1 INTRODUCTION

1.1 Introduction

Fungi are one of the most important sources for biologically active substances. A number of drugs derived from fungal secondary metabolites and their modified analogs have been developed. Two genera of fungi, *Penicillium* and *Cordyceps*, are of interest since they produced many bioactive compounds.

***Penicillium* sp.**, is a genus of fungi imperfect of the form-family Trichomaceae (www.doctorfungus.org). Species of *Penicillium* are recognized by their dense brush-like spore-bearing structures. The conidiophores are simple or branched and are terminated by clusters of flask-shaped phialides. The spores (conidia) are produced in dry chains from the tips of the phialides, with the youngest spore at the base of the chain, and are nearly always green. *Penicillium* sp. is a large genus encountered almost everywhere, and usually the most abundant genus of fungi in soils (www.botany.utoronto.ca).

Cordyceps is a Chinese mushroom used in traditional Chinese medicine. *Cordyceps* (*Clavicipitaceae* Family) is also known as the Chinese caterpillar fungus because it is a parasitic organism that grows on a rare Tibetan caterpillar until the caterpillar dies and the mushroom sprouts from the caterpillar's head. The source of *Cordyceps* used in most modern supplements is not pulverized caterpillar heads, but a strain grown on soybeans or other less disgusting nutrient source. One of the most commonly collected species is *C. militaris* which is one of entomopathogenic fungi infecting lepidopteran insects (www.nifg.org.uk).

1.2 Review of literatures

1.2.1 Chemical constituents from the genera *Penicillium* and *Cordyceps*

The genera *Penicillium* and *Cordyceps* have been extensively investigated from pharmacological point of view. Various compounds have been isolated. Tables 1 and 2 demonstrated compounds isolated from both genera since 1998. For the genus

Penicillium, all microbial metabolites have been reported in the database named *AntiBase* 2002. Important secondary metabolites isolated from *Clavicipitaceae* fungi have been reviewed in the year 2003 (White, *et al.*).

1.2.2 Biological and pharmacological activities

The genus *Penicillium* is well-known for producing a variety of bioactive metabolites, possessing a wide variety of biological properties, for example, plant growth regulators (Kimura, 2000; Macias, 2000; Nakada, 1999), antifeedant (Kosemura, 2002) and antioxidant (Chen, 2002). Fungal metabolites from *Cordyceps* species exhibited interesting biological activities, such as, antitumor (Bok, 1999), cytotoxicity (Kittakoop, 1999) and antimalarial activities (Jaturapat, 2001; Isaka, 2001; Kittakoop, 1999). Biological activities of compounds isolated from both genera since 1998 are also summarized in Tables 1 and 2.

Table 1 Compounds isolated from *Penicillium* species and biological activity

Scientific name	Compound	Structure	Activity	References
P. brevicom-pactum	brevione A	10h	plant growth regulators	Macias, *et al.*, 2000
P. chrysogenum	methyl *ent*-7α-hydroxy-16-keto-beyeran-19-oate	15h	-	Oliveira, *et al.*, 1999
	methyl *ent*-1β,7α-dihydroxy-16-keto-beyeran-19-oate	16h		
	isosteviol	17h		
	17-hydroxyisoste-viol	18h		

Table 1 (Continued)

Scientific name	Compound	Structure	Activity	References
P. citreo-viride B. IFO 6200 and 4692	citreo-γ-pyrone citreothiopyrane A isocitreohybridone C citreohybridone J citreohybridone K citreohybridone L	10i 11i 11h 12h 13h 14h	plant growth inhibitor -	Nakada, *et al.*, 1999 Kosemura, *et al.*, 2002
P. citrinum F5	2,3,4-trimethyl-5,7-dihydroxy-2,3-dihydrobenzofuran gentisic acid	5i 6i	antioxidant	Chen, *et al.*, 2002
P. crustosum Thom	penitrem A thomitrem A thomitrem E	6c 7c 8c	-	Rundberget and Wilkins, 2002
P. cyclopium	conidiogenol conidiogenone	8h 9h	conidiation inducing activity	Roncal, *et al.*, 2002
P. decumbens	decumbenone A decumbenone B versiol	4e 5e 6e	fungal melanin inhibitor	Fujii, *et al.*, 2002
P. dipodomyis	dipodazine	18c	-	Sorensen, *et al.*, 1999
P. fellutanum	fellutanine A fellutanine B fellutanine C fellutanine D	13c 14c 15c 16c	-	Kozlovsky, *et al.*, 2001

