Tetrahedron, 1965, Vol. 21, pp. 1529 to 1536. Pergamon Press Ltd. Printed in Northern Ireland

TRITERPENE COMPOUNDS–VII[1]

THE CONSTITUTION OF MELALEUCIC ACID

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(*Received* 19 *January* 1965)

Abstract—Melaleucic acid, the triterpene dicarboxylic acid isolated from *Melaleuca* species, has been shown to be 3β-hydroxylup-20(29)-en-27,28-dioic acid (IIa).

MELALEUCIC acid occurs in the barks of three *Melaleuca* species, *M. rhaphiophylla* Schau., *M. cuticularis* Labill. and *M. viminea* Lind. and was characterized as a pentacyclic hydroxytriterpene dicarboxylic acid[2] having non-equivalent carboxyl groups, an equatorial hydroxyl and a vinylidene group.

Hydrogenation of the vinylidene group afforded derivatives of dihydromelaleucic acid which showed negative molecular rotation differences when compared with the corresponding derivatives of melaleucic acid. These differences were similar to, but larger than, those between corresponding derivatives of dihydrobetulinic acid and betulinic acid[3] (Table 1).

TABLE 1. MOLECULAR ROTATION DIFFERENCES*

Compound	$10^{-2}[M]_D$	Dihydro-compound	Δ
Melaleucic acid	+ 92 (py.)	−205 (py.)	−297
Melaleucic acid acetate	+ 63 (EtOH)	−238 (py.)	−301
Methyl melaleucate	+ 82	− 72	−154
Methyl melaleucate acetate	+221	− 89	−310
Methyl 3-oxolup-20-en-27,28-dioate	+246	+111	−135
Betulinic acid	− 36 (py.)	−128 (diox.)[4]	−164
Betulinic acid acetate	+100	− 60	− 160
Methyl betulinate	− 38	− 90	−128
Methyl betulinate acetate	− 92	− 67	−159
Methyl betulonate	+145	− 38	−107

* All values for betulinic acid and derivatives are from ref. 3 except for dihydrobetulinic acid.[4]

This suggested that melaleucic acid had the lupane skeleton and the structure I was proposed in the preliminary communication.[2] However, this structure was eliminated on the grounds of reactions described below and the revised structure (IIa) is proposed.

[1] Part VI. C. S. Chopra, M. W. Fuller, K. J. L. Thieberg, D. C. Shaw, D. E. White, S. R. Hall and E. N. Maslen, *Tetrahedron Letters* 1847 (1963).
[2] H. R. Arthur, A. R. H. Cole, K. J. L. Thieberg and D. E. White, *Chem. & Ind.* 926 (1956).
[3] D. H. R. Barton and E. R. H. Jones, *J. Chem. Soc.* 659 (1944).
[4] L. Ruzicka, M. Brenner and E. Rey, *Helv. Chim. Acta* **24**, 515 (1941).

Experimental details of previous work[2] together with degradative reactions which support structure IIa are now presented.

Methyl melaleucate (IIb) was oxidized by osmium tetroxide to a triol which was cleaved by periodic acid to the hydroxynorketone (III); ν_{max} 1723, shoulders at 1715; 1354 cm^{-1} consistent with the formulation as a methyl ketone (cf. 20-oxo-30-norlupan-3β-ol, ν_{max} 1712, 1354 cm^{-1}).[5] The acetate of the hydroxynorketone (III) afforded a benzylidene derivative which was converted, by successive reduction, dehydration and ozonolysis, to the acetoxytrisnorketone (IV) with the expected ν_{max} 1711 (methoxycarbonyl); 1733 (acetate and methoxycarbonyl), shoulder at 1742 (5-ring ketone); 1414 cm^{-1} (CH_2 adjacent to 5-ring ketone). This clearly established that melaleucic acid contained an isopropenyl group on a 5-membered ring, with a methylene group adjacent to the point of attachment.

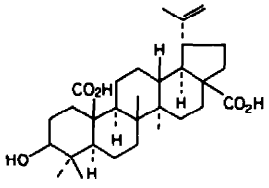
The keto-ester (IIc) obtained by the mild oxidation[6] of methyl melaleucate was converted, by sulphuric acid in acetic acid, into the keto-lactone (V), ν_{max} 1778 (γ-lactone), 1718 (methoxycarbonyl), 1708 cm^{-1} (6-ring ketone). The γ-lactone and 6-ring carbonyl frequencies were identical with those of betulonic lactone[7] and the low methoxycarbonyl frequency was assigned to the less reactive ester group. This was confirmed when keto-lactone (V) was reduced by LAH to the trihydroxy ester (VI) without attack upon the hindered methoxycarbonyl group.

