Journal of Chromatography, 494 (1989) 23–30
Biomedical Applications
Elsevier Science Publishers B V , Amsterdam — Printed in The Netherlands

CHROMBIO 4877

LONG-CHAIN FATTY ACIDS AND ALCOHOLS FROM GERBIL MEIBOMIAN LIPIDS

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(First received April 17th, 1989, revised manuscript received May 25th, 1989)

SUMMARY

Capillary gas chromatography–mass spectrometry was used to identify constituent fatty acids, alcohols and steroids from gerbil meibomian glands Over 80 compounds representing about 90% of the total fraction were identified The major steroid was cholesterol accompanied by a lower concentration of 3β-hydroxy-5α-cholestane Fatty acids with chain lengths from 12 to 27 carbon atoms were present, they had predominantly straight, *iso* or *anteiso* chains with major concentrations in the C_{15}–C_{18} and C_{25}–C_{27} regions Unsaturated acids had mainly 16 and 18 carbon atoms The fatty alcohols were mainly branched-chain with the majority of compounds having chain lengths of 25–27 carbon atoms Several alcohols, both branched and unsaturated, were found with chains of up to 33 carbon atoms long The profile was similar to that found earlier in other species but with lower concentrations of mono-unsaturated compounds than were found in rats and humans Di-hydroxy compounds, on the other hand, tended to be more abundant although still of low relative concentration

INTRODUCTION

The meibomian glands, located on the outer margins of the eye-lid, secrete an oily fluid over the tear film to prevent evaporation Studies on the composition of meibomian secretions from several species have revealed a complex pattern of wax and sterol esters with fatty acids and alcohols of several distinct structural types [1] Dominant among these are the unusual branched-chain acids Chain lengths of the fatty acids show a biphasic distribution with peaks in the C_{15}–C_{18} and C_{25}–C_{27} regions, but with considerable species variation in the abundancies of individual compounds Alcohols tend to be concentrated in the C_{25}–C_{30} region In previous studies from this and other laboratories, mei-

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bomian secretions from cow [2], rat [3], mouse [4], human [3] and rabbit [5] have been examined This paper presents results from an investigation into meibomian extracts from the gerbil

EXPERIMENTAL

Reference compounds

Straight-chain saturated (10 0–24 0), mono-unsaturated (16 1 and 18 1), and di-unsaturated (18 2, Δ-9,12) acids were obtained from Sigma (Poole, U K) The saturated acids were reduced with lithium aluminium hydride, in diethyl ether, to the corresponding alcohols Fatty acids from the *iso* and *anteiso* series (14 0–21 0) were obtained from Applied Science Labs (State College, PA, U S A) and were reduced to the corresponding alcohols as above 1,2-Diols (12 0, 14 0 and 16 0) were from Aldrich Chemical (Gillingham, U K) and steroids were from Sigma

Preparation of meibomian extracts

The meibomian glands were dissected from the eye lids of ten gerbils (Mongolian, male, 40 g), washed with ethyl acetate to remove skin-surface lipids and crushed under ethyl acetate (1 ml) to express the meibomian fluid as described previously [4] The solution was collected and the glands were washed further with ethyl acetate (2×1 ml) The washings were combined and centrifuged to remove debris, and the solvent was removed with a stream of nitrogen Hydrolysis of this solution was achieved by heating at 80 °C for 8 h with 1 ml of a 1 1 (v/v) mixture of 1 *M* aqueous potassium hydroxide solution and ethanol After cooling and acidification with 1 *M* sulphuric acid, the meibomian constituents were extracted with ethyl acetate (3×1 ml) and concentrated to 1 ml for storage at −20 °C Aliquots (0 1 ml) of this solution were converted into trimethylsilyl (TMS), [$^2H_9$]TMS [6], methyl ester–TMS, picolinyl–TMS and nicotinate–TMS derivatives for examination by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) as described earlier [3,4]

Gas chromatography

GC data were recorded with a Hewlett-Packard 5890A gas chromatograph (FID) fitted with a 50 m×0 3 mm bonded-phase OV-1 fused-silica capillary column, film thickness 0 52 μm (Hewlett-Packard) Helium at 2 0 ml/min was used as the carrier gas and the split–splitless injector was used in the split mode with a split ratio of 15 1 Injector and detector temperatures were both 300 °C and the column oven was temperature programmed from 130 to 380 °C at 2 °C/min Results were recorded with a Servoscribe chart recorder and quantitative data were recorded with a Hewlett-Packard 3390A recording integrator

