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An insecticidal mixture of tetramethylcyclohexenedione isomers from *Kunzea ambigua* and *Kunzea baxterii*

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Abstract

A mixture of isomers, all 4-[1-(5,7-dihydroxy-6-methyl-4-oxo-2-phenyl-chroman-8-yl)-3-methyl-butyl]-5-hydroxy-2,2,6,6-tetramethyl-cyclohex-4-en-1,3-diones, which comprises a pair of epimers, each of which is a pair of conformers, has been isolated from the hexane extract of the aerial parts of *Kunzea ambigua* and *K. baxterii* (Myrtaceae). The mixture exhibits moderate insecticidal activity in comparison with natural pyrethrum extract. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Myrtaceae; Kunzea; Tetramethylcyclohexenedione; Flavanone; Insecticide

1. Introduction

During the search for insecticidal compounds in plants, we have chosen to explore the Myrtaceae family. Of the species reported in a comprehensive review (Grainge and Ahmed, 1988) 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. The *Kunzea* genus is endemic to Australasia and is recognised mainly for antimicrobial activity, e.g. Kanuka oil ((Porter and Wilkins, 1998). In addition, tetramethylcyclohexenediones have been isolated from various species of this genus (Bloor, 1992; Khambay et al., 1999b, c) and we have recently shown them to be insecticidal. In this study, we have examined two further species of *Kunzea*, *K. ambigua* (Sm) Druce and *K. baxterii* Schauer, and shown that they contain structurally related insecticidal compounds.

2. Results and discussion

Bioassay-guided fractionation on silica gel eluted with hexane–Et_2O combinations, of the hexane extract of the leaves and stems resulted in the isolation of a mixture (**1**).

Structures were determined from HRMS and NMR data. The molecular ion peak at 500.2461 corresponds to $C_{31}H_{36}O_7$. Examination of the ^{13}C (Table 1) and ^{1}H NMR spectra in conjunction with information from DEPT spectra showed four sets of peaks with relative intensities indicating four contributing components in 10:5:4:3 ratio. The ^{1}H NMR spectrum for the major component exhibited three broad singlets at δ 13.97, 11.77 and 9.91 attributed to three acidic hydroxyl groups. Peaks corresponding to five protons in the aromatic region δ 7.41–7.47 were observed, along with seven methyl peaks, five of which were singlets (δ 2.07, 1.50, 1.41 and 1.35) and two doublets (δ 0.87 and 0.86). In addition there were seven multiplets (δ 5.43, 4.33, 3.03, 2.89, 2.07, 1.80 and 1.41). The ^{13}C NMR data for the major component showed peaks characteristic of the syncarpic acid-derived tetramethylcyclohexenedione system already observed in other products from *Kunzea* species (Bloor, 1992; Khambay et al., 1999 b,c), that is: four methyl groups at δ 27.1, 26.2, 24.2 and 22.1; on quaternary carbons at δ 55.2 and 48.6; two carbonyl groups at δ 212.0, 203.3; and one tetrasubstituted ethylenic group (one of the substituents being oxygen) at δ

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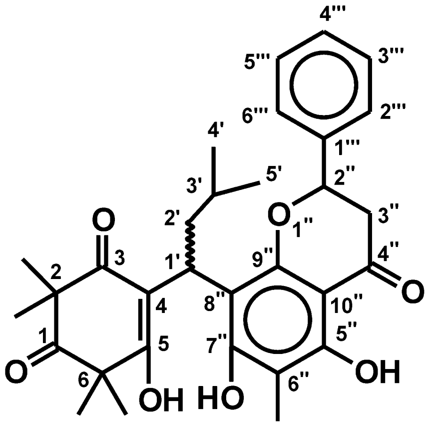

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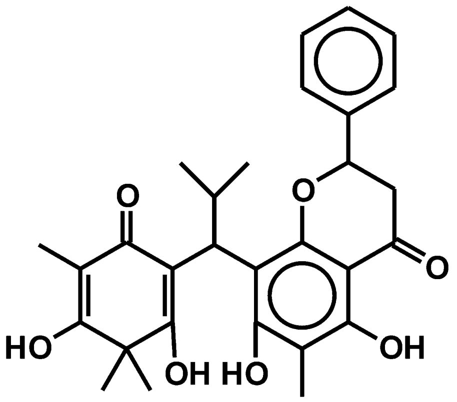

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Table 1
^{13}C NMR data for **1** (the four isomers) in $CDCl_3$ (100 MHz, δ values)[a]

