Insecticidal Activity of *Maytenus* Species (Celastraceae) Nortriterpene Quinone Methides against Codling Moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae)

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The insecticidal effects of nortriterpene quinone methides (pristimerin, tingenonee, and 20-α-hydroxytingenone) are reported for the first time. The natural products were isolated from *Maytenus* sp. (Celastraceae) and their effects tested on larvae of codling moth (*Cydia pomonella*, Lepidoptera: Tortricidae). The three metabolites produce the same effects on codling moth larvae that azadirachtin does, although at higher concentrations. 20-α-Hydroxytingenone was the most active compound, showing lethal, antifeedant, and insect growth regulation activities. Pristimerin showed also a high antifeedant activity together with its molt effect suppression. Tingenone showed the lowest activity. The differences in the activity of the three products are related to the structure of the E ring.

Keywords: *Maytenus; Cydia pomonella; nortriterpene quinone methides; antifeedant; mortality; insect growth regulation*

INTRODUCTION

The use of some plant species to cure illnesses goes back to ancient human civilizations and can even be seen in the behavior of some animals such as cats and dogs. People also learned soon that some plant species are less often attacked by phytophagous species or that they can better resist their attack than others. Mainly through trial and error, people developed procedures to extract, purify, and use the plant substances responsible for these actions. Thanks to modern chemistry, the structures of many of these biologically active agents are now known, and systematic studies on the natural products that protect plants from pests have become a recognized common activity within the fields of chemistry and pest control (Hedin and Hollingworth, 1997). Among these natural products, pyrethrins from *Chrysanthemum cinerariaefolium*, which have insecticidal properties, and azadirachtin from neem tree (*Azadirachta indica*), which exhibits antifeedant, repellent, and insect growth regulator (IGR) activities, are the most important ones until now (Harborne, 1993). More recently, Arnason and co-workers have described a group of novel spirotriterpenoids with insect antifeedant activity, and the annonaceous acetogenins have become one of the most rapidly growing classes of bioactive natural products (Hedin et al., 1997).

The plants of the genus *Maytenus* (Celastraceae) have a long history in traditional medicine (González et al., 1982). Many bioactive metabolites have been isolated from these plants, such as maytansinoids with insecticide activity (Madrigal et al., 1985), sesquiterpene pyridine alkaloids with insect antifeedant and immunosuppressive activities, sesquiterpene polyesters with antitumor-promoting activity (Shirota et al., 1994), quinoid triterpenes and triterpenes dimers with antimicrobial activities (González et al., 1996a), sesquiterpenes presenting antifeedant activity (González et al., 1993), and nortriterpene quinone methides, which show antimicrobial activity (González et al., 1996b). Despite the huge diversity of plant metabolites showing insecticide activity, no nortriterpene quinone methide presenting insecticide activity has been described until now.

The aim of the present work was to determine the effect of three nortriterpene quinone methides (pristimerin, tingenone, and 20-α-hydroxytingenone, Figure 1) isolated from *Maytenus* sp. on the mortality, feeding activity, and development of larvae of codling moth [*Cydia pomonella* (L.), Lepidoptera: Tortricidae], using an extract from neem tree as a control. *C. pomonella* is a polyphagous insect that is a key pest of apples all over the world. Newly hatched larvae may feed on apple leaves for a short time before entering into a fruit. Larval development occurs completely within the attacked fruit. The main damage is due to the deep entries of the larvae, but even small feeding injuries, without an entry, can depreciate the fruit. Several control measures have been developed against it (chemical and nonchemical control measures), but the development of resistance against some insecticides and public concern about environmental risks point to the necessity of other control measures.

