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A novel approach to the rapid assignment of ¹³C NMR spectra of major components of vegetable oils such as avocado, mango kernel and macadamia nut oils

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Assignment of ¹³C nuclear magnetic resonance (NMR) spectra of major fatty acid components of South African produced vegetable oils was attempted using a method in which the vegetable oil was spiked with a standard triacylglycerol. This proved to be inadequate and therefore a new rapid and potentially generic graphical linear correlation method is proposed for assignment of the ¹³C NMR spectra of major fatty acid components of apricot kernel, avocado pear, grapeseed, macadamia nut, mango kernel and marula vegetable oils. In this graphical correlation method, chemical shifts of fatty acids present in a known standard triacylglycerol is plotted against the corresponding chemical shifts of fatty acids present in the vegetable oils. This new approach (under carefully defined conditions and concentrations) was found especially useful for spectrally crowded regions where significant peak overlap occurs and was validated with the well-known ¹³C NMR spectrum of olive oil which has been extensively reported in the literature. In this way, a full assignment of the ¹³C{1H} NMR spectra of the vegetable oils, as well as tripalmitolein was readily achieved and the resonances belonging to the palmitoleic acid component of the triacylglycerols in the case of macadamia nut and avocado pear oil resonances were also assigned for the first time in the ¹³C NMR spectra of these oils. Copyright © 2009 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

Keywords: NMR; ¹³C; olive oil; apricot kernel oil; avocado pear oil; grapeseed oil; macadamia nut oil; mango kernel oil; marula oil; tripalmitolein

Introduction

Nuclear magnetic resonance (NMR) spectroscopy has found application in the identification and quantitative determination of the major fatty acid components of vegetable oils, in particular olive oil.^[1-5] One of the significant advantages of NMR spectroscopy is that, unlike other analytical techniques, it does not in general require extraction, separation or chemical modification of the vegetable oil to be analyzed. On the other hand the relatively low sensitivity of NMR spectroscopy limits its use to major and minor components of vegetable oils, rendering it generally unsuitable for analyzing trace components. Of the numerous vegetable oils available, olive oil has been the most widely studied by NMR spectroscopy, presumably in view of its high-value and wide consumption. This has resulted in the need for methods of authentication and the detection of adulteration of olive oil with other vegetable oils such as hazelnut and sunflower oils.^{[6-8] 1}H NMR spectroscopy has been used in the analysis of olive oil for measuring diglyceride content, determinations of squalene, cyclo-arthenol and Mg-depleted chlorophyll, and analysis of sterols as well as other components responsible for the taste and aroma of olive oil such as acetic acid, trans-2-hexenal and formaldehyde.^[1,2] Although GC (gas chromatography) is the most commonly used technique for the qualitative and quantitative determination of fatty acid residues in vegetable oils, ¹³C NMR spectroscopy can also be used for analyzing these major components.^[1-5] The distinct advantage of using ¹³C NMR

spectroscopy in this context is that no chemical derivatization of the sample is required whereas for GC the fatty acid methyl esters must be prepared from the triacylglycerols before analysis is undertaken. ¹³C NMR spectroscopy has been successfully used to determine quantitatively the major fatty acid residues in olive oils^[5,7] and moreover can give direct information about the positional distribution of the fatty acids on the glycerol backbone. Much success has been found in detecting adulteration of olive oil by other oils using ¹³C NMR spectroscopy. In conjunction with chemometric methods ¹³C NMR spectroscopy can also be used to distinguish between geographic and cultivar-based differences of olive oils.^[1-3,9]

We have recently become interested in the analysis of other high-value vegetable oils, including apricot kernel, avocado pear, grapeseed, macadamia nut, mango kernel and marula oils. To our knowledge, the ¹³C NMR spectra of these oils have not been examined or fully assigned to date. The conventionally used method for the full assignment of ¹³C NMR spectra of vegetable

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Figure 1. ¹³C NMR spectrum of macadamia nut oil in CDCI₃ with an expansion of the crowded 29 ppm region.



Figure 2. ¹³C NMR spectrum of tripalmitolein in CDCl₃ with an expansion of the crowded 29 ppm region and numbered structure.

oils is by means of standard addition (spiking) of the vegetable oil with a standard triacylglycerol.^[10] Comparison of the resulting ¹³C NMR spectra of the unspiked and spiked vegetable oil leads to the assignment of the ¹³C resonances in the NMR spectrum of the different fatty acid components present in the vegetable oil. Besides this method having some practical disadvantages, we found that it was not possible to achieve a full, unambiguous assignment of the ¹³C NMR spectra of the desired oils using this technique. Desirous of developing a rapid method for the accurate assignment of the ¹³C resonances of the various major components in a vegetable oil, we developed and tested a graphical linear correlation method for the assignment of the ¹³C resonances of a vegetable oil. The technique was tested and validated with extra-virgin olive oil, for which the ¹³C NMR spectrum has been well characterized in the literature. Using this approach one could easily achieve the full assignment of the ^{13}C NMR spectra of six locally produced South African vegetable oils in CDCl3 solution.

Experimental

Materials and sample preparation

Standard triacylglycerols, tripalmitin, tripalmitolein, tristearin, triolein and trilinolein were purchased from Sigma-Aldrich and used without further purification (≥99% purity). Samples of olive oil were provided by Brenn-o-Kem (Wolseley, South Africa). Apricot kernel, avocado pear, grapeseed, macadamia nut, mango kernel and marula oils were supplied by Specialized Oils (Paardeneiland, Cape Town, South Africa). All oils were filtered before use. For storage, the oils were flushed with nitrogen gas.



Figure 3. Change in chemical shift for different types of carbon atoms plotted against the mass of oil per 1 g of solution.

¹³C NMR data collection and processing

Approximately 100 µl of each oil in 700 µl of CDCl₃ with TMS as reference was used for NMR analysis at 25 °C. ¹³C NMR spectra were run on a 400 MHz Varian ^{Unity}Inova NMR spectrometer operating at 100 MHz for ¹³C. Acquisition parameters similar to those recommended by Mannina *et al*.^[10] were used for collecting the ¹³C NMR spectra: number of points 256 K; spectral width 195 ppm; relaxation delay: 7 s; acquisition time 4.5 s. When processing, a line-broadening of -0.092 and Gaussian enhancement of 0.7 was used to optimize the resolution of the spectra. The precision of the procedure is estimated by the average half height of the resonances in the crowded region which is at most 0.7 Hz (0.007 ppm). In fact if we do the procedure with the graphical linear correlation method we get even better results, and a precision of ± 0.004 ppm.

GC analysis of vegetable oils

Methyl esterification of vegetable oils for GC analysis^[11] was carried out as follows: Sodium (0.5 g) was dissolved in 100 ml methanol. The sodium methoxide solution (0.3 g) together with 2 g of the specific vegetable oil was placed in a vial and heat sealed. The heat sealed vial was left for 2 h at 85–90 °C in an oil bath, with occasionally shaking. GC analysis of the vegetable oil samples was carried out to determine their fatty acid content in order to compare with the ¹³C NMR spectroscopy data obtained. 20 µl of each sample was diluted with 1 ml of dichloromethane and 1 µl portions were injected into a HP 5890 Series 2 GC equipped with a fused silica capillary (30 m × 0.25 mm i.d., 0.2 mm film thickness) coated with a 100% cyanopropylpolysiloxane non-bonded phase. A temperature programmed elution from 40 to 240 °C at a rate of 4 °C/min was used.

Results and Discussion

The striking superficial resemblance of the ${}^{13}C{}^{1}H$ NMR spectrum of a typical macadamia nut oil in CDCl₃ shown in Fig. 1 to that of a ${}^{13}C{}^{1}H$ NMR spectrum of olive oil, suggests that similar major triacylglycerols are present in all these oils. In order to aid the rapid but unambiguous identification of the individual major triacylglycerols present in all six vegetable oils from their ${}^{13}C{}^{1}H$ NMR spectra we examined a series of oils and developed a simple method with which to assign all ${}^{13}C{}^{1}H$ resonances of the major triacylglycerols in such oils. GC analysis was first used to determine the fatty acid components of the triacylglycerols. Although GC analysis is the accepted analytical technique for

Table 1.	Assignment of ¹³ C NMR spectrum of trip	almitolein
Carbon	Position on glycerol backbone	Chemical shift (ppm)
C1	sn 1′, 3′	173.205
	sn 2′	172.795
C2	sn 1′, 3′	34.021
	sn 2′	34.185
C3	sn 1′, 3′	24.879
	sn 2′	24.879
C4	sn 1′, 3′	29.082
	sn 2′	29.044
C5	sn 1′, 3′	29.167
	sn 2′	29.188
C6	sn 1′, 3′	29.100
	sn 2′	29.115
C7	sn 1′, 3′	29.695
	sn 2′	29.709
C8	sn 1′, 3′	27.162
	sn 2′	27.162
C9	sn 1′, 3′	129.691
	sn 2′	129.665
C10	sn 1′, 3′	129.984
	sn 2′	129.995
C11	sn 1′, 3′	27.220
	sn 2′	27.224
C12	sn 1′, 3′	29.729
	sn 2′	29.729
C13	sn 1', 3'	29.984
_	sn 2′	29.986
C14	sn 1′, 3′; sn 2′	31.783
C15	sn 1′, 3′; sn 2′	22.655
C16	sn 1′, 3′; sn 2′	14.094
CHO		68.877
CH ₂ O		62.086

fatty acid compound analysis and quantification in vegetable oils, it has the main disadvantage of being a destructive and timeconsuming technique since chemical modification (esterification) of the triacylglycerols is required before analysis, unlike ¹³C{¹H} NMR spectroscopy. GC analysis of the oils indicated that apricot kernel, avocado pear, grapeseed, macadamia nut, mango kernel and marula oils contained the same major fatty acid components, namely oleic acid, palmitic acid, linoleic acid and stearic acid found in olive oil, although palmitoleic acid not detected in any olive oil samples was observed to be present in macadamia nut and avocado pear oil in significant amounts. With the exception of tripalmitolein, the ¹³C NMR spectra of the other triacylglycerols present in the six vegetable oils have previously been assigned by Mannina et al.^[10] The full assignment of the ¹³C NMR spectrum of a sample of pure tripalmitolein (Fig. 2) was carried out by methodologies given by Mannina et al.^[10] for other triacylglycerols, and comparison of chemical shift trends observed for mainly triolein (Table 1).

