CHAPTER IV

Experimental

Melting points are uncorrected. Petroleum ether used had b.p. 60-80°. All optical rotations were measured in chloroform solution unless otherwise stated. NMR spectra were taken in Varian-60 spectrophotometer. IR spectra recorded were taken in Perkin-Elmer spectrophotometer 337. UV spectra were observed in Zeiss VSU-1 spectrophotometer.

Extraction

Dried and powdered trunk bark and stem (2.0 kg) of *Aleurites montana*, was extracted with benzene in a Soxhlet apparatus for 18 hours. From the extract benzene was distilled off and a gummy residue (9.0 gm) was obtained. The gummy residue was dissolved in ether (1 litre) and the ether solution was washed with 10% aqueous sodium hydroxide solution (3 x 300 ml) and with water till neutral. The neutral ether solution was dried (Na_2SO_4) and the ether evaporated. The gummy residue (4.6 gm) constituted the neutral portion was investigated separately (see Chapter V).

The aqueous alkaline layer was thoroughly shaken with ether to remove any neutral material that might be present. The aqueous layer was acidified with cold and dilute 10% hydrochloric acid (1 litre) when some insoluble solids separated out. The acidified portion was extracted with ether, washed with water till neutral and then dried (Na_2SO_4). Ether was removed when a gummy residue (1.0 gm)

was obtained. To the latter dissolved in ether (150 ml) was added a solution of diazomethane in ether prepared from nitrosomethylurea (700 mg) and was kept overnight. Next day excess of diazomethane was destroyed with acetic acid. The ether solution was washed with water, 10% sodium bicarbonate solution and again with water till neutral and then dried (Na_2SO_4). Evaporation of the ether yielded a gummy residue (600 mg).

Chromatography of the above gummy material (600 mg) : Isolation of acetyl methyl aleuritolate 36 and methyl betulinate 41

The above crude ester (600 mg.) dissolved in benzene (6 ml) was placed over a column of alumina (40.0 gm deactivated with 1.5 ml of 10% aqueous acetic acid). The chromatogram was developed with petroleum ether and was eluted with following solvents (table I).

Table I

Chromatography of the above gummy material (600 mg)

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether (300 ml)	1-6	Oil (300 mg) becomes solid m.p. 232-5° on digestion with petroleum ether
Petroleum ether:benzene (4:1) (200 ml)	7-10	Nil
Petroleum ether:benzene (3:2) (300 ml)	11-16	Solid (200 mg) m.p. 216-8° on digestion with methanol

Further elution with more polar solvents did not yield any crystalline material

Isolation of acetyl methyl aleuritolate 36

Fractions 1-6 (table I) were combined (300 mg) dissolved in benzene (4 ml) and was rechromatographed over a column of alumina (20 gm. deactivated with 0.7 ml of 10% aqueous acetic acid). The chromatogram was developed with petroleum ether (table II).

Table II

Chromatography of the above residue (300 mg)

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether (100 ml)	1-2	Oil (150 mg)
Petroleum ether (300 ml)	3-8	Gummy residue (110 mg) which gave crystalline solid, m.p. 235-8° on digestion with petroleum ether

Further elution with more polar solvents did not afford any crystalline material

The combined solids from fractions 3-8 (table II) were collected by filtration and after several crystallisations (three times) from a mixture of chloroform and methanol, fine needle shaped crystals of m.p. 241-43°, $(\alpha)_D$ + 23.08° was obtained. The melting point could not be raised by further crystallisation.

Found: C, 76.88; H, 10.06%

$C_{33}H_{52}O_4$ requires: C, 77.34; H, 10.15%.

UV (in 95% ethanol) - No absorption in the region 220-300 mμ.

IR ($CHCl_3$): ν_{max} 1735 (broad, -O-$COCH_3$,-$COOCH_3$), 1245 (-O-$COCH_3$), 820 (trisubstituted double bond) cm^{-1} (fig. 1).

NMR (60 Mc): 5.50 δ (triplet, 1H, vinyl proton, trisubstituted double bond), 4.46 δ (1H, H-C-O-OCH_3), 2.04 (singlet 3H, -O-$COCH_3$), and several sharp signals between 0.8 to 1.65 δ accounting for 21 protons (seven methyl groups) (fig. 2).

Mass: Molecular ion peaks (m/e): 512 (M^+), 493 (M^+-CH_3), 452 (M^+-CH_3COOH), 437 (M^+ - (CH_3COOH + CH_3), 344 (52, chart V), 329 (54, chart V), 248 (56 chart V), 189 (57, chart V), 262 (60, chart V), 233 (62, chart V)(fig. 4).

