TABLE I

X-ray Diffraction Patterns of Tristearin in Ethyl Acetate

Sample number	Sample conc. (wt %)	Short spacings (A)				Chain packing
1	11.3	4.6	4.2	3.8	3.7	$\beta' + \beta$
2	5.9	–	4.2	3.8	–	β
3	2.7	–	4.2	3.8	–	β
4	0.5	4.6	4.2	3.8	3.7	$\beta' + \beta$

Spectroscopic evidence so far obtained indicates that the molecular structure of the triglyceride molecules remained unchanged during the course of fixation. Infra-red spectra of tristearin, before and after fixation were identical. The infra-red spectrum of fractionated Canola exhibited an additional band near 980 cm^{-1} after fixation which can be due to the formation of monoester as a product of the reaction of a double bond with osmium tetroxide. Reaction of the double bonds in unsaturated fats with osmium tetroxide has been postulated by other researchers (10) and various structures have been proposed; the most generally accepted involves four coordinate osmium VI. The formation of such a structure does not, however, change the morphology of the crystals which is evident from the identical appearance of the spherulites in the micrographs showing the β-form of tristearin (Fig. 3) and that of fractionated Canola (Fig. 4). C^{13} spectra obtained for the β-form of tristearin after fixation also confirmed that the molecular structure remained essentially intact.

This simple and fast method for crystal fixation provides a technique that is very promising for studying fats and fat products using the high resolution capabilities of scanning electron microscopy.

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Lipid Methodology – Chromatography and Beyond. Part II. GC/MS, LC/MS and Specific Enzymic Hydrolysis of Glycerolipids

A. KUKSIS,* J.J. MYHER and **L. MARAI,** Banting and Best Department of Medical Research, University of Toronto, Ontario, Canada

ABSTRACT

The combination of specific enzymic degradation with GC/MS or LC/MS identification and quantitation of enantiomeric diacylglycerols and reverse isomers has greatly improved the methods of structural analysis of triacylglycerols, so that in many instances complete characterization of both major and minor species is possible. The techniques described for the analysis of triacylglycerols and sn-1,2- and sn-2,3-diacylglycerols are also applicable to the X-1,3-diacylglycerols and X-1-monoacylglycerols following coversion to triacylglycerols by acylation with appropriate fatty acids. For many applications, however, a combination of specific enzymic hydrolysis with a GLC analysis of the products on polar capillary columns may be adequate for the identification and quantitation of the major molecular species of both triacylglycerols and diacylglycerols.

INTRODUCTION

In Part I of this series (1) we demonstrated that a combination of either GLC or HPLC with mass spectrometry was necessary for an unequivocal identification of the chemical composition and molecular association of the fatty acids in the resolved triacylglycerol and diacylglycerol species. Except for certain reverse isomers of diacylglycerols, these techniques do not allow the identification of positional isomers or enantiomers of acylglycerols. In the past, positional distribution of fatty acids in triacylglycerols has been determined by the stereospecific analysis of Brockerhoff (2), but this method gives no information about the molecular association of the fatty acids, unless the triacylglycerol mixture first has been resolved into individual molecular species, which is impractical. Likewise, no indication of molecular association is obtained by the positional analysis of fatty acids in the glycerophospholipids commonly performed by phospholipase A_2 (3). Myher and Kuksis (4) have proposed a method of stereospecific analysis which overcomes this difficulty. In their routine the X-1,2-diacylglycerols are converted into the corresponding X-1,2-diacylphosphatidylcholines, which are then subjected to a stepwise stereospecific release of the sn-1,2- and the sn-2,3-diacylglycerol moieties by digestion with phospholipase C. The enantiomeric diacylglycerols then can be submitted to a complete chromatographic resolution. The problem of a physical separation of the reverse isomers remains, but their proportion can be determined by collecting enantiomerically pure molecular species of diacylglycerols by HPLC and subjecting them to hydrolysis with pancreatic lipase in the form of the tertiary-butyldimethylsilyl (t-BDMS) ethers.

*To whom correspondence should be addressed.

