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Isolation, characterisation and synthesis of an insecticidal tetramethyltetrahydrochromenedione-spiro-bicyclo[3.1.1]cycloheptane from two species of Myrtaceae

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Abstract—A new moderately insecticidal compound, named ficifolidione, has been isolated from the hexane extract of the aerial parts of two species of Myrtaceae, *Eucalyptus ficifolia* and *Kunzea ericoides*. Its structure, (1′*R*,2′*R*,4*R*)-4-isobutyl- 6,6,8,8-tetramethyl-2,3,4,8-tetrahydrochromene-5,7-dione-2-spiro-2′-6,6-dimethyl-bicyclo[3.1.1]heptane (**1**), was confirmed by synthesis from syncarpic acid, isovaleraldehyde and (1*S*)-β-pinene.
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1. Introduction

During the search for insecticidal compounds in plants, we have chosen to explore the Myrtaceae family. Insecticidal activity has been found in several species, including *Kunzea ambigua* and *K. baxterii*,[1] *K. ericifolia*,[2] and *Callistemon viminalis*,[3] and the compounds responsible for the activity have been isolated and characterised. They all contain the tetramethylcyclohexenedione (or similar) moiety. Examination of two further species of Myrtaceae is reported here. Compounds with a range of biological activities have been isolated from many *Eucalyptus* species,[4] but they do not include *E. ficifolia* (red flowering gum). *K. ericoides* (kanuka) is the source of a commercial essential oil,[5] and has been shown to contain two tetramethylcyclohexenediones with antiviral activity.[6]

2. Results and discussion

Hexane extracts from *E. ficifolia* and *K. ericoides* were insecticidal. Fractionation by column chromatography with bioassay monitoring led to the isolation of the same compound in both cases. The structure was established from HRMS and NMR data (Table 1). The molecular ion peak at 386.28122 corresponds to $C_{25}H_{38}O_3$. Consideration of the ^{13}C NMR spectrum in conjunction with information from DEPT spectra showed a set of 10 peaks characteristic of the syncarpic acid-derived tetramethylcyclohexenedione system already observed in other products from species[1,2,5,6] of *Kunzea* i.e. four methyl groups at δ 22.3, 24.3, 25.5 and 26.3 on quaternary carbons at δ 48.0 and 55.4, two carbonyl groups at δ 197.9 and 213.5, and a tetrasubstituted ethylenic group (one of the substituents being oxygen) at δ 113.0 and 168.9. The remaining 15 carbon atoms included four methyl groups, two of which were doublets in the ^1H spectrum, correlated to a CH peak at δ 1.67, and therefore arising from a $CHMe_2$ group. The other methyl groups, singlets at δ 0.99 and 1.28, were therefore on a quaternary carbon. The remaining carbon atoms were present as $5CH_2$s, 3 aliphatic CHs, and 1 oxygenated quaternary C at δ 84.2. The COSY spectrum indicates two separate coupled systems. A CH_2 group (δ 2.12 and 1.43) is coupled to the CH at δ 2.70, which is in turn coupled to a second CH_2 group (δ 0.92 and 1.70). Both of these protons show additional couplings to the CH of the $CHMe_2$ group at δ 1.67. Other NMR data were essentially identical to those reported for the 9-carbon bicyclic spiro-attached group in the robustadials[7] from *E. robusta*.

These observations suggest that ficifolidione (**1**) is structurally related to robustadial B (**2**). Support for this carbon skeleton came from completing our NMR analysis with

