129. *Constituents of* Eugenia maire *A. Cunn. Part I. A Trimethyl Ether of Ellagic Acid and Mairin, a new Triterpene.*

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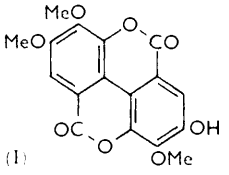
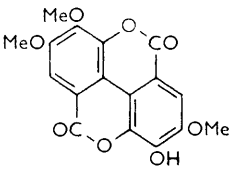
A new, naturally occurring, trimethyl ether of ellagic acid, β-sitosterol, and mairin, a new dihydroxytriterpene, have been isolated from the trunk bark of *Eugenia maire* A. Cunn. The ether has been identified as 3,3′,4-tri-*O*-methylellagic acid (I).

Eugenia maire A. Cunn. (Maori name, " Maire-tawake "; Fam. Myrtaceae) is a tree found in the lowland forests of New Zealand where it is endemic and the only member of the genus in the country. By extraction of the trunk bark with methanol a sparingly soluble phenol, m. p. 293—294°, was isolated in 0·64% yield. It was also isolated in lower yield (0·03%) from the trunk wood but was absent in both the root bark and wood. Analyses, determination of equivalent weight by potentiometric titration, and solubility in aqueous sodium carbonate agreed for a compound, $C_{17}H_{12}O_8$, possessing three methoxyl groups and an activated phenolic group but no carboxylic acid function. It formed a monoacetate, a monobenzoate, and a monomethyl ether, each of which was insoluble in cold alkali and showed no hydroxyl absorption in the infrared spectra. The absence of normal carboxyl or ester groups was shown by recovery of the phenol after attempted formation of ketonic derivatives and after prolonged treatment with alkali under mild conditions in the absence of oxygen. The presence of an αβ-unsaturated lactone was shown by a strong carbonyl band [1] at 1754 cm.$^{-1}$. In potentiometric titrations, however, the compound did not behave as a typical lactone and its similarity in this respect,[2-4] its ultraviolet spectrum, and its other properties [4] suggested a close relationship to ellagic acid.

The identification of its methyl ether as tetra-*O*-methylellagic acid showed that it was a trimethyl ether of ellagic acid, and this was confirmed by mild demethylation with concentrated sulphuric acid to give ellagic acid. Only two structures (I and II)



(I)



(II)

are possible for the trimethyl ether and, at the beginning of the investigation there was no record of it in the literature. Distinction between the structures was possible from the expected ionic behaviour of the free hydroxyl group in each case. The 3,3′-hydroxyl groups of ellagic acid have greater acidity than those in the 4,4′-positions,[2,4,5] and Jurd has shown it is possible to ionise, selectively, the two pairs of hydroxyl groups by the use of bases of varying strength, thus obtaining characteristic shifts of the absorption peaks of the ultraviolet spectrum. When the 3,3′-hydroxyl groups only are free a change of the short-wavelength peak occurs in the presence of sodium acetate. Ionisation of the 4,4′-hydroxyl groups, however, requiring a stronger base, occurs in the presence of sodium ethoxide which causes a bathochromic shift of the long-wavelength band. The spectrum of our phenol was unchanged in the presence of sodium acetate, strongly suggesting that

[1] Bellamy, " The Infra-red Spectra of Complex Molecules," Methuen and Co. Ltd., London, 2nd edn. 1958, p. 178.
[2] Schweitzer, *Collegium*, 1933, 149; *Chem. Abs.*, 1933, **27**, 3107.
[3] Sunthankar and Jatkar, *J. Indian Inst. Sci.*, 1938, **21**, *A*, 189.
[4] Hathway, *Nature*, 1956, **177**, 747.
[5] Jurd, *Chem. and Ind.*, 1959, 261; *J. Amer. Chem. Soc.*, 1959, **81**, 4610.

the free hydroxyl group was in the 4-position, as in (I). It was desirable, however, to distinguish between the possible structures by unequivocal synthesis.

