MAMMALIAN EXOCRINE SECRETIONS. XVIII: CHEMICAL CHARACTERIZATION OF INTERDIGITAL SECRETION OF RED HARTEBEEST, *Alcelaphus buselaphus caama*

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Abstract
Gas chromatography, coupled gas chromatography-mass spectrometry (electron impact mode and chemical ionization with methane as reactant gas), gas chromatography-infrared spectroscopy, and derivatization techniques were used to identity 53 compounds in the interdigital secretion of the red hartebeest, *Alcelaphus buselaphus caama*. These compounds included alkanes, isoalkanes, alcohols, ketones, carboxylic acids, oxiranes, furanoid linalool oxides, and a large number of branched and unbranched saturated and unsaturated aldehydes. The secretion probably plays a role in demarcation of territories by dominant bulls.

Key Words
Red hartebeest, *Alcelaphus buselaphus*, semiochemicals, pheromones, mass spectra, branched-chain aldehydes, territorial marking.

INTRODUCTION

The red hartebeest, *Alcelaphus buselaphus*, is a member of the alcelaphine group of antelopes (Family Bovidae, Tribe Alcelaphini). Two main subspecies are distinguished: the North African Hartebeest, *Alcelaphus buselaphus buselaphus*, now extinct, and the Red Hartebeest, *Alcelaphus buselaphus caama*, a relatively rare antelope mostly encountered in the southern African subregion, from Namibia southeastwards to the Botswana border and in the north of the Northern Cape Province along the Botswana border.

Their habitat is grassland, semidesert bush savanna, and open woodland. They avoid the more closed types of woodland (Kok, 1975; Skinner and Smithers, 1990). Adult male red hartebeest have a mass of about 150 kg. Females are slightly smaller with a mean mass of 120 kg. High humped shoulders, sloping backs, and elongated heads, held high on their upright necks, characterize the shape of the body and head. Hairs are reddish-brown in general, with dark and black patches on the forehead, on the front of the shoulder, on forelegs, and hind legs. Usually, males are territorial and herd females, actively defending their territory against other trespassing males.

The social structure consists of harem herds (groups of females with their offspring, a dominant bull, and young bulls), bachelor herds (males of all ages), and, to a small extent, territorial bulls without followers (Kok, 1975). In territorial behavior, visual and olfactory techniques are used for demarcation. Olfactory demarcation is carried out by feces and urine, since harem bulls frequently lie down on dung patches, typical behavior by which their char- acteristic odor is spread.

It is claimed that ground horning by bulls has a territorial function because it could facilitate transfer of preorbital secretion to the ground. Pawing and scratching with the foreleg before lying down is regularly performed by both sexes. This behavior also could play a role in marking by transferring secretion from the interdigital glands, situated between the front hooves, to the ground. In general, these glands occur in both sexes, but are more productive in territorial bulls (Kok, 1975). The chemical compositions of the preorbital and interdigital secretions of the red hartebeest are unknown.

This antelope is of interest from a nature preservation point of view, and the development of a semiochemical lure that can be used in translocation operations could play a future role in its conservation. Identification of the constituents of the interdigital secretion of the red hartebeest reported here is a first step toward development of such a lure.

METHODS AND MATERIALS

General.

All Pyrex glassware was throughly cleaned with water and organic solvents, and heated at 500°C in an annealing oven to remove any trace of organic contaminants. Syringes and other apparatus that could not be heated to 500°C were cleaned with dichloromethane (Merck, Residue Analysis Grade). Interdigital secretions were extracted and extracts diluted, when necessary, with this solvent.

Collection and Sample Preparation

Interdigital secretions were collected individually from four male and four female red hartebeest captured in Kimberly, Northern Province, South Africa, during August 1999. Surgical gauze squares (ca. 25×25 mm) consisting of several layers of surgical gauze were extracted for five hr with dichloromethane (Residue Analysis Grade), dried in an atmosphere of activated charcoal purified N_2, and stored in glass-stoppered bottles.

Interdigital secretion was collected by rolling a gauze square around the tip of dressing forceps, inserting the forceps into the interdigital cavity, and collecting the secretion by rotating the forceps while removing it from the cavity. This procedure does not seem to be painful to the animals because they did not twitch

during the procedure, nor attempted to kick themselves free from their handlers. The gauze pads with the secretion were stored at −30°C in glass bottles with Teflon-lined screw caps until used for analysis.

To extract the organic constituents of the secretion from the gauze, just enough CH_2C1_2 to wet the gauze was added to one or more gauze pads in a suitable glass vial. The vial was closed with a screw cap with a Teflon-lined septum and was centrifuged for 1 min at 1500 rpm to enhance contact between the gauze and the small volume of solvent by compressing the gauze in the vial, after which the contents were sonicated for 2 min. The extract was separated from the gauze by centrifuging in a sintered glass filter insert suspended in a 5-ml Reacti-Vial.

If a more concentrated solution was required, the solvent was evaporated by placing the vial containing the extract in a 2-1 glass beaker covered with aluminum foil and purging the solvent vapor from the beaker with a weak stream of purified (activated charcoal) nitrogen. Care was taken not to blow the purge gas directly into the vial containing the extract.

Analytical Methods

Gas chromatographic (GC) analyses were carried out on a Carlo Erba 5300 (Mega) gas chromatograph equipped with a flame ionization detector (FID) and a split/splitless injector. The FID was operated at 280°C. Glass capillary columns (40 m × 0.3 mm), drawn from Duran 50 glass (Schott, Mainz, Germany), were coated in our laboratory with the following phases: (1) PS-089-OH (DB-5 equivalent, film thickness 0.25 µm, (2) OV-1701-OH, film thickness 0.375 µm, and (3) Carbowax 20M, film thickness 0.25 µm. Hydrogen was used as carrier gas at a linear velocity of 50 cm/sec.

Samples were injected in split mode (injector temperature 220°C) with an oven temp of ≤30°C. After injection, the oven was heated ballistically to 40°C, then at 2°C from 40–280°C. Temperature programming and data acquisition were started at 40°C. A programming rate of 4°C/min was used in GC analyses of synthetic compounds and intermediates. Low-resolution electron impact mass spectra (EI-MS) were obtained at 70 eV on a Carlo Erba QMD 1000 GC-MS instrument using the above columns and conditions with He as carrier gas (linear velocity 28.6 cm/sec).

