^{13}C Nuclear Magnetic Resonance Spectroscopic Analysis of the Triacylglycerol Composition of *Biota orientalis* and Carrot Seed Oil

Marcel S.F. Lie Ken Jie*, C.C. Lam, and Mohammed Khysar Pasha

Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong

ABSTRACT: ^{13}C nuclear magnetic resonance (NMR) spectroscopic analysis of the whole oil (triacylglycerols) of *Biota orientalis* seeds confirms the presence of oleate [18:1(9*Z*)], linoleate [18:2(9*Z*,12*Z*)], linolenate [18:3((9*Z*,12*Z*,15*Z*)], 20:3 (5*Z*,11*Z*, 14*Z*), 20:4(5*Z*,11*Z*,14*Z*,17*Z*), and saturated fatty acids in the acyl groups by comparing the observed carbon shifts with previously established shift data for model triacylglycerols. This technique shows that the saturated, 20:3 and 20:4 fatty acids are distributed mainly in the α-acyl positions, whereas oleate, linoleate, and linolenate are randomly acylated to the α- and β-positions of the glycerol "backbone." Stereospecific hydrolysis of the *Biota oil* with pancreatic lipase, followed by chromatographic analysis of fatty esters, reveals the presence of trace amounts of 16:0(0.7%), 18:0(0.5%), 20:3 (0.4%), and 20:4 (1.3%) in the β-position of the glycerol "backbone," which are undetectable by ^{13}C NMR technique on the whole oil. Semi-quantitative assessment of the ^{13}C NMR signal intensities gives the relative percentages of the fatty acid distribution as: saturated 16:0, 18:0 (12.0% α-acyl), oleate (7.7% α-acyl 8.7 % β-acyl), total linoleate and linolenate (31.7% α-acyl; 24.2% β-acyl), total 20:3 and 20:4 (15.7% α-acyl). The ^{13}C NMR spectroscopic analysis of carrot seed oil identifies the presence of saturated (18:0), 18:1(6*Z*), 18:1(9*Z*), and 18:2(9*Z*,12*Z*). The saturated fatty acid is found in the α-acyl positions. Semi-quantitative assessment of the signal intensities gives the relative percentages of the fatty acids as: 18:0 (4.5% α-acyl), 18:1(6*Z*) (49.6% α-acyl; 19.7% β-acyl), oleate (6.5% α-acyl; 8.6% β-acyl) and linoleate (5.2% α-acyl; 6.9% β-acyl).
JAOCS 73, 557–562 (1996).

KEY WORDS: *Biota orientalis* seed oil, carrot seed oil, ^{13}C NMR, 5,11,14,17-eicosatetraenoic acid, 5,11,14-eicosatrienoic acid, fatty acid composition, petroselinic acid, triacylglycerol, triglyceride.

Applications of ^{13}C nuclear magnetic resonance (NMR) spectroscopy to the structural elucidation of butterfat, vegetable oils, fish oils, animal fats, and partially hydrogenated vegetable oils recently have been reviewed by Gunstone (1). We have reported the ^{13}C NMR spectroscopic properties of over 70 synthetic triacylglycerol molecules that contained saturated, olefinic, acetylenic, and polyunsaturated acyl groups (2–5). This technique permits the elucidation of the distribution of the different acyl groups on the glycerol "backbone" and the position of the unsaturated center(s) in the acyl groups of a triacylglycerol. In this paper we describe the results obtained by ^{13}C NMR analysis of *Biota orientalis* (6) and carrot (*Daucus carota*) seed oil. The main objectives of this project were (i) to reconfirm the presence of the reported fatty acids in *Biota* seed oil, (ii) to identify and semi-quantitate the fatty acids in carrot seed oil, and (iii) to determine the fatty acid distribution on the glycerol "backbone" of both oils.

EXPERIMENTAL PROCEDURES

Materials. Biota orientalis (also known as *Platycladus orientalis*, *Thuja orientalis*, or *Arbor vitae*) seeds were purchased from herbal shops in Guangzhou (China). This seed is used in traditional Chinese herbal treatment. Carrot seeds were purchased locally from vegetable seed suppliers.

