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New secondary metabolites from *Croton sparsiflorus* Morong

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Abstract: Sparsifloamide (**1**), a new sphingolipid, and sparsifloside (**2**), a new diglyceride galactoside, have been isolated from the ethyl acetate soluble fraction of the 80% ethanolic extract of the whole plant of *Croton sparsiflorus* Morong. Their structures were assigned by ^{1}H- and ^{13}C-NMR spectra and DEPT, COSY, NOESY, HMQC, HMBC, and ESI-MS experiments.

Key words: *Croton sparsiflorus*, Euphorbiaceae, sphingolipid, diglyceride

1. Introduction

The genus *Croton* (Euphorbiaceae) comprises well over 1300 species growing as trees, shrubs, and herbs in tropical and subtropical regions of both hemispheres.[1] Its various species are reported to possess diverse medicinal properties.[1] One of its species is *Croton sparsiflorus* Morong (syn. *C. bonplandianus*), which is a woody shrub growing in sandy clay soil in Asia and South America. In Pakistan it grows in the Punjab and Sind provinces.[2] It is used as a potent hypotensive agent[3] and for the treatment of a variety of ailments like fever, inflammation, hypertension,[4] and it causes sharp fall in blood pressure.[5] Different extracts of this plant show antibacterial activity.[6] Sifting of the literature revealed that alkaloids,[7,8] diterpenes,[9] nonapeptide,[10] 1-*O*-methyl *cis*-inositol,[8] and amides[11] have previously been reported from this species. The chemotaxonomic and ethnopharmacological importance of the genus *Croton* prompted us to carry out further phytochemical studies on *C. sparsiflorus*. As a result, we herein report the isolation and structural elucidation of a sphingolipid named as sparsifloamide (**1**) and a diglyceride galactoside named as sparsifloside (**2**), respectively. The structures of these compounds are presented in Figure 1.

2. Experimental

General: Column chromatography (CC) was performed on silica gel (70-230 mesh, E. Merck, Darmstadt, Germany). TLC was carried out on precoated silica gel G-25-UV$_{254}$ plates (E. Merck) with detection at 254 and 366 nm or by spraying ceric sulfate in 10% H_2SO_4 (heating). The HPLC was carried out on LC-908W-C-60 (Japan Analytical Industry Co. Ltd., Tokyo, Japan). GC-MS was performed on an HP5890 gas chromatograph–mass spectrometer using a 5% diphenyl-polysiloxane/95% dimethyl-polysiloxane HP5-MS capillary column, column temperature ranging from 80 to 250 °C at 5 °C/min, helium used as carrier gas, and EI mode at 70 eV for mass. Optical rotations were measured on a JASCO DIP-360 polarimeter (JASCO,

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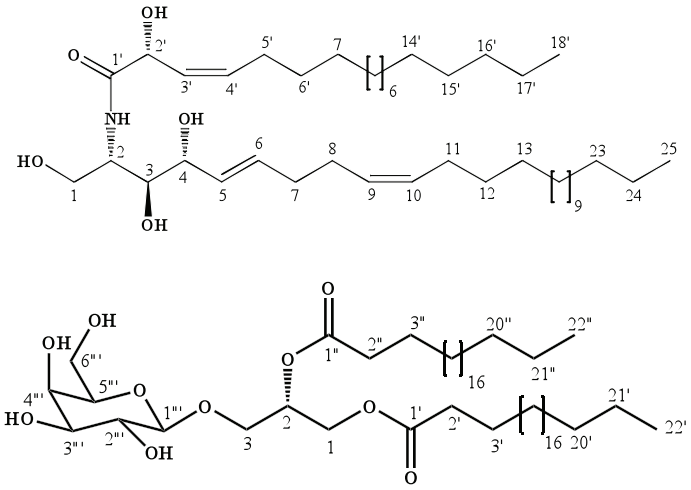

Figure 1. Structures of sparsifloamide (**1**) and sparsifloside (**2**).

Tokyo, Japan). UV spectra were recorded on a Hitachi UV-3200 spectrophotometer (Hitachi, Tokyo, Japan), while the IR spectra were recorded on a Shimadzu FTIR-8900 spectrometer (Shimadzu, Kyoto, Japan) as KBr pellets. One- and 2-dimensional NMR spectra were recorded on an AM-500 spectrometer (Bruker BioSpin, Fällanden, Switzerland) in C_5D_5N. The chemical shifts are given in ppm (δ) relative to tetramethylsilane as an internal standard, and scalar couplings are reported in *Hz*. Mass spectra (El and HR-EI) were obtained in electron impact mode on Finnigan MAT-112 and MAT-113 spectrometers (Finnigan, Waltham, MA, USA), HRESI-MS was measured on QSTAR XL spectrometers, and ions are given in m/z (%).

