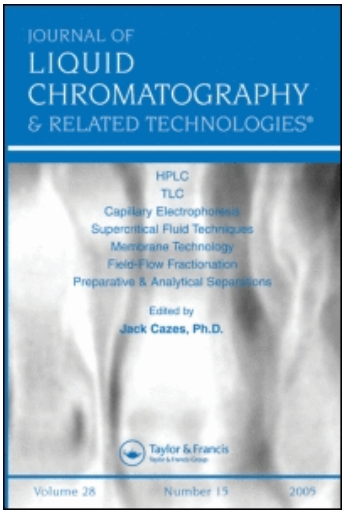

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SEPARATION OF MONOACYLGLYCEROL ENANTIOMERS AS URETHANE DERIVATIVES BY CHIRAL-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

High Performance Liquid Chromatographic (HPLC) separation of monoacylglycerol enantiomers, as [di-]3,5-dinitrophenyl isocyanate (urethane) derivatives, was carried out on a chiral column containing N-(R)-1-(α–naphthyl)ethylaminocarbonyl-(S)-valine chemically bound to γ-aminopropyl silanized silica as stationary phase.In addition to the separation of commercial rac-, sn-2- and sn-3-monoacylglycerols with saturated and unsaturated acyl groups, the analysis of natural source mixtures of sn-1(3)-monoacylglycerols obtained by partial chemical and enzymatic deacylation, was reported. These natural source monoacylglycerols, originated from peanut and cottonseed oil triacylglycerols isolated by combination of argentation-TLC and reversed-phaseHPLC. They were first fractionated by HPLC according to chain length, unsaturation and partially according to positional isomerism (sn-2- separated from sn-1(3)-monoacylglycerol) and then derivatized. An isocratic elution at ambient temperature with mixtures of hexane- ethylene dichloride- ethanol was used for the separation of the monoacylglycerol-isomers, which were detected by their refractive indices or their UV absorption. The sn-1- and sn-3-isomers of a racemic mixture were very well separated, so as the sn-2- and sn-3-isomers. On the other hand, the separation between the sn-2- and sn-3-isomers was generally incomplete. The observed elution order of the three

isomers was sn-1- followed by sn-2- and finally sn-3-monoacylglycerol. In complex mixtures, the separation of the isomers differing by two acyl carbon number or one double bond was very poor. The elution order indicates that for each isomer the retention time increases with decreasing chain length and increasing unsaturation of the constituent fatty acid. The relationships between the logarithmic retention volumes against the partition number in each homologous series of isomers were practically linear. The enantiomer composition of racemic mixtures, calculated from peak areas was very close to the composition expected, indicating that chiral-phase HPLC analysis of monoacylglycerol enantiomers could be used to study the stereospecific distribution of fatty acids in natural oil triacylglycerols.

INTRODUCTION

Theoretically the best method for studying the stereospecific distribution of fatty acids in the triacylglycerol molecules would be to determine the fatty acid profiles of the three sn-1, sn-2 and sn-3 monoacylglycerol stereoisomers unspecifically formed by an appropriate deacylation procedure.

Natural triacylglycerols generally generate mixtures of monoacylglycerol isomers too complex to be analyzed in one step. Analysis by reversed-phase HPLC allows separations according to chain length, unsaturation and partially to position isomerism since sn-2-isomers can be separated from the sn-1 and sn-3 groups together eluting [1-3]. The different monoacylglycerol isomers are sufficiently well separated to be collected with minor contamination.

The group of sn-1(3)-isomers need further analysis for the two enantiomers to be separated. Ôi et al. [4-6] have developed different chiral stationary phases for high-performance liquid chromatography (HPLC) which show characteristic enantioselectivities for enantiomer derivatives of amino-acids, carboxylic acids and alcohols. Two of these phases present as chiral selectors, N-(S)-2-(4-chlorophenyl)isovaleroyl D-phenyl glycine and N-(R)-1-(α–naphthyl)ethylaminocarbonyl-(S)-Valine respectively. Both have been used by Takagi and coworkers [7-10] to separate monoalkyl- and monoacylglycerol enantiomers previously derivatized with 3,5-dinitrophenyl isocyanate (urethane derivatives)

Regarding results obtained by these authors, we have worked out separations of monoacylglycerol optical isomers as urethane derivatives by chiral phase HPLC. The

method was more particularly applied to mixtures obtained after partial chemical deacylation of natural triacylglycerols. Results show that urethane derivatives of monoacylglycerol enantiomers can be accurately analyzed by this method, both qualitatively and quantitatively.

