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THE ANTIFEEDANT ACTIVITY OF CLERODANE DITERPENOIDS FROM *TEUCRIUM*

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Key Word Index—*Teucrium*, *Ajuga*, Labiatae; *Spodoptera littoralis*, *Heliothis armigera*, antifeedant activity, clerodane diterpenoids

Abstract—Clerodane diterpenoid compounds from *Teucrium* (Labiatae) were assayed for antifeedant activity against larvae of *Spodoptera littoralis* and *Heliothis armigera* The functional groups responsible for antifeedant activity are discussed

INTRODUCTION

This paper forms part of an intensive study to analyse the importance of various functional groups within the molecules of plant-derived compounds, responsible for antifeedant activity against some economically important lepidopterous pests [1–5] We investigate here the activity of a range of clerodane compounds isolated from medicinal plants in the genus *Teucrium* (Labiatae) [6]. Within the Labiatae, many diterpenoids have been shown to be potent antifeedants including ajugarin I, isolated from *Ajuga remota* [7] Only a few of the clerodanes isolated from *Teucrium* have been assessed for antifeedant activity [4, 8, 9]. The results of these studies have shown that the antifeedant activity of these compounds lies in the configuration of both the side chain and its decalin fragment. The compounds studied here enable us to test further the effects of small changes in the molecular structure of compounds on inhibiting feeding in the larvae of two polyphagous species of Lepidoptera

RESULTS AND DISCUSSION

The results (Table 1) show that, overall, compounds **1–19** affected the feeding behaviour of *S littoralis* more than *H. armigera*, although the most active compound, **3**, at the higher concentration tested, was more active against *H armigera*. Ajugarin I (**1**) was the only compound to show significant antifeedant activity against both species. Of the five compounds **1**, **2**, **5**, **9** and **15** that significantly decreased feeding in *S. littoralis*, **2** was the only compound to be inactive at the lower concentration. The data clearly show that small changes of structure (Fig 1) can cause large changes in antifeedant activity. It is also apparent that both the side chain at C-9 and the decalin portion of the molecule play a role in the activity, yet no clear picture emerges concerning the precise functional groups required for significant antifeedant activity In line with our previous studies [4], the epoxide group at the C-4 position combined with the C-5 methyl acetoxy substitutions are important These substitutions are present in the active antifeedant compounds **1**, **3**, **5**, **9** and **10** but they are also present in the less active compounds **4**, **6–8**, **11** and **12** This variation in activity indicates the importance of slight changes to the substitutions made in the rest of the molecule, as illustrated by comparing the activity ascribed to compounds **5** and **6** where the orientation of the hydroxy group at C-6 influences activity. Previous results [4] reported that a C-6 keto group was associated with poor activity **4**. However, antifeedant activity occurs if this keto group is accompanied by a neighbouring polar hydroxy group **9** but not if is accompanied by an acetoxy group **12**

Antifeedant activity can be present in compounds in which complete structural changes have occurred within the decalin unit (**15**) and (**16**) For example, in compound **15**, where the epoxide and acetates are replaced by a bridging butenolide ring, activity is present However, activity decreased when the butenolide ring is accompanied by a polar hydroxy group at C-7 along with a change in the orientation of the side chain at C-12 **14**. A similar comparison can be made between compounds **16** and **17**; both contain a butenolide ring between C-4 and C-5, but when the ring is accompanied by a hydroxy group, this time at C-6 **17**, antifeedant activity decreased

One final conclusion that can be drawn from these data, is that no single substitution on the decalin portion of the molecule can be ascribed to being essential for antifeedant activity against the insects tested

EXPERIMENTAL

Insects Spodoptera littoralis (Boisduval) and *Heliothis armigera* (Hubner) were reared on a bean-based diet [12] at Birkbeck College, at 26+/−1° in a 16L 8D photoperiod

Compounds Ajugarin I (**1**) was a gift from Professor Kubo, University of California. Compounds **2–6** and **8–19** were from Professor Savona, Palermo and Professor Rodriguez, Madrid. Compound **7** was prepared from teucjaponin A at Imperial College. Ajugarin I (**1**) was isolated from *Ajuga remota* [7].