Table 1 (Continued)

Scientific name	Compound	Structure	Activity	References
P. cf. montanense	xestodecalactone A xestodecalactone B xestodecalactone C	1d 2d 3d	active against the yeast *Candida albicans*	Edrada, *et al.*, 2002
P. multicolor	8-*O*-methyl-sclerotiorinamine	7i	SH2 domain antagonist	Nam, *et al.*, 2000
P. roqueforti	(+)-aristolochene	1h	-	Demyttenaere, *et al.*, 2002
P. solitum	solistatin	4d	-	Sorensen, *et al.*, 1999
P. sp.	communesin B communesin C communesin D	1c 2c 3c	antiprolifera-tive activity	Jadulco, *et al.*, 2003
P. sp.	7-deacetoxyyanu-thone A 2,3-hydro-7-deace-toxyyanuthone A farnesylhydroqui-none farnesylquinone	1b 2b 4g 5g	cytotoxicity antibacterial activity against MRSA	Li, *et al.*, 2003
P. sp.	ravenic acid	8i	-	Michael, *et al.*, 2002
P. sp.	polyketide 1 polyketide 2	1e 2e	against plant pathogen *Sclerotinia sclerotiorum*	Stierle and Ganser, 1999
P. sp.	coruscol A	3e	-	Kagata, *et al.*, 2000

Table 1 (Continued)

Scientific name	Compound	Structure	Activity	References
P. sp.	preaustinoid A preaustinoid B verruculogen	6h 7h 17c	antibacterial activity	Geris dos Santos and Rodrigues-Fo, 2002
P. sp. (Strain #386)	penicillazine	5f	-	Lin, *et al.*, 2000
P. sp. No. 13	peniamidienone penienone penidilamine	3b 4b 9i	plant growth inhibitor	Kimura, *et al.*, 2000
P. sp.	sculezonone A sculezonone B herqueinone	2g 3g 12g	-	Komatsu, *et al.*, 2000
P. thiersii	thiersindole A thiersindole B thiersindole C	9c 10c 11c	cytotoxicity antibacterial activity against MRSA and multidrug resistant *S. aureus*	Li, Gloer and Wicklow, 2003
P. thymicola	serantrypinone alantrypinone daldinin D fumiquinazoline F	4c 5c 1g 12c	-	Ariza, *et al.*, 2001 Larsen, *et al.*, 1998

Table 2 Compounds isolated from *Cordyceps* species and biological activity

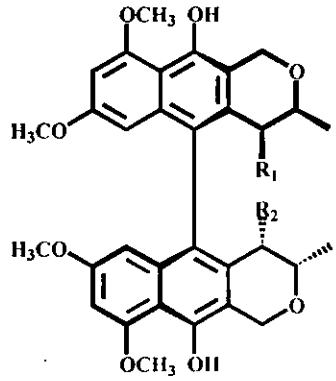
Scientific name	Compound	Structure	Activity	References
C. militaris	adenine	1i	-	Huang,
	adenosine	2i		*et al.*, 2003
	cordycepin	3i		
	hypoxanthine	4i		
C. nipponica	cordypyridone A	1f	antimalarial	Isaka, *et al.*,
BCC 1389	cordypyridone B	2f	activity	2001
	cordypyridone C	3f		
	cordypyridone D	4f		
C. pseudomili-	bioxanthracene 1	1a	antimalarial	Jaturapat,
taris BCC 1620	bioxanthracene 2	2a	activity	*et al.*, 2001
	bioxanthracene 3	3a		
	bioxanthracene 4	4a		
	bioxanthracene 5	5a		
	bioxanthracene 6	6a		
	bioxanthracene 7	7a		
	bioxanthracene 8	8a		
	bioxanthracene 9	9a		
	bioxanthracene 10	10a		
	bioxanthracene 11	11a		
	oxanthracene 12	12a		
	oxanthracene 13	13a		
	cordyanhydride A	13i		
	cordyanhydride B	14i		
C. sinensis	5α,8α-epidioxy-24-(*R*)-methylcholesta-6,22-dien-3β-D-glucopyranoside	2h	antitumor	Bok, *et al.*, 1999

Table 2 (Continued)

