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Analysis of 2,4,6-Nonatrienal Geometrical Isomers from Male Flea Beetles, *Epitrix hirtipennis* and *E. fuscula*

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Geometrical isomers of 2,4,6-nonatrienal have been reported from a variety of food- and insect-related sources. It was discovered recently that the eggplant flea beetle, *Epitrix fuscula*, uses the (2*E*,4*E*,6*Z*) and (2*E*,4*E*,6*E*) isomers as components of its male-produced aggregation pheromone. Here, we learned that the related species, *E. hirtipennis*, also emits a blend of 2,4,6-nonatrienals, including isomers not previously characterized. Patterns in emission and response suggest a pheromonal function. In an effort to acquire standards to aid in identification, we found that exposing (2*E*,4*E*,6*E*)-2,4,6-nonatrienal (or other available 2,4,6-nonatrienals) to light readily generated a mixture of six geometrical isomers. Configurations of these were determined by NMR, and chromatographic properties (GC and HPLC) were documented. On the basis of chromatographic comparison to these standards, the most abundant, new compound from *E. hirtipennis* was concluded to be (2*E*,4*Z*,6*Z*)-2,4,6-nonatrienal. Minor components from both *E. hirtipennis* and *E. fuscula* were also characterized. The analytical approach given here would also be of use in the food industry, where 2,4,6-nonatrienals are important as aroma compounds.

KEYWORDS: 2,4,6-Nonatrienal; *Epitrix hirtipennis*; *Epitrix fuscula*; pheromone; food volatiles; photoisomerization; GC; HPLC; NMR

INTRODUCTION

2,4,6-Nonatrienals have been detected in a wide array of foods and are important as aroma compounds. Three geometrical isomers have been characterized: (2*E*,4*E*,6*E*,)-2,4,6-Nonatrienal was reported from dried beans (*1*), cooked spinach (*2*), and oat flakes (*3*); (2*E*,4*E*,6*Z*)-2,4,6-nonatrienal was found to occur in oat flakes (*3*) and black tea (*4*); and (2*E*,4Z,6*E*,)-2,4,6-nonatrienal was detected in oat flakes (*3*). Syntheses, mass spectra, and NMR spectra were reported for these isomers (*1, 3*). (2*E*,4*E*,6*Z*)-2,4,6-Nonatrienal is one of the most odor-active aroma compounds ever detected in food (*3*). There have been other reports of 2,4,6-nonatrienals in foods with unspecified double-bond configurations (*5–9*). The 2,4,6-nonatrienals in food can arise from the oxidation of linolenic acid by lipoxygenases (*10, 11*). Schuh and Schieberle (*3*) demonstrated that 2,4,6-nonatrienal can be formed by either enzymatic degradation or autoxidation of linolenic acid.

2,4,6-Nonatrienals have also been reported from insects. The (2*E*,4*E*,6*Z*) and (2*E*,4*E*,6*E*) isomers were identified from the eggplant flea beetle, *Epitrix fuscula* (Crotch) (Coleoptera: Chrysomelidae) as components of the male-produced aggregation pheromone, and their syntheses and mass- and NMR spectra were reported (*12*). Traps baited with these nonatrienals attracted significantly more *E. fuscula* males and females than did unbaited control traps.

Here, we report that the tobacco flea beetle, *Epitrix hirtipennis* (Melsheimer) (*13*) was also attracted to the *E. fuscula* pheromone in a field test, suggesting the species have related pheromones and prompting us to examine volatile emissions from *E. hirtipennis*. A blend of 2,4,6-nonatrienals was found, one main component of which had not been previously characterized. Geometrical isomer standards were needed to aid identification but were not commercially or otherwise available. Synthesis of the eight possible isomers would not be trivial, especially since these aldehydes are very susceptible to photoisomerization (*12, 14*). However, this tendency to isomerize in light proved to be very useful to us: Six of the eight possible 2,4,6-nonatrienal geometrical isomers were readily generated from a single isomer by exposure to light; these were characterized by MS, NMR, GC, and HPLC and thus could serve as the needed standards. One of these matched the unknown isomer from *E. hirtipennis*. Therefore, the project led to a convenient approach for identification of 2,4,6-nonatrienal isomers, from insects or food materials.

