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GERMACROLIDES FROM *PERYMENIUM BERLANDIERI**

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Key Word Index—*Perymenium berlandieri*; Compositae; Heliantheae; sesquiterpene lactones; germacrolides.

Abstract—Two germacrolides have been isolated from the aerial parts of *Perymenium berlandieri*. Their structures were established as 15-acetylstenophyllolide[15-acetoxy-9β-hydroxygermacra-1(10),4,11(13)-trien-6α,12-olide] and 15-acetoxy-8α-hydroxycostunolide[15-acetoxy-8α-hydroxygermacra-1(10),4, 11(13)-trien-6α, 12-olide] by spectroscopic means and chemical transformations. A guaianolide was formed by spontaneous cyclization of 15-acetoxy-8α-hydroxycostunolide.

INTRODUCTION

The secondary metabolites isolated from the *Perymenium* species analysed so far have been kaurane derivatives [1–3] and sesquiterpene lactones [2–5]. The lactones are structurally varied and include eudesmanolides [4, 5], melampolides [2, 5] as well as germacrolides [2, 3]. In a continuation of our chemical studies of the genus *Perymenium*, we have analysed the aerial parts of *P. berlandieri* DC. This investigation resulted in the isolation of the new natural product 15-acetylstenophyllolide (**1**) and 15-acetoxy-8α-hydroxycostunolide (**3**) which had been obtained from cnicin [6] and isolated from *Dicoma schinzii* [7]. Compound **3** undergoes a spontaneous cyclization process yielding the guaianolide **7**.

RESULTS AND DISCUSSION

Compound **1** showed IR absorption indicative of the presence of hydroxyl (3475 cm^{-1}), α,β-unsaturated-γ-lactone (1762 cm^{-1}), saturated ester (1739 cm^{-1}) and double bond (1662, 966 cm^{-1}) functionalities. Its structure was formulated as that of a germacrolide on the basis of the characteristic ^1H NMR signals for H-1, H-5, H-6, H-9, H-13, H-14 and H-15, which were assigned by COSY experiments (Table 1). The coupling constants of H-5, H-6 and H-13 established a *Z*-geometry for the C-4 double bond and a *trans*-C-6 lactone ring closure. The position of the acetoxy group was inferred from the chemical shift of the H-15 singlet signal, while the free hydroxyl group was located on C-9 with a β-orientation taking into account the values of the H-9 coupling constants (J = 10.5 and 3 Hz). The ^{13}C NMR signals are in agreement with structure **1** for this compound.

Upon acetylation **1** gave **2**, which had been previously reported as stenophyllolide diacetate [8] or idomain [9]. The structure of this compound was corroborated by X-ray analysis [8]. Comparison of the spectral data of these diacetates established their identity and therefore corroborated the structure of **1** as 15-acetylstenophyllolide.

Compound **3** was obtained as crystals and its structure established as the previously reported 15-acetoxy-8α-hydroxycostunolide [6, 7] from its spectral data. Additional proof for structure **3** was provided by the preparation of the oxo derivative **4**, in whose ^1H NMR spectrum the C-9 protons appeared as an AB system. Furthermore, the H-7 signal was shifted to lower field (δ4.02) as expected for an allylic proton, α to a carbonyl.

On standing, the colourless crystals of **3** became yellowish. TLC revealed its partial transformation into a mixture, with the major component being a less polar compound, which could not be isolated. Acetylation of this mixture gave the derivatives **5** and **6**. The structure of **5** was formulated as 8α,15-diacetoxycostunolide from its spectral data. Derivative **6** was determined to be an 8,15-diacetoxyguaianolide based on the following evidence. Its ^1H NMR spectrum did not show the characteristic signals for a germacradiene (H-1, H-5 and vinylic methyl group), instead, two broad singlets at δ5.11 and 5.02 showed the presence of an exomethylene group attached to C-10 (^{13}C NMR: δ142.1 *s*, C-10; δ116.6 *t*, C-14). Furthermore, the signals for H-1 and H-5 were located at δ2.92 and 2.34, respectively (COSY spectrum), and signals for C-1 and C-5 appeared as doublets at δ48.6 and 46.8, respectively. The signals for the C-15 protons, geminal to the acetoxy group, appeared as the AB part of an ABX system at δ4.23 and 4.09, and were coupled with

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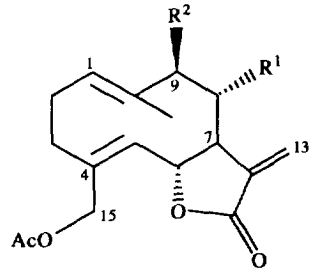
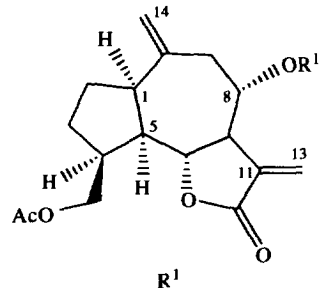


