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NEW CEMBRENE DITERPENES FROM THE RESINS OF *EREMOPHILA* SPECIES

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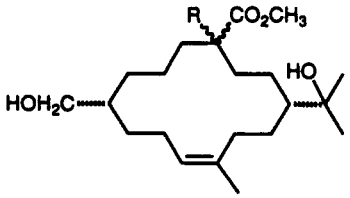
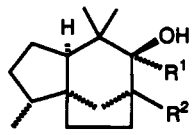
ABSTRACT.—The isolation of two new cembrene diterpenes, **1** from the resin of *Eremophila gilesii* and **6** from the resins of *Eremophila metallicorum* and *Eremophila viscida* var., is described. The structures of the cembrenes were assigned by chemical and spectroscopic methods.

Eremophila species (Myoporaceae) are probably the most abundant resin plants of the Eremean region in Western Australia (1). Many of the desert-adapted species produce copious quantities of resin which are mainly composed of flavones and oxygenated diterpenes (2). In continuation of our work on the phytochemistry of this ecologically important genus, we have investigated the secondary metabolites produced by three of its members, *Eremophila gilesii* F. Muell., *Eremophila metallicorum* S. Moore, and a variety of *Eremophila viscida* Endl. *E. gilesii* is the name given to a species complex within which different races are recognized (R.J. Chinnock, State Herbarium of South Australia, Adelaide, South Australia; private communication, 1987). Members of this complex occur throughout most of the mainland states, regenerate effectively, are highly ornamental (3), and are regarded as important medicinal plants by the Aboriginal people of Central Australia (4). The species *E. metallicorum* and *E. viscida* are restricted to Western Australia and are drought tolerant (3). One of the simpler diterpene skeletons encountered in *Eremophila* resins is that of the 14-membered-ring cembrenes. The cembrenes present in *Eremophila* species are unusual in containing trisubstituted double bonds with the cis-configuration with respect to the ring carbons (5–8). In this report, we present evidence for the structures of two new compounds containing this stereochemically unique class of diterpenes.

Extraction of leaves and branchlets of *E. gilesii* (Western Australian race) with Me_2CO, and separation of the extract by vacuum liquid chromatography (vlc), yielded fractions consisting mainly of one component [**1**]. Methylation followed by centrifugal preparative chromatography (cpc) of the product gave a homogeneous sample of the ester, **2**, $C_{23}H_{38}O_5$. The ^{1}H-nmr spectrum included signals for two tertiary methyls (δ 1.00, 1.23), a vinylic methyl (δ 1.67), an acetoxy methyl (δ_H 2.02), and a carbomethoxyl group (δ 3.67). The presence of a primary acetoxy group was indicated by signals for a methylene group at δ 4.21 (AB part of ABX system). Other signals at δ 3.88 (m) and 5.11 (t, J=7 Hz) were attributed to an oxymethine proton and a vinylic proton, respectively.

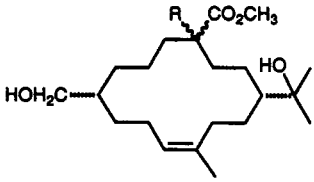
The ^{13}C-nmr spectrum of **2** contained only four signals for sp^2-carbons, therefore the compound is bicyclic. The presence of an oxygen heterocycle was indicated by two carbons (δ 82.6, s; 76.7, d) sharing the only oxygen atom not involved in ester groups. The chemical shift and multiplicities of the remaining carbon suggested a cembrene skeleton. This was confirmed by treatment of **2** with $LiAlH_4$ to give the diol **3** whose structure has been defined as (11*Z*)-3,15-epoxycembr-11-ene-18,19-diol by X-ray diffraction studies (6). The remaining point of ambiguity was the relative positioning of the two pendant ester groups. A choice in favor of that shown in **2** was possible by comparing its ^{13}C-nmr spectrum with those of **4**, which contains a primary acyloxy group at C-4, and **5**, in which a carbomethoxyl group is at this position (see Table 1) (7). In particular, C-4 resonated at δ 42.8 and 42.2 in **1** and **4**, respectively, but at δ 51.5 in **5**. Thus the original metabolite is assigned the structure of (11*Z*)-18-acetoxy-15-

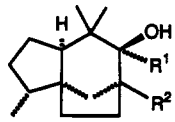




1 $R^1=CO_2H$, $R^2=CH_2OAc$
2 $R^1=CO_2CH_3$, $R^2=CH_2OAc$
3 $R^1=R^2=CH_2OH$
4 $R^1=CHO$, $R^2=CH_2OCOCH_2CO_2CH_3$; Δ^7
5 $R^1=R^2=CO_2CH_3$

