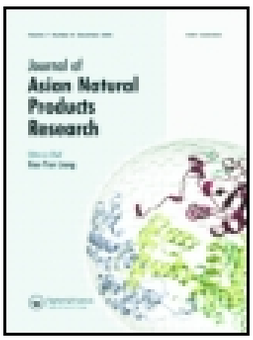

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The major zeaxanthin dipalmitate derivatives from wolfberry

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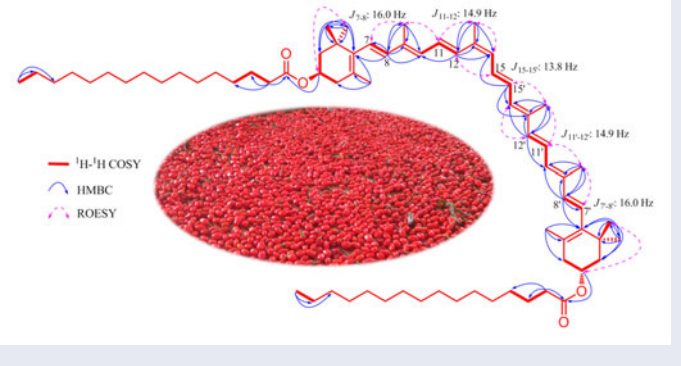
ABSTRACT

Zeaxanthin dipalmitate (**3**) and two zeaxanthin dipalmitate derivatives, including one new compound (**1**), were obtained from wolfberry [the fruit of *Lycium barbarum* L. (Solanaceae)]. Their structures were unambiguously elucidated by spectroscopic analyses. Compound **2** is isolated from the genus *Lycium* for the first time, and its 1D/2D NMR data are firstly reported. All the compounds belong to carotenoids which are a kind of major bioactive constituents in wolfberry and are also responsible for wolfberry's red color.



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Lycium barbarum L.; Solanaceae; wolfberry; carotenoids; zeaxanthin dipalmitate

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1. Introduction

Carotenoids are isoprenoid compounds (mostly C_{40}) with polyene chains that may contain up to 15 conjugated double bonds [1]. Carotenoids play an important role in human nutrition and health, providing provitamin A and having antioxidative, anticancer, and hepatoprotective activities [1–3]. However, carotenoids generally cannot be produced in the human body, which are synthesized by photosynthetic organisms as well as some non-photosynthetic bacteria and fungi [4, 5]. The fruit of *Lycium barbarum* L. (Solanaceae), named wolfberry, is known to be a rich source of carotenoids, and the predominant carotenoid is zeaxanthin dipalmitate [5–7]. Some minor carotenoids, mainly esterified constituents, were also detected from wolfberry by LC-MS, including β-cryptoxanthin monopalmitate and its two isomers, zeaxanthin monopalmitate and its two isomers, and zeaxanthin [7–9]. Due to the low polarity and complex structure of carotenoids, especially asymmetric esterified compounds, the purification and identification are difficult. The phytochemical study on carotenoids in wolfberry still remains inadequate [10].

In our previous phytochemical study on water-soluble constituents of wolfberry, 72 compounds were reported [11–14]. Herein, the investigation of lipid-soluble constituents of wolfberry led to the identification of zeaxanthin dipalmitate (**3**) and its two geometrical isomers (**1** and **2**) (Figure 1). **1** is a new compound, and **2** is isolated from the genus *Lycium* for the first time. **2** was only detected in *Physalis alkekengi* L. belonging to the family of Solanaceae by LC–MS in the past, and its 1D/2D NMR data are firstly reported herein [15]. Details of isolation and structure identification of **1**−**3** are reported in this paper.

