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NEO-CLERODANE DITERPENOID INSECT ANTIFEEDANTS FROM *AJUGA REPTANS* cv CATLINS GIANT

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Key Word Index—*Ajuga reptans*; Labiatae; *neo*-clerodane diterpenoids; 14,15-dehydroajugareptansin; 3β-hydroxyajugavensin B; 3α-hydroxyajugamarin F4; ajugareptansin; insect antifeedant.

Abstract—Three new *neo*-clerodane diterpenoids (14,15-dehydroajugareptansin, 3β-hydroxyajugavensin B and 3α-hydroxyajugamarin F4), have been isolated from aerial parts of *Ajuga reptans* cv Catlins Giant, together with the known compound, ajugareptansin. The structures were determined by ^{1}H and ^{13}C NMR spectroscopy and MS. Insect antifeedant testing of all four compounds revealed that 14,15-dehydroajugareptansin had significant activity against sixth stadium larvae of *Spodoptera littoralis*. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

The Labiatae family is a rich source of *neo*-clerodane diterpenoids [1]. Some of these compounds isolated from species of *Ajuga* [2], *Teucrium* [3, 4], *Scutellaria* [5, 6] and *Salvia* [7] have been shown to have insect antifeedant properties [2, 8]. We have extended the scope of these studies by re-examining *Ajuga reptans* L., a common herb found throughout Europe. In previous studies of this plant, the *neo*-clerodane diterpenoids ajugareptansin [9] and ajugareptansone A [10] were isolated but neither has been shown to exhibit insect antifeedant activity [8]. However, it has been noted subsequently that ethanol extracts of *A. reptans* do have significant antifeedant activity [4]. In the present paper, the isolation and characterisation of three new *neo*-clerodane diterpenoids from *A. reptans* cv Catlins Giant and their effect on the feeding behaviour of *Spodoptera littoralis* larvae are reported.

RESULTS AND DISCUSSION

Acetone extraction of the aerial parts of *Ajuga reptans* cv Catlins Giant, followed by silica gel chromatography and preparative TLC, yielded three new *neo*-clerodane diterpenoids, 14,15-dehydroajugareptansin (**1**), 3β-hydroxyajugavensin B (**2**) and 3α-hydroxyajugamarin F4 (**3**), in addition to the known compound ajugareptansin (**4**). ^{1}H and ^{13}C NMR spectra obtained for **4** were in agreement with the previously published assignments for ajugareptansin [9], although only a partial set of ^{1}H NMR assignments was given in that report. Assignment of the ^{1}H NMR spectrum of ajugareptansin was therefore completed by analysis of COSY data. These results are included in Table 1 for reference.

Analysis of the ^{1}H and ^{13}C NMR spectra of **1** indicated clearly that the compound was of the *neo*-clerodane diterpenoid class and furthermore, closely related to ajugareptansin. In the ^{1}H NMR spectrum, two coupled 1H resonances at δ 6.42 (*dd*, J = 2.4 Hz) and 4.75 (*dd*, J = 2.4 Hz), were distinctive and could be assigned to H-15 and H-14, respectively, based on sequential COSY connectivities. The remainder of the resonances in the ^{1}H NMR spectrum were characterised by chemical shift and coupling constant parameters similar to those of ajugareptansin, with the exception of H-13 and H-16, which were downfield-shifted, as is evident from Table 1. All assignments were verified by connectivity data from a COSY experiment. Two resonances at δ 101.8 and 146.6 in the ^{13}C NMR spectrum were readily assigned to C-14 and C-15, respectively, and should be compared with corresponding chemical shift values of 34.1 and 67.7 for these carbon atoms in ajugareptansin [9]. Overall, the NMR data indicated that **1** differed from ajugareptansin only by the presence of a double bond between C-14 and C-15. The empirical formula of

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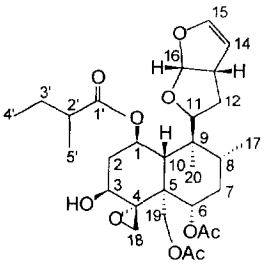

