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NEO-CLERODANE INSECT ANTIFEEDANTS FROM *SCUTELLARIA ALPINA* SUBSP. *JAVALAMBRENSIS*

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Key Word Index—*Scutellaria alpina* subsp. *javalambrensis*; Labiatae; neo-clerodane diterpenoids; 11-deacetylscutalpin D; *Spodoptera littoralis*; antifeedant activity.

Abstract—Seven previously known neo-clerodanes have been isolated from *Scutellaria alpina* subsp. *javalambrensis* together with a new diterpenoid, 11-deacetylscutalpin D, whose structure was established as (13*S*)-19-acetoxy-4α,18;8β,13-diepoxy-11β-hydroxy-6α-tigloyloxy-neo-clerodan-15,16-olide by chemical and spectroscopic means. The antifeedant activity of some of the isolated diterpenoids (scutalpins B–D) was assessed against larvae of *Spodoptera littoralis* and one of them (scutalpin C) showed very potent activity. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

In continuation of our systematic studies on neo-clerodane diterpenoids from *Scutellaria* species [1–6], we have carried out further investigations of *S. alpina* subsp. *javalambrensis*. In a previous study [4] three neo-clerodanes (scutalpins B–D, **1**–**3**, respectively) were isolated from this plant. Extraction of a large quantity of the aerial parts of this species allowed the isolation of large amounts of the previously isolated constituents (**1**–**3**) [4] together with minor quantities of four already known neo-clerodanes and a new diterpenoid, 11-deacetylscutalpin D (**7**). We report here on the isolation and identification of these substances and the effects of some of the isolated compounds on the feeding behaviour of larvae of *Spodoptera littoralis*.

RESULTS AND DISCUSSION

Repeated chromatography of the acetone extract of the aerial parts of *S. alpina* subsp. *javalambrensis* provided scutalpins B–D (**1**–**3**, respectively, see Experimental) [4] together with minor quantities of scutalpins G (**4**), I (**5**) and J [5, 7] and scutecolumnin C (**6**) [1], all of which had been isolated previously from *Scutellaria* species, and a new neo-clerodane derivative, 11-deacetylscutalpin D (**7**), the structure of which was established.

Compound **7** ($C_{27}H_{38}O_9$) showed hydroxyl absorption (3540 cm^{-1}) in the IR spectrum, and its ^1H and ^{13}C NMR spectra (Tables 1 and 2) were almost identical to those of scutalpin D (**3**) [4].

The observed differences were consistent with the presence in **7** of an 11β-hydroxyl group [$\delta_{H\text{-}11\alpha}$ 4.11 *dt*, $J_{11\alpha,12\alpha} = J_{11\alpha,OH} = 3.7$ Hz, $J_{11\alpha,12\beta} = 12.7$ Hz, only one OAc at δ 2.08, 3H, *s*; δ_C 71.6 *d* (C-11), 171.2 *s* and 21.2 *q* (OAc)] instead of the 11β-acetoxyl substituent of **3** [$\delta_{H\text{-}11\alpha}$ 5.32 *dd*, $J_{11\alpha,12\alpha} = 4.2$ Hz, $J_{11\alpha,12\beta} = 12.9$ Hz, two OAc at δ 2.05, 3H, *s* and 2.04, 3H, *s*; δ_C 72.6, *d* (C-11), 171.1 *s*, 170.2 *s*, 21.1 *q* (two carbons) (OAc)].

Oxidation of a sample of **7**, containing minor quantities of scutecolumnin C (**6**), yielded a keto derivative **8** ($\delta_{C\text{-}11}$ 204.5 *s*, C-12 methylene protons as an AB system at δ 2.76 and 2.64, $J_{gem} = 14.8$ Hz; no hydroxyl absorption in its IR spectrum), thus confirming that the new diterpenoid (**7**) possessed a hydroxyl group at C-11. In addition, scutecolumnin C (**6**) was oxidized to the corresponding 19,2α-δ-lactone derivative (**9**), a substance not previously described.