The introduction of the stereospecific analysis via phospholipase C along with the pancreatic lipolysis of the t-BDMS ethers of diacylglycerols has greatly expanded the repertoire of methods for structural analysis of acylglycerols, so that in many instances complete identification at least of the major molecular species has become practical. In the following we review the present limits of the combined application of chromatography with mass spectrometry and enzymic positional analysis.

Structural Analysis of Acylglycerols

In Part I (1) we considered the advantages of using phospholipase C for releasing the diacylglycerol moieties of natural glycerophospholipids for subsequent GC/MS or LC/MS analysis and of pancreatic lipase for determining the reverse isomer ratios in the t-BDMS ethers of the diacylglycerols.

The first attempts to determine the molecular association of fatty acids in the enantiomeric diacylglycerol moieties of triacylglycerols were made by pyrolysis GLC of the sn-2,3-diacylphosphatidylphenols (5) resulting from the specific enzymic hydrolysis of rac-1,2-diacylphosphatidylphenols generated in the Brockerhoff analysis (2). This approach has yielded the carbon number distribution of the sn-2,3-diacylglycerols from several fats (6,7) and oils (8). The carbon number distribution of the sn-1,2-diacylglycerols is obtained by subtraction of the sn-2,3-enantiomer distribution from the carbon number distribution of the total sn-1,2(2,3)-diacylglycerols. Figure 1 compares the carbon number distribution of the sn-2,3-diacylglycerols of an abnormal human milk fat to that of the sn-1,2-diacylglycerols obtained by subtraction of the sn-2,3-diacylglycerols from the sn-1,2(2,3)-diacylglycerols (7). It is obvious that the sn-2,3-diacylglycerols possess a shorter overall chain length because of the concentration of the shorter chain acids in the sn-3-position. On the basis of comparable results obtained on pyrolysis of intact phosphatidylcholines of blood serum in the hot injector port of the gas chromatograph, it was suggested (9) that the series of

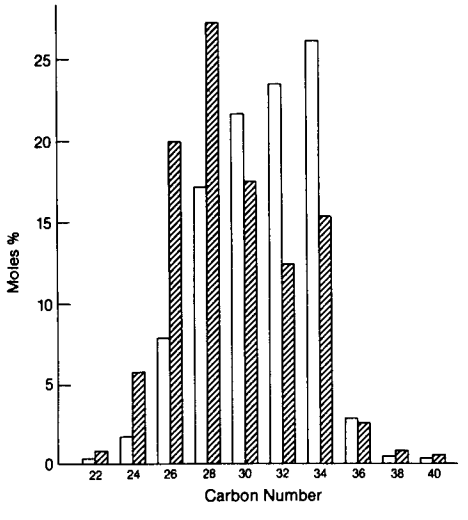

FIG. 1. Carbon number distribution of the sn-1,2- and sn-2,3-diacylglycerol moieties of milk triacylglycerols of Type 1 hyperlipemia patient. Open bars, sn-1,2-diacylglycerols; Hatched bars, sn-2,3-diacylglycerols. Sn-2,3-diacylglycerol distribution was determined by direct GLC of the corresponding phosphatidylphenols; the distribution of the sn-1,2-diacylglycerols was derived by subtraction from the carbon number distribution of the sn-1,2(2,3)-diacylglycerols as obtained by direct GLC of the corresponding phosphatidylphenols. The carbon numbers represent diacylglycerols with a total of 22-40 acyl carbons. GLC conditions were as given elsewhere (7).

the carbon numbers was due to the formation of the propyleneglycol diesters, which corresponded to the diacylglycerols in the original phosphatidylcholine. This tentative identification subsequently was confirmed by GC/MS of the pyrolysis product by Perkins and Johnston (10). Thus, dipalmitoylphosphatidylcholine yielded a mass spectrum corresponding to that of dipalmitoylglycerol with readily discernible $[M-18]^+$ ion at m/z 550, and an $[RCO+128]^+$ ion at m/z 367, and smaller ions for $[RCOOH]^+$ and $[RCO]^+$ at m/z 256 and 239, respectively. The spectra exhibited a base peak at m/z 98 ($C_6H_{10}O$), which was believed to be formed by the 6,7-cleavage of the acyl moiety. There have been no specific attempts to determine the molecular species of the sn-1,2- or the sn-2,3-diacylglycerol moieties of any triacylglycerols by this method, but the potential exists. The pyrolysis of the glycerophospholipids in the injector port of the gas chromatograph, however, is not suitable for a subsequent resolution of the molecular species on polar capillary columns (11,12).

