CHAPTER IV

DISCUSSION

In this present work, the chloroform extract of dried powderd roots of *Atherolepis pierrei* Cost. var. *glabra* Kerr (Asclepiadaceae) was separated by chromatographic technique and afforded five isolated compounds. The structure elucidation of the compounds was performed on the basis of interpretation of their spectral data, and further confirmed by comparison these data with those reported in the literature.

The first and least polar compound, AP-1, was obtained as colorless crystals and identified as " heptatriacontane".

The electron impact mass spectrum (EIMS) (Figure 3.19) exhibits a molecular ion peak at *m/z* 520 (1.20%) and therefore suggests a tentative molecular formula of $C_{37}H_{76}$. The IR spectrum (Figure 3.16) shows the characteristic of normal alkanes. Two distinct bands occuring at 2957 and 2849 cm^{-1} are the C-H stretching vibrations of sp^3. The bending vibration of the C-H bonds in the methylene group occures at 1465 cm^{-1} ; and the band resulting from the methylene rocking vibration appears near 720 cm^{-1} for straight-chain alkanes of seven or more carbon atoms (Silverstein, 1981).

The ^{1}H-NMR spectrum of AP-1 (Figure 3.17) shows the signals of 70 methylene and 6 methyl protons.

The structure is confirmed by the analysis of the mass fragmentation. The straight-chain alkanes always shows weak molecular ions and typical series of $(C_nH_{2n+1})^+$ and to a lesser extent $(C_nH_{2n-1})^+$ the fragmentation pattern is characterized by clusters of peaks , and the corresponding peaks of each cluster are 14 (CH_2) mass units apart (Silverstein, 1981).

The second compond, AP-6, was obtained as pale yellow crystals with characteristic odor.

The EIMS of AP-6 (Figure 3.24) exhibits a molecular ion at *m/z* 152 (91.11%) consistent with a molecular formula of $C_8H_8O_3$. The UV absorption at 276 nm (logε 3.67) (Figure 3.20) shows the characteristic of aldehyde with *p*-substituent of hydroxy or alkoxy groups (Scott, 1964). The IR spectrum (Figure 3.21) confirms the presence of the aldehyde at υ_{max} 1700, and 1238 cm^{-1}; and also suggest hydroxyl group at υ_{max} 3,300 cm^{-1}(broad).

AP-6 can be assigned as a known aromatic aldehyde, 2-hydroxy-4-methoxy benzaldehyde or 4-methoxy salicylaldehyde (Pouchert and Behnke, 1993) through analysis of its ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum (Figure 3.22-3.23) provides the signals of 3 aromatic protons, in addition to 1 methoxy, 1 hydroxy, and 1 aldehyde groups. The ^{13}C NMR spectrum (Figure 3.24) shows the signals of 1 carbonyl carbon, 1 methoxy carbon, and 6 aromatic carbons. From DEPT (Figure 3.25), we can conclude that AP-6 has 1 methyl and 1 carbonyl group, 3 methine and 4 quaternary carbons.

The signals of the olefinic protons in ^1H NMR spectrum at 6.34 ppm (d,*J* = 2.4 Hz), 6.45 ppm (dd, *J*=2.4,8.8 Hz) and 7.34 ppm (d, *J*=8.8 Hz) are assigned as H-3 ,H-5 and H-6, respectively. The remaining signals are the signals of 4-OCH_3(3.77 ppm, s), 1-CHO (9.62 ppm, s), and 2-OH (11.4 ppm, s).

The ^{13}C NMR spectrum supports this structure. The aromatic carbon signals at 100.58, 108.30, 115.09, 135.19, 164.44, and 166.76 ppm are assigned as 5-CH, 3-CH, 1-C, 6-CH, 2-C, and 4-C, respectively. The signals at 55.63, and 194.33 ppm are assigned to the methoxy groups, and carbonyl carbon, respectively.

