Three New Sesquiterpene Butenolides from *Eremophila* Species

Yana M. Syah, Emilio L. Ghisalberti,* Brian W. Skelton, and Allan H. White

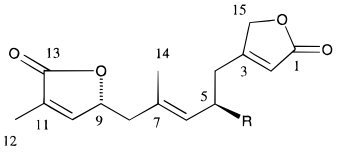
Department of Chemistry, University of Western Australia, Nedlands, Western Australia 6907, Australia

Received August 23, 1996[⊗]

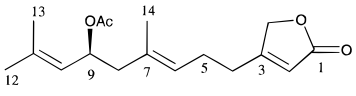
Two new (**1**and **2**) and one known (**3**) sesquiterpene bis-butenolides have been isolated from the aerial parts of *Eremophila homoplastica* (Myoporaceae). The structures of the new compounds were determined by spectroscopic studies and confirmed by single-crystal X-ray crystallography of one (**2**) of the new compounds. Spectroscopic evidence for the structure of a new related sesquiterpene butenolide (**4**) from *E. forrestii* is presented. The isolation of the phenylethanoid glycosides, verbascoside and poliumoside, from *E. forrestii* is noted.

Eremophila R. Br. (Myoporaceae) is a genus of hardy, perennial shrubs and small trees that occur throughout the arid and semiarid regions of the mainland states of Australia.[1] A number of species have been regarded as important medicinal plants by the Aboriginal people of Central Australia.[2] Since many species are tolerant to drought, fire, frost, salinity, and grazing, present interest in this genus is centered on its potential in rangeland revegetation and minesite rehabilitation programs.[1,3]

In continuation of the work on the phytochemistry and bioactive metabolites of this genus,[4] we have investigated the major metabolites produced by *E. forrestii* F. Muell. and *E. homoplastica* (S. Moore) C. Gardner, two species that occur in the arid regions of Western Australia.[2] The isolation and evidence for the structures of four sesquiterpene butenolides (**1**−**4**), which include three new examples (**1**, **2**, and **4**), is the subject of this report. The isolation of 4′,7-dimethoxyflavone from *E. homoplastica* and two phenylethanoid glycosides, verbascoside and poliumoside, from *E. forrestii* is also described.



1 R = OH
2 R = OAc
3 R = H



4

Air-dried powdered leaves of *E. homoplastica* were extracted with acetone, and the crude extract was fractionated by VLC. Purification of fractions enriched in one compound by centrifugal TLC yielded four compounds that are discussed below. The least polar compound, obtained as an oil, had a molecular formula $C_{15}H_{18}O_4$ (HRMS) and spectroscopic properties consistent with those described for the sesquiterpene *bis*-butenolide (**3**) previously isolated from a new species of *Eremophila*.[5]

A second compound (**1**), isolated as an oil, showed a molecular formula (HRMS) for $C_{15}H_{18}O_5$ ($M^+ + 1$ at *m/z* 279.1244). The NMR spectral parameters were similar to those of compound **3** with the exception that one of the hydrogens at C5 in **3** (δ_H 2.22−2.32, m) had been replaced by a hydroxyl group in compound **1** with consequent deshielding of the remaining methine proton (δ_H 4.59). The ^{13}C NMR spectrum showed the expected deshielding of the *ipso* and α-carbons. The structure of this compound was assigned as shown in **1**, with the relative stereochemistry being established by reference to the third compound isolated. The third compound was readily deduced to be the corresponding acetate derivative **2** from an HRMS measurement ($C_{17}H_{20}O_6$) and NMR spectroscopic properties (δ_H 1.97, 3H, s, H5; δ_C 20.9, s; 169.8, s). The relative stereochemistry of compound **2**, and, by inference, that of compound **1**, was resolved by single-crystal X-ray diffraction studies (see below). With this result in hand, the absolute stereochemistry of compounds **1** and **2** (5*R*,9*R*) could be assigned from that originally deduced for the known bis-butenolide (**3**).[5] The last compound isolated was identified as 4′,7-dimethoxy-5-hydroxyflavone.