Scientific name	Compound	Structure	Activity	References
	5α,6α-epoxy-24-(*R*)-methylcholesta-7,22-dien-3β-ol	3h		
	ergosteryl-3-*O*-β-D-glucopyranoside	4h		
	22,23-dihydroergosteryl-3-*O*-β-D-glucopyranoside	5h		
	adenine	1i		
	adenosine	2i		
	cordycepin	3i		
	hypoxanthine	4i		
C. sp. BCC 1681	cordytropolone	12i	antimalarial activity	Isaka, *et al.*, 2001
C. unilateralis	erythrostominone	6g	antimalarial activity cytotoxicity	Kittakoop, *et al.*, 1999
	deoxyerythrostominone	7g		
	4-*O*-methylerythrostominone	8g		
	epierythrostominol	9g		
	deoxyerythrostominol	10g		
	3,5,8-trihydroxy-6-methoxy-2-(5-oxohexa-1,3-dienyl)-1,4-naphthoquinone	11g		

1.2.3 Structures of compounds in Tables 1 and 2

Bioxanthracenes and oxanthracenes



1a: $R_1 = R_2 = OH$: bioxanthracene 1

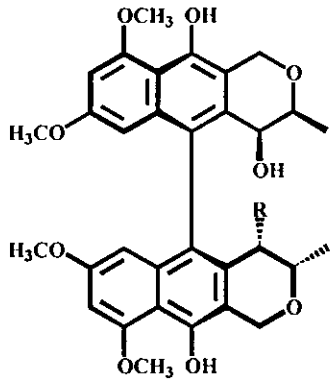
2a: $R_1 = OAc$, $R_2 = OH$: bioxanthracene 2

3a: $R_1 = R_2 = OAc$: bioxanthracene 3

4a: $R_1 = OH$, $R_2 = H$: bioxanthracene 4

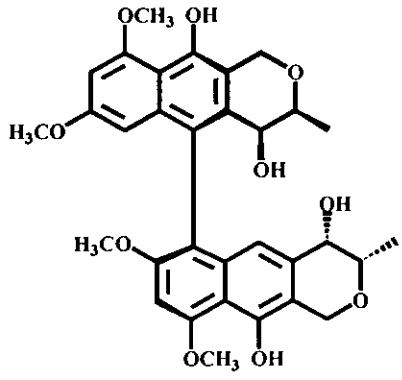
5a: $R_1 = OAc$, $R_2 = H$: bioxanthracene 5

