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Mass Spectrometry of the Lithium Adducts of Diacylglycerols Containing Hydroxy FA in Castor Oil and Two Normal FA

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Abstract Castor oil can be used in industry. The molecular species of triacylglycerols containing hydroxy fatty acids (FA) in castor oil have been identified. We report here the identification of twelve diacylglycerols (DAG) containing hydroxy FA in castor oil using positive ion electrospray ionization mass spectrometry of the lithium adducts. They were RR (diricinolein, R is ricinoleate), RL, RS, R-diOH18:0, R-diOH18:1, R-diOH18:2, R-triOH 18:0, R-triOH18:1, R-triOH18:2, diOH18:0-diOH18:1, diOH18:1-diOH18:1 and diOH18:1-diOH18:2. The MS^2 fragment ions, $[M + Li - FA]^+$ and $[FA + Li]^+$, from the lithium adducts of DAG containing hydroxy FA (one or two hydroxy FA), were used for the identification. The additional fragment ions from the neutral losses of FA lithium salts $[M + Li - FALi]^+$ were used for the identification of eleven DAG containing two normal FA in a soybean oil bioconversion product. The MS^2 fragment ions from the neutral losses of FA lithium salts $[M + Li - FALi]^+$ were not detected from the DAG containing hydroxy FA. The DAG containing FA with more hydroxyl groups than the other FA on the same DAG molecule tended to have a prominent fragment ion $[FA + Li]^+$ and an undetectable fragment ion $[M + Li - FA]^+$ while the FA was the more hydroxylated FA. Also the less hydroxylated FA of a DAG tended to have a prominent fragment ion $[M + Li - FA]^+$ and an undetectable fragment ion $[FA + Li]^+$ while the FA was the less hydroxylated FA.

Keywords Mass spectrometry · Lithium adducts · Diacylglycerols · Hydroxy fatty acids · Castor oil · Soybean oil

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Introduction

Triacylglycerols (TAG) containing hydroxy fatty acids (FA), e.g., castor oil, have many industrial uses such as the manufacture of biodegradable aviation lubricant, plastic, paint, nylons and cosmetics, because of the hydroxyl group on the FA constituents. Castor oil is the only commercial source of TAG containing hydroxy FA. Diacylglycerols (DAG) containing hydroxy FA can also be used in industry. DAG are the intermediate molecules in the biosynthesis of TAG and phospholipids. We have earlier detected a small amount (0.14 %) of diricinolein (RR), DAG containing two ricinoleic acids (OH18:1), in castor oil by HPLC [1]. RR was later identified by mass spectrometry (MS) [2]. We report here the identification of twelve DAG containing hydroxy FA (one or two hydroxy FA) in castor oil by the MS.

Positive ion electrospray ionization mass spectrometry (ESI-MS) of lithium adducts of TAG containing three normal (non-hydroxy) FA has been reported [3–5]. The same method has also been used for the identification of TAG containing hydroxy FA in castor oil [2, 6–11]. The MS^2 spectra of the lithium adducts of TAG containing three normal FA showed the prominent fragment ions from the neutral losses of the three constituent FA $[M + Li - FA]^+$ or $[M + Li - RCO_2H]^+$, and the three constituent FA lithium salts $[M + Li - FALi]^+$ or $[M + Li - RCO_2Li]^+$

[4, 5]. The fragmentation pathways of these ions have been proposed [4, 5]. The MS^2 spectra of the lithium adducts of TAG containing hydroxy FA in castor oil showed the fragment ions from the neutral losses of three constituent FA $[M + Li - FA]^+$, an aldehyde $[M + Li - C_6H_{13}CHO]^+$ and a ketene $[M + Li - C_5H_{11}CH{=}C{=}O]^+$, and FA lithium adducts $[FA + Li]^+$ [2, 7–11]. We have reported the MS^2 spectra of five DAG containing hydroxy FA in castor oil [8, 10, 11] and the characteristic fragment ions were similar to those of the TAG containing hydroxy FA in castor oil. The MS^2 spectra of the lithium adduct of DAG containing two normal FA have been rarely reported. The MS^2 spectrum of the lithium adduct of distearin (SS) showed the fragment ions $[M + Li - FALi]^+$ and $[M + Li - FA]^+$ [12]. We report here the identification of twelve DAG containing hydroxy FA in castor oil by MS^2 of the lithium adducts of DAG. The MS^2 spectra were compared with those of DAG containing two normal FA in the soybean oil bioconversion product [13].