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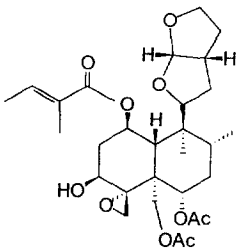

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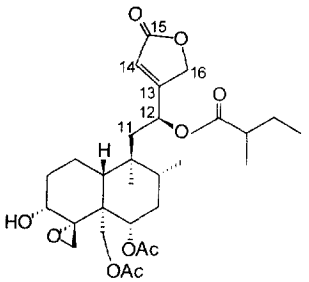

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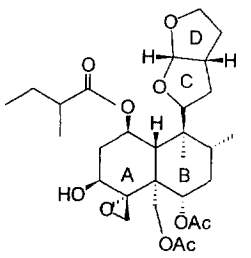

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$C_{29}H_{42}O_{10}$ proposed for **1** was confirmed by MS data, with a $[M]^{+\cdot}$ ion recorded at *m/z* 550. Compound **1** is therefore 14,15-dehydroajugareptansin, a new *neo*-clerodane diterpenoid.

The ^1H NMR spectrum of **2** was also similar to that of ajugareptansin, with the notable exception of three distinctive resonances at δ 6.81 (1H, *qq*, $J = 7.0$ and 1.5 Hz), 1.82 (3H, *d*, $J = 7.0$ Hz) and 1.86 (3H, *br s*). In the corresponding COSY spectrum of **2**, a cross-peak was identified between the resonances at δ 6.81 and 1.82. These features indicated the presence of a tigloyl ester group. The remaining resonances in the ^1H NMR spectrum of **2** were assigned unambiguously using COSY data and, as inspection of Table 1 indicates, are very similar to those obtained for ajugareptansin, particularly in the B, C and D rings of the structure. Assignment of H-1, H-2 and H-3 in the A ring confirmed that, as with **1** and **4**, compound **2** was substituted by an ester group at C-1 and a β-OH at C-3. However, in contrast to ajugareptansin, the ester functionality at C-1 is that of a tigloyl, rather than a 2-methylbutyroyl group. An empirical formula of $C_{29}H_{42}O_{10}$ proposed for **2** was confirmed by MS data, with a $[M]^{+\cdot}$ ion recorded at *m/z* 550. Compound **2** is therefore a second new *neo*-clerodane diterpenoid, and has been assigned the trivial name 3β-hydroxyajugavensin B, based on the known compound ajugavensin B isolated previously from *A. genevensis* [11, 12].

The determination of the molecular structure of **3** required the acquisition of a more extensive set of ^1H and ^{13}C NMR experiments, as it was recognised from the outset that while this compound was clearly of the *neo*-clerodane diterpenoid class, its structure differed significantly from **1**, **2** and ajugareptansin (**4**). A subset of resonances in the ^1H NMR spectrum at δ 2.34 (1H, *sextet*, $J = 7.0$ Hz), 1.46, 1.69 ($2 \times 1H$, $2 \times m$), 0.89 (3H, *t*, $J = 7.5$ Hz) and 1.14 (3H, *d*, $J = 7.0$ Hz), where appropriate proton connectivities were verified by COSY data, indicated that **3** was substituted with a 2-methylbutyroyl group. In addition, it was clear that **3** comprised the typical functional groups characteristic of *neo*-clerodane diterpenoids, with ^1H NMR resonances at δ 2.71 (*d*, $J = 4.0$ Hz) and 2.82 (*d*, $J = 4.0$ Hz) indicating a C-18 epoxide, 4.19 (*d*, $J = 12.1$ Hz) and 4.84 (*d*, $J = 12.1$ Hz) a C-19 CH_2OAc moiety, 1.94 (*s*) and 2.07 (*s*), the methyl groups of two acetate moieties, 0.82 (*d*, $J = 5.9$ Hz), a C-17 methyl and 0.74 (*s*), a C-20 methyl. In the COSY spectrum, the epoxide resonance at δ 2.81 exhibited a weak ^4J connectivity to a single proton resonance at 4.03 (*dd*, $J = 11.4$ and 4.8) which in turn correlated with a CH_2CH_2CH fragment with resonances at δ 1.24, 2.25 (*i*), 1.77, 1.97 ($i+1$) and 1.55 ($i+2$), respectively. The multiplicities of the carbon atoms were also verified through use of DEPT and HMQC data. The fragment could be uniquely assigned to a C-3 bearing hydroxyl group, C-2, C-1 and C-10, respectively, which further established that the 2-methylbutyroyl ester function was not located

