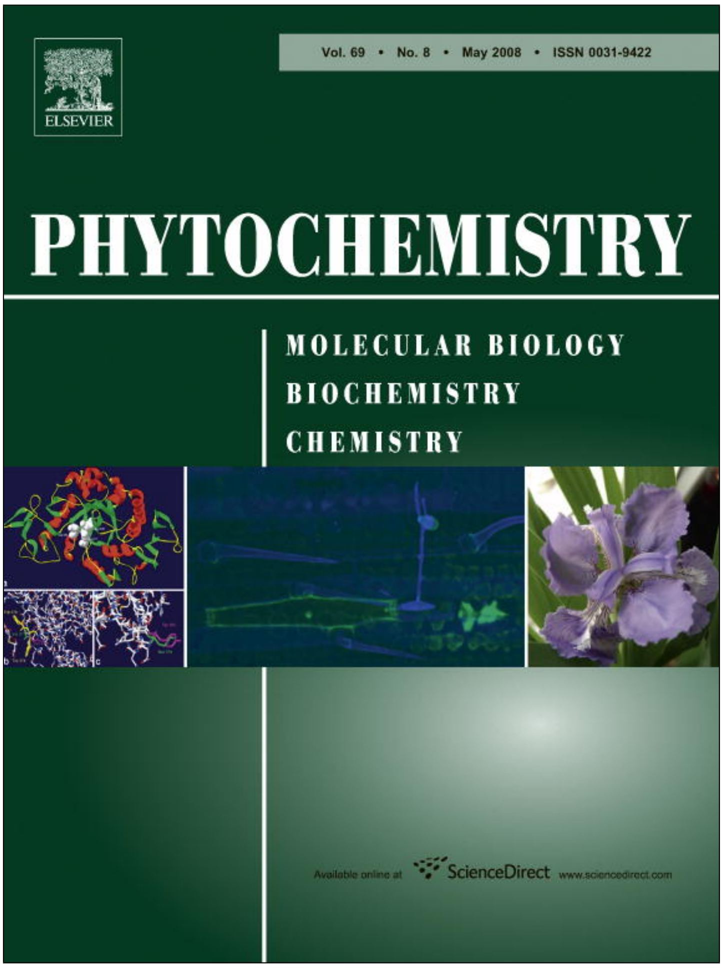

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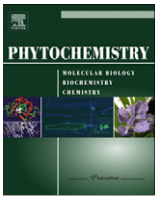

Eudesmanes from *Pluchea sagittalis*. Their antifeedant activity on *Spodoptera frugiperda*

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ABSTRACT

Eudesmane-type sesquiterpenoids 3α-(2,3-epoxy-2-methylbutyryloxy)-4α-formoxy-11-hydroxy-6,7-dehydroeudesman-8-one (**1**) and 3α-(2,3-epoxy-2-methylbutyryloxy)-4α,7α,11-trihydroxyeudesman-8-one (**2**), together with 10 known structurally related eudesmanes were isolated from the $CHCl_3$ extract of aerial parts of *Pluchea sagittalis* (Lamarck) Cabrera. Their structures were deduced by extensive application of 1 and 2D NMR spectroscopic techniques and high and low resolution CIMS. X-ray crystallographic analysis of the known compound 3α-(2,3-epoxy-2-methylbutyryloxy)-4α-formoxycuauthemone (**9**) is reported here for the first time, and confirms the structural features for the series of the reported eudesmanes. All eudesmanes were tested for their antifeedant activity by incorporating them to an artificial diet of larvae of the polyphagous insect *Spodoptera frugiperda* at a concentration of 100 ppm. Our results, from feeding choice tests, indicated that most of the compounds deter larval feeding at the cited concentration.

1. Introduction

The genus *Pluchea* (Asteraceae) comprises 80 species distributed mainly in North and South America, Africa, Asia and Australia (Anderberg, 1994). Previous chemical investigations on the genus have shown the presence of eudesmane-type sesquiterpenoids (Chiang et al., 1979; Ahmad et al., 1990, 1991; Uchiyama et al., 1991; Guilhon and Müller, 1996, 1998a,b; Mahmoud, 1997) as well as monoterpenes, lignan glycosides, triterpenoids, (Chakravarty and Mukhopadhyay, 1994) and flavonoids (Ahmed et al., 1987; Scholz et al., 1994), although their bioactivity has been rarely reported. Decoctions of aerial parts of *Pluchea sagittalis* (Lamarck) Cabrera are widely used in traditional medicine of South America for digestive diseases (Soraru and Bandoni, 1979). In addition, anti-inflammatory and antioxidant effects produced by CH_2Cl_2 and aqueous extracts of *P. sagittalis* have been reported (Perez-García et al., 1996, 2005).