The procedure of Myher and Kuksis (4) for generating enantionmeric diacylglycerols from triacylglycerols takes advantage of the differential rates of hydrolysis of the sn-1- and sn-3-phosphorylcholine derivatives of diacylglycerols by phospholipase C. In the proposed routine, rac-1,2-diacylglycerols are randomly formed from triacylglycerols by Grignard degradation (13). The rac-1,2-diacylglycerols are isolated and converted to the corresponding phosphatidylcholines, which are then subjected to a stepwise hydrolysis with phospholipase C from *Clostridium perfringens*. Under the selected experimental conditions the sn-1,2-enantiomer is hydrolyzed within 2 min, at which time the released sn-1,2-diacylglycerols are recovered, while the residual sn-2,3-diacylphosphatidylcholines are submitted to a further hydrolysis to release the sn-2,3-diacylglycerols. Figure 2 shows the molecular species profiles of the sn-1,2-, sn-2,3- and x-1,3-diacylglycerols of the triacylglycerols of natural peanut oil as obtained by GLC on polar capillary columns (14). The native oil shows a higher proportion of the longer chain species in the sn-2,3- and in the x-1,3-diacylglycerols than in the sn-1,2-diacylglycerols, which is due to the preferential location of the longer chain fatty acids in the sn-3 position in this oil. The different diacylglycerols of the randomized oil possess identical molecular species distributions. We have described elsewhere (14) the complete composition of the molecular species of the diacylglycerols as obtained by GC/MS along with the reconstituted composition of the original peanut oil triacylglycerols. We have used a similar stereospecific method for the determination of the structure of lard (4) and of human plasma triacylglycerols (15). Figure 3 compares the composition of the molecular species of the sn-1,2- and sn-2,3-diacylglycerols of lard as obtained by GC/MS (4). Clearly this fat is also asymmetric as indicated by the large differences in the relative quantities of the different molecular species in the two enantiomeric moieties. The palmitic acid in the sn-2-position is combined with markedly different fatty acids in the sn-1- and sn-3-positions. In many instances the stereospecific degradation of triacylglycerols in combination with GLC analyses of the molecular species of the diacylglycerols on polar capillary columns is adequate for the determination of essentially complete molecular structure. Figure 4 shows the GLC profiles of the sn-1,2- and sn-2,3-diacylglycerols derived from cocoa butter (16) as obtained on a polar capillary column (11). There are minor but significant differences in the composition of the molecular species, and this oil also cannot be considered to be symmetrical or random.

The stereospecific resolution of the sn-1,2- and sn-2,3-diacylglycerols via rac-phosphatidylcholine and phospho-

