J Am Oil Chem Soc (2012) 89:1785–1792
DOI 10.1007/s11746-012-2083-1

ORIGINAL PAPER

Ratios of Regioisomers of Minor Acylglycerols Less Polar than Triricinolein in Castor Oil Estimated by Mass Spectrometry

Jiann-Tsyh Lin · Grace Q. Chen

Received: 13 September 2011 / Revised: 4 January 2012 / Accepted: 7 February 2012 / Published online: 27 May 2012

Abstract We have recently reported the identification of forty new minor molecular species of acylglycerols containing hydroxy fatty acids less polar than triricinolein by electrospray ionization mass spectrometry of the lithium adducts. The ratios of regioisomers of triacylglycerols (ABC and AAB types) and tetraacylglycerols (AAAB type) identified were estimated by the relative abundances of the fragment ions from the neutral losses of fatty acids as α,β-unsaturated fatty acids at the *sn*-2 position. The order of the contents of regioisomers of triacylglycerols with the fatty acids at the *sn*-2 position are: nonhydroxy fatty acids > monohydroxy fatty acids > dihydroxy fatty acids > trihydroxy fatty acids. For tetraacylglycerols (AAAB type) such as ricinoleoylricinoleoyl–ricinoleoyl–oleoyl–glycerol (RRRO), ricinoleoylricinoleoyl chain was predominately at the *sn*-2 position, while ricinoleate was not detected at the *sn*-2 position.

Keywords Castor oil · Triacylglycerols · Estolide · Tetraacylglycerols · Ricinoleate · hydroxy fatty acids · Regioisomers · *Ricinus communis* L.

Introduction

Ricinoleate (R, OH18:1), a monohydroxy fatty acid, has many industrial uses such as the manufacture of biodegradable lubricants, plastics, paints and cosmetics. Castor oil is the only commercial source of ricinoleate. Ricinoleate occurs as acylglycerols in castor oil and about 90 % of its fatty acids is ricinoleate [1]. We had quantified the molecular species of acylglycerols in castor oil, and triricinolein (RRR) was about 70 % of castor oil [2]. Some acylglycerols more polar than triricinolein containing polyhydroxy fatty acids have been identified by mass spectrometry [3–5]. Polyhydroxy fatty acids can be used in industry similar to that of ricinoleate with different physical properties such as viscosity, pour point, melting point, heat of fusion, solubility, crystal structure and polymorphism [6]. We have recently identified the minor triacylglycerols less polar than triricinolein by mass spectrometry and proposed biosynthetic pathway of castor oil to include the triacylglycerols containing polyhydroxy fatty acids and tetraacylglycerols [7]. The pathway will help to produce the desired triacylglycerols for industrial uses by metabolic engineering.

We have estimated the ratios of regiospecific isomers of some triacylglycerols containing hydroxy fatty acids in castor oil by electrospray ionization mass spectrometry using the lithium adducts of triacylglycerols and the fragment ions from the losses of fatty acids as α,β-unsaturated fatty acids specific at the *sn*-2 position [8–11]. Using the same method, the ratios of regioisomers of triacylglycerols containing normal fatty acids in olive oil was also estimated [12]. Regiospecificity affects the physical property and the human absorption of triacylglycerols. The ratios of regioisomers is thus important for industrial uses. We report here the ratios of regioisomers of minor triacylglycerols less polar than triricinolein.

The ratios of regioisomers of triacylglycerols have been estimated by mass spectrometry using the fact that the neutral loss of fatty acid from the *sn*-2 position is energetically less favored in comparison with that from *sn*-1,3 positions [13–16]. However regioisomeric triacylglycerol

J.-T. Lin (✉) · G. Q. Chen
Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, CA 94710, USA
e-mail: jiann.lin@ars.usda.gov

standards were needed for making calibration curves and the estimation of the ratios of regioisomers were limited to the standards (AAB type triacylglycerols) available. Our method used here and earlier [8–11] has no such limitations. Our method can be used for AAB and ABC types of triacylglycerols as well as AAAB type tetraacylglycerols without the standards.

Experimental Procedure

HPLC Fractionation of the Molecular Species of Acylglycerols in Castor Oil

The fractionation of the molecular species of acylglycerols in castor oil (Sigma, St. Louis, MO, USA) was as previously reported [2]. Chromatographic fractionation was performed using a Waters HPLC (Waters Associate, Milford, MA, USA) and a C_{18} analytical column (Gemini, 250 × 4.6 mm, 5 μ, C18, Phenomenex, Torrance, CA, USA). One milligram of castor oil in ethanol (50 µl) was chromatographed at 22 °C (room temperature) with a linear gradient from 100 % methanol to 100 % 2-propanol in 40 min, at a 1 mL/min flow rate, and detected at 205 nm (Fig. 1). Fractions were collected every half minute and corresponding fractions were pooled from 15 HPLC runs. HPLC fractions were used for MS studies. The final methanol solutions of samples were prepared for direct infusion into the mass spectrometer by combining approximately one fourth of each HPLC fraction with 50 µL of a methanol solution of 100 mM lithium acetate and diluting to a total volume of 250 µL.

Electrospray Ionization Mass Spectrometry (ESI-MS)

An LCQ Advantage ion-trap mass spectrometer (MS 2.0) with Xcalibur 2.0 SR2 software (ThermoFisher Scientific, San Jose, CA, USA) was utilized for MS analysis of the various molecular species of acylglycerols. The infusion at a 2.5 µL/min flow rate from a syringe (250 µL) pump produced stable singly-charged positive lithiated parent ions which were subsequently fragmented for MS^2, MS^3 and MS^4 analysis. Electrospray ionization source conditions were as follows: sheath gas flow rate, 10 arbitrary units (au); aux/sweep gas flow rate, 0 au; spray voltage, 4 kV; capillary temperature, 200 °C; capillary voltage, 5 V; tube lens offset, 15 V. Scan conditions were as follows: isolation width, 1.5 *m/z*; normalized collision energy, 27–42 %; scan ranges, 100–1,500 *m/z*. Acquire time was usually 3 min.

