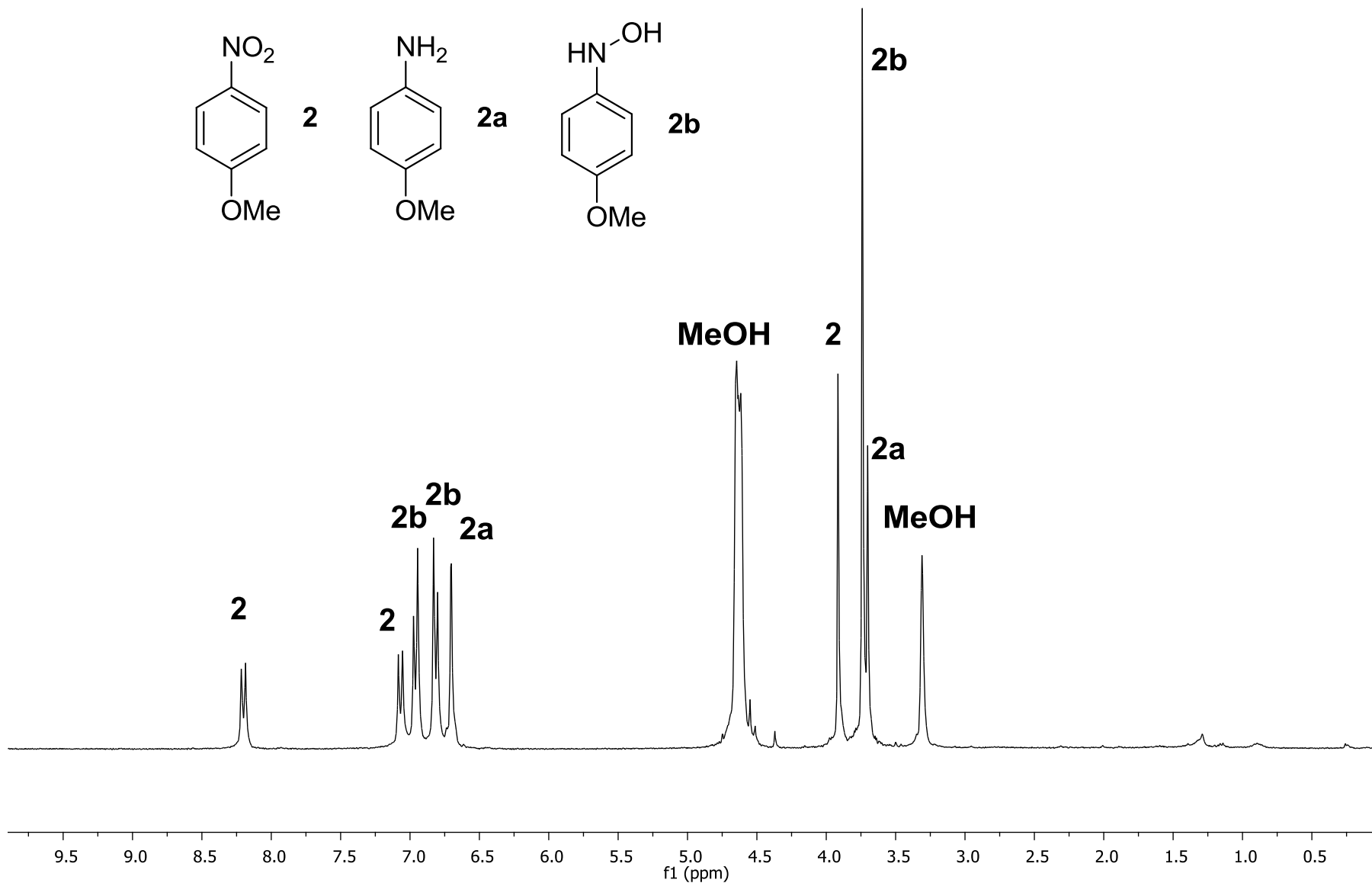
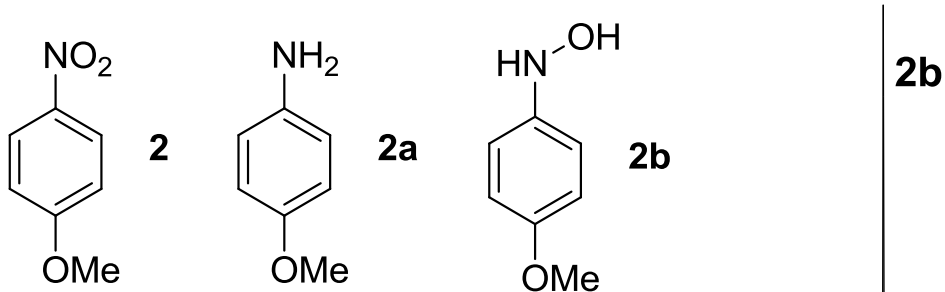
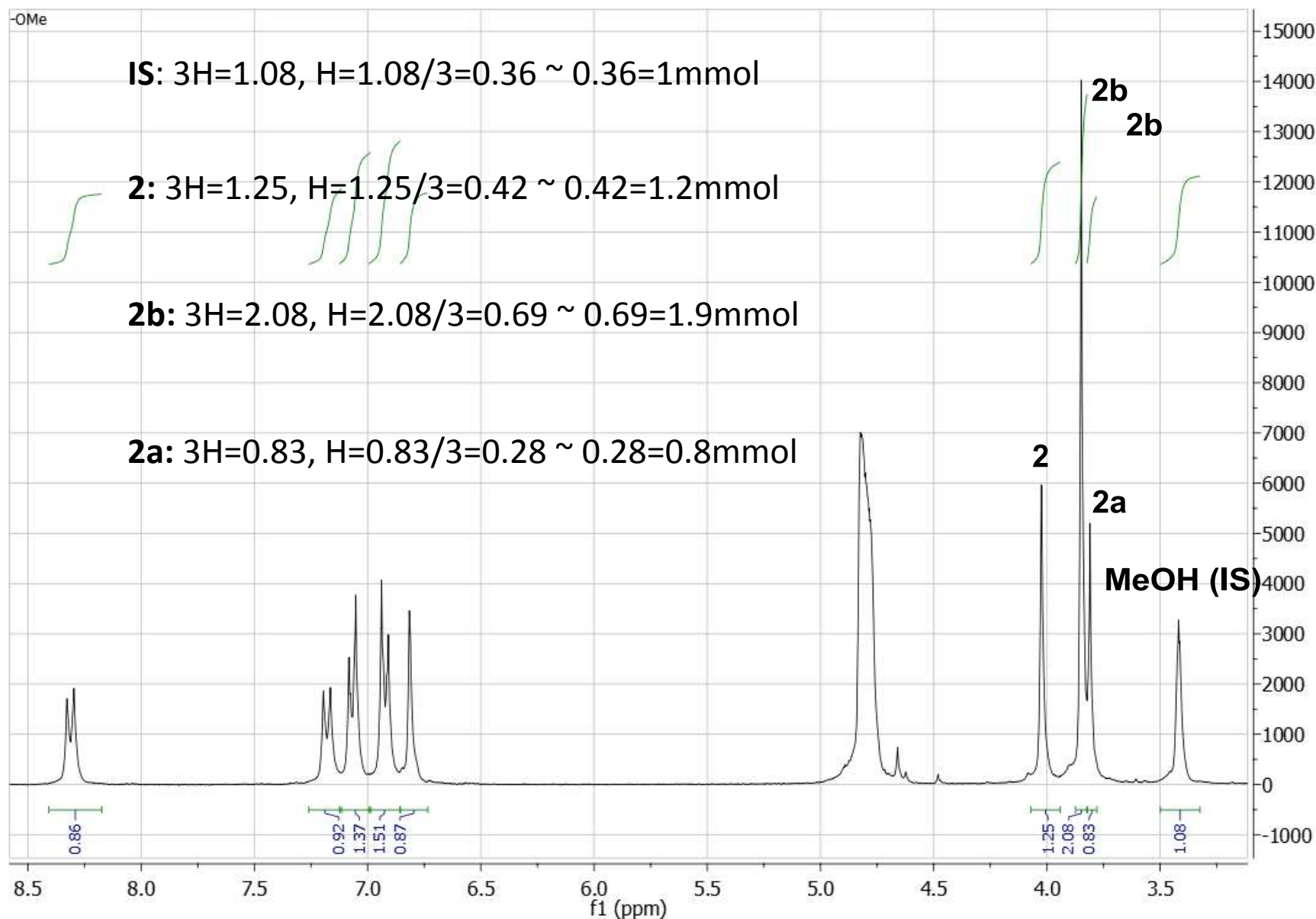


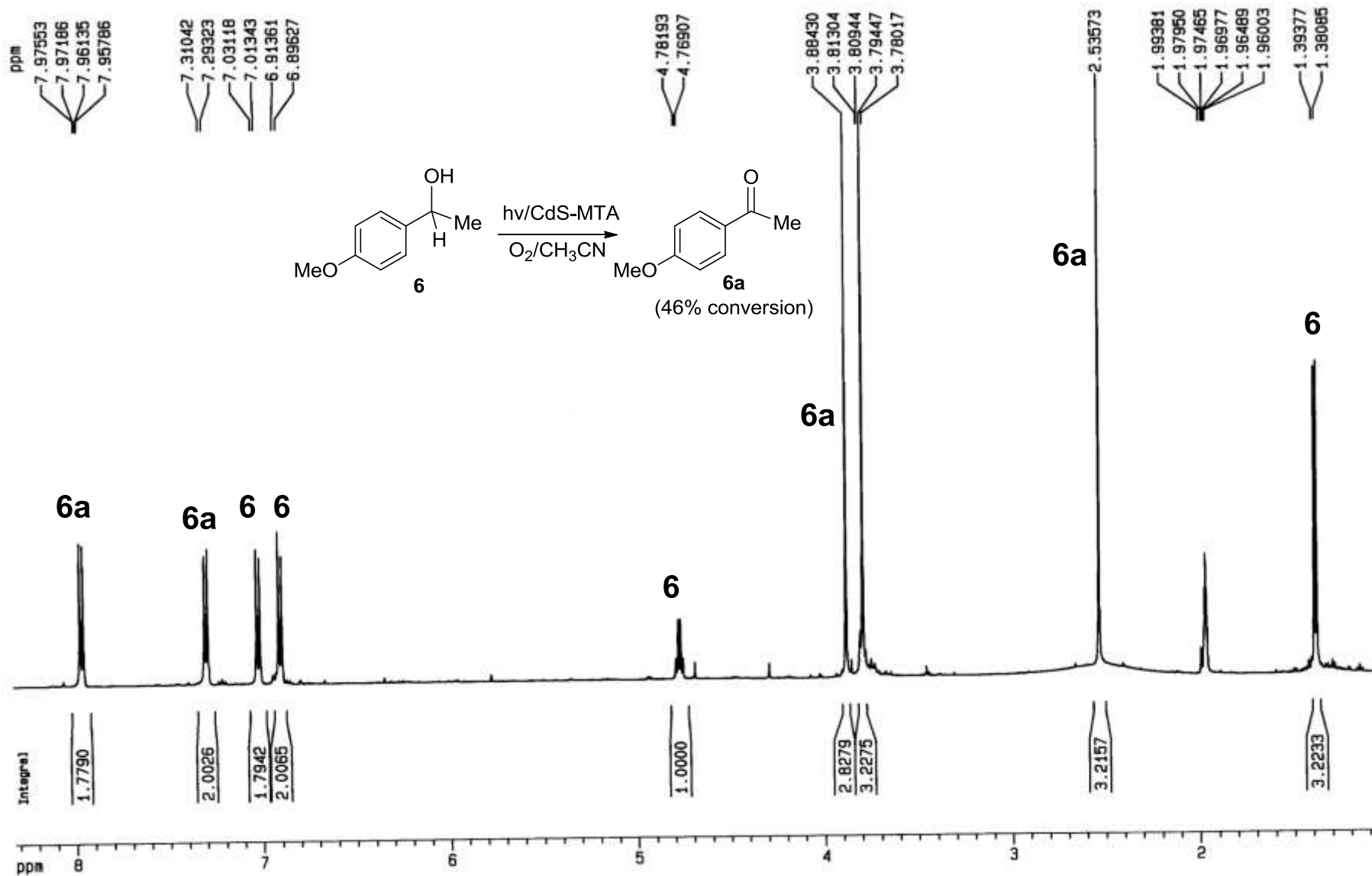
# $^1\text{H-NMR}$ Φασματοσκοπία: ποσοτικοποίηση



# $^1\text{H-NMR}$ Φασματοσκοπία: ποσοτικοποίηση



# $^1\text{H-NMR}$ Φασματοσκοπία: ποσοτικοποίηση



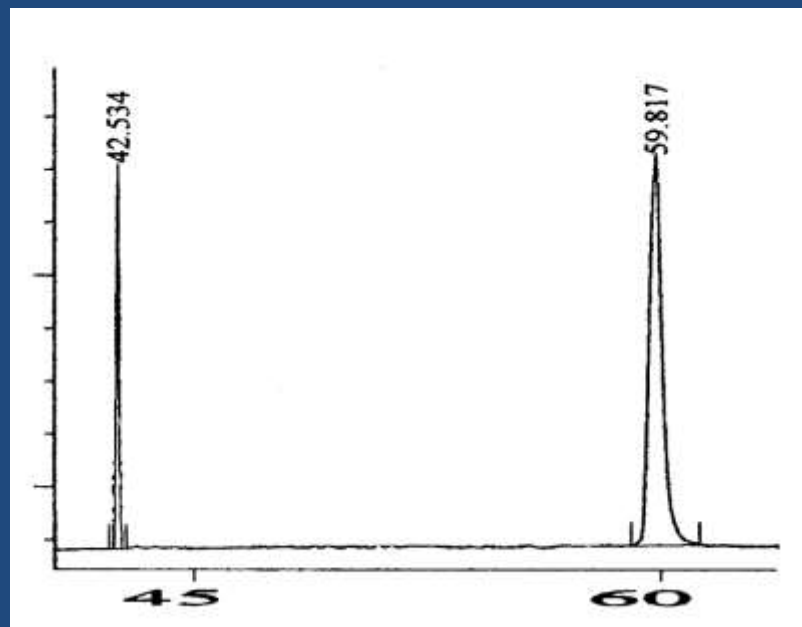
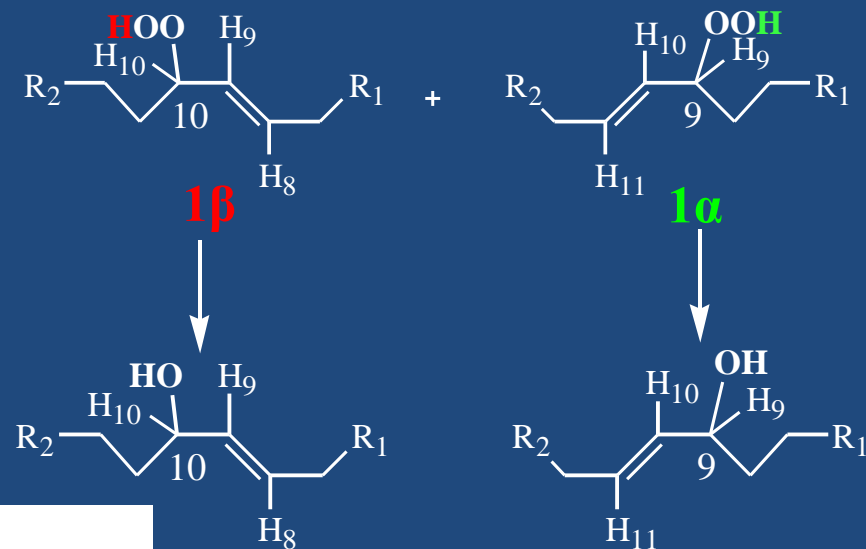
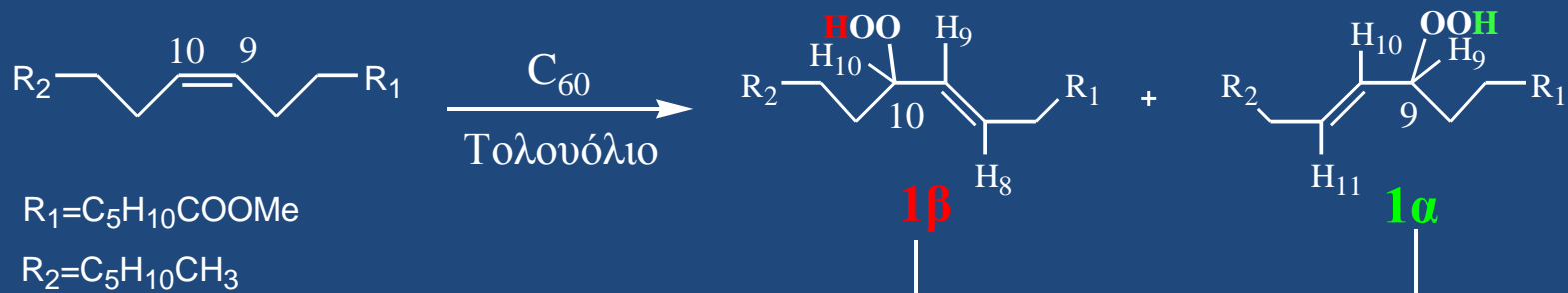
# Δομή Μερικών Λιπαρών Οξέων

