CHAPTER II

RESULTS AND DISCUSSION

Chromatographic separation of *Chaetomium brasiliense, C. bostrychodes, and C. siamense* led to the isolation of nineteen compounds. Twelve compounds from *C. brasiliense*, seven compounds from *C.bostrychodes*, and eight compounds from *C. siamense*. The result of these isolations are as follows :-

2.1 Extraction and Isolation

2.1.1 *C. brasiliense*

Sequential extraction of air-dried mycelial mats of *C. brasiliense* (300 g) with hexane, EtOAc, and MeOH gave three crude extracts, crude hexane 6.8 g (2.27%), crude EtOAc 17.8 g (5.93%), and crude MeOH 20.6 g (6.87%), respectively. Chromatographic separation by FCC and PLC of all crude extracts using hexane, CH_2Cl_2, EtOAc, and MeOH as solvent systems gave twelve compounds, **1.1-1.12**. They were ergosterol **(1.1)** 104.2 mg, 24(*R*)-5α,8α-epidioxyergosta-6-22-dien-3β-ol **(1.2)** 7.4 mg, mollicellin H **(1.3)** 14 mg, mollicellin J **(1.4)** 54 mg, mollicellin K **(1.5)** 155.1 mg, mollicellin L **(1.6)** 25.2 mg, mollicellin M **(1.7)** 7.8 mg, mollicellin B **(1.8)** 67.6 mg, mollicellin C, **(1.9)** 13.2 mg, mollicellin E **(1.10)** 66.6 mg, mollicellin N **(1.11)** 16 mg and mollicellin F **(1.12)** 21.3 mg as summarized in table 2.1.

2.1.2 *C. bostrychodes*

Sequential extraction of air-dried mycelial mats of *C. bostrychodes* (119 g) by the same method as 2.1.1 gave three crude extracts, crude hexane 1.5 g (1.26%), crude EtOAc 2.1 g (1.76%), and crude MeOH 4.5 g (3.78%). Chromatographic separation by FCC of crude extracts gave seven compounds, **2.1-2.7**. They were ergosterol (**2.1**) 44.6 mg, 24(*R*)-5α,8α-epidioxyergosta-6-22-dien-3β-ol (**2.2**) 52 mg, ergosterylpalmitate (**2.3**) 8.7 mg, chaetoviridin A (**2.4**) 145.2 mg, chaetoviridin F (**2.5**) 9.5 mg, chrysophanol (**2.6**) 3.6 mg, and emodin (**2.7**) 9.5 mg as summarized in table 2.1.

2.1.3 *C. siamense*

Sequential extraction of air-dried mycelial mats of *C. siamense* (135 g) by

the same method as 2.1.2 gave three crude extracts, crude hexane 3.6 g (2.67%), crude EtOAc 4.9 g (3.63%), and crude MeOH 7.6 g (5.63%). Chromatographic separation by FCC of crude extracts gave eight compounds, **3.1-3.8**. They were ergosterol **(3.1)** 37.9 mg, 24(*R*)-5α,8α-epidioxyergosta-6-22-dien-3β-ol **(3.2)** 136.2 mg, ergosterylpalmitate **(3.3)** 116.7 mg, cochliodone D **(3.4)** 13.3 mg, chaetoviridin A **(3.5)** 983.5 mg, chaetoviridin F **(3.6)** 15.5 mg, chaetoviridin G **(3.7)** 10.9 mg and chrysophanol **(3.8)** 2 mg as summarized in table 2.1.

Table.2.1 Weight and percentage of compounds from *Chaetomium* spp.

Compound	1. *C. brasiliense* mg, (%)	2. *C. bostrychodes* mg, (%)	3. *C. siamense* mg, (%)
ergosterol **(1.1), (2.1), (3.1)**	104 (0.0034%)	44.6 (0.0387%)	37.9 (0.0274%)
24(R)-5α,8α-epidioxyergosta-6-22-dine-3α-ol **(1.2), (2.2), (3.2)**	7.4 (0.0025%)	52.0 (0.0437%)	136.2 (0.0454%)
mollicellin H **(1.3)**	14 (0.0047%)		
mollicellin J **(1.4)**	54 (0.0018%)		
mollicellin K **(1.5)***	155.1 (0.0517%)		
mollicellin L **(1.6)***	25.2 (0.0083%)		
mollicellin M **(1.7)***	7.8 (0.023%)		
mollicellin B **(1.8)**	67.6 (0.0018%)		
mollicellin C **(1.9)**	13.2 (0.0045%)		
mollicellin E **(1.10)**	66.6 (0.022%)		
mollicellin N **(1.11)***	16.2 (0.0054%)		
mollicellin F **(1.12)**	21.3 (0.007%)		
ergosterylplamitate **(2.3), (3.3)**		8.7 (0.0073%)	116.7 (0.0087%)
cochliodone D **(3.4)**			13.3 (0.0096%)
chaetoviridin A **(2.4),(3.5)**		145.2 (0.0122%)	983.5 (0.729%)
chaetoviridin F **(2.5), (3.6)**		9.5 (0.079%)	15.5 (0.0115%)
chaetoviridin G **(3.7)**			10.9 (0.0014%)
chrysophanol **(2.6), (3.8)**		3.6 (0.0302%)	2.0 (0.0014%)
emodin **(2.7)**		9.5 (0.0079%)	

* new compounds

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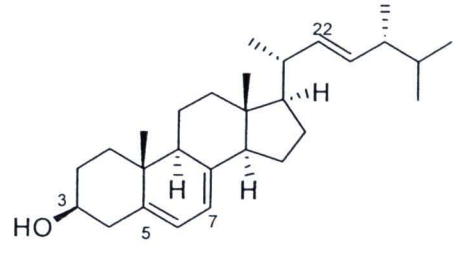
2.2 Structural Identification

2.2.1 Compounds isolated from *C. brasiliense*

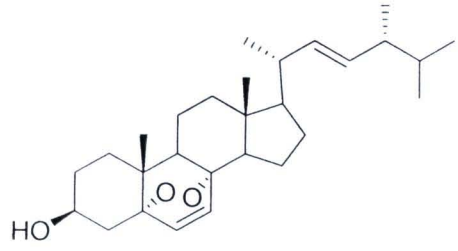
Compound **1.1** was obtained as a white solid. The IR spectrum (Figure 1 in Appendix) showed a broad absorption band at 3429 cm^{-1} indicated the O-H stretching of a hydroxyl group. A weak absorption band at 3044 and 1655 cm^{-1} due to its C-H and C=C stretching, repectively. A set of absorption bands at 2953, 2870 and 1458, 1382, 1368 cm^{-1} was characterized as saturated C-H stretching and bending, repectively. A medium absorption band at 1032 cm^{-1} was assigned to C-O stretching. The ^{1}H-NMR and ^{13}C-NMR spectra (Figures 2 and 3 in Appendix, respectively) showed the characteristic signals of a steroid unit. The ^{1}H NMR spectral data showed the resonance signals of four olefinic protons at δ 5.58 (dd, *J*=5.6, 2.4 Hz), 5.38 (dd, *J*=5.6, 2.4 Hz), 5.22 (dd, *J*=15.6, 7.0 Hz), and 5.18 (dd, *J*=15.6, 7.0 Hz). An oxymethine (CH_3) proton displayed at δ 3.66 (m) while signals between δ 2.50-1.10 were assigned to methane and methylene protons. The resonance signals of the methyl protons were displayed at δ 1.03 (d, *J*=6.4 Hz), 0.94 (s), 0.91 (d, *J*=6.8 Hz), 0.84 (d, *J*=6.4 Hz), 0.82 (d, *J*=6.4 Hz), and 0.63 (s). Comparison of the NMR spectral data[73], and mixed-TLC with the authetic sample indicated that compound **1.1** was ergosterol.



ergosterol **(1.1)**

Compound **1.2** was obtained as a white solid. The IR spectrum (Figure 7 in Appendix) showed a O-H stretching of a hydroxyl group at 3301 cm^{-1}. The absorption bands due to C-H and C=C stretching of alkenes were displayed at 3080 and 1655 cm^{-1}. A set of absorption bands at 2955, 2871 and 1458, 1377 cm^{-1} was characterized as saturated C-H stretching and bending, respectively. An absorption band at 1044 cm^{-1} was assigned to C-O stretching. Comparision of the NMR spectral data (Figures 5 and 6 in Appendix) with those of known sterol[74] and mixed-TLC with

the authetic sample indicatied that compound **1.2** was 24(*R*)-5α,8α-epidioxyergosta-6-22-diene-3β-ol.

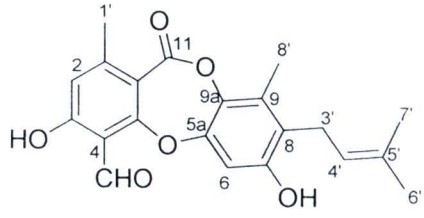


24(*R*)-5α.8α-epidioxyergosta-6-22-diene-3β-ol **(1.2)**

Compounds **1.3–1.12** are depsidones derivatives. Their structural identification is as follows :-

Compound **1.3** was isolated as a white solid. Its molecular formula $C_{21}H_{20}O_6$. The IR spectrum suggested the presence of OH groups (3397 cm^{-1}) and aromatic aldehyde (1640 cm^{-1}). The ^{13}C-NMR spectrum showed 21 signals (Table 2.2). The IR band at ν_{max} 1700 cm^{-1} and the ^{13}C- NMR signal at δ_c 162.3 (C-11) indicated the presence of a conjugated ester C=O group. The low-field singlet signal (δ 11.90) was assigned as a chelated OH involving the carbonyl of an aldehyde group (δ 10.53) at the *ortho* position. A prenyl group could be concluded from the ^{1}H-NMR signals at δ_H 1.66 (s, Me-6′), 1.74 (s, Me-7′), 3.28 (d, *J*=6.7 Hz, H-3′), and 4.90 (d, *J*=6.7 Hz, H-4′) (Table 2.2), and the HMBC correlations of Me-6′ and Me H-7′ with C-3′ and C-4′; H-3′ with C-3′ and C-4′; and H-2 with C-4, C-11a, and C-1′. Two benzene moieties were evident from the twelve ^{13}C-NMR signals between δ_C 104.6 and 164.5 indicated one ester C=O and two olefinic C-atoms in the prenyl group. A Me group at δ_H 2.46 was located at C-1 on basis of HMBC correlation of Me-1′ with δ_C 153.0 (C-1), 116.8 (C-2) and 112.9 (C-11a). The HMBC correlation of δ_H 10.5 (C-2′) with δ_C 110.2 (C-4) and 164.5 (C-3). The C-atom at δ_C 148.1 (C-5a) and 135.8 (C-9a) were oxygenated. The remaining C-atom δ_C 162.3 (C-11) could only be adjacent to C-11a). Based on the analysis of the above data and from a comparison of the physical properties with those reported in the literature[17], compound **1.3** was deduced to be a known natural product, mollicellin H. The complete assignments of

the ^{1}H and ^{13}C NMR signals of **1.3** were established from the HMBC and NOESY are shown in Table 2.2.



mollicellin H (**1.3**)

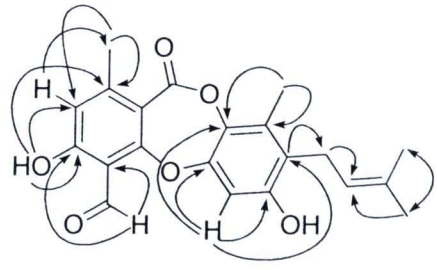


Figure 2.1 Selected HMBC correlations of mollicellin H (**1.3**).

