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Identification of (12-Ricinoleoylricinoleoyl)diricinoleoylglycerol, an Acylglycerol Containing Four Acyl Chains, in Castor (*Ricinus communis* L.) Oil by LC-ESI-MS

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An acylglycerol (AG) containing four acyl chains, (12-ricinoleoylricinoleoyl)diricinoleoylglycerol (RRRR), was positively identified for the first time in a natural source in castor oil using electrospray ionization tandem mass spectrometry (ESI-MS/MS). HPLC-purified RRRR from castor oil was subjected to ion trap and high-resolution ESI-MS/MS. The precursor and fragment ions of $[RRRR + Na]^+$ showed the expected masses, and the sodiated fragment ions of both diacylglycerols and fatty acids were detected. Because fragment ions of fatty acids from $[AG + NH_4]^+$ adducts cannot be detected by ESI-MS/MS, $[AG + Na]^+$ adducts are more informative. Radiolabeled triricinolein (RRR) was incorporated into RRRR in castor microsomes, indicating that RRRR is biosynthesized in castor bean. This newly identified and biosynthesized RRRR represents a new AG subclass of tetra-acylglycerols (or acylacyldiacylglycerol).

KEYWORDS: Castor oil; ricinoleate; acylglycerol; tetra-acylglycerol; acylacyldiacylglycerol; biosynthesis; HPLC; *Ricinus communis* L.

INTRODUCTION

Acylglycerols (AG), including the subclasses of triacylglycerols, diacylglycerols, and monoacylglycerols, from various natural sources are the most abundant acyl lipid class. Ricinoleate (R), a hydroxy fatty acid (FA), has many industrial uses, such as for the manufacture of aviation lubricant, plastics, paints, and cosmetics. Ricinoleate occurs as AG in castor oil, and ~70% of AG in castor oil is triricinolein (triricinoleoylglycerol, RRR) (*1*). Glycerol contains three hydroxyl groups to which up to three FA can be directly esterified to form AG. A fourth FA can be attached to a hydroxyl group on acyl chains. We have positively identified for the first time in a natural source an AG containing four acyl chains, (12-ricinoleoylricinoleoyl)-diricinoleoylglycerol (RRRR, **Figure 1**), in castor oil using electrospray ionization tandem mass spectrometry (ESI-MS/MS). AG containing four acyl chains have been previously suggested or tentatively identified (*2*−*9*). However, this new acyl lipid subclass (tetra-acylglycerols or acylacyldiacylglycerol) was not included in the new comprehensive classification system for lipids (*10*). The positive identification of an AG in a new subclass will add the new subclass into the comprehensive classification system for lipids. The new acyl lipids will eventually become useful.

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 in castor bean has been established (*11*, *12*), and key enzymatic steps on the pathway driving ricinoleate into RRR have been identified (*11*, *13*) (**Figure 2**). This information can be used to develop transgenic plants that produce seed oil containing RRR without the toxic substances. The key enzymatic steps identified were lysophosphatidylcholine acyltransferase (incorporates oleate) (*13*), oleoyl-12-hydroxylase, phospholipase A_2, and diacylglycerol acyltransferase (*11*). 2-Oleoylphosphatidylcholine (PC) is the direct precursor of 2-ricinoleoyl-PC, the substrate for oleoyl-12-hydroxylase (*11*). The cDNA for oleoyl-12-hydroxylase, the enzyme catalyzing the hydroxylation of oleate to ricinoleate, has been cloned from castor and expressed in tobacco, resulting in accumulation of low levels of ricinoleate in seed oil (*14*). Expression of this enzyme in a transgenic *Arabidopsis thaliana* plant was improved but still resulted in low amounts of hydroxy FA (*15*) compared to castor oil (20 vs 90% ricinoleate in castor oil). With the identification of RRRR in castor oil, it is desirable to overproduce RRRR in transgenic seed because of its higher ricinoleate content.

We recently reported the identification and quantification of 16 molecular species of AG in castor oil by C_{18} HPLC and evaporative light scattering detector (ELSD) (*1*). The major AG were triricinolein (RRR, 71%), diricinoleoyloleoylglycerol (RRO, 9%), and diricinoleoyllinoleoylglycerol (RRL, 7%). Mass

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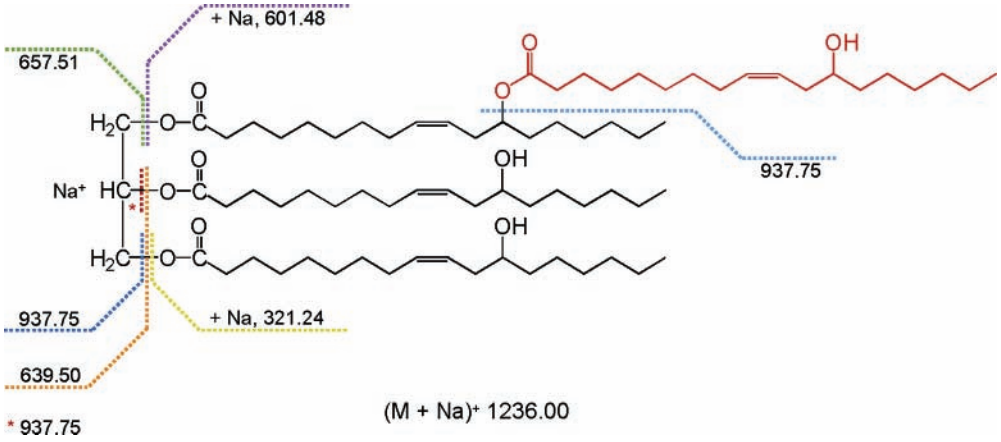

