Hydrocarbon and multibranched ester waxes from the uropygial gland secretion of grebes (Podicipediformes)

Jürgen Jacob[1]

Biochemisches Institut für Umweltcarcinogene, D-2070 Ahrensburg/Holst., West Germany

Abstract The uropygial gland secretion of some grebes (Podicipediformes) has been shown to contain saturated and unsaturated aliphatic hydrocarbons and monoester waxes. While ester waxes are common constituents of preen gland secretions, nonisoprenoid hydrocarbons have not been detected hitherto. The wax constituents are very complex, belonging to several multibranched homologous series, including unusual acids with ethyl branches. The waxes were identified by gas–liquid chromatography–mass spectrometry and equivalent chain length comparisons. A method for the prediction of equivalent chain length values of unknown methyl esters is offered. The results are discussed from a chemotaxonomic viewpoint.

Supplementary key words branched fatty acids · branched alcohols · GLC increments · chemotaxonomy

Instead of the numerous sebaceous glands occurring in the skin of mammals, birds possess a single, but large, gland of this type, the uropygial gland. Its secretion is spread all over the plumage by the bird's bill and prevents water penetration. From a number of investigations, however, it is obvious that we have to look for additional functions, and it seems that the bacteriostatic and fungistatic properties of preen wax constituents play a role in the protection of the bird's skin against microorganisms.

Uropygial gland secretions from a large number of birds have been analyzed and the qualitative chemical composition has been correlated with the phylogenetic relationship within the class Aves (1). Although several species of some of the more than 30 orders have been investigated, the order Podicipediformes (grebes), which has 19 different species, has not been studied hitherto. This paper presents the composition of the preen gland secretions of *Podiceps* species, two of which inhabit Europe and Asia (*Podiceps ruficollis* (little grebe) and *P. cristatus* (great crested grebe)), and two species from South America (*P. rolland* (white-tufted grebe) and *P. occipitalis* (silvery grebe)). These two groups have been geographically isolated for a long time and it was of interest to determine whether there were differences in preen wax composition.

MATERIAL AND METHODS

The glands were excised from freshly killed adult male animals (*P. ruficollis* and *P. cristatus* from Schleswig-Holstein, Germany; *P. rolland* and *P. occipitalis* from Lago Fagnano/Tierra del Fuego, Argentina and Las Coles/Magellanes, Chile). Each gland was extracted with 60 ml of chloroform–methanol 2:1 (v/v). After addition of water (20 ml) the lower layer contained all the lipid material. The separation into single lipid classes was performed by column chromatography on silica gel (10 g of silica gel Woelm, containing 14.5% water). Hydrocarbons were eluted with 70 ml of cyclohexane, ester waxes with 100 ml of cyclohexane–benzene 9:1 (v/v), triglycerides with 50 ml of benzene–chloroform 9:1 (v/v), and the more polar lipids with 50 ml of chloroform–methanol 9:1 (v/v). The homogeneity of all fractions was determined by thin-layer chromatography using commercially available plates (E. Merck, Darmstadt) and the solvent systems of carbon tetrachloride–chloroform 1:1 (v/v) or pure chloroform.

Reesterification of the ester waxes was performed with 5% methanolic HCl and the resulting methyl esters and alcohols were separated by silica gel chromatography. Alcohols were oxidized with CrO_3–acetic acid in cyclohexane and the resulting acids were esterified as above. The efficiency of all column chromatography fractionations was monitored by thin-layer chromatography.

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

[1] Requests for reprints should be sent to Priv. Doz. Dr. Jürgen Jacob, Biochemisches Institut für Umweltcarcinogene, D-2070 Ahrensburg/Holst., Sieker Landstrasse 19, West Germany.

TABLE 1. Composition of the uropygial gland secretion from four Podicipediformes species

Lipid	*Podiceps occipitalis*[a]	*Podiceps rolland*	*Podiceps ruficollis*	*Podiceps cristatus*[a]
Crude lipid material (mg)	181.6 (212.0)	203.0	65.6	117.7 (92.6)
Hydrocarbons (%)	25.1 (21.1)	48.1	19.9	
Monoester waxes (%)	43.7 (42.6)	32.6	74.8	96.2 (92.0)
Triglycerides (%)	24.6 (31.3)	10.6	2.1	3.1 (7.2)
Polar lipids (%)	6.6 (5.0)	8.7	3.2	0.7 (0.8)

[a] Figures in parentheses are from a second specimen.