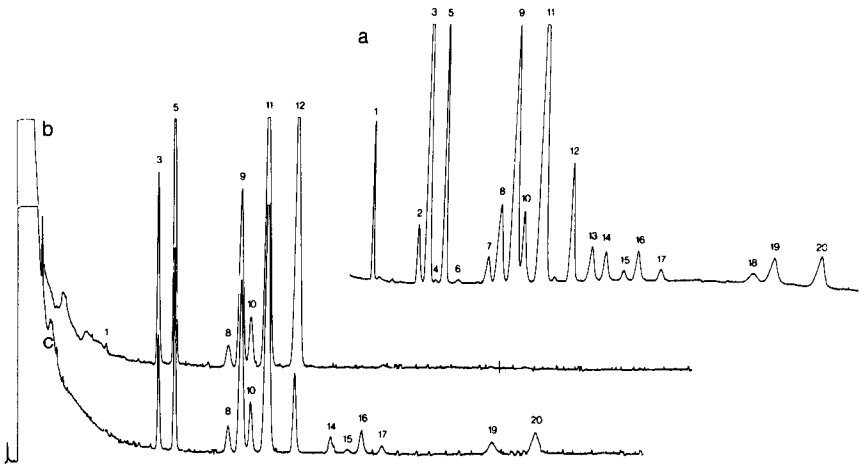

FIG. 2. GLC profiles of the sn-1,2- and sn-2,3-diacylglycerol moieties of triacylglycerols from peanut oil. a, TMS ethers of X-1, 3-diacylglycerols; b, TMS ethers of sn-1,2-diacylglycerols; c, TMS ethers of sn-2,3-diacylglycerols. Peak identity: 1, 16:0 16:0; 2, 16:0 18:0; 3, 16:0 18:1; 4, unknown; 5, 16:0 18:2; 6, 17:0 18:1; 7, 18:0 18:0 + 16:0 20:0; 8, 18:0 18:1; 9, 18:1 18:1; 10, 18:0 18:2; 11, 18:1 18:2; 12, 18:2 18:2; 13, 16:0 22:0 + 18:0 20:0; 14, 18:1 20:1; 15, 18:1 20:1; 16, 18:2 20:0; 17, 18:2 20:1; 18, 18:0 22:0 + 16:0 24:0; 19, 18:1 22:0; 20, 18:2 22:0. GLC conditions as described elsewhere (11).

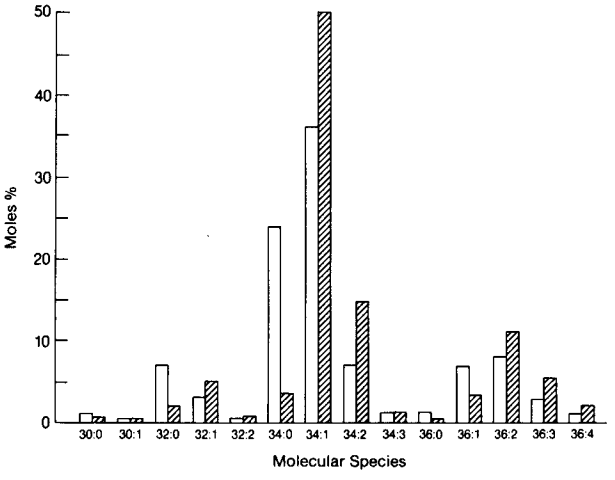

FIG. 3. GC/MS profiles of sn-1,2- and sn-2,3-diacylglycerol moieties of lard triacylglycerols. Open bars, sn-1,2- diacylglycerols; hatched bars, sn-2,3-diacylglycerols. The molecular species are identified by the number of acyl carbons:number of double bonds. GC/MS conditions as described elsewhere (4).

lipase C can be equally well applied to the analysis of the X-1,2-diacylglycerols occurring in the free form in various tissue extracts. We have used this approach in a reexamination of the stereochemical course of acylation of sn-2-monoacylglycerols to diacylglycerols by rat intestinal villus cells using 2-lauroylglycerol and [^{2}H] 3 palmitic acid as substrates (17). The newly synthesized X-1,2-diacylglycerols were recognized on the basis of the content, positional distribution and molecular association of the fatty acids by GLC with mass spectrometry in combination with stereospecific analysis. It was found that the free X-1,2-[^{2}H] 3palmitoyllauroylglycerols contained an average of 74% of the sn-1,2- and 26% of sn-2,3- enantiomers, which were used for triacylglycerol formation in the same proportion (Table I). The stereospecific separation of the enantiomers was performed with very small amounts of material using appropriately selected carrier rac-diacylglycerols. The method is applicable to both long and medium chain length diacylglycerols, but combinations of short and medium and short and long chain lengths in the diacylglycerols thus far have not been examined. The reverse isomer composition of individual diacylglycerol peaks

