



**UNIVERSITI PUTRA MALAYSIA**

**CHEMICAL CONSTITUENTS OF *GLYCOSMIS CALCICOLA* AND  
*G. RUPESTRIS* (*RUTACEAE*) AND THEIR BIOLOGICAL  
ACTIVITIES**

**CHEW YEAN L I N G**

**FSAS 1995 7**

**CHEMICAL CONSTITUENTS OF *GLYCOSMIS CALCICOLA* AND  
*G. RUPESTRIS* (RUTACEAE) AND THEIR BIOLOGICAL  
ACTIVITIES**

By

**CHEW YEAN LING**

**Thesis Submitted in Fulfilment of the  
Requirements for the Degree of Master of Science  
in the Faculty of Science and Environmental Studies,  
Universiti Pertanian Malaysia**

**June 1995**



## ACKNOWLEDGEMENTS

I would like to take this opportunity to express my sincere appreciation and gratitude to Assoc. Prof. Dr. Mawardi Rahmani and Assoc. Prof. Dr. Sariah Meon for the invaluable advice and guidance throughout the course of this project, and Assoc. Prof. Dr. Mohd. Aspollah Hj. Sukari for his constant support.

I would also like to acknowledge the cooperation and assistance of the staff of the Chemistry Department of UPM: En. Nazri Ahmad, En. Zainal Abidin Kasim, En. Mohamad Abu Samah, En. Zainal Zahari Zakaria, En. Nordin Ismail and En. Zainuddin Samadi.

Last but not least, I wish to thank Prof. Norio Aimi of Chiba University for the running of  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectra, Mr. S. Anthony Samy of the Biology Department of UPM and En. Ahmad Zainuddin Ibrahim of UKM for identifying and collecting the plant materials, and En. Khir Rani and the staff of the Plant Protection Department of UPM for their help and cooperation during the bioassay study.



## TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	11
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF PLATES	xi
LIST OF ABBREVIATIONS	x
ABSTRACT	xi
ABSTRAK	xiii
<b>PART I      EXTRACTION AND ISOLATION OF CHEMICAL COMPONENTS FROM GLYCOSMIS CALCICOLA AND G. RUPESTRIS (RUTACEAE)</b>	
INTRODUCTION.....	2
LITERATURE REVIEW.....	3
OBJECTIVES OF STUDY.....	17
RESULTS AND DISCUSSION.....	18
<i>Glycosmis Calcicola</i> B.C. Stone.....	18
<i>Glycosmis Calcicola</i> (Langkawi Island)...	18
<i>Glycosmis Calcicola</i> (Templer Park).....	36
<i>Glycosmis Rupestris</i> Ridley.....	55
<i>Glycosmis Rupestris</i> ((Stem Bark).....	55
<i>Glycosmis Rupestris</i> (Leaves).....	83
CONCLUSION.....	84



EXPERIMENTAL.....	85
General Materials and Methods.....	85
Extraction of <i>Glycosmis Calcicola</i> (Langkawi Island).....	87
Isolation of GC1 (44).....	87
Isolation of Sitosterol (48).....	89
Isolation of GC6.....	90
Extraction of <i>Glycosmis Calcicola</i> (Templer Park).....	91
Isolation of Flindersine (49).....	91
Isolation of Desmethoxyzanthophylline (50).....	93
Extraction of <i>Glycosmis Rupestris</i> .....	
Isolation of Glycomaurin (22).....	94
Isolation of 7-methoxyglycomaurin (51).....	96
Isolation of Des-N-methylacronycine (53).....	97
Isolation of Stigmast-7-enol (54).....	98
Isolation of GC15.....	99

**PART II      ANTIFUNGAL ASSAYS AGAINST *CHAONEPHORA*  
*CUCURBITARIUM*, *COLLETOTRICHUM CAPSICI*  
AND *C. GLOEOSPORIOIDES***

