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ACARICIDAL AND INSECTICIDAL ACTIVTIES OF CADINA-4,10 (15)-DIEN-3-ONE

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Key Word Index—*Hyptis verticillata*; Labiatae; cadina-4,10(15)-dien-3-one; *Boophilus microplus*; *Cylas formicarius elegantulus*; acaricidal; insecticidal.

Abstract—The novel assignment of ^{13}C and ^{1}H NMR data for cadina-4,10(15)-dien-3-one obtained from *Hyptis verticillata* is presented. The study revealed that cadina-4,10(15)-dien-3-one possesses chemosterilant activities against the economically important cattle tick, *Boophilus microplus*, and toxic action against adult *Cylas formicarius* the most destructive pest of sweet potato (*Ipomoea* sp.).

INTRODUCTION

The wild mint *Hyptis verticillata* Jacq. is reported to be effective in alleviating colds, marasmus and gout in African and Caribbean folk medicine [1–4], however, the insecticidal and acaricidal properties of its extract are unknown. The growth regulatory and insecticidal properties of cadina-4,10(15)-dien-3-one, **1**, isolated from *H. verticillata* were evaluated against adult female ticks (*Boophilus microplus*) and both sexes of the weevil *Cylas formicarius*. *Boophilus microplus* is of considerable economic importance because of its ability to transmit disease-causing organisms in cattle [5], while *C. formicarius* inflicts over U.S.$7 million loss per year to the sweet potato (yam) industry in the southern U.S. [6]. The high percentage of infertile eggs produced by ticks treated with cadina-4,10(15)-dien-3-one prompted us to investigate the inhibitory effects of lipid sequestration in eggs oviposited by adult female *B. microplus*. Inhibition of lipid uptake and metabolism are two of the major causes of infertile egg production in ticks and insects [7–10]. In addition to the abovementioned investigations this study also provides for the first time a total proton and carbon (^{13}C) NMR assignment of **1** obtained from a plant species in high yield. Cadina-4,10(15)-dien-3-one was previously thought to exist only in trace quantities in oils from *Lavendula officinalis* and *Teucrium susitanicum* [11, 12].

RESULTS AND DISCUSSION

Structural elucidation of cadina-4,10(15)-dien-3-one

The high-resolution electron impact mass spectrum revealed the compound to be a sesquiterpene of formula of $C_{15}H_{22}O$ ($[M]^+ = 218.1672$). Fourier transform infrared spectroscopy (FTIR) suggested the presence of an α,β-unsaturated carbonyl system with absorptions at 1680 and 1650 cm^{-1}. The absorption at 890 cm^{-1} was characteristic of an exocyclic double bond. Data generated from the ^{13}C NMR spectrum and DEPT experiments confirmed the presence of five double bond equivalents. Four olefinic signals (105.38, 135.45, 146.45 and 149.73 ppm) and the carbonyl at δ199.80 implicated a bicyclic molecule. The singlet at δ6.8 (1H) belonged to a vinylic hydrogen β to the 3-carbonyl at δ199.8. There was no proton assignable to that borne the α-carbon of the conjugated system, however, a doublet of doublets at δ1.75 (J = 2, 4 Hz) proved to be due to a methyl group on a double bond. There were two methyl doublets at δ0.72 (J = 12 Hz) and δ0.93 (J = 12 Hz) along with a one hydrogen sextet at δ2.2 (J = 4 Hz, H-12). This, in conjunction with biogenetic information available for this class of terpene, demonstrated the occurrence of an isopropyl unit. The methylene group at δ105.38 in the ^{13}C spectrum was typical of an exocyclic double bond, while the two methine singlets at δ4.45 and 4.68 were characteristic of terminal olefinic centres.

$^{1}H,^{13}C$-Heteronuclear correlation spectroscopy (HETCORR) and proton–proton homonuclear correlation spectroscopy (COSY) data were used to determine the connectivities and ring sizes of **1**. The HETCORR spectrum showed that the ^{13}C signal at 146.45 ppm bore a hydrogen which resonated at δ6.8 (1H, s). From the ^{1}H–^{1}H COSY experiment, it was observed that the proton appearing at δ6.8 was coupled to another at δ1.92, which from the HETCORR spectrum was attached to a carbon at δ45.34. The proton at δ1.92 was adjacent to hydrogens at δ1.32 and 2.34, which the HETCORR data showed to be two methylenes at δ45.21 and 45.36, respectively. The fact that the single proton at spectrum