The injector temperature was set at 220°C, the interface at 250°C, and the ion source at 180°C. A scan rate of 0.9 sec per scan with an interval of 0.1 sec between scans was employed. Chemical ionization mass spectra, with methane as reactant gas [CI(CH_4)-MS] were obtained on the same GC-MS instrument. The methane flow into the ion source was regulated to keep the source housing pressure at an optimum value of 8×10^{-5} torr. Chiral GC and GC-MS analyses were done with a capillary column (30 m × 0.2 mm) coated with OV-1701-OH

containing 13% (w/w) of the chiral selector heptakis(2,3-di-*O*-methyl-*6*-*O*-*tert*-butyldimethylsilyl)-β -cyclodextrin (synthe- sized in our laboratory) at a film thickness of 0.25 μm, using hydrogen and helium, respectively, as carrier gasses.

Solid-phase microextraction (SPME) was used to extract the volatile compounds from the headspace in a 50-ml vial containing the interdigital secretion on gauze pads as well as some hairs extracted from the interdigital cavity. The 100-μm polydimethylsiloxane SPME fiber was exposed to the secretion for 60 min at room temperature (22°C). Enriched volatiles were desorbed from the fiber for 5 min at an injector temperature of 220°C, cryotrapped on the column for 30 sec with dry ice, and analyzed by GC and GC-MS using the specified columns and conditions. Mass spectral data were also obtained using a solventless sample introduction technique.

About 10–15 hairs from the interdigital cavity were inserted into a glass injector liner. To circumvent the oxidation of sensitive compounds at 220°C in the presence of oxygen, the liner containing the hairs was introduced into the injector at 40°C, and the injector was heated to 220°C over a period of 4 min. The desorbed volatile organic compounds were cryotrapped on the column with dry ice. The liner and septum were quickly replaced by a clean liner and septum after which the cryotrapping was terminated and the oven temperature programs and data acquisition were started.

GC-IR data were obtained with an HP 5890 series II GC equipped with an HP IRD 5965B IR detector. Column 2, specified above, and a temperature program of 4°C/min from 40°C to 250°C were used. The temperature of the injection port was set at 250°C and the transfer line and flow cell at 220°C. The sample was injected splitless with a purge delay of 10 sec. ^{1}H and ^{13}C NMR spectra of synthetic compounds and intermediates were recorded at 299.905 and 75.42 MHz, respectively, at 25°C on a Varian VXR 300 NMR spectrometer.

Lithium Aluminium Hydride Reduction

An aliquot of the interdigital secretion extract was reduced with excess $LiAlH_4$ in diethyl ether. Thus, a sample (4 μl) of the concentrated extract was transferred to a Reacti-Vial. The solvent was gently evaporated. To remove residual CH_2C1_2, two drops of diethyl ether were added to the vial and evaporated in a slow stream of nitrogen. This step was repeated, after which the residue was diluted with a few drops of ether and treated with two drops of a saturated solution of $LiAlH_4$ in ether. The reaction mixture was sealed and heated to 40°C for 1 hr, cooled, diluted with ether (1 ml), cooled in ice, and treated with a drop of distilled water. The mixture was centrifuged and the ether supernatant was removed with a 100-μl syringe and concentrated for GC-MS analysis.

Pyrrolidide Derivatives

A small sample (ca. 8 μl) of the extract was concentrated under nitrogen, The residue was dissolved in diethyl ether (1 ml, containing 10% methanol), and

treated with diazomethane-diethyl ether solution until the mixture remained yellow. The mixture was concentrated under a stream of N_2.

The resulting mixture of methyl esters was dissolved in pyrrolidine (1 ml) and acetic acid (100 µl), and the solution was heated at 100°C for 30 min (Andersson and Holman, 1974). After cooling to room temperature, the amides were taken up in ether (2 ml), and the solution was washed three times with 2 M HC1 and three times with distilled water. The organic phase was concentrated for GC-MS analysis under a stream of nitrogen.

Trimethylsilyl Derivatives

Approximately 5 µl of the extract were treated with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) (Pierce, Rockford, IL), and the solution was heated at 40°C for 30 min. The resulting mixture of trimethylsilyl ethers was immediately subjected to GC-MS analysis (Donike, 1969).

Reference Compounds

Some of the compounds identified in the interdigital secretion of the red hartebeest were available from previous research projects, whereas others were obtained commercially. The following compounds were synthesized.

2-Methylundecane (29)

Wurtz condensation of 1-bromoheptane and 1-bromo-3-methylbutane (Stander et al., 2002) gave a mixture of 2-methylundecane [MS *m/z* (%): 170 (1), 155 (1), 127 (4), 126 (5), 113 (2), 99 (5), 97 (3), 85 (23), 84 (3), 71 (48), 70 (7), 69 (6), 57 (86), 56 (18), 55 (16), 43 (100), 42 (19), 41 (44)], 2,7-dimethyloctane, and tetradecane.

6-Methyl-1-heptanol (27)

A solution of phosphorous tribromide (13.5 g, 50 mmol) in dichloromethane (10 ml) was added over 30 min at −20°C to a solution of 4-methyl-1-pentanol (5.11 g, 50 mmol) and pyridine (0.5 g, 6 mmol) in dichloromethane (10 ml) (Tang et al., 1995) at −20°C. The reaction mixture was stirred for another 4 hr at 0°C, carefully treated with water (5 ml), and the organic product was washed with saturated sodium bicarbonate solution and brine.

Isolation of the organic product in the usual manner and distillation (70°C/53 mm Hg) gave 1-bromo-4-methylpentane (4.6 g, 56%). A solution of 1-bromo-4-methylpentane (4.5 g, 27 mmol) in dry THF (25 ml) was added drop-wise to magnesium turnings (648 mg, 27 mmol) in dry THF (15 ml), and the reaction mixture was stirred for 3 hr. Anhydrous CuI (500 mg, 2.6 mmol) was added to the resulting Grignard reagent at −10◦C, and the reaction mixture was treated with gaseous ethylene oxide (6.2 g, 141 mmol), introduced by means of a tube with its tip about 15 mm above the surface of the mixture. The reaction mixture was stirred overnight at −20◦C, then hydrolyzed with water (3 ml), the resulting solid

material was filtered off and washed repeatedly with hexane, and the combined filtrate and washings were washed with HCl (3M) and brine.

Work-up gave 6-methyl-1-heptanol **(27)** (2.7 g, 47%). MS: 98 (2), 97 (19), 84 (18), 83 (6), 70 (25), 69 (58), 68 (19), 57 (36), 56 (100), 55 (76), 43 (77), 41 (72).

