251. *Extractives from the New Zealand Myrtaceae. Part VII.* Neutral and Phenolic Compounds from the bark of* Leptospermum scoparium.

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The bark of *L. scoparium* (Manuka) contains long-chain aliphatic esters, *p*-coumaric esters, long-chain aliphatic alcohols, β-sitosterol, a triterpene hydroxy-lactone, a triterpene hydroxy-ester, and a triterpene diol.

The genus *Leptospermum* is represented in New Zealand by *L. ericoides* (A. Rich), *L. sinclarii*, and *L. scoparium*; the last is the most abundant and widely distributed native tree in New Zealand. The bark of *L. ericoides* [1] contains the triterpene acids, betulic acid, ursolic acid acetate, and an acid $C_{30}H_{48}O_4$. Corbett and McDowall [2] reported the isolation of the triterpene acids, ursolic acid acetate, betulic acid, and oleanolic acid from a hexane extract of the outer bark of *L. scoparium*, and in the present Paper we report an investigation of the neutral and phenolic components (*ca.* 22%) of similar extract.

The major component of the neutral fraction was a mixture of long-chain aliphatic esters. Chromatography on alumina gave a series of fractions with steadily increasing melting point. Analysis of the first and last fractions indicated the molecular formulæ $C_{42}H_{84}O_2$ and $C_{54}H_{108}O_2$, respectively. The ester mixture was saponified and the acid fraction formed was converted into hydrocarbons, by the method of Downing *et al.*,[3] which were analysed by gas–liquid chromatography. The acid fraction was thus shown to consist of tetracosanoic, hexacosanoic, and octacosanoic acids. The alcohol fraction formed in the saponification was oxidized to an acid mixture which was esterified; gas chromatography of the mixture of methyl esters showed the alcohol fraction to consist of octadecan-1-ol, eicosan-1-ol, docosan-1-ol, tetracosan-1-ol, hexacosan-1-ol, and octacosan-1-ol.

The extracts from which the acids and the aliphatic esters had been removed were separated into neutral and phenolic components by chromatography on neutral alumina. Analysis of the phenolic component, as ester, indicated the formula $C_{30}H_{50}O_3$, which was apparently confirmed by the analysis of the acetyl and methyl derivatives. Saponification of the ester gave *p*-coumaric acid, and an alcohol fraction, m. p. 77—78°. The alcohol fraction was converted into a mixture of methyl esters; gas–liquid chromatography of these showed the alcohol fraction to contain the same six alcohols as the alcohol fraction from the aliphatic esters. It is surprising that this phenolic fraction was not isolated with the acid fraction; no trace of it could be detected in this fraction.[2] Although *p*-coumaric acid and its esters have frequently been identified in plant extractives,[4] there

* Part VI, *Austral. J. Chem.*, 1963, **16**, 191.

[1] Corbett and McCrow, *J. Sci. Food Agric.*, 1959, **10**, 29.
[2] Corbett and McDowall, *J.*, 1958, 3715.
[3] Downing, Kranz, and Murray, *Austral. J. Chem.*, 1960, **13**, 80.
[4] Bonner, "Plant Biochemistry," Academic Press, New York, 1952.

does not appear to be a report of the isolation of an ester mixture of this type. It is quite probable that such mixtures are not uncommon. Brooker [5] reported the isolation of an ester of ferulic acid, and considered the alcohol derived from it to be a mixture of ceryl and lignoceryl alcohols. The melting point of the alcohol and its reported infrared spectrum are very similar to those of the alcohol mixtures described in this Paper.

Chromatography of the remaining neutral material on alumina gave fractions from which a mixture of long-chain alcohols, β-sitosterol, a triterpene hydroxy-lactone, a triterpene ester, and a triterpene diol were identified by standard methods.

The melting point and infrared spectrum of the alcohol were similar to those of the mixture of alcohols isolated in the saponification of the esters. Although it was not examined by gas chromatography, it was clearly a similar mixture, and was possibly an artefact resulting from hydrolysis of the esters during the extraction processes.

The triterpene hydroxy-lactone and hydroxy-ester, which were present in very small amounts, could not be identified with known compounds.

The triterpene diol was also a trace constituent of the extract. The melting point of the purest sample was 222—223°, and the infrared spectrum was very similar to that of betulin (m. p. 252°) with which the diol is tentatively identified.

Experimental

Melting points were taken on a Kofler block, and are corrected. Alumina refers to Merck, standardized according to Brockmann.

