Chemical composition of uropygial gland secretions of owls

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Abstract The compositions of the uropygial gland secretions of the long-eared owl, eagle owl, and barn owl have been determined. The waxes of the first two owls, which are closely related, are composed of 2-alkyl-substituted fatty acids and *n*- or monomethyl-branched alcohols with even-numbered branching positions. In addition, some dimethyl-substituted alkanols were observed. In contrast to these waxes, the secretion of the barn owl is composed of 3-methyl- and 3,5-, 3,7-, 3,9-, 3,11-, 3,13-, and 3,15-dimethyl-branched fatty acids and *n*- as well as monomethyl-substituted alkanols branched at positions 2, 3, and 4. The mass spectra of esters of 2-alkyl-substituted fatty acids are discussed.

Supplementary key words long-eared owl · eagle owl · barn owl · 2-alkyl fatty acids · 2-ethyl fatty acids · 2-propyl fatty acids · 2-butyl fatty acids · branched fatty acids · chemotaxonomy · birds

Among the many investigations of preen gland fats, there is only one relatively old paper dealing with the uropygial gland secretion of owls (1). The authors investigated the preen waxes of several birds by thin-layer chromatography, two of which were species of the order Strigiformes, namely the Tengmalm's owl (*Aegolius funereus*) and the long-eared owl (*Asio otus*). No further attempt was made to elucidate the structures of these waxes.

In several investigations we have been able to demonstrate a certain relationship between the composition of uropygial gland secretion and the position of birds in the natural system, leading to a chemotaxonomy of birds (2–8). We therefore were interested in investigating the preen wax composition of owls. The present paper deals with the waxes from three species of the order Strigiformes: long-eared owl (*Asio otus*), eagle owl (*Bubo bubo*), and barn owl (*Tyto alba*).

Abbreviations: GLC, gas–liquid chromatography; MS, mass spectrometry; ECL, equivalent chain length; DEGS, diethylene glycol succinate.

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MATERIALS AND METHODS

The uropygial glands of the species investigated were extracted with chloroform–methanol 2:1; after addition of water the chloroform phase was cautiously evaporated. The crude lipids were fractionated by column chromatography (9) (silica gel Woelm, 9% water content), and pure waxes were eluted with cyclohexane–benzene 9:1 (98 mg, *A. otus;* 325 mg, *B. bubo;* 33 mg, *T. alba*). The waxes were subjected to methanolysis, and the methyl esters and alcohols were purified by column chromatography. The

TABLE 1. Fatty acid composition of the uropygial gland wax from the long-eared owl (*Asio otus*)

Fatty Acid	%
2-Ethyldecanoic	0.1
-undecanoic	0.1
-dodecanoic	0.4
-tridecanoic	0.3
-tetradecanoic	4.7
-pentadecanoic	1.9
-hexadecanoic	14.1
-heptadecanoic	1.2
-octadecanoic	1.6
2-Ethyl-substituted fatty acids, total	(24.4)
2-Propyldecanoic	0.2
-undecanoic	0.1
-dodecanoic	1.1
-tridecanoic	0.1
-tetradecanoic	3.2
-pentadecanoic	0.8
-hexadecanoic	4.0
-heptadecanoic	0.5
2-Propyl-substituted fatty acids, total	(10.0)
2-Butyloctanoic	0.1
-nonanoic	0.2
-decanoic	3.1
-undecanoic	1.3
-dodecanoic	9.6
-tridecanoic	3.0
-tetradecanoic	18.4
-pentadecanoic	4.0
-hexadecanoic	15.9
2-Butyl-substituted fatty acids, total	(55.6)
Unidentified	10.0

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TABLE 2. Fatty acid composition of the uropygial gland wax from the eagle owl (*Bubo bubo*)

