

CHAPTER V



Acanthus ilicifolius and Acanthus ebracteatus

Experimental

The extraction previously described for O. diffusa was applied to A. ilicifolius and A. ebracteatus. It gave a crude petroleum ether extract of 345 g. (0.6 % by wt. from 57 kg.) and a crude ethanol extract of 1065 g. (1.87 % by wt.). The crude ethanol extract, after partition between chloroform and water, gave 197g. (1.87 % by wt.) of crude chloroform soluble fraction and 856 g. (1.5 % by wt.) of aqueous fraction. The aqueous fraction, after being concentrated under reduced pressure, was dissolved in methanol. It gave 720 g. (1.26 % by wt.) insoluble inorganic salt and 130.8 g. (0.23 % by wt.) of methanol soluble fraction of monosaccharide.

The crude petroleum-ether extract, after evaporating solvent in vacuo, was chromatographed on the standardized alumina : : 90 (activity II-III, E. Merck, Darmstadt), using light petroleum ether as the initial eluting solvent, changing to diethyl ether by gradual introduction of the latter. Finally, the column was stripped with methanol (the method was the same as for O. diffusa, see table III). Each eluted fraction was monitored by TLC using concentrated sulphuric acid for detection and equivalent fractions were combined.

All waxes, oil, and tars were discarded. The crude petroleum ether extract gave only two major components which were designated as compound A and B.

Compound A

The combined eluted fractions of 5-10 % ether:petroleum ether from No. 5 to No. 12 on standing deposited colourless amorphous crystals which were filtered off and washed with a little cold light petroleum ether. On recrystallization from benzene-petroleum ether, colourless amorphous crystals (180 mg., 0.15 % by wt.) were obtained, m.p. 87-88°C. It gave negative tests with Liebermann-Burchard reagent, potassium permanganate solution and bromine in carbon tetrachloride.

Spectroscopic data:

IR (KBr pellet) ν_{\max} . 3350, 2915, 2845, 1475,
1460, 1060, 735, 720, cm^{-1} .

Analytical data: found: C 82.34 %, H 14.48 %

calc. for $\text{C}_{30}\text{H}_{62}\text{O}$: C 82.11 %, H 14.24 %

Preparation of Compound A Acetate

Compound A (80 mg.) was mixed with acetic anhydride (1 cm.)³ and a few drops of pyridine. The mixture was heated on a water steam bath, with occasional shaking, for two hours. The cooled reaction mixture was then poured, with vigorous stirring, into 20 cm.³ of ice water. Stirring was continued until the excess

acetic anhydride was hydrolyzed. The precipitated acetate (83 mg.) was filtered off, washed thoroughly with water and purified by recrystallization from petroleum ether as colourless crystals (75 mg., 86 %). m.p. 74-75°C.

Spectroscopic data:

IR (KBr pellet) $\gamma_{\max.}$ 1740, 1230, 1030 cm^{-1}

Preparation of Compound A Benzoate

50 mg. of the compound A were mixed with 2 cm.^3 of pyridine and 1.5 cm.^3 of redistilled benzoyl chloride in a 50 cm.^3 round bottomed flask, the mixture was heated under reflux, with occasional shaking, for one and a half hours. 25 cm.^3 of 5 % sodium hydrogen-carbonate solution was added to the cold reaction mixture. The resulting mixture was cooled in an ice bath until the precipitate was formed. The solid was filtered and washed with cold water until no more pyridine was left. It was recrystallized from petroleum ether as amorphous solids (46 mg., 74 %) m.p. 67-68°C.

Spectroscopic data:

IR (KBr pellet) $\gamma_{\text{mzx.}}$ 2950, 2870, 1725, 1608,
1585, 1465, 1290, 1130,
700, 690, cm^{-1}

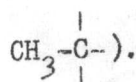
Compound B

The combined eluted fractions of 40 % ether:petroleum ether from No. 27 to No. 36 on standing deposited colourless needle crystals which were filtered off and washed with a little cold light petroleum ether. On recrystallization from petroleum ether or ethanol, colourless needle-shaped crystals (1.87 g., 1.56 % by wt.) were obtained, m.p. 168-169 C. It gave positive tests with Liebermann-Burchard reagent, potassium permanganate solution and bromine in carbon tetrachloride.