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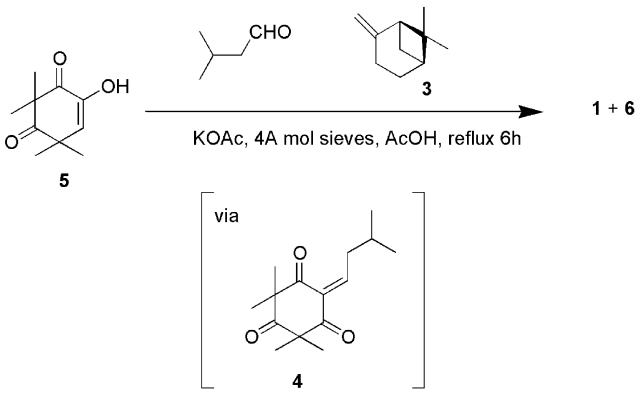
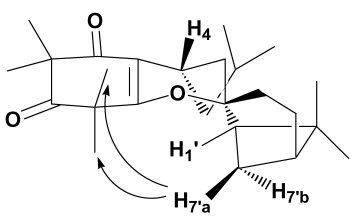
Table 1. ^{1}H and ^{13}C NMR data and HMBC correlations for ficifolidione (**1**)

No.	^{13}C δ_C	^{1}H δ (mult., J (Hz))	HMBC correlations from proton to carbon resonances
2	84.2 (s)	–	
3	39.6 (t)	1.43 (dd, 14.0, 8.8), 2.12 (dd, 6.6, 11.8)	113.0, 84.2, 51.3, 42.2, 113.0, 84.2, 51.3, 42.2
4	25.5 (d)	2.70 (ddd, 10.3, 8.8, 6.7, 3.5)	168.9, 113.0, 42.2
4a	113.0 (s)	–	
5	197.9 (s)	–	
6	55.4 (s)	–	
7	213.5 (s)	–	
8	48.0 (s)	–	
8a	168.9 (s)	–	
9	25.5 (q)	1.31 (s)	Overlaps H-12
10	24.3 (q)	1.33 (s)	213.5, 168.9, 48.0
11	26.3 (q)	1.28 (s)	213.5, 197.9, 55.4, 22.3
12	22.3 (q)	1.31 (s)	Overlaps H-9
1′	51.3 (d)	2.11 (t, 5.5)	40.4, 38.2, 39.6
3′	28.0 (t)	1.75–1.9 (m)	Overlaps H-4′
4′	24.9 (t)	1.75–1.9 (m)	Overlaps H-3′
5′	40.4 (d)	1.96 (m)	51.3, 38.2, 27.3
6′	38.2 (s)	–	
7′	26.9 (t)	1.58 (d, 10.2), 2.25 (ddt, 1.2, 10.2, 6.1)	84.2, 51.3, 40.4, 38.2, 24.9, 84.2, 51.3, 40.4, 38.2, 24.9
8′	23.8 (q)	0.99 (s)	51.3, 40.4, 38.2, 27.3
9′	27.3 (q)	1.28 (s)	51.3, 40.4, 38.2, 23.8
1″	42.2 (t)	0.92 (ddd, 13.1, 10.2, 3.9) 1.70 (m)	113.0, 113.0, 39.6
2″	25.6 (d)	1.67 (m)	42.2
3″	24.1 (q)	0.88 (d, 6.3)	42.2, 20.9
4″	20.9 (q)	0.96 (d, 6.3)	42.2



Scheme 1. Synthesis of compounds **1** and **5**.



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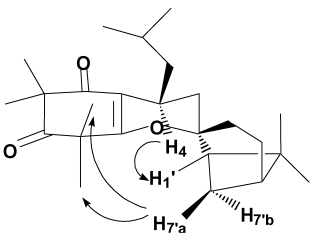
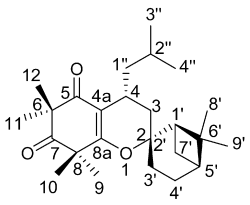


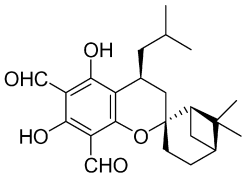
6

Figure 1. Key gradient NOE correlations observed for **1** and **5**.

gradient HMBC and gradient HMQC experiments, but stereochemistry was not determined at this stage.



