Diesters of 3-hydroxy fatty acids produced by the uropygial glands of female mallards uniquely during the mating season

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Abstract The uropygial gland secretions produced by female mallards (*Anas platyrhynchos*) throughout the year were analyzed by thin-layer chromatography and combined gas-liquid chromatography and mass spectrometry. Most of the year, the secretion was composed of wax esters. With the beginning of the mating season in the middle of March, a polar component appeared which became the dominant and sole component of the secretion through April and May and as the mating season ended in June, wax esters became the sole component of the secretion. The polar components were identified to be diesters of *n*-C_8, *n*-C_{10}, and *n*-C_{12} 3-hydroxy acids with *n*-C_{16} and *n*-C_{18} alcohols and *n*-C_6 to C_{16} even chain acids. Immediately after the diester-producing period the female uropygial glands produced very long chain wax esters composed of fatty acids longer than C_{12}. By the end of August, shorter chain wax esters composed of C_6 and C_{12} acids became the dominant components of the secretion and this composition, previously considered characteristic of mallards, remained constant until March. The observed disappearance of the short chain waxes during the postnuptial period is similar to that in males. The dramatic changes in the composition of the uropygial glands similar to those observed in the female mallards during the mating season have not yet been observed in any other species. — **Kolattukudy, P. E., S. Bohnet, and L. Rogers.** Diesters of 3-hydroxy fatty acids produced by the uropygial glands of female mallards uniquely during the mating season. *J. Lipid Res.* 1987. **28:** 582–588

Supplementary key words wax esters

Sebaceous glands in animals are known to generate a wide variety of lipids that perform many different functions that are not well understood (1–3). Whereas numerous diminutive glands are distributed throughout much of the mammalian skin, birds contain only one large uropygial gland (preen gland) located at the base of the tail feathers (4, 5). The composition of the complex mixture of lipids produced by these glands has been used for chemotaxonomic purposes (4–6). That caution should be exercised in such use of the composition was suggested first by the observation that both chain length and diastereoisomer composition of the alkane-2,3-diols of the chicken uropygial gland changed with the developmental stage of the bird (7). More recently, it was found that the composition of the wax esters in the uropygial gland of the male mallards changed dramatically during postnuptial molt (8). The cause of this change was suggested to be a suppression of the expression of the gene for S-acyl fatty acid synthase thioesterase (9). In view of these results and a report that products of female mallard glands showed pheromonal activity (10), we examined the changes in the structure and composition of the uropygial lipids of adult female mallards throughout the year. The results reported in this paper show that during the breeding season the wax esters of the gland are replaced by diesters of 3-hydroxy fatty acids. Following this period, long chain wax esters similar to those found in the males in eclipse are found also in the female, and after the summer, short chain esters reappear as the major products.

MATERIALS

Mallard ducks (*Anas platyrhynchos*) were purchased from the Ted-Mar licensed game farm, Puyallup, WA, and Whistling Wings, Inc., Hanover, IL, and maintained in outdoor cages on a high-energy breeder ration. The crude lipid was obtained by gently squeezing the uropygial gland; the exudate was weighed and stored at −20°C until further workup. A group of five birds was used and the lipid samples were pooled. The compositional analysis

Abbreviations: GLC, gas-liquid chromatography; MS, mass spectrometry.

