JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Regiospecific Identification of 2-(12-Ricinoleoylricinoleoyl)-1,3-diricinoleoyl-*sn*-glycerol in Castor (*Ricinus communis* L.) Oil by ESI-MS^4

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(12-Ricinoleoylricinoleoyl)diricinoleoylglycerol (RRRR), a tetraacylglycerol, was identified earlier in castor oil. Using ESI-MS^4, 95% of the 12-ricinoleoylricinoleoyl chain was identified at the *sn*-2 position of the glycerol backbone of RRRR. Regiospecific location of the 12-ricinoleoylricinoleoyl chain of RRRR on the glycerol backbone was identified and quantified by the ions from the losses of the acyl chains at the *sn*-2 position as α,β-unsaturated fatty acids from the lithium adduct of RRRR. The regiospecific location was confirmed by hydrolysis of RRRR using *sn*-1,3 specific lipase. By comparison to the mass spectrum of 1-*O*-palmityl-2,3-palmitoyl-*rac*-glycerol containing one ether bond, the 12-ricinoleoylricinoleoyl chain of RRRR is indeed the ester bond between the two ricinoleoyl chains, not the ether bond formed from the two hydroxyl groups of the two ricinoleoyl chains. The structure of RRRR is 2-(12-ricinoleoylricinoleoyl)-1,3-diricinoleoyl-*sn*-glycerol.

KEYWORDS: Castor oil; ricinoleate; 2-(12-ricinoleoylricinoleoyl)-1,3-diricinoleoyl-*sn*-glycerol; tetraricinolein; tetraacylglycerol; regiospecific; 1-*O*-palmityl-2,3-palmitoyl-*rac*-glycerol; ESI-MS; lipase; *Ricinus communis* L.

INTRODUCTION

Ricinoleate, a hydroxyl fatty acid (FA), has many industrial uses such as the manufacture of aviation lubricant, plastic, paint, and cosmetics. Ricinoleate occurs as acylglycerol (AG) in castor oil, and about 70% of castor oil is triricinolein (triricinoleoylglycerol, RRR) (*1*). We have recently identified (12-ricinoleoylricinoleoyl)diricinoleoylglycerol (RRRR, **Figure 1**), a tetraacylglycerol, in castor oil (*2*) by high-resolution ESI-MS^2 of sodium adduct. RRRR was the first positively identified tetraacylglycerol. The biosynthesis of RRRR was proven the first time among the proposed tetraacylglycerols (*2*). The content of RRRR in castor oil is about 0.5% (*2*). Castor oil is the only commercial source of ricinoleate. However, castor bean contains the toxin, ricin, and potent allergens, which make it hazardous to grow, harvest, and process. It would be desirable to produce ricinoleate from a transgenic oilseed lacking these toxic components. The biosynthetic pathway of RRR and RRRR in castor bean has been reported, and the key enzymatic steps driving ricinoleate into RRR and RRRR have been identified (*3, 4*). This information can be used to develop transgenic plants that produce seed oil containing RRR and RRRR without the aforementioned toxic substances. With the identification of RRRR in castor oil, it is desirable to overproduce RRRR in transgenic seed because of its higher ricinoleate content compared to that of RRR. The intact RRRR has different mass, size, and physical properties, for example, viscosity and pour point, compared to RRR and other triacylglycerols, accounting for its potential utility in industry, for example, lubricants, polymers, plastics, paints, and cosmetics (*5*).

Regiospecific location of the non-ricinoleoyl chain on the glycerol backbone of the molecules of diricinoleoyl-acylglycerols in castor oil was recently identified using electrospray ionization−MS^3 of lithium adducts (*3*). The content of 1,3-diricinoleoyl-2-oleoyl-*sn*-glycerols (ROR) among the three stereospecific isomers, RRO, ROR, and ORR, combined was about 91%. The contents of other 1,3-diricinoleoyl-2-acylglycerols among the three stereospecific isomers combined were as follows: 1,3-diricinoleoyl-2-linoleoyl-*sn*-glycerol, 95%; 1,3-diricinoleoyl-2-linolenoyl-*sn*-glycerol, 96%; 1,3-diricinoleoyl-2-stearoyl-*sn*-glycerol, 96%; 1,3-diricinoleoyl-2-palmitoyl-*sn*-glycerol, 78%; and 1,3-diricinoleoyl-2-lesqueroloyl-*sn*-glycerol, 31%. These nonhydroxyl normal fatty acids were predominately at the *sn*-2 position of triacylglycerols in castor oil. Transgenic inhibition of phospholipase C hydrolysis of phosphatidylcholine might be used to block the incorporation of nonhydroxyl normal fatty acids into triacylglycerols, thus increasing the content of ricinoleate in seed oil. The regiospecific identification is important in the study of the lipid biosynthesis. The regiospecific location of the 12-ricinoleoylricinoleoyl chain on the glycerol backbone of RRRR is reported here.

In our earlier identification of RRRR (*2*), high-resolution ESI-MS^2 of the sodium adduct of RRRR used could not exclude the possibility that the ether bond was formed from the two

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10.1021/jf072750z
Published on Web 04/29/2008

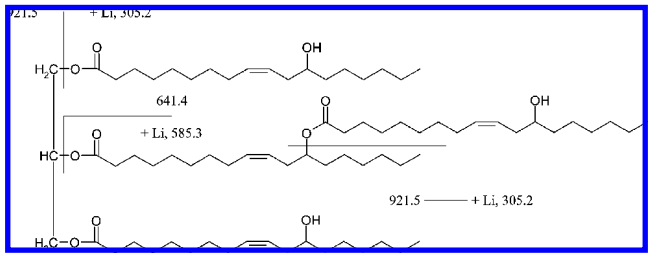

Figure 1. Structure of 2-(12-ricinoleoylricinoleoyl)-1,3-diricinoleoyl-*sn*-glycerol (RRRR, associated with Li^+). The fragment ions shown were those of the ESI-MS^2 of $[RRRR + Li]^+$ at 1219.6 of **Figure 2**, except *m*/*z* at 305.2. The calculated exact mass of monoisotopic $[M + Li]^+$ is 1220.02; the exact mass of RRRR is 1213.01.

hydroxyl groups of the two ricinoleoyl chains on the 12-ricinoleoylricinoleoyl chain of RRRR. In this paper we exclude that possibility.