MATERIALS AND METHODS

Plant Material. Plants belonging to the genus *Maytenus* were collected in San Lorenzo (Paraguay), and a voucher specimen (Ortiz no. 1376) is kept in the Herbarium of the Departamento de Botánica, Facultad de Ciencias Químicas,

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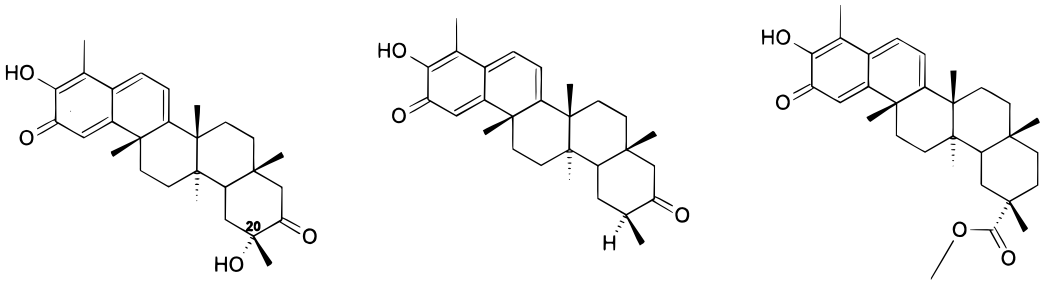

20-α-hydroxytingenone tingenone pristimerin

Figure 1. Chemical structures corresponding to the three nortriterpene quinone methides isolated from the root bark of a Celastraceae species (C20 is labeled in the 20-α-hydroxytingenone structure).

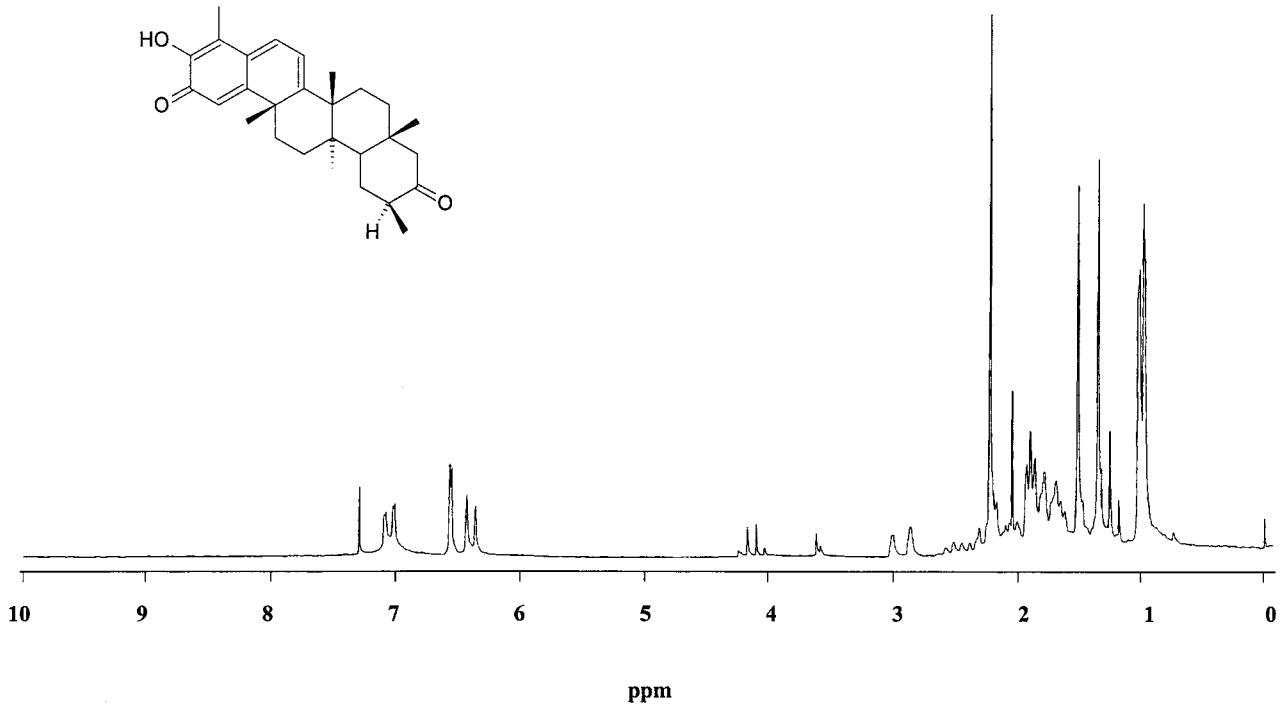

Figure 2. ^{1}H NMR spectrum corresponding to a solution of tingenone in $DCCl_3$. Spectrum was recorded in a 100 MHz spectrometer using TMS as internal standard.

