Synthesis and Field Tests of Analogues of the Housefly Pheromone (Z)-9-Tricosene

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Analogues of the housefly pheromone (Z)-9-tricosene have been synthesized and tested. All of the analogues have the same chain length as (Z)-9-tricosene, but they have a functional group substituted for one or more carbons. The field results suggest that steric effects correlate with pheromone activity in the analogues.

The isolation and identification of (Z)-9-tricosene (1) as an attractant pheromone for the male housefly, *Musca domestica*, was reported by Carlson et al. (1971). These workers demonstrated that 1, which they named "musculure", was attractive to male houseflies in laboratory tests (Carlson et al., 1974) and to male and female houseflies in field tests (Carlson and Beroza, 1973). These workers also prepared hydrocarbon analogues of 1 which contained a 9-alkyne or trans 9-alkene function instead of the cis 9-alkene group of 1 or which were longer or shorter than 1. Although many of these analogues retained some attractant activity, few were as active as 1 itself (Carlson et al., 1974).

We have synthesized a number of analogues of 1 also. Unlike previous work, however, we have retained the 23-atom chain length by substituting one or more heteroatoms or other functional groups into the skeleton of 1. We have also carried out field tests to determine the relative attractant activity of these analogues. Our goals were to learn more about the molecular features of 1 which make it an effective pheromone and, perhaps, to synthesize an analogue of 1 which would be nearly as effective an attractant but which would be less expensive to synthesize. Such a result could lead to an artificial housefly attractant that could be used to bait poison or traps, thus reducing the need to disperse nonspecific pesticides over a large area.

EXPERIMENTAL SECTION

Instruments. Proton NMR spectra were recorded on a Varian EM-360L NMR spectrometer. The solvent was CDCl$_3$ with Me$_4$Si as internal standard. Infrared spectra were recorded neat on a Perkin-Elmer 283 spectrophotometer. Kugelrohr distillations were carried out with an apparatus supplied by Aldrich Chemical Co. Microanalyses were conducted by Atlantic Microlab, Inc., Atlanta, GA.

Synthesis of (Z)-14-Tricosen-2-one (2). The ketone was synthesized by a modification of the method of Cargill and Rosenblum (1972). Erucic acid (29.2 g, 0.085 mol, Pfaltz and Bauer) and *o*-phenanthroline (5 mg, Fisher) were dissolved in anhydrous ether (100 mL) in a three-necked flask equipped with a mechanical stirrer and reflux condenser and arranged for N$_2$ atmosphere. Methyllithium (120 mL, 0.17 mol, 1.44 M by titration, Alfa) in ether was added by syringe at a rapid rate while the solution was stirred vigorously. The mixture was refluxed for 1 h and cooled and was added dropwise to a rapidly stirred dilute HCl solution. The ether and aqueous layers were separated. The aqueous phase was extracted with ether. The

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combined ether portions were washed with saturated NaHCO$_3$, washed with water, and dried over anhydrous MgSO$_4$. Removal of the solvent and distillation (210–215 °C at 1 torr) gave 25.4 g (87%) of 2 as an oil [literature bp 140 °C at 0.1 torr; Cargill and Rosenblum (1972)]: NMR δ 0.9–2.6 (m, 42 H, –CH$_3$–, –CH$_2$–, and –COCH$_3$), 5.28 (t, J = 4.5 Hz, 2 H olefinic); IR 3005 (olefinic C–H) and 1720 cm^{-1} (C=O).

