Journal of Chromatography, 425 (1988) 11–24
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4014

FATTY ACID PROFILES OF SEBACEOUS TRIGLYCERIDES BY CAPILLARY GAS CHROMATOGRAPHY WITH MASS-SELECTIVE DETECTION

Z.M.H. MARZOUKI*

Department of Clinical Biochemistry, Faculty of Medicine and Allied Sciences, King Abdulaziz University, Jeddah 21413 (Saudi Arabia)

A.M. TAHA

Medical Chemistry Department, Faculty of Medicine and Allied Sciences, King Abdulaziz University, Jeddah 21413 (Saudi Arabia)

and

K.S. GOMAA

Biochemistry Department, Faculty of Science, Girls Section, King Abdulaziz University, Jeddah 21413 (Saudi Arabia)

(First received July 16th, 1987; revised manuscript received October 16th, 1987)

SUMMARY

Fatty acid methyl esters prepared from the triglyceride fraction of skin surface lipids from six adult human males were chromatographed on a 50-m fused-silica column coated with the highly polar cyanopropylpolysiloxane phase. This permitted the resolution of double-bond positional and geometric isomers. By means of mass-selective detection, 33 saturated and 14 unsaturated fatty acid chain types were analysed. Interpretation of the mass spectra combined with precise calculation of equivalent chain length values permitted the identification of 22 saturated and all of the unsaturated chain types. Quantification by integration of total-ion and selected-ion chromatograms revealed marked variation in the triglyceride fatty acid composition between different subjects. The greatest variation was observed in the concentrations of even-carbon-numbered iso-branched acids, which ranged from 1.5 to 11% of the saturated and from 1.9 to 12.7% of the monounsaturated acids. The anteiso chain structures constituted 4–9% of the saturated and 3–6% of the unsaturated members. Fatty acids with 4-methyl branch showed the least variation, in the range 5.7–7.4%. Other methyl-branched acids made up 4–10% of the saturated group, but were not detected in the unsaturated acids fraction. Two 18:1 fatty acids were identified (a Δ^8 and a Δ^9), which possibly have different anatomical origins. Similarly, two 18:2 fatty acids (linoleic and a 2,3-dimethyl derivative) were identified. A 2-methyl C_{17} acid, probably of bacterial origin, was detected.

0378-4347/88/$03.50

INTRODUCTION

Human skin surface lipids (SSLs) are a very complex mixture of different lipid classes, which have been investigated using a variety of analytical techniques [1–4]. Fatty acid chain types present in human and animal SSLs have been reported to involve extensive branching and include members with unusual positions of unsaturation [1, 3, 5–8]. The average class composition of the lipid mixture is [1–4]: squalene, 10–15%; wax esters, 20–31%; glycerides, 29–41%; free fatty acids, 12–33%; cholesterol, 1–2.5%; sterol esters, 2–3%. The lipid present on the skin surface is actually a mixture of sebum and of lipid derived from the keratinizing epidermis [1–3, 5]. Sebum is thought to be the sole source of the wax esters and squalene. Triglycerides constitute a sizable percentage of sebum, but have been also shown to be contributed by the epidermis to the SSL film [1–3, 5]. Free fatty acids are normally absent from freshly produced sebum and were shown to arise by subsequent hydrolysis exclusively of triglycerides [9]. However, the relative contributions of sebum and epidermal lipids to the skin surface pools of cholesterol and cholesterol esters have been difficult to define. Until recently, these two SSL components were considered to arise exclusively from epidermal sources [1–3, 9, 10]. However, after it was demonstrated that the human epidermis contains insignificant amounts of cholesterol esters [11], this concept has been reevaluated. Recent work demonstrated that cholesterol, free fatty acids and triglycerides are released to the skin by the epidermis, and that *the sebaceous glands do produce cholesterol esters* [12].

This special interest in the SSLs in general, and in the anatomical origin of their component lipids in particular, is stimulated by the established role of sebum in the aetiology of many skin disorders, principally acne vulgaris. Recently, Downing and coworkers [13–15] have reported significant variation in the ratio of differently branched saturated and unsaturated fatty acid chains among normal individuals. These authors utilized a 50-m capillary column coated with a non-polar phase for the resolution of fatty acid methyl esters (FAMEs) prepared from sebaceous wax esters. The FAMEs were previously fractionated into saturated and monounsaturated chains. In view of the constancy of composition of fatty acids for each individual, and the wide variation among different individuals, certain suggestions were advanced that assigned a conceivable role of SSLs in olfactory recognition of human beings and discussed the possible genetic control of the synthesis of sebaceous fatty acids [13–15]. Since SSL triglycerides are derived both from sebum and epidermis, it seemed of interest to find out the extent of variation in the proportions of the diverse branched chain types among glyceride-derived fatty acids. Furthermore, identification of chain types in these previous publications [13–15] was based exclusively on retention time data, utilizing the equivalent chain length (ECL) values. Accordingly it was thought worthwhile to substantiate the identification process by using a mass-selective detector, which gives standard 70-eV mass spectra.

In the present work both fractionated and unfractionated FAMEs prepared from the SSL triglyceride fraction of six individuals were analysed on a 50-m column coated with a highly polar phase. The column was capable of resolving

geometric and positional double-bond isomers, which helped in assigning the possible anatomical origin of some fatty acids.

