# CHAPTER IV

RESULTS AND DISCUSSION

4.1 Characteristics of *Psilocybe samuiensis*

4.1.1 Macroscopic structure examination

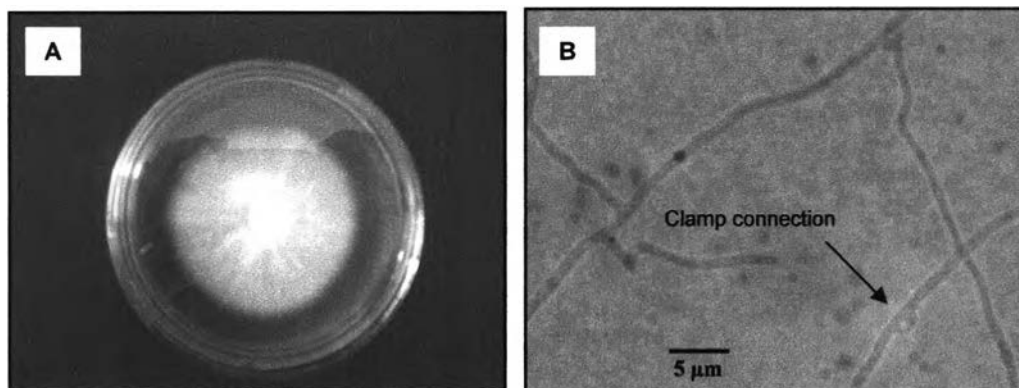The culture of *Psilocybe samuiensis* was whitish mycelium on PDA and MEA and did not produced spores.

4.1.2 Microscopic structure examination

Spores print and dry specimens of *Psilocybe samuiensis* from Koh Samui, Surat Thani Province, Thailand were provided from J. W., Allen. in July 2004. The spores were mounted in lactophenol-cotton blue and observed by light microscopy, and dry specimen was used for Scanning electron microscopy. The semipermanent slide of spores and scanning electron micrograph is shown in Figure 4.1. These results indicated that the spores was purplish brown in deposit, rhomboid to subrhomboid and the scanning electron micrograph showed 4-spored basidia with broad flattened germ pore. These characteristics was according to descriptions of *Psilocybe samuiensis*, which described before in section 2.4.4.
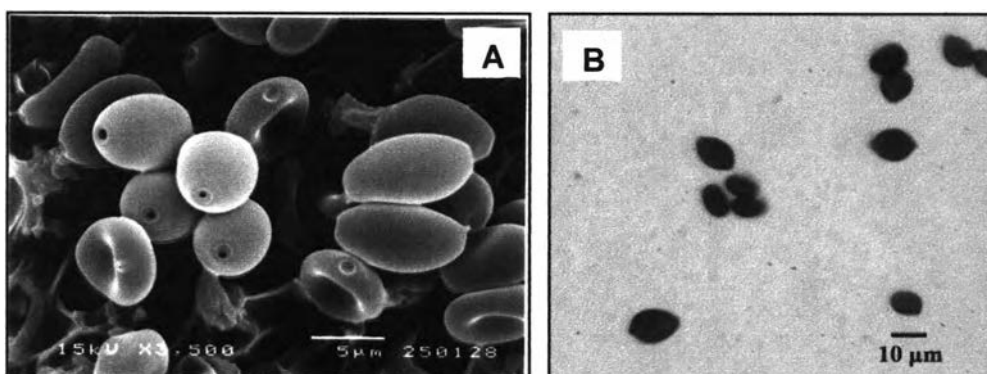
Figure 4.1 (A) Scanning electron micrograph of *Psilocybe samuiensis* basidiospore (Bar=5 μm)

(B) Basidiospore of *Psilocybe samuiensis* prepared from semipermanent slide.

The semipermanent slides (from slide culture) of *Psilocybe samuiensis* were mounted in lactophenol-cotton blue and observed by light microscopy. Colony morphology and slide culture of *Psilocybe samuiensis* is shown in Figures 4.2.

Figure 4.2 (A) Culture on MEA (14days)

(B) Clamp connection of *Psilocybe samuiensis* from slide culture

Classical identification of fungi is based on observing characteristics. Assignment of morphological species can be based on colony surface texture, hyphal structures and sporulating structures. The results showed that the fungus have white mycelia, and clamp connection (special septal structures are usually formed in *Psilocybe* mushrooms). The fungus was identified as basidiomycete fungi, belonging to the genera *Psilocybe*.

4.1.3 Analysis DNA of *Psilocybe samuiensis*

The ITS sequence of *Psilocybe samuiensis* was 654 bp., containing a part of the 18S, ITS_1, 5.8 and 28 rDNA, as shown in Figure 4.3. This sequence was compared with reported ITS sequences in the gene bank. The result revealed that ITS region of this fungus had highest sequence similarity of 93% with *Psilocybe fasciata* (Accession No.AB158635, Score=686 bits) and of 93% with *Psilocybe semilanceata* (Accession No.AJ519801, Score=680 bits, Accession No.AJ519800, Score=676 bits, Accession No.AJ519799, Score=674 bits, Accession No.AJ519797, Score=674, Accession No.AJ519798, Score=583 bits). The identification based on molecular and morphological characteristics indicated that the species of this fungus was *Psilocybe samuiensis*. Since this species was identified as a new species from Koh Samui,

Thailand in 1991, reported by Allen (Allen, 1991) and the ITS sequence had not reported yet.

AGGTTCGTGG	TGACCTGCGG	AGGATCATTA	TTGAATGAAC
TTGACTTGGT	TGTAGCTGGT	TCTCTCGAGG	ACATGTGCTC
ACCTTGTCAT	CTTTATCTAT	CCACCTGTGA	ACTTTTTGTA
GACTTGGAAC	TAGTGAACGG	GAAAGCATGC	TTTCCTTGAA
GCTACACCAG	GCCTATGTTT	TCATATACCC	CAAAGAATGT
AACAGAATGT	ATTGTATGGC	CTTGTGCCTA	TAAATCTATA
CAACTTTCAG	CAACGGATCT	CTTGGCTCTC	GCATCGATGA
AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA
TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	CTTGCGCTCC
TTGGTATTCC	GAGGAGCATG	CCTGTTTGAG	TGTCATTAAA
TTCTCAACCT	TACCAGCTTT	TGCTGATAAT	GGCTTGGATG
TGGGGGCTTC	ATTTTGCTGG	CTTAGGTCGG	CTCCCCTCAA
ATGCATTAGC	TGGTACCCCG	CGTGGAACCG	TCTATTGGTG
TGATAATTAT	CTACGCCGTG	GACATCTACA	TAAATGGGCT
TGTACTGCTT	CTAACCGTCC	NTTCACTGGA	CAATACAATG
ACAATTTGAC	CTCAAATCAG	GTAGGACTAC	CCGCTGAACT
TAAGCATATC	ATAA		

Figure 4.3 Nucleotide sequences of partial 18S region, complete ITS region of *Psilocybe samuiensis*, containing a partial of the 18S, ITS1, 5.8S and 28S rDNA.

