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Systematic characterisation of long-chain aliphatic esters of wool wax by gas chromatography–electron impact ionisation mass spectrometry☆

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Abstract

A detailed structural characterisation of the aliphatic high-molecular-mass esters extracted from raw wool based on high-temperature gas chromatography–electron impact ionisation mass spectrometry is described. The raw wool esters extracted are in the range of C_{37} to C_{54} (i.e., molecular mass 550–788). The selected ion chromatogram exhibited four isomers for the esters with an odd number of carbon atoms (*i*:*a*, *i*:*n*, *a*:*n* and *n*:*n*) and five for those with an even number of carbon atoms (*i*:*i*, *a*:*a*, *i*:*n*, *a*:*n* and *n*:*n*). Isomeric structural elucidation is discussed with respect to the long-chain fatty acid and long-chain fatty alcohol structures, on the basis of chromatographic retention behaviour and mass spectral information.

Keywords: Waxes; Retention behaviour; Lanolin; Fatty acids; Fatty alcohols; Esters

1. Introduction

Wool wax, known as lanolin, is a complex mixture of high-molecular-mass lipidic compounds [1–8]. It is an important ingredient for the manufacture of cosmetics, toiletries and pharmaceuticals [2–5,7,9,10]. Its specific use can be determined only by the characterisation of its individual classes of components.

Previously published papers dealing with lanolin characterisation have mainly focused on either the individual fatty acids and alcohols obtained by complete hydrolysis of the lanolin ester mixture or the global lipidic classes [6,11–14]. Only limited data have been reported on aliphatic esters structures because of their extremely high complexity. More than 10 000 mono-esters may result from the combination of lanolin alcohols and lanolin acids, assuming a completely randomised combination and no preferential esterification in lanolin biosynthesis in the sheep sebaceous gland [7].

The aim of the present work is to study by gas chromatography–mass spectrometry (GC–MS), the

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specificity of the ester biosynthesis process in which high-molecular-mass fatty acids combine with fatty alcohols. Therefore, we have developed a high-temperature capillary gas chromatography–electron impact ionisation mass spectrometry (HT-GC–EI-MS) method for the structural characterisation and quantification of the long-chain aliphatic esters obtained from lanolin by supercritical fluid extraction (SFE) combined with gel permeation chromatography (GPC) fractionation. Ester structures were elucidated using chromatographic retention data and the EI-MS information obtained by GC–MS.

2. Experimental

2.1. Samples

Lanolin samples were isolated from raw wool by SFE with carbon dioxide (CO_2) in the presence of a modifier, according to a previously described procedure [15]. Real samples enriched in aliphatic esters were selected for this study. The SFE extract was collected in an ethyl acetate–cyclohexane (1:1) mixture and filtered through a 0.45 μm nylon membrane. Filtered samples were fractionated using an LC system (see Section 2.2).

2.2. Instrumentation

The LC system used was from Shimadzu (Kyoto, Japan) equipped with a Rheodyne high-pressure valve with a 100 μl loop, LC-10AT pumps, a UV detector SPD-10AV, SCL-10A and Class-VP software. The GPC column used was 450 mm×10 mm I.D. and packed with Bio Beads SX-3, 200–400 mesh from Bio-Rad (Hercules, CA, USA). Ethyl acetate–cyclohexane (1:1) at a 2 ml min^{-1} flow-rate as the mobile phase was used and the first fraction was collected in the time interval from 0 to 8 min.

The HT-GC–MS analyses were performed using a Fisons MD 800 mass spectrometer (Fisons, Loughborough, UK). The instrument was operated in the EI mode at 70 eV. Chromatographic conditions were as follows: injection was in the splitless mode at 330 °C. The GC column was a polyimide-clad polycarborane–dimethylsiloxane column (HT 5, SGE, Ringwood, Victoria, Australia) of 10 m×0.32 mm I.D. and 0.10 μm film thickness. Column temperature was programmed from 90 °C (1 min) to 240 °C at 10 °C min^{-1} and then to 360 °C at 5 °C min^{-1} and holding the final temperature for 5 min (total analysis time 45 min). Helium was the carrier gas at an inlet pressure of 12 p.s.i. (1 p.s.i=6894.76 Pa) (i.e., 1 ml min^{-1} flow-rate). Transfer line and ion source temperatures were held at 320 and 230 °C, respectively.

3. Results and discussion

The identification of the long-chain esters was based on (a) the interpretation of mass spectra of the individual compounds obtained by GC–MS, and (b) the chromatographic retention data.

The characterisation of the wool long-chain esters presents great difficulty because of the high number of compounds, as aliphatic or steryl ester type, which may occur in the samples [2–5,7,8,10,16]. The wool wax aliphatic alcohols and acids consist of (*normal*) (*n*) *iso* (*i*) and *anteiso* (*a*) series [2,4,5,10,16]. The *iso* series contains an isopropyl terminal group and the *anteiso*, a secondary butyl terminal group. Compounds containing an odd carbon number comprise *n* and *a* structures and the ones with an even carbon number, *n* and *i* structures. As a consequence, in the ester biosynthesis process, there is a distinct possibility of aliphatic ester formation by the combination of acids and alcohols of different structure and size.

We propose the following nomenclature:

(1) [O], [E] for the aliphatic esters containing an odd or even number of carbon atoms, respectively.

(2) [O_i], [E_i] for the aliphatic acid and alcohol moiety containing an odd or even number of carbon atoms, respectively, where the *i* value can be 1 or 2 and denotes the acid or alcohol moiety, respectively.

(3) a_i, i_i, n_i the *anteiso*, *iso* and *normal* structures, where $i=1$ for the acid chain and $i=2$ for the alcohol chain.

