



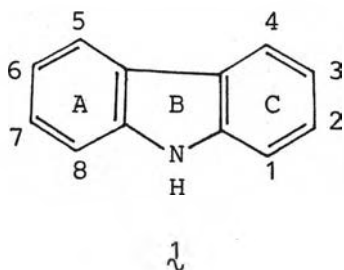
CHAPTER I

INTRODUCTION

Carbazole Alkaloids

Carbazole alkaloids were isolated as early as 1872 from coal tar by Graebe and Glazer. Its occurrence in plants was not reported until 1964-65 when the structure of murrayanine, the first member of carbazole alkaloids, was reported. The distribution of carbazole alkaloids in plant is at present limited to the family Rutaceae. The plants in this family which have been investigated and reported to contain such alkaloids are *Murraya koenigii* Spreng., *Murraya exotica* L. [*M. paniculata* (L.) Jack], *Glycosmis pentaphylla* (Retz.) DC., *Clausena heptaphylla* Wt. and Arn., *Clausena lansium* (Lour) Skeels (1), *Clausena indica* Oliv (2), and *Clausena anisata* Willd (3). There are also carbazole derivatives hyellazole and chlorohyellazole in blue-green algae (4).

Classification of Carbazole Alkaloids



Many structurally different carbazole alkaloids was reported; all are based on the parent carbazole nucleus (1), but they differ in the number of carbon atoms attached to nucleus. The group of

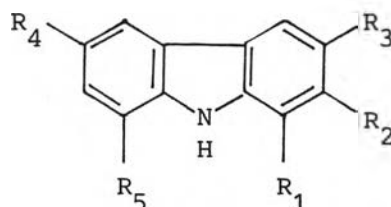
carbazole alkaloids is sometimes analyzed in terms of the number of isoprene units linked to an indole nucleus, so three basic groups of carbazole alkaloids have been recognized (1). The first group, represented by murrayanine, has an indole unit plus one isoprene unit, the second group [e.g. heptaphylline] has an indole unit plus two isoprene units, and the third group [e.g. mahanimbine] has an indole unit plus three isoprene units.

According to the number of carbons in skeleton, Chakraborty classified these alkaloids into three main groups (5) :

1. Members with C-13 carbon skeleton

This group of carbazole alkaloids contains 12 carbon atoms of carbazole nucleus and one carbon atom attached to position 3, which appears to be methyl, aldehyde, acid, or ester. There can be other substitution on other positions of carbazole nucleus, for example, on position 1, 2, 6, or 8. A number of alkaloids in this group are shown in table 1.

Table 1 Structures of Presently Known C-13 Carbazoles



	R ₁	R ₂	R ₃	R ₄	R ₅
3-Methyl carbazole	H	H	CH ₃	H	H
Murrayanine	OCH ₃	H	CHO	H	H
Mukoeic acid	OCH ₃	H	COOH	H	H
Glycozoline	H	H	CH ₃	OCH ₃	H
Glycozolidine	H	OCH ₃	CH ₃	OCH ₃	H
Mukonine	OCH ₃	H	COOCH ₃	H	H
Mukonidine	H	OH	COOCH ₃	H	H
Mukoline	H	H	CH ₂ OH	H	OCH ₃
Mukolidine	H	H	CHO	H	OCH ₃

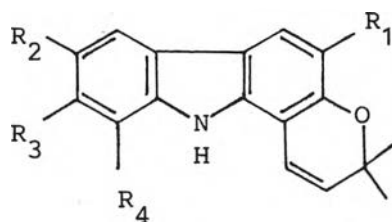
2. Members with C-18 carbon skeleton

This group of alkaloid contains 18 carbon atoms in the molecule. Due to the structures of molecules, this group can be divided into two sub-groups :

2.1 2,2-Dimethyl- Δ^3 -pyrano carbazole

The alkaloids in this sub-group are listed in Table 2.

Table 2 Structures of Presently Known 2,2-Dimethyl Δ^3 -Pyrano Carbazoles



	R ₁	R ₂	R ₃	R ₄
Girinimbine	CH ₃	H	H	H
Myrrayacine	CHO	H	H	H
Koenimbine	CH ₃	OCH ₃	H	H
Koenine	CH ₃	OH	H	H
Koenigicine	CH ₃	OCH ₃	OCH ₃	H
Heptazoline	OCH ₃	CH ₃	H	H
Koenigine	CH ₃	OCH ₃	OH	H

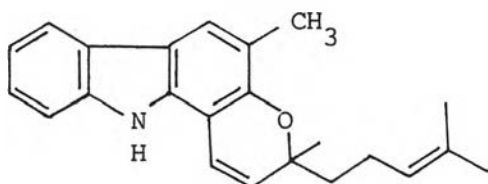
2.2 Dimethylallyl carbazoles

The alkaloids in this sub-group are shown in

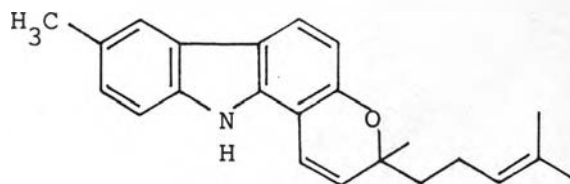
Table 3.

3. Members with C-23 carbon skeleton

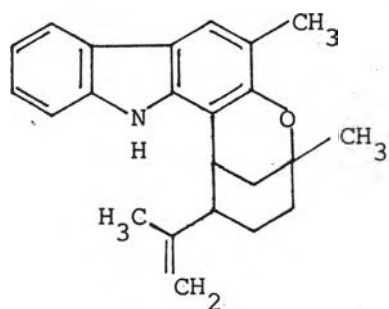
The alkaloids in this group are shown in Fig. 1