With the aim of developing a rapid method with which to determine the major triacylglycerols present in apricot kernel, avocado pear, grapeseed, macadamia nut, mango kernel and marula oils using $^{13}C\{^{1}H\}$ NMR spectroscopy we initially investigated the use of the procedure developed for mainly olive oils^[10] which involves the addition of standard triacylglycerols

Table 2. Assignment of ¹³ C NMR resonances of the major fatty acid residues in olive oil							
		Decitien en elected	Chaminal	Chemical shift (ppm) Literature assignments ^[3,4,13]			
Section	Assignment	Position on glycerol backbone	Chemical shift (ppm) measured	Vlahov et al. ^[3]	Sacchi <i>et al</i> . ^[4]	Shaw et al. ^[13]	
А	P1	sn 1′, 3′	173.236		173.27	173.113	
	V/E		173.223			173.101	
	01	sn 1′, 3′	173.204		173.2	173.084	
	L1	sn 1′, 3′	173.194		173.17	173.075	
	01	sn 2'	172.837		172.83	172.688	
	L1	sn 2′	1/2./8/		1/2.//	1/2.6/9	
В	L13	sn 2′	130.198	130.15	130.22	130.105	
	L13	sn 1′, 3′	130.191	130.15	130.22	130.097	
	010	sn 2'	130.016	129.96	130.04	129.945	
	010	sn 1′, 3′	130.001	129.94	130.02	129.93	
	L9	sn 1°, 3°	129.984	129.91	129.98	129.9	
	L9 V/E	511 2	129.956	129.69	150.01	129.074	
	V/E		129.910				
	09	sn 1′. 3′	129.702	129.65	129.69	129.64	
	09	sn 2'	129.676	129.63	129.72	129.614	
	L10	sn 2′	128.095	128.07	128.12	128.053	
	L10	sn 1′, 3′	128.077	128.05	128.11	128.035	
	L12	sn 1′, 3′	127.911	127.89	127.94	127.874	
	L12	sn 2′	127.899	127.88	127.93	127.862	
С	СНО		68.911	68.90		68.8852	
	CH2O		62.108	62.06		62.0478	
D	02/L2	sn 2′	34,206	34.16		34,1408	
-	P2	sn 1′, 3′	34.064	34.01		33.9972	
	UK	,	34.054				
	O2/L2	sn 1′, 3′	34.041	33.990		33.9758	
Е	P14		31.959	31.940	34.26	31.9283	
_	016		31.938	31.920	34.2	31.9074	
	UK		31.818	31.80 (UK)			
	L16		31.555	31.53	34.15	31.5167	
F	012		29.796	29.770		29.7586	
	07	sn 2′	29.745				
	P10		29.735	29.720 (UK)		29.6637	
	O7/P12	sn 1′, 3′	29.731				
	P11		29.718	29.68 (UK)			
	P8		29.693	29.64 (UK)			
	P7	sn 1′, 3′	29.653			29.6637	
	L7	sn 2′	29.650			29.6203	
	L/	sn 1′, 3′	29.635	20.54		20 5200	
	014	$\sin 2'$	29.560	29.54		29.5289	
	D14 D5	5111,5	29.556	29.54		29.5269	
	P13		29.308	29.49		29.4740	
	115		29.357	29.36		29 3419	
	013		29.356	29.34		29.3156	
	015		29.353	29.34		29.3156	
	P6		29.302	29.28		29.2686	
	O5/L5	sn 2′	29.225	29.20		29.1869	
	O5/L5	sn 1′, 3′	29.203	29.18		29.1656	
	O6/L6	sn 2′	29.153	29.10			
	P4		29.145	29.10			
	O6/L6	sn 1′, 3′	29.136	29.10			
	04	sn 1′, 3′	29.114	29.10		29.0717	
	L4	sn 1′, 3′	29.108	29.10		29.0717	
	04/L4	sn 2'	29.075	29.05		29.0324	

				Chemical shift	t (ppm) Literature assi	gnments ^[3,4,13]
Section	Assignment	Position on glycerol backbone	Chemical shift (ppm) measured	Vlahov et al. ^[3]	Sacchi <i>et al</i> . ^[4]	Shaw et al. ^[13]
G	011	sn 2′	27.249	27.22	27.31	27.1477
	011	sn 1′, 3′	27.245	27.22	27.31	27.1477
	L14		27.226	27.20	27.22	27.1657
	L8		27.214	27.18		27.1815
	08		27.194	27.16	27.31	27.2009
Н	L11		25.653	25.62	25.67	25.6084
	O3	sn 2′	24.908	24.88		24.8605
	L3	sn 2′	24.898	24.88		24.8605
	P3		24.890	24.86	24.84	24.8466
	O3	sn 1′, 3′	24.869	24.84	24.8	24.8239
	L3	sn 1′, 3′	24.861	24.84	24.7	24.8239
I	P15		22.720	22.70 (S17)*	22.82	22.6841 (S17)*
	017		22.711	22.70	22.8	22.6743
	L17		22.602	22.58	22.61	22.5656
J	P16		14.133	14.09 (S18)*	14.13	14.0824 (S18)*
	O18		14.127	14.09	14.13	14.0769
	UK		14.119			
	L18		14.081	14.05	14.09	14.037

O, olein; P, palmitin; L, linolein; S, saturated; UK, unknown; V/E, vaccenin or eicosenoin.

* The chemical shifts of the omega 1, 2 and 3 carbons (P14, 15 and 16) have been correlated with the corresponding omega carbons in the literature.

(triolein, tristearin, etc.) to the sample of vegetable oil and using deconvolution of the ¹³C{¹H} NMR spectrum collected to detect enhancement of particular resonances.^[7,8,10] Although this approach has been successfully applied to olive and other oils in the literature, its limitations include the time-consuming nature (requiring repeated ¹³C NMR spectra to be acquired for each standard triacylglycerol added) and the use of expensive but potentially unavailable pure triacylglycerol standards. In our hands moreover, a major limitation with this standard addition approach was that the complete assignment of the spectra of the six oils of interest was not always possible. Although a large number of ${}^{13}C{}^{1}H$ resonances in the spectrum could be assigned by inspection and comparison with data in the literature, the method of standard additions could not be applied to all regions in the $^{13}\text{C}\{^{1}\text{H}\}$ spectrum, notably in the spectrally crowded 29 \pm 1 ppm region in which several CH₂ carbon resonances are found resulting in significant spectral overlap (Fig. 1). Spiking the oil with a standard in this crowded region leads to the expected intensity increases of specific resonances, but unfortunately can also result in significant loss of resolution due to spectral overlap in this region making clear assignments of individual resonances very difficult if not impossible. This is probably the reason why this region of the ¹³C{¹H} spectrum of olive oil has not been fully assigned in the literature to our knowledge. A more invidious problem with the standard additions method is small but detectable concentration dependence of ¹³C{¹H} chemical shifts of triacylglycerols and thus the possibility of this leading to peak overlap on addition of the standard; in the case of olive oils a limited concentration dependence of ${}^{13}C{}^{1}H$ has been reported by Mannina et al. [12] It is obvious that such concentration dependence of ¹³C{¹H} chemical shifts may lead to ambiguity when using the standard addition method for the assignment of the ¹³C spectra of vegetable oils. In

this context we propose a new approach to aid in the rapid and reliable assignment of ¹³C NMR spectra in vegetable oils.

Assignment of $^{13}\mathrm{C}$ NMR spectra using the graphical linear correlation method

The proposed method is based on the reasonable expectation that the ¹³C{¹H} chemical shifts of a fatty acid residue of a particular triacylglycerol in a given solvent at a specified concentration should all be affected, to a first approximation, in a similar manner by the factors responsible for the observed concentration dependence. Moreover it may be expected that saturated sp^3 carbon atoms might be differently affected to unsaturated sp² carbon atoms for a given fatty acid residue (vide infra). On this basis it would be reasonable to expect that $^{13}\mbox{C}{^1\mbox{H}}$ shifts of all carbon resonances of a fatty acid of a pure, standard triacylglycerol (e.g. triolein, tripalmitin, etc.) would be linearly correlated to the corresponding fatty acid residues of the triacylglycerols in a vegetable oil mixture in a given solvent at a specified concentration range. To test this expectation the dependence of the ${}^{13}C{}^{1}H$ chemical shifts of concentration changes has been determined for olive oil in CDCl₃ and we found that for sp³ carbon atoms the method is satisfactorily independent on concentration of the vegetable oil within the concentration range of approximately 0.10-0.20 g oil/g of CDCl₃ solution. Similar trends were found for the other vegetable oils. For each concentration of vegetable oil, the ¹³C{¹H} shifts of selected carbon atoms were found to result in linear trends with identical gradients but slightly differing intercepts at 'zero' concentration, which indicates that at a practical concentration range of approximately 0.10–0.20 g oil/g of CDCl₃ solution, all aliphatic ¹³C{¹H} shifts are affected to the same degree, and importantly in a linear fashion as a result of small concentration changes.

This can be clearly seen in Fig. 3 where the chemical shifts of an aliphatic sp^3 carbon L5 of trilinolein is shown to change linearly with a change in concentration from 0.095 to 0.20 g oil/g solution.

Interestingly the concentration dependence of resonances of sp²-type carbon atoms (e.g. olefinic and carbonyl carbon atoms) shows a rather more complex non-linear trend (Fig. 3). This suggests that linear concentration dependences for such ¹³C{¹H} shifts and thus correlations cannot generally be expected for such

carbon atoms. As these resonances are in non-crowded spectral regions they are generally easily assigned by inspection in most vegetable oils making this effect not too serious a limitation. Nevertheless we have found that within a limited concentration range (approximately 0.10–0.20 g oil/1 g of CDCl₃ solution) the proposed graphical linear correlation method can, as a first approximation, also be used for assignments of sp²-type carbon resonances. Comparing the graphs of the sp² and sp³ (Fig. 3)

