Phytochemistry 69 (2008) 2856–2861

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Glycerogalactolipids from the fruit of *Lycium barbarum*

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ARTICLE INFO

Article history:
Received 23 May 2008
Received in revised form 21 July 2008
Available online 30 October 2008

Keywords:
Lycium barbarum
Solanaceae
Goji berries
Glycerogalactolipids

ABSTRACT

Four glycerogalactolipids (**1–4**), together with 11 other previously known homologues were isolated from the fruit of *Lycium barbarum*. Their structures were elucidated by chemical analyses including regio-selective enzymatic, alkaline and acidic hydrolyses and spectroscopic methods involving GCMS, HRESIMS and 1D and 2D NMR, respectively.

1. Introduction

Lycium barbarum L. (Solanaceae) is distributed from the south-east of Europe to China (Mabberley, 2000). It is a well known traditional Chinese medicine, recorded as Gouqizi in the Chinese pharmacopoeia (The Pharmacopoeia Commission, 2005). Goji berries have a long history of use for the treatment of eye problems, skin rashes, psoriasis, allergies, insomnia, chronic liver disease, diabetes, tuberculosis, and kidney disorders. There are many ways that people consume this fruit for example; eating raw, drinking juice and/or smoothies, mixed with tea, and added to trail mix, cereals, muffins, energy bars or soups. The pharmacological activities associated with *L. barbarum* include hypoglycemic, immunomodulation, anti-hypertension, lipotropic, protecting hepatic function, anti-aging, anti-fatigue, antioxidant and so on (Yu et al., 2006). In spite of a number of phytochemical and bioactivity related reports on saccharides of *L. barbarum* fruit (Yoshiko et al., 2004; Lin et al., 2008), its non-polar constituents have been rarely explored. A comprehensive phytochemical investigation of the non-polar constituents of Goji berries was carried out as part of our program to identify chemical and/or biomarkers of the dietary supplements. In this report, the isolation and characterization of four new and 11 known glycerogalactolipids are described. Their structures were determined by chemical methods including regio-selective enzymatic, alkaline and acidic hydrolyses and spectroscopic analyses involving GCMS, HRESIMS and 1D and 2D NMR.

2. Results and discussion

The sugar constituents of the concentrated methanolic extract of Goji berries were removed by precipitation with acetonitrile. Fifteen glycerogalactolipids were separated from the acetonitrile soluble part by repeated column chromatography over normal and reversed-phase (RP-C_{18}) silica gel. Compound **1** was obtained as a colorless gum. The absorption bands observed at 3380 and 1737 cm^{-1} in its IR spectrum indicated the presence of hydroxyl and ester functions. The quasimolecular ion in the negative HRESIMS of **1** at *m/z* 1209.7792 $[M + Cl]^-$ corresponded to the molecular formula of $C_{67}H_{114}O_{16}$. The ^{13}C NMR spectrum showed five well differentiated groups of resonances between δ_C 10–40 (alkyl chain), δ_C 60–75 (13 methine and methylene carbons of saccharides and glycerol), δ_C 101.2 and 105.7 (two anomeric carbons), δ_C 125–135 (12 olefinic carbons), and about δ_C 174 (three acid carbonyl carbons). The oxygenated carbon resonances at δ_C 63.7 (CH_2), 71.2 (CH) and 68.3 (CH_2) suggested a glycerol moiety. The above spectroscopic data indicated that **1** was a glyceroglycolipid containing a disaccharide moiety and three fatty acid units. The olefinic resonances centered at δ_H 5.49 in the ^1H NMR spectrum can be attributed to the isolated double bonds in the fatty acid residues. An intense multiplet at about δ_H 1.25 corresponded to the aliphatic methylene protons, whereas the resonances at δ_H 4.0–6.0 accounted for disaccharide and glycerol moieties. The ^1H and ^{13}C NMR spectroscopic data assignment of **1** was facilitated by comparison with those of identical published compounds (Reshef

