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On the Chemistry of Preen Gland Waxes of Waterfowl

GÖRAN ODHAM AND EINAR STENHAGEN*

Departments of Plant Physiology and Medical Biochemistry, University of Göteborg, Göteborg, Sweden

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Many species of birds spend a large part of their lives swimming, diving, or resting on water and are able to do so because of their waterproof plumage. The water-repellent property of the plumage is due to the texture of the feathers and the presence of a waterproofing wax that is produced by a sebaceous gland situated at the bird's rear.

The existence of this *Glandula uropygialis*, or preen gland, has long been known. The first published observations are those of Friedrich II (Hohenstaufen) (1194–1250), whose work "De Arte Venandi cum Avibus" was printed in 1596.[1] The first chemical analysis of preen gland material was undertaken by de Jonge[2] in 1879; he found that in ducks and geese the secretion contained wax esters, *i.e.*, esters of fatty acids and long-chain alcohols. These results were confirmed by Röhmann in 1904.[3] The literature to 1928 concerning the preen gland in birds has been reviewed by Hou.[4]

Weitzel and coworkers[5] reinvestigated the lipids of the preen gland of the domestic goose in 1952 and showed that the wax consisted mainly of esters of 1-octadecanol with optically active saturated branched-chain acids. A tetradecanoic acid was the major acid component. The structure of this acid was subsequently determined by degradative methods by Murray,[6] who showed that the C_{14} acid most likely possessed the structure of a 2,4,6,8-tetramethyldecanoic acid.

Shortly afterwards Murray left the field and, as work on branched-chain fatty acids from bacteria has been pursued for a long time in our laboratory and the structural similarity between the C_{14} acid from the goose and C_{32}-mycocerosic acid (2D,4D,6D,8D-tetramethyloctacosanoic acid[7,8]) from the tubercle bacillus was intriguing, it was easy to follow his suggestion that we should continue the work in this field.

All previous work appears to have been carried out on whole glands. No one apparently had studied the free-flowing secretion. With the free-flowing secretion contamination by compounds from the tissues is avoided. The uropygial gland is a paired organ on the rump of the bird forming a small protuberance around the two excretory openings. Around them are arranged two small downy tufts which in the living bird are soaked with secretion. The collection of secretion was performed by scraping the tufts with a spatula. The material was usually collected every second day. The amount of material that could be collected from each bird varied from a few milligrams up to about 200 mg each time. For the goose about 40 mg of secretion was obtained without difficulty. The secretion was usually slightly yellowish and contained, in addition to the oily substance, traces of water and some cell fragments. The secretion of the uropygial gland resembles that of the sebaceous glands in being of the destructive type, *i.e.*, the whole cells of the glandular epithelium are successively transformed into secretion.

Analytical Work

The material collected was filtered, and further purification was carried out by liquid chromatography on silicic acid. For the goose this gave the results shown in Figure 1. About 90% of the material consists of wax, the rest comprising many minor components. The hydrolysis of the wax was carried out in a homogeneous solution of KOH in a mixed solvent of water, ethanol, and benzene (previously hydrolysis had been carried out by sodium methoxide in a nonaqueous medium which may cause partial racemization of the α carbon[9]). The acidic material was esterified with methanol–sulfuric acid.

The gas chromatograms of the esters showed only two components, which turned out to be the C_{14} and C_{15} tetramethyl-substituted acids previously found by Weitzel, *et al.*,[5] and Murray.[6] The structures of the acids were deduced from the mass spectra.[10] The alcohol component was found to consist of 1-octadecanol (99.5%), as had previously been found by

(1) F. Hohenstaufen, 2nd, "De Arte Venandi cum Avibus," Augsburg, 1596.
(2) D. de Jonge, *Z. Physiol. Chem.*, **3**, 225 (1879).
(3) F. Röhmann, *Beitr. Chem. Physiol. Pathol.*, **5**, 110 (1904).
(4) H. C. Hou, *Chin. J. Physiol.*, **2**, 345 (1928).
(5) G. Weitzel, A.-M. Fretzdorff, and J. Wojahn, *Z. Physiol. Chem.*, **291**, 46 (1952).
(6) K. E. Murray, *Aust. J. Chem.*, **15**, 510 (1962).
(7) C. Asselineau, J. Asselineau, R. Ryhage, S. Ställberg-Stenhagen, and E. Stenhagen, *Acta Chem. Scand.*, **13**, 822 (1959).
(8) G. Odham, E. Stenhagen, and K. Waern, *Ark. Kemi*, **31**, 533 (1970).
(9) J. Kenyon and D. P. Young, *J. Chem. Soc.*, 216 (1940).
(10) G. Odham, *Ark. Kemi*, **21**, 379 (1963).

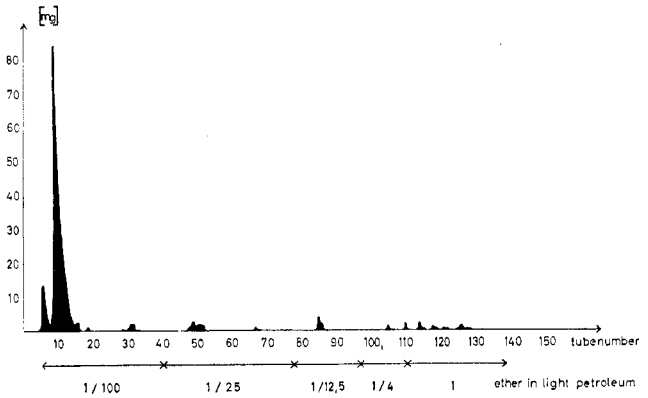

Figure 1. Liquid chromatogram of the wax of the preen gland of the goose on silicic acid.