| Όνομασία         | Αρ. Ατόμων<br>Άνθρακα | Δομή  |
|------------------|-----------------------|---|
| <b>Κορεσμένα</b> |                       |   |
| Παλμιτικό        | 16                    | $\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$  |
| Στεατικό         | 18                    | $\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$  |
| Αραχιδικό        | 20                    | $\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$  |
| <b>Ακόρεστα</b>  |                       |   |
| Παλμιτελαϊκό     | 16                    | $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$ (cis)                               |
| Ελαϊκό           | 18                    | $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$ (cis)                               |
| Λινελαϊκό        | 18                    | $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$<br>(cis,cis) |
| Αραχιδονικό      | 20                    | $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_4\text{CH}_2\text{CH}_2\text{COOH}$<br>(όλοι cis)        |

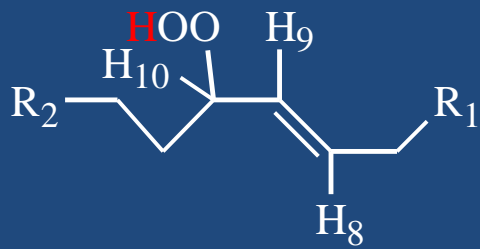
# Περιεκτικότητα λιπών και ελαίων σε λιπαρά οξέα

| Πηγή                | <u>Κορεσμένα Λιπαρά Οξέα (%)</u> |                              |                             | <u>Ακόρεστα Λιπαρά Οξέα (%)</u> |                              |
|---------------------|----------------------------------|------------------------------|-----------------------------|---------------------------------|------------------------------|
|                     | C <sub>14</sub><br>Μυριστικό     | C <sub>16</sub><br>Παλμιτικό | C <sub>18</sub><br>Στεατικό | C <sub>18</sub><br>Ελαϊκό       | C <sub>18</sub><br>Λινελαϊκό |
| <b>Ζωικά Λίπη</b>   |                                  |                              |                             |                                 |                              |
| Λαρδί               | 1                                | 25                           | 15                          | 50                              | 6                            |
| Βούτυρο             | 11                               | 25                           | 11                          | 25                              | 5                            |
| Ανθρώπινο<br>λίπος  | 4                                | 25                           | 8                           | 46                              | 10                           |
| <b>Φυτικά Έλαια</b> |                                  |                              |                             |                                 |                              |
| Αραβοσιτέλαιο       | 1                                | 10                           | 4                           | 35                              | 45                           |
| Ελαιόλαδο           | 1                                | 5                            | 5                           | 80                              | 7                            |
| Φυστικέλαιο         | -                                | 7                            | 5                           | 60                              | 20                           |
| Λινέλαιο            | -                                | 5                            | 3                           | 20                              | 20                           |

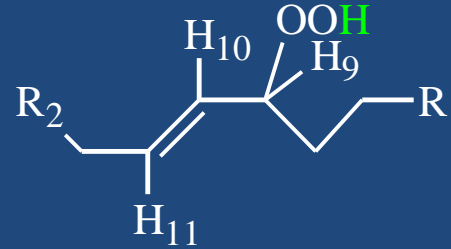
# Ανάλυση υπεροξειδίων του ελαϊκού μεθυλεστέρα με GC



# Φάσμα $^1\text{H-NMR}$ Προϊόντων Φωτοξείδωσης του 1 με Φωτοευαισθητοποιητή $\text{C}_{60}$



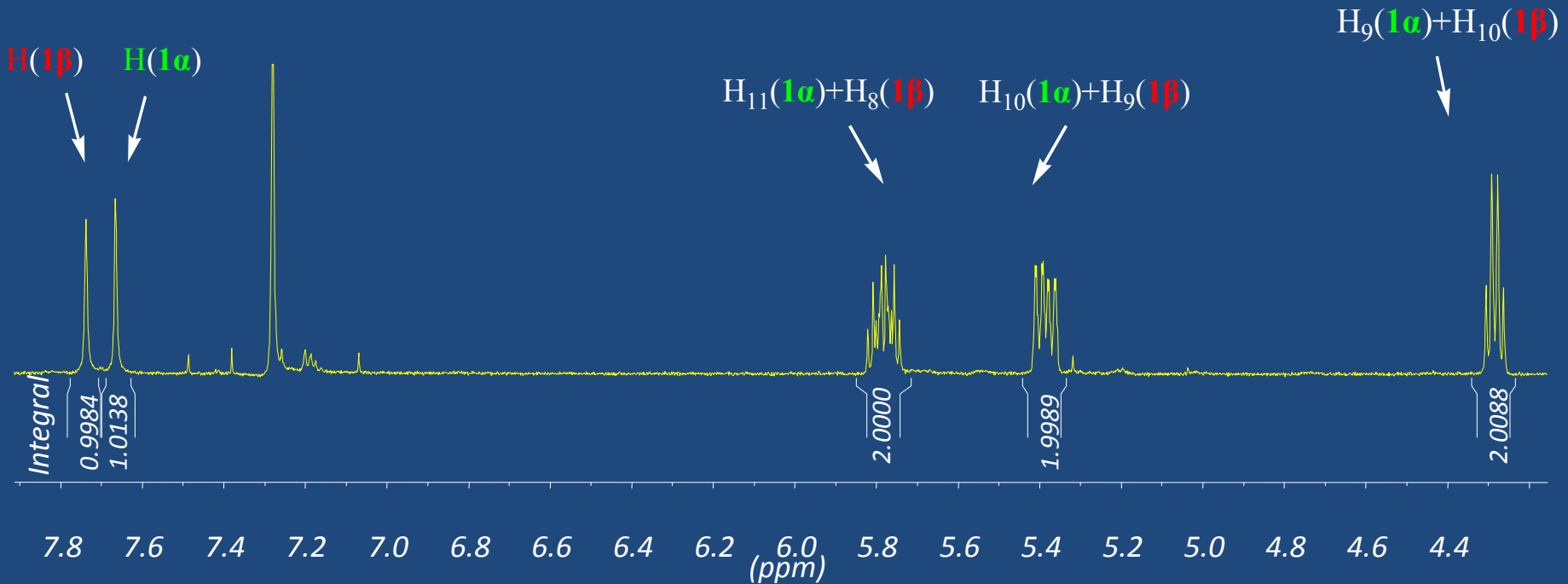
**1β**



**1α**

$\text{R}_1 = \text{C}_5\text{H}_{10}\text{COOMe}$

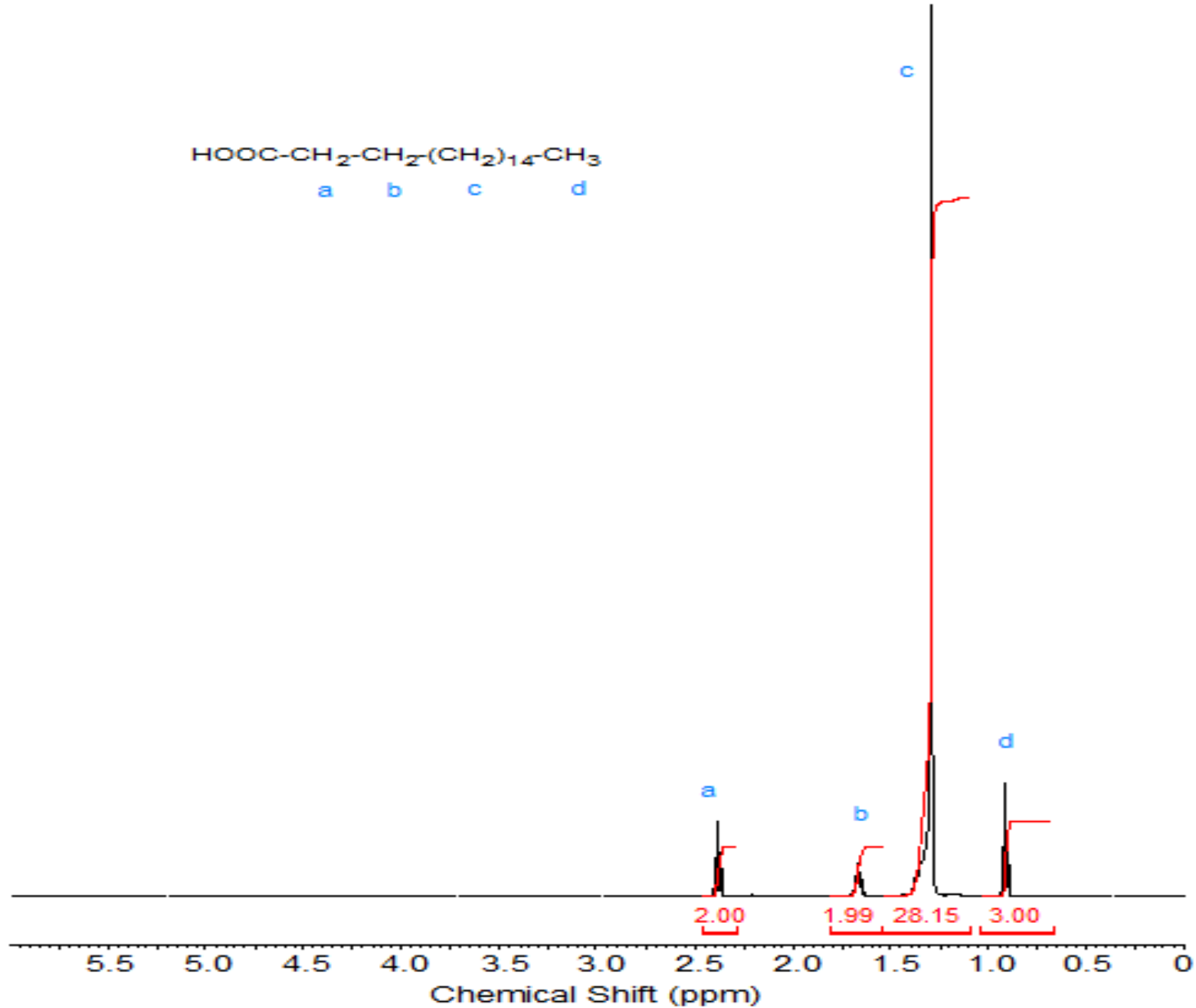
$\text{R}_2 = \text{C}_5\text{H}_{10}\text{CH}_3$



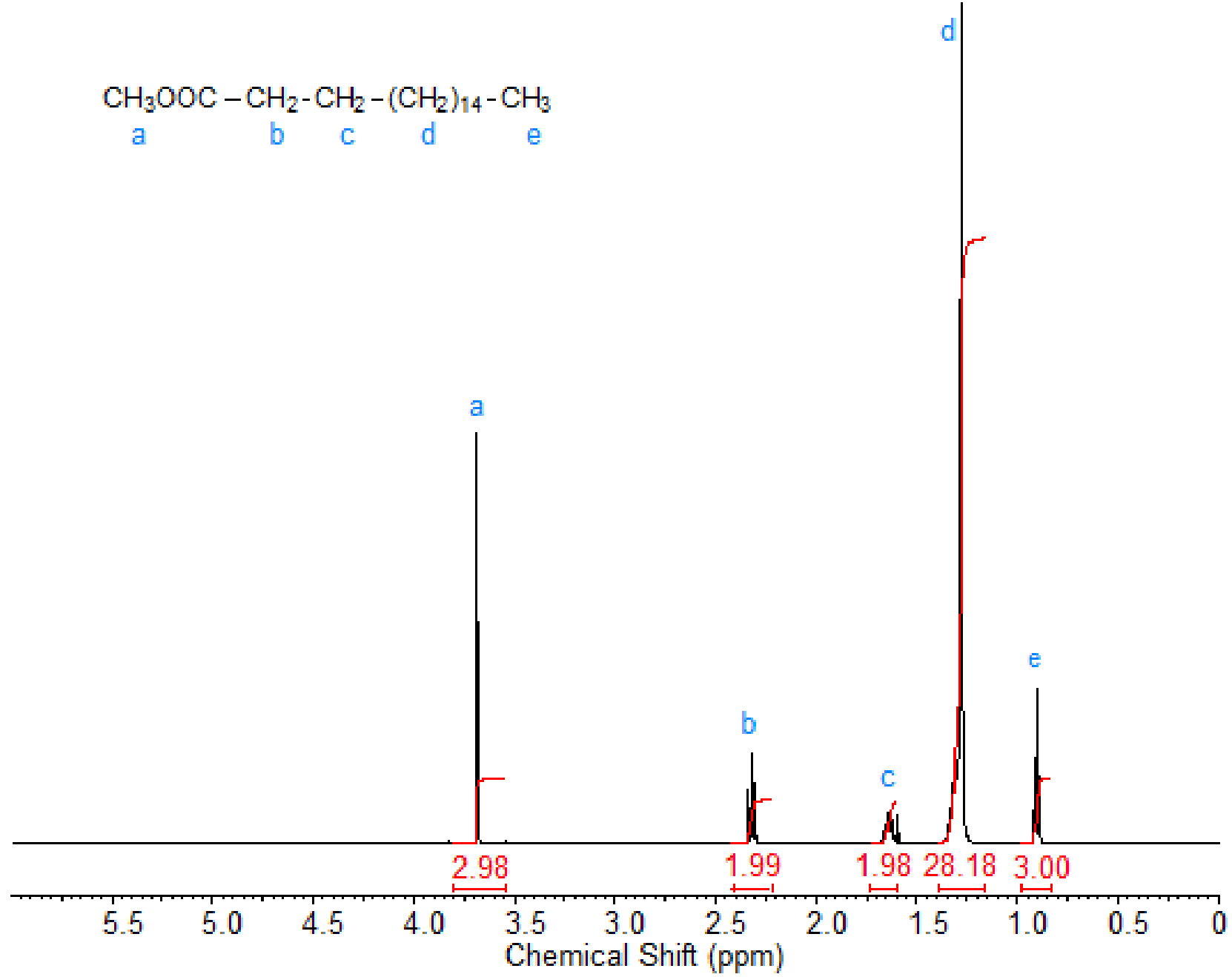
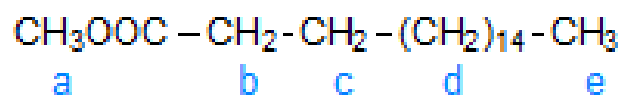
# **$^1\text{H}$ -NMR SPECTROSCOPY OF FATTY ACIDS AND THEIR DERIVATIVES**