EXPERIMENTAL

Samples

Monoacylglycerols were from commercial and natural sources :

i) Optically active sn-3-monopalmitoylglycerol (sn-3-16:0) was from Fluka AG (Buchs, Switzerland). Rac-1-monopalmitoylglycerol (rac-1-16:0), rac-1-monostearoylglycerol (rac-1-18:0), rac-1-monooleoylglycerol (rac-1-18:1), rac-1-monolinoleoylglycerol (rac-1-18:2) were purchased from Serdary (London, Ontario, Canada), as were optically inactive sn-2-isomers, namely sn-2-monopalmitoylglycerol (sn-2-16:0) and sn-2-monooleoylglycerol (sn-2-18:1). These monoacylglycerols were used without prior purification.

ii) Natural source monoacylglycerol samples were prepared by partial chemical deacylation [11, 12] and enzymatic deacylation [13] of oil triacylglycerols. Those were isolated from peanut oil and cottonseed oil by a combination of argentation-TLC and reversed-phase HPLC [14-16]. They comprised palmitoyldioleoylglycerol (16:0 18:1 18:1), trioleoylglycerol (18:1 18:1 18:1), palmitoyloleoyllinoleoylglycerol (16:0 18:1 18:2), dioleoyllinoleoylglycerol (18:1 18:1 18:2), oleoyldilinoleoylglycerol (18:1 18:2 18:2) isolated from peanut oil. Palmitoyloleoyllinoleoylglycerol was also isolated from cottonseed oil for comparison with peanut oil and with results previously obtained [17]. Monoacylglycerols were separated from the other hydrolysis products by TLC on silicagel G (Merck, Darmstadt, Germany) impregnated with 5 % (w/w) boric acid [11, 18]. The plates were developed with petroleum ether / diethyl ether (50 / 50 ,v/v). The monoacylglycerol mixtures were fractionated by reversed-phase HPLC according to chain length, to unsaturation and partially according to positional isomerism, i.e. the sn-2 isomers were separated from the group of sn-1(3)-isomers, as previously described [3]. The sn-1(3)-monoacylglycerols were collected at the outlet of the detector and derivatized.

Preparation of urethane derivatives

Monoacylglycerol 3,5-dinitrophenyl urethane derivatives were prepared according to the procedure described by Ôi and Kitahara for derivatization of chiral alcohols [19] and adapted to monoacylglycerols by Takagi and Itabashi [7, 9, 10].

Twenty μmol (ca 7 mg) of monoacylglycerols were dissolved in 450 μl of dry toluene in a 0.5 ml glass vial with teflon linked screw cap. To this solution were added 45 μl (ca 10 mg) of 3,5-dinitrophenyl isocyanate powder (Sumitomo, Osaka, Japan) and 45 μl of dry pyridine. The mixture was heated at 70 °C for 1 h in an oven (or left for 3 h without heating) with occasional shaking. At the end of the reaction, the solution was cooled and the solvent was removed under nitrogen. The resulting urethane derivatives were dissolved in 0.2 ml of chloroform and purified by TLC on silicagel 60 F 254 precoated plates (20 cm x 20 cm, 0.25 mm thick) from Merck. The plates containing a fluorescence indicator were previously activated for 1 h at 110 - 120 °C in an oven. They were developed using a mixture of hexane / ethylene dichloride (or dichloromethane) / ethanol (40/10/3, v/v/v). The plates were dried under nitrogen. The monoacylglycerol derivatives were revealed under UV light (254 nm), delineated and the corresponding silicagel was scraped off the plate. The urethane derivatives were extracted from the adsorbent with diethyl ether.

Alternatively the crude urethane derivatives of monoacylglycerols were purified by reversed-phase HPLC instead of TLC. In this case, at the end of the derivatization reaction, the mixture of urethane derivatives, remaining reagent, toluene and pyridine was left to decant. The limpid upper phase was filtered through hyperfine glasswool. The solvent was evaporated under nitrogen and the monoacylglycerol derivatives were dissolved in chloroform for storage or in acetonitrile (or the mixture corresponding to the HPLC mobile phase) for fractionation.

