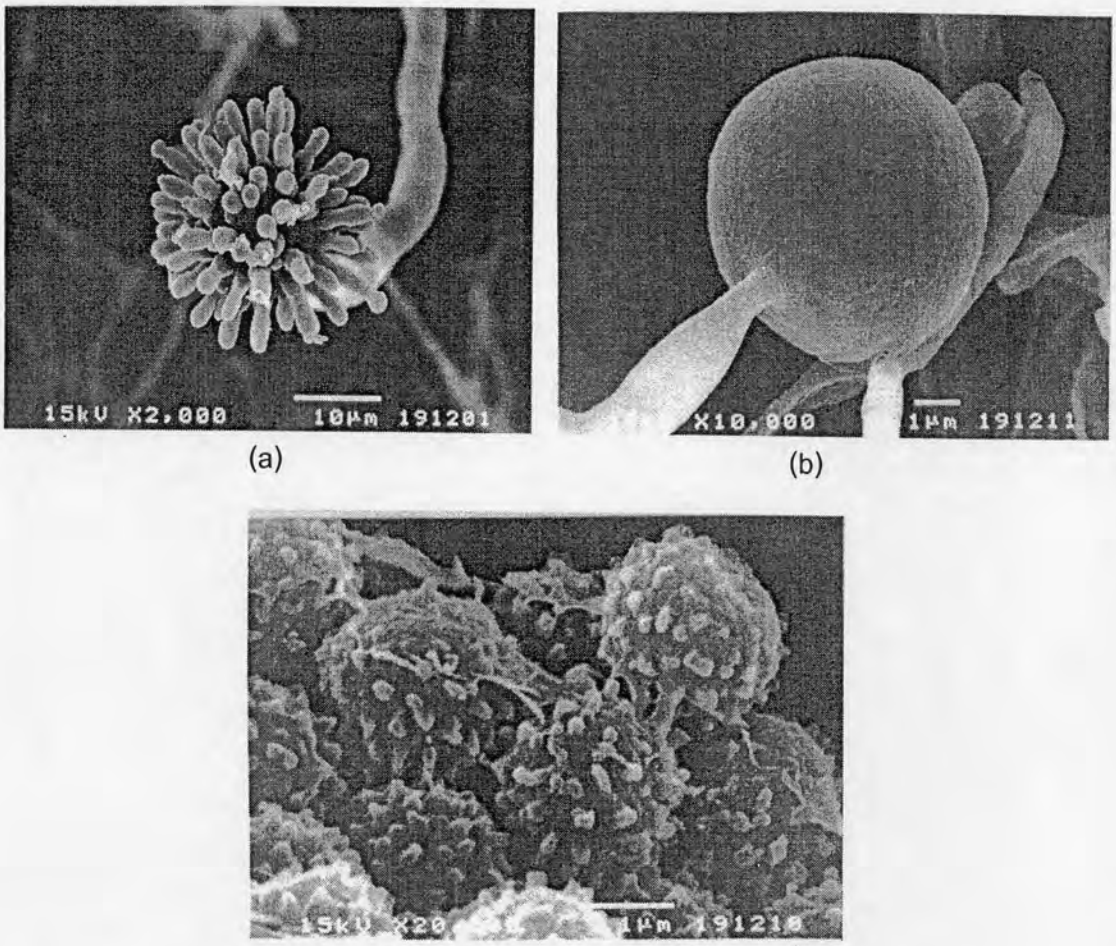
4.2.2 Identification of *Emericella variecolor* by scanning electron microscope (SEM)

Emericella variecolor was identified by scanning electron micrograph. Characteristic of asexual state, conidia and conidiophore, and sexual state ascus, ascospore and Hulle cells were shown in Figure 4.3.



(a) (b)

(c)

Figure 4.4 Scanning Electron Micrograph (SEM) of the asexual apparatus of *Emericella variecolor* (a) conidia and conidiopore (Bar = 10 μm) (b) Hulle cell (Bar = 1 μm) (c) sexual spore: ascospores (star shape) (Bar = 1 μm) (Bar =1 μm)

4.3 Growth curve of *Emericella variecolor* in culture media

Emericella variecolor was cultured in 250 ml flask containing 100 ml of MEB, MCzpek-Dox and Czapek-Dox and culture at room temperature for 60 days. The culture broth were filtered through filter paper (Whatman No.1) and mycelia were measured the cell mass for growth profile as shown in Figure 4.5.

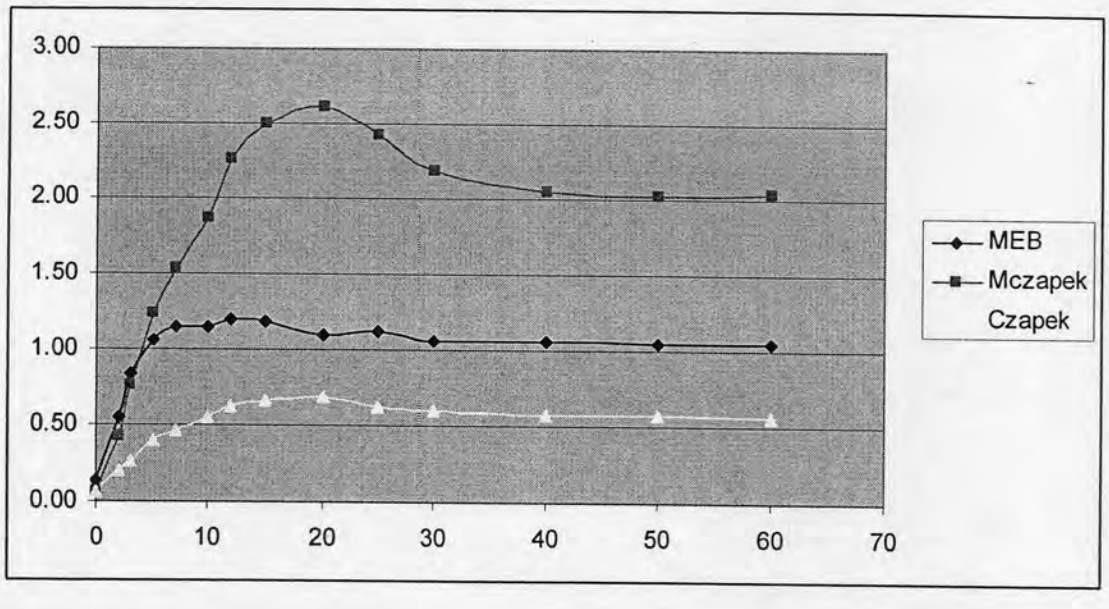


Figure 4.5 Growth profile of *Emericella variecolor* culture in Malt extracts broth (MEB), Malt Czapek-Dox (MCzB) and Czapek-Dox broth (CzB).

4.4 The metabolite from *Emericella variecolor*

4.4.1 Metabolites from EtOAc crude extract of mycelium cultured in MEB

Isolation procedure of EtOAc crude extract of *Emericella variecolor* mycelium cultured in MEB by silica gel column chromatography was collected. Total 385 fractions were obtained from isolation of EtOAc crude extract of mycelium (MEB). Compound A was crystallized from combined fraction 29-39, eluted with 15 % EtOAc in hexane. After filtration and washing with hexane and ethyl acetate, respectively, compound A (11 mg) was obtained as a white solid. Compound B obtained in fraction 46-60, eluted with 20 % EtOAc in hexane was crystallized during evaporation. The precipitate was filtered and then washed with hexane and ethyl acetate, respectively, to give compound B (10 mg) as a white crystal: Fraction 121-138 eluted with 35 % EtOAc in hexane was crystallized from chloroform to obtain compound C1 (11 mg) as a colorless crystal.

Compound A

Fraction 29-39 eluted with 15 % EtOAc in hexane then filtered and washed by hexane and ethyl acetate, respectively to obtain a white solid of compound A (11 mg).

White solid, m.p. 228-230 °C;

$[\alpha]_D^{25}$ +8 ($CHCl_3$, c 0.3);

EIMS (EI 70 eV) *m/z* 370 [M^+, 53 %], 355 (23), 327 (32), 257 (12), 246 (15), 203 (29), 189 (100), 175 (24), 161 (40), 147 (52), 135 (40), 121 (45), 107 (60), 95 (50), 93 (48), 81 (42), 67 (28) and 55 (24);

λ_{max} ($CHCl_3$) (ε) 245 (5617) nm;

ν_{max} (KBr) 3435 (br.s), 2945 (w), 2855 (w), 1745 (m), 1649 (m), 1563 (m), 1411 (m), 1264 (w) and 1022 (w) cm^{-1};

δ_H ($CDCl_3$, 400 MHz) 5.94 (1H, d, 4.4 Hz, 2-H), 4.97 (1H, dd, 4.8 and 4.8 Hz, 6-H), 4.75 (s, 1H, H-24), 4.73 (1H, s, 24-H), 2.89 (1H, d, 12.0 Hz, 4-H), 2.71 (1H, dd, 6.8 and 7.6 Hz, 1-H), 2.31 (1H, m, 5-H), 2.26 (1H, m, 18-H), 2.11 (1H, m, 5-H), 2.09 (1H, m, 1-H), 2.06 (1H, m, 8-H), 1.97 (1H, m, 8-H), 1.90 (1H, m, 16-H), 1.87 (1H, m, 4-H), 1.75 (3H, s, 25-H), 1.57 (1H, m, 13-H), 1.54 (1H, m, 16-H), 1.50 (1H, m, 9-H), 1.48 (1H, m, 17-H), 1.46 (1H, m, 12-H), 1.32 (3H, s, 21-H), 1.31 (1H, m, 13-H), 1.30 (1H, m, 17-H), 1.28 (1H, m, 12-H), 1.26 (1H, m, 10-H), 1.20 (1H, m, 12-H), 1.17 (1H, m, 14-H), 0.90 (s, 3H, 22-H) and 0.84 (3H, s, 23-H) ppm;

δ_C ($CDCl_3$, 100 MHz) 173.19 (20-CO), 150.16 (2-CH), 148.12 (19-C), 138.74 (7C), 125.28 (3-C), 123.68 (6-CH), 109.61 (24-CH_2), 54.43 (14-CH), 49.63 (10-CH), 47.59 (18-CH), 45.85 (15-C), 42.64 (1-CH_2), 41.15 (13-CH_2), 40.37 (8-CH_2), 39.17 (12-CH_2), 37.92 (11-C), 34.85 (4-CH_2), 27.69 (16-CH_2), 27.35 (5-CH_2), 24.13 (22-CH_3), 22.27 (9-CH_2), 21.03 (17-CH_2), 19.98 (25-CH_3), 15.98 (21-CH_3) and 15.57 (23-CH_3) ppm.

Compound A as a white solid m.p. 228-230 °C, which showed the M^+ ion at 370 in EIMS and on the basis of NMR analysis assigned the structure of compound A.

The IR spectrum of compound A showed that the absorption peaks assigned as in **Table 4.1** and indicated absorption bands of a OH stretching vibration at 3435 cm^{-1}, CH stretching vibration at 2945 and 2855 cm^{-1}, C=O stretching vibration at 1745 cm^{-1}, C=C stretching vibration at 1649 and 1563 cm^{-1}.

Table 4.1 The IR absorption bands assignment of compound A

Wave number (cm^{-1})	Intensity	Tentative assignment
3435	Broad, Strong	O-H stretching vibration of carboxylic acid
2945, 2855	Weak	stretching vibration of CH_2, CH_3
1745	Weak	C=O stretching vibration of carbonyl
1649, 1563	Medium	C=C stretching vibration of olefin
1411	Medium	C-H bending vibration of CH_2, CH_3
1264, 1022	Weak	C-O stretching vibration of carbonyl group
656	Weak	C-H out of plane bending vibration

The mass spectrum showed the molecular ion of compound A at *m/z* 370. The ^{1}H-NMR spectrum of stellatic acid indicated that it possesses 3 olefinic protons (δ 4.73, 4.75 and 5.94 ppm) and four methyl group (δ 1.32, 0.90, 0.84 and 1.75 ppm).

The ^{13}C-NMR showed 25 signals including six signals of olefinic carbons (δ 109.61, 123.68, 125.28, 139.74, 148.12 and 150.16 ppm), a carbonyl carbon at δ 173.19 ppm, four methyl signals (δ 15.57, 15.98, 19.98, 24.13 ppm), ten C_{sp3} methylene signals (δ 22.27, 22.35, 27.35, 34.85, 39.17, 40.37, 41.15, 42.64, 54.43 ppm), two methine signals (δ 47.59 and 49.63 ppm) and two quarternary carbons (δ 37.92 and 45.85 ppm).

On the basis of spectroscopic data including ^{1}H, ^{13}C, gHSQC, gHMBC, gCOSY, TOCSY and NOESY, the long range correlations of gHMBC and NOESY was observed as shown in **Figure 4.6** and **Figure 4.7**, respectively. The specific optical rotation of stellatic acid, $[\alpha]_D^{25}$ +8 ($CHCl_3$, c 0.3), was corresponded to the previous report [Lit (Quereshi et. al., 1980) $[\alpha]_D^{25}$ +13.5 ($CHCl_3$, c 0.3)]. The chemical structure of compound A was assigned as a known compound, stellatic acid (**Figure 4.8**).

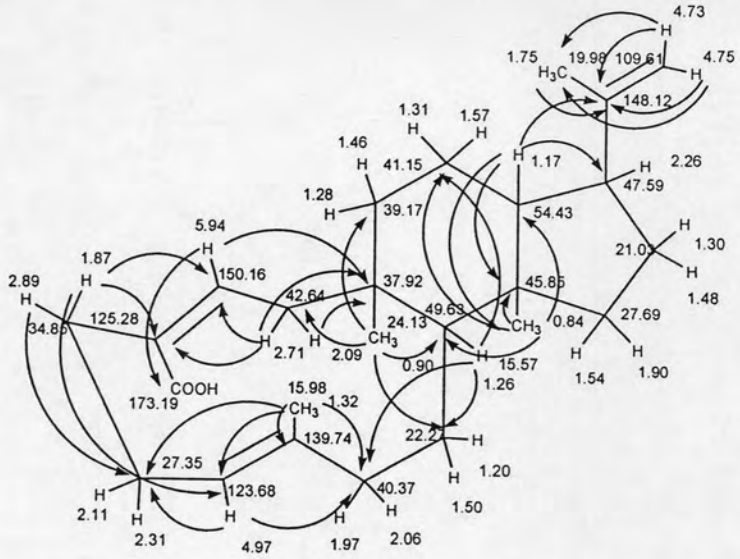


Figure 4.6 The gHMBC correlation of compound A

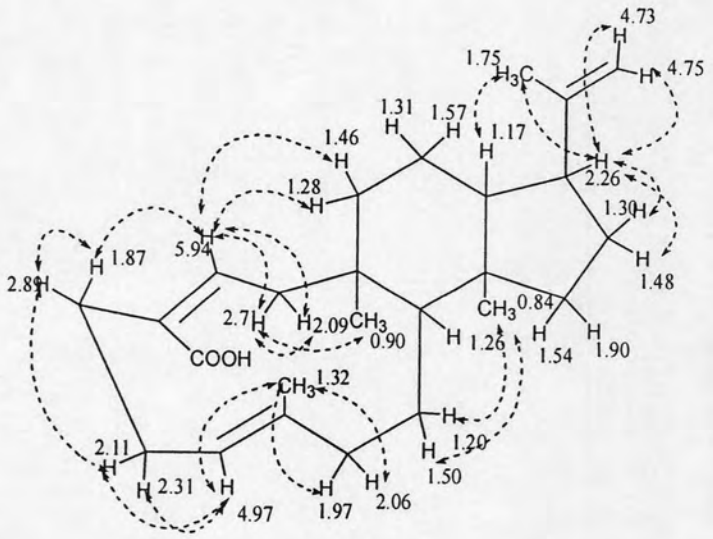


Figure 4.7 The NOESY correlation of compound A

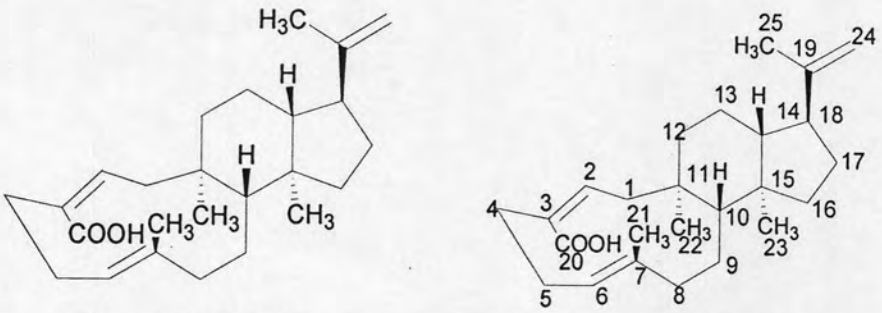


Figure 4.8 Structure and position of stellatic acid.

Compound B

Fraction 46-60 eluted with 20 % EtOAc in hexane was filtered and washed by hexane and ethyl acetate, respectively to give a colorless crystal as compound B (10 mg).

m.p. 167-168 °C;

$[\alpha]_D^{20}$ -84($CHCl_3$, c 0.1);

ESIMS: $[M+H]^+$ 397.3421;

λ_{max} 325(967), 345 (864) nm;

ν_{max} (KBr) 3427 (br, s), 2956 (s), 2871 (s), 1654 (w), 1553 (w), 1459 (m), 1382 (m), 1370 (m), 1241 (w), 1158 (w), 1127 (w), 1111 (w), 1058 (m), 1039 (m), 983 (m), 969 (m), 834 (w), 802 (w) cm^{-1};

^{1}H-NMR ($CDCl_3$, 400 MHz): 5.56 (1H, dd, *J* = 1, 5.2 Hz, H-6), 5.38 (1H,dd, *J* = 1, 5.2 Hz, H-7), 5.19 (1H, m, H-22), 5.18 (1H, m, H-23), 3.63 (1H, H-3), 2.47(1H, ddd, J = 2.8, 4.8,14.0 Hz, H-4), 2.27 (1H, dd, J =12.4, 13.6, m, H-4), 2.07(1H, m, H-12), 2.04 (1H, m, H-20), 1.98 (1H, m, H-9),1.92 (1H, m, H-2), 1.91 (1H, m, H-1), 1.90 (1H, m, H-14), 1.72 (1H, m, H-16), 1.64 (1H, m, H-11), 1.64 (1H, m, 15), 1.58 (1H, m, H-11), 1.50 (1H, m, H-2), 1.47(1H, m, H-25),1.35 (1H, m, H-15), 1.32 (1H, m, H-1), 1.28 (1H, m, H-16), 1.25 (1H, m, H-17), 1.24 (1H, m, H-12), 1.03 (3H, d, *J* = 6.4 HzH-27), 0.93(3H, s, H-19), 0.90 (3H, d, J = 6.8 Hz, H-28), 0.82 (3H, d, *J* = 6.4 Hz, H-26), 0.82 (3H, *J* = 6 Hz, H-21), 0.62 (3H, s, H-18) ppm;

^{13}C-NMR ($CDCl_3$, 100 MHz): 141.37 (s, C-8), 139.77 (s, C-5), 135.56 (d, C-23), 131.94 (d, C-22), 119.57 (d, C-6), 116.26 (d, C-7), 70.44 (d, C-3), 55.68 (d, C-17), 54.54 (d, C-14), 46.21 (d, C-9), 42.80(d, C-24), 42.80 (s, C-13), 40.73 (t, C-4), 40.45 (d, C-20), 39.05 (t, C-12), 38.35 (t, C-1), 37.00 (s, C-10), 33.07 (d, C-25), 31.93 (t, C-2), 28.30 (t, C-16), 22.99 (t, C-15), 21.09 (q, C-27), 21.09 (t, C-11), 19.96 (q, C-26), 19.65 (q, C-21), 17.61 (q, C-28), 16.27 (q, C-19), 12.04 (q, C-18) ppm.

Compound B as a colorless crystal, m.p167-168 °C, which showed molecular ion of $[M+H]^+$ at 397.3421 that indicated molecular weight 396, IR adsorption peaks were indicated absorption peaks were indicated absorption bands a OH stretching vibration at 3427 cm^{-1}(br), C=C stretching vibration at 1654 and 1553 $cm^{-1}$and C-O stretching

vibration at 1241, 1158, 1127, 1111, 1058 and 1039 cm^{-1}. The IR absorption assignment of compound B was shown in **Table 4.2**.

Table 4.2 The IR absorption bands assignment of compound B

Wave number (cm^{-1})	Intensity	Tentative assignment
3464	Broad, Strong	O-H stretching vibration of hydroxyl
2970, 2927	Weak	stretching vibration of CH_2, CH_3
1753	Weak	C=O stretching vibration of carbonyl
1659, 1555	Medium	C=C stretching vibration of olefin
1415	Medium	C-H bending vibration of CH_2, CH_3
1250, 1015	Weak	C-O stretching vibration of hydroxyl

The ^{1}H-NMR spectrums of compound B showed the important signals that indicated four olefinic protons at δ_H 5.56, 5.38, 5.19 and 5.18, ppm, six methyl groups at δ_H 1.03, 0.93, 0.90, 0.82, 0.82 and 0.62 ppm, a proton attached to oxygenated carbon at 3.63 and proton the ring and side chain of ergosterol at 2.47-1.24.

The ^{13}C NMR spectrums of ergosterol showed 28 carbon signals consisting of six olefinic carbons at δ_C 141.37, 139.77, 135.56, 131.94, 119.37 and 116.26 ppm, six methyl carbons at δ_C 21.09, 19.96, 19.65, 17.61, 16.27 and 12.04 ppm and a carbon attached heteroatom (CH-OH) at 70.44 ppm.

Abraham and Monasterios, 1974 reported the ^{13}C NMR spectrum of ergosterol compared with compound A showed in **Table 4.3**. NMR data of compound B were similar to ergosterol. In comparison with ^{13}C NMR data and $[\alpha]_D^{21}$ -130.7° of ergosterol reported by Pruess,Peterson, and Fred, 1932). The chemical structure of compound A was assigned as a known compound, ergosterol (**Figure 4.9**).

Table 4.3 Comparison of 1H-NMR spectrum of ergosterol and compound B

Position	Compound A	Ergosterol
1	38.35	38.4
2	31.93	32.0
3	70.44	69.5
4	40.73	40.5
5	139.77	140.5
6	119.57	119.2
7	116.26	116.5
8	141.37	140.4
9	46.21	46.3
10	37.00	37.0
11	21.09	21.0
12	39.15	39.2
13	42.80	42.8
14	55.54	54.4
15	22.99	22.9
16	28.30	28.1
17	55.68	55.8
18	12.04	11.6
19	16.27	15.8
20	40.45	40.3
21	19.65	19.2
22	131.94	132.0
23	135.56	135.8
24	42.80	42.8
25	33.07	33.0
26	19.96	19.5
27	21.09	20.8
28	17.61	17.2

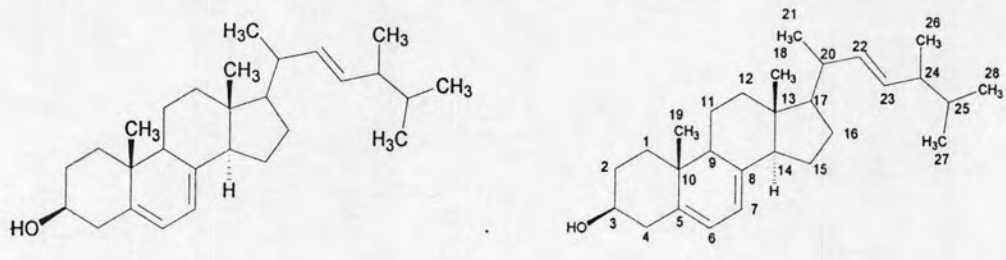


Figure 4.9 Structure and position of ergosterol

Compound C1

Fraction 121-138 eluted with 35 % EtOAc in hexane was crystallized by chloroform to obtain a colorless crystal as compound C1 (11 mg).

m.p. 235-236°C(dec);

$[\alpha]_D^{20}$ -220°($CHCl_3$, c 0.1);

HRESIMS $[M+Na]^+$ = 431.1834 (calc for $C_{25}H_{28}O_5Na$ 431.183451);

λ_{max} (EtOH) (ε) 308.05 (335), 252 (1329); ν_{max} (KBr) 3464 (br.s), 2970 (w), 2927 (w),1753 (m), 1711 (w), 1659 (m), 1555 (m), 1415 (w), 1250 (w), 1015 (w) nm;

^{1}H-NMR ($CDCl_3$, 400 MHz) 6.80 (1H, s, H-6'), 6.76 (1H, d, *J* = 10.4 Hz, H-1), 5.87 (1H, d, *J* = 10.4 Hz, H-2), 4.70 (1H, s, H-9'), 4.62 (1H, s, H-9'), 4.55 (1H, d, 10 Hz, H-1'), 4.45 (1H, d, *J* = 9.2 Hz, H-1'), 3.63 (1H,s, H-11), 2.87 (brs, OH-9), 2.32 (1H, d, *J* = 11.6 Hz, H-12), 2.06 (1H, dd, *J* = 6, 14 Hz, H-5), 2.00 (1H, m, H-6), 1.75 (1H, m, H-7), 1.72 (1H, m, H-6), 1.51 (1H, m, H-7), 1.29 (1H, d, *J* = 12 Hz, H-12), 1.27 (3H, s, H-10'), 1.20 (3H, s, H-13), 1.09 (3H, s, H-15), 1.04 (3H, s, H-14) ppm;

^{13}C-NMR ($CDCl_3$, 100 MHz) 202.71 (s, C-3), 202.25 (s, C-4'), 166.22 (s, C-8'), 152.66 (s, C-7'), 150.03 (d, C-1), 144.41 (d, C-6'), 138.29 (s, C-3'), 126.32 (d, C-2), 103.03 (t, C-9'), 82.00 (s, C-9), 72.32 (t, C-1'), 73.20 (d, C-11), 57.48 (s, C-2'), 51.26 (s, C-8), 50.19 (s, C-5'), 47.85 (t, C-12), 44.46 (s, C-4), 43.64 (s, C-10), 41.09 (d, C-5), 25.05 (q, C-15), 24.93 (t, C-6), 22.21 (q, C-14), 19.60 (q, C-10'), 17.52 (q, C-13), 16.88 (t, C-7) ppm.