Table 1. ^{1}H NMR chemical shift data for the *neo*-clerodane diterpenoids **1** to **4** (δ in $CDCl_3$, 30°). Resonance assignments for methylene groups are given as "a" and "b", where a indicates the resonance with the most downfield δ value

H	1	2	3	4
1a	5.60 *ddd*	5.79 *ddd*	1.97	5.63 *m*
1b	—	—	1.77	—
2a	2.47	2.46 *ddd*	2.25	2.46
2b	1.66	1.82	1.24	1.74
3	4.23 *dd*	4.24 *dd*	4.03 *dd* (11.4, 4.8)	4.26 *dd* (8.9, 6.4)
6	4.83 *dd* (11.6, 4.6)	4.82 *dd* (11.3, 4.6)	4.78 *dd* (10.3, 5.5)	4.82 *dd* (11.3, 4.6)
7a	1.67	1.68	1.65–1.55	1.66
7b	1.53	1.49	1.65–1.55	1.54
8	2.07	1.86	1.64	2.06
10	2.42 *d* (9.2)	2.31 *d* (7.6)	1.55	2.38 *d* (8.9)
11a	4.54 *dd* (11.3, 4.6)	4.45 *dd* (10.7, 6.4)	2.13 *dd* (16.5, 9.5)	4.58 *dd* (11.0, 6.1)
11b	—	—	1.53 *d* (16.5)	—
12a	1.93	2.03	5.61 *br d* (9.2)	2.04
12b	1.57	1.43	—	1.54
13	3.46 *m*	2.65 *m*	—	2.78*m*
14a	4.75 *dd* (2.4)	2.04	5.92 *s*	2.14
14b	—	1.57	—	1.63
15	6.42 *dd* (2.4)	3.77 *m*	—	3.85 *m*
16a	6.02 *d* (6.4)	5.55 *d* (5.2)	4.85 *dd* (17.6, 1.8)	5.65 *d* (5.2)
16b	—	—	4.69 *dd* (17.6, 1.8)	—
17	0.90 *d* (6.4)	0.88 *d* (6.7)	0.82 *d* (5.9)	0.89 *d* (6.4)
18a	2.91 *br s*	2.98 *d* (4.3)	2.82 *d* (4.0)	2.92 *br s*
18b	2.91 *br s*	2.94 *d* (4.3)	2.71 *d* (4.0)	2.92 *br s*
19a	5.04 *d* (12.8)	5.03 *d* (12.8)	4.84 *d* (12.1)	5.04 *d* (12.8)
19b	4.21 *d* (12.8)	4.18 *d* (12.8)	4.19 *d* (12.1)	4.20 *d* (12.8)
20	0.82 *s*	0.89 *s*	0.74 *s*	0.82 *s*
2′	2.32 *sextet* (7.0)	—	2.34 *sextet* (7.0)	2.29 *sextet* (7.0)
3′a	1.50–1.62 *m*	6.81 *qq* (7.0, 1.5)	1.69	1.68
3′b	1.50–1.62 *m*	—	1.46	1.48
4′	0.88 *t* (7.6)	1.82 *d* (7.0)	0.89 *t* (7.5)	0.90 *t* (7.3)
5′	1.08 *d* (7.0)	1.86 *br s*	1.14 *d* (7.0)	1.12 *d* (7.0)
$OCOCH_3$	1.94 *s*	1.94 *s*	1.94 *s*	1.94 *s*
	2.10 *s*	2.10 *s*	2.07 *s*	2.10 *s*

