Journal of Chromatography, 274 (1983) 305—312
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1582

Note

Separation of *erythro* and *threo* forms of alkane-2,3-diols from the uropygial gland of the quail by glass capillary column gas chromatography

J.H. ABALAIN*, D. PICART and F. BERTHOU

Laboratoire de Biochimie, ERA No. 784 du CNRS, Faculté de Médecine, 22 Avenue Camille Desmoulins, 29200 Brest (France)

R. OLLIVIER

Laboratoire de Synthèse Hétéro Organique, U.E.R. Sciences, Avenue Le Gorgeu, 29200 Brest (France)

and

Y. AMET, J.Y. DANIEL and H.H. FLOCH

Laboratoire de Biochimie, Faculté de Médecine, 22 Avenue Camille Desmoulins, 29200 Brest (France)

(First received July 9th, 1982; revised manuscript received November 9th, 1982)

The uropygial or preen gland is the unique but large sebaceous gland which is present in birds. Its lipidic secretion is spread all over the plumage by the bill to prevent water penetration. It seems that the preen waxes play a role in the bacteriostatic and fungistatic protection of the bird's skin against micro-organisms. In addition, it has been found that the uropygial gland produces pheromones involved in sexual behaviour in ducks [1, 2].

Intensive chemical investigations of the composition of the lipidic secretion have been performed. It is now well established that the secretion consists of mono or diester waxes and is species-specific [3, 4]. In the quail and the Galliformes the lipidic secretion is mainly composed of alkane-2,3-diol diesters [5—8].

The present paper describes the separation and identification of *erythro* and *threo* forms of the alkane diols from the Japanese quail *Coturnix coturnix*

0378-4347/83/$03.00 © 1983 Elsevier Science Publishers B.V.

Japonica by capillary gas–liquid chromatography (GLC) and mass spectrometry (MS).

MATERIALS AND METHODS

Animals

Male Japanese quails (*Coturnix coturnix Japonica*) were purchased from R. Salaun (Lannilis, France) and killed when 6–7 weeks old. Food (Guyomarc'h, Vannes, France) and water were offered ad libitum. Temperature and humidity were constant and lights were on a long-day cycle (18 h light–6 h darkness).

Reagents

The extraction solvents were obtained from E. Merck (Darmstadt, G.F.R.). N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Pierce (Rotterdam, The Netherlands). 2-Bromododecane was obtained from Sigma (St. Louis, MO, U.S.A.). C_{14} to C_{34} *n*-alkanes were purchased from Fluka (Buchs, Switzerland).

Isolation and derivatization of diols

Animals were killed by cervical decapitation and the glands were quickly removed. Each gland was opened and washed with 20 ml of hexane. The separation into main lipid classes was performed by column chromatography. The extracts were applied directly to a 200 × 15 mm silica gel (70–200 mesh) chromatographic column. Three eluent fractions were obtained with 100 ml of hexane, *n*-hexane–diethyl ether (95:5, v/v) and diethyl ether–methanol (50:50, v/v). The homogeneity of all fractions was checked by thin-layer chromatography (TLC) on silica gel using precoated plates (HF 60/254 Merck) with *n*-hexane–diethyl ether–acetic acid (80:20:1, v/v) as mobile phase. The wax fraction was found in the hexane–diethyl ether eluate and its yield was 85% by weight of the total extract. Subsequently the wax fraction was subjected to alkaline hydrolysis in potassium hydroxyde and ethanol–benzene–water (5:2:1, v/v) at 80°C for 2 h. The unsaponifiable alcoholic fraction was extracted by 200 ml of hexane. TMS (trimethylsilyl ethers) derivatives were prepared by heating the alcoholic fraction with the mixture BSTFA–pyridine (20:1, v/v) for 1 h at 60°C and subjected to gas chromatography without further purification.

Synthesis of threo-2,3-dodecanediol from 2-bromododecane

2-Dodecene was prepared from 2-bromododecane according to the method of Sturtz and Rio [9]. After distillation, proton nuclear magnetic resonance (NMR) revealed the presence of two compounds: 15% of 1-dodecene and 85% of *trans*-2-dodecene. The olefins were oxidized without any further purification using osmium tetraoxide according to the following procedure: 100 mg of olefins were added to an equal weight of OsO_4 in the presence of *tert.*-butanol; a brown coloration appeared immediately and after 1 h 2 ml of H_2O_2 were added; the mixture was allowed to stand overnight, 35 ml of diethyl ether and 2 g of sodium sulfite were added and the mixture was allowed to stand 1 h; the aqueous layer was then discarded and the diethyl ether layer was evaporated.

