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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 Jaenicke

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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.



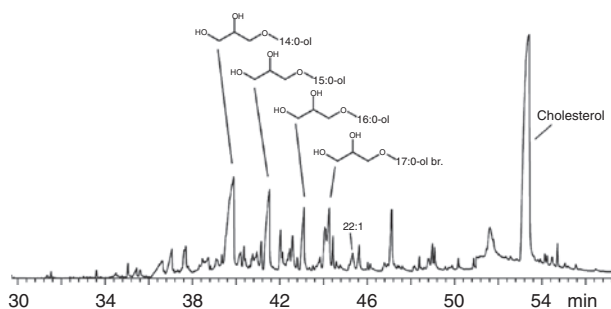
**Figure 1:** An adult female *Loxocemus bicolor* (total length=90 cm). Photograph by T. Barker (Vida Preciosa International, Inc., Boerne, TX, USA).

## 2 Results

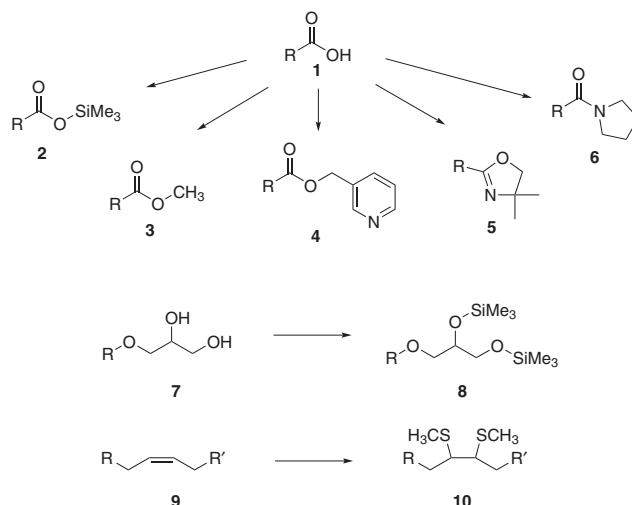
The analysis of the scent gland secretions revealed the presence of many peaks in the total ion chromatograms. The bad peak shapes leading to largely overlapping peaks suggested the presence of a high percentage of compounds with active hydrogens, such as alcohols and acids (Figure 2). Therefore, the 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*.