at C-1 as found previously in compounds **1**, **2** and **4**. Assignment of the remaining connectivities in the COSY spectrum indicated that the side-chain substituent at C-9 was not a furofuran ring but rather a β-butenolide. Furthermore, it was evident that the two-carbon bridge between the butenolide ring and ring B was a CH_2CHOR rather than CH_2CH_2 fragment, with a ^{1}H chemical shift value for the H-12 resonance of δ 5.61 (*br d*, J = 9.2 Hz). This indicated that **3** was substituted at C-12 by the 2-methylbutyroyl ester group. Use of HMQC and DEPT data in combination with the ^{1}H NMR resonance assignments facilitated the assignment of the ^{13}C NMR spectrum and indicated an empirical formula of $C_{29}H_{42}O_{10}$ for **3**, which was verified by observation of a $[M+H]^+$ signal in the mass spectrum at m/z 551.

The configuration of the hydroxyl group at C-3 of **3** is a matter of some interest and calls for comment, as the overwhelming majority of *neo*-clerodane diterpenoids isolated from *Ajuga* are characterised by a β-OH group at this position [1]. Comparison between the ^{13}C NMR assignments for C-2 and C-18 of **3**, at δ 32.0 and 42.5, with those of a *neo*-clerodane diterpenoid such as ajugacumbin D, which has identical A ring structure to **3** but with known 3β-OH substitution, reveals corresponding ^{13}C chemical shift values of 24.7 and 48.1, respectively [13]. This indicates that the configuration of the C-3 hydroxyl in **3** should be assigned as α rather than β, a premise supported by data recently published for a furano-diterpene from *Croton hovarum*, which contains a 3α-OH group, and where the ^{13}C chemical shift value for C-2 is δ 30.2 [14]. Compound **3** is therefore a third new *neo*-clerodane diterpenoid, and has been assigned the trivial name 3α-hydroxyajugamarin F4, based on the known compound ajugamarin F4 isolated previously from *A. decumbens* [15].

The antifeedant activity of compounds **1** to **4** was assessed by using a choice bioassay against sixth stadium larvae of *S. littoralis* [5]. Table 2 shows the results of the bioassays and summarises additional data for related *neo*-clerodane diterpenoids from *Ajuga* and *Scutellaria* spp. The results indicate that the antifeedant activity of **1** is enhanced significantly

Table 2. Antifeedant indices for compounds **1**–**4** and a range of related *neo*-clerodane diterpenoids against *S. littoralis* larvae

Compound	Antifeedant index† (mean ± SEM) Concentration applied (ppm) 100	25
14,15-Dehydroajugareptansin (**1**)	92 ± 5.5*	60 ± 15.9*
3β-Hydroxyajugavensin B (**2**)	0.3 ± 30.1	—
3α-Hydroxyajugamarin F4 (**3**)	−32 ± 13.9	—
Ajugareptansin (**4**)	−41 ± 19.6	—
Ajugarin I	43 ± 7.3*[a]	26 ± 14.8*[b]
Clerodin	74 ± 8.4*[c]	24 ± 9.8*[b]
14,15-Dihydrojodrellin T	63 ± 7.8*[b]	44 ± 4.5*[b]
Jodrellin A	92 ± 7.6*[d]	53 ± 13.3*[d]
Jodrellin B	100 ± 0.0*[b]	83 ± 10.3*[b]

† Antifeedant index [(C − T)/(C + T)] × 100, 10 replications. Significance (Wilcoxon signed ranks test): * = $P < 0.05$. [a][3], [b][5], [c][16] and [d][17].