Universidad Nacional de Asunción, Paraguay. The species remains unknown until now.

Extraction and Isolation. The bark of the plant roots was extracted with *n*-hexane/diethyl ether (1:1) in a Soxhlet apparatus. The extract was repeatedly chromatographed on Sephadex LH-20 and silica gel using solvent mixtures of *n*-hexane/chloroform/methanol (2:1:1) and *n*-hexane/ethyl acetate, respectively, to afford pristimerin, tingenone, and 20-α-hydroxytingenone. All solvents were glass distilled.

Compounds were identified by considering their ^{1}H NMR and FT-IR spectra. Their purities were assessed by TLC analysis using SiO_2 plates. A mixture of *n*-hexane/ethyl acetate/formic acid (7:3:0.02) was used for pristimerin and tingenone analyses, whereas *n*-hexane/ethyl acetate/formic acid (5:5:0.02) was used for 20-α-hydroxytingenone.

Work Solutions. The three isolated natural products were dissolved in reagent grade acetone and serially dissolved at the required concentration. A commercial product made with neem tree extract (Mubel, Fertimet S.L., Spain) was used as control. This commercial product contains 2% of azadirachtin.

Insect Population. A *C. pomonella* laboratory colony reared on a semisynthetic diet (Esteban, 1975) was used in the experiments. It was collected from an abandoned orchard in Lleida (Spain) in 1993 and has been reared in the laboratory since then.

Bioassays. Fifty microliters of a solution of each product on reagent grade acetone was topically applied on the upper surface of a 1 × 1 × 1 cm diet cube. One newly hatched larva was deposited on the treated surface, covered with a gelatin capsule size 00 (0.5 cm^2), and allowed to feed for 10 days. Ten diet cubes were placed in a glass Petri dish sealed with Parafilm M and stored at 22 °C and a 16:8 h (light/dark) photoperiod. Each larva was observed 5 and 10 days later, recording the number of cephalic capsules (as a way of measuring the larval development) and whether it was alive and had fed. The bioassay was replicated three times, so 30 larvae per concentration and chemical were tested.

The concentrations tested were 20, 40, and 80 mg/mL (pristimerin and tingenone); 1, 2, 4, 6, 8, 12, 16, 18, and 20 mg/mL (20-α-hydroxytingenone); and 2 and 20 mg/mL (azadirachtin). Fifty microliters of acetone (reagent grade) was applied to the controls.

Statistical Analyses. Analyses of variance of the data followed by mean comparison (LSD test) were carried out by using the ANOVA procedure from the SAS program (SAS Institute, 1987). Percentages were transformed to arcsin prior to analysis. Mean lethal concentration (LC_{50}) was computed, when possible, using probit analysis by means of POLO program (Robertson and Preisler, 1991).

