Syntheses and Behavioral Evaluations of Fluorinated and Silylated Analogs of Raspberry Ketone as Attractants for the Melon Fly, *Bactrocera cucurbitae* (Coquillett)[1]

V. Casaña-Giner, J. E. Oliver,[2] E. Jang[3] and L. Carvalho[3]

USDA/ARS, Chemicals Affecting Insect Behavior Laboratory, B-007, BARC-W, Beltsville, MD 20705 USA

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Abstract Cuelure, the acetate of raspberry ketone, has for many years been the only practical lure for male melon flies, *Bactrocera cucurbitae* (Coquillett). Outdoor olfactometer and field tests of several new analogs of raspberry ketone were conducted in Hawaii. Of the newly synthesized compounds, only 4-(4-((trimethylsilanyl)oxy)phenyl)butan-2-one, the trimethylsilyl ether of raspberry ketone, was highly attractive for male melon flies, being statistically equivalent to cuelure in a 2-d field test. None of several fluorinated analogs of raspberry ketone were nearly as attractive as raspberry ketone itself. The recently reported attractant zingerone did not show significant attractiveness to either male or female melon flies.

Key Words Melon fly, *Bactrocera cucurbitae,* male lures, fluorinated analogs, silylated analogs

The melon fly, *Bactrocera cucurbitae* (Coquillet), is one of the most destructive agricultural pests of cucurbit and other crops, attacking more than 125 host plants (Metcalf 1990). Its distribution includes Africa, India, Southeast Asia, and many Pacific Islands including Hawaii (Kuba et al. 1996). Heavy infestations can result in complete crop loss, as sometimes happens in Hawaii and other countries. Because of infestations in past years, California has restricted the intrastate movement of certain melon fly host articles (California Department of Food and Agriculture 1999).

Cuelure (acetic acid 4-(3-oxobutyl)phenyl ester) (Beroza et al. 1960) is the most commonly employed attractant for melon flies and several other *Bactrocera* spp. It is the acetate of the naturally-occurring melon fly attractant, raspberry ketone (4-(4-hydroxyphenyl)butan-2-one, also called rheosmin (Metcalf 1990)). Raspberry ketone itself is very attractive to melon flies, but is released too slowly to be an adequate bait in traps. Metcalf and Metcalf (1992) estimated that cuelure was released about 20x faster than raspberry ketone. Cuelure has been widely used for monitoring and less frequently for population reduction; for example, it was reported that the population of male melon flies was reduced by 99% by cuelure-impregnated fiberboard blocks (2.25 blocks per ha impregnated with 14 g of a 5% naled wt/wt cuelure solution). In a more favorable melon fly host habitat, however, a rate of 13.5 fiberboard blocks per

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[2] To whom offprint requests are addressed (oliverj@ba.ars.usda.gov).

[3] USDA/ARS, Pacific Basin Agricultural Research Center, P.O. Box 4459, Stainback Highway, Kulani Rd, Hilo, HI 96720.

ha was necessary (Cunningham and Steiner 1972). In spite of its improvement over raspberry ketone, low volatility is considered to be a limitation of cuelure, and a more effective attractant could reduce the number of traps and the amounts of bait and insecticide required.

Several fluorinated analogs of raspberry ketone and some of their synthetic intermediates **(1-14)** (Fig. 1) were evaluated for their ability to maintain attractiveness while promoting enhanced volatility and/or more favorable release properties. Silyl ethers of alcohols are routinely employed to improve gas chromatographic behavior, and the trimethylsilyl and *tert*-butyldimethylsilyl ethers of raspberry ketone **(15** and **16)** were also prepared and tested. In addition, two bicyclic analogs **(17** and **18)** were prepared and evaluated. The latter can be perceived as resulting from bond formation between a side chain carbon or oxygen and the aromatic ring of raspberry ketone. Finally, we also examined 4-(4-hydroxy-3-methoxyphenyl)butan-2-one **(19)** (synonyms: zingerone, vanillylacetone). Zingerone is a naturally-occurring analog of raspberry ketone, recently reported as attractant for *B. cucurbitae* and several other *Bactrocera* species (Tan 2000). Khoo and Tan (2000) reported that zingerone-fed male flies may be more successful in attracting and courting mates compared with cuelure-fed and cuelure-deprived males. Herein we describe results of outdoor olfactometer tests of the new compounds as well as of a field assay of the most effective analog, 4-(4-((trimethylsilanyl)oxy)phenyl)butan-2-one **(15)**, against the melon fly.

