Identification of the Less Common Isologous Short-Chain Triacylglycerols in the Most Volatile 2.5% Molecular Distillate of Butter Oil

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The isologous short-chain triacylglycerols of the most volatile 2.5% distillate of butter oil were resolved by reversed-phase high-performance liquid chromatography (HPLC) with mass spectrometry. The molecular species were identified by means of the $[MH]^+$ and the $[MH-RCOOH]^+$ ions in positive chemical ionization mode. A set of empirically determined incremental elution factors was found that could be used to calculate the accurate elution order of natural butterfat triacylglycerols when analyzed by reversed-phase HPLC. The triacylglycerols were also resolved by temperature-programmed gas–liquid chromatography on capillary columns coated with polar liquid phases. The high polarity of the columns provided separation of triacylglycerols on the basis of the degree of unsaturation, as well as on the nature of the shortest acyl chain, with the isologous species having the shortest chainlength eluting last. Both saturated and unsaturated triacylglycerols containing normal and branched-chain odd-carbon fatty acids in combination with short-chain acids were identified, and over 150 molecular species were quantitated.

KEY WORDS: Elution factors, gas–liquid chromatography, high-performance liquid chromatography, molecular species, on-line mass spectrometry, polarizable liquid phase, quantitation, reversed-phase.

We have previously reported the general elution sequence of the complex short-chain triacylglycerols by polar capillary gas–liquid chromatography (GLC) (1) and reversed-phase high-performance liquid chromatography (HPLC) with mass spectrometry (LC/MS) (2). A preliminary segregation of the triacylglycerol mixture into small groups of molecular species by silver ion thin-layer chromatography ($AgNO_3$-TLC) helped to identity the major components. Numerous triacylglycerol species, however, remained unidentified because of poor resolution and small amounts of mass. Of particular interest was the presence of triacylglycerols containing two or three short-chain fatty acids and the determination of their relative order of elution. In the present study, we have avoided the problem of insufficient mass of the low-molecular weight components by using the most volatile 2.5% redistillate of butter oil as the sample and by subjecting it to detailed reversed-phase LC/MS analysis. Using this approach, we have identified several new series of butterfat triacylglycerols and established their elution sequence on both polar capillary GLC and reversed-phase HPLC. The new results should be helpful in the identification of short-chain triacylglycerols in natural butterfat and bovine milk fat.

MATERIALS AND METHODS

Butter oil distillates. The first (R-1) and the fourth (R-4) most volatile 2.5% distillates, derived by molecular redistillation of the original most volatile 10% cut, had been obtained by distilling 777 pounds of butter oil (Distillation Products Industries, Rochester, NY), and the general properties have been described (3). The sample of native butter was from another earlier analysis (4). Synthetic short- and long-chain triacylglycerols, including normal and branched (iso and anteiso) and odd- and even-carbon number chains, were available in the laboratory from earlier work (5).

Solvents and reagents. All reagents and solvents were of analytical or chromatographic grade and were obtained from reputable laboratory supply houses. Acetonitrile was from Fluka (Basel, Switzerland), and propionitrile was from Romil (Loughborough, England). Acidified methanol and *n*-butanol for transesterification of fatty acids were prepared by addition of 5 mL sulfuric acid to 100 mL of the anhydrous alcohol.

TLC. TLC was performed as previously described (6). Triacylglycerols of both short and long chainlengths were recovered from silica gel by extraction with chloroform/methanol (2:1, vol/vol).

GLC and GLC with mass spectrometry (GC/MS). GLC analyses of fatty acid butyl esters and intact triacylglycerols by carbon number were performed on nonpolar capillary columns (1), while the separations based on both carbon and double-bond number of the fatty acids were done on polar (1) and of the triacylglycerols on polarizable (1,7) capillary columns as previously described in detail.

HPLC and LC/MS. The reversed-phase HPLC analyses were performed with a Hewlett-Packard (Palo Alto, CA) Model 1050 liquid chromatograph equipped with a Supelcosil LC-18 reversed-phase column (25 cm × 0.46 cm i.d.; Supelco, Mississauga, Ontario, Canada) coupled to a Varex ELSD II light-scattering detector (Varex, MD). The column was operated at a flow rate of 1 mL/min (90 min) with a linear gradient of 10–90% isopropanol in acetonitrile. For LC/MS, the triacylglycerols were resolved by reversed-phase HPLC with a linear gradient of 10–90% propionitrile in acetonitrile (1.5 mL/min) in a Hewlett-Packard Model 1084B liquid chromatograph with chemical ionization MS for peak monitoring (8). On-line LC/MS was done with a Hewlett-Packard Model 5985B quadrupole mass spectrometer interfaced with the Hewlett-Packard 1084B liquid chromatograph *via* a direct liquid inlet interface (8), as previously described. About 1% of the column effluent was admitted to the mass spectrometer, and the ion current was monitored in positive chemical ionization mode. Mass spectra were acquired every 7 s in the 200–900 mass range over the entire elution profile. Single ion plots were extracted from the total spectra by means of appropriate computer programs.

