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Structure–activity relationships of natural and synthetic *neo*-clerodane diterpenes from *Teucrium* against Colorado potato beetle larvae

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Abstract

Treatment of potato leaf disks with ten *neo*-clerodane diterpenes isolated from *Teucrium* and with ten of their synthetic derivatives, resulted in a significant antifeedant activity against *Leptinotarsa decemlineata* larvae in both choice and no-choice assays for nine of the natural products and three of the hemisynthetic compounds. Changes in the antifeedant activity were associated with certain structural features of the compounds and some significant aspects of the structure–activity relationship are discussed.

Keywords: Leptinotarsa decemlineata; Colorado potato beetle; Labiatae; *Teucrium*; *Neo*-clerodane diterpenes; Antifeedants

1. Introduction

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is an economically important pest that has developed resistance to most classes of synthetic insecticides (Forgash, 1981, 1985). Despite widespread development of resistance, particularly in the northeastern United States, chemical insecticides remain the major control method (Hare, 1990). Recently, interest has grown for the search of antifeedants of plant origin as a promising source of new control agents (Alford, Cullen, Storch, & Bentley, 1987; Hough-Goldstein, 1990; Huang, Chung, Bentley, & Alford, 1995).

Neo-clerodane diterpenes have attracted interest in the last years on account of their biological activities against some economically important pests (Merritt & Ley, 1992; Piozzi, 1994; Rodríguez-Hahn, Esquivel, & Cárdenas, 1994). The most abundant source of this kind of compounds are the plants belonging to the genus *Teucrium* (Labiatae), from which about 180 *neo*-clerodanes have been isolated (Piozzi, Rodríguez, & Savona, 1987; Merritt & Ley, 1992; Piozzi, 1994; Rodríguez-Hahn et al., 1994). *Neo*-clerodanes from *Teucrium* and some of their derivatives reduced feeding in larvae of some economically important lepidopteran (Blaney, Simmonds, Ley, & Jones, 1988; Simmonds et al., 1989; Simmonds & Blaney, 1992; Rodríguez et al., 1994), coleopteran (Ortego, Rodríguez, & Castañera, 1995) and orthopteran pests (Hanson, Rivett, Ley, & Williams, 1982).

We report here on the effects of ten natural *neo*-clerodanes from *Teucrium* and ten synthetic derivatives on the feeding behaviour of larvae of the Colorado potato beetle in order to elucidate the functional groups responsible for antifeedant activity.

2. Results and discussion

Among the compounds tested against Colorado potato beetle larvae there are two groups clearly different from a structural point of view: 19-nor-*neo*-clerodane derivatives (**1–6**) and *neo*-clerodane diterpenes in their own right (**7–20**, see Fig. 1).

All the compounds belonging to the 19-nor-*neo*-clerodane class (**1-6**) possess a 9-spiro-20,12-γ-lactone grouping with a β-furyl substituent at γ-position and an α,β-unsaturated γ-lactone in which the C-4, C-5, C-6 and C-18 carbon atoms of the *neo*-clerodane framework are involved. All these substances showed strong antifeedant

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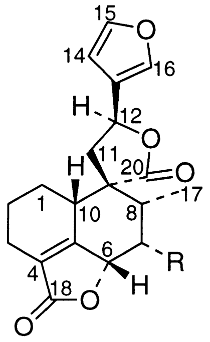

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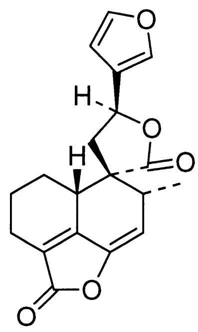
3 R=H
6 R=OAc



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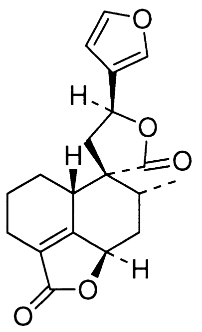

