CHAPTER IV

RESULTS AND DISCUSSION

The dried aerial part of *Andrographis paniculata* Nees. were extracted with 95 % Ethanol. The extract was partitioned between water and chloroform fraction was separated between hexane and chloroform. The chloroform extract was then separated by chromatographic technique to afford two pure compounds, namely Andrographolide (4)and 14-deoxy-11,12-didehydroandrographolide(6). The structural characterizations of these compounds were based on data from the IR, NMR and Mass spectra. The structures of these compounds were further confirmed by comparison of their physical properties with the data reported in the literature. The large amount of Andrographolide (4) obtained in this study provided a good oppotunity to study the chemistry and spectral properties of this group of compounds. Hence, Several related diterpene lactone compounds were prepared using andrographolide as the starting material.

1. Structure characterization of isolated compounds.

1.1 Identification of compound 4

Compound 4 was obtained as colorless plates from fraction C-3 (35.50 g, 0.35 %)in Table 2. The GCMS of compound 4 (Figure 6) revealed its molecular ion peak at *m/z* 350. The mass fragmentation pattern was shown in scheme 4. The IR spectrum (Figure 7) indicated :

3450-3000 CM^{-1} (O-H stretch)

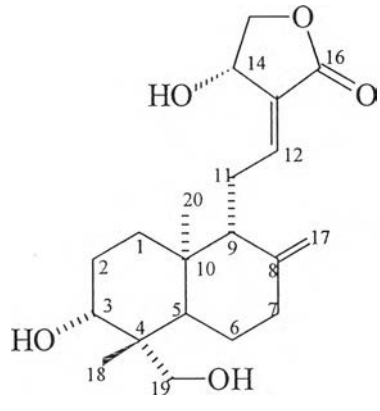
1735 CM^{-1} (C=O stretch of α, β-unsaturated-γ-lactone)

1680 CM^{-1} (C=C stretch of conjugated C=C)

1650 CM^{-1} (C=C stretch of exocyclic methylene)

970 CM^{-1} (C-H out of plane stretch of exocyclic methylene)

The assignments of this compound have been reported by Takakuni Matsuda et al ; 1994. The ^{1}H and ^{13}C assignments are summarized in Table 3.

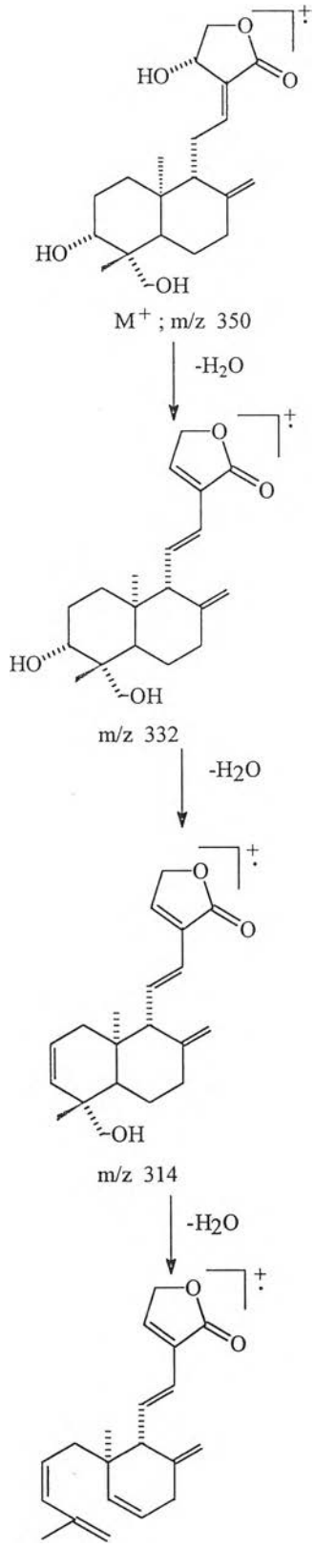


Compound 4

Table 3. The ^{1}H and ^{13}C NMR spectral data of compound 4 (in DMSO-d_6)

Position	^{1}H		^{13}C
	δ (ppm)	Multiplicity,J(H_z)	δ (ppm)
1	1.21	m	36.5
2	1.70	m	27.8
3-CH	3.24 [a)]	m	78.4
4	-	-	42.2
5	1.21	m	54.4
6	1.70	m	23.9
7	2.33	m	37.5
8	-	-	147.5
9	1.94	td (4.58,4.88,4.88)	55.5
10	-	-	38.5
11	4.39	dd (6.10,5.80)	23.9
12	6.63	td (1.52,1.53,1.83)	146.2
13	-	-	128.9
14	5.05	d (4.88)	64.5
15	4.13,3.85	dd (3.05,2.75),(30.5,2.74)	74.3
16	-	-	169.9
17	4.82,4.63	(brs), (brs)	108.2
18-CH_3	1.09	s	23.0
19	3.24 [a)]	m	62.6
20 CH_3	0.66	s	14.7
3-OH	2.48	m	-
14-OH	5.70	d (6.1)	-
19-OH	4.91	brt	-

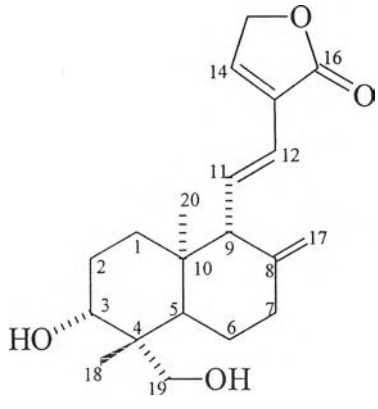
a) obscured by overlapping with other signals.



Scheme 4. Mass fragmentation of compound 4

1.2 Identification of compound 6

Compound 6 was obtained as colorless needles, from fraction C-2(24.0 g ,0.24 %) in Table 2.



Compound 6

The EIMS of compound 6 (Figure 13) revealed its molecular ion peak at *m/z* 332. The mass fragmentation pattern was shown in scheme 5. The IR spectrum (Figure 14) indicated :

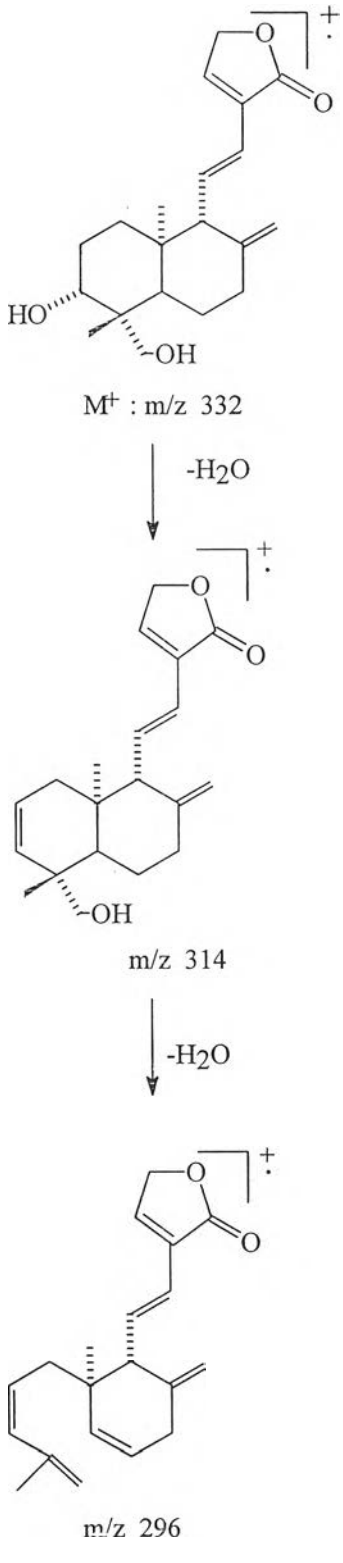
3000	CM^{-1}	(O-H stretch)
1754	CM^{-1}	(C=O stretch of α, β-unsaturated-γ-lactone)
1640	CM^{-1}	(C=C stretch of exocyclic methylene)
900	CM^{-1}	(C-H out of plane stretch of exocyclic methylene)

This compound was assigned as a known 14-deoxy-11,12-didehydroandrographolide(6). The ^{1}H and ^{13}C assignments of this compound have been reported by Takakuni, Matsuda. et al ; 1994. The ^{1}H and ^{13}C assignments are summarized in Table 4.

Table 4. The ^{1}H and ^{13}C NMR spectral data of compound 6 (in $CDCl_3$)

Position	^{1}H		^{13}C
	δ(ppm)	Multiplicity,*J*(Hz)	δ(ppm)
1	1.50	d	38.6
2	1.70	m	28.2
3-CH	3.48 [a)]	brt	80.8
4	-	-	43.0
5	1.15	m	54.7
6	1.70	m	23.1
7	2.47	m	36.7
8	-	-	147.9
9	2.29	m	61.7
10	-	-	38.3
11	6.85	q	135.8
12	6.18	d	121.0
13	-	-	129.1
14	7.20	br t	142.7
15	4.74	br s	69.6
16	-	-	172.0
17	4.74,4.50	d	109.1
18-CH_3	1.22	s	22.8
19	4.18	d	64.2
20 CH_3	0.82	s	16.0
3-OH	2.95	br	-
19-OH	3.28	d	-

a) obscured by overlapping with other signals.



