CHAPTER II

HISTORICAL

Alkaloids Isolated from Species of *Erythrophleum*

The *Erythrophleum* alkaloids were firstly studied by Gallois and Hardy in 1875 from *Erythrophleum guineense* G. Don bark and *E. couminga* Baill. leaves and seeds (Dalma, 1954). To date, there are about fourty alkaloids of which complete structures are known from only six *Erythrophleum* species. Most of the alkaloids were isolated from the bark extract. Arya (1962) reported the alkaloidal amount in the bark of various species as shown in Table 1.

Table 1

Alkaloidal amount of some *Erythrophleum* species

Plant	Source	Alkaloid (%)
Erythrophleum africanum Harms.	Tanganyika	0.04
E. chlorostachys Baill.	Queensland	0.25
E. fordii Oliv.	Viet Nam	0.37
E. guineense G. Don	Zanzibar	0.77
E. ivorense A. Chev.	West Nigeria	0.31
E. lasianthum Crob.	Portuguese E. Africa	0.26

The alkaloids reported to be distributed in genus *Erythrophleum* are summarized in Table 2.

Table 2

Distribution of *Erythrophleum* alkaloids

Plant	Alkaloid	Reference
Erythrophleum africanum Harms. (bark)	Cassamidine	Jansson and Cronlund, 1976.
	Erythrophlamine	
	Norerythrophlamide	
	Norerythrostachamide	
E. couminga Baill. (bark)	Cassaine	Cronlund and Oguakwa, 1975; Ruzicka *et al.*, 1945 b.
	Cassaidine	
	Coumingidine	Schlittler, 1941.
	Coumingine	Cronlund and Oguakwa, 1975; Ruzicka *et al.*1945 a, b.
	Norcoumingide	Cronlund and Oguakwa, 1975.
	19-Nor-4-dehydro-cassaidine	Oguakwa and Cronlund, 1976.
	Cassamine	Cronlund and Oguakwa, 1975.
	Norcassamide	
	Cassamidine	
	Erythrophlamine	
	Erythrophleguine	
	3-Hydroxynorerythro-suamide	

Table 2 (continued)

Plant	Alkaloid	Reference
Erythrophleum chlorostachys Baill. (bark)	Cassaidine	Loder *et al.*, 1974; Falkiner *et al.*, 1975.
	Norcassaidine	Falkiner *et al.*, 1975.
	Norcassaidide	Loder *et al.*, 1974.
	Norerythrostachaldine	Falkiner *et al.*, 1975; Loder and Nearn, 1975 b.
	3β-Acetoxynorerythrostachaldine	
	Cassamidine	Falkiner *et al.*, 1975; Loder *et al*, 1974.
	Norcassamidine	
	Norcassamidide	Loder *et al.*, 1974.
	Norerythrophlamine	Falkiner *et al.*, 1975.
	3β-Acetoxynorerythrophlamine	
	Norerythrophlamide	Loder *et al.*, 1974.
	Norerythrostachamine	Falkiner *et al.*, 1975.
	3β-Acetoxynorerythrostachamine	
	Norerythrostachamide	Loder *et al.*, 1974.
	Norerythrosuamine	Falkiner *et al.*, 1975.
	3β-Acetoxynorerythrosuamine	Loder and Nearn, 1975 a.

Table 2 (continued)

Plant	Alkaloid	Reference
Erythrophleum chlorostachys Baill. (leaf)	β-Dimethylaminoethylcinnamate	Griffin *et al.*, 1971.
	N-2-Hydroxyethyl-N-methylcinnamamide	
	N-2-Hydroxyethyl-N-methyl-*trans*-*p*-hydroxycinnamamide	
	N-2-Hydroxyethylcinnamamide	
E. fordii Oliv. (bark)	Cassaine	Arya, 1962.
E. guineense G. Don (bark)	Cassaine	Dalma, 1939; Clarke, 1971; Lindwall *et al.*, 1965.
	Cassaidine	Lindwall *et al.*, 1971; Ruzicka and Dalma, 1940.
	Coumidine	Thorell *et al.*, 1968.
	Coumingine	Lindwall *et al.*, 1965.
	Cassamine	
	Norcassamide	Friedrich-Fiechtl and Spitteller, 1971; Loder *et al.*, 1972.
	Cassamidine	Thorell *et al.*, 1968.

Table 2 (continued)

Plant	Alkaloid	Reference
Erythrophleum guineense G. Don (bark)	Norcassamidide	Friedrich-Fiechtl and Spitteller, 1971; Loder *et al.*, 1972.
	Erythrophlamine	Engel and Tondeur, 1948 & 1949.
	Erythrophleguine	Clarke, 1971; Lindwall *et al.*, 1965.
	Erythrosuamine	Thorell *et al.*, 1968.
	Norerythrosuamide Dehydronorerythrosuamide	Friedrich-Fiechtl and Spitteller, 1971; Loder *et al.*, 1972.
E. ivorense A. Chev. (bark)	Cassaine	Cronlund and Sandberg, 1971.
	19-Hydroxycassaine 3-(3-Methylcrotonyl)-cassaine	Cronlund, 1973.
	Norcassaide	Cronlund and Sandberg, 1971; Loder *et al.*, 1972.
	Cassaidine Coumidine	Cronlund and Sandberg, 1971.
	Ivorine	Ottinger *et al.*, 1965.
	Cassamine	Cronlund and Sandberg, 1971; Cronlund, 1973.

Table 2 (continued)

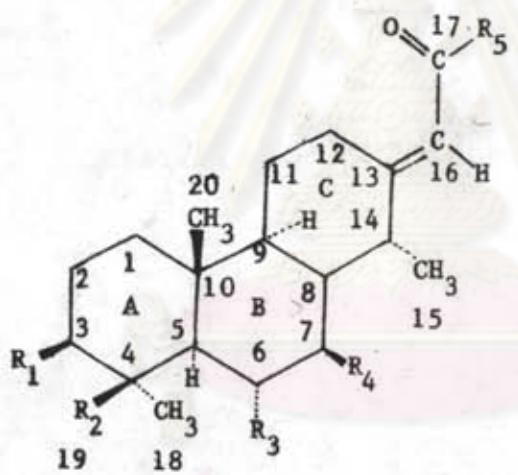
Plant	Alkaloid	Reference
Erythrophleum ivorense A. Chev. (bark)	Norcassamide	Cronlund and Sandberg, 1971; Loder *et al.*, 1972; Loder and Nearn, 1972.
	Cassamidine	Cronlund and Sandberg, 1971.
	Erythrophlamine	
	Norerythrophlamide	Cronlund and Sandberg, 1971; Loder *et al.*, 1972; Loder and Nearn, 1972.
	Erythrophleguine	Cronlund and Sandberg, 1971.

Chemistry of *Erythrophleum* Alkaloids

Research on the chemistry of *Erythrophleum* alkaloids was begun in 1935 when Dalma succeeded in isolating three crystalline alkaloids (cassaine, cassaidine and norcassaidine) and an amorphous one (homophleine) from the bark of *Erythrophleum guineense* G. Don (Dalma, 1954). Subsequently extensive chemical investigations of this group of alkaloids have been undertaken in several laboratories and led to the structural elucidation of those which have been isolated.

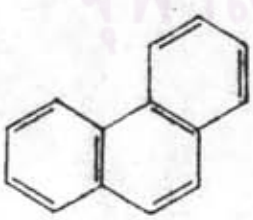
A. Basic Structure of *Erythrophleum* Alkaloids (1).

Most of the alkaloids obtained from the species of *Erythrophleum* are, in general, secondary or tertiary aminoethanol esters, or amides of α,β-unsaturated monocarboxylic acid of tricyclic diterpene series containing a perhydrophenanthrene skeleton (3). The tricyclic skeleton of these alkaloids is classified for cassane group (4) of diterpenoids. In this thesis, numbering system of diterpenoid portion follows the proposal of McCrindle (1965). The basic and correlative structures of *Erythrophleum* alkaloids are shown in Figure 1.

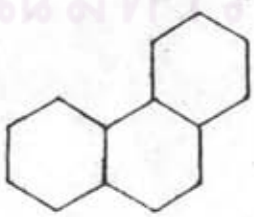


R_5 = $OCH_2CH_2N(CH_3)_2$ or
$OCH_2CH_2NH(CH_3)$ or
$N(CH_3)CH_2CH_2OH$

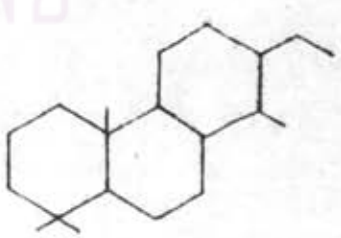
(1) Basic structure



(2) Phenanthrene



(3) Perhydrophenanthrene

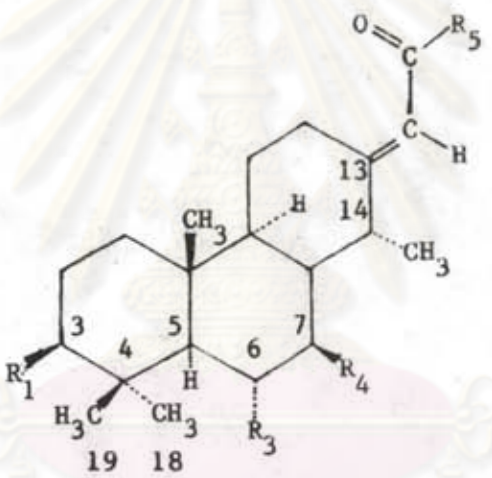


(4) Cassane

Figure 1. Basic and correlative structures of *Erythrophleum* alkaloids.