Peak identification. The HPLC/MS analysis of the triacylglycerol species makes use of a data base consisting of mass spectrometric ions $[M]^+$ and $[MH-RCOOH]^+$, as well as the corresponding elution times. This extremely powerful method allows identification of species that have the same elution times but different mass

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spectra, or species that have the same spectra but different elution times. An example of the latter is the pair consisting of 4:0-*n*15:0-16:0 and 4:0-*br*15:0-16:0. Mass chromatograms of all possible ions were generated from the run file. The peak intensities and elution times for each ion mass were measured and entered into an EXCEL spreadsheet (Microsoft) with a Macintosh SE computer (Apple Computer, Inc., Itasca, IL). The data were then sorted according to elution times, and the molecular species were identified manually, according to previously described principles (8).

RESULTS

Fatty acid composition. Table 1 compares the fatty acid composition of the most volatile 2.5% redistillate (R-1), the 2.5% redistillation residue (R-4) and of a reference butterfat sample. It is seen that in comparison to R-4 and the reference butter, R-1 is significantly enriched in 4:0, 6:0, 8:0 and 10:0, and greatly reduced in 16:0, 18:0 and 18:1, while relatively little change has occurred in the proportion of 12:0 and 14:0.

GLC of triacylglycerols. Figure 1 compares the GLC elution profiles of R-1 as obtained on the polar SP-2380 liquid phase by temperature programming over the range 240–260°C and on the high-temperature polarizable methyl 65% phenyl liquid phase by a stepwise temperature programming over the range 40–360°C. Due in part to the shallower temperature gradient, the more polar SP-2380 liquid phase gives a more complete resolution. However, the corresponding peaks are readily recognized in both chromatograms and can be matched quantitatively, as shown in Table 2. In most instances, each peak contains more than one major component, as indicated in Figure 1. The peaks were tentatively identified on the basis of previous work with the R-4 distillate, which was subjected to $AgNO_3$-TLC prior to capillary GC/MS (1) with the methyl 65% phenylsilicone liquid phase. Table 2 indicates good qualitative and quantitative agreement between the two different GLC columns. Only the major species are identified by the fatty acids present in the triacylglycerol molecules, the minor ones being noted only by carbon and double-bond numbers. There is no resolution of enantiomers or reverse isomers. Likewise, no distinction is made between *cis*- and *trans*- isomers of the monoenoic species.

HPLC of triacylglycerols. Figure 2 compares the HPLC elution profiles of R-1 as obtained on the same reversed-phase column with a linear gradient of 10–90% isopropanol in acetonitrile and a light-scattering detector (last peak 40% isopropanol), and a linear gradient of 10–90% propionitrile in acetonitrile and total ion current

TABLE 1

Fatty Acid Composition of Molecular Distillates of Butter Oil and a Reference Butter Sample (mol %)

Fatty acids	Butter oil distillates[a]		Reference butter
	R-1	R-4	
4:0	21.84	16.70	8.76
6:0	8.57	6.67	3.34
8:0	5.93	2.29	2.44
9:0	0.09	0.00	0.00
10:0	8.89	3.22	4.80
10:1	0.79	0.34	0.47
11:0	0.12	0.04	0.10
12:0	7.85	3.10	5.11
12:1	0.10	0.00	0.00
13:0*br*	0.11	0.04	0.00
13:0*n*	0.18	0.09	0.16
14:0*i*	0.23	0.17	0.21
14:0*n*	14.92	10.81	13.39
14:1	0.54	0.73	1.02
15:0*i*	0.25	0.30	0.36
15:0*ai*	0.45	0.56	0.60
15:0*n*	1.16	1.25	1.34
16:0*i*	0.16	0.29	0.23
16:0*n*	17.80	28.03	32.36
16:1	0.75	1.39	1.58
17:0*i*	0.25	0.29	0.23
17:0*ai*	0.20	0.41	0.43
17:1		0.30	0.00
17:0*n*	0.30	0.64	0.58
18:0*i*	0.15	0.36	0.06
18:0*n*	2.83	7.16	7.35
18:1*t*	0.35		1.01
18:1n-9	4.79	13.67	11.40
18:1n-7	0.12	0.08	0.00
18:2	0.29	0.97	0.95
20:0		0.00	0.00
18:3		0.00	0.29

[a]R-1, the first; and R-4, the fourth most volatile 2.5% distillates.

