The Structure of Preen Gland Waxes from Pelecaniform Birds Containing 3,7-Dimethyloctan-1-ol – an Active Ingredient against Dermatophytes

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Uropygial Gland Secretion, Antimicrobial Activity, Dermatophytes, Pelecaniformes, Branched Fatty Acids

A biologically active principle has been isolated from the uropygial gland secretion of the gannet (*Sula bassana*) exhibiting growth inhibitory activity for Gram-positive bacteria, yeasts, moulds and, in particular, for dermatophytes.The feather lipids of this species consist of monoester waxes predominantly composed of 3,x-dimethylalkanols esterified with fatty acids belonging to homologous series of 2- and 3-methyl-, 2,x- and 3,x-dimethyl- as well as 2,x,y- and 3,x,y-trimethyl-substituted alkanoic acids. For chemosystematic reasons other pelecaniform species (*Pelecanus crispus, Pelecanus onocrotalus, Phaeton lepturus)* and one gaviiform species (*Gavia stellata)* have also been analysed. The pattern of their preen wax constituents suggest to assume that the Pelecaniformes are polyphyletic with gannets and cormorants being closely related, whereas pelicans and tropic birds are different, both from the former and from each other. Pelicans resemble very much the grebes, while tropic birds seem to be more closely related to the charadriiform, alciform, and possibly to the gaviiform birds.

Introduction

In contrast to other vertebrates, birds possess a singular holocrine sebaceous gland (uropygial or preen gland) the secretion of which is distributed to the bird's plumage with the help of the bill. In the past, it has been assumed that the main or exclusive purpose of this is to make the plumage waterproof and to improve the swimming ability (Friedrich v. Hohenstaufen, 1596; Paris, 1913; Weitzel, 1951). Rutschke (1960), however, demonstrated that the stability of the plumage against water is independent from its impregnation by preen waxes and a number of experiments in which the gland had been extirpated, confirmed these findings in principle (Ida 1931; Elder 1954). This assumption is even more likely if the huge variety of the lipid composition found for various bird species is regarded. The high species-specificity of this composition which also has been used for a chemosystematic classification of birds (Jacob 1977, 1982; Jacob and Ziswiler, 1982) may indicate that preen gland lipids meet also other physiological requirements.

For many alkyl-substituted fatty acids and alcohols antibacterial and antimycotic properties have been reported (Stanley *et al.,* 1929; Breusch 1964,1969; Schildknecht and Koob, 1971) and effects of the feather waxes on the growth of dermatophytes have been described in a series of papers by Pugh (Pugh, 1966, 1971, 1972; Pugh and Evans, 1970a, 1970b).

Birds from the order Pelecaniformes combining the families tropic birds (Phaetontidae), pelicans (Pelecanidae), gannets and boobies (Sulidae), cormorants (Phalacrocoracidae), darters (Anhingidae) and fregate birds (Fregatidae) have not yet been analysed for their uropygial gland secretions. This study presents for the first time data on their chemical composition and compares them to that of species from related orders such as penguins (Sphenisciformes), tubenoses (Procellariiformes), divers (Gaviiformes), and grebes (Podicipediformes).

One of the constituents, 3,7-dimethyloctan-1-ol, which is commercially available in large quantities has been tested for a variety of biological activities and exhibited growth inhibition for Gram-positive

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bacteria, yeasts, moulds and, in particular, for dermatophytes. It is likely that these effects may also be observed for the numerous homologues found in the preen constituents listed here.

Material and Methods

Cleanup and chemical analysis of the waxes: The uropygial glands were excised from naturally died zoo animals and extracted with 60 ml chloroform/methanol (2:1;v/v) each. After addition of 20 ml water to the extract, the lower layer, containing all lipid material, was evaporated to dryness. Thin-layer chromatography on silica gel plates (DC-Fertigplatten, Kieselgel 60, E. Merck) indicated the presence of monoester waxes and traces of triglycerides (contaminants from the adjacent tissue of the gland). The monoester waxes were separated by column chromatography on silica gel (Woelm, 14.5% water content) eluting with 100 ml cyclohexane/benzene (9:1; v/v). They were transesterified with 5% methanolic HCl to give fatty acid methyl esters and alcohols. After chromatography of the mixture on silica gel (see above) methyl esters were eluted with 100 ml cyclohexane/benzene (3:1; v/v) and alcohols with 50 ml chloroform/methanol (95:5; v/v). The latter were oxidised with CrO_3/acetic acid in cyclohexane for 12 h (Jacob and Zeman, 1970) resulting in fatty acids which then were esterified as above.

The GC separations of both fractions were carried out with a 25 m x 0.32 mm NB 54 capillary column (Nordion; 0.25 μm) under isothermal conditions (column temperature: 160 °C or 200 °C; injection port and detector temperature: 200 °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 against chain length with reference standards of unbranched fatty acid methyl esters (*n*-C_{10}–C_{20}).

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. Alternatively, a NERMAG R-10–10 quadrupole instrument operating at 70 eV has been used.

Microbiological test system: for the determination of the antimicrobial effect the following three test methods have been used.