4.2 Effects of media on growth of *Psilocybe samuiensis*

Psilocybe samuiensis was grown on five media, Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Yeast Extract Agar (YEA), Corn Meal Agar (CMA) and Sabouraud Dextrose Agar (SDA), for two weeks at room temperature. Colonial morphology of the fungal is shown in Figures 4.4. The result indicated that suitable media for the cultivation of *Psilocybe samuiensis* was PDA and MEA. In this research MEA was selected for cultivation and determined growth rate of *Psilocybe*

samuiensis, because the thickness of colony of *Psilocybe samuiensis* on MEA was higher than PDA (see table 4.1).
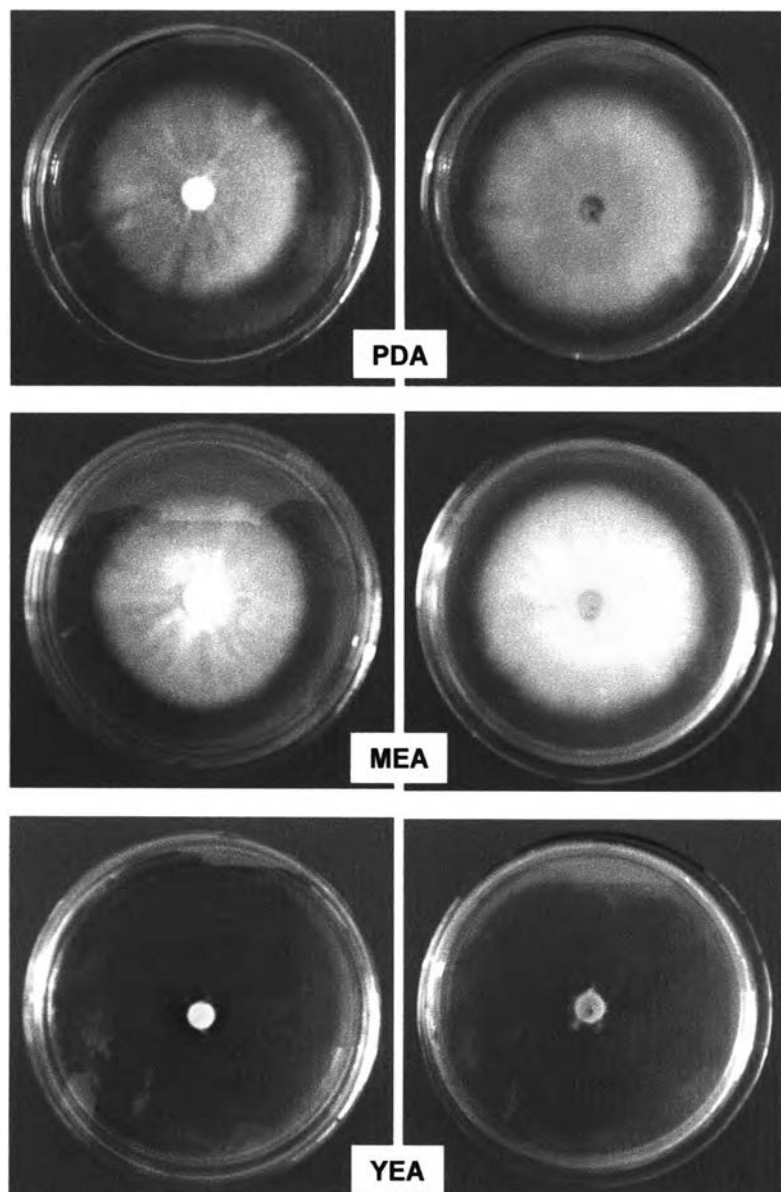
Figure 4.4 Colonial morphology characteristics of *Psilocybe samuiensis* on PDA, MEA, YEA, CMA and SDA media incubated at room temperature for 2 weeks. Appearance on top view (left) and bottom view (right).
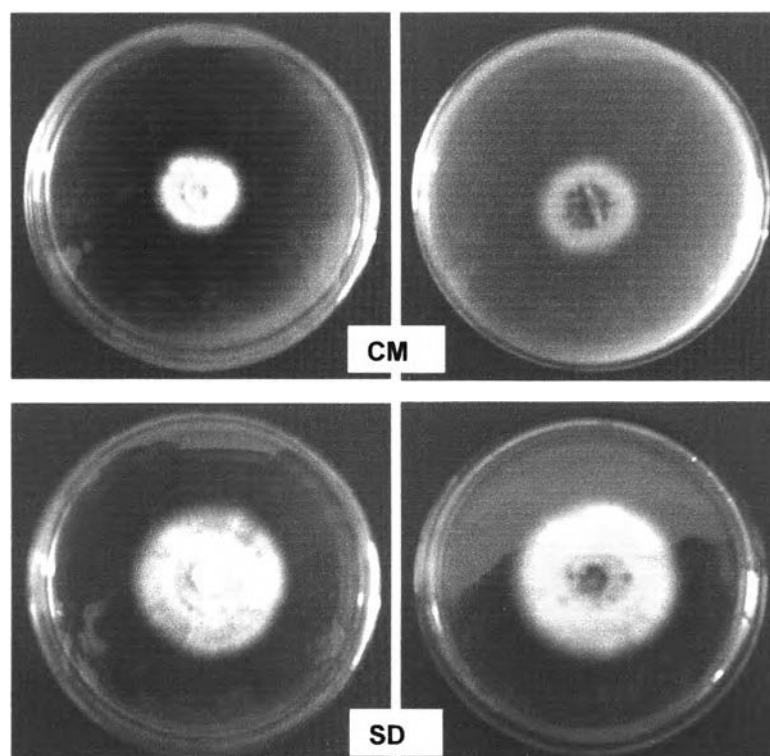
Figure 4.4 (continued) Colonial morphological characteristics of *Psilocybe samuiensis* on PDA, MEA, YEA, CMA and SDA media incubated at room temperature for 2 weeks. Appearance on top view (left) and bottom view (right).

Table 4.1 Colonial size and morphological characteristics of *Psilocybe samuiensis* on PDA, MEA, YEA, CMA and SDA media incubated at room temperature for 2 weeks.

Media	Colonyl size (Ø cm)	Thickness of colony	Colony morphological characteristics
PDA	6.8	+ + +	Growing slowly, slightly translucent and loose mycelia, smooth round margin
MEA	6.5	+ +	Growing slowly, slightly translucent and loose mycelia, smooth round margin
YEA	1.1	+	Non forming colony
CMA	2.6	+ + +	Growing very slow, white and dense mycelia, ringle surface, smooth round margin
SDA	4.8	+ + +	Growing slowly, slightly translucent and loose mycelia with radial growth, smooth round margin

Note: + + + refer to the highest thickness, + + refer to the medium thickness, + refer to the least thickness

Psilocybe samuiensis was cultivated in MEB and incubated at room temperature to determined growth rate. The results were displayed in a plot of dry weight of mycelia (g) and times (week). The results indicated that *Psilocybe samuiensis* started the stationary phase at the fifth week. A plot of growth rate of *Psilocybe samuiensis* is shown in Figure 4.5.
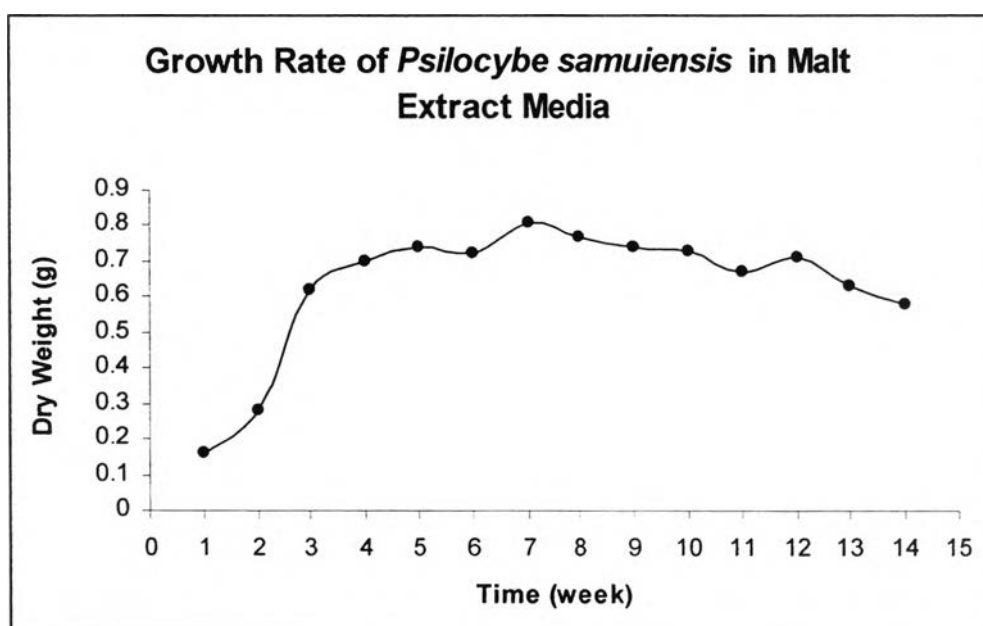
Figure 4.5 Growth curve of *Psilocybe samuiensis*

4.3 Cultivation and extraction of *Psilocybe samuiensis* metabolites

Psilocybe samuiensis was cultivated in 250 ml Erlemeyer flasks containing 100 ml of Malt Extract Broth (x110) malt. The mycelia were extracted with MeOH, then partitioned with hexane, EtOAc and MeOH, respectively. Then, the concentrated broth was extracted with hexane, EtOAc, and MeOH, respectively.

