Conformational Analysis of Triacylglycerols by Means of Nuclear Magnetic Resonance and Molecular Mechanics

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The conformational structures around the C-C-C bonds of the glycerol group of three triacylglycerols [C₃H₅(OCOR)₃ where the R is CH₃, n-C₃H₇, and n-C₁₇H₃₅, respectively] in CDCl₃ solution were investigated by proton nuclear magnetic resonance (¹H-NMR) and molecular mechanics calculations (revised QCMP/MM2 force field calculations). The fractional populations of the conformers as determined by both methods showed close agreement. Irrespective of the chain lengths of the acyl group, the three oxygens in the glycerol unit were in the gauche configurations. The most preferred conformation has been shown to be the trans-trans configuration around C-C-C bonds and to constitute about 20% of the possible conformers with lower energy. Both anisochronous methylene protons and the effect of acyl chain length on the glycerol unit have been demonstrated by lanthanide induced shifts in the presence of Eu(fod)₃.

Introduction

Glycerol is a chemical component common to various kinds of vegetable oils, lipids, membranes and enzymes. The esters are usually composed of acyl chains which make the molecular structures complex.

Pertinent arguments on the aggregation behaviors of lipids were given with carbon-13 relaxation times¹⁻². NMR spectroscopy is also suitable for the investigation of the conformation around glycerol unit. In fact, Govil and his coworkers³ have reported stable conformations, (gauche-gauche) and (gauche-trans), of glycerol trivaleate based on ¹H-NMR coupling constants and on the data of potential energy calculations⁴,⁵ with reference to crystal studies⁶,⁷. The relationships between conformational preference of phospholipids and coordination numbers of the phosphorus atom were examined with highly resolved NMR spectra⁸.

In the present studies, all possible staggered conformations with minimum energies, arising by rotations around C₁-C₂, C₂-C₃ and C₂-O₁ in the glycerol unit (Fig.-1) have been taken into consideration to analyze spin-spin coupling constants. Fractional populations for these possible conformers have been estimated for the first time, and compared with those calculated by molecular mechanics.

Experimental

Triacylglycerol, tributyrlylglycerol, tristearoylglycerol and tris (6,6,7,7,8,8,8-heptafluoro-2,2-dimethyloctane-3,5-dionato) europium (III) [Eu(fod)₃] purchased from WAKO Pure Chemical Industries, Ltd. were of chemical pure grade and used without further purification.

Proton NMR spectra were run on a JNM-PMX60 NMR spectrometer (JEOL) at 25°C. Samples were made up with triacylglycerol of 0.4 M in 0.5mL of chloroform-d containing 1% tetramethylsilane (TMS).

Fig.-1 The molecular structure and the numbering schemes of triacylglycerol (TAG). Consistent with the notation and nomenclature of Sundaralingam⁹, the torsion angles in the glycerol backbone are referred to as θ₁ and θ₂, while those in the fatty acid chains are labelled as (α₁-α₃), (β₁-β₃) and (γ₁-γ₃).
A revised QCMP/MM2 program was used for the calculation of energy of each conformers with atomic coordinates composed of 41, total number of atoms including lone pair electrons. Torsion angles and bond lengths were varied until minimum energies were obtained.

**Results and Discussion**

$^1$H-NMR spectra of triacetylglycerol (TAG), tributyrylglycerol (TBG), and tristearoylglycerol (TSG) in CDCl$_3$ are presented in Fig.-2. All peaks in the spectra have been assigned based on the chemical shift, integral peak intensity, and multiplicity of the spin-spin couplings. In each spectrum the complex pattern of peaks over 4.0 to 5.5 ppm is attributable to the five protons of the glycerol unit. The quintet-like peaks with a center about 5.3 ppm are analyzed to be triplets of triplet of the methine proton as shown in Fig.-3.

The spin–spin coupling constants and the chemical shift differences between geminal protons, $H_A$ and $H_B$, are given in Table-1. These values are in agreement with the data of glycerol trivalerate. The values of spin–spin coupling constants, $J(A, H_A)$, $J(A, H_B)$, and $J(B, H_B)$, are independent of the acyl chain length of the triacylglycerols.

It is reasonable to assume that three staggered conformations arising by rotations around the C–C bond in glycerol unit are of minimum energy. These three conformations around $\theta_1$ and $\theta_2$ are represented in Fig.-4. The observed coupling constants are expressed by summation of $J_A(k)$ and $J_B(k)$ weighted with the fractional populations of the three conformers.

<table>
<thead>
<tr>
<th>Table-1</th>
<th>Spin-spin coupling constants and chemical shift differences between $H_A$ and $H_B$ of glycerol moiety in three triacylglycerols.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerol</td>
<td>$^{2}J(A, H_B)$</td>
</tr>
<tr>
<td>(C$_3$)</td>
<td>11.8</td>
</tr>
<tr>
<td>(C$_3$)</td>
<td>11.7</td>
</tr>
<tr>
<td>(C$_3$)</td>
<td>11.8</td>
</tr>
</tbody>
</table>

$a$ Data are given in Hz units on a 60 MHz spectrometer using CDCl$_3$ solution at 25°C.

