Asian Journal of Chemistry Vol. 14, No. 2 (2002), 957–960

Alcyonacean Metabolites VIII–Antibacterial metabolites from *Labophytum crassum* of the Indian Ocean.

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Bioassay guided fractionation of the ethyl acetate soluble fraction of the soft coral *Lobophytum crassum* yielded a cembranoid diterpene [(7E, 11E,1R,2S,3R,4R,14S)-14-acetoxy-3,4-epoxycembra-7,11,15-triene-17,2-olide,1] as the potent anti-bacterial metabolite exhibiting activity against *Pseudomonas aeroginosa, Staphylococcus epidermis, Staphylococcus aureus* and *Bacillus subtilis*. A new ceramide (2) with moderate antibacterial activity besides two known polyhydroxysterols and batyl alcohol were also isolated.

Key Words: Antibacterial, Metabolites, *Lobophytum crassum*, Indian ocean.

INTRODUCTION

Alcyonaceans (soft corals; phylum: Coelenterata) of the genus *Lobophytum* have been known to accumulate cembranoid diterpens which are structurally unique and pharmacologically diverse.[1] It was reported that the soft corals release eggs along with considerable quantity of toxic cembranoid diterpenes. These diterpenes might be providing the chemical defence for the eggs and colonies and confer significant ecological advantage to soft corals.[2] In a continuing search for bioactive metabolites from Alcyonaceans,[3–9] we have carried out bioactivity guided fractionation of the soft coral, *Lobophytum crassum*, collected fom the Rameswaram coast of the Indian ocean and the results are described in this paper.

EXPERIMENTAL

Melting points were determined on a Mel Temp apparatus and are uncorrected. IR spectra on a Perkin Elmer BX FTIR spectrometer, ^{1}H NMR spectra on a AMX 400 MHz NMR spectrometer and mass spectra on a VG micromass and JEOL SX 1021 DA–6000 mass spectrometer. Optical rotation data were recorded on an Autopole Polarimeter. TLC was carried out on silica gel (ACME) thin layers. Petroleum ether is the fraction of bp 60–80°C.

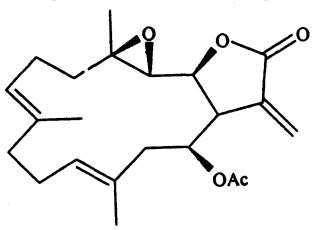
Collection of the soft coral: Specimens of the soft coral were collected at the Rameswaram coast of the Indian Ocean during June 2000. Freshly collected specimens (10 Kg. wet wt.) were washed with fresh water to remove salt deposits and other adhering materials, cut into thin slices and soaked them in aqueous ethanol (95%). The specimens were identified as *Lobophytum crassum* by Dr. Phil

Laila Impex Communication # 15.

Alderslade (Northern Territory Museum of Arts and Sciences, Darwin, Australia. Voucher specimens were on deposit at NTM of Australia NTMC13105).

Extraction and bioassay guided fractionation: The specimens of the soft coral were cut in to thin slices and soaked in aqueous ethanol (95%) at the site of collection. After keeping the soft coral for a month in aqueous ethanol, the ethanolic solution was decanted. The coral was extracted six times more with methanol, solvent was removed from the extract under reduced pressure and the combined concentrate was partitioned with water and ethyl acetate. The ethyl acetate layer was dried over anhydrous sodium sulphate, which on concentration gave a dark green residue (105 g).

The residue from the ethyl acetate solubles were subjected to bioassay guided fractionation (antibacterial activity) by using SiO_2 column with hexane and mixtures of hexane and ethyl acetate as eluents with increasing polarity. Combined fractions were tested for their antibacterial activity and selected fractions which showed antibacterial activity were purified further to obtain cembranoid diterpene (**1**, 560 mg), new ceramide (**2**, 60 mg), two known polyhydroxysterols, (24S)–ergostane-1β,3β,5α,6β-tetraol (130 mg) and (24S)–ergostane-1β,3β,5α, 6β-tetraol-25-monoacetate (750 mg) in addition to batyl alcohol (150 mg).