Extraction.—The fibrous outer bark of *L. scoparium* was stripped from a tree growing near Dunedin, cut into short lengths, milled to a fine powder (3·1 kg.), and extracted (soxhlet) for 48 hr. with hexane (10 l.). The extract was evaporated to dryness, and the amorphous solid (270 g.), extracted with ether (2 l.), left a residue (A) of aliphatic esters (25 g.). The ethereal solution was extracted repeatedly with 20% sodium hydroxide (3 × 200 c.c.) until there was no further separation of sparingly soluble sodium salts. The remaining ethereal solution gave, after evaporation of the solvent, a waxy solid (B) (27 g.).

Aliphatic Esters.—The ether-insoluble ester mixture (A) had m. p. 67—69° (from ethyl acetate), $\nu_{max.}$ 1740 and 1165 (ester), 735, and 722 cm.$^{-1}$ (chain of CH_2 groups). It (1·0 g.) was dissolved in hexane, and run on to a column of alumina (70 g.). Benzene aliquots (200 c.c.) eluted a series of fractions showing a regular gradation in m. p. from 65 to 81°. Each fraction crystallized from ethyl acetate in plates (Found for 1st fraction: C, 81·2; H, 13·5. $C_{42}H_{84}O_2$ requires C, 81·2; H, 13·2%. Found for last fraction: C, 82·5; H, 13·5. $C_{54}H_{108}O_2$ requires C, 82·2; H, 13·8%). The infrared spectrum of each fraction showed strong ester bands at 1740 and 1165 cm.$^{-1}$.

Saponification of Aliphatic Esters.—The esters (13·5 g.) were heated under reflux with 0·5N-ethanolic potassium hydroxide (250 c.c.) for 6 hr. and the hot solution was poured into 2N-sulphuric acid (200 c.c.). The solid which separated was dissolved in ether (1·5 l.) and extracted with 2N-sodium hydroxide (250 c.c.), and the insoluble sodium salts were filtered off. The sodium salts were extracted with acetone under reflux for 2 hr. and the extract added to the ether solution of the alcohols. Acidification of a solution of the sodium salts in 70% ethanol gave the acid fraction (5·2 g.), m. p. 77—78°. Evaporation of the ethereal solution gave the alcohol fraction (6·1 g.), m. p. 73—74°.

Conversion of the Acid Fraction into Hydrocarbons.[3]—The acid fraction (3·0 g.) was heated under reflux with methanol (250 c.c.) containing concentrated sulphuric acid (2·5 c.c.) for 6 hr. The solution was poured into water (200 c.c.) and the precipitated ester dissolved in ether (300 c.c.). The ethereal extract, which was washed with 2N-sodium hydroxide (2 × 25 c.c.) and water and dried (Na_2SO_4), gave, after removal of the solvent, the methyl esters (2·1 g.). The esters (1·6 g.) in anhydrous ether (50 c.c.) were added to lithium aluminium hydride (0·3 g.) in ether (25 c.c.), and the mixture heated under reflux for 3 hr. The unreacted hydride was destroyed with ethyl acetate, the solution washed with dilute sulphuric acid, and the solvent evaporated, to give the alcohols (1·4 g.). The alcohols (1·215 g.), finely powdered iodine (0·7 g.), and red phosphorus (0·16 g.) were heated on a steam-bath at 100° for 1½ hr. Excess of iodine

[5] Brooker, *New Zealand J. Sci.*, 1959, **2**, 212.

was removed at 100°/12 mm. The residue was dissolved in hexane (300 c.c.), the solution washed with water and dried (Na_2SO_4), and the solvent evaporated. The recovered product (1·4 g.) had m. p. 47—50° (Found: C, 64·2; H, 10·9; I, 25·4. Calc. for $C_{26}H_{53}I$: C, 63·4; H, 10·8; I, 25·8%). The iodide (1·4 g.) was dissolved in anhydrous ether (100 c.c.), and an excess of lithium aluminium hydride (0·3 g.) was added. The mixture was heated under reflux for 1 hr. and kept overnight at room temperature. The unreacted hydride was destroyed with ethyl acetate, the solution washed with dilute sulphuric acid, and the solvent evaporated, to give a product (0·82 g.). This product (0·2 g.) was heated under reflux with 0·5N-ethanolic potassium hydroxide for 1 hr. to hydrolyse possible traces of unchanged iodides. The solution was then diluted with water (100 c.c.) and extracted with hexane (3 × 50 c.c.). Removal of the hexane gave a product which was dissolved in pentane and run on to a column of alumina (10 g.). Pentane eluted the hydrocarbons (0·08 g.), m. p. 68—70° (Found: C, 84·9; H, 14·8. Calc. for $C_{26}H_{54}$: C, 85·2; H, 14·8%). This was submitted to gas–liquid chromatography (Table 1) in a column of 3% (w/w) Apiezon L on Johnson 60—80 mesh C22 firebrick, packed in 18 in. of 5 mm. (o.d.) copper tubing coiled after packing. The retention times of these hydrocarbons plotted on a log scale against carbon numbers gave a straight line, and thus confirmed their identification.[6]

TABLE 1.

