UNIVERSITI PUTRA MALAYSIA

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

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

By

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LIST OF ABBREVIATIONS

br	broad
$CHCl_3$	chloroform
$CDCl_3$	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 ^{1}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.

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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}H-NMR, ^{13}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 (desmethoxyzanthophylline) 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 annuum 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 desmethoxyzanthophylline.

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 spesis 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

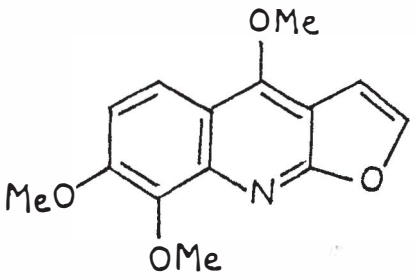
Glycosmis is a genus of forty species of small trees and shrubs of the family Rutaceae, found mostly in South-East Asia and South of China. Fourteen species are reported to occur in Malaysia (Whitmore, 1983). *G. calcicola* B.C. Stone and *G. rupestris* Ridley are shrubs which grow on limestone rocks 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°C and three new acridone alkaloids: noracronycine (2), $C_{19}H_{17}O_3N$, m.p. 200°C; des-N-methylacronycine (3), $C_{19}H_{17}O_3N$, m.p. 268-270°C and des-N-methylnoracronycine (4), $C_{18}H_{15}O_3N$, m.p. 246°C. In 1969, Chakraborty reported the isolation of glycozoline (5), $C_{14}H_{13}NO$, m.p. 181-182°C, a carbazole derivative.



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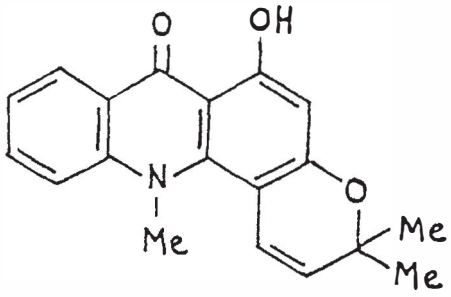

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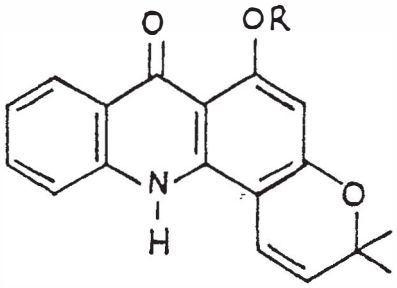
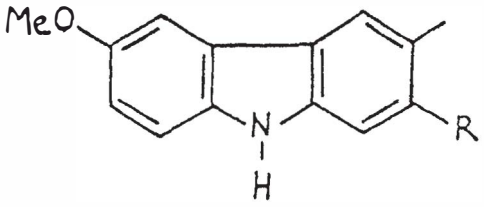


(3) R = Me

(4) R = H

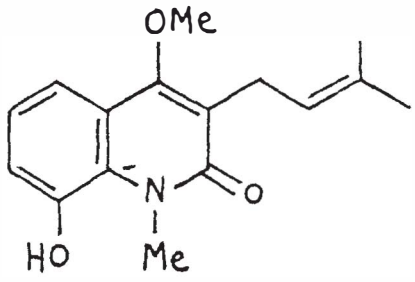


(5) R = H

(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°C and 3-methycarbazole (9), $C_{13}H_{11}N$, m.p. 207°C. This is the first report of the isolation of carbazole from a plant source.



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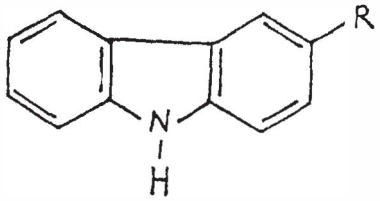
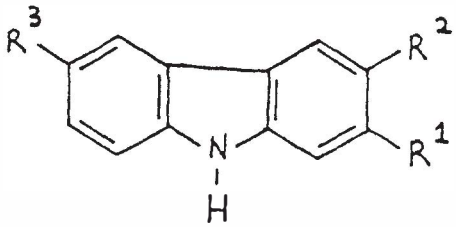


(8) R = H

(9) R = Me

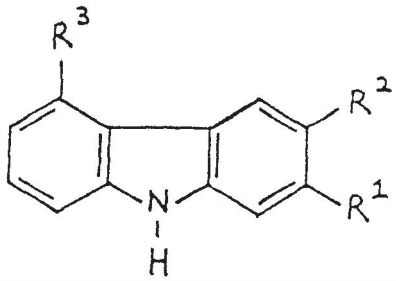
An antibacterial carbazole alkaloid, glycozolidol (10), $C_{14}H_{13}NO_2$, m.p.240°C was discovered from the root of *G. pentaphylla* by Bhattacharyya et al. (1985). The compound has been found to be active against some Gram-positive and

