Surface Lipids of the Silverfish (*Lepisma saccharina* L.)

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Z. Naturforsch. **52c,** 109–113 (1997); received October 4/October 29, 1996

Insects, Zygentoma, Cuticular Lipids, Chemotaxonomy, 3-Methylalkanes

Surface lipids obtained from the silverfish by short-term solvent extraction contain aliphatic hydrocarbons, monoester waxes, cholesteryl esters, triglycerides, free cholesterol, and free fatty acids. Together, cholesteryl esters and free cholesterol account for >30% of the total lipids.

As found for other arthropods living in aquatic or moist environments unsaturated homologues predominate among the acidic constituents.

The cuticular lipid composition of silverfish resembles that of other more primitive arthropod forms such as stoneflies and dragonflies.

Introduction

Similar to the feather waxes for the vertebrate class Aves (Jacob, 1978) cuticular lipids have turned out to be specific chemotaxonomic characters for the invertebrate class of insects which may help to clarify systematic problems (Jacob and Hanssen, 1986, for review see Lockey, 1988). Though numerous data on the cuticular lipids from the higher developed insect orders have been published (for review see Nelson and Blomquist, 1995) the primitive forms of the wingless insects (Diplura, Protura, Collembola, Archaeognatha, Zygentoma) have not yet been studied in detail. An inventory of the lipids from the silverfish (*Lepisma saccharina* L., order Zygentoma) has been published by Kinsella (1969) in which the total body lipids were analysed. However, the author did not report on the occurrence of any hydrocarbons which are considered to be chemosystematically important constituents. According to the extraction procedure applied, his results differ markedly from those reported here. In our study the animals were immersed for only a few seconds in order to obtain preferrentially the surface lipids which were compared to those reported for species from two other more primitive insect orders, Odonata (Jacob and Hanssen, 1979) and Plecoptera (Armold *et al.,* 1969).

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Material and Methods

Trapped silverfish (550 individuals, total weight 1.426 g) were immersed for 10 sec in chloroform and the extract filtered and evaporated. The residue (18.7 mg) was redissolved in cyclohexane and separated into five fractions by silica gel chromatography (5 g; 9.8% water content). Hydrocarbons were eluted with cyclohexane (70 ml), monoester waxes plus cholesteryl esters with cyclohexane/benzene (9:1; v/v; 100 ml), an intermediary fraction with cyclohexane/benzene (1:1; v/v; 60 ml), triglycerides with benzene/chloroform (2:1; v/v; 50 ml), and more polar lipids with chloroform/methanol (2:1; v/v; 50 ml). Free fatty acids were extracted from a chloroform solution of the latter with 2 N methanolic NaOH from which they were recovered after acidification with conc. HCl by extraction with cyclohexane. The residue was separated into alcohols and more polar lipids by preparative thin-layer chromatography.

Waxes, cholesteryl esters, triglycerides, and free fatty acids were transesterified with 5% methanolic HCl. In case of monoester waxes and cholesteryl esters the resulting fatty acid methyl esters and alcohols or cholesterol were separated by column chromatography on silica gel, eluting methyl esters with cyclohexane/benzene (3:1; v/v;

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Table I: Composition of the lipids extracted from a total of 550 silverfish individuals (*Lepisma saccharina* L.).

Lipid class	Amount extracted [mg]	% of total
Hydrocarbons	1.0	5.4
Monoester waxes + cholesteryl esters	1.3	7.0
Intermediary fraction	0.1	0.5
Triglycerides	4.0	21.4
Free alcohols	0.2	1.1
Cholesterol	5.3	28.3
Free fatty acids	5.8	31.0
Polar lipids	1.0	5.3
Total	18.7	100.0

100 ml) and alcohols with chloroform/methanol (95:5; v/v; 50 ml). The latter were oxidized by treatment with CrO_3/acetic acid in cyclohexane for 12 h (Jacob and Zeman, 1970) resulting in the corresponding fatty acids which then were esterified as above.

The GC separation of all fractions was carried out with a 25 m x 0.32 mm NB 54 capillary column (Nordion; 0.25 μm) under isothermical conditions (column temperature: 180 °C or 250 °C; injection port and detector temperature 200 °C or 250 °C) using a Delsi DI 700 instrument adapted to an electronic integrator system Shimadzu CR 3A. Equivalent chain-lengths (ECL values) were obtained from a semi-logarithmic plot of the retention times versus chain length with reference standards of either unbranched fatty acid methyl esters (n-C_{10}-C_{20}) or n-alkanes (C_{16}-C_{28}).

Gas chromatography/mass spectrometry combination (GC/MS) was carried out with a Varian MAT 112S instrument using the above GC equipment and conditions. Mass spectra were recorded at 70 eV and 200 °C ion source temperature.

