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CHEMICAL STUDIES ON THE ANOBIIDAE: SEX PHEROMONE OF THE DRUGSTORE BEETLE, *STEGOBIUM PANICEUM* (L.) (COLEOPTERA)†

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Abstract—Chemical and spectroscopic evidence is presented to show that 2,3-dihydro-2,3,5-trimethyl-6-(1-methyl-2-oxobutyl)-4H-pyran-4-one (10) is the sex pheromone produced by the female drugstore beetle, *Stegobium paniceum* L.

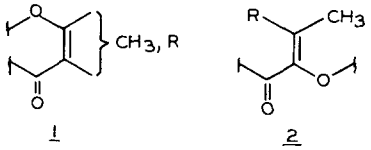
Anobiid beetles are serious pests of a wide variety of commodities and because of their clandestine nature are often difficult to detect until their populations have exceeded economic threshold levels. Many workers have demonstrated the value of insect pheromones as population monitoring tools, especially at sub-economic threshold levels,[1] and the availability of such chemicals for noxious anobiids would undoubtedly be a valuable addition to pest management programs for these insects.

Earlier work from our group,[2] as well as from England[3] has clearly established the presence of a female derived sex pheromone in the anobiid beetle *Stegobium paniceum* L. which elicits precopulatory searching behavior by the males of the species. The isolation and purification of this pheromone was reported.[2] This paper sets forth our conclusion regarding the gross structural features of this rather novel chemical.

The molecular formula of the pheromone was deduced as $C_{13}H_{20}O_3$ on the basis of its high resolution mass spectrum (M^+: *m/e* 224.1413, calcd. 224.1412) as described previously.[2] This formula requires four unsaturation equivalents, three of which are suggested from intense absorption bands in the IR spectrum (liquid film) at 1730 and 1673 cm^{-1} (two CO groups) and at 1615 cm^{-1} (>C=C<).[2] The remaining unsaturation equivalent must be either a second C=C double bond, a third CO or a ring. That it is the latter is evident from the proton-decoupled CMR data which shows 13 carbon resonances. Off-resonance CMR data allows the observed resonances to be assigned to five methyl carbons, one methylene carbon, three methine carbons, two tetrasubstituted olefinic carbons and two CO carbons (Table 1).[4] The resonance positions of the CO carbons demand that one be an aliphatic ketone (207.4 ppm) and that the other be a conjugated ketone (196.9 ppm). Since it was previously shown[2] that no hydroxyls are present in the molecule, the remaining O atom must be included in an ether linkage.

Five distinct sets of resonances are discernable in the 90 MHz PMR spectrum obtained in $CDCl_3$ (Fig. 1). All chemicals shifts, multiplicities, and coupling constants were obtainable either from direct inspection, employment of a shift reagent (tris(dipivalomethanoto) europium), or from appropriate decoupling experiments and are listed in Table 2. Chemical shifts and coupling constants obtained in deuterobenzene are also listed in Table 2.

The sharp three proton singlet at δ 1.76 in the PMR spectrum was assigned to a Me group attached to the C=C double bond. The UV spectrum of the pheromone ($\lambda_{max}^{95\%\ ethanol}$: 272 nm, ϵ = 8400; λ_{max}^{hexane}: 266 nm, ϵ = 8600) requires that the double bond be in conjugation not only with one of the CO groups but also with the alkoxyl substituent.[5] This structural feature was also consistent with the intense IR absorption bands at 1673 and 1615 cm^{-1} of the pheromone.[2] The partial structure neighboring to the double bond is suggested to be either **1** or **2** aside from the geometric configuration.



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The nine-line signals in the Me region of the PMR (Fig. 1) were readily assignable to the remaining four Me groups: a 3H doublet at δ 1.01 (J = 6.4 Hz), A 3H triplet at δ 1.03 (J = 7.0 Hz), a 3H doublet at δ 1.27 (J = 7.0 Hz) and a 3H doublet at δ 1.28 (J = 6.8 Hz).

