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NOVEL ANTIFEEDANT AND INSECTICIDAL COMPOUNDS FROM AVOCADO IDIOBLAST CELL OIL

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Abstract—Several insecticidal compounds have been identified by bioassay-driven fractionation of avocado, *Persea americana* Mill, idioblast cell oil. A flash chromatography fraction of the oil showed substantial toxicity to early instars of the generalist insect herbivore, *Spodoptera exigua* (Hübner) (100% mortality after seven days). Following further fractionation, five biologically active compounds, 2-(pentadecyl)furan, 2-(heptadecyl)furan, 2-(1*E*-pentadecenyl)furan, 2-(8*Z*,11*Z*-heptadecadienyl)furan, and the triglyceride triolein, were identified. Several minor components were also tentatively identified, including 2-(1*Z*-pentadecenyl)furan, 2-(1*E*-heptadecenyl)furan, and 2-(1*E*,8*Z*,11*Z*-heptadecatrienyl)furan. Several 2-alkylfurans of this type have been reported previously from avocado (*Persea* spp.) and have received the common name of avocadofurans. The major compounds were tested individually for toxic and growth inhibitory effects. Individually, the compounds had low to moderate toxicity. Of these, 2-(pentadecyl)furan had the greatest effects, with an LC_{50} value of 1031 μg/g. At concentrations of 600 μg/g or higher in diets, larval growth was inhibited by >70% compared to controls. The analogous 2-(heptadecyl)furan had an LC_{50} value of 1206 μg/g, and also significantly reduced larval growth (>75% versus controls) at concentrations of >600 μg/g. The unsaturated analogs 2-(1*E*-pentadecenyl)furan and 2-(8*Z*,11*Z*-heptadecadienyl)furan were less toxic. Triolein was only weakly toxic, with an LC_{50} value of 10,364 μg/g diet. Larval growth was inhibited only at concentrations of 7000 μg/g or higher. The potential of avocadofurans in insect control is discussed.

Key Words—*Spodoptera exigua*, *Persea americana*, avocado, idioblast cells, avocadofuran, 2-(pentadecyl)furan, 2-(heptadecyl)furan, triolein.

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INTRODUCTION

Avocados, *Persea americana* Mill (Lauraceae), are oleaginous fruit in which oil levels in the mesocarp (or flesh) vary from 1–2% (fresh weight) early in the season to over 30% late in the season (Biale and Young, 1971). The oils are valuable nutritionally as a source of energy, vitamins, and unsaturated lipids, with the edible portion of the fruit being rich in oleic, palmitic, linoleic, and palmitoleic acids (Biale and Young, 1971). Avocado oils also are used extensively in cosmetics (Anonymous, 1980).

Compounds isolated from avocado fruit also have been reported to have various types of biological activity. For example, 1-acetoxy-2-hydroxy-4-oxo-heneicosa-(12*Z*,15*Z*)-diene (persin) extacted from fresh avocado leaves induced vomiting and inhibited growth of fourth-instar *Bombyx mori* L. (Chang et al., 1975; Murakoshi et al., 1976) at a concentration of 200 μg/g within two days. Prusky et al. (1982) subsequently isolated persin from peels of unripe avocado fruit and characterized its antifungal activity against *Colletotrichum gloeosporioides* Penz. In addition, Prusky et al. (1991) found that a related compound, 1-acetoxy-2,4-dihydroxy-*n*-heptadec-16-ene, isolated from the peel and flesh of unripe avocado fruit, also was active as a fungicide. A further study identified a series of related compounds with antifungal activity, including 1,2,4-trihydroxyheptadec-16-yne, 1,2,4-trihydroxy-*n*-heptadec-16-ene, and 1-acetoxy-2,4-dihydroxyheptadec-16-yne (Adikaram et al., 1992).

All aforementioned compounds were isolated from crude homogenates of plant tissues rather than from specific cells or structures. In contrast, our work has focused on chemicals present in a specific cell type. Idioblast cells are specialized cells that markedly differ from other constituents of the same tissue in form, structure, and contents (Esau, 1967). Avocado leaves, seeds, roots (Armstrong, 1964), and fruit (Platt-Aloia et al., 1983; Platt-Aloia and Thomson, 1992) contain specialized idioblast oil cells scattered throughout the avocado mesocarp, composing approximately 2% of the tissue volume (Cummings and Schroeder, 1942). Moreover, the cells have been reported to contain an oil that differs from other lipids found in the fruit mesocarp (Platt and Thomson, 1992).

In recent studies, Kobiler et al. (1993) demonstrated the antifungal activity of two compounds present in these idioblast oil cells to the fungus, *C. gloeosporioides*. Rodriguez-Saona and Trumble (1996) found that the crude oil extracted from idioblast cells was not only toxic to early (LC_{50} = 1600 μg/g) and late instars (LC_{50} = 5100 μg/g) of the herbivore, *Spodoptera exigua* (Hübner), but also deterred feeding. Rodriguez-Saona et al. (1997) subsequently isolated and identified persin from idioblast cell oil by bioassay-driven fractionation and demonstrated its detrimental effects to *S. exigua* larvae. Persin inhibited larval growth when mixed with artificial diet at concentrations of 200 μg/

g. At concentrations of 400 μg/g or above, persin significantly deterred feeding and reduced larval weight by more than 70%.

A second, less polar fraction from the initial flash chromatography fractionation of the idioblast cell oil was also toxic to *S. exigua* larvae. The objectives of the present study were: (1) to identify the biologically active compound(s) in this fraction, (2) to synthesize the active compound(s), and (3) to test the synthetic compound(s) for mortality and growth effects on *S. exigua*.

METHODS AND MATERIALS

Extraction of Idioblast Cell Oil

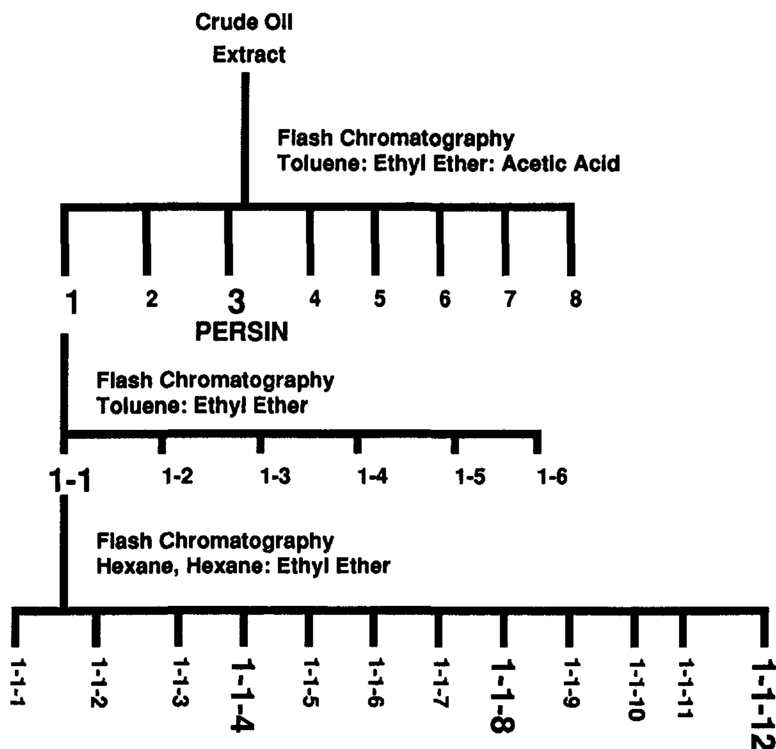
Hass avocado fruit were collected from trees at the South Coast Research and Extension Center, University of California, Santa Ana, California. Idioblast cells were separated from ripe fruit and the oil was extracted as previously described (Rodriguez-Saona and Trumble, 1996).