Material and Methods

Materials

Castor oil, lithium acetate and the standards of DAG of PP, LL and LnLn were obtained from Sigma (St. Louis, MO, USA). High purity methanol and 2-propanol (Honeywell Burdick & Jackson) for LC and MS were purchased from VWR International (West Chester, PA, USA). High purity nitrogen for MS was acquired from Praxair (Oakland, CA, USA). Research grade helium (Praxair) was used as a collision gas of MS. The soybean oil bioconversion product was obtained as described in Ref. 13.

HPLC Fractionation of the Molecular Species of Acylglycerols in Castor Oil and the Soybean Oil Bioconversion Product

The fractionation of the molecular species of TAG and DAG in castor oil and the soybean oil bioconversion product were as previously reported [1]. 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). First, 1 mg of the sample in ethanol (50 μL) was chromatographed at 22 °C (room temperature) with a linear gradient from 100 % methanol to 100 % 2-propanol over 40 min, at a 1 mL/min flow rate, and detected at 205 nm. Fractions were collected every 30 s and corresponding fractions were pooled from seven 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 half of each HPLC fraction collected 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

An LCQ Advantage ion-trap mass spectrometer (MS 2.0) with Xcalibur 2.0 SR2 software (ThermoFisher Scientific, San Jose, CA, USA) was used for MS analysis of the various molecular species of DAG. The infusion at a 2.5 μL/min flow rate from a syringe (250 μL) pump produced stable singly-charged lithiated parent ions which were subsequently fragmented for MS^2 analysis. ESI 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 ; normalized collision energy, 27–42 %; scan ranges, 100–1500 *m/z*. Acquire time was 3 min.

Results and Discussion

Castor oil and the soybean oil bioconversion product containing TAG and DAG of both hydroxy FA and normal FA [13] were fractionated by HPLC. The HPLC fractions were used for the analysis of DAG by positive ion electrospray ionization mass spectrometry (ESI-MS) of the lithium adducts. The hydroxy FA of acylglycerols in castor oil have been identified including the numbers and locations of hydroxyl groups and double bonds on the acyl chains [8, 10, 11]. They were biosynthesized from ricinoleate presumably with the addition of OH groups at C-11 and/or C-13 positions and with the addition of double bonds at the C-13 or C-14 positions [2] or the saturation of double bond at the C-9 position.

Figure 1 shows the MS^2 spectrum of diricinolein (RR) in castor oil from $[M + Li]^+$ at *m/z* 659.4. This is a simple spectrum. The prominent fragment ions were ricinoleate lithium adduct $[R + Li]^+$ at *m/z* 305.1, and from the neutral losses of ricinoleate $[M + Li - R]^+$ at *m/z* 361.1 and an aldehyde $[M + Li - C_6H_{15}CHO]^+$ at *m/z* 545.3. The fragment ion from the neutral loss of the aldehyde was due to the cleavage between C-11 and C-12 on the C18 acyl chain containing a double bond at the C-9 position and a hydroxyl group at the C-12 position, e.g., ricinoleate [8]. This ion was also the common fragment ion on the MS^2 spectra of the lithium adducts of TAG containing hydroxy FA in castor oil [7–12]. None of the MS^2 spectra of the TAG [8–12] and DAG (Figs. 1, 2; Table 1) [8, 10, 11] containing hydroxy FA (one, two or three hydroxy FA) in castor oil showed the fragment ions from the neutral losses