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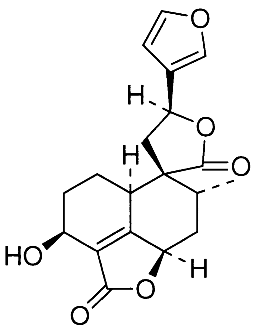

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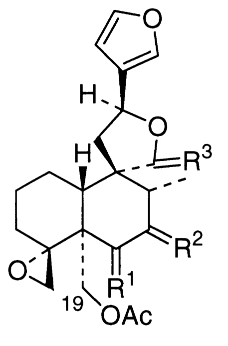
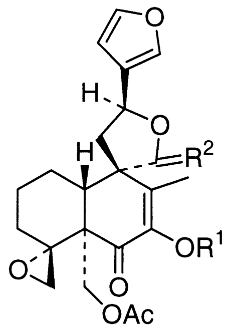
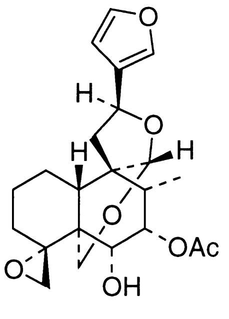


	R1	R2	R3
7	O	αOH,βH	αH,βOAc
8	O	αOH,βH	αH,βOH
9	O	αOAc,βH	αOH,βH
10	O	αOAc,βH	O
11	O	H,H	O
13	O	αOAc,βH	αOMe,βH
17	αOH,βH	O	αOMe,βH
18	αOAc,βH	O	αOMe,βH
19	αOH,βH	O	αH,βOMe
20	αOH,βH	αOAc,βH	O



12 R^1=H, R^2=O
14 R^1=Ac, R^2=αH,βOAc



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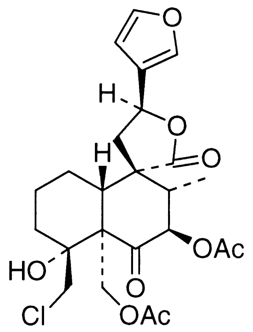

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[a] Natural compounds: **1** (teucrin A), **2** (teuscorolide), **3** (teucvin), **4** (teuflin), **5** (teuflidin), **7** (eriocephalin), **8** (20-deacetyleriocephalin), **10** (capitatin), **11** (19-acetylgnaphalin) and **12** (picropolinone). Hemisynthetic compounds: **6**, **9** and **13-20**.

Fig. 1. Molecular structures of the *neo*-clerodane diterpenes tested against Colorado potato beetle larvae[a].

Table 1
Effect of natural and synthetic *neo*-clerodane diterpenes on the feeding behaviour of fourth instar larvae of Colorado potato beetle

Compound (1000 ppm)	No-choice assay AI[a]	Choice assay AI[b]	Choice/ no-choice SI[c]
1	87.4±3.0	26.8±20.2	69.8±3.8
2	70.4±2.7	53.4±7.1	31.0±4.1
3	85.9±2.5	46.2±9.8	69.4±2.9
4	89.3±1.1	44.1±19.4	55.2±7.7
5	92.9±1.6	57.5±12.6	50.8±6.2
6	91.0±1.4	57.6±15.4	43.8±7.3
7	86.1±1.5	−1.2±18.5	63.7±5.9
8	40.5±4.2	9.5±8.2	13.3±5.2
9	53.5±4.3	28.5±8.7	29.1±7.4
10	89.3±2.8	−10.1±14.8	64.6±5.0
11	86.6±2.7	9.4±20.2	64.4±5.6
12	89.2± 2.0	20.1±17.0	74.8±3.9
13	51.7±2.9	10.7±10.1	30.9±6.7
14	62.6±2.9	31.8±13.4	40.5±6.2
15	30.9±2.8	54.5±8.6	29.7±6.2
16	46.4±2.6	22.8±14.9	40.2±6.4
17	64.7±2.9	49.0±7.6	38.7±6.0
18	58.3±4.1	61.7±7.2	24.6±5.5
19	77.4±1.6	38.0±3.8	33.1±3.5
20	89.9±2.3	0.7±18.7	79.1±2.9

[a] Average antifeedant index $[(C-T)/C]\times 100\% \pm$ standard error ($n=10$). C and T represent consumption of control and treatment disks, respectively.

