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Structure Identification of Triacylglycerols in the Seed Oil of *Momordica Charantia* L. Var. *Abbreviata* Ser.

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Abstract Triacylglycerols (TGs) in the seed oil of *Momordica charantia* L. var. *abbreviata* Ser. (MCV) were separated by non-aqueous reversed-phase (NARP)-HPLC. Many of the TGs contain two different fatty acyl chains, such as palmitic (P), stearic (S), oleic (O), linoleic (L), and conjugated linolenic acid (CLn). Seven pairs of AAB/ABA-type TGs might present in the seed oil of MCV, namely CLnCLnP/CLnPCLn, CLnCLnS/CLnSCLn, CLnCLnO/CLnOCLn, CLnCLnL/CLnLCLn, SSCLn/SCLnS, OOCLn/OCLnO and LLCLn/LCLnL. The positional isomers of a AAB/ABA-type TGs pair yielded mass spectra showing a significant difference in relative abundance ratios of the fragment ions $[AA]^+$ to $[AB]^+$, which were produced by preferred losses of the fatty acid from the 1/3-position compared to the 2-position of the glycerol backbone. The precise stereospecific structures of the predominant regioisomers of TGs in AAB/ABA pairs were identified by atmospheric pressure chemical ionization mass spectrometry (APCI-MS) according to the special relative abundance ratios of the fragment ions $[AA]^+$ to $[AB]^+$. TGs with CLn occupying the sn-2 position in seven pairs of AAB/ABA might be major constituents of the oil, such as CLnCLnS, LCLnL, CLnCLnP, and so on. Some of the TGs which were isolated and collected as fractions from the seed oil of MCV by NARP-HPLC were further analyzed by ^{13}C-NMR. ^{13}C-NMR data of type-AAA TGs containing α-eleostearic acyl have been complemented.

Keywords The seed oil of *Momordica charantia* L. var. *abbreviata* Ser. · Triacylglycerol · Conjugated trienoic acid · NARP-HPLC/APCI-MS · α-Eleostearic acid · Conjugated linolenic acid

Introduction

Recently, the seed oils of *Trichosanthes kirilowii*, *Momordica charantia*, and Pomegranate which are rich in conjugated linolenic acids (CLn. Punicic acid, α-eleostearic acid, and *β*-eleostearic acid are *cis* or *trans* isomers of CLn), and CLn themselves, have attracted interest as colon carcinogenesis inhibitors. Most of the investigations have focused their attention on the anti-colon carcinogenesis activities and mechanisms of the seed oil of *Momordica charantia* and α-eleostearic acid [1–6]. The results of the investigations demonstrated that the cytotoxic action of the fatty acids (FAs) against human tumor cells was attributable to their conjugated trienoic structure [1]. However, this may not be the only reason for cytotoxic effects of lipids containing conjugated trienoic acids. Triacylglycerols (TGs) are the most abundant form of natural lipids in plants and animals. It is generally accepted that the TG molecular structure, including the distribution of FAs among the different stereospecific positions, has an effect on the nutritional, biochemical, and physical properties of lipids [7]. TGs with special stereospecific structures may display special functional properties.

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However, there are few reports about the connections between structures of TGs and their anti-carcinogenesis activities because of TGs complex composition, presence of various isomers, and separation difficulties. Identification of the precise structures of TGs is the basic work for elucidating the connections. ^{13}C-NMR and hydrolysis of the TGs by enzymes or chemical processes and subsequent analysis of the TGs are the normal methods for elucidating the composition of TGs and providing information about the distribution of FAs in lipids. However, the precise structure of an individual species of TGs cannot be elucidated [8–12]. We all know what kinds of different fatty acyl chains a certain TG contains. However, we do not know exactly which one of the fatty acyl chains occupies the 1/3-position or the 2-position on the glycerol backbone. There are many reports about the precise structures of TGs analyzed by non-aqueous reversed-phase (NARP)-HPLC which was coupled to atmospheric pressure chemical ionization mass spectrometry (APCI-MS) [7, 13, 14]. The method makes it possible to elucidate the connections between structures of TGs and their functions. NARP-HPLC/APCI-MS and ^{13}C-NMR were used in this paper to determine the precise structures of TGs in the seed oil of *Momordica charantia* L. var. *abbreviata* Ser. (MCV). MCV is identified as a species of *Momordica charantia.* by the Service Center for Products of Quality in Jiangsu Province, China. ^{13}C-NMR data of type-AAA TG containing α-eleostearic acyl have been complemented.