Compound C1 $[\alpha]_D^{20}$ -220 ($CHCl_3$, c 0.1), had a molecular formula $C_{25}H_{28}O_5$ (431.1834 $[M+Na]^+$, cald 431.1835). The ^{13}C NMR and gHSQC exhibited 25 resonance including four methyl, five methylene, five methine and eleven quarternary carbons. Resonances at δ_c 202.71 and 202.25 suggested the presence of two ketone carbonyl groups, and δ 166.22 suggested a ester carbonyl group, which was also supported the IR absorption bands at 1753 and 1711 cm^{-1}. The presence of an OH group was deduced from the IR absorption band at 3464 cm^{-1}. Resonances at 1659 and 1655 $^{cm-1}$ suggested the presence of olefinic functionality. IR absorption assignment of compound C1 was shown in **Table 4.4**.

Table 4.4 The IR absorption bands assignment of compound C1

Wave number (cm^{-1})	Intensity	Tentative assignment
3464	Broad, Strong	O-H stretching vibration of hydroxyl
2970, 2927	Weak	stretching vibration of CH_2, CH_3
1753	Weak	C=O stretching vibration of carbonyl
1659, 1555	Medium	C=C stretching vibration of olefin
1415	Medium	C-H bending vibration of CH_2, CH_3
1250, 1015	Weak	C-O stretching vibration of hydroxyl

The ^{1}H-NMR spectrum showed the signals of four methyl groups at δ_H 1.27 (s), 1.20 (s), 1.09 (s), 1.04 (s) and five sp_2 protons at 6.80 (s), 6.76 (d, J = 10.4 Hz), 5.87 (d, J = 10.4 Hz), 4.70 (s) and 4.62 (s) a methine protons indicated at δ_H 3.63 (s) ppm.

The 13C NMR spectrums of compound C1 showed 25 carbon signals including carbonyl carbons at δ_C 202.71, 202.25, 166.22 ppm, olefinic carbons at δ_C 152.66, 150.03, 144.41, 138.29, 126.32 , 103.03 ppm, four methyl carbons at δ_C 22.21, 19.60 17.52, 25.05 ppm, four methylene carbons at δ_C 72.32, 47.85, 24.93 and 16.88 ppm, sp_3 methine carbons at δ_C 73.20 and 41.09 ppm, quarternary sp_3 carbons at δ_C 82.00, 43.64, 44.46, 50.19 51.26 and 57.48 ppm.

Detailed analyses of the 1D and 2D NMR spectra indicated the structure of compound C1 and allowed assignment of all proton and carbon signals. HMBC correlations from H1 to C-3, C-5 and C-9 and H-2 to C-10 and H-14 and H-15 to C-3 are indicative of a six member ketone ring. HMBC correlations from H_2-9' to C-11 and C-2' and H-11 to C-8 indicated a five member rings. The HMBC correlations of H-10' to C-12, C-4' and C-6', and H-6 to C-7' and C-8' indicated the fused of skeletal of three rings showed in **Figure 4.10**. The gCOSY, gHMBC and NOESY correlations showed in **Table 4.5**. NOE correlation was shown the stereochemistry of compound C1 in the **Figure 4.11**.The metabolites of *Aspergillus variecolor* was reported the C-25 sesterterpenoid metabolites and biosynthetic pathways. ^{1}H-NMR and ^{13}C NMR spectra were compared with anditomin compound C1 showed in **Table 4.6**. Comparison of $[\alpha]_D$ of anditomin(-81)

and compound C1 (-220) in $CHCl_3$ was negative in the same. ORTEP views was confirmed the structure of emervaridione are shown in **Figure 4.12**. Thus, the structure of compound C1 and anditomin showed in **Figure 4.13** and was assigned as shown,and it was named emervaridione. The structure of emervaridione was shown in **Figure 4.14**.

Table 4.5 gCOSY, gHMBC, NOESY correlation of compound C1

Position	1H-NMR	gCOSY	gHMBC	NOESY
1	6.76	H-2	C-3, C-5, C-9	H-1, H-11, H-13
2	5.87	H-1	C-10	H-2
5	2.06	H-6	C-4, C-6, C-9, C14, C-15	H-15
6a 6b	1.72 2.00	H-5, H-6b, H-7 H-5, H-6a, H-7	C-5, C-8 C-8	H-13, H-14 H-13
7a 7b	1.51 1.75	H-6 H-6	- C-5, C-8, C-9	H-15
11	3.63	-	C-8, C-9, C-10, C-2', C-4', C-7', C-9'	H-1
12a 12b	1.29 2.32	H12b H12a	C-8, C-9, C-5', C-6' C-8, C-9, C-2', C-5', C-6', C-10'	H-12b H-12a
13	1.20	-	C-1, C-5, C-9, C-10	H-1, H-6, H-14
14	1.04	H-15	C-3, C-4, C-5, C-15	H-6a, H-13
15	1.09	H-14	C-3, C-4, C-5, C-14	H-5, H-7a
1'a 1'b	4.45 4.55	H-1'b H-1'a	C-8, C-2', C-9', C-7' C-2', C-7', C-8'	H-1'b H-1'a
6'	6.80	-	C-2', C-4', C-5', C-7', C-8', C-10'	H-10'
9'a 9'b	4.62 4.70	H-9'b H-9'a	C-2', C-11, C-3' C-2', C-11, C-3', C-7'	H-9'b H-9'a
10'	1.27	-	C-12, C-4', C-5', C-6'	H-6'

Table 4.6 Comparison of ^{13}C-NMR chemical shifts of Compound C1 and Anditomin (Simpson., T. J., 1981)

Position	Compound 1	Anditomin
	^{13}C-NMR	^{13}C-NMR
1	150.03	147.4
2	126.32	120.1
3	202.71	165.9
4	44.46	83.6
5	41.09	44.5
6	24.93	21.0
7	16.88	25.7
8	51.26	47.7
9	82.00	61.5
10	43.64	44.4
11	73.20	64.1
12	47.85	49.8
13	17.52	25.9
14	22.21	30.6
15	25.03	23.2
1'	72.32	75.5
2'	57.48	42.6
3'	138.20	148.0
4'	202.25	207.9
5'	50.19	54.1
6'	144.41	30.6
7'	152.66	43.2
8'	166.22	173.8
9'	103.03	111.4
10'	19.60	23.7

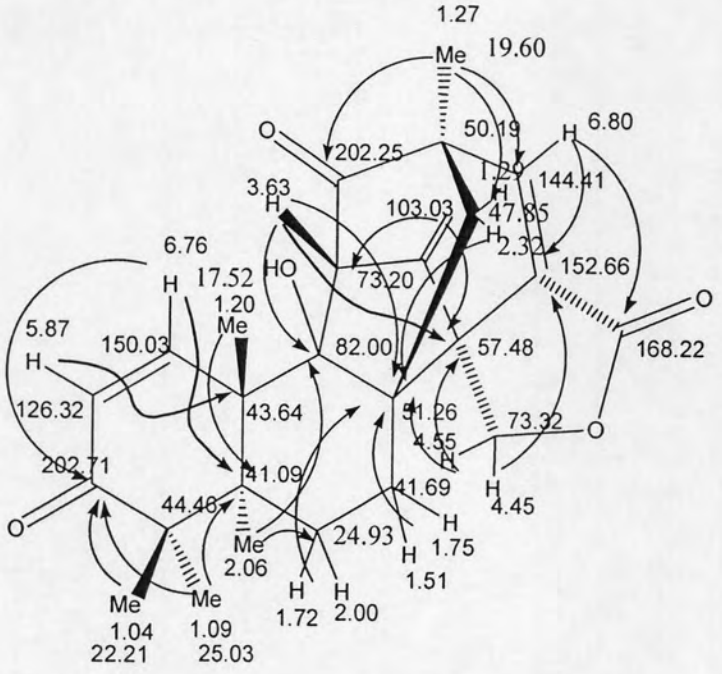


Figure 4.10 gHMBC correlation of compound C1

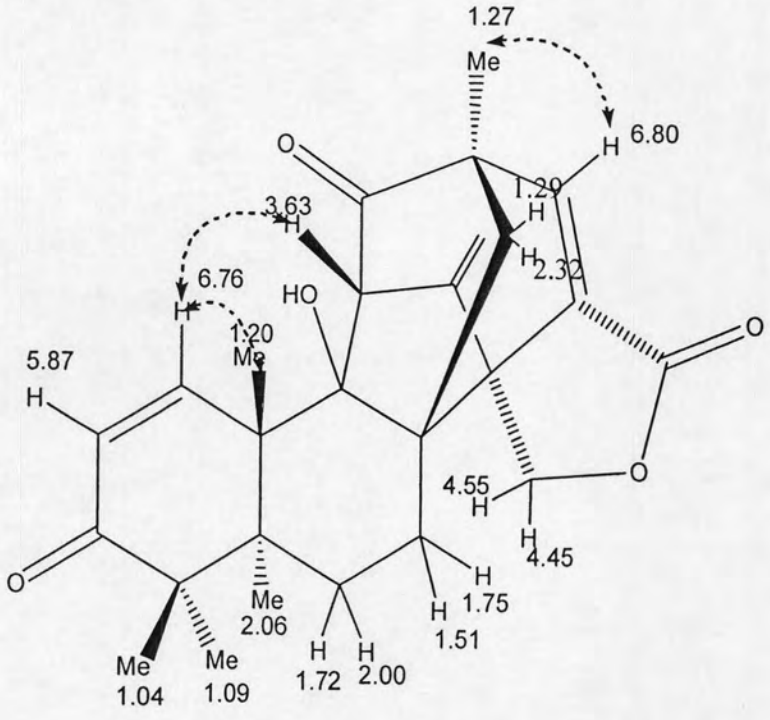


Figure 4.11 NOESY correlation of compound C1

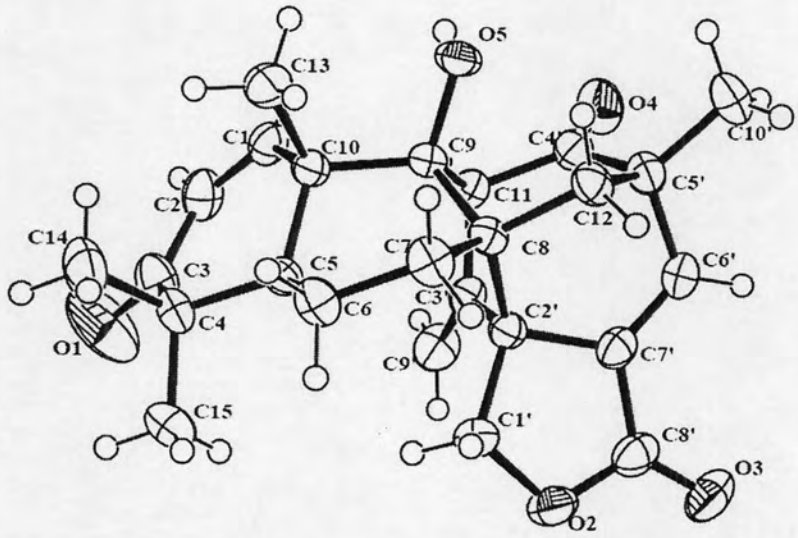
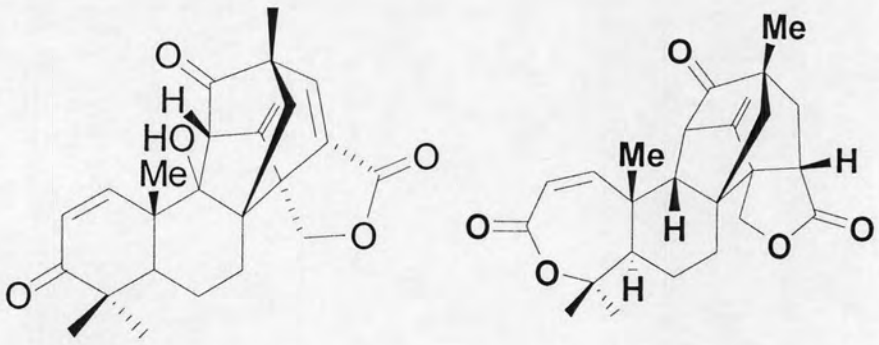


Figure 4.12 ORTEP structure of compound C1



Compound C1 Anditomin

Figure 4.13 Structure of compound C1 and anditomin

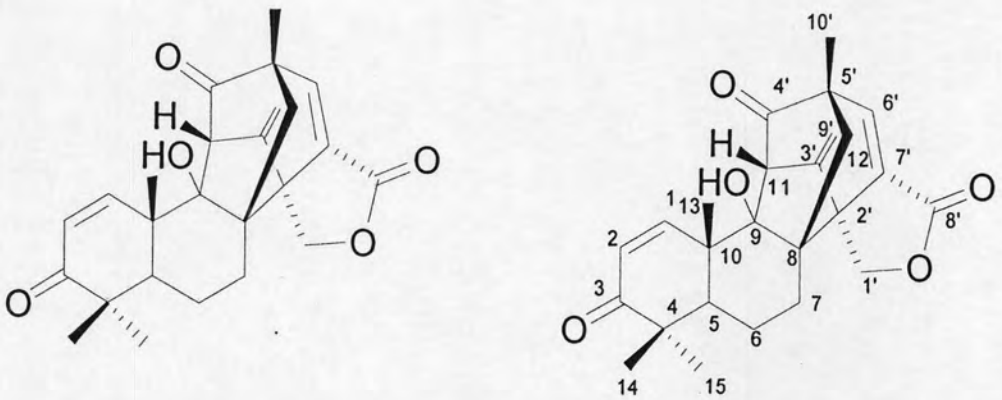


Figure 4.14 Structure and position of emervaridione

4.4.2 Metabolites from EtOAc crude extract of fermentation broth culture in MEB

Isolation procedure of EtOAc crude extract of fermentation MEB by silica gel column chromatography was collected. Total 325 fractions isolated from EtOAc crude extract of broth (MEB). Fraction 18-30 eluted with 50-55% EtOAc in hexane was crystallized with chloroform to obtain compound D1 (10 mg) as a colorless crystal. Fraction 91-112 eluted with 65-70 % EtOAc in hexane was washed by hexane and ethyl acetate, respectively to give compound D2 (12 mg) as a white solid. Fraction132-145 eluted with 75 % EtOAc in hexane was purified by PTLC (silica gel) using 75 % EtOAc in hexane as mobile phase to obtain compound E (4 mg) as brown oil.

Compound D1

Fraction 18-30 eluted with 50-55% EtOAc in hexane was crystallized with chloroform to obtain compound D1 (10 mg) as a colorless crystal. m.p. 156-157 °C;

$[\alpha]_D^{20}$ +46 (c 0.1, EtOH);

(HRESIMS) [M+Na]+ 303.1216, calcd [M+Na]+ 303.1208;

λ_{max} (268, 914), (275, 959), (299, 198); ν_{max} (KBr) 3415(br,s), 2921(w), 2860(w), 1641(w), 1555(m), 1418(w), 1483(w), 1418(w), 1333(w), 1263(w), 1049(w), 1001(w), 933(w), 827(w)

^{1}H-NMR ($CDCl_3$, 400 MHz); 7.27 (1H, t, *J* = 7.5 Hz, H-4), 6.76 (1H, d, *J* = 8.5 Hz, H-5), 6.75 (1H, d, *J* = 7.5 Hz, H-3), 5.58 (1H, m, H-1'), 5.17 (1H, dd, *J* = 2.5, 12.5 Hz, OCH_2-1), 5.14 (1H, dd, *J* = 2.0, 12.5 Hz, OCH_2-1), 3.86 (1H, ddd, *J* = 2.5, 5.0, 8.0 Hz, H-3'), 3.84 (3H, s, OCH_3-2), 3.70 (1H, dd, *J* = 4.5, 5.5 Hz, H-4'), 3.06 (1H, dq, *J* = 2.5, 5.0 Hz, H-6'), 2.96 (1H, dd, *J* = 2.5, 4.0 Hz, H-5'), 2.25 (1H, ddd, *J* = 3.5, 8.5, 14.5 Hz, H-2'), 2.00 (1H, ddd, *J* = 2.5, 7.0, 14.5 Hz, H-2'), 1.33 (3H, d, *J* = 5.0 Hz, H-7') ppm;

^{13}C-NMR($CDCl_3$, 100 MHz); 154.13 (s, C-2), 142.81 (s, C-6), 129.55 (d, C-4), 126.78 (s, C-1), 113.17 (d, C-3), 109.21 (d, C-5), 82.69 (d, C-1'), 71.71 (d, C-4'), 71.10 (t, CH_2O-1), 70.53 (d, C-3'), 58.99 (d, C-5'), 55.24 (q, OCH_3-2), 51.41 (d, C-6'), 37.11 (t, C-2'), 17.21 (q, C-7') ppm.

Compound D1 had the molecular formula $C_{15}H_{20}O_5$ as established by HRESIMS (m/z 303.1216 $[M+Na]^+$, calcd 303.1208), corresponding to six elements of unsaturation. IR adsorption peaks indicated a OH stretching vibration at 3415 (br) cm^{-1}, C=C of aromatic stretching vibration at 1641 and 1555cm^{-1}. On the other hand, no carbonyl bands were present. The IR absorption assignments were was summarized in **Table 4.7**.

Table 4.7 The IR absorption bands assignment of compound D1

Wave number (cm^{-1})	Intensity	Tentative assignment
3415	Strong	O-H stretching vibration of hydroxy
2921, 2860	Weak	stretching vibration of CH_2, CH_3
1641, 1555	Weak, medium	C=C stretching vibration of aromatic
1483, 1418,1333	Weak	C-H bending vibration of CH_2, CH_3
1263, 1049, 1001	Weak	C-O stretching vibration of hydroxyl group
933, 827	Weak	C-H out of planes bending vibration

The ^1H NMR spectrum showed signals at δ_H at 7.27 (t, J = 7.5 Hz, H-4), 6.76 (d, *J* = 8.5 Hz, H-5) and 6.75 (d, *J* = 7.5 Hz, H-3) which were assigned to three adjacent aromatic protons. A methyl doublet (1.33, d, J = 5.0 Hz, H-7'). The ^1H NMR spectrum also exhibited signals assigned to an oxygenated benzylic methylene of OCH_2-1 at δ_H (5.17, dd, *J* = 2.5, 12.5 Hz) and 5.14 (dd, *J* = 2.0, 12.5 Hz). The oxygenated benzylic methine was assigned at 5.58 (m) ppm. Four oxygenated methane (3.86 (ddd, *J* = 2.5, 5.0, 8.0 Hz, H-3'), 3.70 (dd, *J* = 4.5, 5.5 Hz, H-4'), 3.06 (dq, *J* = 2.5, 5.0 Hz, H-6'), 2.96 (dd, *J* = 2.5, 4.0 Hz, H-5'). A signal for a methoxy group appeared at δ_H 3.84 (s) suggested the methoxy was directly attached to aromatic ring. Two methylene sp_3 signals at δ_H 2.25 (ddd, *J* = 3.5, 8.5, 14.5 Hz), 2.00 (ddd, *J* = 2.5, 7.0, 14.5 Hz).

The ^{13}C NMR spectrum showed 15 signals, six of which corresponded to the aromatic system. Since no other double bond was observed, compound D1 must have two rings, presumably a cyclic ether according to the number of oxygen atoms, four oxygenated methines and a benzylic methylene and benzylic methine.

At this point, 2D NMR experiments served to establish the connections between the different structural elements of compound D1. Thus, diagnostic HMBC correlations were observed between the resonance for the hydrogens of the methoxy group and that for the aromatic C-2, between the resonance for the oxygenate benzylic methylene and that for the aromatic C-1 and C-6, and indicated that the benzylic oxygenated methylene was located on the aromatic ring between the methine oxygenated benzylic and methoxy groups. In the gCOSY spectrum, 1H-1H couplings were observed between H_2-2' and H-3', between H-3' and H-4', between H-4' and H-5', between H-5' and H-6', and between H-6' and H_3-7'. The determination of the relative configuration of the four oxygenated methine protons was attempted. The compound D1 was subject to NOE experiments because the differences among the chemical shifts of protons H-3', H-4', H-5', and H-6' was measured. The NOEs observed between H-3' and H-4', between H-4' and H-5', between H-5', H-6' and H3-7' from coupling constant values. The 2D correlation was summarized in **Table 4.8.** gHMBC and NOESY correlation was shown in **Figure 4.15** and **4.16**, respectively. The relative stereochemistry was confirmed by ORTEP structure by x-ray diffraction methods was shown in **Figure 4.17**.

Table 4.8 gCOSY and gHMBC and NOESY correlation of compound D1

Position	1H-NMR	gCOSY	gHMBC	NOESY
3	6.75	H-4	C-1, C-2, C-4	H-4
4	7.27	H-3, H-5	C-2, C-6	H-3, H-5
5	6.76	H-4	C1, C-3	H-4
1'	5.58	H-1'a	-	H-1'a, H-2'b
2'	2.00	H-2'b, H-1', H-3'	C-1', C-4'	H-2'b
	2.25	H-2'a, H-3'	C-1, C-1', C-3'	H-2'a
3'	3.86	H-2'b, H-4'	C-4'	H-2'a, H-2'b
4'	3.69	H-5', H-3'	C-3', C-5', C-6'	-
5'	2.96	H-4'	C-3'	-
6'	3.06	H-7'	C-7'	-
7'	1.33	H-6'	C-5', C-6'	-
CH_2O-1	5.14	CH_2O-1b	C-1, C-6	CH_2O-1b
	5.17	CH_2O-1a	C-1, C-6	CH_2O-1a
OCH_3-2	3.84	-	C-2	-

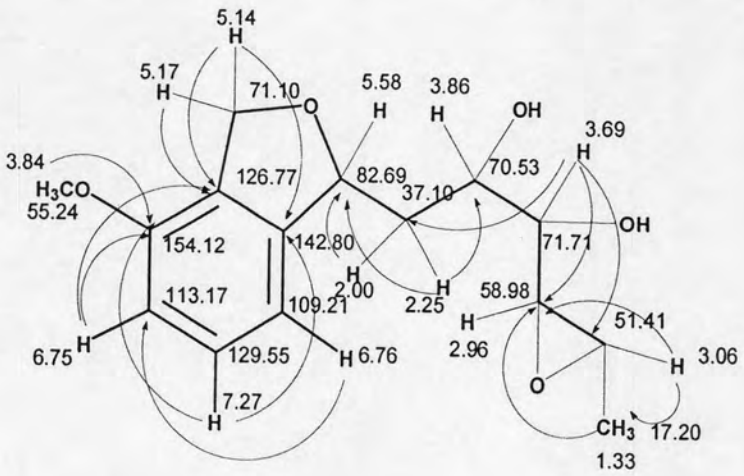


Figure 4.15 gHMBC correlation of compound D1

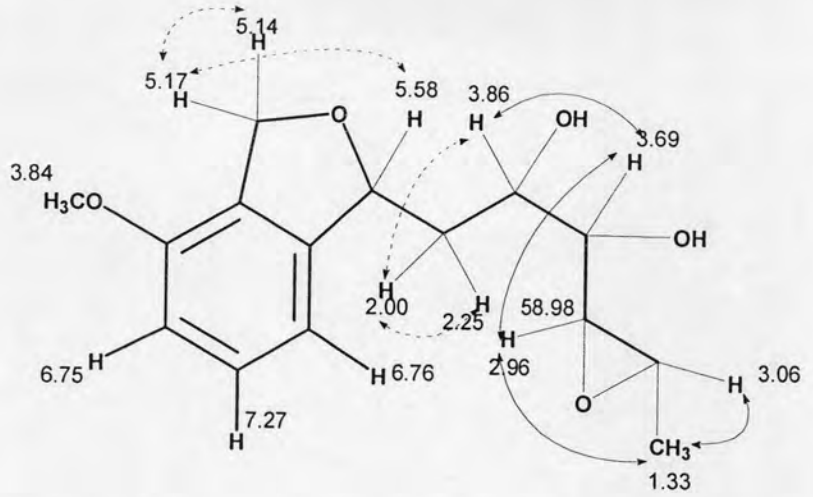


Figure 4.16 NOESY correlation of compound D1

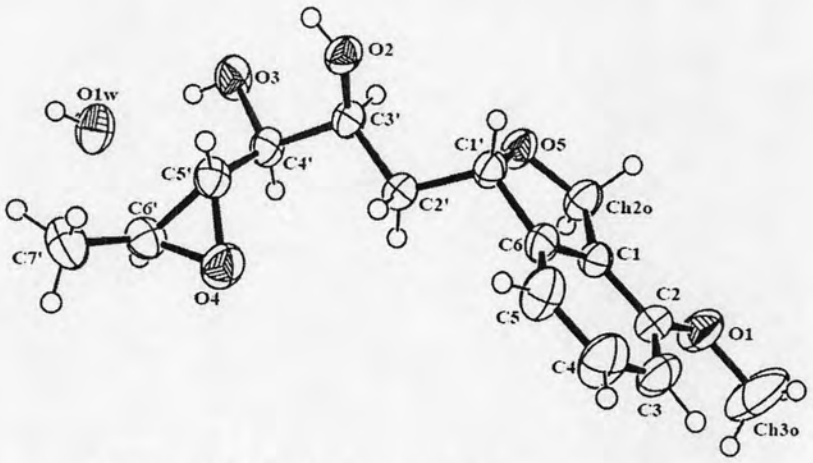
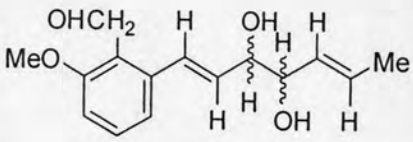


Figure 4.17 ORTEP structure of compound D1

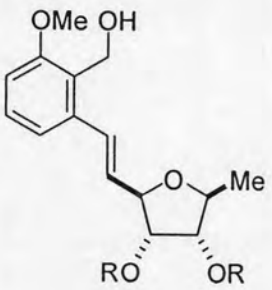
2-Methoxy-6-(3,4-dihydroxyhepta-1,5-dienyl)benzyl alcohol is the common metabolites of *Aspergillus variecolor* reported by Dunn and Johnstone, 1979 and varioxirane and variotriol was found by Malmstrom et. al., 2002. Structure of 2-methoxy-6-(3,4-dihydroxyhepta-1,5-dienyl)benzyl alcohol, varioxirane and varitriol showed in **Figure 4.18.** Based on a hypothetical biogenetic relationship between varioxirane and varitriol , the 3'*R**,4'*R**,5'*S**,6'*R** relative configuration is proposed. The relative configuration at C-3' and C-4' of 2-methoxy-6-(3,4-dihydroxyhepta-1,5-dienyl)benzyl alcohol was not established by Dunn and Johnstone, the biosynthesis of varitriol from 2-methoxy-6-(3,4-dihydroxyhepta-1,5-dienyl)benzyl alcohol, via varioxirane, in *E. variecolor* cannot be ruled out. Enzymatic epoxidation of the 5', 6' double bond of 2-methoxy-6-(3,4-dihydroxyhepta-1,5-dienyl)benzyl alcohol would yield varioxirane and

enzymecatalyzed S_N2 reaction at C-6' in varioxirane by the 3'-OH would yield varitriol. Compound D1 has the related stereochemistry with varitriol and varioxirane in measurement $[\alpha]_D$ of compound D1 (+46) and varitriol (+18.5).