Methods. Oil (1.0 g), isolated from the seeds by solvent extraction, was percolated through a silica gel (15 g) column with a mixture of petroleum ether (b.p. 40–60°C) and diethyl ether (95:5, vol/vol, 150 mL) as eluent. The eluate was evaporated under reduced pressure to give a mixture of triacylglycerols (0.85 g). The ^{13}C NMR spectroscopic analysis was recorded in deuterated chloroform on a JEOL GSX-270 spectrometer (Tokyo, Japan) (4).The fatty acid composition of the same sample of oil was determined by gas–liquid chromatographic (GLC) analysis as described elsewhere (7), and stereospecific hydrolysis of triacylglycerols with pancreatic lipases was performed according to the method reported by Luddy *et al.* (8).

RESULTS AND DISCUSSION

^{13}C NMR analysis of the seed oil of Biota orientalis. The ^{13}C NMR spectrum of the triacylglycerol fraction of *B. orientalis* seed oil shows more than 80 signals. For convenience, we abbreviate a saturated acyl group as Sat, oleate [18:1(9Z)] [18:1(9Z)—the first number denotes the number of carbon atoms in the fatty acid chain; the number after the colon indicates the number of unsaturated centers; the number within the bracket gives the position of the unsaturated center, and

*To whom correspondence should be addressed at the Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong.

the letter describes the type of unsaturation, where Z stands for *cis*-double bond] as O, linoleate [18:1(9Z,12Z)] as L, linolenate [18:3(9Z,12Z,15Z)] as Ln, 20:3(5Z,11Z,14Z) as 20:3, and 20:4(5Z,11Z,14Z,17Z)] as 20:4. Triacylglycerol molecules are described by using either a combination of letters (e.g., LLL for trilinolein) or by using the abbreviated structures of the fatty acid {e.g., $[18:1(9Z)]_3$ for triolein}. The fatty acid composition by GLC analysis of the sample of *B. orientalis* oil used in the ^{13}C NMR analysis is as follows: 16:0 (5.0%), 18:0 (3.9%), 18:1(9Z) (12.8%), 18:2(9Z,12Z) (24.7%), 18:3(9Z,12Z,15Z) (32.3%), 20:0 (trace), 20:1(11Z) (0.6%), 20:2 (5Z,11Z) (0.9%), 20:2(11Z,14Z) (0.9%), 20:3(5Z,11Z, 14Z) (4.5%), 20:3 (11Z,14Z,17Z) (0.6%) and 20:4(5Z,11Z, 14Z,17Z) (10.2%), which is comparable to results reported earlier (6).

There are four regions in the spectrum that provide essential information regarding the structure of the acyl groups and their distribution on the glycerol backbone of triacylglycerols present in seed oils. These regions are: (i) the C-1 carbon shift region (*ca.* 174 ppm); (ii) the C-2 carbon shift region (*ca.* 34 ppm); (iii) the C-3 (*ca.* 24 ppm), allylic (20-27 ppm), ω1 (*ca.* 14 ppm), ω2 (*ca.* 22 ppm) and ω3 (*ca.* 31 ppm) carbon shift regions; and (iv) the olefinic carbon shift region (127–132 ppm).

The C-1 carbon shift region (ca. *174 ppm).* The C-1 carbon shift region of the spectrum shows six distinct signals at 172.791, 172.803, 173.050, 173.192, 173.204, and 173.233 ppm. Referring to the shift data observed for the C-1 carbon atoms of triacylglycerols of type AAA [containing saturated, oleate, linoleate, linolenate, and other polyunsaturated acyl groups (2,4,5)], four of the six signals can be paired, *viz.*, 173.192/172.791 and 173.204/172.803 ppm, with a shift difference value of 0.401 ppm. The remaining two individual signals appear at 173.050 and 173.233 ppm. From the established shift data for LLL, LnLnLn and OOO (5), the first pair of signals at 173.192/172.791 ppm can be assigned to the shifts of the C-1 carbon atoms of L and Ln in the α- and β-acyl chains. The second pair of signals at 173.204/172.803 ppm arises from the shifts of the C-1 carbon atoms of O in the α- and β-acyl chains. The signal at 173.050 ppm is characteristic of the shift of the C-1 carbon atom of an acyl chain containing a (5Z)-olefinic system (i.e., 20:3 and 20:4). The remaining signal in this region at 173.233 ppm is due to the C-1 carbon shifts of a saturated acyl group in the α-acyl positions (2). From these results, Sat, 20:3 and 20:4 are found to be distributed in the α-acyl positions of the glycerol "backbone," while O, L, and Ln are distributed between the α- and β-acyl positions. The assignment of the shifts of the C-1 carbon atoms and their intensities are summarized in Table 1.

