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SERRULATANE DITERPENES FROM A NEW *EREMOPHILA* SPECIES

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Key Word Index—*Eremophila 'phyllopoda'*; Myoporaceae; serrulatane diterpenes; calamanene isoprenologues.

Abstract—Two new diterpenes have been isolated from a new *Eremophila* species. Their structures have been deduced by chemical methods and spectroscopic analysis and have been shown to contain the serrulatane ring system characteristic of the calamenene isoprenologue class of diterpenes. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Eremophila species have been shown to produce a remarkable number of unusual diterpenes which characterize the resin coatings of these plants [1]. Recently, we have found that some polar metabolites from *Eremophila* have interesting cardiotonic activity [2]. In a continuation of our studies on this genus, we had the opportunity of investigating a new species of *Eremophila*. The species, yet to be formally described, has been named *E. 'phyllopoda'* 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 serrulatane diterpenes isolated from the resin of this species.

RESULTS AND DISCUSSION

The air-dried powdered leaves and branchlets of *E. 'phyllopoda'* were extracted with acetone and the crude extract was fractionated by silica gel chromatography into essentially non-polar and polar portions. The non-polar fraction, on further separation by radial chromatography, yielded three compounds which, from their physico-chemical properties, were identified as the lignans (+)-sesamin [3, 4], (+)-episesamin [3, 4] and aptosimon (9-oxosesamin) [5]. It is worth noting, that the structure initially assigned [5] to aptosimon has since been corrected [6]. High field NMR spectral parameters for aptosimon are not available in the literature and have been listed in the experimental section.

The more polar fraction, from TLC, consisted of one major and a minor component which were separated and, as discussed below, have been shown to have structures **1** and **5**. The less polar component (**1**) was assigned a molecular formula of $C_{20}H_{30}O_3$ from HR mass spectrometry and ^{13}C NMR data. The NMR spectra showed the presence of two unsubstituted aromatic carbons in a *meta*- arrangement (δ_H 6.48 and 6.52, *br s*, $W_{h/2}$ 4 Hz; δ_C 122.3, 115.3, *d*), two benzylic methines (δ_H 2.73 and 3.31, *m*), a primary alcohol group (δ_H 3.59, *dd*, J = 10.0, 3.4 Hz, and 3.74, *dd*, J = 10.0, 7.4 Hz, AB part of an ABX, δ_C 68.0, *t*), a secondary alcohol (4.22, *m*, δ_C 67.1, *d*) and a trisubstituted double bond (δ_H 5.15; *tqq*; δ_C 124.7, *d*; 131.4 *s*) associated with two vinylic methyl groups (δ_H 1.69, *d*, J = 0.7 Hz; 1.61, *d*, J = 1.2 Hz; δ_C 25.7, 17.6, *q*). This information allows the deduction that the compound is bicyclic and strongly suggests that it contains the serrulatane skeleton, examples of which have been previously found in *Eremophila* species [1]. The presence of a shielded secondary methyl group (δ_H 0.62, *d*; J = 6.7 Hz) was the most significant difference between the ^1H NMR spectrum of **1** and those of related serrulatanes which show the same methyl group at $\delta_H \sim 1.0$. This shielding effect has been shown to occur when a secondary hydroxyl group is located at C-3 [7]. In fact, COSY experiments showed that the benzylic methine at δ_H 2.73 was coupled to the hydroxymethine (δ_H 4.22). HMQC and HMBC experiments were consistent with the gross structure proposed for **1** and helped in the assignment of the carbon chemical shifts (Table 1). Treatment of **1** with tosyl chloride in pyridine yielded a mixture of monotosylate **2**, ditosylate **3** and the cyclic ether **4**. Spin-decoupling experiments on the monotosylate **2** were used to extract some key coupling constants. In par-

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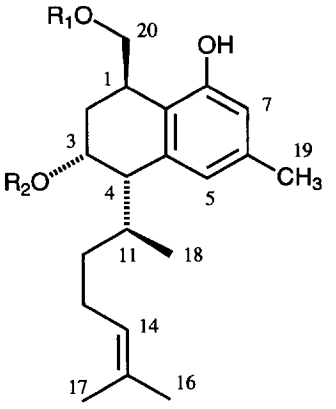

