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New Prostaglandins from the Soft Coral *Sarcophyton ehrenbergi* Marengeller of Andaman and Nicobar Islands of Indian Ocean

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New prostaglandins of PGB-type and hippurins have been isolated from the soft coral *Sarcophyton ehrenbergi* Marengeller, collected from the coasts of Andaman and Nicobar Islands of Indian Ocean along with a pregnane derivative, arachidonic acid and its ethyl ester. Their structures have been determined by spectral data.

Key Words: *Sarcophyton ehrenbergi* Marengeller, Hippurins, Prostaglandins, Soft coral, Indian ocean.

INTRODUCTION

The soft corals of the genus *Sarcophyton* are rich source of variety of metabolites[1-3]. In continuation of our work on the metabolites of marine organisms from Indian Ocean[4-10], a systematic chemical examination of *Sarcophyton ehrenbergi* Marengeller was taken up and several new and known metabolites were isolated. We report herein the isolation of two hippurins **1** and **2** along with six prostaglandins **3-8** of which three (**5**, **7** and **8**) are new to marine sources, a pregnenolone **9**, arachidonic acid **10** and its ethyl ester **11**. Previously compounds **1** and **2** were reported from *Sarcophyton* crassocaule of Andaman and Nicobar Islands of Indian Ocean.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage and are uncorrected. Optical rotation was measured on a JASCO DIP-370 digital polarimeter in $CHCl_3$. IR spectra were recorded in $CHCl_3$ with Perkin-Elmer-841 infrared spectrophotometer. UV spectra were recorded on Roy spectronic 1201 double-beam spectrophotometer.

^1H NMR spectra were recorded in $CDCl_3$ on a Bruker VM-400 (400 MHz FT NMR) spectrometer and ^{13}C NMR on JEOL JNM GX-270 spectrometer. Using TMS as internal standard. MS were determined on a JEOL D-300 (EI/CI) spectrometer. Elemental analysis was carried out on a CARLO ERBA EA 1108-instrument. HPLC separation was performed on water Delta Prep. 3000-instrument.

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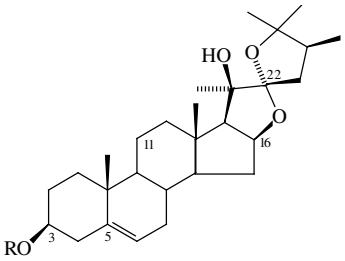
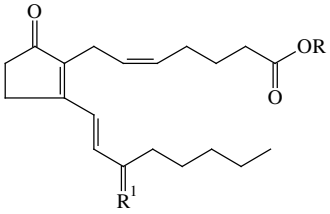


1. R = H

2. R = $OCOCH_3$



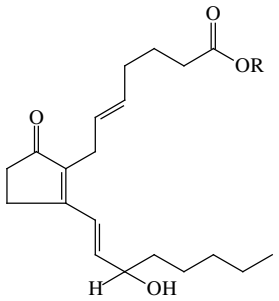
3. R = CH_2CH_3 ; R^1 = 

4. R = H ; R^1 = 

4a. R = CH_3 ; R^1 = 

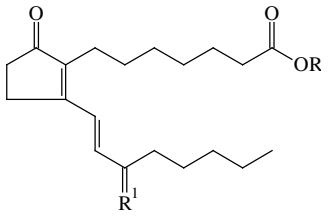
7. R = H ; R^1 = =O

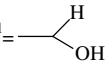
7a. R = CH_3 ; R^1 = =O



5. R = H

5a. R = CH_3

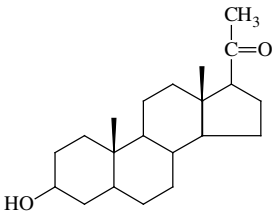


6. R = H ; R^1 = 

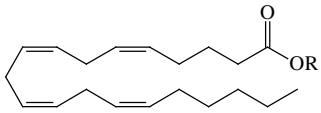
6a. R = CH_3 ; R^1 = 

8. R = H ; R^1 = =O

8a. R = CH_3 ; R^1 = =O



9



10. R = H

11. R = CH_2CH_3

Scheme-I

Collection, extraction and purification: The soft coral *Sarcophyton ehrenbergi* Marengeller was collected on the coasts of Andaman and Nicobar Islands of Indian Ocean. A voucher specimen is on deposit at the Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam. The organisms (0.6 kg dry weight) were cut into thin slices and preserved in EtOH at room-temperature. The EtOH extract was concentrated under reduced pressure. The whole crude mass was fractionated with ethyl acetate and dried over anhydrous $MgSO_4$. Fractionation of the crude

extract was carried out using the column of Sephadex LH-20. Each fraction was repeatedly chromatographed over silica gel columns to yield six pure compounds, **1** (65 mg), **2** (42 mg), **3** (510 mg), **9** (36 mg), **10** (0.5 g) and **11** (2.5 g) and a mixture of prostaglandins (12.8 g).

