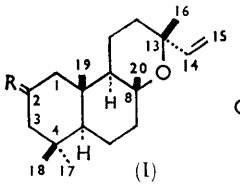
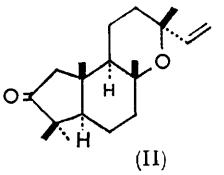
702. *The Diterpenes of* Dacrydium colensoi. *Part II* [1]

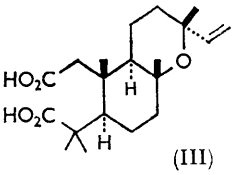
By P. K. Grant, M. H. G. Munro, and (in part) N. R. Hill

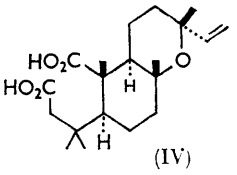
Three oxido-diterpenes have been isolated from *Dacrydium colensoi* and characterised as 2α-hydroxymanoyl oxide (I; R = β-H, α-OH), 2,3-dicarboxy-2,3-secomanoyl oxide (III), and 2-oxo-3-oxamanoyl oxide (V).

Thin layer chromatography (t.l.c.) of the neutral ether extract of the heartwood of *D. colensoi* has shown the presence of at least seventeen compounds, the structures of only three, manoyl oxide (I; R = H_2),[2] 2-oxomanoyl oxide (I; R = O),[3] and colensen-2-one (II), having been reported to date. These are all oxido-diterpenes, the occurrence of which has been reported from only two other sources, *D. biforme* [4] and *Xylia dolabriformis*.[5]


(I)


(II)


(III)


(IV)

On chromatography of the high-boiling fractions of *D. colensoi* extract, a further four diterpenoid compounds were isolated, a hydroxyoxido-diterpene, a lactone, a hydroxy-oxido-oxo-diterpene, m. p. 113—114° (compound A), and a diol, m. p. 169—170° (compound B). Modification of the isolation technique of the lactone (using alkali extraction) resulted in the isolation of a dicarboxylic acid which must have been present in the original extract as an ester. We now report the structure of the hydroxy-diterpene, the dicarboxylic acid, and the lactone.

Hydroxyoxido-diterpene.—The infrared spectrum showed hydroxyl absorption (3400 $cm.^{-1}$) and bands characteristic of an oxide ring (1115, 1078 $cm.^{-1}$) and of a vinyl group (1640, 991, 923 $cm.^{-1}$). On oxidation it gave a ketone identified as 2-oxomanoyl oxide (by mixed m. p. and infrared). Since the alcohol isolated is not identical with 2β-hydroxymanoyl oxide,[6] the lithium aluminium hydride reduction product of 2-oxomanoyl oxide, it must be the epimeric 2α-hydroxymanoyl oxide. Although a sample of the naturally occurring alcohol was not available, its infrared and nuclear magnetic resonance (n.m.r.) spectra were identical with those of 2α-hydroxymanoyl oxide (I; R = β-H, α-OH), prepared by sodium–alcohol reduction of 2-oxomanoyl oxide, a method known to give predominantly the equatorial epimer.[7]

Dicarboxylic Acid.—The acid analysed for $C_{20}H_{32}O_5$. The infrared spectrum showed a typically broad carboxyl OH band (3000 $cm.^{-1}$), carbonyl absorption (1718, 1694 $cm.^{-1}$) and bands characteristic of a vinyl group (991, 921 $cm.^{-1}$) and of an oxide ring (1115, 1075 $cm.^{-1}$). Esterification of the acid with diazomethane gave a diester, $C_{22}H_{36}O_5$, which showed carbonyl absorption at 1741 and 1715 $cm.^{-1}$ and established the presence of two carboxyl groups, thus accounting for all the oxygen atoms.