The assignments of carbons and protons of AP-6 are supported by comparison with the reports by Pouchert and Behnke in The Aldrich Library of ^{13}C and ^1H FT NMR Spectra Volume 2. (1993)

The structure of AP-6 is finally confirmed by the analysis of the mass fragmentation. The loss of H_2O causes the fragment at *m/z* 134 (3.89%) which suggests the presence of a hydroxyl group. The base peak, 151 $(M-1)^+$, indicates the presence of a labile hydrogen atom. The $(M-28)^+$, $(M-29)^+$, and $(M-31)^+$ ions are due to loss of CO, CHO and O-CH_3, respectively (McLafferty, 1980). (Figure 3.48)

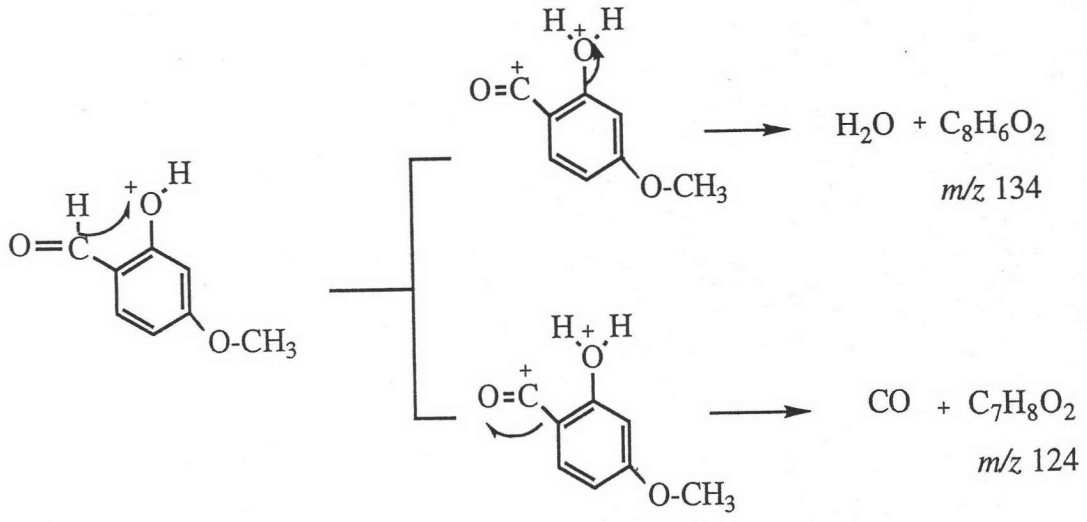

Figure 3.48 Mass fracmentation of AP-6

In addition, three known pentacyclic triterpenoids have been isolated and identified as lupeol acetate (AP-3), α-amyrin acetate (AP-4), and a mixure of α–amyrin acetate and β-amyrin acetate (AP-2). Triterpenoids are an important class of naturally occurring compounds which ^{13}C NMR has proved useful in identifying. Analysis of their spectra provides a wealth of data on stereochemical and functional group substitution effects on ^{13}C chemical shifts in polycyclic system (Knight, 1974).

One of the pentacyclic triterpenoids, AP-3, was obtained as colorless crystals. The EIMS of AP-3 (Figure 3.32) shows a molecular ion peak at *m/z* 426 (0.64%). The IR spectrum (Figure 3.27) exhibits the bands at 3072-2851, 1730, 1641, 1471, 1382, 977, 912, and 718 cm^{-1} indicating the presence of cyclic alkanes, methyl groups and olefinic hydrocarbons.

AP-3 is identified as a known triterpenoid, lupeol acetate, by analysis of the ^{13}C NMR spectrum. The ^{13}C NMR spectrum (Figure 3.30-3.31) shows the signals of 7 quaternary, 6 methine, 11 methylene, and 8 methyl carbons and the chemical shifts are summarized in table 3.8. The assignments of carbons of AP-3 are supported by comparison with the data of lupeol acetate previously reported by Sholichin *et al.*, 1980.

The ^{13}C NMR spectrum of compound AP-3 shows the signals of a carbonyl carbon at 170.97 ppm, eight methyl carbons at 27.94, 21.30, 19.27, 17.99, 16.48, 16.16, 15.97, and 14.50 ppm which are assigned as C-23, CO-$\underline{C}H_3$, C-30, C-28, C-

24, C-25, C-26, and C-27, respectively. The remaining signals are the signals of methylene,methine and quaternary carbons as shown in table 3.8. The integration of ^{1}H NMR shows 52 protons in the structure. ^{1}H NMR spectrum is generally much too complex to allow direct assignment of methylene and methine chemical shifts in triterpenoid derivatives.

Although the mass spectra of lupane derivatives seem to be much less characteristic and only in simplest cases are a few fragments outstanding enough to offer useful information, it was found that this compound is characterized by intense peaks at *m/z* 189, 191, 218. (Figure 3.49) These peaks are important fragments in EIMS technique which show a skeleton structure of lupane type (Budzikiewicz, Djerassi, and Williams, 1964; Ogunkoya, 1981).

