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INSECT ANTIFEEDANT ACTIVITY OF
CLERODANE DITERPENOIDS

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ABSTRACT.—Fourteen clerodane-type diterpenoids isolated from plants in the genera
Baccharis, Teucrium, and Salvia were assayed for antifeedant activity against Tenebrio molitor larvae
in order to establish structure-activity relationships. Among the compounds tested, furano-
diterpenes with α,β-unsaturated-γ-lactone moieties, or C-4-epoxy substitution with C-5-
methylacetoxy or C-12-acyloxy functionalities, exhibited maximal antifeedant and repellent
activities.

It is currently accepted that the accumulation of secondary metabolites in plants can be a consequence of requirements for chemical defense against insects. Recent research carried out in the chemical and biological sciences has resulted in much evidence concerning the defensive role of natural products. These structurally diverse compounds act as growth inhibitors, antifeedants, deterrents, and insecticides (1–3).

Many neo-clerodane diterpenoids with antifeedant activity against insect larvae have been isolated from plants in the families Compositae and Labiatae, and Ajuga, Baccharis, Salvia, and Teucrium species are important sources of these compounds (4,5). In previous papers we reported the isolation of several clerodane diterpenoids from Baccharis and Teucrium species (6–12). Their structural features stimulated us to study their biological activity using Tenebrio molitor L., an insect that is a serious pest in stored flour and grains. We report here the antifeedant and deterrent properties of these compounds, as well as those of two known clerodane diterpenoids isolated from Baccharis rethinodes Meyen and Walpers and Salvia reflexa Horlem and some of their derivatives. Some conclusions about structure-activity relationships are also discussed.

Antifeedant activities were tested according to the bioassay procedure described in the Experimental section. When the antifeedant activities of compounds 1–8 were compared, the more

1   R=H
2   R=OH

3

4

5   **6**   **7**

8

9 R^1=OAc, R^2=H
10 R^1=OH, R^2=H
11 R^1, R^2==O
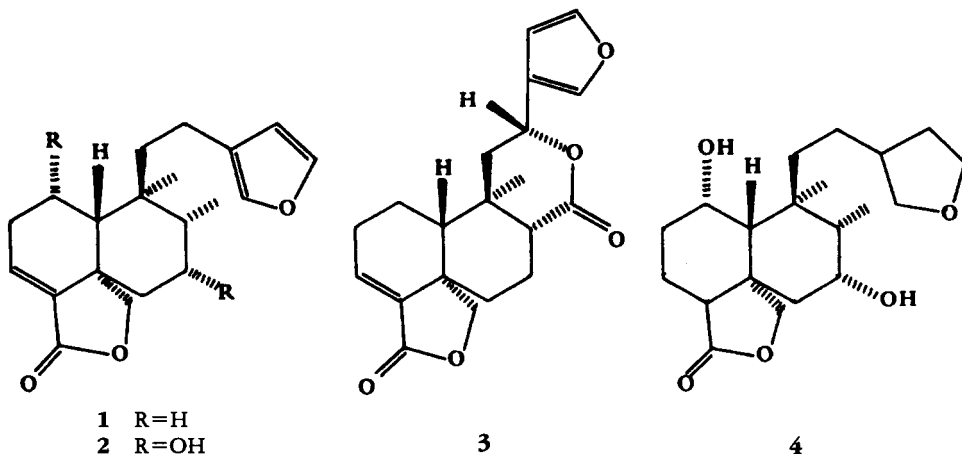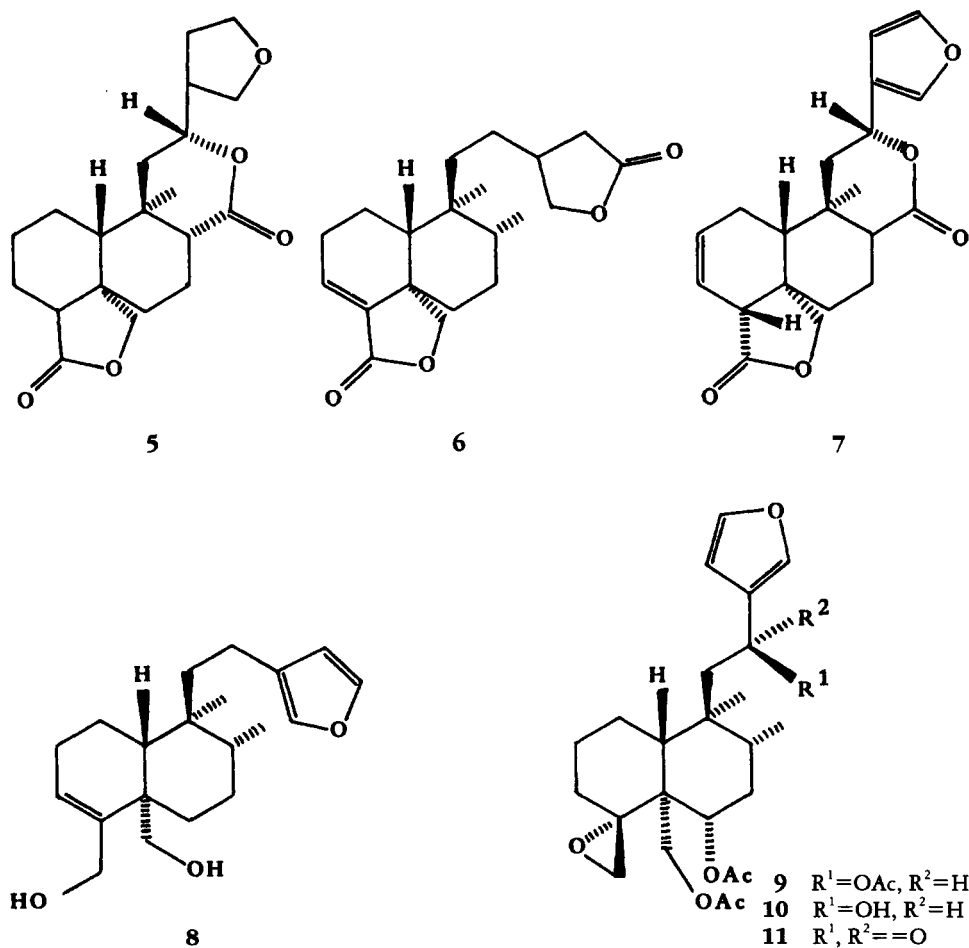
active clerodanes were **1**, **2**, and **3**. The common structural features of these compounds are an α,β-unsaturated-γ-lactone system on the decalin portion of the molecule and a furan ring in the side-chain. Compounds **4** and **5**, which were obtained by catalytic hydrogenation of **2** and **3**, showed loss of activity.