The n.m.r. spectrum of the acid showed a broad two-proton peak at low field (−2·23 τ), due to the carboxyl protons, which disappeared on the addition of D_2O. The typical 13α-vinyl pattern of the oxido-diterpenes was present. Sharp methyl singlets appeared at 9·13, 8·83 (6 protons), 8·75, and 8·67 τ, the two at lowest field being assigned to the

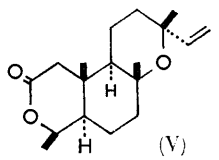
[1] P. K. Grant and R. M. Carman, *J.*, 1962, 3740, to be considered as Part I.
[2] J. R. Hosking and C. W. Brandt, *Ber.*, 1935, **68**, 37.
[3] J. R. Hosking and C. W. Brandt, *Ber.*, 1935, **68**, 286.
[4] R. C. Cambie and L. N. Mander, *N.Z. J. Sci.*, 1964, **7**, 188.
[5] R. A. Laidlaw and J. W. W. Morgan, *J.*, 1963, 644.
[6] L. F. Fieser and M. Fieser, "Steroids," Reinhold, New York, 1959, p. 269.
[7] D. H. R. Barton, *J.*, 1953, 1027; M. Balasubramian and A. D'Souza, *Tetrahedron Letters*, 1963, 1891.

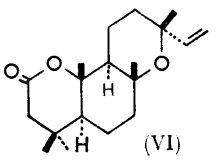
quaternary 8- and 13-methyl groups which are β to the ethereal oxygen. The presence of five quaternary methyl groups excluded the carboxyl groups from replacing the quaternary methyls of the manoyl oxide skeleton. A two-proton singlet (7·62 τ) was assigned to $CH_2 \cdot CO_2H$, a grouping which can only be accommodated by the seco-acids (III) and (IV) which are derived from ring A bond fission.

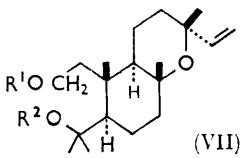
Since the dihydro-derivatives of both these acids have been prepared [1,8] it was possible to distinguish readily between the alternative structures. Hydrogenation of the dicarboxylic acid gave the dihydro-acid, $C_{20}H_{34}O_5$, m. p. 185·5—187°, undepressed on admixture with the synthetic dihydro-2,3-seco-acid. Hence the structure of the dicarboxylic acid is established as 2,3-dicarboxy-2,3-secomanoyl oxide (III). The isolation of this acid is biogenetically interesting as it can be regarded as a possible precursor of the co-occurring nor-oxido-diterpene, colensen-2-one (II).

Lactone.—The solubility of this neutral compound in alkali, from which it was recovered on acidification, characterised it as a lactone. It analysed for $C_{19}H_{30}O_3$, this being confirmed by the mass spectrum which showed the molecular ion at *m/e* 304. The infrared spectrum showed a carbonyl band (1730 cm.$^{-1}$) and bands characteristic of a vinyl group (3090, 1643, 990, 912 cm.$^{-1}$) and of an oxide ring (1122, 1080 cm.$^{-1}$). The frequency of the carbonyl band was consistent with a δ-lactone.

The n.m.r. spectrum of the lactone showed the typical 13α-vinyl pattern. Four of the five methyl signals [9·03, 8·70, 8·66 (6 protons), 8·57 τ] appeared at unusually low field. Since two of these can be assigned to the quaternary 8- and 13-methyl groups, the remaining two methyl groups at low field are probably the result of the proximity of the lactone ring. An AB system centred at 7·37 τ (J_{AB} 16·5 c./sec.) can be assigned to a methylene group adjacent to the lactone carbonyl. As the compound is a δ-lactone, this O–CO–CH_2 grouping can only be part of ring A. Two alternative structures, (V) and (VI), can be assigned to the lactone, (V) being preferred on account of the two low-field methyl signals.


(V)


(VI)



(VII)

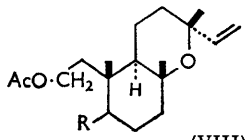
Structure (V) for the lactone was confirmed in the following way. Reduction of the lactone with lithium aluminium hydride gave the diol $C_{19}H_{34}O_3$ (VII; $R^1 = R^2 = H$) [3250 (OH) cm.$^{-1}$], the n.m.r. spectrum of which showed four of the five methyl signals at low field [9·07, 8·84, 8·80, 8·73 (6 protons)], again supporting structure (V) for the lactone. Acetylation of the diol gave the hydroxyacetate (VII; R^1 = Ac, R^2 = H) [3500 (OH), 1734 (C=O) cm.$^{-1}$]. Dehydration of the hydroxyacetate with phosphorus oxychloride–pyridine gave a product, $C_{21}H_{34}O_3$, which was shown by t.l.c. on silver nitrate-impregnated silica gel [9] to be a mixture of two compounds. These were considered to be the isopropenyl form [VIII; R = C(:CH_2)Me] [895 (C:CH_2) cm.$^{-1}$] and the isomeric isopropylidene form (VIII; R = :CMe_2).

