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PALOMINOL, A NEW DITERPENE BASED ON THE DOLABELLANE SKELETON FROM THE CARIBBEAN GORGONIAN OCTOCORALS *EUNICEA CALYCULATA* AND *EUNICEA LACINIATA* [1].

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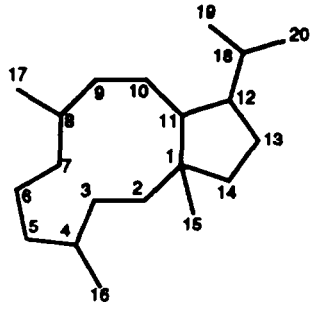
Abstract: A new bicyclic diterpenoid, palominol (**2**), of the dolabellane ring system has been isolated from the Caribbean gorgonians *Eunicea calyculata* and *Eunicea laciniata* . The structure of this new compound was assigned on the basis of chemical and spectral studies, particularly heteronuclear chemical shift correlation methods, homonuclear spin decoupling experiments and nuclear Overhauser enhancement difference spectroscopy (NOEDS).

In connection with our investigations of the constituents of marine invertebrates with biomedical potential around Puerto Rico and its neighboring islands, we made collections of marine octocorals of the order Gorgonacea (phylum Cnidaria) near Vieques Island (February ,1988) and Palomino Key (September,1988). Special attention was given to the collection of those individuals commonly referred to as sea whips [4]. In the Caribbean Sea, sea whips of the genus *Eunicea* (family Plexauridae) have been recognized as an abundant source of structurally unique secondary metabolites possessing a formidable array of biological activities [5]. While cembranoid diterpenes are by far the most common natural constituents among the fifteen species of *Eunicea* found in the Caribbean Sea, an increasing number of a distinct class of non-cembranoid bicyclic diterpenoids have been reported since 1976 [6]. These still relatively uncommon metabolites are regarded as examples of diterpenoids of the dolabellane ring system **1**. This class of constituents feature a bicyclo[9.3.0] carbon skeleton joined in a *trans* fashion that could conceivably originate by cyclization of geranylgeraniol pyrophosphate as proposed by Faulkner [7].

Diterpenes with dolabellane skeleton had been originally isolated from the herbivorous sea hare *Dolabella californica* Sterns [8] and successively from the brown algae *Glossophora galapagensis* Taylor (family Dictyotaceae) [9] upon which the sea hare feeds, *Dictyota dichotoma* (Huds) Lamouroux [10] and *Dilophus fasciola* (Roth) [11]. They have been isolated also from the sea whip *Eunicea calyculata* [12] (Ellis and Solander), from the mollusc *Aplysia dactylomela* [13], the liverwort *Odontoschisma denudatum* (Ness) Dum. [14] and recently the structure determination of three new bioactive dolabellane diterpenes was reported from a Japanese octocoral of the genus *Clavularia* [15].In this paper we wish to report the isolation and structure determination of a new bicyclic dolabellane diterpene, palominol (**2**), from the Caribbean octocorals *Eunicea calyculata* and *Eunicea laciniata*. In addition to this new compound, extracts of both marine animals also yielded known dolabellane derivative **3** as the major constituent in each case [12].



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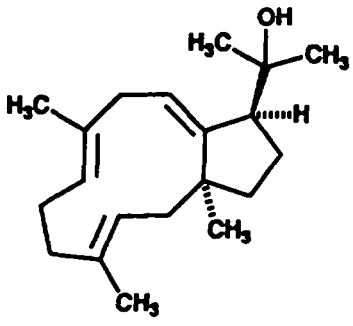

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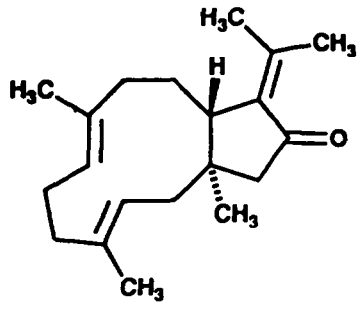