Fatty Acid	%
n-Tetradecanoic	0.1
n-Hexadecanoic	1.9
n-Octadecanoic	0.6
(Total)	(2.6)
2-Methylheptadecanoic	0.1
-octadecanoic	0.4
-eicosanoic	0.6
-docosanoic	0.6
(Total)	(1.7)
2-Ethyltetradecanoic	0.2
-hexadecanoic	3.5
-heptadecanoic	0.6
-octadecanoic	12.2
-nonadecanoic	1.5
-eicosanoic	16.2
-heneicosanoic	0.6
-docosanoic	7.0
-tricosanoic	0.5
(Total)	(42.3)
2,6-Dimethyloctadecanoic	0.6
-nonadecanoic	0.7
-eicosanoic	4.6
-heneicosanoic	2.8
-docosanoic	4.3
(Total)	(13.0)
2,8-Dimethyloctadecanoic	0.5
-nonadecanoic	0.7
-eicosanoic	4.5
-heneicosanoic	2.7
-docosanoic	4.4
(Total)	(12.8)
2,10-Dimethylnonadecanoic	0.7
(Total)	(0.7)
2-Ethyl-6-methylhexadecanoic	0.4
-octadecanoic	1.0
-nonadecanoic	0.6
-eicosanoic	6.7
-heneicosanoic	1.2
(Total)	(9.9)
2,6,12-Trimethylheneicosanoic	1.2
2,6,14-Trimethylheneicosanoic	1.3
(Total)	(2.5)
Unidentified	14.5

TABLE 3. Fatty acid composition of the uropygial gland wax from the barn owl (*Tyto alba*)

Fatty Acid	%
3-Methylundecanoic	1.1
-dodecanoic	2.9
-tridecanoic	11.7
-tetradecanoic	9.2
-pentadecanoic	16.4
-hexadecanoic	9.8
-heptadecanoic	8.2
(Total)	(59.3)
3,5-Dimethyltetradecanoic	0.3
-pentadecanoic	0.2
-hexadecanoic	0.8
-heptadecanoic	0.2
(Total)	(1.5)
3,7-Dimethylundecanoic	0.2
-dodecanoic	0.6
-tridecanoic	1.9
-tetradecanoic	3.1
-pentadecanoic	3.1
-hexadecanoic	1.1
-heptadecanoic	0.2
(Total)	(10.2)
3,9-Dimethylundecanoic	0.4
-dodecanoic	0.3
-tridecanoic	1.0
-tetradecanoic	0.1
-pentadecanoic	3.1
-hexadecanoic	1.1
-heptadecanoic	0.2
(Total)	(6.2)
3,11-Dimethyltridecanoic	3.5
-tetradecanoic	2.0
-pentadecanoic	3.8
-hexadecanoic	1.6
-heptadecanoic	1.0
(Total)	(11.9)
3,13-Dimethylpentadecanoic	5.4
-hexadecanoic	1.9
-heptadecanoic	1.2
(Total)	(8.5)
3,15-Dimethylheptadecanoic	1.4
3,9,11-Trimethylpentadecanoic	1.0

yields of methyl esters and alcohols from *A. otus, B. bubo,* and *T. alba* were 45.3 and 51.9 mg, 36.1 and 29.7 mg, and 17.1 and 15.7 mg, respectively. In the case of *B. bubo,* only 20% of the purified waxes was used for methanolysis. It should be noted that the reesterification reaction is very slow and takes about 10 hr. The alcohols were oxidized by CrO_3 in *t*-butanol–acetic acid–hexane as described previously (10). Only freshly distilled solvents were used. The conditions for MS and GLC were described in a previous paper (7).

Authentic samples of ethyl 2-butyl and 2-ethyl dodecanoates were prepared by condensation of sodium ethyl- or butylmalonic diethyl ester with 1-bromodecane, followed by alkaline hydrolysis and decarboxylation. They were reesterified with 5% methanolic HCl to yield the methyl esters.

RESULTS

The compositions of the uropygial waxes from the owls were determined by GLC after methanolysis with 10% methanolic HCl and are given in Tables 1–5.

All components were identified by comparison of their retention times (or ECL values) on two GLC columns of different polarities (a 9-m glass column with 3% OV-101 on Gas-Chrom Q at 200°C and a 3-m glass column with 5% DEGS on Celite at 185°C; instrument, Perkin-Elmer F-7) and by mass spectrometry (Varian MAT 111 GNOM). The mass spectra of methyl esters of unbranched, 2-, 3-, 4-, 6-, 8-, 10-, 12-monomethyl-, 2,6-, 2,8-, 2,10-, 3,5-, 3,7-, 4,6-, 4,8-dimethyl-, and 2,6,12-trimethyl-substituted fatty acids are well known (11–15).