Little has been published on either the insect antifeedant or toxic effects of eudesmane-type sesquiterpenoids (Srivastava et al., 1990; Faini et al., 1997). The eudesmanoids, 3β-hydroxycostic acid and encelin have shown to display antifeedant activities against larvae of the serious pest *Spodoptera littoralis* "cotton leafworm". In addition, encelin produces strong larvicidal effects on the same pest.

Continuing with our search for bioactive constituents of plant origin, we report herein the isolation and identification of twelve sesquiterpenoids from a Bolivian collection of *P. sagittalis*; the new eudesmane-type sesquiterpenoids **1** and **2** together with the previously described compounds **3** (Arriaga-Giner et al., 1983), **4** (Ahmad et al., 1992b,1998), **5** (Bohlmann et al., 1985), **6** (Guilhon and Müller, 1998b), **7** (Arriaga-Giner et al., 1983), **8** (Ahmad et al., 1989a), **9** (Ahmad et al., 1992a), **10** (Arriaga-Giner et al., 1983; Bohlmann and Mahanta, 1978), **11** (Dominguez et al., 1981; Mukhopadhyay et al., 1983), and **12** (Bolhmann et al., 1980; Mukhopadhyay et al., 1983). Finally, bioassays where conducted in which eudesmanes were incorporated to artificial larval diets of the polyphagous insect *Spodoptera frugiperda* in order to evaluate the influence of these plant constituents on the feeding behavior of larvae. Our results indicated that most of them significantly deter feeding at a concentration of 100 ppm.

2. Results and discussion

The air-dried aerial plant parts were extracted with $CHCl_3$. A combination of column chromatography on silica gel of the $CHCl_3$ extract and preparative HPLC furnished the new compound **1** (Fig. 1). In its CIMS spectrum, the molecular ion peak was not

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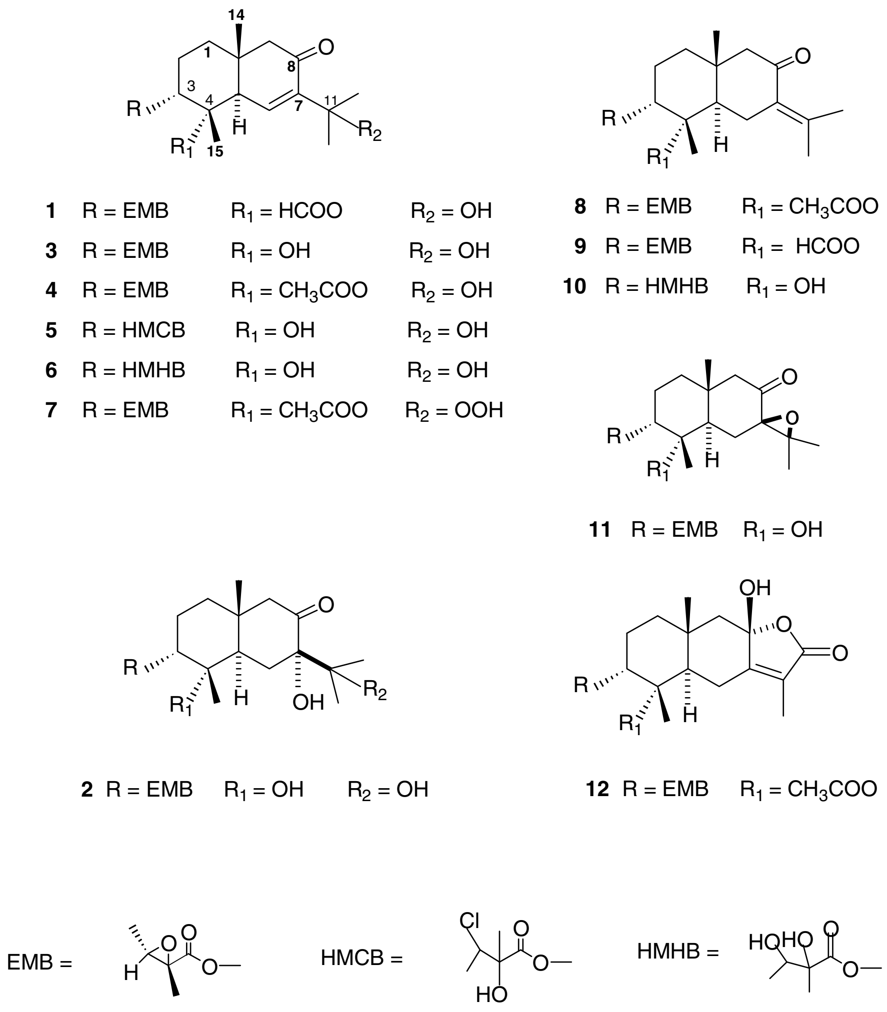