Composition of the mixture of hydrocarbons derived from the acids obtained by the saponification of the aliphatic esters.

Temperature 280 ± 2°; N_2 flow 30 c.c./min.; thermal conductivity detector.

Peak No.	Relative retention time (tetracosane = 1; hexacosane = 1·66)	Fatty acids (as hydrocarbons)	Approx. quantity (%)
1	1·00	Tetracosanoic	11·5
2	1·66	Hexacosanoic	63
3	2·74	Octacosanoic	25·5

Conversion of Alcohol Fraction into Methyl Esters.—Chromic acid solution [7] was added dropwise to a solution of the alcohols (0·9 g.) in acetone (250 c.c.) at 40° in a thermostat, until a persistent orange-brown coloration indicated that the reaction was complete. The addition of water (600 c.c.) caused the precipitation of a gelatinous solid which was filtered off and crystallized from ethyl acetate (0·86 g.), m. p. 74—77°. The methyl ester, made with fresh diazomethane in methanol–ether, formed plates, m. p. 61·5—62·5° (from ethyl acetate) (Found: C, 78·8; H, 12·9. Calc. for $C_{27}H_{54}O_2$: C, 79·0; H, 13·2%). This was submitted to gas–liquid chromatography (Table 2) in a column of 2% (w/w) Apiezon L on 60—80 mesh acid-washed Celite 545, packed in 4 ft. of 5 mm. (o.d.) glass tubing. The retention times of these esters plotted on a log scale against carbon numbers gave a straight line.[6]

TABLE 2.

Composition of the mixture of methyl esters derived from the aliphatic ester alcohols.

Temperature 250 ± 1°; argon flow 50 c.c./min.; β-ray ionization detection.

Peak No.	Relative retention time (methyl stearate = 1)	Alcohols (as methyl esters)	Approx. quantity (%)
1	1·00	Octadecan-1-ol	trace
2	1·71	Eicosan-1-ol	1
3	3·24	Docosan-1-ol	10
4	6·40	Tetracosan-1-ol	27
5	13·10	Hexacosan-1-ol	45
6	22·43	Octacosan-1-ol	17

Isolation of p-*Coumaric Esters.*—The neutral fraction (B) (12 g.) from the original hexane extraction of the bark was dissolved in 1 : 1 hexane–benzene (1 l.) and run on to a column of alumina (400 g.). The column was eluted with ether (10 l.) which removed neutral products (7·8 g.) (C), and then with 1 : 9 ethanol–ether (2 l.) which removed the phenolic esters. These phenolic fractions, dissolved in ether, were re-chromatographed on alumina. Elution with

[6] James and Martin, *Biochem. J.*, 1952, **50**, 697.
[7] Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2548.

1 : 9 ethanol–ether gave a green waxy solid (2·7 g.) which gave a microcrystalline product after crystallization from acetone, and hexane (twice), m. p. 70—80° (Found: C, 78·5; H, 11·2. Calc. for $C_{30}H_{50}O_3$: C, 78·5; H, 11·0%), $\lambda_{max.}$ (in 95% ethanol) 230, 314 mμ (log ε 3·88, 4·13), $\lambda_{min.}$ 256 mμ (log ε 3·31), $\lambda_{max.}$ (in 95% ethanol plus one drop of 2N-sodium hydroxide) 240, 367 mμ (log ε 4·0, 4·27), $\lambda_{min.}$ 276 mμ (log ε 3·60) (values of ε are calculated for an average mol. wt. of 500). Acetylation with acetic anhydride and sodium acetate at 140° for 6 hr. gave an acetate, m. p. 74—78° (from ethanol) (Found: C, 77·0; H, 11·0. Calc. for $C_{32}H_{52}O_4$: C, 76·8; H, 10·5%), $\lambda_{max.}$ 218, 282 mμ (log ε 3·76, 3·96), $\lambda_{min.}$ 243 mμ (log ε 3·37). Methylation with an ethereal solution of diazomethane gave a methyl ether, m. p. 67—68° (from ethanol) (Found: C, 78·4; H, 11·4. Calc. for $C_{31}H_{52}O_3$: C, 78·8; H, 11·1).

