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INSECTICIDAL CHROMENES FROM THE VOLATILE OIL OF *Hemizonia fitchii*[1,2]

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Abstract—Based on field observations of the effects of the resinous tarweed *Hemizonia fitchii* A. Gray (Asteraceae) on mosquito populations in California, the volatile oil of this plant was investigated for insecticidal activity. Analysis of the oil by TLC and capillary GC-MS showed the presence of five major constituents which were identified as the monoterpenoid 1,8-cineole, and the chromenes encecalin, eupatoriochromene (desmethylencecalin), 6-vinyl-7-methoxy-2,2-dimethylchromene, and desmethoxyencecalin. Trace amounts of several volatile fatty acids, alkanes, *p*-coumarate derivatives, additional chromene derivatives, and numerous mono- and sesquiterpenoids were also detected and identified by GC-MS. Fractionation of the oil by preparative TLC and column chromatography afforded the major chromenes, the identities of which were confirmed by NMR and IR spectral data. The chromenes exhibited weak to moderate toxicity against *Culex pipiens* (house mosquito) larvae and *Oncopeltus fasciatus* (large milkweed bug) nymphs. However, no anti-juvenile hormone activity was observed for any of the compounds tested against these insect species.

Key Words—*Hemizonia fitchii*, insecticidal volatile oil, chromenes, 1,8-cineole, *Culex pipiens*, *Oncopeltus fasciatus*, mosquito, milkweed bug.

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INTRODUCTION

In a systematic search for naturally occurring compounds which exhibit effects on the growth, development, and behavior of insect pests, we are currently screening extracts from over 800 species of higher plants found in the western United States. The basis for the present report was a field observation by one of us (E.K.) that resinous *Hemizonia fitchii* A. Gray (Asteraceae) (Fitch's spikeweed, tarweed) growing at the edges of ponds in California had a definite suppressant effect on local mosquito populations. Pond water in contact with *H. fitchii* was observed to be totally devoid of any stage of mosquito. However, nearby ponds, which appeared to be identical chemically, physically, and biologically, with the exception that there was no contact with *H. fitchii*, supported large numbers of all stages of the mosquito *Aedes melanimon*. In addition, the aboveground parts of *H. fitchii* possess a strong, pungent, lachrymatory aroma that was observed in the field to be repellent to insects and spiders, but highly attractive to domestic cats.

Previous phytochemical studies on members of the genus *Hemizonia* have resulted in the identification of an acetylenic thiophene (Bohlmann et al., 1973), sesquiterpenes, diterpenes, benzofurans, benzopyrans (chromenes), *p*-coumarate derivatives, squalene, an alkanol ester, several geraniol esters (Bohlmann et al., 1981), and a number of flavonoids (Proksch et al., 1984). However, no biological studies appear to have been conducted previously with any species of *Hemizonia*.

METHODS AND MATERIALS

Extraction. Aerial parts of *H. fitchii* were collected in June in Oroville, California, at Afterbay of the Feather River Power Water Project. Collected plants were sealed in plastic bags, frozen (−20°C), and airmailed to Salt Lake City, Utah. Upon arrival, steam distillates (2.50 g) were obtained from the frozen whole plant material (160 g, fresh weight) using a modified Clevenger distillation apparatus (24 hr). The plant material was subsequently dried, finely ground with a Wiley mill, and sequentially extracted with hexane, methylene chloride, and methanol. Since bioassay-guided fractionation using larvae of *Culex pipiens* (see below for a description of the bioassay) confirmed that the vast majority of the biological activity resided in the volatile oil fraction, further chromatographic work was confined to the steam distillate fraction.

Gas–Liquid Chromatography (GC) of Volatile Oil. GC analyses were performed with a Varian 1800 gas chromatograph equipped with a flame ionization detector (350°C) using a J & W DB-1 fused silica capillary column (30 m × 0.32 mm ID; 0.25 μm film thickness) with nitrogen as the carrier gas (18 cm/sec). All GC analyses were performed in the split mode (1:25 split ratio) with

the injector temperature at 275°C. The oven temperature was programed from 60° to 230°C at 4°C/min, and peak areas were calculated using a Columbia Scientific Industries Supergrator-2 electronic digital integrator.

