Phytochemistry, 1980 Vol. 19, pp. 1975–1977. Pergamon Press Ltd. Printed in England.

DELTOIDIN A AND B, TWO NEW GERMACROLIDES FROM *EUPATORIUM DELTOIDEUM**

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(*Received* 3 *December* 1979)

Key Word Index—*Eupatorium deltoideum*; Compositae; Eupatorieae; germacrolides; sesquiterpene lactones.

Abstract—Investigation of *E. deltoideum* provided two new germacrolide-type sesquiterpene lactones, deltoidin A and B, besides the known guaianolide ligustrin, and diterpenes and triterpenes.

INTRODUCTION

Several sesquiterpene lactones of the guaianolide, germacrolide and heliangolide types have been isolated from plants of the genus *Eupatorium*, a number of them with cytotoxic and antitumor activity [1–3]. In the present paper we report the isolation and structure elucidation of two new germacrolide-type sesquiterpene lactones named deltoidin A (**1a**) and B (**1b**) from *E. deltoideum* Jacq., besides a known guaianolide ligustrin (**3**) [4], three diterpenes, kaurenoic acid, xilopic acid and kaura-9(11),16-dienoic acid, and the triterpenes, taraxasterol, taraxasteryl acetate and a mixture of taraxasteryl palmitate and stearate.

RESULTS AND DISCUSSION

Deltoidin A (**1a**) $C_{20}H_{26}O_5$, mp 159–161°, $[\alpha]_D$ − 145.1° is a conjugated γ-lactone which showed the typical IR absorptions at 1765 and 882 cm^{-1}, as well as at 1715 and 1645 cm^{-1} indicating the presence of an α,β-unsaturated ester. The MS of **1a** showed a molecular ion at *m/e* 346 and fragmentation peaks at *m/e* 263 ($M^+ - 83$), 246 ($M^+ - 100$), 83 (100%) and 55 which suggested the presence of a 5-carbon ester side chain, which was an angelate group since the vinyl proton signal appeared as a split quartet at δ 6.1 in the ^1H NMR spectrum [5]. The ^1H NMR spectrum (Table 1) showed the two doublets typical of the lactonic exocyclic methylene at 6.33 (J = 3.5 Hz) and 5.70 (J = 3.0 Hz).

The remaining proton signals were assigned from spin–spin decoupling experiments and chemical shift reagent $Eu(fod)_3$ experiments; H-7 was located as a broad signal at 3.22 since irradiation of this signal collapsed the exocyclic methylene doublets to singlets, converted a triplet at 4.44 (J = 9.0 Hz) into a doublet and affected a broadened doublet at 5.74 (partially obscured by H-13b).

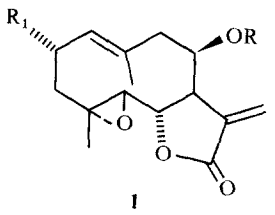
Table 1. ^1H NMR data* of deltoidin A (**1a**) and B (**1b**), and deltoidin A acetate (**1d**)

	1a	**1b**	**1d**
H-1	5.28 *br d* (10)	5.37 *br d* (10)	5.28 *br d* (10)
H-2	—	4.70 *dt* (10, 6)	5.64 *dt* (10, 6)
H-5	2.82 *d* (9)	2.92 *d* (9)	2.90 *d* (9)
H-6	4.41 *t* (9)	4.39 *t* (9)	4.39 *d* (9)
H-7	3.16 *m*	3.22 *m*	3.16 *m*
H-8	5.72 ob†	5.78 ob	5.78 ob
H-13a	6.33 *d* (3.5)	6.37 *d* (3.5)	6.38 *d* (3.5)
H-13b	5.70 *d* (3)	5.72 *d* (3.0)	5.72 *d* (3)
H-14	1.73 *br s*	1.79 *br s*	1.88 *br s*
H-15	1.35 *s*	1.34 *s*	1.38 *s*
H-3′	6.10 *qq* (7, 1)	6.12 *qq* (7, 1.5)	6.13 *qq* (7, 1.5)
H-4′	1.94 *dq* (7, 1)	1.95 *dq* (7, 1.5)	1.96 *dq* (7, 1.5)
H-5′	1.82 *m*	2.01 *m*	1.83 *m*
AcO			2.02 *s*

* Run at 100 MHz in $CDCl_3$ with TMS as internal standard. Values are in ppm (δ). Values in parentheses are coupling constants in Hz.

† ob = signal obscured.

* Contribution No. 535 from Instituto de Química de la U.N.A.M. (México).