Hydrogenation of this isomeric dehydration product gave a mixture of the tetrahydroacetate (IX) and the dihydroacetate (X; R = :CMe_2) which was separated by preparative t.l.c. on silver nitrate-impregnated silica gel. The methyl region of the n.m.r. spectrum of the tetrahydroacetate, $C_{21}H_{38}O_3$, showed three singlets (13-, 8-, and 10-Me, respectively), a triplet (15-Me), and two doublets (*J* 6·8 c./sec.) assigned to the methyl groups of the 5-isopropyl group. This methyl signal pattern is most distinctive and is consistent only with tetrahydroacetate (IX), *i.e.*, with structure (V), for the lactone. The methyl region for the corresponding tetrahydroacetate derived from the alternative lactone structure

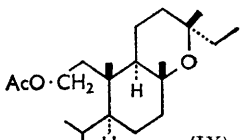
[8] P. K. Grant and N. R. Hill, *Austral. J. Chem.*, 1964, **17**, 66.
[9] T. Norin and L. Westfelt, *Acta Chem. Scand.*, 1963, **17**, 1828.

(VI) would have given a different pattern consisting of four singlets, a triplet, and one doublet.

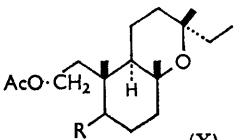
An unusual feature of the separation of the tetrahydroacetate (IX) and the dihydroacetate (X; R = :CMe_2) was the isomerisation of the latter to the isopropenyl form [X; R = C(:CH_2)Me during] the complexing with silver nitrate. This can be attributed



(VIII)



(IX)



(X)

to the relative instability of a double bond exocyclic to a six-membered ring.[10] The infrared spectrum of the mixture before separation showed no absorption due to >C:CH_2 grouping. After the separation the unsaturated acetate isolated, $C_{21}H_{36}O_3$, showed typical >C:CH_2 absorption (892 $cm.^{-1}$) and was assigned structure [X; R = C(:CH_2)Me]. This was confirmed by the n.m.r. spectrum which showed a doublet due to >C:CH_2 (5·25, 5·09 τ) and an allylic methyl signal (8·23 τ). Furthermore, the presence of these signals in the unsaturated acetate is consistent only with structure (V) for the lactone.

Biogenetically the lactone can be considered as arising from a Baeyer–Villiger type oxidation on the co-occurring colensen-2-one (II).

Experimental

Melting points were taken on a Kofler hot-stage apparatus and are corrected. Infrared spectra were measured on a Perkin-Elmer 421 instrument, and n.m.r. spectra were determined at 60 Mc. in deuterochloroform with tetramethylsilane as internal standard. Light petroleum refers to the fraction of b. p. 45—70°. Merck's standardised aluminium oxide was used in chromatography unless otherwise stated.

Isolation Procedure.—The neutral fraction of the heartwood extract of *Dacrydium colensoi* was distilled, all fractions to b. p. 160°/0·15 mm. were removed, and the distillation residue was chromatographed from ether on alumina. Elution with ether gave manoyl oxide, 2-oxomanoyl oxide, and colensenone with the solvent front. Further elution of the column with ether gave a mixture of 2α-hydroxymanoyl oxide and the lactone, 2-oxo-3-oxamanoyl oxide. Continued elution of the column with ether gave compound A followed by compound B. *Compound A* had m. p. 113—114° (from light petroleum) (Found: C, 75·4; H, 10·1. $C_{20}H_{32}O_3$ requires C, 75·0; H, 10·1%), $\nu_{max.}$ (Nujol) 3470 (OH), 1699 (C=O), 1118, 1075 (C–O) $cm.^{-1}$. *Compound B* had m. p. 169—170° after recrystallisation from aqueous methanol and vacuum-sublimation (Found: C, 78·9; H, 10·4. $C_{20}H_{32}O_2$ requires C, 78·9; H, 10·6%), $\nu_{max.}$ (Nujol) 3280 (OH) $cm.^{-1}$. A more convenient method of isolating the lactone used extraction with alkali. The distillation residue (410 g.) in 10% ethanolic sodium hydroxide (30 ml.) was refluxed for 2 hr., the solution diluted to less than 10% ethanol concentration, and then ether extracted to remove the neutral material (375 g.). The aqueous layer was acidified, extracted with ether, and the extracts were shaken with 10% aqueous sodium carbonate. The ethereal solution, after washing and evaporation, gave a brown gum (4·05 g.) from which *2-oxo-3-oxamanoyl oxide* crystallised on standing. The aqueous sodium carbonate layer was acidified, extracted with ether, and the ethereal layer extracted with saturated aqueous sodium hydrogen carbonate. Evaporation of the ethereal layer gave a brown gum (8·8 g.), shown by t.l.c. to consist of two acids. Acidification and ether extraction of the aqueous sodium hydrogen carbonate extract gave a yellow oil (22 g.) which crystallised, on standing, from light petroleum to give *2,3-dicarboxy-2,3-secomanoyl oxide.*

