CHAPTER IV

RESULT AND DISCUSSION

The 1.8 kg of dried leaves of *Artocarpus gomezianus* Wall. ex Trec. was macerated with ethanol. The ethanol extract was then separated by chromatographic technique to afford five pure compounds. The structure elucidations of the isolated compounds were based on data from UV, IR, NMR and Mass spectra, and further confirmed by comparison with the data reported in literature, as discussed below.
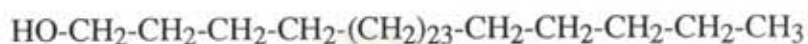
1. Structure Elucidation of AG-1

AG-1 was obtained as white crystals from F-06 (Table 8) by quick column chromatographic technique using sintered glass filter column of silica gel with a gradient system of hexane-chloroform-methanol as eluents to yield 156 mg (8.67×10^{-3} % based on dry weight).

The IR spectrum of AG-1 (Figure 3) exhibited O-H stretching at υ_{max} 3400-3200 cm^{-1}, C-O stretching at υ_{max} 1062 cm^{-1}, C-H stretching of methyl and methylene groups at υ_{max} 2918, 2849 cm^{-1}, C-H bending of methyl and methylene groups at υ_{max} 1473, 1463 cm^{-1} and C-H rocking of methylene groups (long aliphatic chain; $(CH_2)_n$, n>4) at υ_{max} 720 cm^{-1}.

The ^{1}H-NMR spectrum (Figure 4) revealed the presence of a terminal methyl group at δ 0.88 ppm (t, *J*=7.2 Hz) that coupled to a pair of methylene protons with coupling constants of 7.2 Hz. The signals at δ 1.25 (br s) and 1.55 ppm (m) were the signals of 30 methylene units. The signals of protons attached to the oxygenated carbon were assigned at δ 3.63 ppm (t, *J*=7.2 Hz) which were coupled to methylene protons.

The IR and ^{1}H NMR data suggested that AG-1 was a long chain alcohol. The EIMS (Figure 2) showed characteristic fragmentation pattern of long chain compound by the clusters of peaks, and the corresponding peaks of each cluster were 14 (CH_2) mass units apart because the loss of $CH_2=CH_2$ (Silverstein, Bassler and Morril,1991). The peak at *m/z* 449 corresponding to [M-OH]$^+$.

Therefore it is concluded that AG-1 is 1-dotriacontanol ($C_{32}H_{66}O$) which previously isolated from *Leucas aspera* (Misra *et al*, 1992), the structure of which is shown below. The individual ^{13}C atoms in straight-chain hydrocarbon can be predicted by calculation (Silverstein *et al*, 1991). Thus, the proposed carbon assignments of AG-1 are shown in table 10.

$$HO\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}(CH_2)_{23}\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$$
$$1 \quad\ 2 \quad\ 3 \quad\ 4 \quad\quad 5 \quad\quad\quad 6 \quad\ 7 \quad\ 8 \quad\ 9 \quad\ 10$$

1-Dotriacontanol

Table 10 Proposed Carbon Assignments of AG-1

C position	δC (ppm)
1	63.07
2	32.78
3	25.71
4	29.39
5	29.65
6	29.55
7	29.31
8	31.87
9	22.65
10	14.06

2. Structure Elucidation of AG-2

AG-2 was obtained as colorless needles from F-07 (Table 8) by quick column chromatographic tecnique using a sintered glass filter column of siliga gel with a gradient system of hexane-chloroform-methanol as eluents and was recrystallized from hexane to yield 72 mg (4.00×10^{-3} % based on dry weight). It gave green color to Libermann-Burchard's test. Thus, it appeared to be the steroidal compound.