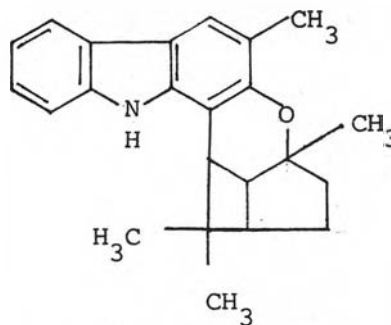
2 mahanimbine



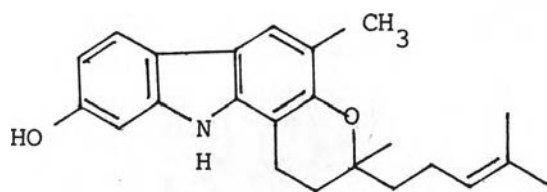
3 isomahanimbine



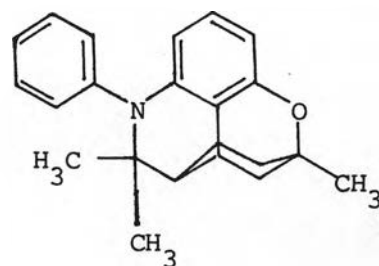
4 cyclomahanimbine



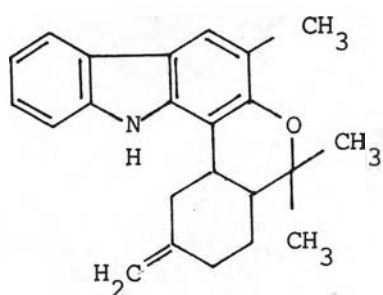
5 bicyclomahanimbine



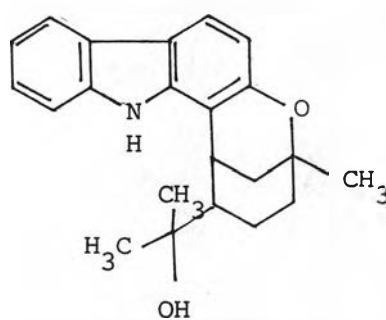
6 mahanine



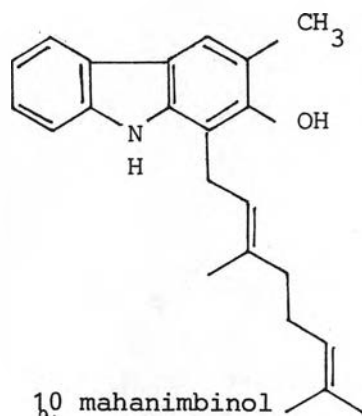
7 mahanimbidine



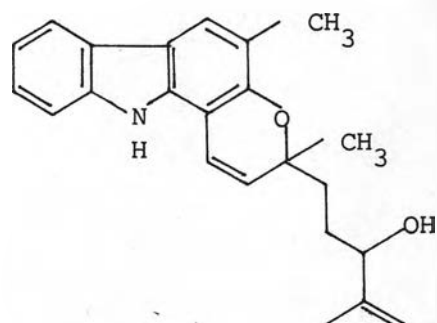
8 murrayazolidine



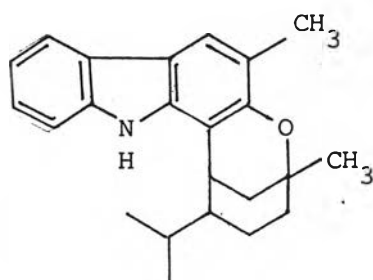
9 murrayazolinine



10 mahanimbinol



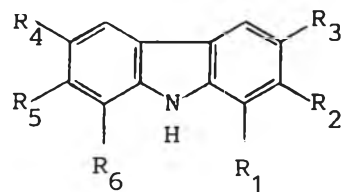
11 mahanimboline



12 exozoline

Fig. 1 Structures of Presently Known C-23 Carbazole

Table 3 Structures of Dimethylallyl Carbazoles



	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
Heptaphylline	$\text{CH}_2\text{CH}=\text{C}\begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix}$	OH	CHO	H	H	H
Heptazoline	$\text{CH}_2\text{CH}=\text{C}\begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix}$	OH	CHO	H	H	OH
6-Methoxyheptaphylline	$\text{CH}_2\text{CH}=\text{C}\begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix}$	OH	CHO	OCH ₃	H	H
Indizoline	OCH ₃	$\text{CH}_2\text{CH}=\text{C}\begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix}$	CHO	H	H	H
Clausanitin	H	OH	CHO	H	$\text{CH}_2\text{CH}=\text{C}\begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix}$	H
Antanisatin	H	OCH ₃	CHO	H	H	$\text{CH}_2\text{CH}=\text{C}\begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix}$

Biosynthesis of Carbazole Alkaloids

Information on the biosynthesis of the carbazole alkaloids is extremely limited. Erdtman, Polpi and Kapil, and Narashimhan (5) conceived that the ring C carrying the extra methyl group may be a contribution from mevalonic acid. Polpi and Kapil (5) carried out feeding experiments to provide evidence in favour of the mevalonate origin of the ring carrying the extramethyl group. Feeding of 2-¹⁴C and 2-³H mevalonic acid to *Murruya koenigii* resulted in the isolation of highly radioactive koenimbine, koenigicine and mahanimbine though the experiment establishing the location of the radioactivity is lacking.

Kapil (1) proposed that 3-methylcarbazole is the key biosynthetic intermediate in the formation of the carbazole alkaloids and that this compound may then be the object of hydroxylation and prenylation reactions (Fig. 2). None of the simple prenylated biogenetic precursors are known, and there is no information as to the mechanism of formations of 3-methyl carbazole. This compound has been obtained recently from *Clausena heptaphylla* Wight and Arn.

Chakraborty carried out the biomimetic hydroxylation studies with 3-methyl carbazole as the substrate (5). Nuclear oxidation of 3-methyl carbazole was carried out with Fenton's reagent as well as with the Udenfriend system (ascorbic acid, ferrous sulfate, H₂O₂, EDTA and molecular oxygen), producing (a) a colourless compound, a dimeric carbazole, (b) 2-hydroxy-3-methyl carbazole, (c) a compound which had colour reactions characteristic for aldehydes and had the uv spectrum characteristic for 3-formyl carbazole system, and (d)

