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Two New Glycosides from the Soft Coral *Sinularia firma*

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Two new glycosides, named Firmacosides A and B, together with known fatty esters, batyl alcohol, $\Delta^{5,20}$ sterol and sphingosine derivatives have been isolated from the soft coral *Sinularia firma* TIX-DUR. On the basis of spectroscopic analysis (^{1}H-NMR, ^{13}C-NMR, ^{1}H–^{1}H COSY, HMQC, HMBC and FAB-MS), Firmacoside A was established as hexadecanyl-1-*O*-*α*-D-arabinopyranosyloxy (1→4)-*α*-D-arabinopyranosyloxy (1→4)-*α*-D-arabinopyranoside (1), and firmacoside B was elucidated as docosanyl-1-*O*-*α*-D-arabinopyranosyloxy (1→4)-3-*O*-acetyl-*α*-arabinopyranosyloxy (1→4)-*α*-D-arabinopyranoside (2).

Key words *Sinularia firma*; firmacoside; soft coral; glycoside

Marine organisms are a potential source of bioactive compounds.[1] Soft corals belonging to genus *Sinularia* have been found to be a rich source of structurally unique and biologically active secondary metabolites.[2,3] In our continuing interest in bioactive secondary metabolites from marine organisms of the Indian Ocean, we have under taken the chemical examination of *Sinularia firma*[4] TIX-DUR collected from the Ramnathpuram, Tamil Nadu coast of India in August 1999. Only a furanosesquiterpene has been reported in the literature[5] of this animal. The present investigation resulted in the isolation and structural characterization of two new glycosides (**1**, **2**) along with known fatty esters (**3**, **4**), batyl alcohol (**5**), $\Delta^{5,20}$ sterol (**6**), sphingosine derivatives (**7**, **8**). These are lipid glycosides whose lipid portion is derived from fatty alcohol linked carbohydrate through glycosidic bond. This type of glycosides have been previously reported from the genus *Cleistopholis* (Annonaceae)[6—8] and were found to possess significant antibacterial[9] activity.

The specimens of the soft coral were cut into thin slices and extracted with methanol. The methanol soluble materials were concentrated under reduced pressure below 50 °C and the dark brown residue was fractionated with ethyl acetate and *n*-butanol successively. The ethyl acetate soluble fraction led to the isolation of six pure compounds (**3**—**8**) while two new glycosides (**1**, **2**) were isolated from *n*-butanol soluble fraction.

Compound **1**, tentatively designated firmacoside A, was obtained as microcrystalline white solid, $[\alpha]_D^{25}$ $-104°$ [c=2.0, $CHCl_3$: MeOH (1 : 1)]. Its positive FAB-MS exhibited molecular ion peak at *m/z* 661 $[M+Na]^+$ consistent with a molecular formula $C_{31}H_{58}O_{13}$. Further fragment ion peaks were observed at *m/z* 397 $[M-241$ (palmityl alcohol)$]^+$, 265 $[397-132]$, 133 $[265-132]$ corresponding respectively, to the successive loss of three pentosyls and one palmityl moiety. The ^{1}H-NMR spectrum of **1** showed a terminal methyl at δ 0.87 (3H, t, J=6.9 Hz), broad methylene singlet at δ 1.25 (26H), and a multiplet at δ 1.60 (2H) as signable to long aliphatic chain. Three anomeric protons appeared at δ 5.16 (1H, d, J=3.0 Hz), 5.41 (1H, d, J=3.0 Hz), and 5.46 (1H, d, J=3.0 Hz), correlated with the anomeric carbon atoms at δ 100.9, 98.8, and 99.1 respectively in HMQC[10] spectrum, suggested that **1** contains three sugar units. Acid hydrolysis of **1** with 2 N HCl in 80% of ethanol afforded *α*-D-arabinopyranose as carbohydrate component, while the aglycone was identified as palmityl alcohol by TLC on comparison with an authentic sample. Starting from the three sugar anomeric protons interpretation of ^{1}H–^{1}H COSY and TOCSY[11] spectra revealed the presence of three pentose rings. All three rings were assigned as *α*-D-arabinopyranoside on the basis of small vicinal coupling constant (J=3.0 Hz) of C-5*α* protons.[12,13] All the anomeric protons were equatorial as judged from vicinal coupling constants of 3.0 Hz. Coupling constants of overlapping proton signals were determined by the HMQC spectrum. The linkage of sugar units was established by HMBC[14] cross peaks between $Ara^2 \cdot H\text{-}1''/Ara^1 \cdot C\text{-}4'$, $Ara^3 \cdot H\text{-}1'''/Ara^2 \cdot C\text{-}4''$ and reverse correlation between $Ara^1 \cdot H\text{-}4'/Ara^2 \cdot C\text{-}1''$, $Ara^2 \cdot H\text{-}4''/Ara^3 \cdot C\text{-}1'''$, while the position of the glycosidic linkage to fatty acid moiety was based on HMBC cross peaks between fatty alcohol H-1a, H-1b/$Ara^1 \cdot C\text{-}1'$ and reverse correlation between $Ara^1 \cdot H\text{-}1'$/Fatty alcohol C-1.