Insects. S. exigua larvae were used in all experiments. Larvae were reared on artificial diet modified from Patana (1969), and maintained at 28 $\pm$ 2°C and 14L:10D photoperiod. The colony was originally collected from Orange County, California, and had new material added within 12 month prior to the study. The age of the cohorts tested was standardized by using neonates within 12 hr of eclosion. All bioassays were maintained at 28 $\pm$ 2°C, 75% relative humidity, and 14L:10D photoperiod with fluorescent lighting.

Isolation and Identification of Active Compounds

Flash chromatography was carried out with 230–400 mesh silica gel (Aldrich, Milwaukee, Wisconsin). Electron impact (70 eV) mass spectra of volatile compounds were taken on a Hewlett-Packard 5890 GC interfaced to a 5970 mass selective detector (carrier gas, helium; head pressure, 105 kPa). A DB5-MS column was used (30 m $\times$ 0.2 mm ID, J&W Scientific, Folsom, California). Chemical ionization (methane) mass spectra were obtained with a 5890 GC (DB5 column, 30 m $\times$ 0.25 mm ID) interfaced to an HP 5989A mass spectrometer. Fast atom bombardment (FAB) spectra were recorded with a VG ZAB-2fHf instrument (VG Instruments, Danvers, Massachusetts), and high resolution exact mass spectra were taken on a VG 7070E double-focusing magnetic sector instrument. ^{1}H NMR spectra were recorded with a QE-300 instrument (General Electric, Fremont, California) in $CDCl_3$.

The crude idioblast cell oil was fractionated as shown in Scheme 1. The initial steps of the fractionation have been previously described (Rodriguez-Saona et al., 1997). The lowest polarity fraction from the initial flash chromat-



Scheme 1.

ographic separation (fraction 1, 3.67 g total weight) was further purified by flash chromatography (5 cm ID × 25 cm column). The column was eluted sequentially with 2 liters each of toluene–ethyl ether 95:5 and 90:10 (v/v). Material remaining on the column was stripped off with ethanol (2 liters). Fractions were checked by thin layer chromatography (TLC) on silica plates developed with toluene–ethyl ether (90:10, v/v). Spots on developed plates were visualized under UV light (254 nm), followed by spraying with H_2SO_4 and charring with a heat gun. Subfractions were combined to yield 6 fractions (1–1 to 1–6), which were concentrated under reduced pressure and then pumped under vacuum (~0.5 mm Hg) to remove traces of solvent. The concentrated fractions were weighed, diluted with acetone to a final volume of 10 ml, and refrigerated at 4°C until bioassayed.

A portion (~75%) of the most active fraction (frac. 1–1, 1.3 g total weight) was fractionated further by flash chromatography (5 cm ID × 25 cm). The

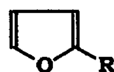
elution solvents used sequentially, were 2 liters of hexane, 1 liter of hexane–ethyl ether (95:5, v/v), 0.4 liter of hexane–ethyl ether (90:10), and 0.4 liter of ethyl ether. Twelve fractions were collected, concentrated, and tested for activity. Subfractions 1-1-4 (0.08 g), 1-1-8 (0.09 g), and 1-1-12 (0.89 g) contained most of the mass of material (6, 7, and 68%, respectively) and were most active in bioassays. Structures of identified compounds are shown in Figure 1.

Fraction 1-1-4

Compound 1 (39.2%). MS (70 eV): 278 (17), 249 (2), 235 (5), 221 (2), 207 (2), 193 (2), 179 (3), 165 (3), 151 (10), 137 (10), 123 (20), 95 (57), 82 (44), 81 (100). CI-MS (methane): 277 (100, M-1), 279 (98, M + 1), 307 (25; M + 29).

Compound 2 (49.4%). MS (70 eV): 276 (25), 247 (1), 233 (1), 219 (2), 191 (1), 177 (2), 163 (3), 149 (8), 135 (12), 121 (9), 107 (61), 94 (100), 81 (19), 79 (24), 77 (19). CI-MS (methane): 275 (61, M-1), 277 (100, M + 1), 305 (25, M+ 29).

Compound 3 (2.8%). MS (70 eV): 276 (35), 247 (2), 233 (2), 219 (3), 191 (3), 177 (2), 163 (4), 149 (9), 135 (15), 121 (11), 107 (60), 94 (100), 81



1: R = $C_{15}H_{31}$

2: R = $C_{13}H_{27}$

3: R = $C_{13}H_{27}$

4: R = $C_{17}H_{35}$

5: R = $C_{15}H_{31}$

6: R = $(CH_2)_7CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3$

7: R = $CH{=}CH(CH_2)_5CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3$

8: R' = $C(O)(CH_2)_7CH{=}CH(CH_2)_7CH_3$

FIG 1. Structures of compounds identified from avocado idioblast cell oil fractions.

(17), 79 (21), 77 (20). CI-MS (methane): 275 (62, M-1), 277 (100, M + 1), 305 (24; M + 29).

Compound 4 (2.6%). MS (70 Ev): 306 (28), 263 (5), 249 (4), 207 (6), 179 (3), 165 (3), 151 (13), 137 (17), 123 (35), 95 (63), 82 (54), 81 (100). CI-MS (methane): 305 (100, M-1), 307 (82, M + 1), 335 (18, M + 29).

Compound 5 (6.0%). MS (70 eV): 304 (31), 247 (2), 219 (1), 193 (1), 177 (3), 163 (4), 149 (11), 135 (16), 121 (9), 107 (48), 95 (20), 94 (100), 81 (20), 79 (20). CI-MS (methane): 303 (65, M-1), 305 (100, M + 1), 333 (19).

^{1}H NMR of Fraction 1-1-4 ($CDCl_3$). δ 0.88 (ragged t, $J \sim 6.9$ Hz), 1.2–1.4 (m), 1.4–1.8 (m), 2.17 (m), 2.61 (t, $J = 7.5$ Hz), 5.96 (d, $J = 1.4$ Hz), 6.13 (d, $J = 1.6$ Hz), 6.18 (t, $J = 1.8$ Hz), 6.27 (distorted dd), 6.33 (distorted dd), 7.29 (m).

Fraction 1-1-8.

Compound 6 (83%). MS (70 eV): 302 (14), 273 (5), 259 (5), 245 (8), 231 (7), 217 (5), 203 (3), 189 (3), 175 (3), 161 (4), 149 (6), 135 (9), 121 (13), 107 (12), 95 (34), 94 (36), 81 (100), 67 (47), 55 (30), 41 (48). HR-MS: Calcd for $C_{21}H_{34}O$: 302.2610; found: 302.2599. ^{1}H NMR: δ 0.89 (3H, t, $J = 7.7$ Hz, CH_3), 1.25–1.5 (14H, m, 7 methylenes), 1.63 (2H, m, $H_{2'}$), 2.05 (4H, m, $H_{7'}$, $H_{13'}$), 2.61 (2H, t, $J = 7.6$ Hz, $H_{1'}$), 2.78 (2H, br. t, $J = 6$ Hz, $H_{10'}$), 5.37 (4H, m, $H_{8',9',11',12'}$), 5.97 (1H, d, $J = 1.3$ Hz, H_3), 6.28 (1H, m, H_4), 7.29 (1H, distorted d, H_5).

Compound 7 (17%). MS (70 eV): 300 (32), 271 (3), 257 (3), 243 (7), 229 (20), 215 (6), 204 (5), 201 (5), 191 (9), 175 (11), 161 (9), 147 (15), 133 (21), 121 (27), 120 (31), 107 (73), 94 (100), 81 (71), 79 (86), 77 (63), 67 (52), 55 (43), 41 (65). HRMS: Calcd for $C_{21}H_{32}O$: 300.2453; found: 300.2451. Diagnostic ^{1}H NMR peaks (from the spectrum of the mixed major and minor compounds): δ 2.18 (2H, br. quart), 6.12 (1H, m), 6.18 (1H, m), 6.34 (1H, br d). Other NMR signals for this compound were obscured under the signals from the major component of this fraction.