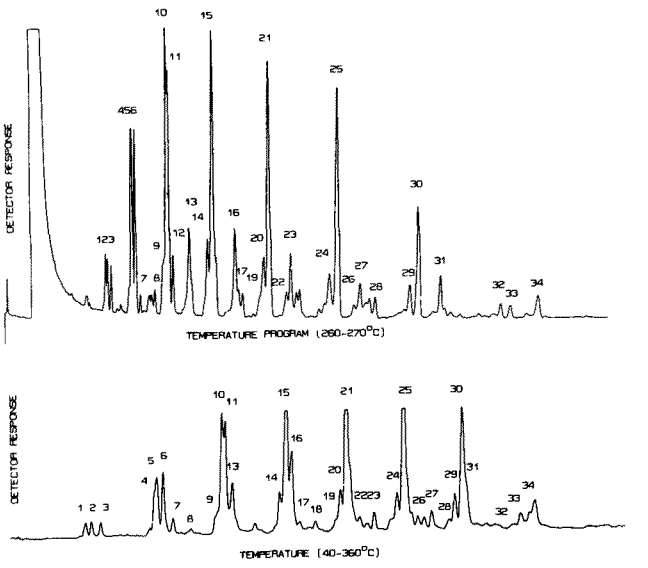

FIG. 1. Triacylglycerol elution profiles of R-1 distillate as obtained by capillary gas–liquid chromatography (GLC) on two liquid phases of different polarities. Peak identification as given in Table 2. GLC conditions: upper panel, SP-2380, Hewlett-Packard (Palo Alto, CA) Model 5880 gas chromatograph equipped with a flexible quartz capillary column (15 m × 0.32 mm i.d.) coated with SP 2380 (a cyanopropylphenylsilicone; Supelco, Bellefonte, PA) liquid phase; temperature program, 240°C (held for 2 min) then 1°C/min to 260°C; lower panel, RSL-300, Hewlett-Packard Model 5880 gas chromatograph equipped with flexible quartz capillary column (25 m × 0.25 mm i.d.) coated with 65% phenylmethylsilicone (Quadrex, New Haven, CT) liquid phase; temperature program, 40°C (held for 5 min), then 50°C/min to 150°C, then 10°C/min to 310°C, and finally 2°C/min to 360°C. Carrier gas, H_2 at 15 psi. Flame-ionization detector at 360°C. Single column compensation of baseline.

TABLE 2

Triacylglycerol Composition of R-1 Distillate as Estimated by Polar Capillary GLC (mole %)[a]

Carbon number	DG + fatty acid composition	Composition of R-1 distillate	
		SP-2380	RSL-300
22	18 + 4	0.36	0.28
22	18 + 4	0.09	0.17
24	20 + 4	1.09	
24	10 + 8 + 6		0.11
24	20 + 4		0.89
24	14 + 4 + 6	0.83	0.87
24	16 + 4 + 4	0.74	0.91
25	21 + 4	0.09	0.11
25		0.22	0.17
26		0.43	
26	10 + 10 + 6		0.39
26	10 + 12 + 4		1.83
26	14 + 8 + 4	5.06	2.53
26	16 + 4 + 6	5.03	3.40
26	18 + 4 + 4	0.30	1.01
27			0.18
27			0.24
27	23 + 4	0.50	0.40
27		0.41	0.40
27		0.46	0.18
28		1.05	0.87
28			1.28
28	14 + 10 + 4	6.31	5.60
28	16 + 8 + 4	5.39	5.29
28	18 + 4 + 6	1.19	3.52
28		0.17	
28		0.37	
29			0.13
29	25 + 4	2.36	0.66
29		0.71	0.15
30	24 + 6	2.19	2.22
30	26 + 4	10.01	9.35
30	18 + 8 + 4	1.68	4.48
30		2.84	0.66
30		0.67	
31		0.55	0.22
31		0.10	0.22
31			0.82
32		0.10	0.33
32	26 + 6	3.05	2.51
32	28 + 4	9.32	8.96
32	28 + 4	0.98	3.09
32:1		2.26	
33			0.91
33	27 + 6		0.52
33	29 + 4		0.90
34	24 + 10	0.30	0.00
34	26 + 8	0.51	0.52
34	28 + 6	2.19	1.77
34	16 + 14 + 4	9.32	8.91
34		0.55	
34:1	30 + 4	1.59	2.10
34		0.56	0.81
34		0.70	
35		0.71	0.65
35		0.22	0.60
35		0.34	1.02
36			0.59
36	16 + 14 + 6	1.59	1.79
36	16 + 16 + 4	5.21	5.98
36		0.29	
36		0.25	
36:1	18:1 + 14 + 4	1.96	2.00
37		0.37	
37	31 + 6	0.27	0.30
37	33 + 4	0.20	0.26
37		0.10	
37		0.24	0.24
37:1	33 + 4	0.14	
38		0.24	
38		0.25	0.40
38	32 + 6	0.83	0.97
38	34 + 4	0.78	0.87
38		0.12	
38:1	32 + 6	0.33	
38		0.22	
38:1	34 + 4	1.54	2.22
39		0.27	0.27
40		1.18	1.18
41		0.04	0.04
42		0.33	0.33
43		0.01	0.01
44		0.16	0.16
SUM		100.00	100.28

[a]R-1, the first most volatile 2.5% distillate; GLC, gas-liquid chromatography; DG, diglyceride.

response in the mass spectrometer. In this instance, the relative order of peak elution is the same, but the peak proportions differ greatly, which is due largely to the variability in the ion current response to the short- and long-chain triacylglycerol species. Table 3 compares the qualitative and quantitative composition of R-1 triacylglycerols as obtained by the two HPLC methods. About 67% of the total peak area was accounted for by the listed major species of triacylglycerols. A more complete compilation of the molecular species is given later. The peak identification was confirmed by LC/MS with 10–90% propionitrile in acetonitrile as eluting solvent. Again, the resolution was matched to the carbon and double-bond numbers (partition numbers) as the reverse isomers and enantiomers were not separated.