4.4 The chemical constituents from fermentation broth of *Psilocybe samuiensis*

A small amount of hexane and EtOAc crudes obtained from extraction of mycelia was subjected to ^{1}H-NMR analysis. The ^{1}H-NMR data showed that the hexane and EtOAc crude mainly consisted of triglyceride and fatty acid. The EtOAc crude extract of broth was isolated by silica gel column chromatography. Compound **1** was obtained in the fraction eluted by 3% methanol in dichloromethane as white solid mixed with yellow viscous liquid. After purification by column chromatography and

crystallization in CH_2Cl_2 and MeOH compound **1** was obtained as colorless crystals. The filtrate was further purified by column chromatography, and crystallization to give more colorless crystals of compound **1**. Totally compound **1** was 736 mg (yield 66.9 mg/L).

The mother liquor from crystallization of **1** was isolated by preparative TLC to afford compound **2** as colorless oil, R_f, 0.55 (10% MeOH in CH_2Cl_2), $[\alpha]_D^{20}$; -51 ($CHCl_3$; c 0.07). Compound **2** may be derived from **1** via the methoxylation. Thus compound **1** was treated with methanol in the presence of p-toluenesulfonic acid. After the purification compound **2** was obtained as colorless oil. The spectral data (^{1}H and ^{13}C NMR spectra) of compound **2** from this reaction were similar to compound **2** from the filtrate of compound **1**. This result suggested that compound **2** may not naturally occur, but it may be caused by methoxylation during purification and crystallization of compound **1**. Chemical structures of these compounds were determined by analyzes of spectroscopic data, including IR, NMR and Mass spectra and X-ray crystallographic data.

4.5 Structural elucidation of isolated compounds from culture of *Psilocybe samuiensis*

4.5.1 Structure elucidation of compound 1

Molecular formula of **1** was established by HR/ES-TOF MS (m/z 293.1732 $[M+Na]^+$; calc. 293.1729) as $C_{15}H_{26}O_4$. The IR spectrum of **1** was showed the vibration of functional groups as summarized in Table 4.2.

Table 4.2 The IR absorption band assignment of compound **1**

Wave number (cm^{-1})	Intensity	Tentative assignment
3425-3352	Broad	O-H stretching vibration of alcohol
2960, 2927, 2872	Strong	C-H stretching vibration of CH_2-, CH_3-
1140, 1102, 1043, 1013	Strong	C-O stretching vibration

The ^{1}H-NMR spectrum (Fig. B2, Table 4.3) of compound **1** showed three methyl signals at δ_H 0.83, 1.13 and 1.34 ppm and five signals of protons on carbons bearing hydroxyl groups at δ_H 3.27, 3.55, 3.42, 3.87 and 5.30 ppm were indicated.

^{13}C-NMR and HSQC NMR spectral data indicated signals of 15 carbons comprising of three methyl carbons at δ_C 11.4, 14.5 and 31.6 ppm, four methylene carbons at δ_C 18.4, 38.6, 61.8 and 73.4 ppm, six methine carbons at δ_C 21.5, 24.2, 30.4, 35.6, 56.6 and 92.9 ppm and two quaternary sp^3 carbons at δ_C 26.5 and 73.4 ppm. The signals at δ_C 92.9, 73.4, 73.4 and 61.8 ppm indicated the signals of carbons which attached with oxygen atom. ^{13}C-NMR spectrum showed no signal of sp^2- and sp- carbon. From molecular formula indicating three degrees of unsaturation, revealed that the molecular structure of **1** should have three cyclic ring.

The information from 1D-NMR and 2D-NMR including COSY (Fig. B4, Table 4.3), HSQC (Fig. B5, Table 4.3), HMBC (Fig. B6, Table 4.3) and NOESY (Fig. B7, Table 4.3) were used to assist the structure assignment of compound **1**.

Table 4.3 The ^{1}H, ^{13}C, HSQC, COSY, HMBC and NOESY spectral data of compound **1**

| Position	δ_C	δ_H	COSY	HMBC	NOESY
1	56.6	1.94 (1H, br s)	H-2	C-2, C5, C6, C9, C-10	H-2, H-14
2	92.9	5.30 (1H, d, 2 Hz)	H-1	C-3, C-5	H-1, -14-H
3	61.8	3.42 (1Ha, dd, J=11.6, 4.4 Hz)	H-3b	C-2, C-4, C-5	H-14
		3.87 (1Hb, dd, J=11.6, 11.6 Hz)	H3a, H-4	C-2, C-4, C-5, C-15	H-6, H-14
4	35.6	2.01 (1H, m)	H-3b, H-15	C-3, C-6, C-15	H-15

**Table 4.3** (continued) The ^{1}H, ^{13}C, HSQC, COSY, HMBC and NOESY spectral data of compound **1**

Position	δ_C	δ_H	COSY	HMBC	NOESY
5	30.4	2.09 (1H, m)	H-6	C-1, C-6	H-13, H-8a
6	21.5	0.93 (1H, t, J=10 Hz)	H-5	C-7	H-3b, H-9b
7	24.2	0.91 (1H, m)	H-8a, H-8b	C-6	H-9b, H-12b
8	18.4	1.53 (1Ha, m)	H-7, H-9b	C-7, C-10	H-5, H-13
		1.69 (1Hb, m)	H-7, H-9a	C-6, C-7, C-9, C-10	H-9a
9	38.6	1.55 (1Ha, dd, J=6.8, 14.4 Hz)	H-8b	C-1, C-7, C-8, C-10 C-14	H-8b
		2.11 (1Hb, dd, J=12.4, 14.0 Hz)	H-8a	C-7, C-8	H-6
10	73.4	-	-	-	-
11	26.5	-	-	-	-
12	73.4	3.27 (1-Ha, d, J=10.8 Hz)	H-12b	C-6, C-7, C11, C-13	H-6
		3.55 (1-Hb, d, J=10.4 Hz)	H-12a	C-6, C-7, C11, C-13	H-7
13	11.4	1.13 (3H, s)	-	C-6, C-7, C11, C-12	H-5, H-8a
14	31.6	1.34 (3H, s)	-	C-1, C-9, C-10	H-1, H-2
15	14.5	0.83 (3H, d, J=7.2 Hz)	H-4	C-3, C-4, C-5	H-4

