Composition of the Surface Lipids from the European Mole *(Talpa europaea)**

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The surface lipids of the European mole consist of squalene, monoester waxes, triglycerides and cholesterol. As shown by GC/MS analysis, the fatty acid moieties of both monoester waxes and triglycerides belong to saturated unbranched, (ω-1)- and (ω-2)-methyl-substituted and unsaturated homologous series. Double bonds are located at the 7/8- and 9/10-position. Apart from the *cis*- also *trans*-isomers were found. The alcohol moieties of the monoester waxes consisted of saturated unbranched, (ω-1)- and (ω-2)-methyl-substituted homologous series exclusively.

Introduction

Surface (skin) lipids of various mammalian species have been analyzed and were found to contain a number of unusual structures when compared to other body lipids such as those present in the abdominal fat tissue. Typically, double bonds were found to be located in the 6-position in human sebum acids in which *cis*-6-hexadecenoic acid predominates among the monounsaturated fatty acids [1]. Branched fatty acids were found as constituents of wax esters, cholesterol esters and triglycerides from rat [2], mouse [3], dog [4], sheep [5], gerbil [6] and other mammalian skin lipids and are obviously significant for the feather impregnation in birds [7].

Although abrasion of the surface lipids may be expected to play an important role in the European mole *(Talpa europaea)* living in subterranean caverns in which he may move along fairly fast, his fur remains in excellent quality. This, in part, might be attributed to specific properties of the skin lipids. The morphology of skin glands of the mole has been thoroughly reviewed by Schaffer [8]. Although the occurrence of sebaceous glands in the prepuce [9], in the auditory canal [10], and in the corner of the mouth [8] have been described no detailed description of general integumental sebaceous glands has been published. Starck [11] emphasized that the mole possesses an extraordinary soft fur without an up-strike, *i.e.*, a homogenously diffuse arrangement of hairs which is in contrast to other fur-bearing mammals with hairs being slantwise implanted into the epidermis.

This paper describes the composition of the lipids obtained from the fur and the paws of the mole by solvent extraction.

Materials and Methods

Three moles were killed by earth traps. Fur was obtained by shaving and paws were cut off. Fur and paws were separately extracted with chloroform:methanol (2:1; v/v; 45 ml) and the mixture diluted with water (15 ml). The hypolayer was evaporated and resulted the crude lipids (7.4–19.3 mg fur lipids and 0.5 mg paw lipids). A preliminary identification of the lipid classes was achieved by thin-layer chromatography using SiO_2-coated plates (E. Merck) and CCl_4:$CHCl_3$ (3:1; v/v) as mobile phase.

The lipids were separated into single classes by column chromatography on SiO_2 (5 g WOELM, 9.1% water content). Hydrocarbons were eluted with cyclohexane (70 ml), monoester waxes with cyclohexane:benzene (9:1; v/v; 100 ml), triglycerides with benzene:chloroform (9:1; v/v; 50 ml) and more polar compounds with chloroform:methanol (9:1; v/v; 70 ml). Free fatty acids were

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separated from the polar lipids by alkaline extraction with methanolic NaOH. Triglycerides and monoester waxes were reesterified with 5% methanolic HCl, and the products purified by SiO_2 column chromatography. Free fatty acids were esterified in the same way. The alcohols were oxidized into the corresponding fatty acids by CrO_3-oxidation in acetic acid/cyclohexane and converted into methyl esters before analyzed by GC/MS.

For the location of the double bonds the monounsaturated fatty acid methyl esters were separated by means of the Hg-II-acetate adduct method [12]. Afterwards they were oxidized with OsO_4 in pyridine/dioxane to the corresponding dihydroxy acid methyl esters which subsequently were converted into their trimethylsilyl ethers by silylation with Trisil/N,O-bis-trimethylsilyl-trifluoroacetamide [13].