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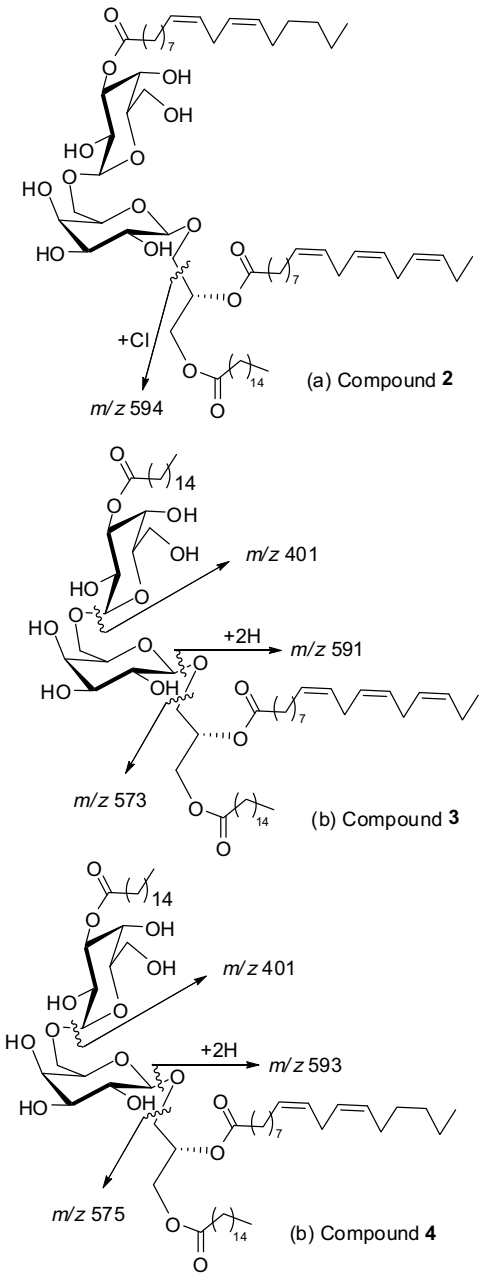
0031-9422/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.phytochem.2008.09.002

et al., 1997; Jung et al., 1996; Murakami et al., 1991) and confirmed by ^1H–^1H COSY, HMQC and HMBC spectra. The down-field shifted C-6′ methylene (δ_C 68.3) indicated the C-1″-C-6′ linkage of the sugar units (Murakami et al., 1991). The down-field shift of H-3″ (δ_H 5.83) and C-3″ (δ_C 75.3) and the up-field shift of C-2″ (δ_C 68.7) and C-4" (δ_C 67.8), when compared with those of the glycerodigalactolipids with no acyl unit at galactose (Murakami et al., 1991; Reshef et al., 1997; Jung et al., 1996), suggested the third fatty acid unit at C-3″. The sugar units were identified as D-galactose by GC analysis of their acetylated thiazolidine derivatives (Hara et al., 1987; Ali and Khan, 2008). The β and α configurations of the galactose units were deduced from the coupling constant values of the anomeric protons at δ_H 4.69 (*d*, *J* = 6.8 Hz, H-1′) and δ_H 5.53 (*d*, *J* = 3.1 Hz, H-1″). The HMBC correlations of H-1′ with C-3 and that of H-1″ with C-6′ confirmed a β-galactose linked to glycerol and α-galactose to C-6′. Alkaline hydrolysis with NaOMe–MeOH of **1** yielded methyl linolenate (methyl 9*z*,12*z*,15*z*-octadecatrienoate) and methyl palmitate (methyl hexadecanoate), which showed same retention times (t_R for methyl linolenate 9.79 min and t_R for methyl palmitate 8.85 min) on GCMS analysis as those of the standards (Sigma–Aldrich). As two peaks were observed in the GCMS analysis, it was concluded that two of the three fatty chains are identical. According to the molecular mass observed in the HRESIMS and the higher intensity of the peak related to methyl linolenate in the GCMS confirmed two linolenoyl and one palmitoyl units in compound **1**. The *Z*-geometry of the double bonds in the fatty acid units was supported by (a) absorption band observed at 721 cm^{-1} in the IR spectrum (about 967 cm^{-1} in case of trans double bond) (b) chemical shifts of the methylene carbons adjacent to the double bonds appeared at δ_C 26–28 in the ^{13}C NMR spectrum of **1**, while those for the *E*-geometry appear at δ_C 32–33 (Jung et al., 1996). Regio-selective enzymatic hydrolysis of **1**, using Lipase type XIII from *Pseudomonoas* sp. (liberating mostly the acyl moiety at C-1 of glycerol) afforded mostly palmitic acid (hexadecanoic acid), which was identified after methanolysis by GCMS analysis (t_R for methyl palmitate 8.85 min). Thus the palmitoyl at C-1 and the two linolenoyl units at C-2 and C-3″ were assigned. The configuration at C-2 was determined to be *S* by comparing the specific rotation sign $[\alpha]_D^{27}$ + 40.0 (*c*, 1.0, MeOH) with that of the identical glyceroglactolipids containing β-D-galactose at C-3 and α-D-galactose at C-6″ (Murakami et al., 1991; Reshef et al., 1997). Consequently, the structure of **1** was determined as (2*S*)-1-*O*-palmitoyl-2-*O*-linolenoyl-3-*O*-[α-D-galactopyranosyl-(1″ → 6′)-(3″-*O*-linolenoyl)-β-D-galactopyranosyl]glycerol.