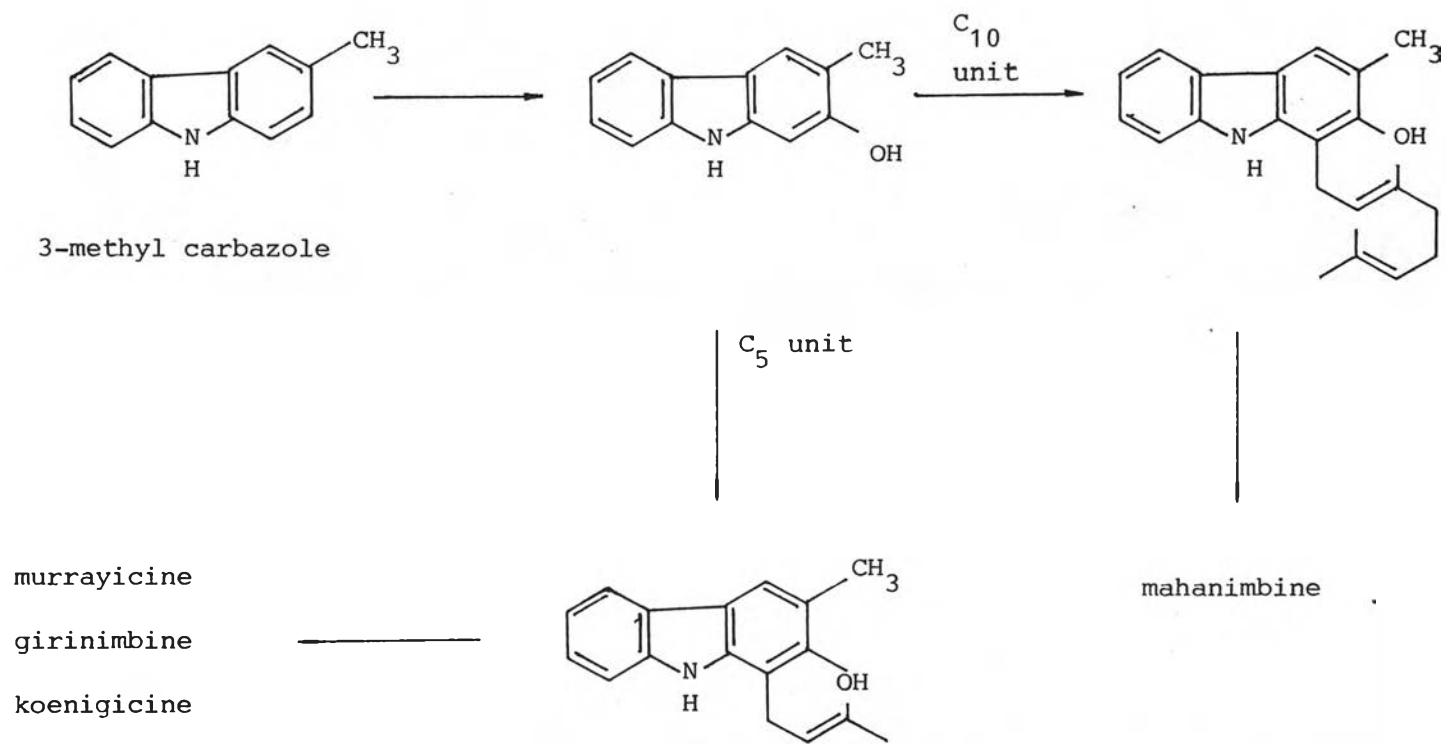
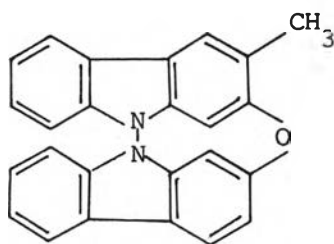


Fig. 2 Biogenesis of Carbazole Alkaloids



1-hydroxy-3-methyl carbazole. The ir spectrum of the dimeric carbazole showed the absence of -NH- or hydroxyl function, but the uv spectrum showed it to be a 2-oxygenated carbazole derivative. From all these data the dimeric compound could be represented by the following structure (13).



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The result of biomimetic oxidation also shows that hydroxylation of 3-methyl carbazole gives 2-hydroxy-3-methyl carbazole as the major hydroxylated product. It appears from the formation of an aldehydic substance that during hydroxylation the aromatic methyl group may be oxidised to formyl group. The lower yield of 1-hydroxy carbazole could probably be due to the fact that it occupies a position meta to the methyl group. These experiments provide a rational basis for the production of larger amount of 2-oxygenated carbazole and support the idea that 3-methyl carbazole is the progenitor of other carbazole alkaloids.

Synthesis of Carbazole Alkaloids

Studies of carbazole alkaloids in plant have been reported in terms of isolation, structure elucidation, and synthesis. Some plants, especially in family Rutaceae, have been investigated; and

the isolated alkaloids were elucidated and synthesized. For the purpose of our studies we can divide these alkaloids into three groups, according to their structures.

1. C-13 carbon skeleton alkaloids

Murrayanine (14) was obtained from the stem bark of *Murraya koenigii*. The uv spectrum of the compound was significantly characteristic for 3-formyl carbazole. On potassium borohydride reduction it afforded an alcohol having uv spectrum characteristic for 1-methoxy carbazole. On zinc dust distillation murrayanine furnished carbazole (1) while its Wolff-Kishner reduction product furnished 3-methyl carbazole confirming the position of the formyl group at 3-position. On decarbonylation this alkaloid furnished 1-methoxy carbazole (Fig. 3) (5).

From these chemical reactions and nmr spectrum which showed two deshielded aromatic protons at 8.09 and four at 7.39 ppm (1), the formulation of murrayanine as 1-methoxy-3-formyl carbazole was advanced which was substantiated by synthesis (Fig. 4) (6). 2-Hydroxymethylene-5-methyl cyclohexanone (15) on condensation with phenyl diazonium chloride (16) under Japp-Klingeman condition gave 4-methyl cyclohexane-1,2-dione-1-phenyl hydrazone (17), which on cyclization with a mixture of acetic acid and hydrochloric acid furnished 1-oxo-3-methyl-1,2,3,4 tetrahydro carbazole (18). On dehydrogenation (18) furnished 1-hydroxy-3-methyl carbazole (19). The phenol (19) on methylation with diazomethane in the presence of methanol furnished 1-methoxy-3-methyl carbazole (20) which brominated with N-bromosuccinimide in the presence of traces of

