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NEW KETODIENES FROM THE INTEGUMENTAL LIPIDS OF THE GUAM BROWN TREE SNAKE, *BOIGA IRREGULARIS*

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ABSTRACT.—A mixture of six new long chain ketodienes, (6Z,26Z)-pentatriacontadien-2-one [1], (8Z,26Z)-pentatriacontadien-2-one [2], (6Z,27Z)-hexatriacontadien-2-one [3], (8Z,27Z)-hexatriacontadien-2-one [4], (6Z,28Z)-heptatriacontadien-2-one [5], and (8Z, 28Z)-heptatriacontadien-2-one [6], has been separated from the cuticular lipids of the Guam brown tree snake *Boiga irregularis*. Their structures were determined by chemical and spectral means.

We have recently reported the isolation, characterization, and synthesis of a series of previously undescribed long-chain saturated and monounsaturated methyl ketones that serve as sex attractiveness pheromones in the red-sided garter snake, *Thamnophis sirtalis parietalis* (1–4). Using this same paradigm, we have begun an investigation of potential skin lipid pheromones from a related colubrid, the brown tree snake, *Boiga irregularis* Merrem (Serpentes: Colubridae). Although native to Australia, Papua New Guinea, and islands of the Western Pacific, the brown tree snake has been inadvertently introduced to Guam (5). With a lack of snake predators on Guam, this snake has managed to increase its numbers which are now estimated at 13,000 per square mile (6). It has been assigned responsibility for the extinction of three species of birds and the drastic reduction in populations of the remaining avian fauna on Guam. The brown tree snake is considered mildly venomous, possessing grooved rear fangs in association with paired venom glands. Currently, about 1 in 1000 hospital visits in Guam is for treatment of brown tree snake bites (7). Hospital records indicate that envenomation of the elderly, persons allergic to the venom, and especially children may result in respiratory distress (7).

Clearly, control of this pest species by manipulation of its reproductive behavior with pheromones is desirable. We have investigated the integumental skin lipids of *B. irregularis* for compounds analogous to those that constitute the sex attractiveness pheromone of the red-sided garter snake. In this report, we describe the identification of a series of long-chain methyl ketones including six new ketodienes, 1–6, from *B. irregularis*. Their pheromonal activity is under active investigation.

$$CH_3 \overset{O}{\underset{}{\parallel}} (CH_2)_m / \diagup (CH_2)_n / \diagup C_8H_{17}$$
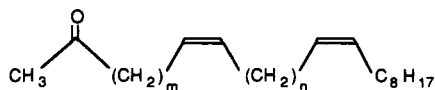
1 $C_{35:6,26}$ (m=3, n=18)   4 $C_{36:8,27}$ (m=5, n=17)

2 $C_{35:8,26}$ (m=5, n=16)   5 $C_{37:6,28}$ (m=3, n=20)

3 $C_{35:6,27}$ (m=3, n=19)   6 $C_{37:8,28}$ (m=5, n=18)

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RESULTS AND DISCUSSION

Preliminary gc-ms examination of the cuticular lipids from female *B. irregularis* revealed the presence of a series of long-chain methyl ketones, which were subsequently isolated by chromatography over alumina (3,4). The initial gc-ms analysis of the methyl-ketone-containing fraction provided the chromatogram shown in Figure 1. Ten
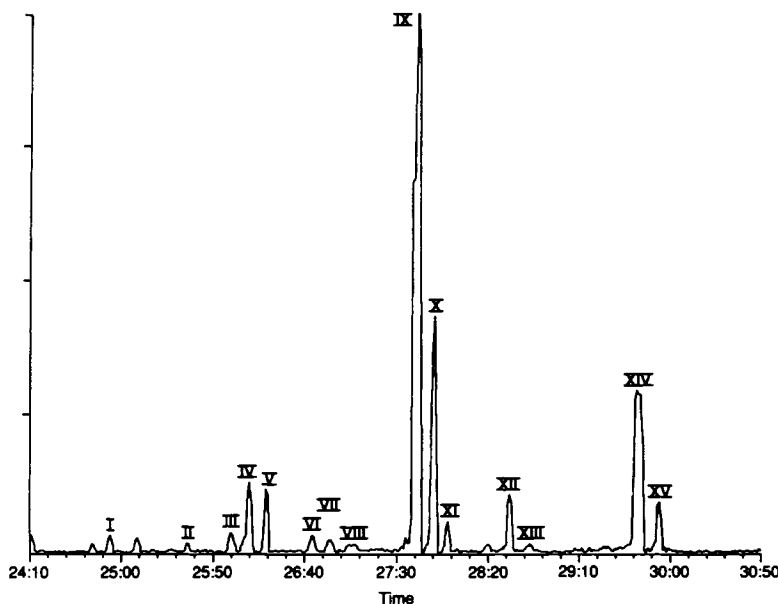

