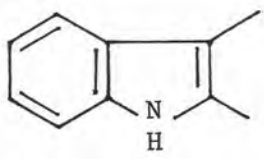
CHAPTER II

HISTORICAL

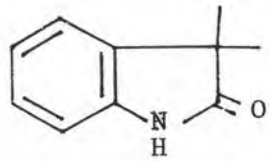
Distribution

1. Indole Alkaloids and Their Occurrence

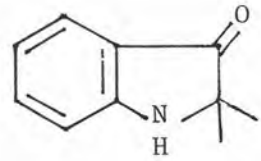
The number of indole alkaloids of known structures today amounts to approximately 1400. Indole alkaloids are defined as the organic compounds containing either the indole nucleus or an oxidized, reduced, or substituted equivalent of it, e.g., oxindole, pseudoindoxyl, dihydroindole, N-acylindole (Kisakürek *et al.*, 1983).



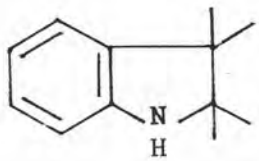
indole



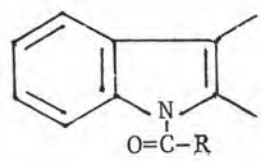
oxindole



pseudoindoxyl

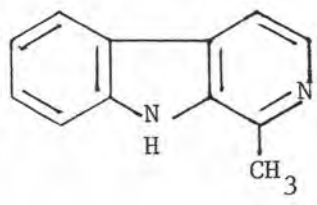


dihydroindole

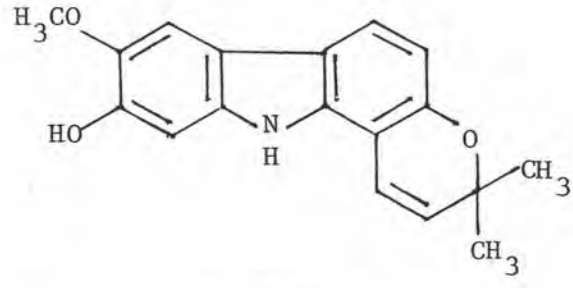


N-acylindole

With respect to their structural features, the indole alkaloids can be divided into two main classes. The first comprises 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, they may occur in many plant families (e.g., harman, obtained from the families Apocynaceae, Chenopodiaceae, Elaeagnaceae, Leguminosae, Loganiaceae, Passifloraceae, Polygonaceae, Rubiaceae, Symplocaceae, and Zygophyllaceae) or be restricted to very few families or to only one (e.g., koenigine obtained only from the Rutaceae).



harman



koenigine

Indole bases of the second class contain two structural elements: tryptamine or tryptophan with an indole nucleus and a C_9-or C_{10}-monoterpene moiety, derived from secologanin. Because of both of the common components and the biogenetic relationships, the occurrence of this 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 (Kisakürek and Hesse, 1980).

2. Alkaloids Isolated from Species of *Gelsemium*

The alkaloids reported to be found in the species of *Gelsemium* are summarized as follows:-

2.1 *Gelsemium sempervirens* (L.) Jaume St.-Hilaire

Stems

14β-hydroxygelsedine : Schun and Cordell, 1985 a.

Roots

sempervirine : Forsyth, Marrian and Stevens, 1945

gelsevirine : Bindra, 1973; Bisset, 1980

gelsedine : Saxton, 1965

gelsemicine : Forsyth *et al.*, 1945

Rhizome and roots

gelsemine : Moore, 1911

Not mentioned

21-oxogelsemine : Nikiforov, Latzel, Varmuza and Wichtl, 1974

14β-hydroxygelsemicine : Schun and Cordell, 1985 a.

gelsemidine : Sayre, 1919

2.2 *G. rankinii* Small

Stems

gelsemine

gelsevirine

21-oxogelsevirine : Schun, Cordell and Garland, 1986

2.3 *G. elegans* Benth.

Leaves

gelsemine : Janot, Goutarel and Cristina, 1953

Leaves and stems

koumine

gelsemine

kounidine : Chou, Wang and Cheng, 1936

Leaves, stems and roots

koumine

kouminicine

kouminidine : Chi, Kao and Huang, 1938; Henry, 1949

Stems

koumine : Janot, *et al.*, 1953

koumidine

gelsemine

gelsevirine

humantenine

gelsenicine

14β-hydroxygelsenicine : S. Sakai, E. Yamanaka, M. Kitajima, M. Yokota, N. Aimi, S. Wongseripipatana and D. Ponglux, in press

Roots

koumine

gelsemine

gelsevirine : Yang and Chen, 1983

19-hydroxydihydrogelsevirine : S. Sakai, E. Yamanaka, M. Kitajima, M. Yokota, N. Aimi, S. Wongseripipatana and D. Ponglux, in press

humantenine

humantenirine

humantenmine

humantenidine : Yang and Chen, 1983

humantendine : Yang and Chen, 1982 a.

Whole plants

sempervirine : Janot *et al.*, 1953

koumine

koumidine

akuammidine

gelsemine

gelsedine : Jin and Xu, 1982

Not mentioned

gelsenicine

gelsenidine

koumicine : Du, Dai, Zhang, Lu and Liu, 1982

3. *Gelsemium* Alkaloids Isolated from Other Botanical Sources

sempervirine	:	*Mostuea brunonis* Didr. var. *brunonis* f. *augustifolia* (Onanga and Khuong-Huu, 1980)
		M. buchholzii Engl. (Saxton, 1965)
		M. stimulans A. Chev. (Saxton, 1965)
gelsemine	:	*M. stimulans* A. Chev. (Saxton, 1965)
gelsemicine	:	*M. brunonis* Didr. var. *brunonis* f. *augustifolia* (Onanga and Khuong-Huu, 1980)
14-hydroxygelsemicine	:	*M. brunonis* Didr. var. *brunonis* f. *augustifolia* (Onanga and Khuong-Huu, 1980)

4. Alkaloids Isolated from Species of *Mostuea*

The alkaloids reported to be found in the species of *Mostuea* are summarized as follows :-

4.1 *Mostuea brunonis* Didr. var. *brunonis* f. *augustifolia*

Leaves and stems

gelsemicine		
mostueine	:	Onanga and Khuong-Huu, 1980

Stems

14β-hydroxygelsemicine	:	Onanga and Khuong-Huu, 1980

Roots

sempervirine : Onanga and Khuong-Huu, 1980

4.2 *M. buchholzii* Engl.

Not mentioned

sempervirine : Saxton, 1965

4.3 *M. stimulans* A. Chev.

Roots

sempervirine

gelsemine : Saxton, 1965

5. Alkaloids Isolated from Species of *Gardneria*

The alkaloids reported to be distributed in genus *Gardneria* are summarized as follows:-

5.1 *Gardneria insularis* Nakai

Roots and stems

gardnerine

gardnutine

18-hydroxygardnutine

gardneramine : Haginiwa, Sakai, Kubo, Takahashi and Taguchi, 1970; Bisset and Phillipson, 1976

5.2 *G. multiflora* Makino

Roots and stems

alkaloid M : Bisset and Phillipson, 1976; Sakai, Aimi, Yamaguchi, Hori and Haginiwa, 1977

chitosenine (alkaloid F) : Bisset and Phillipson, 1976

alkaloid L

alkaloid I

alkaloid N

alkaloid J

exomethylene compound : Bisset and Phillipson, 1976; Sakai *et al.*, 1977

18-demethoxygardmultine : Sakai, Aimi, Yamaguchi, Yamanaka and Haginiwa, 1982

gardmultine (alkaloid E) : Bisset and Phillipson, 1976

18-demethoxygardneramine : Sakai, Aimi, Yamaguchi, Ohhira, Hori and Haginiwa, 1975; Bisset and Phillipson, 1976

18-demethylgardneramine

(alkaloid G)

gardneramine : Bisset and Phillipson, 1976

gardneramine N-oxide : Bisset and Phillipson, 1976; Sakai *et al.*, 1977

gardfloramine

18-demethoxygardfloramine : Sakai *et al.*, 1975; Bisset and Phillipson, 1976

alkaloid H : Bisset and Phillipson, 1976

(structure undetermined)

5.3 *G. nutans* Sieb. et Zucc.

Roots and stems

gardnerine

18-hydroxygardnerine : Aimi, Yamaguchi, Sakai, Haginiwa and Kubo, 1978

gardnutine

18-hydroxygardnutine

gardneramine : Bisset and Phillipson, 1976

18-demethoxygardneramine : Bisset and Phillipson, 1976; Sakai, 1976

5.4 *G. shimadai* Hayata

Roots and stems

chitosenine (alkaloid F)

gardmultine

gardneramine

18-demethylgardneramine : Bisset and Phillipson, 1976

Sakai *et al.* (1977) had also studied alkaloidal constitution of *G. liukiuensis* Hatsushima and proved to be quite similar to that of *G. multiflora* Makino. A leaf sample of *G. angustifolia* Wall. collected from Nepal in 1954, gave an extract (1.9 mg = 0.49 %) which afforded a + + + test; TLC indicated the presence of three major and three minor bases (Bisset and Phillipson, 1976).

Chemistry of the Alkaloids

1. Chemistry of the *Gelsemium* Alkaloids

1.1 Basic structures

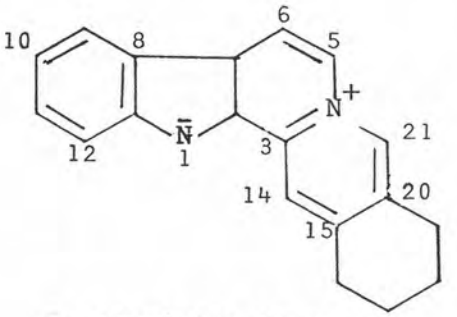
The alkaloids obtained from the species of *Gelsemium*, in general, can be divided into two main groups, indole and oxindole alkaloids which are shown in Figures 1 and 2.

1.1.1 Indole alkaloids

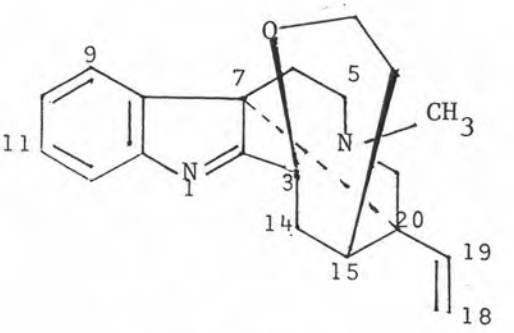
The alkaloids in this group possess three types of basic structures, sempervirine-, koumine- and sarpagine-types.

Figure 1

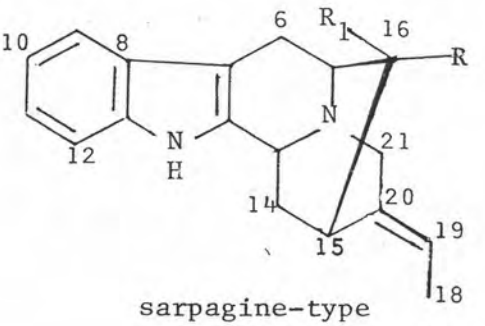
Basic structures of *Gelsemium* indole alkaloids



sempervirine-type



koumine-type



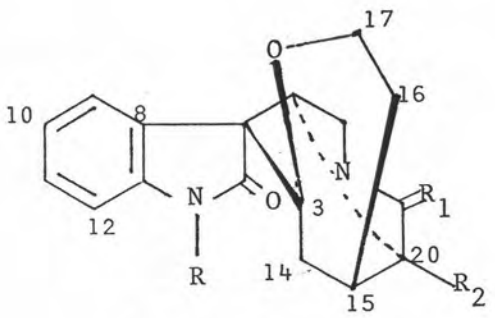
sarpagine-type

1.1.2 Oxindole alkaloids

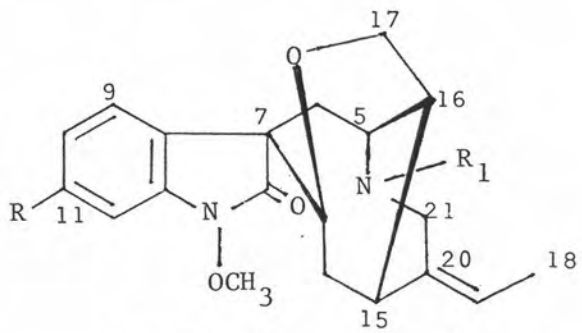
The alkaloids in this group possess three types of basic structures, gelsemine-, humantenine- and gelsedine-types.

Figure 2

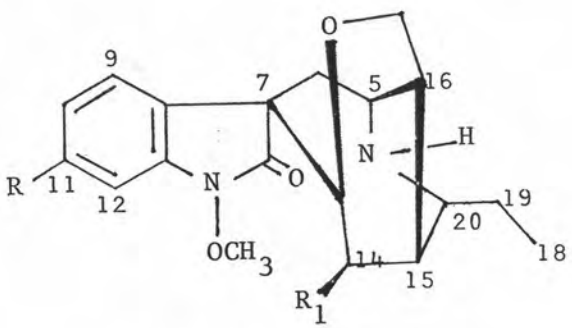
Basic structures of *Gelsemium* oxindole alkaloids



gelsemine-type



humantenine-type



gelsedine-type

1.2 Configurations

Both indole and oxindole alkaloids possess a C(15)-Hα configuration, since these alkaloids are all derived from the monoterpene secologanin. Some of the alkaloids possess double bond between C(19) and C(20), so occurring two configurations, *E* and Z. The alkaloids with *E* configuration possess a C(18) *cis* to C(15) while those with Z configuration possess a C(18) *trans* to C(15), though all alkaloids of known absolute configurations possess *E* configuration. Substitutions at C(14) have been found to be only β-oriented and the substituting group being hydroxy group only.

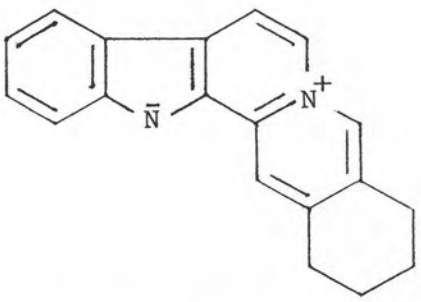
Substitutions at N(a) have been found only in oxindole alkaloids, and the group being only methoxy group. Methyl substitutions at N(b) have been found in both types of alkaloids. Substitutions in aromatic ring have been found only in oxindole alkaloids at C(11), and the only substituting group found is methoxy group. All known oxindole alkaloids possess ether bond between C(17) and C(3).