6 $R^1=CH_2OH$, $R^2=CO_2H$
7 $R^1=CH_2OH$, $R^2=CO_2CH_3$
8 $R^1=R^2=CH_2OH$
9 $R^1=CO_2CH_3$, $R^2=CH_2OH$





10 R=H (as *bis*-TMS ether)
11 R=SPh
12 R=S(O)Ph
13 Δ^4

14 $R^1=CH_3$, $R^2=H$
15 $R^1=H$, $R^2=CH_3$

TABLE 1. ^{13}C-Nmr Spectral Data of Selected Compounds ($CDCl_3$; 20.1 MHz).

Carbon	Compound				
	2[a]	**4**	**5**	**7**[b]	**8**
C-1	45.2	47.0	45.7	49.7	49.7
C-2	34.2	33.6	34.3	21.0	20.6
C-3	76.7	76.0	77.5	24.2	24.5
C-4	42.8	42.2	51.5	42.8	40.0
C-5	24.4[c]	28.4[c]	24.8[c]	28.3	29.5
C-6	24.4[c]	27.7[c]	24.0[c]	24.2	24.5
C-7	31.0	154.7	31.7[d]	30.2	30.0
C-8	43.3	143.5	42.9	38.7	38.9
C-9	30.7	25.4	31.4[d]	28.9	29.2
C-10	26.4	27.7	26.1	26.4	26.5
C-11	126.1	126.2	126.5	123.8	124.6
C-12	135.2	134.2	135.5	136.8	136.3
C-13	29.7	30.1	30.2	31.8	31.8
C-14	27.8	27.7	28.9	28.6	28.5
C-15	82.6	81.8	82.9	74.0	74.1
C-16	28.5	27.2	28.3	28.3	28.1
C-17	22.7	20.7	22.4	26.7	27.5
C-18	65.8	67.6	175.8	176.5	66.7[c]
C-19	176.8	195.2	176.3	65.8	66.4[c]
C-20	22.3	23.3	22.9	23.4	23.4

[a]Methoxyl carbon at δ 51.4; acetoxy carbons at δ 171.3, 20.9.
[b]Methoxyl carbon at δ 51.5.
[c,d]Values in any one column may be interchanged.

hydroxycembr-11-en-19-oic acid [**1**], with the absolute configuration at C-1 assumed to be that common to all *Eremophila* cembrenes (6,7).

Leaves and terminal branches of two samples of *E. metallicorum*, collected 100 km apart, were extracted with Et_2O. Each extract was shown by tlc to contain the same carboxylic acid, **6**, as the major lipophilic metabolite. The same compound was also the major component in the resin of an *Eremophila* species (*E. viscida* var.) obtained from a local nursery. Purification proved difficult even after methylation and the samples from *E. metallicorum* could not be freed from contamination by an isomer. However, the compound from *E. viscida* var. was obtained in a homogeneous form. The structure of this compound, **7**, $C_{21}H_{38}O_4$, was deduced from interpretation of the spectral data. The presence of a carbomethoxyl group (δ_C 176.5, s; 51.5, q; δ_H 3.65, s), two hydroxyl groups that could be etherified with trimethylsilyl chloride, and a trisubstituted double bond (δ_C 136.8, s; 123.8, d) indicated that the compound was monocyclic. In addition, the spectral parameters strongly indicated a cembrene skeleton by comparison with compounds isolated previously. Confirmation of this was obtained by AlH_3 reduction of **7** to the triol **8**, which was also isolated from the neutral portion of the extract. The structure and relative configuration of **8** rest on chemical and X-ray crystallographic studies (5,9).

A distinction between the two possibilities, **7** and **9**, proved difficult. Initially, the carbon chemical shifts in the ^{13}C-nmr spectrum were assigned, by reference to other cembrene model compounds, so as to 'best-fit' the two possibilities. However, the comparison, while slightly favoring **7**, was not decisive. A series of HMBC nmr-experiments, with J varying in value from 10 Hz to 2 Hz, revealed little that was new. Nevertheless, the long-range correlation (J=4 Hz) between the proton α- to the methyl ester (δ_H 2.4, shown to correlate with δ_C 42.8 by HMQC experiments) and the most shielded carbon (δ 20.1; C-2) indicates that the carboxymethyl group is located at C-4. Thus, the structure of (11*Z*)-15,19-dihydroxycembr-11-en-18-oic acid [**6**] is assigned to the original acidic metabolite.