There are two major groups of *Erythrophleum* alkaloids depending on the R_2 group at C-4 of ring A (1).

The first group (5) contains two methyl groups at C-4 (1, R_2 = CH_3) and a hydroxyl group at C-3 (1, R_1 = OH). At C-3, some of the alkaloids are esters having acetate ($OOCCH_3$), 3-methylcrotonate ($OOCCH{=}C(CH_3)_2$), 3-hydroxyisovalerate ($OOCCH_2C(OH)(CH_3)_2$) or hydroxylvalerate ($OOCC_4H_8OH$) group as R_1. The alkaloids in this group are summarized in Table 3.



(5)

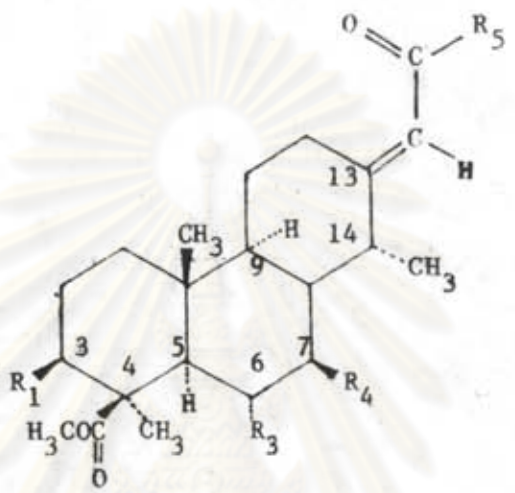
Table 3

Erythrophleum alkaloids containing C-4 dimethyl groups

Alkaloid	R_1	R_3	R_4	R_5
Cassaine	OH	H	=O	$OCH_2CH_2N(CH_3)_2$
6α-Hydroxycassaine	OH	OH	=O	$OCH_2CH_2N(CH_3)_2$
3-(3-Methylcrotonyl)-cassaine	$OOCCH{=}C(CH_3)_2$	H	=O	$OCH_2CH_2N(CH_3)_2$
Norcassaide	OH	H	=O	$N(CH_3)CH_2CH_2OH$
Cassaidine	OH	H	OH	$OCH_2CH_2N(CH_3)_2$
Norcassaidine	OH	H	OH	$OCH_2CH_2NH(CH_3)$
Norcassaidide	OH	H	OH	$N(CH_3)CH_2CH_2OH$
Coumingine	$OOCCH_2C(OH)(CH_3)_2$	H	=O	$OCH_2CH_2N(CH_3)_2$
Norcoumingide	$OOCCH_2C(OH)(CH_3)_2$	H	=O	$N(CH_3)CH_2CH_2OH$
Coumidine	$OOCCH_2C(OH)(CH_3)_2$	H	OH	$OCH_2CH_2N(CH_3)_2$
Coumingidine	$OOCC_4H_8OH$	H	=O	$OCH_2CH_2NH(CH_3)$
Ivorine	$OOCCH{=}C(CH_3)_2$	H	=O	$OCH_2CH_2NH(CH_3)$

References: Clarke, 1971; Cronlund, 1973 a, b; Cronlund and Oguakwa, 1975; Falkiner *et al.*, 1975; Hauth *et al.*, 1965; Loder *et al.*, 1974; Morin, 1968; Ottinger *et al.*, 1965; Thorell *et al.*, 1968.

The second group (6) has one β-carbomethoxy group at C-4 in place of a methyl group (1, R_2= $COOCH_3$). At C-3, some of the alkaloids are esters having acetate group as R_1. The alkaloids in this group are summarized in Table 4.



(6)

Table 4

Erythrophleum alkaloids containing C-4 β-carbomethoxy group

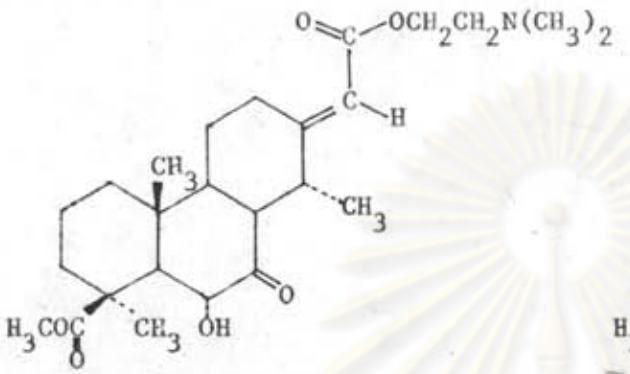
Alkaloid	R_1	R_3	R_4	R_5
Cassamine	H	H	=O	$OCH_2CH_2N(CH_3)_2$
Norcassamine	H	H	=O	$OCH_2CH_2NH(CH_3)$
Norcassamide	H	H	=O	$N(CH_3)CH_2CH_2OH$
Cassamidine	H	H	OH	$OCH_2CH_2N(CH_3)_2$
Norcassamidine	H	H	OH	$OCH_2CH_2NH(CH_3)$
Norcassamidide	H	H	OH	$N(CH_3)CH_2CH_2OH$
Erythrophlamine	OH	H	=O	$OCH_2CH_2N(CH_3)_2$
Norerythrophlamine	OH	H	=O	$OCH_2CH_2NH(CH_3)$
Norerythrophlamide	OH	H	=O	$N(CH_3)CH_2CH_2OH$

Table 4 (continued)

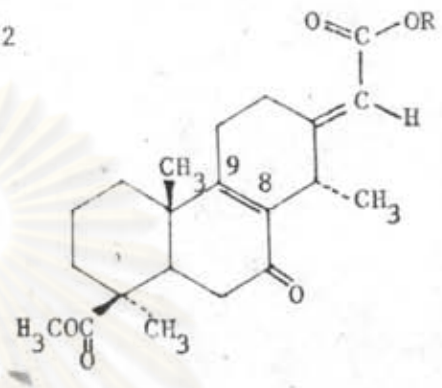
Alkaloid	R_1	R_3	R_4	R_5
3β-Acetoxynorerythrophlamine	$OOCCH_3$	H	=O	$OCH_2CH_2NH(CH_3)$
Erythrostachamine	OH	H	OH	$OCH_2CH_2N(CH_3)_2$
Norerythrostachamine	OH	H	OH	$OCH_2CH_2NH(CH_3)$
Norerythrostachamide	OH	H	OH	$N(CH_3)CH_2CH_2OH$
3β-Acetoxynorerythrostachamine	$OOCCH_3$	H	OH	$OCH_2CH_2NH(CH_3)$
Erythrophleguine	H	OH	=O	$OCH_2CH_2N(CH_3)_2$
Erythrosuamine	H	=O	OH	$OCH_2CH_2N(CH_3)_2$
Norerythrosuamine	H	=O	OH	$OCH_2CH_2NH(CH_3)$
Norerythrosuamide	H	=O	OH	$N(CH_3)CH_2CH_2OH$
Dehydronorerythrosuamide	H	=O	=O	$N(CH_3)CH_2CH_2OH$
3β-Hydroxynorerythrosuamine	OH	=O	OH	$OCH_2CH_2NH(CH_3)$
3β-Hydroxynorerythrosuamide	OH	=O	OH	$N(CH_3)CH_2CH_2OH$
3β-Acetoxynorerythrosuamine	$OOCCH_3$	=O	OH	$OCH_2CH_2NH(CH_3)$

References: Arya and Engel, 1961; Clarke, 1971; Cronlund and Oguakwa, 1975; Falkiner *et al.*, 1975; Jansson and Cronlund, 1976; Lindwall *et al.*, 1965 a, b; Loder *et al.*, 1972; Loder *et al.*, 1974; Loder and Nearn, 1975 a, b; Mathieson *et al.*, 1960; Morin, 1968 and Thorell *et al.*, 1968.

8-Dehydrocassamic acid (8); the hydrolyzed product of erythrophleguine (7); was converted via the acid chloride to 8-dehydrocassamine (9), a member of this group which has not yet been isolated naturally (Clarke, 1971).