EXPERIMENTAL PROCEDURES

Mass Spectrometry. An LCQ Advantage quadrupole ion-trap mass spectrometer with Xcalibur 1.3 software (ThermoFinnigan, San Jose, CA) was utilized for MS analysis of the RRRR fraction collected from castor oil as previously reported (*2*). Direct infusion of 200 μL samples with 20 mM lithium acetate in methanol containing approximately 100 μg of RRRR into the MS at a 2.5 μL/min flow rate from a syringe pump produced stable singly charged lithiated parent ions, which were subsequently fragmented for MS^2, MS^3, and MS^4 analysis. ESI source conditions were as follows: 50 arbitrary units (au) nitrogen sheath gas flow rate, 4.5 kV spray voltage, 250 °C capillary temperature, isolation width of 1.5 *m*/*z*, acquisition time of 3 min, capillary voltage of 38 V, normalized collision energy of 34−39% for MS^2, MS^3, and MS^4 fragmentations, activation time 30 of ms, and activation *Q* of 0.25. Research grade (99.999%) helium was used as collision gas (Praxair, Oakland, CA). 1-*O*-Palmityl-2,3-palmitoyl-*rac*-glycerol (Sigma, St. Louis, MO) in dichloromethane (20 mM lithium acetate) was also used for MS analysis.

Hydrolysis of RRRR Using *sn*-1,3 Specific Laipase. Lipase from *Rhizomucor miehei* (L4277 Sigma, *sn*-1,3 specific) was used for hydrolysis of RRRR. Approximately 0.1 mg of RRRR in 25 μL of ethanol was added to lipase stock solution (1 mL), and the mixture was stirred overnight at 25 °C. The lipid was extracted from the mixture by partition between chloroform (10 mL) and water (10 mL), and the chloroform layer was washed twice with 10 mL of water. The lipid extract was chromatographed for purification. HPLC conditions were the same as previously reported (*3*). Retention times of diricinoleoylglycerol (RR), RRR, and RRRR in this purification HPLC were 3.2, 11.6, and 18.6 min, respectively, according to the HPLC of castor oil performed prior to the purification run (*1*). Fractions were combined as 2.5−9, 9−13, and 17−20 min.

RESULTS AND DISCUSSION

The regiospecific location of the 12-ricinoleoylricinoleoyl chain on RRRR was identified by the ions from the losses of the acyl chains at the *sn*-2 position as α,β-unsaturated fatty acids from the lithium adduct of RRRR fraction collected by HPLC. The regiospecific loss from the fatty acid at the *sn*-2 position as an α,β-unsaturated fatty acid was first reported by Hsu and Turk using the lithium adducts of TAG by ESI-MS with low-energy collisionally activated dissociation (CAD) (*6*). **Figure**