Atom	Isomer 1 (45%)	Isomer 2 (23%)	Isomer 3 (18%)	Isomer 4 (14%)
1	212.0	212.4	Obs	Obs
2	55.2	54.3	Obs	Obs
2-Me-a	22.1	Obs	Obs	Obs
2-Me-b	27.1	27.2	27.0	27.0
3	203.3	203.2	203.4	Obs
4	114.5	114.6	114.7	114.7
5	176.1	176.0	176.7	176.8
6	48.6	49.0	Obs	Obs
6-Me-a	26.2	26.1	26.0	25.9
6-Me-b	24.2	24.3	23.8	24.0
1′	27.6	29.7	27.5	29.3
2′	38.1	37.9	39.0	38.8
3′	26.8	Obs	Obs	Obs
4′	22.6	Obs	Obs	Obs
5′	22.3	Obs[b]	Obs	Obs
2″	78.5	78.6	Obs	Obs
3″	42.9	42.9	42.9	42.9
4″	196.5	196.6	197.1	197.0
5″	158.7	158.6	Obs	Obs
6″	106.8	107.1	107.0	106.6
6″-Me	7.9	8.0	8.3	8.2
7″	164.9	164.7	164.2	164.5
8″	108.6	108.7	Obs	Obs
9″	156.7	157.6	Obs	Obs
10″	101.5	101.6	102.1	101.9
1‴	138.8	138.7	138.7	138.6
2‴, 6‴	128.8	Obs	Obs	Obs
3‴, 5‴	125.8	Obs	Obs	Obs
4‴	128.6	Obs	Obs	Obs

[a] The relative amounts of the four isomers are indicated in the headings.

[b] Obs—denotes peak obscured.

176.1 and 114.5. Peaks at δ 38.1, 27.6, 26.8, 22.6 and 22.3 suggested an *iso*-butyl moiety. Aromatic peaks at δ 164.9, 158.7, 156.7, 108.6, 106.8 and 101.5 corresponded to a 2,4,6-trialkyloxyphenyl group, and peaks at δ 138.8, 128.8 (2C), 128.6 (2C) and 125.8 corresponded to a phenyl ring. Remaining peaks at δ 196.5, 78.5 and 42.9 are typical of the central ring of flavanones, suggesting the structure shown. The ^{13}C data (Table 1) indicate that (**1**) consists of four closely related components. Other mixtures with similar structures (Tsui and Brown, 1996; Khambay et al., 1999c) have been isolated as mixtures of C-1′ epimers. In addition, when hydrogen bonding between the 3-CO and the 7″-OH can occur, the two possible conformers are each sufficiently stable to be separately observable in the NMR spectrum. The situation for (**1**) with four isomers is therefore parallel to that for the metabolite (**2**) isolated from *Baeckea frutescens* (Tsui and Brown, 1996).

The absolute stereochemistry at C-2″ was not elucidated. However, the moiety containing C-2″ is clearly of flavone origin and flavanones have been found in general to have *S* stereochemistry at the centre in question (Harborne and Williams, 1998).

Mixture **1** is insecticidal. The LD_{50} by topical application to mustard beetles, *Phaedon cochleariae*, is 1.0 μg/insect, to the aphid, *Aphis fabae* it is 3.9 μg/insect, and to the thrips, *Thrips tabaci* 15 μg/insect. A dose of 10 μg per insect caused 83% mortality of houseflies, *Musca domestica*. In comparison, the corresponding figures. for commercial pyrethrum extract (25%), an established botanical insecticide, are 0.3, 3.8, 7.9 and 0.01 μg/insect, respectively.

3. Experimental

3.1. General

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 and the DEPT experiment used a standard procedure. Infrared spectra were recorded using a Nicolet Impact 410 FT-IR spectrometer and ultraviolet data were obtained using a Shimadzu UV-160A spectrophotometer. Bioassay procedures have been described previously (Elliott et al., 1987; Khambay et al., 1999a). They involve topical application of droplets containing a range of concentrations of test material in acetone using a microsyringe to adult insects (10 or more insects×2 replicates per concentration). Mortality was assessed 24, 48 or 72 h later, and LD_{50}s were computed using probit analysis.

3.2. Plant material

Foliage from *K. ambigua* (Accession No.1944-23201) and *K. baxterii* (Accession No. 1993-28911) was collected from plants growing in the Royal Botanic Gardens, Kew. Identification was verified by E. Nielughada.