<http://lipidlibrary.aocs.org/nmr/nmr.html>

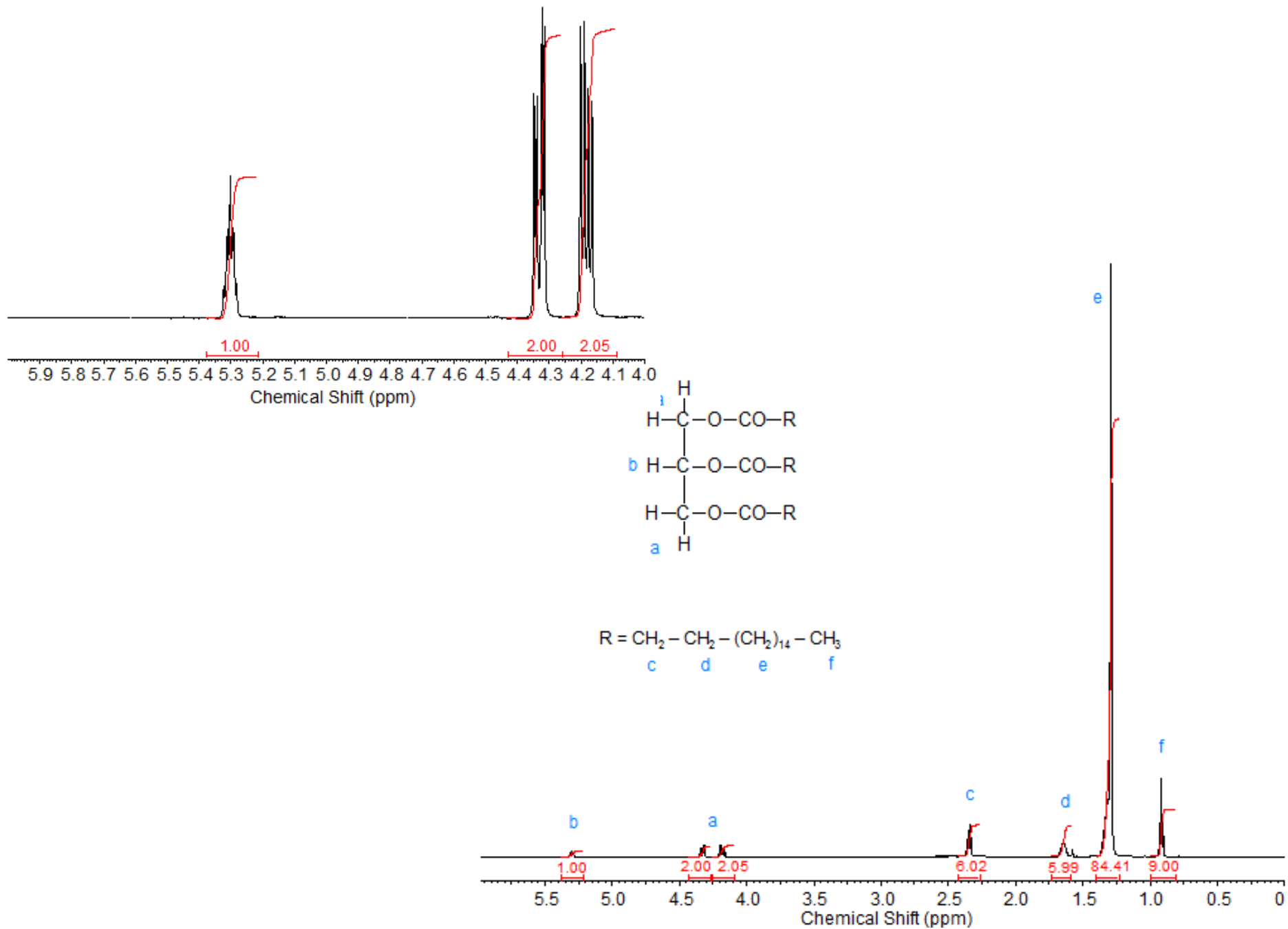




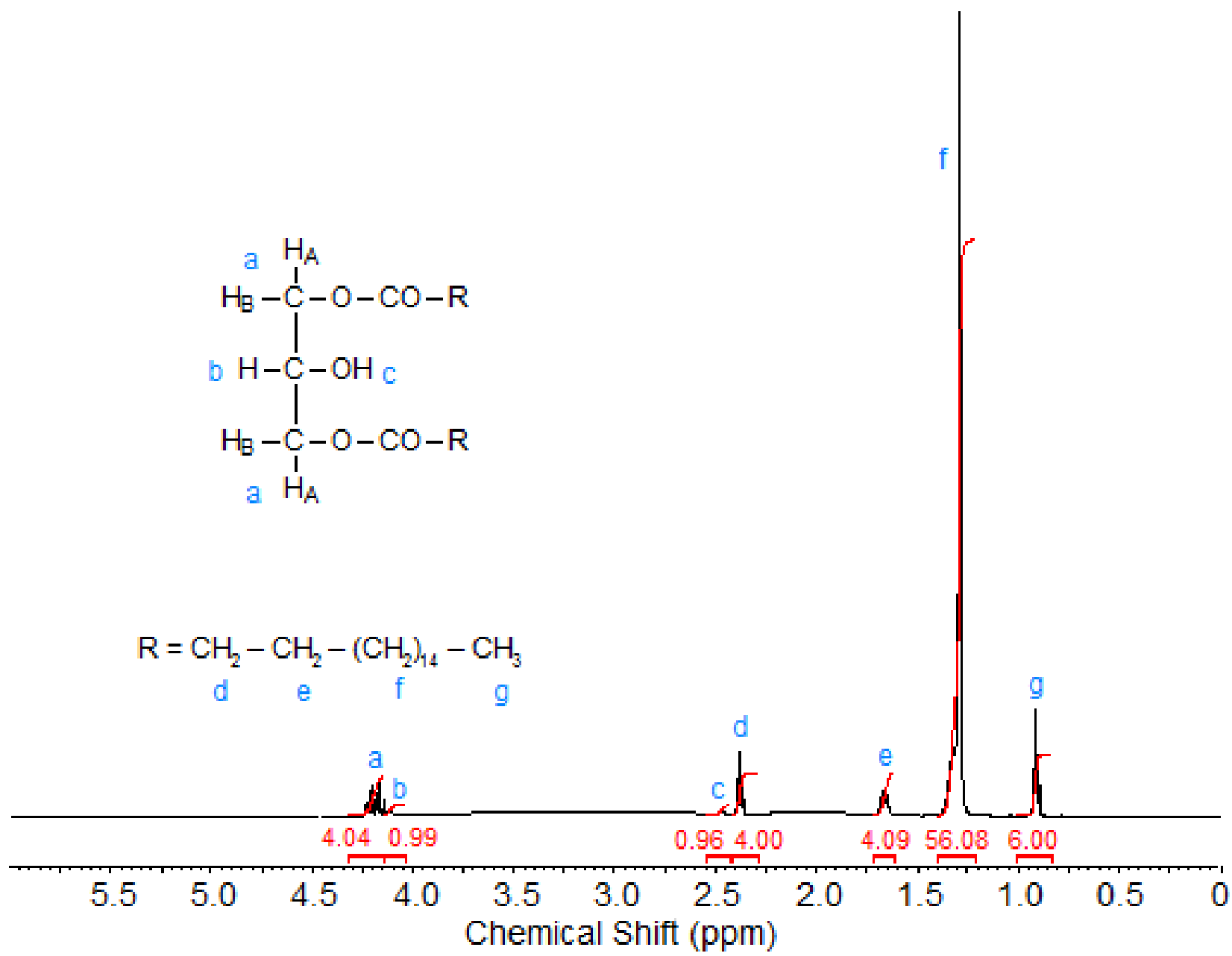
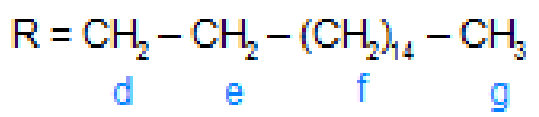
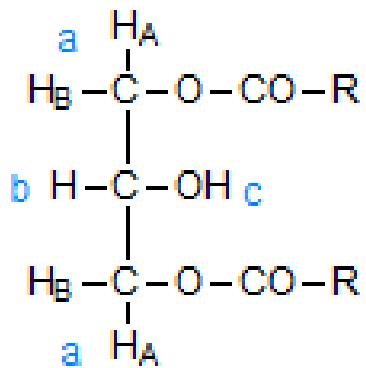
$^1\text{H-NMR}$  spectrum of stearic acid.



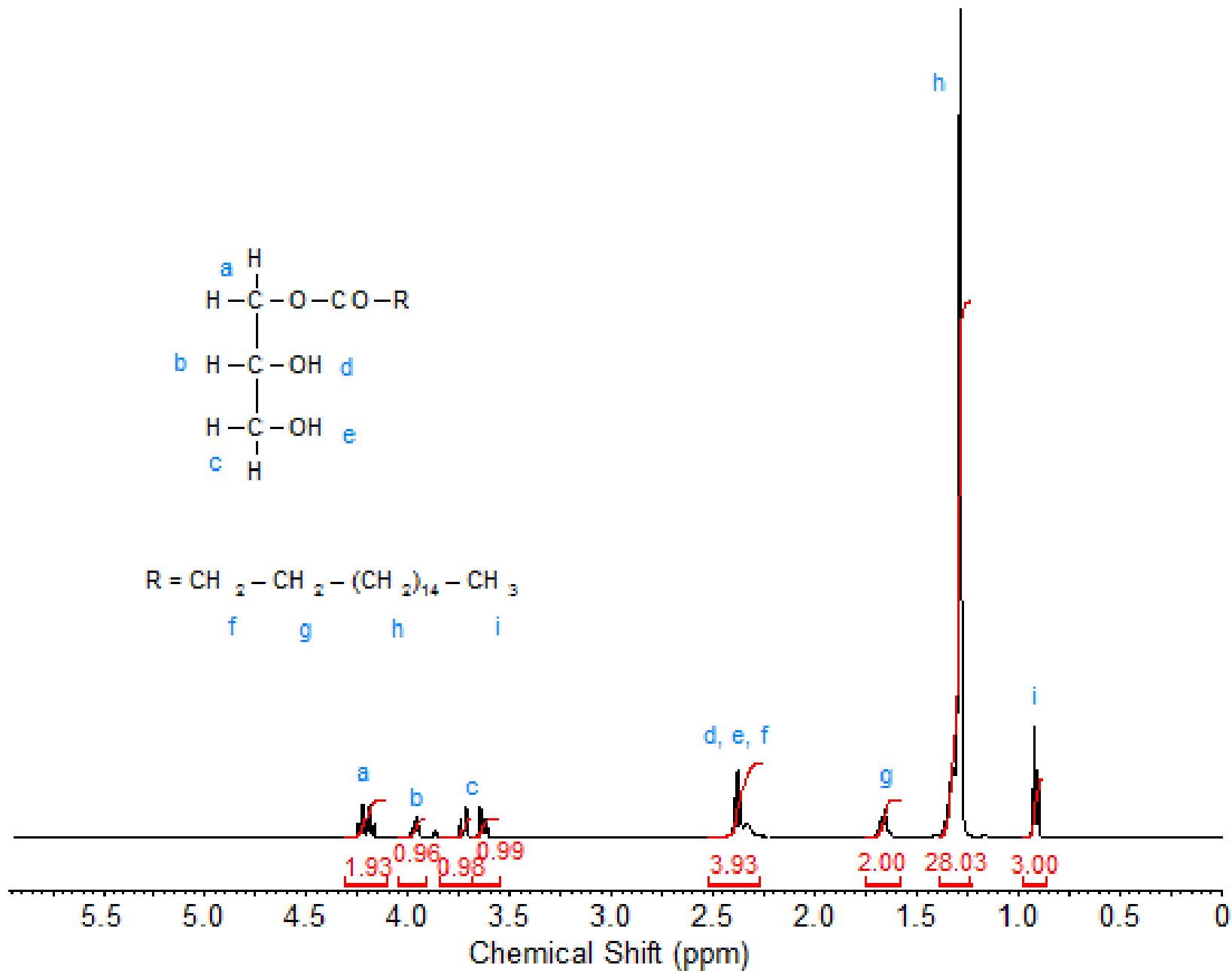
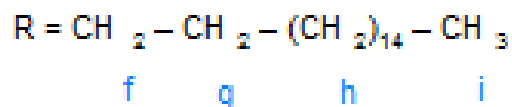
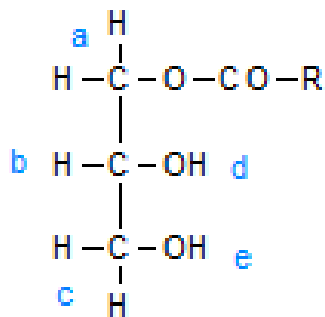
<sup>1</sup>H-NMR spectrum of methyl stearate



$^1\text{H}$ -NMR spectrum of tristearin

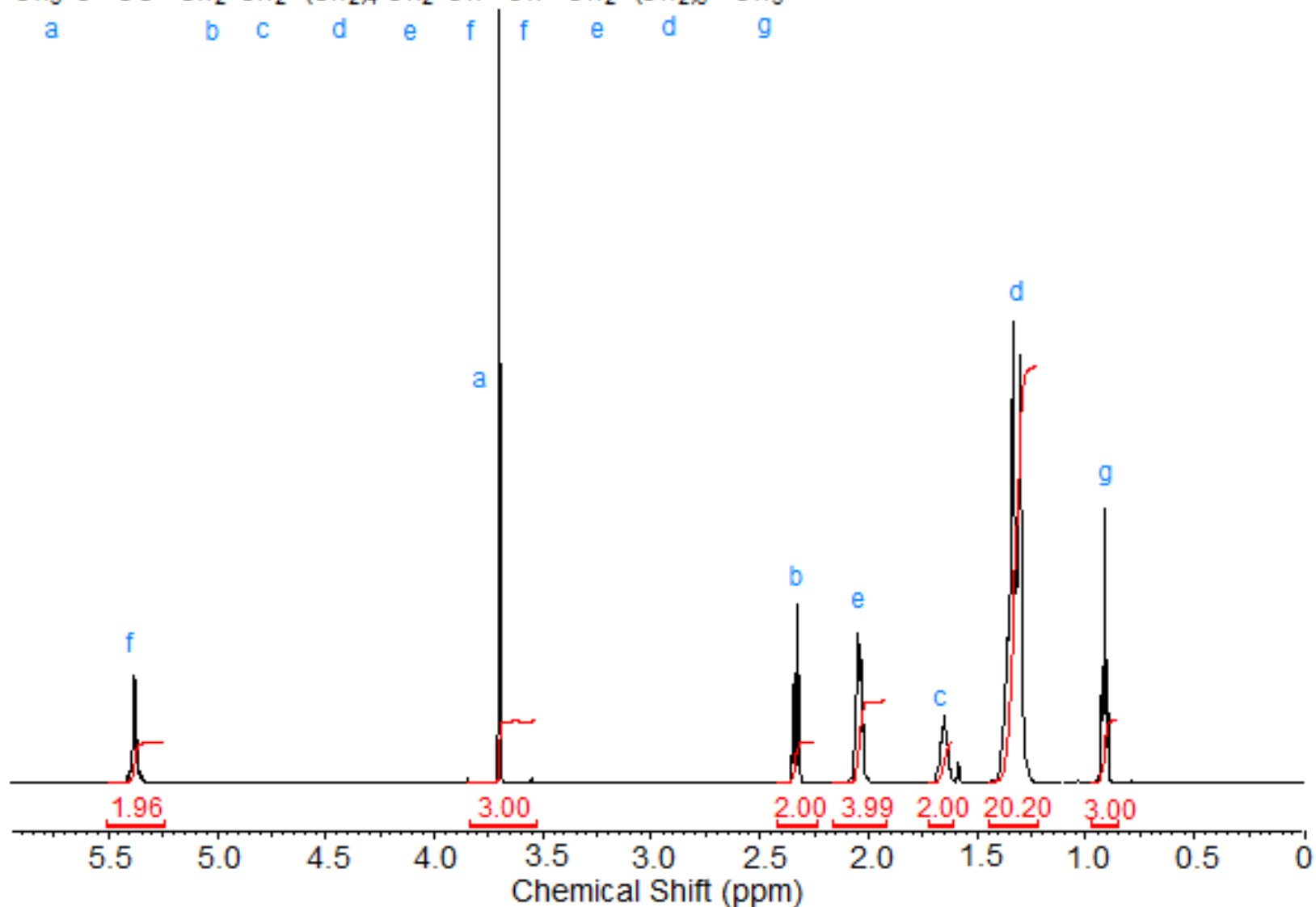
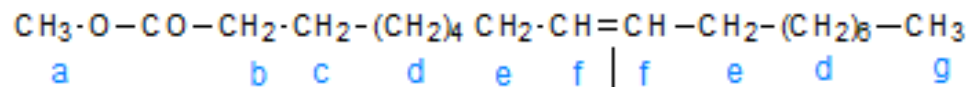


<sup>1</sup>H-NMR spectrum of 1,3-distearin.