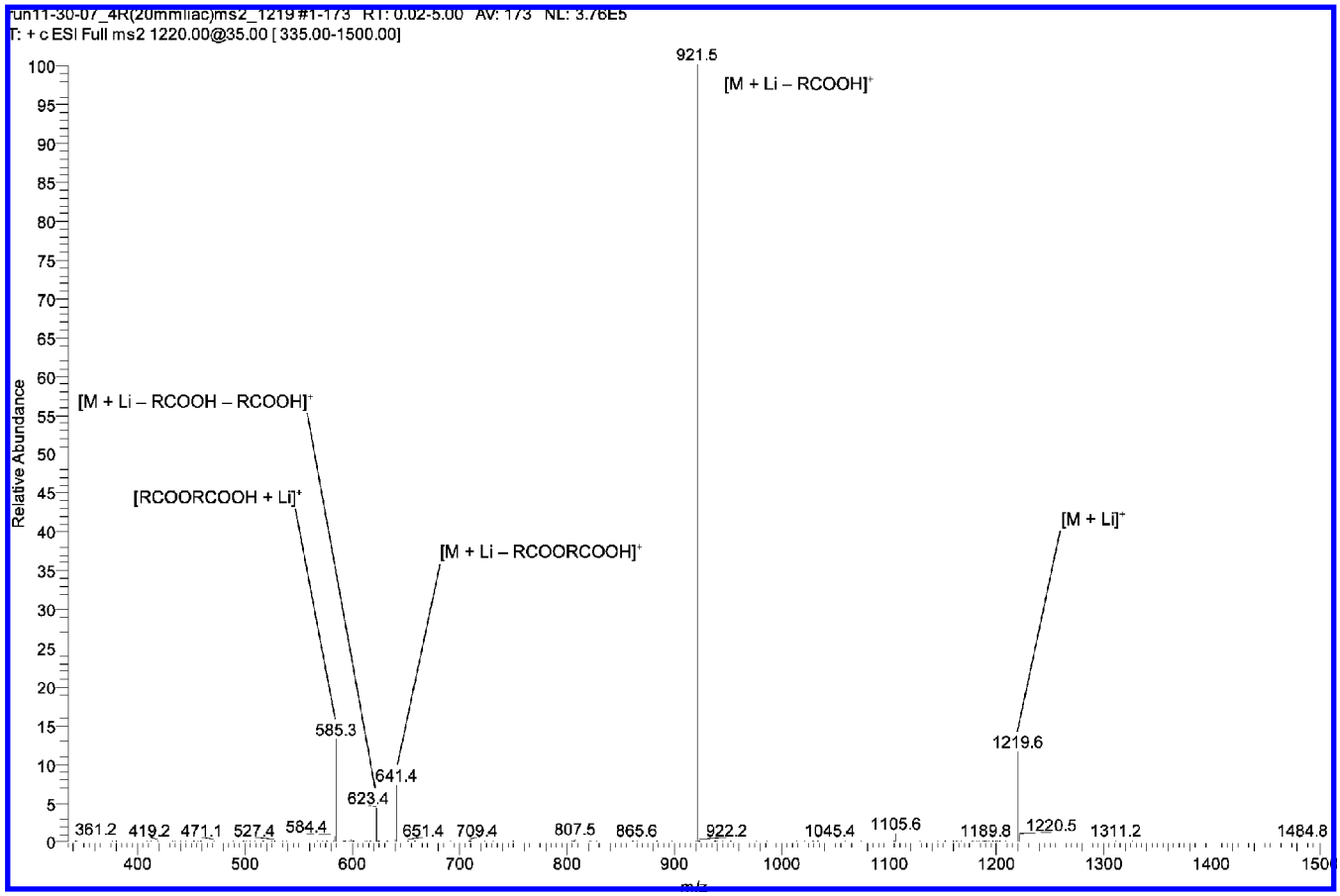

Figure 2. Ion trap mass spectrum of ESI-MS^2 of $[RRRR + Li]^+$ at 1219.6. RRRR is (12-ricinoleoylricinoleoyl)diricinoleoylglycerol. RCOOH is ricinoleic acid. RCOORCOOH is the 12-ricinoleoylricinoleoyl chain of the molecule of RRRR. 12-RCOOH is the ricinoleic acid esterified at the 12-hydroxyl group of ricinoleic acid at the *sn*-2 position. The other possible structure of *m*/*z* 623.4 is $[M + Li - RCOORCOOH - H_2O]^+$, whereas the loss of water is the dehydration of a hydroxyl group at one of the ricinoleates attached on the glycerol backbone.

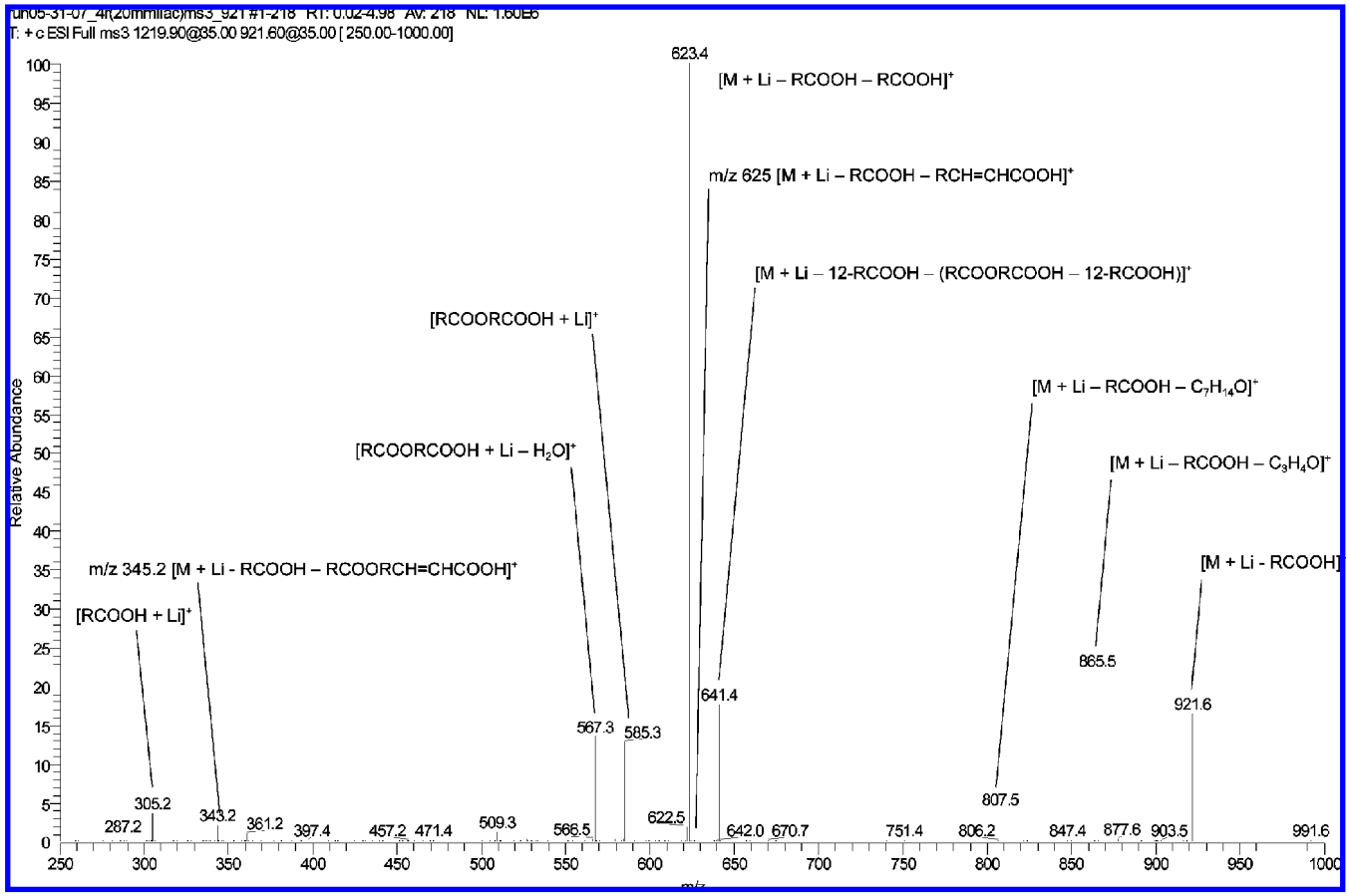

Figure 3. Ion trap mass spectrum of ESI-MS^3 of $[RRRR + Li - RCOOH]^+$ at 921.6. For abbreviations, see **Figure 2**. RCH═CHCOOH is the loss of ricinoleoyl chain at the *sn*-2 position as α,β-unsaturated ricinoleic acid. RCOORCH═CHCOOH is the loss of the ricinoleoylricinoleoyl chain at the *sn*-2 position as ricinoleoyl-α,β-unsaturated-ricinoleoyl chain. $C_7H_{14}O$ is the loss from the cleavage between C-11 and C-12 of the ricinoleoyl chain. C_3H_4O is the loss of glycerol backbone to form acid anhydride from the ricinoleoyl and 12-ricinoleoylricinoleoyl chains. 12-RCOOH is the ricinoleoyl chain attached to another ricinoleoyl chain at the C-12 position to form a 12-ricinoleoylricinoleoyl chain.