Materials and Methods

Compounds tested and chemical syntheses. Gas chromatographic analyses (GC) were conducted with a Shimadzu GC-14A equipped with a flame ionization detector and a J&W fused silica column DB 1 (30 m × 0.25 mm ID, 0.25 μm) with a hydrogen flow of 3 mL/min (initial pressure 140 kPa). The oven temperature was programmed from 70°C (no initial hold) to 280°C at 15°C/min. Gas chromatography-mass spectrometry (GC-MS) was performed on a Hewlett-Packard 5890 GC/MS at 70eV, with a J&W fused silica column HP 5 (30 m × 0.25 mm ID, 0.25 μm), heated at 80°C for 2 min then programmed to 280°C at 15°C/min. Chemical ionization mass spectra, infrared spectra and nuclear magnetic resonance data were collected for confirmation purposes but are not reported here. Mention of trade names or commercial products in this article are solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

All compounds tested (Fig. 1) were of >96% purity as determined by GC analysis. Purchased compounds included cuelure and raspberry ketone (Aldrich, Milwaukee, WI), 1-(4-(trifluoromethyl)phenyl)ethanone **(13)**, and 4-fluoroacetophenone **(14)** (Flurochem, West Columbia, SC) and 4-(4-hydroxy-3-methoxyphenyl)butan-2-one **(19)** (Givaudan, Vernier, Switzerland). 1,1,1-Trifluoro-4-(4-(trifluoromethyl)phenyl)-butan-2-one **(6)** was synthesized by condensation of 1-(4-(trifluoromethyl)phenyl)-ethanone **(13)** with ethyl trifluoroacetate (sodium hydride, refluxing tetrahydrofuran), followed by hydrogenation with 5% Pd on C in acetic acid containing 1% trifluoroacetic acid, a modification of a procedure of Cornier and McCauley (1988). Pure **(6)** was obtained after flash chromatography (80/20 hexanes/ethyl acetate), MS: (m/z, %) 270 (97.5), 251 (40.3), 207 (24.7), 201 (100), 183 (12.1), 173 (54.1), 159 (92.3), 153 (18.0), 152 (13.0), 133 (17.0), 109 (13.9), 42 (11.1). 4,4,4-Trifluoro-1-phenylbutane-