For GLC, 9-m glass columns with 3% OV 101 on GasChrom Q were used. All mass spectra were recorded on a Varian-MAT 111 (GNOM) instrument at 80 eV using combined GLC–MS and GLC columns as above. The complexity of the mixtures required a temperature program of 1°C/min from 160°C to 240°C. All details of the methods used have been reported extensively elsewhere (2).

RESULTS

The amounts of crude lipids extracted from the uropygial glands of the four species investigated and the distribution in different lipid classes (in weight percent) are given in **Table 1.** The hydrocarbons belong to several homologous series (unbranched, 2-, 3-, 5-, 7-, 9-, and 11-monomethyl-, traces of dimethyl-branched, as well as mono- and di-unsaturated hydrocarbons) that have not been detected before in uropygial gland secretions (**Table 2**). The only hydrocarbon previously observed in preen waxes is squalene (3, 4) which, however, is absent in the waxes of the grebes investigated.

The hydrocarbons were identified by their ECL values and by mass spectrometry. The ECL values on a 9-m glass column with 3% OV 101 on GasChrom Q at 230°C were determined before and after hydrogenation, and are shown in **Table 3.** The values are compared with those of methyl esters of methyl-branched fatty acids (with three carbon atoms less, taking the $COOCH_3$ group into account). The ECL increments of both isomeric series (hydrocarbons and esters) are identical if homologous structures are compared, e.g., a 19-C_{20} ($=\omega - 1$) methyl ester is equivalent to a 2-C_{23} hydrocarbon, with both possessing an increment of +0.65; or a 18-C_{20} ($=\omega - 2$) methyl ester is equivalent to a 3-C_{23} hydrocarbon, with both possessing an increment of +0.75; and so forth. Our ECL data differ only a little from those of other authors who obtained results under different conditions (5–9), except for the 2-methyl-branched hydrocarbon for which Jackson et al. (10–12) reported a longer retention time than for the 3-methyl isomer. This is not supported by our findings but confirms the data of Chortyk, Severson, and Higman (13).

The mass spectra of methyl-branched hydrocar-

TABLE 2. Hydrocarbon composition of the uropygial secretion from Podicipediformes species as determined by GLC

Hydrocarbon	*P. occipitalis*[a]	*P. rolland*	*P. ruficollis*
		% of total	
Saturated (total)	(62.2)	(71.6)	(81.6)
Unbranched (total)	(31.3)	(26.7)	(26.7)
n-C_{15-18}	1.1	0.5	0.4
n-C_{19-20}	1.7	1.8	9.1
n-C_{21}	13.9	11.6	8.0
n-C_{22}	1.9	2.0	1.1
n-C_{23}	8.8	7.7	5.8
n-C_{24-27}	3.9	3.1	2.3
Monomethyl-branched (total)	(29.9)	(44.9)	(48.4)
2-C_{19-22}	0.4	1.3	
2-C_{23-25}	1.9	2.4	
3-C_{18-20}	0.3	0.2	0.1
3-C_{21}	1.1	2.9	7.8
3-C_{22}	0.3	2.9	0.7
3-C_{23}	5.0	12.8	9.5
3-C_{24-27}	1.3	1.2	3.4
5-C_{21-24}	1.9	0.6	3.9
5-C_{25}	1.4	4.8	5.7
7-C_{21}	0.8	0.3	
7-C_{23}	2.9	5.7	
7-C_{24-25}	0.5	3.3	
9-C_{21-27}	1.2	0.2	2.4
11-C_{23}	4.7	2.6	
11-C_{24}	0.3	0.4	
11-C_{25}	4.6	1.5	3.3
11-C_{27}			5.2
Other monomethyl-branched not identified	1.3	1.8	6.4
Dimethyl-branched (total)	(1.0)		(6.5)
Mono-unsaturated (total)	(29.2)	(24.9)	(18.4)
$C_{19:1}/C_{20:1}$			0.5
$C_{21:1}$	3.1	5.8	5.7
$C_{22:1}$	4.9	1.4	trace
$C_{23:1}$	15.6	14.1	4.5
$C_{24:1}$	2.0	1.6	4.7
$C_{25:1}$	3.6	2.0	trace
$C_{27:1}$			3.0
Di-unsaturated (total)	(8.6)	(3.5)	
$C_{21:2}$	1.7	0.6	
$C_{23:2}$	3.7	1.8	
$C_{25:2}$	3.2	1.1	

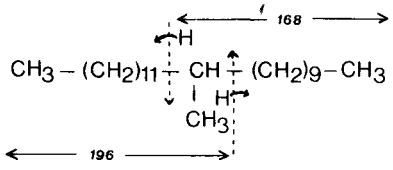
[a] A second specimen of *P. occipitalis* showed similar composition.

