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A NEW CEREBROSIDE FROM THE FRUIT OF *Ziziphus jujuba* var. *spinosa*

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*A new cerebroside, 1-*O-β-*D-glucopyranosyl-(2*S*,3*S*,4*R*,9*E*)-2-[(2′R)-2′-hydroxyeicosanoylamino]-9-tetradecene-1,3,4-triol (**1**), was isolated from the fruits of* Ziziphus jujuba *var.* spinosa*, together with one known compound, dibutyl phthalate. Their structures were elucidated through spectroscopic and chemical methods.*

Keywords: *Ziziphus jujuba* var. *spinosa*, Rhamnaceae, cerebrosides.

Ziziphus jujuba var. *spinosa* (Bunge) Hu ex H. F. Chow is a thorny rhamnaceous plant widely distributed in northern China. Its dried seeds (known as Suanzaoren in China) have been used as a sedative for thousands of years, and many studies on its chemical constituents have been reported [1–5]. Besides the seeds, its sarcocarp is also used as a folk medicine for treating diarrhea and hemorrhage in China [6]. However, compared to the seed, few studies have been performed on its sarcocarp. Considering this fact, we initiated phytochemical studies on the sarcocarp of *Ziziphus jujuba* var. *spinosa.* Previous studies have resulted in the isolation and characterization of several triterpenic acids [7, 8]. In the present paper, we report the isolation and identification of a new cerebroside **1** together with a known compound, dibutyl phthalate (**2**) [9], from the sarcocarp of this plant.

Compound **1** was isolated as a white powder. The molecular formula of **1** was established as $C_{40}H_{77}NO_{10}$ by HR-ESI-MS *m*/*z* 754.5459 $[M + Na]^+$ (calcd 754.5445 for $C_{40}H_{77}NO_{10}Na$) and 1D and 2D NMR spectroscopic analyses. Its MS showed characteristic peaks at *m/z* 702 (an ion formed by McLafferty rearrangement of the olefinic bond), 592 (an ion from *O*-glycoside bond cleavage), and 327 (an ion derived from α-fission of the NH group, Fig. 1). In the ^{13}C NMR data, the carbon resonances at δ 103.6 (C-1″), 73.6 (C-2″), 76.6 (C-3″), 70.1 (C-4″), 77.0 (C-5″), and 61.2 (C-6″) confirmed the glucopyranose moiety. The β-configuration of the glucoside unit was indicated by the anomeric proton at δ_H 4.14 (1H, d, J = 8.0 Hz, H-1″) correlated to the carbon signal at δ 103.6 in the HSQC spectrum. The characteristic signals of an amide linkage (a nitrogen-bearing methine proton at δ_H 4.10, a carbonyl carbon at δ_C 173.9, and a doublet at δ_H 7.52 due to an NH proton) and a long acyl chain (terminal methyl protons at δ_H 0.85) were observed, indicating its glycosphingolipid nature [10].

In the ^1H–^1H COSY spectrum, the correlations of methylene protons at δ 3.81 (H-1a) and 3.66 (H-1b), methine proton 3.37 (H-3) with methine proton at 4.10 (H-2), and methine proton at 3.37 with methine proton at 3.35 (H-4) were observed, which suggested the presence of three hydroxy groups at C-1, C-3, and C-4. An HMBC experimental result was also supported by these assignments. The fatty acid linked to C-2 of the sphingosine has been confirmed by the correlation between H-2 (δ 4.10) and the carbonyl carbon with the signal at δ 173.9. HMBC correlation of the carbonyl carbon with the H-2′ (δ 3.85), which in turn showed correlation with C-2′ (δ 71.1) in HSQC and the proton 2′-OH (δ 5.56) in ^1H–^1H COSY, confirmed the presence of an α-hydroxy fatty acid side chain. When **1** was methanolyzed with methanolic hydrochloric acid, a fatty acid methyl ester (FAME) was obtained together with a long-chain base (LCB). Based on the ESI-MS analysis, the molecular formula of the FAME was established as methylhydroxyeicosanoate ($C_{21}H_{42}O_3$, $[M–H]^-$ at *m/z* 341).

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TABLE 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for Compound **1** (DMSO-d_6, δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
1	69.1 (t)	3.81 (m, H_a), 3.66 (m, H_b)	1′	173.9 (s)	
2	50.0 (d)	4.10 (m)	2′	71.1 (d)	3.85 (m)
3	74.3 (d)	3.37 (m)	3′	34.5 (t)	1.56 (m, H_a), 1.45 (m, H_b)
4	70.7 (d)	3.35 (m)	4′–18′	28.7–31.4 (t)	1.26 (m)
5	34.6 (t)	1.58 (m, H_a), 1.47 (m, H_b)	19′	22.2 (t)	1.26 (m)
6, 7	28.7–31.4 (t)	1.26 (m)	20′	14.1 (q)	0.85 (t, J = 7.3)
8	32.1 (t)	1.95 (m)	1″	103.6 (d)	4.14 (d, J = 8.0)
9	129.9 (d)	5.36 (m)	2″	73.6 (d)	2.94 (m)
10	130.4 (d)	5.36 (m)	3″	76.6 (d)	3.14 (m)
11	32.4 (t)	1.95 (m)	4″	70.1 (d)	3.04 (m)
12	24.6 (t)	1.26 (m)	5″	77.0 (d)	3.09 (m)
13	22.2 (t)	1.26 (m)	6″	61.2 (t)	3.66 (m, H_a), 3.44 (m, H_b)
14	14.1 (q)	0.85 (t, J = 7.3)			