MATERIALS AND METHODS

Field Response of *Epitrix hirtipennis*. As described previously (*12*), field experiments were conducted during 2005 to assess the activity of synthetic pheromone for *E. fuscula*, versus controls. Pheromone lures

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contained (2*E*,4*E*,6*Z*)-2,4,6-nonatrienal (85%) and (2*E*,4*E*,6*E*)-2,4,6-nonatrienal (15%); these were prepared fresh on each day of the study. Examination of beetles in trap catches during the period, August 11−19, (N = 25 for each treatment) revealed considerable numbers of *E. hirtipennis*. These data were submitted to analysis of variance after transformation to $\log(x + 1)$.

Volatiles from *Epitrix* Beetles. Flea beetles, including both *E. hirtipennis* and *E. fuscula*, were netted in the eggplant research plot at NCAUR. Under a microscope, these were separated by species (*13*) and by sex, using the abdominal characteristics described for *Phyllotreta* spp. (*15*). Volatiles were collected from groups of 15−18 male *E. hirtipennis* feeding on eggplant leaves, generally as described previously (*12*), except that the beetles and plants were held in Teflon bottles (500 mL, 6.5 cm diameter × 16 cm height) instead of the usual glass tubes and the Super-Q volatile-collection filter was covered with aluminum foil. These precautions were taken to minimize contact with potentially active glass surfaces and exposure to light. A gentle vacuum drew air through the system at 300 mL/min. The filters were rinsed with 400 μL of hexane every 2−3 days into amber colored vials. Parallel collections were made from *E. fuscula* males at the same time, for comparison. The 39 volatile collections from each species were combined, concentrated to 40 μL, and subsequently used for HPLC and GC. Storage was in the dark at −20 °C. For comparison, 43 collections were also made from *E. hirtipennis* females.

Photoisomerization of 2,4,6-Nonatrienals. A hexane solution of (2*E*,4*E*,6*E*)-2,4,6-nonatrienal (1 mL containing 100 μg, in a clear glass vial with Teflon-lined cap) was exposed to full sunlight outside for 240 min between 9:00 a.m. and 1:00 p.m. As a control, an identical sample was wrapped in aluminum foil to prevent light penetration and then similarly exposed.

Alternatively, similarly prepared samples were placed in a sunlight simulator (Suntest CPS, Atlas, Gainesville, FL). Samples included (2*E*,4*E*,6*Z*)-2,4,6-nonatrienal, (2*E*,4*E*,6*E*)-2,4,6-nonatrienal, and two mixtures previously created by light exposure and then partially purified by HPLC: a 95:5 mixture of (2*Z*,4*E*,6*E*)- and (2*Z*,4*E*,6*Z*)-2,4,6-nonatrienal, and a 15:85 mixture of (2*E*,4*Z*,6*Z*)- and (2*E*,4*Z*,6*E*)-2,4,6-nonatrienal. Each sample vial was placed 21 cm below the light source for 240 min. Temperatures at the sample level did not exceed 35 °C. Total irradiance between 300 and 1100 nm was previously determined (*16*) to be 891.5 W/m^2. Immediately after exposure, samples were analyzed by GC-MS. To characterize isomerization over time, samples of the (2*E*,4*Z*,6*Z*)- plus (2*E*,4*Z*,6*E*)-2,4,6-nonatrienal mixture were analyzed by GC-MS following light exposures of 45, 90, 240, and 450 min.