The diols were submitted to TMS derivatization as described above and subjected to GLC and MS.

Gas—liquid chromatography

Analyses were carried out with a Carlo Erba 2150 (Milan, Italy) gas chromatograph equipped with a flame ionization detector.

The glass capillary column (SE-30 film thickness 0.4 μm; 39 m × 0.3 mm I.D.) was prepared in our laboratory as described by Berthou et al. [10, 11]. The carrier gas was hydrogen at a flow-rate of 2 ml/min. The gas chromatograph was operated under the following conditions: injector and detector temperatures 270° C, column temperature programmed from 130° C to 270° C at a rate of 4° C/min. Injections were carried out according to the split—splitless mode.

Analysis on a packed column was run with a glass column packed with Gas-Chrom Q with 2% OV-1 (3 m × 3 mm I.D.). Conditions for chromatography were the same as described for the capillary column.

Mass spectrometry

Mass spectra were run on a Ribermag R-10-10B (Rueil Malmaison, France) apparatus. Sample introduction was via the GC inlet OV-101 on a glass capillary column (50 m × 0.4 mm I.D.). The temperature was programmed from 130°C to 280°C at 4°C/min. The mass spectra were recorded at an ionization voltage of 70 eV.

RESULTS

Thin-layer chromatography on silica gel of preen secretion of the Japanese quail showed that the major component (85—90%) consisted of waxes and revealed the presence of more polar compounds identified as free fatty acids (unpublished data). The unsaponifiable fraction was converted to TMS ethers and submitted to gas chromatography. It was found to contain nine major doublets and a wide range of minor peaks (Fig. 1). Their structures were determined by mass spectrometry. The two components of doublets exhibited the same spectrum (Fig. 2); in the electron ionization a base peak at *m/z* M—117 resulted from the elimination of CH_3-CH_2-O-TMS. The other fragment ion produced from this cleavage was found at *m/z* 117 but was much weaker than M—117. The molecuar ion was never detected; the heaviest ion observed was M—15 which indicated loss of a CH_3 from the molecular ion. The mass spectrum showed a strong peak at *m/z* 73 characteristic of silyl ethers. These structural data, which are in agreement with those of Sawaya and Kolattukudy [8], suggested that such components were alkane-2,3-diols and that each doublet consisted of diastereoisomers.

In order to confirm the stereochemistry of the diols, a diastereoisomer was obtained by unequivocal synthesis. Oxidation of *trans*-2-dodecene, whose structure was ascertained by proton NMR, provided the *threo* isomer of dodecane-2,3-diol. When compared to the two dodecane diols from the biological extract, the synthetic isomer gave the same retention time as the second compound of the chromatographic doublet.

