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Scent gland constituents of the Middle American burrowing python, *Loxocemus bicolor* (Serpentes: Loxocemidae)

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Abstract: Analysis by gas chromatography/mass spectrometry of the scent gland secretions of male and female Middle American burrowing pythons (*Loxocemus bicolor*) revealed the presence of over 300 components including cholesterol, fatty acids, glyceryl monoalkyl ethers, and alcohols. The fatty acids, over 100 of which were identified, constitute most of the compounds in the secretions and show the greatest structural diversity. They include saturated and unsaturated, unbranched and mono-, di-, and trimethyl-branched compounds ranging in carbon-chain length from 13 to 24. The glyceryl monoethers possess saturated or unsaturated, straight or methyl-branched alkyl chains ranging in carbon-chain length from 13 to 24. Alcohols, which have not previously been reported from the scent glands, possess straight, chiefly saturated carbon chains ranging in length from 13 to 24. Sex or individual differences in secretion composition were not observed. Compounds in the scent gland secretions of *L. bicolor* may deter offending arthropods, such as ants.

Keywords: alcohols; fatty acids; glyceryl monoalkyl ethers; scent gland; snake.

Dedication: In memory of the late Lothar Jänicke

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1 Introduction

Reptiles possess a number of macroscopic integumentary glands, many of which are unique to a particular order or suborder (see [1] for recent bibliography). Snakes (order Squamata, suborder Serpentes) possess a paired exocrine organ, called the scent gland, situated in the base of the tail and opening through two ducts at the margin of the cloacal orifice. Foul-smelling fluids typically are released from this gland when snakes are molested, thus inspiring the frequent suggestion that these secretions repel predators. Other proposed functions for the scent glands include the production by females of courtship deterrents against unpreferred suitors [2, 3].

Chemical analyses reveal that scent gland secretions consist chiefly of proteins [4–6]. Lipids and other low-molecular components, including volatiles that impart the characteristic secretion odors of some species, are also present. Cholesterol [6–11], which is a common tetrapod skin lipid, and carboxylic acids [5–10, 12] are widely documented in scent gland secretions. Nitrogen-containing compounds, including piperidone [12], amines [8, 12], and amides [8, 9], also have been indicated in some taxa. 1-*O*-Monoalkylglycerols have been reported in the secretions of the western diamondback rattlesnake (*Crotalus atrox*) [11].

The Middle American burrowing python (*Loxocemus bicolor* Cope) occurs in moist to dry forests from southwestern Mexico through Guatemala, Honduras, El Salvador, Nicaragua, and into Costa Rica (Figure 1). This fossorial snake feeds predominantly on small vertebrates and reptile eggs [13, 14]. *Loxocemus bicolor* is the sole member of its genus and family, the Loxocemidae. Molecular studies indicate that this snake is a basal alethinophidean, closely allied with or properly placed within the Pythonidae [15, 16]. We chose to investigate the scent gland secretions of *L. bicolor* because of its unique phylogenetic position in a clade of primitive constricting snakes. This secretion proved to be particularly diverse, consisting of more than 300 lipid components.

Figure 1: An adult female *Loxocemus bicolor* (total length = 90 cm). Photograph by T. Barker (Vida Preciosa International, Inc., Boerne, TX, USA).

2 Results

The analysis of the scent gland secretions revealed the presence of many peaks in the total ion chromatograms. The bad peak shapes leading to largely overlapping peaks suggested the presence of a high percentage of compounds with active hydrogens, such as alcohols and acids (Figure 2). Therefore, the samples were analyzed again after derivatization with *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA, Figure 3). This procedure led to better peak separation and allowed the identification of the major compound classes. Over 300 components were observed. Cholesterol was a major constituent of all samples. The other components belonged to the following compound classes, in order of abundance: fatty acids, glyceryl monoalkyl ethers, and alcohols.

2.1 Fatty acids

The acids comprise most of the compounds in the secretions and showed the highest structural diversity. For GC/MS analysis of these acids, the crude extracts were treated with diazomethane to yield the corresponding methyl esters, which improved gas chromatographic behavior

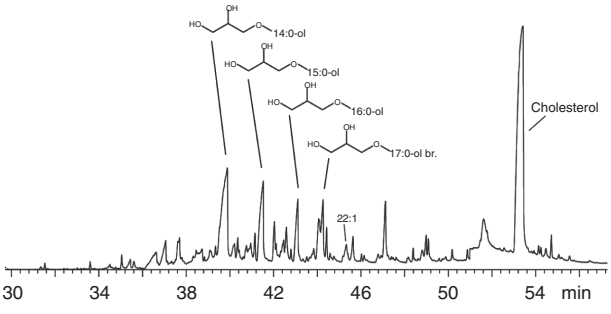

Figure 2: Total ion chromatogram of an extract of the scent gland secretions of *Loxocemus bicolor*.

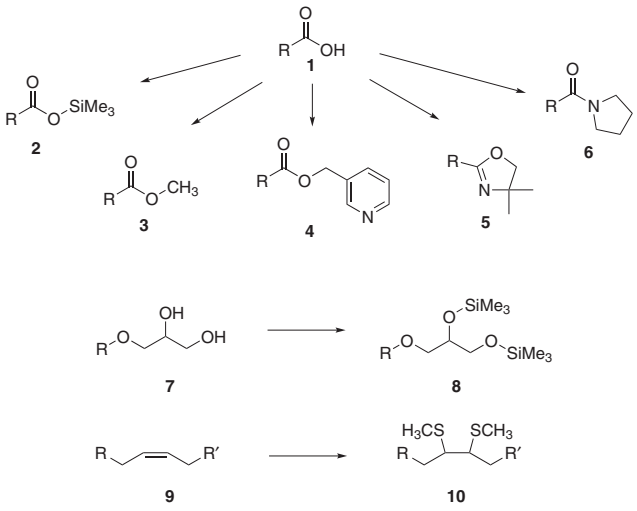

Figure 3: Derivatization reactions performed with extracts for structure elucidation.

(Figure 3). Mass spectra of methyl esters (**3**) allow the detection of several structural features such as methyl groups near the ester group [17] and the number of double bonds in the chain. The retention index *I* can be used to identify methyl groups near the alkyl end of the chain [18]. Nevertheless, internal methyl groups or double-bond positions cannot be determined, and other derivatives must be used to locate such groups. The best results are usually obtained by converting of the acids into 3-pyridiylmethyl esters **4** [19], but other methods such as the formation of dimethyloxazolines **5** [20] or pyrrolidides **6** [21] are also used.