Coupled Gas Chromatography–Mass Spectrometry (GC-MS). GC-MS analysis was performed using a Hewlett-Packard 5973 mass selective detector, interfaced to a Hewlett-Packard 6890 GC (Agilent, Technologies Inc., Santa Clara, CA), equipped with well-maintained cool-on column (COC) and split/splitless inlets. A Stabilwax-DA capillary column (15 m × 0.25 mm, 0.10 μm film thickness, Restek, Bellefonte, PA) was used. This column was chosen for its high polarity, which was beneficial for separation of the geometrical isomers, but it also had a very thin film, which permitted analyses to be completed at low temperatures and minimized any possible thermal degradation. The oven program began at 40 °C for 1 min., increased at a rate of 25 °C /min to 75 °C, and held at 75 °C for 44 min. Unless otherwise noted, COC inlet temperature tracked (was always 3 °C hotter than) the oven. The transfer line temperature was 75 °C.

Samples containing either single isomers or mixtures of two isomers of 2,4,6-nonatrienals were also injected at each of four inlet temperatures: 100, 150, 200, and 250 °C, through both COC and splitless inlets. Comparisons of the chromatograms provided information about thermal degradation. The splitless-type inlet is more common in laboratories but would likely give longer exposure to hot surfaces than the COC inlet.

High-Performance Liquid Chromatography (HPLC). HPLC was done on a Supelcosil LC-SI silica column (25 cm, 0.46 cm ID, 5 μm particle size, Supelco, Bellefonte, PA) attached to a Waters 515 pump (Waters, Milford, MA). The solvent system was 5% ether (distilled to remove the preservative, BHT) in hexane with a flow rate of 2 mL/min. A Waters R401 differential refractometer detector attached to a HP 3396 Series III integrator (Hewlett-Packard, Palo Alto, CA) was used to guide the collection of fractions. Samples were in hexane, and the injection volume was usually 10 μL, containing up to 2 mg of 2,4,6-nonatrienal. Fractions were collected in vials covered in aluminum foil to minimize the exposure to light and were stored at −5 °C until NMR or GC-MS analysis. Laboratory lights were kept as dim as possible. In preparation for NMR analysis of compounds in the photoisomerized sample, a second HPLC pass was sometimes done to remove traces of components eluting in adjacent HPLC fractions.

Nuclear Magnetic Resonance Spectroscopy (NMR). NMR spectra were acquired on a Bruker Avance 500 instrument (Bruker BioSpin Corp, Billerica, MA) with a 5 mm inverse broadband probe with a Z-gradient. Proton, COSY, and HSQC spectra were obtained at 500 MHz in CD_2Cl_2 (this solvent was used instead of $CDCl_3$ to ensure that the solvent signal would be well-separated from sample resonances and also to avoid possible adverse effects of residual acid in the solvent). As previously described (*12*), the COSY spectra of 2,4,6-nontrienals could be used to assign the olefinic proton resonances along the chain, by starting from the readily recognized aldehydic or alkyl ends. Then, the olefinic coupling constants for particular double bonds could be read from the ^{1}H spectrum. Configurations of *E* or *Z* were assigned based on the values of these constants, with *E* corresponding to $J >$ 12.0 Hz and *Z*, to $J <$ 12.0 Hz (*17*). In two cases, GC analyses of NMR samples indicated that pairs of isomers were present, but proton integrations and COSY spectra readily established which resonances belonged to each isomer, and the integrations linked the spectra to the appropriate GC peaks. Samples of (2*E*,4*E*,6*Z*)- and (2*E*,4*E*,6*E*)-2,4,6-nonatrienal, which had previously been run in $CDCl_3$ and C_6D_6 (*12*), were reanalyzed in CD_2Cl_2 during this study for comparison. ^{13}C spectra were not generally acquired due to small sample amounts, and the reported ^{13}C shifts were obtained from HSQC spectra.