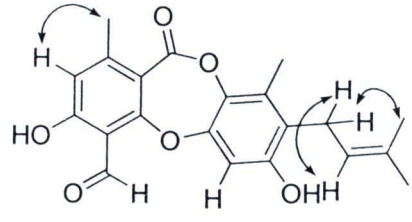


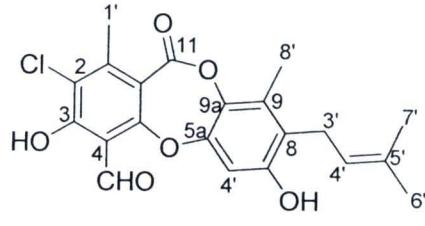
Figure 2.2 Selected NOESY correlations of mollicellin H (**1.3**).

Table 2.2 ^{1}H and ^{13}C NMR spectral data (δ values), NOESY and HMBC correlations of mollicellin H **(1.3)** in $CDCl_3$[a]

position	δ_H	δ_C	DEPT	NOESY	HMBC
1		153.0	C		
2	6.64 (s)	116.8	CH	H-1′	C-4, 11a, C-1′
3		164.5	C		C-2, 3, 4
4		110.2	C		
4a		153.5	C		
5a		148.1	C		
6	6.52 (s)	104.6	CH		C-7, 5a, 8, 9a
7		150.9	C		
8		124.3	C		
9		129.5	C		
9a		135.8	C		
11		162.3	C=O		
11a		112.9	C		
1′	2.46 (s)	21.7	CH_3	H-2	C-1, 2, 4a
2′	10.50 (s)	192.4	CH		C-3, 4
3′	3.28 (d, J=6.7)	25.3	CH_2	H-4′, 7′	C-8, 4′
4′	4.90 (d, J=6.7)	119.9	CH	H-3′, 7′	C-3′, 5′
5′		134.6	C		
6′	1.66 (s)	25.1	CH_3		C-4′, 5′, 7′
7′	1.74 (s)	17.4	CH_3	H-3′, 4′	C-4′, 5′, 6′
8′	2.26 (s)	12.4	CH_3		C-8, 9, 9a
OH-3	11.90 (s)				

[a]Figures in parentheses are coupling constants in Hz.

Compound **1.4** was obtained as a white amorphous powder. The IR spectrum (Figure 14 in Appendix) showed the presence of OH (3410 cm^{-1}), carbonyl ester (1712 cm^{-1}), and conjugated aldehyde (1637 cm^{-1}). The NMR spectra data (Figures 15-20 in Appendix) of compound **1.4** were similar to mollicellin H **(1.3)** (Table 2.3). However, the H-2 in compound **1.3** was replaced by a chlorine atom in compound **1.4** (δ_C 120.9). The Cl-C-2 moiety was determined by comparing its NMR data with those of known mollicellin J[58] as well as the HMBC correlation of Me-1′ with quaternary olefinic C-2 atom at δ 120.9 and δ 114.4 at C-11a (Table 2.4). Based on the analysis of the above data and the comparison of physical properties with those reported in the literature[17], compound **1.4** was deduced to be mollicellin J. The complete assignments of the ^1H and ^{13}C NMR signals of **1.4** were established from the HMBC and NOESY as shown in Figures 2.3 and 2.4, and Table 2.4.



mollicellin J **(1.4)**

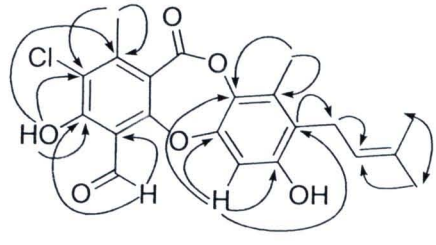


Figure 2.3 Selected HMBC correlations of mollicellin J **(1.4)**.

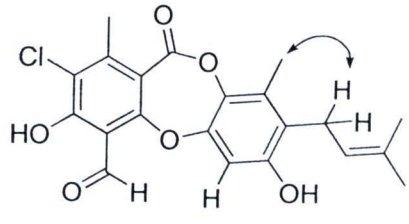


Figure 2.4 Selected NOESY correlations of mollicellin J **(1.4)**.

Table 2.3 ^{1}H and ^{13}C NMR spectral data (δ values) of mollicellin H **(1.3)** and mollicellin J **(1.4)** in $CDCl_3$[a]

position	δ_H		δ_C	
	mollicellin H **(1.3)**	mollicellin J **(1.4)**	mollicellin H **(1.3)**	mollicellin J **(1.4)**
1			**153.0**	**150.2**
2	**6.64 (s)**		**116.8**	**120.9**
3			**164.5**	**160.7**
4			110.2	110.6
4a			**153.5**	**162.1**
5a			148.1	148.6
6	6.52 (s)	6.56 (s)	105.0	105.1
7			152.6	151.6
8			125.6	125.1
9			129.5	130.1
9a			135.2	136.1
11			162.7	162.6
11a			112.9	114.4
1′	2.46 (s)	2.58 (s)	21.7	19.6
2′	10.50 (s)	10.53 (s)	192.4	192.7
3′	3.28 (d, *J*=6.7)	3.33 (d, *J*=6.7)	25.3	25.8
4′	4.90 (d, *J*=6.7)	5.04 (d, *J*=6.7)	119.9	120.3
5′			134.6	135.2
6′	1.66 (s)	1.71 (s)	25.1	25.6
7′	1.74 (s)	1.79 (s)	17.4	17.9
8′	2.26 (s)	2.31 (s)	12.4	12.9
OH-3	11.90 (s)	12.65 (s)		

[a]Figures in parentheses are coupling constants in Hz.

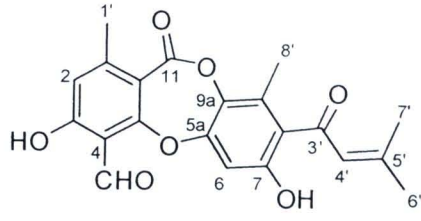
Table 2.4 ^{1}H and ^{13}C NMR spectral data (δ values), DEPT, NOESY, and HMBC correlations of mollicellin J **(1.4)** in $CDCl_3$[a]

position	δ_H	δ_C	DEPT	NOESY	HMBC
1		150.2	C		
2		120.9	C		
3		160.7	C		C-2, 3, 4
4		110.6	C		
4a		162.1	C		
5a		148.6	C		
6	6.56 (s)	105.1	CH		C-7, 5a, 8, 9a
7		151.6	C		
8		125.1	C		
9		130.1	C		
9a		136.1	C		
11		162.6	C=O		
11a		114.4	C		
1′	2.58 (s)	19.6	CH_3		C-1, 2, 11a
2′	10.53 (s)	192.7	CH		C-2, 3, 4
3′	3.33 (d, *J*=6.7)	25.8	CH_2	H-8′	C-7,8, 4′,5′
4′	5.04 (d, *J*=6.7)	120.3	CH		
5′		135.2	C		
6′	1.71 (s)	25.6	CH_3		C-4′, 5′, 7′
7′	1.79 (s)	17.9	CH_3		C-4′, 5′, 6′
8′	2.31 (s)	12.9	CH_3	H-3′	C-8, 9, 9a
OH-3	12.65 (s)				

[a]Figures in parentheses are coupling constants in Hz.

Compound **1.5** was obtained as a white solid, and its molecular formula, $C_{21}H_{18}O_7$, was deduced from the HRESITOFMS (observed *m/z* 383.1161 $[M + H]^+$), indicating 13 degrees of unsaturation. The IR spectrum showed the presence of OH (3407 cm^{-1}), carbonyl ester (1731 cm^{-1}), aromatic aldehyde (1656 cm^{-1}), α,β-unsaturated ketone (1638 cm^{-1}), and aromatic (1594 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra (Table 2.5) indicated 21 signals attributable to 13 sp^2 quaternary (including two carbonyl groups), four sp^2 methine (including an aldehyde group), and four methyl carbons. The ^{13}C NMR data together with the degrees of unsaturation revealed that **1.5** contained two aromatic rings in the molecule. The IR absorption band at 1731 cm^{-1} and the ^{13}C NMR resonance signal at δ 163.7 suggested the presence of a conjugated carbonyl ester group.[17] The ^1H NMR data showed two singlet signals of aromatic protons at δ 6.72 (H-2) and 6.69 (H-6), as well as two aromatic methyl substituents at δ 2.51 (H_3-1′) and 2.53 (H_3-8′). The low-field singlet signal (δ 12.06) was assigned as a chelated OH involving the carbonyl of an aldehyde group (δ 10.53) at the *ortho* position. The ^1H and ^{13}C NMR spectroscopic data of **1.5** were comparable to an analogue, mollicellin H (**1.3**), except for the prenyl group at C-8, which was replaced by a 1-oxo-3-methylbut-2-enyl moiety. This unit was deduced from the ^1H and ^{13}C NMR signals at δ_H 6.31 (s, H-4′), 2.23 (s, Me-6′), and 2.01 (s, Me-7′) and δ_C 196.0 (C-3′), 126.0 (C-4′), 158.3 (H-5′), 21.5 (Me-6′), and 28.1 (Me-7′), and it was located at C-8 on the basis of the HMBC correlations of H-8′ to C-8, C-9, C-9a, and C-3′, and H-6 to C-5a, C-7, C-8, C-9a, and C-3′. The HMBC spectrum of **1.5** also showed correlations of H-1′ to C-1, C-2, C-11, C-4a, and C-11a; H-2 to C-3, C-4, C-11a, C-1′, C-4a, and C-11; the OH proton at C-3 to C-2, C-3, and C-4; H-6 to C-5a, C-7, C-8, and C-9a; the aldehyde proton (H-2′) to C-3 and C-4; the OH proton at C-7 to C-6, C-7, and C-8; H-4′ to C-3′, C-6′, and C-7′; H-6′ to C-4′, C-5′, C-7′, and C-3′; and H-7′ to C-4′, C-5′, and C-6′ to confirm the connectivity in the molecule (Figure 2.5). Surprisingly, the ^3J correlation of the aldehyde proton (H-2′) to C-4a was not observed in the HMBC experiment. Thus, the chemical shift of C-4a was then identified by comparison with those reported for the analogues mollicellins I and J **(1.4)**[17] and also from the ^4J correlations of H-2 and H-1′ in the HMBC spectrum. In addition, the NOESY spectrum of **1.5** demonstrated correlations between aldehyde proton H-2′ and H-6, between H-1′ and H-2, and from H-4′ and H-8′ to H-7′

(Figure 2.6). Chemical transformation of **1.5** by cyclization with MeOH in the presence of *p*-toluenesulfonic acid yielded the product that was identical (mp, IR, NMR, and behavior on TLC) to a natural mollicellin B (**1.8**) (Figure 2.7). On the basis of the above data and Scifider Scholar database 2010, the structure of **1.5** was defined as a new depsidone and has been named mollicellin K.



mollicellin K (**1.5**)

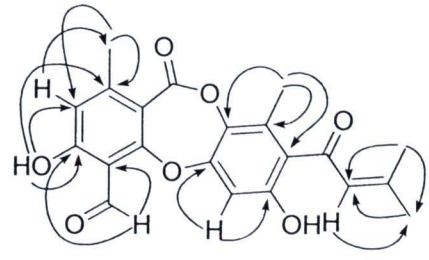


Figure 2.5 Selected HMBC correlations of mollicellin K (**1.5**).