Experimental Procedures

Chemicals and Materials

The seeds were taken from ripened fruits of MCV supplied by Wuxi FuRen Biologic Science and Technology CO., Ltd, Wuxi, China, and were air-dried in a shady place. The Tung seeds were gathered from a suburb of Jishou city, Hunan Province, China. All solvents and reagents were of AR or HPLC-grade.

Extraction and Purification of the Seed Oil of MCV

The total lipids were extracted according to the method of Bligh and Dyer [15] and purified according to AOCS Official Method Cd 20–91 [16] to get a pure TGs mixture. All procedures were performed under an atmosphere of nitrogen whenever possible and in a darkened room.

NARP-HPLC/APCI-MS Analysis

For NARP-HPLC/APCI-MS experiments, a Waters 2695 Alliance HPLC system was connected to a Platform ZMD4000 quadrupole mass spectrometer (Waters, Milford, MA, USA). Samples were dissolved in 2-propanol/hexane (5/4, v/v) at a concentration of ca. 1 mg/mL, and 3 μL was injected into a SunFire C18 column (25 cm × 2.1 mm, 5 μm particle size, Waters, Milford, MA, USA). The column was kept at 35 °C. HPLC program: reservoir A contained acetonitrile and reservoir B contained 2-propanol/hexane (5/4, v/v). A linear gradient from 100% A to 50% A+50% B in 50 min, then to 100% B in 10 min was employed. The flow rate was 0.3 mL/min [14.] The mass spectrometric data were acquired in the range of *m*/*z* 300–1,000 in the positive-ion APCI mode. The APCI probe was kept at 400 °C. The ion source was kept at 120 °C. A 52-V voltage was applied to the cone. Nitrogen was used as the drying, sheath and nebulizing gas. A diode array detector was also used in this study with scan range of 200–400 nm to identify TGs containing conjugated trienoic acids.

^{13}C-NMR Analysis

NMR spectra were recorded on a BRUKER AVANCE 500 Fourier transform NMR spectrometer (Bruker Biospin International AG, Fällanden, Switzerland). Tetramethylsilane (TMS) was used as the internal standard. The analysis was conducted at 300 K, with 500–1,000 scans, using a 200 ppm spectral width, 64 K data points, repetition time of 4 s and a pulse angle of 45 and, zero-filled to 128 K to give a digital resolution of 0.38 Hz/point.

Results and Discussion

Structure Identification of TGs by NARP-HPLC/APCI-MS

The FA composition of TGs in the seed oil of MCV was determined by GC/MS of 2-alkenyl-4,4-dimethyloxazolines prepared by the method of Zhang et al. [17] with 2-amino-2-methylpropanol (AMP). The AMP derivatives of unsaturated fatty acids gave spectra that readily indicated the location of the double bonds in the molecules. The spectra of the unusual fatty acids gave identical fragmentation patterns and, therefore, had the same number of double bonds in the same positions [17]. Relative contents of FAs were calculated by peak area normalization. Five kinds of fatty acids were identified: palmitic acid (P, 16:0) 1.65%, stearic acid (S, 18:0) 31.78%, oleic acid (O, 18:1) 5.52%, linoleic acid (L, 18:2) 3.86%, conjugated trienoic fatty acids with 18 carbons (CLn, 18:3) 55.13%. Unidentified composition was 2.07%. No linolenic acid (Ln, 18:3) was found [18]. Tsuzuki et al. [19] reported that α-eleostearic acid in its free FA form was easily oxidized,

suggesting that use of the free FA form of α-eleostearic acid in food or medicines would result in oxidation before absorption, and would not allow for beneficial bioactivity. Oxidation rate of CLn can be slowed by triacylglycerol esterification.

