Chapter IV

Result and Discussion

The 100 g of dried ground aerial part of Andrographis paniculata Nees. was refluxed with 95% ethanol and fractionated with chloroform and water.

Compound C-2, C-3, C-4 and C-5 were separated from chloroform crude extract. Compound C-5 and C-6 were separated from butanol crude extract. The physicochemical properties of each compound was performed as follow.

1. Compound C-2, 14-deoxy-11,12-didehydroandrographolide

4.53 g (4.53% yield) of white crystal was obtained from fraction 28-43 of chloroform crude extract and recrystallised in chloroform.

Solubility soluble in chloroform, benzene, Ethanol, Methanol ; insoluble in water

Rf value

system			Rf
Chloroform			0.11
Chloroform	: Methanol	9:1	0.93
Chloroform	: Acetone	9:1	0.31
Chloroform	: Ab. Ethanol	85:15	0.95
Methanol	: Benzene	1:1	0.95

Melting point 203-204°C (Balmain and Connolly, 1973)

Molecular formular $C_{20}H_{28}O_4$

Molecular weight 332

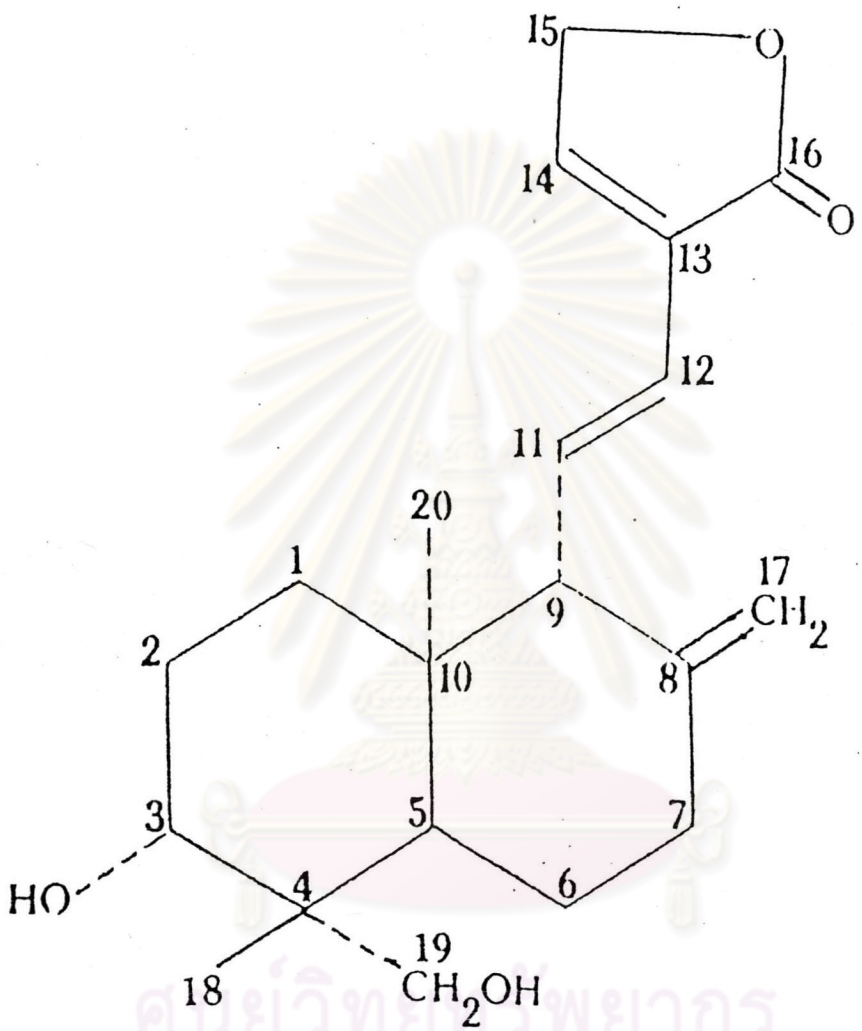


Figure 17 Structure of compound C-2 , 14-deoxy-11,12-didehydroandrographolide

Spectral data

UV spectrum λ_{max} in chloroform = 248 nm

IR spectrum

= 3275 cm^{-1} (O-H stretching)

3050 cm^{-1} (C=C-H stretching)

2975 cm^{-1} (C-H stretching)

1740 cm^{-1} (C=O stretching)

1640 cm^{-1} (C=C)

1100-1050 cm^{-1} (C-O stretching)

Mass spectrum

m/z = m/e (rel. int.)

= 332 (232.6),133.0 (501.6),121.0 (1000.0), 120.0 (683.2), 119.0 (766.8), 109.0 (506.4), 107.0 (517.3), 105.0 (543.3)

Nuclear Magnetic Resonance spectrum

Table 3 Proton chemical shift of compound C-2 in $CDCl_3$

Proton	Chemical shift(ppm)
H - 1 (a, b)	1.15
H - 2 (a, b)	1.80, 1.80
H - 3	3.40
H - 5	1.20
H - 6 (a, b)	1.75, 1.30
H - 7 (a, b)	1.90, 2.40
H - 9	2.30
H - 11	6.75
H - 12	6.10
H - 14	7.15
H - 15 (a, b)	4.90
H - 17 (a,b)	4.85, 4.55
Me - 18	1.20
H - 19 (a,b)	3.30, 4.25
Me - 20	0.65

Assignment of proton signal of 14-deoxy-11,12 didehydroandrographolide was started from the down field signal at 7.15 ppm as H-14. In 2-D COSY spectrum the coupling of H-14 and H-15 was shown.

The second peak in the double bond region was assigned as H-11. The spliting pattern of H-11 was a doublet of doublet with the coupling constant : $J_{11,12}$ = 18 Hz and $J_{11,9}$ = 10 Hz. From coupling constant revealed that H-11 was trans configuration with H-12 and dihedral angle between H-11 and H-9 was 0^{o} or 180^{o}.

From H-11 the assignment of another proton was H-9 that showed direct coupling with H-11 in 2-D spectrum and allylic coupling with H-17(a,b). The H-17 methylene proton could be assigned at 4.85 and 4.55 ppm.

Another allylic coupling of H-17 was the coupling with H-7(a) at 1.90 ppm. From H-7(a) , led to the assignment of geminal proton of H-7 (b) at 2.40 ppm ($J_{7,7}$ = 10 Hz) and the assignment of H-6 (a)vicinal coupling at 1.75 ppm. H-6(b) was identified by the geminal coupling pattern in proton spectrum at 1.3 ppm. and confirmed with direct coupling with H-6 (a) in 2-D COSY expanded scale.