INTRODUCTION.....	102
OBJECTIVES OF STUDY.....	106
LITERATURE REVIEW.....	107
MATERIALS AND METHODS.....	111
Preparation of Plant Extracts.....	111
Preparation of Stock Cultures.....	111
Preparation of Assay Media.....	112
Mycelial Growth and Sporulation.....	112
Spore Germination.....	114
TLC Bioassay.....	115
RESULTS.....	118
Mycelial Growth.....	118
Sporulation.....	121
Spore Germination.....	125
TLC Bioassay.....	127
DISCUSSION AND CONCLUSION.....	131



BIBLIOGRAPHY.....	133
VITA.....	137



## LIST OF TABLES

Table		Page
1	<sup>1</sup> H-NMR Data ( $\delta$ ) of (44) and GC1.....	25
2	<sup>13</sup> C-NMR Chemical Shift Data (ppm) of GC1.....	32
3	<sup>13</sup> C-NMR Chemical Shift Data (ppm) of Flindersine with the Observed Values...	48
4	<sup>13</sup> C-NMR Chemical Shifts (ppm) of Glycomaurin (22).....	63
5	<sup>1</sup> H-NMR Spectral Data ( $\delta$ ) of (22), (51) and (52).....	70
6	Plant Extracts Investigated for Antifungal Activity.....	113
7	Effect of <i>G. calcicola</i> and <i>G. rupestris</i> extracts on Mycelial Growth of <i>C. cucurbitarium</i> , <i>C. capsici</i> and <i>C. gloeosporioides</i> .....	119
8	Effect of <i>G. calcicola</i> and <i>G. rupestris</i> extracts on Spore Germination of <i>C. cucurbitarium</i> , <i>C. capsici</i> and <i>C. gloeosporioides</i> .....	126
9	Antifungal Constituents of Extract B...	129



## LIST OF FIGURES

Figure		Page
1	Schematic Diagram of Extraction and Isolation Procedure of <i>G. calcicola</i> (Langkawi Island).....	19
2	IR Spectrum of (44).....	21
3	<sup>1</sup> H-NMR Spectrum of (44).....	23
4	Mass Spectrum of (44).....	26
5	Mass Spectral Fragmentation Patterns of (44).....	27
6	<sup>13</sup> C-NMR Spectrum of (44).....	29
7	PHSQC Spectrum of (44).....	30
8	PHSQC Spectrum of (44).....	31
9	IR Spectrum of (48).....	34
10	Mass Spectrum of (48).....	35
11	Mass Spectral Fragmentation Patterns of (48).....	37
12	Schematic Diagram of Extraction and Isolation Procedure of <i>G. calcicola</i> (Templer Park).....	38
13	Mass Spectrum of (49).....	40
14	IR Spectrum of (49).....	41
15	<sup>1</sup> H-NMR Spectrum of (49).....	43
16	NOESY Spectrum of (49).....	44
17	Coupling of Various Protons Observed from NOESY Spectrum of (49).....	45
18	<sup>13</sup> C-NMR Spectrum of (49).....	46
19	IR Spectrum of (50).....	49





20	$^1\text{H}$ -NMR Spectrum of (50).....	51
21	Mass Spectrum of (50).....	53
22	Mass Spectral Fragmentation Patterns of (50).....	54
23	Schematic Diagram of Extraction and Isolation Procedure of <i>G. rupestris</i> Stem Bark and Leaves.....	56
24	Mass Spectrum of (22).....	58
25	IR Spectrum of (22).....	59
26	$^1\text{H}$ -NMR Spectrum of (22).....	60
27	$^{13}\text{C}$ -NMR Spectrum of (22).....	62
28	Mass Spectrum of (51).....	65
29	Mass Spectral Fragmentation Patterns of (51).....	66
30	IR Spectrum of (51).....	68
31	$^1\text{H}$ -NMR Spectrum of (51).....	69
32	NOESY Spectrum of (51).....	72
33	Proton Coupling Pattern Observed in NOESY Spectrum of (51).....	73
34	Mass Spectrum of (53).....	75
35	IR Spectrum of (53).....	76
36	$^1\text{H}$ -NMR Spectrum of (53).....	77
37	Mass Spectral Fragmentation Patterns of (53).....	78
38	IR Spectrum of (54).....	81
39	Mass Spectrum of (54).....	82
40	Histogram showing effect of <i>G. calcicola</i> and <i>G. rupestris</i> extracts on mycelial growth of <i>C. cucurbitarium</i> , <i>C. capsici</i> and <i>C. gloeosporioides</i> .....	120
41	Histogram showing effect of <i>G. calcicola</i> and <i>G. rupestris</i> extracts on sporulation of <i>C. capsici</i> and <i>C. gloeosporioides</i> .....	124