Separation of prostaglandins mixture: The mixture of prostaglandins on repeated chromatography over a column of silica gel (100-200 mesh, ACME make) using chloroform-methanol mixtures as eluants afforded a light yellow liquid (11.6 g). This liquid showed several spots on 20 % $AgNO_3$ impregnated silica gel TLC. The mixture was methylated with excess of diazomethane and the methylated product formed was purified and separated by reverse phase HPLC. It afforded five pure compounds **4a-8a** as pale yellow liquids.

Ethyl-15-hydroxy-9-oxo-5 Z,8(12),13E-prostatrienoate (3): Pale yellow liquid, IR (KBr, ν_{max}, cm^{-1}, $CHCl_3$): 3600-3500, 2930, 1735, 1710, 1685, 1520, 1350, 1240 and 970; UV (EtOH): λ_{max} 278 nm (ε 28,600); MS: $M^+ = 362$ ($C_{22}H_{34}O_4$); ^{1}H NMR ($CDCl_3$): δ 5.38(2H, dt like, X in ABX, 5 and 6-H), 3.05 (2H, d, *J* = 5.5 Hz, 7-H), 6.89 (1H, d, *J* = 16.5 Hz ,13-H) , 6.31 (1H, dd, *J* = 16.5, 5.5 Hz, 14-H), 4.33 (1H, m, 15-H) 1.26-1.36 (6H, br s, 17, 18, 19-H), 0.88 (3H, t, *J* = 6.0 Hz, 20-Me), 4.12 (2H, q, *J* = 8.0 Hz, -COOC<u>H</u>$_2$CH$_3$) 1.26 (3H, t, *J* = 8.0 Hz, -COOCH$_2$C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$): 173.9 (C-1), 33.7 (C-2), 25.0 (C-3), 26.5 (C-4), 129.7 (C-5), 123.4 (C-6), 22.4 (C-7),139.6 (C-8), 208.9 (C-9), 33.6 (C-10), 25.6 (C-11), 163.5 (C-12), 126.7 (C-13), 141.2 (C-14), 71.9 (C-15), 37.1 (C-16), 24.7 (C-17), 31.6 (C-18), 21.3 (C-19), 13.9 (C-20), 60.3 (-COO<u>C</u>H$_2$CH$_3$), 14.1 (-COOCH$_2$<u>C</u>H$_3$).

Methyl 15-hydroxy-9-oxo-5 Z,8(12),13E-prostatrienoate (4a): Pale yellow liquid, IR (KBr, ν_{max}, cm^{-1}, $CHCl_3$): 3600-3500, 2940, 1735, 1710, 1680, 1350, 1240 and 970; UV (EtOH): λ_{max} 278 nm (ε 8,400); MS: $M^+ = 348$ ($C_{21}H_{32}O_4$); ^{1}H NMR ($CDCl_3$): δ 5.36 (2H, dt like, X in ABX, 5 and 6-H), 3.05 (2H, d, *J* = 5.5 Hz, 7-H), 6.86 (1H, d, *J* = 16.5 Hz, 13-H), 6.27 (1H, dd, *J* = 16.5, 5.5. Hz, 14-H), 4.31 (1H, m, 15-H), 1.26-1.36 (6H, br s, 17, 18, 19-H), 0.88 (3H, t, *J* = 6.0 Hz, 20-Me), 3.66 (3H, s, -COOCH$_3$).

Methyl 15-hydroxy-9-oxo-5 E,8(12),13E-prostatrienoate (5a): Pale yellow liquid, IR (KBr, ν_{max}, cm^{-1}, $CHCl_3$): 3600-3500, 2940, 1735, 1710, 1680, 1520, 1240 and 970; UV (EtOH): λ_{max} 278 nm (ε 28,600); MS: $M^+ = 348$ ($C_{21}H_{32}O_4$); ^{1}H NMR ($CDCl_3$): δ 5.38 (2H, m, 5 and 6-H) 3.0 (2H, br s, *J* = 5.5 Hz, 7-H), 6.86 (1H, d, *J* = 16.5 Hz, 13-H), 6.27 (1H, dd, *J* = 16.5, 5.5 Hz, 14-H), 4.34 (1H, m, 15-H), 1.26-1.36 (6H, br s, 17, 18, 19-H), 0.88 (3H, t, *J* = 6.0 Hz, 20-Me), 3.66 (3H, s, -COOCH$_3$).

