CHAPTER IV

RESULTS AND DISCUSSION

4.1 Structure elucidation of the isolated compound from the stem bark of *C. oblongifolius.*

4.1.1 Structure elucidation of Compound 1.

The IR spectrum of compound 1 is shown in Fig B1 and the absorption peaks were assigned as shown in Table 4.1. Its IR spectrum showed important absorption bands at 3300-2400 cm^{-1} of the O-H stretching vibration of acid, the absorption bands at 2936 and 2853 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the absorption band at 1688 cm^{-1} of the C=O stretching vibration of carbonyl group and the absorption band at 1469 cm^{-1} of the C=C stretching vibration of alkene. The absorption bands were assigned as shown in Table 4.1.

Table 4.1 The IR absorption bands assignment of Compound 1.

Wave number (cm^{-1})	Intensity	Vibration
3300-2400	broad	O-H stretching vibration of acid
2936, 2853	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1688	strong	C=O stretching vibration of carboxylic acid
1469	medium	C=C stretching vibration of alkene
1258, 1192	medium	C-O stretching vibration
874	medium	C-H out of plane bending vibration

The ^{1}H-NMR spectrum (Fig. B2, Table 4.2) of compound 1 indicated that it possesses two methyl groups at 1.01 and 1.22 ppm and two olefmic protons at 4.76 and 4.82 ppm.

The ^{13}C-NMR spectrum (Fig. B3, Table 4.3) showed 20 lines. The signal at δ 184.9 ppm should be the carbonyl of carboxylic acid. Two signals of olefmic carbons appeared at δ 155.4 and 102.3 ppm. There were two methyl carbon signals at δ 28.1 and 14.9 ppm and three methine carbon signals at δ 56.8, 55.1 and 43.8 ppm. Nine signals of methylene carbons appeared at δ 48.7, 41.1, 40.7, 39.4, 37.8, 32.8, 21.7, 18.9 and 18.0 ppm and three quaternary carbons at δ 44.2, 43.0 and 39.4 ppm.

Its molecular formula was established as $C_{20}H_{30}O_2$, which was confirmed by observing molecular ion at *m/z* 302 (Fig. B4). These data indicated that compound **1** was kaur-16-en-19-oic acid which was reported in the literature [8].

The ^{1}H-NMR and ^{13}C-NMR chemical shifts of compound **1** were compared with those of the kaur-16-en-19-oic acid as shown in Tables 4.2 and 4.3, respectively.

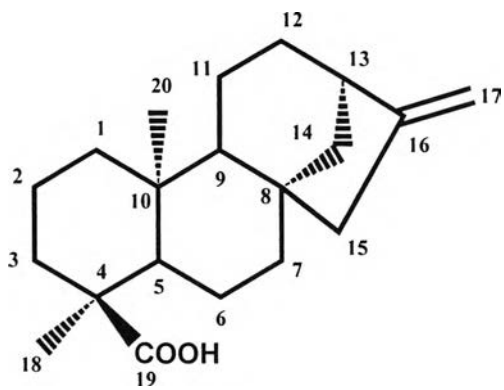


Figure 4.1 Structure of Compound **1**.

Table 4.2 ^{1}H-NMR spectra of Compound **1** and Kaur-16-en-19-oic acid

Position	Chemical shifts (ppm)	
	Kaur-16-en-19-oic acid [8]	Compound **1**
H-1a	0.83 (1H, br)	0.88 (1H, br)
H-1b	0.81 (1H, d, *J*=4)	0.87 (1H, d, *J*=4)
H-2a	1.83 (1H, br)	1.91 (1H, br)
H-2b	1.85 (1H, br)	1.92 (1H, br)
H-3a	1.12 (1H, d, *J*=5)	1.12 (1H, d, *J*=5)
H-3b	2.16 (1H, d, *J*=14)	2.18 (1H, d, *J*=14)
H-4	-	-
H-5	1.06 (1H, d, *J*=7.34)	1.09 (1H, d, *J*=7)
H-6a	1.81 (1H, d, *J*=3.50)	1.87 (1H, d, *J*=3)
H-6b	1.91 (1H, dt, *J*=3, 4)	1.94 (1H, br)
H-7a	1.51 (1H, dt, *J*=3, 4)	1.56 (1H, br)
H-7b	1.44 (1H, br)	1.46 (1H, br)
H-8	-	-
H-9	1.05 (1H, d, *J*=7)	1.05 (1H, d, *J*=7)
H-10	-	-
H-11a	1.60 (1H, br)	1.62 (1H, br)
H-11b	1.59 (1H, br)	1.61 (1H, br)
H-12a	1.47 (1H, br)	1.49 (1H, br)
H-12b	1.61 (1H, br)	1.66 (1H, br)
H-13	2.63 (1H, t, *J*=5, 10)	2.65 (1H, br)
H-14a	1.13 (1H, dd, *J*=5, 11)	1.16 (1H, dd, *J*=5, 11)
H-14b	1.99 (1H, dd, *J*=2, 11)	2.03 (1H, dd, *J*=2, 11)
H-15a	2.05 (1H, br)	2.06 (1H, br)
H-15b	2.04 (1H, d, *J*=2)	2.04 (1H, d, *J*=2)
H-16	-	-
H-17a	4.74 (1H, s)	4.75 (1H, s)
H-17b	4.80 (1H, s)	4.82 (1H, s)
H-18	1.24 (3H, s)	1.22 (3H, s)
H-19	-	-
H-20	0.90 (3H, s)	1.01 (3H, s)

Table 4.3 ^{13}C-NMR spectra of Compound **1** and Kaur-16-en-19-oic acid.

Position	Chemical shifts (ppm)	
	Kaur-16-en-19-oic acid [8]	Compound **1**
C-1	40.6t	40.7t
C-2	19.1t	18.9t
C-3	37.7t	37.8t
C-4	43.7s	43.0s
C-5	57.0d	56.8d
C-6	21.8t	21.7t
C-7	41.3t	41.1t
C-8	44.2s	44.2s
C-9	55.7d	55.1d
C-10	39.7s	39.4s
C-11	18.4t	18.0t
C-12	33.1t	32.8t
C-13	43.8d	43.8d
C-14	39.6t	39.4t
C-15	48.9t	48.7t
C-16	155.6s	155.4s
C-17	103.1t	102.3t
C-18	28.9q	28.1q
C-19	185.1s	184.9s
C-20	15.5q	14.9q

4.1.2 Structure elucidation of Compound 2.

The IR spectrum of compound **2** is shown in Fig B5. Its IR spectrum showed important absorption bands at the absorption bands at 2933 and 2867 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the absorption bands at 1655 and 1615 cm^{-1} of the C=O stretching vibration of unsaturated carbonyl group and the absorption band at 1453 cm^{-1} of the C=C stretching vibration of alkene. The absorption bands were assigned as shown in Table 4.4.

Table 4.4 The IR absorption bands assignment of Compound **2**.

Wave number(cm^{-1})	Intensity	Vibration
2933, 2867	strong	C-H stretching vibration of -CH_3, -CH_2-
1655, 1615	strong	C=O stretching vibration of carbonyl group
1453, 1377, 1259	medium	C=C stretching vibration of alkene
1120	medium	C-O stretching vibration

The ^{1}H-NMR spectrum (Fig. B6, Table 4.5) of compound **2** indicated that it possesses five methyl groups at 1.05, 1.09, 1.17, 1.32 and 1.38 ppm and α,β-unsaturated ketone protons at 5.86 and 7.09 ppm and terminal double bond protons at 4.95 and 5.17 ppm.

The ^{13}C-NMR spectrum (Fig. B7, Table 4.6) showed 20 signals. Four signals of olefinic carbons appeared at δ 157.9, 147.8, 126.2 and 111.1 ppm. The signal at δ 205.7 ppm should be the carbonyl of α,β-unsaturated ketone. There were five methyl carbon signals at δ 29.0, 28.0, 26.0, 21.7 and 15.9 ppm and two methine carbon signals at δ 53.7 and 50.4 ppm. Four signals of methylene carbons appeared at δ 45.0, 35.8, 20.6 and 15.9 ppm and four signals of quaternary carbons at δ 75.4, 74.1, 42.7 and 39.8 ppm.

The mass spectrum of compound **2** (Fig. B8) exhibited a molecular ion at 302 $[M]^+$. The molecular formula, $C_{20}H_{30}O_2$, of compound **2**, defined a DBE of 6, therefore, compound **2** must consisted of one ring of α,β-unsaturated ketone (DBE = 3), one double bond and two rings. These data indicated that compound **2** was *ent*-1,2-dehydro-3-oxomanoyl oxide which was reported in the literature [9].

The ^{1}H-NMR and ^{13}C-NMR chemical shifts of compound **2** were compared with those of the *ent*-1,2-dehydro-3-oxomanoyl oxide as shown in Tables 4.5 and 4.6, respectively.

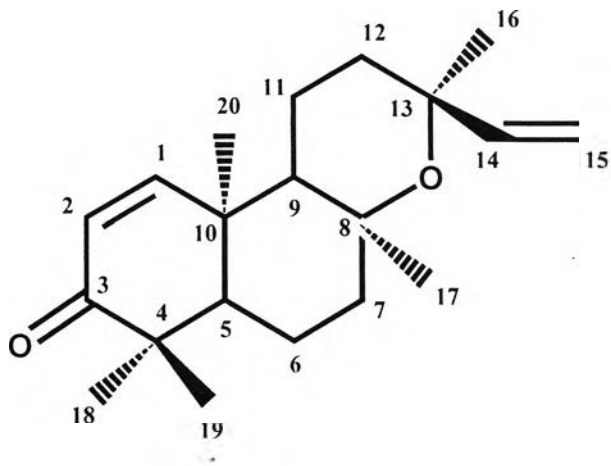


Figure 4.2 Structure of Compound **2**.

Table 4.5 ^{1}H-NMR spectra of Compound **2** and *ent*-1,2-Dehydro-3-oxomanoyl oxide.

Position	Chemical shifts (ppm)	
	ent-1,2-Dehydro-3-oxomanoyl oxide[9]	Compound **2**
H-1	7.10 (1H, d, *J*=10)	7.09 (1H, d, *J*=10)
H-2	5.86 (1H, d, *J* =10)	5.86 (1H, d, *J* =10)
H-3	-	-
H-4	-	-
H-5	1.78 (1H, m)	1.78 (1H, m)
H-6a	1.52 (1H, m)	1.53 (1H, m)
H-6b	1.70 (1H, m)	1.71 (1H, m)
H-7a	1.53 (1H, m)	1.54 (1H, m)
H-7b	1.92 (1H, m)	1.92 (1H, m)
H-8	-	-
H-9	1.62 (1H, m)	1.63 (1H, m)
H-10	-	-
H-11a	1.70 (1H, m)	1.71 (1H, m)
H-11b	1.84 (1H, m)	1.85 (1H, m)
H-12a	1.72 (1H, m)	1.73 (1H, m)
H-12b	1.90 (1H, m)	1.91 (1H, m)
H-13	-	-
H-14	5.89 (1H, dd, *J* = 17, 10)	5.86 (1H, dd, *J* = 17, 10)
H-15a	4.94 (1H, dd, *J* = 10, 1)	4.95 (1H, dd, *J* = 10, 1)
H-15b	5.16 (1H, dd, *J* = 17, 1)	5.17 (1H, dd, *J* = 17, 1)
H-16	1.32 (3H, s)	1.33 (3H, s)
H-17	1.38 (3H, s)	1.39 (3H, s)
H-18	1.16 (3H, s)	1.17 (3H, s)
H-19	1.08 (3H, s)	1.09 (3H, s)
H-20	1.05 (3H, s)	1.06 (3H, s)

Table 4.6 ^{13}C-NMR spectra of Compound **2** and *ent*-1,2-Dehydro-3-oxomanoyl oxide.

Position	Chemical shifts (ppm)	
	ent-1,2-Dehydro-3-oxomanoyl oxide [9]	Compound **2**
C-1	157.6d	157.9d
C-2	125.8d	126.2d
C-3	205.1s	205.7s
C-4	42.3s	42.7s
C-5	53.2d	53.7d
C-6	20.2t	20.6t
C-7	44.6t	45.0t
C-8	75.0s	75.4s
C-9	49.9d	50.4d
C-10	39.4s	39.8s
C-11	15.5t	15.9t
C-12	35.4t	35.8t
C-13	73.7s	74.1s
C-14	147.4d	147.8d
C-15	110.7t	111.1t
C-16	28.6q	29.0q
C-17	25.6q	26.0q
C-18	27.6q	28.0q
C-19	21.3q	21.7q
C-20	15.6q	15.9q

4.1.3 Structure elucidation of Compound 3.

Compound 3 was obtained by column chromatography using 40% ethyl acetate in hexane and recrystallization technique. The structure of compound 3 was elucidated by FT-IR, NMR and Mass spectroscopic data as follows.

The IR spectrum of compound 3 (Fig. B9) showed important absorption bands at 3500-3200 cm^{-1} of the O-H stretching vibration of alcohol, the absorption bands at 2938 and 2867 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the presence of a carbonyl group with corresponding to the strong absorption band at 1712 cm^{-1} and the absorption bands at 1508 cm^{-1} of the C=C stretching vibration of alkene. The IR spectral data of compound 3 are summarized in Table 4.7.

Table 4.7 The IR absorption bands assignment of Compound 3.

Wave number(cm^{-1})	Intensity	Vibration
3500-3200	medium	O-H stretching vibration
2938, 2867	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1712	strong	C=O stretching vibration of carbonyl group
1508	medium	C=C stretching vibration of alkene
1285, 1117	medium	C-O stretching vibration

The ^{1}H-NMR spectrum (Fig. B10, Table 4.8) of compound 3 possessed five methyl group at 1.36, 1.31, 1.10, 1.07 and 0.89 ppm, proton attached with oxygen group at 3.92 ppm and three olefinic protons at 5.91, 5.18 and 4.96 ppm.

The ^{13}C-NMR spectrum (Fig. B11, Table 4.9) showed 20 signals, which the carbonyl group of ketone corresponded to the signal at 215.1 ppm. Two signals of olefinic carbons appeared at δ 147.8 and 110.7 ppm. There were five methyl carbon signals at δ 28.8, 27.4, 24.9, 20.4 and 10.9 ppm, three methine carbon signals at δ 55.3, 51.2 and 42.7 ppm and methine carbon of secondary alcohol at 78.0 ppm. Four signals of methylene carbons appeared at δ 45.1, 35.6, 20.7 and 17.8 and four signals of quaternary carbons at δ 74.7, 73.6, 47.3 and 42.7 ppm.

The mass spectrum of compound 3 (Fig. B12) exhibited a molecular ion at 320 $[M]^+$. The molecular formula, $C_{20}H_{32}O_3$, of compound 3, defined a DBE of 5,

therefore, compound **3** must consisted of one carbonyl, one double bond, and three rings. These data indicated that compound **3** was *ent*-1β-hydroxy-3-oxomanoyl oxide.

The ^{1}H-NMR and ^{13}C-NMR chemical shifts of compound **3** were compared with those of *ent*-manoyl derivative, *ent*-1β-hydroxy-3-oxomanoyl oxide, previously isolated from *C. oblongifolius* [9] as shown in Table 4.8 and 4.9, respectively.

