C type)
	Group B_5 : Diaboline-E-*seco* indole (S_2-C_1) group



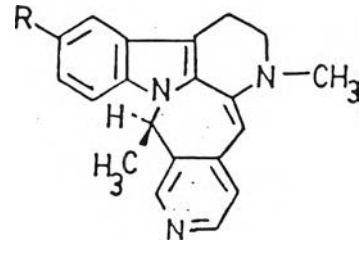
3 E-*seco* indole group (C_1)



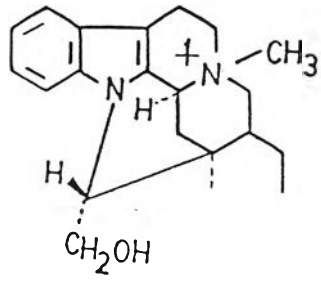
4 Ajmalicine group (C_2)



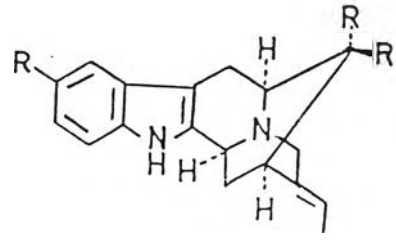
5 Yohimbine group (C_3)



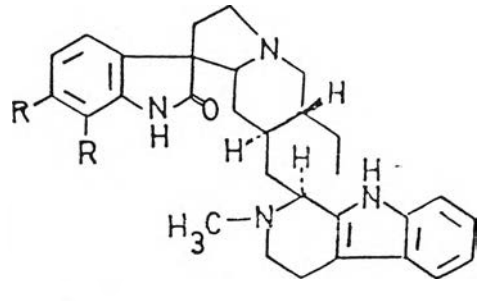
6 Akagerine group (C_4)



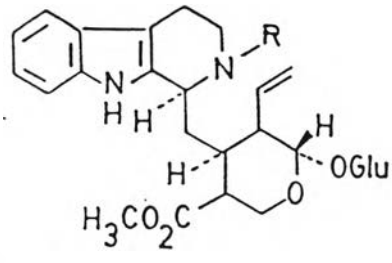
7 Mavacurine group (C_5)



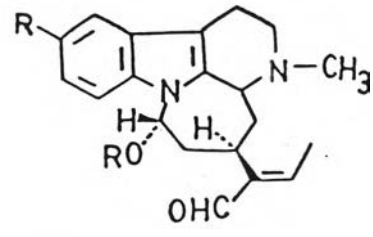
8 Sarpagine group (C_6)



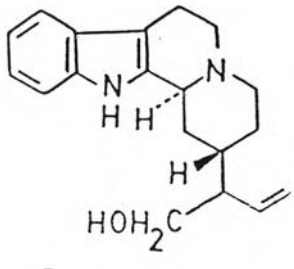
9 Oxindole group (C_7)



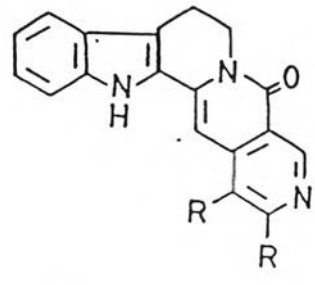
10 Strictosidine group (D_1)



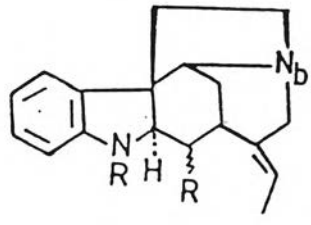
11 Decussine group (D_2)



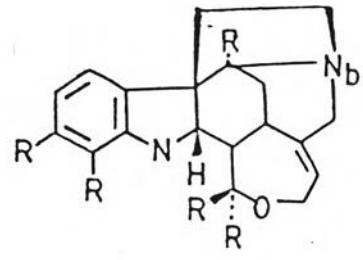
12 Antirhine group (A_1)



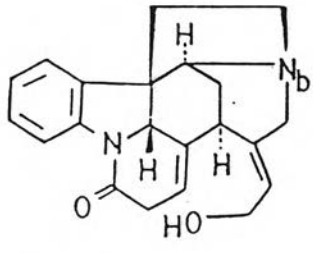
13 Angustine group (A_2)



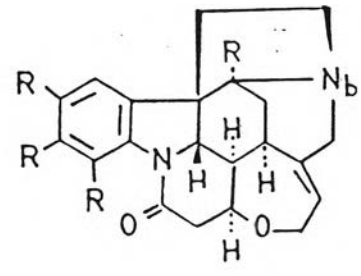
14 Retuline group (S_1)



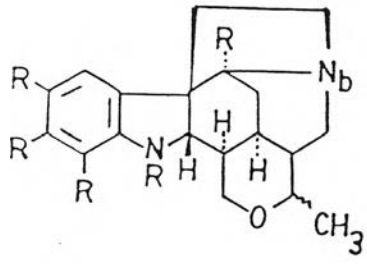
15 Diaboline group (S_2)



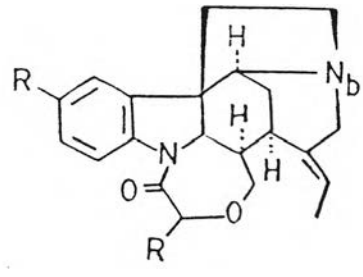
16 Işostrychnine group (S_3)



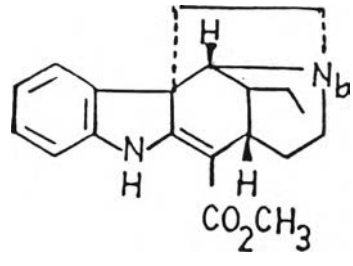
17 Strychnine group (S_4)



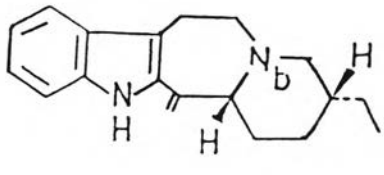
18 Spermostrychnine group (S_5)



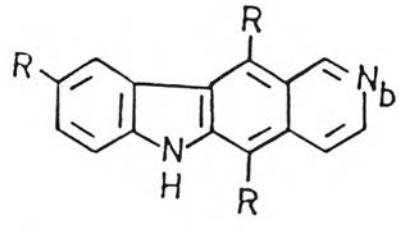
19 Tsilanine group (S_6)



20 Condylocarpine group (A_1)



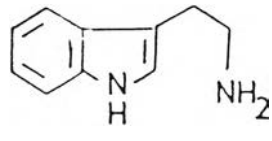
21 Ngouiensine group (M_1)



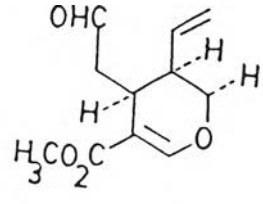
22 Olivacine group (M_2)

Biosynthesis of *Strychnos* alkaloids

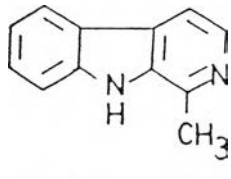
The *Strychnos* alkaloids are derived from the condensation of tryptamine (23) and a $C_{9,10}$-monoterpene moiety, secologanin (24) or other modified secologanin units (Kompis, Hesse, and Schmid, 1971). They are different from simple indole alkaloids such as harman (25) and its derivatives which are not the products of the tryptamine-monoterpene condensation. These simple indole alkaloids are less closely related form a chemotaxonomy point of view and found in only some *Strychnos* species.



23 Tryptamine



24 Secologanin

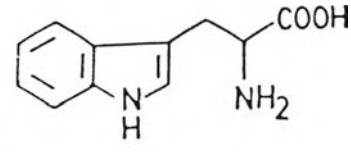


25 Harman

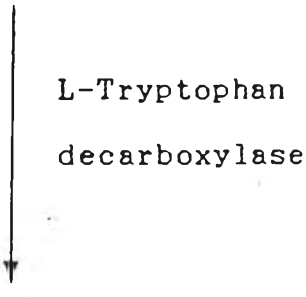
Like other terpenoid indole alkaloids, the biosynthesis of *Strychnos* terpenoid indole alkaloids involves four important pathways as follows.

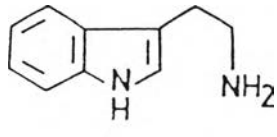
1. The Non-Terpenoid Moiety

The non-terpenoid moiety or tryptamine (23) is the decarboxylation product of L-tryptophan (26). The enzyme that indicated to involve in this process is L-tryptophan decarboxylase (Baxter, and Slaytor, 1972; Scott, and Lee, 1975).



26 L-Tryptophan





23 Tryptamine

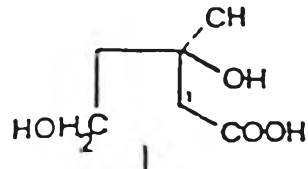
Figure 3 : Formation of tryptamine.

2. The Terpenoid Moiety

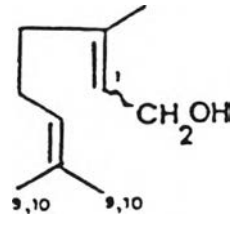
The C_9,C_{10}-monoterpene moiety is derived from secologanin (24). This was first suggested by Thomas (1961). The biosynthetic routes to secologanin (24) require previous transformations which involve the production of geraniol (28) and its *cis*-isomer nerol (29) (Battersby, Brown, Knight et al., 1966; Hall, McCapra, and Money, 1966; Loew, Goeggel, and Arigoni, 1966; Battersby, Byrne et al., 1968; Cordell, 1974) and which later lead to loganin (35) (Battersby, Brown, Kapil et al., 1966; Battersby, Kapil et al., 1968; Loew, and Arigoni, 1968; Battersby, Hall, and Sauthgate, 1969) and secologanin (24) (Battersby, Burnett, and Parsons, 1969a). Mevalonic acid (27) was proved to be a precursor of geranirol (28) (Battersby, Byrne et al., 1968; Coscia, Botta, and Guarnaccia, 1970; Popjak, 1970).