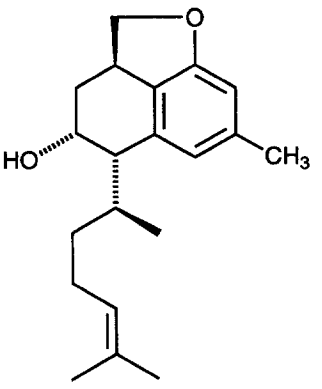

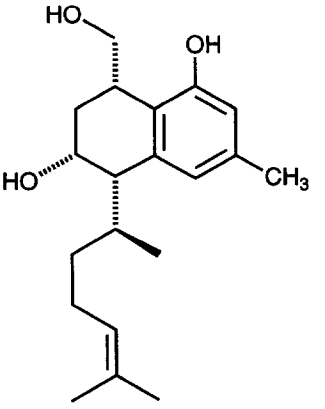

1 $R_1 = H$ $R_2 = H$
2 $R_1 = OTs$ $R_2 = H$
3 $R_1 = OTs$ $R_2 = OTs$

4

5

Table 1. ^{13}C NMR spectral data of compounds **1–5***

C	1	2†	3†	4	5
1	35.4	36.6	33.2	35.8	35.0
2	31.2	30.5	30.4	30.8	31.8
3	67.1	66.4	71.5	66.9	67.1
4	48.6	48.5	48.2	48.6	48.7
5	122.3	121.0	122.2‡	122.6	122.3
6	136.6	136.9	137.7	136.3	136.9
7	115.3	128.9	129.0	114.2	115.8
8	154.4	148.1	147.8	153.3	154.7
9	121.1	126.0	121.5‡	119.7	121.4
10	138.8	140.6	140.7	139.7	138.6
11	30.7	29.6	29.1	30.6	30.7
12	37.3	37.8	37.7	37.4	37.0
13	26.3	26.1	26.1	26.3	26.3
14	124.7	124.5	124.4	124.6	124.7
15	131.4	131.5	131.6	131.6	131.6
16	25.7	25.6	25.7	25.7	25.7
17	17.6	17.7	17.7	17.7	17.7
18	18.9	18.9	18.9	19.0	19.0
19	21.0	21.6	21.1	21.1	21.1
20	68.0	65.7	65.9	48.3	69.1

* $CDCl_3$; 75 MHz except for **1** (125 MHz).
† Signals for the tosyl groups have been omitted.
‡ Values may be interchanged.

ticular, decoupling the C-4 methine proton reduced the H-3 to a *dd* with $J = 10.5$ and 4.5 Hz to the C-2 methylene protons and allowed a coupling of 4.5 Hz to be deduced for the H-3/H-4 interaction. NOESY measurements on **1** clearly indicated interactions between one of the H-20 protons with H-3, H-4 and H-3 and H-5 with the secondary methyl at C-11.

A compound with similar oxygenation pattern, but containing an extra phenolic group at C-7, has been reported previously as a metabolite of *E. duttoni* [7]. A derivative of this compound showed $J_{3,4}$ 4.5, $J_{2a,3}$ 2.8 and $J_{2b,3}$ 11.0 Hz which is consistent with the values 4.5, 4.5 and 10.5 Hz, respectively obtained for the compounds derived from **1**.

The more polar compound (**5**), isolated in much smaller quantities, had an identical molecular formula to **1** and showed NMR spectroscopic properties indistinguishable from those of **1** (Table and Experimental). The only significant differences were due to the methylene protons at C-20 which appeared as a tight AB pattern over a 0.1 ppm range and the chemical shift value of C-20 (δ_C 69.1) compared to that of C-20 (68.0) in **1**. The multiplicity of H-3 (*dt*, $J_{3,4}$ 3.5, $J_{2a,3}$ 3.5 and $J_{2b,3}$ 9.5) was similar to that observed for **1**, suggesting that **5** was epimeric at C-1. Moreover, NOE measurements still showed interaction between H-3 and H-4 but lacked interaction between H-3 and either of the C-20 protons.