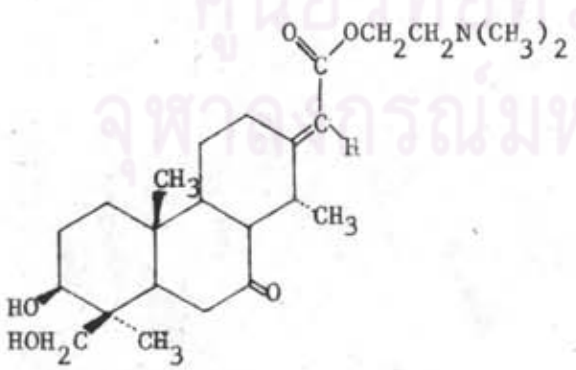
(7) Erythrophleguine



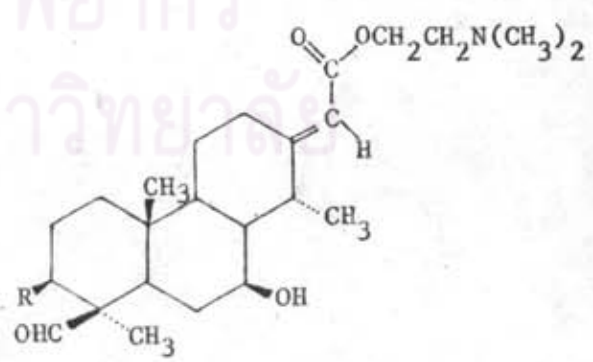
(8) R = H

(9) R = $CH_2CH_2N(CH_3)_2$

There is further a minor group of *Erythrophleum* alkaloids consists of few alkaloids which a hydroxylated methyl group or an aldehyde group is found at C-4 in place of a methyl group. The three alkaloids of this group are 19-hydroxycassaine (10) (Cronlund, 1973 b), norerythrostachaldine (11) and 3β-acetoxynorerythrostachaldine (12) (Falkiner *et al.*, 1975).



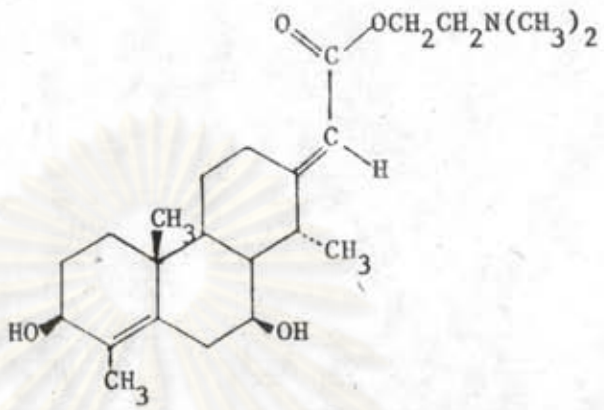
(10) 19-Hydroxycassaine



(11) R = OH

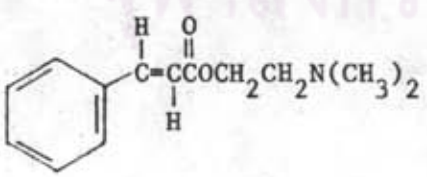
(12) R = $OOCH_3$

19-Nor-4-dehydrocassaidine (13), isolated from *Erythrophleum couminga* Baill., was reported to have one double bond with no methyl group at C-4 (Oguakwa and Cronlund, 1976).

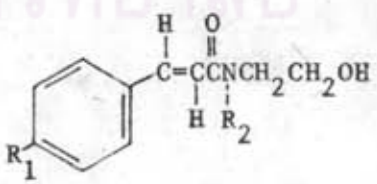


(13) 19-Nor-4-dehydrocassaidine

According to the previous statement on page 14, the *Erythrophleum* alkaloids are derivatives of tricyclic diterpene acids. In four cases, the alkaloids isolated from the leaves of *Erythrophleum chlorostachys* Baill. are secondary or tertiary aminoethanol ester and amides of cinnamic acid, i.e. β-dimethylaminoethyl cinnamate (14), N-2-hydroxyethyl-N-methyl cinnamamide(15), N-2-hydroxyethyl-N-methyl-*trans*-p-hydroxycinnamamide (16) and N-2-hydroxyethylcinnamamide (17) (Griffin *et al.*, 1971).



(14)

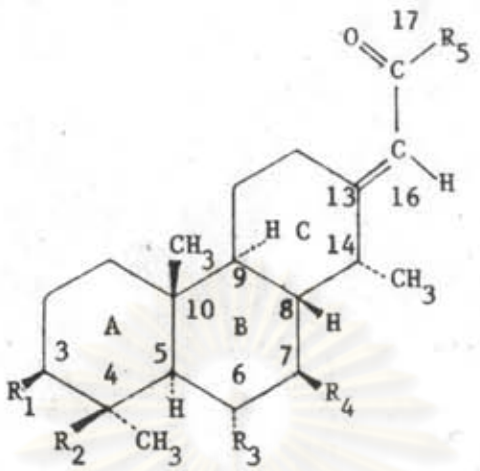


(15) R_1 = H, R_2 = CH_3

(16) R_1 = OH, R_2 = CH_3

(17) R_1 = H, R_2 = H

B. Stereochemistry of *Erythrophleum* alkaloids



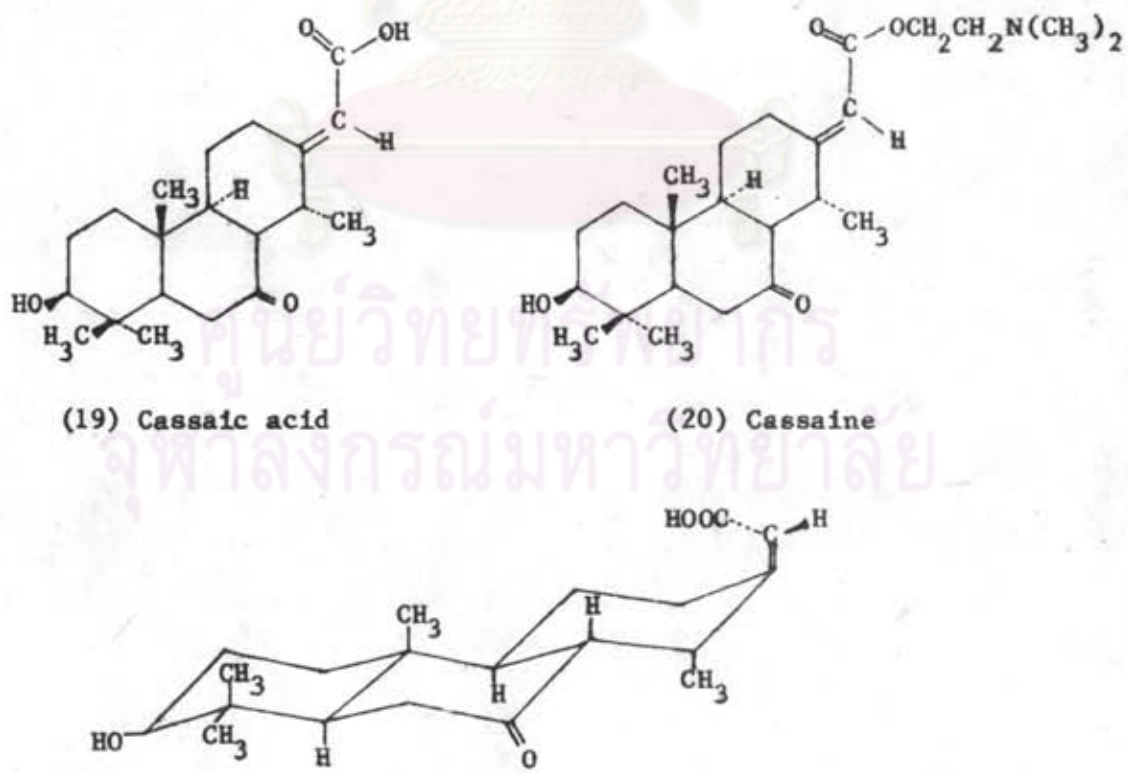
(18)

All naturally occurring *Erythrophleum* alkaloids (18) have asymmetric centers at C-5, C-8, C-9, C-10 and C-14. The configurations of C-5 hydrogen, C-9 hydrogen and C-14 methyl are assigned the α-orientation and those of C-8 hydrogen and C-10 methyl the β-orientation (Chapman *et al.*, 1963; Clarke *et al.*, 1966; King *et al.*, 1958; Mori and Matsui, 1966 and Turner *et al.*, 1959). The alkaloids are known to have geometric isomerization since the double bond between C-13 and C-16 posseses the C-16 carbonyl *trans* to the axial C-14 α-methyl group (Hauth *et al.*, 1965).

Substitutions in ring A have been found at C-3 and C-4. The group at C-3 (R_1) may be β-configuration of hydroxyl (OH), acetate ($OOCCH_3$), 3-methylcrotonate ($OOCCH{=}C(CH_3)_2$), 3-hydroxyisovalerate ($OOCCH_2C(OH)(CH_3)_2$), or hydroxyvalerate ($OOCC_4H_8OH$) group (Cronlund, 1973 a, b; Cronlund and Oguakwa, 1975; Loder and Nearn, 1975 a, b and Turner *et al.*, 1959). The group at C-4 (R_2) may be β-configuration of methyl (CH_3), hydroxymethyl (CH_2OH), formyl (CHO), or carbomethoxyl ($COOCH_3$) group (Chapman *et al.*, 1963; cronlund, 1973 b and Loder and Nearn, 1975 b). In ring B, the

functional group on C-6 (R_3) may be either α-hydroxyl (OH) or ketone (=O) group and that on C-7 (R_4) may be either β-hydroxyl or ketone group (Blessington *et al.*, 1970; Lindwall *et al.*, 1965 b and Thorell *et al.*, 1968).

