Triacylglycerols of Winter Butterfat Containing Configurational Isomers of Monoenoic Fatty Acyl Residues. II. Saturated Dimonoenoic Triacylglycerols

Päivi Laakso and **Heikki Kallio***
Department of Biochemistry and Food Chemistry, University of Turku, FIN-20500, Finland

Triacylglycerols of Finnish winter butterfat containing one saturated and two monoenoic fatty acyl residues were studied. With silver ion high-performance liquid chromatography (HPLC), molecules were separated according to the difference in the configuration of one fatty acyl moiety. The distribution of the saturated *cis,trans*-dimonoenoic and saturated *cis,cis*-dimonoenoic triacylglycerols according to their acyl carbon numbers was compared by means of reversed-phase HPLC and tandem mass spectrometry. Furthermore, two examples of the fatty acid composition of a specified molecular weight species were shown. The fatty acid compositions of corresponding saturated *cis,trans*-dimonoenoic and saturated *cis,cis*-dimonoenoic triacylglycerols were similar; however, there may be differences in the proportions of different fatty acid combinations or in the distribution of fatty acids between primary and secondary glycerol positions.

KEY WORDS: Bovine, butterfat, *cis*-monoenoic fatty acid, molecular species, reversed-phase high-performance liquid chromatography, silver ion chromatography, tandem mass spectrometry, *trans*-monoenoic fatty acid, triacylglycerols.

When molecular structures of triacylglycerols are investigated, demands for high-resolution chromatography increase by increasing complexity of molecules, *i.e.*, the number of double bonds in the fatty acyl residues. This is especially prominent in the case of butterfat because it contains considerable quantities of *trans*-fatty acids in addition to a wide range of molecular weight species of triacylglycerols.

The importance and efficiency of silver ion high-performance liquid chromatography (HPLC) in lipid research has been demonstrated by analyzing configurational isomers of disaturated monoenoic triacylglycerols of butterfat (1). Not even multiple-stage mass spectrometric (MS) analysis can replace silver ion HPLC separation because of its adjustable selectivity. Therefore, combinations of these two techniques may open the most effective prospects in understanding the diversity of lipid molecules produced by nature.

The aim of this study was to outline a scheme for investigations of the configurational isomers of triacylglycerols of more complex natural mixtures. Saturated dimonoenoic triacylglycerols of winter butterfat were analyzed by silver ion HPLC and ammonia negative ion tandem mass spectrometry.

MATERIALS AND METHODS

Samples and reagents. Triacylglycerols of Finnish winter butter were isolated and purified as described earlier (1). Triacylglycerols containing one saturated and two *cis*-monoenoic (SM^cM^c) or one saturated, one *cis*-monoenoic and one *trans*-monoenoic fatty acyl residues (SM^cM^t) were isolated by means of silver ion HPLC as described earlier (1). The isolated fractions were dissolved in 1,2-dichloroethane for HPLC analysis or in hexane for MS analysis. All solvents were of HPLC-grade and were supplied by Merck (Darmstadt, Germany) and Rathburn (Walkerburn, Scotland).

HPLC. Separation of the saturated dimonoenoic triacylglycerols was performed by reversed-phase HPLC with an octadecylsilyl stationary phase and a binary gradient of dichloromethane/1,2-dichloroethane (4:1, vol/vol) and acetonitrile as described earlier (1). Components were detected with a light-scattering detector.

Tandem MS analysis. Mass spectra of triacylglycerols were obtained by negative ion chemical ionization (NICI) with ammonia as reactant gas. Daughter spectra of the derived $[M - H]^-$ ions were produced by collisional activation with argon as described earlier (1,2).