TABLE I

FATTY ACIDS FROM GERBIL MEIBOMIAN GLANDS

Peak	Number of carbon atoms	Chain type	Methylene unit	Percentage of total
1	12	Straight	16 46	0 18
3	13	*Anteiso*	17 18	0 06
4	13	Straight	17 45	0 05
7	14	*Iso*	18 06	0 23
8	14	Δ-?[a]	18 13	0 24
9	14	Δ-?[a]	18 18	0 13
11	14	Straight	18 41	0 82
13	15	*Iso*	19 06	0 38
14	15	*Anteiso*	19 15	2 37
16	15	Straight	19 41	0 39
20	16	*Iso*	20 05	1 12
21	16	Δ-7	20 11	3 60
22	16	Δ-9	20 17	1 88
23	16	Straight	20 41	7 58
29	17	*Iso*	21 03	0 12
30	17	Δ-?[a]	21 07	0 16
31	17	*Anteiso*	21 12	1 65
33	17	Straight	21 38	0 30
38	18	Δ-9,12	22 00	5 05
39	18	*Iso*	22 05	0 35
40	18	Δ-9	22 09	8 79
41	18	Δ-11	22 15	0 73
43	18	Straight	22 37	1 57
46	19	*Iso*	23 02	0 11
48	19	*Anteiso*	23 11	0 30
50	19	Straight	23 37	0 07
55	20	*Iso*	24 01	0 82
59	20	Straight	24 37	0 40
61	21	*Iso*	25 01	0 13
62	21	*Anteiso*	25 10	0 43
64	21	Straight	25 37	0 06
68	22	*Iso*	26 00	0 34
70	22	Straight	26 34	0 24
72	23	*Iso*	26 99	0 03
73	23	*Anteiso*	27 08	0 57
75	23	Straight	27 34	0 15
77	24	*Iso*	28 00	0 70
78	24	*Anteiso*	28 07	0 04
81	24	Straight	28 34	0 77
85	25	*Iso*	29 00	0 18
86	25	*Anteiso*	29 06	1 77
88	25	Straight	29 31	0 30
90	26	*Iso*	29 97	1 00
91	26	*Anteiso*	30 07	0 05
94	26	Straight	30 31	0 36
97	27	*Iso*	31 00	0 07
98	27	*Anteiso*	31 09	1 25
101	27	Straight	31 35	0 10

[a]Unsaturated acids listed as ? gave spectra that were too weak for determination of double bond position

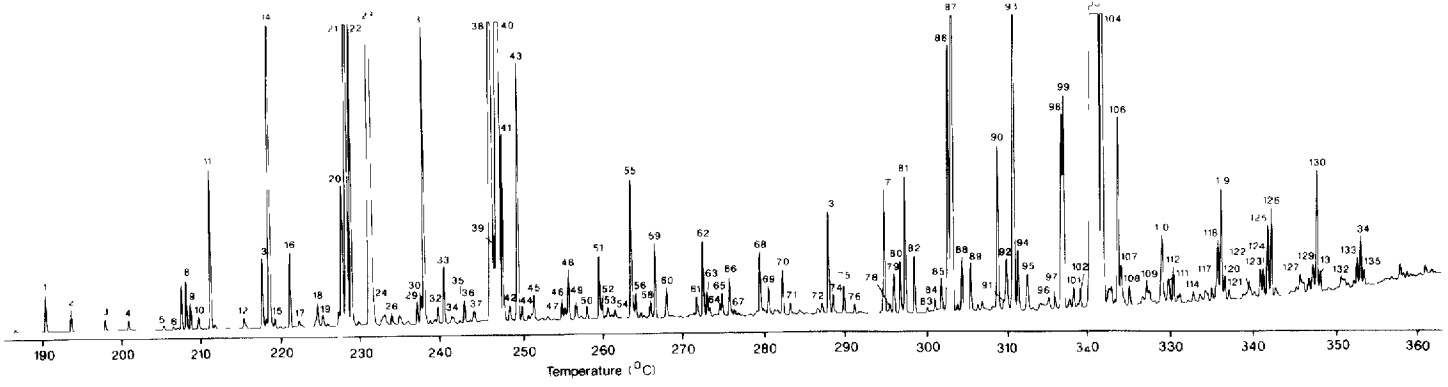

Fig 1 Separation of constituents of hydrolysed gerbil meibomian glands as TMS derivatives on a 50 m×0 3 mm fused-silica OV-1 capillary column Operating conditions are given in the Experimental section