Oxidation of alkenes with OsO_4 has been carried out in pyridine/dioxane as described by Murawski *et al.*(1971).

Results and Discussion

The composition of the crude lipids extractable from silverfish by short-term immersion with chloroform is presented in Table I. The lipid amount per animal may be calculated to be about 34 μg.

Calculation of the equivalent chain lengths of the hydrocarbons from the gas chromatography data indicated the presence of at least 4 homologous series of saturated alkanes: unbranched paraffins (increment 0.00), 3-methylalkanes (increment + 0.75), internally monomethyl-substituted alkanes (e.g. 11-C_{27}; 13-C_{29}; 15-C_{31} etc., increment + 0.35- 0.40) and 3,(ω-2)-dimethyl-substituted alkanes (e.g. 3,25-C_{27}; 3,27-C_{29} etc.,

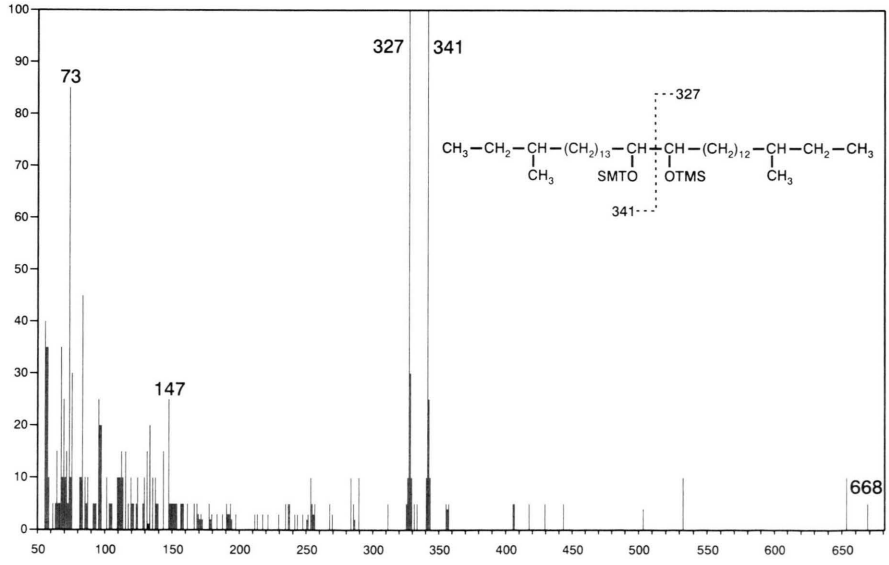

Fig. 1. Mass spectrum of the main representative of alkane diols as OTMS-ether after oxidation of the hydrocarbon fraction with OsO_4 and subsequent trimethylsilylation (identified as 16,17-dihydroxy-3,31-dimethyl-tritriacontane trimethylsilyl ether).

Table II. Quantitative composition of the hydrocarbons from the surface lipids of the silverfish (*Lepisma saccharina* L.) (in% of totals).

	ECL*	% of totals
Saturated		(50.4)
n-alkanes		(6.4)
n-C_{25}	25.00	0.2
n-C_{27}	27.00	2.1
n-C_{29}	29.00	1.5
n-C_{31}	31.00	1.5
n-C_{33}	33.00	0.5
n-C_{35}	35.00	0.6
3-methylalkanes		(13.5)
3-C_{25}	25.69	0.2
3-C_{27}	27.70	7.2
3-C_{29}	29.75	3.7
3-C_{31}	31.75	1.9
3-C_{33}	33.75	0.5
other monomethylalkanes		(2.3)
11-C_{27}	27.30	0.2
13-C_{29}	29.30	0.7
15-C_{31}	31.30	1.4
3,(ω-2)-dimethylalkanes		(28.2)
3,19-C_{21}	22.45	0.1
3,21-C_{23}	24.45	0.4
3,23-C_{25}	26.45	0.2
3,25-C_{27}	28.45	3.6
3,27-C_{29}	30.45	16.0
3,29-C_{31}	32.50	3.7
3,31-C_{33}	34.50	3.2
3,33-C_{35}	36.50	1.0
Unsaturated		(38.4)
*3-methylalkenes***		(6.6)
3-$C_{27:1}$	27.45	0.2
3-$C_{29:1}$	29.45	0.6
3-$C_{31:1}$	31.45	2.6
3-$C_{33:1}$	33.50	2.4
3-$C_{35:1}$	35.50	0.8
*3,(ω-2)-dimethylalkenes***		(31.8)
3,27-$C_{29:1}$	30.22	0.8
3,29-$C_{31:1}$	32.25	4.2
3,31-$C_{33:1}$	34.25	6.9
3,33-$C_{35:1}$	36.25	19.9
Unidentified		(11.2)

* ECL = equivalent chain length (related to *n*-alkanes).
** Tentatively identified.