Compound **2**, a colorless gum, displayed a quasimolecular ion in the negative HRESIMS at *m*/*z* 1211.7951 $[M + Cl]^-$, which corresponded to the molecular formula of $C_{67}H_{116}O_{16}$. Two extra hydrogen atoms in the molecular formula of **2**, as that of **1**, suggested the hydrogenation of one of the double bonds in **2**. The ^1H and ^{13}C NMR spectra of **2** were found to be identical to those of **1** except for resonances of one double bond begin absent in **2**. Similar to **1**, the sugars were identified as β-D-galactopyranose at C-1 and α-D-galactopyranose at C-6″. Alkaline hydrolysis with NaOMe-MeOH of **2** yielded methyl linolenate, methyl linoleate (methyl 9*z*,12*z*-octadecadienoate) and methyl palmitate, which were identified by GCMS analysis on having same retention times (t_R for methyl linolenate: 9.79 min, t_R for methyl linoleate: 9.75 min and t_R for methyl palmitate: 8.85 min) as those of the standards (Sigma–Aldrich). Regio-selective enzymatic hydrolysis of **2** afforded mostly palmitic acid, which was identified after methanolysis by GCMS analysis as a similar manner to that of **1** and helped in assigning the palmitoyl residue at C-1. The fragment ions at *m*/*z* 594 in the negative ESIMS spectrum supported the attachment of linolenoyl at C-2 of the glycerol and linoleoyl at C-3" of β-D-galactose (see Scheme 1).

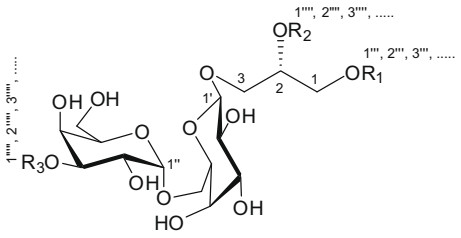


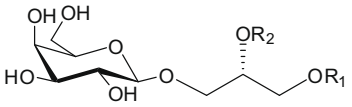
Scheme 1. Fragments observed in ESIMS in (a) negative and (b) positive modes.

The quasimolecular ion observed in the negative HRESIMS of **3** at *m*/*z* 1187.7988 $[M + Cl]^-$ corresponded to the molecular formula of $C_{65}H_{116}O_{16}$. The ^1H and ^{13}C NMR spectra of **3** were identical to those of **1** and **2** except for the olefinic resonances counting for three double bonds in **3**. Alkaline hydrolysis of **3** with NaOMe–MeOH yielded methyl linolenate and methyl palmitate, which were identified by GCMS analysis as for **1**. One linolenoyl and two palmitoyl units were suggested in **3** according to the molecular mass observed in the HRESIMS. The palmitoyl residue at C-1 was supported by the regio-selective enzymatic hydrolysis of **3**, which liberate mostly palmitic acid, identified after methanolysis by GCMS analysis in a similar manner to that of **1**. Thus, it was concluded that one palmitoyl residue was attached to C-1. On the basis of the fragment ions at *m*/*z* 401, 573 and 591 observed in the positive ESIMS (see Scheme 1), linolenoyl at C-2 and palmitoyl at C-3″ moieties were established.

Similar to compounds **1**–**3**, compound **4** was obtained as a colorless gum. A quasimolecular ion in the negative HRESIMS of **4** at *m*/*z* 1189.8124 $[M+Cl]^-$ led to the molecular formula of $C_{65}H_{118}O_{16}$. When the ^1H and ^{13}C NMR spectra of **4** were compared with those of the **1**–**3**, two double bonds were suggested in **4** due to four resonances between δ_C 125 and 135. The methyl linoleate and methyl palmitate obtained from the alkaline hydrolysis of **4** with NaOMe–MeOH were identified by GCMS analysis as for compound **2** and according to the molecular mass one linoleoyl and two palmitoyl units were suggested. The palmitoyl unit at C-1 was established from the regio-selective enzymatic hydrolysis of **4** in a similar manner to that of compounds **1**–**3**. The fragment ions observed at *m*/*z* 401, 575 and 593 in the positive ESIMS spectrum helped in assigning the linoleoyl at C-2 and palmitoyl at C-3″ (see Scheme 1).