Table 1 Antifeedant index $[(C-T)/(C+T)]\%$ of test compounds [mean+/−(S E M)] in dual choice test with glass fibre discs [control (C) versus treatment (T) ($N=20$)]

	Antifeedant index			
	S littoralis		*H armigera*	
Conc ppm Compound	100	10	100	10
1	43 1 (7 31)*	34 5 (6 95)*	39 6 (9 65)*	23 9 (9 86)
2	29 6 (8 96)*	17 9 (9 65)	23 9 (9 62)	2 9 (5 65)
3	32 2 (22 16)	21 7 (15 87)	63 2 (5 41)*	16 9 (7 78)
4	23 7 (6 98)	21 9 (18 04)	11 7 (14 87)	19 5 (7 09)
5	48 9 (5 98)*	43 0 (7 64)*	29 8 (14 95)	23 2 (6 58)
6	12 9 (7 67)	7 5 (6 98)	3 9 (14 98)	3 4 (14 67)
7	10 4 (13 21)	7 4 (10 41)	4 6 (10 21)	3 0 (18 41)
8	6 5 (7 87)	7 5 (9 97)	4 9 (12 98)	8 9 (15 98)
9	48 9 (5 98)*	40 9 (9 76)*	23 9 (14 56)	24 0 (7 22)
10	38 9 (16 98)	33 1 (10 59)	24 8 (16 98)	20 9 (7 98)
11	14 8 (6 98)	12 6 (12 25)	8 8 (8 76)	6 8 (3 71)
12	19 9 (12 87)	18 5 (10 61)	16 9 (12 98)	11 5 (5 68)
13	−9 8 (23 87)	−2 6 (16 85)	2 8 (11 98)	−2 9 (6 98)
14	9 8 (11 87)	8 7 (15 92)	11 9 (5 95)	15 6 (6 52)
15	49 9 (3 87)*	45 4 (9 61)*	29 6 (8 93)	29 2 (8 55)
16	31 8 (11 87)	25 6 (13 51)	18 9 (7 65)	16 7 (13 65)
17	−9 9 (13 76)	−18 6 (17 54)	− 9 (11 87)	−5 8 (5 00)
18	14 8 (6 94)	16 6 (7 42)	9 8 (15 56)	19 6 (6 46)
19	9 6 (11 41)	9 4 (10 62)	8 6 (10 41)	2 4 (3 75)

*Significant difference between the amount eaten of the control and treatment discs (Wilcoxon's matched pairs test $p<0.05$)