Results and Discussion

Castor oil was fractionated by C_{18} HPLC at 0.5 min/fraction as shown in Fig. 1 [7]. Samples of the methanol solutions of the HPLC fractions and lithium acetate were infused into the MS from the syringe pump. HPLC fractions mainly after RRR (retention time 9.0 min) were used for these MS studies. The acylglycerols identified by MS in various HPLC fractions are listed in Table 1 of reference #7. The ratios of the regioisomers of triacylglycerol shown in Table 1was estimated by the relative abundances of the fragment ions from the losses of fatty acids as α,β-unsaturated fatty acids specific at the *sn*-2 position after neutral

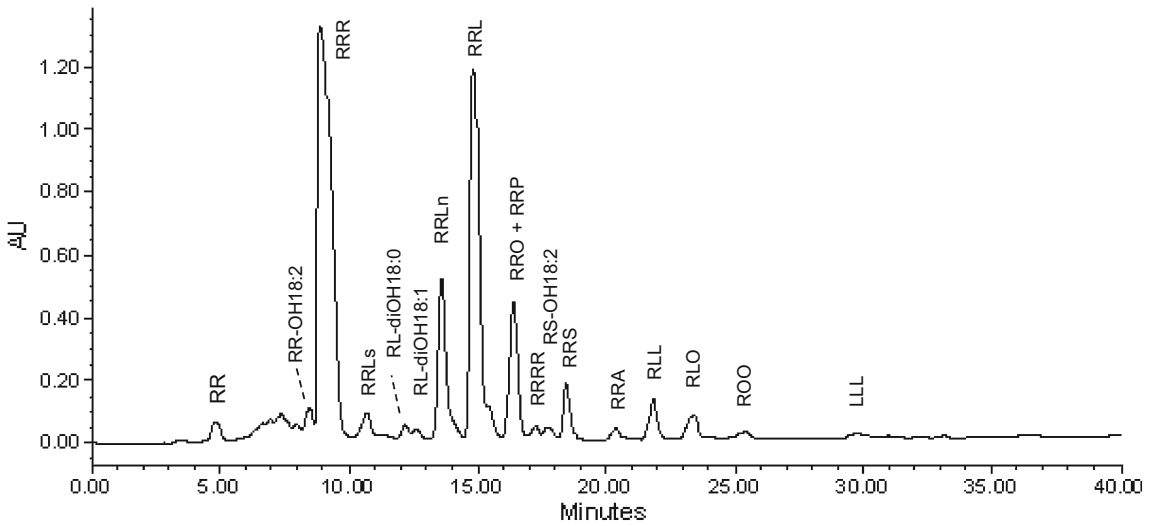

Fig. 1 HPLC chromatogram for fractionation (0.5 min/fraction) of castor oil (1 mg). For HPLC conditions, see experimental procedures. For abbreviations, see Table 1

Table 1 Ratios of regioisomers of acylglycerols in HPLC fractions less polar than triricinolein in castor oil

Fraction #	Acylglycerol	Regioisomers with acyl chain on *sn*-2	Ratio (%)
17	RROH18:2	R:OH18:2	50:50
24	RL-diOH18:0	L:R:diOH18:0	89:9.4:1.3
24	RL-diOH18:1	L:R:diOH18:1	92:7.5:0.5
26	RO-diOH18:1	O:R:diOH18:1	98:2:0.1
28	RO-diOH18:0	O:R:diOH18:0	97:2.5:0.5
28	RP-diOH18:1	P:R:diOH18:1	61:33:6
29	RL-OH18:2	L:R:OH18:2	93:6.5:0.5
32	RO-OH18:2	O:R:OH18:2	97:2.5:0.5
34	RLLs	L:R:Ls	94:4:2
34	RR-20:2	20:2:R	94:6
36	RS-OH18:2	S:R:OH18:2	94:4:2
37	ROLs	O:R:Ls	98:1.5:0.5
38	RR-20:1	20:1:R	91:9
40	RSLs	S:R:Ls	85:10:5
41	RLLn	L:Ln:R	64:34:2
42	RRA	A:R	95:5
43	RLL	L:R	85:15
43	RPLn	P:Ln:R	59:23:18
43	RLPo	Po:L:R	72:27:1
45	ROLn	O:Ln:R	67:32:0.5
45	RRRLn	RR:Ln:R	61:39:0
46	RLP	L:P:R	75:15:10
46	RRB	B:R	92:8
47	ROL	O:L:R	74:25:1
47	RRRL	RR:L:R	x:x
48	RR-23:0	23:0:R	94:6
50	RRRO	RR:O:R	59:41:0
50	ROO	O:R	99:1
50	ROP	O:P:R	96:3:1
52	RLS	S:L:R	62:38:0.2
52	RRRS	RR:S:R	56:44:0
53	ROS	S:O:R	52:39:9
57	RSS	S:R	91:9
57	LLLn	L:Ln	64:36
59	RLB	B:L:R	83:15:2
62	LLP	L:P	65:35
62	LLO	O:L	52:48
65	OLP	O:L:P	46:34:20
65	LOO	L:O	54:46
67	PPO	O:P	82:18
69	OLS	O:L:S	62:27:11
71	SOO	O:S	82:18
72	LSS	S:L	56:21
74	OSS	O:S	96:4

The ratio of regioisomers with RR and L at the *sn*-2 position of RRRL in Fraction #47 could not be estimated, because (RR − R) and L have the same mass

R ricinoleic acid (OH18:0), *Ls* lesquerolic acid (OH20:1), *A* arachidic acid (20:0), *B* behenic acid (22:0), *Po* palmitoleic acid (16:1)

loss of a fatty acid (MS^3) [8, 17, 18]. Regioisomer of ABC type triacylglycerol does not differentiate the *sn*-1 and *sn*-3 positions, e.g., stereoisomers of ABC and CBA are the same regioisomer.