NARP-HPLC fixed with APCI-MS has been used for the analysis of TGs [7, 13, 14]. The advantages of MS are minimal sample preparation, short analysis time, and the possibility to analyze individual species of TGs in mixtures, which is difficult to achieve by other methods. The APCI-MS of TG species typically exhibit a prominent $[M + H]^+$ ion, plus fragment ions of the type $[M + H\text{-RCOOH}]^+$ [also called $(DG)^+$ ions, i.e., diacylglycerol] originating from the loss of one acyl moiety. The molecular masses of the individual compounds can be readily determined from the peaks of $[M + H]^+$ ions and of the characteristic molecular adducts with alkali metal cations—$[M + Na]^+$ (22 *m*/*z* units higher) and $[M + K]^+$ (38 *m*/*z* units higher) [14]. Because of characteristic differences in the masses of the fragment ions produced by subsequent loss of fatty acids with different numbers of double bonds and different masses, the reconstructed ion current chromatograms (RIC) from the total ion current chromatograms (TIC) were reconstructed using characteristic ions of the individual DGs or TGs of the seed oil of MCV to identify TGs, to deconvolve overlapping peaks, and to determine the exact retention times (RT) of the coeluted TGs.

The APCI mass spectra also provided information about the regiospecific distribution of fatty acids on the glycerol backbone. It is possible to identify the major regioisomer of an AAB/ABA pair of TGs by comparing the ratios of relative abundances of $c[AA]^+$ to $c[AB]^+$ [7]. Here, 'AAB' denotes a triacylglycerol containing two identical FAs, such as PPO or SOO, and can be asymmetric (AAB) or symmetric (ABA, e.g., POP or OSO). APCI-MS fragmentation is affected by the distribution of FAs on the glycerol backbone and the loss of the fatty acyl chain located at the sn-2 is energetically disfavored. For highly unsaturated TG species, though the fragmentation will be affected a lot by the degree of unsaturation [7]—that means the loss of the highly unsaturated fatty acyl chain located at the sn-2 is easier than that of ordinary fatty acyl chains, the FAs regiospecific distribution of a TG with a special ratio of relative abundances of $c[AA]^+$ to $c[AB]^+$ can be still identified. Seven pairs of AAB/ABA-type TGs may present in the seed oil of MCV, namely CLnCLnP/CLnPCLn, CLnCLnS/CLnSCLn, CLnCLnO/CLnOCLn, CLnCLnL/CLnLCLn, SSCLn/SCLnS, OOCLn/OCLnO and LLCLn/LCLnL. The precise stereospecific structures of the predominant regioisomers of TGs in AAB/ABA pairs should be identified.

TIC and RIC of ions with *m*/*z* 879.8, 595.5, and 601.6 of the seed oil of MCV were shown in Fig. 1. Two $[DG]^+$s