The spectra of the methyl esters of 2,16-, 2,18-, 3,9-, 3,11-, 3,13-, and 3,15-dimethyl-substituted fatty acids as well as those of 4,14-, 4,16-dimethyl-, and 4,6,14-tri-

TABLE 4. Alcohol compositions[a] of the uropygial gland waxes from the long-eared and eagle owls

Fatty Acid	*Asio otus*	*Bubo bubo*
	%	
n-Tetradecanoic	0.1	
n-Pentadecanoic	0.3	
n-Hexadecanoic	5.5	0.5
n-Heptadecanoic	5.3	0.8
n-Octadecanoic	13.4	9.4
n-Nonadecanoic	1.5	1.0
n-Eicosanoic	2.7	8.2
n-Heneicosanoic		
n-Docosanoic		1.8
n-Tricosanoic		1.3
(Total)	(28.8)	(23.0)
2-Methylhexadecanoic	1.0	
-heptadecanoic	0.9	0.1
-octadecanoic	1.0	3.5
-nonadecanoic	0.3	0.6
-eicosanoic		9.7
(Total)	(3.2)	(13.9)
4-Methyloctadecanoic	3.9	0.3
6-Methylhexadecanoic		0.1
-heptadecanoic	0.1	
-octadecanoic	3.6	
-nonadecanoic	4.0	0.9
-eicosanoic	9.0	
-heneicosanoic	1.5	
-docosanoic	2.7	
(Total)	(20.9)	(1.0)
8-Methylhexadecanoic	3.6	
-nonadecanoic	0.5	
(Total)	(4.1)	
10-Methylhexadecanoic	2.5	0.2
-heptadecanoic	2.1	0.4
-octadecanoic	3.9	0.1
-nonadecanoic	0.5	0.6
-eicosanoic	9.1	0.1
-heneicosanoic	1.5	8.7
-docosanoic	2.7	13.7
-tricosanoic		4.0
-tetracosanoic		4.2
(Total)	(22.3)	(32.0)
12-Methylhexadecanoic	2.5	0.1
-heptadecanoic	1.2	0.2
-octadecanoic	0.4	
-nonadecanoic	1.6	
(Total)	(5.7)	(0.3)
2-Butyltetradecanoic	0.8	
-hexadecanoic	4.6	
(Total)	(5.4)	
2,6-Dimethyleicosanoic		1.0
-docosanoic		1.0
(Total)		(2.0)
2,8-Dimethylnonadecanoic		0.6
-eicosanoic		1.0
-heneicosanoic		0.7
-docosanoic		1.0
(Total)		(3.3)
2,10-Dimethyloctadecanoic		1.3
-nonadecanoic		0.6
-eicosanoic		8.5
-heneicosanoic		2.7
-docosanoic		6.1
-tricosanoic		1.1
-tetracosanoic		1.2
(Total)		(21.5)
2,16-Dimethyloctadecanoic		0.8
2,18-Dimethyleicosanoic		1.9
Unidentified	5.7	

[a] Determined as fatty acids after oxidation with CrO_3.

TABLE 5. Alcohol composition[a] of the uropygial gland wax from the barn owl (*Tyto alba*)

