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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 <sup>1</sup>H- and <sup>13</sup>C-NMR spectra and DEPT, COSY, NOESY, HMQC, HMBC, and ESI-MS experiments.

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

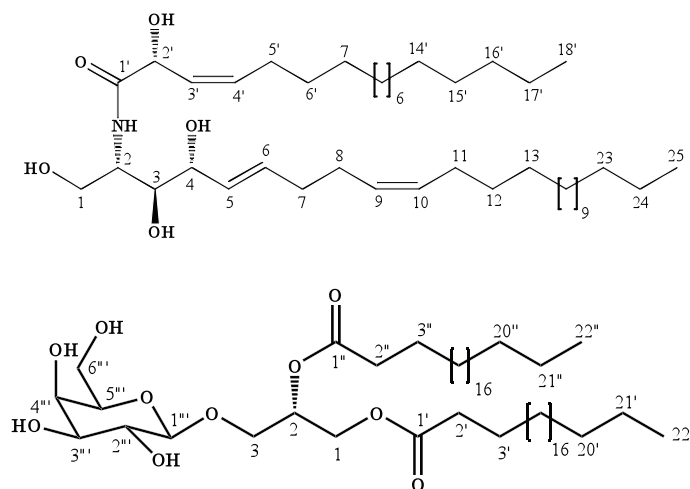
### 1. Introduction

The genus *Croton* (Euphorbiaceae) comprises well over 1300 species growing as trees, shrubs, and herbs in tropical and subtropical regions of both hemispheres.<sup>1</sup> Its various species are reported to possess diverse medicinal properties.<sup>1</sup> 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.<sup>2</sup> It is used as a potent hypotensive agent<sup>3</sup> and for the treatment of a variety of ailments like fever, inflammation, hypertension,<sup>4</sup> and it causes sharp fall in blood pressure.<sup>5</sup> Different extracts of this plant show antibacterial activity.<sup>6</sup> Sifting of the literature revealed that alkaloids,<sup>7,8</sup> diterpenes,<sup>9</sup> nonapeptide,<sup>10</sup> 1-*O*-methyl *cis*-inositol,<sup>8</sup> and amides<sup>11</sup> 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<sub>254</sub> plates (E. Merck) with detection at 254 and 366 nm or by spraying ceric sulfate in 10% H<sub>2</sub>SO<sub>4</sub> (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 ( $\delta$ ) relative to tetramethylsilane as an internal standard, and scalar couplings are reported in *Hz*. Mass spectra (EI 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;  $[\alpha]_D^{25} + 23.7$  ( $c$ 0.04, MeOH); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ): 204 (2.3), 231 (3.1); IR (KBr)  $\nu_{max}$   $cm^{-1}$ : 3421, 3356, 1658, 1626, 1543, 1325;  $^1H$ - 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]<sup>+</sup> (calcd for C<sub>43</sub>H<sub>81</sub>O<sub>5</sub>N, 691.6115).

**Table 1.** <sup>1</sup>H- (C<sub>5</sub>D<sub>5</sub>N, 500 MHz) and <sup>13</sup>C- (C<sub>5</sub>D<sub>5</sub>N, 125 MHz) NMR data, and <sup>1</sup>H-<sup>1</sup>H COSY and HMBC correlations of compound **1**.

Carbons	$\delta_C$	$\delta_H$	<sup>1</sup> H- <sup>1</sup> H COSY	HMBC correlations
1	61.9	4.44 (dd, $J = 4.5, 10.5$ Hz)	H-1 <sub>b</sub> , H-2	C-2, C-3
		4.49 (dd, $J = 7.8, 10.5$ Hz)	H-1 <sub>a</sub> , H-2	C-2, C-3
2	52.9	5.09–5.12 (m)	H-1 <sub>a</sub> , H-1 <sub>b</sub> , 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 <sub>b</sub>	C-5, C-6, C-7, C-10
		1.93 (dt $J = 6.8, 6.0$ Hz)	H-6, H-8, H-7 <sub>a</sub>	
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	1.23–1.30 (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' <sub>b</sub> , H-6'	C-4', C-6'
		2.02–2.04 (m)	H-4', H-5' <sub>a</sub> , H-6'	
6'	26.8	1.74–1.76 (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)  $\lambda_{\max}$  (log  $\epsilon$ ): 204 (2.1), 269 (2.5); IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3520–3450, 1725, 1290; <sup>1</sup>H- and <sup>13</sup>C-NMR: see Table 2; EI-MS  $m/z$  (rel. %): 735 ([M–galactose]<sup>+</sup>, 15), 734 ([M–galactose-H]<sup>+</sup>, 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]<sup>+</sup> (calcd for C<sub>53</sub>H<sub>103</sub>O<sub>10</sub>, 899.7551).