In compounds **2**–**4**, the *Z*-geometry of the double bonds in the fatty acid units, the *S* configuration at C-2, the sugar units as D-galactopyranosyl]glycerol (**7**), (2*S*)-1-*O*-linolenoyl-2-*O*-linoleoyl-3-*O*-[α-D-galactopyranosyl-(1″ → 6′)-β-D-galactopyranosyl]glycerol (**8**), (2*S*)-1-*O*-palmitoyl-2-*O*-linolenoyl-3-*O*-[α-D-galactopyranosyl-(1″ → 6′)-β-D-galactopyranosyl]glycerol (**9**), (2*S*)-1-*O*-palmitoyl-2-*O*-linoleoyl-3-*O*-[α-D-galactopyranosyl-(1″ → 6′)-β-D-galactopyranosyl]glycerol (**10**), (2*S*)-1-*O*-palmitoyl-2-*O*-oleoyl-3-*O*-[α-D-galactopyranosyl-(1″ → 6′)-β-D-galactopyranosyl]glycerol (**11**), (2*S*)-1-*O*-stearoyl-2-*O*-linoleoyl-3-*O*-[α-D-galactopyranosyl-(1″ → 6′)-β-D-galactopyranosyl]glycerol (**12**), (2*S*)-1-*O*-palmitoyl-2-*O*-linolenoyl-3-*O*-β-D-galactopyranosylglycerol (**13**), (2*S*)-1-*O*-palmitoyl-2-*O*-linoleoyl-3-*O*-β-D-galactopyranosylglycerol (**14**) and (2*S*)-1-*O*-palmitoyl-2-*O*-oleoyl-3-*O*-β-D-galactopyranosylglycerol (**15**) by using similar spectroscopic and chemical methods as described for compounds **1**–**4**. Their spectroscopic data and specific optical rotations were compared with those reported in literature for the same compounds (Murakami et al., 1991; Reshef et al., 1997; Jung et al., 1996; Cateni et al., 2001).





1 R_1=Palmitoyl R_2=R_3=Linolenoyl

2 R_1=Palmitoyl R_2=Linolenoyl R_3=Linoleoyl

3 R_1=R_3=Palmitoyl R_2=Linolenoyl

4 R_1=R_3=Palmitoyl R_2=Linoleoyl

5 R_1=R_2=R_3=Palmitoyl

6 R_1=R_2=Palmitoyl R_3=H

7 R_1=R_2=Linolenoyl R_3=H

8 R_1=Linolenoyl R_2=Linoleoyl R_3=H

9 R_1=Palmitoyl R_2=Linolenoyl R_3=H

10 R_1=Palmitoyl R_2=Linoleoyl R_3=H

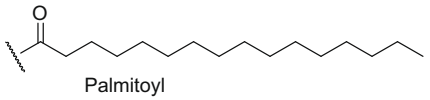
11 R_1=Palmitoyl R_2=Oleoyl R_3=H

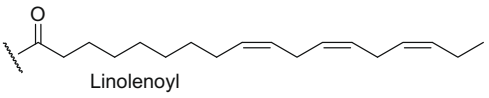
12 R_1=Stearoyl R_2=Linoleoyl R_3=H

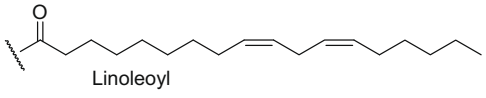
13 R_1=Palmitoyl R_2=Linolenoyl

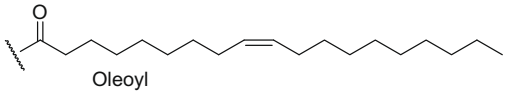
14 R_1=Palmitoyl R_2=Linoleoyl

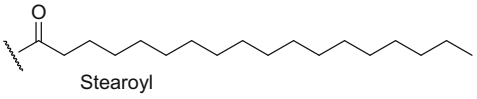
15 R_1=Palmitoyl R_2=Oleoyl











β-D-galactose and α-D-galactose and their positions at C-1 and C-6′, respectively, were determined in a similar manner to those of compound **1**. Accordingly, compounds **2**–**4** were elucidated as (2*S*)-1-*O*-palmitoyl-2-*O*-linolenoyl-3-*O*-[α-D-galactopyranosyl-(1″ → 6′)-(3″-*O*-linoleoyl)-β-D-galactopyranosyl]glycerol (**2**), (2*S*)-1-*O*-palmitoyl-2-*O*-linolenoyl-3-*O*-[α-D-galactopyranosyl-(1″ → 6′)-(3″-*O*-palmitoyl)-β-D-galactopyranosyl]glycerol (**3**) and (2*S*)-1-*O*-palmitoyl-2-*O*-linoleoyl-3-*O*-[α-D-galactopyranosyl-(1″ → 6′)-(3″-*O*-palmitoyl)-β-D-galactopyranosyl]glycerol (**4**). Known compounds **5**–**15** were elucidated as (2*S*)-1-*O*-palmitoyl-2-*O*-palmitoyl-3-*O*-[α-D-galactopyranosyl-(1″ → 6′)-(3″-*O*-palmitoyl)-β-D-galactopyranosyl]glycerol (**5**), (2*S*)-1-*O*-palmitoyl-2-*O*-palmitoyl-3-*O*-[α-D-galactopyranosyl-(1″ → 6′)-β-D-galactopyranosyl]glycerol (**6**), (2*S*)-1-*O*-linolenoyl-2-*O*-linolenoyl-3-*O*-[α-D-galactopyranosyl-(1″ → 6′)-β-