Fatty Acid	%
n-Decanoic	0.1
n-Undecanoic	0.3
n-Dodecanoic	0.8
n-Tridecanoic	1.0
n-Tetradecanoic	0.9
n-Pentadecanoic	0.6
n-Hexadecanoic	tr
n-Heptadecanoic	1.3
(Total)	(5.0)
2-Methylundecanoic	tr
-tridecanoic	tr
-tetradecanoic	0.1
-pentadecanoic	tr
-hexadecanoic	0.1
-heptadecanoic	10.2
-octadecanoic	1.3
(Total)	(11.7)
3-Methyldecanoic	0.3
-undecanoic	2.9
-dodecanoic	tr
-tridecanoic	6.3
-tetradecanoic	5.2
-pentadecanoic	12.9
-hexadecanoic	7.9
(Total)	(35.5)
4-Methyldodecanoic	3.2
-tetradecanoic	tr
-hexadecanoic	1.6
-octadecanoic	6.2
-nonadecanoic	1.2
(Total)	(12.2)
3,7-Dimethyldodecanoic	0.1
-tridecanoic	0.2
-tetradecanoic	0.6
-pentadecanoic	0.4
-hexadecanoic	tr
(Total)	(1.3)
3,9-Dimethyldodecanoic	0.1
-tridecanoic	0.1
-tetradecanoic	0.6
-pentadecanoic	0.4
-hexadecanoic	tr
(Total)	(1.2)
3,11-Dimethyltridecanoic	0.6
-pentadecanoic	2.4
(Total)	(3.0)
3,13-Dimethylpentadecanoic	2.6
-heptadecanoic	2.2
(Total)	(4.8)
3,15-Dimethylheptadecanoic	2.9
4,6-Dimethylhexadecanoic	1.8
-heptadecanoic	0.9
-octadecanoic	0.3
-nonadecanoic	1.0
(Total)	(4.0)
4,8-Dimethylhexadecanoic	1.8
-heptadecanoic	2.2
-octadecanoic	1.3
-nonadecanoic	0.5
(Total)	(5.8)
4,14-Dimethylhexadecanoic	0.6
-octadecanoic	2.0
-nonadecanoic	0.5
(Total)	(3.1)
4,16-Dimethyloctadecanoic	4.0
4,6,14-Trimethylhexadecanoic	0.6
Unidentified	4.9

[a] Determined as fatty acids after oxidation with CrO_3.

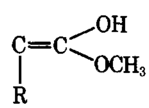
TABLE 6. Typical mass spectrometric fragments of some esters of fatty acids occurring in uropygial gland waxes of owls

Branching Position	McLafferty Ion	$\overset{\oplus}{C}H(CH_3)-(CH_2)_n-CH(CH_3)-(CH_2)_m-COOCH_3$ (=X)	X − CH_3OH	X − CH_3OH − H_2O	Other Typical Ions
2,16	88	297 (m = 0, n = 13)	265	247	
2,18	88	325 (m = 0, n = 15)	293	275	
3,9	74	199 (m = 1, n = 5)	167	149	87 ≪ 74, 125
3,11	74	227 (m = 1, n = 7)	195	177	87 ≪ 74, 153
3,13	74	255 (m = 1, n = 9)	223	205	87 ≪ 74, 181
3,15	74	283 (m = 1, n = 11)	251	233	87 ≪ 74, 209
4,14	74	269 (m = 2, n = 9)	237	219	74 < 87, M − 73
4,16	74	297 (m = 2, n = 11)	265	247	74 < 87, M − 73
3,9,11	74	199 (m = 1, n = 5)	167	149	87 ≪ 74
		241 (m = 1, n = 5, o = 1)[a]	209	191	
4,6,14	74	157 (m = 2, n = 1)	125	(107)	74 < 87, M − 76
		283 (m = 2, n = 1, o = 7)[a]	251	233	M − 73
2-Ethyl	102				M − 59, M − 28
2-Propyl	116				M − 59, M − 42
2-Butyl	130				M − 59, M − 56

[a] With an additional branch according to the formula: $\overset{\oplus}{C}H(CH_3)-(CH_2)_o-CH(CH_3)-(CH_2)_n-CH(CH_3)-(CH_2)_m-COOCH_3$

methyl-branched homologs are analogous to the spectra of the above-mentioned esters and will not be discussed. Their typical mass spectroscopic fragmentations together with those of the compounds discussed below are in Table 6.

The mass spectra of 2-ethyl-, 2-propyl-, and 2-butyl-branched esters are given in Figs. 1–3. Synthetic samples of these compounds gave spectra identical with those of the naturally occurring wax esters. In all three spectra a typical even-numbered ion, 102, 116, or 130, respectively, occurs according to the McLafferty rearrangement ion of the following structure:



R = C_2H_5 (⟶ 102)
= C_3H_7 (⟶ 116)
= C_4H_9 (⟶ 130)

The fragment M − 28 (elimination of ethylene) is very useful in differentiating between 2-ethyl- and 2,2-dimethyl-branched compounds. This fragment is absent in the spectrum of methyl 2,2-dimethyl octanoate recorded by us under the same MS conditions (80 eV). Analogous ions are observed in the mass spectra of propyl- and butyl-substituted esters (M − 42 and M − 56). The substitution at position 2 can also be seen from the M − 59 ion, representing the loss of $—COOCH_3$.