The one proton quartet at δ 3.62 (J = 6.8 Hz) coupled to the doublet Me signal at δ 1.28 was assigned to partial structure **3** with X and Y being electronegative functionally to account for the unusually low chemical shift.

Molecular fragment 4 is suggested as the origin of the eight line multiplet (double quartet) at 4.44 ppm (J_{H,CH_3} = 6.4 Hz, $J_{H,H}$ = 3.4 Hz), since such a low-field chemical shift requires a methine proton adjacent to an electronegative element[5] (likely an O atom). This assignment

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Table 1. CMR spectral data† of the pheromone and its assignment

Carbon Number	Chemical Shift (ppm)	Multiplicity with Off-Resonance Decoupling	Assignment
1	7.947	quartet	CH_3-
2	9.403	quartet	CH_3-
3	9.403	quartet	CH_3-
4	12.862	quartet	CH_3-
5	15.713	quartet	=C-CH_3
6	33.915	triplet	-CH_2-
7	43.804	doublet	-CH-
8	49.264	doublet	-CH-
9	77.112	doublet	-CH-O-
10	109.511	singlet	-C = C-
11	168.908	singlet	
12	196.938	singlet	C=O
13	207.434	singlet	C=O

†25.14 MHz in $CDCl_3$.

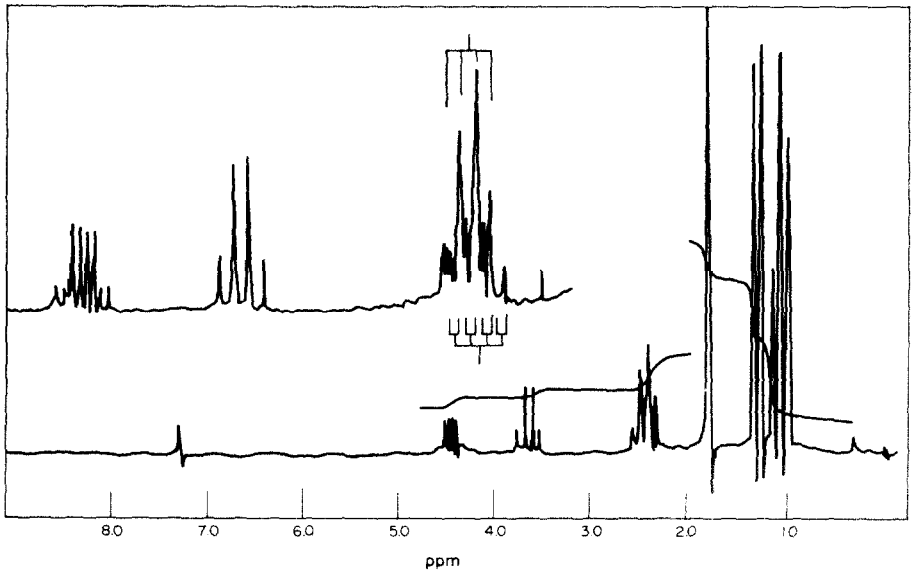


Fig. 1. 90 MHz PMR spectrum of the sex pheromone isolated from female drugstore beetles.

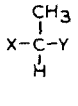
is collaborated by the CMR methine carbon resonance at 77.1 ppm[4] (Table 1).

The remaining three-proton multiplet at *ca.* δ 2.40 contains two sets of resonances: one for a methylene group (quartet) coupled to the methyl triplet at δ 1.03 ($J = 7.0$ Hz), and the other for a methine proton (double quartet) responsible for the 3.4 Hz splitting in the low field double quartet (δ 4.44) as well as the 7.0 Hz splitting in the Me doublet at δ 1.27. All three protons have their chemical shifts in a region likely assignable to protons alpha to a carbonyl group,[5] thus suggesting partial structure **4** and **5**. Employment of the shift reagent [tris(dipivalomethanato)europium] in the PMR measurement of the pheromone resulted in a complete separation of these overlapping resonances with the methine signal now being seen as an eight line double quartet at somewhat lower field than the shifted methylene quartet.

Consideration of the above data allows for six possible gross molecular structures, **6–11**.