Gas chromatography–mass spectrometry

GC–MS data were recorded with a VG 70/70F mass spectrometer interfaced to a Varian 2440 gas chromatograph fitted with a SGE split–splitless injection system The column was a 25 m × 0 2 mm OV-1 bonded-phase fused-silica capillary, film thickness 0 33 μm, terminating 10 mm inside the mass spectrometer ion source Helium at 1 ml/min (measured in the absence of the mass spectrometer vacuum) was used as the carrier gas Operating conditions were injector, transfer line and ion source temperatures, 300, 300 and 280 °C, respectively, column oven temperature, programmed from 150 to 350 °C at 2 °C/min, accelerating voltage, 4 kV, electron energy, 70 eV; trap current, 1 0 mA, scan speed, 1 s/decade. The mass spectrometer was under the control of a VG 11/250 data system and data were recorded and processed with the same system

RESULTS

Fig 1 shows a GC trace of the lipids from the hydrolysed meibomian glands Compounds were identified by their GC retention time, their reactivity towards the various derivatizing reagents such as diazomethane and by GC–MS

TABLE II

FATTY ALCOHOLS FROM GERBIL MEIBOMIAN GLANDS

Peak	Number of carbon atoms	Chain type	Methylene unit	Percentage of total
35	18	Straight	21 58	0 02
49	20	*Iso*	23 23	0 10
52	20	Straight	23 57	0 09
58	21	*Anteiso*	24 29	0 09
76	24	Straight	27 50	0 06
79	25	*Iso*	28 14	0 24
80	25	*Anteiso*	28 25	0 30
82	25	Straight	28 50	0 32
87	26	*Iso*	29 12	3 07
89	26	Straight	29 46	0 26
93	27	*Anteiso*	30 21	2 88
95	27	Straight	30 53	0 26
99	28	*Iso*	31 10	1 07
102	28	Straight	31 53	0 10
106	29	*Anteiso*	32 21	1 18
108	29	Straight	32 47	0 11
110	30	*Iso*	33 09	0 54
119	31	*Anteiso*	24 23	0 54
124	32	*Iso*	35 05	0 16
125	32	Δ-23[a]	35 22	0 43
126	32	Δ-25[a]	35 30	0 48
166	33	Δ-?[b]	36 25	0 74

[a]Identification by TMS derivative mass spectrum and retention time only

[b]Spectrum too weak for double bond position to be determined

TABLE III

SATURATED DIOLS FROM GERBIL MEIBOMIAN GLANDS

Peak	Number of carbon atoms	Chain type	Methylene unit	Percentage of total
26	15	1,2-Straight	20 72	0 06
32	16	—[a]	21 29	0 08
36	16	1,2-Straight	21 62	0 12
42	17	—[a]	22 26	0 10
45	17	1,2-Straight	22 60	0 15
60	19	1,2-Straight	24 54	0 17
63	20	—[a]	25 15	0 13
66	20	1,2-Straight	25 50	0 22
69	21	—[a]	26 11	0 15
71	21	1,2-Straight	26 46	0 06
74	22	—[a]	27 16	0 10

[a]Not determined, spectrum too weak

The positions of the double bonds and methyl branch points in the aliphatic chains of the acids and alcohols were determined by the mass spectra of the picolinyl (acids [7]) and nicotinate (alcohols [8,9]) derivatives, respectively Full details of the identification methods have been published [3,4] Confirmation of structures, by comparison with the GC–MS properties of reference compounds, was made when these compounds were available (see Experimental section) and GC traces were compared with those from the mouse, rat and human reported earlier [3,4] Identified compounds are listed in Tables I (acids), II (alcohols) ad III (diols) Quantitative data are expressed as the percentage of the total lipids of each compound calculated from the peak areas measured with the flame ionization detector Other compounds identified were the steroids cholesterol (peak 103, 25 03% of the total lipids) and 3β-hydroxy-5α-cholestane (peak 104, 4 96%)