EXPERIMENTAL

Collection of samples

SSL samples were collected from six healthy adult male volunteers by wiping the forehead with a small polyurethane sponge moistened with hexane. The subjects were instructed to refrain from using soap or creams on the sampling area for at least 12 h prior to the collection. The lipids were recovered from the sponges by extraction with hexane, and the solvent was removed under nitrogen. The reconstituted samples (1 mg/ml in hexane) were checked by analytical thin-layer chromatography (TLC) [1, 4].

Isolation of triglyceride fatty acids

Aliquots of the collected SSLs (4 mg) were subjected to preparative TLC using 10×20 cm plates coated with 0.5-mm layers of silica gel H (Camag, Muttenz, Switzerland) that had been previously cleaned by development with chloroform–methanol (2:1). The chromatograms were developed successively with hexane (to 19 cm), with benzene–toluene (1:1) to (19 cm), then with hexane–diethyl ether–acetic acid (70:30:1) (to 10 cm). The plates were air-dried for 10 min between each development. After the final drying (ca. 45 min), the chromatograms were sprayed with 2′,7′-dichlorofluorescein (0.1% solution in ethanol) and viewed under UV light (366 nm) to locate the lipid bands. For each sample the band containing the triglycerides [4] was scraped off and the lipids were recovered from the adsorbent by extraction with diethyl ether. After a purity check with TLC [1, 4], the collected triglycerides were transesterified to FAMEs using 0.5 *M* methanolic hydrochloric acid (2 ml) containing benzene (5 drops) and heating at 80°C for 1 h in PTFE-capped vials. Cooling to room temperature was followed by addition of 3 ml of water and 0.5 g of sodium chloride. The resulting FAMEs were extracted with three 1-ml portions of diethyl ether. The extracts were dried over a mixture of anhydrous sodium sulphate–anhydrous sodium carbonate (4:1). For direct gas chromatography (GC) the diethyl ether solution was adjusted to contain ca. 400 ng of total lipids per μl.

Separation of saturated and unsaturated FAMEs

The prepared triglyceride FAMEs were subjected to preparative TLC on 0.5-mm silica gel H plates impregnated with 5% silver nitrate. The chromatograms were developed with toluene and sprayed with 2′,7′-dichlorofluorescein. Lipid bands corresponding to saturated and unsaturated esters (identities were confirmed by analytical TLC using methyl palmitate and methyl palmitoleate as references) were scraped off separately and eluted with diethyl ether (saturated) or diethyl ether–chloroform (2:1) (unsaturated). In either case the solvent was evaporated under nitrogen and the residual FAMEs were reconstituted in hexane to yield a solution containing 300 ng/μl, which was subjected to GC with mass-selective detection (MSD).

Gas chromatography with mass-selective detection

GC was carried out on a fused-silica column (50 m × 0.22 mm I.D.), wall-coated with CP Sil-88 (100% cyanopropylpolysiloxane, Chrompack International, Middelburg, The Netherlands) and installed in HP 5890A gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.). The injection port was in the splitless mode with the purge valve closed for 48 s during injection. The injector temperature was 250°C and the column was maintained at 160°C (isothermal) throughout the analysis. For the proper concentration of sample components at the entrance of the column prior to GC, the initial column temperature during injection was set at 100°C to allow condensation of sample components. The temperature was then quickly raised to 160°C at the rate of 40°C/min taking 90 s after injection (i.e. 42 s after the splitless valve was switched off). A typical sample volume was 0.2–0.5 μl. The carrier gas was helium (99.999+%), with a column head-pressure of 186 kPa giving a linear velocity of 24 cm/s (under these conditions the retention time of butane was found to be 220.3 s). No post-column make-up gas is necessary with MSD, which yields standard 70-eV mass spectra. The instrument was an HP 5970B quadropole mass spectrometer (Hewlett-Packard, Palo Alto, CA, U.S.A.) interfaced with an HP 59970A workstation. The system controller was an HP series 200 computer with 32-bit internal architecture and 8-MHz clock-rate. The transfer line from the chromatograph to the mass-selective detector was maintained at 280°C with an open-split interface configuration. The ionization mode was electron impact (EI) with the electron energy fixed at 70 eV. Calibration of the atomic mass units was via a built-in auto-tune program using perfluorotributylamine as a mass standard (in the m/z range 10–800) and utilizing its peaks at masses 69, 219 and 502 for calibration. Typical settings were: multiplier voltage, 1400 V; repeller, 10.2 V; filament emission current, 0.22 mA; electron energy, 70 eV. The ion source temperature was 200°C, the inlet interface temperature 300°C and the ion source pressure $2.7 \cdot 10^{-4}$–$6.7 \cdot 10^{-4}$ Pa. The detector was turned on 6 min after injection, to permit escape of solvent and tail. Lipid samples were regularly scanned from m/z 40 to 400 (regular sensitivity). In some cases scanning was performed from m/z 50 to 330 (a range that covers up to the molecular ion of C_{20} FAME) to enhance sensitivity (shorter dwell times and hence more scans per second), thus revealing low abundance ions as well as the molecular ion peak in the spectra of FAMEs present in small amounts.