Exhaustive NMR studies (^1H, ^{13}C, HMQC, HMBC and NOESY) established that **7** possessed a relative stereochemistry at its C-4–C-6, C-8–C-11 and C-13 asymmetric centres identical to that of scutalpin D (**3**) [4]. Furthermore, these studies allowed the complete and unequivocal assignment of the ^1H and ^{13}C NMR spectra of **7** and **8**, even distinguishing both methylene protons at C-14 and C-16 (Tables 1 and 2) and confirming that the tigloyloxy group was attached to the C-6 position [HMBC spectrum of **7**: correlation of the carbonyloxy carbons at δ 166.9 *s* (tiglate) and 171.2 *s*

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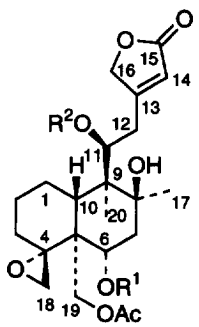
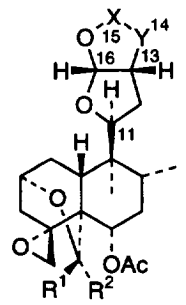
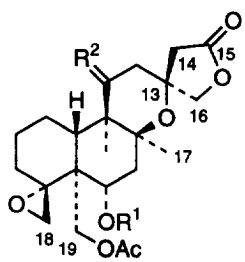


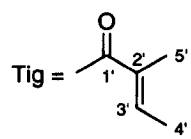
	R^1	R^2
1	Tig	Ac
2	Tig	H
5	Bz	Ac



	R^1	R^2	X-Y
6	OH	H	CH_2-CH_2
9	O		CH_2-CH_2
10	OAc	H	CH=CH
11	$OCOCHMe_2$	H	CH=CH



	R^1	R^2
3	Tig	αH,βOAc
4	Bz	αH,βOAc
7	Tig	αH,βOH
8	Tig	O



(acetate) with the H-6β (δ 5.13 *dd*) and C-19 (δ 4.59 *d* and 4.64 *d*) protons, respectively].

In agreement with the above deductions, acetic anhydride–pyridine treatment of **7** yielded a compound identical in all respects with natural scutalpin D (**3**) [4].

Finally, application of Horeau's method [8] to 11-deacetylscutalpin D (**7**) established an 11*S* absolute stereochemistry for this asymmetric carbon (see Experimental) and, consequently, a neo-clerodane absolute configuration [9] for this new diterpenoid and for scutalpin D (**3**) [4], taking into account that **7** was transformed into **3** (see above)

The antifeedant activity of scutalpins B–D (**1**–**3**, respectively) was assessed using choice and no-choice bioassays against larvae of the lepidopteran *Spodoptera littoralis* [10]. Table 3 shows the results of the bioassays in comparison with the antifeedant activity of jodrellins A (**10**) and B (**11**), which are the most potent clerodane antifeedants so far described [11].

The results indicate that scutalpin C (**2**) is a very potent antifeedant, more so than jodrellin A (**10**), although their structures are quite different in the C-9 side chain and in the conformational constraint of the decalin moiety [11]. Acetylation of the 11β-hydroxyl group of **2** causes a noticeable decrease of the antifeedant activity (Table 3, compound **1**) and the presence of an 8,13-ether bridge (**3**) results in a weak phagostimulant activity.

EXPERIMENTAL

General. Mps uncorr. Plant material was collected in August 1995 at Sierra de Javalambre, Teruel Province, Spain, and voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Complutense University of Madrid, Spain.

Extraction and isolation of the diterpenoids. Dried and finely powdered aerial parts of *Scutellaria alpina* L. subsp. *javalambrensis* Pau (1085 g) were extracted with Me_2CO (3 × 6 l) as previously described [4]. The extract (28 g) was subjected to CC (silica gel, Merck

Table 1. ^{1}H NMR spectral data of compounds 7 and **8** [500 MHz, $CDCl_3$, δ values relative to residual $CHCl_3$ (δ 7.25)]*