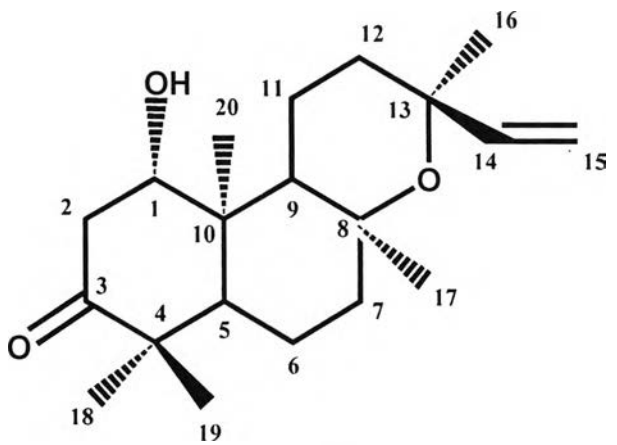


Figure 4.3 Structure of Compound **3**.

Table 4.8 ^{1}H-NMR spectra of Compound **3** and *ent*-1β-hydroxy-3-oxomanoyl oxide.

Position	Chemical shifts (ppm)	
	ent-1β-hydroxy-3-oxomanoyl oxide [9]	Compound **3**
H-1	3.89 (1H, t, *J*=6)	3.92 (1H, t, *J*=6)
H-2a	2.36 (1H, dd, *J* =15, 5)	2.39 (1H, dd, *J* =15, 5)
H-2b	2.92 (1H, dd, *J* =15, 8)	2.95 (1H, dd, *J* =15, 8)
H-3	-	-
H-4	-	-
H-5	1.49 (1H, m)	1.52 (1H, m)
H-6a	1.50 (1H, m)	1.53 (1H, m)
H-6b	1.54 (1H, m)	1.57 (1H, m)
H-7a	1.35 (1H, m)	1.38 (1H, m)
H-7b	1.50 (1H, m)	1.52 (1H, m)
H-8	-	-
H-9	1.52 (1H, m)	1.55 (1H, m)
H-10	-	-
H-11a	1.55 (1H, m)	1.58 (1H, m)
H-11b	2.18 (1H, m)	2.20 (1H, m)
H-12a	1.54 (1H, m)	1.57 (1H, m)
H-12b	1.56 (1H, m)	1.59 (1H, m)
H-13	-	-
H-14	5.87 (1H, dd, *J* = 18, 11)	5.91 (1H, dd, *J* = 18, 11)
H-15a	4.93 (1H, dd, *J* = 11, 2)	4.96 (1H, dd, *J* = 11, 2)
H-15b	5.15 (1H, dd, *J* = 18, 2)	5.18 (1H, dd, *J* = 18, 2)
H-16	1.28 (3H, s)	1.31 (3H, s)
H-17	1.33 (3H, s)	1.36 (3H, s)
H-18	1.08 (3H, s)	1.10 (3H, s)
H-19	1.04 (3H, s)	1.07 (3H, s)
H-20	0.86 (3H, s)	0.89 (3H, s)

Table 4.9 ^{13}C-NMR spectra of Compound **3** and *ent*-1β-hydroxy-3-oxomanoyl oxide.

Position	Chemical shifts (ppm)	
	ent-1β-hydroxy-3-oxomanoyl oxide [9]	Compound **3**
C-1	77.8d	78.0d
C-2	44.9t	45.1t
C-3	214.9s	215.1s
C-4	47.1s	47.3s
C-5	51.0d	51.2d
C-6	20.5t	20.7t
C-7	41.9d	42.1d
C-8	74.5s	74.7s
C-9	55.1d	55.3d
C-10	42.5s	42.7s
C-11	17.6t	17.8t
C-12	35.4t	35.6t
C-13	73.4s	73.6s
C-14	147.6d	147.8d
C-15	110.5t	110.7t
C-16	28.6q	28.8q
C-17	24.9q	25.1q
C-18	27.2q	27.4q
C-19	20.2q	20.4q
C-20	10.7q	10.9q

4.1.4 Structure elucidation of Compound 4.

Compound 4 was obtained as a white crystal by column chromatography using 50% ethyl acetate in hexane and recrystallization technique. The structure of compound 4 was elucidated by FT-IR, NMR and Mass spectroscopic data as follows.

The IR spectrum of compound 4 is shown in Fig B13. The IR spectrum of compound 4 (Fig. B13) showed important absorption bands at 3600-3200 cm^{-1} of the O-H stretching vibration of alcohol, the absorption bands at 2931 and 2857 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the presence of a carbonyl group with corresponding to the strong absorption band at 1712 and 1660 cm^{-1} and the absorption bands at 1448 and 1376 cm^{-1} of the C=C stretching vibration of alkene. The absorption bands were assigned as shown in Table 4.10.

Table 4.10 The IR absorption bands assignment of Compound 4.

Wave number(cm^{-1})	Intensity	Vibration
3600-3200	medium	O-H stretching vibration
2931, 2857	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1712, 1660	strong	C=O stretching vibration of carbonyl group
1448, 1376	medium	C=C stretching vibration of alkene
1285, 1117, 1093	weak	C-O stretching vibration

The ^{1}H-NMR spectrum (Fig. B14, Table 4.11) of compound 4 indicated that it possesses five methyl groups at 1.04, 1.10, 1.16 and 1.39 ppm and α,β-unsaturated ketone proton at 5.88 and 7.13 ppm, proton attached with oxygen group at 3.79 ppm and terminal double bond protons at 5.81, 5.44 and 5.26 ppm.

The ^{13}C-NMR spectrum (Fig. B15, Table 4.12) showed 20 signals, which the carbonyl group of α,β-unsaturated ketone corresponded to the signal at 205.0 ppm. Four signal of olefinic carbons appeared at δ 157.4, 142.6, 126.0 and 115.9 ppm. There were five methyl carbon signals at δ 27.7, 27.5, 25.4, 21.3 and 19.0 ppm and two methine carbon signals at δ 53.3 and 43.6 ppm and methine carbon of secondary alcohol at 70.1 ppm. Three signals of methylene carbons appeared at δ 41.6, 23.0 and 20.2 and four signals of quaternary carbons at δ 76.8, 75.4, 44.7 and 38.8 ppm.

The mass spectrum of compound **4** (Fig. B16) exhibited a molecular ion at 318 $[M]^+$. The molecular formula, $C_{20}H_{30}O_3$, of compound **4**, defined a DBE of 6, therefore, compound **4** must consisted of one ring of α,β-unsaturated ketone (DBE = 3), one double bond, and two rings. These data indicated that compound **4** was *ent*-1,2-Dehydro-12α-hydroxy-3-oxomanoyl oxide which was reported in the literature [6].

The ^{1}H-NMR and ^{13}C-NMR chemical shifts of compound **4** were compared with those of the *ent*-1,2-Dehydro-12α-hydroxy-3-oxomanoyl oxide as shown in Table 4.11 and 4.12, respectively.

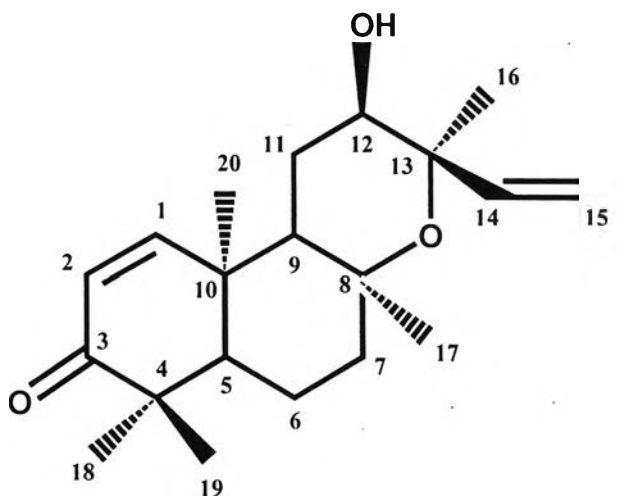


Figure 4.4 Structure of Compound **4**

Table 4.11 ^{1}H-NMR spectra of Compound **4** and *ent*-1,2-Dehydro-12α-hydroxy-3-oxomanoyl oxide.

Position	Chemical shifts (ppm)	
	ent-1,2-Dehydro-12α-hydroxy-3-oxomanoyl oxide. [9]	Compound **4**
H-1	7.13 (1H, d, *J*=10)	7.13 (1H, d, *J*=10)
H-2	5.88 (1H, d, *J* =10)	5.87 (1H, d, *J* =10)
H-3	-	-
H-4	-	-
H-5	1.85 (1H, dd, *J* =12, 3)	1.85 (1H, dd, *J* =12,3)
H-6a	1.52 (1H, m)	1.52 (1H, m)
H-6b	1.72 (1H, m)	1.72 (1H, m)
H-7a	1.58 (1H, m)	1.58 (1H, m)
H-7b	1.92 (1H, m)	1.92 (1H, m)
H-8	-	-
H-9	2.09 (1H, dd, *J* =9, 6)	2.09 (1H, dd, *J* =9, 6)
H-10	-	-
H-11a	1.94 (1H, m)	1.94 (1H, m)
H-11b	1.98 (1H, m)	1.98 (1H, m)
H-12a	3.80 (1H, t, *J* =7)	3.79 (1H, t, *J* =7)
H-12b	-	-
H-13	-	-
H-14	5.81 (1H, dd, *J* = 17, 11)	5.82 (1H, dd, *J* = 17, 11)
H-15a	5.26 (1H, dd, *J* = 11, 2)	5.26 (1H, dd, *J* = 11, 2)
H-15b	5.44 (1H, dd, *J* = 18, 2)	5.44 (1H, dd, *J* = 18, 2)
H-16	1.39 (3H, s)	1.39 (3H, s)
H-17	1.38 (3H, s)	1.38 (3H, s)
H-18	1.16 (3H, s)	1.16 (3H, s)
H-19	1.09 (3H, s)	1.09 (3H, s)
H-20	1.04 (3H, s)	1.04 (3H, s)

Table 4.12 ^{13}C-NMR spectra of Compound **4** and *ent*-1,2-Dehydro-12α-hydroxy-3-oxomanoyl oxide.

Position	Chemical shifts (ppm)	
	ent-1,2-Dehydro-12α-hydroxy-3-oxomanoyl oxide [9]	Compound **4**
C-1	157.3d	157.4d
C-2	125.9d	126.0d
C-3	205.0s	205.1s
C-4	44.6s	44.7s
C-5	53.2d	53.3d
C-6	20.1t	20.2t
C-7	41.5d	41.6d
C-8	75.3s	75.4s
C-9	43.5d	43.6d
C-10	38.7s	38.8s
C-11	22.9t	23.0t
C-12	70.0t	70.1t
C-13	76.7s	76.8s
C-14	142.5d	142.6d
C-15	115.8t	115.9t
C-16	27.4q	27.5q
C-17	25.3q	25.4q
C-18	27.6q	27.7q
C-19	21.2q	21.3q
C-20	18.9q	19.0q

4.2 Biotransformation of substrates by some fungi.

4.2.1 Biotransformation of Compound 1 by *Absidia blakesleeana*.

4.2.1.1 Structure elucidation of Metabolite 1a.

The IR spectrum of metabolite **1a** is shown in Fig B17. Its IR spectrum showed important absorption bands at 3600-3000 cm^{-1} of the O-H stretching vibration of acid and alcohol, the absorption bands at 2937 and 2867 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the absorption band at 1688 cm^{-1} of the C=O stretching vibration of carboxylic acid and the absorption bands at 1462 cm^{-1} of the C=C stretching vibration of alkene. The absorption bands were assigned as shown in Table 4.13.

Table 4.13 The IR absorption bands assignment of Metabolite **1a**.

Wave number (cm^{-1})	Intensity	Vibration
3600-3000	broad	O-H stretching vibration of acid and alcohol
2937, 2867	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1688	strong	C=O stretching vibration of carboxylic acid
1462 and 1248	medium	C=C stretching vibration of alkene
1022	medium	C-O stretching vibration
863	medium	C-H out of plane bending vibration

The ^{1}H-NMR spectrum (Fig. B18, Table 4.14) of Metabolite **1a** indicated that it possesses two methyl groups at 1.14 and 1.24 ppm and two olefinic protons at 4.82 and 4.85 ppm in addition to proton of a secondary alcohol at 3.63 ppm.

The ^{13}C-NMR spectrum (Fig. B19, Table 4.14) and DEPT experiment showed 20 lines. The signal at δ 180.6 ppm should be the carbonyl group of carboxylic acid. Two olefinic carbons appeared at δ154.8 and 102.4 ppm. Two signals of methine carbons appeared at δ 42.4 and 42.0 ppm and a methine carbon of secondary alcohol at 78.0 ppm. Eight signals of methylene carbons appeared at δ 41.2, 38.4, 37.7, 33.2, 31.9, 29.0, 27.4 and 18.8 ppm and three quaternary carbons at δ 51.9, 43.8 and 43.1 ppm and a quaternary carbon of tertiary alcohol at δ 78.9 ppm. There were two methyl carbon signals at δ 28.1 and 17.1 ppm.

The information of 2D-NMR including gCOSY correlations (Fig. B20, Table 4.14), gNOESY correlations (Fig. B21), gHMBC correlations (Fig. B22, Table 4.14) and gHSQC correlations (Fig. B23) supported the structural elucidation of Metabolite **1a**.

The configuration of the hydroxyl group was obtained from the gNOESY-NMR spectrum (Fig. 4.6 and Fig B21) which showed no coupling of the H-7 and H-9 with H-5 resonance (expected for diaxial coupling).

The LC-MS spectrum showed the fragmentation as follows, m/z (ESI-MS) (Fig. B24): 357 $[M+Na]^+$ and HRESIMS m/z: 357.2039 $[M+Na]^+$.

Metabolite **1a** showed a weak molecular ion peak with *m/z* = 334 and the respective sodium adduct at *m/z* = 357, consistant with the molecular formula of $C_{20}H_{30}O_4$, which indicated a DBE of 6. Metabolite **1a** should be consisted of one carbonyl group of carboxylic acid, one double bond in addition to four rings.

From all this spectroscopic data, the new metabolite (**1a**), named ent-(7α,9α)-dihydroxy-kaur-16-en-19-oic acid was proposed. The structure of Metabolite **1a**, is shown in Figure 4.5.

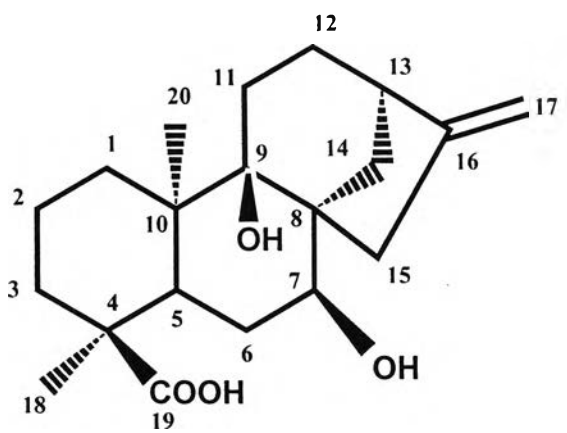


Figure 4.5 Structure of Metabolite 1a.

Table 4.14 The ^{1}H-NMR, ^{13}C-NMR, gCOSY and gHMBC spectral data of Metabolite **1a**.