(4) [x_1:y_2] denoting the isomeric structures of esters, where *x* and *y* can be *a*, *i* or *n* for the acid ($i=1$) or the alcohol ($i=2$) moiety, respectively.

(5) *N* (*k*:*m*) for an ester with *N* total carbon atoms, *k* atoms in the acid and *m* in the alcohol moiety.

The general structural scheme of all the isomeric

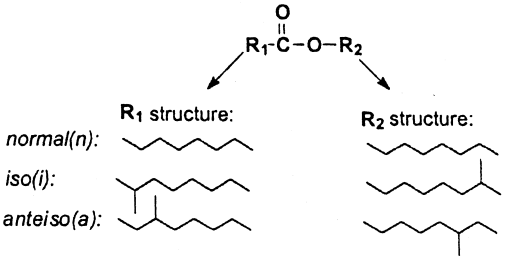
aliphatic esters and the notation used are shown in Fig. 1.

Taking into account that an acid or alcohol of the $[O_i]$ type can have an a_i or n_i structure and that an acid or alcohol of the $[E_i]$ type can have an i_i or n_i structure, the resultant ester formed by biosynthesis can be deduced.

An aliphatic ester [O], with an odd number of carbon atoms, can be formed from an acid $[O_1]$, with an odd number of carbon atoms, and an alcohol $[E_2]$, with an even number of carbon atoms; or from an acid $[E_1]$, with an even number of carbon atoms, and an alcohol $[O_2]$, with an odd number of carbon atoms, as follows:

$$[O] = [O_1]:[E_2] = [a_1, n_1]:[i_2, n_2] = [a_1:i_2] + [a_1:n_2] + [n_1:i_2] + [n_1:n_2] \quad (1)$$

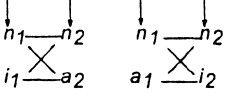
$$[O] = [E_1]:[O_2] = [i_1, n_1]:[a_2, n_2] = [i_1:a_2] + [i_1:n_2] + [n_1:a_2] + [n_1:n_2] \quad (2)$$



Symbols:
k and **m** are the number of carbon atoms in acid and alcohol moiety, respectively.
[E1](if **k** is even) and **[O1]**(if **k** is odd) for acid moiety,
[E2](if **m** is even) and **[O2]**(if **m** is odd), for alcohol moiety,
[E](if **N** is even) and **[O]**(if **N** is odd) for the intact esters.
N(=k+m) is the number of carbon atoms in the intact ester;

Isomeric structures of **[O]** type esters:

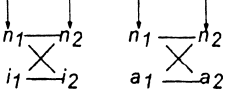
[O] = [E1] + [O2] or [O1] + [E2]



[n1:n2]+ [n1:a2]+ [i1:n2]+ [i1:a2]
[n1:n2]+ [n1:i2]+ [a1:n2]+ [a1:i2]

Isomeric structures of the **[E]** type esters:

[E] = [E1] + [E2] or [O1] + [O2]

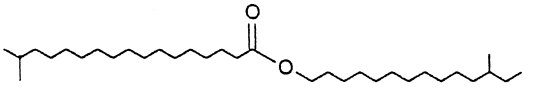


[n1:n2]+ [n1:i2]+ [i1:n2]+ [i1:i2]
[n1:n2]+ [n1:a2]+ [a1:n2]+ [a1:a2]

Ester notation:

-by the carbon atom number: **N(k:m);**
-by the sort of carbon atom number: **[O]=[E1:O2], [O]=[O1:E2], [E]=[E1:E2], [O]=[O1:O2];**
-by the isomer type of acid and alcohol moiety: **[*x1:y2*],** where ***x,y*** can be ***i, n*** or ***n*** isomers, for acid and alcohol moiety, respectively; the indice **1** is for acid and **2** for alcohol moiety.

Example



k=18, m=15, N=33
Symbols: 33(18:15); [O]=[E1:O2]; [*i1:a2*]

Fig. 1. The general structure scheme of aliphatic ester isomers from lanolin sample and the symbolism used.

In the same way, an ester [E], having an even number of carbon atoms, can be synthesised from an acid [O_1] and an alcohol [O_2], or from an acid [E_1] and an alcohol [E_2], as:

$$[E] = [O_1]:[O_2] = [a_1, n_1]:[a_2, n_2] = [a_1:a_2] + [a_1:n_2] + [n_1:a_2] + [n_1:n_2] \quad (3)$$

$$[E] = [E_1]:[E_2] = [i_1, n_1]:[i_2, n_2] = [i_1:i_2] + [i_1:n_2] + [n_1:i_2] + [n_1:n_2] \quad (4)$$

As can be seen from Eqs. (1)–(4), eight different isomeric structures for a given molecular mass are theoretically possible.

3.1. Mass spectra of aliphatic long-chain esters

The base peaks in the mass spectra of the long-chain esters are produced by a rearrangement process involving the transfer of 2H atoms from the alcohol chain to the acid chain giving a protonated acid ion [17,18]. The molecular ion M^+ has an intensity in the range of 20–25%. The base peak gives the number of carbon atoms in the acid moiety, $k=(m/z-33)/14$ and the M^+, the total number of carbon atoms, $N=(M-32)/14$. The mass spectrum of the *normal* ester 38 (16:22) (docosanoylhexadecanoate, $N=38$, $M=564$) in the EI mode (70 eV) is shown in Fig. 2. The base peak is m/z 257 corresponding to the protonated hexadecanoic acid. The other ions in the mass spectrum are: M^+, m/z 564 (21.4%); $O^+\equiv C-(CH_2)_{21}-CH_3$, m/z 353 (12.1%) produced by α-fission of the molecular ion and the ion $[CH_2=CH-(CH_2)_{19}-CH_3]^+$, m/z 308 (5.7%) corresponding to the deprotonated alcohol moiety. Some common ions for saturated hydrocarbons from the series $[C_nH_{2n+1}]^+$ with m/z 57, 71 and 85 can be detected with an abundance of 10–25%.