According to the extensive structural investigation of cassaic acid (19), the hydrolyzed product of the alkaloid cassaine (20), the A/B and B/C ring junctions of perhydrophenanthrene nucleus are *trans-anti-trans* system with equatorial C-3 β-hydroxyl and axial C-14 α-methyl (Linstead *et al.*, 1942 and Morin, 1968). The conformation of all rings are assigned the *chair-chair-chair* arrangement, those configurations with larger number of equatorial bonds at the point of ring fusion are the more stable (Johnson, 1951). The conformation of cassaic acid is illustrated in Figure 2.

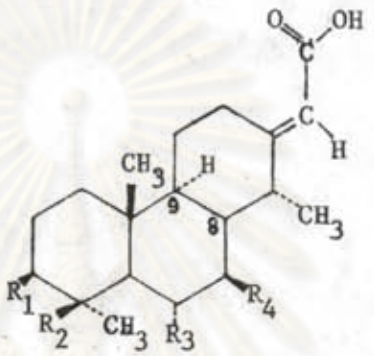


(19) Cassaic acid (20) Cassaine

Figure 2. Conformation of cassaic acid.

C. Hydrolysis of *Erythrophleum* Alkaloids

On mild hydrolysis with diluted mineral acid, these alkaloids afford β-methylaminoethanol and α,β-unsaturated tricyclic diterpenic acids. The diterpenic acids (21) hydrolyzed from *Erythrophleum* alkaloids are summarized in Table 5.



(21)

Table 5

Diterpenic acid from *Erythrophleum* species

Diterpenic acid	R_1	R_2	R_3	R_4	Δ^8 Bond
Cassaic acid	OH	CH_3	H	=O	-
8-Dehydrocassaic acid	OH	CH_3	H	=O	Present
Cassaidic acid	OH	CH_3	H	OH	-
Coumidic acid	$OOCCH_3C(OH)(CH_3)_2$	CH_3	H	OH	-
Coumingic acid	$OOCCH_3C(OH)(CH_3)_2$	CH_3	H	=O	-
Coumingidic acid	$OOCC_4H_8OH$	CH_3	H	=O	-
Erythrostachaldic acid	OH	CHO	H	OH	-
Cassamic acid	H	$COOCH_3$	H	=O	-
8-Dehydrocassamic acid	H	$COOCH_3$	H	=O	Present
Cassamidic acid	H	$COOCH_3$	H	OH	-

Table 5 (continued)

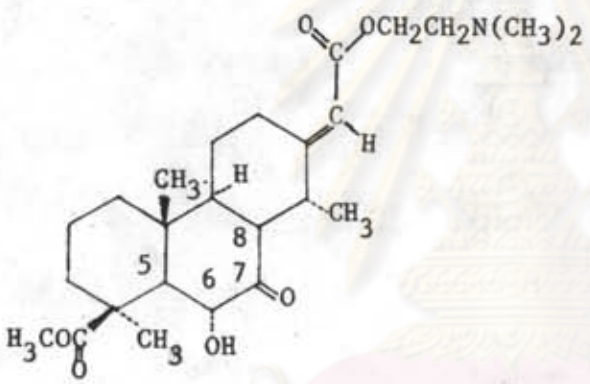
Diterpenic acid	R_1	R_2	R_3	R_4	Δ^8 Bond
Erythrophlamic acid	OH	$COOCH_3$	H	=O	-
Erythrostachamic acid	OH	$COOCH_3$	H	OH	-
Erythrosuamic acid (Cassminic acid)	H	$COOCH_3$	=O	OH	-
Erythrophleadienolic acid	H	$COOCH_3$	H	OH	Present

Reference: Arya, 1962; Blessington *et al.*, 1970; Chapman *et al.*, 1963; Chapman *et al.*, 1965; Clarke, 1971; Dalma, 1954; Gensler and Sherman, 1959; Lindwall *et al.*, 1965; Loder *et al.*, 1974; Loder and Nearn, 1975 b; Mathieson *et al.*, 1960; Morin, 1968; Ruzicka *et al.*, 1941; Schlittler, 1941 and Thorell *et al.*, 1968.

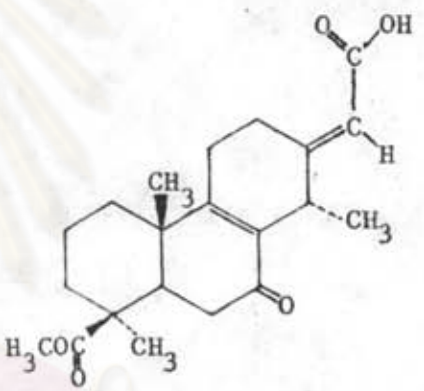
The 3β-esterification in diterpenic acids and alkaloids can undergo hydrolysis with mineral acid or alkali. Coumingine, on acid hydrolysis, yields cassaic acid and in addition β-hydroxyisovaleric acid (Ruzicka *et al.*, 1941) while 3-(3-methylcrotonyl)cassaine yields cassaic acid and 3-methylcrotonic acid (Cronlund, 1973). Coumidine, on mild acid hydrolysis, provides the mixture of coumadic and cassaidic acids (Thorell *et al.*, 1968). Acid hydrolysis of coumingidine generates methylaminoethanol together with coumingidic acid, but its methyl ester, on alkaline hydrolysis forms an acid identified as cassaic acid (Dalma, 1954).

Erythrophleguine (22), the 6-hydroxycassamine, isolated from *Erythrophleum guineense* G. Don having a 6α-hydroxy and a 7-ketone group,

is hydrolyzed and rearranged easily under acid conditions to give 8-dehydrocassamic acid (23) but a 7-hydroxy-6-oxo orientation as in erythrosuamine (26) effectively prevents this rearrangement. The proposed mechanism shown in Figure 3 for the formation of 8-dehydrocassamic acid from erythrophleguine involves a 1,4-elimination of water from the enol (24) to yield another enol (25) (Lindwall *et al.*, 1965 a, b). Similar reaction would explain the proposal that 6-hydroxycassaine (27) might probably exist and would be the reasonable precursor of the isolated 8-dehydrocassaic acid (28) (Clarke, 1971).



(22) Erythrophleguine



(23) 8-Dehydrocassamic acid

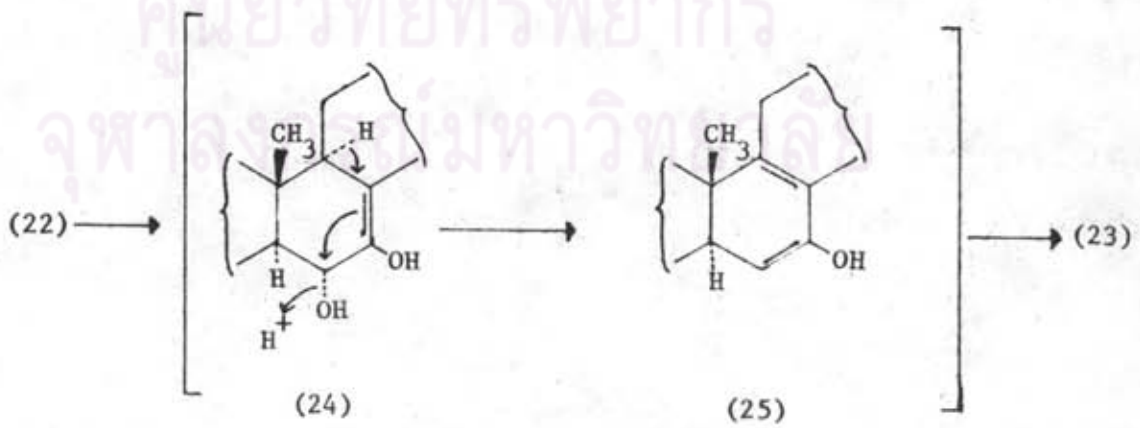
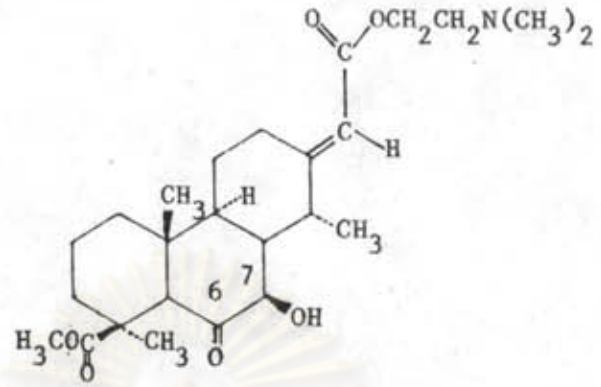
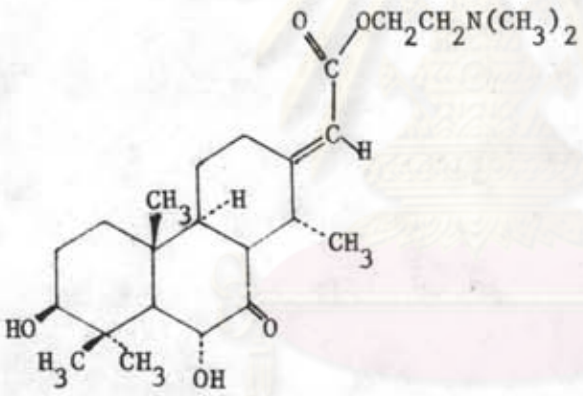