H-5 could be identified by through spece coupling with H-3 at 1.2 ppm. and direct coupling with H-6 (a) and H-6(b) as shown in 2-D spectrum.

H-3 was a proton connected with heteroatom that showed the spectrum at 3.4 ppm as a triplet due to vicinal coupling with H-2(a,b). From 2-D spectrum the coupling of H-3 and H-2(a) was shown. The H-2 (a,b) which was assigned at 1.82 ppm led to position of H-1 (a,b) at 1.15 ppm.

The remain proton peaks in heteroatom coupling region were two doublet of doublet signals at 3.30 and 4.25 ppm. The signal expressed only geminal coupling in 2-D spectrum , so the proton were assigned as H-19 (a) and H-19 (b).

In 2-D spectrum, H-18 methyl proton could be identified by long range coupling with H-19 (a,b) at 1.20 ppm. and the remain methyl group at 0.65 ppm. was assigned as C-20 methyl proton.

Table 4 Carbon-13 chemical shift of compound C-2 in $CDCl_3$

Carbon	Chemical shift(ppm)
C-1	22
C-2	28
C-3	79
C-4	42
C-5	56
C-6	23
C-7	36
C-8	132
C-9	54
C-10	39
C-11	121
C-12	135
C-13	146.5
C-14	146
C-15	70
C-16	174
C-17	107
C-18	24
C-19	63
C-20	15

Assignment of carbon-13 spectrum was started with comparing the spectrum of carbon 13 decoupling spectrum with DEPT 135 spectrum.

From DEPT 135 revealed that there were 6 methine groups, 2 methyl groups and 7 methylene groups.

A methine group in the down field region at 147 ppm was assigned as C-14 . The remain 2 methine groups in unsaturated region at 135 and 121 ppm were assigned as C-11 and C-12 respectively.

A methylene group in unsaturated region at 107 ppm was assigned as C-17.

Carbon atoms in heteroatom coupling region (50-100 ppm) included C-3 at 79 ppm, C-15 at 65 ppm, C-19 at 63 ppm, C-5 at 56 ppm and C-9 at 55 ppm.

The remain carbon signals were C-7, C-2, C-6, C-1 at 37 ppm, 28 ppm, 23 ppm and 22 ppm respectively.

The two methyl groups at 20-25 ppm and 15 ppm were assigned as C-18 methyl and C-20 methyl respectively.

2. Compound C-3, Andrographolide

3.52 g (3.52% yield) of rhombic crystal was obtained from fraction 44-57 of chloroform crude extract and recrystallised in chloroform and methanol

Solubility soluble in methanol, dimethyl sulfoxide, ethyl acetate, ether, acetone, ethanol; insoluble in water.

Rf value

system		Rf
Chloroform : Methanol	9:1	0.56
Chloroform : Methanol	8:1	0.76
Chloroform : Acetone	9:1	0.08
Chloroform : Ab. Ethanol	85:15	0.86
Methanol : Benzene	1:1	0.95

Melting point 230-231°C (Cava et al. , 1962)

Molecular formular $C_{20}H_{30}O_5$

Molecular weight 350

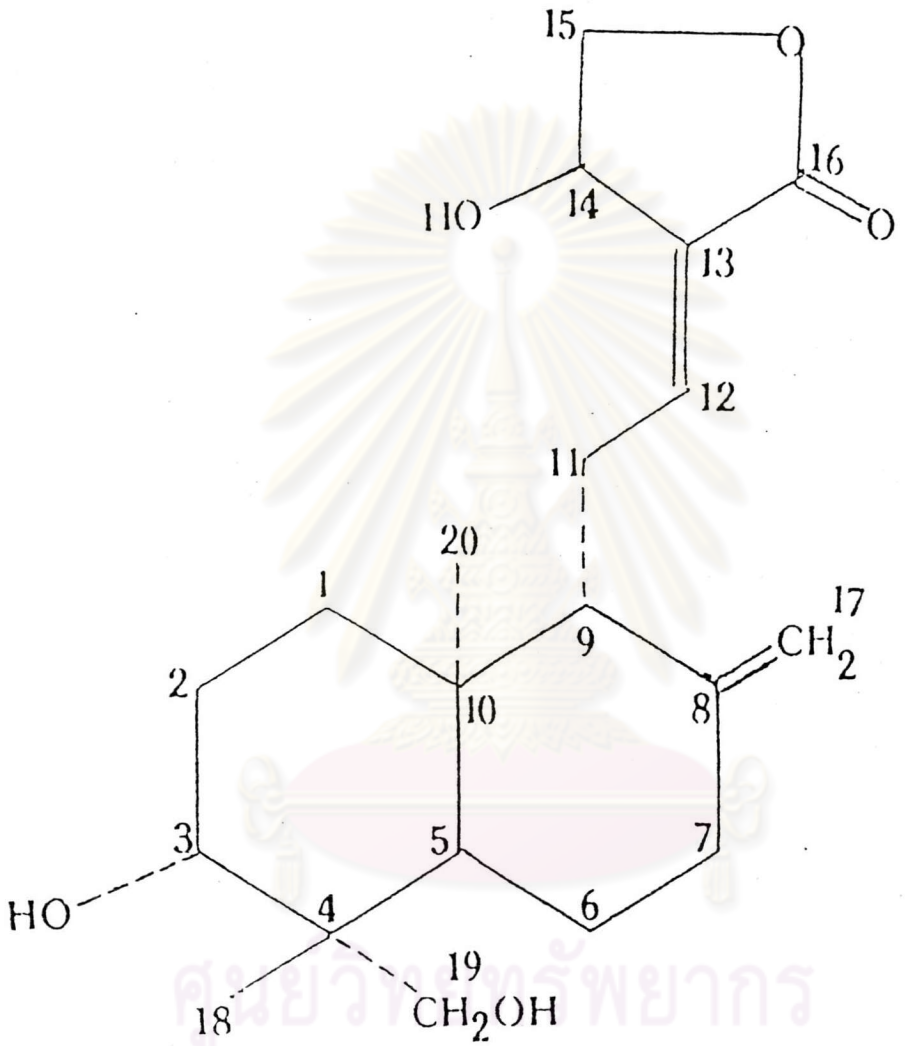


Figure 26 Structure of compound C-3 , Andrographolide

Spectral data

UV spectrum λ_{max} = 233

IR spectrum

= 3450 cm^{-1} (OH stretching)

2950-2900 cm^{-1} (C-H stretching)

1720 cm^{-1} (C=O stretching)

1650 cm^{-1} (C=C)

1250-1050 cm^{-1} (C-O stretching)

Mass spectrum

m/z = m/e (rel. int.)