To confirm this assignment, an attempt was made to convert the ester functionality in **7** into an α,β-unsaturated ester. It was expected that a mixture of compounds containing 3,4- and 4,5-unsaturation would result. Oxidative ozonolysis of this mixture would be expected to generate homoterpenyl ketone from the 3,4-ene compound as observed previously for this arrangement (6,7). To this end, the bis-trimethylsilyl ether derivative [**10**] was sulfenylated and the corresponding mixture of diastereomeric sulfides [**11**] was oxidized to the sulfoxide **12** which underwent elimination on thermolysis. The ^{1}H-nmr spectrum of the product showed that one compound predominated (90%) and included signals at δ 6.78 (t, J=7 Hz) for the β-proton of an α,β-unsaturated ester in which the ester and the vinylic hydrogen are cis. Furthermore, a comparison of the ^{13}C-nmr parameters for the major component with those of (*E*)-3,4-unsaturated cembrenes (7) showed little correlation, suggesting that the compound was a 4,5-ene. The alternative pair, arising from a carbomethoxy group at C-8, were eliminated as a possibility because the 8,9-ene contains a doubly allylic methylene group (C-10), which was not supported by the ^{1}H-nmr spectrum, and the 7,8-ene also correlated poorly with a model system (7). Thus, the major component of the thermolysis product is formulated as shown in **13**. The unexpected regio- and stereoselectivity of the conversion **12** to **13** warrants further investigation. Two sesquiterpene alcohols were identified in the neutral portion of the extracts from *E. metallicorum*. These were shown to be **14** and **15** by ^{1}H-nmr and gc-ms comparison with samples previously obtained from *E. georgei* (10).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Details have been reported previously (11).

PLANT MATERIAL.—A specimen of *E. gilesii* was deposited at the South Australian State Herbarium (RJC 4729), and specimens of *E. metallicorum* (801010 and 801013) have been deposited with the Western Australian Herbarium, Department of Agriculture, with duplicates in the Department of Chemistry. The sample of *E. viscida* var. was obtained from Jandakot Field Nursery in Western Australia.

ISOLATION PROCEDURES.—A sample of *E. gilesii* (W.A. race) (3.6 g) was extracted with Me_2CO. The extract recovered (490 mg) was subjected to vlc and elution with 30–40% EtOAc/petroleum ether gave a fraction (110 mg) which by tlc (Si gel, 40% EtOAc-petroleum ether) appeared to contain one major component (**1**, R_f 0.6). A portion was methylated with ethereal CH_2N_2 and the product was purified by rpc (Si gel, 20% EtOAc/petroleum ether) to give **2** (26 mg).

E. metallicorum fresh leaves and terminal branches (204 g), collected 45 km north of Menzies in Western Australia, were soaked in Et_2O (1.5 liters) overnight and the ethereal extract was partitioned into neutral (4.2 g), 8% aq. $NaHCO_3$-soluble (11.8 g) and 5% aq. NaOH-soluble (3.2 g) fractions. Vlc (Si gel; EtOAc to 10% MeOH; 2% gradient) of the $NaHCO_3$-soluble extract gave fractions (4.5 g; 2 to 4% MeOH/EtOAc) containing mainly one compound [**6**]. This was treated with ethereal CH_2N_2 and the product was subjected to cc (alumina, Act. III). Elution with 40% EtOAc/CH_2Cl_2 yielded the dihydroxy methyl ester **7** (2.9 g) contaminated by an isomer. The neutral fraction was dissolved in Et_2O and on standing gave a crystalline precipitate (140 mg) of the triol [**8**], identical to an authentic sample (5). The NaOH-soluble fraction appeared to consist mostly of flavones and was not investigated further.

A second sample of *E. metallicorum* (154 g), collected 40 km west of Laverton in Western Australia, was extracted in a similar manner to give three fractions; neutral (1.8 g), $NaHCO_3$-soluble (5.8 g), NaOH-soluble (1.89 g). A portion of the $NaHCO_3$-soluble extract (100 mg) was separated by prep. tlc [Si gel; $CHCl_3$-MeOH-AcOH; (90:8:2)] and the major component recovered was treated with ethereal CH_2N_2 to give a sample of **7** similar to that described above. A portion (100 mg) of the neutral fraction was subjected to prep. tlc [Si gel; $CHCl_3$-MeOH-AcOH (95:4:1)]. The major component (28 mg) was an oil. The pentane-soluble portion of this oil was analyzed by gc/ms (glc: SP2001 capillary column; 0.31 mm×25 m; temperature programmed at 6°/min from 95°) which showed it to be a mixture of 7β-hydroxy-2,6,6,8-tetramethyltricyclo[6.2.1.$0^{1,5}$]undecane [**14**] (23%; R_t 10.7 min) and 7β-hydroxyprezizaene [**15**] (77%; R_t 11.6 min).