The C-2 carbon shift region (ca. *34 ppm).* Four signals appear in this region, *viz.*, 34.188, 34.052, 34.023, and 33.428 ppm. The signals at 34.023 and 34.188 ppm can be paired (shift difference of 0.165 ppm). These shifts are due to the C-2 carbon atoms of unsaturated acyl groups (O, L, and Ln) in the

TABLE 1
Chemical Shifts (ppm) of C-1, C-2, C-3, Allylic, ω3, ω2, ω1 Carbon Atoms of *Biota orientalis* Seed Oil[a]

Carbon nucleus	Carbon shifts (ppm)	Assignments
C-1	173.192/172.791	L and Ln (α,β)
	173.204/172.803	O (α,β)
	173.050	20:3 and 20:4 (α)
	173.233	Sat (α)
C-2	34.023/34.188	L, Ln and O (α,β)
	33.428	20:3 and 20:4 (α)
	34.052	Sat (α)
C-3	24.888	L, Ln, O (β) and Sat (α)
	24.853	L, Ln and O (α)
	24.813	20:3, 20:4 (α)
Allylic	27.244	C-11 of O
	27.223, 27.211, 27.192	C-8 of O, L, Ln and C-16 of 20:3
	27.157, 27.145	C-10 and C-7 of 20:3 and 20:4
	26.522	C-4 of 20:3 and 20:4
	25.651, 25.643. 25.637	C-11 of L, Ln and C-13 of 20:3, 20:4
	25.549	C-14 of Ln and C-16 of 20:4
	20.574	C-17 of Ln and C-19 of 20:4
ω3	31.963	Sat
	31.942	O
	31.554	L, 20:3
ω2	22.727	Sat
	22.718	O
	22.609	L, 20:3
ω1	14.298	Ln, 20:3
	14.138	O, Sat
	14.097	L, 20:3

[a]Sat = saturated, O = oleate, L = linoleate, Ln = linolenate, 20:3 = 20:3(5Z,11Z,14Z), 20:4 = 20:4(5Z,11Z,14Z,17Z).

α- and β-acyl positions. Referring to the spectra of $[20:3]_3$ and $[20:4]_3$, reported elsewhere (5), we have found that the shifts of the C-2 carbon atoms in the α/β-acyl positions of these triacylglycerols appear at *ca.* 33.4 ppm. The signal at 33.428 ppm in the spectrum of the *Biota* seed oil is therefore associated with the shift of the C-2 of 20:3 and 20:4 in the α-acyl positions. The remaining signal at 34.052 ppm agrees with the shift of the C-2 carbon atoms of Sat in the α-acyl position (2). From the shifts of the signals in the C-2 region of the spectrum, we are able to confirm that the 20:3, 20:4, and Sat acyl chains occupy the α-acyl positions of the glycerol "backbone." The assignment of these four signals are shown in Table 1.

The C-3, allylic, ω3, ω2, and ω1 carbon shift regions. The assignment of the multitude of signals in these regions is much more difficult than for the two regions discussed above. However, because we have presumed that the fatty acid composition of *Biota* oil is known, most of the signals can be assigned or used to reconfirm the presence of the particular fatty acids present (Table 1). There are three signals in the C-3 region (*ca.* 24 ppm). From the shift data established for the shifts of C-3 carbon atoms of triacylglycerols of type AAA containing Sat, L, Ln, or O, the intense signal at 24.888 ppm is assigned to the shifts of the C-3 carbon atoms of L, Ln, and O in the β-acyl chain and that of the C-3 carbon atoms of Sat in the α-acyl chains. The other intense signal at 24.853 ppm is characteristic of the shifts of the C-3 carbon atoms of L, Ln, and O in the α-acyl chains. The remaining and less intense signal at 24.813 ppm is characteristic of the shift of the C-3 carbon atom of an acyl group with a Δ^5 double bond (i.e., arising from 20:3 and 20:4 in this case) in the α-acyl position.