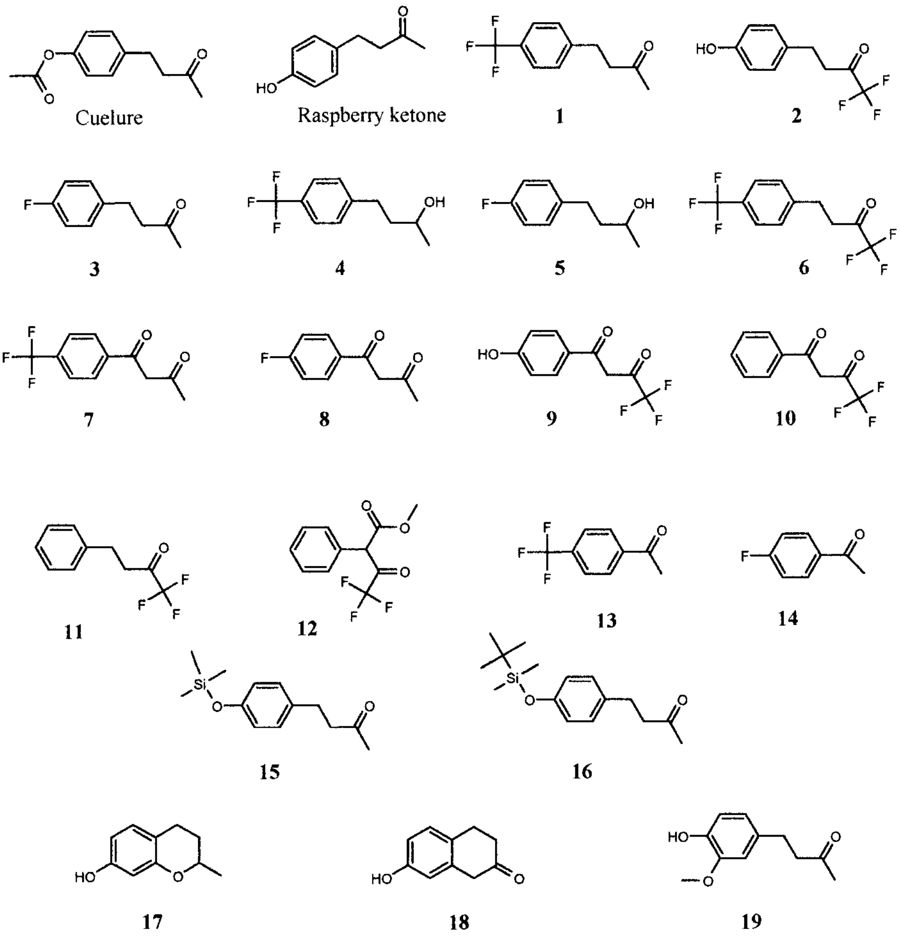

Fig. 1. Compounds tested as melon fly attractants cuelure: acetic acid 4-(3-(oxobutyl)phenyl ester (synonym: cuelure); raspberry ketone: 4-(4-hydroxyphenyl)butan-2-one (synonyms: raspberry ketone, rheosmin); **1:** 4-(4-(trifluoromethyl)phenyl)butan-2-one; **2:** 1,1,1-trifluoro-4-(4-hydroxyphenyl)butan-2-one; **3:** 4-(4-fluorophenyl)butan-2-one; **4:** 4-(4-(trifluoromethyl)phenyl)butan-2-ol; **5:** 4-(4-fluorophenyl)butan-2-ol; **6:** 1,1,1-trifluoro-4,(4-(trifluoromethyl)phenyl)butan-2-one; **7:** 1-(4-(trifluoromethyl)phenyl)butane-1,3-dione; **8:** 1-(4-fluorophenyl)butane-1,3-dione; **9:** 4,4,4-trifluoro-1-(4-hydrooxyphenyl)butane-1,3-dione; **10:** 4,4,4-trifluoro-1-phenylbutane-1,3-dione; **11:** 1,1,1-trifluoro-4-phenylbutan-2-one; **12:** 2-phenyl-4,4,4-trifluoro-3-oxobutyric acid methyl ester; **13:** 1-(4-(trifluoromethyl)phenyl)ethanone; **14:** 4-fluoroacetophenone; **15:** 4-(4-((trimethylsilanyl)oxy)phenyl)butan-2-one; **16:** 4-(4-*tert*-butyldimethylsilanyloxyphenyl)butan-2-one; **17:** 2-methylchroman-7-ol; **18:** 7-hydroxy-3,4-dihydro-1*H*-naphthalen-2-one; **19:** 4-(4-hydroxy- 3-methoxyphenyl)-butan-2-one (synonyms: zingerone, vanillylacetone).