2. Results and discussion

Compound **1** was obtained as dark red amorphous powders. UV spectrum of **1** exhibited absorption maxima at 283, 346, 431, 457, and 483 nm, which were similar to those of the known compound zeaxanthin dipalmitate (**3**), suggesting the presence of the

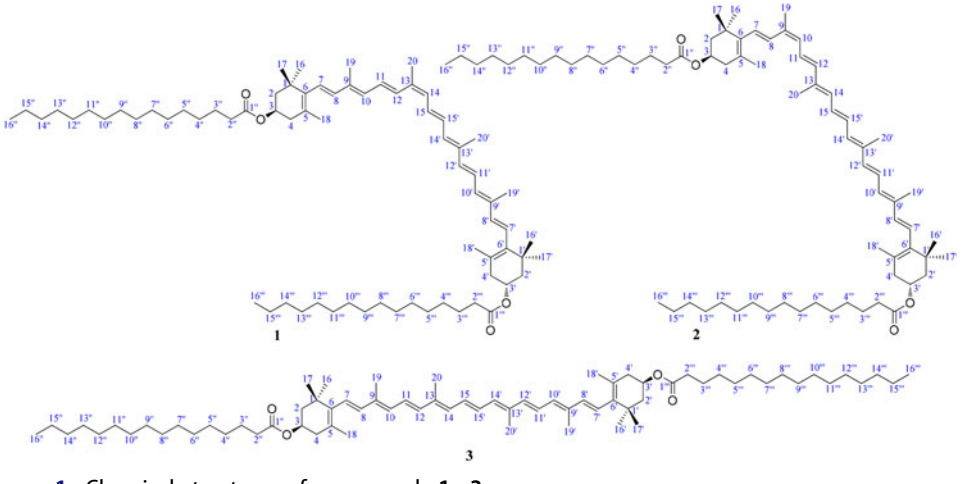

Figure 1. Chemical structures of compounds **1**−**3**.

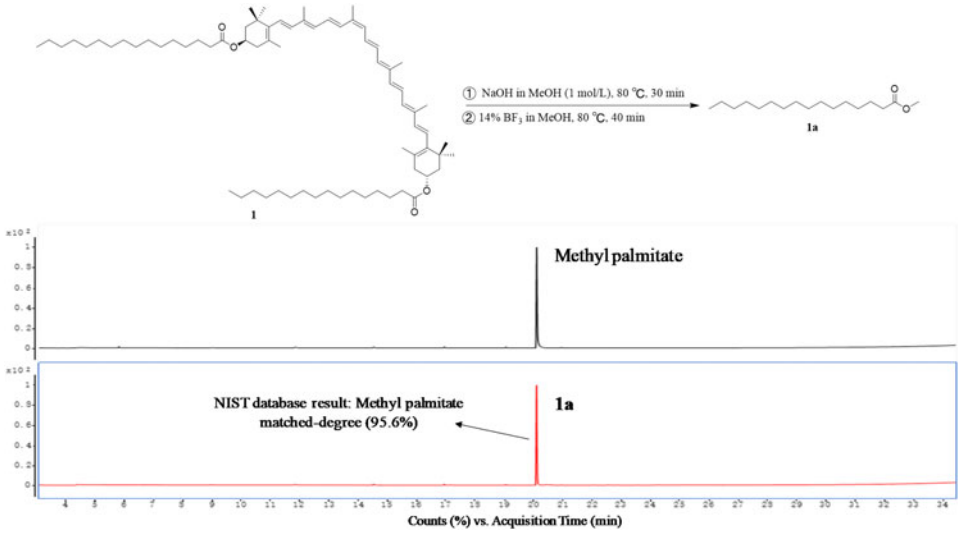

Figure 2. The product (**1a**) prepared from **1** and methyl palmitate were compared by GC–MS.