Preparation and GC–MSD of fatty acid pyrrolidides

Pyrrolidides of fatty acids were prepared by boiling 1mg of the prepared FAMEs with 0.5 ml of redistilled pyrrolidine (BDH) and 0.1 ml of acetic acid for 1 h [16]. They were purified by TLC (hexane–diethyl ether, 1:1) on silica gel G (Macherey and Nagel). The pyrrolidides were gas chromatographed on a quartz capillary column (12.5 m × 0.2 mm I.D.), wall-coated with cross-linked 5% phenylmethyl silicone. The operating conditions for GC–MSD were as follows: temperature programming from 200 to 260°C at 4°C/min; injector temperature 275°C; mass-scan range m/z 50 to 370. All other GC–MSD conditions were identical with those described above for FAMEs.

Evaluation of the gas chromatograms

Identification of peaks was based on two complementary parameters, namely retention times (t_R), from which ECLs were calculated, and mass fragmentation patterns of FAMEs. The ECL values were compared with the values published by Nicolaides and Apon [17] as well as those of Green et al. [13]. Mass spectral identifications of FAMEs using the present quadrupole analyser were based on established interpretation principles [18–20] and were related to previously published data using magnetic [8, 21] as well as quadrupole instruments [22].

The concept of ECLs is based on the observation that for a homologous series of FAMEs chromatographed at constant temperature, a plot of ln t_R versus the number of carbon atoms in the fatty acid chain forms a straight line [23]. The conventional procedure is to plot such a line using the values of t_R and the number of carbons for the straight-chain components of a mixture. By finding the point on the plot corresponding to the t_R of a branched component, the apparent number of carbons (the ECL) of the branched component can then be obtained by interpolation. A 15-carbon iso-branched chain, for example, behaves gas chromatographically as if it had 14.63 carbon atoms. The corresponding ECL for 15-carbon anteiso-branched chain is 14.74. On non-polar stationary phases, such as OV-101, both saturated and monounsaturated FAMEs with similar branches (iso or anteiso) were shown [13] to have identical fractional ECL values. Because of the large number of fatty acid chains in the SSLs, the traditional graphic determination of ECLs is both tedious and insufficiently precise. For this reason, a simple computer program was utilized to fit the data both graphically and numerically according to the equation $ECL = b + m \ln t_R$, where m is the slope and b the intercept of the linear regression relation. A correlation coefficient, r, for the best data fit was calculated to check peak identification. Values of r were always 0.99994 or greater for saturated series, and 0.9998 for the monounsaturated members, giving an accuracy in the calculated ECL to three significant digits. The data acquisition computer/integrator reported the t_R values to 0.001 min with a capability of resolving and separately reporting the areas of peaks differing by 0.005 ECL unit. With strong verification from MS fragmentation data, peak identification using these two combined parameters is believed to be valid.

RESULTS AND DISCUSSION

Fatty acid GC profiles of sebaceous triglycerides

In Fig. 1 (lower trace) the GC profile of a saturated FAME fraction is shown that represents a case with low iso-branched fatty acid chain types. The profile for the unsaturated fraction of the same individual is shown in Fig. 2. The inset upper tracing in Fig. 1 illustrates a portion of another subject's FAME profile that shows high iso-branched chain types.

Identification of normal, iso and anteiso chains

The identification of fatty acid chain types was based on two parameters. These are the retention times, from which the ECL values were calculated (see details in Experimental), and the mass fragmentation patterns of FAMEs. This latter

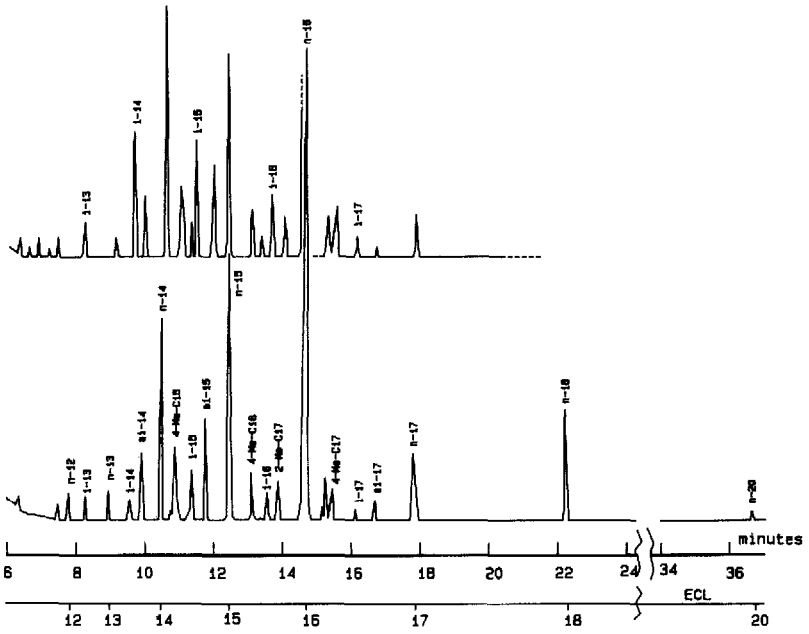

Fig. 1. Gas chromatograms of the saturated fatty acid methyl esters from SSL triglycerides. Main (lower) chromatogram: low iso content (subject 1); upper inset: high iso content (subject 4).