Further differentiation among these structures was achieved via derivatization of the pheromone.

Ozonolysis in either carbon disulfide, pentane, methanol, or ethyl acetate afforded a complex mixture of unidentified products, but ozonolysis in carbon tetra-



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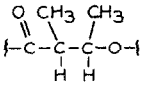

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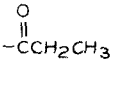

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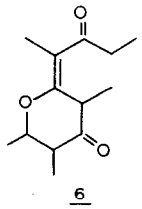

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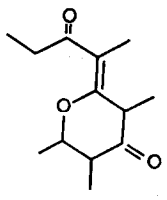

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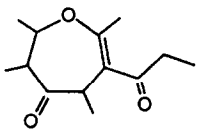

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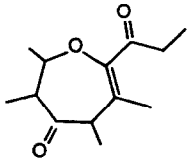

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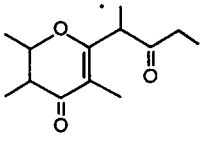

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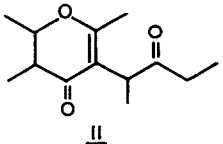

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chloride gave a single product, containing twenty protons by PMR spectroscopy (Fig. 2 and Table 3). Structures **6** and **7** can therefore be excluded from further consideration. The IR spectrum of the ozonolysis product suggested the presence of four carbonyl groups which were tentatively assigned to a α-diketone (1722 cm^{-1}), a normal ketone (1707 cm^{-1}), and an ester (1735 and 1190 cm^{-1}). Determination of the UV spectrum in 0.1N ethanolic sodium hydroxide solution resulted in a bathochromic shift (λ_{max}: 285 nm) suggesting the presence of a β-keto-ester moiety. With the object of confirming the α-diketo-structure, the ozonolysis product was treated with *o*-phenylenediamine to give the corresponding quinoxaline derivative in good yield. In the PMR spec-

Table 2. PMR spectral data of the pheromone

Proton	Multiplicity	Chemical Shift ($\delta^{\#}$) $CDCl_3$	C_6D_6
$-CH_3$ (A)	doublet	1.01	0.76
$-CH_3$ (B)	triplet	1.03	0.92
$-CH_3$ (C)	doublet	1.27	0.82
$-CH_3$ (D)	doublet	1.28	1.16
$-CH_3$ (E)	singlet	1.76	1.73
$-CH_2-$	quartet*	2.42	2.08
-CH- (a)	double quartet*	2.37	1.97
-CH- (b)	quartet	3.62	3.09
-CH- (c)	double quartet	4.44	3.91

Coupling Constant#	in $CDCl_3$	in C_6D_6
$J_{H_a-CH_{3(C)}}$	7.0 Hz	7.6 Hz
$J_{H_b-CH_{3(D)}}$	6.8	6.8
$J_{H_a-H_c}$	3.4	3.7
$J_{H_c-CH_{3(A)}}$	6.4	7.0
$J_{-CH_2-CH_{3(B)}}$	7.0	7.2

* Multiplicity was assigned after separation of a three proton complex multiplet to each component signal by the aid of shift reagent.

\# obtainable either from direct inspection or by appropriate decoupling experiments.