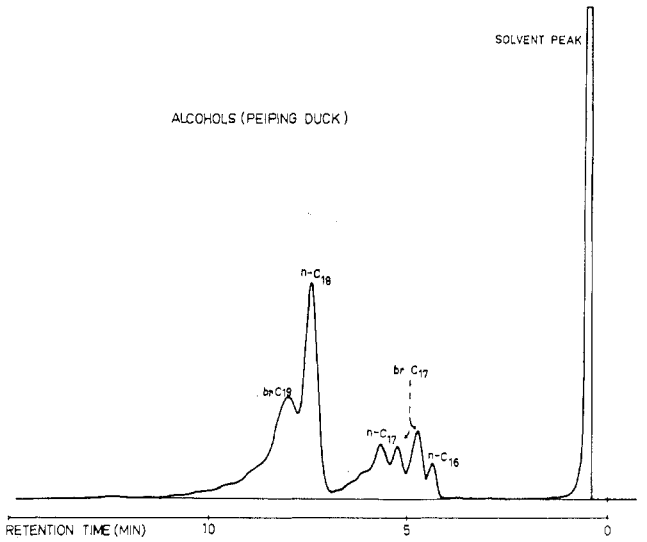

Figure 2. Gas chromatogram of alcohols obtained from the preen gland wax of the Peiping duck; 215°, Reoplex 400 as stationary phase.

Röhmann.[3] The preen gland wax of the goose has thus a rather simple composition with respect to the number of components present.

The preen gland wax of the Peiping duck was next studied.[11] It had already been examined by Weitzel, *et al.*,[12,13] who arrived at the conclusion that 4-methylhexanoic acid was the main acidic component. The alcoholic component was stated to be 1-octadecanol. The reinvestigation using modern techniques (gas chromatography–mass spectrometry combination[14]) gave the results shown in Table I. The structures of the acids were determined through the mass spectra of the methyl and ethyl esters (*cf.* ref 15 and 16). The 2-methyl-substituted acids were levorotatory for sodium D light, with absolute values corresponding to the pure D forms.

The alcohols were subjected to gas chromatography with the results shown in Figure 2. In order to resolve as far as possible the components of the mixture, the alcohols were oxidized by means of oxygen and platinum oxide[17] *via* the aldehydes to the corresponding fatty acids. The methyl esters of the acids were subjected to gas chromatography on a Golay capillary column (Figure 3). Fortunately, the relative retention times for all isomers of monomethyl-substituted C_{19} acids had been determined by Ställberg-Stenhagen[18] (Figure 4), and from these data it is evident that not all of the isomers are separable by gas chromatography. In Figure 5 several of those which can be separated have been added to the mixture. In this way it was possible to show that the mixture contained every possible monomethyl-substituted isomer with the branch on an even-numbered carbon atom. Table II shows the results of the analysis of the alcohols.[11]

Table I
Acids Found as Components of the Preen Gland Secretion of Peiping Duck

Structure	Relative abundance, %
2D-Methylpentanoic acid	0.5
n-Hexanoic acid	1.8
2D-Methylhexanoic acid	33.7
4D-Methylhexanoic acid	40.5
n-Heptanoic acid	0.3
2D-Methylheptanoic acid	3.1
4D-Methylheptanoic acid	2.8
n-Octanoic acid	0.4
2D-Methyloctanoic acid	6.9
{4D-Methyloctanoic acid / 6D-Methyloctanoic acid}	6.9
n-Nonanoic acid	0.3
2D-Methylnonanoic acid	0.5
4D-Methylnonanoic acid	0.2
n-Decanoic acid	0.4
2D-Methyldecanoic acid	0.9
{4D-Methyldecanoic acid / 6D-Methyldecanoic acid}	0.8
	100.0

The analysis of the preen gland waxes of these two waterfowl, the goose and the duck, gave a hint as to the ways in which nature makes a good wax for oiling the plumage. Evidently the wax must be saturated and noncrystallizable. These requirements can be met in two ways, either by the use of a single alcohol and one or two highly branched acids or by a mixture of monomethyl-substituted acids esterified with a mixture of normal and monomethyl-substituted long-chain alcohols (see Table III).

In addition to small amounts of the acids of the goose, the mute swan[19] (*Cygnus olor* L.) was found to possess two trimethyl-substituted acids esterified with

(11) G. Odham, *Ark. Kemi.*, **22**, 417 (1964).
(12) G. Weitzel and K. Lennert, *Z. Physiol. Chem.*, **288**, 251 (1952).
(13) G. Weitzel, A.-M. Fretzdorff, and J. Wojahn, *ibid.*, **291**, 29 (1952).
(14) S. Ställberg-Stenhagen and E. Stenhagen in "Topics in Organic Mass Spectrometry," A. L. Burlingame, Ed., Wiley, New York, N. Y., 1970, p 167.
(15) R. Ryhage and E. Stenhagen, "Mass Spectrometry of Organic Ions," F. W. McLafferty, Ed., Academic Press, New York and London, 1963, Chapter 9.
(16) G. Odham and E. Stenhagen, "Biochemical Applications of Mass Spectrometry," G. Waller, Ed., Wiley–Interscience, New York, N. Y., in press, Chapter 3.
(17) K. Heyns, and L. Blazejewicz, *Tetrahedron*, **9**, 67 (1960).
(18) S. Abrahamsson, S. Ställberg-Stenhagen, and E. Stenhagen, *Progr. Chem. Fats Other Lipids*, **7** (pt 1) (1963).
(19) G. Odham, *Ark. Kemi*, **23**, 431 (1965).