4-Methylpentanal (11)

Oxidation of 4-methyl-1-pentanol with pyridinium dichromate according to Tietze and Eicher (1981) gave 4-methylpentanal. MS: 100 (0.1), 99 (0.1), 85 (1), 82 (1), 81 (2), 72 (12), 71 (7), 67 (11), 58 (16), 57 (100), 56 (98), 55 (19), 44 (12), 43 (78), 41 (76).

3-Methyl-3-butenal (15)

Oxidation of 3-methyl-3-buten-1-ol with pyridinium dichromate (Tietze and Eicher, 1981) gave 3-methyl-3-butenal. Because of its volatility, the product was not isolated but was used as a solution in ether. There was no detectable isomerization to the conjugated isomer. MS: 85 (8), 84 (99), 83 (54), 69 (7), 65 (2), 56 (13), 55 (100), 53 (25), 41 (71), 39 (79).

5-Methylhexanal (17)

This compound was prepared as described by Andersson et al. (1979). Isobutyl magnesium bromide (16.1 g, 0.1 mol) in dry ether was added drop-wise over 20 min to a cold (10°C) stirred solution of acrolein diethyl acetal (13 g, 0.1 mol) and CuBr (0.5 g) in 100 ml THF. After 2 hr at room temperature, the reaction mixture was treated with saturated ammonium chloride, extracted with pentane, dried, and concentrated to give 5-methylhexanal diethyl acetal.

The acetal was refluxed for 1 hr with aqueous acetone (35 ml, 75%) and a few drops of conc. HC1 to hydrolyze the acetal. Standard isolation procedures gave 5-methylhexanal. MS: 96 (28), 86 (9), 81 (39), 71 (51), 70 (44), 57 (23), 56 (9), 55 (72), 44 (67), 43 (100), 41 (69).

6-Methylheptanal (22)

Oxidation of 6-methyl-1-heptanol with pyridinium dichromate (Tietze and Eicher, 1981) gave 6-methylheptanal. MS: 110 (2), 100 (5), 96 (2), 95 (18), 85 (23), 84 (12), 82 (31), 71 (5), 69 (33), 67 (14), 57 (54), 56 (35), 55 (20), 44 (38), 43 (100), 42 (17), 41 (70).

8-Methylnonanal (39)

Reduction of 8-methylnonanoic acid (Sigma Aldrich, Cape Town, South Africa) with $LiAlH_4$ in ether and subsequent oxidation of the alcohol with pyridinium dichromate (Tietze and Eiche 1981) gave 8-methylnonanal in almost quantitative yield. MS: 128 (3), 123 (5), 112 (4), 110 (5), 109 (2), 97 (6), 96 (6), 95 (23), 83 (10), 82 (41), 81 (23), 71 (28), 70 (20), 69 (38), 68 (19), 67 (23), 57 (100), 56 (52), 55 (53), 43 (93), 41 (76).

(E)- and (Z)-8-Methyl-2-Nonenal (45) and (47)

These isomeric aldehydes were synthesized from 1-bromo-5-methylhexane (1-**1**) according to Scheme 1. The starting bromide for the synthesis was obtained by conventional methods from 1-bromo-3-methylbutane *via* alkylation of diethyl malonate, hydrolysis of the alkylation product, decarboxylation of the alkylated malonic acid, reduction of the resulting 5-methylhexanoic acid to 5-methyl-1-hexanol, and bromination with PBr_3. 1-Bromo-5-methylhexane (1-**1**) (37.8 g, 0.21 mol) was condensed with sodium acetylide in liquid NH_3 (500 ml) according to Ziegenbein (1963).

The reaction mixture was stirred for 2 hr, *N*,*N*-dimethylformamide (200 ml) was added, the liquid NH_3 was allowed to evaporate, and the reaction mixture was stirred overnight at room temperature. The unreacted acetylide was hydrolyzed with ice-cold water (200 ml), extracted with pentane, and the extract was washed twice with water, then dried over anhyd. $MgSO_4$. Filtration, concentration, and distillation (bp. 142–143°C) gave 7-methyl-1-octyne (1-**2**) (21.0 g, 80%). MS:109 (22), 95 (12), 82 (35), 81 (97), 79 (35), 69 (47), 68 (25), 67 (100), 57 (14), 56 (27), 55 (52), 54 (18), 53 (22), 43 (91), 41 (87), 39 (43).

7-Methyl-1-octyne (1-**2**) (9 g, 72 mmol) was added drop-wise to a suspension of $NaNH_2$ (9 g, 0.23 mol) in dry ether (80 ml), the reaction mixture was refluxed for 4 hr, poured onto dry ice (60 g), and allowed to stand overnight (Wotiz, 1954). The reaction mixture was treated with water and extracted with ether. The water phase was acidified with phosphoric acid, and extracted with ether. After drying and concentration, the residue (6 g) contained some unreacted 7-methyl-1-octyne and the target compound 8-methyl-2-nonynoic acid (1-**3**). MS: 135 (1), 126 (2), 125 (2), 109 (11), 97 (8), 95 (7), 81 (68), 69 (35), 67 (68), 57 (12), 56 (20), 55 (44), 53 (16), 44 (54), 43 (100), 41 (80). This product was used without further purification.

Reduction of 8-methyl-2-nonynoic acid (1-**3**) (0.14 g) with a clear solution of $LiAlH_4$ in ether for 22 hr at 22°C, and work-up in the conventional manner, gave a mixture of products (100 mg, 77%) containing 8-methyl-2-nonen-1-ol, 8-methyl-1-nonanol, and 8-methyl-2-nonyn-1-ol (91:4:5). 8-Methyl-2-nonen-1-ol (1-**5**): MS: 138 (3), 123 (9), 110 (7), 109 (9), 96 (14), 95 (42), 82 (56), 81 (32), 69 (43), 67 (42), 57 (100), 56, (41), 55 (55), 43 (86), 41 (88), 39 (37), 29 (30). ^{13}C NMR analysis showed that (*Z*)- and (*E*)-8-methyl-2-nonen-1-ol (1-**5a** and 1-**5b**, respectively) were present in a ratio of 1:10. (*E*)-8-methyl-2-nonen-1-ol (1-**5b**): ^{13}C NMR ($CDCl_3$): δ = 22.6 (q, C-9, C-8 Me); 26.91 (t); 27.90 (d, C-8); 29.37 (t); 32.22 (t, C-4); 38.81 (t); 63.79 (t, C-1); 128.75 (d, C-2); 133.57 (d, C-3). (*Z*)-8-methyl-2-nonen-1-ol (1-**5a**): ^{13}C NMR ($CDCl_3$): δ = 58.57 (t, C-1); 128.25 (d, C-2); 133.25 (d, C-3). (Other resonances not assigned.) The mixture of alcohols (1-**5a**) and (1-**5b**) (100 mg) in hexane (250 μl) was added to a suspension of MnO_2 (250 mg) in hexane (250 μl) (Hickinbottom, 1957).