A major problem in the analysis of the snake acids proved to be the large number of compounds present. This led to extensive coelution of compounds, making proper identification difficult. All three derivatization procedures mentioned were performed. It turned out that the analysis of both 3-pyridiylmethyl esters and pyrrolidides were necessary for proper identification, while dimethyloxazolines did not add additional information. To complement this approach, dimethyl disulfide derivatization was used to assign the position of double bonds in addition to 3-pyridiylmethyl esters.

As an example, the identification of a major methyl-branched acid, 14-methyloctadecanoic acid, and a major unsaturated acid, 11-icosenoic acid will be discussed in detail. The various spectra of their derivatives are shown in Figure 4 as examples. These spectra allow the localization of the methyl branch and the location of the double bond in the two compounds. While the spectrum of methyl 14-methyloctadecanoate (Figure 4A) is difficult to interpret a priori [17], the respective pyrrolidide (Figure

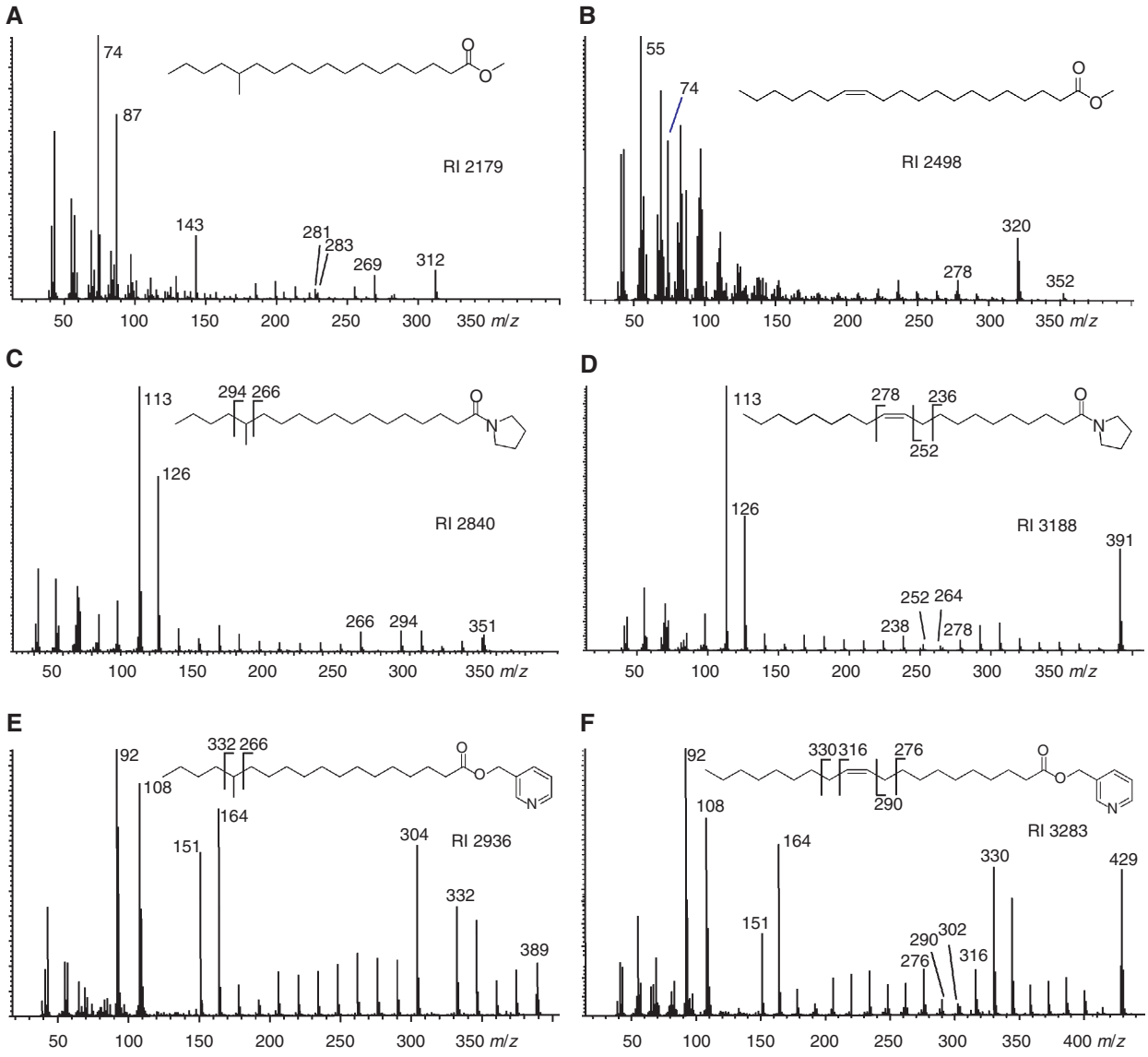

Figure 4: Mass spectra of derivatives of 14-methyloctadecanoic acids (A, C, E) and 11-icosenoic acids (B, D, F), gas chromatographic retention indices and characteristic mass spectrometric cleavages. Methyl esters (A, B), pyrrolidides(C, D), and 3-pyridylmethyl esters (E, F).

4C) lacks ion *m/z* 280 of the ion series $[C_4H_8NCOC_nH_{2n}]^+$. This ion series is formed by sequential alkyl cleavage along the chain. Similarly, the respective 3-pyridiylmethyl ester (Figure 4E) lacks ion *m/z* 318 of the ion series $[C_6H_7NOCOC_nH_{2n}]^+$. The intensities of the neighboring ions in these homologous series (*m/z* 266/294 and *m/z* 304/332, respectively) are enhanced. The gaps in the ion series indicate a methyl branch in the chain that can be located by the ions mentioned at C-14.

The localization of the double bond is more difficult but can also be achieved. The methyl ester (Figure 4B) mass spectrum does not give any hints on the localization of the double bond. The mass spectrum of the pyrrolidide shows 14 amu gaps between the methylene groups, but only 12 amu between *m/z* 252 and 264 (Figure 4D). Obviously, the double bond is near C-11 but cannot exactly be located. Somewhat better results are obtained with the 3-pyridiylmethyl esters (Figure 4E). The strong allylic cleavage ion at *m/z* 330 and the 12 amu gap between *m/z* 290 and 302 locates the double bond at C-11. Nevertheless, a good and clean spectrum is required for reliable identification. This was not always the case for the other acids of the secretion. The most reliable results were obtained by the mass spectra of dimethyl disulfide (DMDS) adducts (**10**) of the unsaturated methyl esters [22, 23]. 11-Icosenoic acid was identified because the mass spectrum of the DMDS adduct of its methyl ester furnished prominent ions at *m/z* 245 $[CH_3SC_{10}H_{20}CO_2CH_3^+]$,

213 [$CH_3SC_{10}H_{20}CO\text{-}CH_3OH^+$] and 173 [$C_9H_{20}SCH_3^+$] [21]. Two isomers, 12-icosenoic acid, characterized by *m/z* 259, 227, and 159, as well as 13-icosenoic acid, characterized by *m/z* 273, 241, and 145, were also present in the secretion.