FIGURE 1.   Gc/ms analysis of the methyl ketones from *Boiga irregularis*. I, 2-hentriacontanone; II, 2-dotriacontanone; III, $C_{33:2}$, $[M]^+$, $m/z = 474$; IV, (24Z)-tritriaconten-2-one; V, 2-tritriacontanone; VI, $C_{34:2}$, $[M]^+$, $m/z = 488$; VII, (25Z)-tetratriaconten-2-one; VIII, 2-tetratriacontanone; IX, $C_{35:2}$, $[M]^+$, $m/z = 502$; X, (26Z)-pentatriaconten-2-one; XI, 2-pentatriacontanone; XII, $C_{36:2}$, $[M]^+$, $m/z = 516$; XIII, (27Z)-hexatriaconten-2-one; XIV, $C_{37:2}$, $[M]^+$, $m/z = 530$; XV, (28Z)-heptatriaconten-2-one.

of the peaks were immediately recognizable as the same long chain saturated and monounsaturated methyl ketones previously identified in the cuticular lipids of the red-sided garter snake, *T. sirtalis parietalis* (3,4), while the remaining peaks (III, VI, IX, XII, and XIV) had mass spectra that indicated methyl ketones with two additional units of unsaturation (Table 1).

In order to determine the number and position of the double bonds in the more unsaturated components, the mixture was treated with dimethyl disulfide in the usual manner (8). This provided a series of tetra(thiomethyl) adducts eluting at the upper temperature limit of the gc column. The ms of the principal (C-35) tetra(thiomethyl)methylketone (Table 2) shows important ions at m/z 145 and 173 together with fragments resulting from the loss of these units with sequential numbers of thiomethyl or methanethiol units. Because of the equivalence of the masses of C=O and CH_2CH_2, the ions at m/z 145 could result following derivatization of a C-6 or a C-28 double bond, while the ion at $m/z = 173$ could result following derivatization of a C-8 or a C-26 double bond, i.e., peak IX could be a mixture of positional diene isomers. The ms of the other tetrathiomethyl derivatives presents the same problem. Unfortunately, hreims data could not be obtained to shed light on this problem, and the N-methoxime derivatives (3,4) that would distinguish the aliphatic from the carbonyl end of the compounds could not be analyzed by gc-ms.

The methylketone mixture was further separated by chromatography on Si gel/

TABLE 1.   Gc/ms Analysis of the Diene Methyl Ketones from *B. irregularis*.

Peak	Rt (min)	Relative %	ms *m/z* (rel. int.) Characteristic Ions
III	26:00	0.9	$[M]^+$ 474 (37), 456 (19), 416 (26), 125 (37), 123 (22), 111 (39), 109 (34), 97 (57), 95 (54), 83 (67), 81 (50), 71 (53), 69 (71), 67 (58), 58 (16), 55 (100).
VI	26:44	0.7	$[M]^+$ 488 (24), 470 (26), 430 (33), 125 (25), 123 (32), 111 (57), 109 (39), 97 (85), 95 (84), 83 (66), 81 (85), 71 (63), 69 (100), 67 (99), 58 (13), 57 (48), 55 (99), 54 (65).
IX	27:42	50.1	$[M]^+$ 502 (14), 484 (10), 444 (14), 125 (39), 111 (31), 109 (29), 97 (45), 95 (53), 83 (59), 81 (59), 71 (50), 69 (71), 67 (60), 58 (20), 57 (42), 55 (100).
XII	28:32	3.3	$[M]^+$ 516 (12), 498 (13), 458 (10), 125 (51), 111 (40), 109 (28), 97 (44), 95 (45), 83 (58), 81 (52), 71 (58), 69 (68), 67 (49), 58 (18), 57 (44), 55 (100).
XIV	29:42	17.8	$[M]^+$ 530 (17), 512 (16), 472 (6), 125 (71), 111 (34), 109 (27), 97 (51), 95 (49), 83 (60), 81 (52), 71 (58), 69 (71), 67 (47), 58 (15), 57 (44), 55 (100).