Fig. 1. Compounds from *Pluchea sagittalis*.

detected. The HRCIMS of the ion at *m/z* 367.2120 assigned to the fragment $[M + 1 - CO]^+$ was consistent with a molecular formula $C_{21}H_{30}O_7$ indicating seven degrees of unsaturation. In the ^{13}C NMR spectrum, signals at δ 199.8, 168.2 and 159.0 were assigned to a ketone and two ester carbonyls, while the peaks at δ 145.5 and 140.6 indicated the presence of a double bond. Therefore, the molecule possesses three rings. One of them was clearly the oxirane of an angelate moiety from the signals at δ 3.09 (1H, *q*, *J* = 5.5 Hz), δ 1.34 (3H, *d*, *J* = 5.5 Hz) and δ 1.56 (3H, *s*) in the ^1H NMR spectrum (Table 1). The remaining two rings were attributable to an eudesmane skeleton through the resonances of the four methyls at δ 1.46, 1.47 (C-12, C-13), 1.03 (C-14) and 1.63 (C-15) in the ^1H NMR spectrum. The location of the CH_3 groups was established by long range correlation cross-peaks in the HMBC spectrum of compound **1** (Table 2). The HMBC spectrum also established that the ester chains were attached to C-3 and C-4. In fact, a cross-peak was observed between the signal assigned to H-3 and the epoxyangelate carbonyl, as well as between the formiate proton and C-4. Additional evidence was provided by the triplet at δ 5.89 (*J* = 3 Hz) in the proton spectrum, assigned to H-3, that indicated that the ester moiety on C-3 lays in an α-orientation consistent with literature data for this type of compound (Bohlmann and Mahanta, 1978). Relative stereochemistry of C-3, C-4 and C-10 was clearly established by NOESY correlations (Fig. 2). In addition, literature data on chemical shifts and coupling constants of H-5, H-6 and CH_3-15 in the proton spectrum are consistent with the proposed the relative stereochemistry of C-4 and C-5 (Ahmad et al., 1989a). The presence of an α,β-unsaturated ketone was deduced by the IR absorption at 1668 cm^{-1} and the band at 239 nm in the UV spectrum. Complete assignment of protons and carbons of **1** was achieved by analyses of HMQC and HMBC spectra and comparison with previously reported spectroscopic data of related compounds (Bohlmann and Mahanta, 1978; Ahmad et al., 1989b,1992c). Confirmation of the relative configuration of the fragment C-1–C-2–C-3 and that of C-5, in the minimum energy conformation of **1** (25.37 kcal/mol, Fig. 4), was accomplished using the PCMODEL program (Burket and Allinger, 1982). These calculations showed that the dihedral angles and coupling constant values for the fragment $CH_2(1)–CH_2(2)–CH(3)$ are: HαC(1)–HαC(2) = −57° (*J* = 3.49 Hz), HαC(1)–HβC(2) = −172° (*J* = 13.48 Hz), HβC(1)–HβC(2) = −57° (*J* = 3.66 Hz), HβC(1)–HαC(2) = 58° (*J* = 3.28 Hz), HαC(2)–HβC(3) = −63° (*J* = 3.44 Hz), and HβC(2)–HβC(3) = 52° (*J* = 2.89 Hz). For the fragment CH(5)–CH(6) PCMODEL calculations showed a dihedral angle of −96° with *J* = 2.71 Hz. The calculated values were in good agreement with the observed coupling constants, as can be seen in Table 1.