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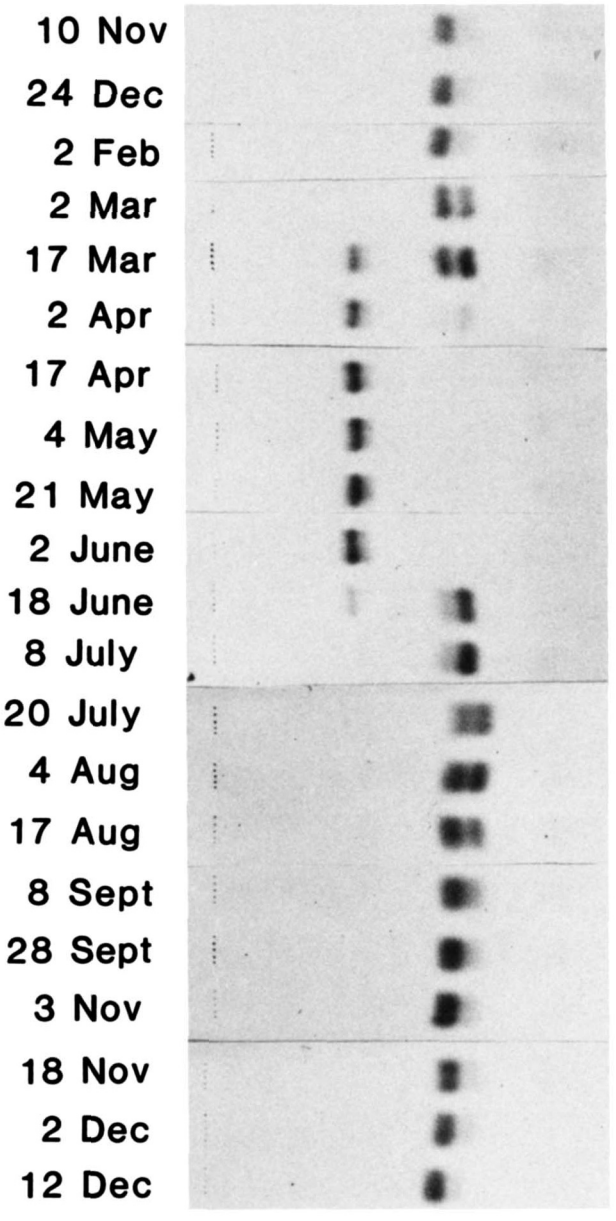

Fig. 1. Thin-layer chromatogram of the uropygial gland secretions of female mallards. The top doublet is composed of monoester waxes and the lower band represents diesters of 3-hydroxy acids. Chromatography conditions are indicated in the text. The origin is at the far left.

was followed for an entire year. A similar analysis was performed for two other seasons but with a less frequent sample collection. Results were very similar.

Analysis of wax samples

The diester and monoester wax portions of the crude lipid were isolated from the lipid exudate by thin-layer chromatography on 1-mm silica gel G plates (20 × 20 cm) with hexane–ethyl ether–formic acid 90:10:1 (v/v). Components were visualized under ultraviolet light after

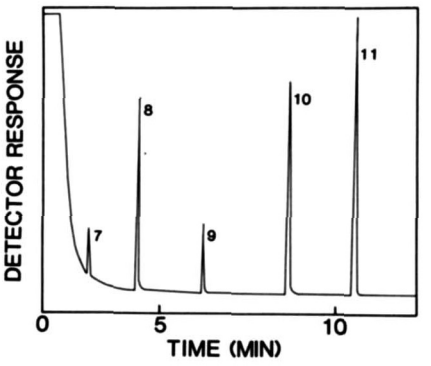

Fig. 2. Gas-liquid chromatogram of trimethylsilyl derivatives of 3-hydroxy acid methyl esters plus fatty alcohols derived from BF_3/methanol transesterification of the diesters obtained from the uropygial glands of female mallards on April 17. Chromatographic conditions are shown in the text; 7, 8, and 9 are 3-hydroxy n-C_8, n-C_{10}, and n-C_{12} acids, respectively; 10,n-C_{16} alcohol; 11,n-C_{18} alcohol.

spraying the plates with 0.1% ethanolic solution of dichlorofluorescein, and the lipids from the two regions were eluted with ethyl ether. An aliquot of the diester wax sample (2–4 mg) was dissolved in 0.5 ml of toluene; 2 to 3 ml of 14% BF_3 in methanol (Sigma Chemical Co., St. Louis, MO) was added and the mixture was refluxed for 3 hr. After the addition of 10 ml of water, the products were thoroughly extracted with chloroform. After removing the solvent by a gentle stream of nitrogen, the residue was subjected to thin-layer chromatography as above. The methyl ester and the combined 3-hydroxy fatty acid methyl ester/fatty alcohol portions were eluted with ethyl ether and chloroform–methanol 2:1, respectively. The 3-hydroxy fatty acid methyl esters and fatty alcohols were converted to their trimethylsilyl derivatives by heating them with 50 μl of N,O-bis (trimethylsilyl)-acetamide (Sigma Chemical Co.) at 90°C for 20 min. Excess reagent