Diffusion test: the test solution was filled into a well cut into the agar medium in a petri dish which had been inoculated with the test organisms. To determine the efficacy the width (in mm) of the resulting inhibition zone was measured after the incubation time.

Dilution test: the test substance in different concentrations was incorporated in the solid medium in a petri dish. The surface of the medium then was inoculated with the test organism. After incubation, the antimicrobial effect was determined by comparing the number of growing colonies with the numbers found on control plates (without antimicrobial agent).

Contact growth inhibition test: the test was carried out as described by Heiss (1973). The inhibition effect is characterized by indices (1 = full inhibition to 6 = no inhibition effect).

The following nutrient media were used for precultivation and the tests: for bacteria (others than *Propionibacterium*) Casein-Pepton medium; for *Propionibacterium* Wilkins Chalgren medium (anaerobic); for all fungi Sabourad Dextrose medium.

The test substance has always been pre-dissolved in ethanol. The given concentrations are finally resulting concentrations of the active substance. Controls were performed with ethanol and without the active substance.

Results

The chemical composition of the wax constituents from the 5 species investigated (*Sula bassana, Pelecanus crispus, P.onocrotalus, Phaeton lepturus, Gavia stellata*) is presented in Tables I–III.

Identification of the individual compounds was achieved by (1) mass spectrometry and (2) by comparing the relative GC retention times calculated as equivalent chain length (ECL) from a semi-logarithmic plot of the retention times (logarithmic) versus chain length (linear) using reference compounds of unbranched fatty acid methyl esters (C_{10}-C_{20}) as calibrants. ECL values along with characteristic mass fragments for homologous series are listed in Table IV. The formation and structure of the various mass spectrometric key fragments have been discussed elsewhere (Zeman and Jacob, 1973; Jacob and Poltz, 1974).

As Tables I–III show there is a great variety of wax constituents present in the species investi-

Table I. Chemical composition of the wax constituents from *Sula bassana* (% of total).

	Fatty acids	Alcohols
2-methyl-substituted	**1.3**	**–**
2-C_{12}	1.1	–
2-C_{13}	0.2	–
3-methyl-substituted	**35.2**	**24.7**
3-C_8	4.7	–
3-C_9	6.9	–
3-C_{10}	11.9	0.2
3-C_{11}	6.1	1.1
3-C_{12}	2.1	2.0
3-C_{13}	1.2	2.7
3-C_{14}	1.3	1.7
3-C_{15}	1.0	8.0
3-C_{16}	–	6.7
3-C_{17}	–	1.7
3-C_{18}	–	0.6
2,x-dimethyl-substituted	**0.5**	**1.8**
2,4-C_8	0.5	–
2,8-C_{12}	–	0.8
2,10-C_{12}	–	1.0
3,x-dimethyl-substituted	**59.9**	**51.0**
3,7-C_8	–	0.3
3,7-C_9	18.2	0.1
3,7-C_{10}	20.1	0.7
3,7-C_{11}	6.7	1.8
3,7-C_{12}	4.2	3.6
3,7-C_{13}	1.0	3.2
3,7-C_{14}	0.8	7.8
3,7-C_{15}	0.4	6.0
3,7-C_{16}	–	5.0
3,7-C_{17}	–	2.2
3,9-C_{11}	4.0	1.6
3,9-C_{12}	1.0	–
3,9-C_{13}	0.3	0.7
3,9-C_{14}	0.2	0.5
3,9-C_{15}	0.4	3.3
3,9-C_{16}	–	1.4
3,9-C_{17}	–	1.1
3,11-C_{12}	0.1	–
3,11-C_{13}	0.6	–
3,11-C_{14}	0.4	1.6
3,11-C_{15}	0.4	0.8
3,11-C_{16}	–	2.1
3,11-C_{17}	–	1.5
3,13-C_{14}	–	1.2
3,13-C_{15}	1.1	1.7
3,13-C_{16}	–	1.8
3,13-C_{17}	–	1.0
2,x,y-trimethyl-subst.	**1.0**	**6.2**
2,4,6-C_{11}	1.0	–
2,6,10-C_{13}	–	1.1
2,6,10-C_{14}	–	1.8
2,6,10-C_{16}	–	3.3
3,x,y-trimethyl-subst.	**1.2**	**10.2**
3,5,7-C_{14}	–	0.6
3,5,7-C_{15}	–	4.7
3,7,9-C_{19}	0.7	–
3,7,9-C_{14}	0.5	–
3,7,11-C_{13}	–	2.9
3,7,11-C_{14}	–	0.9
3,7,11-C_{15}	–	0.7
3,7,11-C_{16}	–	0.4
unidentified	**0.9**	**6.1**

Table II. Chemical composition of the wax constituents from *Phaëton lepturus* and *Gavia stellata* (% of total).