The ^{1}H-^{1}H COSY and HSQC spectral data (Fig B4) showed a methylene proton at δ_H 3.87 ppm and methyl protons at δ_H 0.83 ppm were coupled with methine proton at 2.01 ppm. Thus, it concluded that the carbon at δ_C 35.6 was adjacent to a methyl carbon at δ_C 14.5 ppm and a oxygenated methyl carbon at δ_C 61.8 pmm.
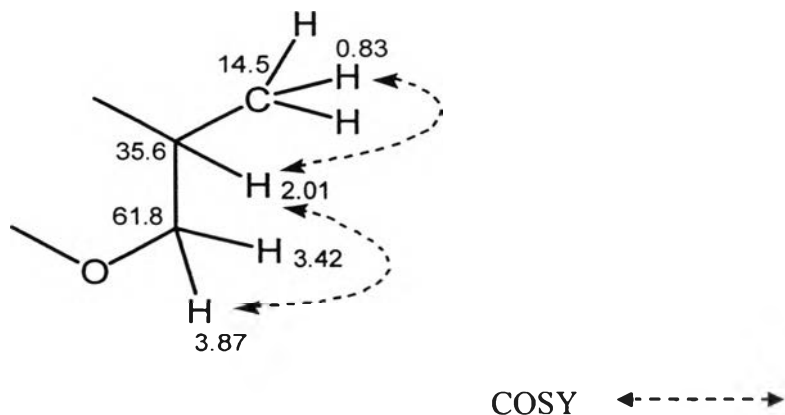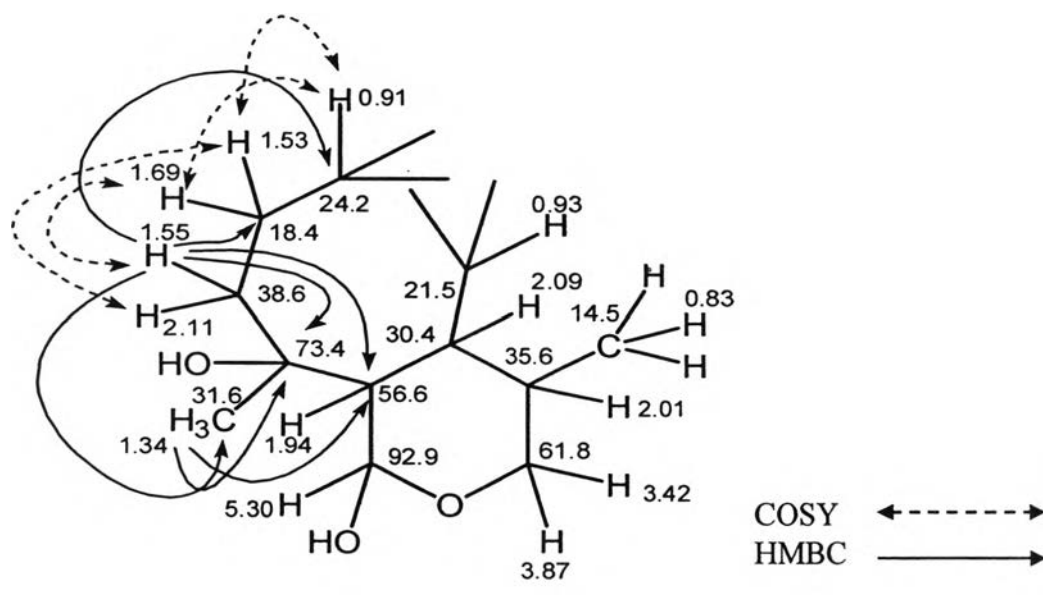

Scheme 4.1 Partial structure part I of compound **1**

The COSY spectrum showed that the proton at δ_H 5.30 ppm coupled with proton at 1.94 ppm, the proton at δ_H 1.94 ppm coupled with the proton at δ_H 2.09 ppm and the proton at δ_H 2.09 ppm coupled with the proton at δ_H 0.93 ppm. These data indicated the connections between the carbons at δ_C 92.9 and 56.6 ppm, 56.6 and 30.4 ppm and 30.4 and 21.5 ppm. The HMBC spectrum showed that the proton at δ_H 5.30 ppm was correlated to carbons at δ_C 61.8 and 30.4 ppm, the proton at δ_H 1.94 ppm was correlated to carbon at δ_C 92.9, 30.4 and 21.5 ppm, the proton at δ_H 2.09 ppm was correlated to carbon at δ_C 21.5 and 56.6 ppm, the proton at δ_H 3.87 ppm was correlated to carbon at δ_C 92.9, 35.6 and 30.4 ppm and the proton at δ_H 2.01 was correlated to carbon at δ_C 21.5 ppm. The chemical shift at δ_C 92.9 was assigned to the acetal group (see Scheme 4.2). The oxygen linkage of carbons at δ_C 92.9 and at δ_C 61.8 were assigned due to the observation of long-range ^{1}H-^{13}C correlations between δ_H 5.3 with δ_C 61.8 and δ_H 3.42 and 3.87 with δ_C 92.8.
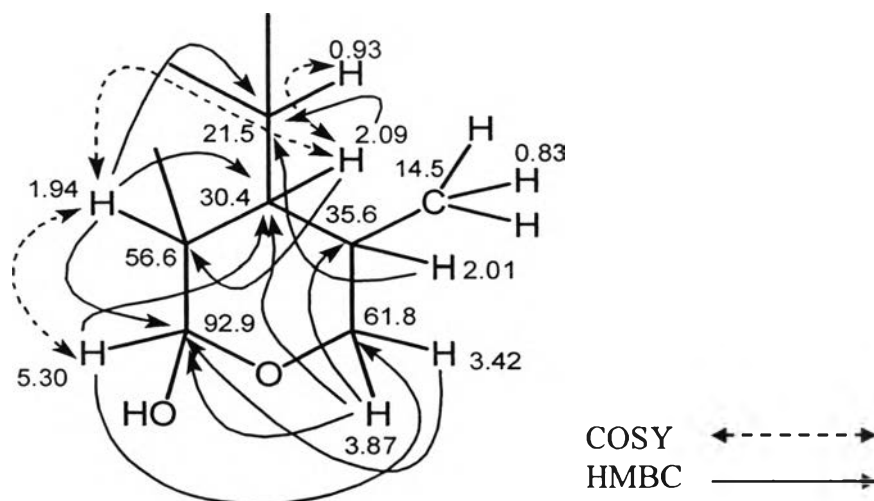
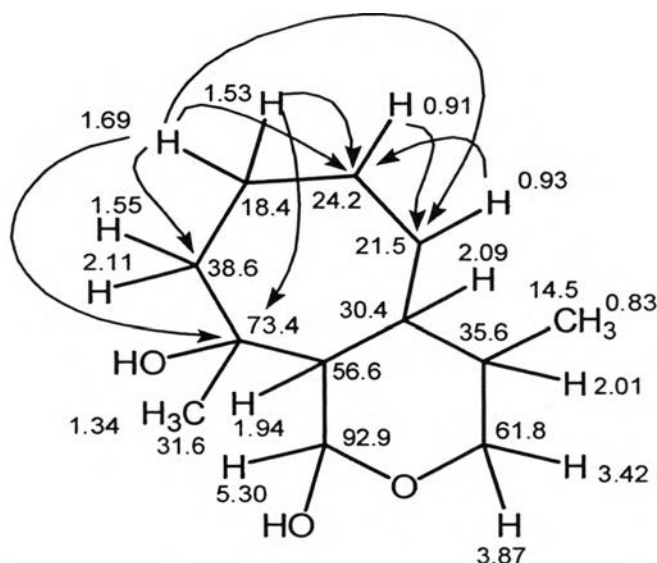**Scheme 4.2** Partial structure part II of compound **1**

The proton at δ_H 1.55 ppm was coupled with the proton at δ_H 1.69 ppm, the proton at δ_H 2.11 ppm was coupled with the proton at δ_H 1.53 ppm and the proton at δ_H 1.53 and 1.69 ppm were coupled with the proton at δ_H 0.91 ppm according to COSY spectrum. These data indicated the connection between the methylene carbons at δ_C 38.6 and 18.4 ppm and the connection between the methylene carbon at δ_C 18.4 and methine carbon at δ_C 24.2 ppm. The HMBC correlations showed that the proton at δ_H 1.55 ppm was correlated to carbon at δ_C 56.6, 73.4, 31.6, 24.2 and 18.4 ppm and the methyl proton at δ_H 1.34 was correlated to carbon at δ_C 73.4 and 56.6 ppm. The ^{13}C-NMR showed the signal of the quaternary carbon which was attached with oxygen atom at δ_C 73.4 ppm (see Scheme 4.3).
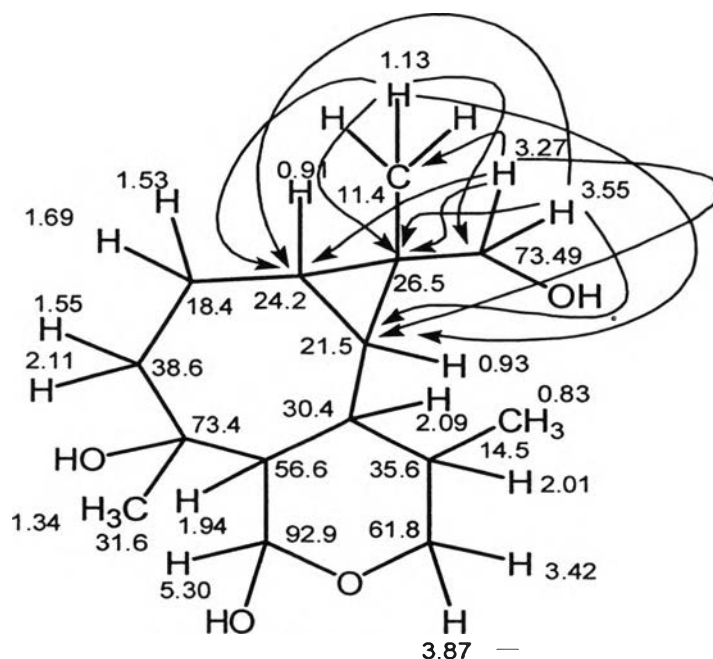