2α-Hydroxymanoyl Oxide.—A mixture (0·2 g.) of 2α-hydroxymanoyl oxide and 2-oxo-3-oxamanoyl oxide, obtained from the chromatography of the distillation residue (above), was rechromatographed on alumina from light petroleum–ether (3 : 1). Elution with light petroleum–ether (1 : 1) gave *2α-hydroxymanoyl oxide* (0·05 g.), m. p. 54·5—56·5° (from light petroleum); mixed m. p. with 2β-hydroxymanoyl oxide 40—45°. Further elution of the column with light

[10] H. C. Brown, *J. Org. Chem.*, 1957, **22**, 439.

petroleum–ether (1 : 1) gave 2-oxo-3-oxamanoyl oxide (0·07 g.). The infrared spectrum (Nujol) of 2α-hydroxymanoyl oxide showed 3400 (OH), 1120, 1070 (C–O), 3090, 1640, 990, 926 ($CH{:}CH_2$) cm.$^{-1}$; proton resonance: methyl signals at 9·12 (6 protons), 9·02, 8·66, 8·60 τ; >CH·OH 5·95 τ (m); vinyl protons H_A 3·90, H_B 4·92, H_C 4·71 τ (J_{AB} 10·8, J_{BC} 1·9, J_{AC} 17·8 c./sec.). The infrared spectrum and n.m.r. spectrum of 2α-hydroxymanoyl oxide were identical with those of synthetic 2α-hydroxymanoyl oxide.

Synthetic 2α-Hydroxymanoyl Oxide.—2-Oxomanoyl oxide (0·77 g.) in propan-1-ol (50 ml.) was refluxed, with the addition of small pieces of sodium, for 3 hr. The product (0·72 g.) was recrystallised from aqueous ethanol to give 2α-hydroxymanoyl oxide, m. p. 54·5—56·5° (Found: C, 78·0; H, 11·2. $C_{20}H_{34}O_2$ requires C, 78·4; H, 11·2%). Oxidation of 2α-hydroxymanoyl oxide: 8N-chromic acid–sulphuric acid was added dropwise to 2α-hydroxymanoyl oxide (0·02 g.) in acetone (1 ml.) until the orange colour persisted. Dilution and ether extraction gave, after recrystallisation from aqueous methanol, 2-oxomanoyl oxide, m. p. 77—78°, undepressed on admixture with 2-oxomanoyl oxide.

2,3-*Dicarboxy*-2,3-*secomanoyl Oxide.*—This acid had m. p. 191—192° (from light petroleum) (Found: C, 68·3; H, 9·3. $C_{20}H_{32}O_5$ requires C, 68·15; H, 9·15%), $\nu_{max.}$ (Nujol) 3000br (OH), 1718, 1694 (C=O), 991, 921 ($CH{:}CH_2$), 1115, 1075 (C–O) cm.$^{-1}$; proton resonance: methyl peaks at 9·13, 8·83 (6 protons), 8·75, 8·67 τ; 1-methylene 7·62 τ (s); vinyl protons H_A 4·20, H_B 5·18, H_C 5·00 τ (J_{AB} 10·4, J_{BC} 2·0, J_{AC} 17·7 c./sec.).