6a: $R_1 = R_2 = H$: bioxanthracene 6

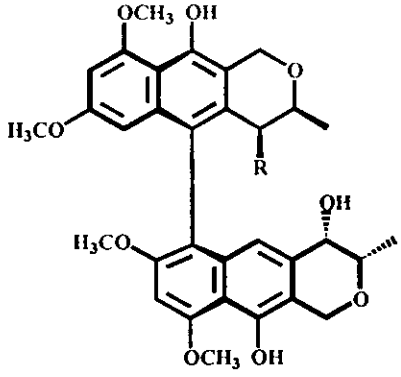


7a: R = OH : bioxanthracene 7

8a: R = H : bioxanthracene 8

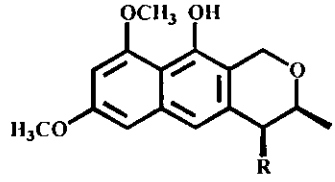


9a: bioxanthracene 9



10a: R = OH : bioxanthracene 10

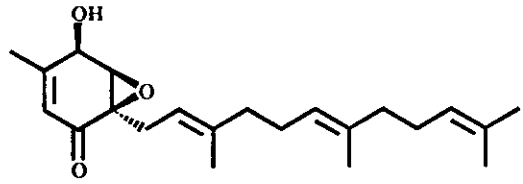
11a: R = H : bioxanthracene 11



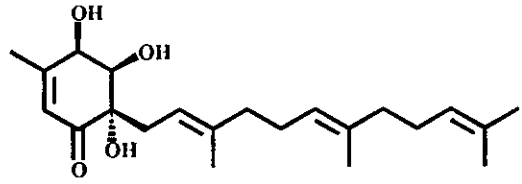
12a: R = OH : oxanthracene 12

13a: R = H : oxanthracene 13

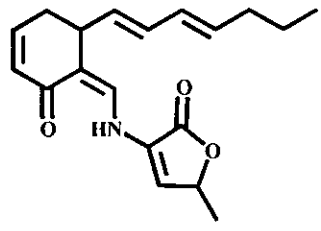
Cyclohexenones



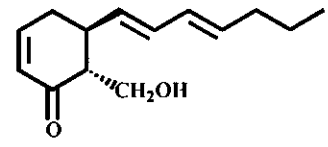
1b: 7-deacetoxyyanuthone A



2b: 2,3-hydro-7-deacetoxyyanuthone A

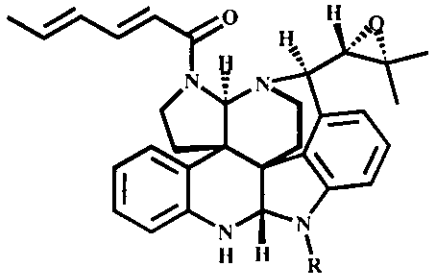


3b: peniamidienone



4b: penienone

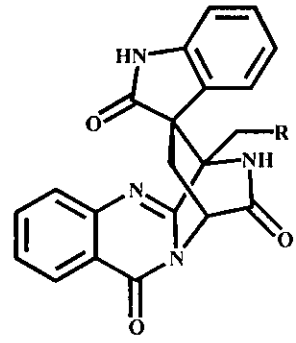
Indoles



1c: R = CH_3 : communesin B

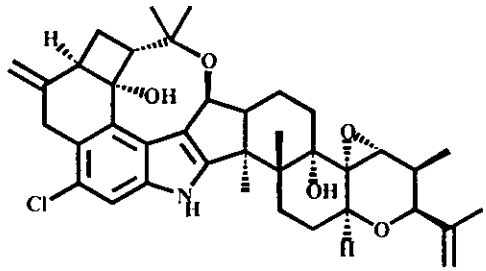
2c: R = H : communesin C

3c: R = CHO : communesin D

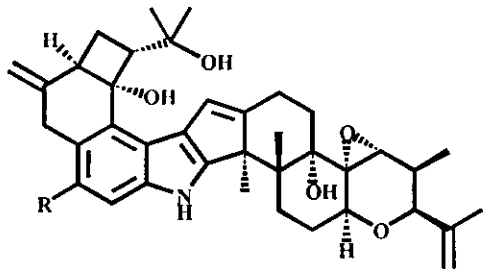


4c: R = OH : serantrypinone

5c: R = H : alantrypinone

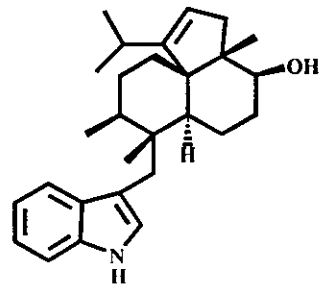


6c: penitrem A