The individual acids were identified by interpretation of the mass spectra of the derivatives as just discussed, according to well established rules [22, 24–29], and by correlation with gas chromatographic retention indices *I* [18, 30]. Over 100 acids were identified. The acids of *L. bicolor* chiefly consist of long-chain, unbranched, saturated, and unsaturated acids, as well as mono-, di-, and trimethyl-branched acids (Table 1). Major constituents are 13-docosenoic acid, docosanoic acid, 15-tricosenoic acid, 15-tetracosenoic acid, and 14-methylhexadecanoic acid. Methyl branching occurs predominately at C-4, C-10, C-12, and C-14. Double bonds prevail at the ω-9 position, a typical location for fatty acids of animal origin. A second double bond can occur in very long tetracosadienoic acids. The two acids **F99** and **F101** (Table 1) represent the two acid types present, those with remote double bonds and those with bishomoconjugated double bonds. Structures of minor amounts of similar acids could not be fully assigned.

Acids with both methyl branches and double bonds occur as well but in minor amounts. Even-numbered carbon chains are dominating, although odd-numbered acids also occur. 2,3-Dihydrofarnesoic acid is the only acid likely originating from the terpene biosynthetic pathway. No attempt was made to elucidate the configuration of the double bonds, but the common (*Z*)-configuration seems most likely. Structures of some of the acids are shown in Figure 5.

A general biosynthetic model explaining the diversity of the acids is shown in Figure 6. Fatty acids are biosynthesized starting from acetyl-coenzyme A (**12**) that is elongated by malonate (**15**) until a typical chain length is reached, e.g. stearic acid (**13**). Dehydrogenation by a Δ9-desaturase leads to oleic acid, (*Z*)-9-octadecenoic acid (**16**), a ω-9 unsaturated acid (Figure 6). Several variations of this pathway obviously occur during biosynthesis of the scent gland acids. Replacement of one malonyl-unit with methylmalonate (**14**) during chain formation leads to methyl groups in the chain. An additional Δ11-desaturase furnishes ω-7 unsaturated acids. The chain can be elongated by additional malonate units or biosynthesis can stop earlier, leading to shorter acids than C_{18}. After elongation, a second desaturation by a Δ7-desaturase leads to dienoic acids. Finally, odd-numbered acids can be formed by using propionate-CoA (**11**) instead of **12** as chain starter, or alternatively, acids can be shortened by α-oxidation by one carbon. Combining all these elements of chain-length diversity from C_{12} to C_{24} including odd-numbered acids, desaturation on a few positions, and methyl groups near the acid group and in the middle/end of the chain leads to the extraordinary diversity in acid structures.

Although variation in the relative proportions of individual compounds occur, neither the acids nor other compounds appeared to be sex specific; the secretion looks remarkably similar for males and females (Figure 7). Furthermore, the composition of the gland does not vary greatly; many compounds were present in all samples analyzed, despite their different origin. It seems, therefore, that the composition of the secretion is relatively stable.

2.2 1-*O*-Alkylglycerols

Glyceryl alkyl ethers in the scent gland secretions were identified by their mass spectra and the trimethylsilyl (TMS) derivatives **8**. The mass spectrum of 1-*O*-tetradecylglyceride and the respective TMS-ether is shown in Figure 8. The mass spectrum of the latter features a base peak at *m/z* 205 [$(CH_3)_3SiOCH_2CHOSi(CH_3)_3^+$], the ion *m/z* 147 indicating the presence of two TMS groups [31], and a small M-15 ion [11, 32]. The ion *m/z* 205 clearly indicates that the terminal alcohol is used in an ether linkage, as do the ions *m/z* 61 and 227 in the original spectrum. Over 40 ethers were present in the secretion in which a homologous series of unbranched, saturated ethers from C_{13} to C_{16} as well as 1-*O*-14-methylhexadecylglycerol dominated. They were accompanied by minor amounts of methyl-branched or unsaturated ethers with longer chains (Table 2 and Figures 8 and 9). The locations of branching points or double-bond positions were not determined; however, *iso*- and *anteiso*-branched compounds were identified based on established rules for calculating their gas chromatographic retention indices (*I*) in long-chain compounds [18, 30].

2.3 Alcohols

A homologous series of 1-alkanols were identified after derivatization with MSTFA. The chain length ranged from C_{13} to C_{24}. Even-numbered alcohols were present in higher amounts than the odd-numbered alcohols, with increasing concentration according to chain length. No branched alcohols could be found; tetracosen-1-ol was identified as the only unsaturated alcohol.

Table 1: Fatty acids occurring in the scent gland secretions of male (M) and female (F) *Loxocemus bicolor*. Relative proportion to the major acid **F87** (100%).