Thin-Layer (TLC) and Open-Column Chromatographies. Analytical and preparative TLC were performed on 20 × 20-cm prescored silica gel GHLF plates (Analtech, Inc.; 0.25 mm) using hexane–diethyl ether–acetic acid (80:20:1, system A; multiple development) and hexane–diethyl ether [3:1 (B) and 4:1 (C)] as solvent systems. Visualization for analytical TLC was accomplished under long- and shortwave ultraviolet (UV) light, followed by spraying with a vanillin–sulfuric acid–ethanol (3 g:1.5 ml:100 ml) spray reagent and heating. For preparative TLC, visualization under UV light revealed the major bands which were subsequently cut from the plates and eluted with acetone.

Additional steam distillates from *H. fitchii* were subjected to column chromatography on silica gel 60 (30–70 mesh ASTM) in a gradient of ether in hexane. Separations were monitored by subjecting eluted fractions to analytical TLC with visualization under UV light, followed by spraying with the vanillin–sulfuric acid spray reagent and heating.

Gas Chromatography–Mass Spectrometry (GC-MS) of Volatile Oil. GC-MS analyses were performed with a Hewlett-Packard model HP-5985 quadrupole gas chromatograph–mass spectrometer, taking mass spectral scans from mass 33 to mass 633 at 800 amu/sec. Chromatographic separations were achieved using a J & W DB-1 fused silica capillary column (26 m × 0.32 mm ID; 1 μm film thickness). All GC-MS analyses were made using a 1:35 split ratio with helium as carrier gas (2 cc/min). The GC column was temperature programed from 70° to 300° at 5°C/min. Compounds were identified by EI (electron impact, 70 eV) mass spectrometry and by their order of elution and relative GC retention times. The identification of 1,8-cineole (I) and several of the trace constituents was aided by the compilations of Jennings and Shibamoto (1980) and Swigar and Silverstein (1981), as well as the EPA/NIH mass spectral data base (Heller and Milne, 1978, 1980).

Proton Nuclear Magnetic Resonance ([^{1}H]NMR) Spectroscopy and Infrared (IR) Spectrophotometry of the Major Chromenes. The identities of the major chromenes were confirmed by 60-MHz [^{1}H]NMR spectroscopy (Varian EM-360) using $CDCl_3$ or benzene-D_6 as solvent and tetramethylsilane (TMS) as internal standard. IR spectrophotometric data (Perkin-Elmer 710B) were consistent with the assigned structures in all cases.

Portions of the volatile oil were acetylated by using acetic anhydride with pyridine as catalyst. Acetylated compounds were separated on silica gel GHLF plates (Analtech, Inc.; 0.25 mm) using hexane–diethyl ether–acetic acid (80:20:1) as the solvent system. Identification of the acetates was accomplished with IR spectral data.

Culex Larvicidal Assay. A susceptible strain of *C. pipiens* originally obtained from the California State Department of Health was used for the larvi-

cidal assay. Animals (all stages of either first or third instar) were counted into test containers (1-oz plastic cups) and treated with a graduated concentration series of 0.1% acetone-diluted test compounds in 10 ml distilled water. First instar larvae were transferred (10 larvae/cup) with a fine-mesh silk cloth. Third instar larvae were transferred (5 larvae/cup) with a 1× 1-in. circle of ordinary window screen. Care was taken to remove excess water before entering the larvae into the test solutions. Following 48 hr of exposure to the treated water at 28°C, 80% relative humidity, and 18 hr daily illumination, LC_{50} values, the lethal concentrations for 50% mortality, were estimated using log probit paper. The assay was repeated three times with four treatments using 5–10 larvae/treatment. Survivors were allowed to complete development in order to observe any developmental effects of sublethal concentrations.

Oncopeltus Topical Assay. Nymphs of the large milkweed bug, *O. fasciatus*, were taken from a laboratory culture maintained on sunflower seeds and water. Animals (all stages of either second or third instar) were temporarily anesthetized with CO_2 and topically treated on the dorsum of the abdomen with 1 μl of an acetone solution of the test compound. The treated insects were transferred to rearing jars with sunflower seeds and water at 28°C, 80% relative humidity, and 18 hr daily illumination for the 8–10 days' duration of the assay period (sufficient time for control insects to undergo two molts). Appropriate controls were kept for each of the treated groups. LD_{50} values, the lethal doses for 50% mortality, were estimated using log probit paper. The assay was repeated three times with four treatments using 5–10 nymphs/treatment. Survivors were allowed to complete development so that any developmental effects of sublethal doses could be observed.