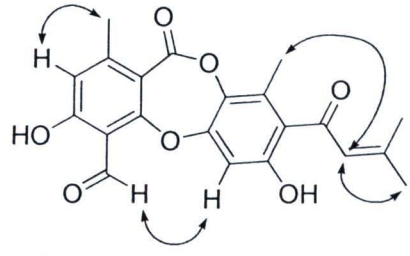
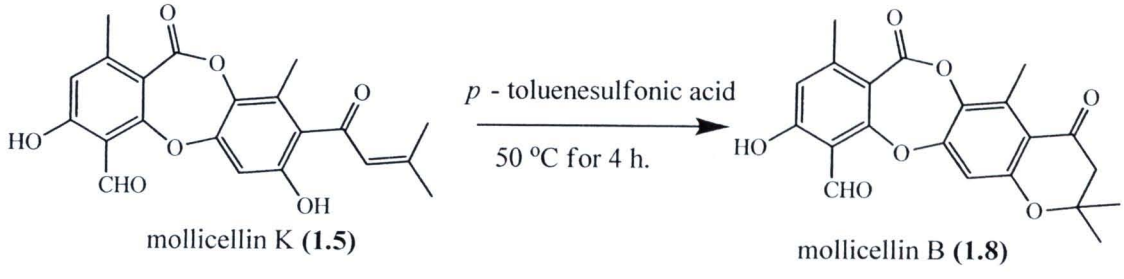


Figure 2.6 Selected NOESY correlations of mollicellin K (**1.5**).



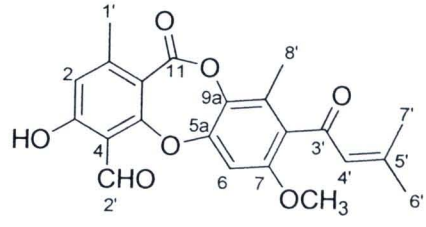
mollicellin K (**1.5**) mollicellin B (**1.8**)

Figure 2.7 Cyclization of mollicellin K (**1.5**) to mollicellin B (**1.8**).

Table 2.5 ^{1}H and ^{13}C NMR spectral data (δ values), DEPT, NOESY and HMBC correlations of mollicellin K **(1.5)** in $CDCl_3$

position	δ_H	δ_C	DEPT	NOESY	HMBC
1		153.4	C		
2	6.72 (s)	117.8	CH		C-3, 4, 11a, 1′,4a, 11
3		165.3	C		C-2, 3, 4
4		110.7	C		
4a		161.6	C		
5a		153.5	C		
6	6.69 (s)	106.8	CH	H-2′	
7		158.3	C		C-6, 7, 8
8		122.3	C		
9		131.1	C		
9a		135.8	C		
11		163.7	C		
11a		112.5	C		
1′	2.51 (s)	22.8	CH_3		C-1, 2, 11, 4a, 11a
2′	10.53 (s)	192.6	CH	H-6	
3′		196.0	C		
4′	6.31 (s)	126.0	CH		C-3′, 6′, 7′
5′		158.6	C		
6′	2.23 (s)	21.5	CH_3		C-4′, 5′, 7′, 3′
7′	2.01 (s)	28.1	CH_3		C-4′, 5′, 6′
8′	2.53 (s)	16.3	CH_3		C-8, 9, 9a, 3′
OH-3	12.06 (s)				
OH-7	10.88 (s)				

Compound **1.6** was obtained as a white solid, and its molecular formula, $C_{22}H_{20}O_7$, was deduced from HRESITOFMS (observed *m/z* 397.1286 $[M + H]^+$), indicating 13 degrees of unsaturation. The IR spectrum showed the presence of OH (3439 cm^{-1}), carbonyl ester (1729 cm^{-1}), aromatic aldehyde (1677 cm^{-1}), α, β-unsaturated ketone (1644 cm^{-1}), and aromatic (1608 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra were similar to those of **1.5**, except for the OH group at C-7, which was substituted by an OCH_3 group (Table 2.6). The complete assignments of the ^1H and ^{13}C NMR signals of **1.6** were established from the HMBC, and NOESY data (Figures 2.8 and 2.9 and Table 2.7). The NOESY spectrum of **1.6** showed correlations between an aldehyde proton H-2′ and H-6; H-6 and the OCH_3 protons at C-7 to support the structure of **1.6**. According to Scifider Scholar Database 2010 **1.6** was defined as a new depsidone and has been named mollicellin L.



mollicellin L **(1.6)**

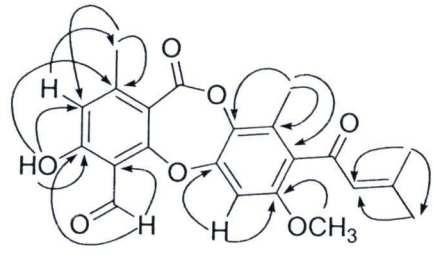


Figure 2.8 Selected HMBC correlations of mollicellin L **(1.6)**.

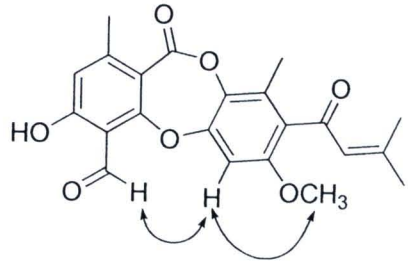


Figure 2.9 Selected NOESY correlations of mollicellin L **(1.6)**.

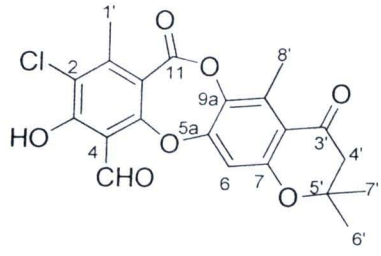
Table 2.6 ^{1}H and ^{13}C NMR spectral data (δ values) of mollicellin K **(1.5)** and mollicellin L **(1.6)** in $CDCl_3$

position	δ_H		δ_C	
	mollicellin K **(1.5)**	mollicellin L **(1.6)**	mollicellin K **(1.5)**	mollicellin L **(1.6)**
1			153.4	154.3
2	6.72 (s)	6.71 (s)	117.8	118.2
3			165.3	165.7
4			110.7	111.2
4a			161.6	162.3
5a			153.5	151.0
6	6.69 (s)	6.57 (s)	106.8	101.6
7			158.3	153.8
8			**122.3**	**129.4**
9			131.1	131.8
9a			135.8	137.1
11			163.7	165.2
11a			112.5	113.3
1′	2.51 (s)	2.52 (s)	22.8	22.8
2′	10.53 (s)	10.60 (s)	192.6	193.3
3′			196.0	194.6
4′	6.31 (s)	6.19 (s)	126.0	126.0
5′			158.6	158.1
6′	2.23 (s)	2.20 (s)	21.5	21.6
7′	2.01 (s)	1.93 (s)	28.1	28.5
8′	2.53 (s)	2.23 (s)	**16.3**	**13.5**
OH-3	12.06 (s)	12.01 (s)		
OH-7	**10.88 (s)**			
OMe-7		**3.76 (s)**		**56.8**

Table 2.7 ^{1}H and ^{13}C NMR spectral data (δ values), DEPT, NOESY, and HMBC correlations of mollicellin L **(1.6)** in $CDCl_3$

position	δ_H	δ_C	DEPT	NOESY	HMBC
1		154.3	C		
2	6.71 (s)	118.2	CH		C-3, 4, 11a, 1′
3		165.7	C		C-2, 3, 4
4		111.2	C		
4a		162.3	C		
5a		151.0	C		
6	6.57 (s)	101.6	CH	H-2′	C-7,5a, 9a,9
7		153.8	C		
8		129.4	C		
9		131.8	C		
9a		137.1	C		
11		165.2	C		
11a		113.3	C		
1′	2.52 (s)	22.8	CH_3		C-1, 2, 11a
2′	10.60 (s)	193.3	CH	H-6	C-2,3,4
3′		194.6	C		
4′	6.19 (s)	126.0	CH		C-3′, 6′, 7′
5′		158.1	C		
6′	2.20 (s)	21.6	CH_3		C-4′, 5′, 7′
7′	1.93 (s)	28.5	CH_3		C-4′, 5′, 6′
8′	2.23 (s)	13.5	CH_3		C-8, 9, 9a, 5a
OH-3	12.01 (s)				
OMe-7	3.76 (s)		CH_3		C-7

Compound **1.7** was obtained as a white solid, $C_{21}H_{17}ClO_7$, as deduced from HRESITOFMS (observed *m/z* 439.0561[M + Na]$^+$ and its ^{37}Cl isotope *m/z* 441.0601 [M + 2 + Na]$^+$), implying 13 degrees of unsaturation. The IR spectrum of **1.7** showed characteristics of OH (3454 cm^{-1}), ester carbonyl (1736 cm^{-1}), aromatic aldehyde (1688 cm^{-1}), conjugated ketone (1652 cm^{-1}) and aromatic (1600 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra revealed 21 signals attributable to 13 sp^2 quaternary (including two carbonyl groups), one sp^3 quaternary, two sp^2 methine (including an aldehyde group), one sp^3 methylene, and four methyl carbons. From these data, **1.7** contained two aromatic rings and a conjugated carbonyl ester as described in **1.5**. The ^{1}H NMR spectrum of **1.7** (Table 2.8) showed only one aromatic singlet signal at δ 6.66 (H-6) and two aromatic methyl substituents at δ 2.60 (H-1′) and 2.67 (H-8′), which were different from **1.5**. The 1-oxo-3-methylbut-2-enyl moiety at C-8 in **1.5** was replaced by a dihydropyrone ring fused to an aromatic ring, as shown by the signals of two geminal methyl groups both at δ 1.41 (H-6′ and H-7′) and one methylene group at δ 2.68 (s, H-4′). The low-field singlet signal at δ 12.68 was assigned as an OH chelated to an aldehyde carbonyl group (δ 10.54) at the *ortho* position as in **1.5**. The structure of **1.7** was constructed by a combination of 2D NMR analyses. The HMBC spectrum demonstrated correlations of H-1′ to C-1, C-2, C-11, C-4a, and C-11a; the OH proton at C-3 to C-2, C-3, and C-4; H-6 to C-5a, C-7, C-8, and C-9a; the aldehyde proton (H-2′) to C-3 and C-4; H-4′ to C-8, C-3′, C-5′,C-6′, and C-7′; H-6′ to C-4′, C-5′, and C-7′; H-7′ to C-4′, C-5′, and C-6′; and H-8′ to C-7, C-5a, C-8, C-9, C-3′, and C-9a (Figure 2.10). In addition, the Cl-C-2 was determined by comparing its ^{13}C NMR spectrum with those reported for mollicellins J[17], K (**1.5**), and B (**1.8**), as well as the HMBC correlation of H-1′ to C-2 (δ_C 121.1). The NOESY spectrum of **1.7** also supported the structure *via* the correlations between H-2′ and H-6 and between H-4′ and H-6′ and H-7′ (Figure 2.11). On the basis of the above data and Scifider Scholar database 2010, the structure of **1.7** was defined as a new depsidone, and it was named mollicellin M.



mollicellin M **(1.7)**

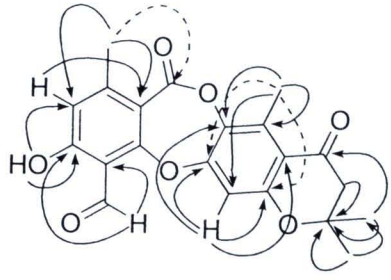


Figure 2.10 Selected HMBC correlations of mollicellin M **(1.7)**.

