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# IDENTIFICATION BY COMBINED GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF CONSTITUENT LONG-CHAIN FATTY ACIDS AND ALCOHOLS FROM THE MEIBOMIAN GLANDS OF THE RAT AND A COMPARISON WITH HUMAN MEIBOMIAN LIPIDS

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## SUMMARY

Constituent long-chain fatty acids and alcohols from the meibomian secretions of the rat were examined as trimethylsilyl (TMS) and methyl ester-TMS derivatives by capillary gas chromatography and by combined gas chromatography–mass spectrometry. The positions of double bonds and methyl branch points were determined by the mass spectra of picolinyl esters and nicotinates for long-chain fatty acids and alcohols, respectively. Fatty acids had chain lengths from $C_{12}$ to $C_{34}$ and were of the straight-chain *iso, anteiso* and monounsaturated types. The unsaturated acids had double bonds in the $\omega$-7 and $\omega$-9 positions. The alcohols had corresponding structures. In common with the constituent acids and alcohols of other meibomian secretions, the chain lengths of the constituents showed a biphasic distribution with maxima around $C_{16}$–$C_{18}$ and $C_{25}$–$C_{27}$. The profile was qualitatively similar to that obtained from human meibomian secretion but with some differences in the relative proportions of certain acids and alcohols.

## INTRODUCTION

The outer margins of the eye-lids are equipped with glands which secrete an oily film over the surface of the eye to reduce evaporation of the tear film. These are known as the meibomian glands and have recently been the subject of several investigations [1] which have shown the secretions to consist of a complex mixture of sterol and wax esters containing straight-chain, branched-chain, unsatu-

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rated and hydroxy fatty acids and alcohols. The relative proportion of these constituents appears to vary considerably with species. Baron and Blough [2], for example, have reported that the major acids of bovine meibomian secretions are *anteiso* acids with chain lengths of 25 and 27 carbon atoms. Human lipids on the other hand appear to contain more unsaturated acids, particularly 18:1, $\Delta$-9 [3,4] although the detailed pattern is subject to considerable inter-individual variation [5]. Hydroxy fatty acids have recently been reported in both steer and human meibomian secretions [6,7]. Secretions from rabbit meibomian glands are somewhat less complex [8] with major differences in the steroid fraction [9] from those found in steer and human meibomian extract. The mouse, on the other hand, produces a profile of lipids more closely related to that found in the human [10]. In this paper we report the results of a structural study on the constituent long-chain fatty acids and alcohols present in meibomian secretions from the rat and a comparison with the corresponding compounds obtained from human meibomian secretions.

## EXPERIMENTAL

### *Reference compounds*

Branched-chain fatty acids (as methyl esters) were obtained from Applied Science Labs. (State College, PA, U.S.A.). Branched-chain alcohols were prepared from these compounds by reduction with lithium aluminium hydride. Straight-chain and unsaturated fatty acids were obtained from Sigma (London) (Poole, U.K.). Reduction of these compounds gave the straight-chain and unsaturated alcohols, respectively.

### *General*

All extractions and hydrolysis reactions were performed in 4-ml screw-capped vials, and Pasteur pipettes were used to transfer solvents. Derivatization reactions were performed in 0.3-ml screw-capped microvials.

### *Extraction of rat meibomian lipids*

The eyelids were removed from eight rats (male, Charles River, Wistar, 200 g) and the meibomian glands were dissected out. These were washed with ethyl acetate to remove surface lipids by allowing them to stand in the solvent (1 ml) for 1 min. They were then slit several times with a scalpel and stood in ethyl acetate (1 ml) for 5 min. The organic solution was removed from the residual tissue which was washed with ethyl acetate (1 ml). The combined ethyl acetate fraction was evaporated to dryness and reconstituted in 1 ml of ethyl acetate for storage in the dark at 4°C. Aliquots of this solution were examined by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) after conversion to derivatives as described below.

### *Extraction of human meibomian lipid*

This was obtained by gentle lid expression and collecting the resulting secretion from the lid margin in a chalazion scoop.