Figure 1. Structure of (12-ricinoleoylricinoleoyl)diricinoleoylglycerol (RRRR, associated with Na) and cleavages of $(M + Na)^+$ by ESI-MS/MS. The red ricinoleoyl chain is the fourth fatty acyl attached to triricinolein (RRR). The stereospecific location of the 12-ricinoleoyl-ricinoleoyl chain attached to the glycerol backbone cannot be determined from these MS/MS data alone. The location of the 12-ricinoleoylricinoleoyl chain on the glycerol backbone can be *sn*-1, -2, or -3. One of the two ricinoleoyl chain losses on $[RRRR + Na - RCOOH - RCOOH]^+$ to form the fragment ion of *m*/*z* 639.50 should be the fourth ricinoleoyl chain (in red), from the comparison of ESI-MS/MS of RRRR (**Figure 5**) and RRR (**Figure 6**) (for detail, see text). The fragment ion of *m*/*z* 639.50 shown in this figure is the loss of two ricinoleoyl chains, not the location of the two chains.

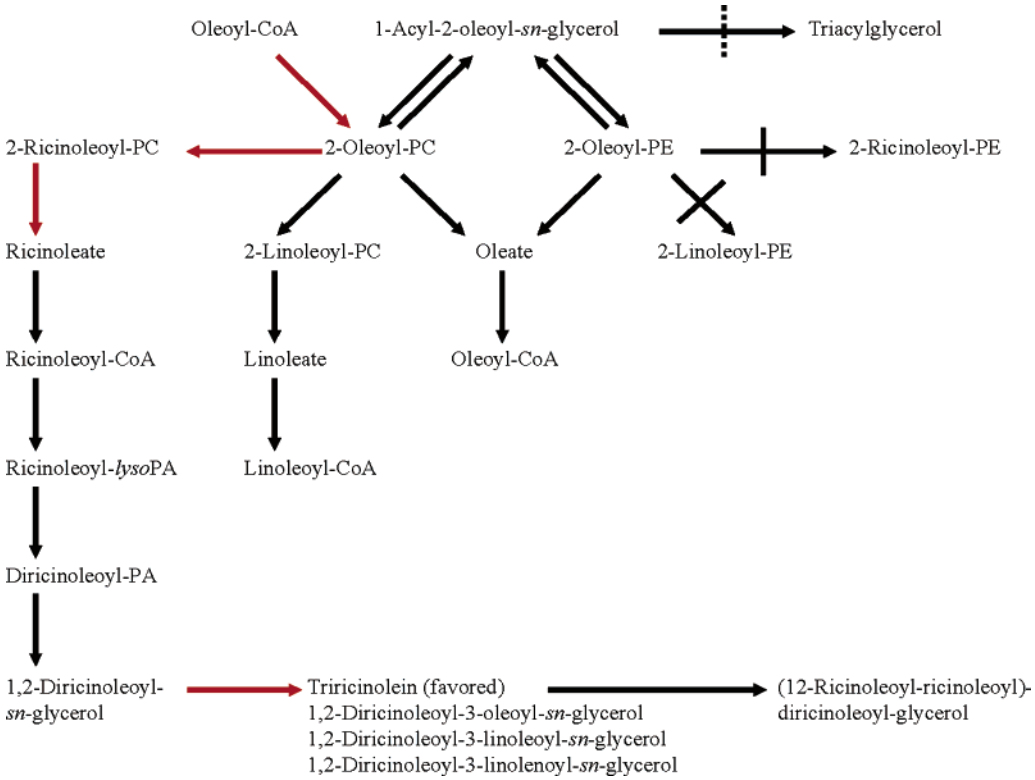

Figure 2. Proposed biosynthetic pathway of castor oil (*11*–*13*). The red arrows show the key enzyme steps driving ricinoleate into acylglycerols. Two arrows show a complete block (arrow with solid bar), and one arrow shows a partial block (arrow with broken bar).

spectrometry (MS) is required for positive identification of the molecular species of AG. Eleven molecular species of AG in castor oil have previously been characterized by MS/MS of the trimethylsilyl derivatives (*16*). We report here the identification of a new AG, RRRR, in castor oil using high-resolution ESI-MS/MS.

MATERIALS AND METHODS

LC-ESI-MS of the Molecular Species of AG in Castor Oil. AG molecular species were separated using a Surveyor HPLC pump, autosampler, and photodiode array (PDA) detector, all from ThermoFinnigan (San Jose, CA). Chromatographic separation was performed on a C_{18} column (Gemini, 250 × 4.60 mm, 5 μm, C18, Phenomenex, Torrance, CA, at 22 °C) with a linear gradient from 100% methanol (Burdick and Jackson) to 100% 2-propanol (Burdick and Jackson) (*1*). A 200 μg/mL sample of castor oil (Sigma Aldrich, St. Louis, MO) in methanol (20 μL) was injected by the autosampler into effluent flowing at 0.4 mL/min to accommodate the ESI constraints of the mass spectrometer. Chromatographic data were also obtained using a PDA detector in series set at 205 nm. An LCQ Advantage ion-trap mass spectrometer (ThermoFinnigan) equipped with an ESI interface (ThermoFinnigan) in positive ion mode was used to detect ions. To promote formation of positively charged NH_4^+ adducts from otherwise neutral AG species, 100 mM ammonium acetate dissolved in methanol was added postcolumn via a T-connector at a 2.5 μL/min flow rate provided by an on-board syringe pump. Mass spectrometer conditions used were as follows: 40 arbitrary units (au) of nitrogen (Praxair, Oakland, CA)