H	7	8	J (Hz)	7	8
1α	1.54 *br qd*	1.74 *m*†	1α, 1β	14.4	†
1β	2.56 *dddd*	1.00 *m*†	1α, 2α	3.9	†
2α	1.91 *m*†	1.95 *m*†	1α, 2β	12.4	13.0
2β	1.44 *br qt*	1.42 *br qt*	1α, 10β	12.4	12.9
3α	2.00 *m*†	2.10 *tdd*	1β, 2α	2.2	†
3β	1.01 *ddd*	1.08 *ddd*	1β, 2β	4.3	4.2
6β	5.13 *dd*	5.36 *dd*	1β, 10β	3.2	2.9
7α	1.80 *dd*	1.95 *dd*	2α, 2β	13.1	13.0
7β	1.70 *dd*	1.74 *m*†	2α, 3α	†	4.2
10β	2.27 *dd*	2.48 *dd*	2α, 3β	2.1	2.1
11α	4.11 *dt*‡	—	2β, 3α	12.4	13.0
12α	2.00 *m*†	2.76 *d*	2β, 3β	4.3	4.2
12β	1.91 *m*†	2.64 *d*	3α, 3β	12.9	13.0
14α§	2.55 *d*	2.65 *d*	3α, 18B	2.3	2.4
14β§	2.95 *d*	3.09 *d*	6β, 7α	11.4	11.4
16α§	4.23 *d*	4.18 *d*	6β, 7β	5.0	5.0
16β§	4.14 *d*	4.12 *d*	7α, 7β	14.2	14.3
Me-17	1.14 *s*	1.04 *s*	11α, 12α	3.7	—
18A¶	2.22 *d*	2.30 *d*	11α, 12β	12.7	—
18B‖	2.99 *dd*	3.10 *dd*	12α, 12β	†	14.8
19A	4.59 *d*	4.56 *d*	14α, 14β	17.2	17.1
19B	4.64 *d*	4.60 *d*	16α, 16β	8.7	9.0
Me-20	1.07 *s*	1.02 *s*	18A, 18B	4.3	4.1
OAc	2.08 *s*	2.09 *s*	19A, 19B	12.3	12.2
3′	6.75 *qq*	6.78 *qq*	3′, 4′	7.1	7.1
Me-4′	1.73 *dq*	1.76 *dq*	3′, 5′	1.5	1.4
Me-5′	1.78 *dq*	1.80 *dq*	4′, 5′	1.0	1.0
OH (C-11)**	2.11 *d*	—	11α, OH**	3.7	—

* Spectral parameters were obtained by first order approximation. All these assignments were in agreement with HMQC, HMBC and NOESY spectra.

† Overlapped signal.

‡ Collapsed into a *dd* after addition of D_2O.

§ These protons were distinguished by NOE experiments (H-14α is the pro *S* hydrogen, H-14β pro *R*, H-16α pro *R*, H-16β pro *S*).

¶ *Exo* hydrogen with respect to ring B.

‖ *Endo* hydrogen with respect to ring B.

** Disappeared after addition of D_2O.

No. 7734, deactivated with 10% H_2O, w/v, 300 g) eluting with a petrol–EtOAc gradient and finally with EtOAc–MeOH (9:1). The fractions eluted with EtOAc–petrol (3:1) contained a complex mixture of diterpenoids which, on repeated CC (silica gel) eluting with petrol–EtOAc (3:2) and CH_2Cl_2–MeOH (49:1), yielded the following compounds in order of increasing chromatographic polarity (EtOAc–petrol, 2:1): scutalpin I (**5**, 35 mg, 0.0032% on dry plant material) [5], scutalpin G (**4**, 40 mg, 0.0037%) [5], scutalpin J (11 mg, 0.001%) [5, 7], scutalpin D (**3**, 230 mg, 0.021%) [4, 5], scutalpin B (**1**, 370 mg, 0.034%) [4, 5], scutalpin C (**2**, 190 mg, 0.017%) [4] and 11-deacetylscutalpin D (**7**, 43 mg, 0.0039%). From the fractions eluted with EtOAc–MeOH, 9:1, after rechromatography (CC, silica gel, EtOAc–petrol, 4:1 as eluent), scutecolumnin C (**6**, 5 mg, 0.00046% on dry plant material) [1] was isolated.

The previously known compounds were identified by their ^{1}H NMR spectra and by comparison (TLC) with authentic samples.

11-Deacetylscutalpin D (**7**). Mp 209–211° (EtOAc–*n*-hexane); $[\alpha]_D^{22}$ +2.8° ($CHCl_3$; *c* 0.437). IR ν_{max}^{KBr} cm^{-1}: 3540 (OH), 3070, 1710, 1650 (tiglate), 1790 (spiro-γ-lactone), 1730, 1270 (OAc), 2980, 1450, 1390, 1180, 1140, 1080, 1030, 990, 970, 920, 840, 730; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2; EIMS (70 eV, direct inlet) *m/z* (rel. int.): 506 $[M]^+$ (0.1), 423 $[M-Tig]^+$ (0.6), 406 $[M-HOTig]^+$ (0.1), 381 (2), 263 (3), 203 (28), 189 (6), 185 (9), 173 (22), 159 (18), 83 (90), 55 (100), 43 (83). $C_{27}H_{38}O_9$ M_r 506.