Hydrolysis of acetyl methyl aleuritolate 36: Isolation of methyl aleuritolate 35

Acetyl methyl aleuritolate 41 (150 mg) was refluxed with 10% methanolic potassium hydroxide solution (10 ml) for four hours. The solution was cooled, acidified with cold 10% hydrochloric acid (20 ml) and extracted with ether. The ethereal layer was washed with water till neutral and then dried (Na_2SO_4). The ether was distilled off and the solid residue obtained was crystallised from methanol. After three crystallisation from methanol it gave a crystalline solid m.p. 208-10°, $(\alpha)_D$ + 11.11°. The melting point could not be raised by further crystallisation.

Found: C, 78.92; H, 10.56%

$C_{31}H_{50}O_3$ requires: C, 79.10; H, 10.71%.

UV (95% ethanol): No absorption in the region 220-300 mμ.

IR ν_{max}^{KBr} 3480 (-OH), 1735 (-$COOCH_3$) 820 (trisubstituted double bond) cm^{-1}.

NMR (60 Mc): 6.50 δ (triplet, 1H, vinyl proton, trisubstituted double bond), 3.54 δ (singlet 3H, -$COOCH_3$) and several sharp peaks between 0.8 to 1.65 δ accounting for 21 protons (seven methyl groups) (fig.3).

The acetyl methyl aleuritolate 36 was refluxed for longer period of times with 10%, 15% and 20% methanolic potassium hydroxide solutions respectively but in each case only methyl aleuritolate 35 was obtained.

CrO_3-Py oxidation of methyl aleuritolate 35: Preparation of methyl aleuritolonate 37

To a complex prepared from chromic acid (400 mg.) and pyridine (12 ml)[62] was added a solution of methyl aleuritolate (0.2 gm) in the same solvent (4 ml), the temperature of the mixture being kept at 0°. The mixture was kept at room temperature for 16 hours, then diluted with methanol (10 ml) and an excess of ethyl acetate was added. The reaction mixture was then filtered from the insoluble solids and the filtrate was concentrated under vacuum. The residue was dissolved in ether (200 ml) and the ether solution was washed successively with dilute hydrochloric acid, 10% aqueous sodium bicarbonate solution and finally with water till neutral and then dried (Na_2SO_4). After removal of ether, a semi solid mass (180 mg) was obtained which was chromatographed over alumina (10 gm., deactivated with 0.4 ml of 10%

aqueous acetic acid). The chromatogram was developed with petroleum ether.

Table III

Chromatography of the above semi solid residue (180 mg): Isolation of methyl aleuritolonate 37

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether (100 ml)	1-2	Oil
Petroleum ether: benzene (9:1) (250 ml)	3-7	Solid (120 mg) m.p. 171-4° on digestion with methanol

Further elution with more polar solvents did not give any material.

The solids from fractions 3-7 (table III) were combined and was crystallised twice from a mixture of chloroform and methanol to afford fine needle shaped crystals of m.p. 174-6°, $(\alpha)_D$ + 11.76°. The melting point could not be raised by further crystallisation.

Found: C, 79.41; H, 16.32%

$C_{31}H_{48}O_3$ requires: C, C 79.48; H, 10.45%.

UV (95% ethanol): λ max 287 mμ (ϵ, 82)

IR ν_{max}^{KBr}: 1705 (carbonyl), 1735 (-$COOCH_3$), 820 (trisubstituted double bond) cm^{-1}.

NMR (60 Mc): 5.58δ(triplet, 1H, vinyl proton, trisubstituted double

bond), 3.58δ (singlet 3H, $-COOCH_3$) and signals between 0.8 to 1.65 δ accounting for 21 protons (seven methyl protons).

Mass: molecular ion peaks (m/e): 468 (M^+), 453 ($M^+ - CH_3$), 300 (53, chart V), 285 (55, chart V), 189 (57, chart V) 233 (62, chart V), 220 (61, chart V) (fig. 5).

Hydrolysis of methyl aleuritolate: preparation of aleuritolic acid 34

To a normal solution of potassium tertiary butoxide in tertiary butanol (prepared from 0.4 gm of potassium in 10 ml dry tertiary butanol), a solution of methyl aleuritolate 41 (150 mg) in 10 ml of dimethyl sulfoxide was added and the reaction mixture was heated on an oil bath at 105° for four hours[49]. The reaction mixture was cooled, diluted with water and then acidified with cold dilute hydrochloric acid (20 ml). The solid that separated out on acidification was taken up in chloroform in a separatory funnel and the chloroform layer was washed with water till neutral and then dried (Na_2SO_4). Evaporation of chloroform afforded a solid which after three crystallisation from a mixture of chloroform and methanol gave an amorphous solid having melting point 300-302° (decomp.).

