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SESQUITERPENE LACTONES FROM *CHAENACTIS DOUGLASII*

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Key Word Index—*Chaenactis douglasii*; Compositae; sesquiterpene lactones; guaianolides; germacranolide.

Abstract—The investigation of the aerial parts of *Chaenactis douglasii* yielded two new closely related guaianolides and one new germacranolide, in addition to two known guaianolides. The structures were elucidated by spectroscopic methods.

INTRODUCTION

Chaenactis douglasii, a native to northwest America, is a species complex with resinous leaves rich in sesquiterpene lactones. As has been suggested [1], its genetic variability seems to be reflected in a variability of its chemical constituents. Previous investigations of the major sesquiterpene lactones from *C. douglasii* yielded different germacranolides from different collections [2, 3]. Eupatoriopicrin, reported as a major constituent from a Texas population [2], was not detected in a further investigation of a Montana population, which yielded eupaformosanin and douglasine as the major constituents.

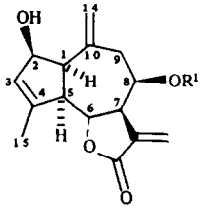
Our investigation of a population in British Columbia yielded two new guaianolides (**1**, **4**) as well as other closely related known guaianolides (**2**, **3**), along with a new germacranolide (**5**). Douglasine [3] was also present in this collection. All of the sesquiterpene lactones identified from *C. douglasii* thus far contain tiglic acid or oxidized tiglic acid side chains at C-8.

RESULTS AND DISCUSSION

The terpenoid mixture obtained from *C. douglasii* methanol–methylene chloride extract yielded sesquiterpene lactones **1**–**5** and douglasine [3] as major constituents upon repeated CC and TLC.

^{1}H NMR spectra of **1**–**5** contained the characteristic methylene doublets (δ 5.40–6.30) for the α-methylene-γ-lactone moiety. All of the compounds also exhibited typical ^{1}H NMR triplets at δ 6.81–6.96 (H-3′) as well as doublets (H-4′) around δ 4.40, indicative of the presence of a tigloyl ester side chain. The position of the H-5′ signal varied depending on the presence of acetylation or hydroxylation at C-5′. In all compounds, hydrogens on C-8 were more deshielded (δ 5.58–5.82) than hydrogens on C-6 (δ 4.49–5.23). This is in agreement with shifts generally observed for an ester group at C-8 and a lactonic hydrogen at C-6 rather than vice-versa.

A 2D-COSY ^{1}H NMR spectrum of 8 β-(4′-hydroxy, 5′-acetoxy tigloyloxy)-preeupatundin (**1**) yielded most of the coupling data while ambiguous couplings were ascertained by individual double irradiation experiments. The coupling constants were obtained from a 2D-J-resolved ^{1}H NMR experiment. A *trans*-diaxial relationship at H-5–H-6–H-7 was apparent from the large $J_{5,6}$ (10.5 Hz) and $J_{6,7}$ (8.5 Hz). The small value for $J_{7,8}$ (2 Hz) and the value for $J_{1,2}$ (6.2 Hz) required H-8 and H-1 to be α-oriented according to Dreiding models. The assignments in Table 1 are in agreement with those for a very similar guaianolide, eupahakonesin [4], for which the same stereochemistry was assigned. As expected, NOEs were observed between H-1 and H-5, H-5 and H-7, H-7 and H-8 while the NOE between H-6 and H-9 called for a boat conformation of the seven-membered ring which would



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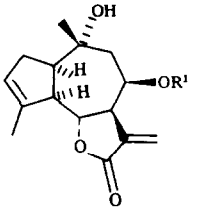

3 $R^1 = R^3$
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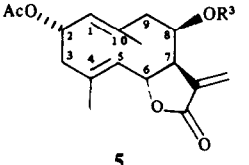

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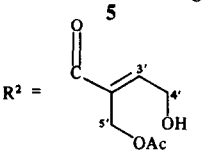

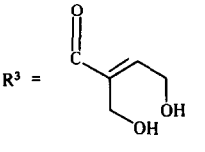

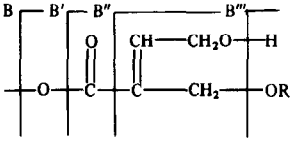

Table 1. ^{1}H NMR spectral data of **1**, **4**, **5** (400 MHz, TMS as internal standard)

