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# A New Cladiellane Diterpenoid from *Eunicella labiata*

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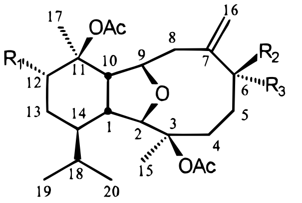
The gorgonian *Eunicella labiata* from Palmones, Spain, contains the known cladiellane diterpenes eunicellin (**1**), palmonine D (**3**), and labiatin B (**4**), together with the new (1*S**,2*Z*,6*E*,10*R**,11*S**,12*S**,13*S**,14*R**)-12,13-diacetoxycladiella-2,6-dien-11-ol (**2**). The structure was elucidated by interpretation of spectral data, and the relative stereochemistry was defined using NOEDS experiments.

Gorgonians have proved to be a rich source of structurally diverse and pharmacologically active natural products.[1] The eunicellin-type compounds that contain a cladiellane diterpene skeleton and a C-2,C-9 ether bridge are characteristic of this group of organisms. Eunicellin (**1**), the first compound possesing a eunicellin-type skeleton, was isolated from the gorgonian *Eunicella stricta*.[2,3] Three other species of the genus *Eunicella* have been studied: *E. verrucosa*,[4−6] *E. cavolini*,[7−9] and *E. labiata*.[10] These studies have stated that the chemistry of the genus *Eunicella* is dominated by the presence of cladiellane diterpenes.

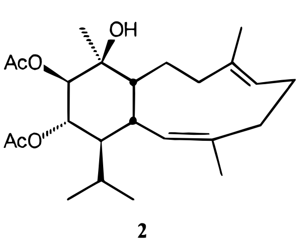
In the course of our investigations of the natural products from marine organisms of the southern coast of Spain, we obtained specimens of *Eunicella labiata* Thomson (Plexauridae). The chemical study of this organism has led to the isolation of four cladiellane diterpenes (**1**−**4**) and is reported in the present paper.



**1** $R_1$ = OAc; $R_2$ = OAc; $R_3$ = H

**3** $R_1$ = H; $R_2$ = $R_3$ = O

**4** $R_1$ = OAc; $R_2$ = $R_3$ = O



**2**

Specimens of *E. labiata* were collected by hand using scuba and were immediately frozen. The coral was extracted with $Me_2CO$, and the resulting extract was concentrated to form an aqueous residue that was extracted with $Et_2O$. Subsequent normal-phase chromatography of the organic phase led to the isolation of the following compounds in order of increasing polarity: eunicellin (**1**, $1.25 \times 10^{-3}$% dry wt), (1*S**,2*Z*,6*E*,10*R**,11*S**,12*S**,13*S**,14*R**)-12,13-diacetoxycladiella-2,6-dien-11-ol (**2**, $5.74 \times 10^{-3}$% dry wt), palmonine D (**3**, $1.25 \times 10^{-3}$% dry wt), and labiatin B (**4**, $2.50 \times 10^{-3}$% dry wt). Compounds **1**,[2,3] **3**,[5] and **4**[10] were identified by comparison of their spectral data with those previously reported.

Compound **2** was isolated as a white amorphous powder. The molecular formula, $C_{24}H_{38}O_5$, was obtained from a high-resolution mass measurement. The IR absorptions at 3300, 1725, and 1220 $cm^{-1}$, together with the $^1H$-NMR signals at 1.98 (3H, s) and 2.10 (3H, s), indicated that **2** was a diacetate bearing a hydroxy group. Analysis of $^1H$-NMR and $^{13}C$-NMR spectra, as well as examination of the connectivities defined by COSY and HETCOR experiments, indicated that compound **2** possessed a cladiellane skeleton.

The $^1H$-NMR signals at $\delta$ 4.85 (1H, d, $J = 6.3$ Hz) and 5.28 (1H, dd, $J = 10.8, 5.2$ Hz), together with the $^{13}C$-NMR signals at $\delta$ 137.1 (s), 135.5 (s), 131.1 (d), and 126.0 (d), were attributable to two trisubstituted double bonds. The olefinic methyl proton signals at 1.76 (3H, br s) and 1.55 (3H, br s), which were correlated in the HETCOR experiment with the $^{13}C$-NMR signals at 24.9 (q) and 17.9 (q), respectively, indicated a different geometry for each double bond.[11] These structural features, together with examination of the molecular formula, indicated that **2** had to be bicyclic and therefore lacked the common ether bridge on the 10-membered ring of most cladiellanes.