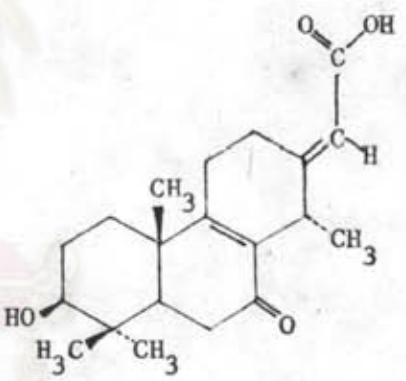
Figure 3. Formation of 8-dehydrocassamic acid



(26) Erythrosuamine



(27) 6-Hydroxycassaine



(28) 8-Dehydrocassaic acid

D. Conversion of *Erythrophleum* Alkaloids

The hydroxyl group in rings A and B of diterpenic portion of *Erythrophleum* alkaloids may be converted by reduction with sodium borohydride ($NaBH_4$) into ketone group which may be reconverted into the hydroxyl group by oxidation with chromic trioxide (CrO_3) in pyridine or chromic acid. Some conversions of *Erythrophleum* alkaloids into their corresponding alkaloids are listed in Table 6.

Table 6

Conversion of *Erythrophleum* alkaloids

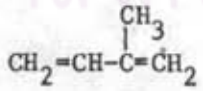
Conversion			Reference
Cassaine	$\underset{CrO_3}{\overset{NaBH_4}{\rightleftharpoons}}$	Cassaidine	Engel, 1959.
Coumingine	$\underset{CrO_3}{\overset{NaBH_4}{\rightleftharpoons}}$	Coumidine	Thorell *et al.*, 1968.
Cassamine	$\xrightarrow{NaBH_4}$	Cassamidine	
Norcassamide	$\xrightarrow{NaBH_4}$	Norcassamidide	Friedich-Fiechtl and Spiteller, 1971.
Norerythrosuamide	$\xrightarrow{CrO_3}$	Dehydronorerythrosuamide	
Norerythrophlamide	$\xrightarrow{NaBH_4}$	Norerythrostachamide	Jansson and Cronlund, 1976.

Biosynthesis

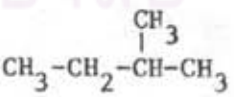
A. Introduction

Nowadays, a great number of research have been undertaken to investigate on how plants produce alkaloids by the use of radioactive tracers. These investigations were based upon structural similarities and also upon the relation to simpler natural products. Experiment on the biosynthesis of *Erythrophleum* alkaloids has been hampered by the shortage of plant materials, in part because of the political unrest in the regions where many of the species are found (Morin, 1968). So the speculative biosynthesis of *Erythrophleum* alkaloids may follow closely that of the diterpenoids because the carbon skeleton of these alkaloids themselves correlates to the cassane group of the diterpenoids.

Terpenoids are the group of compounds of extraordinarity diverse structures, all of them may be formally considered to have originated from fragments whose carbon skeletons correspond to isoprene units (methylbutadiene, 29). The completely saturated form of this five carbon unit is called isopentane (30) (Nicholas, 1973). These units link together in various ways and with different types of ring closures, degree of unsaturation and functional groups (Robinson, 1980).



(29) Isoprene



(30) Isopentane

Almost all of the terpenoids conform to the "biogenetic isoprene rule" firstly developed by Wallach (1914) and refined by Ruzicka *et al.* (1953), a rule which states that a terpene is constituted by union of two or more isoprene units in a "head-to-tail" manner (Nicholas, 1973).

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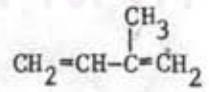

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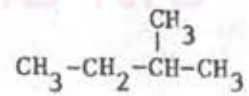

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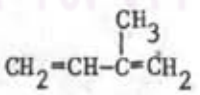

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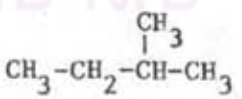

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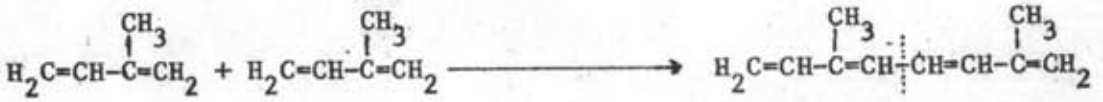
The combination of two isoprene units arranged "head-to-tail" is shown below:



The terpenoid embraces wide varieties of compounds according to the number of isoprene units contained in their molecules as shown in Table 7.

Table 7

General formulae of terpenoids

Class	Number of isoprene unit	General formula
Hemiterpenoids	1	C_5H_8
Monoterpenoids	2	$C_{10}H_{16}$
Sesquiterpenoids	3	$C_{15}H_{24}$
Diterpenoids	4	$C_{20}H_{32}$
Sesterterpenoids (Ophiobolanes)	5	$C_{25}H_{40}$
Triterpenoids	6	$C_{30}H_{48}$
Tetraterpenoids	8	$C_{40}H_{64}$
Polyterpenoids	n	$(C_5H_8)_n$

The generally acceptable biosynthesis pathways of isoprenoid compounds are outline in Figure 4 (Bu'Lock, 1965).

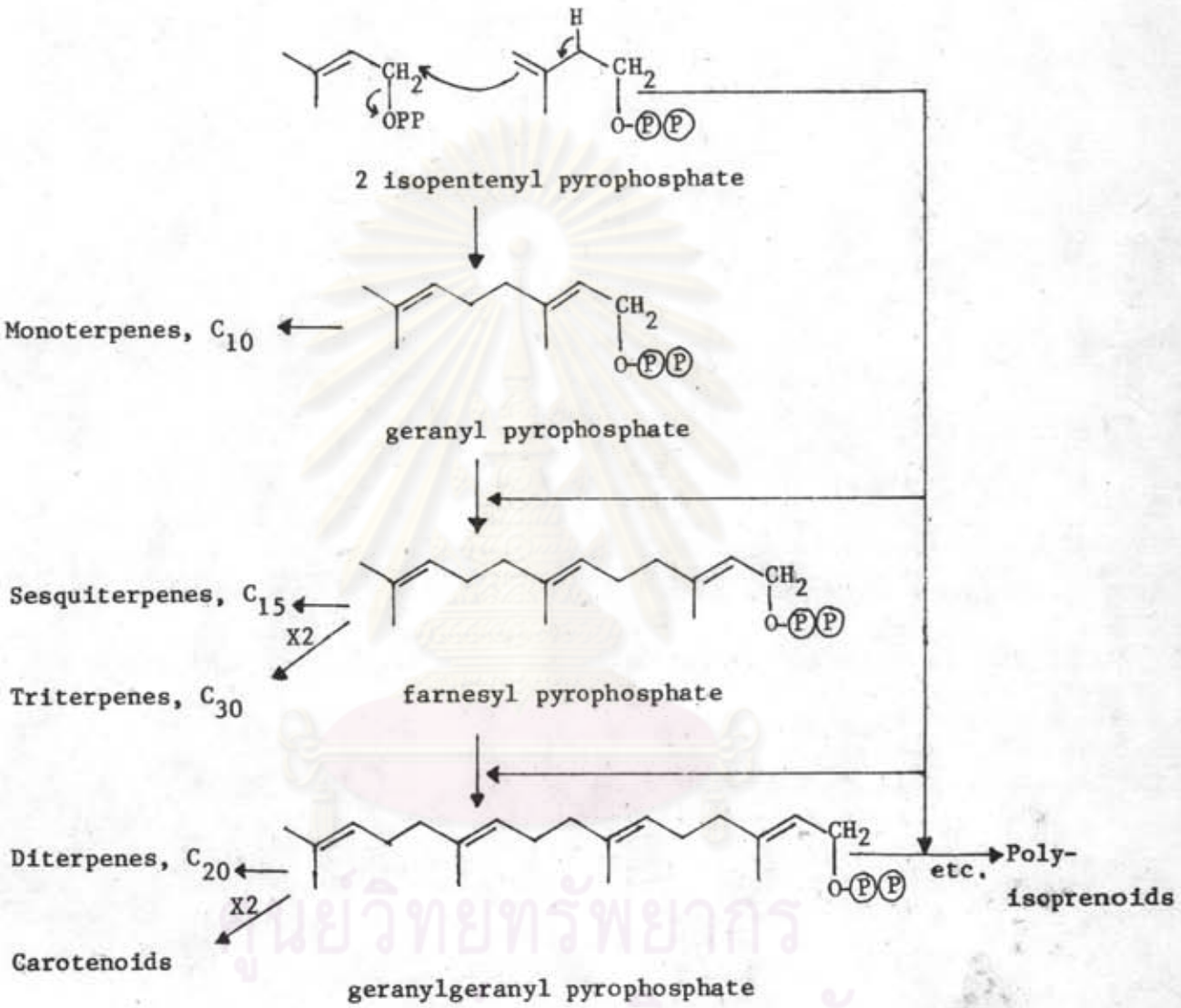


Figure 4. Biosynthesis of isoprenoid compounds.