1,3-dione **(10)** was obtained by condensation of acetophenone with ethyl trifluoroacetate (sodium hydride, refluxing tetrahydrofuran) MS: (m/z, %) 216 (M^+ 62.1) 147 (68.2), 105 (22.3), 77 (32.3), 69 (100), 51 (23.4), 43 (11.7), 40 (25.1). Hydrogenation as described above gave 1,1,1-trifluoro-4-phenylbutan-2-one **(11),** MS: (m/z, %) 202 (M^+ 60.1), 133 (45.9), 107 (19.3), 91 (100), 69 (15.4), 51 (11.3). Similarly, 1-(4-fluorophenyl)butane-1,3-dione **(8)** was obtained from 4-fluoroacetophenone **(14)** and ethyl acetate [MS: (m/z, %) 180 (M^+ 47.1), 179 (21.3), 165 (45.9), 123 (100), 95 (41.6), 85 (12.6), 75 (18.1), 69 (58.8), 43 (41.4)]; it was then converted to 4-(4-fluorophenyl)butan-2-one **(3)** by hydrogenation as described above, MS: (m/z, %) 166 (M^+ 66.6), 151 (15.0), 123 (33.4), 109 (84.4), 103.1 (19.0), 96 (11.8), 83 (10.2), 43 (100). In this case, 4-(4-fluorophenyl)butan-2-ol **(5)** was obtained as a byproduct and separated by flash chromatography (90/10 hexane/ethyl acetate and then 80/20 hexane/ethyl acetate), MS: (m/z %) 168 (M^+ 15.0), 125 (75.8), 83 (10.2), 43 (100), 109 (15), 97 (43.9), 95 (50.2), 69 (19.5), 43 (100). Using the same procedure, condensation-hydrogenation-flash chromatography (85/15 hexane/ether), we obtained 1-(4-(trifluoromethyl)phenyl)butane-1,3-dione **(7)** as the product of condensation of 4-(trifluoromethyl)acetophenone **(13)** with ethyl acetate, MS: (m/z, %) 230 (M^+ 76.4), 229 (40.7), 215 (100), 173 (70.2), 145 (63.2); hydrogenation and flash chromatography (85/15 hexane/ether) provided 4-(4-(trifluoromethyl)phenyl)butan-2 one **(1),** MS: (m/z, %) 216 (M^+ 50.1), 197 (10.2), 174 (18.0), 159 (24.5), 133 (10.1), 43 (100), and 4-(4-(trifluoromethyl)phenyl)butan-2-ol **(4),** MS: (m/z, %) 218 (M^+ 2.1), 200 (10.3), 109 (100), 107 (20.5), 43 (21), 41 (11.1). The synthesis of 4,4,4-trifluoro-1-(4-hydroxyphenyl)butane-1,3-dione **(9)** began with protection of 4-hydroxyacetophenone as its benzyl ether. Condensation with ethyl trifluoroacetate (sodium hydride in refluxing toluene) gave 4,4,4-trifluoro-1-(4-(benzyloxyphenyl)) butane-1,3-dione. Removal of the benzyl group by hydrogenolysis gave 4,4,4-trifluoro-1-(4-hydroxyphenyl)butane-1,3-dione **(9),** MS: (m/z, %) 232 (M^+ 18.1) 214 (22.6), 199 (10.0), 173 (34), 175 (28), 145 (59), 127 (35), 118 (31), 58 (21) and 1,1,1-trifluoro-4-(4-hydroxyphenyl)butan-2-one **(2),** MS: (m/z, %) 218 (M^+ 81.7), 149 (100), 131 (26.4), 107 (22.0), 103 (13.0), 91 (12.2), 76.9 (10.3), 55 (19.4), with reduction of the ketone and the hydrogenolysis of the benzyl ether occurring in the same reaction; diketone **(9)** was separated from monoketone **(2)** by flash chromatography (85/15 hexane/ether). 2-Phenyl-4,4,4-trifluoro-3-oxobutyric acid methyl ester **(12)** was obtained by treating phenylacetyl chloride with trifluoroacetic anhydride in methylene chloride and pyridine, followed by addition of methanol (Boivin et al. 1995), MS: (m/z, %) 247 (12.35), 246 (M^+ 100), 214 (14.6), 201 (27.5), 187 (74.5), 159 (29.6), 149 (58.1), 121 (72.1), 128 (28.3), 109 (53.4), 105 (15.6), 91 (19.1), 90 (37.5), 89 (37.5), 77 (18.6), 69 (14.1), 63 (18.6), 59 (37.3). The silyl ethers of raspberry ketone **(15)** and **(16)** were obtained upon treatment of raspberry ketone with trimethylsilyl chloride and *tert*-butyldimethylsilyl chloride, respectively (Veysoglu and Mitscher 1981), to give 4-(4-((trimethylsilanyl)oxy)phenyl)butan-2-one **(15),** MS (m/z, %) 150 (5.0), 135 (6.4), 107 (15.0), 91 (5.3), 81 (13.4), 79 (20.0), 69 (100), 67 (11), 53 (15.4), 41 (81.2) and 4-(4-(*tert*-butylsilanyloxy)phenyl)butan-2-one **(16),** MS (m/z, %) 148 (20.6), 91 (61.1), 65 (22.3), 57 (100). 2-Methylchroman-7-ol **(17)** was synthesized by cyclodehydration of racemic 4-(3-hydroxybutyl)benzene-1,3-diol with *p*-toluensulfonic acid in toluene (Tsukayama et al. 1994). Flash chromatography gave **(17),** MS: (m/z, %) 164 (M^+ 100), 149 (96.7), 147 (20.2), 135 (14.3), 123 (95.9), 121.1 (24.6), 107 (22.6), 94 (25.6), 91 (14), 77 (15.9), 69 (11.4), 66 (18.8), 65 (17.6), 51 (10.1). 7-Hydroxy-3,4-dihydro-1*H*-naphthalen-2-one **(18),** was obtained by refluxing commercially available

