Antiviral Phloroglucinols from New Zealand Kunzea Species

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ANTIVIRAL PHLOROGLUCINOLS FROM NEW ZEALAND *KUNZEA* SPECIES

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ABSTRACT.—Four acyl-phloroglucinol derivatives showing antiviral activity have been isolated from *Kunzea sinclairii* and *Kunzea ericoides* (Myrtaceae) from New Zealand. The structures of these compounds were deduced from analysis of spectral data. Two of these compounds, **1** and **2**, are the isomers of isobutyryl methoxyresorcinol. The two new compounds, **3** and **4**, were isolated as a mixture and determined to be 4-cyclohexene-1,3-dioxo-5-hydroxy-2,2,6,6-tetramethyl-4- { 1-[2,6-dihydroxy-4-methoxy-3-(3-methyl-1-oxobutyl)phenyl]-3-methylbutyl } and its 2-methyl-1-oxopropyl analogue, respectively.

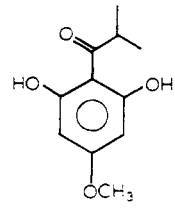
The genus *Kunzea* (Myrtaceae) is represented in New Zealand by two species: *Kunzea ericoides* (A. Rich) Thompson (kanuka) and *Kunzea sinclairii* (Kirk) W. Harris. While the former is a common shrub or small tree, the latter is a low-growing shrub, naturally present only on Great Barrier Island. Various biological activities have been reported for extracts from members of the Myrtaceae family (1), so it was not surprising to discover that extracts of both of these *Kunzea* species demonstrated in vitro antiviral activity in our New Zealand plant screening program.

Bioassay-guided fractionation has led to the isolation and structural identification of the compounds responsible for the activity in both species. An initial partitioning of the aqueous EtOH extract between hexanes and aqueous MeOH yielded two active fractions. The strongly active polar fraction from *K. sinclairii* was investigated first, and the activity was revealed to be associated with two compounds which were isolated by reversed-phase hplc from a fraction dominated by the presence of a series of simple flavonoids. The two active compounds were identified from nmr spectral data to be the two isomers **1** and **2** of isobutyryl methoxyresorcinol. One of these compounds, robustaol B [**2**], has been reported previously from *Eucalyptus robusta* (2), and this class of substituted phloroglucinols has been previously reported as antiviral agents (3). The proof of structure and activity was achieved by synthesis of the isomers from 5-methoxyresorcinol and isobutyrylnitrile using the Hoesch reaction. These two compounds were also present in the extract of *K. ericoides*.

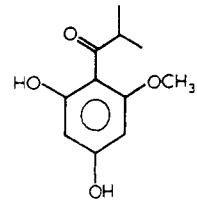
Investigation of the non-polar fraction from *K. ericoides* revealed the activity to be associated with a readily isolable yellow crystalline material (mp 129–132°). Despite the appearance of this material, the nmr and ms (see Table 1 and Experimental) indicated the presence of two compounds in a 4:1 ratio. Efforts directed towards purification of the material, including reversed-phase hplc using a variety of solvent mixtures, were unsuccessful. However, once the general nature of the compounds was determined, the presence of both compounds did not affect the structural elucidation. The two compounds, **3** and **4** could be recognized as phenolic (tlc spray with anisaldehyde) and containing a β-triketone group (^{13}C and ^{1}H nmr).

A comparison of the spectral data for **3** and **4** with those of other phenolic compounds from the Myrtaceae revealed the similarity between these compounds and myrtucommulone A, which was isolated from the common Eurasian shrub, *Myrtus communis* by Kashman *et al.* (4). The paper outlining the structure determination of myrtucommulone A was most helpful in this work.

The major compound, **3**, has a molecular formula of $C_{27}H_{38}O_7$ (hreims found 474.2616, calcd 474.2617). As previously noted, the presence of a β-triketone moiety was suggested by the ^{1}H- and ^{13}C-nmr spectra and comparison of the ^{13}C-nmr shifts



1



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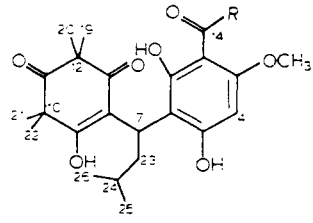
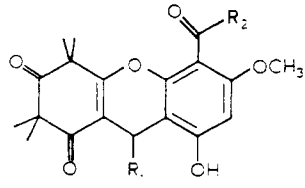
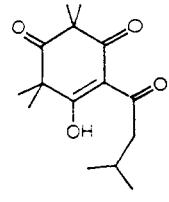


