Impurities in Cue-Lure Attractive to Female Tephritidae

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Acetic acid and acetic anhydride were identified as the contaminants in commercial cue-lure that are responsible for its transient attraction to female Mediterranean fruit flies and female melon flies. In addition, 4-(*p*-hydroxyphenyl)-3-buten-2-one, an intermediate in the commercial manufacture of cue-lure, was separated into its *Z* and *E* isomers. The *Z* isomer, which could be elaidinized to the *E* isomer, was attractive to the females of both species. The *E* isomer occurred in colorless and yellow crystalline forms which attracted mainly female Mediterranean fruit flies.

Cue-lure, 4-(*p*-hydroxyphenyl)-2-butanone acetate, is a powerful attractant for the male melon fly, *Dacus cucurbitae* Coquillet, a serious economic pest in Hawaii and in many other parts of the world. The compound, which is very useful in traps for detecting the insect in the field, is prepared by a Claisen–Schmidt condensation of *p*-hydroxybenzaldehyde and acetone, hydrogenation, and acetylation of the product (Beroza et al., 1960; Alexander et al., 1962). However, Keiser et al. (1976) recently found that under field conditions traps baited with commercial cue-lure also attracted female Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann). They therefore conducted tests in a laboratory olfactometer with wicks impregnated with commercial cue-lure freshly drawn from containers. The freshly baited wicks were indeed attractive to male melon flies and female Mediterranean fruit flies, but if they were left at room temperature for a few days, few or no Mediterranean fruit flies were attracted though large numbers of male melon flies still were. This indicated that a highly volatile impurity or impurities present in commercial cue-lure were responsible for the attraction to Mediterranean fruit flies.

Since attractants for female tephritids, which are serious economic pests in many areas, are badly needed, we decided to isolate and identify the impurity in cue-lure that was attractive to female Mediterranean fruit flies.

EXPERIMENTAL SECTION

Materials. Solvents and chemical reagents used were reagent grade. All boiling points are uncorrected; melting points are corrected. Ir spectra were obtained with KBr discs on a Beckmann Model DK2-A spectrophotometer.

Commercial cue-lure, a yellow oily liquid showing a refractive index ($n^{25}D$) of 1.5077, was purchased from Food Industries Ltd., Bromborough Port, Cheshire, England. It had been prepared by hydrogenating the crude condensation product from *p*-hydroxybenzaldehyde and acetone, decolorizing the hydrogenation product by treatment with charcoal, and acetylating in the conventional manner by refluxing with acetic acid and acetic anhydride. After removal of the excess acetylating agents at 20 mm pressure, the final product was distilled at temperatures between 100 and 170 °C at 1 mm pressure.

Food Industries Ltd. also made a gift of a sample of the crude condensation product (A) (obtained before hydrogenation). It was a rather gummy, yellow solid, mp 60–96 °C.

Vacuum Aeration and Distillation of Cue-lure. Commercial cue-lure (126 g) was subjected to a vacuum pump pressure of 0.5 mm at 60 °C for 15 h during which the volatiles were collected in a trap immersed in a dry ice–acetone bath. The pot temperature was then raised, and the cue-lure was fractionally distilled at the same pressure through a column (30 × 1.2 cm) packed with glass helices.

Fractionation of the Crude Condensation Product. The commercial product (A) (10 g) melting at 60–96 °C was recrystallized from a large volume of benzene to give 6.5 g of a yellow solid; evaporation of the benzene filtrate to a small volume gave 3.0 g of yellow solid. Another recrystallization of the less soluble solid from benzene yielded 6.3 g of pale yellow needles (B), mp 112–113 °C; recrystallization from H_2O of the more soluble solid yielded 2.9 g of small, yellow prisms (C), mp 103–104 °C.

Synthesis of 4-(*p*-Hydroxyphenyl)-3-buten-2-one. To an ice-cold mixture of 25 g of *p*-hydroxybenzaldehyde and 12 g of acetone was added slowly, with stirring, 250 ml of a 20% solution of NaOH. The mixture was allowed to stand overnight at room temperature, treated with H_2O, and acidified to Congo with 10% HCl solution. The resulting orange solid was filtered off, washed with H_2O, and dried. Recrystallization from a large volume of benzene gave 20 g of yellow needles, mp 110–112 °C; the melting point was raised to 112–113 °C by an additional recrystallization from benzene. Evaporation of the benzene filtrates to a small volume gave 7.5 g of pale yellow prisms, mp 103–104 °C, after recrystallization from H_2O.

Bioassay Method. Laboratory tests were conducted with freshly drawn commercial cue-lure, cue-lure allowed to remain uncovered indoors at room temperature for 12–14 days, and pure distilled cue-lure by placing a 0.4–0.5-ml sample on a cotton wick ($^1/_3$ cm) and suspending it in an invaginated glass McPhail trap filled with H_2O. The traps were hung from a horizontally suspended revolving wheel (18 rpm) in a 3-m^3 outdoor olfactometer (Gow, 1954) stocked with ca. 150 000 laboratory-reared adults (both sexes) of melon flies, Mediterranean fruit flies, and oriental fruit flies, *Dacus dorsalis* Hendel.

When other test materials (150 mg each) were bioassayed, they were placed in an Erlenmeyer flask, 1 drop of polyethylene glycol 400 monolaurate (emulsifier, Glyco Products), and 3 ml of ethyl alcohol were added, and the mixture was diluted with H_2O to 150 ml to make a 0.1% emulsion. Then 50 ml of this emulsion was poured into each of three McPhail traps (three replicates) and evaluated in the olfactometer as described.

