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Cladioxazole: a novel sesquiterpene from a marine soft coral of genus *Cladiella*

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Abstract—Chemical investigations on the methanolic extract of *Cladiella* species, collected from Andaman Island, India, yielded a novel sesquiterpene, cladioxazole (**1**). Its structure was established with the aid of extensive spectral studies.
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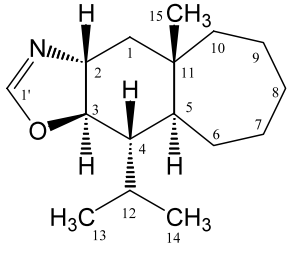
Chemical studies on marine soft corals have yielded several novel bioactive natural products having potential biomedical applications. For instance, *Pseudopterogorgia elisabethae* produces pseudopterosins, pesudopteroxazole, seco-pseudopteroxazole and erogorgiaene. Pseudopterosins have exhibited potent anti-inflammatory and analgesic activities, while pseudopteroxazole and erogorgiaene have shown anti-tuberculosis activity.[1–5] Our recent chemical studies on *Cladiella* sp. collected from Andaman Island, India have resulted in the isolation of a novel sesquiterpene, cladioxazole (**1**), which possesses a novel sesquiterpene skeleton having an oxazole ring incorporated in its structure. This is the first reported isolation of a nitrogenous sesquiterpene from *Cladiella*, a genus, that until now has yielded predominantly eunicellane- and cembrane-type diterpenes.[6–9] This also represents the first report of isolation of a sesquiterpene containing an oxazole ring from a marine soft coral. To our knowledge, only two diterpenes having oxazole ring incorporated in their structures, namely, pseudopteroxazole and seco-pseudopteroxazole have been reported from a marine soft coral *P. elisabethae* to date.[4]

Cladiella sp. was collected from Andaman Island, India and was extracted with methanol. The methanolic extract was re-dissolved in 20% ethanol and 80% water. This aqueous alcoholic extract was extracted with ethyl acetate (2 g) and was loaded onto a silica gel column. This column was eluted with hexane-ethyl acetate (0–100%) and ethyl acetate–methanol (0–100%) to yield several fractions. A fraction obtained on the elution of hexane–ethyl acetate (60:40) was subjected to reverse-phase HPLC using a gradient elution of acetonitrile–water (0–100) to afford cladioxazole (**1**) (4.3 mg) as a colorless oil. The presence of nitrogen in **1** was evident due to a positive Dragendroff's reagent. Its UV spectrum showed a terminal absorption indicating the lack of a conjugated π system in the molecule and IR spectrum displayed intense absorptions at 2904 (CH) and 1595 (C=N) cm^{-1}. The high-resolution electron-impact mass spectrum (HREIMS) of compound **1** showed a molecular ion peak at m/z 249.2089, which corresponds to the molecular formula $C_{16}H_{27}NO$ (calcd 249.2093), and indicated the presence of four degrees of unsaturation in the molecule.



(**1**)

The ^1H NMR spectrum ($CDCl_3$, 500 MHz) of **1** showed two doublets integrating for three hydrogens each at δ 0.86 and 0.91 ($J = 6.5$ Hz) due to the C-13 and C-14 methyl protons, respectively. A three-hydrogen singlet at δ 1.36 was assigned to the C-15 methyl