The IR spectrum of AG-2 (Figure 7) suggested that functional groups of AG-2 were hydroxyl group, methyl group, methylene group and alkyl group. (Table 11)

Table 11   IR Spectrum Assignment of AG-2

Range of Absorption (cm⁻¹)	Intensity	Assignment
3500-3200	medium	O-H stretching of R-OH
2960-2860	high	C-H stretching of CH_3, CH_2
1642	weak	C=C stretching of alkene
1465	medium	C-H bending of CH_3 (asymmetric), CH_2
1381	medium	C-H bending of CH_3 (symmetric)
1062	medium	C-O stretching
840, 802	weak	out-of-plane C-H bending of trisubstituted alkene

AG-2 could be assigned as a known sterol, β-sitosterol through analysis of its
^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum of AG-2 (Figure 8-9) showed the
signals at δ 0.67-1.01 ppm which were the signals of methyl protons that substituted at
C-18, C-19 and at side chain of the steroidal compounds. The signals at δ 1.1-2.3 ppm
were the signals of methylene and methine proton of steroid. The signal at δ 3.52 ppm
(m) was the signal of proton at C-3. The olefinic signal at δ 5.34 ppm (m) could be
assigned as H-6 which was trisubstituted vinyl proton. The ^{13}C NMR spectrum
(Figure 10-11) showed the signals of 3 quaternary, 9 methine, 11 methylene and 6
methyl carbons. The carbon assignments of AG-2 are shown in Table 12.

Both the ^{1}H and ^{13}C NMR spectra were in accordance with those
published previously of β-sitosterol (Ogura, Cordell and Farnsworth, 1977; Robinstein
et al, 1976; Wright *et al*., 1976). Therefore, it was concluded that AG-2 was β-
sitosterol, the structure of which is shown below.

β-sitosterol
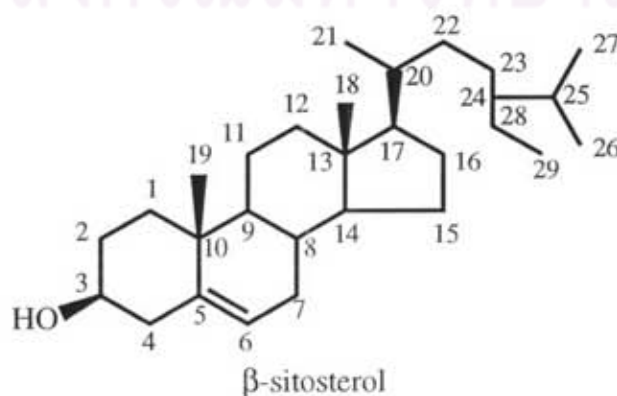
Table 12 Carbon Assignments of AG-2

C position	δ C (ppm)	C position	δ C (ppm)
1	37.27	16	28.25
2	31.67	17	56.07
3	71.80	18	11.87
4	42.31	19	19.40
5	140.77	20	36.15
6	121.71	21	18.78
7	31.91	22	33.95
8	31.91	23	26.09
9	50.14	24	45.84
10	36.51	25	29.16
11	21.10	26	19.82
12	39.78	27	19.05
13	42.31	28	23.08
14	56.78	29	11.99
15	24.31		