Saponification of p-*Coumaric Esters.*—The ester mixture (0·87 g.) was heated under reflux for 3 hr. with 0·5N-ethanolic potassium hydroxide (25 c.c.). Addition of water to the cold solution precipitated a cream solid (0·415 g.), which was filtered off and dried. The filtrate was acidified with 2N-sulphuric acid and extracted with ether (3 × 50 c.c.). Evaporation of the dried (Na_2SO_4) ethereal extract gave a microcrystalline solid (0·19 g.), m. p. 205° (from water) undepressed on admixture with *p*-coumaric acid (Found: C, 65·6; H, 5·3. Calc. for $C_9H_8O_3$: C, 65·8; H, 4·9%). With ethereal diazomethane it gave methyl *p*-methoxycinnamate, m. p. 87—88° (lit.,[8] 89°).

The alcohol fraction of the saponification, m. p. 74—76°, was oxidized with chromic acid [7] to give a mixture of acids, m. p. 75—77°. Methylation of the acid mixture in 1 : 2 ether–methanol with ethereal diazomethane gave the methyl esters, m. p. 60° (Found: C, 78·6; H, 13·0. Calc. for $C_{25}H_{50}O_2$: C, 78·5; H, 13·2%). This was submitted to gas–liquid chromatography (Table 3) as detailed for the methyl esters. The retention times of these esters plotted on a log scale against carbon numbers gave a straight line.[6]

TABLE 3.

Composition of the alcohols obtained by saponification of the *p*-coumaric esters.

Conditions of chromatography as in Table 2.

Peak No.	Relative retention time (methyl stearate = 1)	Alcohols (as methyl esters)	Approx. quantity (%)
1	1·00	Octadecan-1-ol	trace
2	1·91	Eicosan-1-ol	8
3	3·71	Docosan-1-ol	24
4	7·36	Tetracosan-1-ol	31
5	14·00	Hexacosan-1-ol	trace
6	26·33	Octacosan-1-ol	trace

Chromatography of Remaining Mixture of Neutral Substances.—The material (C) (5·0 g.), eluted from the original chromatogram by ether, was dissolved in benzene, run on to a column of alumina (300 g.), and eluted in 200 c.c. aliquots with (a) 20 : 1 benzene–ether (1000 c.c.), fraction 1, (b) 10 : 1 benzene–ether (1000 c.c.), fraction 2, (c) 1 : 1 benzene–ether (100 c.c.), fraction 3, (d) 1 : 1 benzene–ether (200 c.c.), fraction 4, (e) ether (800 c.c.), fraction 5, and (f) 99 : 1 ether–methanol (500 ml.), fraction 6.

Fraction 1 (0·45 g.) was a gum which did not yield any crystalline material.

Fraction 2 (2·0 g.) had m. p. 77—78° (from ethanol). The infrared spectrum was identical with that of the alcohol fraction isolated by saponification of the aliphatic esters.

Fraction 3 (2·03 g.) contained β-sisosterol, m. p. and mixed m. p. 137° (after several crystallizations from ethanol), $[\alpha]_D^{20}$ −35·5° (*c* 1·742 in $CHCl_3$).

Fraction 4 (0·13 g.) was a *hydroxy-lactone* which crystallized from hexane–ethanol in tufts of fine needles, m. p. 278—279°, $[\alpha]_D^{20}$ −4° (*c* 0·75 in $CHCl_3$) (Found: C, 78·3; H, 11·2. $C_{30}H_{50}O_3$ requires C, 78·6; H, 10·9%), $\nu_{max.}$ 3350 (OH) and 1780 cm.$^{-1}$ (lactone).

Fraction 5 (0·09 g.) was an *ester* which formed plates, m. p. 320—325° (decomp.) (from ethyl acetate), $[\alpha]_D^{20}$ +43° (*c* 1·28 in $CHCl_3$) (Found: C, 74·7; H, 10·0; O, 15·6. $C_{31}H_{50}O_5$ requires C, 74·1; H, 10·0; O, 15·9%), $\nu_{max.}$ 3320 (OH), 1738 and 1232 cm.$^{-1}$ (ester).

Fraction 6 (0·2 g.) was dissolved in ether and run on to a column of alumina (6·0 g.). Elution with 199 : 1 ether–methanol (aliquots of 10 c.c.) gave a fraction (0·02 g.), m. p. 200—205°,

[8] Heilbron and Bunbury, "Dictionary of Organic Compounds," Eyre and Spottiswoode, London, 1953, p. 586.

which had m. p. 222—225° after several crystallizations from ethanol, $[\alpha]_D^{20}$ +25° (*c* 0·4 in $CHCl_3$) (Found: C, 80·9; H, 12·8. Calc. for $C_{30}H_{50}O_2$: C, 81·4; H, 11·4%), $\nu_{max.}$ 3350 (OH), 1640, and 887 cm.$^{-1}$ ($>C{:}CH_2$).

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