		RI	M1 ♂	M2 ♂	M3 ♂	F1 ♀	F2 ♀	F3 ♀	F4 ♀	F5 ♀	F6 ♀	F7 ♀
F1	3-Methyldodecenoic acid	1572	2.1	2.1	1.2	2.0	x	1.3	1.1	1.1	0.3	5.2
F2	3-Methyldodecenoic acid	1681	0.6				1.4	1.2	0.6	0.3		2.0
F3	2,3-Dihydrofarnesoic acid	1725	1.1	0.5	0.3	0.2	0.3	0.2	0.4	0.2	0.3	2.0
F4	2-Methyltetradecanoic acid	1762	0.1									tr
F5	3-Methyltetradecanoic acid	1772										tr
F6	4-Methyltetradecanoic acid	1778	0.7								0.3	0.7
F7	Pentadecanoic acid	1830	0.6				0.7					
F8	4,12-Dimethyltetradecanoic acid	1850										1.4
F9	2-Methylpentadecanoic acid	1863										1.0
F10	3-Methylpentadecanoic acid	1868										0.5
F11	6-Methylpentadecanoic acid	1873										tr
F12	8-Methylpentadecanoic acid	1878										1.0
F13	4-Methylpentadecanoic acid	1880	4.6	4.0	5.9	3.2	6.6	4.6	4.4	1.4	4.5	9.6
F14	4,8,12-Trimethyltetradecanoic acid	1883								0.9	0.5	1.0
F15	12-Methylpentadecanoic acid	1888								0.7	0.6	1.9
F16	14-Methylpentadecanoic acid	1894								0.7		1.6
F17	13-Methylpentadecanoic acid	1902	0.6									2.4
F18	Hexadecenoic acid	1905	0.8		1.8	tr	2.1	tr	tr			1.1
F19	2,*x*-Dimethylpentadecanoic acid	1914	0.7	tr	0.9	tr	1.2	1.1	1.2		0.5	3.7
F20	2,*x*-Dimethylpentadecanoic acid	1920	tr									1.4
F21	4,*x*-Dimethylpentadecanoic acid	1924	0.8	tr	0.9	0.9	0.9	1.3	1.2	0.4	0.5	4.0
F22	Hexadecanoic acid	1930	7.5	4.9	4.0	5.0	4.2	3.0	3.2	4.6	2.1	13.2
F23	4,12-Dimethylpentadecanoic acid	1932	tr	tr	tr	0.8	tr	1.0	1.3	0.3	1.0	5.5
F24	2-Methylhexadecanoic acid	1963	3.9	4.0	4.6	2.6	4.0	2.9	3.3	5.0	4.0	8.8
F25	4,*x*,*x*-Trimethylpentadecanoic acid	1969	0.9	?	0.9	0.8	tr	1.1	1.0	0.5	0.5	2.5
F26	10-Methylhexadecanoic acid	1974	13.9	tr	14.8	7.0	17.0	10.5	10.5	11.2	11.9	24.8
F27	4-Methylhexadecanoic acid	1979	13.2	15.9	15.5	12.5	12.9	14.0	13.4	15.0	21.7	29.3
F28	12-Methylhexadecanoic acid	1981	1.4	?	?	0.7	tr	tr	1.1			1.3
F29	15-Methylhexadecanoic acid	1994	1.2	tr	tr	tr	tr	tr	tr	0.8	0.6	1.6
F30	14-Methylhexadecanoic acid	2002	25.4	25.8	19.6	19.9	22.5	20.2	20.4	28.6	27.1	56.9
F31	9-Heptadecenoic acid[a]	2007	1.3	tr	2.7	1.1	2.4	tr	0.9			2.2
F32	11-Heptadecenoic acid[a]	2007										
F33	2,10-Dimethylhexadecanoic acid	2013	1.2	tr	tr	1.1	1.3	1.7	1.6	1.1	2.1	3.7
F34	4,10-Dimethylhexadecanoic acid	2200	1.5	tr	1.6	1.6	1.5	2.5	2.1	1.4	1.9	5.0
F35	4,12-Dimethylhexadecanoic acid	2029	tr	2.2	tr	1.8	tr	1.7	1.6			
F36	Heptadecanoic acid	2031	2.5	tr	1.7	tr	1.9	tr	tr	1.2	1.0	4.7
F37	2,14-Dimethylheptadecanoic acid	2035	0.7	tr	tr	0.8	0.9	1.1	1.2	0.9	0.9	2.8
F38	10,14-Dimethylhexadecanoic acid	3041	5.2	6.3	5.7	5.1	6.5	7.0	7.1	4.4	5.1	16.8
F39	4,14-Dimethylhexadecanoic acid	2051	2.5	3.7	2.7	2.8	2.4	3.8	3.6	1.9	2.4	8.5
F40	4,8,12-Trimethylhexadecanoic acid	2060	0.7	tr	0.7	0.6	tr	0.9	1.0	0.2	0.3	2.4
F41	2-Methylheptadecanoic acid	2063	0.8	tr	tr	tr	1.1	tr	0.8	0.4	0.5	1.8
F42	10-Methylheptadecanoic acid	2072	7.9	6.7	7.7	4.3	11.6	6.9	7.1	4.5	4.5	15.4
F43	12-Methylheptadecanoic acid	2078	4.3	tr	tr	tr	4.9	tr	tr			10.6
F44	4-Methylheptadecanoic acid	2079	4.4	9.4	9.2	7.0	5.6	9.8	9.7	7.4	9.4	10.8
F45	4,10,14-Trimethylhexadecanoic acid	2085	?	10.9	5.0	2.9	1.3	3.2	tr			
F47	14-Methylheptadecanoic acid	2089	18.6	19.4	16.1	17.0	16.7	18.1	18.5	15.4	16.2	47.9
F48	Octadeca-9,12-dienoic acid	2099	1.9	tr	3.9		2.8	1.3	1.3	1.0	0.8	2.3
F49	9-Octadecenoic acid	2105	13.4	13.3	22.4	10.1	22.2	9.5	9.5	21.5	14.5	21.2
F50	11-Octadecenoic acid	2110	5.9	7.7	6.9	4.1	7.5	4.7	4.6	9.5	7.2	10.4
F51	2,14-Dimethylheptadecanoic acid	2119	tr								1.4	
F52	4,10-Dimethylheptadecanoic acid	2120	tr	tr	tr	1.2	1.3	1.5	1.6	2.4	2.9	4.2
F53	10,14-Dimethylheptadecanoic acid	2124	3.0	3.4	3.5	3.3	4.0	4.8	4.9			11.1
F54	Octadecanoic acid	2131	2.7	2.3	1.6	2.1	1.8	1.2	1.3	2.8	1.1	4.1
F55	4,14-Dimethylheptadecanoic acid	2137	1.8	2.1	2.0	2.2	2.2	3.1	3.2	2.4	2.2	7.2

Table 1 (continued)