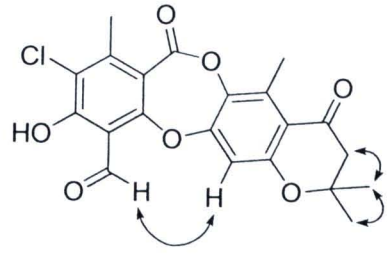
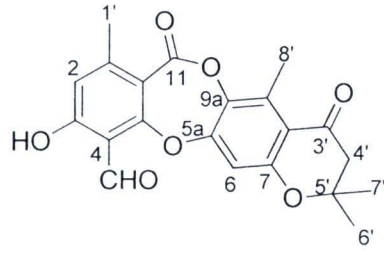


Figure 2.11 Selected NOESY correlations of mollicellin M **(1.7)**.

Table 2.8 ^{1}H and ^{13}C NMR spectral data (δ values), DEPT, NOESY, and HMBC correlations of mollicellin M **(1.7)** in $CDCl_3$

position	δ_H	δ_C	DEPT	NOESY	HMBC
1		150.3	C		
2		121.1			
3		161.0	C		C-2, 3, 4
4		110.6	C		
4a		161.2	C		
5a		154.7	C		
6	6.66 (s)	107.5	CH	H-2′	C-5a, 7, 8, 9a
7		158.6	C		
8		117.2	C		
9		134.4	C		
9a		136.5	C		
11		161.3	C=O		
11a		114.0	C		
1′	2.60 (s)	19.7	CH_3		C-1, 2, 11, 4a, 11a
2′	10.54 (s)	192.5	CH	H-6	C-3, 4
3′		192.5	C=O		
4′	2.68 (s)	50.1	CH_2	H-6′	C-3′,5′,6′, 7′, 8
5′		79.5	C		
6′	1.41 (s)	26.3	CH_3	H-4′	C-4′, 5′, 7′
7′	1.41 (s)	26.3	CH_3		C-4′, 5′,6′
8′	2.67 (s)	14.2	CH_3		C-7, 5a, 8, 9, 3′, 9a
OH-3	12.68 (s)				C-2, 3, 4

Compound **1.8** was obtained as a white solid, $C_{21}H_{18}O_7$, as deduced from HRESITOFMS (observed m/z 405.0953 $[M+Na]^+$), indicating 13 degrees of unsaturation. The IR absorption band (Figure in Appendix) at 1739 cm^{-1} and ^{13}C NMR resonance signal at δ 161.5 suggested the presence of a conjugated carbonyl ester group hydroxyl group (3461 cm^{-1}) aromatic aldehyde (1686 cm^{-1}), conjugated ketone (1651 cm^{-1}) and aromatic (1602 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra (Figures 48-49 in Appendix) revealed 22 signals attributable to 13 sp^2 quaternary (including two carbonyl groups), one sp^3 quaternary, three sp^2 methine (including an aldehyde group), one sp^3 methylene, and four methyl carbons. The ^1H and ^{13}C NMR spectra of **1.8** were similar to those of **1.7** (Table 2.9), except for the chlorine atom at C-2 which was substituted by a proton. The ^1H NMR spectrum (Figure 50 in Appendix) of **1.8** showed two aromatic singlet signal at δ 6.71 (H-2) and 6.66 (H-6) and two aromatic methyl substituents at δ 2.52 (H-1′) and 2.66 (H-8′). The dihydropyrone ring fused to an aromatic ring at C-8, as shown by the signals of two geminal methyl group both at δ 1.41 (H-6′ and H-7′) and one methylene group at 2.67 (H-4′). The low - field singlet signal at δ 12.03 was assigned as an OH chelated to an aldehyde carbonyl group δ 10.53 at the *ortho* position (Table 2.10). The structure of **1.8** was constructed by a combination 2D NMR (Figures 50-52 in Appendix) and analyses. The HMBC spectrum demonstrated correlation of H-1′ to C-1, C-2, C-11, C-4a, and C-11a; H-6 to C-5a, C-7, C-8, and C-9a; the aldehyde proton (H-2′) to C-2, C-3, and C-4; H-4′ to C-3′, C-5′, C-6′, and C-7′; H-6′ to C-4′, C-5′, and C-7′; H-7′to C-4′, C-5′, and C-6′; H-8′ to C-7, C-5a, C-8, C-9, C-3′, and C-9a; and H-2 to C-1, C-2, C-3, and C-4 (Figure 2.12). The NOESY spectrum of **1.8** also supported the structure *via* the correlations between H-2 and H-1′ and between H-6 and H-2′ (Figure 2.13). Therefore, **1.8** was definded as a known mollicellin B. ^1H, ^{13}C NMR, DEPT, and 2D NMR (COSY, HMBC, and NOESY) spectral data of **1.8** are shown in Table 2.9.



mollicellin B **(1.8)**

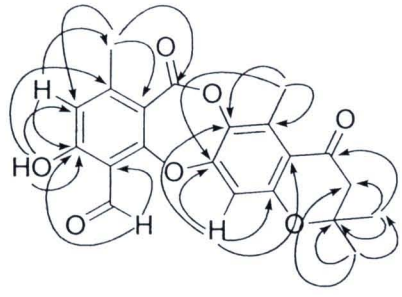


Figure 2.12 Selected HMBC correlations of mollicellin B **(1.8).**

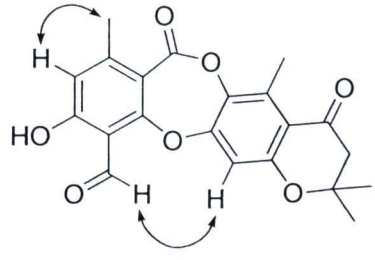


Figure 2.13 Selected NOESY correlations of mollicellin B **(1.8)**.

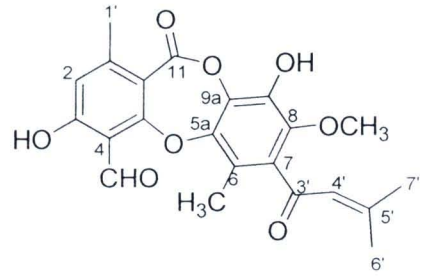
Table 2.9 ^{1}H and ^{13}C NMR spectral data (δ values) of mollicellin M **(1.7)** and mollicellin B **(1.8)** in $CDCl_3$

Position	δ_H		δ_C	
	mollicellin M **(1.7)**	mollicellin B **(1.8)**	mollicellin M **(1.7)**	mollicellin B **(1.8)**
1			150.3	153.6
2		**6.71 (s)**	**121.1**	**117.9**
3			**161.0**	**165.2**
4			110.6	110.7
4a			161.2	161.5
5a			154.7	154.7
6	6.66 (s)	6.66 (s)	107.5	107.5
7			158.6	158.6
8			117.2	117.0
9			134.4	136.7
9a			136.5	163.5
11			161.3	161.3
11a			114.0	112.5
1′	**2.60 (s)**	**2.52 (s)**	**19.7**	**22.2**
2′	10.54 (s)	10.53 (s)	192.5	192.6
3′			192.5	192.5
4′	2.68 (s)	2.67 (s)	50.1	50.1
5′			79.5	79.3
6′	1.41 (s)	1.41 (s)	26.3	26.3
7′	1.41 (s)	1.41 (s)	26.3	26.3
8′	2.67 (s)	2.66 (s)	14.2	14.2
OH-3	12.68 (s)	12.03 (s)		

Table 2.10 ^{1}H and ^{13}C NMR spectral data (δ values), DEPT, NOESY, and HMBC correlations of mollicellin B **(1.8)** in $CDCl_3$

position	δ_H	δ_C	DEPT	NOESY	HMBC
1		153.6	C		
2	6.71 (s)	117.9	CH	H-1′	C-1, 2, 3, 4
3		165.2	C		
4		110.7	C		
4a		161.5	C		
5a		154.7	C		
6	6.66 (s)	107.5	CH	H-2′	C-5a, 7, 8, 9a
7		158.6	C		
8		117.0	C		
9		134.3	C		
9a		136.7	C		
11		163.5	C=O		
11a		112.5	C		
1′	2.52 (s)	22.2	CH_3	H-2	C-1, 2, 11, 4a,11a
2′	10.53 (s)	192.6	CH	H-6	C-2, 3, 4
3′		192.5	C=O		
4′	2.67 (s)	50.1	CH_2		C-3′, 5′, 6′, 7′
5′		79.3	C		
6′	1.41 (s)	14.2	CH_3		C-4′, 5′, 7′
7′	1.41 (s)	14.2	CH_3		C-4′, 5′, 6′
8′	2.66 (s)	26.3	CH_3		C-7, 5a, 8, 9, 3′, 9a
OH-3	12.03 (s)				

Compound **1.9** was obtained as a white solid, and its molecular formula, $C_{22}H_{20}O_8$, was deduced from HRESITOFMS (observed *m/z* 435.0648 $[M + Na]^+$), implying 13 degree of unsaturation. The IR spectrum (Figure 53 in Appendix) showed the presence of presence of hydroxyl group (3389 cm^{-1}), carbonyl ester (1734 cm^{-1}), α, β-unsaturated ketone (1645 cm^{-1}), and aromatic (1559 cm^{-1}) group. The ^{13}C NMR and DEPT (Figures 56 and 57 in Appendix) spectra indicated 22 signals attributable to 13 sp^2 quaternary (including two carbonyl groups), four sp^2 methine (including an aldehyde group), and five methyl carbons. From these data, **1.9** contained two aromatic rings and a conjugated carbonyl ester. The ^1H NMR (Figure 55 in Appendix) data showed one singlet signal of aromatic proton at δ 6.71 (H-2), as well as two aromatic methyl substituents at δ 2.51 (H-1′) and 2.17 (H-8′). The NMR spectra showed signals δ_H 3.73 methyl group and δ_C 63.1 suggested the presence of a methoxyl group. The low–field singlet signal δ 12.17 (OH-3) was assigned as a chelated OH involving the carbonyl of aldehyde group δ 10.83 (H-2′) at the *ortho* position. The HMBC spectrum (Figure in 2.14) exhibited correlations of H-2 to C-3, C-4, C-11a, and C-12; the OH group at C-3 to C-2, C-3, and C-4; the OH group at C-9 to C-8, C-9, and C-9a; H-1′ to C-1, C-2, and C-11a; H-2′ to C-3 and C-4; H-6′ to C-4′, C-5,′ and C-7′; H-7′ to C-4′,C-5′, and C-6′; and H-8′ to C-5a, C-6, and C-7 (Figure 2.14). The NOESY spectrum (Figure 60 in 2.15) of **1.9** showed correlation between H-2 and H-1′ and between H-4′ to H-6′and H-7′ (Figure 2.15). Therefore, **1.9** was definded as a known mollicellin C. ^1H, ^{13}C NMR, DEPT, and 2D NMR (COSY, HMBC, and NOESY) spectral data of **1.9** are shown in Table 2.11.



mollicellin C **(1.9)**

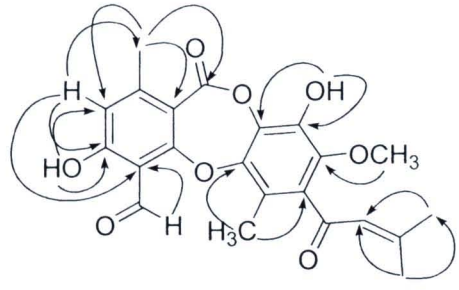


Figure 2.14 Selected HMBC correlations of mollicellin C **(1.9)**.