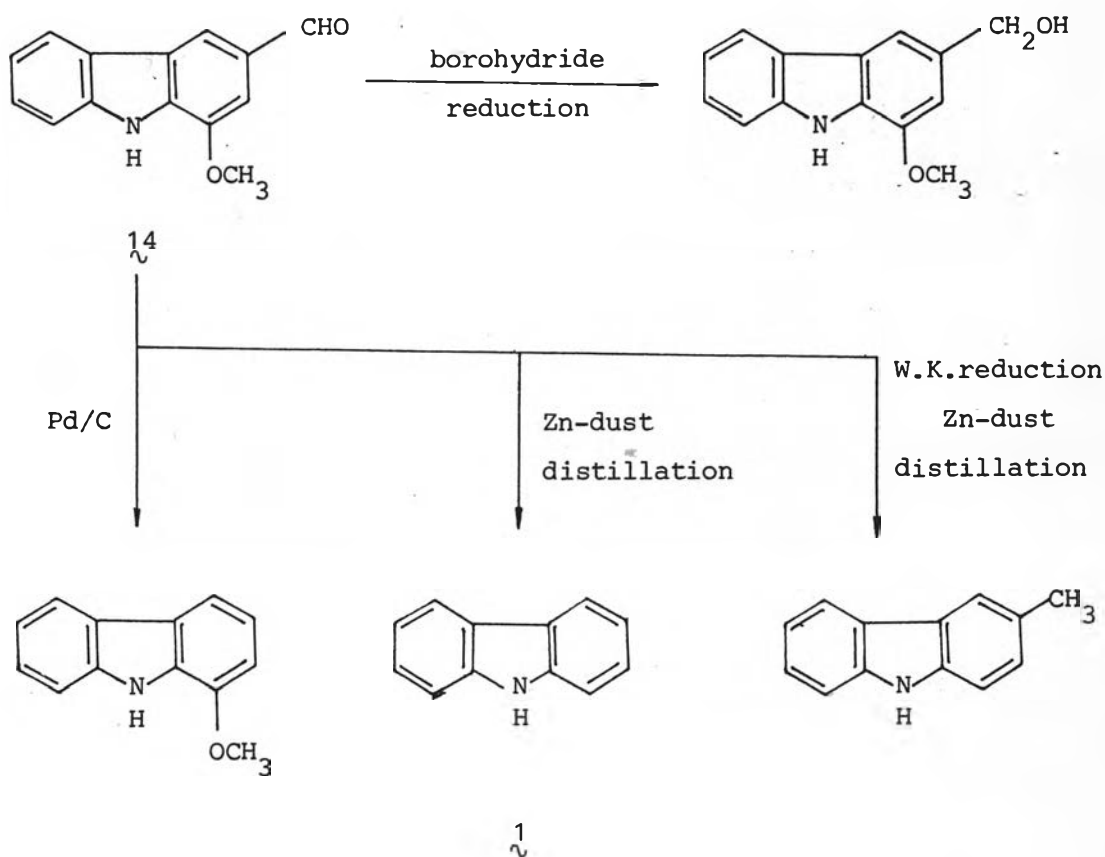


Fig. 3 Chemical Reactions for Structural Elucidation of Murrayanine (14).

benzoyl peroxide and the resulting 1-methoxy-3-bromomethyl carbazole was hydrolyzed *in situ* with caustic potash to 1-methoxy-3-hydroxymethyl carbazole (21). Oxidation of 21 with active MnO₂ furnished murrayanine (14).

Glycozolidine, a member of the C-13 skeleton group, was isolated from root bark of *Glycosmis pentaphylla*. Its structure has been deduced from physical data, degradative reactions, and synthesis. In synthesis of glycozolidine, Chakarboroty (5) developed method for the synthesis of carbazoles from diphenyl amine (Fig. 5). It has been found that the diphenyl amines 22-25 in the presence of elemental iodine

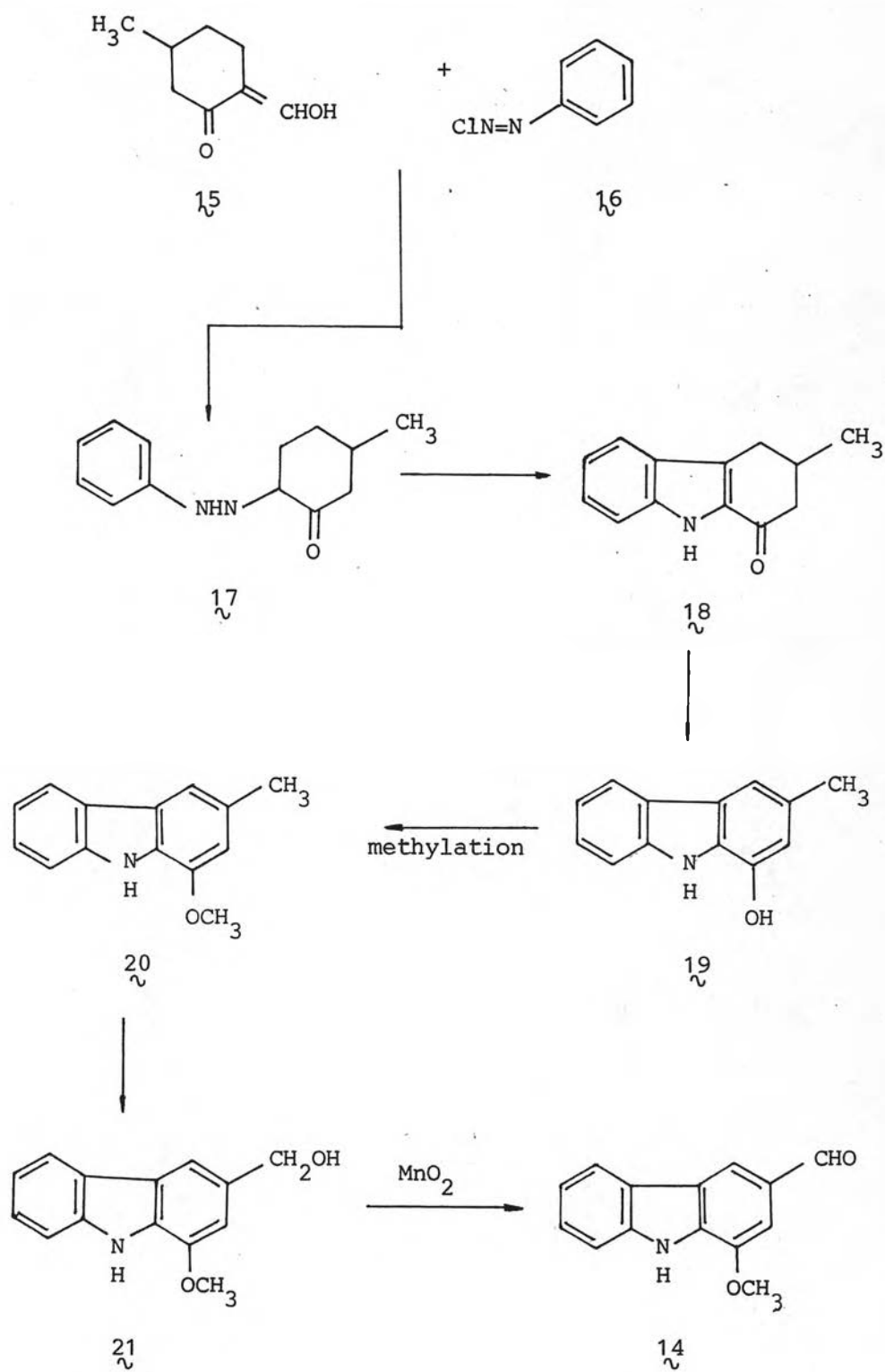
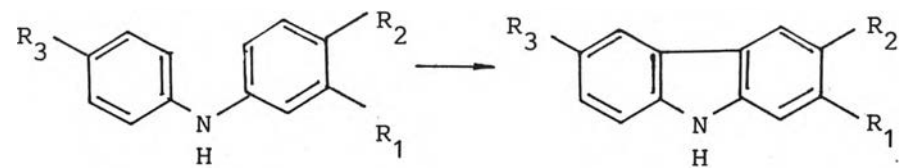