[b] Average antifeedant index $[(C-T)/(C+T)]\times 100\% \pm$ standard error ($n=10$). C and T represent consumption of control and treatment disks, respectively within each arena.

[c] Average suppression index $[(C-T)/C]\times 100\% \pm$ standard error ($n=10$). T represents consumption of both treated and untreated disks in test arenas and C in control arenas that contain untreated leaf disks only.

activity against the larvae in no-choice assays at 1000 ppm (Table 1).

The different behaviour of compounds **1**–**6** as antifeedants in no-choice assays must be attributed to their decalin part, because all the substances possess identical functionality at the C-9 position. The stereochemistry of the closure of the α,*β*-unsaturated 18,6-γ-lactone seems to be a superfluous structural feature for the biological action, because epimers at C-6 like **3** and **4** showed similar activity. When C-6 is an sp^2 carbon as in teuscorolide (**2**), however, the activity decreased drastically. Taking into account the structures of compounds **1**–**6** (Fig. 1), it is reasonable to assume that the antifeedant activity of these substances could be due to their capability for operating as Michael-type acceptors at a molecular level (Jansen & de Groot, 1991). The 18,6-γ-lactone grouping of these diterpenoids should link together with the nucleophilic groups ($-NH_2$, –SH, –S–) of the sense organs used by the larvae to taste the compounds. In fact, teuscorolide (**2**) was the less active of the 19-nor-*neo*-clerodanes tested (**1**–**6**) and it is chemically the less reactive as a Michael acceptor.

The presence of a secondary hydroxyl group in positions C-3 or C-7, neighbouring to the 18,6-γ-lactone, causes an increase in the activity, as in the case of compounds **1** (teucrin A) and **5** (teuflidin), although without significant differences with compounds **3**, **4** and **6**.

Toxic (antibiosis) and deterrent (antixenosis) modes of action have been suggested as responsible for the antifeedant activity of *neo*-clerodanes on Colorado potato beetle. Thus, Ortego et al. (1995) found that the *neo*-clerodane teuscorolide (**2**) acts as a feeding deterrent against Colorado potato beetle larvae, whereas the antifeedant activity of teucrin A (**1**), teucvin (**3**) and eriocephalin (**7**) is likely associated with a toxic mode of action. Likewise, we have observed that teucrin A (**1**) and teucvin (**3**) caused a significant suppression of feeding in the choice/no-choice assay, but only a low discrimination between treated and control disks in the choice tests (Table 1), suggesting that they operate predominantly as antifeedants with a post-ingestive toxic mode of action. On the contrary, disks treated with teuscorolide (**2**) were avoided in choice tests, whereas low suppression activity was induced (Table 1), suggesting that this compound acts as a feeding deterrent. The other 19-nor-*neo*-clerodanes (**4**–**6**) caused both high discrimination in choice tests and high suppression activity Table 1, indicating that these compounds probably exhibit both modes of action. From our data the mode of action from the structural variations of compounds **1**–**6** can not be forecast.

Comparison between the antifeedant indices in choice and no-choice assays suggests that the activity against Colorado potato beetle larvae of the *neo*-clerodane derivatives **7**–**20** was clearly a toxic mode of action for those compounds which were active (see Table 1). The data clearly show that small changes of structure (Fig. 1) can cause large changes in antifeedant activity. The 4α,18-oxirane combined with the C-19 acetoxymethylene group are essential for the activity, as was revealed by comparing the AI values of capitatin (**10**), which possesses these two functionalities and was active and those of the derivatives **15** and **16** (where the C-19 acetoxymethylene is replaced by a 20,12,19-acetal or the 4α,18-epoxide is transformed into a chlorohydrin, respectively), in which the activity decreased. Moreover, the most active compounds (**7**, **10**–**12**, **19** and **20**) possess, apart from the 4α,18-epoxide and 19-acetoxymethylene groups, a secondary alcohol at the equatorial C-6α position (**19** and **20**) or a 6-keto group (**7**, **10**–**12**), and this oxidation at C-6 also influences activity (Simmonds et al., 1989). Additional oxidation at C-7 is not a relevant structural feature, because 19-acetylgnaphalin (**11**, without oxidation at C-7) showed an antifeedant index similar to those of capitatin (**10**), picropolinone (**12**) and compound **20**, all of them having the C-7 carbon oxidised (see Table 1 and Fig. 1). Substitution of the 20,12-γ-lactone of compounds such as **10**–**12**, and **20** by a 20,12-hemiacetal or 20,12-acetal function (derivatives **8**, **9**, **13**–**15** and **17**–**19**,