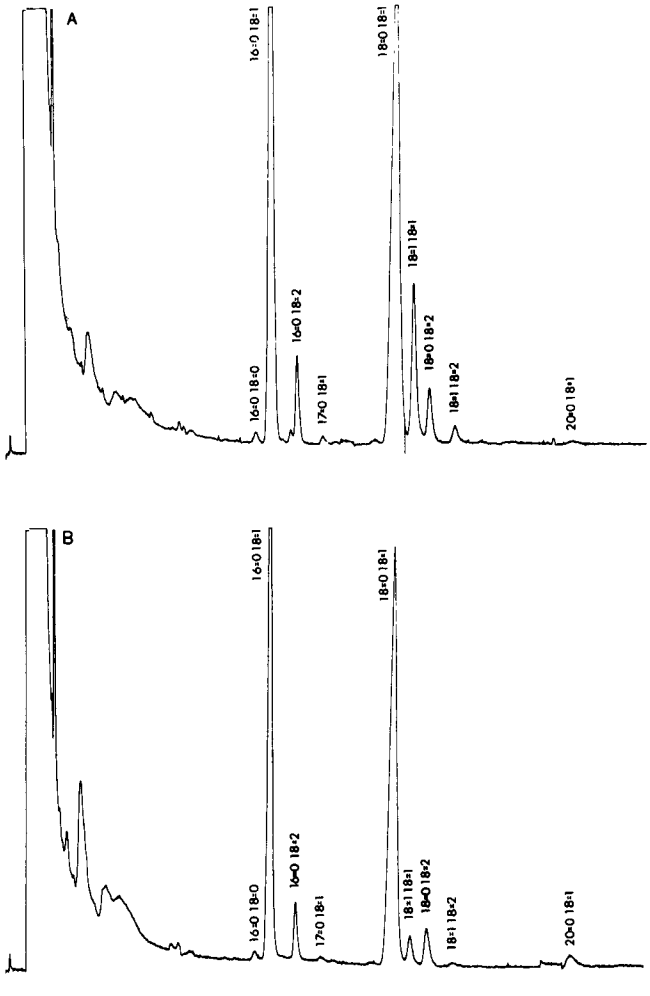

FIG. 4. Resolution of molecular species of the sn-1,2- and sn-2,3-diacylglycerol moieties of cocoa butter triacylglycerols on a polar capillary column (16). A, TMS ethers of sn-1,2-diacylglycerols; B, TMS ethers of sn-2,3-diacylglycerols. Peaks are identified by the pairing of the component fatty acids represented as number of acyl carbons:number of double bonds. Column and GLC conditions as described elsewhere (11).

TABLE I.

Molar Proportions of Free sn-1,2- and sn-2,3-diacylglycerols Generated from Exogenous and Endogenous Substrates by Isolated Intestinal Villus Cells (17)

Molecular Species*	15 min.		30 min	
	sn-1,2-	sn-2,3-	sn-1,2-,	sn-2,3-
	** ***			
12:0 ($^2H_0$) 16:0	19.7 (70)	8.8 (30)	19.2 (73)	7.2 (27)
12:0 ($^2H_3$) 16:0	25.6 (77)	7.7 (23)	26.6 (73)	9.3 (26)
12:0 C_{18}	13.6 (50)	13.5 (50)	8.9 (50)	8.4 (50)
($^2H_0$) 16:0 ($^2H_5$) 16:0	2.6 (73)	1 (27)	4.9 (89)	0.6 (11)
($^2H_3$) 16:0 ($^2H_3$) 16:0	1.9 (66)	1 (34)	6.1 (91)	0.6 (9)
($^2H_3$) 16:0 C_{18}	3.1 (68)	1.5 (32)	3.8 (50)	4.4 (50)

*Exogenous substrates: 2-monolauroylglycerol and ($^2H_3$) palmitic acid in a molar ratio 1:2.
**Results are given as mole % with total products normalized to 100. Each value is an average of two estimates. C_{18} combined total of all fatty acids with 18 carbon atoms.
***Values in brackets represent percentages of the enantiomers in each species.

collected from the reverse phase HPLC columns as the t-BDMS ethers can be determined by hydrolysis with pancreatic lipase, which attacks only the ester group at the primary position, leaving the ester group in the secondary position intact (Myher and Kuksis, 1983, unpublished results). The residual monoacylglycerol t-BDMS ether then can be identified by either GLC as the TMS ether or by reverse phase HPLC as the di-t-BDMS ether, or as the underivatized monoacylglycerolmono-t-BDMS ether. The identification of the fatty acid in the sn-2-position in the form of the t-BDMS ether of the original monacylglycerol guards against contamination, which is a major hazard when analyzing small amounts of free fatty acids. The t-BDMS ethers are resistant to moisture and do not interfere with the pancreatic lipase specificity, although the rate of reactivity is lower than that observed for acylglycerols. The reverse isomer proportions of simple mixtures of sn-1,2- and sn-2,3-diacylglycerols also can be assessed at the phosphatidylcholine stage using phospholipase A_2, which attacks only the sn-1,2-enantiomers (3).