Scheme 4.3 Partial structure part III of compound **1**

The HMBC spectrum showed that the proton at δ_H 1.53 ppm was correlated to carbon at δ_C 73.4 and 24.2 ppm, proton at δ_H 1.69 was correlated to carbon at δ_C 73.4, 38.6, 24.2 and 21.5 ppm, proton at δ_H 0.91 ppm was correlated to carbon at δ_C 21.53 ppm and proton at δ_H 0.93 ppm was correlated to the carbon at δ_C 24.2 (see Scheme 4.4).


Scheme 4.4 Partial structure part IV of compound **1**

The HMBC spectrum showed that the proton at δ_H 3.27 and 3.55 ppm were correlated to carbon at δ_C 24.2, 21.5, 26.5 and 11.4 ppm, proton at δ_H 1.13 ppm was correlated to carbon at δ_C 24.2, 21.5, 73.4 and 26.5 ppm (see Scheme 4.5).
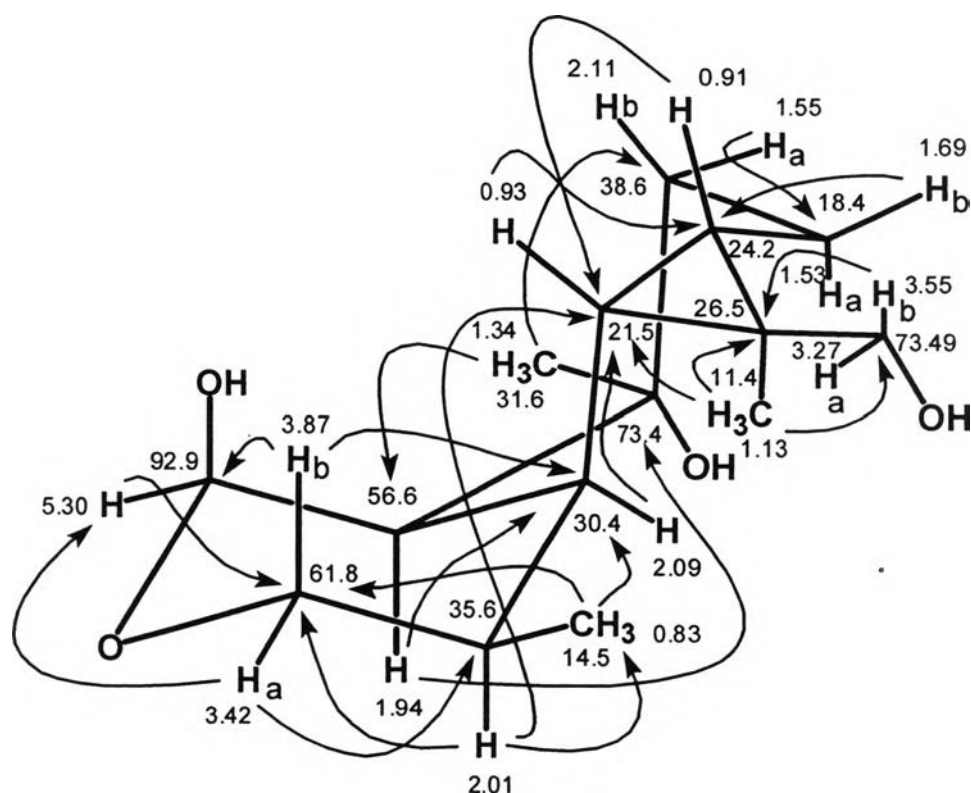

Scheme 4.5 Partial structure part V of compound **1**

Thus the structure of compound **1** was established to be 2,3-secoaromadendrane-2,10,12-triol as shown in Figure 4.6. The long range C-H correlation by HMBC is summarized in Scheme 4.6.
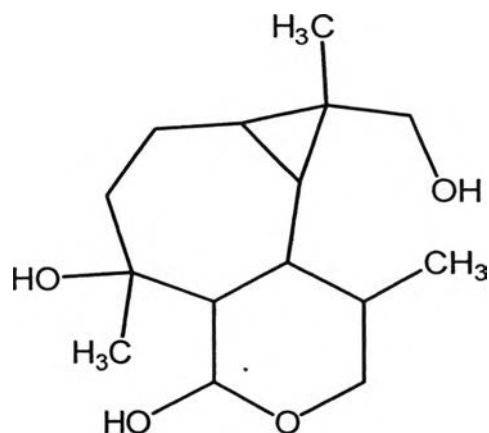
**Figure 4.6** The structure of compound **1**

**Scheme 4.6** The crucial HMBC correlations of compound **1**

The structure search in the literature revealed that compound **1** was a novel compound. The most closely related structures were plagiochiline B (Matsuo et al., 1981), because this compounds have partial structure similar to compound **1** (Table 4.4). From ^{13}C-NMR chemical shift of compound **1** compared with A, the structure of compound **1** was proposed as in Figure 4.6.

Table 4.4 ^{13}C-NMR chemical shift of compound **1** was compared to plagiochiline B

| Position	Chemical shift of ^{13}C NMR	
	Compound 1	Plagiochiline B
1	56.6	50.1
2	92.9	92.0
3	61.8	140.9
4	35.62	116.0
5	30.4	31.6
6	21.5	30.5
7	24.2	27.8
8	18.4	22.1
9	38.6	33.9
10	73.4	60.0
11	26.5	21.7
12	73.4	65.4
13	11.4	24.3
14	31.6	51.6
15	14.5	63.5
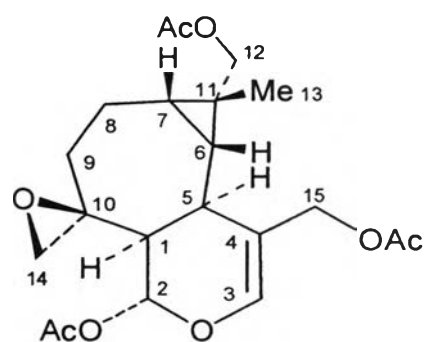
Plagiochiline B
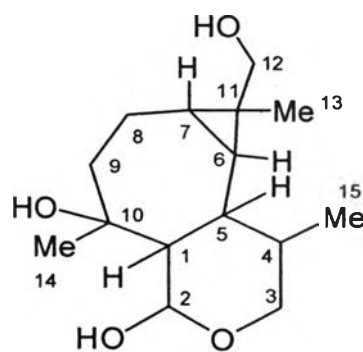
Compound 1

The stereochemistry of compound **1** was asigned by combination of NOESY data and coupling constant. The NOESY revealed crosspeak of H-1(1.94) and H-2 (5.30), of H-1 (1.94) and Me-14 (1.34), of H-2 (5.30) and Me-14 (1.34), of H-3a (3.42) and Me-15 (0.83), of H-3b (3.87) and Me-15 (0.83), of H-3b (3.87) and H-6 (0.93), of H-5 (2.09) and Me-13 (1.13), of H-6 (0.93) and H-12a (3.27), of H-6 (0.93) and H-9b (2.11), of H-7 (0.91) and H-12b (3.55), of H-12a (3.27) and H-12b (3.55) and of H-13 (1.13) and H-8a (1.53). Therefore, compound **1** was established as ent-2,3-secoaromadendrane-2,10,12-triol. Key NOESY correlations in compound **1** are shown in Scheme 4.7.

