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ALLELOCHEMICAL EFFECTS OF EUDESMANE AND EREMOPHILANE SESQUITERPENES ON *Tribolium castaneum* LARVAE

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Abstract—Eight eudesmane and eremophilane sesquiterpenes administered to *Tribolium castaneum* larvae caused different allelochemical effects. Topical application of 3-oxo-γ-costic acid produced the greatest lengthening in the duration of the pupal stage. Morphological deformities were found, specifically when ilicic, costic, and γ-costic acids and costic aldehyde were used. Ilicic acid exhibited the major toxicity 72 hr following topical application. All compounds were significantly toxic at the end point of the experiment (60 days). Treated surface toxicity was lower than when topical assays were carried out. Responses to tessaric acid in choice bioassays had the highest attractive effect. Maximum repellency was caused by the 3-oxo-γ-costic acid. However, experimental series carried out using γ-costic acid, eremophilan-1(10),2,11(13)-trien-12-oic acid, costic aldehyde, and ilicic aldehyde showed no clear response.

Key Words—*Tribolium castaneum*, *Tessaria absinthioides*, allelochemical effects, eudesmanes, eremophilanes, sesquiterpenes, tessaric acid, ilicic acid, 3-oxo-γ-costic acid.

INTRODUCTION

Terpenoids are one of the major phytochemical classes characterized by stereochemical diversity, exceeding that of any other group of plant products (Gershenzon and Croteau, 1999). Certain sesquiterpenoids interfere with normal chemoreceptor

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function and inhibit food uptake by fifth instars of *Pieris brassicae* L. (Lepidoptera: Pieridae) (Schoonhoven and Fu-Shun, 1989). Sesquiterpene lactones, in contrast, have been shown to have deterrent, antifeedant, growth regulator, and toxic activities (Picman, 1986; Srivastara et al., 1990; Chou and Mullin, 1993).

In previous papers, we have reported on the feeding deterrent activities exhibited toward *Tenebrio molitor* (L) (Coleoptera: Tenebrionidae) larvae by some *neo*-clerodane diterpenoids isolated from natural sources, as well as on the role that hydrophobicity and stereoelectronic effects play in their antifeedant bioactivity (Enriz et al., 1994; 2000; Luco et al., 1994; Sosa et al., 1994).

Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) is one of the most economically important insect pests, with a worldwide distribution. Several authors have studied the effects of plant secondary metabolites on it (Harmatha and Nawrot, 1984; Picman, 1986; Isman, 2000).

The present research is aimed toward discovering the allelochemical properties of known sesquiterpenes, and those obtained by chemical transformation, that might be compatible with newer integrated pest control approaches. The biological activity of compounds **1**–**8** (Figure 1) were evaluated using *T. castaneum* as an insect model.

METHODS AND MATERIALS

Chemicals. Tessaric acid (**1**), ilicic acid (**2**), and 3-oxo-γ-costic acid (**4**) (Figure 1) were isolated from aerial parts of *Tessaria absinthioides* (Hook. et Arn.) DC, as previously reported (Kurina Sanz et al., 1997). Compounds **3** and **7** were obtained from **2** by dehydration (Donadel et al., 1998). Compounds **5** was obtained from the eremophylane **1** by reduction using alane($LiAlH_4/AlCl_3$) in THF (Brown and Krishnamurthy, 1979). Derivatives **6** and **8** were prepared by reduction and dehydration of compound **2** (Guerreiro et al., 1979). Identities of all compounds were confirmed by ^{1}H NMR (200.13 MHz), ^{13}C NMR (50.23 MHz), EI-MS, and IR spectral data, as well as optical rotation.

Insects. All experiments were conducted in the laboratory using established colonies of *Tribolium castaneum* Herbst. (Coleoptera: Tenebrionidae). Larvae were reared on a mixture of flour, yeast, and starch (3:3:1) at $25 \pm 1^{\circ}$C, 65% relative humidity, and a 16L:8D photoperiod.

Bioassays

Topical Application. Fifth instars of *T. castaneum* were randomly selected. Four acetone solutions of each compound (10, 20, 40, and 80 μg/μl) were prepared.