The sn-1,2-and sn-2,3-diacylglycerols derived from the synthetic glycerophospholipids by hydrolysis with phospholipase C are also suitable for subsequent LC/MS analysis, as already described in Part 1 of this series (1) for the sn-1,2-diacylglycerols derived from natural glycerophospholipids. There are no differences in the mass spectra between the corresponding species of the sn-1,2- and sn-2,3-diacylglycerols as already noted (1). In comparison to GC/MS, the LC/MS method requires a larger sample, because only 1% of the total effluent is being admitted to the mass spectrometer using the conventional HPLC columns (18). However, the use of minicolumns for the HPLC step eventually may permit working sensitivities more comparable to those obtained by GC/MS, because these columns permit the diversion of a larger proportion of the total effluent to the mass spectrometer.

There are no methods presently available for the physical resolution of enantiomeric X-1,3-diacylglycerols, although the fatty acid composition of the sn-1- and sn-3-positions can be readily determined (19), as can their molecular association (20).

In order to determine the fatty acid composition of the sn-1- and sn-3-positions it is necessary to prepare the 2-phosphorylphenol derivatives, which can be subjected to hydrolysis by phospholipase A_2 to release the fatty acid from the sn-1-position (19). To obtain the fatty acid composition of the sn-1- and the sn-3-positions for each X-1,3-diacylglycerol molecule, the molecular species must first be resolved to the limit of their chromatographic separation. It may be desirable to retain the fatty acids in combination with the glycerol residue for the positional analysis. This can be accomplished by converting the X-1,3-diacylglycerol to a triacylglycerol using a medium chain length fatty acid. The resulting triacylglycerol then can be subjected to a stereospecific analysis via phospholipase C as described above (4). This approach is time-consuming and requires modest amounts of substrate. However, it yields pure diacylglycerol enantiomers containing the sn-1- and sn-3-acyl groups of the original X-1,3-diacylglycerols. Since this method also destroys the molecular association of the fatty acids, it can be applied effectively only to pure molecular species of X-1,3-diacylglycerols.

Like the analysis of the X-1,3-diacylglycerols, that of the X-1-monoacylglycerols presents a problem. Although lipoprotein lipase has been shown to possess specificity for the sn-1-position (21) and lingual lipase for the sn-3-position (22), these enzymes are not suitable for a practical structural analysis even when the X-1-monoacylglycerols are converted into the corresponding triacylglycerols by reacting with an anhydride or acid chloride of a medium chain length fatty acid. The latter derivatives are suitable, however, for a Grignard degradation to the corresponding sn-1,2(2,3)- diacylglycerols, which, after a conversion to the sn-1,2(2,3)-diacylphosphatidylcholines can be degraded by phospholipase C or phospholipase A_2 to reveal the true enantiomeric nature of the original monoacylglycerol.

Structural Analyses of Alkylglycerols

The stereospecific degradation and positional analysis by means of lipases is applicable also to natural alkylacylglycerols, which occur in minor amounts in animal tissues (23), where the alkyl groups are confined to the primary positions. However, the substitution of an alkyl for an acyl group in the phosphatidylcholine molecule is known (24) to decrease the reaction rate greatly. Thus, the sn-1-alkyl-2-acylglycerol may not be released any faster than the sn-2,3-diacylglycerol counterpart from the corresponding phosphatidylcholines by phospholipase C. Should the alkyl group be present at the sn-3-position, the corresponding sn-2-acyl-3-alkylglycerol might be released long after the sn-2,3-diacylglycerols have been degraded. Hence it may be necessary to resolve the alkylglycerols from any acylglycerols prior to the stereospecific analysis. These methods are not suitable for a stereospecific assay of the X-1,2-dialkyl-3-acylglycerols (see below).

The method ought to be applicable to the stereospecific analysis of the free X-1-alkyl-2-acylglycerols believed to be formed by enzymic acylation of the rac-1-alkylglycerols in the intestinal villus cells (25). A conversion of the X-1,2-alkylacylglycerols to the corresponding X-1,2-alkylacylphosphatidylcholines could be followed by a stepwise stereospecific degradation with phospholipase C.