## LIST OF PLATES

Plate		Page
1	Chilli A is a healthy fruit. Chilli B showing symptoms of anthracnose.....	105
2	Effect of CHCl <sub>3</sub> extract B on mycelial growth of <i>Chaonephora cucurbitarium</i> ....	122
3	Effect of CHCl <sub>3</sub> extract B on mycelial growth of <i>Colletotrichum capsici</i> .....	122
4	Effect of CHCl <sub>3</sub> extract B on mycelial growth of <i>Colletotrichum</i> <i>gloeosporioides</i> .....	123
5	Effect of CHCl <sub>3</sub> extract F on spore germination of <i>Colletotrichum capsici</i> ..	128
6	CHCl <sub>3</sub> extract B, GC7 (Flindersine (49)) and GC8 (desmethoxyanthophylline (50)) with some impurities) on TLC plate showing zones of inhibition after spraying with <i>Aspergillus flavus</i> .....	130



## LIST OF ABBREVIATIONS

br	broad
CHCl <sub>3</sub>	chloroform
CDCl <sub>3</sub>	deuterated chloroform
d	doublet
dd	doublet of doublet
dt	doublet of triplet
EtOH	ethanol
FAB	Fast Atomic Bombardment
IR	Infra Red
m	medium (in IR data)
m	multiplet (in <sup>1</sup> H-NMR data)
Me	methyl
MeOH	methanol
m.p.	melting point
MS	Mass Spectrum
NMR	Nuclear Magnetic Resonance
PLC	Preparative Thin Layer Chromatography
ppm	part per million
s	strong
TLC	Thin Layer Chromatography
w	weak



Abstract of the thesis presented to the Senate of  
Universiti Pertanian Malaysia in fulfilment of  
requirements for the degree of Master of Science.

CHEMICAL CONSTITUENTS OF *GLYCOSMIS CALCICOLA* AND  
*G. RUPESTRIS* (RUTACEAE) AND THEIR BIOLOGICAL  
ACTIVITIES

By

CHEW YEAN LING

June 1995

Chairman: Associate Professor Dr. Mawardi Rahmani  
Faculty : Science and Environmental Studies

Investigation of the leaf and twigs extracts of  
*Glycosmis calcicola* (Rutaceae) from two different  
locations has led to the isolation of five compounds.  
The structures of these compounds were elucidated by  
means of spectroscopic methods (IR, UV,  $^1\text{H-NMR}$ ,  $^{13}\text{C-}$   
NMR, MS) and by comparison with previous studies.

*G. calcicola* collected from Langkawi Island  
yielded methylgerambullin, sitosterol and another  
unidentified compound. From another separate



collection of the same plant from Templer Park, a new alkaloid (desmethoxyanthophylline) and flindersine have also been isolated. A similar study on the bark of another species, *G. rupestris*, collected from Langkawi Island afforded a new alkaloid (7-methoxyglycomaurin), glycomaurin, des-N-methylacronycine and stigmast-7-enol, while the leaves of the plant gave an unidentified compound.

The antifungal activities of these plant extracts were evaluated using the poison food and spore germination techniques. The plant extracts exhibited varying degree of fungitoxic response on the growth of the fungal pathogens of chilli (*Capsicum annum L.*). The chloroform extract of *G. calcicola* collected from Templer Park has been shown to be most effective in inhibiting the mycelial growth, sporulation and spore germination of *Chaonephora cucurbitarium*, *Colletotrichum capsici* and *C. gloeosporioides*.

The antifungal compounds in this extract were identified using TLC bioassay technique against *Aspergillus flavus*. The results indicated that the activity of the extract is due to the presence of its major components: flindersine and desmethoxyanthophylline.



Abstrak tesis yang dikemukakan kepada Senat  
Universiti Pertanian Malaysia bagi memenuhi keperluan  
Ijazah Master Sains.