	Phaëton lepturus	*Gavia stellata*
Fatty acids		
2-methyl-substituted	**26.2**	**5.1**
2-C_8	0.2	–
2-C_9	2.0	–
2-C_{10}	5.5	–
2-C_{11}	6.9	0.3
2-C_{12}	9.7	0.7
2-C_{13}	1.0	0.2
2-C_{14}	0.7	1.5
2-C_{15}	–	1.0
2-C_{16}	0.2	0.4
2-C_{17}	–	1.0
4-methyl-substituted	**69.8**	**5.2**
4-C_7	4.5	–
4-C_8	16.0	–
4-C_9	9.0	–
4-C_{10}	24.0	–
4-C_{11}	12.2	–
4-C_{12}	4.1	0.3
4-C_{13}	–	0.2
4-C_{14}	–	0.9
4-C_{15}	–	1.2
4-C_{16}	–	1.9
4-C_{17}	–	0.7
6-methyl-subtituted	**–**	**2.9**
6-C_{16}	–	2.7
6-C_{17}	–	0.2
2,x-dimethyl-substituted	**–**	**9.9**
2,4-C_8	–	0.8
2,4-C_{13}	–	0.2
2,4-C_{14}	–	0.3
2,6-C_{10}	–	0.4
2,6-C_{11}	–	0.2
2,6-C_{12}	–	0.5
2,6-C_{13}	–	0.6
2,6-C_{14}	–	2.4
2,6-C_{15}	–	0.3
2,6-C_{16}		2.0
2,6-C_{17}	–	0.7
2,10-C_{14}	–	0.7
2,10-C_{17}	–	0.6
2,12-C_{15}	–	0.2
4,x-dimethyl-substituted	**–**	**6.3**
4,8-C_{14}	–	1.1
4,8-C_{15}	–	0.3
4,8-C_{16}	–	0.8
4,10-C_{15}	–	0.8
4,10-C_{16}	–	2.2
4,14-C_{16}	–	1.1
2,x,y-trimethyl-subst.	**–**	**64.6**
2,4,6-C_8	–	0.8
2,4,6-C_9	–	58.6
2,4,6-C_{10}	–	0.3
2,4,6-C_{11}	–	0.8
2,4,8-C_{11}	–	0.4
2,4,6-C_{13}	–	0.4

Table II. Continued.

	Phaëton lepturus	*Gavia stellata*
2,4,8-C_{13}	–	0.2
2,4,10-C_{13}	–	0.2
2,4,6-C_{14}	–	0.7
2,4,8-C_{14}	–	0.4
2,4,6-C_{15}	–	0.2
2,4,8-C_{15}	–	0.2
2,4,8-C_{16}	–	1.4
unidentified	**4.0**	**4.2**
Alcohols		
unbranched	**96.8**	**64.9**
n-C_{14}	0.6	0.3
n-C_{15}	0.8	0.6
n-C_{16}	5.8	7.8
n-C_{17}	15.6	10.2
n-C_{18}	70.2	44.7
n-C_{19}	3.8	1.3
branched	**2.2**	**32.7**
2-C_{14}	–	0.2
2-C_{16}	–	2.1
2-C_{17}	–	1.6
2-C_{18}	–	1.1
4-C_{14}	–	0.2
4-C_{15}	–	0.8
4-/6-C_{16}	–	12.6
4-C_{17}	–	1.6
4-C_{18}	–	4.8
8-C_{16}	1.5	–
14-C_{16}	–	5.3
12-C_{17}	–	1.4
10-C_{18}	0.7	–
14-C_{18}	–	0.6
16-C_{18}	–	0.4
unidentified	**1.0**	**2.4**

gated. Homologous series of compounds possessing at least one substituent at the C-3 atom predominate in both wax acids and alcohols from *Sula bassana*. One of these constituents (3,7-dimethyloctan-1-ol) has been tested for antimicrobial activities.

Phaeton lepturus exhibits a simple pattern with 2- and 4-methyl-substituted fatty acids and predominantly unbranched alcohols, whereas *Gavia stellata* contains small amounts of these and other dimethyl-substituted acids but larger percentages of trimethyl-substituted homologues. The amount of branched wax alcohols is also higher in *Gavia* than in *Phaeton*.

Acids with a first ethyl-substituent at C-2 and further methyl-(or ethyl-)substituents at other even-numbered C-atoms are typical characters for

Table III. Chemical composition of the wax constituents from *Pelecanus crispus* and *P. onocrotalus* (% of total).