We had intended to extend the phospholipase C method of resolution of enantiomeric diacylglycerols to the sepa-

ration of the alkylacylglycerol products of acylation of 2-monoalkylglycerols by the monoacylglycerol acyltransferase. It was observed, however, that phospholipase C lost much of its stereospecificity when an alkyl group was substituted for an acyl group at the sn-2-position of the phosphatidylcholine molecules (26). Thus, the initial rates of hydrolysis of the sn-1-acyl-2-alkyl- and sn-2-alkyl-3-acylphosphatidylcholines were only 5:1 and 13:1, compared to those of 100:1 for the corresponding diacyl derivatives. This apparent loss of stereospecificity was related to the overall rate of the enzymatic hydrolysis of these substrates. The more rapidly hydrolyzed lauroyloleyl species allowed a partial resolution of the enantiomers. It is possible that a substitution of a shorter chain alkyl group at the sn-2-position would allow a more effective discrimination between the sn-1,2- and sn-2,3-enantiomers bearing the alkyl group in the sn-2-position. Other experiments with phospholipase D, which also exhibits a marked specificity for the enantiomeric diacylphosphatidylcholines, resulted in a complete loss of stereospecificity upon introduction of an sn-2-alkyl group (26). Since the alkylacylphosphatidylcholines are less soluble than their diacyl analogs, it is possible that the loss of specificity is related to the poor solubilization of these compounds, which may also account for the 500- 1000-fold decrease in the overall reactivity of these compounds. Perhaps another solvent mixture would have yielded higher rates of reactivity. However, the loss of stereospecificity may be due to the absence of appropriate functional groups, since it has been shown that lipoprotein lipase becomes stereochemically incompetent when hydrolyzing sn-1,2-dialkyl-3-acyl and sn-2,3-dialkyl-1-acylglycerols at rates much more comparable to those observed for the corresponding triacylglycerols (27).

Combinations of Other Chromatographic Methods with Enzymic Positional Analysis

In the past, chromatographic methods other than GC/MS and LC/MS have been used in combination with enzymic analyses. The most commonly employed method involved the pancreatic lipase digestion of the triacylglycerol (28) or diacylglycerol (29) fractions resolved by argentation TLC. This method has been particularly widely used for the identification of the molecular species of the sn-1,2-diacylglycerol species derived from natural diacylphosphatides (30). A complete stereospecific analysis of the triacylglycerol fractions derived by $AgNO_3$-TLC of peanut oil has given all the necessary information for establishing the complete structure of all the components present. Specifically, it has been shown (20) that the diene fraction was made up of triacylglycerols containing two oleic acid residues in combination with one residue of either palmitic acid in the sn-1-position and sn-3-position, or one residue of the C_{20} and C_{24} saturated acid in the sn-3-position. Similarly, it was possible to obtain a complete description of the molecular species present in the tetraene fraction (Table II). Sephadex LH-20 separations of milk fat triacylglycerols into fractions of different molecular weight have been used in combination with stereospecific analysis by Mills et al. (31). Although no specific triacylglycerols were identified, the results obtained were suitable for an evaluation of the effect of dietary fat supplementation on the positional distribution of fatty acids in the milk and tissue triacylglycerols of sheep, goats, cows and pigs. A more effective prefractionation of triacylglycerols for subsequent positional analysis has been envisaged by Bezard and Ouedraogo (32), who have combined $AgNO_3$-TLC and HPLC as a means of obtaining simple triacylglycerol fractions for an unequivocal positional analysis.

Clearly, combinations of enzymic positional analysis with chromatographic methods of separation are necessary for complete determination of the structure of natural triacylglycerols and diacylglycerols. On the basis of the knowledge of the exact pairing of the fatty acids in the sn-1,2-, sn-2,3- and sn-1,3-diacylglycerol moieties of even only partially resolved triacylglycerols it is possible to reconstitute the structure of the original triacylglycerols either by inspection (simple mixtures) or by reiterated computer fitting (complex mixtures) to match the carbon number profiles of the original triacylglycerols, or of their subfractions derived by argentation TLC, reversed phase HPLC or both (33). The maximum number of different fatty acids that may be acceptable for an unambiguous determination of the triacylglycerol structure depends on the actual positional distribution. For triacylglycerol mixtures approaching random distribution, the maximum number of different fatty acids per chromatographic fraction should not exceed four. For the determination of the structure of triacylglycerols containing stable isotope labelled fatty acid and glycerol moieties, it is necessary to combine the chromatographic separation and enzymic positional analysis with a mass spectrometric assessment of the chromatographic fractions. This is required for the identification and quantitation of each stable isotope-

TABLE II.