The ketone (VIIa) obtained by the mild oxidation[6] of methyl dihydromelaleucate had ν_{max} 1718, shoulders at 1727, 1710 cm^{-1}; the last being attributed to a 6-ring ketone and the others to the two methoxycarbonyl groups. A positive Zimmermann reaction[8] suggested the presence of a C_3-ketone. This was supported by bromination to the equatorial monobromoketone (ν_{max} 1718, 718 cm^{-1}) which was dehydrobrominated to the conjugated ketone, (ν_{max} 1672 cm^{-1}, λ_{max} 231 mμ (ϵ = 5170).[9] This λ_{max} was only compatible with Woodward's rules[10] if the carbonyl groups were at C_1 or C_3. In view of the positive Zimmermann reaction and the very rare occurrence of 1-hydroxytriterpenoids, C_3-oxygenation was favoured. The equatorial nature of the hydroxyl group in melaleucic acid was demonstrated when the reduction of the ketone, from methyl dihydromelaleucate, by sodium borohydride gave the original alcohol with ν_{max} 3628 cm^{-1} consistent with that of equatorial hydroxyl groups.[11]

The lupane skeleton in melaleucic acid was confirmed as follows. The di-acid chloride (VIIb) of dihydromelaleucic acid acetate was reduced by prolonged treatment with LAH to give as the major product, the triol (VIIc). Acetylation of this triol afforded the diacetate (VIId) which was oxidized by chromic acid in acetic acid–chloroform[12] to the corresponding aldehyde. Huang-Minlon reduction of this aldehyde afforded dihydrobetulin. This evidence confirmed the presence of the lupane skeleton and the location of functional groups at C_3 and C_{28}. The degradation to dihydrobetulin also proved that the secondary hydroxyl group in melaleucic acid was at 3.

At this stage the only remaining feature of the structural elucidation of melaleucic

[5] A. R. H. Cole, personal communication.
[6] R. G. Curtis, Sir Ian Heilbron, E. R. H. Jones and G. F. Woods, *J. Chem. Soc.* 457 (1953).
[7] Sir Ewart R. H. Jones, personal communication.
[8] D. H. R. Barton and P. de Mayo, *J. Chem. Soc.* 887 (1954).
[9] Cf. D. S. Irvine, J. A. Henry and F. S. Spring, *J. Chem. Soc.* 1316 (1955).
[10] R. B. Woodward, *J. Amer. Chem. Soc.* **63**, 1123 (1941); **64**, 76 (1942).
[11] (Miss) I. L. Allsop, A. R. H. Cole, D. E. White and R. S. Willix, *J. Chem. Soc.* 4868 (1956).
[12] A. Zurcher, O. Jeger and L. Ruzicka, *Helv. Chim. Acta* **37**, 2145 (1954).



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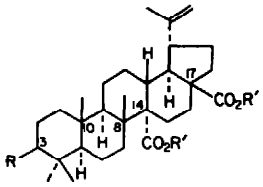
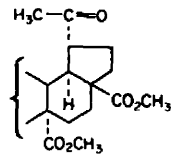


II a ; R=OH, R'=H
II b ; R=OH, R'=CH$_3$
II c ; R=O , R'=CH$_3$
II d ; R=OCOCH$_3$, R'=CH$_3$



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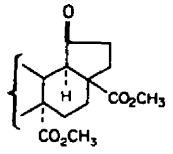

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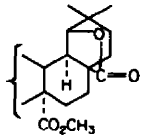

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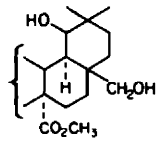

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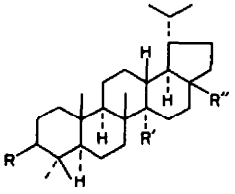
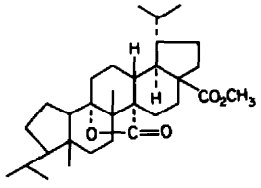
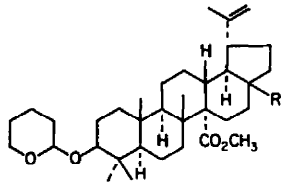


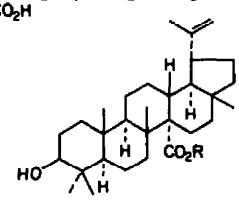
VII a; R=O, R'=R''=CO$_2$CH$_3$
VII b; R=OCOCH$_3$, R'=R''=COCl
VII c; R=OH, R'=R''=CH$_2$OH
VII d; R=OCOCH$_3$, R'=CH$_2$OH, R''=CH$_2$OCOCH$_3$
VII e; R=O, R'=R''=CO$_2$H



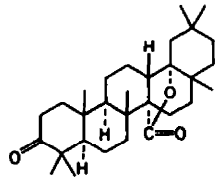
VIII



IX a; R=CO$_2$CH$_3$
IX b; R=CH$_2$OH
IX c; R=CHO



X a ; R=H
X b ; R=CH$_3$



XI

acid was the location of the hindered carboxyl group. There were four possible sites for the location of this group; C_4, C_{10}, C_8 and C_{14}.