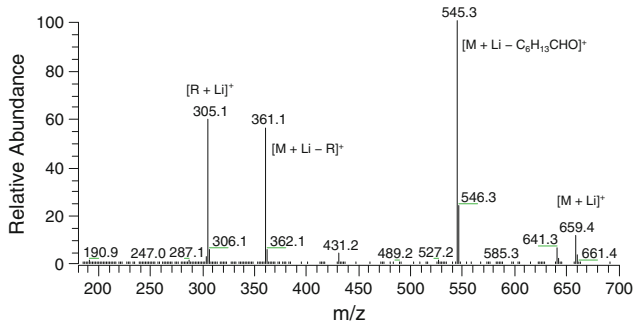

Fig. 1 The MS^2 spectrum of $[RR + Li]^+$ at *m/z* 659.4 from the HPLC fraction #10 of castor oil

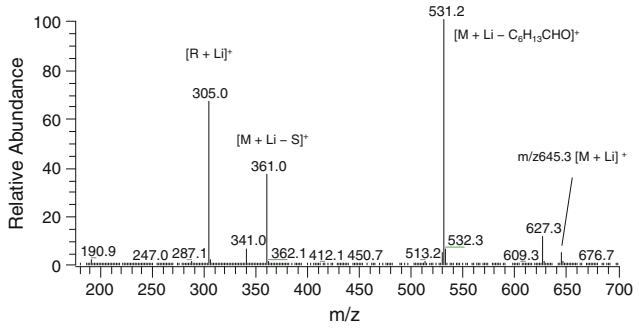

Fig. 2 The MS^2 spectrum of $[RS + Li]^+$ at *m/z* 645.3 from the HPLC fraction #23 of castor oil

of FA lithium salts $[M + Li - FALi]^+$. While the fragment ions from the neutral losses of FA lithium salts $[M + Li - FALi]^+$ were the prominent ions from the TAG and DAG containing all normal FA [5, 6, 12]. We have recently obtained the MS^2 spectra of TAG containing three normal FA in castor oil [2] and they showed the prominent fragment ions of both $[M + Li - FA]^+$ and $[M + Li - FALi]^+$.

Figure 2 is the MS^2 spectrum of ricinoleoyl-stearoyl-glycerol (RS) in castor oil from $[M + Li]^+$ at *m/z* 645.3. The prominent fragment ions were ricinoleate lithium adduct $[R + Li]^+$ at *m/z* 305.0, and from the neutral losses of stearate $[M + Li - S]^+$ at *m/z* 361.0 and an aldehyde $[M + Li - C_6H_{15}CHO]^+$ at *m/z* 531.2. No fragment ions of stearate lithium adduct $[S + Li]^+$ at *m/z* 291.0 and that from the neutral loss of ricinoleate $[M + Li - R]^+$ at *m/z* 347.0 were detected. The MS^2 spectra of DAG containing both hydroxy FA and normal FA (e.g., RS, RL) seems that hydroxy FA tend to show as lithium adducts only and normal FA tend to show as those from the neutral losses of the normal FA only. Both Figs. 1 and 2 are simple spectra. The MS^2 spectra of the TAG including three hydroxy FA and three normal FA and the mix of both showed the fragment ions from the neutral losses of all of the three FA constituents [3, 5, 6, 8–12].

We have identified twelve DAG containing hydroxy FA in castor oil by MS as shown in Table 1. The characteristic ions (*m/z*) and their relative abundance of the DAG are also given. Among the twelve DAG in Table 1, five are newly reported here and they are RL, RS, R-diOH18:2, diOH18:0-diOH18:1 and R-triOH18:0. We did not detect RO in castor oil and oleate is the precursor of ricinoleate [14]. Previously reported were the MS^2 spectra of DAG lithium adducts of R-diOH18:1 [8], diOH18:1-diOH18:1 [10], R-triOH18:1 [10], diOH18:1-diOH18:2 [11], and R-triOH18:2 [11]. The most abundant fragment ions (base peaks) were usually from the neutral losses of aldehyde,

Table 1 MS^2 characteristic ions (*m/z*) and their relative abundance (% in parenthesis) of DAG containing hydroxy FA identified in the HPLC fractions of castor oil