Scheme 5. Mass fragmentation of compound 6

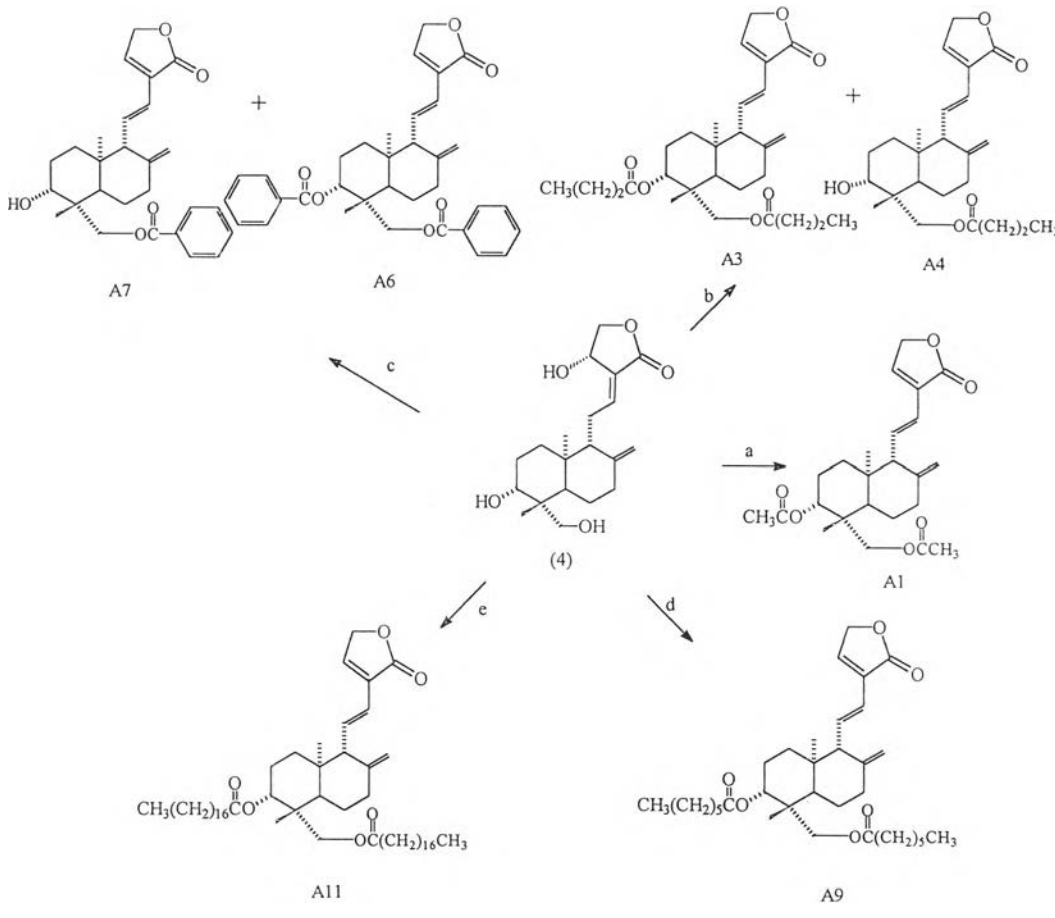
2. Esterification of Andrographolide (4)

Seven related diterpene lactone synthesized compounds were prepared, using andrographolide(4) as the starting material (Scheme 6). The ^{1}H and ^{13}C NMR properties of these synthesized products were extensively studied, using1-D and 2-D NMR techniques.

2.1 Acetylation of Andrographolide (4)

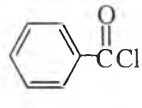
Acetylation of andrographolide (4) was performed with acetic anhydride in pyridine to give compound A1&A2. The mechanism of this reaction is shown in scheme 7. Acetic anhydride was a reactive acylating reagent because of combination of the inductive effect of oxygen substituent on the reactivity of the carbonyl group and the ease with which the tetrahedral intermediate could expel such relatively good leaving group. Acylation of this compound was performed in the presence of an organic base, pyridine. This base served two purpose. It neutralized the protons generated in the reaction and prevented the developement of high acid condition. Pyridine also became directly involved in the reaction as a nucleophile catalyst. It was more nucleophilic than the hydroxy of andrographolide, toward the carbonyl center of acetic anhydride. The product that resulted, an acylpyridinium ion, was in turn more reactive toward the hydroxy group than than the original acetic anhydride (Carey, and Sunberg.1993).

Both compound A1(0.22 g,18.6 %)&A2(0.32 g,27.4%)were obtained as yellow solid from the acetylation of andrographolide in pyridine and dichloromethane respectively. But by preparing at room temperature, the higher yield of A2 was gave. these compounds were 14-deoxy-11,12-didehydroandrographolide diacetate.



a) $CH_3C(=O)OC(=O)CH_3$, Pyridine , Reflux

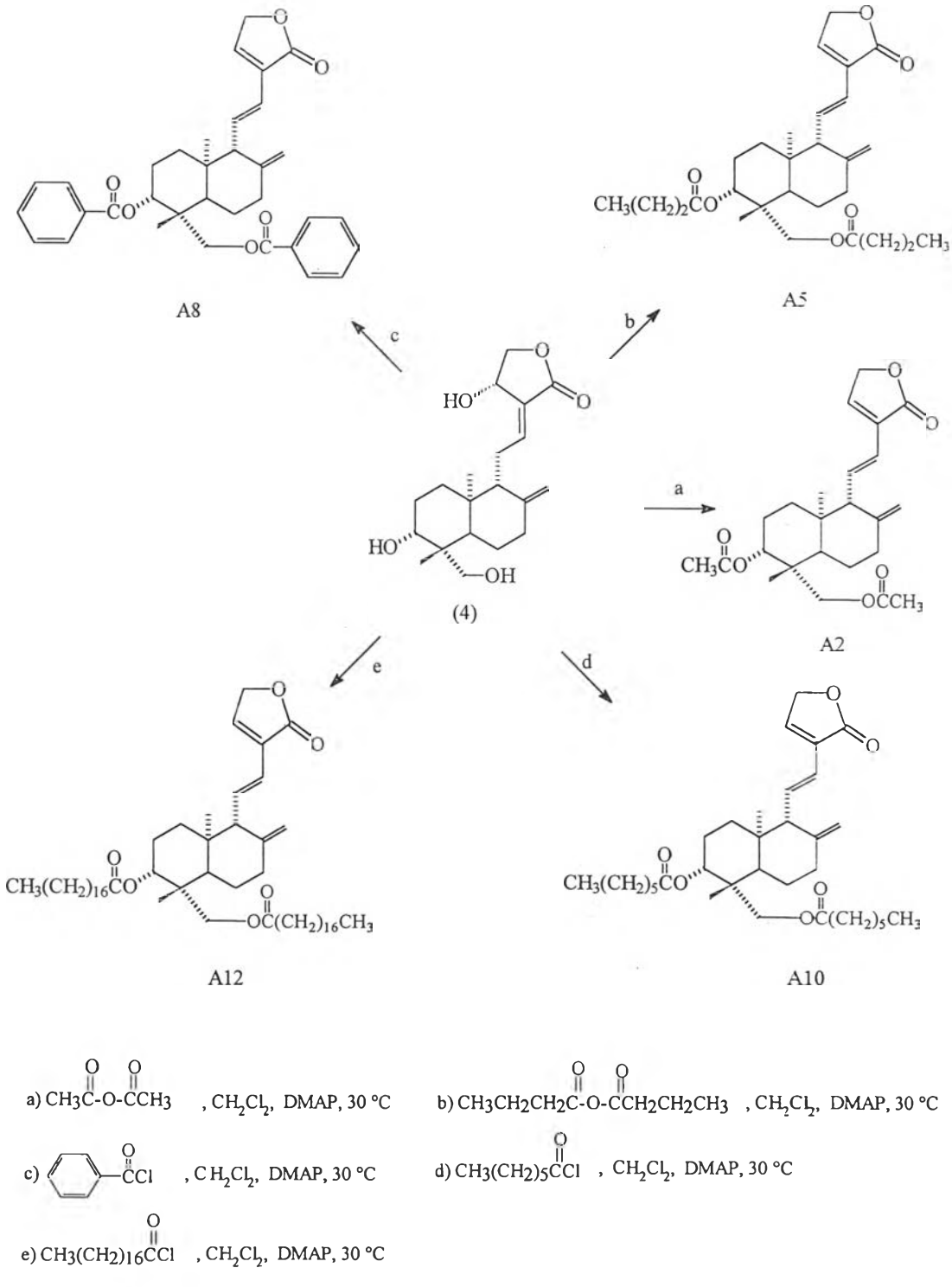
b) $CH_3CH_2CH_2C(=O)OC(=O)CH_2CH_2CH_3$, Pyridine, Reflux

c)  , Pyridine , Reflux

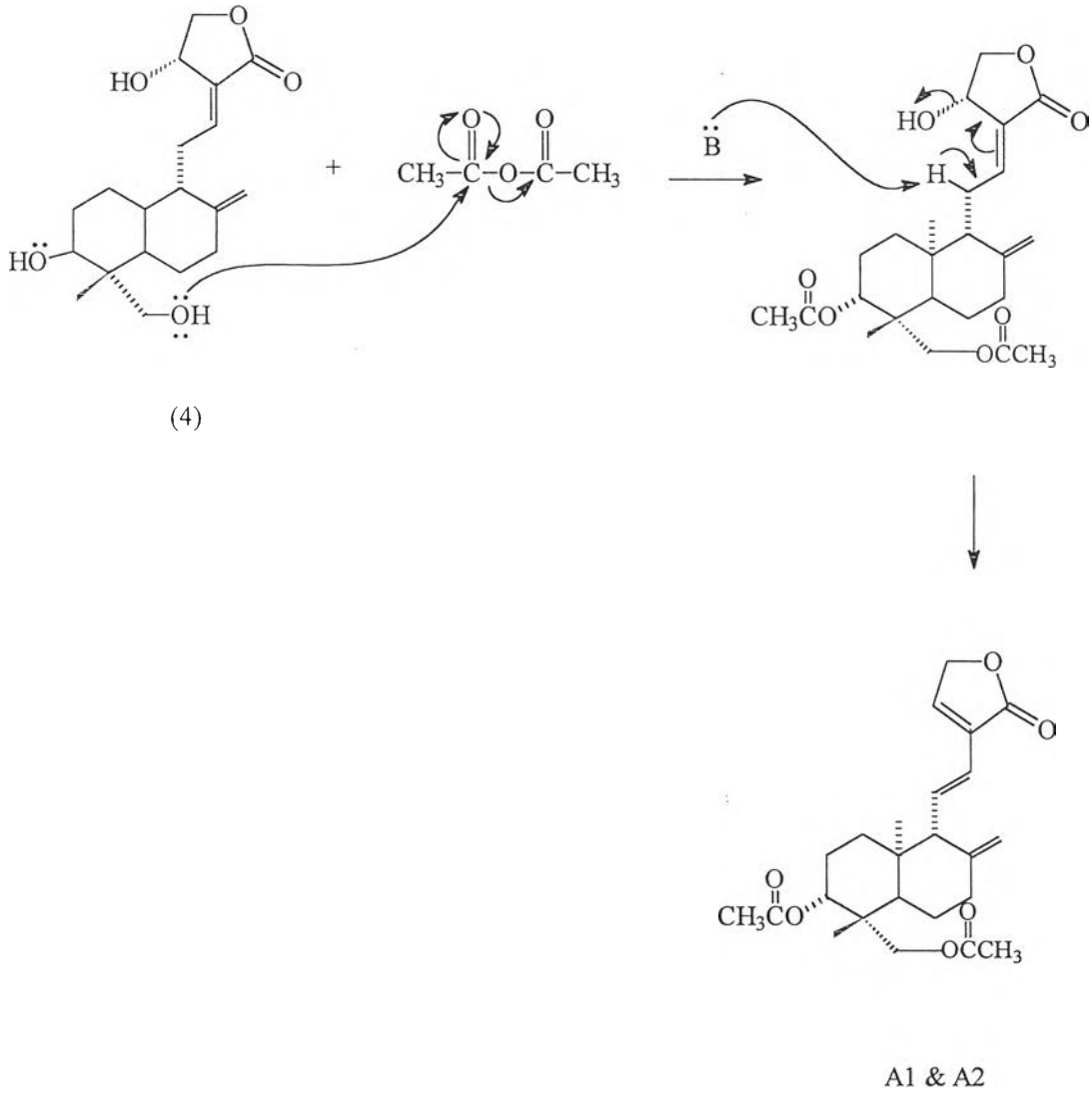
d) $CH_3(CH_2)_5C(=O)Cl$, Pyridine , Reflux

e) $CH_3(CH_2)_{16}C(=O)Cl$, Pyridine , Reflux

Scheme 6. Esterification of Andrographolide (4)