Plant material: The whole flowering plant of *Croton sparsiflorus* (18 kg) was collected from the Thatta district of Sindh Province in April 2009 and was identified by Prof Dr Surraiya Kahtoon, Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen has been deposited in the herbarium (voucher specimen no. 4309 KUH).

Extraction and isolation: The freshly collected whole plant material of *C. sparsiflorus* (18 kg) was shade-dried, cut into small pieces, and extracted with 80% ethanol (3 $\times$ 20 L, 10 days each) at room temperature (r.t.). The combined ethanolic extract was evaporated under reduced pressure at r.t. to yield a residue (300 g), which was suspended in water and successively divided into n-hexane (55 g), CH_2Cl_2 (16 g), AcOEt (6 g), n-BuOH (14 g), and H_2O (200 g) soluble subfractions. The AcOEt soluble subfraction (6 g) was subjected to CC over silica gel eluting with n-hexane-$CHCl_3$, $CHCl_3$, and $CHCl_3$-MeOH in increasing order of polarity. The fraction obtained with $CHCl_3$-MeOH (9.5:0.5) (50 mg) was extracted with acetone, and the acetone insoluble residue was rechromatographed over silica gel and eluted with the same solvent system to afford compound **1** (35 mg). The fractions obtained with $CHCl_3$-MeOH (9.0:1.0) (40 mg) provided a semipure compound. It was also extracted with acetone and the acetone insoluble residue was rechromatographed over silica gel eluting with $CHCl_3$-MeOH (9.2:0.8) to provide compound **2** (22 mg). The purity of the compounds was checked by HPLC over a reverse-phase C_{18} silica gel column, eluting with 90% MeOH in water.

Sparsifloamide (1): Colorless amorphous solid; $[\alpha]_D^{25}$+ 23.7 (c0.04, MeOH); UV (MeOH) λ_{max} (log ε): 204 (2.3), 231 (3.1); IR (KBr) $\nu_{max}\,cm^{-1}$: 3421, 3356, 1658, 1626, 1543, 1325; ^{1}H- and ^{13}C-NMR: see Table 1; EI-MS m/z (rel. %): 691 ($[M]^+$, 9), 677 ($[M–CH_2]^+$, 11), 673 ($[M–H_2O]^+$, 8), 663 ($[M–2CH_2]^+$, 19), 655 ($[M–2H_2O]^+$, 10), 649 ($[M–3CH_2]^+$, 25), 439 (15), 410 (14), 395 (22), 357 (20), 351 (11), 321 (13),

289 (14), 281 (18), 253 (70), 225 (12), 211 (15), 97 (425), 69 (30), 43 (100); HRESI-MS m/z: 691.6109 $[M]^+$ (calcd for $C_{43}H_{81}O_5N$, 691.6115).

Table 1. ^{1}H- (C_5D_5N, 500 MHz) and ^{13}C- (C_5D_5N, 125 MHz) NMR data, and ^{1}H-^{1}H COSY and HMBC correlations of compound **1**.