Spectroscopic data:

IR (KBr pellet)	ν_{\max}	3400-3200, 3100-3000, 1450-1350, 1060, 970, 960, 830, 790, cm^{-1}
NMR (CDCl_3)	δ	5.4 (1H, broad multiplet, >C=CH-CH ₂ -), 5.14 (2H, broad multiplet, >CH-CH = CH-CH<), 3.3-3.8 (1H, broad multiplet, >CH-OH), 1.57 (1H, singlet, >CH-OH), 1.2 (3H, doublet, CH ₃ -CH< , J=6Hz.), 1(3H, singlet, CH ₃ - $\overset{ }{\underset{ }{\text{C}}}$ -), 0.87 (3H, triplet, CH ₃ -CH ₂ -, J=6Hz.), 0.82 (6H, doublet, $\begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix} > \text{CH}-$,

$J = 6\text{Hz.}), 0.7 (3\text{H, singlet,}$



Preparation of Compound B Acetate

Compound B (100 mg.) was mixed with acetic anhydride (2 cm.³) and a few drops of pyridine. The reaction mixture was refluxed on a steam bath for four hours, with occasional shaking. The cooled solution was then poured, with vigorous stirring, into 30 cm.³ of ice water. Stirring was continued until the excess acetic anhydride was hydrolyzed. The precipitated acetate (118 mg.) was collected by filtration, washed thoroughly with ice-cold water, and purified by recrystallization from methanol as colourless plates (102 mg., 92.7 %), m.p. 143-144°C.

Spectroscopic data:

IR (KBr pellet)	$\gamma_{\text{max.}}$	1742, 1270-1250 cm^{-1}
NMR (CDCl_3)	δ	5.37 (1H, broad multiplet, >C = CH-CH ₂ -), 5.1 (2H, triplet like structure, >CH-CH=CH [*] -CH<), 4.9-4.3 (1H, broad multiplet, >CH-OAc), 2.01 (3H, singlet, CH ₃ - $\overset{\text{O}}{\parallel}$ C-O-), 1.01 and 0.7 (6H, singlet, CH ₃ - $\overset{\text{I}}{\underset{\text{I}}{\text{C}}}$ -), 1.03 (3H, doublet CH ₃ -CH, J=6Hz.),

0.85 (6H, doublet, $\begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix} > \text{CH}-$,
 J=6Hz.), 0.84 (3H, triplet,
 CH_3-CH_2- , J=6Hz.).

Hydrogenation of Compound B

20 mg. of 10 % Pd/C catalyst were placed in the hydrogenation vessel and then a 100 mg. of compound II in 30 ml. of absolute alcohol were introduced. The solution should completely cover the catalyst, the stopper was lubricated with an inert grease and inserted into the vessel. The hydrogen gas was introduced into the reaction mixture with mechanical shaker, under 2.06×10^5 n./m.² pressure at such a rate that the temperature did not rise above 25°C . The mixture was shaken for further four hours. The reaction product was then filtered through two filter papers supported on a Buchner funnel. The filtrate was evaporated to dryness on a water bath and the residue was purified by re-crystallization from 95 % ethanol as white colour plates (101 mg., 91.8 %), m.p. $135-136^\circ\text{C}$.

Spectroscopic data:

IR (KBr pellet)	γ_{max}	3400-3200, 2915, 2880
		1475, 1460, 1385, 1060 cm^{-1}
NMR (CDCl_3)	δ	3.55 (1H, broad multiplet,
		$>\text{CH}-\text{OH}$), 1.63 (1H, singlet,

$>\text{CH}-\underline{\text{OH}}$, 0.84 (9H, doublet,
 $\text{CH}_3-\text{CH}<$, $J=6\text{Hz.}$), 0.83 (3H,
 triplet, CH_3-CH_2- , $J=6\text{Hz.}$)

The Chloroform Fraction of A. ilicifolius and A. ebracteatus

The chloroform part was taken from the crude ethanol fraction by equilibration between chloroform and water. After being concentrated under reduced pressure, the crude chloroform soluble fraction (10 g.) was chromatographed on standardized alumina 90 column (200g.). Using 40 % chloroform in petroleum ether as the initial eluting solvent it was changed to chloroform by gradual introduction of the latter. Finally, the column was stripped with methanol. Each eluted fraction was monitored by TLC using concentrated sulphuric acid for detection and equivalent fractions were combined.

Compound C

The combined eluted fraction No. 44 to No. 58 (see Table IV) on standing deposited colourless amorphous crystals which were washed with a little cold methanol. On recrystallization from chloroform-methanol, colourless amorphous solids (780 mg., 0.0014 %) were obtained, m.p. 292°C (decompose). It gave a violet colour with Liebermann-Burchard reagent.

Spectroscopic data:

IR (KBr pellet) ν_{\max} . 3470-3220, 1465, 1385,
1375, 1175, 1100-1030 cm^{-1}

NMR (TFA-D) δ 4.34-4.2, 1.35, 1.02
1.1, 0.75.