This structure was confirmed by the analyses of mass fragmentations. EIMS spectrum (Figure 6) exhibited a weak molecular ion peak at m/z 414 (32%) corresponding to molecular formula $C_{29}H_{50}O$. The proposed fragmentation patterns are shown below.
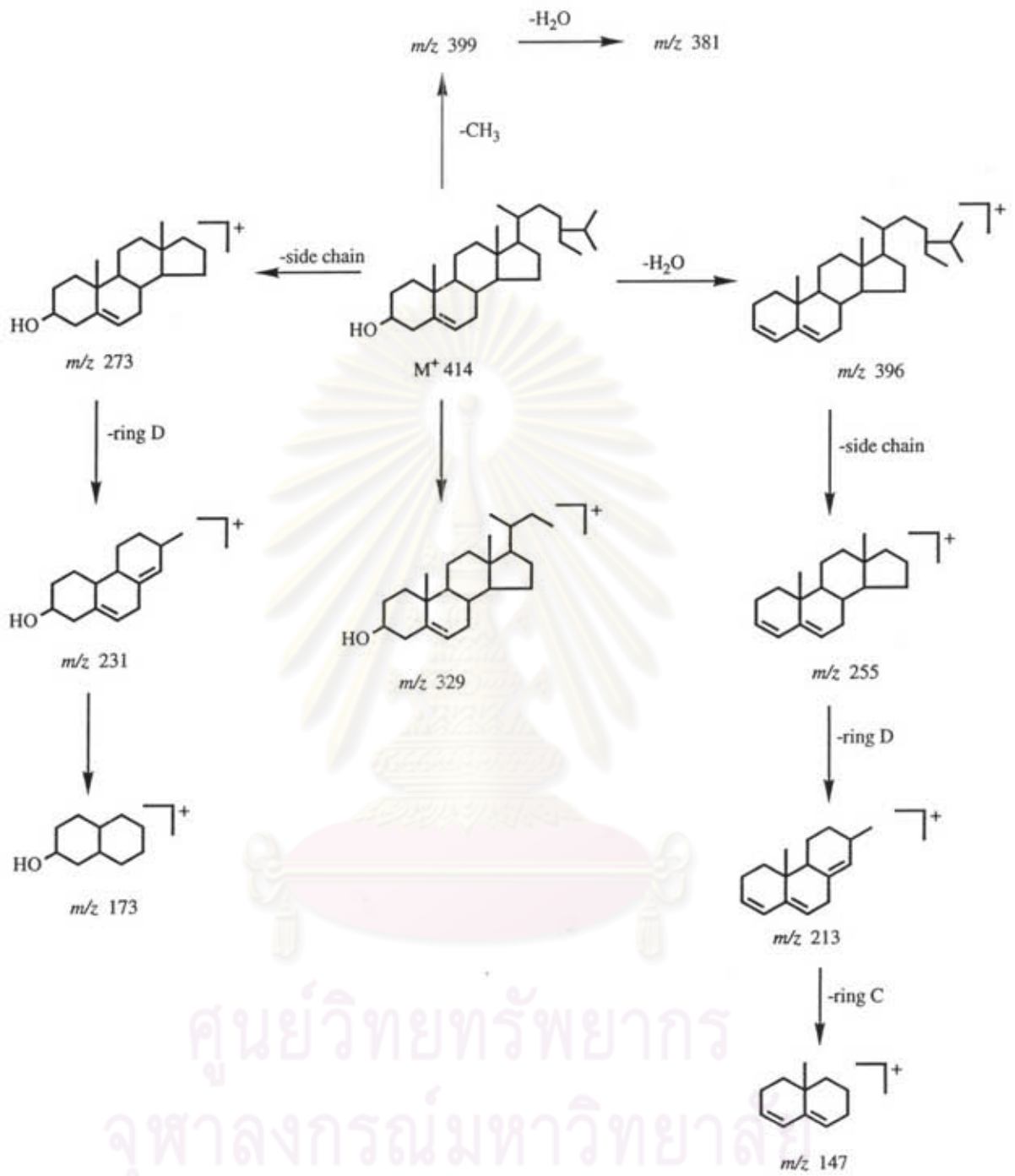
Scheme 10   Mass Fragmentation of AG-2

3. Structure Elucidation of AG-3

AG-3 was obtained as colorless needles from F-04 (Table 8) by repeated chromatographic technique using a silica gel column with a gradient system of 1-50% chloroform in hexane as eluents to yield 69 mg (3.83×10^{-3} % based on dry weight). It gave pink color with Libermann-Burchard's test which indicated that it was a triterpenoid compound.

The EIMS of AG-3 (Figure 12) exhibited the weak molecular ion peak at m/z 468 (7%), suggesting a molecular formula of $C_{32}H_{52}O_2$. The IR spectrum of AG-3 (Figure 13) suggested that functional groups of AG-3 were carbonyl group of ester, methyl group, methylene group and olefinic hydrocarbon (Table 13).