The substitution pattern on the six-membered ring was established as follows. The mutually coupled $^1H$-NMR signals at $\delta$ 5.00 (1H, d, $J = 9.4$ Hz) and 5.34 (1H, dd, $J = 10.4, 9.4$ Hz) indicated the presence of two *trans* acetoxyl groups in C-12 and C-13 positions. The $^1H$-NMR singlet at $\delta$ 1.16 (3H, s) and the $^{13}C$-NMR signals at $\delta$ 23.9 (q) and 75.7 (s) that were assigned to a methyl group and to a quaternary carbon bearing oxygen, respectively, were consistent with the presence of a hydroxy group on C-11.

The location of the double bonds on the 10-membered ring was deduced from a careful examination of the COSY spectrum. Correlation was observed between the H-1 signal at $\delta$ 2.73 (1H, ddd, $J = 12.0, 6.3, 5.9$ Hz) and the olefinic proton signal at $\delta$ 4.85. This latter signal showed allylic coupling with the methyl proton signal at $\delta$ 1.76, which was correlated in a HETCOR spectrum

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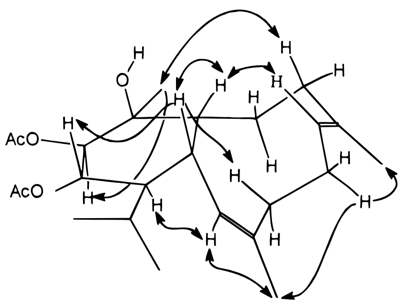

**Figure 1.** NOE correlations for compound **2**.

with the 24.9 quartet, indicating a C-2,C-3 double bond with *Z* geometry.[11] Similarly, the correlations observed between the H-4 and H-5 methylene proton signals and between this latter signal and the olefinic proton signal at $\delta$ 5.28 indicated that the remaining *E* double bond must be located at C-6,C-7.

A series of NOEDS experiments provided confirmation of the proposed structural assignments and defined the relative stereochemistry of **2** (Figure 1). Irradiation of the H-1 signal caused enhancements on the H-10 and H-13 signals, indicating a *cis*-ring junction and an $\alpha$ orientation of the C-13 acetoxyl group. Irradiation of the Me-17 proton signal enhanced the H-12 signal, defining the relative stereochemistry at C-11 and C-12. The orientation of the C-14 isopropyl group is proposed as $\beta$ upon observation of the mutual NOE enhancements produced on irradiation of H-2 and H-14 proton signals. Finally, the proposed geometry for the C-2,C-3 and C-6,C-7 double bonds was confirmed by observation of the enhancements produced on Me-15 and Me-16 signals upon irradiation of the H-2 and H-5$\alpha$ signals, respectively. These data defined the structure of (1*S**,2*Z*,6*E*,10*R**,11*S**,12*S**,13*S**,14*R**)-12,13-diacetoxycladiella-2,6-dien-11-ol (**2**).

In general, the cladiellane diterpenes from gorgonians are highly oxidized compounds. The ten-membered ring commonly presents an ether bridge that links the positions C-2,C-9,[1,9,10,12,13] C-4,C-7,[14] or C-2,C-6,[10] in addition to epoxides, and hydroxy and acetoxyl groups. The new compound **2** represents the first example of a cladiellane diterpene lacking oxygen functionalities on the ten-membered ring.

## Experimental Section

**General Experimental Procedures.** IR spectra were recorded on a Perkin-Elmer 881 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded on a Varian 400 at 400 MHz and 100 MHz, respectively, using $CDCl_3$ as solvent. The resonances of residual $CHCl_3$ at $\delta_H$ 7.26 and $\delta_C$ 77.0 were used as an internal reference for $^{1}$H-NMR and $^{13}$C-NMR spectra, respectively. Mass spectra were measured on a VG 12250 or a Kratos MS 80RFA spectrometer. HPLC separations LiChrosorb Si-60 was used in normal-phase mode using a differential refractometer. An asterisk indicates interchangeable signals. All solvents were distilled from glass prior to use.

