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Structure Analysis of Triacylglycerol Positional Isomers Using Atmospheric Pressure Chemical Ionisation Mass Spectrometry

Hazel R. Mottram and Richard P. Evershed*

School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, UK.

Abstract: Fragmentation of triacylglycerols by atmospheric pressure ionisation allows identification of positional isomers. The relative intensities of the $[M\text{-}RCO_2]^+$ ions provide information on the position of fatty acids within an ABC type triacylglycerol and enable ABA and AAB type molecules to be distinguished. Copyright © 1996 Elsevier Science Ltd

The position of fatty acid substitution on the glycerol backbone of a triacylglycerol (TAG) molecule is of great importance from biochemical[1,2], nutritional[3,4] and biotechnological[5] points of view. However, the ability to unambiguously identify positional isomers of individual TAGs, especially when they are components of complex natural mixtures, is a long standing problem in lipid chemistry. Existing chemical[6] and enzymatic[7] methods, although effective for isolated components or simple mixtures become somewhat limited when studying complex TAG mixtures, such as fats and oils. Whilst argentation chromatography can be used to separate pairs of ABA and AAB type positional isomers[8], this technique gives no direct information on the positions of fatty acyl groups in ABC type TAGs and is also restricted to simple TAG mixtures.

Two mass spectrometric techniques have been shown to give positional information on TAG. Using electron ionisation (EI), Ryhage and Stenhagen[9] noted that whilst $[M\text{-}RCO_2\text{+}14]^+$ ions were observed for fatty acids in positions *sn*-1 and *sn*-3, they were absent for the fatty acid in position *sn*-2. More recently, Kallio and Currie[10] noted that in negative ion chemical ionisation spectra (NICI), the $[M\text{-}H\text{-}RCO_2H]^-$ and related ions occurred primarily with the loss of *sn*-1 and *sn*-3 position fatty acids. However neither EI nor NICI are readily combined with a separation technique capable of resolving complex mixtures of TAGs.

Atmospheric pressure chemical ionisation (APCI) is a relatively new mass spectrometric ionisation technique[11], which has considerable potential for combined HPLC-MS analysis of TAGs. The spectra obtained using APCI are relatively simple due to the low energies involved in the ionisation process. APCI spectra of TAGs typically show a protonated molecular ion $[M+H]^+$, as well as diglyceride ions, $[M\text{-}RCO_2]^+$, which allow identification of the fatty acyl groups present in the individual components either introduced into the source

individually[12], or resolved by reversed phase HPLC[13,14]. However, until now, no preferential fragmentations of fatty acyl moieties which indicate position of substitution have been reported.

In this study, mass spectra of various TAGs were obtained through injection of individual authentic compounds (Sigma) directly into the source *via* the loop injector[15]. The spectra produced showed a protonated molecular ion, $[M+H]^+$, $[M\text{-}RCO_2]^+$ ions caused by loss of fatty acyl moieties ("diglyceride" ions, $[DG]^+$) and in some cases, $[RCO]^+$ ions derived from the fatty acyl moieties themselves. $[M\text{-}RCO_2+18]^+$ ions were also present, albeit at low relative abundance. The relative intensity of the protonated molecular ion increased with the degree of unsaturation of the TAG. The mass spectra of AAA type TAGs are typified by that of 1,2,3-trioleoyl glycerol (OOO[16]), in which three clusters of ions can be seen: the protonated molecular ion at *m/z* 885; a single $[M\text{-}RCO_2]^+$ ion at *m/z* 603 due to loss of oleate and a single $[RCO]^+$ ion at *m/z* 265. The mass spectrum of an ABA type TAG 1,3-distearoyl-2-oleoyl glycerol (SOS) is given in Figure 1. Since two different fatty acids are present, $[RCO]^+$ ions are seen at *m/z* 265 (O^+) and *m/z* 267 (S^+). Likewise, two $[M\text{-}RCO_2]^+$ ions are present corresponding to the loss of stearate (*m/z* 605) and oleate (*m/z* 607), resulting in $[SO]^+$ ($[AB]^+$) and $[SS]^+$ ($[AA]^+$) ions respectively. Similarly, the mass spectrum of an ABC type TAG, 1-myristoyl-2-oleoyl-3-palmitoyl glycerol (MyOP; Figure 2), shows three $[M\text{-}RCO_2]^+$ ions at *m/z* 523, 549 and 577, corresponding to loss of oleate to give $[MyP]^+$, loss of palmitate to give $[MyO]^+$ and loss of myristate to give $[PO]^+$ respectively.