RL-diOH18:0, an ABC type triacylglycerol with three different fatty acids, was identified in the HPLC fractions #24 and #25 (Fig. 1, retention times 11.5–12.5 min) [7]. ABC type triacylglycerol has three regioisomers (and six stereoisomers). The MS^2 spectrum of $[RL\text{-}diOH18:0 + Li]^+$ at *m/z* 939.5 in HPLC fraction #24 showed the fragment ions from the three neutral losses of fatty acids as $[RL\text{-}diOH18:0 + Li - R]^+$ at *m/z* 641.4, $[RL\text{-}diOH18:0 + Li - L]^+$ at *m/z* 659.5 and $[RL\text{-}diOH18:0 + Li - diOH18:0]^+$ at *m/z* 623.4. The MS^3 spectrum (Fig. 2) from $[RL\text{-}diOH18:0 + Li - diOH18:0]^+$ at *m/z* 623.5 showed the regiospecific *sn*-2 fragment ions as $[RL\text{-}diOH18:0 + Li - diOH18:0 - L + 2]^+$ at *m/z* 345.1 and $[RL\text{-}diOH18:0 + Li - diOH18:0 - R + 2]^+$ at *m/z* 327.1. The ratio of the abundances of these two fragment ions was 100:11 and the ratio of the contents of regioisomers, RL-diOH18:0 and LR-diOH18:0 (with L and R at the *sn*-2 position) was also about 100:11. Identification and ratio quantification of the two fatty acids among the three fatty acids at the *sn*-2 position by mass spectrometry are the identification and ratio quantification of the two regioisomers among the total of three regioisomers. It is not necessary on the mean time to know the fatty acid distributions at the *sn*-1 and *sn*-3 positions to estimate the ratio of these two regioisomers because regioisomer does not differentiate the *sn*-1 and *sn*-3 positions.

The MS^3 spectrum (Fig. 3) from $[RL\text{-}diOH18:0 + Li - L]^+$ at *m/z* 659.4 showed the *sn*-2 fragment ions as $[RL\text{-}diOH18:0 + Li - L - R + 2]^+$ at *m/z* 363.1 and $[RL\text{-}diOH18:0 + Li - L - diOH18:0 + 2]^+$ at *m/z* 345.1. The ratio of the abundances of these two fragment ions was 100:14 and the ratio of the contents of regioisomers, RL-diOH18:0 and R-diOH18:0-L (with L and diOH18:0 at the *sn*-2 position) was also about 100:14. From these two ratios of the two regioisomers from the three regioisomers, the ratio of the contents of these three regioisomers, RL-diOH18:0, LR-diOH18:0 and R-diOH18:0-L (with L, R and diOH18:0 at the *sn*-2 position), was estimated as 90.7 %:8.2 %:1.1 %. The third ratio of the two regioisomers was estimated from the abundances of the two *sn*-2 fragment ions in Fig. 4, the MS^3 spectrum from $[RL\text{-}diOH18:0 + Li - R]^+$ at *m/z* 641.4. The *sn*-2 fragment ions were $[RL\text{-}diOH18:0 + Li - R - L + 2]^+$ at *m/z* 363.2 and $[RL\text{-}diOH18:0 + Li - R - diOH18:0 + 2]^+$ at *m/z* 327.1. The ratio of their abundances and contents of RL-diOH18:0 and R-diOH18:0-L (with L and diOH18:0 at the *sn*-2 position) was 100:1.6. This ratio of the two regioisomers together with either one of the two earlier ratios of the two

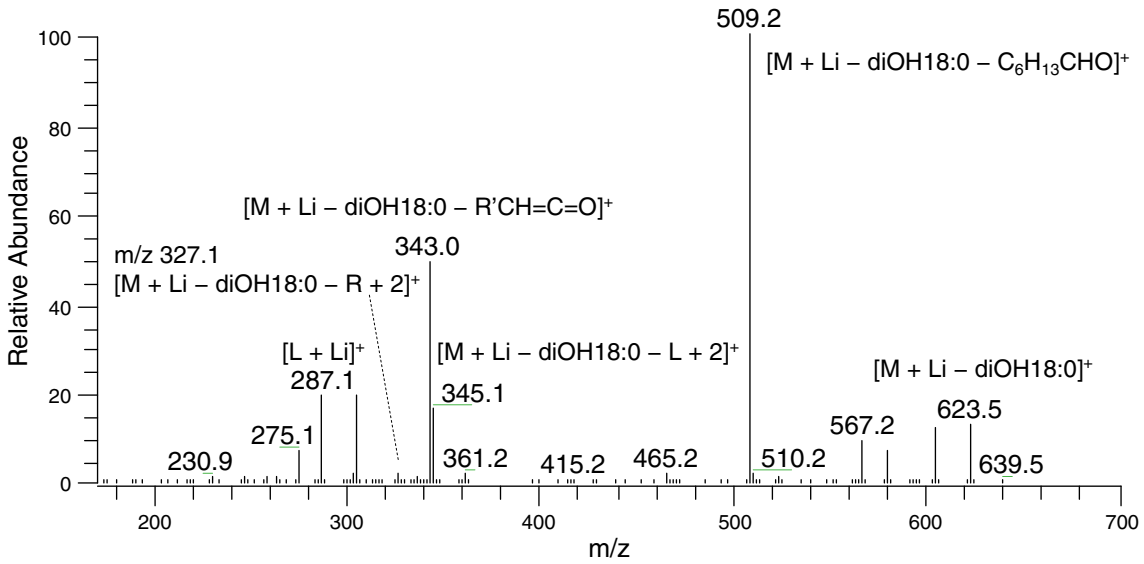

Fig. 2 The MS^3 spectrum of $[RL\text{-}diOH18:0 + Li - diOH18:0]^+$ at *m/z* 623.5 from the HPLC fraction #24 of castor oil. The precursor ion was from the MS^2 of $[RR\text{-}diOH18:0 + Li]^+$ at *m/z* 939.6. R is ricinoleic acid (OH18:1). (R − 2) is α,β-unsaturated ricinoleate from the *sn*-2 position. (L − 2) is α,β-unsaturated linoleate from the *sn*-2 position. R'CH=C=O is a ketene from ricinoleate at the *sn*-1,3 position. $C_6H_{13}CHO$ is the loss as aldehyde from the cleavage between C-11 and C-12 of ricinoleate chain (For proposed fragmentation pathway, see Fig. 6a of reference 3)