Fraction 1-1-12

Reduction with $LiAlH_4$. Two milligrams of the fraction were stirred with 10 mg of $LiAlH_4$ in ether (1 ml) at room temperature for 2 hr. The mixture was cautiously quenched with 1 M aq. HCl and extracted with ether. The dried (Na_2SO_4) extract was analyzed by GC-MS (DB5-MS column, 20 m × 0.2 mm ID, temperature program 50°C for 1 min then 10°/min to 250°C). The retention times and mass spectra of the sample components were compared with those of authentic samples of hexadecyl (palmityl) and *Z*9-octadecenyl (oleyl) alcohols. The mass spectrum and retention time of the third component [tentatively identified as *Z*9-hexadecenyl (palmitoleyl) alcohol] were compared with those of an

authentic standard of Z11-hexadecenyl alcohol, providing close but not exact matches (retention times different by 0.05 min).

Base Hydrolysis. Two milligrams of the fraction were dissolved in 0.5 ml EtOH, and 1 drop of 20% aq. NaOH was added. The mixture was stirred at room temperature 2 hr, then acidified with 1 M HCl and extracted with ether. The dried (Na_2SO_4) extract was analyzed by GC (DB-5, 30 m × 0.32 mm ID, temperature program 50°C for 1 min, 15°/min to 275°C), and the retention times of the sample components were compared with those of authentic standards of oleic, palmitic, and palmitoleic acids.

^{1}H NMR ($CDCl_3$): δ 0.88 (9H, distorted t), 1.2–1.4 (m), 1.61 (~6H, m), 2.02 (12H, m), 2.32 (6H, distorted t, J ~ 7.4 Hz), 4.15 (2H, dd, J = 11.9, 6 Hz), 4.30 (2H, dd, J = 11.9, 4 Hz), 5.27 (1H, m), 5.33 (6H, m). FAB-MS (nitrobenzyl alcohol matrix): highest mass peak cluster centered at m/z 603 ($M-C_{18}H_{35}O_2$).

Bioassays

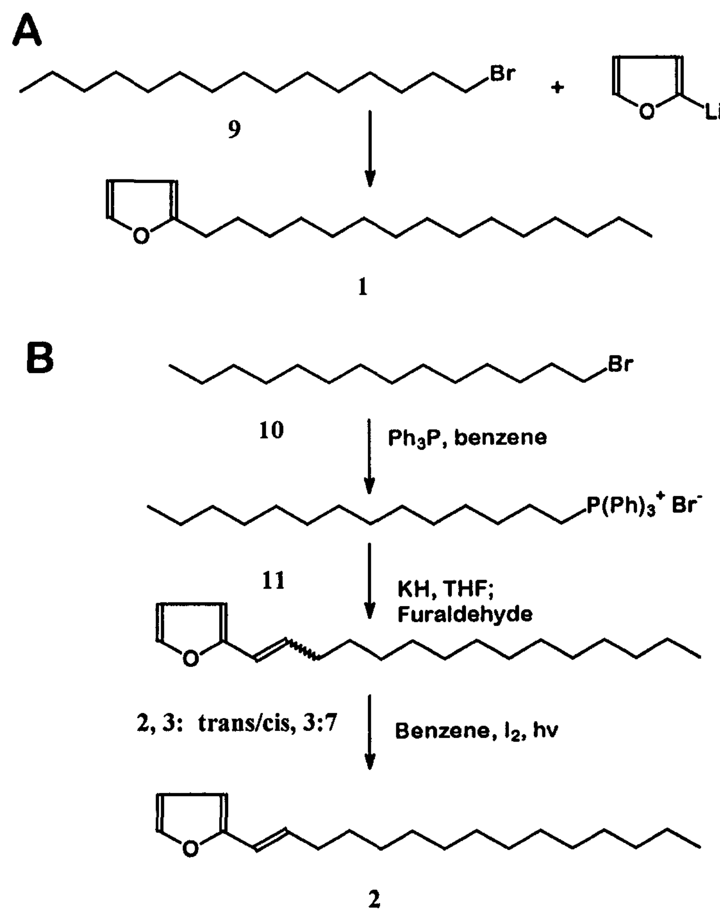
The insecticidal activity of the fractions was tested with artificial diet bioassays. Treated diets were prepared by transferring 750 μl of acetone solutions of each fraction (equivalent to 300 mg crude idioblast cell oil) into 50-ml polypropylene centrifuge tubes (Fisher, Pittsburgh, Pennsylvania), evaporating the acetone, adding 2 ml of 0.1% Tween 80 solution (Fisher), homogenizing with an ultrasonic homogenizer (Cole-Parmer, Chicago, Illinois), and adding artificial diet to produce a final weight of 15 g containing the equivalent of 2% idioblast oil. The mixture was vortexed for 3 min. Control diet was prepared by mixing 2 ml of Tween solution and 13 g of artifical diet to produce a final weight of 15 g. Control and treated diets were poured into 16-well (15.9-mm-diameter and 15.9-mm-deep) bioassay trays (C-D International Inc., Pitman, New Jersey). One neonate was added per well, and trays were placed in an incubator under the previously described conditions. Twenty-four neonates were tested for each fraction and control. Mortality and larval weights were recorded after seven days.

Synthesis of Furan Compounds

Reactions were carried out under a N_2 atmosphere unless otherwise stated. Flash chromatography was carried out with 230–400 mesh silica gel. THF was dried by distillation from sodium-benzophenone ketyl. ^{1}H NMR spectra were obtained on a 270-MHz JEOL NMR spectrometer, in $CDCl_3$ or C_6D_6. ^{13}C NMR spectra were obtained at 67.9 MHz in $CDCl_3$. Infrared spectra were obtained on a Mattson Galaxy 2000 FT-IR with NaCl plates or in carbon tetrachloride solution. Mass spectra (HR-MS and LR-MS) were obtained from the University of California Riverside, Mass Spectroscopy Laboratory.

2-(Pentadecyl)furan (1) (Scheme 2A)

A dry 50-ml round-bottomed flask charged with furan (1.40 g, 20.6 mmol) and THF (10 ml) was cooled to −78°C and *n*-butyllithium (13.6 ml, 1.50 M in hexanes, 20.6 mmol) was added dropwise. The solution was stirred for 30 min at −78°C, then warmed to 0°C and placed in an ice bath for 1 hr. The mixture was then recooled to −78°C and 1-bromopentadecane **9** (5 g, 17.2 mmol) in THF (10 ml) was added dropwise. The resulting solution was stirred for 1 hr, warmed to room temperature, and stirred for 12 hr. The reaction was



Scheme 2.

quenched with a saturated solution of NH_4Cl (5 ml). The organic layer was separated and the aqueous layer extracted with ether (3 × 20 ml). The combined organic layers were washed with saturated $NaHCO_3$ (1 × 20 ml), brine (1 × 20 ml), dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude product was purified by flash chromatography on silica, eluting with hexanes to afford an oily liquid that was recrystallized from methanol (~50 ml/g, cooling to 5°C) to afford 4.8 g (64%) of 2-(pentadecyl)furan (**1**). ^1H NMR: ($CDCl_3$) δ 0.85 (3H, distorted t, J = 7.2 Hz, CH_3), 1.1–1.4 (24H, broad m, 12 methylenes), 1.57 (2H, quintet, J = 7.2 Hz, $H_{2'}$), 2.58 (2H, t, J = 7.2 Hz, $H_{1'}$), 5.99 (1H, dd, J = 1, 3.0 Hz, H_3), 6.29 (1H, dd, J = 1.8, 3.0 Hz, H_4), 7.29 (1H, dd, J = 1, 1.8 Hz, H_5).