Liquid chromatography

The sn-1- and sn-3-isomers were separated on a chiral column, 250 mm L x 4 mm I.D., packed with 5 μm particles of N-(R)-1-(α-naphthyl)ethylaminocarbonyl-(S)-valine chemically bound to γ-aminopropyl silanized silica, OA-4100 (Sumitomo, Osaka, Japan). It was installed on a liquid chromatograph Waters (Milford, MA, USA) comprising a model 6000 A solvent delivery system and a Model 450 variable wavelength UV detector. A Lichrocart 4-4 guard column filled with Lichrosorb Si 60 (Merck) was attached at the column inlet.

The analyses were carried out isocratically using hexane/ ethylene dichloride (or dichloromethane) / ethanol (40/12/3 ,v/v/v) at a constant flow rate of 1.0 ml min^{-1} and at ambient temperature. Peak areas were measured by means of an Enica 21 calculator-integrator (Delsi, Suresnes, France).

Definitions

Separation of monoacylglycerol derivatives were characterized by several parameters :

i) The retention time (min) and the retention volume (ml) corrected from the column void volume.

ii) The separation factor [20] between two peaks expressed as the ratio of their retention times (or volumes).

iii) The resolution factor [20] calculated from the formula :

$$R_s = 2\ (t_{R2} - t_{R1}) / (w_2 + w_1)$$

in which t_R is the retention time of two peaks 1 and 2 (eluting in that order) and w the peak width at baseline.

Monoacylglycerol molecules were characterized by their equivalent carbon number (ECN) calculated from the formula :

$$ECN = CN - 2\ DB\ [21]$$

where CN is the acyl carbon number and DB the acyl double-bond number.

RESULTS AND DISCUSSION

Derivatives

As previously stated [7, 9, 10], the monoacylglycerols react very rapidly to completion with the derivatization 3,5-dinitrophenyl isocyanate reagent provided they were completely dissolved in the solvent (dry toluene) in the presence of pyridine. Moreover this reaction does not induce isomerization. However the urethane derivatives of unsaturated monoacylglycerol are more liable to be degraded than the underivatized molecules, so that they should be analyzed shortly after they have been synthetized.

Separation

Figure 1 shows the chromatograms registered in the analysis of rac-monopalmitoylglycerol (rac-16:0 or sn-1(3)-16:0) (panel A), of the single enantiomer sn-3-monopalmitoylglycerol (sn-3-16:0) (panel B) and of the sn-2-monopalmitoylglycerol (sn-2-16:0) (panel C). The chromatograms show separation of sharp and symmetrical peaks within moderate retention times (less than 20 min). The chromatogram in panel A shows that the rac-16:0, which was eluted as a single peak by reversed-phase HPLC [3] was now separated into two well-separated peaks in the presence of the chiral phase OA-4100. The retention time of sn-3-16:0 (panel B) corresponded to that of the second peak, indicating that the elution order of the two enantiomers was sn-1 and sn-3. The separation of the two antipodes brought about by this chiral phase was very high since the observed separation and resolution factors were 1.35 and 4.31 respectively. It was much better than the separation obtained by Itabashi and Takagi [7-9] in the presence of another chiral phase OA-2100, since the separation and resolution factors were only 1.19 and 2.58 respectively. However these authors recently achieved the same good separation of sn-1 and sn-3 monoacylglycerols [10] as we did on the same chiral phase. This proves that the chiral selector enantioselectivity of N-(R)-1-(α-naphthyl)ethylaminocarbonyl-(S)-valine (OA-4100 from Sumitomo) towards monoacylglycerols is higher than of N-(S)-2-(4-chlorophenyl)isovaleroyl-D-phenylglycine (OA-2100 from Sumitomo).

Analysis of the sn-2-monopalmitoylglycerol (Panel C) showed that this isomer was eluted just after the sn-1-isomer on the chiral phase. The presence of traces of the sn-1- and sn-3-isomers in this sample confirms that the elution sequence of sn-1, sn-2, sn-3 was in that order. It also shows that separation of the sn-1- and sn-2-monopalmitoylglycerols was incomplete. This is more evident in Fig. 2 illustrating the separation of a mixture of the 3 monopalmitoylglycerol isomers. In comparison the chiral-phase OA-2100 allowed a more clearcut separation of the 3 isomers [9].