The conclusion of this compound was supported by a single crystal X-ray determination which showed the relative stereochemistry, as shown in Figure 4.7.
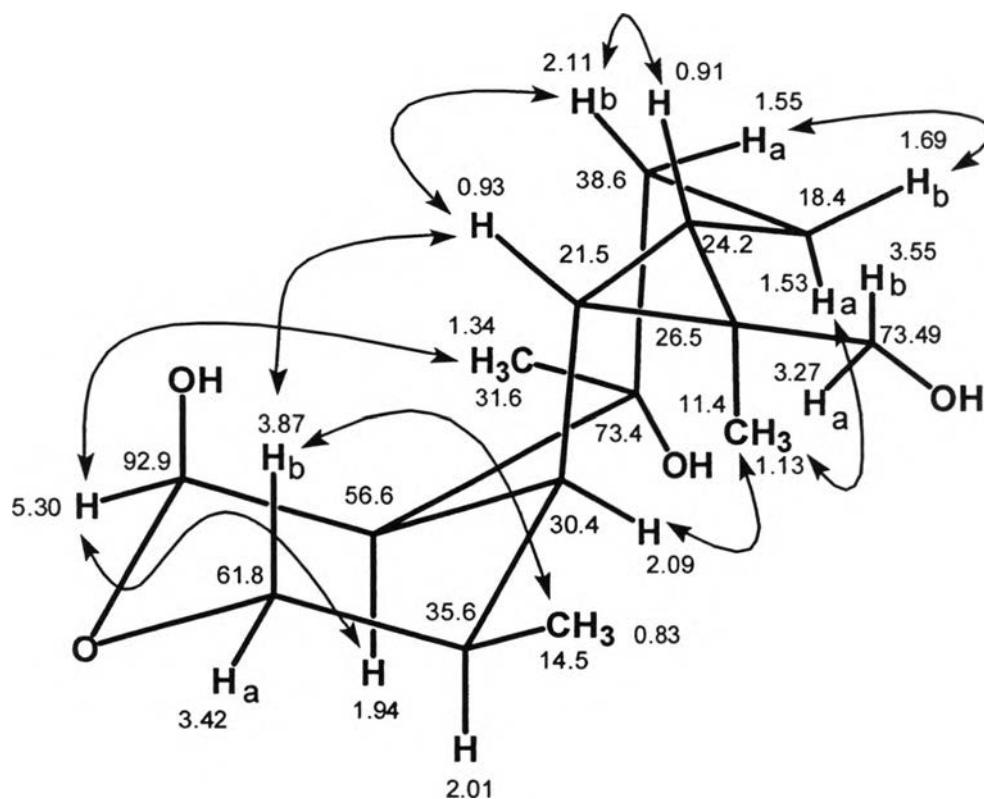
Scheme 4.7 The crucial NOESY correlations of compound **1**
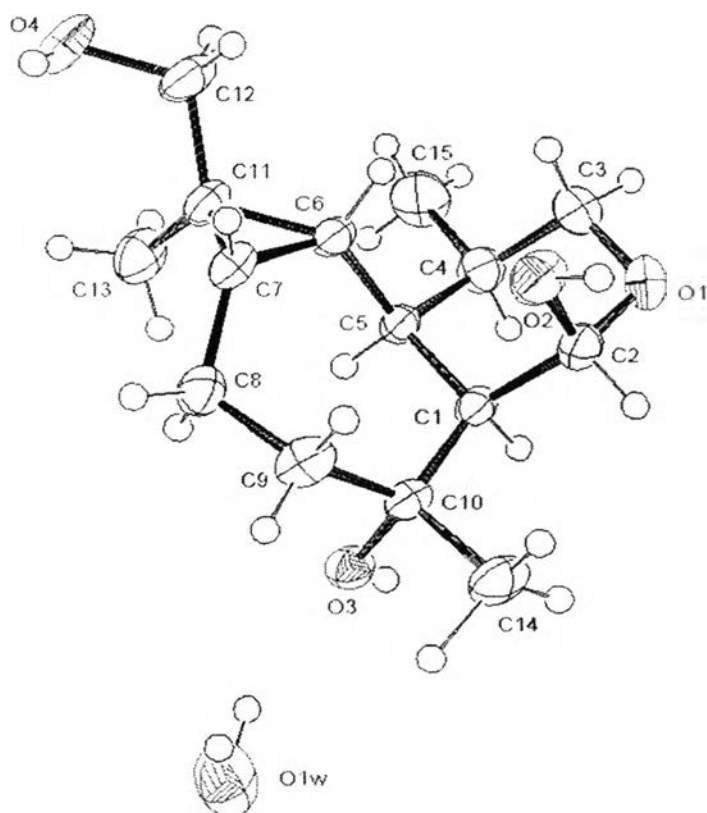
Figure 4.7 The ORTEP drawing of compound **1**

4.5.2 Structure elucidation of compound 2

Compound **2** was isolated as colorless oil whose molecular formula was established as $C_{16}H_{28}O_4$ from HR/ES-TOF MS (a molecular ion ([M+Na]$^+$) with *m/z* 307.1890; calc. 307.1885), which indicated DBE of 3. The IR spectrum was consistent with the presence of hydroxyl functional group (3397 cm^{-1}) and IR spectrum was summarized in Table 4.5.

Table 4.5 The IR absorption band assignment of compound **2**

Wavenumber (cm^{-1})	Intensity	Tentative assignment
3397	Broad	O-H stretching vibration of alcohol
2967, 2931, 2878	Strong	C-H stretching vibration of CH_2-, CH_3-
1129, 1103, 1045	Strong	C-O stretching vibration

The information from 2D-NMR techniques, COSY correlations (Fig. B12, Table 4.6), HSQC correlations (Fig. B13, Table 4.6), HMBC correlations (Fig. B14,

Table 4.6) and NOESY correlations (Fig. B15) were used to assist the structure assignment of compound **2**.

The ^{1}H-NMR spectrum (Fig. B10, Table 4.6) of compound **1** showed three methyl group signals at δ_H 0.83, 1.13 and 1.34 ppm, and one methoxy group at δ_H 3.34 ppm.

The ^{13}C-NMR spectrum (Fig. B11, Table 4.6) and HSQC showed 16 lines of difference carbons. The signals at δ_C 99.6, 73.9, 73.4, 61.8 and 55.3 ppm indicated the signal of carbon which attached with hetero atom. Four signals of methyl carbons appeared at δ_C 55.3, 31.7, 14.4 and 11.5 ppm and two quaternary sp^3 carbon signals at δ_C 73.4 and 26.3 ppm. The signals at δ_C 73.9, 61.8, 38.6 and 18.5 ppm indicated the signal of methylene carbon and signal of methine carbons were displayed at δ_C 99.6, 56.6, 35.6, 30.8, 24.3 and 21.9 ppm. Since the ^{13}C-NMR spectrum showed no signals of *sp^2*- and *sp*- carbon, and from formula indicating three degrees of unsaturation, so the molecular structure should have three cyclic ring.