3. Concluding remarks

This is the first phytochemical investigation on the non-saccharide constituents of the fruit of *Lycium barbarum* L. The glycerogalactolipids reported in this manuscript might be useful for chemical fingerprinting of Goji berries and their related products.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a Rudolph Research Auto Pol IV automatic polarimeter. NMR spectra were recorded on a

Table 1
^{1}H and ^{13}C NMR spectroscopic data for compounds **1**–**4** in C_5D_5N

Position	**1**		**2**		**3**		**4**	
	δ_C	δ_H[a]	δ_C	δ_H[a]	δ_C	δ_H[a]	δ_C	δ_H[a]
1	63.7 *t*	4.43 *m*, 4.69 *m*	63.8 *t*	4.48 *m*, 4.73 *m*	63.7 *t*	4.46 *m*, 4.73 *m*	63.7 *t*	4.48 *m*, 4.73 *m*
2	71.2 *d*	5.68 *m*	71.3 *d*	5.71 *m*	71.2 *d*	5.71 *m*	71.2 *d*	5.71 *m*
3	68.3[b] *t*	4.04 *m*, 4.27 *m*	68.4[b] *t*	4.10 *m*, 4.33 *m*	68.3[b] *t*	4.06 *m*, 4.27 *m*	68.3[b] *t*	4.10 *m*, 4.33 *m*
1′	105.7 *d*	4.69 *d* (6.8)	105.8 *d*	4.75 *d* (7.2)	105.7 *d*	4.73 *d* (6.8)	105.7 *d*	4.75 *d* (7.2)
2′	72.4 *d*	4.38 *m*	72.5 *d*	4.42 *m*	72.4 *d*	4.40 *m*	72.4 *d*	4.42 *m*
3′	74.8 *d*	4.04 *m*	74.9 *d*	4.08 *m*	74.8 *d*	4.06 *m*	74.8 *d*	4.08 *m*
4′	70.1 *d*	4.43 *m*	70.2 *d*	4.58 *m*	70.1 *d*	4.46 *m*	70.2 *d*	4.58 *m*
5′	75.3[b] *d*	4.04 *m*	75.5[b] *d*	4.10 *m*	75.3[b] *d*	4.06 *m*	75.4 *d*	4.10 *m*
6′	68.3[b] *t*	4.04 *m*, 4.38 *m*	68.4[b] *t*	4.10 *m*, 4.42 *m*	68.3[b] *t*	4.06 *m*, 4.40 *m*	68.3[b] *t*	4.10 *m*, 4.42 *m*
1″	101.2 *d*	5.53 *d* (3.1)	101.5 *d*	5.58 *d* (2.8)	101.3 *d*	5.56 *d* (3.2)	101.3 *d*	5.58 *d* (2.8)
2″	68.7 *d*	4.91 *m*	68.9 *d*	4.95 *m*	68.7 *d*	4.93 *m*	68.8 *d*	4.95 *m*
3″	75.3[b] *d*	5.83 *m*	75.5[b] *d*	5.90 *m*	75.3[b] *d*	5.85 *m*	75.3 *d*	5.90 *m*
4″	67.8 *d*	4.91 *m*	68.0 *d*	4.95 *m*	67.9 *d*	4.91 *m*	67.9 *d*	4.95 *m*
5″	72.7 *d*	4.58 *m*	72.8 *d*	4.64 *m*	72.7 *d*	4.62 *m*	72.7 *d*	4.64 *m*
6″	62.3 *t*	4.38 *m*, 4.43 *m*	62.5 *t*	4.42 *m*, 4.48 *m*	62.3 *t*	4.40 *m*, 4.46 *m*	62.3 *t*	4.42 *m*, 4.48 *m*
1‴,2‴,3‴,4‴	174.1 *s*, 173.6 *s*, 173.5 *s*		174.2 *s*, 173.7 *s*, 173.6 *s*		174.1 *s*, 173.7 *s*, 173.5 *s*		174.1 *s*, 173.6 *s*, 173.5 *s*	