Twelve signals appear in the allylic region (20–27 ppm). Because no signal is found in the region of *ca.* 32 ppm, the presence of (*E*)-ethylenic systems in the seed oil can be ruled out. The signal at 27.244 ppm is due to the shift of the C-11 carbon atom of O. The signals at 27.223, 27.211, and 27.192 ppm are recognized as the shifts arising from the C-8 carbon atoms of O, L, Ln, and C-16 of 20:3. The shifts of the C-7 and C-10 carbon atoms of 20:3 and 20:4 are found at 27.145 and 27.157 ppm, respectively. These latter assignments are supported by shift data recorded for the C-7 and C-10 carbon atoms of the triacylglycerol $[20:3]_3$, where these signals appear at 27.148 (C-7) and 27.161 (C-10) ppm (5). The signal at 26.522 ppm is characteristic of the shift of the C-4 carbon atom of 20:3 or 20:4, which contains a Δ^5 double bond. The shifts of the C-11 carbon atom of L, Ln, and C-13 of 20:3 and 20:4 are found at 25.651, 25.643, and 25.637 ppm but cannot be further differentiated. The signal at 25.549 ppm arises from the shifts of the C-14 carbon atom of Ln and that of C-16 of 20:4. The signal at 20.574 ppm is characteristic of the shift of the C-17 of Ln and that of C-19 of 20:4.

The carbon chemical shifts of the ω1 (methyl), ω2, and ω3 carbon atoms appear all as single signals and are not paired. Referring to the results obtained for the mixed triacylglycerol [18:0/18:2(9Z,12Z)/18:1(9Z)] (5), we have noticed the general trend for the shifts of ω3 carbon nuclei, which follow a deshielding order: Sat (31.976 ppm) > O (31.954 ppm) > L (31.567 ppm). This trend is also true for ω2 carbon atoms of Sat, O, and L. With these shift trends in mind, we are able to assign the signal at 31.963 ppm as arising from the shift of the ω3 of Sat, while the signal at 31.942 ppm is due to the shift of the ω3 of O. The remaining ω3 carbon signal at 31.554 ppm is due to the shift of ω3 of L and 20:3. The assignments of the shifts of the ω2 and ω1 carbon atoms are similarly performed by referring to the established data from our previous studies of the corresponding unsaturated triacylglycerols (4,5). The results are summarized in Table 1.

The olefinic carbon shift region (ca. *127–132 ppm).* There are 35 signals in this region of the spectrum. All of these signals can be assigned by referring to data established for the unsaturated triacylglycerols (4,5). The results are summarized in Table 2. The olefinic carbon atoms of 20:3 and 20:4 give only single signals in the spectrum, which supports our above findings that these polyunsaturated (20:3 and 20:4) acyl chains are found only in the α-acyl positions of the glycerol "backbone." The olefinic carbon atoms of Ln (except for C-16) appear in pairs and are readily assigned (Table 2). For instance, the pair of signals at 130.207/130.183 ppm are due to the shifts of the C-9 carbon atoms of Ln in the α- and β-acyl chains, where the shift difference of 0.024 ppm agrees with that found for LnLnLn (difference value 0.026 ppm) (5). For L and O, the olefinic carbon atoms also give rise to pairs of signals. These results indicate that O, L, and Ln are distributed between the α- and the β-acyl positions of the glycerol "backbone."

We can conclude from this study of the ^{13}C NMR spectral analysis of the seed oil of *B. orientalis* that Sat, 20:3, and 20:4 are located mainly in the α-acyl positions of the triacylglycerol, whereas oleate (O), linoleate (L), and linolenate (Ln) are distributed between the α- and β-acyl positions of the glycerol "backbone." From the intensities of the signals, it appears that O, L, and Ln are about equally distributed between the α- and β-acyl positions of the triacylglycerols. To lend support to our conclusion, the stereospecific hydrolysis of *Biota* oil with pancreatic lipase has been performed. GLC analysis of the fatty esters obtained after interesterification of the isolated *sn*-2-mono-acylglycerol shows the following fatty acid composition: 16:0 (0.7%), 18:0 (0.5%), 18:1(24.2%), 18:2(44.5%), 18:3(28.3%), 20:3(0.4%), and 20:4(1.4%). Despite the relatively small amounts of 20:3 and 20:4 revealed in this analysis, the ^{13}C NMR technique remains a reasonably reliable method for providing insight into the types of fatty acids and their distribution in the whole oil.

