

## CHAPTER V

### DISCUSSION

#### Crude hexane extract

The crude hexane extract of the stem barks of *Croton oblongifolius* Roxb. was separated by Column Chromatography using a Silica gel column and a hexane-ethyl acetate gradient system. Two Cembrane diterpenoids (**1-2**) and three Clerodane diterpenoids (**3-5**) were obtained.

#### 5. Structure elucidation of the isolated compounds from the stem barks of *Croton oblongifolius* Roxb.

##### 5.1 Structure elucidation of Compound **1**

Compound **1** was obtained by column chromatography using 10% ethyl acetate-hexane. The structure of this compound was elucidated by using FT-IR, NMR, Mass spectroscopic data and X-ray as follows.

The IR spectrum of Compound **1** is shown in Fig. 5 and the absorption peaks were assigned as summarized in Table 5. Its IR spectrum indicated important absorption bands at 2400-3500  $\text{cm}^{-1}$  (O-H stretching vibration of carboxylic acid), 2955, 2930 and 2868  $\text{cm}^{-1}$  (C-H stretching vibration), 1690  $\text{cm}^{-1}$  (C=O stretching vibration of carbonyl group), and 1644  $\text{cm}^{-1}$  (C=C stretching vibration of olefin).

**Table 5.** The IR absorption band assignment of Compound **1**.

Wave Number (cm <sup>-1</sup> )	Intensity	Tentative assignment
2400-3500	Broad	O-H stretching vibration of carboxylic acid
2955, 2930, 2868	Medium	C-H stretching vibration of CH <sub>3</sub> -, -CH <sub>2</sub> -
1690	Strong	C=O stretching vibration of carbonyl group
1644	Weak	C=C stretching vibration of olefin

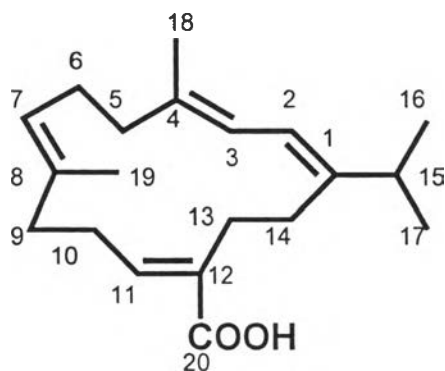
The <sup>1</sup>H-NMR spectrum (Fig. 6, Table 6) of Compound **1** showed the proton signals of an isopropyl group ( $\delta$  1.05 ppm), two vinylic methyl groups ( $\delta$  1.68 and 1.71 ppm) and four olefinic protons ( $\delta$  5.14, 5.91, 6.01 and 6.89 ppm).

The <sup>13</sup>C-NMR spectrum (Fig. 7, Table 7) showed 19 signals. Eight signals of olefinic carbons appeared at  $\delta$  146.5, 145.7, 135.6, 134.8, 132.1, 127.8, 120.0 and 118.6 ppm. The signals at  $\delta$  173.5 ppm should be the carboxyl group of carboxylic acid.

From DEPT-135 (Fig. 8) and DEPT-90 (Fig.9), Compound **1** possesses twenty carbon atoms and twenty nine protons. The EI mass spectrum (Fig. 10) showed the molecular ion peak at  $m/z$  302. If it is assumed that this compound contains only carbons, protons and oxygens, then the molecular formula of C<sub>20</sub>H<sub>30</sub>O<sub>2</sub> (calcd.  $m/z$  = 302.225; High-resolution mass spectroscopy) can be established. The molecular formula, C<sub>20</sub>H<sub>30</sub>O<sub>2</sub>, of this compound indicated six degrees of unsaturation; therefore, Compound **1** must consist of one ring in addition to the four double bonds and one carbonyl group. These data indicated that Compound **1** could be a Cembranoid possessing a 14-membered ring diterpene skeleton. X-ray data confirmed in table 8-11.

It could be concluded that Compound 1 exhibited the  $^{13}\text{C}$ -NMR chemical shifts similar to (1*E*, 3*E*, 7*E*, 11*E*)-1-isopropyl-4, 8-dimethyl-cyclotetradeca-1, 3, 7, 11-tetraene-12-carboxylic acid or Neocrotocembraneic acid. The spectroscopic and physical data of Compound 1 was identical to those reported for Neocrotocembraneic acid obtained from *C. oblongifolius* from Petchaboon province.<sup>8</sup>

Moreover, the single crystal x-ray diffraction analysis also confirmed that Compound 1 was Neocrotocembraneic acid as shown in Fig. 3.



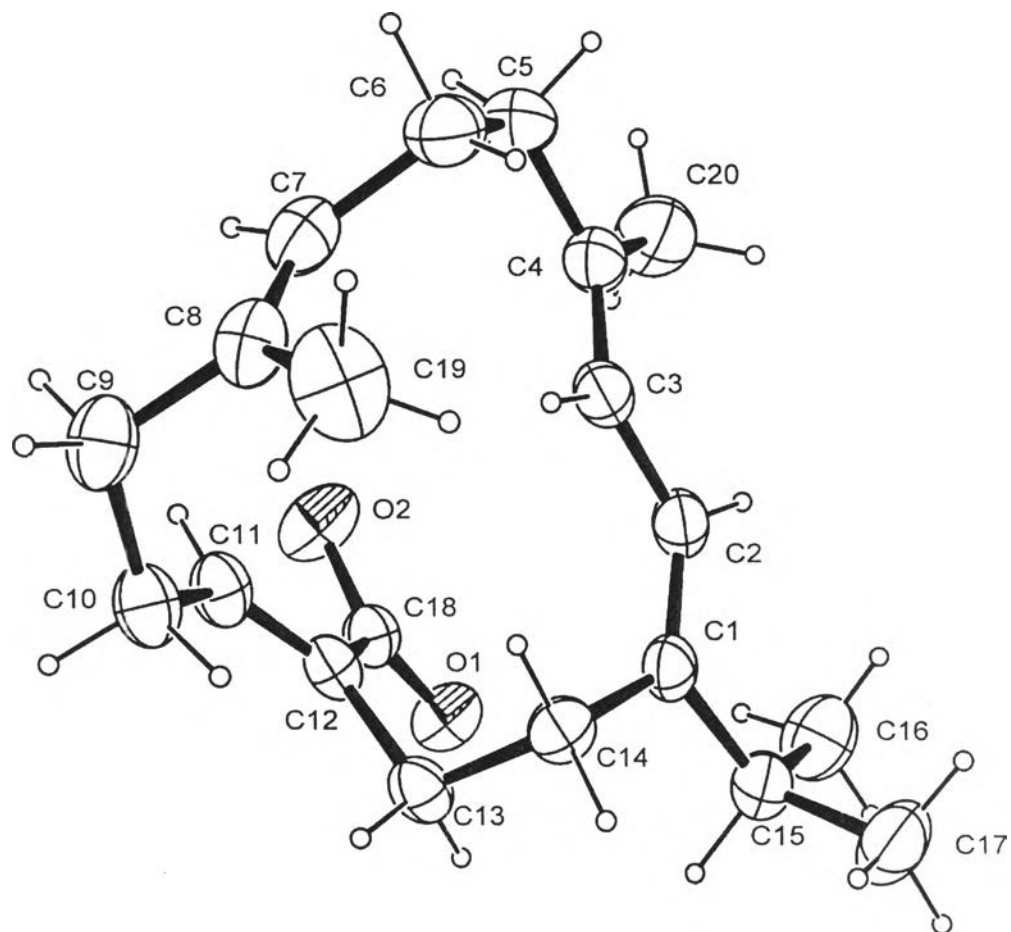
The  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR chemical shifts of Compound 1 and Neocrotocembraneic acid were compared as shown in Table 6 and 7, respectively.