Methyl 15-hydroxy-9-oxo-8(12),13 E-prostadienoate (6a): Pale yellow liquid, IR (KBr, ν_{max}, cm^{-1}, $CHCl_3$): 3600-3500, 2950, 1735, 1710, 1680, 1350 and 970; UV (EtOH): λ_{max} 276 nm (ε 28,400); MS: $M^+ = 350$ ($C_{21}H_{34}O_4$); ^{1}H NMR ($CDCl_3$): δ 6.79 (1H, d, *J* = 16.5 Hz, 13-H), 6.24 (1H, dd, *J* = 16.5, 5.5 Hz, 14-H) 4.31 (1H, m, 15-H), 1.26 (*ca.* 12H, br s, 17,18,19-H), 0.88 (3H, t, *J* = 6.0 Hz, 20-Me), 3.66 (3H, s, -COOCH$_3$).

Methyl 9,15-dioxo-5Z,8(12),13 E-prostatrienoate (7a): Yellow liquid, IR (KBr, ν_{max}, cm^{-1}, $CHCl_3$): 2950, 1735, 1710, 1685, 1665, 1340 and 970 cm^{-1}; UV (EtOH): λ_{max} 296 nm (ε 22,600); MS: M^+ = 346 ($C_{21}H_{30}O_4$); ^1H NMR ($CDCl_3$): δ 5.36 (2H, dt like, X in ABX, 5 and 6-H) 3.11 (2H, d, *J* = 5.5 Hz, 7-H), 6.53 (1H, d, *J* = 16.5 Hz, 13-H), 7.65(1H, d, *J* = 16.5 Hz, 14-H), 1.26 (6H, br s, 17, 18, 19-H), 0.9 (3H, t, *J* = 6.0 Hz, 20-Me), 3.66 (3H, s, -$COOCH_3$).

Methyl 9,15-dioxo-8(12),13 E-prostadienoate (8a): Yellow liquid, IR (KBr, ν_{max}, cm^{-1}, $CHCl_3$): 2940, 1735, 1710, 1685, 1665, 1340 and 960; UV (EtOH): λ_{max} 296 nm (ε 22, 400); MS: M^+ = 348 ($C_{21}H_{32}O_4$); ^1H NMR ($CDCl_3$): δ 6.56 (1H, d, *J* = 16.5 Hz, 13-H), 7.62 (1H, d, *J* = 16.5 Hz, 14-H), 1.26 (br s) 0.9 (3H, t, *J* = 6.0 Hz, 20-Me), 3.66 (3H, s, -$COOCH_3$).

RESULTS AND DISCUSSION

The ethanolic concentrate obtained from the soft coral *Sarcophyton ehrenbergi* Marengeller was extracted with ethyl acetate and the residue from it was partitioned through Sephadex LH-20. Each fraction was then chromatographed over silica gel to yield two known hippurins (**1** and **2**)[11], a pure prostaglandin (**3**), a mixture contaiining five prostaglandins (**4-8**) [see experimental section for isolation], pregnenolone (**9**), arachidonic acid (**10**) and its ethyl ester (**11**). The structures of **9**, **10** and **11** are previously known and well established. Compound **3**: pale yellow liquid; m/z = 362 (M^+, $C_{22}H_{34}O_4$). IR spectrum of the compound showed absorption bands for hydroxyl (3600-3500 cm^{-1}), ester carbonyl (1735 cm^{-1}) and conjugated cyclopentanone system (1710 cm^{-1}). The ^1H NMR spectrum contained signals at δ 1.26 (t, 3H, *J* = 8.0 Hz) and δ 4.12 (q, 2H, *J* = 8.0 Hz) that were assigned to -OCH_2CH_3 protons. It also showed a terminal methyl group at δ 0.88 (t, 3H; *J* = 6.0 Hz). The ^{13}C NMR spectrum showed resonance at δ 173.9 and 208.9, which are assigned to carboxy carbonyl and unsaturated carbonyl carbons. It also showed signals at δ 123.3, 126.7, 129.7, 139.2, 141.2 and 163.5 indicative of one tetra substituted and two disubstituted double bonds. The up field signal at δ 5.38 (2H, dt like) characteristic of vinylic protons of an isolated *cis*-double bond. The proton signals at δ 6.31 (1H, dd, *J* = 16.5, 5.5 Hz) and 6.89 (1H, d, *J* = 16.5 Hz) are due to the conjugated trans double bond protons. The multiplicity of these proton signals also indicated the presence of a hydroxyl group at allylic position. The UV absorption maxima at 278 nm (ε 28, 600) is also suggested the presence of doubly conjugated cyclopentenone system. Based on the above the compound **3** is described as ethyl-5-hydroxy-9-oxo-5 Z,8(12),13 E-prostatrienoate or ethyl ester of prostaglandin B_2 or PGB_2 ethyl ester (**3**). It was reported earlier from the lipid extracts of *Lobophytum carnatum*[12]. It may be an artifact as ethanol was used in the extraction procedure.

