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Chromatographic Behavior of Glycerolipid with Respect to 1, 2-; 2, 1-and 1, 2-; 1, 3-Positional Isomers on Reverse Phase Mode

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This study was conducted to determine why 1, 2-; 2, 1-and 1, 2-; 1, 3-positional isomers of diacyl glycerolipids, including those that are depolarized, are separated on reverse phase HPLC while triglycerides are not.

Interaction forces among the carbon chains of lipid molelcules generated by the Coulomb force of atoms were concluded to be stereochemically well balanced in the case of triglycerides in contrast to diacyl glycerolipids. Possibly, the larger the acyl carbon number, the greater is the lack of conformity of interaction forces among acyl moieties. This is a factor determining the chemical potential of lipid molecules and gives rise to small but reproducible differences in retention on reverse phase HPLC between the 1, 2-and 2, 1-as well as 1, 2-and 1, 3-positional isomers.

1 Introduction

In accordance with the increase in resolving power of high performance liquid chromatography (HPLC), in the reverse phase mode, rules of the chromatographic behavior of triacylglycerol molecular species have been developed originally from partition number[1),2)], equivalent carbon number[3)], method of Goiffon *et al.*[4),5)], theoretical carbon number[6)], matrix model[7)] and relative retention potential theory[8)].

Principally, simple addition theorem of chemical potentials of functional groups (components) in the solute molecule should hold in chromatographic systems. This is called the law of Martin[9)], and can be expressed as,

$$\Delta\mu_B/R\cdot T=\Delta\mu_A/R\cdot T+\Delta\mu_X/R\cdot T \quad (1)$$

where A and B are members of a homologous series differing with the functional group X, μ is the chemical potential, R is the gas constant and T is the absolute temperature. Basically, it can be considered that this simple addition theorem of chemical potentials should hold in all kinds of the chromatographic systems, and therefore, the retention time can be predicted by utilizing this theory[7),8),10),11)]. However, a slight but reproducible deviation in retention time from the theoretically predicted value is often observed. This phenomenon can be attributed to the variation of the three dimensional arrangement of the functional groups *i. e.* stereochemical isomers. Therefore, strictly speaking, it is impossible in a perfect form, to formulate the whole chemical potential of the lipid molecule in a simple addition theorem as in equation (1).

This paper insights the precise chromatographic behavior of 1, 2-; 2, 1-and 1, 2-; 1, 3-positional isomers of glycerolipid on reverse phase HPLC in comparison to symmetric glycerolipid.

2 Experimental

Symmetric glycerolipid standards *e. g.* trioctanoyl-, tridecanoyl-, trilauroyl-, trimyristoyl-, tripalmitoyl-, and trioleoylglycerols were obtained from Sigma Chemical Company, and were applied to reverse phase HPLC under the following conditions: instrument, Hitachi 638-50 liquid chromatograph (Hitachi Co. Ltd., Tokyo, Japan); column, Chemcosorb I-5C18, 30 cm×4 mm (Chemco Ltd., Osaka, Japan); detector, Shodex SE-11 RI monitor (Showa Denko Ltd., Tokyo, Japan); solvent, acetone/acetonitrile (3 : 2, vol/vol); flow, 0.5 mL/min; column temp., room temperature (20~22°C). Peaks appeared on the HPLC chromatogram were collected manually, and each collected fraction (nitrogen gas dried) was esterified as follows by the method of Christopher & Glass modified by Prevot & Mordret[12)] for the fatty acid composition analysis.

Collected fractions were dissolved in 1 mL *n*-hexane, and 0.2 mL of methanolic 2N NaOH solution was added. After the mixture had been shaken, it was stood for 20 s under 50°C and then 0.2 mL of methanolic 2N HCl solution was added. The *n*-hexane layer was collected, then concentrated and subjected to gas liquid chromatographic analysis (GLC). The analytical conditions of GLC were as follows; instrument, Hitachi 063 gas chromatograph (Hitachi Co. Ltd., Tokyo, Japan); column, Unisole 3000 (Gasukuro Kogyo, Tokyo, Japan) 3 m×3 mm glass column; column temp., 205°C; injection temp., 270°C; detector, FID; carrier gas, N_2 40 mL/min. By infering from the result of the fatty acid analysis of each peak, the standard molecular species were confirmed on the HPLC chromatogram, and the relative retention time (RRT) of them were calculated. As for the nonsymmetric glycerolipid, RRT data of Kito *et al.*[13] and Takamura *et al.*[14] were introduced.