Avecado Macado Macadomia Partoni (peno) Cafon Position mitoli (peno) P1 sn 1/3' 173.266 P1 sn 1/3' 173.253 Pat sn 1/3' 173.253 Pat sn 1/3' 173.253 D1 sn 1/3' 173.255 D1A1 sn 1/3' 173.253 D1/Pat sn 1/3' 173.256 D1/Pat/L1 sn 2' 120.201 D1/Pat sn 2' 172.815 D10/Pat/D1 sn 2' 130.001 L1 sn 2' 130.017 U/E 129.823 129.823 D10/Pat0 sn 2' 130.003 U/E 129.823 129.823 D10/Pat0 sn 1/3' 130.007 U/E 129.823 129.823 D10/Pat0 sn 1/3' 129.824 D10 sn 2'.3 129.829 U10 sn 1/3' 129.824 D10 sn 2'.3 129.829 V/E 129.824 D10 sn 2'.3 129.824 V/E 129.826 </th <th colspan="7">Table 3. Assignment of ¹³C NMR resonances of the major fatty acid residues in avocado pear and macadamia nut oil</th>	Table 3. Assignment of ¹³ C NMR resonances of the major fatty acid residues in avocado pear and macadamia nut oil						
P1sn1/3'173,266P1sn1/3'173,258Pa1sn1/3'173,253Pa1sn1/3'173,226L1sn1/3'173,253O1/L1sn1',3'173,226L1sn1',3'173,226L13sn2'122,018L1sn2'172,215O10/Pa10sn1',3'130,003L13sn2'130,017VE129,923O10/Pa10sn1',3'130,003V/E129,825O10/Pa10sn1',3'130,003V/E129,825O10/Pa10sn1',3'120,003V/E129,825O10/Pa10sn1',3'120,003V/E129,825O10/Pa10sn1',3'120,003V/E128,925U10/Pa10sn1',3'129,026O9/Pa9sn2'128,026VE129,928L10sn1',3'128,034VE129,929L10sn1',3'128,034VE129,724L10sn1',3'128,034VE129,724L10sn1',3'128,036VE129,724L12sn1',3'127,99609sn2'128,037CHO62,11110sn1',3'128,057CHO62,111110sn1',3'128,056CHO127,996121sn1',3'128,056CHO13,933122sn1',3'34,056L1631,935124sn1',3'34,056L1631,931125sn1',3'34,056L1631,931	Carbon	Position	Avocado pear oil (ppm)	Carbon	Position	Macadamia nut oil (ppm)	
Pa1sn1/3'173.253Pa1sn1',3'173.243O1sn1/3'173.255O1/L1sn1',3'173.265L1sn1',3'173.265O1/Pa1/L1sn2'172.818O1/Pa1sn2'172.825L13	P1	sn 1′,3′	173.266	P1	sn 1′, 3′	173.256	
01sn '1.3''73.23501./1sn '1.3''73.226L1sn '2''72.81501./Pa1.0sn 2''73.205L1sn 2''72.81501.0/Pa1.0sn 2''130.017L1sn 2''130.02201.0/Pa1.0sn 2''130.033L13sn 2''130.15519sn 2''139.825010/Pa1.0sn 1'.3''130.031V/E'139.825010/Pa1.0sn 1'.3''129.96209/Pa9sn 1'.3''129.825010/Pa1.0sn 1'.3''129.96209/Pa9sn 2''129.82510sn 1'.3''129.96209/Pa9sn 2''129.82510sn 1'.3''129.96209/Pa9sn 2''129.82510sn 1'.3''129.96209/Pa9sn 2''129.82610sn 1'.3''129.962Cl0sn 1'.3''127.90512sn 1'.3''129.92L10sn 1'.3''127.90509sn 1'.3''129.92Cl0sn 1'.3''127.906110sn 1'.3''128.075O2/Pa2.2sn 1'.3''127.90512sn 1'.3''128.075O2/Pa2.2sn 1'.3''140.8112sn 1'.3''128.91Pa2sn 1'.3''140.8112sn 1'.3''128.91'128.91'13.91'13.9113'129.91Pa2sn 1'.3''13.91'13.9114sn 1'.3''128.91Pa1sn 1'.3''13.911504.92sn 1'.	Pa1	sn 1′,3′	173.253	Pa1	sn 1′, 3′	173.243	
11sn 1',3'173.22601/Pa10sn 2'172.81801/Pa10sn 2'172.8251.13130.00111sn 2'130.002010/Pa10(.9)sn 2'130.001113sn 1',3'130.022010/Pa10(.9)sn 1',3'130.003010/Pa10sn 2'130.017V/E129.825010/Pa10sn 2'130.017V/E129.825010/Pa10sn 2'129.98909/Pa9sn 1',3'129.70519sn 2'129.96209/Pa9sn 1',3'129.70519sn 1',3'129.96209/Pa9sn 1',3'128.07419sn 1',3'129.96209/Pa9sn 1',3'128.07419sn 1',3'129.96209/Pa9sn 1',3'128.07419sn 1',3'129.709112sn 1',3'128.07509sn 1',3'129.076CHQsn 2'42.08609/Pa9sn 2'128.07502/Pa2.12sn 1',3'34.05610sn 1',3'128.07502/Pa2.12sn 1',3'34.056112sn 1',3'34.056Pa14sn 1',3'34.056112sn 1',3'34.056Pa14sn 1',3'31.9330,L/Pa2sn 1',3'34.056Ia14sn 1',3'29.7630,L/Pa2sn 1',3'34.056Ia14sn 1',3'29.7630,L/Pa2sn 1',3'34.056Ia14sn 1',3'29.7630,L/Pa2sn 1',3'34.056Pa12sn	01	sn 1′,3′	173.235	01/L1	sn 1′, 3′	173.226	
01/Pa1sn 2'172.8151313130.001L1sn 1'.3'130.022010/Pa10.9sn 1', 3'130.033L13sn 1'.3'130.022010/Pa10.9sn 1', 3'129.923010/Pa10sn 2'130.017V/E129.825010/Pa10sn 1'.3'130.03V/E129.825010/Pa10sn 1'.3'129.96209/Pa9sn 1'.3'129.67919sn 2'129.96209/Pa9sn 2'128.07419sn 1'.3'129.96209/Pa9sn 2'128.074V/E129.96210sn 1'.3'127.909V/E129.9705L12sn 1'.3'127.90909sn 1'.3'129.706L12sn 1'.3'127.90909sn 1'.3'129.704L12sn 1'.3'127.90909sn 1'.3'129.678CH0sn 2'63.07L10sn 2'128.092CH20sn 2'34.065L12sn 1'.3'128.07502/Pa2.12sn 1'.3'34.065L12sn 1'.3'127.91P2sn 1'.3'34.065L12sn 1'.3'34.065Pa1413.93113.931P2sn 1'.3'34.065Pa1413.15531.811P32sn 1'.3'34.065L1631.85131.811P32sn 1'.3'34.056L1631.81129.763P1431.812Pa12sn 1'.3'29.763P1431.812Pa12sn 1'.	L1	sn 1′,3′	173.226	O1/Pa1/L1	sn 2′	172.818	
L1sn 2'172.815010/Pa10sn 2'130.017L13sn 1',3'130.025010/Pa10(L9sn 1', 3'130.030L13sn 2'130.017V/E129.822010/Pa10sn 2'130.017V/E129.822010/Pa10sn 2'130.017V/E129.822L9sn 1',3'129.030V/E129.825L9sn 1',3'129.92209/Pa9sn 1',3'129.705L9sn 1',3'129.922L10sn 2'128.074VE129.92L10sn 1', 3'128.074VF129.92L10sn 1', 3'128.074VF129.92L12sn 1', 3'128.074Og/Pa9sn 1', 3'128.074L12sn 1', 3'128.074Og/Pa9sn 2'128.074L12sn 1', 3'127.896Og/Pa9sn 2'128.074CH2Osn 2'44.086Og/Pa9sn 1', 3'128.07502/Pa2/L2sn 1', 3'34.056L12sn 1', 3'128.07502/L2sn 1', 3'34.056CH2Osn 1', 3'34.056L613.15531.557OL/L2sn 1', 3'34.056L1631.55731.557OL/L2sn 1', 3'39.55O1229.72829.738Pa1431.55108/Pa7sn 1', 3'29.759Pa1431.55108/Pa7sn 1', 3'29.751Pa1431.55108/Pa7sn 1', 3' <t< td=""><td>O1/Pa1</td><td>sn 2′</td><td>172.825</td><td>L13</td><td></td><td>130.201</td></t<>	O1/Pa1	sn 2′	172.825	L13		130.201	
L13sn 1/3'130.202010Pa10/L9sn 1/3'13003L13sn 2'130.195Usn 2'129.923010Pa10sn 2'130.003V/E129.825010Pa10sn 1/3'129.06209/Pa9sn 1/3'129.079L9sn 1/3'129.92L10sn 2'128.079VE129.92L10sn 1/3'128.074VE129.709L12sn 1/3'127.09609sn 1/3'129.704L12sn 1/3'127.09609sn 1/3'129.0704L12sn 1/3'127.09609/Pa9sn 2'128.072CHO68.907110sn 2'128.072CHO4.005L10sn 1/3'128.074L12sn 1/3'34.065L12sn 1/3'128.07502/Pa2/L2sn 1/3'34.065L12sn 1/3'128.07502/Pa2/L2sn 1/3'34.065L12sn 1/3'127.91P134.05531.935L12sn 1/3'34.065P1431.93531.935P2sn 1/3'34.065P1431.93531.55O2/L2sn 1/3'34.065P1431.93531.55O2/L2sn 1/3'34.065P1431.95531.55O2/L2sn 1/3'34.065P1431.95531.55O2/L2sn 1/3'34.065P1431.95531.55O2/L2sn 1/3'32.75P14Sn 1/3'29.755 <td>L1</td> <td>sn 2′</td> <td>172.815</td> <td>O10/Pa10</td> <td>sn 2′</td> <td>130.017</td>	L1	sn 2′	172.815	O10/Pa10	sn 2′	130.017	
L13sn 2'130.19519sn 2'129.823O10/Pa10sn 2'130.017V/E129.825O10/Pa10sn 1',3'129.08909/Pa9sn 1',3'129.055L9sn 2'129.98909/Pa9sn 1', 3'129.075L9sn 1',3'129.09209/Pa9sn 2'128.093V/E129.824L10sn 1', 3'128.074Pa9129.079L12sn 1', 3'127.07609sn 1', 3'129.076L12sn 2'127.86609/Pa9sn 2'128.072CH2Osn 2'34.06509/Pa9sn 1', 3'128.072CH2Osn 1', 3'34.065L12sn 1', 3'128.072CH2Osn 1', 3'34.055L12sn 1', 3'128.073O2/Pa2/L2sn 1', 3'34.055L12sn 1', 3'34.065Pa131.95331.933P2sn 1', 3'34.065Pa1431.95531.55OL/Pa2sn 1', 3'34.065L1631.95729.752O1631.934Pa12sn 1', 3'29.751Pa1431.955O1229.732Pa1431.955O2/Pa2sn 1', 3'29.751Pa1431.954Pa12sn 1', 3'29.751Pa1431.951O8/Pa2sn 1', 3'29.751Pa1431.951O8/Pa2sn 1', 3'29.751Pa12sn 2' <td>L13</td> <td>sn 1′,3′</td> <td>130.202</td> <td>O10/Pa10/L9</td> <td>sn 1′, 3′</td> <td>130.003</td>	L13	sn 1′,3′	130.202	O10/Pa10/L9	sn 1′, 3′	130.003	
010/Pa10sn 2'130.017V/E129.82010/Pa10sn 1',3'129.96209/Pa9sn 1',3'129.763L9sn 1',3'129.96209/Pa9sn 2'128.073L9sn 1',3'129.962L10sn 2'128.073V/E129.704L10sn 1',3'127.09609sn 1',3'129.704L12sn 1',3'127.89609/Pa9sn 2'128.073L12sn 1',3'127.89609/Pa9sn 2'128.073CHO62.1110sn 1',3'128.07502/Pa2/L2sn 1',3'34.065L12sn 1',3'128.07502/Pa2/L2sn 1',3'34.056L12sn 1',3'128.07502/Pa2/L2sn 1',3'34.056CHO68.91302/L2sn 1',3'34.05531.955OL/Pa2sn 1',3'34.056Pa1431.95131.955OL/Pa2sn 1',3'34.056L1631.5131.955OL/Pa2sn 1',3'34.056L1631.5531.555O2/L2sn 1',3'34.056L1631.5531.555O2/L2sn 1',3'34.056L1631.5531.555O16sn 1',3'32.7531.95531.55531.53O17sn 2'32.756Pa7/P1sn 1',3'32.75Pa12sn 1',3'29.756Pa7/P1sn 1',3'32.75P1430.63L1630.95P14 <td>L13</td> <td>sn 2′</td> <td>130.195</td> <td>L9</td> <td>sn 2′</td> <td>129.923</td>	L13	sn 2′	130.195	L9	sn 2′	129.923	
O10/Pa10sn 1',3'130.003V/E129.822L9sn 2'129.96209/Pa9sn 1',3'129.057V/E129.96209/Pa9sn 2'128.093V/E129.924L10sn 2'128.093V/E129.924L10sn 1',3'127.091Pa9sn 1',3'129.709L12sn 1',3'127.991O9/Pa9sn 1',3'129.709L12sn 1',3'127.996O9/Pa9sn 1',3'129.678CHC68.0716.111L10sn 1',3'128.092CH2Osn 2'3.42.08L12sn 1',3'128.092CH2Osn 2'3.40.65L12sn 1',3'128.092CH2Osn 1',3'3.40.65L12sn 1',3'127.91P2sn 1',3'3.40.65CH2Osn 1',3'127.91P2sn 1',3'3.40.55CH2Osn 1',3'34.065Pa1sn 1',3'3.40.55CH2Osn 1',3'34.065Pa1sn 1',3'3.40.55OL/Pa2sn 1',3'34.065Pa1sn 1',3'3.9.33Pa2sn 1',3'34.056Pa1sn 1',3'2.9.79.2O16sn 1',3'3.40.55Pa12sn 2', 2.9.73.1P14Sn 1',3'3.9.85P12sn 2', 2.9.73.12.9.73.1P14sn 1',3'2.9.73.1P14sn 1',3'2.9.73.1P12sn 1',3'2.9.73.1P14sn 1',3'2.9.73.1P12sn 1',	O10/Pa10	sn 2′	130.017	V/E		129.825	
L9sn 2'129,98909/Pa9sn 1',3'129,703L9sn 1',3'129,96209/Pa9sn 2'129,679V/E129,292L10sn 2'128,074Pa9129,879L10sn 1',3'128,074Pa9129,704L12sn 1',3'127,09909sn 1',3'129,704L12sn 1',3'127,09609/Pa9sn 2'126,078CHOsn 2'68,007L10sn 2'128,092CH2sn 2'34,005L10sn 1',3'128,07502/Pa2/L2sn 1',3'34,065L12sn 1',3'127,919P2sn 1',3'34,065L12sn 1',3'127,919P2sn 1',3'34,065L12sn 1',3'34,00502/L2sn 1',3'34,065CHO62,114P1431,95331,933P2sn 1',3'34,005L1631,933P2sn 1',3'34,065L1631,931P3sn 1',3'31,934P12sn 1',3'29,763P1431,935O1229,76329,763P1431,934P412sn 1',3'29,731P3sn 1',3'29,763P3,711sn 1',3'29,731P3sn 1',3'29,763P3,711sn 1',3'29,721O7sn 2'29,732P629,73429,731P1/Pa7sn 1',3'29,732P829,554P1/Pa7sn 1',	O10/Pa10	sn 1′,3′	130.003	V/E		129.822	
L9 sn 1,3' 129,962 09/Pa9 sn 2' 128,074 VE 129,824 L10 sn 1,' 3' 128,084 Pa9 129,824 L10 sn 1,' 3' 129,096 09 sn 1,' 3' 129,079 L12 sn 1,' 3' 127,096 09/Pa9 sn 2' 129,079 L12 sn 1,' 3' 127,096 10 sn 2' 128,072 CHQ 6.11 110 sn 1,' 3' 128,073 CHQ 6.11 110 sn 1,' 3' 128,073 CHQ sn 1,' 3' 34,065 112 sn 1,' 3' 127,91 P2 sn 1,' 3' 34,065 112 sn 1,' 3' 127,91 P2 sn 1,' 3' 34,065 112 sn 2' 127,898 Pa2 sn 1,' 3' 34,043 CH2O 62,114 P14 31,935 91,92 31,935 O1/A2 sn 1,' 3' 34,056 L16 31,55 O2/L2 sn 1,' 3' 34,042 UK 30,897 P14 31,934 Pa12 sn 1,' 3' 29,763 P14 31,935 O12 29,763 91,97 P14 31,935 O12 29,763	L9	sn 2′	129.989	O9/Pa9	sn 1′, 3′	129.705	
V/E 129.92 L10 sn 2' 128.093 V/E 129.824 L10 sn 1', 3' 129.099 Pa9 sn 1', 3' 129.079 L12 sn 1', 3' 127.099 09 sn 1', 3' 129.074 L12 sn 2' 127.896 09/Pa9 sn 2' 128.092 CH20 68.907 110 sn 1', 3' 128.092 CH20 sn 2' 34.086 L12 sn 1', 3' 127.91 P2 sn 1', 3' 34.086 L12 sn 1', 3' 127.898 Pa2 sn 1', 3' 34.045 CH20 62.913 02/L2 sn 1', 3' 34.045 CH20 62.913 02/L2 sn 1', 3' 34.045 CH20 sn 1', 3' 34.056 P14 31.811 Pa2 sn 1', 3' 34.056 P14 31.811 Pa2 sn 1', 3' 34.056 L16 31.811 Pa2 sn 1', 3' 34.056 L16 31.811 Pa2 sn 1', 3' 34.056 L16 31.931	L9	sn 1′,3′	129.962	O9/Pa9	sn 2′	129.679	
V/E 129,824 L10 sn 1',3' 128,074 Pa9 129,709 L12 sn 1',3' 127,909 09 sn 2' 129,704 L12 sn 2' 128,050 09/Pa9 sn 2' 128,072 CH0 68,907 L10 sn 2' 128,075 O2/Pa2/L2 sn 1', 3' 34,065 L12 sn 1', 3' 127,91 P2 sn 1', 3' 34,065 L12 sn 1', 3' 127,91 P2 sn 1', 3' 34,065 L12 sn 2' 127,898 Pa2 sn 1', 3' 34,065 CH20 62,114 P14 31,933 19,935 OL/Pa2 sn 1',3' 34,065 P14 31,851 OL/Pa2 sn 1',3' 34,065 P14 31,851 OL/L2 sn 1',3' 34,065 P14 31,851 OL/L2 sn 1',3' 34,065 P14 31,851 OL/L2 sn 1',3' 34,042 UK 30,897 P14 31,934 P12 sn 1',3' 29,735 P14	V/E		129.92	L10	sn 2′	128.093	
Pa9I29.709L12sn 1', 3'127.099O9sn 1', 3'129.704L12sn 2'127.896O9/Pa9sn 2'128.092CHO68.907L10sn 1', 3'128.075O2/Pa2/L2sn 1', 3'34.065L12sn 1', 3'127.918Pa2sn 1', 3'34.065L12sn 2'127.898Pa2sn 1', 3'34.056CHO68.913O2/L2sn 1', 3'34.056CH2O5n 1', 3'34.065Pa1431.953OL,Pa2sn 1', 3'34.065Pa1431.953OL,Pa2sn 1', 3'34.065Pa1431.953OL,LPa2sn 1', 3'34.065Pa1431.953OL,LPa2sn 1', 3'34.065Pa1431.953OL,LPa2sn 1', 3'34.065Pa1431.955O2/L2sn 1', 3'34.065Pa1431.955O2/L2sn 1', 3'34.065Pa1431.955O2/L2sn 1', 3'34.065Pa12sn 2'P1431.955O1229.755D16J1.954Pa12sn 1', 3'P12sn 1', 3'29.755Pa14sn 1', 3'P12sn 2'29.763Pa17sn 1', 3'P12sn 1', 3'29.756Pa7/P11sn 1', 3'P32sn 1', 3'29.732Pa29.689O7/P12/Pa7sn 1', 3'29.756Pa7/P11sn 1', 3'P3429.756P13I1529.565 </td <td>V/E</td> <td></td> <td>129.824</td> <td>L10</td> <td>sn 1′, 3′</td> <td>128.074</td>	V/E		129.824	L10	sn 1′, 3′	128.074	
O9 sn 1',3' 129,704 L12 sn 2' 127,896 O9/Pa9 sn 2' 128,092 CHO 68,077 L10 sn 1', 3' 128,092 CH2O 50,211 L10 sn 1', 3' 128,075 O2/Pa2/L2 sn 2' 34,085 L12 sn 1', 3' 127,91 P2 sn 1', 3' 34,065 L12 sn 2' 22,12 sn 1', 3' 34,065 CHO 68,913 O2/L2 sn 1', 3' 34,043 CHO 62,114 P14 31,955 01,Pa2 sn 1',3' 34,043 CHQ sn 1',3' 34,056 L16 31,551 02/L2 sn 1',3' 31,551 O2/L2 sn 1',3' 34,056 L16 31,551 02/L2 sn 2' 29,763 P14 31,955 O12 29,793 P10 sn 1', 3' 29,753 P14 31,951 O2/Pa2/L2 sn 1', 3' 29,731 P3 P12 sn 1', 3' <t< td=""><td>Pa9</td><td></td><td>129.709</td><td>L12</td><td>sn 1′, 3′</td><td>127.909</td></t<>	Pa9		129.709	L12	sn 1′, 3′	127.909	
O9/Pa9sn 2'129.678CHO68.907L10sn 2'128.092CH2O62.11L10sn 1', 3'128.075O2/Pa2/L2sn 2'L12sn 1', 3'127.91P2sn 1', 3'L12sn 2'127.898Pa2sn 1', 3'L12sn 2'62.114P1431.955CH2O62.114P1431.955OL,Pa2sn 1', 3'34.065Pa1431.935OL,Pa2sn 1', 3'34.065Pa1431.935OL,Pa2sn 1', 3'34.065Pa1431.935O2/L2sn 1', 3'34.065Pa1431.811Pa2sn 1', 3'34.065Pa1431.815O2/L2sn 1', 3'34.056L1631.937Pa431.935O12sn 2'29.792O1631.934Pa12sn 1', 3'29.792Pa1431.812Pa12sn 1', 3'29.792Pa12sn 2'29.793P10sn 1, 3'29.792Pa12sn 1', 3'29.793P39.7129.714Pa12sn 1', 3'29.724P329.72429.743P10/Pa7sn 1', 3'29.732P829.743P10/Pa7sn 1', 3'29.732P329.744P10/Pa7sn 1', 3'29.732P329.744P10/Pa7sn 1', 3'29.732P329.745P1129.743P929.74329.745P12sn 1', 3'29.75	09	sn 1′,3′	129.704	L12	sn 2′	127.896	