The leaves and terminal branches of a sample of *E. viscida* var. (23.2 g) were soaked in Me_2CO for 3 days to yield an oil (2.7 g) which was fractionated into 8% aq. $NaHCO_3$-soluble (1.48 g) and neutral extracts (0.60 g). A portion (700 mg) of the acidic material was treated with CH_2N_2 and separated by cpc. Elution with EtOAc-hexane (1:3) yielded the hydroxy ester **7** (150 mg).

Acetoxy ester **2**.—Oil, M^- 394.2721. Calcd for $C_{23}H_{38}O_5$: 394.2719. Glc (column OV-101, 0.31 mm×25 m WCOT capillary; temperature programmed 120° at 2°/min from 120°): single peak at R_t 8.3 min; ^{1}H nmr (80 MHz, $CDCl_3$) δ 5.11 (1H, t, J=6.5 Hz, H-11), 4.21 (2H, AB part of ABX, H-19), 3.67 (3H, s, OCH_3), 2.02 (3H, s, acetoxymethyl protons), 1.67 (3H, s, H_3-20), 1.23 and 1.00 (each a 3H s, H_3-16, H_3-17); ^{13}C-nmr see Table 1; eims *m/z* 394 $[M]^-$ 4, 334 (6), 215 (11), 175 (18), 163 (14), 162 (11), 161 (14), 123 (53), 110 (100), 107 (58), 95 (67), 93 (69), 81 (86), 69 (84).

Dihydroxy ester **7**.—Oil, bp 145–155° (bath)/0.07 mm, $[\alpha]_D$ +63° (c=4.9; $CHCl_3$); found: C, 71.0; H, 10.9; $[M-18]^+$, 336.265; $C_{21}H_{38}O_4$ requires, C, 71.1; H, 10.8%; $C_{21}H_{36}O_3$ requires, 336.266; ir ν max (CCl_4) 3630, 1745 cm^{-1}; ^{1}H nmr (90 MHz, $CDCl_3$) δ 5.19 (1H, br s, H-11), 3.65 (3H, s, OCH_3), 3.60–3.38 (2H, m, H_2-19), 2.84 (2H, 2OH), 1.70 (3H, br s, H_3-20), 1.23 and 1.16 (each 3H, s, H_3-16 and H_3-17); ^{13}C nmr see Table 1; eims *m/z* 336 $[M-18]^+$ (19), 318 (17), 305 (21), 294 (13), 150 (17), 135 (23), 121 (35), 109 (37), 95 (55), 82 (45), 81 (55), 69 (33), 69 (100).

CORRELATION OF **2** WITH DIOL **3**.—A solution of **2** (38 mg) in dry Et_2O was treated with excess $LiAlH_4$ under N_2 at room temperature for 18 h. The product (28 mg) recovered was identical to an authentic sample of the epoxy diol **3** (6).

CORRELATION OF **7** WITH TRIOL **8**.—A solution of **7** (38 mg) in dry THF (20 ml) was added to a slurry of $LiAlH_4$ (240 mg) and $AlCl_3$ (168 mg) in THF (10 ml) and the mixture was stirred under N_2 at 38° for 22 h. The product (280 mg) recovered crystallized from aqueous MeOH as prisms and was identical to an authentic sample of the triol **8** (5).

CONVERSION OF **7** TO THE 4,5-ENE ANALOGUE **13**.—The dihydroxy methyl ester **7** (1.42 g) in dry pyridine (10 ml) was treated with trimethylsilylchloride (10 ml) and hexamethyldisilazane (20 ml) under N_2 at 80° for 4 h. Solvent and excess reagents were removed by distillation *in vacuo* and the residual oil was