3.3. Extraction and isolation

The ground air-dried leaves and stems of *K. ambigua* (36 g) were extracted with hexane (3×500 ml) at room temperature to give a dark green residue (0.77 g) which was chromatographed on silica gel eluting with petrol ether–diethyl ether (7:3). The biologically active fraction (0.12 g) was subjected to preparative TLC on silica gel eluting with petrol ether–diethyl ether (1:1). This gave a fraction exhibiting biological activity (20 mg) which was further purified by flash column chromatography on silica gel using petrol ether–diethyl ether (1:1) to afford mixture **1,** (16 mg, 0.044%). Ground air-dried leaves and stems of *Kunzea baxterii* (215 g) similarly extracted with hexane and chromatographed also afforded mixture **1** (0.18 g, 0.084%) containing similar proportions of the four components.

(1): light brown oil; $[\alpha]_D^{25}$ −55.4° (*c* 3.24 in $CDCl_3$); IR ($CHCl_3$) ν_{max} 3029, 2981, 2654, 2870, 1710, 1627, 1468, 1384 cm^{-1}; UV ($CHCl_3$) λ_{max} 299.4, 241.8 nm; ^1H NMR ($CDCl_3$, 400 MHz) (peaks listed are for the major component) δ 13.97 (1H, *s*, OH-5), 11.77 (1H, *s*, OH-7″), 9.91 (1H, *s*, OH-5″), 7.42–7.45 (5H, *m*, H-2‴, H-3‴, H-4‴, H-5‴ H-6‴), 5.43 (1H, *dd, J*=12.7, 2.9 Hz, H-2″), 4.33 (1H, *t*, *J*=7.6 Hz, H-1′), 3.03 (1H, *dd, J*=17.1, 12.7 Hz, H-3″a), 2.89 (1H, *dd, J*=17.6, ca 3 (obs) Hz, H-3″b), 2.07 (3H, *s*, Me-6″), 2.07 (1H, *m*, H-2′a), 1.80 (1H, *m*, H-2′b), 1.50 (3H, *s*, Me-6), 1.41 (1H, *m*, H-3′), 1.41 (3H, *s*, Me-2), 1.35 (3H, *s*, Me-6), 1.34 (3H, *s*, Me-2), 0.87 (3H, *d, J*=6.3 Hz, Me-3′), 0.86 (3H, *d, J*=5.9 Hz, Me-3′); ^{13}C NMR peaks for the 4 components are listed in Table 1; EIMS *m/z* 520 $[M]^+$ (5), 445 (10), 338 (20), 323 (13), 283 (17), 270 (100), 250 (19), 193 (40), 179 (50), 166 (50), 138 (60), 70 (90), 41 (80); HRMS *m/z* 520.2461 (calc. for $C_{31}H_{36}O_7$ 520.2461).

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References

Bloor, S.J., 1992. Antiviral phloroglucinols from New Zealand *Kunzea* species. Journal of Natural Products 55, 43–47.

Elliott, M., Farnham, A.W., Janes, N.F., Johnson, D.M., Pulman, D.A., 1987. Synthesis and insecticidal activity of lipophilic amides. 1. Introductory survey and discovery of an active synthetic compound. Pesticide Science 18, 191–201.

Grainge, M., Ahmed, S., 1988. Handbook of Plants with Pest-Control Properties. John Wiley & Sons, New York.

Harborne, J.B., Williams, C.A., 1998. Anthocyanins and other flavonoids. Natural Product Reports 15, 631–652.

Khambay, B.P.S., Batty, D., Cahill, M., Denholm, I., Mead-Briggs, M., Vinall, S., Niemeyer, H.M., Simmonds, M.S.J., 1999a. Isolation, characterization and biological activity of naphthoquinones from *Calceolaria andina* L. Journal of Agriculture and Food Chemistry 47, 770–775.

Khambay, B.P.S., Beddie, D.G., Hooper, A.M., Simmonds, M.S.J., Green, P.W.C., 1999b. New insecticidal tetradecahydroxanthenediones from *Callistemon viminalis*. Journal of Natural Products 62, 1666–1667.

Khambay, B.P.S., Beddie, D.G., Simmonds, M.S.J., Green, P.W.C., 1999c. A new insecticidal pyranocyclohexenedione from *Kunzea ericifolia*. Journal of Natural Products 62, 1423–1424.

Porter, N.G., Wilkins, A.L., 1998. Chemical, physical and antimicrobial properties of essential oils of *Leptospermum scoparium* and *Kunzea ericoides*. Phytochemistry 50, 407–415.

Tsui, W.Y., Brown, G.D., 1996. Unusual metabolites of *Baeckea frutescens*. Tetrahedron 52, 9735–9742.