Table 13 IR Spectrum Assignment of AG-3

| Range of Absorption (cm^{-1})	Intensity	Assignment
3073	weak	=C-H stretching of alkene
2941-2854	high	C-H stretching of CH_3, CH_2
1736	high	C=O stretching of ester
1640	weak	C=C stretching of alkene
1455	medium	C-H bending of CH_3 (asymmetric), CH_2
1367	medium	C-H bending of CH_3 (symmetric
1247	high	C-O-C stretching of acetate ester
1025-877	medium	out-of-plane C-H bending of alkene

AG-3 could be assigned as the known pentacyclic triterpene lupeol acetate, by analyses of its ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum of AG-3 (Figure 14-15) showed signals δ 0.79-1.03 ppm which were the signal of methyl proton that substituted at C-23, C-24, C-25, C-26, C-27 and C-28. The signal at δ 1.68 ppm was the signal of methyl group that substituted at olefinic carbon. The signal at δ 2.04 ppm was a singlet for an acetyl proton. The signal at δ 4.47 ppm (m) was the signal of proton at C-3. The olefinic signal at δ 4.57 ppm (br s) and 4.69 ppm (br s) were vinyl protons (H_2-30).

The ^{13}C NMR spectrum (Figure 16) suggested the presence of 1 carbonyl ester carbon, 8 methyl carbons, 11 methylene carbons, 6 methine carbons and 6 quaternary carbons. The carbon assignments of AG-3 are shown in Table 14

Table 14 Carbon Assignments of AG-3

C position	δ C (ppm)	C position	δ C (ppm)
1	38.39	17	42.98
2	23.70	18	48.00
3	80.96	19	48.29
4	37.78	20	150.93
5	55.38	21	29.83
6	18.20	22	39.98
7	34.21	23	27.94
8	40.84	24	16.48
9	50.35	25	16.16
10	37.07	26	15.97
11	20.93	27	14.50
12	25.10	28	17.99
13	38.04	29	19.27
14	42.81	30	109.34
15	27.43	Methyl	21.30
16	35.56	Carbonyl ester	170.97

The assignments of protons and carbons were confirmed by comparison of the recorded data of lupeol acetate previously reported by Shieh and Lin (1992) and Sholichin *et al* (1980). Thus, AG-3 was determined as lupeol acetate and structure is shown below.

Lupeol acetate
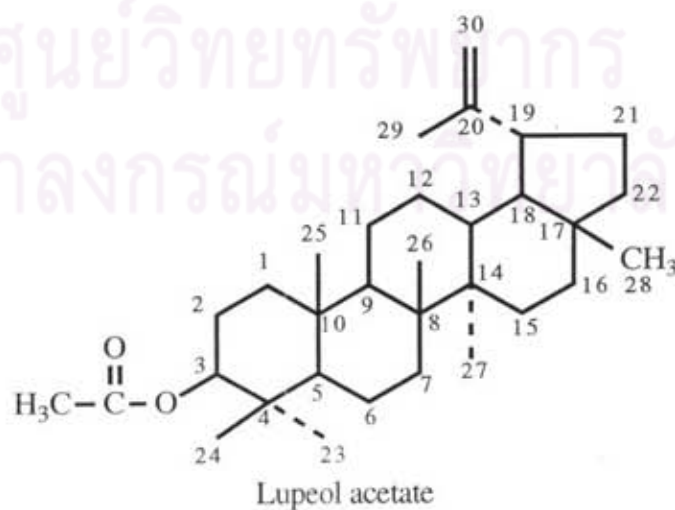
The structure was confirmed by the analyses of mass framentations from EIMS (Figure 12). It was found that lupane derivatives are characterized by an intensed peak at m/z 189 (in case where there is an iso-propenyl group in ring E) and m/z 218 (Scheme 11). These peaks are an important fragments in EIMS technique which show a skeleton structure of lupane type (Buzikiewiez *et al*, 1963; Buzikiewiez *et al*, 1964; Ogunkoya, 1981).

### 4. Structure Elucidation of AG-4

AG-4 was obtained as colorless needles from fraction number 148-198 (Table 9) by repeated chromatographic technique using silica gel column with hexane : ethylacetate (49:1) as eluents to yield 25 mg (1.39×10^{-3} % based on dry weight). It gave pink color to Libermann-Burchard's test. Thus, it appeared to be the triterpenoid compound.