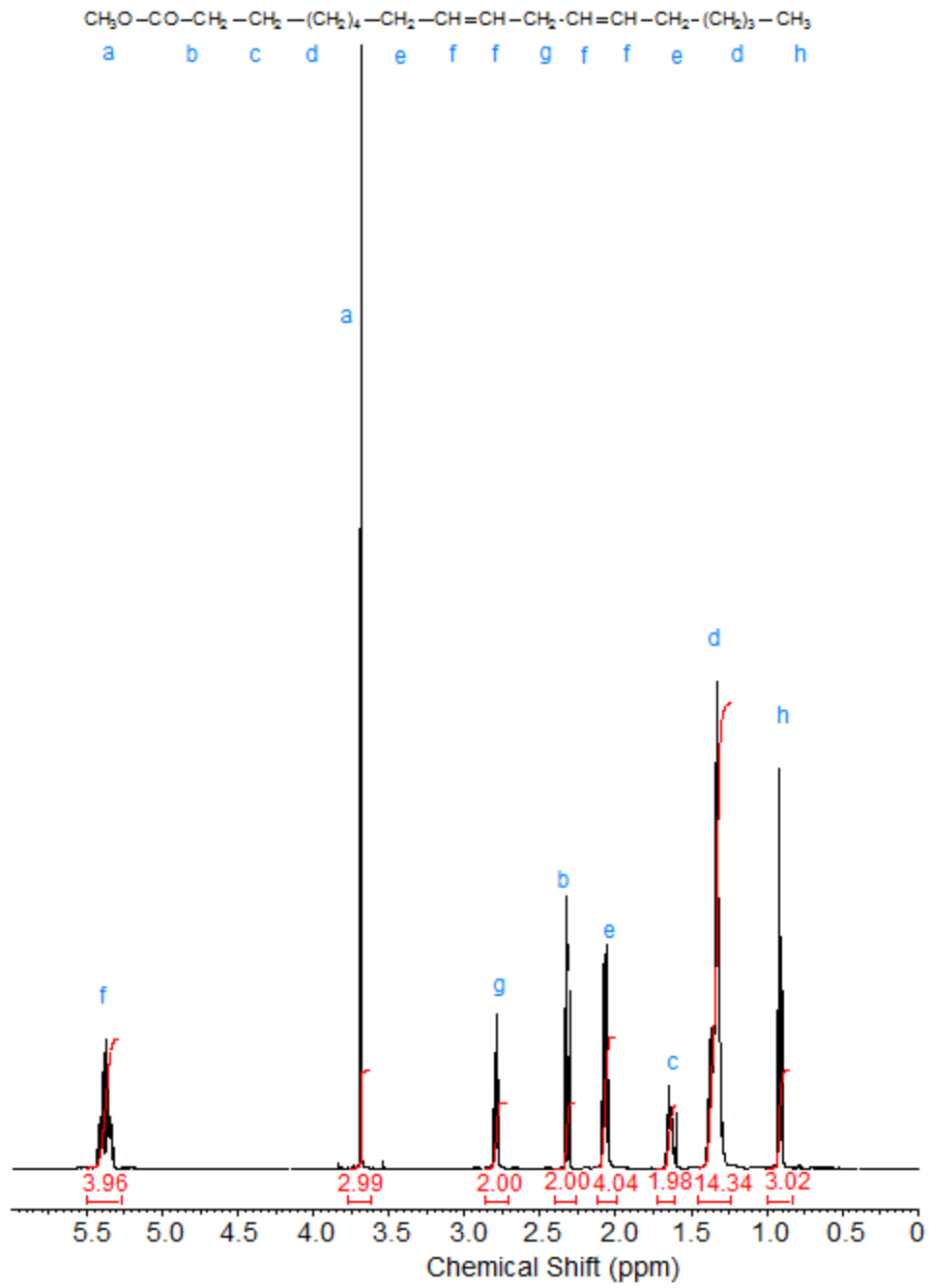


$^1\text{H}$ -NMR spectrum of 1-monostearin.

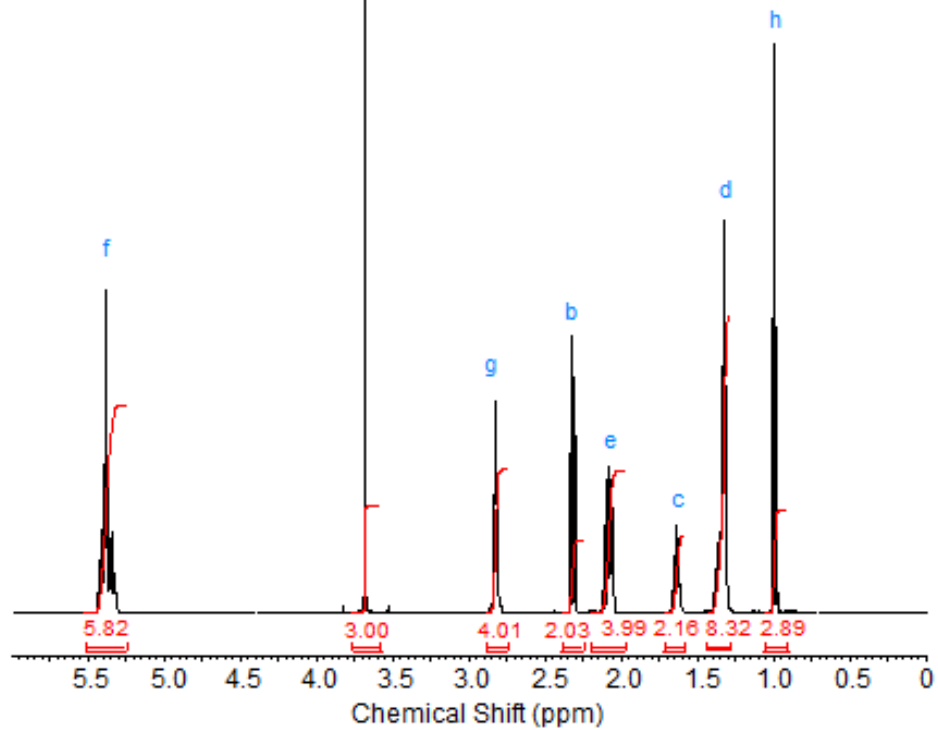
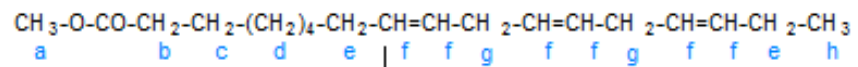
# Non-Conjugated Double Bonds



$^1\text{H-NMR}$  spectrum of methyl oleate



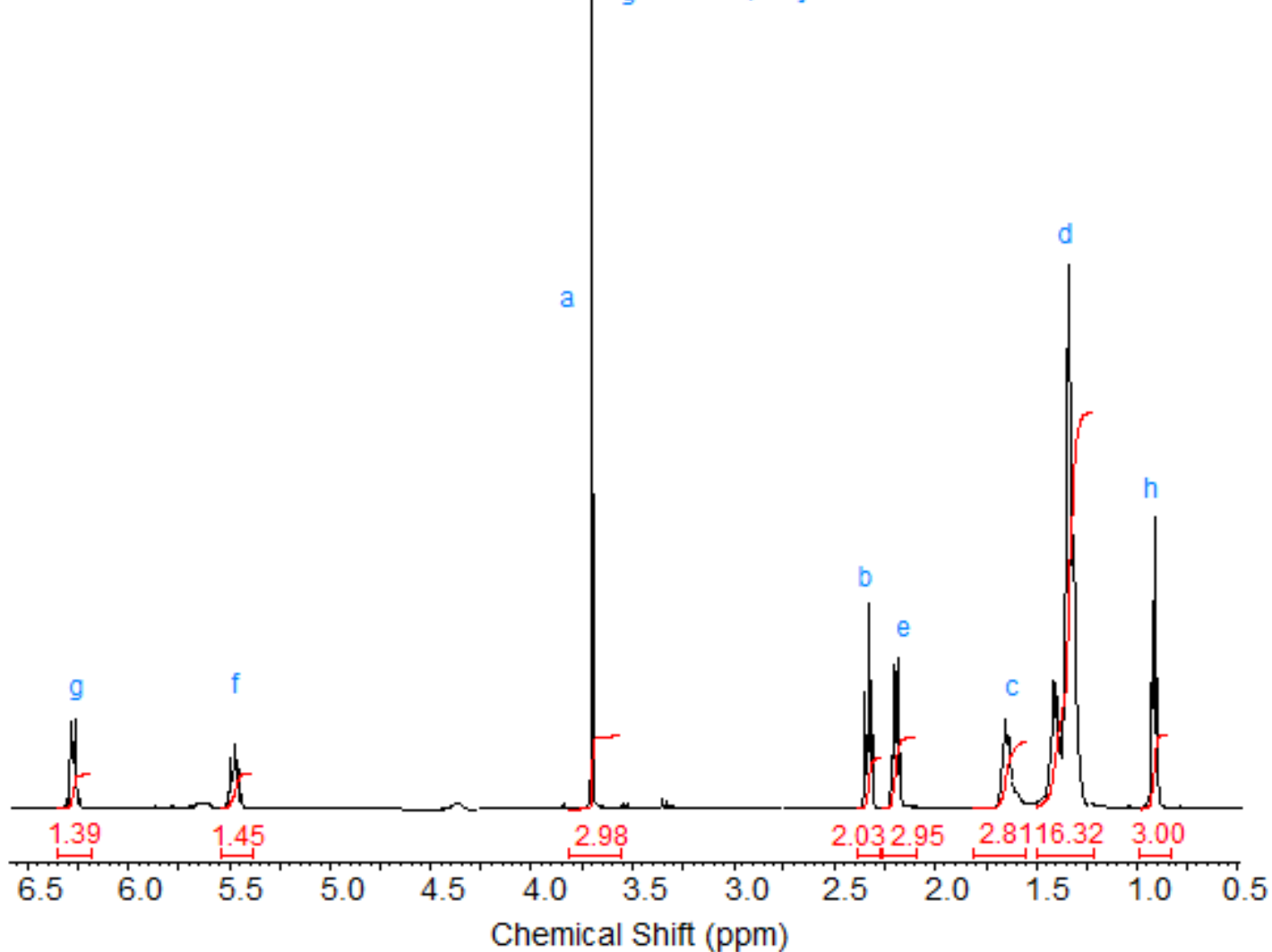
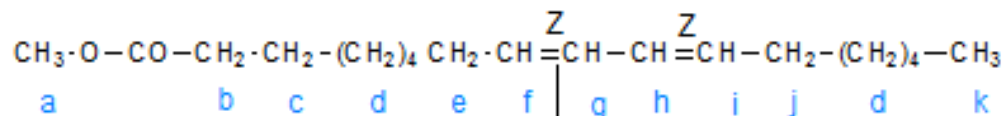
$^1\text{H}$ -NMR spectrum of methyl linoleate.



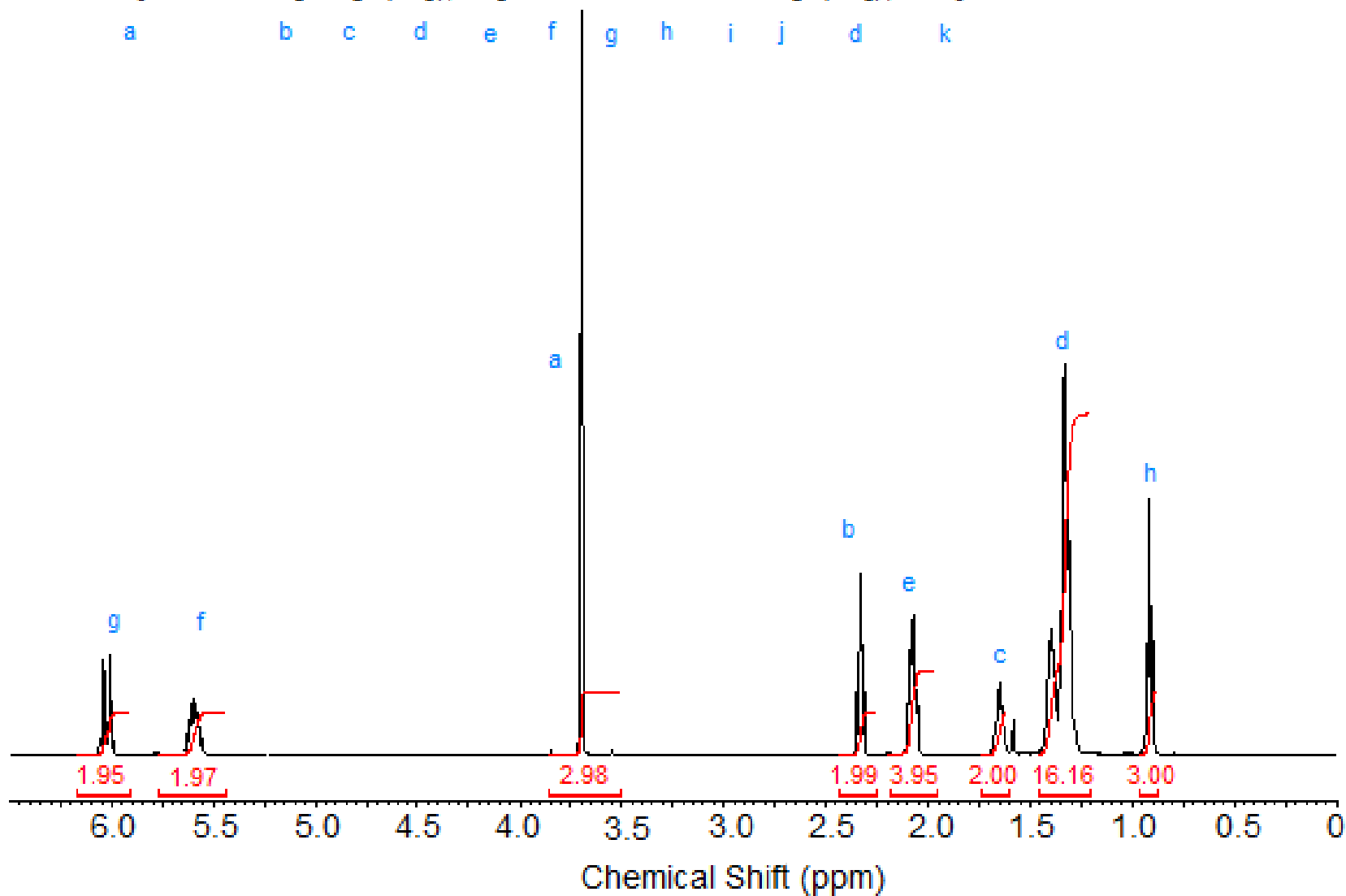
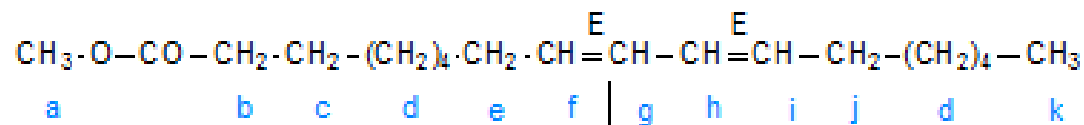
$^1\text{H-NMR}$  spectrum methyl  $\alpha$ -linolenate