2 shows the ion trap mass spectrum of ESI-MS^2 of the lithium adduct of RRRR, $[M + Li]^+$ at *m/z* 1219.6. Similar to that of the sodium adduct of RRRR (*2*), the fragment ions of $[M + Li - RCOOH]^+$ at *m/z* 921.5, of $[M + Li - RCOORCOOH]^+$ at *m/z* 641.4, of $[M + Li - RCOOH - RCOOH]^+$ at *m/z* 623.4, and of $[RCOORCOOH + Li]^+$ at *m/z* 585.3 were shown. The other possible structure of the ion at *m/z* 623.4 was $[M + Li - RCOORCOOH - H_2O]^+$. In this MS^2 spectrum, the diagnostic ions reported by Hsu and Turk (*6*) were not detected, for example, $[M + Li - RCOOH - RCOORCH═CHCOOH]^+$ at *m/z* 345.2, $[M + Li - RCOORCOOH - RCH═CHCOOH]^+$ at *m/z* 345.2, and $[M + Li - RCOOH - RCH═CHCOOH]^+$ at *m/z* 625.5. These diagnostic ions are shown in the MS^3 and MS^4 spectra of **Figures 3**, **4**, and **5**.

In MS^2 studies, the losses of fatty acid from the precursor ion of triacylglycerol adducts were mostly at *sn*-1,3 positions (*7*). **Figure 2** shows that the abundance of $[M + Li - RCOOH]^+$ at *m/z* 921.6 was much larger than that of $[M + Li - RCOORCOOH]^+$ at *m/z* 641.5 with a ratio of 100:7, indicating that the 12-ricinoleoylricinoleoyl chain was predominately at the *sn*-2 position.

However, because part of the precursor ion $[M + Li - RCOOH]^+$ at *m/z* 921.6 in **Figure 2** was from the loss of 12-RCOOH on the 12-ricinoleoylricinoleoyl chain as shown below, the ions $[M + Li - RCOOH]^+$ at *m/z* 921.6 and $[M + Li - RCOORCOOH]^+$ at *m/z* 641.5 together were not suitable for regiospecific quantification as previously reported (*7*).

Figure 3 shows the ion trap mass spectrum of ESI-MS^3 of $[M + Li - RCOOH]^+$ at *m/z* 921.6. The ion $[M + Li - 12\text{-}RCOOH - (RCOORCOOH - 12\text{-}RCOOH)]^+$ at *m/z* 641.4 was from the loss of 12-ricinoleate followed by the loss of remaining ricinoleate on the 12-ricinoleoylricinoleoyl chain alone. The abundance of the ion $[M + Li - 12\text{-}RCOOH - (RCOORCOOH - 12\text{-}RCOOH)]^+$ at *m/z* 641.4 was much less than that of the ion of $[M + Li - RCOOH - RCOOH]^+$ at *m/z* 623.4, because the latter had three potential precursors contributed to it. The precursor ion, $[M + Li - RCOOH]^+$ at *m/z* 921.6, was the mixture from the loss of ricinoleate attached to glycerol and from the loss of ricinoleate attached to another ricinoleate on the 12-ricinoleoylricinoleoyl chain. Unlike triacylglycerols containing only nonhydroxyl normal fatty acids (*6*), the diagnostic ions from the loss of fatty acid at the *sn*-2 position as α,β-unsaturated fatty acids, $[M + Li - RCOOH - RCH═CHCOOH]^+$ at *m/z* 625.5 and $[M + Li - RCOOH - RCOORCH═CHCOOH]^+$ at *m/z* 345.2, were not detected. Therefore, MS^3 of $[M + Li - RCOOH]^+$ at *m/z* 921.6 as shown in **Figure 3** cannot be used for regiospecific identification. The loss of 114 ($C_7H_{14}O$) as the ion of $[M + Li - RCOOH - C_7H_{14}O]^+$ at *m/z* 807.5 from the cleavage between C-11 and C-12 of the ricinoleoyl chain has previously been reported for TAG-containing ricinoleate (*3, 8*). The ion $[M + Li - RCOOH - C_3H_4O]^+$ at *m/z* 865.5 in **Figure 3** was from the loss of the glycerol backbone to form acid anhydride. The fragmentation pathway to form acid anhydride was recently proposed (*3*).

