Boll Weevil Feeding Deterrents from Tung Oil

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α-Eleostearic acid and *erythro*-9,10-dihydroxy-1-octadecanol acetate have been identified as the components responsible for the feeding deterrency of tung oil to the adult boll weevil, and methods have been developed for isolating large quantities of the acid from the oil and for synthesizing *erythro*-9,10-dihydroxy-1-octadecanol acetate. Although α-eleostearic acid is too unstable for practical use as a feeding deterrent under field conditions, its methyl ester is much more stable and equally effective as a deterrent.

The tung tree, *Aleurites fordii* Hemsl. (family Euphorbiaceae), is a native of China, where the oil expressed from the nut (tung oil or Chinese wood oil) was used for centuries as a waterproofing agent. The seedlings were first introduced into the United States in 1903, and the resulting tung oil industry in this country grew remarkably for 60 years because the oil's excellent drying qualities made it eminently suitable for use in the production of varnishes and resins [for a comprehensive history of tung oil and its uses, see Fondrobert (1951)]. When less expensive synthetic products for the paint and varnish industries became available in 1963, the large number of tung plantations declined, and only a few remain in existence. Most of the tung oil now used (20–30 million lb annually) in this country is imported (Koehn, 1978; Pryde, 1979).

Hardee and Davich (1966) reported the presence in tung oil of an unidentified feeding deterrent for the boll weevil, *Anthonomus grandis grandis* Boheman (order Coleoptera), the most destructive insect pest of cotton in the United States and Mexico. The deterrent appeared to be stable, easily soluble in water, resistant to moderate heat, and only slowly volatile. Although an effective feeding preventive for the boll weevil would be extremely valuable to the cotton grower, this report was not further investigated. Because of its great potential for practical use in eliminating damage to growing cotton by this pest, we decided to attempt the isolation and identification of the feeding deterrent in tung oil.

EXPERIMENTAL SECTION

Materials. Tung nuts used for extraction of the oil were generously provided by LaRow Plantation, Picayune, MS. Imported tung oil used for large-scale extraction of antifeedant was purchased from Alnor Oil Co., Valley Stream, NY. Seeds of *Catalpa ovata* Don were obtained from the Medicinal Plant Resources Laboratory, U.S. Department of Agriculture, Beltsville, MD, and pomegranate seeds (*Punica granatum* L.) were supplied by the Horticultural and Special Crops Laboratory, USDA Northern Regional Research Center, Peoria, IL. All solvents used were reagent grade, except for the hexane, which was free of UV-absorbing materials (Burdick and Jackson Laboratories, Muskegon, MI), and the ether, which was anhydrous. IR spectra were determined in KBr disks, and only the major bands are quoted. UV spectra were recorded in absolute ethanol. ^{1}H NMR spectra were obtained at 60 MHz by using Me_4Si as an internal standard. Gas chromatographic (GC) determinations were performed with a Packard Model 417 gas chromatograph using a glass column (91 × 0.2 cm i.d.) packed with 2% OV-101 on Chromosorb W-HP: temperature 165 °C; N_2 gas flow 30 mL/min. GC–MS spectra were determined with a Hewlett-Packard 5992A instrument. Column chromatographic separations were carried out on Bio-Sil HA silica gel (–325 mesh; Bio-Rad Laboratories, Richmond, CA) prewashed with hexane.

Bioassay Method. The laboratory bioassay method described by Hardee and Davich (1966) was used to monitor the fractionation. Briefly, an appropriate solvent was used to prepare a 1% solution of the test fraction or compound, and an unpunctured, debracted bud from a greenhouse-grown cotton plant was dipped momentarily in the solution. Ten 1- or 2-day-old adult boll weevils, unfed from time of emergence or starved for 24 h, were placed in a Petri dish with one treated bud and one control bud (dipped in solvent only) and held for 4 h. Five control and five test dishes (five replicates) were prepared for each test fraction or compound. The number of feeding punctures per bud was counted with a dissecting microscope.

