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APPENDIXES

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Table 3 Rf Value of Four Diterpenoid Compounds on Silica Gel 60 GF 254, the Detection was Iodine Vapour.

Developing Solvent	Rf Value of C-2	Rf Value of C-3	Rf Value of C-4	Rf Value of C-5
Chloroform	0.11	0.00	0.00	0.00
Chloroform:Methanol ( 9 : 1 )	0.93	0.71	0.52	0.33
Chloroform:Methanol ( 8 : 1 )	0.92	0.76	0.54	0.38
Chloroform:Acetone ( 9 : 1 )	0.31	0.08	0.00	0.00
Chloroform:Ab.Ethanol ( 85 : 15 )	0.95	0.86	0.65	0.40
Methanol : Benzene ( 1 : 1 )	0.95	0.95	0.92	0.90

C-2 = Dehydroandrographolide

C-3 = Andrographolide

C-4 = Neoandrographolide

C-5 = Deoxyandrographolide-19 $\beta$ -D-glucoside

Table 4 Values of Peak Height and Peak Area versus Concentration of Dehydroandrographolide (C-2).

Conc. mg/ml	Peak <sup>a</sup> Height	%RSD	Peak <sup>a</sup> Area	%RSD	Retention <sup>a</sup> Time (min)
0.10	7.50	0.00	3153008	5.08	18.90
0.20	16.00	0.00	6363986	4.20	18.99
0.30	25.00	0.00	8977526	5.03	18.92
0.57	47.75	0.74	12409832	7.85	18.97
0.97	82.90	0.17	24819664	6.85	18.93

Mobile phase methanol:water (42:58)

UV-detector 255 nm, Sensitivity 0.1 AUFS

<sup>a</sup> Mean value of two experiments

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Table 5 Values of Peak Height and Peak Area versus  
Concentration of Andrographolide (C-3).

Conc. mg/ml	Peak <sup>a</sup> Height	%RSD	Peak <sup>a</sup> Area	%RSD	Retention <sup>a</sup> Time (min)
0.10	26.25	0.46	3883575	5.77	6.10
0.21	51.00	0.00	7058243	0.35	6.14
0.31	77.00	0.00	10464085	0.53	6.10
0.42	102.30	0.35	13708028	0.67	6.12

Mobile phase methanol:water (42:58)

UV-detector 255 nm, Sensitivity 0.1 AUFS

<sup>a</sup> Mean value of two experiments

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Table 6 Values of Peak Height and Peak Area versus Concentration of Neoandrographolide (C-4).

Conc. mg/ml	Peak <sup>a</sup> Height	%RSD	Peak <sup>a</sup> Area	%RSD	Retention <sup>a</sup> Time (min)
0.10	7.65	0.92	3812080	4.24	25.34
0.15	11.50	0.69	5602853	4.82	25.41
0.20	15.50	0.00	9055768	8.25	25.45
0.30	23.25	0.52	12619403	10.18	25.38

Mobile phase methanol:water (42:58)

UV-detector 220 nm, Sensitivity 0.1 AUFS

<sup>a</sup> Mean value of two experiments

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Table 7 Values of Peak Height and Peak Area versus Concentration of Deoxyandrographolide-19 $\beta$ -D-glucoside (C-5).

Conc. mg/ml	Peak <sup>a</sup> Height	%RSD	Peak <sup>a</sup> Area	%RSD	Retention <sup>a</sup> Time (min)
0.02	0.87	5.34	115436	4.32	11.38
0.04	1.85	2.38	174490	6.40	11.36
0.08	3.56	0.84	303505	1.33	11.53
0.10	4.28	0.65	377199	5.90	11.34
0.20	8.62	0.00	754398	5.91	11.51

Mobile phase methanol:water (42:58)

UV-detector 255 nm, Sensitivity 0.1 AUFS

<sup>a</sup> Mean value of two experiments

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Table 8 Precision of Peak Height and Peak Area for Dehydroandrographolide (C-2).

Injection	Retention Time (min)	Peak Height	Peak Area
1	18.90	25.00	9267630
2	18.97	25.00	9249534
3	18.99	25.00	9815490
4	18.92	25.00	10011864
5	18.93	24.50	9298894
Mean		24.90	9528682.40
Std.Dev		± 0.22	± 358677.40
%RSD		0.89	3.76

Sample size 20  $\mu$ l of dehydroandrographolide (C-2)  
 0.30 mg/ml in methanol, mobile phase methanol:water  
 (42:58), UV-detector 255 nm, sensitivity 0.1 AUFS.

Table 9 Precision of Peak Height and Peak Area for Andrographolide (C-3).

Injection	Retention Time (min)	Peak Height	Peak Area
1	6.10	80.80	10496886
2	6.12	79.80	10453658
3	6.12	80.80	10479508
4	6.10	80.80	10528822
5	6.14	79.90	10396380
Mean		79.92	10471051
Std.Dev.		± 0.64	± 49886.65
%RSD		0.80	0.48

Sample size 20  $\mu$ l of andrographolide (C-2) 0.31 mg/ml in methanol, mobile phase methanol:water (42:58), UV-detector 255 nm, sensitivity 0.1 AUFS.

Table 10 Precision of Peak Height and Peak Area for  
Neoandrographolide (C-4).

Injection	Retention Time (min)	Peak Height	Peak Area
1	25.34	16.50	8610762
2	25.56	16.20	7802274
3	25.41	16.00	7767920
4	25.45	16.00	7647274
5	25.38	16.20	8046282
Mean		16.18	7974902.40
Std.Dev.		± 0.20	± 383860.37
%RSD		1.27	4.81

Sample size 20  $\mu$ l of neoandrographolide (C-4) 0.20 mg/ml in methanol, mobile phase methanol:water (42:58), UV-detector 220 nm, sensitivity 0.1 AUFS.

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Table 11 Precision of Peak Height and Peak Area for Deoxyandrographolide-19 $\beta$ -D-glucoside (C-5).

Injection	Retention Time (min)	Peak Height	Peak Area
1	11.36	3.73	311948
2	11.53	3.62	299466
3	11.34	3.60	319550
4	11.38	3.70	317538
5	11.51	3.60	306888
Mean		3.65	311078
Std.Dev.		$\pm$ 0.06	$\pm$ 8164.83
%RSD		1.67	2.62

Sample size 20  $\mu$ l of deoxyandrographolide-19 $\beta$ -D-glucoside (C-5) 0.08 mg/ml, mobile phase methanol:water (42:58), UV-detector 255 nm, sensitivity 0.1 AUFS.

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Table 12 Contents of Dehydroandrographolide (C-2) in the Leaves of *Andrographis paniculata*, Sampling Monthly.

Month	Wt. of Crude Powder Taken (mg)	Peak Height	% Amount (w/w)
JAN.	250.09	2.94	3.95
	250.60	2.96	3.98
			$\bar{X} = 3.97$
			% RSD = 0.53
FEB.	249.90	3.36	4.16
	250.91	3.39	4.20
			$\bar{X} = 4.18$
			% RSD = 0.68
MAR.	250.35	3.87	5.12
	250.47	3.90	5.16
			$\bar{X} = 5.14$
			% RSD = 0.55
APR.	250.72	6.93	7.27
	250.80	6.98	7.32
			$\bar{X} = 7.30$
			% RSD = 0.48
MAY.	249.43	5.24	4.41
	250.22	5.50	4.44
			$\bar{X} = 4.43$
			% RSD = 0.50
JUN.	250.28	4.53	5.49
	250.68	4.57	5.54
			$\bar{X} = 5.52$

Table 12 (Cont.)

Month	Wt. of Crude Powder Taken (mg)	Peak Height	% Amount (w/w)
JUL.	250.26	4.96	% RSD = 0.64
	250.41	5.01	5.63
			5.69
			$\bar{X} = 5.66$
AUG.	250.35	3.15	% RSD = 0.79
	250.75	3.19	3.56
			3.60
			$\bar{X} = 3.58$
SEP.	250.83	1.19	% RSD = 0.79
	251.19	1.20	1.44
			1.45
			$\bar{X} = 1.45$
OCT.	250.51	0.56	% RSD = 0.55
	250.93	0.57	0.68
			0.69
			$\bar{X} = 0.69$
NOV.	250.62	0.69	% RSD = 0.51
	251.36	0.70	0.83
			0.84
			$\bar{X} = 0.84$
DEC.	251.08	0.50	% RSD = 0.84
	250.98	0.51	0.60
			0.61
			$\bar{X} = 0.61$
			% RSD = 0.58

Table 13 Contents of Andrographolide (C-3) in the Leaves of *Andrographis paniculata*, Sampling Monthly.

Month	Wt. of crude Powder Taken (mg)	Peak Height	% Amount (w/w)
JAN.	250.09	3.02	1.16
	250.60	3.05	1.17
			$\bar{X} = 1.17$
			% RSD = 0.60
FEB.	249.90	2.10	0.81
	250.91	2.13	0.82
			$\bar{X} = 0.82$
			% RSD = 0.86
MAR.	250.35	2.60	1.00
	250.47	2.63	1.01
			$\bar{X} = 1.01$
			% RSD = 0.70
APR.	250.72	5.45	1.98
	250.80	5.51	2.00
			$\bar{X} = 1.99$
			% RSD = 0.71
MAY.	249.43	8.63	3.00
	250.22	8.69	3.02
			$\bar{X} = 3.01$
			% RSD = 0.47
JUN.	250.28	7.80	3.12
	250.68	7.85	3.14
			$\bar{X} = 3.13$

Table 13 (Cont.)

Month	Wt. of Crude Powder Taken (mg)	Peak Height	% Amount (w/w)
JUL.	250.26	9.00	% RSD = 0.45 3.42
	250.41	9.05	3.44
			$\bar{X}$ = 3.43
AUG.	250.35	11.25	% RSD = 0.41 4.26
	250.75	11.30	4.28
			$\bar{X}$ = 4.27
SEP.	250.83	11.83	% RSD = 0.33 4.84
	251.19	11.90	4.87
			$\bar{X}$ = 4.86
OCT.	250.51	13.48	% RSD = 0.44 5.56
	250.93	13.58	5.60
			$\bar{X}$ = 5.58
NOV.	250.62	14.70	% RSD = 0.51 6.00
	251.36	14.80	6.04
			$\bar{X}$ = 6.02
DEC.	251.08	10.95	% RSD = 0.47 4.42
	250.98	11.00	4.44
			$\bar{X}$ = 4.43
		% RSD = 0.32	



Table 14 Contents of Neoandrographolide (C-4) in the  
Leaves of *Andrographis paniculata*, Sampling  
Monthly.