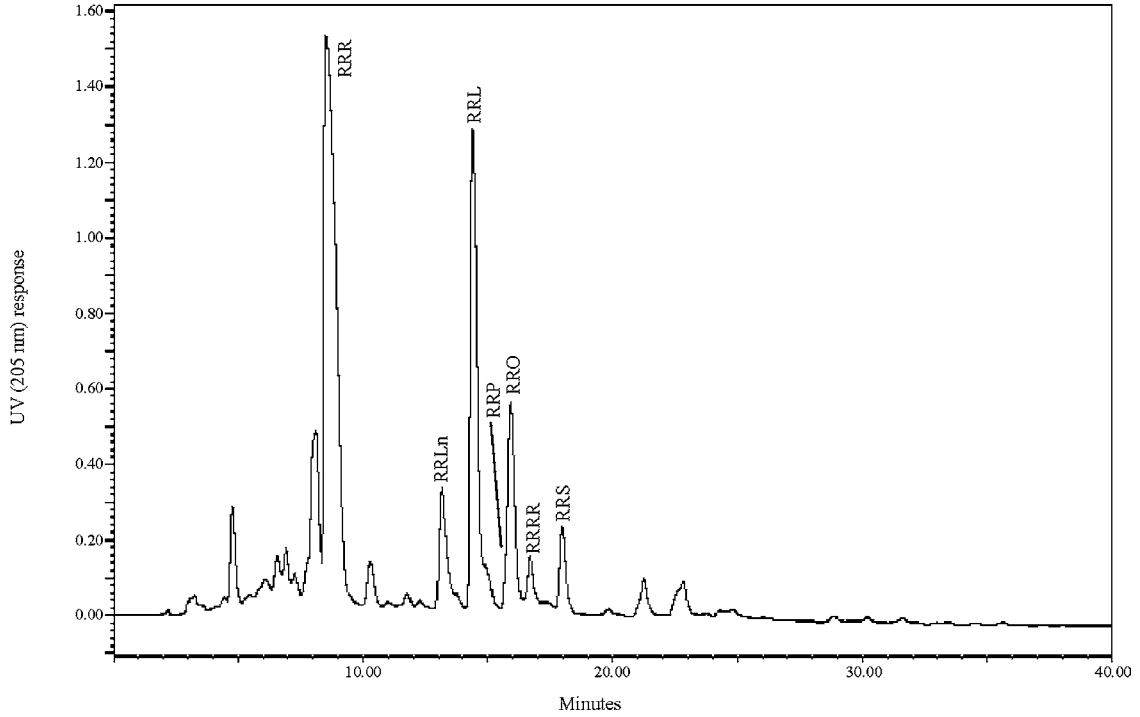

Figure 3. HPLC chromatogram of castor oil (1 mg). RRRR was fraction collected (0.5 min/fraction) as fraction 34. Analytical column (C_{18}, Gemini, at 22 °C), linear gradient of methanol to 2-propanol in 40 min, flow rate of 1 mL/min, and absorbance at 205 nm were used. For abbreviations of AG: R, ricinoleic acid; Ls, lesquerolic acid; Ln, linolenic acid; L, linoleic acid; O, oleic acid; P, palmitic acid; S, stearic acid; RRRR, see **Figure 1**.

sheath gas flow rate; 4.6 kV spray voltage; 31 V capillary voltage; 265 °C capillary temperature; *m*/*z* 300−2000 mass range; and 3 min acquisition time.

ESI-MS/MS of RRRR and RRR. An LCQ Advantage ion-trap mass spectrometer system (ThermoFinnigan) as described above was utilized for MS/MS analysis with slightly different conditions to optimize the additional fragmentation of the RRRR and RRR fractions collected from castor oil (**Figure 3**). Direct infusion of a 200 μg/mL sample dissolved in methanol at a 2.5 μL/min flow rate from a syringe pump, into an isocratic methanol (Burdick & Jackson) effluent flow (0.4 mL/min) from the HPLC system, as described above, produced stable MS/MS spectra. MS conditions were as follows: 50 au of nitrogen (Praxair) sheath gas flow rate; 4.5 kV spray voltage; 250 °C capillary temperature; *m*/*z* 3.0 isolation width; *m*/*z* 300−2000 mass range; 3 min acquisition time; 38 V capillary voltage; and 40% relative collision energy. Research grade (99.999%) helium (Praxair) was used as a collision gas. The spectrum of ESI-MS/MS of RRRR is shown in **Figure 4**.