Extraction and Fractionation of Tung Oil. Previous extraction of tung nuts consecutively with pentane, ethyl ether, and 95% ethanol had shown that all of the deterrent activity was removed with pentane (Jacobson et al., 1978). Tung nuts (4 kg) were therefore ground in a Wiley mill and extracted overnight with pentane in Soxhlet extractors. Removal of solvent at 28 °C under reduced pressure (15 mm) yielded 1.2 kg of odorless yellow oil. One hundred grams of this oil was chromatographed on a column (8 × 47 cm) of silica gel by eluting successively with 10-L portions of hexane, increasing concentrations of ether in hexane (5:95, 10:90, 25:75, and 50:50), and finally ether alone. The following two active fractions were obtained following removal of the solvent at 15 mm Hg of pressure: (A) 2.5 g of yellow wax eluted with hexane–ether (95:5) and (B) 5.0 g of yellow oil eluted with hexane–ether (50:50). The wax was dissolved in 25 mL of acetone, and the solution was cooled to –25 °C, precipitating a colorless crystalline solid that was recrystallized once from pentane to give 1.8 g of deterrent A, shining plates, mp 49 °C. The oil (B) was rechromatographed on a column (4 × 45 cm) of silica gel, eluting with 2-L portions of hexane–ether (75:25) and ether alone; only the latter eluate contained active material, consisting of 2.0 g of white solid; recrystallization from dilute ethanol gave 1.9 g of crystalline deterrent B, mp 54 °C.

RESULTS AND DISCUSSION

The chromatographic fractions obtained from crude tung oil are shown in Table I, together with the number of boll weevil punctures permitted by each fraction. *Pure* deterrent A permitted 3 punctures (15 for the control), and *pure* deterrent B permitted 7 punctures (60 for the control).

Identification of Deterrent A. Colorless plates, mp 49 °C, were obtained. The IR spectrum showed strong

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Table I. Silicic Acid Chromatography of Tung Oil and the Effect of the Fractions on Boll Weevils

eluate	appearance	no. of feeding punctures in cotton squares dipped in: tung solution	no. of feeding punctures in cotton squares dipped in: solvent only
hexane	colorless wax	12	7
hexane–ether (95:5)	yellow wax (A)	5	28
hexane–ether (90:10)	yellow oil	14	7
hexane–ether (75:25)	colorless solid	19	29
hexane–ether (50:50)	yellow oil (B)	12	60
ether	yellow oil	24	13

bands at 1706 (CO_2H), 993 and 969 (*t*-CH=CH in a cis,-trans,trans-conjugated system), and 760 cm^{-1} (long chain); UV λ_{max} 261, 271, 281 nm. The NMR spectrum showed a complex multiplet centered at ~4.0 tau. These data were highly indicative of a long-chain acid with a conjugated triene grouping (Crombie and Jacklin, 1957; Hopkins, 1965). Oxidative cleavage of 100 mg of deterrent A with $KMnO_4$–$NaIO_4$ (Lemieux and von Rudloff, 1955) yielded pentanoic and 1,9-nonanedioic (azelaic) acids, suggesting that the unsaturation was in the 9, 11, and 13 positions. On hydrogenation with Pt catalyst, the deterrent afforded a colorless solid, mp 70 °C, identified as octadecanoic (stearic) acid by mixed melting point with an authentic sample. The deterrent thus appeared to be a straight-chain C_{18} acid with double bonds in the 9, 11, and 13 positions, and it remained only to determine the geometric configuration. Ready formation of a crystalline maleic anhydride adduct, mp 64–65 °C, established the fact that at least two of the double bonds were trans conjugated, in agreement with the IR spectrum; that the third double bond had the cis configuration was confirmed by elaidinization of the deterrent to the all-trans compound, mp 72 °C, with iodine and UV light. Of the eight possible geometric isomers of 9,11,13-octadecatrienoic acid, only the *Z,E,E* (α-eleostearic acid) and *E,E,Z* isomers fit the data obtained. Comparison of deterrent A with the authentic *Z,E,E* isomer (obtained from ICN Pharmaceuticals, Plainview, NY, and purified by low-temperature recrystallization from pentane) and authentic *E,E,Z* isomer [isolated from *C. ovata* seed oil by the method of Hopkins and Chisholm (1962)], as well as with the *Z,E,Z* isomer [punicic acid; isolated from pomegranate seed oil by the method of Crombie and Jacklin (1957)] and the all-trans isomer, showed without doubt that deterrent A was (*Z,-E,E*)-9,11,13-octadecatrienoic acid; see Table II for the comparative data. Identity was confirmed by preparation of the methyl ester of deterrent A with diazomethane and comparison of its gas chromatograms and mass spectra with those of authentic methyl α-eleostearate.

