# A Direct Method for Regiospecific Analysis of TAG Using α-MAG

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**ABSTRACT:** An analytical procedure was developed for regiodistribution analysis of TAG using α-MAG prepared by an ethyl magnesium bromide deacylation. In the present communication, the deacylation procedure is shown to lead to representative α-MAG, allowing the composition of the native TAG in the α-position to be determined directly. The composition in the β-position can then be estimated from the composition of the α-MAG and TAG according to the formula 3 × TAG − 2 × α-MAG. The estimates are superior to those obtained using the α,β-DAG and Brockerhoff calculations as they come closer to the theoretical value and have smaller SD. The present procedure, first demonstrated on a synthetic TAG, was then successfully applied to the analysis of borage oil, milkfat, and tuna oil.

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Much work has been done to simplify the regiodistribution analysis of TAG using a chemical deacylation (1–4). Since the chemical deacylation makes most of the TAG accessible to a regiodistribution analysis (5–8), difficulties are due to the occurrence of some unavoidable acyl migration, which naturally changes analysis results (9). In order to improve the accuracy of analysis, suitable conditions need to be found to minimize the incidence of isomerization and, therefore, for the Grignard deacylation to produce acylglycerols representative of the original TAG structure. Accordingly, methods in the literature generally make use of α,β-DAG (9–13) or β-MAG (2), with appropriate calculations. A shortcoming is that the FA composition of the α-position is not analyzed directly but obtained by calculation and is therefore subject to cumulative errors of GC analysis (10,13). Some recent methods using both the α- and β-MAG produced from TAG by a Grignard deacylation involve their analysis by HPLC on chiral columns (1,3). However, use of these methods on a routine basis is laborious, since they require special and expensive analytical equipment rather than the standard GC equipment.

In the present communication, we have examined the possibility of producing representative α-MAG, i.e., with a random FA distribution, by an ethyl magnesium bromide deacylation of TAG. The classical procedure of deacylation is modified to (i) find suitable conditions for decreasing isomerization, (ii) determine the regiospecific distribution through the GC analysis of α-MAG, and (iii) corroborate the results obtained with the pancreatic lipase hydrolysis (standardized procedure ISO 6800).

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Abbreviations: DCF, 2′,7′-dichlorofluorescein; POP, *sn*-1,3-dipalmitoleyl-*sn*-2-oleoyl-glycerol.

The method was first demonstrated by the regiodistribution analysis of a racemic *sn*-1,3-dipalmitoleyl-*sn*-2-oleoyl-glycerol (POP), then by the analysis of borage oil, milkfat, and tuna oil.

## EXPERIMENTAL PROCEDURES

*Materials.* Borage oil was obtained from Bertin (Lagny-le-sec, France). Tuna oil was an industrial product from Sea Oil (Beaumont-Hague, France). Milkfat was obtained from Bel (Vendome, France). The racemic synthetic POP (99%+), a crude lipase from porcine pancreas, and a 0.2% solution of 2′,7′-dichlorofluorescein in ethanol (DCF solution) were purchased from Sigma Chemical Company (St. Louis, MO). A 3.0-M solution of ethyl magnesium bromide ($C_2H_5MgBr$) in diethyl ether was obtained from Aldrich (Milwaukee, WI). A methanolic solution of boron trifluoride (10%, wt/vol) was obtained from Fluka (Hauppauge, New York). Silicic acid 60 $F_{254}$ TLC plates were obtained from Merck (Darmstadt, Germany). Anhydrous $NaHCO_3$, boric acid, and all organic solvents were also from Merck.

*Pancreatic lipase hydrolysis.* The standard pancreatic lipase hydrolysis procedure used to determine the FA composition in the β-position of TAG was modified from Luddy *et al.* (14).

*Chemical deacylation of TAG.* Forty milligrams of pure TAG was dissolved in 2 mL of dry diethyl ether. A $C_2H_5MgBr$ solution (670 μL) was added directly using a pipette equipped with a positive displacement mechanism that isolated the aspirated liquid from the air (Gilson, Middleton, WI). The mixture was shaken for 15 s, then 300 μL of glacial acetic acid was added, followed by 5 mL of a 0.4-M boric acid solution in water to stop the reaction. The lipid products (newly formed partial acylglycerols, tertiary alcohols, and residual TAG) were then extracted with the diethyl ether. This extract was washed with a 5-mL solution of aqueous boric acid (0.4 M)/aqueous $NaHCO_3$ (2%), 50:50 (vol/vol) then resolved by TLC without further treatment.