**KANDUNGAN KIMIA DARI *GLYCOSMIS CALCICOLA* DAN  
*G. RUPESTRIS* (RUTACEAE) DAN AKTIVITI BIOLOGINYA**

Oleh

**CHEW YEAN LING**

Jun 1995

Pengerusi: Professor Madya Dr. Mawardi Rahmani  
Fakulti : Sains dan Pengajian Alam Sekitar

Kajian ke atas ekstrak daun dan ranting *Glycosmis calcicola* (Rutaceae) telah menghasilkan lima kompaun. Struktur sebatian tersebut telah dapat ditentukan dengan menggunakan kaedah spektroskopi dan perbandingannya dengan kajian-kajian yang telah dilakukan terdahulu.

*G. calcicola* yang dikumpulkan dari Pulau Langkawi telah memberikan metilgerambulin sitosterol dan satu sebatian yang belum dikenal pasti. Dari pengumpulan berasingan spesies yang sama



di Templer Park, satu alkaloid baru (desmetoksizantofilina) dan flindersina telah berjaya dipencilkan. Kajian yang serupa ke atas kulit batang spesies yang lain, *G. rupestris*, yang dikumpulkan dari Pulau Langkawi telah memberikan satu alkaloid baru (7-metoksiglikomaurin), glikomaurin, des-N-metilakronisin dan stigmast-7-enol, manakala daunnya pula memberikan satu sebatian yang belum dikenal pasti.

Aktiviti antikulat ekstrak tumbuhan tersebut telah dikaji dengan menggunakan teknik 'poison food' dan percambahan spora. Ekstrak tumbuhan yang dikaji menunjukkan aktiviti antikulat yang berbeza terhadap pertumbuhan kulat patogen cili (*Capsicum annuum L.*). Ekstrak klorofom *G. calcicola* yang dikumpulkan dari Templer Park didapati paling berkesan merencat pertumbuhan miselium, pensporalan dan percambahan spora *Chaonephora cucurbitarium*, *Colletotrichum capsici* dan *C. gloeosporioides*.

Sebatian antikulat dalam ekstrak ini telah dapat dikenal pasti dengan menggunakan kaedah biocerakinan TLC terhadap *Aspergillus flavus*. Keputusannya menunjukkan bahawa aktiviti ekstrak tersebut adalah disebabkan oleh kehadiran komponen utamanya: flindersina dan desmetoksizantofilina.



**PART I**

**EXTRACTION AND ISOLATION OF  
CHEMICAL COMPONENTS FROM  
*GLYCOSMIS CALCICOLA*  
AND  
*G. RUPESTRIS*  
(RUTACEAE)**





## INTRODUCTION

*Glycosmis* is a genus of forty species of small trees and shrubs of the family Rutaceae, found mostly in South-East Asia and South of China. Fourteen species are reported to occur in Malaysia (Whitmore, 1983). *G. calcicola* B.C. Stone and *G. rupestris* Ridley are shrubs which grow on limestone rocks and limestone hills respectively. *G. calcicola* can be found in Langkawi, Perak, Selangor and Kelantan whereas *G. rupestris* occurs in Perlis, Kedah, Pulau Adang and Langkawi.

Members of the genus are aromatic and are used medicinally, chiefly the roots (Burkill, 1966). *G. pentaphylla* Corr. is used for the treatment of fever in India, while *G. cochinchinensis* Pirre found in the Dutch Indies is used to treat swollen spleen. A decoction of the leaves and roots of the latter, also, is used as a stimulant to digestion.

