Ziziphorins A and B. New Flavonoids from *Ziziphora tenuior*

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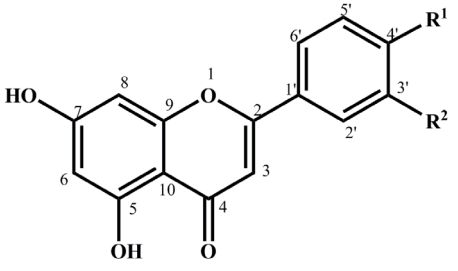
Ziziphorins A (**1**) and B (**2**), new flavonoids, have been isolated from the chloroform-soluble fraction of *Ziziphora tenuior* along with 1-hentetracontanol, β-sitosterol-3-O-β-D-glucoside, ursolic acid, oleanolic acid, and apigenin. Their structures were assigned from spectral studies including 1D and 2D NMR spectroscopic data.

Key words: Ziziphora tenuior, Labiatae, Falvonoids, Ziziphorin A, Zizphorin B

Introduction

The genus *Ziziphora* belongs to the family Lamiaceae and comprises 62 species growing as perennial or annual herbs from South-West Asia to Eastern Europe. In Pakistan this genus is represented by two species. One of these is *Ziziphora tenuior* which grows in Balochistan and Northern Provinces of Pakistan [1]. It is locally used as herbal tea due to its sweet fragrance. The literature revealed that no phytochemical or pharmacological studies have been carried out on this species so far. The chemotaxonomic and ethnopharmacological importance of the genus *Ziziphora* prompted us to carry out phytochemical studies on *Z. tenuior*. As a result, we herein report the isolation and structural elucidation of two new flavonides named as ziziphorin A (**1**) and ziziphorin B (**2**) (Fig. 1), along with the known compounds 1-hentetracontanol (**3**) [2], ursolic acid (**4**) [3], oleanolic acid (**5**) [4], β-sitosterol-3-O-β-glucoside (**6**) [5] (Fig. 2), and apigenin (**7**) [6].



1 R^1 = OCO-$CH_2(CH_2)_{16}CH_3$, R^2 = H
2 R^1 = H, R^2 = OCO-$CH_2(CH_2)_{24}CH_3$
7 R^1 = OH, R^2 = H

Fig. 1. Structures of ziziphorins A (**1**), B (**2**) and apigenin (**7**).

Results and Discussion

The ethanolic extract of *Ziziphora tenuior* (whole plant) was divided into *n*-hexane-, chloroform-, ethyl acetate-, *n*-butanol-, and water-soluble fractions. The chloroform sub-fraction was subjected to a series of column chromatographic techniques to obtain compounds **1** – **7**, respectively.

Ziziphorin A (**1**) was obtained as pale-yellow amorphous powder which gave a violet coloration with $FeCl_3$ for a phenol. The UV spectrum showed characteristic bands of a flavonoid at 269 and 335 nm [7]. Addition of $AlCl_3$/HCl resulted in a bathochromic shift of 32 nm of the band at 269 nm suggesting the presence of a chelated hydroxyl group. On the other hand, a bathochromic shift was also observed on addition of NaOAc revealing the presence of a phenolic group at C-7. The IR spectrum showed the presence of hydroxyl groups (3406 cm^{-1}), an ester moiety (1760 cm^{-1}), a conjugated carbonyl (1670 cm^{-1}), and an aromatic moiety (1600 – 1400 cm^{-1}). The HR-EI-MS showed a molecular ion peak at m/z = 550.3265 corresponding to the molecular formula $C_{34}H_{46}O_6$. This molecular formula was confirmed by broad-band and DEPT ^{13}C NMR spectra which showed 34 signals comprising of one methyl, 17 methylene, 7 methine, and 9 quaternary carbons. The downfield sig-