The HRCIMS spectrum of **2** showed a quasimolecular ion peak at *m/z* 385.2210 $[M + 1]^+$ indicating a molecular formula $C_{20}H_{32}O_7$ and five degrees of unsaturation. No signals for double bonds were detected in the IR or NMR spectra, however, the presence of one ketone and one ester could be inferred from the IR carbonyl absorptions at 1700 and 1734 cm^{-1}, respectively, as well as from

Table 1
^{1}H NMR and ^{13}C NMR spectroscopic data of compounds **1** and **2** (500 MHz, CDCl$_3$)

	Compound **1**		Compound **2**	
	^{1}H	^{13}C	^{1}H	^{13}C
1a	1.55–1.60[a]	31.9	1.60 *td* (13.5, 3.5)	33.5
1b	1.23–1.28[a]	–	1.28 *dt* (13.5, 3.5)	–
2a	2.05 *dq* (16. 3.5)	23.1	1.95 *dq* (15.5, 3.5)	23.3
2b	1.92 *dddd* (16, 13.5, 4.0, 2.5)	–	1.84 *dddd* (15.5, 13.5, 3.5, 2.5)	–
3	5.89 *t* (3.0)	72.9	4.88 *dd* (3.0, 2.5)	78.2
4	–	82.6	–	71.5
5	3.14 *d* (2.0)	59.6	2.42 *dd* (13.5, 3.0)	43.3
6a	6.95 *d* (2.0)	140.6	2.19 *dd* (13.5, 3.0)	28.8
6b	–	–	1.69 *brt* (13.5)	–
7	–	145.5	–	78.5
8	–	199.8	–	212.5
9a	2.43 *d* (15.5)	48.4	2.87 *d* (14.0)	55.6
9b	2.34 *d* (15.5)	–	1.99 *d* (14.0)	–
10	–	39.1	–	37.9
11	–	71.7	–	74.9
12	1.46 s	29.1	1.22 *s*	23.8
13	1.47 *s*	28.7	1.42 *s*	25.0
14	1.03 *s*	18.1	0.91 *s*	18.4
15	1.63 *s*	19.1	1.20 *s*	21.1
OH -7	–	–	3.52 *brs*	–
OH-11	–	–	3.65 *brs*	–
1’	–	168.2	–	169.5
2’	–	59.8	–	60.2
3’	3.09 *q* (5.5)	59.7	3.10 *q* (5.5)	59.8
4’	1.34 *d* (5.5)	13.8	1.35 *d* (5.5)	14.0
5’	1.56 *s*	19.1	1.64 s	19.5
HCOO	7.98 *s*	159.0	–	–

[a] Overlapped signals.

Table 2
HMBC correlations for compounds **1** and **2**

Compound **1**		Compound **2**	
H	C	H	C
2a	4, 10	1a	14
3	1′	1b	5, 2
5	4, 6, 9, 10	2a	4
6	4, 8, 10, 11	3	4, 5, 1′, 15
9a	1, 5, 10, 14	5	4, 6, 10, 14, 15, 9
12	11	6a	7, 5, 10
13	11	6b	4, 5, 10, 11
14	1,5, 9, 10	9a	1, 5, 10, 14
15	3, 5	9b	1, 5, 7, 10
3′	4′	12	7, 11, 13
4′	2′, 3′	13	7, 11, 12
5′	3′	14	1, 5, 9, 10
HCOO	4	15	3, 4, 5
		3’	4′
		4’	3′, 2′
		5’	2′
		OH-11	11,12
		OH-7	6,7

the ^{13}C NMR resonances at δ 212.5 and 169.5, respectively. In addition, signals for an epoxyangelate residue (Table 1) were also observed in the NMR spectra of compound **2**. The remaining two degrees of unsaturation were attributed to the rings of an eudesmane skeleton through the resonances of the four methyls at δ 1.22, 1.42 (C-12, C-13), 0.91 (C-14) and 1.20 (C-15) in the ^{1}H NMR spectrum (Table 1) whose locations could be deduced by exhaustive analysis of the HMBC spectrum of **2** (Table 2). The HMBC spectrum (Table 2) also established that the ester chain was attached to C-3. In fact, a cross-peak was observed between the signal assigned to H-3 and the epoxyangelate carbonyl. The ^{1}H NMR spectrum of **2** (Table 1) showed two broad singlets at δ 3.52 (1H) and 3.65 (1H) assigned to vicinal hydroxyl groups. In the HMBC spectrum the proton resonance at δ 3.65 (OH) correlated with the ^{13}C signals assigned to C-12 and C-13 (δ 23.8 and 25.0 ppm) indicating unambiguously the location of this OH group at C-11. Furthermore, cross-peaks were observed between the proton (OH) signal at δ 3.52 and the ^{13}C resonances at δ 78.5 (assigned to C-7) and δ 28.8 (C-6) indicating that the other OH was located at C-7. Finally, the location of the OH at C-4 was evident by the ^{1}H NMR signal at δ 4.88, assigned to H-3, the singlet at δ 1.20 assigned to CH$_3$-15 and the double doublet at δ 2.42 (H-5). The three mentioned proton resonances of **2** were shielded in comparison with the corresponding ones in compound **1**, because the latter carries an ester at C-4. Complete assignments of the ^{1}H and ^{13}C NMR spectra of **2** were achieved by ^{1}H ^{1}H COSY and HMQC experiments and comparison with spectroscopic data of related compounds (Ahmad et al., 1989b,1992c). Relative stereochemistry of the chiral carbons was established by NOESY and NOE difference spectra. In fact, NOESY correlations indicated that H-2β, CH$_3$-14, CH$_3$-15 and H-6β lay on the same side (β) of the molecule in axial conformations, while H-5, H-1α and H-9α are located on the α side and also in axial conformations (Fig. 3). The α-orientation of the ester chain at C-3 was confirmed by a NOESY correlation of H-3 (β oriented) and