RESULTS

Because of the highly resinous and aromatic nature of the plant material, aerial parts of *H. fitchii* were subjected to steam distillation (24 hr), which afforded a viscous yellow volatile oil. The plant material was then dried, finely ground, and sequentially extracted with hexane, methylene chloride, and methanol. Bioassay of the various extracts with larvae of *C. pipiens* revealed that the vast majority of the biological activity resided in the volatile oil fraction.

Analysis of the volatile oil by TLC and fused silica capillary GC revealed the presence of only five major components. These major constituents were identified by fused silica capillary GC-MS as the monoterpenoid 1,8-cineole (I) (which comprised approximately 25% of the oil), and the chromenes encecalin (II) (the major constituent of the oil at 30%), eupatoriochromene (desmethylencecalin) (III) (17% of the oil), 6-vinyl-7-methoxy-2,2-dimethylchromene (IV) (7% of the oil), and desmethoxyencecalin (V) (6% of the oil) (Figure 1). These five major constituents together accounted for approximately 86% of the oil.

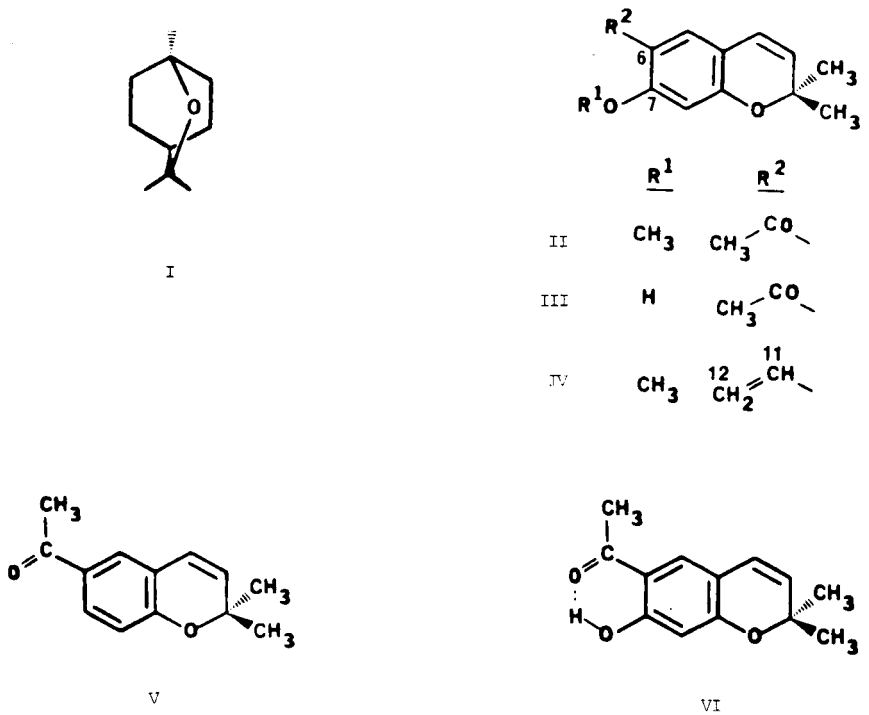

FIG. 1. Structures of the major constituents of the volatile oil of *Hemizonia fitchii* (I–V), and the internally hydrogen-bonded form of III (VI).

The remainder of the oil consisted of a large number of constituents which were present in concentrations of approximately 1% or less. These trace constituents were also detected and identified by GC-MS and included several volatile fatty acids, alkanes, *p*-coumarate derivatives, additional chromene derivatives, and numerous mono- and sesquiterpenoids (Table 1).

Fractionation of the oil by preparative TLC and column chromatography afforded 1,8-cineole (I) and the major chromenes, the identities of which were established by [^{1}H]NMR and IR spectroscopies, as well as by direct probe MS. The spectral data and physical properties of the compounds isolated agreed with literature values in all cases (Bjeldanes and Geissman, 1969; Anthonsen, 1969; Bohlmann and Grenz, 1970, 1977; Steelink and Marshall, 1979; Swigar and Silverstein, 1981; Proksch and Rodriguez, 1982).