The mixture was stirred for 20 hr at room temperature in darkness, then filtered and concentrated to give (*E*)-8-methyl-2-nonenal **(47)** (43 mg, 44%, purity 97.3% (GC-MS). ^{13}C NMR did not show the presence of any (*Z*)-isomer in this product. Another 250 μl of hexane was added to the recovered MnO_2, and the suspension was stirred for a further 36 hr. Work-up as before gave a mixture (4 mg) of the (*E*)- and (*Z*)-aldehydes, that were separable on the apolar PS-089 column.

This material, which also contained traces of the acetylenic alcohol (1–4), was not combined with the main product, but could be used for retention time comparison with the natural (*Z*)-aldehyde **(45)**. (*E*)-8-methyl-2-nonenal **(47)**. MS: 136 (5), 121 (18), 111 (10), 110 (7), 107 (8), 97 (20), 95 (23), 93 (15), 83 (44), 81 (20), 70 (58), 69 (50), 57 (43), 55 (57), 43 (98), 41 (100), 39 (52), 29 (32). ^{13}C NMR ($CDCl_3$): δ = 22.39 (q, C-9, C-8 Me), 26.72 (t), 27.69 (d, C-8), 27.94 (t), 32.65 (t, C-4), 38.49 (t), 133.15 (d, C-2), 159.27 (d, C-3), 194.53 (d, C-1). (Z)-8-Methyl-2-nonenal (**45**). MS: 136 (4), 121 (13), 111 (7), 110 (5), 107 (7), 97 (13), 95 (15), 93 (10), 83 (39), 81 (14), 70 (55), 69 (47), 57 (40), 55 (66), 43 (100), 41 (97), 39 (49), 29 (34).

2-Methyl-2,3-Epoxypentane (Ethyl-2,2-Dimethyloxirane) (8)

2-Methyl-2- pentene (0.8 g, 9.5 mmol) was added in portions over 20 min to a stirred solution of m-chloroperbenzoic acid (MCPBA), (1.9 g, 11 mmol) in chloroform (20 ml) at 0°C (Vogel, 1989). The mixture was stirred overnight in the dark at room temperature, cooled to −10°C and the *m*-chlorobenzoic acid filtered off. The filtrate was washed with $NaHCO_3$ solution (10 ml, 10%), dried over anhydrous sodium sulfate, and concentrated to give the title compound. MS: 85 (34), 71 (10), 59 (100), 58 (85), 57 (53), 43 (86), 42 (80), 41 (89).

Linalool Oxides

cis- and trans-Furanoid linalool oxides (**32a**) and (**32b**), were prepared from linalool according to Scheme 2 (Tang et al., 1995). Linalool (0.15 g, 1 mmol) was added drop-wise over 15 min to a stirred solution of MCPBA (0.21 g, 1.2 mmol) in CH_2Cl_2 (8 ml) at 0°C. The mixture was stirred 5 hr at 0°C and overnight at room temperature.

The solids were filtered off, and the filtrate was washed with aqueous NaOH (10%) and brine, and concentrated to give a mixture of *cis*- and *trans*-furanoid linalool oxides, (**32a**) and (**32b**), and *cis*- and *trans*- pyranoid linalool oxides, (2-**2a**) and (2-**2b**), in a ratio of 42:45:7:6, respectively. The furanoid linalool oxides, (**32a**) and (**32b**) have identical mass spectra. MS:
155 (7), 137 (6), 111 (26), 94 (40), 93 (31), 83 (14), 81 (16), 79 (12), 72 (6), 68 (27), 67 (29), 59 (100), 55 (41), 43 (57), 41 (34), as do the *cis*- and *trans*-pyranoid linalool oxides (2-**2a** and 2-**2b**). MS: 155 (7), 143 (2), 137 (5), 109 (6), 94 (71), 83 (15), 81 (16), 79 (23), 68 (100), 67 (67), 59 (69), 55 (28), 43 (65).

RESULTS AND DISCUSSION

To obtain reliable information on the composition of the interdigital secre- tion of the red hartebeest, three different gas chromatographic sample introduction techniques were used: (1) split injection of an extract of the secretion, (2) SPME, and (3) solventless sample introduction. Because the quality of the GC information and mass spectra obtained from the extract was by far the best, chromatographic and mass spectral data obtained with this method were used as reference data in the characterization of the secretion.

Split injection was also the preferred injection technique because it was difficult to match retention times of the natural and synthetic reference compounds with the solventless sample introduction technique. However, because no solvent is used in the other two sample introduction techniques, they provided valuable information on early eluting compounds that were obscured by the solvent peak when extracts were analyzed. A typical TIC of an interdigital secretion extract of a male hartebeest obtained with the OV-1701 column is shown in Figure 1.

To obtain information on constituents that might coelute from this column, GC-MS analyses were also done with the apolar PS-089 column. The differences in peak shape and order of elution from the two columns provided additional diagnostic information. The secretion from females contained the same compounds, albeit in different relative amounts. A TIC of the headspace volatiles collected by SPME is shown in Figure 2A. The compounds identified in the interdigital secretion of the red hartebeest and the diagnostic methods employed in their identification are listed in Table 1.

Tentative identification of components was based on mass spectra comparisons with NBS, NIST, LECUS, and Wiley libraries. Reduction of samples of the crude secretion with $LiAlH_4$ gave proof of the presence of aldehydes, acids, and ketones, which were reduced to the corresponding alcohols. The molecular masses of a series of saturated and unsaturated aldehydes and carboxylic acids were confirmed by their CI(CH_4) spectra. In some cases, GC-IR provided invaluable information concerning the organic compound class to which some of the unknowns belonged.

