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Theoretical Aspects of the Chromatographic Behavior of *Cis* • *Trans* Isomers and Omega Isomers of Lipids in Polar Stationary Phase Gas Liquid Chromatography and in Reverse Phase High Performance Liquid Chromatography

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Abstract

Theoretical aspects of the chromatographic behavior of *cis* • *trans* isomers and ω isomers in glycerolipids were discussed with an insight into the contribution of dipole moments and van der Waals radiuses of glycerolipid molecules to the chromatographic system. It was considered that when there are plural polar groups in the glycerolipid molecule that generate dipole moment, the whole dipole moment of the molecule can be approximated as a resultant vector of them. And therefore it was concluded that in polar stationary phase chromatography, *cis* residues always give higher chemical potentials than *trans* residues so as to elute later, while on the other hand, in the reverse phase high performance liquid chromatography, it was demonstrated to have the inverse relation. With respect to the ω isomers in glycerolipids, both *cis* and *trans* isomers were theoretically born out to exhibit a minimum retention value when the ethylene unit is located near the center of the carbon chain, and becomes large when it is shifted near the end despite the chromatographic system.

Introduction

We have been trying to apply and develop the classical partition chromatographic theory to the modern analysis of molecular species of lipids. However, authorized specialists have pointed out that our work still does not work with *cis* • *trans* isomers and ω isomers.

This paper reports on the theoretical aspects of the chromatographic behavior of *cis* • *trans* isomers and ω isomers of lipids by taking into consideration the relationship between the dipole moments as well as the van der Waals radiuses of the lipid molecules and the chemical potential of them.

Experimental

Materials

Cis-6-octadecenoic methyl and *trans*-11-octadecenoic methyl were purchased from Serdary Research Laboratories, Inc., *cis*-9-octadecenoic methyl from Wako Pure Chemical Industries, and *trans*-9-octadecenoic methyl from Nu-Chek Prep, Inc.

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These authentic standards were combined into an integral ratio in weight in order to identify each peak by peak area. Ten μl (containing 5 μl acetone) of this solution was injected into the following reverse phase high performance liquid chromatograph.

Light scattering mass detector equipped reverse phase high performance liquid chromatography

Reverse phase HPLC was carried under the following conditions.

Chromatograph: Hitachi 638-50 Liquid Chromatograph, Column: Wakosil-5C18N 300×4 mm and Supersphere RP-18 250×4 mm in tandem, Solvent: acetone/acetonitrile (3 : 2→7 : 1/240 min, linear gradient), flow 0.7 ml/min, Column temp.: 5°C, Detector: Applied Chromatographic Systems 750/14 Light Scattering Mass Detector (Time constant: 5, Attenuation: ×2, Photomultiplier sensitivity: 1, Air pressure of the nebuliser: 0.8 kg/cm^2.

Results

Figure 1 shows the reverse phase HPLC elution profile obtained. The retention time (t_R) of each peak was 36.02 for *cis*-9-octadecenoic methyl, 36.48 for *cis*-6-octadecenoic methyl, 38.66 for *trans*-9-octadecenoic methyl, and 39.28 for *trans*-11-octadecenoic methyl. This supported the data shown in Fig. 2 given by Svensson et al.[1], Aveldano et al.[2] and Borch[3] *i.e.* t_R reaches its minimum when the ethylene unit is near the center of the carbon chain and *trans* isomers always give higher t_R than *cis* isomers in monoenoic acid. This relation is valid even when the *trans* fatty acid exists as a diacylglycerolipid as exhibited by Hullin et al.[4] *e.g.* (*trans* 18 : 1, 18 : 1) elutes after (*cis* 18 : 1, 18 : 1).

On the contrary, polar stationary phase gas-liquid chromatography has been

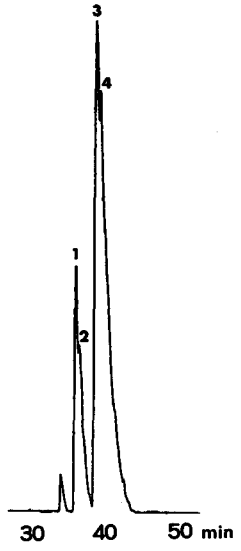

Fig. 1. Reverse phase HPLC elution profile of *cis* and *trans* isomers of octadecenoic methyl.
1 : *cis*-9-octadecenoic methyl ; 2 : *cis*-6-octadecenoic methyl ; 3 : *trans*-9-octadecenoic methyl ; 4 : *trans*-11-octadecenoic methyl.

