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CEDRENE ISOPRENOLOGUES FROM A NEW *EREMOPHILA* SPECIES

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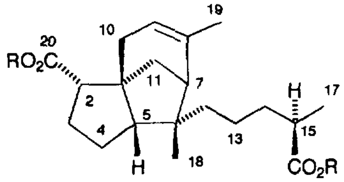
Key Word Index—*Eremophila* "*pungens*"; Myoporaceae; cedrene isoprenologues; diterpenes.

Abstract—Two new diterpenes have been isolated from a new *Eremophila* species (Myoporaceae). Their structures have been deduced by chemical correlation and spectroscopic analysis and have been shown to contain the 2-*epi*-cedrene ring system characteristic of the cedrene isoprenologue class of diterpenes.

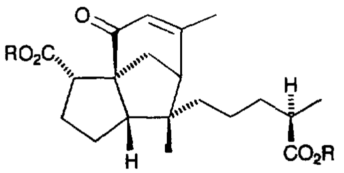
INTRODUCTION

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, in the main, are composed of flavones and oxygenated diterpenes [2]. In continuation of our study of the phytochemistry of *Eremophila* species, we had the opportunity of investigating a new species of *Eremophila*. This species, yet to be formally described, has been named *E.* "*pungens*" by Dr R. Chinnock (The Botanic Gardens of Adelaide, South Australia) who is presently revising the taxonomy of the genus. We now report on the structures of two new diterpenes isolated from the resin of this species and comment on the probable non-enzymatic origin of one of them.

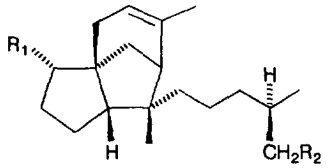
The air-dried powdered leaves and branchlets of *E.* "*pungens*" were extracted with CH_2Cl_2 and the crude extract was fractionated into acidic (10% of dry weight) and neutral fractions (2%) by extraction with aq. $NaHCO_3$. TLC of the acidic fraction showed it to consist mainly of two compounds, shown below to have structures **1** and **2**, which could not be easily separated by normal chromatographic techniques. The mixture was methylated and the methyl esters (**3** and **4**) were separated by centrifugal TLC.



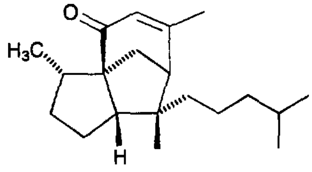
	R
1	H
3	Me



	R
2	H
4	Me



	R_1	R_2
5	CO_2H	OH
6	Me	H



7

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The less polar component (**3**) was assigned a molecular formula of $C_{22}H_{34}O_4$ from interpretation of the MS and ^{13}C NMR data. The NMR spectra showed the presence of two carbomethoxyl groups (δ_H 3.62, 3.65, *s*; δ_C 51.2, 51.7, *q*; 174.6, 177.3, *s*), a tertiary methyl (δ_H 0.97) and a trisubstituted double bond (δ_H 1.62; *d*, $J = 1.7$ Hz; δ_C 24.6, *q*). This information allows the deduction that the compound is tricyclic and the presence of two quaternary sp^3-hybridized carbons (δ_C 51.1, 54.5) strongly suggests that it contains the 2-*epi*-cedrene ring system associated with the cedrane-type diterpenes (e.g. **5**) previously found in *Eremophila* species [3, 4]. In fact, comparison of the ^{13}C NMR spectral data for **3** and **5** showed good agreement between the chemical shifts of C-1 to C-11 (Table 1).

Confirmation of the structure and stereochemistry assigned to the diester (**3**) came from its preparation from the acid alcohol (**5**). Thus, oxidation of **5** with PDC [5] followed by methylation of the derived diacid (**1**) yielded a dimethyl ester identical to **3**. The preparation of **1** allowed us to confirm that the diacid is the real metabolite, since the R_f of the less polar compound in the original acid fraction was identical to that of **1**.

Comparison of the NMR spectral parameters of **3** and the more polar compound ($C_{22}H_{32}O_4$) showed that the latter (**4**) contained a dimethyl ester and an α,β-unsaturated ketone (δ_H 5.79, *br s*; δ_C 200.7, *s*; λ_{max} 244 nm, log ε 3.9). Furthermore, the chemical shifts for C-1 and C-2 (δ_C 66.9 and 43.2) in the ^{13}C NMR spectrum of **4** were significantly shielded and deshielded, respectively, in comparison with the equivalent carbons in the spectrum of the diester (**3**) (δ_C 54.5, and 51.5) (Table 1). This paralleled the chemical shift differences for C-1 ($\Delta\delta$ 13.2 ppm) and C-2 ($\Delta\delta$ 8.3 ppm) observed between the hydrocarbon (**6**) and the conjugated ketone (**7**) prepared previously [4] (Table 1). Thus the more polar diester has the structure shown in **4**. Since in the initial acid extract the two metabolites had similar polarity, it seems likely that **4** represents the dimethyl ester of the original metabolite (**2**).