	Pelecanus crispus	*Pelecanus onocrotalus*
Fatty acids		
monomethyl-subst.	**8.8**	**–**
2-C_{10}	0.3	–
2-C_{12}	0.8	–
2-C_{13}	1.2	–
2-C_{15}	1.9	–
2-C_{17}	0.7	–
3-C_{15}	2.6	–
3-C_{17}	1.3	–
dimethyl-subst.	**1.1**	**–**
2,8-C_{10}	1.1	–
trimethyl-subst.	**17.7**	**39.3**
2,4,6-C_{10}	3.9	0.2
2,4,6-/2,4,8-C_{12}	1.8	0.5
3,5,7-C_{11}	–	0.9
3,5,9-C_{13}	–	2.9
3,7,9-C_{13}	6.6	20.0
3,5,9-C_{15}	–	0.8
3,7,9-C_{15}	–	6.1
3,7,11-C_{15}	3.4	7.9
3,9,11-C_{15}	0.7	–
4,6,8-C_{10}	1.3	–
tetramethyl-subst.	**–**	**18.3**
2,4,6,10-C_{14}	–	0.2
2,4,8,10-C_{14}	–	0.4
3,5,9,11-/3,7,9,11-C_{13}	–	1.6
3,5,9,11-/3,7,9,11-C_{14}	–	9.5
3,5,11,13-/3,7,11,13-/ 3,9,11,13-C_{14}	–	6.6
2-ethyl-x-methyl-subst.	**15.0**	**0.3**
2e, 4m-C_8	–	0.3
2e, 6m-C_8	1.3	–
2e, 6m-C_{10}	6.0	–
2e, 6m-C_{11}	0.6	–
2e, 6m-C_{12}	1.4	–
2e, 10m-C_{13}	2.8	–
2e, 12m-C_{13}	2.9	–
2-ethyl-x,y-dimethyl-subst.	**28.9**	**24.8**
2e, 4,6 dim.-C_9	1.0	–
2e, 4,6 dim.-C_{11}	–	1.5
2e, 6,8 dim.-C_{11}	1.4	–
2e, 4,6 dim.-C_{12}	–	1.3
2e, 6,8 dim.-C_{12}	–	9.8
2e, 6,10 dim.-C_{12}	14.7	10.0
2e, 4,8 dim.-C_{14}	–	2.2
2e, 6,8 dim.-C_{14}	3.2	–
2e, 6,10 dim.-C_{14}	8.6	–
2-ethyl-x,y,z-trimethyl-subst.	**–**	**1.8**
2e, 4,6,10 trim.-C_{12}	–	1.8
2,x-diethyl-subst.	**2.4**	**1.8**
2,6-die-C_{10}	2.4	1.8
2,x-diethyl-y-methyl-subst.	**11.4**	**8.1**
2,4-die-6m-C_8	–	0.1

Table III. Continued.

	Pelecanus crispus	*Pelecanus onocrotalus*
2,4-die-6m-C_9	–	8.0
2,8-die-12m-C_{13}	7.6	–
2,10-die-6m-C_{14}	3.8	–
unidentified	**14.7**	**5.6**
Alcohols		
unbranched	**9.6**	**26.9**
n-C_{18}	2.8	0.5
n-C_{19}	1.5	3.8
n-C_{20}	1.4	7.5
n-C_{21}	3.1	14.9
n-C_{22}	0.8	0.2
dimethyl-subst.	**15.2**	**0.7**
2,6-C_{11}	–	0.1
2,4-C_{12}	0.6	–
2,6-C_{12}	1.1	0.3
2,10-C_{13}	1.0	–
2,6-C_{14}	3.1	0.3
2,6-C_{15}	1.0	–
2,6-C_{16}	1.2	–
2,8-C_{16}	1.0	–
2,10-C_{16}	1.0	–
2,14-C_{16}	0.5	–
4,8-C_{13}	2.0	–
4,10-C_{14}	0.5	–
4,6-C_{15}	2.0	–
4,6-C_{17}	0.2	–
trimethyl-subst.	**62.8**	**18.6**
2,4,6-C_{10}	0.4	0.3
2,4,6-/2,4,8-C_{11}	1.1	0.2
2,4,6-/2,4,8-/2,6,8-C_{12}	8.8	7.2
2,6,10-C_{13}	5.0	–
2,4,6-C_{14}	6.4	3.4
2,4,8-C_{14}	–	1.8
2,6,10-C_{14}	14.7	–
2,4,6-C_{15}	9.2	–
2,6,12-C_{15}	1.8	–
2,4,6-C_{16}	1.6	–
2,6,10-C_{16}	3.5	3.7
2,6,12-C_{16}	6.4	–
2,6,8-C_{17}	0.8	–
3,5,9-C_{13}	–	1.0
3,7,9-C_{13}	3.1	1.0
tetramethyl-subst.	**7.8**	**40.4**
2,4,6,10-C_{12}	–	3.3
2,4,6,10-C_{14}	–	13.1
2,4,6,8-C_{15}	–	0.7
2,6,8,10-C_{15}	3.0	–
2,6,8,14-C_{15}	–	1.7
2,4,6,12-C_{16}	–	12.7
2,6,8,10-C_{16}	4.8	–
2,6,8,12-C_{16}	–	8.9
unidentified	**4.6**	**13.4**

the family Pelecanidae since they were not detected in *Sula bassana* (Sulidae) and *Phaeton* (Phaetontidae).

The antimicrobial effect of 3,7-dimethyloctan-1-ol has been studied with three different test methods against various test organisms (see Tables V and VI). The test methods used led to similar results regarding the spectrum of efficacy and the effective concentration range. A bacteriostatic effect has been found for concentrations > 0.1%. This effect, however, mainly covers Gram-positive bacteria (staphylococci; *Propionibacterium*). A better effect against staphylococci than against *Propionibacterium acnes* has been observed. The activity against Gram-negative bacteria (pseudomonads, *E.coli*) proved to be rather poor or not existing in the given concentration range (0.1–1.0%).