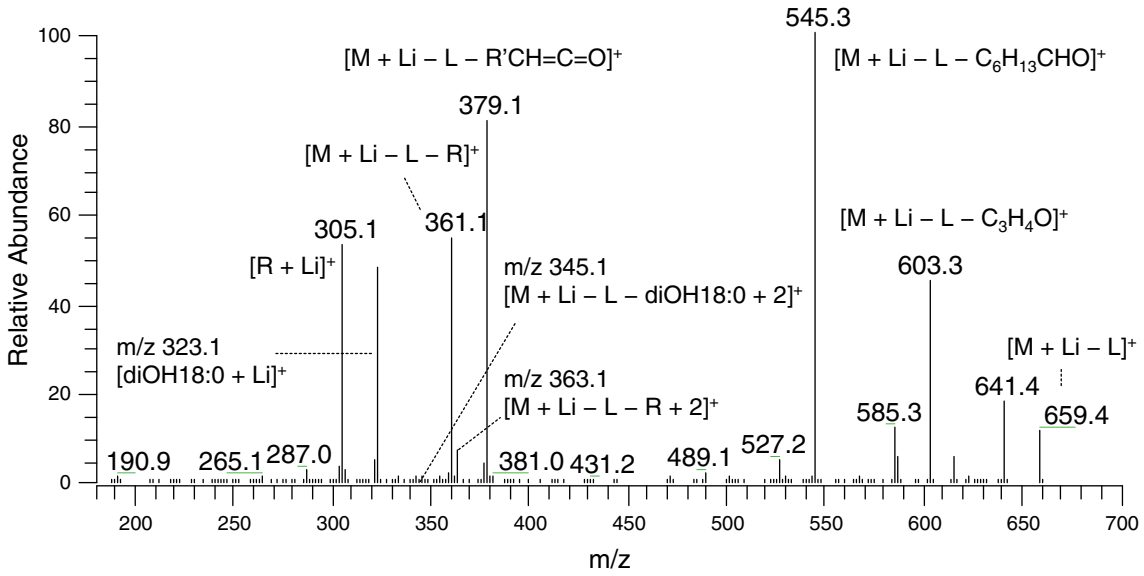

Fig. 3 The MS^3 spectrum of $[RL\text{-}diOH18:0 + Li - L]^+$ at *m/z* 659.4 from the HPLC fraction #24 of castor oil. The precursor ion was from the MS^2 of $[RR\text{-}diOH18:0 + Li]^+$ at *m/z* 939.6. For abbreviations, see Fig. 2. (diOH18:0 − 2) is α,β-unsaturated diOH18:0 from the *sn*-2 position. C_3H_4O is the loss of the glycerol backbone to form acid anhydrides of two fatty acids (For proposed fragmentation pathway, see Figs. 4B and 4C of reference 8)

regioisomers were used to estimate the ratio of the three regioisomers, RL-diOH18:0, LR-diOH18:0 and R-diOH18:0-L (with L, R and diOH18:0 at the *sn*-2 position) as just described, and the ratios were 88.7 %:9.9 %:1.4 % and 88.4 %:10.2 %:1.4 %. Three ratios of the three regioisomers obtained were very close and the contents was averaged as 89.3 % (regioisomer RL-diOH18:0), 9.4 % (regioisomer LR-diOH18:0), and 1.3 % (regioisomer R-diOH18:0-L). We were the first to estimate the ratios of three regioisomers of ABC type triacylglycerols with three different fatty acids [10]. The estimation of other ABC type triacylglycerols were the same as this