The facile isolation of the keto-acid (VIIe) showed that it was not a β-keto-acid, and therefore that the carboxyl group was not at C_4. Of the three remaining possibilities (C_{10}, C_8, C_{14}) for the position of this carboxyl group on the lupane nucleus, C_{14} was favoured,[13,14] since only one triterpene is recorded with oxygenation at C_{25}[15] and none is known with oxygenation at C_{26}.[16]

[13] Cf. A. Brossi, B. Bischof, O. Jeger and L. Ruzicka, *Helv. Chim. Acta* **34**, 244 (1951). R. Tschesche, I. Duphorn and G. Snatzke, *Liebig's Ann.* **667**, 151 (1963).
[14] Cf. P. de Mayo and A. N. Starratt, *Canad. J. Chem.* **40**, 1632 (1962).
[15] D. H. R. Barton, A. Hameed and J. F. McGhie, *J. Chem. Soc.* 5176 (1962).
[16] D. E. White, *Rev. Pure and Applied Chem.* **6**, 191 (1956).

Examination of the NMR spectra[1] of a number of derivatives of melaleucic acid suggested that the hindered carboxyl group was at C_{14}. Hence the structure of melaleucic acid is considered to be 3β-hydroxylup-20(29)-en-27,28-dioic acid (IIa). This has now been confirmed unambiguously by an X-ray crystallographic investigation of methyl melaleucate iodoacetate.[17]

As part of the early work[2] designed to locate the hindered carboxyl group of melaleucic acid, methyl dihydromelaleucate was found to react with sulphuric acid in acetic acid to give a supposed δ-lactone (ν_{max} 1762 cm^{-1}) thought to bridge C_{25}–C_3. In view of the revised structure (IIa) for melaleucic acid, we propose that this lactonization is preceded by skeletal rearrangement, being a complete analogy of the quinovic acid-novic acid rearrangement[18] as had been suggested previously,[19] leading to the γ-lactone (VIII).

In another sequence designed to support the structure IIa, methyl melaleucate (IIb) was converted to the tetrahydropyranylether (IXa)[20] which was reduced by LAH to give the hydroxyester (IXb). The hydroxyester (IXb) was oxidized by chromium trioxide in pyridine[21] to the aldehyde (IXc) which was reduced by the Huang-Minlon[22] procedure to give the hydroxy-acid (Xa). The derived ester (Xb) was oxidized to the keto-ester. Ring E expansion[23] of the keto-ester gave a γ-lactone which must be formulated as (XI).

EXPERIMENTAL

M.ps were recorded on a Kofler stage. Optical rotations, UV and IR spectra were recorded for $CHCl_3$, EtOH and CCl_4 solutions respectively. NMR spectra were recorded, on a Varian A60 high-resolution spectrometer, for $CDCl_3$ solutions with tetramethylsilane as internal reference. All peak positions are recorded on δ-scale. Alumina for chromatography was Peter Spence grade H standardized[24] at activity II. Light petroleum refers to the fraction b.p. 56–60°. Micro-analyses were carried out by the C.S.I.R.O. Microanalytical service in the University of Melbourne.

Isolation of melaleucic acid. The bark of *Melaleuca rhapiophylla* schau., collected in the neighbourhood of South Perth, Western Australia, was dried in a current of air at 35°, cut into pieces less than 2·5 cm square (900 g) and extracted with ether (5 l.) The extract was concentrated to 2·5 l. and treated with NaOH aq (2·5%; 2·5 l.) to precipitate sodium betulinate which was removed by filtration. After separation of the organic layer, the aqueous solution was treated with charcoal, filtered, heated to boiling, acidified with HCl aq and the crude acid (5·8 g; m.p. 347–348°) collected. Further quantities were obtained by repeated extraction of the same bark. Better yields were obtained from powdered bark (e.g. 4·4% from *M. rhaphiophylla* Schau,. 4·0% from *M. vimimea* Lindl. and 2·8% from *M. cuticularis* Labill.).

A solution of the crude acid (5·8 g) in ether was filtered through alumina (Act. III–IV; 70 g) to give *melaleucic acid* (3β-hydroxy-20-en-27,28-*dioic acid*, IIa; 3 g) as prisms from MeOH–water, m.p. 363–364°, $[\alpha]_D$ +19° (*c*, 0·97, pyridine). (Found: C, 73·8; H, 9·1. $C_{30}H_{46}O_5$ requires: C, 74·0; H, 9·5%).