It is possible to determine the individual contribution of esters to every chromatographic peak by mass spectrometric determination of the molecular ion and the base peak, correlated with the retention parameters. We analysed the wool wax (lanolin) aliphatic esters with N in the range 37–54 (molecular mass range 550–788). The chromatograms are shown in Fig. 3 ($N=37$–42), Fig. 4 ($N=43$–48) and Fig. 5 ($N=49$–54).

3.2. Chromatographic separation

3.2.1. Effect of the acid or alcohol moiety structure

A complete resolution for N (total carbon atoms), as is shown in Figs. 3–5, was obtained. To study the branching effects of the acid or alcohol moiety, the ester pairs of the N (k:m) and N (m:k) types were analysed by MS. The latter compound contains the same type of branched chain but in reversed position (the acid chain structure is replaced by the alcohol

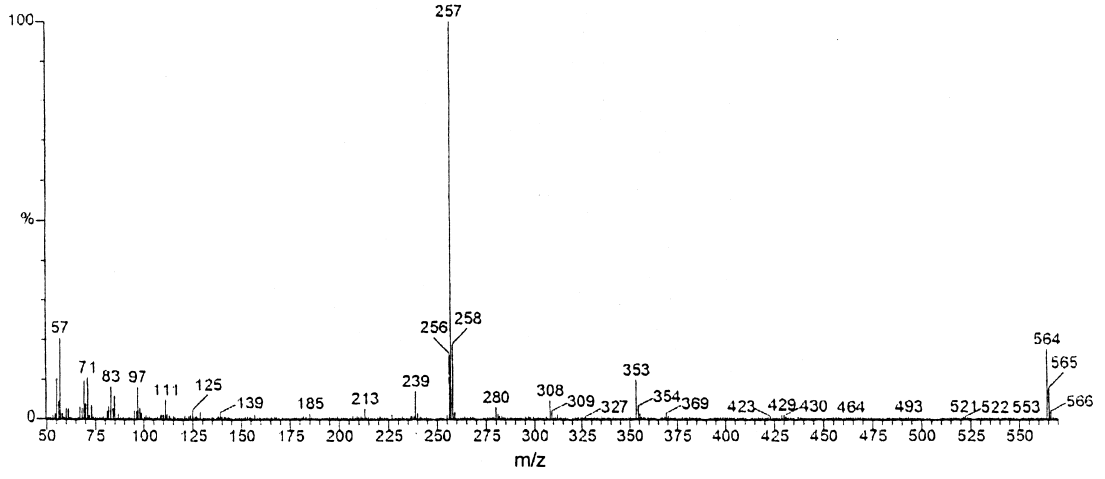


Fig. 2. EI mass spectrum (70 eV) of docosanoyl hexadecanoate, 38 (16:22), $M=564$.