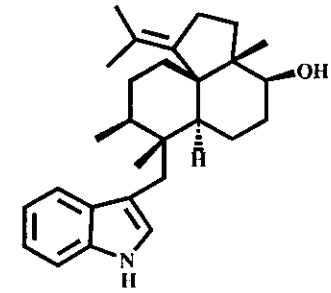


7c: R = Cl : thomitrem A

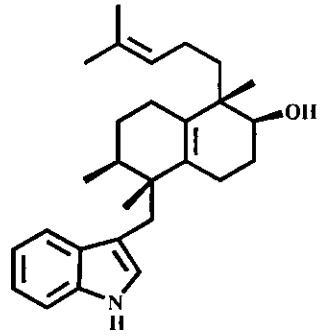
8c: R = H : thomitrem E



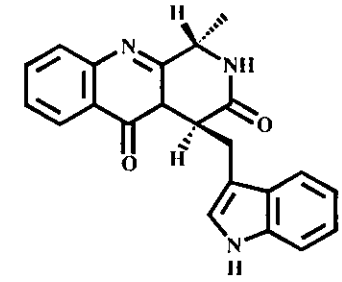
9c: thiersindole A



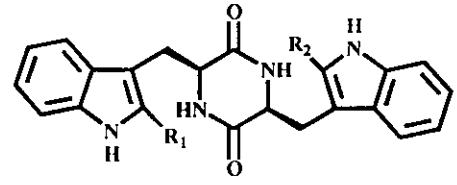
10c: thiersindole B



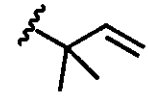
11c: thiersindole C

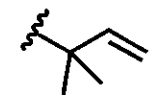


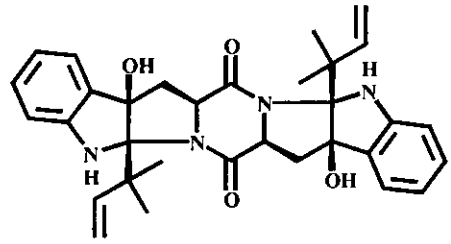
12c: fumiquinazoline F



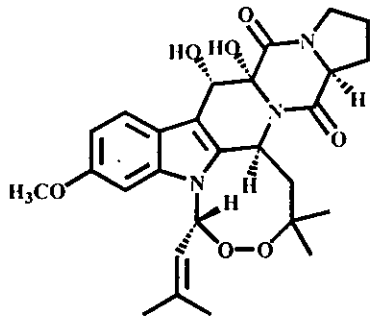
13c: R_1 = H; R_2 = H : fellutanine A

14c: R_1 =  R_2 = H : fellutanine B

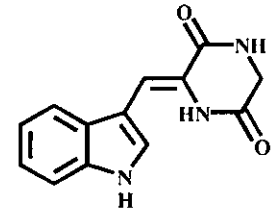
15c: R_1, R_2 =  : fellutanine C



16c: fellutanine D

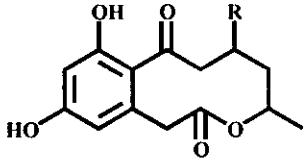


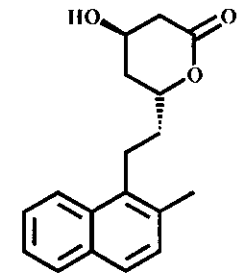
17c: verruculogen



18c: dipodazine

Lactones





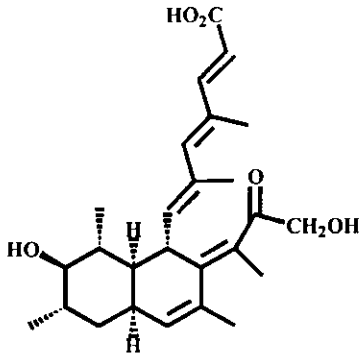
1d: R = H : xestodecalactone A

2d: R = OH; 9,11-*cis* : xestodecalactone B

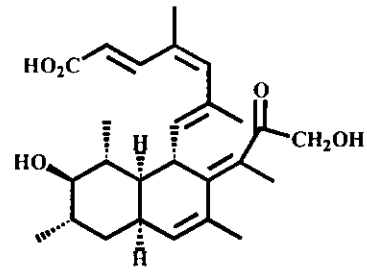
3d: R = OH; 9,11-*trans* : xestodecalactone C