DAG HPLC fractions	$[M + Li]^+$	$[M + Li - FA]^+$	$[FA + Li]^+$	$[M + Li - C_6H_{13}CHO]^+$
RL (F16)	641.4	343.1(2), 361.1(47)	305.1(67). 287.1(2)	527.3(100)
RS (F23)	645.3	347.0(0), 361.0(37)	305.0(68), 291.0(0)	531.2(100)
RR (FIO)	659.5	361.1(56)	305.1(60)	545.3(100)
R-diOH18:2(F9)	673.5	375.2(18), 361.2(6)	305.2(4), 319.2(24)	559.4(100)
R-diOH18:l (F9)	675.5	377.2(43), 361.2(9)	305.2(11), 321.2(100)	561.4(95)
R-diOH18:0(F9)	677.5	379.2(44), 361.2(9)	305.3(11), 323.3(100)	563.4(56)
diOH18:l-diOH18:2(F8)	689.6	375.1(6), 377.2(8)	321.3(11), 319.3(7)	575.3(100)
R-triOH18:2(F8)	689.6	391.2(19), 361.2(4)	305.3(4), 335.2(29)	575.3(100)
diOH18:1-diOH18:1(F8)	691.6	377.2(8)	321.3(19)	577.4(100)
R-triOH18:1(F8)	691.6	393.2(17), 361.2(3)	305.3(1), 337.2(42)	577.4(100)
diOH18:0-diOH18:1(F8)	693.5	377.2(31), 379.2(15)	323.3(35), 321.3(66)	579.4(100)
R-triOH18:0(F8)	693.5	395.2(44), 361.3(0)	305.3(3), 339.3(73)	579.4(100)

On the columns of $[M + Li - FA]^+$ and $[FA + Li]^+$, the *m/z* of two fragment ions are listed when the DAG containing two different FA constituents. The orders of the two *m/z* listed are the same as the orders of the two FA constituents on the name of DAG shown on the DAG column

R ricinoleate

$[M + Li - C_6H_{13}CHO]^+$ as shown in Table 1. The fragment ions of the two FA lithium adducts combined, $[FA + Li]^+$, were usually more abundant than those from the neutral losses of the two FA combined, $[M + Li - FA]^+$. Among the DAG containing two different FA, the fragment ions from the losses of the less hydroxylated FA, $[M + Li - FA]^+$, were more abundant than those from the losses of the more hydroxylated FA. The fragment ions of the lithium adducts, $[FA + Li]^+$, of the less hydroxylated FA were less abundant than those of the more hydroxylated FA. The extreme case is shown as Fig. 2 of RS, a DAG containing both hydroxylated FA and normal FA and both $[M + Li - R]^+$ at *m/z* 347.0 and $[S + Li]^+$ at *m/z* 291.0 were not detectable (Table 1).

We have recently used MS^2 of DAG lithium adducts to identify the DAG containing hydroxy FA in the soybean oil bioconversion product and the presence or lack of $[M + Li - R]^+$ and $[FA + Li]^+$ were similar to those from the DAG containing hydroxy FA in castor oil [13]. Figure 3 is an example of the identification by MS of DAG containing hydroxy FA in the soybean oil bioconversion product. This is the MS^2 spectrum of three DAG, OH18:1-OH:18:1, diOH18:2-S and diOH18:1-O, combined. For the DAG, diOH18:2-S and diOH18:1-O containing both hydroxy FA and normal FA, the fragment ions of hydroxy FA of lithium adducts as $[diOH18:2 + Li]^+$ at *m/z* 319.1 and $[diOH18:1 + Li]^+$ at *m/z* 321.1 were prominent, while the fragment ions from the neutral losses of hydroxy FA $[M + Li - diOH18:2]^+$ at *m/z* 347.1 and $[M + Li - diOH18:1]^+$ at *m/z* 345.1 were either not detected or trace only. For the same two DAG, the fragment ions from the neutral losses of normal FA, $[M + Li - S]^+$ at *m/z* 375.1 and $[M + Li - O]^+$ at *m/z* 377.1, were prominent and the fragment ions of the lithium adducts of these two normal FA, $[S + Li]^+$ at *m/z* 291.1, $[O + Li]^+$ at *m/z* 289.1, were not detected. For DAG OH18:1-OH18:1 containing two monohydroxy FA, both $[OH18:1 + Li]^+$ at *m/z* 305.1 and $[M + Li - OH18:1]^+$ at *m/z* 361.1 were prominent. Again this example showed that the more hydroxylated FA of a DAG tend to have prominent fragment ion $[FA + Li]^+$ and undetectable fragment ion $[M + Li - FA]^+$. Also the less hydroxylated FA of a DAG tend to have prominent fragment ion $[M + Li - FA]^+$ and undetectable fragment ion $[FA + Li]^+$. We have quantified DAG containing two normal FA in the soybean oil bioconversion product by HPLC using an evaporative light scattering detector [13].