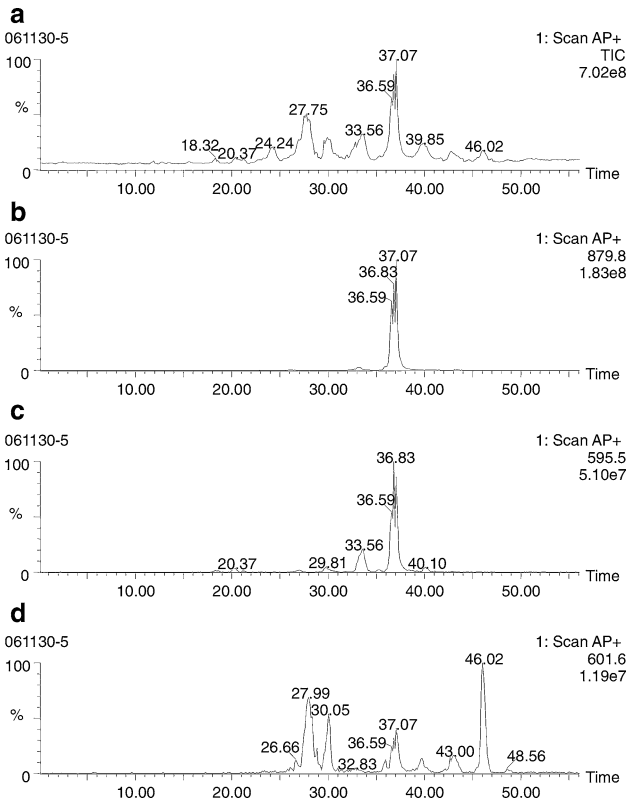

Fig. 1 TIC of the seed oil of MCV (**a**) and RIC at *m*/z 879.8 (**b**), 595.5 (**c**) and 601.6 (**d**) by NARP-HPLC/APCI-MS. MCV *Momordica charantia* L. var. *abbreviata* Ser.

with *m*/*z* 601.6 and 595.5 are characteristic fragment ions, which were produced by TG in the peak of $[M + H]^+$ (*m*/*z* = 879.8) at RT 36.586 min in TIC of the seed oil of MCV. It means that the TG contains two CLns and one S. If the relative abundances of $[CLnS]^+$ ions (*m*/*z* 601.6) is higher than that of $[CLnCLn]^+$ ions (*m*/*z* 595.5), it is difficult for us to determine the sn-2 FA. It is inevitable that the relative abundances of $[CLnS]^+$ ions is higher than that of $[CLnCLn]^+$ ions when S occupies sn-2 position. It is also possible that the relative abundances of $[CLnS]^+$ ions is higher than that of $[CLnCLn]^+$ ions when CLn occupies sn-2 position, because the loss of the highly unsaturated fatty acyl chain located at the sn-2 is easier than that of ordinary fatty acyl chains—the conclusion was deduced from Ref. [7]. However, the relative abundance of $[CLnCLn]^+$ ions is further higher than that of $[CLnS]^+$ ions. The ratio of relative abundances of $[CLnCLn]^+$ ions to $[CLnS]^+$ ions is about 15. This happens only when S rarely occupies sn-2 position. The TG was identified as 1(3),2-di-conjugated linolenoyl-3(1)-stearoyl-sn-glycerol (CLnCLnS) (Shown in Table 1).

There is a peak at RT 33.561 min in TIC of the seed oil of MCV. In fact, it is the peak of coeluted compounds consisting of three kinds of TGs with the same Partition

Table 1 Structure identification of triacylglycerols in the seed oil of MCV by NARP-HPLC/APCI-MS

RT (min)[a]	*m/z*	PN[b]	$[AA]^{+}$[c]	$[AB]^{+}$[d]	$c[AA]^{+}/c[AB]^{+}$[e]	Main regioisomers of triacylglycerols	Relative content (%)[f]
18.319						Non-triacylglycerols	11.04
20.374						Non-triacylglycerols	
24.241						Non-triacylglycerols	
27.025	873.9	36	595.5			CLnCLnCLn	22.10
27.750						Not determined	
29.685	875.9	38	595.5	597.5	1	CLnCLnL, CLnLCLn	5.91
30.049						Not determined	
32.227	877.8	40	599.6	597.6	0.5	LCLnL	12.57
32.831	877.8	40	595.5	599.5	1	OCLnCLn, CLnOCLn	
33.439	851.8	40	595.6	573.5	15	PCLnCLn	
36.586	879.8	42	595.5	601.6	15	CLnCLnS	32.24
36.829	879.8	42	595.5	601.6	15	CLnCLnS	
37.070	879.8	42	595.5	601.6	15	CLnCLnS	
39.855	881.9	44	603.6	599.5		CLnOO, OCLnO	6.69
	881.9	44				LCLnS, LSCLn, CLnLS	
	855.7	44				CLnPO, CLnOP, PCLnO	
42.877	883.8	46				CLnOS,SCLnO,CLnSO	3.17
46.140	885.9	48	607.6	601.6	0.4	SCLnS	4.03
						Not determined	2.25