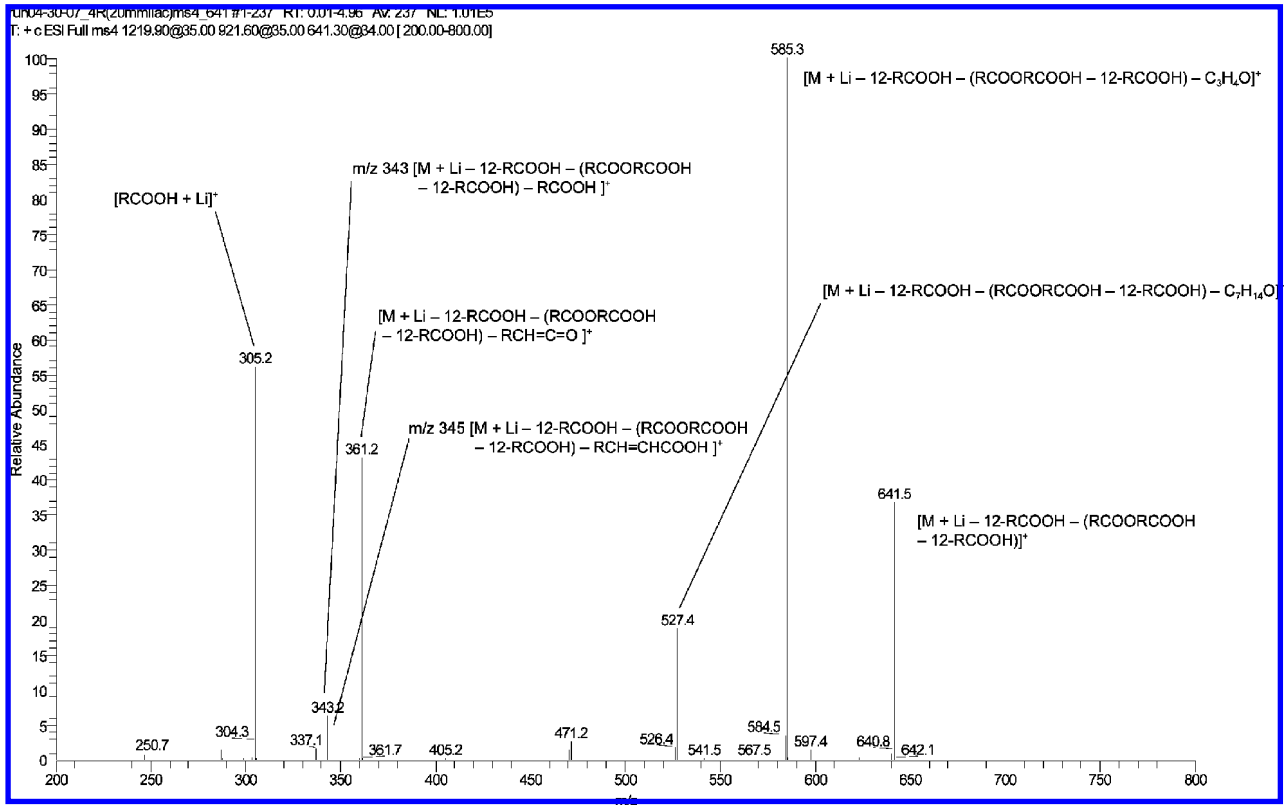

Figure 4. Ion trap mass spectrum of ESI-MS^4 of [RRRR + Li − 12-RCOOH − (RCOORCOOH − 12-RCOOH)]$^+$ at 641.5. For abbreviations, see **Figures 2** and **3**. RCH═C═O is ricinoleoyl ketene.

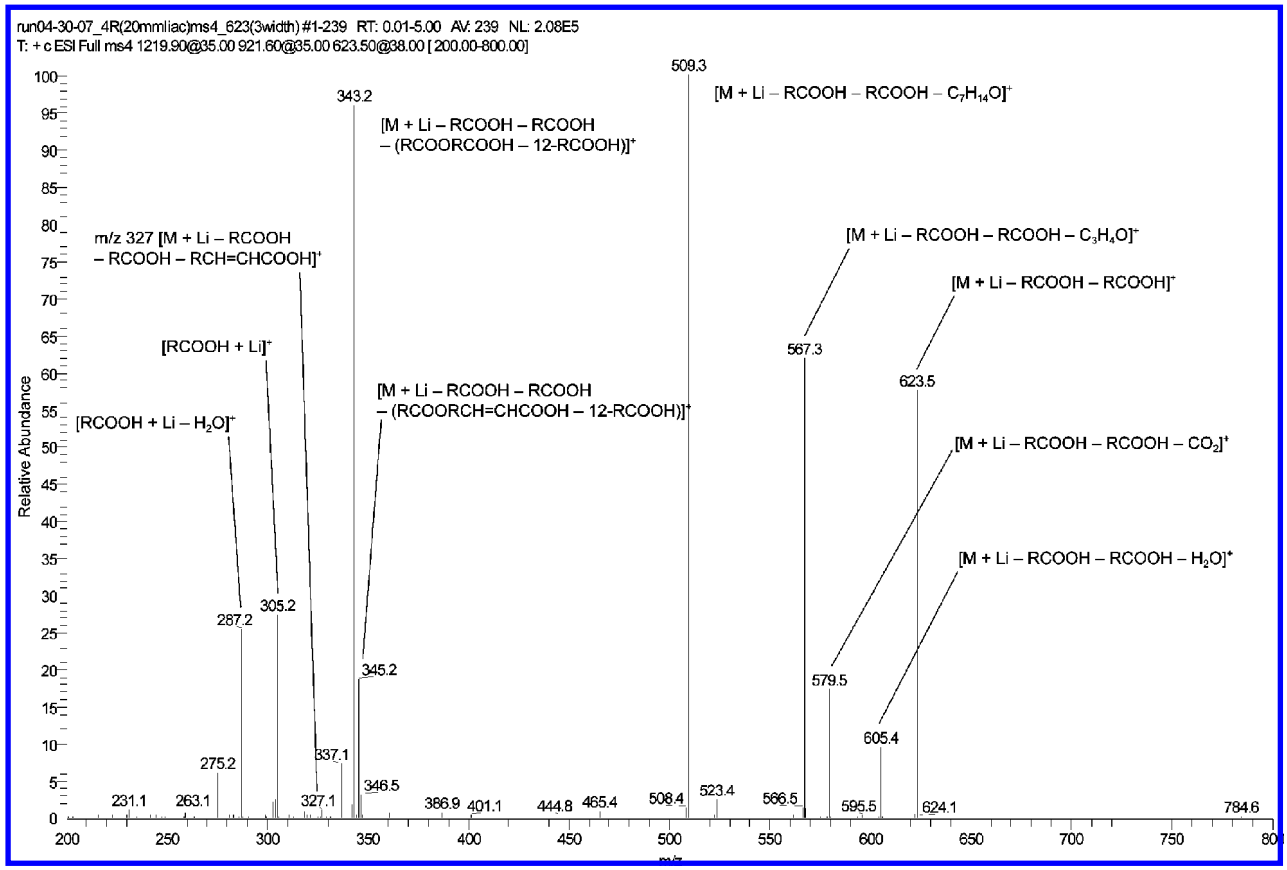

Figure 5. Ion trap mass spectrum of ESI-MS^4 of [RRRR + Li − RCOOH − RCOOH]$^+$ at 623.5. For abbreviations, see **Figures 1**−**3**. (RCOORCH═CHCOOH – 12-RCOOH) is the chain after the loss of ricinoleoyl chain from the 12-ricinoleoylricinoleoyl chain as α,β-unsaturated-ricinoleoyl chain at the *sn*-2 position.