Found:	C, 78.84; H, 10.38%
$C_{30}H_{48}O$ requires:	C, 78.94; H, 10.52%

UV (95% ethanol) no absorption in the region 220-300 mμ.

IR $\nu_{max}^{CHCl_3}$: 3400 (-OH), 3065, 1700 (-COOH), 820 (trisubstituted double bond) cm^{-1}.

Acetylation of aleuritolic acid : Preparation of acetyl aleuritolic acid 38

A mixture of aleuritolic acid (25 mg.), pyridine (2 ml) and acetic anhydride (2 ml) was heated on a water bath for four hours. The reaction mixture was then cooled and poured into ice cold water. A solid precipitated out which was taken in ether (100 ml). The ether layer was washed with water till neutral and then dried (Na_2SO_4). On distilling the solvent a solid was obtained which on crystallisation from a mixture of chloroform and methanol gave acetyl aleuritolic acid m.p. 278-81°.

Found: C, 79.32; H, 10.11%

$C_{32}H_{50}O_3$ requires: C, 79.66; H, 10.37%.

Hydrolysis of methyl aleuritolonate 37 : Preparation of aleuritolonic acid 39

To a normal solution of potassium tertiary butoxide (prepared from 0.4 gm of potassium and 10 ml of dry tertiary butanol), a solution of methyl aleuritolonate (150 mg) in 10 ml of dimethyl sulfoxide was added and the reaction mixture was then heated on an oil bath at 105° for four hours. The reaction mixture was cooled, acidified with cold dilute hydrochloric acid (20 ml) and then extracted with chloroform. The chloroform layer was washed with water and then dried (Na_2SO_4). The solvent was then distilled off, when an amorphous solid m.p. 276-8° was obtained, which after three crystallisation from chloroform-methanol gave crystals of aleuritolonic acid 39 m.p. 280-82°.

Found: C, 79.20; H, 10.3%

$C_{30}H_{46}O_3$ requires: C, 79.29; H, 10.13%.

Saponification of acetyl aleuritolic acid 38

The acetyl compound 38 (20 mg) was gently refluxed with 0.5N methanolic potassium hydroxide solution (25 ml) for 3 hours. The reaction product on acidification gave crude aleuritolic acid which after crystallisation from a mixture of chloroform and methanol gave the pure acid m.p. 300-302° (decomp.), identical with aleuritolic acid prepared by another procedure.

Isomerisation of double bond : Preparation of acetyl methyl oleanolate 43

To (150 mg) of acetyl methyl aleuritolate in glacial acetic acid (35 ml) kept at 90° was added conc. hydrochloric acid (1 ml)[52] and the mixture was heated on a water bath for 15 minutes, during which time the solid dissolved. It was then cooled and then diluted with water. A solid came out which was extracted with ether (100 ml). The ether layer was washed with water till neutral and then dried (Na_2SO_4). Removal of the solvent gave a solid (110 mg) which after crystallisation from a mixture of chloroform and methanol gave crystals m.p. 219-20°, $(\alpha)_D$ + 58.82°. The latter had been identified as acetyl methyl oleanolate, by direct comparison with an authentic specimen (m.m.p., IR and TLC).

Found: C, 76.86; H, 9.83%

Calc. for $C_{33}H_{52}O_4$: C, 77.34; H, 10.15%.

Estimation of double bonds : Perbenzoic acid titration of acetyl methyl aleuritolate:

Acetyl methyl aleutirolate (0.0548 gm) was taken in a 25 ml volumetric flask and dissolved in chloroform (10 ml). A solution of perbenzoic acid in chloroform (5 ml) was pipetted out and added to the solution and the volume made upto 25 ml by addition of chloroform. A blank solution was similarly prepared by taking 5 ml of perbenzoic acid solution as above in a 25 ml volumetric flask and then making up the volume to 25 ml with chloroform. Perbenzoic acid was prepared by the method of Mayer and Mansky[63]. 5 ml of aliquot portions were titrated from each of the above solutions against standard sodium thiosulphate solution as shown in the table IV below.

Strength of sodium thiosulphate solution = 0.0296N.

Results

Table IV

Time interval	Blank	Thio reqd. ml	Reaction mixture	Thio requd.	Diff. in ml	No. of double bonds
5 mins.	Blank (5 ml) + 2% kI (10%) +AcOH (2 ml) +starch soln.	4.7	Aliquot (5 ml)+ 2% K9 (10 ml)+ AeOH (2 ml) starch soln.	4.7	0	0
1 hour	"	4.8	"	4.8	0	0
2 hours	"	4.8	"	4.6	0.2	.27
6 hours	"	4.8	"	4.5	0.3	0.415

Table IV (Contd.)