= 350 (125) , 332 (175) , 121 (875) ,

119 (1000) , 109 (650) , 105 (650) ,

93 (675) , 91 (650)

The m/e 332 reveal that the molecule was dehydrated

Nuclear Magnetic Resonance Spectrum

Table 5 Proton chemical shift of compound C-3 in DMSO-d6

Proton	Chemical shift (ppm.)
H - 1 (a,b)	1.20
H - 2 (a,b)	1.70
H - 3	3.25
H - 5	1.20
H - 6 (a,b)	1.75 , 1.40
H - 7 (a,b)	2.00 , 2.35
H - 9	1.85
H - 11 (a,b)	2.50
H - 12	6.70
H - 14	4.90
H - 15 (a,b)	4.35, 4.10
H - 17 (a,b)	4.80, 4.80
Me- 18	1.10
H - 19 (a,b)	4.15, 3.20
Me- 20	0.65

The 200 MHz ^{1}H spectrum and 2-D COSY 45 spectrum of compound C-3 showed the C-12 olefinic proton as the most deshield proton at 6.70 ppm. It occured as a triplet of doublet due to vicinal coupling with C-11 methylene proton and allylic coupling with H-14 .

The H-14 resonated as a triplet due to coupling with H-15 (a) and H-15 (b) that could be seen in 2-D spectrum.

The H-15(a) and H-15(b) appeared as two double doublets. The H-15 (a) that recieved shielding effect from C-14 OH showed the proton signal at higher field than H-15 (b).

The two H-11(a,b) could be seen in 2-D spectrum due to direct coupling with H-12 . H-9 that had direct coupling with H-11 could be assigned and confirmed with allylic coupling with H-17(a,b)

To begin the assignment of H-7(a,b) was achieved by seeing allylic coupling with H-17(a,b) in 2-D spectrum.

H-7(a) was assigned at chemical shift = 2 ppm. From H-7 (a) led to positioned of H-7(b) at 2.35 ppm. and H-6(a,b) at 1.75 ppm. and 1.4 ppm.

Assignment of H-2 (a,b) and H-1 (a,b) were very difficult. The 2-D spectrum showed coupling of H-3 with H-2 in a cluster of coupling. So the two groups of

coupling at about 1.70 and 1.20 ppm. might include H-1 (a,b), H-2(a,b),and H-6(a,b)

The remain assignments were methylene proton of C-19 , two methyl groups at C-18 and C-20 , three-OH signals of C-14 ,C-3 and C-19.

In proton spectrum, a doublet peak at 5.65 ppm was integrated one proton. Direct coupling of this peak with H-14 could be seen in 2-D COSY , so the peak could be assigned as the OH of C-14.

A doublet peak at 5.1 ppm. was integrated one proton. Direct coupling of this peak with H-3 could be seen in 2-D spectrum , so the peak could be assigned as the OH of C-3.

The remain one proton signal at 3.9 ppm. was assigned as the OH of C-19. The coupling with H-19(a,b) could be seen in 2-D spectrum.

Two methylene proton of C-19 were assigned at chemical shift = 4.1 ppm. and 3.2 ppm respectively.The geminal coupling of the protons could be seen in 2-D spectrum. The chemical shift of the two protons was difference due to 1-3 interaction of one proton with methyl group of C-10 which cause shielding effect to the proton.

The excess one proton at 3.9-3.4 ppm. might be the impurities from water in the molecule. The signal presented as a high intensity peak that did not coupling with any peak in 2-D spectrum.

Table 6 Carbon - 13 chemical shift of compound C-3 in DMSO-d6

Carbon	chemical shift(ppm.)
C-1	22
C-2	27
C-3	79
C-4	42
C-5	56
C-6	37
C-7	38
C-8	129
C-9	58
C-10	39
C-11	24
C-12	147
C-13	147
C-14	65
C-15	75
C-16	169
C-18	23
C-19	63
C-20	15

To begin the assignment of ^{13}C of compound C-3, the ^{13}C decoupling spectrum was compare with DEPT 135 and DEPT 90 spectrum to identify CH, CH_2, CH_3, and quaternary carbon atom (-$\overset{|}{\underset{|}{C}}$-) in molecule.

DEPT 90 spectrum showed only CH in the molecule which are 5 signals. A down field signal at 146 ppm. that was in double bond region could be assigned as C-12 signal.

Three CH signals at chemical shift 50 -100 ppm. revealed that carbon connected with heteroatom. From C,H COSY signal of C-14, C-3, and C-19 could be assigned at 65 ppm.,79 ppm. and 63 ppm. respectively.

The remain two CH groups were the C-9 and C-5 that could be assigned by C,H COSY at 58 ppm.and 56 ppm. respectively.

Two methyl group and eight methylene group could be assigned from DEPT 135. Positive peaks at 15 ppm. and 23 ppm. were assigned as C-20 and C-18 respectively. Negative peaks in double bond region at 103 ppm. could be assigned as C-17. Two negative peaks in heteroatom coupling region could be assigned as C-15 at 75 ppm. and C-19 at 63 ppm. The remain negative peak at high field region could be assigned from C,H COSY as C-7 at 38 ppm., C-6 at 37 ppm. ,C-2 at 27 ppm. , C-11 at 24 ppm. ,and C-1 at 22 ppm.

There were 5 **quaternary** carbon signals. The most down field signal at 169 ppm. was assigned as carbonyl of C-17. There were two **quaternary** carbon atoms in double bond region that could be assigned as C-13 at 147 ppm. and C-8 at 109 ppm. The remain peaks at 42 and 39 ppm. were assigned as C-4 and C-10 respectively.

3. Compound C-4, Neoandrographolide

2.35 g (2.35% yield) of colorless needle crystal was obtained from fraction 28-64 of chloroform crude extract and recrystallised in chloroform and methanol.