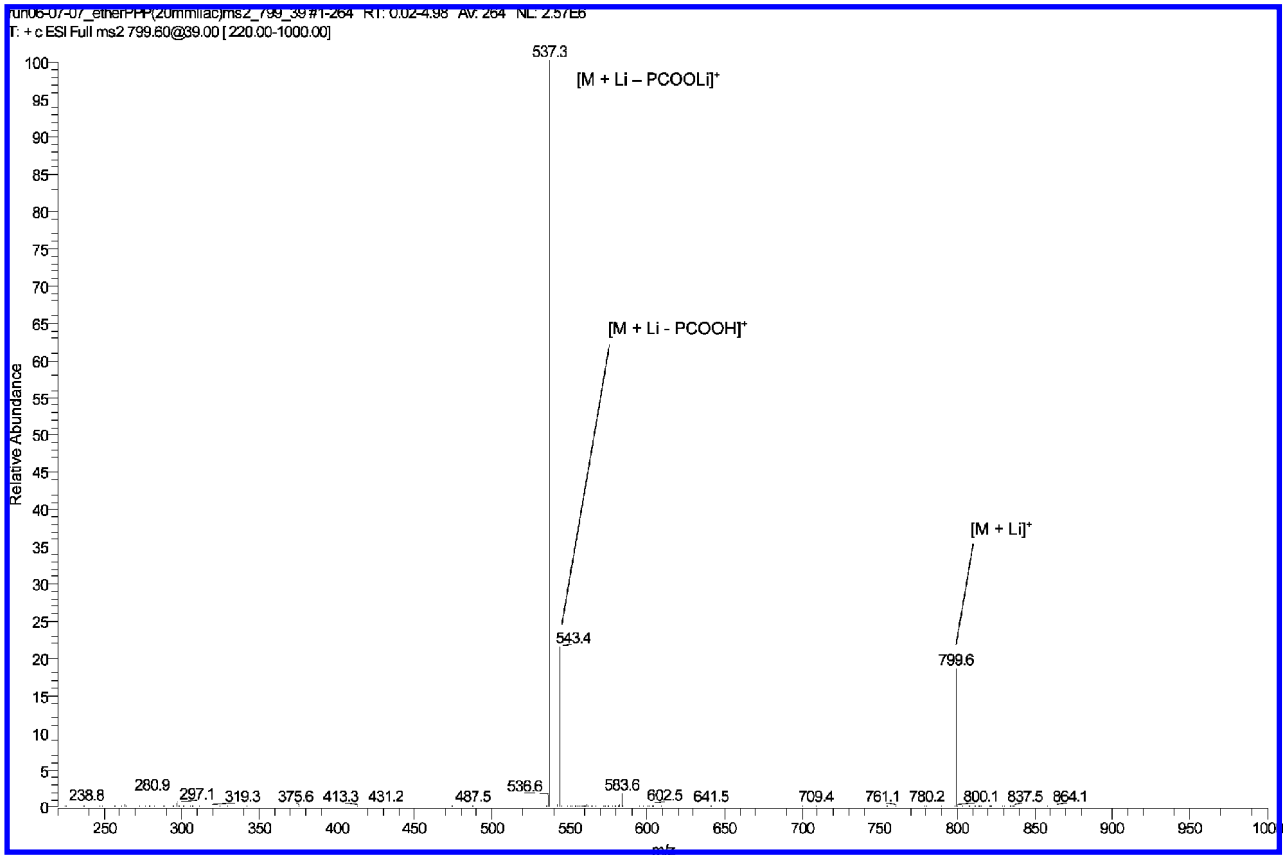

Figure 6. Ion trap mass spectrum of ESI-MS^2 of [aPPP + Li]$^+$ at 799.6. aPPP is 1-*O*-palmityl-2,3-palmitoyl-*rac*-glycerol. PCOOH is palmitic acid.

Figure 3 shows the abundance of [RCOORCOOH + Li]$^+$ at *m/z* 585.3 and [RCOORCOOH + Li − H_2O]$^+$ at *m/z* 567.3 with the absence of [RCOORCH═CHCOOH + Li]$^+$ at *m/z* 583.3. Similar to **Figures 6** and **7** in our earlier paper on 1,3-diricinoleoyl-2-linoleoyl-glycerol (RLR) and 1,3-diricinoleoyl-2-linolenoyl-glycerol RLnR from castor oil (*3*), an abundance of linoleic and linolenic lithium adducts was detected but not α,β-unsaturated linoleic and linolenic lithium adducts. The presence of α,β-unsaturated oleic lithium adduct in **Figure 3** of our earlier paper (*3*) was shown at *m/z* 287.2; however, it should be labeled [RCOOH + Li − H_2O]$^+$, not [OCH═CHCOOH + Li]$^+$. The presence of α,β-unsaturated fatty acid lithium adducts was expected for triacylglycerols containing only nonhydroxyl normal fatty acids (*6*), but not for the triacylglycerols containing ricinoleate (*3*).

Figure 4 shows the ion trap mass spectrum of ESI-MS^4 of [M + Li − 12-RCOOH − (RCOORCOOH − 12-RCOOH)]$^+$ at *m/z* 641.5. The ion [M + Li − 12-RCOOH − (RCOORCOOH − 12-RCOOH) − RCH═CHCOOH]$^+$ at *m/z* 345 from the loss of ricinoleate at the *sn*-2 position as α,β-unsaturated ricinoleate was low. This meant the content of 1-(12-ricinoleoylricinoleoyl)-2,3-diricinoleoyl-*sn*-glycerol (1-RRRR) and 3-(12-ricinoleoylricinoleoyl)-1,2-diricinoleoyl-*sn*-glycerol (3-RRRR) combined was detected and low. The ion trap mass spectrum of ESI-MS^3 of [M + Li − RCOORCOOH]$^+$ at *m/z* 641.5 in **Figure 2** (not reported) was similar to **Figure 4**, because the precursor ions were the same.