4d: solistatin

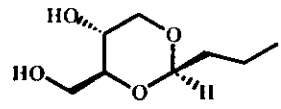
Polyketides



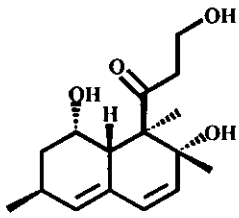
1e: polyketide 1



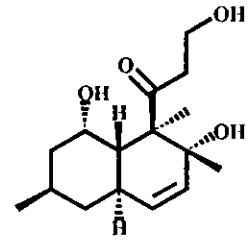
2e: polyketide 2



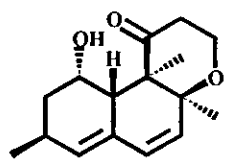
3e: coruscol A



4e: decumbenone A

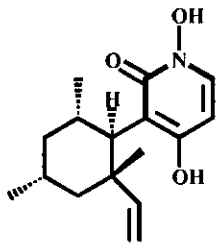


5e: decumbenone B

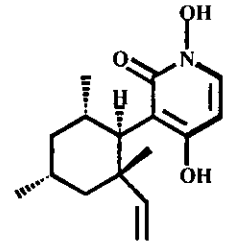


6e: versiol

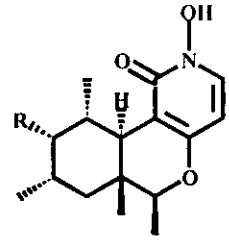
Pyridones



1f: cordypyridone A

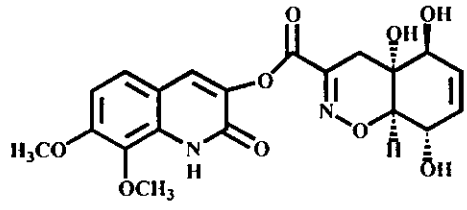


2f: cordypyridone B



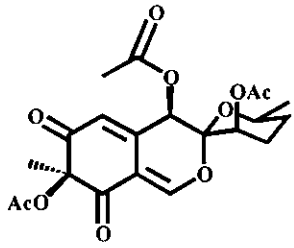
3f: R = H : cordypyridone C

4f: R = OH : cordypyridone D

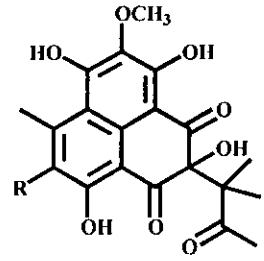


5f: penicillazine

Quinones

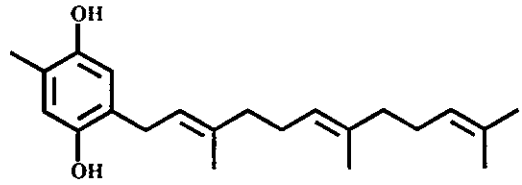


1g: daldinin D

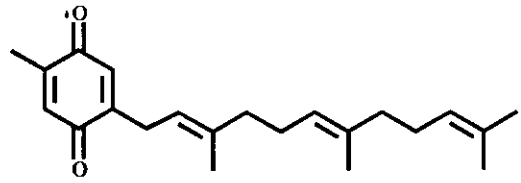


2g: sculezonone A

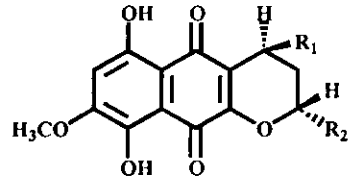
3g: sculezonone B

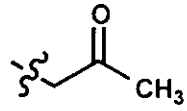


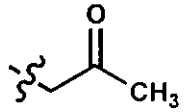
4g: farnesylhydroquinone

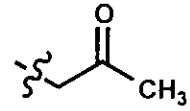


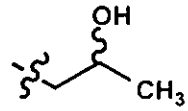
5g: farnesylquinone

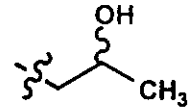


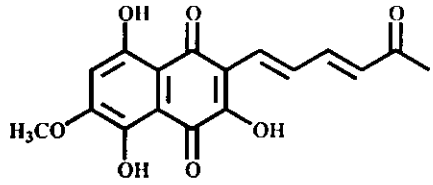
6g: R_1 = OH; R_2 =  : erythrostominone

7g: R_1 = H; R_2 =  : deoxyerythrostominone

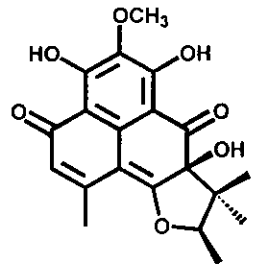
8g: R_1 = OCH_3; R_2 =  : 4-*O*-methylerythrostominone