Similar fractionation of the extract obtained from powdered leaves of *E. forrestii* with acetone yielded a single major metabolite as an oil. The ^{13}C NMR spectrum of this compound ($C_{17}H_{24}O_4$; CIMS, $M^+ + 1 - 60$ at *m/z* 233) showed good correspondence of the chemical shifts for C1-C6 and C15 with those of compound **3**, supporting the presence of the ethylbutenolide moiety. The presence of a 2-methylpropen-1-yl moiety was deduced from NMR measurements (δ_H 1.63, 1.65, each a d; 5.02, m), the assignments being supported by HMBC studies. Moreover, these also showed the presence of allylic acetate, the methine carbon of which was correlated with an sp^2-fully substituted carbon (C7). That this carbon was part of an internal isoprene unit followed from the spectral data. The structure of the metabolite from *E. forrestii*, therefore, is as shown in **4**. The absolute stereochemistry of **4** was tentatively assigned as *S* since the sign and value of its optical rotation ($[\alpha]_D$ −11°) corresponds to that of 4-hydroxydendrolasin (**5**) ($[\alpha]_D$ −15.8°), which was previously isolated from *E. rotundifolia*.[6] From the MeOH extract of *E. forrestii*, the known phenylethanoid diglycoside verbascoside[7] and the triglycoside poliumoside[8] were also isolated. Verbascoside has previously been shown to have cardiotonic activity.[7]

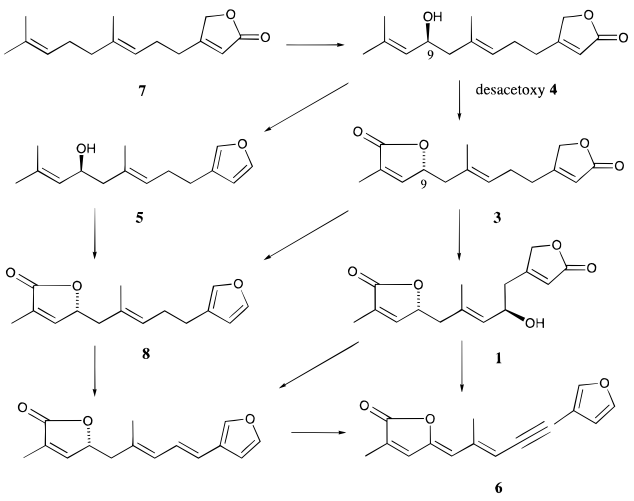
The occurrence of **1**, **3**, and **4** may have some signifi-

* To whom correspondence should be addressed. Tel.: (09) 380-3174. Fax: (09) 380-1005. E-mail: elg@declan.chem.uwa.edu.au.

[⊗] Abstract published in *Advance ACS Abstracts,* January 1, 1997.

S0163-3864(96)00591-5 CCC: $14.00

Scheme 1. Hypothetical Biosynthetic Correlations of *Eremophila* Butenolides



cance with respect to the biosynthesis of freelingyne (**6**), the first naturally occurring acetylenic sesquiterpene described.[9] As shown in Scheme 1, hydroxylation of the simple sesquiterpene butenolide (**7**) would lead to the deacetoxy equivalent of **4**, which could be a precursor of 4-hydroxydendrolasin (**5**) on the one hand and of the bis-butenolide (**3**) on the other. This second conversion requires an inversion at C9 in the formation of the butenolide ring. In turn, compound **3** could lead to the hydroxy bis-butenolide (**1**), a probable precursor of freelingyne (**6**), and/or to freelingnite (**8**). The absolute configuration of freelingnite (**8**), which co-occurs with freelingyne in *E. freelingii*,[10] appears to be the same as that of **3**.[5] Dehydrogenation of compound **8** could then provide freelingyne (**6**).