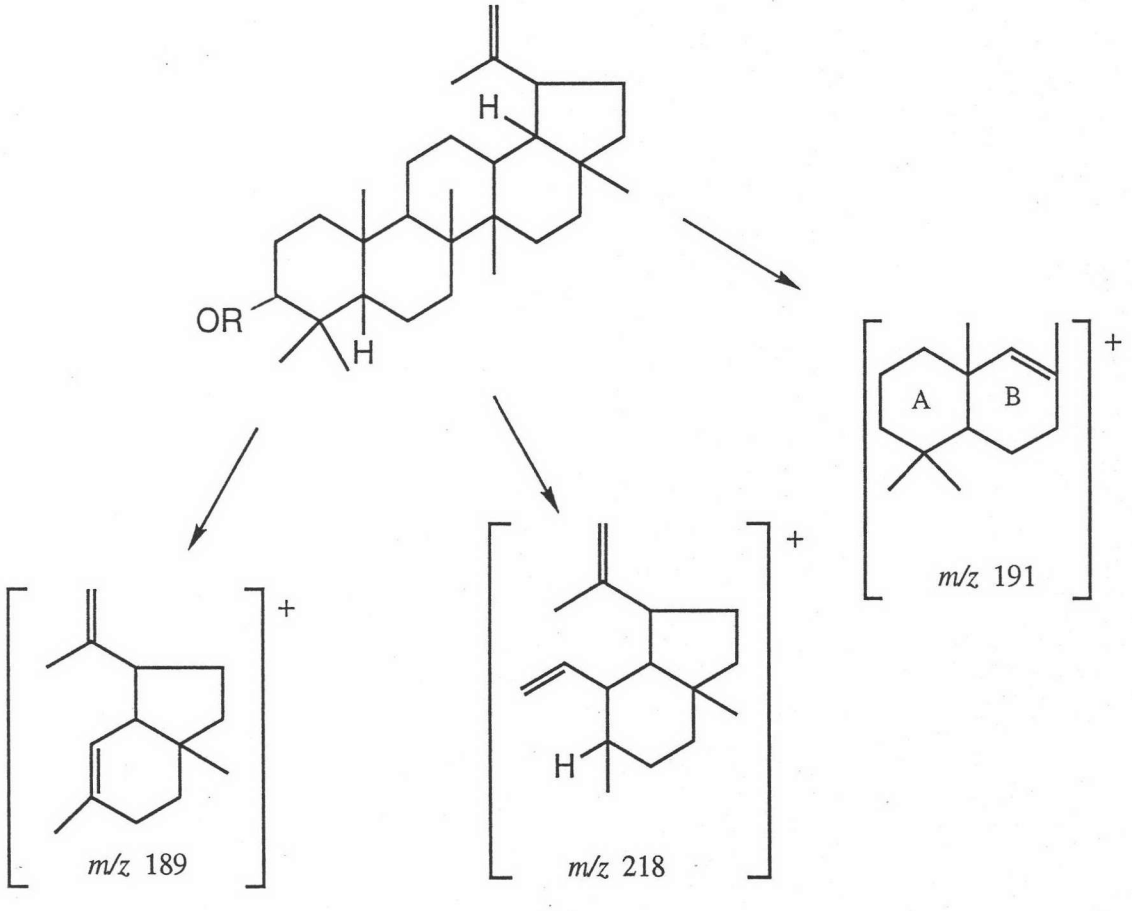

Figure 3.49 Mass fracmentation of AP-3

The isomeric pentacyclic triterpenoids, AP-2 and AP-4 were obtained as colorless needle crystals. The EIMS of AP-2 (Figure 3.36) and AP-4 (Figure 3.47) show a molecular ion peak at *m/z* 468 and suggesting a molecular formula of

$C_{32}H_{52}O_2$. The IR spectra (Figure 3.33, and 3.37) confirm the presence of the carbonyl at 1736 cm^{-1}, cyclic alkane at 2925 and 1455 cm^{-1}, and olefinic at 987, and 1003-1004 cm^{-1}.

From the Distortionless Enhancement by Polarization Transfer (DEPT) we can conclude that AP-4 has contained 7 quaternary carbons, 7 methine, 9 methylene, and 9 methyl groups. Its EIMS revealed a parent peak at 468, corresponding to the molecular formula $C_{32}H_{52}O_{2,}$, and this was confirmed by high resolution electron impact mass spectrum(HREIMS). The base peak at m/z 218 is the characteristic feature of triterpenoid skeleton which includes members of α-(Δ^{12}-ursane) or β-(Δ^{12}-oleanane) amyrin series. The molecular ion undergoes the equivalent of a retro-Diels-Alder fracmentation to furnish a very characteristic peak (*m/z* 218)(Budzikiewicz et al., 1964). (Figure 3.50) The skeleton structure of AP-4 is corresponding to Δ^{12}-ursane rather than Δ^{12}-oleanane due to the member of quaternary carbon, methine, and methyl groups. (Δ^{12}-oleanane has 8 quaternary carbons, 5 methine, and 10 methyl groups which is the skeleton structure of AP-2).