Reversed-phase HPLC elution order of triacylglycerols. Figure 3 shows a series of single ion plots for the major diacylglycerol masses detected in the fragmentation products of triacylglycerols associated with the corresponding partition numbers. The peaks have been produced by a loss of the complementary fatty acids. The double and triple peaks indicate resolution of isologous triacylglycerols within the partition numbers. Thus, the butyrates are retained longer than the caproates, which are retained longer than the caprylates and higher fatty acid-containing triacylglycerols. The order of elution of the isologous triacylglycerols is identical to that noted for these triacylglycerols on the polar capillary columns (as previously mentioned).

Curve fitting. A conventional linear fit of theoretical carbon number (TCN) *vs.* elution time was made impossible by the use of gradient elution analysis. A reiterative curve fitting procedure was used instead. The data set consisted of 150 molecular species and their retention times as determined by LC/MS.

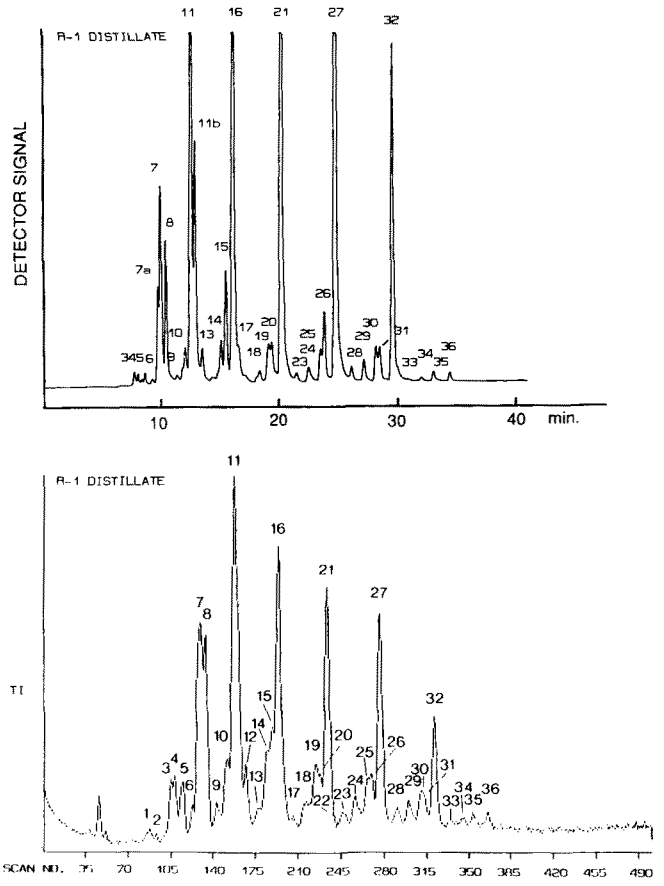

FIG. 2. Triacylglycerol elution profile of R-1 distillate as obtained by reversed-phase high-performance liquid chromatography (HPLC) with two different solvent and detection systems. Peak identification as given in Table 3. HPLC conditions: upper panel, HPLC/light scattering detector, Hewlett-Packard Model 1050 liquid chromatograph equipped with Supelcosil LC-18 column (25 cm × 0.46 cm i.d.) coupled to a Varex ELSD light scattering detector; solvent, a linear gradient of 10–90% isopropanol in acetonitrile (1 mL/min); lower panel, liquid chromatography/mass spectrometry, Hewlett-Packard Model 1084B liquid chromatograph equipped with Supelcosil LC-18 column (25 cm × 0.46 cm i.d.) coupled to Hewlett-Packard Model 5985B quadrupole mass spectrometer *via* a direct liquid inlet interface; solvent, a linear gradient of 10–90% propionitrile in acetonitrile (1.5 mL/min).

TABLE 3

Major Triacylglycerol Composition of R-1 Distillate as Estimated by Reversed-Phase High-Performance Liquid Chromatography (HPLC) and Light-Scattering Detector (LSD) or Liquid Chromatography/Mass Spectrometry (LC/MS) (area %)

Peak number	Molecular species	Triacylglycerol composition	
		HPLC/LSD	HPLC/MS
1			0.5
2			0.1
3	8-12-4:0	0.8	1.5
4	6-14-4:0	0.6	1.8
5	16-4-4:0	0.6	1.9
6	12-8-6:0	0.4	0.7
7	6-14-6:0	2.4	9.3
7	8-14-4:0	4.7	
8	6-16-4:0	3.6	5.2
9	18-4-4:0+	0.4	1.3
10	12-10-6	0.6	2.5
10	14-8-6	1.5	
10	16-6-6:0		
11	14-10-4:0	9.7	15.3
11	16-8-4:0	5.6	
12	18-6-4:0	1.6	2.1
13	12-10-12	0.5	1.3
14	14-10-6	1.9	2.8
15	10-18:1-4:0	3.6	3.1
16	14-12-4:0 + 16-10-4:0	10.9	11.0
17	10-15*n*-6:0	0.7	0.8
18	14-10-8:0	1.0	1.7
19	16-10-6:0	1.7	2.1
20	12-4-18:1	1.7	1.7
21	14-14-4:0	11.4	8.5
23	14-15*br*-4:0	0.6	1.2
24	14-12-8+	1.1	1.9
25	14-14-6:0	1.4	1.8
26	14-4-18:1	2.5	1.7
27	14-16-4:0	14.0	7.4
28	16-15*br*-4:0	0.7	1.1
29	16-15*n*-4:0	1.3	1.4
30	14-16-6:0	1.6	1.5
31	16-18:1-4:0	1.6	0.9
32	16-16-4:0+	7.2	3.6
33			0.5
34			0.5
35	16-16-6	0.8	0.6
36	16-18-4:0	0.7	0.6