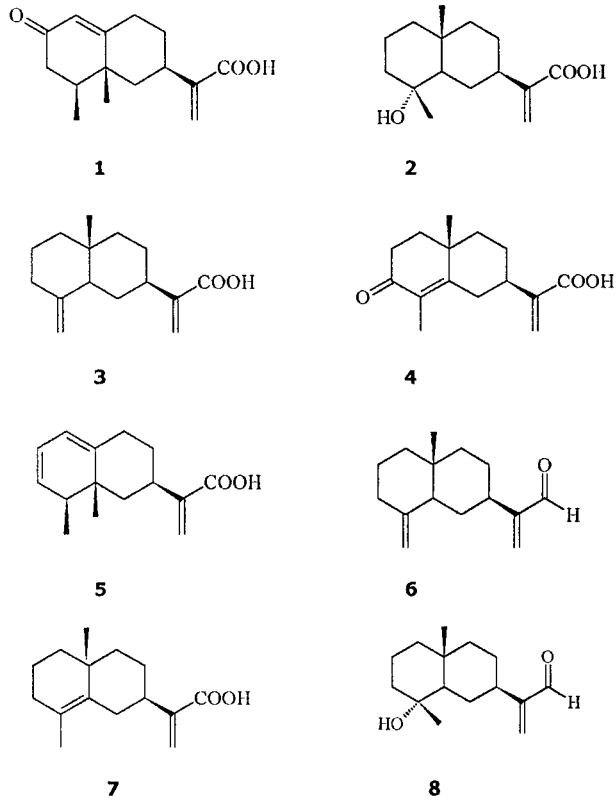

FIG. 1. Structure of compounds **1–8**.

Test solutions were topically applied to the ventral surface of the thoracic segments with a Hamilton microsyringe (1 μl/larvae). Controls were treated with the solvent alone. There were three replicates of 10 larvae for each individual compound tested. After treatments, insects were placed into plastic vials (10 cm diam. $\times$ 7 cm high) containing food and held at 25 $\pm$ 1°C with a 16L:8D photoperiod. Insects were examined daily for 60 days. The duration of the pupal stage (days) was recorded as well as the inhibition of the imaginal molt. Molt inhibition was classified according to the morphofunctional criteria of Pascual et al. (1990) using five basic categories (see description in Table 1 below). This classification represents an arbitrary categorization of a continuum from normal adults, to specimens with different inhibitory effects simply affecting ecdysis and the pre-ecdysial cuticle deposition process.

TABLE 1. RESPONSE OF *T. castaneum* LARVAE TO SESQUITERPENE COMPOUNDS IN TOPICAL BIOASSAYS[a]

Chemical (dose, μg/μl)	*T. castanueum*						
	Duration of pupal instar (days±SEM)	MI					
		0	1	2	3	4	5
Control	9(±0.1)	28	—	—	—	—	—
1 (10)	10 ± 1.0	7	—	—	2	—	2
1 (20)	13 ± 1.3	3	2	—	2	—	—
1 (40)	12 ± 0.3	5	—	—	2	—	1
1 (80)	9 ± 1.5	8	—	—	—	—	2
2 (10)	16 ± 0.2	—	4	—	2	1	—
2 (20)	$16 \pm 0.7^{**}$	8	—	—	1	—	—
2 (40)	$16 \pm 0.4^{**}$	2	3	—	2	—	—
2 (80)	$14 \pm 0.5^{*}$	7	2	—	2	1	2
3 (10)	$16 \pm 0.4^{**}$	3	3	—	1	—	—
3 (20)	13 ± 1.3	8	2	—	3	1	5
3 (40)	11 ± 0.8	8	—	—	—	1	—
3 (80)	15 ± 1.3	4	1	—	2	1	—
4 (10)	15 ± 1.2	5	—	—	—	—	2
4 (20)	9 ± 0.7	5	3	—	2	—	—
4 (40)	$18 \pm 0.4^{***}$	4	3	—	—	—	—
4 (80)	13 ± 0.8	8	—	1	2	3	—
5 (10)	$15 \pm 0.5^{***}$	17	2	1	1	2	—
5 (20)	$15 \pm 0.4^{***}$	19	1	—	2	1	—
5 (40)	$15 \pm 0.5^{***}$	18	—	2	—	—	—
5 (80)	$13 \pm 0.5^{**}$	20	2	—	1	1	—
6 (10)	$15 \pm 0.5^{***}$	20	—	—	1	1	1
6 (20)	$13 \pm 0.6^{**}$	18	3	2	—	1	—
6 (40)	$14 \pm 0.5^{***}$	24	1	—	—	—	—
6 (80)	$15 \pm 0.7^{***}$	15	3	2	3	—	1
7 (10)	$15 \pm 1.2^{**}$	6	2	—	—	3	2
7 (20)	$16 \pm 0.1^{***}$	8	2	—	3	—	1
7 (40)	12 ± 0.9	12	4	—	—	—	2
7 (80)	13 ± 1.1	3	2	1	1	1	—
8 (10)	12 ± 0.6	22	1	—	—	—	—
8 (20)	$14 \pm 0.6^{***}$	21	—	—	—	—	2
8 (40)	$14 \pm 0.7^{**}$	16	—	1	—	2	1
8 (80)	12 ± 0.6	13	1	1	1	—	1