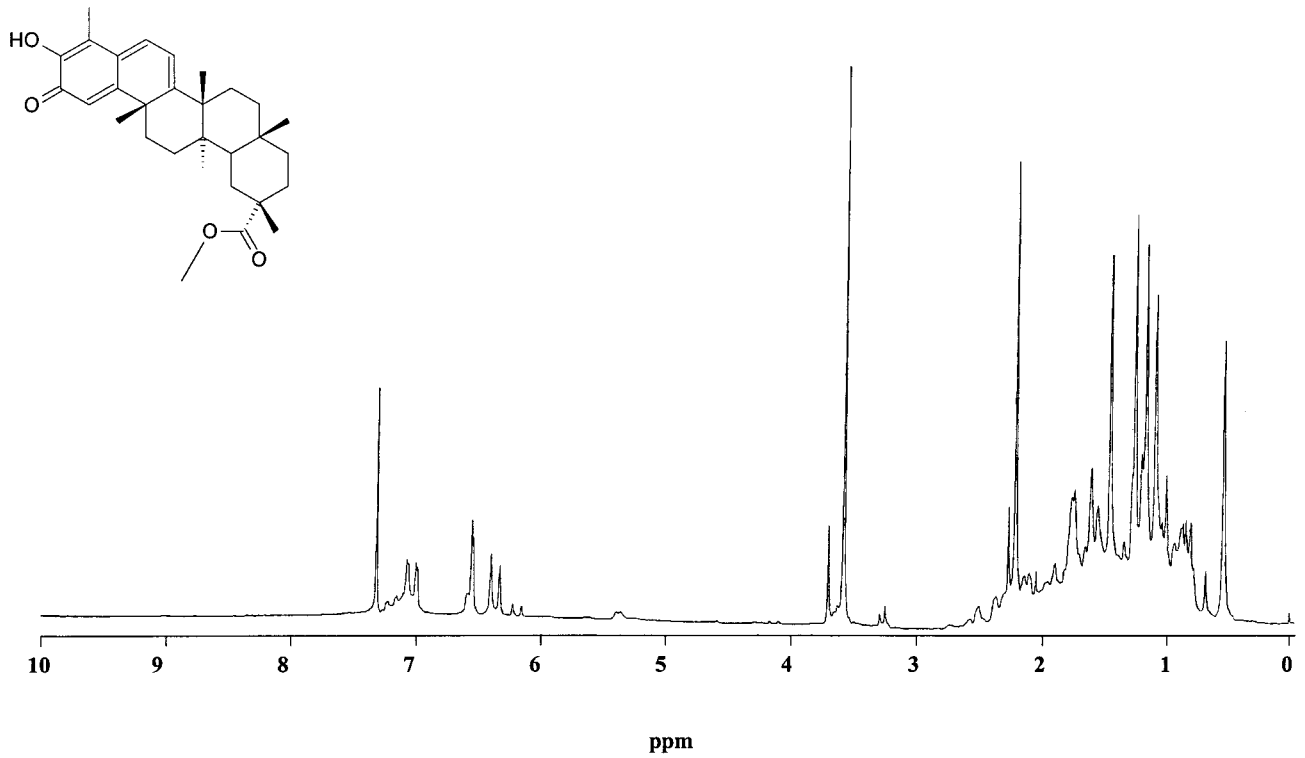

Figure 3. ^{1}H NMR spectrum corresponding to a solution of pristimerin in $DCCl_3$. Spectrum was recorded in a 100 MHz spectrometer using TMS as internal standard.