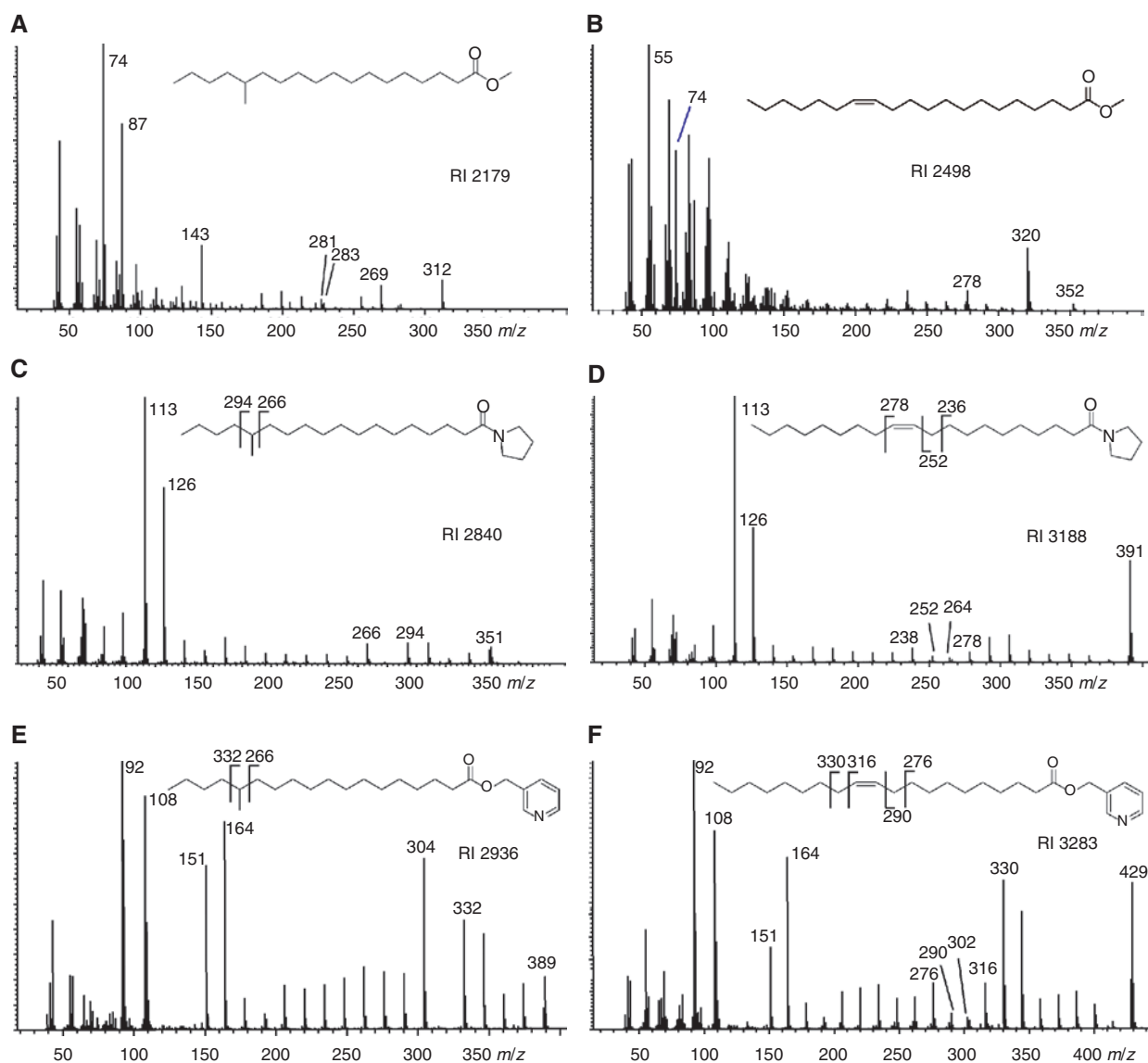


**Figure 3:** Derivatization reactions performed with extracts for structure elucidation.

(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-pyridylmethyl 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-pyridylmethyl 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-pyridylmethyl 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 (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-pyridylmethyl 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-pyridylmethyl 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 (DMS) adducts (10) of the unsaturated methyl esters [22, 23]. 11-Icosenoic acid was identified because the mass spectrum of the DMS adduct of its methyl ester furnished prominent ions at  $m/z$  245  $[CH_3SC_3H_{10}CO_2CH_3]^+$ ,

213  $[\text{CH}_3\text{SC}_{10}\text{H}_{20}\text{CO-CH}_2\text{OH}^+]$  and 173  $[\text{C}_9\text{H}_{20}\text{SCH}_3^+]$  [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  $\omega$ -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  $\Delta^9$ -desaturase leads to oleic acid, (*Z*)-9-octadecenoic acid (**16**), a  $\omega$ -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  $\Delta^{11}$ -desaturase furnishes  $\omega$ -7 unsaturated acids. The chain can be elongated by additional malonate units or biosynthesis can stop earlier, leading to shorter acids than  $\text{C}_{18}$ . After elongation, a second desaturation by a  $\Delta^7$ -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

$\alpha$ -oxidation by one carbon. Combining all these elements of chain-length diversity from  $\text{C}_{12}$  to  $\text{C}_{24}$  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  $[(\text{CH}_3)_3\text{SiOCH}_2\text{CHOSi}(\text{CH}_3)_3]^+$ , 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  $\text{C}_{13}$  to  $\text{C}_{16}$  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  $\text{C}_{13}$  to  $\text{C}_{24}$ . 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%).