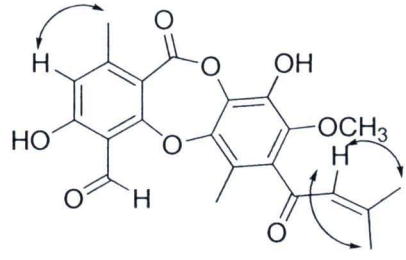
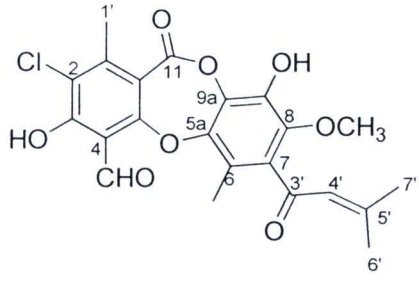


Figure 2.15 Selected NOESY correlations of mollicellin C **(1.9).**

Table 2.11 ^{1}H and ^{13}C NMR spectral data (δ values), DEPT, NOESY, and HMBC correlations of mollicellin C **(1.9)** in $CDCl_3$

Position	δ_H	δ_C	DEPT	NOESY	HMBC
1		153.1	C		
2	6.71 (s)	117.7	CH	H-1′	C-3, 4, 11a, 12
3		165.2	C		C-2, 3, 4
4		110.9	C		
4a		161.4	C		
5a		140.2	C		
6		134.0	C		
7		117.5	C		
8		141.2	C		
9		139.2	C		C-8, 9, 9a
9a		138.7	C		
11		164.5	C=O		
11a		112.7	C		
1′	2.51 (s)	22.1	CH_3		C-1, 2, 11a
2′	10.83 (s)	193.4	CH	H-2	C-3, 4
3′		195.4	CH_3		
4′	6.25 (s)	125.2	C=O	H-6′, 7′	
5′		159.1	C		
6′	2.23 (s)	21.1	C	H-4′	C-4′, 5′, 7′
7′	1.96 (s)	28.0	CH_3	H-4′	C-4′, 5′, 6′
8′	2.17 (s)	12.1	CH_3		C-5a, 6, 7
OH-3	12.17 (s)				C-2,3,4
OMe-8	3.73 (s)	63.1			
OH-9	5.84 (s)				C-8,9,9a

Compound **1.10** was obtained as a white solid, and its molecular formula, $C_{22}H_{19}ClO_8$ was deduced from HRESITOFMS (observed *m/z* 469.0427 $[M + Na]^+$), implying 13 degree of unsaturation. The IR spectrum (Figure 62 in Appendix) showed the presence of hydroxyl group (3382 cm^{-1}), carbonyl ester (1741 cm^{-1}), α, β-unsaturated ketone (1651 cm^{-1}), and aromatic(1566 cm^{-1}) group. The ^1H and ^{13}C NMR spectra of **1.10** were similar to those of mollicellin C (**1.9**), except for the difference of δ_C 121.3 at position 2 which were bonded to chlorine atom (Table 2.12). Therefore, **1.10** was defind as a know mollicellin E. The complete assignments of the ^1H and ^{13}C NMR signals of **1.10** were established from the DEPT, COSY, HMBC, and NOESY are shown in Figures 2.16 and 2.17 and Table 2.13.



mollicellin E (**1.10**)

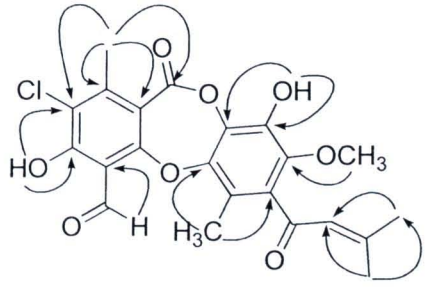


Figure 2.16 Selected HMBC correlations of mollicellin E (**1.10**).

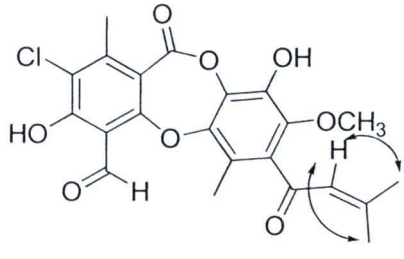


Figure 2.17 Selected NOESY correlations of mollicellin E (**1.10**).

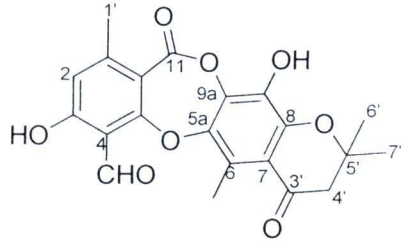
Table 2.12 ^{1}H and ^{13}C NMR spectral data (δ values) of mollicellin C **(1.9)** and mollicellin E **(1.10)** in $CDCl_3$

position	δ_H		δ_C	
	mollicellin C **(1.9)**	mollicellin E **(1.10)**	mollicellin C **(1.9)**	mollicellin E **(1.10)**
1			**153.1**	**149.8**
2	**6.71 (s)**		**117.7**	**121.3**
3			165.2	161.2
4			110.9	110.8
4a			161.4	160.9
5a			140.2	140.0
6			134.0	134.2
7			117.5	117.4
8			141.2	141.2
9			139.2	139.3
9a			138.7	138.8
11			164.5	162.2
11a			112.7	114.2
1′	2.51 (s)	2.57 (s)	**22.1**	**19.5**
2′	10.83 (s)	10.81 (s)	193.4	193.3
3′			195.4	195.2
4′	6.25 (s)	6.24 (s)	125.2	125.1
5′			159.1	159.3
6′	2.23 (s)	2.16 (s)	21.1	21.2
7′	1.96 (s)	1.96 (s)	28.0	28.0
8′	2.17 (s)	2.16 (s)	12.1	12.1
OH-3	12.17 (s)	12.75 (s)		
OMe-8	3.73 (s)	3.72 (s)	63.1	63.1
OH-9	5.84 (s)			

Table 2.13 ^{1}H and ^{13}C NMR spectral data (δ values), DEPT, NOESY, and HMBC correlations of mollicellin E **(1.10)** in $CDCl_3$

position	δ_H	δ_C	DEPT	NOESY	HMBC
1		149.8	C		
2		121.3	CH	H-1′	
3		161.2	C		C-2, 3, 4
4		110.8	C		
4a		160.9	C		
5a		140.0	C		
6		134.2	C		
7		117.4	C		
8		141.2	C		
9		139.3	C		
9a		138.8	C		
11		162.2	C=O		
11a		114.2	C		
1′	2.57 (s)	19.5	CH_3		C-1, 2, 11a
2′	10.81 (s)	193.3	CH	H-2	C-3, 4
3′		195.2	C=O		
4′	6.24 (s)	125.1	C=O	H-6′, 7′	
5′		159.3	C		
6′	2.23 (s)	21.2	CH_3	H-4′	C-4′, 5′, 7′
7′	1.96 (s)	28.0	CH_3	H-4′	C-4′, 5′, 6′
8′	2.16 (s)	12.1	CH_3		C-5a, 6, 7
OH-3	12.75 (s)				
OMe-8	3.72 (s)	63.1	CH_3		

Compound 1.11 was obtained as a white solid, and its molecular formula, $C_{21}H_{18}O_8$, was deduced from HRESITOFMS (observed *m/z* 421.0897 $[M + Na]^+$), implying 13 degrees of unsaturation. The IR spectrum of **1.11** indicated OH (3355 cm^{-1}), ester carbonyl (1738 cm^{-1}), conjugated aldehyde (1688 cm^{-1}), conjugated ketone (1644 cm^{-1}), and aromatic (1574 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra revealed 21 signals attributable to 13 sp^2 quaternary (including two carbonyl groups), one sp^3 quaternary, two sp^2 methine (including an aldehyde group), one sp^3 methylene, and four methyl carbons. The ^1H and ^{13}C NMR spectra of **1.11** (Tables 2.14) showed splitting patterns similar to those of **1.11** with four singlet methyl (δ_H 2.53, 2.59, 1.47, and 1.47), one methylene (δ_H 2.74), and two methine groups (δ_H 6.72 and 10.85). However, groups substituted on the aromatic ring and chromone units of **1.11** were located at different positions than in **1.11**. The HMBC spectrum exhibited correlations of H-1′ to C-1, C-2, C-4a, C-11, and C-11a; H-2 to C-1, C-3, C-4, C-4a, and C-11a; the OH group at C-3 to C-2, C-3, and C-4; the OH group at C-9 to C-8, C-5a, and C-9a; aldehyde proton H-2′ to C-3 and C-4; H-4′ to C-7, C-3′, C-5′, C-6′, and C-7′; H-6′ to C-4′, C-5′, and C-7′; and H-7′ to C-4′, C-5′, and C-6′, confirming the structure of **1.11** (Figure 2.18). The NOESY spectrum of **1.11** showed correlations between H-2 and H-1′ and between H-4′ and H-6′ and H-7′. Unfortunately, the correlation between aldehyde proton H-2′ and C_3-8′ was not observed and the NOE-difference data showed the same correlations of protons as in the NOESY experiment (Figure 2.19). This suggested that the distance between the aldehyde proton (H-2′) and C_3-8′ is more than 4.2 Å. On the basis of the above evidence and Scifider Scholar database 2010, the structure of compound **1.11** was determined to be a new depsidone, and it was named mollicellin N.