2-(Heptadecyl)furan (4)

1-Heptadecanol (10 g, 39.1 mmol) was placed in a dry 25-ml round-bottomed flask attached to a reflux condenser and heated to 60°C under a nitrogen atmosphere. Phosphorous tribromide (10 g, 39.1 mmol) was added dropwise and the resulting solution was stirred for 48 hr. The reaction mixture was then cooled in an ice bath and quenched with a saturated solution of $NaHCO_3$ (10 ml). The aqueous layer was extracted with ether (4 × 10 ml). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude residue was flash chromatographed (hexanes) to afford 7.2 g (57%) of 1-bromoheptadecane as a colorless oil. ^1H NMR ($CDCl_3$): δ 0.85 (3H, t, J = 6.5 Hz), 1.1–1.4 (28H, broad s), 1.86 (2H, quintet, J = 6.8 Hz), 3.41 (2H, t, J = 6.8 Hz). 2-(Heptadecyl)furan **4** was prepared exactly as described above for 2-(pentadecyl)furan, by substituting 1-bromoheptadecane for 1-bromopentadecane. The chromatographed product was recrystallized from methanol as described above to afford 2.5g of 2-(heptadecyl)furan **4** in 48% yield. ^1H NMR ($CDCl_3$): δ 0.86 (3H, distorted t, J = 7.2 Hz, CH_3), 1.1–1.4 (28H, broad m, 14 methylenes), 1.60 (2H, quintet, J = 7.2 Hz, $H_{2'}$), 2.58 (2H, t, J = 7.2 Hz, $H_{1'}$), 5.95 (1H, dd, J = 1.0, 2.7 Hz, H_3), 6.26 (1H, J = 1.8, 2.7 Hz, H_4), 7.30 (1H, dd, J = 1.0, 1.8 Hz, H_5).

2-(1Z-pentadecyl)furan (3) (Scheme 2B)

A mixture of 1-bromotetradecane **10** (10.0 g, 36.1 mmol), triphenylphosphine (10.5 g, 36.1 mmol), and benzene (25 ml) was refluxed for 48 hr, and the mixture was concentrated under reduced pressure to a viscous oil. Addition of ether afforded a white solid, which was dried under vacuum for 24 hr at 65°C, yielding 18.9 g (92%) of tetradecyltriphenylphosphonium bromide **11**. It was used without further purification.

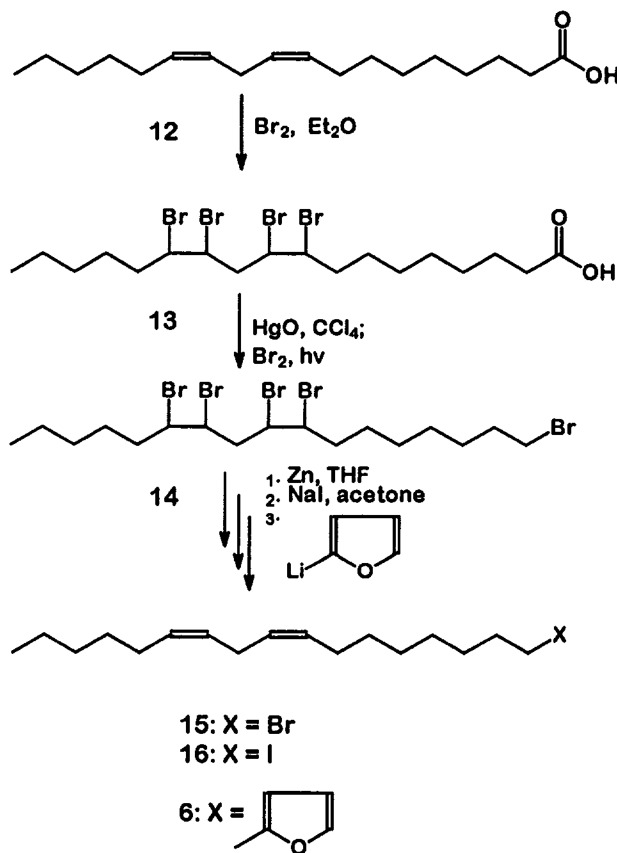
Potassium hydride (2.33 g, 20.2 mmol, 35% oil dispersion) was washed with hexanes (3 × 5 ml) and pumped under vacuum. After 5 min, the flask was filled with N_2, dry THF (50 ml) was added, and the reaction flask cooled in an ice bath. The phosphonium salt **11** (10 g, 18.5 mmol) was added in small portions over a 20-min period. After stirring for 15 min, the resulting yellow solution was warmed to room temperature, stirred for 30 min, and then cooled to −78°C. Freshly distilled furaldehyde (2.1 g, 22.2 mmol) in THF (25 ml) was added dropwise over 15 min. The resulting solution was stirred for 1 hr, warmed to room temperature, and stirred 18 hr. The reaction was quenched with saturated aq. NH_4Cl (50 ml). The organic layer was separated and the aqueous layer was extracted with ether (3 × 20 ml). The combined organic layers were washed with saturated $NaHCO_3$ (1 × 25 ml) and brine (1 × 25 ml), dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude product was purified by flash chromatography (hexanes), to afford 4.2 g (81%) of a mixture of furans **3** and **2** (7:3 *cis/trans* ratio). ^1H NMR of major product: ($CDCl_3$): δ 0.92 (3H, distorted t, J = 7.0 Hz, CH_3), 1.2–1.6 (22H, m, 11 methylenes), 2.45 (2H, apparent dq, J = 1.8 Hz, 7.1 Hz, $H_{3'}$), 5.58 (1H, dt, J = 11.6, 7.1 Hz, $H_{2'}$), 6.18 (1H, dt, J = 7.1, 1.8 Hz, $H_{1'}$), 6.24 (1H, d, J = 3.4 Hz, H_3), 6.40 (1H, dd, J = 3.2, 2.0 Hz, H_4), 7.38 (1H, d, J = 1.7 Hz, H_5).

2-(1E-pentadecenyl)furan(2) (Scheme 2B)

The diastereomeric mixture of 2-(1-pentadecenyl)furans **2** and **3** (1.0 g, 3.4 mmol) was placed in a stirred 2% iodine/benzene solution (10 ml) in an open beaker and irradiated with a fluorescent light. The *cis* to *trans* isomerization was monitored by NMR [scanned unlocked, observing the disappearance of the resonance at δ = 5.58 (1H, dt, J = 11.9 Hz, 6.9 Hz, *cis* isomer) and appearance of the resonance at δ = 6.3 (1H, dt, J = 16.1 Hz, 6.9 Hz, *trans* isomer)]. After ~30 min of irradiation, the reaction mixture was washed thoroughly with saturated sodium thiosulfate. The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated. The crude product was passed through a silica gel plug and eluted with hexanes. The eluate was concentrated, and the oily residue was recrystallized from methanol as described above, filtering cold to afford 0.53 g (57%) of **2** as low-melting white crystals. ^1H NMR (C_5D_5): δ 0.90 (3H, distorted t, J = 7.0 Hz, CH_3), 1.2–1.5 (22H, m, 11 methylenes), 2.05 (2H, apparent q, J = 6.9 Hz, $H_{2'}$), 5.95 (1H, d, J = 3.2 Hz, H_3), 6.16 (1H, m, H_4), 6.20 (1H, d, J = 16.1 Hz, $H_{1'}$), 6.30 (1H, dt, J = 16.1 Hz, 6.9 Hz, $H_{2'}$), 7.06 (1H, s, H_5).