The prostaglandin mixture obtained from the extracts of the *Sarcophyton ehrenbergi* Marengeller was methylated with excess of diazomethane and the methylated product was purified by reverse phase HPLC. It afforded five pure

compounds (**4a-8a**) as pale yellow liquids. The corresponding natural products were identified tentatively as compounds **4-8**. Out of these, two compounds **4** and **6** are known to literature.

Compound 4a: Pale yellow liquid; m/z = 348 (M^+, $C_{21}H_{32}O_4$). It was readily identified as methyl 15-hydroxy-9-oxo-5 Z,8(12),13 E-prostatrienoate (PGB_2 methyl ester, **4a**) by comparison of its spectral data with those reported for the compound isolated earlier from the lipid extracts of *Lobophytum carnatum*[13] and with compound **3**. Therefore the corresponding natural product is PGB_2 (**4**).

Compound 5a: Pale yellow liquid; m/z = 348 (M^+, $C_{21}H_{32}O_4$). It could also be recognized as a methyl derivative of a prostaglandin from its spectral characteristics. The almost identical ^{1}H NMR signals of compound **5a** and **4a** suggesting that the compound **5a** to have the same structure as **4a** excepting the difference in ^{1}H NMR signals of the vinylic protons. The ^{1}H NMR spectrum of **5a** showed a signal at δ 5.38 (2H, m) instead of a dt like (X in ABX) signal at δ 5.36 in **4a** indicated that the compound **5a** and **4a** are geometrical isomers. Thus, the compound **5a** has the *trans* double bond at C-5. Hence, the structure of the compound **5a** is described as methyl 15-hydroxy-9-oxo-5 E,8(12),13 E-prostatrienoate (5-*trans* PGB_2 methyl ester) and the corresponding marine natural product is 5-*trans*-PGB_2 (**5**).

Compound 6a: Pale yellow liquid; m/z = 350 (M^+, $C_{21}H_{34}O_4$). It was also identified as a prostaglandin analog from its spectral data. The ^{1}H NMR spectrum of the compound showed the absence of vinylic proton signals at δ 5.36 (as in compound **4a**) and the presence of a broad singlet at δ 1.26-1.35 approximately integrated to about 12 protons, suggested no further unsaturation in the α-side chain. The remaining ^{1}H NMR signals of the compound **6a** is almost identical with those signals assigned to compound **4a**. Thus, the compound **6a** is described as methyl 15-hydroxy-9-oxo-8(12),13 E-prostadienoate (PGB_1 methyl ester) and the corresponding acid is PGB1 (**6**).

Compound 7a: Pale yellow liquid; m/z = 346 (M^+, $C_{21}H_{30}O_4$). It was also found to be a prostaglandin. The IR spectrum of the compound showed ester carbonyl absorption at 1735 cm^{-1}, absorption for cyclopentanone at 1710 cm^{-1} and unsaturated ketonic absorption at 1685 cm^{-1}. The presence in ^{1}H NMR spectrum, a pair of doublets at δ 6.53 (1H, d, *J* = 16.5 Hz), 7.65 (1H, d, *J* = 16.5 Hz) and the high value of UV absorption maxima at 296 nm (ε 22.600) suggested the presence of a carbonyl group on C-15. Compound **4a** on oxidation with Jones reagent gave compound **7a** from the foregoing analysis the structure of the compound **7a** could be derived as methyl 9,15-dioxo-5 Z,8(12),13 E-prostatrienoate (15-keto PGB_2 methyl ester) and the marine natural product is 15-keto PGB_2 (**7**).

Compound 8a: Pale yellow liquid; m/z = 348 (M^+, $C_{21}H_{32}O_4$). The ^{1}H NMR spectrum of the compound **8a** showed a pair of doublets at δ 6.56 (1H, d, *J* = 16.5 Hz) and 7.62 (1H, d, *J* = 16.5 Hz), which are attributable to a disubstituted double bond. The multiplicities of these proton signals and high value of UV absorption maxima at 296 nm (ε 22.400) indicated the presence of a carbonyl on C-15. The

comparison of ^{1}H NMR data of **8a** and **7a** suggested the absence of Δ^5-double bond in the former and hence the structure of the compound **8a** may be described as methyl 9,15-dioxo-8(12),13E-prostadienoate (15-keto PGB_1 methyl ester). The corresponding marine natural product is 15-keto PGB_1 (**8**).

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