High-Resolution (HR) ESI-MS and MS/MS. Samples were analyzed by HR-ESI-MS using a hybrid quadrupole/time-of-flight (TOF) mass spectrometer (Q-STAR Pulsar I, MDS Sciex/ABI, Toronto, ON, Canada). Two fragment ions generated by the collision-activated dissociation of the +2 charge state of glufibrogen (*m*/*z* 785.8) were used to externally calibrate the TOF mass analyzer. TOF resolution was typically ~8000−9000 fwhm. Eluent fractions of discrete peaks as shown in **Figure 3** were collected by off-line HPLC. Two microliters of a fraction were diluted with 100 μL of HPLC grade methanol and 10 μL of HPLC grade water. Eight microliters of this diluted sample was loaded into an ESI spray capillary (NanoES, Proxeon Biosystems) using a 10-μL gel pipet tip. The spray capillary was then centrifuged briefly and mounted onto the source head, and a backing pressure of 1−8 atm was applied from a 10-mL glass Luer-lock glass syringe. MS instrument parameters were as follows: 1800 V spray voltage; 15 au of current gas; *m*/*z* 100−2000 mass range; 1 s accumulation time; 2 min acquisition time; 50 au of declustering potential; 220 V focusing potential; 15 au second declustering potential; 11 μs ion release delay; and 10 μs ion release width. For MS/MS experiments, the collision gas setting was 5 au and the collision energy setting was 70 au. Ultrahigh-purity nitrogen (99.999%) supplied from a nitrogen generator was used for the curtain and collision gases.

Castor Microsomal Incubation of Radiolabeled Triricinolein (RRR). Microsomes from castor bean were prepared as previously described (*11*, *17*). The incubation mixture in a total volume of 1 mL included sodium phosphate buffer (0.1 M, pH 6.3), CoA-SH (0.5 μmol), NADH (0.5 μmol), ATP (0.5 μmol), $MgCl_2$ (0.5 μmol), catalase (1000 units), and microsomal fraction of endosperm from immature castor bean (15 μL, 0.14 mg of protein). [^{14}C]RRR was prepared by the castor microsomal incubation of [^{14}C]ricinoleate (5.0 μCi, 20 mL incubation), and the [^{14}C]RRR incorporated was purified by HPLC as previously described (*13*). [^{14}C]RRR substrate (0.3 μCi, in 20 μL of ethanol) was added last into a screw-capped tube containing incubation mixture, followed by immediate mixing. The mixture was then incubated in a shaking water bath for 60 min at 22 °C. The incubation was stopped by suspension in 3.75 mL of chloroform/methanol (1:2, v/v), followed by mixing with 0.63 mL of chloroform and 0.63 mL of water. The lower chloroform layer contained the lipid extract. The lipid extract was cochromatograpged with castor oil using an HPLC system previously described for the separation of the molecular species of AG (*18*, *19*).

RESULTS AND DISCUSSION

We have confirmed the identity of the 16 molecular species of AG in castor oil (*1*) using LC-ESI-MS of their ammonium adducts. A low-bleed HPLC column (Gemini, Phenomenex) was used instead of the one used in the earlier study (Ultrasphere C18, Beckman, Fullerton, CA) (*1*). One HPLC peak (RRRR) eluting slightly after RRO (**Figure 3**) was previously misidentified as RRP (diricinoleoylpalmitoylglycerol) (*1*) because of similar retention times and lack of MS data. The $[M + NH_4]^+$ ion from this peak was *m*/*z* 1231.0, which was larger than those of triacylglycerols and agreed with that of RRRR (**Figure 1**). The $[M + NH_4]^+$ ion of RRR was at *m*/*z* 950.8. We used tandem and high-resolution MS to confirm the identity of this HPLC peak. The content of RRRR in castor oil was found to be 0.47% in our previous study (*1*).

The molecular species of AG in castor oil were separated by reversed-phase HPLC as shown in **Figure 3**. The Gemini

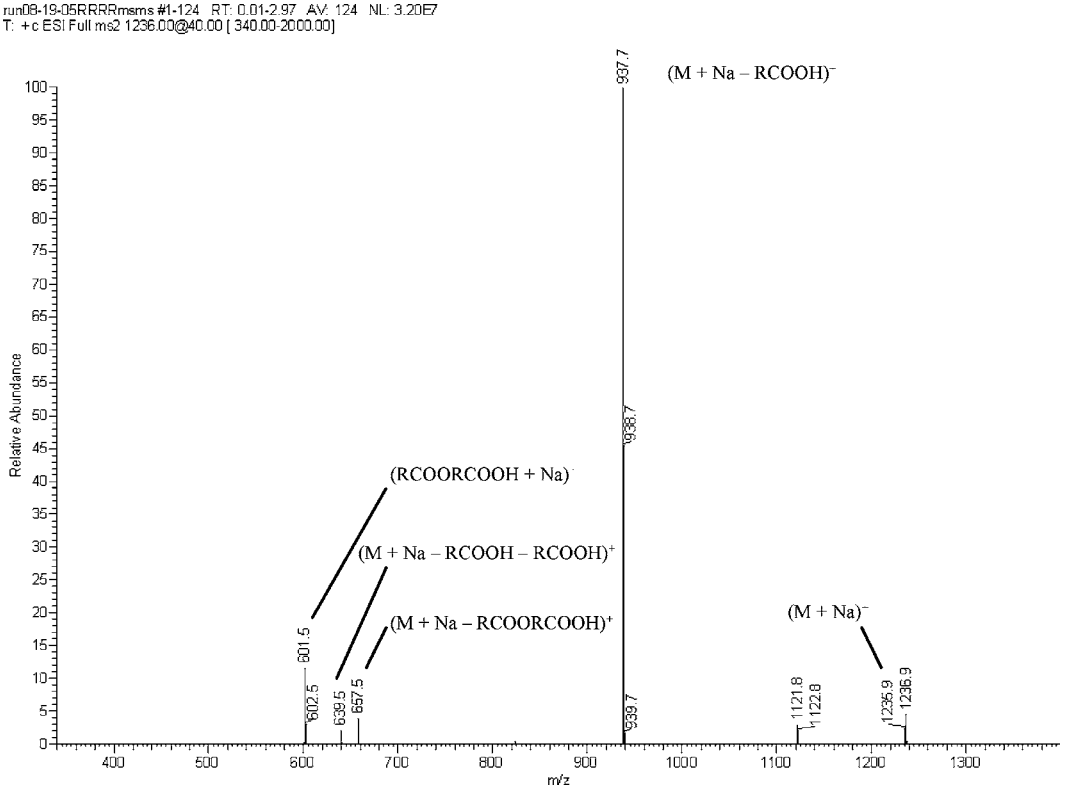

Figure 4. Ion trap mass spectrum of ESI-MS/MS of $(RRRR + Na)^+$ ion at *m*/*z* 1236.0. For abbreviations, see **Figure 1**.

