

CHAPTER III



Acanthus ilicifolius and Acanthus ebracteatus

Discussion and Result

The powdered plants of A. ilicifolius and A. ebracteatus were extracted with light petroleum ether and ethanol. The crude ethanol extract, after evaporating the solvent in vacuo, was equilibrated between chloroform and water. The light petroleum ether extract, after chromatographed on a standardized alumina column, gave two components. The first one (A), m.p. 87-88°C, had the infra-red absorption indicative of hydroxylic group (3350 cm^{-1} and 1060 cm^{-1}). The presence of the hydroxylic group was also confirmed by chemical means. Acetylation and benzoylation of A yielded ester derivatives, m.p. 74-75°C and 67-68°C respectively. Its IR spectrum had an absorption for carbonyl group (1740 cm^{-1} , 1230 cm^{-1} for acetate derivative and 1725 cm^{-1} , 1290 cm^{-1} for benzoate derivative). Furthermore, compound A was identical with myricyl alcohol by its melting point, mixed m.p. and IR spectrum.

The latter (B), m.p. 168-169°C, gave a positive test with Liebermann-Burchard. The infra-red spectrum had an absorption for hydroxylic group (3400-3200 cm^{-1} and 1060 cm^{-1}) and two double bonds (970 cm^{-1} , 960 cm^{-1} and 830 cm^{-1}). The NMR spectrum showed a hydroxylic proton (δ 1.57, 1H, singlet) which disappeared upon shaking with D₂O and two double bonds (δ 5.4, 1H, broad multiplet

and $\delta 5.14$, 2H, broad multiplet). The presence of hydroxylic group and double bonds were also confirmed by chemical means. Acetylation of B yielded acetate derivative, m.p. $143-144^{\circ}\text{C}$, IR absorption at 1742 cm^{-1} (carbonyl stretching vibration) and $1270-1250\text{ cm}^{-1}$ (C-O stretching vibration of acetoxy group), NMR peaks at $\delta 2.01$ (3H, singlet, $\text{CH}_3 - \overset{\text{O}}{\parallel}{\text{C}} - \text{O}$) and $\delta 4.9 - 4.3$ (1H, broad multiplet, $>\text{CH} - \text{OAc}$). Hydrogenation of B yielded hydrogenated product; m.p. $135-136^{\circ}\text{C}$, no IR absorption at 970 cm^{-1} , 960 cm^{-1} , 830 cm^{-1} and NMR absorption at $\delta 5.4$ and $\delta 5.14$. These mean the double bonds in the compound B were completely hydrogenated. In addition, compound B was identical with stigmasterol by its melting point, mixed m.p., IR and NMR spectrum.

The 95 % ethanolic extract of A. ilicifolius and A. ebracteatus, after being concentrated under reduced pressure, was equilibrated between chloroform and water. The chloroform soluble fraction was chromatographed by using standardized alumina 90 as an absorbent and eluted with 20 % methanol in chloroform yielded only one component and appeared as a single spot on TLC, designated as compound C.

Compound C

Compound C crystallized as white amorphous crystal solids from methanol in chloroform, m.p. 292^o C (decompose). Carbon-hydrogen analysis found that it contains C 73.24 %, H 10.72 %. It gave a deep violet-green colour with Liebermann-Burchard, thus indicating the presence of steroid or triterpenoid compound.

The infra-red spectrum of compound C shows a prominent broad absorption band at 3470-3220 cm^{-1} (OH-stretching vibration) and the two absorption bands in the region near 1110-1030 cm^{-1} (C-OH out-of-plane bending vibration) which suggest that compound C is suspected to be a primary and/or secondary alcoholic compound, probably there is more than one hydroxylic group present in the compound C.

The NMR spectrum in deuterio-trifluoroacetic acid (TFA-D) displays a complex signals in the regions near δ 1.02-0.75 and δ 1.35 which correspond with the chemical shifts of methyl protons, methylene protons, and methylenidene protons. The singlet at δ 1.1

disappears upon shaking with D_2O indicating the presence of hydroxylic protons. The broad multiplet in the regions near $\delta 4.35 - 4.2$ is considered to be due to the olefinic protons present in the compound C.

Compound C Acetate

Acetylation of Compound C with acetic anhydride in pyridine gave the acetylated product which crystallized as white amorphous crystalline solids, m.p. $162-163^\circ C$.

The infra-red spectrum of compound C acetate shows a multiplet peaks in the region near $1760-1730\text{ cm}^{-1}$ (carbonyl-stretching vibration) and the broad bands at $1075-1045\text{ cm}^{-1}$ $1265-1225\text{ cm}^{-1}$ (C-O-stretching vibration of acetoxy group). The observation of multiplet bands of carbonyl-stretching vibration indicates that it probably has more than one acetoxy group present in the compound C.

The NMR spectrum in deuteriochloroform ($CDCl_3$) shows a triplet peak at $\delta 2.02$, with equal intensity and slightly different chemical shifts. This indicates that at least three acetoxy groups are present in the compound C acetate, which agrees well with the infra-red spectrum. The signals arising from the remaining protons in the molecule behave similarly to the NMR spectrum of compound C.

Conclusion

The IR, NMR spectral information and chemical test suggest that the structure of compound C is the steroid or

triterpenoid which contains at least three hydroxylic groups and an olefinic double bond. Due to paucity of the spectral data available, compound C could not be further investigated without the mass spectrum (and then the structure of compound C is further studied).

The aqueous fraction, after being equilibrated with chloroform, was concentrated under reduced pressure and then dissolved in methanol. The insoluble precipitate mainly consisted of chloride-salts of sodium 20.625 %, potassium 12.53 %, magnesium 0.003125 %, calcium 0.0008 %, manganese , 0.00075 %, and traces amount of iron , copper , cobalt , zinc , and lead .

The methanol soluble part was preliminary tested with the reagents as follow. It gave a silver mirror with Tollen's reagent, an orange-red colour of cuprous oxide with Benedict's, Barfoed's, and Fehling's solution. These indicated that the methanol soluble part mainly consist of monosaccharides or reducing sugars. Paper chromatography showed two spots which probably were glucose and galactose. The osazone formation of the crude methanol soluble fraction gave glucosazone, m.p. 205°C (decompose) and galactosazone, m.p. $185-186^{\circ}\text{C}$. The acetate formation of the crude methanol soluble fraction yielded glucose-pentacetate, m.p. $112-113^{\circ}\text{C}$ and galactose-pentacetate m.p. $94-95^{\circ}\text{C}$. The acetate and osazone derivatives were found to be identical with authentic glucose and galactose by its melting point, mixed m.p., and infra-red spectra.