Cast Skin Lipids of the Indian Python (*Python molurus bivittatus*, Kühl, 1820)

Jürgen Jacob, Britt Ziemsen
Biochemisches Institut für Umweltcarcinogene, Lurup 4,
D-W-2070 Großhansdorf, Bundesrepublik Deutschland
and
Udo Hoppe
Paul Gerson Unna-Forschungszentrum, Beiersdorf AG,
D-W-2000 Hamburg, Bundesrepublik Deutschland

Z. Naturforsch. **48c**, 80–84 (1993); received September 28, 1992

Skin Lipids, Cast Skin, Snakes, Sterols, Secondary Alcohols

Cast skins of various snakes and in addition one lizard were found to contain 3.5–8.6% extractable lipid material. Lipids obtained from a cast skin of the Indian python were analyzed in detail indicating the presence of hydrocarbons (squalene, cholestadiene and alkanes), monoester waxes, sterol esters, diester waxes, triglycerides, sterols, free fatty acids and even more polar lipids. Among the monoester wax constituents odd-numbered secondary alkanols were found. It is assumed that the above lipids originate from cells of the past integumental generation and that they play an important facilitating role during the sloughing process.

Introduction

Although integumental lipids of various animal classes such as insects [1], mammals [2] or birds [3] have been more or less thoroughly investigated only little is known on the skin lipids of reptiles. A few attempts have been undertaken to analyze lipids from cast snake skin [4–7] indicating the presence of various lipid classes such as hydrocarbons, sterol esters, triglycerides, free alcohols, sterols and fatty acids, but also more polar lipids (phosphatidylethanolamine, -serine, -choline and sphingomyeline). For the red-sided garter snake (*Thamnophis sirtalis parietalis*) both a sex dependence and seasonal variation of the skin lipids obtained by solvent soaking the animals have been demonstrated by Mason *et al.* [8].

In our study the lipids of the cast snake skin from a male Indian python have been analyzed in more detail and evidence is presented that these lipids originate from the inner part of the cast formed during the shedding process.

Materials and Methods

Cast skins of various species as indicated in Table I were separately immersed in chloroform (40 ml) for 20 min after which methanol (20 ml) was added. After another 20 min the extract was evaporated and resulted the crude lipids. Their preliminary characterization was achieved by thin-layer chromatography on silica-coated plates (E. Merck) using the following solvent mixtures as mobile phase: (a) tetrachloromethane/chloroform (2:1; v/v); (b) chloroform; (c) chloroform/methanol (9:1; v/v), and (d) chloroform/methanol/water (13:5:0.8; v/v/v) which allow a good separation of either apolar and polar lipids.

The crude lipids of the python skin (male, 2.80 m in length) were separated into differently polar classes by column chromatography on silica (20 g; WOELM; 9.1% water content). Hydrocarbons were eluted with cyclohexane (280 ml), monoester waxes with cyclohexane/toluene (9:1; v/v; 400 ml), a diester wax fraction with cyclohexane/toluene (1:1; v/v; 240 ml), triglycerides with chloroform/toluene (1:9; v/v; 200 ml), alcohols and sterols along with free fatty acids with chloroform/toluene (1:2; v/v; 200 ml) and more polar lipids with chloroform/methanol (1:1; v/v; 100 ml). Free fatty acids were separated from the alcohols and sterols by alkaline extraction with methanolic NaOH. Monoester waxes, diester waxes and triglycerides were reesterified with 5% methanolic HCl and the products purified by silica column chromatography. Free fatty acids were esterified the same way. Alcohols were (a) converted into trimethylsilyl ethers by treatment with Trisil

Reprint requests to Prof. Dr. Jürgen Jacob.

Verlag der Zeitschrift für Naturforschung,
D-W-7400 Tübingen
0939–5075/93/0100–0080 $ 01.30/0

(N,O-bis-trimethylsilyl-trifluoroacetamide) and (b) oxidized with CrO_3 in tert.-butanol to give fatty acids or ketones.

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 25 m or 50 m glass capillaries coated with CP sil 5 preferentially 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. Alternatively, a NERMAG R-10-10 quadrupole instrument operating at 70 eV equipped with a gas chromatograph Delsi 700 has been used.

Results

For a general comparison the cast skin of various species were extracted to determine their lipid content. As indicated in Table I the latter varies between 3.5% and 8.6%.

Table I. Content of extractable lipids from various cast skins (in % of fresh material).