The more or less pronounced retention of the two antipodes sn-1- and sn-3-monopalmitoylglycerols is governed by their diastereoisomeric interactions with the chiral phase. The interaction was higher with the sn-3-isomer on both phases but the difference was more pronounced with the OA-4100 chiral column. Retention of the sn-2-isomer seems more related to the lesser polarity of the molecule versus the polarity of the chiral phase silica support, as can be observed in TLC on silicagel [22]. This could suggest that between the two chiral phases, OA-4100 is less polar than OA-2100.

Resolution between the sn-1- and the sn-2-isomers could be improved by decreasing the analysis temperature or decreasing the mobile phase polarity (lesser proportion of

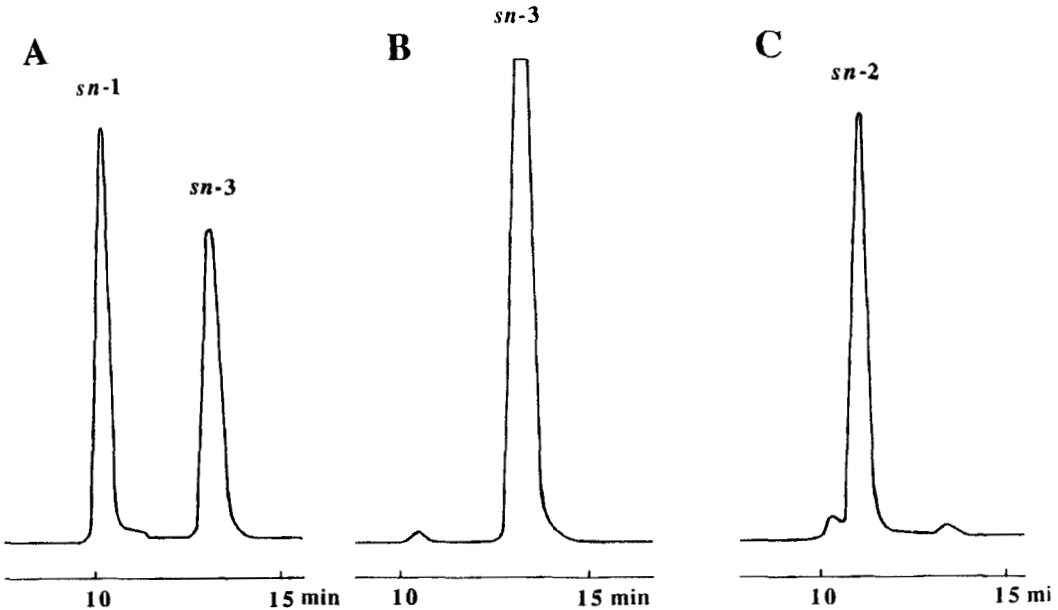

Figure 1. HPLC isocratic analysis of monopalmitoylglycerol isomers as [di-]3,5-dinitrophenyl isocyanate (dinitrophenyl urethane = DNPU) derivatives at ambient temperature and with UV absorption detection (254 nm), on stainless-steel (250 mm x 4 mm i.d.) chiral OA-4100 column. The column was packed with 5 µm particles of N-(R)-1-(α-naphthyl)ethylaminocarbonyl-(S)-valine chemically bonded to γ-aminopropyl silanized silica (Sumipax OA-4100); mobile phase was hexane- ethylene dichloride-ethanol (40:12:3, v/v/v) at 1 ml min^{-1}.
A = *rac*-16:0; B = *sn*-3-16:0; C = *sn*-2-16:0.

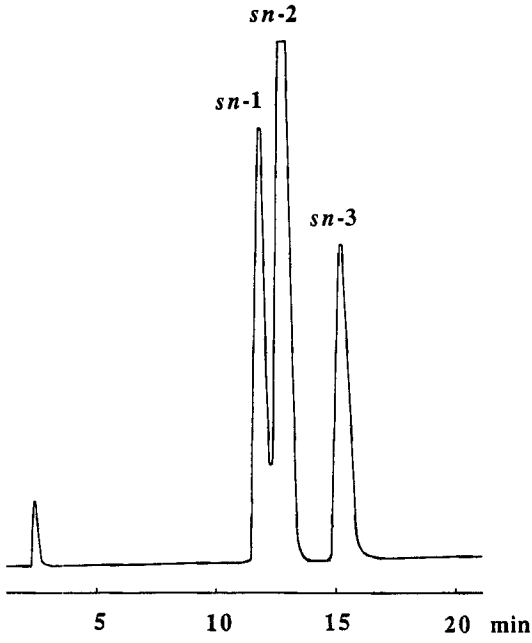

Figure 2. Chiral-phase HPLC separation at ambient temperature or a synthetic mixture of the 3 monopalmitoylglycerol isomers as [di-]3,5-DNPU derivatives on Sumipax OA-4100. Mobile phase was hexane- ethylene dichloride- ethanol (40:12:3, v/v/v) at a flow rate of 1 ml mn^{-1}.

ethanol or / and higher proportion of hexane), but it would be to the detriment of the retention times which would be increased.