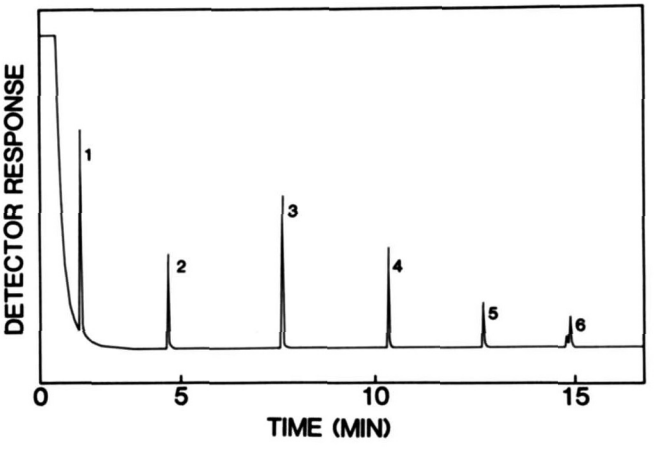

Fig. 3. Gas-liquid chromatogram of fatty acid methyl esters obtained from BF_3/methanol transesterification of the diesters from the uropygial glands of female mallards on April 17. Chromatographic conditions are shown in the text; 1–6 indicate even chain acids from n-C_6–C_{16}.

TABLE 1. The composition of the fatty acids, 3-hydroxy fatty acids, and primary alcohols of the diester waxes from the uropygial gland of female mallard ducks

Component	2 Apr	17 Apr	4 May	21 May	2 June	18 June
			% of total fatty acids			
Fatty acid						
Hexanoic	20.8	23.0			14.5	22.6
Octanoic	15.3	12.7	9.2	13.6	12.7	15.8
Decanoic	22.8	33.9	40.2	37.6	28.7	28.6
Dodecanoic	15.6	16.5	24.3	23.9	16.3	15.6
Tetradecanoic	11.3	6.3	14.8	12.4	11.0	6.0
Hexadecanoic	14.2	7.6	11.5	12.5	16.8	11.5
3-Hydroxy fatty acid						
Octanoic	21.6	13.4	11.2	17.8	12.0	16.5
Decanoic	69.6	69.6	74.0	68.8	75.0	72.8
Dodecanoic	8.8	17.0	14.8	13.4	13.0	10.7
Fatty alcohol						
Hexadecanol	49.5	40.8	44.9	43.5	35.9	42.4
Octadecanol	50.5	59.2	55.1	56.5	64.1	57.6

was removed with a stream of N_2 and the products were dissolved in chloroform prior to GLC–MS. The monoester wax fractions were transesterified with 14% BF_3 in butanol and the butyl esters and alcohols were isolated by thin-layer chromatography and analyzed by combined GLC–MS as described before (8).

TABLE 2. Composition of the acyl portion of the uropygial gland monoester wax collected from female mallard ducks at different times during the year (% of total acid)[a]