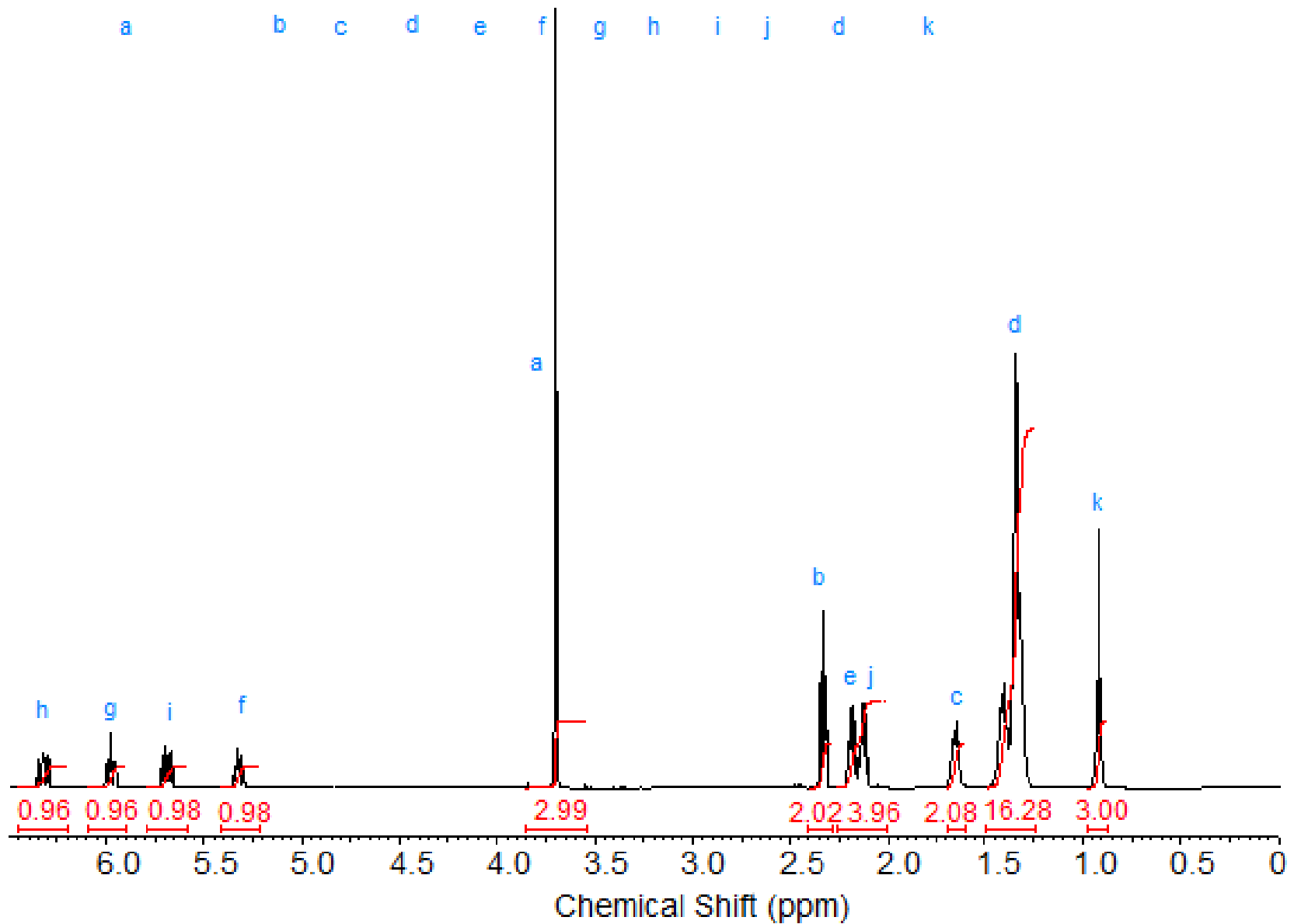
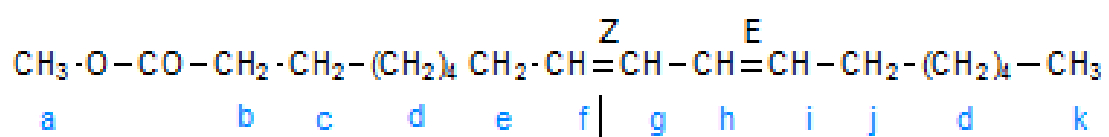
# Coniugated Linoleic Acid (CLA)



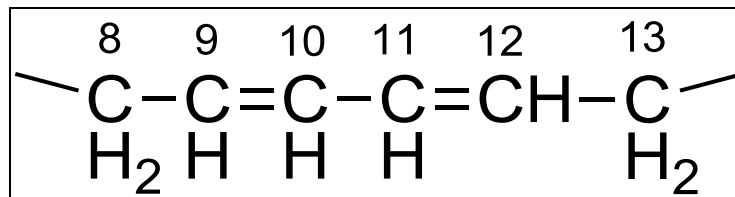
<sup>1</sup>H-NMR spectrum of methyl 9(Z),11(Z)-octadecadienoate



<sup>1</sup>H-NMR spectrum of methyl 9(*E*),11(*E*)-octadecadienoate



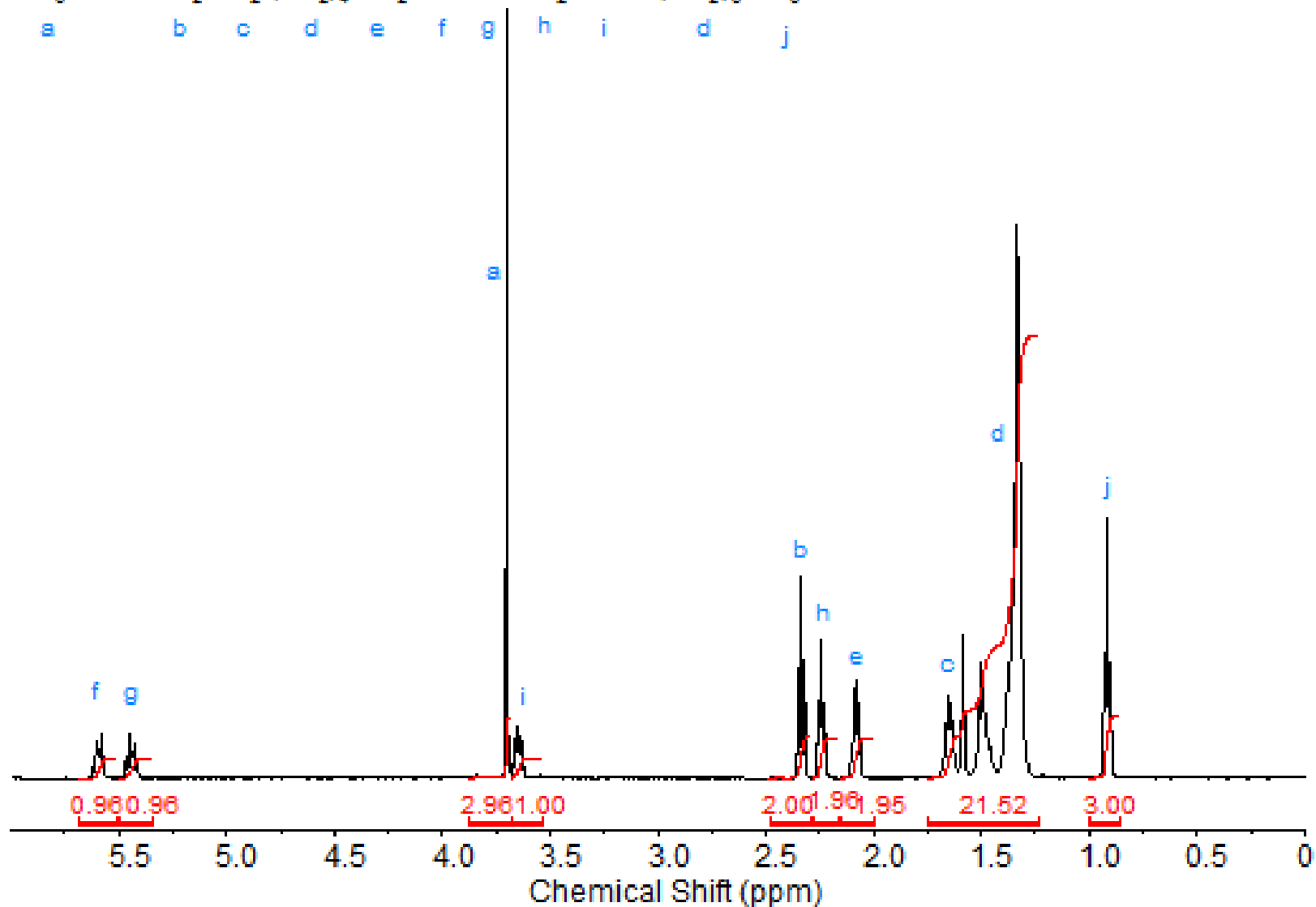
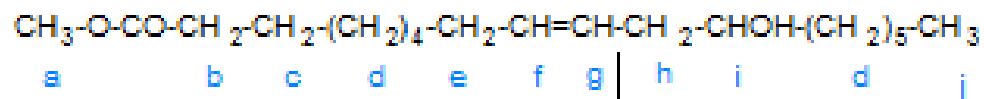
<sup>1</sup>H-NMR spectrum methyl 9(Z),11(E)-octadecadienoate.



**Assignments of characteristic signals in the spectra of 9,11-octadecadienoic acid isomers:**

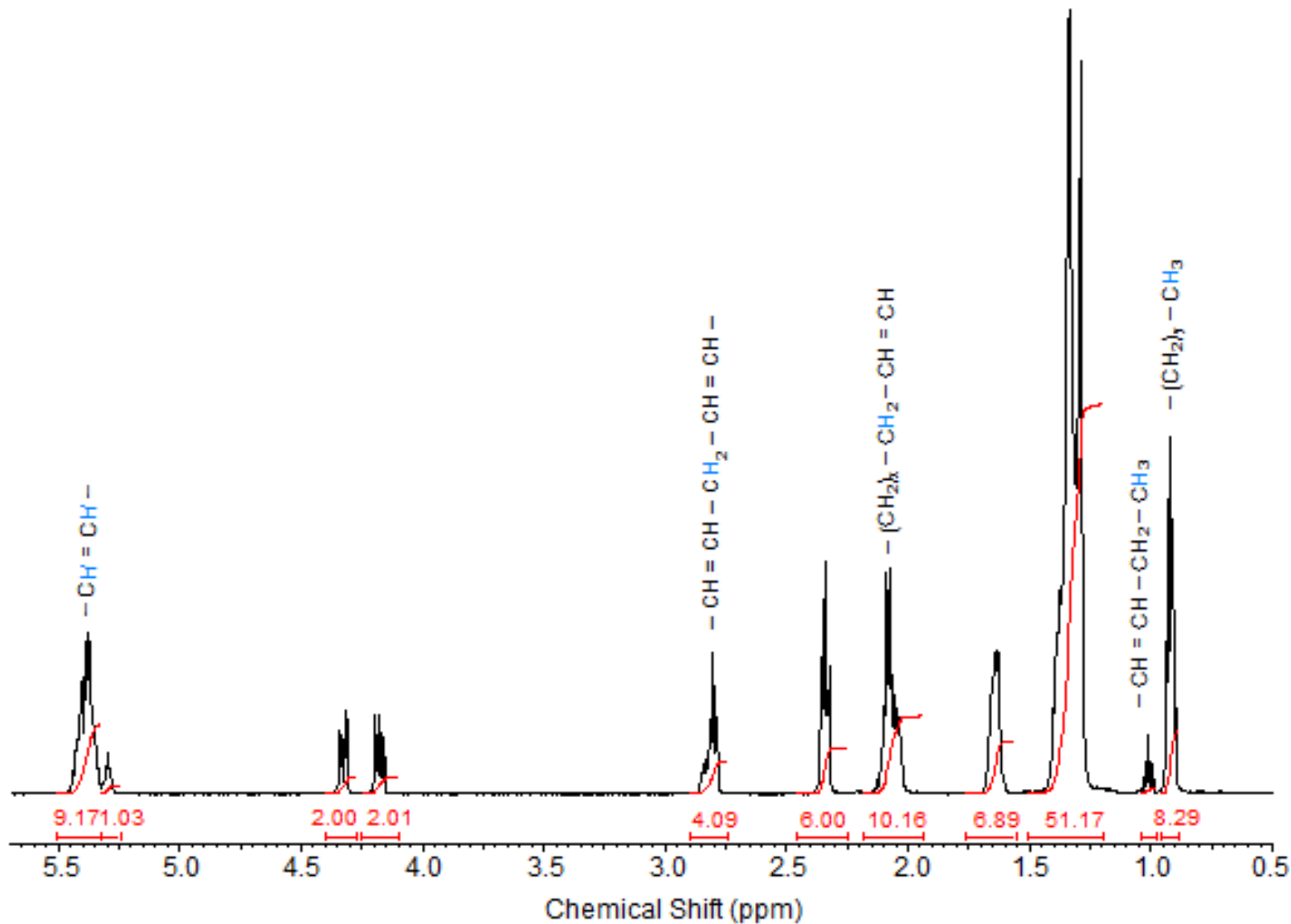
|        | Allylic protons (C8, C13)                     | C9   | C10  | C11  | C12  |
|--------|---|------|------|------|------|
| 9Z,11Z | 2.08  | 5.47 | 6.27 | 6.27 | 5.47 |
| 9E,11E | 2.20  | 5.59 | 6.03 | 6.03 | 5.59 |
| 9Z,11E | 2.18 ( <i>cis</i> ),<br>2.13 ( <i>trans</i> ) | 5.32 | 5.97 | 6.34 | 5.69 |