L10sn 2'128.092CH20	O9/Pa9	sn 2'	129.678	СНО		68.907	
L10sn 1', 3'128.075O2/Pa2/L2sn 2'34.208L12sn 1', 3'127.91P2sn 1', 3'34.065L12sn 2'127.898Pa2sn 1', 3'34.066L12sn 2'68.913O2/L2sn 1', 3'34.036CHO68.913O2/L2sn 1', 3'31.95531.955O,LPa2sn 2'34.208O1611.933P2sn 1', 3'34.065Pa141.811Pa2sn 1', 3'34.056L1631.811Pa2sn 1', 3'34.042UK30.897P1431.955O1229.762O1631.934Pa12sn 1', 3'P1431.812Pa12sn 1', 3'O1631.551O8/Pa7sn 2'P1431.551O8/Pa7sn 2'O1631.551O8/Pa7sn 1', 3'P12sn 2'29.763Pa12sn 1', 3'O1229.773Pi0sn 1', 3'O1229.732P829.714O7sn 2'29.732P829.714O7sn 1', 3'29.732P829.556P10/Pa7sn 1', 3'29.556P1329.554P10/Pa7sn 1', 3'29.65P1329.332P10/Pa7sn 1', 3'29.556P1329.337P10/Pa7sn 1', 3'29.556P1329.337P1129.556P13	L10	sn 2′	128.092	CH2O		62.11	
L12sn 1', 3'127.91P2sn 1', 3'34.065L12sn 2'127.898Pa2sn 1', 3'34.066CHO68.91302/L2sn 1', 3'34.043CH2O62.114P14915O,LPa2sn 1', 3'34.065Pa1431.933P2sn 1', 3'34.065L1631.933P2sn 1', 3'34.065L1631.933P2sn 1', 3'34.062UK31.937P1431.955O1229.792O161.1, 3'31.934Pa12sn 1', 3'P1431.955O1229.763P1431.8151O8/Pa7sn 1', 3'O122.9, 793P10sn 1', 3'P12sn 2'29.763O8/P12sn 1', 3'O122.9, 793P10sn 1', 3'29.721O143.92'29.732P829.731P12sn 2'29.732P829.731P142.9, 732P829.689O7/P12/Pa7sn 1', 3'29.72429.755P112.929.732P829.554P142.9, 556O1529.372P142.9, 556O1529.372P142.9, 556O1529.372P142.9, 556O1529.372P142.9, 556O1529.374P142.9, 556O1529.374P142.9, 556O1529.374P142.9, 556O15 </td <td>L10</td> <td>sn 1′, 3′</td> <td>128.075</td> <td>O2/Pa2/L2</td> <td>sn 2′</td> <td>34.208</td>	L10	sn 1′, 3′	128.075	O2/Pa2/L2	sn 2′	34.208	
L12 sn 2' 127.898 Pa2 sn 1', 3' 34.056 CHO 68.913 OZ/L2 sn 1', 3' 34.043 CH2O 62.114 P14 31.955 OL,Pa2 sn 2' 34.065 Pa14 31.931 P2 sn 1',3' 34.065 Pa14 31.811 Pa2 sn 1',3' 34.056 L16 31.55 O2/L2 sn 1',3' 34.042 UK 30.897 P14 31.935 O12 29.792 O16 31.934 Pa12 sn 2' 29.763 P14 31.812 Pa12 sn 2' 29.763 P14 31.551 08/Pa7 sn 1, 3' 29.751 L16 31.551 08/Pa7 sn 1, 3' 29.731 Pa12 sn 2' 29.763 08/P12 sn 1', 3' 29.731 Pa12 sn 2' 29.743 P9 29.744 29.741 P10/Pa7 sn 2' 29.732 P8 29.669 P11 9.1',3' 29.714 29.505 29.505 P7/L7 sn 1',3' 29.505 P13 29.505 P10/Pa7 sn 1',3' 29.505 29.332 29.332 29.332 <td>L12</td> <td>sn 1′, 3′</td> <td>127.91</td> <td>P2</td> <td>sn 1′, 3′</td> <td>34.065</td>	L12	sn 1′, 3′	127.91	P2	sn 1′, 3′	34.065	
CHO68.91302/L2sn 1', 3'34.043CH2O62.114P1431.955O,L/Pa2sn 2'34.006O1631.933P2sn 1', 3'34.065Pa1431.951Pa2sn 1', 3'34.056L1631.55O2/L2sn 1', 3'34.042UK30.897P1431.955O1229.792O1611.934Pa12sn 2'29.763Pa1431.955O1229.763Pa1431.55108/Pa7sn 1', 3'29.755L1631.55108/Pa7sn 1', 3'29.731Pa12sn 2'29.763Pa17sn 1', 3'29.731Pa12sn 2'29.763Pa7/P11sn 1', 3'29.721Pa12sn 1', 3'29.756Pa7/P11sn 1', 3'29.721Pa12sn 1', 3'29.732P829.73229.743P10/Pa7sn 2'29.732P829.68929.655P7/L7sn 1', 3'29.555O1329.554P829.689P5sn 1', 3'29.552P7/L7sn 1', 3'29.633L1529.332P529.506O1529.33329.333P51329.332P6sn 1', 3'29.292P13229.333P6sn 1', 3'29.292P13129.333Pa5sn 1', 3'29.292P13129.349O5/L5sn 1', 3'29.216P13 <td>L12</td> <td>sn 2'</td> <td>127.898</td> <td>Pa2</td> <td>sn 1′, 3′</td> <td>34.056</td>	L12	sn 2'	127.898	Pa2	sn 1′, 3′	34.056	
CH2062.114P1431.955 $O,L,Pa2$ $sn 2'$ 34.208 O16 31.933 $P2$ $sn 1',3'$ 34.065 $Pa14$ 31.811 $Pa2$ $sn 1',3'$ 34.056 $L16$ 31.935 $O2/L2$ $sn 1',3'$ 34.056 $L16$ 30.957 $P14$ 31.955 O12 29.792 $O16$ 31.934 $Pa12$ $sn 2'$ 29.763 $P14$ 31.812 $Pa12$ $sn 2'$ 29.763 $P14$ 31.812 $Pa12$ $sn 1', 3'$ 29.755 $D16$ 31.934 $Pa12$ $sn 1', 3'$ 29.755 $P14$ 31.812 $Pa12$ $sn 1', 3'$ 29.751 $D16$ $sn 1, 3'$ 29.793 $P10$ $sn 1, 3$ 29.731 $Pa12$ $sn 2'$ 29.763 $08/P12$ $sn 1', 3'$ 29.721 $O12$ $sn 2'$ 29.756 $Pa7/P11$ $sn 1', 3'$ 29.721 $O7$ $sn 2'$ 29.732 $P8$ $r1', 3'$ 29.561 $O7/P12/Pa7$ $sn 1', 3'$ 29.732 $P8$ $r1', 3'$ 29.561 $P11$ 29.715 $O14$ 29.555 29.352 29.721 29.372 $P34$ $p6$ $sn 1', 3'$ 29.352 29.353 29.353 29.354 29.354 $P5$ $sn 1', 3'$ 29.555 $O13$ $r1', 3'$ 29.347 29.347 $P34$ $P34$ $P34$ $P6$ $sn 1', 3'$ 29.298 $P5$ $sn 1', 3'$ 29.353	СНО		68.913	O2/L2	sn 1′, 3′	34.043	
O,L,Pa2sn 2'34.208O1631.933P2sn 1',3'34.065Pa1431.811Pa2sn 1',3'34.056L1631.55O2/L2sn 1',3'34.042UK30.897P141.955O1229.792O1631.934Pa12sn 2'P1431.812Pa12sn 2'O1631.55108/Pa7sn 2'P1431.55108/Pa7sn 2'29.733O1231.55108/Pa7sn 1', 3'29.731O1229.793P10sn 1, 3'29.731Pa12sn 2'29.79308/P12sn 1', 3'29.721Pa12sn 1', 3'29.756Pa7/P11sn 1', 3'29.721O7sn 2'29.732P829.669P10/Pa7sn 1', 3'29.72229.73529.556P1129.715O1429.556P7/L7sn 1', 3'sn2(L)29.655P1329.352P529.566O1529.35329.353P529.555O1329.33129.341P1329.393P6sn 1', 3'29.292O13/P629.353P6sn 1', 3'29.292O13/P629.349O5/L5sn 2', 39.229.216O13/P629.349O5/L5sn 1', 3'29.222	CH2O		62.114	P14		31.955	
P2 sn 1',3' 34.065 Pa14 31.811 Pa2 sn 1',3' 34.056 L16 31.55 O2/L2 sn 1',3' 34.042 UK 30.897 P14 31.955 O12 29.792 O16 sn 1', 3' 31.934 Pa12 sn 2' 29.763 Pa14 J1.551 O8/Pa7 sn 1', 3' 29.743 O12 sn 2' 29.793 P10 sn 1', 3' 29.731 O12 sn 1', 3' 29.743 O8/P12 sn 1', 3' 29.731 O12 sn 2' 29.763 O8/P12 sn 1', 3' 29.729 Pa12 sn 1', 3' 29.731 29.743 29.741 31.934 29.729 Pa12 sn 1', 3' 29.732 P8 29.743 29.741 29.743 29.741 P10/Pa7 sn 2' 29.732 P8 29.659 29.741 29.651 29.651 P10/Pa7 sn 1', 3' 29.755 O14 29.554 <t< td=""><td>O.L.Pa2</td><td>sn 2′</td><td>34.208</td><td>016</td><td></td><td>31.933</td></t<>	O.L.Pa2	sn 2′	34.208	016		31.933	
Pa2 sn 1',3' 34.056 L16 31.55 O2/L2 sn 1',3' 34.042 UK 30.897 P14 31.955 O12 29.792 O16 31.934 Pa12 sn 2' 29.763 Pa14 31.812 Pa12 sn 1', 3' 29.763 D16 . 31.812 Pa12 sn 1', 3' 29.763 D12 . Sn 2' 29.731 29.731 29.731 O12 . 9.793 O8/Pa7 sn 2' 29.731 Pa12 sn 1',3' 29.731 29.731 29.731 29.731 Pa12 sn 1',3' 29.732 Pa17 sn 1',3' 29.729 P12 sn 1',3' 29.732 Pa17 29.731 29.731 O7 sn 2' 29.733 Pa 29.659 29.659 O7/P12/Pa7 sn 1',3' 29.551 014 29.551 29.551 P3 . 1,3'sn2(L) 29.655 91.3	P2	sn 1′.3′	34.065	Pa14		31.811	
O2/L2 sn 1',3' 34.042 UK 30.897 P14 31.955 O12 29.792 O16 sn 2' 29.763 Pa14 31.812 Pa12 sn 1', 3' 29.755 L16 31.51 O8/Pa7 sn 2' 29.731 O12 29.792 31.51 O8/Pa7 sn 2' 29.731 O12 31.51 O8/Pa7 sn 1', 3' 29.731 O12 sn 2' 29.763 O8/P12 sn 1', 3' 29.731 Pa12 sn 2' 29.756 O8/P12 sn 1', 3' 29.721 O7 sn 2' 29.743 29.743 29.721 29.721 O7 sn 2' 29.732 Pa sn 1', 3' 29.721 O7 sn 2' 29.732 Pa 29.651 29.651 P10/Pa7 sn 2' 29.732 Pa 29.651 29.551 P11 29.755 O14 29.555 29.551 29.372 P14 29.555 O13 29.321 29.331 29.331 P5 sn 1',3' 29.355 O13 29.347 P13 2 29.353 Pa 29.216 P13 2 29	Pa2	sn 1′,3′	34.056	L16		31.55	
P1431.955O1229.792O1631.934Pa12sn 2'29.763Pa1431.812Pa12sn 1', 3'29.755L1631.551O8/Pa7sn 2'29.743O1229.793P10sn 1, 329.731Pa12sn 2'29.763O8/P12sn 1', 3'29.729Pa12sn 1', 3'29.756Pa7/P11sn 1', 3'29.721O7sn 2'29.743P929.71429.714P10/Pa7sn 2'29.732P829.659O7/P12/Pa7sn 1', 3'29.728L7/P729.65P1129.715O1429.55429.595P7/L7sn 1', 3'29.651P1329.331L7sn 1', 3'29.653L1529.372O1429.555O1329.33229.332P5sn 1', 3'29.505O1329.333P51329.393P6sn 1', 3'29.331P1329.393P6sn 1', 3'29.321O1429.555O1329.32129.347P1329.393P6sn 1', 3'29.321D1429.555O1329.32129.347P1329.393P6sn 1', 3'29.216O1429.393P6sn 1', 3'29.228L529.393P6sn 1', 3'29.216O1429.393P6sn 1', 3'29.216D1529.373P65sn 2'29.216<	O2/L2	sn 1′.3′	34.042	UK		30.897	
016 31.934 Pa12 sn 2' 29.763 Pa14 31.812 Pa12 sn 1', 3' 29.755 L16 31.551 08/Pa7 sn 2' 29.743 O12 29.793 P10 sn 1, 3 29.731 Pa12 sn 2' 29.763 08/P12 sn 1', 3' 29.729 Pa12 sn 1', 3' 29.756 Pa7/P11 sn 1', 3' 29.721 O7 sn 2' 29.743 P9 29.743 29.721 O7 sn 2' 29.743 P9 29.712 29.743 O7/P12/Pa7 sn 1',3' 29.722 P8 29.651 29.743 O7/P12/Pa7 sn 1',3' 29.728 17/P7 29.651 P11 29.715 014 29.555 29.561 29.392 P7/L7 sn 1',3'(sn2(L) 29.653 115 29.372 29.372 P13 2 29.354 115 29.347 29.347 P13 2 9.555 013 29.347 29.347 P13 2 9.393	P14	,	31.955	012		29.792	
Pa14 31.812 Pa12 sn 1', 3' 29.755 L16 31.551 08/Pa7 sn 2' 29.743 O12 29.793 P10 sn 1, 3 29.751 Pa12 sn 2' 29.763 08/P12 sn 1', 3' 29.729 Pa12 sn 1', 3' 29.756 Pa7/P11 sn 1', 3' 29.721 O7 sn 2' 29.732 P9 29.714 29.714 P10/Pa7 sn 2' 29.732 P8 29.689 29.675 O7/P12/Pa7 sn 1',3' 29.728 L7/P7 29.554 29.554 P11 29.715 O14 29.554 29.555 P7/L7 sn 1',3'sn2(L) 29.659 P13 29.372 Q14 29.555 O13 29.372 29.372 Q14 29.506 O15 29.373 29.373 P13 29.393 P6 sn 1', 3' 29.292 L15 29.393 P6 sn 2' 29.292 Q13/P6 29.333 Pa5 sn 2' 29.216 Q13/P6	016		31.934	Pa12	sn 2′	29.763	
L16 31.551 O8/Pa7 sn 2' 29.743 O12 29.793 P10 sn1,3 29.731 Pa12 sn 2' 29.763 O8/P12 sn 1', 3' 29.729 Pa12 sn 1', 3' 29.756 Pa7/P11 sn 1', 3' 29.721 O7 sn 2' 29.743 P9 29.714 P10/Pa7 sn 2' 29.732 P8 29.689 O7/P12/Pa7 sn 1', 3' 29.728 L7/P7 29.554 P1 29.715 O14 29.554 29.555 P7/L7 sn 1', 3'sn2(L) 29.633 L15 29.372 P4 29.555 O13 29.347 29.347 P13 2 29.393 P6 sn 1', 3' 29.292 P13 2 29.372 O5/L5 sn 2' 29.292 D13/P6 2 29.339 Pa5 sn 2' 29.216 O13/P6 2 29.339 Pa5 sn 2' 29.216 O13/P6 2 29.349 O5/L5 sn 2' 29.216 </td <td>Pa14</td> <td></td> <td>31.812</td> <td>Pa12</td> <td>sn 1′, 3′</td> <td>29.755</td>	Pa14		31.812	Pa12	sn 1′, 3′	29.755	
O12 9.793 P10 sn1,3 29.731 Pa12 sn 2' 29.763 O8/P12 sn 1', 3' 29.729 Pa12 sn 1',3' 29.756 Pa7/P11 sn 1', 3' 29.721 O7 sn 2' 29.743 P9 29.714 P10/Pa7 sn 2' 29.732 P8 29.689 O7/P12/Pa7 sn 1',3' 29.728 L7/P7 29.554 P1 29.715 O14 29.554 29.554 P8 29.689 P5 sn 1',3' 29.505 P7/L7 sn 1',3'sn2(L) 29.653 P13 29.392 L7 sn 1',3' 29.555 O13 29.353 P5 sn 1',3' 29.393 P6 sn 1',3' 29.298 L15 29.372 O5/L5 sn 2' 29.222 O13/P6 29.353 Pa5 sn 2' 29.222 O13/P6 29.349 O5/L5 sn 2' 29.216	L16		31.551	O8/Pa7	sn 2′	29.743	
Pa12 sn 2' 29,763 O8/P12 sn 1', 3' 29,729 Pa12 sn 1', 3' 29,756 Pa7/P11 sn 1', 3' 29,721 O7 sn 2' 29,743 P9 29,714 P10/Pa7 sn 2' 29,732 P8 29,689 O7/P12/Pa7 sn 1',3' 29,756 D14 29,554 P1 29,715 O14 29,555 29,689 P7/L7 sn 1',3'sn2(L) 29,653 L1/5 29,372 P7/L7 sn 1',3'sn2(L) 29,653 P13 29,392 L7 sn 1',3' 29,555 O13 29,353 P5 sn 1',3' 29,555 O13 29,347 P13 2 29,353 P6 sn 1', 3' 29,298 L15 29,393 P6 sn 1', 3' 29,292 D13/P6 2 29,353 Pa5 sn 2' 29,216 O13/P6 2 29,349 O5/L5 sn 1', 3' 29,216	012		29.793	P10	sn1,3	29.731	
Pa12sn 1',3'29.756Pa7/P11sn 1', 3'29.721O7sn 2'29.743P929.714P10/Pa7sn 2'29.732P829.689O7/P12/Pa7sn 1',3'29.728L7/P729.65P1129.715O1429.554P829.689P5sn 1',3'29.505P7/L7sn 1',3'sn2(L)29.65P1329.392L7sn 1',3'29.633L1529.372O1429.555O1329.372O1429.506O1529.347P529.393P6sn 1',3'29.298L1529.372O5/L5sn 2'29.222O13/P629.353Pa5sn 2'29.216O1529.349O5/L5sn 1',3'29.216	Pa12	sn 2′	29.763	O8/P12	sn 1′, 3′	29.729	
O7 sn 2' 29.743 P9 29.714 P10/Pa7 sn 2' 29.732 P8 29.689 O7/P12/Pa7 sn 1',3' 29.728 L7/P7 29.65 P11 29.714 29.728 L7/P7 29.554 P8 29.689 P5 sn 1', 3' 29.505 P7/L7 sn 1', 3'/sn2(L) 29.65 P13 29.392 L7 sn 1', 3' 29.653 L15 29.372 O14 29.555 O13 29.372 O14 29.555 O15 29.347 P13 29.393 P6 sn 1', 3' 29.298 L15 29.372 O5/L5 sn 2' 29.292 O13/P6 29.372 O5/L5 sn 2' 29.222 O13/P6 29.353 Pa5 sn 2' 29.221 O13/P6 29.349 O5/L5 sn 1', 3' 29.216	Pa12	sn 1′,3′	29.756	Pa7/P11	sn 1′, 3′	29.721	
P10/Pa7 sn 2' 29.732 P8 29.689 O7/P12/Pa7 sn 1',3' 29.728 L7/P7 29.65 P11 29.715 O14 29.554 P8 29.689 P5 sn 1', 3' 29.505 P7/L7 sn 1',3'/sn2(L) 29.653 P13 29.392 L7 sn 1',3' 29.555 O13 29.372 O14 29.555 O13 29.372 P13 29.393 P6 sn 1', 3' 29.298 L15 29.393 P6 sn 1', 3' 29.298 L15 29.372 O5/L5 sn 2' 29.222 O13/P6 29.353 Pa5 sn 2' 29.226 O15 29.372 O5/L5 sn 2' 29.216	07	sn 2′	29.743	P9		29.714	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	P10/Pa7	sn 2′	29.732	P8		29.689	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	O7/P12/Pa7	sn 1′,3′	29.728	L7/P7		29.65	
P8 29.689 P5 sn 1', 3' 29.505 P7/L7 sn 1', 3'/sn2(L) 29.65 P13 29.392 L7 sn 1', 3' 29.633 L15 29.372 O14 29.555 O13 29.333 P5 29.506 O15 29.347 P13 29.393 P6 sn 1', 3' 29.298 L15 29.372 O5/L5 sn 2' 29.222 O13/P6 29.353 Pa5 sn 2' 29.216 O15 29.353 Sn 2' 29.222 O13/P6 29.349 O5/L5 sn 1', 3' 29.216	P11		29.715	014		29.554	
P7/L7 sn 1',3'/sn2(L) 29.65 P13 29.392 L7 sn 1',3' 29.633 L15 29.372 O14 29.555 O13 29.353 P5 29.506 O15 29.347 P13 29.393 P6 sn 1', 3' 29.298 L15 29.372 O5/L5 sn 2' 29.222 O13/P6 29.349 Pa5 sn 2' 29.216 O15 29.349 O5/L5 sn 1', 3' 29.216	P8		29.689	P5	sn 1′, 3′	29.505	
L7sn 1',3'29.633L1529.372O1429.555O1329.353P529.506O1529.347P1329.393P6sn 1', 3'29.298L1529.372O5/L5sn 2'29.222O13/P629.353Pa5sn 2'29.216O1529.349O5/L5sn 1', 3'29.2	P7/L7	sn 1′,3′/sn2(L)	29.65	P13	•	29.392	
O1429.555O1329.353P529.506O1529.347P1329.393P6sn 1', 3'29.298L1529.372O5/L5sn 2'29.222O13/P629.353Pa5sn 2'29.216O1529.349O5/L5sn 1', 3'29.2	L7	sn 1′,3′	29.633	L15		29.372	
P529.506O1529.347P1329.393P6sn 1', 3'29.298L1529.372O5/L5sn 2'29.222O13/P629.353Pa5sn 2'29.216O1529.349O5/L5sn 1', 3'29.2	014		29.555	013		29.353	
P1329.393P6sn 1', 3'29.298L1529.372O5/L5sn 2'29.222O13/P629.353Pa5sn 2'29.216O1529.349O5/L5sn 1', 3'29.2	P5		29.506	015		29.347	
L1529.372O5/L5sn 2'29.222O13/P629.353Pa5sn 2'29.216O1529.349O5/L5sn 1', 3'29.2	P13		29.393	P6	sn 1′, 3′	29.298	
O13/P6 29.353 Pa5 sn 2' 29.216 O15 29.349 O5/L5 sn 1', 3' 29.2	L15		29.372	O5/L5	sn 2′	29.222	
O15 29.349 O5/L5 sn 1', 3' 29.2	O13/P6		29.353	Pa5	sn 2′	29.216	
	015		29.349	O5/L5	sn 1′, 3′	29.2	