Month	Wt. of Crude Powder Taken (mg)	Peak Height	% Amount (w/w)
JAN.	250.09	1.35	1.94
	250.60	1.36	1.95
			$\bar{X} = 1.95$
			% RSD = 0.36
FEB.	249.90	1.19	1.50
	250.91	1.20	1.51
			$\bar{X} = 1.51$
			% RSD = 0.47
MAR.	250.35	1.30	1.63
	250.47	1.31	1.64
			$\bar{X} = 1.64$
			% RSD = 0.43
APR.	250.72	0.58	0.60
	250.80	0.60	0.61
			$\bar{X} = 0.61$
			% RSD = 0.58
MAY.	249.43	0.63	0.66
	250.22	0.70	0.67
			$\bar{X} = 0.67$
			% RSD = 0.53
JUN.	250.28	0.64	0.92
	250.68	0.65	0.93
			$\bar{X} = 0.93$

Table 14 (Cont.)

Month	Wt. of Crude Powder Taken (mg)	Peak Height	% Amount (w/w)
JUL.	250.26	0.70	% RSD = 0.76
	250.41	0.71	0.93
			0.94
			$\bar{X}$ = 0.94
AUG.	250.35	0.95	% RSD = 0.75
	250.75	0.96	1.26
			1.27
			$\bar{X}$ = 1.27
SEP.	250.83	1.15	% RSD = 0.55
	251.19	1.16	1.33
			1.34
			$\bar{X}$ = 1.34
OCT.	250.51	1.04	% RSD = 0.53
	250.93	1.05	1.40
			1.41
			$\bar{X}$ = 1.41
NOV.	250.62	0.69	% RSD = 0.50
	251.36	0.70	0.93
			0.94
			$\bar{X}$ = 0.94
DEC.	251.08	1.51	% RSD = 0.75
	250.98	1.50	2.02
			2.01
			$\bar{X}$ = 2.02
			% RSD = 0.35

Table 15 Contents of Deoxyandrographolide-19 $\beta$ -D-glucoside (C-5) in the Leaves of *Andrographis paniculata*, Sampling Monthly.

Month	Wt. of Crude Powder Taken (mg)	Peak Height	% Amount (w/w)
JAN.	250.09	7.74	2.02
	250.60	7.78	2.03
			$\bar{X} = 2.03$
			% RSD = 0.35
FEB.	249.90	14.02	3.21
	250.91	14.06	3.22
			$\bar{X} = 3.22$
			% RSD = 0.22
MAR.	250.35	16.66	3.80
	250.47	16.75	3.82
			$\bar{X} = 3.81$
			% RSD = 0.39
APR.	250.72	5.69	1.18
	250.80	5.74	1.19
			$\bar{X} = 1.19$
			% RSD = 0.59
MAY.	249.43	3.62	0.70
	250.22	3.67	0.71
			$\bar{X} = 0.71$
			% RSD = 0.99
JUN.	250.28	4.60	1.05
	250.68	4.64	1.06
			$\bar{X} = 1.06$



Table 15 (Cont.)

Month	Wt. of Crude Powder Taken (mg)	Peak Height	% Amount (w/w)
			% RSD = 0.66
JUL.	250.26	3.98	0.87
	250.41	4.03	0.88
			$\bar{X} = 0.88$
			% RSD = 0.80
AUG.	250.35	3.60	0.80
	250.75	3.65	0.81
			$\bar{X} = 0.81$
			% RSD = 0.87
SEP.	250.83	2.00	0.53
	251.19	2.06	0.54
			$\bar{X} = 0.54$
			% RSD = 0.65
OCT.	250.51	0.00	0.00
	250.93	0.00	0.00
			$\bar{X} = 0.00$
			% RSD = 0.00
NOV.	250.62	0.00	0.00
	251.36	0.00	0.00
			$\bar{X} = 0.00$
			% RSD = 0.00
DEC.	251.08	4.28	1.10
	250.98	4.24	1.11
			$\bar{X} = 1.11$
			% RSD = 0.63

Table 16 Contents of Four Diterpenoid Compounds in the leaves of *Andrographis paniculata*, Sampling Monthly.

Month	% Amount of Diterpenoide Compounds (w/w)			
	C-2	C-3	C-4	C-5
January	3.97	1.17	1.95	2.03
Febuary	4.18	0.82	1.51	3.22
March	5.14	1.01	1.64	3.81
April	7.30	1.99	0.67	1.19
May	4.43	3.01	0.66	0.71
June	5.52	3.13	0.93	1.06
July	5.66	3.43	0.94	0.88
August	3.58	4.27	1.27	0.81
September	1.45	4.86	1.34	0.54
October	0.69	5.58	1.41	0.00
November	0.84	6.02	0.94	0.00
December	0.61	4.43	2.02	1.11

C-2 = Dehydroandrographolide

C-3 = Andrographolide

C-4 = Neoandrographolide

C-5 = Deoxyandrographolide-19 $\beta$ -D-glucoside

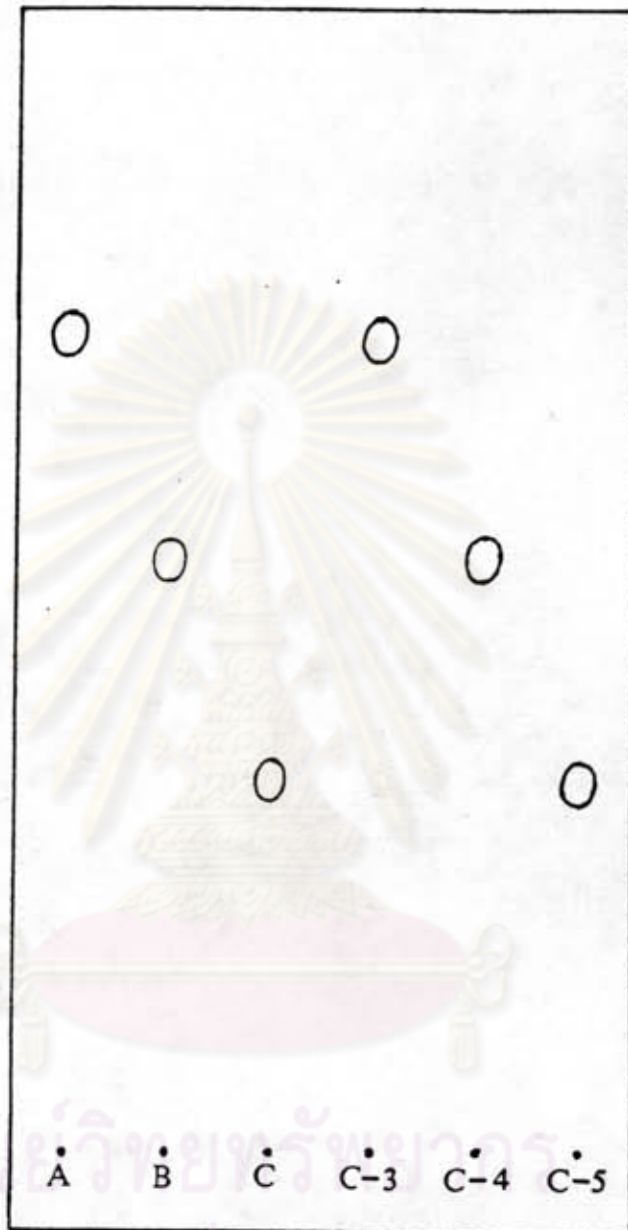


Figure 2 Thin Layer Chromatogram of C-3, C-4, C-5 Compound and Their Authentic Samples in Chloroform : Methanol (9:1)

- A = Authentic of Andrographolide
- B = Authentic of Neoandrographolid
- C = Authentic of Deoxyandrographolide-19 $\beta$ -D-glucoside

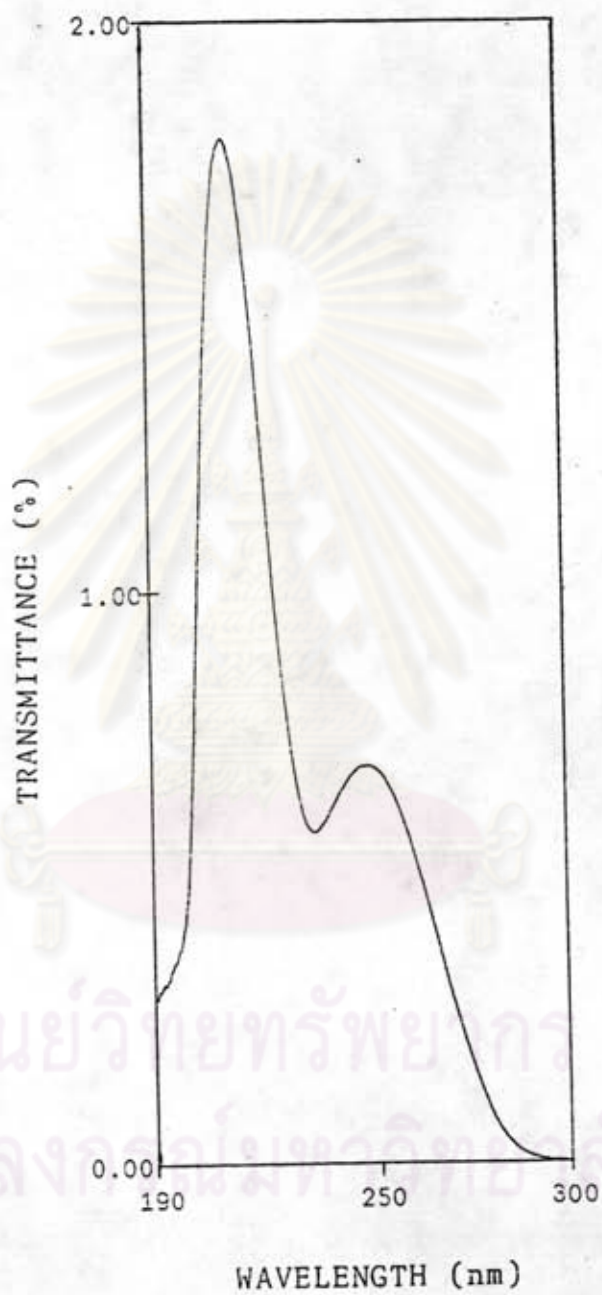


Figure 3 Ultraviolet Absorption Spectra of Dehydroandrographolide (C-2) in Ethanol.