7-methoxy-2-tetralone with sodium thioethoxide in dimethylformamide for 6 h (Kende and Rizzi 1981), MS: (m/z, %) 162 (M^+ 100), 147 (21.6), 133 (70.4), 119 (39.2), 118 (23.5), 91 (52.2), 90 (34.7), 63 (18.4), 44 (35.7).

Insects. Laboratory non-irradiated and irradiated melon fly pupae were obtained from the USDA-ARS-PBARC rearing facility in Honolulu, HI. Sixty mL of pupae (approximately 1,750 insects) were held in cubical cages (30 × 30 × 30 cm) or screened action packers as described in Jang et al. (2001). Flies were given sugar, water and hydrolyzed yeast protein. They were kept at 26°C and LD 12/12 h. Non-irradiated melon flies were released in the rotating outdoor olfactometer. Sterile (irradiated) melon flies (150 Gy, 2 d preemergence) were released in the open field tests. All released flies were at least 10 d old.

Outdoor rotatory olfactometer testing. Preliminary laboratory tests were conducted in a previously described device (Jang et al. 2001) on 19 test compounds plus cuelure, raspberry ketone, and an acetone control. Compounds (1 mg of test compound in 100 µL of acetone) were placed on dental wicks (1.25 cm) inside a Jackson trap with a sticky insert. Traps were randomly placed on each arm of the rotating hub. Tests were conducted from 0900 to 1500 h. Treatments were randomized to minimize positional effects of the treatments. One stock cage (1,750 insects) was released for each test. Temperature ranged from 23 to 26°C and relative humidity was 60 to 70%. Male and female trap captures were recorded at the end of the tests. All of our tests (olfactomer and field tests) measured the number of attracted male and female melon flies, but because of the negligible number of females attracted, only male catches are reported.