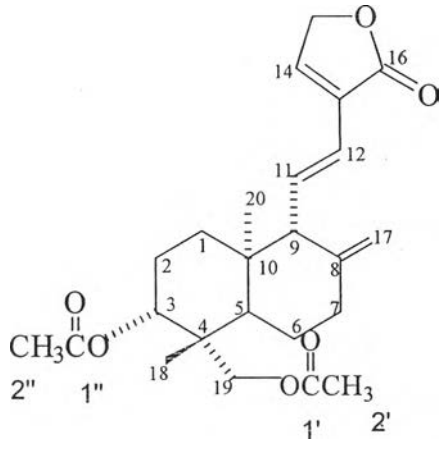


Scheme 6. Esterification of Andrographolide (4) (continued)



(4)

Scheme 7. Acetylation of Andrographolide (4)



Compound A1 = Compound A2

The GCMS of compound A1 (Figure 20) showed the molecular ion peak at *m/z* 416 corresponding to the molecular formular $C_{24}H_{32}O_6$.The mass fragmentation (Scheme 8) was proposed, based on the mass fragmentation of andrographolide. The IR absorption spectrum (Figure 21) showed :

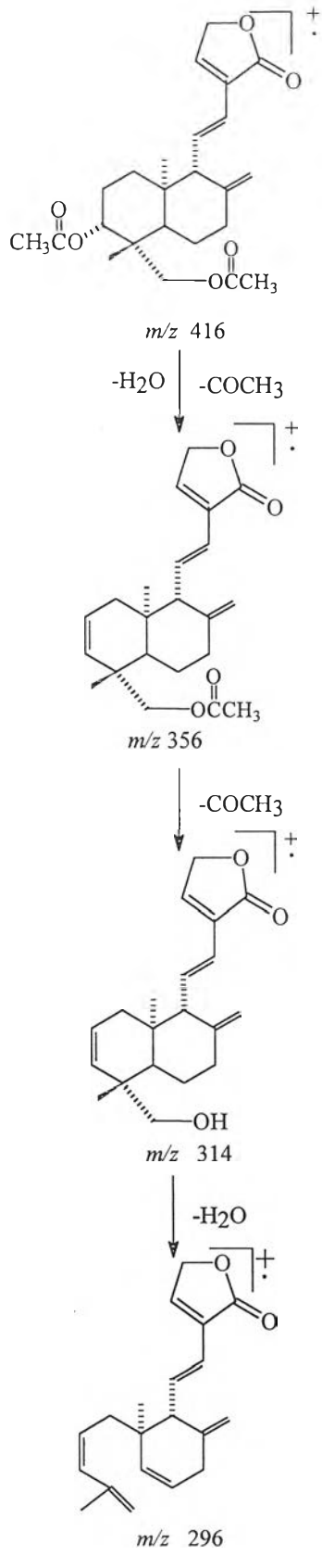
3000-2860	CM^{-1}	(C-H stretch ; aliphatic)
1734	CM^{-1}	(C=O stretch ; ester like carbonyl)
1470-1370	CM^{-1}	(C-H bend)
1247	CM^{-1}	(C-(C=O)-O stretch ; acetate)
1035	CM^{-1}	(C=O stretch)

The proton of compound A1 and carbon of compound A1 & A2 were assigned as 14-deoxy-11,12-didehydroandrographolide diacetate by comparison of their ^{1}H NMR (Figure 22), ^{13}C NMR (Figure25) data with those of Andrographolide and are shown in Table 5.

Table 5. The ^{1}H and ^{13}C NMR spectral data of compound A1 and ^{13}C NMR spectral data of compound A2 (in $CDCl_3$)

Position	compound	A1	A2
	δ_H(ppm) (Multiplicity,*J* in Hz)	δ_C(ppm)	δ_C(ppm)
1	1.19 (s)	38.2	38.2
2	1.61 (m)	24.1	24.1
3-CH	4.54 (m)	80.0	80.0
4	-	41.3	41.3
5	2.27 (d, 10.07)	54.7	54.7
6	1.61 (m)	23.8	23.8
7	1.98 (d, 7.02)	36.7	36.7
8	-	147.8	147.8
9	2.39 (m)	61.7	61.6
10	-	38.6	38.6
11	6.85 (dd, 10.07)	135.5	135.5
12	6.06 (d, 15.56)	121.3	121.3
13	-	129.1	129.1
14	7.10 (br t)	143.2	143.2
15	4.71[a] (d, 1.22)	69.5	69.5
16	-	172.1	172.1
17	4.75[a] (dd, 1.83,1.22),4.71(d,1.22)	109.2	109.2
18-CH_3	0.97 (s)	22.7	22.6
19	4.31,4.09 (d,11.59,11.9)	64.8	64.7
20-CH_3	0.83 (s)	15.2	15.2
1′ C=O	-	170.9	170.8
1″ C=O	-	170.5	170.5
2′2″ CH_3	1.98(d,7.02)	36.7	36.7

a)obscured by overlapping with other signals.

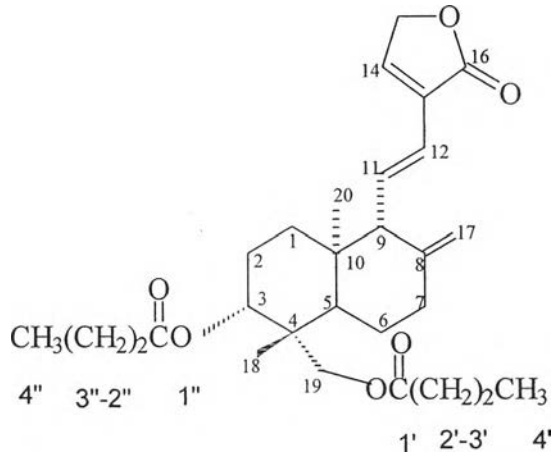


Scheme 8. Mass fragmentation of compound A1 & A2

2.2 Acylation of Andrographolide (4)

2.2.1 Acylation of Andrographolide (4) with Butyric anhydride

Acylation of Andrographolide was achieved by using butyric anhydride The mechanism of the reaction is shown in scheme 9. For reflux reaction gave two products, They were A3 and A4, but at room temperature gave one product, It was A5.

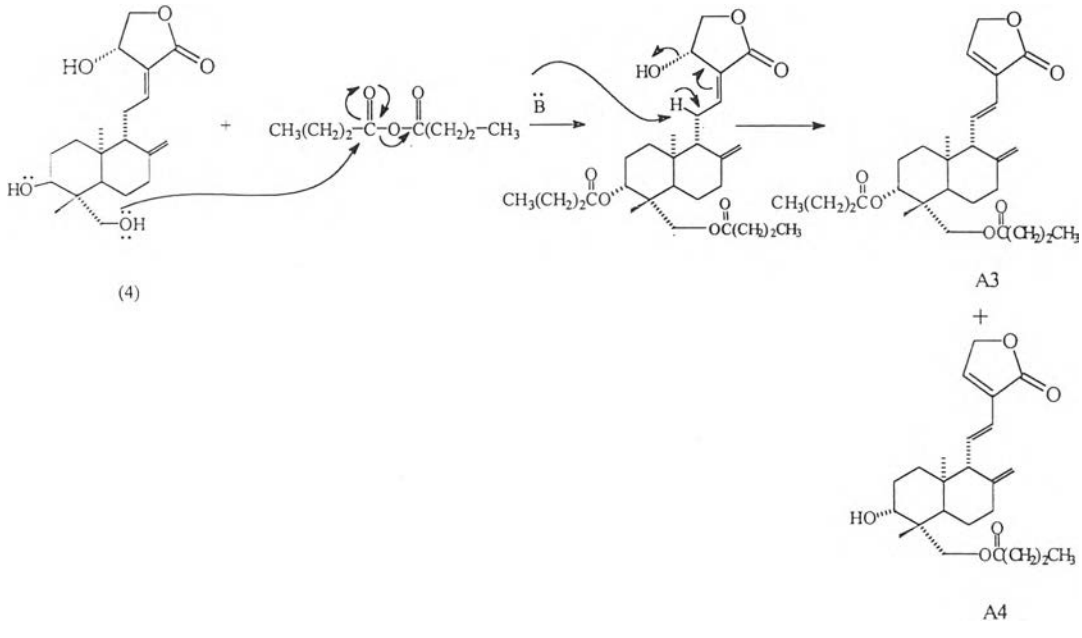


Compound A3 = Compound A5

Both compound A3(0.16 g,15.0 %)& A5 (0.46 g,38.0 %)was obtained as yellow waxy solid from this reaction, but by preparing at room temperature, the higher yield of A5 was obtained. The EIMS (Figure 28) showed the molecular ion at m/z 472 corresponding to the molecular formula $C_{28}H_{40}O_6$. The mass fragmentation of compound A3 & A5 is shown in Scheme 10. The IR spectrum (Figure 29) showed:

3000-2860	CM^{-1} (C-H stretch ; aliphatic)
1756-1729	CM^{-1} (C=O stretch ; ester like carbonyl)
1470-1370	CM^{-1} (C-H bend)
1084	CM^{-1} (C=O stretch)

Compound A3 & A5 were assigned as14-deoxy-11,12-didehydroandrographolide dibutyrate by analysis of its ^{1}H (Figure 30) and ^{13}C NMR (Figure 33) spectra. The complete ^{1}H, ^{13}C NMR of A3 and ^{13}C NMR of A5 were shown in Table 6.