*2-(8Z,11Z-Heptadecadienyl)furan **6** (Scheme 3)*

9,10,12,13-Tetrabromooctadecanoic Acid (13). Bromine (10.6 g, 66.6 mmol) was added dropwise to a rapidly stirred solution of linoleic acid **12** (8.9



Scheme 3.

g, 31.7 mmol) in diethyl ether (264 ml) at 0°C. After stirring for 30 min, the solution was warmed to room temperature and quenched by addition of saturated aq. sodium thiosulfate (20 ml). After stirring for 10 min, the organic layer was separated and the aqueous layer extracted with ether (3 × 20 ml). The combined organic layers were washed with saturated aq. NH_4Cl (2 × 10 ml) and brine (1 × 10 ml), dried over $MgSO_4$, filtered, and concentrated. The crude product was recrystallized from pentane–ether (1 : 1) to afford 16.6 g (85%) of the tetrabromo acid **13** as white crystals. ^1H NMR ($CDCl_3$): δ 0.89 (3H, distorted t, J = 6.3 Hz), 1.1–2.1 (18H, m), 1.60 (2H, m), 1.83 (2H, m), 2.01 (2H, m), 2.34 (2H, t, J = 7 Hz), 2.48 (2H, m).

1,8,9,11,12-Pentabromoheptadecane (14). A 500 ml roundbottom flask, equipped with a reflux condenser and addition funnel, was charged with tetrabromo acid **13** (16.4 g, 27.3 mmol), carbon tetrachloride (135 ml), and mercuric

oxide (8.87 g, 41.0 mmol) and brought to a gentle reflux. Bromine (3.1 g, 41 mmol) was added dropwise over 30 min. After refluxing for 2 hr, the reaction was cooled to room temperature and quenched with a 10% solution of sodium thiosulfate (50 ml). The organic layer was removed and the aqueous layer was extracted with carbon tetrachloride (2 × 20 ml). The combined organic layers were washed with saturated aq. NH_4Cl (2 × 20 ml) and brine (1 × 20 ml), dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by flash chromatography (hexanes) and recrystallized (pentane) at 5°C to afford 10.9 g (63%) of pentabromide **14** as white crystals (mp, 62–64°C). ^1H NMR ($CDCl_3$): δ 0.88 (3H, distorted t, J = 6.5 Hz), 1.1–2.1 (18H, m), 2.50 (2H, m), 3.34 (2H, t, J = 7.3 Hz), 4.0–4.2 (2H, m), 4.32 (2H, m), 4.54 (2H, m).

(8Z,11Z)-1-Bromoheptadecadiene (15). Pentabromide **14** (5.35 g, 8.42 mmol) and zinc powder (1.2 g, 18.5 mmol) in THF (55 ml) were refluxed for 4 hr. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure. The oily residue was extracted into ether–pentane (1:1), the solution was concentrated, and the crude product was purified by flash chromatography (pentanes) to afford 2.6 g (>95%) of the (Z,Z)-diene **15** as a colorless oil. ^1H NMR ($CDCl_3$): δ 0.87 (3H, distorted t, J = 6.9 Hz), 1.2–1.4 (14H, m), 1.82 (2H, quintet, J = 6.9 Hz), 2.03 (4H, apparent q, J = 6.8 Hz), 2.75 (2H, t, J = 5.9 Hz), 3.38 (2H, t, J = 7 Hz), 5.2–5.5 (4H, m).

(8Z,11Z)-1-Iodoheptadecadiene (16). (Z,Z)-Bromodiene **15** (2.67 g, 8.45 mmol) and sodium iodide (3.80 g, 25.3 mmol) in acetone (133 ml) were refluxed 4 hr. The reaction mixture was then cooled to room temp, filtered, concentrated, and extracted with ether (3 × 20 ml). The ether extracts were filtered concentrated, and the residue purified by flash chromatography (pentane) to afford 2.25 g (74%) of the (Z,Z)-iododiene **16**. ^1H NMR ($CDCl_3$): δ 0.87 (3H, distorted t, J = 6.9 Hz), 1.2–1.4 (14H, m), 1.79 (2H, quintet, J = 6.9 Hz), 2.03 (4H, apparent q, J = 6.9 Hz), 2.76 (2H, t, J = 5.9 Hz), 3.17 (2H, t, J = 7 Hz), 5.2–5.5 (4H, m).

2-(8Z,11Z-Heptadecadienyl)furan (6). Furan (1.1 g, 16.1 mmol) in THF (5 ml) was cooled to −78°C and *n*-butyllithium (5.4 ml, 1.5 M in hexanes, 8.1 mmol) was added dropwise. The resulting solution was stirred 30 min at −78°C, warmed to 0°C for 1 hr, and then recooled to −78°C. (Z,Z)-Iododiene **16** (2.94 g, 8.1 mmol) in THF (5 ml) was added via cannula, and the mixture was stirred 2 hr. The reaction was warmed to room temperature and stirred an additional 2 hr, and quenched with saturated aq. NH_4Cl (10 ml). The aqueous layer was separated and extracted with ether (3 × 10 ml). The combined organic layers were washed with saturated $NaHCO_3$ (2 × 10 ml) and brine (1 × 10 ml), dried over $MgSO_4$, filtered, and concentrated. The crude residue was passed through a silica gel plug, eluted with hexane, and then purified by HPLC (Rainin Dynamax column, 2.24 × 25 cm, Rainin Instruments, Emeryville, California) to afford 1.0 g (41%) of furanyl diene **6** as a colorless oil. ^1H NMR ($CDCl_3$):

δ 0.87 (3H, distorted t, J = 7.0 Hz), 1.2–1.4 (14H, m), 1.66 (2H, quintet, J = 6.6 Hz), 2.08 (4H, apparent q, J = 6.4 Hz), 2.64 (2H, t, J = 7.4 Hz), 2.80 (2H, t, J = 5.6 Hz), 5.2–5.5 (4H, m), 5.99 (1H, m), 6.28 (1H, m), 7.30 (1H, m).

Toxicity Studies

The effects of the synthetically produced avocadofuran compounds (>95% purity) and commercially available triolein (Sigma, St. Louis, Missouri) were tested on larval growth and mortality of *S. exigua*. Treated and control diet were prepared as previously described. Preliminary data (see results) showed that of the two compounds identified from subfraction 1-1-4, 2-(1*E*-pentadecenyl)furan **2** had little effect on survivorship and growth of *S. exigua* as compared to the saturated analog, 2-(pentadecyl)furan **1**. Similarly, 2-(8*Z*,11*Z*-heptadecadienyl)furan **6** was of much lower toxicity than 2-(heptadecyl)furan **4**. Thus, we focused our attention on the two saturated compounds. Six different concentrations (0, 600, 750, 900, 1050, and 1200 μg/g) were chosen on the basis of studies with the oil fractions and evaluated for both 2-(pentadecyl)furan and 2-(heptadecyl)furan. Triolein (subfraction 1-1-12) also was tested at 0 (control), 7000, 8000, 9000, 10,000, and 11,000 μg/g. Twenty-four neonates were used per treatment. Bioassays were conducted as previously described (Rodriguez-Saona et al., 1997) and replicated four times for each concentration (i.e., a total of 96 larvae was tested for each concentration). Larval weight, instar, and mortality were recorded after 7 days.

Data Analysis

Effective concentration (EC) values were calculated by subtracting the mean larval weight for each treatment from the mean weight of the controls, and dividing by the mean weight of the controls. Lethal concentration (LC) values were obtained from probit analysis (Finney, 1971). Growth index (GI) and Relative growth index (RGI) values were calculated as described by Zhang et al. (1993), but with an i_{max} of 3 (the stage attained by most control larvae after seven days). Statistical comparisons were conducted with SuperAnova (1989).