RESULTS AND DISCUSSION

The usefulness of silver ion HPLC for fractionating milk fat triacylglycerols according to a difference in the configuration of one fatty acyl residue has been shown (1,3,4). With the silver ion HPLC method introduced by Christie (5), the triacylglycerols of Finnish winter butterfat were separated into six fractions, namely trisaturated (Ag-HPLC Fraction 1), disaturated *trans*-monoenoic (Ag-HPLC Fraction 2), disaturated *cis*-monoenoic (Ag-HPLC Fraction 3), saturated *cis,trans*-dimonoenoic (Ag-HPLC Fraction 4), saturated *cis,cis*-dimonoenoic (Ag-HPLC Fraction 5) and the more unsaturated triacylglycerols (Ag-HPLC Fraction 6). The fatty acid compositions of these fractions are presented in the earlier paper (1). The saturated dimonoenoic triacylglycerols represented 14% of all triacylglycerols present in the winter butterfat studied. The SM^cM^c molecules represented 4.5% and SM^cM^t molecules 9.5% (1).

Saturated dimonoenoic triacylglycerols. The ammonia negative ion chemical ionization spectra of SM^cM^c and SM^cM^t fractions of butterfat triacylglycerols are shown in Figure 1A and B, the ions representing deprotonated triacylglycerols, $[M - H]^-$ ions. Each $[M - H]^-$ ion can be analyzed further by collisional activation. The intensities of the RCO_2^- ions in the daughter spectra of the major $[M - H]^-$ ions 689.7 and 857.7 of both SM^cM^c and SM^cM^t fractions are summarized in Table 1. The fatty acids were identified by the number of acyl carbon atoms and double bonds, and their proportions were calculated according to the uncorrected intensities of the RCO_2^- ions. To achieve information on the distribution of configurational isomers of fatty acyl residues in triacylglycerols, it was necessary to fractionate the sample by silver ion HPLC prior to MS analysis. In addition to *cis*- and *trans*-separation, silver ion HPLC resolved both SM^cM^t and SM^cM^c groups further into several peaks, *e.g.*, based on molecular asymmetry.

Based on reversed-phase HPLC and MS analyses, the distribution of the triacylglycerols according to their acyl carbon numbers did not differ much between saturated

*To whom correspondence should be addressed.