Carbons	δ_C	δ_H	^{1}H-^{1}H COSY	HMBC correlations
1	61.9	4.44 (dd, J = 4.5, 10.5 Hz)	H-1_b, H-2	C-2, C-3
		4.49 (dd, J = 7.8, 10.5 Hz)	H-1_a, H-2	C-2, C-3
2	52.9	5.09–5.12 (m)	H-1_a, H-1_b, H-3	C-1, C-1′, C-3, C-4
3	76.7	4.35 (dd, J = 5.5, 6.9 Hz)	H-2, H-4	C-1, C-2, C-4
4	72.4	4.26–4.29 (m)	H-3, H-5	C-2, C-3, C-5
5	130.3	6.71 (dd$J$ =7.0 15.0 Hz)	H-6, H-4	C-3, C-4, C-6, C-7
6	130.2	6.25 (dt$J$ =6.0, 15.0 Hz)	H-5, H-7	C-4, C-5, C-7, C-8
7	33.9	2.29 (dt$J$ =7.0 6.0 Hz)	H-6, H-8, H-7_b	C-5, C-6, C-7, C-10
		1.93 (dt$J$ =6.8 6.0 Hz)	H-6, H-8, H-7_a	
8	27.8	2.22–2.24 (m)	H-9	C-7, C-9
9	130.3	5.51–5.54 (m)	H-8, H-9	C-7, C-8, C-10, C-11
10	130.2	5.42–4.44 (m)	H-9, H-11	C-8, C-9, C-11
11	27.5	2.05–2.08 (m)	H-9, H-12	C-10, C-12
12	25.8	1.71–1.73 (m)	H-11, H-(13-22)	C-11, C-(13-22)
13–22	30.0–29.5	1.23–130 (br. s)	H-11, H-(13-22)	C-12, C-23, C-24
23	32.1	1.22 (br. s)	H-(13-22), H-24	C-(13-22), C-24, C-25
24	22.9	1.21 (m)	H-23, H-25	C-(13-22), C-23, C-25
25	14.2	0.84 (t, J = 7.0 Hz)	H-24	C-23, C-24
NH	—	8.57 (d, J = 9.0 Hz)	H-2	C-1′, C-2
1′	175.3	—	—	—
2′	72.9	4.62 (dd, J = 6.0 Hz)	H-3′	C-1′, C-3′
3′	130.8	7.62 (dd$J$ =6.0, 15.2 Hz)	H-3′, H-4′	C-1′, C-2′, C-5′
4′	130.6	6.73 (dt$J$ =6.5, 15.2 Hz)	H-3′, H-5′	C-2′, C-5′, C-6′
5′	35.6	2.20–2.23 (m)	H-4′, H-$5'_b$, H-6′	C-4′, C-6′
		2.02–2.04 (m)	H-4′, H-$5'_a$, H-6′	
6′	26.8	1.74–176 (m)	H-5′, H-(7′-15′)	C-4′, C-5′, C-(7′-15)
7′–15′	29.5–30.0	1.23–1.30 (br. s)	H-6′, H-16′	C-5′, C-6′, C-16′
16′	32.1	1.22 (br. s)	H-(7′-15′), H-17′	C-(7′-15′), C-17′, C-18′
17′	22.9	1.21 (br. s)	H-16′, H-18′	C-16′, C-18′
18′	14.2	0.84 (t, J = 7.0 Hz)	H-17′	C-16′, C-17′

Sparsifloside (2): Colorless amorphous solid; $[\alpha]_D^{25}$+ 104 (c 0.04, MeOH); UV (MeOH) λ_{max} (log ε): 204 (2.1), 269 (2.5); IR (KBr) ν_{max} cm^{-1}: 3520–3450, 1725, 1290; ^{1}H- and ^{13}C-NMR: see Table 2; EI-MS m/z (rel. %): 735 ([M–galactose]$^+$, 15), 734 ([M–galactose-H]$^+$, 30), 323 (25), 239 (88), 225 (10), 211 (11), 99 (50), 97 (25), 83 (65), 69 (35), 43 (55); HRESI-MS m/z: 899.7535 $[M+H]^+$ (calcd for $C_{53}H_{103}O_{10}$, 899.7551).

Table 2. ^{1}H- (C_5D_5N, 500 MHz) and ^{13}C- (C_5D_5N, 125 MHz) NMR data, and ^{1}H-^{1}H COSY and HMBC correlations of compound **2**.