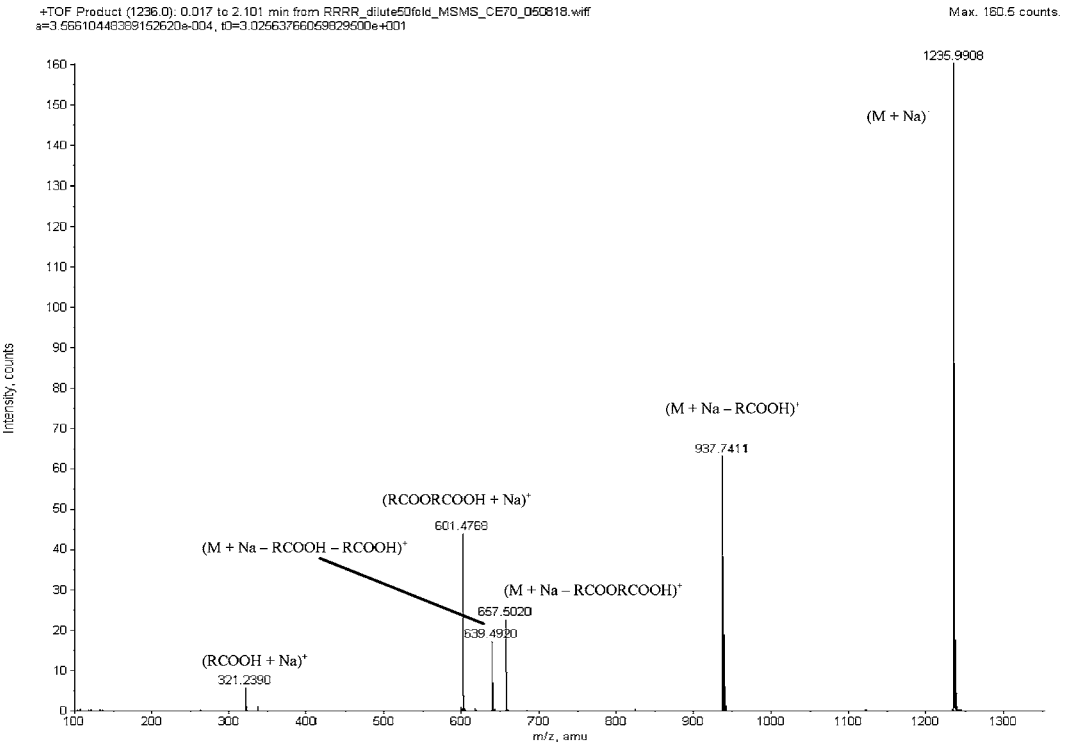

Figure 5. High-resolution mass spectrum of ESI-MS/MS of $(RRRR + Na)^+$ ion at *m*/*z* 1236.0. The collision energy setting was 70 au. For abbreviations, see **Figure 1**.

column used separated RRRR and RRO much better than the Ultrasphere C18 column used previously (*1*). Fraction 34 (from 16.5 to 17 min) was pooled from five replicate runs yielding ~20 μg of RRRR. This RRRR sample was quite pure with only a minor amount of RRO according to the $[M + Na]^+$ ions (not shown), giving $[RRRR + Na]^+$ at *m*/*z* 1236.0 and $[RRO + Na]^+$ at *m*/*z* 939.8. ESI-MS/MS of the precursor ion at *m*/*z* 1236.0 resulted in identical fragment ions on both the lower resolution quadrupole ion trap instrument (**Figure 4**) and the higher resolution hybrid quadrupole time-of-flight (QqTOF) (**Figure 5**).

The HR-ESI-MS/MS spectrum of $[RRRR + Na]^+$ (**Figure 5**, single run) shows the precursor and fragment ions as follows: $[M + Na]^+$ at *m*/*z* 1235.9908 ($C_{75}H_{136}O_{11}Na$, 1235.9980