In our work, the problem of the separation of the sn-1- and sn-2-isomers does not raise since the sn-2-isomers are well separated by reversed-phase HPLC from the sn-1(3)-isomers [3]. Only the latter group, collected at the HPLC column outlet, will be further analyzed by chiral-phase HPLC for compositional determination of the two enantiomers in the mixture. This is probably also the reason why Takagi and Ando [10] only reported enantiomer separation in their last publication, excluding the sn-2-isomers.

Table I reports the separation factors calculated from the chromatograms obtained in the separation of different mixtures of saturated (16:0 and 18:0) and unsaturated (18:1 and 18:2) rac-1- and sn-2-monoacylglycerols.. For each type of monoacylglycerol, the racemic mixture and the sn-2-isomer were first analyzed separately and then mixed. If

TABLE I.

SEPARATION OF MONOACYLGLYCEROL STEREOISOMERS AS URETHANE DERIVATIVES BY CHIRAL-PHASE HPLC

STEREO-ISOMERS[a]	Retention time (min)[b]	Separation factor[c]	Constituent fatty acid[d]	STEREO-ISOMERS	Separation factor[e]	STEREO-ISOMERS	Separation factor[f]
sn-1-18:0	9.25			*sn*-1		*sn*-1	
sn-1-16:0	9.75	1.05	18:0	*sn*-2	1.10	*sn*-3	1.35
sn-1-18:1	9.79	1.00		*sn*-3	1.22		
sn-2-18:0	10.20	1.04		*sn*-1		*sn*-1	
sn-1-18:2	10.36	1.02	16:0	*sn*-2	1.09	*sn*-3	1.35
sn-2-16:0	10.64	1.03		*sn*-3	1.24		
sn-2-18:1	10.71	1.00		*sn*-1		*sn*-1	
sn-2-18:2	11.37	1.06	18:1	*sn*-2	1.09	*sn*-3	1.36
sn-3-18:0	12.48	1.10		*sn*-3	1.24		
sn-3-16:0	13.18	1.06		*sn*-1		*sn*-1	
sn-3-18:1	13.30	1.01	18:2	*sn*-2	1.10	*sn*-3	1.35
sn-3-18:2	13.98	1.05		*sn*-3	1.23		

a) Listed according to elution order.
b) Retention times corrected from column void volume.
c) Ratio of the retention time of a peak to the retention time of the preceding one.
d) Constituent fatty acid of the monoacylglycerol isomers.
e) Separation factor between the two isomer couples (*sn*-2, *sn*-1) and (*sn*-3, *sn*-2).
f) Separation factor between the two *sn*-1 and *sn*-3 enantiomers.

separation of two peaks is considered to be achieved when the separation factor (3 rd column) is at least equal to 1.10 (retention times differing from 10 %) data in theTable show that only one pair of isomers would be completely separated, namely sn-2-18:2 and sn-3-18:0. The other isomers would be only partially separated except sn-1-18:2 and sn-2- 18:0 which eluted practically together, as did the monopalmitoyl- and monooleoylglycerol isomers. Figure 3 illustrates the partial separations observed between