Species	Lipid material [%]
Python reticulatus (reticulated python)	4.1
2nd individual	3.6
Python molurus bivittatus (Indian python)	8.6
Lampropeltis zonata parvirubra (king snake)	3.6
Lampropeltis mexicana (Mexican king snake)	6.6
Lampropeltis spec. (king snake)	6.0
Elaphe situla (leopard snake)	6.0
Elaphe guttata (corn snake)	3.6
Elaphe longissima (Aesculapian snake)	4.4
Thamnophis sirtalis parietalis (red-sided garter snake)	4.5
2nd individual	4.0
Boaedon olivaceus (house snake)	4.6
Telescopus fallax (cat snake)	4.2
Natrix natrix (grass snake)	6.9
Lacerta lepida (eyed lizard)	3.5

Lipid classes

As determined by thin-layer chromatography the lipid composition was complex in all samples analyzed indicating the presence of hydrocarbons, mono- and diester waxes, triglycerides, free alcohols and sterols, free fatty acids, mono- and diglycerides and more polar lipids such as phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine.

In case of the Indian python lipids were separated into seven classes the percentages of which are presented in Table II.

Table II. Percentages and absolute amounts of various lipids obtained by solvent extraction from the cast skin of the Indian python.

Lipid	mg	% of all lipids
Hydrocarbons	3.3	0.7
Monoester waxes	23.6	5.0
Diester waxes	4.7	1.0
Triglycerides	146.3	31.0
Sterols	171.9	36.4
Free fatty acids	29.4	6.2
Polar lipids	93.0	19.7
Total	472.2	100.0

Hydrocarbons

The small hydrocarbon fraction (0.7% of all lipids) mainly consisted of squalene and cholesta-3,5-dien identified by their mass spectra (M = 410 and 368) and their GC retention times when compared with authentic reference standards. Apart from unbranched preferentially odd-numbered alkanes small amounts of hydrocarbons were detected the equivalent chain lengths of which suggest the presence of at least five other homologous series (mono-, di-, tri- and phenyl-substituted alkanes as well as alkenes). The composition of the hydrocarbon fraction is summarized in Table III.

Monoester waxes

Methanolysis of this fraction resulted in fatty acid methyl esters and a polar fraction consisting of sterols, primary and secondary alcohols. While mass spectra of the free primary and secondary alcohols are less characteristic their TMSO derivatives can be readily interpreted. Trimethylsilyl

Table III. Composition of the hydrocarbon fraction from the cast skin lipids of the Indian python (in per cent of the total fraction as detected by GC).

Hydrocarbon	%
Squalene	33.7
Cholesta-3,5-dien	43.8
n-Alkanes (unbranched)	
n-C_{14}	0.2
n-C_{15}	0.4
n-C_{16}	0.8
n-C_{17}	1.1
n-C_{18}	0.9
n-C_{19}	0.9
n-C_{20}	0.6
n-C_{21}	0.4
n-C_{22}	0.3
n-C_{23}	0.3
n-C_{24}	0.5
n-C_{25}	0.5
n-C_{26}	0.4
n-C_{27}	0.6
n-C_{28}	0.7
n-C_{29}	1.3
n-C_{30}	0.7
n-C_{31}	1.0
Total	(11.6)
Alkanes (substituted)*	
Monomethyl-substituted	3.0
Dimethyl-substituted	2.4
Trimethyl-substituted	3.3
Phenyl-substituted	1.0
Alkenes*	1.2
Total	(10.9)

* Tentatively identified based on the equivalent chain lengths.

Table IV. Composition of the monoester wax constituents from the cast skin of the Indian python, in per cent of the total fraction as detected by GC.

Fatty acids	%	Alcohols	%
Saturated (total)	(85.9)	Primary alcohols (total)	(16.2)
n-C_{14}	0.1	*n*-C_{16}	0.2
n-C_{16}	1.7	*n*-C_{17}	0.2
n-C_{17}	0.1	*n*-C_{18}	0.2
n-C_{18}	6.0	*n*-C_{19}	0.1
n-C_{20}	6.0	*n*-C_{20}	0.2
n-C_{21}	0.4	*n*-C_{21}	0.3
n-C_{22}	9.7	*n*-C_{22}	10.2
n-C_{23}	0.2	*n*-C_{23}	0.5
n-C_{24}	7.5	*n*-C_{24}	1.9
n-C_{25}	0.5	*n*-C_{25}	0.2
n-C_{26}	14.9	*n*-C_{26}	0.8
n-C_{27}	0.3	*n*-C_{28}	1.4
n-C_{28}	9.8	Secondary alcohols (total)	(33.7)
n-C_{29}	0.6	*n*-C_{21}	2.4
n-C_{30}	16.1	*n*-C_{23}	5.1
n-C_{32}	9.2	*n*-C_{24}	0.3
n-C_{34}	2.8	*n*-C_{25}	11.6
Unsaturated (total)	(11.4)	*n*-C_{26}	0.3
n-$C_{16:1}$	0.2	*n*-C_{27}	7.5
n-$C_{18:1(a)}$*	5.1	*n*-C_{29}	3.8
n-$C_{18:1(b)}$*	0.6	*n*-C_{31}	2.7
n-$C_{18:2}$	1.5	Sterols (total)	(41.9)
n-$C_{20:1}$	0.3	Cholesterol	20.6
n-$C_{22:1}$	0.6	Cholestanol	17.5
n-$C_{24:1}$	0.3	Ergostanol	0.7
n-$C_{25:1}$	0.1	Ergostenol	0.9
n-$C_{26:1}$	0.2	β-Sitosterol	0.8
n-$C_{28:1}$	0.3	Stigmastanol	1.4
n-$C_{30:1}$	1.5	Unidentified	(8.2)
n-$C_{32:1}$	0.7		
Unidentified	(2.7)		