The structure determination of **2** was carried out as follows. A unique room- temperature diffractometer data set (ENRAF-Nonius CAD-4 instrument; $2\theta/\theta$ scan mode; monochromatic Mo Kα radiation, λ 0.7107$_3$ Å, T ~295 K) was measured within the limit $2\theta_{max} = 50°$, yielding 1686 independent reflections; 868 of these with $I > 3\sigma(I)$ were considered "observed" and used in the full-matrix least-squares refinement without absorption correction after solution of the structure by direct methods. Anisotropic thermal parameters were refined for C, O; $(x, y, z, U_{iso})_H$ were included constrained at estimated values after location in difference syntheses. Neutral atom complex scattering factors were employed, and computation used the XTAL 3.2 program system implemented by Hall.[11] Conventional residuals R *and* R_w on $|F|$ were 0.048 and 0.041 (statistical weights, derivative of $\sigma^2(I) = \sigma^2(I_{diff}) + 0.0004\sigma^4(I_{diff})$). The atomic coordinates for non-hydrogen atoms are given in Table 1. Lists of other parameters have been deposited with the Cambridge Crystallographic Data Centre.[12]

Crystal data: $C_{17}H_{20}O_6$, M_r 320.4; orthorhombic, space group $P2_12_12_1$ (D_2^4, No. 19), a = 14.662(5) Å, b = 11.134(4) Å, c = 10.217(7) Å, V = 1668 Å^3; D_c (Z = 4) 1.28 g·cm^{-3}; F(000) 632; μ_{Mo} 1.0 cm^{-1}; specimen 0.27 × 0.15 × 0.10 mm.

The structure determination from a rather weakly diffracting specimen was otherwise well behaved, the result being consistent with the above stoichiometry, connectivity, and sterochemistry (Figure 1); absolute sterochemistry was adopted from the chemistry. Intramolecular geometries are substantially as expected.

Table 1. Non-Hydrogen Positional and Isotropic Displacement Parameters for **2**

atom	x	y	z	U_{eq} (Å^2)
C(1)	0.5397(5)	−0.2187(7)	0.6841(9)	0.063(3)
O(1)	0.5224(4)	−0.3180(4)	0.7195(7)	0.093(3)
C(2)	0.6249(4)	−0.1489(6)	0.6911(8)	0.056(3)
C(3)	0.6111(4)	−0.0429(6)	0.6358(7)	0.041(2)
C(4)	0.6731(4)	0.0631(6)	0.6163(8)	0.051(3)
C(5)	0.7649(4)	0.0603(5)	0.6829(7)	0.041(2)
C(6)	0.8155(4)	0.1764(6)	0.6606(8)	0.047(3)
C(7)	0.8122(4)	0.2715(6)	0.7369(8)	0.044(3)
C(8)	0.8546(4)	0.3880(6)	0.6945(8)	0.057(3)
C(9)	0.7967(5)	0.4600(6)	0.5956(8)	0.054(3)
O(9)	0.8013(3)	0.4004(4)	0.4705(5)	0.056(2)
C(10)	0.6963(5)	0.4668(6)	0.6227(8)	0.054(3)
C(11)	0.6505(4)	0.4115(6)	0.5302(8)	0.048(3)
C(12)	0.5492(5)	0.3934(7)	0.5145(8)	0.075(3)
C(13)	0.7163(6)	0.3689(6)	0.4317(9)	0.050(3)
O(13)	0.7016(4)	0.3169(5)	0.3318(6)	0.081(3)
C(14)	0.7627(5)	0.2737(7)	0.8650(8)	0.067(3)
C(15)	0.5159(4)	−0.0383(6)	0.5849(7)	0.055(3)
O(15)	0.4760(3)	−0.1506(4)	0.6224(5)	0.059(2)
O(51)	0.8147(3)	−0.0400(4)	0.6242(5)	0.047(2)
C(51)	0.8865(5)	−0.0837(6)	0.6924(8)	0.046(3)
O(52)	0.9093(3)	−0.0429(5)	0.7943(6)	0.073(2)
C(52)	0.9302(4)	−0.1833(6)	0.6233(9)	0.062(3)