Table 1. ^{13}C NMR spectral data of selected compounds*

	Compound				
Carbon	**3**†	**5**	**4**†	**6**	**7**
C-1	54.5	54.6	66.9	54.6	67.8
C-2	51.5	51.5	43.2	41.6	33.3
C-3	29.9	29.9	30.7	35.9	38.2
C-4	23.3	23.3	24.6	23.3	23.9
C-5	60.6	60.9	57.7	60.6	56.8
C-6	51.1	51.3	45.9	51.5	46.9
C-7	48.3	48.5	52.6	48.2	51.8
C-8	140.4	140.6	167.7	141.0	167.4
C-9	119.9	120.1	124.2	120.4	125.1
C-10	42.3	42.3	200.7	41.7	204.0
C-11	34.8	34.2	39.3	35.1	37.2
C-12	35.2	35.8	37.1	33.9	34.9
C-13	22.7	22.5	22.9	22.9	23.0
C-14	34.9	34.9	34.5	40.4	40.1
C-15	39.4	35.8	39.4	28.1	28.1
C-16	174.6	68.3	174.3	22.8	22.7
C-17	17.1	16.7	17.3	22.8	22.7
C-18	24.0‡	24.2‡	24.6‡	24.4‡	25.1‡
C-19	24.6‡	24.6‡	25.5‡	24.6‡	25.5‡
C-20	177.3	179.9	177.0	13.8	14.4

*$CDCl_3$; 75 MHz except for **6** and **7** (20.1 MHz).

†Methoxyl carbon: for **3** at δ51.2, 51.7; for **4** at δ 51.4, 51.5.

‡Values in any one column may be interchanged.

The presence of **1** and **2** in the resin raises the possibility that the latter may be derived from the former by non-enzymatic allylic oxidation. In fact, this transformation had been employed by us in the preparation of cedrenone from cedrene and of the enone (**7**) from the corresponding hydrocarbon [4]. The diterpene metabolites produced by xerophilic *Eremophila* species accumulate on the leaves and branchlets where they are exposed for extended periods of time to the intense sunlight of the natural habitat. It is possible that, under these conditions, metabolites initially exuded onto the leaf surface undergo autooxidative and/or photooxidative modification. An example of autooxidation of a metabolite in the exudate of leaf glandular trichomes of the wild rose *Rosa rugosa* has been noted [6, 7]. Furthermore, we have demonstrated the involvement of a post-biosynthesis photolytic reaction of a diterpene ketone accumulating in the resin of a xerophilic *Beyeria* sp. (Euphorbiaceae) [8, 9].

Diterpenes containing the cedrene isoprenologue skeleton represent one of nine classes of diterpenes discovered in the *Eremophila* genus [2]. Previously only two examples of this class had been isolated from *E. gilesii* and varieties of *E. georgei* [3, 4]. Interestingly, these two species and *E.* "*pungens*" have been placed in the same taxonomic section in the current revision of the genus [Chinnock, pers. commun., 1995].

EXPERIMENTAL

General experimental details have been reported [10].

Isolation of metabolites from Eremophila "pungens". Air-dried powdered leaves and branchlets (50 g) of the plant (specimen number: GSR 204), collected at Yakabindie Station near Wiluna in Western Australia in August 1994, were soaked in CH_2Cl_2 (0.8 l) overnight. The extract was fractionated into $NaHCO_3$-soluble (5 g; 10% of dry wt) and neutral fractions (1.1 g). A portion (1 g) of the acidic fraction was decolorized by filtration through charcoal to give an oil which, from TLC, appeared to consist mainly of two compounds. Attempts to separate the compounds by Si gel chromatography failed. The mixture was dissolved in Me_2CO and treated with CH_3I/K_2CO_3 and the mixture of Me esters were subjected to centrifugal TLC using Si gel. Elution with 5% MeOH/EtOAc afforded fractions of (*a*) ene dimethyl ester (**3**) (20 mg) as colourless oil, $[\alpha]_D - 9°$ ($CHCl_3$; *c* 2.3); ^1H NMR(300 MHz; $CDCl_3$): δ 5.24 (1H, *br m*, H-9), 3.65 (3H, *s*, methoxy protons), 3.62 (3H, *s*, methoxy protons), 1.62 (3H, *d*, $J = 1.7$ Hz, H_3-19), 1.13 (3H, *d*, $J = 7.0$ Hz, H_3-17), 0.94 (3H, *s*, H_3-18); ^{13}C NMR: see Table. EIMS *m/z*; 362 $[M]^+$ (5), 330 (8), 302 (8), 205 (28), 187 (42), 173 (42), 164 (74), 145 (78), 131 (80), 119 (66), 118 (29), 105 (100), 91 (92); and (*b*) the enone dimethyl ester (**4**) (30 mg) as an oil, $[\alpha]_D - 99°$ ($CHCl_3$; *c* 1.6); λ_{max} (EtOH) 244 nm (log ε 3.99). ^{1}H NMR (300 MHz; $CDCl_3$): δ 5.79 (1H, *br m*, H-9), 3.66 (3H, *s*, methoxy protons), 3.58 (3H, *s*, methoxy protons), 1.96 (3H, *d*, $J = 1.3$ Hz, H_3-19), 1.14 (3H, *d*, $J = 7.0$ Hz, H_3-17), 0.92 (3H, *s*, H_3-18); ^{13}C NMR: see Table 1. EIMS *m/z*: 376 $[M]^+$ (39), 345 (36), 316 (17), 312 (13), 290 (42), 243 (42), 230 (24), 215 (56), 187 (42), 180 (50), 161 (100), 148 (56), 145 (38), 135 (26), 131 (30), 121 (32), 119 (23), 117 (23), 105 (27).

Correlation of **5** *with* **3**. The acid alcohol (**5**) (100 mg) in CH_2Cl_2 was treated with PDC (300 mg) according to the method of ref. [5] for 16 hr at room temp. The recovered product (85 mg) was dissolved in Me_2CO, treated with CH_3I/K_2CO_3 and heated under reflux. The product was purified by centrifugal TLC using Si gel. Elution with 5% MeOH/EtOAc gave a sample of the ene dimethyl ester (**3**) whose physical and spectral data was in agreement with that of the sample described above.

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