Semi-quantitative analysis of the fatty acid content and acyl distribution. By taking into consideration the intensities of the signals in the ^{13}C NMR spectrum, Gunstone has shown that it is possible to estimate semi-quantitatively the relative quantities of fatty acids present in a seed oil (9). By considering the intensities of the signals in the C-1 region of the spectrum, the relative percentages and their distribution of the fatty acids are: saturated (12.0% α-acyl), oleate (7.7% α-acyl; 8.7% β-acyl), total linoleate and linolenate (31.7% α-acyl; 24.2%

TABLE 2
Assignment of the Olefinic Carbon Signals in the ^{13}C NMR Spectrum of *Biota orientalis* Seed Oil[a]

Acyl group	Carbon nucleus	Chemical shift (ppm)	Acyl group	Carbon nucleus	Chemical shift (ppm)
20:4(α)	C-18	131.933	Ln (α,β)	C-16	131.924
	C-6	131.029		C-9	130.207/130.183
	C-11	130.108		C-13	128.291/128.301
	C-5	128.365		C-12	128.247/128.234
	C-14	128.229		C-10	127.783/127.802
	C-12	127.828		C-15	127.147/127.140
	C-15,C-17	Overlapped			
20:3(α)	C-6	131.048	Ln (α,β)	C-13	130.189/130.197
	C-11	129.886		C-9	129.986/129.961
	C-5	128.349		C-10	128.096/128.114
	C-12	128.168		C-12	127.930/127.919
	C-14	127.913			
	C-15	Overlapped			
			O (α,β)	C-10	130.011/130.026
				C-9	129.714/129.689

[a]See Table 1 for abbreviations.

β-acyl), total 20:3 and 20:4 (15.7% ω-acyl). These values are comparable to those obtained by GLC analysis.

^{13}C NMR analysis of carrot seed oil. In this part of our study, we presume that the fatty acid composition of carrot seed oil is not known to us. The aim therefore is to apply the results of the ^{13}C NMR results reported earlier (2,4,5) to determine the fatty acid composition and the distribution of the fatty acids in the α- or β-position of the triacylglycerol "backbone." In this exercise, we shall identify the position of the unsaturated center(s) in the acyl chains and semi-quantitate the relative amount of each fatty acid present in the oil. The spectrum of carrot seed oil shows about 50 signals. A general inspection of the spectrum indicates that the oil (triacylglycerols) is made up of saturated and (*Z*)-olefinic fatty acids only. There are no signals in the region of *ca.* 32 ppm, which rules out the presence of (*E*)-olefinic bonds.

The C-1 and C-2 shift regions. There are seven signals in the C-1 region (*ca.* 174 ppm); six of them can be paired: 173.210/172.809 (shift difference 0.401), 173.201/172.799 (shift difference 0.402), and 173.098/172.701 (shift difference 0.397) ppm as arising from the shifts of the C-1 carbon atoms of unsaturated long-chain acyl groups in the α- and β-positions. The single signal at 173.243 ppm corresponds to the shift of the C-1 carbon atom of Sat in the α-acyl chain (2). Referring to the shift values of the C-1 carbon atoms of the (*Z*)-olefinic triacylglycerols of type AAA (4), the first pair of signals at 173.210/172.809 is due to the C-1 shift of a possible Δ^8 or Δ^9 mono-(*Z*)-olefinic positional isomer. The second pair of signals at 173.201/ 172.799 ppm with a shift difference of 0.402 ppm is characteristic of the shift of the C-1 carbon atoms of linoleate (L) in the α- and β-acyl positions, respectively. The ability to differentiate the first from the second pair of signals is substantiated from our published results of the mixed unsaturated triacylglycerols (5). The third pair of signals at 173.098/172.701 ppm (shift difference of 0.397 ppm) is close to the carbon shifts of the C-1 carbon of a (*Z*)-monoethylenic acyl group in the α- and β-position with the double bond located at the Δ^5, Δ^6, or Δ^7 position of the chain (4). From the analysis of the C-1 carbon shift region, we have therefore been able to show that the saturated acyl chains are located in the α-positions only, whereas the mono-olefinic acyl groups and linoleate are distributed in the α- and β-positions. It remains for us to determine the position of the unsaturated centers of the mono-olefinic acyl groups.