2‴,3‴,4‴,5‴	35.0 *t*, 34.7 *t*, 34.5 *t*	2.36 *m*	35.1 *t*, 34.8 *t*, 34.6 *t*	2.39 *m*	35.0 *t*, 34.8 *t*, 34.6 *t*	2.38 *m*	35.0 *t*, 34.7 *t*, 34.5 *t*	2.39 *m*
3‴,4‴,5‴,6‴	25.5[b] *t*, 25.6 *t*	1.62 *br s*	25.6[b] *t*, 25.8 *t*	1.63 *br s*	25.6[b] *t*, 25.7 *t*	1.64 *m*	25.6[b] *t*, 25.7 *t*	1.63 *br s*
8‴	27.8[b] *t*	2.08 *m*	27.9[b] *t*	2.11 *m*	27.9 *t*	2.10 *m*	27.9 *t*	2.11 *m*
8⁗	27.8[b] *t*	2.08 *m*	27.9[b] *t*	2.11 *m*	30.3–29.7[b] *t*	1.26 *m*	30.3–29.7[b] *t*	1.26 *m*
9‴, 10‴, 12‴, 13‴	130.9[b] *d*, 128.9[b] *d*, 127.8[b] *d*	5.49 *m*	131.0 *d*, 130.9 *d*, 130.8 *d*, 129.1[b] *d*,	5.51 *m*	130.9 *d*, 127.8 *d*, 128.9[b] *d*	5.50 *m*	130.8 *d*, 130.7 *d*, 128.7[b] *d*	5.51 *m*
9⁗, 10⁗, 12⁗, 13⁗			128.8[b], 128.0 *d*		30.3–29.7[b] *t*	1.26 *m*	30.3–29.7[b] *t*	1.26 *m*
11‴	26.3[b] *t*	2.91 *m*	26.4[b] *t*	2.93 *m*	26.3 *t*	2.92 *m*	26.3 *t*	2.93 *m*
11⁗	26.3[b] *t*	2.91 *m*	26.4[b] *t*	2.93 *m*	30.3–29.7[b] *t*	1.26 *m*	30.3–29.7[b] *t*	1.26 *m*
14‴	26.2[b] *t*	2.91 *m*	26.3 *t*	2.93 *m*	26.2 *t*	2.92 *m*	27.8 *t*	2.11 *m*
14⁗	26.2[b] *t*	2.91 *m*	27.8 *t*	2.11 *m*	32.5[b] *t*	1.26 *m*	32.5 *t*	1.26 *m*
15‴, 16‴	132.4 *d*, 128.4 *d*	5.49 *m*, 5.44 *m*	132.5 *d*, 128.5 *d*	5.51 *m*, 5.46 *m*	132.4 *d*, 128.4 *d*	5.50 *m*, 5.46 *m*	30.3–29.7[b] *t*	1.26 *m*
15⁗, 16⁗	132.4 *d*, 128.4 *d*	5.49 *m*, 5.44 *m*	30.4–29.8[b] *t*	1.26 *m*	23.3[b] *t*, 14.6[b] *q*	1.26 *m*, 0.87 *t* (6.4)	23.3[b] *t*, 14.6[b] *q*	1.26 *m*, 0.88 *t* (6.4)
17‴,18‴	21.1[b] *t*	2.08 *m*	21.2 *t*, 23.3 *t*	1.26 *m*, 2.11 *m*	21.2 *t*	2.10 *m*	23.2 *t*	1.26 *m*
18‴,18⁗	14.6[b] *q*	0.94 *t* (7.2)	14.6[b] *q*	0.96 *t* (7.2)	14.8 *q*	0.95 *t* (8.0)	14.5 *q*	0.96 *t* (7.2)
14″	32.4 *t*	1.25 *m*	32.5 *t*	1.26 *m*	32.5[b] *t*	1.26 *m*	32.5 *t*, 32.0 *t*	1.26 *m*
15″	23.2 *t*	1.25 *m*	23.3 *t*	1.26 *m*	23.3[b] *t*	1.26 *m*	23.3[b] *t*	1.26 *m*
16″	14.8 *q*	0.87 *t* (6.4)	14.8 *q*	0.88 *t* (6.4)	14.6[b] *q*	0.87 *t* (6.4)	14.6[b] *q*	0.88 *t* (6.4)
Other CH_2	30.3–29.6[b] *t*	1.25 *m*	30.4–29.8[b] *t*	1.26 *m*	30.3–29.7[b] *t*	1.26 *m*	30.3–29.7[b] *t*	1.26 *m*