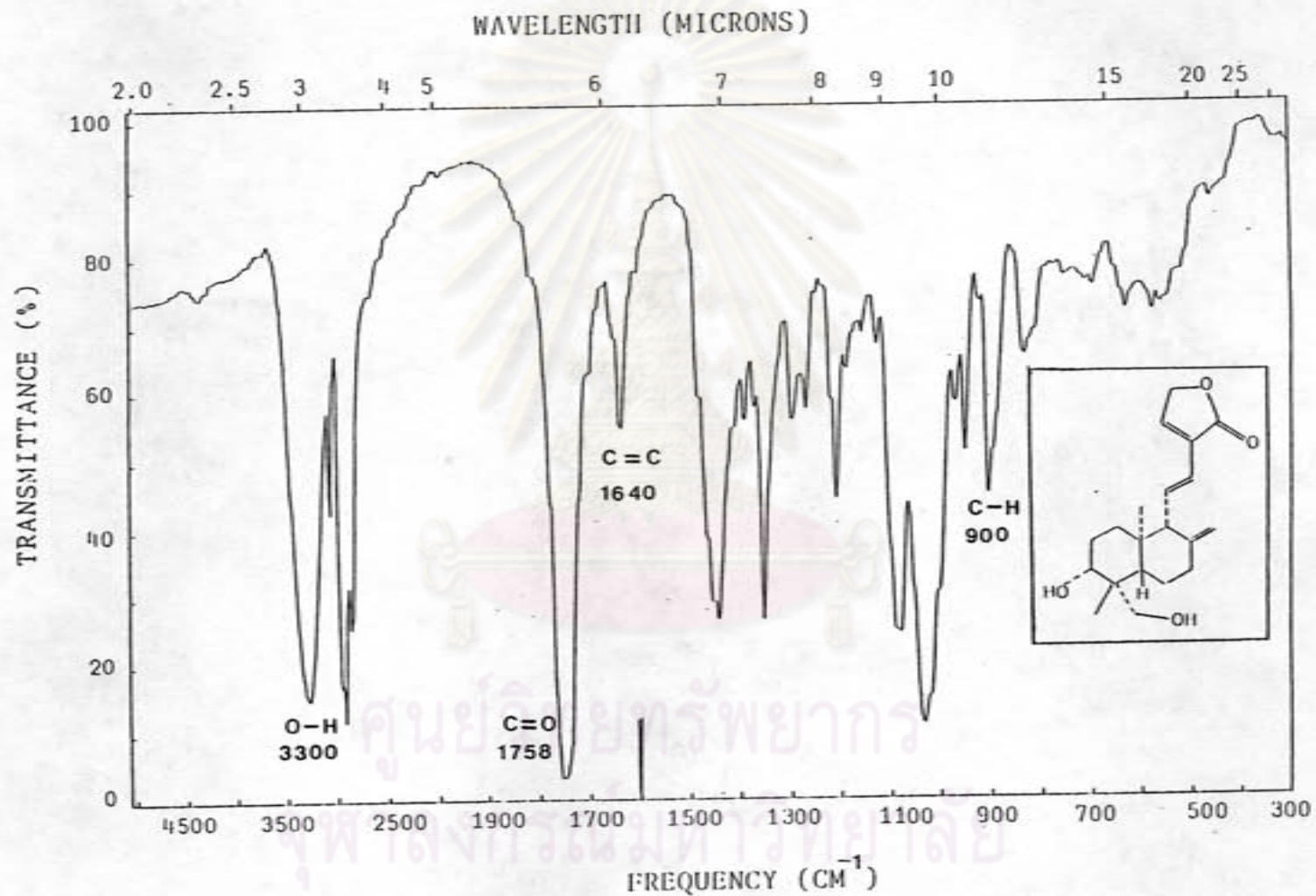


Figure 4 Infrared Absorption Spectra of C-2 Compound.



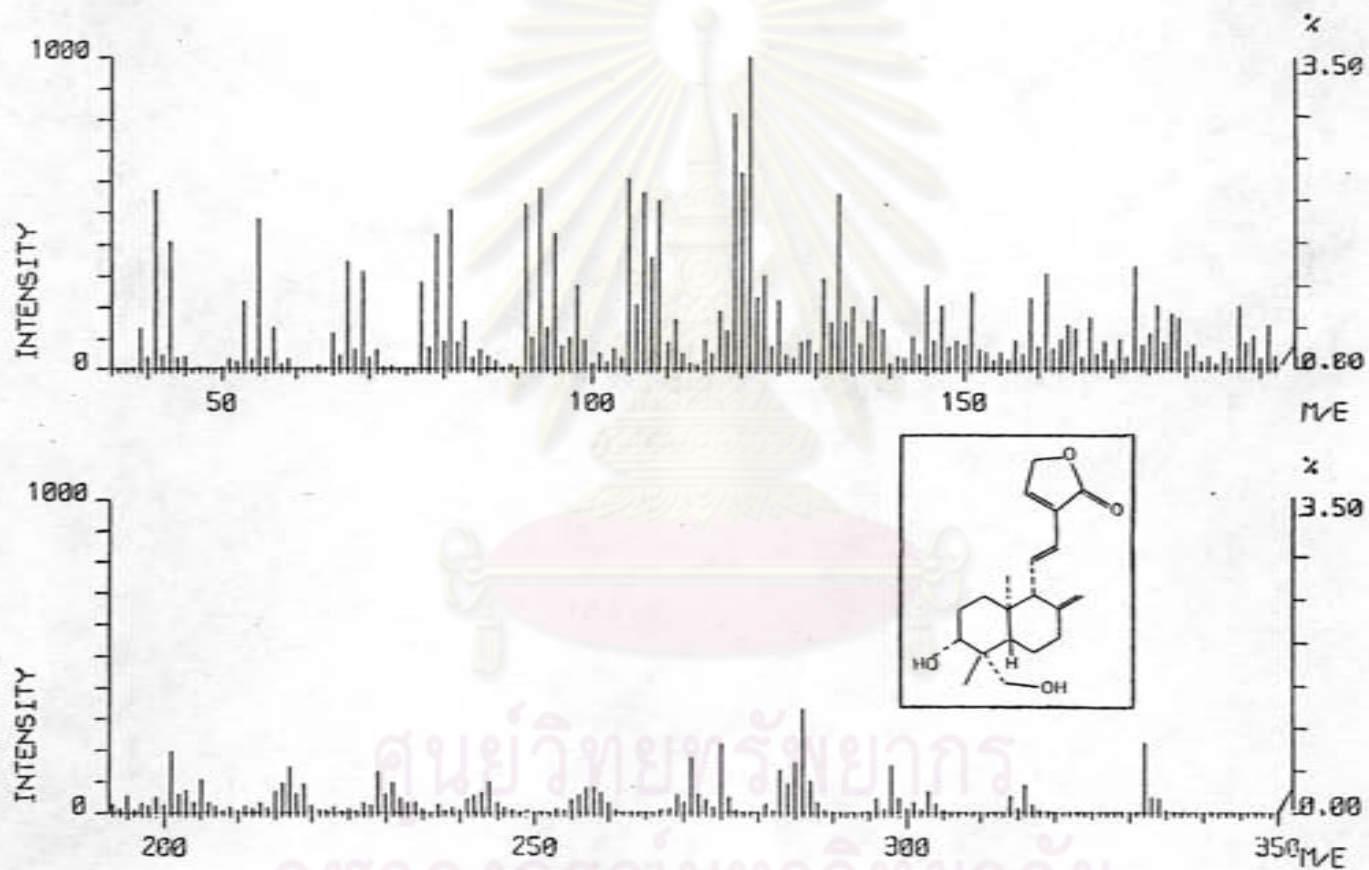


Figure 5 Mass Spectrum of Dehydroandrographolide (C-2).