Note: The values are mean (± SEM); $N = 30$. Data followed by *,**,*** are significantly different from the control ($P < 0.05$, $P < 0.01$, $P < 0.001$, by Kruskal–Wallis test; multiple comparisons Dunn's test). M I, Number of insects showing different levels of molt inhibition (Pascual et al., 1990). 0, Normal adults. 1, Adults with normal levels of sclerotization. First and second pair of wings showing certain degree of deformation. Pupal exuviae attached to the posterior part of the abdomen and to the appendages. 2, Adultoids completely enclosed in pupal exuviae, which is usually ruptured along the midline of the pronotum and in the articulation areas of the legs. Cuticle is well tanned as in normal adults. 3, Pupa with adult cuticular level. Pupal exuviae remains intact. 4, Pupa that suffered a complete inhibition of ecdysis and severe deficiencies in the process of new cuticle deposition. Appendages are partially unsegmented. 5, Pupa that suffered a complete inhibition of ecdysis and no cuticle deposition. The cephalic capsule is not normally formed and the appendages are partially unsegmented and with an abnormal position.—, no individual showing this category.

[a] Effects on the duration of pupal instar (days ± SEM) and different degrees of imaginal moult inhibition.

Insect mortality was recorded after 1, 2, 3, and 60 days (end point of the experiment) after treatment. The mortality percentage was calculated by the equation $[1 - A * B/X * D] * 100$, where A is the number of treated larvae that remain alive at the end of the experiment; B is the number of untreated larvae at the beginning of the experiment; X is the number of treated larvae at the beginning of the experiment; and D is the number of untreated larvae living at the end of the experiment (Henderson and Tilton, 1985).

Contact Toxicity. An aliquot of 10 μl of each solution with 10, 20, 40, and 80 μg/μl concentration was diluted into 1 ml of ethanol. The solution was applied to the surface of a plastic Petri dish (5.5 cm diam. $\times$ 1 cm high) and homogeneously dispersed. Controls were treated with solvent alone. The solvent was allowed to evaporate for 2 hr prior to the introduction of 10 terminal instars of *T. castaneum*. Treated Petri dishes were kept at 25 $\pm$ 1°C with a 16L:8D photoperiod. Each treatment was replicated tree times. Insect mortality was recorded at 1, 2, and 3 days, and mortality (%) was calculated using the Henderson and Tilton (1985) evaluation.

Choice Bioassay. The experiment utilized a two-choice arena based on an area preference test described by Obeng-Ofori and Reichmuth (1997). The test arena consisted of a glass Petri dish (9 cm diam. $\times$ 1.5 cm high) divided in half with adhesive tape (1 mm wide). A 10 μl dose of test solution containing 10, 20, 40, and 80 μg/μl concentration was dissolved in 0.2 ml of acetone. Each solution was applied to half of the Petri dish arena as uniformly as possible with a microsyringe. The control side (the other half of the arena) was treated with solvent alone. Petri dishes were air dried to evaporate the solvent completely. Once the Petri dish was dry, the barrier tape was removed, 10 terminal instars of *T. castaneum* were released at the center of the dish, and then the dishes were covered. Each treatment was replicated 10 times. Bioassays were conducted in complete darkness at 25 $\pm$ 1°C and 65% humidity. After 30 min, a preference index (PI) for larvae in the two-choice bioassay was calculated as $PI = (T - C/\text{Tot})*100$ (Phillips et al., 1993), where T is the number of larvae responding to the treatment; C is the number responding to the control, and Tot is the total number of insects released.

Positive PIs indicate attraction to the treatment and negative PIs indicate repellency; values could theoretically range from -100 for complete repellency, to $+100$ for complete attraction.