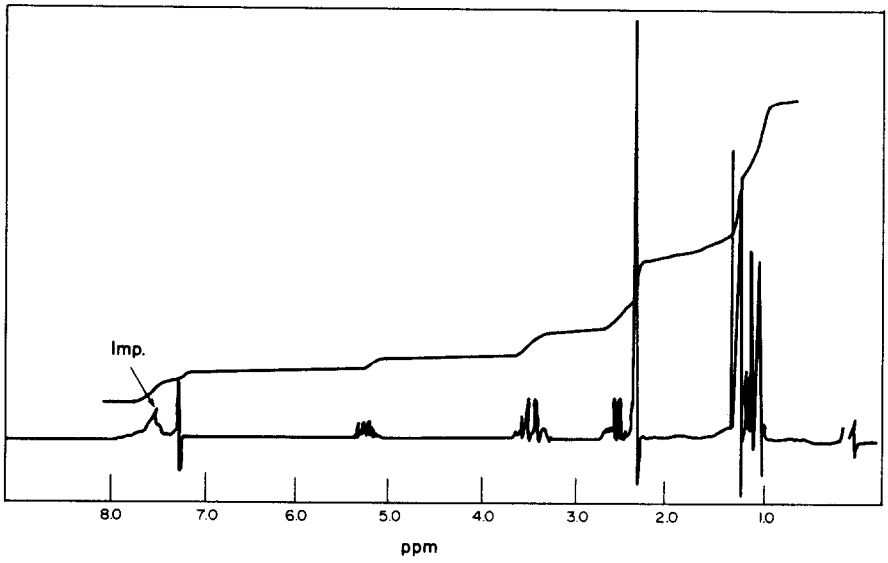


Fig. 2. 90 MHz PMR spectrum of ozonolysis product.

Table 3. PMR spectral data of ozonolysis product and its quinoxaline derivative

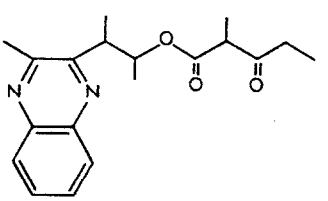
Proton	Multiplicity	Chemical Shift *(δ) Ozonolysis Product	Quinoxaline Derivative
$-CH_3$ (A)	doublet	1.28	1.20
$-CH_3$ (B)	triplet	1.07	1.09
$-CH_3$ (C)	doublet	1.05	1.39
$-CH_3$ (D)	doublet	1.27	1.39
$-CH_3$ (E)	singlet	2.32	2.82
$-CH_2-$	quartet	2.51	2.59
-CH- (a)	double quartet	3.52	3.49
-CH- (b)	quartet	3.44	3.58
-CH- (c)	double quartet	5.26	5.52
aromatic	multiplet	--	7.62-7.80
aromatic	multiplet	--	7.80-7.95

* 90 MHz in $CDCl_3$

Coupling Constant

	Ozonolysis Product	Quinoxaline Derivative
$J_{H_a-CH_{3(C)}}$	7.1 Hz	7.0 Hz
$J_{H_b-CH_{3(D)}}$	7.4	7.0
$J_{H_a-H_c}$	3.5	8.0
$J_{H_c-CH_{3(A)}}$	6.7	6.1
$J_{-CH_2-CH_{3(B)}}$	7.0	6.9

trum of this derivative (Fig. 3 and Table 3) four aromatic protons were observed along with the twenty protons of the ozonolysis product. Mass spectrometric analysis (Fig. 4) of the quinoxaline derivative led to an unambigious structure assignment for the ozonolysis product. Elemental compositions of all prominent fragment ions were confirmed by means of high resolution mass spectrometry. The molecular ion was found at *m/e* 328.1763 (calc. for $C_{19}H_{24}N_2O_3$; 328.1785). The base peak observed at *m/e* 172.1005 was assigned to a fragment ion consisting of a quinoxaline moiety ($C_8H_4N_2$) plus C_3H_8 (calc. for $C_{11}H_{12}N_2$; 172.0999) likely arising from McLafferty rearrangement via a 6-membered transition state containing an aromatic N atom. Two ion peaks containing no O atoms were found at *m/e* 143.0614 and 199.1212, and assigned to CH_3– and C_5H_{11}–units attached to the quinoxaline moiety respectively (calc. for $C_9H_7N_2$; 143.0609, and calc. for $C_{13}H_{15}N_2$; 199.1233). The intense ion peak at *m/e* 57.0350 (calc. for C_3H_5O; 57.0340) collaborated with the ion at *m/e* 299.1404 (calc. for $C_{17}H_{19}N_2O_3$; 299.1395) suggesting the presence of a CH_3CH_2CO-group. The ions at *m/e* 113.0609 and 215.1180 were assigned to $C_6H_9O_2$ (calc. 113.0602) and $C_{12}H_{15}N_2O$ (calc. 215.1182 resulting from α-fission of the ester CO. The *m/e* 113 ion suggested an α-methyl-β-keto-ester moiety in agreement with the conclusions from the base UV spectrum of the ozonolysis product. Taking into account all of the other information these data uniquely define formula **12** as the structure of the quinoxaline derivative.