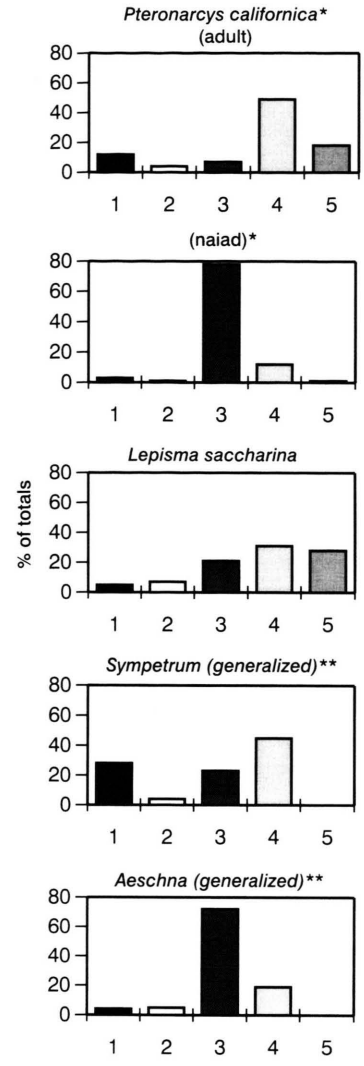

Fig. 2. Comparison of the cuticular lipid composition of *Lepisma saccharina*, *Pteronarcys californica* and of two Odonata genera (*Sympetrum*; *Aeschna* , data generalized from 2 species each). 1 = *n*-alkanes; 2 = monoester waxes + cholesteryl esters; 3 =triglycerides; 4 = free fatty acids; 5 = sterols. (* Data taken from Armold *et al.*, 1969; ** from Jacob and Hanssen, 1979).

increment + 1.50). Both 3-methyl- and 3,(ω-2)-dimethylalkanes display intense (M-29)- and (M-57)-fragments and a very small (or absent) (M-43)-fragment in their mass spectra.

In addition, two homologous series of alkenes could be characterised by their MS and their ECL-values. The mass spectra displayed intense molecular ions and fragments belonging to the structure C_nH_{2n-1} (e.g. *m/z* 41; 55; 69 etc.). The location of substituents cannot readily be recognized although the intense (M-27)-, (M-28) and (M-29)-fragments may indicate a 3-methyl-substitution. Furthermore, the GC increments (+ 0.45 and + 1.25) support the assumption that the two homologous series belong to 3-methyl- and 3,(ω-2)-dimethylalkenes. For instance in case of 3-methyl-tritriacon-

tene (3-$C_{33:1}$) an ECL-value of 33.00–0.25 (for mono-unsaturation) + 0.75 (for 3-methyl-substitution) = 33.50 and in case of 3,31-tritriacontene (3,31-$C_{33:1}$) an ECL-value of 33.00–0.25 (for mono-unsaturation) + 1.50 (for 3- and (ω-2)-methyl-substitution) = 34.25 is to be expected which were actually found.

After hydrogenation of the hydrocarbon fraction with Pd/H_2 in ethanol peaks originally characterised as olefins disappeared and those of the corresponding alkanes increased. Oxidation of this fraction with OsO_4 and subsequent trimethylsilylation resulted in the formation of a series of alkane diol OTMS-ethers the main representative of which could be identified by its mass spectrometric fragmentation as 16,17-dihydroxy-3,31-dimethyl-tritriacontane trimethylsilyl ether as shown in Figure 1.

The quantitative composition of the hydrocarbon fraction is summarised in Table II.

The composition of the free fatty acids and the acidic moieties from the monoester waxes, cholesteryl esters and the triglycerides is fairly similar and rich in monoenoic and dienoic acids the double bonds of which have not been determined. Only small amounts of monomethyl-substituted fatty acids (4-, 6-, and (ω-2)-branched) were found as can be seen from Table III.

Though only marginal amounts of n-alkanols were detected in the polar lipid fraction (2.1%, but 97.9% cholesterol), the homologues were similar to those found in the monoester waxes (Table IV). Cholesterol was identified by its relative retention time (ECL = 27.55 related to *n*-alkanols) and the mass spectrum (M = 386; *m/z* 371; 368; 353; 301; 275; 255; 247; 231; 213; 207) identical with that recorded from the authentic reference compound.