AgNO$_3$ to provide three fractions containing these compounds. Fraction A contained the saturated and monounsaturated methylketones previously identified in *T. sirtalis parietalis*, while both fractions B and C contained peaks III, VI, IX, XII, and XIV.

The ^{1}H-nmr spectra of fractions B and C clearly showed the presence of a methyl ketone (δ 2.13, s), a terminal alkyl methyl group (δ 0.88, t, $J = 7.3$ Hz), olefinic protons (δ 5.35, m), allylic methylene protons (δ 1.97–2.06, m), and a large number of aliphatic methylene groups (δ 1.3–1.25). The Z geometry of all the double bonds was suggested by the lack of the expected E disubstituted double bond absorption at 990–900 cm^{-1} in the ir spectra of these fractions. This was confirmed by the presence of the allylic methylene resonances of a Z double bond at δ 27.2 ppm in their ^{13}C-nmr spectra and by no observable signal at ca. δ 32.7 ppm, characteristic of the allylic methylene carbons of E double bonds (9).

TABLE 2.   Interpretation of Important Fragment Ions in the ms of the Dimethyl Disulfide Derivatives of the C-35 Methyldienones (m/z $[M]^+$ 690).

Fragment m/z (rel. int.)	Assignment
596 (0.5)	$M - (2 \times CH_3S)$
549 (4)	$M - (3 \times CH_3S)$
547 (3)	$M - (2 \times CH_3SH + CH_3S)$
497 (13)	$M - (CH_3SH + 145)$
469 (15)	$M - (CH_3SH + 173)$
451 (11)	$M - (2 \times CH_3S + 145)$
449 (7)	$M - (2 \times CH_3SH + 145)$
423 (16)	$M - (2 \times CH_3S + 173)$
421 (15)	$M - (2 \times CH_3SH + 173)$
373 (8)	$M - (3 \times CH_3SH + 173)$
355 (3)	$M - (3 \times CH_3SH + 145)$
173 (32)	$CH_3(CH_2)_7CHSCH_3$ or $CH_3CO(CH_2)_5CHSCH_3$
145 (27)	$CH_3(CH_2)_5CHSCH_3$ or $CH_3CO(CH_2)_3CHSCH_3$

In addition, the possible presence of at least two positional isomers of the double bond nearer the carbonyl group is indicated by the C-3 methylene and the C-4 methylene resonances. These appeared as a triplet (δ 2.42, J = 7 Hz) and as a triplet of triplets (δ 1.63, J = 7, 7 Hz), respectively, in the ^{1}H spectrum of fraction B. On the other hand, the ^{1}H-nmr spectrum of fraction C showed an overlapping pair of triplets for the C-3 methylene protons and an additional triplet of triplets (δ 1.57, J = 7, 7 Hz) in the region of the C-4 resonances (Figure 2).
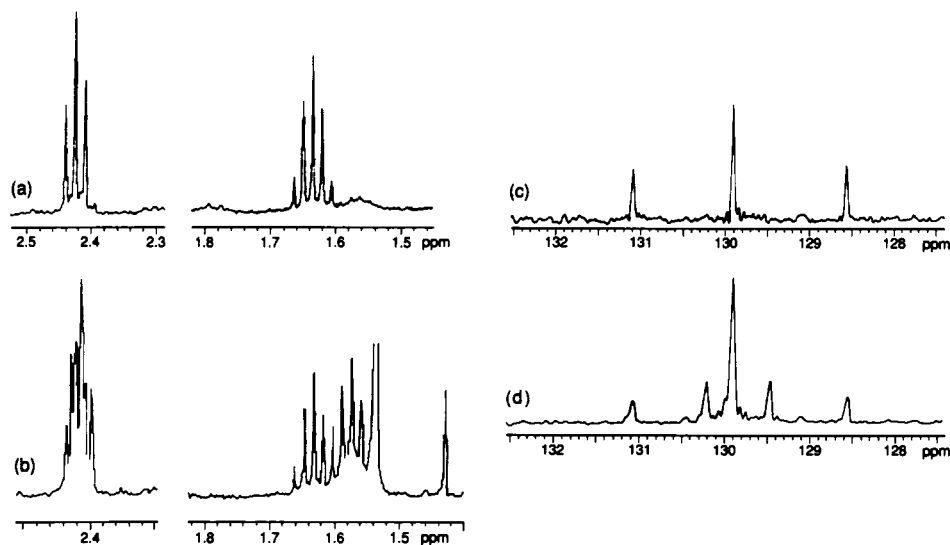