Fig. 4 Synthesis of Murrayanine.



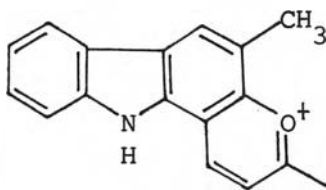
	R ₁	R ₂	R ₃		R ₁	R ₂	R ₃
22	H	H	H	carbazole	H	H	H
23	H	CH ₃	H	3-methyl carbazole	H	CH ₃	H
24	H	CH ₃	OCH ₃	glycozoline	H	CH ₃	OCH ₃
25	OCH ₃	CH ₃	OCH ₃	glycozolidine	OCH ₃	CH ₃	OCH ₃

Fig. 5 Widely Applicable Synthesis of Carbazoles from Diphenylamines

and oxygen cyclise to carbazoles at 350° in a sealed tube,

2. C-18 carbon skeleton alkaloids

Girinimbine(26), the first member of this group, was isolated from the stem bark of *Murraya koenigii*. The uv and ir data showed the compound to belong to the carbazole group. A six proton singlet at 1.42 ppm in nmr spectrum of girinimbine together with the symmetrical doublet at 5.45 ppm and 6.25 ppm suggested the presence of a 2 : 2 dimethyl- Δ^3 -pyran ring in the compound. The mass spectrum of girinimbine showed a high intensity peak at m/e 248 which could be represented by the carbazolopyrillium ion (27).



27

The steps in the synthesis of girinimbine are shown in Fig. 6.

The tetrahydrocarbazole (28), obtained by the condensation of 3-methoxy-4-methylaniline and 2-hydroxycyclohexanone, was dehydrogenated over 5% Pd/C to give 29, which was demethylated with HBr/AcOH to give 30. The phenolic compound (30), on reaction with N-methyl-formanilide and POCl₃, furnished the 1-formyl derivative (31). The dry sodium salt of 31 was heated with methallyl-triphenylphosphonium chloride in DMF solution to furnish girinimbine (26) (7).