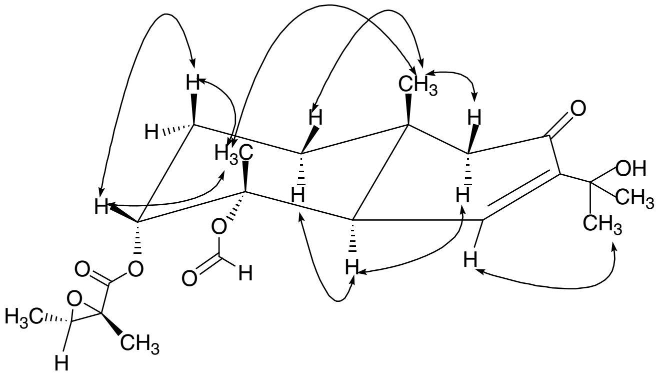

Fig. 2. Partial NOEs observed for compound **1**.

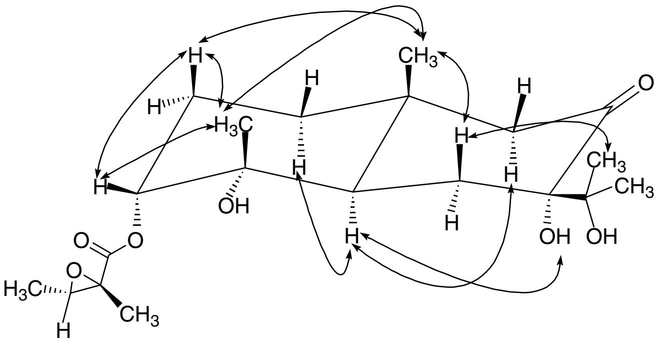

Fig. 3. Partial NOEs observed for compound **2**.

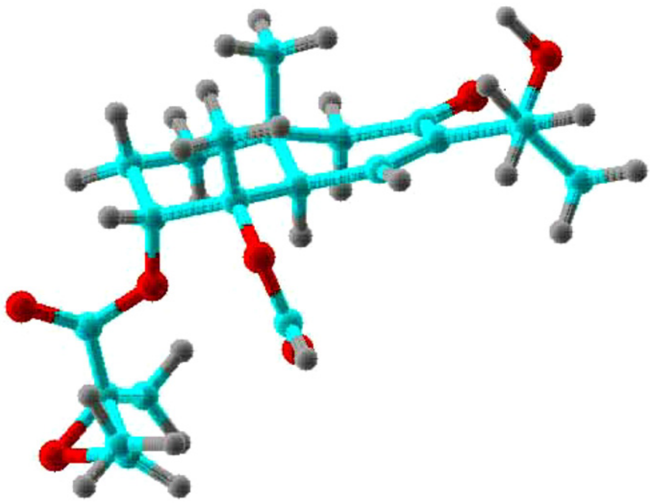

Fig. 4. Minimum energy conformation of **1**.