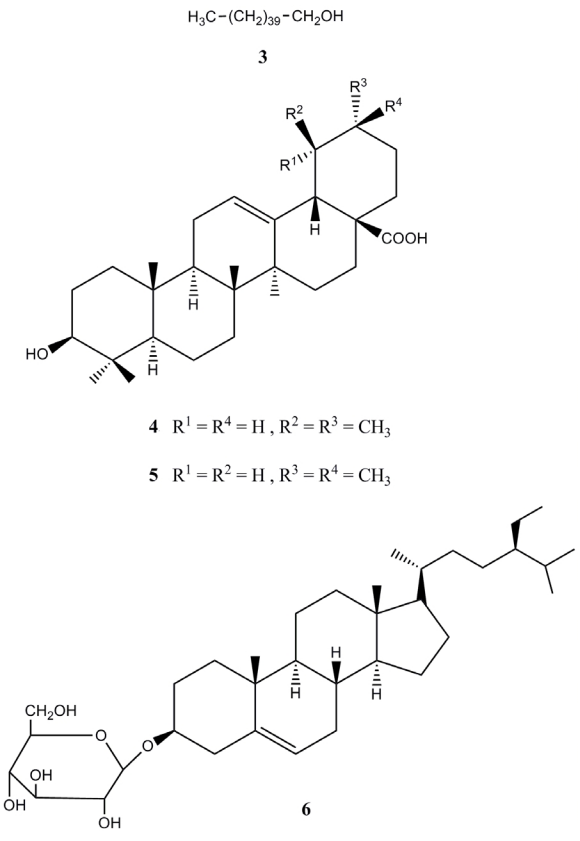


Fig. 2. Compounds **3** – **6**.

nals at δ = 183.9 and 176.7 could be assigned to the carbonyl carbons of a conjugated ketone and ester moiety, respectively. The signals at δ = 166.2, 103.8 and 183.9 were typical of a flavonoid moiety. Other oxygenated aromatic carbons resonated at δ = 163.2, 162.5 and 159.2, respectively. The EI-MS gave, besides the molecular ion peak, diagnostic fragments at m/z = 535 $[M–CH_3]^+$, 521 $[M–CH_2CH_3]^+$ and m/z = 270 [M–nonadecanoyl]$^+$. The retro-Diels Alder fragments at m/z = 152 and 398 showed the presence of two hydroxyl groups in ring A and the nonadecanoyl moiety in ring B. The ^{1}H NMR spectrum showed a singlet at δ = 6.61 which could be assigned to C-3 based on HMBC correlations (Table 1). The protons at δ = 6.47 and 6.20 were assigned to C-6 and C-8 on the basis of their coupling constants and HMBC correlations. The *para*-substituted ring B showed an AA′BB′ pattern with signals at δ = 7.85 (2H, d, J = 8.5 Hz) and 6.92 (2H, d, J = 8.5 Hz). Based on these cumulative evidences, the structure of ziziphorin A (**1**) could be assigned as 4-(5,7-dihydroxy-4-oxo-4*H*-1-benzopyran-2-yl)phenyl-nonadecanoate [4′-*O*-(nonadecanoyl)apigenin].

Ziziphorin B (**2**) was also obtained as a pale-yellow amorphous powder which gave a violet coloration with $FeCl_3$ for a phenol. The UV and IR spectra were similar to those of **1**. The molecular formula was established as $C_{42}H_{62}O_6$ on the basis of the $[M]^+$ peak in the HR-EI-MS at m/z = 662.4929. The broad-band and DEPT ^{13}C NMR spectra showed 42 signals comprising one methyl, 25 methylene, 7 methine, and 9 quarternary carbons. The signals of rings A and C were similar to those of **1**, the only notable difference being observed for ring B. The EI-MS showed characteristic fragments at m/z = 647 $[M–CH_3]^+$ and m/z = 270 [M–heptacosanoyl]$^+$. The retro-Diels Alder fragments at m/z = 152 and 510 revealed that compound **2** differs from **1** only in having a heptacosanoyl group instead of a nonadecanoyl moiety. The ^{1}H MNR spectrum showed similar signals of rings A and C while ring B showed a 1,3-disubstitution pattern [δ = 7.85 (1H, d, J = 9.0), 7.21 (1H, t, J = 9.0), 7.51 (1H, dd, J = 2.4, 2.4), and 6.92 (1H, dd, J = 9.0, 2.4)]. The HMBC correlations were in complete agreement to the assigned structure of ziziphorin B (**2**) as 3-(5,7-dihydroxy-4-oxo-4*H*-chromen-2-yl)phenyl heptacosanoate [3′-*O*-(haptacosanoyl)-5,7-dihydroxyflavone]. Flavonoids carrying long-chain acyl groups are not commonly reported in literature. Previously such compounds have also been synthesized from hesperitin and shown to posses free radical scavenging, anti-elastase and hypocholesterolemic activities [8, 9]. Pharmacological screening of the isolated compounds could not be carried out due to paucity of material.