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was attached to a carbon at 45.34 ppm. The proton at δ1.92 was adjacent to hydrogens at δ1.32 and 2.34, which the HETCORR data showed to be two methylenes at 45.21 and 45.36 ppm respectively. The fact that the single proton at δ1.92 was linked to three other methines, one being vinylic, suggested that this was at a ring junction, with two methine groups forming the bridgehead. The proton at δ2.34 was coupled to hydrogens appearing at δ2.45 and δ2.6, found to be the methylenic pair borne on a carbon resonating at 41.39 ppm. This methylene was not further proton coupled and, from its ^{13}C position, was thought to be adjacent to a carbonyl. Hence, there was a six-membered ring. The other ring system could similarly assigned. The signal at δ1.32 was coupled to protons at δ1.13, 1.77 and 2.2. The HETCORR spectrum established that the proton at δ2.2 was from a CH group at δ26.41 which was further linked to the two secondary methyl groups at δ0.72 and 0.93. This confirmed the existance of the isopropyl group. The protons at δ1.13 and 1.77 belonged to the methylene whose carbon signal resonated at δ26.33.

The assignments of the quaternary centres were determined by analysis of the long-range proton–carbon heteronuclear correlation (COLOC) spectrum. The olefinic proton at δ6.8 demonstrated coupling with carbons at δ45.36, 135.45 and 146.45. The methyl group at δ1.73 showed strong interaction with the carbon at δ135.45 and weaker interactions with carbons at 146.45 and 199.8. Finally the olefinic protons at δ4.45 and 4.68 were connected with carbons at δ35.5, 45.36 and 105.38. Based on biogenesis and the connectivities observed within the molecule, **1** was proposed.

The compound was then subjected to single crystal X-ray diffraction studies because of the ambiguity in the resonance of the vinylic methyl which appeared as a doublet of doublets. This unexpected result was due to long range coupling with hydrogens 5 and 6. The absolute stereochemistry of cadinanes has been determined [13] and the optical rotation of **1** agrees with that of the authentic material [11].

Insecticidal and acaricidal studies

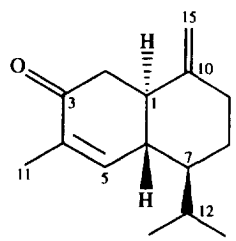
The cadinane was found to be an effective insecticide against *C. formicarius* at a concentration of 3.6 mg g^{-1} insect over 48 hr (Table 1). Data presented in Table 2 indicates that **1** disrupted the oviposition and hatching of *B. microplus* eggs, however, it was not lethal to the adult ticks (Table 3). Thus, doses of 0.45, 0.90 and 1.80 mg g^{-1} tick body weight inhibited oviposition (and egg hatching) by 29.05% (20.0%), 40.70% (30.0%) and 50.0% (75.0%), respectively.

Pyrenone (a commercially available formulation of the pyrethrin, butyl carbityl (6-propylpiperonyl) ether with piperonyl butoxide and petroleum distillate) was approximately twice as effective at inhibition of oviposition in *B. microplus* as the cadinane, but this was due, in part, to the overall toxicity. The reduction in egg laying by Altozar (ethyl (2*E*,4*E*)-3,7,11-trimethyl-2,4-dodecadienoate) was similar to that by **1**, however, the former killed 25% of the ticks at the highest concentration (Table 3).

Table 1. Toxic action of cadina-4,10(15)-dien-3-one against adult *Cylas formicarius elegantulus* (N = 20 in two replicates of 10 each)

Doses (mg per g insect body wt.)	Mortality (%)	
	After 24 hr	After 48 hr
1.8	55.0	70.0
3.6	75.0	90.0
Control	0.0	0.0

Williams [10] revealed that lipid sequestration could be equally as important as protein uptake for the formation of fertile eggs in ticks. Thus, interference with these physiological processes could induce the formation of infertile eggs, a feature common to insect growth regulators [8, 14]. Cadina-4,10(15)-dien-3-one inhibited the metabolism of lipids during embryogenesis of *B. microplus* eggs in a dose related manner (Table 2). Thus, 69.78% lipid was metabolized in the control eggs after 11 days of embryonic development, while 59.61 and 35.92% were utilized by eggs produced by ticks treated with 0.9 and 1.8 mg g^{-1} doses, respectively. It is possible that the inhibition of oviposition was due to the effect of **1** on neuromuscular binding sites, since egg laying in Acarina is a neuromuscular process [15].