Table 3. (Continued)							
Carbon	Position	Avocado pear oil (ppm)	Carbon	Position	Macadamia nut oil (ppm)		
P6	sn 1′,3′	29.299	Pa5	sn 1′, 3′	29.194		
O5/L5	sn 2′	29.222	O6/L6	sn 2′	29.151		
Pa5	sn 2′	29.217	Pa6/L6/P4	sn 2′/sn1,3	29.142		
O5/L5	sn 1′,3′	29.201	O6	sn 1′, 3′	29.135		
Pa5	sn 1′,3′	29.194	Pa6	sn 1′, 3′	29.126		
O6/L6	sn 2′	29.152	O4/Pa4/L4/P4	sn 1′, 3′	29.111		
L6/P4/Pa6	sn 1′,3′	29.145	O4/Pa4/L4	sn 2′	29.072		
06	sn 1′,3′	29.135	Pa13		29.011		
Pa6	sn 2′	29.126	O11/Pa8	sn 2′	27.246		
O4/L4/Pa4	sn 1,3	29.111	011	sn 1′, 3′	27.243		
O4/L4/Pa4	sn 2′	29.073	L14		27.223		
Pa13		29.011	L8		27.212		
O11/Pa8	sn 2′	27.247	08		27.193		
011	sn 1′,3′	27.243	Pa11		27.187		
L14		27.224	L11		25.651		
L8		27.212	O3/Pa3/L3	sn 2′	24.905		
08		27.193	P3		24.888		
Pa11		27.188	O3/Pa3/L3/P3	sn 1′, 3′	24.865		
L11		25.651	P17		22.717		
O3/Pa3	sn 2′	24.906	017		22.707		
L3	sn 2′	24.896	Pa15		22.681		
P3	sn 1′,3′	24.888	L17		22.598		
O3/L3/Pa3	sn 1′,3′	24.866	P16		14.131		
P15		22.717	O18		14.125		
017		22.707	Pa16		14.117		
Pa15		22.682	L18		14.085		
L17		22.599					
P16		14.131					
018		14.125					
Pa16		14.116					
L18		14.084					
O, olein; P, palmitin; Pa, palmitolein; L, linolein; UK, unknown; V/E, vaccenin or eicosenoin; FFA, free fatty acid.							