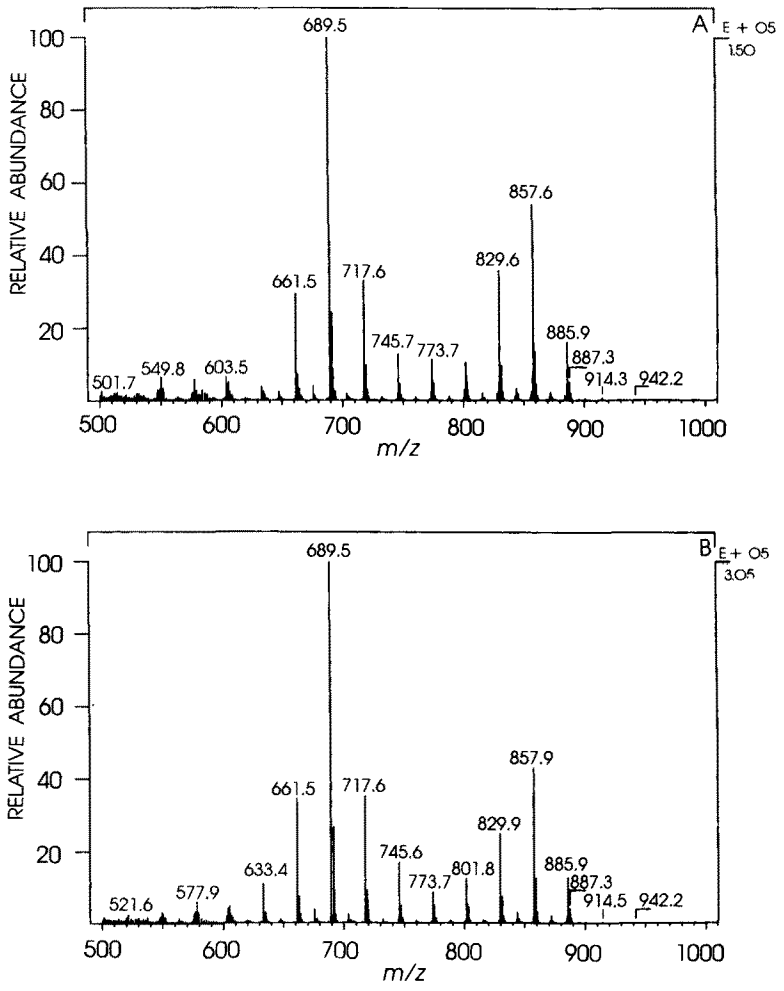

FIG. 1. Mass spectra of the negative ions produced by chemical ionization with ammonia from the triacylglycerol fractions of winter butterfat: (A) saturated *cis,trans*-dimonoenoic (Ag-HPLC Fraction 4) and (B) saturated *cis,cis*-dimonoenoic (Ag-HPLC Fraction 5) triacylglycerols. The ions of the displayed areas represent the deprotonated triacylglycerols, $[M - H]^-$ ions. The experimental conditions are given elsewhere (1). HPLC, high-performance liquid chromatography.

TABLE 1

Two Examples of the Fatty Acid Compositions of Saturated *cis,trans*-Dimonoenoic (SM^cM^t) and Saturated *cis,cis*-Dimonoenoic (SM^cM^c) Triacylglycerols of Winter Butterfat Obtained by Collisional Activation of $[M - H]^-$ Ions by Mass Spectrometry

		$[M - H]^-$			
		m/z = 689.5		*m/z* = 857.7	
Fatty acid[a]	RCO_2^- (*m/z*)	SM^cM^t	SM^cM^c	SM^cM^t	SM^cM^c
4:0	87	21.0	20.8		
6:0	115	2.5	2.1		
14:1	225	trace[b]	0.4		
16:1	253	1.5	1.7	0.3	0.8
16:0	255	1.1	0.4	26.7	30.0
18:2	279	1.4	0.7	0.4	trace[b]
18:1	281	72.0	72.0	70.5	66.7
18:0	283	0.6	1.9	2.0	2.5

[a]Fatty acids were identified only by the number of carbon atoms and double bonds. Identification of the fatty acids and calculation of their proportions were based on the intensities of the uncorrected RCO_2^- ions.
[b]trace, less than 0.2%.

cis,cis- and saturated *cis,trans*-dimonoenoic fractions. However, the distribution obtained by MS (Fig. 2A) was greatly different from that obtained by reversed-phase HPLC (Fig. 2B). Based on the reversed-phase HPLC analysis, approximately 80% of the molecules consisted of species with acyl carbon numbers 50, 52 and 54 compared with 25% obtained by MS. The most abundant acyl carbon number determined by MS was 40, comprising over 30% of the total. These results show clearly the strong discrimination of the intensities of $[M - H]^-$ ions according to molecular weight in the NICI MS analysis, as shown earlier by Kallio and Currie (2).

Sufficient resolution of the ammonia NICI MS made it possible to recognize and analyze minor molecular weight fractions. Butterfat triacylglycerols contained small amounts, 5–6%, of saturated dimonoenoic molecules with an odd number of acyl carbons. Separation of these triacylglycerols is also possible by reversed-phase HPLC, but the identification of such molecules is unreliable without MS confirmation. Therefore, odd acyl carbon