# Unsaturated Hydroxy Fatty Acids



<sup>1</sup>H-NMR spectrum of methyl ricinoleate

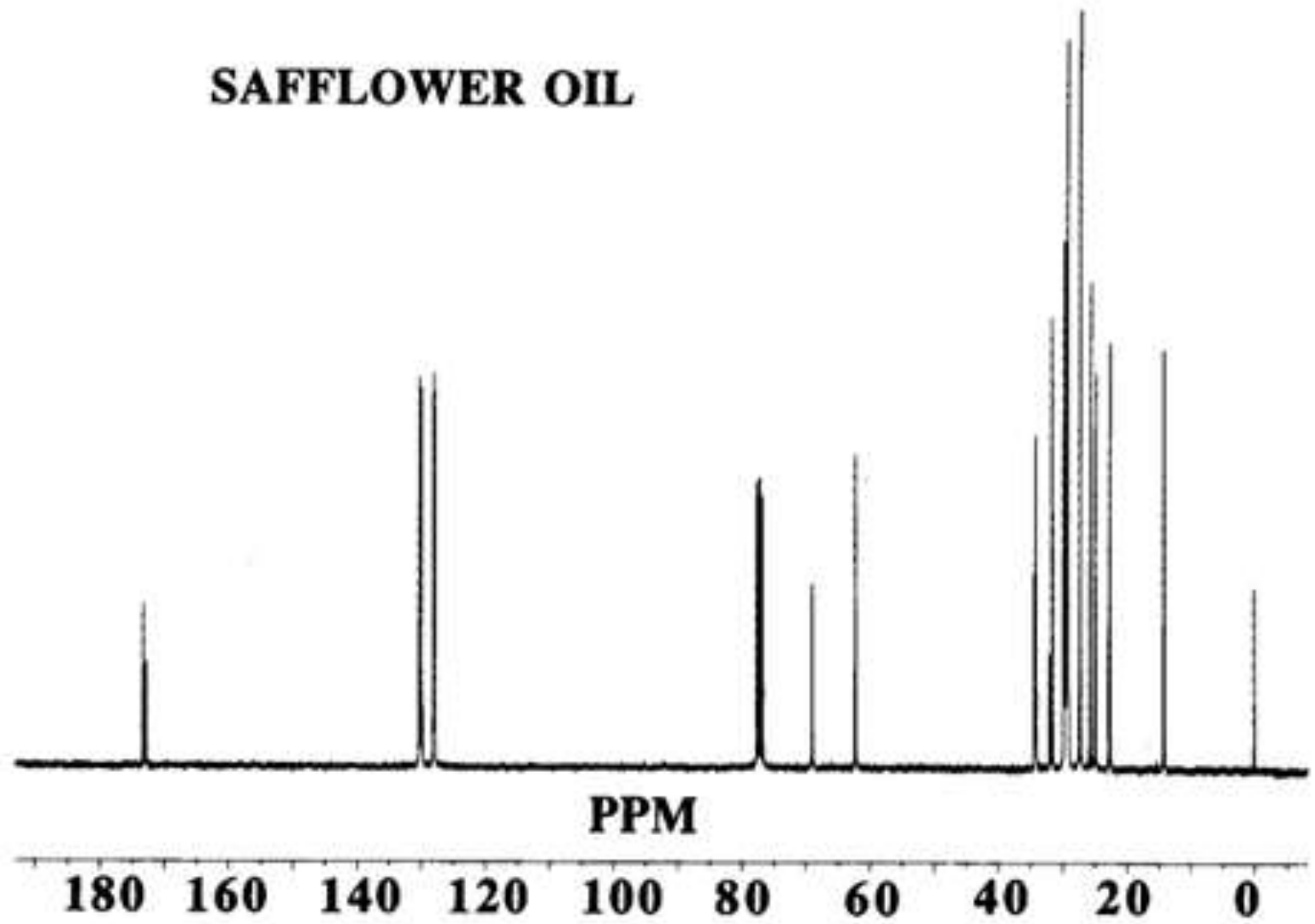
# Quantification by $^1\text{H-NMR}$



$^1\text{H-NMR}$  spectrum of soybean oil

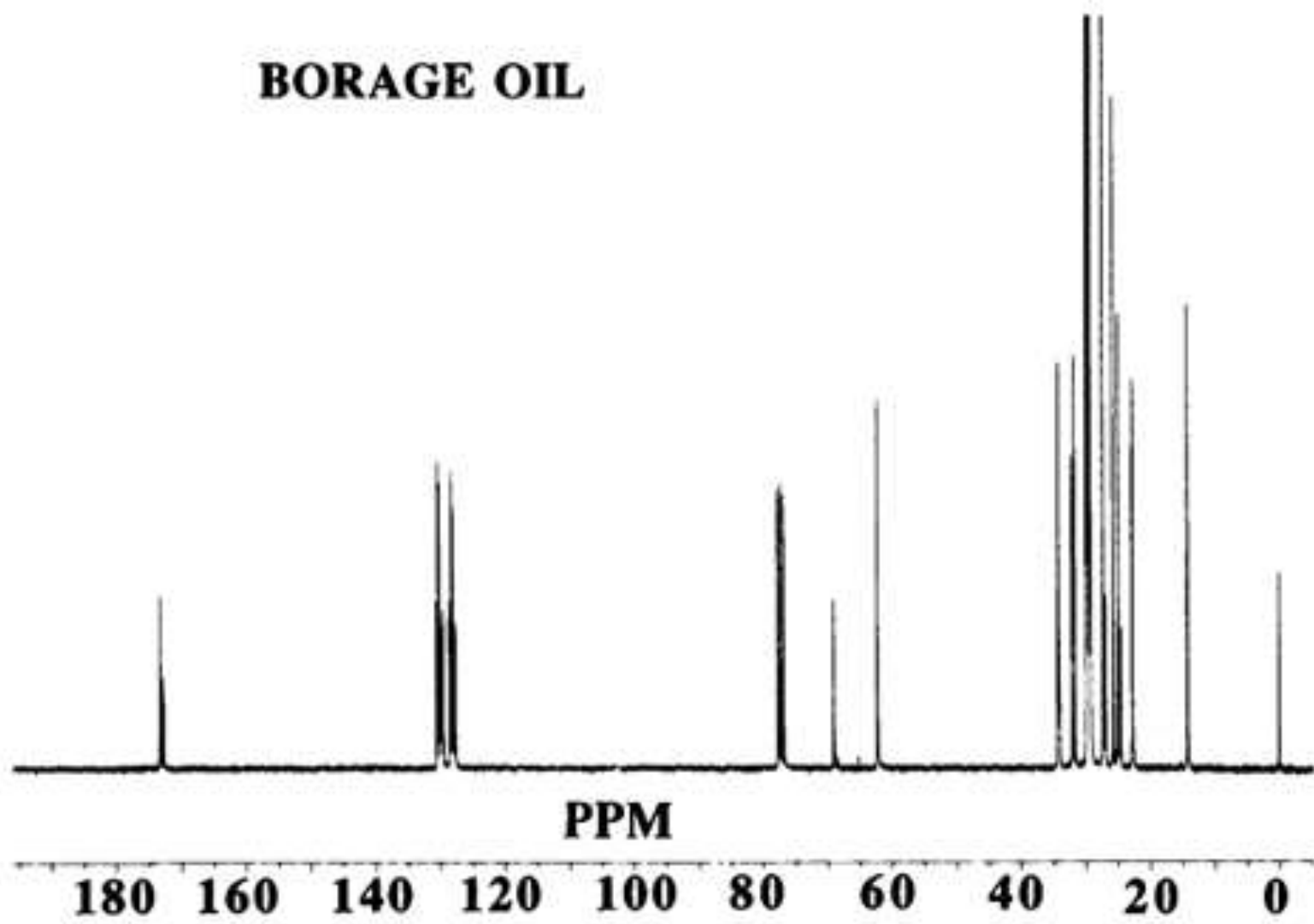
# **$^{13}\text{C}$ -NMR SPECTROSCOPY OF FATTY ACIDS AND DERIVATIVES**

# SAFFLOWER OIL





# BORAGE OIL



**Chemical shifts (ppm) for *cis* and *trans* triacylglycerols containing 5-14:1 and 9-18:1 (are taken from more extensive data provided by Lie Ken Jie *et al.* and by Mannina *et al.***

|            | 5-14:1 <i>cis</i> | 5-14:1 <i>trans</i> | 9-18:1 <i>cis</i> | 9-18:1 <i>trans</i> |
|------------|-------------------|---------------------|-------------------|---------------------|
| 1          | 173.112, 172.718  | 173.154, 172.751    | 173.224, 172.819  | 173.281, 172.869    |
| 2          | 33.443, 33.631    | 33.348, 33.515      | 34.041, 34.206    | 34.055, 34.219      |
| 3          | 24.828, 24.894    | 24.705, 24.773      | 24.876, 24.914    | 24.877, 24.917      |
| 4          | 26.513, 26.497    | 31.388, 31.839      | 29.124, 29.084    | 29.157, 29.110      |
| 5          | 128.168, 128,172  | 128.674, 128.678    | 29.217, 29.239    | 29.087, 29.110      |
| 6          | 131.290, 131.283  | 131.867, 131.859    | 29.147, 29.162    | 28.998, 29.017      |
| 7          | 27.277            | 32.618, 32.627      | 29.742, 29.755    | 29.613, 29.628      |
| 8          | -                 | -                   | 27.202            | 32.591              |
| 9          | -                 | -                   | 129.720, 129.693  | 130.196, 130.173    |
| 10         | -                 | -                   | 130.014, 130.029  | 130.502, 130.517    |
| 11         | -                 | -                   | 27.254            | 32.642              |
| $\omega$ 7 | 29.731, 29.743    | 29.595, 29.611      | 29.809            | 29.690              |
| $\omega$ 6 | 29.362            | 29.248              | 29.370            | 29.224              |
| $\omega$ 5 | 29.560, 29.567    | 29.524              | 29.577            | 29.525              |
| $\omega$ 4 | 29.345            | 29.349              | 29.370            | 29.350              |
| $\omega$ 3 | 31.935            | 31.944              | 31.956            | 31.937              |
| $\omega$ 2 | 22.710            | 22.712              | 22.726            | 22.710              |
| $\omega$ 1 | 14.129            | 14.128              | 14.138            | 14.128              |

Where two shifts are cited these refer to the  $\alpha$  and  $\beta$  chains, respectively; where there is only one figure, this relates to both chains

# Fatty Acids with Conjugated Unsaturation

Chemical shifts (ppm) for the four isomeric 9,11-18:2 acids (Lie Ken Jie *et al.*).

|    | 9c11t  | 9t11c  | 9t11t               | 9c11c               |
|----|--------|--------|---------------------|---------------------|
| 1  | 174.32 | 174.34 | 174.22              | 174.27              |
| 2  | 34.10  | 34.10  | 34.09               | 34.10               |
| 3  | 24.95  | 24.95  | 24.98               | 24.97               |
| 4  | 29.06  | 28.97  | 29.04               | 29.14               |
| 5  | -      | -      | -                   | -                   |
| 6  | -      | -      | -                   | -                   |
| 7  | -      | -      | -                   | -                   |
| 8  | 27.66  | 32.06  | 32.61               | 27.46               |
| 9  | 129.89 | 134.51 | 132.16 <sup>a</sup> | 131.87 <sup>c</sup> |
| 10 | 128.71 | 125.72 | 130.37 <sup>b</sup> | 123.58 <sup>d</sup> |
| 11 | 125.58 | 128.57 | 130.51 <sup>b</sup> | 123.72 <sup>d</sup> |
| 12 | 134.76 | 130.17 | 132.43 <sup>a</sup> | 132.14 <sup>c</sup> |
| 13 | 32.92  | 27.72  | 32.68               | 27.54               |
| 14 | 29.41  | 29.73  | 29.40               | 29.68               |
| 15 | 28.95  | 28.97  | 28.97               | 29.04               |
| 16 | 31.77  | 31.77  | 31.82               | 31.81               |
| 17 | 22.65  | 22.65  | 22.68               | 22.69               |
| 18 | 14.12  | 14.12  | 14.13               | 14.13               |

**Chemical shifts (ppm) listed in increasing numerical value for olefinic carbon atoms in  $\alpha$ -eleostearic acid according to Tulloch and Blaise.**

Tulloch *et al.* (1979)

Blaise *et al.* (1997)

Shift (ppm)

Carbon atom

Shift (ppm)

Carbon atom

125.98

11

125.99

11

128.95

9

128.84

10

130.73

14

130.64

13

131.52

13

131.72

9

132.91

10

132.93

12

134.90

12

135.16

14

# Oils and Fats

Chemical shifts (ppm) of saturated, oleic, linoleic, and linolenic glycerol esters from a study of soybean oil (Vlahov *et al.*).

|    | Saturated |         | Oleic    |         | Linoleic |         | Linolenic |         |
|----|-----------|---------|----------|---------|----------|---------|-----------|---------|
|    | $\alpha$  | $\beta$ | $\alpha$ | $\beta$ | $\alpha$ | $\beta$ | $\alpha$  | $\beta$ |
| 2  | 34.00     |         | 33.98    | 34.14   | 33.98    | 34.14   | 33.98     | 34.14   |
| 3  | 24.82     |         | 24.82    | 24.85   | 24.82    | 24.85   | 24.82     | 24.85   |
| 4  |           |         | 29.06    | 29.03   | 29.06    | 29.03   | 29.06     | 29.03   |
| 5  | 29.27     |         | 29.16    | 29.18   | 29.16    | 29.18   | 29.16     | 29.18   |
| 6  | 29.47     |         |          |         |          |         |           |         |
| 7  |           |         |          |         |          |         |           |         |
| 8  |           |         | 27.17    | 27.17   | 27.17    | 27.17   | 27.17     | 27.17   |
| 9  |           |         | 129.65   | 129.62  | 129.90   | 129.88  | 130.13    | 130.10  |
| 10 |           |         | 129.94   | 129.95  | 128.04   | 128.06  | 127.73    | 127.74  |
| 11 |           |         | 27.17    | 27.17   | 25.56    | 25.56   | 25.56     | 25.56   |
| 12 |           |         | 29.76    | 29.76   | 127.88   | 127.87  | 128.18    | 128.17  |
| 13 |           |         | 29.32    | 29.32   | 130.10   | 130.10  | 128.22    | 128.22  |
| 14 |           |         | 29.53    | 29.53   | 27.17    | 27.17   | 25.51     | 25.51   |
| 15 | 29.37     |         | 29.32    | 29.32   | 29.34    | 29.34   | 127.10    | 127.10  |
| 16 | 31.93     |         | 31.91    | 31.91   | 31.52    | 31.52   | 131.84    | 131.84  |
| 17 | 22.68     |         | 22.67    | 22.67   | 22.57    | 22.57   | 20.54     | 20.54   |
| 18 | 14.08     |         | 14.08    | 14.08   | 14.04    | 14.04   | 14.25     | 14.25   |

C1 signals are not reported; saturated acids were not identified in the  $\beta$  position; unresolved peaks appear at 20.68, 20.66, 20.61, and 20.10; many signals overlap and the same shifts occur several times in this table.