1.2.1 Indole alkaloids

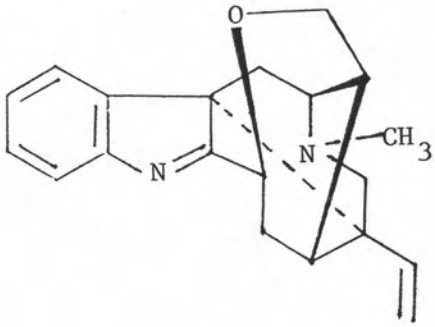
All *Gelsemium* indole alkaloids are summarized in Figure 3.

Figure 3

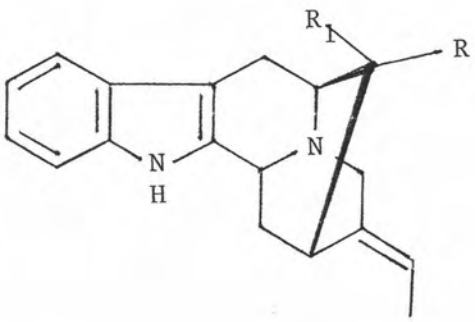
Gelsemium indole alkaloids



sempervirine



koumine



Indole alkaloid	R	R_1
koumidine	-H	$-CH_2OH$
akuammidine	$-CH_2OH$	$-COOCH_3$

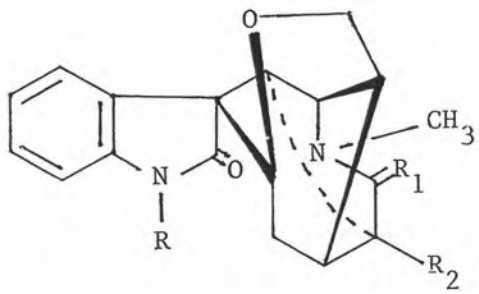
Reference : Silvers and Tulinsky, 1962; Glasby, 1975; Bisset, 1980; Khuong-Huu, Chiaroni and Riche, 1981; S. Sakai, E. Yamanaka, M. Kitajima, M. Yokota, N. Aimi, S. Wongseripipata and D. Ponglux, in press

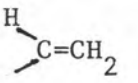
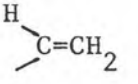
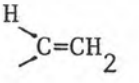
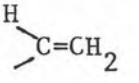
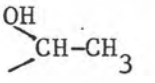
1.2.2 Oxindole alkaloids

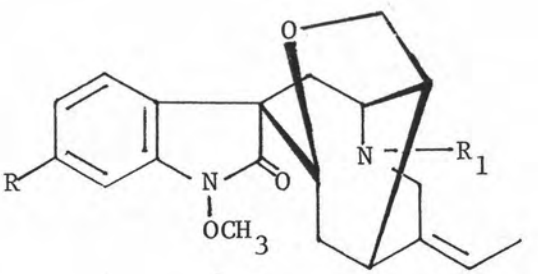
All *Gelsemium* oxindole alkaloids are summarized in Figure 4.

Figure 4

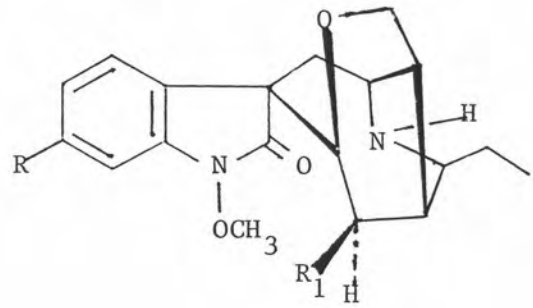
Gelsemium oxindole alkaloids



Oxindole alkaloid	R	R_1	R_2
gelsemine	-H	$-H_2$	
21-oxogelsemine	-H	=O	
gelsevirine	$-OCH_3$	$-H_2$	
21-oxogelsevirine	$-OCH_3$	=O	
19-hydroxygelsevirine	$-OCH_3$	$-H_2$	



Oxindole alkaloid	R	R_1
humantenine	-H	$-CH_3$
humantenirine	$-OCH_3$	-H



Oxindole alkaloids	R	R_1
gelsedine	-H	-H
14 -hydroxygelsedine	-H	-OH
gelsenicine (20-(N-4)-didehydrogelsedine)	-H	-H
14β-hydroxygelsenicine	-H	-OH
gelsemicine	$-OCH_3$	-H
14β-hydroxygelsemicine	$-OCH_3$	-OH

Reference : Nikiforov *et al.*, 1974; Bisset, 1980; Yang and Chen, 1982 a, b; Yang and Chen, 1984 a, b; Schun and Cordell, 1985 a; Schun, *et al.*, 1986; S. Sakai, E. Yamanaka, M. Kitajima. M. Yokota, N. Aimi, S. Wongseripipatana and D. Ponglux, in press

The rest of *Gelsemium* alkaloids mentioned in the literatures, of which chemical structures remain undetermined, are summarized as follows:-

Alkaloid	Chemical Formular	Reference
gelsemidine	-	Sayre, 1919
koumidine	$C_{21}H_{24}N_2O_5$	Chou *et al.*, 1936
kouminicine	-	Glasby, 1975
kouminidine	$C_{19}H_{25}N_2O_4$ (m.p. 299°C)	Glasby, 1975
gelsemidine	$C_{21}H_{26}N_2O_3$ (m.p. 143-145°C)	Du *et al.*, 1982
koumicine	$C_{21}H_{24}N_2O_3$ (m.p. 252-254°C)	Du *et al.*, 1982

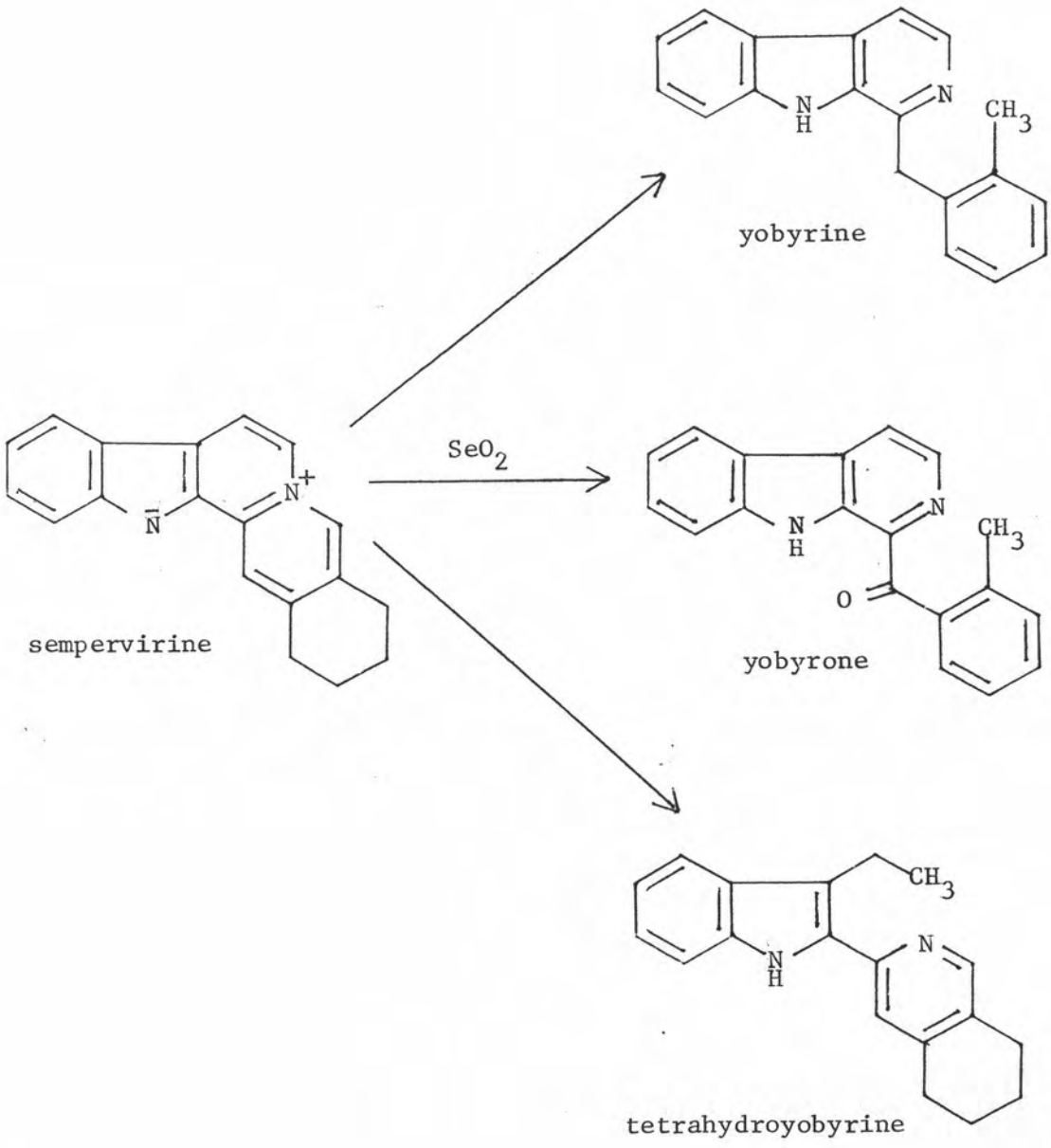
An alkaloid named humantenmine by Yang and Chen (1983) is the same alkaloid as gelsenicine (Chemical Substance Index vol. 103, 3162CS, 1985). Humantendine is 14β-hydroxygelsenicine and humantenidine seems to be the misprinting of humantendine.

1.3 Reactions of *Gelsemium* alkaloids

Moore (1911) stated that gelsemine, on treatment with acetic anhydride, yields acetylgelsemine, $C_{20}H_{20}ON_2$. OAc, prism, m.p. 60-70°C or 106-108°C (dry). When boiled with concentrated hydrochloric acid, gelsemine takes up one molecule of water, forming apogelsemine, $C_{20}H_{24}O_3N_2$ and isoapogelsemine. A third product, in which a molecule of hydrogen chloride has been added, is chloroisoapogelsemine, $C_{20}H_{23}O_2N_2Cl$.

According to Chu and Chou (1940) gelsemine, on treatment with zinc and hydrochloric acid, in the presence of palladium chloride or platinum chloride, yields (a) isogelsemine, an isomer of gelsemine, $C_{20}H_{22}O_2N_2$, which is different from gelsemine in its melting point and specific rotatory power, and (b) an unnamed base, $C_{18}H_{22}O_4N$, m.p. 265°-267°C. On hydrogenation in the presence of platinic oxide, gelsemine and isogelsemine give dihydrogelsemine, $C_{20}H_{24}O_2N_2 . COMe_2$, m.p. 224°-225°C.

When heated sempervirine with selenium at 295°-300°C it is isomerized to yobyrine, and is oxidized by selenium dioxide in boiling xylene to yobyrone. Refluxed with Raney nickel in boiling xylene for 10 hours, it is converted into tetrahydroyobyrine (Henry, 1949).


yobyrine
SeO_2
sempervirine
yobyrone
tetrahydroyobyrine

Gelsemicine does not react with either hydroxylamine or 2,4-dinitrophenylhydrazine (Henry, 1949). According to Forsyth *et al.* (1945) it was not hydrogenated over palladium; it absorbed one molecule of hydrogen rapidly, and a further two more slowly, in acetic acid over Adams' catalyst.

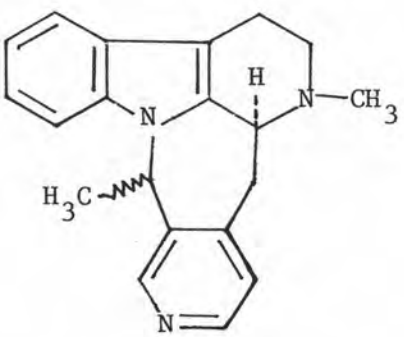
Gelsedine is slowly hydrogenated, in acid solution using Adams' catalyst at slightly elevated temperatures, to a hexahydrodemethoxy derivative, by elimination of the methoxy group and saturation of the benzene ring (Saxton, 1965). The methoxy group is also lost when the alkaloid is reduced with lithium aluminium hydride.

2. Chemistry of *Mostuea* Alkaloids

Only very few studies have been made on the alkaloids of *Mostuea* and much more certain informations are undoubtedly needed. So far reported almost all of the *Mostuea* alkaloids are those found also in the genus *Gelsemium* and their chemistry has already been shown in 1.2. Additional alkaloid in *Mostuea* is of the indole group, i.e. mostueine of which structure is shown in Figure 5.

Figure 5

Additional *Mostuea* alkaloid



mostueine

Reference : Onanga and Khuong-Huu, 1980

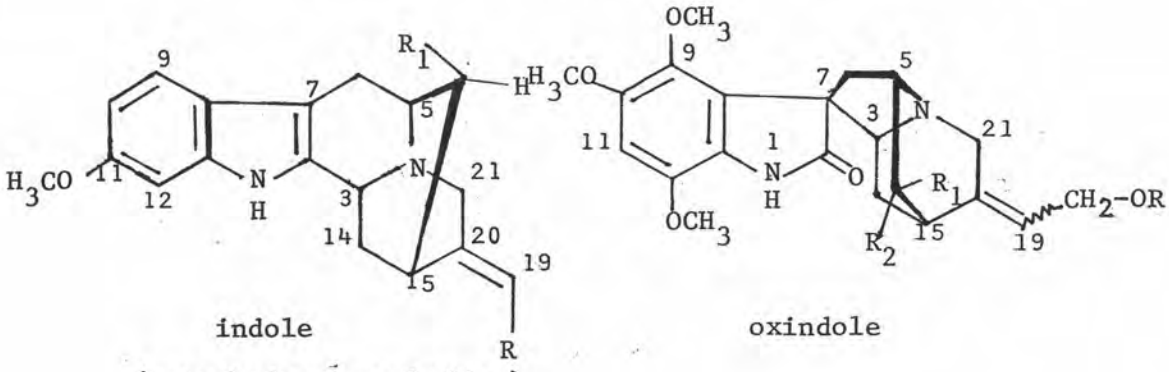
3. Chemistry of *Gardneria* Alkaloids

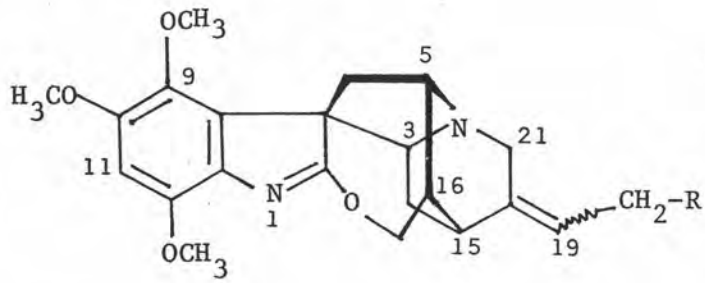
3.1 Basic structures

Gardneria alkaloids possess three basic structures, i.e. indole, oxindole and imino-ether which are shown in Figure 6.