FIGURE 2.   Selected portions of the ^{1}H and ^{13}C nmr spectra of dieneone Fractions B and C: (a) ^{1}H nmr: H-3 and H-4 of Fraction B; (b) ^{1}H nmr: H-3 and H-4 of Fraction C; (c) ^{13}C nmr: olefinic carbons of Fraction B; (d) ^{13}C nmr: olefinic carbons of Fraction C.

This result was verified by the olefinic carbon resonances in the ^{13}C-nmr spectra of fractions B and C (Figure 2). In fraction B, the carbon resonance for an isolated Z double bond appears at δ 129.9 together with resonances at δ 131.1 and 128.6 for the proximal and remote carbons, respectively, of the double bond nearer the carbonyl group. In fraction C, these resonances are joined by resonances at δ 130.2 and δ 129.5 for the proximal and remote resonances, respectively, of a double bond more remote from the carbonyl group.

The position of the double bond near the carbonyl group in these ketodienes was established from analysis of their COSY and relayed COSY nmr spectra. The COSY spectrum of fraction B shows connectivity between the C-4 resonance at δ 1.63 and C-3 resonance at δ 2.42, as well as connectivity between the C-4 resonance and the allylic proton resonance at δ 2.0 so that C-5 is an allylic methylene group. In addition, the resonance at δ 2.0 shows connectivity to the olefinic resonance at δ 5.35 and homoallylic protons at δ 1.34. In a relayed COSY experiment (relay = 1, τ = 0.02), cross peaks were observed arising from relayed connectivity between the resonances at C-3 and C-5 and relayed connectivity between the resonance at C-4 and the olefinic resonance. Thus, the double bond nearest the carbonyl group in the ketodienes of fraction B is a C-6 double bond.

From its 1D ^{1}H- and ^{13}C-nmr spectra, fraction C undoubtedly contains a mixture of two positional isomers of the double bond nearer the carbonyl group. The COSY and relayed COSY spectra of fraction C (Figure 3) show the cross peaks described above that arise from the C-6 double bond along with signals that determine the position of the
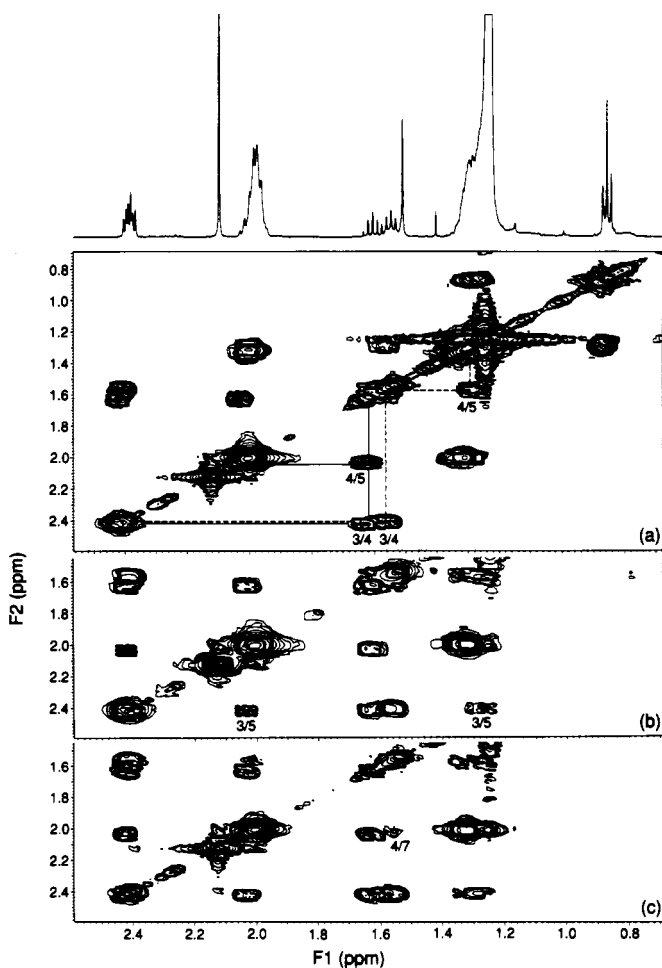
FIGURE 3.   COSY and relayed COSY spectra of fraction C: a, COSY; b, Relayed COSY (relay = 1, $\tau = 0.02$); c, Relayed COSY (relay = 2, $\tau = 0.02$).