3 Results and Discussion

As previously reported[15),16)], a general expression for the chemical potential of the triacylglycerol molecule can be expressed as,

$$\Delta\mu_{TG}=g\{f(c_1,\ d_1,\ \omega_1),\ f(c_2,\ d_2,\ \omega_2),\ f(c_3,\ d_3,\ \omega_3)\} \quad (2)$$

where c and d are acyl carbon number and number of double bonds in each acyl group, respectively. ω is the position of double bonds, and "*g*" is the function of chemical potential given by the three dimensional arrangement of the acyl groups. If we express the chemical potential of each acyl group as $\Delta\mu_1$, $\Delta\mu_2$, $\Delta\mu_3$ for position 1, 2, 3, function (2) can be written as,

$$\Delta\mu_{TG}=g(\Delta\mu_1,\ \Delta\mu_2,\ \Delta\mu_3) \quad (3)$$

This function (3) can give the chemical potential generated by the three dimensional conformation of $\Delta\mu_1$, $\Delta\mu_2$, $\Delta\mu_3$, arranged on the glycerol moiety. Supposing that this three dimensional factor is negligible, function (3) can be rewritten as follows,

$$\Delta\mu_{TG}=\Delta\mu_1+\Delta\mu_2+\Delta\mu_3 \quad (4)$$

and simple addition theorem of chemical potential *i. e.* the law of Martin [see equation (1)] should hold.

Nevertheless, as seen in **Table-1**, 1,2-; 2,1- and 1,2-; 1,3-positional isomers had slight but obviously different RRT, and could be separated by high resolution HPLC. Therefore, formula (4) can be considered as an approximated formula of the gross chemical potential in one triacylglycerol molecule. We will now embody function (3), the "*g*" function, by reviewing the three dimensional molecular structure of glycerolipid. As for a representative form of glycerolipid, we will consider the three dimensional molecular structure of triacylglycerol (all other depolarized glycerolipids such as acetyl-diacylglycerol[16)~21)], diacyl-dinitrobenzoylglycerol[13),14),22)], diacyl-benzoylglycerol[23),24)], and as a matter of course, diacylglycerol derivatives originated from glycerophospholipids are in a wide sense, considered to be the members of triacylglycerol). The three dimensional interactions between $acyl_1$ and $acyl_2$ as well as $acyl_2$ and $acyl_3$ compare to that of $acyl_1$ and $acyl_3$ in the triacylglycerol molecule are considered to be not equal even though the acyl combinations are the same, since the arrangement of these acyl groups are not symmetric against the glycerol moiety. And the reason why 1,2-; 2,1- or 1,2-; 1,3-positional isomers possess different chemical potential *i. e.* in a practical case exhibits different RRT, is attributed to the difference in the degree of interaction between the two acyl groups that are bound in different positions of the glycerol moiety. If we express the interaction degree

Table-1 Relative retention time of the dinitrobenzoyl molecular species including 1,2-;2,1-and 1,2-;1,3-positional isomers with respect to the (1-12 : 0, 2-12 : 0) derivative. *

Molecular species	RRT	log (RRT)
(1–20 : 4, 2–16 : 0)	2.10	0.322
(1–16 : 0, 2–20 : 4)	2.15	0.332
(1–20 : 4, 2–18 : 0)	3.00	0.477
(1–18 : 0, 2–20 : 4)	3.09	0.490
(1–15 : 0, 3–15 : 0)	2.40	0.380
(1–15 : 0, 2–15 : 0)	2.66	0.425
(1–18 : 1, 3–18 : 1)	3.22	0.508
(1–18 : 1, 2–18 : 1)	3.50	0.544
(1–16 : 0, 3–16 : 0)	3.44	0.537
(1–16 : 0, 2–16 : 0)	3.83	0.583

* Transcribed a part from *J. Biochem.*, 98, 327 (1985)[13)].
Relative retention time (RRT) of the (1–12 : 0, 2–12 : 0) derivative is regarded as 1.00.