Synthesis of (Z)-14-Tricosen-6-one (3). A three-necked, round-bottom flask equipped with nitrogen inlet, mechanical stirrer, and condenser was charged with anhydrous ether (125 mL) and magnesium turnings (4.38 g, 0.18 mol), and *n*-pentyl bromide (30.06 g, 0.2 mol, Fisher) was added dropwise. After the reaction was complete, anhydrous CdCl$_2$ (16.5 g, 0.09 mol) was added, and the mixture was cooled on an ice bath for 3 min. The solution was allowed to warm to room temperature and then was refluxed for 1.5 h. It was then cooled on an ice bath and volume loss replaced with benzene. Most of the remaining ether was then distilled from the reaction vessel and was replaced by benzene. Oleoyl chloride (45.15 g, 0.15 mol, Eastman) in benzene (100 mL) was added dropwise with ice bath cooling. The ice bath was removed and the solution was refluxed for 2 h. The cooled reaction mixture was added to dilute sulfuric acid. The layers were separated, and the water layer was extracted twice with benzene. The combined benzene portions were washed twice with saturated NaHCO$_3$ and with saturated NaCl, then dried over anhydrous magnesium sulfate. Removal of benzene and distillation gave 30 g (59%) of an oil: bp 200–210 °C at 2 torr [literature bp 170–174 °C at ca. 0.6 torr; Ho and Wong (1974)]; NMR δ 0.9–2.6 (m, 42 H, –CH$_3$ and –CH$_2$–), 5.28 (t, J = 4.5 Hz, 2 H olefinic); IR 3010 (olefinic C–H), 1720 cm^{-1} (C=O).

Synthesis of Butyl Oleyl Ether (4). Sodium (2.33 g, 0.10 mol) was placed in a three-necked, 250-mL, round-bottom flask equipped with nitrogen inlet, mechanical stirrer, and drying tube, and 1-butanol (36.6 mL, 0.4 mol) was added to the flask. The reaction mixture was left in a hood overnight, by which time evolution of hydrogen had ceased and all of the sodium appeared to have reacted. Oleyl tosylate (17 g, 0.04 mol, made from oleyl alcohol and tosyl chloride) dissolved in 1-butanol (9.15 mL, 0.1 mol) was added. The solution, which began to turn yellow immediately, was packed in ice and allowed to warm to room temperature over 15 h. The reaction mixture was washed with water. The organic phase was dissolved in ether and exracted with 10% Na$_2$CO$_3$ and with saturated NaCl. The ether phase was dried over anhydrous potassium carbonate and filtered, and the solvent was removed on a rotary evaporator. The resulting pale-orange oil was distilled (155–168 °C at 0.6 torr) to yield 8.2 g (64%) of 4 as an oil: NMR δ 0.9–2.2 (m, 38 H, –CH$_3$ and –CH$_2$–), 3.2 (t, J = 6 Hz, 4 H, –CH$_2$–O), 5.28 (t, J = 4.5 Hz, 2 H,

Table I. Structures and Results

no.	structure	no. tests	mean activ	bp[a]
1	$CH_3(CH_2)_7CH=CH(CH_2)_7CH_2CH_2CH_2CH_2CH_2CH_3$	18	4.2	390° [b]
2	$CH_3(CH_2)_7CH=CH(CH_2)_7CH_2CH_2CH_2CH_2C(=O)CH_3$	10	2.0	430°
3	$CH_3(CH_2)_7CH=CH(CH_2)_7C(=O)CH_2CH_2CH_2CH_2CH_3$	8	1.2	405°
4	$CH_3(CH_2)_7CH=CH(CH_2)_7CH_2OCH_2CH_2CH_2CH_3$	20	1.1	380°
5	$CH_3(CH_2)_7CH=CH(CH_2)_7CH_2SCH_2CH_2CH_2CH_3$	15	1.8	415°
6	$CH_3(CH_2)_7CH=CH(CH_2)_7C(=O)OCH_2CH_2CH_2CH_3$	9	1.1	400°
7	$CH_3(CH_2)_7CH=CH(CH_2)_7CH_2CH_2CH_2CH_2C\equiv N$	8	1.0	430°

[a] Experimental boiling points measured at reduced pressure were corrected to atmospheric pressure (Gordon and Ford, 1972, p 36).   [b] Corrected from reduced pressure boiling point given by Cargill and Rosenblum (1972).

olefinic); IR 3010 (olefinic C–H), 1120 cm^{-1} (C–O). Anal. Calcd.: C, 81.41; H, 13.66. Found: C, 81.44; H, 13.68.