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Consequently, the ozonolysis product of the pheromone is 1, 2 - dimethyl - 3, 4 - dioxopentyl - 2 - methyl - 3 - oxopentanoate, and therefore the pheromone is 2, 3 - dihydro - 2, 3, 5 - trimethyl - 6 - (1 - methyl - 2 - oxobutyl) - 4H - pyran - 4 - one (10).

A *cis* stereochemical relationship for the vicinal 2,3-protons (and by extension the 2,3-Me groups) on the dihydropyranone ring of the pheromone is suggested by their 3.4 Hz coupling constant, which is closely analogous to the coupling constants reported for several *cis*-2,3-dimethyl chromanones.[6–8]

The pheromone remains uncharacterized with respect to absolute configurations of its three asymmetric carbons. Even after elucidation of the absolute

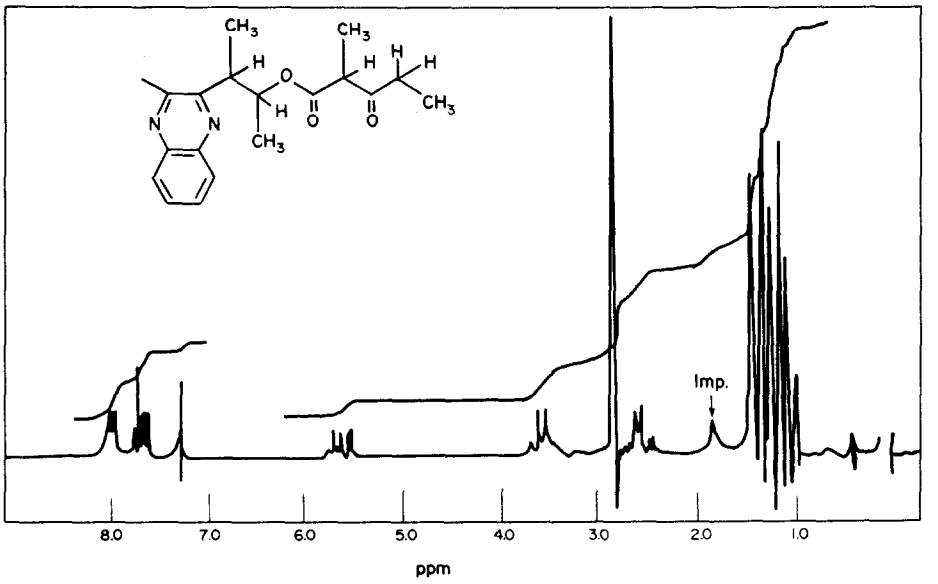

Fig. 3. 90 MHz spectrum of quinoxaline derivative of the ozonolysis product.