Final proof of the structure of most compounds was provided by coinjection of commercially available or synthesized reference compounds. The main compound classes represented in the secretion were alkanes and isoalkanes, primary alcohols, ketones, straight-chain, branched, and unsaturated aldehydes, carboxylic acids, and cyclic ethers. In addition to the straight- and branched-chain hydrocarbons listed in Table 1, the secretion contains traces of 2-methylalkanes from C_{10} to C_{17}.

Methyl branching seems to be characteristic for this secretion as it also was present in other compound classes (e.g., alcohols, aldehydes, and carboxylic

acids). Apart from 2-methylundecane, all the alkanes were commercially available. Compound (**21**) coeluted with synthetic 5-methyl-1-hexanol but the natural compound had a mass spectrum that was also almost identical to that of 3-methyl-1-hexanol. The structure was confirmed to be 5-methyl-1-hexanol by GC-MS analysis of the trimethylsilyl ethers (TMS ethers) of 5-methyl-1-hexanol and the natural compound.

The similarity of the mass spectra of constituents (**21**) and (**27**) and the retention time increment between these two compounds suggested that **27** could also be an isobranched primary alcohol. Constituent (**27**) was confirmed as 6-methyl-1-heptanol by coelution of the natural and synthetic compounds. Aldehydes were the most abundant class of compounds present in the secretion. They are also plentiful in the interdigital secretions of the bontebok, blesbok (Burger et al., 1999a,b), and black wildebeest (Burger et al., unpublished manuscript), albeit in lesser amounts.

In contrast, aldehydes are rather rare in the preorbital secretions of the antelope that have so far been investigated (Stander et al., 2002). In addition to straight-chain saturated and monounsaturated aldehydes, a large number of isobranched analogs also were present in the secretion. The unsaturated aldehydes were only tentatively identified because the mass spectra of their DMDS derivatives did not provide unequivocal diagnostic information.

The isobranched aldehydes higher than 5-methylhexanal are not commercially available, and they are not represented in mass spectra libraries. Characteristically, the molecular ion is not present in the EI spectra of branched aldehydes. Diagnostic information can, however, be derived from ions at $[M-18]^+$ (loss of H_2O), $[M-28]^+$ (loss of C_2H_4), and $[M-33]^+$ (loss of H_2O plus CH_3). The CI (CH_4) spectra of the branched aldehydes contain a prominent $[M-17]^+$ ion formed by the loss of water from the protonated molecular ion.

All of the constituents under discussion were identified as aldehydes in GC-IR library searches. The available information is reconcilable with these compounds being isobranched aldehydes. This interpretation was confirmed by synthesis of the iso-C_6, C_7, C_8, and C_{10} aldehydes and their coelution with consituents (**11**), (**17**), (**22**), and (**39**). Constituent (**28**) was identified as 7-methyloctanal based on its mass spectrum and retention time increment comparison. The identification of a series of compounds **(23)**, **(31)**, **(41)**, and **(50)** as straight chain (*E*)-2-alkenals was confirmed by their coelution with the commercially available compounds. In the case of (*E*)-2-undecenal **(57)**, retention time increment correlation was used. (*E*)-2-Octenal **(31)** coeluted with hexanoic acid on the OV-1701 column, but was well separated from other constituents on the PS-089 column.

A series of isobranched α,β-unsaturated aldehydes was present in the secretion. According to their IR spectra, **(47)** and **(55)** appeared to be unsaturated aldehydes. The IR spectra of constituents **(38)**, **(45)**, and **(54)** contained little diagnostic information due to their low concentration. Ions in their EI mass spectra at $[M-18]^+$ (loss of H_2O), $[M-33]^+$ (loss of H_2O plus CH_3), and ions at $[M+1]^+$ and $(M-17)^+$ in their CI(CH_4) mass spectra were used to determine their molecular masses.

The Z -isomers of these compounds show more prominent ions at *m/z* 70 and *m/z* 83 than the later eluting *E*-isomers. The structures of **(45)** and **(47)** were confirmed by their GC coelution with synthesized (*Z*)- and (*E*)-8-methyl-2-nonenal, respectively. The identification of **(38)**, **(54)**, and **(55)** as (*E*)-7-methyl-2-octenal, (*Z*)-9-methyl-2-decenal, and (*E*)-9-methyl-2-decenal was based on their mass spectral and retention time data. Two branched diunsaturated aldehydes could not be fully characterized.

According to the (M+1) ions in their CI(CH_4) spectra, compounds **(52)** and **(60)** have molecular masses of 138 and 152, respectively, in agreement with the library search of GC-IR spectra, which gave branched, nonadienal, and decadienal as the most likely candidate structures. The mass spectra of their DMDS derivatives did not contain any useful diagnostic information, and these two constituents remain unidentified. The mass spectra of constituents **(46)**, **(53)**, **(65)**, and **(67)** were typical of branched fatty acids, but did not contain ions that can be used to determine the position of branching.

Comparison of mass spectra and retention times of available fatty acid standards allowed identification of constituents **(65)** and **(67)** as 14-methylpentadecanoic acid and 16-methylheptadecanoic acid, respectively. 6-Methylheptanoic acid **(46)** and 7-methyloctanoic acid **(53)** were converted to pyrrolidine derivatives, both of which have abundant $[M-15]^+$ and $[M-43]^+$ ions typical of pyrrolidine derivatives of isobranched fatty acids (Andersson and Holman, 1975).

These two ions are almost undetectable in the straight chain derivatives. Figure 2A shows the TIC of the SPME-sampled headspace volatiles of the secretion, analyzed on the apolar PS-089 column. Many overlapping peaks and contaminants complicated the interpretation of the mass spectra obtained in this analysis. However, this chromatogram shows large peaks of compounds with the typical spectra of the *cis*- and *trans*-pyranoid linalool oxides of which only the *cis* isomer could be found in the extracted secretion.