The MAG were quickly isolated by preparative TLC on precoated silicic acid that was impregnated with a 5% boric acid solution in methanol (wt/vol) to prevent isomerization (15). The plates were developed with a chloroform/acetone/acetic acid solution (85:15:1, by vol) to isolate the *sn*-1(3)-from the *sn*-2-MAG. After development, bands were revealed and visualized with the DCF solution under UV light (366 nm). The following bands were observed: α-MAG ($R_f$ = 0.26); β-MAG ($R_f$ = 0.38); α,β-DAG ($R_f$ = 0.76); α,α′-DAG ($R_f$ = 0.85); tertiary alcohols of the deacylated FA and residual TAG ($R_f$ = 0.95). The silica gel of corresponding bands

was scraped off and transferred to Teflon-lined screw-capped tubes for methyl ester preparation.

*Methyl ester preparation and analysis.* Methyl esters were prepared according to Morrison and Smith (16). A methanolic solution (0.5 to 0.8 mL) of boron trifluoride was added directly to the silica gel, and methylation was performed at 100°C for 60 min.

FAME were analyzed by GLC with a Carlo Erba GC 8000 Top apparatus (Erba Science, Paris, France) equipped with an FID. Methyl esters were separated in a glass capillary column (30 m × 0.32 mm i.d.) coated with CARBOWAX 20M, programmed from 185 to 210°C (4°C/min), and held isothermally for 10 min. Peak areas and percentages were calculated by a Merck D2000 integrator. Areas obtained with the integrator were divided by chain length to yield the mole percentage of FA.

## RESULTS

*Regiodistribution analysis of POP.* The degree of purity of the POP was first verified. Pancreatic lipase hydrolysis confirmed that the β-position was occupied almost entirely by oleic acid (98.5%) and that the α-position was occupied by palmitic acid (99.3%). Results of the analysis of partial acylglycerols obtained from the Grignard degradation of POP are detailed in Table 1. Results of the analysis of the α,β-DAG agreed well with the lipase hydrolysis, certainly within the limits of analysis errors. The α,α′-DAG was contaminated with isomerized α,β-DAG, as evidenced by the content of oleic acid (4.5%). The β-MAG were strongly contaminated with 5.6% palmitic acid that had migrated from the α-position. We observed only 1.8% contamination of α-MAG. Consequently, the results from the α-MAG analysis were closer to the reference value and provided direct analysis of the α-position. The FA distribution in the β-position could then be analyzed by direct determination through the β-MAG or by calculation based on the composition of the different acylglycerols, as demonstrated at the bottom of Table 1. The most accurate estimate was obtained by calculation from 3 × TAG − 2 × α-MAG. The estimates were 96.3 ± 0.9% (mean ± SD) oleic acid.

**TABLE 1**
**Regiospecific Analysis of Synthetic *sn*-1,3-Dipalmitoleyl-*sn*-2-oleoyl-glycerol**

| Method | Compound | Positions | FA composition[a] (mol%[b]) 16:0 | 18:1 |
|---|---|---|---|---|
| | TAG | α, β, α′ | 66.7 | 33.3 ± 0.2 |
| Pancreatic | β-MAG | β | 1.5 | 98.5 ± 0.9 |
| lipase | α,β-DAG | α, β, α′ | 49.8 | 50.2 ± 0.4 |
| | α FA[c] | α, α′ | 99.3 | 0.7 ± 0.4 |
| $C_2H_5MgBr$ | β-MAG | β | 5.6 | 94.4 ± 3.5 |
| | α,β-DAG | α, β, α′ | 49.4 | 50.6 ± 0.1 |
| | α,α′-DAG | α, α′ | 95.5 | 4.5 ± 1.2 |
| | α-MAG | α, α′ | 98.2 | 1.8 ± 0.4 |
| | 4 × α,β-DAG − 3 × TAG | β | −2.5 | 102.5 ± 0.5 |
| | 3 × TAG − 2 × α-MAG | β | 3.7 | 96.3 ± 0.9 |
| | Direct β-MAG | β | 5.6 | 94.4 ± 3.5 |
| | 3 × TAG − 2 × α,β-DAG | α, α′ | 101.3 | −1.3 ± 0.2 |
| | (3 × TAG − β-MAG)/2 | α, α′ | 97.3 | 2.7 ± 1.8 |
| | Direct α-MAG | α, α′ | 98.2 | 1.8 ± 0.4 |