There are five signals in the C-2 shift region. The signals at 34.038 and 34.202 ppm are characteristic of the shifts of the C-2 carbon atoms of a Δ^8 or Δ^9 monounsaturated acyl group and linoleate (L) in the α- and β-acyl positions, respectively. The signal at 34.048 ppm can be assigned to the shift of the C-2 of Sat in the α-position, which supports our earlier deduction. The remaining pair of signals at 33.945/34.104 ppm (with a shift difference of 0.159 ppm) corresponds to the shifts of the C-2 carbon atoms of (*Z*)-unsaturated acyl groups with a double bond at either the Δ^6 or Δ^7 position. This result eliminates the possibility of the presence of a Δ^5 isomer because the recorded shift difference value for the C-2 carbon shifts of $[14:1(5Z)]_3$ is 0.188 ppm (4). The assignment of the C-1 and C-2 carbon signals and their intensities are given in Table 3.

The olefinic carbon shift region. The olefinic region of the spectrum shows eight pairs of signals. The four pairs of signals at 127.926/127.916, 128.111/128.093, 129.984/129.959, and 130.190/130.197 ppm are characteristic of the shifts of the C-10, C-12, C-9 and C-13 carbon atoms, respectively, of linoleate (L) in the α- and β-acyl positions, which match closely the corresponding signal recorded for LLL (5).

The two pairs of signals of about equal intensity at 129.710/ 129.684 (shift difference of 0.026) and 130.009/130.02 (difference of 0.014) match more closely the shift data recorded for the C-9 and C-10 olefinic carbon atoms of triolein (OOO) than those recorded for the C-8 and C-9 olefinic carbon of $[17:1(8Z)]_3$ (4). The Δ^9 position of the olefinic center is confirmed by

TABLE 3
Chemical Shift Values (ppm) of Carbon Atoms of Carrot Seed Oil

Acyl group	Carbon nucleus	Chemical shifts (ppm) (α,β)	Shift difference		Intensity
			Calc.	Ref.	
Sat	C-1	173.243(α)	—	—	3.335
O	C-1	173.210/172.809	0.401	0.401[a]	6.249/8.230
L	C-1	173.201/172.799	0.402	0.400[b]	5.021/6.653
Δ^6	C-1	173.098/172.701	0.397	0.401[b]	47.637/19.012
O and L	C-2	34.202(β)	—	—	5.126
Sat	C-2	34.048(α)	—	—	3.342
O and L	C-2	34.038(α)	—	—	4.885
Δ^6	C-2	33.945/34.104	0.159	0.159[c]	26.308/10.073
(C=C)					
O	C-10	130.009/130.023	0.014	0.015[a]	12.560/15.243
	C-9	129.710/129.684	0.026	0.027[a]	12.156/15.590
(C=C)					
Δ^6	C-7	130.549/130.557[d]	0.008	0.006[c]	100.000/44.116
	C-6	128.949/128.936[d]	0.013	0.011[c]	99.018/39.436
(C=C)					
L	C-13	130.190/130.197	0.007	0.008[b]	16.532/18.188
	C-9	129.984/129.959	0.025	0.026[b]	13.136/13.661
	C-10	128.111/128.093	0.018	0.019[b]	12.382/14.098
	C-12	127.926/127.916	0.010	0.012[b]	14.602/17.163
(Allylic)					
Δ^6	C-8, C-5	27.297 and 26.834	—	—	47.760 and 42.711
O	C-11, C-8	27.262 and 27.208	—	—	11.401 and 10.646
L	C-8, C-14	27.237 and 27.225	—	—	17.605 and 11.785
L	C-11	25.661	—	—	23.028
Δ^6	C-16	31.979	—	—	87.631
O, Sat	C-16	31.960	—	—	17.417
L	C-16	31.572	—	—	16.227
Δ^6	C-17	22.739	—	—	91.900
O, Sat	C-17	22.730	—	—	21.161
L	C-17	22.622	—	—	14.257
Δ^6, Sat, O	C-18	14.146	—	—	77.987
L	C-18	14.099	—	—	11.719