Fatty Acid	24 Dec	2 Feb	2 Mar	17 Mar	2 Apr	17 Apr May 4, 20 2 June[b]	18 June	8 July	20 July	4 Aug	17 Aug	8 Sept	28 Sept	12 Oct	3 Nov
2-Me-C_6	22.8	11.0	13.2	15.7	3.4		2.1	0.6	2.7	7.2	7.4	19.9	14.6	23.6	23.4
4-Me-C_6	24.1	6.0	3.6	4.4	–		–	0.4	2.2	6.8	6.5	28.1	22.3	34.9	27.2
2-Me-C_7	2.4	1.9	3.6	4.9	–		0.9	–	0.7	1.9	3.1	3.4	3.8	2.4	1.9
4-Me-C_7	2.0	–	–	–	–		–	–	–	1.2	1.2	3.2	3.8	2.8	1.8
2-Me-C_8	4.7	2.3	1.2	2.1	–		1.1	0.4	1.7	2.9	3.3	5.3	5.4	5.2	3.2
4-Me-C_8	4.7	0.7	–	–	–		–	–	1.1	2.4	2.5	6.1	7.1	7.2	3.8
2,6-Me_2-C_8	–	–	–	3.1	1.5		1.6	0.7	1.4	1.3	2.9	0.8	1.7	–	–
2-Me-C_9	0.4	0.6	1.0	1.0	–		1.4	0.3	–	0.8	0.5	0.6	1.1	–	–
2,6-Me_2-C_9	–	–	–	1.3	–		–	–	–	–	–	–	–	–	–
2-Me-C_{10}	–	–	–	0.5	–		1.0	0.8	2.4	2.5	3.0	2.4	3.6	1.8	0.7
4-Me-C_{10}	–	–	–	–	–		–	–	–	1.0	0.9	–	2.7	1.6	0.6
n-C_{11}	–	–	–	–	–		–	–	–	–	–	1.6	–	–	–
2,6-Me_2-C_{10}	–	–	–	–	–		1.4	0.8	0.9	0.8	1.4	–	0.8	–	–
2-Me-C_{11}	1.4	–	–	0.9	–		–	–	–	–	0.7	–	–	–	–
4-Me-C_{11}	1.1	–	–	–	–		–	–	–	–	0.6	–	0.9	–	–
2,6-Me_2-C_{11}	–	–	–	0.7	1.7		1.1	0.4	–	–	0.5	–	–	–	–
2-Me-C_{12}	1.0	0.8	0.6	0.8	1.8		1.3	1.7	4.7	3.4	4.2	2.7	4.5	1.5	1.0
4-Me-C_{12}	1.5	0.6	–	–	–		–	0.9	3.1	2.3	2.7	2.3	4.1	2.1	1.6
2,6-Me_2-C_{12}	0.4	0.8	1.2	1.6	2.0		1.2	0.9	1.5	0.9	1.4	0.8	0.9	–	–
2,8-Me_2-C_{12}	1.0	–	–	–	–		–	0.8	1.5	0.9	0.4	–	1.2	–	–
4,8-Me_2-C_{12}	0.4	–	–	–	–		–	–	1.1	–	0.6	–	–	–	–
2-Me-C_{13}	0.3	1.0	1.1	1.3	–		–	0.7	–	0.9	1.2	0.6	1.1	–	0.9
2,6,10-Me_3-C_{12}	0.3	–	3.6	5.9	1.6		2.8	1.0	–	1.2	1.1	–	–	–	–
4-Me-C_{13}	–	–	–	–	–		1.1	0.4	–	–	1.1	–	–	–	–
2,6-Me_2-C_{13}	–	1.4	0.8	1.2	6.6		0.7	0.6	–	–	0.4	–	–	–	–
4,6-Me_2-C_{13}	–	–	–	–	–		1.1	0.6	–	–	–	–	–	–	–
2,6,8-Me_3-C_{13}	–	–	1.6	2.0	2.7		–	0.3	–	0.7	1.6	–	–	–	–
2-Me-C_{14}	1.4	2.6	1.6	1.6	2.9		1.8	2.2	5.7	3.2	4.1	2.3	3.7	1.4	1.8
4-Me-C_{14}	3.0	2.7	0.9	–	–		1.6	2.0	3.0	1.7	2.1	1.9	2.7	3.0	3.6
2,6-Me_2-C_{14}	1.0	1.8	2.1	1.8	2.8		2.0	1.2	1.9	1.3	1.7	0.8	1.1	0.5	1.5

[a] The following components were identified but were not more than 1% of the total acids at any time: 2-methyl-C_5, n-C_6, n-C_7, n-C_8, 2,6-dimethyl-C_7, n-C_{10},2,8-dimethyl-C_{13}, 4,8-dimethyl-C_{13}, 2,6,10-trimethyl-C_{13}, 2-methyl-C_{15} and n-C_{16}. Me, methyl; Me_2, dimethyl; Me_3, trimethyl.
[b] None, only diester.

Gas-liquid chromatography-mass spectrometry

A Hewlett-Packard model 5840 gas chromatograph attached to an HP 5985 mass spectrometer was used to obtain the electron impact mass spectra. Samples were injected on a 25 m × 0.2 mm, OV-1 glass capillary column. All butyl esters and methyl esters were separated with a 2.5-min isothermal period at 80°C followed by an 80–280°C temperature program at 10°C/min. The intact lipids and the trimethylsilyl derivatives of the 3-hydroxy fatty acid methyl esters and fatty alcohols were separated with a 1.0-min isothermal period at 150°C followed by a 150–280°C temperature program at 10°C/min. All analyses were repeated several times but little variation was observed; typical results are shown in the tables and figures.

