New Insecticidal Tetradecahydroxanthenediones from *Callistemon viminalis*

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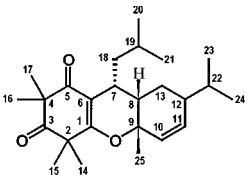
Received February 15, 1999

Two novel epimeric compounds, viminadione A (**1**) and viminadione B (**2**), have been isolated by bioassay-guided fractionation of the aerial parts of *Callistemon viminalis* and their structures (including relative stereochemistry at C-7, C-8, and C-9) elucidated by spectroscopic methods. Viminadione A (**1**) exhibits moderate insecticidal activity in comparison with natural pyrethrum extract. Viminadione B (**2**) is less active.

Continuing our search for insecticidal compounds,[1] we have examined *Callistemon viminalis* Sol. ex Gaertn. (Myrtaceae), a shrub from Australia. Bioassay-guided flash chromatography on Si gel, eluted with hexanes−Et_2O combinations, of the hexane extract of the leaves and stems led to the isolation of two compounds as pale yellow oils. They are previously unknown epimers, which we have named viminadione A (**1**) and viminadione B (**2**).



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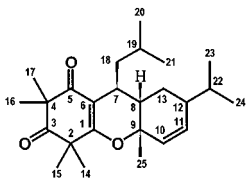

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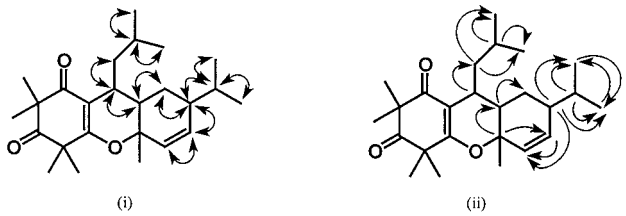
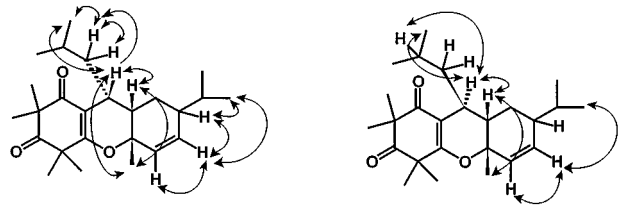
The structure of the more abundant compound (**1**) followed from HRMS and NMR data. The molecular ion peak at 386.2811 corresponds to $C_{25}H_{38}O_3$. Examination of the ^{13}C NMR spectrum in conjunction with information from the DEPT spectra showed a set of 10 peaks characteristic of the syncarpic acid-derived tetramethylcyclohexenedione system already observed in products from *Kunzea* species[1], that is, four methyl groups at δ 23.5, 23.8, 25.8, and 26.8 on quaternary carbons at δ 47.9 and 55.8; two carbonyl groups at δ 198.5 and 213.3; and a tetrasubstituted ethylenic group (one of the substituents being oxygen) at δ 110.2 and 166.9. The remaining 15 carbon atoms included five methyl groups, four of which (at δ 24.2, 21.5, 21.0, and 20.8) were doublets in the ^1H spectrum, correlated to CH peaks at 1.63 or 1.67, and therefore arising from two $CHMe_2$ groups. The remaining methyl group at δ 23.8, correlated to a singlet at 1.32, was therefore on the remaining quaternary carbon (at 76.2, indicating that it is bonded to oxygen). Further correlations of the CH groups, to a CH_2 group at δ 1.11, 1.97, and a CH group at 1.94 respectively, were indicated. The CH_2 was further coupled to a CH at δ 2.98, and thence apparently to another CH at δ 1.87. The remaining carbons were present as a CH_2, and a CH=CH group, suggesting the structure shown. Confirmation of structure **1** came from the long-range C−H correlation data (Figure 1). This, in conjunction with the one-bond C−H COSY information, led to complete assignments for all the peaks observed.



(i) (ii)

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



1 **2**

Figure 2. NOE correlations for **1** and **2**.

Essentially identical arguments established that the less abundant compound, viminadione B (**2**) is an isomer, differing only in the relative stereochemistry between the four chiral centers. The optical rotations ($[\alpha]_D$ +120.3° and −25.5°) for **1** and **2** are as expected for the proposed structures.

