Growth-Inhibitory Activities of Benzofuran and Chromene Derivatives toward *Tenebrio molitor*

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Growth-inhibitory activities of selected natural benzofurans (**4**−**9**), *trans*-cinnamic acid derivatives (**10**−**13**), chromene compounds (**14** and **16**), and some semisynthetic derivatives were determined in last instar larvae of *Tenebrio molitor* via topical administration in Me_2CO. The most inhibitory of the tested compounds were 3-γ,γ-dimethylallyl-*p*-coumaric acid (**10**) and the benzofuran derivative 12-(*p*-cumaroyloxy)-tremetone (**5**), the former compound acting on the pupae and the latter on the last instar larvae. Several developmental deficiencies were observed, and some structure−activity relationships are discussed.

Benzopyrans, benzofurans, prenylated phenols, and acetophenone derivatives are present in many species of higher plants, particularly in the Asteraceae.[1] In this regard, precocene I (**1**) and precocene II (**2**), a special type of chromene lacking the methyl ketone group, were isolated 30 years ago, and their influence on the metamorphosis of certain sensitive insect species, which results in precocious molting, has been intensively studied.[2] The acetylchromene encecalin (**3**) has been reported as the principal chemical defense of the genus *Encelia* (Asteraceae) against herbivorous insects[3−5] and over the past 10 years, complete investigations of the metabolic fate of encecalin administered to insects have been carried out.[6−8]

Recently, it has been reported that the chromene homologue methyl 4-hydroxy-3-(3′-methyl-2′-butenyl) benzoate from *Piper guanacastensis* (Piperaceae) is an important active constituent toward *Aedes atropalpus* L. (Diptera: Culicidae).[9]

The yellow mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) has a high reproductive capacity and attacks several classes of stored grains and flour, making this insect a major pest. Identification of new natural products active against insects enhances our understanding of the chemical defense mechanisms of plants. As part of a study of the interaction between plant secondary metabolites and this insect at the larvae stage,[10−13] we report here the effects of selected natural compounds, previously isolated from species that grow in the central-western semiarid area of Argentina, and semisynthetic aromatic derivatives of these.

T. molitor has a variable number of larval instars and, therefore, selection of larvae by weight is necessary. It has been reported that larvae weighing between 120 and 140 mg belong to the last instar,[14] and for our experiments we used larvae weighing between 113 and 160 mg. In normal development, the pupal stage takes about 9 days,[14] and this period was determined to be 8.6 ± 1.6 days in our controls (see Table 1).

To analyze structure−activity relationships of the assayed compounds they were divided into three main groups; namely, benzofuran derivatives (**4**−**9**), *trans*-cinnamic acid derivatives (**10**−**13**), and benzopyran derivatives (**14**−**17**).

Table 1. Growth Inhibition Activity of Compounds **4**−**18** toward *T. molitor* Last Instar Larvae

compound[a]	percent successfully pupating[b]	duration of pupal stage[c]
4	83.0 (3.1)[d]	10.5 (2.1)[e]
5	26.0 (0.8)[f]	10.5 (1.0)[e]
6	75.0 (6.5)[f]	9.8 (2.2)[g]
7	75.0 (7.8)[f]	8.6 (0.5)[g]
8	73.3 (2.1)[f]	11.2 (2.3)[f]
9	73.0 (8.1)[f]	11.6 (2.1)[f]
10	63.0 (3.4)[f]	13.0 (1.3)[f]
11	50.0 (8.2)[f]	10.2 (1.3)[e]
12	87.0 (3.4)[g]	10.0 (2.1)[e]
13	92.0 (5.6)[g]	8.7 (1.9)[g]
14	77.0 (6.4)[f]	10.2 (0.9)[e]
15	87.0 (7.1)[g]	7.0 (1.4)[g]
16	66.6 (1.7)[f]	11.5 (2.1)[f]
17	87.0 (1.3)[g]	12.1 (2.4)[f]
18	75.0 (3.8)[f]	11.4 (2.1)[f]
control	93.0 (6.1)	8.6 (1.6)

[a] Dose: 120 μg/larvae; topically applied. [b] The values are mean (SE); $n = 60$. Data treated by χ^2 test. [c] The values, in days, are mean (SE); $n = 60$. Data treated by Dunnet test. [d,e,f] Difference significant ($p < 0.05$, $p < 0.01$ and 0.001, respectively) from control by χ^2 test or Dunnet test. [g] No significant difference.