The EIMS spectrum of AG-4 (Figure 17) exhibited a weak molecular ion peak at m/z 426 (4%) and the accurate mass was consistent with the molecular formula $C_{30}H_{50}O$. The IR spectrum (Figure 18) suggested the presence of a hydroxyl group, a methyl group, a methylene group and an alkyl group (Table 15).

Table 15 IR Spectrum Assignment of AG-4

| Range of Absorption (cm^{-1})	Intensity	Assignment
3508	medium	O-H stretching of R-OH
2931-2868	high	C-H stretching of CH_3, CH_2
1650	weak	C=C stretching of alkene
1470, 1454	medium	C-H bending of CH_3 (asymmetric), CH_2
1384	medium	C-H bending of CH_3 (symmetric)
1052	medium	C-O stretching
831,818	weak	out-of-plane C-H bending of trisubstituted alkene

m/z 468

m/z 189

m/z 218
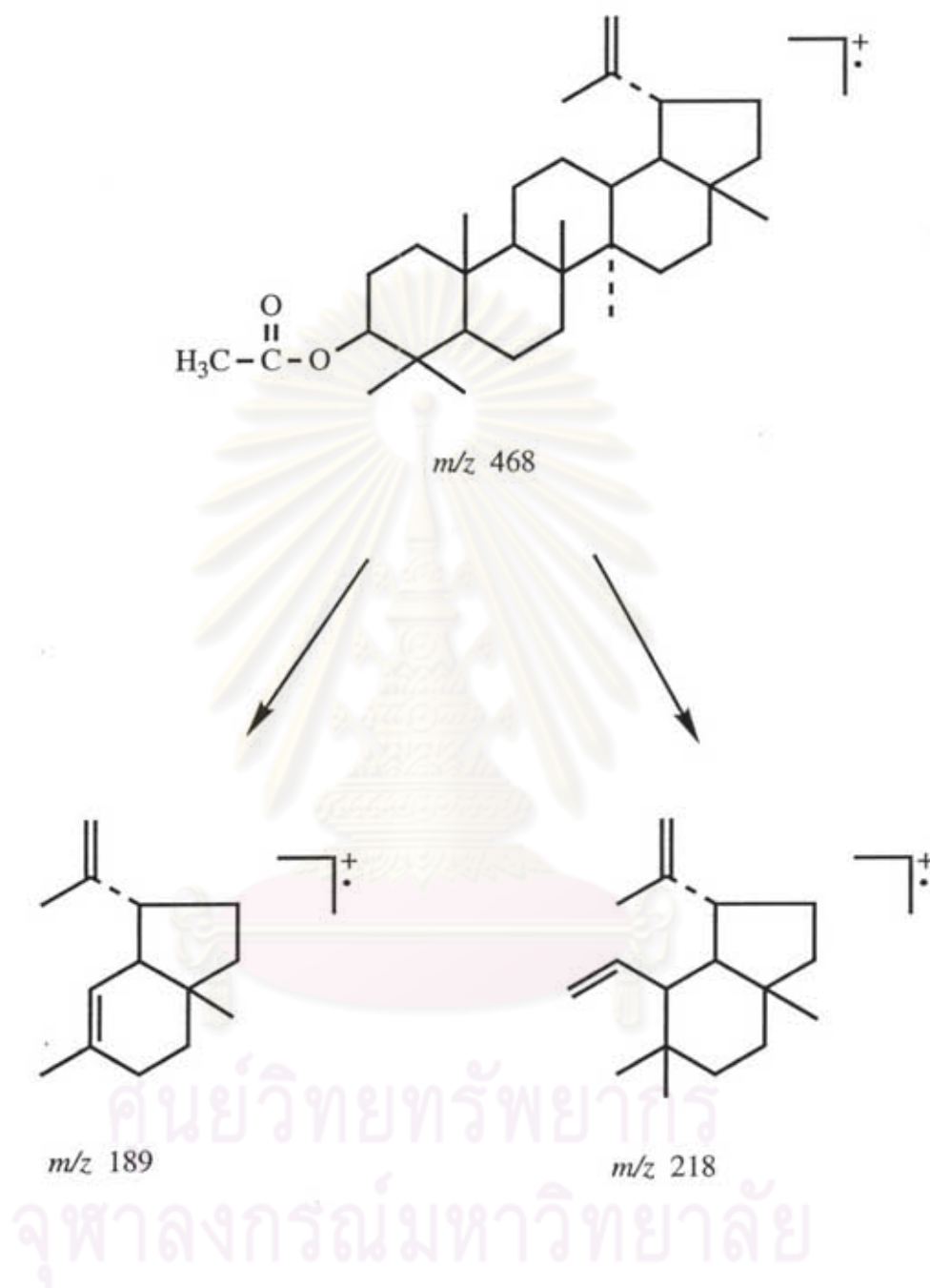
Scheme 11   Mass Fragmentation of AG-3