MCV Momordica charantia L. var. *abbreviata* Ser., *P* palmitic acid, *S* stearic acid, *O* oleic acid, *L* linoleic acid, *CLn* conjugated linolenic acid

[a] Retention times

[b] Partition number

[c,d,e] 'AAB' denotes a triacylglycerol containing two identical FAs, and can be asymmetric (AAB) or symmetric (ABA), $[AA]^{+}$ and $[AB]^{+}$.are diacylglycerol ions originating from the loss of one acyl moiety from triacylglycerol. $c[AA]^{+}/c[AB]^{+}$ is the ratio of relative abundances of $[AA]^{+}$ to $[AB]^{+}$

[f] Relative contents were calculated by peak area normalization. Peak area of triacylglycerols with the same PN was calculated together by the software

number (PN) of 40. The HPLC procedure used in this study was not able to separate these isomers. PN was defined as CN-2DB, where CN is the number of carbon atoms in the acyl chains of acylglycerol and DB is the number of double bonds. By identifying the characteristic ions and comparing the ratios of relative abundances of the individual $[DG]^{+}$s of the coeluted TGs, the exact RTs and structures of three kinds of TGs were determined. At RT 32.227–32.831 min, $[M + H]^{+}$ m/z = 877.7, $[LL]^{+}$ ions (m/z = 599.6) and $[LCLn]^{+}$ ions (m/z = 597.6) are characteristic ions. The ratio of relative abundances of $[LL]^{+}$ to $[LCLn]^{+}$ ions is about 0.5. This happens only when CLn largely occupies sn-2 position. The TG was identified as 1,3-dilinoleoyl-2-conjugated linolenoyl-sn-glycerol (LCLnL). At RT 32.831–33.317 min, $[M + H]^{+}$ m/z = 877.7, m/z of two $[DG]^{+}$ ions are 599.5 $[(CLnO)^{+}]$ and 595.5 $[(CLnCLn)^{+}]$. It means that TG contains two CLns and one O. The ratio of relative abundances of $[CLnCLn]^{+}$ to $[CLnO]^{+}$ ions is about one. The TG was identified as 1(3),2-di-conjugated linolenoyl-3(1)-oleoyl-sn-glycerol (CLnCLnO) and 1,3-di-conjugated linolenoyl-2-oleoyl-sn-glycerol (CLnOCLn). There was more CLnCLnO than CLnOCLn according to the ratio of 1–1. At RT 33.439–34.286 min, $[M + H]^{+}$ m/z = 851.8, characteristic ions are $[CLnCLn]^{+}$ (m/z = 595.6) and $[PCLn]^{+}$ (m/z = 573.5). The ratio of relative abundances of $[CLnCLn]^{+}$ to $[PCLn]^{+}$ ions is about 15. The TG was identified as 1(3),2-di-conjugated linolenoyl-3(1)-palmitoyl-sn-glycerol (CLnCLnP). P rarely occupies the sn-2 position.

Two DG^{+} ions with m/z 601.6 $[(CLnS)^{+}]$ and 607.4 $[(SS)^{+}]$ are characteristic fragment ions, which were produced by TG in the peak of $[M + H]^{+}$ (m/z = 885.9) at RT 46.140 min in TIC of the seed oil of MCV. It means that TG contains one CLn and two Ss. The ratio of relative abundances of $[SS]^{+}$ to $[CLnS]^{+}$ ions is about 0.4. The TG was identified as 1,3-di-stearoyl-2-conjugated linolenoyl-sn-glycerol (SCLnS). Most of CLn occupies sn-2 position.

The molecular masses and the FAs regiospecific distribution of TGs in the seed oil of MCV were determined in this way. Structure identification of TGs in the seed oil of MCV by NARP-HPLC/APCI-MS was shown in Table 1. TGs with CLn which does not occupy sn-2 position in

seven pairs of AAB/ABA, namely CLnPCLn, CLnSCLn, CLnOCLn, CLnLCLn, SSCLn, OOCLn and LLCLn, might be minor constituents of the oil or not present in the oil at all.