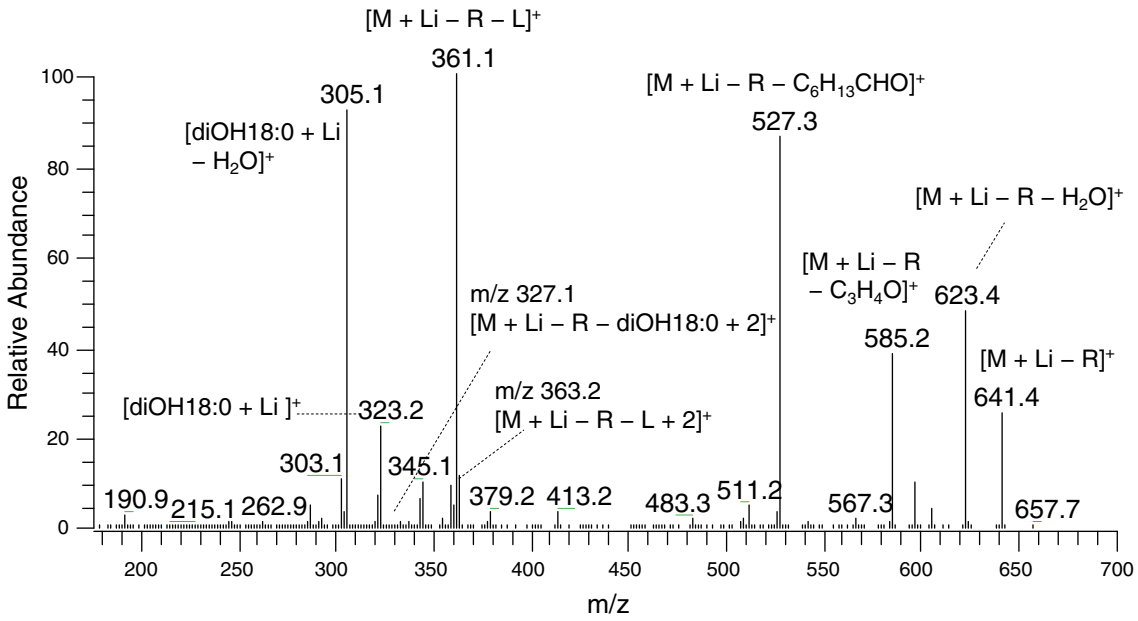

Fig. 4 The MS^3 spectrum of [RL-diOH18:0 + Li − R]$^+$ at *m/z* 641.4 from the HPLC fraction #24 of castor oil. The precursor ion was from the MS^2 of [RR-diOH18:0 + Li]$^+$ at *m/z* 939.6. For abbreviations, see Figs. 2 and 3

example. Since the three ratios of the three regioisomers with L, R and diOH18:0 at the *sn*-2 position were pretty similar (90.7 %:8.2 %:1.1 %, 88.7 %:9.9 %:1.4 % and 88.4 %:10.2 %:1.4 %), sometimes only one ratio without the average of the three ratios was used. Also since the ratios of the three sets were pretty similar, the effect of different acyl chain (normal, monohydroxy and dihydroxy fatty acids) on the cleavage efficiency were pretty similar. Therefore the estimation of the ratio of regioisomers did not include the correction of the cleavage efficiency. Since no regiospecific standards of triacylglycerols and tetraacylglycerols containing hydroxy fatty acids were available, no calibration curve was made for this estimation. According to the three sets of the similar ratios, the estimated ratios in Table 1 were representative and the accuracy might be at about ±10 %.

Figure 4 shows the abundance of [diOH18:0 + Li − H_2O]$^+$ at *m/z* 305.1, a dehydrated fragment ion from [diOH18:0 + Li]$^+$ at *m/z* 323.2 also shown in Fig. 4. This fragment ion at *m/z* 305.1 is different from [R + Li]$^+$ at *m/z* 305.1 in Figs. 2 and 3, because the precursor ion was [M + Li − R]$^+$ at *m/z* 641.4 and there was no ricinoleate chain in the precursor ion. The lithium adducts of the other two fatty acids are shown in Fig. 4, as [diOH18:0 + Li]$^+$ at *m/z* 323.2 and [L + Li]$^+$ at *m/z* 287.1. The lithium adducts of the two fatty acids are also shown as [L + Li]$^+$ at *m/z* 287.1 and [R + Li]$^+$ at *m/z* 305.1 in Fig. 2, and [R + Li]$^+$ at *m/z* 305.1 and [diOH18:0 + Li]$^+$ at *m/z* 323.1 in Fig. 3. The dehydrated fragment ion of [R + Li]$^+$ at *m/z* 305.1 is also shown in Fig. 3 as [R + Li − H_2O]$^+$ at *m/z* 287.0. The dehydrated fragment ion from the lithium adduct of dihydroxy fatty acid was much more abundant that that from the lithium adduct of monohydroxy fatty acid such as ricinoleate.

We have identified some tetraacylglycerols in castor oil [7, 19] and they are RRRR (HPLC fractions #35, 36), RRRLn (fraction #45), RRRL (fraction #46, 47), RRRO (fraction #49, 50), RRRP (fraction #49, 50) and RRRS (fraction #52, 53). The ratio of regioisomers of RRRR (AAAA type tetraacylglycerol) with RR (ricinoleoylricinoleoyl chain) and R at the *sn*-2 position was about 95 %:5 % for RRRR as we reported earlier [9]. The estimation of the ratio of regioisomers of RRRO is described here as example for that of other AAAB type tetraacylglycerols. Figure 5 shows the MS^4 spectrum from the precursor ion [RRRO + Li − R − R]$^+$ at *m/z* 607.4 from HPLC fraction #50 (Fig. 1, retention time from 24.5–25.0 min). From the relative abundances of the fragment ions from the losses of fatty acids as α,β-unsaturated fatty acids specific at the *sn*-2 position in Fig. 5, [RRRO + Li − R − R − (RR − R) + 2]$^+$ at *m/z* 329.1, [RRRO + Li − R − R − O + 2]$^+$ at *m/z* 327.1 and [RRRO + Li − R − R − R + 2]$^+$ at *m/z* 311.1, the ratio of three regioisomers of RR, O and R at the *sn*-2 position was 59 %:41 %:0 %. (RR − R) is the mass after the neutral loss of ricinoleate from ricinoleoylricinoleoyl chain.

We recently reported that oleoyl chain of RRRO was directly attached on the glycerol backbone and no oleoylricinoleoyl (OR) chain was detected [7] on RRRO. Because in the Fig. 9 of reference 7, a large abundance of