Compound **6** contains an α,β-unsaturated-γ-lactone system in the decalin portion, but the furan ring in the side-chain has been replaced by a butanolide function. On the other hand, although compound **7** shows a side-chain similar to the active compound **3**, the C-18–C-19 olide system is not conjugated. Compounds **6** and **7** were inactive. This observation suggests that the simultaneous presence of a furan ring and an α,β-

unsaturated-γ-lactone group are essential for antifeedant activity. Additional support for this conclusion was obtained when compound **1** was subjected to reduction with LiAlH$_4$. Although the resulting diol [**8**] has a β-ethylfuran side-chain, it did not show any activity.

In contrast, observations made using *Spodoptera littoralis* and *Heliothis armigera* (Lepidoptera: Noctuidae) as models (4), suggest that an epoxide group at the C-4 position, together with methylacetoxy substitution at C-5, are important in mediating this antifeedant activity toward insect larvae. At the same time, it is known that slight functional changes in the side-chain at C-9 can cause drastic changes in antifeedant activity (4,5). The same behavior has been observed in our

experiments against *T. molitor* (Coleoptera: Tenebrionidae) using analogous compounds. In the series **9–12**, the most active compounds were **9** and **12**. The former carries an acetoxy group at C-12, while in the latter a γ-lactone system is present in the side-chain. When **9** was selectively hydrolyzed at the C-12 position, the resulting product [**10**] was inactive. The 12-keto derivative **11**, obtained by oxidation of **10**, also did not show activity.

Finally, two furanoditerpenes (**13** and **14**) were assayed and neither showed any activity. This result is not surprising due to the stereochemical changes and the different substitution patterns. Compounds **1–12** belong to the *neo*-clerodane series whereas **13–14** are of the clerodane series.

In order to verify the results described above, a choice test was conducted. Table 1 shows that the most important repellent properties are in agreement with the major antifeedant activity.

Results obtained from the structure-activity correlation studies suggested that among the furanoditerpenes tested, the possession of an α,β-unsaturated-γ-lactone moiety or a C-4-epoxy with C-5-methylacetoxy or C-12-acyloxy substitution are essential for imparting antifeedant and repellent activities in the test systems used in this investigation.

TABLE 1.   Antifeedant and Repellent Activities of Compounds **1–14**.

| Compound	Non-choice test[a] (PFI) (100 ppm)	(SD)	Choice test[b] Percentage
1	30.9[c]	4.8	6.0[c]
2	31.2[c]	4.1	8.0[c]
3	22.6[c]	4.6	7.0[c]
4	48.3	6.6	37.0
5	49.4	2.8	34.0
6	62.6	3.8	41.0
7	47.3	4.6	47.0
8	48.7	5.8	41.0
9	31.4[c]	3.2	8.0[c]
10	62.5	3.3	46.0
11	57.9	5.0	48.0
12	25.0[c]	3.7	8.0[c]
13	57.8	3.8	59.0
14	62.7	6.4	46.0

[a]Statistical significance determined by Anova.
[b]Statistical significance determined by χ^2 test.
[c]Compound deemed as active.