Identification of 2,4,6-Nonatrienals from *Epitrix* Beetles. The volatile collections (above) were analyzed by GC-MS. As a class, 2,4,6-nonatrienals were recognized by mass spectrum (*12*), and the *m*/*z* 136 ion was integrated to determine relative amounts of isomers. The mixture from males for each species was submitted to HPLC, and 1 mL fractions were collected, without regard to detector peaks. Then, each HPLC fraction was analyzed by GC-MS. An unknown 2,4,6-nonatrienal isomer was considered to be identified when it produced the characteristic mass spectrum and matched one of the standards generated through photoisomerization with respect to GC retention and relative HPLC retention.

RESULTS AND DISCUSSION

Field Responses of *E. hirtipennis* and Initial Examination of Volatiles. More *E. hirtipennis* were captured on the traps baited with *E. fuscula* pheromone than on controls. Means were 4.92 ± 1.25 (SE) for the pheromone and 2.72 ± 0.67 (SE) for the control (N = 25). Analysis of variance after transformation indicated a significant difference between treatments, suggesting that *E. hirtipennis* had a pheromone related to that of *E. fuscula* (F = 5.76, df = 1, 17, P = 0.028, considering only the 18 blocks for which total of treatment plus control > 0).

The 2,4,6-nonatrienals in the volatile collections from male *E. hirtipennis* showed differences from *E. fuscula* (**Figure 1**). The profile for *E. fuscula* contained two prominent peaks, as expected (*12*), with the major one being the (2*E*,4*E*,6*Z*) isomer and the minor one, the (2*E*,4*E*,6*E*) isomer. In *E. hirtipennis*, there was evidence for at least four 2,4,6-nonatrienals; two of these coincided with the known (2*E*,4*E*,6*Z*) and (2*E*,4*E*,6*E*) isomers from *E. fuscula*, but two did not (**Figure 1**). The main isomer in *E. hirtipennis* (retention time 25.1 min) was present in, at most, a trace amount from *E. fuscula*, and both species had another minor GC peak at retention time 24.2 min. Appropriate standards would be required for identification of the unknown isomers. Nonatrienals were not detected from

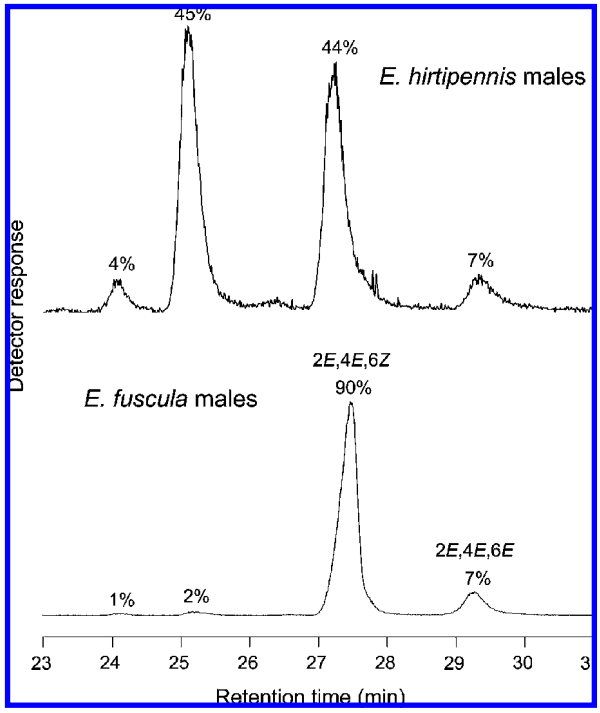

Figure 1. Comparison of 2,4,6-nonatrienals emitted from male flea beetles, *E. hirtipennis* and *E. fuscula* (single-ion GC traces for *m*/*z* 136). The two isomers previously identified from *E. fuscula* are labeled.