Data Analysis. Growth assays were analyzed by using the Kruskal-Wallis test, and Dunn's multiple comparisons test at the $P < 0.05$ level. Data from the first 72 hr (topical application assays) were analyzed using the Kruskal-Wallis test, and Dunn's multiple comparisons test at the $P < 0.05$ level. A χ^2 test was used to analyze data from the end point of the experiment. For contact toxicity assays, data were analyzed using the Kruskal-Wallis test, and Dunn's multiple comparisons test at the $P < 0.05$ level. In response index assays, numbers responding to treatments and controls were subjected to the Kruskal-Wallis test, and Dunn's

multiple comparisons test. PriProbit analysis (Sakuma, 1998) was used to determine ED_{50}, ED_{90}, LD_{50}, LD_{90}, and the corresponding 95% confidence limits.

RESULTS AND DISCUSSION

Although the sesquiterpenes studied possessed similar structures, our results indicate that *T. castaneum* responded quite differently to the related compounds. Tessaric acid (**1**) produced a certain degree of morphological abnormalities, without a significant alteration in the duration of the pupal stage (Table 1). In the topical toxicity test, no significant mortality was observed after 3 days, and the highest mortality was reached by the lower concentration after 60 days of treatment (Table 2). This suggests a toxic, but delayed, action against *T. castaneum* larvae. Similar effects were seen in *T. castaneum* adults exposed to food treated with extracts of *Crambe abyssinica* Hochst ex. R.E. Fr. (Cruciferae) (Tsao et al., 1996).

Compound **1**, which is the the most abundant sesquiterpene in *Tessaria absinthioides*, showed a significant toxicity when it was applied in the contact bioassay at 16.84 μg/cm^2 after 3 days of exposure (Table 3). It shows attractive properties with an ED_{50} (95% CL) = 18.16 μg/cm^2 (11.14–38.26) and an ED_{90}(95% CL) = 103.28 μg/cm^2 (45.04–316.62) (Figure 2), and toxic activity. Therefore, it is not surprising that this secondary metabolite acts as an attracticide. This activity could have important implications in the defense of plants against insect attack (Phillips, 1994).

Ilicic acid (**2**) increased pupal stage duration when applied at 20, 40, and 80 μg/μl dose (topical application), and interfered with larval development (Table 1). This was the only compound to cause significant mortality after 2 days at the lower dose (Table 2). This product was toxic in topical and surface contact bioassays, although the latter had more significant differences after 1, 2, and 3, days (Table 3). This phenomenon may be related to the physicochemical behavior of this compound on different surfaces. For instance, the insects on a treated surface receive a topical application (due to their own movement inside the capsule) as a chronic treatment. However, the topically treated larvae received the application only at the beginning of the assay. The highest mortality occurred at the end point of the experiment (Table 2). This confirms a moderate toxicity during the first 3 days and an acute toxicity over long periods of exposure. The highest mortality occurred in response to the lowest concentration of compound. This could result from higher doses acting as a trigger for the activation of detoxification enzymes, i.e., gluthatione-S-transferase (Wood et al., 1986). Finally, compound **2** (Figure 2) had an ED_{50}(95% CL) = 21.39 μg/cm^2 (16.21–25.32) and an ED_{90}(95% CL) = 36.12 μg/cm^2(29.01–90.32), acting as a moderate repellent against *T. castaneum* larvae.

TABLE 2. MORTALITY OF *T. castaneum* LARVAE TREATED WITH SESQUITERPENES IN A TOPICAL BIOASSAYS 1, 2, AND 3 DAYS AFTER STARTING AND AT END POINT OF EXPERIMENT