The diterpenoids are C_{20} compounds which may be regarded as derived from four isoprenoid residues. The diterpenes are found in acyclic, monocyclic, bicyclic, tricyclic, tetracyclic and pentacyclic forms. According to Rowe *et al.*, basic diterpene hydrocarbon skeletons are shown in Figure 5 (Hanson, 1972).

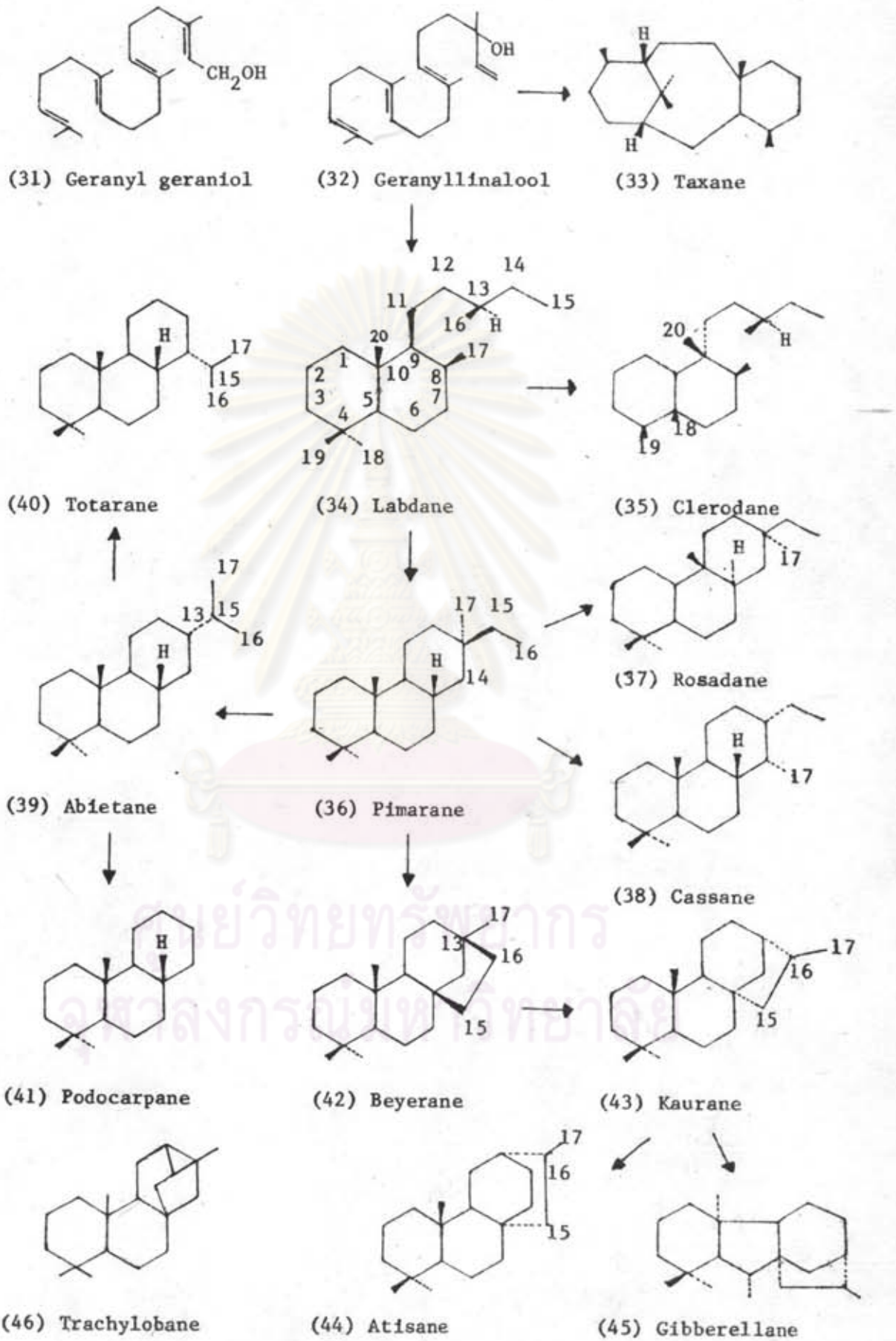


Figure 5. Basic skeletons of diterpenoids.

Like the other terpenoids, they are found in plants or animals as hydrocarbon or in combination with oxygen in the form of hydroxyl, ketone, aldehyde, furan rings, oxides and others (Nicholas, 1973). A uniform numbering system favoring that followed the steroid ring system has been suggested for the diterpenoids (McCrindle and Overton, 1965).

B. Biosynthesis of Diterpenoids

The biosynthesis of diterpenoids involves the following mechanisms:

1. Formation of isopentenyl pyrophosphate.
2. Polymerization of isopentenyl pyrophosphate.
3. Formation of cyclic diterpenoids.

1. Formation of Isopentenyl pyrophosphate

All terpenoid compounds originate from isopentenyl pyrophosphate, which is also known as "activated isoprene". Isopentenyl pyrophosphate is synthesized from acetyl CoA in the same manner by both plants and animals as the following steps (Bu'Lock, 1965; Luckner, 1972):

a) Acetoacetyl CoA (47) is first formed from two molecules of acetyl CoA by "head-to-tail" condensation. This reaction is catalyzed by the enzyme thiolase.

b) A third molecule of acetyl CoA adds to the carbonyl group at position three of acetoacetyl CoA to form 3-hydroxy-3-methylglutaryl CoA (48). The steps (a) and (b) are normally interconvertible. 3-Hydroxy-3-methylglutaryl CoA may be derived from leucine as shown in Figure 7.

c) 3-Hydroxy-3-methylglutaryl CoA is then reduced to an intermediate product mevaldic acid (49). This reaction is practically irreversible and requires NADPH molecule.

d) The enzyme mevaldate reductase transfers the hydrogen stereospecifically from NADPH or NADH to the substrate, 5(R)-[H*]mevalonic acid (50) is thus formed (the tritium atom is marked (*) in Figure 6).

e) Mevalonic acid is then phosphorylated at the primary alcoholic group to form mevalonic acid monophosphate (51) and then in a second reaction step mevalonic acid pyrophosphate (52) is formed.

f) The product of a third phosphorylation at tertiary alcoholic group *via* ATP undergoes concerted elimination of a molecule of water and decarboxylation yields Δ^3 isopentenyl pyrophosphate (53).

These steps are illustrated in Figure 6.

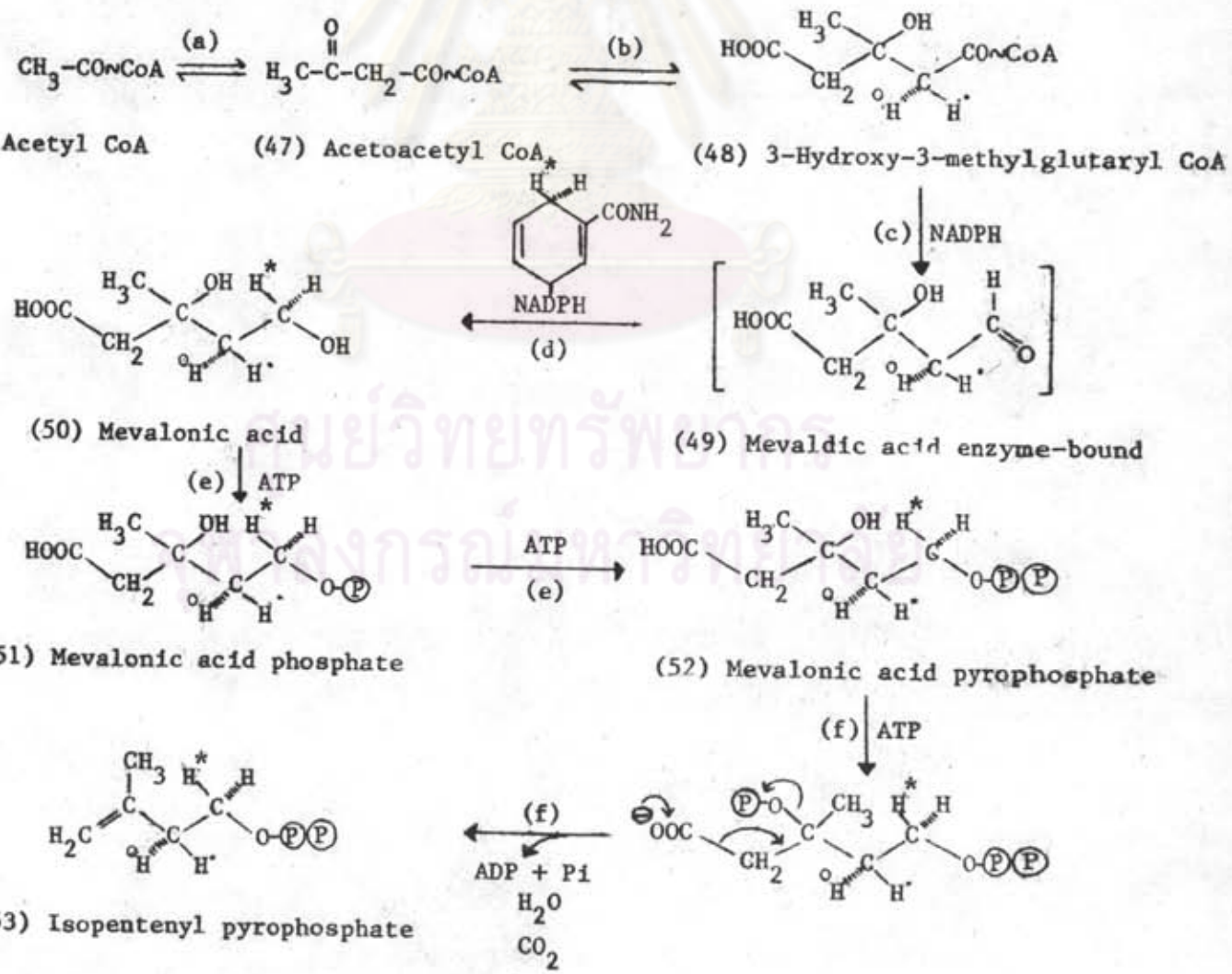


Figure 6. Biosynthesis of isopentenyl pyrophosphate from acetyl CoA.