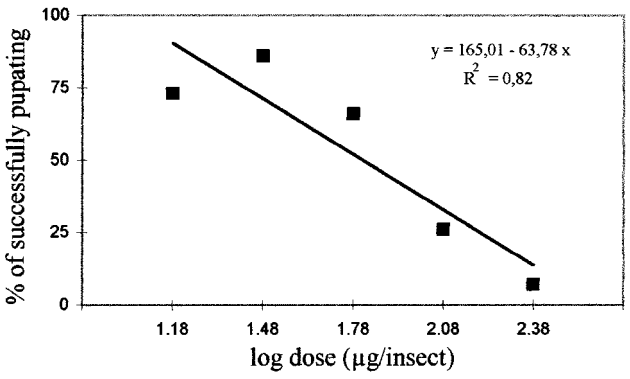

Figure 1. Regression graph of dose/response for compound **5**.

Insects were treated topically, via the abdomen, with the test compounds dissolved in acetone. A single dose of 120 μg/larva was applied. The dosage value was chosen taking into account preliminary results obtained using **5**. This tremetone cumarate showed both inhibition of successful pupation as well as a significant increase in the pupal stage duration at this dosage (Table 1). Results of the dosage increase of **5** are summarized in Figure 1. The percentage of larvae that reached the pupae instar diminished in

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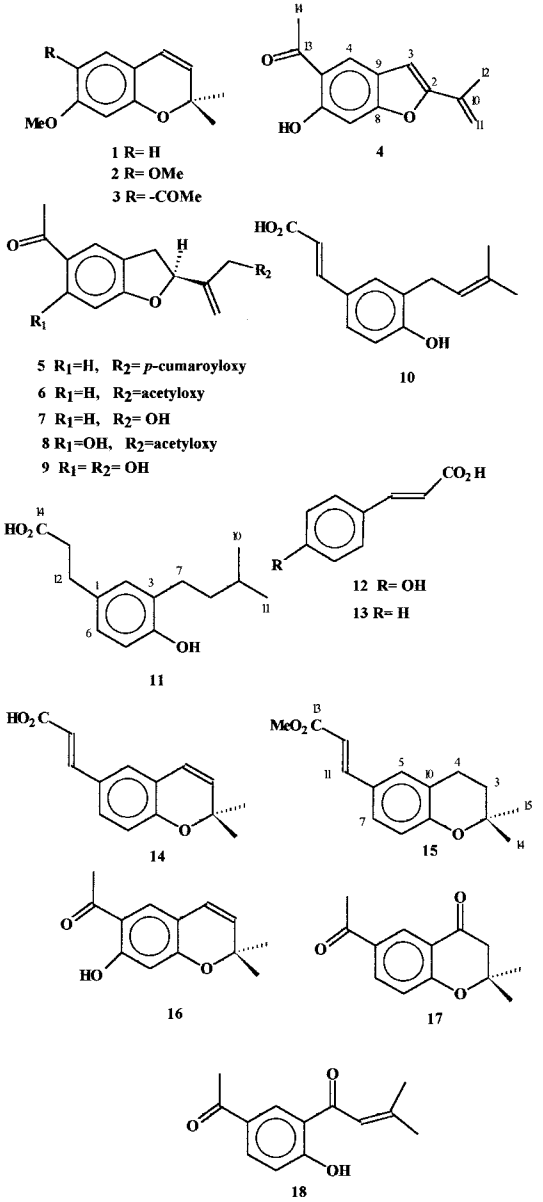
dependence to *log dose* in the regression analysis ($n = 20$ larvae each point), and from these data the $DE_{50} = 63.5$ μg/larva was calculated. To ensure reaching the toxic dose, we used nearly two-fold the DE_{50} of the higher molecular weight compound **5** for all the assayed compounds. In the group of benzofuran derivatives (**4**−**9**), all of the compounds showed significant effects on the ratio of larvae pupating at the end-point of the experiment (30 days), but the most important activity was observed for compound **5**. In this case, only 26% of the treated larvae reached the pupal stage, with the rest typically dead in the course of the first 10 days of the test.



The presence of a bulky aromatic ester group on the side chain at C-2 could be the key functionality for activity. In compounds **6** and **7**, where the *p*-cumaroyloxy group had been replaced by an acetoxyl or hydroxyl group, respectively, this dramatic effect was not observed; however, 25% of the larvae died without pupation. Similar results were achieved with the 6-hydroxy analogues **8** and **9** and euparine (**4**). Another interesting observation made with compound **8** was that with two replicates, two morphologically normal adults had fragments of pupal exuviae on the distal parts of the abdomen, and there were deformities of the elytra. Finally, compounds **4**, **5**, **8**, and **9** showed significant differences from controls in the duration of the pupal instar with prolongation of this stage.

It has been reported that benzofurans are not toxic to other insects species; however, they antagonize the toxicity of the chromene encecalin (**3**).[7] In our study using similar dose:insect weight ratios (0.33 μmol/insect for compound **5**), the benzofurans were toxic by themselves toward *T. molitor*. Recently it was noted that 2-(1′-oxo-5′-methyl-4′-hexenyl)benzofuran derivatives show toxicity to spruce budworm, *Christoneura fumiferana* Clem., larvae at the level of 0.8 μmol per insect.[15] Presumably, as in compound **5**, the bulky group at C-2 is important for the biological response.