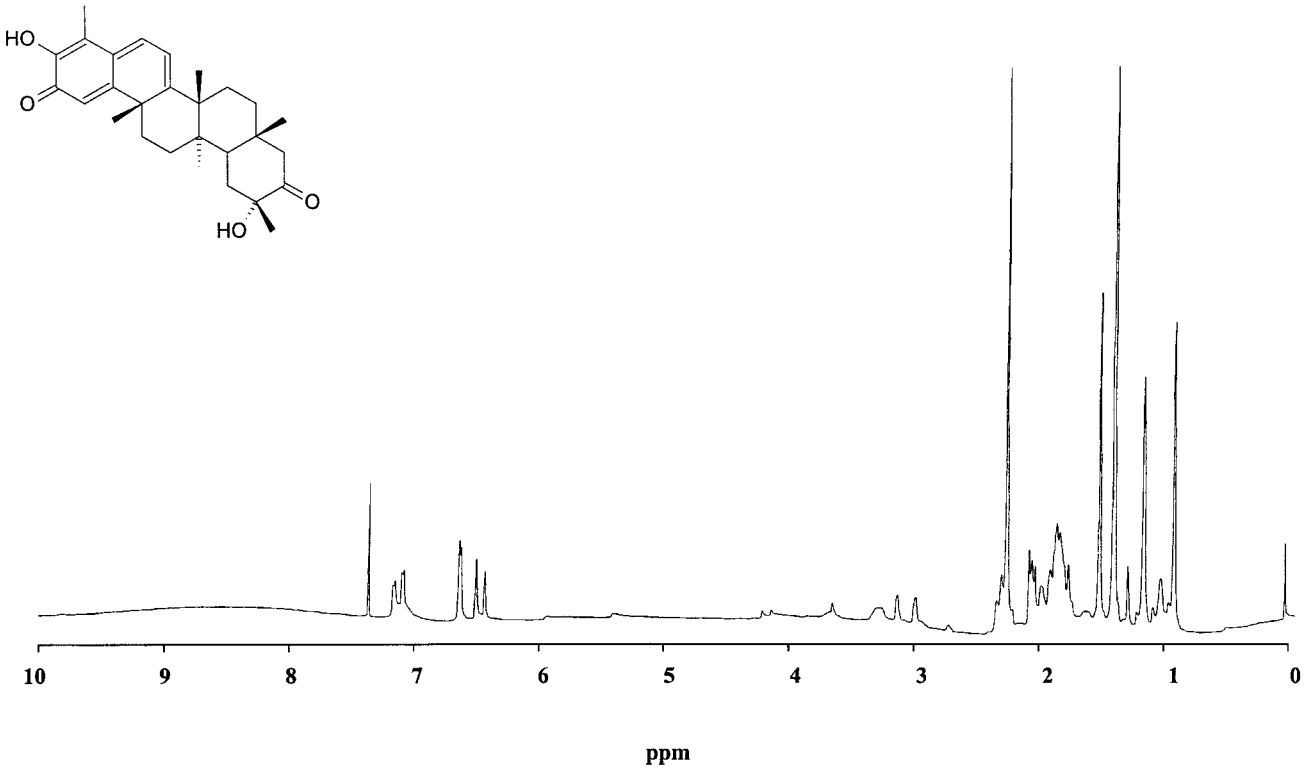

Figure 4. ^{1}H NMR spectrum corresponding to a solution of 20-α-hydroxytingenone in $DCCl_3$. Spectrum was recorded in a 100 MHz spectrometer using TMS as internal standard.

RESULTS AND DISCUSSION

Identification of the Compounds. Figures 2−4 show the ^{1}H NMR spectra of the three compounds. The spectral signals for each compound are in concordance with the ones described in the literature (Gunatilaka et al., 1989). In all cases a single spot were observed by TLC at R_f 0.7 for pristimerin, 0.5 for tingenone, and 0.3 for 20-α-hydroxytingenone.

Effect on Larval Mortality. The mortality of codling moth larvae was concentration-dependent and increased over time. 20-α-Hydroxytingenone and tingenone were the most and least active compounds against codling

Table 1. Mortality of *C. pomonella* Larvae after 5 or 10 Days of Continuous Exposure to Diet Topically Treated with 50 μL of a Solution of 20-α-Hydroxytingenone, Tingenone, or Pristimerin on Acetone at Different Concentrations[a]

concn	20-α-hydroxytingenone		tingenone		pristimerin	
(mg/mL)	5 days	10 days	5 days	10 days	5 days	10 days
0	0.0 ± 0.0	3.3 ± 3.3	0.0 ± 0.0 a	3.3 ± 3.3 a	0.0 ± 0.0 c	3.3 ± 3.3 c
1	3.3 ± 3.3	3.3 ± 3.3				
2	0.0 ± 0.0	0.0 ± 0.0				
4	6.7 ± 3.3	16.7 ± 6.7				
6	10.0 ± 10.0	23.3 ± 18.6				
8	3.3 ± 3.3	43.3 ± 12.0				
12	53.3 ± 14.5	60.0 ± 11.5				
16	60.0 ± 5.8	93.3 ± 3.3				
18	86.7 ± 6.7	93.3 ± 6.7				
20	100.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0 a	0.0 ± 0.0 a	20.0 ± 15.3 bc	60.0 ± 15.3 b
40			3.3 ± 3.3 a	10.0 ± 5.8 a	33.3 ± 3.3 b	60.0 ± 10.0 b
80			13.3 ± 8.8 a	23.3 ± 12.0 a	90.0 ± 5.8 a	100.0 ± 0.0 a
LC_{50}	13.0	8.5				

[a] Mean and SE of the percentage of dead larvae, calculated from 3 replicates of an initial number of 10 larvae each. Means followed by the same letter in the same column are not significantly different ($P > 0.05$, LSD test). LC_{50} calculated by means of probit analysis.