9g: R_1 = OH; R_2 =  : epierythrostominol

10g: R_1 = H; R_2 =  : deoxyerythrostominol

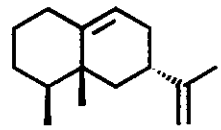


11g: 3,5,8-trihydroxy-6-methoxy-2-(5-oxohexa-1,3-dienyl)-1,4-naphthoquinone

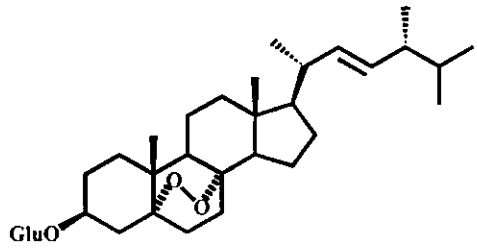


12g: herqueinone

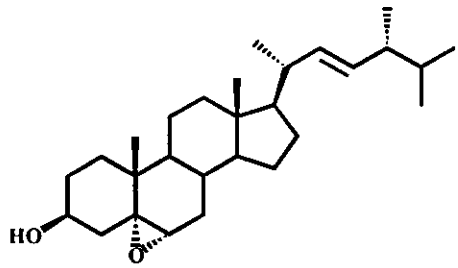
Terpenes



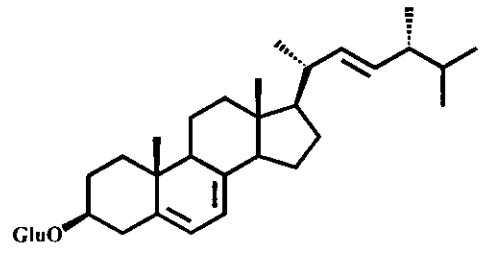
1h: (+)-aristolochene



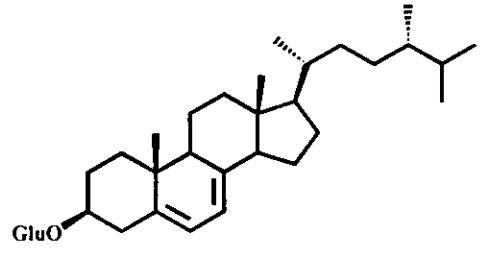
2h: 5α,8α-epidioxy-24(R)-methylcholesta-6,22-dien-3β-D-glucopyranoside



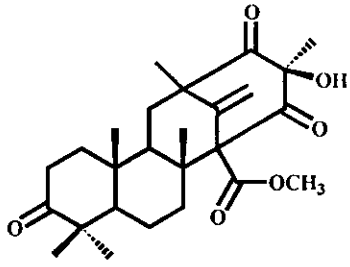
3h: 5α,6α-epoxy-24(R)-methylcholesta-7,22-dien-3β-ol



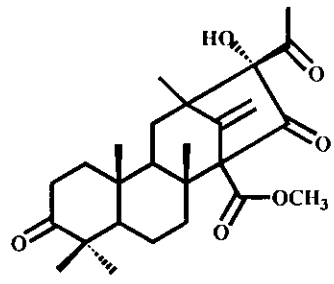
4h: ergosteryl-3-*O*-*β*-D-glucopyranoside



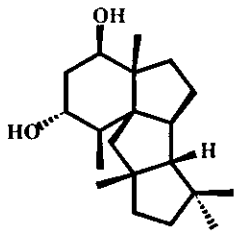
5h: 22,23-dihydroergosteryl-3-*O*-*β*-D-glucopyranoside