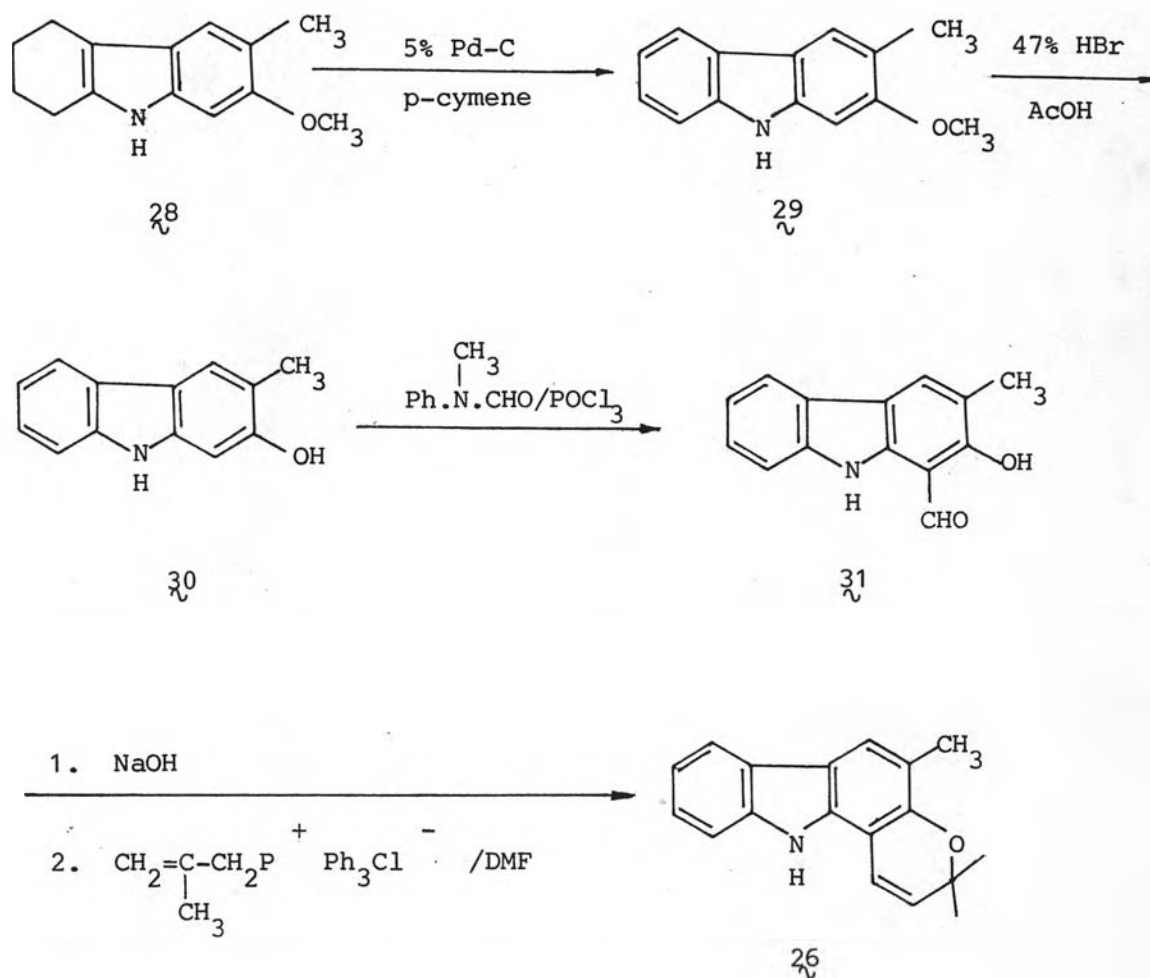


Fig. 6 Synthesis of Girinimbine.

Heptaphylline (32), one member of C-18 carbon skeleton alkaloids, was obtained from the roots of *Clausena heptaphylla*, and again the uv spectrum was similar to that of carbazole-3-aldehyde and this was confirmed by the ir spectrum (8). The nmr spectrum showed the presence of two olefinic methyl groups at 1.66 and 1.83 ppm, an olefinic proton (t, J = 6 Hz) at 5.35 ppm, and a methylene doublet at 3.60 ppm, suggesting a 3,3'-dimethylallyl unit attached to the carbazole nucleus. A phenolic group was placed between the formyl group and the dimethylallyl unit since reaction with polyphosphoric acid gave the isomeric chroman (33). The structure was

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confirmed by synthesis (Fig. 7) (9).

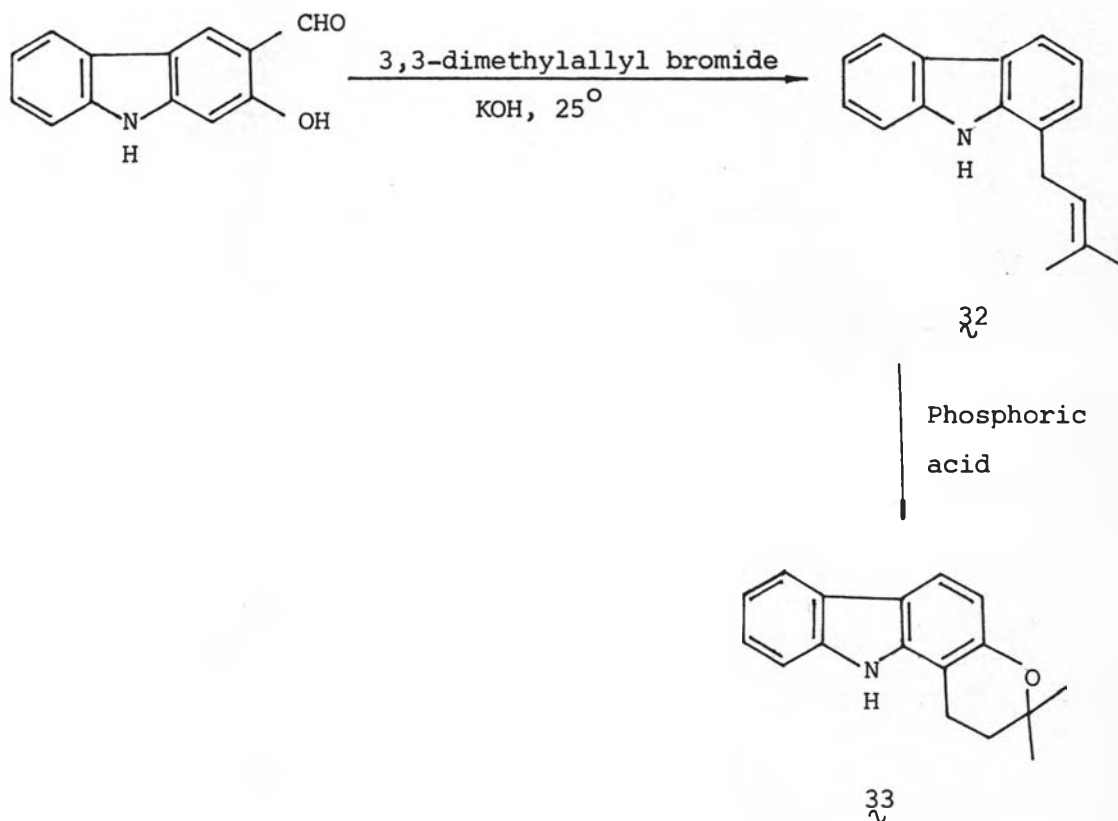


Fig. 7 The Synthesis and Chemical Reaction of Heptaphylline

3. C-23 carbon skeleton alkaloids

Mahanimbine (ζ), the first member of C-23 carbazole alkaloids, was isolated from the stem bark of *Murraya koenigii*. Its uv spectrum was similar to that of girinimbine suggesting the presence of a pyrano carbazole skeleton like girinimbine. This was confirmed by the mass spectral data of mahanimbine when the high intensity peak at m/e 248 characteristic for the carbazole-pyrylium ion (27) was observed. The complete structure of mahanimbine was proposed by Narasimhan (10) from the nmr, uv data and chemical reactions. The structure was confirmed by synthesis (Fig. 8). Mahanimbine

dissolved in benzene and shaken with silica gel (48 hrs.) or Dowex 50W-X8 ion exchange resin (H^+) yielded bicyclomahanimbine (34)

(Fig. 9)(11). The side-chain of mahanimbine undergoes cyclisation even under mild acidic conditions; it is possible that bicyclomahanimbine is in fact an artifact (11).