AG-4 could be assigned as a known pentacyclic triterpene, simiarenol that is 3β-hydroxy-E:B-*friedo*-hop-5-ene (Aplin *et al*, 1966) by the analysis of its ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum (Figure 19-20) showed the signals at δ 0.782-1.140 ppm which were signals of 8 methyl protons, they were H_3-23 (δ 1.045, s), H_3-24 (δ 1.140, s), H_3-25 (δ 0.895, s), H_3-26 (δ 1.006, s), H_3-27 (δ 0.926, s), H_3-28 (δ 0.782, s), H_3-29 (0.888, d, J=6.7 Hz), and H_3-30 (δ 0.829, d, J=6.7 Hz). The signals at δ 1.16-2.16 ppm were the signals of methylene and methine protons. The signal at δ 3.467 ppm (br s) was the signal of the proton at C-3 (3α proton). The olefinic signal at δ 5.615 ppm (ddd, J=1.93, 1.93, 5.81) could be assigned to H-6 which was a trisubstituted vinyl proton. The ^{13}C NMR spectrum (Figure 21-22) indicated thirty carbon signals which were the signals of 8 methyl carbons, 10 methylene carbons, 6 methine carbons and 6 quaternary carbons. The carbon assignments of AG-4 are shown in Table 16.

Table 16   Carbon Assignments of AG-4

C position	δ C (ppm)	C position	δ C (ppm)
1	18.06	16	35.41
2	27.78	17	42.80
3	76.34	18	51.73
4	40.81	19	19.91
5	142.00	20	28.30
6	121.96	21	60.04
7	24.06	22	30.77
8	44.26	23	29.06
9	34.82	24	25.46
10	50.25	25	17.84
11	34.14	26	15.74
12	29.00	27	15.00
13	38.60	28	16.06
14	39.31	29	21.95
15	29.11	30	22.89

The assignments of protons and carbons were supported by comparison with the data previously reported (Chakravarty, 1994; Tulloch, 1977). The structure of AG-4 is shown below.
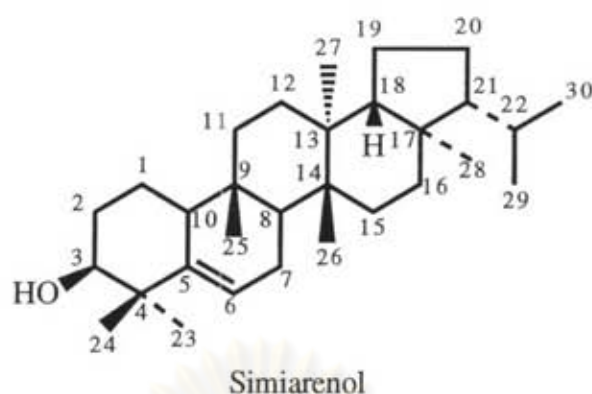
Simiarenol

The structure of AG-4 was finally confirmed by analysis of the mass fragmentations from EIMS (Figure 17). The presence of a Δ^5 double bond and an isopropyl group in simiarenol and its derivative is reflected in their mass spectra by the major fragmentation of these compounds which is the retro-Diels-Alder cleavage of ring B to give the base peak at m/z 274. Loss of the allylic C-26 methyl group give rise to the other major peak at m/z 259. A weak M-43 peak and a strong m/z 231 peak due to the loss of $C_3H_7\cdot$ from the molecular ion and the ion m/z 274, reflect the presence of an isopropyl group in simiarenol. An additional strong peak in the spectrum is m/z 152, which arises from the retro-Diels-Alder cleavage of ring B with charge rotation by the diene fragment. The formation of these fragments can be relationalized as shown in Scheme 12 for simiarenol (Aplin *et al*, 1966; Arthur and Hui, 1965).