Difference NOE and NOESY studies on **1** and **2** (Figure 2) were carried out using benzene-d_6, and the important signals were well spaced. Compound **1** showed a NOE from H-25 to H-7 and weakly to H-8, and also from H-7 to H-8, suggesting that they are all on the same face. The carbon resonances of C-18 and C-13 are shifted upfield in **1** due to compression between them, which can be seen clearly in a model. Compound **2**, however, also showed a NOE between H-8 and both H-7 and H-25, but no effect between H-7 and H-25. This leads us to propose that H-7 is now on the opposite face to H-8 and H-25 but is still able to relax H-8. This configuration also releases the compression between C-13 and C-18, which are shifted downfield by 8.2 and 9.2 ppm, respectively. These considerations led to the relative stereochemistries (at centers 7, 8, and 9) shown for **1** and **2**. No data could be gained to elucidate the relative stereochemistry at C-12 for either compound, so synthetic studies are underway to verify the structures proposed and to determine total relative and absolute stereochemistry.

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10.1021/np990056a CCC: $18.00
Published on Web 11/10/1999

Both compounds exhibited insecticidal activity. LD_{50}s were determined by established procedures[2,3] involving topical application of microdroplets 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. For compound **1**, they were: to houseflies, *Musca domestica*, 1.9 μg/insect; to the aphid, *Aphis fabae*, 5.9 μg/insect; and to the thrips, *Thrips tabaci*, 4.2 μg/insect. Compound **2** was less active, killing only 60% of houseflies at a dose of 10 μg. In comparison, the corresponding figures for pyrethrum extract, an established botanical insecticide, are 0.01, 3.8, and 7.9 μg/insect.

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 one-bond couplings) or 40 ms (for long-range couplings). H−H COSY, DEPT, difference NOE, and NOESY experiments used standard procedures. IR spectra were recorded using a Nicolet Impact 410 FT-IR spectrometer, and UV data were obtained using a Shimadzu UV-160A spectrophotometer. Optical rotations were recorded, using a known concentration of compound in $CHCl_3$, on a Thorn NPL143 polarimeter. The reference sample of pyrethrum extract (pale oil containing 25% of natural pyrethrins) was a gift from AgrEvo Environmental Health, U. K.

Plant Material. Foliage of *Callistemon viminalis* Sol. ex Gaertn. was collected from plant material growing in the Royal Botanic Gardens, Kew (accession no. 1987-1503). The plant was verified by E. NicLughadha.

Extraction and Isolation. The air-dried leaves and stems of *C. viminalis* (1.2 kg) were extracted with hexane (4 × 1.5 l) at room temperature. After evaporation of the solvent, the dark green residue (12.7 g) was chromatographed on Si gel, eluting with hexanes−Et_2O (9:1). The active fraction (1.07 g) was rechromatographed on Si gel eluting with hexanes−Et_2O (19:1) to give two active fractions, the more polar of which (0.24 g) was further purified by flash chromatography on Si gel using hexanes−Et_2O (24:1). The insecticidal fraction (0.12 g, 0.01%) was characterized as viminadione A (**1**), a pale yellow oil, $[\alpha]_D$ +120.3° (*c* 1.12, $CHCl_3$); IR ($CHCl_3$) υ_{max} 3019, 2958, 2933, 2872, 1713, 1642, 1606, 1467, 1385 cm^{-1}; UV ($CHCl_3$) λ_{max} 262 nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.99 (1H, dd, J = 10.1, 3.7 Hz, H-11), 5.75 (1H, dd, J = 10.0, 1.8 Hz, H-10), 2.98 (1H, ddd, J = 11.6, 6.3, 3.5 Hz, H-7), 1.97 (1H, m, H-18), 1.94 (1H, m, H-12), 1.87 (1H, ddd, J = 15.2, 6.5, 3.0 Hz, H-8), 1.70 (1H, m, H-13a), 1.65 (1H, m, H-22), 1.64 (1H, m, H-19), 1.36 (3H, s, H-17^b), 1.32 (3H, s, H-25^a), 1.30 (1H, m, H-13b), 1.29 (3H, s, H-15^a), 1.28 (3H, s, H-16^b), 1.27 (3H, s, H-14), 1.11 (1H, td, J = 11.5, 2.5 Hz, H-18), 1.00 (3H, d, J = 6.4 Hz, H-21^c), 0.98 (3H, d, J = 6.4 Hz, H-23^c), 0.96 (3H, d, J = 6.7 Hz, H-24^c), 0.95 (3H, d, J = 6.7 Hz, H-20^c); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 213.3 (s, C-3), 198.5 (s, C-5), 166.9 (s, C-1), 135.2 (d, C-11), 130.8 (d, C-10), 110.2 (s, C-6), 76.2 (s, C-9), 55.8 (s, C-4), 47.9 (s, C-2), 41.2 (d, C-12), 35.2 (t, C-18), 33.6 (d, C-8), 31.5 (d, C-22), 28.2 (d, C-7), 26.8 (q, C-16^a), 25.8 (q, C-15^b), 24.6 (d, C-19), 24.2 (q, C-20^c), 23.8 (q, C-14), 23.5 (q, C-17^b), 21.6 (q, C-25^a), 21.5 (q, C-24^c), 21.0 (q, C-23^c), 20.8 (q, C-21^c), 20.7 (t, C-13); EIMS *m*/*z* 386 [M]$^+$ (35), 343 (18), 329 (30), 287 (8), 251 (23), 136 (47), 93 (100); HRMS *m*/*z* [M]$^+$ 386.2811 (calcd for $C_{25}H_{38}O_3$, 386.2821).