1



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Confirmation of the proposed carbon skeleton was afforded by a successful synthesis (Scheme 1), using an analogous procedure to that used for robustadial (**2**).[8] (1*S*)-β-pinene (**3**) reacted with the condensation product (**4**) of syncarpic acid (**5**) and isovaleraldehyde formed in situ. In this one-pot Diels–Alder reaction, pinene (**3**) can react with the condensation product (**4**) in two distinct orientations and each can lead to two pairs of diastereomers. However, the isolation of only 2 out of the 8 possible compounds, indicates selectivity in the Diels–Alder reaction. The preferred regioselectivity arises from the oxygen of the oxabutadiene attacking the more substituted end of the pinene double bond. Stereoselectivity with respect to the spirocentre formation arises because the oxabutadiene can only approach one π-face of the double bond, away from the sterically obstructing dimethylated bridge of the pinene. The two products are epimeric at C-4 and arise from the two possible isobutyl group orientations when the single available dieneophile π-face attacks the diene. One of these two products had identical NMR spectra to those of ficifolidione.

Relative stereochemistry of the two Diels–Alder products was deduced by gradient NOE experiments (Fig. 1). Both **1** and **6** showed a NOE between the upfield H-7′ proton and both *gem*-dimethyl groups on C-8, demonstrating identical C-2 stereochemistry. The unnatural isomer **6**, however, also revealed a NOE between H-4 and H-1′. As the absolute stereochemistry of the β-pinene moiety is known, H-1′ is on the back face along with H-4 and this defines C-4 as *S*. The nature identical isomer therefore has the relative stereochemistry shown in structure **1**. The absolute stereochemistry follows from the optical rotation measurement. Both the natural and synthetic samples had positive rotations (albeit different in size due to different levels of purity) so the natural compound must also have the absolute stereochemistry of (1*S*)-β-pinene as shown in structure **1**.

Ficifolidione (**1**) is moderately insecticidal. The LD_{50} by topical application to the aphid, *Aphis fabae*, is 12 μg/insect, and to the thrips, *Thrips tabaci*, is 5.9 μg/insect. For the natural pyrethrum extract (25%) the corresponding values[1] are 3.8 and 7.9. In contrast to pyrethrum extract, ficifolidione was inactive towards the housefly, *Musca domestica*, and the mustard beetle, *Phaedon cochleariae*. A dose of 10 μg per insect caused 45% mortality of the third stadium larvae of the cabbage white, *Pieris brassica*. A dose of 0.2 μg/insect caused mortality 48, 30 and 26% after 48 h to *Aedes aegypti*, *Culex quinquefasciatus* and *Frankliniella occidentalis*, respectively.

3. Experimental

3.1. General

HPLC on SiO_2 used Gilson equipment and Dynamax 60A Si columns. EIMS were obtained using a VG Autospec mass spectrometer. ^1H and ^{13}C NMR spectra were measured with a Bruker AVANCE 500 MHz NMR spectrometer. Infrared spectra were recorded using a Nicolet Impact 410 FT-IR spectrometer and ultraviolet data was obtained using a Shimadzu UV-160A spectrophotometer. Optical rotations were recorded, using a known concentration of compound in chloroform, on a Thorn NPL143 polarimeter.

3.1.1. Plant material. Foliage from *E. ficifolia* F. Muell. (Accession no. 1980–2857) and *K. ericoides* (*A.Rich.*) J. Thompson (Accession no. 1993–2892) were collected from plants growing in the Royal Botanic Gardens, Kew. The identification of the plants was verified by Dr. E. Nielughada.

3.1.2. Bioassay. LD_{50}s were determined by established procedures[9,10] that involved topical application of micro-droplets of acetone solutions (5 different concentrations) to batches of insects (2 batches per concentration, 10 to 15 insects per batch), and assessment of mortality after 24 or 48 h.