MM2 energy minimization procedures [8] revealed an interesting situation. In both **1** and **5** the conformations of the cyclohexene ring approximates to that of a half-chair; except that the C-20 methylene hydroxyl group is pseudoaxial in **1** and pseudoequatorial in **5**. The calculated dihedral angles for C1-C2-C3-C4 were $-61°$ ($-52°$ for **5**), C1-C2-C3-O3, 172° ($-177°$); C3-C2-C1-C10, 48° (20°); C3-C2-C1-C20, $-76°$ (149°) and C2-C3-C4-C5 43° (57°). The close contact distances for **1** were H20a-H3, 2.239 Å; H3-H4, 2.279 Å; H4-H5, 2.536 Å; H2ax-H12 2.113 A[faaN]; for **5**; H1-H2eq, 2.296 A[faaN]; H20a-H2ax, 2.476 Å; H3-H4, 2.324 Å; H4-H5, 2.400 Å and C_{18}H-3-H2ax, 2.010 Å. All of these are in excellent agreement with the NOE observed for the two compounds.

In attempts to study the aerial oxidation of **1**, the compound was heated to 250° under a stream of air. The reaction mixture consisted of starting material and the epimer (**5**) (50%), the latter presumably arising from a reaction in which epimerization occurs via a benzylic radical.

Of the nine classes of diterpenes produced by *Eremophila* species, the serrulatane class appears to be

the most commonly represented one [1]. Interest in this class of compounds has been stimulated by the finding that the *seco*-pseudopterosins, isolated from sea whips *Pseudopterogorgia* spp, and their biogentically related tricyclic congeners, the pseudopterosins (tricyclic structures arising from an extra bond between C-5 and C-13), have potent inflammatory and analgesic activities [9]. It is of some interest to note that the pseudopterosins occur as diastereoisomers with different configuration at C-1 [9] as observed in the present case for the *Eremophila* metabolites **1** and **5**. The absolute configuration of **1** and **5** is tentatively assumed to be that shown in which the asymmetric carbons at C-4 and C-11 retain the configuration established for the serrulatanes [1]. The possibility that these compounds have the enantiomeric configuration at these positions cannot be excluded, a situation which again has a precedent in the pseudopterosin group of compounds [9].

EXPERIMENTAL

General experimental details have been reported [10].

Plant material. Leaves and branchlets of the plant were collected at Woodleigh Station near Wiluna in Western Australia in October 1993 (specimen number: GSR 9301). Specimens have been deposited with Dr R. Chinnock, The Botanic Gardens of Adelaide and State Herbarium.

Isolation of metabolites from Eremophila phyllopoda. Air-dried powdered leaves and branchlets (100 g) of the plant were soaked in Me_2CO (1 l) overnight. The extract (8 g) was fractionated by chromatography on silica gel. The components of a fr. obtained with petrol–EtOAc (4:1) were sepd by radial chromatography. Elution with petrol–diisopropyl ether (4:1) afforded *a*) (+)-episesamin (20 mg), mp 118–120° $[\alpha]_D$ +128.5° ($CHCl_3$; *c* 1.0); lit. [3] mp 122–123°, $[\alpha]_D$+118.6° ($CHCl_3$); ^1H and ^{13}C NMR in agreement with those reported in the literature [4]; and *b*) (+)-sesamin (50 mg), mp 120–121°, $[\alpha]_D$+74.7° ($CHCl_3$; *c* 1.3); lit. [3] mp 122–123°, $[\alpha]_D$ +68.1° ($CHCl_3$); ^1H and ^{13}C NMR in agreement with those reported in the literature [4]. A fr. obtained with petrol–EtOAc (7:3) on further purification yielded aptosimon (15 mg); ^1H NMR ($CDCl_3$, 300 MHz): δ 6.86–6.72 (6H, *m*, H-2,2′,5,5′,6,6′), 5.95 and 5.93 (each 2H, *s*, O-CH_2-O), 5.28 (1H, *d*, *J* = 3.7 Hz, H-7), 5.26 (1H, *d*, *J* = 3.7 Hz, H-7′), 4.30 (1H, *dd*, *J* = 9.5, 6.8 Hz, H-9a), 3.98 (1H, *dd*, *J* = 9.5, 4.7 Hz, H-9b), 3.40 (1H, *dd*, *J* = 9.1, 3.7 Hz, H-8′), 3.18 (1H, *m*, H-8). ^{13}C NMR ($CDCl_3$, 75 MHz): 176.6 (C-9′), 148.3 (C-3), 148.0 (C-3′), 147.9 (C-4′), 147.2 (C-4), 134.3 (C-1), 133.0 (C-1′), 119.0 (C-6), 118.7 (C-6′), 108.5 (C-2), 108.3 (C-2′), 105.9 (C-5), 105.7 (C-5′), 101.4 and 101.1 (2 × O-CH_2-O), 84.3 (C-7), 83.3 (C-7′), 72.6 (C-9), 53.2 (C-8′), 49.9 (C-8).