**Collection, Extraction, and Isolation Procedures.** The gorgonian *E. labiata* (80.1 g dry wt) was collected in Palmones, Spain (5°25′ W– 36°10′ N) in November 1995, and extracted with $Me_2CO$ at room temperature. A voucher specimen is deposited at the Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Cádiz (no. 9604). The $Me_2CO$ solution was evaporated under reduced pressure and the aqueous residue extracted with $Et_2O$. The organic layer was dried over anhydrous $Na_2SO_4$ and the solvent evaporated to give a dark oil (1.7 g), which was chromatographed on a Si gel column eluting with solvents of increasing polarity from hexane to $Et_2O$ and subsequently $CHCl_3$–MeOH (8:2). Fractions eluted with hexane–$Et_2O$ (1:1) were further purified by HPLC (LiChrosorb 5 $\mu$m, 5 mm × 25 cm; hexane–EtOAc, 8:2) to obtain eunicellin (**1**) (1 mg, $1.25 \times 10^{-3}$% dry wt). Fractions eluted with hexane–$Et_2O$ (3:7) contained a mixture of two compounds that were separated by HPLC (LiChrosorb 10 $\mu$m, 10 mm × 25 cm; hexane–EtOAc, 8:2), affording compound **2** (4.6 mg, $5.74 \times 10^{-3}$% dry wt) and palmonine D (**3**) (1 mg, $1.25 \times 10^{-3}$% dry wt). More polar fractions provided labiatin B (**4**) (2 mg, $2.50 \times 10^{-3}$% dry wt) after purification by HPLC (LiChrosorb 5 $\mu$m, 5 mm × 25 cm; hexane–EtOAc, 6:4).

**(1*S**,2*Z*,6*E*,10*R**,11*S**,12*S**,13*S**,14*R**)-12,13-Diacetoxycladiella-2,6-dien-11-ol (2):** amorphous powder; $[\alpha]^{25}_{D}$ −18.3° (*c* 0.4, $CHCl_3$); IR (dry film) $\nu_{max}$ 3300 (OH), 1725 (C=O), 1660 (C=C), 1220 (C–O) cm$^{-1}$; $^{1}$H NMR ($CDCl_3$, 400 MHz) $\delta$ 5.34 (1H, dd, *J* = 10.4, 9.4 Hz, H-13), 5.28 (1H, dd, *J* = 10.8, 5.2 Hz, H-6), 5.00 (1H, d, *J* = 9.4 Hz, H-12), 4.85 (1H, d, *J* = 6.3 Hz, H-2), 2.73 (1H, ddd, *J* = 12.0, 6.3, 5.9 Hz, H-1), 2.45 (1H, m, H-10), 2.33 (1H, ddd, *J* = 12.4, 10.8, 2.4 Hz, H-5$\alpha$), 2.12 (1H, ddd, *J* = 12.4, 12.4, 2.4 Hz, H-4$\beta$), 2.10 (3H, s, $CH_3$CO–), 2.05 (1H, m, H-5$\beta$), 2.01 (1H, m, H-8$\alpha$) 1.98 (3H, s, $CH_3$CO–), 1.90 (1H, m, H-4$\alpha$), 1.84 (1H, m, H-18), 1.71 (1H, m, H-8$\beta$), 1.76 (3H, br s, H-15), 1.55 (3H, br s, H-16), 1.54 (1H, m, H-14), 1.30 (2H, m, H-9), 1.16 (3H, s, H-17), 0.90 (3H, d, *J* = 7.3 Hz, H-19), 0.84 (3H, d, *J* = 7.1 Hz, H-20); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 170.5 (s, $CH_3$*CO*-), 170.4 (s, $CH_3$*CO*-), 137.1 (s, C-7)*, 135.5 (s, C-3)*, 131.1 (d, C-2), 126.0 (d, C-6), 77.4 (d, C-12), 75.7 (s, C-11), 72.5 (d, C-13), 47.5 (d, C-14), 45.4 (d, C-10), 39.7 (t, C-8), 34.9 (d, C-1), 34.1 (t, C-4), 27.4 (d, C-18), 25.0 (t, C-5), 24.9 (t, C-9), 24.9 (q, C-15), 23.9 (q, C-17), 22.3 (q, C-19), 21.4 (q, $CH_3$CO–), 20.8 (q, $CH_3$CO-), 17.9 (q, C-16), 17.3 (q, C-20); EIMS (70 eV) *m/z* (rel int) 406 (1), 388 (1), 346 (3), 286 (27), 268 (15), 243 (65), 109 (100); HREIMS *m/z* 406.2720 calcd for $C_{24}H_{38}O_5$, 406.2716.

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