erythro-Diols of wax from the uropygial gland of the turkey

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ABSTRACT The uropygial (preen) gland secretion of the domestic turkey resembles that of the chicken in consisting mainly of a diester wax. The esterified fatty acids are saturated; they include all members of the *n*-C_{10}–C_{20} homologous series, the C_{17}–C_{19} acids together accounting for 60% of the total. There are four major 2,3-*n*-alkanediols, C_{19}–C_{23}, all having the *erythro* configuration as determined by thin-layer chromatography on boric acid–silica gel and by gas–liquid chromatography. The chicken uropygiols, by contrast, contain *erythro* and *threo* diols. It is suggested that the chicken possesses two biosynthetic enzyme systems for the diols, the turkey only one

SUPPLEMENTARY KEY WORDS chicken · diester wax · *threo* diols · uropygiols · thin-layer chromatography · boric acid–silica gel · gas–liquid chromatography · isomeric acetonides · stereospecific biosynthesis

OF THE SECRETIONS from the uropygial (preen) glands of various species of birds so far examined, most consist of waxes that are esters of monohydric alcohols. The composition of the waxes of representative species among the family Anatidae has been reviewed recently by Odham (1), and a survey by TLC of the uropygial lipids from birds of several orders indicates the presence of monoester waxes (2). By contrast, birds belonging to the order Galliformes secrete esters of diols (2). For the chicken, the major diols have been identified as a mixture of *threo* and *erythro* isomers of 2,3-diols of chain length C_{20}, C_{21}, and C_{22} (3). We report here that the turkey secretes a similar wax, except that only four major diols are present, C_{20}–C_{23}, all with the *erythro* configuration.

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

MATERIALS AND METHODS

The breeds of domestic fowls (Tegel crossbreed; Tegel Ltd., Leppington, N.S.W., Australia) and turkeys (White crossbreed) used in this study are the most common types bred for the meat industry in this country. Intact uropygial glands from freshly slaughtered birds were kindly supplied by a local poultry processor, Diamond Foods Ltd. These were frozen and then opened before the tissue thawed completely. In this way the secretion could be removed as a solid without contamination by tissue fragments. The lipids were extracted with chloroform, each chicken gland yielding about 75 mg and each turkey gland about 50 mg of lipid. After separation by preparative TLC on Silica Gel G (Merck) with light petroleum (bp 55–65° C)–ethyl ether–acetic acid 100:10:1 (solvent A), the individual lipid classes were eluted and weighed.

The wax esters were methanolyzed in BF_3–methanol (4) by heating for 90 min under the conditions recommended for sterol esters. The resultant fatty acid methyl esters were separated from the diols by TLC on Silica Gel G with light petroleum–ethyl ether–methanol–acetic acid 100:20:5:10 (solvent B) (5), and recovered by elution with ethyl ether–methanol 95:5. The diols were converted to acetonides as described by Haahti and Fales (3). For periodate oxidation, 5 mg of the diol fraction was dissolved in 1.0 ml of tetrahydrofuran, 0.2 ml of 0.1 M sodium metaperiodate and 0.1 ml of 0.5% K_2CO_3 were added, and the mixture was left at 20°C for 1 hr. About 0.5 g of Na_2SO_3 was then added and the tetrahydrofuran layer was removed and examined directly by GLC.

The *threo* and *erythro* diols were separated by TLC on Silica Gel G containing 5% (w/w) boric acid (6). The atmosphere was saturated with the solvent light petroleum–ethyl ether 40:60 (solvent C) before the chromatogram was developed.

TABLE 1 COMPOSITION OF LIPIDS FROM UROPYGIAL GLANDS OF CHICKEN AND TURKEY

Lipid Class	Chicken	Turkey
	wt %	
Wax ester	97.3	82.4
(*Fatty acid: diol*)	(*65:35*)	(*62:38*)
Triglyceride	0.4	1.3
Cholesterol	0.6	7.3
Phospholipid	1.7	9.0

Values are means of two samples from each species. The constituents of the wax ester fraction were weighed after methanolysis of about 20 mg and separation of the products by TLC. Cholesterol was identified by spraying the chromatogram with a 10% aqueous solution of phosphotungstic acid and heating at 110°C for 15 min.

TABLE 2 FATTY ACID COMPOSITION OF WAX ESTERS FROM UROPYGIAL GLANDS OF CHICKEN AND TURKEY

Fatty Acid	Chicken	Turkey
	wt %	
10:0	0.7	0.6
11:0	0.2	1.4
12:0	9.6	4.3
13:0	1.1	2.1
14:0	26.6	12.9
15:0	0.8	1.5
16:0	11.5	9.2
17:0	1.2	9.7
18:0	13.3	34.6
19:0	11.5	18.1
20:0	17.9	5.5
21:0	5.6	0.1

The composition of wax from one individual of each species is shown as the mean of estimations on EGSS-X and JXR columns. Fatty acids are denoted as chain length:number of double bonds.

