# CHAPTER III

# EXPERIMENTAL

## 1.Source of Plant Materials

The roots of *Atherolepis pierrei* Cost.var.*glabra* Kerr (Asclepiadaceae) were obtained from Chiang Mai province, Thailand, in May, 1992. Authentification was achieved through comparison with herbarium specimens in the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand.

## 2. General Techniques

### 2.1 Thin-Layer Chromatography (TLC)

| | |
|---|---|
| Technique | : one way, ascending |
| Adsorbent | : Aluminium sheet silica gel 60 F254 (E.Merck) precoated plate |
| Plate size | : 2x7 cm , 4x7 cm |
| Layer thickness | : 0.2 mm |
| Solvent system | : a) *n*-Hexane : Chloroform (2:1, 4:1) |
| | b) *n*-Hexane : Dichloromethane (1:1) |
| | c) *n*-Hexane : Diethyel ether (10:1) |
| | d) *n*-Hexane : Ethyl acetate (10:1) |
| | e) Cyclohexane : Dichloromethane (4:1) |
| | f) Cyclohexane : Benzene (1:2) |
| | g) Cyclohexane : Ethyl acetate (20:1) |

h) Petroleum ether : Dichloromethane (10:1.5)

i) Petroleum ether : Dichloromethane (2:1)

j) Petroleum ether : Ethylacetate (10:1)

k) Chloroform : Benzene (1:3)

Distance : 5.5 cm

Laboratory temperature : 30-35°C

Detection on chromatographic plate :

a) Ultraviolet light (UV)

Compounds which contain unsaturated dienes become visible as quenching spots under uv light at 254 nm on TLC. plate.

b) Chromogenic agents

1. 10% Sulfuric acid in ethanol

Various organic compouds showed characteristic colors after heating (hair dryer).

2. Iodine vapour

Unsaturated organic compounds display yellow-brown spots with iodine vapour.

3. Anisaldehyde-Sulfuric acid spray reagent (1 ml conc. Sulfuric acid is added to a solution of 0.5 ml anisaldehyde in 50 ml acetic acid)

After spraying, the plate was heated (hair dryer) until the spots attain maximum color intensity. Terpenoid compounds exhibit purple pink or pink spots.

4. Liebermann Berchard spray reagent (5 ml acetic anhydride are carefully mixed with 5 ml conc. sulfuric acid. This mixture is added cautiously to 50 ml absolute ethanol)

The plate after spraying was heated (hair dryer). The color reaction is purple pink or pink spots suggesting the presence of terpenoid compounds.

2 2.Column Chromatography (CC)

Adsorbent : Silica Gel 60 (0.040-0.63 mm (E. Merck)
Silica Gel 60 G

Packing of column : Dry packing

Sample loading : A portion of crude extract was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, dried under vacuum; and added to the top of a column.

Solvent system : a) *n*-Hexane

b) Gradient of chloroform in *n*-hexane

c) Gradient of dichloromethane in *n*- hexane

d) Chloroform

e) Gradient of ethyl acetate in chloroform

f) Petroleum ether : Dichloromethane (2:1)

Examination of fractions : The fractions were examined by TLC using uv light and chromogenic agents. The fractions of similar patterns were combined together.

2.3 Physical Constant

Melting Point

Melting points were determined by Gallenkamp Melting Point Apparatus with digital thermometer (uncorrected).

2.4.Spectroscopy

2.4.1 Ultraviolet (UV) Absorption Spectroscopy

Ultraviolet absorption spectra were obtained with a.Lambda Series PECSS UV spectrometer.

2.4.2 Infrared (IR) Absorption Spectroscopy

IR absorption spectra were obtained with a Shimadsu IR-440 infrared spectrometer, using potassium bromide disc.

2.4.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

All the $^{1}H$ NMR and $^{13}C$ NMR spectra were obtained with a Jeol alpha FT NMR spectrometer by operating at 500 and 125.65 MHz respectively and taken by using tetramethylsilane (TMS) as an internal standard and deuterated chloroform as a solvent. The chemical shifts were reported in ppm scale.

2.4.4 Mass Spectroscopy (MS)

Electron-impact mass spectra (EIMS) were obtained by operating at 70 ev with a Hitachi M-60 and with a Fisons VG Trio 2,000 mass spectrometer (using a direct inlet system) and the high resolution mass spectra (HRMS) were measured with a Hitachi RMU-7M mass spectrometer.