*Hydrolysis of lipids*

An aliquot (0.5 ml) of the meibomian gland extract was blown to dryness (nitrogen stream), dissolved in ethanol (0.2 ml) and heated at 80°C for 1 h with 1 *M* aqueous potassium hydroxide (0.05 ml). The cooled solution was then diluted with water (1.5 ml), acidified with 2.5 *M* sulphuric acid (0.5 ml), and the hydrolysed lipids were extracted with ethyl acetate (three times 1 ml). The combined ethyl acetate fraction was washed once with water (about 1 ml), twice with saturated sodium chloride solution (about 1 ml), evaporated to dryness and dissolved in ethyl acetate (2 ml) for storage. Aliquots of this solution were derivatized as described below.

*Preparation of derivatives*

*TMS derivatives.* Aliquots of the unhydrolysed extract (0.1 ml) or the hydrolysed extract (0.25 ml) were blown to dryness and converted into TMS derivatives by heating them at 60°C for 10 min with a mixture of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 8 $\mu$l) and acetonitrile (2 $\mu$l). Samples of the resulting solution were examined by capillary column GC and GC–MS directly.

*[$^2H_9$] TMS derivatives [11].* Samples (0.1 ml) of the hydrolysed extracts were heated at 60°C for 10 min with [$^2H_{18}$]bis(trimethylsilyl)acetamide (5 $\mu$l) and acetonitrile (5 $\mu$l).

*Methyl ester–TMS derivatives.* An aliquot of the hydrolysed extract (0.25 ml) was blown to dryness with a nitrogen stream, mixed with an excess (0.3 ml) of ethereal diazomethane (prepared from Diazald, Aldrich) and allowed to stand at room temperature for 2 min. The solvents were then removed with a nitrogen stream. Fractions derivatized in this way were reacted further with the TMS reagent as described above.

*Nicotinates.* Nicotinates of the alcohols present in 0.25-ml aliquots of the meibomian gland hydrolysate were synthesized by their reaction with 0.1 ml of fresh nicotinoyl chloride [12] as described previously [13,14]. The products were reacted with BSTFA as described above.

*Picolinyl derivatives [15].* Aliquots (0.25 ml) of the hydrolysed sample were blown to dryness and allowed to stand for 1 min at room temperature with thionyl chloride (0.3 ml). The thionyl chloride was removed with a nitrogen stream, and 20 $\mu$l of a 10% solution of 3-pyridyl carbinol in acetonitrile were added. This was allowed to stand at room temperature for 1 min, the acetonitrile was blown off and the alcohols and sterols in the residue were converted into their TMS ethers by heating them for 10 min at 60°C with 10 $\mu$l of BSTFA.

*Reduction with lithium aluminium hydride.* An aliquot (0.25 ml) of the hydrolysed extract was reduced with either lithium aluminium hydride or lithium aluminium deuteride in anhydrous ether using standard procedures. The products were converted into either TMS or nicotinate derivatives for examination by GC and GC–MS.

*Gas chromatography*

GC separations of the TMS derivatives and methyl ester–TMS derivatives of

TABLE I

CONSTITUENTS OF RAT AND HUMAN MEIBOMIAN GLAND HYDROLYSATES

Peaks which were not identified were omitted.