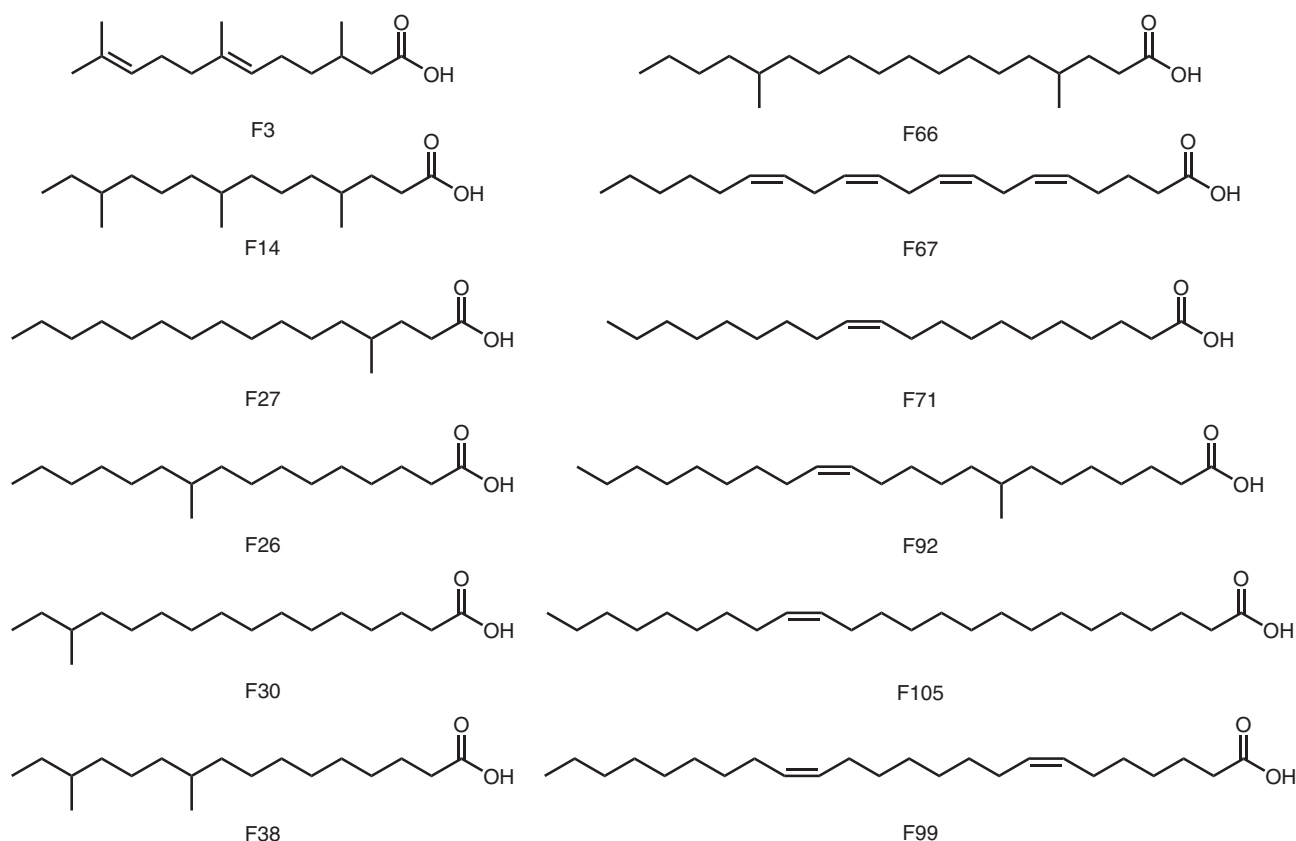
|     |                                    | RI   | M1<br>♂ | M2<br>♂ | M3<br>♂ | F1<br>♀ | F2<br>♀ | F3<br>♀ | F4<br>♀ | F5<br>♀ | F6<br>♀ | F7<br>♀ |
|-----|------------------------------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| F1  | 3-Methyldodecanoic acid            | 1572 | 2.1     | 2.1     | 1.2     | 2.0     | x       | 1.3     | 1.1     | 1.1     | 0.3     | 5.2     |
| F2  | 3-Methyldodecanoic 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 | Hexadecanoic 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 <sup>a</sup>  | 2007 | 1.3     | tr      | 2.7     | 1.1     | 2.4     | tr      | 0.9     |         |         | 2.2     |
| F32 | 11-Heptadecenoic acid <sup>a</sup> | 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<br>♂ | M2<br>♂ | M3<br>♂ | F1<br>♀ | F2<br>♀ | F3<br>♀ | F4<br>♀ | F5<br>♀ | F6<br>♀ | F7<br>♀ |
|------|----------------------------------------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 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 <sup>a</sup> | 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 <sup>a</sup>               | 2313 | 6.0     | 5.9     | 6.2     | 2.9     | 9.9     | 5.3     | 4.8     | 12.7    | 6.1     | 7.2     |
| F74  | 4,X-Dimethyllicosanoic 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-Methyllicosanoic acid                      | 2367 | 4.5     | 5.1     | 4.0     | 2.9     | 8.1     | 5.1     | 4.6     | 7.6     | 1.7     | 7.3     |
| F78  | 14-Methyllicosanoic acid                     | 2373 | 1.9     | 7.5     | 2.7     | 2.4     | 3.5     | 3.9     | 2.6     | 4.2     | 1.2     | 5.7     |
| F79  | 16-Methyllicosanoic 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 <sup>a</sup>            | 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 <sup>a</sup>              | 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 <sup>a</sup>             | 2610 | tr      | ?       | 5.3     | tr      | 5.5     | tr      | 5.1     |         |         | 4.9     |
| F96  | 14-Tricosenoic acid <sup>a</sup>             | 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  | Tetracos-7,15-dienoic acid <sup>b</sup>      | 2667 | 3.8     | ?       | 2.6     | 2.8     | ?       | ?       | tr      | 4.4     | 0.9     | 1.7     |
| F100 | Tetracosadienoic acid <sup>b</sup>           | 2669 | 4.1     | ?       | tr      | 4.8     | 3.0     | 3.7     | 2.6     |         | 1.7     | 1.9     |
| F101 | Tetracos-13,17-dienoic acid <sup>b</sup>     | 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%). <sup>a</sup>Compounds elute together with previous entry in the table. The percentages given represent the amount for both compounds.