Solubility

soluble in methanol, ethanol, acetone, pyridine sparingly soluble in chloroform and water; insoluble in ether and petroleum ether

Rf value

Chloroform	: Methanol	9:1	0.52
Chloroform	: Methanol	8:1	0.54
Chloroform	: Acetone	9:1	0.00
Chloroform	: Ab. Ethanol	85:15	0.65
Methanol	: Benzene	1:1	0.92

Melting point 167-168°C (Chan, Taylor, and Willis, 1968)

Molecular formular $C_{26}H_{40}O_8$

Molecular weight 480

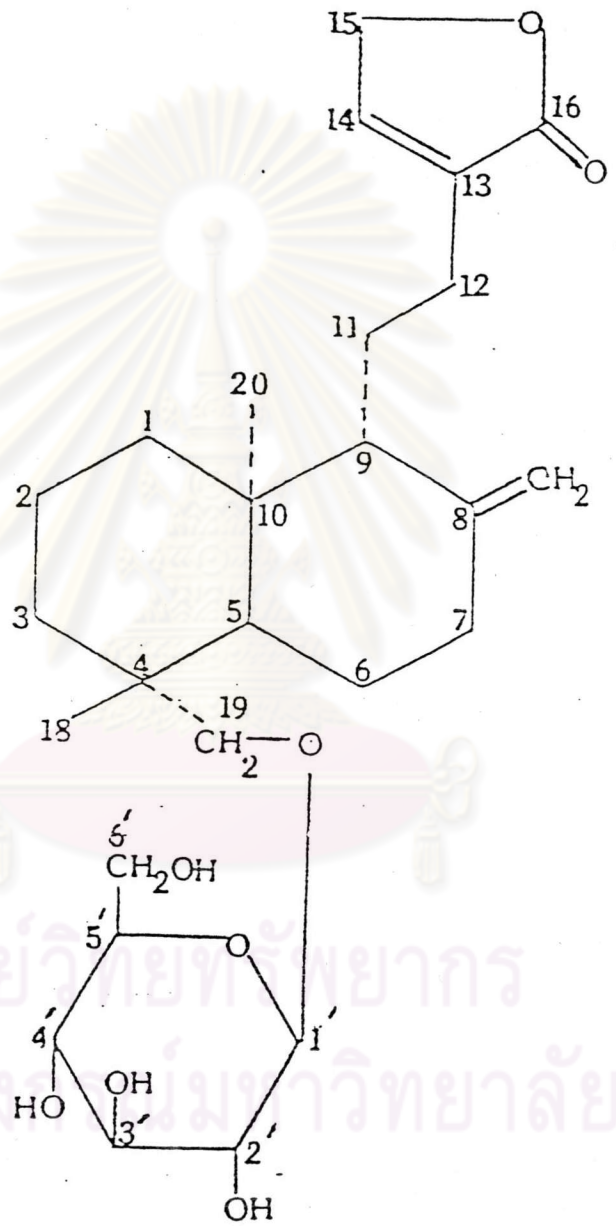


Figure 41 Structure of compound C-4 , Neoandrographolide

Spectral data

UV spectrum λ max = 217.4 nm

IR spectrum

= 3,425 cm^{-1} (OH stretching)

= 2,925-2,850 cm^{-1} (C-H stretching)

= 1,750 cm^{-1} (C=O stretching)

= 1,637 cm^{-1} (C=C)

= 1062-1025 cm^{-1} (C-O stretching)

Mass spectrum

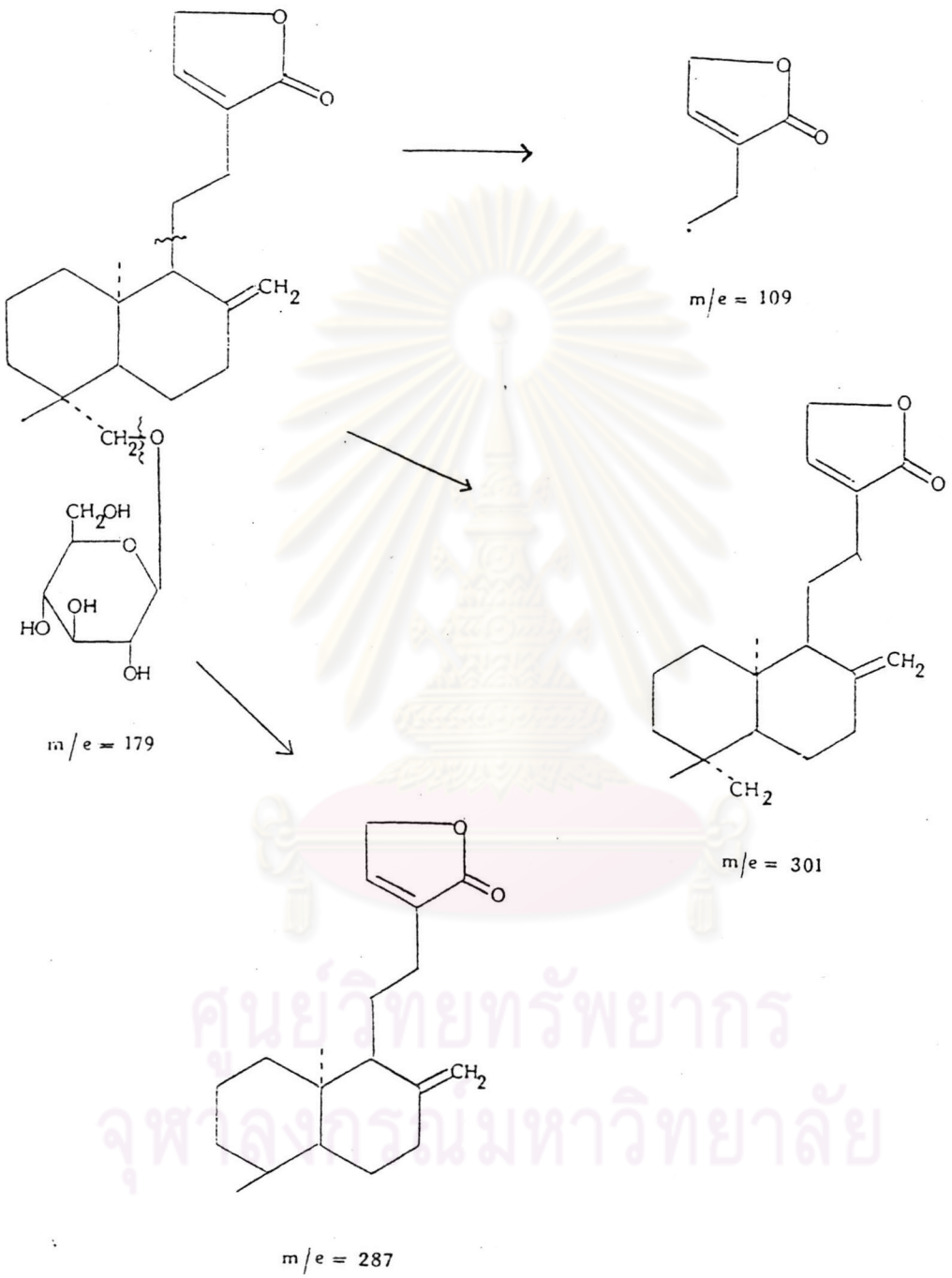
m/z = m/e (rel. int.)