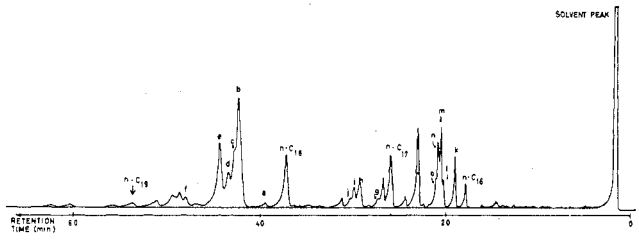

Figure 3. Gas chromatogram of methyl esters of fatty acids derived from the alcohols of the preen gland secretion of the duck: Golay capillary column type R (polypropylene glycol) at 199°.

Table II[a]

Alcohols Found as Components of the Preen Gland Secretion of Peiping Duck

Class	Structure	Relative abundance, %	
Straight chain alcohols	1-Hexadecanol	3.1	
	1-Heptadecanol	3.1	
	1-Octadecanol	34.2	
	1-Nonadecanol	0.5	40.9
Branched C_{17} alcohols	2-Methyl-1-hexadecanol	2.2	
	4-Methyl-1-hexadecanol	3.0	
	6-Methyl-1-hexadecanol + 8-Methyl-1-hexadecanol	0.6	
	10-Methyl-1-hexadecanol	3.0	
	12-Methyl-1-hexadecanol	0.7	
	14-Methyl-1-hexadecanol	4.9	14.4
Branched C_{18} alcohols	2-Methyl-1-heptadecanol	0.4	
	4-Methyl-1-heptadecanol	2.2	
	6-Methyl-1-heptadecanol + 8-Methyl-1-heptadecanol + 10-Methyl-1-heptadecanol	2.0	
	14-Methyl-1-heptadecanol	0.4	5.0
Branched C_{19} alcohols	2-Methyl-1-octadecanol	0.9	
	4-Methyl-1-octadecanol	2.6	
	6-Methyl-1-octadecanol + 8-Methyl-1-octadecanol + 10-Methyl-1-octadecanol	18.5	
	12-Methyl-1-octadecanol	0.9	
	14-Methyl-1-octadecanol	8.9	
	16-Methyl-1-octadecanol	0.9	32.7
Unidentified alcohols C_{18} range		5.0	
Unidentified alcohols C_{19} and C_{20} range		2.0	
		100.0	100.0

[a] Tables II, III, IV, and VI are reproduced through the courtesy of the publishers of *Arkiv för Kemi*, Almqvist och Wiksell, Stockholm.

a mixture of normal-chain and monomethyl-substituted alcohols. The structures of the acids were deduced from the mass spectra as 2,4,6-trimethyloctanoic and 2,4,6-trimethylnonanoic acids. In this case it was possible to show the all-D configuration of the C_{12} acid by direct gas chromatographic comparison with synthetic material (*cf.* section on stereochemistry). The alcohols of the swan are similar to those of the duck, but the swan alcohols contain more (73%) straight-chain material.

Bertelsen has studied several species of swans in order to make a complete study of all the species within this tribe. The whooper swan[20] (*Cygnus cygnus*) was found to possess the same acids as the mute swan. The C_{12} structure dominates quantitatively.

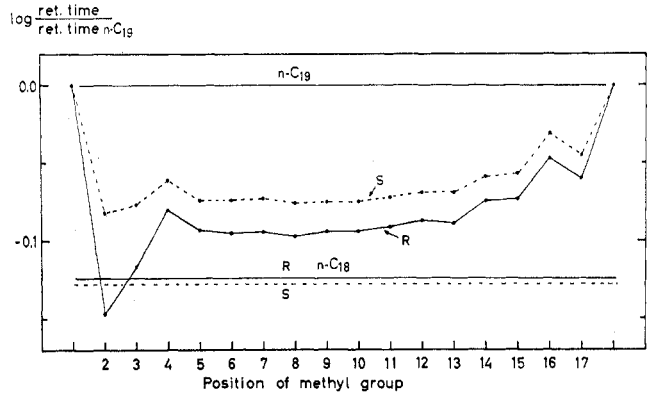

Figure 4. Relative retention time data for methyl esters of monomethyl-substituted acids of the C_{19} series: stationary phase, R = Reoplex 400, S = silicone.

Table III

Acids and Alcohols Found as Components of the Preen Gland Wax of Peiping Ducks and Common Mallards

Structure	Relative abundance, % Peiping duck		Common mallard (*Anas platyrhynchos*)	
A. Acids				
2D-Methylpentanoic acid	0.5		0.2	
n-Hexanoic acid	1.8		12.5	
2D-Methylhexanoic acid	33.7		35.8	
4D-Methylhexanoic acid	40.5		35.4	
n-Heptanoic acid	0.3		0.1	
2D-Methylheptanoic acid	3.1		3.3	
4D-Methylheptanoic acid	2.8		2.3	
n-Octanoic acid	0.4		0.1	
2D-Methyloctanoic acid	6.9		4.6	
4D-Methyloctanoic acid	6.9		5.6	
Acids with longer chains	3.1		0.1	
	100.0		100.0	
B. Alcohols				
1-Hexadecanol	3.1		9.7	
1-Heptadecanol	3.1		8.3	
1-Octadecanol	34.2		43.3	
1-Nonadecanol	0.5		0.7	
		40.9		62.0
Branched C_{17} alcohols	14.4		15.0	
Branched C_{18} alcohols	9.0		3.8	
Branched C_{19} alcohols	34.7		18.5	
		58.1		37.3
	99.0		99.3	

The black-necked swan (*Cygnus melanocoryphorys*) is another member of this tribe. In addition to the structures just mentioned, the acid moiety consists of 2,6-nonanoic and 2,6-undecanoic acids, together with 2,4,6-trimethyl-substituted acids of longer chain length.

The black swan (*Cygnus atratus*) has independently been studied by Edkins in Australia.[21] His results have been confirmed by Bertelsen. The preen gland wax contains 2,4-dimethylhexanoic acid (31.8%) and 2,4-dimethylheptanoic acid (47.5%), the rest consisting of the C_{11} and C_{12} trimethyl-substituted acids.