carbon atoms, it can be seen that as the concentration increases the chemical shifts of the sp² carbon atoms move upfield, while the sp³ carbon atoms chemical shifts move downfield. It is therefore evident that the sp² and sp³ carbon atoms are affected very differently with a change in concentration, and thus they should clearly not be plotted on the same graph.

On the basis of these observations therefore, we have tested a new method for the possible assignment of ¹³C{¹H} resonances of triacylglycerols in vegetable oils by establishing a correlation of the observed $\delta^{13}C{^{1}H}$ of the various peaks of the major components with those of the pure component obtained under similar conditions at similar concentrations. The graphical correlation method therefore consists of correlating the chemical shift values of defined fatty acid carbon atoms in the standard triacylglycerols (triolein, tripalmitin, etc.) with the corresponding peaks present in the ${}^{13}C{}^{1}H$ NMR spectrum of the vegetable oil. Essentially we employ the following modus operandi: Firstly, the ${}^{13}C{}^{1}H{}$ NMR spectrum of the vegetable oil is divided into sections each representing different carbon atoms of the same type present in the triacylglycerols. Section A covers the ¹³C resonances due to the carbonyl sp²-carbon resonances, Section B those of the olefinic sp²-carbons, while Sections D-J shows the crowded spectral region due to aliphatic sp³-carbon ¹³C signals (Table 2).