polyene chain. The molecular ion, $M^{\bullet+}$ at *m/z* 1044.8852 by HR-APCI-MS showed that the molecular formula of **1** was $C_{72}H_{116}O_4$ (15 degrees of unsaturation), which was the same as that of zeaxanthin dipalmitate (**3**). GC–MS analysis of products obtained from alkaline hydrolysis and derivatization reaction exhibited that **1** contained palmitic acid moieties (Figure 2) [16, 17]. The ^{13}C NMR spectrum of **1** showed 72 carbons. Based on the DEPT-135 data, these carbons could be categorized into two carbonyl, 22 aromatic or olefinic carbons (including 14 sp^2 methine carbons), two sp^3 quaternary carbons, two oxygenated sp^3 methine carbons, 32 sp^3 methylene carbons, and 12 methyl carbons. The ^1H NMR spectrum displayed that **1** had 14 aromatic or olefinic protons, two oxygenated sp^3 methine protons, 32 sets of sp^3 methylene protons, and 12 sets of methyl protons. The proton signals were associated with the directly attached carbon atoms in the HSQC experiment. The analysis of the $^1H-^1H$ COSY experiment and the coupling values of the protons (Figure 3) showed the presence of 11 subunits [H_2-2 − H-3 − H_2-4, H-7 − H-8, H-10 − H-11 − H-12, H-14 − H-15 − H-15′−H-14′, H_2-2′−H-3′−H_2-4′, H-7′−H-8′, H-10′−H-11′−H-12′, H_2-2″−H_2-3″−H_2-4″, H_2-15″−H_3-16″, H_2-2‴−H_2-3‴−H_2-4‴, and H_2-15‴−H_3-16‴]. Based on these deduced subunits, molecular formula, degrees of unsaturation, and alkaline hydrolysis [16, 17], the key HMBC correlations shown in Figure 3 deduced the planar structure of **1**. The assignments of all proton and carbon resonances are provided in Table 1.

The coupling values ($J_{7/7',8/8'}$ = 16.0 Hz, $J_{11,12}$ = 14.9 Hz, $J_{15,15'}$ = 13.8 Hz, $J_{11',12'}$ = 14.9 Hz) and the key ROESY correlations (between H-12 and H-15, between H-19 and H-7/H-11, between H-20 and H-11/H-14, between H-12′ and H-14′, between H-19′ and H-7′/H-11′, between H-20′ and H-11′/H-15′) exhibited that the geometrical configurations of the related double bonds in **1** were (7*E*, 9*E*, 11*E*, 13*Z*, 15*E*, 7′*E*, 9′*E*, 11′*E*, 13′*E*). In plant, naturally occurring zeaxanthin possessed only (3*R*,3′*R*) configuration [18, 19]; Further, native zeaxanthin dipalmitate from wolfberry was proved to be only present in the (3*R*,3′*R*) form [20]. Due to the coexistence with zeaxanthin and zeaxanthin dipalmitate, the absolute configuration of **1** was deduced to be

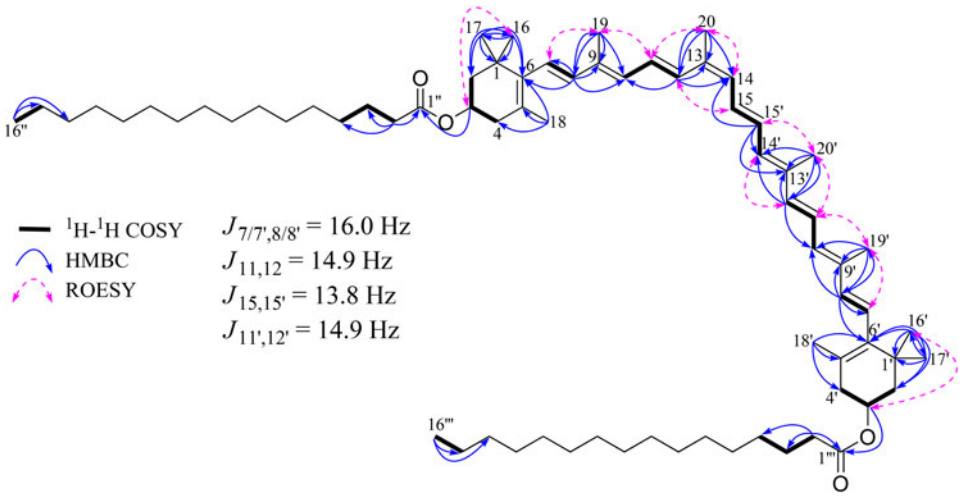

Figure 3. Key 2D NMR correlations of compound **1**.

(3*R*,3′*R*). Therefore, the structure of **1** was identified as (3*R*,3′*R*,13*Z*)-3,3′-*O*-dipalmitoyl-*β*-carotene, and named 13*Z*-zeaxanthin dipalmitate.