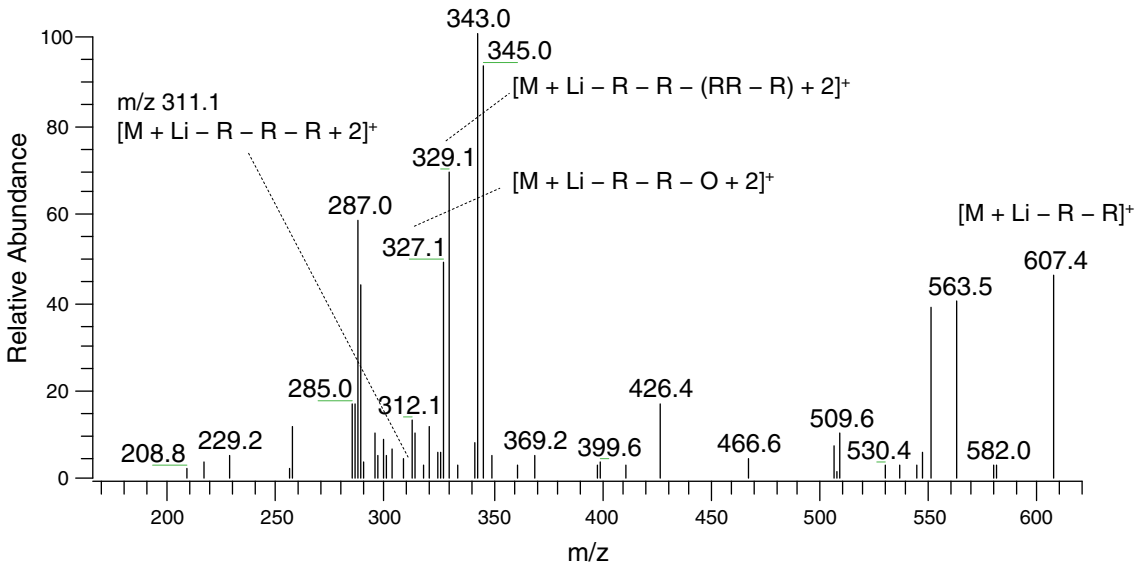

Fig. 5 The MS^4 spectrum of $[RRRO + Li - R - R]^+$ at *m/z* 607.4 from the HPLC fraction #50 of castor oil. The precursor ion was from the MS^2 of $[RRRO + Li]^+$ at *m/z* 1203.8 and MS^3 of $[RRRO + Li - R]^+$ at *m/z* 905.6. The fragment ion $[RRRO + Li - R - R - R'CH{=}C{=}O]^+$ was also at *m/z* 327.1 but its abundance was negligible according to the MS^4 of RRRS

$[RR + Li]^+$ at *m/z* 585.3 in the MS^2 spectrum from $[RRRO + Li]^+$ at *m/z* 1203.8 was shown and no fragment ion of $[OR + Li]^+$ at *m/z* 569.3 was detected. We also proposed that RRRO was biosynthesized from RRO [7]. The ratio of the regioisomers of RRO with O and R at the *sn*-2 position was 91 %:9 % [8]. The ratio of the three regioisomers of RRRO with RR, O and R at the *sn*-2 position was 59 %:41 %:0 %. Apparently the attachment of the third ricinoleoyl chain to the hydroxyl group of the ricinoleoyl chain at the *sn*-2 position was preferred then from those at the *sn*-1,3 positions and the ricinoleoyl chain at the *sn*-2 position of RRO was used up for the attachment. Other tetraacylglycerols identified showed the similar pattern. The RR chain was predominately at the *sn*-2 position and R was not detected at the *sn*-2 position.

The estimation of the ratios of the regioisomers of AAB type acylglycerols is easier than those of the last two examples (ABC and AAAB types). Figure 6 is the MS^3 spectrum of $[RRA + Li - R]^+$ at *m/z* 655.4 from the HPLC fraction #42 of castor oil. A is arachidic acid (20:0). The precursor ion was from the MS^2 of $[RRA + Li]^+$ at *m/z* 953.7. The relative abundances of the fragment ions from the neutral losses of fatty acids as α,β-unsaturated fatty acids at the *sn*-2 position, $[RRA + Li - R - A + 2]^+$ at *m/z* 345.0 and $[RRA + Li - R - R + 2]^+$ at *m/z* 359.0, were used to estimate the ratio of regioisomers of RAR and RRA as 95:5.

The MS^1 of HPLC fraction #17 (Fig. 1, retention time 8.0–8.5 min) showed the molecular ions of $[RR\text{-}OH18{:}2 + Li]^+$ at *m/z* 937.6 and $[RRR + Li]^+$ at *m/z* 939.6. The MS^2 of $[RR\text{-}OH18{:}2 + Li]^+$ at *m/z* 937.6 showed only the two significant fragment ions of $[RR\text{-}OH18{:}2 + Li - R]^+$ at *m/z* 639.4 and $[RR\text{-}OH18{:}2 + Li - OH18{:}2]^+$ at *m/z* 641.4. Figure 7 is the MS^3 of $[RR\text{-}OH18{:}2 + Li - R]^+$ at *m/z* 639.3. The fragment ions from the neutral losses of fatty acids as α,β-unsaturated fatty acids at the *sn*-2 position were $[RR\text{-}OH18{:}2 + Li - R - OH18{:}2 + 2]^+$ at *m/z* 345.0 and $[RR\text{-}OH18{:}2 + Li - R - R + 2]^+$ at *m/z* 343.0. The relative abundances of these two fragment ions in Fig. 7 were used to estimate the ratio of the regioisomers of RR-OH18:2. However the fragment ion at *m/z* 343.0 also include the fragment ion $[RR\text{-}OH18{:}2 + Li - R - OH18{:}2]^+$ at *m/z* 343.0. This complicated the estimation of the ratio of the regioisomers of RR-OH18:2 because of the same *m/z* value of the fragment ions of $[AAB + Li - A - A + 2]^+$ and $[AAB + Li - A - B]^+$. Usually the *m/z* of the fragment ions of $[AAB + Li - A - A + 2]^+$ and $[AAB + Li - A - B]^+$ were not the same and the relative abundance of $[AAB + Li - A - B]^+$ varies [8–12]. The ratio of regioisomers RR-OH18:2 and R-OH18:2-R (with R and OH18:2 at the *sn*-2 position) was about 1:1 with the assumption that the ratio of the abundances of fragment ions $[RR\text{-}OH18{:}2 + Li - R - OH18{:}2 + 2]^+$ at *m/z* 345.0 and $[RR\text{-}OH18{:}2 + Li - R - OH18{:}2]^+$ at *m/z* 343.0 was about 1:1 and the ratio of the abundances of fragment ions $[RR\text{-}OH18{:}2 + Li - R - R + 2]^+$ at *m/z* 343.0 and $[RR\text{-}OH18{:}2 + Li - R - R]^+$ at *m/z* 341.0 was also about 1:1.