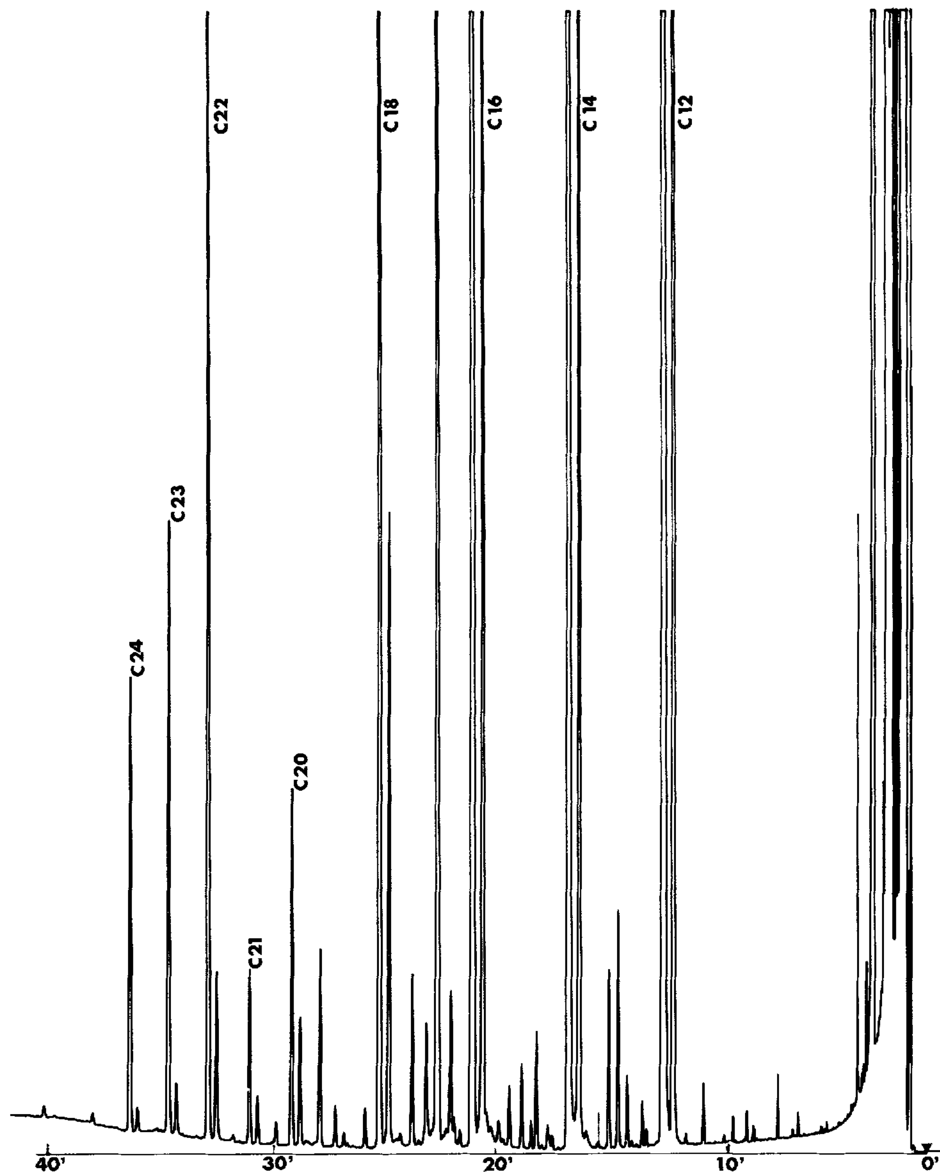

Fig. 1. Separation of alkane-2,3-diols as TMS derivatives by GLC with a glass capillary column. GLC conditons: SE-30 capillary column (39 × 0.3 mm I.D.), temperature programming from 130°C to 270°C at 4°C/min.

The methylene unit values were measured on the capillary column according to the method described by VandenHeuvel et al. [12]. Plots of methylene unit values against alkyl chain length are linear from diols C_{12} to C_{24}. The *erythro* and the *threo* forms of the diols gave two parallel lines. The difference in retention index between the *threo* and *erythro* isomers was shown to be about 20 units on SE-30.

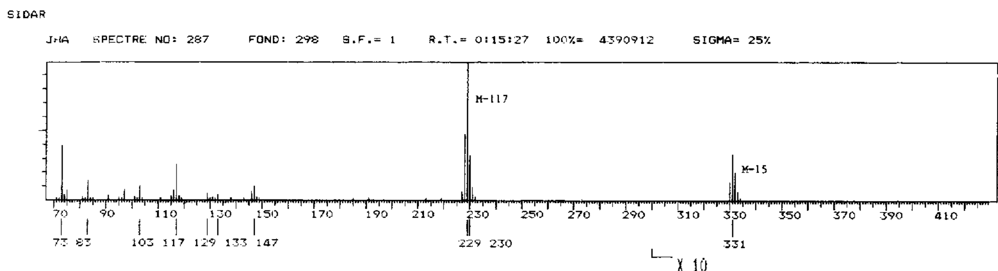

Fig. 2. Mass spectra of TMS ether of C_{12} diol (for experimental conditions see text).

Fig. 3. Gas chromatogram of TMS derivatives on a packed column. GLC conditions: OV-1, 2% packed column (3 m × 3 mm I.D.), temperature programming from 130°C to 270°C at 4°C/min.