Table 6. <sup>1</sup>H-NMR spectra of Compound 1 and Neocrotocembraneic acid

Position	Chemical shifts (ppm)	
	Compound <u>1</u>	Neocrotocembraneic acid
H-1	-	-
H-2	6.01 (1H,d, $J=11.0$ Hz)	6.01 (1H, d, $J=11.0$ Hz)
H-3	5.91 (1H, m)	5.91 (1H, dd, $J=11.0$ Hz)
H-4	-	-
H-5	2.17 (2H, m)	2.15 (2H, m)
H-6	2.24 (2H, m)	2.23 (2H, m)
H-7	5.14 (1H, m)	5.14 (1H,dt, $J=8.0,2.2$ Hz)
H-8	-	-
H-9	2.21 (2H, m)	2.20 (2H, m)
H-10	2.37 (2H, m)	2.38 (2H, m)
H-11	6.89 (1H,t, $J=8.0$ Hz)	6.89 (1H,t, $J=8.0$ Hz)
H-12	-	-
H-13	2.36 (2H, m)	2.36 (2H, m)
H-14	2.26 (2H, m)	2.26 (2H, m)
H-15	2.40 (1H, m)	2.39 (1H, m)
H-16	1.07 (3H, d, $J=6.79$ Hz)	1.05 (3H, d, $J=7.0$ Hz)
H-17	1.03 (3H, d, $J=6.79$ Hz)	1.05 (3H, d, $J=7.0$ Hz)
H-18	1.71 (3H, s)	1.71 (3H, s)
H-19	1.69 (3H, s)	1.68 (3H, s)
H-20	-	-

Table 7.  $^{13}\text{C}$ -NMR spectra of Compound 1 and Neocrotocembraneic acid

Position	Chemical shifts (ppm)	
	Compound <u>1</u>	Neocrotocembraneic acid
C-1	146.5s	146.5s
C-2	118.6d	118.6d
C-3	120.0d	120.0d
C-4	135.6s	135.6s
C-5	37.7t	37.7t
C-6	24.7t	24.7t
C-7	127.8d	127.8d
C-8	134.8s	134.8s
C-9	38.5t	38.5t
C-10	30.5t	30.5t
C-11	145.7d	145.7d
C-12	132.0s	132.1s
C-13	26.7t	26.7t
C-14	29.1t	29.1t
C-15	34.5d	34.6d
C-16	22.2q	22.1q
C-17	22.1q	22.1q
C-18	18.0q	18.0q
C-19	17.4q	17.4q
COOH	173.5s	173.5s



**Figure 3.** ORTEP structure of Compound 1.

**Table 8.** The Crystal data and structure refinement for Compound **1**.Empirical formula  $C_{20}H_{30}O_2$ 

Formula weight 3072.44

Temperature 293(2) K

Wavelength 0.71073 Å

Crystal system, space group Triclinic, P(-1)

Unit cell dimensions  $a = 7.6396(3)$  Å  $\alpha = 95.4180(10)$  deg. $b = 9.7372(5)$  Å  $\beta = 98.2060(10)$  deg. $c = 13.1450(6)$  Å  $\gamma = 98.925(2)$  deg.Volume 949.29(8) Å<sup>3</sup>Z, Calculated density 2, 1.058 Mg/m<sup>3</sup>Absorption coefficient 0.066 m<sup>-1</sup>

F(000) 332

Theta range for data collection 1.58 to 30.40 deg.

Limiting indices  $-10 \leq h \leq 9$ ,  $-13 \leq k \leq 13$ ,  $-18 \leq l \leq 17$ 

Reflections collected / unique 7057 / 5129 [R (int) = 0.0359]

Completeness to theta = 89.5 %

Refinement method Full-matrix least-squares on F<sup>2</sup>

Data / restraints / parameters 5129 / 0 / 272

Goodness-of-fit on F<sup>2</sup> 1.121

Final R indices [I &gt; 2 sigma(I)] R1 = 0.1149, wR2 = 0.3121

R indices (all data) R1 = 0.1867, wR2 = 0.3886

Absolute structure parameter 0.3(8)

Extinction coefficient 0.028(15)

Largest diff. Peak and hole 0.373 and -0.452 e.Å<sup>-3</sup>

**Table 9.** Atomic coordinates ( $\times 10^4$ ) and equivalent isotropic displacement parameters ( $\text{Å}^2 \times 10^3$ ) for Compound **1**.