dried under vacuum for 7 days. The bis-trimethylsilyl ether **10** (1.77 g) was recovered as an oil; ir ν max (CCl_4) 1745 cm^{-1}; ^1H nmr (90 MHz, $CDCl_3$) δ 5.15 (1H, m, H-11), 3.63 (3H, s, OCH_3), 3.38 (2H, m, H_2-19), 1.67 (3H, br s, H_3-20), 1.16 (6H, s, H_3-16 and H_3-17), 0.07 (18H, silyloxymethyl protons); gc-ms (column OV-101, 0.31 mm×25 m WCOT capillary; temperature programmed at 20°/min from 120°), *R*, 9.1 min, eims *m/z* 483 $[M-15]^+$ (2), 440 (5), 408 (2), 318 (2), 159 (3), 147 (3), 131 (100), 73 (22). The bis-ether **10** (1.73 g) in THF (20 ml) was added dropwise to a solution prepared from di-isopropylamine (3.5 ml) in THF (30 ml) and *n*-butyl lithium (15.6 ml, 25 mmol) at −78° under Ar and previously stirred for 30 min, and allowed to react for 30 min. The reaction mixture was allowed to warm to −35° prior to the dropwise addition of a solution of diphenyldisulfide (7.58 g) in THF (30 ml). After stirring for 30 min the reaction mixture was allowed to reach room temperature and was stirred for another 30 min. The solution was diluted with H_2O, acidified with 10% HCl and stirred for 2 h. The product (9.15 g), recovered with Et_2O, was chromatographed by vlc and elution with 50% CH_2Cl_2/petroleum ether and gave unreacted diphenyl disulfide (6.9 g). Elution with EtOAc yielded the diastereomeric mixture of phenyl sulfide **11** (1.39 g) as a pale yellow oil; ir ν max (CCl_4) 3640, 1740 cm^{-1}; ^1H nmr (90 MHz, $CDCl_3$) δ 7.51–7.15 (5H, m, aromatic protons), 5.13 (1H, m, H-11), 3.61 and 3.60 (3H, s, OCH_3), 3.46 (2H, m, H_2-19), 1.69 (3H, br s, H_3-20); 1.22 and 1.15 (each 3H, s, H_3-16 and H_3-17); eims *m/z* 462 $[M]^+$ 1, 444 (7), 426 (1), 334 (13), 218 (14), 147 (17), 135 (24), 121 (30), 109 (56), 93 (48), 81 (100). To a solution of the phenyl sulfide [**11**] (1.36 g) in CH_2Cl_2 (59 ml) under Ar at −78° was added *m*-chloroperbenzoic acid (759 mg) in CH_2Cl_2 (21 ml) and the mixture was stirred for 5 h. The product recovered from the organic layer consisted of a diastereomeric mixture of sulfoxides [**12**] (1.18 g) as an oil; ^1H nmr (90 MHz, $CDCl_3$) δ 7.51 (5H, m, aromatic protons), 5.25 (1H, m, H-11), 3.71 and 3.41 (3H, s, OCH_3), 3.61–3.39 (2H, m, H_2-19), 1.72 (3H, br s, H_3-20); 1.23 and 1.16 (each 3H, s, H_3-16 and H_3-17); eims *m/z* 334 $[M-PhSOH-18]^+$ (21), 316 (6), 302 (37), 291 (16), 262 (23), 218 (36), 186 (28), 161 (30), 147 (52), 135 (51), 125 (100), 109 (77), 93 (74), 81 (88). The mixture of sulfoxides [**12**] (1.2 g) in CCl_4 (41 ml) was heated under reflux in a N_2 atmosphere for 19 h. Evaporation of the solvent followed by cc (alumina, neutral, Act. III) and elution with CH_2Cl_2 gave benzenesulfenic acid (198 mg). Elution with 60% EtOAc/CH_2Cl_2 to 10% MeOH/EtOAc afforded an inseparable mixture of the 3,4- and 4,5-ene esters [**13**] (724 mg, 84%) as a colorless oil, bp 160–170° (bath)/0.15 mm; found: $[M-18]^+$, 334.249, $C_{21}H_{34}O_3$ requires $[M-18]^+$ 334.251; ir ν max (CCl_4) 3640, 1725 cm^{-1}; ^1H nmr (90 MHz, $CDCl_3$) of the major component [**13**] δ 6.78 (1H, m, H-5), 5.27 (1H, m, H-11), 3.71 (3H, s, OCH_3), 3.60 (2H, m, H_2-19), 1.71 (3H, br s, H_3-20), 1.23 and 1.14 (each 3H, s, H_3-16 and H_3-17); eims *m/z* 334 $[M-18]^+$ (5), 316 (2), 302 (11), 213 (8), 147 (21), 135 (23), 121 (32), 107 (41), 93 (49), 81 (100).

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