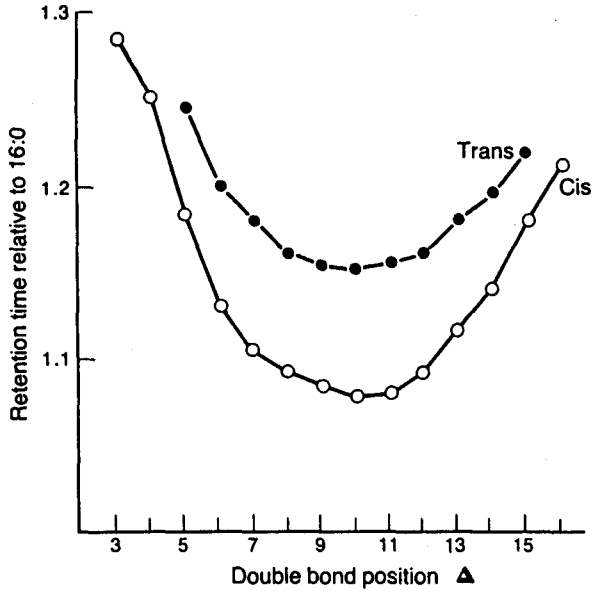

Fig. 2. Retention times relative to hexadecanoic methyl of different positional and geometrical isomers of octadecenoic methyl on reverse phase high performance liquid chromatography[1]. Transcribed from the figure of Svensson et al.[1].

shown to exhibit an inverse relation *i.e. trans* isomers elutes before *cis* isomers as reported by Ackman and Hooper[5], Rakoff and Emken[6], Wijesundera and Ackman[7], and Ratnayake et al.[8], Ratnayake and Peeletier[9], Wolff[10~12].

These inverse relational chromatographic behavior was theorized as follows.

Discussion

From the equation of Martin[7],

$$\Delta\mu_A = RT\ln\alpha \tag{1}$$

can be derived where $\Delta\mu_A$ is the chemical potential of the solute A; α is the partition coefficient, R is the gas constant, and T is the absolute temperature. In this chromatographic system, $\Delta\mu_A$ corresponds to the free energy in transferring the solute A from the stationary phase to the mobile phase.

On the other hand, free energy can be written as,

$$F = E - TS \tag{2}$$

where F is the free energy, E is the initial energy, S is the entropy, and T is the absolute temperature.

The initial energy E of the solute consists of the initial potential energy E' generated by the configuration of the molecule (three-dimensional orientation of the atoms and the orbital), the van der Waals force U, and the repulsive force A among the molecules generated by the excluded volumetric effect. E is considered to be

the sum of E', U and A. Therefore,

$$E = E' + U + A \tag{3}$$

On the other hand, from the formula of Boltzmann, S can be give as.

$$S = k\ln m \tag{4}$$

where k is the Boltzmann constant, m is the state number given by the configuration of the molecule. And the more the portions of the folding in the molecule, the larger the m becomes, resulting in the increase in entropy.

By putting formulae (3) and (4) into (2),

$$F = E' + U + A - kT\ln m \tag{5}$$

can be obtained. This formula (5) is considered to be the basic formula of thermodynamics in chromatography.

We will now consider the free energy of a lipid molecule F_{TG} by applying function (5).

From formula (5), free energy of the lipid molecule when it is in the stationary phase (F_{TG-S}) can be expressed as,

$$F_{TG-S} = E'_{TG-S} + U_{TG-S} + A_{TG-S} - kT\ln m_{TG-S} \tag{6}$$

And that of the mobile phase, F_{TG-M} can be expressed as,

$$F_{TG-M} = E'_{TG-M} + U_{TG-M} + A_{TG-M} - kT\ln m_{TG-M} \tag{7}$$

If we express the difference in potential energy of the lipid molecule between these two phases as Δ,

$$F_{TG-M} - F_{TG-S} \equiv \Delta F_{TG} = \Delta E'_{TG} + \Delta U_{TG} + \Delta A_{TG} - kt\ ln\ (m_{TG-M}/m_{TG-S}) \tag{8}$$

can be obtained.