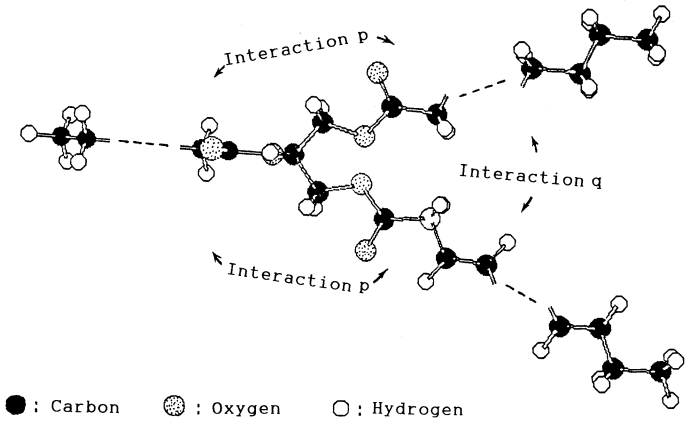

Fig.-1 Interactions between the carbon chains inside glycerolipid molecule.

between $acyl_1$ and $acyl_2$ or $acyl_2$ and $acyl_3$ as "p" and that of between $acyl_1$ and $acyl_3$ as "q" (**Fig.-1**) function (3) can be written as,

$$\Delta\mu_{TG}=g(\Delta\mu_1,\ \Delta\mu_2,\ \Delta\mu_3)$$
$$=\Delta\mu_1+\Delta\mu_2+\Delta\mu_3+p(\Delta\mu_1,\ \Delta\mu_2)$$
$$+p(\Delta\mu_2,\ \Delta\mu_3)+q(\Delta\mu_1,\ \Delta\mu_3) \quad (5)$$

In function (5), terms given by the functions "p" and "q" represent discrepancy from the law of Martin[9]. By utilizing function (5), we can say that ①when $\Delta\mu_1=\Delta\mu_2=\Delta\mu_3$, function "$g$" is symmetric, *i. e.* $\Delta\mu_{TG}$ does not change even though $\Delta\mu_1$, $\Delta\mu_2$, $\Delta\mu_3$ are alternatively exchanged. ②When $\Delta\mu_1=\Delta\mu_3\neq\Delta\mu_2$, function "$g$" is symmetric only for $\Delta\mu_1$ and $\Delta\mu_3$, *i. e.* $\Delta\mu_{TG}$ does not change even though $\Delta\mu_1$ and $\Delta\mu_3$ are alternatively exchanged, but does change when others are exchanged. ③When $\Delta\mu_1=\Delta\mu_2\neq\Delta\mu_3$, function "$g$" is symmetric for $\Delta\mu_1$ and $\Delta\mu_2$ and also for $\Delta\mu_1$ and $\Delta\mu_3$, but is not symmetric for $\Delta\mu_2$ and $\Delta\mu_3$. ④When $\Delta\mu_1\neq\Delta\mu_2=\Delta\mu_3$, function "$g$" is symmetric for $\Delta\mu_2$ and $\Delta\mu_3$ and also for $\Delta\mu_1$ and $\Delta\mu_3$, but is not symmetric for $\Delta\mu_1$ and $\Delta\mu_2$. ⑤When $\Delta\mu_1\neq\Delta\mu_2\neq\Delta\mu_3$, function "$g$" is only symmetric for $\Delta\mu_1$ and $\Delta\mu_3$. By analyzing the data in **Table-1** given by the diacyl-dinitrobenzoylglycerol, it is obvious that in 1, 2-; 2, 1-positional isomers of glycerolipid, $\Delta\mu_{TG}$ gives larger value when $\Delta\mu_1>\Delta\mu_2$, compared to when it is $\Delta\mu_1<\Delta\mu_2$. For example in this table, (1-16 : 0, 2-20 : 4) elutes later than (1-20 : 4, 2-16 : 0), and (1-18 : 0, 2-20 : 4) elutes later than (1-20 : 4, 2-18 : 0). Also, it is obvious that between 1, 2- and 1, 3-isomers, $\Delta\mu_{TG}$ is larger in 1, 2-isomer *e. g.* (1-15 : 0, 2-15 : 0) elutes later than (1-15 : 0, 3-15 : 0), and (1-18 : 1, 2-18 : 1) elutes later than (1-18 : 1, 3-18 : 1), and (1-16 : 0, 2-16 : 0) elutes later than (1-16 : 0, 3-16 : 0). Since we can consider that dinitrobenzoyl derivative is a member of glycerolipid which is represented by triacylglycerol, function (5) should also hold in this (**Table-1**) data. For ease, if we express the ($acyl_1$, $acyl_2$, $acyl_3$) as 1, 2 type, and ($acyl_2$, $acyl_1$, $acyl_3$) as 2, 1- type, and by applying function (5),