A fungistatic effect could be proven against yeasts (*Candida* species, *Torulopsis*), moulds (*Aspergillus, Penicillium*) and dermatophytes (*Trichophyton* species, *Microsporum gypseum*). Whereas the efficacy against yeasts and moulds is basically comparable with that found for Gram-positive bacteria with some variations for different taxonomic groups, the efficacy against dermatophytes is considerably more potent. The measured parameters as well as finding a full inhibition in the dilution test already with a concentration of 0.1% suggests an effective concentration of < 0.1% against this group of fungi.

Discussion

The relationship between pelicans (Pelecanidae) and the other pelecaniform families is not clear though gannets, boobies, cormorants, and anhingas have supposed to be their nearest allies (Elliott, 1992). More recent work on DNA/DNA-hybridization seems to indicate that the shoebilll (*Balaeniceps rex*) is also closely related to the pelicans (Sibley and Ahlquist, 1990). There is also some confusion about the taxonomical position of the Podicipediformes which according to Llimona and del Hoyo, (1992) do not appear to be closely related to any other group of birds, the more than apparent similarities to the Gaviiformes have been considered to be due to convergent evolution. DNA/DNA-hydridization experiments favour a linkage to Ciconiiformes, Procellariiformes, Pele-

Table IV. ECL-values and typical mass spectrometric fragments of fatty acid methyl esters derived from the preen waxes of four pelecaniform and one gaviiform species.

Type of substitution	ECL*	Typical mass fragments
monomethyl-		
2-	16.35	88; M-59
3-	16.40	87 ≪ 74; M-74
4-	16.50	87 ≫ 74; M-73
6-	16.41	M-76
8-	16.40	171 → 139 → 121
10-	16.41	129 ≈ 130; 199 → 167 → 149
12-	16.50	227 → 195 → 177
14- (=[ω-2])	16.75	(M-29) > (M-31) 241 → 209 → 191
dimethyl-		
2,4-	16.85	88; (M-59); (M-87)
2,6-	16.75	88; (M-49); (M-90); 157 → 125
2,8-	16.76	88; 185 → 183 → 135
2,10-	16.76	88; 213 → 181 → 163, (M-146)
2,12-	16.85	88; 241 → 209 → 191
2,14	17.10	88; 269 → 237 → 219
3,7-	16.80	87 ≪ 74; 171 → 139 → 121
3,9-	16.82	87 ≪ 74; 199 → 167 → 149
3,11-	16.85	87 ≪ 74; 227 → 195 → 177
3,13-	16.93	87 ≪ 74; 255 → 223 → 205
4,6-	16.91	87 ≫ 74; (M-73); (M-76); 157 → 125
4,8-	16.90	87 ≫ 74; (M-73); 185 → 153 → 135
4,10-	16.92	87 ≫ 74; (M-73); 213 → 181 → 163
4,14-	17.22	87 ≫ 74; (M-73); 269 → 237 → 219
trimethyl-		
2,4,6-	17.26	88; (M-49); 171 → 139
2,4,8-	17.25	88; 199 → 167 → 149
2,4,10-	17.26	88; 227 → 195 → 177
2,6,8-	17.15	88; (M-90); 157 → 125; 199 → 167 → 149
2,6,10-	17.17	88; (M-90); 157 → 125; 227 → 195 → 177
2,6,12-	17.25	88; (M-90); 157 → 125; 255 → 223 → 205
3,5,7-	17.30	87 ≪ 74; 185 → 153 → 135
3,5,9-	17.30	87 ≪ 74; 213 → 181 → 163
3,7,9-	17.30	87 ≪ 74; 171 → 139 → 121; 213 → 181 → 163
3,7,11-	17.40	87 ≪ 74; 171 → 139 → 121 → 241 → 209 → 191
3,9,11-	17.42	87 ≪ 74; 199 → 167 → 149; 241 → 209 → 191
4,6,8-	17.31	87 ≫ 74; (M-73); (M-76); 199 → 167 → 149
tetramethyl-		
2,4,6,8-	17.66	88; (M-49); (M-87); 171 → 139; 213 → 181 → 163
2,4,6,10-	17.66	88; (M-49); (M-87); 171 → 139; 241 → 209 → 191
2,4,6,12-	17.75	88; (M-49); (M-87); 171 → 139; 269 → 237 → 219
2,6,8,10-	17.56	88; (M-90); 157 → 125; 199 → 167 → 149; 241 → 209 → 191
2,6,8,12-	17.65	88; (M-90); 157 → 125; 199 → 167 → 149; 269 → 237 → 219
2,6,8,14-	17.90	88; (M-90); 157 → 125; 199 → 167 → 149; 297 → 265 → 247
3,5,9,11-	17.71	87 ≪ 74; 213 → 181 → 163; 255 → 223 → 205
3,5,11,13-	17.72	87 ≪ 74; 241 → 209 → 191; 283 → 251 → 233
3,7,9,11-	17.72	87 ≪ 74; 171 → 139; 213 → 181 → 163; 255 → 223 → 205
3,7,11,13-	17.80	87 ≪ 74; 171 → 139; 241 → 209 → 191; 283 → 251 → 233
3,9,11,13-	17.82	87 ≪ 74; 199 → 167 → 149; 241 → 209 → 191; 283 → 251 → 233
2-ethyl-x-methyl-		
2e, 4m-	17.55	102; (M-28); (M-59); 143 → 111
2e, 6m-	17.45	102; (M-28); (M-59); 171 → 139; (M-104)
2e, 10m-	17.46	102; (M-28); (M-59); 227 → 195 → 177
2e, 12m-	17.49	102; (M-28); (M-59); 255 → 223 → 205