The *acetate* was obtained from the acid by the acetic anhydride-pyridine method, and after filtration in $CHCl_3$ solution through alumina, formed prisms from MeOH–water, m.p. 343–344°, $[\alpha]_D$ +12°, (*c*, 0·81, EtOH), ν_{max} 1740, 1683 cm^{-1} (nujol mull). (Found: C, 72·8; H, 9·05. $C_{32}H_{48}O_6$ requires: C, 72·7; H, 9·15%)

[17] S. R. Hall and E. N. Maslen, *Acta crystl.* in press (1965).
[18] D. H. R. Barton and P. de Mayo, *J. Chem. Soc.* 3111 (1953).
[19] D. H. R. Barton, personal communication.
[20] W. E. Parham and E. L. Anderson, *J. Amer. Chem. Soc.* 70, 4187 (1948).
[21] G. I. Poos, G. E. Arth, R. E. Bayler and L. H. Sarett, *J. Amer. Chem. Soc.* 75, 442 (1953).
[22] Huang-Minlon, *J. Amer. Chem. Soc.* 68, 2487 (1946); 70, 2802 (1948).
[23] T. R. Ames, T. G. Halsall and E. R. H. Jones, *J. Chem. Soc.* 450 (1951).
[24] H. Brockman and H. Schodder, *Ber. Dtsch. Chem. Ges.* 74, 73 (1941).

The *benzoate* was obtained, by the benzoyl chloride-pyridine method, as prisms from CH_2Cl_2–MeOH, m.p. 320–321°, $[\alpha]_D$ +28° (*c*, 1·09). (Found: C, 75·3; H, 8·4. $C_{37}H_{50}O_6$ requires: C, 75·2; H, 8·5%).

Methyl melaleucate (IIb). Melaleucic acid (6·8 g) in water (100 ml) containing KOH (3·2 g) was treated three times at 60° with dimethyl sulphate (6 ml) and KOH aq (10%; 70 ml). The mixture was heated under reflux for 10 min when the solid (7·1 g) was collected, dissolved in benzene (100 ml) and filtered through alumina (90 g) in benzene to give the *methyl ester* as needles from MeOH, m.p. 207°, $[\alpha]_D$ +16° (*c*, 1·07), ν_{max} 3628, 1720 (shoulder at 1726), 3070, 1645, 885 cm^{-1}. (Found: C, 74·9; H, 9·7; OCH_3, 12·3. $C_{32}H_{50}O_5$ requires: C, 74·7; H, 9·8; 2xOCH_3, 12·1%). The ester gave a yellow coloration with tetranitromethane.

Methyl maleleucate was acetylated by the acteic anhydride-pyridine method to give the *acetate* (IId) as prisms from MeOH, m.p. 167–168°, $[\alpha]_D$ +40° (*c*, 1·00). (Found: C, 73·4; H, 9·4; OCH_3, 11·4. $C_{34}H_{52}O_6$ requires: C, 73·3; H, 9·4; OCH_3, 11·1%). The same acetate was obtained when melaleucic acid acetate was treated with diazomethane.

Methyl melaleucate iodoacetate. Methyl melaleucate (1·6 g) was treated with chloroacetyl chloride (12 ml) in dioxan (230 ml) at room temp for 18 hr. The addition of water afforded *methyl melaleucate chloroacetate* (1·3 g) as needles from MeOH, m.p. 184–185°, $[\alpha]_D$ +29° (*c*, 2·30). (Found: C, 67·7; H, 8·7; Cl, 6·1. $C_{34}H_{51}O_6Cl$ requires: C, 67·4; H, 8·6; Cl, 6·0%).

The chloroacetate (210 mg) was heated under reflux with KI (2·3 g) in acetone (25 ml) for 3 hr to give the *iodoacetate* (172 mg) as needles from MeOH, m.p. 189–190°, $[\alpha]_D$ +16° (*c*, 1·80). (Found: C, 59·5; H, 7·5; I, 18·6. $C_{34}H_{51}O_6I$ requires: C, 59·8; H, 7·5; I, 18·6%).

Mild oxidation of methyl melaleucate. The ester (1·55 g) in acetone (150 ml) was oxidized by the Jones' reagent[6] (1·28 ml) during 30 min. Excess Na_2CO_3 was added, the solution filtered and evaporated to give a crude product, part of which (393 mg) was adsorbed from light petroleum–benzene (9:1) on alumina (12 g). *Methyl 3-oxolup-20-en-27,28-dioate* (IIc; 298 mg) was eluted in light petroleum–benzene (4:1) and gave cubes from MeOH, m.p. 143–144°, $[\alpha]_D$ +51° (*c*, 1·01). (Found: C, 74·6; H, 9·35. $C_{32}H_{48}O_5$ requires: C, 74·9; H, 9·4%).

Dihydromelaleucic acid. Melaleucic acid (4·66 g) in acetic acid (200 ml) with Adams' catalyst (643 mg) absorbed H_2 (185 ml; calc. for one double bond, 228 ml). *Dihydromelaleucic acid* (4·5 g) formed needles from MeOH–water, m.p. 388–389°, $[\alpha]_D$ −42° (*c*, 0·96, pyridine). (Found: C, 73·9; H, 9·9. $C_{30}H_{48}O_5$ requires: C, 73·7; H, 9·9%).

The corresponding *acetate* formed needles from CH_2Cl_2–MeOH m.p. 359–360°, $[\alpha]_D$ −45° (*c*, 1·00, pyridine). (Found: C, 72·5; H, 9·5. $C_{32}H_{50}O_6$ requires: C, 72·4; H, 9·5%).