Synthesis of Butyl Oleyl Sulfide (5). Sodium (1.5 g, 0.05 mol) was added to n-butyl mercaptan (9.02 g, 0.10 mol, Eastman) in a dry three-necked, 250-mL, round-bottom flask equipped with drying tube, magnetic stirrer, stopper, and nitrogen inlet. The reaction mixture was cooled in an ice bath. As the reaction progressed, the mixture began to solidify, so sufficient (ca. 5 mL) mixed xylenes was added to effect solution. At the end of 6 h, evolution of hydrogen was no longer apparent. Oleyl tosylate (16.94 g, 0.04 mol) was added to the solution with stirring. The flask was packed in ice and allowed to warm over 15 h. The reaction mixture was dissolved in ether and extracted with water, 10% NaHCO$_3$, and saturated NaCl. The ether phase was dried over anhydrous potassium carbonate and filtered, and the ether was removed on a rotary evaporator. Vacuum distillation [165 °C (0.5 torr)–190 °C (0.65 torr)] produced an oil with a faint yellow color and an odor characteristic of mercaptans. Lithium aluminum hydride was added to the oil and it was allowed to sit overnight, then filtered, and redistilled. The product was a faintly yellow oil with a slight mercaptan odor. The yield was 6.6 g (48.4%) collected from 212 °C (2.8 torr) to 223 °C (2.7 torr): NMR δ 0.9–2.3 (m, 38 H, –CH$_3$ and –CH$_2$–), 2.5 (m, 4 H, –CH$_2$–S), 5.3 (t, J = 4.5 Hz, 2 H, olefinic); IR 3005 cm^{-1} (olefinic C–H). Anal. Calcd.: C, 77.57; H, 13.02; S, 9.41. Found: C, 77.53; H, 13.02; S, 9.40.

Synthesis of Butyl Oleate (6). Anhydrous pyridine (6 mL) and n-butyl alcohol (3.0 g, 0.04 mol) were added to a 25-mL, round-bottom flask equiped with a magnetic stirrer. The mixture was cooled to 10 °C and oleoyl chloride (10.0 g, 0.033 mol, Eastman) was added dropwise to the mixture. The reaction mixture was stirred for 30 min and filtered, and the residue was washed with ether. The combination of organic phases was dried over anhydrous sodium sulfate. The volatiles were removed and the residual oil was subjected to kugelrohr distillation. The yield was 9.4 g (84%) collected at 142–152 °C (0.1 torr): NMR δ 0.9–2.4 (m, 38 H, –CH$_3$ and –CH$_2$–), 4.0 (t, J = 6 Hz, 2 H, –CH$_2$–O), 5.28 (t, J = 4.5 Hz, 2 H, olefinic); IR 3005 (olefinic C–H), 1740 (C=O), 1175 cm^{-1} (C–O); literature bp 204–208 °C at 3 torr (Reid et al., 1935).

Synthesis of Erucyl Nitrile (7). The method used was that of Stephens et al. (1955). Erucylamide (10.0 g, 0.03 mol, Pfaltz and Bauer) was added to anhydrous pyridine (20 mL) in a 100-mL, round-bottom flask and stirred for 10 min. Tosyl chloride (5.72 g, 0.03 mol) was added slowly with stirring and cooling. The mixture was stirred for 6 h at room temperature, refluxed for 5 h, then stirred overnight at room temperature. The product mixture was diluted with ether (150 mL) and filtered. The filtrate was washed with 5% HCl, 5% NaHCO$_3$ (2×), and water (2×). It was dried, and the volatiles were removed under vacuum. The resulting oil contained some solid. It was dissolved in hexane (50 mL), cooled to 0 °C, and filtered cold. The hexane was removed under vacuum to

yield an oil. Kugelrohr distillation [175 °C (0.1 torr)] gave 4.3 g (45%) of an oil [literature bp 213–240 °C (13 torr); Reutenauer and Paquot, 1947]: NMR δ 0.9–2.4 (m, 39 H, –CH$_3$ and –CH$_2$–), 5.3 (t, J = 4.5 Hz, 2 H, olefinic); IR 3005 (olefinic C–H), 2225 cm^{-1} (CN).