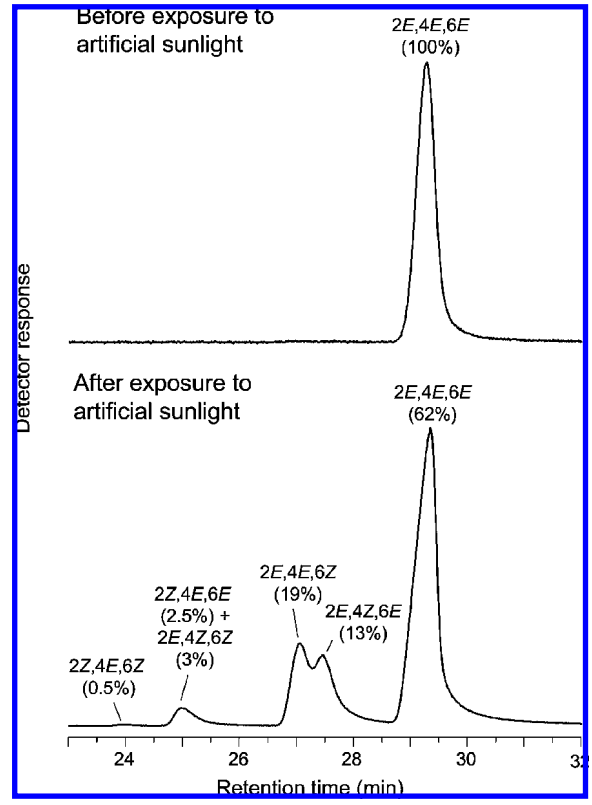

Figure 2. Isomerization of (2*E*,4*E*,6*E*)-2,4,6-nonatrienal induced by sun machine (isomer identifications based on subsequent analysis; see **Figure 3** and **Table 1**).

female *E. hirtipennis*, which is consistent with earlier results for *E. fuscula* (*12*) and supports a pheromonal role for the compounds in *E. hirtipennis*.

Generation and Analysis of 2,4,6-Nonatrienal Geometrical Isomer Standards. After exposure to sunlight, either natural or simulated, a sample of (2*E*,4*E*,6*E*)-2,4,6-nonatrienal gave five GC peaks (**Figure 2**) that were 2,4,6-nonatrienal geometrical isomers, based on mass spectra (all isomers had mass spectra nearly identical to the (2*E*,4*E*,6*Z*) isomer, reported earlier (*12*)). Control samples that were protected from light did not change. The light-exposed mixture produced four peaks when submitted to HPLC, and the GC traces of the four fractions each contained one or two peaks (**Figure 3**). Altogether, there was always evidence for six isomers, some of which coeluted by GC.

The ^{1}H and COSY NMR spectra confirmed that the compounds were all 2,4,6-nonatrienal isomers, and shifts and configurations were assigned (**Table 1**). Fortuitously, there was little overlap of NMR signals between major and minor isomers in HPLC fractions A and B. Comparing results from this study and the previous one (*12*), the ^{1}H shifts in methylene chloride and chloroform were very similar, as would be expected. Our ^{1}H assignments for the (2*E*,4*Z*,6*E*) isomer differed somewhat from those of Schuh and Schieberle (*3*). ^{13}C shifts (measured from HSQC spectra) are also given in **Table 1** to further document the structures.

The (2Z,4Z,6Z)- and (2*Z*,4*Z*,6*E*)-2,4,6-nonatrienals were not detected in the photoisomerized samples. They would probably have shorter GC retention times than the other isomers, based on the observed pattern that the *Z* configuration eluted before *E* for each carbon position (**Figure 3**).