## Regiospecific Analysis of Triacylglycerols

| Acid         | Unsaturation             | $\alpha$ | $\beta$ | Ref.        |
|--------------|--------------------------|----------|---------|-------------|
| 18:0         | -                        | 173.186  | 172.778 | Bergana     |
| 18:1         | $\Delta 9$               | 173.133  | 172.728 | Bergana     |
| 18:2         | $\Delta 9,12$            | 173.111  | 172.706 | Bergana     |
| 18:3         | $\Delta 9,12,15$         | 173.107  | 172.702 | Bergana     |
| 18:3         | $\Delta 6,9,12$          | 172.959  | 172.563 | Bergana     |
| 18:1         | $\Delta 6$               | 173.098  | 172.071 | Lie Ken Jie |
| 20:4         | $\Delta 5,8,11,14$       | 172.893  | 172.510 | Bergana     |
| Nmip#        | $\Delta 5$               | 173.050  | -       | Lie Ken Jie |
| Nmip#        | $\Delta 5$               | 173.03   | -       | Gunstone    |
| 20:5         | $\Delta 5,8,11,14,17$    | 172.896  | 172.513 | Bergana     |
| 20:5         | $\Delta 5,8,11,14,17$    | 172.97   | 172.58  | Gunstone    |
| 20:5         | $\Delta 5,8,11,14,17$    | 172.98   | 172.59  | Aursand     |
| 22:6         | $\Delta 4,7,10,13,16,19$ | 172.403  | 172.025 | Bergana     |
| 22:6         | $\Delta 4,7,10,13,16,19$ | 172.50   | 172.10  | Gunstone    |
| 22:6         | $\Delta 4,7,10,13,16,19$ | 172.51   | 172.11  | Aursand     |
| Saturated    | -                        | 173.247  | -       | Blaise      |
| Erucic       | 13-22:1                  | 173.240  | 172.830 | Blaise      |
| Eicosenoic   | 11-20:1                  | 173.233  | -       | Blaise      |
| Oleic        | 9-18:1                   | 173.215  | 172.807 | Blaise      |
| Conj triene* | 9,11,13-18:3             | 173.206  | 172.799 | Blaise      |
| Sciadonic    | 5,11,14-20:3             | 173.054  | -       | Blaise      |
| Taxoleic     | 5,9-18:2                 | 173.042  | -       | Blaise      |
| Pinolenic    | 5,9,12-18:3              | 173.030  | -       | Blaise      |

Gerhard Knothe  
James A. Kenar

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Utilization Research, Agricultural  
Research Service,  
U.S. Department of Agriculture,  
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## Determination of the fatty acid profile by $^1\text{H-NMR}$ spectroscopy\*

The common unsaturated fatty acids present in many vegetable oils (oleic, linoleic and linolenic acids) can be quantitated by  $^1\text{H}$ -nuclear magnetic resonance spectroscopy ( $^1\text{H-NMR}$ ). A key feature is that the signals of the terminal methyl group of linolenic acid are shifted downfield from the corresponding signals in the other fatty acids, permitting their separate integration and quantitation of linolenic acid. Then, using the integration values of the signals of the allylic and *bis*-allylic protons, oleic and linoleic acids can be quantitated. The procedure was verified for mixtures of triacylglycerols (vegetable oils) and methyl esters of oleic, linoleic and linolenic acids as well as palmitic and stearic acids. Generally, the NMR (400 MHz) results were in good agreement with gas chromatographic (GC) analyses. As the present  $^1\text{H-NMR}$ -based procedure can be applied to neat vegetable oils, the preparation of derivatives for GC would be unnecessary. The present method is extended to quantitating saturated (palmitic and stearic) acids, although in this case the results deviate more strongly from actual values and GC analyses. Alternatives to the iodine value (allylic position equivalents and *bis*-allylic position equivalents) can be derived directly from the integration values of the allylic and *bis*-allylic protons.

**Tab. 2.** Fatty acid composition by two GC methods and <sup>1</sup>H-NMR of vegetable oils and their methyl esters (ME) as well as <sup>1</sup>H-NMR analysis of some prepared triacylglycerol mixtures. The methyl esters were derived directly from the oils for purpose of GC analysis.

| Sample                                  | Method           | C14:0 | C16:0         | C18:0 | C18:1                       | C18:2       | C 18:3 |
|---|------------------|-------|---------------|-------|-----------------------------|-------------|--------|
| <i>Vegetable oil samples</i>            |                  |       |               |       |                             |             |        |
| Cottonseed oil ME                       | GC-1             | 0.73  | 24.69         | 2.60  | 17.77                       | 54.14       | 0.07   |
|   | GC-2             | 0.55  | 26.06         | 2.48  | 16.11+0.8+0.12 <sup>†</sup> | 53.10+0.54* | 0.24   |
| Cottonseed oil                          | NMR <sup>†</sup> | –     | –             | –     | 19.24                       | 49.97       | 0.00   |
| High-oleic safflower oil ME             | GC-1             | 0.00  | 4.87          | 2.10  | 79.18                       | 13.64       | 0.20   |
|   | GC-2             | 0.05  | 5.21          | 1.98  | 79.39+0.35 <sup>†</sup>     | 12.76       | 0.25   |
| High-oleic safflower oil                | NMR <sup>†</sup> | –     | –             | –     | 79.22                       | 12.13       | 0.00   |
| Soybean oil ME                          | GC-1             | 0.00  | 10.73         | 4.66  | 24.01                       | 53.15       | 7.44   |
|   | GC-2             | 0.07  | 11.83         | 4.46  | 22.53+1.33 <sup>‡</sup>     | 52.42       | 7.35   |
| Soybean oil                             | NMR <sup>†</sup> | –     | –             | –     | 24.5                        | 49.99       | 7.88   |
| Mid-oleic sunflower oil ME              | GC-1             | 0.00  | 4.89          | 3.68  | 57.55                       | 33.44       | 0.44   |
| Mid-oleic sunflower oil                 | GC-2             | 0.02  | 5.21          | 1.98  | 57.50+0.43 <sup>§</sup>     | 32.75+0.18* | 0.23   |
|   | NMR <sup>†</sup> | –     | –             | –     | 58.25                       | 31.65       | 0.00   |
| <i>Prepared triacylglycerol samples</i> |                  |       | C16:0 + C18:0 | C18:1 | C18:2                       | C18:3       |        |
| Prepared                                |                  |       | 14.97         | 19.99 | 54.91                       | 10.13       |        |
|   | NMR              |       | 17.73         | 21.31 | 51.25                       | 9.71        |        |
| Prepared                                |                  |       | 15.05         | 25.08 | 59.88                       | 0           |        |
|   | NMR              |       | 16.56         | 25.36 | 58.08                       | 0           |        |
| Prepared                                |                  |       | 14.94         | 50.09 | 0                           | 34.97       |        |
|   | NMR              |       | 16.67         | 50.46 | –0.08                       | 32.95       |        |
| Prepared                                |                  |       | 0             | 25.00 | 64.87                       | 10.13       |        |
|   | NMR              |       | 1.36          | 26.15 | 62.31                       | 10.19       |        |

<sup>†</sup> Total saturates by NMR: Cottonseed oil: 30.79%, high-oleic safflower oil: 8.65%, soybean oil: 17.63%, mid-oleic sunflower oil: 10.10%.

<sup>‡</sup> C18:1Δ9 + C18:1Δ11 + C18:1Δ13.

\* C18:2-all-*cis* + C18:2-all-*trans*.

<sup>§</sup> C18:1Δ9 + C18:1Δ11.



## Research Article

***Cis* and *trans* components of lipids: Analysis by  $^1\text{H}$  NMR and silver shift reagents**Alexia Agiomyrgianaki<sup>1</sup>, Jacqueline Sedman<sup>2</sup>, Frederik R. Van de Voort<sup>2</sup> and Photis Dais<sup>1</sup><sup>1</sup> Department of Chemistry, NMR Laboratory, University of Crete, Crete, Greece<sup>2</sup> Department of Food Science and Agricultural Chemistry, McGill IR Group, McGill University, Ste-Anne-de-Bellevue, QC, Canada

In this study, the methodology of shift reagents was exploited to distinguish *cis* and *trans* unsaturation in oils and fats. The differential binding of silver ions (in the form of AgFOD) to *cis* and *trans* double bonds allowed the separation of the allylic and olefinic proton signals in the  $^1\text{H}$  NMR spectra of mixtures of *cis* and *trans* methyl esters of monoene aliphatic acids and unsaturated triacylglycerol mixtures at low frequency spectrometers (300 MHz). Careful integration of the appropriate proton resonances in the recorded quantitative  $^1\text{H}$  NMR spectra afforded percentage concentrations in very good agreement with the actual values. This  $^1\text{H}$  NMR methodology was validated by analyzing AOCS Laboratory Proficiency Program GC samples containing various percentages of saturated, *cis*-mono unsaturated, and *cis*-polyunsaturated fat as well as *trans* content. This fast and relatively low-cost NMR methodology could be used on line for obtaining nutrition labeling compositional data (NLCD) required for fat-containing food products. Attempts to differentiate lipid molecules with different degree of unsaturation and positional distribution of *cis* double bonds were unsuccessful.

**Table 1.** Actual and <sup>1</sup>H NMR mol% and wt% of the TAG model mixtures

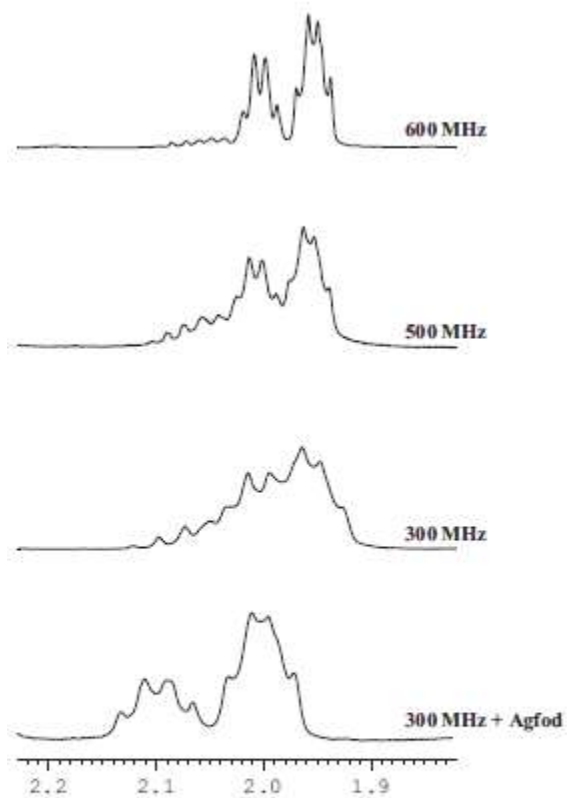
| Sample | Saturates        |               |              | Mono- <i>cis</i> |               |              | Mono- <i>trans</i> |               |              | Poly- <i>cis</i> |               |              |
|--------|------------------|---------------|--------------|------------------|---------------|--------------|--------------------|---------------|--------------|------------------|---------------|--------------|
|        | Actual<br>(mol%) | NMR<br>(mol%) | NMR<br>(wt%) | Actual<br>(mol%) | NMR<br>(mol%) | NMR<br>(wt%) | Actual<br>(mol%)   | NMR<br>(mol%) | NMR<br>(wt%) | Actual<br>(mol%) | NMR<br>(mol%) | NMR<br>(wt%) |
| S1     | 13.47            | 12.72         | 12.57        | 1.97             | 2.41          | 2.45         | 29.66              | 29.46         | 29.97        | 54.90            | 54.66         | 55.01        |
| S2     | 7.03             | 8.01          | 3.95         | 34.57            | 32.96         | 34.19        | 20.86              | 21.70         | 22.51        | 37.53            | 38.27         | 39.35        |
| S3     | 1.95             | 2.50          | 1.04         | 56.01            | 53.67         | 54.55        | 13.80              | 15.43         | 15.68        | 28.25            | 28.50         | 28.73        |
| S4     | 34.02            | 32.79         | 30.38        | 38.85            | 38.49         | 39.62        | 7.43               | 7.83          | 8.06         | 19.69            | 21.53         | 21.94        |
| S5     | 55.42            | 58.84         | 55.79        | 29.27            | 28.03         | 28.17        | 2.24               | 2.24          | 2.24         | 13.07            | 13.87         | 13.80        |
| S6     | 39.35            | 41.08         | 36.98        | 20.86            | 21.13         | 22.09        | 32.07              | 31.21         | 33.34        | 7.72             | 8.25          | 9.15         |
| S7     | 28.02            | 29.64         | 26.24        | 13.72            | 15.77         | 16.31        | 56.15              | 53.54         | 55.37        | 2.10             | 2.04          | 2.08         |
| S8     | 20.62            | 23.74         | 21.55        | 7.19             | 9.47          | 9.64         | 40.09              | 37.65         | 38.33        | 32.10            | 30.15         | 30.48        |

mol% saturates<sub>NMR</sub> = 0.199 + 1.039 × mol% saturates<sub>actual</sub> *R* = 0.997 SD = ± 1.623.

mol% mono-*cis*<sub>NMR</sub> = 1.879 + 0.923 × mol% mono-*cis*<sub>actual</sub> *R* = 0.998 SD = ± 1.007.

mol% mono-*trans*<sub>NMR</sub> = 1.323 + 0.932 × mol% mono-*trans*<sub>actual</sub> *R* = 0.999 SD = ± 0.940.

mol% poly-*cis*<sub>NMR</sub> = 0.664 + 0.983 × mol% poly-*cis*<sub>actual</sub> *R* = 0.998 SD = ± 1.131.



**Figure 1.**  $^1\text{H}$  NMR signals of the allylic protons of a mixture 0.2M of methyl oleate and methyl elaidate dissolved in  $\text{CDCl}_3$  and recorded at different Larmor frequencies. The bottom spectrum was recorded after the addition of 0.1 M AgFOD (silver chelate-to-lipid molar ratio 0.5).

## Classification of Edible Oils by Employing $^{31}\text{P}$ and $^1\text{H}$ NMR Spectroscopy in Combination with Multivariate Statistical Analysis. A Proposal for the Detection of Seed Oil Adulteration in Virgin Olive Oils

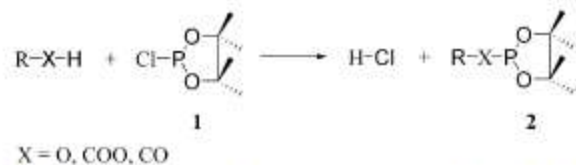
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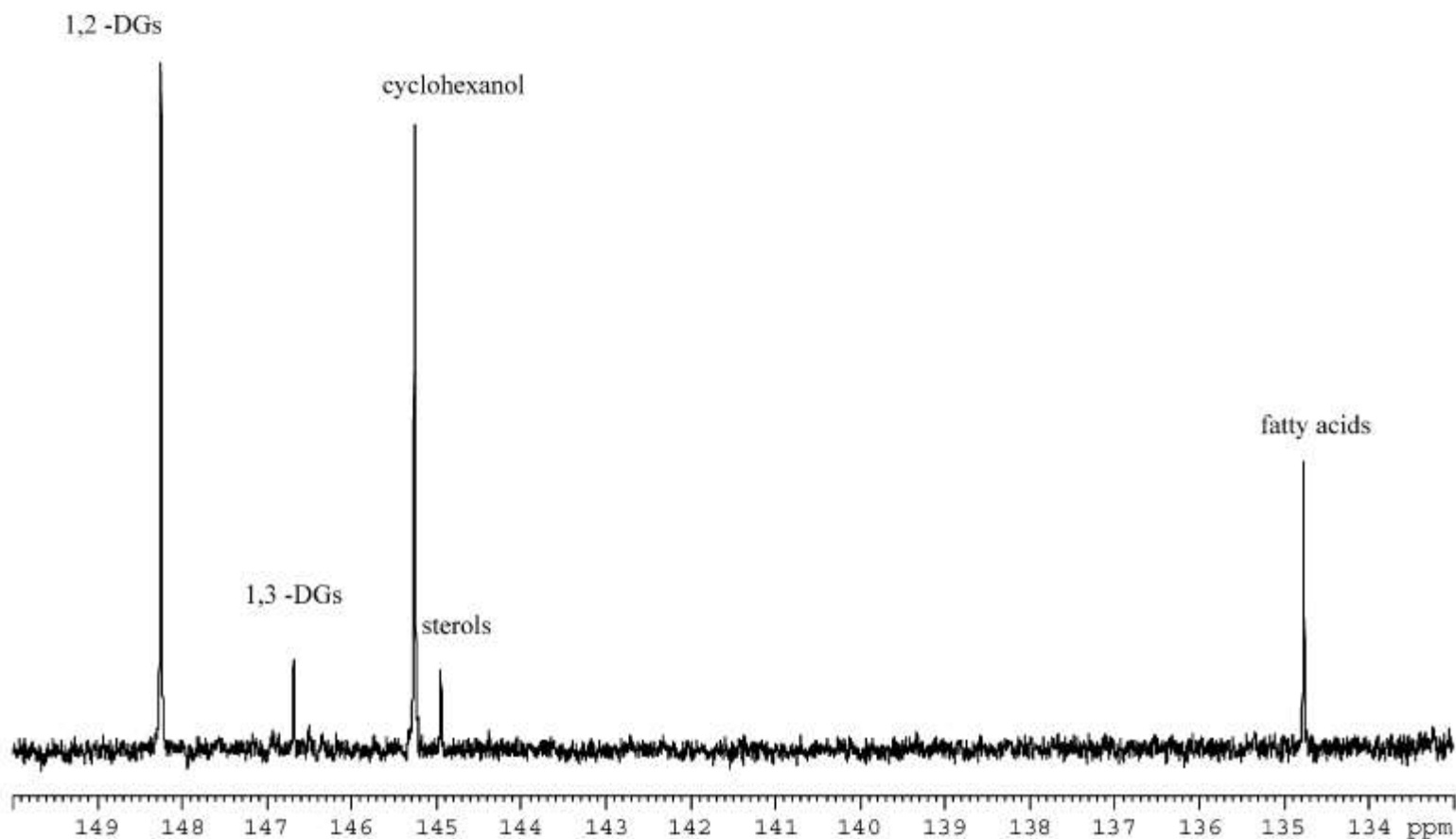
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A combination of  $^1\text{H}$  NMR and  $^{31}\text{P}$  NMR spectroscopy and multivariate statistical analysis was used to classify 192 samples from 13 types of vegetable oils, namely, hazelnut, sunflower, corn, soybean, sesame, walnut, rapeseed, almond, palm, groundnut, safflower, coconut, and virgin olive oils from various regions of Greece. 1,2-Diglycerides, 1,3-diglycerides, the ratio of 1,2-diglycerides to total diglycerides, acidity, iodine value, and fatty acid composition determined upon analysis of the respective  $^1\text{H}$  NMR and  $^{31}\text{P}$  NMR spectra were selected as variables to establish a classification/prediction model by employing discriminant analysis. This model, obtained from the training set of 128 samples, resulted in a significant discrimination among the different classes of oils, whereas 100% of correct validated assignments for 64 samples were obtained. Different artificial mixtures of olive–hazelnut, olive–corn, olive–sunflower, and olive–soybean oils were prepared and analyzed by  $^1\text{H}$  NMR and  $^{31}\text{P}$  NMR spectroscopy. Subsequent discriminant analysis of the data allowed detection of adulteration as low as 5% w/w, provided that fresh virgin olive oil samples were used, as reflected by their high 1,2-diglycerides to total diglycerides ratio ( $D \geq 0.90$ ).