From geraniol (28) and nerol (29), the hydroxylation at C_{10} of both compounds is the primary step (Battersby, Brown, and Payne, 1970). The following stages are proceeded through the oxidation at C_1, C_9 and C_{10} to form a compound with trialdehyde functions (32) which later cyclization to give a cyclopentane intermediate compound (33) (Escher, Loew, and Arigoni, 1970) and then the iridoid skeleton compounds, 7-deoxyloganin (34) and loganin (35) respectively (Battersby, Burnett, and Parsons, 1970; Inouye et al., 1972). The final, loganin (35) is directly

clevaged to give rise to its corresponding *seco*-derivative, secologanin (24) (Guarnaccia, and Coscia, 1971). The overall view of the biosynthetic pathway is shown in figure 4.

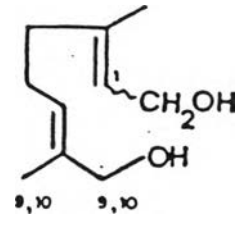


27 Mevalonic acid



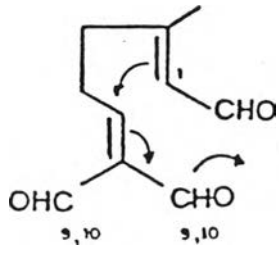
28 Geraniol (*E*-isomer)

29 Nerol (*Z*-isomer)

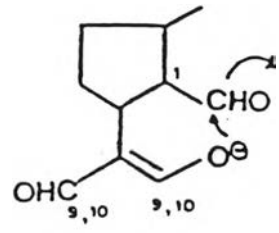


30 10-Hydroxy-geraniol (*E*-isomer)

31 10-hydroxy-nerol (*Z*-isomer)



32 Trialdehyde



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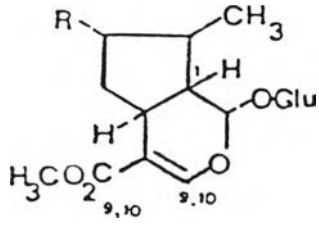
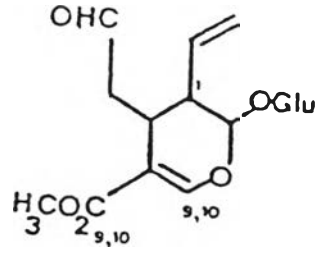


34 7-Deoxyloganin (R =H)

35 Loganin (R = OH)



24 Secologanin

Figure 4 : Hypothetical pathway for the conversion of geraniol(28)and nerol(29)to loganin(35)and secologanin(24)

3. The Key Role Intermediates Strictosidine (36)

The condensation of tryptamine (23) with secologanin (24) yields two epimeric-β-carboline gluco-alkaloids : strictosidine (36) (isovincoside) and vincoside (37) (figure 5) (Battersby, Burnett, and Parsons, 1969b). But only strictosidine (36) has been defined as the precursor of various types of monoterpenoid indole alkaloids including the *Strychnos* alkaloids (Cordell, 1974; Stockigt, and Zenk, 1977; Brown, Leonard and, Sleigh, 1978; Rueffer, Nagakura, and Zenk, 1978; Nagakura, Rueffer, and Zenk, 1979; Stockigt, 1980; Zenk, 1980; Herbert, 1981a; 1981b). The enzyme catalysing the formation of strictosidine (36) is called strictosidine synthase (Stöckigt, and Zenk, 1977; Stöckigt, 1980).

The relationships between strictosidine (36) and the various types of monoterpenoid indole alkaloids are demonstrated in figure 6 (Nagakura, Rüeffer, and Zenk, 1979; Stöckigt, 1980).

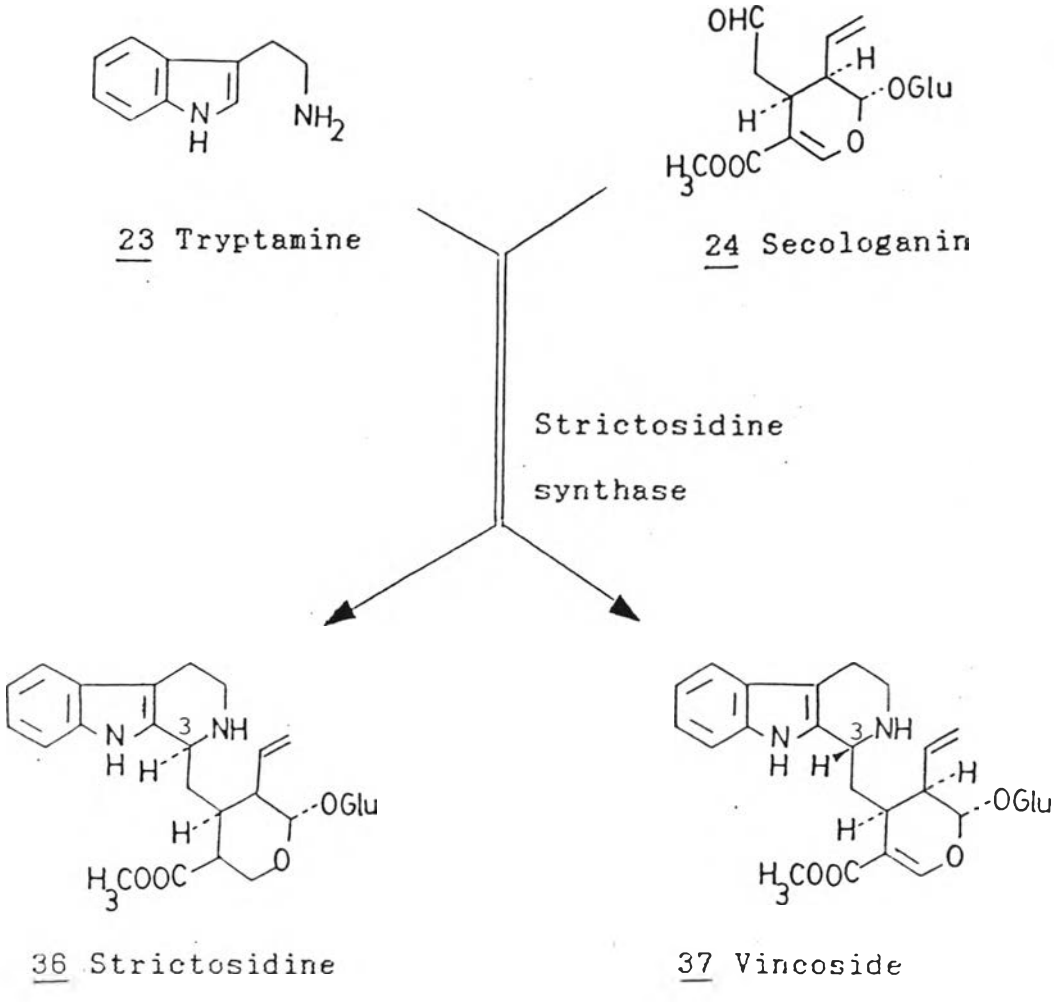


Figure 5 : Formation of strictosidine (36)

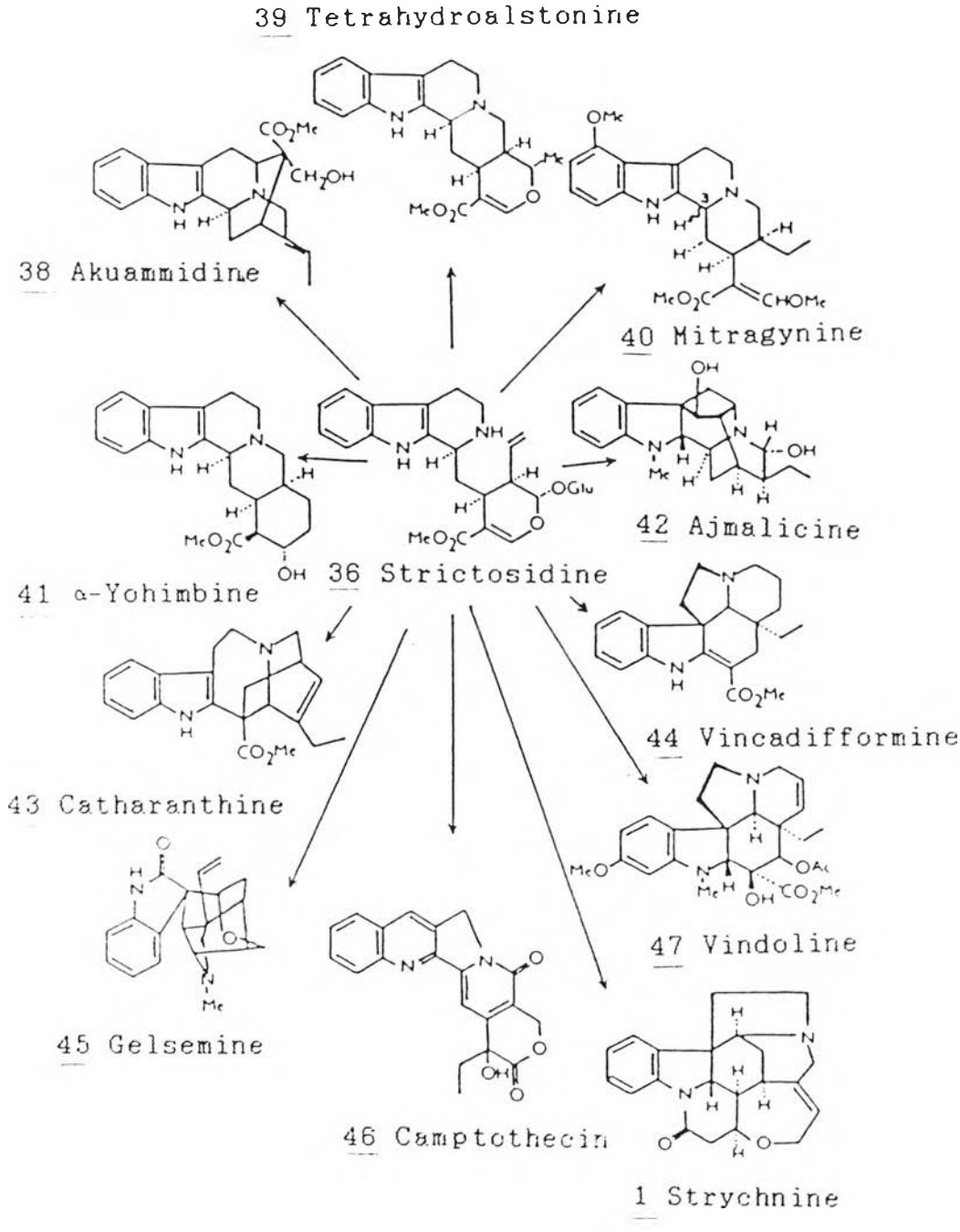


Figure 6 : Strictosidine (36) as a key role intermediate in indole alkaloids biosynthesis.