301 (147.3), 288 (719.3), 287 (1000),

205 (570.8), 179 (315.7), 123 (570.8),

121 (581.9), 104 (753.7), 81 (714.9)

The m/e = 287 may be achieved by fragmentation as followed.



m/e = 109

m/e = 179

m/e = 301

m/e = 287

Nuclear Magnetic Resonance spectrum

Table 7 Proton chemical shift of compound C-4 in DMSO-d6

Proton	Chemical shift (ppm)
H - 1 (a, b)	0.80
H - 2 (a, b)	0.90
H - 3 (a, b)	1.80
H - 5	1.20
H - 6 (a, b)	1.75
H - 7 (a, b)	2.10, 2.30
H - 9	1.60
H - 11 (a, b)	1.85, 1.30
H - 12 (a, b)	2.30, 2.00
H - 14	7.50
H - 15 (a, b)	4.90
H - 17 (a, b)	4.85, 4.65
Me - 18	0.90
H - 19 (a, b)	4.20. 3.30
Me - 20	0.50
H - 1´	4.90
H - 2´	3.10
H - 3´	3.40
H - 4´	3.40
H - 5´	3.97
H - 6´	4.80, 3.75

A suitable entry point to begin the assignment was the down field signal for H-14 which showed coupling with H-15 in 2-D spectrum. From 2-D spectrum H-15 (a,b) could be assigned at 4.8 ppm. The broad singlet at 4.85 and 4.65 ppm were H-17(a) and H-17(b) of the exocyclic vinyl group.

Proton in the 4.2 to 3.1 ppm region arised from the methylene at C-19 and the attached glucose.

H-19(a) was shown as a doublet with geminal coupling constant 10 Hz at 4.20 ppm. and direct coupling with H-19(b) at 3.30 ppm.

Proton of glucose were assigned by comparing with proton and 2-D COSY spectra of glucose. Proton signal at 4.75-4.9 ppm. were integrated five protons that include H-15(a,b), H-17(a), H-1′ and H-6′(a). H-1′ of glucose coupling with H-2′ at 3.1 ppm. , H-2′led to H-3′ and H-4′ at 3.4 ppm , H-4′ led to H-5′ at 3.97 ppm. , H-5′ led to H-6′(a,b) at 4.80 and 3.75 ppm respectively.

The remain proton in the ring was difficult of identified. The assignment might be interchangible.

The assignment of the ring proton started with a signal at 2.1 and 2.3 ppm which had direct coupling with each other and had long range coupling with H-17(a,b) could be assigned as H-7(a,b).

From H-7(a,b) cross peak , led to H-6(a,b) in diagonal at 1.75 ppm. From H-6(a,b) cross peak , led to H-5 in diagonal at 1.2 ppm as showed in 2-D COSY spectrum.

A signal at 1.6 ppm showed long range coupling with H-17(a,b) was assigned as H-9. From H-9 cross peak led to H-11(a,b) in diagonal at 1.3 and 1.85 ppm. In 2-D spectrum the coupling between H-11(a,b) and H-12(a,b) could be seen,so H-12(a,b) were assigned at 2.3 and 2.0 ppm.

The remain protons in position 1,2,3 in the ring are proposed to assigned at 1.8 ppm for H-3(a,b) which nearby the heteroatom of glucose and coupling with H-2(a,b) at 0.9 ppm. H-2(a,b) were proposed to coupling with H-1(a,b) at 1.8 ppm.

Table 8 Carbon-13 chemical shift of compound C-4 in DMSO-d6

Carbon	Chemical shift (ppm)
C - 1	19
C - 2	22
C - 3	25
C - 4	40
C - 5	55.5
C - 6	38
C - 7	38
C - 8	133.5
C - 9	56
C - 10	39
C - 11	36
C - 12	39.5
C - 13	145.5
C - 14	147.5
C - 15	72
C - 16	175
C - 17	107
C - 18	28
C - 19	70
C - 20	16

Carbon	Chemical shift (ppm)
C - 1′	104
C - 2′	77
C - 3′	76
C - 4′	74
C - 5′	70
C - 6′	62

Carbon-13 assignment of compound C-4 started with comparing carbon-13 decoupling spectrum, DEPT 135 and DEPT 90 of C-4 with glucose. Glucose in molecule of C-4 could be assigned as C-1′ = 104 ppm, C-2′ = 77 ppm, C-3′ = 76 ppm C-4′ = 74 ppm, C-5′ = 70 ppm and C-6′ = 62 ppm.

A CH signal in double bond region of the spectrum was assigned as C-14 at 147.5 ppm.

A CH_2 signal in double bond region of the spectrum was assigned as C-17 at 107 ppm.

The quaternary carbon in the down field of the spectrum were assigned as C-16 carbonyl carbon at 175 ppm, C-13 at 145.5 ppm. that is nearby heteroatom and C-8 at 133.5 ppm.

Two CH signals at 55.5 and 56 ppm were assigned as C-5 and C-9 respectively. The chemical shift of two

species were downfield due to the allylic coupling of C-9 with C-17 and C-5 was nearby heteroatom of glucose.

Assignment of the remained carbon atom might be interchangible due to chemical equivalence of the carbon atom. C-12 signal was assigned at 39.5 due to allylic coupling with C-13.

C-11 and C-7 were assigned as 36 and 38 ppm. Both atoms were nearby double bond at C-17.

The remain CH_2 signals were assigned as C-6 at 36 ppm, C-3 at 25 ppm, C-2 at 22 ppm and C-1 at 19 ppm due to effect of the nearby heteroatom.

Two methyl signal at 28 and 16 ppm were assigned as C-18 and C-20 methyl respectively. C-18 was nearby heteroatom so the chemical shift was downfield.

The remain quaternary carbon atoms were C-4 and C-10. C-4 and C-10 were assigned at 41 ppm and 39 ppm respectively C-4 was nearby heteroatom so the chemical shift was downfield. The two signal may be changible due to overlaping with signal of DMSO.