[a]Shift difference from 000 (4). See Table 1 for abbreviations.
[b]Shift difference from LLL (5).
[c]Shift difference from [15.1(6*Z*)]$_3$ (4).
[d]Values reported for [18:1(6*Z*)]$_3$ by Mallet *et al.* (10) are 130.58 (C-7) and 128.99 (C-6).

examining the difference value of the shifts of the olefinic carbon atoms in the same acyl chain, i.e., 130.009 – 129.710 = 0.299 (α-acyl) and 130.023 – 129.684 = 0.339 ppm (β-acyl). These difference values match the established values (0.294 and 0.336 ppm) for the difference in shift values of the C-9 and C-10 carbon atoms in the *same acyl chain* of triolein (OOO) (4). To exclude the possibility of the presence of a Δ^8 isomer, the recorded shift difference values between the C-8 and C-9 carbon atoms in the *same acyl chain* of [17:1(8*Z*)]$_3$ are 0.515 (α-acyl) and 0.562 (β-acyl), which are significantly different from the observed shift difference values in the spectrum of the carrot seed oil. It can therefore be concluded that one of the (*Z*)-olefinic isomers is oleate (O).

There are two intense pairs of signals of about equal intensity at 128.949/128.936 (difference of 0.013) and 130.549/130.557 (difference of 0.008) ppm. The difference of these olefinic carbon shift values in the same acyl chains are 130.549 – 128.949 = 1.600 (α-acyl chain) and 130.557 – 128.936 = 1.621 (β-acyl chain) ppm. From the established shift data for the (*Z*)-olefinic triacylglycerols of type AAA (4), these two pairs of signals can result only from the shifts of the olefinic carbon atoms of a (6*Z*)-isomer. We are therefore able to identify the presence of a long-chain (6*Z*)-olefinic acyl group in carrot seed oil, which we presume to be 18:1(6*Z*). The results of the shifts of the olefinic carbon atoms are given in Table 3. From the olefinic carbon region of the spectrum, we confirm the presence of O, L, and a (6*Z*)-monounsaturated fatty acid in the triacylglycerol molecules.

The allylic, ω1, ω2, and ω3 regions. The presence of linoleate (L) is further confirmed by the signal at 25.661 ppm for the shift of the C-11 carbon atom and also from the characteristic signals at 14.099 (ω1), 22.622 (ω2), and 31.572 (ω3) ppm. This oil does not seem to contain any linolenate (Ln) groups because the related carbon shifts for the triene

system are absent. Because there are only three olefinic acyl groups present in this seed oil, *viz.*, O, L, and a (6Z)-isomer, the allylic carbon shift region is relatively simple compared with that of the *B. orientalis* seed oil. There are seven allylic carbon signals in the spectrum of the carrot seed oil. The signals at 27.297 and 26.834 ppm are characteristic of the shifts of the C-8 and C-5 of the (6Z)-isomer, respectively. The signals at 27.208 and 27.262 ppm are due to the shifts of C-8 and C-11 of oleate (O), respectively. The remaining three signals at 27.237, 27.225, and 25.661 ppm are due to the shifts of C-14, C-8, and C-11 of linoleate (L), respectively. The ω3, ω2, and ω1 carbon signals are readily assigned as summarized in Table 3.

We conclude from this study that carrot seed oil is composed of Sat, a large amount of a (6Z)-isomer, presumably 18:1(6Z), oleate (O), and linoleate (L). To determine the chainlength of the (6Z)-unsaturated isomer, it would be necessary to resort to GC analysis of the methyl ester derivatives of the fatty acids.