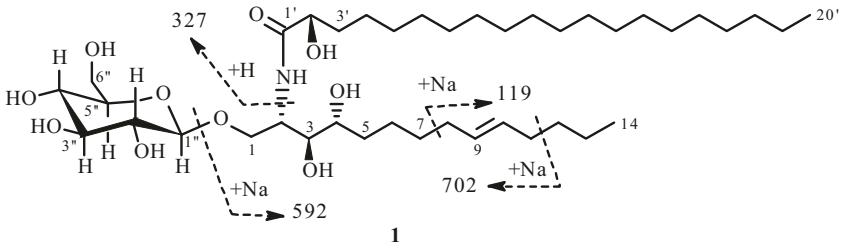

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Fig. 1. ESI-MS/MS fragment analysis of **1**.

The ^{1}H NMR spectrum showed a pair of olefinic protons at δ 5.36 attributable to the presence of one olefinic bond. The position of the double bond in the LCB was determined at C-9 by ESI-MS/MS analysis. Moreover, the *trans*-geometry (*E*) of the double bond was evidenced by the chemical shifts of the allylic carbons at δ 32.1 (C-8) and 32.4 (C-11) [11].

The absolute stereochemistry of C-2 to C-4 were deduced to be 2*S*, 3*S*, and 4*R* by comparing their ^{13}C NMR spectral data of δ 50.0 (C-2), 74.3 (C-3), and 70.7 (C-4) with the reference [12]. The optical rotation of the FAME $[\alpha]_D^{25}$ –3.3° (*c* 0.1, $CHCl_3$) was in agreement with the data reported in the literature [13, 14]. Therefore, the absolute stereochemistry at C-2′ was suggested to be *R*. Based on the above evidences, the structure of compound **1** was established as 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*S*,4*R*,9*E*)-2-[(2′*R*)-2′-hydroxyeicosanoylamino]-9-tetradecene-1,3,4- triol.

EXPERIMENTAL

Optical rotations were recorded on a JASCO P-1020 polarimeter. NMR spectra were recorded on a Bruker AV-500 spectrometer. HR-ESI-MS were measured on a Synapt Q-TOF mass spectrometer. Column chromatography (CC) was carried out on silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, China) or macroporous adsorption resin (D101, Hebei Baoeng Chemical Inc., Cangzhou, China). All solvents used were of analytical grade (Nanjing Chemical Industry Factory).

Plant Material. The fruits of *Z. jujuba* var. *spinosa* were collected from Ningxia of China in September 2008. The material was identified by Prof. Jin-ao Duan of Nanjing University of Chinese Medicine, China.

Extraction and Isolation. The air-dried and hardcore-removed fruits of *Z. jujuba* var. *spinosa* (20 kg) were chipped and refluxed with 80% ethanol twice for 2 h. The 80% EtOH extract was evaporated to dryness under reduced pressure. The residue was suspended in water (10 L) and partitioned sequentially with EtOAc and *n*-butanol (each 5 × 10 L) to yield 558 and 810 g of crude extracts, respectively.

The *n*-butanol extract was chromatographed on a macroporous adsorption resin (D101) column eluting with 30%, 50%, and 95% ethanol. The 30% ethanol eluate was then chromatographed on a silica gel column eluting with a step gradient of EtOAc–MeOH–H_2O (10:1:0.1→1:10:0.1), and compound **2** was isolated. From the 95% ethanol eluate, compound **1** was purified by repeated silica gel column chromatography with EtOAc–MeOH (10:1→1:10) as eluent.

Methanolysis of Compound 1. Compound **1** (ca. 10 mg) was heated with 10% HCl in MeOH (10 mL) at 80°C for 14 h. The reaction mixture was then extracted with *n*-hexane and concentrated to yield a fatty acid methyl ester (FAME): $[\alpha]_D^{25}$–3.3° (*c* 0.1, $CHCl_3$). The FAME was analyzed by ESI-MS, and a deprotonated quasi-molecular ion at *m/z* 341 $[M - H]^-$ was observed, which could be elucidated as methylhydroxyeicosanoate.

1-*O*-*β*-D-Glucopyranosyl-(2*S*,3*S*,4*R*,9*E*)-2-[(2′*R*)-2′-hydroxyeicosanoylamino]-9-tetradecene-1,3,4-triol (1). $C_{40}H_{77}NO_{10}Na$. White powder, mp 179–181°C (MeOH), $[\alpha]_D^{25}$ +10.5° (*c* 0.05, MeOH). ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz), see Table 1. HR-ESI-MS *m/z* 754.5459 $[M + Na]^+$.

Dibutyl Phthalate (2). White gum (MeOH). ESI-MS *m/z* 277 $[M - H]^-$. ^1H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.72 (2H, m, H-3, 6), 7.67 (2H, m, H-4, 5), 4.22 (4H, t, J = 6.5, H-1′, 1″), 1.64 (4H, m, H-2′, 2″), 1.37 (4H, m, H-3′, 3″), 0.91 (6H, t, J = 7.0, H-4′, 4″). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 166.9 (C=O), 130.5 (C-1, 2), 128.7 (C-3, 6), 132.2 (C-4, 5), 65.1 (C-1′, 1″), 30.3 (C-2′, 2″), 19.3 (C-3′, 3″), 13.6 (C-4′, 4″).

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