	R^1	R^2
1	H	OH
2	H	OAc
3	OH	H
4	O	H
5	OAc	H



	R^1
6	Ac
7	H

Table 1. ^{1}H NMR spectral data of **1–6** (200 MHz, $CDCl_3$, TMS as int. standard)

H	1	2	3	4	5	6†
1	5.16 *br dd*	5.27 *br dd*	4.91 *br dd*	5.20 *br dd**	4.8–5.1*	2.92 *dt*
	9.5, 6	9.5, 6	11, 4.5	~ 10, 3.3		7.4, 6.6
5	4.83 *br d*	4.87 *br d*	4.81 *d*	5.24 *d**	4.8–5.1*	2.43 *m**
	10	9	9	10	4.8–5.1*	10.5, 6.6
6	4.59 *dd*	4.61*	4.60 *t*	4.89 *dd*		4.07 *dd*
	10, 9		9	10, 9		10.5, 9.5
7	2.69 *m*	2.74 *br t*	2.81 *br tt*	4.02 *dt*	3.00 *br s*	3.06 *br tt*
		9	9, 3	9, 3	4.8–5.1*	9.5, 3.3
8	1.88 *dt*	1.94 *dd*	4.03 *ddd*			4.91 *m**
	14.5, 10.5	14.5, 10	11, 9, 3			
9	4.22 *dd*	5.23 *dd*	2.64 *br d*	3.44 *d*		2.79 *dd*
	10.5, 3	10, 3	12.6	9.5		13, 5.4
			2.38 *dd*	3.00 *br d*		2.10*
			12.6, 11	9.5		
13	6.30 *d*	6.32 *d*	6.34 *dd*	6.34 *d*	6.34 *dd*	6.23 *d*
	3.5	3.5	3.2, 1.5	3.5	3,5, 1	3.3
13′	5.62 *d*	5.58 *d*	6.31 *dd*	5.47 *d*	5.81 *dd*	5.67 *d*
	3.2	3.2	3, 1.5	3	3, 1	2.4
14	1.41 *d*	1.42 *d*	1.38 *d*	1.50 *s*	1.49 *s*	5.11 *br s*
	1	1	1			5.02 *br s**
15	4.61 *s*	4.61 *s*	4.53 *s*	4.43 *s*	4.58 *s*	4.23 *dd*
						11, 5.5
						4.09 *dd*
						11, 9
OAc	2.10 *s*	2.10 *s*,	2.04 *s*	2.06 *s*	2.08 *s*	2.12 *s*
		2.07 *s*			2.07 *s*	2.03 *s*

*Partially superimposed signals.
†H-4 signal at δ2.54 as multiplet.

a partially superimposed multiplet at δ2.54 (X-proton) assigned to H-4. The relative configuration of **6** was inferred from the observed J values as compared with similar compounds [10, 11], except that of C-4.

From the above data we can infer that germacrolide **3** produced the guaianolide **7**, which upon acetylation gave the derivative **6**. Considering the cyclization reaction depicted in Fig. 1 as a stereospecific process, probably catalysed by traces of acid, in which **3** adopts the preferred UU conformation [12], the stereochemistry at C-4 in **7** and consequently in **6**, can be proposed with the

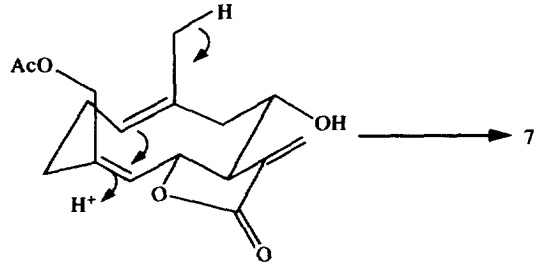


Fig. 1. Acid catalysed rearrangement of **3** to form **7**.

Table 2. ^{13}CNMR spectral data of **1**, **3** and **6** (50 MHz, $CDCl_3$, TMS as int. standard)

C	1	3	6
1	128.6 *d*	128.8 *d*	48.6 *d*[a]
2	26.0 *t*	26.4 *t*	28.2 *t*[b]
3	35.5 *t*	35.2 *t*	28.0 *t*[b]
4	138.1 *s*[a]	138.1 *s*[a]	42.2 *d*
5	130.8 *d*	138.8 *d*	46.8 *d*[a]
6	79.5 *d*	75.9 *d*	77.1 *d*
7	47.3 *d*	54.7 *d*	50.5 *d*
8	35.2 *t*	71.0 *d*	74.1 *d*
9	79.2 *d*	52.5 *t*	41.1 t
10	139.4 *s*[a]	138.8 *s*[a]	142.1 *s*
11	138.4 *s*[a]	136.3 *s*[a]	136.7 *s*
12	170.7 *s*	170.7 *s*	171.0 *s*
13	120.5 *t*	126.8 *t*	123.2 *t*
14	10.7 *q*	17.0 *q*	116.6 *t*
15	61.5 *t*	61.8 *t*	64.1 *t*
MeCO	20.9 *q*	20.9 *q*	21.2 *q*
			21.0 *q*
MeCO	169.7 *s*	170.5 *s*	169.8 *s*

[a,b]Values in any vertical column may be interchanged.