[a]Values are means ± SD of two GC analyses of products from four hydrolysis experiments.
[b]Areas obtained with the integrator were divided by chain length to yield the mole percentage of FA.
[c]Calculated from the equation 2 × α-MAG = 3 × TAG − β-MAG.

Although the FA profile of the α,β-DAG was closer to the FA distribution of the original TAG, the estimates based on the α,β-DAG composition gave negative values. This reflects the larger factors of the equation 4 × α,β-DAG − 3 × TAG than the equations used with α-MAG. The positional analysis obtained by calculation from the α,β-DAG was therefore subject to cumulative experimental errors of GC analysis.

*Regiodistribution analysis of borage oil.* The method was then applied to determine the regiospecific distribution of borage oil. First, pancreatic lipase hydrolysis was used to determine the composition at the β-position through the β-MAG. To verify the validity of the lipase hydrolysis, the TAG composition was recalculated from the composition of α,β-DAG and β-MAG through the equation 3 × TAG = 4 × α,β-DAG − β-MAG (Table 2). Results showed excellent agreement with the data for the composition of TAG (multiple correlation coefficient $R = 0.993$, with a confidence interval of 95%), confirming that the enzymatic deacylation generated a random mixture of representative acylglycerols. Table 2 gives the analysis of partial acylglycerols from the Grignard deacylation compared with the results obtained with pancreatic lipase. Nonreproducible results were obtained for the β-MAG analysis; the FA composition disagreed within 3% (absolute) for the minor component (palmitic acid). The composition in the β-position could then be estimated from the composition of α-MAG and TAG. The estimates compared favorably to those found using the pancreatic lipase procedure, as they came closer to the theoretical value and had smaller SD (Table 3). The composition of the FA at the α-position could be calculated from data for α,β-DAG and TAG or from TAG minus β-MAG. However, a direct analysis of α-MAG was preferable, since the results obtained were more accurate and showed smaller SD than those obtained by calculations from the α,β-DAG (Table 4).

*Regiodistribution analysis of tuna oil and milkfat.* The results obtained by the modified regiodistribution analysis of milkfat and tuna oil, respectively, are presented in Tables 5 and 6. These confirm previously published work on the FA regiodistribution of these substrates for the short- or medium-chain FA ($C_{10}$ or less) (17,18) or for very long chain PUFA (19,20).

Regiodistribution analysis on the basis of the classical procedures involving α,β-DAG or β-MAG is often complicated for fats and oils with a widely diverse FA spectrum, such as milkfat or fish oils. The superposition of elution bands in the TLC technique is the main problem, so this analytical limitation was eliminated in our approach of using boric acid coated

**TABLE 2**
**Regiospecific Analysis of Borage Oil Obtained by Hydrolysis with Pancreatic Lipase and Deacylation with Ethyl Magnesium Bromide**