| Compound | | | | Rat | | Human | |
|---|---|---|---|---|---|---|---|
| Type | Chain length | Chain type | Retention index | Peak No. | Percentage of total | Peak No. | Percentage of total* |
| Acid | 12 | Straight chain | 16.47 | 3 | 0.047 | 1 | < 0.01 |
| Acid | 13 | *Iso* | 17.09 | 5 | < 0.01 | 3 | < 0.01 |
| Acid | 13 | *Anteiso* | 17.18 | 6 | < 0.01 | 4 | < 0.01 |
| Acid | 13 | Straight chain | 17.40 | 7 | < 0.01 | 6 | < 0.01 |
| Alcohol | 14 | Straight chain | 17.63 | 8 | < 0.01 | 7 | 0.027 |
| Acid | 14 | *Iso* | 18.07 | 12 | 0.019 | 10 | 0.044 |
| Acid | 14 | $\Delta$-? | 18.13 | 13 | 0.232 | 11 | 0.057 |
| Acid | 14 | $\Delta$-? | 18.18 | 14 | 0.291 | – | – |
| Alcohol | 14 | *Anteiso* | 18.36 | – | – | 14 | < 0.01 |
| Acid | 14 | Straight chain | 18.44 | 16 | 0.338 | 15 | 0.174 |
| Acid | 15 | *Iso* | 19.00 | 19 | 0.257 | 23 | 0.079 |
| Acid | 15 | *Anteiso* | 19.10 | 20 | 0.171 | 25 | 0.160 |
| Alcohol | 16 | *Iso* | 19.25 | 22 | < 0.01 | 27 | 0.034 |
| Alcohol | 16 | *Anteiso* | 19.33 | 23 | 0.018 | – | – |
| Acid | 15 | Straight chain | 19.40 | 24 | 0.064 | 28 | 0.114 |
| Alcohol | 16 | Straight chain | 19.63 | 25 | < 0.01 | 30 | 0.051 |
| Acid | 16 | *Iso*-$\Delta$-7 | 19.75 | 27 | < 0.01 | 31 | 0.295 |
| Acid | 16 | *Iso* | 20.08 | 31 | 0.766 | 37 | 0.633 |
| Acid | 16 | $\Delta$-7 | 20.15 | 32 | 7.724 | 38 | 0.723 |
| Acid | 16 | $\Delta$-9 | 20.21 | 33 | 1.506 | 39 | 0.826 |
| Alcohol | 17 | *Iso* | 20.29 | 34 | 0.017 | 40 | < 0.01 |
| Acid | 16 | Straight chain | 20.44 | 35 | 3.990 | 42 | 0.927 |
| Alcohol | 17 | Straight chain | 20.65 | – | – | 45 | < 0.01 |
| Acid | 17 | *Anteiso*-$\Delta$-7 | 20.89 | – | – | 48 | 0.541 |
| Acid | 17 | *Iso* | 21.00 | 42 | 0.295 | 51 | 0.093 |
| Acid | 17 | *Anteiso* | 21.10 | 43 | 1.077 | 53 | 2.134 |
| Alcohol | 18 | *Iso* | 21.20 | 45 | 0.023 | 55 | 0.048 |
| Acid | 17 | Straight chain | 21.36 | 47 | 0.088 | 57 | 0.062 |
| Alcohol | 18 | Straight chain | 21.70 | 49 | 0.019 | 60 | 0.067 |
| Acid | 18 | *Iso*-$\Delta$-? | 21.75 | – | – | 62 | 0.284 |
| Acid<br>Acid | 18<br>18 | *Iso*<br>$\Delta$-9,12 | 22.02 | 56 | 2.748 | 67 | 0.748 |
| Acid | 18 | – | 22.07 | 57 | 0.661 | – | – |
| Acid | 18 | $\Delta$-9 | 22.12 | 58 | 7.283 | 69 | 10.744 |
| Acid | 18 | $\Delta$-11 | 22.19 | 59 | 1.009 | 70 | 2.062 |
| Alcohol | 19 | *Anteiso* | 22.34 | – | – | 72 | 0.058 |
| Acid | 18 | Straight chain | 22.40 | 62 | 1.385 | 74 | 0.392 |
| Alcohol | 19 | Straight chain | 22.73 | – | – | 77 | < 0.01 |
| Acid | 19 | *Iso* | 23.00 | 69 | 0.309 | 83 | 0.077 |
| Acid | 19 | *Anteiso* | 23.05 | 70 | 0.397 | 85 | 0.337 |
| Alcohol | 20 | *Iso* | 23.17 | 71 | 0.328 | 86 | 0.568 |
| Acid | 19 | Straight chain | 23.35 | 72 | 0.017 | 89 | 0.058 |
| Alcohol | 20 | Straight chain | 23.53 | 73 | 0.907 | 91 | 0.080 |
| Acid | 20 | *Iso* | 24.00 | 81 | 1.527 | 98 | 0.943 |

TABLE I (continued)