Field testing. Field tests were conducted in a macadamia nut orchard near Hilo, HI. The compounds tested were the trimethylsilyl ether **(15),** cuelure, raspberry ketone and an acetone control. Ten mg of each compound as a solution in 100 µL acetone was placed on a dental wick (1.25 cm) inside a Jackson trap with a sticky insert, and traps were hung within the perimeter canopy of every other tree and every row. There were 10 replicates placed in a randomized complete block design. Five action packers (3500 insects/action packer) were released (one action packer released per row). Temperature was 22 to 27°C and relative humidity was 60 to 90%. Traps were left in the field for 48 h, then male and female trap captures were recorded.

Statistical analyses. Statistical analyses were performed with the STATGRAPHICS (1994) software. Residuals were examined graphically for homogeneity and normality. The distribution of groups of attractants within the same category (similar attraction) was done with arcsine-transformed data, by means of the least significance difference (LSD) test ($P < 0.05$).

Results and Discussion

Because of its natural occurrence and accepted pivotal role in fruit fly taxonomy and evolution (Metcalf 1990), raspberry ketone is a logical departure point for the design of melon fly attractants. Conventional wisdom holds that raspberry ketone enjoys a relatively high inherent attractiveness, but suffers from a lack of volatility. More precisely, whether it is because of limited volatility or unfavorable interactions with a source matrix, raspberry ketone is not released into the air rapidly enough, or in sufficient quantity, to fulfill requirements for an ideal bait. In this regard, cuelure, although not particularly more volatile than raspberry ketone in terms of boiling point, tends to be more rapidly released from dispensers (e.g., cotton wicks) and has come to be the commonly-used attractant.

One of our initial objectives was to look for an analog that would retain structural features required for attractiveness but at the same time be more rapidly released from a bait matrix, either because of higher inherent volatility or more favorable release properties. Fluorine can mimic, at least in some cases, the effect of a hydroxyl group (Welch 1991), and with three non-bonded electron pairs, can also participate in hydrogen bonding. Fluorine atoms are only slightly larger than hydrogen atoms (Dixon and Smart 1991), and multiply-fluorinated compounds are sometimes more volatile than their hydrogen-containing counterparts. Compounds **(1)**, **(2)**, **(3)**, and **(10)** (Fig. 1) were evaluated in this context, along with carbonyl-reduced analogs **(4)** and **(5).** A somewhat analogous study of benzyl acetate derivatives was conducted by Metcalf et al. (1986) who tested the analogs against males of both *B. cucurbitae* and the oriental fruit fly, *B. dorsalis.* They found that a p-hydroxy group on the aromatic ring of benzyl acetate imparted the highest activity, analogous to the required p-OH of raspberry ketone. Virtually all alternative substitutions gave analogs with lower attractiveness. Ortho- and para-fluorobenzyl acetates retained some activity, but considerably less than that of the p-OH. Other fluorine substitutions decreased activity even further.

Similar to the results of Metcalf et al. (1986), all of our fluorine-containing analogs were less attractive than either raspberry ketone or cuelure. Consistent with the earlier conclusions, compound **(3)** (*p*-F substituted for *p*-OH) was the fluorine analog stimulating the highest response in the olfactometer tests. Compound **(5)**, in which the ketone group of **(3)** was reduced to a hydroxyl group, was also attractive. Even the less volatile diketone **(8)** showed attractiveness, in contrast to the other 2,4-diketone analogs illustrated in Figure 1. The remaining fluorine analogs displayed no significant attractiveness for male melon flies.

A few non-fluorinated analogs were also synthesized and evaluated. Neither of the bicyclic analogs **(17** or **18)** was attractive. The rationale for their synthesis was that if a raspberry ketone antennal receptor were to accommodate raspberry ketone in a folded configuration, one or the other of these compounds might be able to fit that receptor.