Scheme 9. Acylation of Andrographolide (4) with Butyric anhydride

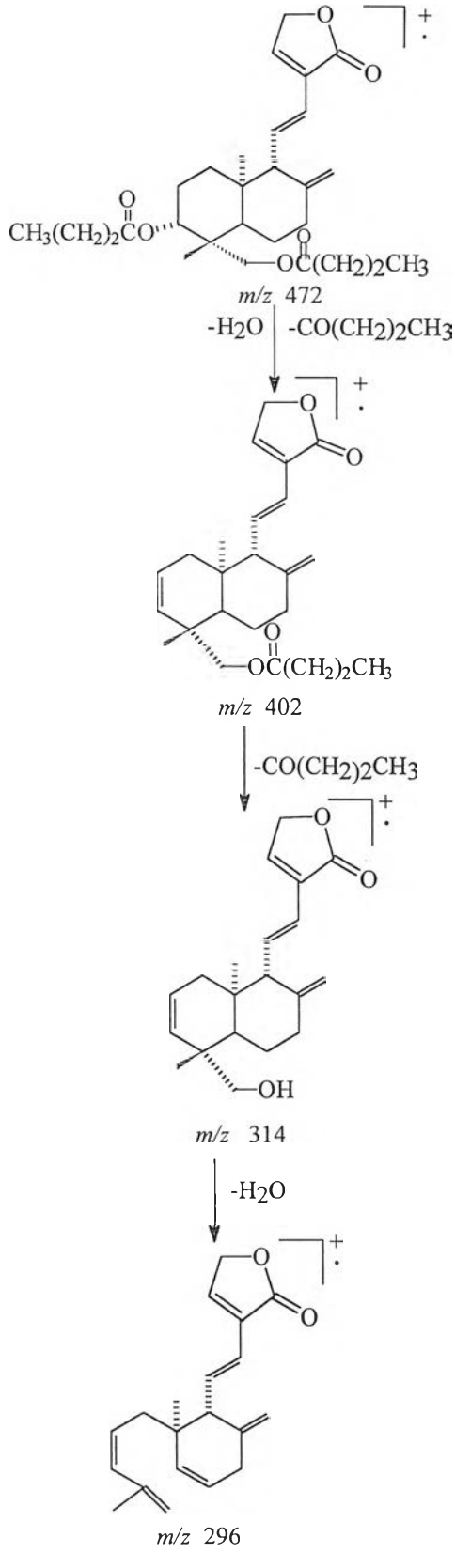
Table 6. The ^{1}H and ^{13}C NMR spectral data of compound A3 and ^{13}C NMR spectral data of compound A5(in $CDCl_3$)

Position	Compound A3		A5
	δ_H(ppm) (Multiplicity,*J* in H_z)	δ_C(ppm)	δ_C(ppm)
1	1.96 (td,4.57,4.58,4.88)	38.3	38.2
2	1.60 (m)	24.2	24.2
3-CH	4.56 (m)	79.8	79.7
4	-	41.4	41.3
5	2.28 (d, 10.07)	54.8	54.7
6	1.60, 1.22 (m)	24.0	23.9
7	1.78 (m)	36.8	36.7
8	-	147.9	147.9
9	2.40 (m)	61.7	61.7
10	-	38.7	38.6
11	6.68 (q)	135.7	135.6
12	6.06 (d,15.56)	121.3	121.3
13	-	129.2	129.1
14	7.09 (br t)	143.1	143.2
15	4.49[a] (d, 1.52)	69.5	69.5
16	-	173.5	173.5
17	4.74[a] (dd,1.83,1.52)	109.2	109.2
18-CH_3	0.95 (s)	22.7	22.7
19	4.24,4.16 (d,11.6,11.91)	64.7	64.7
20-CH_3	0.83 (s)	13.6	13.6
1′ C=O	-	173.1	173.1
1″C=O	-	172.1	172.1
2′ CH_2	2.21 (q)	36.4	36.4

Table 6. The ^{1}H and ^{13}CNMR spectral data of compound A3and ^{13}C NMR spectral data of compound A5(in $CDCl_3$) (continued)

Position	Compound A3		A5
	δ_H(ppm) (Multiplicity,*J* in H_z)	δ_C(ppm)	δ_C(ppm)
2″ CH_2	2.21 (q)	36.8	36.5
3′ CH_2	1.23 (m)	18.4	18.3
3″ CH_2	1.23 (m)	18.5	18.5
4′ CH_3	0.88 (q)	13.7	13.7
4″ CH_3	0.88 (q)	15.2	15.2

a) obscured by overlapping with other signal.

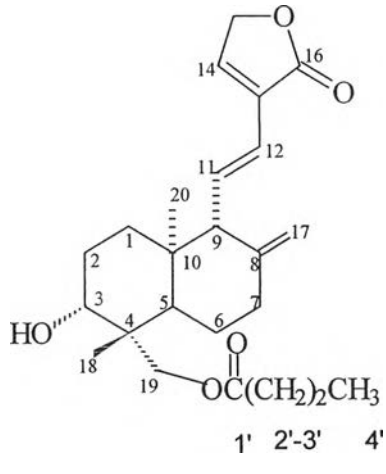


Scheme 10. Mass fragmentation of A3 & A5

Compound A4(0.17 g,17.1 %) was obtained as yellow waxy solid from this reaction. The EIMS (Figure 35) showed the molecular ion at *m/z* 402 corresponding to the molecular formula $C_{24}H_{34}O_5$. The mass fragmentation of compound A4 is shown in Scheme 11. The IR spectrum (Figure 36) showed:

3482	CM^{-1} (O-H stretch)
3000-2860	CM^{-1} (C-H stretch ; aliphatic)
1756-1729	CM^{-1} (C=O stretch ; ester like carbonyl)
1470-1370	CM^{-1} (C-H bend)
1086	CM^{-1} (C=O stretch)

Compound A4 was assigned as14-deoxy-11,12-didehydroandrographolide monobutyrate by analysis of its ^{1}H (Figure 37) and ^{13}C NMR (Figure 40) spectra are shown in Table 7.

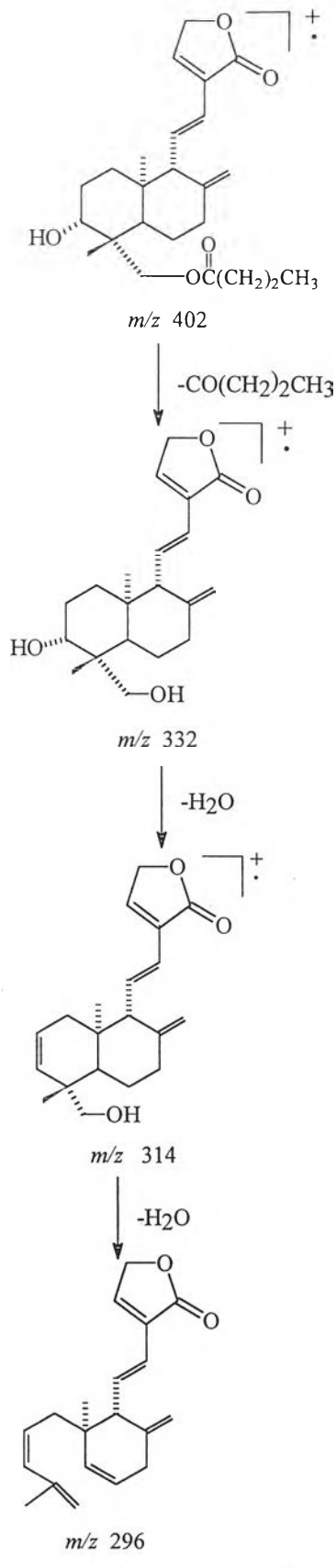


Compound A4

Table 7. The ^{1}H and ^{13}C NMR spectral data of compound A4 (in $CDCl_3$)

Position	^{1}H		^{13}C
	δ(ppm)	Multiplicity, *J* in (H_z)	δ(ppm)
1	2.01	td (5.49,5.18,5.55)	38.5
2	1.61	q	27.7
3-CH	3.29	q	79.2
4	-	-	42.5
5	2.26	t	54.8
6	1.50	m	23.4
7	1.80	m	36.6
8	-	-	147.9
9	2.44	m	61.6
10	-	-	38.7
11	6.85	dd (10.06,10.07)	135.8
12	6.09	d (15.87)	121.1
13	-	-	129.2
14	7.15	br t	142.9
15	4.76[a]	d (1.52)	69.6
16	-	-	173.6
17	4.78[a],4.52	dd (2.14,1.52)	109.1
18-CH_3	1.13	s	22.4
19	4.32,4.15	d,d (11.6, 11.91)	64.8
20-CH_3	0.82	s	13.6
3-OH	3.29	q	-
1′ C=O	-	-	172.2
2′ CH_2	2.26	t	36.3
3′ CH_2	1.22	dd (2.75,2.44)	18.3
4′ CH_3	0.91	t	15.5

a) obscured by overlapping with other signals



Scheme 11. Mass fragmentation of compound A4

2.2.2 Acylation of Andrographolide (4) with Benzoyl chloride

2.2.2.1 Preparation of Benzoyl chloride

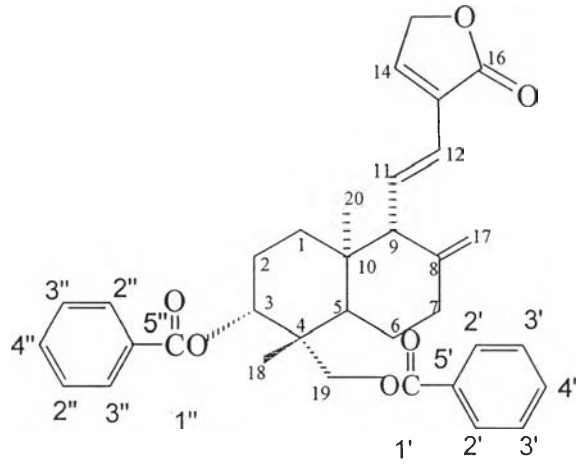
This compound was prepared from benzoic acid and thionyl chloride. The reaction was the general method in the preparation of acyl halide. In the reaction when the benzoyl chloride had been formed, the sulfurdioxide and hydrogen chloride gas were generated. The excess thionyl chloride was easily removed by distillation and the residue, benzoyl chloride, could bed used with redistillation.

The mechanism of the reaction involved nucleophilic substitution by chloride ion on a highly reactive intermediate, an acyl chlorosulfite is shown in scheme 12.

The structure of benzoyl chloride could be confirmed by IR spectrum (Figure 42): at the wavenumber 3100-3000 CM^{-1} represented to C-H stretching of aromatic compound, 2000-3000 CM^{-1} was overtone or combination bands of aromatic compound, 1775 CM^{-1} was C=O stretching of acylchloride, 1724 was fermi resonance band (of C=O stretching, and overtone of 862 CM^{-1}), 1600, 1452 CM^{-1}were C=C stretching of aromatic ring, 862, and 765 CM^{-1} were C-H bending (out-of-plane) of aromatic compound, and 665 CM^{-1} was C=C bending (out-of-plane) of aromatic ring.