Interestingly, exposure of any of the four HPLC fractions (A−D, **Figure 3**) in the sunlight simulator resulted in virtually the same 2,4,6-nonatrienal blend. The isomer proportions in **Figure 3** apparently represent an equilibrium condition. HPLC fraction B (**Figure 3**) produced identical blends for all durations of light exposure (45−450 min). The compounds were surprisingly robust to thermal degradation. No sample changes were seen when injections were made at 200 °C or cooler, through either inlet. However, at 250 °C, two samples did show evidence of degradation, only when the splitless inlet was used: With the (2*E*,4*Z*,6*Z*)- and (2*E*,4*Z*,6*E*)-2,4,6-nonatrienal mixture, there was <1% change in the relative composition of 2,4,6-nonatrienals, but a significant, early eluting peak was produced (GC retention 8.7 min, 44% of the total peak area; MS *m*/*z* (%): 134 (98, M^{+}), 133 (100), 115 (23), 105 (58), 91 (47), 79 (18), 77 (28); the MS library suggested an ethylbenzaldehyde, which could result from thermal cyclization and aromatization). Problems with GC stability were similarly encountered for the (2*E*,4*Z*,6*Z*)- and (2*E*,4*Z*,6*E*)- isomers of methyl 2,4,6-decatrienoate (*18*). With the (2*Z*,4*E*,6*E*)- and (2*Z*,4*E*,6*Z*)-2,4,6-nonatrienal mixture, the 8.7 min peak was not produced, but a very small (1% of the total sample) new peak was seen, 0.7 min earlier than the (2*Z*,4*E*,6*Z*)- isomer and with a typical 2,4,6-nonatrienal mass spectrum. It was likely either (2*Z*,4*Z*,6*Z*)- or (2*Z*,4*Z*,6*E*)-2,4,6-nonatrienal, but further analysis was not possible.

2,4,6-Nonatrienals in *Epitrix* Species. After HPLC and GC-MS analysis, it was concluded that the major 2,4,6-nonatrienals in *E. hirtipennis* were (2*E*,4*Z*,6*Z*)-2,4,6-nonatrienal and (2*E*,4*E*,6*Z*)-2,4,6-nonatrienal (**Table 2**). Very minor amounts (relative to the main isomers) of (2*Z*,4*E*,6*E*)-, (2*Z*,4*E*,6*Z*)-, (2*E*,4*Z*,6*E*)-, and (2*E*,4*E*,6*E*)-2,4,6-nonatrienals were also detected. The typical emission rate of 2,4,6-nonatrienals was 10 ng per male per day (total of all isomers). The result that both species emit (2*E*,4*E*,6*Z*)-2,4,6-nonatrienal could account for the modest attraction of *E. hirtipennis* to the *E. fuscula* baits.

For *E. fuscula*, the volatile collections during this study generally confirmed the previous report (*12*) in that the major isomer was (2*E*,4*E*,6*Z*), followed by (2*E*,4*E*,6*E*). The relative

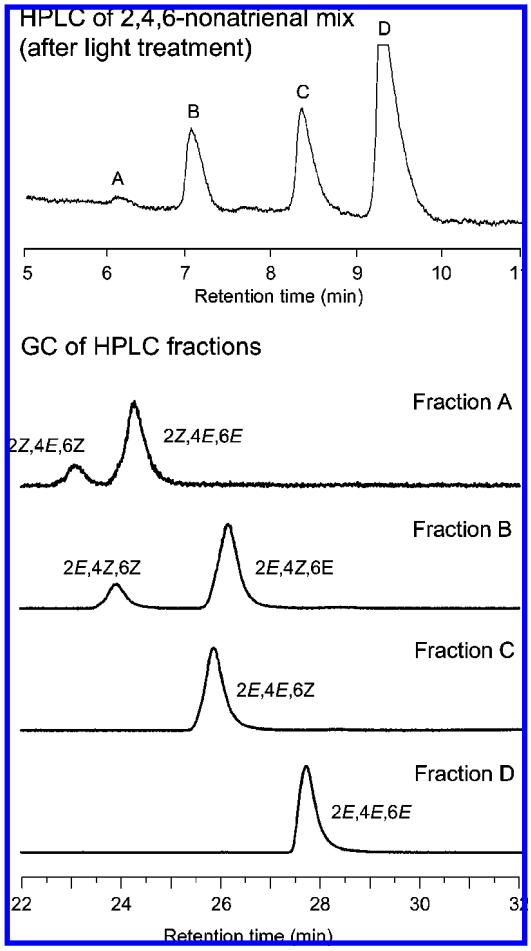

Figure 3. HPLC and GC separation of 2,4,6-nonatrienal isomers generated by treatment of the (2*E*,4*E*,6*E*) isomer with sunlight. Identifications based on NMR (**Table 1**).