The composition of TGs in the seed oil of MCV reported in this paper is not in accordance with the Ref. [20] to some degree. Species difference might be an interpretation.

Compositions of TGs in plant oils are very complex. TGs with the same PN probably display the same retention property on NARP-HPLC, and can not be separated by this NARP-HPLC. APCI-MS was used to deconvolve overlapping peaks, to determine the exact RTs of the coeluted TGs, and to identify structure of TGs. APCI-MS cannot identify TG isomers with different position of double bonds or *cis–trans* isomers. Different TGs consisting of *cis–trans* isomers of CLns were not identified in Table 1, neither were TGs consisting of Ln or CLn. For the later situation, there will be great difference in UV absorbance at 270 nm. No Ln was found in the seed oil of MCV [18]. No significant difference was shown between chromatogram of the seed oil of MCV detected by UV at 270 nm and under scan range of 200–400 nm (not shown).

Structural Identification of TGs by ^{13}C-NMR

Two groups of TGs of the seed oil of MCV in peaks at RT 29.68 and 36.83 min in TIC were isolated and collected by NARP-HPLC, and were determined as CLnCLnL, CLnLCLn and SCLnCLn by APCI-MS and a diode array detector.

To verify the results of structure identification by APCI-MS, the seed oil of MCV and the isolated TGs were analyzed by ^{13}C-NMR respectively.

No ^{13}C-NMR data of type-AAA TGs containing α-eleostearic acyl were found in literature. A kind of TG which was isolated and collected as a fraction from Tung oil by NARP-HPLC was determined as CLnCLnCLn by APCI-MS and a diode array detector. ^{13}C-NMR Data of a type-AAA TG containing α-eleostearic acyl and their assignments are shown in Table 2, and the ^{1}H-NMR data were as follows:

δ_H0.895(H-18), 1.331(H-3,4,5,6,7), 1.588(H-16,17), 2.092(H-8), 2.168(H-15), 2.309(H-2), 4.218(αH on glycerol backbone), 5.262(*β*H on glycerol backbone), 5.384(H-9), 5.676(H-14), 5.987(H-10), 6.117(H-12,13), 6.370(H-11).

For the ^{1}H-NMR data, no signal was shown at δ_H2.73–2.70. It means no Ln presented in the sample. The signal at *δ*2.168(H-15) was assigned as methylene connecting with *trans* FAs. Type-AAA TG containing α-eleostearic acyl was proved.

^{13}C-NMR data of the seed oil of MCV and the isolated TGs were assigned according to the data of ^{13}C-NMR of type-AAA TG containing α-eleostearic acyl and literature [8–12].

Table 2 ^{13}C-NMR chemical shift values of triacylglycerol of type-AAA containing α-eleostearic acyl

	α-Acyl chains	*β*-Acyl chains		α-Acyl chains	*β*-Acyl chains
Glycerol backbone	62.08	68.915	C-10	128.744	
C-1	173.165	172.758	C-11	130.562	
C-2	34.002	34.164	C-12	131.709	131.671
C-3	24.811	24.846	C-13	125.927	
C-4	28.985		C-14	135.135	
C-5	29.103		C-15	32.453	
C-6	29.046		C-16	31.454	
C-7	29.621		C-17	22.195	
C-8	27.779		C-18	13.874	
C-9	132.847				

Table 3 Chemical shift values of C-1, C-2, C-3, allylic, olefinic, *ω*3, *ω*2, *ω*1 carbon signals of the seed oil of MCV

Carbon nucleus	Chemical shifts value	Assignments
C-1	173.196	L and sat (α)
	173.159/172.751	αE (α, *β*)
C-2	34.169/34.007	αE (*β*, α)
	34.037	sat (α)
C-3	24.852/24.816	αE (α, *β*), L and O (α)
Allylic	32.452	C-15 of αE
	27.780	C-8 of αE
Olefinic	135.133	C-14 of αE
	132.853	C-9 of αE
	131.696	C-12 of αE
	131.656	αE
	130.570	C-11 of αE
	128.756	C-10 of αE
	125.917	C-13 of αE
*ω*3	31.902	O, sat
	31.459	αE
*ω*2	22.654	L, O and sat
	22.196	αE
*ω*1	14.057	L, O and sat
	13.867	αE