The MS spectra of DAG containing hydroxy FA (one or two hydroxy FA) were simple as shown in Figs. 1 and 3. The MS spectra of DAG containing two normal FA were more complicated as shown in Fig. 4. Figure 4 is the MS^2 spectrum of DAG, OL, in the soybean oil bioconversion product from $[M + Li]^+$ at *m/z* 625.4 [13]. The most abundant fragment ion was $[M + Li - H_2O]^+$ at *m/z*

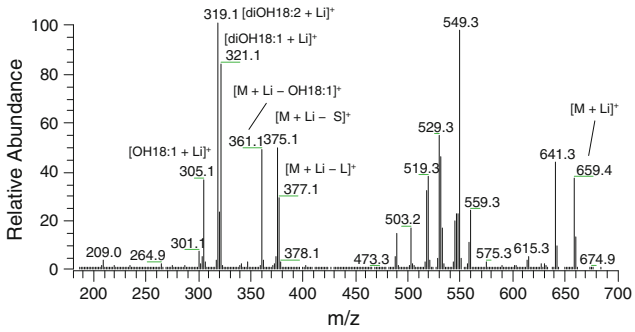

Fig. 3 The MS^2 spectrum of $[M + Li]^+$ at *m/z* 659.4 from the HPLC fraction #18 of the soybean oil bioconversion product. This is the MS^2 spectrum of three DAG, OH18:1-OH:18:1, diOH18:2-S and diOH18:1-O, combined

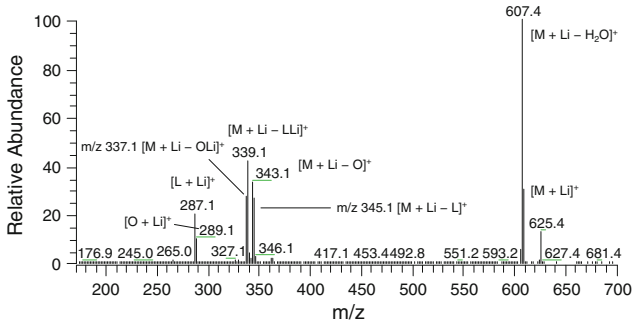

Fig. 4 The MS^2 spectrum of $[OL + Li]^+$ at *m/z* 625.4 from the HPLC fraction #31 of soybean oil bioconversion product

607.4. The other prominent fragment ions were $[M + Li - L]^+$ at *m/z* 345.1, $[M + Li - O]^+$ at *m/z* 343.1, $[M + Li - LLi]^+$ at *m/z* 339.1, $[M + Li - OLi]^+$ at *m/z* 337.1, $[O + Li]^+$ at *m/z* 289.1 and $[L + Li]^+$ at *m/z* 287.1. Their relative abundances are shown in Table 2. Table 2 shows the MS^2 characteristic ions of thirteen DAG containing two normal FA in the soybean bioconversion product (LL and LnLn were the standards). Some of the DAG in Table 2 might not be commercially available (e.g., LP, OL, OP, OS, OA, SA and PB). We have shown that the MS^2 spectrum of PP standard was identical to that of PP in the soybean oil bioconversion product. The relative abundances of the fragment ions are also given in Table 2. Consistently the most abundant fragment ions (base peak) were $[M + Li - H_2O]^+$ and these fragment ions were also shown for the MS^2 spectra of DAG and TAG containing hydroxy FA at much lower abundances (Figs. 1, 2) [7–11]. The relative abundance of $[M + Li - FA]^+$ and $[M + Li - FALi]^+$ were generally more abundant than those of $[FA + Li]^+$.