When compared to other insect orders already analysed for the composition of their cuticular lipids (Fig. 2), *Lepisma* resembles most the adult form of the Plecoptera species *Pteronarcys californica* while its aquatic living naiad differs significantly from that (Armold *et al.*, 1969). Within the Odonata, cuticular lipids of *Lepisma* resemble those of *Sympetrum*, whereas *Aeschna* is more similar to naiads of *P. californica*, though no sterols were found in these genera (Jacob and Hanssen, 1979). All other insect orders investigated so far appear to be more distant in this respect which corresponds to our present understanding of insect systematics.

Table III. Fatty acid composition of various lipid classes from the surface lipids of the silverfish (*Lepisma saccharina* L.) (in % of totals).

	Monoester waxes*	Triglycerides	Free fatty acids
Saturated			
unbranched	(22.5)	(12.2)	(15.6)
n-C_{12}	tr	tr	tr
n-C_{14}	0.9	0.4	0.6
n-C_{15}	0.7	tr	0.2
n-C_{16}	15.3	9.8	12.1
n-C_{17}	0.7	0.1	0.2
n-C_{18}	4.7	1.9	2.5
n-C_{20}	0.2	tr	–
branched	(0.8)	(1.6)	(2.5)
11-C_{13}	–	0.1	–
4-C_{14}	–	0.1	–
6-C_{14}	–	0.2	–
12-C_{14}	0.3	0.2	1.1
6-C_{16}	–	0.4	0.5
14-C_{16}	0.5	0.6	0.9
Unsaturated	(75.2)	(81.5)	(81.9)
16:1	4.3	5.5	5.3
16:2	0.1	0.1	0.1
17:1	0.6	0.2	0.3
18:1	55.9	62.6	60.4
18:2	13.7	12.7	14.6
20:1	0.2	tr	0.4
20:3	0.4	0.4	0.8
Unidentified	(1.5)	(4.7)	(–)

* Including cholesteryl esters.

Table IV. Alcohol composition of the monoester waxes (including cholesteryl esters) and the free alcohols from the surface lipids of *Lepisma saccharina* (in % of totals).

	Monoester waxes*	Free alcohols
n-C_{16}	tr	0.1
n-C_{17}	2.7	0.1
n-C_{18}	0.6	0.1
n-C_{20}	tr	0.1
n-C_{21}	1.3	tr
n-C_{22}	1.3	0.4
n-C_{24}	–	0.3
Cholesterol	88.3	97.9
Unidentified	5.8	1.0

* Including cholesteryl esters.

On the basis of the distribution of cuticular hydrocarbons to distinct structural classes such as n-alkanes, alkenes, 2-, 3- and internally branched hydrocarbons as chemosystematic characters having successfully applied by Lockey and Metcalfe (1988) and Lockey (1991), *Lepisma saccharina* exhibits some resemblance to the stonefly *Pteronarcys californica* inasmuch as 3-methylalkanes are present in both species. They are, however, also present (some 10%) in Odonata species and are, in general, widely distributed to other insect orders as well (Lockey, 1991). To our knowledge, (ω-2),(ω'-2)-dimethylalkanes have not yet been reported as major constituents of cuticular lipids.

Noteworthy, a high content of cholesteryl esters (5%) and free cholesterol (28,3%) is not commonly found in arthropod cuticular lipids though Cherry (1969) reported that for the tick *Boophilus microphus.*

A high ratio of unsaturated to saturated acids was found for both free and triglyceride fatty acids (Fig. 3). This has also been reported for *Pteronarcys californica* (Armold *et al.*,1969) and for two *Aeschna* species (Odonata) living in moist environments, whereas for two *Sympetrum* species living in more arid areas a markedly lower ratio has been reported (Jacob and Hanssen, 1979).

In an early review by Thompson (1973) the fatty acid compositions of 7 insect orders (Lepidoptera, Hemiptera, Orthoptera, Diptera, Hymenoptera, Dictyoptera, Coleoptera) were compared exhibiting the lowest ratio for Hemiptera (0.1) and the highest for Hymenoptera (3.3). These data, however, are of limited value for our study since total body lipids had been regarded rather than cuticular lipids.

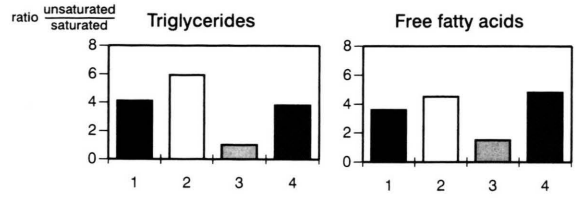

Fig. 3. Ratio unsaturated/saturated fatty acids for the triglycerides and free fatty acids found in the cuticular lipids of *Pteronarcys californica* (adult,(1)), *Lepisma saccharina* (2), *Sympetrum**(3), *Aeschna**(4).* Generalized from two species each.

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