Position	^{13}C-NMR δ, ppm.	^{1}H-NMR δ, ppm.		gCOSY correlations	gHMBC correlations
1	38.4t	1a	1.31 (1H, m)	H-1b	C-4, C-9, C-10
		1b	2.05 (1H, m)	H-1a, H-2a, H-2b	C-8, C-20
2	18.8t	2a	1.49 (1H, m)	H-1b	-
		2b	1.49 (1H, m)	H-1b, H-3a, H-3b	-
3	37.7t	3a	1.08 (1H, m)	H-2a	C-2, C-18
		3b	2.18 (1H, d, *J*=10)	H-2b	C-10
4	43.1s	-		-	-
5	42.0d	5	2.05 (1H, m)	-	C-1, C-6, C-8
6	29.0t	6a	2.06 (1H, m)	H-7a, H-6b	C-2, C-3, C-7, C-15
		6b	2.22 (1H, m)	H-6a	C-10
7	78.0d	7a	3.63 (1H, s)	H-6a	C-5
		7b	-	-	-
8	51.2s	-		-	-
9	78.9s	-		-	-
10	43.8s	-		-	-
11	31.9t	11a	1.51 (1H, m)	H-12a, H-11b	C-3, C-13, C-20
		11b	1.94 (1H, m)	H-11a, H-12b	C-8, C-9, C-12
12	33.2t	12a	1.58 (1H, m)	H-11a, H-12b	C-13, C-14
		12b	1.71 (1H, ddt, *J* = 2, 6, 12 Hz)	H-12a, H-11b	-
13	42.4d	13	2.69 (1H, br, s)	H-14a	-
14	27.4t	14a	1.34 (1H, m)	H-13, H-14b	C-8, C-12, C-10
		14b	1.96 (1H, m)	H-14a	-
15	41.2t	15a	2.06 (1H, m)	H-14a, H-15b, H-17a	C-7, C-14, C-13
		15b	3.08 (1H, d, *J* = 17 Hz)	H-17b	-
16	154.8s	-		-	-
17	102.4t	17a	4.82 (1H, s)	H-15a	C-15
		17b	4.85 (1H, s)	H-15b	C-13
18	28.1q	18	1.24 (3H, s)	-	C-1, C-2, C-3, C-4
19	180.6s	-		-	-
20	17.1q	20	1.14 (3H, s)	-	C-9, C-10, C-11, C-13

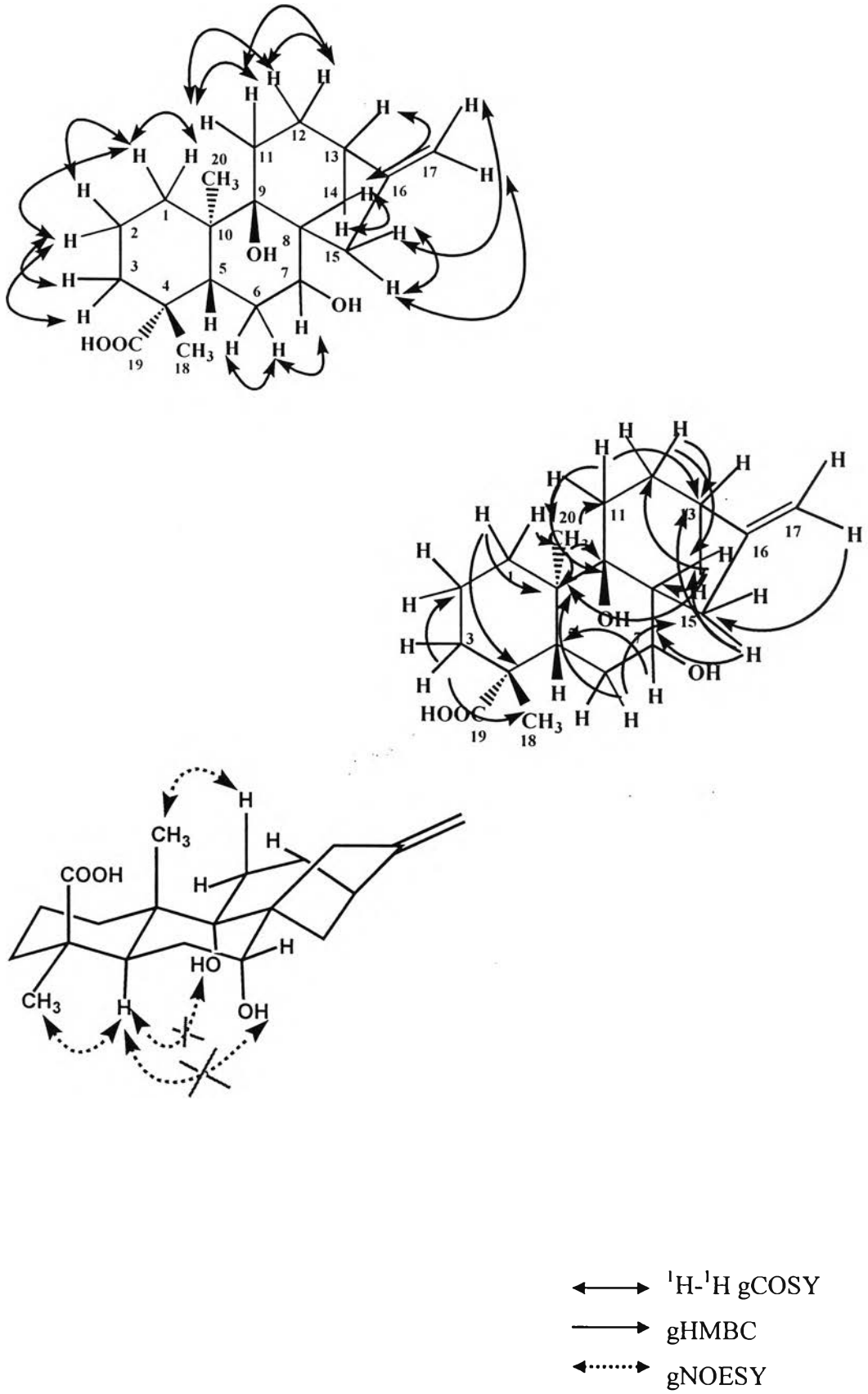


Figure 4.6 Selected ^{1}H-^{1}H gCOSY, gHMBC and gNOESY correlations for Metabolite **1a**.

4.2.1.2 Structure elucidation of Metabolite 1b.

The IR spectrum of Metabolite **1b** is shown in Fig B25. Its IR spectrum showed important absorption bands at 3600-3000 cm^{-1} of the O-H stretching vibration of acid and alcohol, the absorption bands at 2933 and 2863 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the absorption band at 1696 cm^{-1} of the C=O stretching vibration of carboxylic acid and the absorption bands at 1474 cm^{-1} of the C=C stretching vibration of alkene. The absorption bands were assigned as shown in Table 4.15.

Table 4.15 The IR absorption bands assignment of Metabolite **1b**.

Wave number (cm^{-1})	Intensity	Vibration
3600-3000	broad	O-H stretching vibration of acid and alcohol
2933, 2863	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1696	strong	C=O stretching vibration of carboxylic acid
1474 and 1376	medium	C=C stretching vibration of alkene
1162 and 1053	medium	C-O stretching vibration
863	medium	C-H out of plane bending vibration

The ^{1}H-NMR spectrum (Fig. B26, Table 4.16) of Metabolite **1b** indicated that it possesses two methyl groups at 1.19 and 1.20 ppm and two olefmic protons at 4.76 and 4.87 ppm in addition to protons of two secondary alcohol at 3.56 and 4.16 ppm.

The ^{13}C-NMR spectrum (Fig. B27, Table 4.16) and DEPT experiment (Fig. B28) showed 20 lines. The signal at δ 180.4 ppm should be the carbonyl group of carboxylic acid. Two olefmic carbons appeared at δ155.5 and 102.2 ppm. Three signals of methine carbons appeared at δ 53.1, 47.9 and 43.0 ppm and two methine carbons of secondary alcohol at 76.2 and 69.0 ppm. Seven signals of methylene carbons appeared at δ 45.3, 40.8, 40.7, 38.8, 38.2, 28.9 and 19.2 ppm and three quaternary carbons at δ 49.8, 43.4 and 42.5 ppm. There were two methyl carbon signals at δ 28.8 and 15.2 ppm.

The information of 2D-NMR including gCOSY correlations (Fig. B29, Table 4.16), gNOESY correlations (Fig. B30), gHMBC correlations (Fig. B31, Table 4.16)

and gHSQC correlations (Fig. B32) supported the structural elucidation of Metabolite **1b**.

The configuration of the hydroxyl group was obtained from the gNOESY-NMR spectrum (Fig. 4.8 and Fig B30) which showed no coupling of the H-7 with H-5 resonance but coupling of the H-11 with CH_3-20 resonance expected for diaxial coupling.

The LC-MS spectrum showed the fragmentation as follows, m/z (ESI-MS) (Fig. B33): 357 $[M+Na]^+$ and HRESIMS m/z: 357.2036 $[M+Na]^+$.

Metabolite **1b** showed a weak molecular ion peak with *m/z* = 334 and the respective sodium adduct at *m/z* = 357, consistant with the molecular formula of $C_{20}H_{30}O_4$, which indicated a DBE of 6. Metabolite **1b** should be consisted of one carbonyl group of acid, one double bond in addition to four rings.

From all this spectroscopic data, metabolite (**1b**), was identified as *ent*-(7α,11β)-dihydroxy-kaur-16-en-19-oic acid, previously obtained in the microbial transformation of 12-oxygenated and other derivatives of *ent*-kaur-16-en-19-oic acid by *Gibberella Fujikuroi*, Mutant B1-41a [35], however the products were only identified by GC-MS. Therefore, ^1H NMR, ^{13}C NMR spectra are reported for the first time. The structure of Metabolite **1b**, is shown in Figure 4.7.

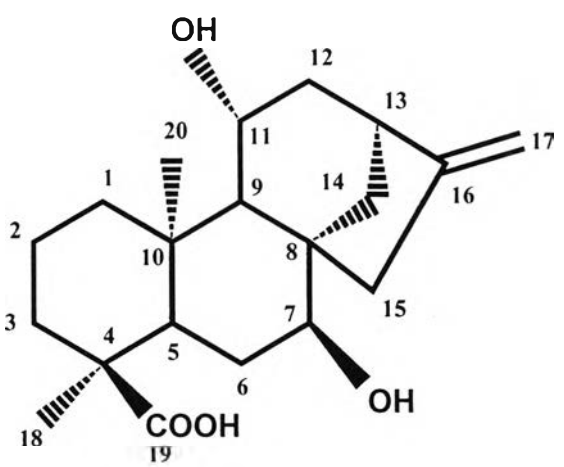


Figure 4.7 Structure of Metabolite **1b**.

Table 4.16 The ^{1}H-NMR, ^{13}C-NMR, gCOSY and gHMBC spectral data of Metabolite **1b**.

Position	^{13}C-NMR δ, ppm.	^{1}H-NMR δ, ppm.		gCOSY correlations	gHMBC correlations
1	38.8t	1a	1.12 (1H, dd, *J*=4, 13)	H-1b	C-2, C-3
		1b	2.06 (1H, d, *J*=9)	H-1a, H-2a, H-2b	C-20
2	19.2t	2a	1.38 (1H, d, *J*=14)	H-1b	-
		2b	1.93 (1H, m)	H-1b, H-3a, H-3b	-
3	38.2t	3a	2.16 (1H, m)	H-2a	C-18
		3b	2.16 (1H, m)	H-2b	C-10
4	43.4s	-		-	-
5	47.9d	5	1.88 (1H, d, *J*=12)	-	C-4, C-6, C-8
6	28.9t	6a	2.00 (1H, m)	H-7a, H-6b	C-2, C-3, C-7
		6b	2.12 (1H, m)	H-6a	C-10
7	76.2d	7a	3.56 (1H, m)	H-6a,H-9	C-5
		7b	-	-	-
8	49.8s	-		-	-
9	53.1d	9	1.96 (1H, d, *J*=7)		C-1, C-10, C-12, C-20
10	42.5s	-		-	-
11	69.0d	11a	4.16 (1H, m)	H-12a	C-12, C-20
		11b	-		-
12	40.8t	12a	1.11 (1H, m)	H-11a, H-12b	C-13, C-14
		12b	2.78 (1H, d, *J* = 13 Hz)	H-12a, H-14b	-
13	43.0d	13	2.71 (1H, br, s)	H-12a	-
14	40.7t	14a	1.77 (1H, m)	H-13, H-14b	C-8, C-13
		14b	1.90 (1H, m)	H-14a	-
15	45.3t	15a	2.25 (1H, m)	H-14a, H-15b, H-17a	C-7, C-14, C-13
		15b	2.25 (1H, m)	H-17b	-
16	155.5s	-		-	-
17	102.2t	17a	4.76 (1H, s)	H-15a, H-17b	C-16
		17b	4.87 (1H, s)	H-15b, H-17a	C-16
18	28.8q	18	1.20 (3H, s)	-	C-1, C-2, C-3, C-4
19	180.4s	-		-	-
20	15.2q	20	1.19 (3H, s)	-	C-9, C-10, C-11, C-13

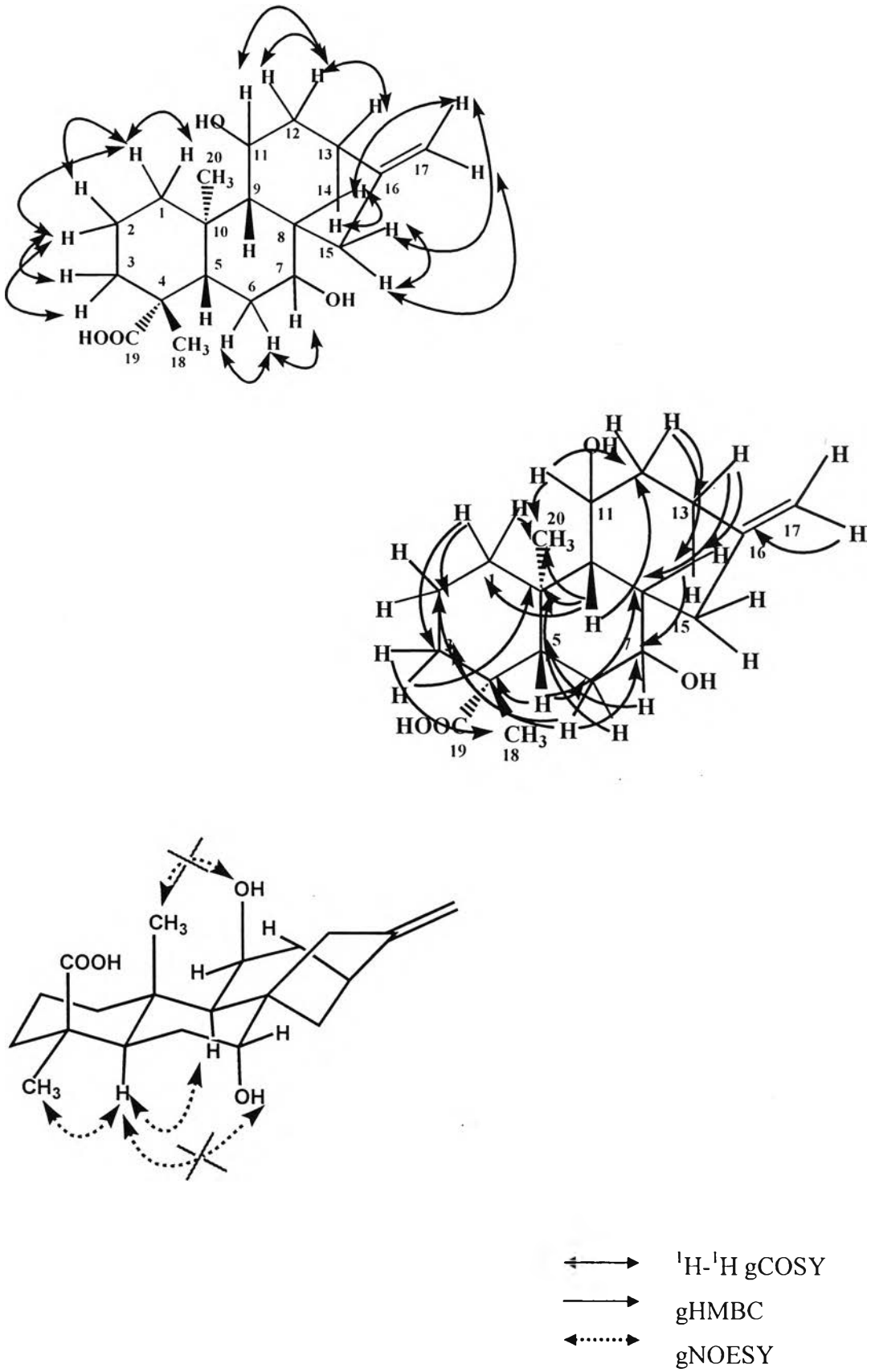


Figure 4.8 Selected ^{1}H-^{1}H gCOSY, gHMBC and gNOESY correlations for Metabolite **1b**.