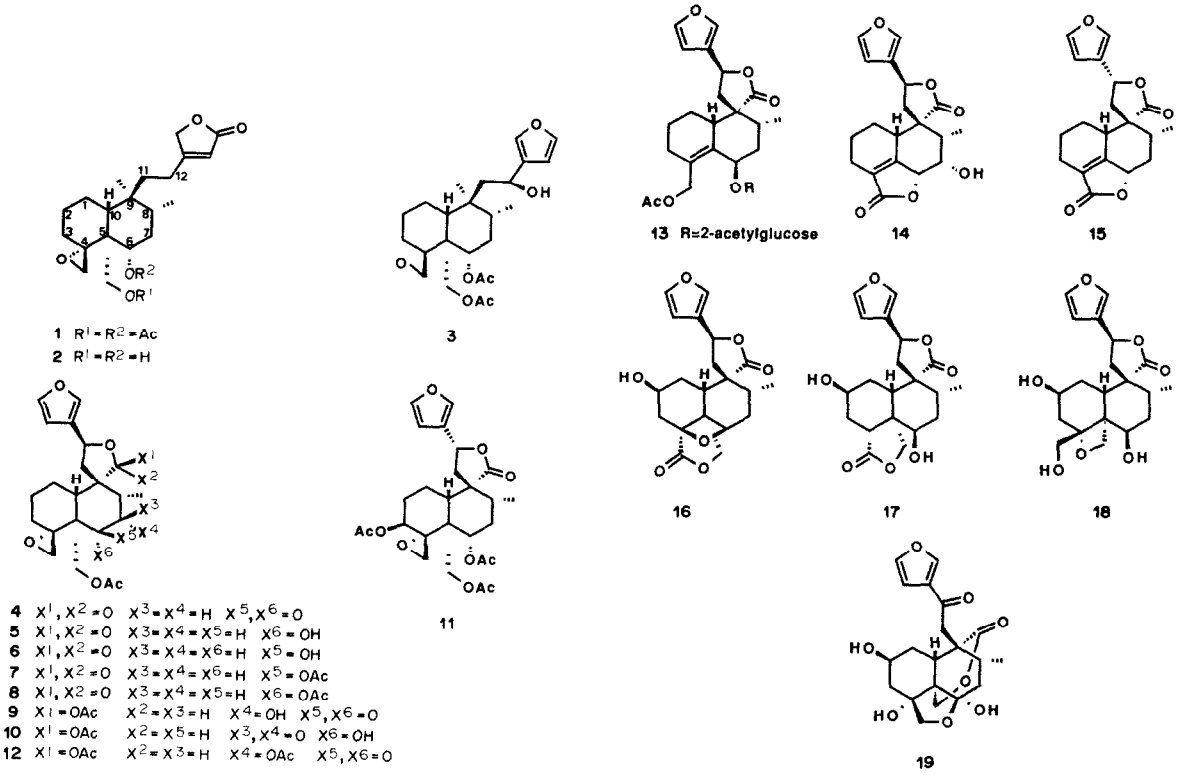

Fig 1 Compounds (**1**) ajugarin I, (**2**) deacetyl ajugarin II, (**3**) 6,19-diacetylteumassilin, (**4**) 19-acetylgnaphalin, (**5**) teucjaponin B, (**6**) teucjaponin A, (**7**) 6-acetylteucjaponin A, (**8**) montanin C, (**9**) eriocephalin, (**10**) isoeriocephalin, (**11**) teupyrenin, (**12**) 7-*O*-acetyleriocephalin, (**13**) teuflavoside, (**14**) teucrin A, (**15**) 12-*epi*-teucvin, (**16**) chamaedroxide, (**17**) dihydroteugin, (**18**) teucroxide, (**19**) 12-ketoteugnaphalodin

Deacetyl ajugarin II (**2**) [13], 6,19-diacetylteumassilin (**3**) [13], teucjaponin A (**6**) [13], also known as montanin F, and montanin C (**8**) [13] were isolated from *Teucrium massiliense* 19-Acetylgnaphalin (**4**), also known as teucrin H3, was isolated from *T hyrcanicum* [14] Teucjaponin B (**5**) was isolated from *T japonicum* [15] Eriocephalin (**9**) was isolated from *T eriocephalum* [16] Isoeriocephalin (**10**) was isolated from *T lanigerum* [17] Teupyrenin (**11**) was isolated from *T pyrenaicum* [18] 7-*O*-acetyleriocephalin (**12**) [19] and 12-ketoteugnaphalodin (**19**) [20] were synthetic Teuflavoside (**13**) [21] and 12-*epi*-teucvin (**15**) [22] were isolated from *T flavum* subsp *glaucum*. Teucrin A (14) [23], chamaedroxide (**16**) [24], dihydroteugin (**17**) (**23**) and teucroxide (**18**) [25], were isolated from *T chaemaedrys*

Antifeedant bioassay The compounds were assessed for antifeedant activity by presenting them on glass fibre discs (Whatman GF/A 2 1 cm diameter) The discs were made palatable by the addition of 100 μl of sucrose (0 05 M) The test compounds were tested at two concentrations, 10 and 100 ppm Final stadium larvae of *Spodoptera littoralis* and *Heliothis armigera* were deprived of food for 4 hr, then placed individually in a Petri dish with two discs, one of which, the control (C) disc, had only sucrose added, the other, the treatment (T) disc, had sucrose and a 100 μl aliquot of one of the test solutions The discs were dried and weighed before being presented to the larvae The duration of the bioassay varied between species but was never longer than 18 hr, so that never more than 50% of any disc was eaten The discs were then reweighed and the Antifeedant Index $[(C-T)/(C+T)]\%$ calculated on the amounts eaten This index identifies both phagostimulants (-ve values) and antifeedants (+ve values)

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