double bond nearer the carbonyl group in the second positional isomer. In the COSY of fraction C, the C-4 resonance of the second isomer at δ 1.57 shows connectivity with the C-3 resonance at δ 2.41 and the aliphatic region at δ 1.32 (C-5). The one-step relayed COSY shows no additional cross peaks associated with the C-4 resonance at δ 1.57 but does contain a cross peak arising from the C-3 region at δ 2.41 and the aliphatic region (C-5) that was not present in the one-step relayed COSY of fraction B. Finally, the two-step relayed COSY shows connectivity between the C-4 resonance at δ 1.57 and the allylic proton region at δ 2.0 (C-7), so that one double bond of the isomers characterized by this C-4 resonance must be at C-8.

Because the position of the isolated double bond further away from the carbonyl group in these ketodienes could not be assigned unambiguously from the above data, a small portion of fraction C was subjected to oxidative cleavage with RuO_4 (10). After methylation of the resulting mixture of carboxylic acids with CH_2N_2, a large quantity of methyl nonanoate was detected along with a mixture of C-18 to C-22 dicarboxylic acid methyl esters. The predominance of methyl nonanoate indicated that for the most part, the more remote double bond is nine carbons removed from the aliphatic end of the ketodienes as in the previously described monounsaturated ketones (3,4). Fractions

B and C from the Si gel/AgNO$_3$ chromatography contain nearly all of the ketodienes detected in the cuticular lipids; peaks IX, XII, and XIV comprised 97% of these compounds. Therefore, peak IX consists of (6Z,26Z)-pentatriacontadien-2-one [1] and (8Z,26Z)-pentatriacontadien-2-one [2], peak XII consists of (6Z,27Z)-hexatriacontadien-2-one [3] and (8Z,27Z)-hexatriacontadien-2-one [4], and peak XIV consists of (6Z,28Z)-heptatriacontadien-2-one [5] and (8Z,28Z)-heptatriacontadien-2-one [6].

Consideration of the relative amounts of the diester degradation products along with the relative amounts of the three predominant ketodiene peaks provided a good approximation of the total ratio of the six major ketodienes 1–6 in fractions B and C (Table 3). Dimethyl octanedioate is derived only from 2 and dimethyl docosanedioate is

TABLE 3.   Approximate relative amounts of ketodienes present in combined fractions B and C.

Component	Total Ketodienes (%)	C-6 ene	C-8 ene	(Ratio)
Peak IX (C-35)	61	1	2	(5:1)
Peak XII (C-36)	7	3	4	(3:2)
Peak XIV (C-37)	30	5	6	(1:1)

derived only from 5. The major dimethyl ester, dimethyl eicosanedioate, is derived from both 1 and 6. Because RuO$_4$ does not discriminate between the C-37 and C-35 ketodienes, the ratio of the total amount of C-35 ketodienes to their diesters is equal to the ratio of the total amount of C-37 ketodienes to their diesters. Because the amount of diester from 2 and 5 is available, along with the relative amounts of total C-35 and C-37 ketodienes in fraction C, the amounts of 1 and 6 in fraction C can be obtained by solving a pair of equations using the relative amounts of the dicarboxylic acid methyl esters. Thus, the ratio of 1 to 2 is 55:45 and the ratio of 5 to 6 is 1:10. Because dimethyl nonadecanedioate and dimethyl heneicosanedioate are derived only from the C-36 ketodienes in fraction C, they indicate a 2:3 ratio of 3 to 4 in fraction C. From these ratios and the relative amounts of C-35, C-36, and C-37 ketodienes, the total C-6 to C-8 olefin ratio in fraction C is 2:3, which matches the ^{1}H-nmr observation for this mixture. The values in Table 3 include the proportionate amounts of 1, 3, and 5 from fraction B, so that the overall ratio of C-6 to C-8 olefin in fractions B and C combined is 5:2.

Although the integumental lipids of B. irregularis contain a mixture of long-chain methyl ketones similar to those utilized by T. sirtalis parietalis as a sexual attractiveness pheromone, the major components of the B. irregularis mixture are the previously undescribed methylketodienes 1–6. Indeed, C-35 ketodienes 1 and 2 (peak IX) and C-37 ketodienes 5 and 6 (peak XIV) predominate the mixture. In addition, small amounts of the analogous C-36 ketodienes 3 and 4 (peak XII) are present along with traces of unidentified C-33 and C-34 ketodienes (peaks III and VI).

These long-chain methyl ketones in the integumental lipids of B. irregularis may serve a variety of functions, such as waterproofing the epidermis and control of microorganisms (11). On the other hand, skin lipid pheromones are believed to be widespread in snakes (12), and the similarity of these structures to those found in the related T. sirtalis parietalis makes them attractive candidates as sex pheromones. We are presently preparing these compounds for comparative field and laboratory bioassays.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were obtained using either a Hewlett-Packard model 5965A FTIR equipped with a model 59970 IRD datasystem or a Perkin-Elmer model 1420 infrared spectrophotometer. ^{1}H-nmr spectra, COSY, and relayed COSY spectra (13–15) were recorded on a Varian VXR-500 instrument. ^{13}C-nmr spectra were recorded on a Varian XL-300 spectrometer. Eims were ob-

tained at an ionizing voltage of 70 eV with a Finnigan model 4500 gc-ms fitted with a 30 m × 0.32 mm i.d. column with a bonded DB-1 phase. Unless otherwise noted, this instrument was programmed from 60 to 100° at 15°/min and then from 100 to 310° at 10°/min and held at that temperature for 15 min. Hreims were obtained using a VG7070F instrument at an ionizing voltage of 70 eV.

EXTRACTION AND ISOLATION OF METHYLKETONES.—A total of 547 mg of crude skin lipids was obtained from the hexane washes of nine female *B. irregularis* captured on Guam and maintained in Plexiglas cages provided with branches for climbing and H_2O ad libitum. A 16:8 photoperiod was maintained with ambient temperature cycling between 26° and 30° daily. Approximately 70% relative humidity was maintained in the room by means of a room humidifier (16). Chromatography over alumina in the usual manner (3,4) provided 14 mg of the mixture of methylketones shown in Figure 1. The mass spectra and retention temperatures of peaks I, II, IV, V, VII, VIII, X, XI, XIII, and XV were identical to those previously described (4).

The mass spectra of peaks III, VI, IX, XII, and XIV are shown in Table 2; hreims exact mass, peak IX m/z $[M]^+$ 502.5118 (calcd for $C_{35}H_{66}O$, 502.5114), peak XII m/z $[M]^+$ 516.5282 (calcd for $C_{36}H_{68}O$, 516.5270), peak XIV m/z $[M]^+$ 530.5448 (calcd for $C_{37}H_{70}O$, 530.5427). Chromatography of this mixture over 4.3 g of 10% $AgNO_3$/Si gel (Analtech) using hexane with increasing amounts of Et_2O (2–8%) as eluent provided fractions A, B, and C, containing methyl ketones. Fraction A contained the previously identified saturated and monounsaturated ketones. Fractions B (5.1 mg) and C (4.3 mg) contained peaks III, VI, IX, XII, and XIV in approximately a 1.5:1:55:6:27 ratio.

Fraction B (containing mainly **1**, **3**, and **5**): ir ν max (gc-ir and $CHCl_3$ solution) (cm^{-1}) 3012, 2932, 2863, 1730, 1459, 1359, 1157; ^1H nmr (500 MHz, CDCl$_3$) δ 5.35 (4H, m), 2.42 (2H, t, $J = 7$ Hz), 2.13 (3H, s), 1.97–2.06 (8H, m), 1.63 (2H, tt, $J = 7$, 7 Hz), 1.25 (28H, br s), 0.88 (3H, t, $J = 7$ Hz); ^{13}C nmr (75 MHz, CDCl$_3$) δ 216.21 (C-2), 131.09 (-CH=CH-), 129.91 (-CH=CH-), 128.56 (-CH=CH-), 43.10 (C-3), 31.92 (-CH_2-CH$_2$-CH$_3$), 29.78, 29.70, 29.58, 29.46, 29.40, 29.33, 27.23 (-CH=CH-CH_2), 26.53 (C-5), 23.78 (C-4), 22.68 (-CH_2-CH$_3$), 14.09 (-CH$_3$).

Fraction C (containing mainly **1**, **2**, **3**, **4**, **5**, and **6**). In addition to the signals observed in Fraction B, the following resonances were observed: ^1H nmr (500 MHz, CDCl$_3$) δ 5.35 (4H, m), 2.41 (2H, t, $J = 7$ Hz), 2.13 (3H, s), 1.97–2.06 (8H, m), 1.57 (2H, tt, $J = 7$, 7 Hz), 1.30 (2H, br s), 1.25 (26H, br s), 0.88 (3H, t, $J = 7$ Hz); ^{13}C nmr (75 MHz, CDCl$_3$) δ 216.21 (C-2), 130.21 (-CH=CH-), 129.91 (-CH=CH-), 129.47 (-CH=CH-), 43.77 (C-3), 31.91 (-CH_2-CH$_2$-CH$_3$), 29.78, 29.70, 29.57, 29.46, 29.41, 29.33, 27.23 (-CH=CH-CH_2-), 27.02 (C-7), 23.78 (C-4), 22.68 (-CH_2-CH$_3$), 14.09 (-CH$_3$) (see Figure 2). A series of COSY and relayed COSY spectra were obtained (Figure 3). In fraction C, the minor component (40%) showed the same connectivity pattern as the COSY spectrum from fraction B.

DERIVATIZATION AND DEGRADATION.—A small portion of the crude methyl ketone mixture was treated with dimethyl disulfide and a catalytic amount of iodine in the usual manner (8). The major long retention time component of the resulting mixture had the mass spectrum: eims m/z (rel. int.) $[M]^+$ 690 (1), 596 (<0.5), 549 (4), 547 (3), 497 (13), 469 (15), 451 (11), 449 (7), 423 (16), 421 (15), 401 (3), 355 (5), 173 (32), 145 (27), 109 (22), 97 (56), 95 (38), 87 (76), 83 (49), 81 (45), 69 (80), 67 (45), 61 (100), 55 (80) (see Table 2). The eims of other components of this mixture had homologous fragmentation patterns including the ions at m/z 145 and 173, although their parent ions were not observed. Attempts to chromatograph the N-methoxime derivatives of this mixture were unsuccessful.

Approximately 2 mg of fraction C was dissolved in 0.5 ml of a 1:1 mixture of CCl_4 and MeCN. This solution was stirred with 1 ml of saturated NaIO$_4$ and <1 mg of RuO$_2$ for 1.5 h. The organic phase was separated, treated with one drop of EtOH, and after 5 min, filtered through a short plug of Florisil. The resulting mixture was treated with a slight excess of ethereal CH_2N_2, and subsequent gc-ms analysis revealed the presence of methyl nonanoate and small amounts of methyl octanoate and methyl decanoate. In addition, the following mixture of five dimethyl dicarboxylic acid esters (17,18) was detected: dimethyl oxtadecanedioate (21%), dimethyl nonadecanedioate (12%), dimethyl eicosanedioate (57%), dimethyl heneicosanedioate (7%), and dimethyl docosanedioate (3%).

ACKNOWLEDGMENTS

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