Thus the structure of Firmacoside A **1** was determined to be hexadecanyl-1-*O*-*α*-D-arabinopyranosyloxy (1→4)-*α*-D-arabinopyranosyloxy (1→4)-*α*-D-arabinopyranoside (**1**), which is a stereoisomer of cervicoside isolated from a Chinese collection of *Sinularia cervicornis*[15] where the sugars are *β*-connected arabinopyranose.

Compound **2**, tentatively designated firmacoside B, was obtained as microcrystalline white solid, $[\alpha]_D^{25}$ $-136°$ [c=1.0, $CHCl_3$: MeOH (1 : 1)] and showed molecular ion peak at *m/z* 766 $[M+2H]^+$ in the positive FAB-MS consistent with a molecular formula $C_{39}H_{72}O_{14}$. Further fragmentation ion peaks were observed at *m/z* 706 $[(M+2H)-CH_3CO_2H]^+$, 438 $[764-326]$, 306 $[438-132]$, 132 $[306-174]$ corresponding respectively, to the successive loss of one acetyl, one pentosyl, one acetyl pentosyl, one pentosyl and one docosanol moiety. Acid hydrolysis of **2** gave *α*-D-arabinopyranose as carbohydrate component, while the aglycone was identified as 1-docosanol by CoTLC comparison with known sample and spectral data comparison with literature.[16] The ^{1}H- and ^{13}C-NMR spectral data of **2** were similar to those of **1**, except for the length of aliphatic chain and position of an acetyl group. The ^{1}H- and ^{13}C-NMR signals were assigned in detail using techniques similar to those used for **1**. The signal of H-3″ was observed in the downfield region when compared to those for **1** in the ^{1}H-NMR spectrum. This observation suggested the location of an acetyl group on the C-3″ of sugar, which was ascertained by the cross peak be-

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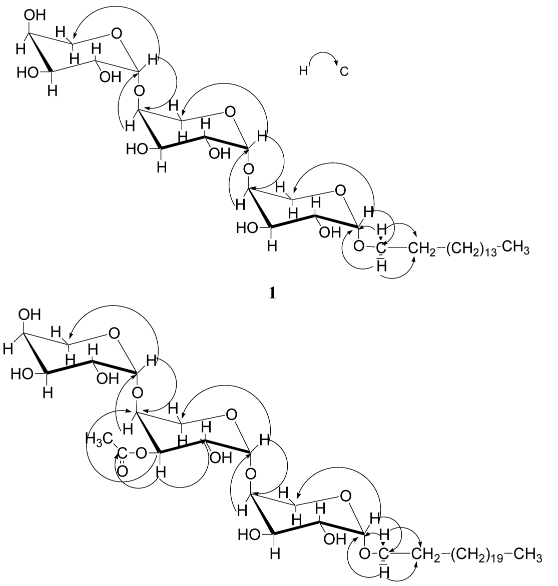
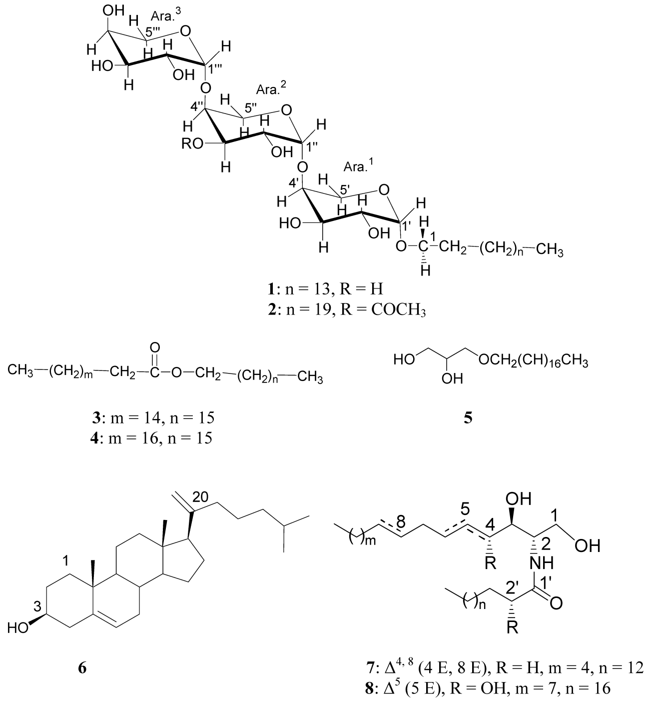


Fig. 1. ^{1}H–^{13}C Long Range Correlations Observed for **1** and **2** in the HMBC Spectra (in Pyridine-d_5, 300 MHz)



1: n = 13, R = H
2: n = 19, R = COCH$_3$

3: m = 14, n = 15
4: m = 16, n = 15

5

6

7: $\Delta^{4,8}$ (4 E, 8 E), R = H, m = 4, n = 12
8: Δ^5 (5 E), R = OH, m = 7, n = 16

Fig. 2. Structures of **1**—**8**

tween δ 5.85 (H-3″) and δ 171.0 (C=O) in the HMBC spectrum.