U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
C(1)	6927(4)	3845(4)	7113(3)	58(1)
C(2)	7546(5)	3019(4)	7790(3)	63(1)
C(3)	6817(5)	1559(4)	7871(3)	63(1)
C(4)	7240(5)	873(5)	8683(3)	71(1)
C(5)	6509(7)	-645(6)	8715(4)	87(1)
C(6)	5271(7)	-1446(5)	7776(4)	87(1)
C(7)	3455(6)	-1006(4)	7603(4)	77(1)
C(8)	2455(6)	-848(4)	6719(31)	72(1)
C(9)	676(6)	-347(5)	6709(4)	82(1)
C(10)	821(5)	1220(5)	6628(3)	70(1)
C(11)	1872(5)	2107(4)	7576(3)	62(1)
C(12)	3057(4)	3277(4)	7631(2)	54(1)
C(13)	3600(5)	3903(4)	6698(3)	59(1)
C(14)	5235(5)	3400(4)	6339(3)	59(1)
C(15)	7885(5)	5314(4)	7072(3)	71(1)
C(16)	9142(7)	5995(5)	8057(4)	99(2)
C(17)	8917(7)	5360(6)	6148(4)	103(2)
C(18)	3878(4)	4002(3)	8662(3)	55(1)
C(19)	3033(28)	-1082(7)	5677(4)	110(2)
C(20)	8456(7)	1584(6)	9660(4)	102(2)
O(1)	4706(4)	5225(3)	8764(2)	72(1)
O(2)	3658(4)	3331(3)	9454(2)	86(1)



**Table 10.** Bond lengths [ $\text{\AA}^0$ ] for Compound **1**.

	Bond lengths [ $\text{\AA}^0$ ]		Bond lengths [ $\text{\AA}^0$ ]
C(1)-C(2)	1.340(5)	C(8)-C(9)	1.513(6)
C(1)-C(14)	1.504(5)	C(9)-C(10)	1.528(6)
C(1)-C(15)	1.512(5)	C(10)-C(11)	1.496(5)
C(2)-C(3)	1.464(5)	C(11)-C(12)	1.330(5)
C(3)-C(4)	1.339(5)	C(12)-C(18)	1.472(5)
C(4)-C(5)	1.504(6)	C(12)-C(13)	1.501(5)
C(4)-C(20)	1.512(6)	C(13)-C(14)	1.529(5)
C(5)-C(6)	1.511(7)	C(15)-C(16)	1.524(7)
C(6)-C(7)	1.508(7)	C(15)-C(17)	1.541(6)
C(7)-C(8)	1.332(6)	C(18)-O(1)	1.244(4)
C(8)-C(19)	1.506(6)	C(18)-O(2)	1.298(4)

**Table 11.** Bond angles [deg] for Compound **1**.

Angles	[ $\text{\AA}^0$ ]	Angles	[ $\text{\AA}^0$ ]
C(2)-C(1)-C(14)	123.2(3)	C(8)-C(9)-C(10)	112.7(3)
C(2)-C(1)-C(15)	122.2(3)	C(11)-C(10)-C(9)	112.9(4)
C(14)-C(1)-C(15)	114.5(3)	C(12)-C(11)-C(10)	128.2(4)
C(1)-C(2)-C(3)	127.8(4)	C(11)-C(12)-C(18)	118.3(3)
C(4)-C(3)-C(2)	125.1(4)	C(11)-C(12)-C(13)	123.5(3)
C(3)-C(4)-C(5)	123.2(4)	C(18)-C(12)-C(13)	118.2(3)
C(3)-C(4)-C(20)	122.3(4)	C(12)-C(13)-C(14)	113.9(3)
C(5)-C(4)-C(20)	114.5(4)	C(1)-C(14)-C(13)	113.2(3)
C(4)-C(5)-C(6)	118.4(4)	O(1)-C(15)-C(16)	115.6(3)
C(7)-C(6)-C(5)	113.6(4)	O(1)-C(15)-C(17)	110.7(4)
C(8)-C(7)-C(6)	129.2(4)	C(16)-C(15)-C(17)	109.4(4)
C(7)-C(8)-C(19)	123.3(4)	O(1)-C(18)-O(2)	121.7(3)
C(7)-C(8)-C(9)	121.3(4)	O(2)-C(18)-C(12)	120.8(3)
C(19)-C(8)-C(9)	115.4(4)	O(2)-C(18)-C(12)	117.4(3)

## 5.2 Structure elucidation of Compound **2**

Compound **2** was obtained by column chromatography using 10% ethyl acetate-hexane and recrystallization technique. The structure of Compound **2** was elucidated by FT-IR, NMR and Mass spectroscopic data as follows.

The IR spectrum of Compound **2** is shown in Fig. 11 and the absorption peaks were assigned as in Table 12. Its IR spectrum showed important absorption bands at 2400-3500  $\text{cm}^{-1}$  (O-H stretching vibration of carboxylic acid), 2960 and 2925  $\text{cm}^{-1}$  (C-H stretching vibration), 1685  $\text{cm}^{-1}$  (C=O stretching vibration of carbonyl group), and 1644  $\text{cm}^{-1}$  (C=C stretching vibration of olefin).

**Table 12.** The IR absorption band assignment of Compound **2**.

Wave Number ( $\text{cm}^{-1}$ )	Intensity	Tentative assignment
2400-3500	Broad	O-H stretching vibration of carboxylic acid
2960,2925	Medium	C-H stretching vibration of $\text{CH}_3$ -, $-\text{CH}_2$ -
1685	Strong	C=O stretching vibration of carbonyl group
1644	Weak	C=C stretching vibration of olefin

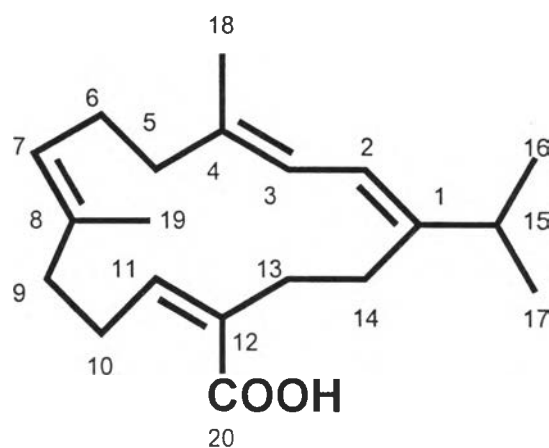
The  $^1\text{H}$ -NMR spectrum (Fig. 12, Table 13) of Compound **2** indicated that it possesses an isopropyl group ( $\delta$  1.04 ppm), two vinylic methyl groups ( $\delta$  1.54 and 1.73 ppm) and four olefinic proton ( $\delta$  5.10, 5.90, 6.01 and 6.03 ppm).

The  $^{13}\text{C}$ -NMR spectrum (Fig. 13, Table 14) showed 19 signals. Eight signals of olefinic carbons appeared at  $\delta$  146.9, 146.3, 135.2, 134.0, 130.9, 125.7, 121.6 and 118.7 ppm.

From DEPT-135 (Fig. 14) and DEPT-90 (Fig. 15), Compound **2** possesses twenty carbon atoms and twenty nine protons. The EI mass spectrum (Fig. 16) showed the molecular ion peak at  $m/z$  302. If it is assumed that this compound contains only carbons, protons and oxygens, then the molecular formula of  $\text{C}_{20}\text{H}_{30}\text{O}_2$  can be established. The molecular formula,  $\text{C}_{20}\text{H}_{30}\text{O}_2$ , of this compound indicated six

degrees of unsaturation; therefore, Compound 2 must consist of one ring in addition to the four double bonds and one carbonyl group. This data indicated that Compound 2 could be a Cembranoid possessing a 14-membered ring diterpene skeleton.