From equation (1) in the case of TG molecule,

$$\Delta\mu_{TG} = RT\ln\alpha \tag{9}$$

should hold.

In the chromatographic system, we can consider that $\Delta\mu_{TG} = \Delta F_{TG}$. Thus from formulae (8) and (9), the following can be obtained;

$$\Delta\mu_{TG} = \Delta F_{TG} = RT\ \ln\ \alpha = \Delta E'_{TG} + \Delta U_{TG} + \Delta A_{TG} - kT\ln(m_{TG-M}/m_{TG-S}) \tag{10}$$

This formula (10) is considered to exhibit the partition chromatographic law of Martin[7] in the manner of thermodynamics.

Under the thermostatic condition, the main factor that governs the partition of lipid molecules in the chromatographic system is considered to be the van der Waals force.

The term ΔU_{TG} in formula (10) can be expressed as,

$$\Delta U_{TG} = U_{\mathrm{dip}.TG-M} + U_{\mathrm{ind}.TG-M} + U_{\mathrm{dis}.TG-M} - (U_{\mathrm{dip}.TG-S} + U_{\mathrm{ind}.TG-S} + U_{\mathrm{dis}.TG-S}) \tag{11}$$

where $U_{\mathrm{dip.}}$, $U_{\mathrm{ind.}}$, $U_{\mathrm{dis.}}$ are potential energies that stand for dipole effect, induction effect, and dispersion effect, respectively.

This formula (11) shoule hold when the phase transition of the lipid molecule is negligible such as in the case of liquid-liquid chromatography. However, in the case when phase transition occurs such as in the case of gas-liquid chromatography,

ven der Waals forces among the lipid molecules themselves should be taken into consideration ;

$$\begin{aligned} i.e.\ \Delta U_{TG} = & U_{\text{dip.}TG-M} + U_{\text{ind.}TG-M} + U_{\text{dis.}TG-M} \\ & + U_{\text{dip.}-(TG)M} + U_{\text{ind.}-(TG)M} + Z_{\text{dis.}-(TG)M} \\ & - (U_{\text{dip.}TG-S} + U_{\text{ind.}TG-S} + U_{\text{dis.}TG-S} + U_{\text{dip.}-(TG)S} \\ & + U_{\text{ind.}-(TG)S} + U_{\text{dis.}-(TG)S}) \end{aligned} \tag{12}$$

where $U_{(TG)M}$ and $U_{(TG)S}$ are the van der Waals forces among the lipid molecules themselves in mobile phase and in stationary phase, respectively.

However, in the gas-liquid chromatographic system, $U_{(TG)M}$ is considered to be negligible. Therefore, formula (12) can be rearranged as follows ;

$$\begin{aligned} \Delta U_{TG-\text{gas}} = & -(U_{\text{dip.}TG-S} + U_{\text{ind.}TG-S} + U_{\text{dis.}TG-S} + U_{\text{dip.}-(TG)S} \\ & + U_{\text{ind.}-(TG)S} + U_{\text{dis.}-(TG)S} \end{aligned} \tag{13}$$

where $\Delta U_{TG-\text{gas}}$ is the van der Waals force in the gas-liquid chromatographic system.

On the other hand, in the liquid-liquid chromatographic system, as aforementioned, we can consider that $U_{(TG)M} \fallingdotseq U_{(TG)S}$. Therefore,

$$\begin{aligned} \Delta U_{TG-\text{liq.}} = & U_{\text{dip.}TG-M} + U_{\text{ind.}TG-M} + U_{\text{dis.}TG-M} \\ & - (U_{\text{dip.}TG-S} + U_{\text{ind.}TG-S} + U_{\text{dis.}TG-S}) \end{aligned} \tag{14}$$

where $\Delta U_{TG-\text{liq.}}$ is the van der Waals force in the liquid-liquid chromatographic system.

Formulae (13) and (14) represent the general formulae of van der Waals forces in gas-liquid and liquid-liquid chromatographic systems, respectively.