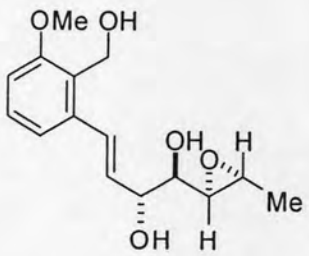
The structure of compound D1, varioxirane and varitriol was compared the biogenetic structure and was assigned as shown,and it was named varioxiranediol. Possible structure of varioxiranediol was shown in **Figure 4.19**. Additionally, Varitriol exhibited selected renal, CNS, and breast cancer cell lines.



2-methoxy-6-(3,4-dihydroxyhepta-1,5-dienyl)benzyl alcohol



Varitriol



Varioxirane

Figure 4.18 The structure of 2-methoxy-6-(3,4-dihydroxyhepta-1,5-dienyl)benzyl alcohol, varitriol and varioxirane

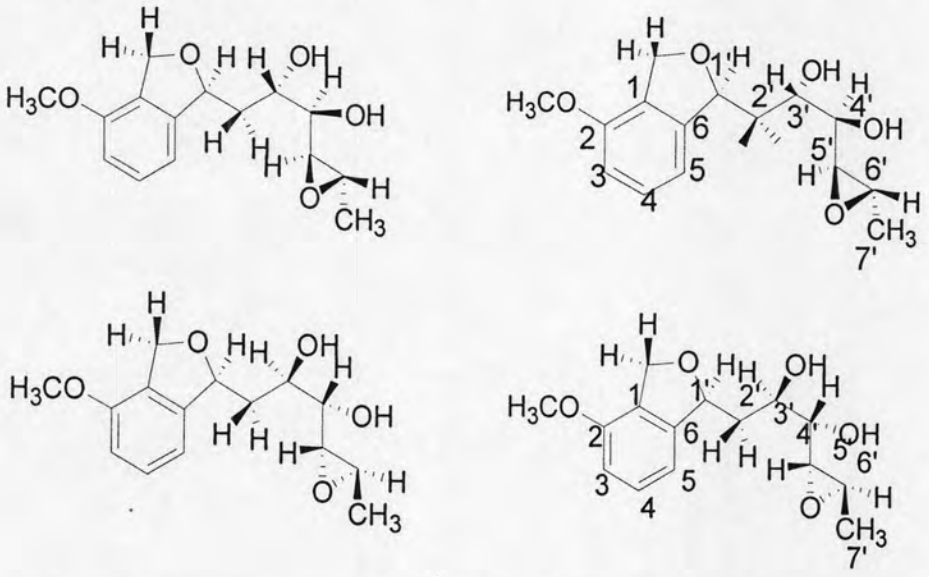


Figure 4.19 Possible structure of varioxiranediol (SSRS or RRSR)

Compound D2

Fraction 91-112 eluted with 65-70 % EtOAc in hexane and washed by hexane and ethyl acetate, respectively to give a white solid of compound D2 (12 mg).

m.p. 119-120 °C;

$[\alpha]_D^{20}$ +8 (c 0.1, EtOH);

HRESIMS $[M+Na]^+$ 321.1322;

λ_{max}(EtOH) (ε) 270 (922), 275 (905) 300 (654); ν_{max} (KBr) 3416 (br, s), 2974 (w), 2924 (w), 2871 (w), 1639 (m), 1595(m), 1559(m), 1486 (w), 1413(w), 1333(w), 1267 (w), 1214 (w), 1051(m), 1004 (m), 935 (w),825 (w), 772 (w) cm^{-1};

^{1}H-NMR ($CDCl_3$, 400 MHz); 7.27 (1H, t, *J* = 8.0 Hz, H-4), 6.79 (1H, d, *J* = 7.5 Hz, H-5), 6.76 (1H, d, *J* = 8.5 Hz, H-3), 5.52 (1H, brd, *J* = 11.0 Hz, H-1'), 5.16 (1H, dd, J = 3.0, 12.0Hz, CH_2O-1'), 5.07 (1H, brd, J = 12.5 Hz, CH_2O-1), 4.14 (1H, dt, *J* = 2.5, 7.5 Hz, H-3'), 4.02 (1H, dq, *J* = 6.0, 6.0 Hz, H-6'), 3.83 (3H, s, OCH_3-2), 3.65 (1H, dd, *J* = 6.5, 6.0 Hz, H-5'), 3.61 (1H, dd, *J* = 6.0, 7.5 Hz, H-4'), 2.37 (1H, ddd, *J* = 2.0, 2.5, 15.0 Hz, H-2'), 1.86 (1H, ddd, *J* = 8.0, 10.5, 15.0 Hz, H-2'), 1.30 (3H, d, *J* = 6.0 Hz, H-7') ppm;

^{13}C-NMR ($CDCl_3$, 100MHz); 154.09 (s, C-2), 143.17 (s, C-6), 129.60 (d, C-4), 125.89 (s, C-1), 113.22 (d, C-5), 109.25 (d, C-3), 84.00 (d, C-1'), 77.33 (d, C-5'), 74.34 (d, C-4'), 72.92 (d, C-3'), 71.41 (t, CH_2O-1), 69.79 (d, C-6'), 55.27 (q, CH_3O-2), 38.63 (t, C-2'), 18.51 (q, C-7') ppm.

Compound D2 had the molecular molecular formula $C_{15}H_{22}O_6$ as established by HRESIMS (m/z 321.1322, calcd 321.1314) corresponding to six elements of unsaturation. IR adsorption peaks indicated a OH stretching vibration at 3416 (br) cm^{-1}, C=C of aromatic stretching vibration at 1639, 1595 and 1559cm^{-1}. On the other hand, no carbonyl bands were present. The IR absorption assignments were summarized in **Table 4.9.**

Table 4.9 The IR absorption bands assignment of compound D2

Wave number (cm^{-1})	Intensity	Tentative assignment
3416	Strong	O-H stretching vibration of hydroxy
2974, 2924, 2871	Weak	stretching vibration of CH_2, CH_3
1639, 1595, 1559	Medium	C=C stretching vibration of aromatic
1486, 1413,1333	Weak	C-H bending vibration of CH_2, CH_3
1267, 1214,	Weak	C-O stretching vibration of hydroxyl group
1051, 1004	Medium	C-O stretching vibration of hydroxyl group
935, 825, 772	Weak	C-H out of planes bending vibration

The ^{1}H NMR spectrum showed signals at δ_H at 7.27 (t, J = 7.5 Hz, H-4), 6.79 (d, *J* = 7.5 Hz, H-5) and 6.76 (d, *J* = 8.5 Hz, H-3) which were assigned to three adjacent aromatic protons. A methyl doublet at δ_H 1.30, d, J = 6.0 Hz, H-7'. The 1H NMR spectrum also exhibited signals assigned to an oxygenated benzylic methylene of OCH_2-1 at δ_H (5.16, dd, *J* = 3.0, 12.0 Hz) and (5.07, brd, *J* = 12.5 Hz). The oxygenated benzylic methine was assigned at δ_H 5.52 (brd, *J* = 11.0 Hz) ppm. Four oxygenated methane (4.14 (dt, *J* = 2.5, 7.5 Hz, H-3'), 4.02 (1H, dq, *J* = 6.0, 6.0 Hz, H-6'), 3.65 (1H, dd, *J* = 6.5, 6.0 Hz, H-5'), 3.61 (1H, dd, *J* = 6.0, 7.5 Hz, H-4'). A signal for a methoxy group appeared at δ_H 3.83 (s) suggested the methoxy was directly attached to aromatic ring. Two methylene sp_3 signals at δ_H 2.37 (ddd, *J* = 2.0, 2.5, 15.0 Hz), 1.86 (ddd, *J* = 8.0, 10.5, 15.0 Hz).

The ^{13}C NMR spectrum showed 15 signals, six of which corresponded to the aromatic system. Since no other double bond was observed, compound D1 must has a rings, presumably a cyclic ether according to the number of oxygen atoms, four oxygenated methines and a benzylic methylene and a benzylic methine.

2D NMR experiments served to establish the connections between the different structural elements of compound D2. Thus, diagnostic HMBC correlations were observed between the resonance for the hydrogens of the methoxy group and that for the aromatic C-2, between the resonance for the oxygenated benzylic methylene and that for the aromatic C-1 and C-6, and indicated that the benzylic oxygenated methylene

was located on the aromatic ring between the methine oxygenated benzylic and methoxy groups. In the gCOSY spectrum, 1H-1H couplings were observed between H_2-2' and H-3', between H-3' and H-4', between H-4' and H-5', between H-5' and H-6', and between H-6' and H_3-7'. The determination of the relative configuration of the four oxygenated methine protons was attempted. The compound D1 was subject to NOE experiments because the differences among the chemical shifts of protons H-3', H-4', H-5', and H-6' was measured. The NOEs observed between H-3' and H-4', between H-4' and H-5', between H-5', H-6' and H3-7' from coupling constant values. The 2D correlation was summarized in **Table 4.10**. gHMBC and NOESY correlation was shown in **Figure 4.20** and **4.21**, respectively.

Compound D2 was measured $[\alpha]_D^{20}$ +8(c 0.1, EtOH) and the coupling constant values of protons at 3', 4', 5', 6' for relative stereochemistry of compound D2 was SSRR or RRSS and was assigned as shown and it was named varitetraol A. Possible structure of varitetraol A was shown in **Figure 4.22**.

Table 4.10 gCOSY and gHMBC and NOESY correlation of compound D2

Position	1H-NMR	gCOSY	gHMBC	NOESY
3	6.76	H-4	C-1, C-2, C-5	OCH_3-2
4	7.27	H-3, H-5	C-2, C-6	-
5	6.79	H-4	C-1, C-3, C-6	H-2'b
1'	5.52	H-2'a	-	H-2'a, H-2'b, H-3'
2'	1.86 2.37	H-2'b, H-1', H-3' H-2'a	C-1', C-C-3' C-3'	H-1', H-2'a H-1', H-2'b, H-3', H-5
3'	4.14	H-4'	C-1'	H-1', H-2'b
4'	3.61	H-3', H-5'	C-2', C-3', C-5'	-
5'	3.65	H-4'	C-3', C-4', C-6', C-7'	-
6'	4.02	H-7'	C-4'	H-7'
7'	1.30	H-6'	C-5', C-6'	H-6'
CH_2O-1	5.07 5.16	CH_2O-1b CH_2O-1a	C-1, C-6 C-1	- -
OCH_3-2	3.83	-	C-2	H-3

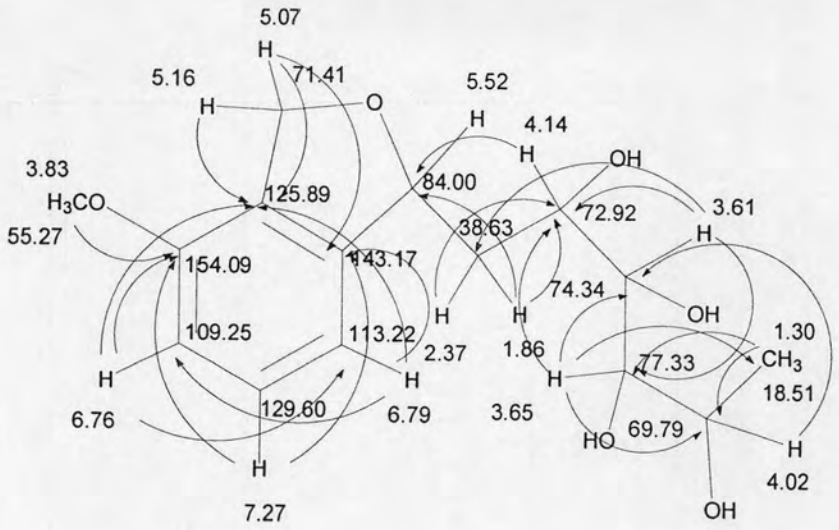


Figure 4.20 gHMBC correlation of compound D2

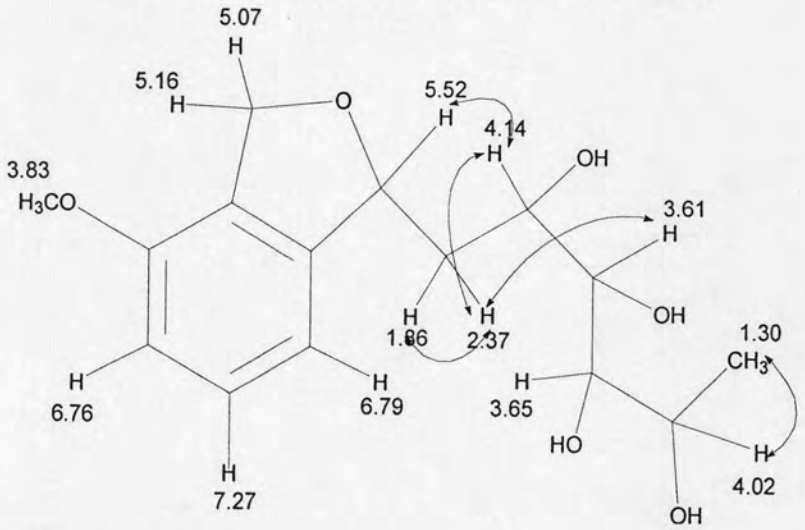


Figure 4.21 NOESY correlation of compound D2

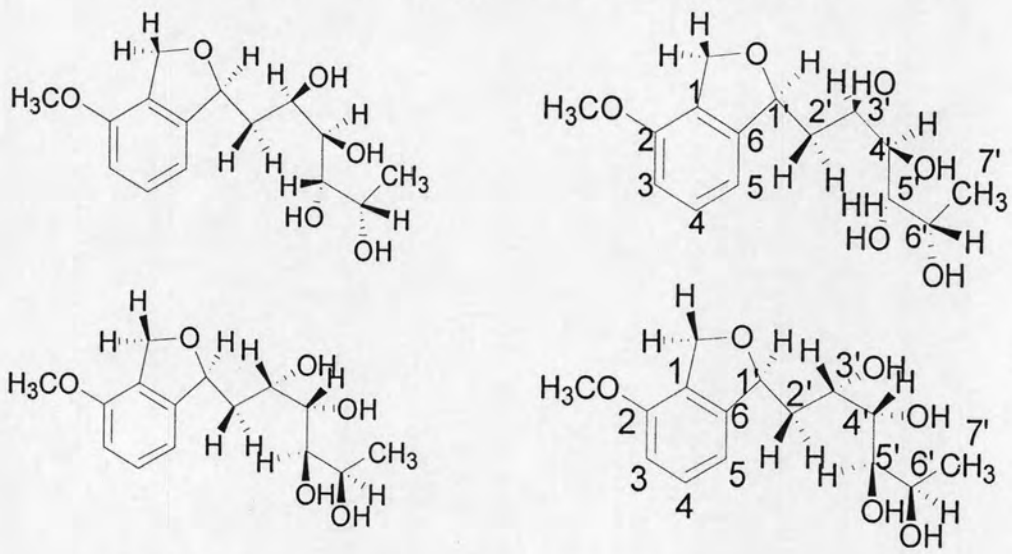


Figure 4.22 Structure and position of varitetraol A (SSRR or RRSS)

Compound E

Fraction132-145 eluted with 75 % EtOAc in hexane then purified by PTLC (silica gel) using 75 % EtOAc in hexane as mobile phase to obtain compound E (4 mg) as brown oil.

$[\alpha]_D^{25}$ -21 (c 0.1,EtOH) ;

ESIMS $[M+Na]^+$ = 179.52;

$\lambda_{max}(\varepsilon)$ 241 (26) nm;

ν_{max} (KBr) 3433(br, s), 2961(w), 2921(w), 2871(w), 1705 (s), 1635(s), 1564 (s), 1412 (s), 1342 (w), 1125 (m), 1080 (m), 1018(w);

^{1}H-NMR (400 MHz, $CDCl_3$); 5.96 (1H, s, H-5), 4.63 (1H, d, *J* = 1.6 Hz, H-3), 4.25 (1H, d, *J* = 2.8 Hz, H-2), 2.47 (2H, dt, H-1'), 1.61 (2H, tq, H-2'), 0.98 (3H, t, J= 7.2 Hz, H-3') ppm;

^{13}C-NMR(100 MHz, $CDCl_3$); 203.20 (s, C-1), 179.16 (s, C-4), 126.34 (d, C-5), 81.58 (d, C-2), 77.57 (d, C-3), 31.74 (t, C-1'), 19.92 (t, C-2'), 13.87 (q, C-3') ppm.

Compound E showed a molecular ion peak at m/z 179.52 $[M+Na]^+$ in the ESIMS and the molecular formula $C_8H_{12}O_3$, IR adsorption implied the presence of hydroxyl (3433 cm^{-1}), carbonyl (1705 cm^{-1}) and olefinic (1635 and 1564 cm^{-1}) functionalities, IR spectrum assignments was summarized in **Table 4.11**.

Table 4.11 The IR absorption bands assignment of compound E

Wave number (cm^{-1})	Intensity	Tentative assignment
3433	Strong	O-H stretching vibration of hydroxy
2961, 2921, 2871	Weak	stretching vibration of CH_2, CH_3
1705	Strong	C=O stretching vibration of carbonyl
1635, 1564	Weak	C=C stretching vibration of olefin
1412, 1342,	Strong, weak	C-H bending vibration of CH_2, CH_3
1125, 1080, 1018	Medium, weak	C-O stretching vibration of hydroxyl group

The ^{1}H-NMR data of compound E showed the presence of a methyl (t) connected with methylene, a sp_2 methine proton, and two oxygenated methine protons that indicated a methyl proton at 0.98 (q) H-3' coupling with 1.61 (tq) H-2'and H-2' coupling with 2.47 (dt) At H-3 at 4.63 (1H, d, J = 1.6 Hz, H-3), coupling with H-2 at 4.25 (1H, d, J = 2.8 Hz, H-2).

The ^{13}C NMR data of compound E revealed 8 carbon signals, due to carbonyl carbons of ketone conjugated double bond, a methyl group, two methine oxygenated carbons and two sp_3 methylene carbons.

On the basis of gHMBC correlations, the methyl group (δ_H 0.98) to the methylene of C1' and C-2'. Correlation of two oxygenated protons at H-2 to C1, C-3 and C-5 and H-3 to C-2, C-4 and C-5 indicated the five member ring connected with propyl chain. From gHMBC correlation assigned the structure in **Figure 4.23**. The chemical structure assigned as a known compound, dihydroterrein in **Figure 4.24**. Terrein was reported by terrein (Dunn et. al., 1975; Malmstrom et. al., 2002).

Table 4.12 gCOSY and gHMBC correlation of compound E

Position	H-NMR	gCOSY	gHMBC
2	4.25	H-3	C-1, C-3, C-5
3	4.63	H-2	C-2, C-4, C-5
5	5.96	-	C-1, C-2, C-3, C-4
1'	2.47	H-2'	C-2, C-3, C-5, C-2', C-3'
2'	1.61	H1', H-3'	C-1', C-3'
3'	0.98	H-2'	C-1', C-2'

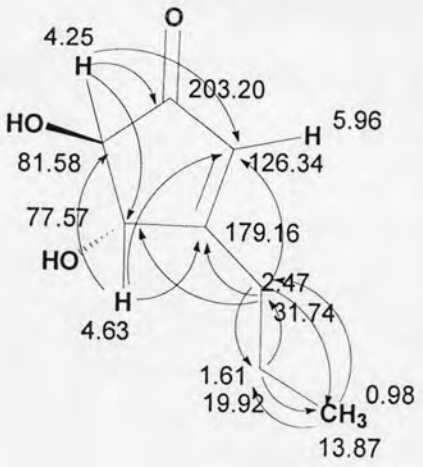


Figure 4.23 gHMBC correlation of compound E

Figure 4.24 Structure and position of dihydroterrein

4.4.3 Metabolites from EtOAc crude extract of mycelium culture in MCzB

Isolation procedure of EtOAc crude extract of *E. variecolor* mycelium cultured in MCzB was shown in **Scheme 3.5**. Total 312 fractions were obtained by silica gel column chromatography. Fraction 23-44 eluted with 15-20 %EtOAc in hexane was washed with hexane and ethyl acetate, respectively to give a white solid of compound A (48 mg). Fraction 45-52 eluted with 20-25% EtOAc in hexane to give compound B (15mg) as white crystal. Compound F1 was precipitate from combined fraction 53-63, eluted with 25 % EtOAc in hexane. After filtration and washing with hexane and ethyl acetate respectively, compound F1 (15 mg) was obtained as a yellow solid. Fraction 71-75 eluted with 25-30 % EtOAc in hexane was washed with hexane and ethyl acetate respectively to obtain compound G1 (10 mg) as orange solid. Compound G2 was precipitated from combined fraction 101-125 eluted with 30-35 % EtOAc in hexane, was precipitated during evaporation. The precipitate was filtered and then washed with hexane and ethyl acetate respectively, to obtain compound G2 (38 mg) as orange solid. Fraction 143-165 eluted with 35-45 % EtOAc in hexane was purified by reverse phase HPLC using methanol as mobile phase to give a orange amorphous of compound H1

(12 mg) from retention time at 17 minutes and a orange solid of compound H2 (8 mg) from retention time at 26 minutes. In addition, Fraction 181-214 eluted with 50 % EtOAc in hexane was washed by ethyl acetate, compound F2 (110 mg) to afford as a yellow solid.

Compound A

Fraction 23-44 eluted with 15-20 %EtOAc in hexane was washed with hexane and ethyl acetate, respectively to give a white solid of compound A (48 mg). Compound A was elucidated by spectroscopic techniques as stellatic acid.

Compound B

Fraction 45-52 eluted with 20-25% EtOAc in hexane to give compound B (15mg) as white crystal. Compound B was analyzed by spectroscopic techniques as ergosterol.