Identification of all lipid constituents was achieved by comparing gas-chromatographic retention times (*via* equivalent chain length indices) with authentic samples as well as by mass spectrometry. Gas chromatography was carried out with 50 m glass capillaries coated with CPsil 5 at 150 °C or 200 °C column temperature (isothermically), at 200 °C injection port and detector temperature by using a Perkin-Elmer Sigma 2 instrument adapted to an electronic integrator Spectra-Physics SP 4100-02. Gas chromatography/mass spectrometry was carried out with a mass spectrometer Varian-MAT 112S instrument adapted to a Perkin-Elmer gas chromatograph as above. Mass spectra were recorded at 70 eV and 200 °C ion source temperature.

Results

The lipids obtained from the fur of three individual moles consisted of almost equal amounts (about 30%) of squalene which actually was the only hydrocarbon detected, monoester waxes, and polar lipids, whereas triglycerides accounted for 10%. The percentages and absolute amounts of the various lipids are presented in Table I. The paw lipids were similarly composed (data not given).

Hydrocarbons

The hydrocarbon fraction consisted almost exclusively of squalene identified by its relative retention time of 27.70 (related to eicosane = 20.00) and its mass spectrum which did not show any difference to that of the authentic reference ($M^+ = 410$).

Monoester waxes

Methanolysis with 5% methanolic hydrochloric acid resulted in fatty acid methyl esters and alcohols which were separated by chromatography on silicagel. They consisted of branched and unbranched saturated and unbranched monounsaturated fatty acids and of unbranched and methyl-branched alcohols as presented in Table II.

(ω-1)-Methyl-branched esters were recognized by the intense (M-15)-fragment and the fragmentation pattern (M-15) → (M-47) → (M-55) according to further elimination of methanol and water. Similarly, (ω-2)-methyl-substituted methyl esters were identified by the ratio (M-29) > (M-31) and the fragment series (M-29) → (M-61) → (M-79). 4-Methyl-branched methyl esters were recognized from the intense (M-73) fragments and the ratio m/z 87 > m/z 74 in their mass spectra. (M-76)-Fragments have been used as a diagnostic fragment in case of 6-methyl-substituted methyl esters and the fragmentation pattern 199 → 167 → 149 for the identification of 10-methyl-substituted methyl esters. Double bonds of the monounsatu-

Table I. Percentages of lipids obtained by solvent extraction from the fur of 3 individual moles (absolute amounts in parenthesis).

Lipid	Sample 1		Sample 2		Sample 3		Mean	s.d.*
	%	[mg]	%	[mg]	%	[mg]	%	%
Hydrocarbons	25.7	(1.9)	27.5	(5.3)	37.4	(4.0)	30.2	20.9
Monoester waxes	33.8	(2.5)	30.7	(5.9)	24.4	(2.6)	29.6	16.2
Triglycerides	10.8	(0.8)	10.4	(2.0)	10.2	(1.1)	10.5	2.9
Polar lipids	29.7	(2.2)	31.4	(6.1)	28.0	(3.0)	29.7	5.7
Total	100.0	(7.4)	100.0	(19.3)	100.0	(10.7)	100.0	

* s.d. = standard deviation (%).

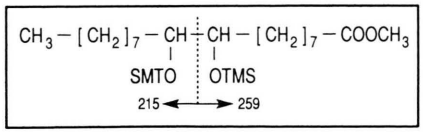
Table II. Composition of the monoester wax constituents from the surface lipids of the European mole *(Talpa europaea)*, in per cent of the total fraction as detected by GC.