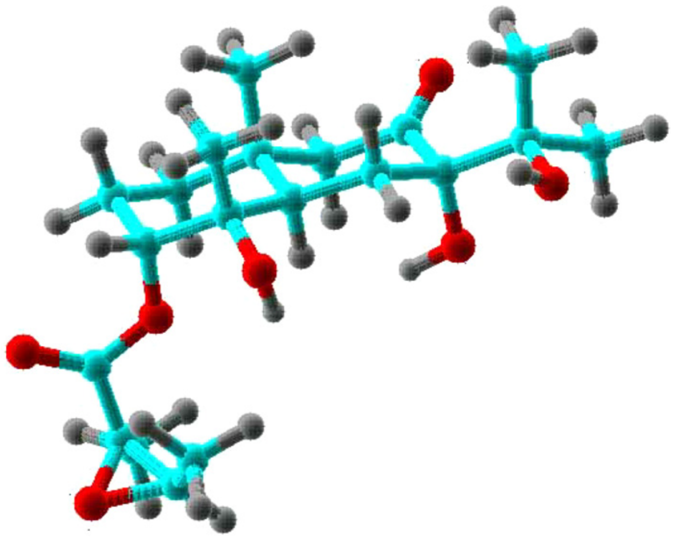

Fig. 5. Minimum energy conformation of **2**.

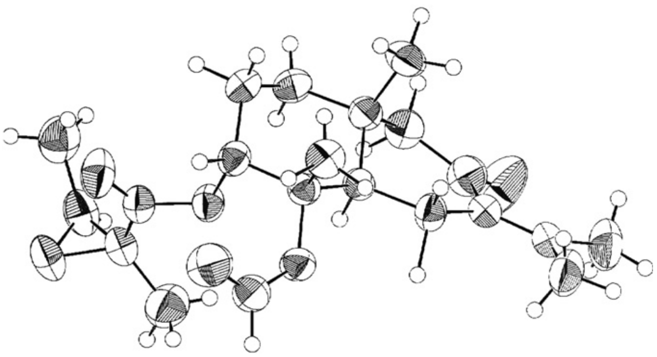

Fig. 6. ORTEP drawing for compound **9**.

CH$_3$-15 and H-2β. The relative stereochemistry at C-7, as depicted, could be established by a NOESY correlation observed between the proton of the OH at C-7 and H-5. Confirmation of the C-7 stereochemistry was accomplished by NOE difference spectroscopy. In fact, irradiation of H-6β produced NOE enhancements of the signals of CH$_3$-12, and CH$_3$-13 indicating that the hydroxyl isopropyl moiety at C-7 cannot be α oriented, therefore, should be in an equatorial β orientation. Additional evidence to the proposed relative configuration of the fragments C-1–C-2–C-3 and that of C-5–C-6–C-7, was provided by J values calculated for protons in the minimum energy conformation of **2**, (24.32 kcal/mol, Fig. 5) using the PCMODEL program that were in good agreement with the observed coupling constants (Table 1). These calculations showed that the dihedral angles and coupling constant values for the fragment CH$_2$(1)–CH$_2$(2)–CH(3) are: HβC(1)–HαC(2) = 56° (J = 3.65 Hz) and HαC(1)–HαC(2) = −59° (J = 3.23 Hz), HαC(1)–HβC(2) = −174° (J = 13.53 Hz), HβC(1)–HβC(2) = −58° (J = 3.37 Hz), HαC(2) –HβC(3) = −60° (J = 3.60 Hz), HβC(2)–HβC(3) = 54° (J = 2.61 Hz). Finally, PCMODEL calculations showed a dihedral angle of −66° with J = 2.35 Hz for the fragment HαC(5)–HαC(6) and the a dihedral angle of 178° with J = 12.34 Hz for HαC(5)–HβC(6).

The known compounds **3**–**12** were identified by their spectroscopic features in comparison with previously reported literature data, cited in the Section 1. Definitive support for structure of **9** was provided by X-ray crystallographic analysis of colorless crystals (ORTEP drawing in Fig. 6). Orthorhombic crystals, obtained from an n-hexane solution belong to the space group $P2_1$. The atomic coordinates and equivalent isotropic displacement parameters, as well as a full list of bond distances and angles, and the structure factor table are deposited as Supplementary material at the Faculty of Pharmaceutical Sciences, Tokushima, Japan and at the Cambridge Crystallographic data Centre.

2.1. Antifeedant effects

None of the tested compounds stimulated feeding. The incorporation of 100 μg of **1**, **7** and **9** per g to the larval diet produced 34%, 52% and 37% of deterrence of feeding, respectively compared to control. Diets treated with the eudesmanes **2**, **3**, **4**, **5**, **8** and **11** also produced 11 to 28% of feeding deterrence, while compounds **10** and **12** had no effect on feeding preferences at 100 μg/g (Table 3).

2.2. Concluding remarks

Although eudesmanes **1**, **7** and **9** of *P. sagittalis* are not as potent insect antifeedants as the natural terpenoid azadirachtin (Blaney et al., 1990), they could be promising precursors to generate series of more antifeedant derivatives from plant sources against *S. frugiperda*, a pest that produces big losses in crops in the north and centre of Argentina.