**Table 2.**  $^1\text{H}$ - ( $\text{C}_5\text{D}_5\text{N}$ , 500 MHz) and  $^{13}\text{C}$ - ( $\text{C}_5\text{D}_5\text{N}$ , 125 MHz) NMR data, and  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC correlations of compound **2**.

Carbons	$\delta_C$	$\delta_H$	$^1\text{H}$ - $^1\text{H}$ COSY	HMBC correlations
1	63.3	4.68 (dd, $J = 12.0, 3.0 \text{ Hz}$ )	H-1 <sub>a</sub>	C-2, C-3
		4.52 (dd, $J = 12.0, 7.0 \text{ Hz}$ )	H-1 <sub>b</sub>	C-2, C-3
2	71.0	5.65–5.71 (m)	H-1 <sub>a</sub> , H-1 <sub>b</sub> , H-3 <sub>a</sub> , H-3 <sub>b</sub>	C-1, C-3
3	68.1	4.37 (dd, $J = 11.0, 5.0 \text{ Hz}$ )	H-3 <sub>a</sub>	C-1, C-2, C-1'''
		4.06 (dd, $J = 11.0, 7.0 \text{ Hz}$ )	H-3 <sub>b</sub>	C-1, C-2, C-1'''
1'	173.3	–	–	–
1''	173.2	–	–	–
2'	34.5	2.36 (t, $J = 7.0 \text{ Hz}$ )	H-3'	C-1', C-3', C-4'
2''	34.2	2.36 (t, $J = 7.0 \text{ Hz}$ )	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	1.63–1.65 (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'' C-1''	14.3	0.85 (t, $J = 7.0 \text{ Hz}$ )	H-(4'-21'), H-(4''-21'')	C-20', C-21', C-20'', C-2'', C-3'', C-22''
OH	–	8.55 (s)	–	–
1'''	105.8	4.83 (d, $J = 7.5 \text{ Hz}$ )	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, $J = 6.0, 3.5 \text{ Hz}$ )	H-2''', H-4'''	C-2''', C-4'''
4'''	70.1	4.56 (d, $J = 3.5 \text{ Hz}$ )	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<sub>2</sub>O, and extracted with *n*-hexane. Evaporation of the *n*-hexane fraction of **1** provided methyl (2*R*, 3*E*)-2-hydroxyoctadec-3-enoate (1.9 mg),  $[\alpha]_D^{25}$ -19.2 (*c* 0.4, CHCl<sub>3</sub>), <sup>12</sup> EI-MS *m/z*: 312 ([M]<sup>+</sup>, 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]<sup>+</sup> peak at *m/z* 354 in GC-MS. The aqueous layer was neutralized by addition of Ag<sub>2</sub>CO<sub>3</sub> and concentrated in vacuo. The residue was purified by CC to afford a mixture of the  $\alpha$ - and  $\beta$ -anomers of methyl D-galactoside. These were identified by TLC, (CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O 12:7:1): R<sub>f</sub> 0.66 ( $\beta$ ) and 0.63 ( $\alpha$ ), optical rotation  $[\alpha]_D + 80$  (*c* = 0.04, H<sub>2</sub>O), as well as EI-MS (*m/z* 194 [M]<sup>+</sup>).

### 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  $Et_2O$  ( $3 \times 30$  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  $m/z$  146 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 ( $3421\text{ cm}^{-1}$ ), amine ( $3356\text{ cm}^{-1}$ ), and olefinic ( $1635\text{ cm}^{-1}$ ) functionalities. The characteristic IR absorptions at  $1658$  and  $1543\text{ cm}^{-1}$  suggested that compound **1** was a secondary amide derivative.<sup>13</sup>

