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(−)-1,12-OXAGUAI-10(15)-ENE: A SESQUITERPENE FROM *ERIOSTEMON FITZGERALDII*

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Key Word Index—*Eriostemon fitzgeraldii*; Rutaceae; coumarin; lignan; sesquiterpene; (−)-1,12-oxaguai-10(15)-ene; 3,4-dimethoxycinnamaldehyde; chemotaxonomy.

Abstract—Examination of the aerial parts of *Eriostemon fitzgeraldii* has afforded two sesquiterpenes, (−)-guaiol and the novel (−)-1,12-oxaguai-10(15)-ene, the lignan (−)-suchilactone, the phenylpropene derivatives methyleugenol and 3,4-dimethoxy cinnamaldehyde, and the simple coumarin umbelliferone. The possible chemotaxonomic significance of these isolates in the genus *Eriostemon* is discussed.

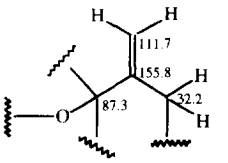
INTRODUCTION

Eriostemon fitzgeraldii C. R. P. Andr. (Rutaceae), a small, compact undershrub found extensively between Esperance and Norseman, Western Australia [1], has not previously been studied. As part of an on-going chemotaxonomic survey of the genus *Eriostemon* [2–7], we wish to report on the major isolates and to discuss their chemotaxonomic significance.

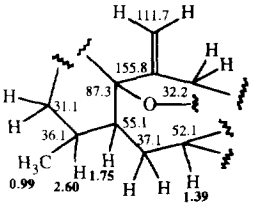
RESULTS AND DISCUSSION

From an *n*-hexane extract of the aerial parts of *Eriostemon fitzgeraldii* the simple coumarin umbelliferone [8, 9], two sesquiterpenes, (−)-guaiol [10] and (−)-1,12-oxaguai-10(15)-ene (**1**), a lignan (−)-suchilactone [11], and the phenylpropene derivatives methyleugenol [12, 13] and 3,4-dimethoxycinnamaldehyde (**2**) have been isolated. A search of the *Dictionary of Natural Products* [14] failed to find previously published information on either **1** or, surprisingly, **2**. All the known compounds were characterized by direct comparison of their physical and spectroscopic characteristics with those published in the literature. The two novel compounds were characterized by spectroscopic means.

Compound **1** was visualised on TLC as a dark-brown spot after heating with vanillin–H_2SO_4. The high-resolution EIMS revealed the empirical formula $C_{15}H_{24}O$. The ^{1}H NMR spectrum displayed signals for an exomethylene, three methyls, one secondary and two tertiary, a shielded proton (δ0.66) as a doublet of triplets and a single proton (δ2.60) as a multiplet. In addition to these signals, it also showed a range of complex multiplets in the regions δ1.20–2.30. ^{1}H–^{1}H COSY-45 and TOCSY (total correlation spectroscopy) experiments [15] allowed identification of spin-coupling systems. A broad band decoupled ^{13}C NMR spectrum showed signals for 15 carbons which with DEPT-135 and DEPT-90 experi-



1a



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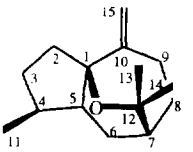

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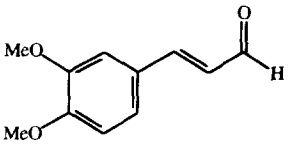

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ments [15] revealed the presence of three quaternary carbons, two oxygen bearing and one olefinic, three methines, one exomethylene, five methylenes, and three methyl carbons. An HC-COBIdec. spectrum [16] displayed C–H direct connectivities and thus confirmed the carbon resonances for respective methine and methyl groups.