Table IV. Continued.

Type of substitution	ECL*	Typical mass fragments
2-ethyl-x,y-dimethyl-		
2e, 4,6-dim-	17.91	102; (M-28); (M-59); 185→153→135
2e, 4,8-dim-	17.90	102; (M-28); (M-59); 213→181→163
2e, 6,8-dim-	17.81	102; (M-28); (M-59); (M-104); 213→181→163
2e, 6,10-dim-	17.82	102; (M-28); (M-59); (M-104); 241→209→191
2-ethyl-x,y,z-trimethyl-		
2e, 4,6,10-trim-	18.30	102; (M-28); (M-59); 185→153→135; 255→223→205
2,x-diethyl-		
2,6-die-	18.10	102; (M-28); (M-59); (M-118); 185→153→135
2,x-die, y-methyl		
2,4-die, 6m-	18.56	102; (M-28); (M-59); 157→125; 199→167→149
2,8-die, 12m-	18.55	102; (M-28); (M-59); 213→181→163; 283→251→233
2,10-die, 6m-	18.65	102; (M-28); (M-59); 171→139; 255→223→205

* Equivalent chain length for branched fatty acid methyl esters containing 16 carbon atoms in the main chain related to methyl hexadecanoate (= 16.00).

Table V. Efficacy of 3,7-dimethyloctan-1-ol against bacteria.

Concentration	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Propionibacterium acnes*	*Pseudomonas aeruginosa*	*Pseudomonas putida*	*E. coli*
			Contact growth inhibition*			
1%	2	1	4	6	6	6
0.5%	5	n.t.	n.t.	n.t.	n.t.	n.t.
0.1%	6	6	6	6	6	6
			Diffusion test (inhibition zone in mm)			
1%	7.1	6,4	2.6	0	2.2	2.1
0.5%	7.0	n.t.	n.t.	n.t.	0	1
0.1%	1.2	2.1	1.3	0	1.1	1.2
			Dilution test (solid medium)*			
1%	1					6
0.5%	1					6
0.1%	6					6

* 1: full inhibition to 6: no inhibition, n.t.: not tested.

caniformes, and Sphenisciformes (Sibley and Ahlquist, 1990).

Taking the results of this study together and comparing them to previously published data as illustrated in Fig. 1, it appears that

(1) Pelecaniformes are a polyphyletic assemblage of species, since there is no uniform pattern of the wax constituents;

(2) the families Sulidae and Phalacrocoracidae (containing a complex mixture of highly branched acids with the first methyl-substituent located at C-3; data not shown) are related to the penguins (Sphenisciformes) and the tubenoses (Procellariiformes), all exhibiting similar patterns of their wax constituents;

(3) the Pelecanidae resemble the Podicipediformes more than any other group;