3 R=-$CH_2CH(Me)_2$
4 R=-$CH(Me)_2$





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5 $R_1=R_2$=-$CH_2CH(Me)_2$
6 R_1=-$CH_2CH(Me)_2$, R_2=-$CH(Me)_2$
7 $R_1=R_2$=-$CH(Me)_2$

with syncarpic acid (5), and the syncarpic acid moiety of uvafzelin (6) confirmed the β-triketone in **3** to be a syncarpic acid unit. It is of note that the essential oil of *K. ericoides* contains leptospermone [**8**] (7), which also has a syncarpic acid unit. The six other signals in the aromatic region of the ^{13}C-nmr spectrum (five quaternary carbons, one CH) could be readily assigned to a di-C-substituted phloroglucinol moiety. The rest of the structure could be determined from analysis of ^{1}H-^{1}H COSY nmr spectra run in $CDCl_3$ or C_6D_6. The latter solvent enabled many of the signals due to the major and minor components of this mixture to be separated. A 1H triplet (4.39 ppm, shifts given in $CDCl_3$) was coupled to a pair of methylene protons (2.20, 1.80 ppm) which showed a further coupling pattern consistent with their being adjacent to an isopropyl group. A second isopropyl group was also present, again coupled to another methylene group (2.89 ppm, d, 2H). The chemical shift of this second methylene group indicated it was adjacent to a carbonyl group. The combination of these partial structures then logically leads to the structure shown for **3**. The CH linked to the isobutyl group forms the connection between the syncarpic acid and phloroglucinol groups, and the isopentanoyl group is the second C-substituent of the phloroglucinol. In the mass spectrum, the loss of syncarpic acid to give a peak at 292 mu ($C_{17}H_{24}O_4$) confirmed the placement of the MeO group on the phloroglucinol. The mass spectral fragmentation pattern is very similar to that observed for myrtoucommulone A. Thus, **3** is 4-cyclohexene-1,3-dioxo-5-hydroxy-2,2,6,6-tetramethyl-4-{1-[2,6-dihydroxy-4-methoxy-3-(3-methyl-1-oxobutyl)phenyl]-3-methylbutyl}.

TABLE 1. ^{1}H-nmr Data for Compounds **3** and **5**.[a]

Proton	Compound	
	3	**5**[b]
H-4	6.04 s	6.26 s
H-7	4.39 t (7.3)	4.23 t
H-15	2.89 d (6.3) (2H)	2.72 AB (2H)
H-16	2.22 m	2.25 m
H-17, -18	0.99 d (6.6 6H)	1.00 d 6.7
		0.98 d 6.7
H-19 to H-22	1.33 s (6H)	1.50 s (3H)
	1.40 s (3H)	1.43 s (3H)
	1.49 s (3H)	1.39 s (3H)
		1.37 s (3H)
H-23	1.80 m	1.4 (obsc)
	2.15 m	
H-24	1.40 m	1.4 (obsc)
H-25, -26	0.85 m (6H)	0.86 d 6.2
		0.83 d 6.2
OMe	3.87 s (3H)	
OH	10.40 s	
	11.60 s	
	15.25 s	

[a]300 MHz, $CDCl_3$.
[b]For clarity the same numbering system is used for **5** and **3**.

The identification of the minor compound, **4**, was possible from the ms of the mixture. A peak at 460 mu corresponded to **3** with one less methylene group giving a molecular formula for **4** of $C_{26}H_{36}O_7$ (found 460.2461, calcd 460.2461). A small ion at 385 mu corresponded to the loss of H_2O and an isobutyl group from the molecular ion, thus indicating that the acyl substituent on the phloroglucinol ring in **4** is an isobutyryl group.

This was confirmed by analysis of the minor signals in the ^{1}H-nmr spectrum of the mixture measured in C_6D_6. A septet at 3.50 ppm was coupled only to a broad methyl resonance at 1.10 ppm. The chemical shift of the former signal places the proton adjacent to a carbonyl; therefore the methyl is part of an isobutyryl group. The remaining signals due to **4**, although showing minor variation in chemical shift, have the same coupling pattern as that described for **3**.

Thus, **4** is 4-cyclohexene-1,3-dioxo-5-hydroxy-2,2,6,6-tetramethyl-4-{1-[2,6-dihydroxy-4-methoxy-3-(2-methyl-1-oxopropyl)phenyl]-3-methylbutyl}.