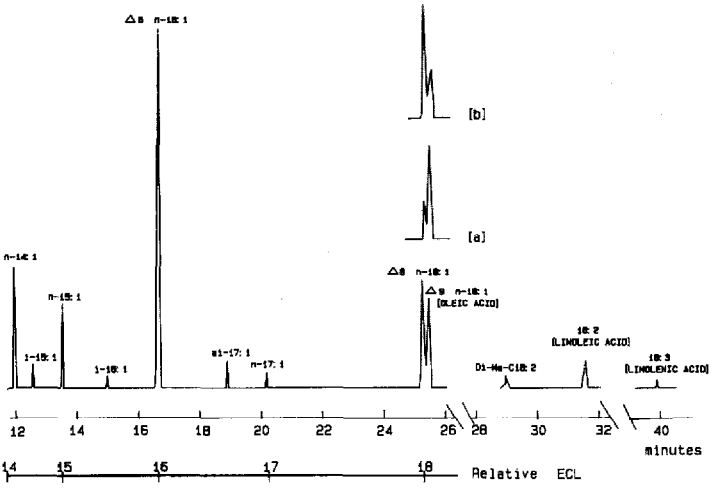

Fig. 2. Gas chromatogram of the monounsaturated fatty acid methyl esters from SSL triglycerides (subject 4). Inset a: two 18:1 acids showing high oleic acid content (subject 1). Inset b: two 18:1 acids showing high Δ^8 acid content (subject 2).

parameter was given special consideration because some previous reports [24, 25] considered the mass spectra of FAMEs of normal and iso- and anteiso-branched chains to be too similar to allow dependable identification. Fatty acid pyrrolidides were claimed to yield more differentiated spectra in the high mass range [24]. It can be seen from Figs. 3 and 4 that the high mass regions in the

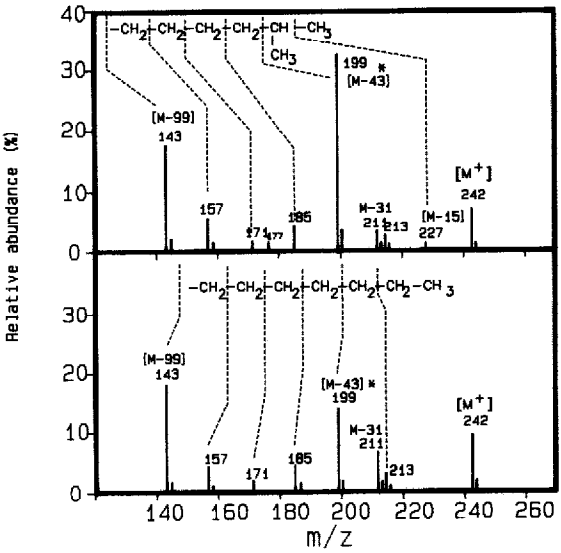

Fig. 3. The high mass region of the spectra of normal (lower) and iso (upper) 14:0 fatty acid methyl esters. Quadrupole spectrometer, EI at 70 eV.

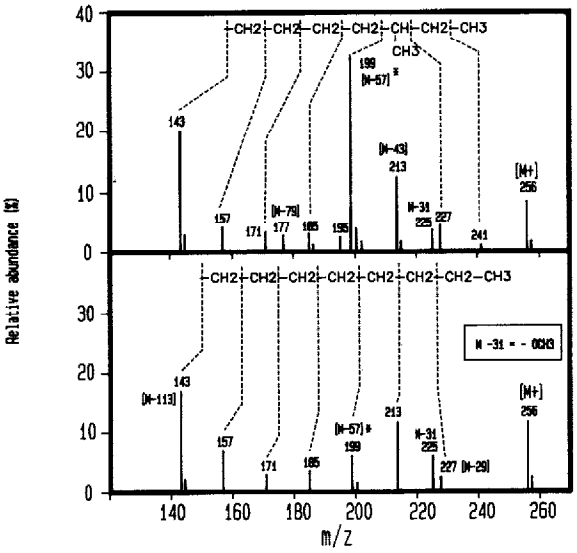

Fig. 4. The high mass region of the spectra of normal (lower) and anteiso (upper) 15:0 fatty acid methyl esters. Quadrupole spectrometer, EI at 70 eV.

spectra of normal as well as iso- and anteiso-branched FAMEs are sufficiently differentiated to permit reliable identification of each chain type. The distinction between the spectra is based primarily on the intensities of M−43 and M−57 peaks, with added confirmation from the relative intensities of M−29 and M−31 ion fragments. The spectrum of the iso C_{14} FAME (Fig. 3, upper part) shows in addition to the small peak caused by elimination of the terminal methyl (M−15, *m/z* 227) a high abundance ion fragment at *m/z* 199 (M−43) corresponding to

the loss of the stable isopropyl group [18–20] caused by the iso branching. The same fragment is much less intense in the spectra of either the normal C_{14} FAME (Fig. 3, lower part), or the anteiso C_{15} FAME (Fig. 4, upper part, now the fragment M – 43 is at *m/z* 213 because of the different molecular masses). Differences in abundance amount to ca. 20%. This is to be compared with differences of 0.5–1% in relative abundances of key ions in the high mass region of pyrrolidide derivatives of C_{15} fatty acids reported by Andersson and Holman [24]. Similarly, the spectrum of anteiso C_{15} FAME (Fig. 4, upper part) is characterized by an intense M – 57 ion corresponding to the loss of the stable fragment at the branch (butyl group) [18–20]. The same fragment is very small in the spectra of normal C_{15} FAME (Fig. 4, lower part at the same *m/z* of 199) or of normal C_{14} FAME (now at *m/z* 185) or of iso C_{14} FAME (also at *m/z* 185) (Fig. 3). The mass spectra of other normal, iso or anteiso FAMEs present in a standard mixture (standard mixture GLC-110, Supelco, Bellefonte, PA, U.S.A.) confirmed that peaks resulting from fragmentation at the carbon carrying the methyl branch itself are very small. Conversely, the peak resulting from loss of the smallest possible fragment immediately before the branch is very intense.