Positional Distribution of Fatty Acids in the Dienoic and Tetraenoic Triacylglycerols of Natural Peanut Oil*

Fatty Acids	Sn-glycerol Position 1-	2-	3-	Major Triacylglycerols			Content
*011***							
16:0	25.3	4.0	20.0	18:1	18:1	16:0	17.5
18:0	8.1	1.5	7.9	18:1	18:1	18:0	7.5
18:1	65.9	93.0	37.3	18:1	18:1	18:1	37.3
20:0	0.1	0.2	8.7	18:1	18:1	20:0	8.7
20:1	0.2	0.1	3.0	16:0	18:1	20:1	2.5
				18:0	18:1	20:1	0.5
22:0	0.2	0.8	15.4	18:1	18:1	22:0	15:4
24:0	0.1	0.3	6.7	18:1	18:1	24:0	6.7
112							
18:1	69.2	47.0	87.8	18:1	18:2	18:1	53.0
18:2	30.8	53.0	12.2	18:2	18:1	18:1	30.8
				18:1	18:1	18:2	12:2

*Original analyses as reported elsewhere (20). 011 and 112 represent molecules species of triacylglycerols containing one saturated and two monounsaturated and two monounsaturated and one diunsaturated fatty acids, respectively.

labelled moiety of the triacylglycerol molecule as well as for a determination of its molecular association with any other labelled or unlabelled moieties of the total molecule. The latter requirement places further demands upon the analytical system, and the resulting problems are discussed in the concluding part of this series (34).

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♣Lipid Methodology – Chromatography and Beyond. Part III. Analyses of Natural and ^{2}H-Labeled Glycerolipids by GC/MS and LC/MS with Specific Enzymic Hydrolyses

A. KUKSIS, J.J. MYHER and **L. MARAI,** Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada

ABSTRACT

In the concluding part of this series, the authors review recent attempts to adopt combinations of chromatography with other complementary analytical techniques to the study of stable isotope-labeled molecules as tracers of glycerolipid metabolism. It is shown that LC/MS in combination with specific enzymic hydrolyses has special advantages for this purpose. Using deuterium-labeled non-lipid precursors, effective labeling of both newly synthesized fatty acids and glycerol has been obtained and their molecular association and positional distribution (fatty acids) in the newly formed glycerolipid molecules has been determined as an indicator of the metabolic pathways involved. The above experimental routines extend the analytical lipid methodology beyond the capabilities of chromatography and radio-chromatography with or without complementary enzymatic analyses.

The studies reviewed in the present part and in the previous two parts of this series provide outlines for a potential practical assessment of the various metabolic pathways of glycerolipids, including the identification and quantitation of the true precursor and product pools involved in specific biosynthetic or degradative transformations. Such investigations have not been possible in the past by chromatographic or radio-chromatographic means. Some of the problems that remain may be subject to solution by means of GC/MS/MS and LC/MS/MS. It is hoped that the new and improved methodology will be matched in the future by comparable advances in the sampling of plasma and cellular components, in selected isotope labeling of the de novo products, and by improvements in the overall design of the metabolic experiments. Although the extension of lipid methodology beyond chromatography has greatly simplified the demands of the experimental design, it is obvious that improved experimental design and sampling techniques will result in further advances in the quality of the observations and in the understanding of lipid metabolism.

*To whom correspondence should be addressed.

INTRODUCTION

In Parts I (1) and II(2) of this series we reviewed the application of GC/MS and LC/MS to the resolution and quantitation of molecular species of both diacyl- and triacylglycerols. Furthermore, in Part II (2) we discussed how an identification and quantitation of positional isomers and enantiomers becomes possible when the above techniques are combined with specific enzymic hydrolysis. In Part III, which is the concluding article in this series, we wish to