Compound F1

Fraction 53-63 eluted with 25 % EtOAc in hexane was filtered and washed by hexane and ethyl acetate, respectively to obtain compound F1 (15 mg) as a yellow solid.

m.p. 219-220°C;

$[\alpha]_D^{20}$ -38 (c 0.1, $CHCl_3$);

EIMS *m/z* 494 [M^+, 8%], 451 (6), 434 (16), 423 (16), 363(100), 347 (12), 333 (14), 307 (10), and 293 (8);

λ_{max} ($CHCl_3$): (ε) 385 (10167), 294 (12953), 270 (42563), 250 (36428) nm;

ν_{max} (KBr) cm^{-1}: 3447 (OH), 2921, 1746 (C=O), 1637, 1559, 1470, 1423, 1369, 1236, 1077, 1018, and 828 cm^{-1}:

^{1}H-NMR($CDCl_3$,400 MHz) δ_H (ppm): 1.23 (3H, s, CH_3 -17), 1.31 (3H, s, CH_3-18), 1.89 (3H, s, CH_3 -23), 2.08 (3H, s, OAc-25), 2.35 (3H, s, CH_3-24), 2.72 (1H, s, H-20), 3.17 (1H, d, *J* = 8.0 Hz, H-15), 4.31 (1H, dd, *J* = 3.2, 11.2 Hz, H-19b),4.55 (1H, d, *J* = 11.2 Hz, H-19a), 4.63, (1H, d, *J* = 8.0 Hz,H-14b), 4.76 (1H, s, H-22b), 4.81 (1H, s, H-22a), 6.83(1H, d, *J* = 8.4 Hz, H-2), 6.90 (1H, s, H-25), 7.26 (1H, s,H-5), 7.66 (1H, d, *J* = 8.4 Hz, H-3), 13.14 (1H, s, 1-OH) ppm;

^{13}C-NMR (100 MHz, CDCl) δ_C (ppm):183.2 (C-13), 170.0(-O*C*CH$_3$), 162.2 (C-1), 152.5 (C-10), 151.6 (C-11), 150.3 (C-7), 141.4 (C-21), 138.0 (C-6), 135.1 (C-3), 120.3 (C-5), 116.2 (C-12), 115.5 (C-9), 114.9 (C-8), 112.8 (C-22), 110.8 (C-2), 109.1 (C-4), 76.1 (C-14), 66.7 (C-15), 65.5 (C-25), 63.8 (C-19), 57.8 (C-16), 42.4 (C-20), 24.8 (C-17), 22.4 (C-23), 21.3 (-OC*CH$_3$*), 19.8 (C-18), 17.4 (C-24) ppm.

The molecular formula $C_{28}H_{30}O_8$ of compound F1 was deduced from EIMS (m/z 494) the basis of NMR analysis assigned the structure of 14-methoxy-tajixanthone-25-acetate. IR adsorption implied the presence of hydroxyl (3447 cm^{-1}), carbonyl (1746 cm^{-1}) and olefinic (1637 and 1559 cm^{-1}) functionalities. IR spectrum assignments were summarized in **Table 4.13**.

Table 4.13 The IR absorption bands assignment of compound F1

Wave number (cm^{-1})	Intensity	Tentative assignment
3447	Strong	O-H stretching of alcohol
2921	Weak	C-H stretching vibration of CH_2, CH_3
1746	Medium	C=O stretching vibration of carbonyl group
1637 and 1559	Medium	C=C stretching vibration of aromatic ring
1470, 1423 and 1369	Weak	bending vibration of CH_2, CH_3
1236,1077 and 1018	Medium	C-O stretching vibration
828	Medium	C-H out of plane bending vibration

The ^{1}H-NMR spectrum of compound F1 showed the signals of four methyl groups at δ_H 1.26, 1.34, 1.92 and 2.38 ppm, a methoxy group at δ_H 3.37 ppm and a acetoxy group at δ_H 2.11 ppm, three aromatic proton at δ_H 6.86, 7.29 and 7.69 ppm, two olefinic proton at δ_H 4.79 and 4.84 ppm and a hydroxy group at δ_H 13.14 ppm.

The ^{13}C-NMR data of compound F1 showed 28 signals consisting of a carbonyl carbon appeared at δ_C 183.16 ppm, an acetoxy carbon at δ_C 170.01 ppm, methyl carbons appeared at δ_C 19.84, 24.82, 17.39 and 22.42 ppm, a methoxy carbon at δ 56.76 ppm and a methyl of acetoxy at δ_C 21.26 ppm, aromatic carbons appeared at δ 109.11, 110.82, 114.90, 115.49, 116.21, 120.30, 135.10, 137.96, 150.33, 151.58, 152.49 and 162.61 ppm. Two signals of olefinic carbons at δ_C 112.80 and 141.44 ppm and five sp^3 carbons at δ_C 42.44, 63.77, 65.49, 66.69 and 76.08 ppm.

The NMR spectroscopic data (^{1}H, ^{13}C gHMBC and NOESY) showed in **Table 4.14** and molecular ion with m/z 494 could assign the structure of compound F1 as a known compound, 14-methoxy tajixanthone 25-actate. The ^{1}H and ^{13}C data and $[\alpha]_D^{20}$ of compound F1 were compared with 14-methoxy tajixanthone 25-actate. The gHMBC and NOESY correlations were illustrated in **Figure 4.25** and **4.26**, X-ray ORTEP structure was shown in **Figure 4.27** and the structure and position of 14-methoxy tajixanthone 25-actate was shown **Figure 4.28**.

Table 4.14 The correlation of gHMBC and NOESY of compound F1

Position	^{13}C-NMR	^{1}H-NMR	g-HMBC	NOESY
1	162.21 (s)	-	-	-
2	110.82 (d)	6.86 (d)	C-1, C-4, C-9	H-3
3	135.10 (d)	7.69 (d)	C-10, C-14	H-2
4	109.11 (s)	-	-	-
5	120.30 (d)	7.29 (s)	C-7, C-11, C-12, C-24	H-24
6	137.96 (s)	-	-	-
7	150.33 (s)	-	-	-
8	114.90 (s)	-	-	-
9	115.49 (s)	-	-	-
10	152.54 (s)	-	-	-
11	151.58 (s)	-	-	-
12	116.21 (s)	-	-	-
13	183.16 (s)	-	-	-
14	76.08 (d)	4.66 (d)	C-3, C-4, C-10, C-15, C-16, OCH_3-C-14	H-15, H18, OCH_3-C14
15	66.69 (d)	3.20 (d)	C-14, C-16, C-18, OCH_3-C14	H-14, H-17, OCH_3-C14
16	57.82 (s)	-	-	-
17	24.82 (q)	1.26 (s)	C-14, C-15, C-16	H-15, H-18, OCH_3-C14
18	19.84 (q)	1.34 (s)	C-15, C-16, C-17	H-14, C-17
19	63.77 (t)	4.34 (dd)	C-20, C-21C-25	H-20
		4.58 (dd)	C-7, C-20, C-21,C-25	H-20, H-22
20	**42.4 (d)**	**2.75 (s)**	**C-8, C-25, C-21, C-22, C-23**	**H-19, , H-23, H-25**
21	141.44 (s)	-	-	-
22	112.80 (t)	4.79 (s)	C-20, C-21, C-23	H-19, H-21, H-25,
		4.84 (s)	C-20, C-21, C-23	H-20, H-23
23	22.42 (q)	1.92 (s)	C-20, C-21, C-22, C-25, OC=O$\underline{C}H_3$	H-20, H-22, H-25, OC=O$\underline{C}H_3$
24	17.39	2.38	C-5, C-6, C-7	H-5, O=$COCH_3$
25	65.49 (d)	6.93 (s)	C-7, C-8, C-12, C-19, C-20, C-21, $\underline{OC}$=OCH_3,	H-20, H-22, H-23
OH-C25	-	13.14 (s)	-	-
OCH_3-C14	56.77 (q)	3.37 (s)	C-14, C-15	H-15
$\underline{OC}$=OCH_3	170.01 (s)	-	-	-
OC=O$\underline{CH_3}$	21.27 (q)	2.11 (s)	C-23, C-25, $\underline{OC}$=OCH_3	H-23

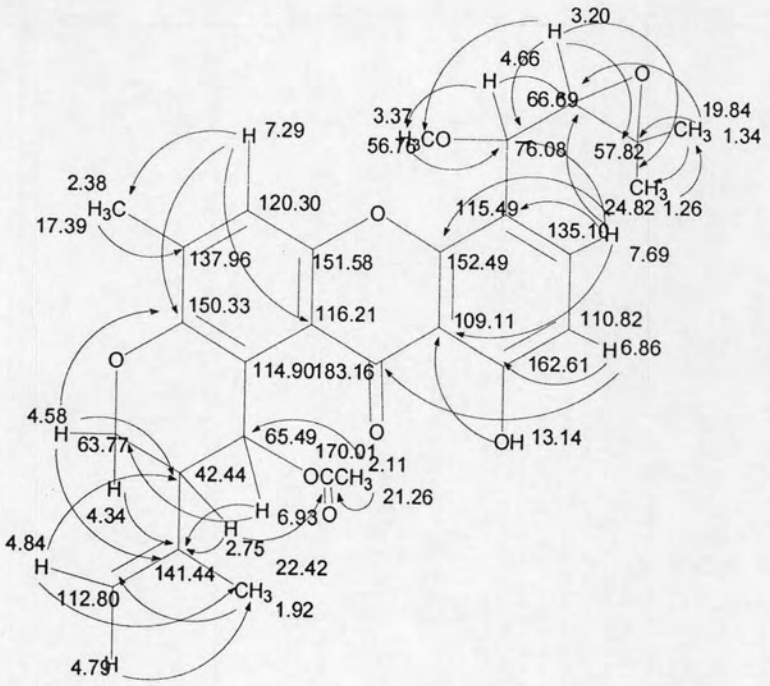


Figure 4.25 The gHMBC correlation of compound F1

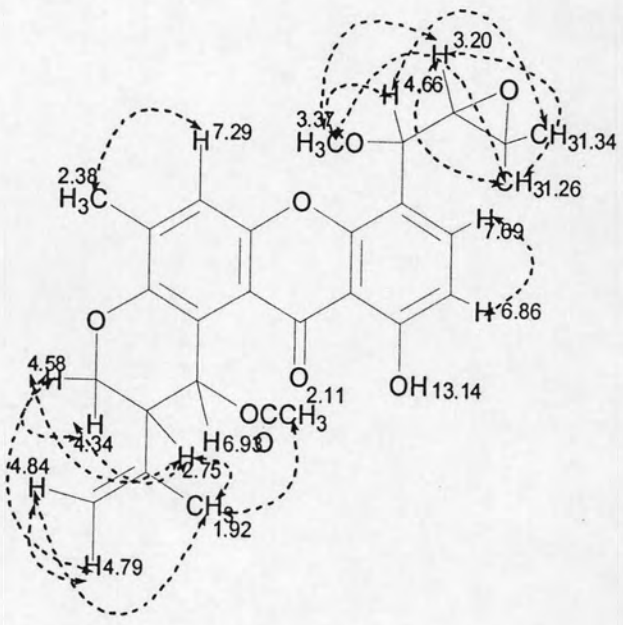


Figure 4.26 The NOESY correlation of compound F1

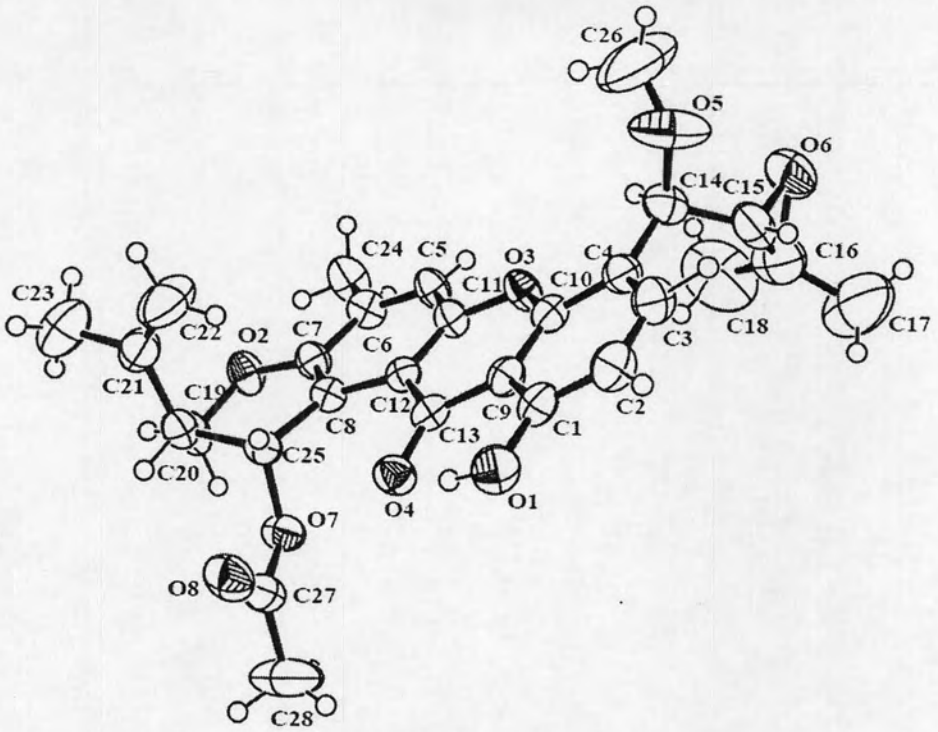


Figure 4.27 ORTEP structure of compound F1

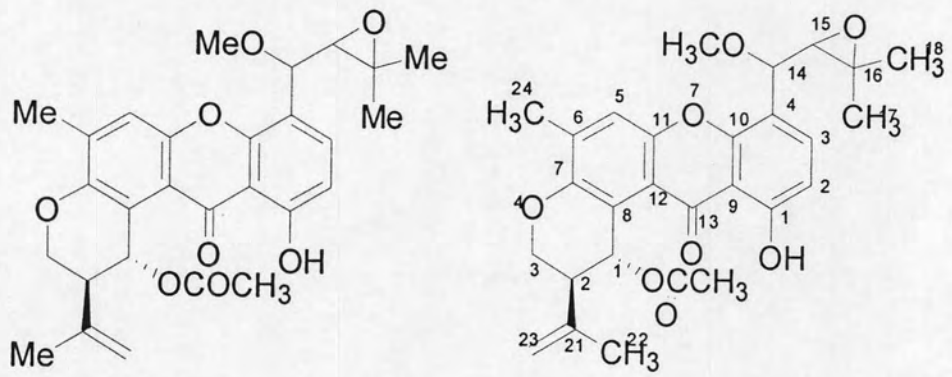


Figure 4.28 Structure and position of 14-methoxytajixanthone-25-acetate

Compound G1

compound G1 was obtained from *E. variecolor* mycelia cultured in MCzB isolated from EtOAc crude extract eluted with 25-30 % EtOAc in hexane and washed by hexane and ethyl acetate, respectively.

m.p. 207-208 °C;

ESIMS ($[M+Na]^+$ = 321.08, $[2M+Na]^+$ =619.16, $[3M+Na]^+$ = 917.25);

λ_{max} ($CHCl_3$): (ε) 270 (9611), 280 (9387), 303 (4865)420.07 (3859) nm;

ν_{max} (KBr) 3430 (br), 3087 (w), 2933(w), 2845(w), 1665 (w), 1625(s), 1598(s), 1558(m), 1493(m), 1456(m),1363(m), 1327(s), 1263(s), 1226(s), 1167(w), 1131(w), 1054(w), 1011(w), 941(w), 882(w), 842(w) cm^{-1};

^{1}H-NMR (400 MHz) 13.08 (1H, s, OH-1), 7.56 (1H, s, H-4), 7.45 (1H, d, *J* = 1.6 Hz, H-7), 7.06 (1H, s, H-2), 6.78 (1H, d, *J* = 2.0 Hz, H-2), 4.02 (3H, s, OCH_3-6), 3.98 (3H, s, OCH_3-8), 2.42 (3H, s, CH_3-3)

^{13}C-NMR (100 MHz) 187.48 (s, C-9), 182.98 (s, C-10), 165.25 (s, C-8), 162.94 (s, C-6), 162.61 (s, C-1), 146.93 (s, C-3), 137.67 (s, C-10a), 132.30 (s, C-4a), 124.80 (d, C-2), 120.00 (d, C-4), 115.20 (s, C-8a), 114.76 (s, C-9a), 104.71 (d, C-7), 103.93 (d, C-5), 56.61 (q, OCH_3-6), 56.05 (q, OCH_3-8), 21.97 (q, CH_3-3)

Compound G1 as a orange solid, m.p. 207-208 °C, which showed molecular ion of TOFMS ($[M+Na]^+$ = 321.08, $[2M+Na]^+$ =619.16, $[3M+Na]^+$ = 917.25) that indicate molecular weight 298, IR adsorption peaks were indicated absorption bands a OH stretching vibration at 3430(br) cm^{-1}, C=O stretching at 1665 and cm^{-1}, C=C of aromatic stretching vibration at 1625, 1598 and 1558 cm^{-1}, C-H stretching of aromatic at 3087 cm^{-1}, C-H stretching of CH_3 at 2933 and 2845 cm^{-1}, C-H bending vibration (m), 1493, 1456,1363, 1327, cm^{-1} and C-O stretching vibration at 1263, 1226, 1167, 1131, 1054, 1011 cm^{-1} and C-H out of plane at 941, 882, 842 cm^{-1}. IR spectrum was shown in **Table 4.15.**

Table 4.15 The IR absorption bands assignment of compound G1.

Wave number (cm^{-1})	Intensity	Tentative assignment
3430	Strong	O-H stretching vibration of hydroxy
3004, 2921, 2851	Weak	stretching vibration of CH_2, CH_3
1665	Weak	C=O stretching vibration of carbonyl
1632, 1592, 1558	Weak	C=C stretching vibration of aromatic
1493,1456 1363	Strong, medium	C-H bending vibration of CH_2, CH_3
1263, 1226,	Medium	C-O stretching vibration
1167 1131, 1054,	Strong	C-O stretching vibration
1011	Weak	C-O stretching vibration
882, 842	Weak	C-H out of planes bending vibration

The 1H-NMR spectrums of compound G1 showed the important peaks that indicated two hydrogen bonding of hydroxyl protons at 13.08 ppm, a methyl proton at 2.42 ppm, a methoxy proton at 4.02 and 3.98 ppm, and aromatic proton at 7.56 , 7.45 and 7.06 ppm.

The 13C NMR spectrums of compound G1 were 16 carbon signals, carbonyl carbons of ketone at 187.48 and 182.98 ppm, a methyl carbon at 21.97 ppm, two methoxy carbon at 56.61 and 56.05 ppm, twelve carbon of aromatic at 165.25, 162.94, 162.61, 146.93, 137.67, 132.30, 124.80, 120.00, 115.20, 114.76, 104.71 and 103.93 ppm.

Waser, et. al., 2005 reported the hydroxylated anthraquinone emodin and endocrocin and 1-hydroxy-6,8-dimethoxy-3-methyl-9,10-anthraquinone was obtained by using improved procedure of Hassall's deprotection of tri-O-methyl emodin. ^{1}H-NMR spectrum of 1-hydroxy-6,8-dimethoxy-3-methyl-9,10-anthraquinone and anthraquinone 3 nearly every peaks was compared in **Table 4.17** and confirmed structure of compound G1 with gHMBC correlation showed in **Table 4.16** and Figure 4.41, respectively. The data were similar to each other and the structure and position of 1-hydroxy-6,8-dimethoxy-3-methylanthraquinone was shown **Figure 4.30.**

Table 4.16 gHMBC correlation of compound G1.

Position	H-NMR	gHMBC
2	7.06	C-1, C-4, C-9a, CH_3-3,
4	7.56	C-2, C-9a, C-10, CH_3-3
5	7.45	C-7, C-8a, C-10
7	6.78	C-5, C-6, C-8, C-8a
OH-1	13.08	C-1, C-2, C-9a
CH_3-3	2.42	C-2, C-3, C-4,
OCH_3-6	4.02	C-6
OCH_3-8	3.98	C-8

Table 4.17 Comparison of 1H-NMR spectrum of compound G1 and 1-hydroxy-6,8-dimethoxy-3-methyl-9,10-anthraquinone.

Position	Anthraquinone 3	1-hydroxy-6,8-dimethoxy-3-methyl-9,10-anthraquinone
2	7.06	7.08 s
4	7.56	7.57 d, s
5	7.45	7.46 d, J = 2.3
7	6.78	6.79, J = 2.3
OH-1	13.08	13.09, s
CH_3-3	2.42	2.43, s
OCH_3-6	4.02	4.03, s
OCH_3-8	3.98	3.99, s

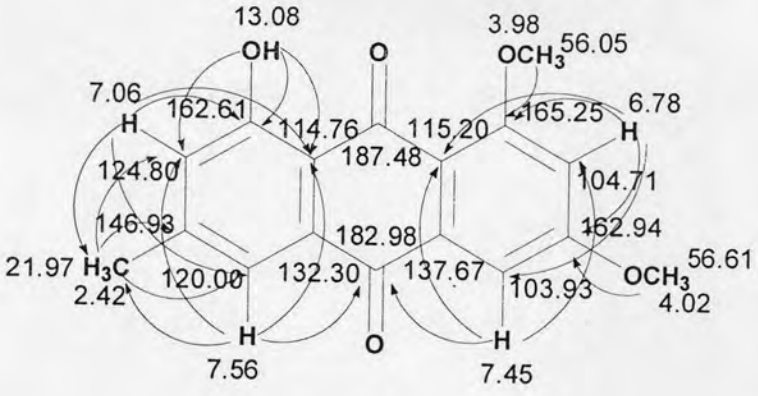


Figure 4.29 gHMBC correlation of compound G1.

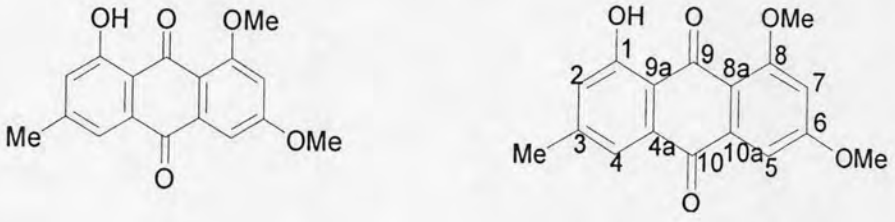


Figure 4.30 Structure and position of 1-hydroxy-6,8-dimethoxy-3-methylanthraquinone.

Compound G2

compound G2 (38 mg) obtained from *Emericella variecolor* mycelia cultured in MCzB and isolated from EtOAc crude extract eluted with 30-35 % EtOAc in hexane and washed by hexane and ethyl acetate, respectively, and was obtained from fermentation broth (MCzB) isolated from EtOAc eluted with 25-30 % EtOAc in hexane and washed by ethyl acetate.

m.p. 224-225 °C;

HRESIMS ($[M+H]^+$ = 315.0843, $[2M+Na]^+$ = 651.1464) (calculate; $[M+H]^+$ 315.0868, $[2M+Na]^+$ 651.1478);

$\lambda_{max}(CHCl_3)$: (ε) 282.96 (12984), 308 (3890), 408.97 (2685); ν_{max} (KBr) 3432(brs), 3004 (w), 2921(w), 2851(w), 1732, 1632(s), 1592(m), 1552(m), 1486(w), 1420 (w), 1340(m), 1270(m), 1217(w), 1154(w),1117(s), 875(w), 795(w) nm;

^{1}H-NMR ($CDCl_3$) 12.88(1H, s, OH-4), 7.69 (1H, s, H-8), 7.58 (1H, s, H-1), 7.07 (1H, s, H-3), 4.09 (3H, OCH_3-7), 4.03 (3H, OCH_3-5), 2.44 (3H,s, CH_3-2) ppm;

^{13}C-NMR 187.56 (s, C-10), 182.03 (s, C-9), 162.60 (s, C-4), 151.90 (s, C7), 147.65 (s, C-2 and s, C-5), 144.94 (s, C-6), 132.47 (C-9a), 127.78 (s, C-8a), 124.32(d, C-

3), 120.23 (d, C-1 and s, C-10a), 114.62 (s, C4a), 106.58 (d, C-8), 61.89 (q, OCH_3-5), 56.69 (q, OCH_3-7), 29.70 (q, CH_3-2) ppm.

Compound G2 as a orange solid, m.p. 224-225 °C, which showed molecular ion of HRTOFMS ($[M+H]^+$ = 315.0843, $[2M+Na]^+$ = 651.1464 that indicate molecular weight 314, IR adsorption peaks were indicated absorption bands a OH stretching vibration at 3432(br) cm^{-1}, C=O stretching at 1732 cm^{-1}, C=C of aromatic stretching vibration at 1632, 1592 and 1552 cm^{-1}, C-H bending vibration 1486, 1420 and 1340 cm^{-1} and C-O stretching vibration at 1270, 1217, 1154 and 1117cm^{-1}. IR absorption bands showed in Table 4.18.

Table 4.18 The IR absorption bands assignment of compound G2

Wave number (cm^{-1})	Intensity	Tentative assignment
3432	Strong	O-H stretching vibration of hydroxy
3004, 2921, 2851	Weak	stretching vibration of CH_2, CH_3
1732	Weak	C=O stretching vibration of carbonyl
1632, 1592, 1552	Medium	C=C stretching vibration of aromatic
1486,1420 1340	Medium, weak	C-H bending vibration of CH_2, CH_3
1270, 1217, 1154	Medium, weak	C-O stretching vibration of hydroxyl group
1117	Strong	C-O stretching vibration of hydroxyl group
875, 795	Weak	C-H out of planes bending vibration

The 1H-NMR spectrums of compound G2 showed the important peaks that indicated two hydrogen bonding of hydroxyl protons at 12.88 ppm, a methyl proton at 2.44 ppm, a methoxy proton at 4.09 and 4.03 ppm, and aromatic proton at 7.69 , 7.58 and 7.07 ppm.