The structures of known compounds **3** – **7** were elucidated through a comparison of their physical and spectral data with those reported in literature [2 – 6].

Experimental Section

General experimental procedures

Column chromatography was carried out using silica gel (230 – 400 mesh, E. Merck, Darmstadt, Germany). TLC was performed with precoated silica gel F_{254} plates (E. Merck, Darmstadt, Germany) and detection was done at 254 and 366 nm, and by spraying with ceric sulfate in 10 % H_2SO_4 (heating). The UV spectra were recorded on a Hitachi UV-3200 spectrophotometer while the IR spectra were recorded as KBr pellets on a Jasco 302-A spectrometer. Mass spectra (EI and HR-EI-MS) were measured in an electron impact mode on Finnigan MAT 12 or MAT 312 spectrometers, and ions are given in m/z (%). The ^{1}H and

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data and HMBC correlations of **1** and **2** recorded in CD_3OD.

	Compound **1**			Compound **2**		
C No.	δ_H	δ_C	HMBC (^{1}H-^{13}C)	δ_H	δ_C	HMBC (^{1}H-^{13}C)
2	–	166.2	–	–	166.1	–
3	6.61	103.2	C-2, C-4, C-10, C-1′	6.59	104.2	C-2, C-4, C-10, C-1′
4	–	183.9	–	–	183.5	–
5	–	162.5	–	–	161.8	–
6	6.20	100.3	C-5, C-7, C-10, C-8	6.20	100.1	C-5, C-7, C-10, C-8
7	–	159.2	–	–	159.4	–
8	6.47	95.3	C-7, C-9, C-10, C-6	6.45	95.1	C-7, C-9, C-10, C-6
9	–	166.0	–	–	165.7	–
10	–	105.1	–	–	105.2	–
1′	–	123.2	–	–	123.5	–
2′	7.85	129.8	C-1′, C-3′, C-4′, C-2	7.51	110.5	C-1′, C-3′, C-4′, C-2
3′	6.92	117.0	C-1′, C-2′, C-4′	–	150.1	–
4′	–	163.2	–	6.92	116.8	C-2′, C-3′, C-5′
5′	6.92	117.0	C-1′, C-2′, C-4′	7.21	129.5	C-1′, C-4′, C-6′
6′	7.85	129.8	C-1′, C-3′, C-4′, C-2	7.85	121.8	C-1′, C-2′, C-5′
1″	–	176.8	–	–	177.1	–
2″	2.34	34.9	C-1″, C-3″, C-4″	2.32	34.9	C-1″, C-3″, C-4″
3″	1.61	26.1	C-2″, C-4″	1.61	26.1	C-2″, C-4″
4″	1.28	30.8	–	1.28	30.6	–
5″	//	27.1	–	//	26.7	–
6″	//	27.5	–	//	27.6	–
7″	//	29.3	–	//	29.4	–
8″	//	30.2	–	//	30.2	–
9″	//	30.4	–	//	30.4	–
10″	//	30.5	–	//	30.5	–
11″	//	30.7	–	//	30.5	–
12″	//	30.8	–	//	30.6	–
13″	//	30.8	–	//	60.7	–
14″	//	29.2	–	//	30.7	–
15″	//	28.2	–	//	30.8	–
16″	//	28.3	–	//	30.8	–
17″	1.28	33.1	–	//	30.8	–
18″	1.28	23.7	C-17″, C-19″	//	30.8	–
19″	0.86	14.5	C-18″, C-17″	//	30.8	–
20″				//	30.8	–
21″				//	30.8	–
22″				//	29.2	–
23″				//	28.2	–
24″				//	28.1	–
25″				1.28	33.1	–
26″				1.28	23.8	C-27″, C-25″
27″				0.86	14.5	C-26″, C-25″

^{13}C NMR spectra were recorded on a Bruker AMX-400 spectrometer in deuterated solvents. The 2D NMR spectra were recorded on a Bruker AMX 400 NMR spectrometer. Chemical shifts are in ppm (δ), relative to tetramethylsilane as an internal standard, and scalar couplings are reported in Hz.