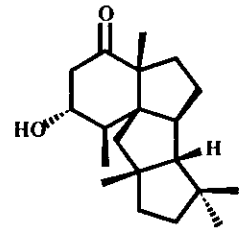
6h: preaustinoid A



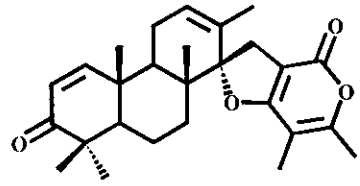
7h: preaustinoid B



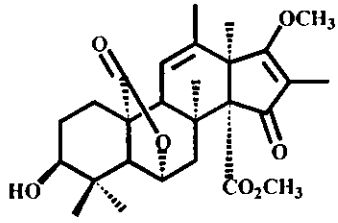
8h: conidiogenol



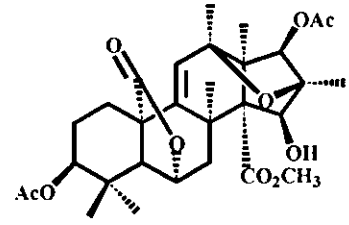
9h: conidiogenone



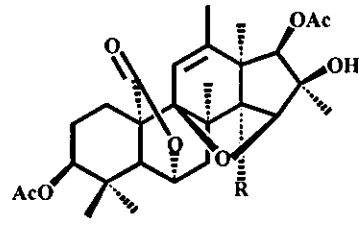
10h: brevione A



11h: isocitreohybridone C

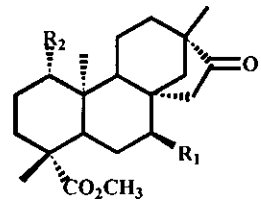


12h: citreohybridone J



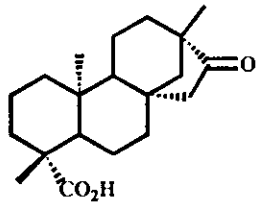
13h: R = CO_2Me : citreohybridone K

14h: R = CH_2OAc : citreohybridone L

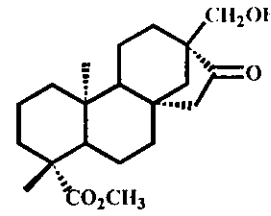


15h: R_1 = OH; R_2 = H : methyl *ent*-7α-hydroxy-16-ketobeyeran-19-oate

16h: R_1 = R_2 = OH : methyl *ent*-1β,7α-dihydroxy-16-ketobeyeran-19-oate

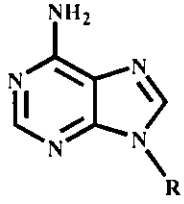


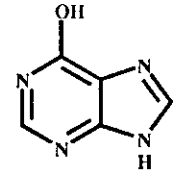
17h: isosteviol



18h: 17-hydroxyisosteviol

Miscellaneous



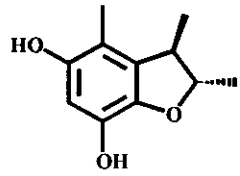


1i: R = H : adenine

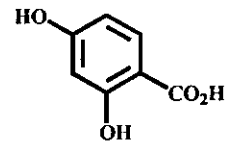
4i: hypoxanthine

2i: R = ribose : adenosine

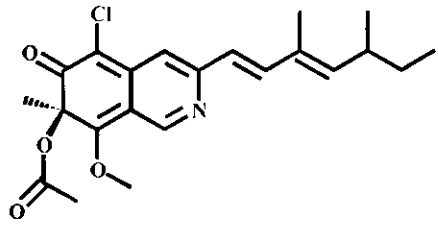
3i: R = 3-deoxyribose : cordycepin



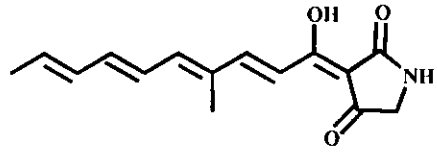
5i: 2,3,4-trimethyl-5,7-dihydroxy-2,3-dihydrobenzofuran



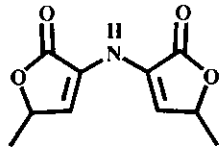
6i: gentisic acid



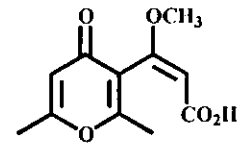
7i: 8-*O*-methylsclerotiorinamine



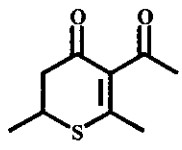
8i: ravenic acid



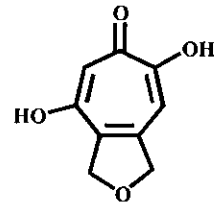
9i: penidilamine



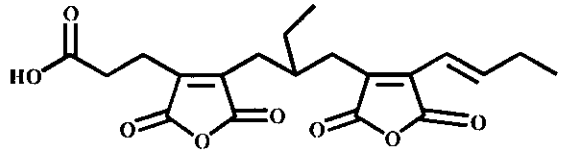
10i: citreo-γ-pyrone



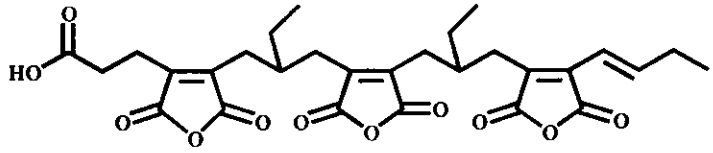
11i: citreothiopyrane A



12i: cordytropolone



13i: cordyanhydride A



14i: cordyanhydride B

1.3 The objectives

Malaria, a serious endemic disease in many parts of Africa, Latin America and Oceania, affects 5% of the world's population. The mortality is over 1 million deaths each year. Because of problems of drug resistance, there has been an urgent need for the discovery of new chemical class of antimalarial leads.

Penicillium sp. BCC 7540 and *C. militaris* BCC 2816, isolated from soils, showed significant antimalarial activity against *Plasmodium falciparum* (K1, multidrug resistant strain). The broth extract of *Penicillium* sp. BCC 7540 (IC_{50} 2.12 μg/mL) exhibited a better activity than the mycelial extract (IC_{50} 4.86 μg/mL). Broth and mycelial extracts of *C. militaris* BCC 2816 showed antimalarial activity with IC_{50}

values of 12.00 and 16.00 μg/mL, respectively, while those of the strain BCC 2819 showed less antimalarial activity. However, its broth and mycelial extracts showed significant anti-cancer (NCL-H187) activity with IC_{50} values of 4.50 and 16.00 μg/mL, respectively. Therefore, we were interested in searching bioactive metabolites from these culture extracts with the hope that additional new compounds with better antimalarial activity against *P. falciparum* will be isolated. This research involved purification and structure elucidation of the chemical constituents from *Penicillium* sp. BCC 7540, *C. militaris* BCC 2816 and BCC 2819.