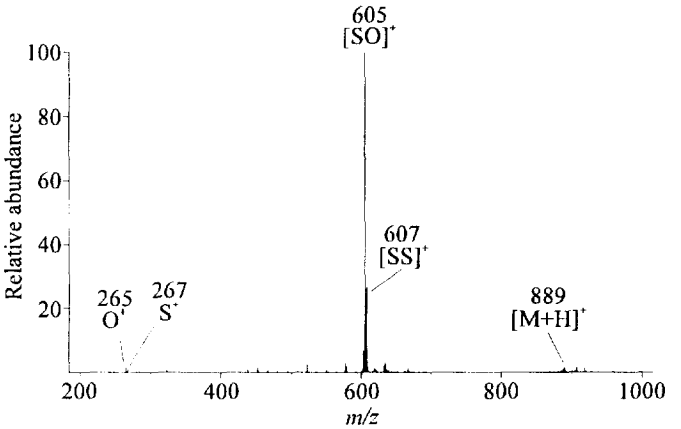

Figure 1. The APCI mass spectrum of 1,3-distearoyl-2-oleoyl glycerol (SOS).

The relative intensities of the $[M\text{-}RCO_2]^+$ ions give information on the position of fatty acids within the TAG. For example, the least abundant $[M\text{-}RCO_2]^+$ ion corresponds to loss of the fatty acid from the *sn*-2 position, since this is energetically less favourable than losing a fatty acid from the *sn*-1 or *sn*-3 position. In the spectrum of MyOP (Figure 2), the least abundant $[M\text{-}RCO_2]^+$ ion is formed by the loss of oleate from the *sn*-2 position, leaving a MyP^+ ion (*m/z* 523). The two more abundant $[M\text{-}RCO_2]^+$ ions are due to loss of My (*sn*-1(3)) and P (*sn*-3(1)) giving $[OP]^+$ and $[MyO]^+$ ions respectively.

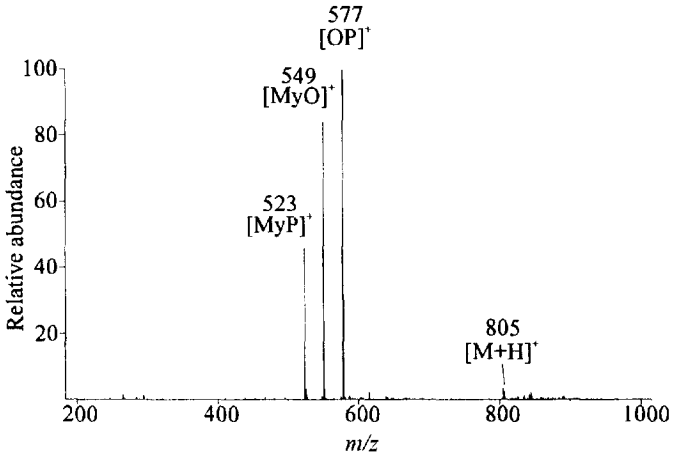

Figure 2. The APCI mass spectrum of 1-myristoyl-2-oleoyl-3-palmitoyl glycerol (MyOP).

Positional isomers of difunctional TAGs can also be distinguished using the $[M\text{-}RCO_2]^+$ ions. ABA and AAB type TAGs will give the same $[M\text{-}RCO_2]^+$ ions, i.e. $[AA]^+$ and $[AB]^+$. However, the ratio of $[AA]^+$:$[AB]^+$ will be lower for the ABA isomer, since formation of the 1,2- isomer of the $[AB]^+$ ion requires less energy than that involved in generating the analogous 1,3-ion from the AAB isomer. This has been confirmed by analysing two isomeric pairs of TAGs (SOS/SSO and POP/PPO). The results of replicate loop injections (Table 1) indicate that AAB TAGs have a $[AA]^+$:$[AB]^+$ ratio of around 1, whereas ABA TAGs have a markedly lower ratio.