Thus, the structure of Firmacoside B was determined to be docosanyl-1-*O*-α-D-arabinopyranosyloxy-(1→4)-3-*O*-acetyl-α-D-arabinopyranosyloxy-(1→4)-α-D-arabinopyranoside (**2**).

Compounds **3**—**8** were identified as hexadecyl hexadecanoate[17] (**3**), hexadecyl octadecanoate[18] (**4**), batyl alcohol[19] (**5**), Cholesta-5,20-diene-3β-ol[20] (**6**), *N*-palmityl-2-amino-1,3-dihydroxy octadeca-4,8-diene[21] (**7**), and *N*-(2′-hydroxyeicosanoyl)-1,3,4-trihydroxy-2-aminoheptadec-5-ene[22] (**8**), respectively, based on their physical and spectral data.

Table 1. ^{1}H- (600 MHz) and ^{13}C-NMR (75 MHz) Spectral Data for Compounds **1** and **2** in Pyridine-d_5

Position	**1**		**2**	
	δ_C	δ_H[a)]	δ_C	δ_H[a)]
Fatty alcohol				
1	68.6	3.76 (td, 9.6, 6.6) 3.45 (td, 9.6, 6.6)	68.8	3.84 (td, 9.6, 6.6) 3.53 (td, 9.6, 6.6)
2	32.2	1.63 (m)	32.2	1.60 (m)
3—15	23.0 to 30.0	1.29 (br s)	23.1—30.1	1.26 (br s)
16	14.4	0.87 (t, 6.9)		
17—21		—		
22		—	14.1	0.85 (t, 6.6)
Arabinose-1				
1′	100.9	5.16 (d, 3.0)	100.9	5.18 (d, 3.6)
2′	70.8	4.41[b)]	70.4	4.50 (dd, 10.6, 3.0)
3′	70.1	4.46[b)]	70.1	4.59 (dd, 10.2, 3.6)
4′	75.82	4.38[b)]	75.7	4.57[b)]
5′	60.6	4.58 (dd, 12.6, 3.0) 4.06 (dd, 12.6, 3.0)	60.4	4.14 (dd, 12.6, 3.0) 4.00 (dd, 12.6, 3.0)
Arabinose-2				
1″	98.8	5.41 (d, 3.0)	98.7	5.52 (d, 3.6)
2″	71.1	4.56 (dd, 9.0, 3.6)	67.6	4.72 (dd, 10.2, 5.6)
3″	70.1	4.51 (dd, 9.0, 3.0)	73.1	5.85 (dd, 10.8, 3.0)
4″	75.81	4.32 (br d)	74.8	4.57[b)]
5″	61.2	4.66 (dd, 12.0, 3.0) 4.00 (dd, 12.0, 3.0)	61.2	4.82 (dd, 12.6, 2.4) 4.15 (dd, 12.6, 2.4)
3″-Ac		—	21.2	2.06 (s)
		—	171.2	—
Arabinose-3				
1‴	99.1	5.46 (d, 3.0)	100.4	5.40 (d, 3.6)
2‴	70.6	4.60[b)]	70.5	4.64 (dd, 9.6, 3.6)
3‴	70.8	4.48 (dd, 9.6, 2.4)	71.3	4.57[b)]
4‴	70.2	4.40 (br d)	70.4	4.47[b)]
5‴	64.8	4.59 (dd, 12.6, 3.0) 4.11 (dd, 12.6, 3.0)	64.8	4.48 (dd, 11.4, 2.4) 4.10 (dd, 11.4, 2.4)

a) Multiplicity and *J* values in Hz are given in parentheses. *b*) Overlapped.