Table 2. Antifeedant Activity of 20-α-Hydroxytingenone, Tingenone, or Pristimerin on *C. pomonella* Larvae[a]

concn	20-α-hydroxytingenone		tingenone		pristimerin	
(mg/mL)	5 days	10 days	5 days	10 days	5 days	10 days
0	6.7 ± 6.7	6.7 ± 6.7	6.7 ± 6.7 bc	6.7 ± 6.7 b	6.7 ± 6.7 b	6.7 ± 6.7 b
1	6.7 ± 6.7	6.7 ± 6.7				
2	36.7 ± 12.0	3.3 ± 3.3				
4	46.7 ± 24.0	33.3 ± 6.7				
6	83.3 ± 16.7	53.3 ± 12.0				
8	100.0 ± 0.0	80.0 ± 11.5				
12	96.7 ± 3.3	90.0 ± 5.8				
16	100.0 ± 0.0	100.0 ± 0.0				
18	100.0 ± 0.0	100.0 ± 0.0				
20	100.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0 c	0.0 ± 0.0 b	96.7 ± 3.3 a	93.3 ± 3.3 a
40			10.0 ± 0.0 b	3.3 ± 3.3 b	83.3 ± 16.7 a	83.3 ± 16.7 a
80			100.0 ± 0.0 a	93.3 ± 6.7 a	90.0 ± 5.8 a	90.0 ± 5.8 a

[a] Mean and SE of the percentage of larvae that after 5 or 10 days of continuous exposure had not fed on diet topically treated with 50 μL of a solution of each compound on acetone at different concentrations, calculated from 3 replicates of an initial number of 10 larvae each. Means followed by the same letter in the same column are not significantly different ($P > 0.05$, LSD test).

Table 3. Growth Regulation Activity of 20-α-Hydroxytingenone, Tingenone, or Pristimerin on *C. pomonella* Larvae[a]

concn	20-α-hydroxytingenone		tingenone		pristimerin	
(mg/mL)	5 days	10 days	5 days	10 days	5 days	10 days
0	0.2 ± 0.2 a	1.5 ± 0.2 a	0.2 ± 0.2 b	1.5 ± 0.2 b	0.2 ± 0.2 a	1.5 ± 0.2 a
1	0.1 ± 0.0 a	1.2 ± 0.2 a				
2	0.1 ± 0.0 a	1.4 ± 0.2 a				
4	0.0 ± 0.0 a	0.6 ± 0.1 b				
6	0.0 ± 0.0 a	0.6 ± 0.2 b				
8	0.0 ± 0.0 a	0.2 ± 0.1 cd				
12	0.0 ± 0.0 a	0.3 ± 0.1 bc				
16	0.0 ± 0.0 a	0.0 ± 0.0 d				
18	0.0 ± 0.0 a	0.0 ± 0.0 d				
20	0.0 ± 0.0 a	0.0 ± 0.0 d	0.7 ± 0.2 a	3.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 b
40			0.3 ± 0.0 ab	1.4 ± 0.1 b	0.0 ± 0.0 a	0.0 ± 0.0 b
80			0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 a	0.0 ± 0.0 b

[a] Mean and SE of the number of head capsules of *C. pomonella* larvae alive after 5 or 10 days of continuous exposure to diet topically treated with 50 μL of a solution of each compound on acetone at different concentrations, calculated from 3 replicates of an initial number of 10 larvae each. Means followed by the same letter in the same column are not significantly different ($P > 0.05$, LSD test).

moth larvae, respectively. Mortality produced by tingenone was nonsignificantly different from the mortality observed in the acetone-treated control. The mortality caused by 20-α-hydroxytingenone reached 100% 5 days after exposure, the LC_{50} ranging from 13.0 mg/mL (5 days later) to 8.5 mg/mL (10 days later). Pristimerin also caused a 100% mortality, but at a much higher concentration (80 mg/mL) than 20-α-hydroxytingenone did (Table 1). Codling moth larvae treated with any concentration of 20-α-hydroxytingenone or pristimerin were smaller than control larvae, which only happened to larvae treated with the highest concentration of tingenone. The mortality caused by the neem extract after 5 days reached 100% at a concentration of 20 mg of azadirachtin/mg (Table 4). Only 20-α-hydroxytingenone was, then, as active as azadirachtin at this concentration.

Because mortality can be a result of product toxicity or can be due to the lack of feeding activity, the antifeedant effect of the compounds was studied.