$$\Delta\mu_{TG}(1,2)=g(\Delta\mu_1,\ \Delta\mu_2,\ \Delta\mu_3)$$
$$=\Delta\mu_1+\Delta\mu_2+\Delta\mu_3+p(\Delta\mu_1,\ \Delta\mu_2)$$
$$+p(\Delta\mu_2,\ \Delta\mu_3)+q(\Delta\mu_1,\ \Delta\mu_3) \quad (6)$$
$$\Delta\mu_{TG}(2,1)=g(\Delta\mu_2,\ \Delta\mu_1,\ \Delta\mu_3)$$
$$=\Delta\mu_2+\Delta\mu_1+\Delta\mu_3+p(\Delta\mu_2,\ \Delta\mu_1)$$
$$+p(\Delta\mu_1,\ \Delta\mu_3)+q(\Delta\mu_2,\ \Delta\mu_3) \quad (7)$$

As mentioned above, $\Delta\mu_{TG(1,2)}>\Delta\mu_{TG(2,1)}$ when $\Delta\mu_1>\Delta\mu_2$.

Therefore,

$$\Delta\mu_{TG(1,2)}-\Delta\mu_{TG(2,1)}=p(\Delta\mu_2,\ \Delta\mu_3)-p(\Delta\mu_1,\ \Delta\mu_3)$$
$$+q(\Delta\mu_1,\ \Delta\mu_3)-q(\Delta\mu_2,\ \Delta\mu_3)$$
$$>0$$
$$=q(\Delta\mu_1,\ \Delta\mu_3)-q(\Delta\mu_2,\ \Delta\mu_3)$$
$$-\{p(\Delta\mu_1,\ \Delta\mu_3)-p(\Delta\mu_2,\ \Delta\mu_3)\}>0 \quad (8)$$

On the other hand, since 1, 2- and 1, 3-positional isomers correspond to the exchange of $acyl_2$ and $acyl_3$ residues,

$$\Delta\mu_{TG(1,2)}=g(\Delta\mu_1,\ \Delta\mu_2,\ \Delta\mu_3)$$
$$=\Delta\mu_1+\Delta\mu_2+\Delta\mu_3+p(\Delta\mu_1,\ \Delta\mu_2)$$
$$+p(\Delta\mu_2,\ \Delta\mu_3)+q(\Delta\mu_1,\ \Delta\mu_3) \quad (9)$$
$$\Delta\mu_{TG(1,3)}=g(\Delta\mu_1,\ \Delta\mu_3,\ \Delta\mu_2)$$
$$=\Delta\mu_1+\Delta\mu_3+\Delta\mu_2+p(\Delta\mu_1,\ \Delta\mu_3)$$
$$+p(\Delta\mu_3,\ \Delta\mu_2)+q(\Delta\mu_1,\ \Delta\mu_2) \quad (10)$$

Since $\Delta\mu_{(1,2)}>\Delta\mu_{(1,3)}$ as aforementioned,

$$\Delta\mu_{TG(1,2)}-\Delta\mu_{TG(1,3)}=p(\Delta\mu_1,\ \Delta\mu_2)-p(\Delta\mu_1,\ \Delta\mu_3)$$
$$+q(\Delta\mu_1,\ \Delta\mu_3)-q(\Delta\mu_1,\ \Delta\mu_2)>0$$
$$=q(\Delta\mu_1,\ \Delta\mu_3)-q(\Delta\mu_1,\ \Delta\mu_2)$$
$$-\{p(\Delta\mu_1,\ \Delta\mu_3)-p(\Delta\mu_1,\ \Delta\mu_2)\}>0 \quad (11)$$