During the investigation in this direction Jurd [6] prepared 3,3',4-tri-*O*-methylellagic acid (I) by an unambiguous synthesis. The recorded melting points for this compound (283°) and its acetate (251°) differed somewhat from those of our product (293—294°) and its acetate (264—265°), although spectral shifts [7] were identical for both compounds. 4-*O*-Acetyl-3,3',4-tri-*O*-methylellagic acid prepared by us by Jurd's method had, however, a melting point of 264°, identical with that of our acetate while a mixed-melting-point determination and comparison of the infrared spectra proved the identity. Deacetylation of the synthetic product led to 3,3',4-tri-*O*-methylellagic acid, m. p. 293—294°, identical with the natural phenol. Further, samples of 3,3',4-tri-*O*-methylellagic acid and its acetate kindly supplied by Dr. L. Jurd, possessed infrared spectra identical with those of the natural product and its acetate, respectively.

Bate-Smith has shown [8] that ellagic acid occurs in 42 families belonging to 15 of the 40 orders of dicotyledonous plants. The family Myrtaceae belongs to the group (Myrtiflorae) which exhibits the most systematic distribution, ellagic acid being found in all families and most of the species, where it is usually associated with myricetin and leucodelphinidin. Unexpectedly, neither of these two compounds was detected in the wood and bark of *Eugenia maire* when extracts were examined chromatographically,[4,8] nor was ellagic acid.

After separation of this phenol, β-sitosterol and a new triterpene, mairin, $C_{30}H_{48}O_3$, m. p. 295—296°, were isolated from the trunk-bark extract. Mairin is neutral and forms a diacetate and a dibenzoate, each of which shows no hydroxyl absorption in its infrared spectrum. It contains no carbonyl group so that the remaining oxygen atom must be present as an ether. Further structural work is in progress.

Experimental

Analyses were by Dr. A. D. Campbell and associates, University of Otago, New Zealand. Infrared spectra were measured for KBr discs with a Beckman IR2 instrument, and ultraviolet spectra for EtOH solutions with a Beckman DU instrument. Ultraviolet spectral shifts were measured by the method of Jurd and Horowitz.[9] Optical rotations were measured for $CHCl_3$ solutions, unless otherwise stated.

3,3,4-*Tri*-O-*methylellagic Acid* (*Natural*).—Ground, air-dried trunk bark (7·1 kg.) of *Eugenia maire* A. Cunn. was extracted (Soxhlet) in batches with methanol for 100—120 hr., fresh solvent being used after 40 hours' extraction. Separation of deposited solid from the initial extracts and from the concentrated mother-liquors gave crude *product.* That obtained from the second extract was less contaminated and was worked up separately. Repeated crystallization from ethylene glycol and then from dioxan gave colourless needles (total yield 46 g.), m. p. 293—294°, with slow sublimation from 288° [Found (sample crystallized from dimethylformamide): C, 59·25; H, 4·0; (sample crystallized * from ethylene glycol): C, 58·9; H, 4·0; OMe, 21·4; (sample crystallized * from dioxan; dried at 100°): C, 59·0, 59·4; H, 3·5, 3·2; OMe, 24·9, 25·4; (sample crystallized * from dioxan; dried at 150°): C, 59·2, 59·3, 59·0; H, 3·35, 3·5, 3·6; OMe, 25·4, 25·1. $C_{17}H_{12}O_8$ requires C, 59·3; H, 3·5; 3OMe, 27·5%],† $\lambda_{max.}$ 249 (log ε 4·64), 355 (sh), and 370 mμ (log ε 4·07), $\nu_{max.}$ 3436 (OH), 2985, 2899, 1462, 1359, 1245, 1175, 1129 (OMe [10]),

* For analysis samples were crystallized four times.

