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TERPENOID METABOLITES OF THE MARINE OCTOCORAL *EUNICEA LACINIATA*

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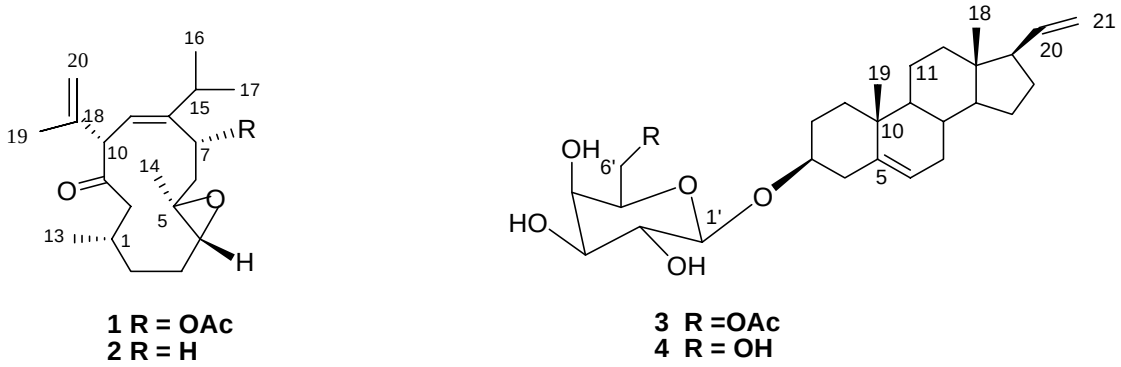
Abstract - A chemical investigation of the Caribbean marine octocoral *Eunicea laciniata* collected along the south coast of Barbados afforded a new cubitane diterpenoid (**1**), a new steroidal glycoside (**3**), along with two known diterpenes. Their structures were determined by 1D and 2D NMR spectral experiments.

Marine octocorals of the genus *Eunicea,* are a rich source of natural products that are biologically active as well as structurally unique.These invertebrates are known to elaborate diterpenoids of a number of structural types including cembrane, dolabellane, cubitane and elemane-type diterpenoids. This genus is also a good source of sterols and metabolites of mixed biogenesis.[2,3] In continuation of our studies of marine invertebrates from the Caribbean,[4] we have investigated the sea whip *Eunicea laciniata* (Duchassaing and Michelotti), collected along the south coast of Barbados. No previous studies were reported on *E. laciniata* from the eastern Caribbean. This led to the isolation of a new cubitane diterpene (**1**), a new steroidal glycoside (**3**) and two known dolabellane diterpenes (**5**) and (**6**) (Figures 1 and 4).

Compound (**1**) was obtained as white needles and the molecular formula $C_{22}H_{34}O_4$, was established by HREIMS. Its IR spectrum showed absorptions assigned to ester (1733 cm^{-1}), ketone (1707 cm^{-1}), and epoxide

group (1238 cm^{-1}). The ^{1}H NMR spectrum had resonances due to olefinic protons at δ 5.80 (1H, d, *J* = 11.0 Hz, H-9), δ 4.90 (1H, t, *J* = 1.0 Hz, H-20a) and 4.86 (1H, d, *J* = 1.0 Hz, H-20b), the latter two being part of a terminal double bond. An oxymethine proton had a resonance at δ 5.98 (dd, *J*= 11.7, 3.6 Hz, H-7). In addition, there were five methyl signals at δ 1.70 (t, *J* = 1.0 Hz, H_3-19), δ 1.17 (d, *J* = 6.9 Hz, H_3-16), 1.14 (d, *J* = 0.6 Hz, H_3-14), 1.09 (d, *J* = 6.5 Hz, H_3-13) and 1.08 (d, *J* = 6.7 Hz, H_3-17) in addition to an acetate methyl singlet at δ 2.00 (H_3-22). Evaluation of the ^{13}C NMR spectrum showed a saturated ketone at δ 209.5 (C-11), an ester carbonyl at δ 169.6 (C-21), an epoxide ring with carbon resonances at δ 57.7 (C-5) and 60.2



