Terpenes from *Eunicea laciniata* and *Plexaurella nutans*

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A new 7,8–epoxydolabella–3(E)–12(18)–diene (1), diterpenoid together with three known compounds were isolated from a Honduras gorgonian *Eunicea laciniata*. The relative stereochemistry of **1** was established by spectroscopic studies and the antiprotozoal and antimicrobial activities of dolabellane diterpenoids **2–4** are reported. Three known cadinane type sesquiterpenes were also isolated from Honduras gorgonian *Plexaurella nutans*.

Keywords: diterpenes, sesquiterpenes, honduras gorgonian

Gorgonians are sea whips or fans belonging to the order Gorgonacea of the phylum *Cnidaria*. The interest in the chemistry of gorgonians has been stimulated by the novelty of the compounds isolated from these marine invertebrates as well as their promising biological activities.[1-4] In our study, two gorgonian samples, *Eunicea laciniata* and *Plexaurella nutans* were collected from Honduras and their ethanol extracts were investigated separately since they exhibited inhibition of *Plasmodium falciparum* and promising antimicrobial activity against several bacterial strains. The ethanol extract of the freeze–dried material of *E. laciniata* was subjected to silica gel flash chromatography followed by preparative thin layer chromatography and reverse phase HPLC to afford compounds **1–4**.

7,8–Epoxydolabella–3(E)–12(18)–diene (**1**) was obtained as a white solid (2 mg, 0.014% of dry weight). Its molecular formula $C_{20}H_{32}O$ was established by HRESIMS 327.2328 $[M+Ka]^+$ (calcd. 327.2328). The ^{13}C NMR signals at δ 66.1 (d), and 60.9 (s) in conjunction with ^1H NMR resonances at δ 2.90 (1H, d, J = 8.8 Hz) and 1.35 (3H, s) showed that **1** possesses a methyl substituted epoxide group.[5] Additional NMR signals suggested that the molecule possessed two unconjugated olefinic double bonds as indicated by carbon signals at δ_C149.6 (s), 137.2 (s), 136.4 (s) and 125.1 (d) and three olefinic methyl groups at δ 2.25 (3H, s), 1.92 (3H, s), and 1.56 (3H, s) and one bridgehead methyl at δ 1.18 (3H, s). One allylic methine proton at δ 2.68 (1H, d, J = 12.0 Hz), epoxide ring proton at δ 2.9 (1H, d, J = 8.8 Hz) and olefinic proton as AB double doublet at δ 5.44 (1H, J = 12.0, 4.4 Hz) were observed in the ^1H NMR spectrum. These features are consistent with a dolabellane epoxide skeleton when compared to the established structure of dollabellane **2** and related compounds.[3,5,6] Furthermore, the ^1H NMR and ^{13}C NMR spectra of **1** and **2** were similar except for those differences due to their functionalities.[5] The ^1H NMR showed a significant change for the H–7 signal. The broad doublet due to H–7 of **2** at δ 4.88 was changed to a broad doublet at δ 2.90, which was assigned to H–7 of **1**. The doublet at δ 2.78 due to H–11 and double doublet at δ 5.19 due to H–3 of trienone **2** remain virtually unchanged in **1**. In the ^{13}C NMR, two olefinic carbon signals of trienone **2** assigned for C–7 and C–8 were changed and appeared at δ 66.1 (d), 60.9 (s) corresponding to epoxide C–7 and C–8. Other carbon signals at δ 149.6 (s), 137.2 (s), 136.4 (s), and 125.1 (d) assigned for two olefinic double bonds C–3/C–4 and C –12/C–18 remain unchanged in **1**. No significant differences in the chemical shifts of the H–15, H–16, H–19, and H–20 methyl proton signals of **2** and **1** were observed in their ^1H NMR spectra. Hence there are no significant differences in the basic skeleta of these two molecules except some differences in functionality. The relative stereochemistry of **1** was determined by a NOESY experiment in which there was no NOE correlation between H–15 and H–11 indicating a Z orientation of these groups. Furthermore, the ^1H NMR and ^{13}C NMR chemical shifts for H–15 of **1** and **2** and related molecules were similar, indicating that the orientation of H–15 in **1** is α as in **2**.[3,5,6] The position of the epoxide was assigned at C–7/C–8 with the help of ^1H NMR and ^{13}C NMR chemical shifts and is supported by NOE correlations between H–3, H–15, and H–7 on the α – face of the molecule and HMBC correlation between H–17 with C–8, C–7. Similarly the position of double bond assigned at C–3/C–4 is supported by the NOE correlation of H–3 with H–15 and H–7 and HMBC correlation of H–16 with C–3 and C–4. No correlation was observed between methyl H–17 and H–7 indicating these groups are *trans* in the same plane. Thus, the relative stereochemistry of **1** was assigned as shown in Fig. 1.