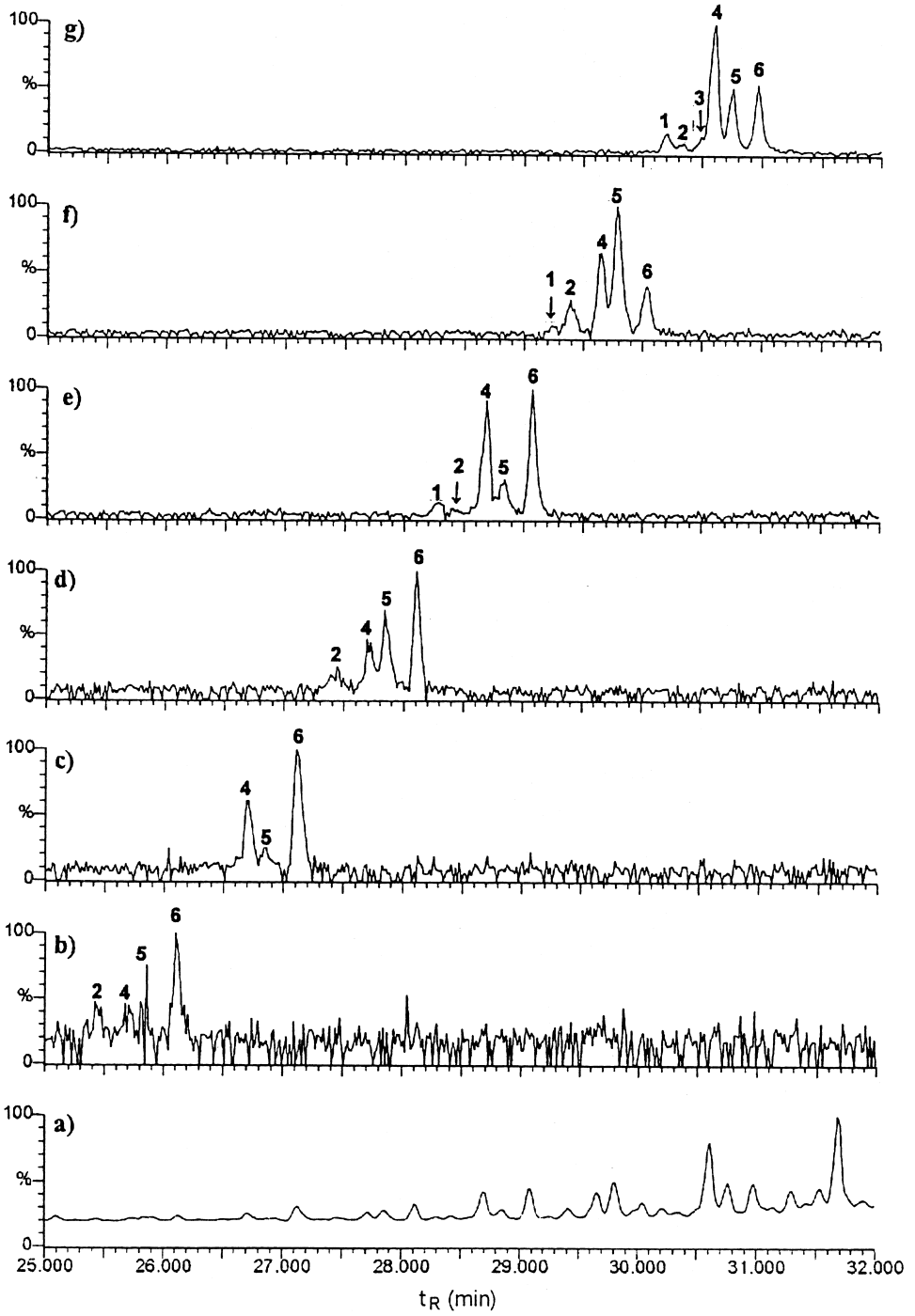


Fig. 3. GC–MS chromatogram of the aliphatic esters in the range of $N=37$–42 (M 550–620): (a) Total ion current (TIC), (b) m/z 550 ($N=37$), (c) m/z 564 ($N=38$), (d) m/z 578 ($N=39$), (e) m/z 592 ($N=40$), (f) m/z 606 ($N=41$), and (g) m/z 620 ($N=42$). Peaks 1–6 are described in Table 1. GC–MS conditions are described in the Experimental section.

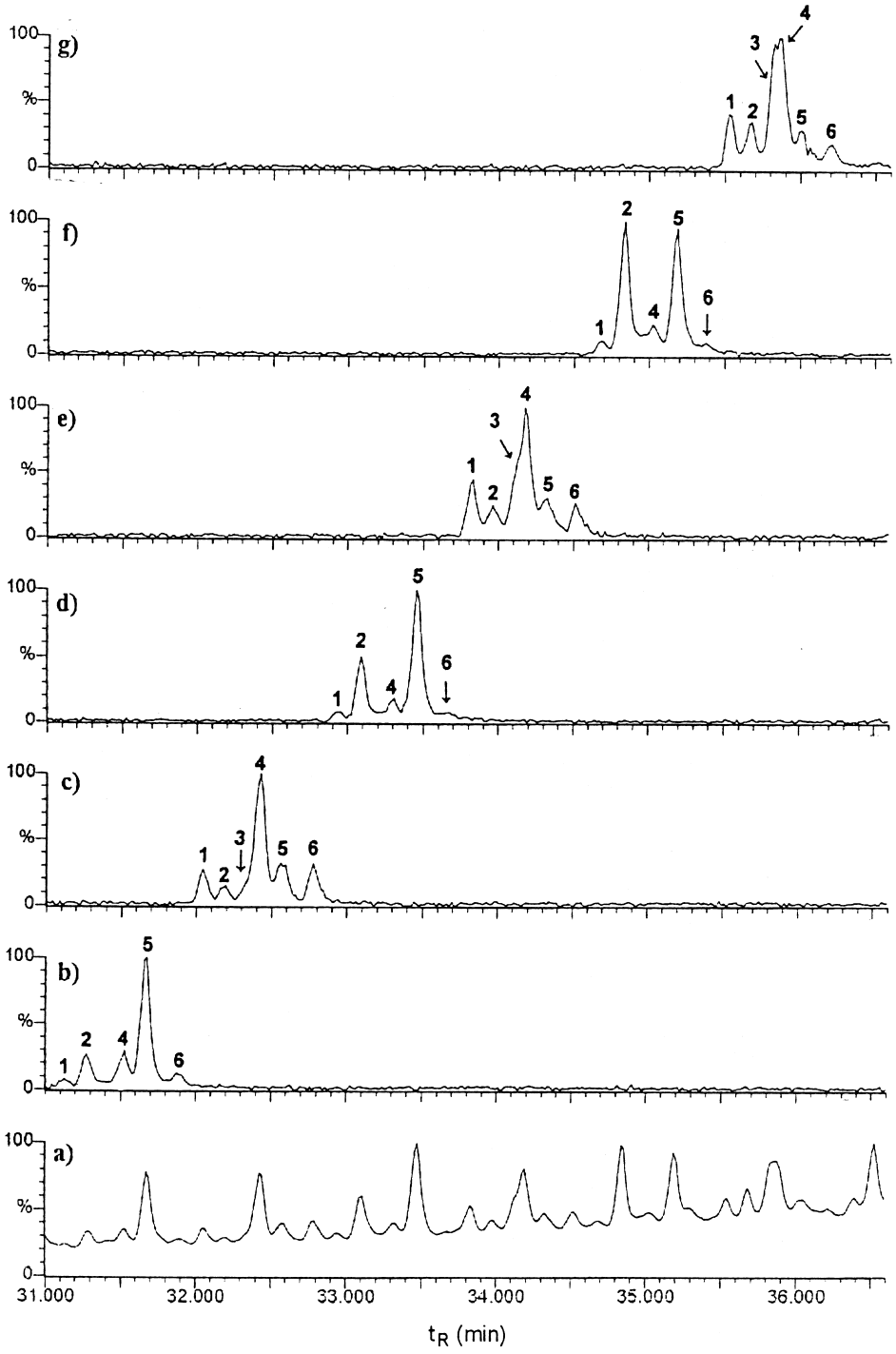


Fig. 4. GC–MS chromatogram of the aliphatic esters in the range of $N=43–48$ (M 634–704): (a) TIC, (b) m/z 634 ($N=43$), (c) m/z 648 ($N=44$), (d) m/z 662 ($N=45$), (e) m/z 676 ($N=46$), (f) m/z 690 ($N=47$), and (g) m/z 704 ($N=48$). Peaks 1–6 are described in Table 1. GC–MS conditions as in the Experimental section.