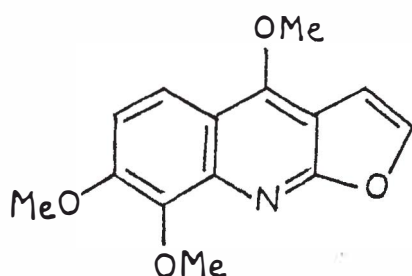


## LITERATURE REVIEW

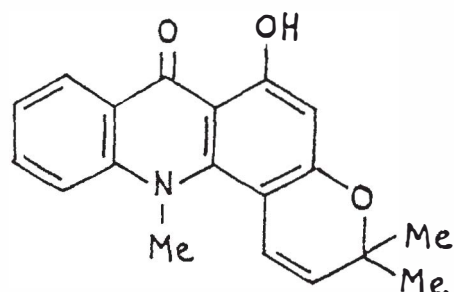
A number of studies on the chemical constituents of *Glycosmis* species have been reported. The species which have been investigated in detail include *G. pentaphylla*, *G. mauritiana*, *G. bilocularis*, *G. arborea*, *G. cyanocarpa* and *G. chlorosperma*. These investigations revealed the presence of various compounds belonging to the alkaloid, coumarin and amide groups.

One of the plants that had been exhaustively investigated was *G. pentaphylla* and it furnishes a number of acridone, carbazole and quinolone bases. The root bark has been reported to elaborate these alkaloids. In 1966, Govindachari et. al isolated a known compound, skimmianine (1),  $C_{14}H_{13}O_4N$ , m.p.  $176^{\circ}C$  and three new acridone alkaloids: noracronycine (2),  $C_{19}H_{17}O_3N$ , m.p.  $200^{\circ}C$ ; des-N-methylacronycine (3),  $C_{19}H_{17}O_3N$ , m.p.  $268-270^{\circ}C$  and des-N-methylnoracronycine (4),  $C_{18}H_{15}O_3N$ , m.p.  $246^{\circ}C$ . In 1969, Chakraborty reported the isolation of glycozoline (5),  $C_{14}H_{13}NO$ , m.p.  $181-182^{\circ}C$ , a carbazole derivative.

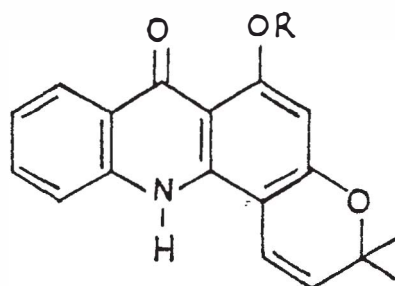




(1)

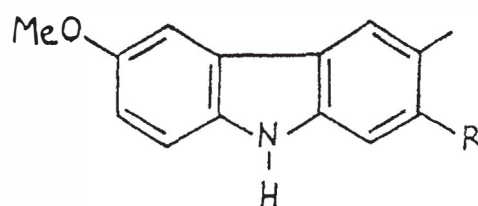


(2)



(3) R = Me

(4) R = H

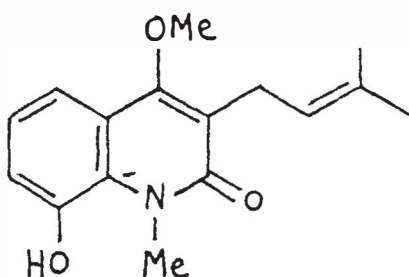


(5) R = H

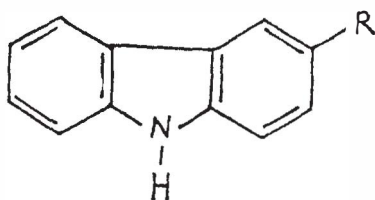
(6) R = OMe

In continuation of their studies on the carbazole alkaloids of *G. pentaphylla*, Chakraborty et al. (1974) reported the structural studies of glycozolidine (6), m.p. 160-162°C from the root bark of this plant. Subsequently, Das and Chowdhury (1978) discovered a novel quinolone alkaloid, glycosolone (7),  $C_{16}H_{19}NO_3$ , m.p. 159°C. Further

study on the root bark of this species by Chowdhury et al. (1987) afforded carbazole (8),  $C_{12}H_{19}N$ , m.p.  $245^{\circ}C$  and 3-methylcarbazole (9),  $C_{13}H_{11}N$ , m.p.  $207^{\circ}C$ . This is the first report of the isolation of carbazole from a plant source.



