A New Insecticidal Pyranocyclohexenedione from *Kunzea ericifolia*

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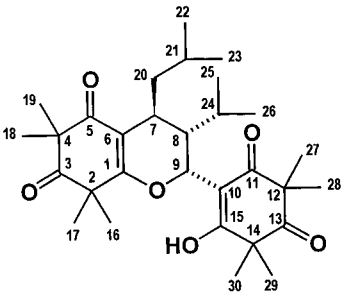
A new compound (**1**), named ericifolione, has been isolated from the hexane extract of the aerial parts of *Kunzea ericifolia* Rehb. (Myrtaceae) by bioassay-guided fractionation. It exhibits moderate insecticidal activity in comparison with natural pyrethrum extract.

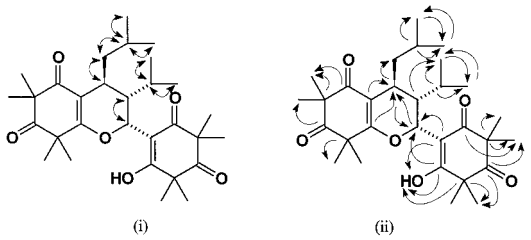
During the search for insecticidal compounds in plants, we have chosen to explore the Myrtaceae family. Of the species reported in a comprehensive review[1] as having pest control properties, less than 1% were from this family, but it is not clear whether this was due to the relative lack of attention to the family, or to the paucity of active compounds therefrom. In this study we report the isolation of a novel compound from *Kunzea ericifolia*, a shrub native to New Zealand. Bioassay-guided chromatography of the hexane extract of the leaves and stems, initially by flash chromatography and subsequently by HPLC on silica gel, led to the isolation of a novel compound, which we have named ericifolione (**1**). Its structure was determined mainly by NMR spectroscopy and by comparison with a compound previously isolated from *K. ericoides*.[2]



1

Ericifolione is insecticidal. LD_{50} values were determined by established procedures[3,4] that involved topical application of microdroplets of acetone solutions (5 different concentrations) to batches of insects (2 batches per concentration, 10−15 insects per batch), and assessment of mortality after 24 or 48 h. They were to houseflies, *Musca domestica*, 1.4 μg/insect, to the aphid, *Aphis fabae*, 5.2 μg/insect, and to the thrips, *Thrips tabaci*, 5.3 μg/insect. A dose of 10 μg killed 97% of mustard beetles, *Phaedon cochleariae*. In comparison, the corresponding figures for pyrethrum extract, an established botanical insecticide, are 0.01, 3.8, 7.9, and 0.3 μg/insect, respectively.

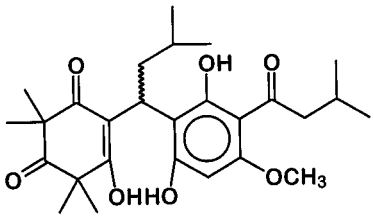
The establishment of its structure followed from HRMS and NMR data. The molecular ion peak at 500.3151 corresponds to $C_{30}H_{44}O_6$. Examination of the ^{13}C and ^{1}H NMR spectra in conjunction with information from DEPT spectra showed two sets of peaks, each of 10 atoms, suggesting the presence of two closely similar moieties i.e.,



(i) (ii)

Figure 1. Correlations observed for **1** by 2-D NMR. (i) H−H COSY. (ii) Long-range C−H COSY shown as C → H.

eight methyl groups at δ 21.3, 23.7, 24.5, 24.9, 25.0, 25.1, 25.4, and 26.7 ppm, four quaternary carbons at δ 47.3, 48.6, 55.2, and 56.2 ppm, four carbonyl groups at d 196.9, 197.8, 211.4, and 212.0, and two tetrasubstituted ethylenic groups (one of the substituents being oxygen in each case) at δ 107.1, 116.7, 167.4, and 175.0. These were thought to indicate the presence of two syncarpic acid-derived tetramethylcyclohexenedione systems of the type already observed in compound **2** (Figure 1), isolated from *K. ericoides*[2] and other plant species.[5] The peaks reported for



2

this system in compound **2** are at 48.6, 55.1 (q), 212.1, 203,1 (C═O), 114.7, 176.6 (C═C−O), and between 22.3 and 27.0 (Me groups). Slight differences between the two sets of 10 peaks observed for ericifolione showed that it was not a symmetrical dimer. The remaining 10 carbon atoms included four methyl groups, each a doublet in the ^{1}H spectrum, correlated to CH peaks at 1.74 or 1.87, and therefore arose from two $CHMe_2$ groups. Further correlation of one of these CH groups (that at 1.74) to a CH_2 group at 1.35, 1.40, and thence to a CH at 2.81 and of a CH group (at 5.55) only to the CH at 1.87 suggested structure **1**. The H−H COSY data alone were not fully conclusive because of the overlap between two CH peaks (both close to 1.87), but unequivocal confirmation of the structure as **1** came from the long-range C−H correlation data, summarized in Figure 1. This, in conjunction with the 1-bond C−H COSY information, also led to a complete assignment for all the peaks observed. A peak in the mass spectrum, at *m*/*z* 250,

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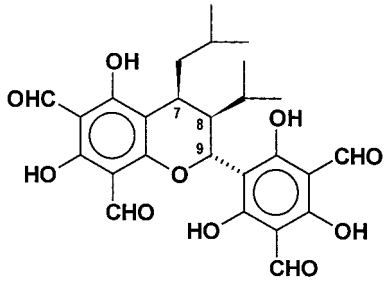
Table 1. Comparison of ^{1}H Signals (δ) for Compound **1** and the Sideroxylonals A and B