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Fresh specimens (171g, wet) of *Eunicea calyculata* collected at Punta Arenas (Vieques, Puerto Rico) were triturated in a blender with methanol to yield after concentration a crude extract (11.50g) which comprised 6.7% of the total weight of the coral. Further partitioning with hexane against water yielded an extract (4.65g) which represented 40.4% of the animal's organic content. About half of the hexane extract which showed only marginal antibacterial activity against Gram negative bacteria was chromatographed successively on a Bio-Beads S-X2 column (toluene), a Sephadex LH-20 column (1:1 $MeOH/CH_2Cl_2$) and a silica gel column by stepwise elution with hexane-ethyl acetate mixture. The relatively nonpolar diterpenoid-containing fractions were combined and subsequently purified by standard high-performance liquid chromatography (HPLC) using a C-18 silica gel column (85:15 $MeOH/H_2O$). The major component (141.3 mg; 2.4% of the crude extract) was the least polar and consisted of a colorless oil which exhibited strong UV activity. This secondary metabolite was subsequently identified as known triene **3** on the basis of its ^{13}C-^{13}C homonuclear shift correlation 2D spectrum (**INADEQUATE**) [16] combined with additional spectral studies. The minor component (69.0 mg; 1.2% of the crude extract) consisted of a non-crystalline UV inactive solid which on the basis of spectroscopic data including two-dimensional NMR and chemical degradation reactions, was identified as new dolabellane diterpene, palominol (**2**).

The extraction of trienes **2** and **3** from a small specimen of *Eunicea laciniata* (25g; wet) was conducted without homogenization of the animal. The coral was stored overnight in excess methanol at room temperature and the resulting suspension was decanted and evaporated under vacuum to give a green residue. The procedure was repeated twice more and after filtration the crude extract (1.47g, 5.9% of the weight of the coral) was chromatographed on silica gel using 5% ethyl acetate in hexane to obtain pure triene **3** (0.23g; 15.6%). Further elution yielded somewhat impure alcohol **2** which after rechromatography by reverse phase HPLC (90:10 $MeOH/H_2O$) was isolated pure (0.10g, 6.8%).

The triene **3** analyzed for $C_{20}H_{30}O$ by high-resolution mass spectrometry and showed ^{1}HNMR, ^{13}CNMR, infrared and UV absorptions which subsequently defined the oil as being identical with material isolated earlier by Fenical and Look also from the Caribbean gorgonian *Eunicea calyculata* [12]. However, it was the ^{13}C-^{13}C homonuclear shift correlation 2D spectrum (**INADEQUATE**) of the oily constituent which established its chemical structure and enabled also the assignment of all resonances in the NMR spectra. The **INADEQUATE** spectrum of **3** showed the cross peaks of

sixteen pairs of carbons as shown in Fig. 1. Correlated ^{1}H-^{13}C spectra which allowed separation of chemical shift and spin coupling information in weakly coupled spin systems along with **COSY** spectra and simple homonuclear spin decoupling experiments, further elucidated the connections between C-3 and C-4, C-7 and C-8 and C-13 and C-14, respectively. Connection of each carbon pair clarified the sequence of carbon atoms and permitted the assignment of all carbon and hydrogen atoms in **3** unambiguously (see **Table I**).

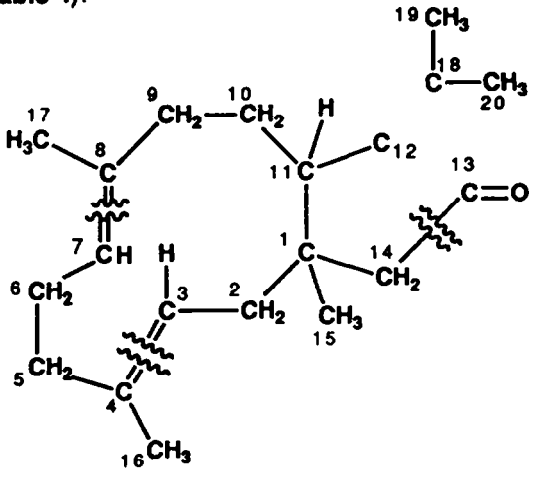

Fig. 1. The 2D INADEQUATE spectrum of triene **3** showed the cross peaks of sixteen pairs of carbons. INADEQUATE: points, 2,048; other parameters, similar to those for ^{13}C-^{1}H correlation spectra (*ca* 400 mg of sample, *ca* 48 h accumulation).

The more polar constituent, palominol (**2**), has the molecular formula $C_{20}H_{32}O$ from the high resolution mass measurement of the molecular ion. Its IR spectrum displayed a strong band at 3440 cm^{-1} (broad, hydroxyl group) and the ^{13}CNMR spectrum confirmed the presence of three trisubstituted double bonds [154.17 (s), 134.52 (s), 133.39 (s), 128.60 (d), 125.46 (d) and 122.52 (d)]. Hence palominol (**2**), having five degrees of unsaturation, must possess a carbobicyclic skeleton. In the ^{1}HNMR spectrum of **2**, five Me signals appeared as singlets at δ 1.60 and 1.48 (vinyl methyls), 1.39 and 1.35 (methyl groups on a carbon atom bearing oxygen) and 1.14 (methyl at a bridgehead or at a side-chain junction). Moreover, three olefinic protons associated with three trisubstituted double bonds were observed as a broad singlet at δ 5.45, a doublet of doublets centered at δ 5.18 (J = 4.8, 11.5 Hz) and a broad doublet at δ 4.83 (J = 10.4 Hz).