TABLE 3. Comparison of ECL values of branched hydrocarbons and branched methyl esters

Hydrocarbon	ECL	Fatty Acid Methyl Ester	ECL
n-C_{23}	23.00	n-C_{20}	20.00
2-C_{23} (ω-1)	23.65	19-C_{20} (ω-1)	20.65
3-C_{23} (ω-2)	23.75	18-C_{20} (ω-2)[a]	20.75
5-C_{23} (ω-4)	23.50	16-C_{20} (ω-4)	20.50
7-C_{23} (ω-6)	23.45	14-C_{20} (ω-6)	20.44
9-C_{23} (ω-8)	23.41	12-C_{20} (ω-8)	20.40
11-C_{23} (ω-10)	23.38	10-C_{20} (ω-10)	20.38
$C_{23:1}$ (Δ^9)	22.75	$C_{20:1}$ (Δ^9)	19.75
$C_{23:2}$	22.70	$C_{20:2}$ ($\Delta^{9,12}$)	19.70

[a] (ω-2) indicates that the methyl branch is located at the last but two carbon atom.

bons show characteristic even-numbered fragments due to the branching position; e.g., in the case of 11-methyltricosane the peak at *m/e* 196 is larger than that at *m/e* 197 and the peak at *m/e* 168 is larger than that at *m/e* 169, according to Jackson (14) (**Scheme 1**).



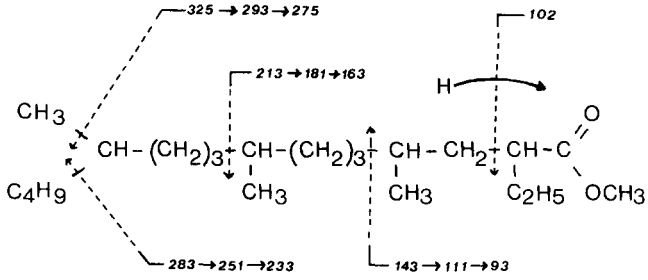
Scheme 1

3-Methylalkanes can be recognized from the large (M − 29) fragment and 2-methylalkanes from the relatively large (M − 43) fragment (15).

Permanganate–periodate oxidative cleavage of mono-unsaturated hydrocarbons indicated a double bond in the 9-position, although minor amounts of positional isomers cannot be excluded. The double bond positions of the di-unsaturated alkanes have not been investigated.

The compositions of the monoester waxes are very complex, containing a large number of homologues belonging to different isomeric series for both the wax acids and the wax alcohols (**Tables 4** and **5**).

Mass spectra of mono- and polymethyl-branched fatty acid esters of the type occurring in the waxes reported here have been discussed elsewhere (1). Ethyl-branched as well as ethyl-dimethyl-branched esters similar to those mentioned above have already been recorded (16, 17). The ethyl branch at C-2 can be readily recognized from the McLafferty ion which is shifted from *m/e* 74 to *m/e* 102 and from the fragment M − 28 resulting from C_2H_4 elimination. Additional methyl branches at C-4 are indicated by the series *m/e* 143 $\xrightarrow{-CH_3OH}$ 111 $\xrightarrow{-H_2O}$ 93, whereas a second methyl branch at C-8 can be recognized from the series *m/e* 213 $\xrightarrow{-CH_3OH}$ 181 $\xrightarrow{-H_2O}$ 163, and a third branch at C-12 yields (in case of 2-ethyl-4,8,12-trimethyl-C_{16}) the two series *m/e* 283 $\xrightarrow{-CH_3OH}$ 251 $\xrightarrow{-H_2O}$ 233 and *m/e* 325 $\xrightarrow{-CH_3OH}$ 293 $\xrightarrow{-H_2O}$ 275 (**Scheme 2**).