MCV Momordica charantia L. var. *abbreviata* Ser., *sat* saturated, *O* oleate, *L* linoleate, *αE* α-eleostearic acid

^{13}C-NMR data of the seed oil of MCV and their assignments are shown in Table 3. CLn, e.g., α-eleostearic acid, was the main composition (above 50%) of the seed oil of MCV. Saturated FAs (sat), O, and L were unresolved at chemical shift value because of weak signals [8]. Signals of C-1 obviously showed that sat and L only distributed on α

Table 4 Chemical shift values of C-1, C-2, C-3, allylic, $\omega 3$, $\omega 2$, $\omega 1$ carbon signals of TGs of the seed oil of MCV at RT 29.68 min

Carbon nucleus	Chemical shifts value	Assignments
C-1	173.226/172.806	αE and L (α, β)
C-2	34.028/34.188	αE and L (α, β)
C-3	24.866	αE and L (β)
Allylic	32.736	αE
	32.453	αE
	27.799	αE
	27.224	C-8 of L
$\omega 3$	31.916	L
	31.522	αE
$\omega 2$	22.671	L
	22.210	αE
$\omega 1$	14.080	L
	13.889	αE

MCV Momordica charantia L. var. *abbreviata* Ser., *sat* saturated, *O* oleate, *L* linoleate, *αE* α-eleostearic acid

Table 5 Chemical shift values of C-1, C-2, C-3, allylic, $\omega 3$, $\omega 2$, $\omega 1$ carbon signals of TG of the seed oil of MCV at RT 36.83 min

Carbon nucleus	Chemical shifts value	Assignments
C-1	173.236	sat(α)
C-2	34.031	sat(α)
C-3	24.855	αE
Allylic	32.699	αE
	32.393	αE
$\omega 3$	31.913	sat
$\omega 2$	22.670	sat
$\omega 1$	14.082	sat
	13.940	αE

MCV Momordica charantia L. var. *abbreviata* Ser., *Sat* saturated, *O* oleate, *L* linoleate, *αE* α-eleostearic acid

position of glycerol backbone, and CLn distributed on both α and β positions of glycerol backbone. It was proved by signals of C-2 and C-3. That means TGs with CLn occupying sn-2 position in Table 1 were the dominant TGs in the seed oil of MCV.

Chemical shift values of carbon signals of TGs of the seed oil of MCV at RT 29.68 min are shown in Table 4. The TGs at RT 29.68 min were identified as CLnCLnL and CLnLCLn in Table 1. Chemical shift value of carbon signals showed that L distributed on both α and β positions of glycerol backbone. Identification of 1(3),2-di-conjugated linolenoyl-3(1)-linoleoyl-sn-glycerol (CLnCLnL) and 1,3-di-conjugated linolenoyl-2-linoleoyl-sn-glycerol (CLnLCLn) by APCI-MS was proved to be reliable.

Chemical shift values of carbon signals of TGs of the seed oil of MCV at RT 36.83 min are shown in Table 5. The TGs at RT 36.83 min were identified as CLnCLnS in Table 1. Chemical shift value of carbon signals showed that only sat distributed on α position of glycerol backbone. Identification of 1(3),2-di-conjugated linolenoyl-3(1)-stearoyl-sn-glycerol (CLnCLnS) by APCI-MS was proved to be reliable. As shown in Table 5, signals of S were stronger than those of CLn. No signal of CLn was seen on C-1 and C-2.

TGs in the seed oil of MCV were separated by NARP-HPLC. Structure of TGs in the seed oil of MCV was identified by APCI-MS and ^{13}C-NMR. The results suggest that structure identification of TG by APCI-MS is reliable. Composition and structure of the TGs in the seed oil of MCV can be elucidated with data shown in Table 1.

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