Semi-quantitative analysis of the fatty acid content and acyl distribution. The signals for the C-1 carbon atoms of Sat, O, L, and the (6Z)-isomer are fully resolved. From the intensities of the C-1 signals, the relative percentages and the distribution of the fatty acids are found as: saturated (3.5%, α-acyl), oleate (6.5% α-acyl; 8.6% β-acyl), linoleate (5.2% α-acyl; 6.9% β-acyl), (6Z)-isomer (49.6% α-acyl; 19.7% β-acyl). Analysis of the methyl esters of carrot seed oil by gas–liquid chromatography (on OV-101) gave the following results: Sat (18:0) = 4.5%, L = 12.5%, mixture of oleate and 18:1(6Z) = 83.0% Methyl oleate and the methyl ester of the (6Z)-isomer are not separated by GLC on OV-101 stationary phase, which indicates that the (6Z)-isomer is a positional isomer of 18:1. The overall results obtained by ^{13}C NMR spectroscopy and gas–liquid chromatography for the fatty acid components are comparable. ^{13}C NMR spectroscopy is able to characterize the various fatty acids and indicate the relative amount present in the α- and β-acyl positions of the glycerol "backbone."

ACKNOWLEDGMENTS

The Lipid Research Fund and the Research Grants Committee of the University of Hong Kong and the Research Grant Council of Hong Kong provided financial assistance.

REFERENCES

1. Gunstone, F.D., High Resolution ^{13}C NMR Spectroscopy of Lipids, in *Advances in Lipid Methodology*—Two, edited by W.W. Christie, The Oily Press, Dundee, 1993, pp. 1–68.
2. Lie Ken Jie, M.S.F., C.C. Lam, and B.F.Y. Yan, ^{13}C NMR Studies on Some Synthetic Saturated Glyerol Triesters, *J. Chem. Res. (S) 12–13*:(M) 250–272 (1992).
3. Lie Ken Jie, M.S.F., C.C. Lam, and B.F.Y. Yan, ^{13}C Nuclear Magnetic Resonance Studies on Some Synthetic Acetylenic Glycerol Triesters, *Ibid. (S) 141*:(M) 1023–1045 (1993).
4. Lie Ken Jie , M.S.F., and C.C. Lam, ^{13}C Nuclear Magnetic Resonance Spectroscopic Studies of Triacylglycerols of type AAA Containing (*Z*)- and (*E*)-Monoethylenic Acyl Groups, *Chem. Phys. Lipids 78*:15–27 (1995).
5. Lie Ken Jie, M.S.F., and C.C. Lam, ^{13}C NMR Studies of Polyunsaturated Triacylglycerols of Type AAA and Mixed Triacylglycerols Containing Saturated, Acetylenic and Ethylenic Acyl Groups, *Ibid. 78*:1–13 (1995).
6. Lie Ken Jie, M.S.F., C.Y.C. Choi, A. Berger, and R.G. Berger, Re-Examination of the Fatty Acid Composition of *Biota orientalis* Seed Oil by GCMS of the Picolinyl Ester Derivatives, *J. Chromatogr. 543*:257–261 (1991).
7. Lie Ken Jie, M.S.F., M.S.K. Syed-Rahmatullah, C.K. Lam, and P. Kalluri, Ultrasound in Fatty Acid Chemistry, Synthesis of a Novel l-Pyrroline Fatty Ester Isomer from Methyl Ricinoleate, *Lipids 29*:889–892 (1994).
8. Luddy, F.E., R.A. Barford, S.F. Herb, P. Magidman, and R.W. Riemenschneider, Pancreatic Lipase Hydrolysis of Triglycerides by a Semimicro Technique, *J. Am. Oil Chem. Soc. 41*: 693–696 (1964).
9. Gunstone, F.D., The ^{13}C-NMR Spectra of Oils Containing γ-Linolenic Acid, *Chem. Phys. Lipids 56*:210–217 (1990).
10. Mallet, J.F., E.M. Gaydou, and A. Archavlis, Determination of Petroselinic Acid in *Umbelliflorae* Seed Oils by Combined GC and ^{13}C NMR Spectroscopy Analysis, *J. Am. Oil Chem. Soc. 67*:607–610 (1990).

[Received May 11, 1995; accepted January 16, 1996]