Time interval	Blank	Thio reqd. ml	Reaction mixture	Thio reqd.	Diff. in ml	No. of double bonds
16 hours	Blank (5 ml) +2% KI (10%) +AcOH (2 ml) +starch soln.	4.8	Aliquot (5 ml)+2% K9 (10 ml)+ AeOH (2 ml) starch soln.	4.3	0.5	0.693
26 hours	"	4.8	"	4.1	0.7	0.972
36 hours	"	4.8	"	4.1	0.7	0.972
48 hours	"	4.8	"	4.1	0.7	0.972

It was found that one mole of perbenzoic acid was consumed within 26 hours, showing thereby that acetyl methyl aleuritolate 35 and hence aleuritolic acid contains one double bond.

Attempted hydrogenation of methyl aleuritolate

To methyl aleuritolate (200 mg) dissolved in ethyl acetate (40 ml) was added 10% palladium-on-charcoal catalyst (100 mg.) and the mixture was stirred at room temperature in an atmosphere of hydrogen. No absorption of hydrogen took place even after six hours. It was then filtered and the filtrate was evaporated to dryness. The residue m.p. 206-8° (190 mg), on crystallisation from methanol gave fine crystals m.p. 208-10° and was found to be unchanged in its mixed m.p. with methyl aleuritolate.

Lithium aluminium hydride reduction of methyl aleuritolate : Preparation of myricadiol 63

To methyl aleuritolate (150 mg) dissolved in dry dioxan (15 ml) was added lithium aluminium hydride (75 mg) and the reaction mixture was heated on a water bath for four hours. After the reaction was over excess of lithium aluminium hydride was decomposed carefully with moist ether and then a saturated solution of sodium sulphate was added to the reaction mixture. The ethereal solution was washed with water and dried over anhydrous sodium sulphate. After removal of the solvent a solid residue (140 mg) was obtained, which was chromatographed over alumina (10 gm.) deactivated with 0.4 ml of 10% aqueous acetic acid.

Table V

Chromatography of the above residue (140 mg)

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether (100 ml)	1-2	Nil
Petroleum ether:benzene (3:1) (100 ml)	3-4	Nil
Petroleum ether:benzene (1:1) (100 ml)	5-6	Nil
Petroleum ether:benzene (1:3) (100 ml)	7-8	Nil
Benzene (300 ml)	9-14	Solid (130 mg) m.p.259-61°
Further elution with more polar solvent did not afford any solid		

The solids from fractions 9-14 (table V) were combined (130 mg) and after crystallisation twice from a mixture of chloroform and methanol it gave fine needle shaped crystals of myricadiol m.p. 265-67°, indistinguishable from an authentic sample of myricadiol by IR comparison and m.m.p. determination.

Found: C, 81.23; H, 10.51%

Calc. for $C_{30}H_{50}O_2$; C, 81.39; H, 11.38%.

Acetylation of myricadiol : Preparation of myricadiol diacetate 64

Myricadiol (200 mg.) in pyridine (2 ml) was mixed with acetic anhydride (5 ml) and the mixture was heated on a water bath for 5 hours. On working up in the usual manner followed by crystallisation from a mixture of chloroform and methanol colorless needle shaped crystals of m.p. 251-52°, $(\alpha)_D$ - 3° were obtained. The compound showed no depression in m.p. when mixed with an authentic sample of myricadiol diacetate. The IR spectra were also identical and they showed the same R_f on a silica gel chromatoplate. (For IR comparison see fig. 6).

Found: C, 78.04; H, 9.89%

Calc. for $C_{34}H_{54}O_4$; C, 77.56; H, 10.26%

Isomerisation of myricadiol diacetate 27 : Preparation of erythrodiol diacetate 66

Myricadiol diacetate (100 mg.) was isomerised by heating with a mixture of acetic acid (25 ml) and conc. hydrochloric acid (1 ml)

for half an hour[52]. The reaction mixture was poured in ice cold water. The precipitate was taken up in chloroform. The chloroform extract after being washed with water (till neutral) was dried (Na_2SO_4) and the solvent removed. The solid obtained (90 mg) was crystallised from methanol, affording crystals m.p. 184-6°, $(\alpha)_D$ + 56.2° and was found to be identical with an authentic sample of erythrodiol diacetate (m.m.p., R_f values and I.R. comparison)(lit.[64] m.p. 185° $(\alpha)_D$ 59.4°).