The less polar biologically active fraction (0.28 g) was further purified by flash chromatography on Si gel using hexanes−Et_2O (24:1). The insecticidal fraction (0.027 g, 0.0023%) was characterized as viminadione B (**2**), a pale yellow oil, $[\alpha]_D$ −25.5° (*c* 1.32, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3031, 2965, 2937, 2871, 1718, 1612, 1471, 1389 cm^{-1}; UV ($CHCl_3$) λ_{max} 264 nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.84 (1H, dd, J = 10.2, 3.9 Hz, H-11), 5.50 (1H, dd, J = 10.2, 2.2 Hz, H-10), 2.56 (1H, dt, J = 9.9, 3.3 Hz, H-7), 2.01 (2H, m, H-8, H-12), 1.76 (1H, m, H-19), 1.67 (1H, m, H-22), 1.60 (2H, m, H-13), 1.46 (3H, s, H-25), 1.45 (1H, m, H-18a), 1.38 (3H, s, H-14), 1.35 (1H, m, H-18b), 1.33 (9H, s, H-15, H-16, H-17), 1.00 (3H, d, J = 6.6 Hz, H-20), 0.95 (3H, d, J = 6.6 Hz, H-23), 0.92 (3H, d, J = 6.6 Hz, H-24), 0.90 (3H, d, J = 6.3 Hz, H-21); ^{13}C NMR δ 213.5 (s,C-3), 198.1 (s, C-5), 168.6 (s, C-1), 134.6 (d, C-11), 131.0 (d, C-10), 113.0 (s, C-6), 77.7 (s, C-9), 55.6 (s, C-4), 47.5 (s, C-2), 44.0 (t, C-18), 39.5 (d, C-12), 38.3 (d, C-8), 31.7 (d, C-7), 31.6 (d, C-22), 29.1 (t, C-13), 27.4 (q, C-25), 26.1 (d, C-19), 25.3 (q, C-17^a), 25.0 (q, C-15^a), 24.7 (q, C-14), 24.0 (q, C-21), 23.5 (q, C-16^a), 21.5 (q, C-20), 20.4 (q, C-23), 20.3 (q, C-24); EIMS *m*/*z* 386 [M]$^+$ (19), 343 (13), 329 (33), 287 (8), 251 (23), 136 (69), 93 (100); HMRS *m*/*z* [M]$^+$ 386.2819 (calcd for $C_{25}H_{38}O_3$, 386.2821).

Acknowledgment. The authors thank S. Maniar, M. Hedges, and J. Orgill for technical assistance. The study received financial support from BTG International Ltd., IACR−Rothamsted, and RBG, Kew, received grant-aided support from the Biotechnology and Biological Sciences Research Council and the Ministry of Agriculture, Food and Fisheries UK, respectively.

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NP990056A