The *benzoate* formed prisms from MeOH, m.p. 329–331°, $[\alpha]_D$ −6°, (*c*, 1·09). (Found: C, 74·8; H, 8·8. $C_{37}H_{52}O_6$ requires: C, 75·0; H, 8·8%).

Methyl dihydromelaleucate. Methyl melaleucate (2·8 g) in acetic acid (50 ml) was reduced, over Adams' catalyst (300 mg), by H_2. The *dihydro-ester* was eluted from alumina (10 g) by light petroleum–benzene (1:1) to give needles from CH_2Cl_2–MeOH, m.p. 256·5°, $[\alpha]_D$ −14° (*c*, 0·91), ν_{max} 3628, 1727 (shoulder at 1720) cm^{-1}. (Found: C, 74·2; H, 10·1. $C_{32}H_{52}O_5$ requires: C, 74·4; H, 10·1%).

The corresponding *acetate* was obtained as plates from MeOH, m.p. 219–220°, $[\alpha]_D$ −16° (*c*, 1·63). (Found: C, 73·3; H, 10·0. $C_{34}H_{54}O_6$ requires: C, 73·1; H, 9·7%).

Mild oxidation of methyl dihydromelaleucate. The dihydro-ester was oxidized by the procedure used for methyl melaleucate. *Methyl 3-oxolupan-27,28-dioate* (VIIa) formed needles from CH_2Cl_2–MeOH, m.p. 198–199°, $[\alpha]_D$ +23° (*c*, 0·96), ν_{max} 1718 (shoulders at 1727, 1710) cm^{-1}. (Found: C, 74·9; H, 9·65. $C_{32}H_{50}O_5$ requires: C, 74·7; H, 9·8%). The Zimmermann reaction[8] afforded a violet coloration, changing to pink upon dilution.

The reduction of this keto-ester (400 mg), by $NaBH_4$ (180 mg) in dioxan (30 ml), afforded only methyl dihydromelaleucate, m.p. 256° undepressed after admixture with an authentic sample.

Mild oxidation of dihydromelaleucic acid. Dihydromelaleucic acid was oxidized by the procedure used for methyl melaleucate. *3-Oxolupan-27,28-dioic acid* (VIIe) formed rosettes of needles from MeOH–water, m.p. 367–368°, $[\alpha]_D$ 0° (*c*, 0·69, pyridine), ν_{max} 1706, 1685 cm^{-1} (nujol mull). (Found: C, 74·2; H, 9·35. $C_{30}H_{46}O_5$ requires: C, 74·0; H, 9·5%).

Oxidation of methyl melaleucate by osmium tetroxide. The ester (2·1 g) in pyridine (2 ml) and ether (100 ml) was treated with OsO_4 (1 g) for 7 days. The product was heated under reflux for 6 hr with KOH (5 g) and mannitol (5 g) in benzene (30 ml), EtOH (30 ml) and water (15 ml). The glycol (2·2 g) was isolated through ether adsorbed, from benzene on alumina (50 g). Elution with

$CHCl_3$ afforded *methyl* 3β-20,29-*trihydroxylupan*-27,28-*dioate* (1·1 g) as needles from MeOH–water, m.p. 237–238°, $[\alpha]_D$ 0° (*c*, 0·86, pyridine), ν_{max} 3606 cm^{-1}. (Found: C, 70·1; H, 9·8. $C_{32}H_{52}O_7$ requires: C, 70·0; H, 9·55%).

Cleavage of methyl 3β,20,29-*trihydroxylupan*-27,28-*dioate*. The trihydroxy-ester (810 mg) in EtOH (150 ml) was cleaved by periodic acid (3·8 g) in water (45 ml) during 12 hr. Solvents were removed *in vacuo* and the product (750 mg) was adsorbed from benzene on alumina (20 g). *Methyl* 3β-*hydroxy*-20-*oxo*-30-*norlupan*-27,28-*dioate* (III; 560 mg) was eluted by benzene–$CHCl_3$ (3:1) and formed needles from MeOH–water, m.p. 208–209°, $[\alpha]_D$ −13° (*c*, 1·01), ν_{max} 3629, 1723 (shoulder at 1715) cm^{-1}. (Found: C, 72·05; H, 9·1. $C_{31}H_{48}O_6$ requires: C, 72·1; H, 9·4%).

The corresponding *acetate* (acetic anhydride–pyridine method) formed plates from MeOH, m.p. 247–248°, $[\alpha]_D$ −9° (*c*, 0·77), ν_{max} 1725 (shoulder at 1732, 1715) cm^{-1}. (Found: C, 70·8; H, 8·9 $C_{33}H_{50}O_7$ requires: C, 70·9; H, 9·0%).

This keto-acetate was recovered unchanged after treatment with perbenzoic acid for 14 days at room temp.