Figure 6 Thin Layer Chromatogram of AC-1 and AC-2 in Chloroform

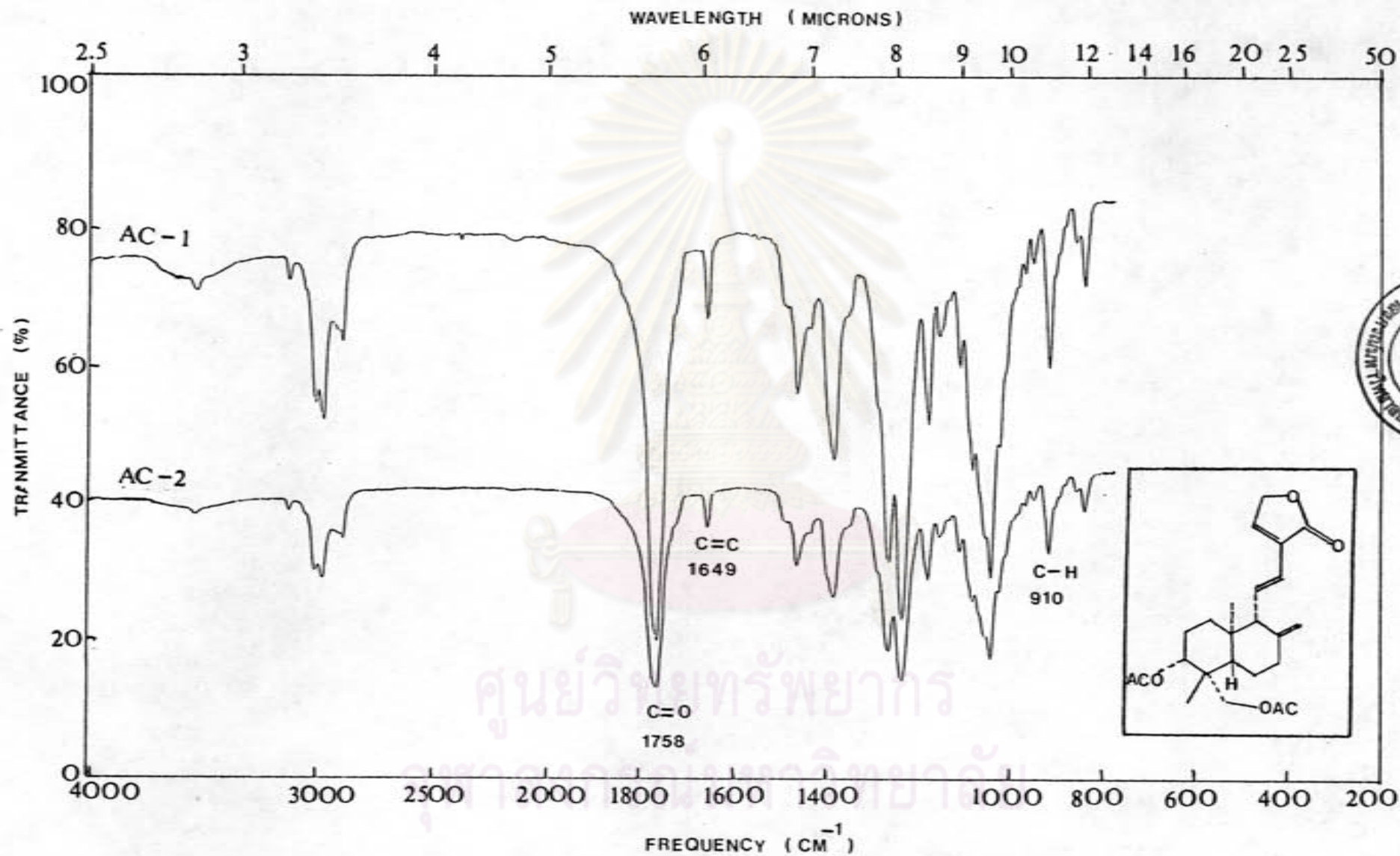


Figure 7 Infrared Absorption Spectrum of AC-1 and AC-2

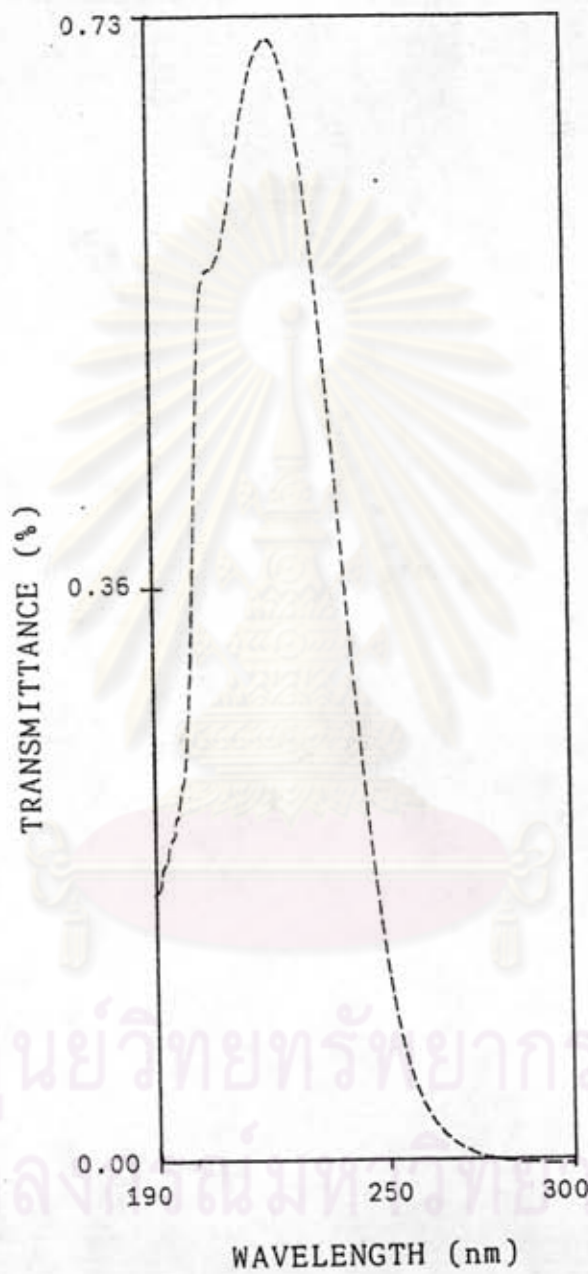


Figure 8 Ultraviolet Absorption Spectra of Andrographolide (C-3) in Ethanol.

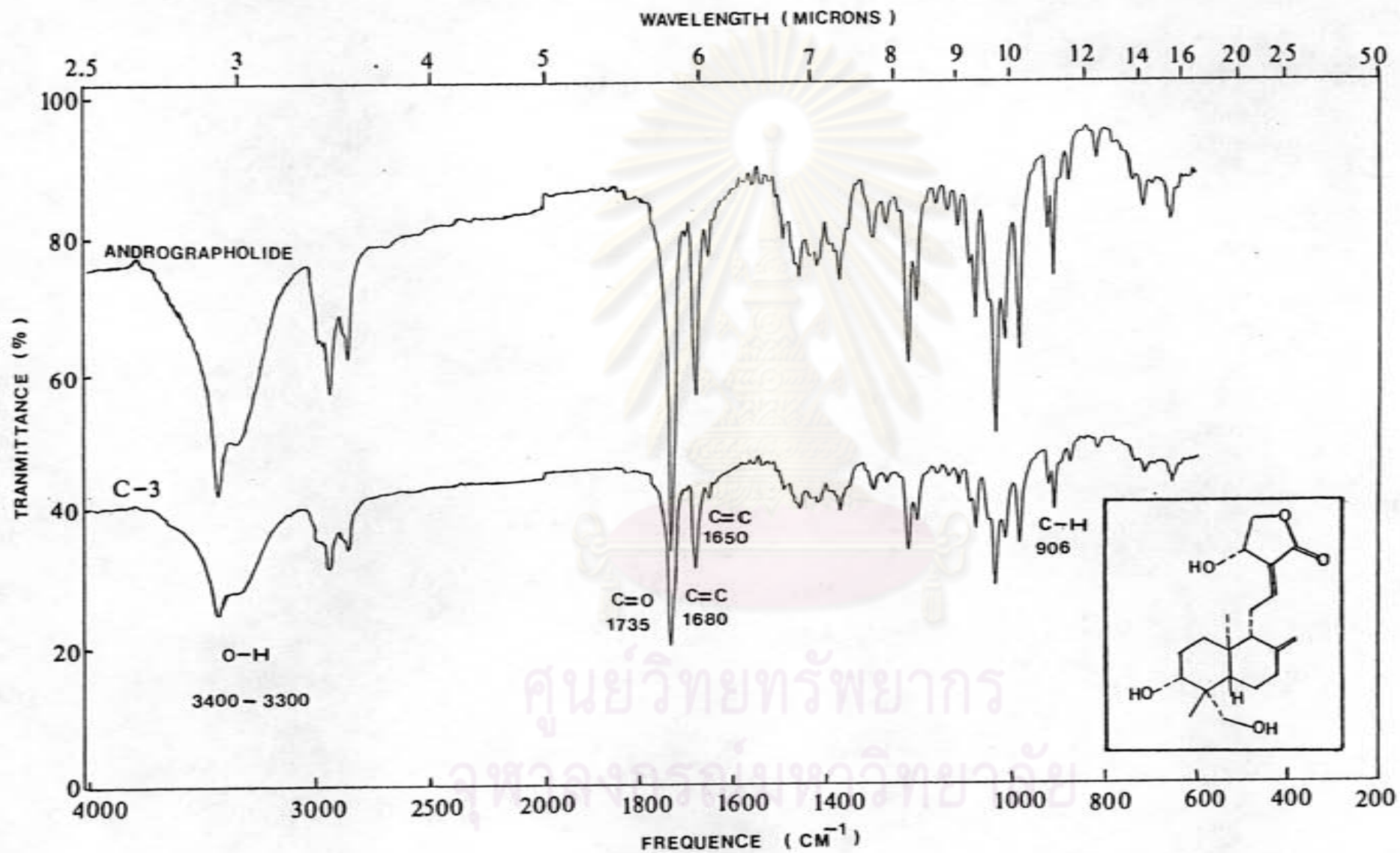


Figure 9 Infrared Absorption Spectrum of Andrographolide and C-3 Compound.

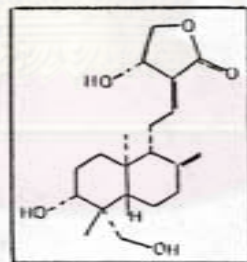
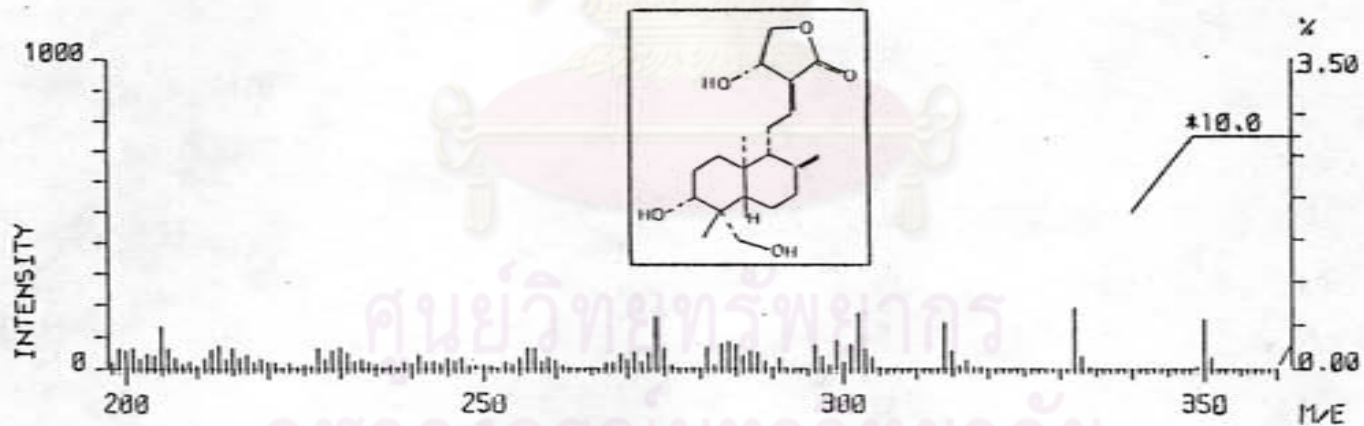
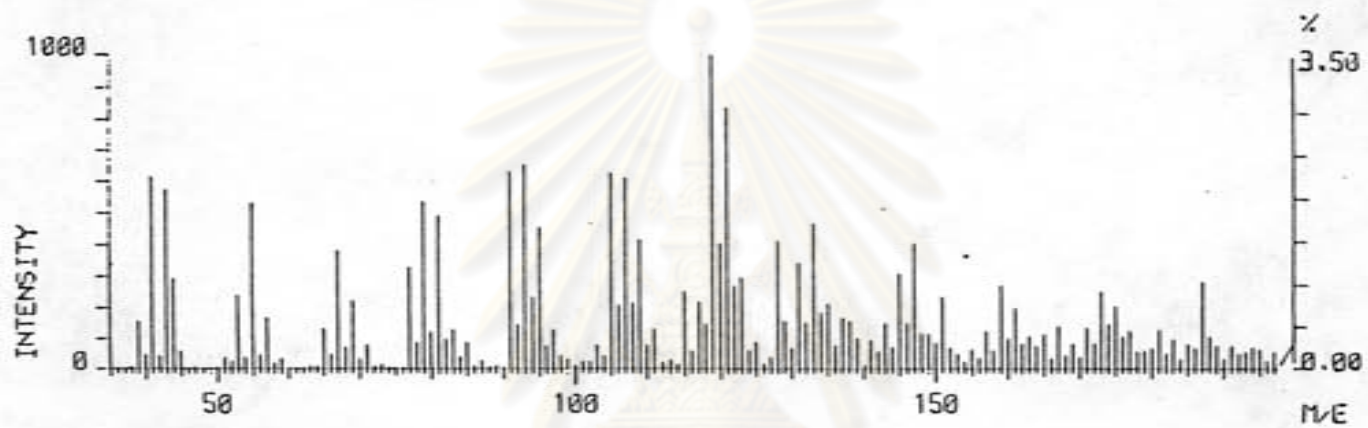


Figure 10 Mass Spectrum of Andrographolide (C-3).