TABLE 3 COMPOSITION (PEAK AREA %) OF UROPYGIOL ACETONIDES FROM CHICKEN AND TURKEY BY GLC ON JXR AT 207°C

Retention Time	Chicken		Turkey	
	Peak No.	%	Peak No.	%
min				
2.55			A	1.2
3.53			B	15.5
4.93			C	27.0
5.90	1	7.8		
6.90	2	13.9	D	41.2
8.22	3	15.7		
9.65	4	18.5	E	15.1
11.45	5	23.9		
13.35	6	20.3		

The Varian Aerograph model 204-B chromatograph used for GLC had an all-glass system, on-column injection, and flame ionization detection. Pyrex columns (6 ft × 1.8 mm I.D.) were packed with, (*a*) 3% JXR (a methyl silicone) on 100–120 mesh Gas-Chrom Q, (*b*) 10% EGSS-X (ethylene glycol succinate polyester combined with a methyl silicone) on 100–120 mesh Aeropak 30 (Varian Aerograph), and (*c*) 20% Apizeon L (hydrocarbon) on 100–120 mesh Gas-Chrom CLA. Materials other than Aeropak were obtained from Applied Science Laboratories Inc. (State College, Pa.) Retention times were measured from the appearance of the acetone solvent. Fatty acid compositions (wt %) were calculated from the products of peak height and retention time (7). Quantitative results with fatty acid standards KA, KB, KC, and KD (Applied Science) agreed with the stated composition data with a relative error less than 4% for major components (>10% of total mixture) and less than 8% for minor components (<10% of total mixture). We assumed that the composition of diol acetonide mixtures could be calculated with similar accuracy.

RESULTS AND DISCUSSION

Since to some extent the identification of the diols in the turkey depended on the characterization of chicken uropygiols by Haahti and Fales (3), we treated the uropygial gland lipids from chicken and turkey similarly for comparison.

Silica gel TLC of the uropygial lipids secreted by the turkey showed that they resembled those of the chicken (Table 1). The predominant lipids in each species were wax esters which had an R_f in solvent A of 0.44 compared with R_f 0.55 for simple monoesters such as cetyl palmitate.

After methanolysis of the wax esters, the fatty acids from the chicken and turkey glands were found to belong to the same homologous series. Table 2 shows the fatty acid composition of waxes from one individual of each species. The amount of C_{10} and C_{12} acids in the chicken waxes varied considerably, certain individuals having ten times as much C_{10} and twice as much C_{12} as the example given. In these cases the composition closely resembled the pooled sample analyzed by Haahti and Fales (3). We regard these variations as of little significance.

GLC of the chicken uropygiols as their acetonides on the nonpolar column JXR confirmed that six major components were present, each comprising >7% of the total (Table 3). The turkey uropygiol acetonides yielded one minor peak and four major ones, the last two of which coincided with peaks 2 and 4 of the chicken. Plotting log retention times on JXR vs. known chain lengths of the uropygiols from the chicken gives two parallel lines (Fig. 1*a*). Of the five peaks given by uropygiol acetonides from the turkey (Table 3), peaks D and E have retention times that fall on the upper line, and if this line is extrapolated to lower chain-lengths the retention times of peaks A, B, and C fall on it at positions corresponding to C_{19}, C_{20}, and C_{21}, respectively.

TABLE 4 COMPOSITION (PEAK AREA %) OF UROPYGIOL ACETONIDES FROM CHICKEN AND TURKEY BY GLC ON EGSS-X AT 207°C

Retention Time	Chicken		Turkey	
	Peak No.	%	Peak No.	%
min				
2.00			A	1.5
2.60			B	16.0
3.35	1	8.1	C	28.0
4.33	2	28.9	D	38.8
5.60	3	41.3	E	15.7
7.22	4	21.7		

This suggests that the turkey uropygiols form a homologous series, C_{19}–C_{23}, all members of which have the same steric configuration, whereas the chicken diols constitute both *erythro* and *threo* series.

GLC of the turkey acetonides on an EGSS-X column again yielded five peaks (Table 4). The areas of these corresponded to those obtained on JXR (Table 3) and the log retention times gave a straight-line plot with the assumed chain lengths (Fig. 1*b*). The chicken acetonides yielded only four peaks on this column, instead of six as on JXR. Three of these coincided with turkey acetonide peaks and the fourth seemed to be a higher homologue (Fig. 1*b*). This unexpected reduction in the number of peaks was judged to be due to coincidence of one stereoisomer with the opposite stereoisomer of the next homologue. Support for this explanation is given by the facts that the combined areas of JXR peaks 2

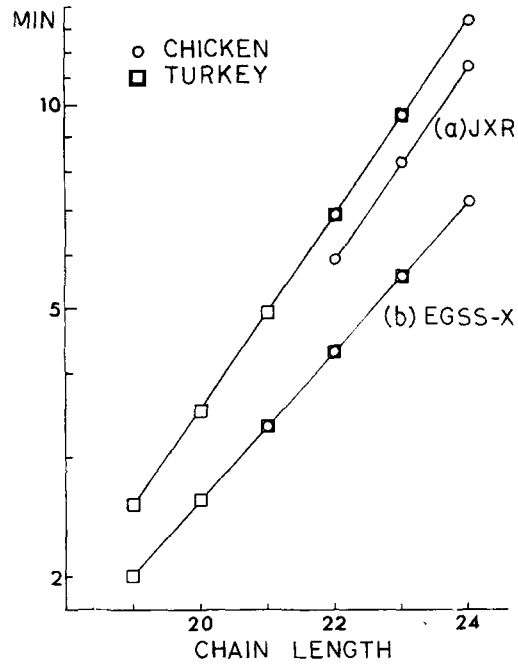

FIG. 1. Log retention time of uropygiol acetonides vs. uropygiol chain length on (*a*) JXR and (*b*) EGSS-X columns.