3.1.3. Extraction and isolation. The ground, air-dried leaves and stems of *E. ficifolia* (300 g) were extracted with hexane (3×1 l). After evaporation of the solvent, the residue (5.5 g) was separated by column chromatography on SiO_2, (hexanes/diethyl ether 9:1). The biologically active fraction (0.67 g) was rechromatographed on SiO_2, eluting with (hexanes/diethyl ether 13:1) to give a biologically active fraction (0.06 g) which was further purified by HPLC on SiO_2, (hexane/ethyl acetate 15:1) to afford ficifolidione (**1**, 18 mg, 0.006%). The ground, air-dried leaves and stems of *K. ericoides* (50 g) extracted and purified similarly also afforded ficifolidione (**1**, 13 mg, 0.0026%) as a colourless oil: $[\alpha]_D$=+35.9° (*c*=0.92, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 2958, 2870, 1710, 1644, 1604, 1472, 1384; UV ($CHCl_3$, nm) λ_{max} 266; HRMS Obsd; *m/z* $[M]^+$ 386.28122. Calcd for $C_{25}H_{38}O_3$: 386.28210. ^1H and ^{13}C NMR spectra: as shown in Table 1.

3.1.4. Synthesis. Syncarpic acid[11] (0.2 g, 1.1 mmol), isovaleraldehyde (0, 19 g, 2.2 mmol), (1*S*)-(−)-β-pinene (0.6 g, 4.4 mol), potassium acetate (0.011 g, 0.11 mmol) and 4A molecular sieve (1 g) in acetic acid (4 ml) were heated under reflux for 6 h. On cooling, the solvent was removed under reduced pressure, dichloromethane (10 ml) was added, and the resultant mixture filtered and washed with water (10 ml) and solvent evaporated under reduced pressure. The product was purified by column chromatography on SiO_2 (hexanes/diethyl ether 10:1) to afford two fractions as colourless oils.

First fraction (0.12 g, 28%)(**1**): $[\alpha]_D^{25}$=+64.2 (*c*=1.81, $CHCl_3$) had spectroscopic data essentially identical to those of the natural product.

Second fraction (0.11 g, 26%)(**6**): $[\alpha]_D^{25}$=−103.4 (*c*=2.08, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 0.87 (3H, d, *J*=6.6 Hz, H-3″), 0.89 (1H, m, H-1″a), 0.93 (3H, d, *J*=6.3 Hz, H-4″), 0.96 (3H, s, H-8′), 1.22 (3H, s, H-9′), 1.24 (3H, s, C-11), 1.26 (3H, s, C-12), 1.29 (3H, s, C-9), 1.36 (3H, s, C-10), 1.47 (1H, d, *J*=9.5 Hz, H-7′a), 1.55 (1H, dd, *J*=14.3, 10.4 Hz, H-3a), 1.72 (1H, dsept, *J*=6.4, 4.1 Hz, H-2″), 1.75 (1H, ddd, *J*=11.0, 10.6, 3.3 Hz, H-1″b), 1.85–2.05 (4H, m, H_2-3′, H_2-4′), 1.95 (1H, m, H-5′), 2.10 (1H, m, H-7′b), 2.13 (1H, t, *J*=5.8 Hz, H-1′), 2.23 (1H, dd, *J*=14.3, 6.6 Hz, H-3b), 2.63 (1H, dddd, *J*=10.4, 10.1, 6.6, 3.3 Hz, H-4); ^{13}C NMR (100 MHz) δ 20.6 (q, C-4″), 22.7 (q, C-11), 23.2 (q, C-8′), 23.9 (q, C-10), 24.2 (q, C-3″), 24.7 (d, C-4′), 25.3 (t, C-2″), 25.9 (q, C-12), 26.1 (t, C-7′), 26.2 (q, C-9), 26.3 (d, C-4), 27.5 (q, C-9′), 30.4 (t, C-3′), 38.1 (s, C-6′), 39.1 (t, C-3), 40.5 (d, C-5′), 42.1 (t, C-1″), 46.7 (d, C-1′), 48.1 (s, C-8), 55.2 (s, C-6), 84.8 (s, C-2), 112.3 (s, C-4a), 169.6 (s, C-8a), 198.1 (s, C-5), 213.6 (s, C-7).

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