Carbons	δ_C	δ_H	^{1}H-^{1}H COSY	HMBC correlations
1	63.3	4.68 (dd, J = 12.0, 3.0 *Hz*)	H-1$_a$	C-2, C-3
		4.52 (dd, J = 12.0, 7.0 *Hz*)	H-1$_b$	C-2, C-3
2	71.0	5.65–5.71 (m)	H-1$_a$, H-1$_b$, H-3$_a$, H-3$_b$	C-1, C-3
3	68.1	4.37 (dd, J = 11.0, 5.0 *Hz*)	H-3$_a$	C-1, C-2, C-1‴
	68.1	4.06 (dd, J = 11.0, 7.0 *Hz*)	H-3$_b$	C-1, C-2, C-1‴
1′	173.3	–	–	–
1″	173.2	–	–	–
2′	34.5	2.36 (t, J = 7.0 *Hz*)	H-3′	C-1′, C-3′, C-4′
2″	34.2	2.36 (t, J = 7.0 *Hz*)	H-3″	C-1″, C-3″, C-4″
3′	25.2	1.63–1.65 (m)	H-(4′-21′)	C-1′, C-2′, C-4′
3″	25.3	1.63–165 (m)	H-(4″-21″)	C-1″, C-2″, C-4″
4′–19′	29.4–30.0	1.23–1.26 (br. s)	H-22′, H-22″	C-2′, C-3′, C-22′
				C-2″, C-3″, C-22″
20′, 20″	32.1	1.23–1.26 (br. s)	H-22′, H-22″	–
21′, 21″	22.9	1.23–1.26 (br. s)	H-22′, H-22″	–
22′, 22″	14.3	0.85 (t, J = 7.0 *Hz*)	H-(4′-21′), H-(4″-21″)	C-20′, C-21′, C-20″,
C-1″				
OH	–	8.55 (s)	–	–
1‴	105.8	4.83 (d, J = 7.5 *Hz*)	H-2‴	C-3, C-2‴
2‴	72.3	4.41–4.44 (m)	H-1‴, H-3‴	C-1‴, C-3‴
3‴	75.3	4.15 (dd, J = 6.0, 3.5 *Hz*)	H-2‴, H-4‴	C-2‴, C-4‴
4‴	70.1	4.56 (d,J = 3.5 *Hz*)	H-3‴, H-5‴	C-2‴, C-3‴
5‴	77.2	4.05–4.07 (m)	H-4‴, H-6‴	C-1‴, C-6‴, C-4‴
6‴	62.3	4.43–4.45 (m)	H-5‴	C-4‴, C-5‴
		4.35–4.37 (m)		C-4‴, C-5‴

Methanolysis of 1 and 2: A solution of compound **1** or **2** (3 mg) in MeOH (4 mL) containing 1 N HCl (2 mL) was refluxed for 5 h, concentrated under reduced pressure, diluted with H_2O, and extracted with n-hexane. Evaporation of the n-hexane fraction of **1** provided methyl (2R, 3E)-2-hydroxyoctadec-3-enoate (1.9 mg), $[\alpha]_D^{25}$-19.2 (c 0.4, $CHCl_3$),[12] EI-MS m/z: 312 ($[M]^+$, 15), 253 (27), 111 (25), 97 (80) 69 (75), 57 (45), 43 (100). The sphingosine base could not be isolated due to lack of material. In the case of **2**, the residue obtained from n-hexane was subjected to CC over silica gel and eluted with n-hexane:AcOEt (9.6:0.4) to obtain methyl docosanoate, which gave the $[M]^+$ peak at m/z 354 in GC-MS. The aqueous layer was neutralized by addition of Ag_2CO_3 and concentrated in vacuo. The residue was purified by CC to afford a mixture of the α- and β-anomers of methyl D-galactoside. These were identified by TLC, ($CHCl_3$-MeOH-H_2O 12:7:1): R_f 0.66 (β) and 0.63 (α), optical rotation $[\alpha]_D$+ 80 (c = 0.04, H_2O), as well as EI-MS (m/z 194 $[M]^+$).

3. Oxidative cleavage of double bonds

To a solution of **1** (6 mg) in acetone were added a 0.04 M solution of K_2CO_3 (3 mL), an aqueous solution of 0.025 M $KMnO_4$ (18 mL), and 0.09 M $NaIO_4$. The reaction was allowed to proceed at 37 °C for 18 h. After acidification with 5 N H_2SO_4, the solution was decolorized with a 1 M solution of oxalic acid and extracted with Et_2O (3 × 30 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. The resulting mixture of carboxylic acids was methylated with ethereal solution of diazomethane and analyzed by GCMS, which gave clusters of peaks including peaks at m/z 270 for methyl hexadecanoate (methyl palmitate), m/z 256 for methyl pentadecanoate, and m/z146 for dimethyl succinate, respectively.

4. Results and discussion

The 80% ethanolic extract of the whole plant of *C. sparsiflorus* was suspended in water and divided into n-hexane, $CHCl_3$, AcOEt, n-BuOH, and H_2O soluble fractions. Column chromatographic techniques were applied to the ethyl acetate soluble fraction to obtain compounds **1** and **2**, respectively.