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protons. A one-proton doublets of double doublet resonated at δ 3.65 ($J_1 = 10.2$ Hz, $J_2 = 9.8$ Hz, $J_3 = 0.3$ Hz) was assigned to the C-3 methine proton. The C-2 methine proton resonated at δ 3.02. The downfield chemical shift values for H-3 and H-2 indicated the presence of geminal oxygen and nitrogen functionalites on these carbon atoms, respectively. The C-1′ olefinic proton resonated at δ 7.69. The COSY-45° spectrum was recorded to assign the ^{1}H NMR chemical shift assignments and to establish the partial structures of this new metabolite. A very careful interpretation of COSY-45° and TOCSY spectra revealed that compound **1** has two spin systems. The first spin system was due to the C-1′ methine proton and this proton does not show cross-peaks in the COSY-45° spectrum which suggested that this methine proton is sandwiched between two heteroatoms, i.e. oxygen and nitrogen. The second partial structure (Fig. 1) was traced from the C-3 methine proton (δ 3.65), which showed cross-peaks with the C-2 methine proton (δ 3.02). The latter showed vicinal coupling with the C-1 methylene protons (δ 1.98 and 1.77). H-3 also exhibited COSY-45° interactions with the C-4 methine proton (δ 2.10), which in turn showed ^{1}H–^{1}H spin correlations with the C-12 methine proton (δ 1.99). H-12 showed cross-peaks with C-13 (δ 0.86) and C-14 (δ 0.91) methyl protons. H-4 also showed vicinal couplings with the C-5 methine proton (δ 1.95). H-5 in turn exhibited cross-peaks with the C-6 methylene protons (δ 1.77 and 1.65), which in turn showed ^{1}H–^{1}H spin correlations with the C-7 methylene protons (δ 1.60 and 1.40), and this further showed vicinal couplings with the C-8 methylene protons (δ 1.52 and 1.34). H-8 showed cross-peaks with the C-9 methylene protons (δ 1.46 and 1.28). The COSY-45° interactions of C-9 methylene protons with the C-10 methylene protons (δ 1.37 and 1.19) were also observed in the spectrum.

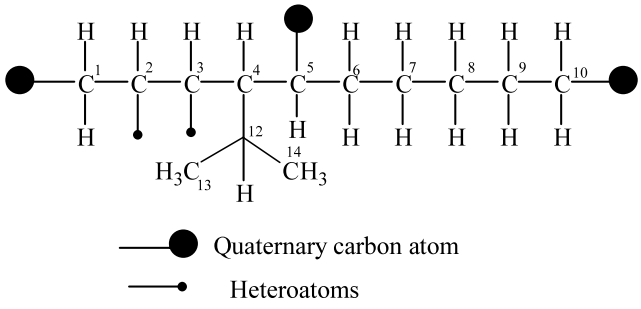

Figure 1. Partial structure of **1** obtained from COSY-45° spectrum.

Table 1. ^{1}H, ^{13}C NMR chemical shift assignments, ^{1}H/^{13}C one-bond shift correlations as determined from HMQC spectra and HMBC interactions of compound **1**

Carbon no.	^{1}H δ	^{13}C δ	Multiplicity[a]	Key HMBC interactions
1	1.98	27.3	CH_2	C-2, C-11 and C-15
	1.77	–		
2	3.02	55.2	CH	C-1, C-3 and C-11
3	3.65	72.4	CH	C-2, C-4 and C-5
4	2.10	46.2	CH	C-3, C-5 and C-11
5	1.95	40.3	CH	C-4, C-6 and C-11
6	1.77	30.1	CH_2	C-5, C-7, C-8 and C-11
	1.65			
7	1.60	27.8	CH_2	C-5, C-6 and C-7
	1.40	–		
8	1.52	26.5	CH_2	C-6, C-7 and C-9
	1.34			
9	1.46	25.4	CH_2	C-8 and C-10
	1.28			
10	1.37	24.8	CH_2	C-8 and C-9
	1.19			
11	–	32.1	-C-	–
12	1.99	35.0(d)	CH	C-4, C-13 and C-14
13	0.86	14.6(q)	CH_3	C-4, 12 and C-14
14	0.91	19.4(q)	CH_3	C-4 and C-12
15	1.36	14.1(q)	CH_3	C-5 and C-11
1′	7.69	152.7	CH	C-1, C-2, C-3 and C-4

[a] Multiplicity was determined from DEPT spectrum. All spectra were recorded in $CDCl_3$.

The ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of **1** showed the resonances of all 16 carbon atoms and DEPT experiment was also performed to establish the multiplicity of each signal in the ^{13}C NMR spectrum. It revealed the presence of three methyl, six methylene and six methine carbon atoms. Subtraction of the DEPT spectrum from the broadband ^{13}C NMR spectrum indicated the presence of one quaternary carbon atom in the molecule. It resonated at δ 32.1 and was assigned to the C-11. Its chemical shift value indicated that C-11 is an sp^3 hybridized carbon atom. Two downfield aliphatic signals at δ 55.2 and 72.4 were due to the C-2 and C-3 carbon atoms, respectively. Their downfield chemical shift values suggested the presence of geminal nitrogen and oxygen functionalities, respectively. The ether nature of an oxygen atom substituted at C-3 was also determined by recording the ^{1}H NMR spectrum of compound **1** in pyridine-d_5 in which the C-3 methine proton showed induced paramagnetic shift from δ 3.65–3.67. It has been reported in the literature that a pronounced shift of $\approx$0.2 ppm was observed in case of protons adjacent to a hydroxyl group when ^{1}H NMR spectrum was recorded in pyridine-d_5.[10] C-1′ signal resonated at δ 152.7, and its downfield chemical shift value indicated that C-1′ is an sp^2 hybridized carbon atom and flanked by a nitrogen and an oxygen atoms. The HMQC spectrum was also recorded to establish the ^{1}H/^{13}C one-bond shift correlation of compound **1**. Complete ^{13}C NMR chemical shift assignments and ^{1}H/^{13}C one-bond shift correlations of all protonated carbon atoms of compound **1** as determined from HMQC spectrum are shown in Table 1.

The HMBC spectrum was very useful to establish a gross structure of compound **1** from a partial structure deduced from the COSY-45° and TOCSY spectra. The C-1 methylene (δ 1.98 and 1.77) and C-10 methylene (δ 1.37 and 1.19) protons showed long-range heteronuclear couplings with C-5 (δ 40.3) and C-11 (δ 32.1). H-4 (δ 2.10) and H-6 (δ 1.77 and 1.65) also exhibited

cross-peaks with C-5 and C-11. The C-15 methyl protons (δ 1.36) also showed HMBC interactions with C-4 (δ 46.2) and C-5. From these HMBC interactions, it is evident that C-1 is connected with C-10 through a quaternary carbon atom (C-11) and C-4 is bonded with C-6 through a tertiary carbon atom (C-5). The connectivity between C-5 and C-10 is also evident from these HMBC observations. Other important HMBC interactions of compound **1** are shown in Table 1. The interpretation of spectral data obtained from the combination of ^{1}H, ^{13}C, COSY-45° and HMBC spectra helped us to propose the tricarbocyclic skeleton for this new natural product. The tricarbocyclic skeleton was also indicative from HREIMS, which provided the molecular formula $C_{16}H_{27}NO$ and indicated the presence of four degrees of unsaturation. Three of them were accounted for a tricarbocyclic skeleton and remaining fourth degree of unsaturation was due to the presence of a double bond in an oxazole ring.

The stereochemistry at various chiral centers in compound **1** was established with the aid of ^{1}H NMR coupling data and the NOESY spectrum. The C-3 methine proton, resonated as a doublets of double doublet at δ 3.65 and showed diaxial couplings with C-2 ($J=9.8$ Hz) and C-4 ($J=10.2$ Hz). The *trans* diaxial couplings of H-3/H-2 and H-3/H-4 permitted us to establish an α-stereochemistry for H-3 and a β-stereochemistry for H-2. The long-range 'W' coupling between H-3 and H-5 ($J=0.3$ Hz) also favored α-stereochemistry for H-3. This assumption was further supported by the NOESY spectrum in which C-3 methine proton (δ 3.65) showed a strong NOE with the C-5 methine proton (δ 1.95). This indicated a *cis* relationship between H-3 and H-5. H-2 (δ 3.02) showed cross-peaks with the C-4 methine proton (δ 2.10). H-2 and H-4 also showed NOE cross-peaks with the C-15 methyl protons (δ 1.36). This NOE data suggested that H-2, H-4 and H_3-15 have similar orientations. The combination of ^{1}H NMR coupling and NOESY spectral data helped us to assume β-orientations for H-2, H-5 and H_3-15. This can only be possible in this spatial proximity. These spectroscopic studies helped us to propose structure **1** for this new natural product.

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