Benzylidene derivative of methyl 3β-*acetoxy*-20-*oxo*-30-*norlupan*-27-28-*dioate*. The keto-acetate (760 mg) in $CHCl_3$ (5 ml), benzene (5 ml) and EtOH (5 ml) was treated for 24 hr with benzaldehyde (0·5 ml) and EtONa (from Na, 330 mg) in abs. EtOH (8 ml). The product was isolated through ether and re-acetylated (acetic anhydride–pyridine method) to give an oil (1·0 g) which was steam-distilled to remove the excess of benzaldehyde. The product was filtered from the aqueous residue to give the *benzylidene derivative* as cubes from MeOH, m.p. 212–213°, λ_{max} 290 mμ (ε, 23000). (Found: C, 74·05; H, 8·15. $C_{40}H_{54}O_7$ requires: C, 74·3; H, 8·4%).

Elimination of side-chain from the benzylidene derivative. The benzylidene derivative (410 mg) was reduced by aluminium isopropoxide (2 g) in isopropanol (60 ml). The distillate (30 ml) was collected during 7 hr. The remaining isopropanol was removed *in vacuo*, the residue acidified and the product isolated through ether.

Dehydration of this alcohol was accomplished by heating under reflux in acetic acid (20 ml) for 5·5 hr and, after the addition of acetic anhydride (20 ml), for a further 30 min. MeOH (30 ml) was added to the cooled solution, and the solvents were removed *in vacuo*. The crude product (360 mg) in ethyl acetate was treated, at −18°, with ozonized O_2 for 15 min. The resultant ozonide was destroyed by the addition of Zn dust and acetic acid, the mixture being stirred for 1 hr at 50°. The product was isolated through ether and filtered, in benzene, through alumina (10 g) to give *methyl* 3β-*acetoxy*-19-*oxo*-20,29,30-*trisnorlupan*-27-28-*dioate* (IV) as needles from MeOH, m.p. 223–224°, $[\alpha]_D$ +23° (*c*, 1·22), ν_{max} 1733 (shoulder at 1742), 1711, 1414 cm^{-1}. (Found: (C, 70·1; H, 8·7. $C_{31}H_{46}O_7$ requires: C, 70·2; H, 8·7%).

Lactonization of methyl 3-*oxolup*-20-*en*-27,28-*dioate*. The keto-ester (IIc; 1·01 g) in benzene (10 ml) was treated with H_2SO_4 (9 ml) in acetic acid (80 ml) at room temp. for 25 days. The product was isolated through ether and adsorbed on alumina (Act. IV; 25 g). Elution with light petroleum–benzene (1:1) gave *methyl* 3-*oxo*-(18α)-*oleanan*-28,19β-*olide* 27-*oate* (V) as needles from MeOH m.p. 250–251°, $[\alpha]_D$ +119° (*c*, 0·38), ν_{max} 1778, 1718 (shoulder at 1708) cm^{-1}. (Found: C, 74·4; H, 9·2; O, 15·9. $C_{31}H_{46}O_5$ requires: C, 74·7; H, 9·3; O, 16·0%).

Reduction of methyl 3-*oxo*-(18α)-*oleanan*-28,19β-*olide*-27-*oate*. The ketolactone (277 mg) was heated under reflux with LAH (340 mg) in ether (70 ml) for 2·5 hr. After the cautious addition of water, the product was isolated through ether to give *methyl* 3β,19β,28-*trihydroxy*-(18α)-*oleanan*-27-*oate* (VI; 274 mg) as fine needles from benzene, m.p. 262–263°, $[\alpha]_D$ +26° (*c*, 0·39), ν_{max} 3620 (ε, 160), 3340, 1709 cm^{-1} ($CHCl_3$). (Found: C, 73·4; H, 10·2; O, 16·0. $C_{31}H_{52}O_5$ requires: C, 73·8; H, 10·4; O, 15·85%).

Acetylation (acetic anhydride–pyridine method) afforded the corresponding diacetate, *methyl* 3β,28-*diacetoxy*-19β-*hydroxy*-(18α)-*oleanan*-27-*oate* as cubes from MeOH–water, m.p. 228–229°, $[\alpha]_D$ +14° (*c*, 0·70). (Found: C, 69·4; H, 9·5; O, 21·1. $C_{35}H_{56}O_7 \cdot H_2O$ requires: C, 69·3; H, 9·6; O, 21·1%).

The diacetate, in acetone, was oxidized by the Jones' reagent and the crude product was hydrolysed by KOH in MeOH to give *methyl* 3β,28-*dihydroxy*-19-*oxo*-(18α)-*oleanan*-27-*oate* as needles from benzene, m.p. 179–180°, $[\alpha]_D$ +51°, (*c*, 0·94), ν_{max} 3623, 1704 cm^{-1} ($CHCl_3$). (Found: C, 74·3; H, 10·0. $C_{31}H_{50}O_5$ requires: C, 74·1; H, 10·0%).