4. Compound C-5, 14-deoxyandrographolide -19β-D-glucoside

535 mg (0.54% yield) of colorless rhombic. crystal was obtained from fraction 58-64 of chloroform crude extract and fraction 27-40 of butanol crude extract and recrystallised in ethyl acetate and methanol.

Solubility soluble in methanol, ethanol ; sparingly soluble in chloroform, water ; insoluble in ether.

Rf value

system			Rf
Chloroform	: Methanol	9:1	0.33
Chloroform	: Methanol	8:1	0.38
Chloroform	: Acetone	9:1	0.00
Chloroform	: Ab. Ethanol	85:15	0.40
Methanol	: Benzene	1:1	0.90

Melting point 187-188°C (Fujita et al. ,1984)

Molecular formular $C_{26}H_{39}O_9$

Molecular weight 495

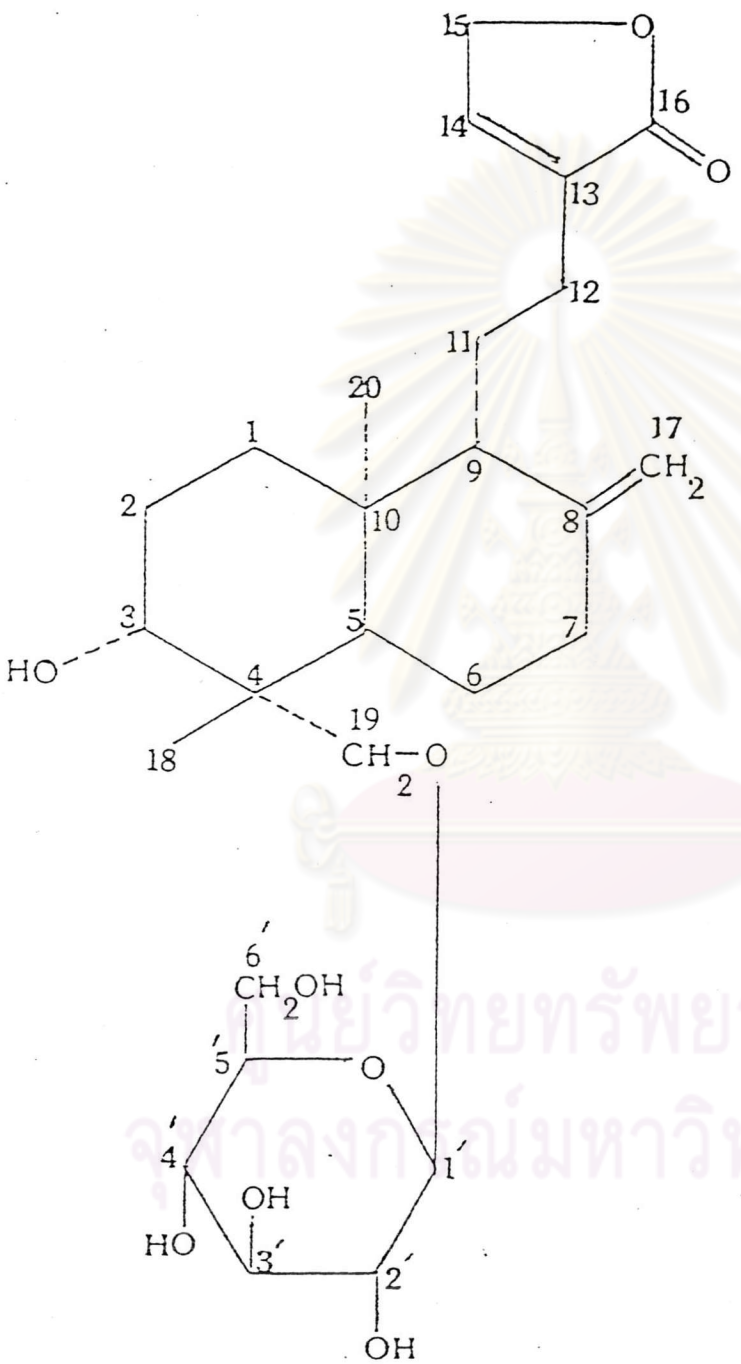


Figure 53 Structure of compound C-5,14-deoxyandrographolide-19β-D-glucoside

Spectral data

UV spectrum λ_{max} in methanol 212 nm

IR spectrum

= 3400-3300 cm^{-1} (O-H stretching)

= 2925-2350 cm^{-1} (C-H stretching)

= 1725 cm^{-1} (C=O stretching)

= 1661.5 cm^{-1} (C=C)

= 1225-1050 cm^{-1} (C-O stretching)

Mass spectrum

m/z = m/e (rel. int.)

= 121 (841.2) ,119 (842.1) ,109 (708.7),60 (857.8) ,55 (703.4),44 (804.3), 43 (1000)

Nuclear Magnetic Resonance spectrum

Table 9 Proton chemical shift of compound C-5 in DMSO-d6

Proton	Chemical shift (ppm.)
H - 1 (a,b)	1.10
H - 2 (a,b)	1.60
H - 3	3.20
H - 5	1.00
H - 6 (a,b)	1.80 , 1.25
H - 7 (a,b)	2.30 , 2.10
H - 9	2.00
H - 11 (a,b)	2.40 , 2.05
H - 12 (a,b)	2.30 , 1.80
H - 14	7.45
H - 15 (a,b)	4.85, 4.60
H - 17 (a,b)	4.80 , 4.60
Me - 18	1.20
H - 19 (a,b)	3.90 , 3.30
Me - 20	0.80
H - 1´	4.90
H - 2´	3.25
H - 3´	3.40
H - 4´	3.40
H - 5´	4.00
H - 6´ (a,b)	4.85 , 2.95

Proton assignment of compound C-5 started with a down field signal at 7.45 ppm. as H-14 which has direct coupling with H-15(a) and H-15(b) at 4.85 ppm. and 4.05 ppm. Two singlets peak at 4.80 and 4.60 ppm. were assigned as H-17 (a) and H-17 (b) respectively.

The assignment of the remain methylene protons could be interchangible.