It could be concluded that Compound 2 exhibited the  $^{13}\text{C}$ -NMR chemical shifts similar to (1*E*, 3*E*, 7*E*, 11*Z*)-1-isopropyl-4, 8-dimethyl-cyclotetradeca-1, 3, 7, 11-tetraene-12-carboxylic acid or Crotoembraneic acid. The spectroscopic and physical data of Compound 2 was identical to those reported for Crotoembraneic acid obtained from *C. oblongifolius* from Petchaboon province.<sup>8</sup>



The  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR chemical shifts of Compound 2 and Crotoembraneic acid were compared as shown in Table 13 and 14, respectively.

Table 13.  $^1\text{H}$ -NMR spectra of Compound 2 and Crotoembraneic acid

Position	Chemical shifts (ppm)	
	Compound <u>2</u>	Crotoembraneic acid
H-1	-	-
H-2	6.01 (1H,d, m)	6.03 (1H, d, $J=11.0$ Hz)
H-3	5.89 (1H, dd, m)	5.90 (1H, dd, $J=11.0$ Hz)
H-4	-	-
H-5	2.15 (2H, m)	2.15 (2H, m)
H-6	2.18 (2H, m)	2.20 (2H, m)
H-7	5.08 (1H, dt, m)	5.10 (1H,dt, $J=6.4,1.2$ Hz)
H-8	-	-
H-9	2.15 (2H, m)	2.15 (2H, m)
H-10	2.69 (2H, m)	2.70 (2H, m)
H-11	5.98 (1H, m)	6.01 (1H,t, $J=6.5$ Hz)
H-12	-	-
H-13	2.39 (2H, m)	2.41 (2H, m)
H-14	2.39 (2H, m)	2.41 (2H, m)
H-15	2.31 (1H, m)	2.34 (1H, m)
H-16	1.03 (3H, d, $J=6.85$ Hz)	1.04 (3H, d, $J=6.7$ Hz)
H-17	1.00 (3H, d, $J=6.85$ Hz)	1.04 (3H, d, $J=6.7$ Hz)
H-18	1.73 (3H, s)	1.73 (3H, d, $J=0.9$ Hz)
H-19	1.54 (3H, s)	1.54 (3H, s)
H-20	-	-

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**Table 14.**  $^{13}\text{C}$ -NMR spectra of Compound **2** and Crotocebraneic acid

Position	Chemical shifts (ppm)	
	Compound <b>2</b>	Crotocebraneic acid
C-1	146.3s	146.9s
C-2	118.7d	118.7d
C-3	121.6d	121.6d
C-4	135.2s	135.2s
C-5	39.2t	39.2t
C-6	25.1t	25.1t
C-7	125.7d	125.7d
C-8	134.0s	134.0s
C-9	38.5t	38.6t
C-10	26.4t	26.4t
C-11	146.7d	146.3d
C-12	130.9s	130.9s
C-13	33.6t	33.6t
C-14	28.7t	28.7t
C-15	33.7d	33.8d
C-16	22.1q	22.1q
C-17	22.1q	22.1q
C-18	17.0q	17.0q
C-19	15.8q	15.8q
COOH	173.6s	174.1s

### 5.3 Structure elucidation of Compound **3**

Compound **3** was obtained by column chromatography using 20% ethyl acetate-hexane. The structure of Compound **3** was elucidated by FT-IR, NMR and Mass spectroscopic data as follows.

The IR spectrum of Compound **3** is shown in Fig. 17 and the absorption peaks were assigned as in Table 15. Its IR spectrum showed important absorption bands at 3500-2400  $\text{cm}^{-1}$  (O-H stretching vibration of alcohol), 2945  $\text{cm}^{-1}$  (C-H stretching vibration), 1054  $\text{cm}^{-1}$  (C-O stretching vibration).

**Table 15.** The IR absorption band assignment of Compound **3**

Wave Number ( $\text{cm}^{-1}$ )	Intensity	Tentative assignment
3500-2400	Broad	O-H stretching vibration of alcohol
2945	Medium	C-H stretching vibration of $\text{CH}_3$ -, $-\text{CH}_2$ -
1054	Weak	C-O stretching vibration

The  $^1\text{H}$ -NMR spectrum (Fig. 18, Table 16) of Compound **3** indicated that it possesses five methyl groups at 0.72, 0.81, 1.03, 1.58, and 1.64 ppm., two vinylic proton at 5.25 and 5.39 ppm., and one allylic proton at 4.13 ppm.

The  $^{13}\text{C}$ -NMR spectrum (Fig. 19, Table 17) showed 20 signals. Four signals of olefinic carbons appeared at  $\delta$  110.0, 125.6, 140.3 and 141.5 ppm.

DEPT 135 experiments (Fig. 20) showed eight methylene carbons at 17.7, 24.1, 28.8, 32.7, 33.1, 36.5, 37.8, and 59.4 ppm. and four methyl carbons at 15.9, 16.5, 17.3 and 19.8 ppm, which indicated that the carbon signals at 36.9, 40.1, 139.9 and 141.0 ppm. were quaternary.

DEPT 90 experiments (Fig. 21) indicated the presence of four sp carbons at 37.3, 44.6, 122.8 and 123.1 ppm.

The MS spectrum showed the fragmentation as follows,  $m/z$  (EIMS) (Fig. 22): 290 [ $\text{M}^+$ , 10 %], 257 (5), 189 (65), 121 (55), 109 (70), 107 (100), 95 (90) and 93 (40).

The molecular formula,  $C_{20}H_{34}O$ , of this compound indicated four degrees of unsaturation; therefore, Compound **3** must consist of two ring and two double bonds. This data indicated that Compound **3** could be a Clerodane diterpene skeleton.

It could be concluded that Compound **3** exhibited the  $^{13}C$ -NMR chemical shifts similar to *ent*-3, 13-Clerodadien-15-ol or Kolavenol. The  $^1H$ -NMR and  $^{13}C$ -NMR data of Compound **3** were compared to those reported for Kolavenol obtained from *Hardwickia pinnata* and roots of *Solidago elongata*<sup>29</sup> as shown in Table 16 and 17, respectively.