4. The derivation of *Strychnos* alkaloids

The typical biosynthtic route of *Strychnos* alkaloids has been indicated by Battersby, and Hall (1969); Schlatter et al. (1969); Scott, Cherry, and Qureshi (1969); Heimberger, and Scott (1973). The overall pathway has proceeded *via* strictosidine (36), geissoschizine (48), dehydropreakuammicine (49) and Wieland-Gumlich aldehyde (50). It seems that this route must be the central alkaloids biosynthesis pathway in *Strychnos* species. However in more recent works (Kan-Fan, and Husson, 1979; Rüeffer et al., 1979; Stöckigt, Höfle, and Pfitzner, 1980; Herbert, 1981a; 1981b) suggested that geissoschizine (48) seems to involve in the pathway after two intermediates, 4,21-dehydrocorynantheine aldehyde (51) and 4,21-dehydrogeissoschizine (52), and the relationships among the intermediates in the biosynthesis of *Strychnos* alkaloids are improved and demonstrated in figure 7.

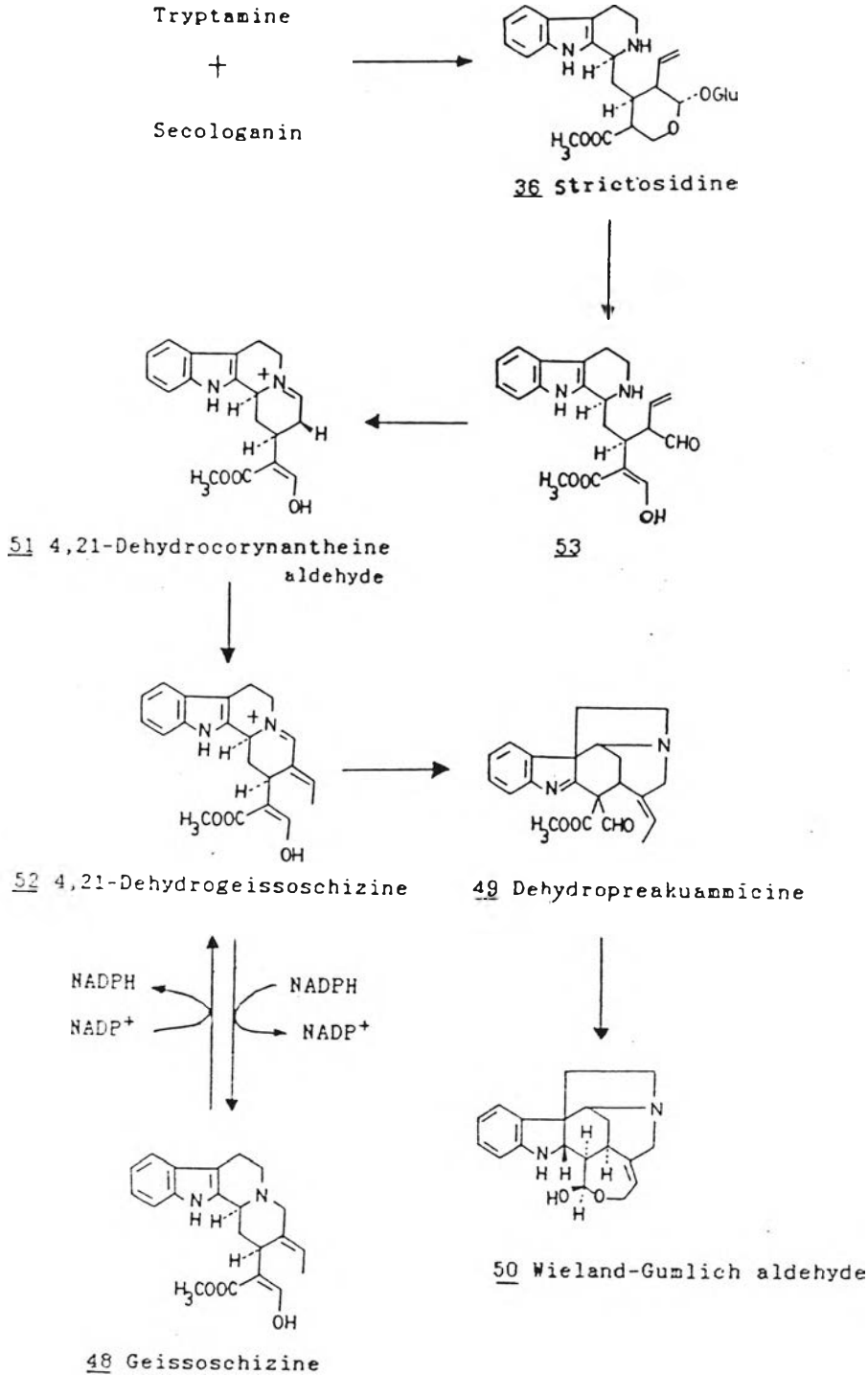
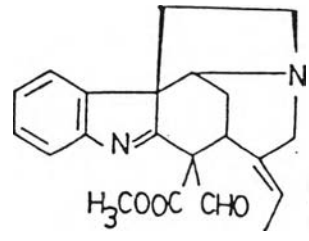


Figure 7 : Overall view of biosynthesis of the *Strychnos* alkaloids.

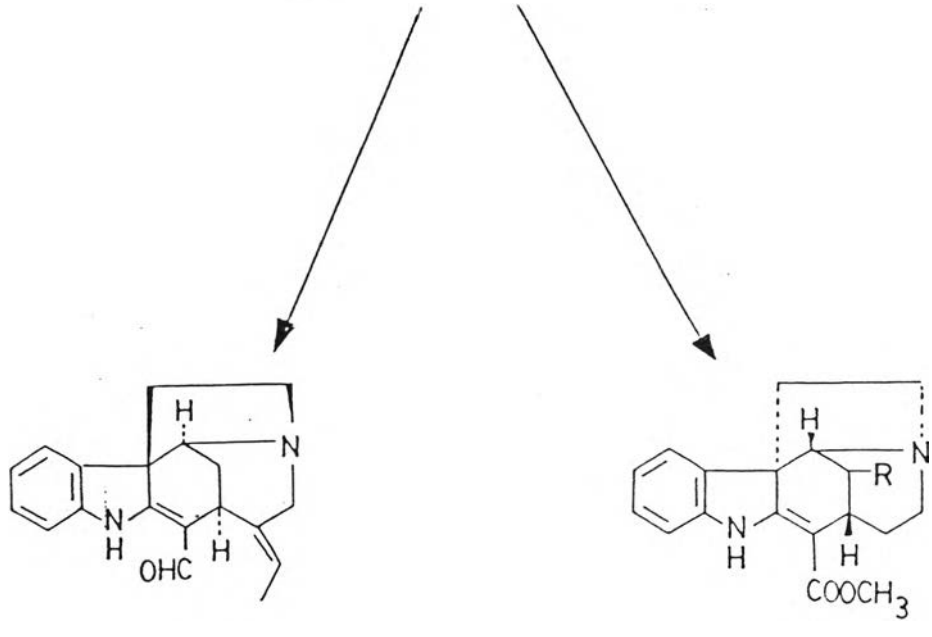
On the pathway, the groups of *Strychnos* alkaloids that classified by Charoendee Pingsuthiwong (1986) can be demonstrated. The *Strychnos* alkaloids that derived from strictosidine (36) are strictosidine group (10) (D_1) and angustine group (V_2) (13). The biosynthesis of these alkaloids are demonstrated by Bisset (1980). From the second intermediate 53, by opening of the C_{17}-O-C_{21} bond of strictosidine (36), serveral *Strychnos* alkaloids that derived are E-*seco* indole group (C_1) (3) and oxindole group (C_7) (9) with the usambarensine skeleton (Bisset, 1980), yohimbine group (C_3) (5) (Cordell, 1974), akagerine group (C_4) (6) (Bisset, 1980; Rolfsen, Bohlin et al., 1978), decussine group (D_2) (11) (Rolfsen et al., 1981), and antirhine group (V_1) (12) (Bisset, 1980).

About the two proposed intermediates, 4,21-dehydrocorynantheine aldehyde (51) has not been accepted to be a precursor of any indole alkaloids while the later, 4,21-dehydrogeissoschizine (52) is considered as the important branch point intermediate (Kan-Fan, and Husson 1979; Rüeffer et al., 1979) and produces sarpagine (C_6) (8) and ajmalicine groups (C_2) (4) (Cordell, 1974; Rüeffer et al., 1979; Stöckigt, Höfle, and Pfitzner, 1980; Herbert, 1981b). Geissoschizine (48) is the next recognized intermediate which Bisset (1980) pointed to it as a precursor of E-*seco* indole group (C_1) (3) and mavacurine group (C_5) (7).