RESULTS

Isolation and Identification of Active Compounds

The least polar fraction from the first two flash chromatographic steps (fractions 1-1, Scheme 1) produced 100% larval mortality (Figure 2). In contrast, larvae fed on diet augmented with fractions 1-3 and 1-4 were significantly larger than control larvae (Figure 2B, $P < 0.05$). Further fractionation (Scheme

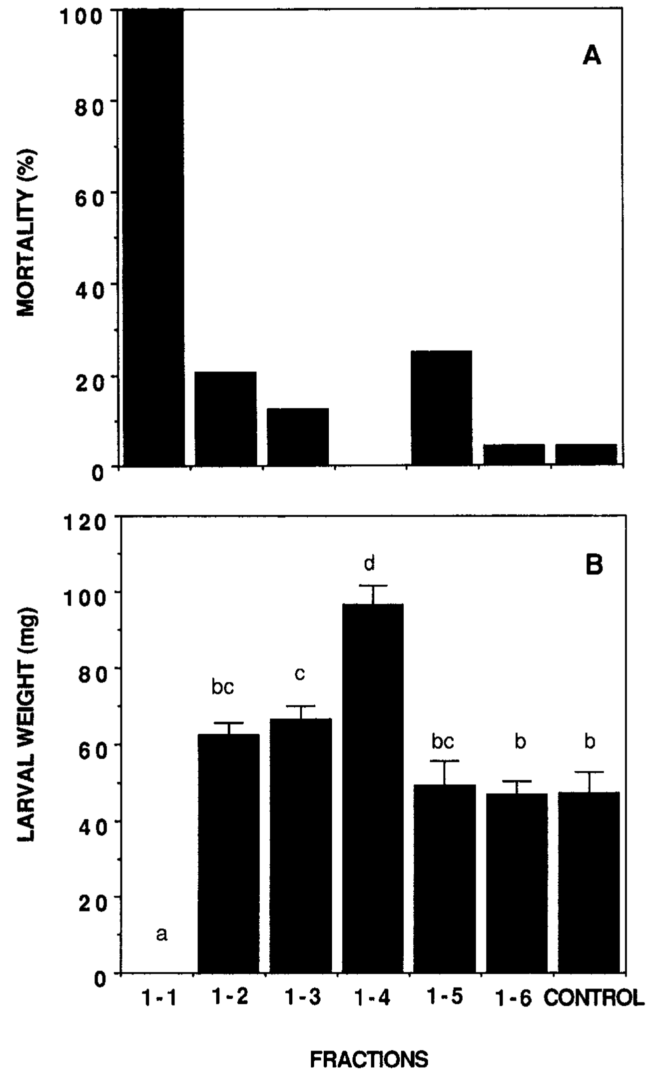


FIG 2. Percent mortality (A) and weight (B) of *S. exigua* larvae fed control diet and diet containing flash chromatography fractions of avocado idioblast cell oil. Different letters indicate statistical differences between treatments (Tukey's pairwise comparisons, $P < 0.05$). Fractions were bioassayed at concentrations equivalent to 20 mg crude oil per ml artificial diet.

1) of fraction 1-1 yielded three active subfractions (1-1-4, 1-1-8, and 1-1-12; Figure 3). These subfractions each significantly reduced larval weight compared to control larvae in feeding bioassays (Figure 3B, $P < 0.05$), although larval mortality was <30% (Figure 3A).

The least polar fraction, 1-1-4, consisted of two major and three minor components. The first major component gave a significant molecular ion (*m/z* 278, 17%), corresponding to a possible molecular formula of $C_{19}H_{34}O$, with three sites of unsaturation. The assignment of the molecular ion was confirmed by methane CI-MS, which gave large ions at *m/z* 277 and 279, and an M + 29 ion at *m/z* 307. The base peak in the EI spectrum (*m/z* 81) and a large fragment at *m/z* 95 (57%) were diagnostic for the monosubstituted 2-alkyl furans known to occur in avocado extracts (e.g., Weyerstahl et al., 1993). Furthermore, the entire series of ions from *m/z* 81 to 249, each separated by 14 mass units, was clearly visible, indicative of a saturated alkyl side chain. The furan ring accounted for all three sites of unsaturation, corroborating that the C-15 alkyl side chain was fully saturated. The ^{1}H NMR spectrum of the fraction showed peaks typical of a furan ring with a single alkyl substituent in position 2 (e.g., Kashman et al., 1969a,b), and no signals from methine protons or doublets from methyl branches, indicating that the alkyl chain was unbranched. Thus, this component was identified as 2-(pentadecyl)furan **1** (Figure 1), previously tentatively identified from avocado on the basis of MS data (Weyerstahl et al., 1993). The identification was confirmed by synthesis.

The second major component of this fraction gave a molecular ion at *m/z* 276, confirmed by methane CI-MS, suggesting it was an unsaturated analog of compound 1. The EI-MS showed a base peak at *m/z* 94 and a large fragment at *m/z* 107 (61%), diagnostic for a 2-substituted furan conjugated with a C=C double bond (Weyerstahl et al., 1993), and a series of ions, each separated by 14 mass units, from *m/z* 107 to 247, indicative of a saturated alkyl chain. The presence of the 1,2-disubstituted olefin conjugated to the furan ring and its geometry (*E*) were confirmed by comparison of multiplets at δ 6.13, 6.18, and 6.35 in the ^{1}H NMR spectrum of the fraction with published spectral data from authentic 2-(1*E*-pentadecenyl)furan (Fraga and Terrero, 1996). Corroboration of the proposed structure, 2-(1*E*-pentadecenyl)furan **2** (Figure 1), was obtained by synthesis.

The first of the minor components (2.8%), which eluted on GC (DB5-MS column) between the two major components, gave a molecular ion at *m/z* 276 in EI-MS and had an EI-MS very similar to that of 2-(1*E*-pentadecenyl)furan. Thus, the structure of this compound was tentatively assigned as the isomeric 2-(1*Z*-pentadecenyl)furan **3** (Figure 1), which was confirmed by synthesis. The *Z* isomer had a diagnostic one-proton multiplet at δ 5.58 (dt, J = 11.9, 6.9 Hz) due to the distal olefinic proton. To our knowledge, this is the first report of this compound.

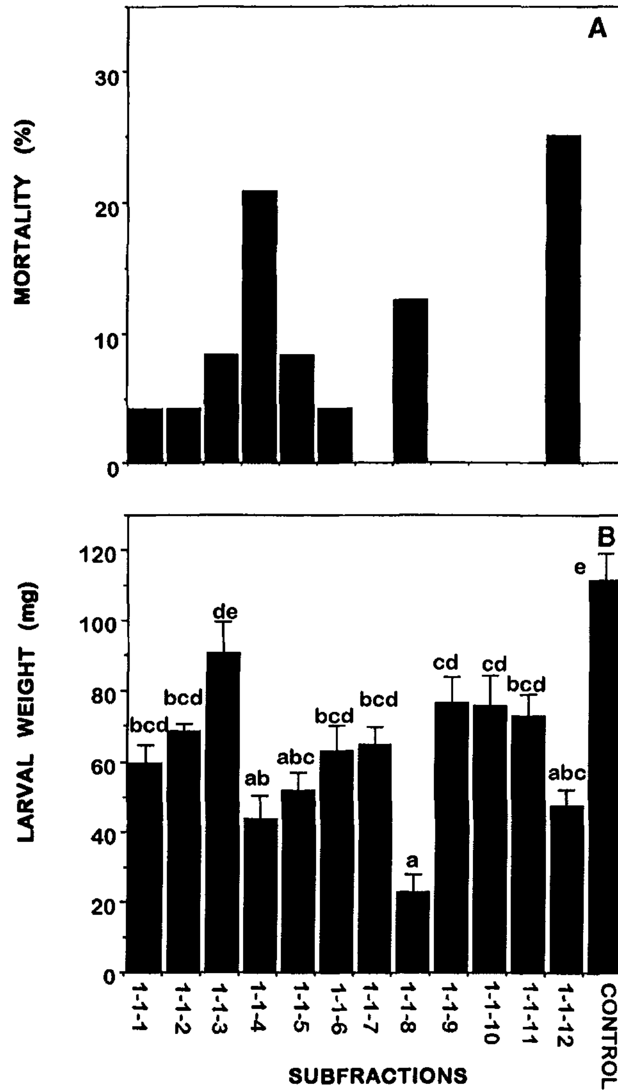


FIG 3. Percent mortality (A) and weight (B) of *S. exigua* larvae fed control diet and diet containing subfractions of fraction 1-1. Different letters indicate statistical differences between treatments (Tukey's pairwise comparisons, $P < 0.05$). Fractions were bioassayed at concentrations equivalent to 20 mg crude oil per milliliter artificial diet.