We will now consider the theoretical aspects of the chromatographic behavior of *cis* • *trans* isomers and ω isomers by introducing formulae (13) and (14).

The *cis.trans* isomers and ω isomers each have the same functional groups. However, they differ in three-dimensional orientations or in positions of the functional groups.

When the two lipid molecules have the same functional groups, but differ only in three-dimensional orientations or in positions, the only factors that affect the van der Waals force might be considered to be the dipole moment and the van der Waals radius of the lipid molecule. These two factors are considered to exert the resolutions of *cis* • *trans* isomers and ω isomers.

We will embody the theoretical aspects of the resolutions of *cis* • *trans* isomers and ω isomers.

If we differentiate formula (13) by regarding them as a function of D_{TG} (Dipole moment of the lipid molecule) and by considering that the van der waals radiuses are the same in order to simplify the formula*,

$$\begin{aligned} & \partial \Delta \mu_{TG-\text{gas}} / \partial D_{TG} = \partial \Delta U_{TG-\text{gas}} / \partial D_{TG} \\ & = \{2(D_S^{**2} + 2D_{TG}^2)/3KT + \alpha_S + 2\alpha_{TG}\} 2D_{TG}/r^6 \end{aligned} \tag{15}$$

can be obtained, since there are relations that $U_{\text{dip.}} = -2D_1^2 D_2^2 / 3KT\ r^6$, $U_{\text{ind.}} = -(\alpha_1 D_2^2 + \alpha_2 D_1^2)/r^6$, and $U_{\text{dis.}} = -3\alpha_1\alpha_2\, h\nu_1 h\nu_2 / 2(h\nu_1 + h\nu_2) r^6$ where D_1 and D_2 are the dipole moment of each molecule, α_1 and α_2 are the polarizability of each

* Even though the van der Waals radiuses are not the same, inequalities (17) and (18) are valid.

** Dipole moment of the stationary phase.

molecule, K is the Boltzmann constant, T is the absolute temperature, h is the Planck constant, ν_1 and ν_2 are the oscillation frequency of the orbitals, and r refers to the distrance (Combination of the radius) between the molecules.

In formula (14), $D_M > D_S \fallingdotseq 0$ should hold in case of reverse phase HPLC, since mobile phase is polar. And we can regard that $U_{\text{dip.}TG-S} \simeq 0$.

Therefore, if we differentiate formula (14) by regarding them as a function of D_{TG}, and by regarding that van der Waals radiuses are the same in order to simplify the formula*,

$$\begin{aligned}\partial \Delta\mu_{TG-\text{liq.}}/\partial D_{TG} &= \partial \Delta U_{TG-\text{liq.}}/\partial \Delta D_{TG} \\ &= (\alpha_S - \alpha_M - 2{D_M}^2/3KT)2D_{TG}/r^6\end{aligned} \tag{16}$$

Since D, K, T, α, r are all >0 in function (15),

$$\partial \Delta\mu_{TG-\text{gas}}/\partial \Delta D_{TG} = \{2({D_S}^2 + 2{D_{TG}}^2)/3KT + \alpha_S + 2\alpha_{TG}\}2D_{TG}/r^6 > 0 \tag{17}$$

should hold.

On the other hand, the following relations should hold in function (16);

$$\alpha_S - \alpha_M - 2{D_M}^2/3KT < 0 \rightarrow \partial \Delta\mu_{TG-\text{liq.}}/\partial \Delta\mu_{TG} < 0 \tag{18}$$

$$\alpha_S - \alpha_M - 2{D_M}^2/3KT > 0 \rightarrow \partial \Delta\mu_{TG-\text{liq.}}/\partial \Delta\mu_{TG} > 0 \tag{19}$$

In the reverse phase mode, the molecules of the mobile phase are polar, therefore, D_M is large and inequality (18) should hold.

After all, variable changes in dipole moment of the lipid molecule, are considered to introduce; ① In the case of polar stationary phase gas-liquid chromatography, in accordance with the increase in D_{TG}, $\Delta\mu_{TG-\text{gas}} \equiv \log(RRT)_{\text{gas}}$** increases; ② In the case of reverse phase liquid-liquid chromatography, in accordance with the increase in D_{TG}, $\Delta\mu_{TG-\text{liq.}}$*** $\equiv \log(RRT)_{\text{liq.}}$ decreases.