Preliminary studies on the Coscoroba swan (*Cos-*

(20) O. Bertelsen, personal communication.
(21) E. Edkins, personal communication.

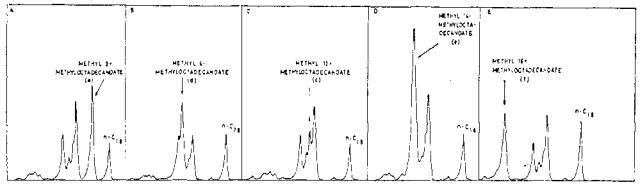

Figure 5. Gas chromatograms obtained after adding known methyl esters of C_{19} monosubstituted acids. Conditions same as for Figure 3.

coroba coscoroba) indicate a close resemblance to the mute and the whooper swans.

The common eider (*Somateria molissima*)[22] gave a wax that separated into two fractions on chromatography on silicic acid (Figure 6). It was found that the wax in the larger peak (A) possesses branched hydrocarbon chains, whereas those in peak B had normal chains. Peak B contained normal fatty acids bound to normal-chain alcohols, preferably 1-hexadecanol, whereas peak A contained the branched-chain acids ester-bound to normal-chain (preferably 1-octadecanol) as well as branched-chain alcohols. Table IV shows the acids present. No monomethyl-substituted acids are found, and the acids with three or four methyl side chains are the same as for the mute swan. A number of dimethyl-substituted acids are present.

The feather wax of the barnacle goose[22] (*Branta leucopsis*) is very different from that of the true geese. The component acids are all trimethyl substituted, and one of these appears to be a stereoisomer, possibly 2L,4D,6D-trimethyloctanoic acid, of the main component, 2D,4D,6D-trimethyloctanoic acid (*cf.* section on stereochemistry).

The white-faced whistling duck[22] (*Dendrocygna viduata*) is intermediate between the true geese (Anser) and the swans. The red-breasted merganser[22] (*Mergus serrator*) produces essentially 2,4,6-trimethylnonanoic acid (97%), whereas the muscovy duck[22] (*Cairina moschata*) has almost exclusively (99%) 2,4,6-trimethyloctanoic acid. In the last-mentioned case chromatography of the wax on deactivated alumina gave two fractions, A and B, as shown in Figure 7. Peak A, making up about two-thirds of the material, was identified as the unsaturated hydrocarbon squalene. The alcohol moiety contained only normal chain C_{18} and C_{20} alcohols.

Large amounts of squalene (87%) have also been found in the preen gland wax of the Magpie goose (*Anseranas semipalmata*), by Edkins.[21] This species takes an isolated position in the systematics in that it constitutes the only member of the subfamily Anseranatinae of the family of Anatidae. The chemical composition of the wax is remarkably different from that of all other species in the family. Only straight-chain acids (C_7–C_{12}) and alcohols (C_{10}–C_{16}) are found.

The flamingo (*Phoenicopterus ruber* L.) stands, in respect to systematics, between the orders of Anseriformes and Ciconiiformes. The composition of the

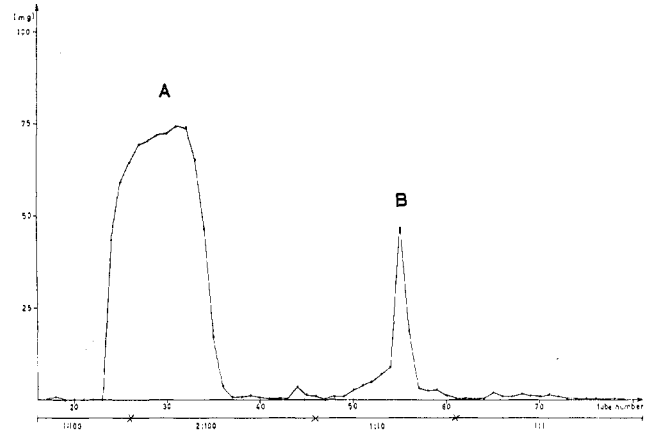

Figure 6. Liquid chromatogram of secretion from the common eider on silicic acid.

Table IV
Acids Found as Components of the Preen Gland Wax of Common Eider

Structure	Relative abundance, %
Branched Acids	
2,6-Dimethyloctanoic acid	18.2
2D,4D,6D-Trimethyloctanoic acid	12.6
4,6-Dimethyloctanoic acid	4.9
2,4-Dimethylnonanoic acid	1.3
2D,4D,6D-Trimethylnonanoic acid	27.0
2,6-Dimethyldecanoic acid	7.8
2,8-Dimethyldecanoic acid	2.7
2D,4D,6D,8D-Tetramethyldecanoic acid	2.1
2,6,8-Trimethyldecanoic acid	8.6
2,6-Dimethylundecanoic acid	3.1
2D,4D,6D,8D-Tetramethylundecanoic acid	1.5
Unidentified material	5.9
Normal Acids	
n-Hexanoic acid	0.1
n-Heptanoic acid	0.1
n-Octanoic acid	1.4
n-Nonanoic acid	0.2
n-Decanoic acid	2.1
n-Undecanoic acid	0.1
n-Dodecanoic acid	0.3
	100.0

preen gland secretion of this bird was studied by Bertelsen,[23] who found a mixture of dimethyl- and trimethyl-substituted fatty acids (Table V and Figure 8). It is remarkable that in the last-mentioned groups of acids there is positional isomerism; for example, 2,4,6-trimethyldecanoic and 2,6,8-trimethyldecanoic acid coexist.