TABLE 7. Occurrence of different types of fatty acids and alcohols in uropygial gland waxes of owls

Fatty Acids	*Asio otus*	*Bubo bubo*	*Tyto alba*
Fatty acids			
Unbranched		−	
2-Methyl-branched		−	
2,*x*-Dimethyl-branched		++	
2-Ethyl-branched	++	+++	
2-Ethyl-*x*-methyl-branched		+	
2-Propyl-branched	+		
2-Butyl-branched	+++		
2,*x*,*y*-Trimethyl-branched		−	
3-Methyl-branched			+++
3,*x*-Dimethyl-branched			+++
3,*x*,*y*-Trimethyl-branched			−
Alcohols			
Unbranched	++	++	+
2-Methyl-branched	−	+	+
4-Methyl-branched	−	−	+
Other monomethyl-branched (6-, 8-, 10-, 12-)	+++	+++	
2-Butyl-branched	+		
2,*x*-Dimethyl-branched		+++	
3-Methyl-branched			+++
3,*x*-Dimethyl-branched			+
4,*x*-Dimethyl-branched			+
4,*x*,*y*-Trimethyl-branched			−

Symbols: −, 0–5%; +, 5–20%, ++, 20–30%; +++, >30%.

DISCUSSION

The uropygial gland waxes of the three owls investigated have very strange compositions. In two cases, 2-alkyl-substituted fatty acids were observed, of which 2-propyl- and 2-butyl-substituted fatty acids were heretofore unknown as naturally occurring compounds. 2-Methyl-branched fatty acids have been shown to be common in uropygial waxes. Esterified with n-alkanols, they have been discussed as chemotaxonomically relevant substances in the family Corvidae of the order Passeriformes (7, 16); furthermore, they are common in the order Anseriformes,

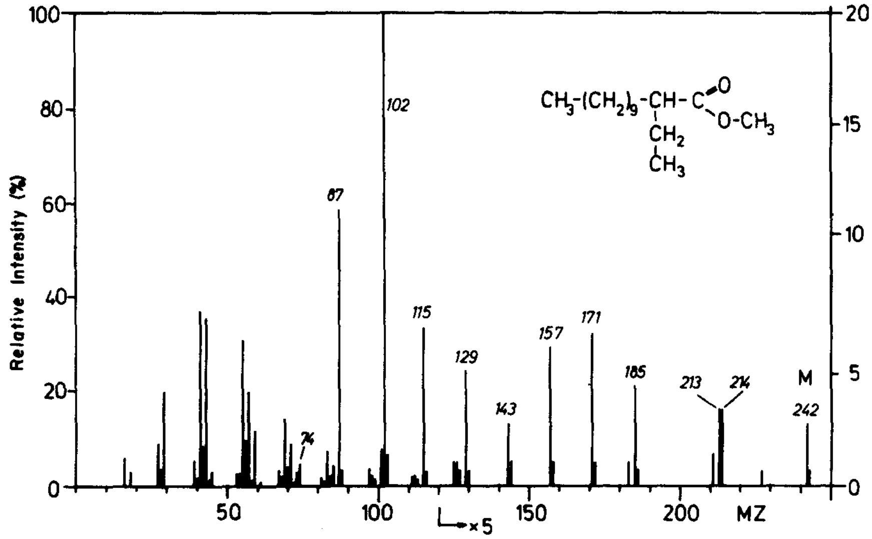


Fig. 1. Mass spectrum of methyl 2-ethyl dodecanoate.