		RI	M1 ♂	M2 ♂	M3 ♂	F1 ♀	F2 ♀	F3 ♀	F4 ♀	F5 ♀	F6 ♀	F7 ♀
F56	Methyloctadecenoic acid	2151	1.1	tr	1.0	0.9	1.6	1.5	0.8	0.3	0.3	1.7
F57	Trimethylheptadecanoic acid	2155								0.6	0.4	1.9
F58	12-Methyloctadecanoic acid	2175	3.5	3.1	3.2	2.4	4.9	3.1	3.4	5.2	2.4	6.5
F59	4-Methyloctadecanoic acid	2179	0.6	0.5	0.4	0.3	0.5	1.3	0.9	1.2	0.8	1.1
F60	14-Methyloctadecanoic acid	2183	20.9	22.2	18.4	19.6	21.6	22.1	21.9	29.5	23.3	46.0
F61	16-Methyloctadecanoic acid	2203	2.9	7.0	3.1	2.3	5.1	2.1	2.6	8.9		6.6
F62	9,10,11-Nonadecenoic acid	2207	1.8	2.2	4.1	0.9	6.3	1.1	1.2	6.9	1.9	2.7
F63	10,14-Dimethyloctadecanoic acid[a]	2216	3.3	3.0	3.9	3.2	5.2	4.9	5.0	9.2	3.5	10.0
F64	8,14-Dimethyloctadecanoic acid	2216										
F65	6,14-Dimethyloctadecanoic acid	2222										1.9
F66	4,14-Dimethyloctadecanoic acid	2230	3.0	3.5	2.8	2.8	4.8	3.9	3.8	7.2	2.5	8.1
F67	Arachidonic acid	2264	1.9	2.2	5.3	1.8	7.6	3.5	4.4	3.4	1.5	6.0
F68	10-Methylnonadecanoic acid	2269	1.1	tr	1.5	tr	2.8	tr	1.3	4.1	1.4	3.9
F69	14-Methylnonadecanoic acid	2278	2.2	3.2	2.5	2.0	4.4	3.2	3.2	6.0	1.7	6.8
F70	16-Methylnonadecanoic acid	2290	1.6	tr	1.0	0.9	1.9	1.0	1.2	5.8	1.1	5.2
F71	11-Icosenoic acid	2306	5.3	4.0	7.2	1.6	10.9	2.8	2.8	11.9		7.0
F72	12-Icosenoic acid	2309	4.3	4.7	4.5	3.2	5.3	3.9	3.7	5.7	7.4	5.9
F73	13-Icosenoic acid[a]	2313	6.0	5.9	6.2	2.9	9.9	5.3	4.8	12.7	6.1	7.2
F74	4,*X*-Dimethylicosanoic acid	2313										
F75	8,14-Dimethylnonadecanoic acid	2321	tr									1.3
F76	Icosanoic acid	2331	10.7	6.5	5.2	6.0	7.8	5.5	5.7	11.1	2.5	10.5
F77	8-Methylicosanoic acid	2367	4.5	5.1	4.0	2.9	8.1	5.1	4.6	7.6	1.7	7.3
F78	14-Methylicosanoic acid	2373	1.9	7.5	2.7	2.4	3.5	3.9	2.6	4.2	1.2	5.7
F79	16-Methylicosanoic acid	2381	7.5	8.2	6.4	7.4	8.5	7.0	7.2	7.9	3.2	14.0
F80	13-Henicosenoic acid	2408	18.3	23.2	25.0	13.7	29.0	18.3	18.7	21.0	15.9	29.2
F81	12-Henicosenoic acid[a]	2408										
F82	Henicosanoic acid	2431	6.1	3.6	3.2	4.1	4.7	3.2	3.6	4.7	1.0	4.9
F83	8-Methylhenicosanoic acid	2468	4.4	4.2	3.9	3.5	6.4	4.0	4.2	7.1	1.2	6.5
F84	14-Methylhenicosanoic acid	2472									1.9	3.9
F85	16-Methylhenicosanoic acid	2475	tr	?	tr	tr	tr	1.3	1.0	10.3	0.6	8.6
F86	12,16-Docosadienoic acid	2499	5.2	4.1	3.5	3.2	3.4	5.7	4.8	7.8	2.3	5.2
F87	13-Docosenoic acid	2516	100	100	100	100	100	100	100	100	100	100
F88	15-Docosenoic acid	2524	18.0	21.1	18.5	19.7	19.1	19.0	18.5	19.6	22.8	22.0
F89	14-Docosenoic acid[a]	2524										
F90	Docosanoic acid	2532	23.5	10.7	8.9	17.0	10.0	12.7	13.1	6.8	3.7	9.0
F91	8-Methyl-7-docosenoic acid	2546	1.8	4.9	1.8	1.9	2.4	2.3	2.5	1.7		2.1
F92	8-Methyl-13-docosenoic acid	2554	1.5		1.5	1.9	1.8	1.7	1.7			
F93	*X*-Methyldocosanoic acid	2573								1.6		1.1
F94	8-Methyldocosanoic acid	2584	3.2	7.9	3.5	3.7	3.8	3.0	3.1			1.8
F95	13-Tricosenoic acid[a]	2610	tr	?	5.3	tr	5.5	tr	5.1			4.9
F96	14-Tricosenoic acid[a]	2610										
F97	15-Tricosenoic acid	2612	23.0	23.7	18.3	25.0	18.0	21.5	16.9	10.7	6.1	18.0
F98	Tricosanoic acid	2631	1.5	2.3		1.1	1.1	1.5	1.5			
F99	Tetracosa-7,15-dienoic acid[b]	2667	3.8	?	2.6	2.8	?	?	tr	4.4	0.9	1.7
F100	Tetracosadienoic acid[b]	2669	4.1	?	tr	4.8	3.0	3.7	2.6		1.7	1.9
F101	Tetracosa-13,17-dienoic acid[b]	2674	6.2	47.1	23.1	5.6	tr	?	3.7	0.7		1.1
F104	Tetracosadienoic acid	2681	5.0	3.3	3.1	4.1	2.8	3.4	2.4	1.6	0.9	2.5
F105	15-Tetracosenoic acid	2694	83.7	68.3	72.6	99.0	69.9	74.5	74.8	43.1	33.0	62.1
F106	Tetracosanoic acid	2732	2.6	tr	tr	1.9	1.2	1.5	0.9	0.8	0.9	0.8

RI, retention index of the respective methyl ester; Tr, trace component. Absence of entries denotes not detectable above detection limit (0.1%). [a]Compounds elute together with previous entry in the table. The percentages given represent the amount for both compounds. [b]A group of at least four, probably more acids. The overlapping peaks of the different derivatives did not allow full structural assignment. Two major components are 7,17-tetracosadienoic acid, eluting earlier, and 13,17-tetracosadienoic acid, eluting later.