Frs (900 mg), obtained from chromatography of the original extract by elution with petrol–EtOAc (1:3) and EtOAc, were combined and subjected to radial chromatography (10–20% MeOH–$CHCl_3$) to yield the epimeric trihydroxy-serrulatanes (**1**) (600 mg) and (**5**) (15 mg) in order of elution.

1β-Trihydroxyserrulatane (**1**). Gum, $[\alpha]_D$+63.0° ($CHCl_3$; *c* 1.0). ^1H NMR ($CDCl_3$, 500 MHz): δ 6.52 (1H, *br s*, H-7), 6.48 (1H, *br s*, H-5), 5.15 (1H, *tqq*, *J* = 7.1, 1.2, 0.7 Hz, H-14), 4.22 (1H, *m*, H-3), 3.74 (1H, *dd*, *J* = 10.0, 7.4 Hz, H20a), 3.59 (1H, *dd*, *J* = 10.0, 3.4 Hz, H-20b), 3.31 (1H, *m*, H-1), 2.73 (1H, *m*, H-4), 2.20 (3H, *s*, H_3-19), 2.11 (1H, *m*, H-11), 2.04 (2H, *m*, H-13), 2.02 and 1.73 (each 1H, *m*, H-2a,b), 1.69 (3H, *d*, *J* = 0.7 Hz, H_3-16), 1.61 (3H, *d*, *J* = 1.2 Hz, H_3-17), 1.55 and 1.33 (each 1H, *m*, H-12a,b), 0.62 (3H, *d*, *J* = 6.7 Hz, H_3-18). ^{13}C NMR: Table 1; assignments were made with the aid of HMQC and HMBC measurements. EI-HRMS: $[M]^+$ found: 318.22057; $C_{20}H_{30}O_3$ requires: *m/z* 318.21950. EI-MS *m/z* (rel. int.): 318 (M^+, 14), 300 (5), 268 (12), 218 (24), 213 (13), 200 (14), 187 (59), 185 (48), 175 (26), 171 (26), 161 (28), 159 (28), 149 (11), 147 (24), 91 (100), 69 (63).

1α-Trihydroxyserrulatane (**5**). Gum, $[\alpha]_D$+48.1° ($CHCl_3$; *c* 0.8). ^1H NMR ($CDCl_3$, 300 MHz): δ 6.54 (1H, *br s*, H-7), 6.51 (1H, *br s*, H-5), 5.14 (1H, *tqq*, *J* = 7.0, 1.2, 0.7 Hz, H-14), 4.20 (1H, *dt*, *J* = 9.5, 3.5, 3.5 Hz, H-3), 3.70 (2H, AB *m*, H_2-20), 3.33 (1H, *m*, H-1), 2.72 (1H, *br d*, *J* = 3.5 Hz, H-4), 2.21 (3H, *s*, H_3-19), 2.15–1.95 (3H, *m*, H-2a, H-13), 1.70–1.50 (1H, *m*, H-2b), 1.68 (3H, *d*, *J* = 0.7 Hz, H_3-16), 1.61 (3H, *d*, *J* = 1.2, H_3-17), 1.40–1.15 (2H, *m*, H-12), 0.65 (3H, *d*, *J* = 6.7 Hz, H_3-18). EI-HRMS: $[M]^+$ found: 318.21843; $C_{20}H_{30}O_3$ requires: *m/z* 318.21950.