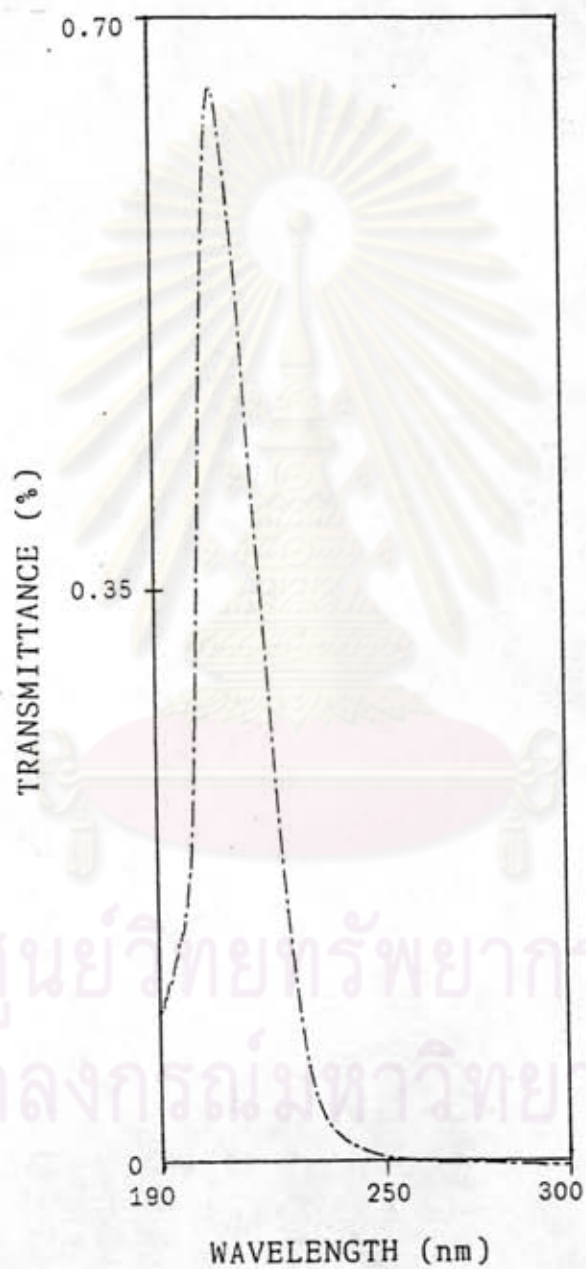


Figure 11 Ultraviolet Absorption Spectra of Neoandrographolide (C-4) in Ethanol.

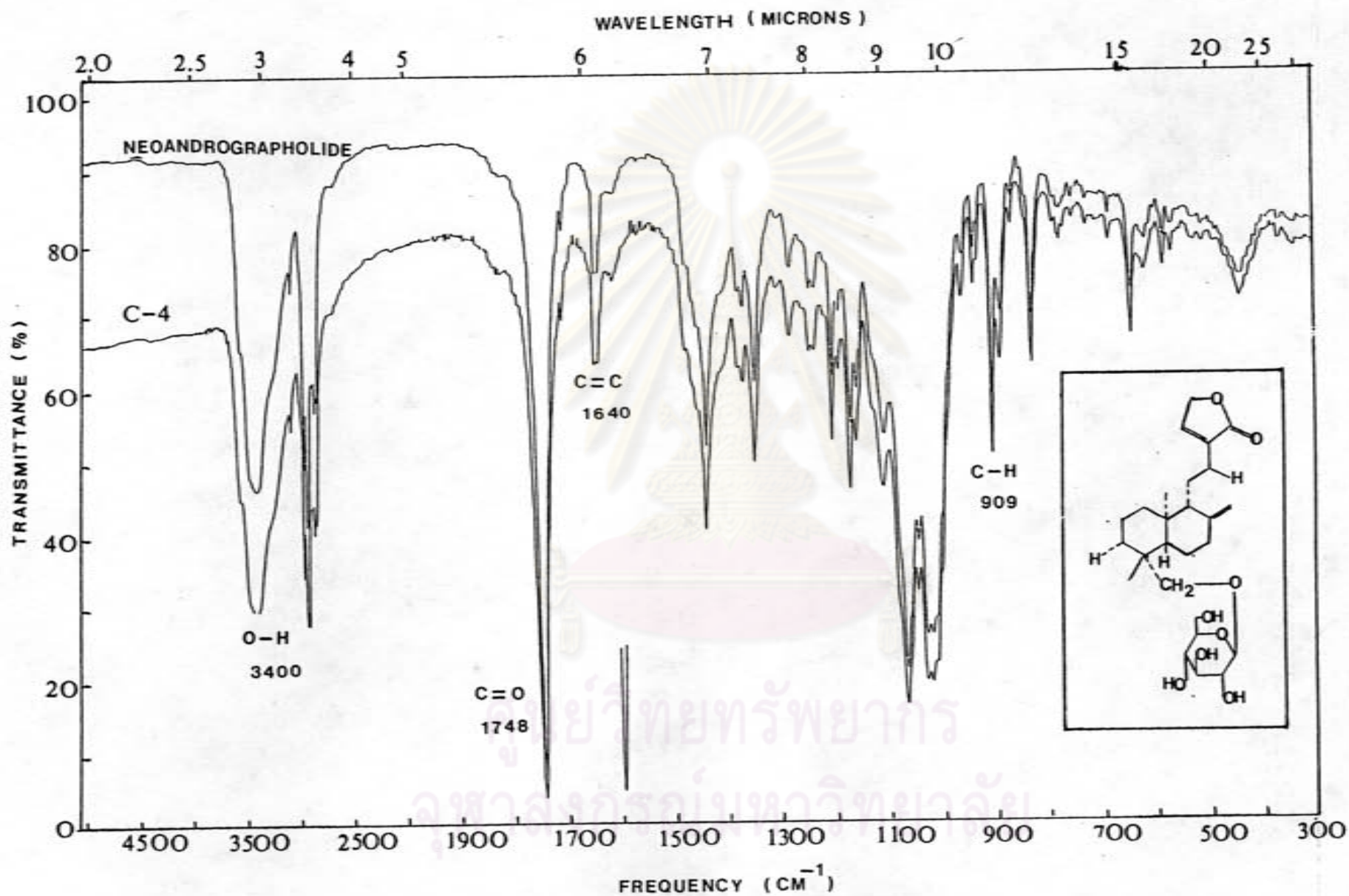


Figure 12 Infrared absorption Spectrum of Neoandrographolide and C-4 Compound.



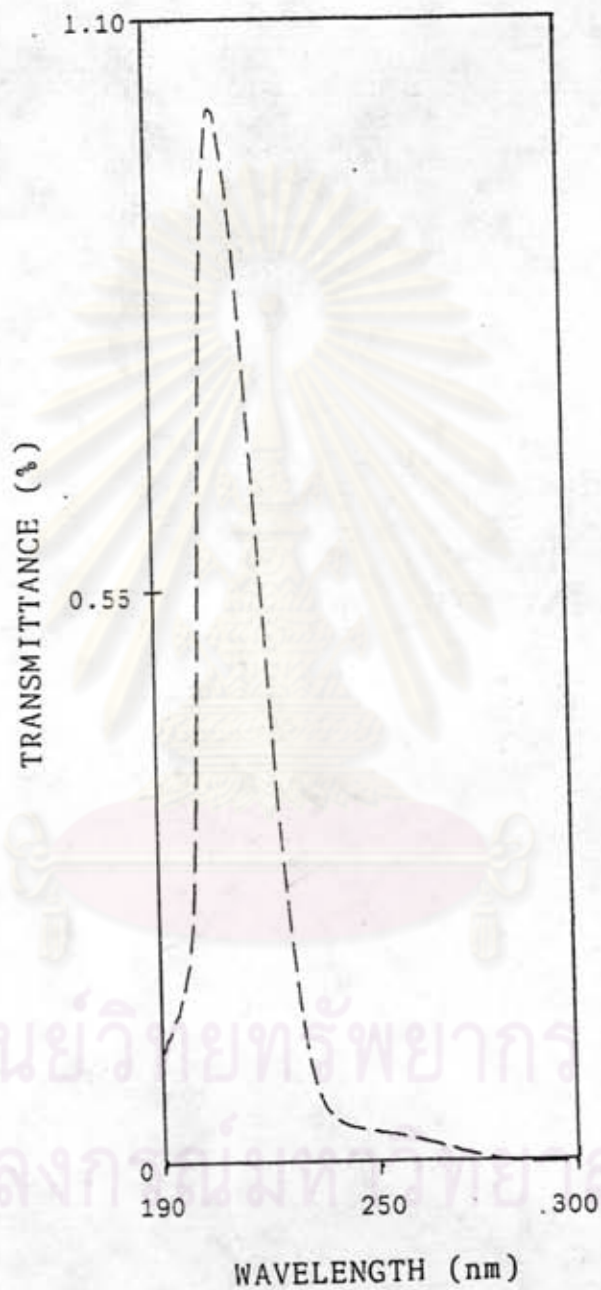


Figure 13 Ultraviolet Absorption Spectra of Deoxyandrographolide-19 $\beta$ -D-glucoside (C-5) in Ethanol.