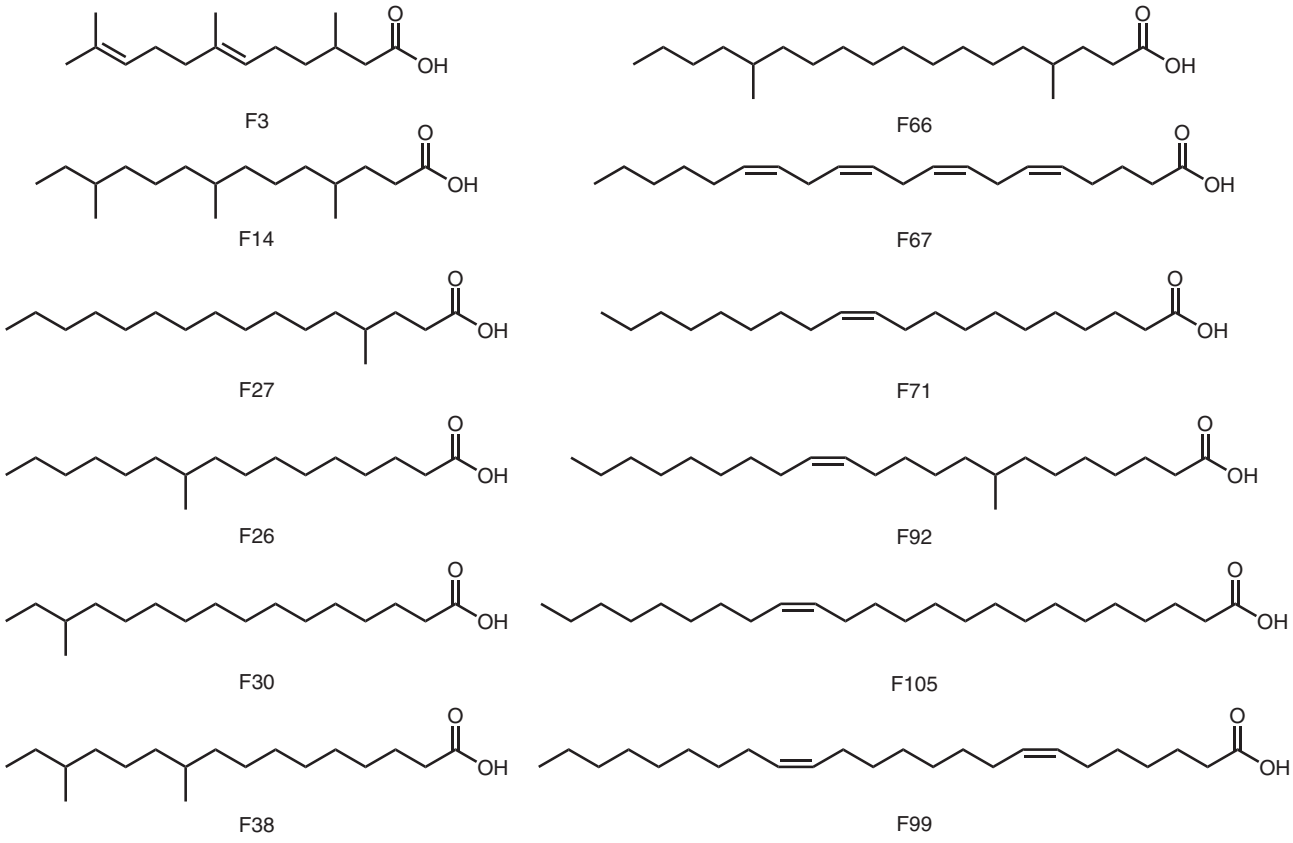

Figure 5: Representative structures for various types of acids found in *Loxocemus bicolor*. The *Z*-configuration of the double bonds is tentative.

3 Discussion

Carboxylic acids evidently are ubiquitous scent gland products of snakes, having been documented in colubrids [12], boids [6, 8, 12], pythonids [12], elapids [7], viperids [9, 10], and leptotyphlopids [5]. These compounds typically possess 2–26 carbon atoms and chiefly feature saturated or monounsaturated straight chains. Lower molecular weight acids (C_4 and C_5) with a single methyl branch [8, 12], hydroxypropanoic acid [6, 8], methylbenzoic acid [9], and phenylacetic as well as phenylpropanoic acids [7–9] also have been documented in some species. The fatty acids of *Loxocemus bicolor* include some of the straight-chain compounds reported in other snakes, but distinctly contain more than 65 mono-, di-, and trimethyl-branched compounds, and many compounds with one, two, or three double bonds. The significance of this structural diversity is open to speculation. The Texas blindsnake (*Leptotyphlops dulcis*), another fossorial snake, discharges repellent cloacal fluids, including scent gland secretions, when attacked by ants [33]. Blum et al. [5] suggested that fatty acids in the secretions of *L. dulcis*, which they identified as 13 straight-chain C_{12} to C_{20} compounds, act against ants as insecticides or by exploiting out-of-context semiochemical responses. Fatty acids of *L. bicolor* should be tested for similar allomonal properties against ants and other offending leaf-litter arthropods.

Glyceryl monoethers possessing *n*-alkyl residues ranging in carbon-chain length from 12 to 20, chiefly C_{14}, C_{16}, and C_{18} chains, were reported in the scent gland secretions of male and female western diamondback rattlesnakes (*Crotalus atrox*) [11]. Young et al. [34]., however, failed to observe bands corresponding to this compound class in thin-layer chromatograms of the secretions of two pitvipers (Crotalinae), the eastern diamondback rattlesnake (*C. adamanteus*) and the Florida cottonmouth (*Agkistrodon piscivorus conanti*). Our analysis of *L. bicolor* affirms 43 glyeryl alkyl monoethers as scent gland products, revealing C_{13} to C_{24} straight-chain or methyl-branched alkyl chains. Glyceryl ethers, mostly showing saturated C_{15} to C_{22} *n*-alkyl chains, have also been observed in the femoral gland secretions of a lacertid lizard (*Acanthodactylus boskianus*) [32]. These compounds may occur widely among squamate reptiles.

We observed straight chain alcohols in the scent gland secretions of *L. bicolor* ranging in carbon-chain length from 13 to 24. This compound class has not previously been described from snake scent glands. However,