H		**1** (CD_2Cl_2)	**4** (CD_2Cl_2)	**5** ($CDCl_3$)
1		3.16 *br dd* (8, 6.2)*	2.58 *br dd* (8, 8)	4.96 *br d* (10)
2	α	4.69 *dd* (6.2, 1)	2.26 *m*	5.21 *m*
	β			
3	α	5.73 *br s*	5.53 *br s*	2.55 *m*
	β			2.39 *m*
5		2.67 *br dd* (8, 10.5)	2.73 *br dd* (7.5, 11)	4.98 *br d* (10)
6		4.63 *dd* (10.5, 8.5)	4.49 *dd* (11, 9)	5.24 *dd* (9, 10)
7		3.22 *m*	3.94 *m*	2.98 *m*
8		5.58 *ddd* (2, 6.6, 7.4)	5.72 *ddd* (7, 7, 2.5)	5.82 *m*
9	α	2.79 *dd* (13.6, 7.4)	2.32 *dd* (14, 7)	2.35 *dd* (5, 14)
	β	2.83 *dd* (13.6, 6.6)	1.93 *dd* (14, 7)	2.84 *m*
13a		6.22 *d* (3.5)	6.17 *d* (3)	6.29 *d* (3.5)
13b		5.52 *d* (3)	5.42 *d* (3)	5.64 *d* (3)
14a		5.09 *br s*	1.28 *s*	1.78 *br s*
14b		5.12 *br s*		
15		1.97 *s*	1.90 *br s*	1.54 *br s*
3′		6.96 *t* (6)	6.96 *t* (6)	6.89 *t* (6)
4′		4.40 *d* (6)	4.40 *d* (6)	4.41 *d* (6)
5′a		4.74 *d* (12)	4.75 *d* (12)	4.34 *br s*
5′b		4.84 *d* (12)	4.81 *d* (12)	
OAc		1.97 *s*	1.98 *s*	2.13 *s*

* Coupling constants (Hz) are given in parentheses.

put the 8-*O*-tigloyl moiety in the equatorial position. This conformation is also in accordance with $J_{8,9\alpha}$ (7.4) and $J_{8,9\beta}$ (6.6) which require H-8 to make 15° and 135° angles with H-9α and H-9β, respectively.

The structures of 8β-(4′, 5′-dihydroxytigloyloxy)-preeupatundin (**2**) and 8β-(4′,5′-dihydroxytigloyl)-cumambrin B (**3**) were confirmed by comparison of their ^{1}H NMR and mass spectral data with that in the literature [5, 6].

The structure of 8 β-(4′-hydroxy, 5′-acetoxytigloyl)-cumambrin-B (**4**) was deduced by comparison of its spectral data with that of **3**. The signals for H-5′ (**4**) were shifted downfield to δ 4.75 and δ 4.81 due to the acetoxyl group on C-5′. The α-orientation of the 10-hydroxy groups on **3** and **4** caused large downfield shifts of the H-7 signals compared to the spectra of **1** and **2**, indicating **3** and **4** exist mainly as the boat conformation in solution.

The ^{1}H NMR spectrum of (**5**) contained two olefinic methyl signals at δ 1.54 and 1.78. An acetyl signal was observed at δ 2.13 while the tigloyl group signals appeared at 6.89 (H-3′), 4.41 (H-4′) and 4.34 (H-5′) indicating that the acetyl group is not on the tigloyloxy side chain. These observations combined with the mass spectral fragments at 420 $[M]^+$, 402 $[M-H_2O]^+$, 360 $[M-HOAc]^+$ and 288 $[M-(B+H)]^+$ called for a germacranolide with one acetyloxy and one tigloyloxy side chain. The ^{1}H NMR chemical shifts for the medium ring hydrogens of **5** were close to those obtained for a similar germacranolide, 2α-hydroxyeupatolide 8-*O*-acetate [7], except for the 2β hydrogen signal which, due to acetylation at C-2, was shifted downfield (δ 5.21). With double irradiation experiments, couplings could be followed from H-1 through H-3 and H-5 through H-9. The large $J_{1,2}$ (10 Hz) agreed best with a 2α-orientation of the acetoxy group if a conformation with both the C-14 and C-15 methyls above the plane was assumed as was the case with 2α-acetoxyeupatolide [8]. The narrow multiplet for H-8 required a small $J_{7,8}$ which meant, as is the case with all compounds from *C. douglasii*, that H-8 is α-oriented.