More encouraging results were realized with silyl ethers of raspberry ketone. In the olfactometer, trimethylsilyl ether **(15)** was 71% as attractive as raspberry ketone and 67% as attractive as cuelure. More importantly, the attractiveness of **(15)** in a field test was comparable to that of cuelure (no statistical difference in the LSD test ($\alpha = 0.05$)), and greater than that of raspberry ketone. Melon fly catches/trap/48 h were ($\bar{X} \pm$ SEM): trimethylsilyl ether **(15)** 141 ± 25; raspberry ketone 70 ± 23; cuelure 170 ± 30; acetone (5.4 ± 2.8). The ANOVA indicated that there are differences between the four compounds tested ($F = 21.17$, $p < 0.0001$), placing **(15)** and cuelure in a homogeneous group in the LSD test ($p < 0.05$). Raspberry ketone and the blank are in heterogeneous (independent) groups (with respect to all the other compounds) according to the LSD test. These latter results illustrate that completely parallel results between olfactometer and field results are not always obtained. In this case, cuelure and raspberry ketone were comparably attractive in the olfactometer (Table 1), both appearing more attractive than silyl ether **(15)**, but, in the field test, **(15)** and cuelure were comparably attractive, both catching at least twice the number of flies as raspberry ketone.

An unresolved issue is that of release rate vs. inherent attractiveness. Metcalf and

Table 1. Attraction of male melon flies to candidate attractants

Compound	Mean ± SEM	ANOVA groups*	Std. dev.
1	13 ± 2.43	e	5.43
2	11 ± 2.83	f	6.32
3	40 ± 8.45	c	18.9
4	21 ± 9.75	cd	21.8
5	39 ± 9.43	c	21.09
6	23 ± 5.77	d	12.91
7	18 ± 1.34	de	3
8	32 ± 7.64	c	39.46
9	16 ± 1.81	de	4.04
10	18 ± 3.90	de	8.72
11	14 ± 3.13	de	7
12	17 ± 2.11	de	4.72
13	12 ± 2.20	ef	4.93
14	31 ± 7.99	cd	17.86
15	94 ± 2.83	b	6.32
16	41 ± 7.83	c	17.5
17	19 ± 8.68	cd	19.4
18	15 ± 9.53	de	21.3
19	23 ± 3.98	cd	8.9
raspberry ketone	132 ± 17.98	a	40.21
cuelure	140 ± 15.19	a	33.96
acetone	16 ± 3.25	de	7.27

* Compounds with the same letter are not significantly different ($n = 4$, $P < 0.05$).

Metcalf (1992) reported that cuelure was released ~20 times faster than raspberry ketone from filter papers treated with 0.1 µg, and suggested that cuelure, as the acetate ester of raspberry ketone, was hydrolytically labile and that its activity probably resulted from hydrolysis in moist air to generate raspberry ketone. However, this theory has been questioned by various investigators as discussed in our recent paper (Casaña-Giner et al. 2003); for example, our analyses of material recovered from cotton wicks that had been maintained outdoors during an unusually rainy summer in Maryland indicated only about 7 to 15% conversion of cuelure to raspberry ketone in 50 d. In contrast, trimethylsilyl ethers, particularly of phenolic compounds, are known to be easily hydrolyzed (Greene and Wuts 1991), and although we have not conducted detailed studies on the hydrolysis of **(15)**, when cotton wicks impregnated with **(15)** were suspended in a chamber maintained at ~100% humidity, hydrolysis of **(15)** to raspberry ketone was complete within 3 to 4 d. The field test with **(15)** described in this paper was of only 2 d duration, and the facile conversion of **(15)** to raspberry ketone just mentioned seems to predict that **(15)** would not outperform raspberry ketone over a multi-week test. Because organosilicon compounds are not known to occur naturally, there is little reason to expect inherent attractiveness from a trimethylsilyl ether, and therefore it's plausible that the role of the trimethylsilyl group of **(15)** could be that suggested by Metcalf and Metcalf (1992) for the acetate of cuelure—to facilitate the transport of raspberry ketone from the dispensing matrix into the atmosphere.

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