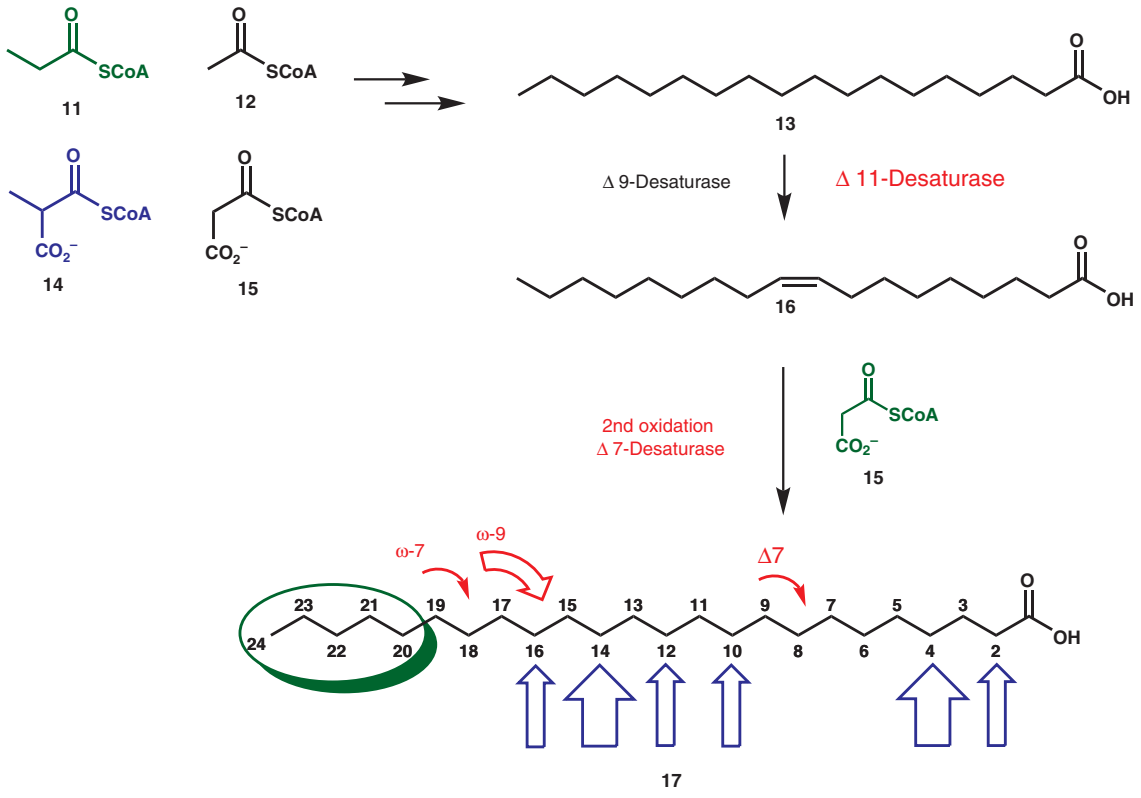

Figure 6: Biosynthetic features leading to a diversity of fatty acids. Black: standard biosynthetic pathway to saturated and unsaturated fatty acids. Blue: the incorporation of methylmalonate (**14**) leads to methyl groups at certain positions of the chain. Red: additional oxidation leads to double bonds at preferentially at ω-9 and ω-7. Dienoic acids are formed by an additional double-bond introduced, e.g., at Δ7. Green: The chain length can vary because of additional chain elongation with malonate (**15**). Odd numbered acids can be formed from a propionate starter (**11**) or α-oxidation of an acid (not shown). Thickness of arrows in **17** indicated relative importance of the modification at a certain position.

alcohols possessing chains of more than 30 carbons have been observed in extracts of the shed or intact epidermis of colubrid snakes [35, 36]. Such long-chain compounds are among the nonpolar lipids that may contribute to the transepidermal water barrier of the epidermis. The significance of alcohols in the scent gland secretions is unclear.

4 Experimental part

4.1 Methods

Scent gland secretions were collected from three male and seven female snakes (total lengths = 61–127 cm) maintained on rodents at the Memphis Zoo (TN, USA) and Vida Preciosa International, Inc. (Boerne, TX, USA). Most specimens were captured in Central America, possibly Honduras; two females were reared in captivity from these wild-caught individuals. Snakes were restrained while manual pressure was applied to the base of the tail. The emerging stream of scent gland fluids was directed into glass vials to which several milliliters of dichloromethane was added. Samples were kept frozen until analysis.

4.2 GC/MS analyses

GC/MS analyses were performed on an HP7890A GC connected to an HP5975C mass selective detector fitted with an HP-5ms fused silica capillary column (30 m, 0.22 mm i.d., 0.25 µm film, Agilent Technologies, USA). Helium served as carrier gas. Conditions were as follows: injection volume 1 µL, transfer line 300 °C, injector 250 °C, electron energy 70 eV. Linear retention indices were determined from a homologous series of *n*-alkanes (C_8–C_{32}).

4.3 Derivatizations

4.3.1 Silylation

N-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) (50 µL) was added to 100 µL of an extract, followed by

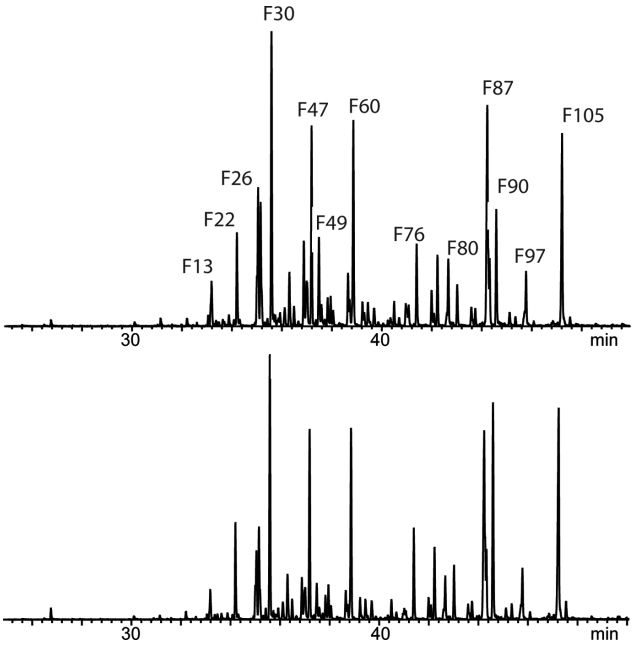

Figure 7: Fatty acids detected in scent gland secretions of male M3 (upper trace) and female F1 (lower trace) *Loxocemus bicolor*. The extract was methylated to convert acids into methyl esters. The ion trace *m/z* 74, characteristic for methyl esters of fatty acids, is shown to exclude other compounds.

heating for 1 h at 60 °C. Excess MSTFA and solvent were removed under a gentle stream of nitrogen to a volume of 5 µL. Finally, dichloromethane was added (100 µL) and the derivatized extract was analysed by GC/MS.

4.3.2 Methylation

N-methyl-*N*-nitroso-*p*-toluenesulfonamide (0.41 M in 1:1 diethyl ether/diethylethylenglykol monoethyl ether, 1 eq.) was added to a 5-mL vial equipped with a Teflon-lined septum cap carrying a small teflon tube as gas outlet. The tubing was connected to a second vial serving as gas washer. This vial was empty and carried a second Teflon tube as gas outlet. This tube was connected to a third vial, carrying diethyl ether. The latter vial was cooled with ice. KOH (1.1 eq, 0.37 M solution in 1:1 methanol/water) was added to the first tube by a syringe. The produced yellow was trapped in the diethyl ether vial. Subsequently, the yellow solution was added to 100 µL of an extract until the gas formation ceased. The derivatized extract was analyzed by GC/MS. *Caution*: Pure diazomethane is explosive.