Gram-negative bacteria. In the same year, he and Chowdhury reported the structure of another carbazole alkaolid, 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



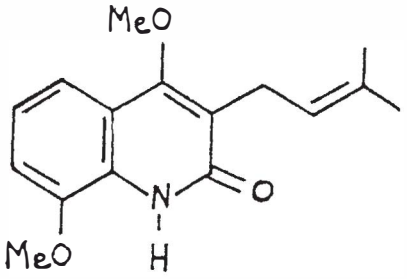
(12) R^1 = H; R^2 = Me; R^3 = OMe

(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°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°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°C and an amide, glycomide (**17**), $C_{14}H_{13}NO$, m.p. 120°C. In 1979, they isolated a new quinazoline alkaloid, glycophymoline (**18**), $C_{16}H_{14}N_2O$, m.p. 165°C.

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



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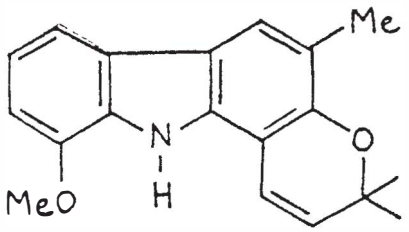

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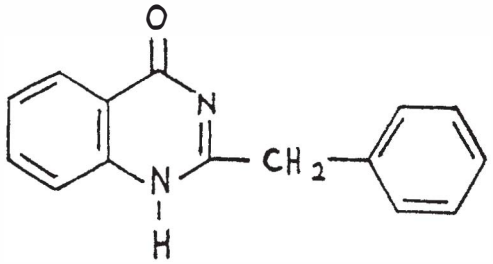

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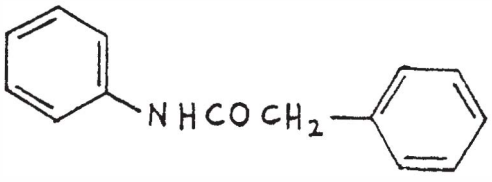

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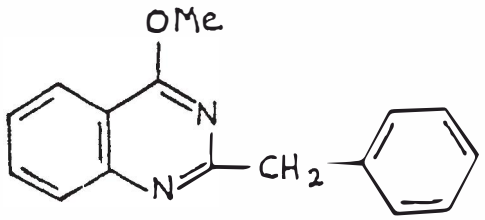

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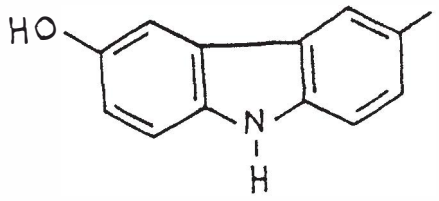
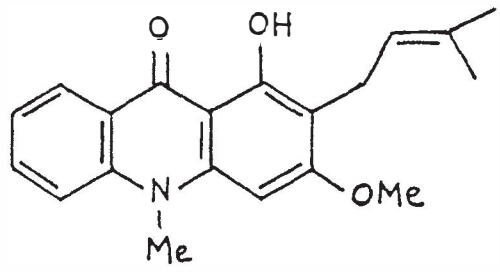


(19)

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).



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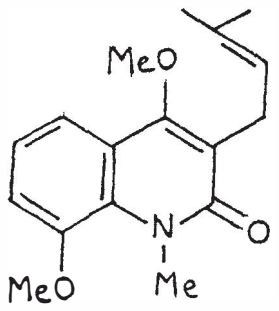

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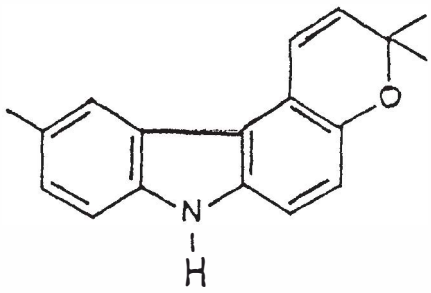

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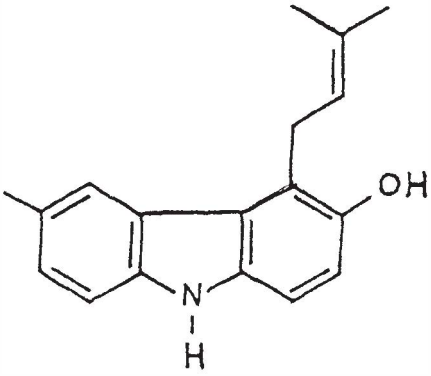

(23)

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.