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Structures of hydroxy fatty acids as the constituents of triacylglycerols in Philippine wild edible mushroom, *Ganoderma lucidum*

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ABSTRACT

Edible Philippine mushrooms including *Ganoderma lucidum* have many health benefits. We have recently reported the identities and the contents of 77 molecular species of acylglycerols containing hydroxy fatty acids (HFA) in this mushroom. The structures of these HFA were proposed using the electrospray ionization mass spectrometry of the lithium adducts of acylglycerols in the HPLC fractions of the lipid extract. The proposed structures of the HFA were $OH^{13}19{:}1^{10}$, $OH^{13}19{:}1^{14}$, $2OH^{11,12}18{:}0$, $2OH^{11,12}18{:}1^{9}$, $2OH^{11,12}18{:}2^{9,13}$, $3OH^{11,12,13}18{:}1^{9}$ and $3OH^{11,12,13}18{:}1^{14}$. The locations of the hydroxyl groups and double bonds were near the middle of the HFA chains. The structures were the same or similar to that of HFA in castor oil except that the HFA of odd numbered carbon atoms were not detected in castor oil. OH18:1 was identified in the mushroom and its structure was not $OH^{12}18{:}1^{9}$ (ricinoleate) as that in castor oil. Ricinoleate and/or HFA (OH18:1) structurally similar to ricinoleate was likely the biological precursors of these HFA in mushroom.

1. Introduction

Some Philippine wild edible mushrooms have been studied for their nutritional and functional activities. Fruiting bodies of mushrooms like *Schizophyllum commune, Lentinus tigrinus, Lentinus sajor-caju, Ganoderma lucidum, Collybia reinakeana* and *Paneaolus antillarium* exhibited anti-diabetic, antibacterial, anti-inflammatory, antioxidant, anti-hypertensive, and anti-coagulative properties (Reyes et al., 2004; Dulay et al., 2014, 2015; Eguchi et al., 2014). The high contents of HFA (hydroxy fatty acids) and the presence of HFA of odd numbered carbon atoms in this Philippine mushroom (Hou et al., 2017a, 2017b) might be related to one or more of its health benefits.

We have recently reported the identification and quantification of seventy-seven molecular species of acylglycerols (AG) containing HFA in Philippine wild edible mushroom, *Ganoderma lucidum* (Hou et al., 2017b). We used mass spectrometry of the HPLC fractions of the lipid extract to obtain the masses of HFA, *m/z* of $[HFA + Li]^+$, and the numbers of hydroxyl groups $[HFA + Li - n\ H_2O]^+$ (n = 1, 2, or 3). Therefore, we obtained the simple formula of the HFA, e.g., 2OH18:2 with two hydroxyl groups, 18 carbon atoms and two double bonds. The MS fragment ions other than the dehydration of HFA, $[HFA + Li - n\ H_2O]^+$, could be used to elucidate the structures of HFA. We report here the structures of some HFA in this Philippine mushroom. This report is the continuation of our lipid analysis of Philippine mushroom (Hou et al., 2017b).

2. Material and methods

The source of mushroom strain, mass production, and extraction of lipids were the same as our recent reports (Hou et al., 2017a).

2.1. HPLC fractionation of the molecular species of acylglycerols in mushroom lipid extract

The mushroom lipid extract (1 mg in 50 μL ethanol) was fractioned using a C_{18} analytical column with a linear gradient from 100% methanol to 100% 2-propanol in 40 min, at 1 mL/min flow rate (Lin et al., 1997). UV detector at 205 nm was used for HPLC fractionation. Fractions were collected every 30 s and corresponding fractions were pooled from eight 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 tenth of each HPLC fraction with 50 μL of methanol solution of 100 mM lithium acetate and diluting to a total volume of 250 μL.

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2.2. 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 lithiated parent ions which were subsequently fragmented for MS^2, MS^3, and MS^4 analysis.

3. Results and discussion

The mushroom lipid extract was fractionated by C_{18} HPLC (Hou et al., 2017b). MS spectra of HFA were obtained from the HPLC fractions of mushroom lipid extract and were usually the MS^3 or MS^4 of the molecular species of TAG, $[TAG + Li]^+$. The *m/z* of the fragment ions of HFA as $[HFA + Li]^+$ usually can be detected at the MS^2 spectrum of the *m/z* of the molecular species of triacylglycerol as $[TAG + Li]^+$. This fragment ion of $[HFA + Li]^+$ at the MS^2 was used to obtain the mass spectrum of HFA (MS^3).