To begin with, a set of six triacylglycerol species, spanning carbon numbers 32 to 42, was chosen as an initial reference set. All species were made up of saturated fatty acids having a minimum chainlength of 10 carbons, and the TCN values for these species were simply equal to the sum of acyl carbons. A second-order polynominal (quadratic) was used to fit the TCN values to the elution times of the basis set, and then the TCN values of all the species with elution times within the range of this basis set were determined from the equation. Average correction factors for 4:0 and 6:0 were then determined for all species falling within this range.

Next, these correction factors were used to calculate TCN values for all the species in the earlier part of the chromatogram that are made up of 4:0, 6:0 and long-chain (>10 carbons) fatty acids. The TCN values and elution times of 20 species with TCN values from 22.14 to 32.56 were then fitted by means of a fourth-order (quartic) polynominal. This equation was used to derive experimental TCN values for all the remaining species, which could then be used to derive the average correction factors for the monoenoic, branched-chain and remaining short-chain fatty acids. In the first cycle, we found that 10:0, contrary to the original assumption, required a TCN correction factor of 0.08 rather than 0. Therefore, the whole procedure was repeated with this correction factor included. The final correction factors for the saturated and monoenoic fatty acids are listed in Table 4, along with the equations used to calculate the TCN values. The form of the equations and the nomenclature were taken from the work of El-Hamdy and Perkins (9).

Figure 4 shows a plot of the observed HPLC elution time *vs.* the TCN for the 150 saturated and monounsaturated triacylglycerols of the R-1 distillate. The validity

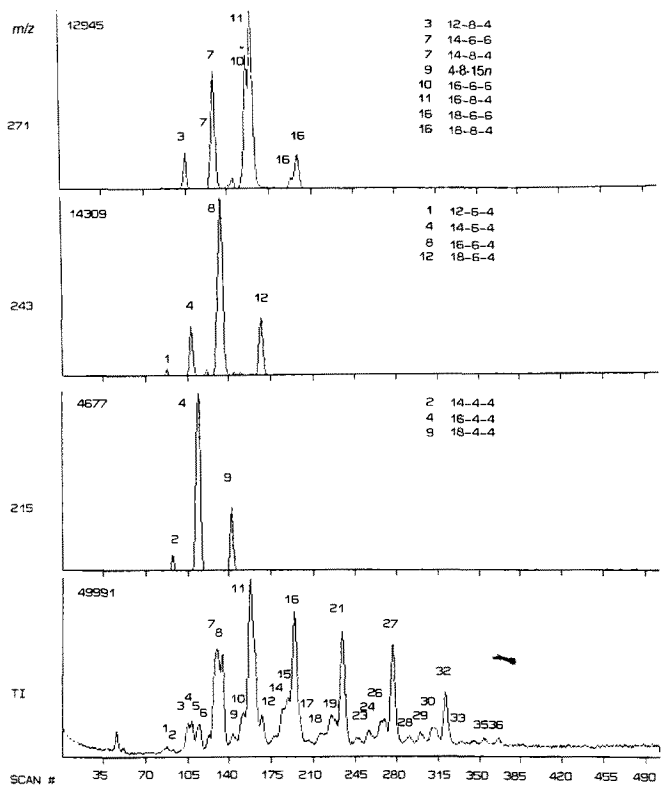

FIG. 3. Mass chromatograms of major diacylglycerol fragment ions, ([MH−RCOOH]$^+$), as obtained by liquid chromatography/mass spectrometry (LC/MS) of the triacylglycerols of R-1 distillate. *m/z*, Mass of diacylglycerol fragment ions; TI, total positive chemical ionization current. Numbers in upper left-hand corner of each ion plot indicate the ion count for the highest peak in the profile. LC/MS conditions as given in Figure 2. Single ion plots based on data retrieved from the computer.

of the equations and the correction factors is demonstrated by the fact that all points fall close to a smooth continuous curve. Therefore, the elution order of all the species is correctly predicted by means of the eleven correction factors given in Table 4.

TABLE 4

Incremental HPLC Elution Factors as Determined by Curve Fitting for Saturated and Monounsaturated Triacylglycerols of Butterfat[a]

Fatty acid	Correction factors (F_i)
2:0	1.660
4:0	1.070
6:0	0.560
8:0	0.250
10:0	0.080
>10:0	0.000
br:0	−0.380
10:1	0.290
12:1	0.000
14:1	−0.210
16:1	−0.300
18:1	−0.340

[a]HPLC, high-performance liquid chromatography; TCN, theoretical carbon number; ECN (equivalent chain length) = CN − 2n; TCN = ECN + SUM (F_i), where CN = sum of the acyl carbons; n = number of double bonds per molecule.