Sparsifloamide (**1**) was obtained as a colorless amorphous solid. The high resolution EI-MS showed the molecular ion peak $[M]^+$ at m/z 691.6105, which led to deduction of the molecular formula $C_{43}H_{81}O_5N$ having 4 degrees of unsaturation. The peaks at m/z 673.6001 and 655.5893 were due to the successive losses of water molecules. The UV spectrum showed the absorption maxima at 204 and 231 nm. The IR absorptions showed the presence of the hydroxyl (3421 cm^{-1}), amine (3356 cm^{-1}), and olefinic (1635 cm^{-1}) functionalities. The characteristic IR absorptions at 1658 and 1543 cm^{-1} suggested that compound **1** was a secondary amide derivative.[13]

In the ^{1}H-NMR spectrum (Table 1), the proton of secondary amide nitrogen showed a doublet at δ_H 8.57 (d, J = 9.0 Hz). The proton signals at δ_H 7.62 (dd J =6.0, 15.2 Hz), 6.73 (dd J =6.5, 15.2 Hz) 6.71 (dd J =7.0, 15.0 Hz), 6.25 (dt J =6.0, 15.0 Hz), 5.51–5.54 (m), and 5.42–4.44 (m) were attributed to 3 disubstituted double bonds. The upfield region showed a broad signal for 4 methylene groups in the range of δ_H 1.90–2.29 and 2 methylene groups in the range of δ_H 1.71–1.76, while the rest of the methylene protons resonated at δ_H 1.21–1.32 (br. s, 23 × CH_2). A triplet for 2 terminal methyl groups was observed at δ_H 0.84 (t, J = 7.0 Hz, 6H). The characteristic sphingolipid azomethine proton appeared at δ_H 5.09–5.12 (m). Two oxymethylene protons resonated at d_H 4.49 (dd, J = 7.8, 10.5 Hz) and 4.44 (dd, J = 4.5, 10.5 Hz), and 3 resonances appeared for oxymethine protons at δ_H 4.62 (d, J = 6.0 Hz), 4.35 (dd, 5.5, 6.9 Hz), and 4.26–4.29 (1H, m), confirming that compound **1** is a fatty acid amide of a sphingosine derivative.[13] The ^{13}C-NMR spectra (BB decoupled and DEPT) (Table 1) showed the signal of an amide carbonyl carbon at δ_C175.3, whereas the olefinic carbons resonated at δ_C 130.8, 130.6, 130.3, and 130.2. The characteristic signal of azomethine carbon appeared at δ_C52.9, while an oxymethylene carbon resonated at δ_C 61.9 along with 3 resonances of oxymethine carbons at δ_C76.7, 72.9, and 72.4. The methylenes of the aliphatic chains resonated in the range of δ_C 22.9-35.6 with the 2 terminal methyl carbons at δ_C 14.2.

In the ^{1}H-^{1}H COSY spectrum (Table 1), the azomethine proton at δ_H 5.09–5.12 showed correlations with oxymethylene protons at δ_H 4.49 (H_b-1) and 4.44 (H_a-1) as well as with the oxymethine proton at δ_H 4.35 (H-3). The latter proton further correlated with another oxymethine proton at δ_H 4.26–4.29 (H-4), revealing the position of 2 hydroxyl groups at C-3 and C-4, respectively. The position of the third hydroxyl group was confirmed at C-2′ by HMBC correlations of the oxymethine proton H-2′ at δ_H 4.62 with the amide carbonyl carbon (δ_C175.3) as well as the olefinic carbon (δ_C 130.8), the latter allowing us to assign the double bond

to C-3. The larger coupling constants between H-3′ and H-4′ (J = 15.2) revealed the E configuration of the double bond. The acid methanolysis of compound **1** furnished the known methyl ester of fatty acid characterized as methyl (2R, 3E)-2-hydroxyoctadec-3-enoate, $[M]^+$ peak in EI-MS at m/z 312, $[\alpha]_D^{25}$-19.2.[12]