RESULTS

Thin-layer chromatographic examination of the lipids produced by adult females of mallard ducks revealed dramatic seasonal variation (**Fig. 1**). Until late February, a single wax ester fraction with an R_f identical to that shown by the wax esters of males was observed with the gland products of the females. In late March to early April, a much more polar component began to appear and this component became the dominant product of the gland with no detectable wax ester fraction by the end of April. At the end of June and into July, wax esters reappeared but the R_f of the wax ester band was higher than that of the wax found prior to the mating season and similar to the long chain wax esters found in the males

TABLE 2. (continued)

Fatty Acid	24 Dec	2 Feb	2 Mar	17 Mar	2 Apr	17 Apr May 4, 20 2 June[b]	18 June	8 July	20 July	4 Aug	17 Aug	8 Sept	28 Sept	12 Oct	3 Nov
2,8-Me_2-C_{14}	1.2	0.8	2.3	2.7	5.4		–	0.4	1.1	1.2	0.6	0.9	0.9	–	1.3
2,10-Me_2-C_{14}	0.4	2.4	3.0	2.5	3.5		1.5	0.9	–	–	1.2	–	–	–	–
4,6-Me_2-C_{14}	2.2	2.3	2.1	1.4	–		2.5	2.7	2.9	1.9	2.1	1.7	1.8	2.3	2.6
2,6,10-Me_3-C_{14}	–	3.4	4.6	4.5	6.0		2.1	1.1	1.3	–	1.2	–	–	–	0.6
2,6,8-Me_3-C_{14}	–	2.2	3.6	3.1	6.1		–	–	–	–	–	–	–	–	–
4,8-Me_2-C_{14}	1.9	1.3	–	–	–		2.0	2.0	1.1	1.2	0.9	1.6	–	–	0.5
2,6-Me_2-C_{15}	0.7	2.0	–	–	–		–	0.7	1.3	0.7	0.8	–	–	–	–
2,8-Me_2-C_{15}	0.6	0.7	–	–	–		2.4	1.2	1.7	1.1	0.1	–	–	–	–
2,4,8-Me_3-C_{15}	–	–	0.9	0.7	1.6		–	–	–	–	0.7	–	–	–	–
4,6-Me_2-C_{15}	2.2	2.7	6.2	2.6	7.5		5.4	8.6	3.9	4.3	3.8	–	1.0	1.4	2.2
2,10-Me_2-C_{15}	–	–	0.6	0.6	4.8		–	0.4	–	–	–	–	0.7	–	0.7
4,8-Me_2-C_{15}	0.8	1.1	1.4	1.3	–		2.6	2.6	2.1	2.0	1.4	–	–	–	–
4,10-Me_2-C_{15}	0.5	0.6	0.8	0.6	–		1.3	1.3	1.2	1.1	0.6	–	–	–	–
4,12-Me_2-C_{15}	–	–	–	–	–		2.5	2.1	2.2	1.0	1.3	–	–	–	–
4-Me-C_{15}	–	2.8	1.9	–	–		0.9	0.6	–	1.5	–	–	–	–	–
2,6,10-Me_3-C_{15}	–	–	0.6	1.1	–		–	–	–	–	–	–	–	–	–
2,6,8-Me_3-C_{15}	–	–	–	1.8	2.9		–	–	–	–	–	–	–	–	–
2-Me-C_{16}	0.9	3.6	2.8	1.6	–		3.7	3.4	5.2	2.8	3.2	1.1	1.4	0.8	1.4
2,6-Me_2-C_{16}	–	4.3	2.9	1.4	2.4		2.6	2.1	3.4	1.4	1.6	1.7	0.7	–	2.6
2,8-Me_2-C_{16}	1.7	2.4	1.1	0.7	1.2		1.0	0.8	1.7	0.6	0.6	0.7	–	–	0.7
2,10-Me_2-C_{16}	0.8	0.9	2.0	1.2	2.4		2.3	1.8	–	1.3	1.4	–	–	–	–
4,6-Me_2-C_{16}	–	2.8	2.1	1.0	1.3		4.5	5.3	3.1	3.2	–	0.6	–	–	–
4,8-Me_2-C_{16}	2.5	3.3	–	–	–		3.0	3.3	3.2	2.1	2.6	1.9	1.6	1.5	2.8
4,10-Me_2-C_{16}	2.7	–	2.6	1.4	1.9		2.9	2.9	3.0	2.2	1.7	1.8	1.4	1.9	3.1
2-Me-C_{17}	0.4	2.4	–	–	4.7		5.5	5.4	5.4	4.1	2.8	–	–	–	–
2,6,10-Me_3-C_{16}	–	1.2	4.0	2.7	–		–	–	–	–	–	–	–	–	–
2,6,8-Me_3-C_{16}	1.1	–	1.7	0.8	–		1.4	1.1	–	0.8	0.6	–	–	–	1.5
4,12-Me_2-C_{16}	1.8	1.4	1.0	0.5	–		4.1	4.9	2.9	2.9	1.6	–	–	–	–
2,6-Me_2-C_{17}	–	3.5	–	3.3	1.6		–	–	–	–	–	–	–	–	–
2,8-Me_2-C_{17}	–	–	3.5	–	3.6		2.2	1.5	1.2	1.5	1.2	–	0.7	–	–
4,6-Me_2-C_{17}	–	3.6	–	–	2.7		6.0	6.1	6.2	3.8	2.4	–	–	0.9	1.4
4,8-Me_2-C_{17}	1.2	3.9	5.0	3.3	5.0		4.9	0.5	4.9	4.1	2.3	–	–	–	–
4,10-Me_2-C_{17}	–	–	–	–	–		1.7	–	–	–	0.7	–	–	–	–
4,12-Me_2-C_{17}	–	–	–	–	–		1.2	6.2	–	–	0.4	–	–	–	–
2,6-Me_2-C_{18}	–	0.8	2.6	1.5	2.9		0.7	1.6	1.4	1.3	–	–	–	–	–
4,X-Me_2-C_{17}	–	3.0	–	–	–		0.9	0.3	0.9	0.9	–	0.9	–	1.0	1.3
4,Y-Me_2-C_{17}	–	0.9	–	–	–		–	0.4	–	0.6	–	1.3	–	–	–
4,Z-Me_2-C_{17}	0.4	–	2.9	0.6	–		–	1.2	–	1.0	–	0.9	–	–	–
4,8-Me_2-C_{18}	–	–	–	–	–		–	1.5	–	–	–	–	–	–	–
2,8-Me_2-C_{18}	–	–	–	–	–		–	1.1	–	–	–	–	–	–	–
Unidentified	0.9	3.5	0.6	3.7	3.4		3.9	7.2	3.1	4.0	4.0	1.4	1.3	1.4	–