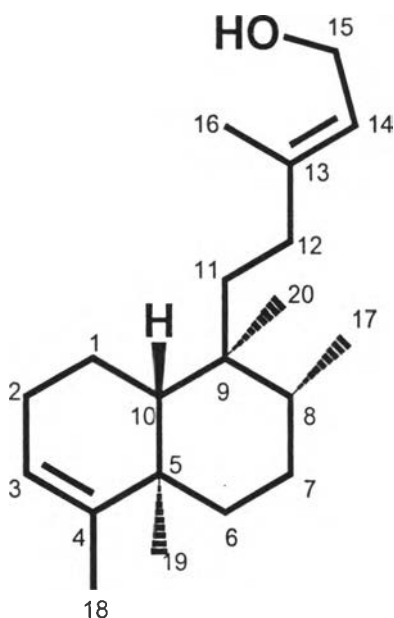


Table 16.  $^1\text{H}$ -NMR spectra of Compound 3 and Kolavenol

Position	Chemical shifts (ppm)	
	Compound <u>3</u>	Kolavenol
H-1	-	-
H-2	-	-
H-3	5.25 (1H, br s)	5.19 (1H, br s)
H-4	-	-
H-5	-	-
H-6	-	-
H-7	-	-
H-8	-	-
H-9	-	-
H-10	-	-
H-11	-	-
H-12	-	-
H-13	-	-
H-14	5.39 (2H, t, $J=6.76$ Hz)	5.39 (2H, t, $J=6.8$ Hz)
H-15	4.13 (1H, d, $J=6.74$ Hz)	4.13 (1H, d, $J=6.8$ Hz)
H-16	1.66 (1H, s)	1.68 (1H, s)
H-17	0.78 (1H, d, $J=6.1$ Hz)	0.81 (1H, d, $J=6.0$ Hz)
H-18	1.52 (1H, d, $J=1.5$ Hz)	1.58 (1H, d, $J=1.6$ Hz)
H-19	1.03 (1H, s)	0.99 (1H, s)
H-20	0.72 (1H, s)	0.72 (1H, s)



Table 17.  $^{13}\text{C}$ -NMR spectra of Compound 3 and Kolavenol

Position	Chemical shifts (ppm)	
	Compound <u>3</u>	Kolavenol
C-1	17.7t	18.2t
C-2	28.8t	26.8t
C-3	122.8d	120.4d
C-4	141.0s	144.4s
C-5	36.9s	38.1s
C-6	37.8t	36.7t
C-7	32.7t	27.5t
C-8	37.3d	36.2d
C-9	40.1s	38.6s
C-10	44.6d	46.4d
C-11	36.5t	36.8t
C-12	24.1t	32.8t
C-13	139.9s	140.9s
C-14	123.1d	122.8d
C-15	59.4t	59.4t
C-16	33.1t	16.5t
C-17	15.9q	15.9q
C-18	16.5q	17.9q
C-19	17.3q	19.9q
C-20	19.8q	18.3q

## 5.4 Structure elucidation of Compound 4

### Characterization of Compound 4

Compound 4 was obtained by column chromatography using 30% ethyl acetate-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 (Fig. 23) showed the presence of a carboxylic group with corresponding to the broad absorption band between 3500 to 2600  $\text{cm}^{-1}$  and the strong absorption band at 1697  $\text{cm}^{-1}$  due to the carboxylic acid carbonyl stretching. The IR spectral data of Compound 4 are summarized in Table 18.

**Table 18.** The IR absorption bands assignment of Compound 4.

Wave Number ( $\text{cm}^{-1}$ )	Intensity	Tentative assignment
3400-2600	Broad	O-H stretching vibration of carboxylic acid
2960, 2865	Strong	C-H stretching vibration of $\text{CH}_3$ -, $-\text{CH}_2$ -
1672	Strong	C=O stretching vibration of carbonyl group
1629, 1410	Weak	C=C stretching vibration of olefin
1250	Strong	C-O stretching vibration

The  $^1\text{H}$ -NMR spectrum (Fig. 24, Table 19) of Compound 4 possessed three methyl groups at 0.75(3H), 0.81(3H) and 1.24(3H) ppm, three olefinic protons of furanoid groups at 7.35, 7.20 and one vinylic proton at 6.87 ppm.

The  $^{13}\text{C}$ -NMR spectrum (Fig. 25, Table 20) showed 20 lines, which the carbonyl group of carboxylic acid corresponded to the signal at 172.7 ppm.

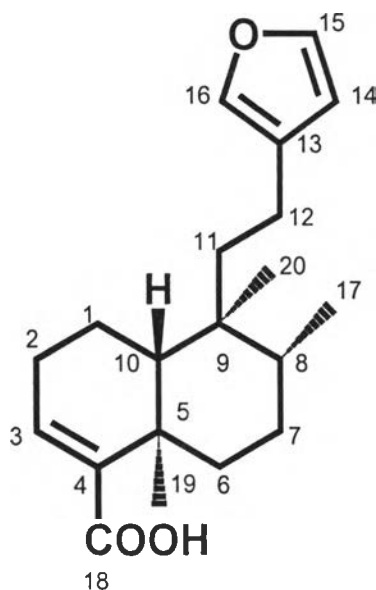
DEPT 135 experiments (Fig. 26) showed six methylene carbons at 17.5, 18.2, 27.3, 27.5, 35.8 and 38.6 ppm. and three methyl carbons at 15.9, 18.3 and 20.5 ppm, which indicated that the carbon signals at 37.6, 38.8, 125.5, 141.5 and 172.7 ppm were quaternary.

DEPT 90 experiments (Fig. 27) indicated the presence of four sp carbons at 111.0, 138.4, 140.3 and 142.7 ppm.

The MS spectrum showed the fragmentation as follows,  $m/z$  (EIMS) (Fig. 28): 316[ $M^+$ ], 299(10), 283(6), 221(18), 203(23), 137(22), 125(100), 96(53), 95(40), 81(55).

Compound **4** showed a molecular ion with  $m/z = 316$  ( $C_{20}H_{28}O_3$ ), which indicated a DBE of 7. Compound **4** must consist of one carbonyl group of carboxylic acid, one ring of furan (DBE=3) in addition to the one double bonds.

It could be concluded that Compound **4** exhibited the  $^1H$ -NMR and  $^{13}C$ -NMR chemical shifts similar to 5-*epi*-Hardwickiic acid or Hardwickiic acid. The spectroscopic and physical data of Compound **4** was identical to those reported for Hardwickiic acid obtained from *Hardwickia pinnata* and roots of *Solidago rugosa*.<sup>43</sup>



The  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR chemical shifts of Compound **4** and Hardwickiic acid were compared as shown in Table 19 and 20, respectively.