1 R = OAc
2 R = H

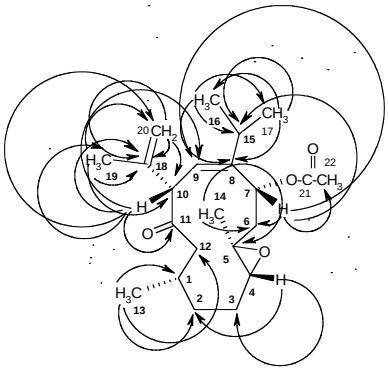
3 R =OAc
4 R = OH

Figure 1 Structures of **1** – **4**

(C-4), and a secondary alcohol with a carbon at δ 70.2 (C-7). These structural features accounted for the four oxygen atoms in the molecular formula. The presence of isopropenyl and isopropyl groups were supported by HMBC correlations for the methyl protons at δ 1.70 and the olefinic protons at δ 4.90 and δ 4.86 on the one hand and between the methyl protons at δ 1.08 and 1.17 (Figure 2). The oxymethine proton at δ 5.98 showed HMBC correlations to the ester carbonyl at δ 169.6, an olefinic carbon at δ 127.1, a methylene carbon at δ 42.4 (C-6) and a methine carbon at δ 28.2 (C-15). This confirmed the attachment of the acetyl residue to C-7. T-ROESY cross peaks (Figure 3) were observed between the oxymethine proton at δ 5.98 (H-7) and the methylene proton at δ 1.42(H-6b), the methine proton at δ 4.71(H-10) and the epoxymethine proton at δ 3.07 (H-4). Hence H-7 is β-orientated and the structure of compound (**1**) was thus assigned as 7(*R**)-

acetoxy-4(*R**),5(*R**)-epoxy-11-keto-1(*S**),10(*S**)-cubata-8(*Z*),18(20)-iene. The deacetyl analogue (**2**) along with other minor metabolites, were previously isolated from specimens of *Eunicea laciniata* collected in Belize, the Bahamas Islands and the Tobago Cays.[5-7]

Compound (**3**) was isolated as a white semi-solid and the molecular formula $C_{29}H_{44}O_7$, was deduced from ESIMS and ^{13}C NMR spectral data. The IR spectrum showed absorbances at 3417 cm^{-1} and 1740 cm^{-1}, indicative of hydroxyl and carbonyl functionalities respectively. The ^1H NMR spectrum had two singlets at δ 1.02 (3H) and 0.61 (3H) due to the C-18 and C-19 methyl groups, in addition to a singlet at δ 2.08 for an acetate. Protons at δ 4.98 (H-21a), 4.97 (C-21b), 5.77 (H-20) and 5.38 (H-6) were all assigned as olefinic protons. Additionally, six signals at δ 3.68 (H-5'), 3.89 (H-4'), 3.62 (H-2', H-3'),4.38 (H-6'a), 4.29 (H-6'b) and 4.34 (H-1') suggested that compound (**3**) possessed a galactose moiety as determined by COSY and T-ROESY experiments. HREIMS on an intense peak at 282 *m/z* as seen in the LREIMS established a molecular



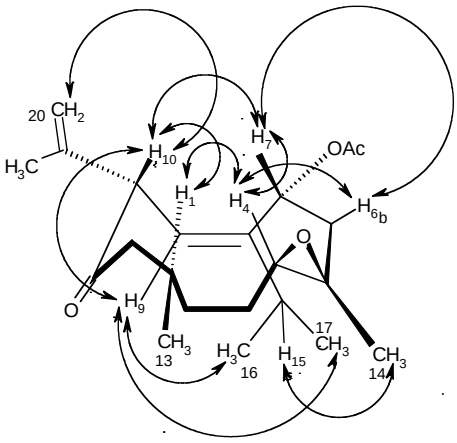
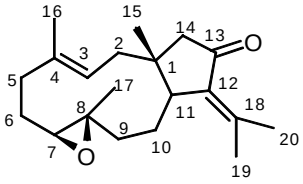


Table 3 HMBC Connectivities for Compound (**1**) Table 4 T-ROESY Connectivities for Compound (**1**)

formula of $C_{21}H_{30}$. This, along with the ^{13}C and ^1H NMR spectra confirmed that the aglycone of compound (**3**) was pregnadienol. The HSQC spectrum revealed that the anomeric proton at δ4.34 was directly bonded to the carbon at δ101.4, while HMBC correlations between both methylene protons at δ4.38 and 4.29 and

the carbonyl carbon at δ 171.1 of the acetate group enabled the attachment of the acetate group on C-6' of the sugar moiety. The stereochemistry of the glycosidic bond was determined as β on the basis of the coupling constant of H-1 (J = 7.7 Hz) and the absence of coupling between the protons H-1 and H-3α.[8] The closely related 3β-pregna-5,20-dienyl-β-D-galactopyranoside (**4**), was previously reported from *Pseudoplexaura wagenaari* and the sugar moiety was identified as galactose and assigned the D configuration with the use of X-Ray methods. The stereochemistry shown for the pregnane-derived aglycone in compound (**3**) was obtained after comparison with the ^{13}C and ^{1}H NMR resonances of the aglycone in **4**.[9] Compound (**3**) was thus named 6'-*O*-acetyl-3β-pregna-5,20-dienyl-β-galactopyranoside; the corresponding 4'-*O*-acetyl analogue was previously isolated from an undescribed *Eunicea* specimen.[10]