Field Testing Pocedures. The field test procedure was similar to a method reported by Carlson and Beroza (1973). Commercial (Aeroxon) adhesive-coated paper strips (4 × 300 cm) were used as fly traps. The attractant (50 ± 5 mg) was dissolved in 0.5 mL of hexane in a vial. About 1 mL of ground corn cobs sifted through wire mesh was added to the solution, and the solvent was allowed to evaporate overnight. The resulting 1 mL of ground cobs was sprinkled over the adhesive strip at the field site. Ground cobs treated only with hexane sprinkled on other adhesive strips served as controls.
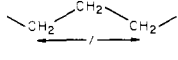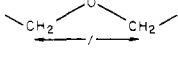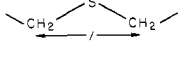
The test site was a horse stable near Huntersville, NC. Four stalls of equal size opened to the outside on the east and west sides of the 13 × 12 m structure. These stalls were protected by a roof extending 2 m beyond each side of the stable. The test strips were hung vertically from the roof beams and were about 2.5 m above the ground and 0.5 m from the outer wall of the stable. Eight testing sites, four on each side of the stable, were selected so that each was directly in front of a stall. The baited and unbaited (control) strips were hung 1.3 m apart. The baited strips were at least 2 m apart. The compounds were initially assigned test positions at random, but almost all of the analogues were tested in all eight locations. Four compounds were tested on each side of the stable each day. Test strips were installed about 9:00 a.m. and retrieved at the same time the following day. Initially, tests were conducted every weekday during June and July, 1978, but later tests were carried out only every other day. The relative attractant power of a strip was taken as the number of flies caught by a baited strip in a 24-h period divided by the average number of flies caught by all the unbaited strips on that side of the stable during the same 24-h period.

RESULTS AND DISCUSSION

Table I lists the compounds synthesized and tested in this study. All of them have the same chain length as 1, but each has a one or two atom substitution for CH$_2$ or CH$_3$ groups in 1. Although only a small portion of the muscalure structure is modified in these analogues, a variety of functional groups are included. These compounds allow us to study the effect of molecular modification of at least portions of 1, even though the same functional group substitutions might have different effects at other positions.

Four of the analogues contain a 17-atom fragment identical with a portion of 1. This fragment is contained in oleic acid, so it was a logical starting point in the synthesis of these analogues. The ketone 3 and ester 6 were synthesized from oleoyl chloride, and the ether 4 and sulfide 5 were made from oleyl alcohol. Two of the ana-

Table II. Some Physical Properties of (Z)-9-Tricosene Heteroatomic Analogues[a]

compound	X	chain structure in C_{18}–C_{20} position	C–X bond length, Å	CXC bond angle, deg	l, Å	local dipole moment (D)[b]	X van der Waals radius, Å	activity rating
1	CH_2	(CH₂ chain, CH₂ branch)	1.54	112.6	2.55	<0.1	2.0	4.2
4	O	(CH₂ chain, O branch)	1.43	110	2.34	1.15	1.4	1.1
5	S	(CH₂ chain, S branch)	1.82	109	2.96	1.54	1.85	1.8

[a] Physical constants are obtained from tables in Gordon and Ford (1972), pp 108–112.   [b] Estimates based on small molecule values (n-C_3H_8, $C_2H_5OC_2H_5$, $C_2H_5SC_2H_5$) tabulated in the "CRC Handbook of Chemistry and Physics" (1969).

logues have a 21-atom portion in common with 1, and it is contained in erucic acid. The nitrile 7 was obtained by dehydration of commercially available erucylamide. The ketone 2 was synthesized from erucic acid via a modification of the procedure reported by Cargill and Rosenblum (1972). All of these syntheses began with Z starting materials which should not have isomerized under the experimental conditions. Carlson and Beroza (1973) reported using a synthetic mixture of 85% Z and 15% E 9-tricosene and noted that using pure Z isomer did not significantly improve activity. Although we did not determine precise E/Z ratios of the analogues synthesized, small concentrations of E isomers should not significantly affect our results.

We conducted field tests of the natural pheromone (obtained as 97% Z isomer from Aldrich Chemical Co.) and of our analogues during the summer of 1978. The value of mean activity listed in Table I reflects the number of flies caught by adhesive strips baited with the test compound relative to the number of flies caught by unbaited test strips. An activity ratio of 1 means that the compound tested did not increase the number of flies caught by adhesive strips. All but one of the analogues have a mean activity greater than 1, but none of the analogues are as effective as (Z)-9-tricosene in attracting flies. Muscalure and the analogues appeared to be equally attractive to both males and females in our field tests. This result is consistent with the finding of Carlson and Beroza (1973) for field tests of muscalure.