The other two types of *Strychnos* alkaloids, A- and S-types are derived from dehydropreakaummicine (49). Bisset (1980) explained that the *Strychnos* alkloids with A-type are derived by lossing of the aldehyde function and rearrangement of the terpenoid portion of dehydro-preakaummicine (49) (figure 9) . On the other hand, loss-ing of carbomethoxyl function but without rearrangement of the molecule can lead to the next recognized intermediat-ed, nor-C-fluorocurarine (54), which is the precursor of Wieland-Gumlich aldehyde (50) and all S-type alkaloids including the bisindole alkaloids with S-S type. While the other type of bisindole alkaloids, S-C type, was demonstrated to be derived from the coupling between C- and S-types alkaloids (figure 9) (Rapepol Bavovada, 1983).



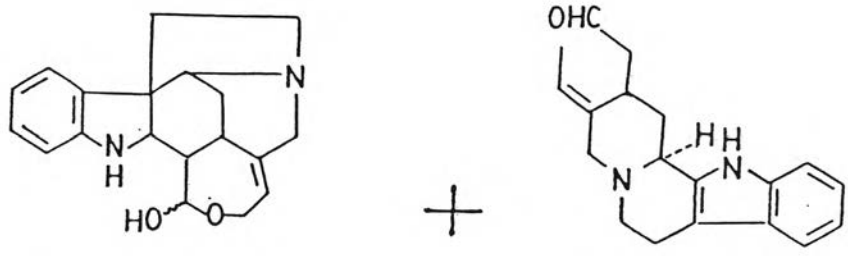
49 Dehydropreakuammicine



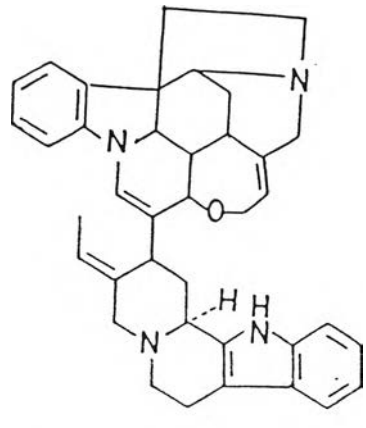
54 Nor-C-fluorocurarine

A-type alkaloid

Figure 8 : Alkaloids derived from dehydropreakuammicine(49)



50 Wieland-Gumlich aldehyde + 55 Geissoschizal



56 Longicaudatine

Figure 9 : Biosynthesis of longicaudatine (56), the S-C type alkaloid.

Pharmacology of *Strychnos* alkaloids

Strychnos species have been long used in folk medicine and in arrow and dart poisons throughtout the world. In America, because of their strong muscle-relaxant activity, the *Strychnos* species are well known to be used as a major component in preparation of some curares (Marini-Bettòlo, 1959). Only some *Strychnos* have been investigested to yield the convulsive alkaloids, strychnine (1) and brucine (2) (Marini-Bettòlo et al., 1972). A few of the African *Strychnos* are consumed as curarizing arrow-poisons, while some species possess central-nervous system stimulant action (Bisset, and Leeuwenberg, 1968; Bohlin, Ali, and Sandberg, 1974; Verpoorte, 1976; Bohlin, 1978; Rolfsen, Hakizadeh et al., 1978). Asian *Strychnos* species are also used for producing arrow and dart poisons to cause convulsive death (Bisset, 1966; Bisset, and Woods, 1966), only some weak curare-like action is observed (Lin, 1952; Bisset et al., 1977). Moreover, they are valuable for the several medicinal application such as appitizer, tonic, antipyretic, and anthelmintic. The review of the ethanobotany have been published by Bisset (1970; 1974).

The major constituents in *Strychnos* species are indole alkaloids. Many studies have been carried out to establish the types and the structures of the alkaloids responsible for their pharmacological and toxicological activities, not only muscle relaxant and convulsive activity but other actions are also described.

1. Muscle-relaxant activity

Muscle-relaxant effects of *Strychnos* species may be subdivided into muscle-relaxant and truely curarizing (Ohiri, et al., 1983). Muscle-relaxant is a weak action on neuro-muscular junctions while curarizing activity is a phenomenological term describing neuro-muscular block of impulse transmission of the motor end-plates as a result of inhibition of acetylcholine, the result is complete paralysis of the skeletal or striated muscle apparatus. This effect is the main activity of American *Strychnos* species. Many of bis-quaternary indole alkaloids have been detected corresponding to this action (Marini-Bottòlo, 1959). Up till now, this activity has been found in other types of *Strychnos* alkaloids too.

a) Bis-quaternary indole alkaloids (58-66)

These alkaloids are potent curarizing agents, the presence of two quaternary nitrogens in a single molecule being responsible for the high activity. The activity depends on the distance between the quaternary nitrogens (Ohiri et al., 1983). For optimal activity, the distance must be about 14 A°. Whereas the distance decreases, the activity decreases too. The precense of hydroxyl group at C-18 will increase the activity. The more polar alkaloids have greater neuro-muscular activity than the less polar alkaloids.

This curarizing activity of bis-quaternary indole alkaloids from *Strychnos* species is similar to the well-known bis-benzylisoquinoline alkaloid d-tubocurarine (57) from *Chondrodendron tomentosum* Ruiz.et Pav. of the family Menispermaceae. Their mechanism are non-depolarizing competitive antagonism of acetylcholine for postsynaptic receptors and a weak activity for presynaptic receptor at the neuro-muscular junctions. The effect is antagonized by a small dose of neostigmine, but in large doses, it will prolong paralytic action.

The bis-quaternary indole alkaloids may be divided into three groups according to the transformation at the central eight-membered ring between the monomers of the molecule (Ohiri et al., 1983).

a.1 Toxiferine group (58-60)

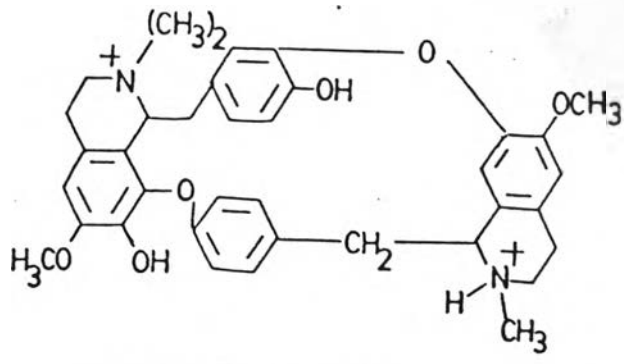
Alkaloids in this group show slow onset of paralysis with a long duration. Toxiferine (58) has the most potent activity which even more than d-tubocurarine (57).

a.2 Curarine group (61-63)

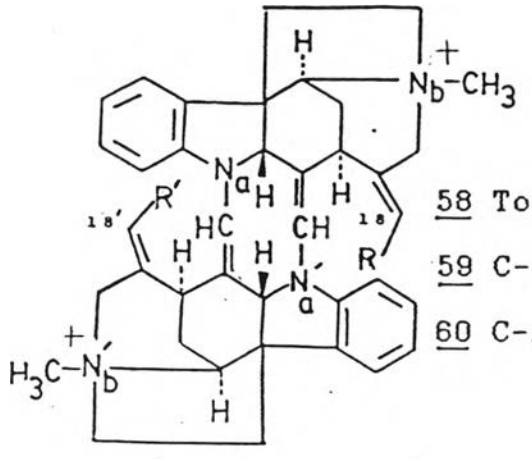
The action of alkaloids in this group is sustained in moderate duration. C-curarine (62) is the most potent member and being more potent than d-tubocurarine (57). The most active effect of this group is possessed by an ether oxygen in the central eight-membered ring.

a.3 Calebassine group (64-66)

The action of this group is less potent than d-tubocurarine (57) and has a short duration. The low activity may be explained by the fact that the presence of the C-C bridge in the central eight-membered ring reduces the distance between the quaternary nitrogen down to 8.6 A°.



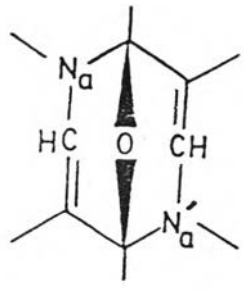
57 d-Tubocurarine



58 Toxiferine ; R = R′= OH

59 C-Dihydrotoxiferine ; R = R′= H

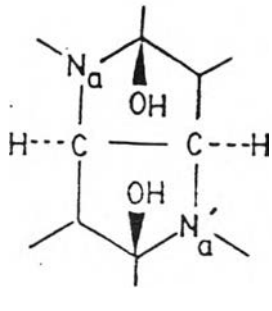
60 C-Alkaloid H ; R = H, R′=OH



61 C-Alkaloid E ; R = R′ = OH

62 C-Curarine ; R = R′= H

63 C-Alkaloid G ; R =H, R′= OH



64 C-alkaloid A ; R = R′= OH

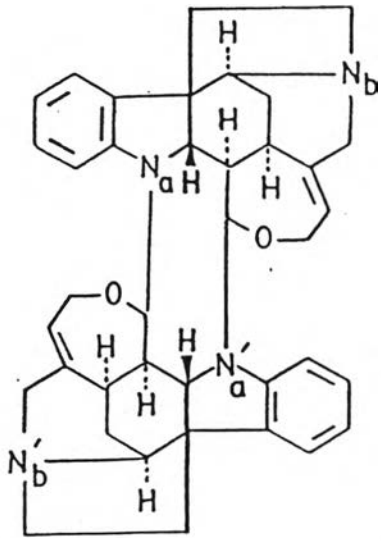
65 C-Calebassine ; R = R′= H

66 C-Alkaloid F ; R = H, R′= OH

b) Bis-tertiary indole alkaloids

The alkaloids that have been studied are caracurine V (67), its *N*-oxide (68-69) (Verpoorte, and Baerheim Svendsen, 1978) and bisnor-dihydrotoxiferine (70) (Ohiri et al., 1983).