We have recently reported the MS^3 spectrum of $[OH18:1 + Li]^+$ at *m/z* 305.2 from the MS^2 spectrum of $[PP\text{-}OH18:1 + Li]^+$ at *m/z* 855.6 from the HPLC fraction #57 of mushroom lipid extract (Fig. 5 of Hou et al. (2017b)). We have also reported earlier the MS spectrum of ricinoleate standard ($OH^{12}18:1^9$, Sigma, from castor oil) (Fig. 7 of Lin et al. (2009)) with the fragment ion of $[OH18:1 + Li - C_6H_{13}CHO]^+$ at *m/z* 191.1, from the cleavage of C_{11-12}. The structures of these two OH18:1 were different, because the mass spectrum of the OH18:1 in the mushroom did not show the fragment ion of *m/z* 191.1. The locations of the hydroxyl group and double bond of the OH18:1 in the mushroom was unknown because there was no significant fragment ion in the mass spectrum, except the fragment ion from dehydration at *m/z* 287.2 (Hou et al., 2017b). The distance between the hydroxyl group and double bond of the OH18:1 in mushroom was not the same as that of ricinoleate (two single bonds apart). Three MS spectra of $[OH18:1 + Li]^+$ from different molecular species of TAG in the mushroom were obtained and they were identical and no ricinoleate was detected in the mushroom. Similarly, due to the lack of significant fragment ions of the mass spectrum of $[OH18:2 + Li]^+$ at *m/z* 303.1 (MS^3), the locations of the hydroxyl group and the two double bonds on this C_{18} chain could not be elucidated (HPLC fraction #45, LP-OH18:2, Table 1 of Hou et al. (2017b)). In castor oil, monohydroxy FA were identified as $OH^{12}18:1^9$ (ricinoleic acid), $OH^{12}18:2^{9,13}$ and $OH^{14}20:1^{11}$ (lesquerolic acid) (Lin and Chen, 2012). In lesquerella oil, monohydroxy FA were identified as $OH^{12}18:1^9$ (ricinoleic acid), $OH^{12}18:2^{9,14}$, $OH^{12}18:3^{9,14,16}$, $OH^{14}20:1^{11}$ (lesquerolic acid) and $OH^{14}20:2^{11,17}$ (auricolic acid) (Lin and Chen, 2013).

Fig. 1 is the MS^3 spectrum of $[OH19:1 + Li]^+$ at *m/z* 319.2 from the MS^2 spectrum of $[PP\text{-}OH19:1 + Li]^+$ at *m/z* 869.7 from the HPLC fraction #48 of the mushroom lipid extract. Part of the Fig. 1 (the top chromatogram panel) was published (Fig. 6 of Hou et al. (2017b)) and the simple formula of the FA as OH19:1 was proposed recently (Hou et al., 2017b). We would like to propose here the structure of this fatty acid using the proposed fragmentation mechanisms as shown in Fig. 1A-C. Fig. 1A is for the fragment ion at *m/z* 205 and the proposed structure is $OH^{13}19:1^{10}$. Fig. 1B is for the fragment ion at *m/z* 207, and the proposed structures was $OH^{13}19:1^{14}$. Fig. 1C is for the fragment ion at *m/z* 235, and the proposed structure is $OH^{13}19:1^{14}$. The proposed structures of OH19:1 were the mixture of $OH^{13}19:1^{10}$ and $OH^{13}19:1^{14}$. This confirmed that the FA was actually OH19:1, the HFA of the odd numbered carbon atoms. The ratio of the contents of $PP\text{-}OH^{13}19:1^{10}$ and $PP\text{-}OH^{13}19:1^{14}$ could be estimated by the ratio of the fragment ion intensities of *m/z* 205 and *m/z* 207 (plus *m/z* 235). Both fragment ions of *m/z* 207 and *m/z* 235 were from the same FA $OH^{13}19:1^{14}$, the fragment ion intensities of these two ions should be combined for the estimation of the ratio of the contents of $OH^{13}19:1^{10}$ and $OH^{13}19:1^{14}$. Since the content of PP-OH19:1 was reported as 0.17% in the