In the second group of compounds (*trans*-cinnamic acid derivatives), **10**−**13**, the simple and ubiquitous acids **12** and **13** were essentially inactive; on the other hand, the 3-γ,γ-dimethylallyl-*p*-coumaric acid (**10**) showed both activities, toxic on the last instar larvae as well as being the most active on the pupal stage. Of particular interest in this set of products was the change in activity of **10** when hydrogenated to **11**. The hydrogenated acid-phenolic **11** weakly affected the duration of the pupal instar but showed enhanced larval toxicity.

Bearing in mind the activity of **10**, we then prepared derivatives **14** and **15**. The first was obtained from **10** by a cyclization reaction with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[16] and the second via a halo-cyclization reaction to give the 3-bromine derivative of **15** (epimeric mixture), by way of the thermodynamically preferred ring closure, followed by hydride reduction.[17] This series of reactions was adopted in order to preserve the carboxylic unsaturated side chain. The semisynthetic compounds **14** and **15** belong in the benzopyran series (**14**−**17**).

In this group of products, both acetylbenzopyrans (**16** and **17**) were active at the pupal stage, but only the chromene **16** (demethylencecalin) was significantly toxic to the larvae. It is noteworthy that a C-3−C-4 double bond in the pyrane ring is essential for the toxic or antihormonal activity.[7] This is the site of attack for mixed function oxidases that epoxidize the double bond leading to a more soluble *trans*-3,4-diol. On the other hand, the highly reactive benzylic position of the oxirane ring can be attacked by nucleophilic functional groups, for example, the thiol function of enzymes quenching the enzyme activity.[8] The absence of heterocycle unsaturation in the chroman **15** could be the reason for lack of activity.

In about 3% of the larvae treated with compounds **16**−**18** we found multiple morphological abnormalities. There were adultoid insects showing tarsi and antennae partly unsegmented (**16**); severe deficiencies in the process of new cuticle deposition (**17**), and adultoid insects enclosed in pupal exuviae but showing mobile appendages (**18**). None of the control larvae showed any of these macroscopic defects in their development.

This report extends previous studies demonstrating that *T. molitor* L. is an insect sensitive to plant natural products.[10−12] In this case some acetophenone derivatives and compounds with the chromene skeleton were active as insect-growth regulators. It would be desirable to extend this investigation to other insect species before drawing more general conclusions.

Experimental Section

General Experimental Procedures. ^{1}H NMR spectra (ppm, δ) were recorded in $CDCl_3$ at 200.13 MHz, ^{13}C NMR spectra were obtained at 50.23 MHz; COSY, XH−CORR, and

COLOC experiments were obtained using standard Bruker software. Column chromatography was performed on Si gel G 70−230 mesh and Kieselgel 60 H using mixtures of *n*-hexane−EtOAc, in increasing polarities, as solvent. TLC was carried out on Si gel 60 F_{254} 0.2-thick plates using C_6H_6−1,4-dioxane−AcOH, 30:5:1 (v:v:v) as solvent.

Insect. A stock culture of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae was maintained on wheat bran in plastic boxes at 24 ± 1° with a 16:8 (L:D) photoperiod.[10] A voucher specimen is deposited at the Cátedra de Entomología, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina.

Test Compounds. Euparin (**4**) was isolated from leaves of *Flourensia oolepis* (Compositae), as previously reported.[18] From the aerial parts of *Parastrephya lepidophylla* (Wedd) Cabr., 12-(*p*-cumaroyloxy)-tremetone (**5**) and 12-acetyloxy-tremetone (**6**) were isolated.[19] The derivative **7** was prepared from **6** by KOH−MeOH hydrolysis followed by acidification with dilute HCl and extraction with Et_2O. The known compounds **8**, **9**, and **16**−**18** were isolated from aerial parts of *Ophryosporus axilliflorus* (Griseb) Hieron.[20] From *Baccharis grisebachii* Hyeronimus, 3-γ,γ-dimethylallyl-*p*-coumaric acid (**10**) and **12** were obtained.[21] Compounds **11**, **14**, and **15** were prepared from **10** as described below. Compound **13** was a commercial sample.