For the next step of this discussion, we will consider formulae (13) and (14) as a function of r_{TG} (The van der Waals radius of glyceride).

If we denote the van der Waals radiuses of the mobile phase molecule by r_M, that of the stationary phase molecule by r_S, and that of the lipid molecule by r_{TG}, the van der Waals radius between the mobile phase molecule and the lipid molecule can be approximated as $r_M + r_{TG}$; that between the stationary phase molecule and the lipid molecule can be approximated as $r_S + r_{TG}$; and that between the lipid molecules can be approximated as $2r_{TG}$. We can consider that $r_S \fallingdotseq r_M$. And by supposing that $r_M + r_{TG} \fallingdotseq r_S + r_{TG}$, the differentiated formulae of (13) and (14) with respect to r_{TG} can be obtained as follows;

$$\begin{aligned}\partial \Delta\mu_{TG-\text{gas}}/\partial r_{TG} &= 6\Delta U_{TG-\text{gas}}/\partial r_{TG} = 6\{(U_{\text{dip.}TG-S} \\ &\quad + U_{\text{ind.}TG-S} + U_{\text{dip.}TG-S})/(r_{TG} + r_S)\} \\ &\quad + 6\{(U_{\text{dip.-}(TG)S} + U_{\text{ind.-}(TG)S} \\ &\quad + U_{\text{dis.-}(TG)S})/r_{TG}\}\end{aligned} \tag{20}$$

$$\begin{aligned}\partial \Delta\mu_{TG-\text{liq.}}/\partial r_{TG} &= \partial \Delta U_{TG-\text{liq.}}/\partial r_{TG} = 6\{(U_{\text{dip.}TG-S} \\ &\quad + U_{\text{ind.}TG-S} + U_{\text{dis.}TG-S})/(r_{TG} + r_S)\} \\ &\quad - 6\{(U_{\text{dip.}TG-M} + U_{\text{ind.}TG-M} \\ &\quad + U_{\text{dis.}TG-M})\}/(r_{TG} + r_M)\end{aligned} \tag{21}$$

* Even though the van der Waals radiuses are not the same, inequalities (17) and (18) are valid.

** Relative retention time in gas chromatography.

*** Relative retention time in liquid chromatography.

can be obtained.

Since r_{TG}, r_S, r_M, are all positive and the potential energy of the van der Waals attraction is negative (*i.e.* U<0),

$$\begin{aligned}\partial \Delta\mu_{TG-\text{gas}}/\partial r_{TG} = 6\{(&U_{\text{dip.}TG-S} + U_{\text{ind.}TG-S} \\ &+ U_{\text{dis.}TG-S})\}/(r_{TG}+r_S)\} \\ &+6\{(U_{\text{dip.}-(TG)S} + U_{\text{ind.}-(TG)S} \\ &+ U_{\text{dis.}-(TG)S})\}/r_{TG} < 0\end{aligned} \tag{22}$$

should hold.

If we define the partition coefficient α as $\alpha \equiv N_M/N_S$where N_M and N_S denote the molar partition of the lipid molecules in the mobile phase and in the stationary phase, respectively,

$$\begin{aligned}&U_{\text{dip.}TG-S} + U_{\text{ind.}TG-S} + U_{\text{dis.}TG-S} \\ &= U_{\text{dip.}TG-M} + U_{\text{ind.}TG-M} + U_{\text{dis.}TG-M} < 0\end{aligned}$$

should hold, since when $\alpha=1$, N_M is equal to N_S.

And when $\alpha>1$, $N_M>N_S$ should hold. Therefore,

$$\begin{aligned}0 > U_{\text{dip.}TG-S} + U_{\text{ind.}TG-S} + U_{\text{dis.}TG-S} > U_{\text{dip.}TG-M} + U_{\text{ind.}TG-M} \\ + U_{\text{dis.}TG-M}\end{aligned}$$

should also hold ($\because$ the potential energy of the van der Waals force is negative *i.e.* U<0).

