

¹³C-NMR SPECTROSCOPY OF FATTY ACIDS AND DERIVATIVES

Regiospecific Analysis of Triacylglycerols

Fatty acid analysis is the starting point for most analytical studies of lipids and is generally carried out by gas chromatography of methyl esters obtained from the lipid by methanolysis. Sometimes information is needed about the fatty acids attached to the α (*sn*-1/3) and the β (*sn*-2) hydroxyl groups of glycerol. Methods of getting these data involve lipases, chemical reactions and several chromatographic procedures. These can be extended to distinguish between the *sn*-1 and 3-positions. However, besides requiring skilled effort, these methods fail with acids having double bonds close to the carboxyl group. ¹³C-NMR spectroscopy provides an alternative way of distinguishing between acids in the α and β chains though it will not distinguish between the two α chains (but see McNeill). The method is normally based on the clusters of signals for the acyl (C1) carbon atom. To use the NMR spectrum for regiospecific analysis, two requirements must be met.

(i) It is first necessary to distinguish two clusters of signals related to acids in the α chain and the β chain. This is not a problem as there are usually clusters corresponding to these two environments and separated from each other by about 0.4 ppm.

(ii) Within each of these two clusters, there must be separate signals for each acid or group of acids. This criterion is most easily met when the acids have unsaturation close to the carboxyl group, and acids with $\Delta 4$, $\Delta 5$, or $\Delta 6$ unsaturation are readily handled in this way. Some acids falling into this category are of considerable significance and include docosahexaenoic acid (DHA $\Delta 4,7,10,13,16,19$), eicosapentaenoic acid (EPA $\Delta 5,8,11,14,17$), arachidonic acid (AA $\Delta 5,8,11,14$), γ -linolenic acid (GLA $\Delta 6,9,12$), and petroselinic acid ($\Delta 6$). The C1 signal will not normally distinguish between acids from the same double bond group such as EPA and AA both of which are $\Delta 5$ acids. The method can be applied to oils with the more common unsaturated acids having $\Delta 9$ unsaturation such as oleic and linoleic acids, but only with the best NMR spectrometers and with data acquisition over periods of time longer than usual. Vlahov has described simpler procedures for obtaining this spectroscopic information for common vegetable oils.

Examples:

The absolute value chemical shifts vary slightly in different laboratories but the differences between the α and β shifts remain constant. More important are the different chemical shifts between the different acids in the α chain or in the β chain. Values in the Table show, for example, why it is harder to distinguish oleic, linoleic, and linolenic acyl groups from each other than, say, EPA and DHA. Examples of the ways in which this procedure is applied are given in the papers from which these chemical shifts are taken. They include DHA, EPA, GLA, petroselinic acid, and Δ -5 non-methylene interrupted polyenoic acids.

Redden *et al.* compared the NMR method of *sn*-2 analysis for oils containing GLA with alternative Grignard deacylation procedures and demonstrated good agreement between all the results. Some of the C1 chemical shifts for acids or esters containing $\Delta 4$, $\Delta 5$, and $\Delta 6$ acids are listed in the Table.

Using values of 173.10 and 172.70 ppm obtained from tripalmitin, 173.07 and 172.67 from triolein, and 173.06 and 172.66 from trilinolein and an improved procedure for collecting the spectra (proton decoupled spectra with full NOE, relaxation delay of 15 sec, 128 scans) Vlahov reported a regiospecific analyses of six vegetable oils (sunflower, peanut, grapestone, corn, hazel, and olive fruit).

Table 1

Acid	Unsaturation	α	β	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
Lin/linolenic	9,12 and 9,12,15	173.204	172.796	Blaise

non-methylene-interrupted polyenes

* triene acids with conjugated unsaturation

References

- Aursand, M., Jorgensen, L. and Grasdalen, H. Positional distribution of $\omega 3$ fatty acids in marine lipid triacylglycerols by high-resolution ¹³C nuclear magnetic resonance spectroscopy. *J. Am. Oil Chem. Soc.*, **72**, 293-297 (1995).
- Bergana, M.M. and Lee, T.W. Structure determination of long-chain polyunsaturated triacylglycerols by high-resolution ¹³C nuclear magnetic resonance. *J. Am. Oil Chem. Soc.*, **73**, 551-556 (1996).
- Blaise, P., Wolff, R. and Farines, M. Regiospecific study of vegetable oil triacylglycerols by chemical cleavage and high-resolution NMR ¹³C. *Oleagineux Corps gras Lipides*, **4**, 135-141 (1997).
- Gunstone, F.D. and Seth, S. A study of the distribution of eicosapentaenoic acid and docosahexaenoic acid between the α and β glycerol chains in fish oils by ¹³C-NMR spectroscopy. *Chem. Phys. Lipids*, **72**, 119-126 (1994).
- Gunstone, F.D., Seth, S. and Wolff, R.L. The distribution of $\Delta 5$ polyene acids in some pine seed oils between the α - and β -chains by ¹³C-NMR spectroscopy. *Chem. Phys. Lipids*, **78**, 89-96 (1995).
- Lie Ken Jie, M.S.F., Lam, C.C. and Pasha, M.K. ¹³C nuclear magnetic resonance spectroscopic analysis of the triacylglycerol composition of *Biota orientalis* and carrot seed oil. *J. Am. Oil Chem. Soc.*, **73**, 557-562 (1996).

- Redden, P.R., Lin, X. and Horrobin, D.F. Comparison of the Grignard deacylation TLC and HPLC methods and high resolution ¹³C-NMR for the *sn*-2 positional analysis of triacylglycerols containing γ -linolenic acid. *Chem. Phys. Lipids*, **79**, 9-19 (1996).
- Vlahov, G., Schiavone, C. and Simone, N. Triacylglycerols of the olive fruit (*Olea europaea* L.): characterisation of mesocarp and seed triacylglycerols in different cultivars by liquid chromatography and ¹³C-NMR spectroscopy. *Fett/Lipid*, **101**, 146–150 (1999).
- Vlahov, G. Regiospecific analysis of natural mixtures of triglycerides using quantitative ¹³C nuclear magnetic resonance of acyl chain carbonyl carbons. *Mag. Res. Chem.*, **36**, 359-362 (1998).

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