| | | | FA composition[a] (mol%) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Compound | Position | 16:0 | 18:0 | 18:1 | 18:2 | 18:3 | 20:1 | 22:1 |
| | TAG | α, β, α′ | 11.7 (0.2) | 4.0 (0.1) | 18.7 (0.1) | 38.2 (0.1) | 21.5 (0.1) | 3.7 (0.1) | 2.1 (0.1) |
| Pancreatic lipase | β-MAG | β | 1.3 (0.1) | 0.4 (0.1) | 18.2 (0.3) | 42.6 (0.5) | 37.0 (0.3) | 0.4 (0.1) | 0.0 (0.0) |
| | α,β-DAG | α, β | 10.4 (0.2) | 3.2 (0.1) | 17.1 (0.1) | 38.7 (0.1) | 27.0 (0.5) | 2.4 (0.1) | 1.2 (0.2) |
| | TAG[b] | α, β, α′ | 13.3 (0.4) | 4.1 (0.1) | 16.7 (0.3) | 37.5 (0.7) | 23.7 (0.7) | 3.1 (0.1) | 1.6 (0.3) |
| $C_2H_5MgBr$ | α,β-DAG | α, β, α′ | 9.2 (0.2) | 3.1 (0.1) | 18.1 (0.2) | 39.4 (0.2) | 26.0 (0.3) | 2.7 (0.1) | 1.4 (0.1) |
| | β-MAG | β | 4.0 (3.0) | 2.1 (2.1) | 18.0 (1.5) | 39.8 (2.3) | 35.5 (1.9) | 0.4 (0.3) | 0.1 (0.2) |
| | α-MAG | α, α′ | 16.9 (0.9) | 6.0 (0.1) | 19.5 (0.2) | 36.9 (0.5) | 12.7 (0.3) | 5.2 (0.2) | 2.8 (0.3) |
| | TAG[c] | α, β, α′ | 12.6 (1.5) | 4.7 (0.9) | 19 (1.0) | 37.9 (1.6) | 20.3 (1.1) | 3.6 (0.8) | 1.9 (0.3) |

[a]Values are means ± SD of two GC analyses of products from four hydrolysis experiments.
[b]Recalculated from the equation 3 × TAG = 4 × α,β-DAG − β-MAG.
[c]Recalculated from the equation 3 × TAG = β-MAG + 2 × α-MAG.

**TABLE 3**
**Results of Regiospecific Analysis of Borage Oil According to the Pancreatic Lipase Hydrolysis and Ethyl Magnesium Bromide Deacylation: β-Position**

| | FA composition[a] (mol%) | | | | | | |
|---|---|---|---|---|---|---|---|
| Compound | 16:0 | 18:0 | 18:1 | 18:2 | 18:3 | 20:1 | 22:1 |
| β-MAG[b] | 1.3 (0.1) | 0.4 (0.1) | 18.2 (0.3) | 42.6 (0.5) | 37.0 (0.3) | 0.4 (0.1) | 0.0 (0.0) |
| 4 × α,β-DAG − 3 × TAG | 1.7 (1.0) | 0.2 (0.2) | 16.3 (0.8) | 42.8 (0.8) | 39.7 (1.3) | −0.2 (0.1) | −0.5 (0.2) |
| β-MAG[c] | 4.0 (3.0) | 2.1 (2.1) | 18.0 (1.5) | 39.8 (2.3) | 35.5 (1.9) | 0.4 (0.3) | 0.1 (0.2) |
| 3 × TAG − 2 × α-MAG | 1.3 (1.7) | 0.1 (0.2) | 17.2 (0.4) | 41.1 (1.1) | 39.0 (0.6) | 0.7 (0.5) | 0.7 (0.6) |

[a]Values are means ± SD of two GC analyses of products from four hydrolysis experiments.
[b]From Table 2, Pancreatic lipase.
[c]From Table 2, $C_2H_5MgBr$.

**TABLE 4**
**Results of Regiospecific Analysis of Borage Oil According to the Pancreatic Lipase Hydrolysis and Ethyl Magnesium Bromide Deacylation: α-Position**

| | FA composition[a] (mol%) | | | | | | |
|---|---|---|---|---|---|---|---|
| Compound | 16:0 | 18:0 | 18:1 | 18:2 | 18:3 | 20:1 | 22:1 |
| (3 × TAG − β-MAG)/2[b] | 16.9 (0.1) | 5.8 (0.1) | 18.9 (0.2) | 36.2 (0.2) | 13.8 (0.2) | 5.3 (0.0) | 3.1 (0.0) |
| 3×TAG − 2 × α,β-DAG | 16.7 (0.5) | 5.9 (0.1) | 19.9 (0.4) | 36.0 (0.4) | 12.4 (0.7) | 5.6 (0.1) | 3.4 (0.1) |
| α-MAG[c] | 16.9 (0.9) | 6.0 (0.1) | 19.5 (0.2) | 36.9 (0.5) | 12.7 (0.3) | 5.2 (0.2) | 2.8 (0.3) |
| (3 × TAG − β-MAG)/2 | 15.6 (1.5) | 4.9 (1.0) | 19.0 (0.7) | 37.5 (1.2) | 14.5 (0.9) | 5.3 (0.2) | 3.1 (0.1) |