$b$ Numbers denote carbon numbers of the aliphatic chain.
Table-2  Fractional populations for nine conformers around the two C-C bonds of glycerol moiety.

<table>
<thead>
<tr>
<th>(θ₁, θ₂)</th>
<th>Populations (%) by NMR⁴</th>
<th>Steric energyb (kcal/mol)</th>
<th>Populations (%) by MM²⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>I II (g−, g+)⁶</td>
<td>16</td>
<td>15.9321</td>
<td>9</td>
</tr>
<tr>
<td>I II (g−, t)</td>
<td>17</td>
<td>15.5513</td>
<td>17</td>
</tr>
<tr>
<td>I III (g−, g−)</td>
<td>8</td>
<td>16.0354</td>
<td>8</td>
</tr>
<tr>
<td>II I (t, g+)</td>
<td>17</td>
<td>15.5513</td>
<td>17</td>
</tr>
<tr>
<td>II II (t, t)</td>
<td>17</td>
<td>15.3593</td>
<td>24</td>
</tr>
<tr>
<td>II III (t, g−)</td>
<td>8</td>
<td>16.0442</td>
<td>8</td>
</tr>
<tr>
<td>III I (g+, g+)</td>
<td>8</td>
<td>16.0354</td>
<td>8</td>
</tr>
<tr>
<td>III II (g+, t)</td>
<td>8</td>
<td>16.0442</td>
<td>8</td>
</tr>
<tr>
<td>III III (g+, g−)</td>
<td>3</td>
<td>16.7903</td>
<td>2</td>
</tr>
</tbody>
</table>

a Populations were calculated by using P(I)=0.04, P(II)=0.41 and P(III)=0.19 (cf. Text).
b MM 2 calculations were carried out for triacetylglycerols (TAG).
c Populations were calculated with the Boltzmann distribution under the NMR experimental condition at 25°C.
d See Figs. -4 and 5

Fig.-4  Newman projections around the bonds θ₁ and θ₂, and values of component coupling constants estimated by using a modified Karplus equation.¹²

Karplus equation refined with (i) three-bond ¹H-¹H spin-spin coupling constant with the H-C-C-H torsion angle, (ii) the electronegativity of substituent and (iii) its orientation relative to the coupling protons¹⁰. Thus, eqs. 1~3 are written with the values, Jₐ(I)=2.8, Jₐ(II)=0.9, Jₐ(III)=10.7, Jₐ(IV)=10.7, Jₐ(V)=5.0 Hz, and the observed vicinal coupling constants, J(Hₐ, H₃)=4.4 and J(Hₐ, H₄)=5.7 Hz.

J(Hₐ, H₂)=P(I)Jₐ(I)+P(II)Jₐ(II)
+P(III)Jₐ(III) (1)
J(Hₐ, H₄)=P(I)Jₐ(I)+P(II)Jₐ(II)
+P(III)Jₐ(III) (2)
P(I)+P(II)+P(III)=1 (3)

Solving eqs. 1~3 gives P(I), P(II) and P(III) to be 0.40, 0.41 and 0.19, respectively.

There are nine possible conformers for the combination of the rotations around θ₁ and θ₂. Combinations of P(I)~P(III) can afford to calculate the fractional populations of these conformers. The results are given in Table-2.

Molecular mechanics (QCMP/MM2 force field) calculations have been conducted for these nine staggered conformations with respect to the C-C-C bond of glycerol group in TAG. Minimum energies for these conformations were obtained. The typical results for (g−, g+) conformation are given in Table-3.¹²

Another three possible torsion angles around β₁, or C₃-O₁ bond give 27 conformers in all, but MM2 calculations have revealed that the additional variations give less than 1 % change in the fractional populations. Thus, both NMR analyses and MM2 calculations have proved that (t, t), (t, g+), (g−, t) and (g−, g+) are major four conformers (see Fig.-5). Especially the (t, t) conformer has the least energy due to the least steric
interactions between the acyl chains at C₁ and C₃.

Based on classical potential functions (CPF), Govil and his coworkers¹⁰ selected (θ₁, θ₃) = (g⁻, g⁺), (g⁺, t) and (g⁺, g⁺) as preferred conformations of glycerol trivaletrate. The former two conformers coincide with our results, but the most stable (t, t) conformation is not included in their results. Their calculations commence with the data of crystal structures where neither (t, t) nor (g⁻, g⁻) is given. The potential energy of (g⁻, g⁺) must be equal to its mirror image, (g⁺, g⁺). Moreover, the identifications of H₆a and H₆b in the glycerol group, |(H₆a) - (H₆b)|, vary with the acyl chain length (Table-1). The acyl chains become bundled states in non-polar solvent such as benzene¹⁰ at high concentrations, and finally, it is suggested that compact conformations, (g⁺, g⁺) and (g⁺, t) are preferred in solid states.