These compounds were recently identified in the castor sacs of the North American beaver, *Castor canadensis*, by Tang et al. (1995). The linalool oxides were synthesized as a mixture of isomers by treating linalool with *m*-chloroperbenzoic acid according to Scheme 2 to give the furanoid linalool oxides,

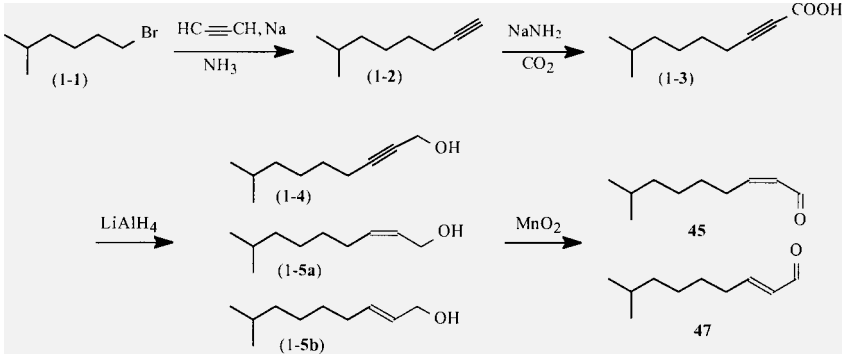
(**32a**) and (**32b**), as well as the pyranoid linalool oxides (2-**2a**) and (2-**2b**), the formation of the furanoid oxides being considerably favored. The *cis*-furanoid linalool oxide and constituent **(32a)** coeluted on GC, proving its identity.

It was concluded that constituent (**32b**), which was not found in extracts but was detected in the headspace of the secretion, is the *trans*-furanoid linalool oxide. The order in which the furanoid linalool oxide enantiomers elute from a chiral column coated with OV-1701-OH containing the chiral selector heptakis (2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-*β*-cyclodextrin, has been determined by Kreis et al. (1996). By comparison of the chiral GC and GC- MS analyses of the natural linalool oxides and the synthetic compounds, *trans*- (2*R*,5*R*)-, *cis*-(2*R*,5*S*)-, and *cis*-(2*S*,5*R*)-linalool oxide were found to be present in the secretion in a ratio of 2.5:1:1.5, respectively, in the headspace of samples of the secretion collected on gauze.

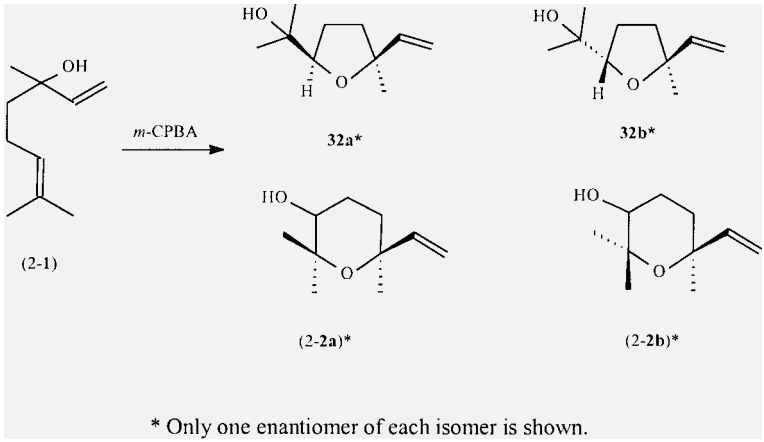
The solventless sample introduction technique used in this study suffers from the problem that it is difficult to control the sample size. This resulted in peak broadening and overlapping of peaks so that it was impossible to obtain interpretable mass spectra from this analysis. Although the information obtained with this method was only used as supporting evidence, the solventless sample introduction of large secretion samples revealed the presence of compounds that were tentatively identified as 2-butylfuran, 2-pentylfuran, and 2-hexylfuran with base peaks at *m/z* 81, and the *γ*-lactones, hexan-4-olide, heptan-4-olide, and octan-4-olide, with base peaks at *m/z* 85.

Because of insufficient diagnostic information in their mass spectra, these identifications remain tentative. Using large sample sizes, several other trace constituents with base peaks at *m/z* 85 were observed in the secretion, but full identification was not possible.

Scheme



SCHEME 1. Synthesis of (*Z*)- and (*E*)-8-methyl-2-nonenal.



SCHEME 2. Synthesis of furanoid and pyranoid linalool oxides.

Figures

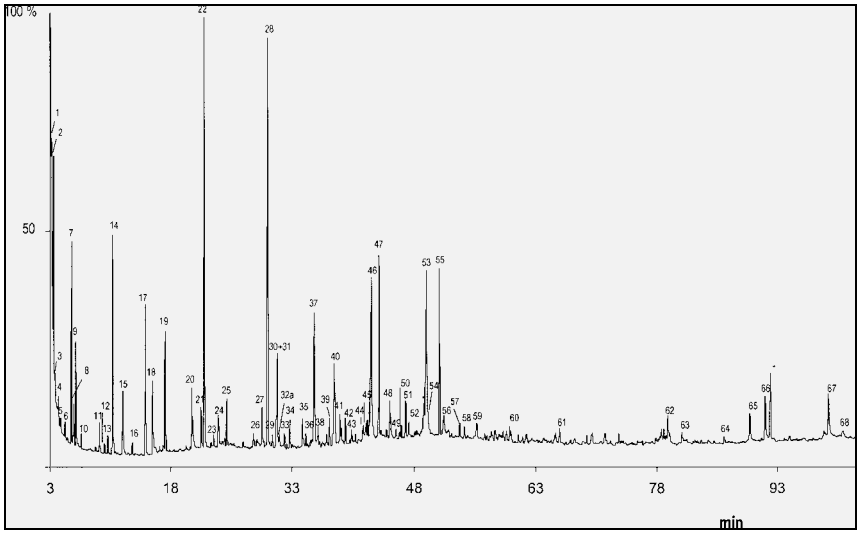


FIG. 1. Total ion chromatogram of an interdigital secretion extract of male red hartebeest, Al- celaphus buselaphus caama analyzed on the OV-1701-OH column, programmed at 2°C/min from 40°C to 280°C. Constituents are numbered as in Table 1. ∗Impurities.

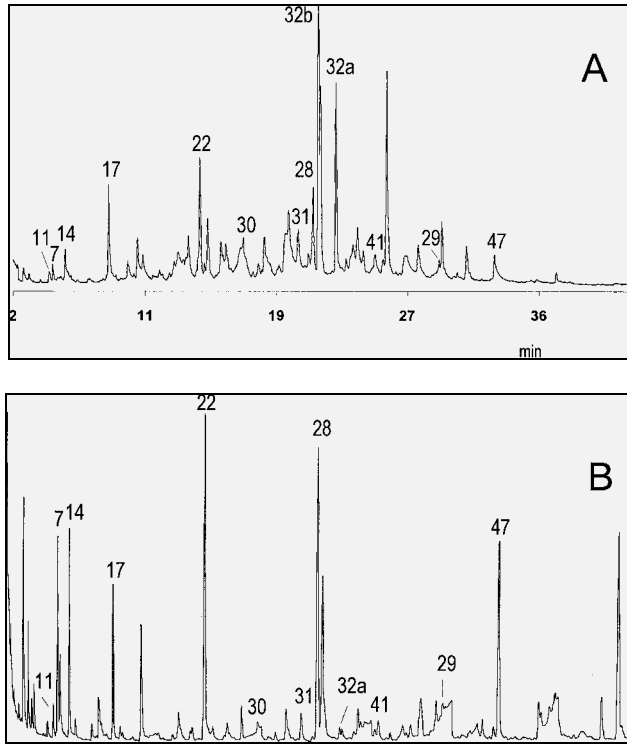


FIG. 2. Total ion chromatogram of (A) an SPME sample of the headspace of the interdigital secretion of male red hartebeest, *Alcelaphus buselaphus caama* and (B) an extract of the secretion. GC conditions for both analyses: PS-089-OH column programmed at 2°C/min from 40°C to 280°C. Constituents are numbered as in Table 1.