Figure 6

Basic structures of *Gardneria* alkaloids





imino-ether

3.2 Configurations

All types of alkaloids possess double bond between C(19) and C(20), thus occurring two configurations, *E* and *Z*, as those of *Gelsemium* alkaloids. Only *E* configuration is found in the indole group, but both are found in the oxindole and imino-ether groups. In the indole-group, the configuration of H at C(3) is α.

According to Bisset (1980) the tetrahydro-β-carboline-type alkaloids of indole group possess a methoxy group at C(11) on their aromatic rings. There are three methoxy substitutions on the aromatic ring at C(9), C(10) and C(12) of all and some of the alkaloids in the

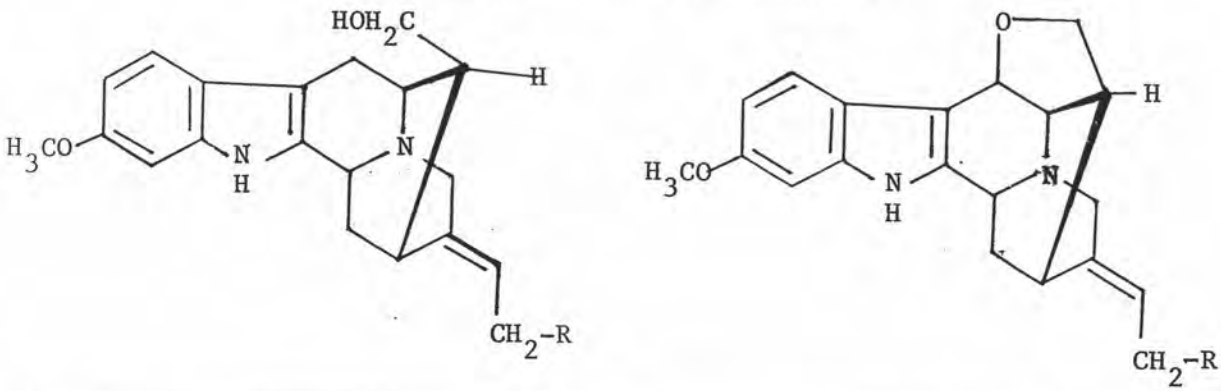
oxindole and imino-ether group, respectively. The 9,10-methylenedioxy-12-methoxy substitution pattern on the aromatic ring may also be found in the imino-ether alkaloids. These patterns have not yet been observed in any other genera neither belonging to Loganiaceae nor to other families. In the oxindole group, there are substitutions at C(16) and also dimeric alkaloids, formed by an oxindole and an imino-ether alkaloids, are found.

3.2.1 Indole alkaloids

The alkaloids in this group are summarized in Figure 7.

Figure 7

Gardneria indole alkaloids



indole alkaloid	R	indole alkaloid	R
gardnerine	-H	gardnutine	-H
18-hydroxygardnerine	-OH	18-hydroxygardnutine	-OH

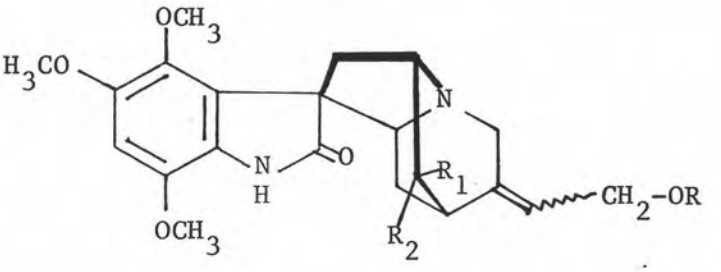
Reference : Bisset and Phillipson, 1976; Sakai, 1976; Aimi *et al.*, 1978

3.2.2 Oxindole alkaloids

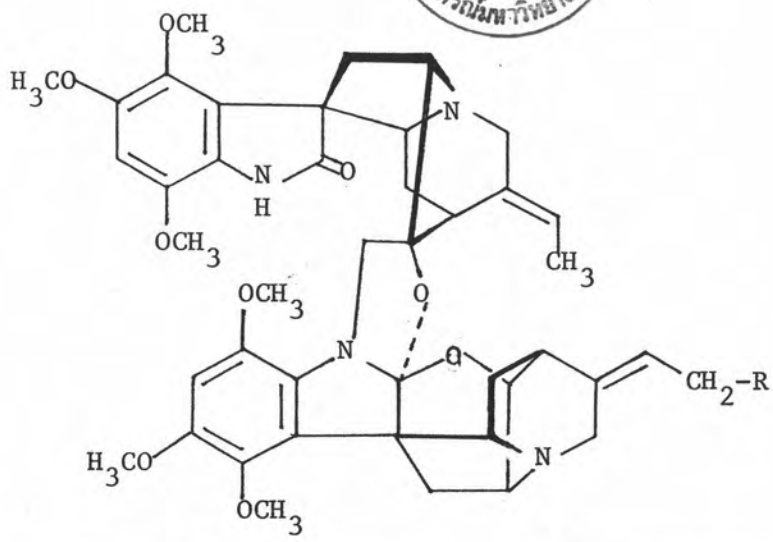
The alkaloids in this group are summarized in Figure 8.

Figure 8

Gardneria oxindole alkaloids



Oxindole Alkaloid	R	R_1	R_2	C(19)
alkaloid M	-H	-H	$-CH_2OH$	*Z*
chitosenine (alkaloid F)	-H	-OH	$-CH_2OH$	*E*
alkaloid L	-H	$-CH_2OH$	-H	*Z*
alkaloid I	$-CH_3$	-H	$-CH_2OH$	*Z*
alkaloid N	$-CH_3$	-OH	$-CH_2OH$	*Z*
alkaloid J	$-CH_3$	$-CH_2OH$	-H	*Z*
exomethylene compound	$-CH_3$	$=CH_2$		*Z*



Dimeric alkaloid	R
demethoxygardmultine	-H
gardmultine	$-OCH_3$

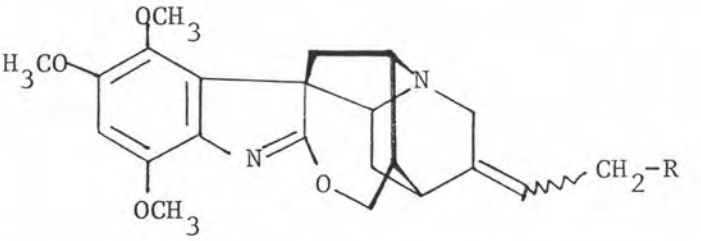
Reference : Bisset and Phillipson, 1976; Sakai, 1976; Sakai *et al.*, 1977; Aimi *et al.*, 1978; Sakai *et al.*, 1982

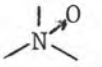
3.2.3 Imino-ether alkaloids

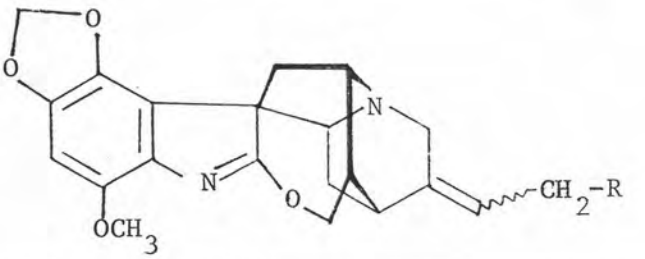
The alkaloids in this group are summarized in Figure 9.

Figure 9

Gardneria imino-ether alkaloids



Imino-ether alkaloid	R	C(19)	N(b)
18-demethoxygardneramine	-H	*E*	
18-demethylgardneramine	-OH	*Z*	
gardneramine	$-OCH_3$	*Z*	
gardneramine N-oxide	$-OCH_3$	*Z*	



Imino-ether alkaloid	R	C(19)
18-demethoxygardfloramine	-H	*Z* or *E*
gardfloramine	$-OCH_3$	*Z* or *E*

Reference : Bisset and Phillipson, 1976; Sakai, 1976; Sakai *et al.*, 1977

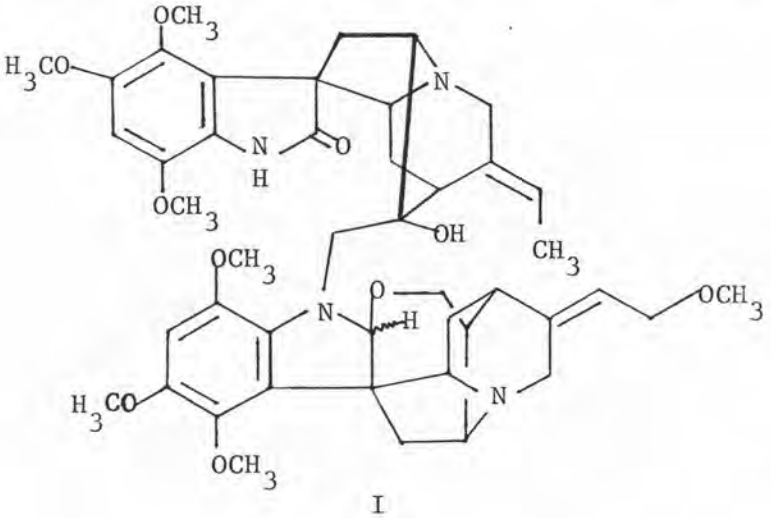
3.3 Conversion of *Gardneria* Alkaloids.

Sakai, Kubo and Haginiwa (1969) stated that, upon oxidation with CrO_3/H_2SO_4 in acetone or with t-BuOCl gardnerine gave gardnutine and reverse reaction was performed by reduction with lithium aluminium hydride. With HBr/AcOH and Zn/AcOH, 18-hydroxygardnutine has been converted to gardnutine. These conversions are summarized below (Sakai, 1976) :-

gardnerine $\underset{\text{ii}}{\overset{\text{i}}{\rightleftharpoons}}$ gardnutine $\underset{\text{iv}}{\overset{\text{iii}}{\longleftarrow}}$ 18-hydroxygardnutine

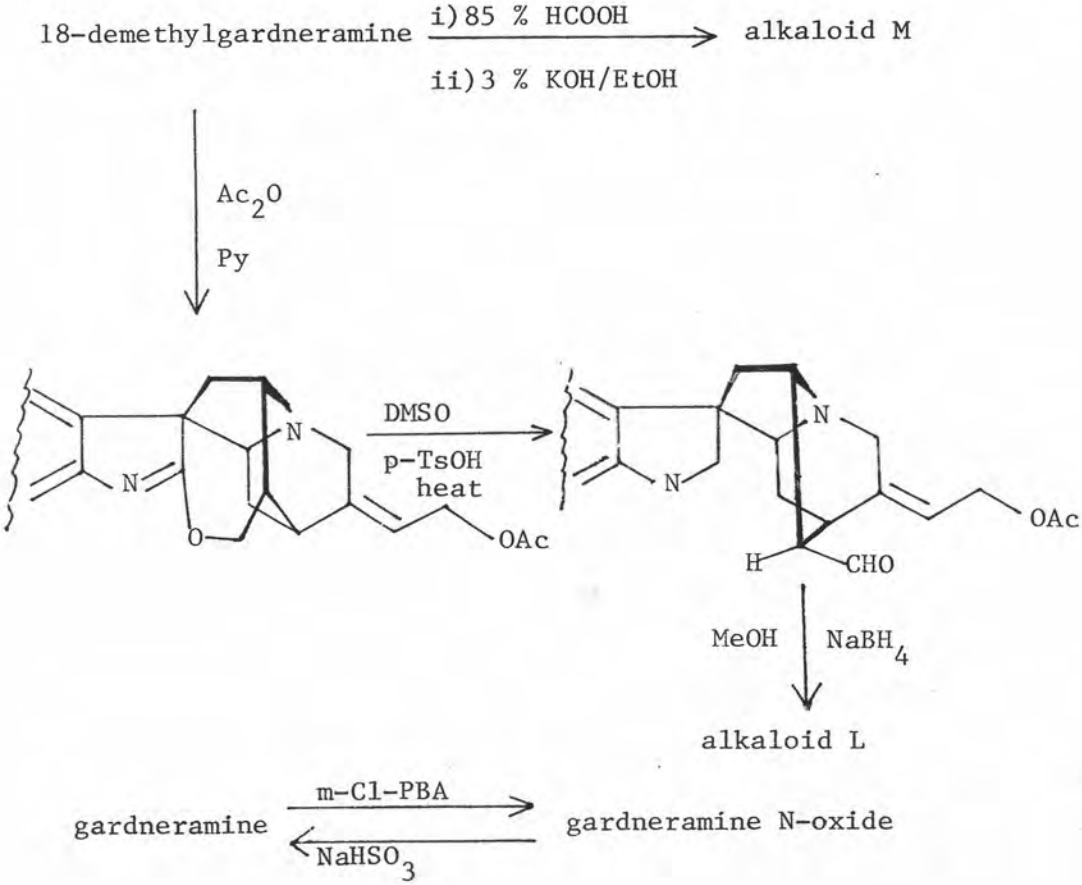
i) CrO_3/H_2SO_4 or t-BuOCl ii) $LiAlH_4$ iii) HBr/AcOH iv) Zn/AcOH

Aimi *et al.*(1978) stated that reduction of 18-hydroxy-gardnutine with lithium aluminium hydride yielded 18-hydroxygardnerine. According to Sakai *et al.*(1982), compound I was derived from gardmultine on reduction with sodium borohydride in acetic acid. Periodic oxidation of I in methanol gave a complex mixture of products from which gardneramine, chitosenine norketone, and gardmultine were isolated . Chitosenine was oxidized with periodic acid to give the same norketone.