Only chromene III yielded a monoacetate derivative, confirming it to be the only compound bearing a hydroxyl group. However, this hydroxyl group was barely observed in the IR spectrum of eupatoriochromene (III). In addition, the hydroxyl proton was at 12.87 ppm in the [^{1}H]NMR spectrum of III, and it took

TABLE 1. ANALYSIS OF VOLATILE OIL OF *HEMIZONIA FITCHII* BY GC-MS[a]

Compound	M^+ (*m/z*)	R_t (min)	Peak area (% of total)
Volatile fatty acids			
Isobutyric acid	88	2.75	0.26
Isovaleric acid	102[b]	4.22	0.11
2-Methylbutyric acid	102[b]	4.63	0.19
Monoterpenes			
Sabinene	136	7.62	0.20
Unidentified	120[c]	8.00	0.21
p-Cymene	134	8.87	0.14
1,8-Cineole (I)	154	9.18	25.07
Linalool	154[b] (136[c])	10.88	0.65
α-Terpineol isomer	154[b] (136[c])	12.77	0.25
Unidentified	168[c]	12.90	0.62
p-Cymen-9-ol	150	13.12	0.35
Naphthalene[d]	128	13.22[e]	0.39[e]
4-Terpineol	154		
Unidentified	136[c]	14.13	0.20
Unidentified	142[c]	14.38	0.40
Unidentified	198[c]	15.75	0.27
p-Cymen-7-ol (cuminyl alcohol)	150	16.07	0.29
Sesquiterpenes			
β-Caryophyllene[f]	204[b] (189[c])	20.45	0.31
Farnesol or isomer[f] ($C_{15}H_{26}O$)	222	23.08	0.53
Farnesol or isomer[f] ($C_{15}H_{26}O$)	222	23.20	0.30
Nerolidol or isomer[f] ($C_{15}H_{26}O$)	222[b] (204[c])	23.58	1.08
Unidentified	150[c]	24.28	0.28
Chromenes			
Desmethoxyencecalin (V)	202	25.37	6.47
6-Vinyl-7-methoxy-2,2-dimethylchromene (IV)	216	26.00	7.42
Encecalin isomer[f]	232	26.80	0.27
Evodionol isomer[f]	248	26.90	0.45
Eupatoriochromene (Desmethylencecalin) (III)	218	28.14	17.34
Encecalol isomer[f]	234	28.78	1.12
Encecalin (II)	232	30.27	29.63
Miscellaneous constituents			
trans-Methyl coumarate-*p*-dimethyl allyl ether	246	32.10	0.15
Palmitic acid	256	32.32	1.30
cis-Methyl coumarate-*p*-dimethyl allyl ether	246	34.05	0.64
Unidentified	168[b,c]	34.77	1.01
Alkanes			
Pentacosane (*n*-$C_{25}H_{52}$)	352	42.25	0.77
Heptacosane (*n*-$C_{27}H_{56}$)	380	45.30	0.74
Nonacosane (*n*-$C_{29}H_{60}$)	408[b]	48.18	0.19

[a]See text for GC-MS conditions.
[b]Molecular ion not observed by EI-MS.
[c]Highest mass ion observed.
[d]Unlikely as a natural product; probably an isolation artifact or impurity.
[e]Unresolved mixture.
[f]Tentative compound identification based on MS and relative R_t data (authentic standards not available).

18 min of vigorous shaking with deuterium oxide to completely exchange this proton. Unexpectedly, the acetate ester derivative of III appeared to be more polar than its parent compound, as shown by its lower R_f value (0.0) in the TLC systems used. This finding, together with the IR and [^{1}H]NMR spectral evidence, confirmed that the hydroxyl group in III is strongly internally hydrogen-bonded. This gives the compound the character of an ether-like "third ring," making it less polar than encecalin (II) and desmethoxyencecalin (V) (see Figure 1, VI). Similar chromatographic behavior has been noted with the strongly internally hydrogen-bonded proton of plumbagin (Kubo et al., 1983).

1,8-Cineole (I). R_f (system C) 0.60 (no color observed under longwave UV light; light blue color after spraying with vanillin–sulfuric acid reagent and heating); MS, *m/z* 154 (M^+, $C_{10}H_{18}O$, 67%), 139 ($M-CH_3$, 51), 136 ($M-H_2O$, 10), 125 (15), 121 (11), 111 (78), 108 (98), 96 (40), 93 (68), 84 (70), 81 (100), 71 (66), 69 (49), 55 (34), 43 (79).