## 3.Extraction and Isolation

3.1 Extraction

The dried coarsely powdered roots of *Atherolepis pierrei* Cost. var.*glabra* Kerr (3 kg) were marcerated twice for two-day period, with chloroform (7.5 L). After combination, the extracts were evaporated under reduced pressure to yield 90 gm of a crude chloroform extract. After this process, the marc was extracted three times for two-day period, with methanol (7.5 L). The combined extracts were evaporated under reduced pressure to give 121 gm of brown syrupy mass.

3.2 Isolation

The crude chloroform extract (90 gm) was dissolved in a small quantity of chloroform, triturated with silica gel G (90 gm), and dried under vacuum. It was divided into three equal portions. Each portion was purified by quick column chromatographic technique using a sintered glass filter column of silica gel G (11x5 cm). The eluents were shown below;

a) *n*- Hexane

b) 10%-80% Chloroform in *n*-hexane

c) Chloroform

d) 10%-50% Ethyl acetate in chloroform

Fractions of fifty ml were collected and examined by thin layer chromatography (TLC). Fractions containing similar pattern were combined together as shown in Table 3.1.

Table 3.1 Solvent systems used in column chromatography of crude extract.

| Fraction | Eluent | Remark |
|---|---|---|
| 1-7 (afforded Fraction A) | *n*-hexane | colorless solution |
| 8-13(afforded Fraction B) | gradient of chloroform in *n*-hexane (0% , 10%, 20%) | pale yellow solution to yellow solution |
| 14-30 (afforded Fraction C) | gradient of chloroform in *n*-hexane (20%, 30%,...,to 60%) | yellow solution |
| 31-43 (afforded Fraction D) | gradient of chloroform in *n*-hexane ; (60%, 70%, 80%); chloroform; 10% ethyl acetate in chloroform | yellow solution to pale-orange solution |

Methanol was used to wash the column until the eluates were diluted and clear.

Fraction A was dried under reduced pressure, and further purified by recrystallization from *n*-hexane to give colorless flakes 2.57 gm (0.086%). The isolate was designated as AP-1, and was identified as heptatriacontane.

Fraction B (10.4 gm) was dried and subsequently dissolved in a small volume of chloroform and then triturated with siliga gel 60 (10 gm). This mixture was dried under vacuum and divided into three portions. They were fractionated by the column chromatographic technique using a column of siliga gel (3x31.5 cm). The eluents were used in the order as shown below:-

a) *n*-Hexane

b) 1-10% Chloroform in *n*-hexane

The fractional volume was about 20 ml the separation was monitored by TLC using *n*-hexane : chloroform (4:1) as developing solvent. Fractions giving similar chromatographic pattern were combined . The results were shown in Table 3.2

Table 3.2 Solvent systems used in column chromatography of residue B.

| Fraction | Eluent | Remark |
|---|---|---|
| 1-21 | *n*-hexane | colorless solution |
| 22-55 | gradient of chloroform in *n*-hexane (0.5% ,1% ,2% 3%) | pale-yellow solution |
| 57-67 | gradient of chloroform in *n*-hexane (3% ,4%) | yellow solution |
| 68-103 | gradient of chloroform in *n*-hexane (4%, 5%, 10%) | yellow solution |

A yellow compound was crytallized from fractions 68-103. It was then recrystallized from a mixture of cholroform and methanol as colorless crystals (AP-3, 1.0gm), and was identified as lupeol acetate.

Fraction C (33.9 gm) after drying, was seperated by quick column chromatographic technique using a sintered glass filter column of silica gel G (7.5x3.5 cm) with *n*-hexane and a gradient of dichloromethane in *n*-hexane, as eluent. Fractions of forty ml were collected and combined after examining with TLC using *n*-hexane : chloroform (4:1) as developing solvent. The results were shown in Table 3.3

Table 3.3 Solvent systems used in column chromatography in fraction C.

| Fraction | Eluent | Remark |
|---|---|---|
| 1-15 (afforded a residue C-1) | n-hexane; 5% of $CH_2Cl_2$ in *n*-hexane | colorless solution to pale-yellow solution |
| 16-33 (afforded a residue C-2) | gradient of $CH_2Cl_2$ in *n*-hexane (10% , 15%, 20%, 25%) | pale yellow solution |
| 34-47 (afforded a residue C-3 ) | gradient of $CH_2Cl_2$ in *n*-hexane (25% ,30% ,35% ,40% ) | yellow solution |
| 48-67 (afforded a residue C-4) | gradient of $CH_2Cl_2$ in *n*-hexane (40%, 45%, 50%, 55%, 60%) | yellow solution |

A crude compound, from residue C-1 was further purified by recrystallization from a mixture of chloroform and methanol to afford colorless crystals (1.74 gm). The isolate was designated as AP-3, and identified as lupeol acetate.