	Mortality (%)[a]															
	10 μg/μl				20 μg/μl				40μg/μl				80μg/μl			
Chemical	24 hr	48 hr	72 hr	60 days	24 hr	48 hr	72 hr	60 days	24 hr	48 hr	72 hr	60 days	24 hr	48 hr	72 hr	60 days
Control	3 ± 3.3	3 ± 3.3	3 ± 3.3	4 ± 2.4	3 ± 3.3	3 ± 3.3	3 ± 3.3	4 ± 2.4	3 ± 3.3	3 ± 3.3	3 ± 3.3	4 ± 2.4	3 ± 3.3	3 ± 3.3	3 ± 3.3	4 ± 2.4
1	3 ± 3.3	20 ± 5.8	20 ± 5.8	70 ± 15.2§	0 ± 0.0	27 ± 9.0	38 ± 9.2	63 ± 8.8§	6 ± 3.3	10 ± 5.7	13 ± 8.8	53 ± 8.8§	3 ± 3.3	10 ± 5.7	20 ± 9.8	60 ± 5.7§
2	16 ± 12.0	45 ± 12.6*	69 ± 9.6*	86 ± 6.6§	10 ± 5.7	20 ± 5.8	24 ± 3.0	66 ± 14.5§	20 ± 5.8	27 ± 9.0	38 ± 9.2*	56 ± 12.0§	10 ± 5.7	17 ± 8.9	31 ± 5.8	66 ± 3.3§
3	0 ± 0.0	10 ± 5.7	13 ± 3.1	76 ± 3.3§	3 ± 3.3	6 ± 3.3	6 ± 3.3	66 ± 3.3§	3 ± 3.3	13 ± 3.1	13 ± 3.3	53 ± 3.3§	0 ± 0.0	3 ± 3.3	13 ± 3.3	76 ± 8.8§
4	3 ± 3.3	13 ± 3.1	13 ± 3.1	66 ± 14.5§	13 ± 8.8	16 ± 12.0	34 ± 8.2	66 ± 3.3§	3 ± 3.3	6 ± 3.3	27 ± 2.5	76 ± 14.5§	3 ± 3.3	10 ± 5.7	17 ± 6.4	56 ± 3.3§
5	3 ± 3.3	3 ± 3.3	3 ± 3.3	36 ± 6.6§	0 ± 0.0	0 ± 0.0	0 ± 0.0	33 ± 8.8§	6 ± 3.3	10 ± 8.8	10 ± 8.8	43 ± 3.3§	0 ± 0.0	0 ± 0.0	0 ± 0.0	30 ± 5.7§
6	0 ± 0.0	0 ± 0.0	0 ± 0.0	33 ± 3.3§	0 ± 0.0	0 ± 0.0	0 ± 0.0	30 ± 10.0§	0 ± 0.0	0 ± 0.0	0 ± 0.0	16 ± 6.6	0 ± 0.0	0 ± 0.0	0 ± 0.0	40 ± 5.7§
7	6 ± 6.6	6 ± 6.6	6 ± 6.6	73 ± 8.7§	3 ± 3.3	20 ± 5.8	34 ± 2.9	66 ± 12.0§	13 ± 3.1	20 ± 5.8	20 ± 5.8	46 ± 3.3§	0 ± 0.0	20 ± 5.8	41 ± 5.9*	83 ± 3.3§
8	0 ± 0.0	0 ± 0.0	0 ± 0.0	26 ± 3.3§	0 ± 0.0	0 ± 0.0	0 ± 0.0	30 ± 5.7§	0 ± 0.0	0 ± 0.0	0 ± 0.0	46 ± 8.8§	0 ± 0.0	0 ± 0.0	3 ± 3.3	53 ± 3.3§

[a] Values are mean $\pm$ SEM ($N = 30$). Mean $\pm$ SEM followed by * are significantly different from the control ($P < 0.05$, Kruskal – Wallis test; multiple comparisons Dunn's test. Mean $\pm$ SEM followed by §are significantly different from the control ($P < 0.01$, (χ^2 test).

TABLE 3. MORTALITY OF *T. castaneum* LARVAE TREATED WITH SESQUITERPENES IN A CONTACT BIOASSAYS 1, 2, AND 3 DAYS AFTER STARTING THE EXPERIMENT