Derivatives **8** *and* **9**. Oxidation (CrO_3–pyridine, 100 mg: 2 ml) of **7** contaminated with **6** (50 mg, in 2 ml pyridine) for 24 hr at room temp. gave a mixture of **8** and **9**, which was subjected to CC (silica gel, EtOAc–petrol, 4:1) yielding **8** (15 mg) and **9** (8 mg).

Compound **8**. Mp 259–261° (EtOAc–*n*-hexane); $[\alpha]_D^{23}$ −45.0° ($CHCl_3$; *c* 0.028). IR ν_{max}^{KBr} cm^{-1}: 3060,

Table 2. ^{13}C NMR spectral data of compounds **7** and **8** [125.7 MHz, $CDCl_3$, δ values relative to the solvent (δ_{CDCl_3} 77.0)]*

C	7	8	C	7	8
1	22.1 *t*	22.7 *t*	15	173.3 *s*	171.9 *s*
2	25.1 *t*	25.1 *t*	16	77.4 *t*	76.8 *t*
3	32.6 *t*	32.7 *t*	17	24.1 *q*	24.9 *q*
4	64.9 *s*	64.9 *s*	18	48.6 *t*	49.2 *t*
5	45.4 *s*	44.4 *s*	19	62.3 *t*	62.3 *t*
6	68.4 *d*	69.0 *d*	20	16.7 *q*	12.8 *q*
7	38.5 *t*	37.9 *t*	OAc	171.2 *s*	170.8 *s*
8	81.3 *s*	82.4 *s*		21.2 *q*	21.1 *q*
9	42.5 *s*	56.0 *s*	1′	166.9 *s*	166.7 *s*
10	43.0 *d*	45.6 *d*	2′	128.9 *s*	128.7 *s*
11	71.6 *d*	204.5 *s*	3′	136.8 *d*	137.4 *d*
12	37.7 *t*	44.7 *t*	4′	14.2 *q*	14.3 *q*
13	77.7 *s*	79.6 *s*	5′	12.0 *q*	12.0 *q*
14	43.9 *t*	44.2 *t*			

* Multiplicities were determined from the HMQC spectra. All these assignments were in agreement with HMQC and HMBC spectra.

1710, 1660 (tiglate), 1790 (spiro-γ-lactone), 1740, 1260 (OAc), 1710 (ketone), 2970, 1460, 1390, 1230, 1180, 1090, 1030, 1010, 910, 840, 730; ^1H NMR: Table 1; ^{13}C NMR: Table 2; EIMS (70 eV, direct inlet) *m/z* (rel. int.): 504 $[M]^+$ (0.1), 421 $[M-Tig]^+$ (0.8), 381 (0.1), 331 (1), 203 (8), 185 (6), 171 (9), 119 (8), 105 (22), 91 (8), 83 (100), 55 (62), 43 (44). $C_{27}H_{36}O_9$ M_r 504.

Compound **9**. Mp 242–245° (EtOAc–*n*-hexane); $[\alpha]_D^{22}$ −15.8° ($CHCl_3$; *c* 0.12). IR ν_{max}^{KBr} cm^{-1}: 1760 (δ-lactone), 1740, 1250 (OAc), 2990, 2940, 1460, 1430, 1370, 1325, 1240, 1125, 1090, 1075, 1010, 975, 930, 880, 830, 800; ^1H NMR (300 MHz, $CDCl_3$): δ 5.62 (1H, *d*, J = 5.0 Hz, H-16), 4.69 (1H, *m*, $W_{1/2}$ = 7.5 Hz, H-2β), 4.68 (1H, *dd*, $J_{6\beta,7\alpha}$ = 12.2 Hz, $J_{6\beta,7\beta}$ = 4.9 Hz, H-6β), 4.01 (1H, *dd*, $J_{11\alpha,12A}$ = 11.0 Hz, $J_{11\alpha,12B}$ = 5.7 Hz, H-11α), 3.86 (2H, *m*, 2H-15), 3.18 (1H, *d*, $J_{18B,18A}$ = 4.1 Hz, H_B-18), 2.83 (1H, *m*, $W_{1/2}$ = 18 Hz, H-13β), 2.58 (1H, *d*, $J_{18A,18B}$ = 4.1 Hz, H_A-18), 2.52 (1H, *m*, $W_{1/2}$ = 30 Hz, H-3α), 2.04 (3H, *s*, OAc), 1.40 (1H, *ddd*, $J_{7\beta,7\alpha}$ = 12.9 Hz, $J_{7\beta,6\beta}$ = 4.9 Hz, $J_{7\beta,8\beta}$ = 3.0 Hz, H-7β), 1.03 (3H, *s*, Me-20) and 0.88 (3H, *d*, $J_{17,8\beta}$ = 6.8 Hz, Me-17); EIMS (70 eV, direct inlet) *m/z* (rel. int.): 406 $[M]^+$ (0.04), 346 $[M-HOAc]^+$ (0.05), 323 (1), 294 (2), 234 (1), 159 (3), 157 (4), 119 (4), 113 [C-9 side chain]$^+$ (100), 105 (3), 91 (6), 83 (7), 69 (57), 55 (17), 43 (20), 41 (9). $C_{22}H_{30}O_7$ M_r 406.