An additional confirmation could be based on the relative abundance of M – 31 (loss of methoxy group) and M – 29 (loss of ethyl group) [18–20]. The relative abundance of M – 31 was fairly constant in the spectra of all FAMEs examined because it is due to a common fragment. Relative to M – 31, the intensity of M – 29 (loss of ethyl) was smaller in normal and iso chains but was larger in the spectra of anteiso only (compare Figs. 3 and 4). This is due to the relative stability of the resulting large tertiary carbon fragment now carrying the methyl branch [18–20], for example at *m/z* 227 in the spectrum of anteiso C_{15} FAME (Fig. 4).

The reason that such simple differences in the spectra of normal and branched FAMEs were not sufficiently exploited by some previous investigators may be related to the fact that ion intensities for magnetic analysers [24, 25] and the quadrupole analyser that we used are not necessarily comparable [26].

Identification of 4-methyl branched chain type

The identification of 4-methyl branched fatty acids was relatively easy. The mass fragmentation pattern clearly showed the diagnostic intensity relation *m/z* 87 > *m/z* 74. The usual intensity relation of these peaks is *m/z* 74 > *m/z* 87 as in the spectra of normal, iso, anteiso and all other internally branched methyl chains found in the examined samples. When this unusual intensity relation (87 > 74) was observed, it always coincided with members having an ECL fraction of 0.5 (e.g. 14.5, 15.5, 16.5). This is in line with previously reported ECL values of 4-methyl branched FAMEs [21].

Detection of branched C_{17} fatty acid probably from a bacterial source

The identification of the 2-methyl-C_{17} fatty acid chain did not depend on the ECL value. The FAME of this acid eluted shortly before normal C_{16} with a calculated ECL of 15.82 (Table I). This value did not correspond to any of the previously reported ECL fractions of SSL fatty acids [8, 13]. The mass spectrum (Fig. 5) showed a base peak at *m/z* 88 and a well defined M^+ ion at *m/z* 284,

TABLE I

COMPOSITION (%) OF TRIGLYCERIDE FATTY ACIDS FROM SIX SAUDI MALES - SATURATES

Peak No.	ECL*	MS identification**	Subject No.					
			1	2	3	4	5	6
1	12.00	n-12	0.35	0.22	0.26	–	–	–
2	12.22	–	0.48	0.37	0.34	0.52	0.28	0.31
3	12.67	i-13	0.58	0.61	0.66	0.43	0.45	0.55
4	13.00	n-13	0.72	0.32	0.78	0.48	0.67	0.51
5	13.50	4-Me-C_{14}	0.26	0.57	0.55	0.31	0.48	0.22
6	13.65	i-14	0.67	2.25	0.83	6.51	1.77	0.58
7	14.00	n-14	12.22	11.51	12.10	11.06	14.53	13.87
8	14.20	–	0.52	0.71	0.21	1.65	1.11	1.09
9	14.44	–	0.69	2.10	1.15	1.91	1.50	1.43
10	14.50	4-Me-C_{15}	2.87	2.37	3.15	2.92	3.17	2.76
11	14.64	i-15	0.33	0.52	0.78	1.5	0.48	0.88
12	14.73	ai-15	2.86	5.51	4.15	7.66	3.63	2.56
13	15.00	n-15	13.68	13.76	12.43	10.01	12.85	13.33
14	15.22	–	0.22	0.75	0.51	0.80	–	0.16
15	15.43	–	0.80	0.42	0.28	0.65	0.51	–
16	15.50	4-Me-C_{16}	1.14	1.25	1.97	1.33	0.9	1.8
17	15.58	–	0.73	–	–	0.68	–	–
18	15.63	i-16	0.45	2.85	1.91	4.12	0.59	0.63
19	15.72	ai-16	0.52	0.18	0.22	0.20	0.15	0.29
20	15.82	2-Me-C_{17}	0.90	0.78	1.12	0.68	0.93	0.89
21	16.00	n-16	44.04	38.35	41.68	33.67	42.26	43.83
22	16.05	–	0.53	0.41	–	0.71	–	–
23	16.14	–	0.29	0.52	0.71	1.31	0.25	–
24	16.41	–	0.35	0.65	0.44	0.61	0.31	0.86
25	16.50	4-Me-C_{17}	1.45	2.47	1.71	2.10	1.61	1.93
26	16.52	–	1.21	2.22	2.06	1.25	0.95	1.49
27	16.63	i-17	0.04	–	–	0.07	–	–
28	16.72	ai-17	0.63	1.55	1.32	1.39	1.07	1.21
29	17.00	n-17	3.18	3.54	2.85	2.20	2.54	3.26
30	17.20	–	0.59	0.11	0.32	0.26	0.61	0.51
31	17.62	i-18	0.36	–	–	0.27	0.31	0.22
32	18.00	n-18	5.38	4.89	4.76	3.16	3.95	4.33
33	20.00	n-20	0.84	–	0.53	–	0.71	0.21
Total			99.88	100.24	99.78	100.09	98.57	99.71