4.2.1.3 Structure elucidation of Metabolite 1c.

The IR spectrum of Metabolite **1c** is shown in Fig B34. Its IR spectrum showed important absorption bands at 3600-3000 cm^{-1} of the O-H stretching vibration of acid and alcohol, the absorption bands at 2960 and 2914 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the absorption band at 1688 cm^{-1} of the C=O stretching vibration of carboxylic acid and the absorption bands at 1446 cm^{-1} of the C=C stretching vibration of alkene. The absorption bands were assigned as shown in Table 4.17.

Table 4.17 The IR absorption bands assignment of Metabolite **1c**.

Wave number (cm^{-1})	Intensity	Vibration
3600-3000	broad	O-H stretching vibration of acid and alcohol
2960, 2814	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1688	strong	C=O stretching vibration of carboxylic acid
1446 and 1384	medium	C=C stretching vibration of alkene
1182 and 1069	medium	C-O stretching vibration
867	medium	C-H out of plane bending vibration

The ^{1}H-NMR spectrum (Fig. B35, Table 4.18) of Metabolite **1c** indicated that it possesses two methyl groups at 1.10 and 1.18 ppm and two olefinic protons at 4.77 and 4.80 ppm in addition to protons of two secondary alcohol at 3.37 and 3.51 ppm.

The ^{13}C-NMR spectrum (Fig. B36, Table 4.18) and DEPT experiment (Fig. B37) showed 20 lines. The signal at δ 180.2 ppm should be the carbonyl group of carboxylic acid. Two olefinic carbons appeared at δ 155.4 and 102.0 ppm. Three signals of methine carbons appeared at δ 49.2, 45.1 and 43.9 ppm and two methine carbons of secondary alcohol at 82.1 and 76.6 ppm. Seven signals of methylene carbons appeared at δ 45.2, 39.3, 35.9, 33.5, 29.3, 28.6 and 19.8 ppm and three quaternary carbons at δ 48.6, 44.3 and 42.4 ppm. There were two methyl carbon signals at δ 27.8 and 10.7 ppm.

The information of 2D-NMR including gCOSY correlations (Fig. B38, Table 4.18), gNOESY correlations (Fig. B39), gHMBC correlations (Fig. B40, Table 4.18) and gHSQC correlations (Fig. B41) supported the structural elucidation of Metabolite **1c**.

The configuration of the hydroxyl group was obtained from the gNOESY-NMR spectrum (Fig. 4.10 and Fig B39) which showed no coupling of the H-7 with H-5 resonance but showed coupling of the H-1 with CH_3-20 resonance expected for diaxial coupling.

The MS spectrum showed the fragmentation as follows, m/z (EI MS) (Fig. B42): 357 $[M+Na]^+$ and HRESIMS m/z: 357.2036 $[M+Na]^+$.

Metabolite **1c** showed a weak molecular ion peak with *m/z* = 334 and the respective sodium adduct at *m/z* = 357, consistant with the molecular formula of $C_{20}H_{30}O_4$, which indicated a DBE of 6. Metabolite **1c** should be consisted of one carbonyl group of acid, one double bond in addition to four rings.

Moreover, the structure of Metabolite **1c** was also confirmed by X-ray diffraction analysis, which is shown in Fig. 4.11. X-ray diffraction data are presented in appendix C.

From all this spectroscopic data, the new Metabolite (**1c**), named ent-(1β,7α)-dihydroxy-kaur-16-en-19-oic acid was proposed. The structure of Metabolite **1c**, is shown in Figure 4.9.

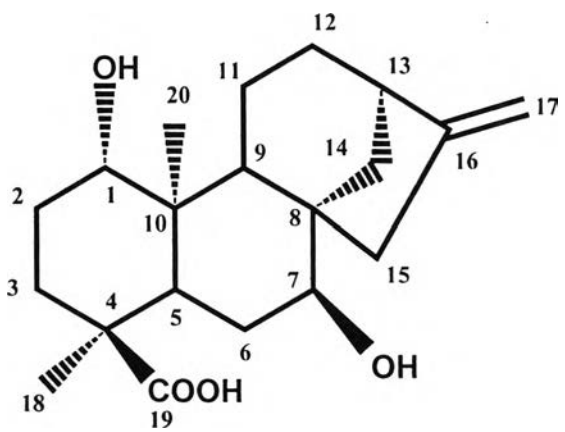


Figure 4.9 Structure of Metabolite **1c**.

Table 4.18 The ^{1}H-NMR, ^{13}C-NMR, gCOSY and gHMBC spectral data of Metabolite **1c**.

Position	^{13}C-NMR δ, ppm.	^{1}H-NMR δ, ppm.	gCOSY correlations	gHMBC correlations
1	82.1d	1 3.37 (1H, dd, *J*=5, 12)	H-2a, H-2b	C-2, C-10, C-12, C-20
2	29.3t	2a 1.54 (1H, m)	H-1	-
		2b 2.00 (1H, m)	H-1	C-1,C-3
3	35.9t	3a 1.18 (1H, m)	H-3b	C-4, C-19, C-18
		3b 2.14 (1H, m)	H-3a	C-5
4	42.4s	-	-	-
5	45.1d	5 1.68 (1H, br, s)	-	-
6	28.6t	6a 1.93 (1H, m)	H-7a, H-6b	C-7, C-16
		6b 2.17 (1H, m)	H-6a	C-5
7	76.6d	7a 3.51 (1H, s)	H-6a	-
		7b -	-	-
8	48.6s	-	-	-
9	49.2d	9 1.74 (1H, d, *J*=7)	-	C-1, C-10, C-11, C-12, C-14, C-15, C-20
10	44.3s	-	-	-
11	19.8t	11a 2.88 (1H, d, *J*=3)	H-12a, H-11b	C-13
		11b 2.85 (1H, d, *J*=4)	H-11a, H-12b	-
12	33.5t	12a 1.44 (1H, m)	H-11a, H-12b	-
		12b 1.68 (1H, br, s)	H-12a, H-11b	-
13	43.9d	13 2.64 (1H, br, s)	H-14a	-
14	39.3t	14a 1.20 (1H, m)	H-13, H-14b	C-5
		14b 1.88 (1H, br, s)	H-14a	C-16
15	45.2t	15a 1.71 (1H, m)	H-14a, H-15b, H-17a	C-3, C-4, C-6,C-7,C-10
		15b 2.25 (1H, m)	H-17b	C-16, C-17
16	155.4s	-	-	-
17	102.0t	17a 4.77 (1H, s)	H-15a	C-16
		17b 4.80 (1H, s)	H-15b	
18	27.8q	18 1.18 (3H, s)	-	C-3, C-4, C-5, C-7
19	180.2s	-	-	-
20	10.7q	20 1.10 (3H, s)	-	C-1, C-5

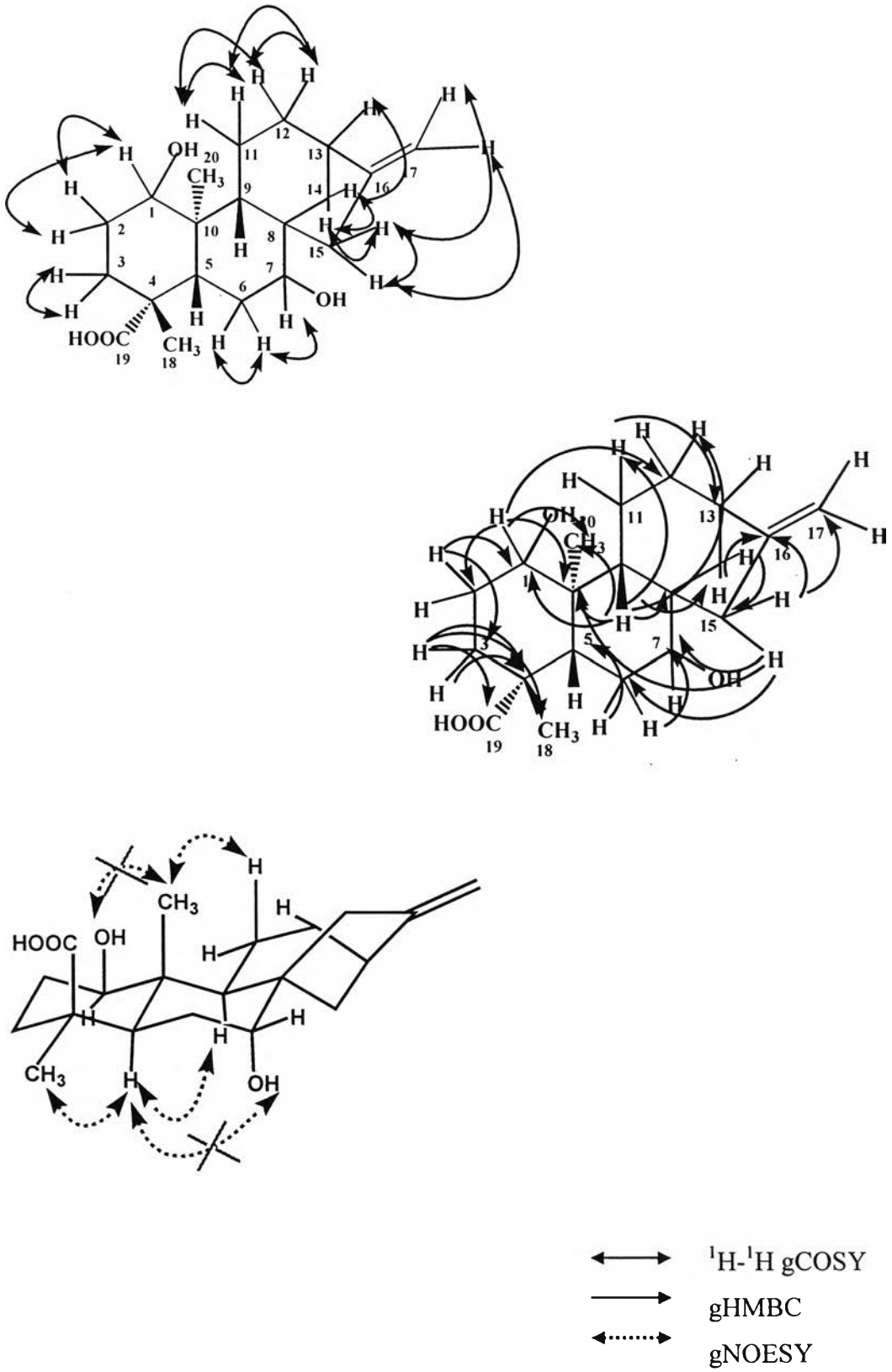


Figure 4.10 Selected ^{1}H-^{1}H gCOSY, gHMBC and gNOESY correlations for Metabolite **1c**.

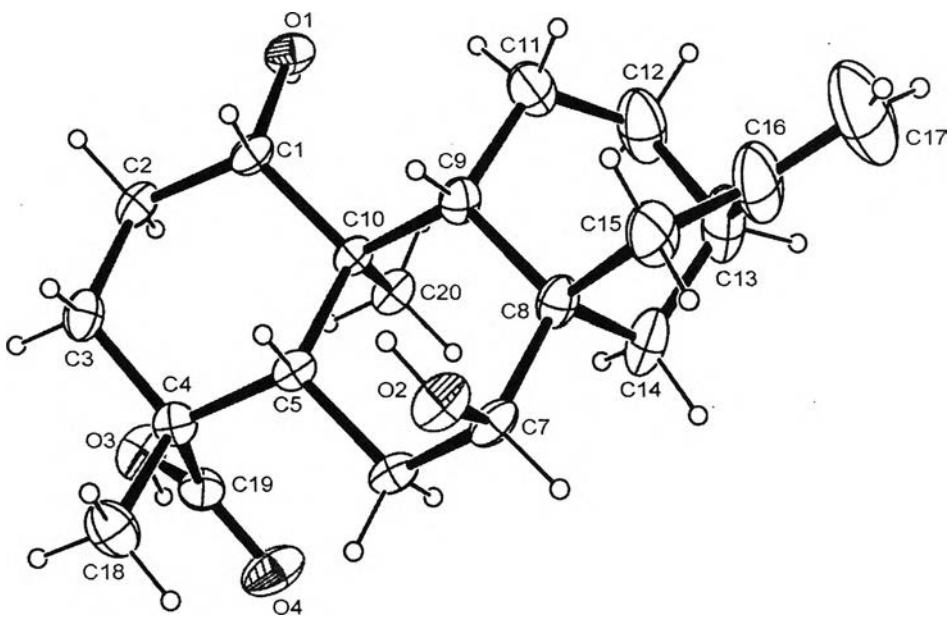


Figure 4.11 The computer generated ORTEP drawing for Metabolite **1c**.

4.2.1.4 Structure elucidation of Metabolite 1d.

The IR spectrum of Metabolite **1d** is shown in Fig B43. Its IR spectrum showed important absorption bands at 3600-3000 cm^{-1} of the O-H stretching vibration of acid and alcohol, the absorption bands at 2949 and 2859 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the absorption band at 1696 cm^{-1} of the C=O stretching vibration of carboxylic acid and the absorption bands at 1439 cm^{-1} of the C=C stretching vibration of alkene. The absorption bands were assigned as shown in Table 4.19.

Table 4.19 The IR absorption bands assignment of Metabolite **1d**.

Wave number (cm^{-1})	Intensity	Vibration
3600-3000	broad	O-H stretching vibration of acid and alcohol
2949, 2859	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1696	strong	C=O stretching vibration of carboxylic acid
1439 and 1388	medium	C=C stretching vibration of alkene
1162 and 1038	medium	C-O stretching vibration
886	medium	C-H out of plane bending vibration

The ^{1}H-NMR spectrum (Fig. B44, Table 4.20) of Metabolite **1d** indicated that it

possesses two methyl groups at 1.01 and 1.20 ppm and two olefinic protons at 4.85 and 4.99 ppm in addition to proton of a secondary alcohol at 3.57 ppm.