An HMBC spectrum (Table 1) [17] identified long range H–C correlations. The exomethylene (H_2-15) protons displayed ^2J coupling to the olefinic quaternary carbon (C-10), ^3J to a quaternary carbon bearing oxygen (C-1) and to the methylene (C-9), and thus formed the partial structure **1a**. The methyl doublet (H_3-11) showed ^2J coupling to a methine (C-4), and ^3J to a methylene (C-3) and to another methine (C-5), the proton of which exhibited ^2J correlations to the methine (C-4), methylene (C-6) and to the quaternary (C-1), and ^3J to the methylene (C-3) and to the methine (C-7). Thus, the partial structure **1a** could be extended to **1b**. The methyls (H_3-13 and H_3-14) displayed ^2J correlation to the oxygenated quaternary carbon (C-12) and showed mutual ^3J coupling between themselves (therefore *geminal*) and another ^3J correlation with the methine (C-7) which in turn showed ^2J coupling to a methylene (C-8) and this methylene was also correlated to another methylene group (H_2-9). As the high-resolution EIMS showed the presence of only one oxygen, both the quaternary carbons bearing oxygen must share the same oxygen. Thus, the structure of this compound was elucidated as **1**. The relative stereochemistry of this compound was established from a NOESY experiment (Fig. 1).

Compound **2** was visualized on TLC as a quenching spot under UV (254 nm) and produced a blue colour after being sprayed with vanillin-H_2SO_4 and heated to 100° for 15 min. The high-resolution EIMS revealed the empirical formula $C_{11}H_{12}O_3$. The UV and IR spectra indicated an aromatic compound and in the IR spectrum an absorption band at 1732 cm^{-1} suggested the presence of an alkyl aldehyde [18]. The presence of this group was confirmed by the ^1H doublet (δ9.65) in the ^1H NMR spectrum which also displayed signals for three aromatic protons arranged in an ABD pattern, *trans*-olefinic protons showing a doublet (J = 16 Hz) and a doublets of a doublet (J = 8, 16 Hz) and two 3H singlets assignable to two aromatic methoxyl groups. The placement of the methoxyl groups at C-3 and C-4 of the aromatic ring, respectively, was confirmed from NOE difference experiments where an irradiation of the methoxyl group at δ3.88 enhanced the signal for H-5 (δ7.04) and that of the methoxyl group at δ3.89 enhanced the *meta* coupled H-2 (δ7.36). On this basis the structure of this compound was unambiguously assigned as **2**.

Methyleugenol, lignan and the guaiol-type sesquiterpenes are reported for the first time from the genus *Eriostemon* which predominantly produces different types of coumarins and to a lesser extent alkaloids [2–7]. *Eriostemon fitzgeraldii* is assigned to sect. *Corynonema* [1]. A second species of this section, *E. pinoides* P. G. Wilson, has been investigated in our laboratory by HPLC (Sarker, S. D., unpublished) and from this prelimi-

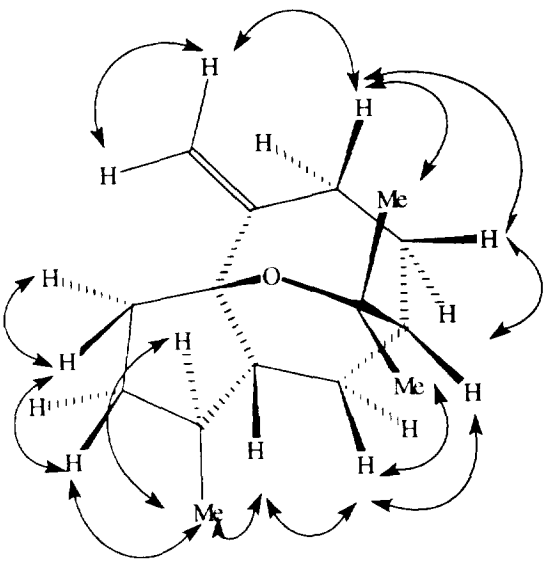

Fig. 1. Relative stereochemistry of **1** based on NOESY experiments.