When $\alpha<1$, $N_M<N_S$ should hold. Therefore,

$$\begin{aligned}U_{\text{dip.}TG-S} + U_{\text{ind.}TG-S} + U_{\text{dis.}TG-S} < U_{\text{dip.}TG-M} + U_{\text{ind.}TG-M} \\ + U_{\text{dis.}TG-M} < 0\end{aligned} \tag{25}$$

should also hold. In general, the partition coefficient of the lipid molecule is considered to be $\alpha<1$, so relation (25) is the usual case. And we can obtain the following inequality;

$$\begin{aligned}\partial \Delta\mu_{TG-\text{liq.}}/\partial r_{TG} = \partial \Delta U_{TG-\text{liq.}}/\partial r_{TG} = 6\{&U_{\text{dip.}TG-S} \\ &+ U_{\text{ind.}TG-S} + U_{\text{dis.}TG-S} - (U_{\text{dip.}TG-M} \\ &+ U_{\text{ind.}TG-M} + U_{\text{dis.}TG-M})\}/r_{TG} < 0\end{aligned} \tag{26}$$

($\because$ We considered that $r_S \fallingdotseq r_M$ for reasons of convenience)

From equations (22) and (26), we can derive that both in polar stationary phase gas-liquid chromatography and in reverse phase liquid-liquid chromatography, $\Delta\mu_{TG}$ becomes smaller in accordance with the increase in van der Waals radius; *i.e.* the larger the r_{TG}, the smaller the $\Delta\mu_{TG-\text{gas}} \equiv \log(\text{RRT})_{\text{gas}}$ or the $\Delta\mu_{TG-\text{liq.}} \equiv \log(\text{RRT})_{\text{liq.}}$ becomes.

So far, correlations between the dipole moment as well as the van der Waals radius of the lipid molecule and its chmical potential have been demonstrated. And we can develop this theory into a chromatographic behavitor of the following lipid isomers.

Cis • Trans Isomers

When there are plural polar groups in the molecule that generate dipole moment, the whole dipole moment of the molecule can be approximated as a resultant vector of them *i.e.* if we denote the dipole moment of the polar groups as

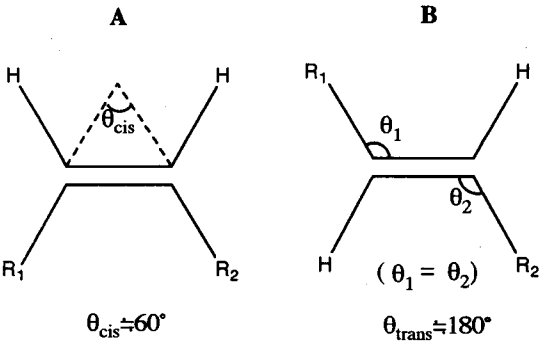

Fig. 3. Configuration of *cis* and *trans* monoethylenic fatty acid isomers.

D_1 and D_2, and let θ be the angle between D_1 and D_2,

$$D=\sqrt{D_1^2+D_2^2+2D_1D_2\cos\theta}$$

should hold, where D is the whole dipole moment of the molecule.

In case of *cis* and *trans* isomers, as illustrated in Fig. 3, θ_{cis} is approximately 60°, while on the other hand, θ_{trans} is almost 180° since θ_1 is equal to θ_2 as shown in the same figure. If we denote the dipole moment of R_1 and R_2 in Fig. 3 as D_1 and D_2, respectively, the dipole moment of *cis* and *trans* isomers can be expressed as;

$$D_{cis}=\sqrt{D_1^2+D_2^2+D_1D_2}$$
$$D_{trans}=\sqrt{D_1^2+D_2^2-2D_1D_2}=D_1-D_2$$

(When $D_1=D_2$, $D_{trans}=0$)

Since D is positive, $D_{cis}>D_{trans}$ should hold. Therefore, in polar stationary phase gas-liquid chromatography,

$$\Delta\mu_{cis-\text{gas}}>\Delta\mu_{trans-\text{gas}}$$
$$\therefore \log(\text{RRT})_{cis-\text{gas}}>\log(\text{RRT})_{trans-\text{gas}}$$

And in reverse phase liquid-liquid chromatography,

$$\Delta\mu_{cis-\text{liq.}}<\Delta\mu_{trans-\text{liq.}}$$
$$\therefore \log(\text{RRT})_{cis-\text{liq.}}<\log(\text{RRT})_{trans-\text{liq.}}$$

ω isomers

Strictly speaking, the orientation between the lipid molecule is not always settled. And even under defined conditions, the orientation between those are always changing at each moment. So statistical dynamics should be emplyed for a perfect discussion. However, we will settle for a certain oriented condition in order to make the discussion simpler. ① In case of *cis* type, there are two representative situations as illustrated in Fig. 4. Figure 4A shows the orientation when the ethylene unit is located in the center of the molecule, and Fig. 4B shows when the ethylene unit is located near the end of the carbon chain.