Residue C-2 (3.27gm) was recrystallized from a mixture of chloroform and methanol to give colorless crystals (3.02 gm). The TLC chromatogram, using *n*-hexane : ethyl acetate (10:1) of these colorless crystals showed that C-2 was a mixture of two major compounds. This residue was further separated by the chromatographic technique using a column of silica gel (3x20 cm) with *n*-hexane : chloroform (4:1) as eluent. The fractions were collected approximately 25 ml. Fractions of similar TLC behaviour were combined.

A colorless compound was crystallized from fractions 1-25. It weighed 0.25 gm, was designated as AP-3, and was identified as lupeol acetate.

AP-3 which was identified as lupeol acetate yielded totally 2.99 gm. (0.099% ).

Another colorless compound while was obtained from fractions 36-54 was recrystallized from *n*-hexane as colorless needles. The pure compound (0.246 gm, 0.0082%) was designated as AP-4, and was subsequently identified as α–amyrin acetate.

A yellow mass from residue C-3 was recrystallized from a mixture of chloroform and methanol as colorless crytals. The TLC chromatogram, using various developing solvents showed that there was only one compound. These colorless crystals yielded 4.86 gm (0.16%), was designated as AP-2, and was identified as a mixture of α–amyrin acetate and β-amyrin acetate.

Residue D (15.09 grams) was equally divided into five portions, and separated by column chromatography, using a column of silica gel 60 (3x23 cm) with petroleum ether : dichloromethane (2:1) as an eluent. Fraction of forty-five ml were collected. The fractions were examined by TLC, using *n*-hexane : $CH_2Cl_2$ (1:1) as developing solvent. The fractions showing the same pattern were combined.

A pale-orange compound with characteristic odor, from fractions 20-29 was obtained as pale-yellow crystals (1.34gm, 0.045%). It was designated as AP-6, and was identified as 2-hydroxy-4-methoxy benzaldehyde.

4. Identification of the Isolated Compounds

Each isolate was identified through analysis of its hRf values, melting point, ultraviolet absorption, infrared absorption, nuclear magnetic resonace and mass spectra in comparison with previously published data.

4.1 Identification of AP-1

AP-1 was obtained as colorless crystals. It is less soluble in *n*-hexane and petroleum ether, insoluble in ethyl acetate, ethanol and methanol.

Melting Point

63-67°C

Molecular Weight

520 (Mass spectrometry)

Infrared (IR) Absorption Spectrum (KBr disc)

(Figure 3.16)

$\upsilon_{max}$ 2957 ,2849 , 1465 , 722 cm.-1

Nuclear Magnetic Resonance (NMR) Spectra

a) Proton ($^1H$) NMR Spectrum (in $CDCl_3$, 500 MHz)

(Figure 3.17)

Table 3.4 $^1H$-NMR Assignment of AP-1

| H Position | δ (ppm) | Multiplicity |
|---|---|---|
| $CH_3$ | 0.87 | t |
| $CH_2$ | 1.20-1.35 | - |

b) Carbon ($^{13}C$) NMR Spectrum (in $CDCl_3$, 125.65 MHz)

(Figure 3.18)

Table 3.5 $^{13}C$-NMR Assignment of AP-1

| Carbon Position | Chemical shift (δ, ppm) |
|---|---|
| 1,1'-$CH_3$ | 14.04 |
| 2,2' | 22.64 |
| 4,4' | 29.31 |
| 5 | 29.65 |
| 3,3' | 31.88 |

Mass Spectrum (EIMS) (Figure 3.19)

*m/z* (% relative intensity)