EXPERIMENTAL

TEST ORGANISM.—*T. molitor* L. (Coleoptera: Tenebrionidae) larvae were obtained from Facultad de Ciencias Agrarias (U.N. Cuyo) and a stock culture was maintained on bran in plastic boxes at $24 \pm 1°$ with a 16:8 (light-dark) photoperiod.

TEST COMPOUNDS.—Diterpenes **1** and **2** were obtained from *Baccharis crispa* Sprengel as described previously (6,7). From aerial parts of *B. rethinodes* collected in Valle de Las Leñas-Malargue, Mendoza, Argentina (voucher Del Vitto-Petenatti No. 1367 UNSL), bacchotricuneatin A [**3**] was isolated (13). Compounds **4** and **5** were prepared from **2** and **3** by catalytic hydrogenation (6). Compound **6** was isolated from *B. triangularis* Haumann (8). Leaves of *Salvia reflexa* collected in Juana Koslay, San Luis, Argentina (voucher Del Vitto-Petenatti No. 6250 UNSL), were extracted

12   13   14
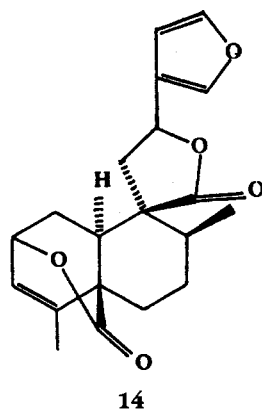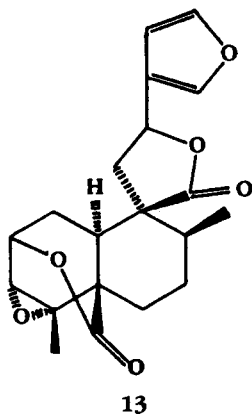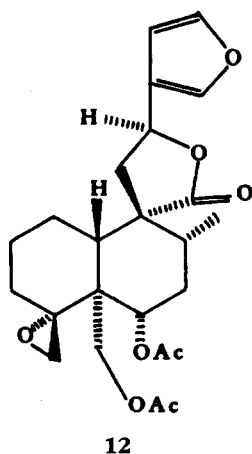
with Me_2CO and the resulting residue was chromatographed over Si gel using a hexane/$CHCl_3$ gradient. After several purifications, **7** was shown to be identical in all respects with salviarin (14). The diol **8** was prepared from **1** as follows: 30 mg of **1** in 30 ml of THF were refluxed for 3 h with 60 mg of $LiAlH_4$; after the usual work-up, 12 mg of kingidiol **8** were obtained (9,15). Compounds **9** and **12** were isolated from *Teucrium grisebachii* Hook. & Arn. (10). 6,19-Diacetylteumassilin **10** was obtained from **9**: 200 mg of **9** were dissolved in MeOH (30 ml) and 100 mg of NaH (60% paraffin oil) were added in small portions over 5 min. After 10 min at 0°, the usual work-up furnished a mixture which was purified by cc over Si gel and 120 mg of **10** (16) was recovered. Compound **11** was obtained from **10** (75 mg) by treatment with Jones' reagent in the usual way (17). After crystallization from EtOAc/petroleum ether, 35 mg of **11** (16) were recovered. Clerodanes **13** and **14** were isolated from *B. artemisioides* Hook. & Arn. as described previously (11,12).

ANTIFEEDANT BIOASSAYS.—Carrot (*Daucus carota* L. var. *sativa* DC., field cultured) slices (2.5 cm diameter and 0.5 cm thick) were coated with 100 μl/slice of test emulsions containing the different compounds. Emulsions were prepared at a concentration of 100 ppm in a mixture of H_2O-MeOH-Me_2CO (90:5:5) containing Triton CS-7 (0.1% by volume) (18). Before use, the emulsions were treated by ultrasonic irradiation for 5 min. Untreated carrot slices were coated with a solvent blank. After drying, six control slices and six treated slices were weighed and separately placed in plastic boxes with third instar *Tenebrio molitor* larvae, twenty for each test. The slices were removed, reweighed, and renewed every 24 h for ten days. Calculations of the amounts of treated or control slices eaten were made by subtracting the weight of the remaining slices from the initial weight of the appropriate test. The activity was expressed as percentage of feeding inhibition (PFI) according to (19). A test result of 50 indicates equal consumption of treated and untreated slices while lower numbers indicate antifeedant activity. This experiment was repeated eight times, in duplicate, for each of the compounds assayed.

$$PFI = \frac{(\% \text{ treated slices consumed})}{(\% \text{ treated slices consumed} + \% \text{untreated slices consumed})} \times 100$$

The test data were subjected to analyses of variance (block design-Anova) followed by means comparisons, and the results are shown in Table 1.

CHOICE TEST.—This procedure was performed using three treated and three untreated carrot slices which were placed in a plastic box, and twenty larvae were placed in the center of the container. Observations were made after 24 h and the results are expressed as the percentage of larvae

under the treated slices (Table 1). Each value is based on an average of five experiments with two repetitions per experiment.

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