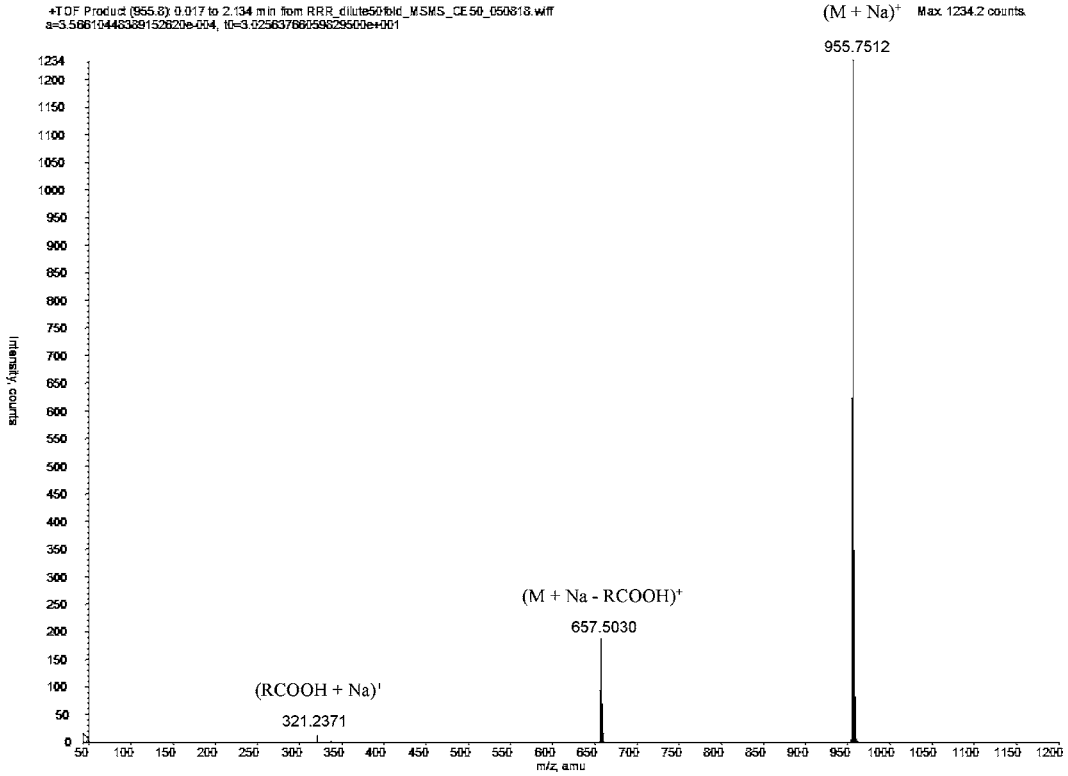

Figure 6. High-resolution mass spectrum of ESI-MS/MS of $(RRR + Na)^+$ ion at *m/z* 955.76. The collision energy setting was 50 au. RRR is triricinolein. For abbreviations, see **Figure 1**.

[exact]; mass error, −0.0072); $[M + Na - RCOOH]^+$ at *m/z* 937.7411 ($C_{57}H_{102}O_8Na$, 937.7472 [exact]; mass error, −0.0061); $[M + Na - RCOORCOOH]^+$ at *m/z* 657.5020 ($C_{39}H_{70}O_6Na$, 657.5070 [exact]; mass error, −0.0050); $[M + Na - RCOOH - RCOOH]^+$ at *m/z* 639.4920 ($C_{39}H_{68}O_5Na$, 639.4964 [exact]; mass error, −0.0044); $[RCOORCOOH + Na]^+$ at *m/z* 601.4768 ($C_{36}H_{66}O_5Na$, 601.4808 [exact]; mass error, −0.0040); $[RCOOH + Na]^+$ at *m/z* 321.2390 ($C_{18}H_{34}O_3Na$, 321.2406 [exact]; mass error, −0.0016). These fragmentations are graphically shown in **Figure 1**. The precursor and fragment ions (and their exact masses) confirm the structure and elemental composition of RRRR as shown in **Figure 1**. However, the stereospecific location of the 12-ricinoleoylricinoleoyl chain attached to the glycerol backbone cannot be determined from these MS/MS data alone. The MS^3 on $[M + Na - RCOOH - RCOOH]^+$ at *m/z* 640 and $[M + Na - RCOORCOOH]^+$ at *m/z* 657 was not done because they were relatively weak in intensity as shown in **Figure 4** and thus would be difficult to obtain MS^3 for them. The masses of the precursor and fragment ions in ESI-MS/MS of an ether linkage between the two 12-hydroxyls at the fourth chain linkage will be the same as those of RRRR, and therefore we cannot exclude the possibility of the ether linkage at the fourth chain. However, this is unlikely given standard plant lipid biochemistry.

Three separate MS and MS/MS experiments (high resolution) were performed for the RRRR fraction collected in addition to the single run, and the instrument was externally recalibrated prior to each measurement. The mean and standard deviation of the measured mass of the $[RRRR + Na]^+$ molecular ion were as follows: *m/z* 1235.9968 ± 0.0037 ($C_{75}H_{136}O_{11}Na$, 1235.9980 [exact]; error, −0.97 ppm). The average and standard deviation of the *m/z* of the $[RRRR + Na]^+$ precursor ion and fragment ions for MS/MS measurements were as follows: precursor ion at *m/z* 1235.9940 ± 0.0028 ($C_{75}H_{136}O_{11}Na$, 1235.9980 [exact]; error, −3.2 ppm); fragment ion at *m/z* 937.7445 ± 0.0030 ($C_{57}H_{102}O_8Na$, 937.7472 [exact]; error, −2.9 ppm); fragment ion at *m/z* 657.5046 ± 0.0024 ($C_{39}H_{70}O_6Na$, 657.5070 [exact]; error, −3.7 ppm); fragment ion at *m/z* 639.4933 ± 0.0011 ($C_{39}H_{68}O_5Na$, 639.4964 [exact]; error, −4.8 ppm); fragment ion at *m/z* 601.4788 ± 0.0019 ($C_{36}H_{66}O_5Na$, 601.4808 [exact]; error, −3.3 ppm); fragment ion at *m/z* 321.2394 ± 0.0004 ($C_{18}H_{34}O_3Na$, 321.2406 [exact]; error, −3.7 ppm). As expected, the averages of three runs have lower mass errors than the single run.