abundance of the (2*E*,4*E*,6*E*) isomer in the present study (7%) was lower than previously (26%), perhaps because of the greater protection of volatile collections from light. Very minor amounts of the (2*Z*,4*E*,6*E*), (2*Z*,4*E*,6*Z*), (2*E*,4*Z*,6*E*), and (2*E*,4*Z*,6*Z*) isomers, not identified previously from *E. fuscula*, were also noted.

At this point it is unclear whether the minor isomers were actually emitted by the beetles or were artifacts of handling/degradation. However, the relatively small amount of the (2*E*,4*E*,6*E*) isomer in the beetle samples, which is the most abundant after light treatment (**Figure 2**), argues for most of the minor isomers not being artifacts.

The behavioral effects of the 2,4,6-nonatrienal blend from *E. hirtipennis* may be difficult to assess in the field. We have been unable to formulate any *Z* isomers of these aldehydes so that degradation (by photoisomerization or some other mechanism) does not occur in the field. Furthermore, it is not known whether the natural blend from *Epitrix* beetles survives unchanged as pheromonal communication takes place. While the time interval between emission and detection by other beetles downwind is probably quite short, pheromonal communication nevertheless occurs during daylight hours when photoisomerization would be likely. Concerns about isomerization in the field also arose regarding methyl (2*E*,4*E*,6*Z*)-2,4,6-decatrienoate, a known attractant for the stink bug, *Halyomorpha halys* (*19*).

Scope of Analysis Method for 2,4,6-Nonatrienals. At this point, only six of the eight possible geometrical isomers of 2,4,6-nonatrienal have been characterized. It appears that a focused synthetic approach would be required to determine the properties of the (2*Z*,4*Z*,6*Z*) and (2*Z*,4*Z*,6*E*) isomers, such as the comprehensive work done by Khrimian (*18*) for the chemically related methyl 2,4,6-decatrienoates.

Nevertheless, if any of the present 2,4,6-nonatrienal isomers can be obtained, all six can be readily prepared by exposing the sample to light, and these can be used as chromatographic standards to aid in the identification of unknowns. One complication is that no GC conditions were found that would give baseline separation of all six isomers. However, if both HPLC and GC runs are made consecutively, then unambiguous identification of any of the six isomers is possible by comparison of chromatographic retentions. Mass spectrometry can confirm that unknowns belong to the 2,4,6-nonatrienal class. We have noted that GC retention times shifted over months and after the column was removed and reinstalled several times (e.g.,

Table 1. ^{1}H and ^{13}C Data for 2,4,6-Nonatrienal Geometrical Isomers[a]