*Fractions are explained in the text.
**ai = anteiso; i = iso; n = normal; Me = methyl branch (number = position).

indicating a C_{17} fatty acid (i.e. FAME with 18 carbons). This premature elution (before a C_{16} chain) suggested a non-linear retention time response, characteristic of substitution near the carboxyl end of the fatty acid chain [20]. This conclusion was in line with the presence of the base peak at m/z 88, which is diagnostic of 2-methyl substitution [21, 27].

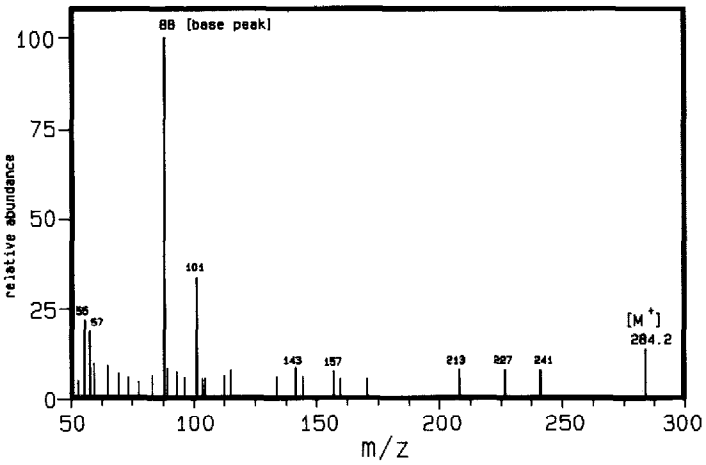

Fig. 5. Mass spectrum of methyl 2-methylhexadecanoate (ECL 15.82) showing characteristic peaks. Quadrupole spectrometer, EI at 70 eV.

A 2-methyl-C_{17} fatty acid chain has been reported (without an ECL value or details of the mass spectrum) by Nordstrom et al. [22] to be present in follicular casts' triglycerides. The origin of this chain is open to speculation. However, a bacterial contribution is likely as the incidence of 2-methyl groups in the fatty acids of the sterile vernix caseosa is low, if they are present at all [28].

Identification of monounsaturated chains

The MS identification of unsaturated FAMEs was based on established previous reports [18–20, 26, 29]. The diagnostic peak in the monounsaturated FAMEs is the molecular ion M^+. This ion and the ion fragments resulting from M−32, M−74 and M−116, coupled with a change in the base peak from m/z 74 (characteristic of saturated FAMEs [18–20]) to m/z 55, can be taken as identification. The position of the double bond, however, cannot be directly discerned from the spectrum of the FAME. In this respect the fatty acid pyrrolidides are superior as identification derivatives [16]. Using these derivatives (which are prepared from FAMEs) it was possible to confirm the position of unsaturation for $C_{16:1}$ in the SSLs as Δ^6. For 18:1 the high-resolution column used permitted the identification of two double-bond positional isomers. The earlier eluting component was found to be Δ^8 and the later eluting component was confirmed from added standard as well as from pyrrolidide analysis to be oleic acid (Δ^9).

The position of the methyl branch in monounsaturated FAMEs was based exclusively on ECL values since the spectra of these esters were too complicated to permit reliable identification on the basis of mass fragmentations. In agreement with previous findings, these monounsaturated fatty acids were found not to contain chain structures with internal methyl branching or to include C_{14}-iso or C_{15}-anteiso components.

Identification of di- and triunsaturated chains

Di- and triunsaturated FAMEs yielded spectra consistent with previous reports [26–29]. Sometimes weak but always discernible, the diagnostic M^+ was

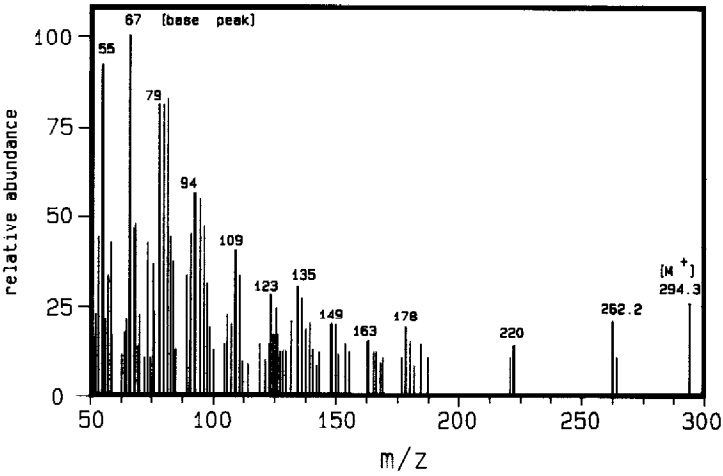

Fig. 6. Mass spectrum of fatty acid methyl ester of dimethyl-18:2 acid (ECL 19.35). Quadrupole spectrometer, EI at 70 eV.

present in the spectra of both types. The base peak changes from *m/z* 55 in the monounsaturated to *m/z* 67 in the diunsaturated (Fig. 6) or to *m/z* 79 in the triunsaturated FAMEs. Dimethyl branching of the 18:2 acid was inferred only from a spectral library computer-search and match. When taken together with strong confirmation from accurate determination of ECL values, these MS identifications were considered reliable.