Preparation of 11 from 10. A solution of **10** (100 mg, 0.42 mmol) in EtOAc (30 mL) was stirred under H_2 at 30 °C overnight in the presence of Pd 5% on activated carbon. The catalyst was removed by filtering through Celite, and the filtrate was evaporated to give 95 mg of the pure **11** as an oil: ^{1}H NMR ($CDCl_3$, 200.13 MHz) δ 6.94 (1H, br s, H-2), 6.88 (1H,d, J = 10.8 Hz, H-5), 6.67 (1H, dd, J = 10.8, 2.5 Hz, H-6), 2.90 (2H, t, J = 6.0 Hz, H-13), 2.61 (4H, m, H-7 and H-12), 1.58 (2H, m, H-8), 1.50 (1H, m, H-9), 0.93 (6H, d, J = 5.4 Hz, H-10 and H-11); ^{13}C NMR ($CDCl_3$, 50.23 MHz) δ 179.1 (C-14), 151.8 (C-4), 132.1 (C-1), 129.8 (C-2), 129.1 (C-3), 126.4 (C-6), 115.3 (C-5), 38.9 (C-13), 35.9 (C-7), 29.8 (C-12), 27.8 (C-9), 27.7 (C-8), 22.4 (C-10 and C-11).

Preparation of 14 from 10. To a stirring solution of compound **10** (100 mg, 0.42 mmol) in 10 mL of 1,4-dioxane−C_6H_6 (1:1) at room temperature, under N_2, was added DDQ (290 mg, 1.26 mmol).[16] After 1 day in darkness, the solvent was evaporated under reduced pressure and the residue dissolved in Et_2O. The organic solution was washed with brine until the aqueous phase became colorless, dried (Na_2SO_4), and concentrated under reduced pressure. After column chromatography purification, 80 mg of **14**, identical in all respects with previous reports, were recovered.[22]

Preparation of 15 from 10. The halo-cyclization reaction was carried out under N_2. The methyl ester of **10**, 400 mg (1.6 mmol), was prepared in the usual way with CH_2N_2−Et_2O and dissolved with stirring in 5 mL of dry MeCN at −20 °C. After 15 min, 660 mg (1.6 mmol) of 2,4,4,6-tetrabromo-2,5-cyclohexadienone, suspended in 10 mL of the same solvent, was added.[17] The heterogeneous reaction was stirred for 3 h, until starting material was not detectable by TLC, and was allowed to warm to 30 °C. The reaction was quenched by addition of brine and extracted twice with Et_2O. The combined organic layers were washed, dried, and concentrated. After flash chromatographic purification, 350 mg of the epimeric 3-bromine derivative of **15** was obtained. The dehalogenation reaction was carried out by refluxing the mixture in C_6H_6 with addition of *n*-Bu_3SnH (2.6 eq) and a catalytic amount of 2,2′-azobis(2-methylpropionitrile) for 3 h. After the usual work up and separation by column chromatography, 120 mg of **15** was obtained (oil): IR ν_{max} 1710, 1632, 1601, 1440, 1250, 1068, 620 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200.13 MHz) δ 7.62 (1H, d, J = 15.5 Hz, H-11), 7.32 (1H, dd, J = 9.0, 2.2 Hz, H-7), 7.25 (1H, d, J = 2.2 Hz, H-5), 6.79 (1H, d, J = 9.0 Hz, H-8), 6.29 (1H, d, J = 15.5 Hz, H-12), 3.80 (3H, s, OMe), 2.79 (2H, t, J = 6.6 Hz, H-4), 1.82 (2H, t, J = 6.6 Hz, H-3), 1.34 (6H, s, H-14 and H-15); ^{13}C NMR ($CDCl_3$, 50.23 MHz) δ 173.0 (C-13), 156.3 (C-9), 144.9 (C-11), 129.9 (C-7), 127.3 (C-5), 126.1 (C-10), 121.2 (C-6), 117.8 (C-12), 114.5 (C-8), 75.0 (C-2), 51.5 (OMe), 32.5 (C-3), 26.8 (C-14 and C-15), 22.3 (C-4).

Bioassays. Bioassays were performed with last instar larvae of *T. molitor* based on live weight (113−160 mg). For each compound, test solutions (in Me_2CO) were topically applied to ventral abdominal segments with a microsyringe (2 μL/larva; equivalent to 120 μg of the assayed compound). Controls were treated with the solvent alone. For each individual compound there were two replicates of 10 larvae each, and the study was repeated three times. After treatment insects were placed individually in Petri dishes (5 cm diameter) and held at 24 ± 1° with a 16:8 (L:D) photoperiod. A moistened piece of cotton was used to preserve humidity. Insects were not fed during this assay. The number of larvae that successfully pupated as well as the duration of the pupal stage (in days) were recorded every 24 h for 30 days (end-point of the experiment). Results are shown in Table 1.

Statistical Analysis. Data of the percent of larvae successfully pupating was treated by χ^2. Prior to statistical analysis of the duration of pupal stage, it was proven, on the basis of the Shapiro-Wilk test, that the patterns come from a population with approximately normal distribution. The homogeneity of variances was determined by the Bartlett test. The mean length of pupal stage (in days) for the different treatments was analyzed by using the Dunnet test.[23]

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