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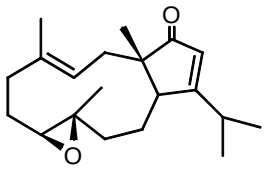

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Figure 4 Structures of **5** and **6**

The dolabellane diterpenoids (**5**) and (**6**) (Figure 2) were isolated as white needles and the spectroscopic data for these compounds were in accord with those reported previously.[11, 12] Compound (**5**) can be regarded as a chemotaxonomic marker for *E. laciniata* as can be judged from previous investigations of this organism.[5-7, 11, 12]

EXPERIMENTAL

General Experimental Procedures -- Melting points were determined using a Fisher/Johns melting point apparatus and were uncorrected. UV spectra were obtained on a Hewlett-Packard 8452A spectrophotometer in MeOH. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter in $CHCl_3$ solutions. IR spectra

were recorded on a Nicolet Nexus 870 FT-IR spectrophotometer as a thin film. ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Varian UNITY 500 MHz spectrometer in $CDCl_3$ solutions using TMS as an internal standard. MS spectra were recorded on a VG 70-250S mass spectrometer operating at 70 eV; *m/z* values are reported for significant peaks. In the FAB mode, this same instrument was used to obtain FAB spectra in an ethylene glycol matrix. HPLC was performed using a Beckman instrument with a Supelcosil LC-18 column (25 cm x 21.2 mm, 5 m).

Animal Materials -- The *E. laciniata* specimen was collected at a depth of 25-40 ft from the Patch Reef at Needhams Point on the south coast St Michael in June 1999. It was identified by Renate Goodridge of the Centre for Environmental and Marine Resources UWI, Barbados. A voucher specimen is kept in the Department of Biological and Chemical Sciences, University of the West Indies, Cave Hill Campus, Barbados.

Extraction and Isolation – The gorgonian specimen (dry weight 170 g) was extracted using acetone (900 mL) at 24 ºC for 15 h. The collected filtrate (800 mL) was evaporated and extracted using CH_2Cl_2 (3 x 150 mL). The CH_2Cl_2 extract (17 g) was dissolved in 100 ml methanol-H_2O (9:1) and extracted with hexane (3 x 75 mL). The hexane extract (7 g) was flash chromatographed on silica gel eluting first with hexane-acetone (9:1) and continued by increasing the ratio of acetone to give eight major fractions. Fraction #2 was separated by preparative TLC using hexane-acetone (20:1), to give compound (**1**) (30 mg). Reverse-phase HPLC (70:30, MeOH-H_2O) on fraction #3 (0.25 g) yielded compounds **(5**) (48.5 mg) and (**6**) (9.2 mg). The aqueous methanol fraction was chromatographed on Sephadex LH-20 using MeOH-CH_2Cl_2 (1:1) as eluent followed by preparative TLC using hexane-acetone (4:1) as the mobile phase to give compound (**3**) (3 mg).

Compound (**1**). White needles, mp 78-80 C; $[\alpha]_D$ +140.3 (*c* 0.06, $CHCl_3$); IR $_{max}$ (film) 1733, 1707, 1238, 954 cm^{-1}; UV λ_{max} (MeOH) 212 nm (log ε 3.45); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 5.98 (1H, dd, *J* = 11.7, 3.6 Hz, H-7), 5.80 (1H, d, *J* = 11.0 Hz, H-9), 4.90 (1H, t, *J* = 1.3 Hz, H-20a), 4.86 (1H, d, *J* = 0.8 Hz, H-20b), 4.71 (1H, d, *J* = 11.0 Hz, H-10), 3.07 (1H, dd, *J* = 8.8, 4.8 Hz, H-4), 2.73 (1H, dd, *J* = 12.2, 4.1 Hz, H-12a),