Certain of these regions are easily assigned by inspection using the relative percentages of each fatty acid residue present in the oil as determined by GC analysis and can be approximately related (If necessary quantitative ¹³C{¹H} NMR spectra can be recorded to confirm this (see ref 5).) to resonance intensities in the NMR spectrum, which further assists in assigning such additional resonances by inspection. In addition it is known from the work of Vlahov et al.^[3] that carbons from a saturated fatty acid chain is generally observed somewhat further upfield than the equivalent carbon in an unsaturated fatty acid chain. These concepts can be applied in order to assign Sections A, B, E, I and J. Subsequently by plotting the ¹³C{¹H} chemical shifts of each of all unambiguously assigned ¹³C{¹H} resonances of a particular fatty acid residue in the vegetable oil on the y-axis against the ${}^{13}C{}^{1}H{}$ shift of that resonance in the standard triacylglycerol along the x-axis, one obtains a remarkably linear correlation as shown for instance for trilinolein in macadamia nut oil in Fig. 4.

This highly linear correlation thus facilitates the assignment of the remaining ${}^{13}C{}^{1}H$ resonances, which were not directly assignable by inspection particularly in a spectrally crowded region encompassing the aliphatic carbon resonances. It is thus possible by using a previously assigned ${}^{13}C{}^{1}H$ shift of the pure triacylglycerol (obtained from the literature or preferably from a



Figure 4. Linear correlation obtained from plotting the chemical shifts of the sp³ carbon resonances belonging to standard trilinolein against those in macadamia nut oil.

 $^{13}C{^{1}H}$ spectrum of a sample of pure triacylglycerol recorded in the same sample and approximate concentration plotted as ordinate) to predict the corresponding chemical shift value of the corresponding $^{13}C{^{1}H}$ shift of that component in the oil as abscissa (Fig. 4).

In this way all the relevant ¹³C resonances can be assigned to the appropriate carbon of the fatty acid residues, particularly in the crowded spectral region of the ¹³C{¹H} NMR spectrum. This method is most suitable for application in regions D, F, G and H where significant spectral overlap occurs and for the same sp³ carbon type. Moreover the degree of linearity obtained by regression analysis (as measured by the regression coefficient r^2) is sensitive to the correct assignment since miss-assignments result in the rapid deviation of the r^2 from close to 1, typically correct assignments lead to r^2 values of 0.999. This linear method is only used for the sp³ hybridized carbons as the carbonyl and olefinic sp² carbon can easily be assigned by inspection. Indeed the sp² and sp³ carbons should not be used on the same linear graph as a result of their non-linear concentration dependence shown previously (Fig. 3), although for very similar concentrations of standard triacylglycerol and vegetable oil in the same solvent remarkable linear correlations of the ¹³C{¹H} shifts for all carbon types also result. The limitation due to the variable concentration dependencies of the ${}^{13}C{}^{1}H$ shifts of sp² and carbonyl type carbon atoms is not a serious limitation in this context, since resonances resulting from such carbon atoms are generally easily assigned by inspection of other methods.

Validation and assignment of ¹³C{¹H} of vegetable oils

Assessment and validation of the linear correlation method described here was first carried out by application to the well-known ${}^{13}C{}^{1}H$ spectrum of olive oil, previously assigned and reported extensively in the literature ${}^{[3,4,13]}$ as shown in Table 2. A comparison of the assignment obtained using the graphical linear correlation method with those from the literature confirms that these ${}^{13}C{}^{1}H$ assignments agree well with those found by

other researchers for all carbon atoms of each fatty acid residue of the major triacylglycerols in olive oil. The various ${}^{13}C{}^{1}H$ chemical shift values for spectral regions A, B, C, E, I and J correspond well with published data^[3,4,13] with some exceptions in other regions: C-2 of all fatty acid residues present in section D corresponds reasonably well with the literature,^[4] however the resonances of the C-2 atoms of triolein and trilinolein in the α positions of the glycerol backbone were clearly separated in our $^{13}C{^{1}H}$ spectrum and could therefore be separately assigned: Shaw et al. found only one resonance representing both these carbon atoms, presumably due to fortuitous overlap. Although most assignments in spectral section G correlate with published data,^[3] we also observed two resolved resonances for C-8 of the olein residue, indicating separate peaks for C-8 in the α and β positions, whereas Shaw *et al.* report only a single resonance for this carbon atom. The same pertains for C-3 of the olein and linolein residues in spectral region H, for which separate resonances could be observed and assigned. Upon first glance, region F appears more difficult to assign unambiguously which probably accounts for why several of these resonances have not previously been assigned in the literature to our knowledge. Nevertheless using our proposed correlation method, it becomes relatively straight forward to assign some of these resonances, some of the unassigned resonances in this section could also be due to the vaccenin or eicosenoin residues or possibly some other saturated residues not previously reported or detected by ${}^{13}C{}^{1}H$ NMR (due to their low concentrations) which are likely to mostly overlap with the palmitin ${}^{13}C{}^{1}H{}$ resonances. Since palmitin is the major saturated component present in olive oil, the assignment of the saturated resonances was carried out using the tripalmitin chemical shifts as a reference. As expected for olive oil, saturated fatty acid residues, for instance for palmitin, are known not to be present in the β position of a triacylglycerol.^[1] This is thought to be due to the observation that in natural olive oils saturated fatty acid residues such as palmitin are found at the β position of the triacylalycerols in amounts of less than 2%. Indeed, the presence of substantial amounts of palmitin in the β position of the glycerol