Tosylation of **1**. A soln of **1** (300 mg) in CH_2Cl_2 was treated with tosyl chloride (0.8 g) and DMAP (0.5 g) for 16 hr. The product recovered, which appeared from TLC to contain mainly three products, was subjected to radial chromatography (silica gel; elution with petrol with incremental addition of $CHCl_3$) to afford the monotosylate **2** (60 mg), the ditosylate **3** (40 mg) and the cyclic ether **4** (30 mg).

Monotosylate (**2**). Gum, ^1H NMR ($CDCl_3$, 300 MHz): δ 7.7 and 7.25 (each 2H, AA′BB′, tosyl protons), 6.89 (1H, *d*, *J* = 1.2 Hz, H-7), 6.78 (1H, *br s*, H-5), 5.12 (1H, *tqq*, *J* = 7.0, 1.2, 0.8 Hz, H-14), 4.21 (1H, *m*, H-3), 3.69 (1H, *br d*, *J* = 10 Hz, H-20a), 3.37 (1H, *br t*, *J* = 10 Hz, H-20b), 2.87 (1H, *m*, H-1), 2.78 (1H, *br d*, *J* = 4.5 Hz, H-4), 2.39 (3H, *s*, tosyl methyl), 2.24 (3H, *s*, H_3-19), 1.68 (3H, *d*, *J* = 0.8 Hz, H_3-16), 1.60 (3H, *d*, *J* = 1.2 Hz, H_3-17), 0.34 (3H, *d*, *J* = 6.9 Hz, H_3-18); ^{13}C NMR: Table 1.

Ditosylate (**3**). Gum, ^1H NMR ($CDCl_3$, 300 MHz): δ 7.70, 7.62, 7.30, 7.24 (each 2H, two AA′BB′ systems, tosyl protons), 6.91 (1H, *br d*, *J* = 1.0, H-7), 6.75 (1H, *br s*, H-5), 5.10 (1H, *tqq*, *J* = 7.0, 1.2, 0.7 Hz, H-14), 4.06 (1H, *m*, H-3), 4.01 (1H, *dd*, *J* = 9.7, 3.6 Hz, H-20a), 3.73 (1H, *t*, *J* = 9.7, 9.7 Hz, H-20b), 2.98 (1H, *m*, H-1), 2.73 (1H, *br s*, H-4), 2.43, 2.40 (each 3H, *s*, tosyl methyl). 2.25 (3H, *s*, H_3-19), 1.68 (3H, *br s*, H_3-

16), 1.60 (3H, *br s*, H_3-17), 0.29 (3H, *d*, $J = 6.9$ Hz, H_3-18); ^{13}C NMR: Table 1.

Cyclic ether (**4**). Gum, $[\alpha]_D = +0.13°$ ($CHCl_3$; *c*, 0.7); ^1H NMR ($CDCl_3$, 300 MHz): δ 6.53 (1H, *br s*, H-7), 6.40 (1H, *br s*, H-5), 5.15 (1H, *tqq*, $J = 7.0$, 1.2, 0.7 Hz, H-14), 4.29 (1H, *dt*, $J = 9.8$, 4.1, 4.1 Hz, H-3), 3.93 (1H, *dd*, $J = 6.6$, 1.0 Hz, H-20a), 3.44 (1H, *m*, H-1), 3.47 (1H, *dd*, $J = 6.6$, 1.5 Hz, H-20b), 2.81 (1H, *br t*, $J = 3.5$, H-4), 2.23 (3H, *s*, H_3-19), 2.23–1.98 (4H, *m*, H2a, 11, 13), 1.69 (3H, *d*, $J = 1.2$ Hz, H_3-16), 1.61 (3H, *d*, $J = 0.7$ Hz, H_3-17), 1.70–1.50 (2H, *m*, H-2b,12a), 1.35 (1H, *m*, H-12b), 0.64 (3H, *d*, $J = 6.9$ Hz, H_3-18). EI-HRMS: $[M]^+$ found: *m/z* 300.20780; $C_{20}H_{28}O_2$ requires: *m/z* 300.20893.

Conversion of **1** *to* **5**. A sample of **1** (20 g) in a porcelain dish was heated to 250° under a stream of air for 30 min. TLC analysis showed that the product consisted of a mixt. of **1** and **5** and some polymeric material. Chromatographic sepn of the epimers, as described above, yielded the two compounds whose identity was established by comparison of the spectral data.

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