Methyl 3-*oxolup*-1-*en*-27,28-*dioate*. The keto-ester (VIIa; 610 mg) and N-bromosuccinimide (350 mg) in CCl_4 (20 ml) were heated under reflux for 9 min in the light of a 250 watt lamp. The

solution was filtered and evaporated to yield an oil (620 mg). A similar product was obtained by bromination with Br_2 (1 mole) in acetic acid.

The crude product gave the bromoketone as an amorphous powder from MeOH, m.p. 136–137°, $[\alpha]_D$ +47° (*c*, 0·87), ν_{max} 1718, 718 cm^{-1}. (Found: C, 64·3; H, 8·1; Br, 13·1. $C_{32}H_{49}O_5Br$ requires: C, 64·7; H, 8·3; Br, 13·5%).

This bromoketone (620 mg) was heated under reflux in collidine (6 ml). After 2 hr the solution was filtered and acidified by HCl. The product (210 mg) was isolated through ether and adsorbed, from light petroleum, on alumina (6 g). Elution with light petroleum–benzene (4:1) gave the *conjugated ketone* (42 mg) as needles from MeOH, m.p. 189–190°, ν_{max} 1726, 1719, 1672 cm^{-1}, λ_{max} 231 mμ (ε, 5170). (Found: C, 74·9; H, 9·4. $C_{32}H_{48}O_5$ requires: C, 75·0; H, 9·4%).

Methyl 2,2-*dibromo*-3-*oxolupan*-27,28-*dioate*. The keto-ester (VIIIa; 352 mg) in acetic acid (6 ml) reacted with Br_2 (2·3 mole) in acetic acid (6 ml) at room temp for 12 hr. The solvent was removed *in vacuo* to give the *dibromoketone* (360 mg) as needles from CH_2Cl_2–MeOH, m.p. 156–157° $[\alpha]_D$ +98° (*c*, 0·98), ν_{max} 1730, 1712, 760 cm^{-1}. (Found: C, 57·0; H, 7·0; Br, 23·8. $C_{32}H_{48}O_5Br_2$ requires: C, 57·1; H, 7·1; Br, 23·8%).

Lupan-3β,27,28-*triol* (VIIc). Dihydromelaleucic acid acetate (500 mg) was heated under reflux in $SoCl_2$ (55 ml) for 1·5 hr. The excess of $SoCl_2$ was removed *in vacuo*, last traces being removed by flushing with dry benzene (25 ml).

The residue, in dioxan (100 ml), was heated under reflux with LAH (1·5 g) for 70 hr. Water was then added and the product was isolated through ether and adsorbed on alumina (10 g). Elution with benzene–ether (1:1) gave the *triol* (310 mg) as needles from benzene–light petroleum, m.p. 182–183°, $[\alpha]_D$ −32° (*c*, 2·2). (Found: C, 78·1; H, 11·4. $C_{30}H_{52}O_3$ requires: C, 78·2; H, 11·4%). No coloration was observed with tetranitromethane.

Dihydrobetulin. Lupan-3β, 27,28-triol (146 mg) was acetylated (acetic anhydride–pyridine method to give a crude product (175 mg) which was filtered through alumina (3 g) in light petroleum to give 3β,28-*diacetoxylupan*-27-*ol* (VIId) as needles from MeOH–water, m.p. 156–157°, $[\alpha]_D$ −26° (*c*, 1·2), ν_{max} 3625, 1732 cm^{-1}. The NMR spectrum showed singlets at 1·97, 2·00 (6H, acetoxy groups). (Found: C, 75·1; H, 10·3. $C_{34}H_{56}O_6$ requires: C, 75·0; H, 10·4%).

To an ice-cooled solution of the diacetate (VIId; 100 mg) in $CHCl_3$ (4 ml) and acetic acid (40 ml) was added during 10 min a solution of CrO_3 (225 mg) in water (2 ml) and acetic acid (6 ml). After 75 min at room temp MeOH was added, the mixture diluted with water and extracted with ether. The organic layer was washed with 3% KOH aq and evaporated to give the crude aldehyde (62 mg).

The aldehyde (62 mg) and hydrazine hydrate (80%; 2 ml) in EtOH (6 ml) were heated under reflux for 2·5 hr. KOH (1 g) and diethylene glycol (5 ml) were added and the mixture distilled until the internal temp reached 205°. The solution was then heated under reflux for 12 hr, diluted with water, acidified and extracted with ether to give a curde product (40 mg) which was adsorbed on alumina (Act III; 2 g). Elution with benzene–ether (4:1) gave dihydrobetulin (22 mg) as needles from EtOH–water, m.p. and m.m.p. with an authentic specimen 277–278°, $[\alpha]_D$ −20° (*c*, 0·64, pyridine). (lit.[25] m.p. 279–280°, $[\alpha]_D$ 22°). The IR spectrum was identical with that of the authentic specimen.