Variations in pheromone analogue activity may be due to microscopic properties (molecule–receptor interactions) or macroscopic properties (volatility, etc.) or both. Although all of our analogues are quite similar to 1, there are differences in their relative volatilities. Table I lists the expected boiling points at atmospheric pressure for the compounds studied. With the exception of 4, the analogues are estimated to boil from 10 to 40 °C higher than 1. Decreased volatility may tend to make attractant activity less apparent since the concentration of attractant in the air around an adhesive strip would be smaller for a less volatile chemical. Furthermore, the change in concentration as a function of distance from the test strip would be less pronounced, so any attractant receptor system in the flies which depends on concentration gradients in the air should be less effective. For the analogues listed in Table I, however, there appears to be little correlation between boiling points and activity values. We infer that volatility differences among these compounds are less important in determining activity than are specific molecule–receptor interactions produced by the various functional group incorporations.

Functional group incorporations alter the parent pheromone both sterically and electronically. One goal of the present work was to determine whether activity could be correlated better with one or the other property. Considering only the molecular shapes first, compounds 1, 5, and 7 are unbranched structures, while compounds 2, 3, and 6 contain a C=O "branch". Carlson et al. (1974) noted that a methyl branch on the penultimate carbon of (Z)-9-tricosene reduced the attractant activity to 29% of the activity of 1. The penultimate carbonyl in 2 reduces the activity to 48% of that of 1. Such a result could be attributed to the smaller "size" (van der Waals radius) of the carbonyl oxygen relative to a methyl group. Carlson et al. did not report the effects of branching at C-18 of 1, but the ketone 3 and the ester 6 show very little activity.

Comparison of compounds 1, 4, and 5 indicates that substitution of a single heteroatom for a methylene unit at the C-19 position of 1 decreases attractant effectiveness. Table II shows some effects expected from these substitutions. Even though the C-18–C-20 separation and approximate local dipole moment of the ether more closely match the corresponding properties of 1 than do the same properties of 5, the sulfide shows greater activity. Of the data listed, the van der Waals radius of the heteroatom seems to be the only factor one could use to correlate structure with activity.

It seems intuitively unlikely that steric effects alone should account for the variation in activity among our analogues, and consideration of the nitrile 7 supports this skepticism. The internuclear separation between C-21 and N in 7 is about 2.59 Å, very close to the C-21–C-23 distance (2.55 Å) expected for 1. The lack of activity of 7 implies that the electronic effect of the terminal cyano group may be important in reducing the response of flies to this chemical. Because the geometry of the terminal portion of 7 is linear and not segmented tetrahedral, however, steric factors may still be important.

In conclusion, steric factors appear to correlate with the activity of our (Z)-9-tricosene analogues although further work would be needed to determine the generality of the relationship. A practical result of our work is the finding that some substitution in the (Z)-9-tricosene structure is possible without destroying all attractant activity. Because 2 is a precursor of 1 in one synthetic scheme and because the synthesis of 5 requires only inexpensive reagents, these analogues might be used to help control fly populations in spite of their having lower attractant activity than (Z)-9-tricosene.

LITERATURE CITED

Cargill, R. L., Rosenblum, M. G., J. Org. Chem. 37, 3971 (1972).
Carlson, D. A., Beroza, M., Environ. Entomol. 2, 555 (1973).
Carlson, D. A., Doolittle, R. E., Beroza, M., Rogoff, W. M., Gretz, G. H., J. Agric. Food Chem. 22, 194 (1974).
Carlson, D. A., Mayer, M. S., Silhacek, D. L., James, J. D., Beroza, M., Bierl, B. A., Science 174, 76 (1971).
Gordon, A. J., Ford, R. A., "The Chemist's Companion", Wiley, New York, 1972.

Ho, T.-L., Wong, C. M., *Can. J. Chem.* **52,** 1923 (1974).
Reutenauer, G., Paquot, C., *Compt. Rend.* **224,** 478 (1947).
Reid, E. E., Cockerile, F. O., Meyer, J. D., Cox, M. W., Jr., Ruhoff, J. R., *Org. Synth.* **15,** 51 (1935).
Stephens, C. R., Bianco, E. J., Pilgrim, F. J., *J. Am. Chem. Soc.* **77,** 701 (1955).

