**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-C₁₀-C₂₅ homologous series, the C₁₇-C₂₅ acids together accounting for 60% of the total. There are four major 2,3-n-alkanediols, C₁₉-C₂₃, 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

**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 BF₃-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-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% K₂SO₃ were added, and the mixture was left at 20°C for 1 hr. About 0.5 g of Na₂S₂O₃ 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.
Aeropak 30 (Varian Aerograph), and (c) combined with 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 uropygials by Haathi 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 Rf in solvent A of 0.44 compared with Rf 0.55 for simple monoesters such as cetyl palmitate.

After methanalysis 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 C19 and C20 acids in the chicken waxes varied considerably, certain individuals having ten times as much C19 and twice as much C20 as the example given. In these cases the composition closely resembled the pooled sample analyzed by Haathi and Fales (3). We regard these variations as of little significance.

GLC of the chicken uropygials 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 uropygial 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 uropygials from the chicken gives two parallel lines (Fig. 1a). Of the five peaks given by uropygial 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 C19, C20, and C21, respectively.
TABLE 4 COMPOSITION (Peak Area %) OF UROPYGIOL ACETONIDES FROM CHICKEN AND TURKEY BY GLC ON EGSS-X AT 207°C

<table>
<thead>
<tr>
<th>Retention</th>
<th>Chicken Peak No.</th>
<th>%</th>
<th>Turkey Peak No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td>A</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.60</td>
<td>B</td>
<td>16.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.35</td>
<td>C</td>
<td>28.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.33</td>
<td>D</td>
<td>28.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.60</td>
<td>E</td>
<td>41.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.22</td>
<td></td>
<td>21.7</td>
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</table>

This suggests that the turkey uropygiols form a homologous series, C₁₉-C₂₃, 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. 1b). 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. 1b). 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 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 C₁₇-C₂₁, 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 (Rₛ 0.38) for diols from the turkey and two bands (Rₛ 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 (Rₛ 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 OsO₄ oxidation (3). On boric acid TLC, the erythro isomer had an Rₛ 0.38; the threo isomer, Rₛ 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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