The mean value of the van der Waals radius can be obtained as follows; When

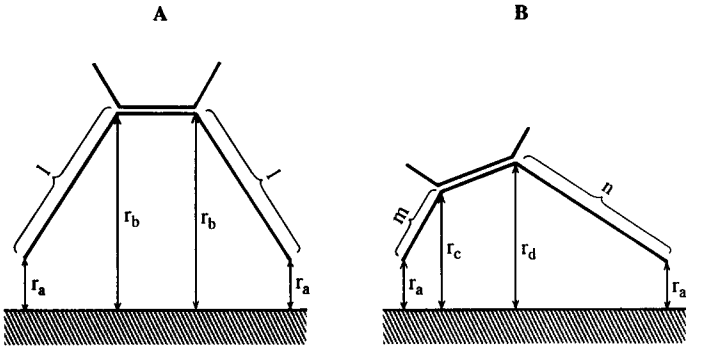

Fig. 4. System for locating *cis* monoethylenic fatty acid with respect to the stationary phase. A : In the case when the ethylene unit is at the center of the carbon chain, and B : when the ethylene unit is near the end of the carbon chain. r_a : van der Waals radius at the end of acyl carbon chain ; r_b, r_c, r_d : van der Waals radiuses at the ethylene unit ; l, m, n : carbon number (when it is $m+n=21$).

the ethylene unit is located in the center of the acyl carbon chain, the mean value of the van der Waals radius will be ;

$$\bar{r}_1=(r_a+r_b)/2$$

On the other hand, when the ethylene unit is located near the end of the acyl carbon chain, the mean value of the van der Waals radius will be ;

$$\begin{aligned}\bar{r}_2&=[\{m(r_a+r_c)/2\}+\{n(r_a+r_d)/2\}]/(m+n)\\&=\{m(r_a+r_c)+n(r_a+r_d)\}/2(m+n)\end{aligned}$$

Suppose that $m+n=2l$, the following relations should hold among l, m, n and r_b, r_c, r_d ;

when $m<l<n\rightarrow r_c<r_d<r_b$ should hold ;

And when $m=n=l\rightarrow r_c=r_d=r_b$ should hold.

Therefore, when $|n-m|=0$, $\bar{r}_2$ becomes maximum and should be the same with $\bar{r}_1$. And when $|n-m|=2l$, $\bar{r}_2$ becomes minimum.

As exhibited in inequalities (22) and (26), in accordance with the increase in van der Waals radius, $\Delta\mu\equiv\log(RRT)$ decreases. For this reason, we can conclude that in *cis*-monoenoic acid, the retention value becomes minimum when the ethylene unit is located in the center of the carbon chain, and becomes large when it is shifted near the end despite the phase type of the chromatographic system.

② Incase of *trans* type, two different situations can be considered as illustrated in Fig. 5. Figure 5A shows the orientation when the ethylene unit is located in the center of the molecule, the Fig. 5B shows when the ethylene unit is located near the end of the carbon chain. Under the Fig. 5A orientation, the van der Waals radiuses at two ends of the carbon chain *i.e.* r_a *and* r_e are considered to be equal to the van der Waals radiuses at the corresponding ends of the ethylene unit (points A, E and A', E', respectively, in Fig. 5B). Therefore, the van der Waals radius $\bar{r}_3$ of when the ethylene unit is located in the center of the carbon chain can be written as,