compared with that of **4**, although the two compounds differ only by one degree of unsaturation in the furofuran ring. The tetrahydrofurofuran side chain present in **1** is also a structural feature of several other active compounds, namely clerodin, 14,15-dihydrojodrellin T and jodrellins A and B. Indeed, **1** maintains a significant level of activity at a concentration of 25 ppm comparable to that of jodrellin A. It is of interest to note in this context that compounds **2** and **4**, which show low levels of activity in this bioassay, both contain hexahydrofurofuran side chains. This functional group has been considered by some authors to be important for antifeedant activity, but only in those cases where the *trans*-decalin ring is not conformationally distorted [8, 18]. In ajugareptansin, for example, ring A appears to adopt a skew boat conformation as a result of steric hindrance between the C-1 and C-9 substituents [19], which may contribute to its low activity. Developing a comprehensive understanding of the relationship between structure and activity for the compounds isolated in the present study, will however require a more detailed description of their three-dimensional structures in solution.

EXPERIMENTAL

Plant material

A. reptans cv Catlins Giant was grown at the Royal Botanic Gardens, Kew, and a voucher specimen deposited in the Herbarium (Kew Accession Number 1987–2717).

General

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 30° using either a JEOL EX 270 MHz or Bruker 400 MHz spectrometer. FAB-MS (positive mode); 3-nitrobenzyl alcohol matrix. EIMS; 70 eV, JEOL JMS-DX300 spectrometer. Prep. TLC (Merck Si gel 60, 2.0 mm layer thickness, elution with Me_2CO).

Extraction and isolation of diterpenoids

250 g of freeze-dried, finely powdered aerial material (excluding flowers) was extracted with Me_2CO (3 × 1 l) at room temp. for 2 days. The resulting extract after solvent evapn (15 g) was chromatographed successively by sequential CC (3 ×) on Si gel with increasingly polar elutions of hexane–$CHCl_3$, hexane–EtOAc and $CHCl_3$–MeOH. At each stage, column fractions were assayed according to the bioassay method described below, and active fractions selected for further purification. The resulting fractions were evapd to dryness to give a residue of 2.8 g which was redissolved in a minimum vol. of 1:1 hexane–EtOAc and re-chromatographed on Si gel eluting with hexane–EtOAc 3:1 (200 ml), 2:1 (3500 ml), and 1:1 (1500 ml). Compounds **1** and **4** eluted in the hexane–EtOAc (2:1) fractions while **2** and **3** eluted in the hexane–EtOAc (1:1) fractions. **1** and **4** were further separated from other components by prep. TLC using $CHCl_3$–MeOH (10:1). Final purification in three stages by prep. TLC using $CHCl_3$–MeOH (10:1), TLC using hexane–EtOAc (5:7) and TLC using $CHCl_3$–EtOAc (1:2) gave essentially pure **1** (3.5 mg). A similar method was used to obtain pure **4** (17.1 mg).

The pooled fractions containing **2** and **3** were separated by CC on Si gel eluting with $CHCl_3$ (1 l), $CHCl_3$–Me_2CO 10:1 (380 ml), 8:1 (900 ml) and 7:1 (540 ml). **2** eluted in the 10:1 fractions and **3** in the 8:1 fractions. **2** was further purified from contaminating material using CC on Si gel eluting with increasingly polar gradients of $CHCl_3$–MeOH. Final purification by three stages of TLC, using $CHCl_3$–Me_2CO (5:1) followed by $CHCl_3$–EtOAc (1:2) and hexane–EtOAc (5:7), gave pure **2** (5.3 mg). **3** was further purified by CC on Si gel using the same elution system as with **2**.

Final purification by two stages of TLC using $CHCl_3$-Me_2CO (5:1) and then hexane-EtOAc (5:7) gave **3** in the form of white platelet crystals (20.7 mg). Terpenoids were visualised throughout as blue spots on thin layer chromatograms by spraying with ammonium molybdate and 10% sulphuric acid (5 g in 100 ml), followed by heating.