| Compound | | | | Rat | | Human | |
|---|---|---|---|---|---|---|---|
| Type | Chain length | Chain type | Retention index | Peak No. | Percentage of total | Peak No. | Percentage of total* |
| Acid | 20 | $\Delta$-11 | 24.05 | 82 | 0.078 | 99 | 0.135 |
| Acid | 20 | $\Delta$-13 | 24.16 | 83 | 0.363 | 100 | 0.344 |
| Alcohol | 21 | *Iso* | 24.22 | 84 | < 0.01 | 101 | < 0.01 |
| Alcohol | 21 | *Anteiso* | 24.30 | 86 | 0.073 | 103 | 0.382 |
| Acid | 20 | Straight chain | 24.37 | 87 | 0.088 | 104 | 0.047 |
| Acid | 21 | *Iso* | 25.00 | 91 | 0.133 | 110 | 0.059 |
| Acid | 21 | *Anteiso* | 25.08 | 92 | 0.465 | 111 | 0.676 |
| Alcohol | 22 | *Iso* | 25.15 | 93 | 0.129 | 112 | 0.538 |
| Alcohol | 22 | *Anteiso* | 25.23 | 94 | < 0.01 | 113 | < 0.01 |
| Alcohol | 22 | $\Delta$-15 } | 25.30 | 95 | 0.087 | 114 | 0.147 |
| Acid | 21 | Straight chain } | | | | | |
| Alcohol | 22 | Straight chain | 25.50 | 98 | 0.144 | 116 | 0.089 |
| Acid | 22 | *Iso* | 26.00 | 103 | 0.527 | 122 | 0.719 |
| Acid | 22 | $\Delta$-13 | 26.08 | 104 | 0.059 | 123 | 0.155 |
| Acid | 22 | $\Delta$-15 | 26.16 | 105 | 0.306 | 124 | 0.370 |
| Alcohol | 23 | *Anteiso* | 26.29 | 106 | 0.058 | 125 | 0.650 |
| Acid | 22 | Straight chain | 26.37 | 107 | 0.138 | 126 | 0.045 |
| Alcohol | 23 | Straight chain | 26.55 | – | – | 128 | < 0.01 |
| Acid | 23 | *Iso* | 27.00 | 110 | 0.077 | 133 | 0.145 |
| Acid | 23 | *Anteiso* | 27.06 | 111 | 0.197 | 135 | 0.638 |
| Alcohol | 24 | *Iso* | 27.14 | 112 | 0.562 | 136 | 4.210 |
| Alcohol | 24 | $\Delta$-15 | 27.23 | 113 | 0.124 | 137 | 0.898 |
| Alcohol | 24 | $\Delta$-17 | 27.31 | 114 | 0.429 | 138 | 1.158 |
| Alcohol | 24 | Straight chain | 27.51 | 116 | 0.412 | 140 | 0.818 |
| Acid | 24 | *Iso* | 28.00 | 119 | 0.865 | 147 | 1.694 |
| Acid | 24 | $\Delta$-15 | 28.10 | 120 | 0.240 | 148 | 0.475 |
| Alcohol | 25 | *Iso* } | 28.17 | 121 | 1.065 | 149 | 1.468 |
| Acid | 24 | $\Delta$-17 } | | | | | |
| Alcohol | 25 | *Anteiso* | 28.28 | 122 | 0.692 | 151 | 4.155 |
| Acid | 24 | Straight chain | 28.37 | 123 | 0.357 | 152 | 0.086 |
| Alcohol | 25 | Straight chain | 28.52 | 124 | 0.032 | 153 | 0.033 |
| Acid | 25 | *Iso* | 29.00 | 129 | 0.220 | 157 | 0.140 |
| Acid | 25 | *Anteiso* | 29.07 | 131 | 1.099 | 159 | 1.920 |
| Alcohol | 26 | *Iso* | 29.15 | 132 | 10.293 | 160 | 6.207 |
| Alcohol | 26 | $\Delta$-17 | 29.30 | 133 | 0.892 | 161 | 1.093 |
| Alcohol | 26 | $\Delta$-19 | 29.33 | 134 | 0.615 | 162 | 1.119 |
| Alcohol | 26 | Straight chain | 29.48 | 136 | 1.397 | 164 | 0.385 |
| Acid | 26 | *Iso* | 30.00 | 141 | 1.244 | 169 | 1.768 |
| Acid | 26 | $\Delta$-17 | 30.13 | 142 | 0.143 | 171 | 0.243 |
| Alcohol | 27 | *Iso*? | 30.18 | 143 | 0.684 | 172 | 0.425 |
| Acid | 26 | $\Delta$-19 | 30.21 | 144 | 0.756 | 172 | 0.425 |
| Alcohol | 27 | *Anteiso* | 30.28 | 145 | 1.725 | 173 | 1.857 |
| Acid | 26 | Straight chain | 30.36 | 146 | 0.121 | 174 | 0.135 |
| Acid | 27 | *Iso* | 31.00 | 150 | 0.064 | 176 | 0.047 |
| Acid | 27 | *Anteiso* | 31.13 | 151 | 0.304 | 178 | 1.567 |
| Alcohol | 28 | *Iso* | 31.17 | 152 | 0.724 | 179 | < 0.01 |

(Continued on p. 258)