* Tentatively identified as 9- (a) and 11-isomer (b).

ethers of primary alcohols exhibit an intense (M-15) fragment whereas those of secondary ones may be identified by the intense *m/z* 117 formed by the cleavage next to the substituent which eliminates the fragment TMS−O−CH−CH_3.

Apart from this key fragment a molecular ion (M^+) and a fragment (M-15) are present though occurring in weak intensity. The mass spectra of the sterols detected were identical with those of authentic standards and also exhibited the same relative GC retention times. The constituents of the monoester waxes are listed in Table IV. Even-numbered individuals predominate in both, fatty acids and the primary alcohols, whereas odd-numbered are the main constituents of the secondary alcohols.

Diester waxes

After methanolysis of the very small diester wax fraction fatty acid methyl esters, alcohols, sterols and small amounts of 2-hydroxy fatty acid methyl esters were found, though only the fatty acids were quantified (Table V).

Triglycerides

The triglyceride acids consisted predominantly of stearic and eicosanoic acid and minor amounts of even-numbered higher homologues. The pattern differs markedly from that found in case of the monoester waxes.

Table V. Composition of the fatty acids resulting from methanolysis of the diester waxes, triglycerides and free fatty acids from the cast skin lipids of the Indian python, in per cent of each fraction as determined by GC.

	Diester waxes	Fatty acid from triglycerides	Free fatty acids
Saturated (total)	(87.3)	(99.6)	(38.0)
n-C_{14}	0.5	0.4	–
n-C_{15}	0.3	0.1	–
n-C_{16}	13.6	5.6	14.6
n-C_{17}	0.8	0.2	3.3
n-C_{18}	17.0	51.7	16.7
n-C_{19}	0.2	0.3	–
n-C_{20}	9.1	21.2	1.1
n-C_{21}	0.4	0.2	–
n-C_{22}	7.8	8.8	2.3
n-C_{23}	0.2	0.3	–
n-C_{24}	7.3	5.4	–
n-C_{25}	0.3	0.2	–
n-C_{26}	12.2	1.7	–
n-C_{27}	0.2	0.1	–
n-C_{28}	6.1	0.7	–
n-C_{29}	0.1	0.1	–
n-C_{30}	7.4	0.9	–
n-C_{32}	3.0	0.9	–
n-C_{34}	0.8	0.2	–
n-C_{36}	–	0.6	–
Unsaturated (total)	(12.7)	(0.4)	(62.0)
n-$C_{18:1}$	8.5	0.4	60.1
n-$C_{20:1}$	–	–	0.3
n-$C_{22:1}$	–	–	1.6
n-$C_{18:2}$	4.2	–	–

Free fatty acids

This fraction mainly contained octadecenoic (60.1%), palmitic (14.6%) and stearic acid (16.7%). The pattern resembles that found in epidermal fat tissue of many vertebrates.

Sterols

The main fraction of the cast skin lipids was found to consist of sterols the composition of which is listed in Table VI. Two series of homologues were detected, one deriving from cholesterol and the other from its dihydro derivative 5α-cholestan-3β-ol. In addition, Table VI presents the main mass spectral fragments detected, the molecular weight and the GC retention time relative to *n*-alkanols (eicosanol = 20.00). No free alkanols were detected in this fraction.

Polar lipids

This fraction has not been further separated, phosphatidylserine, -ethanolamine and -choline, however, could be definitely characterized by thin-layer chromatography.