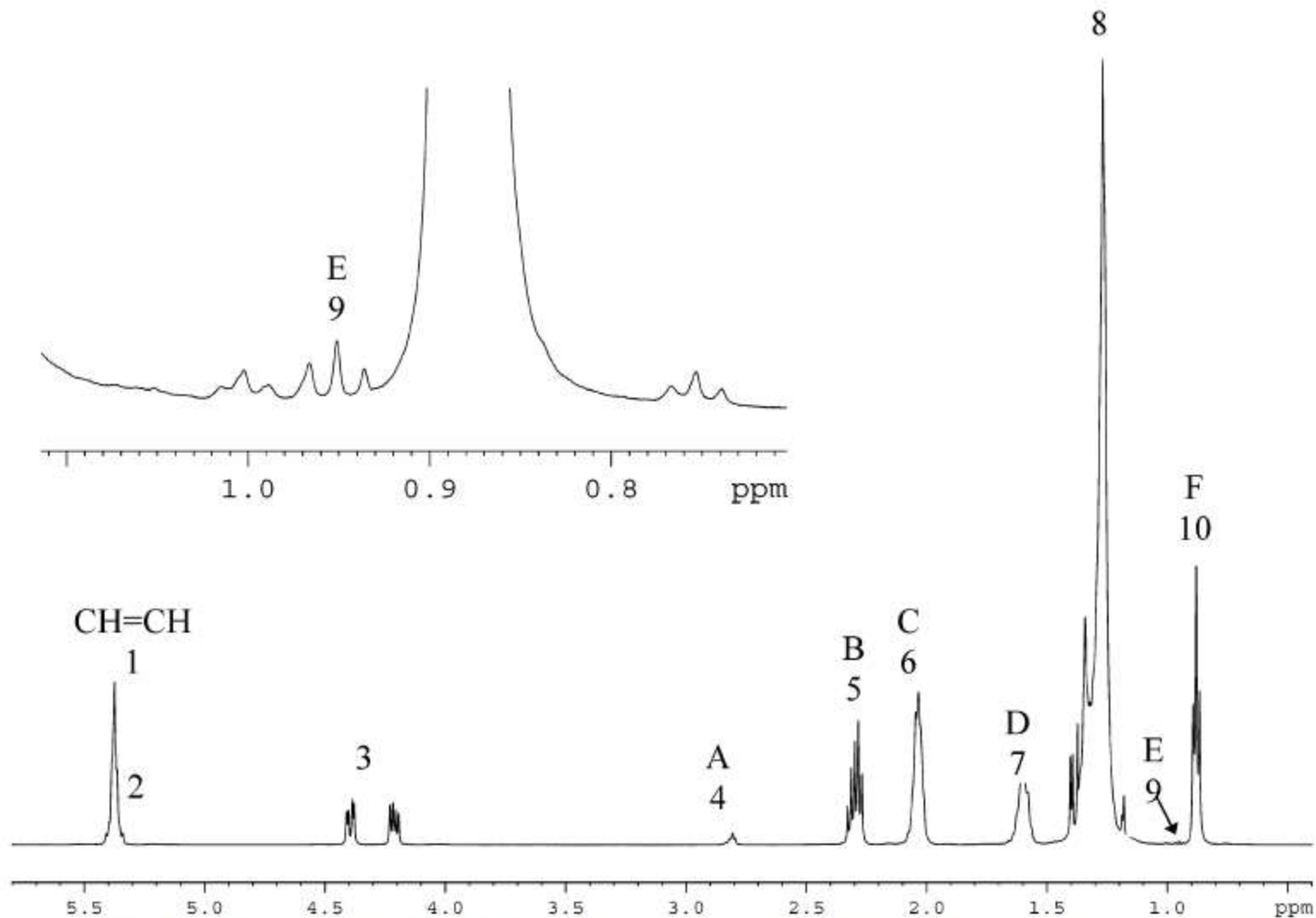
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**Figure 1.** Reaction of hydroxyl groups of diglycerides with 2-chloro-4,4,5,5-tetramethyldioxaphospholane (**1**).



**Figure 2.** 202.2 MHz  $^{31}\text{P}$  NMR spectrum of virgin olive oil. The region where the phosphitylated total sterols, diglycerides, and free fatty acids absorb is illustrated. The phosphitylated cyclohexanol is used as internal standard.

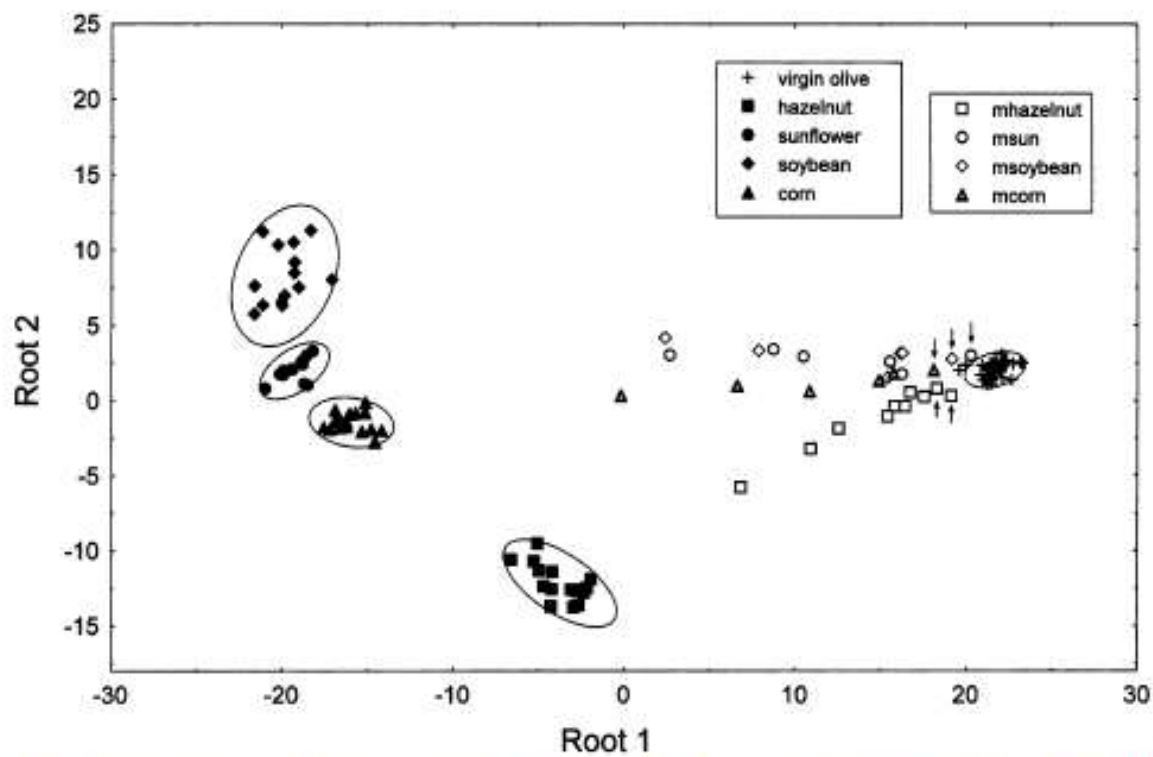


**Figure 3.** 500 MHz <sup>1</sup>H NMR spectrum of virgin olive oil. The inset shows expansion of the spectrum where the methyl protons (signal E) of the linolenyl chain appear. The assignments of all signals are summarized in **Table 1**.

**Table 2.** Mean Values and Standard Deviations of the Variables for 13 Types of Vegetable Oils

| oil       | 1,2-DGs     | 1,3-DGs     | total DGs   | <i>D</i>    | total sterols | acidity     | oleic acid   | linoleic acid | linolenic acid | SFA          | IV <sup>a</sup> |
|-----------|-------------|-------------|-------------|-------------|---------------|-------------|--------------|---------------|----------------|--------------|-----------------|
| olive     | 1.71 ± 0.29 | 0.17 ± 0.09 | 1.88 ± 0.31 | 0.91 ± 0.04 | 0.12 ± 0.03   | 0.21 ± 0.12 | 77.13 ± 2.48 | 7.89 ± 1.36   | 0.47 ± 0.10    | 14.51 ± 1.65 | 80.58 ± 1.93    |
| soybean   | 0.33 ± 0.07 | 0.74 ± 0.20 | 1.08 ± 0.25 | 0.33 ± 0.04 | 0.21 ± 0.04   | 0.03 ± 0.01 | 24.63 ± 1.68 | 50.11 ± 0.94  | 7.30 ± 0.73    | 17.98 ± 0.68 | 127.35 ± 2.27   |
| sunflower | 0.50 ± 0.08 | 1.01 ± 0.17 | 1.51 ± 0.24 | 0.33 ± 0.02 | 0.16 ± 0.04   | 0.01 ± 0.02 | 26.06 ± 1.79 | 60.78 ± 2.65  | 0.61 ± 0.26    | 12.37 ± 0.36 | 129.12 ± 1.82   |
| corn      | 0.98 ± 0.14 | 2.16 ± 0.19 | 3.14 ± 0.31 | 0.31 ± 0.02 | 0.33 ± 0.03   | 0.05 ± 0.03 | 31.14 ± 1.18 | 52.51 ± 1.91  | 1.83 ± 0.72    | 14.59 ± 0.90 | 120.49 ± 2.32   |
| hazelnut  | 0.87 ± 0.15 | 1.85 ± 0.36 | 2.72 ± 0.50 | 0.32 ± 0.02 | 0.07 ± 0.03   | 0.12 ± 0.07 | 77.04 ± 6.85 | 12.95 ± 6.13  | 0.29 ± 0.15    | 9.51 ± 0.82  | 89.91 ± 4.40    |
| sesame    | 0.74 ± 0.14 | 1.55 ± 0.23 | 2.29 ± 0.36 | 0.32 ± 0.01 | 0.17 ± 0.05   | 0.06 ± 0.02 | 42.68 ± 0.85 | 38.74 ± 0.91  | 0.88 ± 0.21    | 16.98 ± 2.98 | 105.81 ± 1.05   |
| groundnut | 0.73 ± 0.07 | 1.62 ± 0.08 | 2.36 ± 0.15 | 0.31 ± 0.01 | 0.15 ± 0.02   | 0.04 ± 0.05 | 57.75 ± 0.24 | 20.65 ± 0.45  | 1.02 ± 0.22    | 20.59 ± 0.25 | 87.48 ± 0.49    |
| walnut    | 1.20 ± 0.16 | 2.27 ± 0.26 | 3.47 ± 0.47 | 0.35 ± 0.01 | 0.09 ± 0.02   | 0.00        | 16.81 ± 1.33 | 57.66 ± 1.59  | 13.69 ± 0.58   | 11.84 ± 0.52 | 150.09 ± 1.94   |
| rapeseed  | 0.30 ± 0.05 | 0.61 ± 0.11 | 0.91 ± 0.14 | 0.33 ± 0.03 | 0.21 ± 0.04   | 0.02 ± 0.02 | 62.82 ± 0.50 | 16.87 ± 0.29  | 11.93 ± 0.09   | 8.39 ± 0.29  | 112.99 ± 0.88   |
| almond    | 0.66 ± 0.09 | 1.16 ± 0.11 | 1.82 ± 0.18 | 0.33 ± 0.03 | 0.17 ± 0.04   | 0.00        | 57.51 ± 1.43 | 25.24 ± 0.61  | 0.63 ± 0.08    | 8.78 ± 0.89  | 94.42 ± 1.32    |
| safflower | 0.52 ± 0.04 | 1.19 ± 0.07 | 1.71 ± 0.09 | 0.30 ± 0.02 | 0.10 ± 0.02   | 0.04 ± 0.03 | 13.92 ± 0.14 | 73.41 ± 0.18  | 0.71 ± 0.04    | 11.96 ± 0.18 | 139.80 ± 0.31   |
| palm      | 1.32 ± 0.18 | 5.61 ± 0.69 | 6.93 ± 0.87 | 0.19 ± 0.01 | 0.01 ± 0.01   | 0.14 ± 0.02 | 38.38 ± 1.12 | 8.26 ± 0.25   | 0.51 ± 0.09    | 52.84 ± 3.12 | 47.63 ± 0.48    |
| coconut   | 1.48 ± 0.51 | 3.07 ± 1.01 | 4.55 ± 1.52 | 0.33 ± 0.01 | 0.06 ± 0.03   | 0.04 ± 0.01 | 7.31 ± 2.30  | 0.32 ± 0.16   | 0.37 ± 0.06    | 92.00 ± 2.23 | 6.12 ± 0.47     |

<sup>a</sup> Iodine value.



**Figure 5.** Plot of discriminant functions roots 1 and 2 for five types of edible oils. Virgin olive oil, hazelnut oil, corn oil, and sunflower oil are shown by crosses and solid symbols. Four sets of mixtures of 5, 10, 15, 20, 35, and 50% w/w of virgin olive oils with hazelnut (mhazelnut), sunflower (msun), soybean (msoybean), and corn (mcom) oils and one set of mixtures of 5, 10, 15, and 20% of virgin olive oils with hazelnut oils (mhazelnut) are denoted by open symbols. Arrows indicate mixtures of 5% w/w of seed oils in virgin olive oils.