What is important in function (8) is that it is held under the condition when the relation of the acyl

groups are $\Delta\mu_1 > \Delta\mu_2 > \Delta\mu_3$. Function (8) was,

$$\Delta\mu_{TG(1,2)} - \Delta\mu_{TG(2,1)} = q(\Delta\mu_1, \Delta\mu_3) - q(\Delta\mu_2, \Delta\mu_3) - \{p(\Delta\mu_1, \Delta\mu_3) - p(\Delta\mu_2, \Delta\mu_3)\} > 0$$

$$\therefore \quad q(\Delta\mu_1, \Delta\mu_3) - q(\Delta\mu_2, \Delta\mu_3) > p(\Delta\mu_1, \Delta\mu_3) - p(\Delta\mu_2, \Delta\mu_3) \qquad (12)$$

$$\text{or} \quad q(\Delta\mu_1, \Delta\mu_3) - p(\Delta\mu_1, \Delta\mu_3) > q(\Delta\mu_2, \Delta\mu_3) - p(\Delta\mu_2, \Delta\mu_3)$$

And also, what is important in function (11) is that it is held under the condition when the relation of the acyl groups are $\Delta\mu_1 > \Delta\mu_3$ and $\Delta\mu_2 > \Delta\mu_3$. Function (11) was,

$$\Delta\mu_{TG(1,2)} - \Delta\mu_{TG(1,3)} = q(\Delta\mu_1, \Delta\mu_3) - q(\Delta\mu_1, \Delta\mu_2) - \{p(\Delta\mu_1, \Delta\mu_3) - p(\Delta\mu_1, \Delta\mu_2)\} > 0$$

$$\therefore \quad q(\Delta\mu_1, \Delta\mu_3) - q(\Delta\mu_1, \Delta\mu_2) > p(\Delta\mu_1, \Delta\mu_3) - p(\Delta\mu_1, \Delta\mu_2) \qquad (13)$$

$$\text{or} \quad q(\Delta\mu_1, \Delta\mu_3) - p(\Delta\mu_1, \Delta\mu_3) > q(\Delta\mu_1, \Delta\mu_2) - p(\Delta\mu_1, \Delta\mu_2)$$

These inequalities (12) and (13) each have the same acyl combinations between the "p" term function and the "q" term function. For example, in inequality (12), it has ($\Delta\mu_1$, $\Delta\mu_3$) in combination with ($\Delta\mu_2$, $\Delta\mu_3$). And in inequality (13), it has ($\Delta\mu_1$, $\Delta\mu_3$) in combination with ($\Delta\mu_1$, $\Delta\mu_2$). Therefore, we can say that ①the interaction degree between the acyl groups given by the variable change in chemical potential of the acyl moieties, *e. g.* increase in carbon number or number of double bonds, is $q > p$; *i. e.* the changes in interaction rate between $acyl_1$ and $acyl_3$ is larger compared to those of $acyl_1$ and $acyl_2$ or $acyl_2$ and $acyl_3$. ②The interaction degree depends on the disparity of chemical potentials among the acyl moieties *i. e.* it depends on the non symmetric degree of the molecular form; And even when $\Delta\mu_1 + \Delta\mu_2 + \Delta\mu_3$ is constant, the larger the disparity of the chemical potentials among the acyl moieties, the more $\Delta\mu_{TG}$ becomes large.