RRN, triacylglycerols containing two ricinoleate and one normal fatty acid (non-hydroxylated), in Table 1 (e.g., RR-20:2, RR-20:1, RRA, RRB and RR-23:0) shows that the regioisomers with normal fatty acids at the *sn*-2

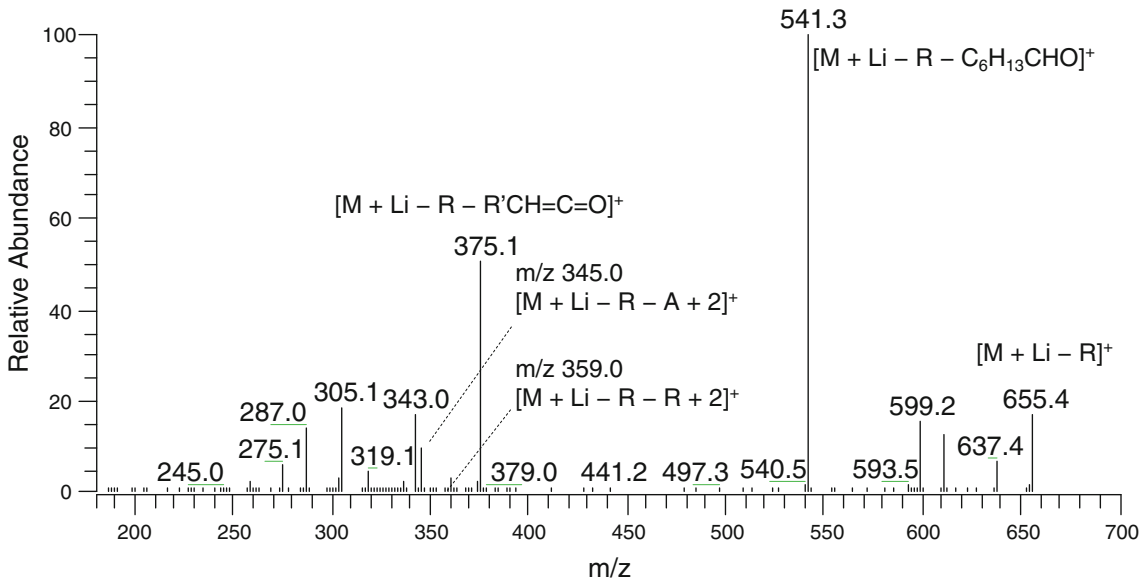

Fig. 6 The MS^3 spectrum of $[RRA + Li - R]^+$ at *m/z* 655.4 from the HPLC fraction #42 of castor oil. The precursor ion was from the MS^2 of $[RRA + Li]^+$ at *m/z* 953.7. For abbreviations, see Figs. 2 and 3. *A* is arachidic acid (20:0)

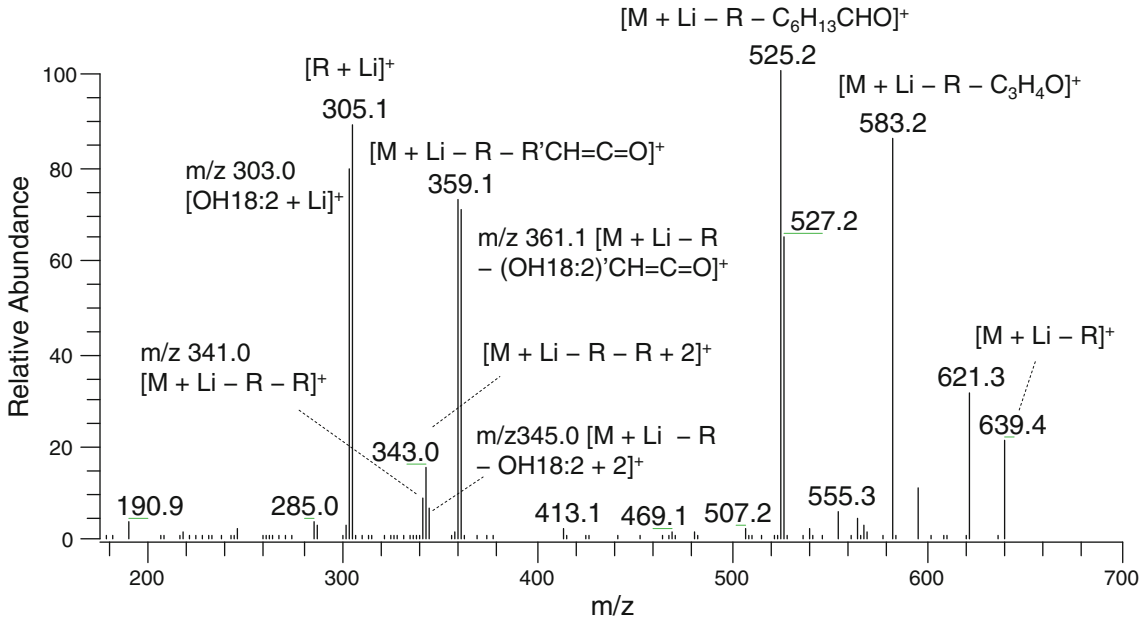

Fig. 7 The MS^3 spectrum of $[RR\text{-}OH18{:}2 + Li \setminus OH18{:}1]^+$ at *m/z* 639.3 from the HPLC fraction #17 of castor oil. The precursor ion was from the MS^2 of $[RR\text{-}OH18{:}2 + Li]^+$ at *m/z* 937.6. For abbreviations, see Figs. 2 and 3. (OH18:2)'CH=C=O is a ketene from OH18:2 at the *sn*-1,3 position