Although these three triacylglycerols have equal spin-spin coupling constants, the chemical shift differences between methylene protons, H₆a and H₆b, in the glycerol group, |(H₆a) - (H₆b)|, vary with the acyl chain length (Table-1). The

![Fig.-5 Conformational structure of triacetylglycerol (TAG) estimated by using a MM2 force field calculation.](image)

![Fig.-6 ¹H-NMR spectra of triacetylglycerol (TAG) with successive addition of Eu(fod)₃. Eu (fod)₃/TAG ratios (mol/mol) are (a) 0, (b) 0.1, (c) 0.3, (d) 0.5.](image)

![Fig.-7 ¹H-NMR spectra of tributyrylglycerol (TBG) with successive addition of Eu(fod)₃. Eu (fod)₃/TAG ratios (mol/mol) are (a) 0, (b) 0.3, (c) 0.4, (d) 0.5 and (e) 0.6.](image)
larger difference of chemical shifts for the longer chain seems to originate from larger difference of distance between either H₆ or H₇ and the carbonyl oxygen. Further discussion are possible from the following experiments with shift reagents.

Two series of ¹H-NMR spectra of TAG and TBG with addition of Eu(fod)$_3$ of 0.02-0.3 M are shown in Figs.-6 and -7, respectively. The dependence of chemical shifts on the amount of added Eu(fod)$_3$ are plotted in Figs.-8 and -9.

The singlet methyl peak in Fig.-6 is splitted into two peaks. The ratio of peak areas of the two peaks is 2:1 which is independent of the added amount of Eu(fod)$_3$. A similar phenomenon is observed at the methylene protons next to the carbonyl in the acyl group of TBG [Fig.-7(b)]. In both cases the peaks of methyl or methylene attached to the a, γ-esters shift more than those of the β-ester. The results suggest that coordination of europium is slightly more favorable for the less hindered secondary a, γ-esters than for the tertiary β-ester. The shift ratios of a, γ to β are 1:0.9 for methyl protons of TAG, and 1:0.96 for methylene protons of TBG.

The largest shift has been observed at H₂ which may be subjected to dipolar shifts by europium at one β- and two a and γ-esters. On the other hand, dipolar effects on H₆ or H₇ originate from one β- and only one a- or γ-positions. The effect of Eu(fod)$_3$ coordinated to a( or γ)-ester on γ (or a) –CH₃ of TBG and CH₃ of TAG are negligible because the distance between them is more than 7 Å. The difference of the acyl chains reflects on the two peaks of the anisochronous methylene protons, H₆ and H₇ in TAG, of which induced shifts are larger than those in TBG. The results are demonstrated as steeper slopes in Fig.-8 compared with those in Fig.-9. The steric effects caused by the acyl chains also account for the phenomenon that the peak of H₆ shifts down field crossing over that of H₇ of TBG (Fig.-7).

Acknowledgements

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References

8) G.H.W.M. Meulendijks, W. Van Es, J.W. de Haan, and H.M. Buck, Eur. J. Biochem., 157,
核磁気共鳴と分子力学の手法による
トリアシルグリセリんの
コンホーメーション解析
小杉 善雄・松原 康史

CDCl₃中、3種のトリグリセリド [C₆H₄ (OCOR)₃] ここで R=CH₃, n-C₃H₇, n-C₁₇H₃₅ の C-C-C 結合のまわりのコンホーメーションをプロトン NMR と分子力学計算（改良 QCMP/MM2 力場計算法）によって検討した。両手法によって求めたコンホーメーションの割合は良く一致した。アシル鎖の長さに関係なく、グリセリン単位の3つの酸素はゴーシュ型である。もっとも好ましいコンホーメーションは、C-C-C 結合について trans-trans 配置であり、可能な低エネルギーのコンホーメーションの約 20%を占めることができた。非等時性のメチレンプロトンとグリセリン単位に及ぼすアシル鎖の長さの影響について Ed(fod)₃ に存在するランダニド誘起シフトによって明確にした。

国際油脂情報
☆アメリカの大麦育種研究者、アルゼンチンで受賞
5人のアメリカの大麦育種研究者で、世界の大麦生産に貢献したとの理由で、世界大麦研究会議において受賞し。E. Hartwig, R. Bernard, R. Cooper, J. Wilcox（いずれもアメリカ農務省）、及びアーカンソー大学の C. Caviness の5氏が銀鎄の額を受けとった。ブエノスアイレスで開催された3月の大会には、760人以上の参加者のもと、約500の研究発表が行われた。
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☆栄養モニター法案導入
新栄養モニター法案が、テキサス州民主党議員 K. Garza 氏とイリノイ州共和党議員 E.R. Madigan 氏によって、アメリカ下院に提出された。包括全米栄養モニター制度法案は、レーガン前大統領によって拒否された法律と同様な内容である。新法案は同国の食習慣を10年間にわたって評価を要求するもので、高年層、若年層及び家族を持たない人々を対象とするものとなる。