Table 2
Effective concentration (EC_{50}) of natural and synthetic *neo*-clerodane diterpenes to inhibit feeding of fourth instar larvae of Colorado potato beetle by 50% in no-choice tests

Compound	EC_{50} (ppm)	Confidence interval[a]
1	73	29–128
2	394	237–803
3	94	50–157
4	91	51–138
5	53	26–81
6	84	43–136
7	286	189–415
10	207	123–299
11	102	62–152
12	108	66–154
19	>300	–
20	95	55–148

[a] Lower and upper values of 95% confidence interval.

Fig. 1) cause, in general, a noticeable decrease in activity, except for eriocephalin (**7**) (see Tables. 1–2).

The behaviour of compounds **7**–**20** seems to indicate that their antifeedant activity is related predominantly to the presence of the 4α,18-oxirane, the oxidation at C-6 and the 19-acetoxy substituent, whereas the 20,12-γ-lactone is also important, but not decisive, for the biological activity. The activity could be due to the reactivity of the 4α,18-epoxide towards nucleophiles. This reactivity is strongly influenced by the neighbouring functions at the C-6 and C-19 positions (Rodríguez et al., 1994), thus causing noticeable differences in the antifeedant action of each compound.

The results achieved in this work on the biological action of *neo*-clerodane derivatives against Colorado potato beetle larvae, confirm previous studies on the activity of these compounds against other insect species (Hanson et al., 1982; Blaney et al., 1988; Simmonds et al., 1989; Simmonds & Blaney, 1992; Rodríguez et al., 1994) and revealed remarkable differences of specificity among insect species.

It is of interest to indicate that among the twelve more active compounds against Colorado potato beetle larvae (**1**–**7**, **10**–**12**, **19** and **20**) nine of them are natural products (**1**–**5**, **7** and **10**–**12**). This finding illustrates the high efficiency of plants in the synthesis of chemical defenses against herbivores and suggests that the whole molecular structure of a secondary metabolite is important for a more effective biological action.