TABLE I (continued)

| Compound | | | | Rat | | Human | |
|---|---|---|---|---|---|---|---|
| Type | Chain length | Chain type | Retention index | Peak No. | Percentage of total | Peak No. | Percentage of total* |
| Alcohol | 28 | Δ-19 | 31.30 | 153 | 0.168 | 180 | 0.347 |
| Alcohol | 28 | Δ-21 | 31.45 | 154 | 0.475 | 181 | 1.130 |
| Alcohol | 28 | Straight chain | 31.50 | 156 | 0.104 | 183 | 0.123 |
| Steroid | – | Cholesterol | 31.84 | 157 | 24.699 | 184 | 23.933 |
| Acid | 28 | Δ-19 | 32.12 | 160 | 0.235 | 186 | 0.304 |
| Acid | 28 | Δ-21 | 32.19 | 161 | 1.172 | 187 | 0.605 |
| Steroid | – | 5α-Cholest-7-ene-3-β-ol | 32.39 | 165 | 1.90 | 191 | 0.608 |
| Alcohol | 30 | Δ-21 | 33.23 | 173 | 0.484 | 199 | 0.445 |
| Alcohol | 30 | Δ-23 | 33.30 | 174 | 0.743 | 200 | 1.125 |
| Acid | 30 | Δ-21 | 34.10 | 181 | 0.252 | 207 | 0.182 |
| Acid | 30 | Δ-23 | 34.20 | 182 | 0.960 | 208 | 0.488 |
| Alcohol | 32 | Δ-23 | 34.14 | 188 | 0.795 | 216 | 0.303 |
| Alcohol | 32 | Δ-25 | 35.30 | 189 | 0.706 | 217 | 0.140 |
| Acid | 32 | Δ-23 | 36.14 | 192 | 0.795 | 222 | 0.193 |
| Acid | 32 | Δ-25 | 36.21 | 193 | 0.317 | 223 | 0.060 |
| Alcohol | 34 | Δ-25 | 37.33 | 195 | 0.074 | – | – |
| Alcohol | 34 | Δ-27 | 37.37 | 196 | 0.040 | – | – |
| Acid | 34 | Δ-25 | 38.23 | 200 | 0.060 | – | – |
| Acid | 34 | Δ-27 | 38.30 | 201 | 0.058 | – | – |
| Total | | | | | 95.531 | | 90.466 |

*Compounds with a composition of <0.01% were not integrated.

the hydrolysed meibomian gland samples were performed with a Hewlett-Packard 5890A gas chromatograph fitted with a flame-ionization detector and a 50 m×0.2 mm (I.D.) bonded-phase fused-silica capillary column coated with 0.52-μm OV-1 (Hewlett-Packard Ultra column). Helium at 2 ml $min^{-1}$ was used as the carrier gas and the column was programmed at 2°C $min^{-1}$ from 100 to 380°C. The sample was introduced by the split technique with a 10:1 split ratio and data were recorded with a Hewlett-Packard 3390A recording integrator. Peak areas were measured by the integrator and quantities of the individual compounds are listed in Table I and are expressed as percentages of the total integrated area.

*Gas chromatography–mass spectrometry*

Packed-column GC–MS analyses were performed with a VG Micromass 12B mass spectrometer interfaced to a VG 2050 data system and via a glass jet separator to a Varian 2440 gas chromatograph. The GC column was 2 m×2 mm (I.D.) glass, packed with 3% SE-30 on 100–120 mesh Gas Chrom Q (Applied Science Labs., State College, PA, U.S.A.). Operating conditions were: column oven, temperature programmed from 150°C to 340°C at 4°C $min^{-1}$; injector, separator and ion source temperatures, 300, 300 and 280°C, respectively; carrier gas, helium at

30 ml min$^{-1}$; accelerating voltage, 2.5 kV; electron energy, 25 eV; trap current, 100 $\mu$A; scan, 3 s decade$^{-1}$, exponential, repetitive.

Capillary column GC–MS was performed with a VG Micromass 7070F mass spectrometer interfaced to a Varian 2440 gas chromatograph and to the above data system. The column was a 50 m$\times$0.3 mm (I.D.) fused-silica OV-1 (film thickness, 0.52 $\mu$m) capillary terminating inside the ion source. Operating conditions for the column were as above, those for the mass spectrometer were: electron energy, 70 eV; trap current, 1 mA; accelerating voltage, 4 kV. Spectra were obtained at a scan speed of 1 s decade$^{-1}$. Single-ion chromatograms were recorded with the mass spectrometer tuned to $m/z$ 103 or $m/z$ 74.