	Mortality (%)[a]											
	4.21μg/cm^2			8.42μg/cm^2			16.84μg/cm^2			33.68μg/cm^2		
Chemical	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
Control	0 ± 0.0	3 ± 3.3	3 ± 3.3	0 ± 0.0	3 ± 3.3	3 ± 3.3	0 ± 0.0	3 ± 3.3	3 ± 3.3	0 ± 0.0	3 ± 3.3	3 ± 3.3
1	0 ± 0.0	10 ± 0.3	10 ± 0.3	3 ± 3.3	10 ± 5.7	20 ± 0.6	0 ± 0.0	13 ± 6.6	$30 \pm 5.1^*$	13 ± 3.3	14 ± 4.0	20 ± 0.6
2	6 ± 3.3	$30 \pm 9.5^*$	$34 \pm 2.8^*$	6 ± 3.3	13 ± 6.6	$33 \pm 2.6^*$	6 ± 3.3	23 ± 5.8	$41 \pm 4.7^{**}$	$23 \pm 6.6^*$	$23 \pm 6.3^*$	30 ± 9.5
3	$16 \pm 3.3^*$	20 ± 10.0	31 ± 1.0	0 ± 0.0	6 ± 3.2	10 ± 0.3	0 ± 0.0	6 ± 3.2	13 ± 3.1	6 ± 3.3	20 ± 0.6	$51 \pm 3.1^{**}$
4	16 ± 6.6	21 ± 6.7	24 ± 4.4	0 ± 0.0	10 ± 0.3	24 ± 4.4	0 ± 0.0	6 ± 3.2	10 ± 0.3	6 ± 3.3	10 ± 0.3	23 ± 5.8
5	3 ± 3.3	3 ± 3.3	3 ± 3.3	0 ± 0.0	0 ± 0.0	0 ± 0.0	3 ± 3.3	3 ± 3.3	3 ± 3.3	16 ± 12.0	16 ± 12.0	16 ± 12.0
6	0 ± 0.0	3 ± 3.3	3 ± 3.3	3 ± 3.3	3 ± 3.3	3 ± 3.3	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
7	0 ± 0.0	10 ± 0.3	30 ± 5.1	$13 \pm 3.3^*$	$20 \pm 0.6^*$	$41 \pm 1.4^*$	6 ± 3.3	10 ± 5.7	20 ± 0.6	0 ± 0.0	10 ± 5.7	20 ± 5.4
8	0 ± 0.0	0 ± 0.0	0 ± 0.0	3 ± 3.3	3 ± 3.3	3 ± 3.3	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0

[a] Values are mean $\pm$ SEM ($N = 30$). Means $\pm$ SEM followed by * are significantly different from the control ($P < 0.05$, Kruskal – Wallis test; multiple comparisons Dunn's test).

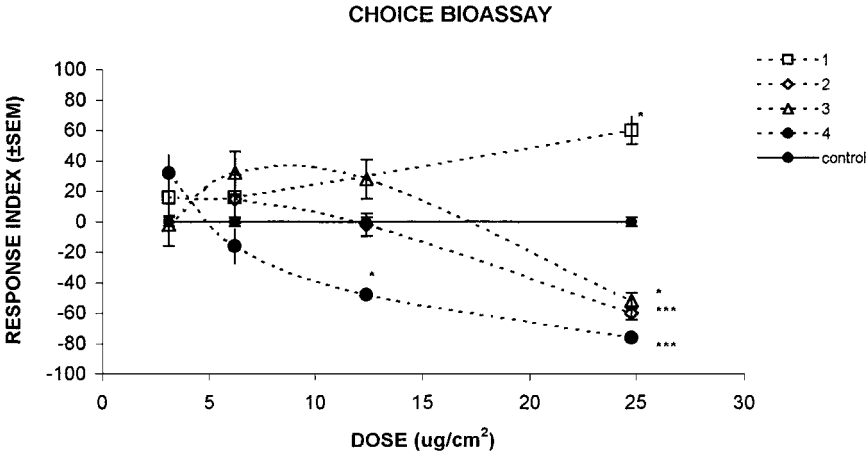

FIG. 2. Mean response index of *T. castaneum* terminal instar larvae to diferent doses of compounds in two-choice bioassays (numbers associated with particular symbols correspond to the number of the structure as seen in Figure 1. 1 = tessarie acid; 2 = ilicic acid; 3 = costic acid; 4 = 3-oxo-λ-costic acid) Response index = $(T - C/Tot) \times 100$; T is the number responding to treatment, C is the number responding to the control, and *Tot* is the total number of larvae released. There were significant differences ($P < 0.001$, Kruskal-Wallis test; multiple responding to control at two doses. Significant differences between treatment and control responses indicated by $^{*}(P < 0.05)$ $^{**}(P < 0.01)$ $^{***}(P < 0.001)$; $N = 10$ bioassays at each dose.

Costic acid (**3**) only affected the pupation period at the 10 μg/μl dose, with different kinds of deformities including severe deficiencies in development of the cephalic capsule, lack of cuticle deposition, and abnormal disposition of legs and wings (Table 1). Mortality was significant at 60 days only (Table 2). When the contact surface biotest was carried out for compound **3**, mortality was 51% after 3 days. The repellent activity, $ED_{50} = 24.65$ μg/cm^2 and $ED_{90} = 28.53$ μg/cm^2, should be considered provisional, because the confidence limits could not be calculated due to variation (Figure 2).