[a] Multiplicity not clear for some signals due to overlapping.
[b] Overlapped in same column; δ in ppm, J in Hz.

Varian AS 400 or Bruker DRX-400 NMR spectrometers in C_5D_5N. ESIMS and HRESIMS data were obtained on an Agilent Series 1100 SL mass spectrometer. GCMS analyses were carried out on a ThermoQuest Trace 2000 GC, equipped with a single split/splitless capillary injector, a ThermoQuest AS2000 autosampler, and an Agilent DB-5ms column (30 m × 0.25 mm × 0.25 μm). Gravity column chromatography (CC) was performed using silica gel (J.T. Baker, 40 μm for flash chromatography) and reversed-phase RP-C_{18} silica (Polarbond, J.T. Baker). Thin layer chromatography (TLC) was carried out on silica gel 60 F_{254} plates (Merck, Germany). Waring Heavy Duty Blender was used for extraction. Sugar samples, Lipase from *Pseudomonoas* sp. type XIII, and L-cysteine methyl ester hydrochloride were purchased from Sigma–Aldrich (St. Lousis, MO).

4.2. Plant material

Fruit of *Lycium barbarum* L. (Goji berries) was purchased online from www.911healthshop.com under the product name "Authentic Tibetan Himalayan Dried Goji Berries". The authentication was done by Dr. V. Joshi, Plant Taxonomist at the National Center for Natural Products Research, University of Mississippi, where the specimen (No. 3469) has been deposited.

4.3. Extraction and isolation

Fruit of *Lycium barbarum* (4.8 kg) was extracted with MeOH (5.0 L × 1, 4.0 L × 4) at room temperature by using a blender. The combined extracts were evaporated under reduced pressure to afford a dark-brown gummy residue (A, 2.4L, 2.9 kg). The residue A (2.8 kg) was dissolved in MeOH and precipitated with CH_3CN. Following removal of the precipitates, the solution was evaporated under reduced pressure to give part B (523 g). A portion of part B (500 g) was subjected to flash silica gel CC (40 μm, 1040 g, column (95 mm × 370 mm), $CHCl_3$–MeOH, gradients of 19:1 (5 L), 9:1 (5 L), 17:3 (8 L), 4:1 (5 L), 0:1 (6 L)] to afford seven fractions B1–B7. Fraction B3 (5.7 g) was appeared to flash silica gel CC (40 μm, 603 g, column, 45 mm × 960 mm), eluted with $CHCl_3$–MeOH, gradients of 19:1 (2 L), 93:7 (3 L), 23:2 (2 L), 9:1 (1 L), 15:2 (1 L), 0:1 (1 L)] to afford 12 sub-fractions B3a–B3l. Compounds **13** (30.6 mg, 0.00064%), **14** (52.4 mg, 0.00109%) and **15** (10.8 mg, 0.00022%) were obtained from sub-fraction B3h (1.28 g) by repeated CC [flash silica gel (40 μm, 250 g), column (40 mm × 550 mm), $CHCl_3$–MeOH, 24:1 (1 L) and 19:1 (1 L) and RP-C_{18} (155 g), column (25 mm × 620 mm), MeOH (1.4 L)]. Compounds **1** (18.7 mg, 0.00039%), **2** (6.5 mg, 0.00013%), **3** (21.0 mg, 0.00045%), **4** (14.1 mg, 0.00029%) and **5** (14.2 mg, 0.0003%) were obtained from fraction B3i (0.76 g) by repeated CC [flash silica gel (40 μm, 130 g), column (25 mm × 650 mm), EtOAc–$CHCl_3$–MeOH–H_2O, 30:16:5:1 (0.6 L) and RP-C_{18} (155 g), column (25 mm × 620 mm), MeOH (3.6 L)]. Fraction B5 (8.36 g) was subjected to CC [flash silica gel (40 μm, 316 g), column (50 mm × 390 mm), EtOAc–$CHCl_3$–MeOH–H_2O, 60:32:12:1 (1.9 L), 15:8:4:1 (0.9 L), 6:4:4:1 (1 L)] to afford 10 sub-fractions B5a–B5j. Compounds **6** (18.3 mg, 0.00038%), **7** (71.8 mg, 0.0015%), **8** (44.3 mg, 0.00092%), **9** (171.0 mg, 0.00356%), **10** (168.5 mg, 0.00351%), **11** (17.9 mg, 0.00037%), and **12** (11.6 mg, 0.00024%) were obtained from sub-fraction B5g (1.25 g) by repeated CC [flash silica gel (40 μm, 130 g), column (25 mm × 650 mm), EtOAc–$CHCl_3$–MeOH–H_2O, 30:16:5:1 (0.6 L) and RP-C_{18} (155 g), column (25 mm × 620 mm), MeOH (3.3 L)].

4.3.1. (2S)-1-O-palmitoyl-2-O-linolenoyl-3-O-[α-D-galactopyranosyl-(1″ → 6′)-(3″-O-linolenoyl)-β-D-galactopyranosyl]glycerol (**1**)

Colorless gum; $[\alpha]_D^{27}$ + 40.0 (*c*, 1.0, MeOH); IR (NaCl) ν_{max} 3380, 3012, 2924, 2853, 1737, 721 cm^{-1}; for ^1H and ^{13}C spectroscopic analyses, see Table 1; HRESIMS, *m*/*z* 1209.7792 $[M + Cl]^-$, $C_{67}H_{114}O_{16}$ + Cl, requires 1209.7795.

4.3.2. (2S)-1-O-palmitoyl-2-O-linolenoyl-3-O-[α-D-galactopyranosyl-(1″ → 6′)-(3″-O-linoleoyl)-β-D-galactopyranosyl]glycerol (**2**)

Colorless gum; $[\alpha]_D^{27}$ + 39.0 (*c*, 1.0, MeOH); IR (NaCl) ν_{max} 3370, 3010, 2924, 2853, 1738, 722 cm^{-1}; for ^1H and ^{13}C spectroscopic analyses, see Table 1; HRESIMS, *m*/*z* 1211.7951 $[M + Cl]^-$, $C_{67}H_{116}O_{16}$ + Cl, requires 1211.7952.