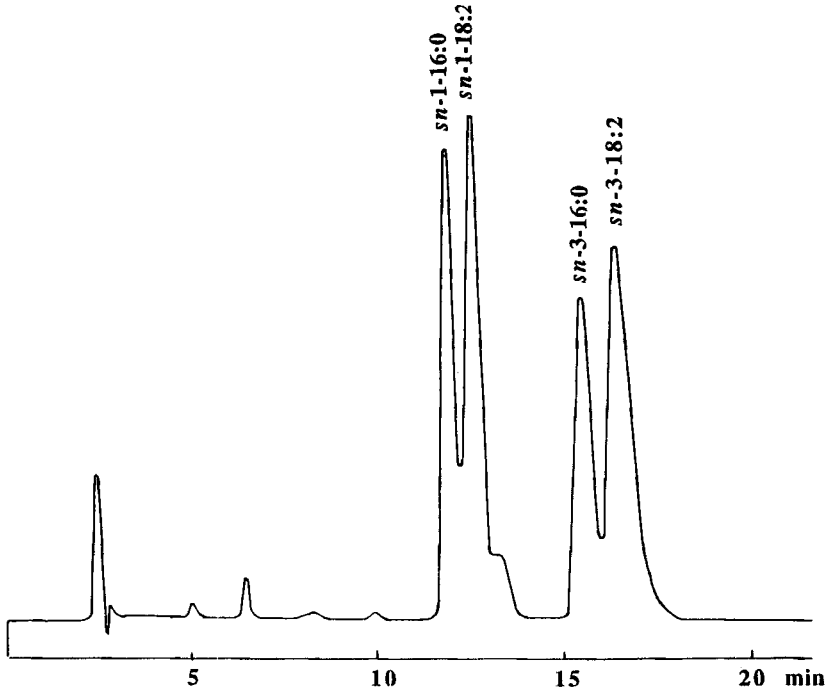

Figure 3. Carbon number separation of a mixture of monopalmitoyl (*sn*-1-16:0, *sn*-3-16:0) and monolinoleoylglycerol (*sn*-1-18:2, *sn*-3-18:2) enantiomers, as [di-]3,5-DNPU derivatives on Sumipax OA-4100. The minor satellite peak emerging after *sn*-1-18:2 represents *sn*-2-18:2. Mobile phase was hexane- ethylene dichloride- ethanol (40:12:3, v/v/v) at a flow rate of 1 ml min^{-1}.

the two sn-1 and sn-3 isomers series of monopalmitoyl-, and monolinoleoylglycerols. The chromatogram shows that monoacylglycerols were eluted into two distinct groups corresponding to the two isomers. In each group the elution order was the same and was according to increasing degree of unsaturation.

With simpler mixtures comprising the 3 isomers of only one monoacylglycerol, separation of the 3 isomers was generally complete, except for monopalmitoyl- and monooleoylglycerols as was also illustrated in Figure 2 for monopalmitoylglycerols. Between the two sn-1 and sn-3 antipodes of a same monoacylglycerol the separation was always observed very good (last column of Table I) and did not vary with the constituent fatty acid in the four studied monoacylglycerols.

In our work on stereospecific analysis of triacylglycerols, samples to be analyzed by chiral-phase HPLC only comprise the two sn-1- and sn-3-isomers of the same monoacylglycerol after fractionation by reversed-phase HPLC [3]. Their very complete resolution allow peak areas to be accurately determined, without any cross-contamination between peaks.

Separation between the sn-1 and sn-2-isomers neither varied with chain length nor unsaturation, remaining partially incomplete (separation factors of 1.09 to 1.10).Because of the high resolution of the two sn-1 and sn-3 enantiomers, the separation between the sn-2 and sn-3 isomers was very good (separation factors of 1.22 to 1.24).

The elution order of the 12 isomers reported in the 1st column of Table I indicates that for each isomer type the retention time (2nd column) increased with decreasing chain length and increasing unsaturation of the constituent fatty acid, that is with increasing polarity of the fatty acid chain. For the two studied saturated monoacylglycerols (16:0 and 18:0), the effect of chain length increase was higher for the sn-3-isomer (5,6 %) and lower for the sn-2-isomer (4,6 %). For the 3 fatty acids of the same chain length and of increasing number of double-bonds (18:0, 18:1, 18:2), retention time increased from 5.0 to 6.6 % by adding a double-bond to the saturated fatty acid and from 5.1 to 6.2 % by adding another double-bond.

From 18:0 to 18:1, the retention time increase was maximum for the sn-3-isomers (6.6 %) and minimum for the sn-2-isomers (5.0 %) but the reverse was true from 18:1 to 18:2 (5.1 and 6.2 % increase for the sn-3 and sn-2-isomers respectively). The change in the molecule polarity between a saturated and a monounsaturated fatty acid and between a monounsaturated and a diunsaturated fatty acid did not identically affect each type of isomer.