I

Refluxing gardneramine with diluted hydrochloric acid yielded chlorine-containing oxindole compounds, catalyzed dehydrochlorination of the compounds afforded 18-demethylgardneramine (alkaloid G)(Sakai, Aimi, Kubo, Kitagawa, Shiratori and Haginiwa, 1971). According to Sakai *et al.*(1977) alkaloids L and M can be derived from 18-demethylgardneramine, and gardneramine N-oxide from gardneramine, pathways of the conversions are summarized as follows:-

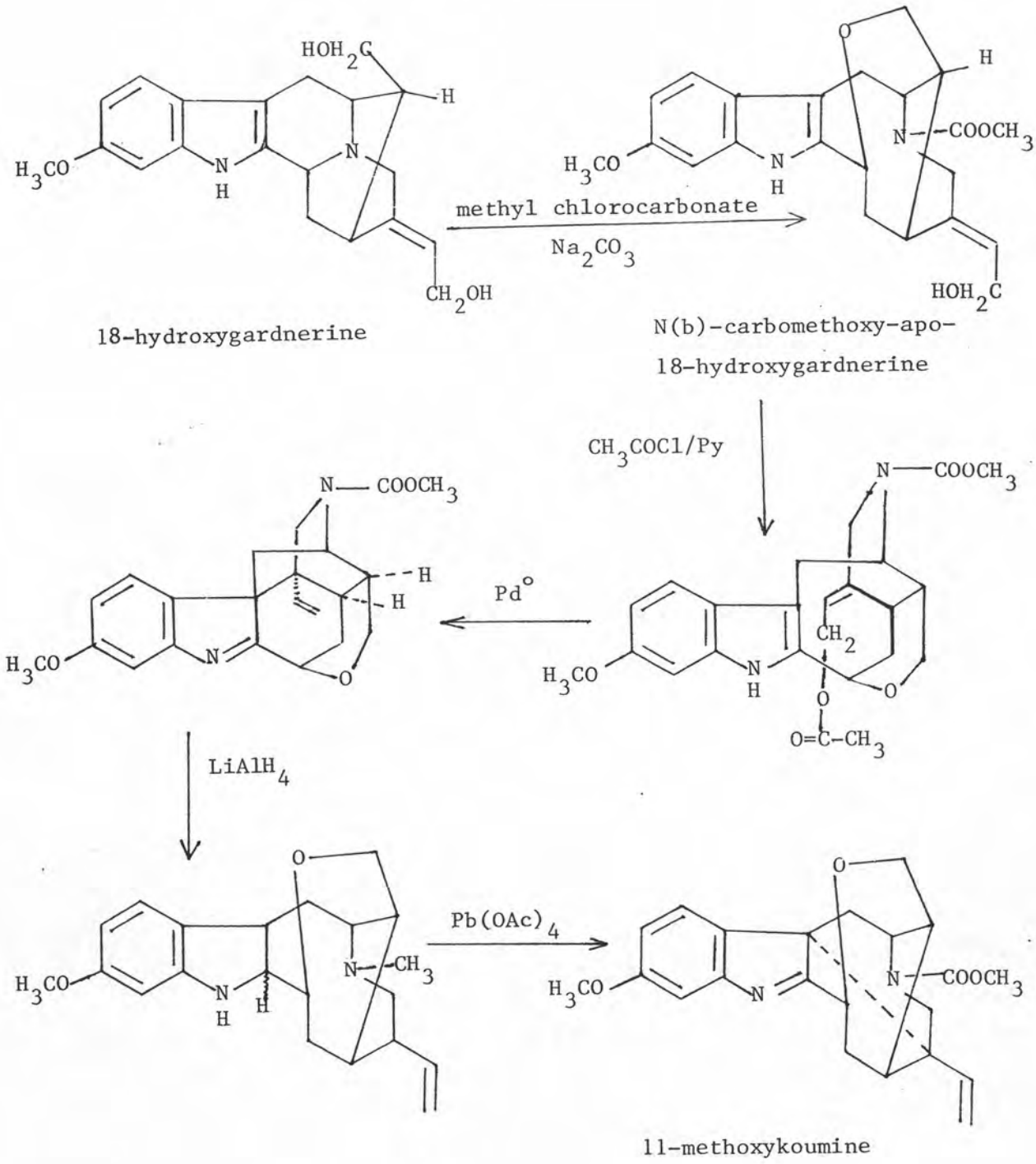


4. Transformation of *Gardneria* Alkaloids to *Gelsemium* Alkaloids.

11-Methoxykoumine was derived from 18-hydroxygardnerine through C-C intramolecular bond formation by Pd catalysis between the indole part and allylic cation in an indole alkaloid. The synthetic pathway of 11-methoxykoumine is shown in Figure 10 (Sakai, Yamanaka, Kitajima, Yokota, Aimi, Wongseripipatana and Ponglux, 1986).

Figure 10

Synthetic pathway of 11-methoxykoumine



Biogenesis

1. Biogenesis of Indole Alkaloids

The biogenesis of indole alkaloids has excited the interest of organic chemists for many years and early speculations were reviewed by Robinson in 1955. Since then radioactive tracer studies have shown that tryptophan is the precursor of the indole portion of the majority of indole alkaloids. Tryptophan itself is derived from shikimic acid (Jackson and Smith, 1968). The other portion of indole alkaloids is C_9-or C_{10}-monoterpene moiety, derived from secologanin and secologanin itself is derived from mevalonate (Kompis, Hesse and Schmid, 1971).

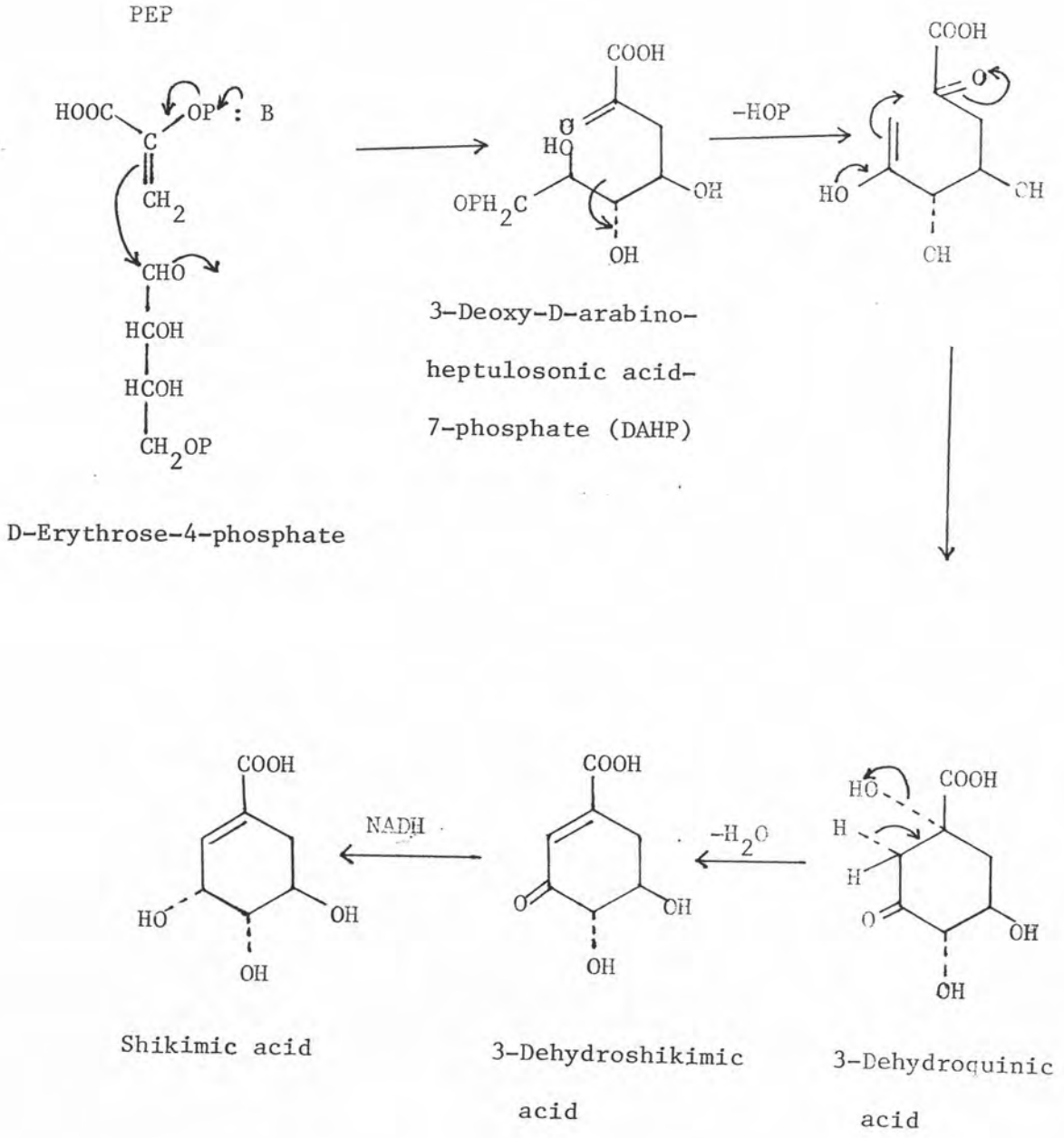
1.1 Formations of shikimic acid and tryptamine

1.1.1 Formation of shikimic acid

Figure 11 summarizes the biogenetic pathways of shikimic acid. An analysis of the distribution of a ^{14}C label in shikimic acid, biosynthesized from specifically labelled ^{14}C-glucose in *E. coli* led to the proposition that erythrose-4-phosphate starts the biosynthetic sequence leading to shikimic acid by condensation with phosphoenol pyruvic acid (PEP) to 3-deoxy-D-arabino-heptulosonic acid-7-phosphate (DAHP). Elimination of phosphoric acid gives the ketone, formally in its enol form, that cyclizes to 3-dehydroquinic acid. Further elimination of water and reduction then gives shikimic acid (Torssell, 1983).

Figure 11

Formation of shikimic acid



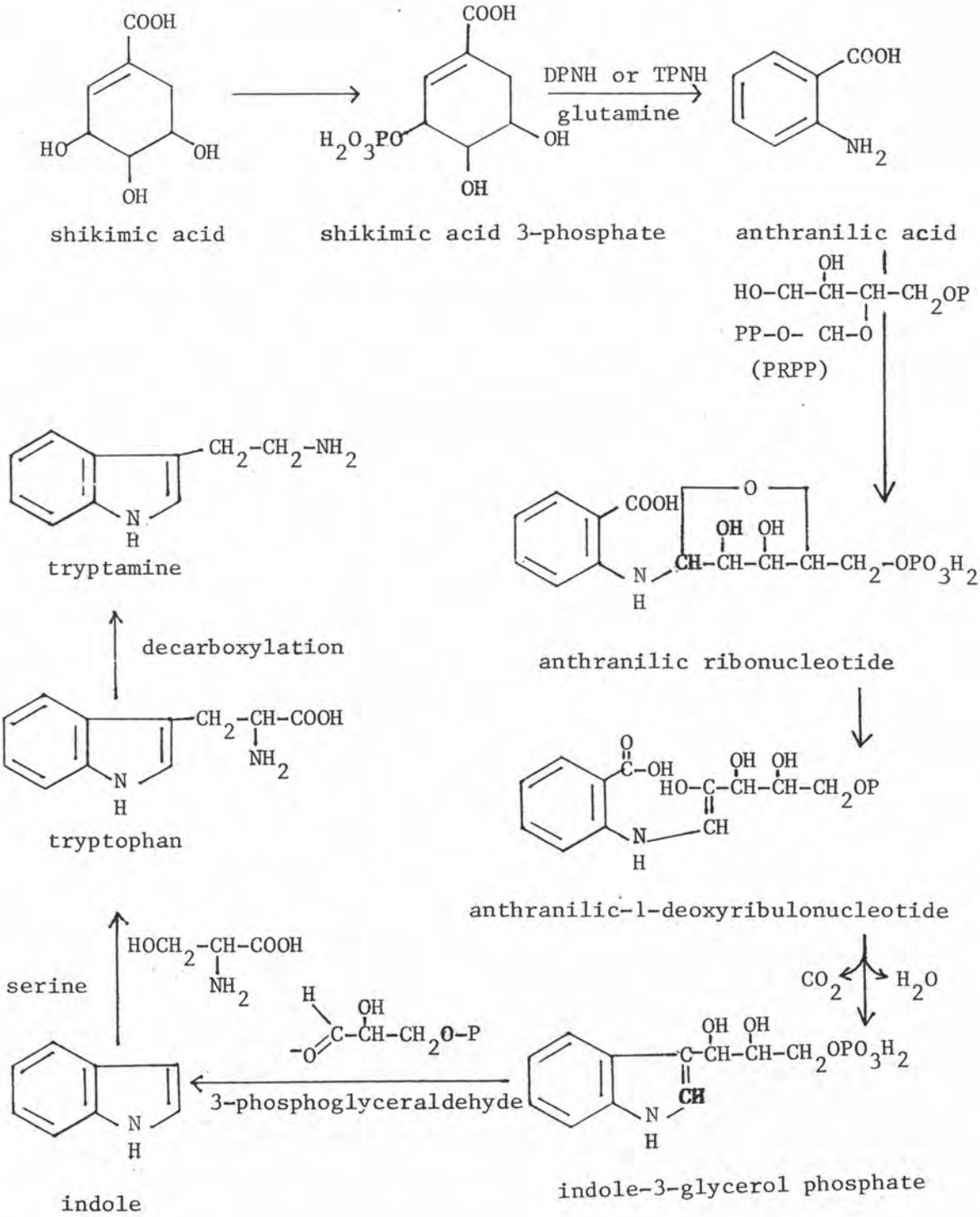
1.1.2 Formation of tryptamine

Robinson had originally suggested that the two nitrogens and the aromatic portion of all of the then-known indole alkaloids originate from tryptophan via its decarboxylation product, tryptamine. This was later experimentally proved (Kompis, *et al.*, 1971).