2.2.2.2 Acylation of Andrographolide (4)

Compound A6 & A8 were prepared from andrographolide and benzoyl chloride at 80 ^{o}C, but A7 was prepared at room temperature(30^{o}C). The mechanism of the reaction is shown in scheme 13.

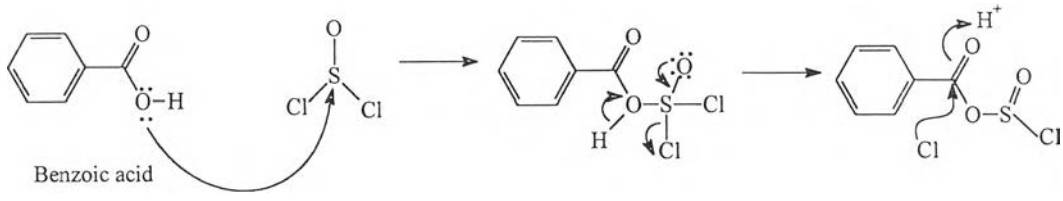


Compound A6 = Compound A8

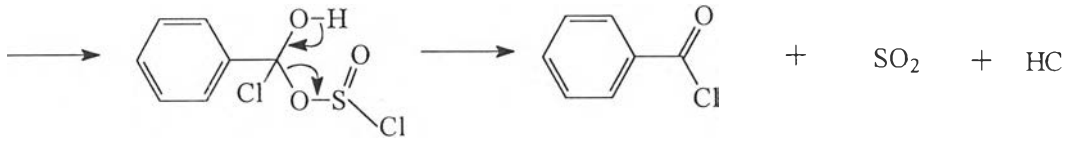
Compound A6(0.14g, 9.0 %)& A8(0.52 g, 33.6 %)were obtained as yellow solid from the reaction, but by preparing at room temperature, the higher yield of A8 was gave. The EIMS (Figure 43) showed the molecular ion at 540 corresponding to the molecular formula $C_{34}H_{36}O_6$. The mass fragmentation of compound A6 & A8 were shown in scheme 14. The IR spectrum (Figure 44) showed:

3100-2300	CM^{-1} (C-H stretch ; Aromatic)
2980-2840	CM^{-1} (C-H stretch ; methylene)
1750-1715	CM^{-1} (C=O stretch of α, β unsat ; benzoate ester)
690	CM^{-1} (out-of-plane ring C=C bend)

Compound A6 was assigned as 14-deoxy-11,12-didehydroandrographolide dibenzoate by analysis of its ^1H (Figure 45) and ^{13}C NMR (Figure 49) assignments of this compound were obtained by ^1H-^1H COSY (Figure 51) experiments and compound A8 was assigned as 14-deoxy-11,12-didehydroandrographolide dibenzoate by comparison of its' Rf value on TLC (Chloroform : Acetone = 35 :1)with that of A6. The complete ^1H , ^{13}C of A6 was shown in Table 8.

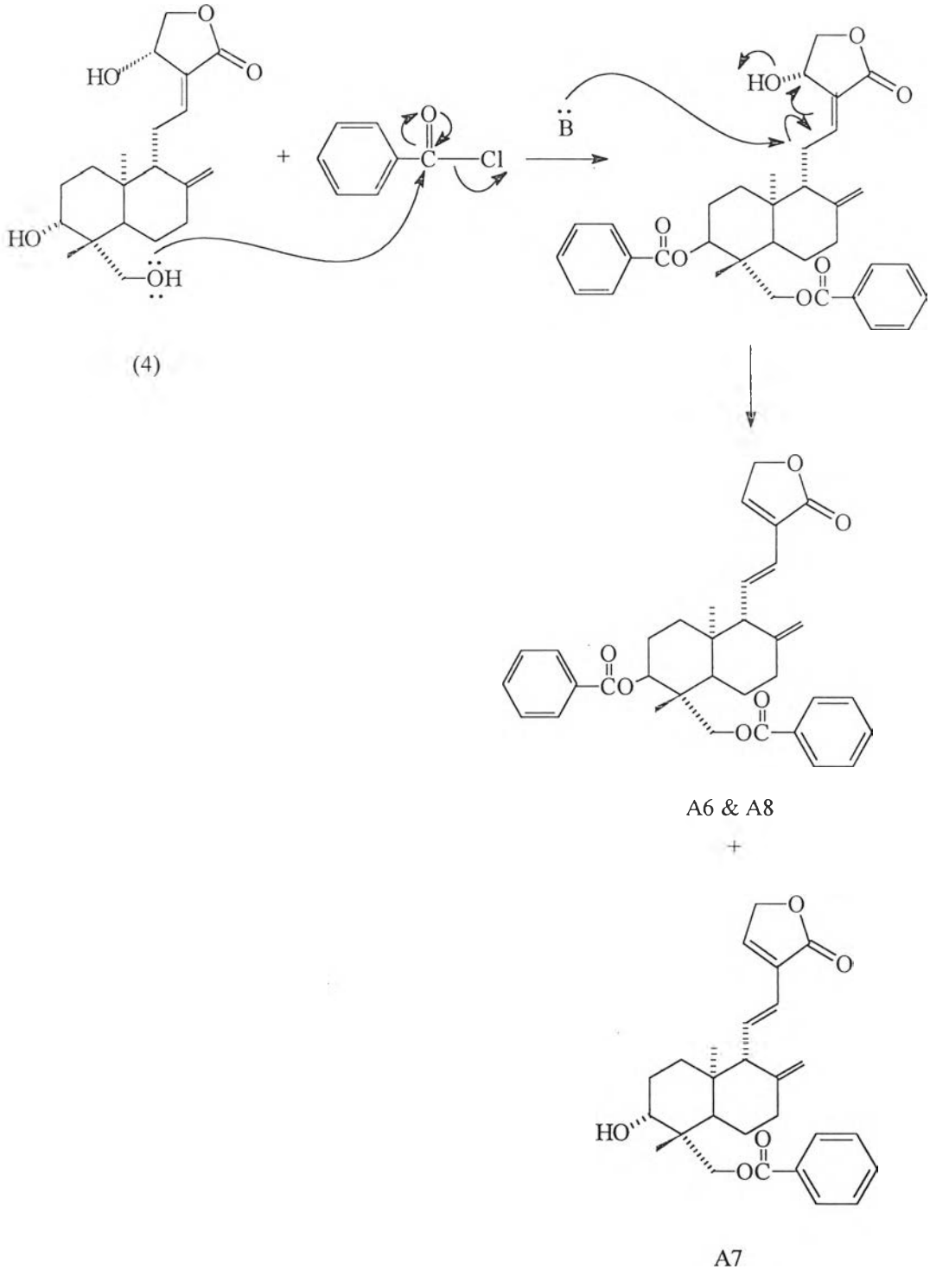


Acylchlorosulfite



Benzoyl chloride

Scheme 12. The reaction mechanism of formation of Benzoyl Chloride



Scheme 13. Acylation of Andrographolide (4) with Benzoyl chloride

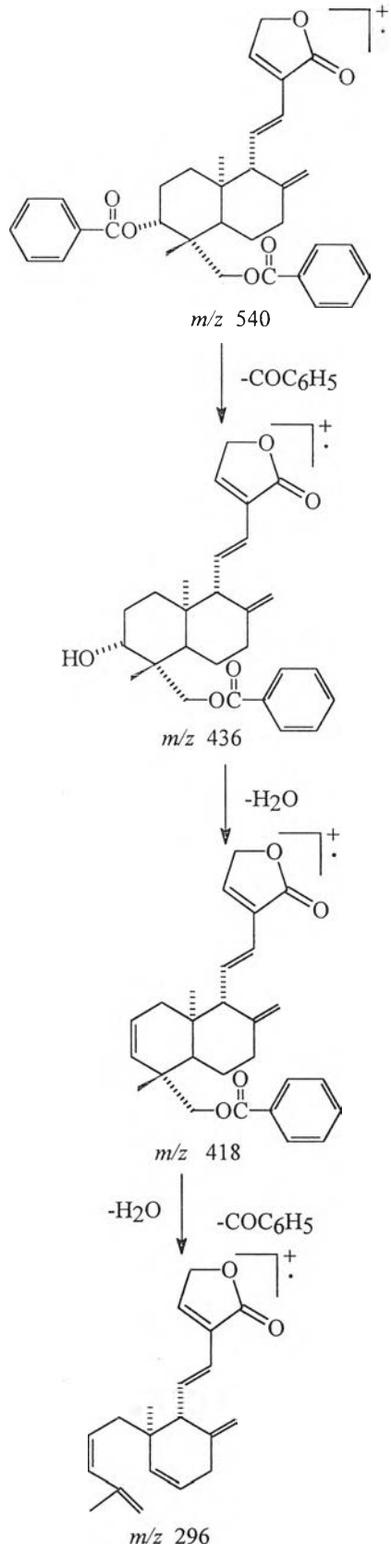
Table 8. The ^{1}H and ^{13}C NMR spectral data of compound A6 (in $CDCl_3$)

Position	^{1}H		^{13}C
	δ(ppm)	Multiplicity, *J* in (H_z)	δ(ppm)
1	1.72	td (4.27,3.97,4.27)	38.3
2	1.65	m	24.3
3-CH	4.94	q	80.7
4	-	-	42.2
5	2.42	d (9.77)	55.1
6	1.72	td(4.27,3.97,4.27)	23.9
7	1.89	m	36.6
8	-	-	147.7
9	2.52	m	61.6
10	-	-	38.7
11	6.96	q	135.6
12	6.17	d (15.87)	121.4
13	-	-	132.8
14	7.18	br t	143.1
15	4.83	d (1.53)	69.5
16	-	-	172.1
17	4.82[a],4.59	d (2.14),d(1.52)	109.4
18-CH_3	1.23	s	22.7
19	4.78,4.54[a]	dd (11.9,11.6)	65.1
20-CH_3	0.99	s	15.4
1′ C=O	-	-	166.7
1″ C=O	-	-	166.2
2′ CH	7.97	ddd (1.23,1.22,1.53)	129.7
2″ CH	7.97	ddd (1.23,1.22,1.53)	129.6

Table 8. The ^{1}H and ^{13}C NMR spectral data of compound A6(in $CDCl_3$) (continued)

Position	^{1}H		^{13}C
	δ(ppm)	Multiplicity, *J* in (H_z)	δ(ppm)
3′ CH	7.36	dd (7.63,7.32)	128.3
3″ CH	7.22	dd (7.63,7.32)	128.2
4′ CH	7.52	dddd (7.63,6.41)	130.3
4″ CH	7.44	dddd (7.63,7.32)	130.2
5′,5″ C	-	-	129.2

a) obscured by overlapping with other signals.