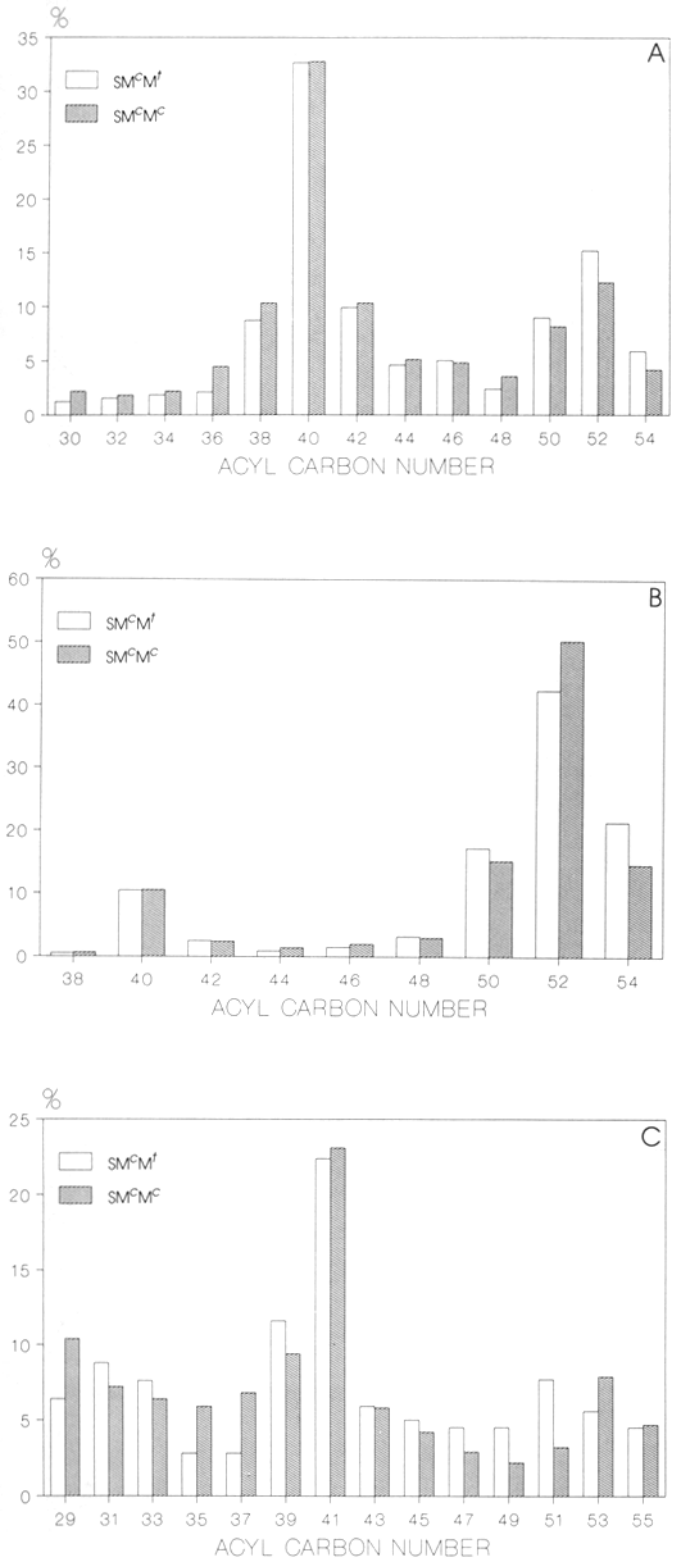

FIG. 2. Distribution of the saturated *cis,trans*-dimonoenoic (SM^cM^t) and saturated *cis,cis*-dimonoenoic (SM^cM^c) triacylglycerols according to (A) the intensities of the uncorrected $[M - H]^-$ ions in relation to their even acyl carbon numbers, analyzed by mass spectrometry (MS), (B) the uncorrected areas of the chromatographic peaks obtained by reversed-phase high-performance liquid chromatography, in relation to their acyl carbon numbers and (C) the intensities of the uncorrected $[M - H]^-$ ions in relation to their odd acyl carbon numbers, analyzed by MS.