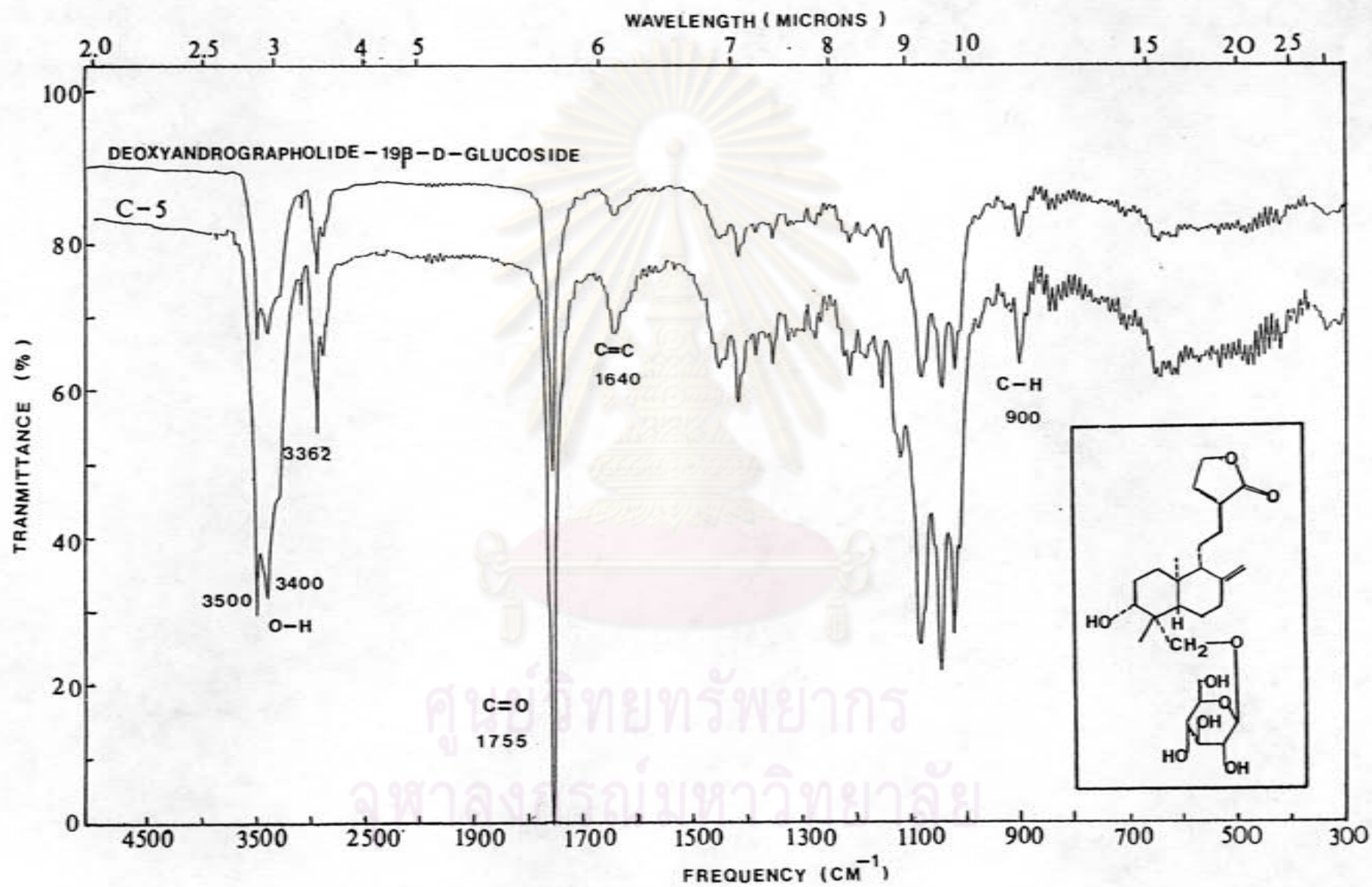


Figure 14 Infrared Absorption Spectrum of Deoxyandrographolide-  
-19 $\beta$ -D-glucoside and C-5 Compound



Figure 15 High Pressure Liquid Chromatogram of Four Diterpenoids Standard. Column  $\mu$ -bondapak  $C_{18}$ , methanol:water (42:58) as mobile phase, flow rate 2 ml/min, UV-detector 255,220 nm, sensitivity 0.1 AUFS andrographolide (Rt=5.69), deoxyandrographolide-19 $\beta$ -D glucoside (Rt=11.34), dehydroandrographolide (Rt=18.90) and neoandrographolide (Rt=25.34).



Figure 16 High Pressure Liquid Chromatogram of Methanolic Extract from the leaves of *Andrographis paniculata* 10 mg/ml in methanol. Column U-bondapak C<sub>18</sub>, methanol: water (42:58) as mobile phase, flow rate 2 ml/min, UV-detector 255,220 nm, sensitivity 0.1 AUFS. andrographolide (Rt=5.67), deoxyandrographolide-19 $\beta$ -D-glucoside (Rt=11.34), dehydroandrographolide (Rt=18.85), and neoandrographolide (Rt= 25.25).

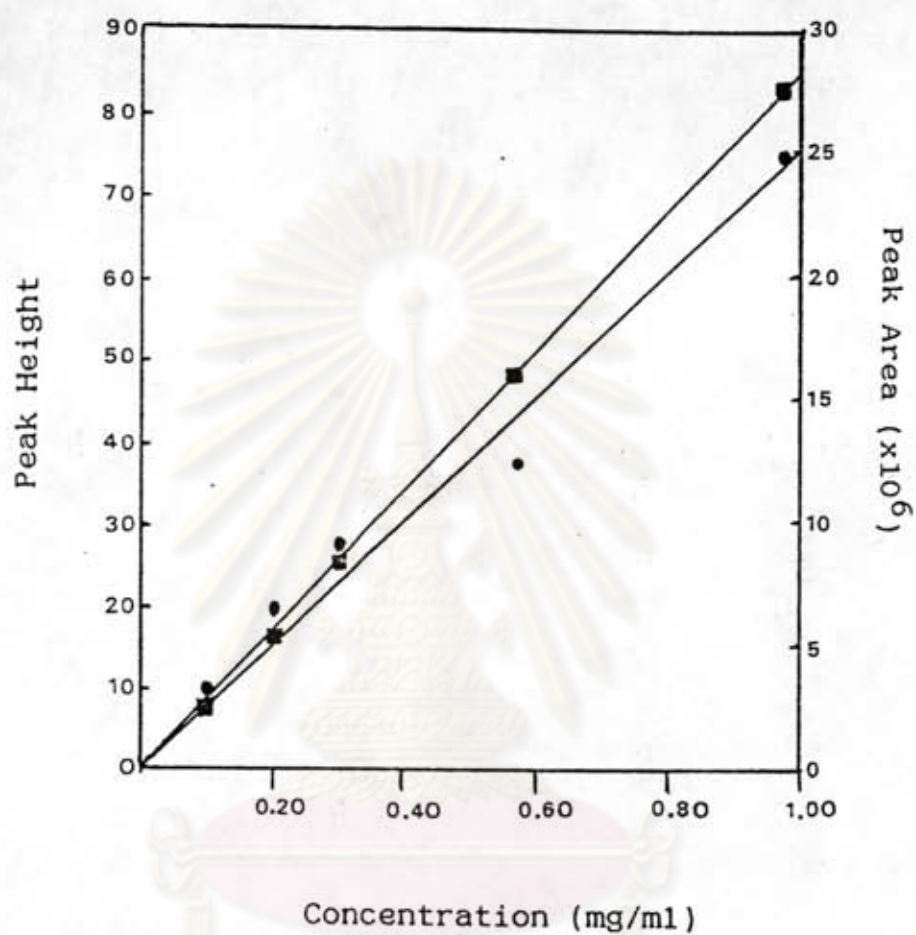


Figure 17 Calibration Curve of Dehydroandrographolide (C-2)

Key : ■ = Peak Height, R = 0.9990

• = Peak Area, R = 0.9709

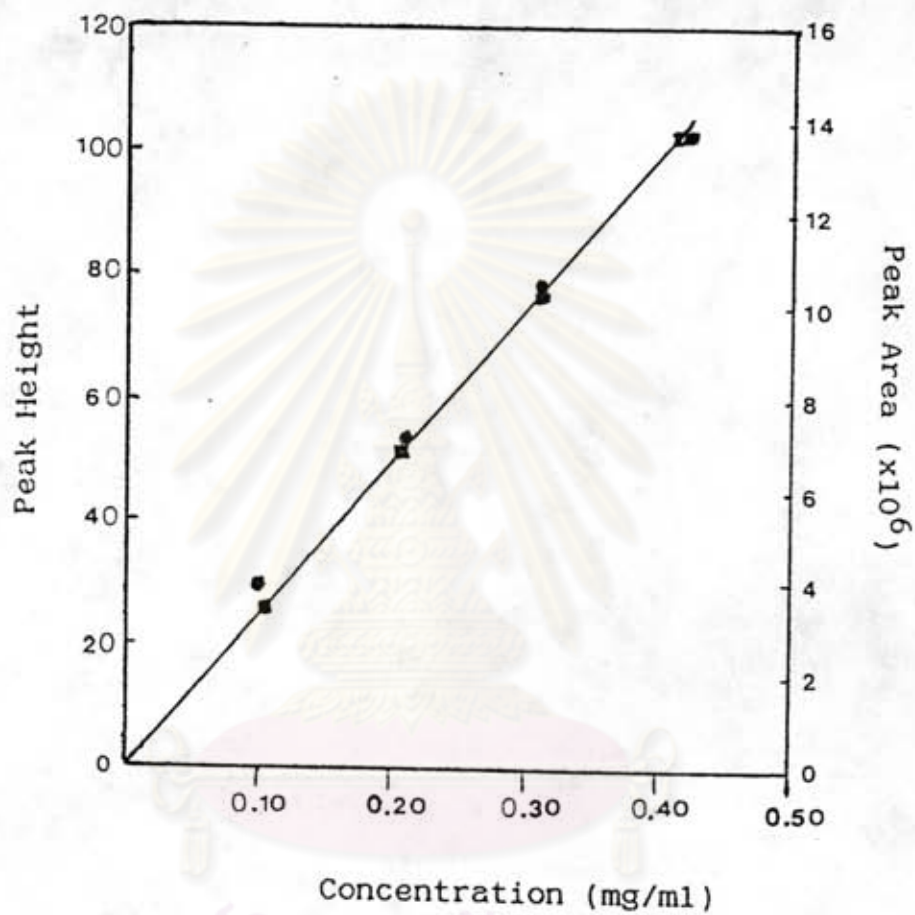


Figure 18 Calibration Curve of Andrographolide (C-3).