Proton signals at 2.4-2.2 ppm. was integrated 3 protons . In 2-D spectrum ,the signal at 2.2 ppm. and 2.3 ppm. showed long range coupling with H-17(a,b) , so these signals were assigned as H-7(a) and H-9 respectively. A remain proton signal at 2.3-2.4 ppm. might be H-12(a,b)

Proton signal at 2.1-2.0 ppm. was integrated 2 proton. In 2-D spectrum , this signal showed direct coupling with the signal at 2.3-2.4 ppm.,so the protons could be assigned as H-7(b) and H-12 (b) respectively.

Proton signals between 1.8-1.65 ppm. were integrated 4 protons . In 2-D spectrum , these signals showed direct coupling with protons at 2.3 ppm.,so the protons were proposed to be H-11 (a,b) and H-6 (a,b).

H-2(a,b) protons could be identified by direct coupling with H-3 at 1.5-1.6 ppm. as seen in 2-D spectrum.

The signal at 2.15-1.6 ppm. led to assigned H-1 (a,b) at 1.0-1.1 ppm. The proton signals at 1.25-1.0 ppm. were integrated 6 proton . These signal might include C-18 methyl , H-1(a,b) . The remain 1 proton about 1.2 ppm. which showed direct coupling with protons at 1.6-1.8 ppm. could be assigned as H-5 .

Table 10 Carbon - 13 Chemical shift of compound C-5 in DMSO-d6

Carbon	Chemical shift(ppm.)
C - 1	22
C - 2	25
C - 3	78
C - 4	42
C - 5	56
C - 6	24.5
C - 7	37
C - 8	132
C - 9	55
C - 10	28
C - 11	40
C - 12	39
C - 13	148
C - 14	147
C - 15	71
C - 16	175
C - 17	106
C - 18	24
C - 19	61
C - 20	15

Carbon	Chemical shift(ppm.)
C - 1′	103
C - 2′	77
C - 3′	73.5
C - 4′	73.5
C - 5′	70
C - 6′	61

Carbon -13 assignment of C-5 started by comparing carbon - 13 decoupling spectrum with DEPT 135 and DEPT 90 to identified CH_3, CH_2, CH and C quaternary in the molecule.

Comparing carbon 13 spectrum and DEPT 90 spectrum between C-5 and glucose revealed that carbon of glucose were assigned as C-1′ = 103 ppm, C-2′ = 77 ppm, C-3′ = 73.5 ppm., C-4′ = 73.5 ppm., C-5′ = 70 ppm. and C-6′ = 61 ppm.

From DEPT 90 , a down field CH signal at 146 ppm. was assigned as C-14. Methine carbons that coupling with heteroatom were C-3 at 78 ppm., C-15 at 71 ppm. (overlap with glucose peak), C-19 at 61 ppm., C-5 at 56 ppm. and C-9 at 55 ppm. respectively.

The remain signal of five quaternary carbon atoms at 175 ppm., 148 ppm., 132 ppm., 42 ppm.,and 28 ppm. were assigned as C-16, C-13, C-8, C-4 and C-10 respectively.

The remain methylene signals were assigned as C-11 at 40 ppm., C-12 at 39.5 ppm., C-7 at 37 ppm., C-2 at 25 ppm. and C-1 at 22 ppm.

Two methyl groups at 24 ppm. and 15 ppm. were assigned as C-18 and C-20 respectively

5. Compound C-6, Andrographiside

260 mg (0.26% yield) needle colorless crystal was obtained from fraction 51-61 in n-butanol crude extract and recrystallized in ethyl acetate and methanol.

Solubility soluble in methanol, ethyl acetate ethanol; less soluble in chloroform and water; insoluble in ether.

Rf value

system			Rf
Chloroform	: Methanol	85:15	0.21
Chloroform	: Acetone	1:1	0.05
Acetone	: Methanol	8:2	0.78
Methanol:Ethyl acetate:Chloroform		5:3:2	0.74
Ether	: Methanol	7:3	0.57

Melting point 195-197 ^{o}C (Gupta, Choudhury, and Yadava, 1990)