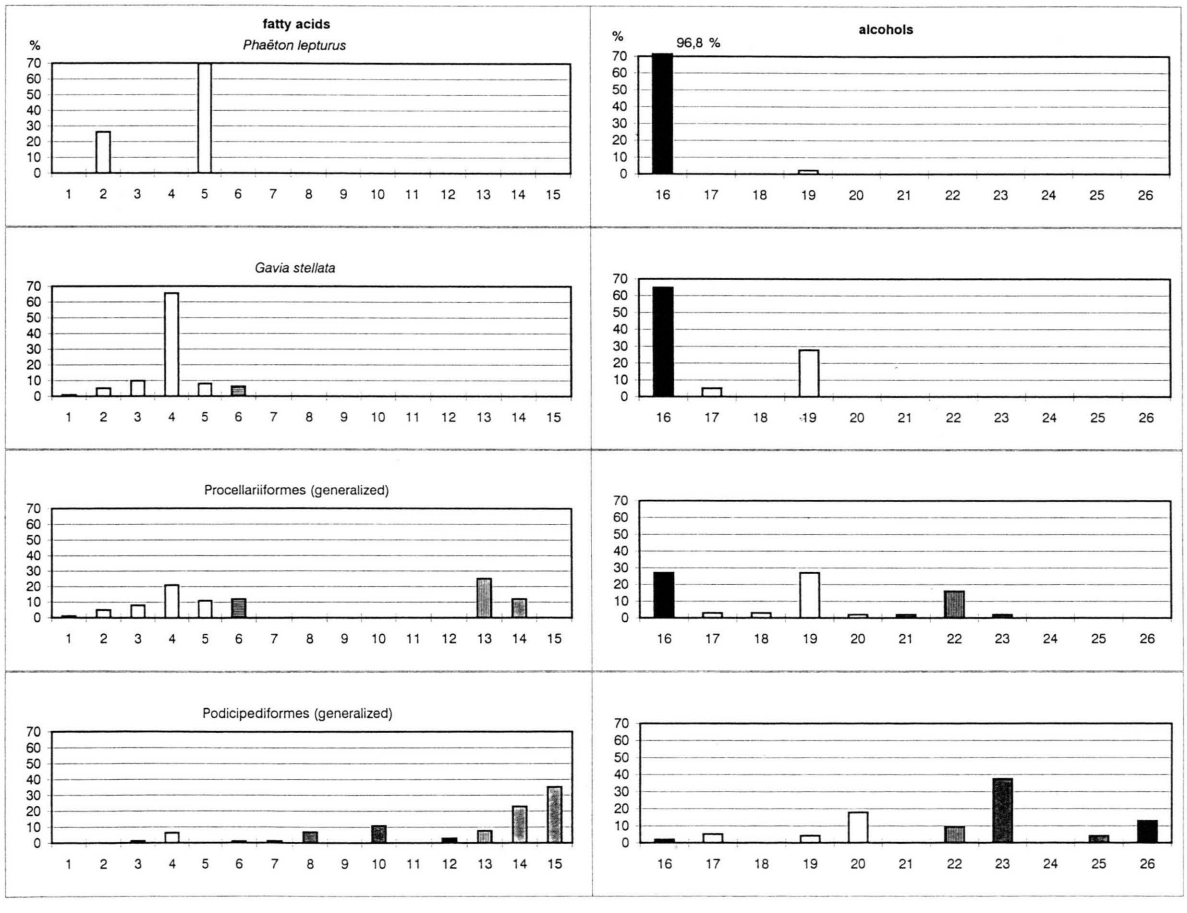
(4) *Phaeton* is isolated within the Pelecaniformes and rather exhibits similarities to the Gaviiformes along with which they may be related to the Charadriiformes.

The antimicrobial effect determined for 3,7-dimethyloctan-1-ol is a growth inhibiting effect. A bactericidal resp. fungicidal effect could not be established in a suspension test in concentrations up to 2% (data not shown). The effective concen-

Table VI. Efficacy of 3,7-dimethyloctan-1-ol against fungi.

Concentration	*Candida albicans*	*Candida parapsilosis*	*Candida guiliermondii*	*Torulopsis candida*	*Aspergillus niger*	*Penicillium verrucosum*	*Trichophyton rubrum*	*Trichophyton mentagrophytes*	*Microsp. gypseum*
				Contact growth Inhibition*					
1%	2	4	2	3	3	3	1	1	1
0.5%	3	n.t.	n.t.	n.t.	n.t.	n.t.	1	1	1
0.1%	6	6	6	6	6	6	6	6	6
				Diffusion test (inhibition zone in mm)					
1%	5.4	2.4	3.4	6.2	12.6	5.9	>30	>30	>30
0.5%	3.3	n.t.	n.t.	n.t.	0	n.t.	>30	>30	0
0.1%	0	0	0	0	0	0	0	0	0
				Dilution test (solid medium)*					
1%	1				1		1	1	1
0.5%	1				1		1	1	1
0.1%	6				6		1	1	1

* 1: full inhibition to 6: no inhibition, n.t.: not tested.