Key : ■ = Peak Height, R = 0.9998  
● = Peak Area, R = 0.9925

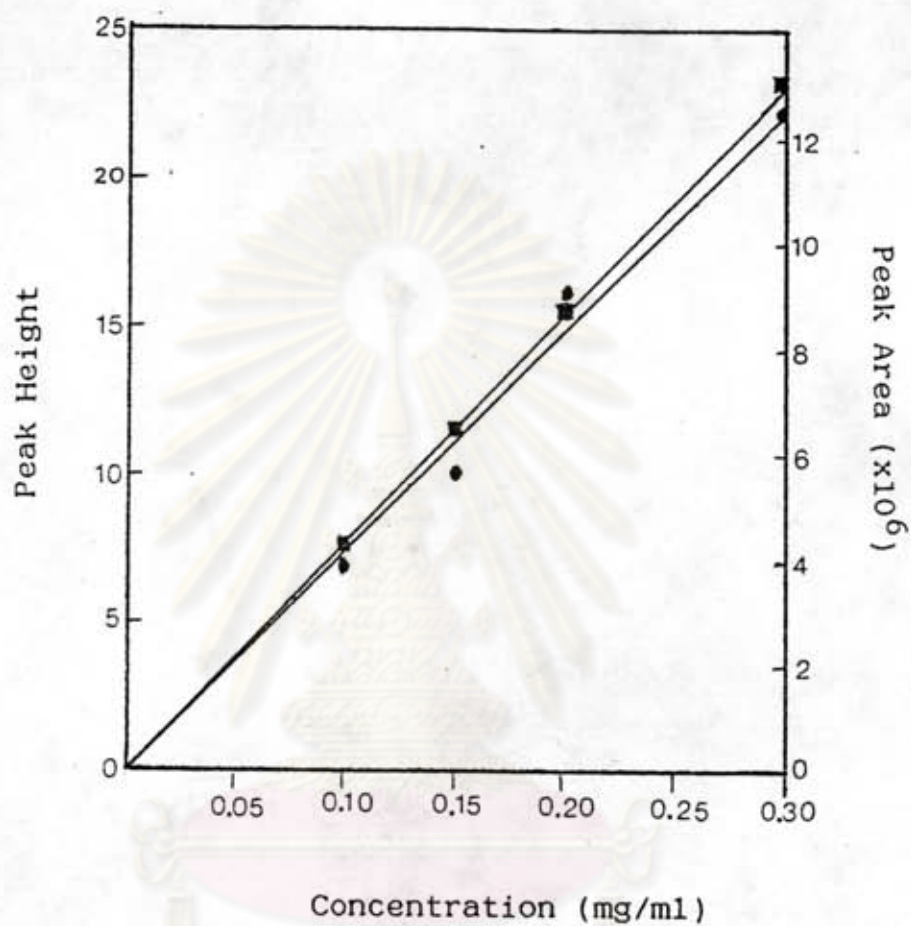


Figure 19 Calibration Curve of Neoandrographolide (C-4).

Key : ■ = Peak Height, R = 0.9998

• = Peak Area, R = 0.9886

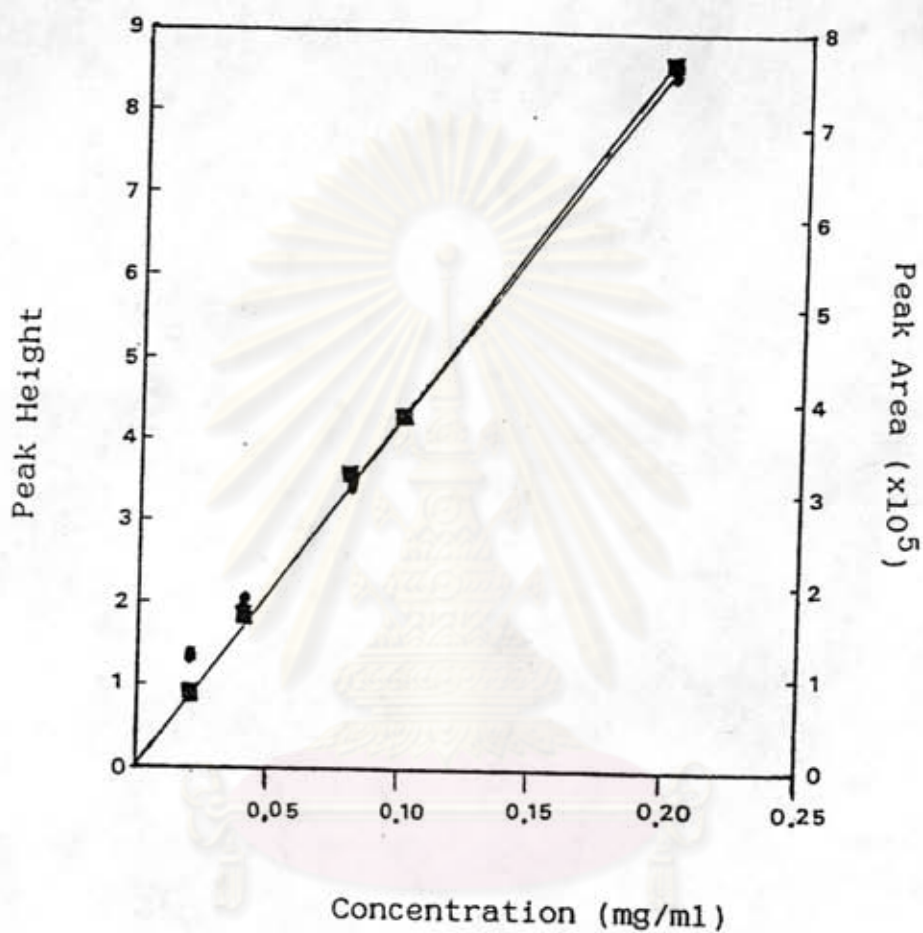


Figure 20 Calibration Curve of Deoxyandrographolide-19 $\beta$ -D-glucoside (C-5).

Key : ■ = Peak Height, R = 0.9991  
● = Peak Area, R = 0.9917



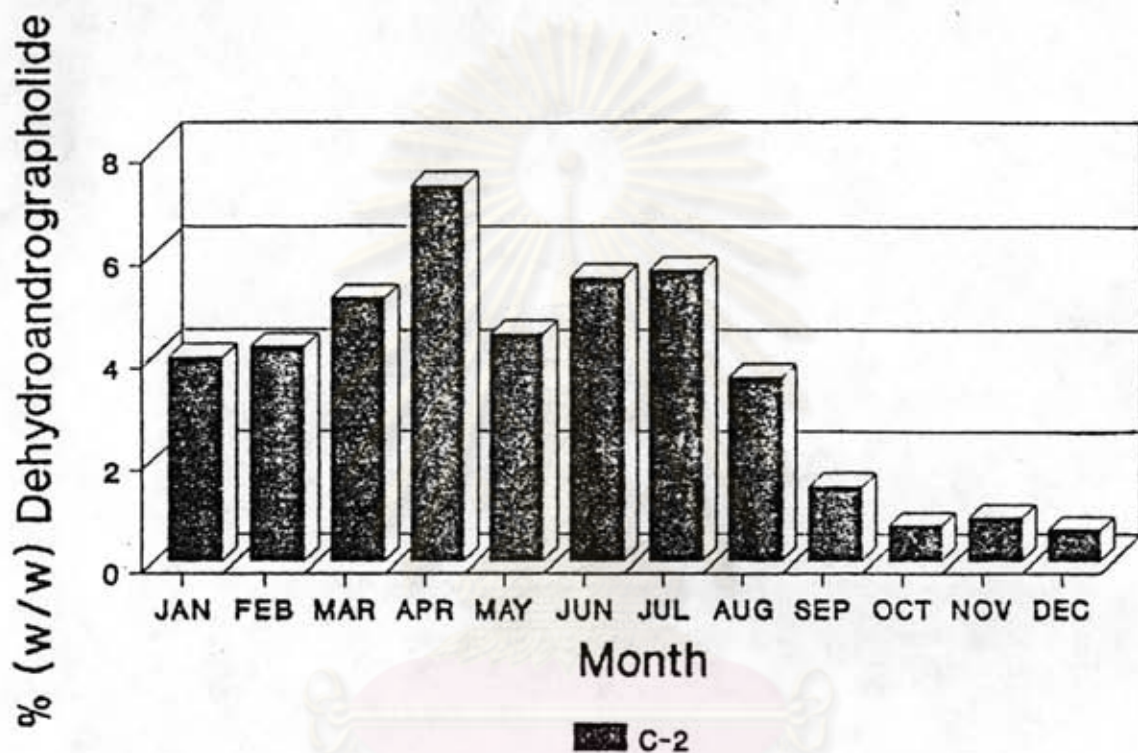


Figure 21 Contents of Dehydroandrographolide (C-2) in the leaves of *Andrographis paniculata*, Sampling Monthly.

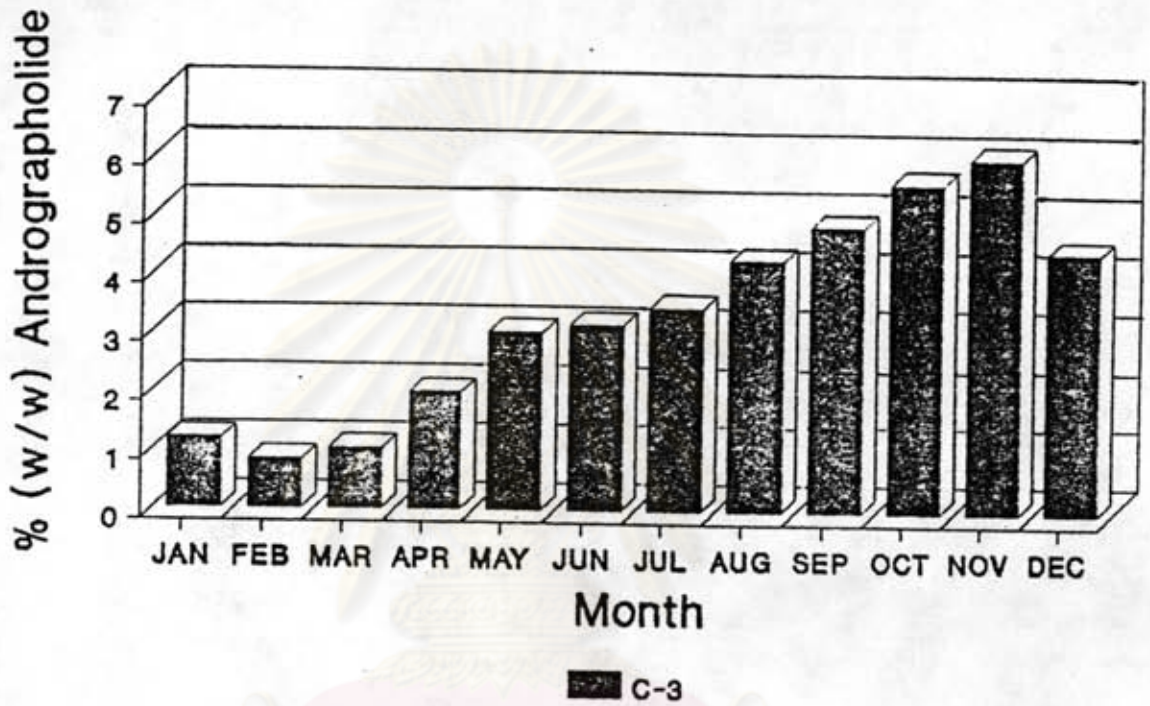


Figure 22 Contents of Andrographolide (C-3) in the Leaves of *Andrographis paniculata*, Sampling Monthly.