Similarly to the structural elucidation of **1**, compounds **2** and **3** were identified as 9*Z*-zeaxanthin dipalmitate (CAS: 2125473-36-9; Supporting Information Table S2) and zeaxanthin dipalmitate (CAS: 144-67-2; Supporting Information Table S3), respectively. 9*Z*-zeaxanthin dipalmitate (**2**) was only tentatively inferred from *Physalis alkekengi* by LC–MS [15]. It is the first time for **2** to be reported from the genus *Lycium,* and it is also the first time to report its 1D/2D NMR data.

Compared with zeaxanthin dipalmitate (**3**), the carbon resonances near the *Z*-double bond of **1** and **2** obviously shifted. C-12 in **1** and C-8 in **2** distinctly shifted to upfield, while C-20 in **1** and C-19 in **2** significantly moved to downfield (Table 1). Compared to **3**, the obvious shifts of C-12 and C-20 in **1** were caused by *γ*-effect of the large substituent groups at C-14. Similarly, the distinct shifts of C-8 and C-19 in **2** were also caused by *γ*-effect of the large substituent groups at C-10 compared to **3**.

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded on an Anton Paar MCP 200 high precision intelligent polarimeter (Anton Paar Co. Ltd, Graz, Austria). UV data were measured on a JASCO V-550 UV/Vis spectrometer (Jasco International Co. Ltd, Tokyo, Japan). IR data were recorded using a NICOLET IS5 FT-IR spectrometer (Thermo Fisher Scientific Inc., Sunnyvale, USA). The GC-MS data were obtained on an Agilent Technologies 7890B GC system equipped with an Agilent Technologies 5977A MSD, and an Agilent Technologies 7693 Autosampler using an Agilent HP-5ms Ultra Inert column (30 m × 250 μm × 0.25 μm) (Agilent Technologies Inc., CA, USA). The HR-APCI-MS spectra were obtained on a Thermo-fisher LTQ Orbitrap XL hybrid mass

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data of **1**−**3** in $CDCl_3$ (δ in ppm, J in Hz).