Figure 5 shows the ion trap mass spectrum of ESI-MS^4 of [M + Li − RCOOH − RCOOH]$^+$ at *m/z* 623.5. The ions from the loss of fatty acid at the *sn*-2 position as α,β-unsaturated fatty acids were [M + Li − RCOOH − RCOOH − (RCOORCH═CHCOOH − 12-RCOOH)]$^+$ at *m/z* 345.2 and [M + Li − RCOOH − RCOOH − RCH═CHCOOH]$^+$ at *m/z* 327.1. (RCOORCH═CHCOOH − 12-RCOOH) was the α,β-unsaturated ricinoleoyl chain at the *sn*-2 position after the loss of ricinoleoyl chain from the 12-ricinoleoylricinoleoyl chain. The ion at *m/z* 345.2 was from 2-(12-ricinoleoylricinoleoyl)-1,3-diricinoleoyl-*sn*-glycerol (2-RRRR), whereas the ion at *m/z* 327.1 was from 1-(12-ricinoleoylricinoleoyl)-2,3-diricinoleoyl-*sn*-glycerol (1-RRRR) and 3-(12-ricinoleoylricinoleoyl)-1,2-diricinoleoyl-*sn*-glycerol (3-RRRR) combined. We assume a linear relationship of the abundances of these two ions and the contents of these isomers. According to the relative abundances of these two ions, the content of 2-RRRR among the three isomers combined was 95% (or >95%). Because the relative abundance of the ion *m/z* at 327.1 shown in **Figure 5** was very low and was close to the background, the content could be >95%.

It is interesting that the content of 2-RRRR among the three isomers was similar to the content of 1,3-diricinoleoyl-2-acylglycerol (RAcR) containing one nonhydroxyl fatty acid (*3*) in castor oil. It opened the possibility that the biosynthesis of 2-RRRR is similar to that of RAcR involving the steps of 1,2-diricinoleoyl-glycerol-3-phophocholine to 1-ricinoleoyl-2-ricinoleoylricinoleoyl-glycerol-3-phosphocholine, phospholipase C, and then diacylglycerolacyltransferase to RRRR. Radiolabeled triricinolein (RRR) was used earlier in a castor microsomal incubation (*2*), and the radiolabel was incorporated into diricinolein (RR), diricinoleoyloleoylglycerol (ROR), diricinoleoyllinoleoylglycerol (RLR), and RRRR. Apparently, lipase hydrolysis was involved in the formation of RR, ROR, and RLR in the incubation. The biosynthesis of RRRR is not clear yet.

RRRR (**Figure 1**) was recently identified by high-resolution ESI-MS^2 of the sodium adduct of the HPLC fraction of castor oil (*2*). The bond of the fourth ricinoleate attached to the hydroxyl group of the other ricinoleoyl chain was an ester bond as shown in **Figure 1**. However, it did not exclude the possibility