The 13C NMR spectrums of compound G2 showed 16 carbon signals, carbonyl carbons of ketone at 187.56 and 182.03 ppm, a methyl carbon at 21.93 ppm, two methoxy carbon at 61.89 and 56.69 ppm, twelve carbon of aromatic at 162.60, 151.90 (147.65x2), 144.94, 132.47, 127.78, 124.32, (120.23 x2), 114.62, 106.58 ppm.

Barba et. al., 1994 reported cassanes and anthraquinone from *Chamaecrista greggii* and 4,6-dihydroxy-5,7-dimethoxy-2-methylanthraquinone was isolated from roots and barks of *Chamaecrista greggii*. 1H-NMR spectrum of 4,6-dihydroxy-5,7-dimethoxy-2-methylanthraquinone and compound G2 nearly every peaks that showed in **Table 4.20** and confirms structure of anthraquinone with gHMBC showed in the **Table 4.19** and **Figure 4.31**, respectively. The data were similar to each other and the structure and position of 4,6-dihydroxy-5,7-dimethoxy-2 –methylanthraquinone was shown **Figure 4.32.**

Table 4.19 gHMBC Correlation of compound G2

Position	H-NMR	gHMBC
1	7.58	C-3, C-9, C-4a
3	7.07	C-1, C-4, C-4a, CH_3-2
8	7.69	C-6, C-7, C-9, C-8a, C-10a
CH_3-2	2.44	C-1, C-2, C-3
OH-4	12.88	C-3, C-4, C-4a
OCH_3-5	4.03	C-5
OCH_3-7	4.09	C-7

Table 4.20 Comparison of ^{1}H-NMR spectrum of 4,6-dihydroxy-5,7-dimethoxy-2-methylanthraquinone and compound G2

Position	Compound G2	4,6-dihydroxy-5,7-dimethoxy -2-methylanthraquinone
1	7.58 s	7.58 br, d (2)
3	7.07 s	7.07 br, d (2)
8	7.69 s	7.60 s
2-Me	2.44 s	2.44 br s
4-OH	12.88 s	12.97 s
5-OMe	4.03 s	4.02 s
7-OMe	4.09 s	4.09 s

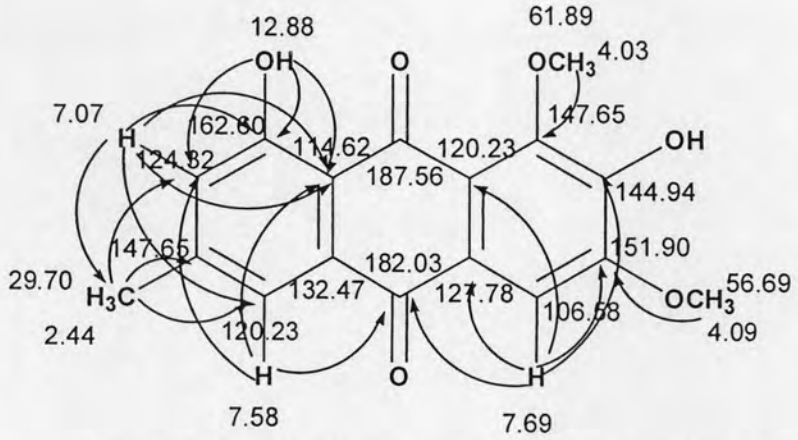


Figure 4.31 gHMBC correlation of compound G2

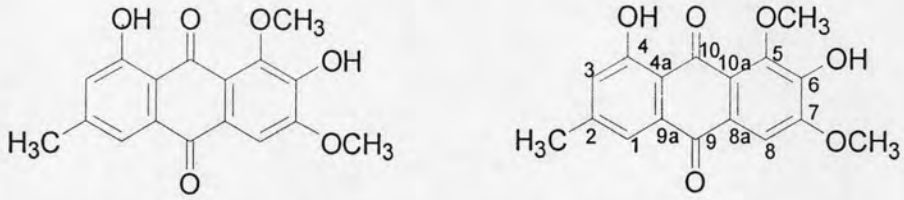


Figure 4.32 Structure and position of 4,6-dihydroxy-5,7-dimethoxy-2 –methyl anthraquinone

Compound H1

compound H1 obtained from *Emericella variecolor* mycelia culture in MCzB and isolated from EtOAc eluted with 35-45 % EtOAc in hexane and was purified by HPLC reverse phase column chromatography using methanol as eluent, compound H1 afforded from retention time at 17 minutes.

m.p. 229-230 °C;

$[\alpha]^{20}_{D}$ +171° (c 0.1, $CHCl_3$);

$[M+H]^+$ 679.3629;

λ_{max} ($CHCl_3$) (ε) (287.98, 22971.4), (434.99, 8848.5) nm:

ν_{max} (KBr) 3549(br, s), 3473 (br, s), 3415(br, s) , 3233(br, w), 2957(m), 2924(m), 2866(w), 1718(w), 1665(w), 1616(s), 1569(w), 1443(m), 1412(m), 1378(m), 1311(m), 1278(s), 1200(w), 1164(w), 1098 (w),1040(w) cm^{-1};

^{1}H-NMR ($CDCl_3$,400 MHz) δ_H: 12.32 (1H, s, 1'-OH), 12.01 (1H, s, 8'-OH), 7.57(1H, s, H-5'), 7.37(1H, s, H-4'), 7.03(1H, s, H-7'), 5.72(1H, d, *J* = 5.6 Hz, H-11), 5.21(1H, dd, *J* = 7.2, 15.2 Hz, H-23), 5.12(1H, dd, *J* = 8.0, 15.2 Hz, H-22), 5.03(1H, s, H-

7), 4.77(1H, s, H-6), 4.00(1H, m, H-3), 2.43(3H, s, 6'-CH_3) , 2.33(2H, m, H-12), 2.26(1H, m, H-1), 2.21(1H, d, *J* = 14.8, H-14), 2.21(1H, m, H-12), 2.17(1H, dd, *J* = 1.6, 11.6 Hz, H-4), 2.08(1H, m, H-2), 2.01(1H, m, H-20), 1.90(1H, brd, *J* = 13.6 Hz, H-1), 1.82(1H, dd, *J* = 6.8, 13.2 Hz, H-24), 1.71(1H, m, H-16), 1.68(1H, m, H-2), 1.66(1H, m, H-15), 1.63(1H, dd, *J* = 11.2, 14.0 HZ, H-4), 1.45(1H, dt, *J* = 7.2, 12.8 Hz, H-25), 1.33(2H, m, H-15), 1.31(1H, m, H-16), 1.23(3H, d, J = 9.2 Hz, H-19), 1.29(1H, m, H-17), 1.00(3H, d, *J* = 6.4 Hz, H-21), 0.89(3H, d, *J* = 6.4 Hz, H-28), 0.81(3H, d, *J* = 6.8 Hz, H-27), 0.79(3H, d, *J* = 6.8 Hz, H-26), 0.55(3H, s, H-18) ppm.

^{13}C-NMR ($CDCl_3$, 100 MHz) δ_C : 193.42(s, C-9'), 181.19(s, C-10'), 162.38(s, C-8'), 153.54(s, H-1'), 148.57(s, C-6'), 147.41(s, C-3'), 140.99(s, C-8), 139.32(s, C-9),135.94(s, C-2'), 135.24(d, C-22), 133.40(s, C-5a'), 132.24(d, C-23), 126.03(s, C-4a'), 124.20(d, C-11), 123.98(d, C-7'), 121.09(d, C-5'), 115.09(d, C-7), 113.77(s, C-8a'), 110.89(d, C-4'), 110.89(s, C-1a') 78.05(s, C-5), 73.62(d, C-6), 66.42(d, C-3), 55.87(d, C-17), 50.87(d, C-14), 42.78(d, C-24), 42.53(s, C-13), 42.00(t, C-12), 42.00(s, C-10), 40.30(d, C-20), 35.40(t, C-4), 33.04(d, C-25), 30.51(t, C-2), 29.45(t, C-1), 28.64(t, C-16), 24.20(q, C-19), 22.88(t, C-15), 22.19(q, CH_3-C6'), 20.68(q, C-21), 19.94(q, C-27), 19.60(q, C-26), 17.62(q, C-28), 11.62(q, C-18) ppm.

Compound H1 as a orange plate solid, m.p. 229-230 °C, which showed the $[M+H]^+$ at m/z 679.3629 in HRTOFMS and on the basis of NMR analysis assigned the structure of compound H1.

The IR spectrum of compound H1 showed that absorption peaks were assigned as summarized in Table and indicated absorption bands were a O-H stretching vibration at 3549, 3473 and 3415 cm^{-1}, C-H stretching vibration of aromatic ring at 3233 cm^{-1}, C-H stretching vibration at 2957, 2924, 2866 cm^{-1}, C=O stretching vibration at 1718 cm^{-1}, C=C stretching vibration at 1665, 1616 and 1569cm^{-1}, CH bending vibration at 1443, 1412, 1378, 1311 cm^{-1}, C-O stretching vibration at 1278, 1200, 1164, 1098,1040cm^{-1}. IR absorption assignment showed in **Table 4.21**.

Table 4.21 The IR absorption bands assignment of compound H1

Wave number (cm^{-1})	Intensity	Tentative assignment
3549, 3473, 3415, 3233	Strong	O-H stretching vibration of hydroxy
2957, 2924, 2866	Medium, weak	stretching vibration of CH_2, CH_3
1718	Weak	C=O stretching of carbonyl
1665, 1616, 1569	Strong, Weak	C=C stretching vibration of olefin and aromatic
1443, 1412, 1378	Medium	C-H bending vibration of CH_2, CH_3
1311, 1278, 1200,	Medium, weak	C-O stretching vibration of hydroxyl group
1164, 1098, 1040	Weak	

The ^{1}H-NMR spectrum of compound H1 indicated that it possesses two phenolic hydroxy proton at 12.32 and 12.01 ppm three aromatic proton at 7.57, 7.37 and 7.03 ppm, seven proton of methyl at 2.43, 1.23, 1.00 0.89, 0.81, 0.79, 0.55 ppm, four olefinic proton at 5.72, 5.21, 5.12 and 5.03 ppm, two proton connecting with heteroatom at 4.77 and 4.00 ppm, proton of hydrocarbon (CH_2 and CH) at 2.43-1.20 ppm.

The ^{13}C-NMR spectrum showed 43 signals assigned that 15 carbon of anthraquinone and 28 carbon of steroid and indicated that two carbon of carbonyl ketone at 193.42 and 181.19 ppm, two phenolic hydroxy carbon appeared at δ 162.38 and 153.54 ppm, seven methyl carbon at 24.20, 22.19, 20.68, 19.94, 19.60, 17.62, 11.62 ppm, six olefinic carbon at at 140.99, 139.32, 135.24 132.24, 124.20, and 115.09 ppm, ten aromatic carbon at 148.57, 147.41, 135.94, 133.40, 126.03, 123.98, 121.09, 113.77, 110.89, 110.89 ppm.

Compound H1 composed of anthraquinone and steroid. Anthraquinone part were likely to 1,2,3,8-tetrahydroxyemodin or 7-hydroxyemodin was shown in **Figure 4.35**. Comparison of 1H-NMR chemical shifts of anthraqionone part of compound H1 and 7-hydroxyemodin shown in **Table 4.22**. The structure was confirmed the structure by gHMBC and NOESY in **Figure 4.33** and **4.34**. The structure and position of evanthrasterol A showed in **Figure 4.36**.

Table 4.22 Comparison of 1H-NMR chemical shifts of anthraqionone part of compound H1 and 7-hydroxyemodin.

Position	Anthraquinone part	7-hydroxyemodin
4	7.37	7.17, s
5	7.57	7.30,d , J = 1.5 Hz
7	7.03	6.98
6-CH3	2.43	2.28
1-OH	12.32	-
8-OH	12.01	-

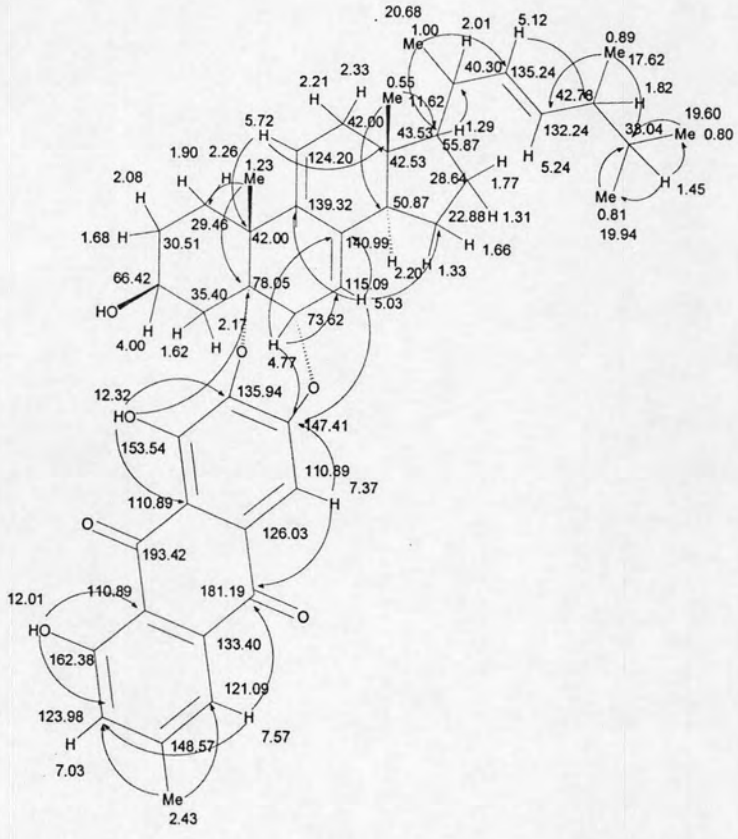


Figure 4.33 gHMBC correlation of compound H1

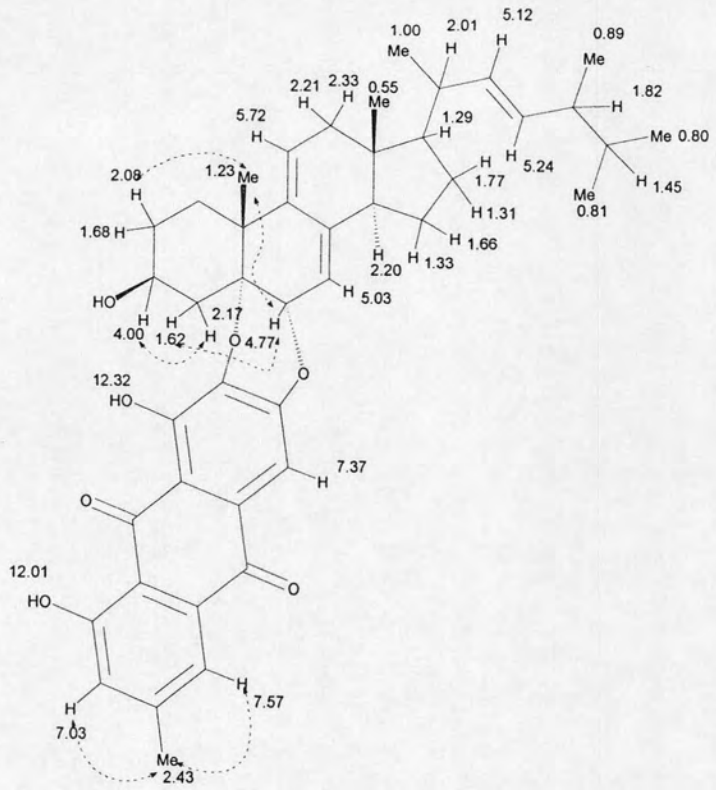


Figure 4.34 NOESY correlation of compound H1

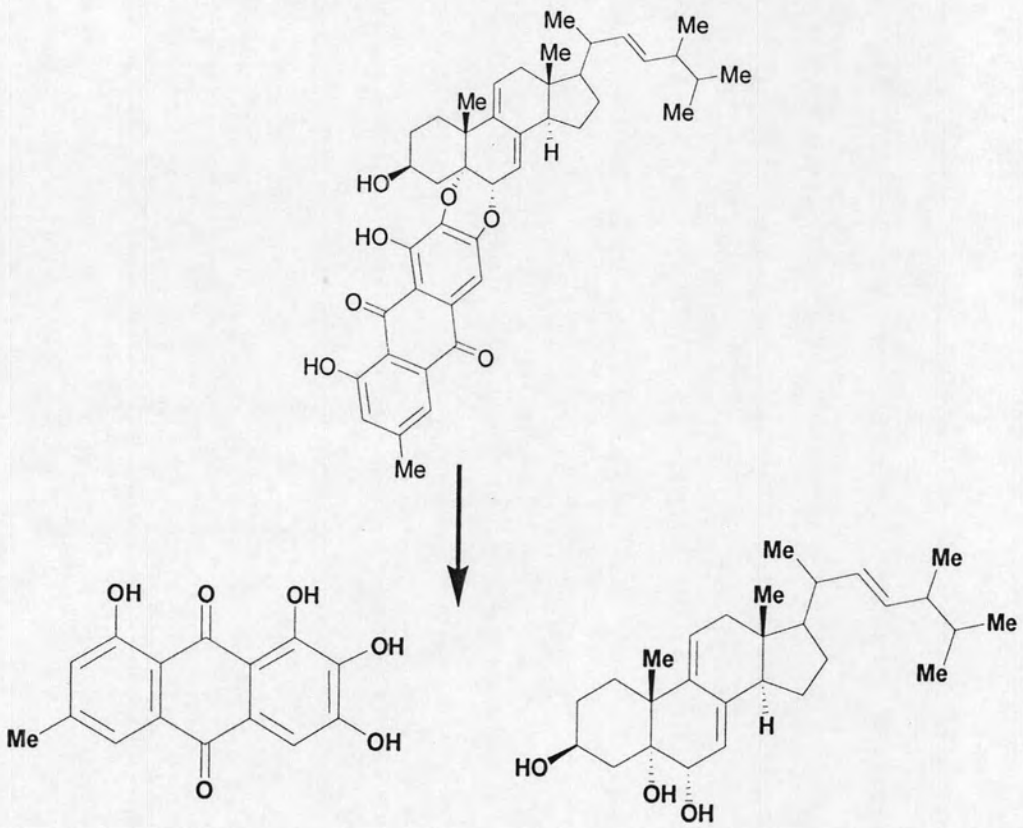


Figure 4.35 Composition of compound H1

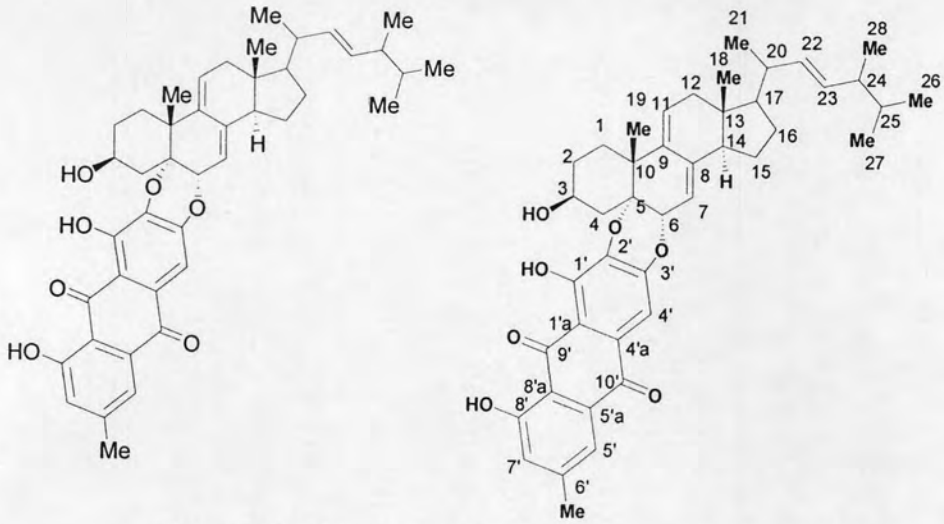


Figure 4.36 Structure and position of evanthrasterol A

A sterol portion fused at C-5 and C-6 to an atrovenetin-like part, have been isolated from a fungus *Sirococcus* sp. that causes Siricoccus shoot blight of spruce. Sirosterol is an adduct of ergosterol and atrovenetin. Dehydroazasirosterol is an adduct of 9(1l)-dehydroergosterol and an azaatrovenetin (Ayer and Ma, 1992). Structure of siroterol, ergoterol and atrovenetin showed in **Figure 4.37**.

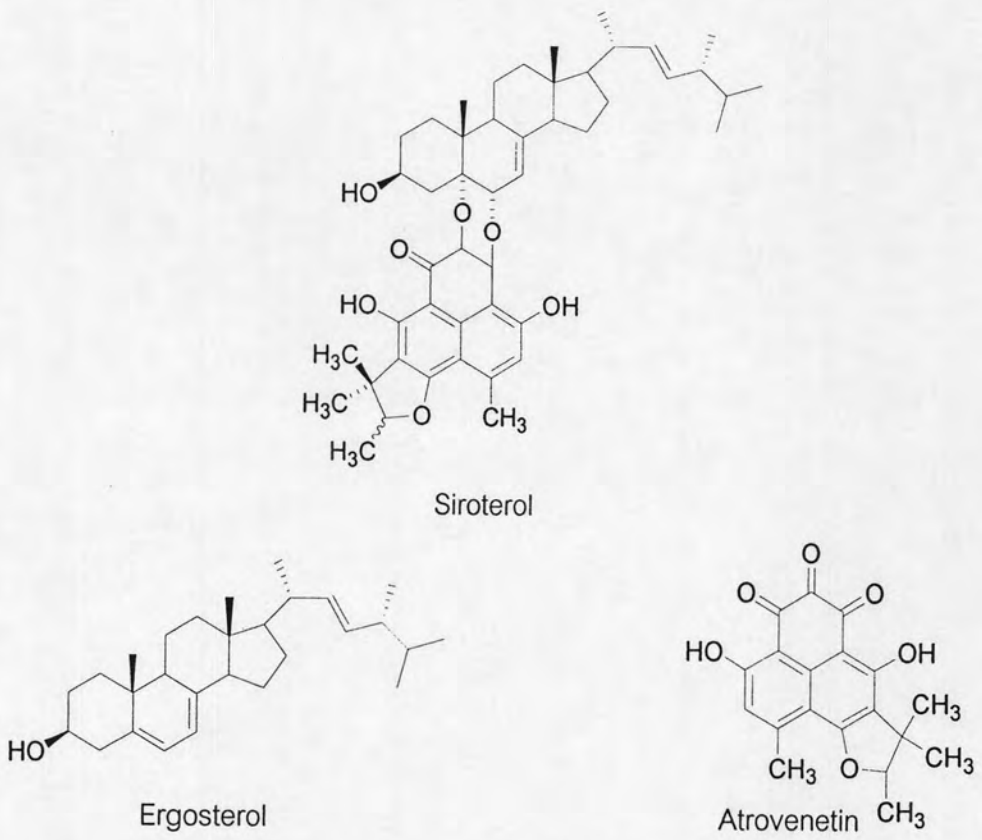


Figure 4.37 Structure of sirostrerol, ergosterol and atrovenetin

Compound H2

compound H2 obtained from *Emericella variecolor* mycelia culture in MCzB and isolated from EtOAc eluted with 35-45 % EtOAc in hexane and was purified by HPLC reverse phase column chromatography using methanol as eluent, compound H1 afforded from retention time at 26 minutes

m.p. 242-243 °C;

$[M+H]^+$ 695.3594,

$[\alpha]^{20}_D$ +172° (c 0.1, $CHCl_3$);

λ_{max} ($CHCl_3$): (ε) (274.95, 15975), 292.06, 18424.5), 493.06, 9443), 515.04, 8094), 529, 6837) nm;

ν_{max} (KBr) 3546(br,s), 3476(br,s), 3412(br,s), 3236(br, w), 2951(s), 2924(s), 2863(m), 1723(w), 1595(s), 1415(s), 1336(s), 1308(s), 1263(s), 1194(m), 1161(m), 1122(m), 1092(m), 1034(m) cm^{-1};

^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 13.48(s, OH-5'), 12.48(1H, s, OH-1'), 12.25(1H, s, OH-8'), 7.43(1H, s, H-4'), 7.06(1H, s, H-7'), 5.72(1H, d, *J* = 5.6 Hz, H-11), 5.17(1H, dd, *J* = 7.2, 15.2 Hz, H-23), 5.13 (1H, dd, *J* = 7.6, 15.2 Hz, H-22), 5.03 (1H, s, H-7) , 4.78(1H, s, H-6), 3.99(1H, ddd, H-3), 2.37 (3H, s, Me-6'), 2.25(1H, m H-12), 2.15 (1H, m, H-12), 2.15 (1H, m, H-14), 2.06(2H, m, H-4), 2.04(1H, m, H-2), 1.92(1H, m, H-20), 1.83 (1H, m, H-1), 1.75 (1H, m, H-24), 1.45 (1H, sept, J = 6.4 Hz, H-25), 1.36 (2H, m, H-16), 1.28 (2H, m, H-15), 1.25 (1H, m, H-1),1.24 (1H, m H-17), 1.23 (3H, s, H-19), 1.00 (3H, d, J = 6.4 Hz, H-21), 0.88 (, 0.81 (3H, d, *J* = 6.8 Hz, H-27) , 0.79 (3H, d, *J* = 6.4 Hz, H-26), 0.55 (3H, s, H-18) ppm;

^{13}C-NMR (100 MHz, CDCl) δ_C (ppm): 189.50 (s, C-9'), 185.85 (s, C-10'), 157.46 (s, C-5'), 157.11 (s, C-8'), 153.51 (s, C-1'), 147.28 (s, C-3'), 141.07 (s, C-8), 140.91 (s, C-6'), 139.29 (s, C-9), 136.17 (s, C-2'), 135.23 (d, C-22), 132.25 (d, C-23), 128.51 (d, C-7'), 125.76 (s, C-4a'), 124.26 (d, C-11), 115.03 (d, C-7), 111.72 (s, C-5a'), 111.32 (s, C-1a'), 110.80 (s, C-8a'), 110.24 (d, C-4'), 78.17 (s, C-5), 73.61 (d, C-6), 66.41 (d, C-3), 55.87 (d, C-17), 50.87 (d, C-14), 42.78 (d, C-24), 42.53 (s, C-13), 41.99 (t, C-12), 40.30 (s, C-10), 35.42 (t, C-4), 33.03 (d, C-25), 30.56 (t, C-2), 29.41 (t, C-1), 28.62 (t, C16), 24.20 (q, C-19), 22.87 (t, C-15), 20.67 (q, C-21), 19.92 (q, C-27), 19.59 (q, C-26), 17.61 (q, 28), 16.52 (q, Me-C-6'), 11.62 (q, C-18) ppm.