## RESULTS AND DISCUSSION

### *Compound identification techniques*

Compound identification was based on the GC–MS properties of TMS and methyl ester–TMS derivatives, picolinyl esters and nicotinates. Spectra of reference compounds were obtained from available compounds. Retention increment and mass spectrometric differences between the TMS and methyl ester–TMS derivatives gave clear differention between acids and alcohols and peak identity was further confirmed with the aid of single-ion chromatograms of $m/z$ 103 for alcohols and $m/z$ 74 for acids using the methyl ester–TMS derivatives. Furthermore, structural identity between corresponding acids and alcohols was confirmed by reduction of the acids in the total extract to alcohols with lithium aluminium deuteride. Fractions were examined as TMS esters or as nicotinates. The deuterium incorporation enabled the fraction of the alcohol peak arising from reduction of an acid to be determined. Mass spectra of the picolinyl [15] and nicotinate [14] derivatives provided unambiguous structural information regarding the positions of methyl branch points and double bonds.

### *Constituents of meibomian lipids*

Fig. 1 shows the GC profile of the TMS derivatives of the constituent long-chain fatty acids and alcohols from the rat meibomian lipids. Peak identity and relative compositions are given in Table I for the 95.531% of the constituents that was identified. Samples taken from different batches of rats at different times gave qualitatively equivalent results. Tests of the relative responses given by the long-chain fatty acids and alcohols at the flame-ionization detector showed these to be similar with the fatty acid methyl esters giving a 13% greater response than the corresponding TMS ethers of alcohols having an equivalent chain length. The results shown in Table I have not been corrected for this difference. The major constituent was cholesterol (peak 157). The acids and alcohols were mainly members of five series having straight chains, *iso* and *anteiso* branched chains and $\omega$-7 and $\omega$-9 monounsaturated chains. The most abundant acids had chain lengths in the $C_{16}$ range whereas the alcohols tended to have longer chains with the *iso*-$C_{26}$ alcohol (peak 132, 10.293%) being the most abundant. Compounds with the longest chains, $C_{28}$ and above, were only found with monounsaturation and an even number of carbon atoms. One diunsaturated acid (linoleic acid, peak

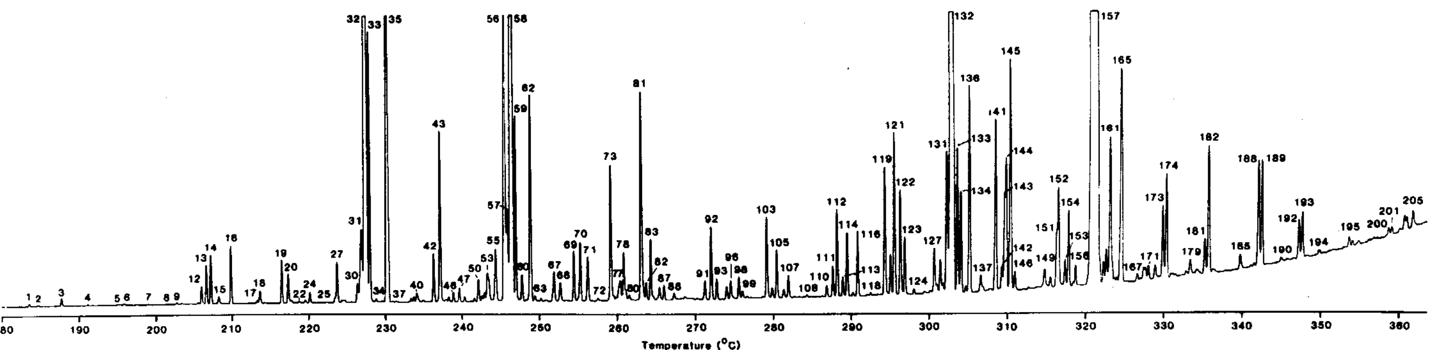


Fig. 1. Separation of constituent acids, alcohols and sterols from hydrolysed meibomian lipid from the rat. Compounds were chromatographed as TMS derivatives on a 50 m × 0.32 mm bonded-phase OV-1 fused-silica capillary column, film thickness 0.52 $\mu$m. This was temperature-programmed from 100 to 380 °C at 2 °C $min^{-1}$. Peak identification is given in Table I.