Scheme 2

Additional information can be derived from the ECL values. From a large number of data we found that ECL increments of branched esters can be added, yielding the increments of polyalkyl-branched esters. By this method the retention times of unknown esters can be predicted. For example, the relative retention time of the hitherto unknown methyl 2-ethyl-4,8-dimethylundecanoate or methyl 2-ethyl-4,8,12-trimethylhexadecanoate are 13.05 and 18.35, respectively (predicted values, 13.10 and 18.38). Some other examples are given in **Table 6.**

DISCUSSION

The uropygial gland waxes of the grebes investigated are the most complex known so far, possessing multibranched wax acids and alcohols which belong to a large number of homologous series including 2-ethyl- and 2-ethyl-polymethyl-branched compounds. They are even more complex than those of Sphenisciformes (18) and Procellariiformes (19) by possessing additional hydrocarbons. Hydrocarbons of non-isoprenoid structure as detected in the species investigated have not previously been reported to occur in preen waxes. The hydrocarbons have an average chain length of C_{23} and thus differ significantly from the fatty acids with their average chain length of about C_{15} (alcohols about C_{15}–C_{16}). From this no biogenetic relationship between hydrocarbons and fatty acids can be seen.

All four Podicipediformes species are closely related chemotaxonomically although hydrocarbons are absent in *Podiceps cristatus*. The constituents of the ester waxes (fatty acids and alcohols) derive from the

TABLE 4. Composition of the fatty acids of the uropygial gland waxes from some Podicipediformes species as determined by GLC

Fatty Acid	*P. occipitalis*[a]	*P. rolland*	*P. ruficollis*	*P. cristatus*[a]
		% of total		
Unbranched (total)		(1.1)		
n-C_{18}		1.1		
Monomethyl-branched (total)	(22.0)	(0.2)	(8.4)	
2-C_{13}		0.2		
3-C_{9-13}	10.7	trace	2.4	
3-C_{14-18}	11.3		6.0	
Dimethyl-branched (total)	(42.5)	(6.8)	(46.8)	(0.5)
2,6-C_{10-15}	1.9		2.0	
2,8-C_{12}	0.5			
2,10-C_{14-15}	1.8	1.0		
3,5-C_{12-15}	5.0	3.7	3.0	0.2
3,7-C_{9-16}	8.4	0.1	5.0	0.3
3,9-C_{11-15}	6.7		10.0	
3,11-C_{13-17}	18.2	2.0	20.2	
3,13-C_{15-17}			3.8	
3,15-C_{16-17}			2.8	
Trimethyl-branched (total)	(22.8)	(43.0)	(42.9)	(51.7)
2,4,6-/2,4,8-C_{10}		0.1		0.1
2,4-8-C_{14}	4.5			
2,6,10-C_{12-14}	11.4	2.8		
3,5,9-/3,7,9-C_{11-13}		4.8	1.7	42.1
3,5,7-/3,7,11-/3,9,11-C_{13}		0.6	4.2	
3,5,7-/3,5,9-/3,7,9-C_{14}		6.7	7.3	
3,5,11-/3,9,11-C_{14}		2.5	3.8	2.0
3,7,11-C_{14}		1.4	7.5	2.4
3,5,9-C_{15}	2.7		3.9	1.4
3,5,11-C_{15}	0.6	7.9	2.7	1.3
3,7,9-C_{15}	3.0	7.6		1.4
3,7,11-C_{15}	0.6	2.0	2.6	1.0
3,7,13-C_{15}		0.9		
3,9,13-C_{15}			6.0	
3,5,7-/3,7,11-/3,9,13-/3,9,15-C_{16}		1.3	3.2	
4,6,12-C_{16}		2.8		
4,10,14-C_{16}		1.6		
Tetramethyl-branched (total)		(8.1)		(1.8)
2,4,8,10-C_{14}		2.0		
2,4,8,12-C_{15}		2.7		
2,4,6,14-/2,4,8,14-/2,6,8,12-C_{15}				1.8
3,5,9,13-/3,7,9,13-C_{15}		3.4		
2-Ethyl-branched (total)	(4.6)		(0.2)	
2e-C_{8-12}	4.6		0.2	
2-Ethyl-x-methyl-branched (total)	(6.3)	(14.4)		(6.8)
2e-4m-/2e-6m-C_{8-10}	2.9	0.1		3.3
2e-6m-/2e-8m-/2e-10m-C_{11}		0.4		0.7
2e-6m-/2e-8m-C_{12}	3.4	0.3		0.3
2e-6m-/2e-8m-/2e-10m-C_{14}				1.3
2e-6m-C_{15}				1.2
2e-14m-C_{15-17}		11.2		
4e-14m-C_{16}		2.4		
2-Ethyl-x,y-dimethyl-branched (total)		(13.2)		(29.8)
2e-4,6-di-m-C_{10}		0.3		1.2
2e-4,8-di-m-C_{11}				8.0
2e-4,10-di-m-C_{11}		2.4		
2e-4,8-di-m-C_{12}		4.3		
2e-6,8-di-m-C_{12}				5.8
2e-4,8-di-m-C_{13}		1.3		
2e-6,8-di-m-/2e-6,10-di-m-C_{13}		1.0		9.9
2e-6,8-di-m-/2e-6,10-di-m-/2e-6,12-di-m-C_{14}		2.1		3.2
2e-6,10-di-m-/2e-6,12-di-m-C_{15}				1.1
2e-4,12-di-m-/2e-6,12-di-m-/2e-8,12-di-m-/ 4e-6,14-di-m-C_{16}		1.8		0.6