$$\bar{r}_3=(r_a+r_e)/2$$

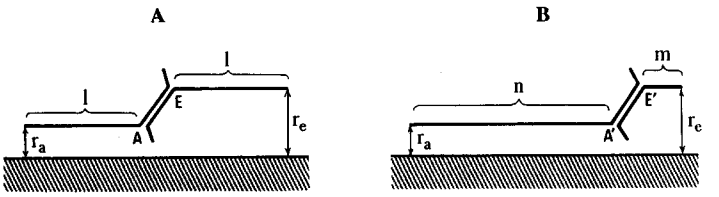

Fig. 5. System for locating *trans* monoethylenic fatty acid with respect to the stationary phase.
A : In the case when the ethylene unit is at the center of the carbon chain, and B : when the ethylene unit is near the end of the carbon chain. r_a, r_e : van der Waals radiuses at the end of acyl carbon chain ($r_a < r_e$) ; l, m, n : carbon number (when it is $m+n=21$, $n \geqq m$).

And the van der Waals radius $\bar{r}_4$ of when the ethylene unit is located near the end of the carbon chain can be written as,

$$\bar{r}_4 = (nr_a + mr_e)/(n+m) \tag{27}$$

Since $r_a < r_e$ and $n+m=2l$ (constant), the longer the m, the larger $\bar{r}_4$ becomes. And $\bar{r}_4$ reaches the maximum when $m=n=l$. (Mathematically, $\bar{r}_4$ becomes much larger when $m>n$ in equation (29). However, this type of orientation is physically very precarious so as to rotate 180°with respect to the alkyl chain axis, and as a result $\bar{r}_4$ becomes smaller compared to when it is $m=n=l$.) Therefore, as it is the same with the *cis* alkyl chain, the following relations should hold.

When $|n-m|=0$, $\bar{r}_4$ reaches the maximum and becomes the same with $\bar{r}_3$.
When $|n-m|=2l$, $\bar{r}_4$ reaches the minimum.

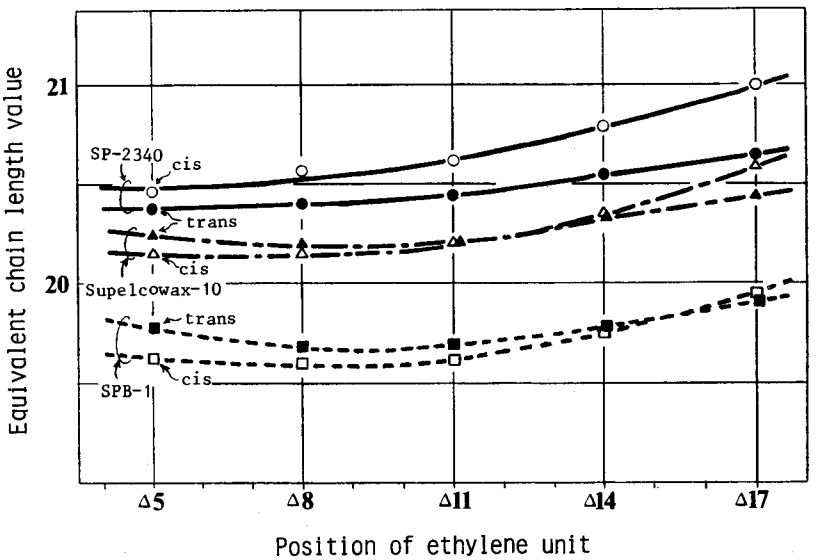

Fig. 6. Equivalent chain length values of the *cis* and *trans* monoethylenic fatty acid isomers derived from icosapentaenoic acid.
Data of Wijesundera and Ackman[7] was used.

We can conclude that with respect to ω isomers, both *cis* and *trans* isomers show the same chromatographic behavior *i.e.* the retention value becomes minimum when the ethylene unit is located in the center of the carbon chain, and becomes large when it is shifted near the end despite the chromatographic sysjen (See Fig. 2).

We did not discuss the apolar stationary phase of gas-liquid chromatography or the moderately polar stationary phase gas-liquid chromatography.

The reason we did not touch on this point is because there are critical points that sequence in elution of *cis* and *trans* isomers go into reverse with respect to the ω isomers (Fig. 6)[7]. This phenomenon can be attributed to the changes in contribution rates of dipole moment of the stationary phase relatively to that of the solute and the van der Waals radius of the solute to the chemical potential of the chromatographic system.

However, it was considered to be too complicated to involve this elucidation in this paper.

This must be reported at a later date.

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