Compound H2 as a yellow plate solid, m.p. 242-243 °C, which showed the $[M+H]^+$ at m/z 695.3594 in HRTOFMS and on the basis of NMR analysis assigned the structure of compound H2.

The IR spectrum showed that absorption peaks were assigned as summarized in Table and indicated absorption bands were a O-H stretching vibration at 3546, 3476, 3412cm^{-1}, C-H stretching vibration of aromatic ring at 3236 cm^{-1}, C-H stretching vibration at 2951, 2924, 2863 cm^{-1}, C=O stretching vibration at 1723 cm^{-1}, C=C stretching vibration at 1595 cm^{-1}, CH bending vibration at 1415, 1336, 1308cm^{-1}, C-O stretching vibration at 1263, 1194, 1161, 1122, 1092, 1034 cm^{-1}. IR spectrum was shown in **Table 4.23**.

Table 4.23 The IR absorption bands assignment of compound H2

Wave number (cm^{-1})	Intensity	Tentative assignment
3546, 3476, 3412, 3236	Strong	O-H stretching vibration of hydroxy
2951, 2924, 2863	Medium, weak	stretching vibration of CH_2, CH_3
1723	Weak	C=O stretching of carbonyl
1595	Strong, Weak	C=C stretching vibration of olefin and aromatic
1415, 1336, 1308,	Medium	C-H bending vibration of CH_2, CH_3
1263, 1194, 1161,	Medium, weak	C-O stretching vibration of hydroxyl group
1122, 1092, 1034	Weak	

The ^{1}H-NMR spectrum of compound H2 indicated that it possesses two phenolic hydroxy proton at 13.48, 12.28, 12.25 ppm, two aromatic proton at 7.06 and 7.43 ppm, seven proton of methyl at 2.37,1.23 , 0.55 , 0.88 , 0.81 , 0.79 , 1.00 ppm, four olefinic proton at 5.72, 5.17, 5.13 and 5.03 ppm, two proton connecting with heteroatom at 4.78 and 3.99 ppm. Proton of hydrocarbon (CH_2 and CH) at 2.25 -1.20 ppm.

The ^{13}C-NMR spectrum showed 43 signals assigned that 15 carbon of anthraquinone and 28 carbon of isteroid and indicated that two carbon of carbonyl

ketone at 193.42 and 181.19 ppm, three phenolic hydroxy carbon appeared at δ 157.46, 157.11, 153.51 ppm, seven methyl carbon at 24.20, 20.67, 19.92, 19.59, 17.61, 16.52 11.62 ppm, six olefinic carbon at 140.91, 139.29, 135.23, 132.25, 124.26, 115.03 ppm, nine aromatic carbon at 147.28, 141.07, 136.17, 128.51, 125.76, 111.72, 111.32, 110.80 110.24 ppm.

The structure was confirmed the structure by gHMBC and NOESY in **Figure 4.38** and **4.39**, respectively. The structure and position of evanthrasterol B showed in **Figure 4.40**.

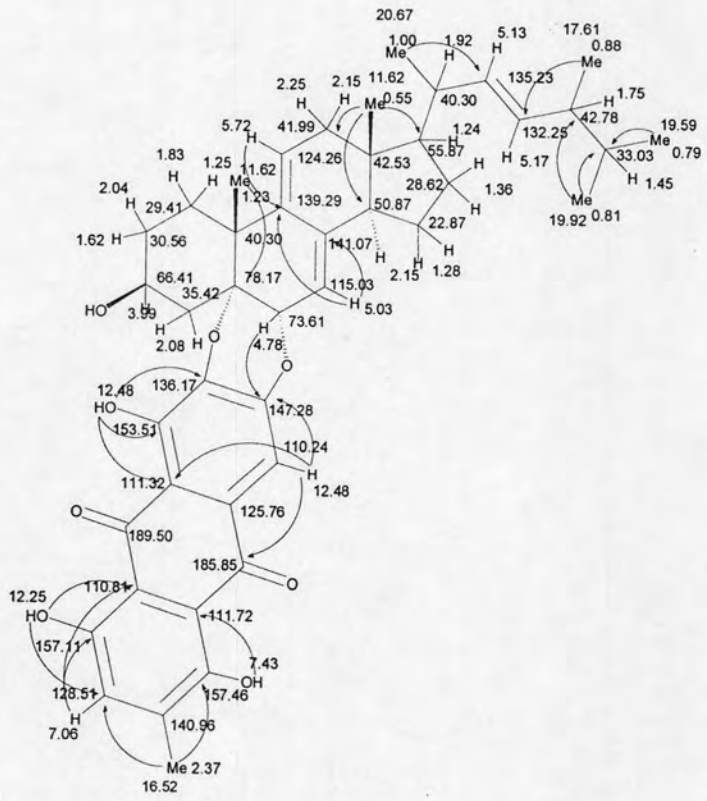


Figure 4.38 gHMBC correlation of compound H2

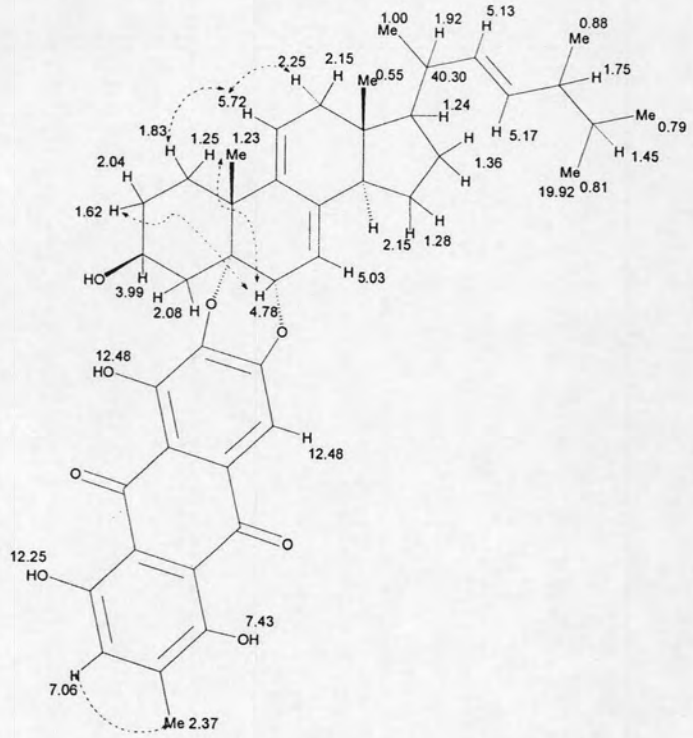


Figure 4.39 NOESY correlation of compound H2

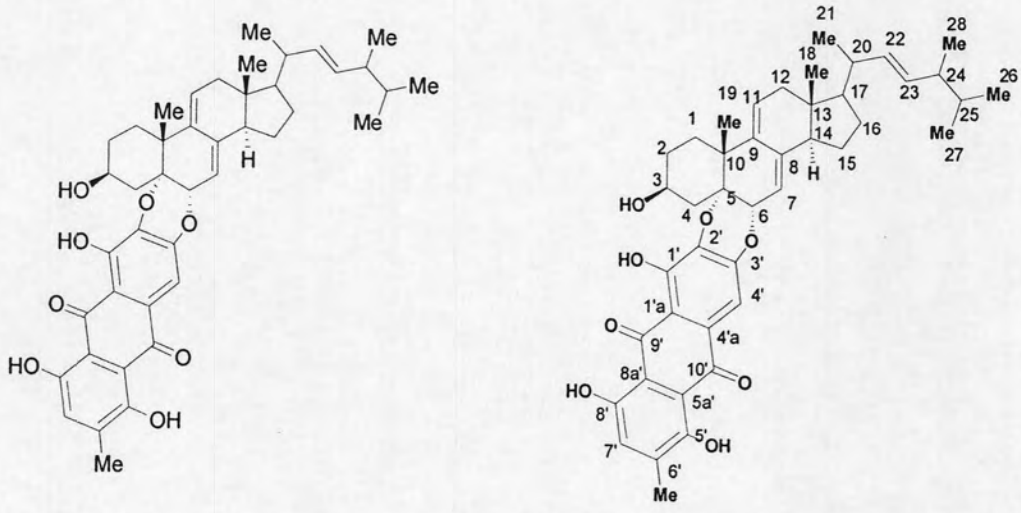


Figure 4.40 Structure and position of evanthrasterol B

Compound F2

compound F2 was afforded from *Emericella variecolor* mycelia cultured in MCzB isolated from EtOAc crude extract eluted with 50 % EtOAc in hexane and washed by ethyl acetate.

m.p. 194-195 °C;

$[\alpha]^{20}_{D}$ -76° (c 0.23, $CHCl_3$);

λ_{max} ($CHCl_3$): (ε) 245 (26928), 255 (33475), 270 (45646), 280 (39579), 300 (13253), and 400 (9002) nm;

ν_{max} (KBr) cm^{-1}: 3486 (OH), 3073, 2976, 2883, 1797 and 1738 (C=O), 1645, 1571, 1474, 1345, 1244, 1046 and 1026 (C-O-C), 898 and 820 cm; EI MS m/z+440 [M, 14%], 409 (14), 398 (8), 371 (44), 333 (100), 283(46), 271 (22), 255 (56), 242 (8), 225 (56), and 59 (39) nm;

^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 1.34 (3H, s, CH_3-17), 1.39 (3H, s, CH_3-18), 1.82 (3H, s, CH_3-23), 2.28 (3H,s, CH_3-24), 2.37 (1H, s, 16-OH), 2.44 (1H, s, 15-OH), 2.63 (1H, dd, *J* = 10.8, 14.0 Hz, H-14b), 2.69 (1H, s, H-20), 3.16 (1H, dd, *J* = 1.2, 14.0 Hz, H-14a), 3.70 (1H, d, *J* =10.8 Hz, H-15), 4.31 (1H, dd, *J* = 2.8, 10.8 Hz, H-19b), 4.41 (1H, dd, J = 2.0, 10.8 Hz, H-19a), 4.53 (1H, s, H-22b), 4.77 (1H, s, H-22a), 4.98 (1H, d, *J* = 4.0 Hz, 25-OH),5.34 (1H, s, H-25), 6.72 (1H, d, *J* = 8.4 Hz, H-2), 7.19 (1H,s, H-5), 7.49 (1H, d, J = 8.4 Hz, H-3), 12.54 (1H, s, 1-OH) ppm;

^{13}C-NMR (100 MHz, CDCl) δ_C (ppm): 184.3 (C-13), 160.3(C-1), 153.1 (C-10), 151.9 (C-11), 149.5 (C-7), 142.5 (C-21), 138.5 (C-6), 138.3 (C-3), 120.8 (C-8), 119.1 (C-5),116.8 (C-12), 116.3 (C-9), 112.3 (C-22), 109.9 (C-2),109.2 (C-4), 77.7 (C-15), 72.9 (C-16), 64.5 (C-19), 63.2(C-25), 44.8 (C-20), 32.0 (C-14), 26.5 (C-17), 23.6 (C-18),22.6 (C-23), 17.4 (C-24) ppm.

Compound F2 as yellow needle crystals m.p. 194-195 °C, which showed the M^+ ion at *m/z* 440 in EIMS and on the basis of NMR analysis assigned the structure of tajixanthone hydrate.

The IR spectrum of compound F2 showed that absorption peaks were assigned as summarized in **Table 4.24** and indicated absorption bands were a O-H stretching vibration at 3486 cm^{-1}, C-H stretching vibration of aromatic ring at 3073 cm^{-1}, (C-H stretching vibration at 2976, 2883 and 1797 cm^{-1}, C=O stretching vibration at 1738 cm^{-1}, C=C stretching vibration at 1645 and 1571 cm^{-1}, CH bending vibration at 1474 cm^{-1}, C-O stretching vibration at 1345, 1244, 1046 and 1026 cm^{-1}, C-H out of plane bending vibration at 898, 820, 750 cm^{-1}.

Table 4.24 The IR absorption bands assignment of compound F2

Wave number (cm^{-1})	Intensity	Tentative assignment
3486	Broad, Strong	O-H stretching vibration of alcohol
3073	Weak	C-H stretching vibration of aromatic
2976, 2883	Weak	ring
1797, 1738	Weak	C-H stretching vibration of CH_2, CH_3
1645, 1571	Medium	C=O stretching vibration
1474	Strong	C=C stretching vibration of olefinic
1345,1244	Medium	bending vibration of parafinic
1046, 1026	Strong	C-O stretching vibration
898, 820, 750	Medium	C-O stretching vibration
		C-H out of plane bending vibration

The ^{1}H-NMR spectrum of compound F2 indicated that it possesses one phenolic hydroxy proton (δ_H 12.57 ppm), three aromatic protons (δ_H 7.52, 7.22 and 6.75 ppm), two olefinic protons (4.56 and 4.80 ppm) two methylene protons (δ 2.64 ppm with 3.22 ppm and δ_H 4.34 ppm with 4.44 ppm) three methine protons (δ 2.72, 3.74 and 5.37 ppm) and four methyl groups (δ_H 1.36, 1.42, 1.85 and 2.34 ppm) and three hydroxy proton of alcohol (δ_H 2.40, 2.47 and 5.01 ppm).

The ^{13}C-NMR spectrum showed 25 signals indicated that the phenolic hydroxy carbon appeared at δ_C 160.31 ppm, the aromatic carbons appeared at δ 109.21, 109.92, 116.26, 116.79, 119.15, 120.81, 138.28, 138.56, 149.54, 151.95 and 153.10 ppm, two signals of olefinic carbons at δ_C 112.33 and 142.47 ppm, the signal at δ 184.29 ppm should be the carbonyl group and the sp^3 carbon signals at δ 17.44, 22.57, 23.59, 26.52, 32.00, 44.80, 63.16, 64.49, 72.90 and 77.70 ppm.

^{13}C-NMR data of compound F2 and tajixanthone hydrate were compare in **Table 4.25** and gHMBC and NOESY correlation showed in **Tablee 4.26**. Two-dimensional NMR techniques were used to assist the structure assignment of compound F2 were assigned by gHMBC and NOESY spectra assigned in **Figure 4.41** and **4.42**,

respectively. The data were similar to each other and the structure and position of tajixanthone hydrate was shown **Figure 4.43.**

Table 4.25 Comparison of ^{13}C-NMR chemical shifts of compound F2 and Tajixanthone hydrate

Position	Compound 9	Tajixanthone hydrate
	δ_c	δ_c
1	160.31 (s)	159.5 (s)
2	109.92 (d)	109.5 (d)
3	138.28 (d)	137.7 (d)
4	109.21 (s)	108.7 (s)
5	119.15 (d)	118.7 (d)
6	138.56 (s)	137.9 (s)
7	149.54 (s)	149.0 (s)
8	120.81 (s)	120.4 (s)
9	116.79 (s)	116.3 (s)
10	153.10 (s)	152.4 (s)
11	151.95 (s)	151.3 (s)
12	116.26 (s)	115.9 (s)
13	184.29 (s)	183.5 (s)
14	32.00 (t)	31.9 (t)
15	77.70 (d)	77.4 (d)
16	72.90 (s)	72.6 (s)
17	26.52 (q)	26.3 (q)
18	23.59 (q)	23.6 (q)
19	64.49 (t)	64.3 (t)
20	44.80 (d)	44.6 (d)
21	142.47 (s)	141.9 (s)
22	112.33 (t)	111.8 (t)
23	22.57 (q)	22.4 (q)
24	17.4 (q)	17.3 (q)
25	63.16 (d)	62.9 (d)
CH3-6	17.44 (q)	17.3 (q)
OH-C4	-	-
OH-C15	-	-
OH-C16	-	-

Table 4.26 The correlation of gHMBC and NOESY of compound F2

Position	^{13}C-NMR	^{1}H-NMR	g-HMBC	NOESY
1	160.31 (s)	-	-	-
2	109.92 (d)	6.75 (d)	C-7a, C-9, C-11, C-11a	H-9
3	138.28 (d)	7.52 (d)	C7a, C-9, C-10, C-11, C-11a, C-1'	H-10
4	109.21 (s)	-	-	-
5	119.15 (d)	7.22 (s)	C-6, C-7, C-8, C-11, C-24	H-24
6	138.56 (s)	-	-	-
7	149.54 (s)	-	-	-
8	120.81 (s)	-	-	-
9	116.26 (s)	-	-	-
10	153.10 (s)	-	-	-
11	151.95 (s)	-	-	-
12	116.79 (s)	-	-	-
13	184.29 (s)	-	-	-
14	32.00 (t)	2.64 (dd)	C-3, C-10	H-2
		3.22 (dd)	C-3, C-10	H-3
15	77.70 (d)	3.74 (d)	-	H-14, H-18
16	72.90 (s)	-	-	-
17	26.52 (q)	1.36 (s)	C-15, C-16, C-18	H-14, H-18
18	23.59 (q)	1.42 (s)	C-15, C-16, C-17	H-14, H-15, H-17
19	64.49 (t)	4.34 (dd)	C-21, C-22, C-25	H-19, H-22
		4.44 (dd)	C-7, C-20, C-21, C-25	H-19, H-22
20	44.80 (d)	2.72 (br)	C-22, C-25	H-19, H-25
21	142.47 (s)	-	-	-
22	112.33 (t)	4.56 (s)	C-20, C-21, C-23	H-19
		4.80 (s)	C-20, C-22, C-23	H-22
23	22.57 (q)	1.85 (s)	C-20, C-21, C-22	25-OH
24	17.44 (q)	2.34 (s)	C-5, C-6, C-7, C-8	H-6, 1-OH
25	63.16 (d)	5.37 (s)	C-7, C-8, C-19, C-20	H-20, H-22
OH-1	-	5.01 (s)	C-20, C-21, C-25	H-22, H-25
OH-C4	-	12.57 (s)	C-2, C-3, C-9	-
OH-C15	-	2.40 (s)	C-15, C-16	H-14
OH-C16	-	2.47 (s)	C-14	H-14

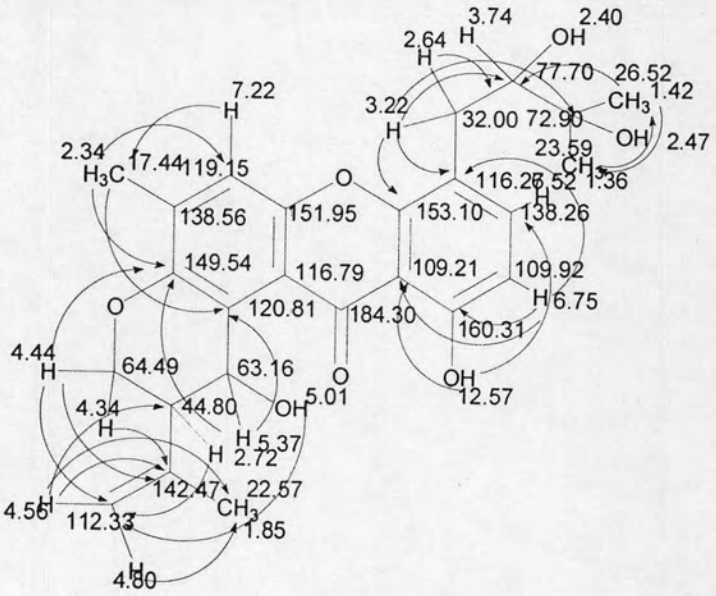


Figure 4.41 The gHMBC correlation of compound F2

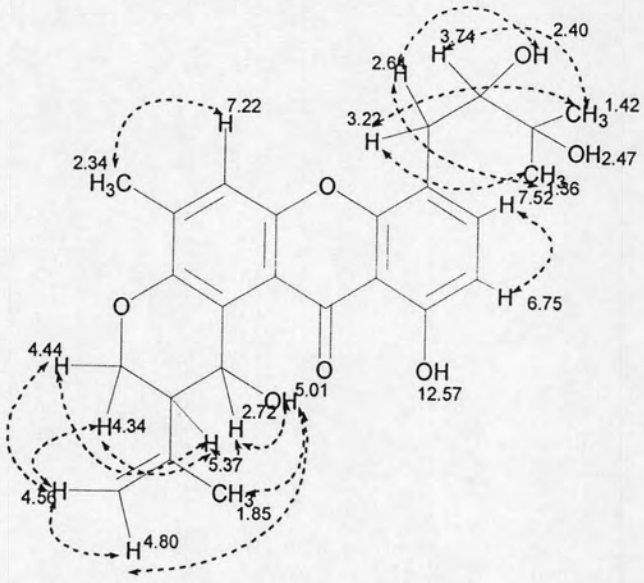


Figure 4.42 The NOESY correlation of compound F2

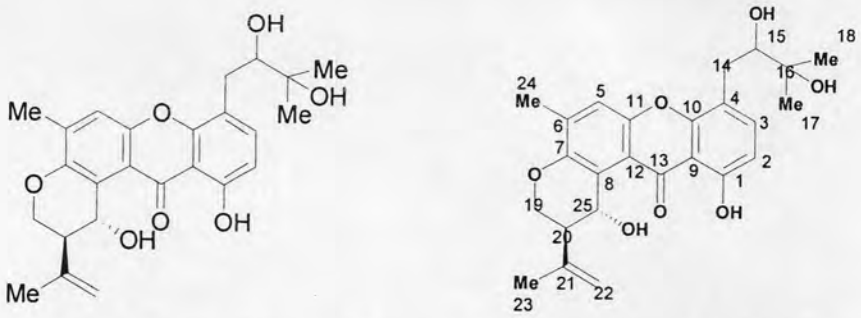


Figure 4.43 Structure and position of tajixanthone hydrate

4.4.4 Metabolites from EtOAc crude extract of fermentation broth cultured in MCzB

Isolation procedure of EtOAc crude extract of MCzB fermentation broth by silica gel column chromatography to give 330 fraction isolated from EtOAc crude extract of broth (MCzB), compound G3 (4 mg) was obtained from fraction 34-48 eluted with 20-25 % EtOAc in hexane and washed by hexane and ethyl acetate, respectively, compound G2 (7 mg) was obtained from fraction 49-52 eluted with 25-30 % EtOAc in hexane and washed by ethyl acetate and compound C2 (4 mg) was obtained from fraction 81-110 eluted with 50 % EtOAc in hexane and washed by hexane and ethyl acetate, respectively and compound D3 (13 mg) was obtained from fraction 191-205 eluted with 90-100 % EtOAc in hexane and washed by hexane and ethyl acetate, respectively.

Compound G3

compound G3 was obtained from fermentation broth (MCzB) of the fungus and isolated from EtOAc crude extract eluted with 20-25 % EtOAc in hexane and washed by hexane and ethyl acetate, respectively.

m.p. 239-240;

HRTOFMS $[M-H]^-$ 299.0561 (calculate$[M-H]^-$ 299.0557);

λ_{max} (CHCl3)(ε): 278 (20085), 307 (7888), 435 (8448); ν_{max} (KBr) 3430(br), 2954 (w), 2924 (w), 2851(w), 1732 (w), 1625 (s), 1566 (m), 1486 (w), 1456(w), 1397(s), 1263(m), 1208(w), 1156(w), 1125(w), 1046(m), 970 (w), 760(m), 717(m) nm;

^{1}H-NMR ($CDCl_3$) 12.43 (1H, s, OH-8), 12.05 (1H, s, OH-1), 7.52 (1H, s, H-5), 7.28 (1H, s, H-4), 7.00 (1H, s, H-7), 3.96 (3H, s, OCH_3-3), 2.37 (3H, s, CH_3-6)

^{13}C-NMR($CDCl_3$) (125 MHz) 191.06 (s, C-9), 181.96 (s, C-10), 161.99 (s, C-8), 156.75 (s, C-2), 148.37 (s, C-6), 139.68 (s, C-3), 133.08 (s, C-1, C10a), 129.50 (s, C-4a), 124.13 (d, C-7), 121.10 (d, C-5), 113.66 (s,C-8a), 110.52 (s, C-9a), 109.50 (d, C-4), 60.67 (q, OCH_3-3), 21.93 (q, CH_3-6)

Compound G3 as a orange solid, m.p. 239-240 °C, which showed molecular ion of HRTOFMS $[M-H]^-$ 299.0561 that indicate molecular weight 300, IR adsorption peaks were indicated absorption bands a OH stretching vibration at 3430 (br) cm^{-1}, C=O stretching at 1732 cm^{-1}, C=C of aromatic stretching vibration at 1625 and 1566 cm^{-1}, C-H bending vibration 1486, 1456, 1397 cm^{-1} and C-O stretching vibration at 1263, 1208, 1156, 1125 and 1046 cm^{-1} and C-H out of plane at 970, 760 and 717 cm^{-1}. IR absorption assignment of compound G3 was shown in **Table 4.27**.