520 (M+, 1.20) , 506 (0.64) , 492 (0.84), 478 (0.40), 450 (0.62), 436 (0.67),422 (0.77), 408 (0.63), 394 (0.71), 380 (0.88), 366 (0.78), 352 (1.27), 338 (0.85) , 324 (0.81) , 310 (0.81), 295 (4.10), 281 (4.29), 267 (4.87), 253 (5.12), 239 (5.81), 225 (6.17), 211 (7.39) , 197 (8.76), 183 (9.69),169 (10.83), 155 (11.46), 141 (14.84), 127 (17.41), 114 (2.40), 100 (2.20), 86 ( 5.42),72 (4.16), 58 ( 3.95 ), 44 (2.61),29(6.42)

Therefore it is concluded that AP-1 is heptatriacontane ($C_{37}H_{76}$), the structure of which is shown below

$CH_3$-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_{15}$-$CH_2$-$CH_2$-$CH_2$-$CH_3$

1 2 3 4 5 4' 3' 2' 1'

4.2 Identification of AP-6

AP -6 was obtained as pale yellow crystals with characteristic odor. It is soluble in chloroform and ethyl acetate.

hRf Value (Figures 3.1-3.5)

The hRF values given are obtained from the following sytems:-

a) *n*-Hexane : Diethyl ether (10:1) = 22

b) *n*-Hexane : $CHCl_3$ (2:1) = 35

c) Petroleum ether : $CH_2Cl_2$ (2:1) = 36

d) *n*-Hexane : $CH_2Cl_2$ (1:1) = 39

e) *n*-Hexane : Ethyl acetate (10:1) = 67

Melting Point

37-40°C (*Ref. 41°C Guenther, 1957*)

Molecular Weight

152 (Mass Spectrometry)

Ultraviolet (UV) Absorption Spectrum (in MeOH)

( Figure 3.20)

$\lambda max$ = 276.00 mm ($log\varepsilon$ = 3.67)

Infrared (IR) Absorption Spectrum (KBr disc)

(Figure 3.21)

$\upsilon_{max}$ 3481-3327 (broad), 2926, 2851, 1760, 1500,1238 $cm^{-1}$

Nuclear Magnetic Resonance (NMR) Spectra

a) Proton ($^1H$) NMR Spectrum (in $CDC1_3$, 500 MHz)

(Figure 3.22-3.23)

Table 3.6 $^1H$-NMR Assignment of AP-6

| H Position | δ (ppm) | Multiplicity | *J* (Hz) |
|---|---|---|---|
| O-$CH_3$ | 3.77 | s | - |
| 3 | 6.34 | d | 2.4 |
| 5 | 6.45 | dd | 2.4,8.8 |
| 6 | 7.34 | d | 8.8 |
| CHO | 9.62 | s | - |
| OH | 11.40 | s | - |

b) Carbon ($^{13}C$) NMR Spectrum (in $CDC1_3$, 125.65 MHz)

(Figure 3.24-3.25)

Table 3.7 $^{13}C$-NMR Assignment of AP-6

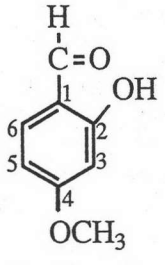
| Carbon Position | Chemical shift (δ,ppm) |
|---|---|
| O-$CH_3$ | 55.63 |
| C-3 | 100.58 |
| C-5 | 108.30 |
| C-1 | 115.09 |
| C-6 | 135.19 |
| C-2 | 164.44 |
| C-4 | 166.76 |
| CHO | 194.33 |

Mass Spectrum (EIMS) (Figure 3.26)

*m/z* (% relative intensity )

152 (91.11), 151 (100.00), 134 (3.89),124 (1.11),122 (1.11), 121 (1.11), 108 (12.78),95 (23.33), 75(2.78), 63 (13.89), 53 (14.44)

These data are in good agreement with the published values of 2-hydroxy-4-methoxy benzaldehyde (Pouchert and Behnke, 1993), a known aromatic aldehyde isolated from various species in Asclepiadaceae. Therefore it was concluded that AP-6 is 2-hydroxy-4-methoxybenzaldehyde (4-methoxysalicylaldehyde), the structure of which is shown below.