CH_2OAc residue β-oriented. Guaianolides **6** and **7** are both unstable and undergo total decomposition.

Perymenium berlandieri showed a chemical composition in accordance with that observed in other *Perymenium* species, in which C-14 and/or C-15-oxygenated germacrolides are present.

EXPERIMENTAL

Dried and powdered aerial parts (480 g) of *Perymenium berlandieri* DC collected in Morelos State, México (voucher specimen deposited at the National Herbarium MEXU 597604), were extracted with Me_2CO. After solvent evapn 19.25 g of extract were obtained. This extract was dissolved in MeOH–H_2O(4:1) and extracted with hexane. The remaining MeOH soln was evapd and H_2O (50 ml) added. The resulting aq soln was extracted with EtOAc. After solvent evapn, 6.3 and 8.25 g of residues were obtained from the hexane and EtOAc frs, respectively. The EtOAc fr. was subjected to CC (silica gel, Hexane–EtOAc gradient elution). Frs eluted with hexane–EtOAc (7:3) contained **1**, which was purified by CC (silica gel hexane–Me_2CO, 17:3) and percolation through activated charcoal. Compound **1** (714.5) mg was obtained as a liquid. $[\alpha]_D$ + 75.86 ($CHCl_3$; *c* 0.261); IR ν_{max}^{film} cm^{-1}: 3475, 1762, 1739, 1662, 1375, 1235, 1144, 1029, 966, 756; MS 70 eV *m/z* (rel. int.): 306 $[M]^+$ ($C_{17}H_{22}O_5$) not observed. 289 (2), 264 (1), 246 (6), 228 (9), 91 (40), 43 (100). Compound **3** (802.3 mg) was isolated from frs. eluted with hexane–EtOAc (4:1) and purified by crystallization from EtOAc–hexane. Crystals, mp 85–87°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3503, 1757, 1742, 1652, 1445, 1374, 1144, 1026, 963; MS 70 eV *m/z* (rel. int.): 306 $[M]^+$ ($C_{17}H_{22}O_5$) (< 1), 246 (2), 228 (4), 91 (22), 55 (34), 43 (100).

Acetylation of compound **1**. A soln of **1** (46.2 mg) in pyridine (0.5 ml) and Ac_2O (0.5 ml) was left to stand at room temp. for 3 hr, then poured into ice. After the usual work-up and crystallization from EtOAc–hexane, 22.4 mg of **2** were obtained as crystals which at 155–158° softened but did not melt and above 200° decomposed. $[\alpha]_D$ + 71.8 ($CHCl_3$; *c* 0.212); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1766, 1732, 1664, 1372, 1139, 1023, 971; MS 70 eV *m/z* (rel. int.): 348 $[M]^+$ ($C_{19}H_{24}O_6$) not observed, 289 (23), 247 (10), 229 (21), 201 (12), 91 (27), 43 (100).

Oxidation of compound **3**. Jones reagent was added dropwise to a stirred soln of **3** (62.3 mg) in Me_2CO (5 ml) at 0°. Compound **4** (17.2 mg) was obtained after the usual work-up and CC (silica gel, hexane–EtOAc) as a liquid; MS 70 eV *m/z* (rel. int.): 304 $[M]^+$ ($C_{17}H_{20}O_5$) unobserved, 276 (3), 91 (43), 69 (48), 43 (100).

Acetylation of compound **3**. A soln of 214.3 mg of **3** (partially decomposed) in pyridine (2 ml) and Ac_2O (2 ml) was left to stand at room temp. for 90 min and then poured into ice. The reaction mixture was worked-up as usual. TLC revealed the presence of two major components, which were purified by CC (silica gel, hexane–EtOAc 7:3). The more polar **5**, was a liquid (46.1 mg); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765, 1740, 1657, 1450, 1373, 1142, 1045, 960; MS 70 eV *m/z* (rel. int.): 348 $[M]^+$ ($C_{19}H_{24}O_6$) not observed, 306 (< 1), 289 (< 1), 246 (1), 228 (4), 91 (16), 79 (11), 43 (100). Compound **6** (37.6 mg) was also obtained; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1766, 1737, 1642, 1450, 1369, 1143, 1023, 960, 910; MS 70 eV *m/z* (rel. int.): 348 $[M]^+$ ($C_{19}H_{24}O_6$) unobserved, 306 (< 1), 228 (16), 91 (13), 79 (9), 43 (100).

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