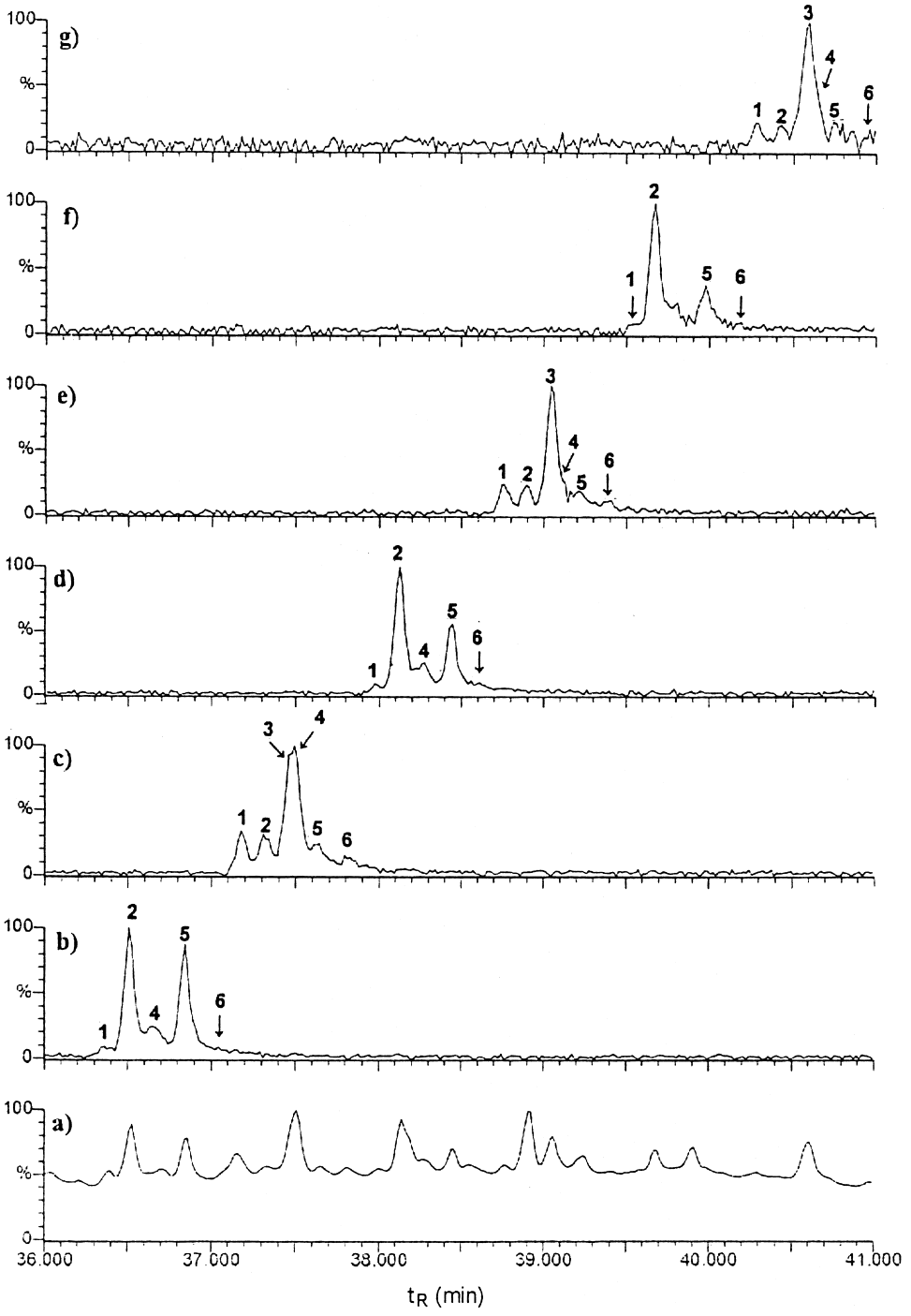


Fig. 5. GC–MS chromatogram of the aliphatic esters in the range of $N=49$–54 (M 718–788): (a) TIC, (b) m/z 718 ($N=49$), (c) m/z 732 ($N=50$), (d) m/z 746 ($N=51$), (e) m/z 760 ($N=52$), (f) m/z 774 ($N=53$), (g) m/z 788 ($N=54$). Peaks 1–6 are described in Table 1. GC–MS conditions as in the Experimental section.

chain structure, and vice versa) relative to the former.

The simultaneous registration of the ion with m/z 327, base peak of the compound 47 (21:26), with m/z 397, base peak for the compound 47 (26:21), in the region of $M^+ = 690$ ($N = 47$), gives two superimposed profiles, as shown in Fig. 6. This observation is consistent with the hypothesis that the effect of chain branching (*iso* or *anteiso*) on the retention time is the same for both positions, i.e., the acid or alcohol moieties. As a consequence, from Eqs. (1)–(4), the following equivalent pairs of structures have the same retention time:

$$[a_1{:}i_2]\ (\text{from Eq. (1)}) = [i_1{:}a_2]\ (\text{from Eq. (2)}) = [a{:}i] \quad (5)$$

$$[n_1{:}i_2]\ (\text{Eq. (1)}) = [i_1{:}n_2]\ (\text{Eq. (2)}) = [i{:}n] \quad (6)$$