Discussion

Snakes shed their skin in regular intervals depending on size, age and optimal living conditions. The morphology of this process has been studied by Maderson [9], Maderson *et al.* [10] and Landmann [11, 12]. The skin of the snake is arbitrary separated into six layers ("Oberhäutchen", β-, mesos-, α-, lacunary and clear layer) which are renewed within the following shedding cycle. During the shedding process the mature outer generation – the cast – is striped off. The two generations cohere by a lobular gearing of the old clear layer with the new "Oberhäutchen". Near the end of the renewal phase the cells of the clear layer become horny and those of the β-layer loose their membranes, whereas the lacunary layer shrinks in this phase. It may be assumed that this process as well as the presence of cellular lipids released during

Table VI. Composition of the free sterols from the cast skin lipids of the Indian python, in per cent of the total fraction as determined by GC.

Sterol	Mole weight	Masses detected	ECL*	%
Cholesterol	386	386; 371; 368; 353; 301; 275; 255; 247; 231; 213	27.65	66.7
5α-Cholestan-3β-ol	388	388; 373; 370; 355; 262; 248; 233; 217; 215; 165	27.85	26.5
Desmosterol (24-Dehydrocholesterol)	384	384; 369; 351; 300; 271; 253; 213	28.00	0.3
Cholest-7-en-3β-ol or Cholest-5-en-3α-ol	386	386; 371; 353; 273; 255; 247; 231; 229; 213	28.05	0.4
Ergost-5-en-3β-ol	400	400; 385; 382; 367; 315; 289; 273; 255; 231; 213	28.60	1.4
Ergostan-3β-ol	402	402; 387; 369; 276; 248; 234; 233; 215; 165	28.65	1.5
Cholestadienol (5,7?)	384	poor spectrum	28.80	0.2
Stimast-5-en-3β-ol (β-Sitosterin)	414	414; 396; 381; 329; 303; 273; 255; 231; 229; 213	29.35	1.2
Stigmastanol (Dihydro-β-sitosterin)	416	416; 355; 276; 262; 230; 215; 165	29.45	1.8

* ECL = Equivalent chain length related to eicosanol = 20.00.

cornification of the clear layer essentially facilitate sloughing. These lipids almost exclusively originate from the old skin generation rather than from other integumental sources, which may be concluded from the fact that extracting the integumental surface of a python having shed several weeks ago yielded only 1 mg crude lipids (data not shown) – in spite of its large size (2.80 m in length). Furthermore, it is well known that integumental glands producing lipids are rare in snakes [13, 14]. Further investigations on the physical properties of the lipids extracted from the cast skin of the Indian python are presently underway.

Acknowledgement

The authors like to thank Dr. D. Vinyard for supplying them with the python skin cast and fruitful discussions.

[1] K. H. Lockey, Comp. Biochem. Physiol. **89B,** 595–645 (1988).
[2] D. T. Downing, Mammalian Waxes, in: Chemistry and Biochemistry of Natural Waxes (P. E. Kolattukudy, ed.), pp. 17–48, Elsevier, Amsterdam, Oxford, New York 1976.
[3] J. Jacob and V. Ziswiler, The Uropygial Gland, in: Avian Biology, **Vol. VI** (D. D. Farner, J. R. King, and K. C. Parkes, eds.), pp. 199–324, Academic Press, New York 1982.
[4] D. G. Ahern and D. T. Downing, Lipids **9,** 8–14 (1974).
[5] J. B. Roberts and H. B. Lillywhite, Science **207,** 1077–1079 (1980).
[6] C. S. Birkby, P. W. Wertz, and D. T. Downing, Comp. Biochem. Physiol. **73B,** 239–242 (1982).
[7] R. R. Burken, P. W. Wertz, and D. T. Downing, Comp. Biochem. Physiol. **81B,** 315–318 (1985).
[8] R. T. Mason, J. W. Chinn, and D. Crews, Comp. Biochem. Physiol. **87B,** 999–1003 (1987).
[9] P. F. A. Maderson, J. Zool. **146,** 98–113 (1965).
[10] P. F. A. Maderson, B. A. Flaxman, S. I. Roth, and G. Szábo, J. Morph. **136,** 191–210 (1972).
[11] L. Landmann, J. Morph. **162,** 93–126 (1979).
[12] L. Landmann, The Skin of Reptiles: Epidermis and Dermis, in: Biology of the Integument II (Vertebrates) (J. Bereiter-Hahn, A. G. Matoltsy, and K. S. Richards, eds.), pp. 150–187, Springer Verlag, Berlin, Heidelberg, New York, Tokyo 1986.
[13] O. von Wettstein, Die Haut der Reptilien, in: Studium generale, pp. 335–349 (1964).
[14] D. Vinyard, Die Präanal- und Femoralorgane der Echsen als Sonderbildungen der Squamatenepidermis – ein Überblick über ihre Morphologie, Biochemie und Funktion, Dissertation, 328 p., Hamburg 1990.