TABLE 4. (*Continued*)

Fatty Acid	*P. occipitalis*[a]	*P. rolland*	*P. ruficollis*	*P. cristatus*[a]
2-Ethyl-x,y,z-trimethyl-branched (total)		(4.8)		(6.6)
2e-4,6,12-tri-m-/2e-4,8,12-tri-m-/2e-6,8,12-tri-m-C_{15}		2.4		4.8
2e-6,8,12-tri-m-/2e-4,8,14-tri-m-C_{16}		2.4		1.8
Unidentified	1.8	8.4	1.7	2.8

[a] Second specimen of *P. occipitalis* and *P. cristatus* showed very similar compositions. Abbreviations: m-, methyl-; e-, ethyl-; di-m-, dimethyl-; tri-m-, trimethyl.

same homologous series and thus show great similarities. The fatty acids possess the first branch in either the 2-, 3-, or 4-position and differ only in the degree of substitution, which increases in the direction *P. occipitalis* → *P. ruficollis* → *P. rolland* → *P. cristatus*. The alcohols, on the other hand, possess the first branch at C-2 or C-4, whereas 3-alkyl-alkanols were not detected. The degree of substitution increases in the direction *P. occipitalis* → *P. rolland* → *P. ruficollis* → *P. cristatus*. The great similarity between *P. occipitalis*, *P. rolland*, and *P. ruficollis* is surprising considering that the two South American species have been geographically isolated from the European form for a long time. The data reconfirm that, genetically, the uropygial gland wax composition is a very stable parameter.

In comparison to birds belonging to other orders, the Podicipediformes have a close relationship to the family Phalacrocoracidae (order Pelecaniformes) when investigated by this method.[2] Further relationships seem to exist with Procellariiformes and Sphenisciformes, the preen wax constituents of which

TABLE 5. Composition of the alcohols of the uropygial gland waxes from some Podicipediformes species as determined by GLC

Alcohol	*Podiceps occipitalis*[a]	*P. rolland*	*P. ruficollis*	*P. cristatus*[a]
		% of total		
Unbranched (total)	(4.9)	(2.3)		(0.8)
n-C_{16}	3.6	1.5		
n-C_{18}	1.3	0.8		0.8
Monomethyl-branched (total)	(31.4)	(5.9)		
2-C_{14-17}	17.2	3.5		
4-C_{14-17}	9.3	2.4		
10-C_{16}	4.9			
Dimethyl-branched (total)	(56.7)	(18.9)	(28.1)	(5.6)
2,6-C_{13-17}	11.2		7.6	1.4
2,10-C_{14}	1.9			0.3
2,10-/2,12-C_{15-17}	17.3	12.4	15.7	3.4
4,6-/4,8-C_{16-17}	5.4	1.0		0.5
4,10-C_{14}	1.6			
4,10-/4,12-C_{15-16}	19.3	5.5		
4,10-C_{17}			4.8	
Trimethyl-branched (total)	(6.1)	(55.2)	(59.1)	(58.6)
2,4,8-C_{11}				0.2
2,4,6-/2,4,8-C_{12}				0.5
2,4,8-C_{13-14}				3.1
2,4,6-/2,4,8-C_{16}				0.4
2,6,8-C_{13-15}		0.6	5.5	5.5
2,6,10-C_{12-16}	6.1	10.5	13.4	18.7
2,6,12-C_{14-17}		1.6	16.2	17.6
2,8,10-C_{14}				0.4
2,8,12-C_{16-17}		10.4	6.2	7.1
2,10,14-C_{15-17}		7.1	15.2	0.8
2,10,16-C_{17}		2.7		
4,6,10-C_{15-16}		5.0		1.7
4,6,12-C_{16}		6.5		
4,10,12-/4,10,14-C_{16}		6.1	2.6	1.5
4,10,14-/4,10,16-C_{17}		4.7		1.1
Tetramethyl-branched (total)		(7.0)	(10.3)	(34.3)
2,4,8,12-/2,6,8,12-C_{14}				6.1
2,4,8,10-/2,6,8,12-C_{15}		7.0	7.3	20.1
2,6,8,12-/2,6,10,14-C_{16}			3.0	8.1
Unidentified	0.9	10.7	2.5	0.7