From these hypothesis, the followings can be derived. ①In case of tri (same acyl) glycerol (simple triacylglycerol), there are no disparities in interaction forces among the acyl moieties ; therefore, $\Delta\mu_{TG}$ is considered to have an exact linear relationship between the total acyl carbon number (CN); *i. e.* CN has an exact linear relationship between log (RRT)*. ②In case of diacyl glycerolipids (DG), even though when it is the same acyl type, disparity in interaction forces between $acyl_1$ and $acyl_3$ and that of $acyl_1$ and $acyl_2$ should be observed since the molecular structure is not symmetric in this case; *i. e.* strictly speaking, CN (or acyl carbon number) does not have an exact linear relationship between $\Delta\mu_{TG}$ (though no doubt they have an approximate linear relationship). Theoretically, in this case, owing to the increase in disparity in interaction forces, increment $\Delta\mu_{TG}$ [$\equiv\Delta$log (RRT)] should slightly increase in accordance with the increase in carbon numbers (*c. f.* in case of simple triacylglycerol, increment should be constant). ③In comparison to diacyl type, triacyl type is well balanced in the arrangement of $\Delta\mu_1$, $\Delta\mu_2$, $\Delta\mu_3$ *i. e.* triacyl is more symmetric than diacyl. Concretely, the values of $|\Delta\mu_1 - \Delta\mu_3|$ and $|\Delta\mu_2 - \Delta\mu_3|$ are triacylglycerol < DG. And because of this, 1, 2-; 2, 1-or 1, 2-; 1, 3 -positional isomers of triacylglycerol are hard to be resolved than DG.

Table-2 Logarithm of relative retention time of the simple triacylglycerol molecular species with respect to trioleoylglycerol* and their differences among the carbon number variations.

Molecular species	log (RRT)	Δlog (RRT)
(8 : 0, 8 : 0, 8 : 0)	0.658)**	
(10 : 0, 10 : 0, 10 : 0)	1.031)**	0.373
(12 : 0, 12 : 0, 12 : 0)	1.384)**	0.353
(14 : 0, 14 : 0, 14 : 0)	1.736)**	0.352
(16 : 0, 16 : 0, 16 : 0)	2.085)**	0.349

* Relative retention time (RRT) of trioleoylglycerol is regarded as 100.0.

** Subtraction *i.e.* differences in log (RRT).

Table-3 shows the differences in log (RRT) among the carbon number variations of molecular species examined. Though the value itself differs at the most at the decimal point level, it is clear cut that the increments of $\Delta\mu_{TG}$ [$\equiv\Delta$log (RRT)] increase in accordance with the increases in CN.

Table-4 shows the differences in log (RRT) with respect to di (same acyl) glycerolipid. As can be seen in this table, the increment of $\Delta\mu_{TG}$ [$\equiv\Delta$log (RRT)] increases in accordance with the increase in CN *e. g.* (1-12 : 0, 2-12 : 0)→(1-

* The glycerol moiety itself is not symmetric. So to be exact, the larger the CN, the more the triacylglycerol molecules become perfectly symmetric. In case of simple triacylglycerol, the larger the CN, the smaller the increment of $\Delta\mu_{TG}$ so as to converge to a constant value *i. e.* the slope of CN *vs.* log (RRT) becomes perfectly constant (**Table-2**).

Table-3 Logarithm of relative retention time of the dinitrobenzoyl molecular species with respect to (1-12 : 0, 2-12 : 0) derivative* and their differences among the carbon number variations.

[Sn-2] \ [Sn-1]	14:0	16:0	18:0	18:1	18:2 (16:1)	20:1	(18:1)
16:0	0.4249	0.5832 (0.1583)	0.7474 (0.1642)	0.5551 (0.1923)	0.4048 (0.1503)		
		(0.0281)	(0.0281)	(0.0262)	(0.0228)		
18:1		0.5551 (0.1503)	0.7193 (0.1642)	0.5289 (0.1904)	0.3820 (0.1469)	0.6857 (0.1568)	0.5289
		(0.1503)	(0.1546)	(0.1469)	(0.1440)		
18:2 (16:1)		0.4048 (0.0764)	0.5647 (0.1599)	0.3820 (0.1827)	0.2380 (0.1440)		
		(0.0764)	(0.0818)	(0.0724)	(0.0707)		
20:4	0.1818 (0.1063)	0.3284 (0.1466)	0.4829 (0.1545)	0.3096 (0.1733)	0.1673 (0.1423)		
	(0.1063)	(0.1162)	(0.1212)	(0.1193)	(0.1066)		
20:5	0.0755 (0.1367)	0.2122 (0.1495)	0.3617 (0.1714)	0.1903 (0.1296)	0.0607		
		(0.2192)	(0.2294)	(0.2145)			
22:4		0.4314 (0.1281)	0.5911 (0.1597)	0.4048 (0.1863)			
		(0.1281)	(0.1272)	(0.1192)			
22:5		0.3096 (0.0666)	0.4639 (0.1543)	0.2856 (0.1783)			
		(0.0666)	(0.0694)	(0.0603)			
22:6		0.2430 (0.1515)	0.3945 (0.1692)	0.2253 (0.1354)	0.0899		