Table 4.6 The ^{1}H, ^{13}C, HSQC, COSY and HMBC spectral data of compound **2**

Position	δ_C	δ_H	COSY	HMBC	NOESY
1	56.6	1.89 (1H, br s)	H-2	C-2, C-6, C-9, C-10	H-2,H-14
2	99.6	4.70 (1H, d, *J*=2.4 Hz)	H-1	C-3, C-5, C-16	H-1, H-14, H-16
3	61.8	3.32 (1H, dd, *J*=11.6, 4.8 Hz)	H-3b	C-2, C-4, C-5	-
		3.64 (1H, dd, *J*=11.6, 11.6 Hz)	H-3a, H-4	C-2, C-4, C-5, C-15	H-6
4	35.6	1.95 (1H, m)	H-3b	C-3, C-5, C-6, C-15	H-15
5	30.8	1.96 (1H, m)	H-6	C-6	H-8a, H-13

**Table 4.6** (continued) The ^{1}H, ^{13}C, HSQC, COSY and HMBC spectral data of compound **2**

| Position	δ_C	δ_H	COSY	HMBC	NOESY
6	21.9	0.84 (1H, m)	H-5	C-4, C-7, C-12	H-3b, H-9b
7	24.3	0.82 (1H, m)	H-8a, H-8b	C-6, C-12	H-9b
8	18.5	1.47 (1H, d, J=12.8 Hz)	H-7, H-9b	C-7, C9, C-10	-
		1.64 (1H, m)	H-7, H-9a	C-6, C-7, C-9, C-10	H-9a
9	38.6	1.47 (1H, b d, J=14.0 Hz)	H-8b	C-1, C-7, C-8, C-10, C-14	H-8b
		2.03 (1H, dd, J=12.4, 14.0 Hz)	H-8a	C-7, C-8	H-6
10	73.4	-	-	-	-
11	26.3	-	-	-	-
12	73.9	3.21 (1H, d, J=11.2 Hz)	H-12b	C-6, C-7, C-11, C-13	-
		3.51 (1H, d, J=11.2 Hz)	H-12a	C-6, C-7, C-11, C-13	-
13	11.5	1.08 (3H, s)		C-6, C-7, C-11, C-12	H-5
14	31.7	1.24 (3H, s)		C-8, C-9, C-10	H-2, H-2
15	14.4	0.77 (3H, d, J=6.8 Hz)	H-4	C-3, C-4, C-5	H-4
16	55.3	3.34 (3H, s)		C-2	H-2

By comparison of spectral data of **2** with those of **1** (Table 4.7), compound **2** was similar to of **1**, except that compound **2** had one more methyl of the methoxy group. The long-range ^{1}H-^{13}C correlation (the HMBC) between methoxy protons at δ_H 3.34 and carbon at δ_C 99.6 indicated that the methoxy group was connected to the C-2 (δ_C 99.6) on the axial position.

Table 4.7 ^{13}C NMR chemical shift of compound **2** and compound **1**

Position	Chemical shift of ^{13}C-NMR	
	Compound **1**	Compound **2**
1	56.6	56.6
2	92.9	99.6
3	61.8	61.8
4	35.6	35.6
5	30.4	30.8
6	21.5	21.9
7	24.2	24.3
8	18.4	18.5
9	38.6	38.6
10	73.4	73.4
11	26.5	26.3
12	73.4	73.9
13	11.4	11.5
14	31.6	31.7
15	14.5	14.4
16	-	55.3
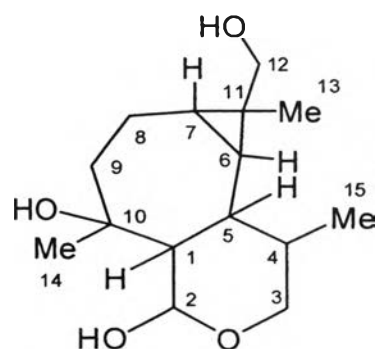
Compound **1**
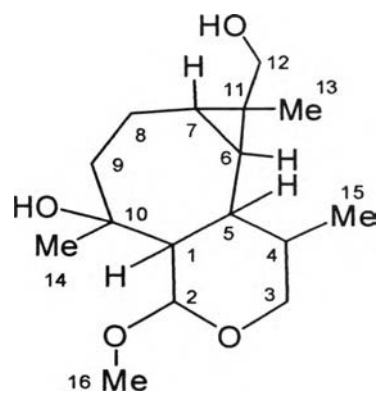
Compound **2**

The relative configuration of compound **2** was determined on the basis of NOESY spectra. The NOESY revealed crosspeak of H-1 (1.89) and H-2 (4.70), of H-2 (4.70) and Me-14 (1.24), of H-2 (4.70) and Me-16 (3.34), of H-3b (3.64) and Me-14 (1.24), of H-3b (3.64) and H-6 (0.84), of H-5 (1.96) and Me-13 (1.10), of H-12a (3.21) and H-6 (0.84) and of H-12b (3.51) and H-7 (0.82).

Thus, the structure of compound **2** was proposed to be *ent*-2,3-secoaromadendrane-2-methoxy-10,12-diol. The ^{13}C NMR chemical shift of compound **2** was compared with those of compound **1** to confirm the structure (Table 4.7).