The sphingosine base is concluded to be of 25 carbons. The olefinic carbon resonances at δc 130.2 and 130.3 suggested the presence of a 5,6-double bond by analogy of similar carbon resonances in astrocerebroside A obtained from *Astropecten latespinosus*.[14] It could further be confirmed through COSY and HMBC correlations. The olefinic proton at δ_H 6.71 showed COSY correlation with another olefinic proton (δ_H 6.25) as well as with H-4 (δ_H 4.26–4.29). It also showed HMBC correlations with C-3 (δ_C 76.6), C-4 (δ_C 72.4), C-6 (δ_C 130.2), and C-7 (δ_C 33.9). The olefinic proton at δ_H 6.25 showed ^2J correlations with C-5 (δ_C 130.3) and C-7 (δ_C 33.9) and ^3J correlations with C-4 (δ_C 72.4) and C-8 (δ_C 27.8), and it could subsequently be assigned to C-6. The remaining olefinic protons at δ_H 5.42–4.44 and 5.51–5.54 showed ^3J and ^2J correlations with C-8 (dc 27.8), allowing us to assign the remaining double bond to C-9. The positions of olefinic bonds in the base chain were further confirmed by the fragmentation peaks in the EI-MS at m/z 265, 237, and 211 due to McLafferty rearrangement. The diagnostic fragment ion peaks at m/z 410 and 281 resulted from the cleavage of amide bond, confirming the lengths of fatty acid and base chains being of 18 and 25 carbons, respectively (Figure 2). The positions of the double bonds were also confirmed by permanganate/periodate oxidative cleavage, which yielded a mixture of carboxylic acids. Subsequent methylation and GCMS gave clusters of peaks including $[M]^+$ peaks at m/z 270, 256, and 146 corresponding to methyl esters of palmitic acid, pentadecanoic acid, and dimethyl succinate, respectively. The smaller coupling constant between H-9 and H-10 ($W_{1/2}$ = 3.3 Hz) indicated *cis*-geometry of the double bond at C-9, subsequently confirmed by the signals of the allylic methylenes at δ_C 27.8 (C-8) and δ_C 27.5 (C-11), respectively. The chemical shift of the proton at δ_H 5.09–5.12 (H-2) and the carbon signals at δ_C 61.9 (C-1), 52.9 (C-2), 76.7 (C-3), 72.4 (C-4), 175.3 (C-1′), and 72.9 (C-2′) were very close to phytoceramides having 2′R, 2S, 3S, and 4R-stereochemistry,[15,16] revealing the same relative configuration at C-2, C-3, C-4, and C-2′, which was further supported by the NOESY spectrum; the azomethine proton at δ_H 5.09–5.12 (H-2) showed correlations with H-2′ at δ_H 4.62 and H-4 at δ_H 4.26-4.29. On the other hand, H-3 at δ_H 4.35 showed correlations with the amide proton at δ_H 8.57. On the basis of this evidence, the structure of sparsifloamide (**1**) could be assigned as *(2R,3E)-N-[(1S,2S,3R,4E,8Z)-2,3-dihydroxy-1-(hydroxymethyl)-4,8-tetracosadienyl]-2-hydroxy-3-octadecenamide* (Figure 1).

Sparsifloside (**2**) was obtained as a colorless amorphous solid. The HRESI-MS showed a quasimolecular ion $[M+H]^+$ peak at m/z 899.7535, which corresponded to the molecular formula $C_{53}H_{103}O_{10}$. The IR spectrum showed the presence of hydroxyl (3520–3450 cm^{-1}), ester (1725 cm^{-1}), and ether (1290 cm^{-1}) functionalities. The ^{1}H-NMR spectrum (Table 2) showed the signals of an oxymethine proton at δ_H 5.65–5.71 and 4 oxymethylene protons at δ_H 4.68 (dd, J = 12.0, 3.0 Hz), 4.52 (dd, J = 12.0, 7.0 Hz), 4.37 (dd, J = 11.0, 5.0 Hz), and 4.06 (dd, J = 11.0, 7.0 Hz), respectively. The anomeric proton showed the doublet at δ_H 4.83 (d, J = 7.5 Hz) and the oxymethine and oxymethylene protons of a hexose unit resonating in the range of δ_H 4.05–4.56. The signals of 2 methylene groups appeared at δ_H 2.36 (d, J = 7.0 Hz), suggesting their attachments with the carbonyl carbons. The signal of 2 further methylene groups were observed at δ_H 1.63–1.65 (m), while the rest of the methylene groups of the hydrocarbon chain resonated in the range of δ_H 1.23–1.26 (br. s, 36 × CH_2). The terminal methyl groups appeared at δ_H 0.85 (t, J = 7.0 Hz, 6H). The larger coupling constant of the anomeric proton allowed us to assign the β-configuration to the hexose moiety. The ^{13}C-NMR spectra