<sup>b</sup>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

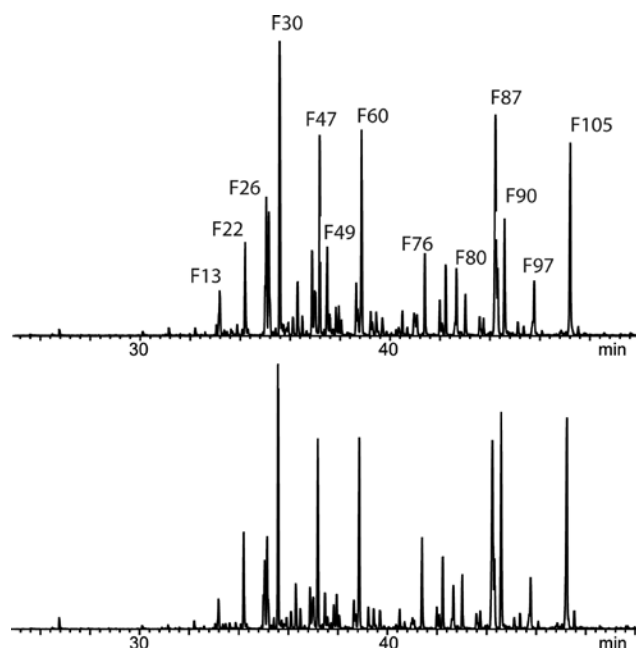
Carboxylic acids evidently are ubiquitous scent gland products of snakes, having been documented in colubrids [12], boids [6, 8, 12], pythonids [12], elapids [7], viperids [9, 10], and leptotyphlopids [5]. These compounds typically possess 2–26 carbon atoms and chiefly feature saturated or monounsaturated 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 glyceryl 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 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  $\mu$ L. Finally, dichloromethane was added (100  $\mu$ L) 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  $\mu$ L 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