mollicellin N **(1.11)**

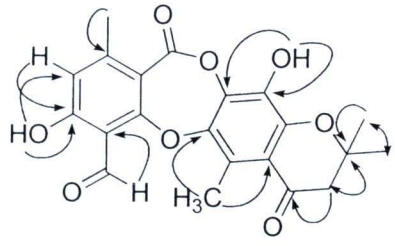


Figure 2.18 Selected HMBC correlations of mollicellin N **(1.11).**

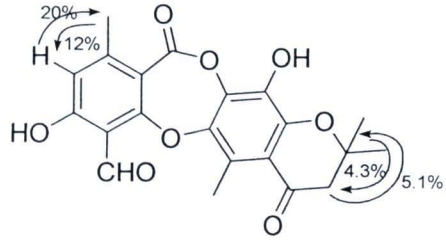
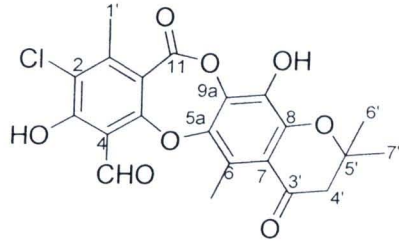


Figure 2.19 Selected NOESY correlations of mollicellin N **(1.11).**

Table 2.14 ^{1}H and ^{13}C NMR spectral data (δ values), NOESY, and HMBC correlations of mollicellin N **(1.11)** in $CDCl_3$

Position	δ_H	δ_C	DEPT	NOESY	HMBC
1		153.2	C		
2	6.72 (s)	117.9	CH	H-1′	C-3
3		165.3	C		C-2,3, 4
4		111.0	C		
4a		163.7	C		
5a		137.5	C		
6		122.6	C		
7		115.8	C		
8		146.2	C		
9		142.0	C		C-9
9a		135.4	C		
11		161.4	C=O		
11a		112.6	C		
1′	2.53 (s)	22.1	CH_3	H-2	C-1, 2, 11a
2′	10.85 (s)	195.4	CH		C-2, 3, 4
3′		192.0	C=O		
4′	2.74 (s)	50.4	CH_2	H-6′, 7′	C-7, 3′, 5′, 6′, 7′
5′		80.9	C		
6′	1.47 (s)	26.4	CH_3	H-4′	C-4′, 5′, 7′
7′	1.47 (s)	26.4	CH_3	H-4′	C-4′, 5′, 6′
8′	2.59 (s)	13.1	CH_3		C-7, 3′, 5′, 6, 7′
OH-3	12.19 (s)				
OH-9	5.67 (s)				C-8, 5a, 9a

Compound **1.12** was obtained as a white solid, and its molecular formula, $C_{21}H_{17}ClO_8$, was deduced from HRESITOFMS (observed *m/z* 455.0318 $[M + Na]^+$), implying 13 degrees of unsaturation. The IR spectrum (Figure 78 in Appendix) of **1.12** indicated OH (3400 cm^{-1}), ester carbonyl (1741 cm^{-1}), conjugated aldehyde (1688 cm^{-1}), conjugated ketone (1644 cm^{-1}), and aromatic (1565 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra (Figure 80 and 81 in Appendix) revealed 21 signals attributable to 13 sp^2 quaternary (including two carbonyl groups), one sp^3 quaternary, two sp^2 methine (including an aldehyde group), one sp^3 methylene, and four methyl carbons. The ^1H and ^{13}C NMR spectra of **1.12** were similar to those **1.11,** except for the proton at C-2, which was substituted by chlorine atom (δ_C 121.1) (Table 2.15). The complete assignments of the ^1H and ^{13}C NMR signals of **1.12** were established from the DEPT, COSY, HSQC, HMBC, and NOESY data (Tables 2.16). The HMBC spectrum (Figure 83 in Appendex) exhibited correlations of the OH group at C-3 to C-2, C-3, and C-4; the OH group at C-9 to C-8, C-9, and C-9a; aldehyde proton H-2′ to C-2 and C-4a; H-4′ to C-6, C-3′, and C-5′; H-6′ to C-4′, C-5′, and C-7′; and H-7′ to C-4′, C-5′, and C-6′. Therefore, **1.12** was defind as a known mollicellin F. The complete assignments of the ^1H and ^{13}C NMR signals of **1.12** were established from the HMBC, and NOESY are shown in Figures 2.20 and 2.21 and Table 2.16.



mollicellin F **(1.12)**

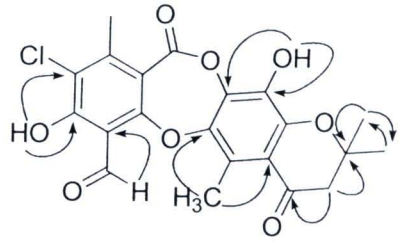


Figure 2.20 Selected HMBC correlations of mollicellin F **(1.12)**.

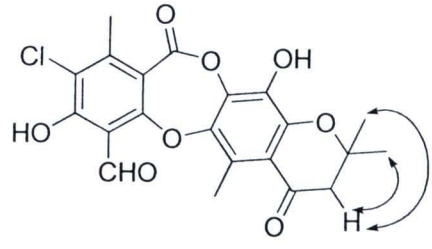


Figure 2.21 Selected NOESY correlations of mollicellin F **(1.12)**.

Table 2.15 ^{1}H and ^{13}C NMR spectral data (δ values) of mollicellin N **(1.11)** and mollicellin F **(1.12)** in $CDCl_3$

position	δ_H		δ_C	
	mollicellin N **(1.11)**	mollicellin F **(1.12)**	mollicellin N **(1.11)**	mollicellin F **(1.12)**
1			153.2	149.8
2	**6.72 (s)**		**117.9**	**121.1**
3			165.3	161.0
4			111.0	110.9
4a			163.7	161.3
5a			137.5	137.3
6			122.6	122.6
7			115.8	115.9
8			146.2	146.2
9			142.0	142.0
9a			135.4	135.4
11			161.4	161.4
11a			112.6	114.1
1′	2.53 (s)	2.53 (s)	22.1	19.5
2′	10.85 (s)	10.78 (s)	195.4	195.1
3′			191.2	191.9
4′	2.74 (s)	2.68 (s)	50.4	50.4
5′			80.9	81.1
6′	1.47 (s)	1.40 (s)	26.4	26.4
7′	1.47 (s)	1.40 (s)	26.4	26.4
8′	2.59 (s)	2.52 (s)	13.1	13.0
OH-3	12.19 (s)	12.77 (s)		
OH-9	5.67 (s)	5.64 (s)		

Table 2.16 ^{1}H and ^{13}C NMR spectral data (δ values), NOESY and HMBC correlations of mollicellin F **(1.12)** in $CDCl_3$

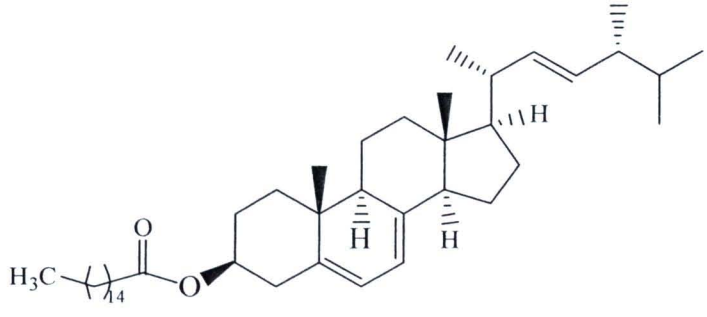
position	δ_H	δ_C	DEPT	NOESY	HMBC
1		149.8	C		
2		121.1			
3		161.0	C		C-2, 3, 4
4		110.9	C		
4a		161.3	C		
5a		137.3	C		
6		122.6	C		
7		115.9	C		
8		146.2	C		
9		142.0	C		C-8, 9, 9a
9a		135.4	C		
11		161.4	C=O		
11a		114.1	C		
1′	2.53 (s)	19.5	CH_3		
2′	10.78 (s)	195.1	CH		C-2, 4a
3′		191.9	C=O		
4′	2.68 (s)	50.4	CH_2	H-6′, 7′	C-3′, 5′, 6
5′		81.1	C		
6′	1.40 (s)	26.4	CH_3	H-4′	C-4′, 5′, 7′
7′	1.40 (s)	26.4	CH_3	H-4′	C-4′, 5′, 6′
8′	2.52 (s)	13.0	CH_3		C-5a, 6, 7, 9, 9a
OH-3	12.77 (s)				C-2, 3, 4
OH-9	5.64 (s)				C-8, 9, 9a

2.2.2 Compounds isolated from *C. bostrychodes*

Compound 2.1 was obatained as a white solid. Comparision of the NMR spectral data[73], and mixed-TLC with compound **1.1** indicated that compound **2.1** was ergosterol.

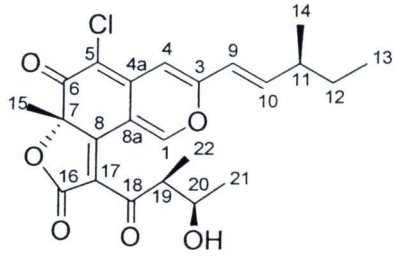
Compound 2.2 was obatained as a white solid. Comparision of the NMR spectral data[74], and mixed-TLC with compound **1.2** indicated that compound **2.2** was 24(*R*)-5α,8α-epidioxyergosta-6-22-diene-3β-ol.

Compound 2.3 was obatained as amorphous solid. The IR spectrum (Figure 85 in Appendix) showed a very weak absorption band at 3040 cm^{-1} indicated the C-H stretching of alkene. A set of absorption bands at 2960, 2910, 2860, and 1465, 1360, 1375 cm^{-1} were characterized as saturated C-H stretching and bending, respectively. A strong adsorption band at 1745 cm^{-1} was assigned to C=O of ester, while the C-O stretching appeared at 1180 cm^{-1}. The ^{1}H-NMR spectrum of **2.3** (Figure 86 in Appendix) showed main principle resonance signals similar to those of ergosterol **(1.1)**, except a broad singlet at δ 1.23 and a triplet at δ 1.00 which were corresponded to the long chain hydrocarbon moiety. The ^{13}C-NMR spectrum (Figure 87 in Appendix) showed resonance signals similar to those of **1.1**, except the signals at δ 173.3 and 72.5 which were consistent with the ester group. The methylene groups of the long chain hydrocarbon appeared at δ 29.7. Comparision of the NMR spectral data[9], and mixed-TLC with the authetic sample indicated that compound **2.3** was deduced to be ergosterylpalmitate.



ergosterylpalmitate **(2.3)**

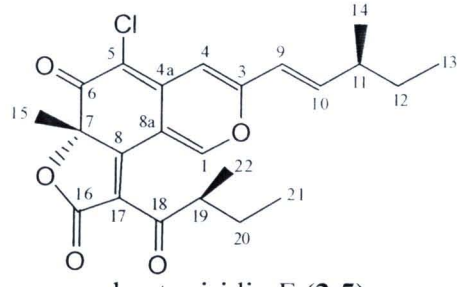
Compound 2.4 was obtained as orange crystals. Its IR spectrum (Figure 88 in Appendix) showed a broad absorption band at 3436 cm^{-1} corresponding to the O-H stretch of a hydroxyl group. The weak absorption band at 3108 cm^{-1} was assigned to C-H stretching of the alkene. The strong absorption band at 1774 cm^{-1} was characterized as an α, β-unsaturated-γ-lactone while conjugated ketones appeared at 1678 and 1619 cm^{-1}. The ^{1}H and ^{13}C NMR spectra (Figures 89 and 90 in Appendix) indicated the presence of three carbonyl, six sp^2 quaternary, four sp^2 methine, one sp^3 quaternary, three sp^3 methine, one sp^3 methylene, and five methyl carbons. The ^{13}C NMR spectrum showed conjugated ketone δ 183.4 and 201.2 and lactone carbonyl δ 167.9 carbons. The ^{1}H NMR spectrum revealed a low-field resonance of an olefinic proton at δ 8.79, three olefinic protons at δ 6.62, 6.58, and 6.10 and five signals of methyl groups at δ 1.70, 1.18, 1.10, and 0.92. The structure of **2.4** was then elucidated to be an azaphilone bearing a five–membered ring lactone. Analysis of the above data and comparison of the NMR spectral data,[9] as well as mixed-TLC with the authetic sample indicated that compound **2.4** was deduced to be the known natural product, chaetoviridin A.