Scutalpin D (**3**) *from compound* **7**. Ac_2O-pyridine (1:1, 2 ml) treatment of **7** (10 mg) at room temp. for 48 hr in the usual manner, gave a compound (8 mg, after chromatographic purification and crystallization from EtOAc–*n*-hexane) which was identical in all respects [mp 271–273°; $[\alpha]_D^{21}$ +3.7° ($CHCl_3$; *c* 0.191), ^1H NMR, TLC] with natural scutalpin D [**3**, mp 270–272°; $[\alpha]_D^{25}$ +2.3° ($CHCl_3$; *c* 0.898)] [4].

Application of Horeau's method to 11*-deacetylscutalpin D* (**7**). This was performed in the usual manner [8]. Compound **7** (24.54 mg, 0.0485 mmol) and (±)-α-phenylbutyric anhydride (35.20 mg, 0.1135 mmol) in dry pyridine (2 ml) was kept at 20° for 18 hr. α_1 = −0.190, α_2 = −0.064; partial resolution: $\alpha_1 - 1.1\alpha_2$ = −0.126. Configuration 11*S*.

Antifeedant bioassay of compounds **1–3**. Larvae of the lepidopteran *Spodoptera littoralis* (Boisduval) which were 24–36 hr into the final stadium were deprived of food for 4 hr before being used in the bioassay. Compounds **1–3** were applied to glass-fibre discs (Whatman GF/A, 2.1 cm diameter) which had been treated with 100 μl sucrose (50 mM). In the choice bioassay larvae were placed individually into Petri dishes with two glass-fibre discs. One disc acted as the control and the other disc, the treatment disc, was treated additionally with 100 μl of a soln (100 ppm) containing one of the test compounds. The dried discs were weighed before being presented to the larvae. The larvae were removed when they had eaten

Table 3. Effect of compounds **1–3**, **10** and **11** on the feeding behaviour of larvae of *Spodoptera littoralis*

	Antifeedant activity	
Compound	Choice bioassay (antifeedant index mean ± S.E.M.)*	No-choice bioassay (LD_{50}, ppm)†
1	26.9 ± 11.01	> 1000
2	96.8 ± 1.17	< 1
3	−1.5 ± 11.29	930
10 (jodrellin A)‡	92 ± 7.6	—
11 (jodrellin B)‡	100 ± 0.0	—

* Antifeedant index [(C−T)/(C+T)]% of compounds tested at 100 ppm, 10 replications per compound.

† LD_{50} is the estimated concentration required to decrease by 50% the amount of a treated disc eaten in 16 hr, relative to the sucrose control. Fifteen replications per concentration per compound.

‡ Data previously published in ref. [11].

approximately 50% of one of the discs, which took 8–24 hr. In the no-choice bioassay larvae were presented with treatment discs which had been treated with 100 μl of a soln containing a test compound at one of six concns (0.01, 0.1, 1, 10, 100 or 1000 ppm). This bioassay was terminated after 16 hr, the time taken to consume 50% of the control discs.

After terminating the bioassays the discs were reweighed. In the choice bioassay the antifeedant index [(C–T)/(C+T)] % was calculated, where C and T represent the weight of the control and treatment discs eaten, respectively. In the no-choice bioassay the amount eaten of the discs treated with different concns was calculated and used to estimate the conc required to decrease feeding by 50% (LD_{50}), relative to the sucrose control.

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