This is the first report on the presence of highly oxygenated eudesmanes in a collection of *P. sagittalis* and the first report on the presence of eudesmanes esterified with formic acid in species of the genus *Pluchea*; in fact, eudesmanes **9** (the major) and **1** carry

Table 3
Effects of pure compounds on the feeding behavior of *Spodoptera frugiperda* larvae

Feeding deterrence index	
Compounds	Choice test 100 μg/g of diet
1	33.73 ± 12.2
2	13.66 ± 8.3
3	20.0 ± 5.6
4	28.44 ± 5.8
5	10.88 ± 3.7
7	52.02 ± 16.1
8	19.21 ± 3.3
9	37.31 ± 14.5
10*	0.1 ± 0.02
11	13.20 ± 2.1
12*	0.02 ± 0.006

[a] Values within a column indicate the feeding deterrence index ± SEM ($n = 20$). Feeding deterrence index = [100 × (1–T/C)], where C and T represent the amount of control and treated diets, respectively, consumed during the test.

* Not significant differences ($P > 0.05$, Tukey multiple range test) in consumption of control and treated diets.

a formiate at C-4. Interestingly, among the most active compounds of our collection were the two mentioned formiates. As shown in Table 3, only the hydroperoxy eudesmane **7** was more antifeedant than **1** and **9**.

3. Experimental

3.1. General

NMR spectra were recorded on a Bruker AC spectrometer operating at 500 MHz for ^{1}H and 125 for ^{13}C with TMS as internal standard in $CDCl_3$. Optical rotations were measured with a HORIBA SEPA-300 high sensitive polarimeter at 26–29 °C with $CHCl_3$ as a solvent. The mass spectra including high-resolution mass spectra were recorded on a JEOL JMS AX 500 spectrometer (HRCIMS). Specific rotations were measured on a JASCO DIP-1000 polarimeter with $CHCl_3$ as solvent. For HPLC separation of mixtures, Gilson, Waters and Konik equipments were used. Detection was accomplished by the use of UV and refractive index detectors. Columns: (A) Phenomenex Ultremex C18 (5 μm, 10 mm i.d. × 250 mm) and (B) Phenomenex Ultremex C8 (5 μm, 10 mm i.d. × 250 mm). Retention time was measured from the solvent peak.

3.2. Plant material

Aerial parts of *Pluchea sagittalis* (Lamarck) Cabrera were collected at the flowering stage in December of 2004 in Tarija, Bolivia (a voucher specimen, LIL N° 603515 is deposited in the herbarium de la Fundación Miguel Lillo, Tucumán, Argentina).

3.3. Extraction and isolation

Flowers and leaves (311 g) were extracted at room temperature for 7 days with $CHCl_3$ (2 × 3 l) to give 19.0 g of residue after solvent removal in a rotary evaporator (yield 6.1%). The extract was then suspended in EtOH (100 ml) at 55 °C, diluted with H_2O (150 ml) and extracted successively with hexane (2 × 150 ml) and $CHCl_3$ (2 × 150 ml). The second $CHCl_3$ extract was evaporated under reduced pressure and furnished a residue (7.0 g) which was subjected to silica gel CC (70–230 Mesh) with $CHCl_3$ and increasing amounts of EtOAc (0–100%) and finally MeOH, as eluents, to give 15 fractions of 10 ml each.

Frs. VI–VIII (1.4 g), which eluted with a mixture of $CHCl_3$–EtOAc (4:1), were combined and submitted to HPLC (Column B, MeOH–H_2O 2:1, 2 ml min^{-1}) to give compounds **8** (100 mg, R_t 16 min), **9** (120 mg, R_t 13 min) and a mixture which was purified further by HPLC (Column A, MeOH–H_2O 2:1, 2 ml min^{-1}) to give compound **7** (16 mg, R_t 23 min).