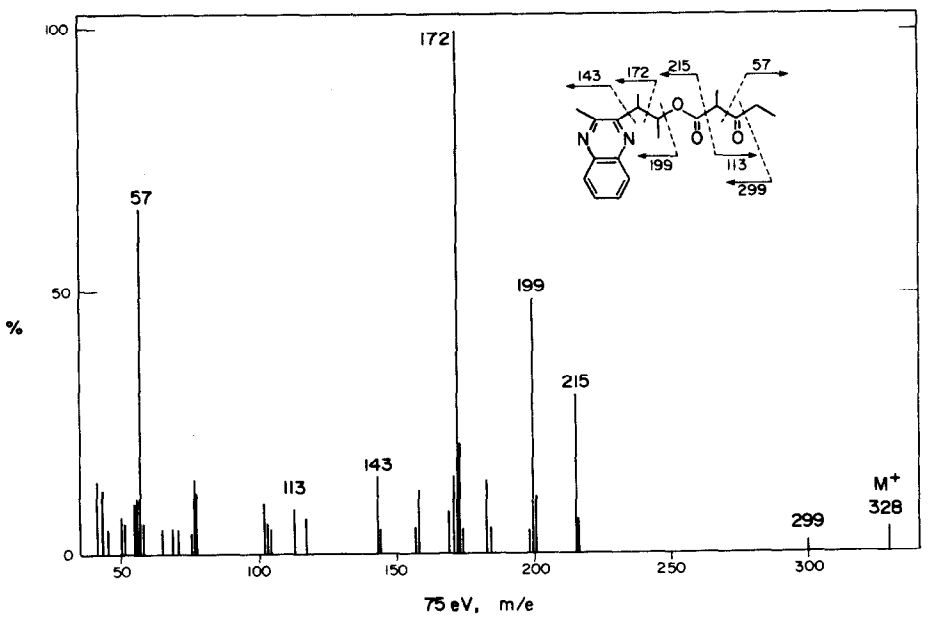

Fig. 4. 75 eV mass spectrum of the quinoxaline derivative of the ozonolysis product.

configuration, however, the final proof of the correctness of our analysis must await a total synthesis of the pheromone and a demonstration of its equal biological activity with the natural pheromone.

EXPERIMENTAL

PMR spectra were recorded at 90 MHz on either a Bruker H-90E FT spectrometer or a Hitachi R-22 NMR Spectrometer with TMS as internal standard using $CDCl_3$ or C_6D_6 as solvent. Accurate mass measurements were obtained on either a Hitachi RMU-7M002 or JEOL JMS-01SG-2 high resolution mass spectrometer. Low resolution mass spectra were obtained on a Hitachi RMS-4 mass spectrometer coupled with a K-53 gas chromatograph or a Finnigan 1015 mass spectrometer via direct probe inlet. CMR spectra were recorded at 25.14 MHz in $CDCl_3$ using a JEOL JNM-PS-100 NMR spectrometer. IR spectra refer to films and were obtained on a Beckman IR-33, Shimadzu IR-400 or Hitachi EPI-G-3 spectrometer. UV spectra were measured on a Varian 635 or a Shimadzu UV-300 spectrophotometer in the solvent indicated. CD spectra were obtained in hexane using a Cary 60 spectropolarimeter equipped with a 6003D CD attachment. GLC analyses were performed on a Beckman GC-4, Varian Model 1800, Yanako 550F or G-80 gas chromatograph equipped with flame ionization detectors.

Isolation of the pheromone. Isolation of the pheromone from about 214,000 female equivalents was accomplished following the method previously described.[2] Recrystallization from hexane yielded the pure pheromone (35.2 mg) as prisms (m.p. 52.5–53.5°). The physico-chemical and biological properties of this material were completely identical to those described in the previous paper.[2] ν_{max}(film): 2980(s), 2930(s), 2880(m), 1730(s), 1673(s), 1615(s), 1455(s), 1387(s), 1345(s), 1260(w), 1214(m), 1185(w),

1168(w), 1146(s), 1118(m), 1092(w), 1052(m), 1000(m), 968(m), 916(w), 860(w), 835(w), 810(w), 780(w), 760(w), 710(m), 560(w) cm^{-1}. High resolution MS(75eV) *m/e*: 97.0649(C_6H_9O), 109.0672(C_7H_9O), 111.0445($C_6H_7O_2$), 112.0538 ($C_6H_8O_2$), 113.0606($C_6H_9O_2$), 124.0872($C_8H_{12}O$), 139.0794 ($C_8H_{11}O_2$), 168.1117($C_{10}H_{16}O_2$), 224.1413(M^+, $C_{13}H_{20}O_3$). UV: λ_{max}^{Hexane} 266 nm, ϵ = 8,600, $\lambda_{max}^{95\%\ EtOH}$ 272 nm, ϵ = 8,400. CD: 260 nm, Φ = +30,121; 285 nm, Φ = −43,076; 345 nm, Φ = −2869; 360 nm, Φ = −1379. CMR and PMR see Tables 1 and 2, and Fig. 1.