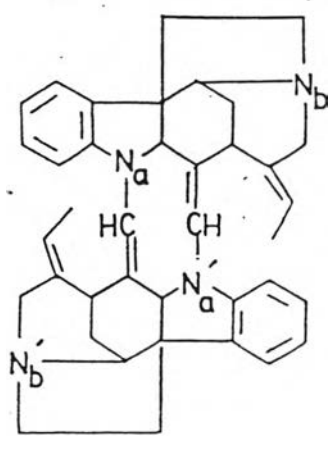
Caracurine V (67) shows a weak muscle-relaxant activity which is not antagonized by choline-esterase inhibitors. The *N*-oxide, both mono- and di-*N*-oxides (68-69), are less toxic and active than the parent alkaloid. Bisnor-dihydrotoxiferine (70) also has a muscle-relaxant activity.



67 Caracurine V

68 Caracurine V *N*-oxide

69 Caracurine V di-*N*-oxide

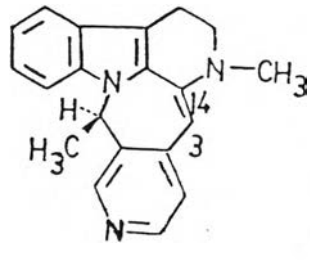


70 Bisnor-dihydrotoxiferine

c) Decussine group

Decussine (71) has a pronounced muscle-relaxant activity (Rolfsen, Olaniyi, and Sandberg, 1980) which can not be antagonized by neostigmine. While 3,14-dihydro-decussine (72) gives only a weak effect. The pharmacolo - gical results of these alkaloids point to a possible role of the 3,14 double bond for the muscle-relaxant effect (Rolfsen et al., 1981).

This alkaloid type may act as inhibitors of the enzyme choline acetyltransferase (ChAc) which is responsi-ble for the synthesis of acetylcholine. It is found that styryl-pyridine analogs possess anti-ChAc activity. It is necessary to have the electron-donor properties of the benzene ring conjugated with the electron-acceptor proper-ties of the pyridine ring through a double or triple bond to produce a thin, flat molecule. This hypothesis fits into the pronounced muscle-relaxant activity of decussine (71) and 3,14-dihydrodecussine (72) (Ohiri et al., 1983).



71 Decussine

72 3,14-Dihydrodecussine

d) Other type alkaloids

d.1 Quaternary alkaloids

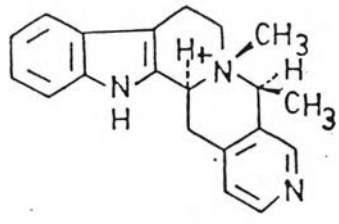
Some quaternary *Strychnos* alkaloids have pronounced muscle-relaxant activity.

- Malindine (73) shows a strong activity which can not be antagonized by neostigmine (Olaniyi, Rolfsen, and Verpoorte, 1981).

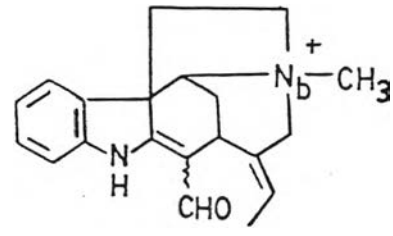
- Fluorocurarine (74), the only one quaternary alkaloids with retuline skeleton group, and the mavacurine type alkaloids (C-mavacurine (75) and C-fluorocurine (76)) have a weak curare activity (Waser, 1972, quoted in Ohiri et al., 1983).

- 11-Methoxy-macusine A (77) shows muscle-relaxant activity (Verpoorte, Bohlin et al., 1983).

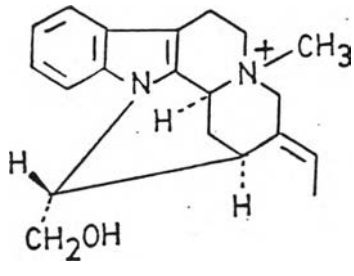
- The synthesis quaternary strychnine alkaloids (78-79) are shown to cause a muscle-relaxant effect instead of a convulsive effect (Karrer, Eugster, and Waser, 1949; Iskander, and Bohlin, 1978).



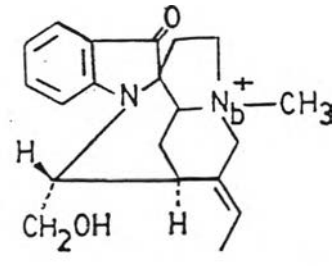
73 Malindine



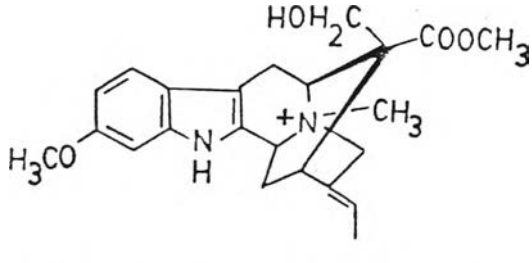
74 Fluorocurarine



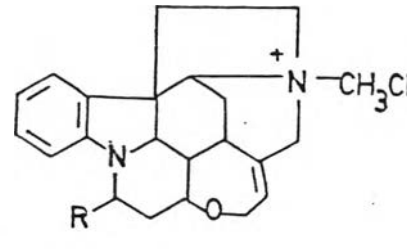
75 C-Mavacurine



76 C-Fluorocurine



77 11-Methoxy-macusine A



78 R = =O

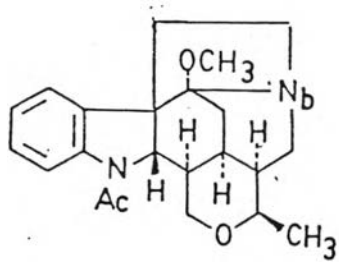
79 R = H, H

d.2 Tertiary alkaloids

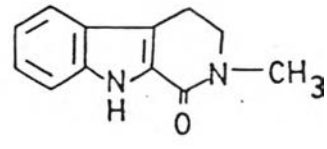
- *O*-Methyl-N_a-acetyl-strychnosplendine (80) shows the strong muscle-relaxant activity *in vivo* and *in vitro* (Goonetilleke, Rolfsen, and Rajapakse, 1980; Weeratunga et al., 1984).

- Strychnocarpine (81) shows a weak muscle-relaxant action (Rolfsen et al., 1980).

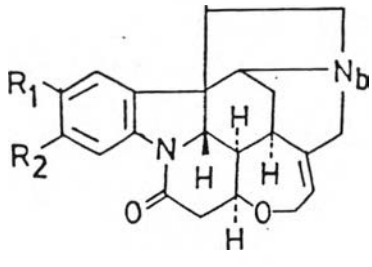
- In the structure-activity-relationship studies of strychnine derivatives, the synthesis 10- and 11-acetamido-strychnine (82-83) are pronounced muscle-relaxant activity instead of convulsive effect (Bohlin, Ali, and Iskander, 1975). An increasing of the chain length of the 10-carboxamido function causes an increasing of this activity (Bohlin, and Iskander, 1978).



80 *O*-Methyl-N_a-acetyl-strychnosplendine



81 Strychnocarpine



82 10-Acetamido-strychnine ; R_1 = NHAc, R_2 = H

83 11-Acetamido-strychnine ; R_1 = H, R_2 = NHAc

2. Convulsive activity

Convulsive activity is well-known as the action of arrow and dart poisons from Asian *Strychnos* species. The activity may be resolved into clonic and tonic convulsants. Clonic convulsants are an alternating contraction and relaxation of the muscles whereas tonic convulsants are sustained rigidity of the muscle. These effects are the results of the antagonistic effect on glycine, an important inhibitor transmitter in the spinal cord (Ohiri et al., 1983). This leads to enhance relax reponses and, in large dose, tonic convulsants occure.

The *Strychnos* alkaloids that responsible for this activity are described as follows.

a) Strychnine group

According to Ohiri et al. (1983), this alkaloid group can be rearranged to 3 series.

a.1 Normal series

The well-known alkaloid strychnine (1) is prototype. It was first isolated from the seed and bark of *Strychnos nux-vomica* L. in 1819. Strychnine (1) and 12-hydroxy-strychnine (84) are the strongest convulsive activity *Strychnos* alkaloids. The phenolic hydroxyl group in 12-hydroxy-strychnine (84) is of minor importance for the pharmacological effect but it strongly forms hydrogen-bond to the amide carbonyl. The effect is such

as to give absorptive properties of 12-hydroxy-strychnine (84) similar to those of strychnine (1).

Strychnine (1) alkaloids especially in this series have been several studies for their structure-activity-relationships which can be concluded as follows.

- Free hydrogen at 10- and 11- positions are important to the receptor. While the free hydrogen at 10-position is more important.

- Free tertiary nitrogen is important for their activity. The activity decreases if *N*-oxide is formed. If it is quaternized, the convulsive activity will decrease or absent but show a muscle-relaxant effect. The explaination is that the quaternization prevents passage through the blood brain barrier to the active site in central-nervous system.

- The 19,20-double bond is important for the activity.

- The amide lactam ring is necessary to give optimal activity.