All the above waterfowl belong to the family of Anatidae of the order of Anseriformes. It was considered useful to obtain more experience outside the Anatidae; one species belonging to water birds (Charadriiformes), *i.e.*, the oyster catcher (*Haematopus ostralegus* L.), was therefore studied.[24] The component acids consist of a mixture of monomethyl- and dimethyl-sub-

(22) G. Odham, *Ark. Kemi*, **27**, 263 (1967).

(23) O. Bertelsen, *ibid.*, **32**, 17 (1970).
(24) H. Karlsson and G. Odham, *ibid.*, **31**, 143 (1969).

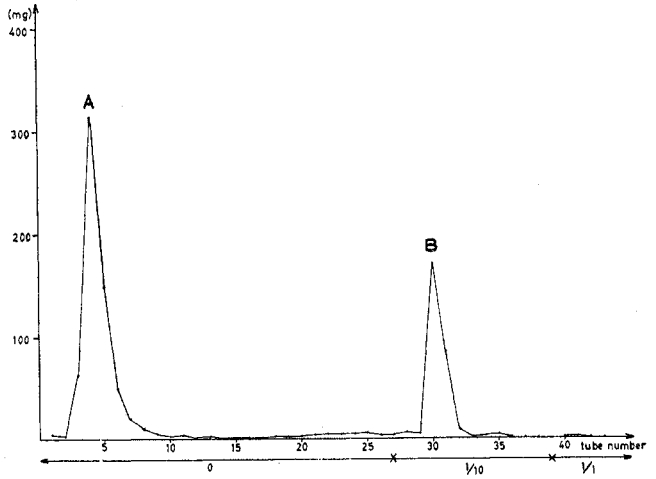

Figure 7. Liquid chromatogram of secretion from the muscovy duck on deactivated aluminum oxide.

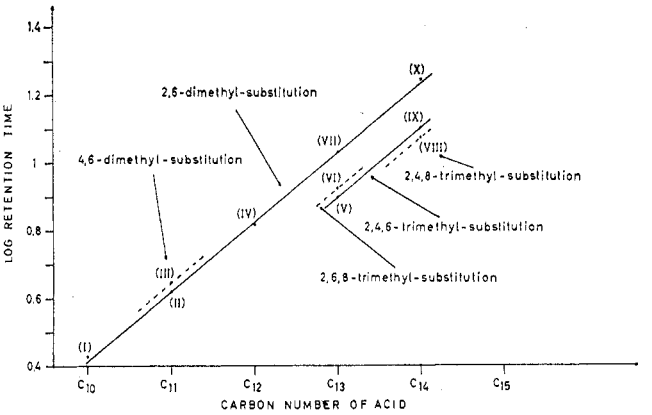

Figure 8. Relative retention times of methyl esters of fatty acids from the wax of the flamingo plotted on a log scale against their carbon number.

Table V

Acids Found as Components of the Preen Gland Wax of Flamingo

Structure	Relative abundance, %
2,6-Dimethyloctanoic acid	1.7
2,6-Dimethylnonanoic acid	29.5
4,6-Dimethylnonanoic acid	3.8
2,6-Dimethyldecanoic acid	1.6
2,4,6-Trimethyldecanoic acid	3.3
2,6,8-Trimethyldecanoic acid	7.1
2,6-Dimethylundecanoic acid	2.8
2,4,8-Trimethylundecanoic acid	22.8
2,4,6-Trimethylundecanoic acid	27.3
2,6-Dimethyldodecanoic acid	0.1
	100.0

stituted structures, as shown in Table VI. The optical rotation of the ester mixture was $[\alpha]^{20}D$ +9.5°, and as approximately 86% of the mixture can be regarded as 2-methyl-substituted as far as the optical rotation is concerned, it is evident that the acids possess an L configuration, in contrast to those found in the preen glands of Anseriformae (*vide infra*). The alcohols were a mixture of normal-chain and branched-chain isomers. 16-Methyl-1-octadecanol made up 10% of the alcohol moiety.

Steric Configurations of Methyl-Branched Wax Acids

Methyl-substituted fatty acids can exist in two or more stereoisomeric forms depending on the number of methyl branches present. The 2,4,6-trimethylnonanoic acid, which is present in several species, exists in eight stereoisomeric forms, of which only one represents the correct structure of the natural acid found as major component. The optical rotation of the latter indicated a D configuration at C-2. It was shown by gas chromatography (Figure 9) of a mixture of the four optically active isomers with the 6D configuration (2D,4D,6D; 2D,4L,6D; 2L,4D,6D; and 2L,4L,6D), each prepared by total synthesis,[25] and of the C_{12} acid from the mute swan that all three asymmetric carbon atoms of the latter possess the D configuration.[26] The levo-

(25) G. Odham, *Ark. Kemi*, **27**, 231 (1967).
(26) G. Odham, *ibid.*, **27**, 251 (1967).

Table VI

Acids Found as Components of the Preen Gland Wax of Oyster Catcher[24]

Structure	Relative abundance, %
2-Methylhexanoic acid	8.5
2-Methylheptanoic acid	3.7
2-Methyloctanoic acid	12.4
2,6-Dimethyloctanoic acid	3.4
2-Methylnonanoic acid	3.7
2,6-Dimethylnonanoic acid	2.5
2-Methyldecanoic acid	16.0
4-Methyldecanoic acid + 2,6-Dimethyldecanoic acid	5.1
2,8-Dimethyldecanoic acid	4.5
2-Methylundecanoic acid	5.5
4-Methylundecanoic acid + 2,6-Dimethylundecanoic acid	1.1
2,8-Dimethylundecanoic acid	1.8
2-Methyldodecanoic acid	13.0
4-Methyldodecanoic acid	4.0
2,8-Dimethyldodecanoic acid	4.1
2,10-Dimethyldodecanoic acid	2.1
2-Methyltridecanoic acid	3.0
2-Methyltetradecanoic acid	5.6
	100.0

rotation of 2-methylhexanoic acid and 4-methylhexanoic acid from the Peiping duck indicates the D configuration at the active center. However, there are indications that stereoisomers also exist. For example, the C_{12} 2D,4D,6D-trimethyl-substituted acid from the swan is accompanied by a minute amount of a stereoisomer (component B).[26] The same is true also for the C_{11} acid. The gas chromatogram (Figure 10) of a large quantity of the natural acids mixed with synthetic isomers shows that component B is 2L,4D,6D-trimethylnonanoic acid (or the enantiomer of this acid).