Molecular formular $C_{26}H_{40}O_{10}$

Molecular weight 512

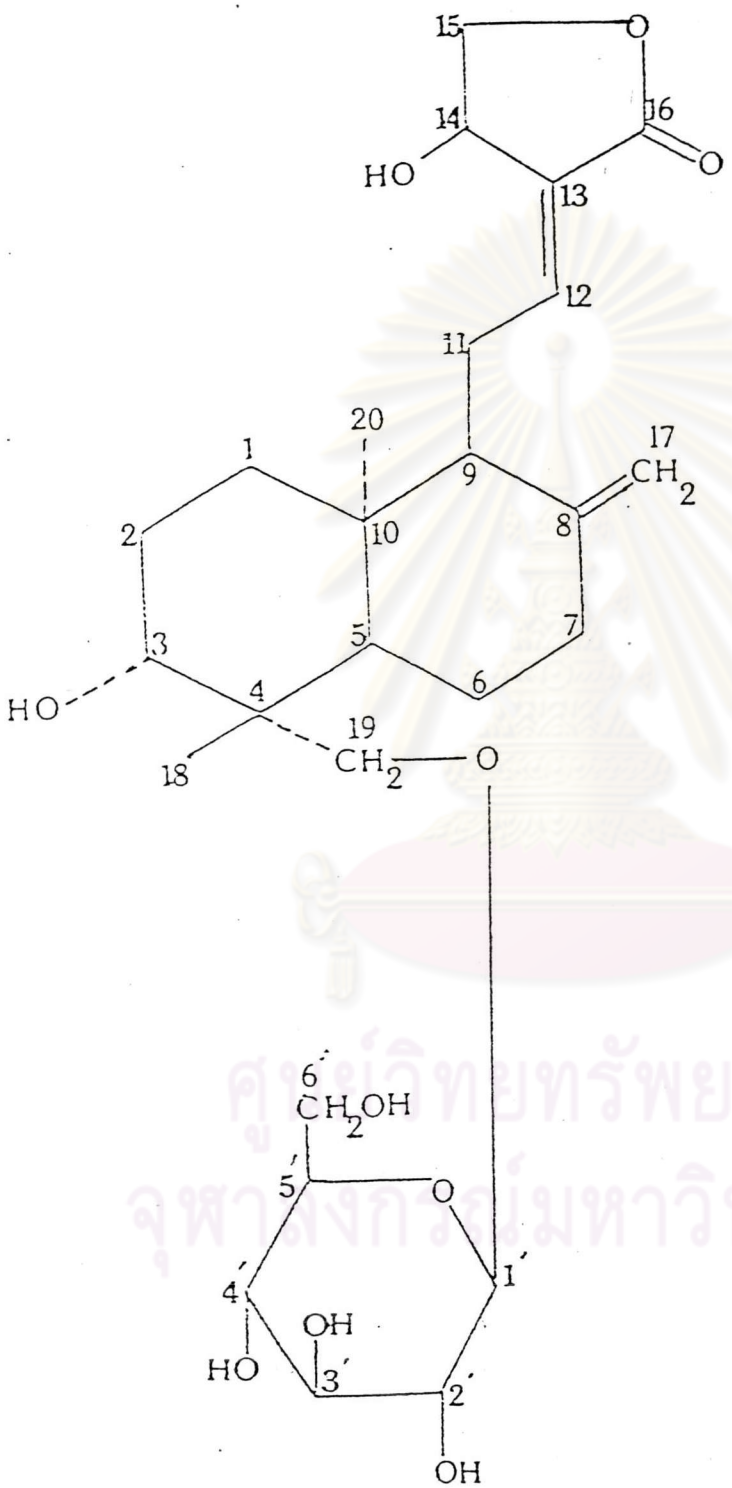


Figure 61 Structure of compound C-6, Andrographiside

Spectral data

UV spectrum λ_{max} in methanol = 223 nm.

IR spectrum

= 3400 cm^{-1} (O-H stretching)

= 2900-2850 cm^{-1} (C-H stretching)

= 1720 cm^{-1} (C=O stretching)

= 1670 cm^{-1} (C=C)

= 1300-1100 cm^{-1} (C-O stretching)

Mass spectrum

m/z = m/e (rel. int.)

= 333 (68.4), 60 (1000), 44 (929.2), 43 (708.4)

Nuclear Magnetic Resonance spectrum

Table 11 Proton Chemical shift of compound C-6 in DMSO-d6

Proton	Chemical shift(ppm)
H - 1 (a,b)	1.15
H - 2 (a,b)	1.55
H - 3	3.10
H - 5	1.25
H - 6 (a,b)	1.7, 1.25
H - 7 (a,b)	2.3, 1.8
H - 9	1.85
H - 11 (a,b)	2.45
H - 12	6.65
H - 14	4.90
H - 15 (a,b)	4.40, 4.00
H - 17 (a,b)	4.60, 4.60
Me -18	1.15
H - 19	3.95, 3.45
Me -20	0.75
H - 1′	4.90
H - 2′	3.25
H - 3′	3.40
H - 4′	3.40
H - 5′	4.0
H - 6′ (a,b)	4.7, 3.0

The assignment of compound C-6 started from the down field signal of H-12 at 6.6 ppm which had splitting pattern as a triplet due to coupling with H-11. From 2-D spectrum direct coupling of H-12 with H-11 and allylic coupling of H-12 with H-14 was shown.

The proton of H-14 was assigned as a doublet of triplet due to coupling with two proton of C-15 and one proton of OH of C-14, so a doublet at 5.75 ppm and two doublet of doublet at 4.45 and 4.10 were assigned as OH of C-14 , H-15(a) and H-15(b) respectively.

Two singlet peaks that coupling with each other at 4.80 and 4.60 ppm are H-17(a) and H-17(b).

The C-19 methylene group could be identified from glucose coupling region by long range coupling with C-18 methyl. The signal presented at 3.90 ppm was H-19(a) and 3.50 ppm was H-19(b).

In 2-D spectrum direct coupling between H-12 and H-11(a,b) was shown. The position of H-11 (a,b) led to assignment of H-9 in at 1.85 ppm.

The remain part of the molecule were H-1 (a,b) H-2(a,b), H-6(a,b), H-7(a,b) and H-3.

One signal in heteroatom coupling region that showed coupling with proton in the range 1-2 ppm. was assigned as H-3 (chemical shift = 3.1 ppm.). In 2-D spectrum , the cross peak of H-3 had direct coupling with H-2(a,b) in diagonal at 1.55 ppm. From H-2(a,b) cross peak, led to assignment of H-1(a,b) in diagonal at 1.15 ppm.

H-7 (a) could be identified by allylic coupling with H-17(a,b) at 2.3 ppm and coupling with H-7(b) at 1.8 ppm. The cross peak of H-7(b) led to H-6 (a,b) in diagonal at 1.70 and 1.25 ppm. The cross peak of H-6(a,b) led to assignment of H-5 in diagonal at 1.25 ppm.

Table 12 Carbon 13 Chemical shift of compound C-6 in DMSO-d6

Carbon	Chemical shift
C - 1	23.0
C - 2	28.7
C - 3	65
C - 4	42.5
C - 5	55.0
C - 6	37.6
C - 7	38
C - 8	128.7
C - 9	57.3
C - 10	40
C - 11	23.7
C - 12	146.7
C - 13	147.5
C - 14	73.75
C - 15	77.5
C - 16	170
C - 17	107.5
C - 18	25
C - 19	70
C - 20	15

Carbon	Chemical shift(ppm)
C - 1′	103
C - 2′	77.5
C - 3′	73.75
C - 4′	70
C - 5′	65
C - 6′	61.25

Assignment of carbon-13 started by comparing between carbon-13 decoupling spectrum with DEPT 135 and DEPT 90 of compound C-6 and glucose.

In DEPT 135 spectrum positive peaks revealed CH and CH_3 signals and negative peaks revealed CH_2 signals. Comparing with C-13 decoupling and DEPT 90 of glucose spectrum could be identified glucose signals at 103 ppm, 77.5 ppm, 73.75 ppm, 70 ppm, 65 ppm and 61.25 ppm.

Two methyl group at 25 ppm and 15 ppm were assigned as C-18 and C-20 methyl respectively.

The down field CH at 147.5 ppm was assigned as C-12 and the down field CH_2 at 107.5 ppm was assigned as C-17 in the molecule.

Carbon signals in heteroatom coupling region were CH_2 at 77.5 ppm and 70 ppm and CH at 73.75 ppm, 65

ppm, 55.0 and 53.7 ppm which could be assigned to C-15, C-19 methylene carbons and C-14, C-3, C-5 and C-9 methine carbons respectively.

The remain spectrum in high field region were C-11, C-7, C-2, C-6 and C-1. The assignment were achieved by comparing the signal with C,H COSY of andrographolide.

From carbon-13 decoupling spectrum, five quaternary carbon atoms were identified. C=O presented at 170 ppm, C-13 at 147.5 ppm, C-8 at 128.7 ppm, C-4 at 42.5 ppm and C-10 at 40 ppm.