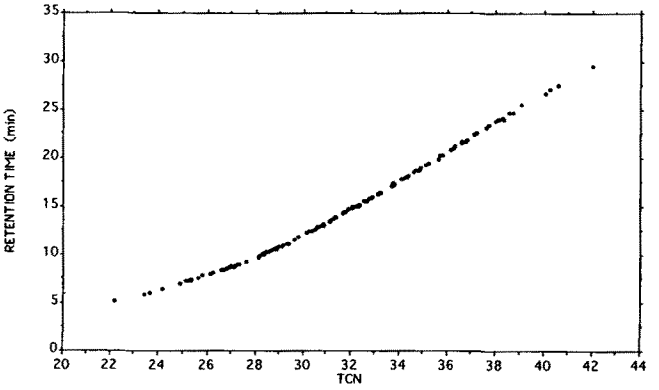

FIG. 4. A plot of elution time *vs.* calculated theoretical carbon number (TCN) of 150 saturated and monounsaturated triacylglycerols of R-1 distillate.

Quantitation. Table 5 gives the composition of the saturated triacylglycerols of the R-1 distillate as estimated by reversed-phase LC/MS. The area percentages that are derived from the sum of the diacylglycerol fragment intensities vary with the exact nature of the molecular species (10).

Table 6 gives the composition of the monounsaturated triacylglycerols of the R-1 distillate as estimated by reversed-phase LC/MS. The area percentages are not corrected for differences in fragment ion response. The saturates made up 77% and the monounsaturates 22% of the total distillate. Altogether, over 150 different molecular species were identified without regard for positional and enantiomeric isomers.

DISCUSSION

In this study, 150 triacylglycerol species of a butterfat fraction have been accounted for in the form of specific molecular structures of triacylglycerols and quantitated. The identification was possible largely because of the on-line combination of reversed-phase HPLC with MS, which allowed a direct matching of the peak retention times with triacylglycerol composition, including those of unresolved triacylglycerol species. By means of correction factors determined for each of the short-chain and monoenoic fatty acids, it is possible to calculate the precise elution order of all the molecular species of triacylglycerols. The order of elution of the isologous triacylglycerols from the reversed-phase column requires comment. The triacylglycerols containing the shortest-chain fatty acid would have been anticipated to be slightly more polar than the other members of an isologous series, and therefore should have been eluted first during reversed-phase HPLC. The fact that it was eluted last suggests that residual polarity of the column support may have contributed to the separation and order of elution of the isologous triacylglycerols. Because the present chromatographic and mass-spectrometric methods did not permit resolution of the reverse isomers and enantiomers, it was not possible to assign the positional distribution of the fatty acids. However, previous studies (11,12), including a recent reanalysis by chiral-phase HPLC (13), have shown that the short-chain

TABLE 5

Saturated Triacylglycerol Composition of R-1 Distillate as Estimated by Reversed-Phase Liquid Chromatography/Mass Spectrometry