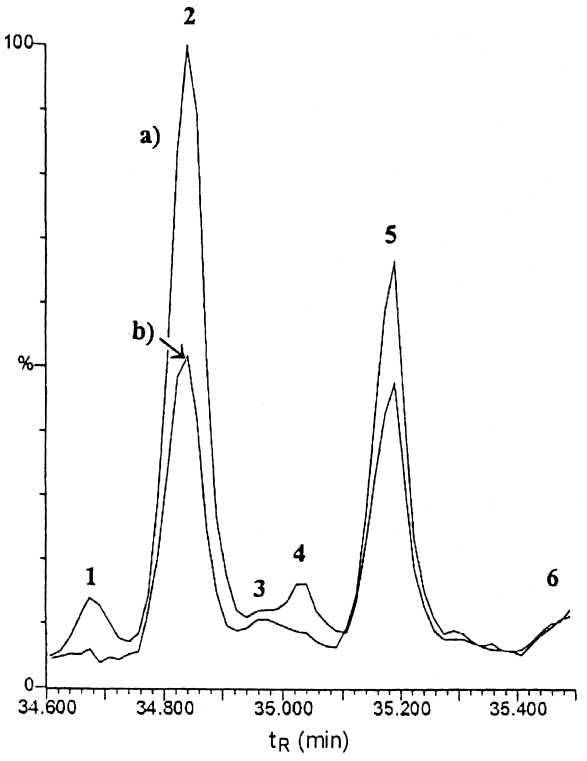


Fig. 6. Mass chromatogram of the base peaks for symmetrical compounds: (a) m/z 327, compound 47 (21:26); (b) m/z 397, compound 47 (26:21). Peaks 1–6 are described in Table 1. GC–MS conditions as in the Experimental section.

$$[a_1{:}n_2]\ (\text{Eq. (1)}) = [n_1{:}a_2]\ (\text{Eq. (2)}) = [a{:}n] \quad (7)$$

$$[a_1{:}n_2]\ (\text{Eq. (3)}) = [n_1{:}a_2]\ (\text{Eq. (3)}) = [a{:}n] \quad (8)$$

$$[i_1{:}n_2]\ (\text{Eq. (4)}) = [n_1{:}i_2]\ (\text{Eq. (4)}) = [i{:}n] \quad (9)$$

Taking into account that the latter terms from Eqs. (1)–(4) correspond to the same structure [n:n], Eqs. (1–9) show that for an ester of [O] type four structures are resolved chromatographically, and for an ester of [E] type, five structures are resolved by GC, as can be seen from Eqs. (10) and (11):

$$[\text{O}] = [i{:}a] + [i{:}n] + [a{:}n] + [n{:}n] \quad (10)$$

$$[\text{E}] = [i{:}i] + [a{:}a] + [i{:}n] + [a{:}n] + [n{:}n] \quad (11)$$

There are six distinct ester structures: [i:n], [a:n] and [n:n] common for both types of esters [O] and [E], [i:i] and [a:a] specific only for [E] esters and [i:a] specific only for [O] esters.

Fig. 7 shows the chromatographic position of all isomeric peaks for the compounds with $M^+ = 676$ ($N = 46$). The ion of structure [i:a] (characteristic for the odd esters) is present probably because the *a* compounds are of minor contribution in either the even acid or the alcohol moieties; or because there are small quantities of *i* compounds in the odd acid or the alcohol moiety.

Specifying the retention behaviour using the terminology of the equivalent chain length (ECL) is very useful for compound identification [19,20]. This parameter is independent of operating conditions. The ECL values for *normal* esters [of (n:n) type] are integers equal to their carbon atom number [$(\text{ECL})_N^{(n:n)} = N$]. The ECL for branched esters is less than for the corresponding *normal* homologues. The ECL value for an isomer (x:y) containing ($N+1$) carbons can be written as:

$$(\text{ECL})_{N+1}^{(x:y)} = (\text{ECL})_N^{(n:n)} + (\text{FCL})^{(x:y)} = N + (\text{FCL})^{(x:y)} \quad (12)$$

where $(\text{FCL})^{(x:y)}$ is the fractional chain length (fraction of the carbon atom number attributed to the methyl branch) [19]. FCL is a fractional number in the range of 0.00–1.00 and is independent of N. For the (n:n) isomers, the FCL is 1.00. Similarly, taking

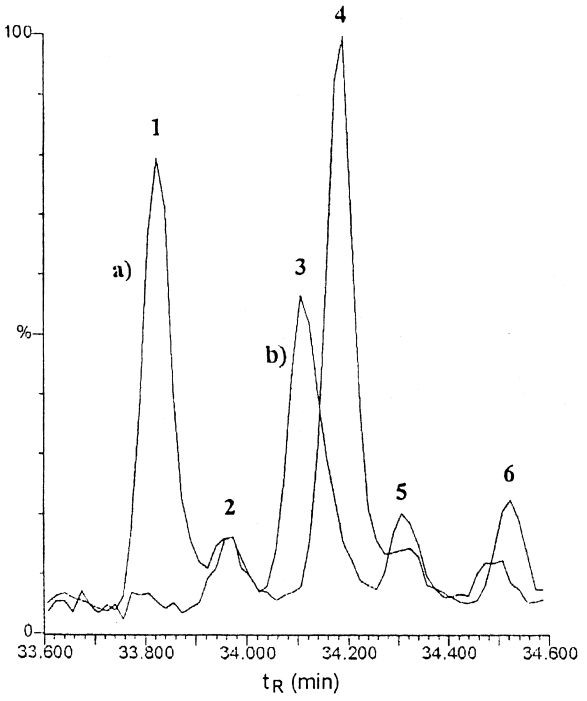


Fig. 7. Isomeric position peaks for the esters of $M^{+\cdot}=676$ ($N=46$); (a) m/z[base peak of compound 46 (20:26)]; (b) m/z 383 (base peak of compound 46 (25:21)]. Peaks 1–6 are described in Table 1. GC–MS conditions as in the Experimental section.

as a reference the *normal* compound with N carbon atoms, the FCL parameters for a *normal* compound containing $N+1$ carbon atoms is 1.00, but it is a fractional number (in the range of 0.00–1.00) for the others.