Bohlin discussed in his Ph.D. thesis (1978) for the pharmacological activity of strychnine (1) and its derivatives that these compounds had at least two components of action, muscle-relaxant and convulsant. The major effect obtained when tested in vivo will be deter-

mined by the balance between these two effects. It was dependent upon the physico-chemical properties of compounds.

a.2 Pseudo series (85)

The pseudo series (85) are slightly less action than strychnine (1). Because their 3-α-hydroxyl group get a lesser fitness with the receptor.

a.3 *N*-methyl-*sec*-pseudo series (86)

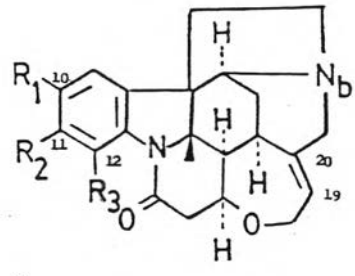
This series are less active than strychnine (1). Because the 3-keto group extrudes from the back of the molecule causing a less satisfactory fitness with the receptor. They show only clonic convulsive action.

b) Diaboline group (15)

This group has the molecular structure analogous to strychnine group (17). But the opening amide lactam ring decreases the potency and shows only clonic convulsive action (Ohiri et al., 1983).

c) Spermostrychnine group (18)

This group has the same convulsive activity as the diaboline group (15) (Ohiri et al., 1983).

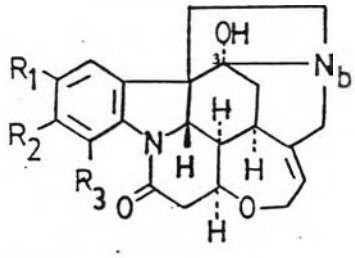


1 Strychnine ;

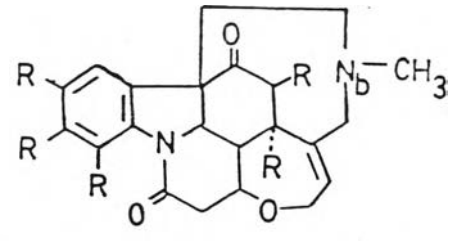
$R_1 = R_2 = R_3 = H$

84 12-Hydroxy strychnine

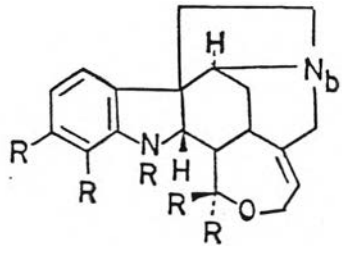
$R_1 = R_2 = H$, $R_3 = OH$



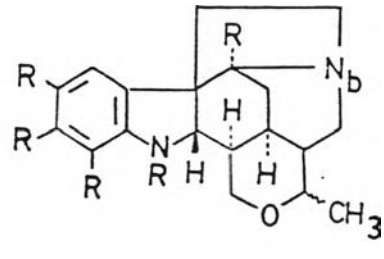
85 Pseudo series



86 *N*-methyl-*sec*-pseudo-series



15 Diaboline group



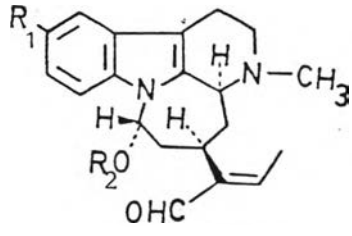
18 Spermostrychnine group

d) Other type alkaloids

- The alkaloid akagerine (87) and its derivatives (90-93) are the potent convulsive agents but less potency than strychnine (1) (Rolfsen, Bolhin et al., 1978; Rolfsen, Olaniyi, and Hylands, 1980).

- Macusine B (92) shows only clonic convulsant *in vivo* (Leonard, 1965a; 1965b).

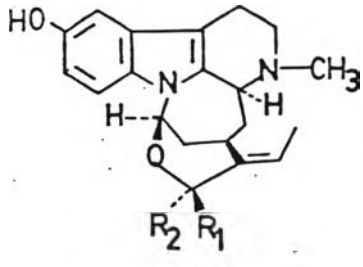
- (+)-Tubotaiwine (93) shows only a weak clonic convulsant in vivo (Bohlin et al., 1979).



87 Akagerine ; $R_1 = R_2 = H$

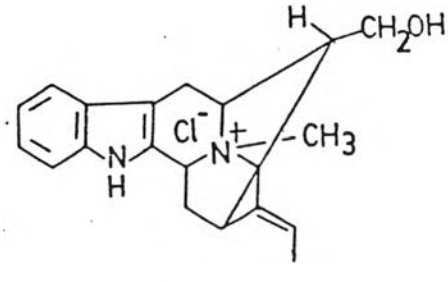
88 17-*O*-Methylakagerine ;
$R_1 = H$, $R_2 = CH_3$

89 10-Hydroxy-17-*O*-methyl-akagerine ; $R_1 = OH$, $R_2 = CH3$

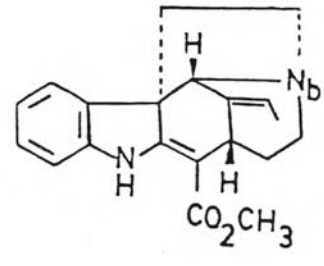


90 10-Hydroxy-21-*O*-methyl-kribine ; $R_1 = OCH_3$, $R_2 = H$

91 10-Hydroxy-epi-21-*O*-methyl-kribine ; $R_1 = H$, $R_2 = OCH_3$



92 Macusine B



93 (+)-Tubotaiwine

3. Cytotoxic activity

a) Usambarane skeleton alkaloids (95-106)

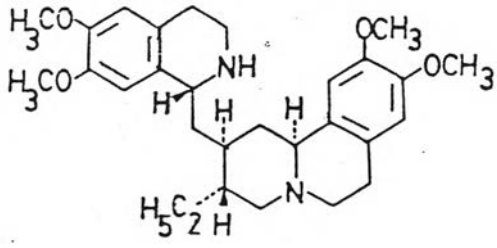
Usambarane skeleton is the skeleton type of E-*seco* indole group (3) and oxindole group (9) of corynanthean type alkaloids (Charoendee Pingsuthiwong, 1986). These alkaloids have a high cytotoxic activity (Leclercq et al., 1986). This fact can be related to the relationship between the structure of these alkaloids and emetine (94) which possess well-known cytotoxicity. All of which result from condensation of a monoterpenoid unit and an amino unit. Strychnopentamine (95) is the most active compound.

The structure-activity-relationships of these usambarane skeleton are dicussed as follows.

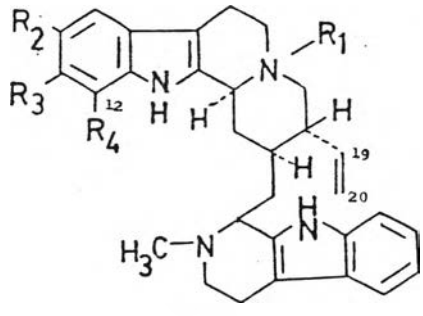
- The *N*-methyl-pyrrolidine group on C-12 increase the activity (Tits et al., 1984). This causes strychnopentamine (95) and strychnophylline (105) to be more potent than 11-hydroxy-usambarine (99) and strychnofoline (106) repectively.

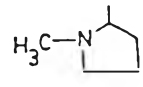
- The quaternization of the alkaloids strongly decrease the cytotoxic activity.

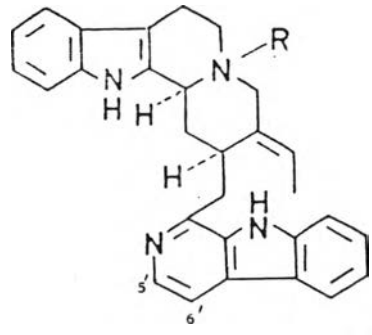
- The 5',6'-dihydro derivative is more active than the one with the extra double bond.



94 Emetine



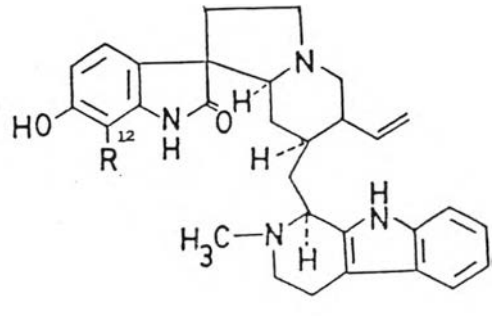
		R1	R2	R3	R4	
95	Strychnopentamine	-	H	OH		
96	Dihydro-usambarine	-	H	H	H	18,19-dihydro
97	Usambarine	-	H	H	H	
98	10-Hydroxy-usambarine	-	OH	H	H	
99	11-Hydroxy-usambarine	-	H	OH	H	
100	N_b-Methyl-10-hydroxy-usambarine	CH_3	OH	H	H	
101	N_b-Methyl-11-hydroxy-usambarine	CH_3	H	OH	H	

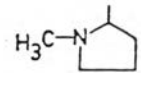


102 Dihydro-usambarensine ; R = -, 5',6'-dihydro

103 Usambarensine ; R = -

104 N_b-Methyl-usambarensine ; R = CH_3



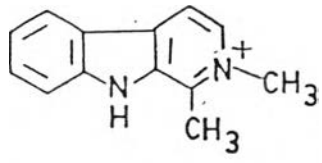
105 Strychnophylline ; R = 

106 Strychnofoline ; R = H

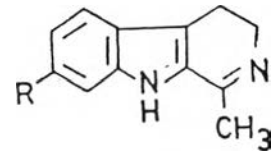
b) Other alkaloids

Other *Strychnos* alkaloids that have been investigated for the cytotoxic activity are melinonine F (107) (Bassleer et al., 1982 , 1983 ; Caprasse, and Angenot, 1982), harmine (108), harmol (109), harmaline (110), N_b-methyl-harmalane (111), 4,21-dehydro-geissoschizine (52), akagerine (87), dolichantoside (114), isodolichantoside (115), methylantirhine (116) (Leclercq et al., 1986), alstonine (112), serpentine (113) (Beljanski, and Bugiel, 1979, quoted in Ohiri et al., 1983), diaboline (117), henningsoline (118), holstiine (119) (Hokanson, 1976), ellipticine (120) and 9-methoxyellipticine (121) (Neuss, 1980), bisnor-dihydrotoxiferine (70) (Melo et al., 1987)

There are no discussion about the structure-activity-relationships of these alkaloids. And some of them are inactive in other report or very less active in the report, such as strychnan type, ajamalicine and bis-indole alkaloids. The only alkaloids that have certain activity are melinonine F (107) and ellipticine alkaloids (120-121) which are the well-known cytotoxic agents. These alkaloids have a planar heterocyclic ring which can be inserted between DNA base pairs. The effect is inhibition of DNA, RNA or protein synthesis (Neuss, 1980; Bassleer et al., 1982, 1983; Caprasse, and Angenot, 1982).