Peak number	Elution time	Molecular species	Diacylglycerol (DG) fragments	Triacylglycerol (TG) (area %)
	5.08	12-4-4	TG20:0	trace
1	5.89	10-8-4	4 + 8, 4 + 10	0.08
1	6.07	12-6-4	4 + 6, 4 + 12, 6 + 12	0.31
2	6.39	14-4-4	4 + 4, 4 + 14	0.18
3,4	7.23	10-10-4	4 + 10, 10 + 10	0.72
3	7.29	8-12-4	4 + 4, 4 + 14	1.32
4	7.54	6-14-4	4 + 6, 4 + 14, 6 + 14	2.07
5	7.98	16-4-4	4 + 4, 4 + 16	2.38
5	8.15	4-6-15*br*	4 + 15	0.07
5	8.43	4-6-15*n*	4 + 6, 4 + 15, 6 + 15	0.25
6	8.47	6-10-10	6 + 10	0.21
6	8.54	12-8-6	6 + 8, 6 + 12, 8 + 12	0.72
7	8.72	6-14-6	6 + 14	0.39
7	8.82	4-10-12	4 + 10, 4 + 12	3.03
7	8.93	8-14-4	4 + 14, 4 + 8, 8 + 14	5.82
8	9.26	6-16-4	4 + 6, 4 + 16, 6 + 16	8.12
8,9	9.72	6-6-15*n*	6 + 6, 6 + 15	0.16
9	9.92	18-4-4	4 + 4, 4 + 18	0.80
9	9.96	4-6-17*br*	6 + 17, 4 + 6	0.21
9	10.09	4-8-15*n*	4 + 15, 4 + 8	0.31
9	10.22	8-8-12	8 + 8, 8 + 12	0.10
10	10.39	12-10-6	6 + 10, 6 + 12	0.92
10	10.39	4-6-17*n*	4 + 6, 4 + 17, 6 + 17	0.08
10	10.53	14-8-6	6 + 8, 8 + 14, 6 + 14	2.10
11	10.86	12-12-4	4 + 12, 12 + 12	0.72
10	10.91	16-6-6	6 + 6, 6 + 16	4.16
11	11.02	14-10-4	4 + 10, 4 + 14, 14 + 10	8.70
11	11.19	16-8-4	4 + 8, 4 + 16, 8 + 16	5.78
12	11.59	18-6-4	4 + 6, 4 + 18, 6 + 18	0.19
12	11.62	12-14-2	2 + 12, 12 + 14	0.19
12	11.82	6-8-15*n*	6 + 15, 8 + 15	0.02
12	11.83	4-10-15*br*	4 + 10, 4 + 15, 10 + 15	0.03
13	12.26	4-10-15*n*	4 + 10, 4 + 15, 10 + 15	0.38
13	12.41	8-10-12	8 + 10, 8 + 12, 10 + 12	0.31
13	12.43	4-8-17*n*	4 + 17	0.01
13	12.54	8-8-14	8 + 14, 8 + 8	0.20
14	12.80	6-12-12	6 + 12, 12 + 12	0.16
14	12.84	14-10-6	6 + 10, 6 + 14, 10 + 14	1.53
15	13.04	6-8-16	6 + 8, 6 + 16, 8 + 16	3.24
16	13.47	14-12-4	4 + 12, 4 + 14, 12 + 14	5.07
16	13.46	6-6-18	6 + 6, 6 + 18	0.39
16	13.53	4-10-16	4 + 10, 4 + 16, 10 + 16	5.64
16	13.72	10-15*br*-6	6 + 15, 10 + 15	0.01
16	13.81	4-8-18	4 + 8, 4 + 18, 8 + 18	1.01
17	14.32	6-10-15*n*	6 + 15, 10 + 15	0.02
17	14.41	4-12-15*br*	4 + 15, 12 + 15, 4 + 12	0.12
17	14.43	4-14-13*br*	4 + 13, 13 + 14	0.01
17	14.46	4-10-17*br*	4 + 17, 10 + 17, 4 + 10	0.10
18	14.90	4-14-13*n*	4 + 13, 13 + 14	0.02
18	14.95	4-12-15*n*	4 + 12, 4 + 15, 12 + 15	0.39
18	15.02	4-10-17*n*	4 + 17, 4 + 10	0.07
18	15.03	10-10-12	10 + 10, 10 + 12	0.27
18	15.13	14-10-8	8 + 10, 14 + 10, 8 + 14	0.42
19	15.56	6-12-14	6 + 12, 6 + 14, 12 + 14	1.03
19	15.61	16-10-6	6 + 16, 6 + 10	1.26
21	16.26	4-12-16	4 + 16, 4 + 12, 12 + 16	5.02
21	16.29	14-14-4	4 + 14, 14 + 14	3.41
21	16.38	4-10-18	4 + 10, 4 + 18	0.81
21	16.40	6-12-15*br*	6 + 15, 2 + 15	0.03
23	17.13	2-14-16	2 + 14, 14 + 16	0.35
23	17.19	4-12-17*br*	4 + 17	0.02
23	17.28	4-14-15*br*	4 + 15, 14 + 15	0.22
23	17.37	4-16-13*br*	4 + 13	0.04
24	17.84	4-12-17*n*	4 + 17	0.06
24	17.86	4-14-15*n*	4 + 14, 4 + 15, 14 + 15	0.62
24	17.91	10-12-12	12 + 12	0.10

(continued)

TABLE 5 (continued)

Peak number	Elution time	Molecular species	Diacylglycerol (DG) fragment	Triacylglycerol (TG) (area %)
24	17.94	14-12-8	8 + 12, 8 + 14, 12 + 14	0.30
25	18.58	6-12-16	6 + 16	0.19
25	18.64	14-14-6	6 + 14, 14 + 14	0.75
25	18.65	6-10-18	6 + 10, 6 + 18	0.13
27	19.32	14-16-4	4 + 14, 4 + 16, 14 + 16	7.43
27	19.33	4-12-18	4 + 12, 4 + 18, 12 + 18	0.49
27	19.44	6-14-15*br*	6 + 15, 14 + 15	0.04
27	19.92	6-14-15*n*	14 + 15	0.02
28	20.22	2-16-16	16 + 16, 2 + 16	0.17
28	20.31	16-15*br*-4	4 + 16, 4 + 15, 15 + 16	0.40
29	20.87	10-12-14	10 + 12, 12 + 14	0.16
29	20.90	16-15n-4	4 + 15, 15 + 16	n.d.
29	21.12	8-14-14	8 + 14, 14 + 14	0.10
30	21.62	14-16-6	6 + 14, 6 + 16, 14 + 16	0.52
30	21.67	6-12-18	6 + 12, 6 + 18, 12 + 18	0.08
32	22.39	4-14-18	4 + 14, 4 + 18, 14 + 18	0.17
32	22.43	16-16-4	4 + 16, 16 + 16	3.08
32	22.51	6-16-15*br*	6 + 15, 15 + 16	0.04
33	23.21	6-16-15*n*	6 + 15, 6 + 16, 15 + 16	0.04
33	23.39	4-16-17*br*	4 + 17, 4 + 16	0.08
33	23.39	4-18-15*br*	4 + 15	0.02
34	23.86	12-12-14	12 + 12, 12 + 14	0.04
34	23.90	10-14-14	10 + 14, 14 + 14	0.01
34	24.02	4-16-17*n*	4 + 17, 4 + 16	0.04
34	24.05	4-18-15*n*	4 + 15	0.03
34	24.20	8-14-16	8 + 14, 14 + 16	0.06
35	24.67	6-16-16	6 + 16, 16 + 16	0.34
35	24.69	6-14-18	6 + 14, 6 + 18, 14 + 18	0.06
36	25.52	16-18-4	4 + 16, 4 + 18, 16 + 18	0.42
	26.76	10-14-16	10 + 14, 10 + 16	0.04
	27.15	8-16-16	8 + 16, 16 + 16	0.03
	27.57	6-16-18	6 + 16, 16 + 18	0.03
	29.60	12-14-16	12 + 14	0.01
SUM				100.00