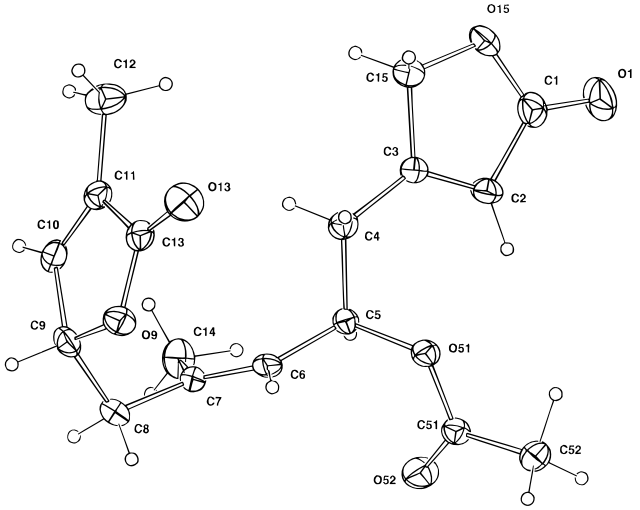


Figure 1. Molecular projection of **2**; 20% thermal ellipsoids are shown for the non-hydrogen atoms, hydrogen atoms having arbitrary radii of 0.1 Å.

Experimental Section

General Experimental Procedures. Experimental details have been reported.[13]

Plant Material. Leaves and stems of *E. homoplastica* and *E. forrestii* were collected 400 m and 3 km south, respectively, of Wangarri Nature Reserve in Western Australia in December 1994. The identification was verified by Dr. R. Chinnock, The Botanic Gardens of Adelaide and State Herbarium, North Terrace, Adelaide, South Australia, with whom specimens have been deposited (*E. homoplastica*, 214GSR; *E. forrestii*, 213GSR).

Extraction and Isolation of Sesquiterpenes. Air-dried powdered leaves (100 g) of *E. homoplastica* were steeped in acetone overnight. A portion (8 g) of the extract (16 g) obtained was chromatographed by VLC using Si gel (120 g) and eluting with a stepwise gradient from light petroleun−CH_2Cl_2 (1:1) to CH_2Cl_2−MeOH (9:1). Individual fractions were combined according to the major compound they contained, and these combined

fractions were individually subjected to circular TLC (light petroleum−EtOAc; 1:1) to yield the bis-butenolide (**3**) (70 mg), the hydroxy bis-butenolide (**1**) (270 mg), the acetoxy bis-butenolide (**2**) (640 mg), and 4′,7-dimethoxy-5-hydroxyflavone (20 mg). Air-dried powdered leaves (80 g) of *E. forrestii* were steeped in acetone overnight. The extract (5.8 g) recovered was chromatographed by VLC using Si gel (120 g). Elution with light petroleum−EtOAc (7:3) yielded a yellow fraction that was filtered through charcoal to give the acetoxy butenolide (**4**) (790 mg). Extraction of the residual plant material with MeOH gave an extract (9.7 g) that on VLC using Si gel (150 g) and elution with EtOAc−10% MeOH afforded fractions of verbascoside (1.5 g). Elution with EtOAc−25% MeOH gave poliumoside (100 mg).

Hydroxy bis-butenolide 1: oil; $[\alpha]_D$ +1° (*c* 1.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.74 (3H, d, J = 1.3 Hz, H_3-12), 1.87 (3H, t, J = 1.3 Hz, H_3-14), 2.22 (1H, ddd, J = 14.0, 8.0, 1.0 Hz, H-8a), 2.39 (1H, ddd, J = 14.0, 5.0, 1.0 Hz, H-8b), 2.53−2.61 (2H, m, H_2-4), 4.59 (1H, ddd, J = 8.5, 7.1, 5.2 Hz, H-5), 4.81 (2H, m, H_2-15), 4.97 (1H, m, H-9), 5.31 (1H, dq, J = 8.6, 1.2 Hz, H-6), 5.88 (1H, quint, J = 1.6 Hz, H-2), 7.00 (1H, dq, J = 1.6, 1.4 Hz, H-10); ^{13}C NMR (75 MHz, $CDCl_3$) 10.6 (C-12), 17.1 (C-14), 36.4 (C-4), 43.1 (C-8), 66.4 (C-5), 74.0 (C-15), 79.2 (C-9), 115.5 (C-2), 130.3 (C-11), 130.4 (C-6), 134.1 (C-7), 148.3 (C-10), 167.2 (C-3), 174.0 (C-1), 174.2 (C-13); CIHRMS *m*/*z* 279.1244, 261.1063 (calcd for $C_{15}H_{19}O_5$, 279.1232; calcd for $C_{15}H_{17}O_4$ 261.1025); EIMS *m*/*z* 278 [M^+] (1), 260 (1), 242 (1), 181 (42), 163 (34), 135 (20), 97 (100), 79 (10), 69 (34).