Ettre [13] has shown that the separation number TZ was related to the number of effective theoretical plates N by $TZ = a\sqrt{N} - 1$. The separation number between *erythro* and *threo* isomers was 5.4 for a complete resolution of 1.5. Accordingly the required N for $a = 1.5$ was only 1300 theoretical plates for a capacity factor of 5. Such an efficiency could easily be obtained on a

TABLE I

COMPOSITION (PEAK AREA %) OF TMS ETHER DIOLS FROM THE UROPYGIAL GLAND OF THE JAPANESE QUAIL

Values are means of observations on a group of 20 animals ± S.D. ($p < 0.05$).

Chain length	Percentage composition		
	Total	*Erythro* form	*Threo* form
C_{12}	37.75 ± 2.62	10.17 ± 0.77	27.58 ± 2.00
C_{13}	0.47 ± 0.05	0.13 ± 0.02	0.34 ± 0.04
C_{14}	12.80 ± 0.85	3.29 ± 0.30	9.51 ± 0.55
C_{15}	0.26 ± 0.03	0.08 ± 0.02	0.18 ± 0.05
C_{16}	14.84 ± 1.07	3.68 ± 0.30	11.16 ± 0.82
C_{17}	0.39 ± 0.04		0.39 ± 0.04
C_{18}	9.85 ± 0.96	2.24 ± 0.20	7.61 ± 0.79
C_{19}	0.32 ± 0.02	0.09 ± 0.01	0.23 ± 0.02
C_{20}	1.16 ± 0.12	0.30 ± 0.03	0.86 ± 0.10
C_{21}	0.69 ± 0.07	0.13 ± 0.02	0.56 ± 0.07
C_{22}	6.71 ± 0.76	0.49 ± 0.10	6.22 ± 0.67
C_{23}	4.45 ± 0.64	0.20 ± 0.05	4.25 ± 0.45
C_{24}	3.15 ± 0.46	0.17 ± 0.04	2.98 ± 0.43

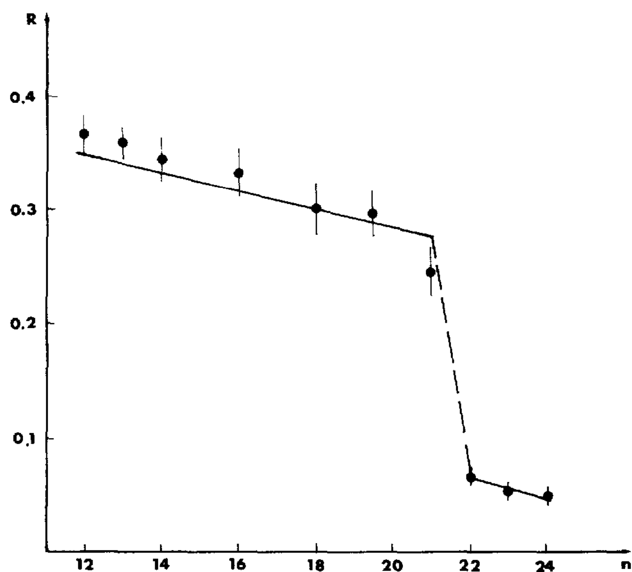

Fig. 4. Ratio of *erythro* to *threo* for each diol present in the wax esters from the uropygial gland of Japanese quail. Each value represents the mean ± S.E.M. of observations on a group of 20 male quails.

packed column. The *threo/erythro* separation was actually satisfactory as shown in Fig. 3. We found that the major advantage of using a capillary column was the shorter time required for the separation of all the diastereoisomers.

Table I shows the diol composition in the wax esters from the Japanese quail. The C_{12} diol was the major component followed by C_{14}, C_{16}, C_{18}, C_{22}, C_{23} and

C_{24} diols. Contents of odd-numbered diols were very small. Primary alcohols from C_{13} to C_{21} represented less than 5% of the unsaponifiable fraction. The percentage of erythro and threo diastereoisomers for each doublet was analyzed (Fig. 4). The ratio of *erythro* and *threo* forms was about 0.35 for the shorter-chain diols between C_{12} and C_{21}; but for chain lengths of C_{22} to C_{24} this ratio became very low; the *erythro* form of the diol was almost undetectable.

DISCUSSION

The TMS ethers of diols appeared to be very convenient derivatives for analysis by GLC. The preparation was simple and quick, no purification was required prior to injection and results could be reproduced easily.