We have found only one MS^2 spectrum of DAG containing two normal FA (SS) recently reported as far as we aware by Bowden et al. [12]. The fragment ions of SS were the same as those shown in Table 2. In this recent report,

Table 2 MS^2 characteristic ions (*m/z*) and their relative abundance (% in parenthesis) of DAG containing two normal FA in soybean oil bioconversion product (LnLn and LL were the standards)

DAG	$[M + Li]^+$	$[M + Li - FA]^+$	$[M + Li - FALi]^+$	$[FA + Li]^+$	$[M + Li - H_20]^+$
LnLn	619.4	341.1(49)	335.1(30)	285.1(22)	601.4(100)
LL	623.4	343.1(24)	337.1(38)	287.1(20)	605.4(100)
LP	599.4	319.1(20), 343.1(30)	313.1(47), 337.1(26)	287.1(20), 263.1(4)	581.4(100)
OL	625.4	343.1(33), 345.1(26)	337.0(18), 339.1(42)	289.1(10), 287.1(21)	607.6(100)
PP	575.4	319.1(39)	313.0(80)	263.1(8)	557.3(100)
OP	601.4	319.1(20), 345.1(23)	313.1(44), 339.1(33)	289.1(10), 263.1(3)	583.4(100)
OO	627.4	345.1(50)	339.1(69)	289.1(19)	609.4(100)
SP	603.4	319.1(21), 347.1(25)	313.1(45), 341.1(49)	291.1(5), 263.1(4)	585.4(100)
OS	629.5	347.1(22), 345.1(24)	341.1(44), 339.1(31)	289.1(9), 291.1(3)	611.4(100)
SS	631.4	347.1(30)	341.1(59)	291.1(6)	613.4(100)
OA	657.4	375.1(25), 345.1(20)	369.1(39), 339.0(26)	289.1(8), 319.1(14)	639.4(100)
SA	659.4	375.1(20), 347.1(22)	369.1(28), 341.1(28)	291.1(2), 319.1(?)	659.4(100)
PB	659.4	403.1(32), 319.1(34)	397.1(58), 313.0(44)	263.1(2), 347.1 (?)	659.4(100)

On the columns of $[M + Li - FA]^{+\cdot}$ $[M + Li - FALi]^+$ and $[FA + Li]^+$, the *m/z* of two fragment ions are listed when the DAG containing two different FA constituents. The orders of the two *m/z* are the same as the orders of the two FA on the name of DAG shown on the DAG column. A is arachidic acid, 20:0. B is behenic acid, 22:0. DAG are arranged in the order of the HPLC fractions. SA and PB have the same mass and were eluted in the same HPLC fraction. Their *m/z* values were obtained from the same mass spectrum. Fragment ions of SA and PB had two 347.1 and two 319.1 and the two relative abundances combined are given on the column of $[M + Li - FA]^+$

fragment ions were also selected for the detection of seven DAG containing normal FA using selected reaction monitoring (SRN) [12].

Conclusions

We have used the MS^2 fragment ions of $[M + Li - FA]^+$ and $[FA + Li]^+$ for the identification of DAG containing hydroxy FA (one or two hydroxy FA) in castor oil. The additional fragment ions, $[M + Li - FALi]^+$, were used for the identification of DAG containing two normal FA. The MS^2 spectra of DAG containing hydroxy FA (Figs. 1, 2) were simple compared to those of DAG containing two normal FA (Fig. 4), mainly because the fragment ions from the neutral losses of FA lithium salts, $[M + Li - FALi]^+$, were not detectable for the DAG containing hydroxy FA. The MS^2 spectra of TAG of lithium adducts showed a similar pattern.

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