The tendency of the species within the family of Anatidae to synthesize optically active branched fatty acids with an all-D configuration appears not to be a general feature. Preliminary investigations of the acid moiety of the wax of the magellan penguin revealed that the biosynthesis here leads to the presence of about equal portions of 2D,4D,6D- and 2L,4D,6D-trimethyl-substituted acids.

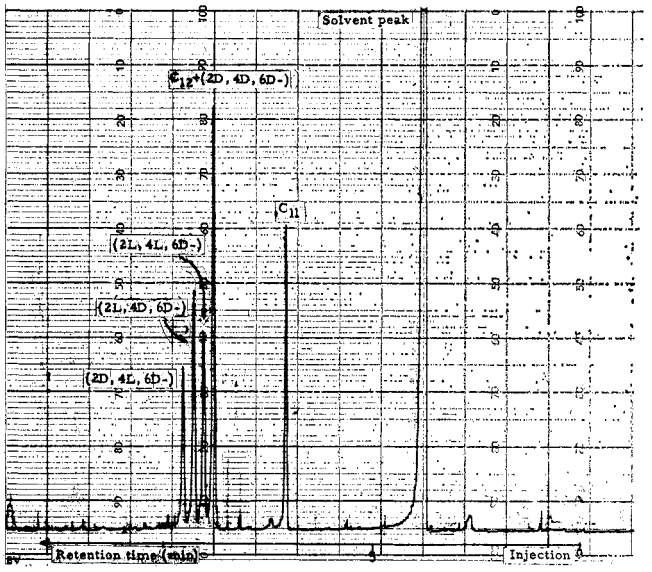

Figure 9. Gas chromatogram of the four stereoisomers of 2,4,6D-trimethylnonanoic acid with added methyl esters of the acids from the mute swan: Golay capillary column type R (polypropylene glycol) at 151°.

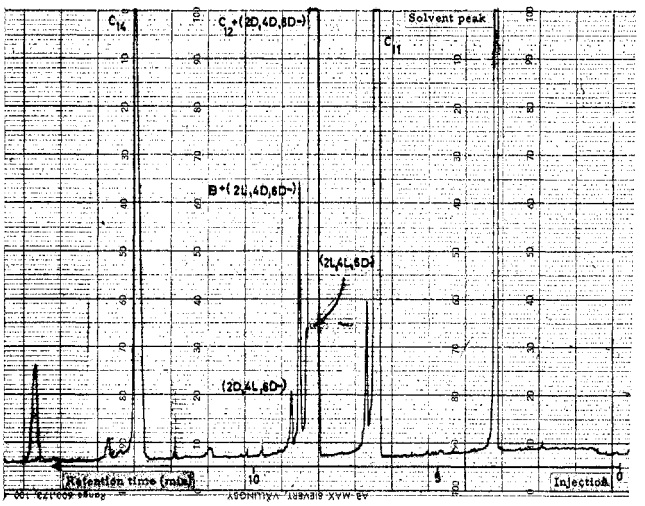

Figure 10. Gas chromatogram of the four stereoisomers of 2,4,6D-trimethylnonanoic acid with an added large quantity of the methyl esters of the acids from the mute swan: Golay capillary column type R (polypropylene glycol) at 151°.

The acids of the oyster catcher (*Haematopus ostralegus* L.) constitute an even more striking example. As already mentioned, the mixture of the methyl esters is dextrorotatory ($[\alpha]^{20}D$ +9.5°). About 86% of the total acid moiety can be regarded as 2-methyl-substituted with respect to optical rotation since the second branch in the dimethyl-substituted structures is situated near the middle of the chain.[21] It is known that 2-methyl-substituted acids with an L configuration at the asymmetric center are dextrorotatory. Accordingly, most of the structures present possess the L configuration at C-2.

Biosynthesis

The branched acids are biosynthetically built up from smaller fragments in a manner similar to the biosynthesis of normal fatty acids. Lederer, *et al.*,[27] have shown that the 2,4,6,8-tetramethyldecanoic acid from the preen gland wax of the greylag goose is derived from acetate and propionate units with C-3 in the propionate molecule forming the methyl branch. This anteiso structure is thus built up from one acetate and four propionate molecules. The acetate forms the end ethyl group. Analogously, the next higher "homolog," 2,4,6,8-tetramethylundecanoic acid, possessing a propyl end group, is derived from five propionate units. Similar experiments by the authors indicate that 2-methylhexanoic acid and 4-methylhexanoic acid, constituting the major acid components in the wax from the Peiping duck, are biosynthesized in an analogous manner. The butyl end group of the former acid is thus formed from two acetate units and the molecule completed by addition of a propionate unit.

No experiments regarding the biosynthesis of the branched alcohols have so far been performed. However, there are indications[11] that monomethyl-substituted acids are first synthesized through the mechanism described and that the acids in a later step are reduced to the corresponding alcohols.

The mode of formation presented above explains why the branched acids and alcohols always have the methyl side chains at even-numbered carbon atoms.

Nothing is so far known about the enzyme systems responsible for the biosynthesis. The preen gland is, however, very specific in its biosynthetic function of producing branched molecules. One has therefore an excellent opportunity here to study, on an enzymatic level, the biosynthesis of branched-chain fatty acids and alcohols in environments apparently not complicated by a large number of other enzymatic activities.