carbon position	2*Z*,4*E*,6*Z*	2*Z*,4*E*,6*E*	2*E*,4*Z*,6*Z*	2*E*,4*Z*,6*E*	2*E*,4*E*,6*Z*	2*E*,4*E*,6*E*
			^{1}H Shifts and Coupling Constants			
1 (1H, d)	10.20	10.19	9.63	9.63	9.58	9.56
2 (1H, dd)	5.84	5.81	~6.14	~6.14	6.16	6.13
3 (1H, dd)	7.04	6.99	7.65	7.64	7.22	7.17
4 (1H, dd)	7.20	7.14	6.28	6.16	6.47	6.40
5 (1H, dd)	6.93	6.61	6.79	6.46	7.04	6.71
6 (1H, dd)	6.18	6.28	6.60	6.70	6.14	6.24
7 (1H, dt)	5.81	6.11	5.87	6.11	5.82	6.13
8 (2H, p)	2.32	2.23	2.32	2.26	2.32	2.23
9 (3H, t)	1.06	1.08	1.07	1.10	1.07	1.08
$J_{1,2}$	7.6	8.0	8.0	8.0	8.0	8.0
$J_{2,3}$	10.9[c]	10.9[c]	15.1[b]	15.1[b]	15.2[b]	15.2[b]
$J_{3,4}$	12.4	12.3	11.8	12.0	11.2	11.1
$J_{4,5}$	14.5[b]	14.3[b]	11.0[c]	11.0[c]	14.9[b]	14.9[b]
$J_{5,6}$	11.4	10.7	11.5	11.6	11.6	10.6
$J_{6,7}$	11.0[c]	15.1[b]	11.5[c]	15.0[b]	10.7[c]	15.2[b]
$J_{7,8}$	7.6	6.6	7.5	6.8	7.9	6.5
$J_{8,9}$	7.5	7.4	7.5	7.4	7.5	7.4
			^{13}C Shifts			
1	190.0	189.9	193.2	193.2	193.5[d]	193.5
2	126.4	126.0	131.4	131.4	131.0[d]	130.8
3	147.0	147.3	146.1	146.2	151.9	152.1
4	125.3	123.5	125.8	124.2	129.6	127.7
5	137.5	143.1	133.3	139.6	137.5	143.2
6	127.0	128.6	122.1	124.1	127.3[d]	128.8
7	140.6	143.7	140.6	143.9	140.7	143.9
8	21.3	26.0	21.0	26.1	21.6	26.0
9	12.8	12.8	13.5	12.9	13.8	12.8

[a] Solvent was CD_2Cl_2, except where otherwise indicated. [b] Evidence for an *E* double bond (J > 12 Hz). [c] Evidence for a *Z* double bond (J < 12 Hz). [d] Data for sample in $CDCl_3$ (see ref *12*) because overlapping peaks in CD_2Cl_2 sample did not allow unambiguous assignment; however, shifts in these solvents generally agreed to within 0.3 ppm whenever spectra were acquired in both.

Table 2. Analysis Results for *E. hirtipennis* and *E. fuscula*

HPLC fraction (min after injection)	GC retention (min)	isomer (% of total)
	E. hirtipennis	
9.0−10.0	23.56	2*Z*,4*E*,6*Z* (3.4%)
	24.88	2*Z*,4*E*,6*E* (0.6%)
10.0−12.0	24.37	2*E*,4*Z*,6*Z* (46%)
	26.89	2*E*,4*Z*,6*E* (3%)
13.0−14.0	26.41	2*E*,4*E*,6*Z* (40%)
15.0−16.0	29.14	2*E*,4*E*,6*E* (7%)
	E. fuscula	
9.0−10.0	23.67	2*Z*,4*E*,6*Z* (0.6%)
	25.06	2*Z*,4*E*,6*E* (0.4%)
10.0−11.0	24.64	2*E*,4*Z*,6*Z* (2%)
	26.98	2*E*,4*Z*,6*E* (4%)
12.0−13.0	26.40	2*E*,4*E*,6*Z* (86%)
14.0−15.0	28.46	2*E*,4*E*,6*E* (7%)

compare retention times in **Figures 2** and **3**), and HPLC retention times were variable (compare **Figure 3** and **Table 2**), due at least in part to different batches of solvent. However, chromatographic retentions were stable for analyses done over a short span of time (e.g., on the same day) as long as overloading was avoided. Thus, it is recommended that both the light-generated standards and the unknowns be analyzed at similar concentrations and within a short time of each other, both on the HPLC and the GC. The use of internal standards as retention time markers could also be helpful. With appropriate precautions, the approach presented here can be used to identify both insect-derived and plant-derived 2,4,6-nonatrienals.

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Dr. A. Konstantinov of the USDA-ARS-Systematic Entomology Laboratory, Beltsville, MD, kindly verified the identity of *E. hirtipennis*. Voucher specimens were kept for deposit in the Smithsonian collection.

LITERATURE CITED

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