Dimethyl Ester of 2,3-*Dicarboxy*-2,3-*secomanoyl Oxide.*—The acid, on esterification with diazomethane gave 2,3-*dicarboxy*-2,3-*secomanoyl oxide dimethyl ester*, m. p. 59·5—61·5° (from aqueous acetone) (Found: C, 69·2; H, 9·6. $C_{22}H_{36}O_5$ requires C, 69·4; H, 9·5%), $\nu_{max.}$ (Nujol) 1741, 1715 (C=O) 1146 (ester C–O), 3100, 1641, 992, 921 ($CH{:}CH_2$) cm.$^{-1}$; proton resonance: methyl peaks at 9·15, 8·79 (6 protons), 8·76 τ (6 protons); CH_3–CO–O 6·41 τ (6 protons); 1-methylene 7·70 τ (s).

Dihydro-2,3-*dicarboxy*-2,3-*secomanoyl Oxide.*—The acid (III) (0·31 g.) in ethyl acetate (20 ml.) was hydrogenated over Adams catalyst. One mol. of hydrogen was absorbed to give dihydro-2,3-dicarboxy-2,3-secomanoyl oxide (0·3 g.), m. p. 185—187° (from hexane) undepressed on admixture with the synthetic acid and having superimposable infrared spectra when measured in solution, $\nu_{max.}$ (CCl_4) 3000br (OH), 1718, 1693 (C=O) cm.$^{-1}$.

Dihydro-2,3-*dicarboxy*-2,3-*secomanoyl Oxide Dimethyl Ester.*—The dihydro-acid (0·075 g.) and the synthetic dihydro-acid (0·097 g.) were methylated with diazomethane. Recrystallisation from aqueous acetone gave the *esters*, m. p. 54·5—56·5°, undepressed on admixture (Found: C, 69·2; H, 10·1. $C_{22}H_{38}O_5$ requires C, 69·1; H, 10·0%), $\nu_{max.}$ (CCl_4) 1746, 1731 (C=O), 1150 (ester C–O) cm.$^{-1}$.

2-*Oxo*-3-*oxamanoyl Oxide.*—This lactone had m. p. 173—174° from light petroleum (Found: C, 74·5; H, 10·1. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ (Nujol) 1726 (C=O), 3090, 1643, 990, 912 ($CH{:}CH_2$), 1122, 1080 (C–O) cm.$^{-1}$; proton resonance: methyl signals at 9·03, 8·70 (6 protons), 8·66, 8·57 τ; CH_2–CO–O as AB system H_A 7·35, H_B 8·11 τ (J_{AB} 16·5 c./sec.); vinyl protons H_A 4·15, H_B 5·10, H_C 4·90 (J_{AB} 10·3, J_{BC} 1·8, J_{AC} 17·0 c./sec.).

Lithium Aluminium Hydride Reduction of 2-*Oxo*-3-*oxamanoyl Oxide* (V).—2-Oxo-3-oxamanoyl oxide (3·05 g.) in dry tetrahydrofuran (25 ml.) was added dropwise to an excess of lithium aluminium hydride in dry tetrahydrofuran (75 ml.) and the solution refluxed for 5 hr. The excess of hydride was destroyed by the careful addition of ethyl acetate, saturated sodium sulphate solution was added, and the solution extracted with ether (4 × 50 ml.). The extract was washed, dried (Na_2SO_4), and evaporated under a vacuum to give the product (2·89 g.) which recrystallised from light petroleum to give the *diol* (VII; $R^1 = R^2 = H$), m. p. 136—138° (Found: C, 73·25; H, 11·1. $C_{19}H_{34}O_3$ requires C, 73·5; H, 11·0%), $\nu_{max.}$ (Nujol) 3250br (OH) cm.$^{-1}$; proton resonance: methyl peaks at 9·03, 8·70, 8·66 (6 protons), 8·57 τ; 1-methylene 7·73 τ (AB system J_{AB} = 16·5 c./sec.); CH_2·OH 6·14 τ (broad triplet); vinyl protons H_A 4·15, H_B 5·10, H_C 4·90 (J_{AB} 10·3, J_{BC} 1·8, J_{AC} 17·0 c./sec.).

Acetylation of the Diol (VII; $R^1 = R^2 = H$).—The diol (2·06 g.) in pyridine–acetic anhydride (20 ml.; 1 : 1) was set aside overnight. The product (2·24 g.) was chromatographed on alumina (65 g.) from light petroleum–ether (3 : 2). Elution with light petroleum–ether (3 : 2) gave the hydroxyacetate (VII; R^1 = Ac, $R^2 = H$) as an oil (1·78 g.). Apart from the infrared 3500 (OH), 1734 (C=O) 1245 (ester C–O) cm.$^{-1}$, the hydroxyacetate was not characterised.