Experimental

General Method Melting points were taken in open capillaries on an electrically heated melting point apparatus Complab and are uncorrected. Optical rotations were determined on an Autopol III automatic polarimeter using the sodium D-line (C in g/100 ml). IR spectra were recorded on a Perkin-Elmer RX-1 spectrophotometer using either KBr pellets or in neat. The FAB-MS were recorded using a beam of Argon (2—8 ev) on Jeol SX 102/DA-6000 mass spectrometer. 1D and 2D NMR spectra were recorded on Varian Inova 600 MHz and Brucker DRX 300 MHz FT-NMR instruments with TMS as an internal standard. Analytical HPLC was carried out on Shimadzu, UV SPD-10 AVP system using RP-18 (Shim-pack RRC-ODS 20 mm×25 cm) column. GLC was carried out in Perkin Elmer Auto System XL using steel column (2 m×4 mm, packed with 3% OV-225 on Gas chrom Q: FID temperature 80 °C; 3 °C/min to 250 °C, N$_2$ at 25 ml/min as carrier gas). TLC was run on Merck aluminium Pre-coated silica gel 60 F$_{254}$ plates. Column chromatography was carried out with Merck silica gel 60—120, 100—200, and 320—400 mesh.

Animal Material The soft coral *Sinularia firma* was collected from Ramnathpuram, Tamil Nadu coast of India in August 1999 and transported to the laboratory dipped in MeOH. A voucher specimen is preserved at the herbarium of Central Drug Research Institute, Lucknow, India with Botany Serial number 339.

Extraction and Isolation The MeOH in which fresh soft coral (10.0 kg) was transported to the laboratory was drained off. The material was chopped into small pieces, filled in glass percolators and extracted with MeOH (3×4 l) at room temperature. The combined MeOH extract was concentrated under reduced pressure on rotatory evaporator below 50 °C to give a residue (70.0 g) which was suspended in H$_2$O (500 ml). The suspension was extracted successively with EtOAc (3×500 ml), *n*-BuOH (3×500 ml) and the solvent was removed under reduced pressure to afford EtOAc soluble (19.8 g) and *n*-BuOH soluble (23.4 g) fractions respectively. The EtOAc soluble (19.8 g) fraction was subjected to column chromatography over silica gel (100—200 mesh) using a gradient of *n*-hexane : $CHCl_3$ and $CHCl_3$: MeOH with increasing proportion of $CHCl_3$ and MeOH respectively. Fraction eluted with *n*-hexane : $CHCl_3$ (60 : 40 v/v) furnished compound **3** (250 mg) and **4** (200 mg). Fraction eluted with *n*-hexane : $CHCl_3$ (25 : 75) furnished compound **5** (400 mg). Fraction eluted with $CHCl_3$: MeOH (99 : 1 v/v) yielded compound **6** (300 mg). Fraction eluted with $CHCl_3$: MeOH (95 : 5 v/v) afforded compound **7** (25 mg) and **8** (65 mg).

Similarly, *n*-BuOH fraction (23.4 g) was subjected to flash chromatography over silics gel (320—400 mesh) column using gradient elution of $CHCl_3$: MeOH : H_2O with increasing proportion of MeOH and H_2O. Fraction eluted with $CHCl_3$: MeOH : H_2O (88 : 11.4 : 0.6 v/v) afforded compound **2** (70 mg). Fraction eluted with $CHCl_3$: MeOH : H_2O (86 : 13.3 : 0.7 v/v) yielded compound **1** (160 mg).

Compound **1**: ^{1}H- and ^{13}C-NMR (pyridine-d_5) data see Table 1. IR ν_{max} (KBr) cm^{-1}: 3399, 2921, 1597, 1077. FAB-MS *m/z*: 661 $[M+Na]^+$, 397, 265, 241 and 133. Microcrystelline white solid, mp 184 °C, $[\alpha]_D^{25}$ $-104°$ [c=2.0, $CHCl_3$: MeOH (1 : 1)].

Compound **2**: ^{1}H- and ^{13}C-NMR (pyridine-d_5) data see Table 1. IR ν_{max} (KBr) cm^{-1}: 3400, 2921, 1730, 1597, 1076. FAB-MS *m/z*: 766 $[M+2H]^+$, 706, 438, 306, and 132. Microcrystelline white solid, mp 190 °C, $[\alpha]_D^{25}$ $-136°$ [c=1.0, $CHCl_3$: MeOH (1 : 1)].

Acid Hydrolysis of 1 and 2 The solution of compound **1** (5 mg) in 2 N HCl in 80% *v/v* of ethanol (1 ml) was refluxed for three 3 h. After this, water was added in the reaction mixture and ethanol was evaporated, it was again refluxed for one more hour. Reaction mixture was then extracted with chloroform; Organic layer was identified as palmityl alcohol by Co TLC with authentic sample, NMR, and mass data. The aqueous phase was neutralized with Amberlite IR 410 CO_3^{2-} resin, filtered and concentrated under reduced pressure to give a residue which was identified as α-D-arabinopyranose by comparison of TLC with authentic sample. Following this procedure, **2** (5 mg) was subjected to acid hydrolysis to yield α-D-arabinopyranose in aqueous layer and an aglycon identified from organic layer as docosanol by Co TLC with authentic sample, NMR, and mass data.

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