The assignment was further verified using modelling studies. All calculations were performed on Silicon Graphics Octane2 workstation, with modeling software from Accelrys Inc., USA (Insight II 2000 and Discover 2.98). All the simulations were performed using a distance dependent dielectric. NMR derived distance restraints were introduced during the MD simulations with a force constant of 20 kcal/mol/rad^2. Starting with a minimised structure, the temperature of the system was raised to 500 K by a slow 'heating' in 100 K steps with sufficient equilibration at each step using the CVFF force field. This was followed by dynamics for 100 ps and the trajectory was sampled at intervals of 1 ps. These structures were then cooled to 300 K, in steps of 100 K, with short molecular dynamics run at each new temperature. The 100 structures were then energy minimized by steepest descents, followed by conjugate

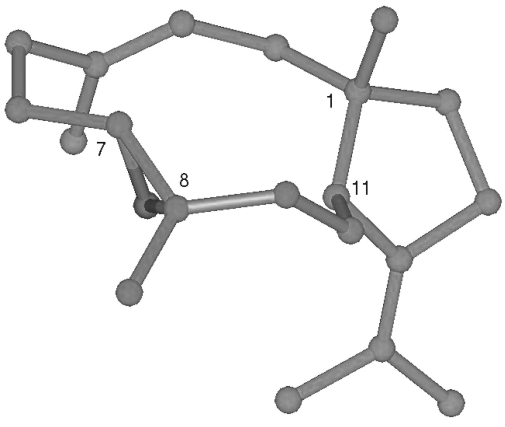

Fig. 1 Conformation of **1** obtained using NOESY derived distance restraints.

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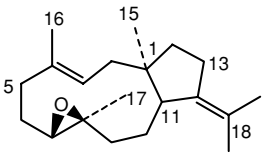

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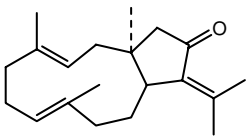

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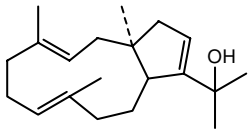

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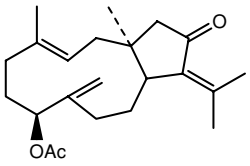

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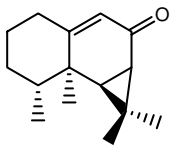

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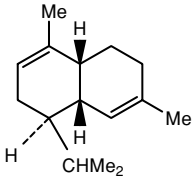

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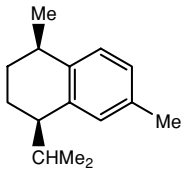

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gradients and ended with the Newton-Raphson (VA09A) method to a gradient of 0.001 kcal/mol/Å or lower. At this stage, the NMR derived distance restraints were removed, and all the 100 conformations were again subjected to the same minimisation protocol as given above. The structures were analysed and the lowest energy conformation of **1** obtained without any restraints which appeared to maintain important inter-proton distances in accordance with the NMR data. This supported the relative stereochemical assignments (Fig. 1).

The dolabellanetrienone (**2**)[5] did not show antimalarial or antimicrobial activity, but did show moderate antileishmanial activity at an LC_{50} and LC_{90} of 23 and 45 μg/ml, respectively. The dolabellanetrienol (**3**)[6] showed moderate antimicrobial activity against the tested organisms with IC_{50} (μg/ml) for *Candida neoformans, Staphylococcus aureus*, and MRS to be 25, 30, and 35 respectively and did not show any antimalarial activity though the antileishmanial activity at IC_{50} and IC_{90} (μg/ml) was found to be 18 and 41 respectively. The dolabellanetrienone acetate (**4**)[3] did not show any antimicrobial or antimalarial activity.

It is interesting to note that both the trienone (**2**) and acetate (**4**) were inactive against these microorganism while trienol (**3**) showed some activity against similar organisms. Dolabellane diterpenoids having a good antimicrobial and/or antileishmanial activities may be obtained by proper replacement of functionality in the parent compound.

The ethanol extract of freeze dried material of *P. nutan*s was subjected to silica gel flash chromatography followed by preparative thin layer chromatography and reverse phase HPLC to afford three related cadinane type sesquiterpenes.[7-11] They were identified as 9–aristoline-8–one (**5**),[10,11] (+)-α–muurolene (**6**)[12] and 1*R*, 4*R*–calamenene (**7**)[13] from their identical ^{1}H NMR and ^{13}C NMR spectral data with the reported values.