Dehydration of the Hydroxyacetate (VII; R^1 = Ac, $R^2 = H$).—The hydroxyacetate (1·47 g.) in pyridine (10 ml.) was added to phosphorus oxychloride (6 ml.) in pyridine (35 ml.), set aside

overnight, and poured on to ice (100 g.). Ether extraction gave a product (1·44 g.) which was chromatographed on alumina (45 g.) from light petroleum. Elution with light petroleum–ether (19 : 1) gave a mixture of the unsaturated acetates. (VIII; R = :CMe_2) and [VIII; R = C(:CH_2)Me] (Found: C, 75·2; H, 10·3. $C_{21}H_{34}O_3$ requires C, 75·4; H, 10·25%), $\nu_{max.}$ (film) 1742 (C=O), 3075, 1640, 895 (C:CH_2), 3075, 1640, 992, 918 (CH:CH_2) cm.$^{-1}$.

Hydrogenation of the Mixture of Unsaturated Acetates (VIII; R = :CMe_2) and [VIII; R = C(:CH_2)Me].—The mixed unsaturated acetates (1·08 g.) in ethyl acetate (25 ml.) were hydrogenated over Adams catalyst. The uptake of hydrogen corresponded to 1½ mol. The product (1·05 g.), which showed no C=C absorption in the infrared but gave two spots on t.l.c. (using silver nitrate-impregnated silica gel), was chromatographed on Woelm grade I acid alumina (68 g.) from light petroleum. Elution with light petroleum–ether (49 : 1) gave a mixture (0·44 g.) of the isopropyl acetate (IX) and the isopropylidene acetate (X; R = :CMe_2) showing no C=C absorption in the infrared. Further elution of the column with light petroleum–ether (97 : 3) gave a purer sample (0·4 g.) of the isopropylidene acetate. Samples of these two fractions (0·25 and 0·24 g., respectively) were purified by preparative scale t.l.c. on silver nitrate-impregnated silica gel, light petroleum–ether (4 : 1) being used to develop the plates. The bands, which were identified by spraying the plates with water, were scraped off and eluted with ether to give the isopropyl acetate (IX) (0·155 g.) as a brown oil and the isopropenyl acetate [X; R = C(:CH_2)Me] (0·135 g.) as a brown oil. The *isopropyl acetate* (IX) (0·155 g.) was chromatographed on Woelm grade I acid alumina (3·5 g.) from light petroleum. Elution with light petroleum–ether (19 : 1) gave a colourless oil which was distilled (100°/0·09 mm.) (Found: C, 74·9; H, 11·5. $C_{21}H_{38}O_3$ requires C, 74·5; H, 11·3%), $\nu_{max.}$ (film) 1740 (C=O) cm.$^{-1}$; proton resonance: methyl peaks at 9·28, 9·19 (doublet, J 6·8 c./sec.), 9·16 (triplet, J 7·4 c./sec.), 9·03 (doublet, J 6·8 c./sec.), 8·85, 8·76 τ; CH_3·CO·O 8·02 τ; CH_2·OAc 5·98 τ (triplet, J 7·8 c./sec.).

The *isopropenyl acetate* (0·135 g.) was chromatographed on Woelm grade I acid alumina (3·6 g.) from light petroleum. Elution with light petroleum–ether (19 : 1) gave a colourless oil which was distilled (100°/0·08 mm.) (Found: C, 74·8; H, 11·0. $C_{21}H_{36}O_3$ requires C, 74·95; H, 10·8%), $\nu_{max.}$ (film) 1738 (C=O) 3070, 1635, 892 (C:CH_2) cm.$^{-1}$; proton resonance: methyl peaks at 9·28, 9·15 (triplet, J 5·4 c./sec.), 8·82, 8·79, 8·35 τ; CH_3·CO·O 8·02 τ; CH_2·OAc 5·92 τ (triplet, J 7·8 c./sec.); C:CH_2 5·25, 5·09 τ.

The authors thank Professor C. Djerassi for measuring the mass spectrum, and Dr. B. R. Davis for measuring the n.m.r. spectra. They are grateful to the N.Z. University Grants Committee for the award of a Fellowship (to M. H. G. M.) and to the Research Committee for financial assistance.

Chemistry Department, University of Otago,
Dunedin, New Zealand. [*Received, January 14th,* 1965.]