† In methylated ellagic acid and hexahydrobiphenic acid derivatives normal Zeisel determination has been found to give low results; this has been attributed to incomplete removal of methoxyl groups (Herzig and Pollak, *Monatsh.*, 1908, **29**, 263; Mayer, *Z. analyt. Chem.*, 1954, **141**, 345). Since our trimethylellagic acid is readily demethylated by concentrated sulphuric acid, incomplete removal of methoxyl groups is probably due to the observed insolubility rather than to stability of the O–methyl bond.

[6] Jurd, *J. Amer. Chem. Soc.*, 1959, **81**, 4606.
[7] Jurd, Palmer, Stitt, and Shoolery, *J. Amer. Chem. Soc.*, 1959, **81**, 4620.
[8] Bate-Smith, *Chem. and Ind. B.I.F. Review*, 1956, R32.
[9] Jurd and Horowitz, *J. Org. Chem.*, 1957, **22**, 1618; see also refs. 5 and 7.
[10] Briggs, Colebrook, Fales, and Wildman, *Analyt. Chem.*, 1957, **29**, 904.

1754 (lactone CO) cm.$^{-1}$. The single lactone peak and the lower frequency than that of ellagic acid (1695 cm.$^{-1}$) are in accord with the findings of Stitt, Gong, Palmer, and Schoolery [10a] for substituted ellagic acids.

Rapid potentiomeric titrations of a purified dioxan solution with 0·083N-aqueous potassium hydroxide, in an atmosphere of nitrogen, gave an equiv. wt. of 341 (average of five determinations ($C_{17}H_{12}O_8$ requires *M*, 344). Potentiometric back-titration in an atmosphere of nitrogen, with 0·062N-aqueous hydrogen chloride gave an equivalent weight of 340 (average of four determinations). In common with ellagic acid and its derivatives, trimethylellagic acid yields yellow solutions in alkali and, reacting in a tautomeric quinonoid form,[4] can be oxidised by air, resulting in further hydroxylation and intermolecular linking.[11] For this reason accurate results in potentiometric titrations can only be obtained by the exclusion of oxygen.

The freshly purified phenol became a very pale yellow on exposure to light and air. It is slowly soluble in methanol, ethanol, and acetone but freely soluble in pyridine, butan-1-ol, cyclohexanol, and phenol. It is insoluble in camphor (Rast) or camphorquinone. In pyridine solution it gives a brown colour with alcoholic ferric chloride.

Extraction of the ground aerial wood of *Eugenia maire* (200 g.) and working up of the methanol-insoluble portion of the extract gave the same material (60 mg.), m. p. and mixed m. p. 293—294° (identical infrared spectrum).

Acetylation with acetic anhydride–pyridine (100° for 8 hr. or 115° for 3 hr.; after shorter periods reaction was incomplete), acetic anhydride–sulphuric acid (100°, 1 hr.), or acetic anhydride–perchloric acid (room temp., ½ hr.) yielded a *monoacetate* which crystallized from dioxan or acetic anhydride as colourless needles, m. p. 264—265° (Found: C, 58·9, 59·2; H, 3·4, 3·6; OMe, 23·4; Ac, 12·6. $C_{19}H_{14}O_9$ requires C, 59·1; H, 3·65; 3OMe, 24·0; Ac, 11·15%), $\lambda_{max.}$ 248 (log ε 4·48), 344 (log ε 3·88), and 357 mμ (log ε 3·90), $\nu_{max.}$ 1736 (lactone CO), 1770 (acetyl CO) cm.$^{-1}$.

With benzoyl chloride–pyridine (100°, 2½ hr.) it gave an oil which after trituration with ether and crystallization from aqueous dioxan gave a *monobenzoate*, colourless needles, m. p. 254—255° (Found: C, 64·1, 64·0; H, 3·6, 3·6; Bz, 22·6. $C_{24}H_{16}O_9$ requires C, 64·3; H, 3·6; Bz, 23·5%).