Quantification of chain types

Based on the above identifications, the detailed and quantitative analyses of the sampled SSL triglyceride fatty acids are shown in Tables I–III for the saturated fraction, monounsaturated fraction and polyunsaturated FAMEs, respectively. The quantification shown in Tables I and II was based exclusively on the integration of the total ion current (TIC). The TIC response is generally considered equivalent to that from flame ionization detection (FID) [26]. Accordingly, these data could be compared with previously published results on sebum analysis, which were based on FID [13–15]. Similarly, the amounts of linoleic acid (18:2) shown in Table III were calculated on the basis of TIC integration. On the other hand, the relatively minute amounts of the suspected 2,3-dimethyl-18:2 acid and of the 18:3 (linolenic) acid shown also in Table III were computed by considering both the TIC integration as well as the integration of a selected group of ions. This technique was utilized in order to evaluate, then to minimize, the effect of background interference, which is usually augmented in the quantification of minor components. The groups of ions selected were in the relatively wide *m/z* ranges of 67–178 and 67–207 for the 2,3-dimethyl-18:2 and the 18:3 acids, respectively. These ranges included ca. 80% of the total ions (see e.g. Fig. 6) with the added advantage of excluding ions of common impurities appearing in the *m/z* range 40–60 – notably ions at *m/z* 55 and 57 (see Fig. 6). However, no precise calibration of the detector response was performed prior to the integration of

TABLE II

COMPOSITION (%) OF TRIGLYCERIDE FATTY ACIDS FROM FOUR MALE SAUDIS - MONOUNSATURATES

For ECL fractions and MS identification abbreviations, see Table I.

Peak No.	ECL	MS identification	Subject No.			
			1	2	4	5
1	14.00	n-14	9.65	6.54	8.66	7.75
2	14.65	i-15	0.44	–	1.25	0.50
3	15.00	n-15	5.11	7.65	6.32	5.53
4	15.63	i-16	1.89	4.47	11.83	6.43
5	16.00	n-16	59.43	58.36	47.62	51.82
6	16.63	i-17	0.23	0.12	0.35	0.25
7	16.72	ai-17	5.64	3.15	5.75	5.51
8	17.00	n-17	4.87	5.77	3.88	6.15
9	17.62	i-18	–	–	0.84	1.35
10	17.95	18:1w10*	6.14	4.92	6.65	8.84
11	18.00	n-18.00	5.73	9.02	6.37	5.72
Total			99.13	100.00	99.52	99.85

*18:1w10 is the Δ^8 isomer of oleic acid (see text).

TABLE III

CLASS COMPOSITION (%) OF TRIGLYCERIDE FATTY ACIDS FROM FOUR MALE SAUDIS

Lipid class	Subject No.			
	1	2	4	5
Total saturates	49.8	56.4	45.5	55.7
Total unsaturates*	50.2	43.6	55.5	44.3
18:2 Linoleic (ECL 19.86)	0.82	0.68	1.38	0.61
18:2 2,3-Dimethyl (ECL 19.35)	0.09	–	0.13	–
18:3 (ECL 21.10)	Trace	–	0.09	–
Ratio 16:0/16:1	0.73	0.86	0.58	1.03
Ratio 18:0/18:1	0.45	0.45	0.20	0.34

*Total unsaturates include the amounts of 18:2 and 18:3 shown.

these two selected-ion ranges. Consequently, the amounts of these two components shown in Table III are not necessarily comparable with any previous data based on integration of FID response.

Data in Tables I–III represent the averages of triplicate analyses of two different samples taken three weeks apart from each individual. There were minor variations between the two sets of samples.

The analyses in Tables I and II show that among the six subjects tested two trends are exhibited. In one group, exemplified by subjects 2 and 4, the amounts of iso-even fatty acid chains, particularly C_{14} and C_{16}, are up to ten-fold higher than the same chain type in the other four subjects. In the second group, represented by subjects 1, 3, 5 and 6, the amounts of iso-even fatty acid chains are much lower. The odd-carbon iso- and anteiso-branched acids each showed less variation among the two subgroups. Only about three-fold variation is displayed among the subjects tested.

These data are qualitatively similar to the reports of Downing and co-workers [13–15]. However, the variations were much greater in these earlier investigations where the concentration range among different individuals for even-carbon iso acids was 1–22% of the monounsaturated fatty acids and 1–13% of the saturated acids. The ranges for the anteiso structures were 3–7.5% and 3–13.5%, respectively [13–15]. These differences between the present work and previous reports are undoubtedly related to the anatomical sources of the lipid samples of both investigations. In the earlier work only the fatty acids from the wax esters (which are produced exclusively by the sebaceous glands) were analysed. Since triglyceride fatty acids are derived from both sebaceous and epidermal sources, it may be inferred that the epidermally originating fatty acids do not vary much between individuals with regard to these branched types. This is to be expected since epidermal lipids are believed to be derived from circulating lipids and thus do not contain the unusual fatty acid profile characteristic of sebum wax esters as well as of sebum triglycerides [30].