	1		2		3	
No.	δ_C	δ_H[a]	δ_C	δ_H[a]	δ_C	δ_H[a]
1/1′	36.7		36.7		36.7	
2/2′	44.1	1.77, br d (12.1), Ha 1.57/1.58, t (12.1), Hb	44.0/44.1	1.78, Ha 1.57/1.59, t (11.9), Hb	44.1	1.78, ddd (12.1, 3.3, 1.6), Ha 1.58, t (12.1), Hb
3/3′	68.1	5.07	68.1	5.08	68.1	5.07
4	38.5	2.43*[4], dd (16.9, 4.8), Ha 2.10, dd (16.9, 10.4), Hb	38.5	2.46, dd (17.0, 5.5), Ha 2.12, dd (17.0, 9.9), Hb	38.5	2.44, dd (16.8, 5.6), Ha 2.11, dd (16.8, 9.4), Hb
5	125.8		125.9		125.7	
6	137.8		138.2		137.9	
7	125.2*[1]	6.08, d (16.0)	127.1	6.11	125.3	6.09, d (16.1)
8	138.6	6.13, d (16.0)	130.9	6.66, d (15.9)	138.6	6.13, d (16.1)
9	135.5		134.1		135.5	
10	131.4	6.21, d (11.6)	129.9	6.07, d (11.2)	131.4	6.16, d (11.6)
11	126.2	6.64, dd (14.9, 11.6)	123.6	6.73, dd (14.9, 11.2)	124.9	6.64, dd (14.9, 11.6)
12	129.4	6.89, d (14.9)	136.9	6.29, d (14.9)	137.6	6.36, d (14.9)
13	134.8		136.4		136.4	
14	130.9	6.11	132.5*[7]	6.24*[11], br d (9.5)	132.6	6.26, br d (9.8)
15	128.8	6.80, t (13.8)	129.9*[8]	6.63	130.1	6.63, dd (9.8, 2.8)
16	28.5	1.11, s	28.5	1.12, s	28.5	1.11, s
17	30.0	1.07*[5], s	30.0	1.08, s	30.0	1.08, s
18	21.5	1.72*[6], s	21.6	1.76, s	21.5	1.72, s
19	12.7*[2]	1.96, s	20.7	1.97, s	12.7*[12]	1.96, s
20	20.7	1.99, s	12.7*[9]	1.97, s	12.8*[12]	1.97, s
4′	38.5	2.44*[4], dd (16.9, 4.8), Ha 2.10, dd (16.9, 10.4), Hb	38.5	2.43, dd (17.2, 5.5), Ha 2.10, dd (17.2, 10.0), Hb	38.5	2.44, dd (16.8, 5.6), Ha 2.11, dd (16.8, 9.4), Hb
5′	125.8		125.7		125.7	
6′	137.8		137.8		137.9	
7′	125.6*[1]	6.08, d (16.0)	125.3	6.08, d (15.7)	125.3	6.09, d (16.1)
8′	138.6	6.13, d (16.0)	138.6	6.13, d (15.7)	138.6	6.13, d (16.1)
9′	135.5		135.5		135.5	
10′	131.4	6.16, d (11.3)	131.4	6.16, d (11.5)	131.4	6.16, d (11.6)
11′	124.7	6.63, dd (14.9, 11.3)	124.8	6.63	124.9	6.64, dd (14.9, 11.6)
12′	137.7	6.36, d (14.9)	137.6	6.36, d (14.9)	137.6	6.36, d (14.9)
13′	136.3		136.4		136.4	
14′	132.5	6.24, d (13.8)	132.6*[7]	6.25*[11], br d (9.5)	132.6	6.26, br d (9.8)
15′	129.2	6.56, t (13.8)	130.1*[8]	6.63	130.1	6.63, dd (9.8, 2.8)
16′	28.5	1.11, s	28.5	1.11, s	28.5	1.11, s
17′	30.0	1.08*[5], s	30.0	1.07, s	30.0	1.08, s
18′	21.5	1.73*[6], s	21.5	1.72, s	21.5	1.72, s
19′	12.8*[2]	1.96, s	12.8*[9]	1.97, s	12.7*[12]	1.96, s
20′	12.8*[2]	1.97, s	12.9*[9]	1.97, s	12.8*[12]	1.97, s
1″/1‴	173.6		173.6		173.6	
2″/2‴	34.7	2.28, t (6.7)	34.7	2.28/2.29, t (7.4)	34.7	2.28, t (7.0)
3″/3‴	25.0	1.62, quint (6.7)	25.0	1.62	25.0	1.62, quint (7.0)
4″/4‴	29.1*[3]	1.26	29.1*[10]	1.25	29.6*[13]	1.26
5″/5‴	29.7*[3]	1.26	29.7*[10]	1.25	29.6*[13]	1.26
6″/6‴	29.6*[3]	1.26	29.6*[10]	1.25	29.3*[13]	1.26
7″/7‴	29.7*[3]	1.26	29.7*[10]	1.25	29.7*[13]	1.26
8″/8‴	29.3*[3]	1.26	29.3*[10]	1.25	29.7*[13]	1.26
9″/9‴	29.7*[3]	1.26	29.7*[10]	1.25	29.1*[13]	1.26
10″/10‴	29.6*[3]	1.26	29.6*[10]	1.25	29.6*[13]	1.26
11″/11‴	29.5*[3]	1.26	29.5*[10]	1.25	29.4*[13]	1.26
12″/12‴	29.7*[3]	1.26	29.7*[10]	1.25	29.7*[13]	1.26
13″/13‴	29.3*[3]	1.26	29.3*[10]	1.25	29.3*[13]	1.26
14″/14‴	31.9	1.26	31.9	1.25	31.9	1.26
15″/15‴	22.7	1.28	22.7	1.31	22.7	1.29
16″/16‴	14.1	0.88, t (6.7)	14.1	0.88, t (6.7)	14.1	0.88, t (6.9)

*Assignment may be interchanged. [a]The indiscernible signals due to overlap or having the complex multiplicity are reported without designating multiplicity.

spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source (Thermo Fisher Scientific Inc.). The NMR spectra were acquired with Bruker AV 400 and 600 spectrometers (Bruker BioSpin Group, Faellanden, Switzerland) using the solvent signals ($CDCl_3$: δ_H 7.26/δ_C 77.0) as internal standards. The analytical HPLC was performed on a Shimadzu HPLC system equipped with a LC-20AT pump, a SPD-M20A DAD, a CTO-10AS VP column oven, and a SIL-20A autosampler (Shimadzu Inc., Kyoto, Japan) using a Cosmosil Packed Cholester column ($4.6 \times 250\,mm^2$, $5\,\mu m$) (Nacalai Tesque Inc., Kyoto, Japan). The preparative HPLC was performed on a Shimadzu LC-6-AD liquid chromatography (Shimadzu Inc.) with a SPD-20A detector using a Cosmosil Packed Cholester column ($20.0 \times 250\,mm^2$, $5\,\mu m$). Column chromatography (CC) was performed on silica gel ($200-300$ mesh, Haiyang Chemical Co. Ltd, Qingdao, China).

3.2. Plant material

The fruit of *Lycium barbarum* L. (Solanaceae) was collected (Zhongning County, Ningxia Hui Autonomous Region, China) and identified by one of the authors (Ying Wang) in 2016. A voucher specimen (LYBA-2016-NX-ZN) was deposited in the Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou, China.

3.3. Extraction and isolation

The dried wolfberry (45.0 kg) was cold-soaked and extracted four times with 100 L of $CHCl_3$ for 24 h each time to yield an extract (244.3 g). The extract was subjected to open silica gel CC ($15.0 \times 100.0\,cm^2$) using a successive elution of cyclohexane-CH_2Cl_2-MeOH (96:4:0, 93:7:0, 90:10:0, 80:20:0, 70:30:0, 60:40:0, 50:50:0, 40:60:0, 0:100:0, 0:50:50, 0:0:100, v/v/v), yielding fractions F1 – F19. A portion (0.2 g) of fraction 7 (18.0 g) was isolated using preparative HPLC [45% methyl tert-butyl ether – MeOH, 10 ml/min] to yield **3** (t_R: 31.2 min, 120.0 mg), **2** (t_R: 35.6 min, 24.5 mg), and **1** (t_R: 39.0 min, 27.3 mg).

3.3.1. Structural characterization of new compound (13Z-zeaxanthin dipalmitate, 1)

Dark red amorphous powders; $[\alpha]_D^{25}$ – 66.0 (*c* 0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 283 (4.06), 346 (4.24), 431 (4.54), 457 (4.65), 483 (4.57) nm; IR (KBr) ν_{max} 2923, 2853, 1732, 1466, 1364, 1172, 966 cm^{-1}; ^1H and ^{13}C NMR spectral data see Table 1; HR-APCI-MS (positive): *m/z* 1044.8852 $[M]^{\bullet+}$ (calcd. for $C_{72}H_{116}O_4$, 1044.8868).

3.4. Alkaline hydrolysis

Alkaline hydrolysis was performed using the method described by Pintea and Blas *et al.* with minor modifications [16, 17]. Compound **1** (5.0 mg) was dissolved in hexane (0.5 ml) and saponified with 2.0 ml of 1.0 mol/L NaOH in MeOH for 30 min at 80 °C. Then, 1.0 ml of 14% BF_3 in MeOH was directly added to the reaction mixture

for 40 min at 80 °C and then cooled. 3.0 ml of hexane and 5.0 ml of water saturated with NaCl were added and then the mixed solution was centrifuged at 1000 rpm for 5 min. Upper organic layer was filtered through a 0.22 μm membrane filter and transferred into a GC vial for analysis.

The GC oven temperature was programmed as follow: initial temperature 50 °C for 2 min and an increase rate of 8.0 °C/min up to 310 °C. Helium was used as a carrier gas with flow rate at 1.0 ml/min. The inlet temperature was set at 270 °C. The injection volume was 1.0 μl with split radio of 100:1. The mass spectrometer was operated in electron impact (EI) mode (70 eV). The transfer line temperature was set at 280 °C and the ion source temperature at 230 °C. The compounds were identified by comparison of mass spectra and retention time with those of reference standard (methyl palmitate) and those available in libraries (NIST database).

Disclosure statement

No potential conflict of interest was reported by the authors.

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