Plant material

The whole plant of *Ziziphora tenuior* Linnaeus (syn. *Z. acutifolia* Montb) (40 kg) was collected from Ziarat valley of Balochistan province of Pakistan and identified by Prof. Dr. Rasool Bakhsh Tareen, Plant Taxonomist, Department of Botany, University of Balochistan, Quetta, where a voucher specimen has been deposited in the herbarium (Voucher specimen no. ZT-RBT-06-08-BUH).

Extraction and isolation

The freshly collected whole plant material of *Ziziphora tenuior* (40 kg) was shade-dried, ground, and extracted with ethanol (3×40 L, 10 d each). The combined ethanolic extract was evaporated at r. t. under reduced pressure to yield a residue (850 g), which was divided into *n*-hexane- (300 g), $CHCl_3$- (100 g), EtOAc- (80 g), *n*-BuOH- (120 g) and water-soluble (250 g) sub-fractions. The chloroform-soluble fraction was subjected to column chromatography over silica gel eluting with *n*-hexane-$CHCl_3$ and $CHCl_3$-CH_3OH in in-

creasing order of polarity. The fractions which eluted with *n*-hexane-$CHCl_3$ (9.4 : 0.6) afforded compound **3** (30 mg). The fractions which eluted with *n*-hexane-$CHCl_3$ (6.0 : 4.0) were a binary mixture (50 mg), which was rechromatographed over silica gel, eluting with *n*-hexane-$CHCl_3$ (7.0 : 3.0 and 6.5 : 3.5) to obtain compounds **4** (21 mg) and **5** (19 mg), respectively. The fraction obtained by elution with $CHCl_3$-MeOH (9.9 : 0.1) afforded compound **6** (35 mg). The fraction obtained by elution with $CHCl_3$-MeOH (9.8 : 0.2) was a mixture, which was triturated with dry acetone to afford a crystalline acetone-insoluble compound which could be identified as apigenin (**7**) (15 mg). The acetone-soluble fraction was freed from the solvent, and the residue was chromatographed over silica gel eluting with $CHCl_3$-MeOH (9.9 : 0.1) providing ziziphorin A (**1**) (11 mg) and ziziphorin B (**2**) (8 mg) from the top and the tail fraction, respectively.

*Ziziphorin A (**1**)*

Pale-yellow amorphous powder. – UV (MeOH): $\lambda_{max}(\log \varepsilon_{max})$ = 269 (2.8), 335 (2.6) nm. – IR (KBr): ν_{max} = 3406 (OH), 1760 (ester CO), 1670 (conjugated carbonyl), 1600 – 1400 (aromatic moiety) cm^{-1}. – ^1H NMR and ^{13}C NMR: see Table 1. – MS (EI, 70 eV): m/z (%) = 550 (8), 535 (10), 521 (11), 398 (15), 368 (13), 313 (8), 270 (100), 242 (17), 152 (20), 117 (16), 57 (30), 43 (38), 28 (98). – HRMS ((+)-EI): m/z = 550.3265 (calcd. 550.3295 for $C_{34}H_{46}O_6$, $[M]^+$).

*Ziziphorin B (**2**)*

Pale-yellow amorphous powder. – UV (MeOH): $\lambda_{max}(\log \varepsilon_{max})$ = 267(3.1), 330(2.8) nm. – IR(KBr): ν_{max} = 3401 (OH), 1763 (ester CO), 1670 (conjugated carbonyl), 1600 – 1400 (aromatic moiety) cm^{-1}. – ^1H NMR and ^{13}C NMR: see Table 1. – MS (EI, 70 eV): m/z (%) = 662 (4), 647 (5), 550 (10), 510 (12), 508 (8), 480 (7), 393 (10), 342 (20), 313 (13), 299 (15), 270 (80), 242 (14), 152 (23), 117 (18), 79 (100), 57 (80), 43 (50). – HRMS ((+)-EI): m/z = 662.4529 (calcd. 662.4547 for $C_{42}H_{62}O_6$, $[M]^+$).

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