107 Melinonine F



108 Harmine

R = OCH_3, 5,6-dehydro

109 Harmol ;

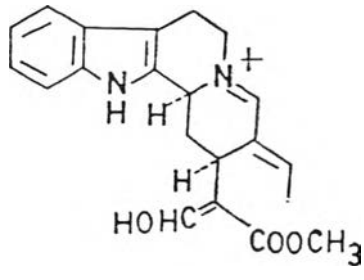
R = OH, 5,6-dehydro

110 Harmaline ;

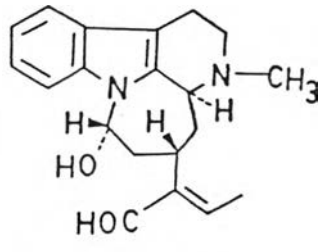
R = OCH_3

111 N_b-Methyl-harmalane ;

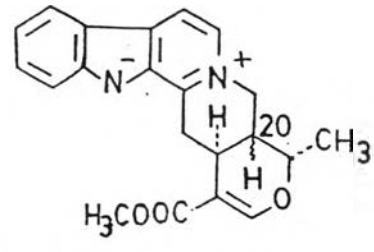
R = H, Nb-CH_3



52 4,21-Dehydrogeissoschizine

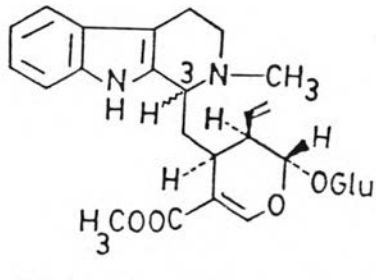


87 Akagerine



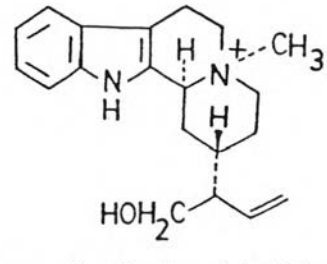
112 Alstonine ; 20-α -H

113 Serpentine ; 20-β -H

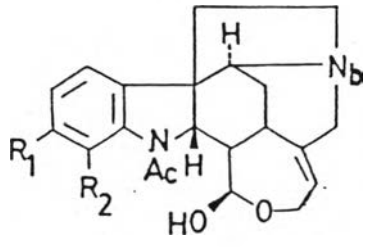


114 Dolichantoside ; 3-α -H

115 Isodolichantoside ; 3-β -H



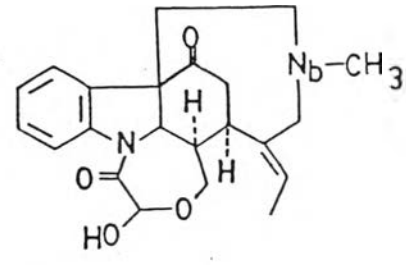
116 Methylantirhine



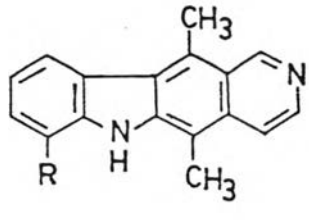
117 Diaboline ; $R_1 = R_2 = H$

118 Henningsoline ;

$R_1 = OCH_3$, $R_2 = OH$



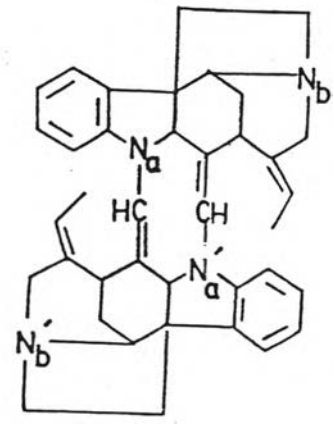
119 Holstiine



120 Ellipticine ; R = H

121 9-Methoxyellipticine ;

$R = OCH_3$



70 Bisnor-dihydrotoxiferine

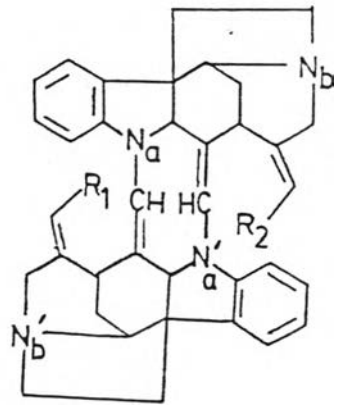
4. Antimicrobial activity

The screening of antimicrobial activity of some plants belonging to the Apocynaceae and Loganiaceae were carried out and some *Strychnos* species were shown to possess this activity. The alkaloids that responsible for the action are bis-tertiary indole alkaloids and ellipticine type alkaloids (Verpoorte, Beek et al., 1983).

a) Bis-tertiary indole alkaloids

The alkaloids that exhibit antimicrobial activity are bisnor-dihydrotoxiferine (70), bisnor-C-alkaloid H (123) and caracurine V (67). The di-*N*-oxides of bis-nordihydro-toxiferine (122) and caracurine V (67) have a little activity. It was concluded that these alkaloids exhibit a bacteriostatic effect rather than a bactericidal effect (Verpoorte et al., 1978).

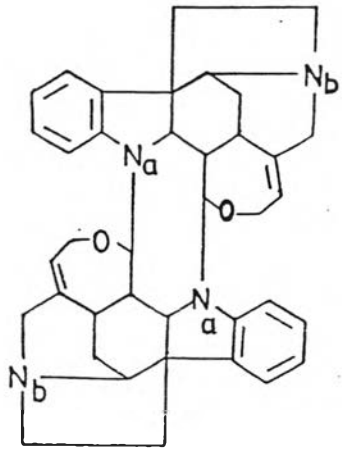
For their antimicrobial spectra, caracurine V (67) is active against *Echerichia coli, Pseudomonas aeruginosa* and *Staphylococcus* species (Verpoorte et al., 1978). Bisnor-dihydrotoxiferine (70) is a board antimicrobial spectrum against gram-positive, gram negative, acid-fast bacteria and fungi but relatively weak (Melo et al., 1987).



70 Bisnor-dihydrotoxiferine ;
$R_1 = R_2 = H$

122 Bisnor-dihydrotoxiferine di-*N*-oxide

123 Bisnor-C-alkaloloid H ;
$R_1 = H$, $R_2 = OH$



67 Caracurine V

69 Caracurine V di-*N*-oxide

b) Ellipticine derivative alkaloids (120)

It has no discussion about the structure and activity for ellipticine type alkaloids (120). But they are found as a major component of *Strychnos dinklagei* Gilg which was a species among the active species in the screening of antimicrobial activity of some plants belonging to the Apocynaceae and Loganiaceae (Verpoorte, Beek et al., 1983).

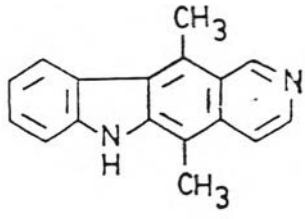
c) Usambarane skeleton alkaloids (124-136)

Some of these alkaloids are found in *Strychnos dale* De Wild. which was one of the most active species during the screening (Verpoorte, Beek et al., 1983). The alkaloids show antimicrobial activity against *Staphylococcus aureus, Mycobacterium smegmatis, Bacillus subtilis* and *Echerichia coli*. 5′.6′-Dihydro-usambarensine (102) possesses amoebicide property *in vitro* against *Entamoeba histolytica* (Ohiri et al., 1983). The structure-activity-relationships of these skeleton alkaloids are concluded as follows (Caron et al., 1988).

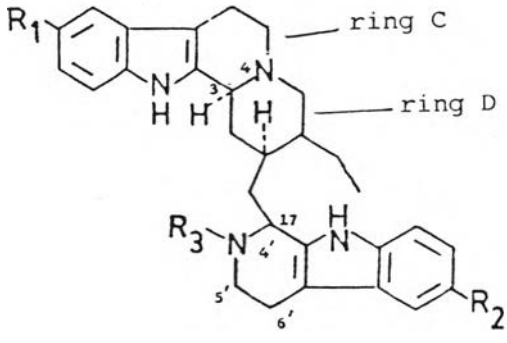
- The introduction of a 3,4- or 17,4′- double bond in a carboline moiety lowers the activity.

- The oxygenated substitutions on benzene ring of indole moiety reduce the activity. But *N*-methylation on amino unit will counteract this activity.

- The stereochemistry of C/D ring junction is important for the activity.