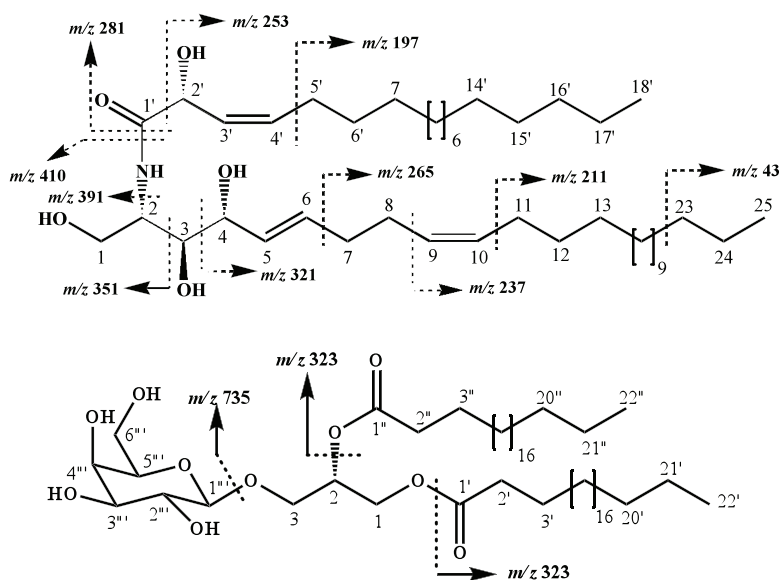
In the  $^1H$ -NMR spectrum (Table 1), the proton of secondary amide nitrogen showed a doublet at  $\delta_H$  8.57 (d,  $J = 9.0\text{ Hz}$ ). The proton signals at  $\delta_H$  7.62 (dd  $J = 6.0, 15.2\text{ Hz}$ ), 6.73 (dd  $J = 6.5, 15.2\text{ Hz}$ ), 6.71 (dd  $J = 7.0, 15.0\text{ Hz}$ ), 6.25 (dt  $J = 6.0, 15.0\text{ Hz}$ ), 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  $\delta_H$  1.90–2.29 and 2 methylene groups in the range of  $\delta_H$  1.71–1.76, while the rest of the methylene protons resonated at  $\delta_H$  1.21–1.32 (br. s,  $23 \times CH_2$ ). A triplet for 2 terminal methyl groups was observed at  $\delta_H$  0.84 (t,  $J = 7.0\text{ Hz}$ , 6H). The characteristic sphingolipid azomethine proton appeared at  $\delta_H$  5.09–5.12 (m). Two oxymethylene protons resonated at  $\delta_H$  4.49 (dd,  $J = 7.8, 10.5\text{ Hz}$ ) and 4.44 (dd,  $J = 4.5, 10.5\text{ Hz}$ ), and 3 resonances appeared for oxymethine protons at  $\delta_H$  4.62 (d,  $J = 6.0\text{ Hz}$ ), 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.<sup>13</sup> The  $^{13}C$ -NMR spectra (BB decoupled and DEPT) (Table 1) showed the signal of an amide carbonyl carbon at  $\delta_C$  175.3, whereas the olefinic carbons resonated at  $\delta_C$  130.8, 130.6, 130.3, and 130.2. The characteristic signal of azomethine carbon appeared at  $\delta_C$  52.9, while an oxymethylene carbon resonated at  $\delta_C$  61.9 along with 3 resonances of oxymethine carbons at  $\delta_C$  76.7, 72.9, and 72.4. The methylenes of the aliphatic chains resonated in the range of  $\delta_C$  22.9–35.6 with the 2 terminal methyl carbons at  $\delta_C$  14.2.

In the  $^1H$ - $^1H$  COSY spectrum (Table 1), the azomethine proton at  $\delta_H$  5.09–5.12 showed correlations with oxymethylene protons at  $\delta_H$  4.49 ( $H_b$ -1) and 4.44 ( $H_a$ -1) as well as with the oxymethine proton at  $\delta_H$  4.35 ( $H$ -3). The latter proton further correlated with another oxymethine proton at  $\delta_H$  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  $\delta_H$  4.62 with the amide carbonyl carbon ( $\delta_C$  175.3) as well as the olefinic carbon ( $\delta_C$  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 (2*R*, 3*E*)-2-hydroxyoctadec-3-enoate,  $[M]^+$  peak in EI-MS at  $m/z$  312,  $[\alpha]_D^{25}$  -19.2.<sup>12</sup>