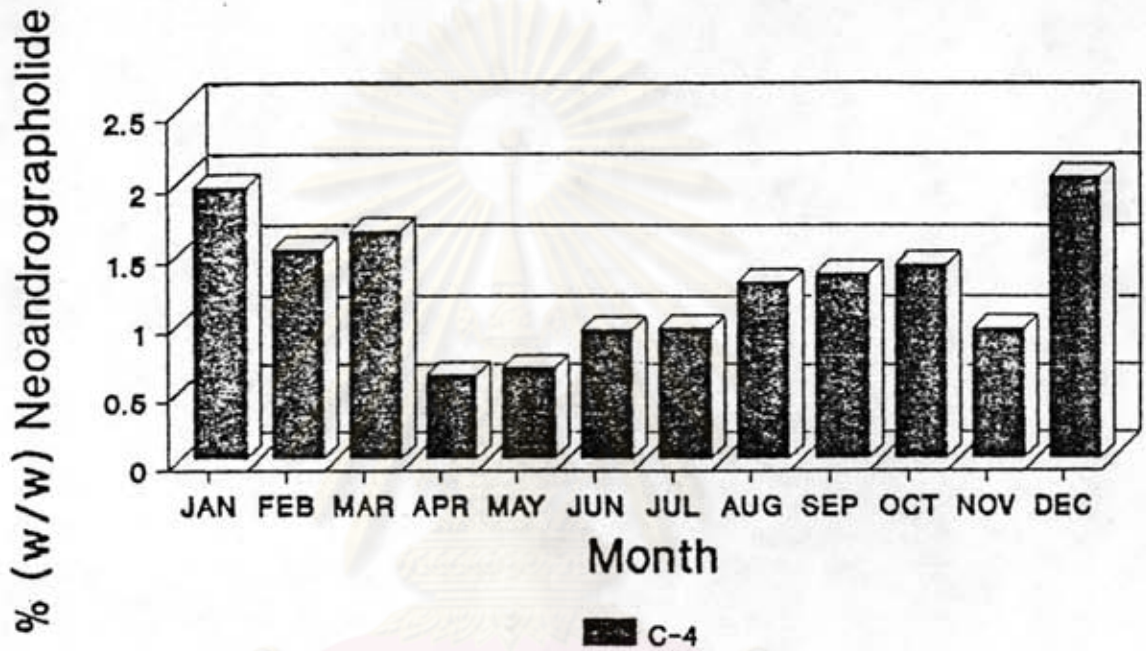


Figure 23 Contents of Neoandrographolide (C-4) in the leaves of *Andrographis paniculata*, Sampling Monthly.

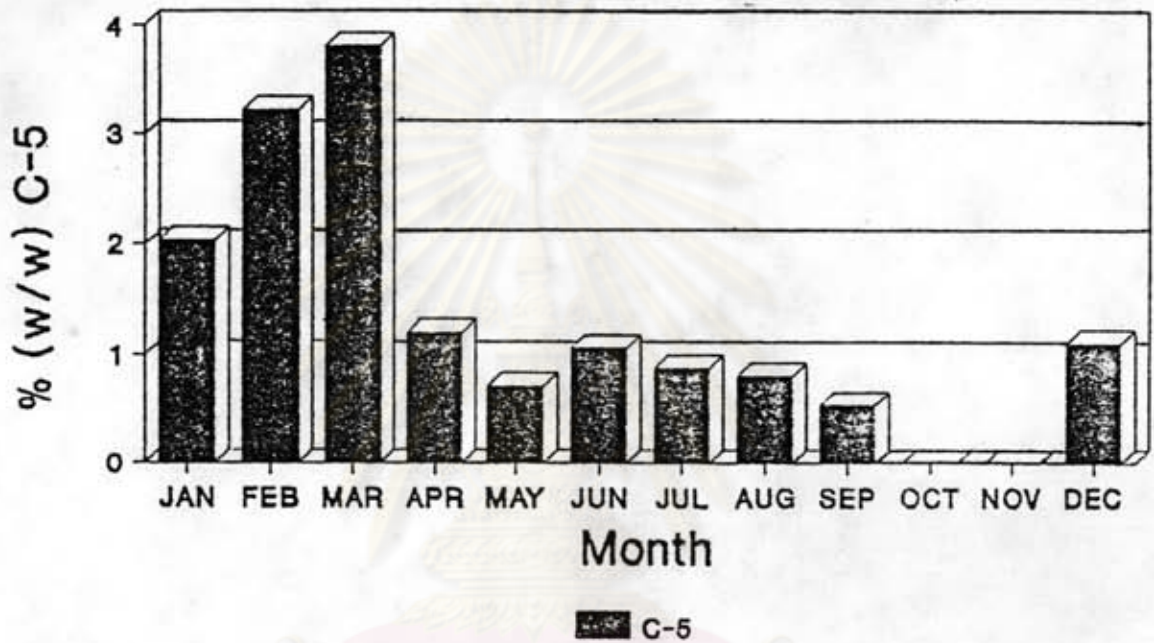


Figure 24 Contents of Deoxyandrographolide-19 $\beta$ -D-glucoside (C-5) in the leaves of *Andrographis paniculata*, Sampling Monthly.

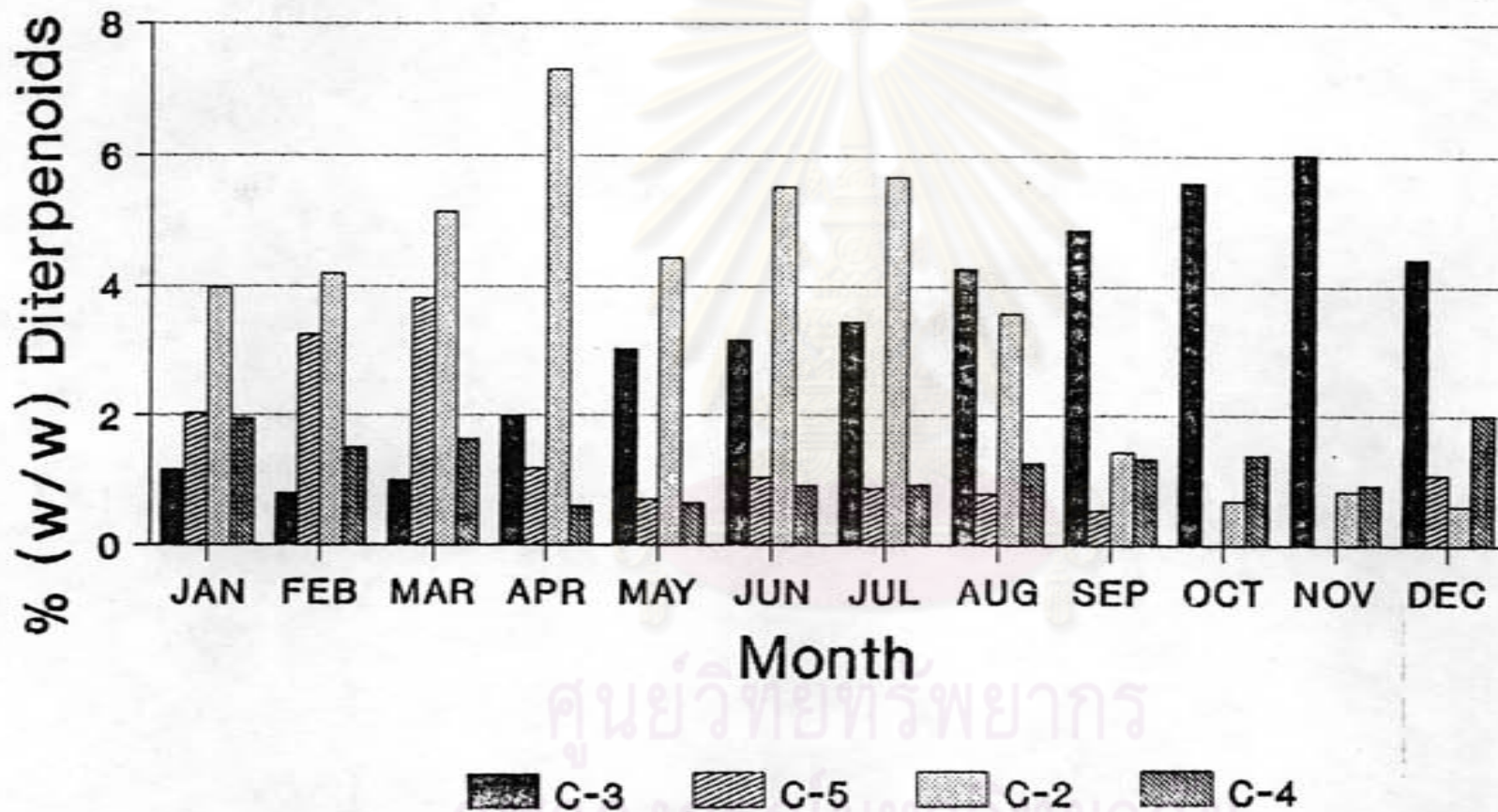


Figure 25 Contents of Four Diterpenoid Compounds in the leaves of *Andrographis paniculata*, Sampling Monthly.

### Statistical Evaluation of the Results

The reproducibility of the analysis was evaluated by the determination of the relative standard deviation (RSD) as follows.

$$\bar{X} = \frac{\sum X_i}{n}$$

$$SD = \sqrt{\frac{\sum (X_i - \bar{X})^2}{n - 1}}$$

$$\% \text{ RSD} = \frac{SD}{\bar{X}} \times 100$$

$\bar{X}$  = Mean

$X_i$  = The value of the  $i^{\text{th}}$  observation

$n$  = The number of observations

SD = The standard deviation

RSD = The relative standard deviation

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