[a]Values are means ± SD of two GC analyses of products from four hydrolysis experiments.
[b]From Table 2, Pancreatic lipase.
[c]From Table 2, $C_2H_5MgBr$.

**TABLE 5**
**Experimental[a] and Literature[b] Values for Principal FA Distribution Between α- and β-Positions of Milkfat**

| | FA composition (mol%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Position | 4:0 | 6:0 | 8:0 | 10:0 | 12:0 | 14:0 | 16:0 | 18:0 | 18:1 | 18:2 |
| α-Position | 11.4 (10.2–17.5) | 6.1 (5.5–7.4) | 2.2 (2.0–3.3) | 3.3 (3–4) | 3.6 (3–3.7) | 9.7 (8–10.2) | 27.1 (19.5–28) | 11.5 (5.5–11.9) | 15.1 (14.6–26.5) | 1.2 (0.4–2.0) |
| β-Position | —[c] (—) | 0.4 (0.1–1) | 1.9 (1–2.9) | 6.9 (3–7.8) | 7.6 (6–8.1) | 20.3 (18–23) | 42.9 (32–36.7) | 6.2 (3–10) | 9.6 (7.2–19) | 1.1 (0.6–4.0) |

[a]Values are means ± SD of five replicate analyses.
[b]Literature data are in parentheses.
[c]Concentrations lower than 0.1% are not reported.

**TABLE 6**
**Experimental[a] and Literature[b] Values for FA Distribution Between α- and β-Positions of Tuna Oil**

| FA | FA composition (mol%) | |
|---|---|---|
| | β-Position | α-Position |
| 14:0 | 6.4 ± 0.7 (3.5–7.6) | 4.3 ± 0.6 (3.0–4.7) |
| 15:0 | 0.9 ± 0.4 (0.7–1.5) | 1.9 ± 0.5 (0.9–1.5) |
| 16:0 | 18.5 ± 1.8 (8.9–16.7) | 18.5 ± 1.8 (13.5–22.3) |
| 16:1n-7 | 5.7 ± 0.9 (5.5–7.1) | 5.7 ± 0.7 (5.5–7.8) |
| 16:2 | 2.8 ± 0.2 (2.7–3.1) | 1.3 ± 0.2 (1.4–1.6) |
| 17:0 | 0.3 ± 0.0 (0.2–0.5) | 1.2 ± 0.0 (0.7–1.4) |
| 18:0 | 0.1 ± 0.0 (0.4–1.3) | 7.3 ± 0.1 (2.3–7.5) |
| 18:1n-9 | 5.9 ± 0.4 (6.1–7.8) | 20.2 ± 0.4 (16.7–24.9) |
| 18:1n-7 | 1.0 ± 0.1 (1.1–2.1) | 3.2 ± 0.5 (3–3.5) |
| 18:2n-6 | 1.4 ± 0.2 (1.1–2.5) | 1.6 ± 0.1 (1.7–2.5) |
| 18:3n-6 | —[c] (—) | 0.5 ± 0.0 (—) |
| 18:3n-3 | 0.5 ± 0.0 (0.6–0.7) | 0.6 ± 0.1 (0.6–0.8) |
| 18:4n-3 | 0.9 ± 0.1 (1.4–1.7) | 0.6 ± 0.1 (0.7–0.9) |
| 20:1n-11, n-13 | 0.3 ± 0.0 (0.2–0.4) | 0.2 ± 0.1 (0.1–0.5) |
| 20:1n-9 | 0.5 ± 0.0 (0.4–0.5) | 2.1 ± 0.2 (1.1–1.3) |
| 20:2n-6 | 0.1 ± 0.0 (0.1–0.3) | 0.5 ± 0.1 (0.3–0.4) |
| 20:3n-6 | 0.2 ± 0.1 (0.1–0.2) | 0.1 ± 0.1 (0.0–0.1) |
| 20:4n-6 | 2.4 ± 0.2 (2.3–2.6) | 1.6 ± 0.2 (1.5–2) |
| 20:5n-3 | 8.9 ± 0.9 (6.7–8.1) | 6.3 ± 0.5 (6.0–8.2) |
| 22:0 | — (—) | 0.4 ± 0.0 (—) |
| 22:1n-11, n-13 | 0.1 ± 0.0 (0.1–0.3) | 0.6 ± 0.1 (0.5–0.7) |
| 22:4n-6 | 0.3 ± 0.0 (0.2–0.3) | 0.6 ± 0.2 (0.2–0.4) |
| 22:5n-6 | 2.0 ± 0.3 (1.9–2.3) | 1.3 ± 0.3 (1.3–1.5) |
| 22:5n-3 | 1.4 ± 0.3 (1.4–1.8) | 1.5 ± 0.1 (0.9–1.4) |
| 22:6n-3 | 38.4 ± 2.5 (32.7–46.3) | 13.5 ± 1.9 (12.4–24.9) |
| Others | 1.0 | 4.4 |