Cadina-4, 10(15)-dien-3-one, 1

EXPERIMENTAL

Freshly collected leaves and stems of *Hyptis verticillata* were harvested in January 1992 from a single isolated population growing in Barbican (Kingston, Jamaica). A voucher specimen was deposited in the herbarium of the Department of Botany (U.W.I., Mona; accession #33483). The plant material was chopped and repeatedly extracted with CH_2Cl_2 at 27° until the extract was pale green.

The combined extracts were pooled and concentrated *in vacuo* to provide a dark green gum (17.6 g, 1.7% yield from the green plant). The crude gum was chromatographed on silica gel (Merck, 40–63 μm diameter). Elution of the column with mixtures of increasing concns of EtOAc–hexane gave five fractions. Fr. 1, eluted in 5% EtOAc–hexane, was evaporated to a yellow wax-like residue (2.6 g). Elution with 10% EtOAc–hexane yielded fr. 2 (1.9 g) as a dark green gum. Fr. 3 eluted in 20%

Table 2. Effects of cadina-4,10(15)-dien-3-one on the metabolism of lipids during the embryogenesis of *Boophilus microplus* eggs

Doses (mg g^{-1} tick body wt.)	Mean egg wt. (mg) ± SE	Mean lipid wt. (mg) ± SE	Lipid content (mg g^{-1})	% lipid metabolized
Data after 0 hr of embryogenesis				
0.9	52.0 ± 3.4	4.21 ± 0.02	80.96	0
1.8	24.0 ± 7.0	1.72 ± 0.08	75.06	0
Control*	61.0 ± 0.0	5.55 ± 0.02	90.98	0
Data after 264 hr of embryogenesis				
0.9	37.00 ± 5.0	1.21 ± 0.8	32.70	59.61
1.8	21.00 ± 6.5	1.01 ± 0.008	48.18	35.92
Control*	51.30 ± 9.4	1.41 ± 0.04	27.49	69.78

*Control = 8.0 μl of acetone.

Table 3. Effects of cadina-4,10(15)-dien-3-one, pyrenone and Altozar on the survivability of fully engorged adult female *Boophilus microplus*

Doses (mg g^{-1} tick body wt.)	% mortality at 96 hr	Mean egg wt. (mg) ± SE	% IO* (after 21 days)	% IH†
Cadina-4,10(15)-dien-3-one				
0.45	0.0	63.57 ± 2.0	27.1	20.0
0.90	0.0	51.67 ± 5.7	40.7	30.1
1.80	0.0	40.00 ± 8.4	50.0	75.1
Pyrenone				
0.45	26.0	39.21 ± 4.7	55.0	65.0
0.90	40.0	13.07 ± 8.5	85.0	78.0
1.80	62.5	0.00 ± 0.0	100.0	100.0
Altozar				
0.50	0.0	53.68 ± 9.5	38.4	10.0
1.00	15.0	47.93 ± 6.3	45.0	34.0
2.00	25.0	37.56 ± 12.8	56.9	63.5
Control‡	0.0	87.14 ± 10.8	0.0	0.0

*IO, Inhibition of oviposition.
†IH, Inhibition of hatching.
‡Control, 8.0 μl of acetone.

EtOAc in hexane and afforded a green powder (2.1 g). Further elution with 30% EtOAc in hexane gave fr. 4 (4.8 g), a light green solid. Finally, fr. 5 eluted with 50% EtOAc–hexane and, after removal of the eluant under reduced pressure, yielded a green gum (1.1 g).

All five fractions were subjected to TLC and their profiles viewed in UV light. Fr. 2, which showed an intense purple fluorescent band at 254 nm (R_f 0.4 in 10% EtOAc–hexane), was examined further.