4.3.3 3-Methylpyridyl esters

About 100 µL dichloromethane extract was treated with 20 µL oxalyl chloride for 1 day in a 2-mL vial. Excess oxalyl chloride was removed by evaporation with a stream of nitrogen. One drop 3-pyridinemethanol and 100 µL dichloromethane were added. The mixture was heated for 1 h in a heating block at 60 °C, followed by GC/MS analysis.

4.3.4 Pyrrolidides

About 100 µL dichloromethane extract was treated with 50 µL of a 9:1 mixture of pyrrolidine and pyridine in a 2-mL vial. After 1 h at room temperature, the 100 µL saturated $NaHCO_3$ solution was added. The organic phase was removed, dried with molecular sieve, and analyzed by GC/MS.

4.3.5 Dimethyl disulfide derivatives

Dimethyl disulfide (DMDS) adducts were obtained by stirring equal amounts of freshly distilled DMDS and

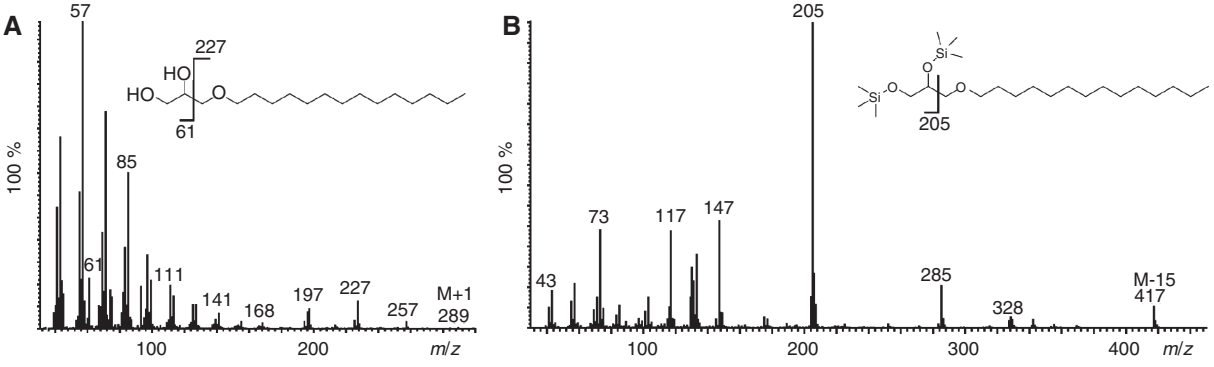

Figure 8: Mass spectra of 1-O-Tetradecylglyceride (A) and its bistrimethylsilyl derivative (B). Characteristic mass spectrometric cleavages are indicated.

Table 2: 1-*O*-Alkylglycerols in the scent gland secretions of *Loxocemus bicolor*.

Number	Alkyl residue	*I*	Conc.
G1	Tridecyl	2212	xxx
G2	12-Methyltridecyl	2280	x
G3	Tetradecyl	2312	xxx
G4	*X*-Methyltetradecyl	2338	x
G5	13-Methyltetradecyl	2365	x
G6	12-Methyltetradecyl	2375	x
G7	Pentadecyl	2403	xxx
G8	*X*-Methylpentadecyl	2432	x
G9	*X*-Methylpentadecyl	2435	x
G10	14-Methylpentadecyl	2460	x
G11	13-Methylpentadecyl	2471	x
G12	Hexadecyl	2499	xxx
G13	14-Methylhexadecyl	2568	xxx
G14	Heptadecyl	2594	x
G15	*X*-Methylheptadecyl	2618	x
G16	*X*-Methylheptadecyl	2637	x
G17	*X*-Methylheptadecyl	2642	x
G18	16-Methylheptadecyl	2648	x
G19	15-Methylheptadecyl	2664	x
G20	Octadecenyl	2667	x
G21	Octadecyl	2689	xx
G22	*X*-Methyloctadecyl	2717	x
G23	*X*-Methyloctadecyl	2727	x
G24	*X*-Methyloctadecyl	2727	x
G25	*X*-Methyloctadecyl	2737	x
G26	16-Methyloctadecyl	2759	x
G27	Nonadecyl	2785	xx
G28	*X*-Methylnonadecyl	2799	x
G29	18-Methylnonadecyl	2827	x
G30	Icosenyl	2858	x
G31	Icosenyl	2862	x
G32	Icosenyl	2866	x
G33	Icosyl	2883	xx
G34	*X*-Methylicosyl	2933	x
G35	Henicosyl	2977	x
G36	Docosenyl	3052	x
G37	Docosenyl	3055	x
G38	Docosenyl	3058	x
G39	Docosenyl	3061	x
G40	Docosyl	3075	x
G41	Tricosenyl	3150	x
G42	Tetracosadienyl	3233	x
G43	Tetracosenyl	3247	xx

Alkyl residue: Aliphatic part of the ether; *I*, gas chromatographic retention index; conc., relative concentration: xxx, major component; xx, minor component; and x, trace component.

100 µL of a natural extract with 5 µL of a 5% I_2-solution in diethyl ether at 60 °C overnight. Then excess I_2 was removed with saturated aqueous $Na_2S_2O_3$. The organic phase was separated and the aqueous phase was extracted twice with 100 µL pentane. The combined organic phases were dried with NaCl and reduced to a volume of 50 µL. The derivatized extract was then analyzed by GC/MS.

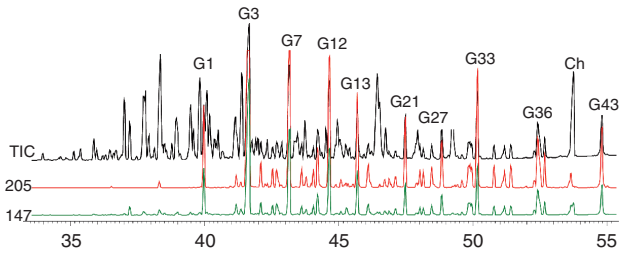

Figure 9: TIC of a scent gland secretion extract of *Loxocemus bicolor* silylated with MSTFA. Ions traces characteristic of 1-*O*-alkylglycerols, *m/z* 147 and *m/z* 205, are shown. Important peaks are annotated according toTable 2. Ch, cholesterol.

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