2-Hydroxy-4-methoxy benzaldehyde

4.3 Identification of AP-3

AP-3 was obtained as colorless crystals. It is soluble in chloroform and ethyl acetate and insoluble in methanol and ethanol.

hRf value ( Figure 3.6-3.10)

The hRf values given are obtained from the following systems:-

a) Cyclohexane : Benzene (1:2) = 16

b) Cyclohexane : $CH_2Cl_2$ (4:1) = 51

c) n-Hexane : $CHC1_3$ (4:1) = 52

d) Petroleum ether : $CH_2Cl_2$ (10:1.5) = 54

e) Cyclohexane : Ethyl acetate (20:1) = 84

Color Reaction

AP-3 gave pink color with Liebermann-Burchard's test which indicated that it was a triterpenoid compound.

Molecular Weight

468

Infrared (IR) Apsorption Spectrum (KBr disc)

(Figure 3.27)

$\upsilon_{max}$ 3072, 2917, 2851, 1730, 1641 1471, 1382, 977, 912, 718 $cm^{-1}$

Nuclear Magnetic Resonance (NMR) spectrum

a) Proton ($^1H$) NMR Spectrum (in $CDCl_3$, 500 MHz)

(Figure 3.28-3.29)

b) Carbon ($^{13}C$) NMR Spectrum (in $CDCl_3$, 125.65 MHz)

(Figure 3.30-3.31)

Table 3.8 $^{13}C$-NMR Assignment of AP-3

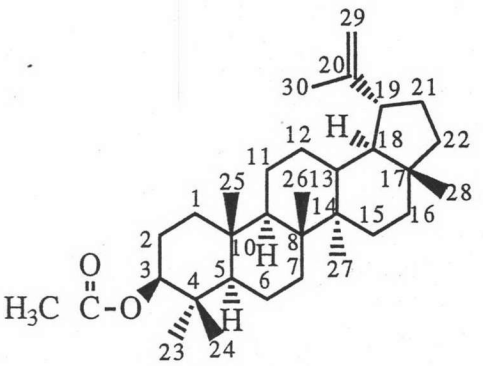
| C- Position | δ (ppm) | Category | C- Position | δ (ppm) | Category |
|---|---|---|---|---|---|
| CO-$CH_3$ | 170.97 | C | C-16 | 35.56 | $CH_2$ |
| C-20 | 150.93 | C | C-7 | 34.21 | $CH_2$ |
| C-29 | 109.34 | $CH_2$ | C-21 | 29.83 | $CH_2$ |
| C-3 | 80.96 | CH | C-23 | 27.94 | $CH_3$ |
| C-5 | 55.38 | CH | C-15 | 27.43 | $CH_2$ |
| C-9 | 50.35 | CH | C-12 | 25.10 | $CH_2$ |
| C-19 | 48.29 | CH | C-2 | 23.70 | $CH_2$ |
| C-18 | 48.00 | CH | CO-CH3 | 21.30 | $CH_3$ |
| C-17 | 42.98 | C | C-11 | 20.93 | $CH_2$ |
| C-14 | 42.81 | C | C-30 | 19.27 | $CH_3$ |
| C-8 | 40.84 | C | C-6 | 18.20 | $CH_2$ |
| C-22 | 39.98 | $CH_2$ | C-28 | 17.99 | $CH_3$ |
| C-1 | 38.39 | $CH_2$ | C-24 | 16.48 | $CH_3$ |
| C-13 | 38.04 | CH | C-25 | 16.16 | $CH_3$ |
| C-4 | 37.78 | C | C-26 | 15.97 | $CH_3$ |
| C-10 | 37.07 | C | C-27 | 14.50 | $CH_3$ |

Mass Spectrum (EIMS) (Figure 3.32)

m/z (% relative intensity)

409 (8.28), 408 (6.69), 297 (5.73), 257 (5.22),299 (10.70), 218 (24.33), 203 (22.93), 190( 27.07), 189 (51.46), 187 (14.65), 175 (21.66), 163(21.02), 161 (26.11), 149 (23.63) , 147 (28.60), 137 (30.57), 135 (43.95), 123 (38.22), 121 (54.78), 109 (51.27),107 (41:40), 95 (47.13), 93 (34.33), 83 (25.48), 81 (51.66), 71 (52.16), 69 (100.00), 67(54.46) ,57 (76.75)

These data are in good agreement with the published values of lupeol acetate, a known triterpenoid isolated from *Stevia rebaudiana* of the family Compositae (Scholichin, Yamasaki, and Kasai, 1980). Therefore it was concluded that AP-3 is lupeol acetate, the structure of which is shown below.