Fatty acids	ECL*	%	Alcohols	ECL*	%
Saturated unbranched (total)		(12.5)	Saturated unbranched (total)		(45.7)
n-C_{12}	12.00	0.4	*n*-C_{10}	10.00	trace
n-C_{14}	14.00	1.4	*n*-C_{11}	11.00	0.1
n-C_{15}	15.00	0.5	*n*-C_{12}	12.00	0.2
n-C_{16}	16.00	5.1	*n*-C_{13}	13.00	0.3
n-C_{17}	17.00	0.6	*n*-C_{14}	14.00	2.9
n-C_{18}	18.00	3.9	*n*-C_{15}	15.00	3.9
n-C_{19}	19.00	trace	*n*-C_{16}	16.00	18.9
n-C_{20}	20.00	0.5	*n*-C_{17}	17.00	1.8
n-C_{21}	21.00	0.1	*n*-C_{18}	18.00	14.9
			n-C_{20}	20.00	2.7
Saturated branched (ω-1) (total)		(11.4)	Saturated branched (ω-1) (total)		(20.4)
9-C_{10}	10.65	0.1	10-C_{11}	11.65	0.2
12-C_{13}	13.65	0.2	11-C_{12}	12.65	0.7
13-C_{14}	14.65	2.0	12-C_{13}	13.65	0.2
14-C_{15}	15.65	2.4	13-C_{14}	14.65	0.2
15-C_{16}	16.65	2.8	14-C_{15}	15.65	8.9
16-C_{17}	17.65	3.1	15-C_{16}	16.65	6.5
17-C_{18}	18.65	0.8	16-C_{17}	17.65	3.5
			17-C_{18}	18.65	0.2
Saturated branched (ω-2) (total)		(9.1)	Saturated branched (ω-2) (total)		(31.3)
10-C_{12}	12.75	0.2	8-C_{10}	10.75	0.4
11-C_{13}	13.75	0.1	10-C_{12}	12.75	0.3
12-C_{14}	14.75	1.5	11-C_{13}	13.75	0.1
13-C_{15}	15.75	1.2	12-C_{14}	14.75	3.3
14-C_{16}	16.75	6.1	13-C_{15}	15.75	1.9
			14-C_{16}	16.75	23.5
			15-C_{17}	17.75	1.0
			16-C_{18}	18.75	0.8
Saturated branched (others) (total)		(0.9)			
4-C_{11}	11.50	0.1			
4-/6-C_{14}	14.50	0.1			
6-/10-C_{15}	15.52	0.2			
4-/6-/10-C_{16}	16.51	0.3			
4-/6-/10-C_{17}	17.50	0.2			
Unsaturated unbranched (total)		(66.1)	unidentified		2.6
cis-9-$C_{12:1}$	11.70	0.1			
cis-9-$C_{13:1}$	12.70	0.1			
cis-7-$C_{14:1}$	13.68	0.2			
cis-9-$C_{14:1}$	13.70	0.3			
cis-7-$C_{15:1}$	14.68	0.2			
cis-9-$C_{15:1}$	14.70	0.2			
cis-9-$C_{16:1}$	15.70	8.0			
trans-9-$C_{16:1}$**	15.80	1.3			
cis-9-$C_{17:1}$	16.70	2.8			
trans-9-$C_{17:1}$**	16.80	0.3			
cis-9-$C_{18:1}$	17.70	38.9			
trans-9-$C_{18:1}$**	17.80	10.5			
cis-11-$C_{18:1}$	17.72	3.2			

* ECL = equivalent chain length. In case of alcohols ECL-values of the corresponding fatty acid methyl esters, which were obtained after CrO_3-oxidation, have been given.

** Tentatively identified.

rated fatty acid methyl esters were found to be located preferentially in the 9-position as indicated by the intense *m/z* 259 resulting from the cleavage between the two OTMS-substituted carbon atoms according to:



in case of the OTMS-ether obtained from octadecenoic acid methyl ester after OsO_4-oxidation and subsequent silylation. Analogous fragments of *m/z* 231 and *m/z* 287 were found for double bonds in 7- or 11-position, respectively. Fragments analogous to the above *m/z* 215 were found for methyl esters with shorter chains, *e.g. m/z* 201; 187; 173 etc. From the GC retention time these acids were identified as *cis*-isomers. In case of $C_{16:1}$ and $C_{18:1}$ additional smaller peaks with greater retention times but identical mass spectra have been detected which co-eluted with the corresponding *trans*-isomers and which have been tentatively identified as such compounds.