**Table 19.**  $^1\text{H}$ -NMR spectra of Compound **4** and Hardwickiic acid

Position	Chemical shifts (ppm)	
	Compound <b>4</b>	Hardwickiic acid
H-1	2.04m	2.04m
H-2	2.33m	2.33m
H-3	6.86s	6.86s
H-4	-	-
H-5	-	-
H-6	2.43m	2.43m
H-7	1.28m	1.28m
H-8	1.55m	1.55m
H-9	-	-
H-10	1.49m	1.49m
H-11	1.72m	1.72m
H-12	2.30m	2.30m
H-13	-	-
H-14	7.17s	7.17s
H-15	7.33, t, $J=1.5$ Hz	7.33, t, $J=1.5$ Hz
H-16	6.24s	6.24s
H-17	0.81, d, $J=7$ Hz	0.81, d, $J=7$ Hz
H-18	-	-
H-19	1.27s	1.27s
H-20	0.80s	0.80s

Table 20.  $^{13}\text{C}$ -NMR spectra of Compound 4 and Hardwickiic acid

Position	Chemical shifts (ppm)	
	Compound <u>4</u>	Hardwickiic acid
C-1	35.8t	35.8t
C-2	18.2t	18.2t
C-3	140.3d	140.3d
C-4	141.5s	141.5s
C-5	37.6s	37.6s
C-6	38.7t	38.7t
C-7	27.3t	27.3t
C-8	36.3d	36.3d
C-9	38.8s	38.8s
C-10	46.7s	46.7s
C-11	17.5t	17.5t
C-12	27.5t	27.5t
C-13	125.6s	125.6s
C-14	110.0d	110.0d
C-15	142.7d	142.7d
C-16	138.4d	138.4d
C-17	15.9q	15.9q
C-18	172.5s	172.5s
C-19	20.5q	20.5q
C-20	18.3q	18.3q

## 5.5 Structure elucidation of Compound 5

### Characterization of Compound 5

Compound 5 was obtained by column chromatography using 45% ethyl acetate-hexane and recrystallization technique. The structure of Compound 5 was elucidated by FT-IR, NMR and Mass spectroscopic data as follows.

The IR spectrum of Compound 5 (Fig. 29) showed the presence of a ketone group with corresponding to the strong absorption band at  $1784\text{ cm}^{-1}$  and the absorption band at  $1650\text{ cm}^{-1}$  of the C=C stretching vibration of olefin. The IR spectral data of Compound 5 are summarized in Table 21.

**Table 21.** The IR absorption bands assignment of Compound 5.

Wave Number ( $\text{cm}^{-1}$ )	Intensity	Tentative assignment
3146	Medium	=C-H stretching vibration
2960, 2865	Strong	C-H stretching vibration of $\text{CH}_3$ -, $-\text{CH}_2$ -
1784	Strong	C=O stretching vibration of carbonyl group
1650	Weak	C=C stretching vibration of olefin
1250	Strong	C-O stretching vibration

The  $^1\text{H}$ -NMR spectrum (Fig. 30-34, table 22) of Compound 5 possessed methyl group at 0.82(3H) ppm, methyl group of ester at 3.6 ppm, and three olefinic protons of furanoid groups at 8.01, 7.42 and one vinylic proton at 6.73 ppm.

The  $^{13}\text{C}$ -NMR spectrum (Fig. 35, Table 23) showed 21 lines, which the carbonyl group of ester corresponded to the signal at 193.6 ppm.

DEPT 135 experiments (Fig. 36) showed six methylene carbons at 20.1, 22.1, 27.3, 33.2, 46.5 and 71.4 ppm. and two methyl carbons at 19.2, and 51.4 ppm, which indicated that the carbon signals at 39.6, 45.1, 128.6, 137.8, 169.0, 174.0 and 193.6 ppm were quaternary.

DEPT 90 experiments (Fig. 37) indicated the presence of six sp carbons at 46.7, 48.7, 108.5, 136.2, 144.3, and 147.1 ppm.

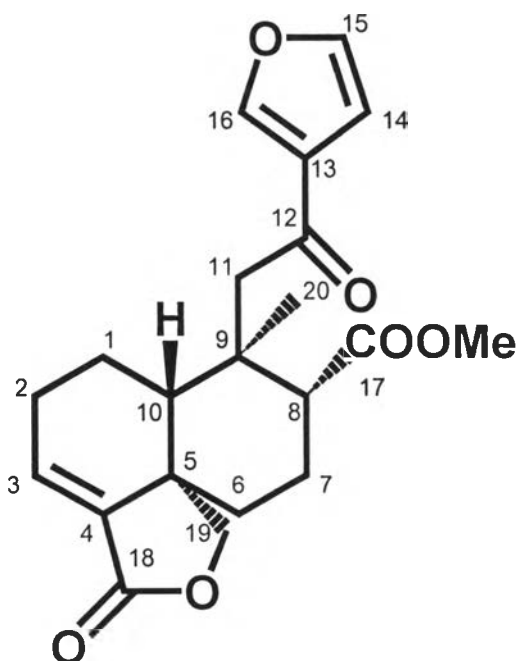
COSY-NMR spectrum (Fig. 38-39), NOESY-NMR spectrum (Fig. 40-41), HMQC-NMR spectrum (Fig. 42-44) and HMBC-NMR spectrum (Fig.45-49).

The MS spectrum showed the fragmentation as follows,  $m/z$  (EIMS) (Fig. 50): 372[ $M^+$ ], 341(15), 263(38), 245(53), 145(78), 110(60), 95(100), and 91(22).

Compound **5** showed a molecular ion with  $m/z = 372$  ( $C_{21}H_{24}O_6$ ), which indicated a DBE of 10. Compound **5** should be consisted of two carbonyl group of ester, one ring of furan (DBE=3), one carbonyl group of ketone, in addition to one double bond and three rings. X-ray data confirmed in table 24-27.

It could be concluded that Compound **5** exhibited the  $^1H$ -NMR and  $^{13}C$ -NMR chemical shifts similar to those of the neo-clerodane-type diterpenoids, methyl-15, 16-epoxy-12-oxo-3, 13(16), 14-neo-clerodatrien-18, 19-olide-17-carboxylate or Nasimalun A, previously isolated from *Barringtonia racemosa* Blume.<sup>30</sup>

Moreover, the single crystal x-ray diffraction analysis also confirmed that Compound **5** was Nasimalun A as shown in Fig. 4.



The  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR chemical shifts of Compound **5** and Nasimalun A were compared as shown in Table 22 and 23, respectively.