The ^{13}C-NMR spectrum (Fig. B45, Table 4.20) showed 20 lines. The signal at δ 180.5 ppm should be the carbonyl group of carboxylic acid. Two olefinic carbons appeared at δ155.3 and 102.2 ppm. Two signals of methine carbons appeared at δ 48.0 and 47.2 ppm and a methine carbon of secondary alcohol at 76.2 ppm. Eight signals of methylene carbons appeared at δ 45.2, 43.8, 40.4, 39.7, 37.8, 29.0, 19.6 and 19.0 ppm and three quaternary carbons at δ 45.1, 42.9 and 38.9 ppm and a quaternary carbon of tertiary alcohol at δ 79.3 ppm. There were two methyl carbon signals at δ 27.9 and 14.8 ppm.

The information of 2D-NMR including gCOSY correlations (Fig. B46, Table 4.20), gHMBC correlations (Fig. B47, Table 4.20) and gHSQC correlations (Fig. B48) supported the structural elucidation of Metabolite **1d**.

The LC-MS spectrum showed the fragmentation as follows, m/z (ESI-MS) (Fig. B49): 357 $[M+Na]^+$.

Metabolite **1d** showed a weak molecular ion peak with *m/z* = 334 and the respective sodium adduct at *m/z* = 357, consistant with the molecular formula of $C_{20}H_{30}O_4$, which indicated a DBE of 6. Metabolite **1d** should be consisted of one carbonyl group of acid, one double bond in addition to four rings.

From all this spectroscopic data, metabolite (**1d**), was identified as ent-(7α,13)-dihydroxy-kaur-16-en-19-oic acid by comparison of their spectral properties with those previously reported in the metabolism of steviol by the mutant B1-41a of *Gibberella fujikuroi* [36]. The structure of Metabolite **1d** is shown in Figure 4.12.

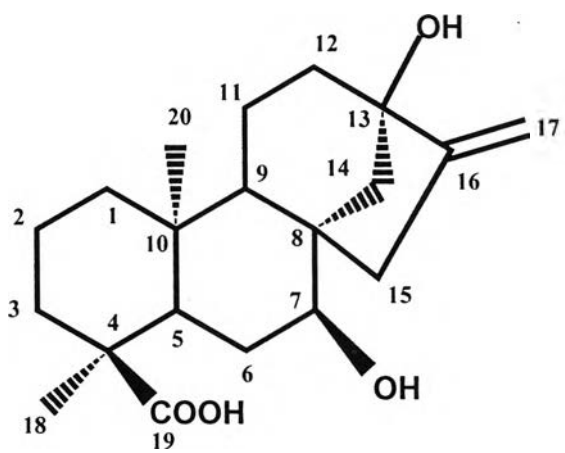
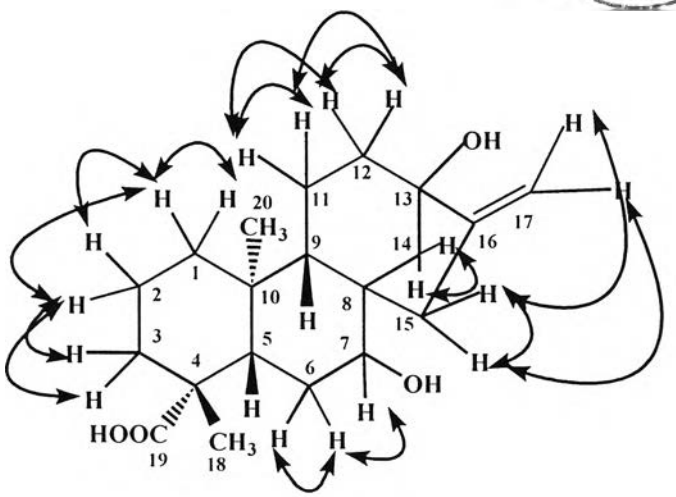
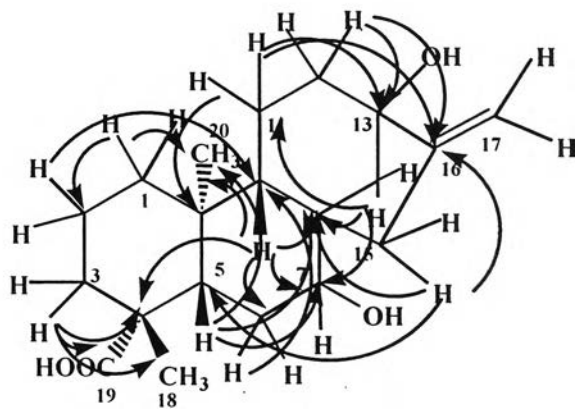


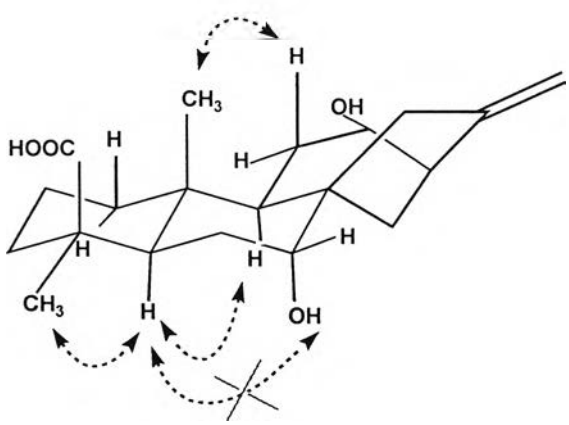
Figure 4.12 Structure of Metabolite **1d**.

Table 4.20 The ^{1}H-NMR, ^{13}C-NMR, gCOSY and gHMBC spectral data of Metabolite **1d**.

Position	^{13}C-NMR δ, ppm.	^{1}H-NMR δ, ppm.		gCOSY correlations	gHMBC correlations
1	40.4t	1a	0.96 (1H, dt, *J*=4, 13)	H-1b	C-2, C-20
		1b	1.88 (1H, m)	H-1a, H-2a, H-2b	C-9
2	19.0t	2a	1.48 (1H, m)	H-1b	-
		2b	1.96 (1H, m)	H-1b, H-3a, H-3b	-
3	37.8t	3a	1.09 (1H, dt, *J*=4, 13)	H-3a	C-4, C-18, C-19
		3b	2.18 (1H, m)	H-3b	-
4	42.9s	-		-	-
5	48.0d	5	1.43 (1H, d, *J*=8)	-	C-7, C-8, C-13, C-14, C-20
6	29.0t	6a	2.02 (1H, m)	H-7a, H-6b	-
		6b	2.13 (1H, m)	H-6a	C-9
7	76.2d	7a	3.57 (1H, s)	H-6a	-
8	45.1s	-		-	-
9	47.2d	9	1.78 (1H, d, *J*=14)	-	C-4, C-6, C-7, C-8, C-19, C-20
10	38.9s	-		-	-
11	19.6t	11a	1.57 (1H, m)	H-12a, H-11b	C-10
		11b	1.82 (1H, m)	H-11a, H-12b	C-5, C-13, C-16
12	39.7t	12a	1.53 (1H, m)	H-11a, H-12b	-
		12b	1.82 (1H, m)	H-12a, H-11b	C-5, C-13, C-16
13	79.3s	13	-	-	-
14	45.2t	14a	1.35 (1H, m)	H-14b	C-5,C-7,C-8,C-11,C-18
		14b	1.96 (1H, m)	H-14a	-
15	43.8t	15a	2.23 (1H, d, *J*=18)	H-15b	C-5, C-8, C-16
		15b	2.40 (1H, d, *J*=17)	H-15a	C-5, C-8, C-16
16	155.3s	-		-	-
17	102.2t	17a	4.85 (1H, s)	H-15a, H-17b	-
		17b	4.99 (1H, s)	H-15b, H-17a	-
18	27.9q	18	1.20 (3H, s)	-	C-1, C-2, C-3, C-7, C-11, C-19
19	180.5s	-		-	-
20	14.8q	20	1.01 (3H, s)	-	C-2, C-4, C-12







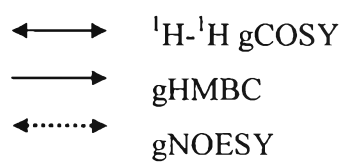


Figure 4.13 Selected ^{1}H-^{1}H gCOSY, gHMBC and gNOESY correlations for Metabolite **1d**.

4.2.2 Biotransformation of Compound 1 by *Rhizopus oligosporus.*

4.2.1.1 Structure elucidation of Metabolite 1e.

The IR spectrum of Metabolite **1e** is shown in Fig B50. Its IR spectrum showed important absorption bands at 3600-3000 cm^{-1} of the O-H stretching vibration of acid and alcohol, the absorption bands at 2925 and 2890 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the absorption band at 1688 cm^{-1} of the C=O stretching vibration of carboxylic acid and the absorption bands at 1450 cm^{-1} of the C=C stretching vibration of alkene. The absorption bands were assigned as shown in Table 4.21.

Table 4.21 The IR absorption bands assignment of Metabolite **1e**.

Wave number (cm^{-1})	Intensity	Vibration
3600-3000	broad	O-H stretching vibration of acid and alcohol
2925, 2890	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1688	strong	C=O stretching vibration of carboxylic acid
1450 and 1279	medium	C=C stretching vibration of alkene
1193 and 1038	medium	C-O stretching vibration
867	medium	C-H out of plane bending vibration

The ^{1}H-NMR spectrum (Fig. B51, Table 4.22) of Metabolite **1e** indicated that it possesses two methyl groups at 1.10 and 1.18 ppm and two olefinic protons at 4.79 and 4.83 ppm in addition to proton of a secondary alcohol at 3.56 ppm.

The ^{13}C-NMR spectrum (Fig. B52, Table 4.22) showed 20 lines. The signal at δ 180.0 ppm should be the carbonyl group of carboxylic acid. Two olefinic carbons appeared at δ 155.1 and 103.7 ppm. Three signals of methine carbons appeared at δ 49.1, 47.2 and 43.7 ppm and a methine carbon of secondary alcohol at 77.1 ppm. Eight signals of methylene carbons appeared at δ 45.3, 40.4, 38.7, 37.8, 33.5, 29.7, 19.1 and 17.9 ppm and three quaternary carbons at δ 48.3, 43.2 and 39.4 ppm. There were two methyl carbon signals at δ 28.7 and 15.5 ppm.

The information of 2D-NMR including gCOSY correlations (Fig. B53, Table 4.22), gHMBC correlations (Fig. B54, Table 4.22) and gHSQC correlations (Fig. B55) supported the structural elucidation of Metabolite **1e**.

The LC-MS spectrum showed the fragmentation as follows, m/z (ESI-MS) (Fig. B58): 357 $[M+Na]^+$.

Metabolite **1e** showed a weak molecular ion peak with *m/z* = 334 and the respective sodium adduct at *m/z* = 357, consistant with the molecular formula of $C_{20}H_{30}O_4$, which indicated a DBE of 6. Metabolite **1e** should be consisted of one carbonyl group of acid, one double bond in addition to four rings.

From all this spectroscopic data, metabolite (**1e**), was identified as *ent*-7α-hydroxy-kaur-16-en-19-oic acid, previously obtained in the biotransformation of **1** with *R. stolonifer* [14]. The structure of Metabolite **1e,** is shown in Figure 4.14.

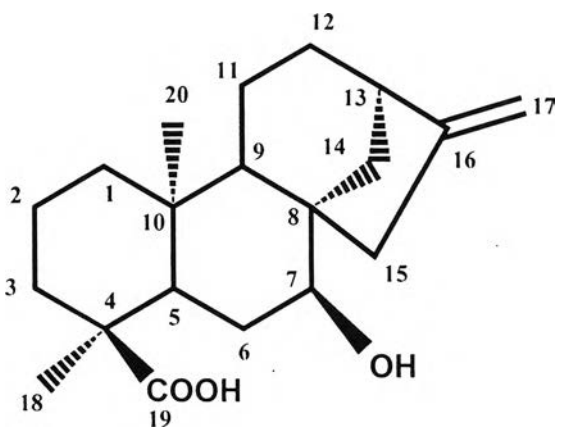


Figure 4.14 Structure of Metabolite **1e**.

Table 4.22 The ^{1}H-NMR, ^{13}C-NMR, gCOSY and gHMBC spectral data of Metabolite **1e**.

Position	^{13}C-NMR δ, ppm.	^{1}H-NMR δ, ppm.		gCOSY correlations	gHMBC correlations
1	40.4t	1a	0.96 (1H, dt, *J*=4, 13)	H-1b	C-2
		1b	1.88 (1H, m)	H-1a, H-2a, H-2b	-
2	19.1t	2a	1.48 (1H, m)	H-1b	-
		2b	1.96 (1H, m)	H-1b, H-3a, H-3b	-
3	37.8t	3a	1.09 (1H, dt, *J*=4, 13)	H-3a	C-4
		3b	2.18 (1H, m)	H-3b	-
4	43.2s	-		-	-
5	49.1d	5	1.43 (1H, d, *J*=8)	-	C-4, C-7
6	29.7t	6a	2.02 (1H, m)	H-7a, H-6b	-
		6b	2.13 (1H, m)	H-6a	C-7
7	77.1d	7	3.57 (1H, s)	H-6a	C-5, C-9
8	48.3s	-		-	-
9	47.2d	9	1.78 (1H, d, *J*=14)	-	C-4, C-7
10	39.4s	-		-	-
11	17.6t	11a	1.57 (1H, m)	H-12a, H-11b	C-10
		11b	1.82 (1H, m)	H-11a, H-12b	-
12	33.5t	12a	1.53 (1H, m)	H-11a, H-12b	-
		12b	1.82 (1H, m)	H-12a, H-11b	C-15
13	43.7d	13	-	H-14a	-
14	38.7t	14a	1.35 (1H, m)	H-13, H-14b	C-7
		14b	1.96 (1H, m)	H-14a	-
15	45.3t	15a	2.23 (1H, d, *J*=18)	H-15b, H-17a	C-7, C-9, C-16
		15b	2.40 (1H, d, *J*=17)	H-15a, H-17b	
16	155.1s	-		-	-
17	103.7t	17a	4.85 (1H, s)	H-15a	C-16
		17b	4.99 (1H, s)	H-15b	-
18	28.7q	18	1.20 (3H, s)	-	C-3, C-4, C-5,
19	180.0s	-		-	-
20	15.5q	20	1.01 (3H, s)	-	C-5, C-9