As the *threo* and *erythro* isomers are not available, the assignment of the stereochemistry of the doublet was based upon the synthesis of the *threo* dodecane-2,3-diol. Moreover, the structure was confirmed by the chromatographic behaviour.

The *erythro* isomer of the diol had a retention time shorter than that of the *threo* form. The two diastereoisomers would not be expected to be well separated on a non-polar liquid phase such as SE-30. Advances towards understanding the separation mechanisms for diastereoisomers have shown that the chromatographic resolution depends upon the conformational immobility around a C—C bond with asymmetric centers [14]. The transformation of alkane-2,3-diols into TMS derivatives maximized the geometric differences between the two isomers. We assumed that the *threo* isomer of the diol presented a greater spatial bulk than the *erythro* form and that the surface interaction of the *threo* isomer with the stationary phase was then stronger than that of the *erythro* form and its retention index was therefore higher.

The major diols in the preen gland of the Japanese quail were found to be C_{12}, C_{14}, C_{16} and C_{18}. They were very different from those obtained by Sawaya and Kolattukudy [8], who found C_{22}, C_{23} and C_{24} as major components in the uropygial gland of the Japanese quail. Such a difference between our results and those of others could be due to the fact that those authors used GC in an isothermal mode (205°C) and that our results were obtained with a temperature programme from 130°C to 270°C. We assumed that the separation of *erythro* and *threo* forms would be better performed with a programmed temperature and that the shorter chains would be detected at lower temperatures.

Hansen et al. [15] suggested that the *erythro* and *threo* forms of the diols were synthesized by two distinct enzyme systems each producing one stereoisomeric series. According to the hypothetical pathway for biosynthesis of alkane-2,3-diols, a fatty aldehyde was condensed with hydroxyethylthiamine pyrophosphate to give an acyloin which was reduced to a diol [16]. We found that the proportion of the *erythro* form in each diol up to a chain length of C_{21} remained more or less constant; but for longer chains this proportion decreased dramatically. It was assumed that for long chains (more than C_{21}) the reduction step became difficult because of the chain length, and only the *threo* form could be synthesized. This would indicate that diols were produced by one enzyme system which was not stereospecific.

ACKNOWLEDGEMENT

The authors wish to thank Professor Sturtz for his valuable help and useful comments during the preparation of synthetic diol.

REFERENCES

1 J. Jacob, J. Balthazart and E. Schoffeniels, Biochem. Syst. Ecol., 7 (1979) 149.
2 J. Balthazart and E. Schoffeniels, Naturwissenschaften, 66 (1979) 55.
3 J. Jacob and J. Poltz, Lipids, 10 (1975) 1.
4 J. Jacob, in P.E. Kolattukudy (Editor), Chemistry and Biochemistry of Natural Waxes, Elsevier, Amsterdam, 1976, p. 93.
5 E.R.O. Haahti and H.M. Fales, J. Lipid Res., 8 (1967) 131.
6 E. Edkins and I.A. Hansen, Comp. Biochem. Physiol., 39B (1971) 1.
7 M. Gamo and K. Saito, Comp. Biochem. Physiol., 39B (1971) 151.
8 W.N. Sawaya and P.E. Kolattukudy, Biochemistry, 11 (1972) 4398.
9 G. Sturtz and M.Y. Rio, Bull. Soc. Chim. Fr., (1974) 2180.
10 F. Berthou, D. Picart, L.G. Bardou and H.H. Floch, J. Chromatogr. Sci., 12 (1974) 662.
11 F. Berthou, Thèse de Doctorat en Sciences Physiques, Université de Bretagne Occidentale, Brest, 1977, pp. 18 and 63.
12 W.J.A. VandenHeuvel, W.L. Gardiner and E.C. Horning, J. Chromatogr., 26 (1967) 387.
13 L.S. Ettre, Chromatographia, 8 (1975) 281.
14 B.L. Karger, R.L. Stern, W. Keane, B. Halper and J. Westley, Anal. Chem., 39 (1967) 229.
15 I.A. Hansen, B.K. Tang and E. Eokins, J. Lipid Res., 10 (1967) 267.
16 P.E. Kolattukudy and T.J. Walton, Prog. Chem. Fats Other Lipids, 13 (1972) 119.