position were predominated (more than 90 %) among the two regioisomers. This agreed with our previous report of RRO, RRL, RRLn and RRS (more than 90 %), while RRP was 78 % [8]. There are many examples of ABC (three regioisomers) and AAB (two regioisomers) types of triacylglycerols containing hydroxy fatty acids in Table 1 showing the predominate regioisomers with normal fatty acids at the *sn*-2 position. Among them, 24 were above 90 % and three were below 90 %, e.g., RL-diOH18:0 (89 %), RP-diOH18:1 (61 %), RLL (85 %). Comparing between ricinoleate and dihydroxy fatty acids among the five triacylglycerols also containing normal fatty acids

listed on the top of Table 1, ricinoleate is more common at the *sn*-2 position than dihydroxy fatty acids. This agreed with our previous report among the six triacylglycerols containing no normal fatty acid [10]. Trihydroxy fatty acids were almost 100 % at the *sn*-1,3 positions in the four triacylglycerols reported recently [11]. The order of the contents of regioisomers of triacylglycerols with the fatty acids at the *sn*-2 position are: nonhydroxy fatty acids > monohydroxy fatty acids > dihydroxy fatty acids > trihydroxy fatty acids.

References

1. Achaya KT, Craig BM, Youngs CG (1964) The component fatty acids and glycerides of castor oil. J Am Oil Chem Soc 41:783–784
2. Lin JT, Turner C, Liao LP, McKeon TA (2003) Identification and quantification of the molecular species of acylglycerols in castor oil by HPLC using ELSD. J Liq Chromatogr Relat Technol 26:773–780
3. Lin JT, Arcinas A, Harden AH (2009) Identification of acylglycerols containing dihydroxy fatty acids in castor oil by mass spectrometry. Lipids 44:359–365
4. Lin JT, Chen GQ (2010) Acylglycerols containing trihydroxy fatty acids in castor oil and the regiospecific quantification of triacylglycerols. J Am Oil Chem Soc 87:1371–1379
5. Lin JT, Chen GQ (2011) Identification of diacylglycerol and triacylglycerol containing 11,12,13-trihydroxy-9,14-octadecadienoic acid in castor oil. New Biotechnol 28:203–208
6. Foubert I, Dewettinck K, Van de Walle D, Dijkstra AJ, Quinn PJ (2007) Physical properties: structural and physical characteristics. In: Gunstone FD, Harwood JL, Dijkstra AJ (eds) The lipid handbook, 3rd edn. Chapter 8. CRC Press, Taylor & Francis Group, Boca Raton, pp 535–590
7. Lin JT, Chen GQ (2012) Identification of minor acylglycerols less polar than triricinolein in castor oil by mass spectrometry. J Am Oil Chem Soc. doi:10.1007/s11746-012-2082-2
8. Lin JT, Arcinas A (2007) Regiospecific analysis of diricinoleoylacylglycerols in castor (*Ricinus communis* L.) oil by electrospray ionization-mass spectrometry. J Agric Food Chem 55:2209–2216
9. Lin JT, Arcinas A (2008) Regiospecific identification of 2-(12-ricinoleoylricinoleoyl)-1,3-diricinoleoyl-*sn*-glycerol in castor (*Ricinus communis* L.) oil by ESI-MS^4. J Agric Food Chem 56:3616–3622
10. Lin JT (2009) Ratios of regioisomers of triacylglycerols containing dihydroxy fatty acids in castor oil by mass spectrometry. J Am Oil Chem Soc 86:1031–1035
11. Lin JT, Chen GQ (2010) Acylglycerols containing trihydroxy fatty acids in castor oil and the regiospecific quantification of triacylglycerols. J Am Oil Chem Soc 87:1371–1379
12. Lin JT, Arcinas A (2008) Analysis of regiospecific triacylglycerols by electrospray ionization-mass spectrometry^3 of lithiated adducts. J Agric Food Chem 56:4909–4915
13. Leskinen HM, Suomela J, Kallio HP (2010) Quantification of triacylglycerol regioisomers by ultra-high-performance liquid chromatography and ammonia negative ion atomospheric pressure chemical ionization tandem mass spectrometry. Rapid Commun Mass Spectrom 24:1–5
14. Leskinen H, Suomela J-P, Kallio H (2007) Quantification of triacylglycerol regioisomers in oils and fat using different mass spectrometric and liquid chromatographic methods. Rapid Commun Mass Spectrom 21:2361–2373
15. Jakab A, Jablonkai I, Forgacs E (2003) Quantification of the ratio of positional isomer dilinoleoyl-oleoyl glycerols in vegetable oils. Rapid Commun Mass Spectrom 17:2295–2302
16. Byrdwell WC (2005) The bottom-up solution to the triacylglycerol lipidome using atmospheric pressure chemical ionization mass spectrometry. Lipids 40:383–417
17. Hsu FF, Turk J (1999) Structural characterization of triacylglycerols as lithiated adducts by electrospray ionization mass spectrometry using low-energy collisionally activated dissociation on a triple stage quadrupole instrument. J Am Soc Mass Spectrom 10:587–599
18. Hsu FF, Turk J (2010) Electrospray ionization multiple-stage linear ion-trap mass spectrometry for structural elucidation of triacylglycerols: assignment of fatty acyl groups on the glycerol backbone and location of double bond. J Am Soc Mass Spectrom 21:657–669
19. Lin JT, Arcinas A, Harden LR, Fagerquist CK (2006) Identification of (12-ricinoleoylricinoleoyl)diricinoleoylglycerol, an acylglycerol containing four acyl chains, in castor oil by LC-ESI-MS. J Agric Food Chem 54:3498–3504