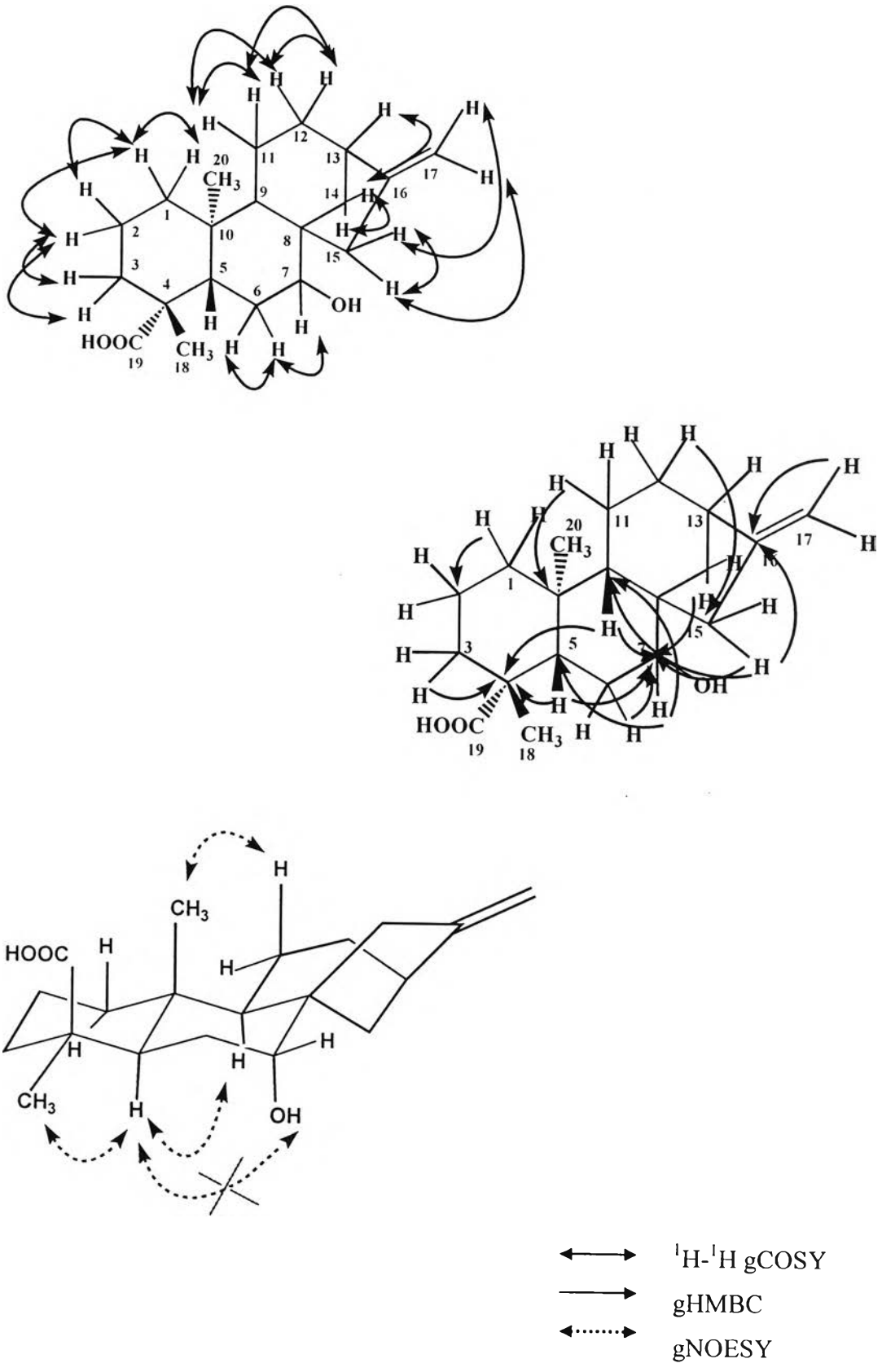


Figure 4.15 Selected ^{1}H-^{1}H gCOSY, gHMBC and gNOESY correlations for Metabolite **1e**.

4.2.2.2 Structure elucidation of Metabolite 1f.

The IR spectrum of Metabolite **1f** is shown in Fig B57. Its IR spectrum showed important absorption bands at 3600-2800 cm^{-1} of the O-H stretching vibration of acid, the absorption bands at 2933 and 2823 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the absorption band at 1680 cm^{-1} of the C=O stretching vibration of carboxylic acid and the absorption bands at 1450 cm^{-1} of the C=C stretching vibration of alkene. The absorption bands were assigned as shown in Table 4.23.

Table 4.23 The IR absorption bands assignment of Metabolite **1f**.

Wave number (cm^{-1})	Intensity	Vibration
3600-2800	broad	O-H stretching vibration of acid
2933, 2823	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1680	strong	C=O stretching vibration of carboxylic acid
1450, 1240	medium	C=C stretching vibration of alkene
1026	medium	C-O stretching vibration
847	medium	C-H out of plane bending vibration

The ^{1}H-NMR spectrum (Fig. B58, Table 4.24) of Metabolite **1f** indicated that it possesses two methyl groups at 1.15 and 1.21 ppm and two protons of a primary alcohol at 3.35 and 3.46 ppm.

The ^{13}C-NMR spectrum (Fig. B59, Table 4.24) and DEPT experiment (Fig. B60) showed 20 lines. The signal at δ 181.2 ppm should be the carbonyl group of carboxylic acid. Two signals of methine carbons appeared at δ 50.7 and 41.0 ppm. Eight signals of methylene carbons appeared at δ 40.2, 38.9, 37.8, 33.6, 30.3, 29.0, 20.7, and 22.7 ppm and a methylene carbon of primary alcohol at δ 70.5 ppm. Three quaternary carbons appeared at δ 49.5, 45.2 and 44.8 ppm and two quaternary carbons of tertiary alcohols at δ 81.1 and 79.2 ppm. There were two methyl carbon signals at δ 29.6 and 18.1 ppm.

The information of 2D-NMR including gCOSY correlations (Fig. B61, Table 4.24), gNOESY correlations (Fig. B62), gHMBC correlations (Fig. B63, Table 4.24)

and gHSQC correlations (Fig. B64) supported the structural elucidation of Metabolite **1f**.

The LC-MS spectrum showed the fragmentation as follows, m/z (ESI-MS) (Fig. B65): 375 $[M+Na]^+$ and HRESIMS m/z: 375.2138 $[M+Na]^+$.

Metabolite **1f** showed a weak molecular ion peak with *m/z* = 352 and the respective sodium adduct at *m/z* = 375, consistant with the molecular formula of $C_{20}H_{32}O_5$, which indicated a DBE of 5. Metabolite **1f** should be consisted of one carbonyl group of acid in addition to four rings.

From all this spectroscopic data, the new compound (**1f**), named *ent*-(9α,16β,17)-trihydroxy-kaur-16-en-19-oic acid was proposed. The structure of Metabolite **1f**, is shown in Figure 4.16.

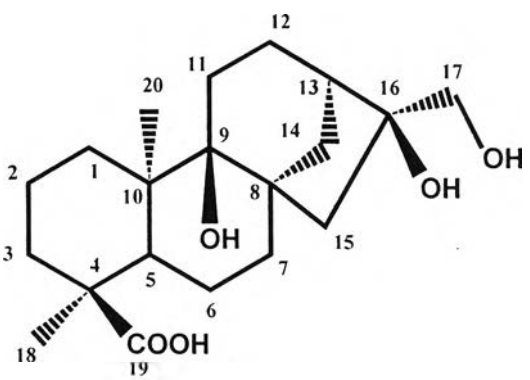


Figure 4.16 Structure of Metabolite **1f**.

Table 4.24 The ^{1}H-NMR, ^{13}C-NMR, gCOSY and gHMBC spectral data of Metabolite **1f**.

Position	^{13}C-NMR δ, ppm.	^{1}H-NMR δ, ppm.		gCOSY correlations	gHMBC correlations
1	38.9t	1a	1.05 (1H, m)	H-1b	C-3, C-18
		1b	2.11 (1H, m)	H-1a, H-2a, H-2b	-
2	20.9t	2a	1.43 (1H, m)	H-1b	-
		2b	1.43 (1H, m)	H-1b, H-3a, H-3b	-
3	37.8t	3a	1.15 (1H, m)	H-3a	C-2, C-20
		3b	1.84 (1H, m)	H-3b	-
4	44.8s	-		-	-
5	50.7d	5	1.70 (1H, m)	-	C-3, C-4, C-6, C-19
6	22.7t	6a	1.82 (1H, m)	H-7a, H-6b	C-8, C-19
		6b	1.82 (1H, m)	H-6a	-
7	40.2t	7a	1.23 (1H, m)	H-6a	C-9, C-16, C-19
		7b	2.27 (1H, m)	-	C-15
8	49.5s	-		-	-
9	81.1s	-		-	-
10	45.2s	-			-
11	30.3t	11a	1.71 (1H, m)	H-12a, H-11b	C-10, C-16
		11b	1.96 (1H, dt, *J*=5, 10)	H-11a, H-12b	C-8, C-10, C-12, C-13, C-16
12	29.0t	12a	1.56 (1H, m)	H-11a, H-12b	C-9, C-11
		12b	1.93 (1H, m)	H-12a, H-11b	C-8
13	41.0d	13	2.07 (1H, d, *J*=2)	H-14a	-
14	33.6t	14a	1.52 (1H, m)	H-13, H-14b	-
		14b	1.85 (1H, m)	H-14a	-
15	47.0t	15a	1.08 (1H, m)	H-15b	C-8, C-9, C-13, C-16, C-17
		15b	2.22 (1H, m)	H-15a	C-5, C-7, C-9, C-16
16	79.2s	-		-	-
17	70.5d	17a	3.35 (1H, d, *J*=11)	-	C-9
		17b	3.46 (1H, d, *J*=11)	-	
18	29.6q	18	1.21 (3H, s)	-	C-1, C-2, C-4, C-5, C-19
19	181.2s	-		-	-
20	18.1q	20	1.15 (3H, s)	-	C-5, C-9, C-10, C-14, C-16

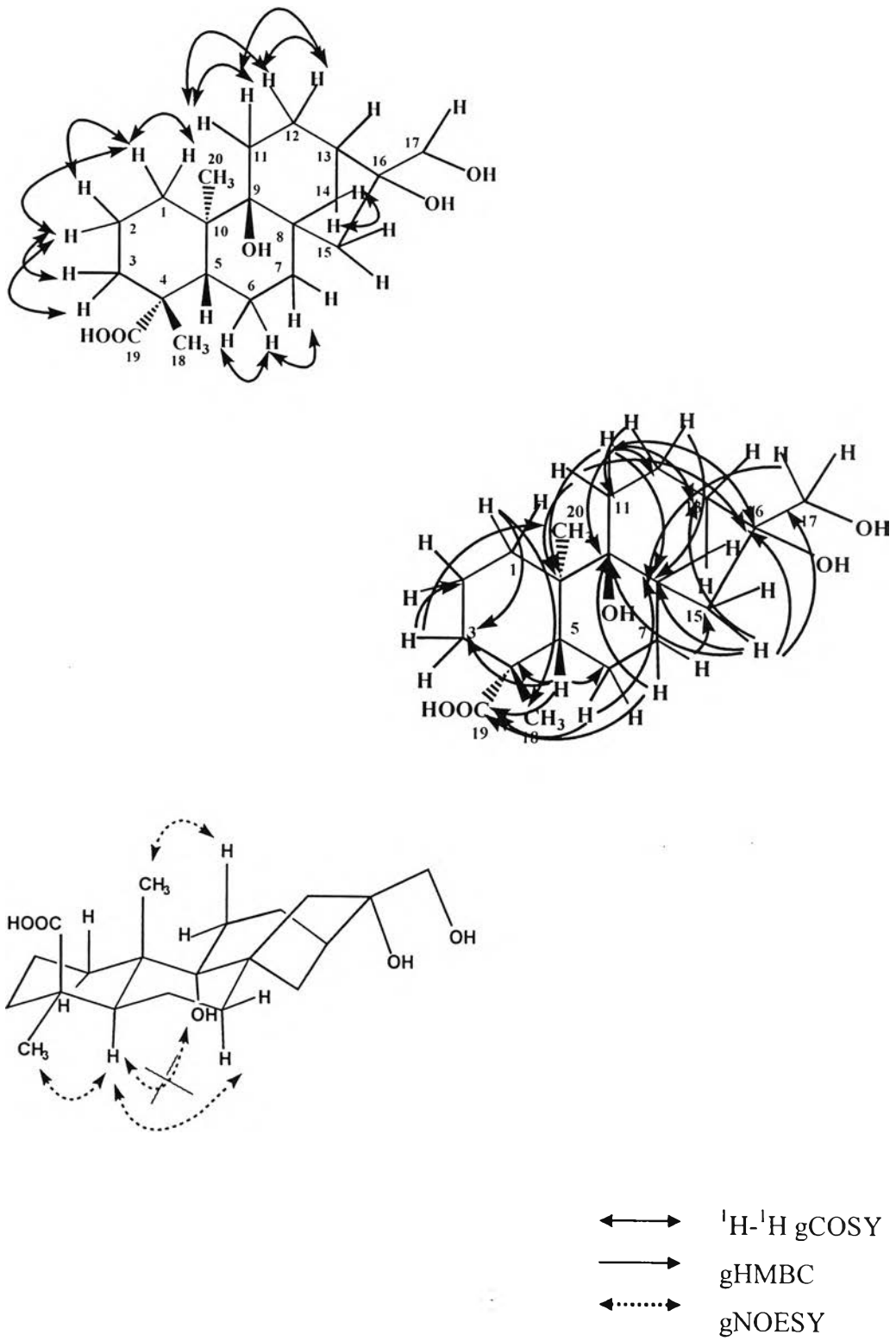


Figure 4.17 Selected ^{1}H-^{1}H gCOSY, gHMBC and gNOESY correlations for Metabolite **1f**.

4.2.4. Biotransformation of Compound 2 by *Rhizopus oligosporus.*

4.2.4.1 Structure elucidation of Metabolite 2a

The IR spectrum of Metabolite **2a** (Fig. B66) showed important absorption bands at 3600-3200 cm^{-1} of the O-H stretching vibration of alcohol, the absorption bands at 2935 and 2862 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the absorption bands at 1699 and 1652 cm^{-1} of the C=O stretching vibration of unsaturated carbonyl group and the absorption band at 1453 cm^{-1} of the C=C stretching vibration of alkene. The IR spectral data of Metabolite **2a** are summarized in Table 4.25.

Table 4.25 The IR absorption bands assignment of Metabolite **2a**

Wave number(cm^{-1})	Intensity	Vibration
3600-3200	medium	O-H stretching vibration
2935, 2862	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1699, 1652	strong	C=O stretching vibration of carbonyl group
1453, 1381	medium	C=C stretching vibration of alkene
1268, 1108	weak	C-O stretching vibration

The ^{1}H-NMR spectrum (Fig. B67, Table 4.26) of Metabolite **2a** possessed five methyl groups at 1.38, 1.28, 1.21, 1.11 and 1.19 ppm, proton attached to carbon bearing oxygen group at 4.21 ppm, α,β-unsaturated ketone protons at 5.85 and 7.77 ppm and terminal double bond protons at 6.06, 5.44 and 5.13 ppm.

The ^{13}C-NMR spectrum (Fig. B68, Table 4.26) and DEPT experiment (Fig. B69) showed 20 lines. Four signals of olefinic carbons appeared at δ 159.4, 147.8, 124.6 and 113.0 ppm. The signal at δ 204.5 ppm should be the carbonyl of α,β-unsaturated ketone. Two signals of methine carbons appeared at δ 58.1 and 53.3 ppm and a methine carbon of secondary alcohol at 65.0 ppm. Three signals of methylene carbons appeared at δ 43.5, 42.8 and 20.1 ppm. Four quaternary carbons appeared at δ 74.5, 73.5, 42.3 and 40.1 ppm. There were five methyl carbon signals at δ 31.5, 27.8, 27.3, 21.5 and 19.3 ppm.

The information of 2D-NMR including gNOESY correlations (Fig. B70), gHMBC correlations (Fig. B71, Table 4.26) and gHSQC correlations (Fig. B72) supported the structural elucidation of Metabolite **2a**

The configuration of the hydroxyl group was obtained from the gNOESY-NMR spectrum (Fig. 4.18 and Fig B70) which shows coupling ofthe H-11 with H-17 and H-20 resonance expected for diaxial coupling.