Table 4.27 The IR absorption bands assignment of compound G3

Wave number (cm^{-1})	Intensity	Tentative assignment
3430	Broad, Strong	O-H stretching vibration of hydroxy
2954, 2924,	Weak	stretching vibration of CH aromatic
2851	Weak	stretching vibration of CH_2, CH_3
1732	Weak	C=O stretching vibration of carbonyl
1625, 1566	Strong, Medium	C=C stretching vibration of aromatic
1486, 1456,	Weak	C-H bending vibration of CH_2, CH_3
1397, 1263,	Strong, medium	C-O stretching vibration of hydroxyl group
1208, 1156, 1125, 1046	Medium and weak	C-O stretching vibration of hydroxyl group
970, 760, 717	Weak and medium	C-H out of planes bending vibration

The 1H-NMR spectrums of anthraquinone 1 showed the important peaks that indicated two hydrogen bonding of hydroxyl protons at 12.43 and 12.05 ppm, a methyl proton at 2.37 ppm, a methoxy proton at 3.96 ppm, and aromatic proton at 7.52 , 7.28 and 7.00 ppm.

The 13C NMR spectrums of compound G3 showed 16 carbon signals, carbonyl carbons of ketone at 191.06 and 181.96 ppm, a methyl carbon at 21.93 ppm, a methoxy carbon at 60.67 ppm, twelve carbon of aromatic at 161.99, 156.75, 148.37, 139.68, (133.08x2), 129.50, 124.13, 121.10, 113.66, 110.52 and 109.50 ppm. From gHMBC correlation H-4 and H-5 correlated with C-10 that structure was confirmed in the position.

Compound G3 confirms structure by gHMBC showed in the **Table 4.28** and **Figure 4.45**, respectively. The data were similar to each other and the structure and position of 4,6-dihydroxy-5,7-dimethoxy-2 –methylanthraquinone was shown **Figure 4.44**.

From literature review (Roberge and Brassard, 1981) reported reactions of ketone Acetals, the 1H-NMR and m.p. of 1,3,8-Trihydroxy-2-methoxy-6-methylanthraquinone and 1,2,8-Trihydroxy-3-methoxy-6-methylanthraquinone (Dermoglaucin) was compared with compound G3. ^{1}H-NMR spectrums of three protons at position H-4, H-5 and H-7, of ,3,8-Trihydroxy-2-methoxy-6-methylanthraquinone at 7.08, 7.23 and 6.96 ppm, respectively, 1,2,8-Trihydroxy-3-methoxy-6-methylanthraquinone (Dermoglaucin) at 7.16, 7.29 and 6.94 ppm, respectively and anthraquinone 1 at 7.52, 7.28 and 7.00 ppm, respectively. Comparison of m.p. of 1,3,8-Trihydroxy-2-methoxy-6-methylanthraquinone,1,2,8-Trihydroxy-3-methoxy-6-methyl anthraquinone (Dermo glaucin) (Roberge and Brassard, 1981) and compound G3 were 270-275, 235-236 and 239-240 °C, respectively. Therefore, compound G3 was likely with 1,2,8-Trihydroxy-3-methoxy-6-methylanthraquinone (Dermoglaucin) showed in **Table 4.30** and structure and position of 1,2,8-Trihydroxy,-3-methoxy-6-methyl anthraquinone (Dermoglaucin) was shown in **Figure 4.45**.

Table 4.28 gHMBC Correlation of compound G3

Position	H-NMR	gHMBC
4	7.52	C-2, C-3, C-9a, C-10
5	7.28	C-7, C-8a, C-10
7	7.00	C-5, C-8a
OH-1	12.05	-
OCH_3-3	3.96	C-3
CH_3-6	2.37	C-5, C-6, C-7
OH-8	12.43	-

Table 4.29 Comparison of compound G3 with 3,8-Trihydroxy-2-methoxy-6-methylanthraquinone and 1,2,8-Trihydroxy-3-methoxy-6-methylanthraquinone (Dermoglaucin).

Position	Compound G3	3,8-Trihydroxy-2-methoxy-6-methylanthraquinone	Dermoglaucin
4	7.52, s	7.08, s	7.16, s
5	7.28, s	7.23, d, J = 2.0 Hz	7.29,d, *J* = 1.5 Hz
7	7.00, s	6.96, br, s	6.94, d *J* = 1.5 Hz
OH-1	12.05, s	11.76	-
OCH3-3	3.96, s	-	3.89, s
OCH3-2		3.87, s	
OCH3-6	2.37, s	2.31, s	2.36,s
OH-8	12.43, s	12.06, s	-

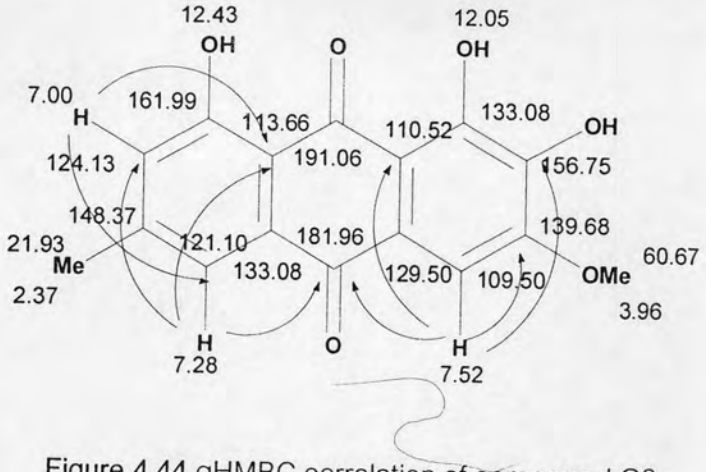


Figure 4.44 gHMBC correlation of compound G3

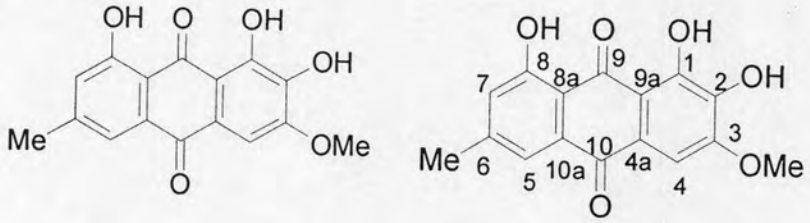


Figure 4.45 Structure and position of 1,2,8-Trihydroxy,-3-methoxy-6-methyl anthraquinone (Dermoglaucin)

Compound G2

compound G2 (7 mg) was obtained from fraction 49-52 eluted with 25-30 % EtOAc in hexane and washed by ethyl acetate.

Compound C2

A novel compound, compound C2 was obtained from fermentation broth (MCzB) of *Emericella variecolor* isolated from EtOAc crude extract eluted with 50 % EtOAc in hexane and washed by hexane and ethyl acetate, respectively.

m.p. 201-202 °C (dec);

$[\alpha]_D^{20}$ -72 ($CHCl_3$, c 0.24);

λ_{max} (ε) – ; HRESIMS $[M+H]^+$ 427.2050;

ν_{max} (KBr) 3418 (br,s), 2961 (s), 2929 (s), 2874 (m), 1711 (s), 1668 (s), 1457 (m), 1385 (m), 1376 (m), 1243 (s), 1222 (s), 1151 (s), 1108 (s), 1094 (s), 1055 (s), 964(m), 939 (m), 903 (m) nm;

^{1}H-NMR (400 MHz): 7.26 (1H, d, *J* = 10 Hz, H-1), 7.00 (1H, s, H-6') , 5.87 (1H, d, *J* = 10 Hz, H-2), 4.65 (1H, d, *J* = 9.2 Hz, H-1'), 4.02 (1H, d, *J* = 9.2 Hz, H-1'), 2.65 (1H, d, J = 19.2 Hz, H-11), 2.48 (1H, d, J = 19.2 Hz, H-11), 2.31 (1H, d, J = 10.4 Hz, H-5), 2.20 (1H, d, J = 15.6 Hz, H-12) , 1.62 (1H, m, H-7), 1.59 (2H, m, H-6), 1.45 (1H, d, J= 15.6 Hz, H-12), 1.43 (3H, s, H-9'), 1.29 (3H, s, H-10'), 1.23 (1H, m, H-7), 1.20 (3H, s, H-13), 1.11 (3H, s, H-15), 1.06 (3H, s, H-14)

^{13}C-NMR (100 MHz): 204.52 (s, C-3), 200.54 (s, C-4'), 167.79 (s, C-8'), 155.61 (d, C-1), 147.88 (d, C-6'), 129.91 (s, C-7'), 126.37 (d, C-2), 110.00 (s, C-9), 84.20 (s, C-3'), 67.32 (t, C-1'), 55.76 (s, C-2'), 53.72 (s, C-8), 51.33 (t, C-11), 51.33 (s, C-5'), 46.59 (d, C-5), 44.50 (s, C-4), 41.71 (s, C-10), 38.27 (t, C-12), 31.00 (t, C-7), 28.40 (q, C-15), 27.80 (q, C-9'), 22.36 (q, C-10'), 21.34 (q, C-14), 19.04 (q, C-13), 18.63 (t, C-6)

Compound C2 as a white crystals, which showed molecular ion of $[M+H]^+$ at *m/z* 427.2050 that indicate molecular weight 426, IR adsorption peaks were indicated absorption bands a OH stretching vibration at 3418 cm^{-1}(br) , C=O stretching at 1711 cm^{-1}, C=C stretching vibration at 1668 $cm^{-1}$and C-O stretching vibration at 1243, 1222, 1151, 1108, 1108, 1094, and 1055 cm^{-1} and C-H out of plane bending vibration at 964, 939 and 903 cm^{-1} and showed in **Table 4.30**.

Table 4.30 The IR absorption bands assignment of compound C2

Wave number (cm^{-1})	Intensity	Tentative assignment
3418	Broad, Strong	O-H stretching vibration of hydroxyl
2961, 2929, 2874	Strong and medium	stretching vibration of CH_2, CH_3
1711	Medium	C=O stretching vibration of carbonyl
1668	Strong	C=C stretching vibration of olefin
1457, 1385, 1376	Medium	C-H bending vibration of CH_2, CH_3
1250, 1015	Strong and medium	
	Medium	C-O stretching vibration of hydroxyl

The mass spectrum showed molecular weight that indicate molecular weight 408. The 1H-NMR spectrums of compound C2 showed the important peaks that indicated three olefinic protons at δ_H 7.26, 7.00 and 5.87 ppm. Five methyl groups indicated at δ_H 1.43, 1.29, 1.20, 1.11 and 1.06 ppm, methylene protons indicated at δ_H (4.65 and 4.02) (CH_2-O-C=O), (1.62 and 1.59) (CH_2-CH_2), (2.65 and 2.48) CH_2-C=O.

The 13C NMR spectrums of compound C2 showed 25 carbon signals, carbonyl carbons at δ_C 204.52, 200.54 and 167.79 ppm, olefinic carbons at δ_C 155.61, 147.88, 129.91 and 126.37, ppm, four methyl carbons at δ_C 28.40, 27.80, 22.36, 21.34 and 19.04 ppm, methylene carbon at δ_C 67.32, 51.33, 38.27, 31.00 and 18.63 ppm, methine carbons at δ_C 46.59 ppm, quarternary carbons at δ_C 110.00 (OC-OH) , 84.20, 55.76, 53.72, 2x 51.33, 44.50 and 41.71ppm.

Compound C2 showed 25 carbons and showed the structure likely to sesterterpene, andilesin (Simpson et. al., 1981) and compared the ^{13}C-NMR spectrum in **Table 4.32.** On the basis of spectroscopic data including ^{1}H, ^{13}C, gHSQC, gHMBC, gCOSY, TOCSY and NOESY shown in **Table 4.31**, the long range correlations of gHMBC and NOESY was observed as shown in **Figure 4.46** and **Figure 4.47**, respectively. The chemical structure of andilesin and compound C2 was compared and assigned as a novel compound, emervaridionin **Figure 4.48** and **Figure 4.49**, respectively.

Table 4.31 gCOSY, gHMBC, NOESY correlation of Compound C2

Position	1H-NMR	gCOSY	gHMBC	NOESY
1	7.26	H-2	C-3, C-5	H-2
2	5.87	H-1	C-4, C-10	H-1
5	2.31	H-6	C-10, C-4, C-14	-
6	1.59	H-5	-	-
7a 7b	1.23 1.62	H-7b H-7a	- C-12	- -
11a 11b	2.48 2.65	H-11b H-11a	C-3', C-4', C-9' C-2', C-3', C-4'	- -
12a 12b	1.45 2.20	H-12b H-12a	C-7, C-4', C-10' C-7, C-8, , C-9, C-4', C-5', C-6'	H-10
13	1.20	-	C-1, C-5, C-9, C-10	H-12b
14	1.06	-	C-3, C-4, C-5, C-15	H-15
15	1.11	-	C-3, C-4, C-5, C-14	H-14
1'a 1'b	4.02 4.65	H-1'b H-1'a	C-8, C-3' C-9, C-C-3', C-8, C-2'	H-1'b, H-6 H-1'a, H-12a
6'	7.00	-	C-12, C-5', C-2', C-4', C-8' , C-10'	H-10'
9'	1.43	-	C-3', C-4'	H-12b
10'	1.29	-	C-12, C-4', C-5', C-6'	H-6'

Table 4.32 Comparison of ^{13}C-NMR chemical shifts of Compound C2 and Andilesin (Simpson., T. J., 1981).

Position	Compound C2	Andilesin
1	155.61	149.5
2	126.37	119.4
3	204.52	166.2
4	44.50	83.5
5	46.59	42.2
6	18.63	22.8
7	31.00	31.7
8	53.72	45.3
9	110.00	56.4
10	41.71	43.8
11	51.33	38.7
12	38.27	52.7
13	19.04	22.4
14	21.34	30.2
15	28.40	23.5
1'	67.32	69.2
2'	55.76	43.1
3'	84.20	56.8
4'	200.54	216.5
5'	51.33	55.0
6'	147.88	32.3
7'	129.91	35.5
8'	167.79	176.2
9'	27.80	19.5
10'	22.36	16.8

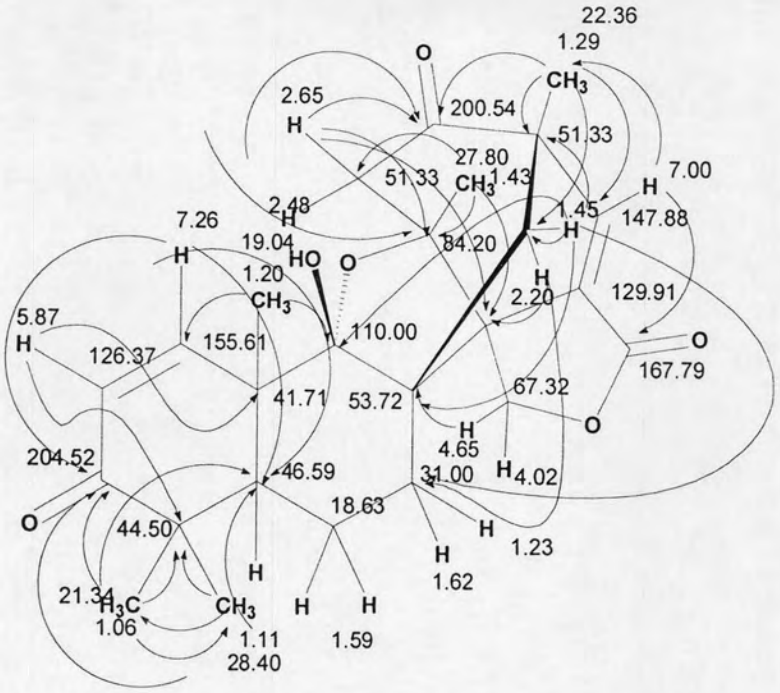


Figure 4.46 gHMBC correlation of Compound C2

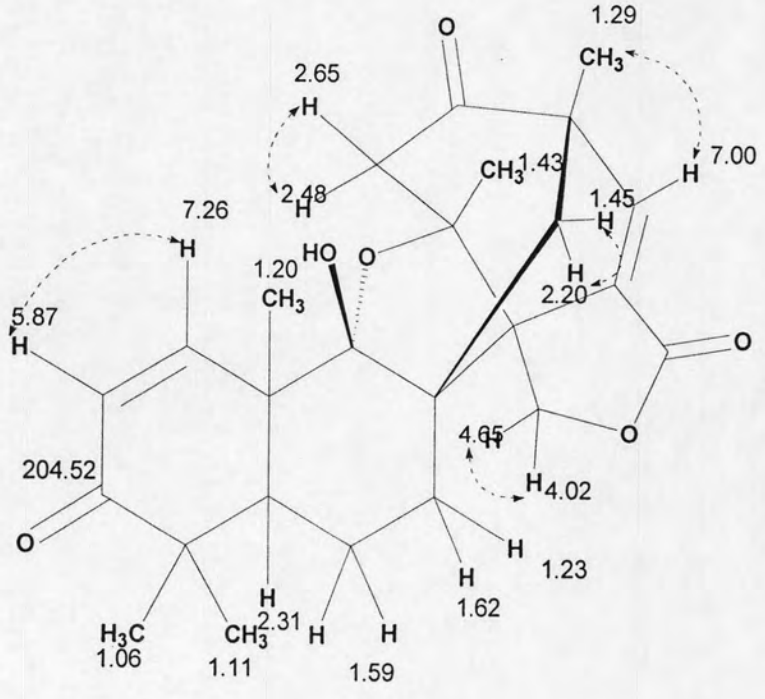


Figure 4.47 NOESY correlation of Compound C2

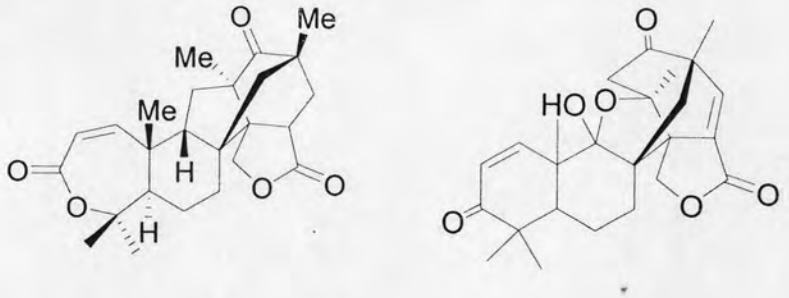


Figure 4.48 Comparison of andilesin and compound C2

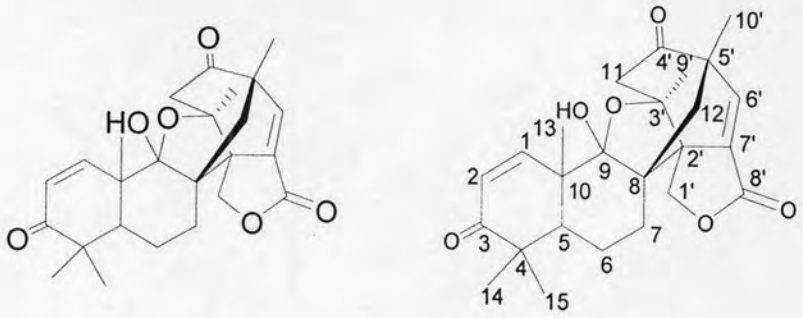


Figure 4.49 Structure and position of emervaridionin

Compound D3

Novel metabolites, compound D3 (13 mg) was obtained from fermentation btoth (MCzB and CzB) and isolated from crude EtOAc extract eluted with 70-100 % EtOAc in hexane and washed by hexane and ethyl acetate, respectively.

m.p. 151-152 °C ;

$[M+H]^+$ 299.1648;

$[\alpha]_D^{20}$ -15°(0.1, EtOH);

λ_{max} (ε) 268 (1201), 274 (1228) nm;

ν_{max} (KBr) 3416 (br, s), 3283 (br, s), 2957 (m), 2914 (m), 2871 (m)1602 (m), 1486 (s), 1443 (m), 1410 (w),1363 (w), 1303 (w), 1263 (s), 1058 (s), 955 (w), 895 (w), 765 (m), 709 (m) cm^{-1};

^{1}H-NMR : 7.26 (1H, t, 7.5 Hz, H-4), 6.78 (1H, d, *J* = 7.5 Hz, H-5), 6.76 (1H, d, *J* = 8.5 Hz, H-3), 5.48 (1H, dd, *J* = 1.5, 10.5 Hz, H-1'), 5.16 (1H, dd, *J* = 2.5, 12.0 Hz, CH_2O-1), 5.07 (1H, d, *J* = 12.0 Hz, CH_2O-1), 4.20 (1H, ddd, *J* = 2.0, 6.0, 9.5 Hz, H-3'), 4.03 (1H, dq, *J* = 6.5, 6.5 Hz, H-6'), 3.83 (3H, s, CH_3O-2), 3.75 (1H, dd, *J* = 1.5, 6.0 Hz, H-4'), 3.70 (1H,

dd, J = 1.5, 5.0 Hz, H-5'), 2.26 (1H, ddd, J = 2.0, 2.5, 14.5 Hz, H-2'), 1.81 (1H, ddd, J = 9.5, 10.0, 14.5 Hz, H-2'), 1.29 (3H, d, J = 6.5 Hz, H-7') ppm;

^{13}C-NMR : 154.15 (s, C-2), 143.07 (s, C-6), 129.58 (d, C-4), 126.02 (s, C-1), 113.12 (d, C-5), 109.30 (d, C-3), 84.92 (d, C-1'), 73.52 (d, C-3'), 73.12 (d, C-5'), 72.62 (d, C-4'), 71.41 (t, CH_2O-1), 70.19 (d, C-6'), 55.26 (q, OCH_3-2), 39.14 (t, C-2'), 19.38 (q, C-7') ppm.

Compound D3 as a white crystals which showed molecular ion of HRTOFMS of $[M+H]^+$ at m/z 299.1648 that indicated the molecular weight in calculated at 280, IR adsorption peaks were indicated a OH stretching vibration at 3416 and 3283 (br) cm^{-1}, C=C of aromatic stretching vibration at 1602 cm^{-1}, C-H stretching of CH hydrocarbon at 2957, 2914 and 2871 cm^{-1}C-H bending vibration (m), 1486, 1443, 1410 and 1303 cm^{-1} and C-O stretching vibration at 1263 and 1058 cm^{-1} and CH out of plane bending vibration at 955, 895, 765 and 709 cm^{-1} showed in **Table 4.33**.

Table 4.33 The IR absorption bands assignment of compound D3

Wave number (cm^{-1})	Intensity	Tentative assignment
3416, 3283	Strong	O-H stretching vibration of hydroxy
2957, 2914, 2871	Medium	stretching vibration of CH_2, CH_3
1602,	Medium	C=C stretching vibration of aromatic
1486, 1443,	Strong, medium	C-H bending vibration of CH_2, CH_3
1410, 1333	Weak	C-H bending vibration of CH_2, CH_3
1263, 1058,	Strong	C-O stretching vibration of hydroxyl group
955, 895, 765, 709	Weak, medium	C-H out of planes bending vibration

The 1H-NMR spectrums of compound D3 showed the important peaks that indicated a methoxy proton at 3.83 ppm, three aromatic proton 6.76, 7.26, and 6.78 ppm at C-3, C-4 and C-5 respectively, methylene proton CH_2O-1 at 5.16, 5.07 ppm, methylene proton at CH_2-2' at 2.26 with J = 2.0, 2.5, 14.5 Hz and 1.81 ppm with J = 9.5, 10.0 and 14.5 Hz, a methyl proton at 1.29 (q) H-7' coupling with 4.03 (d), H-6', J = 6.5 Hz, H-6' coupling with H-5' and H-7' at 4.03 (dq) J = 6.5 and 6.5 Hz, H-5' coupling with

H-4' and H-6' at 3.70 (dd) with J = 1.5 and 5.0 Hz, H-4' coupling with H-3' and 5' at 3.75(dd) with J = 1.5 and 5.0 Hz, H-3 coupling with CH_2-2' and H-4' at 4.20 (ddd) with J = 2.0, 6.0 and 9.5 Hz.