DISCUSSION

Over 80 compounds were identified in the hydrolysed fraction of these secretions, these accounted for about 90% of the material present (calculated as a percentage of the total integrated peak area from the gas chromatogram) The chromatogram showed a fairly typical profile of acids and alcohols with *iso* and *anteiso* compounds dominating the branched-chain fraction *iso* Compounds tended to have an even number of carbon atoms in the chain whereas *anteiso* compounds had predominantly odd numbers of carbon atoms Major fatty acids had chain lengths in the C_{15}–C_{18} and in the C_{25}–C_{27} regions as shown in Fig 2 Fewer unsaturated acids and alcohols were observed than in the secretion from rat and human but very-long-chain unsaturated alcohols with

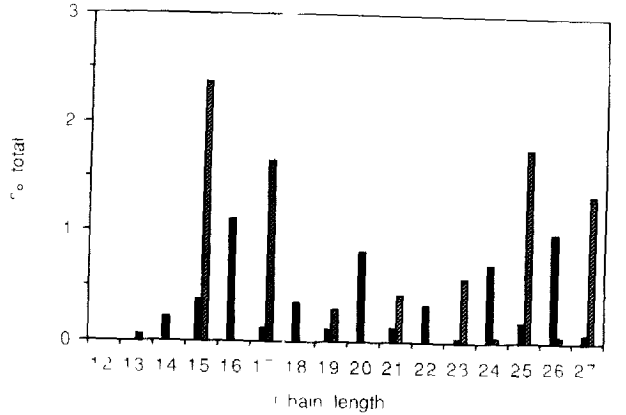

Fig 2 Profile of the branched-chain fatty acids from gerbil meibomian glands expressed as a percentage of the total extract ■ = *iso*, ▨ = *anteiso*

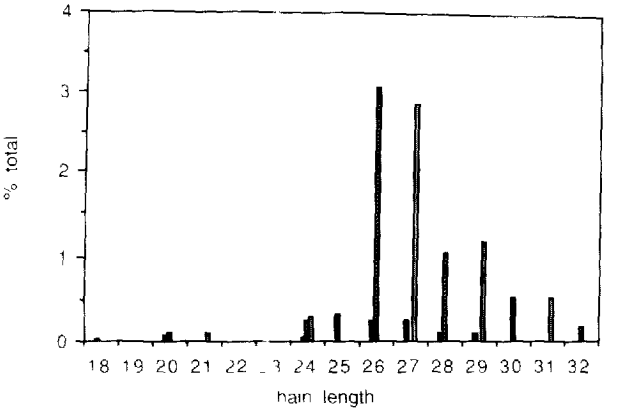

Fig 3 Profile of the fatty alcohols from gerbil meibomian glands expressed as a percentage of the total extract ■ = Straight chain, ▨ = *iso*, ▩ = *anteiso*

chain lengths of over 30 carbon atoms were found The saturated alcohols were concentrated in the C_{26}–C_{31} region (Fig 3) with the most abundant alcohols being *iso*-26 0 and *anteiso*-27 0 This contrasted with the human [3] where the *iso*-24 0, *anteiso* 25 0 and *iso*-26 0 alcohols were the most abundant In the rat, most of the alcohol fraction was accounted for by the *iso*-26 0 alcohol

Several dihydroxy compounds were detected as summarised in Table III Their mass spectra (TMS derivatives) were characterised by abundant $[M-CH_2OTMS]^+$ ions and weaker ions at *m/z* 103 ($[CH_2OTMS]^+$) and 205 ($[TMSOCH_2{=}CHOTMS]^+$) [8]. The *n*-C_{16} diol (peak 48) was further characterised by comparison with a reference sample The other straight-chain 1,2-diols were identified by their methylene unit [10] values and mass spectra (TMS derivatives) Other diols appeared to be branched-chain 1,2-diols but

their spectra (nicotinate derivatives) were too weak to permit confirmation 1,2-Diols of this type have been observed previously in meibomian fluid but the α,ω-types, observed in steer and human meibomian fluid [11], were not observed

CONCLUSIONS

This work has shown that the profile of constituent acids and alcohols from gerbil meibomian glands is similar to that previously observed from other species but with some differences in the relative concentration of certain constituents In particular, the biphasic profile of compounds with maxima in the C_{14}–C_{18} and C_{25}–C_{29} regions is present, but the relative concentration of dihydroxy compounds is higher than is usually observed in other species

ACKNOWLEDGEMENTS

This work was supported by the Wellcome Trust The mass spectrometer was originally bought with a grant from the Medical Research Council

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