The sphingosine base is concluded to be of 25 carbons. The olefinic carbon resonances at  $\delta_C$  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*.<sup>14</sup> It could further be confirmed through COSY and HMBC correlations. The olefinic proton at  $\delta_H$  6.71 showed COSY correlation with another olefinic proton ( $\delta_H$  6.25) as well as with H-4 ( $\delta_H$  4.26–4.29). It also showed HMBC correlations with C-3 ( $\delta_C$  76.6), C-4 ( $\delta_C$  72.4), C-6 ( $\delta_C$  130.2), and C-7 ( $\delta_C$  33.9). The olefinic proton at  $\delta_H$  6.25 showed  $^2J$  correlations with C-5 ( $\delta_C$  130.3) and C-7 ( $\delta_C$  33.9) and  $^3J$  correlations with C-4 ( $\delta_C$  72.4) and C-8 ( $\delta_C$  27.8), and it could subsequently be assigned to C-6. The remaining olefinic protons at  $\delta_H$  5.42–4.44 and 5.51–5.54 showed  $^3J$  and  $^2J$  correlations with C-8 ( $\delta_C$  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  $\delta_C$  27.8 (C-8) and  $\delta_C$  27.5 (C-11), respectively. The chemical shift of the proton at  $\delta_H$  5.09–5.12 (H-2) and the carbon signals at  $\delta_C$  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*, 2*S*, 3*S*, and 4*R*-stereochemistry,<sup>15,16</sup> 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  $\delta_H$  5.09–5.12 (H-2) showed correlations with H-2' at  $\delta_H$  4.62 and H-4 at  $\delta_H$  4.26–4.29. On the other hand, H-3 at  $\delta_H$  4.35 showed correlations with the amide proton at  $\delta_H$  8.57. On the basis of this evidence, the structure of sparsifloamide (**1**) could be assigned as (2*R*,3*E*)-*N*-[(1*S*,2*S*,3*R*,4*E*,8*Z*)-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  $^1H$ -NMR spectrum (Table 2) showed the signals of an oxymethine proton at  $\delta_H$  5.65–5.71 and 4 oxymethylene protons at  $\delta_H$  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  $\delta_H$  4.83 (d,  $J = 7.5$  Hz) and the oxymethine and oxymethylene protons of a hexose unit resonating in the range of  $\delta_H$  4.05–4.56. The signals of 2 methylene groups appeared at  $\delta_H$  2.36 (d,  $J = 7.0$  Hz), suggesting their attachments with the carbonyl carbons. The signal of 2 further methylene groups were observed at  $\delta_H$  1.63–1.65 (m), while the rest of the methylene groups of the hydrocarbon chain resonated in the range of  $\delta_H$  1.23–1.26 (br. s,  $36 \times CH_2$ ). The terminal methyl groups appeared at  $\delta_H$  0.85 (t,  $J = 7.0$  Hz, 6H). The larger coupling constant of the anomeric proton allowed us to assign the  $\beta$ -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  $\delta_C$  173.3 and 173.2 for the ester moieties. An oxymethine carbon showed a signal at  $\delta_C$  71.0 and oxymethylene carbons appeared at  $\delta_C$  68.1 and 63.3, suggesting a glycerol moiety.<sup>17,18</sup> The methylene carbons observed at  $\delta_C$  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  $\delta_C$  22.9–32.1 along with the terminal methyl carbons at  $\delta_C$  4.3. The anomeric carbon resonated at  $\delta_C$  105.8, and the oxymethine and oxymethylene carbons of the hexose unit were observed in the range of  $\delta_C$  62.3–77.2. The hexose moiety was identified as  $\beta$ -D-galactose by the comparison of NMR data with the reported NMR data in the literature,<sup>19</sup> further confirmed by acid methanolysis of **2**, which furnished a glycone that could be identified as  $\alpha$ - and  $\beta$ -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  $^1H$ - $^1H$  COSY spectrum (Table 2), the oxymethine proton at  $\delta_H$  5.65–5.71 showed correlations with the oxymethylene protons at  $\delta_H$  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 ( $\delta_H$  5.65–5.71) showed the  $^2J$  correlations with C-3 at  $\delta_C$  68.1 and C-1 at  $\delta_C$  63.3, as well as  $^3J$  correlation with the carbonyl carbon C-1'' at  $\delta_C$  173.2. The anomeric proton at  $\delta_H$  4.83 showed  $^3J$  correlation with the oxymethylene carbon at  $\delta_C$  68.1. The oxymethylene protons at C-1 ( $\delta_H$  4.68, 4.52) showed  $^3J$  correlations with the carbonyl carbon C-1' at  $\delta_C$  173.3 and C-3 at  $\delta_C$  68.1. The oxymethylene protons at C-3 ( $\delta_H$  4.37, 4.06) showed correlations with the oxymethylene C-1 at  $\delta_C$  63.3 as well as the anomeric carbon C-1''' at  $\delta_C$  105.8, revealing the attachment of *O*- $\beta$ -D-galactoside moiety to C-3 (Figure 2). The attachment of *O*- $\beta$ -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.<sup>18</sup> Consequently, sparsifloside (**2**) is confirmed as a diglyceride *O*- $\beta$ -D-galactoside and its structure could be assigned as 2(*S*)-1-*O*-docosanoyl-2-*O*-docosanoyl-3-*O*- $\beta$ -D-galactopyranosylglycerol (Figure 1).



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