3. Experimental

3.1. Test compounds

The *neo*-clerodane diterpenes and their synthetic derivatives Fig. 1 were available from previous studies. Teucrin A (**1**), teucvin (**3**), teuflin (**4**) and teuflidin (**5**) were obtained from *Teucrium chamaedrys* L. as was described previously (Fernández-Gadea, Pascual, Rodríguez, & Savona, 1983; Rodríguez et al., 1984). Teuscorolide (**2**) was isolated from *T. scorodonia* L. and prepared from **1** by treatment with acetic anhydride-sodium acetate (Marco, Rodríguez, Savona, & Piozzi, 1982). Compound **6** (7-*O*-acetylteucrin A) was obtained by acetic anhydride–pyridine treatment of **1** (Savona, García-Alvarez, & Rodríguez, 1982). Eriocephalin (**7**) and its 20-deacetyl derivative (**8**) were isolated from *T. eriocephalum* L. and *T. lanigerum* Lag. (Fayos et al., 1979; Fernández-Gadea, Rodríguez, Savona, & Piozzi, 1984). Compound **9** was obtained by treatment of **7** with sodium *tert*-butoxide (de la Torre et al., 1994). Capitatin (**10**) was isolated from *T. polium* L. subsp. *capitatum* Arcangeli (Márquez et al., 1980; Fernández, Rodríguez, Savona, & Pozzi, 1986). 19-Acetylgnaphalin (**11**) is a common constituent of *Teucrium* species (Piozzi et al., 1987; Merritt & Ley, 1992; Piozzi, 1994; Rodríguez-Hahn et al., 1994) and the material used in this work was isolated from *T. gnaphalodes* L'Her. (Savona, Paternostro, Piozzi, & Rodríguez, 1979). Picropolinone (**12**) was extracted from *T. polium* L. (Márquez & Valverde, 1979; Fernández et al., 1986). Compounds **13**, **15** and **17**–**19** were prepared from **7** by different chemical reactions (Domínguez, Mössner, de la Torre, & Rodríguez, 1998). Compound **14** was obtained (Domínguez et al., 1998) by acetic anhydride–pyridine treatment of 7,8-dehydro-eriocephalin (Hueso-Rodríguez et al., 1986). The chlorohydrin derivative **16** (Hundal & Martínez-Ripoll, 1996) was obtained by treatment of 19-acetylteupolin IV (de la Torre, Piozzi, Rizk, Rodríguez, & Savona, 1986) with hydrochloric acid. Finally, compound **20** was obtained by sodium borohydride reduction of **10** (Márquez et al., 1980).

3.2. Insects

A colony was established by collecting over 200 adults of Colorado potato beetle from a potato field located in Toledo (Spain) in 1996. The laboratory colony was reared on potato plants, *Solanum tuberosum* cv. Kennebec, at 22±2°C, 90±10% rh and 16:8 h (light:dark) photoperiod in an environmental chamber.

3.3. Feeding experiments. General

The arena for the assays consisted of plastic petri dishes (15×90 mm), coated on their bottom half with about 20 ml of a 2.5% agar solution (Escoubas, Lajide, & Mitzutani, 1993). Potato leaf disks (1.77 cm^2) were cut with a cork borer No. 15 and fit into holes punched in the agar layer. The disks were treated on the upper surface with 12 μl of an acetone solution containing the test compound or the solvent carrier alone. After complete evaporation of the solvent, newly emerged fourth instar larvae (less than 24 h old) were starved for 6 h and placed in each dish in a growth chamber at 26 ± 0.5°C and 85 ± 10% rh, where they were allowed to feed.

Feeding indices were calculated on a dry weight basis. For each assay, initial dry weight of the leaf disks was estimated with 100 leaf disks to calculate the ratio of fresh to dry weight. At the end of the experiment, the uneaten leaf disks were oven dried at 60°C for 2 days and weighed.

3.4. Choice and no-choice feeding assays

Potato leaf disks were treated with 1000 ppm of the test compounds. In the choice assay, three treated and three control disks were alternatively arranged in each arena. For the no-choice assays, six treated or control disks were used. One newly emerged fourth instar larvae was placed in each dish. Feeding was terminated after the consumption of 50% of the disks in the choice assay and 75% of the control disks in the no-choice assay. Ten replicates per treatment were used in both assays.

The antifeedant index (AI) was calculated in the no-choice assay by the equation $[(C-T)/C]*100\%$ (Bentley, Leonard, Stoddard, & Zalkow, 1984) and in the choice assay by the equation $[(C-T)/(C+T)]*100\%$ (Simmonds et al., 1989), where C and T represent the consumption in control and treated disks, respectively. The suppression index (SI) was determined by the equation $[(C-T)/C]*100\%$, where T represents consumption of both treated and untreated leaf disks in choice arenas and C the consumption in control arenas that contain untreated leaf disks only (Raffa & Frazier, 1988).

No-choice assays were also performed to assess the effective concentration of the test compounds required to inhibit feeding of Colorado potato beetle larvae by 50% (EC_{50}). Potato leaf disks were treated with 0, 30, 100, 300 and 1000 ppm. EC_{50} values of each compound and 95% fiducial limits were obtained by probit analysis using POLO-PC (LeOra Software, 1987).

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