3-oxo-γ-costic acid (**4**) showed one of the highest repellency activities in the two-choice bioassay, with an ED_{50}(95% CL) = 13.46 μg/cm^2 (9.84–17.08) and ED_{90}(95% CL) = 39.5 μg/cm^2 (27.56–96.32) (Figure 2). According to this, this product could play an important role in plant defense. A toxic effect was not evident after 3 days of treatment, but toxic action was confirmed after 60 days of exposure (Table 2).

Both 3-oxo-γ-costic acid (**4**) and tessaric acid (**1**) have an α, β-unsaturated ketone function in the A ring of the decaline moiety, with an electrophilic-β-carbon. This electrophilic carbon could act as an acceptor in a Michael reaction with

nucleophilic functional groups such as sulfhydryl, hydroxyl, and amino groups from proteins. These kinds of electrophilic functional groups play an important role in the bioactivity of sesquiterpene lactones against many insects (Harmatha and Nawrot, 1984; Arnason et al., 1986; Picman, 1986; Chou and Mullin, 1993; Carrizo et al., 1998). Our studies indicate that compound **4** interferes with the normal development of *T. castaneum*, significantly increasing the duration of the pupal stage at 40 μg/μl dose (Table 1).

Neither eremophilan-1(10),2,11(13)-trien-12-oic acid (**5**) nor the aldehyde **6** showed activity in the two-choice bioassay. These compounds produced a significant elongation of the duration of the pupal stage at every concentration evaluated, as well as a series of morphological alterations (Table 1). This suggests that they could produce an antifeedant effect resulting in reduction in growth or death by starvation. Likewise, Carrizo et al. (1998) found a similar response to benzofuranes by *T. molitor* larvae as did Isman and Rodriguez, (1983) using *Heliothis zea* Booddie (Lepidoptera: Noctuidae) larvae treated with sesquiterpene esters. The presence of morphogenetic defects could be related to action as an insect growth regulator.

γ-Costic acid (**7**) did not show activity in the two-choice bioassay. At the end point of the topical experiment, the mortality was significantly increased at all concentrations (Table 2). Some alterations in the length of the pupal stage as well as many morphological abnormalities (Table 1) were detected for this product.

Compound **8** was the only compound to produce a dose-related effect in topical application assays. This toxic response was reached at the end point of the experiment, LD_{50} (95% CL) = 61.19 μg/μl and LD_{90} (95% CL) = 1935.68 μg/μl (confidence limits could not be calculated due to variation) (Table 2). Significant alterations in the duration of the pupal stage were produced. As mentioned above, the highly reactive β-position of the C-7 side-chain unsaturated carbonyl system could act as an alkylation agent. In the two-choice bioassay, ilicic aldehyde (**8**) exhibited an attractive activity at the lower doses. However, we were unable to detect a trend when higher doses were used.

The lack of a dose–response effect on growth and toxicity by contact biotests made it inaccurate to estimate the ED_{50}, ED_{90} or the LD_{50}, LD_{90} values, respectively.

Several reports deal with morphological alterations mediated by secondary natural products. Bowers and Thompson (1963), Thompson and Uebel (1966), and Low et al. (1968), using *Oncopeltus fasciatus* (Dallas) (Heteroptera: Lygaeidae) and *T. molitor* L. specimens treated with related sesquiterpenoids, found similar morphological abnormalities. More recently, these kind of results were studied by Pascual (1990) and Quennedey (1998) when the response of *T. molitor* was treated with azadirachtin and 20-hydroxyecdysone. These authors showed how the compounds were acting at the endocrine level, producing morphological

abnormalities. In agreement with the results obtained in our study, we postulate that some of the sesquiterpenes evaluated here could affect the endocrine system. New investigations at the microscopic, enzymatic, and cytological level are necessary in order to determine whether the abnormalities observed in the present studies are in agreement with hormonal disorders.

The deleterious properties of plant natural products could be related to an antifeedant effect and the subsequent death by starvation. Our results are consistent with those found by Alfatafta and Mullin (1992), in which eudesmanoic acid showed antifeedant action against *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). These authors documented a reduction in the food uptake by at least 50%.

In summary, the results reported here allow us to confirm the hypothesis that the compounds studied alter development in *T. castaneum* larvae. In general, the natural products evaluated here affect larval survival, but only after a long period of exposure. Finally, it would be desirable to extend this investigation to other insect species before drawing more general conclusions.

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