Table 2. ^{13}C NMR data for compounds **1** and **4**, (CD_2Cl_2)

C	**1**	**4**
1	53.8 *d*	55.1 *d**
2	79.1 *d*	34.3 *t*
3	129.9 *d*	125.8 *d*
4	147.3 *s*	143.4 *s*
5	56.4 *d*	55.3 *d**
6	81.3 *d*	80.1 *d*
7	48.3 *d*	47.1 *d*
8	69.0 *d*	68.5 *d*
9	39.3 *t*	38.6 *t*
10	142.1 *s*	73.4 *s*
11	134.9 *s*	135.4 *s*
12	169.6 *s*	170.1 *s*
13	122.2 *t*	121.5 *t*
14	119.4 *t*	32.7 *q*
15	17.4 *q*	17.6 *q*
1′	165.5 *s*	165.6 *s*
2′	127.4 *s*	127.1 *s*
3′	147.6 *d*	147.7 *d*
4′	59.6 *t*	59.41 *t*
5′	58.2 *t*	58.2 *t*
OAc (Me)	21.0 *q*	20.9 *q*
OAc(C=O)	171.4 *s*	171.3 *s*

* Interchangeable.

Preliminary bioassays done with **1**, **3**, **4** and douglasine

on P 815 mastocytoma cells showed cytotoxic activity by **1**, **3**, and **4** (unpublished data) with levels close to that of parthenin [9]. Detailed bioassay results will be published shortly.

EXPERIMENTAL

C. douglasii (Hook) H & A was collected in August 1986 from Princeton, B. C., Canada. Voucher specimens are deposited in the herbarium, Department of Botany, The University of British Columbia, Canada.

Fresh leaves (920 g) were first macerated in MeOH (2 l) and subsequently extracted with CH_2Cl_2 (5 l). The mixed crude extracts were dried *in vacuo* and treated with lead(II) acetate as described in ref. [10]; 10.4 g of terpenoid mixt. thus obtained was subjected to CC (silica gel). 50 (100 ml) fractions were collected using MeOH–CH_2Cl_2 (1:24). **4** (156 mg) was obtained from frs 26–27 and purified by prep. TLC (silica gel) with EtOAc–petrol (2:1) and then with petrol–Me_2CO (2:1) eluting repeatedly. Frs 28–29 were sepd by flash CC (petrol–Me_2CO, 2:1). Frs 20–26 of the 26 (10 ml) fractions contained **1** (320 mg) which was purified by prep. TLC (silica gel) with petrol–Me_2CO (2:1). Frs 32–33 of the initial CC were rechromatographed over silica gel (Et_2O–Me_2CO, 6:1). Frs 15–17 of the 20 (15 ml) fractions obtained yielded **5** (4 mg), purified by prep. TLC (EtOAc). Frs 36–40 of the initial CC contained douglasine, previously reported as a new sesquiterpene lactone from this plant [3]. Frs 41–48 of the initial CC contained **2** and **3** as major constituents. Prep. TLC (MeOH–CH_2Cl_2) yielded 21 mg **2** and 76 mg **3**.

(**1**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600(OH), 1764 (γ-lactone), 1759 (tigloyl ester), 1736 (OAc); EIMS (probe) 70 eV, *m/z* (rel. int.): 401 $[M-OH]^+$ (0.6), 400 $[M-H_2O]^+$ (0.5), 358 $[M-B''']^+$ (0.2), 340 $[M-B'''\text{-}H_2O]^+$ (1.8), 261 $[M-B']^+$ (1.5), 244 $[M-(B+H)]^+$ (13.7), 226 $[M-(B+H)-H_2O]^+$ (21.2), 157 $[B']^+$ (25.9), 97 $[B'-B''']^+$ (88.3), 69 $[B''-B''']^+$ (100.0), 43 $[Ac]^+$ (91.9).

(**4**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3600 (OH), 1762 (γ-lactone), 1738 (OAc), 1718 (tigloyl ester); EIMS (probe) 70 eV, *m/z* (rel. int.): 420 $[M]^+$ (2.1), 402 $[M-H_2O]^+$ (0.2), 360 $[M-B''']^+$ (0.5), 342 $[M-B'''-H_2O]^+$ (0.3), 246 $[M-(B+H)]^+$ (14.5), 228 $[M-(B+H)-H_2O]^+$ (47.0), 157 $[B']^+$ (38.3), 97 $[B'-B''']^+$ (100.0), 69 $[B''-B''']^+$ (40.9).

(**5**). Colourless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440 (OH), 1764 (γ-lactone), 1738 (OAc), 1730 (tigloyl ester).

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