(7)

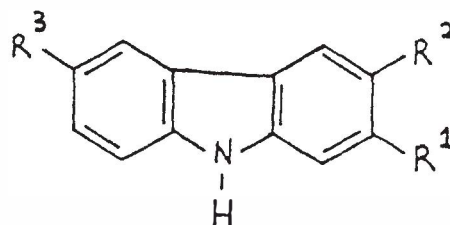


(8) R = H

(9) R = Me

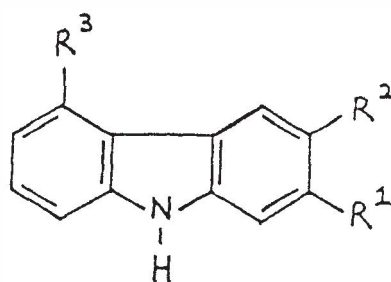
An antibacterial carbazole alkaloid, glycozolidol (10),  $C_{14}H_{13}NO_2$ , m.p.  $240^{\circ}C$  was discovered from the root of *G. pentaphylla* by Bhattacharyya et al. (1985). The compound has been found to be active against some Gram-positive and

Gram-negative bacteria. In the same year, he and Chowdhury reported the structure of another carbazole alkaloid, glycozolidal (11), m.p. 185°C. In 1992, Jash et al. isolated two carbazole alkaloids, glycozolicine (12),  $C_{14}H_{13}NO$ , m.p. 135°C and glycosinine (13),  $C_{14}H_{11}NO_2$ , m.p. 185°C from the same source .



(10)  $R^1 = OMe$ ;  $R^2 = Me$ ;  $R^3 = OH$

(11)  $R^1 = R^3 = OMe$ ;  $R^2 = CHO$



(12)  $R^1 = H$ ;  $R^2 = Me$ ;  $R^3 = OMe$

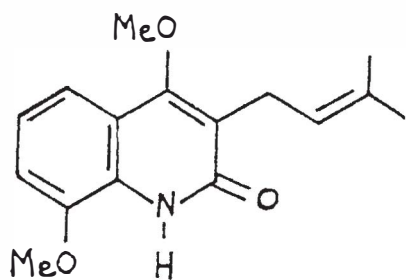
(13)  $R^1 = OMe$ ;  $R^2 = CHO$ ;  $R^3 = H$

An investigation of the leaves of *G. pentaphylla* by Bhattacharyya and Chowdhury (1985) led to the isolation of a quinolone alkaloid, glycolone (14),  $C_{16}H_{19}NO_3$ , m.p.  $118^{\circ}C$ . Further chemical study on the leaves of this species by Kamaruzzaman et al. (1989) afforded a carbazole alkaloid, mupamime (15),  $C_{19}H_{19}NO_2$ , m.p.  $152^{\circ}C$ . This is the first  $C_{18}$  alkaloid reported from the genus *Glycosmis*.

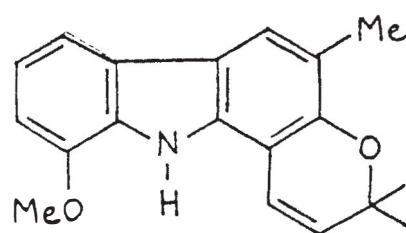
Sarkar and Chakraborty (1977) undertook the examination of the flower heads of *G. pentaphylla* from which they reported the isolation and structure of glycophymine (16),  $C_{15}H_{12}N_2O$ , m.p.  $254^{\circ}C$  and an amide, glycomide (17),  $C_{14}H_{13}NO$ , m.p.  $120^{\circ}C$ . In 1979, they isolated a new quinazoline alkaloid, glycophymoline (18),  $C_{16}H_{14}N_2O$ , m.p.  $165^{\circ}C$ .

Work on the seeds of *G. pentaphylla* by Mukherjee et al. (1983) had yielded a carbazole derivative, glycozolinine (19), m.p.  $231-232^{\circ}C$ . From physical and chemical evidence, its structure was identified as 6-hydroxy-3-methylcarbazole.