during eclipse. By August, the wax ester region revealed two bands presumably representing both the short and long type wax esters previously observed in male mallards; by September only the wax esters with the lower R_f could be detected.

The polar component that appeared during the breeding season showed only one band in the thin-layer chromatogram. Upon treatment with methanol containing 14% BF_3, followed by thin-layer chromatography, three components were found. One with an R_f of 0.57 corresponded to that of methyl esters of fatty acids, and the other two, which were close together, showed R_f values corresponding to those of hexadecanol and methyl 3-hydroxydodecanoate. These two components were recovered together and subjected to combined gas–liquid chromatography and mass spectrometry as their trimethylsilyl derivatives (**Fig. 2**). The three components with shorter retention times were identified as 3-hydroxy-C_8, C_{10}, and C_{12} fatty acids by their retention times and mass spectra (11). The major ions at the high mass region corresponded to M^+–15, M^+–31, M^+–47, and M^+–73, and the expected α-cleavage on either side of the trimethylsiloxy function gave major diagnostic ions. Furthermore, the spectrum of one of the homologues matched precisely with that obtained with authentic methyl 3-hydroxydodecanoate. The components with the larger retention times were identified as fatty alcohols by their mass spectra.

Assuming that the polar compound found in the gland during the breeding season was a diester of a 3-hydroxy acid, equimolar amounts of fatty acids, fatty alcohols, and 3-hydroxy acids should be present. However, the flame ionization detector gave less response from the 3-hydroxy acids than expected from such a stoichiometry (Fig. 2). When authentic hexadecanol and 3-hydroxydodecanoate were used as quantitative standards, it was found that the hydroxy acid gave considerably less flame ionization detector response than a corresponding amount of fatty alcohol. When calculated on the basis of the responses obtained with the authentic standards, it was found that the diester fraction yielded equimolar amounts of fatty alcohols and 3-hydroxy acids. Since the fatty acids contained acids as short as C_6, they were analyzed both as their methyl and butyl esters to avoid possible complications resulting from the losses due to volatility. On the basis of their retention times and mass spectra, the fatty acids were identified to be *n*-fatty acids with even number of carbon atoms from C_6 to C_{16} (**Fig. 3**). Quantitative analysis showed that all of the fatty acids together were equimolar to the fatty alcohols and 3-hydroxy fatty acids. This 1:1:1 stoichiometry confirmed that the polar component that appeared in the preen gland only during the breeding season was a diester of 3-hydroxy fatty acids.