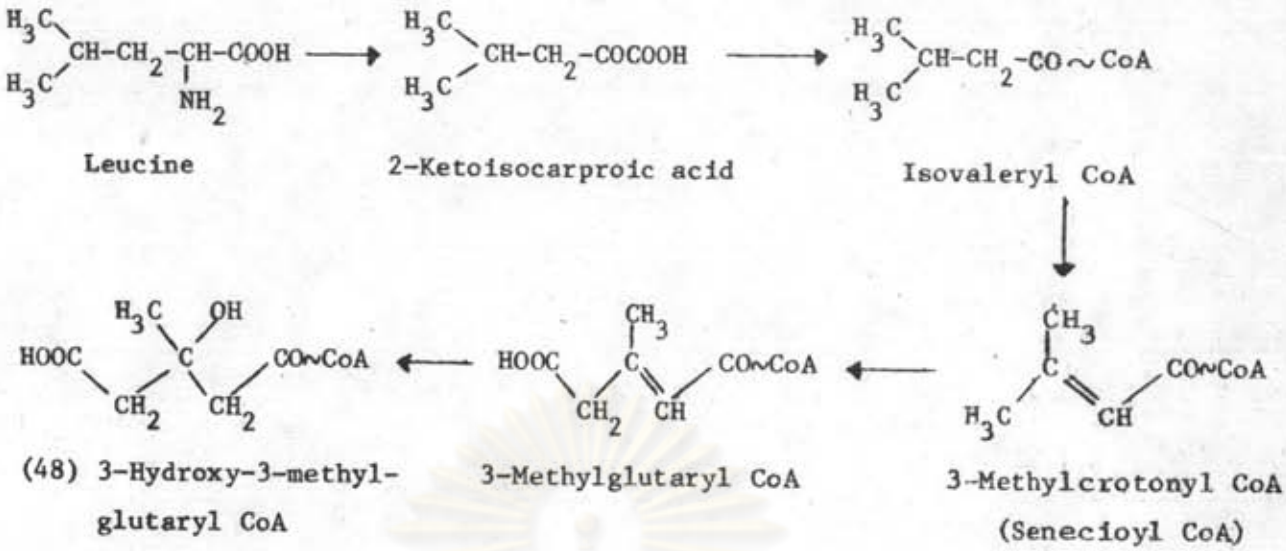


Figure 7. Conversion of leucine to 3-hydroxy-3-methylglutaryl CoA.

2. Polymerization of Isopentenyl pyrophosphate

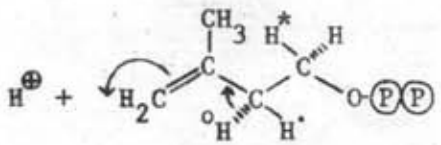
The formation of diterpenoids takes place by the polymerization of several molecules of isopentenyl pyrophosphate. These reactions of polymerization are shown in Figure 8 and described below (Luckner, 1972).

a) By the shift of the double bond of isopentenyl pyrophosphate catalyzed by isopentenyl pyrophosphate isomerase, this yields 3,3-dimethyl-allyl pyrophosphate (54) which serves as a starter molecule for this polymerization both in plants and animals. As an allylic ester, 3,3-dimethylallyl pyrophosphate or the derived cation is an effective electrophilic alkylating agent. The elimination of a hydrogen atom in this reaction at C-2 is strictly stereospecific. The α-hydrogen atom (^{o}H) is always eliminated.

b) One molecule of dimethylallyl pyrophosphate then serves as an acceptor for one molecule of isopentenyl pyrophosphate. The pyrophosphate group is then lost from the starter molecule. The condensation may be considered as a nucleophilic substitution by the CH_2 group of isopentenyl pyrophosphate. The substitution causes an inversion of

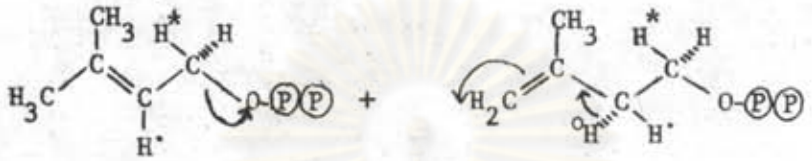
configuration at C-1 of the starter molecule since the CH_2 group of isopentenyl pyrophosphate opposite the pyrophosphate group enters the molecule from the side in a concerted reaction. During the resulting shift of the double bond, occurring simultaneously with the new C-C bonding, the ^{o}H atom at C-2 is lost. The resulting monoterpene is geranyl pyrophosphate, C_{10} (55).

c) Since geranyl pyrophosphate is an allylic ester, the process can be repeated by a similar mechanism, generating farnesyl pyrophosphate, (56), and this is then converted to geranylgeranyl pyrophosphate, C_{20} (57). Configurations around all the double bonds in these compounds are *trans*.



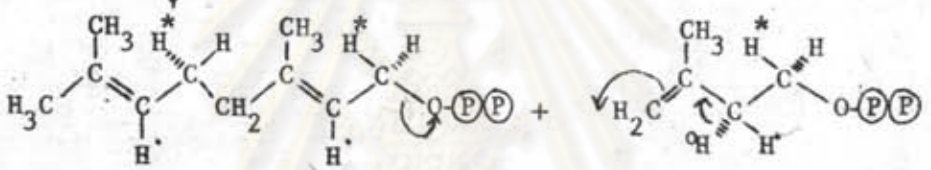
Isopentenyl pyrophosphate

(a)



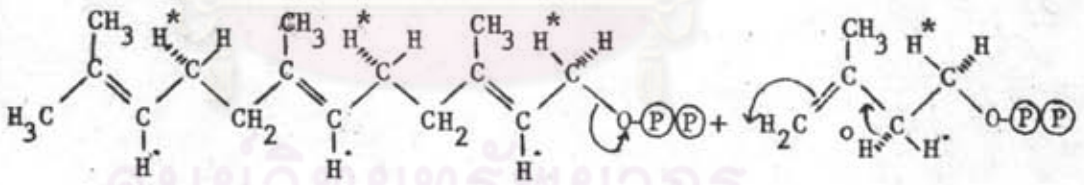
(54) Dimethylallyl pyrophosphate

(b)



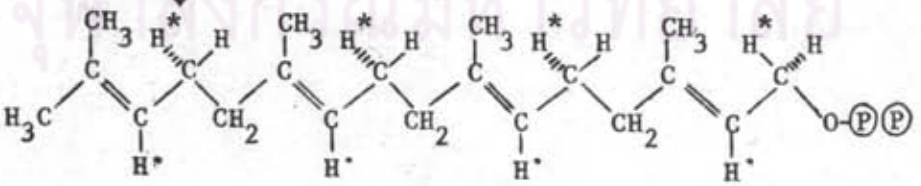
(55) Geranyl pyrophosphate, C_{10}

(c)



(56) Farnesyl pyrophosphate, C_{15}

(c)

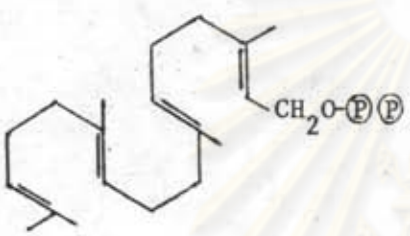


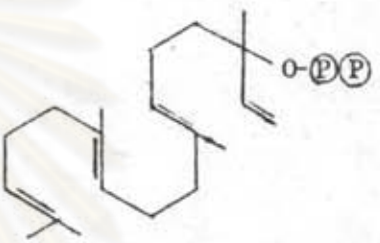
(57) Geranylgeranyl pyrophosphate, C_{20}

Figure 8. Polymerization of isopentenyl pyrophosphate.