The second minor component (2.6%) gave an EI molecular ion at *m/z* 306, with a base peak at *m/z* 81, a large fragment at *m/z* 95, and a series of ions separated by 14 mass units (some weak ions in the series were missing), from *m/z* 95 to 263. On the basis of the spectral similarities to compound 1, this compound was assigned the previously unknown structure 2-(heptadecyl)furan **4** (Figure 1), which was confirmed by synthesis.

The final component in the fraction (6%) gave a molecular ion at *m/z* 304, a base peak at *m/z* 94, and a large fragment at *m/z* 107, indicative of a furan conjugated to a 1,2-disubstituted olefin. The mass spectrum was otherwise very similar to that of 2-(1*E*-pentadecenyl)furan. Consequently, the compound was tentatively assigned the structure 2-(1*E*-heptadecenyl)furan **5** (Figure 1).

The second active fraction, 1-1-8, was composed of a major and a minor component (ratio ~5:1). The major component gave a significant molecular ion at *m/z* 302 (14%), a base peak at *m/z* 81, and significant fragments at *m/z* 94 and 95 (36 and 34%) in EI-MS, again indicative of a substituted furan with an alkyl chain at the two position. The high-resolution exact mass measurement provided a molecular formula of $C_{21}H_{34}O$, corresponding to five sites of unsaturation, three of which were accounted for by the furan ring. The ^1H NMR spectrum contained a multiplet corresponding to four very similar olefin protons (δ 5.37), and a broad two-proton triplet at δ 2.78, assignable to a bisallylic methylene group. These data suggested that the two double bonds in the side chain might be in a (Z,Z)-1,4-diene structural unit typical of fatty acids. There were no multiplets indicative of branch points, allowing a tentative assignment of the structure as the known compound 2-(8Z,11Z-heptadecadienyl)furan **6** (Figure 1) (Murakoshi et al., 1976), and this structure was confirmed by synthesis.

The minor component of this fraction gave a molecular ion at *m/z* 300, with a molecular formula of $C_{21}H_{32}O$, suggesting that it was an analog of the major component with one further unsaturation. The base peak at *m/z* 94 and a large fragment at *m/z* 107 (73%) indicated that the additional double bond was conjugated with the furan ring, and this was supported by multiplets in the ^1H NMR of the mixed fraction at δ 6.12, 6.18, and 6.34, typical of a 1,2-disubstituted double bond of *E* configuration conjugated to the furan ring. No other peaks were seen in the olefin region of the ^1H NMR spectrum, other than those attributable to the major component, indicating that the signals due to the protons on the two remaining double bonds of the minor compound were hidden under the multiplet at δ 5.37. There data suggested that this compound had a (Z,Z)-1,4-diene structure also. On the sum of the evidence, the structure of the minor component of this fraction is tentatively proposed as 2-(1*E*,8*Z*,11*Z*-heptadecatrienyl)furan **7** (Figure 1).

The third fraction, 1-1-12, did not elute from the GC under standard conditions (max. oven temperature 300°C). On reverse-phase TLC plates, the com-

pound did not migrate with MeOH solvent, but did move up the plate with mixed MeOH–methylene chloride solvent mixtures, indicating a highly lipophilic compound. The compound was readily base hydrolyzed to produce primarily oleic acid, with smaller amounts of palmitic and palmitoleic acids. Similarly, reduction with $LiAlH_4$ produced oleyl alcohol, with lesser amounts of palmityl and palmitoleyl alcohols. These data were suggestive of a glyceride structure. The highest mass ion cluster seen in the FAB mass spectrum was centered at m/z 603, in the molecular weight range suggestive of a diglyceride. However, on silica gel TLC plates developed with hexane–ethyl acetate (10:1), 1,2-diolein and 1,3-diolein standards had R_f values of 0.10 and 0.14, respectively, versus the 0.51 R_f value of the unknown that corresponded with a triolein standard. Comparison of the ^1H NMR spectrum with an authentic standard confirmed that the major component of the fraction was indeed triolein **8** (Figure 1). In particular, there were two multiplets at δ 4.15 and 4.30, assignable to two pairs of diastereotopic protons on carbons 1 and 3 of the glyceride, with large geminal couplings to each other (J = 11.9 Hz) and smaller, unequal couplings (6 and 4 Hz, respectively) to the single proton (δ 5.27) on carbon 2 of the glyceride. The six olefinic protons appeared as an undifferentiated multiplet at δ 5.33.

The minor components of this fraction were not conclusively identified, but the evidence suggests that they are mixed triglycerides, with either palmitic or palmitoleic acids substituting for one or more of the oleic acid moieties of triolein.

Synthesis of Furan Compounds

Syntheses of the saturated C_{15} and C_{17} 2-(alkyl)furans **1** and **4** were achieved by the coupling of the appropriate bromoalkanes with 2-lithiofuran in THF. Both of the low-melting saturated alkylfurans could be recrystallized in methanol, allowing easy purification on multigram scale.

2-(1*E*-pentadecenyl)furan **2** and its *Z* isomer **3** were prepared by Wittig reaction of the ylide prepared from tetradecyl triphenylphosphonium bromide with furfural (Scheme 2B), to afford a 7:3 mixture of *Z* and *E* isomers (Lie Ken Jie and Lam, 1978). Photochemical isomerization of the mixed isomers in benzene with iodine catalysis (Ikedawa et al., 1970), followed by recrystallization from methanol, gave the *E* isomer **2** in greater than 97% purity.

In the first step of the synthesis of doubly unsaturated furan **6** (Scheme 3), linoleic acid **12** was treated with bromine to afford the tetrabromo acid **13**. Compound **13** was decarboxylated by a modification of the Hunsdiecker reaction to give pentabromide **14**. Regeneration of the diene moiety with zinc powder in THF yielded bromo diene **15**, which was converted to the **16**, and then

coupled with 2-lithiofuran to afford the doubly unsaturated avocadofuran **6** (Murakoshi et al., 1976).

Toxicity Studies

Table 1 and Figure 4 show the growth inhibitory and mortality effects of the most insecticidal compounds found in each of the three active subfractions (1-1-4, 1-1-8, and 1-1-12; Figure 3). 2-(Pentadecyl)furan **1**, present in subfraction 1-1-4, was the most active of the two major compounds from that subfraction. Furan **1** significantly inhibited larval growth, and reduced larval development by >70% at concentrations above 600 μg/g (Table 1). The LC_{50} [95% Fiducial limits (FL)] was 1031 μg/g (988–1084 μg/g) of furan **1** in diet, with a log dose-probit regression line slope of 7.44 ± 0.84 (Figure 4).