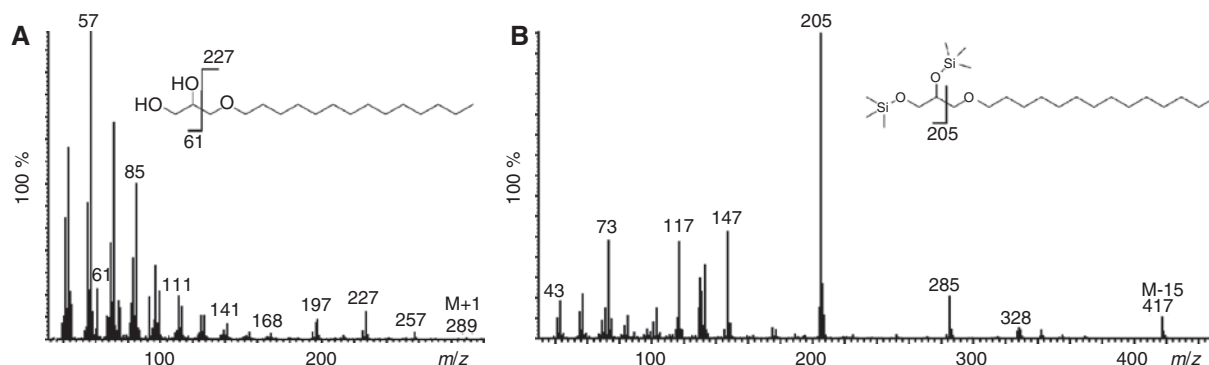
About 100  $\mu$ L dichloromethane extract was treated with 20  $\mu$ L oxalyl chloride for 1 day in a 2-mL vial. Excess oxalyl chloride was removed by evaporation with a stream of nitrogen. One drop 3-pyridinemethanol and 100  $\mu$ L dichloromethane were added. The mixture was heated for 1 h in a heating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

#### 4.3.4 Pyrrolidides

About 100  $\mu$ L dichloromethane extract was treated with 50  $\mu$ L of a 9:1 mixture of pyrrolidine and pyridine in a 2-mL vial. After 1 h at room temperature, the 100  $\mu$ L saturated  $\text{NaHCO}_3$  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



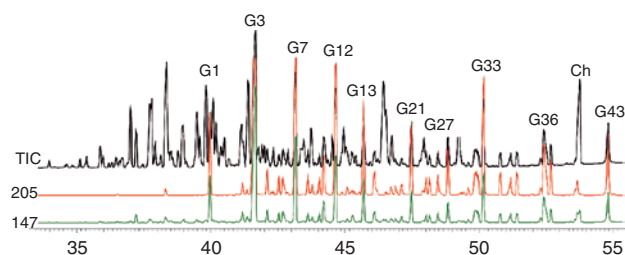
**Figure 8:** Mass spectra of 1-O-Tetradecylglyceride (A) and its bistrimethylsilyl derivative (B). Characteristic mass spectrometric cleavages are indicated.

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

| Number | Alkyl residue              | <i>I</i> | Conc. |
|--------|----------------------------|----------|-------|
| G1     | Tridecyl                   | 2212     | xxx   |
| G2     | 12-Methyltridecyl          | 2280     | x     |
| G3     | Tetradecyl                 | 2312     | xxx   |
| G4     | <i>X</i> -Methyltetradecyl | 2338     | x     |
| G5     | 13-Methyltetradecyl        | 2365     | x     |
| G6     | 12-Methyltetradecyl        | 2375     | x     |
| G7     | Pentadecyl                 | 2403     | xxx   |
| G8     | <i>X</i> -Methylpentadecyl | 2432     | x     |
| G9     | <i>X</i> -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    | <i>X</i> -Methylheptadecyl | 2618     | x     |
| G16    | <i>X</i> -Methylheptadecyl | 2637     | x     |
| G17    | <i>X</i> -Methylheptadecyl | 2642     | x     |
| G18    | 16-Methylheptadecyl        | 2648     | x     |
| G19    | 15-Methylheptadecyl        | 2664     | x     |
| G20    | Octadecenyl                | 2667     | x     |
| G21    | Octadecyl                  | 2689     | xx    |
| G22    | <i>X</i> -Methyloctadecyl  | 2717     | x     |
| G23    | <i>X</i> -Methyloctadecyl  | 2727     | x     |
| G24    | <i>X</i> -Methyloctadecyl  | 2727     | x     |
| G25    | <i>X</i> -Methyloctadecyl  | 2737     | x     |
| G26    | 16-Methyloctadecyl         | 2759     | x     |
| G27    | Nonadecyl                  | 2785     | xx    |
| G28    | <i>X</i> -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    | <i>X</i> -Methylcosyl      | 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  $\mu$ L of a natural extract with 5  $\mu$ L 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  $\mu$ L pentane. The combined organic phases were dried with NaCl and reduced to a

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

volume of 50  $\mu$ L. The derivatized extract was then analyzed by GC/MS.

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