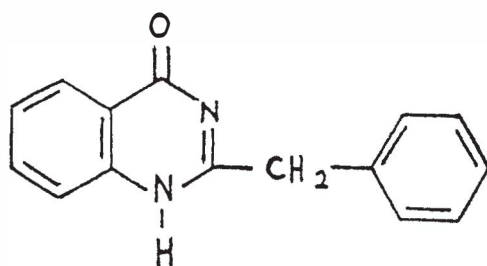




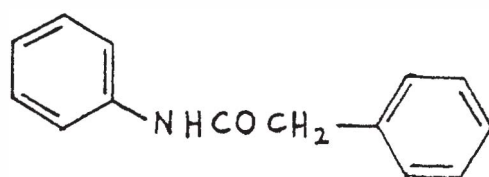
(14)



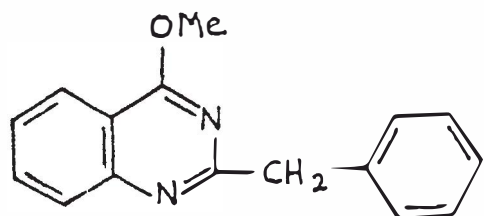
(15)



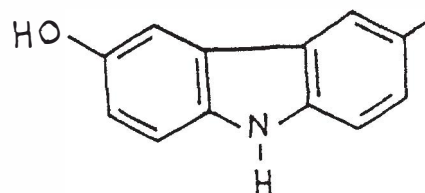
(16)



(17)

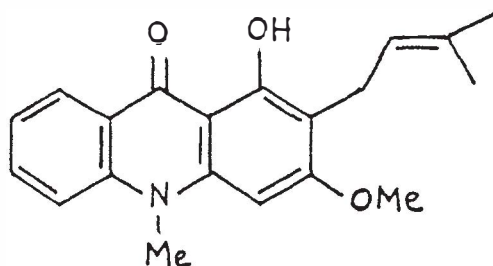


(18)

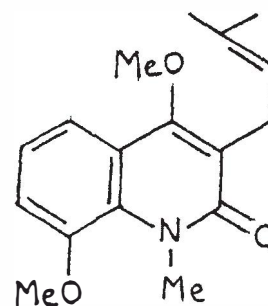


(19)

A chemical investigation of the roots of *G. mauritiana* by Rastogi et al. (1980) resulted in the isolation of two alkaloids: 1-hydroxy-3-methoxy-2-(3-methylbut-2-enyl)-*N*-methylacridan-9-one (20), m.p. 134-136°C and 4,8-dimethoxy-3-(3-methylbut-2-enyl)-*N*-methyl-2-quinolone (21). The stem bark of *G. mauritiana* collected in Giritale, north central of Sri Lanka was shown to contain carbazole alkaloids: glycomaurin (22), m.p. 195-196°C and glycomaurrol (23), m.p. 149-150°C by Kumar et al. (1989).

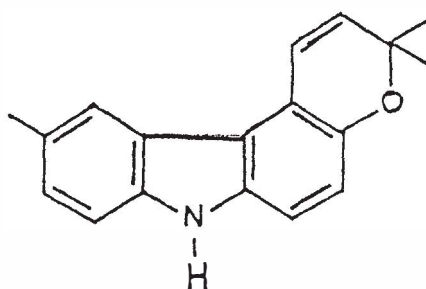


(20)

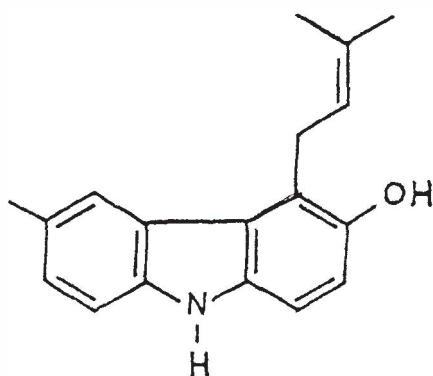


(21)





(22)



(23)

A new 5-hydroacridone was isolated by Bowen et al. (1978) from the leaves of *G. bilocularis*. It was characterized as 1,5-dihydroxy-2,3-dimethoxy-10-methyl-9-acridone (24), m.p. 206-207°C. In addition, they also isolated five known compounds: arborine (25), m.p. 154-156°C; arborinine (26), m.p. 174-176°C; skimmianine (1), kokusaginine (27), m.p. 170-172°C and glycerine (28), m.p. 145-146°C.