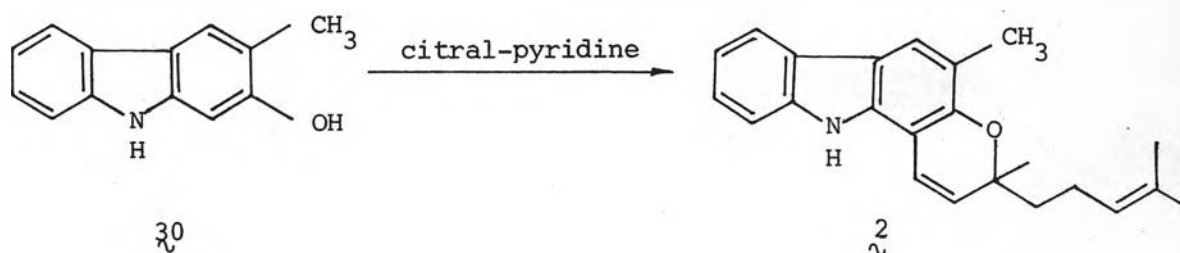


Fig. 8 Synthesis of Mahanimbine

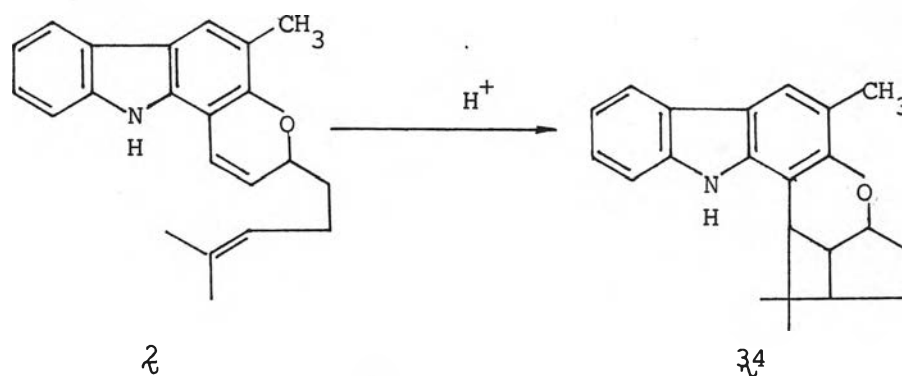


Fig. 9 Conversion of Mahanimbine (2) into Bicyclomahanimbine (34)

Pharmacological Activities of Carbazole Alkaloids

The antibiotic properties of the carbazole alkaloids and some related products have been tested by the agar cup assay method using Sabourad's medium against *Microsporium gypseum*, *Trichophyton*

rubrum, *Epidermophyton floccusum*, *Candida albicans*, *Candida tropicalis*, *Staphylococcus aureus*, and *Escherichia coli*. Glycozoline, glycozolidine, murrayanine, girinimbine, mahanimbine, and heptazoline were active at a concentration of 10 µg/ml. The most significant action was observed with 6-hydroxy-3-methyl carbazole which could inhibit the growth of *Trichophyton rubrum* at 10 µg/ml. Girinimbine was active against *Nocardia asteroides* at a concentration of 30 µg/ml (5). Mahanimbine, koenimbine and N-methylmahanimbine were tested for their anticancer activity; reports indicated that all of them are inactive. Mahanimbine was also tested for its antimalarial activity, and the result was negative (12).

Occurrence of Carbazole Alkaloids in Plants

It is evident that carbazole alkaloids have been found only in the plants of family Rutaceae. Table 4 shows the occurrence of the different types of carbazole alkaloids in different genera(5).

Table 4 Distribution of Carbazole Alkaloids

Genus	Alkaloids
<i>Glycosmis</i>	C ₁₃
<i>Clausena</i>	C ₁₃ and C ₁₈
<i>Murraya</i>	C ₁₃ , C ₁₈ and C ₂₃

Presently known carbazole alkaloids which were isolated from Rutaceous plants are shown in table 5.

Table 5 Known Occurrence of Carbazole Alkaloids in plants

Alkaloids	Sources	References
Antanisatin	<i>Clausena anisata</i> (Willd)	3
Clausanitin	<i>Clausena anisata</i> (Willd)	3
Cyclomahanimbine	<i>Murraya koenigii</i> Spreng.	11
Exozoline	<i>Murraya exotica</i> Linn.	13
Girinimbine	<i>Murraya koenigii</i> Spreng.	14
Glycozoline	<i>Glycosmis pentaphylla</i> (Retz.) D.C.	14
Glycozolidine	<i>Glycosmis pentaphylla</i> (Retz.) D.C.	14
Heptaphylline	<i>Clausena heptaphylla</i> Wt. and Arn.	8
	<i>Clausena harmandiana</i> Pierre.	15
	<i>Clausena lansium</i> (Lour.) Skeels	16
Heptazoline	<i>Clausena heptaphylla</i> Wt. and Arn.	17
Indizoline	<i>Clausena indica</i> Oliv.	2
Isomahanimbine	<i>Murraya koenigii</i> Spreng.	12
Koenidine	<i>Murraya koenigii</i> Spreng.	12
Koenigine	<i>Murraya koenigii</i> Spreng.	12
	<i>Clausena heptaphylla</i> Wt. and Arn.	17
	<i>Micromelum zeylanicum</i>	18
Koenimbine	<i>Murraya koenigii</i> Spreng.	12
Koenine	<i>Murraya koenigii</i> Spreng.	12
	<i>Clausena heptaphylla</i> Wt. and Arn.	17
Lansine	<i>Clausena lansium</i> (Lour.) Skeels	16

Table 5 (continue)

Alkaloids	Sources	References
Mahanimbidine	<i>Murraya koenigii</i> Spreng.	11
(Murrayazoline)	<i>Murraya exotica</i> Linn.	19
Mahanimbine	<i>Murraya koenigii</i> Spreng.	12
	<i>Murraya exotica</i> Linn.	19
Mahanimbinol	<i>Murraya koenigii</i> Spreng.	20
Mahanimboline	<i>Murraya koenigii</i> Spreng.	20
Mahanine	<i>Murraya koenigii</i> Spreng.	12
	<i>Clausena heptaphylla</i> Wt. and Arn.	17
6-Methoxyheptaphylline	<i>Clausena indica</i> Oliv.	21
3-Methylcarbazole	<i>Clausena heptaphylla</i> Wt. and Arn.	22
	<i>Clausena indica</i> Oliv.	23
Mukonidine	<i>Murraya koenigii</i> Spreng.	4
Murrayacine	<i>Clausena heptaphylla</i> Wt. and Arn.	24
	<i>Murraya koenigii</i> Spreng.	25
Murrayanine	<i>Clausena heptaphylla</i> Wt. and Arn.	26
Murrayazolinine	<i>Murraya koenigii</i> Spreng.	27