Effect on Feeding Activity. The three compounds tested showed an antifeedant activity on codling moth larvae, 20-α-hydroxytingenone being the most active (the antifeedant activity was observed at a concentration of 2 mg/mL 5 days after exposure to topically treated diet) and tingenone being the least active (the antifeedant activity was observed only at a concentration of 80 mg/mL). Any larvae fed on diet treated with 20-α-hydroxytingenone at a concentration of 16 mg/mL or higher (Table 2). The antifeedant effect decreased over

Table 4. Mortality, Antifeedant Activity, and Growth Regulation Activity of an *A. indica* Extract (Mubel) on *C. pomonella* Larvae[a]

azadirachtin concn (mg/mL)	mortality		antifeedant activity		growth regulation	
	5 days	10 days	5 days	10 days	5 days	10 days
0	23.3 ± 18.5 a	43.3 ± 26.0 a	30.0 ± 20.9 a	30.0 ± 20.9 a	0.3 ± 0.3 a	1.3 ± 0.3 c
2	50.0 ± 15.3 a	66.7 ± 8.8 a	76.7 ± 14.5 b	70.0 ± 15.3 b	0.0 ± 0.0 a	0.3 ± 0.2 b
20	100.0 ± 0.0 b	100.0 ± 0.0 b	100.0 ± 0.0 b	100.0 ± 0.0 c	0.0 ± 0.0 a	0.0 ± 0.0 a

[a] Means and standard error of the percentage of dead larvae (mortality), of the percentage of larvae that had not fed on the diet (antifeedant effect), and of the number of head capsules per larva (growth regulator activity) after 5 or 10 days of continuous exposure to diet topically treated with 50 μL of a solution at different concentrations, calculated from 3 replicates of an initial number of 10 larvae each. Means followed by the same letter in the same column are not significantly different ($P > 0.05$, LSD test). Concentrations refer to the azadirachtin content of the solution.

time; more larvae fed on treated diet after 10 days of exposure than did after 5 days (Table 2). The neem extract showed a great antifeedant activity, even at a concentration of 2 mg azadirachtin/mL (Table 4).

All of the codling moth larvae that died 5 days after exposure (for the three metabolites) or 10 days after exposure (in the case of all the concentrations of 20-α-hydroxytingenone) had not fed on the diet.

Effect on Larval Development. The three metabolites slowed the development of codling moth larvae, expressed as the number of head capsules at a given date (Table 3). The effect of 20-α-hydroxytingenone and tingenone increased with the concentration applied; no larvae molted at the highest concentrations (20 mg/mL for 20-α-hydroxytingenone and 80 mg/mL for tingenone). The effect of pristimerin was also very high; no larvae were able to molt at all the concentrations tested. Whether or not this effect is due to a juvenile hormone like activity is now under study. The neem extract also slowed the development of codling moth larvae, but at a lower concentration than the three metabolites from *Maytenus* did (Table 4).

The structure−activity study of the action of the tested nortriterpenes shows the proton lack on the C20 is an essential feature of the active molecules. Thus, the hydroxylation of this carbon in the 20-α-hydroxytingenone dramatically increases the activity found. The enhancement of the bioactivity as a result of the hydroxylation of a tertiary carbon is a fact already described. Thus, it is well-known that α-ecdisone must be hydroxylated in its 20-position to become β-ecdisone, the true molting insect hormone (Hoffmann and Porchet, 1984). Recently, González-Coloma et al. (1998) have described the antifeedant activity of *Delphinium* diterpenoid alkaloids. These authors have found that the presence of hydroxy groups in some positions of these substances enhances drastically the activity against *Spodoptera littoralis* (CPB) and *Leptinotarsa decemlineata* (Say).

In summary, the three metabolites from *Maytenus* sp. produce the same effects on codling moth larvae that the neem extract does, although at higher concentrations. 20-α-Hydroxytingenone is the most active compound among the three studied. Pristimerin shows also a high antifeedant activity together with its molt effect suppression. Finally, tingenone presents the lowest activity. On the contrary, when tested as antitumoral and antibacterial agents, 20-α-hydroxytingenone was the least active compound, exhibiting no genotoxic and antibiotic effects (González et al., 1988; Shirota et al., 1994).

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