Encecalin (II). R_f (C) 0.23 (bright blue under longwave UV light; bright red after spraying with vanillin–sulfuric acid reagent and heating); IR, ν_{max} (neat, NaCl plates) 3050, 2975, 1660, 1605, 1285 cm^{-1}; MS, *m/z* 232 (M^+, $C_{14}H_{16}O_3$, 17%), 217 ($M-CH_3$, 100), 187 ($217-OCH_2$, 10), 185 ($217-CH_3OH$, 7), 174 ($217-CH_3CO$, 8), 145 (4), 144(4), 115(6), 101 (9); [^{1}H]NMR (60 MHz, $CDCl_3$)δ 1.43 (6H, s, $2-CH_3$), 2.56 (3H, s, $-COCH_3$), 3.88 (3H, s, $-OCH_3$), 5.59 (1H, d, $J_{3,4}$ = 10, H—3), 6.36 (1H, d, $J_{4,3}$ = 10, H—4), 6.46 (1H, s, H—8), 7.62 (1H, s, H—5), (benzene-d_6)δ 1.32 (6H, s, $2-CH_3$), 2.53 (3H, s, $-COCH_3$), 3.37 (3H, s, $-OCH_3$), 5.32 (1H, d, $J_{3,4}$ = 10, H—3), 6.21 (1H, d, $J_{4,3}$ = 10, H—4), 6.36 (1H, br s, H—8), 7.87 (1H, s, H—5).

Eupatoriochromene (desmethylencecalin) (III). R_f (C) 0.38 (yellow-green under longwave UV light; light blue after spraying with vanillin–sulfuric acid reagent and heating); IR, ν_{max} (KBr) 3050, 2920, 1630, 1370 cm^{-1}; MS, *m/z* 218 (M^+, $C_{13}H_{14}O_3$, 26%), 203 ($M-CH_3$, 100), 185 ($203-H_2O$, 20), 160 ($203-CH_3CO$, 4); [^{1}H]NMR (60 MHz, $CDCl_3$)δ 1.43 (6H, s, $2-CH_3$), 2.53 (3H, s, $-COCH_3$), 5.62 (1H, d, $J_{3,4}$ = 10, H—3), 6.32 (1H, d, $J_{4,3}$ = 10, H—4), 6.37 (1H, br s, H—8), 7.37 (1H, s, H—5), 12.87 (1H, D_2O-exchangeable, s, —OH, intramolecularly H-bonded).

6-Vinyl-7-methoxy-2,2-dimethylchromene (IV). R_f (C) 0.73 (no color observed under longwave UV light; purple color after spraying with vanillin–sulfuric acid reagent and heating); IR, ν_{max} (neat, NaCl plates) 3060, 2975, 1735, 1630, 1620 (C=C), 1500, 1295, 1130 cm^{-1}; MS, *m/z* 216 (M^+, $C_{14}H_{16}O_2$, 23%), 201 ($M-CH_3$, 100), 185 ($M-OCH_3$, 20), 158 ($185-CH_2{=}CH$, 5); [^{1}H]NMR (60 MHz, $CDCl_3$)δ 1.45 (6H, s, $2-CH_3$), 3.87 (3H, s, $-OCH_3$), 5.19 (1H, dd, J_{cis} = 11, J_{gem} = 2, H—12), 5.58 (1H, d, $J_{3,4}$ = 10, H—3), 5.65 (1H, dd, J_{trans} = 18, J_{gem} = 2, H—12), 6.37 (1H, d, $J_{4,3}$ = 10, H—4), 6.47 (1H, s, H—8), 7.05 (1H, dd, J_{trans} = 18, J_{cis} = 11, H—11), 7.20 (1H, s, H—5).

Desmethoxyencecalin (V). R_f (C) 0.28 (no color observed under longwave

TABLE 2. 48-HOUR *CULEX PIPIENS* LARVICIDAL BIOASSAY

Compound tested	Instar tested	LC_{50} (ppm)
6-Vinyl-7-methoxy-	1st	1.8
2,2-dimethylchromene (IV)	3rd	3.8
Encecalin (II)	1st	3.0
	3rd	6.6
Eupatoriochromene (III)	1st	6.4
	3rd	13.0
1,8-Cineole (I)	3rd	[a]

[a]No effect observed to 20 ppm.