Some Chemotaxonomic Aspects

The high species specificity in the biosynthesis of the compounds in the preen gland secretion suggests that these can be employed for taxonomic purposes.

The somewhat intermediate position taken by the Tadorna species as regards morphology, ecology, and ethology within the family Anatidae made a study of their preen gland secretion interesting from the taxonomic point of view. *Tadorna ferruginea* Pall. (ruddy shelduck) and *Tadorna tadorna* L. (common shelduck) were therefore studied. The results are shown in Table VII. A comparison between the secretions of the greylag goose (*Anser anser*), the common mallard (*Anas platyrhynchos*), and the mute swan (*Cygnus olor*) reveals that the Tadorna species possess the same fatty acids as the mute swan (*cf.* Table VII), but with respect to relative abundance the discrepancies are larger than among the Tadorna species themselves. With regard to the alcohols the shelducks differ considerably from the mute swan. The secretion of the latter contains about 27% of monomethyl-substituted alcohols, whereas the Tadorna species produces such alcohols in trace (about 1%) amounts only. In this

(27) R. Noble, R. Stjernholm, D. Mercier, and E. Lederer, *Nature*, **199**, 600 (1963).

Table VII
Comparison of the Acids of the Tadorna Species with Those of the Mute Swan

Structure	Relative abundance, % *Tadorna ferruginea*	*Tadorna tadorna*	*Cygnus olor*
2D,4D,6D-Trimethyloctanoic acid	28.8	18.2	42.0
2D,4D,6D-Trimethylnonanoic acid	66.6	78.1	44.5
2D,4D,6D,8D-Tetramethyldecanoic acid	2.2	2.5	11.4
2D,4D,6D,8D-Tetramethylundecanoic acid	2.4	1.2	2.1
	100.0	100.0	100.0

respect they resemble the goose and differ entirely from the ducks.

The chemical composition of the preen gland secretions of *Tadorna ferruginea* and *Tadorna tadorna* shows such large similarity that a close relationship between the two species may be suspected. Furthermore, the two Tadorna species appear to constitute intermediates between the species of Anser and Cygnus.

Delacour and Scott[28] write that the Tadornini are "closely related to the river ducks (Anatini), to which they are linked by species that form a gradual transition between them." Lorenz[29] also considers that the Tadornini are more linked to other ducks (Anatinae) than to "true geese" (Anserini).

The greylag goose (*Anser anser*), the mallard, and the muscovy duck (*Cairina moschata*) are domesticated forms which differ in habitus and habits from the wild forms. The greylag goose is probably the oldest domesticated waterfowl; it appears on Egyptian frescoes about 4000 years old. The mallard was kept for food at the time of the Roman empire and was fully domesticated in Europe during the middle ages. With regard to the muscovy duck, Delacour and Scott[28] write "the Spaniards found them already fully domesticated by the Indians of Peru and Columbia, and no one knows how long they had been so kept by them." The domesticated forms usually have greatly increased in size, and most of them have lost their ability to fly. The neck has become longer, and many forms have taken up a more erect posture. Color mutations have occurred, and changes in behavior are also common; for example, they have become polygamous and lay many more eggs than their wild ancestors.

The Peiping duck and its wild ancestor, the mallard (*Anas platyrhynchos*), were considered a suitable pair for comparison, since the preen gland secretion of the former is very specific and complex both with respect to acids and alcohols. The Peiping duck is a yellowish white, large Chinese breed which stands fairly erect. It has existed for a long time in China and came to Europe and America in about 1850. Table III shows the result of the comparative study. Both qualitatively and quantitatively the compositions are virtually identical. It is thus apparent that the pattern of the wax components has not changed during the gradual evolution from the wild species into the present form of the Peiping duck. In other words, the enzymatic system which controls the biosynthesis of the specific preen gland components is genetically conservative and has remained unaltered during the time many other mutations have taken place.

New Possibilities for the Rehabilitation of Oiled Seabirds

Release of petroleum from tankers is a constant threat to seabirds, especially along seaboards or in inland seas. The grounding of the Torrey Canyon in March 1967 was a major disaster for the bird life on the Cornish coast. A month later over 8000 birds, mainly guillemots (*Uria aalge*), razorbills (*Alca torda*), and shags (*Phalacrocorax aristotelis*), had been collected and taken to centers for rehabilitation (*cf.*, *e.g.*, ref 30 and 31). The problem has recently been accentuated in the Baltic where the sea traffic now includes tankers of more than 100,000 tons.

Experience gained in the study of oiled seabirds has shown that both water repellency and heat insulation, two very important functions of the plumage, are highly affected by the oil. After oiling, the bird's ability to fly decreases or is totally lost, and feeding becomes difficult or impossible. The risk of poisoning by toxic sulfur compounds in the oil is also very real. Post mortems have shown that oil is frequently present in the digestive tract, presumably as a result of preening. Oil poisoning changes the natural bacterial flora and is often followed by fungal infections of the intestinal organs.

When detergents are used to wash oiled seabirds, the natural feather wax is removed as the solubility and emulsifying properties of the feather wax and the contaminating oil are almost identical. Due to the importance of wax in maintaining water repellency and heat insulation, no seabird can be returned to its natural environment until the wax has been replaced in one way or another.

Since guillemots in particular were affected by the Torrey Canyon disaster and nothing was known about the composition of their preen gland wax, we were asked by the ICI to investigate the wax. Capillary gas chromatography showed the presence of more than 100 fatty acids and about the same number of alcohols. We reproduce the gas chromatogram in Figure 11 as an illustration of the highly complex mixtures encountered in many birds. The inset is an expansion of the gas chromatogram between normal-chain C_8 and C_{12} methyl esters. There are, for example, at least 12 components with retention times between those of methyl undecanoate and methyl dodecanoate. If all possible combinations between acids and alcohols exist, the number of wax molecule structures exceeds 10,000.