Table 1 shows the experimental FCL values (as average values obtained for the $N=44$ and 46 isomers). The resolution obtained on the separation of the six isomeric structures is better than 1 except for the separation of the peaks 3 and 4 ([a:a] and [i:n]), for which resolution is in the range of 0.5–1.0 (see Fig. 7).

Table 1
Fractional chain length (FCL) for ester isomers as the mean value obtained for $N=44$ and 46 compounds

Peak No.	Structure	FCL	Ester type	
1	*i*:*i*	0.19	E	
2	*i*:*a*	0.36		O
3	*a*:*a*	0.51	E	
4	*i*:*n*	0.61	E	O
5	*a*:*n*	0.76	E	O
6	*n*:*n*	1.00	E	O

i, *a* and *n* denote the *iso*, *anteiso* and *normal* structures, respectively, of the acid and alcohol moieties.

As can be seen only from the chromatographic retention parameters, it is not possible to see if the branched structure is on the acid or the alcohol moiety. The complete structural elucidation can be obtained by using chromatographic data in conjunction with mass spectral information. As previously stated, the base ion gives the carbon number of the acid (k), and the molecular ion M^+ gives the total number of carbon atoms N, thus the carbon atom number of the alcohol moiety (m) can be deduced. If k and m are known, the branched structure of the acid and alcohol moiety also can be found: *i* and *n* for [O] and *a* and *n* for [E] types.

3.2.2. Effect of the relative size of the acid and alcohol chain (k:m)

A shift of the peaks containing the same N and the same structure but different values for k and m (carbon atom distribution on the acid and alcohol chain, respectively) was observed (the ''chain shift''). The shift is in the direction of the lower retention time when the k value is increasing. The position of the peaks m/z 313 (20:26), m/z 285 (18:28) and m/z 257 (16:30) for compounds with $k+m=46$ ($M=676$) are shown in Fig. 8. All these compounds have the same isomer structure (the acid and alcohol moieties are of even type). The shift effect $\Delta t/\Delta k$ is in the order of 0.006 min. This means that the retention time is changed by 0.006 min when the chain length is changed by one carbon atom (the sum $k+m$ is unchanged). The chain shift leads to an increase of peak width (decrease of the resolution when Δk is large) when the isomer number is increasing.

On the basis of Table 1 and mass spectral information, the structural distribution of the wool wax aliphatic esters in the molecular mass range 550–788 ($N=37$–54) was obtained. The results are shown in Table 2.

3.3. Isomeric ester distribution

Using isomer abundance as a criterion for both

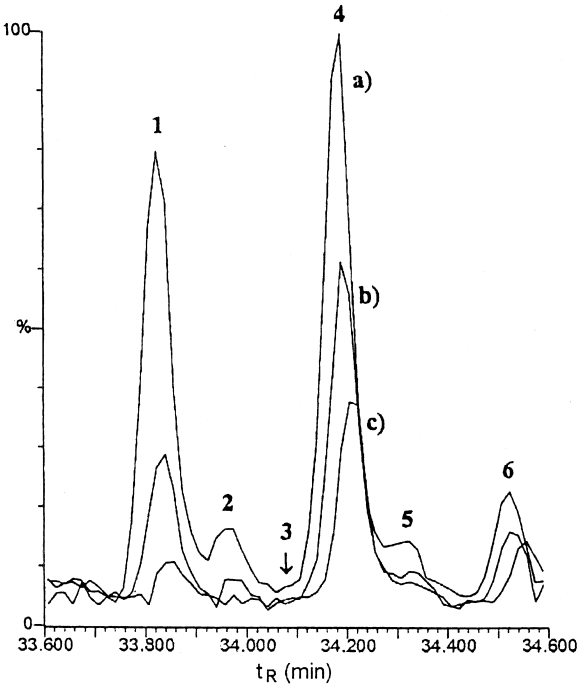


Fig. 8. Peak shift by the effect of the carbon atom distribution in the acid and alcohol moiety (*k*:*m*): (a) *m*/*z* 313, compound 46 (20:26); (b) *m*/*z* 285, 46 (18:28); (c) *m*/*z* 257, 46 (16:30). Peaks 1–6 are described in Table 1. GC–MS conditions as in the Experimental section.

types of esters, the three regions of the chromatogram can be seen.

3.3.1. Isomer distribution for odd esters ([O] type esters)

(a_1) The $N=37$–39 range (the unbranched linear chain region): for the esters with $N=37$ or 39, the major isomeric abundance corresponds to the [*n*:*n*] structure, being peak 6 the base peak. The structures [*n*:*i*] (peak 4, Fig. 3) and [*n*:*a*] (peak 5, Fig. 3) also have important contributions.

The mass spectrometric analysis of the individual esters shows that all compounds of higher abundance have the acid chain of type *n*. The contribution of type [*a*:*n*] and [*i*:*n*] was not detected (only [*n*:*a*] and [*n*:*i*]). This observation is consistent with the fact that for $N=37$ and 39, in the biosynthetic process preferentially combines acids and alcohols which produce esters with minimally branched chains (only one branched chain, on either the acid or the alcohol moiety). The combinations of [*i*:*i*] and [*a*:*a*] type (two branched chains, acid and alcohol moiety) are present only in very small abundances (under 8%). The dominant contribution in this region is given by the compounds 37 (15:22) and 39 (15:24).

(a_2) The $N=41$–45 range (the single branched chain region): in this *N* range, the contribution of the [*n*:*a*] type isomers (peak 5) is dominant. The main contribution is given by the compounds 41 (16:25), 43 (16:27) and 45 (18:27). In every situation, the acid chain is of *normal* type. The increase of the relative abundance of [*i*:*a*] isomers (peak 2, Figs 3 and 4) with an *N* increase should be noted (23% in the compounds of $N=41$ to 62% in the compounds of $N=45$). Peak 6 is decreasing from 49% ($N=41$) to 6.7% ($N=45$).