TABLE 5 GLC OF ACETONIDES FORMED AFTER SEPARATION OF *erythro*- AND *threo*-UROPYGIOLS BY BORIC ACID TLC

Mobility of Uropygiol	JXR, 210°C				EGSS-X, 210°C	
	Chicken		Turkey			
	Peak No.	R_t	Peak No.	R_t	Chicken	Turkey
	min		*min*		*min*	
R_f 0.38 (*erythro*)			A	2.35		1.97
			B	3.23		2.55
			C	4.43		3.23
	2	6.20	D	6.20	4.10	4.10
	4	8.60	E	8.60	5.27	5.27
	6	11.85			6.70	
R_f 0.57 (*threo*)	1	5.23			3.23	
	3	7.20			4.10	
	5	9.93			5.27	

Peak numbers of chicken uropygiols correspond to those in Table 3. R_t, retention time of acetonides.

and 3 (Table 3) correspond to that of EGSS-X peak 2 (Table 4), and JXR peaks 4 and 5 to EGSS-X peak 3. The fact that the number of peaks from turkey diol acetonides was the same on JXR as on EGSS-X was taken as further evidence for the presence of only one stereoisomeric form. To confirm our conclusion about the chain lengths of the turkey uropygiols, we compared the aldehydes produced by periodate cleavage with reference aldehydes by GLC and found them to have chain lengths of C_{17}–C_{21}, as expected.

TLC on Boric Acid-Impregnated Plates

Stereoisomers of vicinal glycols may be separated by chromatography of their borate complexes (8). Preparative boric acid TLC of the uropygiols gave one band (R_f 0.38) for diols from the turkey and two bands (R_f 0.38 and 0.57) for diols from the chicken. Each band was recovered and converted to acetonides for GLC on JXR and EGSS-X (Table 5).

We assumed that the more polar diols (R_f 0.38) were the *erythro* isomers, and since these were converted to *cis*-acetonides, it was the *cis* isomers that were eluted more slowly during GLC. As this behaviour was characteristic of the turkey diols, it was important to be certain that our assumption about configuration was justified. Methyl *erythro*- and *threo*-9,10-dihydroxystearates were synthesized from methyl oleate, and methyl elaidate, respectively, by OsO_4 oxidation (3). On boric acid TLC, the *erythro* isomer had an R_f 0.38; the *threo* isomer, R_f 0.57. The corresponding *cis* and *trans*-acetonides had retention times relative to methyl stearate as follows: EGSS-X at 180°C, 4.95 and 3.73; JXR at 180°C, 2.73 and 2.32; Apiezon L at 250°C, 2.27 and 1.88.

These data confirm that the uropygiol *trans*-acetonides are eluted earlier than the *cis* isomers on both polar and nonpolar columns, in a manner analogous to the *trans*-

and *cis*-epoxides (9). Since this order of elution is the reverse of that of methyl oleate and methyl elaidate on nonpolar columns, the GLC peaks shown by Haahti and Fales (3) should be redesignated.

It seems likely, then, that the turkey uropygial gland synthesizes *erythro*-uropygiols stereospecifically. Possibly, the uropygiols in the chicken are produced by a different type of enzyme system which is not stereospecific. However, the composition of the uropygiol mixture suggests a slight preference for longer carbon chains in the *threo* series (peaks 1, 3, and 5 in Table 3) which is less evident in the *erythro* series. This may indicate that the chicken has two distinct enzyme systems, each producing one stereoisomeric series, while the turkey has only a single system.

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References

1. Odham, G. 1967. *Ark. Kemi.* **27:** 295.
2. Haahti, E. O. A., K. Lagerspetz, T. Nikkari, and H. M. Fales. 1964. *Comp. Biochem. Physiol.* **12:** 435.
3. Haahti, E. O. A., and H. M. Fales. 1967. *J. Lipid Res.* **8:** 131.
4. Morrison, W. R., and L. M. Smith. 1964. *J. Lipid Res.* **5:** 600.
5. Hansen, I. A. 1965. *Comp. Biochem. Physiol.* **15:** 27.
6. Thomas, A. E., III, J. E. Scharoun, and H. Ralston. 1965. *J. Amer. Oil Chem. Soc.* **42:** 789.
7. Bartlet, J. C., and J. L. Iverson. 1966. *J. Ass. Offic. Agr. Chem.* **49:** 21.
8. Morris, L. J. 1963. *J. Chromatog.* **12:** 321.
9. Morris, L. J., R. T. Holman, and K. Fontell. 1961. *J. Lipid Res.* **2:** 68.