The amino acid tryptophan is derived from shikimic acid. By mean of a kinase reaction, shikimic acid is formed to be shikimic acid 3-phosphate. A reduction involving DPNH or TPNH and a transfer of an amino group from glutamine to the ring are involved in the formation of anthranilic acid. In the next phase of the sequence, the formation of the pyrrole ring, phosphoribosyl pyrophosphate (PRPP) provided the two necessary carbon atoms while the carbonyl carbon of anthranilic acid is lost. The immediate product of the interaction of PRPP and anthranilate is anthranilic ribonucleotide, which appears to form anthranilic 1-deoxyribulonucleotide. Ring closure, with accompanying production of CO_2 and H_2O gives rise to indole-3-glycerol phosphate. Many enzymes catalyze the reversible formation of free indole and triose phosphate or condensation of serine and indole to form tryptophan (Kompis, *et al.*, 1971). The reaction is illustrated in Figure 12.

Figure 12

Formation of tryptamine



1.2 Formation of loganin and secologanin

Figure 13 illustrates the main steps of the biosynthesis of loganin and secologanin from mevalonate. Three molecules of acetyl coenzyme A are condensed with the aid of β-ketoacylthiolase to form 3-hydroxy 3-methylglutaryl coenzyme A (HMG CoA). With a hemithioacetal intermediate, HMG CoA is specifically reduced to (R)-(+)-mevalonic acid. Sequential phosphorylation to 5-phosphomevalonic acid, a plant metabolite, and 5-pyrophosphomevalonic acid, also a plant metabolite, followed by *trans* elimination afford isopentenylpyrophosphate. 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. Stereoselective loss of the pro-4*S* (in (R)-(+)-mevalonic acid) proton from isopentenylpyrophosphate in the coupling-elimination reaction with dimethylallylpyrophosphate produces geranylpyrophosphate, in which the pro-4*S* hydrogens of the two mevalonate units are completely lost. The methyl group of dimethylallylpyrophosphate are not 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 (R)-(+)-mevalonic acid. A *cis-trans* isomerization of the 2,3-double bond of geranylpyrophosphate to give nerylpyrophosphate, in which the hydrogen at C(2) of the former is retained in the latter, and hydroxylation of the latter at C(10) to give 10-hydroxyneryl-pyrophosphate. The route between 10-hydroxynerylpyrophosphate to deoxyloganin is not well understood. Hydroxylation at C(7) of deoxyloganin occurs stereospecifically to give loganin, and ring opening of loganin gives secologanin (Cordell, 1974 and 1981 b).

Figure 13

Formations of loganin and secologanin

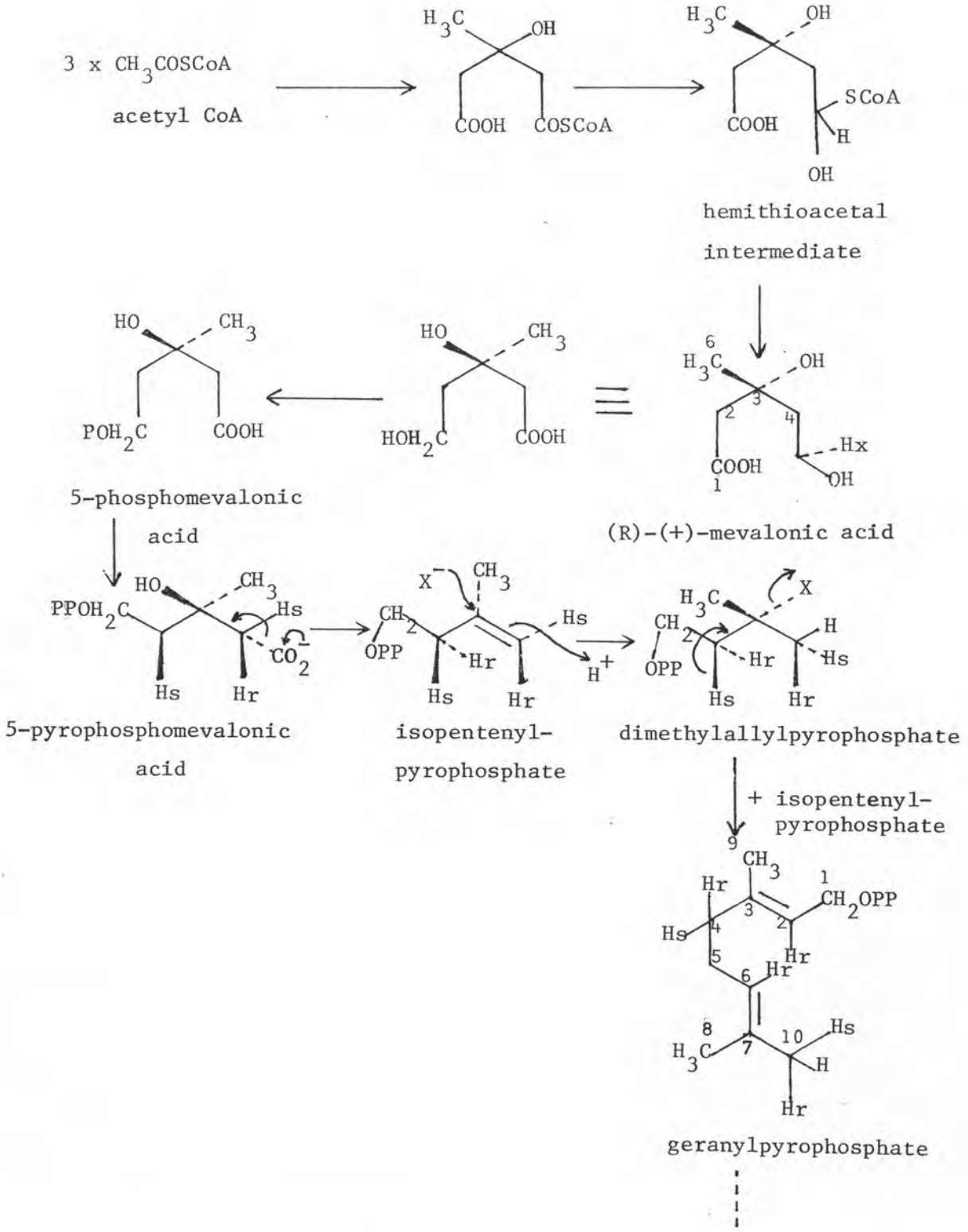
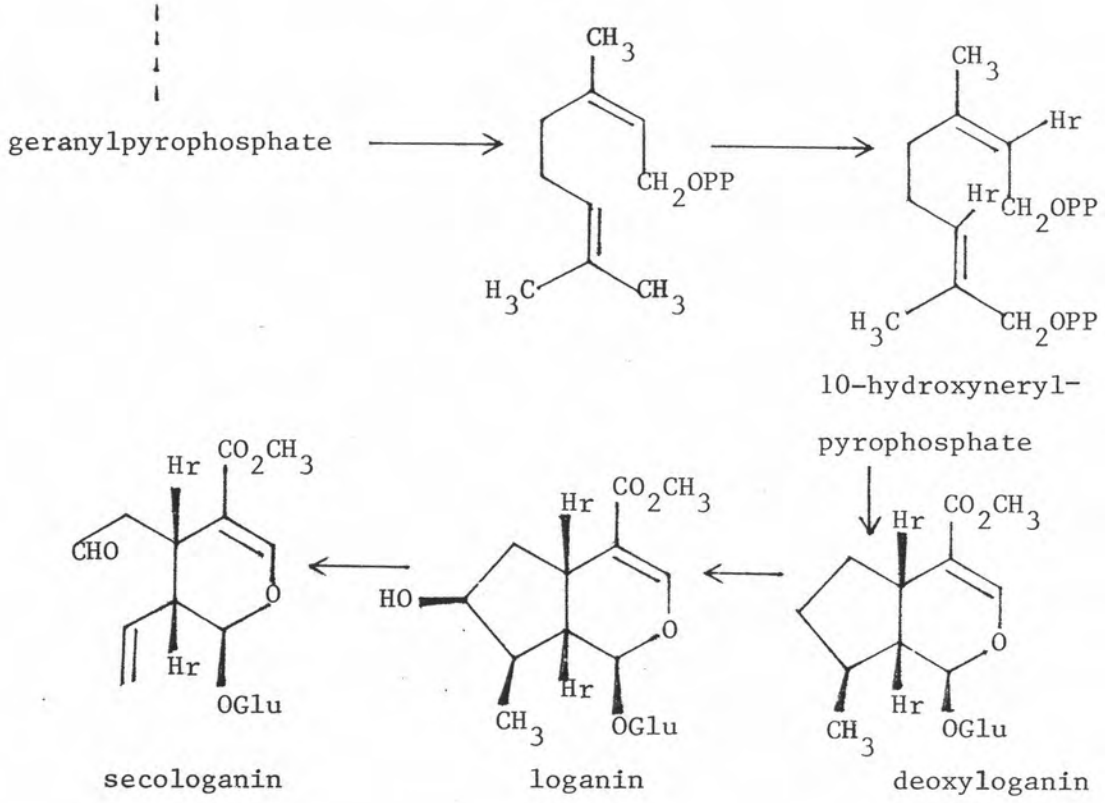


Figure 13 (Continued)



1.3 Formations of variable skeletal types of indole alkaloids

Indole alkaloids with a C_9-or C_{10}-monoterpene moiety are classified into the following subgroups: corynanthean (C), vincosan (D), vallesiachotaman (V), strychnan (S), aspidospermatan (A), eburnan (E), plumeran (P), and ibogan (J) types. In a simplified manner, the biogenetic relationships of these main skeletal types are shown in Figure 14. As an established fact compound D3a* is obtained from condensation of tryptamine, or in some other case tryptophan with secologanin. All of the main skeletal types can be derived from D3a. Skeleton D3a can be converted to compound 1 by opening of the C(17)-O-C(21) bond via $2b^3$. From compound 1, compounds $2b^1$, $2b^2$, $2b^3$, and 2c can be obtained without rearrangement, or structure 2a by rearrangement of the secologanin portion of the molecule. Ring formation between C(2) and C(3) leads to compound 2b. Intermediates $2b^1$, $2b^2$, and $2b^3$ differ from each other only through rotation about the C(14)-C(15) and C(15)-C(16) bonds respectively. Ring closures between C(21) and N(b) in $2b^1$, and between C(17) and N(b) in $2b^2$ give rise to the main corynanthean-type skeleton C3a and the main vallesiachotaman-type V3 respectively. A new additional bond between C(17)-OH and C(21) in $2b^3$ yields the basic skeleton of vincosan group D3a. Intermediate 2c is obtained by ring closure between C(21) and N(b) in 1. An additional ring closure between C(16) and C(2) in 2c yields A3, the fundamental skeleton of the aspidospermatan group. Starting with A3, S4 is obtained by another ring formation between C(3) and C(7). On the other hand, ring closure between C(21) and C(7) yields A4.