2.57 (1H, dd, *J* = 13.6, 11.7 Hz, H-6a), 2.55 (1H, m, H-15), 2.29 (1H, m, H-1), 2.10 (1H, ddd, *J* = 10.8, 10.8, 5.0 Hz, H-3a), 2.00 (3H, s, H-22), 1.94 (1H, dd, *J* = 12.2, 11.8 Hz, H-12b), 1.70 (3H, t, *J* = 0.8 Hz, H-19), 1.42 (1H, dd, *J* = 13.6, 3.6 Hz, H-6b), 1.27 (2H, m, H-2a,b), 1.24 (1H, m, H-3b), 1.17 (3H, d, *J* = 6.9 Hz, H-16), 1.14 (3H, d, *J* = 0.6 Hz, H-14), 1.09 (3H, d, *J* = 6.5 Hz, H-13), 1.08 (3H, d, *J* = 6.7, H-17); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 209.5 (C-11), 169.6 (C-21), 143.0 (C-8), 142.3 (C-18), 127.1 (C-9), 113.8 (C-20), 70.2 (C-7), 60.2 (C-4), 57.7 (C-5), 54.2 (C-10), 51.5 (C-12), 42.4 (C-6), 32.3 (C-2), 28.9 (C-1), 28.2 (C-15), 25.9 (C-17), 24.7 (C-3), 22.0 (C-16), 21.2 (C-22), 21.0 (C-19), 20.4 (C-13), 18.4 (C-14); EIMS *m/z* [M - HOAc]$^+$ 302 (58), 287 (29), 279 (63), 259 (92), 241 (27), 231 (56), 203 (34), HREIMS *m/z* [M - HOAc]$^+$ 302.2258 (calcd for $C_{22}H_{34}O_4$ –HOAc, 302.2246).

Compound (**3**). White semi-solid; $[\alpha]_D$ –193.7 (*c* 0.09, $CHCl_3$); IR ν_{max} (film) 3417, 1740, 1462 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 5.77 (1H, ddd, *J* = 16.5, 10.5, 7.7 Hz, H-20), 5.38 (1H, br d, *J* = 5.2 Hz, H-6), 4.98 (1H, d, *J* = 8.6 Hz, H-21a), 4.97 (1H, d, *J* = 7.4 Hz, H-21b), 4.38 (1H, m, H-6'a), 4.34 (1H, d, *J* = 7.7 Hz, H-1'), 4.29 (1H, dd, *J* = 11.4, 6.6 Hz, H-6b'), 3.89 (1H, s, H-4'), 3.68 (1H, t, *J* = 6.2 Hz, H-5), 3.62 (1H, m, *J* = H-3), 3.57 (1H, m, H-3), 2.38 (1H, m, H-4a), 2.27 (1H, m, H-4b), 2.08 (3H, s, $OCOCH_3$), 2.02 (1H, m, H-7a), 1.96 (1H, m, H-17), 1.96 (1H, m, H-2a), 1.88 (1H, ddd, *J* = 12.5, 7.5, 2.5 Hz, H-1a), 1.80 (1H, m, H-16a), 1.72 (1H, m, H-8), 1.69 (1H, m, H-15a), 1.63 (1H, m, H-2b), 1.58 (1H, m, H-16b), 1.56 (1H, m, H-11a), 1.48 (1H, s, H-7b), 1.43 (1H, m, H-11b), 1.22 (1H, m, H-15b), 1.07 (2H, m, H-12a,b), 1.06 (1H, m, H-1b), 1.02 (3H, s, H-19), 1.02 (1H, m, H-14), 0.95 (1H, m, H-9), 0.61 (3H, s, H-18); ^{13}C- NMR ($CDCl_3$, 125 MHz) δ 171.1 ($O\underline{C}OCH_3$), 140.5 (C-5), 139.8 (C-20), 122.1 (C-6), 114.6 (C-21), 101.4 (C-1'), 79.4 (C-3), 73.4 (C-3'), 72.1 (C-5'), 72.0 (C-2'), 68.2 (C-4'), 62.8 (C-6'), 55.9 (C-14), 55.4 (C-17), 50.5 (C-9), 43.4 (C-13), 38.8 (C-4), 37.4 (C-12), 37.3 (C-8), 37.2 (C-1), 36.9 (C-10), 32.0 (C-7), 29.7 (C-2), 27.3 (C-16), 25.0 (C-15), 21.0 ($OCO\underline{C}H_3$), 20.7 (C-11), 19.4 (C-19), 12.7 (C-18); EIMS *m/z* 282 (100), 213 (9), 189 (29), 161 (12), 60(6); HRFABMS *m/z* [M + Na]$^+$ 527.3109 (calcd for $C_{29}H_{44}O_7Na$, 527.3087).

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