Triglycerides

The fatty acid pattern of the triglycerides resembles that of the monoester waxes. However, *cis*-9,12-octadecadienoic acid and minor amounts of 3-methyltetradecanoic acid have been detected as additional constituents. No 4-, 6- and 10-methyl-substituted acids were found in this fraction. The OTMS-ether of the OsO_4-oxidation product of the above octadecadienoic acid methyl ester exhibited the same mass spectrum as has been previously shown by Murawski *et al.* [13] for the *cis*-9,12-isomer. The composition of the triglyceride acids is presented in Table III.

Polar lipids

This fraction consisted almost exclusively of free cholesterol and to about 3% of cholesta-4,6-diene-3β-ol as identified by mass spectrometry and comparison with the fragmentation pattern of an authentic sample. In addition, minor amounts of free alcohols and free fatty acids with structures also to be found in the fractions mentioned above have been detected.

Table III. Composition of the triglyceride fatty acids from the surface lipids of the european mole *(Talpa europaea)*, in per cent of the total fraction as detected by GC.

	ECL*	%
Saturated unbranched (total)		(19.2)
n-C_{13}	13.00	0.1
n-C_{14}	14.00	1.1
n-C_{15}	15.00	0.9
n-C_{16}	16.00	6.2
n-C_{17}	17.00	0.7
n-C_{18}	18.00	5.9
n-C_{20}	20.00	0.8
n-C_{22}	22.00	3.5
Saturated branched (ω-1) (total)		(9.7)
12-C_{13}	13.65	0.1
13-C_{14}	14.65	1.4
14-C_{15}	15.65	2.8
15-C_{16}	16.65	3.0
16-C_{17}	17.65	1.5
17-C_{18}	18.65	0.9
Saturated branched (ω-2) (total)		(9.8)
12-C_{14}	14.75	1.0
14-C_{16}	16.75	7.9
16-C_{18}	18.75	0.9
Saturated branched (others) (total)		(0.7)
3-C_{14}	14.40	0.4
unidentified	14.65	0.3
Unsaturated unbranched (total)		(60.6)
cis-7-$C_{14:1}$	13.68	0.1
cis-9-$C_{14:1}$	13.70	0.1
cis-7-$C_{16:1}$	15.68	1.3
cis-9-$C_{16:1}$	15.70	7.2
trans-9-$C_{16:1}$**	15.80	1.0
cis-9-$C_{17:1}$	16.70	2.1
cis-9-$C_{18:1}$	17.70	23.5
trans-9-$C_{18:1}$**	17.80	12.1
cis-11-$C_{18:1}$	17.72	3.3
cis-9,12-$C_{18:2}$	17.60	3.4
cis-9-$C_{20:1}$	19.70	1.4
cis-11-$C_{20:1}$	19.72	0.7
trans-9-$C_{20:1}$**	19.80	1.4
cis-9-$C_{22:1}$	21.70	3.0

* ECL = equivalent chain length.
** *trans*-isomers were tentatively identified.

Discussion

Integumental lipids are a rich source of unusual fatty acids and alcohols. (ω-1)- and (ω-2)-methyl-substituted fatty acids account for some 20% of the monoester waxes of mouse and rat [2, 3] and

for even more in case of sterol esters in these species and in the gerbil [6]. In the mole this type of fatty acid accounts for about 20% of the monoester waxes and the triglycerides, while 50% of the wax alcohols correspond to these structures. Surprisingly, a similar lipid pattern has been reported to occur in the 'neutral lipids' of the earthworm *(Lumbricus terrestris)* [14]. Whether this is accidental or linked to a subterranean living habit of these species is not yet clear. Possibly branched fatty acids protect the integument against pathogenic soil microorganisms – a hypothesis which has generally been stressed for skin lipids since many of them exhibit bacteriostatic and/or fungistatic properties. It should also been mentioned that (ω-1)- and (ω-2)-methyl-substituted alcohols and acids which are major constituents of the bovine and human meibomian secretion have been claimed to play an important role as a hydrophobic barrier to prevent water loss from the tear film which covers the eye [15, 16].

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