Figure 14

Formations of variable skeletal types of indole alkaloids

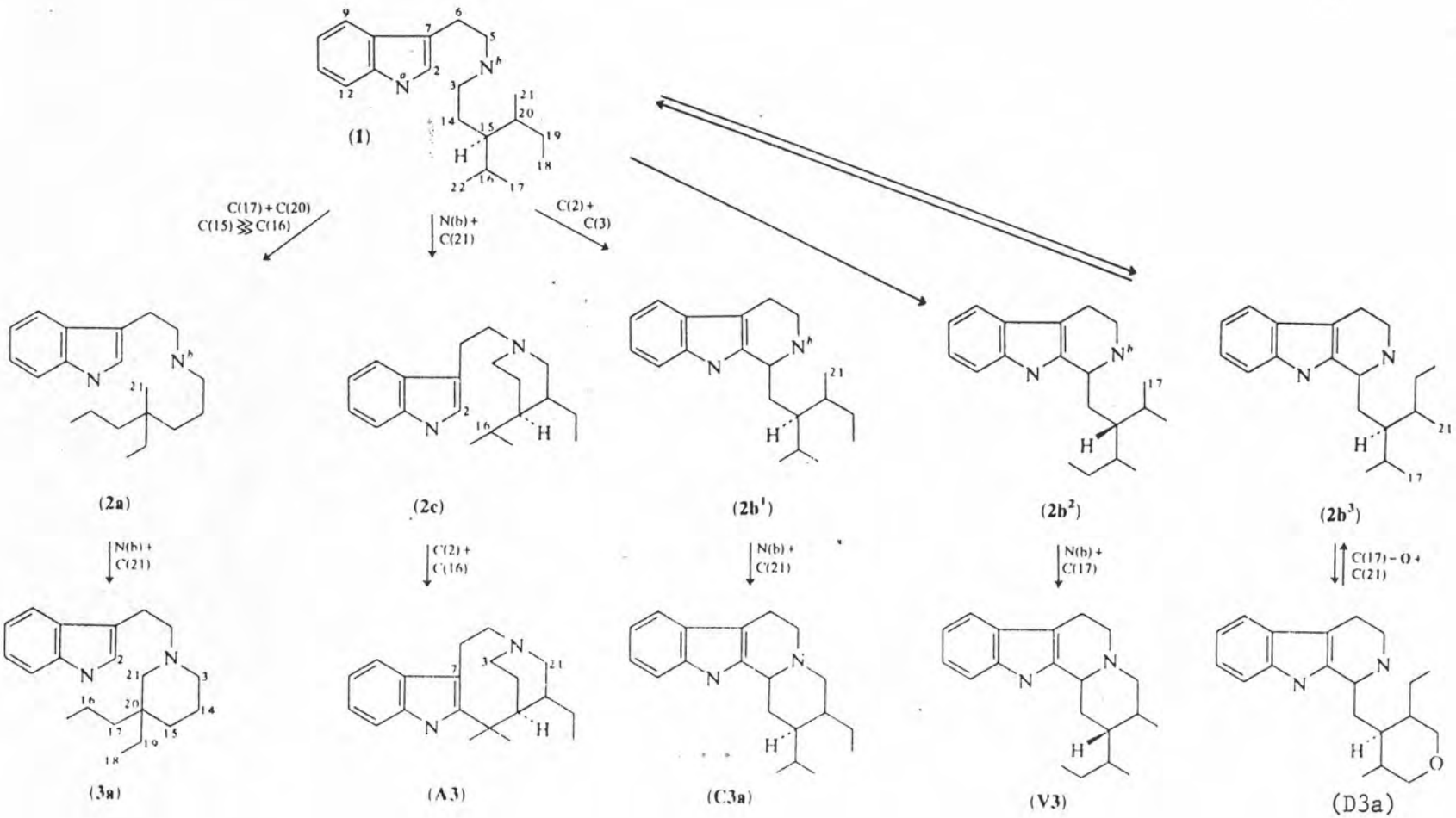
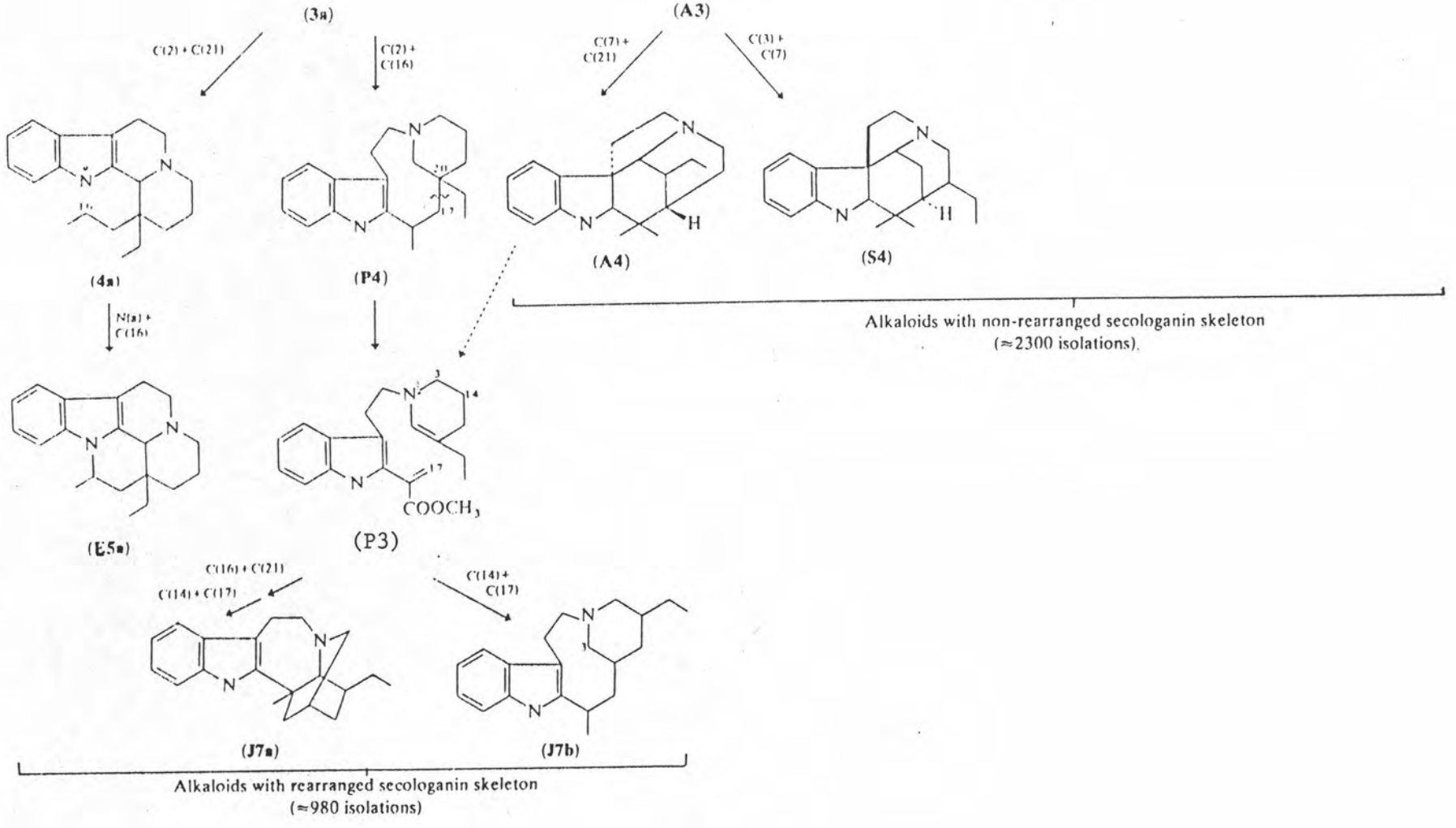


Figure 14 (continued)



Intermediate 2a is derived from 1 by cleavage of the C(15)-C(16) bond followed by the formation of a new bond at C(17)-C(20). Ring closure between C(21) and N(b) lead to 3a, from which 4a and the main skeleton of plumeran group P4 can be derived by additional ring closures (C(2)-C(21) and C(2)-C(16), respectively). Ring closure (N(a)-C(16)) in 4a yields E5a, the main skeleton of the eburnan group. Cleavage of the C(17)-C(20) bond in P4 forms P3. By further reactions, the main skeletons of ibogan groups J7a and J7b can be derived from P3. Further reactions are necessary, starting from C3a, D3,S4, A4, E5a, P4, and J7a, to form derivatives of various other skeletal types (Kisakürek *et al.*, 1983).

As shown in Figure 14, the main skeletal types of indole alkaloids can be divided biogenetically into two main groups: the C, D, V, S, and A types containing a skeleton with a nonrearranged secologanin moiety and the E, P, and J types with a rearranged secologanin moiety. This classification is confirmed, in addition to the common structural features, by the fact that all of the C-, D-, V-, S-, and A-types alkaloids-with known absolute configuration-show the same absolute configuration at C(15) as secologanin at C(7).

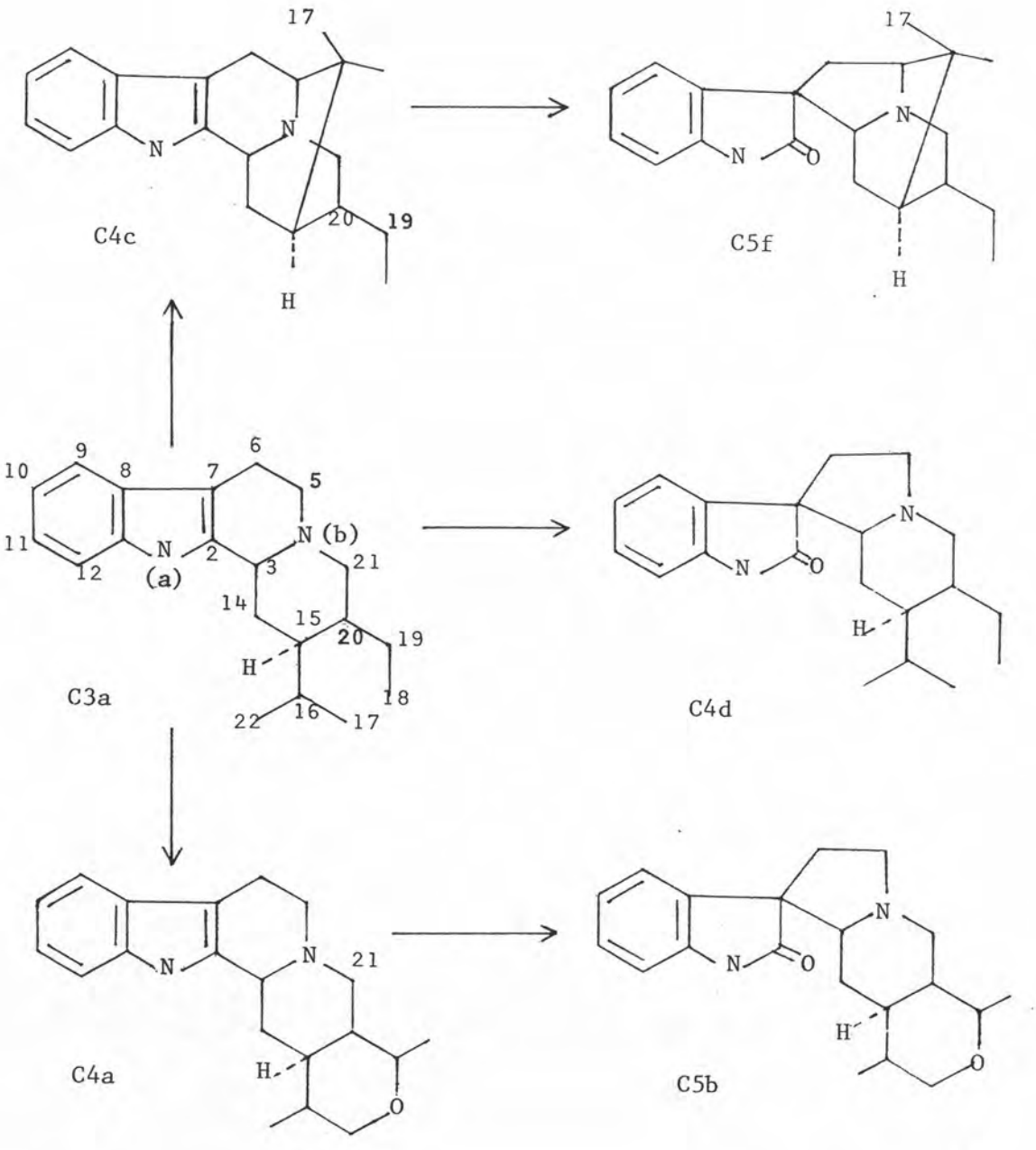
*The numbers after the abbreviations correspond to the number of steps (oxidation, bond formation, cyclization, rotation followed by bond formation or cyclization) necessary to derived a certain skeletal variation starting from the compound 1. Chemical or enzymatic reactions follow, and as a result, a compound chemically more complex than 1 results. The number of steps can be seen immediately by the

structure numbers: e.g. by this definition J7 is more complex compared with 1 than P4 (Figure 14). But P4 is higher than 1 in the rank of chemical complexity.

In the corynanthean type, the C(2) oxidations with skeletal rearrangement produce alkaloids containing the oxindole chromophore = C3a $\longrightarrow$ C4d, C4a $\longrightarrow$ C5b, C4c $\longrightarrow$ C5f (Figure 15) (Kisakurek *et al.*, 1983).

Figure 15

Formations of oxindole alkaloids

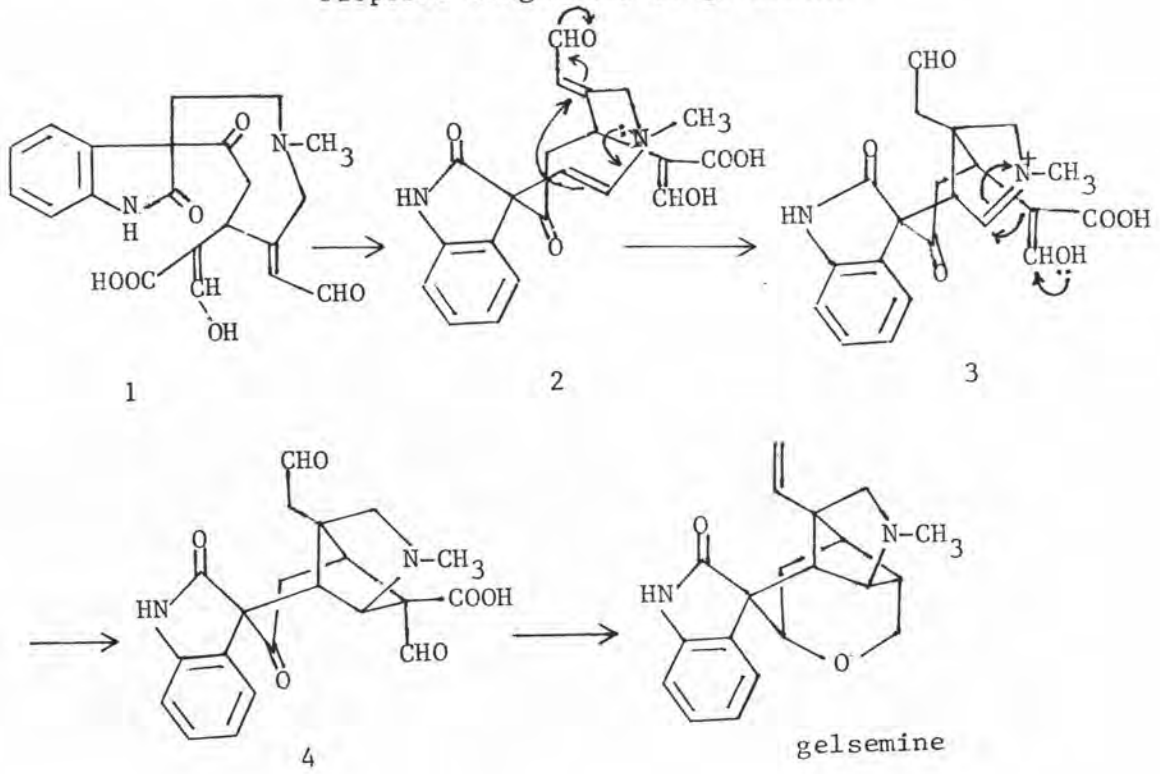


2. Biogenesis of *Gelsemium* alkaloids

Gelsemium species produce a number of alkaloids having an elaborate ring system. So far, only few studies have been made on the biogenesis of *Gelsemium* alkaloids. Conroy and Chakrabarti (1959) suggested a biogenetic route of the major alkaloid, gelsemine, as shown in Figure 16. They proposed an intermediate 1 which derived from tryptamine according to the accepted principles. Further dehydrogenation at N(b) gives 2; Michael addition of the enamine to the conjugated system established the quaternary carbon and formed the five-membered ring enclosing N(b) 3. The intermediate 3 was disposed to internal Mannich condensation to give 4, whence decarboxylation, completion of the oxide ring and adjustment of oxidation state resulted in gelsemine.