Methylation. The natural product (500 mg.) in 2N-sodium hydroxide (10 c.c.) was shaken with dimethyl sulphate (1·5 c.c.) for 3 hr. at room temperature. Crystallization of the precipitated solid from ethylene glycol gave pale yellow needles (426 mg.) of the methyl ether, m. p. 341—342°. The same derivative was prepared by use of diazomethane in methanol–ether at 0° for 24 hr. (98% yield) and by prolonged heating (60 hr.) of a suspension in dry acetone with dimethyl sulphate and anhydrous potassium carbonate (95% yield). The m. p. was undepressed by a sample of tetra-*O*-methylellagic acid and the infrared spectra were identical (Found: C, 59·8, 59·7; H, 3·7, 3·75; OMe, 29·3. Calc. for $C_{18}H_{14}O_8$: C, 60·3; H, 3·9; 4OMe, 34·6%).

Demethylation. The natural product (500 mg.) was heated under reflux with concentrated sulphuric acid (10 c.c.) at 140° for 10 min. and the cooled solution poured into water. Crystallization of the flocculent precipitate from dimethylformamide gave a solvate of ellagic acid (410 mg.) as pale yellow needles, m. p. >360° [Found: C, 53·65; H, 4·5; OMe, 0. Calc. for $C_{14}H_6O_8(C_3H_7ON)_2$: C, 53·6; H, 5·0%], $\lambda_{max.}$ 255 (log ε 4·64), 352 (sh), and 366 mμ (log ε 4·03). The infrared spectrum was identical with that of an authentic sample crystallized from the same solvent. Acetylation with acetic anhydride–anhydrous sodium acetate (100°, ½ hr.) gave the tetra-acetate which crystallized as needles, m. p. and mixed m. p. 341—343° (Found: C, 56·2; H, 3·3. Calc. for $C_{22}H_{14}O_{12}$: C, 56·2; H, 3·0%). The retention of solvent is usual and has received notice by a number of workers.[12]

3,3′,4-*Tri*-O-*methylellagic Acid Monoacetate.*—Ellagic acid tetra-acetate was converted into ellagic acid diacetate, pale yellow needles, m. p. 327—330° (lit.,[6] m. p. 325—327°), by Jurd's method.[6] Methylation of a dry acetone suspension of the diacetate (2·0 g.) with dimethyl sulphate (4 c.c.) and anhydrous potassium carbonate (3·0 g.) for 3 hr. and working up by Jurd's method gave 4,4′-di-*O*-acetyl-3,3′-di-*O*-methylellagic acid (1·4 g.), m. p. 302—304° (lit.,[6] m. p. 304—305°), and, from the acetone solution, 4-*O*-acetyl-3,3′,4′-tri-*O*-methylellagic acid (120 mg.),

[10a] Stitt, Gong, Palmer, and Schoolery, *J. Amer. Chem. Soc.*, 1959, **81**, 4615.
[11] Feist and Bestehorn, *Arch. Pharm.*, 1925, **263**, 16.
[12] Goldschmidt, *Monatsh.*, 1905, **26**, 1139; Kunz-Krause, *Arch. Pharm.*, 1921, **259**, 193; Zetzsche and Graef, *Helv. Chim. Acta*, 1931, **14**, 240; Cambie, *New Zealand J. Sci.*, 1959, **2**, 257).

needles (from dioxan), m. p. 263—264°. The m. p. was undepressed on admixture with a sample prepared by Dr. L. Jurd (m. p. 251°) or by the acetate of the natural product (identical infrared spectra) (Found: C, 59·4; H, 3·7; Ac, 11·6. Calc. for $C_{19}H_{14}O_9$: C, 59·1; H, 3·65; Ac, 11·15%).

3,3′,4-*Tri*-O-*methylellagic Acid* (*Synthetic*).—Treatment of a suspension of the monoacetate (80 mg.) in 50% aqueous methanol (10 c.c.) with 5% aqueous potassium hydroxide (5 c.c.) by Jurd's method [6] gave 3,3′,4-tri-*O*-methylellagic acid (30 mg.), colourless needles, m. p. 293—294° after repeated crystallization from dioxan. The m. p. was undepressed on admixture with a sample prepared by Dr. L. Jurd (m. p. 283°) or by the natural product (identical infrared spectra).