The ^{13}C NMR spectrums of compound D3 showed 15 carbon signals, a methyl carbon at 19.38 ppm, a methoxy carbon at 55.26 ppm, and methylene carbon at 71.41 and 39.14 at OCH_2-1and C-2', respectively, the side chain of CH-OH at C-3', C-4', C-5' and C-6' at 73.52, 72.62, 73.12, 70.19 ppm, respectively. Confirmation of correlation by gCOSY and gHMBC showed in **Table 4.34**. The correlation of gHMBC and NOESY was shown in **Figure 4.50** and **Figure 4.51**, respectively. The chemical structure and position of compound D3 was assigned as a novel compound, varitetrail B (RRRR or SSSS) in **Figure 4.52**.

Table 4.34 gCOSY and gHMBC and NOESY correlation of compound D3

Position	1H-NMR	gCOSY	gHMBC	NOESY
3	6.76	H-4	C-1, C-2, C-5	H-4
4	7.26	H-3, H-5	C-2, C5, C-6	H-3, H-5
5	6.78	H-4	C-3, C-6, C-1'	H-4
1'	5.48	H-2'a, CH_2O-1b	-	H-2'b, H-3'
2'	1.81 2.26	H-1',H-2'b H-2'a	C-1', C-3' C-3'	H-2'b, H-3', H-5' H-1', H-2'a, H-3', H-4'
3'	4.20	H-2'a, H-4'	C-1', C-4'	H-2'a
4'	3.75	H-3', H-5'	C-2', C-3', C-6'	H-2'b, H-3', H-7'
5'	3.70	H-4', H-6'	C-3', C-6', C-7'	H-2'a, H-6', H-7'
6'	4.03	H-5', H-7'	C-3', C-4', C-7'	H-5', H-7'
7'	1.29	H-6'	C-5', C-6'	H-4', H-5', H-6'
CH_2O-1	5.07 5.16	CH_2O-1b CH_2O-1a, H-1'	C-1, C-2, C-6 C-1, C-6	CH_2O-1b CH_2O-1a
OCH_3-2	3.83	-	C-2	-

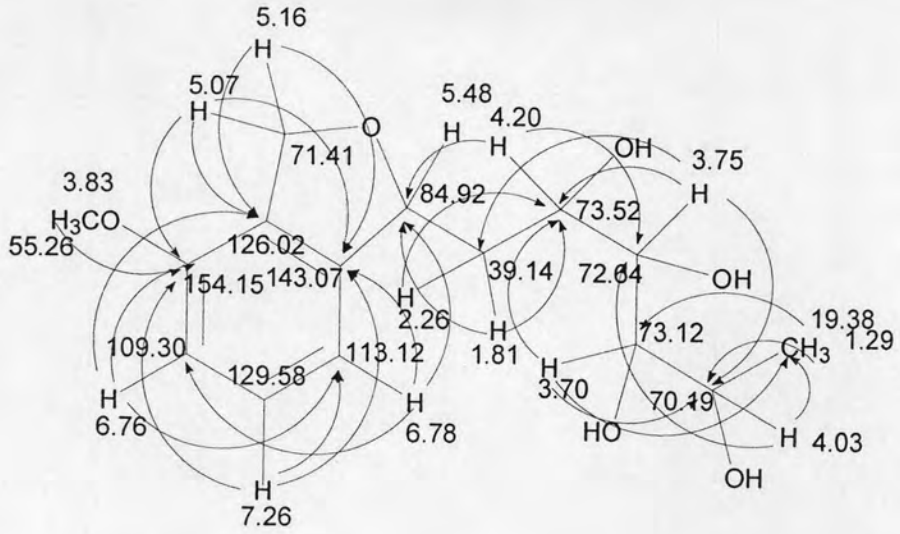


Figure 4.50 gHMBC correlation of compound D3

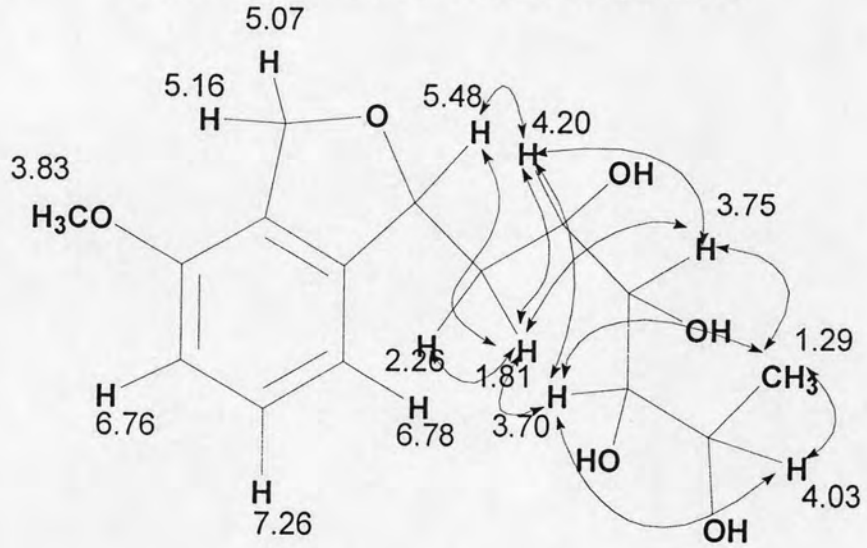


Figure 4.51 NOESY correlation of compound D3

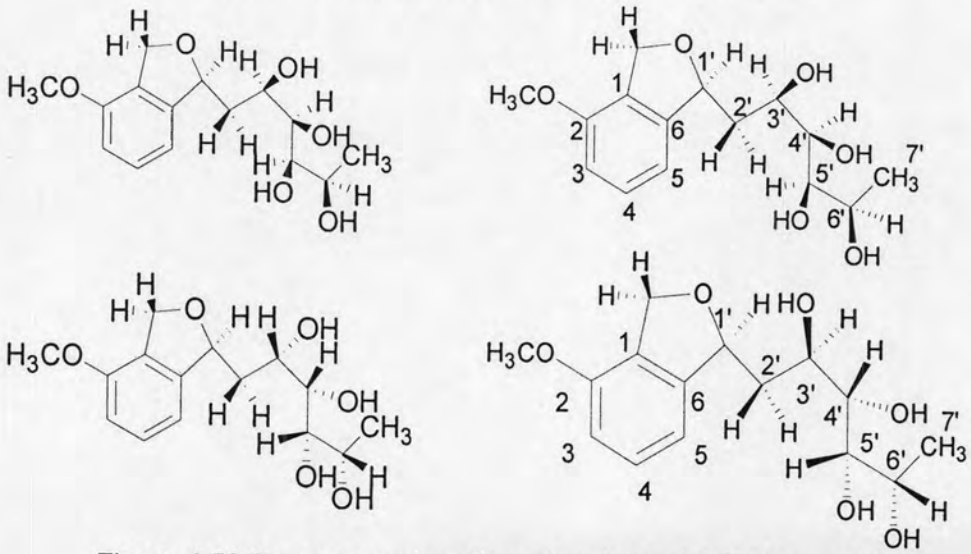


Figure 4.52 Structure and position of varitetraol B (RRRR or SSSS)

4.4.5 Metabolites from EtOAc crude extract of mycelium cultured in CzB

From isolation procedure of EtOAc crude extract of mycelium culture in CzB of *Emericella variecolor* by silica gel column chromatography to give 280 fraction isolated from EtOAc crude extractof mycelium (CzB), compound A (12 mg) obtained from fraction 36-48 eluted with20-25% EtOAc in hexane and washed by hexane and ethyl acetate, respectively and compound B (10 mg) obtained from fraction 49-56 eluted with 25 % EtOAc in hexane and washed by hexane and ethyl acetate, respectively.

Compound A

compound A (12 mg) obtained from fraction 36-48 eluted with20-25% EtOAc in hexane and washed by hexane and ethyl acetate, respectively. Compound A was analyzed by spectroscopic techniques as stellatic acid.

Compound B

compound B (10 mg) obtained from fraction 49-56 eluted with 25 % EtOAc in hexane and washed by hexane and ethyl acetate, respectively. Compound B was elucidated the structure as ergosterol.

4.4.6 Metabolites from EtOAc crude extract of fermentation broth cultured in CzB

From isolation procedure of EtOAc crude extract of CzB broth of *Emericella variecolor* by silica gel column chromatography to give 340 fraction isolated from EtOAc crude extract of broth (CzB), compound D3 (24 mg) obtained from fraction 168-190 eluted with 70-90 EtOAc in hexane and washed by hexane and ethyl acetate, respectively and compound D4 (12 mg) obtained from fraction 216-240 eluted with 90-100 % EtOAc in hexane and washed by hexane and ethyl acetate, respectively.

Compound D3

compound D3 (24 mg) obtained from fraction 168-190 eluted with 70-90 EtOAc in hexane and washed by hexane and ethyl acetate, respectively. Compound D3 was analyzed by spectroscopic techniques, the structure as varitetraol B.

Compound D4

A Novel compound, compound D4 (12 mg) obtained from fermentation broth (CzB) isolated from EtOAc eluted with 90-100 % EtOAc in hexane and washed by hexane and ethyl acetate, respectively.

m.p. 157-158 °C ;

$[\alpha]_D^{20}$ +26° (0.1, EtOH);

HRTOFMS $[M+H]^+$ 299.1487, $[M+Na]^+$ 321.1306;

λ_{max} (EtOH): (ε) 266 (1189), 274 (1368), 291 (122) nm;

ν_{max} (KBr) 3323 (br, s), 3007 (w), 2951 (m), 2921 (m), 2834 (m), 1735 (w), 1606 (m), 1489 (m), 1356 (w)1310 (w), 1267 (s), 1230 (m), 1054 (s), 1001 (m), 955 (w), 892 (m), 848 (w), 765 (m), 709 (m), 642 (m) cm^{-1};

^{1}H-NMR : 7.27 (1H, dd, J= 8.0, 7.5 Hz, H-4), 6.77 (1H, d, *J* = 7.5 Hz, H-5) , 6.76 (1H, d, *J* = 8.0 HzH-3), 5.57 (1H, m, H-1'), 5.14 (1H, dd, *J* = 2.5, 12,.5 Hz, CH_2O-1), 5.03 (1H, dd, *J* = 1.5, 12.5 Hz, CH_2O-1) , 4.01 (1H, dq, *J* = 5.0, 6.5 Hz, H-6'), 3.91 (1H, ddd, *J* = 3.0, 5.5, 9.0 Hz, H-3'), 3.84 (3H, s, OCH_3-2), 3.79 (1H, dd, *J* = 1.0, 5.5 Hz, H-4'), 3.72 (1H, dd, *J* = 1.0, 5.0 Hz, H-5'), 2.21 (1H, ddd, *J* = 3.0, 9.0, 14.5 Hz, H-2')*, 2.00 (1H, ddd, *J* = 3.5, 7.5, 15.0 Hz, H-2')*, 1.28 (3H, d, *J* = 6.5 Hz, H-7') ppm;

^{13}C-NMR : 154.14 (s, C-2), 142.75 (s, C-6), 129.58 (d, C-4, 126.72 (s, C-1), 113.19 (d, C-3), 109.28 (d, C-5), 82.68 (d, C-1'), 72.93 (d, C-5')*, 72.33 (d, C-4')*, 71.35 (d, C-3')*, 71.11(t, CH_2O-1), 70.45 (d, C-6')*, 55.24 (q, OCH_3-2), 38.30 (t, C-2'), 19.09 (q, C-7') ppm.

Compound D4 as a white crystals which showed molecular ion of HRTOFMS at $[M+H]^+$ 299.1487, $[M+Na]^+$ 321.1306 that indicated the molecular weight in calculated at 298, IR adsorption peaks were indicated a OH stretching vibration at 3323 cm^{-1}, C-H of aromatic stretching vibration at 3007 cm^{-1}, C-H stretching of CH hydrocarbon at 2951, 2921 and 2834 cm^{-1}, C=C stretching of aromatic at 1735 and 1606 cm^{-1}, C-H bending vibration (m), 1489, 1356 and 1310 cm^{-1} and C-O stretching vibration at 1267, 1230, 1054 and 1001 cm^{-1} and CH out of plane bending vibration at 955, 892, 848, 765, 709 and 642 cm^{-1} and the IR spectrum showed in Table 4.35.

Table 4.35 The IR absorption bands assignment of compound D4

Wave number (cm^{-1})	Intensity	Tentative assignment
3323,	Strong	O-H stretching vibration of hydroxy
3007, 2951, 2921, 2834	Medium	stretching vibration of CH_2, CH_3
1735	Weak	C=O stretching of carbonyl
1606	Medium	C=C stretching vibration of olefin
1489, 1310,	Medium, weak	C-H bending vibration of CH_2, CH_3
1267, 1230, 1054, 1001	Strong, medium	C-O stretching vibration of hydroxyl group
955, 892, 848, 765, 709, 642	Medium, weak	C-H out of planes bending vibration

The 1H-NMR spectrums of compound D4 showed the important peaks that indicated a methoxy proton at 3.84 ppm, three aromatic proton 6.76, 7.27, and 6.77 ppm at C-3, C-4 and C-5 respectively, methylene proton CH_2O-1 at 5.14, 5.03 ppm, methylene proton at CH_2-2' at 2.21 with J = 3.0, 9.0, 14.5 Hz and 2.08 ppm with J = 3.5, 7.5 and 15.0 Hz, a methyl proton at 1.28 (d) H-7' coupling with H-6', J = 6.5 Hz, H-6' coupling with H-5' and H-7' at 4.01 (dq) J = 5.0 and 6.5 Hz, H-5' coupling with H-4' and H-6' at 3.72(dd) with J = 1.0 and 5.0 Hz, H-4' coupling with H-3' and 5' at 3.79(dd) with J = 1.5 and 5.5 Hz, H-3 coupling with CH_2-2' and H-4' at 3.91 (ddd) with J = 3.0, 5.5 and 9.0 Hz.

The ^{13}C NMR spectrums of compound D4 showed 15 carbon signals, a methyl carbon at 19.09 ppm, a methoxy carbon at 55.24 ppm, and methylene carbon at 71.11 and 38.30 at OCH_2-1and C-2', respectively, the side chain of CH-OH at C-3', C-4', C-5' and C-6' at 71.35, 72.33, 72.93, 70.45 ppm, respectively. Confirmation of correlation by gCOSY and gHMBC showed in **Table 4.36**. The correlation of gHMBC and NOESY was shown in **Figure 4.53** and **Figure 4.54**; respectively. The chemical structure and position of compound D4 was assigned as a novel compound, varitetraol C (RRSS or SSRR) in **Figure 4.55**.

Table 4.36 gCOSY and gHMBC and NOESY correlation of compound D4

Position	1H-NMR	gCOSY	gHMBC	NOESY
3	6.76	H-4	C-1, C-2, C-5	H-4
4	7.27	H-3, H-5	C-2, C-5, C-6	H-3, H-5
5	6.77	H-4	C-1, C-3, C-6	H-4
1'	5.57	H-2'b	-	H-2'b
2'	2.00	H-2'b	C-1', C-4'	H-2'b
	2.21	H-1, H-2'a, H-3'	C-1', C-3'	H-1, H-2'a
3'	3.91	H-2'b	-	*
4'	3.79	H-5'	C-2'	*
5'	3.72	H-4', H-6'	-	*
6'	4.01	H-5', H-7'	C-4'	*
7'	1.28	H-6'	C-5', C-6'	*
CH_2O-1	5.03	CH_2O-1b	C-1, C-6	CH_2O-1b
	5.14	CH_2O-1a	C-1	CH_2O-1a
OCH_3-2	3.84	-	C-2	-

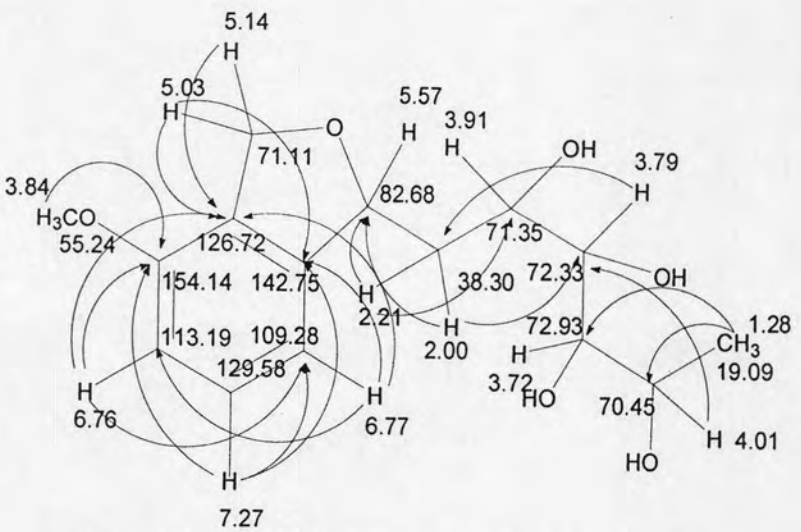


Figure 4.53 gHMBC correlation of compound D4

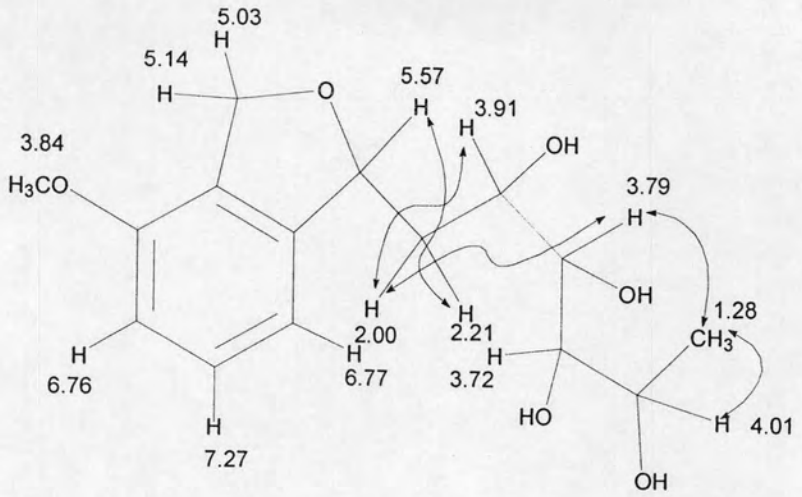


Figure 4.54 NOESY correlation of compound D4

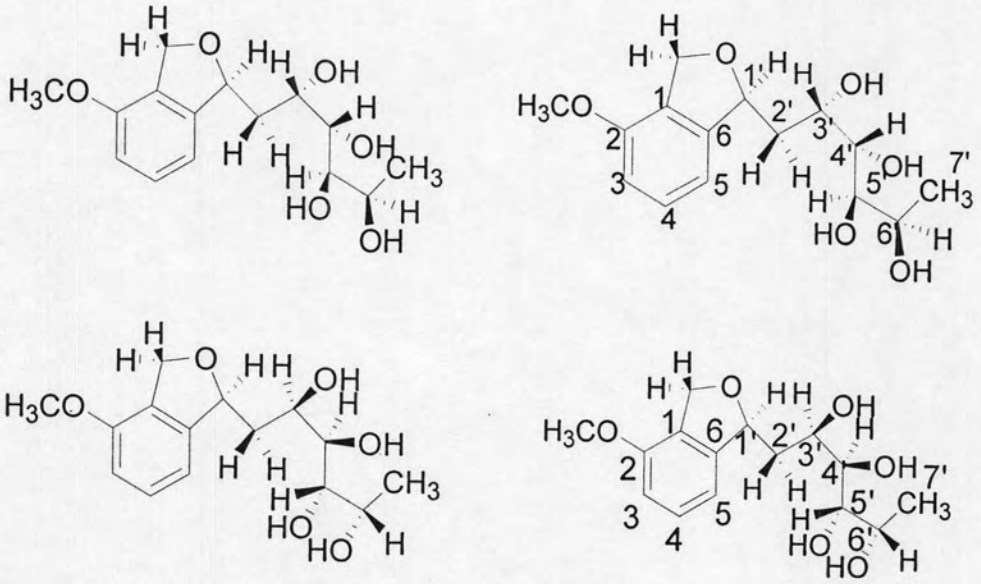


Figure 4.55 Sturcture and position of varitetraol C (RRSS or SSRR)

4.5 Effects of culture media on secondary metabolites production

Malt extract broth consists of glucose as carbon source for growth of fungi and malt extract (Himedia) and peptone as nitrogen sources for secondary metabolites production. *E. variecolor* produced the common metabolites and some sesterterpenoid metabolite. Malt Czapek-Dox broth consists of sucrose as carbon source for growth of fungi and malt extract (Himedia) and $NaNO_3$ as organic and inorganic nitrogen sources for secondary metabolites production and mineral sources as cofactor in metabolites production. *E. variecolor* produced the aromatic compounds such as anthraquinones and xanthones but Czapek-Dox broth lack of malt extract for organic nitrogen sources, *E. variecolor* produced only common metabolites. The Diels–Alder construction to generate a complex array of natural products. Enzymes generally catalyze reactions by stabilizing the structure and charge of the developing transition state was described by Stocking and Williams, 2003.

4.6 Metabolites from mycelium and fermentation broth of three media.

Metabolites was isolated from *E. variecolor* culture in three culture media and mycelium and fermentation broth were extracted from EtOAc to obtain the metabolites showed in Table 4.37.

Table 4.37 Metabolites from mycelium and fermentation broth of three media.

Compound	Culture media					
	Malt extract broth		Malt Czapek-Dox broth		Czapek-Dox broth	
	Mycelium	Broth	Mycelium	Broth	Mycelium	Broth
A	✓	×	✓	×	✓	×
B	✓	×	✓	×	✓	×
C1	✓	×	×	×	×	×
C2	×	×	×	✓	×	×
D1	×	✓	×	×	×	×
D2	×	✓	×	×	×	×
D3	×	×	×	✓	×	✓
D4	×	×	×	×	×	✓
E	×	✓	×	×	×	×
F1	×	×	✓	×	×	×
F2	×	×	✓	×	×	×
G1	×	×	✓	×	×	×
G2	×	×	✓	✓	×	×
G3	×	×	×	✓	×	×
H1	×	×	✓	×	×	×
H2	×	×	✓	×	×	×

A = stellatic acid B = ergosterol C1 = Emervaridione C2 = Emervaridionin

D1 = Varioxiranediol D2 = Varitetraol A D3 = Varitetraol B D4 = Varitetraol C

E = Dihydroterrein F1 = 14-methoxytajixanthone-25-acetate F2 = Tajixanthone hydrate

G1 = 1-hydroxy-6,8-dimethoxy-3-methylanthraquinone

G2 = 4,6-dihydroxy-5,7-dimethoxy-2 –methylanthraquinone

G3 = 1,2,8-Trihydroxy,-3-methoxy-6-methylanthraquinone (Dermoglaucin)

H1 = Evanthrasterol A H2 = Evanthrasterol B

4.7 Biological activity tests

4.7.1 Cytotoxic activity test against cancer cell lines

In *vitro* cytotoxiv activity of compound A-H2 against five cell lines, including HEP-G2 (hepatoma), SW 620 (colon), CHAGO (lung), KATO-3 (gastric), BT474 (breast) cancer was reported in Table 4.38.

Table 4.38 Cytotoxic activity against cell lines of compound A-H2

Compound	IC_{50} μg/ml (nM)				
	HEP-G2 (hepatoma)	SW 620 (colon)	CHAGO (lung)	KATO-3 (gastric)	BT474 (breast)
A	>10	>10	>10	>10	>10
B	n/a	n/a	n/a	n/a	n/a
C1	>10	>10	>10	>10	>10
C2	n/a	n/a	n/a	n/a	n/a
D1	>10	>10	>10	>10	>10
D2	>10	>10	>10	>10	>10
D3	>10	>10	>10	>10	>10
D4	>10	>10	>10	>10	>10
E	n/a	n/a	n/a	n/a	n/a
F1	8.7 (17.6)	7.1 (14.4)	>10	5.7 (11.5)	6.0 (12.1)
F2	7.2 (16.4)	6.0 (13.6)	5.1 (11.6)	4.8 (10.9)	5.4 (12.3)
G1	n/a	n/a	n/a	n/a	n/a
G2	n/a	n/a	n/a	n/a	n/a
G3	n/a	n/a	n/a	n/a	n/a
H1	>10	>10	>10	>10	>10
H2	>10	>10	>10	>10	>10

IC_{50} was the minimum concentration of 50 % inhibitory activity.

n/a = no analysis

compound A-H2 (see in 4.5)

The results showed that all of compounds except **F1** and **F2** were inactive against cancer cell lines. Xanthone derivatives **F1** and **F2** seem to inhibit selectively against gastric and beast cancer cell14-methoxy-tajixanthone-25-methanoate **F1** exhibited moderate cytotoxic activity against KATO-3 (gastric) and BT 474 (breast) cell lines with IC_{50} 11.5 and 12.1 μM, respectively which other cell lines were lesser activity. Tajixanthone hydrate exhibited cytotoxic activity against five cell lines with IC_{50} 16.4, 13.6, 11.6, 10.9, and 12.3 μM, respectively (Pornpakakul et. al., 2006).

4.7.2 Antimicrobial activity test.

The isolated compound, compound G1, G2, G3, H1 and H2 were tested for antimicrobial activity showed no antimicrobial activity for 5 microorganisms.

4.7.3 Antioxidant activity test.

The metabolites of *Emericella variecolor*, compound G1, G2, G3, H1 and H2 were tested for free radical scavenging test for antioxidant activity showed inactive all of them.