[a]Values are means ± SD of five replicate analyses.
[b]Literature data are in parentheses.
[c]Concentrations lower than 0.1% are not reported.

silica plates in isolating the α-MAG fraction. The strong affinity of the hydroxyl groups in the α-MAG fraction—independent of their compositions—resulted in the lowest $R_f$ values, which allowed an easy separation and contamination-free recovery of the α-MAG.

## DISCUSSION

The present method gives an accurate analysis of the α-position in the original TAG. The α-MAG are present in sufficient quantities and a random FA distribution is obtained. This point deserves particular attention. As early as 1966, Yurkowski and Brockerhoff (9) reported that the MAG (α- and β-MAG) from the Grignard deacylation reaction could not be used for analytical purposes, since their FA compositions were not representative of the original TAG structure. Extensive acyl migration took place during the deacylation procedure. In 1993, Becker *et al.* (2) discussed the positive effect of an excess amount of allyl magnesium bromide reagent to produce representative β-MAG.

Based on these observations, we modified deacylation conditions from the usual procedure by using a higher ratio of ethyl magnesium bromide to TAG for a shorter reaction time. Under these conditions, more acyl shifting was observed from the α- to β-position (5.6% contamination of β-MAG) than from the β- to α-position (1.8% contamination of α-MAG). These results are opposite those usually observed for this type of analysis (2,9,11) and for chemical equilibrium between the α- and β-positions (21). However, as reported in the literature (4,22), deacylation is not random with regard to FA position on the glycerol molecule. Ethyl magnesium bromide exhibited a higher selectivity toward the α-position than did allyl magnesium bromide, and the Grignard reagents generated opposite results regarding MAG production. Thus, the proportion of α-MAG (30–35%) produced by ethyl magnesium bromide may have been overemphasized with a higher ratio of reagent and a shorter reaction time, leading to the formation of minute amounts of β-MAG (less than 10%).

The present procedure allows oils and fats that are not amenable to analysis with pancreatic lipase to be directly accessible to a regiodistribution analysis, without resorting to calculations. This method is particularly useful with TAG containing short-chain FA ($C_{10}$ or less) or very long chain PUFA, as no significant selectivity was observed with respect to chain length or unsaturation of FA in the partial deacylation of TAG (2,22).