Figure 4 shows the relationship between log retention volume (V_R) and the equivalent carbon number (ECN) in each homologous series of isomers. For the four studied fatty acids the relationship was practically linear and the 3 straight lines traced from the 2 saturated fatty acids (16:0, 18:0) were practically parallel. However the retention volumes of the unsaturated monoacylglycerols were a little higher than predicted by the relationship, especially for the monolinoleoyglycerols (18:2) and the retention volume of the sn-3-isomers seemed to increase a little more rapidly with increasing ECN than those of the sn-1- and sn-2-isomers.

Between the sn-1, sn-2 and sn-3 isomers of a given monoacylglycerol the relationships can be approximately expressed by the following equations in the analytical conditions used :

$$\log V_R \text{ (sn-3)} = \log V_R \text{ (sn-1)} + 0.131$$
$$\log V_R \text{ (sn-2)} = \log V_R \text{ (sn-1)} + 0.040$$
$$\log V_R \text{ (sn-3)} = \log V_R \text{ (sn-2)} + 0.091.$$

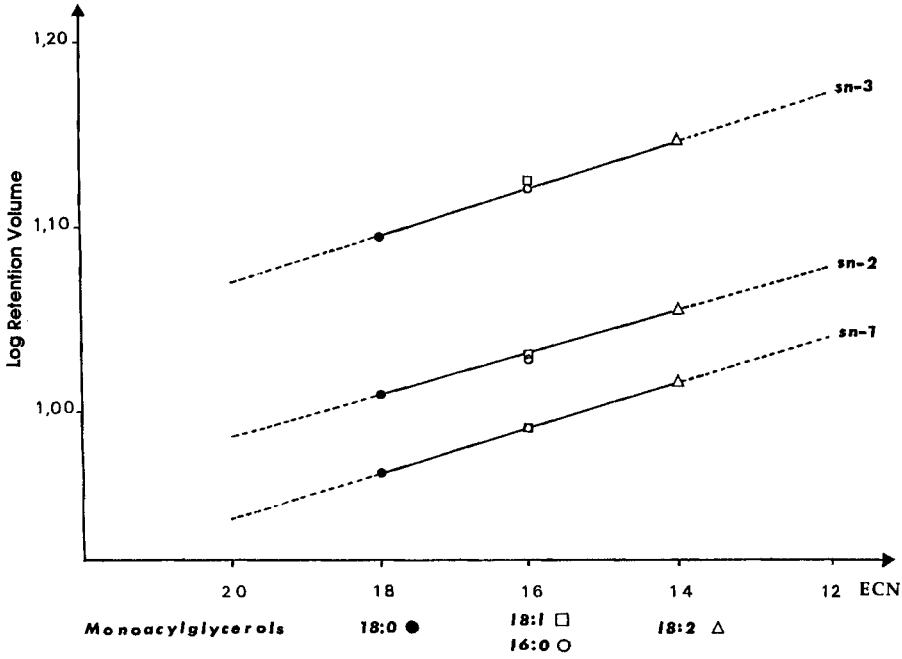

Figure 4. Plot of logarithm of retention volume versus partition number of monoacylglycerol enantiomers analyzed by chiral-phase HPLC as urethane derivatives. Retention volume (ml) = retention time (min) corrected from the column void volume x solvent flow-rate (ml min^{-1}). Equivalent Carbon Number (ECN) was calculated from the formula : ECN = CN - 2 DB, where CN is the total number of acyl carbon atoms and DB the total number of double bonds of the constituent fatty acid.

These 3 equations determine 3 parallel straight lines.

Such relationships are interesting to be established in an attempt to identify monoacylglycerol isomers by their retention volumes or conversely to predict the retention volumes of known isomers.

Data in Figure 4 and in Table I show that the retention volume of a sn-2-monoacylglycerol is practically identical to that of a sn-1 monoacylglycerol of the same chain length but with 2 double-bonds (for example sn-2-18:0 and sn-1-18:2) or of the same unsaturation but with 4 additional carbon atoms (for example sn-2-18:0 and sn-1-14:0). These two couples of isomers constitute two critical pairs whose constituents are

uneasily separable from each other. This would also be the case with the couples sn-2-16:0 or sn-2-18:1 and sn-1-12:0 or sn-1-18:3 or with the couple sn-2-20:0 and sn-1-16:0 (or sn-1-18:1).