14,15-*Dehydroajugareptansin* (**1**). ^{1}H NMR: Table 1. ^{13}C NMR (67.8 MHz, $CDCl_3$); δ 11.3 (C-4′), 14.4 (C-5′), 15.8 (C-17), 19.0 (C-20), 2×21.0 ($OCOCH_3$), 26.9 (C-3′), 32.5, 33.3 (C-7, C-8), 33.9 (C-2), 38.1 (C-12), 40.7, 40.9 (C-9, C-2′), 43.3 (C-18), 44.7 (C-5) 46.0 (C-13), 51.7 (C-10), 61.3 (C-19), 63.6 (C-3), 66.6 (C-4), 69.3 (C-1), 71.4 (C-6), 83.3 (C-11), 101.8 (C-14), 108.2 (C-16), 146.6 (C-15), 169.7, 170.2 (2×$OCOCH_3$), 175.5 (C-1′). EIMS (probe) 70 eV, *m/z* (rel. int.): 550 [M$^+$] (2), 423 (6), 367 (10), 219 (8), 187 (17), 171 (14), 145 (13), 119 (9), 111 (48), 95 (12), 85 (53), 69 (19), 57 (100), 55 (27), 43 (82), 41 (23).

3β-*Hydroxyajugavensin B* (**2**). ^{1}H NMR: Table 1. EIMS (probe) 70 eV *m/z* (rel. int.): 550 [M$^+$] (0.4), 450 (3), 421 (15), 380 (5), 361 (1), 201 (7), 187 (8), 159 (8), 145 (6), 113 (100), 107 (6), 91 (6), 83 (66), 69 (73), 55 (60), 43 (64), 41 (15).

3α-*Hydroxyajugamarin F*4 (**3**). ^{1}H NMR: Table 1; ^{13}C NMR (67.8 MHz, $CDCl_3$); δ 11.5 (C-4′), 15.3 (C-17), 15.8 (C-5′), 17.0 (C-20), 20.4 (C-1), 2×21.0 ($OCOCH_3$), 26.8 (C-3′), 32.0 (C-2), 32.5 (C-7), 35.4 (C-8), 39.5 (C-9), 2×40.7 (C-11, C-2′), 42.5 (C-18), 45.3 (C-5), 48.7 (C-10), 61.5 (C-19), 65.7 (C-3), 66.0 (C-12), 67.5 (C-4), 70.4 (C-16), 71.7 (C-6), 116.0 (C-14), 168.5 (C-13), 169.7, 170.5 (2×$OCOCH_3$), 172.4 (C-15), 175.6 (C-1′). FAB-MS (positive) *m/z*: 551 [M+H]$^+$.

Ajugareptansin (**4**). ^{1}H NMR: Table 1: FAB-MS (positive) *m/z*: 553 [M+H]$^+$.

Insect

Spodoptera littoralis (Boisduval) were reared on a maize-based diet [20], at 26±2° and in an 18L:8D photoperiod.

Antifeedant bioassay

This assay has previously been used to investigate the antifeedant activity of *neo*-clerodane diterpenoids from *Teucrium* [3], *Scutellaria* [5] and *Salvia* [7]. Compounds **1** to **4** were assayed for antifeedant activity by presenting them on glass-fibre discs (Whatman GF/A, 2.1 cm diameter), made palatable by application of 100 μl of sucrose (0.05 M). After drying, 100 μl of the test compound at 100 or 25 ppm was added to treatment discs (10 replications per treatment) and the discs redried and weighed. Sixth stadium larvae of *S. littoralis* were deprived of food for 4 h, then placed individually in a Petri dish with a treatment and a control disc. The insects were removed either after 50% of either disc had been consumed or after 14 h. The discs were then reweighed and an antifeedant index calculated as $[(C-T)/(C+T)]\times 100$, where C and T are the weights of control and treatment discs consumed, respectively. The index identifies both phagostimulants (negative values) and antifeedants (positive values).