[a] Second specimens of *P. occipitalis* and *P. cristatus* showed very similar compositions.

[2] Jacob, J., unpublished results.

TABLE 6. Measured and predicted relative retention times (ECL values) for some unusual 2-ethyl branched fatty acid methyl esters

Methyl Ester	Increment for 2-ethyl-branch	ECL for the lower branched ester	ECL predicted	ECL found
2e-4m-C_8	+1.10	4m-C_8 = 8.65	9.75	9.70
2e-6m-C_8	+1.10	6m-C_8 = 8.75	9.85	9.90
2e-6m-C_{10}	+1.10	6m-C_{10} = 10.55	11.65	11.66
2e-4,8-di-m-C_{11}	+1.10	4,8-di-m-C_{11} = 12.00	13.10	13.05
2e-6,10-di-m-C_{14}	+1.07	6,10-di-m-C_{14} = 15.00	16.07	16.00
2e-4,8,12-tri-m-C_{15}	+1.05	4,8,12-tri-m-C_{15} = 16.33	17.38	17.35
4e-14m-C_{16}	+1.48 (for 4e-)	14m-C_{16} = 16.73	18.21	18.25

[a] The increments of the 2-ethyl-branch depend a little on the chain length.

Abbreviations: e-, ethyl-; m-, methyl-; di-m-, dimethyl-; tri-m-, trimethyl-.

also show mono- and multibranched fatty acids with a first branch in the 2-, 3-, or 4-position and minor amounts of 2-ethyl-branched fatty acids. This agrees with the fact that all these orders are closely linked in many taxonomic systems (20).

Phylogenetically young species so far investigated by this method have very simple uropygial gland secretions, e.g., the secretions of weaver-birds (Ploceidae) contain predominantly one wax (21); secretions of other Passeriformes species contain waxes that are composed of only one homologous series of fatty acids, e.g., finches contain only 3-methyl-branched acids (22, 23). The variety of wax constituents increases with the phylogenetic age of a species. From this viewpoint the Podicipediformes must be a group of birds that arose very early in the evolutionary process.

Although the biological function of preen waxes is still under discussion, we are convinced that they do not serve exclusively for feather impregnation. Moreover the relatively high content of mono- and di-unsaturated hydrocarbons also contradicts this hypothesis because these compounds can be readily oxidized in air if distributed as a surface film. We also know that mono-unsaturated hydrocarbons serve as pheromones in insects; they may have a similar function in birds.

I am grateful to Dr. M. Rossi and Dr. Olivero from the Ministerio de Economia in Buenos Aires and to the Ministerio de Defensa of Chile in Punta Arenas as well as to Instituto de la Patagonia in Punta Arenas for permission to collect the biological material and for generous support of the work abroad. I am greatly indebted to Mr. K. H. Siemers and the Hamburgische Stiftung and to the Universitätsgesellschaft Hamburg who supported this investigation.

Manuscript received 24 January 1977 and in revised form 26 May 1977; accepted 1 September 1977.