Fr. XII (2.1 g), which eluted with $CHCl_3$–EtOAc (7:3), was processed again on silica gel using $CHCl_3$ and increasing amounts of EtOAc (0–100%) and finally MeOH to give six fractions. Fr. 4 (113 mg) was processed on HPLC (Column B, MeOH–H_2O 3:2, 1.5 ml min^{-1}) to give compounds **1** (38 mg, R_t 30 min), **6** (1 mg, R_t 32 min) and mixture submitted to a new HPLC process (Column A, MeOH–H_2O 1:1, 1.5 ml min^{-1}) to give compounds **11** (2.5 mg, R_t 22 min) and **12** (2.8 mg, R_t 17 min). Fr. 5 (590 mg) that eluted with a $CHCl_3$–EtOAc (55:45) was processed by HPLC (Column B, MeOH–H_2O 2:3, 1.5 ml min^{-1}) to give compounds **10** (3.6 mg, R_t 16 min), **2** (6 mg, R_t 12 min), **3** (60 mg, R_t 18 min), **4** (13 mg, R_t 20 min), and **5** (2.8 mg, R_t 25 min).

3.3.1. Compound **1**

3α-(2,3-epoxy-2-methylbutyryloxy)-4α-formoxy-11-hydroxy-6,7-dehydroeudesman-8-one. Oil; [α_D26] + 0.74 (c 0.02, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540, 1738, 1668; For ^{1}H and ^{13}C NMR spectra, see Table 1; CIMS (*iso*-butane) m/z (rel. int.): 367 $[(M + H) CO]^+$ (25), 349 $[(M + H) HCOOH]^+$ (90), 233 $[(M + H) HCOOH CH_3CHOC(CH_3)COOH]^+$ (42). HRCIMS 367.2120 $[(M + H) - CO]^+$ (calc. for $C_{20}H_{31}O_6$ 367.2112).

3.3.2. Compound **2**

3α-(2,3-epoxy-2-methylbutyryloxy)-4α,7α,11-trihydroxyeudesman-8-one. Oil; [α_D26] + 0.31° (c 0.02, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540, 1734, 1700; For ^{1}H and ^{13}C NMR spectra, see Table 1; CIMS (*iso*-butene) m/z (rel. int.): 385 $[M + H]^+$ (10), 367 $[(M + H) H_2O]^+$ (81), 349 $[(M + H) 2H_2O]^+$ (75), 326 $[(M + H) (CH_3)_2COH]^+$ (100), 233 $[(M + H) 2H_2O\ CH_3CHOC(CH_3)COOH]^+$ (68). HRCIMS: 385.2210 $[M + H]^+$ (calc. for $C_{20}H_{33}O_7$ 385.2217).

3.4. X-ray crystallographic analysis of **9**

X-ray crystallographic analysis was carried out on a Mac Science Bruker Nonius diffractometer, Data collection: Dip image plate. Cell refinement; Scalepack (HKL). Data reduction: Maxus. The program used to refine structure: SHELXL-97. The molecular formula was $C_{21}H_{30}O_6$, Molecular weight = 378.465, orthorhombic, $P2_12_12_1$, $a = 10.2260$ (4) Å, $b = 11.6850$ (6) Å, $c = 17.1570$ (12) Å. $\alpha = 90°$, $\beta = 90°$, (=90°, $V = 2050.1(2)$ Å^3 $Z = 4$, $D_x = 1.226$ Mg m^{-3}, D_m not measured, λ = (Mo Kα; graphite monochromator) = 0.71073, μ = 0.089 mm^{-1}, $T = 298$ K, absorption correction: sphere, $\theta_{max} = 25.63°$, 3528 measured reflections, refinement on F^2, $R(all) = 0.0591$, $R(gt) = 0.0559$, $\omega R(ref) = 0.1571$, $\omega R(gt) = 0.1456$, $S(ref) = 1.104$, 3498 reflections, 244 parameters, only coordinates of H atoms refined, $(\Delta/\sigma)_{max} = 0.000$, $\Delta\rho_{max} = 0.253$ eÅ^3, $\Delta\rho_{min} = -0.384$ eÅ^3, extinction corrections: none.

3.5. Feeding preference test

A portion of artificial diet was mixed with acetone and, after solvent removal *in vacuo*, this portion was employed as control diet. Another portion was mixed with an acetone solution of each test compound (treatment), in order to leave 100 μg of treatment per g of diet. After evaporation of the solvent, 125 mg of control and the same amount of treated diet were placed in a glass tube. Between the two diet portions a larva was introduced in the tube. The larva was allowed to choose the diet and, after 48 h, the remaining diets (control and treated) were weighted. The experiment was carried out in 20 replicates. To evaluate the feeding behavior a "feeding deterrence index" was calculated as FDI = (1–T/C)100, where C and T represent the amounts eaten of control and treated diets, respectively.

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Appendix A. Supplementary data

^{1}H and ^{13}C NMR spectra of Compounds **1** and **2** on request to the corresponding author. Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.phytochem.2008.02.020.

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Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk.