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MAMMALIAN EXOCRINE SECRETIONS[1]: IX. CONSTITUENTS OF PREORBITAL SECRETION OF ORIBI, *Ourebia, ourebi*

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Abstract—Using gas chromatography–mass spectrometry in conjunction with ancillary techniques such as chemical ionization with different reactant gases, determination of the position of double bonds by means of dimethyl disulfide derivatization, and finally gas chromatographic and mass spectrometric comparison with authentic synthetic material, 75 constituents were identified in the preorbital secretion of the male oribi, *Ourebia ourebi*. The secretion contains compounds with long-chain, unbranched structures similar to those found in many other preorbital secretions but with a finite volatility range, in contrast to the seemingly endlessly increasing chain lengths typical of other preorbital secretions.

Key Words—*Ourebia ourebi*, Bovidae, mammalian semiochemicals, mammalian pheromones, exocrine secretion, preorbital secretion, dimethyl disulfide derivatization, skipped dienes, chemical-ionization mass spectrometry, NMR.

INTRODUCTION

Cutaneous glands producing odoriferous substances that act as semiochemicals occur in most mammalian species. They are common in carnivores, rodents, and in most ungulate groups, but are also found in many other orders (Schaffer, 1940). The glands may be situated in many different regions of the body, and they may have different functions. For example, the scent glands described in

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[1] The heading of the series has been changed as the semiochemical activity of these secretions cannot always be demonstrated.

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the black-tailed deer, *Odocoileus hemionus columbianus*, include preorbital (Albone, 1984), interdigital, metatarsal (Müller-Schwarze, 1971), and tarsal glands (Müller-Schwarze, 1971; Müller-Schwarze and Müller-Schwarze, 1975).

Volatile substances may be released into the air by diffusion from the gland, but in most cases described, objects are marked with secretions from these glands. Scent marking with glandular exudate is often associated with territorial behavior (Eisenberg and Kleiman, 1972) and many bovid species utilize secretions from their preorbital glands for this purpose.

The exocrine secretions of several South African antelope species have been studied in considerable detail in the Laboratory for Ecological Chemistry at the University of Stellenbosch. Some of them, such as the grysbok and the grey duiker cause considerable damage in vineyards, orchards, and pine plantations in parts of South Africa (Bigalke, 1974). A research program was therefore initiated to identify the semiochemicals produced in these preorbital glands and to investigate the feasibility of reducing damage by the use of synthetic pheromones. The first stage of the program was the chemical characterization of the preorbital gland secretion of the grysbok. Although the ritual involved in marking objects with preorbital gland secretions undoubtedly plays an important part in the behavior of male grysbok, no experimental evidence could be found that the olfactory stimuli released from the deposited material serve as deterrents signalling territorial occupancy to other males (Novellie et al., 1984).

Preliminary behavioral studies with other antelope species indicated that the response-guided strategy (Albone, 1984) was unlikely to lead to a complete understanding of the function of the various constituents of the preorbital secretions of these animals in their territorial and social behavior, and it became clear that more background information would be needed on the preorbital secretions from individual conspecific animals, as well as from related and unrelated species.

One of the species selected for this purpose is the oribi, *Ourebia ourebi*, which belongs to the tribe Raphicerini. Other members of this tribe are the grysbok, *Raphicerus melanotis*, and the steenbok, *R. campestris*. The oribi normally inhabits grasslands, open plains, and thickly bushed country (Kenmuir and Williams, 1975) and is found in the eastern Cape, Natal, southeastern Transvaal, Mozambique, and southeastern Zimbabwe (Walker, 1982). They are normally seen in pairs or in small groups (Kenmuir and Williams, 1975).

There are six scent glands on the oribi's body, namely preorbital glands, tracts of glandular skin on the forelegs covered with long hair and on the hindlegs covered with shorter brushes, inguinal glands, pedal glands, as well as ear glands (Kingdon, 1982). In the oribi, marking with the preorbital glands is associated mainly with territorial and sexual activity (Kingdon, 1982). Only males mark with their well-developed preorbital glands (Smithers, 1986), while females induce the male's marking behavior (Kingdon, 1982). The male oribi's preor-

bital secretion consists of a mucoid emulsion containing black insoluble material and small quantities of volatile organic compounds.

In this paper, the identification of the volatile organic constituents of the preorbital gland secretion of the oribi, and the synthesis of some of them, are reported.

METHODS AND MATERIALS

General. All Pyrex glassware used in the handling of biological material and extracts, as well as in the preparation of reference compounds, was heated to 500°C in an annealing oven to remove any traces of organic material. Dichloromethane (Merck, Residue Analysis Grade) was analyzed gas chromatographically and found to be pure enough for extraction purposes when used in small quantities. Syringes, stainless-steel needles, etc., were cleaned by rinsing with this solvent.

Analytical Methods. Gas chromatographic (GC) analyses were carried out with Carlo Erba 4200 and 5300 gas chromatographs equipped with flame ionization detectors, Grob split-splitless injectors, and glass columns coated by the Laboratory for Ecological Chemistry with a 0.25-μm film of the apolar stationary phase PS-089, a silanol-terminated 95% dimethyl-5% diphenylsiloxane copolymer. Helium was used as carrier gas at a linear velocity of 28.6 cm/sec at 40°C. The flame ionization detector was operated at 280°C and the injector was normally used at 220°C. Samples were injected in the split mode, and the volatiles entering the column were thermally focused at ca. 30°C and subsequently analyzed using a temperature program of 2°C/min. For the analysis of synthetic products and intermediates, a 4°C/min programming rate was used. Methyl heptanoate was used as an internal standard in a quantitative analysis of the preorbital secretion.

Electron impact (EI) mass spectra were recorded at 70 eV on a Carlo Erba QMD 1000 GC-MS instrument, using the column and conditions described above. Chemical ionization (CI) mass spectra were obtained with methane and nitric oxide as reactant gases.

^{1}H and ^{13}C NMR spectra of synthesized compounds were obtained on a Varian VXR-300 spectrometer operating at 299.9 MHz for the observation of ^{1}H and at 75.42 MHz for the observation of ^{13}C, respectively. All spectra were recorded at 25°C. ^{13}C Multiplicities were determined by means of the APT technique. Two-dimensional ^{1}H–^{1}H correlation spectra (COSY) were obtained using the pulse sequence: D1-90°-(t1 + D3)-60°-AQ (Bax et al., 1981), with D3 = 0.06 sec to enhance long-range coupling effects. Two-dimensional ^{1}H–^{13}C correlation spectra (HETCOR) were obtained by means of the pulse sequence as described previously (Wilde and Bolton, 1984). Where necessary

^{13}C assignments were verified by means of a long-range ^{1}H–^{13}C heteronuclear correlation experiment (Reynolds et al., 1989). The ^{13}C spectrum of the whole, unprocessed secretion was obtained using a 67° pulse angle and a pulse repetition time of 0.8 sec. A total of 29,600 transients were accumulated. The ^{13}C chemical shifts were measured in benzene-d_6 as lock solvent relative to tetramethylsilane as internal reference.

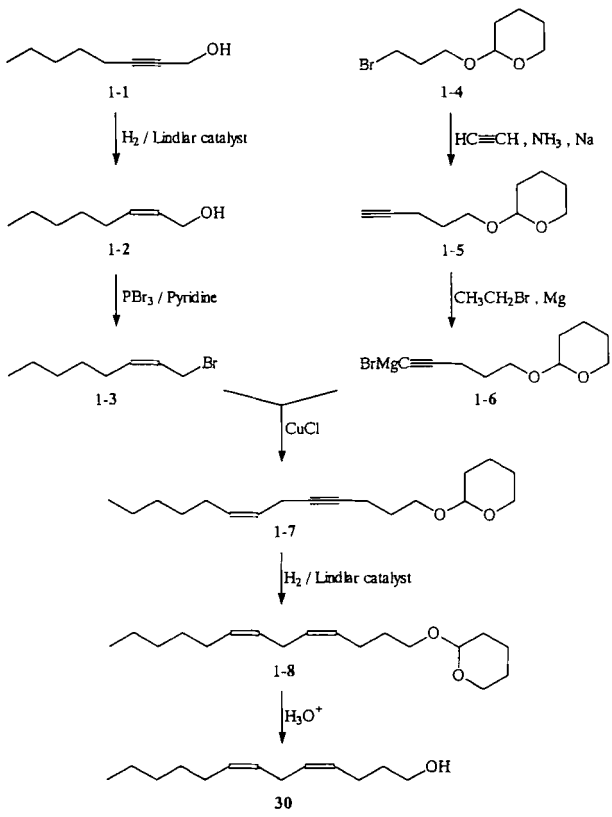
Infrared spectra were recorded on a Mattson Galaxy Series FT-IR 3000 instrument, equipped with a Roland DXY-1100 plotter.

Collection and Sample Preparation. Preorbital gland secretion was collected from a netted, sexually mature male animal by scooping the exudate from the preorbital cavity with a tubular PTFE scoop fitted with a PTFE plunger with which the material was ejected from the scoop into a Reacti-Vial. The organic volatiles were extracted by stirring each collected sample with dichloromethane (ca. 100 μl), using a thin (0.5 mm) glass rod to produce a homogeneous suspension, centrifuging the suspension at 2000–3000 rpm for ca. 15 min, removing the dichloromethane extract from underneath the supernatant water and mucus layer with a 100-μl syringe, and transferring it to a clean Reacti-Vial. Most extracts could be used without subsequent concentration. When more concentrated solutions were required, the solvent was removed in a slow stream of purified (activated charcoal) nitrogen.

Dimethyl Disulfide Derivatization. The derivatization was carried out using two methods. Following the method described by Buser et al. (1983), an aliquot (10 μl) of a dichloromethane extract of the preorbital secretion was concentrated in a 1-ml Reacti-Vial using a slow stream of purified nitrogen. The residual material was dissolved in hexane (50 μl) and treated with 5 μl of iodine solution (60 mg/ml of diethyl ether) and 50 μl of dimethyl disulfide (DMDS). The Reacti-Vial was sealed using a PTFE-faced rubber septum and the reaction mixture left at 40°C for 15 hr in the oven of a gas chromatograph. The reaction was quenched with an aqueous solution of sodium thiosulfate (5%). The organic and aqueous layers were separated by centrifuging for a few minutes at 2000 rpm, after which the organic layer was transferred to a clean Reacti-Vial. The solution was concentrated to 5 μl for GC-MS analysis. In the alternative method, described by Vincenti et al. (1987), a similar procedure is employed except for the substitution of hexane with carbon disulfide as solvent, a higher reaction temperature (60°C), and a longer reaction time (40 hr).

Reference Compounds. Compounds required for comparison with constituents of the preorbital secretion that are not commercially available were synthesized from authentic starting materials.

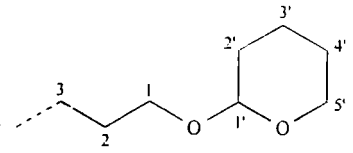
(4*Z*,7*Z*)-4,7-Tridecadien-1-ol, **30,** was synthesized according to Scheme 1. Partial hydrogenation of 2-octyn-1-ol 1-**1** (Taylor and Strong, 1950) with Lindlar catalyst (Augustine, 1965) gave pure (*Z*)-2-octen-1-ol 1-**2** in 80% yield; bp 97–



SCHEME 1. Synthesis of (4Z,7Z)-4,7-tridecadien-1-ol.

98°C (14 mm Hg); ^{13}C NMR ($CDCl_3$): Table 1. Bromination of the octenol 1-**2** with phosphorus tribromide in the presence of pyridine (Taylor and Strong, 1950) gave (Z)-1-bromo-2-octene 1-**3**, containing 9.8% of the *E* isomer (GC-MS), in 81% yield; bp 89–90°C (16 mm Hg). 5-Tetrahydropyranyloxy-1-pentyne 1-**5** was synthesized from 1-bromo-3-tetrahydropyranyloxypropane 1-**4** and sodium acetylide according to the method of Vaughn et al. (1937) in 60% yield; bp 116–118°C (28–30 mm Hg); ^{13}C NMR ($CDCl_3$): Table 1. Coupling of the bromooctene 1-**3** with the acetylenic Grignard reagent 1-**6** in the presence of CuCl, according to the method described by Osbond et al. (1961), gave 1-tetrahydropyranyloxy-7-tridecen-4-yne 1-**7** in 72% yield; ^{13}C NMR ($CDCl_3$):

TABLE 1. ^{13}C NMR SPECTRAL ASSIGNMENT FOR SYNTHETIC INTERMEDIATES IN SCHEME 1



Position	Compounds		
	1-2	1-5	1-7
1	58.55(t)	65.78(t)[a]	65.78(t)
2	133.10(d)i[b]	28.75(t)[a]	28.76(t)
3	128.46(d)i[b]	15.36(t)[a]	15.37(t)
4	27.43(t)	83.95(s)	83.93(s)
5	29.32(t)	68.49(d)	79.22(s)
6	31.45(t)		17.19(t)
7	22.54(t)		124.98(d)
8	14.04(q)		131.31(d)
9			27.10(t)
10			29.23(t)
11			31.49(t)
12			22.57(t)
13			14.06(q)
1'		98.79(d)	98.78(d)
2'		30.69(t)[a]	30.69(t)
3'		19.53(t)[a]	19.52(t)
4'		25.53(t)	25.53(t)
5'		62.15(t)[a]	62.14(t)

[a] According to HETCOR.
[b] i denotes interchangeable assignments.

Table 1. Partial hydrogenation of 1-tetrahydropyranyloxy-7-tridecen-4-yne with Lindlar catalyst followed by deprotection of the resulting skipped diene derivative with hydrochloric acid (Le Roux, 1980), gave 5.84 g (38% overall yield from 1-**1**) of a mixture of 4,7-tridecadien-1-ol isomers containing 32% of the required Z,Z isomer **30**. Chromatography on 7% $AgNO_3$-coated silica gel gave pure (GC) (4Z,7Z)-4,7-tridecadien-1-ol **30**: ^{13}C NMR ($CDCl_3$): Table 2.

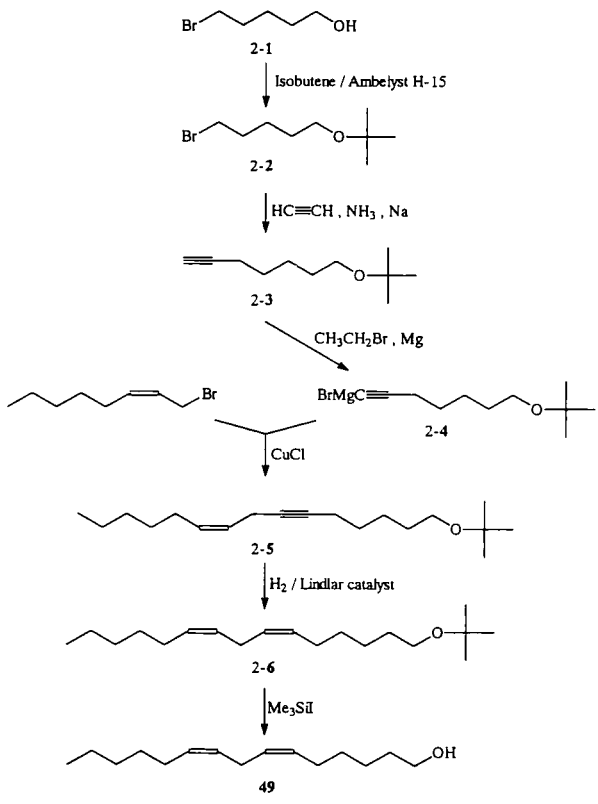
(6Z,9Z)-6,9-Pentadecadien-1-ol, **49**, was prepared according to Scheme 2. Treatment of 5-bromo-1-pentanol 2-**1** with isobutene and Amberlyst H-15 as catalyst (Alexakis et al., 1988) gave 5-bromo-1-*tert*-butoxypentane 2-**2** in quan-

TABLE 2. ^{13}C NMR SPECTRAL ASSIGNMENT FOR SYNTHETIC ALKADIENOLS

Position	Compounds		
	30	**49**	(**9Z**, **12Z**)-9,12-Octadecadien-1-ol
1	62.55(t)	62.98(t)	63.06(t)
2	32.55(t)	32.70(t)	32.83(t)
3	23.60(t)	25.41(t)	25.77(t)
4	129.17(d)i[a]	29.45(t)	29.53(t)i
5	127.70(d)i	27.18(t)i	29.43(t)i
6	25.62(t)	129.80(d)ii	29.27(t)i
7	128.92(d)i	127.80(d)iii	29.68(t)ii
8	130.44(d)	25.66(t)	27.25(t)
9	27.24(t)	128.20(d)iii	130.13(d)iii[b]
10	29.34(t)	130.25(d)ii	127.96(d)iv[b]
11	31.54(t)	27.24(t)i	25.67(t)[b]
12	22.59(t)	29.35(t)	128.03(d)iv[b]
13	14.07(q)	31.54(t)	130.22(d)iii[b]
14		22.58(t)	27.25(t)
15		14.06(q)	29.38(t)ii
16			31.56(t)
17			22.60(t)
18			14.08(q)

[a]i–iv denote interchangeable assignments.
[b]According to HETCOR.

titative yield; ^{13}C NMR ($CDCl_3$): Table 3. 1-*tert*-Butoxy-6-heptyne 2-**3** was synthesized from 2-**2** in a manner analogous to the preparation of 1-**5** in 97% yield; ^{13}C NMR ($CDCl_3$): Table 3. 1-*tert*-Butoxy-(*Z*)-9-pentadecen-6-yne 2-**5** was prepared from the acetylenic Grignard reagent 2-**4** and 1-bromo-2-octene by the procedure used for the preparation of 1-**7** in 87% yield; ^{13}C NMR ($CDCl_3$): Table 3. Partial hydrogenation of 2-**5** with Lindlar catalyst gave 1-*tert*-butoxy-6,9-pentadecadiene as a mixture of *Z,Z*, *Z,E*, *E,Z*, and *E,E* isomers containing 62% of the *Z,Z* isomer 2-**6**; ^{13}C NMR ($CDCl_3$): Table 3. Deprotection of the mixture of 1-*tert*-butoxy-6,9-pentadecadiene isomers with Me_3SiI according to the method of Jung and Lyster (1977), proceeded quantitatively to give 3.40 g of the isomeric 6,9-pentadecadienols in an overall yield of 68% from the starting compound 5-bromo-1-pentanol 2-**1**. Column chromatography on 7% $AgNO_3$-coated silica gel gave the pure (6*Z*,9*Z*)-6,9-pentadecadien-1-ol **49**; ^{13}C NMR ($CDCl_3$): Table 2.

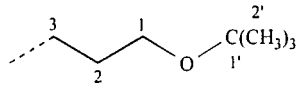


SCHEME 2. Synthesis of (6Z,9Z)-6,9-pentadecadien-1-ol.

(9Z,12Z)-9,12-Octadecadien-1-yl formate, **74,** was synthesized in 92% yield by the reduction of linoleic acid methyl ester with $LiAlH_4$ followed by the esterification of the resulting (9Z,12Z)-9,12-octadecadien-1-ol with formic acid at room temperature for 5 hr. The reaction product was purified by bulb-to-bulb distillation to give (9Z,12Z)-9,12-octadecadien-1-yl formate **74** in 95% yield; bp 165°C (0.015 mm Hg). The ^{13}C NMR data for this and other formates synthesized according to this method, are given in Table 4.

(9Z,12Z)-9,12-Octadecadien-1-yl acetate, **77**, was prepared by the esterification of (9Z,12Z)-9,12-octadecadien-1-ol with acetyl chloride in the presence of pyridine in 99% yield; bp 159°C (0.007 mm Hg). The ^{13}C NMR data for

TABLE 3. ^{13}C NMR SPECTRAL ASSIGNMENT FOR SYNTHETIC INTERMEDIATES IN SCHEME 2



Position	Compounds				
	2-**1**	2-**2**	2-**3**	2-**5**	2-**6**
1	62.44(t)	61.22(t)	61.38(t)	61.48(t)	61.60(t)
2	31.59(t)	29.81(t)	30.20(t)	30.27(t)	30.65(t)
3	24.44(t)	25.06(t)	25.51(t)	25.61(t)	26.00(t)
4	32.50(t)	32.75(t)	28.46(t)	29.01(t)i[a]	29.60(t)
5	33.76(t)	33.80(t)	18.42(t)	18.82(t)[b]	27.23(t)i
6			84.63(s)	79.92(s)ii	130.04(d)ii
7			68.14(d)	78.50(s)ii	127.95(d)iii
8				17.19(t)[b]	25.65(t)
9				125.02(d)[b]	128.09(d)iii
10				131.33(d)[b]	130.22(d)ii
11				27.09(t)	27.26(t)i
12				29.09(t)i	29.37(t)
13				31.49(t)	31.55(t)
14				22.56(t)	22.59(t)
15				14.05(q)	14.08(q)
1'		72.52(s)	72.46(s)	72.44(s)	72.42(s)
2'		27.58(q)	27.59(q)	27.60(q)	27.60(q)

[a] i–iii denote interchangeable assignments.
[b] According to HETCOR.

this and other acetates synthesized according to this method are listed in Table 5.

(4Z,7Z)-4,7-Tridecadienal, **22,** was obtained by the oxidation of the dienol **30** on pyridinium chromate-silica gel in 57% yield according to the procedure described by Singh et al. (1979). The ^{13}C NMR data for the aldehydes synthesized for comparison by this method, are given in Table 6.

RESULTS AND DISCUSSION

Although the oribi enjoys a widespread distribution in the eastern parts of sub-Saharan Africa, its distribution is patchy, and it is therefore a relatively rare

TABLE 4. ^{13}C NMR SPECTRAL ASSIGNMENT FOR SYNTHETIC ALKADIENYL FORMATES

Position	Compounds		
	35	53	74
1	63.43(t)	64.00(t)	64.10(t)
2	28.44(t)	28.47(t)	28.53(t)
3	23.50(t)	25.50(t)	25.83(t)
4	129.52(d)i[a]	29.21(t)	29.39(t)i
5	127.50(d)ii	27.06(t)	29.20(t)i
6	25.60(t)	129.56(d)i	29.17(t)i
7	128.14(d)ii	127.80(d)ii	29.63(t)ii
8	130.52(d)i	25.66(t)	27.22(t)
9	27.24(t)	128.45(d)ii	130.07(d)iii
10	29.33(t)	130.30(d)i	127.93(d)iv
11	31.54(t)	27.24(t)	25.65(t)
12	22.59(t)	29.36(t)	128.06(d)iv
13	14.07(q)	31.55(t)	130.22(d)iii
14		22.59(t)	27.22(t)
15		14.07(q)	29.36(t)ii
16			31.55(t)
17			22.58(t)
18			14.07(q)
1′	161.07(d)	161.11(d)	161.16(d)

[a]i–iv denote interchangeable assignments.

TABLE 5. ^{13}C NMR SPECTRAL ASSIGNMENT FOR SYNTHETIC ALKADIENYL ACETATES

Position	Compounds		
	42	**58**	**77**
1	63.96(t)	64.56(t)	64.64(t)
2	28.54(t)	28.56(t)	28.64(t)
3	23.60(t)	25.61(t)	25.93(t)
4	129.28(d)i[a]	29.29(t)	29.43(t)i
5	127.59(d)ii	27.09(t)	29.25(t)i
6	25.59(t)	129.66(d)i	29.23(t)i
7	128.42(d)ii	127.83(d)ii	29.66(t)ii
8	130.45(d)i	25.66(t)	27.23(t)
9	27.23(t)	128.37(d)ii	130.09(d)iii
10	29.34(t)	130.28(d)i	127.94(d)iv

TABLE 5. Continued

Position	Compounds		
	42	**58**	**77**
11	31.54(t)	27.23(t)	25.66(t)
12	22.59(t)	29.36(t)	128.05(d)iv
13	14.06(q)	31.55(t)	130.22(d)iii
14		22.59(t)	27.23(t)
15		14.07(q)	29.37(t)ii
16			31.56(t)
17			22.59(t)
18			14.08(q)
1′	171.11(s)	171.14(s)	171.19(s)
2′	20.97(q)	20.98(q)	21.01(q)

[a]i–iv denote interchangeable assignments.

TABLE 6. ^{13}C NMR SPECTRAL ASSIGNMENT FOR SYNTHETIC ALKENALS AND ALKADIENALS

Position	Compounds				
	10	**23**	**34**	**22**	**43**
1	202.63(d)	202.78(d)	202.84(d)	202.00(d)	202.55(d)
2	43.31(t)	43.89(t)	43.91(t)	43.74(t)	43.79(t)
3	22.10(t)	22.02(t)	22.09(t)	20.10(t)	21.72(t)
4	26.48(t)	28.80(t)	28.99(t)i	129.86(d)i	29.14(t)
5	128.19(d)	29.45(t)i	29.08(t)i	127.28(d)ii	26.93(t)
6	131.43(d)	26.96(t)	29.52(t)ii	25.62(t)	129.21(d)i
7	27.22(t)	129.35(d)	27.11(t)	127.38(d)ii	127.71(d)ii
8	29.35(t)	130.32(d)	129.58(d)	130.68(d)i	25.66(t)
9	31.51(t)	27.20(t)	130.17(d)	27.25(t)	128.71(d)ii
10	22.56(t)	29.42(t)i	27.21(t)	29.31(t)	130.36(d)i
11	14.05(q)	31.53(t)	29.45(t)ii	31.53(t)	27.24(t)
12		22.58(t)	31.55(t)	22.58(t)	29.35(t)
13		14.06(q)	22.59(t)	14.07(q)	31.54(t)
14			14.08(q)		22.59(t)
15					14.08(q)

[a]i and ii denote interchangeable assignments.

to $(M+30)^+$ in the CI(NO) mass spectra of the saturated formates was used to determine the molecular mass of the formates. This ion is formed by addition of a nitrosonium ion to the formate. The M^+ and $(M-46)^+$ ions are the base peaks in the mass spectra of the di- and monounsaturated formates, respectively. Formates were distinguished from other long-chain constituents by the presence in their CI(CH_4) mass spectra of a $(M+1-HCOOH)^+$ ion, which forms the base peak.

The acetates present in the secretion were characterized by a characteristic ion at *m/z* 61 (Sharkey et al., 1959; Beynon et al., 1961). In addition, the saturated acetates also have an ion at *m/z* 116, formed by cleavage of the bond between C-3 and C-4 with rearrangement of one hydrogen atom. The presence of an acetate moiety was confirmed by the presence of $(M+1-CH_3COOH)^+$ ions in the CI(CH_4) mass spectra of these compounds. The $(M+1)^+$ and $(M+NO)^+$ ions in their CI(CH_4) and CI(NO) spectra, respectively, were used to determine the molecular mass of these constituents.

The aldehydes present in the secretion were distinguished by an ion at *m/z* 44 accompanied by $(M-CH_2{=}CH_2)^+$ and $(M-CH_2{=}CHOH)^+$ ions in the high mass range of their EI mass spectra. The $(M-1)^+$ ion constitutes the base peak in these CI(NO) mass spectra, which also contain $(M+NO)^+$ and $(M-H_2O)^+$ ions, whereas the ion at *m/z* 44 has a very low abundance (<ca. 1%) in the spectra of the mono- and diunsaturated aldehydes.

The position of the double bonds in the monounsaturated alcohols, aldehydes, formates, and acetates present in the secretion was determined by GC-MS analysis of an extract of the secretion that had been subjected to the dimethyl disulfide (DMDS) derivatization procedure described by Buser et al. (1983). A total ion current chromatogram of organic material extracted from the secretion and subjected to DMDS derivatization is shown in Figure 2. The EI mass spectra of the bis(methylthioether) derivatives exhibited easily recognizable molecular and less abundant $(M-CH_3S)^+$ ions. The position of the double bonds was derived using the diagnostic ions A^+, B^+, and C^+, as shown in Table 7.

The configuration of the double bonds was established by coinjection of an extract of the secretion with a number of synthetic reference compounds after it had been confirmed that the *E* and *Z* isomers of these compounds could be separated on the available capillary column. Although the concentration of many of the unsaturated compounds in the preorbital secretion is below the detection limit of the NMR instrument, it was possible to confirm the presence of a double bond with *Z* configuration in the major constituents because of a resonance at δ 27.2 in the ^{13}C NMR spectrum of the whole secretion and the total absence of a resonance at δ ca. 33. It is known that the carbon atoms of the allylic methylene group in long-chain unbranched compounds resonate at δ ca. 27 in the *Z* isomers and at δ ca. 33 in the *E* compounds (Breitmaier et al., 1979; Wenkert et al., 1976).

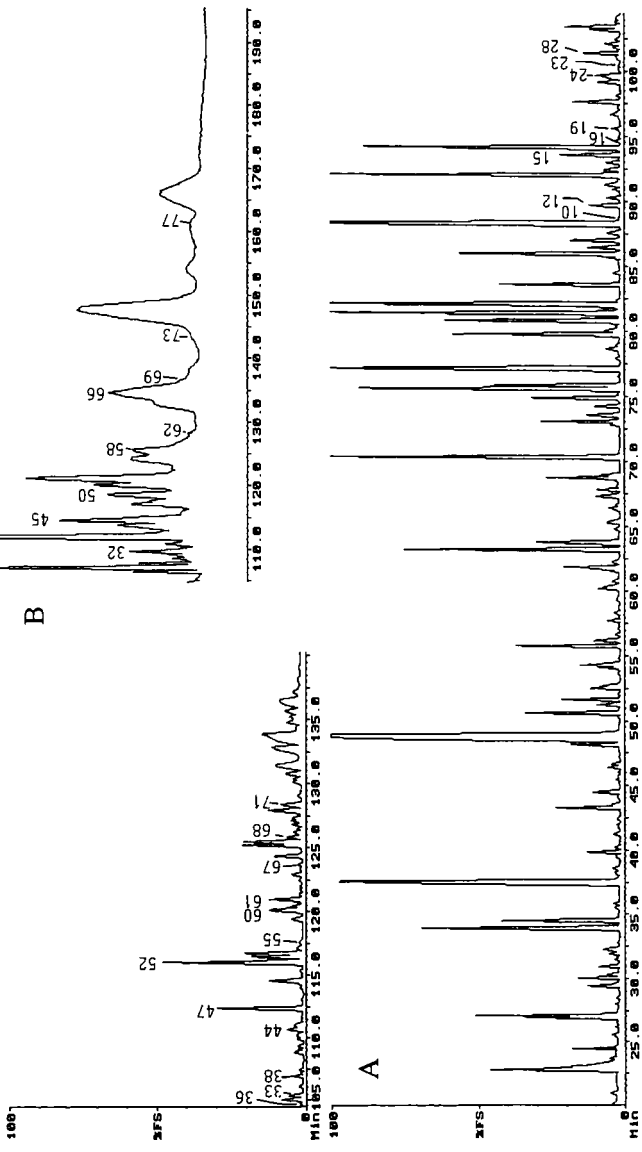
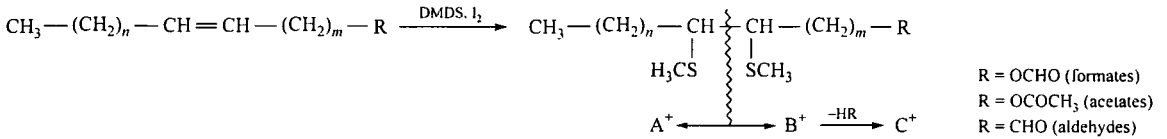


FIG. 2. Total ion chromatogram of an extract of the preorbital secretion of the oribi subjected to dimethyl disulfide derivatization. (A) at 40°C, (B) at 60°C.

TABLE 7. DIAGNOSTIC IONS IN EI MASS SPECTRA OF DMDS DERIVATIVES OF MONOUNSATURATED COMPOUNDS PRESENT IN PREORBITAL SECRETION OF ORIBI



Compound	Compound No. in Figures 1 and 2	Diagnostic ion (%, normalized abundance)				
		M^+	$(M-47)^+$	A^+	B^+	C^+
(Z)-4-Decen-1-yl formate	12	278(9)	231(2)	131(100)	147(3)	101(38)
(Z)-5-Undecen-1-yl formate	19	292(10)	245(2)	131(100)	161(10)	115(26)
(Z)-6-Dodecen-1-yl formate	28	306(10)	259(2)	131(100)	175(14)	129(16)
(Z)-5-Tridecen-1-yl formate	36	320(16)	273(−)	159(100)	161(17)	115(43)
(Z)-7-Tridecen-1-yl formate	38	320(9)	273(3)	131(100)	189(37)	143(11)
(Z)-8-Tetradecen-1-yl formate	47	334(10)	287(3)	131(100)	203(68)	157(11)
(Z)-9-Pentadecen-1-yl formate	55	348(12)	301(3)	131(100)	217(96)	171(6)
13-Methyl-(Z)-8-pentadecen-1-yl formate	60	362(10)	315(3)	159(97)	203(78)	157(7)
(Z)-4-Decen-1-yl acetate	15	292(13)	245(1)	131(100)	161(3)	101(85)
(Z)-5-Undecen-1-yl acetate	24	306(4)	259(1)	131(44)	175(5)	115(25)
(Z)-6-Dodecen-1-yl acetate	33	320(10)	273(1)	131(100)	189(12)	129(49)
(Z)-7-Tridecen-1-yl acetate	44	334(11)	287(2)	131(100)	203(30)	143(36)
(Z)-8-Tetradecen-1-yl acetate	52	348(10)	301(2)	131(100)	217(61)	157(31)
(Z)-9-Pentadecen-1-yl acetate	61	362(9)	315(1)	131(100)	231(76)	171(22)
(Z)-8-Hexadecen-1-yl acetate	67	376(9)	329(2)	159(94)	217(70)	157(34)
(Z)-10-Hexadecen-1-yl acetate	68	376(7)	329(1)	131(97)	245(73)	185(23)
(Z)-9-Heptadecen-1-yl acetate	71	390(4)	343(−)	159(41)	231(39)	171(12)
(Z)-5-Undecenal	10	262(6)	215(−)	131(46)	131(46)	
(Z)-6-Dodecenal	16	276(10)	229(2)	131(100)	145(18)	
(Z)-7-Tridecenal	23	290(9)	243(3)	131(92)	159(9)	

Attempts to determine the position of the double bond in the unsaturated alcohols were unsuccessful as their DMDS derivatives could not be found in the total ion chromatogram of the derivatized secretion. This was probably due to the low concentration of these alcohols in the secretion. The assumption, based on the structures of the other constituents of the secretion, that the double bond in these compounds is situated between C-6 and C-7 from the methyl end of the carbon chain, was confirmed by retention time comparison of the natural constituents with the authentic synthetic alcohols.

In the EI spectrum of component **60,** the $(M-HCOOH)^+$ ion at *m/z* 222 is weaker than the molecular ion at *m/z* 268, and the base peak appears at *m/z* 70. These two phenomena distinguish this monounsaturated formate from other alkenyl formates present in the secretion. The $(M+1)^+$ and $(M+1-HCOOH)^+$ ions at *m/z* 269 and 223 in its CI(NO) spectrum as well as the $(M+NO)^+$, M^+, and $(M-HCOOH)^+$ ions at *m/z* 298, 268, and 222, respectively, in its CI(NO) spectrum confirm that component **60** is a monounsaturated hexadecen-1-yl formate. In the EI spectrum of its DMDS derivative, the presence of the diagnostic ions at *m/z* 159, 203, and 157 suggested the presence of a C-8–C-9 double bond. Synthetic (*Z*)-8-hexadecen-1-yl formate was coinjected with the oribi secretion, and the retention time of component **60** was found to be much shorter than that of the reference compound. On account of this difference in retention time, component **60** was assumed to be a branched monounsaturated formate. According to Brown et al. (1954), Friedel et al. (1956), Dolejs et al. (1968), and Bowen and Maccoll (1984), the base peak in the EI mass spectra of anteisoalcohols appears at *m/z* 70. Component **60** was therefore assumed to be an anteisoformate and, by combining all the relevant information, was identified as 13-methyl-(*Z*)-8-pentadecen-1-yl formate. This was not, however, confirmed by comparison with synthetic material.

According to the EI, CI(CH_4), and CI(NO) mass spectra, component **59** was assumed to be a pentadecen-1-yl acetate. The DMDS derivative of 7-pentadecen-1-yl acetate was found in the EI total ion chromatogram of the derivatized oribi preorbital secretion. In addition, the retention time of component **59** is shorter than the retention time of component **61,** (*Z*)-9-pentadecen-1-yl acetate, the difference between the retention times of these two components being only slightly longer than the difference between the retention times of (*Z*)-8- and (*Z*)-10-hexadecen-1-yl acetate, components **67** and **68**, respectively. It was therefore reasonable to assume that component **59** could be 7-pentadecen-1-yl acetate. However, the retention time of the (*Z*)-7-pentadecen-1-yl acetate reference, which was coinjected with oribi secretion, was longer than that of component **59.** The question arises as to why the DMDS derivative of 7-pentadecen-1-yl acetate was unequivocally identified in the EI total ion chromatogram of the DMDS-derivatized secretion but could not be detected in the EI total ion chromatogram of the secretion itself. The reason may possibly be that

7-pentadecen-1-yl acetate is actually present in the secretion, but in a low concentration and that it is obscured in the EI total ion chromatogram by other components. However, component **59** is not 7-pentadecen-1-yl acetate and could therefore only be characterized as an unidentified pentadecenyl acetate.

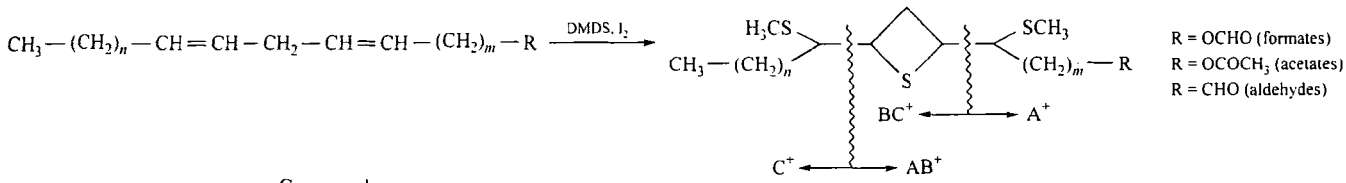
The molecular mass and functionality of the diunsaturated compounds present in the secretion were determined from EI, CI(CH_4), and CI(NO) mass spectral data.

The DMDS derivatization method for the determination of the position of double bonds as described by Buser et al. (1983) produced only two mono-DMDS derivatives of a single diunsaturated acetate, component **50.** Their EI spectra both have a molecular ion at *m/z* 346. Typical diagnostic ions appear at *m/z* 171 (A^+), 175 (B^+), 115 ($B^+ - CH_3COOH$) in one of the spectra, suggesting that one double bond in component **50** is in the 5 position. The diagnostic ions appear at *m/z* 131 (A^+), 215 (B^+), and 155 ($B^+ - CH_3COOH$) in the spectrum of the second DMDS derivative, indicating that the other double bond in component **50** is in the 8 position. Component **50** was therefore concluded to be 5,8-tetradecadien-1-yl acetate. The formation of the mono-DMDS derivative of a diunsaturated acetate has been described by Tonini et al. (1986) in a paper on the sex pheromone of the leopard moth. The DMDS derivatization procedure described by Vincenti et al. (1987), in which a higher reaction temperature and a longer reaction time are used, was therefore employed as an alternative method. According to Vincenti et al. (1987) and Carlson et al. (1989), the structures of the DMDS derivatives of compounds containing a diene system depend on the number of carbon atoms between the two double bonds. Information furnished by the partial derivatization of the skipped diene system of component **50,** 5,8-tetradecadien-1-yl acetate, according to Buser et al. (1983), proved useful in the interpretation of the more complex results produced by the complete derivatization according to Vincenti et al. (1987).

The reaction of a skipped diene with two molecules of DMDS gives four-membered cyclic thioethers substituted with two alkyl chains, each containing a methylthio group α to the ring. The mass spectrometric fragmentation of the DMDS derivatives of constituents containing a skipped diene system is shown in Table 8.

Information on the molecular mass and functionality of a number of alkadienals was supplied by the presence of $(M+1)^+$ and $(M+1-H_2O)^+$ ions in their CI(CH_4) mass spectra. However, only one DMDS derivative of an alkadienal, that of 5,8-tetradecadienal **32,** could be detected in the total ion current chromatogram of the derivatized secretion. The molecular ion, which is absent from the mass spectra of the derivatized alkadienyl acetates and formates, appears at *m/z* 334 in the mass spectrum of the derivatized dienal **32.** The ion at *m/z* 287 is formed by the loss of a methylthio radical from the molecular ion. The diagnostic ions at *m/z* 203 [AB^+ and BC^+], 155 [$(AB-CH_3SH)^+$ and

TABLE 8. DIAGNOSTIC IONS IN EI MASS SPECTRA OF DMDS DERIVATIVES OF DIUNSATURATED COMPOUNDS PRESENT IN PREORBITAL SECRETION OF ORIBI



Compound	Compound No. in Figures 1 and 2	Diagnostic ion (%, normalized abundance)											
		M'	(M-47)'	(M-48)'	(M-95)'	AB'	(AB-48)'	(AB-RH)'	(AB-48-RH)'	BC+	(BC-48)+	A+	C+
(5Z,8Z)-5,8-Tetradecadien-1-yl acetate	50	378(−)	331(7)	330(17)	283(8)	247(−)	199(—)	187(−)	139(84)	203(7)	155(51)	175(1)	131(22)
(6Z,9Z)-6,9-Pentadecadien-1-yl acetate	58	392(−)	345(15)	344(36)	297(20)	261(−)	213(3)	201(−)	153(60)	203(16)	155(100)	189(−)	131(31)
(7Z,10Z)-7,10-Hexadecadien-1-yl actate	66	406(−)	359(6)	358(14)	311(13)	275(−)	227(4)	215(1)	167(30)	203(7)	155(39)	203(7)	131(16)
(8Z,11Z)-Heptadecadien-1-yl acetate	73	420(−)	373(4)	372(6)	325(6)	289(2)	241(4)	229(3)	181(10)	203(5)	155(35)	217(5)	131(7)
(9Z,12Z)-9,12-Octadecadien-1-yl acetate	77	434(−)	387(3)	386(4)	339(5)	303(−)	255(−)	243(14)	195(5)	203(4)	155(22)	231(6)	131(−)
(5Z,8Z)-5,8-Tetradecadien-1-yl formate	45	364(−)	317(10)	316(27)	269(16)	233(1)	185(5)	187(−)	139(36)	203(11)	155(73)	161(5)	131(18)
(7Z,10Z)-7,10-Hexadecadien-1-yl formate	62	392(−)	345(3)	344(7)	297(6)	261(−)	213(2)	215(−)	167(4)	203(2)	155(18)	189(2)	131(11)
(8Z,11Z)-8,11-Heptadecadien-1-yl formate	69	406(−)	359(6)	358(25)	311(18)	275(−)	227(13)	229(−)	181(27)	203(2)	155(28)	203(2)	131(16)
(5Z,8Z)-5,8-Tetradecadienal	32	334(32)	287(4)	286(−)	239(−)	203(29)	155(50)			203(29)	155(50)	131(100)	131(100)

$(BC-CH_3SH)^+$], and 131 [A^+ and C^+] provide evidence that the double bonds are in the 5 and 8 positions. Component **32** was therefore identified as a 5,8-tetradecadienal. All of the alkadienals were found to contain a skipped diene system. Their structures and the configuration of the double bonds were confirmed by GC retention time comparison with synthetic compounds, employing coinjection techniques.

The EI mass spectra of the four components, **30, 40, 49,** and **57,** are similar to those of the alkadienals except for the presence in their spectra of the ion at *m/z* 31, which is typical for alcohols and formates. The prominent $(M+1)^+$ and $(M+1-H_2O)^+$ ions in their CI(CH_4) spectra indicated that they could be alkadienols. Although the $(M-3)^+$ ion, normally present in the CI(NO) spectra of primary alcohols, does not appear in an appreciable abundance in their CI(NO) mass spectra, the presence of a primary hydroxyl function in these compounds is confirmed by the presence of $(M-1)^+$ and $(M-2+NO)^+$ ions and the absence of a $(M-OH)^+$ ion. No DMDS derivative of an alkadienol could, however, be located in the total ion chromatogram shown in Figure 2 and, as a working hypothesis, these compounds were assumed to contain skipped diene systems similar to those found in the formates and acetates. This assumption was confirmed by coinjection of the secretion with the synthetic compounds.

In contrast to the other compound types, only saturated carboxylic acids are present in the secretion. The compounds identified in the preorbital secretion of the oribi are listed in Table 9 together with quantitative data and information on the methods used in their identification.

(4*Z*,7*Z*)-4,7-Tridecadien-1-ol, **30,** was synthesized using published procedures as outlined in Scheme 1. The bromination of the alkenol 1-**2** with phosphorus tribromide and pyridine gave (*Z*)-1-bromo-2-octene 1-**3** containing 10% of the *E* isomer. Condensation of this bromide with an excess of the acetylenic Grignard reagent 1-**6** in the presence of CuCl under reflux conditions, followed by partial hydrogenation and acid catalyzed removal of the protective groups, gave a mixture of 4,7-tridecadien-1-ol isomers. Column chromatography on 7% $AgNO_3$-coated silica gel was used to separate the product isomers and to isolate (4*Z*,7*Z*)-4,7-tridecadien-1-ol, **30.**

(6*Z*,9*Z*)-6,9-Pentadecadien-1-ol, **49,** was synthesized according to Scheme 2. In this synthesis the alcohol function was protected as its *tert*-butyl ether (Alexakis et al., 1988). The use of Ac_2O and $FeCl_3$ for the conversion of *tert*-butyl ethers to the corresponding acetates has been reported (Alexakis et al., 1988; Ganem and Small, 1974; Gross and Watt, 1987; Alexakis and Duffault, 1988). However, this method proved unsuccessful in this synthesis. Me_3SiI as deprotection reagent (Jung and Lyster, 1977), chosen for its reported high yield and limited reaction time, afforded complete cleavage of the ether in 6 min at room temperature. Column chromatography on 7% $AgNO_3$-coated silica gel was used to isolate pure (6*Z*,9*Z*)-6,9-pentadecadien-1-ol, **49.**

TABLE 9. COMPOUNDS IDENTIFIED IN PREORBITAL SECRETION OF ORIBI

No. in Figure 1	Compounds	Analytical methods	Quantity (ng/animal)
3	1-Nonanol	a,b,c,f[a]	0.16
9	1-Decanol	a,b,c,f	0.10
2	1-Octyl formate	a,b,f	0.03
7	1-Nonyl formate	a,b,c,f	0.33
14	1-Decyl formate	a,b,c,f	0.58
20	1-Undecyl formate	a,b,c,f	1.79
29	1-Dodecyl formate	a,b,c,f	4.21
39	1-Tridecyl formate	a,b,c,f	7.64
48	1-Tetradecyl formate	a,b,c,f	9.56
56	1-Pentadecyl formate	a,b,c	2.51
64	1-Hexadecyl formate	a,b,c,f	0.66
6	1-Octyl acetate	a,b,f	[b]
13	1-Nonyl acetate	a,b,c,f	0.19
18	1-Decyl acetate	a,b,c,f	0.55
27	1-Undecyl acetate	a,b,c,f	0.51
37	1-Dodecyl acetate	a,b,c,f	1.47
46	1-Tridecyl acetate	a,b,c	5.88
54	1-Tetradecyl acetate	a,b,c,f	14.87
63	1-Pentadecyl acetate	a,b,c	18.60
70	1-Hexadecyl acetate	a,b,c,f	5.21
75	1-Heptadecyl acetate	a,b,c	0.50
1	Nonanal	a,b,c,f	0.02
4	Decanal	a,b,c,f	0.02
11	Undecanal	a,b,c,f	0.06
17	Dodecanal	a,b,c,f	0.30
26	Tridecanal	a,b,c	0.33
65	Hexadecanoic acid	a,b,c,f	0.94
72	Heptadecanoic acid	a,b,c,f	0.57
76	Octadecanoic acid	a,b,f	0.31
8	(Z)-4-Decen-1-ol	a,b,c	0.20
21	(Z)-6-Dodecen-1-ol	a,b,c,f	0.27
31	(Z)-7-Tridecen-1-ol	a,b,f	0.28
41	(Z)-8-Tetradecen-1-ol	a,b,c,f	1.32
5	(Z)-3-Nonen-1-yl formate	a,b,c	[b]
12	(Z)-4-Decen-1-yl formate	a,b,c,d	0.11
19	(Z)-5-Undecen-1-yl formate	a,b,c,d,f	0.34
28	(Z)-6-Dodecen-1-yl formate	a,b,c,d,f	2.07
36	(Z)-5-Tridecen-1-yl formate	a,b,c,d	0.17
38	(Z)-7-Tridecen-1-yl formate	a,b,c,d,f	1.13
47	(Z)-8-Tetradecen-1-yl formate	a,b,c,d,f	7.91
55	(Z)-9-Pentadecen-1-yl formate	a,b,c,d,f	0.31
60	13-Methyl-(Z)-8-pentadecen-1-yl formate	a,b,c,d,e	[c]

TABLE 9. Continued

No. in Figure 1	Compounds	Analytical methods	Quantity (ng/animal)
15	(Z)-4-Decen-1-yl acetate	a,b,c,d	0.38
24	(Z)-5-Undecen-1-yl acetate	a,b,c,d,f	0.49
33	(Z)-6-Dodecen-1-yl acetate	a,b,c,d,f	1.00
44	(Z)-7-Tridecen-1-yl acetate	a,b,c,d,f	1.43
52	(Z)-8-Tetradecen-1-yl acetate	a,b,c,d,f	10.20
59	[d]-Pentadecen-1-yl acetate	a,b,c	[c]
61	(Z)-9-Pentadecen-1-yl acetate	a,b,c,d,f	2.89
67	(Z)-8-Hexadecen-1-yl acetate	a,b,c,d,f	0.97
68	(Z)-10-Hexadecen-1-yl acetate	a,b,c,d,f	1.05
71	(Z)-9-Heptadecen-1-yl acetate	a,b,c,d	0.53
10	(Z)-5-Undecenal	a,b,d,f	0.02
16	(Z)-6-Dodecenal	a,b,c,d	0.55
23	(Z)-7-Tridecenal	a,b,d,f	0.27
34	(Z)-8-Tetradecenal	a,b,d,f	1.10
30	(4Z,7Z)-4,7-Tridecadien-1-ol	a,b,c,f	1.60
40	(5Z,8Z)-5,8-Tetradecadien-1-ol	a,b,c	5.80
49	(6Z,9Z)-6,9-Pentadecadien-1-ol	a,b,c,f	7.63
57	(7Z,10Z)-7,10-Hexadecadien-1-ol	a,b,c	1.18
25	(3Z,6Z)-3,6-Dodecadienyl-1-formate	a,b	0.05
35	(4Z,7Z)-4,7-Tridecadienyl-1-formate	a,b,c,f	1.25
45	(5Z,8Z)-5,8-Tetradecadienyl-1-formate	a,b,c,d	6.87
53	(6Z,9Z)-6,9-Pentadecadienyl-1-formate	a,b,c,f	40.37
62	(7Z,10Z)-7,10-Hexadecadienyl-1-formate	a,b,c,d	1.86
69	(8Z,11Z)-8,11-Heptadecadienyl-1-formate	a,b,c,d	7.57
74	(9Z,12Z)-9,12-Octadecadienyl-1-formate	a,b,c,f	0.30
42	(4Z,7Z)-4,7-Tridecadien-1-yl acetate	a,b,c,f	4.88
50	(5Z,8Z)-5,8-Tetradecadien-1-yl acetate	a,b,c,d	14.24
58	(6Z,9Z)-6,9-Pentadecadien-1-yl acetate	a,b,c,d,f	16.83
66	(7Z,10Z)-7,10-Hexadecadien-1-yl acetate	a,b,c,d	35.72
73	(8Z,11Z)-8,11-Heptadecadien-1-yl acetate	a,b,c,d	7.06
77	(9Z,12Z)-9,12-Octadecadien-1-yl acetate	a,b,c,f	4.70
22	(4Z,7Z)-4,7-Tridecadienal	a,b,c,f	0.94
32	(5Z,8Z)-5,8-Tetradecadienal	a,b,c,d	13.30
43	(6Z,9Z)-6,9-Pentadecadienal	a,b,c,f	18.67
51	(7Z,10Z)-7,10-Hexadecadienal	a,b,c	0.55

[a] a, low-resolution EI mass spectral data; b, CI(CH_4) mass spectral data; c, CI(NO) mass spectral data; d, EI mass spectral data of dimethyl disulfide derivative; e, published data; f, retention time comparison.

[b] Unresolved peak; total quantity 0.04 ng.

[c] Unresolved peak; total quantity 2.26 ng.

[d] Position and configuration of the double bound uncertain.

The formates were prepared by esterification of the corresponding alcohols with formic acid at room temperature, and the acetates were obtained by acetylation of the alcohols with acetyl chloride and pyridine in ether. The unsaturated aldehydes were prepared by oxidation of the corresponding alcohols with pyridinium chromate on silica gel in dichloromethane in the presence of a small quantity of acetic acid.

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