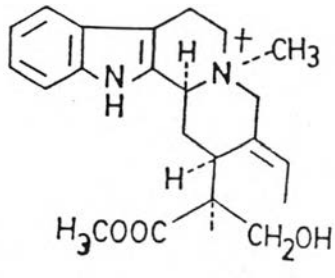
120 Ellipticine



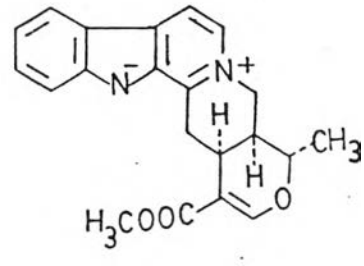
	Structure name	C-3	Configuration at C-17	C-20	Junction C(19) = C(20)	C/D-ring	Substituents R^1	R^2	R^3
87	usambarensine	3αH	—	—	*E*	*cis*	H	H	—
102	tchibangensine (= dihydrousambarensine)	3αH	—	—	*E*	*cis*	H	H	—
124	tetrahydrousambarensine	3αH	17αH	—	*E*	*cis*	H	H	H
125	tetrahydrousambarensine	3αH	17βH	—	*E*	*cis*	H	H	H
126	*epi*-tetrahydro-usambarensine	3βH	unknown	—	*E*	*trans*	H	H	H
127	*epi*-tetrahydro-usambarensine	3βH	opposite of 5	—	*E*	*trans*	H	H	H
128	10'-hydroxytetrahydro-usambarensine	3αH	17αH	—	*E*	*cis*	H	OH	H
129	10'-hydroxytetrahydro-usambarensine	3αH	17βH	—	*E*	*cis*	H	OH	H
130	10,10'-dimethoxytetra-hydrousambarensine	3αH	17αH	—	*Z*	*trans*	OCH_3	OCH_3	H
131	10-hydroxy-10'-dimethoxytetra-hydrousambarensine	3αH	17αH	—	*Z*	*trans*	OH	OCH_3	H
132	10,10'-dihydroxytetra-hydrousambarensine	3αH	17αH	—	*Z*	*trans*	OH	OH	H
133	10,10'-dimethoxy-*N*-methyl-tetrahydrousambarensine	3αH	17αH	—	*Z*	*trans*	OCH_3	OCH_3	CH_3
134	10'-hydroxy-10-methoxy-*N*-methyl-	3αH	17αH	—	*Z*	*trans*	OCH_3	OH	CH_3
135	10,10'-dihydroxy-*N*-methyl-tetrahydrousambarensine	3αH	17αH	—	*Z*	*trans*	OH	OH	CH_3
136	10-hydroxy-10'-methoxy-*N*-methyl-tetrahydrousambarensine	3αH	17αH	—	*Z*	*trans*	OH	OCH_3	CH_3

d) Corynanthean type alkaloids

Diploceline (137), the E-*seco* indole group, shows a weak antimicrobial activity towards *Staphylococcus aureus* and *S. haemolyticus*. The portion that contained alstonine (112) was more active than that of diploceline (137). But no tests could be performed owing to lack of material (Coune, 1980, quoted in Ohiri et al., 1983).



137 Diploceline



112 Alstonine

5. Hypotensive activity

a) Corynanthean type

a.1 Ajmalicine group

Alstonine (112), and serpentine (113) have hypotensive and α-adrenolytic properties (Tits, 1982 quoted in Ohiri et al., 1983).

a.2 Sarpagine group

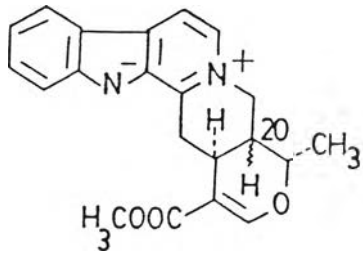
Macusine B (92) (Leonard, 1965a; 1965b), and Normacusine B (138) (Ohiri et al., 1983) have hypotensive effect *in vivo*.

b) Strychnan type

Diaboline (117) has a potent hypotensive action and also exhibits depressant effect on isolated heart (Singh, and Kapoor, 1976).

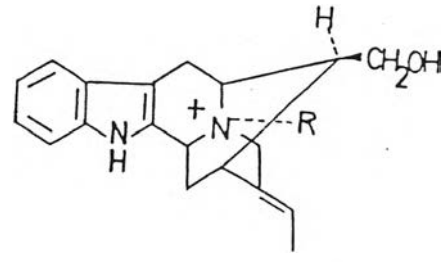
c) Bis-indole alkaloids

Longicaudatine (56) has strong reserpine-like activity (Massiot et al., 1983).



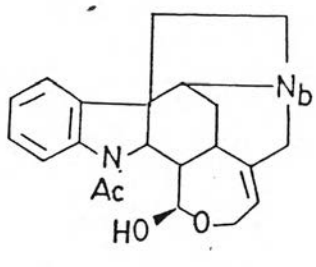
112 Alstonine ; 20-α-H

113 Serpentine ; 20-β-H

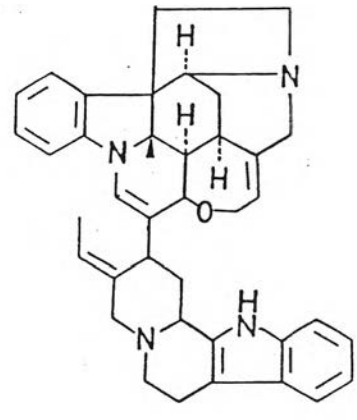


92 Macusine B ; R = CH_1

138 Normacusine B ; R = CH_2OH



117 Diaboline



56 Longicaudatine

6. Other activities

- Bisnor-dihydrotoxiferine (70) is a sedative in consequence of its marked depressant action on the central-nervous system (Bernauer et al., 1963, quoted in Ohiri et al., 1983).

- Normacusine B (138) is a sedative *in vivo* in mice (Ohiri et al., 1983)

- Macusine B (92) is a competitive inhibitor of 5-HT (Leonard, 1965a; 1965b).

- Strychnocarpine (81) is a stimulator of the central 5-HT receptor (Rolfsen et al., 1980)

- Harman (25) and its derivatives act as inhibitors of monoamine oxidase (Tits, 1982, quoted in Ohiri et al., 1983)

- Usambarensine (103) presents atropine-like and spasmolytic activities (Angenot et al., 1975; Dubois et al., 1974, quoted in Ohiri et al., 1983)

Overall pharmacological activity of various groups and types of *Strychnos* alkaloids could be summerized in table 3.

Table 3 *Strychnos* alkaloids and their activities

alkaloids	-1-	-2-	-3-	-4-	-5-	-6-
Non-terpenoid indole						
Harman derivatives (108-111)			/			a
Melinonine F (107)			/			
Strychnocarpine (81)	/					b
Monomeric indole alkaloids						
Corynanthean-type						
E-*seco* indole group						
Diploceline (137)				/		
4,21-Dehydrogeissoschizine (52)			/			
Usambarensine derivatives (95-104, 124-136)			*	*		
Usambarensine (97)						c
Ajmalicine group						
Alstonine (112)			/	/	/	
Serpentine (113)			/		/	
Akagerine group						
Akagerine derivatives (87-91)		*				
Akagerine (87)			/			

Table 3 (continue)

alkaloids	-1-	-2-	-3-	-4-	-5-	-6-
Mavacurine group						
C-Mavacurine (75)	/					
C-Fluorocurine (76)	/					
Sarpagine group						
11-Methoxy-macusine A (77)	/					
Macusine B (92)		/			/	e
Normacusine B (138)					/	d
Oxindole group						
Strychnophylline (105)			/			
Strychnofoline (106)			/			
Vincosan-type						
Decussine group						
Decussine (71)	*					
3,14-Dihydro-decussine (72)	/					
Strictosidine group						
Dolichantoside (114)			/			
Isodolichantoside (115)			/			
Vallesiachotanan-type						
Methylantirhine (116)			/			
Strychnan-type						
Retuline group						
Fluorocurarine (74)	/					

Table 3 (continue)

alkaloids	-1-	-2-	-3-	-4-	-5-	-6-
Diaboline group						
Diaboline derivatives (15)		/				
Diaboline (117)			/		*	
Henningsoline (118)			/			
Strychnine group						
Strychnine derivatives (17)		*				
Synthesis quaternary-derivative (78-79)	/					
Synthesis 10- and 11-acetamidostrychnine (82-83)	/					
Spermostrychnine group						
Spermostrychnine - derivatives (18)		/				
O-Me-N_a-Ac--strychnosplendine (80)	/					
Tsilanine group						
Holstiine (119)			/			
Aspidospermatan-type						
(+)-Tubotaiwine (93)		/				
Miscellaneous-type						
Ellipticine derivatives (120-121)			*	*		

Table 3 (continue)

alkaloids	-1-	-2-	-3-	-4-	-5-	-6-
Bis-tertiary alkaloids						
Strychnan-Strychnan type						
Retuline-Retuline group						
Bisnor-dihydrotoxiferine (70)	/		/	*		d
Bisnordihydrotoxiferine-di-*N*-oxide (122)				/		
Bisnor-C-alkaloid H (123)				/		
Diaboline-Diaboline group						
Caracurine V (67)	/			*		
Caracurine V *N*-oxide (68)	/					
Caracurine V di-*N*-oxide (69)	/			/		
Strychnan-Corynanthean type						
Longicaudatine (56)					*	
Bis-quaternary alkaloids (58-66)	*					

-1- = muscle-relaxant activity
-2- = convulsant activity
-3- = cytotoxic activity
-4- = antimicrobial activity
-5- = hypotensive activity
-6- = others activity

a = MAOI
b = 5-HT stimulant
c = spasmolytic
d = sedative
e = 5-HT inhibitor
/ = moderate-less activity
* = strong activity

From the table, it can be noticed as follows.

- The non-terpenoid indole alkaloids have cytotoxic activity as a main action.

- The corynanthenan-type alkaloids have been shown several activities. The interested alkaloids are usambarensine derivatives which show strong antimicrobial and cytotoxic activity, and akagerine derivatives which are the potent convulsive agents.

- Decussine is the only vincosan-type alkaloids that has a strong muscle-relaxant activity.

- The vallesiachotaman-type and aspidospermatan-type alkaloids have only weak cytotoxic and convulsive activities, respectively.

- The strychnan-type alkaloids are the recognized convulsive agents. Diaboline shows a strong hypotensive effect.

- The ellipticine derivatives of miscellaneous-type alkaloids are recognized cytotoxic agents and investigated to have antimicrobial activity.

- The bis-tertiary indole alkaloids, strychnan-strychnan type have been proved for their antimicrobial action while some muscle-relaxant activity are also observed. The only one strychnan-corynanthean type, longicaudatine, has strong reserpine-like activity.

- The bis-quaternary indole alkaloids are the recognized curarizing agents.