β-*Sitosterol*.—The methanolic mother-liquors from the initial extract of the bark were reduced in volume, yielding a coloured solid and finally a black tar. The solid was extracted with benzene, leaving further tri-*O*-methylellagic acid as an insoluble residue, and the extract was chromatographed on activated alumina (P. Spence & Co., grade H). Crystallization from aqueous methanol of fractions eluted from the column with benzene gave plates of β-sitosterol (100 mg.), m. p. 135—136° (Found: C, 82·4; H, 12·4. Calc. for $C_{29}H_{50}O,\frac{1}{2}H_2O$: C, 82·2; H, 12·1%). The acetate, prepared by the use of acetic anhydride–pyridine (100°, 30 min.), had m. p. and mixed m. p. 124—125° (identical infrared spectra). Extraction of the black tar with ether and chromatography of the extract in benzene on alumina yielded further β-sitosterol (20 mg.).

Mairin.—The remaining tar was extracted repeatedly with cold 10% aqueous sodium carbonate which separated ellagitannins.[13] The alkali-insoluble residue, after repeated crystallization from methanol (charcoal) and separation from waxes was comprised mainly of long-chain esters, yielded long colourless needles, of *mairin* (5·0 g.), m. p. 295—296°, with sintering from 285°, $[\alpha]_D^{23}$ +10·6° (*c* 0·71), +10·65° (*c* 1·85 in pyridine) [Found: C, 77·2; H, 10·1. $C_{30}H_{48}O_3,\frac{1}{2}CH_3{\cdot}OH$ requires C, 77·3; H, 10·7. Found (for sample dried to constant wt.): C, 79·3; H, 10·5%; *M* (Rast), 453, 464. $C_{30}H_{48}O_3$ requires C, 78·9; H, 10·6%; *M*, 457], $\nu_{max.}$ 3584 (OH), 3472 (OH), 2959 (CH_3) cm.$^{-1}$.

Mairin gave a violet colour in the Liebermann–Burchard reaction but negative Zimmermann, Salkowski, and Rosenheim tests. It gave no colour with tetranitromethane, did not decolorize dilute potassium permanganate or bromine in chloroform, and was recovered after being heated under reflux with methanolic potassium hydroxide for 1 hr.

Mairin (150 mg.) with acetic anhydide–pyridine (100°, 1 hr.) gave a *diacetate* (110 mg.) which crystallized from aqueous methanol as thick plates, m. p. 289—290°, $[\alpha]_D^{23}$ +25·2 (*c* 1·11) (Found: C, 75·5; H, 9·7. $C_{34}H_{52}O_5$ requires C, 75·7; H, 9·2%).

Mairin (200 mg.) with benzoyl chloride–pyridine (100°, 1 hr.) gave an oil which was triturated with aqueous sodium hydrogen carbonate and then with light petroleum to yield a crystalline *dibenzoate*. Recrystallization from aqueous methanol–dioxan gave prisms (190 mg.), m. p. 331—333°, $[\alpha]_D^{23}$ +45·4° (*c* 1·24) (Found: C, 78·9, 78·2; H, 9·5, 8·9. $C_{42}H_{56}O_5$ requires C, 78·7; H, 8·8%).

We are indebted to Dr. L. Jurd for samples of 3,3′,4-tri-*O*-methylellagic acid and its acetate and to Dr. R. C. Cooper for identification and assistance in the collection of *Eugenia maire* samples. Assistance is gratefully acknowledged from the Chemical Society, the Rockefeller Foundation of New York, the Australian and New Zealand Association for the Advancement of Science, and the Research Grants Committee of the University of New Zealand.

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[13] Ware, *Analyst*, 1925, **50**, 384.