Potential significance of the ratio of Δ^8/Δ^9 18:1 acids

The relative contributions of epidermal and sebaceous sources to the SSL triglyceride pool cannot be estimated from the above-mentioned inter-individual variations of methyl branched acids. However, the relative amounts of the two 18:1 acids could give an indication of the contribution from these two sources. Although the majority of sebaceous monounsaturates are of the Δ^6 type [3, 6–8,13, 26, 30], the 18:1 of sebaceous follicles has been shown to be exclusively of the Δ^8 configuration [8, 22]. Hence any Δ^9 (oleic acid) present in the SSLs is in all probability arising from the dermal sources. Consequently, the relative amounts of these two 18:1 acids may be taken as suggestive of the proportions of epidermal and sebaceous lipids in the SSLs for each individual. Three cases were met with where both Δ^8 and Δ^9 acids were present in comparable amounts or else one isomer predominated (Fig. 2 and insets). Constancy of the Δ^8/Δ^9 ratio for each individual in the two samples analysed was evident even in the limited number of samples examined. Work is in progress on a larger number of samples with greater frequency and longer time between samplings to confirm these preliminary findings. However, at present it can be suggested that the ratio of Δ^8 to Δ^9 18:1 acids could be one of the important factors in assigning the anatomical origin of fatty acids in the SSL pool.

ACKNOWLEDGEMENT

This work was supported by a grant from King Abudlaziz City for Science and Technology, Riyadh, Kingdom of Saudi Arabia, Grant No. AR-06-95.

REFERENCES

1 D.T. Downing, in J.C. Touchstone and R. Roger (Editors), Thin-layer Chromatography, Quantitative Environmental and Clinical Applications, Wiley Interscience, New York, 1980, p. 495.
2 J.L. Burton, in R. Marks (Editor), Investigative Techniques in Dermatology, Blackwell, Oxford, 1979, p. 1.
3 N. Goetz, K. Kaba and P. Boré, J. Chromatogr., 233 (1982) 19.
4 Z.M.H. Marzouki, A.M. Taha and K.S. Gomaa, Trop. Geogr. Med., in press.
5 D.T. Downing, M.E. Stewart, P.W. Wertz, S.W. Cotton, W. Abraham and J.S. Strauss, J. Invest. Dermatol., 88 (1987) 2.
6 N. Nicolaides, Science, 186 (1974) 19.
7 N. Nicolaides, Lipids, 6 (1971) 901.
8 N. Nicolaides, H.C. Fu, M.N.A. Ansari and G. Rice, Lipids, 7 (1972) 506.
9 D.T. Downing, J.S. Strauss and P. Pochi, J. Invest. Dermatol., 53 (1969) 322.
10 R.S. Greene, D.T. Downing, P.E. Pochi and J.S. Strauss, J. Invest. Dermatol., 54 (1970) 240.
11 G.M. Gray and H.J. Yardley, J. Lipid Res., 16 (1975) 434.
12 M.E. Stewart, A.M. Benoit, D.T. Downing and J.S. Strauss, J. Invest. Dermatol., 82 (1984) 74.
13 S.C. Green, M.E. Stewart and D.T. Downing, J. Invest. Dermatol., 83 (1984) 114.
14 M.E. Stewart and D.T. Downing, J. Invest. Dermatol., 84 (1985) 501.
15 M.E. Stewart, M.W. McDonnel and D.T. Downing, J. Invest. Dermatol., 86 (1986) 706.
16 B.A. Andersson and R.T. Holman, Lipids, 9 (1973) 185.
17 N. Nicolaides and J.M.B. Apon, Biomed. Mass Spectrom., 4 (1977) 337.
18 F.W. McLafferty, Interpretation of Mass Spectra, Benjamin, Reading, MA, 2nd ed., 1973.
19 F.W. McLafferty and R. Venkataraghavan, Mass Spectral Correlations, American Chemical Society, Washington, DC, 2nd ed., 1982.
20 J.A. McCloskey, Methods Enzymol., 14 (1969) 382.
21 J. Jacob, J. Chromatogr. Sci., 13 (1975) 415.
22 K.M. Nordstrom, J.N. Labows, K.J. McGinley and J.J. Leyden, J. Invest. Dermatol., 86 (1986) 700.
23 A.T. James and A.J.P. Martin, Biochem. J., 50 (1952) 679.
24 B.A. Andersson and R.T. Holman, Lipids, 10 (1975) 716.
25 S. Abrhamsson, S.S. Stenhagen and E. Stenhagen, in R.T. Holman (Editor), Progress in the Chemistry of Fats and Other Lipids, Vol. 7, Pergamon Press, New York, 1963, p. 41.
26 G.R. Waller and O.C. Dermer (Editors), Biochemical Applications of Mass Spectrometry, First Supplemental Volume, Wiley Interscience, New York, 1980.
27 E. Stenhagen and H.A. Boekenoogen, Analyses and Characterization of Oils, Fats and Fat Products, Interscience, London, 1968.
28 N. Nicolaides, Lipids, 9 (1973) 85.
29 L.P. Alexander and J.B. Justice, Jr., J. Chromatogr., 342 (1985) 1.
30 D.T. Downing, J. Am. Acad. Dermatol., 14 (1986) 221.