* Transcribed a part from *Lipids*, 21, 356 (1986)[14]. Numerical values under the bars are the subtraction *i. e.* differences in log (RRT).

14 : 0, 2-14 : 0)<(1-14 : 0, 2-14 : 0)→(1-16 : 0, 2-16 : 0), (1-14 : 0, 2-14 : 0)→(1-15 : 0, 2-15 : 0)<(1-15 : 0, 2-15 : 0)→(1-16 : 0, 2-16 : 0), (1-15 : 0, 2-15 : 0)→(1-16 : 0, 2-16 : 0)<(1-16 : 0, 2-16 : 0)→(1-17 : 0, 2-17 : 0).

4 Appendix

4·1 Origin of the Interaction Forces between the Acyl Groups

The three dimensional molecular structure is considered to be stable when its potential energy is minimum. Therefore, three dimensional structure of the molecule will be settled, when the repulsion forces by electrons (repulsions by the

Table-4 Logarithm of relative retention time of the di (same acyl) dinitrobenzoyl molecular species with respect to (1-12 : 0, 2-12 : 0) derivative* and their differences among the carbon number variations.

Molecular species	RRT	log (RRT)	Δlog (RRT)
(1-12 : 0, 2-12 : 0)	1.00	0	
			)** 0.274/2=0.137
(1-14 : 0, 2-14 : 0)	1.88	0.274	
			)** 0.151
(1-15 : 0, 2-15 : 0)	2.66	0.425	
			)** 0.158
(1-16 : 0, 2-16 : 0)	3.83	0.583	
			)** 0.164
(1-17 : 0, 2-17 : 0)	5.59	0.747	

* Transcribed a part from *J. Biochem.*, 98, 327 (1985)[13]. Relative retention time (RRT) of (1-12 : 0, 2-12 : 0) derivative is regarded as 1.00.

** Subtraction *i. e.* differences in log (RRT).

Coulomb forces) of the atoms that construct the molecule is minimum. This general law can also be adopted to glycerolipids. If there is an imbalance of the carbon chain length between the acyl groups in the lipid molecule, three dimensional metamorphosis of the acyl groups shall occur so as to reach to the minimum Coulomb force. This will result in the changes in van der Waals forces in the chromatographic system employed. And as a result, increment of $\Delta\mu_{TG}$ [$\equiv$log (RRT)] will not become constant in accordance with the changes in acyl carbon number. This change in van der Waals force which is caused by the three dimensional metamorphosis of the lipid molecule is considered to generate the interaction forces between acyl groups. If we disregard these interactions (*i. e.* changes in van der Waals force which is caused by the three dimensional metamorphosis), the law of Martin[9] should hold.

This paper has been reached to notice the important contribution of the van der Waals force in the chromatographic system for lipid analysis. Details will be discussed in the next paper.

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グリセロ脂質の立体構造よりみた1,2-;2,1-ならびに1,2-;1,3位置異性体の逆相クロマトグラフ系における挙動

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リン脂質の誘導体を含むジアシルグリセロ脂質の1,2-;1,3位置異性体がODS系逆相クロマトグラフィーでは分離されるのに対して，トリアシルグリセリンではそれらの分離が困難である理由について考察した。その結果，クーロン力の反発によるアシル基間の相互作用のバランスがトリアシルグリセリンではよいのに対して，ジアシル型脂質ではこのバランスがわるく，炭素鎖がのびるほどこの傾向が強くなり，クロマト系における化学ポテンシャルにも影響を与えて1,2-;1,3-位置異性体が分離されるものと結論した。