Identification of Deterrent B. Colorless needles, mp 54 °C, $C_{20}H_{40}O_4$, were obtained. The IR spectrum showed strong bands at 3299 (OH), 1740 (primary ester), 1300 and 1110 (secondary OH), 1240 ($-OCOCH_3$), and 750 cm^{-1} (long chain); no unsaturation was indicated, and the compound failed to absorb H_2. UV absorption was absent. These data were suggestive of a long-chain, aliphatic, primary acetate with two secondary OH groups, and rapid reaction of a sample of the compound with HIO_4 showed that these OH groups were vicinal; the oxidation produced nonanal and 9-(acetyloxy)nonanal (Bjostad et al., 1980), which corresponded with the products obtained by microozonolysis of oleyl acetate. It thus appeared that the deterrent was 9,10-dihydroxy-1-octadecanol acetate, and this was confirmed by $LiAlH_4$ reduction to give 9,10-dihydroxy-1-octadecanol, mp 127 °C, identical with an authentic sample of the erythro triol synthesized from oleyl alcohol by the method of Okimoto and Swern (1977). In contrast, *threo*-9,10-dihydroxy-1-octadecanol showed mp 82 °C (Okimoto and Swern, 1977). Deterrent B was thus identical with *erythro*-9,10-dihydroxyoctadecan-1-ol acetate; this was confirmed by synthesis (described below).

Table II. Comparison of Melting Points, Significant UV and IR Absorption, and Maleic Anhydride (MA) Adducts of 9,11,13-Octadecatrienoic Acids

configuration	mp, °C	UV (λ_{max}), nm	IR, cm^{-1}	MA adduct mp, °C
Z,E,E	49	261, 271, 281	993, 969	64–65
E,E,Z	32	261, 271, 281	986, 957	73–74
Z,E,Z	44	264, 274, 285	987, 937	
E,E,E	72	259, 268, 279	994	77–78

Synthesis of Deterrent B. Five grams of oleic acid (Kahlbaum, >99% pure) was reduced with $LiAlH_4$ to 4.9 g of oleyl alcohol, bp 140 °C (0.3 mmHg), n_D^{25} 1.4608, which was then converted quantitatively to oleyl acetate, bp 139 °C (0.1 mmHg), n_D^{25} 1.4493, by refluxing with acetyl chloride and pyridine in benzene solution. OsO_4 catalyst solution (Daniels and Fischer, 1963) was prepared by dissolving 1 g of OsO_4 in 122 mL of *t*-BuOH (purified with $KMnO_4$) and adding dropwise 30% H_2O_2 until a stable pale green color resulted. A mixture of 2 mL of catalyst solution and 2 mL of 30% H_2O_2 was added to a solution of 2 g of oleyl acetate in 16 mL of acetone and 4 mL of ether, and the resulting brown solution was stirred at 30 °C for 24 h and freed of solvent on a Rinco evaporator at 15-mmHg pressure. Pentane was added and the separated solid was cooled, filtered off, and crystallized from dilute ethanol, yielding 1.8 g of *erythro*-9,10-dihydroxy-1-octadecanol acetate as colorless needles, mp 54 °C; the melting point of a mixture with deterrent B was undepressed.

Batch Preparation of α-Eleostearic Acid from Tung Oil. A solution of 134 g of tung oil and 54 g of KOH in 500 mL of 95% ethanol was refluxed for 4 h in a nitrogen atmosphere and then cooled in an ice bath and acidified with 10% H_2SO_4. The upper oily layer was extracted with ether, and the ether solution was dried (Na_2SO_4). The solvent was evaporated, and the acidic residue was taken up in acetone and cooled to −20 °C to give 75 g (56%) of pure α-eleostearic acid, mp 49 °C. This compares very favorably with the yield (36%) of the acid, mp 47–48 °C, obtained by Crombie and Jacklin (1957) after 6-fold recrystallization of saponified tung oil from ethanol and pentane.