Lactonization of methyl dihydromelaleucate. The ester (1·01 g) in benzene (10 ml) was treated with a mixture of acetic acid (80 ml) and H_2SO_4 (9 ml) at room temp. After 15 days, on dilution with water the product (990 mg) was isolated through ether and filtered in light petroleum–benzene (1:1) through alumina (30 g) to give the γ-*lactone* (VIII; 675 mg) as prisms from MeOH, m.p. 184–185°, $[\alpha]_D$ −11° (*c*, 0·70), ν_{max} 1762, 1726. (Found: C, 76·75; H, 9·6. $C_{31}H_{48}O_4$ requires: C, 76·8; H, 10·0%).

Dihydropyran with methyl melaleucate. The ester (1·0 g) was treated with dihydropyran (2 ml) and conc. HCl (2 drops) during 20 min, with shaking, at 0° and the mixture was then allowed to stand at room temp for 12 hr and evaporated to dryness. The product (1·2 g) was filtered, in light petroleum, through alumina (20 g) to give *methyl* 3β-*tetrahydropyranyloxylup*-20-*en*-27,28-*dioate* (IXa; 650 mg) as prisms from EtOH, m.p. 211–212°, $[\alpha]_D$ +54° (*c*, 1·02). (Found: C, 73·9; H, 10·0. $C_{37}H_{58}O_6$ requires: C, 74·2; H, 9·8%). The IR spectrum showed no hydroxyl absorption.

3β-*Hydroxylup*-20-*en*-27-*oic acid* (Xa). Methyl melaleucate tetrahdyropyranyl ether (2·1 g) was

[25] E. Josephy and F. Radt, *Elsevier's Encyclopedia of Organic Chemistry* Vol. 14: p. 569. Elsevier, New York (1940); Vol. 14 Supplement, p. 1135s (1952).

reduced by LAH in ether under reflux (2 hr) to give *methyl 28-hydroxy-3β-tetrahydropyranyloxylup-20-en-27-oate* (IXb; 1·1 g) as needles from acetone, m.p. 136–137°, $[\alpha]_D$ +75° (*c*, 0·97). C, 76·0; H, 10·4. $C_{36}H_{58}O_5$ requires: C, 75·8; H, 10·2%).

This alcohol (1·1 g) was oxidized by CrO_3 (1·1 g) in pyridine (20 ml) during 12 hr. The mixture was diluted with water and extracted with ether to give *methyl 28-oxo-3β-tetrahydropyranyloxylup-20-en-27-oate* (IXc; 950 mg) as needles from acetone–EtOH, m.p. 128–129°, $[\alpha]_D$ +32° (*c*, 0·86). (Found: C, 76·3; H, 10·1. $C_{36}H_{56}O_5$ requires: C, 76·1; H, 9·9%).

The aldehyde (600 mg) was reduced under the Huang-Minlon conditions (see above, for dihydrobetulin), to give *3β-hydroxylup-20-en-27-oic acid* (470 mg) as needles from MeOH–water, m.p. 308–309°, $[\alpha]_D$ +29° (*c*, 0·93). (Found: C, 78·7; H, 10·6. $C_{30}H_{48}O_3$ requires: C, 78·9; H, 10·5%).

The acid was treated with KOH and dimethyl sulphate in aqueous acetone to give methyl *3β-hydroxylup-20-en-27-oate* (Xb) as needles from MeOH–water, m.p. 190–191°, $[\alpha]_D$ +37° (*c*, 1·20). (Found: C, 79·2; H, 10·6; OCH_3, 7·0. $C_{31}H_{50}O_3$ requires: C, 79·15; H, 10·6; OCH_3, 6·6%).

Mild oxidation of methyl 3β-hydroxylup-20-en-27-oate. The ester (250 mg) was oxidized by the Jones' reagent (1·2 ml) during 1·5 hr to give *methyl 3-oxolup-20-en-27-oate* (71 mg) as prisms from CH_2Cl_2–MeOH, m.p. 177–178°, $[\alpha]_D$ +25° (*c*, 0·97), ν_{max} 1710 cm^{-1}. (Found: C, 79·4; H, 10·3. $C_{31}H_{48}O_3$ requires: C, 79·5; H, 10·3%).

Lactonization of methyl 3-oxolup-20-en-27-oate. A solution of the keto-ester (400 mg) in benzene (5 ml) was treated with a mixture of glacial acetic acid (40 ml) and H_2SO_4 (7 ml). The solution was warmed to 50° and then kept at 20° for 15 days. The resultant brown solution (green fluorescence) was poured into water and the product was isolated through ether and adsorbed on alumina (10 g). Elution with light petroleum–benzene (1:4) gave *3-oxo-oleanan-27,18α-olide* (XI; 133 mg) as needles from MeOH, m.p. 251–252°, $[\alpha]_D$ +147° (*c*, 0·81), ν_{max} 1772, 1708 cm^{-1}. (Found: C, 79·4; H, 10·3. $C_{30}H_{44}O_3$ requires: C, 79·2; H, 10·2%).

Acknowledgments.—The authors wish to thank Dr. P. R. Jefferies and Dr. G. V. Baddeley for valuable discussions.