Isolation of cadina-4,10(15)-dien-3-one, **1**. The fr. was subjected to prep TLC using 10% EtOAc–hexane. The fluorescent band was collected and eluted from the silica gel with acetone. Removal of the solvent *in vacuo* gave a light green solid (877 mg, 0.08% yield from the green plant) which was redissolved in EtOAc and purified by HPLC using a preparative silica column (Perkin Elmer, 25 × 2.1 cm, 10 μm). The column was eluted with an isocratic mixture of 7% EtOAc–hexane at a flow rate of 10 ml min^{-1}. Column eluants were monitored at 245 nm. The fraction eluting after 13.6 min was collected and solvent removed. The resulting compound was recrystallized from acetone to give colourless needles, mp 79–80°, $[\alpha]_D$ = + 127° (*c* = 1.6, $CHCl_3$). Lit. [11] mp 69–70°, $[\alpha]_D$ = + 112° (*c* = 0.316, $CHCl_3$); UV (MeOH): λ_{max} 238 nm (log ε = 3.98); IR ν_{max} cm^{-1}: 2950, 1680, 1650, 887; HRMS (EI): *m/z* (%) 218.1672 (75) [$C_{15}H_{22}O$], 147 (100); ^{1}H NMR (200.13 MHz $CDCl_3$): δ0.72 (3H, *d*, *J* = 12 Hz, H-14), 0.92 (3H, *d*, *J* = 12 Hz, H-13), 1.75 (3H, *dd*, *J* = 2 Hz, *J* = 4 Hz, H-11), 2.2 (1H, sextet, *J* = 4 Hz, H-12), 4.46 (1H, *s*, H-15), 4.68 (1H, *s*, H-15), 6.80 (1H, *s*, H-5); ^{13}C NMR (50.32 MHz $CDCl_3$): δ15.22 (CH_3-14), 16.04 (CH_3-13), 21.53 (CH_3-11), 26.33 (CH_2-8), 26.41 (CH-12), 35.5 (CH_2-9), 41.39 (CH_2-2), 45.21 (CH-7), 45.34 (CH-6) 45.36 (CH-1), 105.38 (CH_2-15), 135.4 (C-4), 146.45 (CH-5), 149.7 (C-10), 199.8 (C-3). Crystallographic data from **1** are deposited at the Cambridge centre.

Insecticidal assay. Two-week-old adult *Cylas formicarius elegantulus* (Summer) weighing 50 ± 2.5 mg each were used for bioassay. Insects were cultured on

sweet potato tubers (*Ipomoea* sp.) in the laboratory at 25 ± 2° and 65–68% relative humidity (RH). A 4.5% (w/v, 9.0 mg in 0.2 ml acetone) stock solution was prepared for cadina-4,10(15)-dien-3-one. From the above 2.0 μl (0.09 mg) and 4.0 μl (0.18 mg) aliquots were topically applied to 20 adult *C. formicarius* in two replicates of 10 each using a Hamilton microapplicator. Twenty insects treated with 4.0 μl of acetone only served as the controls. The number of dead insects was recorded at 24 and 48 hr after treatment.

Acaricidal assay. The assay was performed on fully engorged adult female *Boophilus microplus* (Canest.) weighing 200 ± 10.0 mg within 4 hr of collection from the local abattoir. From the 4.5% (w/v) stock solution of **1** prepared for insecticidal assay above 4.0 μl (0.9 mg g^{-1} tick body wt.) and 8.0 μl (1.8 mg g^{-1} tick body wt.) samples were topically applied to the dorsa of 20 fully engorged adult female *Boophilus microplus* (Canest.) in two replicates of 10 each. The control ticks were treated with 8 μl of acetone only. The treated and control ticks were kept in Petri dishes at room temperature (27 ± 2.0°) and 55 to 60% RH. The number of dead ticks was recorded. These were removed from the petri dishes and the survivors allowed to lay eggs. After 21 days eggs from each replicate were weighed, pooled and placed in a test tube which was covered with a moist cotton plug. Every third day the cotton plug was moistened. The hatching success of the eggs was determined after 6 weeks of incubation by removing the larvae from the top of the test tubes, and mixing the residues at the bottom throughly. Three equal subsamples weighing 0.1 g each were taken and the number of empty egg shells and unhatched eggs were counted at × 100 magnification. The inhibition of oviposition and hatching was determined as described previously [16].

Lipid determination. Total lipids were determined gravimetrically [17] on eggs at different stages of embryogenesis. Eggs were homogenized for 10 min and the homogenate filtered through glass wool into a 10.0 ml glass-stoppered centrifuge tube. The filtrate was shaken for 5.0 min with 1.0 ml of 1.0% (w/v) NaCl solution and then centrifuged for 5.0 min at 1500 rpm. The upper methanol–water–salt layer was removed with a capillary pipette. The lower phase was evaporated to dryness under reduced pressure in pre-weighted 10.0 ml round-bottomed flasks. The weight of the flasks and their contents were found and the percentage lipid determined. All samples were analysed in triplicate.

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