chaetoviridin A **(2.4)**

Compound 2.5 was obtained as a red solid. The IR spectrum of **2.5** (Figure 96 in Appendix) exhibited the vinylic proton absorption band at 3107 cm^{-1}. The absorption band at 1769 cm^{-1} was assigned to an α,β-unsaturated-γ-lactone whereas the characteristic of an α,β-conjugated ketone presented at 1682 and 1620 cm^{-1}. The ^{1}H and ^{13}C NMR together with DEPT spectral data (Figures 97-99 in Appendix) indicated twenty three carbons attributable to nine sp^2 quaternary, four sp^2 methine, one sp^3 quaternary, two sp^3 methine, two sp^3 methylene, and five methyl carbons. On comparison of the ^{1}H and ^{13}C NMR spectral data (Table 2.17) of **2.5** with chaetoviridin A **(2.4)**, the data showed that they were similar, except for an

absence of an oxymethine proton at C-20 which was replaced by a methylene group δ_H 1.51, 1.25; δ_C 26.5 (Table 2.17). On the basis of the above data, the structure of **2.5** comparison of the NMR spectral data, and mixed-TLC with the authetic sample indicated that compound **2.5** was chaetoviridin F.



chaetoviridin F **(2.5)**

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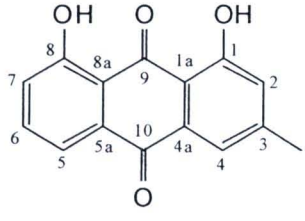
Table 2.17 ^{1}H and ^{13}C NMR spectral data (δ values) of chaetoviridin A **(2.4)** and chaetoviridin F **(2.5)** in $CDCl_3$[a]

Position	δ_H		δ_C	
	chaetoviridin A **(2.4)**	chaetoviridin F **(2.5)**	chaetoviridin A **(2.4)**	chaetoviridin F **(2.5)**
1	8.79 (s)	8.74 (s)	151.5 (d)[b]	151.8 (d)[b]
3			157.1 (s)	157.5 (s)
4	6.58 (s)	6.50 (s)	105.4 (d)	105.5 (d)
4a			139.7 (s)	140.0 (s)
5			108.9 (s)	109.0 (s)
6			183.4 (s)	183.9 (s)
7			87.6 (s)	87.8 (s)
8			162.7 (s)	164.0 (s)
8a			110.4 (s)	110.5 (s)
9	6.10 (d, 15.7)	6.04 (d, 15.6)	119.7 (d)	119.9 (d)
10	6.62 (dd, 15.7, 8.0)	6.57 (dd, 15.6, 7.8)	148.1 (d)	148.3 (d)
11	2.30 (s)	2.23 (sept, 7.0)	38.9 (d)	39.1 (d)
12	1.42 (quint, 7.4)	1.39 (quint, 7.2)	29.1 (t)	29.3 (t)
13	0.92 (t, 7.4)	0.85 (t, 7.5)	11.7 (q)	11.9 (q)
14	1.10 (d, 6.7)	1.03 (d, 6.8)	19.2 (q)	19.4 (q)
15	1.70 (s)	1.64 (s)	26.2 (q)	26.3 (q)
16			167.9 *(s)*	167.9 (s)
17			125.1 (s)	124.5 (s)
18			201.2 (s)	201.2 (s)
19	3.62 (quint, 7.4)	3.64 (sext, 6.2)	50.9 (d)	45.1 (d)
20	3.89 (quint, 6.6)	1.51 (m), 1.25 (m)	70.9 (d)	26.5 (d)
21	1.18 (d, 6.5)	0.72 (t, 7.6)	13.5 (q)	11.7 (q)
22	1.18 (d, 6.5)	1.06 (d, 6.4)	21.4 (q)	14.3 (q)

[a]Figures in parentheses are coupling constants in Hz.

[b]Multiplicities were determined by analyses of HSQC spectrum.

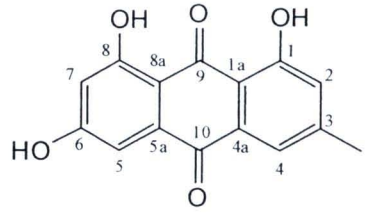
Compound 2.6 was obtained as orange needles. The IR spectrum (Figure 104 in Appendix) showed the O-H stretching of hydroxyl group at 3433 cm^{-1}. A set of medium absorption bands at 2925, 2854 and 1474, 1452, 1369 cm^{-1} was assigned to C-H stretching and bending, respectively. The characteristic absorption bands of an aromatic system appeared at 3036, 1605, and 1567 cm^{-1} while the absorption bands at 1676 and 1627 cm^{-1} were characteristic of chelating carbonyl ketones. The ^{1}H and ^{13}C NMR spectra showed the signals pattern of anthraquinone sketon. The ^{1}H NMR spectrum (Figure 105 in Appendix) indicated the presence of two hydroxyl groups as singlet signals at δ 12.05 and 11.94. The signals of the aromatic protons appeared as two singlets at δ 7.57 (H-4) and 7.02 (H-2), two doublet of doublets at δ 7.74 (H-5) and 7.22 (H-7), and one triplet at δ 7.60 (H-6). A methyl substituent attached to the 3-position of the aromatic ring was displayed as a singlet at δ2.40. The ^{13}C NMR spectrum (Figure 106 in Appendix) showed signals at δ 162.7 and 162.4 for C-1 and C-8 on the aromatic ring which corresponded to the hydroxyl bearing carbons at the 1 and 8 positions. The resonance signals at δ 192.5 and 181.9 were assigned to the carbonyl carbon atoms at C-9 and C-10 of anthraquinone, respectively. By comparison of the NMR spectral data [75], and mixed-TLC with the authetic sample, compound **2.6** was deduced to be chrysophanol.



chrysophanol **(2.6)**

Compound 2.7 was obtained as an orange solid. The IR spectrum (Figure 111 in Appendix) showed characteristic absorption bands of a hydroxyl group at 3367 cm^{-1}, an aromatic ring at 3050 and 1588 cm^{-1}, and a chelating carbonyl ketone at 1630 cm^{-1}. The ^{1}H and ^{13}C NMR spectral features (Figures 112 and 113 in Appendix) of **2.7** were similar to those of chrysophanol (**2.6**) except for the presence

of the hydroxyl group at δ 165.4 (C-6) (Table 2.18). The DEPT spectrum (Figure 114 in Appendix) showed two carbonyl, eight quaternary, four methine and one methyl carbons. Base on this spectroscopic evidence and a comparison of the NMR spectral data[75], as well as mixed-TLC with the authetic sample, compound **2.7** was identified as a known emodin.



emodin **(2.7)**

Table 2.18 ^{1}H and ^{13}C NMR spectral data (δ values) of chrysophanol **(2.6)** and emodin **(2.7)** in $CDCl_3$ and CD_3OD, respetively[a]

position	δ_H		δ_C	
	chrysophanol **(2.6)**	emodin **(2.7)**	chrysophanol **(2.6)**	emodin **(2.7)**
1			162.7	162.0
1a			113.7	113.6
2	7.02 (s)	7.05 (s)	124.3	124.3
3			149.3	148.1
4	7.57 (s)	7.55 (s)	121.3	121.0
4a			133.6	133.1
5	7.74 (dd, 7.4, 0.7)	7.21 (d, 2.4)	119.9	109.4
5a			133.2	135.2
6	7.60 (t, 7.3)		136.9	165.4
7	7.22 (dd, 9.9, 0.7)	6.60 (d, 2.8)	124.5	108.4
8			162.4	165.0
8a			**115.9**	**108.3**
9			192.5	190.3
10			181.9	182.5
1-OH	11.94 (s)	12.18 (s)		
3-CH_3	2.40 (s)	2.44 (s)	22.2	21.8
6-OH		12.28 (s)		
8-OH	12.05 (s)			

[a]Figures in parentheses are coupling constants in Hz.

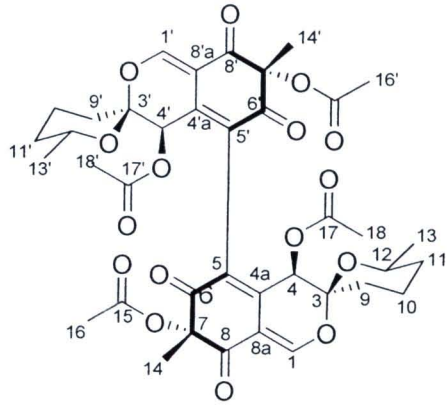
2.2.3 Compounds isolated from *C. siamense*

Compound 3.1 was obatained as a white solid. Comparison of the NMR spectral data[73], and mixed-TLC with compound **2.1** indicatied that compound **3.1** was ergosterol.

Compound 3.2 was obatained as a white solid. Comparison of the NMR spectral data[74], and mixed-TLC with compound **2.2** indicated that compound **3.2** was 24(*R*)-5α,8α-epidioxyergosta-6-22-diene-3β-ol.

Compound 3.3 was obatained as a amorphous solid. Comparision of the NMR spectral data[9], and mixed-TLC with compound **2.3** indicated that compound **3.3** was also ergosterylpalmitate.

Compound 3.4 was obtained as pale yellow crystals. The IR spectrum (Figure 121 in Appendix) showed absorption bands at 3057, 1597, and 1559 cm^{-1} due to C-H and C=C stretching. The characteristic absorption bands of ester appeared at 1756 band 1738 cm^{-1} whereas the absorption bands at 1706 and 1672 cm^{-1} were assigned to an α,β-unsaturated and an α,β,γ,δ-conjugated ketone groups. The ^{1}H and ^{13}C NMR and DEPT spectra (Figures 119 and 120 in Appendix) indicated the presence of only 19 signals attributable to four carbonyl, three sp^2 quaternary, one sp^2 methine, two sp^3 quaternary, two sp^3 methine (oxymethines, δ 69.5, 68.2), three sp^3 methylene and four methyl canbons. On the analysis of the above data and comparison of the NMR spectral data as well as mixed-TLC with the authetic sample, compound **3.4** was cochliodone D which was previously isolated from *C. cochliodes* CTh05.[27]

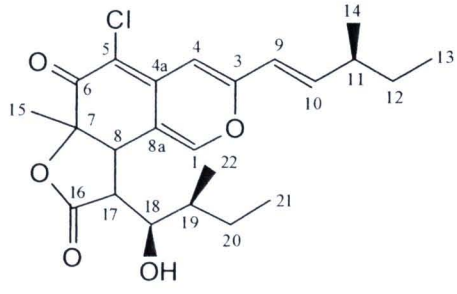


cochliodone D **(3.4)**

Compound 3.5 was obatained as orange crystals. Comparision of the NMR spectral data[9], and mixed-TLC with compound **2.4** indicated that compound **3.5** was chaetoviridin A.