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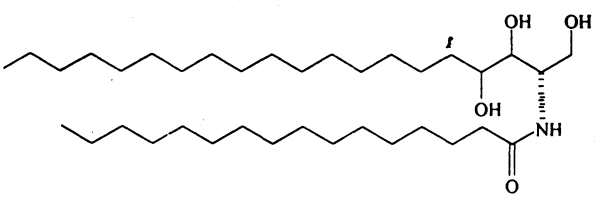

2

1,3,4-Trihydroxy-2-[(hexadecanoyl) amino] eicosane (**2**, 20 mg). Colourless solid from a mixture of hexane and ethyl acetate, m.p. 120–22°C, $[\alpha]_D$ + 7.9° (c0.5, MeOH); IR (KBr) ν_{max}: 33.28 and 1624 cm^{-1}. ^1H NMR (d_5-pyridine): δ 4.51 (2H, brs, H-1), 5.12 (1H, m, H-2), 4.32 (1H, m, H-3), 4.41 (1H, m, H-4), 1.85 (2H, m, H-5), 2.47 (2H, brt, J= 7.4 Hz, H-2′), 1.72 (2H, m, H-3′), 8.35 (1H, d, J= 8.5 Hz, NH), 0.87 (6 H, t, J = 6.9 Hz, H-16′ and H-20), 1.30 (52H, brs, H-6 and H-19); ^{13}C NMR (d5-pyridine) δ 62.0 (C-1), 53.5 (C-2), 76.5 (C-3), 72.8 (C-4), 33.7 (C-5), 173.1 (C-1′), 36.6 (C-2′), 26.4 (C-3′), 14.0 (C-16′ and C-20′), 31.9, 30.1, 29.8, 29.7, 29.6, 29.5, 29.4, 26.2 and 22.7 (C-6 to C-19 and C-4′ to C-15′); FABMS m/z [M^+ + H], 584 (100), [M^+ + H—H_2O], 566 (17), 300 (17), 284 (50), 154 (100), 138 (100), 120 (40).

Identity of the known compounds, (7E,11E,1R,2S,4R,14S)-14-acetoxy-3,4-epoxycembra-7,11,15-triene-17,2-olide (**1**)[9]; (24S)-ergostane-1β,3β,5α,6β-tetraol[9]; (24S)-ergostane-1β,3β,5α,6β-tetraol 25-monoacetate[9] and batyl alcohol[9] was ascertained further by direct comparison with the authentic samples (mmp, co-TLC and spectral data).[9]

Antibacterial activity: Antibacterial activity was carried out using Agar cup–plate diffusion method[10]. **1** exhibited significant antibacterial activity against *Pseudomonas aeroginosa, Staphylococcus epidermis, Staphylococcus aureus* and *Bacillus subtilis* and **2** showed moderate activity and the results are reported in Table-1.

TABLE-1
ANTIBACTERIAL ACTIVITY OF **1** AND **2**

Compound	Concenttration (μg/mL)	Test organisms (Zone of inhibition in mm)			
		Pseudomonas aeroginosa	*Staphylococcus epidermis*	*Bacillus subtilis*	*Staphylococcus aureus*
Ethyl acetate soluble fraction	500	8.5	10.0	12.0	16.0
	200	Slight	9.0	10.0	13.0
	50	Slight	8.5	8.5	11.0
1	200	14.0	16.5	17.5	19.5
	100	10.0	12.0	18.0	12.0
	50	9.0	12.0	16.0	11.0
	10	9.0	10.0	13.0	9.0
	1	—	—	10.0	—
2	200	11.0	13.5	13.5	13.0
	100	10.0	10.0	9.0	9.5
	50	9.5	9.5	8.5	9.5
Ampicillin	1	9.0	17.0	17.0	16.0