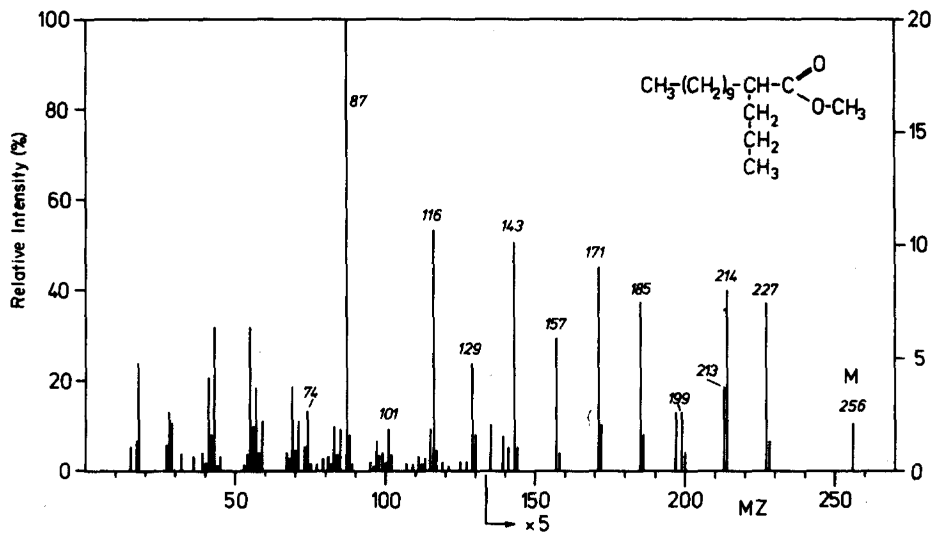


Fig. 2. Mass spectrum of methyl 2-propyl dodecanoate.

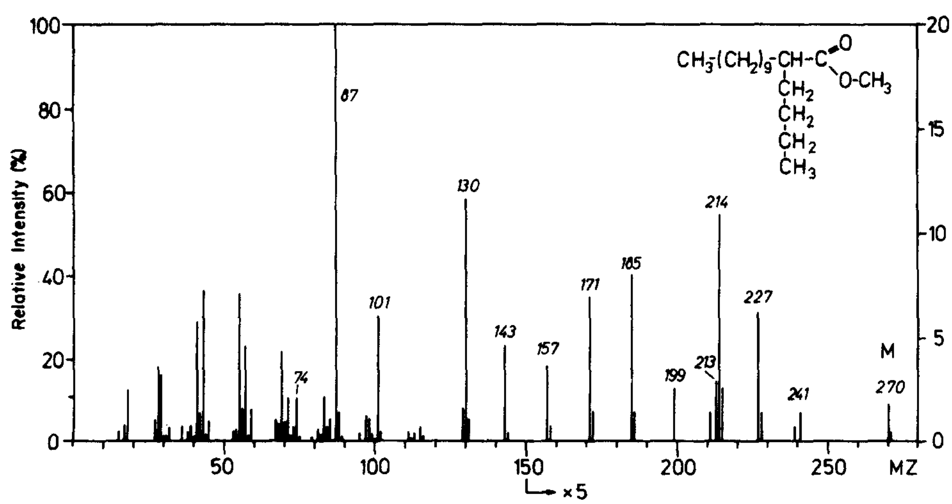


Fig. 3. Mass spectrum of methyl 2-butyl dodecanoate.

generally accompanied by 2,x-dimethyl- and 2,x,y-trimethyl-substituted fatty acids.

Since 2-ethyl-substituted fatty acids occur in the preen waxes of long-eared and eagle owls and also in that of the tawny owl (*Strix aluco*) (as a casual investigation showed), higher 2-alkyl-branched fatty acids can be considered as predominant constituents of preen secretions of owls. It should be mentioned, however, that we have now isolated very similar acids from the preen wax of tits (Paridae). Although nothing is known about the biosynthesis of these acids, butyryl-, valeroyl-, and caproyl CoAs perhaps could be precursors via carboxylation to ethyl-, propyl-, and butylmalonyl CoAs, respectively.

Table 7 shows the qualitative composition of the waxes investigated. As can be seen, the barn owl (*Tyto alba*) differs significantly from the other owls by producing mainly 3-methyl-branched compounds in contrast to the other owl waxes, which have alkyl branches at position 2. This agrees very well with the opinion of zoologists that *Tyto alba* is not in the same family as *Asio otus* and *Bubo bubo*. The wax type observed in *T. alba* is closely related to the waxes of the cuckoo (5) and several passeriforms (e.g., Fringillidae, Emberizidae) (2, 10, 17). This relationship is still not well understood.