Lupeol-3-acetate

4.4 Identification of AP-2 and AP-4

AP-2 and AP-4 were obtained as colorless needle crystals. They are soluble in chloroform and ethyl acetate, insoluble in methanol and ethanol.

hRF value ( Figure 3.11-3.15)

The hRF values given are obtained from the following system:-

a) Cyclohexane : $CH_2Cl_2$ (4:1) = 26

b) Petroleum ether : $CH_2Cl_2$ (10:1.5) = 27

c) $CHCl_3$ : Benzene ( 1: 3 ) = 29

d) *n*-Hexane : $CHCl_3$ ( 4: 1 ) = 30

e) Petroleum ether : Ethyl acetate ( 10: 1) = 78

Color Reaction

AP-2 and AP-4 gave pink color with Liebermann-Burchard's test, suggestively that they were triterpenoid compounds.

Melting Point of AP-4

205-208.5°C *(Ref. 225 - 226°C Windholz, 1983)*

Melecular Weight

468 (Mass spectrometry)

Infrared (IR) Absorption Spectra (KBr disc)

AP-2 (Figure 3.33)

$\upsilon_{max}$ 3445, 3100-2925, 1736, 1455, 1368, 4954,1004-987, 716 $cm^{-1}$

AP-4 (Figure 3.37)

$\upsilon_{max}$ 3451, 2954-2846, 1736, 1454, 1367, 1244, 1003-969, 729 $cm^{-1}$

Nuclear Magnetic Resonance ( NMR ) Spectrum

a) Proton ($^1H$) NMR Spectrum of AP-4 (in $CDCl_3$, 500 MHz)

(Figure 3.38-3.39)

b) Carbon ($^{13}C$) NMR Spectrum of AP-2 (in $CDC1_3$ , 125.65 MHz)

(Figure 3.34-3.35)

Table 3.9 $^{13}C$-NMR Assignment of AP-2

| C- Position | δ (ppm) | Category | C- Position | δ (ppm) | Category |
|---|---|---|---|---|---|
| CO-$CH_3$ | 170.9 | C | C-7 | 32.6 | $CH_2$ |
| C-13 | 145.1 | C | C-17 | 32.5 | C |
| C-12 | 121.6 | CH | C-20 | 31.0 | C |
| C-3 | 80.9 | CH | C-15 | 28.4 | $CH_2$ |
| C-5 | 55.3 | CH | C-23 | 28.1 | $CH_3$ |
| C-9 | 47.5 | CH | C-28 | 27.3 | $CH_3$ |
| C-18 | 47.2 | CH | C-16 | 26.1 | $CH_2$ |
| C-19 | 46.7 | $CH_2$ | C-27 | 25.9 | $CH_3$ |
| C-14 | 41.7 | C | C-30 | 23.6 | $CH_3$ |
| C-8 | 39.7 | C | C-2 | 23.6 | $CH_2$ |
| C-1 | 38.2 | $CH_2$ | C-11 | 23.3 | $CH_2$ |
| C-4 | 37.6 | C | CO-CH3 | 21.3 | $CH_3$ |
| C-22 | 37.1 | $CH_2$ | C-6 | 18.2 | $CH_2$ |
| C-10 | 36.8 | C | C-24 | 16.8 | $CH_3$ |
| C-21 | 34.7 | $CH_2$ | C-26 | 16.7 | $CH_3$ |
| C-29 | 33.3 | $CH_3$ | C-25 | 15.5 | $CH_3$ |

c) Carbon ($^{13}C$) NMR Spectrum of AP-4 (in $CDCl_3$, 125.65 MHz)

(Figure 3.40-3.41)