The HR-ESI-MS/MS spectrum of $[RRR + Na]^+$ from triricinolein (RRR) is shown in **Figure 6**. The precursor and fragment ions are as follows: $[M + Na]^+$ at *m/z* 955.7512 ($C_{57}H_{104}O_9Na$, 955.7578 [exact]; mass error, −0.0066); $[M + Na - RCOOH]^+$ at *m/z* 657.5030 ($C_{39}H_{70}O_6Na$, 657.5070 [exact]; mass error, −0.0040); and $[RCOOH + Na]^+$ at *m/z* 321.2371 ($C_{18}H_{34}O_3Na$, 321.2406 [exact]; mass error, −0.0035). The fragmentations of precursor ions $[RRRR + Na]^+$ and $[RRR + Na]^+$ were similar. This is the first report of acyl ions, for example, $[RCOOH + Na]^+$, in the ESI-MS/MS spectrum of AG.

During an MS/MS experiment, a dissociative loss from the precursor ion will usually produce a fragment ion that is stabilized such that a subsequent dissociative loss from the fragment ion is less probable. However, the fragment ion $[RRRR + Na - RCOOH - RCOOH]^+$ at *m/z* 639.50 shown graphically in **Figure 1** and observed in **Figures 4** and **5** is the result of dissociative loss of *two* ricinoleoyl chains, presumably sequentially. In contrast, the fragment ion $[RRRR + Na - RCOORCOOH - RCOOH]^+$ at *m/z* 359.26 ($C_{21}H_{36}O_3Na$) was not observed in **Figures 4** and **5**. Similarly, MS/MS of $[RRR + Na]^+$ did not produce the fragment ion $[RRR + Na - RCOOH - RCOOH]^+$ at *m/z* 359.26 ($C_{21}H_{36}O_3Na$) as shown in **Figure 6**. Therefore, one of the two ricinoleoyl chain losses on $[RRRR + Na - RCOOH - RCOOH]^+$ was the fourth ricinoleoyl chain attached to a ricinoleoyl chain, and the other ricinoleoyl chain

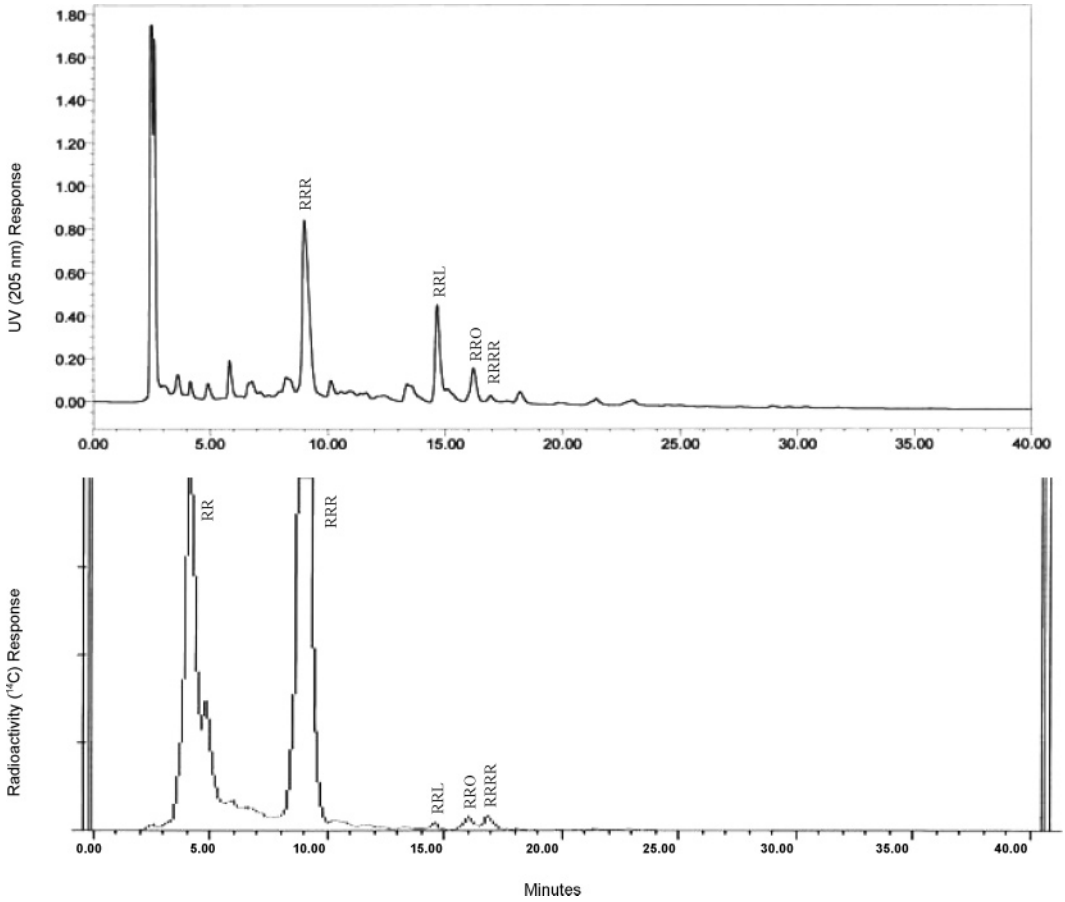

Figure 7. HPLC chromatograms of lipid extract of the incubation products of radiolabeled triricinolein (RRR) in castor microsomes cochromatographed with castor oil. For incubation conditions, see ref *13*. For HPLC conditions, see **Figure 3**. UV (205 nm) chromatogram was obtained from an absorbance detector, and radiochromatogram was obtained from a flow scintillation analyzer. Both detectors were used in series.

loss was one of the two chains attached to the glycerol backbone (**Figure 1**). The fragment ion $[RRRR + Na - RCOORCOOH]^+$ at *m*/*z* 657.5020 in **Figure 5** has the same mass (within experimental error) as the fragment ion $[RRR + Na - RCOOH]^+$ at *m*/*z* 657.5030 shown in **Figure 6**, which confirms identical elemental composition and presumably identical structure.