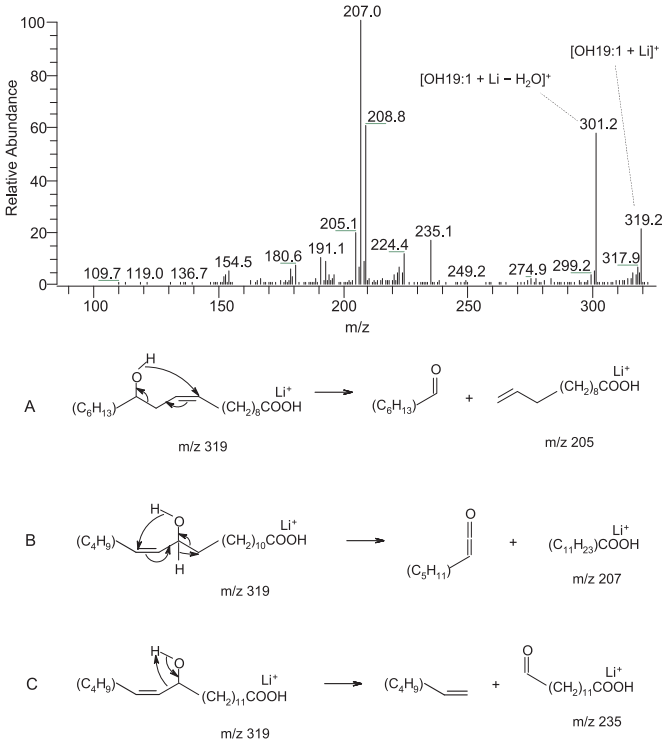

Fig. 1. MS^3 spectrum of $[OH19:1 + Li]^+$ at *m/z* 319.2 from the MS^2 spectrum of $[PP\text{-}OH19:1 + Li]^+$ at *m/z* 869.7 from the HPLC fraction #48 of the mushroom lipid extract. A, B and C were the proposed fragmentation mechanisms of fragment ions of *m/z* 205, 207 and 235.

mushroom lipid extract (Hou et al., 2017b), the contents of $PP\text{-}OH^{13}19:1^{10}$ and $PP\text{-}OH^{13}19:1^{14}$ were estimated as 0.03% and 0.14% respectively. PP-OH19:1 was one of the higher contents of 77 molecular species of acylglycerols in the mushroom lipid extract (Hou et al., 2017b). The structure of $OH^{13}19:1^{10}$ was similar to that of ricinoleate ($OH^{12}19:1^9$) from castor oil except the addition of one carbon atom. We did not detect odd carbon numbered FA in lesquerella oil (Lin and Chen, 2013) and castor oil (Lin et al., 2009, 2013a; Lin and Chen, 2010, 2011, 2012), with the exception of finding the triacylglycerol RR-23:0 in castor oil (Lin and Chen, 2012), R is ricinoleate. We also did not detect odd carbon numbered FA in the polyol oil produced from soybean oil by *Pseudomonas aeruginosa* (Hou et al., 2015).

Fig. 2 is the MS^3 spectrum of $[2OH18:1 + Li]^+$ at *m/z* 321.2 from the MS^2 spectrum of $[P\text{-}2OH18:2\text{-}2OH18:1 + Li]^+$ at *m/z* 927.7 from the HPLC fraction #26 of the mushroom lipid extract. Fig. 2 is very similar to Fig. 5 in Lin et al. (2009), the mass spectrum of $[2OH18:1 + Li]^+$ at *m/z* 321.2 from castor oil. Therefore, the structure of this FA is proposed as $2OH^{11,12}18:1^9$, the same as the 2OH18:1 in castor oil. Fig. 2 shows the fragment ions of *m/z* 207.0 and 209.0. Their proposed

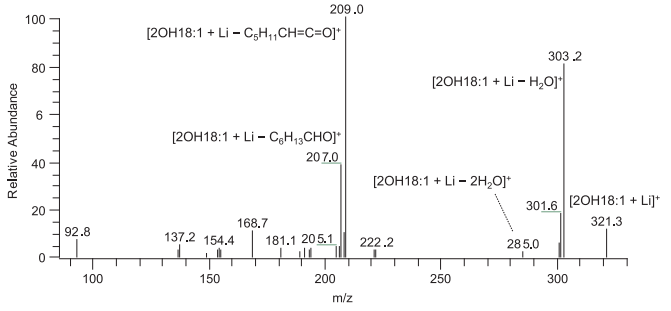

Fig. 2. MS^3 spectrum of $[2OH18:1 + Li]^+$ at *m/z* 321.2 from the MS^2 spectrum of $[P\text{-}2OH18:2\text{-}2OH18:1 + Li]^+$ at *m/z* 927.7 from the HPLC fraction #26 of the mushroom lipid extract.