Table 1. HMBC correlations in **1**

	^{13}C	
Protons	^2J	^3J
H-2	31.1 (C-3), 87.3 (C-1)	36.1 (C-4), 55.1 (C-5)
H-5	36.1 (C-4), 37.1 (C-6)	52.3 (C-7), 31.1 (C-3)
H-6	52.3 (C-7), 55.1 (C-5)	32.9 (C-8), 73.8 (C-12)
H-7	37.1 (C-6)	
H-9	32.9 (C-8), 155.8 (C-10)	111.7 (C-15), 52.3 (C-7)
3H-11	36.1 (C-4)	31.1 (C-3), 55.1 (C-5)
3H-13	73.8 (C-12)	28.1 (C-14), 52.3 (C-7)
3H-14	73.8 (C-12)	26.3 (C-13), 52.3 (C-7)
2H-15	155.8 (C-10)	32.2 (C-9), 87.3 (C-1)

nary study also appears to lack the complex coumarins characteristic of most *Eriostemon* species.

Wilson [1] observes that species of this section 'show morphological diversity' from typical *Eriostemon*. *Eriostemon fitzgeraldii* was in fact once assigned to *Phebalium*, as *P. apricum* (Diels) Ewart and Rees and it is noteworthy that the presence of sesquiterpenes of the type found here is more characteristic of *Phebalium* [19].

EXPERIMENTAL

UV spectra were in EtOH and IR spectra as KBr discs/film. NMR spectra were obtained on a Bruker AMX-400 instrument using standard Bruker microprograms. For HMBC experiments ^2J and ^3J coupling was set for approximately 7 Hz. High resolution EIMS were obtained by direct probe insertion at elevated temp. (120–140°) and at 70 eV. Silica gel 60 H (Merck 7736) and silica gel (Merck 7734), respectively, for vacuum liquid chromatography (VLC) and CC, and Silica gel 60-PF_{254} (Merck 7749) for TLC and circular PTLC (Chromatotron) were used. Sephadex LH20 (Sigma 82H0368) was used for gel-filtration. Petrol stands for petroleum ether (40–60°) throughout this text.

Plant material. The aerial parts of *Eriostemon fitzgeraldii* (voucher: PERTH 01655817, at the Western Australian Herbarium) were collected about 60 km south of Norseman, in south-western Western Australia; as an understorey shrub in mallee woodland vegetation on reddish-brown loam over laterite.

Extraction. Powdered plant material (470 g) was extracted in a Soxhlet with, successively, *n*-hexane, $CHCl_3$ and MeOH and the extracts were concentrated using a rotary evaporator at a maximum temperature of 40°.

Isolation of compounds. VLC of concd *n*-hexane extract (10.2 g), eluting with solvents of increasing polarity starting from petrol (100%) via EtOAc to MeOH (100%) yielded a number of frs. Methyleugenol (234 mg) was isolated from the VLC fraction (5% EtOAc in petrol) by Chromatotron, eluting with petrol–EtOAc mixt. of increasing polarity and collecting the quenching band (254 nm). The VLC fraction (20% EtOAc in petrol) was subjected to CC (100% $CHCl_3$) and frs of 4 ml vol. were collected. PTLC ($CHCl_3$–EtOAc, 90:10) of the CC frs 17–26 yielded (−)-guaiol (183 mg). VLC frs (22–40% EtOAc in petrol) were combined and subjected to CC (100% $CHCl_3$) and frs of 4 ml volume were collected. PTLC (*n*-hexane–EtOAc, 80:20, double development) of the CC frs 6–8 afforded to lignan (−)-suchilactone (15.3 mg) and **2** (5.5 mg). The VLC fraction (50% EtOAc in petrol) was run through a Sephadex column eluting with 0.5% MeOH in $CHCl_3$ and fractions of 4 ml vol. were collected. Sephadex frs 10–16 were combined and subjected to CC (100% $CHCl_3$), and again frs of 4 ml vol. were collected. PTLC ($CHCl_3$–EtOAc, 80:20) of CC frs 9–20 produced **1** (36.0 mg). Umbelliferone was isolated from the VLC fractions (65 70% EtOAc in petrol) by PTLC ($CHCl_3$–EtOAc, 80:20).