5. Structure Elucidation of AG-5

AG-5 was obtained as colorless needles from F-09 by repeated quick column chromatographic technique using silica gel with the gradient system of chloroform-ethylacetate-methanol as eluents to yield 3.02 g (1.68×10^{-1} % based on dry weight).

In its HR-FAB-MS (dithiodiethanol + NaCl) (Figure 23), AG-5 displayed a $[M+Na]^+$ ion as a pseudo-molecular ion at m/z 295.0787, corresponding to the formula $C_{12}H_{16}O_7$ Na (Calcd. for 295.0793 a.m.u.), and therefore suggesting a molecular formula of $C_{12}H_{16}O_7$. The UV apsorption at λ_{max} 224 (log ϵ 3.82) and 286 (log ϵ 3.32) (Figure 25) showed the characteristic of phenolate anion with *p*-substituent of hydroxy group. The IR spectrum (Figure 26) confirmed the presence of hydroxyl group at υ_{max} 3372 cm^{-1} (broad). The band at υ_{max} 1611, 1513 and 1461 cm^{-1} indicated the presence of aromatic ring. The bands at υ_{max} 1223 cm^{-1} and 833 cm^{-1} suggested the presence of phenolic moiety and 1,4 disubstituted benzenoid.

(M^+) *m/z* 426

(M^+) *m/z* 426

m/z 274

m/z 231

*m/z* 152

-Me•

m/z 259

Scheme 12   Mass Fragmentation of AG-4

AG-5 could be assigned as known phenolic glycoside, 4-hydroxyphenyl-β-D-glucopyranoside, the trivial name arbutin, by the analysis of it ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum (Figure 27-28) provided the signals of 4 olefinic or aromatic protons and 5 hydroxy groups. The ^{13}C NMR spectrum (Figure 29) suggest the presence of 6 aromatic carbons and 6 oxygenated sp^3 carbons. From DEPT (Figure 30), the spectrum showed that AG-5 has 2 quaternary carbons, 9 methine carbons and 1 methylene carbon.

The downfield region of the ^{1}H NMR spectrum (Figure 27-28) showed the presence of a phenolic proton at δ 8.97 ppm.(s), along with aromatic protons appearing at δ 6.85 ppm (2H, d, J=8.55 Hz) and 6.65 ppm. (2H, d, J=8.55 Hz). Further analysis of the ^{1}H-NMR spectrum in more upfield area revealed typical signals of a sugar moiety, with an anomeric proton (H-1') at δ 4.62 ppm (d, J=7.24 Hz) and 10 protons in the region δ 5.20-3.13 ppm. The sugar unit was derived from β-D-glucose, as evident from the resonance of H-2' (δ 3.17, ddd, J=9.21, 7.24, 5.26 Hz), OH-2' (δ 5.20, d, J=5.26 Hz), H-3' and H-5' (δ 3.20-3.25, overlapping), OH-3' (δ 5.00, d, J=5.26 Hz), H-4' (δ 3.11, ddd, J=9.21, 8.55, 5.26 Hz), OH-4' (δ 4.94, d, J=5.26 Hz), H_2-6' (δ 3.45, ddd, J=11.84, 5.26, 5.26 Hz and δ 3.67, ddd, J=11.84, 5.26, 1.97 Hz), OH-6' (δ 4.51, t, J=5.26 Hz) in the COSY spectrum. (Figure 31-34).