Both $[M + Na]^+$ (*20*−*22*) and $[M + NH_4]^+$ (*23*−*28*) adducts of AG have been used to interpret ESI-MS/MS. In **Figures 4**−**6**, both diacylglycerol fragment ions and acyl ions from $[M + Na]^+$ were shown and attached to Na^+, whereas $[M + NH_4]^+$ produced diacylglycerol fragment ions without NH_4^+ attached (*23*−*28*). The diacylglycerol fragment from $[M + Na]^+$ is $[M + Na - RCOOH]^+$, whereas that from $[M + NH_4]^+$ is $[M + NH_4 - RCOONH_4]^+$. The cleavage of $[M + Na]^+$ showed both sides of intact fragments as sodiated ions (**Figures 1** and **4**−**6**), whereas the cleavage of $[M + NH_4]^+$ showed the diglyceride ions without the acyl ions (*23*−*28*). Therefore, ESI-MS/MS spectra of $[M + Na]^+$ of AG are more structurally informative than those of $[M + NH_4]^+$. The recently reported ESI-MS/MS spectra of $[M + Na]^+$ of AG showed the diacylglycerol fragment ions of both $[M + Na - RCOOH]^+$ and $[M + Na - RCOONa]^+$ (*21*, *22*), whereas **Figures 4**−**6** are simple and do not show the $[M + Na - RCOONa]^+$ ions. The spectra shown in **Figures 4**−**6** are clean, and only a few significant *m*/*z* values are present above the background, almost all of which are interpretable.

AG with four acyl chains have previously been tentatively identified (*2*−*9*) as estolides. In sclerotia of the rye ergot, the AG containing four to nine acyl chains including ricinoleate and nonhydroxylated FA have been suggested (*9*). However, as far as we know, no further study of these ricinoleate-containing AG was reported. RRRR was not previously suggested in sclerotia of the rye eregot (*9*) and is reported here for the first time. For positive identification of RRRR, we have shown the clean spectrum of HR-MS/MS with significant original precursor ion (nonhydrogenated), diglyceride and acyl ions, and their exact masses. The previous reports of AG with four acyl chains (*2*−*9*) were either tentative or suggestive.

We would like to know whether RRRR in castor oil was biosynthesized in castor bean or formed after harvest, processing, and storage of the oil. AG containing five ricinoleate (RRRRR) was not detected in castor oil in this study using LC-MS of $[M + NH_4]^+$ (data not shown). The $[RRRRR + NH_4]^+$ ion at *m*/*z* 1511.3 was not detected. Thus, it is unlikely that RRRR in castor oil was formed after the harvest of castor bean due to nonbiochemical polymerization. [^{14}C]Ricinoleate (5.0 μCi) was incubated with castor microsomes (20-mL incubation) as described earlier (*13*). Many molecular species of AG were formed including [^{14}C]RRR as shown in the C_{18} HPLC radiochromatogram (*13*). We used a Gemini C_{18} column here because RRRR can be well separated from RRO (**Figure 3**). The [^{14}C]RRR (0.3 μCi) fraction was collected and incubated with and without unlabeled ricinoleate (10 μg) and coenzyme A (0.38 mg) in castor microsomal incubation. [^{14}C]RRRR was identified in the lipid extract by HPLC cochromatography with castor oil. The retention time of RRRR as detected at 205 nm matched that of a peak in the radiochromatogram (**Figure 7**). About 1300 dpm was incorporated into RRRR. This [^{14}C]RRR incubation was repeated with the same results. The radiochro-

matogram also shows incorporation of radioactivity into of RRO and RRL. Apparently, [^{14}C]RRR was also hydrolyzed by lipase in castor microsomes to [^{14}C]diricinolein, and then endogenous oleoyl-CoA and linoleoyl-CoA were incorporated by diacylglycerolacyltransferase (DGAT). It is likely that RRRR is biosynthesized from RRR by a triacylglycerolacyltransferase, which is similar to DGAT because of the similar biochemical reactions.

We have positively identified RRRR in castor oil and have shown that RRRR was biosynthesized in castor bean. RRRR is the first AG containing four acyl chains positively identified. RRRR represents a new subclass of AG as tetra-acylglycerol (or acylacyldiacylglycerol). This new subclass of AG has previously been called tetraester triglycerides (*2*, *4*), tetra-acid triglycerides (*3*), tetra-acid glycerides (*5*), and tetra-acyl triglycerides (*7*, *8*). The addition of an acyl chain on one of the three acyl chains resulting in a tetra-acylglycerol will change the mass, size, and physical properties of the molecule from triacylglycerol, and eventually tetra-acylglycerol can be useful in industry. Transgenic plants targeted at producing RRRR can be developed to obtain seed oil with higher ricinoleate content than the seed oil from transgenic plants targeted to produce RRR.

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