The MS spectrum showed the fragmentation as follows, m/z (EI MS) (Fig. B74): 318 [M^+], 285 (6), 233 (10), 215 (5), 192 (10), 149 (25), 124 (100), 96 (75) and 55 (30).

Metabolite **2a** showed a molecular ion with m/z = 318 ($C_{20}H_{30}O_3$), which indicated a DBE of 6, therefore, Metabolite **2a** should be consisted of one ring of α,β-unsaturated ketone (DBE = 3), one double bond, in addition to two rings.

From all this spectroscopic data, the new compound (Metabolite **2a**), named *ent*-1,2-Dehydro-11α-hydroxy-3-oxomanoyl oxide was proposed. The structure of Metabolite **2a**, is shown in Figure 4.18.

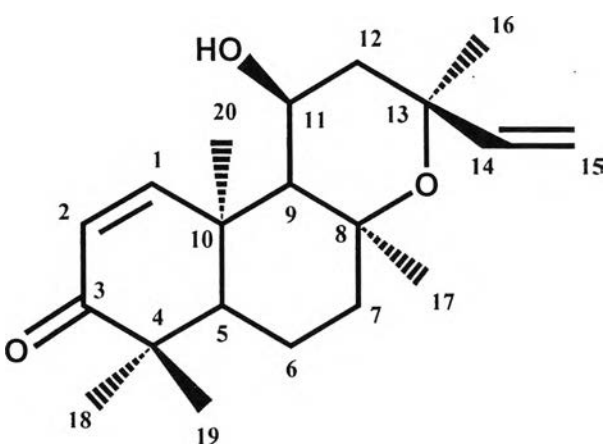


Figure 4.18 Structure of Metabolite **2a**

Table 4.26 The ^{1}H-NMR, ^{13}C-NMR, gCOSY and gHMBC spectral data of Metabolite **2a.**

Position	^{13}C-NMR δ, ppm.	^{1}H-NMR δ, ppm.		gCOSY correlations	gHMBC correlations
1	159.4d	1	7.77 (1H, d, *J*=10)	H-2	C-3
2	124.6d	2	5.85 (1H, d, *J*=10)	H-1	C-10
3	204.5s	3	-	-	-
4	42.3s	4	-	-	-
5	53.3d	5	1.82 (1H, m)	-	-
6	20.1t	6a	1.51 (1H, m)	H-5, H-6b, H-7a	-
		6b	1.74 (1H, m)	H-6a, H-7b	-
7	42.8t	7a	1.57 (1H, m)	H-7b	-
		7b	1.97 (1H, m)	H-7a	-
8	74.5s		-	-	-
9	58.1d	9	1.78 (1H, m)	H-11	C-8, C-10, C-11, C-17, C-20
10	40.1s		-	-	-
11	65.0d	11	4.21 (1H, m)	H-9, H-12b	C-13
12	43.5t	12a	2.03 (1H, m)	H-12b	C-9, C-11
		12b	2.48 (1H, m)	H-11, H-12a	C-13, C-14, C-16
13	73.5s		-	-	-
14	147.8d	14	6.06 (1H, dd, *J* = 17,10 Hz)	-	C-13
15	113.0t	15a	5.13 (1H, dd, *J* = 11, 1 Hz)	-	C-13, C-14
		15b	5.44 (1H, dd, *J* = 17, 2 Hz)	-	-
16	31.5q	16	1.38 (3H, s)	-	C-12, C-13, C-14
17	27.8q	17	1.28 (3H, s)	-	C-7, C-8, C-9
18	27.3q	18	1.21 (3H, s)	-	C-3, C-4, C-5
19	21.5q	19	1.11 (3H, s)	-	C-3, C-4, C-5
20	19.3q	20	1.19 (3H, s)	-	C-5, C-9, C-10

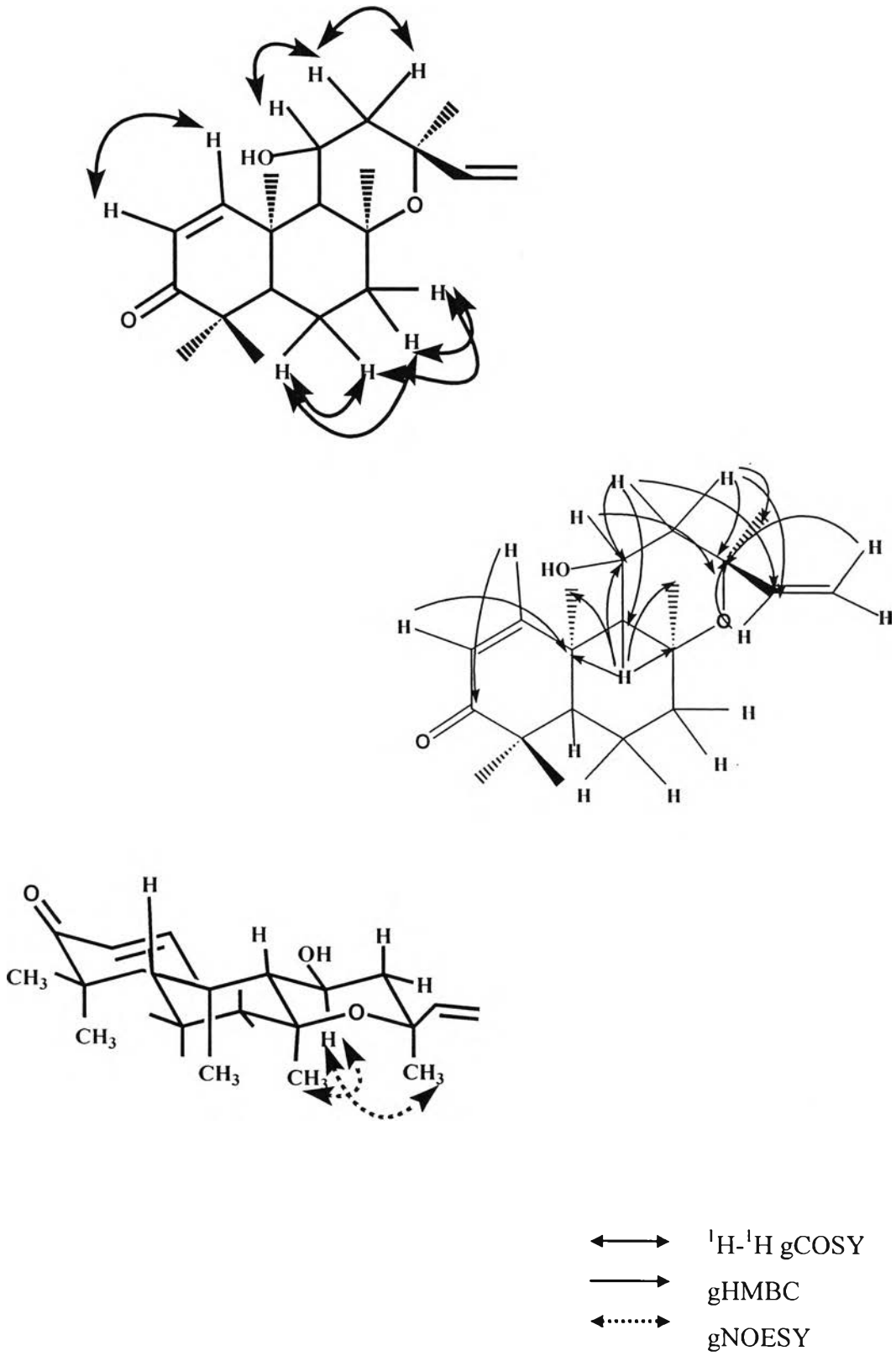


Figure 4.19 Selected ^{1}H-^{1}H gCOSY, gHMBC and gNOESY correlations for Metabolite **2a**

4.2.2.2 Structure elucidation of Metabolite 2b

The IR spectrum of Metabolite **2b** (Fig. B74) showed important absorption bands at 3300-3000 cm^{-1} of the O-H stretching vibration of alcohol, the absorption band at 2928 and 2867 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the presence of a carbonyl group with corresponding to the strong absorption band at 1694 and 1653 cm^{-1} and the absorption bands at 1455 and 1377 cm^{-1} of the C=C stretching vibration of alkene. The IR spectral data of Metabolite **2b** are summarized in Table 4.27.

Table 4.27 The IR absorption bands assignment of Metabolite **2b**

Wave number(cm^{-1})	Intensity	Vibration
3300-3000	medium	O-H stretching vibration
2928, 2867	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1694, 1653	strong	C=O stretching vibration of carbonyl group
1455, 1377	medium	C=C stretching vibration of alkene
1285	weak	C-O stretching vibration

The ^{1}H-NMR spectrum (Fig. B75, Table 4.28) of Metabolite **2b** possessed five methyl groups at 1.31, 1.30, 1.18, 1.14 and 1.08 ppm, proton attached to carbon bearing oxygen group at 4.10 ppm and two olefinic protons at 7.79 and 5.85 ppm.

The ^{13}C-NMR spectrum (Fig. B76, Table 4.28) and DEPT experiment (Fig. B77) showed 20 lines. Two signals of olefinic carbons appeared at δ 159.5 and124.6 ppm. The signal at δ 204.5 ppm should be the carbonyl of α,β-unsaturated ketone. Two signals of methine carbons appeared at δ 59.0 and 53.1 ppm and two methine carbon of secondary alcohol at 63.2 and 58.6 ppm. Three signals of methylene carbons appeared at δ 42.3, 41.5 and 20.0 ppm and a methylene carbon of primary alcohol at 58.6 ppm. Four quaternary carbons appeared at δ 89.0, 74.0, 44.8 and 39.8 ppm. There were five methyl carbon signals at δ 28.4, 27.2, 27.0, 21.5 and 19.4 ppm.

The information of 2D-NMR including gCOSY correlations (Fig. B78, Table 4.28), gNOESY correlations (Fig. B79), gHMBC correlations (Fig. B80, Table 4.28) and gHSQC correlations (Fig. B81) supported the structural elucidation of Metabolite **2b**

The configuration of the hydroxyl group was obtained from the gNOESY-NMR spectrum (Fig. 4.21 and Fig B79) which shows coupling of the H-11_{ax} with H-18 and H-20 resonance expected for diaxial coupling.

The MS spectrum showed the fragmentation as follows, m/z (EI MS) (Fig. B82): 352 [M^+], 334 [M-H_2O], 301 (5), 291 (70), 233 (22), 149 (25), 124 (100), 96 (85) and 55 (25).

Metabolite **2b** showed a molecular ion with m/z = 352 ($C_{20}H_{30}O_5$), which indicated a DBE of 6. Metabolite **2b** should be consisted of one ring of α,β-unsaturated ketone (DBE = 3) and two rings.

From all this spectroscopic data, the new compound (Metabolite **2b**), named *ent*-(11α,14ξ,15)-trihydroxy- 3-oxomanoyl oxide was proposed. The structure of Metabolite **2b**, is shown in Figure 4.20.

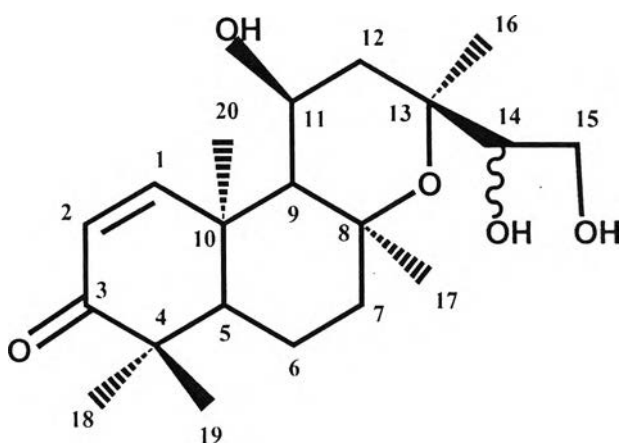


Figure 4.20 Structure of Metabolite **2b**

Table 4.28 The ^{1}H-NMR, ^{13}C-NMR, gCOSY and gHMBC spectral data of Metabolite **2b**

Position	^{13}C-NMR δ, ppm.	^{1}H-NMR δ, ppm.	gCOSY correlations	gHMBC correlations
1	159.5d	1 7.79 (1H, d, *J*=10)	H-2	C-3, C-10
2	124.6d	2a 5.85 (1H, d, *J*=10)	H-1	C-9, C-10
3	204.5s	-	-	-
4	44.8s	-	-	-
5	53.1d	5 1.84 (1H, dd, *J*=12, 2)	-	C-4, C-10, C-18, C-19, C-20
6	20.0t	6a 1.50 (1H, m)	H-5, H-6b, H-7a	C-5, C-7
		6b 1.70 (1H, m)	H-6a, H-7b	C-8, C-10
7	42.3t	7a 1.37 (1H, m)	H-7b	C-6
		7b 1.81 (1H, m)	H-7a	C-8, C-9, C-15
8	74.0s	-	-	-
9	59.0d	9 1.62 (1H, m)	H-11	C-1, C-5, C-7, C-8, C-10, C-11, C-17, C-18
10	39.8s	-	-	-
11	63.2d	11 4.10 (1H, m)	H-9, H-12b	C-9, C-10, C-13
12	41.5t	12a 2.06 (1H, m)	H-12b	C-11, C-14
		12b 2.50 (1H, m)	H-11, H-12a	C-13, C-14, C-16
13	89.0s	-	-	-
14	58.6d	14 2.81 (1H, m)	H-15	C-13
15	46.1d	15a 2.81 (1H, m)	-	-
		15b 3.06 (1H, m)	H-14	-
16	28.4q	16 1.30 (3H, s)	-	C-12, C-13, C-14
17	27.0q	17 1.31 (3H, s)	-	C-7, C-8
18	27.2q	18 1.14 (3H, s)	-	C-1, C-5, C-9
19	21.5q	19 1.18 (3H, s)	-	C-3, C-5, C-9, C-20
20	19.4q	20 1.08 (3H, s)	-	C-3 , C-5, C-11, C-19