The other major component present in subfraction 1-1-4, 2-(1*E*-penta-

TABLE 1. GROWTH INHIBITORY AND MORTALITY EFFECTS OF SYNTHETIC COMPOUNDS FROM AVOCADO IDIOBLAST OIL CELLS TO *S. exigua* LARVAE

Concentration (μg/g)	7-Day larval weight (mg, mean ± SE)	7-Day instar (mean ± SE)[a]	GI[b]	EC (%)[b]	Mortality (%)
2-(Pentadecyl)furan					
600	8.50 ± 0.95b	2.29 ± 0.07c	0.69	72.89	9.37
750	4.44 ± 0.45ab	2.04 ± 0.07bc	0.55	85.83	18.75
900	3.03 ± 0.35ab	1.74 ± 0.07ab	0.26	90.35	32.29
1,050	1.41 ± 0.15a	1.61 ± 0.07a	0.25	95.50	54.17
1,200	1.32 ± 0.19a	1.56 ± 0.09a	0.15	95.78	71.87
2-(Heptadecyl)furan					
600	6.44 ± 0.44b	2.25 ± 0.06c	0.73	79.46	2.08
750	4.03 ± 0.44ab	1.95 ± 0.06b	0.62	87.14	4.20
900	3.19 ± 0.30a	1.87 ± 0.06b	0.56	89.84	10.42
1,050	2.02 ± 0.16a	1.65 ± 0.07ab	0.46	93.55	16.67
1,200	1.28 ± 0.16a	1.43 ± 0.08a	0.23	95.93	54.17
Triolein					
7,000	10.84 ± 1.42ab	2.35 ± 0.08c	0.64	65.43	18.75
8,000	17.67 ± 2.05b	2.70 ± 0.10bc	0.67	43.64	26.04
9,000	4.88 ± 0.65a	1.91 ± 0.08ab	0.39	84.44	40.63
10,000	5.79 ± 0.96a	1.87 ± 0.10a	0.37	81.52	42.71
11,000	8.82 ± 2.22ab	1.95 ± 0.13a	0.28	71.85	59.37
Control	31.35 ± 2.13c	3.19 ± 0.07d	1.03		3.57

[a] Treatments followed by the same letter within each compound are not significantly different from each other (Tukey's pairwise comparisons, $P < 0.05$).
[b] GI = growth index, EC = effective concentration; see text for calculations.

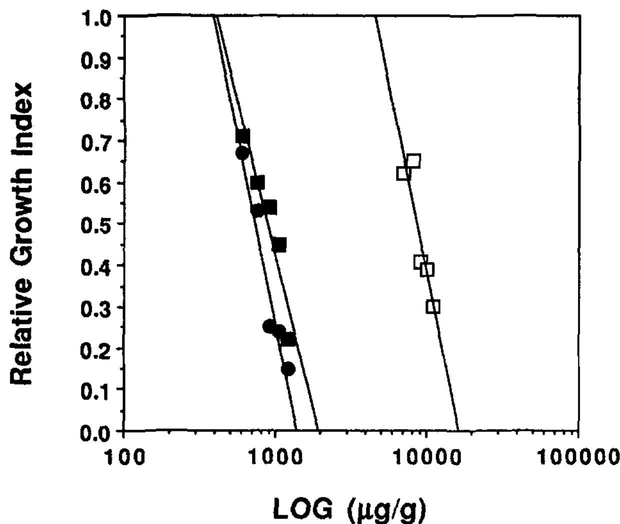


FIG. 4. Effects of synthetic avocadofurans or triolein on *S. exigua* larval development. Solid circles: response to 2-(pentadecyl)furan ($y = 5.66 - 1.79 * \log(x)$, $r^2 = 0.94$); solid squares: response to 2-(heptadecyl)furan ($y = 4.82 - 1.47 * \log(x)$, $r^2 = 0.89$); open squares: response to triolein ($y = 7.68 - 1.83 * \log(x)$, $r^2 = 0.86$).

decyl)furan **2**, showed biological activity only at concentrations approximately three times higher than furan **1**, inhibiting *S. exigua* growth at concentrations of 3500 µg/g or higher. At 3500 µg/g, larval growth was reduced by 57.3% compared to the controls, but mortality was only 12.5%.

2-(Heptadecyl)furan **4** was present as a minor component of subfraction 1-1-4, and it significantly inhibited larval growth (Table 1). Although mortality was low (<15%) at concentrations between 600 and 900 µg/g, larval development and growth were significantly reduced (>75%) at these concentrations (Table 1). The LC_{50} (95% FL) of 2-(heptadecyl)furan in diet was 1206 µg/g (1165–1273 µg/g), with a log dose-probit regression line slope of 14.03 ± 2.29 (Figure 4).

The major component in subfraction 1-1-8, 2-(8Z,11Z-heptadecadienyl)furan **6**, was active only at concentrations (1600 µg/g or higher) twice as high as the saturated analog **4**. At 1600 µg/g, *S. exigua* growth was inhibited by 54.51% compared to control larvae, but mortality was <5%.

Triolein, the major component in subfraction 1-1-12, significantly inhibited larval growth at relatively high concentrations (Table 1), but larval weight was not linearly correlated with concentration. Concentrations of 700 µg/g or higher significantly reduced larval weight and development (Table 1). The LC_{50} (95%

FL) of triolein in diet was 10,364 μg/g (9813–11,277 μg/g), with a log dose-probit regression line slope of 5.75 ± 0.94 (Figure 4).

DISCUSSION

Bioassay-driven fractionation of crude avocado idioblast oil resulted in the identification of five compounds that were toxic to larvae of *S. exigua*. Four of these belonged to the previously identified class of compounds known as avocadofurans, which, with one exception (the aquatic plant *Elodea canadensis* Michaux) (Previtera et al., 1985), have been found only in plants in the genus *Persea*. The fifth compound, the common triglyceride triolein, is a general constituent of avocado mesocarp and composes 15.8% of the total triglyceride composition of avocado mesocarp 12 days after flowering (Gaydou et al., 1987). Three other previously unknown avocadofurans (**3**, **5**, and **7**) were identified as minor components of fractions 1-1-4 and 1-1-8.

Kashman et al. (1969a, b) first reported the avocadofurans as a new class of phytochemicals. They isolated 2-(trideca-12-ynyl)furan and 2-(trideca-12-enyl)furan from *P. americana* fruit and seeds. Magalhaes et al. (1970) subsequently identified several other 2-alkylfurans with C_{13} mono- and diunsaturated side chains from methanol extracts of avocado seeds [*Persea gratissima* Gärtn. (syn. *P. americana*)] from Brazil. Several other avocadofurans have been identified since then (e.g., Néeman et al., 1970; Murakoshi et al., 1976; Weyerstahl et al., 1993; Fraga and Terrero, 1996).

The avocadofurans have received limited screening for biological activity to date. Néeman et al. (1970) tested a group of eight new long-chain aliphatic compounds from avocados, and some derivatives, for activity against 13 species of bacteria and a yeast and reported that 2-(trideca-12-enyl)furan inhibited growth of *Bacillus subtilis* (Ehrenberg) Cohn and *Staphylococcus aureus* Rosenbach. Murakoshi et al. (1976) tested 2-(8Z,11Z-heptadecadienyl)furan **6** produced by acid-catalyzed dehydration of persin from avocado leaves against silkworm larvae, *B. mori*, and found no activity at concentrations in the diet up to 300 μg/g. We found that *S. exigua* larvae were only moderately susceptible; a decrease in survivorship was found only at concentrations of **6** in excess of 1600 μg/g.

Although the avocadofurans we tested are structurally similar, our studies showed differences in their toxicity and growth inhibition effects. The two saturated avocadofurans **1** and **4** were more toxic and growth inhibitory to *S. exigua* than either of the unsaturated compounds **2** and **6**. Of these, **1** was the most toxic. Furthermore, these avocadofurans appear to be active as antifeedants at substantially lower (sublethal) concentrations, as demonstrated by the growth inhibition effects.

To our knowledge, triolein or other triglycerides have never been reported to have deleterious effects on insects, and the toxicity exhibited by triolein at levels of about 1% in artificial diet was unexpected. Furthermore, studies in progress indicate strong synergism between triolein and the avocadofurans (C. Rodriguez-Saona and J. Tromble, unpublished). The results of these studies will be published in due course.

At this early stage, the potential for avocadofurans as lead compounds for a new class of insecticides is unknown. There is no information available as to their nontarget (especially mammalian) toxicity, chemical stability, photostability, and phytotoxicity. However, the fact that the structures are simple and can be made in a single step from readily available precursors may provide impetus for further investigations.

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