Found: C, 77.61; H, 10.69%

Calc. for $C_{34}H_{54}O_4$: C, 77.56; H, 10.26%.

Saponification of erythrodiol diacetate 63 : Generation of erythrodiol 66

The above diacetate 29 (25 mg) was hydrolysed with ethanolic potassium hydroxide (10 ml, 5% soln.) by heating on a water bath for three hours. After working up in the usual manner and crystallisation from a mixture of chloroform and methanol gave crystals, m.p. 229-31°, $(\alpha)_D$ + 73.6° and was found to be identical with erythrodiol (m.m.p. and TLC).

Found: C, 81.814; H, 11.50%

Calc. for $C_{30}H_{50}O_2$: C, 81.45; H, 11.31%

Isolation of methyl betulinate 40

Solids obtained from fractions 11-16 (table I) were combined (20 mg), m.p. 216-20°, and were then crystallised repeatedly (four

times) from a mixture of chloroform and methanol to afford shining colorless needles of the methyl betulinate m.p. 220-22°, $(\alpha)_D$ + 1.4°, identical with an authentic sample (m.m.p.).

Found : C, 78.91; H, 10.80%

Calc. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71%.

UV: no absorption in the region 220-300 mμ.

IR: ν_{max}^{KBr} 3540 (-OH), 1724 (-COOMe), 1658 (unsaturation), 1470 (-C-Me) and 890 cm^{-1} (exocyclic methylene) cm^{-1}.

NMR (60 Mc) : 4.6-4.8 δ(two doublet, $=CH_2$), 3.75 δ(singlet, -COOMe), 2.00 δ(singlet, -CHOH), 1.75 δ(sharp singlet, $H_2C=\underset{CH_3}{C}-C-$ for three protons) and 1.00 δ(a tall singlet accounting for 15 protons, 5 methyl groups).

Preparation of betulinic acid

To a normal solution of potassium tertiary butoxide (10 ml), a solution of methyl betulinate (150 mg) in dimethyl sufoxide (10 ml) was added and the reaction mixture was heated under reflux condition on an oil bath for four hours. After working up in the usual manner it afforded amorphous solid m.p. 299-302°, identical with betulinic acid.

Acetyl betulinic acid

Betulinic acid (150 mg) was acetylated by heating with pyridine (5 ml) and acetic anhydride (5 ml). After usual work up and crystallisation from a mixture of chloroform and methanol colourless

needles of acetyl betulinic acid was obtained, m.p. 281-83° (decomp.), $(\alpha)_D$ + 18.7° (pyridine).

Found: C, 76.83; H, 9.50%

Calc. for $C_{32}H_{50}O_4$ C, 77.06; H, 10.04%.

Preparation of acetyl methyl betulinate

Methyl betulinate (450 mg) was acetylated with pyridine (25 ml) and acetic anhydride (10 ml) in the usual manner and the crude solid (400 mg) thus obtained was chromatographed over alumina (25 gm) deactivated with 1 ml of 10% aqueous acetic acid.

Table VI

Chromatography of the crude solid (400 mg)

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether (100 ml)	1-2	Nil
Petroleum ether:benzene (9.1) (200 ml)	3-6	Solid m.p. 102-4°, on digestion with methanol

Further elution with more polar solvents did not yield any material

Solids from fractions 3-6 (table VI) were combined and crystallised from chloroform and methanol mixture to give crystals of acetyl methyl betulinate m.p. 200-202° identical with an authentic sample of methyl betulinate.

Found: C, 77.37; H, 10.13%

Calc. for $C_{33}H_{52}O_4$: C, 77.34; H, 10.15%.

Preparation of methyl betulonate 42

Oxidation of methyl betulinate (300 mg) by cromium trioxide-pyridine complex method prepared from cromium trioxide (600 mg) and pyridine (15 ml) and subsequent work up in the usual manner gave a solid residue (200 mg) which was purified by chromatography on a column of alumina (20 gm) deactivated with 0.6 ml of 10% aqueous acetic acid.

Table VII

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether (300 ml)	1-6	Solid (0.18 gm) m.p. 156-58° on digestion with methanol

Further elution with more polar solvents did not give any solid material.

The solids from fractions 1-6 (table VII) were combined and crystallised from ether and methanol (thrice) in rods m.p. 164-6°, $(\alpha)_D$ + 42°. The ester gave positive Zimmermann reaction and was found to be identical with an authentic sample of methyl betulonate (m.m.p.).

Found: C, 79.04; H, 9.65%

$C_{31}H_{48}O_5$ requires: C, 79.48; H, 10.25%.