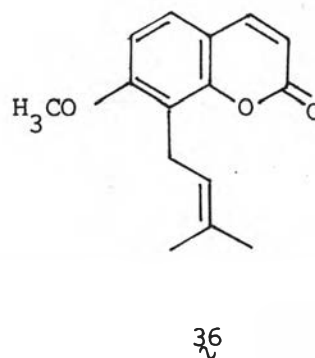
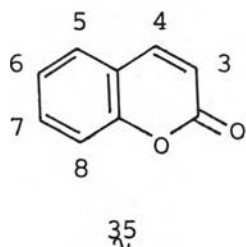
Coumarins

Coumarins were originally isolated from plants. They were found widely spread in various plant families, such as Leguminosae, Umbelliferae, Labiatae, and Rutaceae. They have also been isolated from microorganisms and animals (28).

Isolated coumarins can be classified into 5 groups due to their structures (29) :

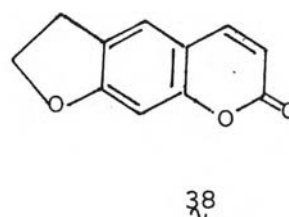
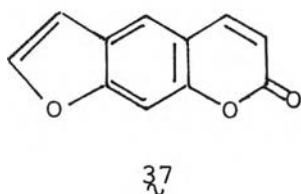
1. Simple coumarins

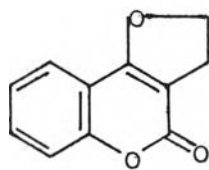
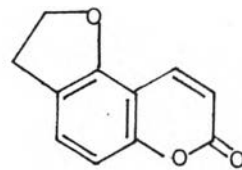
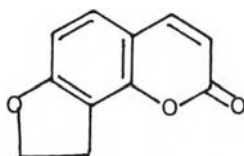
This group of coumarins is possessed a 2-H-1-benzopyran-2-one, a coumarin nucleus (35). The substitution may occur on the benzene ring at position 5, 6, 7 or 8. Osthol (36) is an example of coumarins in this group (30).



2. Furanocoumarins

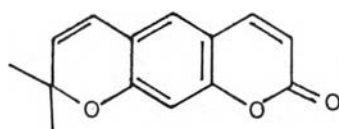
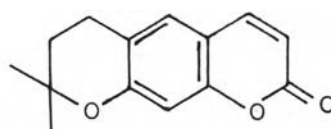
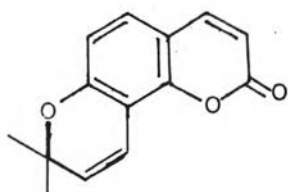
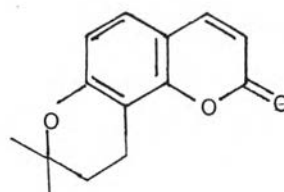
This group of coumarins consists of the furan ring fused to the coumarin nucleus at the various positions to form linear furanocoumarins or angular furanocoumarins. The examples of coumarins in this group are psoralene type (37) and dihydrofuranocoumarin (38), linear furanocoumarins, and dihydrofuranocoumarin (39, 40, 41), angular coumarins.



39
~40
~41
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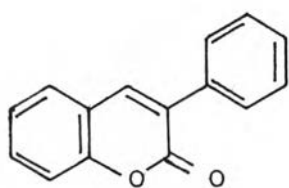
3. Pyranocoumarins

Coumarins in this group have a pyran ring fused to the coumarin nucleus at various position to form linear pyranocoumarins : xanthyletin type (linear) (42), dihydroxanthyletin type (43), or angular pyranocoumarins : seselin type (44), dihydroseselin type (45).

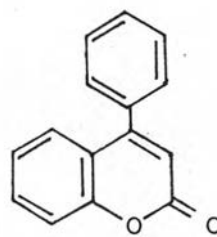
42
~43
~44
~45
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4. Phenylcoumarins

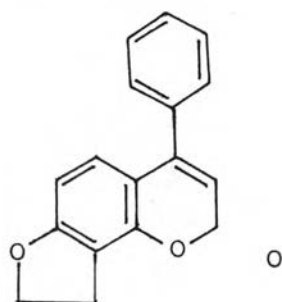
There is phenyl substituted at position 3 or 4 in the coumarin nucleus. They can be divided into 6 types : 3-phenylcoumarin (46), 4-phenylcoumarin (47), 4-phenyldihydroangelicin (48), 3-phenylxanthyletin (49), 3-phenylseselin (50), and 4-phenylseselin (51).



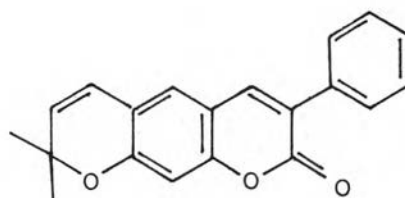
46



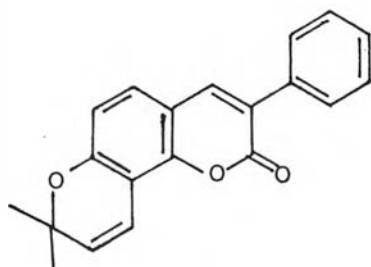
47



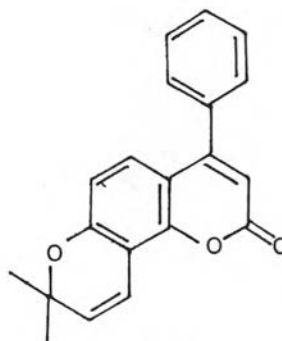
48



49



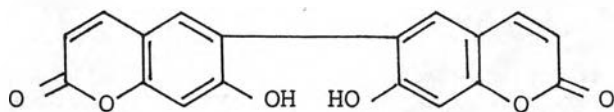
50



51

5. Bicoumarins

Coumarins in this group consist of two coumarin nuclei in their structures. Bicoumol (52) is an example of coumarins in this group.



52
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More information about structure determination, biosynthesis, synthesis, and physiological activities of coumarins can be obtained from reference 31.