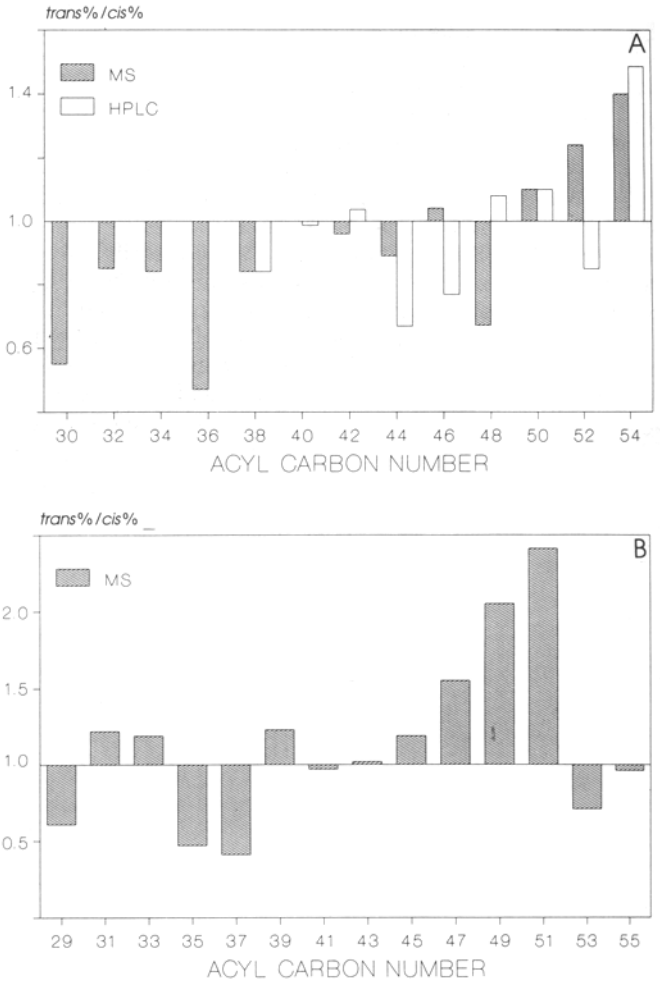

FIG. 3. The ratios of the proportion of the saturated *cis,trans*- to saturated *cis,cis*-dimonoenoic triacylglycerols within each (A) even acyl carbon number and (B) odd acyl carbon number. Based on mass spectrometric (MS) analysis, the ratio was calculated with the proportions of the corresponding $[M - H]^-$ ions. Based on reversed-phase high-performance liquid chromatography (HPLC) analysis, the ratio was calculated with the proportions of the corresponding chromatographic peaks.

number molecules were not differentiated from molecules with an even number of acyl carbons when analyzed by reversed-phase HPLC. The distribution profile of the saturated dimonoenoic triacylglycerols with an odd number of acyl carbons (Fig. 2C) differed somewhat from that of the corresponding molecules with an even number of acyl carbons, when analyzed by MS. There was no great difference between saturated *cis,cis*- and saturated *cis,trans*-dimonoenoic fractions. Triacylglycerols with 41 acyl carbons were most abundant, representing over 20% of the total. The proportions of small-molecular weight triacylglycerols were higher than in the corresponding triacylglycerols containing an even number of acyl carbons.

The ratios of the proportion of SM^cM^t to SM^cM^c fration within each acyl carbon numbers are shown in Figure 3A and B. The tendencies shown by the ratios are not as clear as those from disaturated monoenoic triacylglycerols presented earlier (1). This may be partly due to the small differences between the SM^cM^t and SM^cM^c fractions.

Slight increases of the ratios toward the greater acyl carbon numbers can be presumed.

There are no distinct differences in the fatty acid compositions between corresponding SM^cM^t and SM^cM^c triacylglycerols (Table 1). However, there may be differences in the proportions of different fatty acid combinations or in the distribution of fatty acids between the *sn*-1/*sn*-3 and *sn*-2 glycerol positions, which can be further studied by tandom mass spectrometry.

There is hardly any knowledge on the distribution of geometrical isomers of fatty acyl residues on triacylglycerols. However, such information is important, because *cis*- and *trans*-fatty acids most probably have different biological effects due to their physicochemical properties. In addition to ruminants, *trans*-fatty acids are found in hydrogenated oils, which are widely consumed, *e.g.*, as margarines. The combination of silver ion HPLC and MS analyses makes it possible to obtain regiospecific information (*sn*-1/*sn*-3 *vs. sn*-2) on the distribution of geometrical fatty acyl isomers on natural mixtures of triacylglycerols.

REFERENCES

1. Laakso, P., and H. Kallio, *J. Am. Oil Chem. Soc. 70*:1049 (1993).
2. Kallio, H., and G. Currie, *Lipids 28*:207 (1993).
3. Christie, W.W., *Rev. Fr. Corps Gras 38*:155 (1991).
4. Laakso, P.H., K.V.V. Nurmela and D.R. Homer, *J. Agric. Food Chem. 40*:2472 (1992).
5. Christie, W.W., *J. High Resolut. Chromatogr. Chromatogr. Commun. 10*:148 (1987).

[Received February 5, 1993; accepted May 5, 1993]