UV light); MS, *m/z* 202 (M^{+}, $C_{13}H_{14}O_2$, 10%), 187 ($M-CH_3$, 100), 171 ($M-OCH_3$, 1), 158 (187-29, 1), 144 ($187-CH_3CO$, 17), 128 (2), 115 (9).

The toxicities of the major components isolated from the volatile oil to *C. pipiens* (house mosquito) larvae are shown in Table 2. The activity of each of the components was about twofold more against first instar than against third instar larvae. Thus, the most active of the compounds tested, 6-vinyl-7-methoxy-2,2-dimethylchromene (IV), had an LC_{50} value of 1.8 ppm against first instar and 3.8 ppm against third instar larvae. The activity against first and third instar larvae of encecalin (II) was LC_{50} = 3.0 ppm and 6.6 ppm, respectively, and that of eupatoriochromene (III) was LC_{50} = 6.4 ppm and 13.0 ppm, respectively. Survivors were allowed to continue development to the adult stage. Although more deaths occurred with time, no effects were observed on development to subsequent larval stadia, pupal formation, or adult emergence.

Three commercially available volatile organic acids which we identified in the volatile oil, namely isovaleric, isobutyric, and 2-methylbutyric acids, were also assayed against third instar *C. pipiens* larvae. We found no activity of these acids to concentrations as high as 250 ppm.

The results of an additional assay of the *Hemizonia* chromenes with *O. fasciatus* (large milkweed bug) nymphs are shown in Table 3. In this assay, encecalin (II) was found to be the most active compound. Topical applications of encecalin (II) caused 50% mortality to second and third instar nymphs at 10 μg and 11 μg, respectively. 6-Vinyl-7-methoxy-2,2-dimethylchromene (IV) had LD_{50} values against second and third instar nymphs of 23 μg and 35 μg, respectively. Eupatoriochromene (III) had no effect on second and third instar nymphs at concentrations up to 100 μg and 200 μg, respectively. Desmethoxyencecalin (V) was not tested due to insufficient quantities available. Survivors were allowed to continue development to the adult stage. Although more deaths occurred with time, no effects were observed on development to subsequent stadia, number of stadia, or adult emergence.

TABLE 3. *ONCOPELTUS FASCIATUS* TOPICAL ASSAY[a]

Compound tested	Instar tested	LD_{50} (μg)
Encecalin (II)	2nd	10 μg
	3rd	11 μg
6-Vinyl-7-methoxy-	2nd	23 μg
2,2-dimethylchromene (IV)	3rd	35 μg
Eupatoriochromene (III)	2nd	[b]
	3rd	[c]

[a] Assay period 8–10 days, sufficient time for control insects to undergo two molts.
[b] No effect observed to 100 μg.
[c] No effect observed to 200 μg.

DISCUSSION

Encecalin (II) and eupatoriochromene (desmethylencecalin) (III) have previously been reported as constituents of *H. fitchii* (Bohlmann et al., 1981). 6-Vinyl-7-methoxy-2,2-dimethylchromene (IV), desmethoxyencecalin (V), and 1,8-cineole (I) have not previously been reported as constituents of *H. fitchii,* although they have been identified in other members of the Asteraceae (Bohlmann and Grenz, 1977; Bohlmann and Jakupovic, 1978; Steelink and Marshall, 1979; Bohlmann et al., 1981, 1982, 1983; Proksch and Rodriguez, 1982, 1983). However, to our knowledge, this is the first report of the facile isolation of these chromenes as constituents of a volatile oil.

Chromene derivatives isolated from other members of the Asteraceae have previously been shown to cause various effects in insects (Bowers et al., 1976; Bowers, 1982a,b; Wisdom and Rodriguez, 1982; Proksch and Rodriguez, 1983; Proksch et al., 1983; Rodriguez, 1983; Wisdom et al., 1983). The most biologically active of these chromenes are the precocenes, 6,7-dimethoxy-2,2-dimethylchromene (or ageratochromene) and 7-methoxy-2,2-dimethylchromene. The precocenes have a number of biological effects against insects, including chemosterilant and antijuvenile hormone activities (Bowers, 1981). For example, the precocenes have been shown to induce precocious metamorphosis in *Oncopeltus* nymphs through specific cytotoxic destruction of the parenchymal cells of the corpus allatum (the gland which secretes juvenile hormone) (Bowers et al., 1982). However, we tested the *Hemizonia* chromenes on *Oncopeltus* for antijuvenile hormone activity but did not observe any precocious metamorphosis. In addition, Cupp et al. (1977) reported preimaginal developmental effects of one of the precocenes (ageratochromene) on *Aedes aegypti,* including inhibited pupation and adult emergence. We did not, however, observe any developmental effects by the *Hemizonia* chromenes on *Culex* larvae. Therefore, the