3. Formation of Cyclic Diterpenoids

All of the presently known cyclic diterpenoids are considered to be derived, as a result of the Ruzicka biogenetic isoprenoid rule, from geranylgeranyl pyrophosphate (58) or geranyllinaloyl pyrophosphate (59), either by direct cyclization or by secondary rearrangements (Nicholas, 1973).





(58) Geranylgeranyl pyrophosphate (59) Geranyllinaloyl pyrophosphate

Geranylgeranyl pyrophosphate is converted to bicyclic and tricyclic derivatives. Cyclization is catalyzed by enzymes and initiated by protonation. The cyclic precursor shown is the bicyclic labdane type names (+)-labdadienyl pyrophosphate (60). From its mode of formation with the first three isoprene units folded in "chair-like" conformation, this necessarily has the typical *trans-anti-trans* stereochemistry, but not for all diterpenoids (Richards and Hendrickson, 1964; Bu'Lock, 1965; Luckner, 1972). Their summary is shown in Figure 9.

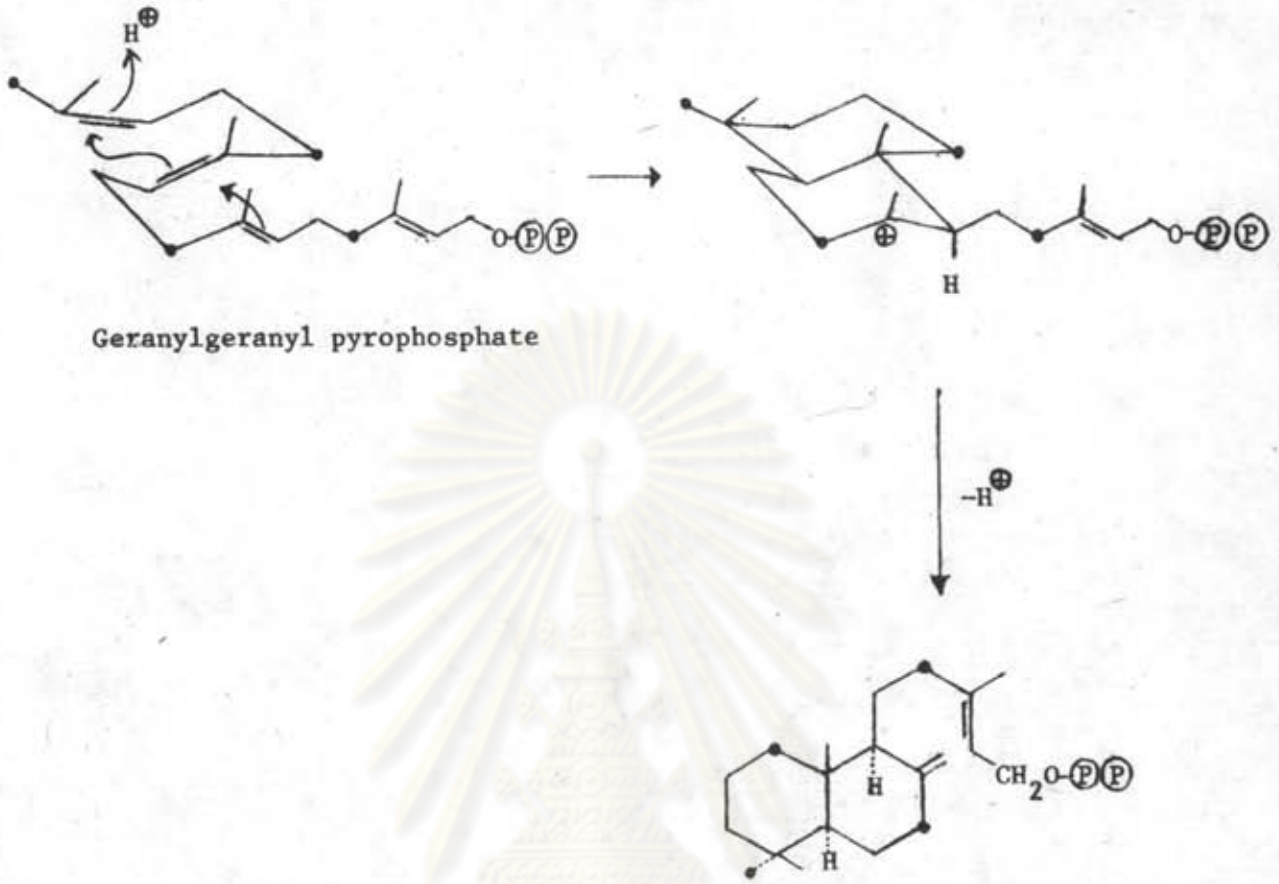


Figure 9. Biosynthesis of a bicyclic precursor of diterpenoids.

The precursor from figure 9 (60) is converted to the allylic cation (61) by the ionization of the terminal nucleophilic (e.g., $-O\text{Ⓟ}\text{Ⓟ}$). That will lead from labdane skeleton, with a 1,2-shift of the methyl group, to the parent skeleton of pimarane, cassane, abietane, kaurane, beyerane, trachylobane and atisane types. Compounds of these types are outlined in Figure 10 (Nicholas, 1973; Richards and Hendrickson, 1964). From Figure 10, the cassane group which related to *Erythrophleum* alkaloids has a modified pimarane skeleton in which the C-17 methyl group has migrated from C-13 to C-14.

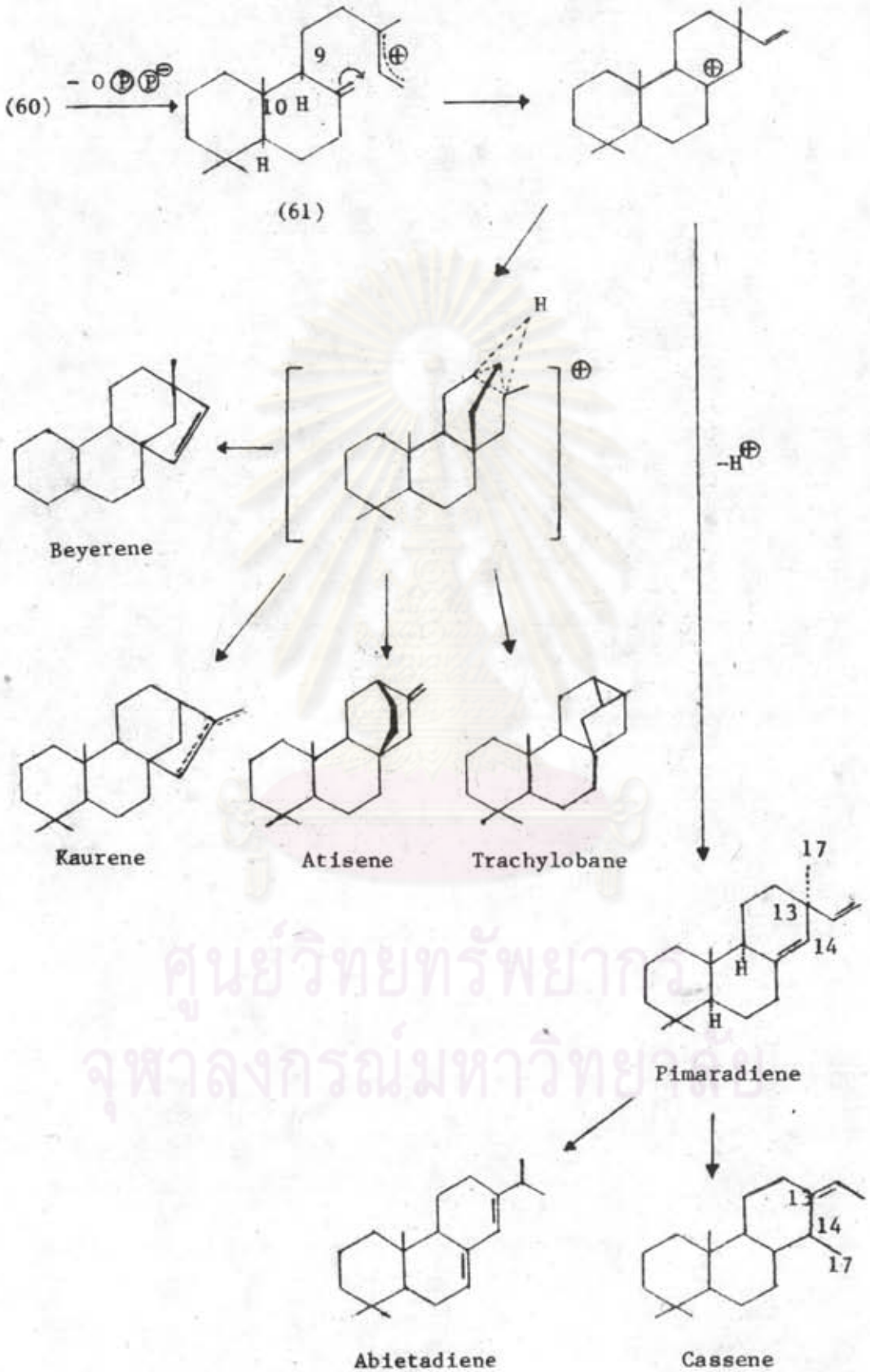


Figure 10, Biosynthesis of cyclic diterpenoids.