In each test, the number of flies of each species caught in the trap during a 1-h exposure of the test sample was compared with the number caught in traps containing water only.

RESULTS AND DISCUSSION

Freshly drawn cue-lure attracted both male melon flies and female Mediterranean fruit flies. Aged cue-lure on wicks (allowed to remain at room temperature for a few

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Table I. Attraction of Acetic Acid and Acetic Anhydride to Tephritidae in an Outdoor Olfactometer

	Total no. of flies attracted[a]					
	Mediterranean fruit flies		Melon flies		Oriental fruit flies	
Formulation	♂	♀	♂	♀	♂	♀
Water (check)	7	15	15	14	5	8
Acetic acid (0.1%)	122	211	225	323	100	146
Acetic acid (1.0%)	43	84	146	195	78	70
Acetic anhydride (0.1%)	62	255	313	429	109	126
Acetic anhydride (1.0%)	11	24	45	52	32	43

[a] Total of three replicates.

days) attracted few or no female Mediterranean fruit flies but continued to attract large numbers of male melon flies. Total catches of female Mediterranean fruit flies due to blank wicks, wicks treated with 0.5 ml of fresh cue-lure, and wicks treated with 0.5 ml of aged cue-lure were 45, 895, and 227, respectively, within 4 h. In another test, fresh cue-lure attracted 1697 female Mediterranean fruit flies in 3 h, and aged cue-lure attracted 256 females.

The trapped volatiles obtained during vacuum aeration of commercial cue-lure consisted of 1.03 g (0.8% based on cue-lure) of pale yellow liquid (n^{25}D 1.3725) possessing a strong odor of acetic acid. Ir spectrophotometry indicated a mixture of acetic acid containing ca. 10% of acetic anhydride, which was verified by microdistillation of the volatiles to give 810 mg of acetic acid (bp 118 °C, n^{25}D 1.3692; anilide, mp 114 °C), and 79 mg of acetic anhydride (bp 139 °C, n^{25}D 1.3886) that was readily convertible to acetic acid by treatment with H_2O. These compounds are undoubtedly traceable to their use in the acetylation step during the manufacture of cue-lure, as well as to possible slight hydrolysis of the finished product. In the bioassay tests (Table I), both compounds (at 0.1% in H_2O) were attractive to female Mediterranean fruit flies and acetic anhydride was somewhat more attractive than acetic acid. The 1% concentrations of the compounds were somewhat repellent compared with the 0.1% concentration. Also, both compounds were attractive to female melon flies.

Distillation of the cue-lure after it had been freed of its volatiles yielded 116.5 g of pure cue-lure as a colorless, odorless liquid, bp 133–135 °C (0.6 mm), n^{25}D 1.5084. The material was highly attractive to male melon flies but did not attract female Mediterranean fruit flies.

Crystalline compounds melting at 112–113 °C (B) and at 103–104 °C (C) were obtained from the crude condensation product submitted by Food Industries Ltd. They both analyzed for $C_{10}H_{10}O_2$. The ir spectra were identical except for the presence of a strong band at 970 cm^{-1} (trans unsaturation) in the spectrum of compound B. The compounds were shown to be the *E* and *Z* isomers, respectively, of 4-(*p*-hydroxyphenyl)-3-buten-2-one by comparison with authentic synthesized specimens and by isomerization of the *Z* isomer into the *E* isomer through treatment with uv light in the presence of a trace of iodine (Jacobson, 1954). As reported by McGookin and Sinclair (1928), the *E* isomer occurred in two crystalline forms of identical melting point (lemon-yellow needles from benzene and colorless needles from ethanol). Isomerization of the

Table II. Attraction of the Geometric Isomers of 4-(*p*-Hydroxyphenyl)-3-buten-2-one to Tephritidae in an Outdoor Olfactometer

	Total no. of flies attracted[b]					
	Mediterranean fruit flies		Melon flies		Oriental fruit flies	
Isomer[a]	♂	♀	♂	♀	♂	♀
Water (check)	8	14	3	3	8	5
Z	426	675	104	119	53	78
Water (check)	62	40	36	46	78	73
E (colorless form)	273	796	130	128	134	108
E (yellow form)	308	617	87	53	57	60

[a] Concentration of 0.1% in water. [b] Total of three replicates.

Z isomer consistently gave the colorless form of the *E* isomer. Acidification of the condensation mixture of *p*-hydroxybenzaldehyde and acetone to pH 6.0 gave only the *E* isomer of 4-(*p*-hydroxyphenyl)-3-buten-2-one; acidification to pH 2.0–3.0 yielded a mixture of both geometric isomers (Zemplen et al., 1945).

The results of the bioassay tests in the outdoor olfactometer with the *Z* and *E* isomers of 4-(*p*-hydroxyphenyl)-3-buten-2-one are shown in Table II. Although the *Z* isomer was attractive to females of both the Mediterranean fruit fly and the melon fly, the *E* isomer (colorless or yellow form) attracted mainly female Mediterranean fruit flies.

The highly volatile impurities in commerical cue-lure that are attractive to female Mediterranean fruit flies thus appear to be solely acetic acid and acetic anhydride. Perhaps of greater interest is the fact that this investigation of the cue-lure intermediates resulted in the discovery that the *Z* and *E* isomers of 4-(*p*-hydroxyphenyl)-3-buten-2-one have considerable attraction for female fruit flies. This finding has led to an extensive investigation of the synthesis and attraction of several series of phenols and phenolic derivatives for female fruit flies, the results of which will be reported elsewhere.

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