presence of a vinyl or a methylketone moiety, such as found in the *Hemizonia* chromenes, rather than a methoxy substituent, such as found in the precocenes, results in a loss of antijuvenile hormone activity. This conclusion is similar to that of Rodriguez (1983) and coworkers (Proksch et al., 1983), who found moderate insecticidal activity, but no antijuvenile hormone activity with encecalin or eupatoriochromene isolated from *Encelia* species. In fact, Bowers (1982a,b) found that alkoxy substitution of the chromene aromatic ring in the 6th and especially the 7th positions was necessary for antijuvenile hormone activity.

We found the *Hemizonia* chromenes [especially 6-vinyl-7-methoxy-2,2-dimethylchromene (IV)] to be moderately toxic to the *Culex* mosquito larvae, although no antijuvenile hormone activity was observed. These chromenes are therefore probably at least partially responsible for the observed suppressant effect of *H. fitchii* on the mosquito populations in the California ponds. The role of these compounds as defense chemicals in host-plant resistance thus seems apparent. However, in light of the organosoluble nature and expected low water-solubility of the chromenes, other compounds from *Hemizonia* may also be found to contribute to the suppressant effects on mosquito populations. For example, although we found some of the volatile constituents of *H. fitchii,* including 1,8-cineole, and isovaleric, isobutyric, and 2-methylbutyric acids, to be inactive as mosquito larvicides, they may possibly act as repellents to the *Culex* adults. Although we have not yet tested for this possibility, certain unsaturated fatty acids have been reported as ovipositional repellents against *Culex quinquefasciatus* (Hwang et al., 1983). In addition, 1,8-cineole has been reported to repel American cockroach adults (Verma and Meloan, 1981; Maugh, 1982; Scriven and Meloan, 1984), and other compounds isolated from *Hemizonia,* such as acetylenes (Bohlmann et al., 1973) have been isolated from other sources with effects on insects (Jermy et al., 1980). Furthermore, 1,8-cineole may also play an ecologically significant role as an allelopathic substance, since it is known to be a very effective phytotoxin (Muller and Chou, 1972).

Although assays with other insects should be conducted, it does not seem from an economic standpoint that the *Hemizonia* chromenes themselves are of sufficient potency to warrant adaptation into pest management strategies. However, the relative ease of extraction of the *Hemizonia* chromenes, coupled with the availability of *Hemizonia* plant material, make these compounds useful as models for new semisynthetic insecticides. That slight structural differences greatly affect the activity of the chromenes is evident both by comparison of the activities of the precocenes with the *Hemizonia* chromenes, and by comparison of the activities of the *Hemizonia* chromenes among themselves. For example, eupatoriochromene (desmethylencecalin) (III) is structurally very similar to encecalin (II) but is less polar (as shown by its higher R_f value) and much less active against insects than II. The nonpolar character of III is due to the internally hydrogen-bonded hydroxy group (Figure 1, VI), which may also be less

likely to give rise to the reactive epoxide intermediate which is the cytotoxic agent responsible for antijuvenile hormone activity (Brooks et al., 1979; Jennings and Ottridge, 1979; Pratt et al., 1980; Bowers et al., 1982). A similar argument has been proposed to explain the weaker feeding deterrent activity of encecalin (II) as compared to the precocenes (Wisdom et al., 1983). It has also been proposed that chromenes exhibiting free hydroxy groups (such as III) could be more rapidly detoxified (presumably by conjugation and elimination) than compounds bearing methoxy groups (such as the precocenes and II) (Proksch et al., 1983). Thus, semisyntheses utilizing the *Hemizonia* chromenes as starting materials might take advantage of slight structural modifications to enhance insecticidal activity to an economically feasible level.

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