Figure 16

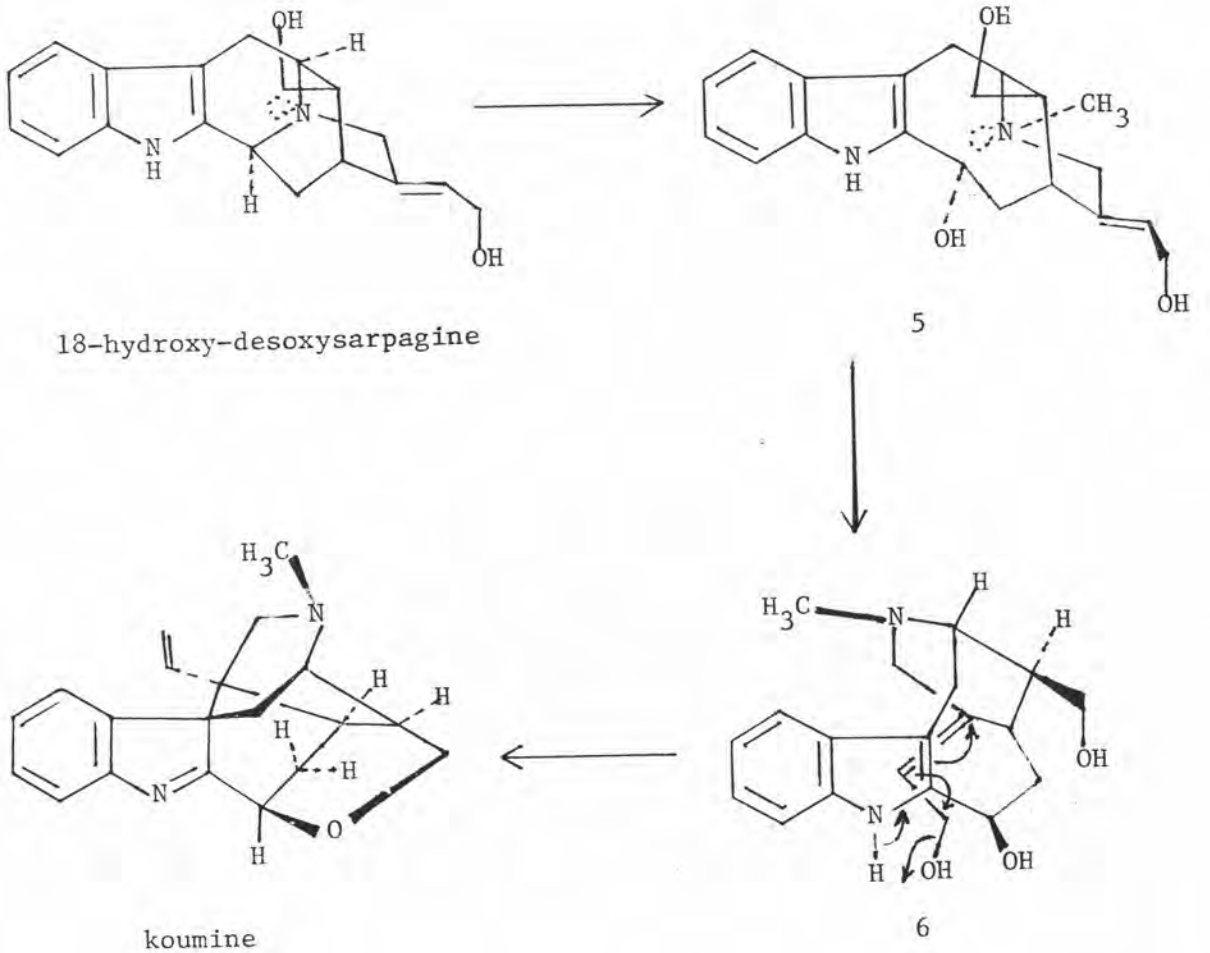
Proposed biogenesis of gelsemine



Lounasmaa and Koskinen (1982) proposed a biosynthetic route of koumine as shown in Figure 17. They suggested that the formation of koumine started with oxidative bond rupture between C(3) and N(b) of 18-hydroxy-desoxysarpagine, giving rise to the compound 5. Repulsive forces between the nitrogen lone pair electrons and the newly introduced hydroxy function forced the intermediate to capture the conformation 6 which was further stabilized by hydrogen bonding of the 18-hydroxy group with the indole N-hydrogen. Expulsion of water and electron pair migrations as depicted would then give rise to the alkaloid koumine.

Figure 17

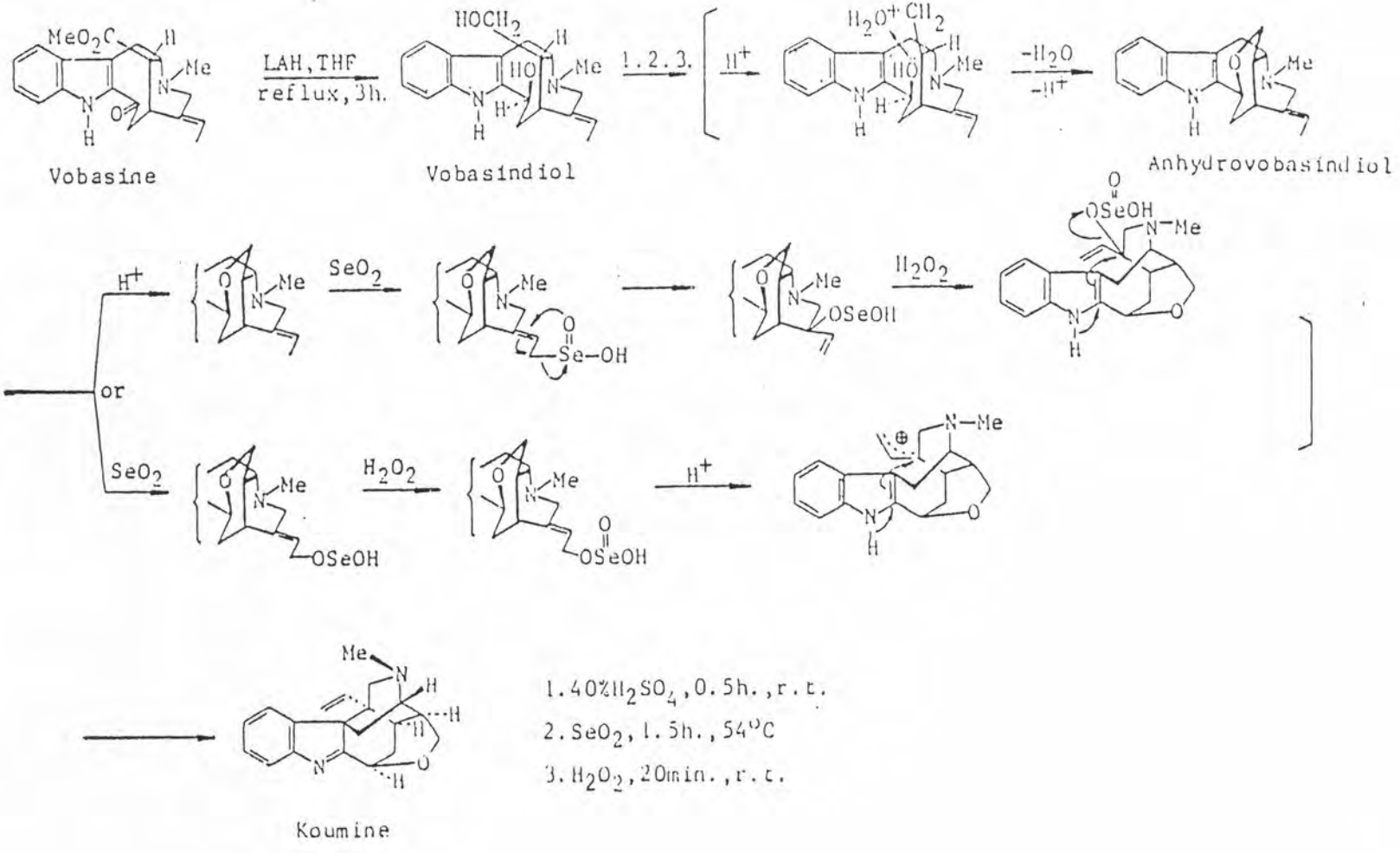
Proposed biogenesis of koumine



Zhujin and Qiansheng (1986) reported a partial synthesis of koumine from a natural alkaloid vobasine which suggested a propable biogenetic link and might well be the precursor of koumine. They reported a two-step process having anhydrovobasindiol as an intermediate.

Figure 18

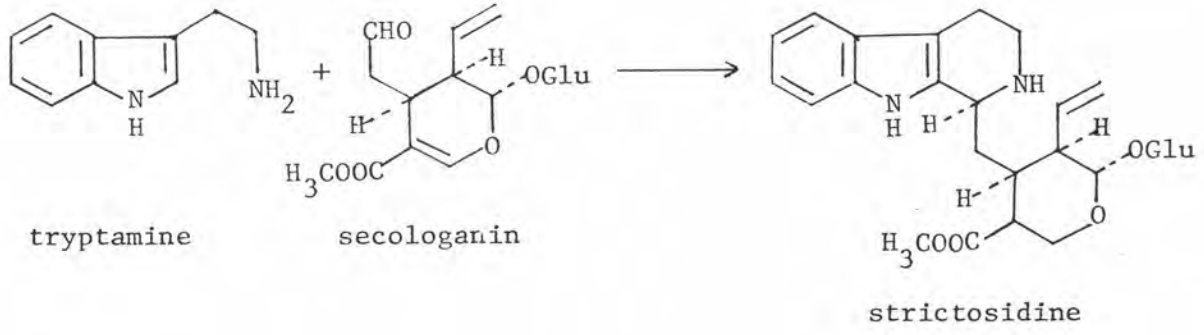
Partial synthesis of koumine



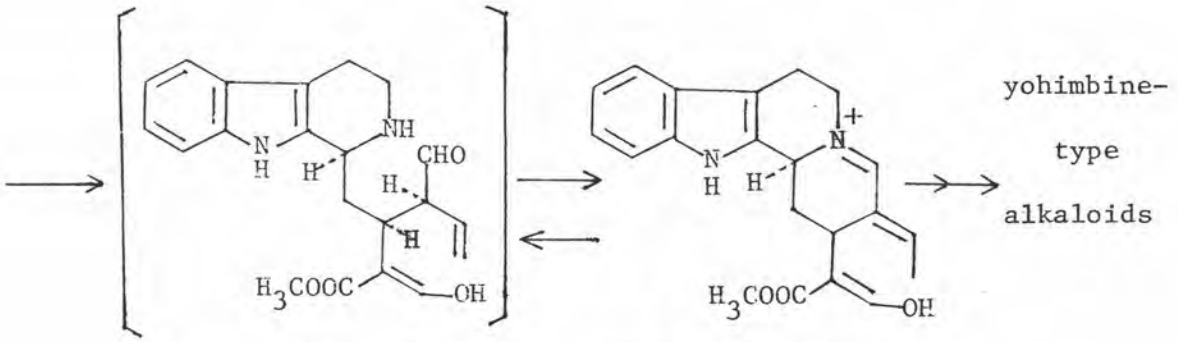
On the biogenetic studies of yohimbine-type alkaloids, dehydrogeissoschizine is known as the important intermediate. Sakai, Yamanaka, Kitajima, Yokota, Aimi, Wongseripipatana and Ponglux (1986, in press) proposed a tentative biogenetic route of *Gelsemium* alkaloids as follows. Figure 19 shows an intermediate B on the biosynthetic route of sarpagine- and ajmaline-type alkaloids and be metabolized to *Gelsemium* alkaloids : humantenine (Figure 20), and koumine and gelsevirine (Figure 21). After the deformylation of the intermediate B, C(21)-norsarpagine-type alkaloid 7 will be metabolized to gelsedine-type alkaloids, as shown in Figure 22.

Figure 19

Tentative biogenetic route of *Gelsemium* alkaloids : intermediates A and B



tryptamine secologanin strictosidine



yohimbine-type alkaloids

intermediate A dehydrogeissoschizine

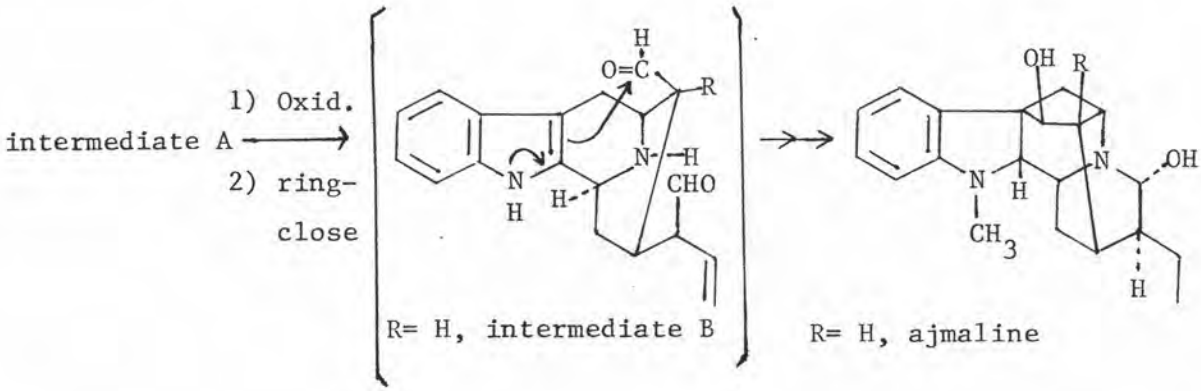


Figure 20

Tentative biogenetic route of *Gelsemium* alkaloids :