Scheme 14. Mass fragmentation of compound A6 & A8

Compound A7(0.52 g, 33.6 %) was obtained as orange solid from the reaction. The EIMS(Figure 52) showed the molecular ion at *m/z* 436 corresponding to the molecular formula $C_{27}H_{32}O_5$. The mass fragmentation of compound A7 is shown in scheme15. The IR spectrum (Figure 53) showed:

3500-3450	CM^{-1}	(O-H stretch)
3100-3000	CM^{-1}	(C-H stretch ; Aromatic)
2980-2840	CM^{-1}	(C-H stretch ; methylene)
1750-1715	CM^{-1}	(C=O stretch of α, β unsat ; benzoate ester)
690	CM^{-1}	(out-of-plane ring C=C bend)

Compound A7 was assigned as14-deoxy-11,12-didehydroandrographolide monobenzoate by analysis of its ^1H (Figure 54) and ^{13}C NMR (Figure 57) assignments of this compound were obtained by ^1H-^1H COSY(Figure59)experiments. The complete ^1H and ^{13}C of A7 are shown in Table 9.

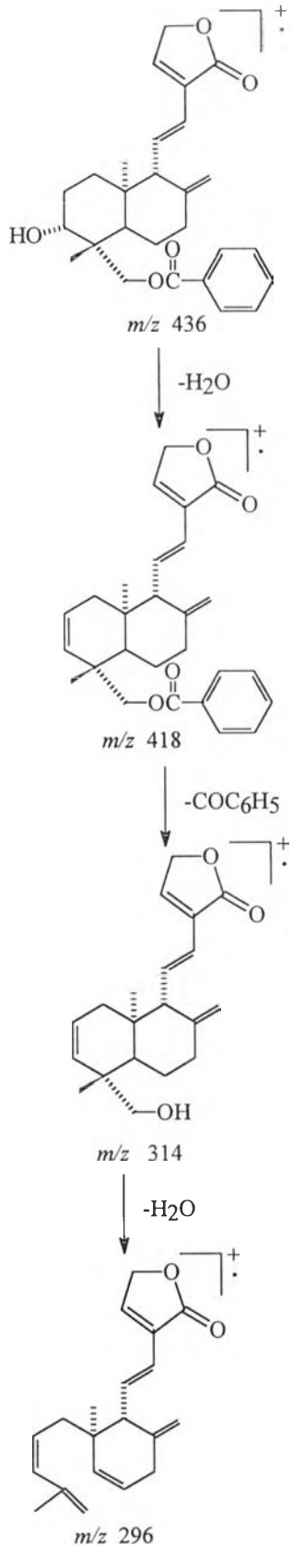
Table 9. The ^1H and ^{13}C NMR spectral data of compound A7 (in $CDCl_3$)

Position	^1H		^{13}C
	δ(ppm)	Multiplicity, *J* in (H_z)	δ(ppm)
1	1.76	m	38.6
2	1.60	m	27.8
3-CH	3.39	dd(4.58)	79.2
4	-	-	43.0
5	2.36	d (10.07)	54.9
6	1.60	m	23.7
7	1.76	m	36.7
8	-	-	147.9
9	2.49	m	61.7
10	-	-	38.8

Table 9. The ^{1}H and ^{13}C NMR spectral data of compound A7 (in $CDCl_3$) (continued)

Position	^{1}H		^{13}C
	δ(ppm)	Multiplicity, *J* in (H_z)	δ(ppm)
11	6.90	dd (10.38,10.07)	135.8
12	6.14	d(15.87)	121.2
13	-	-	133.0
14	7.18	br t	142.8
15	4.55	d (1.52)	69.6
16	-	-	172.2
17	4.81[a]	dd (1.83,1.53)	109.2
18-CH_3	1.28	s	22.6
19	4.61,4.40	dd (11.9,11.89)	65.4
20-CH_3	0.91	s	15.5
3 OH	3.39	dd(4.58)	-
1 ′ C=O	-	-	166.6
2′ CH	8.00	d (1.22)	129.7
3′ CH	7.44	dd (7.93,7.33)	128.4
4'CH	7.56	m	130.2
5'C	-	-	129.3

a) obscured by overlapping with other signals.



Scheme 15. Mass fragmentation of compound A7

2.2.3 Acylation of Andrographolide (4) with Heptanoyl chloride

2.2.3.1 Preparation of Heptanoyl chloride

Similar to the preparation of Benzoyl chloride, The Heptanoyl Chloride was prepared from heptanoic acid and thionyl chloride. The reaction was the general method in the preparation of acyl halide. In the reaction was the heptanoyl chloride had been formed, the sulfurdioxide and hydrogen chloride gas were generated. The excess thionyl chloride was easily removed by distillation, The heptanoyl chloride was purified by redistillation.

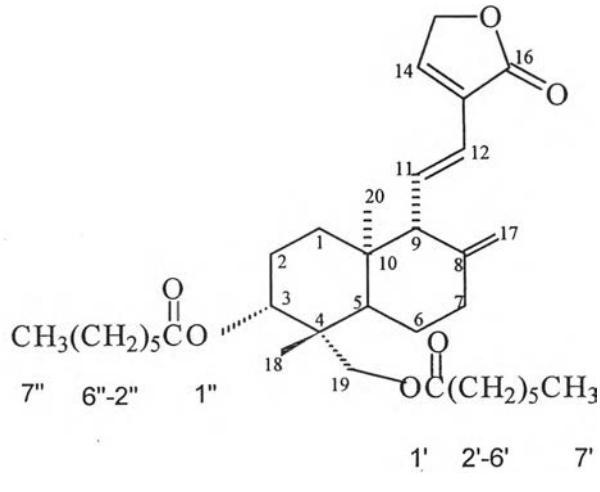
The structure of Heptanoyl chloride could by confirmed by IR spectrum (Figure 60) :

3000-2860, 1470, 1383 CM^{-1} were mineral oil

1818-1785 CM^{-1} (C=O stretching of acylchloride)

2.2.3.2 Acylation of Andrographolide (4)

Compound A9 and A10 were prepared from Andrographolide (4) and heptanoyl chloride at 80 °C and 30 °C respectively. The mechanism of the reaction is shown in Scheme 16.



Compound A9 = Compound A10

Compound A9(0.14 g,9.1%)&A10(0.42 g,26.4%)were obtained as orange waxy solid from the reaction, but by preparing at room temperature, the higher yield of A10 was gave. The EIMS (Figure 61) showed the molecular ion at 556 corresponding to the molecular formula $C_{34}H_{52}O_6$. The mass fragmentation of compound A9&A10 are shown in scheme 17. The IR spectrum (Figure 62) showed :

3000-2860	CM^{-1}	(C-H stretch)
1764-1739	CM^{-1}	(C=O stretch ; ester like carbonyl)
1470-1370	CM^{-1}	(C-H bend)
1165	CM^{-1}	(C=O stretch)

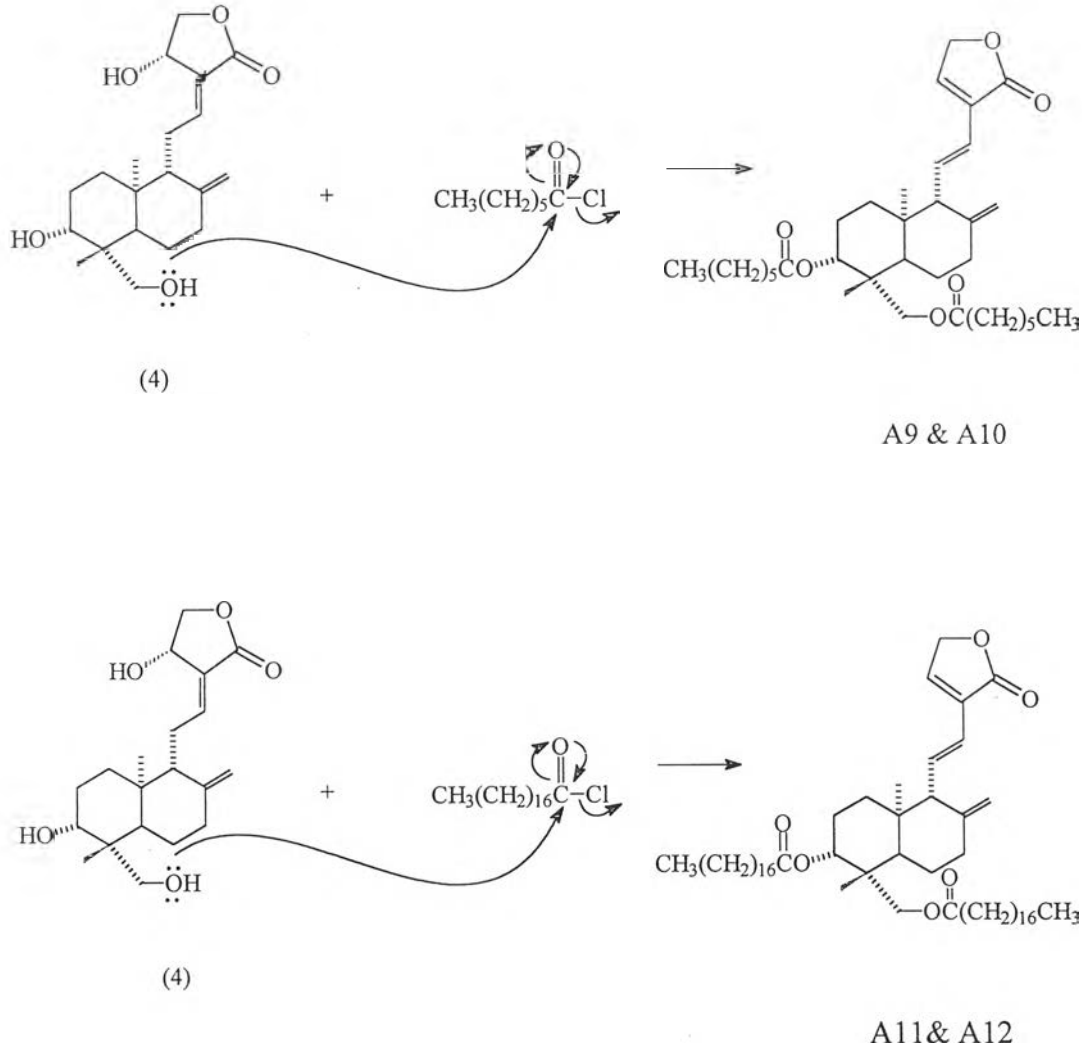
Compound A9 was assigned as 14-deoxy-11,12-didehydroandrographolide diheptanoate by analysis of its ^1H(Figure 63)and ^{13}C NMR (Figure 66)spectra. A10 was assigned as 14-deoxy-11,12-didehydroandrographolide diheptanoate by comparison of its' Rf value on TLC(Chloroform: Acetone =35:1)with that of A6. The complete ^1H and ^{13}C of A9 are shown in Table 10.