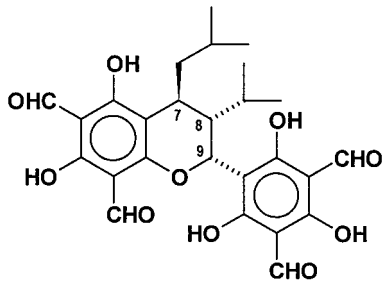
position	compound **1**	sideroxylonal A (**3**)	sideroxylonal B (**4**)
7	2.81, dd (8.3, 1.4)	3.38, dt (6.6, 3.7)	2.98, dd (10.3, 1.5)
8	1.87, m	2.40, ddd (11.7, 8.8, 3.7)	2.02, dd (2.2, 1.5)
9	5.55, d (2.5)	5.95, d (11.7)	5.92, d (1.5)

was consistent with the structure deduced, because it would be expected to arise by retro-Diels−Alder fragmentation of the dimer, involving cleavage of the 7−8 and O-9 bonds.

Structure **1** contains three chiral centers and has an observed optical activity ($[\alpha]_D$ −35.6°). Comparison with the NMR data for the related sideroxylonals A and B (**3** and **4**)[6] allows conclusions because the relative stereochemistry at these three centers has been established in these aromatic analogues. Thus, while the ^{1}H and ^{13}C shifts for the central regions of the molecule are generally similar, significant variations in the coupling constants between the central CH protons were observed when the stereochemistry at C-8 is inverted in the sideroxylonals (Table 1). Clearly, **1** has the same relative stereochemistry as sideroxylonal B but the absolute chemistry is yet to be elucidated.



Sideroxylonal A (**3**)



Sideroxylonal B (**4**)

Experimental Section

General Experimental Procedures. EIMS were obtained using a VG-Autospec. ^{1}H and ^{13}C NMR spectra were measured with a JEOL GX-400 spectrometer. C−H correlations were established using a waiting time of 1.9 ms (for 1-bond couplings) or 40 ms (for long-range couplings). H−H COSY and DEPT experiments used standard procedures. IR spectra were recorded using a Nicolet Impact 410 FT-IR spectrometer and ultraviolet data were obtained using a Shimadzu UV-160A spectrophotometer. HPLC was conducted using a Gilson system on Dynamax 60A columns with UV detection by a 1000S diode array detector (Applied Systems) set at 210, 230, 254, and 280 nm. Optical rotations were recorded, using a known concentration of compound in $CHCl_3$, on a Thorn NPL143 polarimeter. Bioassay procedures have been described previously.[3,4]

Plant Material. The foliage of *K. ericifolia* Rehb. was collected from plants growing in the Royal Botanic Gardens, Kew (accession no. 1993-1871). The plant was verified by E. NicLughadha.

Extraction and Isolation. The air-dried leaves and stems of *K. ericifolia* (100 g) were ground to a fine powder and extracted with hexane (3 × 1l). After evaporation of the solvent, the dark green residue (1.1 g) was chromatographed on silica gel, eluting with hexane−Et_2O (9:1). The biologically active fraction (0.15 g) was rechromatographed on silica gel, eluting with hexane−EtOAc (9:1) followed by HPLC also on silica gel eluting with hexane−EtOAc (9:1) to afford compound **1** (0.04 g, 0.04%).

Ericifolione 1: colorless oil; $[\alpha]_D$ −35.6° (*c* 0.16, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2962, 2928, 2872, 1706, 1628, 1466, 1382 cm^{-1}; UV ($CHCl_3$) λ_{max} 259 nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.01 (1H, s, OH), 5.55 (1H, d, J = 2.5 Hz, H-9), 2.81 (1H, dd, J = 8.3, 1.4 Hz, H-7), 1.87 (2H, m, H-8, H-24), 1.74 (1H, m, H-21), 1.52 (6H, s, H-18, H-29), 1.44 (3H, s, H-17), 1.40 (3H, s, H-28), 1.39 (6H, s, H-19, H-30), 1.35−1.40 (2H, m, J = 6.8 Hz, H-20), 1.35 (3H, s, H-27), 1.07 (3H, d, J = 6.4 Hz, H-23), 0.99 (3H, d, J = 6.8 Hz, H-22), 0.95 (3H, d, J = 6.8 Hz, H-25), 0.75 (3H, d, J = 6.8 Hz, H-26); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 212.0 (s, C-3), 211.4 (s, C-13), 197.8 (s, C-5), 196.9 (s, C-11), 175.0 (s, C-15), 167.4 (s, C-1), 116.7 (s, C-6), 107.1 (s, C-10), 79.4 (d, C-9), 56.2 (s, C-12), 55.2 (s, C-4), 48.6 (s, C-14), 47.3 (s, C-2), 44.7 (t, C-20), 41.4 (d, C-8), 27.2 (d, C-7), 26.7 (d, C-24), 26.7 (s, C-28), 25.4 (s, C-17), 25.3 (d, C-21), 25.1 (s, C-29), 25.0 (d, C-18), 24.9 (s, C-27), 24.5 (s, C-19), 24.3 (s, C-22), 23.8 (s, C-25), 23.7 (s) and 21.3 (s) (C-30 and C-16), 21.0 (s, C-23), 19.6 (s, C-26); EIMS *m*/*z* 500 $[M]^+$ (9), 487 (10), 458 (9), 457 (9), 443 (5), 305 (11), 275 (20), 250 (42), 236 (38), 70 (83), 43 (100); HRMS *m*/*z* 500.3151 (calcd for $C_{30}H_{44}O_6$ 500.3138);

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