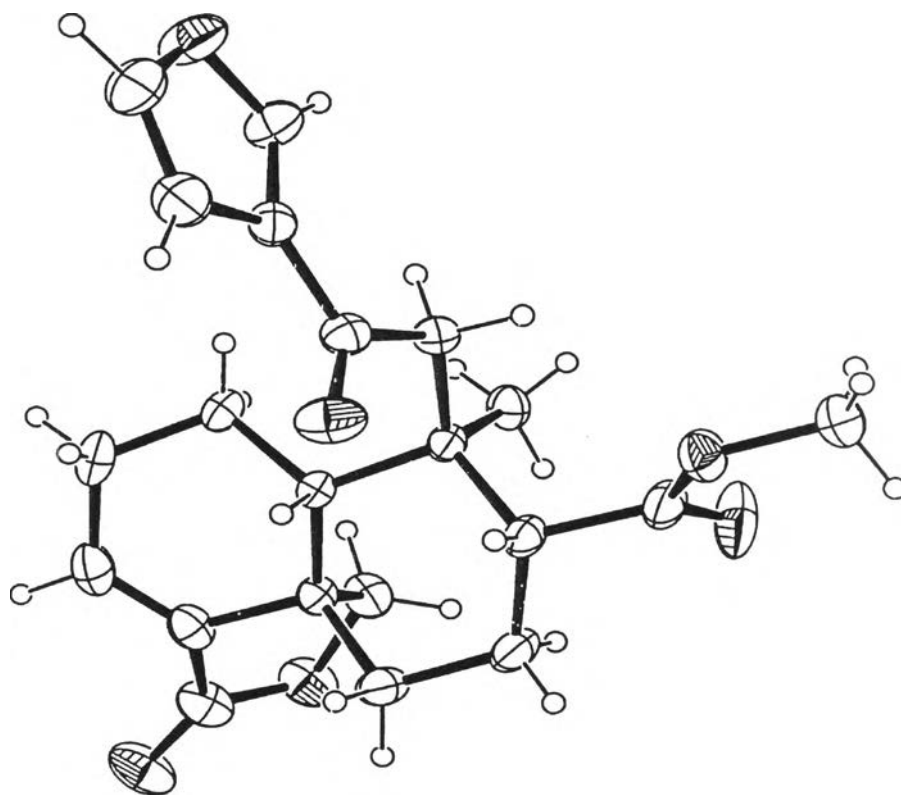
**Table 22.**  $^1\text{H}$ -NMR spectra of Compound **5** and Nasimalun A

Position	Chemical shifts (ppm)	
	Compound <b>5</b>	Nasimalun A
H-1	1.64 (1H, d, $J=11.0$ Hz)	1.64 (1H, d, $J=11.0$ Hz)
H-2	2.28m	2.28m
H-3	6.74 (1H, d, $J=8.0$ Hz)	6.74 (1H, d, $J=8.0$ Hz)
H-4	-	-
H-5	-	-
H-6	1.98 (1H, d, $J=13.4$ Hz)	1.98 (1H, d, $J=13.5$ Hz)
H-7	2.03 (1H, d, $J=11.5$ Hz)	2.03 (1H, d, $J=13.5$ Hz)
H-8	3.21 (1H, d, $J=13.1$ Hz)	3.21 (1H, d, $J=13.5$ Hz)
H-9	-	-
H-10	2.73 (1H, d, $J=10.9$ Hz)	2.73 (1H, d, $J=11.0$ Hz)
H-11	3.04 (1H, d, $J=18.0$ Hz)	3.04 (1H, d, $J=18.0$ Hz)
H-12	-	-
H-13	-	-
H-14	6.73 (1H, d, $J=2.0$ Hz)	6.73 (1H, d, $J=2.0$ Hz)
H-15	7.42 (1H, d, $J=1.9$ Hz)	7.42 (1H, d, $J=2.0$ Hz)
H-16	8.01s	8.01s
H-17	-	-
H-18	-	-
H-19	4.33 (1H, d, $J=7.9$ Hz)	4.33 (1H, d, $J=8.0$ Hz)
H-20	0.82s	0.82s
COOMe	3.6s	3.6s



Table 23.  $^{13}\text{C}$ -NMR spectra of Compound 5 and Nasimalun A

Position	Chemical shifts (ppm)	
	Compound <u>5</u>	Nasimalun A
C-1	20.1t	20.1t
C-2	27.3t	27.3t
C-3	136.2d	136.2d
C-4	137.8s	137.8s
C-5	45.1s	45.1s
C-6	33.2t	33.2t
C-7	22.1t	22.1t
C-8	48.7d	48.7d
C-9	39.6s	39.6s
C-10	46.7d	46.7d
C-11	46.5t	46.5t
C-12	193.6s	193.6s
C-13	128.6s	128.6s
C-14	108.5d	108.5d
C-15	144.3d	144.3d
C-16	147.1d	147.1d
C-17	174.0s	174.0s
C-18	169.0s	169.0s
C-19	71.4t	71.4t
C-20	19.2q	19.2q
COOMe	51.4q	51.4q



**Figure 4.** ORTEP structure of Compound **5**.

**Table 24.** The Crystal data and structure refinement for Compound **5**.Empirical formula  $C_{21}H_{24}O_6$ 

Formula weight 372.40

Temperature 293(2) K

Wavelength  $0.71073 \text{ \AA}^0$ 

Crystal system, space group Orthorhombic, P2(1)2(1)2(1)

Unit cell dimensions  $a = 10.10890(10) \text{ \AA}^0$   $\alpha = 90 \text{ deg.}$  $b = 10.14330(10) \text{ \AA}^0$   $\beta = 90 \text{ deg.}$  $c = 18.2541(2) \text{ \AA}^0$   $\gamma = 90 \text{ deg.}$ Volume  $1871.73(3) \text{ \AA}^{03}$ Z, Calculated density 4,  $1.322 \text{ Mg/m}^3$ Absorption coefficient  $0.096 \text{ m}^{-1}$ 

F(000) 792

Theta range for data collection 2.23 to 30.46 deg.

Limiting indices  $-14 \leq h \leq 14$ ,  $-14 \leq k \leq 11$ ,  $-25 \leq l \leq 20$ 

Reflections collected / unique 13816 / 5403 [R(int) = 0.0181]

Completeness to theta = 30.46 97.3 %

Refinement method Full-matrix least-squares on  $F^2$ 

Data / restraints / parameters 5403 / 0 / 341

Goodness-of-fit on  $F^2$  0.873Final R indices [ $I > 2 \sigma(I)$ ]  $R1 = 0.0364$ ,  $wR2 = 0.1126$ R indices (all data)  $R1 = 0.0425$ ,  $wR2 = 0.1203$ 

Absolute structure parameter 0.3(8)

Extinction coefficient 0.022(4)

Largest diff. Peak and hole 0.208 and  $-0.120 \text{ e.\AA}^{-3}$

**Table 25.** Atomic coordinates ( $\times 10^4$ ) and equivalent isotropic displacement parameters ( $\text{Å}^2 \times 10^3$ ) for Compound 5.