humantenine and humantenirine

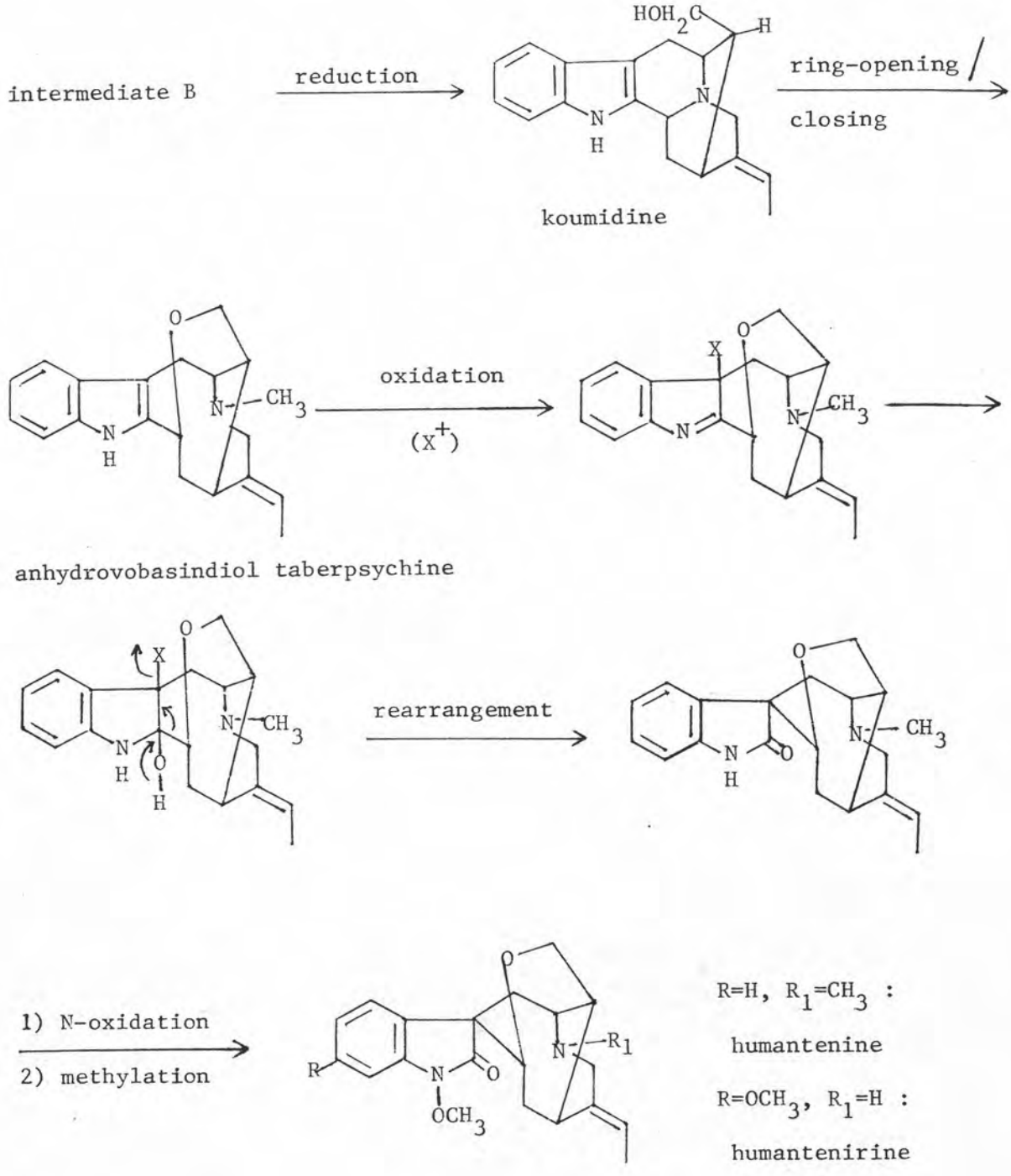


Figure 21

Tentative biogenetic route of *Gelsemium* alkaloids :

koumine and gelsevirine

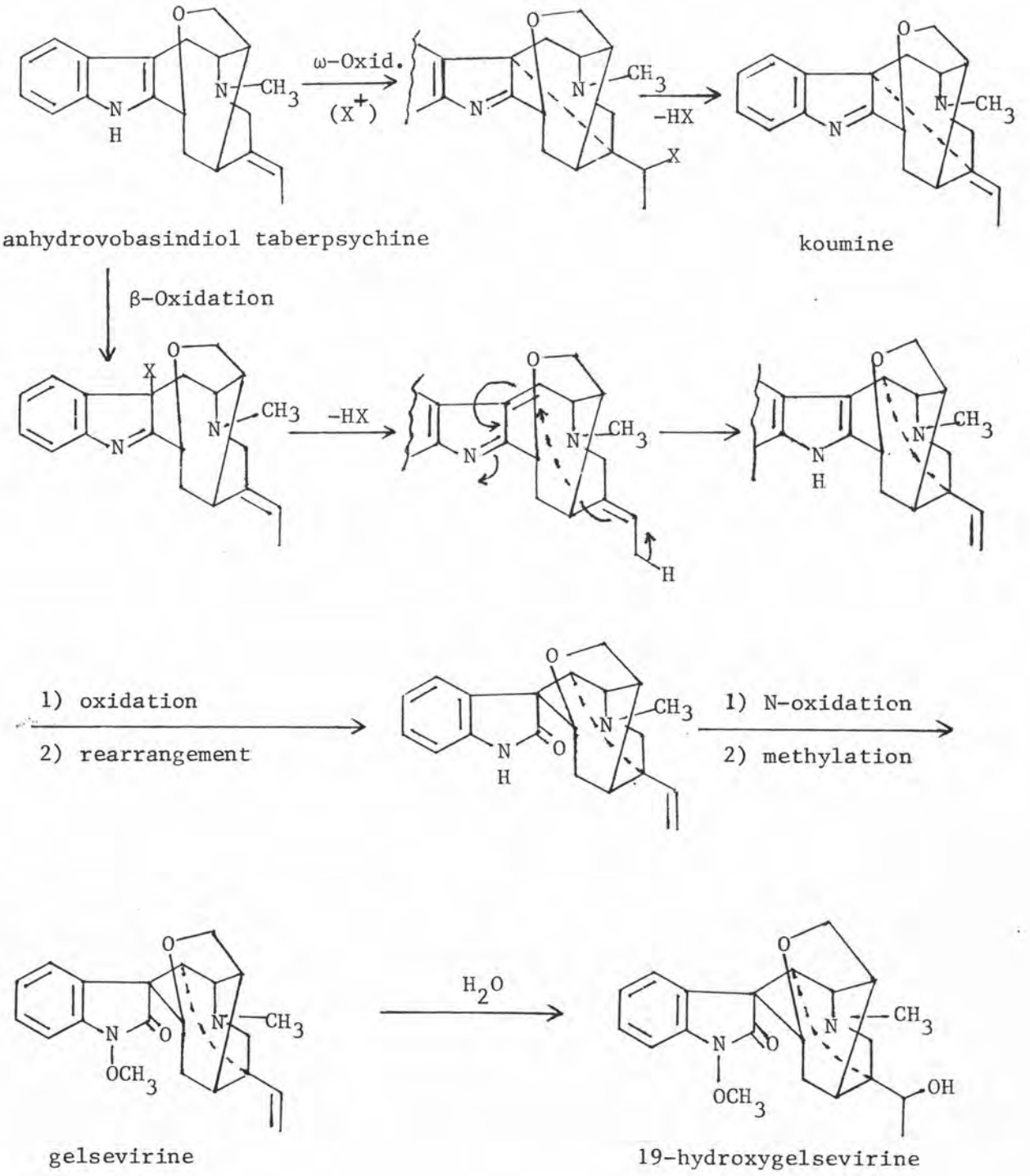
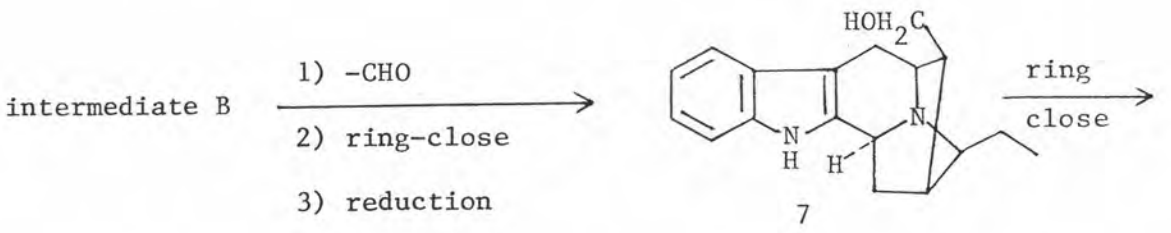
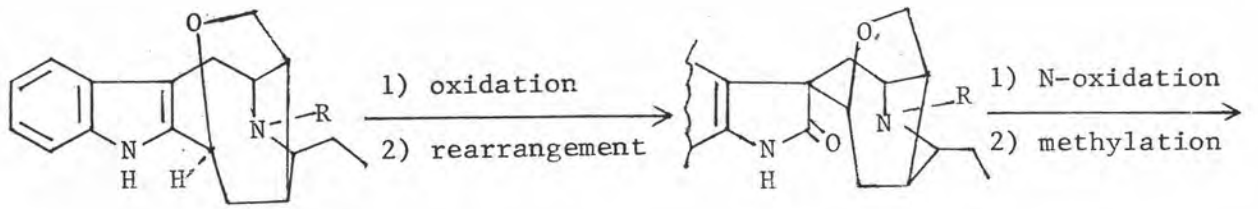


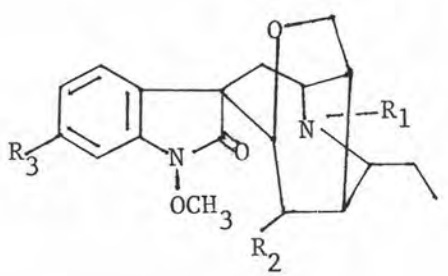
Figure 22

Tentative biogenetic route of *Gelsemium* alkaloids :

gelsedine-type alkaloids





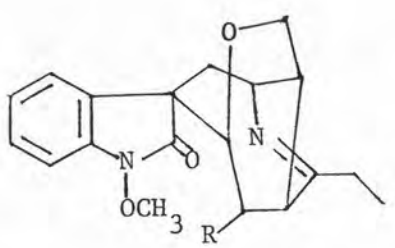


$R_1=R_2=R_3=H$: gelsedine

$R_1=R_2=H$, $R_3=OCH_3$: gelsemicine

$R_1=H$, $R_2=OH$, $R_3=OCH_3$:

14-hydroxygelsemicine



R=H : gelsenicine

R=OH : 14-hydroxygelsenicine

Pharmacology

1. Pharmacological Activities of *Gelsemium* alkaloids

1.1 Effect on Cardiovascular System

Injection into a dog of 0.2 mg gelsemine-HCl per kg provokes a fall in blood pressure and a rise in respiratory movements. Gelsemine reinforces the blood-pressure activity of adrenaline and suppresses almost completely its apnoeic action (Raymond-Hamet, 1937). According to Moisset and Espanes (1938 a) gelsemine produces a slight vasoconstriction in the kidneys but not in the spleen of the chloralosed dog. A 1:5000 of gelsemine solution stops the frog heart, weaker solutions decrease the amplitude of the contractions. Washing with Ringer solution restores the activity of the stopped heart. Atropine does not modify the action of gelsemine; adrenaline and barium chloride inhibit its action. Gelsemine has no parasympathomimetic or parasympatholytic action on the heart (Moisset and Espanes, 1938 b). Its stimulating action on cardiac muscle is like that of atropine (Tamba, 1921).

In the perfusion of the frog, toad or turtle hearts gelsemicine-HCl in concentrations of 1 to 2 mg % produced a primary stimulation followed by a depression of the rate and amplitude of the contractions. A much higher concentration (about 4 mg %) was required to cause this action when the vagal endings were previously paralyzed with atropine. The drug had no action on the spleen or aorta on the peripheral vessels of the nose, intestine, kidney or leg (Hou, 1932 a). Sempervirine is much more active than gelsemine or gelsemicine on the action of inhibit the cholinesterase of nervous tissue and of serum (Vincent and lagreu, 1951).

1.2 Effects on Intestine, Uterus and Urinary Bladder

Gelsemicine-HCl in small concentrations caused a slight increase of tone and slight inhibition of pendulum movements of both the isolated intestine and uterus. Larger concentrations lowered the tone and decreased the movements of the intestine but the tone of the uterus was increased. Neither large nor small concentrations has any effect on the urinary bladder muscles. There was a mutual antagonism between gelsemicine and pilocarpine, physostigmine or barium, but none between it and atropine or adrenaline. Neither ergotoxine nor atropine altered the action of gelsemicine. Similar but less marked results were obtained with the intact intestines and uteri of anesthetized dogs (Hou, 1932 b).

1.3 Effects on Other Organs

Gelsemine injected into the ventral lymph sac of toads and frogs paralyzed the skeletal muscles. The effect was of medullary origin and not due to heterochronism (Moisset and Epsanes 1938 c). Gelsemine given intraperitoneally or orally has marked analgesic activity in doses far below the toxic range, in the study of the analgesic action of various drugs by the method of electrical stimulation of the nervous structures in the incisor teeth of rabbits (Eicher, Hertle and Staib, 1957).

The main symptoms of toxicity of gelsemicine in mammals are depressed respiration, tremors, incoordination of movement, paralysis of extremities, convulsions, urination, defecation, retchings and salivation. Death apparently results from respiratory failure. The

minimum lethal dose in mg per g is for frogs (injection into anterior lymph sac) 0.02 to 0.03, for rats (subcutaneous or intraperitoneal injection) 0.00010 to 0.00012, for rabbits (intravenous injection) 0.00005 to 0.00006 and for dogs (intravenous injection) 0.0005 to 0.001 (Hou, 1931). The toxicities of gelsemine, aconitine, and pseudaconitine (as halides) were compared, weight by weight, in mice, rats, guinea pigs and rabbits. The alkaloids were injected intravenously in mice, rats and rabbits, but subcutaneously in guinea pigs. The order of toxicity of the 3 substances varies from one species to another. The results show that in mice, gelsemicine > aconitine > pseudaconitine; in rats gelsemicine > pseudaconitine > aconitine; in rabbits, pseudaconitine > aconitine > gelsemicine; and in guinea pigs, pseudaconitine > aconitine > gelsemicine (Chen, Anderson and Robbins, 1938).

According to Du *et al.* (1982) gelsenicine is the most toxic alkaloid found in *Gelsemium elegans* Benth. Its intraperitoneal LD_{50} value is 185 μg/kg for mouse.

2. Pharmacological Activities of *Gardneria* Alkaloids

Gardneramine, compared with gardnerine, gardnutine, and hydroxy-gardnutine, is the most potent alkaloid inhibiting the contraction of the urinary bladder induced by electrical stimulation of the pelvic nerves. Its potency was about one-half of that of hexamethonium, and the effect was short duration. Gardneramine depressed the contraction induced by intraarterial dimethylphenylpiperazinium, with no antagonising action to the acetylcholine-induces contraction. All four *Gardneria* alkaloids had only a weak effect on local anesthetic action in the isolated frog sciatic

nerve preparation (Harada, Ozaki and Ohno, 1979).

Effect on neuromuscular transmission was examined in the rat limb preparation *in situ*. Gardneramine inhibited the gastrocnemius contractions elicited by electrical stimulation of the sciatic nerve, but excerted little or no inhibition on the contractions elicited by direct stimulation of the muscle. The inhibitory effect of gardneramine was a little stronger than that of hexamethonium and was very weak when compared with that of *d*-tubocurarine. Gardnerine augmented both contractions elicited by nerve and muscle stimulation. Gardnutine and hydroxygardnutine showed a long-lasting depressive effect on both contractions (Harada and Ozaki, 1976).