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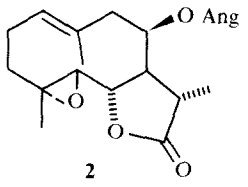
	R	R_1
a	Ang	H
b	Ang	OH
c	Tig	H
d	Ang	OAc
e	4-OH-Ang	H

Ang = Angeloyl Tig = Tigloyl



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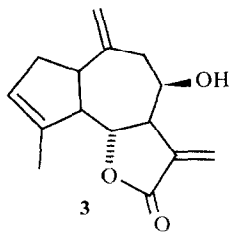

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Thus the signals at 4.44 and 5.74 were assigned to H-6 and H-8 respectively. Conversely irradiation at the frequency of H-8 and H-13b converted the H-7 signal to a doublet of doublets (J = 8, 3.5 Hz). A doublet of doublets at 2.36 (J = 15, 2 Hz) was converted to a doublet (J = 15 Hz) and this signal was assigned to one of the C-9 protons. Irradiation at the frequency of H-6 (4.44) converted the H-7 signal into a broad singlet and a doublet at 2.82 (J = 9 Hz) was modified into a singlet thus assigning this signal to H-5. Conversely irradiation of this signal (2.82) collapsed the H-6 signal into a doublet.

According to the above data, the structure of deltoidin A can be represented by **1a**. The stereochemistry of the lactone ring and H-5 was deduced from the coupling constant values. The large allylic coupling constant (3.5, 3.0) between H-7 and H-13 suggested a *trans*-fused γ-lactone according to Samek's rule [6]. Furthermore, assuming that H-7 is generally α in all sesquiterpene lactones isolated from plants, the large value of $J_{6,7}$ (9 Hz) and $J_{5,6}$ (9 Hz) required that H-6 be β and H-5 be α and therefore the lactone ring had to be *trans*-fused. Concerning the stereochemistry at C-8 the small value of $J_{7,8}$ (< 1 Hz) indicated that the ester side chain was β-oriented. Moreover the chemical shift of H-8 (5.74) was in accord with a germacrolide with a C-8β ester side chain as we have commented before [7]. Finally, comparison of the reported ^{1}H NMR spectral parameters of eupassopilin (**1e**) isolated from *E. hyssopifolium* [2] with those of deltoidin A indicated close similarities.

Reduction of deltoidin A (**1a**) with sodium borohydride gave the dihydro derivative (**2**), its ^{1}H NMR spectrum showed a new secondary methyl group doublet at 1.30 (J = 7.0 Hz) and lacked the exocyclic methylene signals.

Deltoidin B (**1b**) $C_{20}H_{26}O_6$, mp 170–171°, $[\alpha]_D$ −87.8° showed IR absorption at 3450 cm^{-1} indicating the presence of hydroxyl groups as well as the typical absorption of an α,β-unsaturated γ-lactone at 1765 cm^{-1} and an α,β-unsaturated ester at 1715 cm^{-1}. The ^{1}H NMR spectrum (Table 1) was similar to that of **1a**, but in addition deltoidin B had one proton signal triplet of doublets at 4.70 (J = 10, 6 Hz), which shifted downfield to 5.64 upon acetylation. Consequently deltoidin B contained a secondary hydroxyl group which was placed at C-2 since the chemical shift of the proton signal on the carbon bearing the hydroxyl group indicated it to be allylic and, indeed, irradiation at the frequency of this signal collapsed the broad doublet at 5.37 (H-1) to a broad singlet. Concerning the stereochemistry of the OH group, the large coupling constant of H-2 (10 Hz) indicated a *trans*-diaxial relationship between this proton and H-1 and H-3. Therefore the OH group must be α. The stereochemistry of the lactone ring was assumed to be the same as in deltoidin A on the same grounds. Consequently deltoidin B can be represented as structure **1b**.

A later fraction obtained during the isolation of deltoidin A (**1a**), afforded a crystalline compound, mp 121–125°, which was shown to be the tiglate **1c**, since the ^{1}H NMR spectrum displayed an extra multiplet at 6.76 due to the vinylic proton of the tigloyl moiety. This compound, which we had named deltoidin C (**1c**), was recently isolated from *E. serotinum* [8]. Comparison of the reported ^{1}H NMR spectrum showed it to be very similar to that of deltoidin A (**1a**).

EXPERIMENTAL

Eupatorium deltoideum Jacq. was collected in Mexico City at U.N.A.M., 30 July 1979. Quijano 26; voucher at Herbarium of Instituto de Biologia (U.N.A.M.), Mexico. Dried leaves (396 g) were extracted with $CHCl_3$; the residue after elimination of the solvent was dissolved in 1 l. MeOH and treated with 5% lead acetate soln (500 ml), filtered, concd *in vacuo* to remove most of the MeOH and extracted 3 × with $CHCl_3$. From the combined $CHCl_3$ extracts 6.0 g of crude syrup were obtained and chromatographed over 150 g Si gel using $CHCl_3$ and mixtures of $CHCl_3$–EtOAc (2.5, 5.0, 10.0, 20.0 and 80.0%) as eluant; 250 ml fractions were taken and all fractions monitored by TLC.