(a_3) The $N=47$–53 range (the two branched chain region): in the range of $N=47$–53, the chromatogram profile is dominated by the structure [*i*:*a*] (peak 2, Figs. 4 and 5), corresponding to a two branched chain ester structure (branched acid and alcohol moiety). The structure [*n*:*a*], peak 5, has also an important contribution. The relative intensity of peak 5 decreases with increasing *N* (82% for $N=47$ to 29% for $N=53$). The main contribution to the base peak is given by the compounds 47 (20:27), 49 (22,27), 51 (24,27) and 53 (26,27).

The characteristic configuration of the [O] type esters is the change of the isomeric structures with the increase of *N*, from [*n*:*n*] (unbranched structure, 100% for $N=37$) to [*i*:*a*] (two branched structure, 100% for $N=53$).

3.3.2. Isomeric distribution of even esters ([E] type esters)

(b_1) The $N=38$–40 range (the unbranched linear chain region): the main contribution to the ester peaks with $N=38$ or 40 consists of structures [*n*:*n*] (peak 6, Fig. 3) and [*n*:*i*] (peak 4, Fig. 3). Peak 6 is of 100% abundance and peak 4 of 48% and 82%, respectively. The main contribution is given by compounds 38 (14:24) and 40 (16:24). The preference for the minimally-branched compounds is a characteristic of this region.

(b_2) The $N=42$–50 range (the single branched chain region): the base peak is peak 4 with [*n*:*i*] structure. The contribution of the structures [*n*:*n*] (peak 6, Figs. 3 and 4) decreases from 58% ($N=42$)

Table 2
The ester isomeric distribution (% from the highest peak) in the range of $N=37–54$; $N=$total carbon atom; $k=$the carbon atom number on acid moiety; $\Delta k=k$ range; $c=$concentration (%, v/v); *i*, *a*, and *n* denote the *iso*, *anteiso* and *normal* structures, respectively, of the acid and alcohol moieties

N (M)	c (%)	Isomer distribution						
		Δk	*i*:*i* (1)	*i*:*a* (2)	*a*:*a* (3)	*i*:*n* (4)	*a*:*n* (5)	*n*:*n* (6)
37 (550)	0.47	14–18	0.0	8.0	0.0	35.2	67.6	100
38 (564)	1.35	14–18	0.0	0.0	5.5	46.7	8.9	100
39 (578)	2.21	14–19	0.0	11.9	0.0	42.8	52.4	100
40 (592)	3.95	14–20	2.2	0.0	4.4	82.2	33.3	100
41 (606)	4.78	14–20	7.0	23.2	0.0	72.1	100	48.8
42 (620)	6.21	14–22	17.8	4.4	11.1	100	48.9	57.8
43 (634)	7.29	14–20	11.4	36.4	0.0	29.5	100	13.6
44 (648)	7.87	14–26	26.6	13.3	13.5	100	31.1	30.4
45 (662)	9.41	15–27	13.3	62.2	0.0	15.5	100	6.7
46 (676)	9.02	16–27	44.4	26.7	62.2	100	28.9	22.2
47 (690)	10.0	14–29	11.4	100	0.0	31.8	81.8	4.5
48 (704)	8.21	16–28	40.0	24.4	71.1	100	24.4	15.5
49 (718)	7.56	16–29	6.7	100	0.0	22.2	80.0	2.2
50 (732)	6.41	18–30	37.8	26.7	88.8	100	15.5	6.7
51 (746)	6.08	18–31	2.5	100	0.0	17.8	51.1	2.2
52 (760)	4.23	21–30	20.0	17.8	100	48.9	11.1	6.7
53 (774)	2.91	20–33	3.3	100	0.0	4.4	28.9	0.0
54 (788)	2.02	23–33	15.5	13.3	100	33.3	11.1	3.3

to 15% ($N=50$). The isomers of major contributions are 42 (16:26), 44 (18:26), 46 (20:26), 48 (22:26) and 50 (24:26). The increase in the relative abundance of the structures [*a*:*a*] (peak 3, Figs. 3 and 4) is to be noted. The abundance of these isomers increases from 11% ($N=42$) to 89% ($N=50$).

(b_3) The $N=52–54$ range (the two branched chain region): for the $N=52$ and $N=54$ esters, the major contribution is of the isomers with [*a*:*a*] structure, peak 3 (100%), having both moieties branched (acid and alcohol). The base contribution is given by the compounds 52 (25:27) and 54 (27:27). The abundance of the structures [*i*:*n*] (peak 4, Fig. 5) decreases from 49% ($N=52$) to 33% ($N=54$). The contribution of the structures [*i*:*i*] (peak 1) is small, in the range 15–20%.

4. Conclusions

The most abundant aliphatic esters, obtained from wool wax by SFE, are in the carbon number range from 37 to 54. The corresponding carbon atom number of the acid moiety is from 14 to 33 and for the alcohol moiety from 19 to 33.

The isomeric structure for every *N*, resulting from the *n*, *i* and *a* structures of the acid and alcohol moieties, can be determined from the chromatographic profile and mass spectral information. When *N* increases from 37 to 54, the major isomeric contribution changes from the [*n*:*n*] structure (peak 6) to [*a*:*n*] (peak 5) and [*i*:*a*] (peak 2) structures, for the odd type esters. For the even type esters, the increase of *N* changes the major isomeric contribution from [*n*:*n*] (peak 6) to [*i*:*n*] (peak 4) and [*a*:*a*] (peak 3) structures.

The carbon number in the acid moiety, for the major contributing compounds, is under 50% [as $(k\times100)/(k+m)$]. It increases from 40.5 to 50% when *N* increases from 37 to 54.

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