Acetoxy bis-butenolide 2: crystalline; mp 82−84 °C; $[\alpha]_D$ −3° (*c* 1.8, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.75 (3H, d, J = 1.5 Hz, H_3-12), 1.84 (3H, t, J = 1.8 Hz, H_3-14), 1.97 (3H, s, acetoxyl methyl), 2.25 (1H, ddd, J = 14.5, 7.0, 0.5 Hz, H-8a), 2.37 (1H, ddd, J = 14.5, 8.0, 0.5 Hz, H-8b), 2.62 (1H, ddd, J = 15.5, 5.0, 1.5 Hz, H-4a), 2.74 (ddd, J = 15.5, 7.0, 1.5 Hz, H-4b), 4.75 (1H, dd, J = 17.7, 1.8 Hz, H-15a), 4.79 (1H, dd, J = 17.7, 1.8 Hz, H-15b), 4.92 (1H, m, H-9), 5.20 (1H, brd, J = 9.0 Hz, H-6), 5.60 (1H, ddd, J = 9.0, 7.0, 5.0 Hz, H-5), 5.84 (1H, quint, J = 1.5 Hz), 6.94 (1H, quint, J = 1.5 Hz, H-10); ^{13}C NMR (75 MHz, $CDCl_3$) 10.4 (C-12), 17.8 (C-14), 33.7 (C-4), 42.6 (C-8), 68.1 (C-5), 77.7 (C-15), 79.1 (C-9), 117.7 (C-2), 125.6 (C-6), 130.3 (C-11), 136.6 (C-7), 147.8 (C-10), 165.0 (C-3), 173.3 (C-1), 173.7 (C-13), 20.9 and 169.8 (acetoxy group), assignments were aided by HMBC measurements; EIHRMS *m*/*z* 320.12527 (calcd for $C_{17}H_{20}O_6$, 320.12599); EIMS *m*/*z* 320 [M^+] (3), 278 (4), 260 (10), 181 (80), 163 (100), 135 (26), 119 (29), 117 (10), 107 (21), 105 (19), 97 (95).

Bis-butenolide 3: oil; $[\alpha]_D$ +36° (*c* 3.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.65 (3H, brd, J = 1.3 Hz, H_3-12), 1.85 (3H, t, J = 1.7 Hz, H_3-14), 2.27 (1H, m, H-5), 2.22−2.32 (2H, m, H_2-8), 2.42 (2H, brt, J = 7.4 Hz, H_2-4), 4.69 (2H, d, J = 1.8 Hz, H_2-15), 4.92 (1H, m, H-9), 5.19 (1H, tq, J = 7.0, 1.7 Hz, H-6), 5.79 (1H, quint, J = 1.6 Hz, H-2), 6.97 (1H, quint, J = 1.5 Hz, H-10); ^{13}C NMR (125 MHz, $CDCl_3$) 10.5 (C-12), 16.7 (C-14), 25.6 (C-5), 28.2 (C-4), 43.2 (C-8), 73.0 (C-15), 79.5 (C-9), 115.5 (C-2), 126.4 (C-6), 130.0 (C-11), 131.7 (C-7), 148.3 (C-10), 169.9 (C-3), 173.9 (C-1), 174.0 (C-13); EIHRMS *m*/*z* 262.12155 (calcd for $C_{15}H_{18}O_4$, 262.12051); EIMS *m*/*z* 262 [M^+] (1.5), 199 (4), 171 (15), 165 (100), 150 (18), 127 (9), 119 (23), 109 (14), 107 (14), 105 (11), 98 (61), 97 (100); essentially identical to that previously described.[5]