Table 1. Relative Abundances of $[AA]^+$ and $[AB]^+$ Ions in ABA and AAB Type Triacylglycerols.

AAB	$[AA]^+$:$[AB]^+$	± standard deviation (n=8)	**ABA**	$[AA]^+$:$[AB]^+$	± standard deviation (n=8)
SSO	1.07	± 0.16	SOS	0.29	± 0.12
PPO	0.95	± 0.30	POP	0.20	± 0.08

In conclusion, atmospheric pressure chemical ionisation has been shown to be an effective technique for the mass spectrometric analysis of triacylglycerols, since it allows identification of constituent fatty acyl moieties, and also gives valuable information on the position at which these fatty acids are esterified to the glycerol backbone of the TAG. The capacity to determine position of substitution of fatty acids directly from APCI spectra represents a significant advance over existing mass spectrometric techniques, since the technique is readily amenable for use in conjunction with the liquid chromatographic techniques which are the most effective methods currently available for the resolution of complex mixtures of TAGs. Analysis of 20 vegetable oils has shown that structural isomers can be readily characterised in natural mixtures of commercial and nutritional importance[17].

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REFERENCES AND NOTES

1. Aoyama, T.; Fukui, K.; Taniguchi, K.; Nagaoka, S.; Yamamoto, T.; Hashimoto, Y. *J. Nutr.* **1996**, *126*(1), 225-231.
2. Pufal, D. A.; Quinlan, P. T.; Salter, A. M. *Biochim. Biophys. Acta* **1995**, *1258*(1), 41-48.
3. Martin, J. C.; Bougnoux, P.; Antoine, J. M.; Lanson, M.; Couet, C. *Lipids* **1993**, *28*(7), 637-643.
4. Zampelas, A.; Williams, C. M.; Morgan, L. M.; Wright, J.; Quinlan, T. *Brit. J. Nutr.* **1994**, *71*(3), 401-410.
5. Valenzuela, A.; Nieto, S. *Grasas Aceites* **1994**, *45*(5), 337-343.
6. Brockerhoff, H.; Yurkowski, M. *J. Lipid Res.* **1966**, *7*, 62-64.
7. Christie, W. W.; Nikolovadamyanova, B.; Laakso, P.; Herslof, B. *J. Am. Oil Chem. Soc.* **1991**, *68*(10), 695-701.
8. Adlof, R. O. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1995**, *18*, 105-107.
9. Ryhage, R.; Stenhagen, E. *J. Lipid Res.* **1960**, *1*, 361-390.
10. Kallio, H.; Currie, G. *Lipids* **1993**, *28*(3), 207-215.
11. Covey, T. R.; Lee, E. D.; Bruins, A. P.; Henion, J. D. *Anal. Chem.* **1986**, *58*(14), 1451.
12. Byrdwell, W. C.; Emken, E. A. *Lipids* **1995**, *30*(2), 173-175.
13. Neff W. E.; Byrdwell W. C. *J. Am. Oil Chem. Soc.* **1995**, *72*(10), 1185-1191.
14. Neff W. E.; Byrdwell W. C. *J. Liq. Chromatogr.* **1995** *18*(20), 4165-4181.
15. Waters 600MS quaternary solvent delivery system coupled to a Finnigan MAT TSQ700 fitted with an APCI source. 25 µl of 0.1 mg ml^{-1} solution of authentic TAG in propionitrile injected *via* loop injector. Propionitrile mobile phase (distilled over P_2O_5 before use) at a flow rate of 1 ml min^{-1}. Vaporiser temperature of 450°C, capillary temperature of 280°C, corona current of 5 µA, high purity nitrogen sheath gas at 60 psi and auxillary gas at 20 ml min^{-1}. Spectra obtained by scanning Q2 over the range *m/z* 200 to 1000, with scan cycle time of 2 seconds.
16. The following abbreviations are used to represent fatty acids: My, myristic, tetradecanoic (14:0); P, palmitic, hexadecanoic (16:0); S, stearic, octadecanoic (18:0); O, oleic, *cis*-9-octadecenoic (18:1). TAG are represented by their constituent fatty acids.
17. Mottram, H. R.; Evershed, R. P. Unpublished results.

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