Table 10. The ^1H and ^{13}C NMR spectral data of compound A9 (in $CDCl_3$)

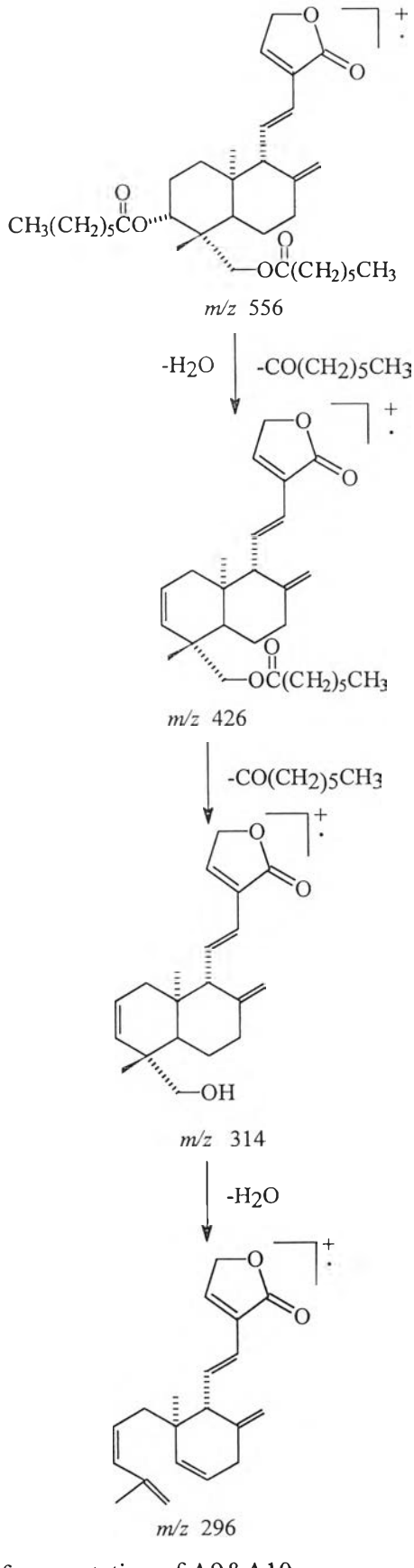
Position	^1H		^{13}C
	δ(ppm)	Multiplicity, *J* in (H_z)	δ(ppm)
1	2.25	q	38.14
2	1.26	brd	24.13
3-CH	4.20	dd (3.05, 2.75)	79.6
4	-	-	41.3
5	2.35	d (10.08)	54.6
6	1.26	m	23.9
7	2.25	m	36.7
8	-	-	147.6
9	2.42	m	60.9
10	-	-	38.4

Table 10. The ^{1}H and ^{13}C NMR spectral data of compound A9(in $CDCl_3$) (continued)

Position	^{1}H		^{13}C
	δ(ppm)	Multiplicity, *J* in (H_z)	δ(ppm)
11	5.48	t	137.7
12	4.77	m	120.0
13	-	-	120.1
14	6.40	ht	137.9
15	4.46	d (1.22)	66.8
16	-	-	173.7
17	4.52	dd (1.53,1.52)	109.2
18-CH_3	0.98	brd	22.6
19	4.26	d (11.29)	64.7
20-CH_3	0.85	m	14.0
1′ C=O	-	-	173.3
1″ C=O	-	-	170.443
2′2″–6′6′CH_2	1.60,1.26,2.25	q,br,q	34.6,34.5
(Nine-CH_2groups)			31.4,31.4
			25.0,25.0
			38.3,28.8
			22.6
7′7″ CH_3	1.26	br	22.4



Scheme 16. Acylation of Andrographolide (4) with Heptanoyl chloride and Stearoyl chloride



Scheme 17. Mass fragmentation of A9&A10

2.2.4 Acylation of Andrographolide (4) with Stearoyl chloride

2.2.4.1 Preparation of Stearoyl chloride

Similar to the preparation of stearoyl chloride, The stearoyl chloride was prepared from stearic acid and thionyl chloride. The reaction was the general method in the preparation of acyl halide. In the reaction when the stearoyl chloride had been formed, the sulfur dioxide and hydrogen chloride gas were generated. The excess thionyl chloride was easily removed by distillation, The stearoyl chloride was purified by redistillation.

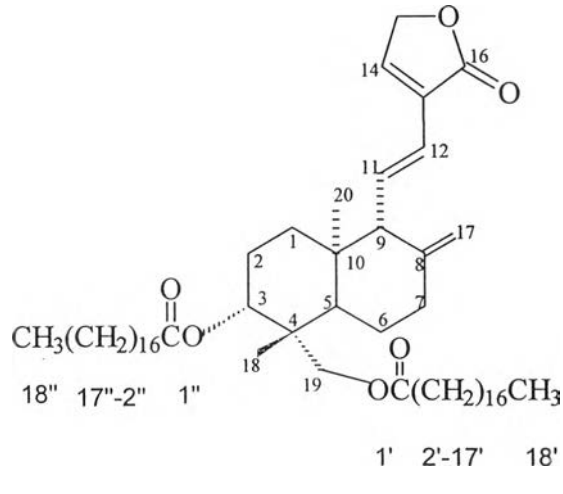
The structure of stearoyl chloride could by confirmed by IR spectrum (Figure 68) : at

3000-2860, 1470, 1383 CM^{-1} were mineral oil

1818-1785 CM^{-1} (C=O stretching of acylchloride)

2.2.4.2 Acylation of Andrographolide (4)

Compound A11 and A12 were prepared from andrographolide and stearoyl chloride at 80 °C and 30 °C respectively. The mechanism of the reaction is shown in Scheme 16.



Compound A11 = Compound A12

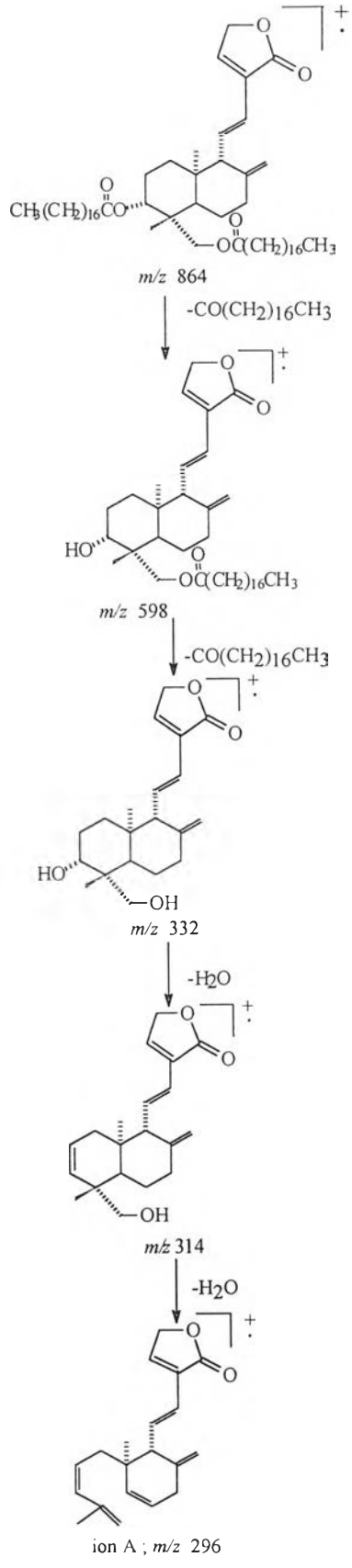
Compound A11(0.24 g, 9.6 %)& A12(0.41 g, 16.7%) were obtained as yellow waxy solid from the reaction, but by preparing at room temperature, the higher yield of A12 was gave. The EIMS (Figure 69) showed the molecular ion at 864 corresponding to the molecular formula $C_{56}H_{96}O_6$. The mass fragmentation of compound A11&A12 were shown in scheme 18. The IR spectrum (Figure 70) showed:

3000-2860	CM^{-1}	(C-H stretch)
1764-1738	CM^{-1}	(C=O stretch ; ester like carbonyl)
1470-1370	CM^{-1}	(C-H bend)
1165	CM^{-1}	(C-O stretch)

Compound A11& A12 were assigned as 14-deoxy-11,12-didehydroandrographolide distearoate by analysis of its ^{1}H (Figure 71) and ^{13}C NMR (Figure 74) spectra. The complete ^{1}H and ^{13}C NMR of A11 and ^{13}C NMR of A12 were shown in Table 11.