Table 3.10 $^{13}C$-NMR Assignment of AP-4

| C- Position | δ (ppm) | Category | C- Position | δ (ppm) | Category |
|---|---|---|---|---|---|
| CO-$CH_3$ | 170.98 | C | C-7 | 32.83 | $CH_2$ |
| C-13 | 139.60 | C | C-21 | 31.22 | $CH_3$ |
| C-12 | 124.28 | CH | C-28 | 28.72 | $CH_3$ |
| C-3 | 80.92 | CH | C-23 | 28.04 | $CH_2$ |
| C-18 | 59.01 | CH | C-15 | 28.04 | $CH_2$ |
| C-5 | 55.23 | CH | C-16 | 26.57 | $CH_2$ |
| C-9 | 47.61 | CH | C-2 | 23.57 | $CH_2$ |
| C-14 | 42.03 | C | C-11 | 23.34 | $CH_2$ |
| C-22 | 41.51 | $CH_2$ | C-29 | 23.20 | $CH_3$ |
| C-8 | 39.99 | C | C-30 | 21.38 | $CH_3$ |
| C-19 | 39.62 | CH | CO-CH3 | 21.29 | $CH_3$ |
| C-20 | 39.58 | CH | C-6 | 18.22 | $CH_2$ |
| C-1 | 38.43 | $CH_2$ | C-27 | 17.49 | $CH_3$ |
| C-4 | 37.68 | C | C-24 | 16.84 | $CH_3$ |
| C-10 | 36.76 | C | C-26 | 16.72 | $CH_3$ |
| C-17 | 33.72 | C | C-25 | 15.71 | $CH_3$ |

Table 3.11 $^{1}H$ Chemical shifts for AP-4 (from HETCOR)

| Hydrogen position | Category | δ(ppm) | Hydrogen position | Category | δ(ppm) |
|---|---|---|---|---|---|
| 1 | $CH_2$ | 1.62 | 19 | CH | 1.28 |
| 2 | $CH_2$ | 1.61 | 20 | CH | 0.84 |
| 3 | CH | 4.47 | 21 | $CH_3$ | 1.33 |
| 5 | CH | 0.82 | 22 | $CH_2$ | 1.26, 1.41 |
| 6 | $CH_2$ | 1.50 | 24 | $CH_3$ | 0.98 |
| 7 | $CH_2$ | 1.34, 1.52 | 25 | $CH_3$ | 0.95 |
| 9 | CH | 1.53 | 26 | $CH_3$ | 0.85 |
| 11 | $CH_2$ | 1.88 | 27 | $CH_3$ | 0.77 |
| 12 | CH | 5.09 | 28 | $CH_3$ | 0.76 |
| 15 | $CH_2$ | 0.85 | 29 | $CH_3$ | 1.04 |
| 16 | $CH_2$ | 0.98 | 30 | $CH_3$ | 0.88 |
| 18 | CH | 1.30 | CO-CH3 | $CH_3$ | 2.02 |

Mass Spectrum

a) EIMS of AP-2

(Figure 3.36)

*m/z* (% relative intensity)

468 (3.04), 219 (18.62), 218 (100.00) , 203 (18.20), 189 (16.68), 135 (13.94),123 (10.00),122 (10.13), 121 (10.21), 109 (12.81), 107 (10.51), 95 (13.85), 81 (12.17), 69 (16.92), 55 (11.62), 43 (13.72)

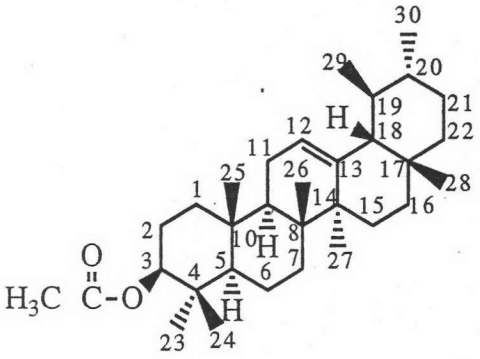
b) HREIMS of AP-4

(Figure 3.47)

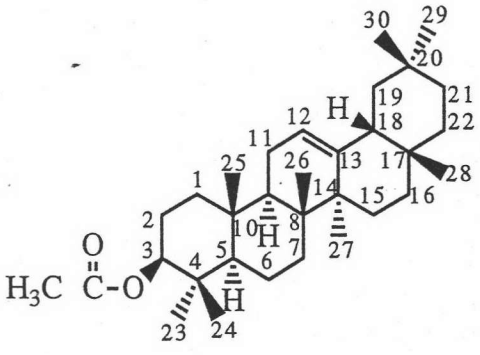
*m/z* (% relative intensity)

468 (3.03), 219 (27.27), 218 (100.00), 135 (22.63), 69 (22.42), 43 (22.42)

These data are in excellent agreement with the published values of α- and β-amyrin acetate (Seo, Tomta, and Tori, 1975). Therefore, it was concluded that AP-2 is β- amyrin acetate and AP-4 is α-amyrin acetate, the structures of which are shown below.




α-Amyrin-3-acetate




β-Amyrin-3-acetate