Weast, R. C., Ed., "Handbook of Chemistry and Physics", 50th ed, Chemical Rubber Co., Cleveland, OH, 1969, pp E-70–E-71.

Wine Headspace Analysis. Reproducibility and Application to Varietal Classification

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Wine headspace (HS) volatiles were collected by displacement and analyzed by gas chromatography (GC), GC–MS, and sensory evaluation of the split GC effluent. Over 77% of the peak areas quantified in triplicate had coefficients of variation <0.10. Nineteen peaks were selected for principal component analysis (PCA) and stepwise discriminant analysis (SDA). The first principal component (PC 1), although accounting for only 38% of the variance, separated 10 of the 11 White Riesling wines (R) from the nine Chardonnays (C) and four French Colombards (F). Weighted >0.50 on PC 1 were were eight components, some of which had fruity, floral descriptions, consistent with the aroma of R. Weighted <-0.50 on PC 1 were three "fruity" esters, also consistent with the characteristic aromas of C and F. Interestingly, linalool, often thought to be important in R, was not weighted heavily on PC 1. By SDA, the 24 wines were sorted successfully into three varietal categories by discriminant functions using five components.

To distinguish among grape varieties, multivariate statistical techniques, including discriminant and cluster analyses, have been successfully applied to volatile composition, obtained by solvent extraction (Schreier et al., 1976; Rapp and Hastrich, 1978; Rapp et al., 1978), to trace element composition (Siegmund and Bachmann, 1978), and to a combination of compositional data including elemental composition, alcohols, and total acidity (Kwan and Kowalski, 1978). However, to investigate the important differences in the aromas of wines from different grape varieties, it is more appropriate to analyze the volatiles in the headspace at equilibrium concentrations and, further, to concentrate upon those components which are of sensory significance.

To evaluate the quantitative differences in volatile composition among wines, the technique of headspace analysis by displacement has been used by several workers (Bertuccioli and Montedoro, 1974, 1975; Bertuccioli and Viani, 1976; Coope, 1977; Noble, 1978). Sensory analysis of gas chromatographic effluent (GC–sniff) has been used by several investigators to evaluate the "aroma significance" of separated components in coffee (Tassan and Russell, 1974), bilberries (Von Sydow et al., 1970), peaches (Spencer et al., 1978), and wine (Noble, 1978; Rapp et al., 1978).

Although the volatiles of White Riesling have been studied extensively by extraction procedures (Van Wyck et al., 1967a,b,c; Shreier and Drawert, 1974, 1976; Schreier et al., 1974, 1975, 1977), no volatile investigations of

Table I. Description of Wines Evaluated

grape variety	code	vintage year	grape source[a] (county)	producer[b]
White Riesling	1	73	Santa Barbara	UC
	2	74	Butte	UC
	3	74	Napa[c]	UC
	4	74	Napa[c]	UC
	5	74	Sonoma[d]	UC
	6	74	Sonoma[d]	UC
	7	75	Napa[e]	UC
	8	75	Napa[e]	UC
	9	74	Napa	Comm
	10	74	Napa	Comm
	11	73	Napa	Comm
Chardonnay	12	73	Alameda	UC
	13	75	Alameda[f]	UC
	14	75	Alameda[f]	UC
	15	75	Napa	UC
	16	75	Napa[g]	UC
	17	75	Napa[g]	UC
	18	73	Salinas	UC
	19	73	Salinas	Comm
	20	75	Sonoma	UC
French Colombard	21	73	Mendocino	Comm
	22	74	Solano	UC
	23	nonvintage	Fresno	Comm
	24	75	Colusa	UC

[a] Wines made from the same vineyard have a common superscript. [b] UC = University of California, David Winery; Comm = commercial.

Chardonnay and French Colombard wines have been reported.

In this study, headspace volatiles of wine of three *Vitis vinifera* grape varieties, White Riesling, Chardonnay, and French Colombard, were analyzed by conventional gas chromatography [GC(FID)], by gas chromatography–mass

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