Table 4.	¹³ C NMR	assignments	for grapesee	ed and apr	icot kernel oil
Carbon	Position	Grapeseed oil (ppm)	Carbon	Position	Apricot kernel oil (ppm)
FFA		173.813	P1		173.244
P1		173.252	UK		173.231
01	sn 1′,3′	173.221	01	sn1′, 3′	173.212
L1	sn 1′,3′	173.21	L1	sn1′, 3′	173.202
UK		172.812	L1	sn 2′	172.805
L1/01	sn 2′	172.801	01	sn 2′	172.794
UK		145.14	L13	sn1′, 3′	130.203
L13	sn 2′	130.204	L13	sn 2′	130.195
L13	sn 1′,3′	130.196	O10	sn 2′	130.016
010	sn 2′	130.018	010	sn1′, 3′	130.002
010	sn 1′,3′	130.002	L9	sn 2′	129.987
L9	sn 1′,3′	129.988	L9	sn1′, 3′	129.962
L9	sn 2′	129.962	09	sn1′, 3′	129.703
09	sn 1′,3′	129.703	09	sn 2′	129.677
09	sn 2′	129.676	L10	sn1′, 3′	128.092
L10	sn 2′	128.091	L10	sn 2′	128.074
L10	sn 1′,3′	128.072	L12	sn 2′	127.908
L12	sn 1′,3′	127.906	L12	sn1′, 3′	127.897
L12	sn 2′	127.894	CHO		68.908
CHO		68.908	CH2O		62.106
CH2O		62.109	02	sn 2′	34.204
L2/02		34.196	L2	sn 2′	34.199
P2		34.06	P2		34.062
L2/02	sn 1′,3′	34.032	02	sn1′, 3′	34.04
P14		31.948	L2	sn1′, 3′	34.034
016		31.927	P14		31.953
L16		31.545	016		31.932
UK		30.899	UK		31.811
012		29.787	L16		31.549
07	sn 2′	29.737	UK		30.895
O7/P10	sn 1′,3′	29.725	012		29.791
P12		29.718	UK		29.762
P11		29.708	07	sn 2′	29.742
P8		29.683	O7/P10,12	sn1′, 3′	29.727
L7	sn 2′	29.641	P11		29.713
L7	sn 1′,3′	29.627	P8	sn 2′	29.687
014		29.548	L7	sn1′, 3′	29.645
P5		29.499	L7	sn 2′	29.631
P13		29.386	014		29.553
L15		29.367	UK		29.528
013		29.347	P5	sn1′, 3′	29.503
015		29.341	UK		29.47
P6		29.293	P13		29.391
L5/05	sn 2′	29.215	L15		29.371
L5/05	sn 1′,3′	29.194	015,13	sn 1′,3′	29.346
L6/06	sn 2′	29.147	P6		29.298
L6/O6/P4	sn 1′,3′	29.133	O5/L5	sn 2′	29.22
L4/04	sn 1′,3′	29.1	O5/L5	sn1′, 3′	29.199
L4/04	sn 2′	29.062	O6/L6/P4	sn 2′	29.15
011	sn 2′	27.242	06/L6	sn1′, 3′	29.133
011	sn 1′,3′	27.238	04	sn1′, 3′	29.11
UK		27.231	L4	sn1′, 3′	29.105
L14		27.219	04	sn 2′	29.071
L8		27.208	L4	sn 2′	29.067
08		27.188	UK		29.01
L11		25.647	011		27.242

Table 4.	(Continue	ed)			
Carbon	Position	Grapeseed oil (ppm)	Carbon	Position	Apricot kernel oil (ppm)
03	sn 2′	24.901	L14		27.222
L3	sn 2′	24.892	L8		27.211
P3		24.883	08		27.191
O3	sn 1′,3′	24.861	L11		25.65
L3	sn 1′,3′	24.854	03	sn 2′	24.905
P15		22.713	L3	sn 1′, 3′	24.896
017		22.703	P3		24.887
L17		22.595	03	sn 1′, 3′	24.865
P16		14.131	L3	sn 2′	24.858
O18		14.125	P15		22.716
L18		14.084	017		22.706
			UK		22.699
			UK		22.682
			L17		22.598
			UK		14.131
			018		14.125
			P16		14.118
			L18		14.085
O, olein; P, palmitin; Pa, palmitolein; L, linolein; UK, unknown; V/E, vaccenin or eicosenoin; FFA, free fatty acid.					

backbone in olive oil in particular indicates adulteration.^[1] The good agreement between our assignments derived from the graphical correlation method and published data for olive oils, acceptably validates the proposed method.

Using the proposed graphical correlation method developed here, we were able to fully assign the ¹³C NMR spectra of of all six vegetable oils in CDCl₃ solution without recourse to the method of standard additions. The maximum relative error determined for the ${}^{13}C{}^{1}H$ shifts of sp³ carbon atoms is $\pm 0.004 \text{ ppm}$ and for the sp² carbon atoms is $\pm 0.036 \text{ ppm}$ under our conditions. The assignments for macadamia nut and avocado pear oils are shown in Table 3, grapeseed and apricot kernel oils in Table 4 and mango kernel and marula oils in Table 5, and the ease with which these were carried out is an indication of the simplicity of the method presented earlier. In principle, under carefully controlled conditions we believe that this simple correlation method of assignment may compliment the more time-consuming standard additions methodology, as well as more elaborate spectroscpic methods such as ¹³C-¹³C correlations spectroscopy (e.g. INADEQUATE), and be suitable for the assignment of ¹³C{¹H} spectra of similar vegetable oils not previously studied by NMR spectroscopy, something currently underway in our laboratory.

Conclusions

In conclusion, the use of simple linear correlations between ¹³C NMR shifts of triacylglycerol fatty acid components in vegetable oils against the corresponding chemical shifts of the standard triacylglycerols in the same solvent at concentration ranges of between 0.10 and 0.20 g oil/1 g solution proves to be a simple method to identify and accurately assign the ¹³C NMR spectra of the major components in such oils, particularly in crowded spectral regions. These major components included

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Table 5.	¹³ C NMR assignments for mango kernel and marula oil						
		Mango					
Carbon	Position	kernel oil (ppm)	Carbon	Position	Marula oil (ppm)		
		170.007			172.072		
UK		178 885	FFA FFA		173.873		
FFΔ		173 898	PTA D1	sn 1′ 3′	173.042		
FFA		173.866	01	sn 1′ 3′	173.205		
UK		173,723	11	sn 1′, 3′	173.223		
P1		173.287	01	sn 2'	172.825		
01	sn 1′,3′	173.255	L1	sn 2′	172.812		
L1	sn 1′,3′	173.244	L13	sn 2′	130.186		
O1	sn 2′	172.844	L13	sn 1′, 3′	130.178		
L1	sn 2′	172.834	010	sn 2′	130.013		
UK		145.079	O10	sn 1′, 3′	129.999		
L13		130.199	L9	sn 1′, 3′	129.973		
O10	sn 1′,3′	130.017	L9	sn 2′	129.948		
010	sn 2′	130.005	UK		129.717		
L9	sn 1′,3′	129.989	UK	1/ 2/	129.707		
L9	sn 2	129.962	09	sn 1′, 3′	129.701		
	cn 2'	129.725	09	$\sin 2'$	129.075		
09	511.2	129.703	L10	$\sin 1/3'$	120.105		
09	sn 1′.3′	129.679	112	sn 1′, 3′	127.920		
L10	5111,5	128.094	L12	sn 2′	127.903		
L10		128.077	CH2O		68.939		
L12		127.912	UK		68.332		
L12		127.9	UK		65.041		
СНО		68.921	CHO		62.127		
DAG		68.349	02/L2	sn 2′	34.212		
DAG		65.051	UK		34.105		
CH2O		62.114	P2		34.069		
02/L2	sn 2′	34.215	02/L2	sn 1′, 3′	34.047		
UK		34.122	UK		34.020		
UK		34.106	UK D14		34.005		
P2	rn 1/2/	34.07	P14 016		31.976		
	5111,5	33.047	116		31.955		
UK		33 958	012		29.810		
P14		31.963	07	sn 2′	29.757		
016		31.941	UK		29.754		
L16		31.557	UK		29.747		
012		29.798	07	sn 1′, 3′	29.743		
P10,12/O7		29.741	P10		29.736		
P11		29.726	P12		29.727		
P9		29.711	P11		29.719		
P8		29.699	UK		29.711		
UK	1/ 2/	29.679	P9		29.704		
P//L/	sn 1′,3′	29.661	P8		29.690		
	sn 2	29.637	UK	cn 2/	29.670		
014	sn 1/ 2/	29.032 29.565	L/ P7	511 2	29.001 29.647		
014	sn 2 ⁷	29.505	17	sn 1′3′	29.643		
P5	5112	29.515	UK	511 1 7 5	29,578		
UK		29.48	UK		29.576		
P13		29.402	UK		29.573		
L15		29.378	014		29.571		
013		29.361	UK		29.525		
015		29.357	P5		29.491		
P6		29.307	UK		29.415		
UK		29.292	P13		29.391		

Table 5.	(Continued	1)				
Carbon	Position	Mango kernel oil (ppm)	Carbon	Position	Marula oil (ppm)	
O5/L5	sn 2′	29.229	O13/L15		29.370	
O5/L5	sn 1′.3′	29.206	015		29.363	
UK	,	29.187	UK		29.318	
06/L6	sn 1′,3′	29.158	P6		29.301	
P4/L6	sn 1′,3′	29.149	O5/L5	sn 1′, 3′	29.239	
O6	sn 2′	29.141	UK		29.226	
04	sn 1′,3′	29.119	O5/L5		29.217	
L4	sn 2′	29.117	UK		29.201	
UK		29.103	UK		29.196	
UK		29.088	O6/L6	sn 1′, 3′	29.164	
O4/L4	sn 2′	29.08	UK		29.158	
011	sn 1′,3′	27.252	O6/L6	sn 1′, 3′	29.148	
011	sn 2′	27.248	P4		29.139	
UK		27.242	04		29.126	
L14		27.228	L4	sn 2′	29.119	
L8		27.217	UK		29.110	
UK		27.208	UK		29.093	
O8		27.199	04/L4	sn 1′, 3′	29.088	
UK		27.188	011		27.260	
UK		27.183	011		27.256	
L11		25.654	UK		27.249	
O3/L3	sn 2′	24.91	L14		27.236	
P3		24.892	L8		27.223	
UK		24.878	08		27.204	
O3/L3	sn 1′,3′	24.87	UK		27.197	
UK		24.753	UK		27.188	
UK		24.737	L11	sn 2′	25.662	
P15		22.723	O3		24.917	
017		22.713	L3	sn 1′, 3′	24.906	
L17		22.604	P3		24.900	
P16		14.133	O3/L3		24.878	
O18		14.127	UK		24.742	
L18		14.087	UK		24.727	
			P15		22.734	
			017		22.724	
			L17		22.615	
			P16		14.137	
			O18		14.132	
			L18		14.092	
O, olein; P, palmitin; Pa, palmitolein; L, linolein; UK, unknown; V/E, vaccenin or eicosenoin; FFA, free fatty acid.						

oleic, linoleic and palmitic fatty acids in all six oils and palmitoleic fatty acid in macadamia nut and avocado pear oils. This method has been validated with the well-known ¹³C NMR spectrum of olive oil, and has been used to fully assign the ¹³C NMR spectra of the triacylglycerol fatty acid residues of six previously unassigned vegetable oils, apricot kernel, avocado pear, grapeseed, macadamia nut, mango kernel and marula oils. This method is reasonably robust and rapidly leads to accurate ¹³C NMR assignments in mixtures of triacylglycerols at reasonable concentrations in similar vegetable oils not previously examined, without having to resort to time-consuming additions of pure standards.

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Supporting information

Supporting information may be found in the online version of this article.

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