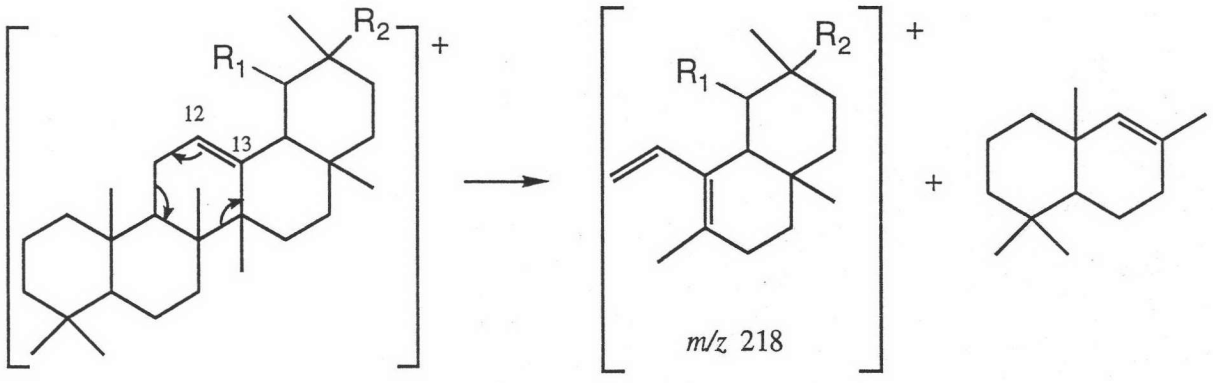

Figure 3.50 Mass fracmentation of AP-2 and AP-4

Through comparison with the data published for the ^{13}C NMR spectra (Seo, Tomita, and Tori, 1975) of urs-12 enes, AP-4 is in complete agreement with α-amyrin-3 acetate. In additionally, AP-2 is β-amyrin-3-acetate. Apart from C-29 and C-30 methyl groups, α- and β-amyrin-3- acetate have a common structure and stereochemistry over the pentacyclic ring system (Knight, 1974). Both have 3-acetate substitutent on ring A. Assignments of the ^{13}C NMR spectrum of α-amyrin acetate are summarized in table 3.10. The signals at 170.98, 31.22, 28.72, 23.20, 21.38, 21.29, 17.49, 16.84, 16.72, and 15.71 ppm are assigned as a carbonyl carbon, and methyl carbons at C-21, C-28, C-29, C-30, CO-$\underline{C}H_3$, C-27, C-24, C-26, and C-25, respectively. The remaining signals are the signals of methylene, methine, and quaternary carbons.

In this investigation, an attempt to completely assign all the ^{13}C NMR signals was made the results from analysis of the DEPT spectrum of AP-4 suggested that the published (Seo, Tomita, and Tori, 1975) assignment should be revised. The assignment of C-15 at 28.7 ppm is doubtful since C-15 is a methylene carbon and the carbon at 28.7 ppm is a methyl group . Alternatively, the carbon at 28.1 ppm was reported to be C-28 (methyl) although the DEPT spectrum is strongly indicated that it was a methylene carbon.

In addition, the assignments of C-11 (methylene) and C-27 (methyl) in the previous report (Seo, Tomita, and Tori, 1975) should be reversed.

Although ^{1}H NMR spectra of triterpenoids are generally much too complex to allow direct assignment of methylene and methine chemical shifts, recent development of two-dimensional NMR spectroscopy has provided a number of new NMR assignment techniques which are useful in the area of natural products chemistry, particularly in the field of triterpenoids (Reynolds, McLean, and Poplawski, 1986). Thus we can assign proton chemical shifts of this compound easily by using HETCOR spectra. The ^{1}H chemical shifts of AP-4 are assigned from direct ^{1}H-^{13}C connectivities determined using HETCOR and summarized in table 3.11.

From the assignment of ^{13}C NMR spectrum of α-amyrin acetate (AP-4), the remaining signals of β-amyrin acetate in the mixture of α-and β-amyrin acetate (AP-2) could be assigned as in Table 3.9.