Table IV

Column Chromatography of the Crude Chloroform Soluble Extract

Eluting solvent	Eluted fractions [*]	Remark
40% CHCl ₃ in P.E.	1- 8	waxes and oil
75% CHCl ₃ in P.E.	9-18	dark tarry oil
Chloroform	19-27	dark tarry oil
5% MeOH in CHCl ₃	28-34	dark tarry oil
10% MeOH in CHCl ₃	35-40	dark tarry oil
20% MeOH in CHCl ₃	41-43	dark tarry oil
	44-58	Compound C (780 mg. 0.0014%)
50% MeOH in CHCl ₃	59-63	oil & tar
MeOH	64-70	tar

*About 500 cm.³ of each fraction were collected

N.B. The percentage yields of the extracted compounds were based on the dried plant weight (57 kg.)

Preparation of Compound C Acetate

Compound C (100 mg.) was mixed with acetic anhydride (1 cm.³) and a few drops of pyridine. The mixture was heated on a steam bath, with occasional shaking, for four hours. The cooled solution was then poured, with vigorous stirring, into 25 cm.³ of ice water. Stirring was continued until the excess acetic anhydride was hydrolyzed. The precipitated acetate (120 mg.) was filtered off, washed thoroughly with water until no more pyridine was left and purified by recrystallization from methanol-chloroform as colourless crystal plates (110 mg, 85 %), m.p. 162-163°C.

Spectroscopic data:

IR (KBr pellet)	$\gamma_{\max.}$	1760-1730, 1385-1370 1265-1225, 1175, 907 1075-1045 cm ⁻¹ .
NMR (CDCl ₃)	δ	5.5-5 (broad multiplet), 4.3-4.1 (broad multiplet), 2.02 (triplet with equal intensity), 1.5 (singlet), 0.96 (singlet), 0.67 (singlet), 0.55 (singlet).

The aqueous part, after partition with chloroform, was concentrated under reduced pressure and then dissolved in methanol. The insoluble precipitate was obtained as an inorganic salt. The chemical test showed that it mainly consisted of sodium chloride and potassium chloride. The atomic absorption spectra showed that it was a 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, after evaporating the solvent in vacuo, was chromatographed on Whatman No. 1 and No. 3 paper by descending method, using n-butanol:benzene:pyridine:water (5:1:3:3) as developing solvent and sprayed with aniline-hydrogen phthalate reagent. (39) The two brown colour spots were obtained and their R_f values were 0.24 and 0.2. The preliminary test of the methanol soluble parts gave a red to violet ring colour with Molisch's reagent, a silver mirror with Tollen's reagent, and a orange-red colour of cupous oxide with Barfoed's, Benedict's, and Fehling's solution

The Formation of Osazone of Methanol Soluble Fraction

Crude methanol soluble extract 10 g., 4.5 g. of pure white phenylhydrazine hydrochloride (AR quality), 5 g, of crystallized sodium acetate and 60 cm.³ of water were placed in a 200 cm.³ round bottomed flask, to which a reflux condenser was attached. The solution was heated on a steam bath for two hours. It was allowed

to cool at room temperature and poured, with vigorous stirring, into 200 cm.³ of ice-cold water. The product was filtered with suction, washed well with cold water and cold ethanol successively. It was fractionally crystallized from ethanol; a yellow solid deposited which was further recrystallized with ethanol for 2-3 times. The yellow needle-shaped crystals (3.2g.) were obtained and its m.p. 205°C decompose. The mother liquor was concentrated and equal volume of water was added and solid precipitated as yellow crystal. It was recrystallized from aqueous methanol for several times as yellow crystals (720 mg.), m.p. 185-186°C.

The Formation of Acetate of Methanol Soluble Fraction

Grinded together in a dry porcelaine or glass mortar 8 g. of anhydrous sodium acetate and 10 g. of crude methanol extract and the mixture was placed in a 200 cm.³ round bottomed flask. 50 cm.³ of acetic anhydride were added. The flask was fitted with a reflux condenser and heated on a water bath till a clear solution was obtained (about 30 mins., the reaction mixture was shaken from time to time). The heating was continued for further 2 hours. The reaction mixture was poured into 500 ml. of ice-cold water in a beaker. The solid lumps were broken up and allowed to stand with occasional stirring for about one hour. The solid was filtered at the pump, washed well with cold water, and purified by fractionally crystallization from methanol. The solid deposited as white crystals and it was further recrystallized with methanol-water for several times.

The needle crystals (3.32 g.), were obtained, m.p. 112-113°C. The mother liquor was concentrated and an equal volume of water was added. The precipitated solid was recrystallized from aqueous-methanol, colourless needle-rod-shaped crystals (1.725 g.) were obtained m.p. 94-95°C.