Experimental

General experimental procedures

The ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ and MeOH–d_4 in 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR. The HRMS spectra were measured using a Bioapex FTESI–MS with electrospray ionisation. Optical rotations were measured with a JASCO DIP–310 digital polarimeter. TLC analysis were carried out on precoated silica gel G_{254} or aluminium oxide ALOX–100 UV_{254} 500 μm.

Collection, identification and taxonomy

The gorgonian specimens were collected from the bay island Utila in coastal Honduras and voucher specimens are deposited in the Department of Medicinal Chemistry, University of Mississippi. The voucher specimen [HOND-12-01] fits the description of *Eunicea (Euniceopsis) laciniata* Duchassaing & Micholotti, 1860 (Phylum Cnidaria, Class Anthozoa, Order Gorgonacea, Family Plexauridae) as described in Bayer (1961, p. 140).[14] It consists of a 2-cm branch tip, which is dark brown in alcohol, clavate, and ~1.5-cm diameter at the tip. The calyces are low warts with eight lobes that have a distinct upturned lower lip. The anthocodia crown is well developed with a strong chevron and collarette of spicules; the axial sheath has violet spindles ~0.3 mm in length; the middle rind has long white spindles up to 2.0 mm in length and 0.25 mm in width; and the outer layer has clubs ~0.110 to 0.13 mm in length, and unilateral foliate spindles. The voucher specimen [HOND-30-01] fits the description of *Plexaurella nutans* (Duchassaing & Michelotti) 1860 (Family Plexauridae) as described in Bayer (1961, p. 172).[14] It consists of a 2-cm branch tip, which is light brown in alcohol, slightly clavate, and ~1.5-cm diameter. The calyces are low warts with a closed slit aperarture. The anthocodial spicules are blunt rods from 0.10 to 0.37 mm in length; the middle layer spicules consist of spindles, triradiates and quadriradiates that are slender and ~0.3 mm in length; and the surface layer has capstans ~0.05 mm in length.

Extraction and isolation

The gorgonian *E. laciniata* was stored frozen until extracted. The lyophilized *E. laciniata* (35.6 g, dry wt) was crushed, homogenised and then extracted with ethanol at room temperature. The extract was concentrated under reduced pressure and the resultant extract (3.8 g) was subjected to SiO_2 gel column chromatography and eluted with increasing polarity of hexane–EtOAc. The fractions containing bicyclic diterpenoid of the dolabellane ring system **1–4** were further separated by preparative tlc and HPLC to afford pure compounds.

The freeze dried material of *P. nutans* (81.3 g, dry wt) was extracted exhaustively with EtOH. The organic extract was evaporated to give a residue (8.7 g), which was separated by silica gel column chromatography using increasing polarity $CHCl_3$–MeCN to afford a series of fractions. The fractions containing sesquiterpene were further separated by preparative TLC and HPLC to afford pure 9–aristoline-8–one, (+)-α –muurolene, and 1*R*, 4*R*–calamenene.

7,8–Epoxydolabella–3(E)–12(18)–diene (**1**): White powder; $[\alpha]_D^{25}$ + 31.6 (c 0.7 $CHCl_3$), ^{1}H NMR ($CDCl_3$): δ 5.44 (1H, dd, *J* = 12, 4.4 Hz, H-3), 2.90 (1H, d, *J* = 8.8 Hz, H-7), 2.68 (1H, d, *J* = 12 Hz, H-11), 2.25 (3H, s, H-19), 1.96 (2H, m, H-13), 1.94 (2H, m, H-5), 1.92 (3H, s, H-20), 1.56 (3H, s, H-16), 1.46 (2H, m, H-6), 1.38 (2H, m, H-9), 1.35 (3H, s, H-17), 1.27 (2H, m, H-10), 1.25 (2H, m, H-14), 1.18 (3H, s, H-15); ^{13}C NMR ($CDCl_3$): 149.6 (s, C-18), 137.2 (s, C-12), 136.4 (s, C-4), 125.1 (d, C-3), 66.1 (d, C-7), 60.9 (s, C-8), 54.7 (t, C-14), 42.5 (d, C-11), 41.4 (s, C-1), 40.2 (t, C-2), 38.4 (t, C-9), 37.3 (t, C-5), 30.1 (t, C-13), 27.9 (t, C-6), 23.7 (q, C-15), 23.2 (t, C-10), 19.2 (q, C-17), 19.1 (q, C-20), 16.1 (q, C-16); HRESIMS, *m/z* = 327.2328, $C_{20}H_{32}O$ [M + Ka]$^+$ requires *m/z* 327.2328.

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