The chain length composition of all components of the diester collected throughout the breeding season was determined. In sharp contrast to the monoester wax composition, no branched compounds were detected in any of the diester components. In the 3-hydroxy acid fraction, *n*-C_{10} was the dominant component with smaller quantities of C_8 and C_{12} (**Table 1**). Intermediate chains dominated also in the acids (Table 1). Fatty alcohols, on the other hand, contained mainly C_{16} and C_{18} components. All three components showed the same composition at all times during the breeding season.

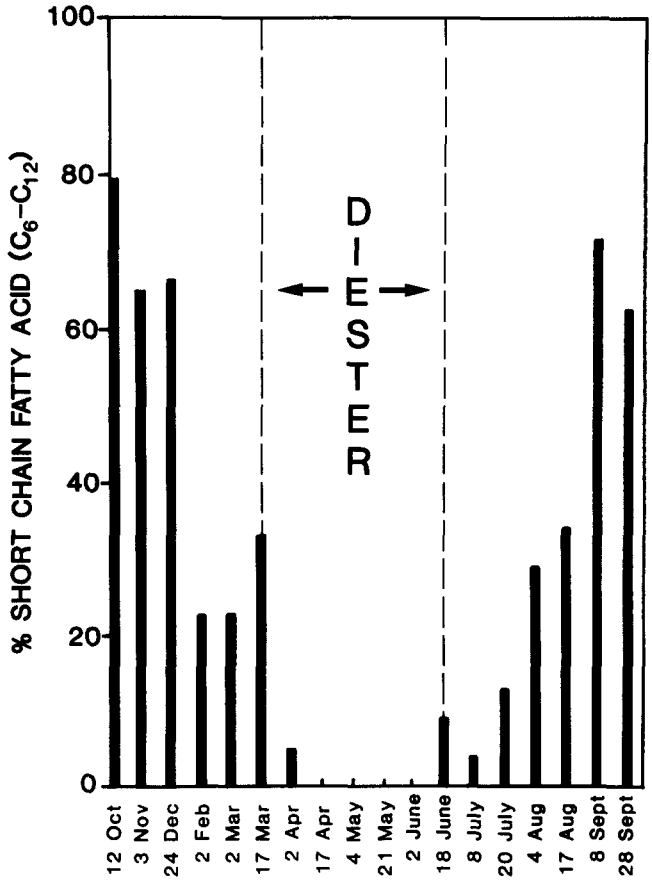

Fig. 4. Changes in the short chain acid content of wax esters from the uropygial gland of female mallards during the year. The dates of collection of the lipids from the gland are indicated. During the periods from April 17 to June 2 only diesters were present.

To determine the composition of the two components that migrated with R_f values similar to wax esters in the thin-layer chromatograms, both fractions were separately transesterified with butanol and the products were subjected to thin-layer chromatography. In both cases, only fatty alcohols and butyl esters of fatty acids were detected and thus both fractions constituted wax esters. Since quantitative separation of the two types of wax esters was not readily achieved by thin-layer chromatography, both fractions were taken together and analyzed by combined gas–liquid chromatography and mass spectrometry after transesterification. The butyl esters derived from the wax esters were identified by their mass spectra as indicated before (8). The complex mixtures contained 83 different

TABLE 3. The composition of the primary alcohols of the monoester wax from the uropygial gland of female mallard ducks