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Note added in proof

A report describing the characterisation of **2**, 3β-hydroxyajugavensin B, appeared (*Phytochemistry*, 1997, **45**, 121) after the present paper was accepted for publication. The authors isolated the compound from the aerial parts of *Ajuga orientalis* and named it ajugorientin, although it is a simple derivative of the known compound ajugavensin B [11, 12]. Their ^{1}H NMR data is in general in good agreement with our complete assignment obtained using both 1D and 2D methods, although the δ values reported for H-7b and H-8 should be reversed. The compound was not tested for biological activity as an insect antifeedant. These results are included in Table 2 of the present work.

REFERENCES

1. Rodríguez-Hahn, L., Esquivel, B. and Cárdenas, J. In *Progress in the Chemistry of Organic Natural Products*, Vol. 63, ed. W. Hertz, G. W. Kirby, R. E. Moore, W. Steglich and Ch. Tamm. Springer-Verlag, Vienna, 1994, p. 107.
2. Camps, F. and Coll, J., *Phytochemistry*, 1993, **32**, 1361.
3. Simmonds, M. S. J., Blaney, W. M., Ley, S. V., Savona, G., Bruno, M. and Rodríguez, B., *Phytochemistry*, 1989, **28**, 1069.
4. Simmonds, M. S. J. and Blaney, W. M. In *Advances in Labiatae Science*, ed. R. M. Harley and T. Reynolds, Royal Botanic Gardens, Kew, 1992, p. 375.
5. Cole, M. D., Anderson, J. C., Blaney, W. M., Fellows, L. E., Ley, S. V., Sheppard, R. N. and

Simmonds, M. S. J., *Phytochemistry*, 1990, **29**, 1793.
6. Rodríguez, B., de la Torre, M. C., Rodríguez, B., Bruno, M., Piozzi, F., Savona, G., Simmonds, M. S. J., Blaney, W. M. and Perales, A., *Phytochemistry*, 1993, **33**, 309.
7. Simmonds, M. S. J., Blaney, W. M., Esquivel, B. and Rodríguez-Hahn, L., *Pesticide Science*, 1996, **47**, 17.
8. Belles, X., Camps, F., Coll, J. and Piulachs, M. D., *Journal of Chemical Ecology*, 1985, **11**, 1439.
9. Camps, F., Coll, J., Cortel, J. and Messeguer, A., *Tetrahedron Letters*, 1979, 1709.
10. Camps, F., Coll, J. and Cortel, A., *Chemistry Letters*, 1981, 1093.
11. Malakov, P. Y., Papanov, G. Y., de la Torre, M. C. and Rodríguez, B., *Phytochemistry*, 1991, **30**, 4083.
12. Malakov, P. Y., Papanov, G. Y., Perales, A., de la Torre, M. C. and Rodríguez, B., *Phytochemistry*, 1992, **31**, 3151.
13. Min, Z., Wang, S., Zheng, Q., Wu, B., Mizuno, M., Tanaka, T. and Iinuma, M., *Chemical and Pharmaceutical Bulletin*, 1989, **37**, 2505.
14. Krebs, H. C. and Ramiarantsoa, H., *Phytochemistry*, 1996, **41**, 561.
15. Shimomura, H., Sashida, Y. and Ogawa, K., *Chemical and Pharmaceutical Bulletin*, 1989, **37**, 988.
16. Blaney, W. M., Simmonds, M. S. J., Ley, S. V. and Jones, P. S., *Entomologia Experimentalis et Applicata*, 1988, **46**, 267.
17. Anderson, J. C., Blaney, W. M., Cole, M. D., Fellows, L. E., Ley, S. V., Sheppard, R. N. and Simmonds, M. S. J., *Tetrahedron Letters*, 1989, **30**, 4737.
18. Kojima, Y. and Kato, N., *Tetrahedron*, 1981, **37**, 2527.
19. Solans, X., Miravitlles, C., Declercq, J. P. and Germain, G., *Acta Crystallographica*, 1979, **B35**, 2732.
20. Simmonds, M. S. J., Blaney, W. M. and Schoonhoven, L. M, *Journal of Insect Physiology*, 1992, **38**, 249.