TABLE 6

Monounsaturated Triacylglycerol Composition of R-1 Distillate as Estimated by Reversed-Phase Liquid Chromatography/Mass Spectrometry

Peak number	Molecular species	Diacylglycerol (DG) fragments	Triacylglycerol (area%)
1	4-6-14:1	DG20:1	0.42
3	6-6-14:1	DG20:1	0.36
3,4	4-8-14:1	DG18:1	0.17
4	4-12-10:1	DG14:1, DG22:1	4.03
4	4-6-16:1	DG20:1, DG22:1	0.31
5	4-4-18:1	DG22:1	4.92
6	6-8-14:1	DG20:1	0.38
6	6-12-10:1	DG16:1	0.71
6	6-6-16:1	DG22:1	0.82
7	4-10-14:1	DG18:1, DG24:1	1.55
7	4-8-16:1	DG20:1, DG24:1	0.38
7	4-14-10:1	DG24:1	16.90
7	4-6-18:1	DG22:1	3.55
9	4-15-10:1	DG14:1, DG25:1	0.51
9	6-10-14:1	DG20:1, DG24:1	0.70
10	6-8-16:1	DG22:1	0.24
10	6-6-18:1	DG24:1	5.84
10	6-14-10:1	DG16:1	2.69
10	6-12-14:1	DG18:1	1.46
10	4-10-16:1	DG20:1	1.34

(continued)

TABLE 6 (continued)

Peak number	Molecular species	Diacylglycerol (DG) fragments	Triacylglycerol (area%)
11	4-8-18:1	DG22:1	8.40
11	4-14-12:1	DG16:1	0.00
11	4-16-10:1	DG14:1, DG26:1	8.53
13	4-15-12:1	DG27:1	0.14
13	6-12-14:1	DG20:1	0.19
13	6-10-16:1	DG22:1	0.22
14	6-8-18:1	DG24:1	1.30
14	6-8-18:1	DG26:1	1.84
14	8-14-10:1	DG18:1	0.45
15	6-16-10:1	DG26:1	0.68
15	4-12-16:1	DG28:1, DG20:1	0.61
15	4-16-12:1	DG28:1, DG16:1	1.90
15	4-14-14:1	DG28:1, DG18:1	1.87
15	4-10-18:1	DG28:1, DG22:1	2.90
16	4-16-12:1	DG16:1	0.77
16	2-16-14:1	DG16:1	1.11
16	4-18-10:1	DG14:1, DG28:1	1.36
18	4-15-14:1	DG29:1	0.16
18	6-12-16:1	DG22:1, DG28:1	0.21
18	6-10-18:1	DG24:1, DG28:1	1.93
18	6-14-14:1	DG20:1	0.43
19	8-16-10:1	DG18:1, DG26:1	0.52
20	4-14-16:1	DG20:1	0.53
20	4-12-18:1	DG22:1	1.15
20	4-16-14:1	DG18:1	2.32
23	4-15-16:1	DG31:1	0.19
24	8-10-18:1	DG28:1	0.22
24	6-12-18:1	DG24:1, DG30:1	0.58
24	6-16-14:1	DG20:1, DG30:1	1.03
26	4-14-18:1	DG22:1	2.33
26	4-16-16:1	DG20:1	1.43
26	4-18-14:1	DG18:1	0.15
28	4-15-18:1	DG33:1, DG22:1	0.13
29	6-14-18:1	DG24:1, DG32:1	0.68
29	6-16-16:1	DG22:1	0.48
30	4-18-16:1	DG20:1, DG34:1	4.93
31	4-18-16:1	DG20:1, DG34:1	0.35
34	6-16-18:1	DG24:1, DG34:1	0.47
35	4-18-18:1	DG22:1, DG36:1	0.25
			100.00

fatty acids C_2–C_6 are exclusively confined to the *sn*-3 position of the triacylglycerol molecule, while the C_8 and longer-chain fatty acids are found in progressively increasing amounts in the *sn*-1 and *sn*-2 positions. Conventional stereospecific analyses have demonstrated that oleic and linoleic acids are preferentially associated with the *sn*-2 position.

Aside from an intellectual satisfaction, the present study provides valuable information for the identification of the short-chain triacylglycerols in natural butterfat and in bovine and other ruminant milk fats. Needless to say, however, that total reliance on the chromatographic retention times does not provide dependable identification of any GLC or HPLC peaks. On-line MS in combination with polar capillary GLC or reversed-phase HPLC would appear to be necessary for this purpose. Recently, however, tandem MS has shown excellent promise for the identification of triacylglycerols in natural butterfat (14).

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