Conc.: 500, 200, 100, 50, 10 and 1 μg/ 0.05 mL in methanol; Cup dia.: 8 mm

RESULTS AND DISCUSSION

The residue from the ethyl acetate soluble portion of the methanol extractives of the soft coral *Lobophytum crassum* was found to possess significant antibacterial activity against *Pseudomonas aeroginosa, Staphylococcus epidermis, Staphylococcus aureus* and *Bacillus subtilis* (Table 1). Bioassay guided fractionation of the residue through chromatography over silica gel and recrystallization of the selected fractions resulted in the isolation of cembranoid diterpene (**1**) as the major metabolite, a new ceramide (**2**), (24S)-ergostane-1β,3β,5α,6β-tetraol[9] and (24S)-ergostane-1β,3β,5α,6β-tetraol 25-monoacetate[9] and batyl alcohol.[9]

Diterpene **1** was obtained as colurless crystals from methanol, m.p. 144–46°C, $[\alpha]_D$ – 254° (c = 0.35, $CHCl_3$) and analysed for $C_{22}H_{30}O_5$ [m/z: 314 (M^+-AcOH)]. The physical, spectral and optical notation data of **1** are in good agreement with those recorded for (7E, 11E, IR, 2S, 3R, 4R, 14S)-14-acetoxy-3, 4-epoxycembra-7, 11, 15-triene-17,2-olide (**1**) isolated earlier from *Lobophytum denticulatum*[9]. The identity was further ascertained by direct comparison with the authentic sample[9]. Diterpene **1** was reported to possess cytotoxic activity and potential inhibitor of Farnasyl protein transferase activity.[11] During the present study, we

have found that it exhibits excellent antibacterial activity against *Pseudomonas aeroginosa, Staphylococcus epidermis, Staphylococcus aureus* and *Bacillus subtilis* and the results are presented in Table 1.

Compound **2** was obtained as colourless solid from a mixture of hexane and ethyl acetate, mp. 120–122°C, $[\alpha]_D + 7{\cdot}9°$ (c.5, MeOH) and analysed for $C_{36}H_{73}NO_4$ [FABMS M/z: 584 ($M^+ + H$)]. Its IR (KBr) spectrum showed bands at 3328 (hydroxyl and NH) and 1624 cm^{-1} (amide carbonyl). ^1H NMR spectrum of **2** exhibited signals at δ 4.51 (2H, brs), 4.32 (1H, m) and 4.41 (1H, m) corresponding to hydroxymethylene and hydroxymethine protons, respectively. It showed signals also at δ 8.35 (1H, d. J=6.9 Hz.), assignable to an amide NH and a series of signals at δ 2.47 (2H, brt, J=7.4 Hz), 1.85 (2H, m), 1.72 (2H, m), 1.30 (52H, brs) and 0.87 (6H, t, J=6.9 Hz) suggestive of a long fatty acid unit and an alkyl chain characteristic of ceramides.[12] The ^{13}C NMR data of **2** supported the presence of an amide carbonyl (δ 173.1), hydroxymethylene (δ 62.0), two hydroxymethines (δ 76.5 and 72.8) and complex group of signals between signals (δ 36.6, 33.7, 31.9, 30.1, 30.0, 29.8, 29.7, 29.6, 29.5, 29.4, 26.2, 22.7 and 14.0) characteristic of ceramides.[12].

The FABMS of **2** showed ions at m/z: 584 ($M^+ + H$), 566 ($M^+ + H—H_2O$), in addition to m/z 282. The formation of ion at m/z 282 is the suggestive of palmitic acid unit in ceramides.[12] Based on the above, the structure of **2** could be deduced as 1,3,4-trihydroxy-2-[(hexadecanoyl)amino] eicosane. **2** was found to be new addition to the class of ceramides and it showed moderate antibacterial activity and the results are presented in Table-1.

ACKNOWLEDGEMENTS

The authors thank Sri G Gangaraju Laila Impex, Vijayawada for encouragement, SIF, IISc Bangalore and CDRI Lucknow for proton NMR and mass spectral data and Mrs. R. Jayalakshmi for antibacterial activity studies.

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(Received: 19 December 2001; *Accepted*: 15 February 2002) AJC-2615