Alcohol	24 Dec	2 Mar	17 Apr May 4, 20 2 June[a]	18 June	8 July	17 Aug	8 Sept	12 Oct	3 Nov
				% of total alcohols					
Hexadecanol	5.0	5.6		2.2	1.1	3.9	4.7	5.3	6.9
Heptadecanol	4.3	2.8		2.1	2.5	3.1	5.2	5.4	6.1
Octadecanol	32.0	40.1		47.8	55.5	54.0	43.9	39.5	31.7
Nonadecanol	1.2	2.2		2.3	2.7	2.7	1.4	1.3	1.0
Eicosanol	0.8	2.2		6.0	5.6	3.0	–	–	–
2-Methyl hexadecanol	2.7	1.6		1.0	1.2	1.4	2.3	2.6	2.8
4-Methyl hexadecanol	5.6	1.9		–	–	1.3	4.3	6.2	7.4
6-Methyl hexadecanol	6.1	2.9		1.6	1.7	2.5	5.8	6.2	7.3
2-Methyl heptadecanol	–	–		–	–	–	–	–	1.3
4-Methyl heptadecanol	6.2	3.7		2.1	1.3	2.0	4.5	5.1	6.3
6-Methyl heptadecanol	3.5	1.7		–	–	–	2.1	3.2	3.2
4-Methyl octadecanol	6.3	6.5		5.8	5.5	5.2	6.5	6.2	6.0
6-Methyl octadecanol	15.5	18.7		19.5	17.1	16.2	16.0	15.1	13.9
4-Methyl nonadecanol	3.6	3.5		3.0	2.1	2.1	3.2	2.7	3.0
6-Methyl nonadecanol	0.9	1.2		1.2	–	–	–	–	1.0
Unidentified alcohols, C_{17} and C_{18} range	2.1	–		–	–	–	–	–	–
Unidentified alcohols, C_{19} and C_{20} range	4.1	5.3		5.1	3.7	2.7	–	1.1	2.1

[a]Only diester.

n, mono-, di-, and trimethyl branched fatty acids. Even after ignoring components that did not at any time during the year reach at least 1% of the total acids, 70 fatty acids were found (**Table 2**). In general, the fatty acids found in the wax esters of the females were similar to those found in the males (8). The wax esters containing the shorter chain acids began to show some changes prior to the appearance of longer chain wax esters. As the time for disappearance of wax esters and appearance of diesters approached by the end of March, 4-methyl and 4,x-dimethyl acids began to disappear; no 4,x,y-trimethyl acid was found but 2,6,x-trimethyl acids were present (Table 2). Since diesters are not made in the male glands at any time during the year (8), an analogous situation was not previously observed. During the breeding season of April and May, no wax ester was found and when esters began to appear by the end of June, the acyl portion showed much higher chain lengths (**Fig. 4**). During August the shorter chain acids began to reappear and by September the short chain acids became the dominant components.

Fatty alcohol portions of wax esters were analyzed by combined gas–liquid chromatography and mass spectrometry as their trimethyl silyl ethers and as butyl esters of the fatty acids derived by CrO_3 oxidation of the alcohols. At all times, *n*-C_{16} to *n*-C_{20} and mono- and dimethyl branched alcohols were the major components (**Table 3**). Only relatively minor compositional changes were noted in this fraction.

DISCUSSION

The uropygial gland lipids of female mallards showed three separate types of composition, presumably representing three physiological states. During the major portion of the year, September to February, the adult female had wax esters containing short chain acids. This composition has been considered the characteristic one for mallards (4, 6, 12, 13). During the breeding season these monoester waxes were replaced by diesters of 3-hydroxy fatty acids. After the breeding season, the females went into eclipse similar to that previously reported for males, generating monoesters of long chain fatty acids. When eclipse was over, the shorter chain esters returned as the major products. The change from the shorter monoesters to the longer monoesters during eclipse and the return to the short esters after eclipse was most probably caused by the suppression of the gene that codes for S-acyl fatty acid synthase thioesterase as previously observed with the male mallards (9). In fact, SDS gel electrophoresis of the proteins from females showed that the 30 kDa thioesterase band virtually disappeared from the gland (data not shown). The biochemical mechanism by which the monoesters are replaced by the diesters remains obscure. In this case, the structural changes involve synthesis of a new class of compounds, namely the 3-hydroxy acids, and the exclusion of any branched components. Such dramatic biochemical changes would probably involve changes in

the expression of more than one gene. An intriguing question is how monoester wax synthesis is prevented even when fatty acid and alcohol synthesis continue for the production of the diester waxes. Appearance of a new metabolic compartment in which the diesters are generated during the breeding season could explain the observed dramatic changes in the lipid composition of the uropygial gland. Since intermediate length 3-hydroxy fatty acids constitute the unique component of the diester "pheromones" characteristic of the breeding season, it is tempting to speculate that a peroxisome type microbody might play a role in the production of this unusual lipid. However, no experimental evidence is available concerning the biochemical mechanisms involved in such changes.

We thank Greg Wichelns for maintaining the birds used in this study. Scientific paper 7652, project 2001, College of Agriculture and Home Economics, Washington State University. This work was supported by a grant GM-18278 from the National Institutes of Health.

Manuscript received 6 November 1986 and in revised form 10 January 1987.

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