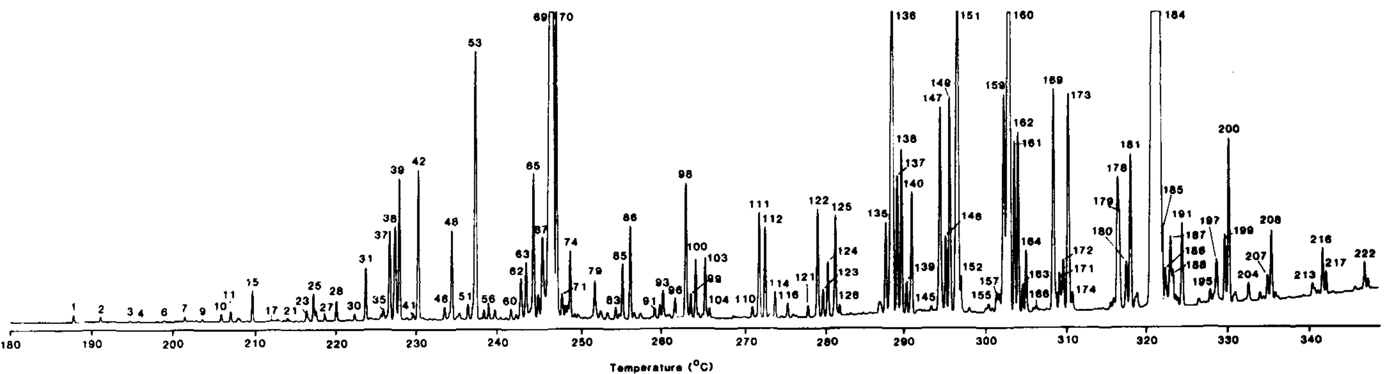


Fig. 2. Separation of constituent acids, alcohols and sterols from hydrolysed meibomian lipid from the human under the same conditions as those for Fig. 1. Peak identification is given in Table I.

56) was found and trace quantities of unsaturated branched acids were also present.

Peak 73 was a 1,2-diol but was not fully characterised as its bis(nicotinate) derivative had a retention time similar to that of the *iso*-$C_{26}$ alcohol and the resulting mixed spectrum could not be interpreted. Some of the peaks eluting just ahead of the major $C_{18}$ acids also appeared to be diols but were not characterised further.

Two other steroids were found in addition to cholesterol. Peak 165 was identified as 5$\alpha$-cholest-7-ene-3-$\beta$-ol (lathosterol) TMS ether by a comparison of its retention time [16] and mass spectrum [17] with those from the literature. This steroid has also been reported as a constituent of rat sebum [18]. In addition, one of the peaks in the 167–172 region appeared to be a methyl sterol but it was not further characterised.

Fig. 2. shows a corresponding chromatogram of the constituent acids, alcohols and sterols from a representative human meibomian gland secretion recorded under the same conditions as those used for the rat meibomian extract. Peak identification is again given in Table I. The quantities of the compounds found were very similar to those reported for other human meibomian secretions [4]. Qualitatively, this profile was very similar to that from the rat in that the acids and alcohols present were very similar. Quantitatively, however, there were some major differences. The human lipid had a higher relative concentration of *iso*-$C_{24}$ and *anteiso*-$C_{25}$ alcohols whereas lipid from the rat had a much higher concentration of the 16:1 acid. The profile from the mouse, reported in our previous study, did not have the same large range of monounsaturated alcohols that was found in these samples although they were present in low concentration. In addition, the relatively large $C_{18}$ diol found in the rat extract was not present in that from the human.

Of the species whose meibomian secretions have been examined (human, steer, mouse, rat and rabbit), most show a profile similar to the ones reported in this paper in that they show a biphasic distribution of chain lengths with similar branched-chain and unsaturated acids and alcohols. The rabbit, however, has a much more simple profile without significant concentrations of unsaturated compounds. The steroid profile is also very different.

No attempt was made to separate the sterol and wax esters from the unhydrolysed lipids or to determine their compositions. However, spectra of intact wax esters were recorded from an unhydrolysed sample. These showed that the major components corresponded to esterification between the most abundant acids and alcohols. Thus the major peak was a $C_{42}$ ester containing the 16:1 acid and the *iso*-$C_{26}$ alcohol.

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