The ^{13}C NMR spectrum (Figure 29) supported this structure. The aromatic carbon signals at δ 153.12, 151.28, 118.40 and 116.18 ppm were assigned as C-1, C-4, C-3,5 and C-2,6, respectively. The remaining signals were the signals of sugar moeity by the signals at δ 102.38, 77.41, 77.11, 73.75, 70.25 and 61.18 ppm were assigned as C-1', C-3', C-5', C-2', C-4' and C-6' respectively.

The ^{1}H NMR and ^{13}C NMR spectrum (in pyridine-d_5) of AG-5 were identical with the data previously reported by Kubo and Ying (1992). Thus, AG-5 was determined as 4-hydroxyphenyl-β-D-glucopyranoside or arbutin which the structure is shown below. The proton and carbon assignments of AG-5 are summerized in Table 17.
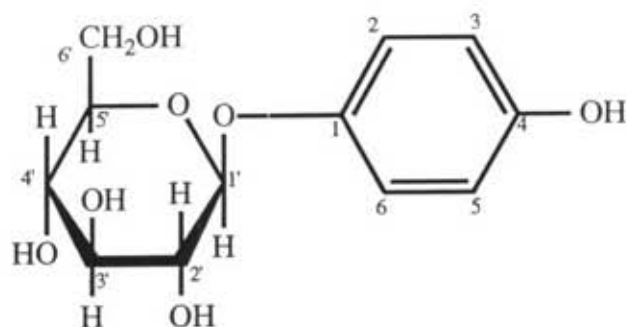
4-Hydroxyphenyl-β-D-glucopyranoside
(Arbutin)

Table 17 Proton and Carbon Assignments of AG-5

Position	δ H (multiplicity, J (Hz))	δ C (ppm)
1		153.12
2,6	6.65 (d, 8.55)	116.18
3,5	6.85 (d, 8.55)	118.40
4	8.97 (OH-4, s)	151.28
1'	4.62 (d, 7.24)	102.38
2'	3.17 (ddd, 9.21, 7.24, 5.26)	73.75
	5.20 (OH-2', d, 5.26)	
3'	3.20-3.25 (overlap with H-5')	77.41
	5.00 (OH-3', d, 5.26)	
4'	3.13 (ddd, 9.21, 8.55, 5.26)	70.25
	4.94 (OH-4', d, 5.26)	
5'	3.20-3.25 (overlap with H-3')	77.11
6'a	3.45 (ddd, 11.84, 5.26, 5.26)	61.18
b	3.67 (ddd, 11.84, 5.26, 1.97)	
	4.51 (OH-6', t, 5.26)	

This structure was confirmed by the analyses of mass fragmentations. (Scheme 13) from EIMS (Figure 24). The base peak at m/z 110 dued to loss of sugar moiety.
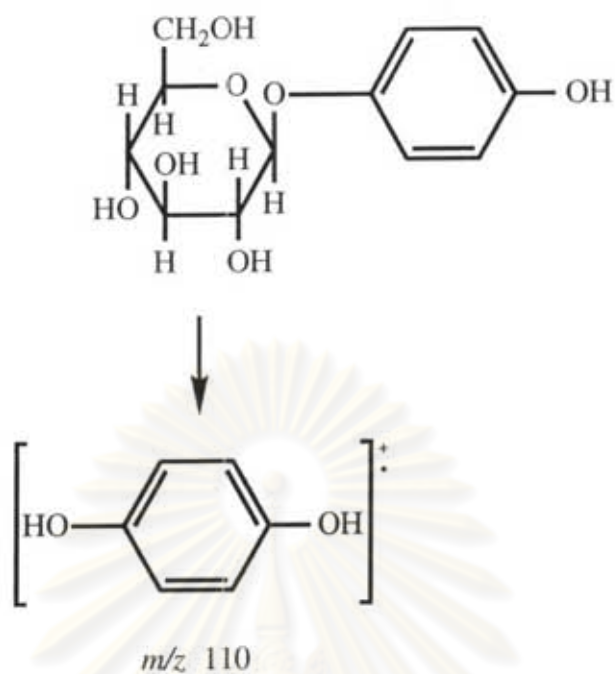
Scheme 13   Proposed Mass Fragmentation of AG-5