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REFERENCES

1. Haahti, E., K. Lagerspetz, T. Nikkari, and H. M. Fales. 1964. Lipids of the uropygial gland of birds. *Comp. Biochem. Physiol.* **12:** 435–437.
2. Jacob, J., and A. Zeman. 1971. Über Bürzeldrüsensekrete von Finkenvögeln. Vergleichende Untersuchung der Bürzellipide vom Grunfink (*Carduelis chloris*), Gimpel (*Pyrrhula pyrrhula*) ünd Hänfling (*Carduelis cannabina*). *Z. Naturforsch.* **26b:** 1352–1356.
3. Zeman, A., and J. Jacob. 1972. Vergleichende Untersuchung der Bürzeldrüsenlipide von Möwen. Zusammensetzung der Bürzellipide der Lachmöwe (*Larus ridibundus*), Heringsmöwe (*Larus fuscus*) und Silbermöwe (*Larus argentatus*). *Z. Anal. Chem.* **261:** 306–309.
4. Jacob, J., and A. Zeman. 1972. Das Bürzeldrüsensekret der Ringeltaube (*Columba palumbus*). *Hoppe-Seyler's Z. Physiol. Chem.* **353:** 492–494.
5. Jacob, J., and J. Poltz. 1972. Das Bürzeldrüsensekret des Kuckucks (*Cuculus canorus*). *Hoppe-Seyler's Z. Physiol. Chem.* **353:** 1657–1660.
6. Jacob, J., and A. Zeman. 1973. Zur Chemotaxonomie der Alkenvögel. Die Zusammensetzung der Bürsellipide des Tordalks (*Alca torda*), der Trottel- und Ringellumme (*Uria aalge*) und der Gryllteiste (*Cepphus grylle*). *Z. Naturforsch.* **28c:** 78–82.
7. Jacob, J., and G. Grimmer. 1973. Zur Chemotaxonomie der Rabenvögel. Zusammensetzung der Bürzeldrüsensekrete der Dohle (*Coloeus monedula*), Raben- (*Corvus corone corone*) und Nebelkrähe (*Corvus corone cornix*). *Z. Naturforsch.* **28c:** 75–77.
8. Jacob, J., and J. Poltz. 1973. Chemotaxonomische Untersuchungen an Limikolen: Die Zusammensetzung des Bürzeldrüsensekretes von Austernfischer, Rotschenkel, Knutt und Alpenstrandläufer. *Biochem. Syst.* **1:** 169–172.
9. Jacob, J., and G. Grimmer. 1970. Das Bürzeldrüsensekret des Papageientauchers (*Fratercula arctica*). *Z. Naturforsch.* **25b:** 54–56.
10. Jacob, J., and A. Zeman. 1970. Die Bürzeldrüsenlipide des Haussperlings (*Passer domesticus*). *Z. Naturforsch.* **25b:** 984–988.
11. Ryhage, R., and E. Stenhagen. 1960. Mass spectrometric studies IV. Esters of monomethyl-substituted long chain carboxylic acids. *Ark. Kemi.* **15:** 291–315.
12. Karlsson, H., and G. Odham. 1969. Studies on feather waxes of birds VIII. The chemical composition of the wax in the free flowing secretion from the preen gland of the oystercatcher (*Haematopus ostralegus* L.). *Ark. Kemi.* **31:** 143–158.
13. Odham, G. 1965. Studies on feather waxes of birds III. The chemical composition of the wax in the free flowing secretion from the preen gland of the mute swan (*Cygnus olor*). *Ark. Kemi.* **23:** 431–451.
14. Jacob, J., and A. Zeman. 1971. Das Bürzeldrüsensekret des Eissturmvogels (*Fulmarus glacialis*). *Z. Naturforsch.* **26b:** 33–40.
15. Jacob, J., and A. Zeman. 1971. Das Bürzeldrüsensekret des Blässhuhns (*Fulica atra*). *Z. Naturforsch.* **26b:** 1344–1351.
16. Jacob, J., and A. Glaser. 1970. Das Bürzeldrüsensekret der Saatkrähe (*Corvus frugilegus*). *Z. Naturforsch.* **25b:** 1435–1437.
17. Poltz, J., and J. Jacob. 1974. Bürzeldrüsensekrete bei Ammern (Emberizidae), Finken (Fringillidae) und Webern (Ploceidae). *J. Ornithol.* In press.