## REFERENCES


1. Takagi, T., and Ando, Y. (1991) Stereospecific Analysis of Triacyl-*sn*-glycerols by Chiral High-Performance Liquid Chromatography, *Lipids 26,* 542–547.
2. Becker, C.C., Rosenquist, A., and Holmer, G. (1993) Regiospecific Analysis of Triacylglycerols Using Allyl Magnesium Bromide, *Lipids 28,* 147–149.
3. Ando, Y., and Takagi, T. (1999) Micro Method for Stereospecific Analysis of Triacyl-*sn*-glycerols by Chiral-Phase High-Performance Liquid Chromatography, *J. Am. Oil Chem. Soc. 70,* 1047–1049.
4. Angers, P., and Arul, J. (1999) A Simple Method for Regiospecific Analysis of Triacylglycerols by Gas Chromatography, *J. Am. Oil Chem. Soc. 76,* 481–484.
5. Entressangles, B., Pasero, L., Savary, P., Sarda, L., and Desnuelle, P. (1961) Influence de la nature des chaînes sur la vitesse de leur hydrolyse par la lipase pancréatique, *Bull. Soc. Chim. Biol. 43,* 581–585.
6. Lawson, L.D., and Hughes, B.G. (1988) Triacylglycerol Structure of Plant and Fungal Oils Containing γ-Linolenic Acid, *Lipids 23,* 313–317.
7. Brockerhoff, H. (1965) Stereospecific Analysis of Triglycerides: An Analysis of Human Depot Fat, *Arch. Biochem. Biophys. 110,* 586–590.
8. Bottino, N.R., Vandenburg, G.A., and Reiser, R. (1967) Resistance of Certain Long-Chain Polyunsaturated Fatty Acids of Marine Oils to Pancreatic Lipase Hydrolysis, *Lipids 2,* 489–493.
9. Yurkowski, M., and Brockerhoff, H. (1966) Fatty Acid Distribution of Triglycerides Determined by Deacylation with Methyl Magnesium Bromide, *Biochim. Biophys. Acta 125,* 55–59.
10. Brockerhoff, H. (1967) Stereospecific Analysis of Triglycerides: An Alternative Method, *J. Lipid Res. 8,* 167–169.
11. Christie, W.W., and Moore, J.H. (1969) A Semimicro Method for the Stereospecific Analysis of Triglycerides, *Biochim. Biophys. Acta 176,* 445–452.
12. Christie, W.W. (1982) *Lipid Analysis*, pp. 155–161, Pergamon Press, Oxford.
13. Brockerhoff, H. (1971) Stereospecific Analysis of Triglycerides, *Lipids 6,* 942–956.
14. Luddy, F.E., Barfield, R.A., Herb, S.F., Magidman, P., and

Riemenschneider, R.W. (1963) Pancreatic Lipase Hydrolysis of Triglycerides by a Semimicro Technique, *J. Am. Oil Chem. Soc. 41,* 693–696.
15. Thomas, A.E., III, Scharoun, J.E., and Ralston, H. (1965) Quantitative Estimation of Isomeric Monoglycerides by Thin-Layer Chromatography, *J. Am. Oil Chem. Soc. 42,* 789–792.
16. Morrison, W.R., and Smith, L.M. (1964) Preparation of Fatty Acid Methyl Esters and Dimethylacetals from Lipids with Boron Fluoride–Methanol, *J. Lipid Res. 5,* 600–608.
17. Christie, W.W., and Claperton, J.L. (1982) Structures of the Triglycerides of Cow's Milk, Fortified Milks (including infant formulae), and Human Milk, *J. Soc. Dairy Technol. 35,* 22–24.
18. Ozenne, C. (1993) Biofaçonnement de la matière grasse laitière, Ph.D. Thesis, University of Montpellier II, France, pp. 119–132.
19. Ando, Y., Ota, T., and Yazawa, K. (1996) Stereospecific Analysis of Triacyl-*sn*-glycerols in Docosahexaenoic Acid-rich Fish Oils, *J. Am. Oil Chem. Soc. 73,* 483–487.
20. Ando, Y., Satake, M., and Takahashi, Y. (2000) Reinvestigation of Positional Distribution of Fatty Acids in Docosahexaenoic Acid-rich Fish Oil Triacyl-*sn*-glycerols, *Lipids 35,* 579–582.
21. Larsson, K., and Quinn, P.J. (1994) Physical Properties: Structural and Physical Characteristics, in *The Lipid Handbook*, 2nd edn., pp. 443–446, Chapman and Hall, London.
22. Angers, P., and Arul, J. (2000) Determination of Selectivity of Grignard Reagents in Partial Deacylation Reactions of Triacylglycerols by Gas Chromatography, *inform 11,* S58.

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