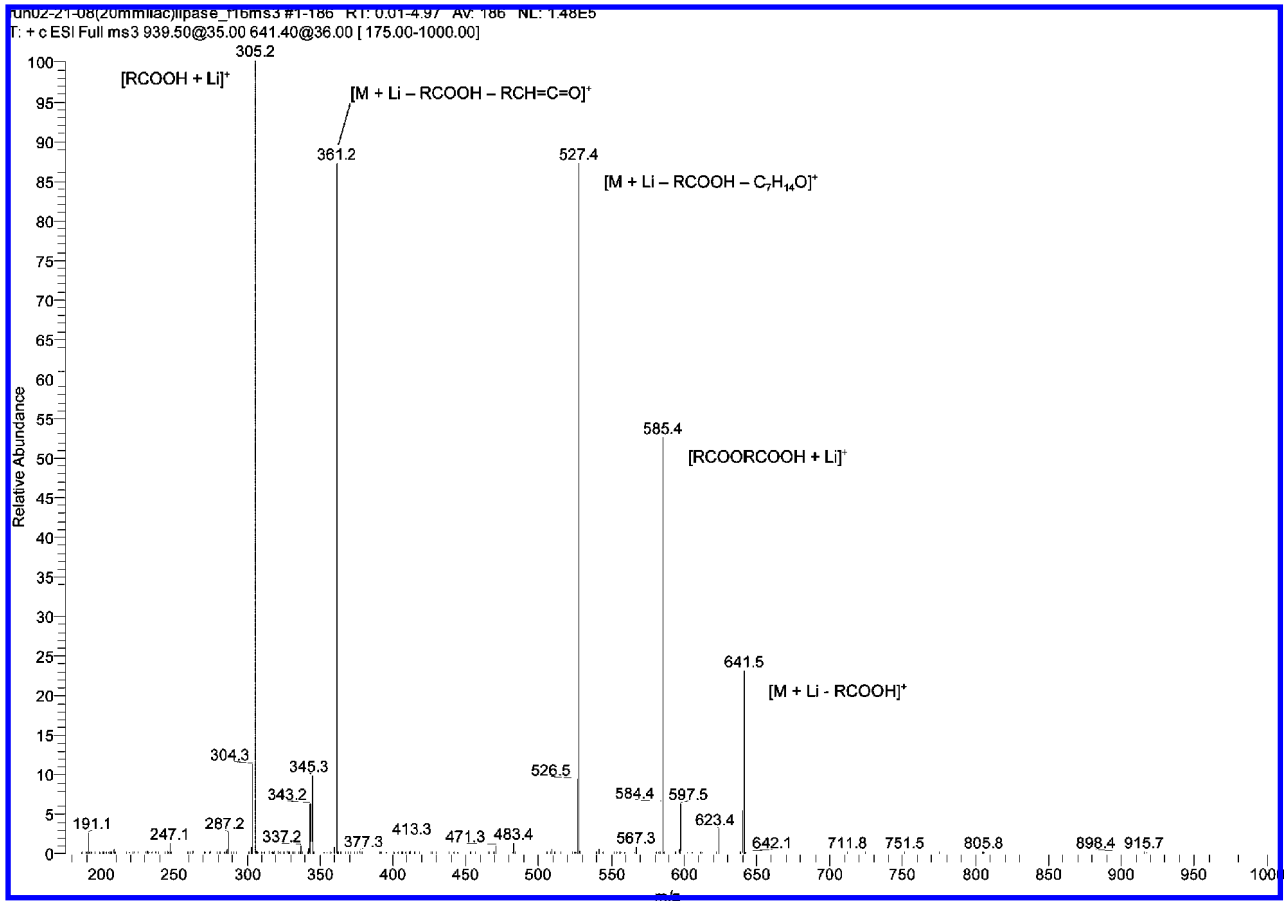

Figure 7. Ion trap mass spectrum of ESI-MS^3 of $[M + Li - RCOOH]^+$ at *m/z* 641.5 from the hydrolyte of RRRR by *sn*-1,3 specific lipase. M is 1-ricinoleoyl-2-ricinoleoylricinoleoyl-glycerol. For abbreviations, see **Figures 1**−**4**.

that the fourth bond was an ether bond between the two hydroxyl groups of the two ricinoleoyl chains, because the masses of the fragment ions of the ester and ether were the same. We assumed the aforementioned bond was an ester bond. We would like to prove that the fourth bond of RRRR was indeed an ester bond as shown in **Figure 1**.

Figure 6 shows the ion trap mass spectrum of ESI-MS^2 of the lithium adduct of 1-*O*-palmityl-2,3-palmitoyl-*rac*-glycerol (alkylPPP or aPPP), $[M + Li]^+$ at *m/z* 799.6. Two prominent fragment ions are shown in **Figure 6** as $[M + Li - PCOOH]^+$ at *m/z* 543.4 and $[M + Li - PCOOLi]^+$ at *m/z* 537.3. Ions from the neutral loss of lithium salt, for example, PCOOLi in **Figure 6**, were common in the ESI-MS of the lithium adducts of triacylglycerols containing only nonhydroxyl normal fatty acids (*6*). However, they were very rare for the triacylglycerols containing ricinoleate (*3*), for example, $[M + Li - RCOOH - RCOOH - (RCOORCOOLi - RCOOH)]^+$ at *m/z* 337.1 in **Figure 5**. The loss of palmityl chain at the ether bond as $[M + Li - POH]^+$ at *m/z* 557.3 was not detected. In this ion trap mass spectrometry condition, the fragment ion from the loss of alcohol at the ether bond (*9*) was impossible, whereas the loss of acid at the ester bond was common.

Figure 3 shows the ion $[M + Li - 12\text{-}RCOOH - (RCOORCOOH - 12\text{-}RCOOH)]^+$ at *m/z* 641.4 from the initial loss of 12-RCOOH. Because the ether bond could not be broken in this condition, whereas the ester bond could be, the 12-ricinoleoylricinoleoyl bond must be the ester bond. In addition, there were many ions shown in **Figures 4** and **5** from the loss of 12-RCOOH from 12-ricinoleoyricinoleoyl chain, for example, $[M + Li - RCOOH - RCOOH - (RCOORCOOH - 12\text{-}RCOOH)]^+$ at *m/z* 343.2 and $[M + Li - RCOOH - (RCOORCH{=}CHCOOH - 12\text{-}RCOOH)]^+$ at *m/z* 345.2 in **Figure 5**.

We would like to confirm that the 12-ricinoleoylricinoleoyl chain is predominately attached at the *sn*-2 position of RRRR using *sn*-1,3 specific lipase. The hydrolyte of RRRR by the lipase was purified by HPLC. The MS^1 spectrum of the hydrolyte (fraction 9–13 min) of RRRR showed the presence of 1-ricinoleoyl-2-ricinoleoylricinoleoyl-glycerol, $[M + Li]^+$ at *m/z* 939.5 (base peak), but could not exclude the possibility of triricinolein $[RRR + Li]^+$, also at *m/z* 939.5. The MS^1 spectrum from the fraction of 17–20 min did not detect RRRR, $[M + Li]^+$ at *m/z* 1219.6, and it showed that the lipase hydrolysis was completed. The MS^1 spectrum from the fraction of 2.5–9 min did not detect 1,2-diricinoleoyl glycerol (RR), $[M + Li]^+$ at *m/z* 659.5, and it suggested that the ricinoleoyl chain was not at the *sn*-2 position of RRRR. The MS^2 spectrum of $[M + Li]^+$ at *m/z* 939.5 showed the only prominent ion $[M + Li - RCOOH]^+$ at *m/z* 641.5 (base peak) and the minor ions (about 1%) of $[RCOORCOOH + Li]^+$ at *m/z* 585.4 and $[RCOOH + Li]^+$ at *m/z* 305.2. **Figure 7** shows the MS^3 spectrum of the prominent ion $[M + Li - RCOOH]^+$ at *m/z* 641.5. The prominent ion $[RCOORCOOH + Li]^+$ at *m/z* 585.4 (**Figure 7**) was from 1-ricinoleoyl-2-ricinoleoylricinoleoyl-glycerol $[M + Li]^+$ at *m/z* 939.5, not from RRR at *m/z* 939.5. The MS^2 spectrum of $[RRR + Na]^+$ at *m/z* 955.76 has been reported (*2*). These MS spectra confirmed that the 12-ricinoleoylricinoleoyl chain is attached at the *sn* -2 position of RRRR in castor oil.

From these ion trap MS studies of the lithium adducts of RRRR, aPPP, and the hydrolyte of RRRR by *sn*-1,3 specific lipase, we have concluded that the 12-ricinoleoylricinoleoyl chain of RRRR was at least 95% at the *sn*-2 position of glycerol

backbone and that the bond between these two ricinoleoyl chains within the 12-ricininoleoylricinoleoyl chain was an ester bond. The structure of RRRR in castor oil is indeed as shown in **Figure 1**.

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Received for review September 14, 2007. Revised manuscript received February 27, 2008. Accepted February 27, 2008.

JF072750Z