Acetoxy butenolide 4: oil; $[\alpha]_D$ −11° (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.59 (3H, brs, H_3-14), 1.63 (3H, d, J = 1.5 Hz, H_3-13), 1.65 (3H, d, J = 1.5 Hz, H_3-12), 1.92 (3H, s, acetoxyl methyl), 2.10 (1H, dd, J = 13.7, 5.9 Hz, H-8a), 2.20−2.28 (2H, m, H_2-4), 2.25 (1H, m, H-8b), 2.38 (1H, t, J = 7.3 Hz, H-5), 4.66 (2H, m, H_2-15), 5.02 (1H, dsept, J = 9.1, 1.5 Hz, H-10), 5.07 (1H, brt, J = 7.0 Hz, H-6), 5.55 (1H, ddd, J = 9.1, 7.7, 6.3 Hz, H-9), 5.77 (1H, quint, J = 1.5 Hz, H-2); ^{13}C NMR (125 MHz, $CDCl_3$) 16.4 (C-14), 18.3 (C-13), 25.5 (C-5, C-12), 28.3 (C-4), 45.0 (C-8), 69.5 (C-9), 73.3 (C-15), 115.4 (C-2), 123.3 (C-10), 125.1 (C-6), 133.2 (C-7), 137.2 (C-11), 170.1 (C-3), 174.0(C-1), 21.1 and 170.2 (acetoxy group), assignments were aided by HMBC measurements; CIMS *m*/*z* 233 [$M^+ + 1 - CH_3CO_2H$]; EIMS *m*/*z* 232 (6), 166 (32), 135 (14), 127 (28), 107 (22), 98 (70), 93 (22), 85 (100), 79 (14).

4′,7-Dimethoxy-5-hydroxyflavone: yellow crystals; mp 172−174 °C (lit.[14] 170−173 °C); ^{1}H and ^{13}C NMR spectral properties were in agreement with the structure.

Verbascoside; identified by comparison with a standard sample.[7]

Poliumoside: amorphous powder; $[\alpha]_D$ −40° (*c* 1.0, MeOH) (lit.[8] $[\alpha]_D[\alpha]_D$ −80°, MeOH); spectroscopic properties identical to those described previously.[8]

Acknowledgment. We are grateful to Dr. R. Chinnock, The Botanic Gardens of Adelaide and State Herbarium, North Terrace, Adelaide, and Dr. G. Richmond for taxonomic information. One of us (Y.M.S.) acknowledges financial support from the AusAID Scholarship Scheme.

References and Notes

(1) Richmond, G. S.; Ghisalberti, E. L. *Econ. Bot.* **1994**, *48*, 35−59.
(2) Ghisalberti, E. L. *J. Ethnopharmacol.* **1994**, *44*, 1−9.
(3) Elliot, W. R.; Jones, D. L. *Encyclopaedia of Australian Plants Suitable for Cultivation*; Lothian Publications: Melbourne, 1984; Vol. 3, pp 428−464.
(4) Ghisalberti, E. L. *Phytochemistry* **1994**, *35*, 7−33.
(5) Dastlik, K. A.; Forster, P. G.; Ghisalberti, E. L.; Jefferies, P. R.; *Phytochemistry* **1989**, *28*, 1425−1426.
(6) Dimitriadis, E.; Massy-Westropp, R. A. *Aust. J. Chem.* **1980**, *33*, 2729−2736.
(7) Pennacchio, M.; Syah, Y. M.; Alexander, E.; Ghisalberti, E. L. *J. Ethnopharmacol.* **1996**, *53*, 21.
(8) Andary, C.; Wylde, R.; Heitz, A.; Rascol, J. P.; Roussel, J. L.; Lafitte, C. *Phytochemistry* **1985**, *24*, 362−364.
(9) Massy-Westropp, R. A.; Reynolds, G. D.; Spotswood, T. M. *Tetrahedron Lett.* **1966**, 1939−1946.
(10) Knight, D. W.; Pattenden, G. *Tetrahedron Lett.* **1975**, 1115−1116.
(11) Hall, S. R., Flack, H. D., Steward, J. M., Eds. *The XTAL 3.2 Reference Manual*; Universities of Maryland, Geneva, and Western Australia, 1992.
(12) Hydrogen coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained, upon request, from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.
(13) Almassi, F.; Ghisalberti, E. L.; Narbey, M. J.; Sivasithamparam, K. *J. Nat. Prod.* **1991**, *54*, 396−402.
(14) Biftu, T.; Stevenson, R. *J. Chem. Soc., Perkin Trans. 1* **1978**, 360−364.

NP9605910