Although pure α-eleostearic acid may be stored in a freezer (−15 °C) without change for several months, it is highly unstable at room temperature, polymerizing after 2 h. This instability and the fact that exposure to sunlight rapidly converts the α to the β acid precludes its practical use as a boll weevil feeding deterrent on cotton plants in the field. This prompted us to prepare the methyl and ethyl esters of α-eleostearic acid in the hope that one of these derivatives would be more stable and active as a feeding deterrent.

Synthesis of Esters of α-Eleostearic Acid. Methyl α-eleostearate was prepared by refluxing, for 2 h in a nitrogen atmosphere, a solution of 5 g of the acid in 50 mL of methanol containing 1 drop of concentrated H_2SO_4, removing the solvent by evaporation at 15 mmHg and 30

°C, pouring the residue into several volumes of H_2O, and extracting with several portions of ether. The dried (Na_2SO_4) solution was freed of solvent and distilled to give a quantitative yield (5.25 g) of the methyl ester as a pale yellow liquid, bp 162 °C (1.0 mmHg), bp 210 °C (12 mmHg), n_D^{25} 1.5000. The compound exhibited strong blue fluorescence in UV light.

Ethyl α-eleostearate was similarly prepared in quantitative yield by using ethanol instead of methanol; it showed bp 190 °C (4.0 mmHg), n_D^{25} 1.4900.

Although ethyl α-eleostearate did not deter feeding by the boll weevil (35 punctures; 39 punctures for the control), methyl α-eleostearate was as effective a feeding deterrent as the free acid, permitting only 9 punctures as compared with 60 for the control. Furthermore, the methyl ester is much more stable than the acid, remaining a mobile, active liquid for at least 1 week at room temperature and indefinitely under ordinary refrigeration (10 °C); UV irradiation at room temperature resulted in slow conversion (over 1 week) to the inactive all-trans methyl ester, bp 169 °C (1.0 mmHg), n_D^{25} 1.5037.

The high activity of methyl α-eleostearate and *erythro*-9,10-dihydroxy-1-octadecanol acetate as boll weevil feeding deterrents in laboratory bioassay trials indicates that they have considerable potential for use under practical conditions to prevent damage to cotton plants by this serious pest. However, only actual field testing can confirm this. Such tests are planned on cotton crops in Mississippi and Mexico during the next growing season. In the meantime, patent applications have been filed covering the isolation of α-eleostearic acid and *erythro*-9,10-dihydroxy-1-octadecanol acetate from tung oil and the activity of the latter compound and methyl α-eleostearate as boll weevil feeding deterrents. Toxicological tests with these compounds are also under way.

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Effect of Impurities on the Delayed Neurotoxicity of *O*-(4-Bromo-2,5-dichlorophenyl) *O*-Ethyl Phenylphosphonothioate Administered Orally to Hens

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The delayed neurotoxicity of technical and purified *O*-(4-bromo-2,5-dichlorophenyl) *O*-ethyl phenylphosphonothioate administered orally to hens was compared under different conditions. The technical material was neurotoxic at 750 mg/kg while the purified compound was neurotoxic at 1000–1250 mg/kg. The chemical composition of the technical material was analyzed for organophosphate contaminants. Impurities present as more than 0.1% were examined for delayed neurotoxic activity. *O,O*-Diethyl phenylphosphonothioate, its oxon analogue, and *O,O*-diethyl (4-chlorophenyl)phosphonothioate were 5–10 times more potent as delayed neurotoxins than the parent compound. These impurities evidently potentiate the delayed neurotoxicity of ethyl leptophos and may potentiate the delayed neurotoxicity of other *O*-ethyl phenylphosphonothioate pesticides as well.

The organophosphorus pesticide *O*-(4-bromo-2,5-dichlorophenyl) *O*-methyl phenylphosphonothioate (leptophos) has been shown to cause delayed neurotoxicity in many species of animals (Report of the Leptophos Advisory Committee, 1976). Its delayed neurotoxic potential was significantly increased by oxidation to its oxon and by photolytic debromination to desbromoleptophos. It was also suggested that differences in the delayed neurotoxic potential of technical leptophos reported from several studies may have been due to potentiating impurities (Sanborn et al., 1977).

In a recent study of the delayed neurotoxicity of structural analogues related to leptophos, it was reported that substitution of the methoxy moiety with a longer chain alkoxy group, e.g., ethoxy or propoxy, abolished

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