Treatment of the mixture of **3** and **4** with *p*-TsOH in refluxing C_6H_6 yielded two compounds separable by reversed-phase hplc. Compound **5**, the dehydration product of **3**, has a simplified ms with a base peak at 399 mu, while compound **6** is the dehydration product of **4** with a base peak at 385 mu. Clearly the loss of the substituent at the carbon bridging the syncarpic acid and phloroglucinol units is highly favored. Compounds **5** and **6** are analogues of myrtucommulone B [7].

The placement of the MeO substituent at C-3 in **3–6** is based upon the absence of the distinctive H-bonded phenolic absorption in the ^{1}H-nmr spectra of **5** and **6**. The free hydroxyl at C-5 cannot interact with a carbonyl group and is seen as a broad signal at 6.0 ppm more typical of a free phenol.

Compound **4** can be considered to be a condensation product of leptospermone [8] and robustaol B [2]. The observed antiviral activity of **3** and **4** can be attributed to the acyl phloroglucinol part of the larger molecules.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Reversed-phase cc and Si gel chromatography were performed according to Blunt *et al.* (8) and Coll and Bowden (9), respectively. Nmr spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C on a Bruker AC300 spectrometer. Ms was recorded on a VG70-250S mass spectrometer. Mp's are uncorrected. Voucher specimens of plants sampled have been deposited in the DSIR Herbarium, Lincoln, New Zealand (CHR).

EXTRACTION AND ISOLATION OF **1** AND **2**.—*K. sinclairii* leaves and twigs (82 g), collected from experimental plots at DSIR Land Resources, Lincoln (CHR 455869), were dried, mechanically shredded, and extracted at room temperature with EtOH-H_2O (4:1). This extract was subjected to reversed-phase flash chromatography (H_2O/MeOH gradient), and the fractions eluted between MeOH-H_2O (8:2) and MeOH-H_2O (9:1) were combined and further chromatographed on Si gel. The initial fractions eluted with CH_2Cl_2 were fractionated by reversed-phase hplc [MeOH-H_2O (75:25)] to yield **1** (7.0 mg) and **2** (5.0 mg).

SYNTHESIS OF **1** AND **2**.—These compounds were synthesized from 5-methoxyresorcinol and isobutyryl nitrile using the method described by Gulati *et al.* (10). The products were subjected to reversed-phase hplc to give, in low yield, **1** and **2**. The ^{1}H-nmr spectra and antiviral activity of the two products were identical to those of the natural products.

2,6-Dihydroxy-4-methoxyisobutyrophenone [**1**].—Colorless oil; ^{1}H nmr ($CDCl_3$) 1.18 (6H, d, 6.7 Hz, H-9, H-10), 3.79 (3H, s, MeO), 3.85 (1H, sept, 6.7 Hz, H-8), 5.93 (2H, s, H-3, H-5), 14.00 (1H, s, OH).

4,6-Dihydroxy-2-methoxyisobutyrophenone [**2**].—Colorless oil; ^{1}H nmr ($CDCl_3$) 1.15 (6H, d, 6.8 Hz, H-9, H-10), 3.75 (1H, sept, 6.8 Hz, H-8), 3.87 (3H, s, MeO), 5.92 (1H, d, 2.2 Hz, H-3 or H-5), 6.00 (1H, d, 2.2 Hz, H-3 or H-5); ^{13}C nmr (Me_2CO-d_6) 210.5 q (C-7), 168.6 q, 165.5 q, 164.2 q [C-2, -4, -6), 104.9 q (C-1), 96.9 CH, 91.9 CH (C-3, C-5), 56.1 Me (MeO), 39.9 CH (C-8), 19.5 Me (C-9 and C-10).

EXTRACTION AND ISOLATION OF **3** AND **4**.—*K. ericoides* leaves and twigs (150 g), collected from Southern Wairarapa, were mechanically shredded and soaked overnight in hexane at room temperature followed by a similar extraction with CH_2Cl_2. The two extracts were combined and subjected to Si gel chromatography (hexane/EtOAc gradient). The fractions containing **3** and **4** (identified on Si gel tlc as an orange/red spot on heating after anisaldehyde/H_2SO_4 spray) were combined and crystallized from MeOH/hexanes to give the mixture of **3** and **4** (140 mg) as yellow crystals, mp 129–132°.