(−)-1,12-*Oxaguai*-10(15)-*ene* (**1**). Oil. $[\alpha]_D$ −35 (c = 0.2, $CHCl_3$); IR γ_{max} (film) cm^{-1}: 2932, 2838, 2359, 1455, 1373, 1299, 1250, 1125, 1011, 915, 898, 1132, 785. ^1H NMR (400 MHz, $CDCl_3$): δ5.01 (1H, *s*, H-15), 4.91 (1H, *s*, H-15), 2.60 (1H, *m*, H-4), 2.32 (1H, *m*, H-9β), 2.06 (1H, *m*, H-9α), 1.94 (1H, *m*, H-8β), 1.84 (1H, *m*, H-6α), 1.80 (2H, *m*, H-2β, H-6β), 1.75 (1H, *m*, H-5), 1.68 (1H, *m*, H-3α), 1.39 (1H, *m*, H-7), 1.22–1.19 (2H, *m*, H-3β, H-8α), 1.18 (3H, *s*, Me-14), 1.14 (3H, *s*, Me-13), 0.99 (3H, *d*, J = 7 Hz, Me-11), 0.66 (1H, *dt*, J = 12.3, 10.4 Hz, H-2α). ^{13}C NMR (400 MHz, $CDCl_3$): δ155.8 (*s*, C-10), 111.7 (*t*, C-15), 87.3 (*s*, C-1), 73.8 (*s*, C-12), 55.1 (*d*, C-5), 52.3 (*d*, C-7), 37.1 (*t*, C-6), 36.1 (*d*, C-4), 32.9 (*t*, C-8), 32.2 (*t*, C-9), 31.1 (*t*, C-3), 28.1 (*q*, C-14), 26.3 (*q*, C-13), 25.5 (*t*, C-2), 16.1 (*q*, C-11). Found: $[M]^+$ 220.1828; $C_{15}H_{24}O$ requires 220.1827. EIMS *m/z* (rel. int.): 220 $[M]^+$ (16), 205 $[M - CH_3]^+$ (9), 191 (3), 177 (11), 165 (11), 163 (14), 159 (15), 149 (19), 135 (100).

3,4-*Dimethoxycinnamaldehyde* (**2**). Amorphous UV λ_{max} nm (log ε): 329 (3.90), 304 (3.98), 262 (3.94) 231 (3.88); IR γ_{max} (KBr) cm^{-1}: 1732, 1671, 1596, 1464, 1270, 1130, 1023, 971, 805, 759. ^1H NMR (400 MHz, acetone-d_6): δ9.65 (1H, *d*, J = 7.7 Hz, CHO), 7.59 (1H, *d*, J = 16 Hz, CH=CH–CHO), 7.36 (1H, *d*, J = 1.6 Hz, H-2), 7.27 (1H, *dd*, J = 8.2, 1.6 Hz, H-6), 7.04 (1H, *d*, J = 8.2 Hz, H-5), 6.68 (1H, *dd*, J = 16, 7.7 Hz, CH=CH–CHO), 3.89 (3H, *s*, 3-OMe), 3.88 (3H, *s*, 4-OMe). Found: $[M]^+$ 192.0771; $C_{11}H_{12}O_3$ requires 192.0786;. EIMS *m/z* (rel. int.): 192 $[M]^+$ (100), 191 $[M - H]^+$ (15), 177 $[M - CH_3]^+$ (23), 161 $[M - CH_3O]^+$ (81), 149 (18), 133 (9), 121 (12).

(−)-*Guaiol.* Colourless prisms from MeOH. Mp 90–92° (lit. 90–91°, [10]); $[\alpha]_D$ −31 (c = 4.0, 95% EtOH); IR, ^1H NMR. ^{13}C NMR and EIMS data as reported [10].

(−)-*Suchilactone.* Needles from 95% EtOH. Mp 128–130° (lit. 129–130° [11]); UV, IR, ^1H NMR, ^{13}C NMR and EIMS data as reported [11].

Methyl eugenol. Oil. UV, IR, ^1H NMR, ^{13}C NMR and EIMS data as reported [12, 13].

Umbelliferone. Needles from H_2O. Mp 230–232°. UV, IR, ^1H NMR and EIMS data as reported [8, 9].

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