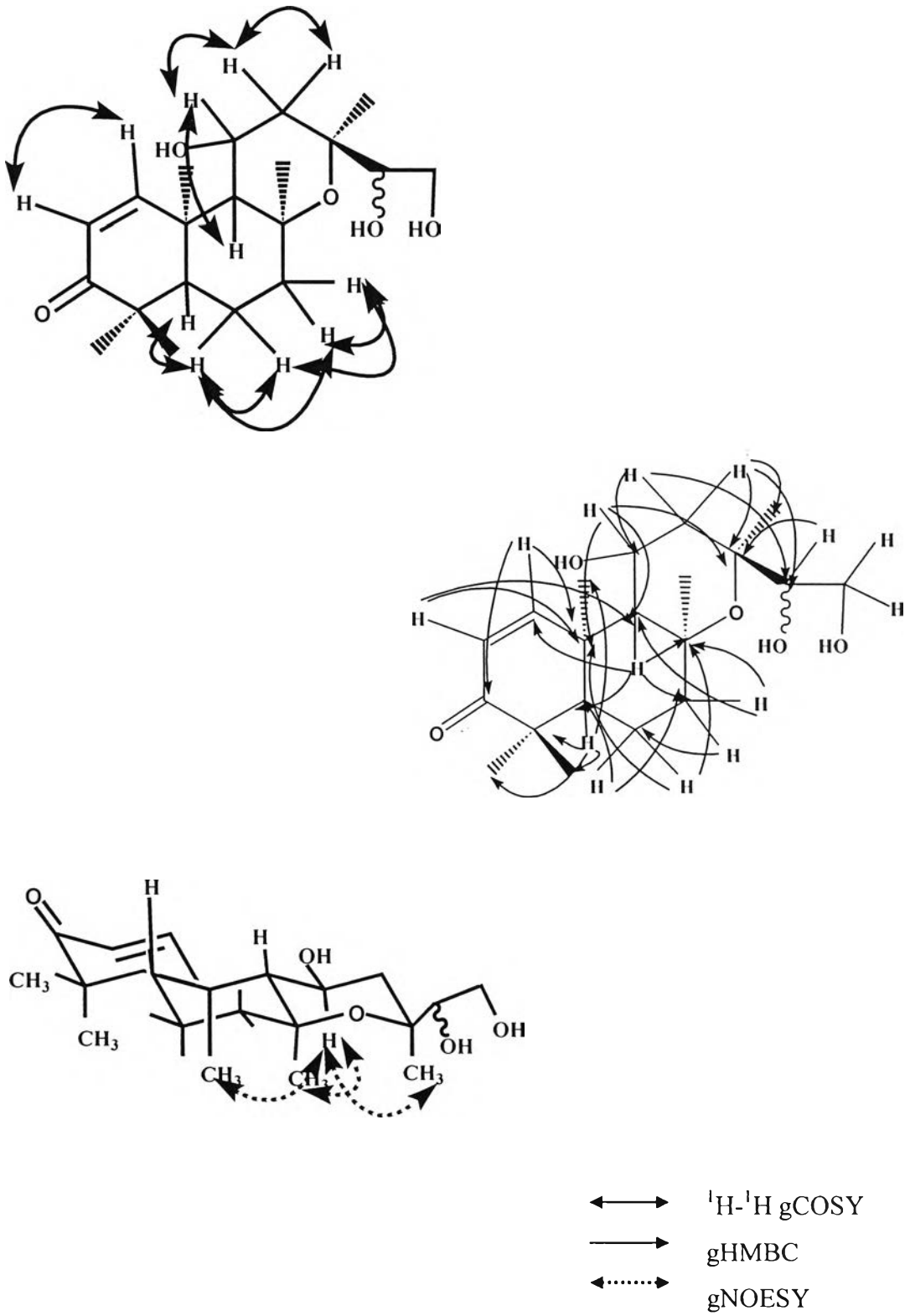


Figure 4.21 Selected ^{1}H-^{1}H gCOSY, gHMBC and gNOESY correlations for Metabolite **2b**

4.2.5. Biotransformation of Compound 2 by *Rhizopus stolonifer.*

4.2.5.1 Structure elucidation of Metabolite 2c

The IR spectrum of Metabolite **2c** is shown in Fig. B83. Its IR absorption bands at 3500-3200 cm^{-1} indicated the O-H stretching vibration of alcohol, the absorption band at 2946 and 2890 cm^{-1} indicated the C-H stretching vibration of methyl and methylene group, the absorption band at 1695 cm^{-1} belonged to the C=O stretching vibration of carbonyl group and the absorption band at 1450 and 1383 cm^{-1} indicated the C=C stretching vibration of alkene. The absorption bands were assigned as shown in Table 4.29.

Table 4.29 The IR absorption bands assignment of Metabolite **2c**

Wave number(cm^{-1})	Intensity	Vibration
3500-3200	medium	O-H stretching vibration of alcohol
2946, 2890	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1695	strong	C=O stretching vibration of carbonyl group
1450, 1383	medium	C=C stretching vibration of alkene
1285	weak	C-O stretching vibration

The ^{1}H-NMR spectrum (Fig. B84, Table 4.30) of Metabolite **2c** showed five methyl groups at 1.36, 1.26, 1.14, 1.07 and 1.07 ppm, and proton attached to carbon bearing oxygen group at 4.03 ppm.

The ^{13}C-NMR spectrum (Fig. B85, Table 4.30) and DEPT experiment (Fig. B86) showed 20 lines. Two signal of olefinic carbons appeared at δ 148.1 and 112.5 ppm. The signal at δ 216.7 ppm should be the carbonyl of ketone. Two signals of methine carbons appeared at δ 61.9 and 55.6 ppm and a methine carbon of secondary alcohol at 64.9 ppm. Five signals of methylene carbons appeared at δ 43.5, 42.8, 38.5, 34.0 and 21.4 ppm. Four quaternary carbons appeared at δ 89.0, 74.0, 44.8 and 39.8 ppm. There were five methyl carbon signals at δ 31.5, 27.3, 26.2, 20.7 and 15.7 ppm.

The information of 2D-NMR including gNOESY correlations (Fig. B87), gHMBC correlations (Fig. B88, Table 4.30) and gHSQC correlations (Fig. B89) supported the structural elucidation of Metabolite **2c**.

The configuration of the hydroxyl group was obtained from the gNOESY-NMR spectrum (Fig. 4.23 and Fig B87) which shows coupling of the H-11 with H-17 and H-20 resonance expected for diaxial coupling.

The MS spectrum showed the fragmentation as follows, m/z (ESI MS) (Fig. B92): 320 [M^+], 305 (90), 287 (50), 269 (28), 235 (27), 193 (25), 135 (34), 109 (68), 69 (75) and 55 (100).

Metabolite **2c** showed a molecular ion with m/z = 320 ($C_{20}H_{32}O_3$), which indicated a DBE of 5, therefore, Metabolite **2c** should be consisted of one ring of ketone (DBE = 2), one double bond, in addition to two rings.

From all this spectroscopic data, the new compound (Metabolite **2c**), named *ent*-11α-hydroxy-3-oxomanoyl oxide was proposed. The structure of Metabolite **2c**, is shown in Figure 4.22.

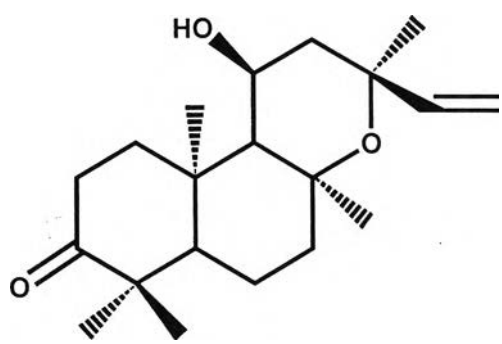


Figure 4.22 Structure of Metabolite **2c**

Table 4.30 The ^{1}H-NMR, ^{13}C-NMR, gCOSY and gHMBC spectral data of Metabolite **2c**

Position	^{13}C-NMR δ, ppm.	^{1}H-NMR δ, ppm.		gCOSY correlations	gHMBC correlations
1	38.5t	1a	1.65 (1H, m)	H-2a	C-3
		1b	2.10 (1H, m)	H-2b	-
2	34.0t	2a	2.37 (1H, m)	H-1a	C-3
		2b	2.59 (1H, m)	H-1b	-
3	216.7s	3	-	-	-
4	47.5s		-	-	-
5	55.6d	5	1.51 (1H, m)	-	-
6	21.4t	6a	1.07 (1H, m)	H-5, H-6b, H-7a	-
		6b	1.07 (1H, m)	H-6a, H-7b	-
7	42.8t	7a	1.57 (1H, m)	H-7b	-
		7b	1.91 (1H, m)	H-7a	
8	73.4s		-	-	
9	61.9d	9	1.54 (1H, m)	H-11	C-8, C-10, C-11, C-17, C-20
10	37.4s		-	-	-
11	64.9d	11a	4.03 (1H, m)	H-9, H-12b	C-13
12	43.5t	12a	1.94 (1H, m)	H-12b	C-9, C-11
		12b	2.40 (1H, m)	H-11, H-12a	C-13, C-14, C-16
13	74.2s		-	-	-
14	148.1d	14	6.05 (1H, dd, *J* = 17,11 Hz)	-	C-13
15	112.5t	15a	5.10 (1H, dd, *J* = 10, 1 Hz)	-	C-13, C-14
		15b	5.42 (1H, dd, *J* = 1, 2 Hz)	-	-
16	31.5q	16	1.26 (3H, s)	-	C-12, C-13
17	27.3q	17	1.36 (3H, s)	-	C-7, C-8, C-9
18	26.2q	18	1.14 (3H, s)	-	C-5, C-9, C-19
19	20.7q	19	1.07 (3H, s)	-	C-5, C-9, C-18
20	15.7q	20	1.07 (3H, s)	-	C-1, C-5, C-9, C-10

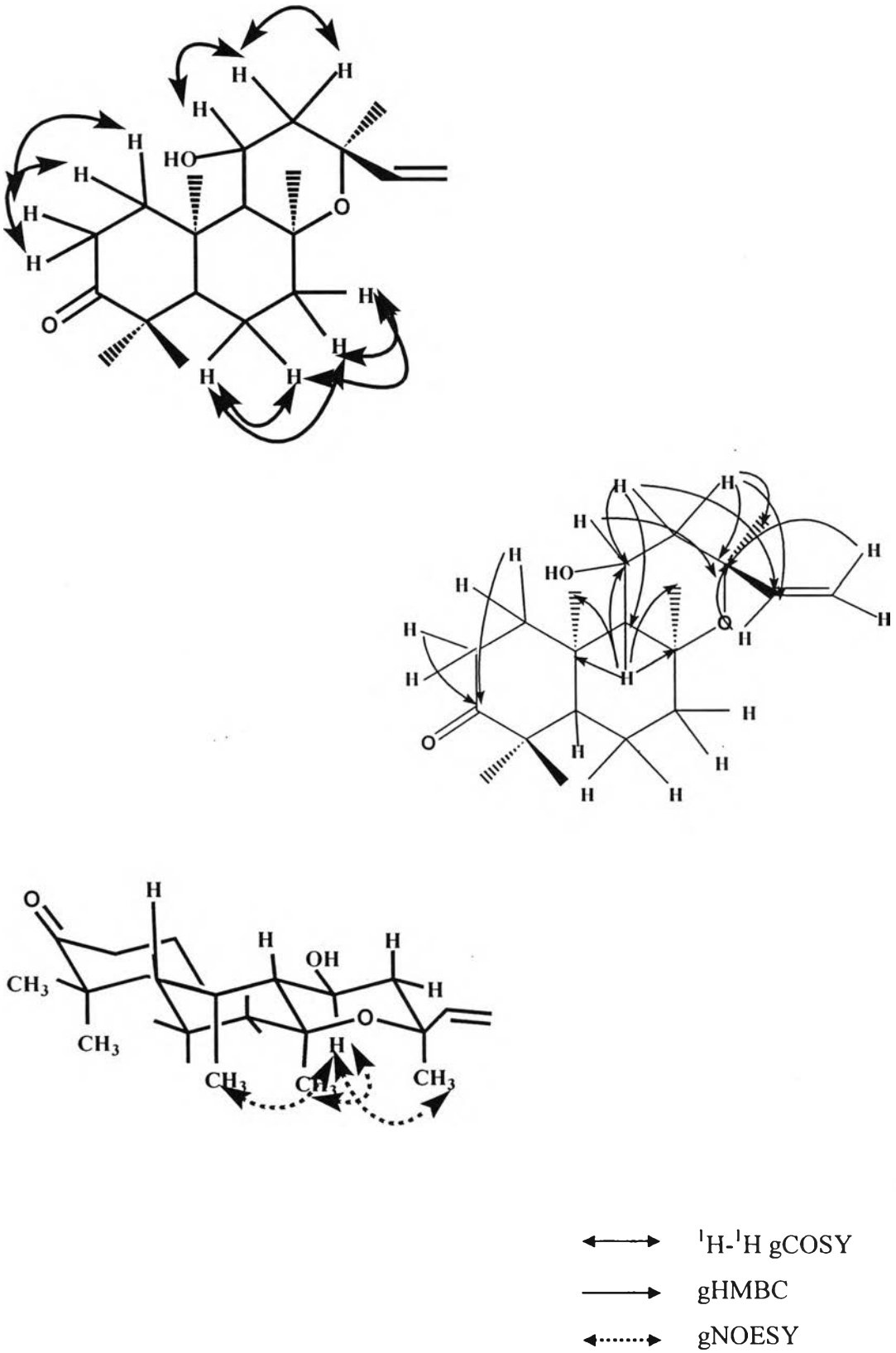


Figure 4.23 Selected ^{1}H-^{1}H gCOSY, gHMBC and gNOESY correlations for Metabolite **2c**.

4.2.6. Biotransformation of Compound 1 by *Mucor plumbus*.

The biotransformation of Compound **1** by *Mucor plumbus* gave Metabolite **2a** and **2b.**

The results of these biotransformations indicate that:

1. The biotransformation of **1** by *A. blakesleeana* was focused only on dihydroxylation in the A, B and C rings.
2. The stereospecific hydroxylation at C-7 (ent-α face) of this kuaranoic acid **1** was obtained by *Absidia blakesleeana, Rhizopus oligosporus* and *Aspergillus ficuum*
3. Our preliminary research work on biotransformation of **1** with *Aspergillus niger* also gave the same pattern of hydroxylation (Punnapayak et al., 2002).
4. This is the first report on the transformation of 1 to 2-5 by *A. blakesleeana.* Whereas *R. oligosporus* which belongs to the same class as *A. blakesleeana*, showed mono- and tri-hydroxylation of ring B, C and D of 1 and hydroxylation of the double bond.
5. *A. blakesleeana* and *A. niger* (Punnapayak et al., 2002) have in common the non-functionalization of the double bond of the molecule, as was observed with *R. oligosporus.*
6. It is also of interesting to note that 1α,7β, 7β,9β and 9β,16β,17 hydroxylation by fungi, to the best of our knowledge, have not been described previously.
7. No biotransformation of *ent*-1,2-dehydro-3-oxomanoyl oxide **2** could be observed with any *Streptomyces* strain tested.
8. The result of the biotransformation of **2** by three fungi indicated that there is a marked preference for hydroxylation at C-11α resulted from the biotransformation of **2** by the three fungal strain selected.
9. Moreover, *R. oligosporus* and *M. plumbeus* were able to functionalize the 14-15 double bond.

4.3 Antimicrobial activity

The isolated metabolites were evaluated for their antimicrobial activities by microdilution broth susceptibility test against *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *C. albicans*

ATCC 10231. These compounds were examined in the concentration range of 1000-0.48 μg/ml. The lowest concentration of the compound that inhibited growth of test microorganisms is recorded as the minimal inhibitory concentration (MIC). Antimicrobial activity of isolated compounds is presented in Table 4.31.

Table 4.31 MIC of antimicrobial activities of isolated metabolites.

Compound	MIC(μg/ml)				
	B. subtilis ATCC 6633	*S. aureus* ATCC 25923	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*C. albicans* ATCC 10231
Compound 1	62.5	125	-	-	-
Metabolite 1b	-	-	-	-	-
Metabolite 1d	-	-	-	-	-
Metabolite 1e	500	-	-	-	-
Amoxicillin	-	0.77	0.96	-	-

- : inactive

The results showed that metabolite **1e** inhibited growth of *B. subtilis* ATCC 6633 with the MIC of 500 μg/ml. Metabolite **1e** had only slightly antimicrobial activity against *B. subtilis* ATCC 6633. On the other hand, Metabolite **1b** and metabolite **1d** did not show any antimicrobial activities.