4-Cyclohexene-1,3-dioxo-5-hydroxy-2,2,6,6-tetramethyl-4-{1-[2,6-dihydroxy-4-methoxy-3-(3-methyl-1-oxobutyl)phenyl]-3-methylbutyl}[**3**] *and 4-cyclohexene-1,3-dioxo-5-hydroxy-2,2,6,6-tetramethyl-4-{1-[2,6-dihydroxy-4-methoxy-3-(2-methyl-1-oxopropyl)phenyl]-3-methylbutyl}*[**4**].—Ir ν max cm^{-1} 3200 (br), 2950, 2930, 2870, 1740, 1718, 1620, 1585; eims *m/z* [M]$^+$ **3** 474 (2), [M]$^+$ **4** 460 (2), 399 (6), 385 (1), 292 (10), 277 (6), 224 (20), 167 (100); ^{13}C nmr ($CDCl_3$) (shifts given for **3** only) 212.1 (C-11), 206.0 (C-14), 203.1 (C-13), 176.6 (C-9), 164.6, 164.6, 162.0 (C-1, -3, -5), 114.7 (C-8), 108.8, 104.5 (C-2, -6), 93.9 (C-4), 55.5 (MeO), 55.1 (C-12), 52.5 (C-15), 48.6 (C-10), 38.1 (C-23), 27.9, 26.8, 25.4 (C-7, -16, -24), 27.0, 26.1, 24.3, 22.9, 22.9, 22.6, 22.4, 22.3 (C-17–C-22, C-25, -26); ^{1}H nmr see Table 1; uv λ max (EtOH) nm 292 (28,300), 212 (27,820).

PREPARATION OF **5** AND **6**.—Several milligrams of the mixture of **3** and **4** were dissolved in C_6H_6, a small amount of *p*-TsOH was added, and the mixture was refluxed for 2.5 h. The reaction mixture was applied directly to a preparative tlc plate and eluted with hexane-EtOAc (7:3). The major product was collected and further fractionated by reversed-phase hplc [H_2O-MeOH (5:95)] to yield **5** and **6**.

1,3-Dioxo-4,9-dihydro-8-hydroxy-6-methoxy-2,2,4,4-tetramethyl-5-(3-methyl-1-oxobutyl)-9-(2-methylpropyl)-1H-xanthene [**5**].—$C_{27}H_{36}O_6$; eims *m/z* [M − C_4H_9]$^+$ 399 (100), [M − C_4H_9 − C_4H_6O]$^+$ 329 (20); uv λ max (EtOH) 282 (10,800), 208 (29,100); ir ν max cm^{-1} 3400 (br), 1720, 1650; ^{13}C nmr ($CDCl_3$) 212.2 (C-11), 202.7 (C-13), 198.0 (C-14), 168.1 (C-9), 156.4, 155.0, 148.9 (C-1, -3, -5), 113.4, 112.8, 106.4 (C-2, -6, -8), 95.7 (C-4), 56.0 (C-12), 55.9 (MeO), 54.2 (C-15), 47.5 (C-10), 46.3 (C-23), 31.0 (C-7), 25.4, 25.0, 24.8, 24.7, 24.6, 24.2, 23.5, 23.1, 22.8, 22.7 (C-16–C-22, C-24–C-26); ^{1}H nmr see Table 1.

1,3-Dioxo-4,9-dihydro-8-hydroxy-6-methoxy-2,2,4,4-tetramethyl-5-(2-methyl-1-oxo-propyl)-9-(2-methylpropyl)-1H-xanthene [**6**].—$C_{26}H_{34}O_6$; eims *m/z* [M − C_4H_9]$^+$ 385 (100), [M − C_4H_9 − C_4H_6O]$^+$ 315 (35); uv λ max (EtOH) 286 (13,100), 210 (32,000); ir ν max cm^{-1} 3400 (br), 1720, 1650; ^{1}H nmr ($CDCl_3$) 6.27 (s, H-4), 4.23 (brt, H-7), 3.77 s (3H, MeO), 3.42 (sept, H-15), 1.49 (s, 3H), 1.40 (s, 3H),

1.39 (s, 3H), 1.36 (s, 3H, H-19–H-22), 1.19 (d, 3H), 1.17 (d, 3H, H-16, H-17), 0.87 (d, 3H), 0.84 (d, 3H, H-25, H-26).

ANTIVIRAL ACTIVITY.—In vitro antiviral assays vs. *Herpes simplex* Type 1 and Polio Type 1 viruses were performed at the Department of Chemistry, University of Canterbury, New Zealand. The antiviral assay used is similar to the method described by Schroeder *et al.* (11). Compounds **1** and **2** showed whole (17 mm) well inhibition of cytopathic effect of either virus at 5 μg/disk. The mixture of **3** and **4** showed similar activity at 40 μg/disk.

ACKNOWLEDGMENTS

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