Deltoidin A (**1a**). Fraction 3, eluted with $CHCl_3$, was rechromatographed over 50 g Si gel fraction 22 of this chromatography provided 53 mg of **1a**, mp 159–161° $[\alpha]_D$ − 145.1° ($CHCl_3$); UV λ_{max}^{EtOH}: 213 nm (ε = 16, 317); IR ν_{max}^{Film} cm^{-1}: 1765, 1715, 1645, 882; MS *m/e*: 346 (M^+) ($C_{20}H_{26}O_5$), 263 ($M^+ - C_5H_7O$), 246 ($M^+ - C_5H_8O_2$), 188 ($C_{12}H_{12}O_2$), 83 (C_5H_7O, 100%); 55 (C_4H_7). Fraction 5 after purification by TLC (Et_2O–petrol, 1:1, 3 × afforded 15 mg of **1c**. A small collection of *E. deltoideum* (114 g) made on October 1977 was extracted first with petrol, then with $CHCl_3$ and the resultant extracts chromatographed on a Si gel column. From the chromatography of the petrol extract (2.32 g), taraxasterol, taraxasteryl acetate, a mixture of taraxasteryl palmitate and stearate, kaurenoic acid, xilopic acid, and kaura-9(11),16-dienoic acid were isolated, as well as deltoidin A(**1a**). From the chromatography of the $CHCl_3$

extract, ligustrin (**3**), a small amount of deltoidin A (**1a**) and deltoidin B (**1b**) were isolated.

Reduction of deltoidin A (**1a**). To a soln of deltoidin A (100 mg) in 15 ml MeOH was added 100 mg $NaBH_4$ and the mixture allowed to react for 2 hr. After dilution with H_2O and acidification with 5% aq. HCl, the aq. phase was extracted with EtOAc, then washed with H_2O, dried and concd; the residue was purified by TLC yielding 28 mg of **2** as a gum. IR ν_{max}^{Film} cm^{-1}: 1780, 1715, 1650; MS *m/e*: 348 (M^+), 265 ($M^+ - C_5H_7O$), 248 ($M^+ - C_5H_8O_2$), 83 (C_5H_7O, 100%), 55 (C_4H_7); ^1H NMR ($CDCl_3$): δ5.25 (*br d*, $J = 10$ Hz, H-1), 2.74 (*d*, $J = 9$ Hz, H-5), 4.27 (*t*, $J = 9$ Hz, H-6), 5.40 (*br d*, $J = 5$ Hz, H-8), 1.30 (*d*, $J = 7$ Hz, H-13), 1.70 (*br s*, H-14), 1.35 (*s*, H-15).

Deltoidin B (**1b**). Chromatography fraction 15 eluted with EtOAc, was purified by TLC (C_6H_6–EtOAc, 2:3) to yield **1b** which was crystallized from Et_2O–petrol, mp 170–171°; $[\alpha]_D$ − 87.8° ($CHCl_3$); UV λ_{max}^{EtOH} nm: 214 ($\varepsilon = 20\,996$); IR ν_{max}^{Film} cm^{-1}: 3450, 1765, 1715, 1645, 880; MS *m/e*: 362 (M^+), 344 ($M^+ - H_2O$), 279 ($M^+ - C_5H_7O$), 262 ($M^+ - C_5H_8O_2$), 83 (C_5H_7O, 100%), 55 (C_4H_7).

Deltoidin B acetate (**1d**). **1b** (30 mg), 1 ml Ac_2O and 0.5 ml Py were combined and left at room temp. The reaction being monitored by TLC. When the reaction was completed, excess of Ac_2O and Py were removed under high vacuum and the resultant residue purified by TLC (Et_2O–petrol, 1:1, developed 3×) yielding the acetate **1d**, which was crystallized from Et_2O, mp 197–199°. UV λ_{max}^{EtOH} nm: 218 ($\varepsilon = 10\,100$); IR ν_{max}^{Film} cm^{-1}: 1765, 1720, 1645, 890; $[\alpha]_D$ −71.6° ($CHCl_3$); MS *m/e*: 404 (M^+), 362 ($M^+ - C_2H_2O$), 345 (M − AcO), 304 ($M - C_5H_8O_2$), 83 (C_5H_7O, 100%), 55 (C_4H_7), 43 (C_2H_3O).

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