COSY, HMBC correlations and NOESY correlation spectrum are summarized in Scheme 4.8, 4.9 and 4.10, respectively.
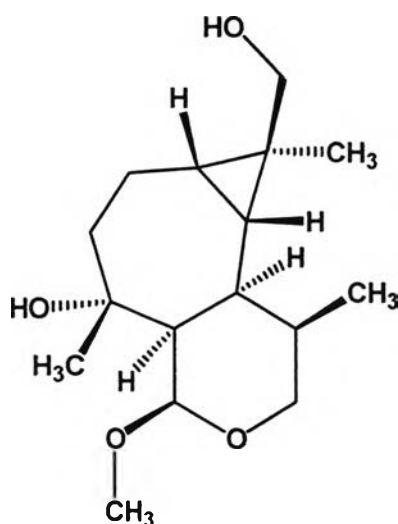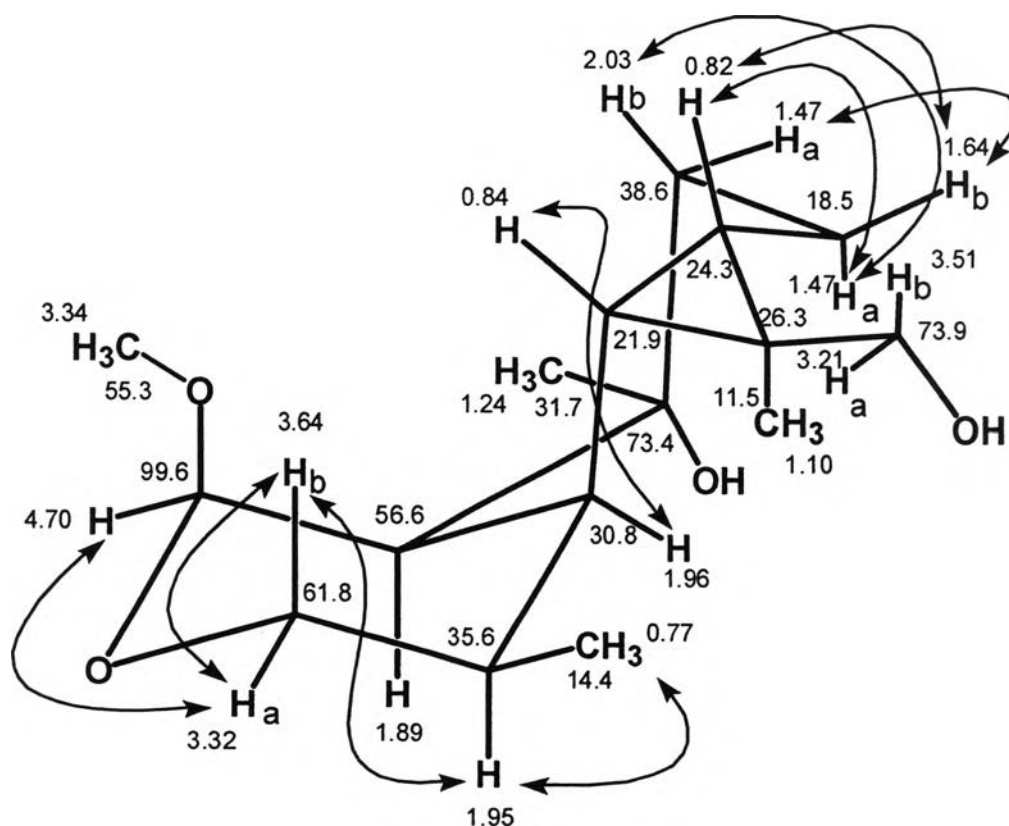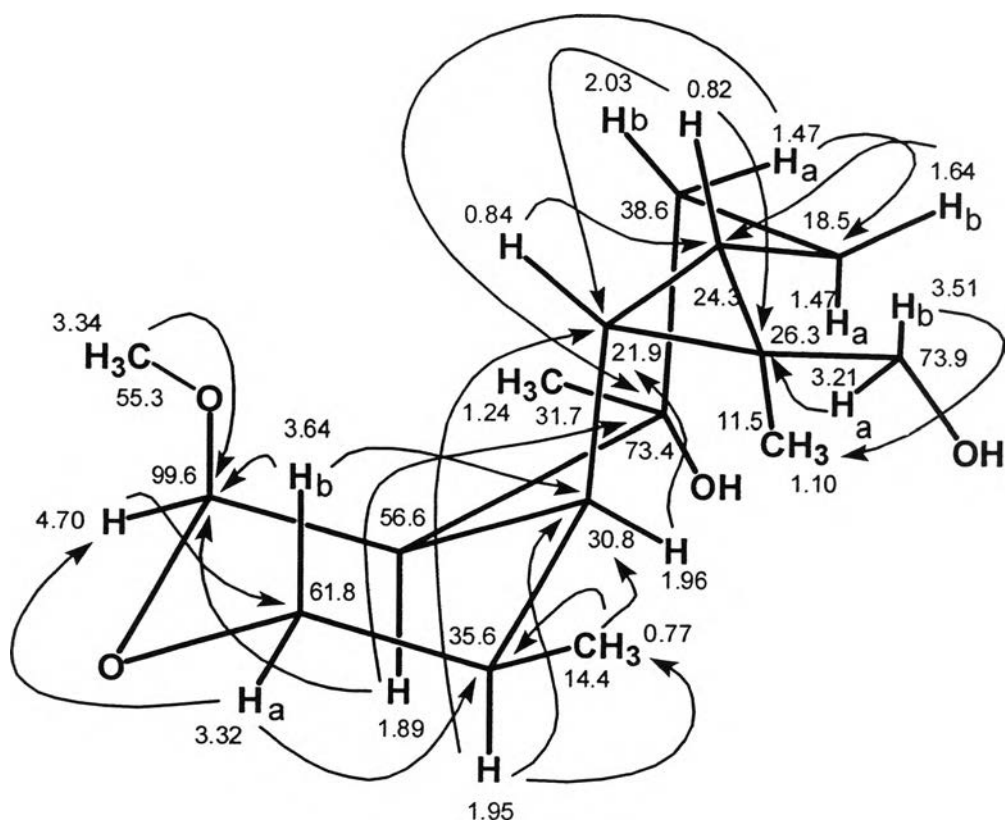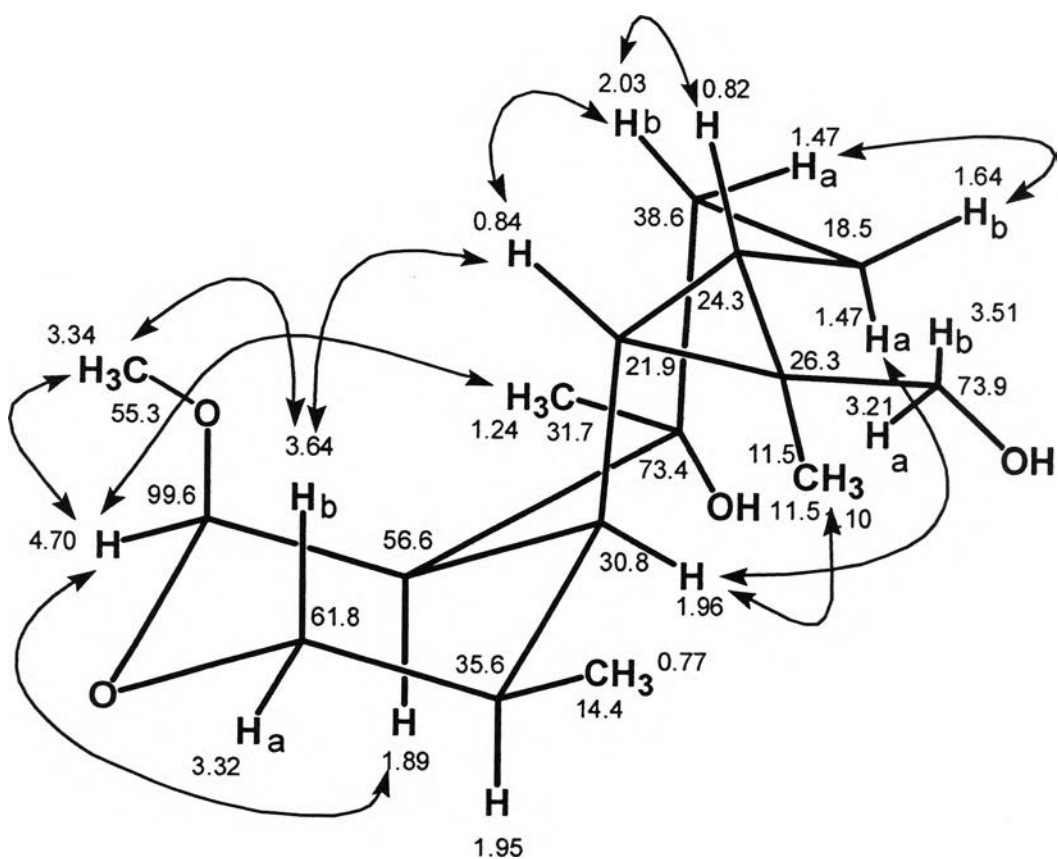

Figure 4.8 The structure of compound **2**

**Scheme 4.8** The COSY correlations of compound **2**

Scheme 4.9 The crucial HMBC correlations of compound **2**

Scheme 4.10 The crucial NOESY correlations of compound **2**

The results of mass spectrometric experiment in acidic matrix (H_2O-MeOH-Formic acid) showed molecular ion m/e 235.33 $[M+H]^+$ (Figure B17). It indicated that compound **2** may be unstable in acidic medium and methoxy and hydroxy group at C-2 and C-10, consequentially removed to give compound **3** (see Figure 4.9).
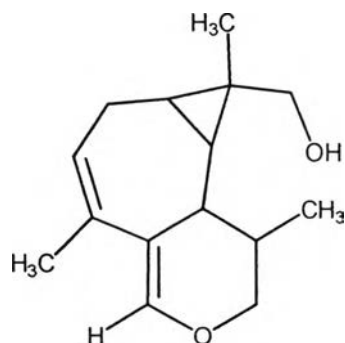
Figure 4.9 The proposed structure of compound **3**

The previous literature revealed that *ent*-secoaromadendrane usually has been isolated from liverwerts and some *ent*-secoaromadendrane was reported as biological active compound such as plagiochiline A from *Plagiochila yokogurensis*. This compound has antifeeding effect on African army worm (Asakawa et al., 1980). Moreover 9α-acetoxyovalifoline isolated from *Plagiochila senidecurrens* was a plant growth inhibitor (Matsuo et al., 1981) (See others in Appendix C).

4.6 Test of *Psilocybe samuiensis* chemical constituents on antimicrobial activity and cytotoxicity

4.6.1 Antimicrobial activity test

Compound **1** was tested for antimicrobial activity assay. Microorganisms used in antimicrobial activity assay were *Bacillus subtilis* ATTC 6633, *Staphyllococcus aureus* ATTC 25923, *Escherichia coli* ATTC 25922, *Pseudomonas aeruginosa* ATTC 27853 and *Candida albicans* ATTC 10231. Streptomycin and Captan (1μg/ml) were used as controlled substances for bacteria and yeast, respectively. This experiment showed that compound **1** was inactive against those five microorganisms.

4.6.2 Cytotoxicity test

Compound **1** was tested for cytotoxic activity towards 5 human tumer cell lines which were HEP-G2 (hepatoma), SW620 (colon), Chago (lung), KATO-3 (gastric) and BT474 (breast) *in vitro* using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. The results indicated that compound **1** was inactive against those five cell lines.