U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
C(1)	41(1)	-205(1)	2578(1)	44(1)
C(2)	-1185(1)	537(2)	2845(1)	63(1)
C(3)	-1003(1)	1008(2)	3617(1)	59(1)
C(4)	188(1)	1157(1)	3906(1)	46(1)
C(5)	1492(1)	915(1)	3526(1)	37(1)
C(6)	2433(1)	2110(1)	3585(1)	48(1)
C(7)	3734(1)	1813(2)	3200(1)	54(1)
C(8)	3565(1)	1422(1)	2389(1)	42(1)
C(9)	2529(1)	284(1)	2265(1)	35(1)
C(10)	1267(1)	663(1)	2700(1)	33(1)
C(11)	1294(1)	151(1)	1438(1)	41(1)
C(12)	1341(1)	1207(1)	1082(1)	42(1)
C(13)	393(1)	799(1)	509(1)	42(1)
C(14)	-424(2)	1668(2)	79(1)	58(1)
C(15)	-1111(2)	912(2)	-377(1)	69(1)
C(16)	127(2)	-433(2)	256(1)	56(1)
C(17)	4915(1)	1031(2)	2093(1)	53(1)
C(18)	452(2)	1008(2)	4702(1)	58(1)
C(19)	1987(2)	-200(2)	4036(1)	50(1)
C(20)	3078(1)	-1068(1)	2502(1)	47(1)
C(21)	6392(2)	1128(3)	1095(1)	69(1)
O(1)	1514(1)	192(1)	4767(1)	65(1)
O(2)	-124(2)	1428(2)	5233(1)	83(1)
O(3)	5129(1)	1468(1)	1417(1)	58(1)
O(4)	1423(2)	2376(1)	1240(1)	62(1)
O(5)	-796(2)	-394(1)	-284(1)	73(1)
O(6)	5721(1)	395(2)	2424(1)	93(1)

**Table 26.** Bond lengths [ $\text{\AA}^0$ ] for Compound 5.

	<b>Bond lengths [<math>\text{\AA}^0</math>]</b>		<b>Bond lengths [<math>\text{\AA}^0</math>]</b>
C(1)-C(2)	1.530(2)	C(9)-C(11)	1.5527(15)
C(1)-C(10)	1.5359(15)	C(11)-C(12)	1.5409(17)
C(2)-C(3)	1.500(3)	C(12)-O(4)	1.2228(18)
C(3)-C(4)	1.322(2)	C(12)-C(13)	1.4782(17)
C(4)-C(18)	1.484(2)	C(13)-C(16)	1.359(2)
C(4)-C(5)	1.5102(16)	C(13)-C(14)	1.440(2)
C(5)-C(10)	1.5452(14)	C(14)-C(15)	1.329(3)
C(5)-C(6)	1.5445(17)	C(15)-O(5)	1.373(3)
C(5)-C(19)	1.5485(17)	C(16)-O(5)	1.3582(17)
C(6)-C(7)	1.521(2)	C(17)-O(6)	1.203(2)
C(7)-C(8)	1.5420(17)	C(17)-O(3)	1.3285(18)
C(8)-C(17)	1.5203(17)	C(18)-O(2)	1.2083(19)
C(8)-C(9)	1.5750(16)	C(18)-O(1)	1.361(2)
C(9)-C(20)	1.509(17)	C(19)-O(1)	1.4709(17)
C(9)-C(10)	1.5516(14)	C(21)-O(3)	1.4468(19)

Table 27. Bond angles [deg] for Compound 5.

Angles	[A <sup>o</sup> ]	Angles	[A <sup>o</sup> ]
C((2)-C(1)-(C10)	108.97(11)	C(1)-C(10)-C(5)	110.85(9)
C(3)-C(2)-C(1)	110.93(12)	C(1)-C(10)-C(9)	116.56(9)
C(4)-C(3)-C(1)	121.48(13)	C(5)-C(10)-C(9)	114.76(8)
C(3)-C(4)-C(18)	122.81(13)	C(12)-C(11)-C(9)	118.59(10)
C(3)-C(4)-C(5)	126.38(12)	O(4)-C(12)-C(13)	118.86(12)
C(18)-C(4)-C(5)	106.02(12)	O(4)-C(12)-C(11)	122.91(12)
C(4)-C(5)-C(10)	110.25(9)	C(13)-C(12)-C(11)	118.22(11)
C(4)-C(5)-C(6)	112.22(10)	C(16)-C(13)-C(14)	105.36(13)
C(10)-C(5)-C(6)	106.83(8)	C(16)-C(13)-C(12)	128.77(12)
C(4)-C(5)-C(19)	97.13(10)	C(14)-C(13)-C(12)	125.85(13)
C(10)-C(5)-C(19)	120.91(10)	C(15)-C(14)-C(13)	106.74(16)
C(6)-C(5)-C(19)	109.35(11)	C(14)-C(15)-O(5)	110.95(14)
C(7)-C(6)-C(5)	110.15(11)	O(5)-C(16)-C(13)	110.80(14)
C(6)-C(7)-C(8)	113.52(11)	O(6)-C(17)-O(3)	122.35(14)
C(17)-C(8)-C(7)	108.06(10)	O(6)-C(17)-C(8)	124.67(13)
C(17)-C(8)-C(9)	110.76(11)	O(3)-C(17)-C(8)	112.98(12)
C(7)-C(8)-C(9)	113.57(9)	O(2)-C(18)-O(1)	121.59(16)
C(20)-C(9)-C(10)	111.89(9)	O(2)-C(18)-C(4)	131.51(18)
C(20)-C(9)-C(11)	105.90(10)	O(1)-C(18)-C(4)	106.83(12)
C(10)-(9)-C(11)	109.84(8)	O(1)-C(19)-C(5)	104.11(11)
C(20)-C(9)-C(8)	111.86(10)	C(18)-O(1)-C(19)	109.93(11)
C(10)-C(9)-C(8)	106.99(9)	C(17)-O(3)-C(21)	116.22(15)
C(11)-C(9)-C(8)	110.40(9)	C(16)-O(5)-C(15)	106.15(13)