TABLE 1. COMPOUNDS IDENTIFIED IN INTERDIGITAL SECRETION OF THE RED HARTEBEEST

No. in Fig. 1	Compound	EI mass spectral data, *m/z* (%)[a]	Identification	Relative quantities (%) Male	Female
	Alkanes				
2	2-Methylhexane		a,c,g	3.4	2.6
4	Heptane		a,c	0.5	0.3
7	2-Methylheptane		a,c,g,i	2.8	3.6
10	Octane		a,c	0.2	0.3
12	2-Methyloctane		a,c	0.1	0.2
29	2-Methylundecane		a,c	0.3	0.1
	Alcohols				
5	2-Methyl-3-buten-2-ol		a,c,i	0.3	0.2
16	2-Methyl-2-hexanol		a,c	0.2	0.3
21	5-Methyl-1-hexanol		a,c,f,j	1.0	1.0
27	6-Methyl-1-heptanol		a,c,g	0.9	1.0
	Aldehydes				
1	2-Methyl-2-propenal		a,c,g	4.3	0.9
3	Butanal		a,c	0.8	0.1
6	3-Methylbutanal		a,c	0.3	0.2
9	Pentanal		a,b,c,d,g,i	1.7	1.4
11	4-Methylpentanal		a,c,d,i	0.4	0.4
14	Hexanal		a,b,c,d,g,i,j	3.6	3.5
15	3-Methyl-3-butenal		a,b,c,g,i,j	1.4	1.7
17	5-Methylhexanal		a,b,c,d,g,i,j	2.7	1.7
19	Heptanal		a,b,c,d,g	2.1	1.9
22	6-Methylheptanal		a,b,c,d,g,i,j	8.0	5.5
23	(*E*)-2-Heptenal	112 (3), 111 (1), 97 (6), 94 (4), 84 (12), 83 (55), 70 (30), 69 (30), 68 (32), 57 (46), 56 (41), 55 (65), 41 (100)	a,b,c,d,i,j	0.3	0.3
25	Octanal		a,b,h	0.9	0.9
28	7-Methyloctanal		a,b,d,g,h,i	8.2	8.1
31	(E)-2-Octenal	111 (3), 108 (3), 98 (13), 97 (13), 95 (9), 93 (9), 83 (58), 82 (42), 70 (83), 69 (42), 58 (57), 57 (56), 55 (88), 43 (72), 41 (100), 39 (55)	a,c,h	*b*	*b*
34	Nonanal		a,b,c,d,i,j	0.6	0.4
38	(*E*)-7-Methyl-2-octenal	125 (5), 122 (3), 107 (26), 97 (13), 96 (24), 95 (9), 84 (12), 83 (34), 81 (26), 70 (63), 69 (43), 57 (32), 56 (32), 55 (59), 43 (94), 41 (100)	a,b,g,h	0.5	0.6
39	8-Methylnonal		a,b,c,d,g	0.7	0.5
41	(*E*)-2-Nonenal	122 (1), 112 (2), 111 (6), 97 (13), 96 (18), 83 (45), 70 (62), 69 (44), 57 (37), 55 (80)	a,b,c,d,g,i	0.6	0.7

TABLE 1. CONTINUED

No. in Fig. 1	Compound	EI mass spectral data, *m/z* (%)[a]	Identification	Male	Female
45	(*Z*)-8-Methyl-2-nonenal	136 (2), 121 (11), 111 (8), 110 (7), 107 (6), 97 (11), 95 (16), 93 (11), 83 (54), 81 (15), 71 (68), 70 (69), 69 (41), 57 (39), 55 (69), 43 (83), 41 (100)	a,b,c,g,h	0.5	0.7
47	(*E*)-8-Methyl-2-nonenal	136 (2), 121 (14), 111 (8), 110 (6), 108 (5), 107 (7), 97 (16), 95 (19), 93 (12), 84 (10), 83 (33), 81 (15), 79 (10), 70 (49), 69 (44), 67 (18), 57 (44), 56 (33), 55 (57), 43 (100), 41 (96)	a,b,c,g,h,i	4.0	5.6
50	(*E*)-2-Decenal	136 (1), 121 (4), 111 (8), 110 (9), 107 (8), 98 (16), 97 (17), 83 (40), 70 (70), 69 (38), 57 (33), 56 (34), 55 (74), 43 (70), 41 (100)	a,b,c,h	0.3	0.4
52	Unidentified (nonadienal)	137 (1), 111 (12), 96 (52), 95 (59), 81 (19), 68 (36), 67 (100), 65 (13), 59 (27), 55 (33), 53 (31), 43 (63), 41 (67), 39 (53)	a,b,g	0.5	0.5
54	(*Z*)-9-Methyl-2-decenal	150 (2), 135 (11), 124 (8), 111 (8), 109 (7), 107 (7), 98 (10), 97 (13), 95 (17), 85 (62), 83 (87), 81 (41), 70 (100), 69 (49), 67 (23), 57 (57), 55 (66), 43 (65), 41 (79)	a,b,g,h	0.8	1.2
55	(*E*)-9-Methyl-2-decenal	150 (3), 135 (19), 124 (7), 121 (6), 111 (12), 109 (9), 107 (12), 97 (20), 95 (20), 83 (70), 82 (31), 70 (93), 69 (76), 67 (29), 57 (91), 55 (78), 43 (86), 41 (100)	a,b,g,h	3.8	5.7
57	(E)-2-Undecenal	150 (2), 124 (10), 121 (17), 111 (11), 98 (16), 97 (18), 83 (64), 82 (35), 70 (100), 69 (44), 57 (64), 56 (23), 55 (69), 43 (39), 41 (78)	a,g,h	0.3	0.6
	Ketones				
20	3-Methyl-2-hexanone	85 (4), 72 (25), 57 (11), 55 (3), 43 (100)	a,b,h	1.6	3.6
	Acids				
24	Pentanoic acid		a,b,c,d	0.7	1.0
30	Hexanoic acid		a,b,c,d	2.8[b]	3.0[b]
40	Heptanoic acid		a,b,c,d,j	2.9	3.6
46	6-Methylheptanoic acid		a,b,d,e,j	6.8	3.8
48	Octanoic acid		a,b,c,d,j	1.1	0.8