Table 11. The ^{1}H and ^{13}C NMR spectral data of compound A11and ^{13}C NMR spectral data of compound A12(in $CDCl_3$)

Position	compound A11		A12
	δ_H(ppm) (Multiplicity, *J* in H_z)	δ_C(ppm)	δ_C(ppm)
1	2.28(q)	38.3	38.2
2	1.68 (m)	24.2	24.2
3	4.61 (m)	79.8	79.7
4	-	41.4	41.4
5	2.35 (d, 1.07)	54.8	54.7
6	1.62,(m)	24.0	24.0
7	2.28(q)	36.8	36.7
8	-	147.7	147.9
9	2.46 (m)	61.8	61.7
10	-	38.7	38.6
11	6.95 (q)	135.7	135.6
12	6.14 (d, 15.56)	121.4	121.3
13	-	129.2	129.1
14	7.15 (br t)	143.2	143.1
15	4.56 (d, 1.52)	69.6	69.5
16	-	173.7	173.7
17	4.81 (dd, 1.53)	109.3	109.2
18-CH_3	1.02 (s)	15.2	15.2
19	4.31,4.22 (d, 11.9)	64.7	64.7
20-CH_3	0.88 (t)	14.1	14.1
1′ C=O	-	173.4	173.3
1″ C=O	-	172.2	173.1
2′2″ -17′17″	1.26 (s)	34.7,34.6	34.7,34.5
(Thirty-two		31.9,29.7	31.9,29.6
CH_2 Groups)		29.7,29.5	29.6,29.5
		29.4,29.3	29.3,29.3
		29.2,25.1	29.2,29.2
		25.0,22.7	29.2,25.0
			24.7,22.6
18′ 18″ CH_3	0.88 (t)	14.1	14.1



Scheme 18. Mass fragmentation of A11 & A12

Table 12. Summary of the ^{1}H NMR data of compound 4, 6, A1, A3, A4, A6, A7, A9 and A11 (in $CDCl_3$)

Position	^{1}H NMR, δ(ppm)								
	4	6	A1	A3	A4	A6	A7	A9	A11
1	1.21	1.50	1.19	1.98	2.01	1.72	1.16	2.25	2.28
2	1.70	1.70	1.61	1.60	1.61	1.65	1.60	1.26	1.62
3-CH	3.24	3.48	4.54	4.56	3.29	4.94	3.39	4.20	4.61
4	-	-	-	-	-	-	-	-	-
5	1.21	1.15	2.27	2.28	2.26	2.42	2.36	2.35	2.35
6	1.70	1.70	1.61	1.60	1.50	1.72	1.60	1.26	1.62
7	2.33	2.42	1.98	1.78	1.80	1.89	1.76	2.25	2.28
8	-	-	-	-	-	-	-	-	-
9	1.94	2.29	2.39	2.40	2.44	2.52	2.49	2.42	2.46
10	-	-	-	-	-	-	-	-	-
11	4.39	6.85	6.85	6.86	6.85	6.96	6.90	5.48	6.95
12	6.63	6.18	6.06	6.06	6.09	6.17	6.14	4.77	6.14
13	-	-	-	-	-	-	-	-	-

Table 12. Summary of the ^{1}H NMR data of compound 4 , 6, A1, A3 , A4 , A6 ,A7 , A9 and A11 (in $CDCl_3$) (continued)

Position	^{1}H NMR, δ(ppm)								
	4	6	A1	A3	A4	A6	A7	A9	A11
14	5.05	7.20	7.10	7.09	7.15	7.18	7.18	6.40	7.15
15	4.13,3.85	4.84,4.50	4.71	4.73	4.76	4.59	4.55	4.46	4.56
16	-	-	-	-	-	-	-	-	-
17	4.82,4.63	4.74	4.75,4.49	4.74,4.49	4.78,4.52	4.82	4.81	4.52	4.81
18-CH_3	1.09	1.22	0.97	0.95	1.13	1.23	1.28	0.98	1.02
19	3.24	4.18	4.31,4.09	4.24,4.16	4.32,4.15	4.78,4.54	4.61,4.40	4.26	4.31,4.22
20-CH_3	0.66	0.82	0.83	0.83	0.82	0.99	0.91	0.85	0.88
3-OH	2.48	2.95	-	-	3.29	-	3.39	-	-
14-OH	5.70	-	-	-	-	-	-	-	-
19-OH	4.91	3.28	-	-	-	-	-	-	-
2'CH	-	-	-	-	-	7.97	8.00	-	-
2"CH	-	-	-	-	-	7.97	-	-	-

Table 12. Summary of the ^{1}H NMR data of compound 4, 6, A1,A3, A4, A6, A7, A9 and A11(in $CDCl_3$) (continued)

Position	^{1}H NMR, δ(ppm)								
	4	6	A1	A3	A4	A6	A7	A9	A11
3'CH	-	-	-	-	-	7.36	7.44	-	-
3"CH	-	-	-	-	-	7.22	-	-	-
4'CH	-	-	-	-	-	7.52	7.56	-	-
4"CH	-	-	-	-	-	7.44	-	-	-
2'CH_2	-	-	-	2.21	2.26	-	-	-	-
2"CH_2	-	-	-	2.21	-	-	-	-	-
3'CH_2	-	-	-	1.23	1.22	-	-	-	-
3"CH_2	-	-	-	1.23	-	-	-	-	-
2'2"-6'6"CH_2	-	-	-	-	-	-	-	1.60,126, 2.25	-
2'2"-17'17"CH_2	-	-	-	-	-	-	-	-	1.26
2' CH_3	-	-	1.98	-	-	-	-	-	-
2"CH_3	-	-	1.98	-	-	-	-	-	-

Table 12. Summary of the ^{1}H NMR data of compound 4, 6, A1,A3, A4,A6, A7,A9 and A11 (in $CDCl_3$) (continued)

Position	^{1}H NMR, δ(ppm)								
	4	6	A1	A3	A4	A6	A7	A9	A11
4'CH_3	-	-	-	0.88	0.91	-	-	-	-
4"CH_3	-	-	-	0.88	-	-	-	-	-
7' CH_3	-	-	-	-	-	-	-	1.26	-
7"CH_3	-	-	-	-	-	-	-	1.26	-
18' CH_3	-	-	-	-	-	-	-	-	0.88
18"CH_3	-	-	-	-	-	-	-	-	0.88

Table 13. Summary of the ^{13}C NMR data of compound 4, 6, A1,A2, A3, A5, A4 , A6,A7, A9, A11 and A12 (in $CDCl_3$)

Position	^{13}C NMR , δ(ppm)											
	4	6	A1	A2	A3	A5	A4	A6	A7	A9	A11	A12
1	36.5	38.6	38.2	38.2	38.3	38.2	38.5	38.3	38.6	38.1	38.3	38.2
2	27.8	28.2	24.1	24.1	24.2	24.2	27.7	24.3	27.8	24.1	24.2	24.2
3-CH	78.4	80.8	80.0	80.0	79.8	79.7	79.2	80.7	79.2	79.6	79.8	79.7
4	42.2	43.0	41.3	41.3	41.4	41.3	42.5	42.1	43.0	41.3	41.4	41.4
5	54.4	54.7	54.7	54.7	54.8	54.7	54.8	55.1	55.0	54.6	54.8	54.7
6	23.9	23.1	23.8	23.8	24.0	23.9	23.4	23.9	23.7	23.9	24.0	24.0
7	37.5	36.7	36.7	36.7	36.8	36.7	36.6	36.7	36.7	36.7	36.8	36.7
8	147.5	147.9	147.8	147.8	147.9	147.9	147.9	147.7	147.9	147.6	148.0	147.9
9	55.5	61.7	61.5	61.6	61.7	61.7	61.6	61.6	61.7	60.9	61.8	61.7
10	38.5	38.3	38.6	38.6	38.7	38.6	38.7	38.7	38.8	38.4	38.7	38.6
11	23.9	135.8	135.5	135.5	135.7	135.6	135.8	135.6	135.8	137.7	135.7	135.6
12	146.2	121.0	121.3	121.3	121.3	121.3	121.1	121.5	121.2	120.0	121.4	121.3
13	128.9	129.1	129.1	129.1	129.2	129.1	129.2	132.8	133.0	120.1	129.2	129.1

Table 13. Summary of the ^{13}C NMR data of compound 4, 6,A1,A2,A3,A5,A4,A6, A7, A9, A11and A12 (in $CDCl_3$) (continued)

Position	^{13}C NMR , δ(ppm)											
	4	6	A1	A2	A3	A5	A4	A6	A7	A9	A11	A12
14	64.5	142.7	143.2	143.2	143.1	143.2	142.9	143.1	142.8	137.9	143.2	143.1
15	74.3	69.6	69.5	69.5	69.5	69.5	69.6	69.5	69.6	66.8	69.6	69.5
16	169.9	172.0	172.1	172.1	173.5	173.5	173.6	172.1	172.2	173.7	173.7	173.7
17	108.2	109.1	109.2	109.2	109.2	109.2	109.2	109.4	109.2	109.2	109.3	109.2
18-CH_3	23.0	22.8	22.7	22.6	22.7	22.7	22.4	22.7	22.6	22.6	15.2	15.2
19	62.6	64.2	64.8	64.7	64.7	64.7	64.8	65.1	65.4	64.7	64.7	64.7
20-CH_3	14.7	16.0	15.2	15.2	13.6	13.6	13.6	15.4	15.5	14.0	14.1	14.1
1' C=O	-	-	170.9	170.8	173.2	173.2	172.2	166.7	166.6	173.4	173.4	173.3
1"C=O	-	-	170.5	170.5	172.1	172.1	-	166.2	-	170.4	172.2	172.1
5' C	-	-	-	-	-	-	-	129.2	129.3	-	-	-
5" C	-	-	-	-	-	-	-	129.2	-	-	-	-
2'CH	-	-	-	-	-	-	-	129.7	129.7	-	-	-
2"CH	-	-	-	-	-	-	-	129.6	-	-	-	-

Table 13. Summary of the ^{13}C NMR data of compound 4, 6,A1,A2,A3,A4,A6,A7, A9,A11 and A12 (in $CDCl_3$) (continued)

Position	^{13}C NMR , δ(ppm)											
	4	6	A1	A2	A3	A5	A4	A6	A7	A9	A11	A12
3'3"CH	-	-	-	-	-	-	-	128.2	-	-	-	-
4'CH	-	-	-	-	-	-	-	130.3	130.2	-	-	-
4"CH	-	-	-	-	-	-	-	130.2	-	-	-	-
2'CH_2	-	-	-	-	36.4	36.4	36.3	-	-	-	-	-
2"CH_2	-	-	-	-	36.8	36.5	-	-	-	-	-	-
3'CH_2	-	-	-	-	18.4	18.3	18.3	-	-	-	-	-
3"CH_2	-	-	-	-	18.5	18.5	-	-	-	-	-	-
2'2"-	-	-	-	-	-	-	-	-	-	34.6,34.5		-
6'6"CH_2										31.4,31.4		
										25.0,25.0		
										28.8,38.3		
										22.6		

Table 13. Summary of the ^{13}C NMR data of compound 4, 6, A1,A2,A3,A5,A4,A6,A7,A9,A11 and A12 (in $CDCl_3$) (continued)

Position	^{13}C NMR , δ(ppm)											
	4	6	A1	A2	A3	A5	A4	A6	A7	A9	A11	A12
2'2"-17'17"CH_2	-	-	-	-	-	-	-	-	-	-	34.1,34.6	34.7,34.5
											31.9,29.7	31.9,29.6
											29.7,29.5	29.6,29.5
											29.4,29.3	29.3,29.3
											29.3,25.1	29.2,29.2
											25.0,22.7	29.2,25.1
												24.9,24.6
2'2"CH_3	-	-	36.7	36.7	-	-	-	-	-	-	-	-
4'4"CH_3	-	-	-	-	13.7	13.7	15.5	-	-	-	-	-
7'7"CH_3	-	-	-	-	-	-	-	-	-	22.4	-	-
18'18"CH_3	-	-	-	-	-	-	-	-	-	-	14.1	14.1