REFERENCES

1. Jacob, J. 1976. Bird waxes. *In* Chemistry and Biochemistry of Natural Waxes. P. E. Kolattukudy, editor. Elsevier, Amsterdam. 94–146.
2. Jacob, J. 1975. TLC, GLC and MS of complex lipid mixtures from uropygial secretions. *J. Chromatogr. Sci.* **13:** 415–422.
3. Odham, G. 1967. Studies on feather waxes of birds. VI. Further investigation of the free flowing preen gland secretion from species within the family of Anatidae. *Ark. Kemi.* **27:** 263–288.
4. Edkins, E., and I. A. Hansen. 1972. Wax esters secreted by the uropygial glands of some Australian waterfowl, including the magpie goose. *Comp. Biochem. Physiol.* **41B:** 105–112.
5. Stránský, K., M. Streibl, and F. Šorm 1966. Über Naturwachse IV. Über einen neuen Typ verzweigter Paraffine aus dem Wachs der Honigbiene (*Apis mellifera*). *Coll. Czech. Chem. Commun.* **31:** 4694–4702.
6. Mold, J. D., R. E. Means, R. K. Stevens, and J. M. Ruth. 1966. The paraffin hydrocarbons of wool wax. Homologous series of methyl alkanes. *Biochemistry.* **5:** 455–461.
7. Cavill, G. W. K., D. V. Clark, M. E. H. Howden, and S. G. Wyllie. 1970. Hydrocarbons and other lipid constituents of the bull ant, *Myrmecia gulosa. J. Insect Physiol.* **16:** 1721–1728.
8. Cavill, G. W. K., and E. Houghton. 1973. Hydrocarbon constituents of the Argentine ant, *Iridomyrmex humilis. Austr. J. Chem.* **26:** 1131–1135.
9. Han, J., E. D. McCarthy, and M. Calvin. 1968. Hydrocarbon constituents of the blue-green algae *Nostoc muscorum, Anacystis nidulans, Phormidium luridum* and *Chlorogloea fritschii. J. Chem. Soc. (C).* 1968: 2785–2791.
10. Jackson, L. L., M. T. Armold, and F. E. Regnier. 1974. Cuticular lipids of adult fleshflies, *Sarcophaga bullata. Insect Biochem.* **4:** 369–379.
11. Blailock, T. T., G. J. Blomquist, and L. L. Jackson. 1976. Biosynthesis of 2-methylalkanes in the crickets *Nemobius fasciatus* and *Gryllus pennsylvanicus. Biochem. Biophys. Res. Commun.* **68:** 841–849.
12. Jackson, L. L., and G. J. Blomquist. 1976. Insect waxes. *In* Chemistry and Biochemistry of Natural Waxes. P. E. Kolattukudy, editor. Elsevier Amsterdam. 201–233.
13. Chortyk, O. T., R. F. Severson, and H. C. Higman. 1975. Chromatographic determination of hydrocarbon waxes in tobacco leaf and smoke. *Beitr. Tabakforsch.* **8:** 204–210.
14. Jackson, L. L. 1970. Cuticular lipids of insects: II. Hydrocarbons of the cockroaches *Periplaneta australasiae, Periplaneta brunnea* and *Periplaneta fuliginosa. Lipids.* **5:** 38–41.
15. Soliday, C. L., G. J. Blomquist, and L. L. Jackson. 1974. Cuticular lipids of insects. VI. Cuticular lipids of the grasshoppers *Melanoplus sanguinipes* and *Melanoplus packardii. J. Lipid Res.* **15:** 399–405.
16. Jacob, J., and J. Poltz. 1974. Chemical composition of uropygial gland secretions of owls. *J. Lipid Res.* **15:** 243–248.
17. Poltz, J., and J. Jacob. 1974. Waxes of the uropygial gland secretion of birds of the genus *Parus. Biochim. Biophys. Acta.* **360:** 348–356.
18. Jacob, J. 1976. Uropygial gland lipids of penguins. *Biochem. Syst. Ecol.* **4:** 209–213.
19. Jacob, J. 1976. Chemotaxonomical relationships between penguins and tubenoses. *Biochem. Syst. Ecol.* **4:** 215–221.
20. Cuisin, M. 1972. *In* Das Leben der Vögel I/II Enzyklopädie der Natur Bd. 12/13. J. Dorst. Edition Rencontre, Lausanne.
21. Poltz, J., and J. Jacob. 1973. Bürzeldrüsensekrete von Webervögeln (*Ploceidae*). *Z. Naturforsch.* **28c:** 449–452.
22. Jacob, J., and A. Zeman. 1971. Über Bürzeldrüsensekrete von Finkenvögeln. Vergleichenden Untersuchung der Bürzellipide vom Grünfink (*Carduelis chloris*), Gimpel (*Pyrrhula pyrrhula*) and Hänfling (*Carduelis cannabina*). *Z. Naturforsch.* **26b:** 1352–1356.
23. Poltz, J., and J. Jacob. 1974. Bürzeldrüsensekrete bei Ammern (*Emberizidae*), Finken (*Fringillidae*) und Webern (*Ploceidae*). *J. Ornithol.* **115:** 119–127.