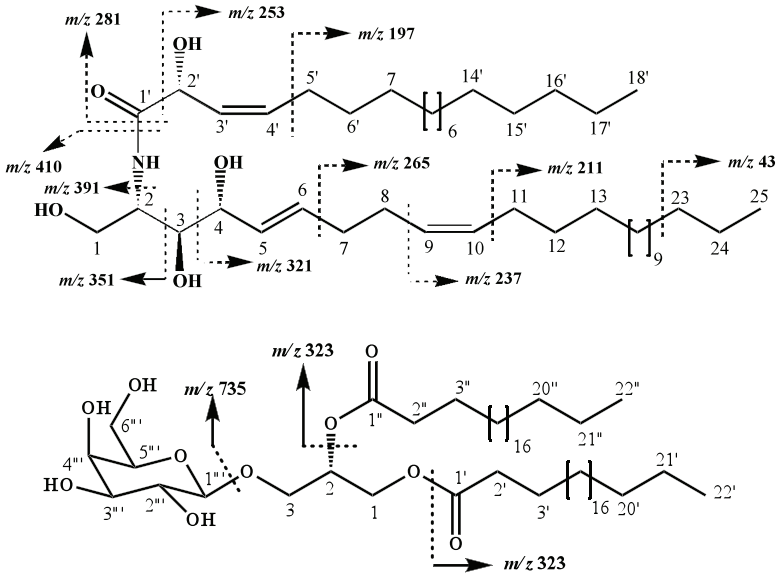

Figure 2. Mass fragmentation of sparsifloamide (**1**) and sparsifloside (**2**).

(BB decoupled and DEPT) showed 2 carbonyl carbon signals at δ_C 173.3 and 173.2 for the ester moieties. An oxymethine carbon showed a signal at δ_C 71.0 and oxymethylene carbons appeared at δ_C 68.1 and 63.3, suggesting a glycerol moiety.[17,18] The methylene carbons observed at δ_C 34.5 and 34.2 were assigned to those adjacent to the carbonyl carbons of ester functionalities. Further methylene carbons of the hydrocarbon chain resonated in the range of δ_C 22.9–32.1 along with the terminal methyl carbons at δ_C 4.3. The anomeric carbon resonated at δ_C 105.8, and the oxymethine and oxymethylene carbons of the hexose unit were observed in the range of δ_C 62.3–77.2. The hexose moiety was identified as β-D-galactose by the comparison of NMR data with the reported NMR data in the literature,[19] further confirmed by acid methanolysis of **2**, which furnished a glycone that could be identified as α- and β-anomers of methyl D-galactoside through the sign of its optical rotation ($[\alpha]_D^{25}+$ 80) as well as co-TLC. The aglycone could be identified as methyl docosanoate (m/z 354 $[M]^+$) through GC-MS. The identity of fatty acid could also be confirmed by EI-MS fragmentations, showing a fragment peak at m/z 323 due to the cleavage of docosanoyl moiety.

In the ^{1}H-^{1}H COSY spectrum (Table 2), the oxymethine proton at δ_H 5.65–5.71 showed correlations with the oxymethylene protons at δ_H 4.68, 4.52, 4.37, and 4.06, revealing the presence of a glycerol moiety. In the HMBC experiment, the oxymethine proton at H-2 (δ_H 5.65–5.71) showed the ^2J correlations with C-3 at δ_C 68.1 and C-1 at δ_C 63.3, as well as ^3J correlation with the carbonyl carbon C-1$''$ at δ_C 173.2. The anomeric proton at δ_H 4.83 showed ^3J correlation with the oxymethylene carbon at δ_C 68.1. The oxymethylene protons at C-1 (δ_H 4.68, 4.52) showed ^3J correlations with the carbonyl carbon C-1$'$ at δ_C 173.3 and C-3 at δ_C 68.1. The oxymethylene protons at C-3 (δ_H 4.37, 4.06) showed correlations with the oxymethylene C-1 at δ_C 63.3 as well as the anomeric carbon C-1$'''$ at δ_C 105.8, revealing the attachment of O-β-D-galactoside moiety to C-3 (Figure 2). The attachment of O-β-D-galactoside moiety was also confirmed by the downfield shift of C-3 compared to C-1. The configuration was established to be S by comparing the sign of optical rotation with those of reported diglyceride galactoside.[18] Consequently, sparsifloside (**2**) is confirmed as a diglyceride O-β-D-galactoside and its structure could be assigned as 2(S)-1-O-docosanoyl-2-O-docosanoyl-3-O-β-D-galactopyranosylglycerol (Figure 1).

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