(28) J. Delacour and P. Scott, "The Waterfowl of the World," Vol. I–IV, Country Life Ltd., London, 1954–1964.
(29) K. Lorenz, *J. Ornithol.*, **3**, 194 (1941).
(30) W. R. P. Bourne, J. D. Parrack, and G. R. Potts, *Nature*, **215**, 1123 (1967).
(31) J. V. Beer, *Wildfowl*, **19**, 120 (1968).

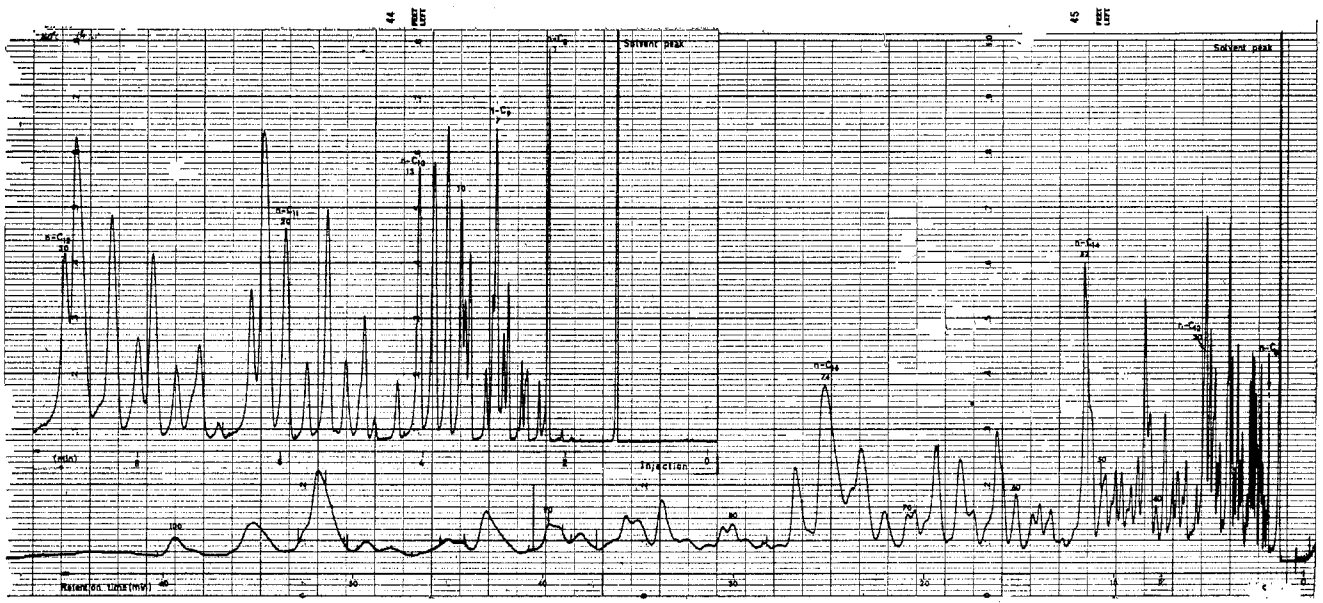

Figure 11. Gas chromatogram of methyl esters of fatty acids from the guillemot: Golay type R capillary column at 168°. Inset: Expanded region of the methyl esters from n-C_8 to n-C_{12} at 160° (H. Karlsson and G. Odham, unpublished).

As mentioned, the preen gland produces about 50 mg of wax per day, which compensates for the natural loss, and the plumage usually contains a few grams of preen gland wax. It is obvious that it takes a very long time for the bird to replace all the wax after cleaning. The species specificity and the sometimes very complex nature of the wax, as exemplified above, makes it essential to use a more simple composition for replacement purposes.

In connection with an accidental release of oil in the harbor of Göteborg, attempts were made to clean 150 oiled swans with an emulsion of triolein in water (Tremalon B). Synthetic wax (Pur-Cellin oil, Dragoco, Holtzminden, Germany) was subsequently sprayed on the plumage. In practice the spraying technique was not very satisfactory; overdoses were given, resulting in a plumage with the same properties as the original oiled plumage.

To overcome this problem a new cleaning agent, Larodan 127,[32] with which the waxing takes place during cleaning (a method similar to that sometimes used in cleaning cars), was formulated. The preparation consists of a dispersion of hydrophilic lipid crystals in water, made by a special procedure[33] with the commercially available synthetic wax mentioned above included in the hydrophobic regions of the lipid crystal matrix. The wax mainly contains the octadecyl ester of 2-methylhexanoic acid and the hydrophilic lipid is the 1-monoglyceride of dodecanoic acid. The proportions of the three components, monoglyceride, wax, and water, were adjusted on the basis of practical tests so that the final product consisted of 20% monoglyceride and 2% wax in water. Larodan thus contains two natural lipids.

Larodan has been used on a large scale in Scandinavia, in Gävle, for example, where about 75 birds belonging to the family of Anatidae were successfully cleaned and returned to their natural environment within a fortnight.

(32) K. Larsson, English Patent 1,174,672 (1970).
(33) K. Larsson and G. Odham, *Mar. Pollut. Bull.*, 1, 122 (1970).

The Thermal Addition of Carbon–Carbon Multiple Bonds to Strained Carbocyclics

PAUL G. GASSMAN

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

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The many similarities which exist in the chemical and physical properties of olefins and small carbocyclic rings have intrigued chemists for decades. In particular, cyclopropane, with 27.6 kcal/mole of strain energy, parallels propene in many of its chemical reactions. Both undergo hydrogenation to give propane. Both add hydrogen bromide. Both are protonated by strong mineral acids. Moreover, the vinylic hydrogens of