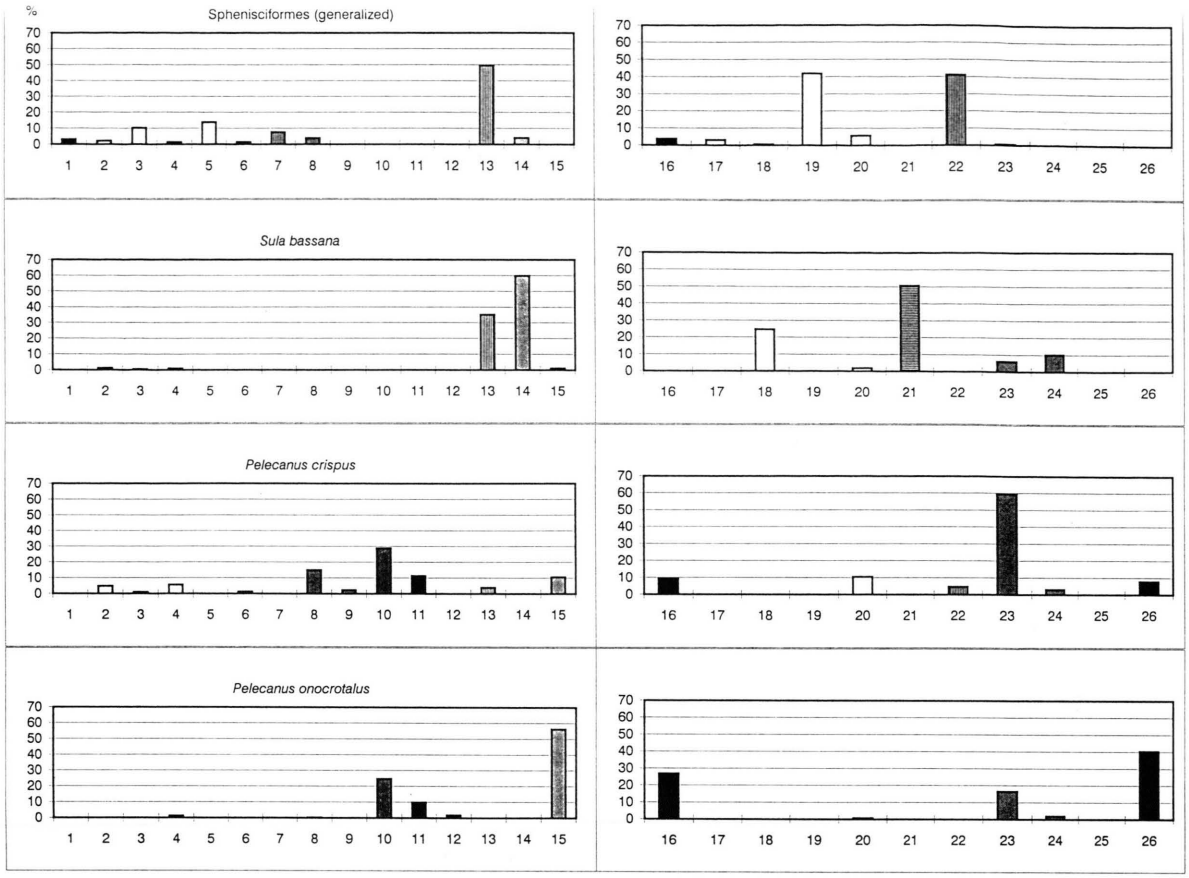


Fig. 1. Percentage of the uropygial gland wax constituents from the species investigated in comparison to generalized patterns for Sphenisciformes (taken from Jacob,1976a, 1976b; Jacob and Hoerschelmann, 1981, 1984), Procellariiformes (taken from Jacob and Zeman, 1971; Jacob, 1976b; Jacob and Hoerschelmann, 1982), and Podicipediformes (taken from Jacob, 1978).
Fatty acid types (totals set to 100%): 1 (unbranched); 2 (2-methyl-subst.); 3 (2,x-dimethyl-subst.); 4 (2,x,y-tri- and 2,x,y,z-tetramethyl-subst.); 5 (4- or 6-methyl-subst.); 6 (4,x-di- and 4,x,y-trimethyl-subst.); 7 (2- and 4-ethyl-subst.); 8 (2-ethyl-x-methyl- and 4-ethyl-x-methyl-subst.); 9 (2,x-diethyl-subst.); 10 (2-ethyl-x,y-dimethyl-subst.); 11 (2,x-diethyl-y-methyl-subst.); 12 (2-ethyl-x,y,z-trimethyl-subst.); 13 (3-methyl-subst.); 14 (3,x-dimethyl-subst.); 15 (3,x,y-tri- and 3,x,y,z-tetramethyl-subst.);
Alcohol types (totals set to 100%): 16 (unbranched); 17 (2-methyl-subst.); 18 (3-methyl-subst.); 19 (other monomethyl-subst.); 20 (2,x-dimethyl-subst.); 21 (3,x-dimethyl-subst.); 22 (4,x- and other dimethyl-subst.); 23 (2,x,y-trimethyl-subst.); 24 (3,x,y-trimethyl-subst.); 25 (4,x,y-trimethyl-subst.); 26 (2,x,y,z-tetramethyl-subst.)

trations as shown in Tables V and VI should be understood as 'relative results' since all test systems contain a concentration gradient due to diffusion effects. The exact concentration limits have not been defined. Since the diffusion distance is different in the various methods slightly different results have been obtained. The results can, however, be used to directly compare the efficacy against the different groups of microorganisms. The predominant effect against Gram-positive bacteria and dermatophytes may give an indication for the physiological role of the substance.

The activity of 3,7-dimethyloctan-1-ol against dermatophytes confirms findings by Pugh (1972) who reported on effects of feather waxes from *Turdus merula, Phasianus colchicus* and *Coturnix spp.* on the growth of keratinophilic fungi such as *Arthroderma curreyi, Chrysosporium keratinophilum* and *Ctenomyces serratus*, both stimulating and inhibitory. For instance, feather waxes from *Tur-*

dus stimulated the growth of *A.curreyi* but inhibited that of *Ch.keratinophilum*. It is also well known that infections of birds by dermatophytes frequently occur on the head possibly in consequence of the fact that this region for anatomical reasons is less efficiently impregnated with preen waxes.

Though not yet tested it is suggested that other homologues listed in Tables I–III also will display antimicrobial potencies since e.g. for some 3- and 4-methyl-substituted acids inhibtion effects towards *Trichophyton, Aspergillus* and *Candida* have been found (data not shown).

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