Data in Figure 4 and Table I also indicate that the difference of retention volume between the sn-1 and sn-3-isomers of a given monoacylglycerol is so high (ca 3.5 ml) that for a wide range of monoacylglycerols, all the sn-1-isomers would always elute earlier than the sn-3-isomers. Table I shows that for 4 monoacylglycerols (16:0, 18:0, 18:1, 18:2) the retention times ranged from 9.25 to 10.36 min for the sn-1 isomers and from 12.48 to 13.98 min for the sn-3 isomers. This is also illustrated in Figure 3 for the 16:0 and 18:2 sn-1 and sn-3 isomers. Figure 4 shows that the sn-1-monoacylglycerol of ECN=10 (such as 10:0 or 18:4) would still elute earlier than the sn-3-18:0. Takagi and Ando [10] also observed this pecularity for the 18:0, 18:1 18:2 and 18:3 series.

Data reported in this work show that only sn-1 and sn-3-isomers differing from 10 units in ECN (10 carbon atoms or 5 double-bonds or any combination of the the two) would practically elute together. Except in very complex oils with a wide range of fatty acids of very different chain lengths (bovine milk fat) or of very different degrees of unsaturation (fish oils), the range of fatty acids encountered in most natural oils will be generally narrow enough to allow complete separation of the sn-1 and sn-3-isomers. However in each series of enantiomers the critical pairs, such as monopalmitoyl- and monooleoylglycerols (16:0 and 18:1) will be very incompletely separated so that prior fractionation by reversed-phase HPLC [3] is still needed.

From the overall results reported here, it follows that on the chiral phase, the separation of monoacylglycerols differing from 2 carbon atoms or 1 double-bond is low when compared to separation of enantiomers, but the retention times (or volumes) are very moderate. It is possible to highly improve separation as demonstrated by Takagi and Ando [10] by increasing the column length and decreasing the solvent flow rate, but the retention was considerably increased, since for the sn-3-18:0 the retention volume was ca 150 ml for a retention time of 5 hours.

The alternative we choose was to proceed to a prior fractionation of the monoacylglycerols by HPLC [3] in very moderate retention times, before enantiomer analysis on a chiral phase in conditions still allowing low retention times. The combined method additionally solves the problem of critical pairs resolution.

Detection

Since no commercial urethane derivatives of monoacylglycerol enantiomer were available, the quantitative aspect of chiral-phase HPLC analysis of the monoacylglycerol

TABLE II

ANALYSIS BY CHIRAL-PHASE HPLC OF RACEMIC MONOACYLGLYCEROL ENANTIOMERS AS URETHANE DERIVATIVES

Racemic monoacylglycerols	18:2		16:0		18:1		18:0	
Enantiomers	*sn*-1	*sn*-3	*sn*-1	*sn*-3	*sn*-1	*sn*-3	*sn*-1	*sn*-3
mol % (a)	49.6	50.4	50.2	49.8	49.9	50.1	50.4	49.6
	± 0.3		± 0.1		± 0.2		± 0.2	

Results are means of n = 3 analysis ± S.E.

(a) Peak area percentages.

stereoisomers was studied using commercial racemic monoacylglycerols known to contain an equal proportion of sn-1 and sn-3 isomers. After derivatization they were analyzed on the chiral column and detected by their absorption in UV light. Proportion (Percentile) of the two enantiomers in the mixtures, calculated from the registered peak areas, is reported in Table II. Data show that the values obtained were very close to those expected i.e. 50:50, since the maximum difference was lower than 1 %. They demonstrate that UV absorption was of the same magnitude for each enantiomer. They also show that no isomerization occured during the derivatization procedure. These two conditions were a prerequisite for accurate analysis of monoacylglycerol enantiomers.

These results confirm others [9] also obtained with rac. monoacylglycerols separated on another type of chiral column and detected in UV light.

CONCLUSION

We have shown in this work that monoacylglycerol enantiomers could be accurately analyzed by high performance liquid chromatography on a chiral phase both qualitatively and quantitatively, provided the mixture of monoacylglycerol isomers were previously fractionated into simple mixtures of sn-1(3)-isomers [3]. The HPLC methods of fractionation and analysis of the monoacylglycerol isomers will now be used to study the stereospecific distribution of fatty acids in natural triacylglycerols by means of the monoacylglycerols generated by enzymatical and chemical deacylation.

Results obtained will be compared to those determined by studying the diacylglycerols formed by partial chemical deacylation [15, 16] in order to check the reliability of the monoacylglycerol method which is simpler to use than the diacylglycerol method.

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