4.3.3. (2S)-1-O-palmitoyl-2-O-linolenoyl-3-O-[α-D-galactopyranosyl-(1″ → 6′)-(3″-O-palmitoyl)-β-D-galactopyranosyl]glycerol (**3**)

Colorless gum; $[\alpha]_D^{27}$ + 41.0 (*c*, 1.0, MeOH); IR (NaCl) ν_{max} 3375, 3011, 2923, 2852, 1737, 721 cm^{-1}; for ^1H and ^{13}C spectroscopic analyses, see Table 1; HRESIMS, *m*/*z* 1187.7988 $[M + Cl]^-$, $C_{65}H_{116}O_{16}$ + Cl, requires 1187.7952.

4.3.4. (2S)-1-O-palmitoyl-2-O-linoleoyl-3-O-[α-D-galactopyranosyl-(1″ → 6′)-(3″-O-palmitoyl)-β-D-galactopyranosyl]glycerol (**4**)

Colorless gum; $[\alpha]_D^{27}$ + 40.0 (*c*, 1.0, MeOH); IR (NaCl) ν_{max} 3377, 3010, 2923, 2853, 1737, 722 cm^{-1}; for ^1H and ^{13}C spectroscopic analyses, see Table 1; HRESIMS, *m*/*z* 1189.8124 $[M + Cl]^-$, $C_{65}H_{118}O_{16}$ + Cl, requires 1189.8108.

4.4. Sugar analysis

Compound **1** (3.0 mg) was hydrolyzed with 2N HCl (1 mL) for 3 h at 95 °C. The reaction mixture was cooled, neutralized with NH_4OH and partitioned between EtOAc and H_2O. The aqueous layer after drying was dissolved in pyridine (0.3 mL) and 0.1 M L-cysteine methyl ester hydrochloride in pyridine (1.0 mL) was added. The mixture was heated at 60 °C for 1 h. An equal volume of Ac_2O was added with heating continued for another 1 h (Hara et al., 1987; Ali and Khan, 2008). Acetylated thiazolidine derivatives were subjected to GC analysis [Capillary Column: DB-5 ms (30 m × 0.25 mm × 0.25 μm); Carrier gas He; Pressure: 19.98 psi; Injection temperature 250 °C; Detection temperature 280 °C; Column temperature: 70 °C 1 min, 20 °C/min to 300 °C]. The sugars were identified as D-galactose by comparing its retention time (t_R D-galactose 13.48 min) with acetylated thiazolidine derivatives prepared in a similar way from the standard D-galactose (Sigma–Aldrich). Compounds **2**–**15** were treated in a similar manner.

4.5. Alkaline hydrolysis

A solution of compound **1** (2.7 mg) in 5% dry NaOMe–MeOH (2 mL) was stirred at room temperature for 5 h. The reaction mixture was neutralized with 2N HCl–MeOH and partitioned between MeOH and *n*-hexane. The *n*-hexane layer containing the fatty acid methyl esters was analyzed by GCMS [Capillary Column: DB-5 ms (30 m × 0.25 mm × 0.25 μm); Carrier gas He; Pressure: 19.98 psi; Injection temperature 250 °C; Detection temperature 280 °C; Column temperature: 70 °C 1 min, 20 °C/min to 300 °C]. Alkaline hydrolysis of compounds **2**–**15** was performed similarly.

4.6. Enzymatic hydrolysis

A solution of compound **1** (1.0 mg) and Lipase type XIII (from *Pseudomonoas* sp., 1.3 mg, 19.5 units) in 0.7 mL dioxane-H_2O (1:1) was stirred and incubated at 37 °C for 3 h. The reaction was quenched by 5% HOAc (0.15 mL) and MeOH (1.5 mL) was added to the reaction mixture. Following removal of the solvent the residue was dissolved in dry MeOH (0.5 mL) and 1 drop of $BF_3{\cdot}OEt_2$ was added. The mixture was stirred at room temperature for 1.5 h and analyzed by GCMS (see alkaline hydrolysis). Enzymatic

hydrolysis of compounds **2**–**15** was performed in a similar way to that of described for compound **1**.

Acknowledgements

Thanks to China Scholarship Council and the United States Food and Drug Administration (FDA) grant number FD-U-002071-06 for financial support. The authors are grateful to Dr. V. Joshi for identification of the plant material, Dr. X. Li for useful suggestions, and Dr. B. Avula and Dr. D. Slade for conducting the mass and GCMS spectra, respectively.

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