Compound 3.6 was obatained as a red solid. Comparision of the NMR spectral data[9], and mixed-TLC with compound **2.5** indicated that compound **3.6** was also chaetoviridin F.

Compound 3.7 was obtained as a yellow solid. The IR spectrum (Figure 127 in Appendix) showed a broad absorption band of a hydroxyl group at 3460 cm^{-1}. It also presented medium absorption bands of a γ-lactone at 1788 cm^{-1} and α, β-conjugated ketones at 1649 cm^{-1} while the resonance absorption at 1570 cm^{-1} was assigned to a conjugated C=C stretching. The ^{1}H and ^{13}C NMR spectra and DEPT experiments (Figures 128-130 in Appendix) indicated the presence of twenty three signals classified as two carbonyl, four sp^2 quaternary, four sp^2 methine, one sp^3 quaternary, five sp^3 methine, two sp^3 methylene and five methyl carbons. The ^{1}H and ^{13}C NMR spectra of **3.7** were similar to those of chaetoviridin F **(3.6),** except that **3.7** showed extra three methine protons of H-8 (δ 3.42), H-17 (δ 3.19) and H-18 (δ 3.93), instead of the alkene at C-8 and carbonyl C-18 (Table 2.19). On the basis of the above data, the NMR spectral data, the comparison and mixed-TLC with the authetic sample indicated that compound **3.7** was chaetoviridin G.



chaetoviridin G **(3.7)**

Table 2.19 ^{1}H and ^{13}C NMR spectral data (δ values) of chaetoviridin F **(3.6)** and chaetoviridin G **(3.7)** in $CDCl_3$[a]

Position	δ_H		δ_C	
	chaetoviridin F **(3.6)**	chaetoviridin G **(3.7)**	chaetoviridin F **(3.6)**	chaetoviridin G **(3.7)**
1	8.74 (s)	7.57 (s)	151.8 (d)[b]	144.4 (d)[b]
3			157.5 (s)	156.9 (s)
4	6.50 (s)	6.50 (s)	105.5 (d)	104.6 *(d)*
4a			140.0 (s)	142.4 (s)
5			109.0 (s)	109.0 (s)
6			183.9 (s)	185.8 (s)
7			87.8 (s)	82.4 (s)
8		3.42 (dd, 12.0, 1.6)	164.0 (s)	46.0 (d)
8a			110.5 (s)	116.6 (s)
9	6.04 (d, 15.6)	6.06 (d, 15.6)	119.9 (d)	120.4 (d)
10	6.57 (dd, 15.6, 7.8)	6.50 (dd, 15.6, 8.0)	148.3 (d)	146.1 (d)
11	2.23 (sept, 7.0)	2.26 (sept, 7.0)	39.1 (d)	38.8 (d)
12	1.39 (quint, 7.2)	1.43 (quint, 7.6)	29.3 (t)	29.2 (t)
13	0.85 (t, 7.5)	0.90 (t, 7.6)	11.9 (q)	11.7 (q)
14	1.03 (d, 6.8)	1.08 (d, 6.8)	19.4 (q)	19.4 (q)
15	1.64 (s)	1.33 (s)	26.3 (q)	18.7 (q)
16			167.9 (s)	173.7 (s)
17		3.19 (dd, 12.0, 8.0)	124.5 (s)	43.6 (s)
18		3.93 (dd, 8.0, 3.4)	201.2 (s)	74.2 (s)
19	3.64 (sext, 6.2)	2.10 (m)	45.1 (d)	37.2 (d)
20	1.51 (m), 1.25 (m)	1.48 (m), 1.38 (m)	26.5 (d)	26.8 (d)
21	0.72 (t, 7.6)	0.98 (t, 8.0)	11.7 (q)	11.6 (q)
22	1.06 (d, 6.4)	0.98 (d, 6.8)	14.3 (q)	12.4 (q)

[a]Figures in parentheses are coupling constants in Hz.

[b]Multiplicities were determined by analyses of HSQC spectrum.

Compound 3.8 was obtained as an orange solid. Comparision of the NMR spectral data[75], and mixed-TLC with compound **2.6** indicated that compound **3.8** was emodin.

2.3 Bioactivity assays

The isolated depsidones **1.3-1.12** were tested for antimalarial (*Plasmodium falciparum*), antituberculosis (*Mycobacterium tuberculosis*), antifungal (*Candida albicans*) and cytotoxicity against NCI-H187, KB, and BC1 cell lines. The results of the antimalarial and antimycobacterial activities of the isolated compounds corresponded to the preliminary screening tests for the crude extracts. Seven depsidones **1.4-1.10** displayed activity against *P. falciparum* with IC_{50} ranging from 1.2-9.1 μg/mL. Only mollicellin K **(1.5)** exhibited antituberculosis activity against *M. tuberculosis* of IC_{50} 1.2 μg/mL. The result of antifungal show that **1.5, 1.9,** and **1.10** were active against *Candida albicans* with IC_{50} values of 49.9, 39.7, and 1.2 μg/mL, respectively.

The isolated compounds were also tested for their cytotoxicity against several cancer cell lines. Eight depsidones **1.3-1.6**, **1.8, 1.9,** and **1.11-1.12,** were cytotoxicity against KB cell with IC_{50} raning from 1.9-46.3 μg/mL. Only **1.5** were cytotoxicity against BC1 with 6.8 μg/mL. All compounds were cytotoxicity against NCI-H187 cell with IC_{50} raning 0.35-23.3 μg/mL as shown in table 2.20.

Depsidones **1.5** and **1.7-1.12** also significantly exhibited cytotoxicity against five cholangiocarcinoma cell lines (KKU-100, KKU-M139, KKU-M156, KKU-M213, and KKU-M214) with IC_{50} value ranging from 2.5 to 15.7 μg/mL. It should be noted that all compounds exhibited IC_{50} values against KKU-100 ranging from 4.5 to 6.5 μg/mL which more cytotoxic than the control drug ellipticine (Table 2.21). Unfortunely **1.3** , **1.4**, and **1.6** were not tested due to the limited samples.

Among isolated compounds, mollicelline K **(1.5)** was active against all tests and was the most active compound.

Table 2.20 Bioactivities activities of isolated compounds from *C. brasiliense*

compound	Antimalarial	anti-TB	antifungal	cytotoxicity IC_{50} (μg/mL)		
	IC_{50} (μg/mL)	MIC (μg/mL)	IC_{50} (μg/mL)	KB[a]	BC1[b]	NCI-H187[c]
1.3	nd	inactive	nd	16.6	nd	3.9
1.4	4.9	inactive	nd	29.1	nd	23.3
1.5	1.2	12.5	1.2	1.9	6.8	0.35
1.6	3.4	inactive	inactive	33.9	nd	9.5
1.7	2.9	inactive	inactive	inactive	inactive	0.68
1.8	4.7	inactive	inactive	37.1	nd	14.7
1.9	9.1	inactive	49.9	46.3	inactive	3.1
1.10	3.2	inactive	39.7	Nd	nd	1.0
1.11	inactive	inactive	inactive	25.9	nd	13.5
1.12	inactive	inactive	nd	37.9	nd	13.1
artmisinin	0.0001					
isoniazid		0.05				
kanamycin sulfate		2.5				
amphotericin B			0.034			0.32
ellipticine				0.36	0.26	

[a]Human epidermoid carcinoma in the mouth,

[b]Human breast cancer cells,

[c]Human small cell lung cancer cells,

nd = not determined

inactive at > 50 μg/mL

Table 2.21 Bioactivities activities of isolated compounds from *C. brasiliense* against Cholangiocarcinoma

compound	cytotoxicity (IC_{50}, µg/mL)				
	KKU-100[a]	KKU-M139[b]	KKU-M156[c]	KKU-M213[d]	KKU-M214[e]
1.5	4.46±0.08	13.94±2.87	6.65±1.15	nd	4.92±0.26
1.7	6.28±0.94	8.55±3.20	6.04±0.15	13.19±1.17	4.51±0.18
1.8	4.63±0.01	11.66±2.70	15.66±0.88	6.94±1.02	4.50±0.39
1.9	5.12±0.17	2.51±0.36	4.18±0.11	3.2±0.52	3.35±0.04
1.10	4.83±0.05	5.03±0.16	5.22±0.08	4.44±0.05	7.81±1.13
1.11	6.46±1.24	4.34±0.08	2.90±0.07	3.00±0.36	0.05±0.50
1.12	5.21±0.09	5.21±0.04	5.36±0.15	4.98±0.05	4.40±0.02
ellipticine	7.11±0.09	1.21±0.03	2.02±0.11	0.30±0.001	0.21±0.04

[a] Poorly differentiated adenocarcinoma,
[b] Squamous carcinoma,
[c] Moderately differentiated adenocarcinoma,
[d] Adenosquamous carcinoma,
[e] Moderately differentiated denocarcinoma,
nd = not determined

In addiotional, crude hexane and crude EtOAc as well as pure compounds **1.6, 1.8, 1.9,** and **1.10** from *C. brasiliense* were tested for inhibition of platelet aggregation and PAF receptor binding. The results showed that all tested samples were weakly active for AA, ADP, and collagen, as shown in Table (2.22). While **1.3** , **1.4**, **1.5**, **1.7**, **1.11,** and **1.12** were not tested due to the limited samples.

Table 2.22 Percentage inhibition of crude extracts and mollicellins **(1.6), (1.8), (1.9),** and **(1.10)**

sample	AA	collagen	ADP
crude hexane	25.0±1.5	36.0±0.5	31.6±0.4
crude EtOAc	35.4±0.7	31.2±1.6	30.0±1.2
1.6	16.9±0.7	26.8±0.9	nd
1.8	21.7±0.3	37.2±0.6	nd
1.9	37.9±0.5	21.4±1.1	nd
1.10	8.8±0.6	8.4±0.6	nd
aspirin	99.4±0.0	39.5±0.6	42.2±0.4

nd = not determined

Evaluation of the ability of the compounds to inhibit PAF receptor binding clearly shown that all compounds were weak inhibitory effect with percentage inhibition values ranging from 7 to 28%, as shown in Table (2.23).

In conclusion, mollicellins K **(1.5)**, B **(1.8),** C **(1.9)**, and F **(1.12)** had no effect on inhibition of platelet aggregation induced by AA, collagen, ADP, and PAF receptor binding.

Table 2.23 Inhibitory effects of the mollicellins of *C. brasiliense* on the PAF recepter binding to rabbit platelets

sample[a]	% inhibition
crude hexane	16.93
crude EtOAc	21.36
1.6	11.94
1.8	23.40
1.9	7.02
1.10	28.89
cedrol	71.02

[a] concentration of sample in reaction mixture =18.2 μg/mL.