TABLE 1. CONTINUED

No. in Fig. 1	Compound	EI mass spectral data, *m/z* (%)[a]	Identification	Relative quantities (%) Male	Female
53	7-Methyloctanoic acid		a,b,d,e,j	6.3	3.9
56	Nonanoic acid		a,b,c,d,j	0.8	0.3
63	Tetradecanoic acid		a,c	0.4	—
64	Pentadecanoic acid		a,c	[c]	—
65	14-Methylpenta-decanoic acid		a,b,c,d	1.0	1.6
66	Hexadecanoic acid		a,b,c,d	1.8	1.6
67	16-Methylhepta-decanoic acid		a,b,c,d	2.1	4.1
68	Octadecanoic acid		a,b,c,d	0.3	1.9
44	Cyclohexane-carboxylic acid		a,c,g	0.5	0.6
	Cyclic ethers				
8	2-Methyl-2,3-epoxypentane	100 (3), 85 (17), 71 (19), 59 (100), 58 (100), 57 (35), 43 (92), 41 (89), 39 (51)	a,c,i	0.6	1.1
32a	*cis*-Furanoid linalool oxide	155 (11), 137 (12), 111 (28), 94 (38), 93 (32), 83 (19), 81 (19), 79 (17), 72 (20), 68 (36), 67 (39), 59 (100), 55 (49), 43 (92), 41 (46)	a,c,i	0.3	0.6
32b	*trans*-Furanoid linalool oxide	155 (5), 137 (8), 111 (30), 94 (44), 93 (42), 83 (18), 81 (30), 79 (19), 72 (9), 68 (39), 67 (45), 59 (100), 55 (42), 43 (63), 41 (33)	a,i	[d]	[d]
	Unidentified				
13	Unidentified	100 (2), 85 (78), 60 (24), 57 (20), 43 (77), 41 (100)		0.4	0.4
18	Unidentified	142 (3), 127 (3), 100 (5), 84 (20), 83 (13), 82 (13), 72 (33), 57 (18), 55 (15), 43 (100)		1.6	2.4
26	Unidentified	83 (5), 71 (100), 57 (17), 55 (28), 43 (38), 41 (44)		0.4	0.1
33	Unidentified	102 (11), 100 (6), 85 (5), 71 (12), 61 (22), 59 (92), 57 (13), 43 (100)		0.3	1.4
35	Unidentified	99 (23), 85 (10), 71 (100), 59 (20), 53 (16), 43 (94)		0.7	0.9
36	Unidentified	95 (4), 71 (100), 69 (11), 57 (17), 55 (21), 43 (23), 41 (44)		0.3	0.3
37	Unidentified	142 (3), 127 (1), 109 (6), 99 (18), 84 (21), 83 (13), 72 (17), 59 (11), 57 (11), 55 (16), 43 (100)		3.5	4.5

TABLE 1. CONTINUED

No. in Fig. 1	Compound	EI mass spectral data, *m/z* (%)[a]	Identification	Male	Female
42	Unidentified	109 (5), 97 (6), 83 (5), 71 (100), 69 (20), 57 (26), 55 (50), 41 (58)		0.5	0.4
43	Unidentified	127 (9), 109 (22), 100 (5), 85 (22), 83 (18), 69 (5), 67 (7), 58 (25), 43 (100), 41 (39)		0.3	1.0
49	Unidentified	130 (4), 113 (2), 99 (17), 72 (18), 59 (62), 57 (13), 43 (100)		0.3	0.6
51	Unidentified	123 (2), 111 (2), 98 (2), 95 (6), 71 (100), 69 (26), 67 (14), 57 (27), 55 (40), 43 (57), 41 (60)		0.9	0.7
52	Unidentified (a nonadienal)	137 (1), 111 (12), 96 (52), 95 (59), 81 (19), 68 (36), 67 (100), 65 (13), 59 (27), 55 (33), 53 (31), 43 (63), 41 (67), 39 (53)	a,b,g	0.5	0.5
58	Unidentified	177 (8), 139 (7), 123 (24), 109 (8), 98 (20), 95 (20), 83 (34), 81 (19), 71 (98), 69 (43), 57 (39), 55 (85), 43 (100), 41 (90)		0.4	0.2
59	Unidentified	129 (16), 111 (6), 86 (12), 85 (8), 59 (100), 43 (47)		0.5	0.7
60	Unidentified (a decadienal)	125 (8), 109 (10), 97 (51), 95 (23), 85 (75), 81 (100), 79 (35), 71 (28), 67 (31), 59 (97), 57 (32), 43 (74), 41 (98), 39 (59)	a,b,g	0.5	1.8
61	Unidentified	168 (8), 142 (8), 125 (27), 111 (27), 109 (36), 107 (28), 96 (21), 95 (42), 93 (21), 83 (29), 77 (10), 69 (52), 55 (62), 43 (100)		0.5	0.4
62	Unidentified	210 (3), 195 (3), 177 (6), 153 (22), 137 (9), 135 (19), 121 (3), 111 (3), 97 (16), 83 (27), 69 (28), 55 (23), 43 (100)		0.8	1.3

Note: a, GC-MS analysis; b, CI (CH_4) spectra; c, retention time comparison with synthetic compounds; d, reduction with $LiA1H_4$; e, EI-spectra of pyrrolidine derivatives; f, EI-spectra of TMS derivatives; g, GC-IR analysis; h, retention time increment comparison; i, observed with SPME sample enrichment; j, observed with solventless sample introduction.

[a] MS data are included for compounds that were not identified and for a few compound types that are not well represented in spectra libraries.

[b] Coeluting compounds; the % for hexanoic acid includes (*E*)-2-octenal.

[c] Traces.

[d] Observed only with SPME sample enrichment.

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