Ozonolysis of the pheromone. To the isolated pheromone (15 mg) dissolved in CCl_4 (1.5 ml), ozone gas was bubbled at 0° for 2 min, during which time all of the pheromone was consumed as assessed by GLC monitoring. After passing air in the mixture to purge remaining ozone, triphenylphosphine crystals (37 mg) were added, immediately producing a yellow color. After evaporation of the solvent *in vacuo*, the resulting crystalline mass was repeatedly extracted with cold pentane. The combined pentane extracts upon evaporation gave an oily residue (15.5 mg, 81% yield). ν_{max}(film): 3400(broad), 2980(s), 2940(s), 2870(w), 1735 (shoulder), 1722(shoulder), 1707(vs), 1630(w), 1455(s), 1415(w), 1382 (m), 1355(m), 1190(s), 1110(m), 1070(m), 910(m), 865(w), 720(m), 695(w) cm^{-1}. GC-MS(12eV) *m/e*; 43(10%), 44(6%), 55(47%), 56(8%), 57(43%), 83(100%, base peak), 84(8%), 86(16%), 113(13%), 126(9%), 127(7%), 213(6%), M^+ was not observed. GLC (column 15% PEG-20M 75 cm × 3 mm id., at 150°, carrier gas; He 30 ml/min) Rt: 12.4 min. (under the same conditions, pheromone; Rt: 14.9 min and dodecyl acetate; Rt: 3.6 min), GLC (column 5% OV-17, 75 cm × 3 mm id., at 120°, carrier gas; He 30 ml/min (under the same conditions, pheromone; Rt: 6.3 min and dodecyl acetate; Rt: 7.2 min). TLC (SiO_2–HF_{254}, Merck 0.25 mm thickness, $CHCl_3$); Rf: 0.53 (pheromone Rf: 0.36). UV; $\lambda_{max}^{EtOH\text{-}0.1N\ NaOH}$ 285 nm. PMR see Table 3 and Fig. 2.

The quinoxaline derivative of ozonolysis product. To the ozonolysis product (15.5 mg) dissolved in EtOAc (0.5 ml) *o*-phenylenediamine (20 mg) in EtOAc (1.0 ml) was added, and kept for 24 hr at room temp. Upon evaporation of the solvent the resulting material was directly transferred to a silicic acid column (5g) and eluted with a mixture of benzene and EtOAc (90:10). Monitoring by GLC and TLC, the resulting quinoxaline derivative was isolated and collected. After evaporation of the solvent the quinoxaline derivative (16 mg) was obtained as a pale yellow syrup (80% yield). ν_{max}(film): 3055(w), 2980(s), 2940(m), 2870(w), 1735(vs), 1711(vs), 1562(w), 1485(s), 1455(s), 1405(w), 1380(s), 1355(w), 1322(m), 1255(m), 1225(w), 1195(s), 1180(s), 1120(w), 1095(w), 1065(s), 1000(s), 965(m), 903(w), 870(w), 833(w), 785(vs), 760(vs) cm^{-1}. Single focus MS(70 eV) *m/e*; 41(14%), 43(12%), 45(5%), 50(7%), 51(6%), 55(10%), 56(11%), 57(65%), 58(6%), 65(5%), 69(5%), 71(5%), 75(5%), 76(14%), 77(13%), 102(10%), 103(6%), 104(6%), 113(9%), 117(7%), 143(15%), 144(5%), 157(6%), 158(12%), 169(9%), 170(5%), 171(15%), 172(100%, base peak), 173(22%), 174(5%), 183(14%), 184(5%), 198(5%), 199(49%), 200(11%), 215(30%), 216(7%), 299(3%), 328(5%, M^+). GLC (column 15% PEG-20M, 75 cm × 3 mm i.d. at 180°, carrier gas; He 30 ml/min) Rt: 14.2 min (under the same conditions, ozonolysis product; Rt: 3.0 min, *o*-phenylene-diamine; Rt: 4.55 min). TLC(SiO_2-HF_{254}, 0.25 mm thickness, $CHCl_3$); Rf 0.29 (ozonolysis product Rf 0.53). PMR see Table 3 and Fig. 3.

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