The NMR features of palominol clearly suggest a close relatedness to triene **3** in which the NMR bands of the enone functionality have disappeared and new bands for an isopropyl alcohol side chain at C-12 and a new trisubstituted olefin at C-10 have appeared in place. The presence of two signals in the ^{1}HNMR at δ 1.39 and 1.35 ppm, together with a signal in the ^{13}CNMR spectrum at 71.56 ppm due to a quaternary carbon, indicated the presence of an isopropyl alcohol moiety. Evidence to support the location of the isopropyl alcohol chain as shown stems from the **COSY** spectra and also from simple homonuclear decoupling experiments. Irradiation of the methine

Table I. ^{13}C- and ^{1}H-NMR Data for Trienes 2[a,b] and 3[a,b,c].

Carbon	2; δ_C ppm	2; δ_H ppm (J in Hz)	3; δ_C ppm	3; δ_H ppm (J in Hz)
1	47.38(s)	—	41.09(s)	—
2	40.79(t)	1.55 (1H, dd, 4.7, 11.5)	40.26(t)	1.58 (1H, m)
		2.14 (1H, m)		2.10 (1H, m)
3	125.46(d)	5.18 (1H, dd, 4.8, 11.5)	124.94(d)	5.20 (1H, dd, 5.0, 11.2)
4	134.52(s)	—	135.66(s)	—
5	38.26(t)	2.11 (1H, m)	39.91(t)	2.25 (2H, m)
		2.20 (1H, m)		
6	24.47(t)	2.07 (1H, m)	24.34(t)	2.15 (2H, m)
		2.28 (1H, m)		
7	128.60(d)	4.83 (1H, br d, 10.4)	130.38(d)	4.90 (1H, br d, 10.1)
8	133.39(s)	—	131.80(s)	—
9	47.90(t)	1.90 (1H, dd, 3.3, 13.5)	38.34(t)	2.21 (2H, m)
		2.20 (1H, m)		
10	122.52(d)	5.45 (1H, br s)	28.07(t)	1.75 (1H, m)
				1.55 (1H, m)
11	154.17(s)	—	41.70(d)	2.80 (1H, br d, 11.2)
12	46.17(d)	2.35 (1H, br d, 10.8)	138.16(s)	—
13	26.22(t)	1.98 (1H, m)	207.11(s)	—
		1.30 (1H, m)		
14	40.03(t)	2.12 (1H, m)	54.91(t)	2.10 (1H, m)
		2.21 (1H, m)		2.33 (1H, m)
15	22.73(q)	1.14 (3H, s)	23.22(q)	1.19 (3H, s)
16	16.16(q)	1.48 (3H, s)	15.57(q)	1.41 (3H, s)
17	15.47(q)	1.60 (3H, s)	16.17(q)	1.61 (3H, s)
18	71.56(s)	—	148.04(s)	—
19	31.90[d](q)	1.35 (3H, s) [d]	24.50(q)	1.79 (3H, s)
20	31.86[d](q)	1.39 (3H, s) [d]	21.42(q)	2.18 (3H, s)

[a] $^{1}HNMR$ spectra were recorded in $CDCl_3$ at 300 MHz. Assignments were aided by COSY spectra and homonuclear spin-decoupling experiments. J values are reported in hertz, and the chemical shifts are given in δ units (parts per million downfield from TMS).

[b] $^{13}CNMR$ spectra were recorded at 75 MHz in $CDCl_3$. Multiplicities were obtained by Attached Proton Test (APT) sequences and assignments were made on the basis of heteronuclear chemical shift correlation methods, comparison to known models and in the case of 3 an INADEQUATE spectrum. The δ values are in parts per million downfield from TMS.

[c] For the original $^{1}HNMR$ and $^{13}CNMR$ data of triene 3 see reference 12.

[d] Signals within a column may be reversed.

proton at C-12 (δ 2.35, br d) revealed that only one set of diastereotopic methylene protons (those at C-13 absorbing at δ 1.98 and δ 1.30) was influenced by the irradiation. Had the alkyl chain been located at C-13 two sets of diastereotopic methylenes would have been affected.

That the new unsaturation encompasses carbon atoms C-10 and C-11 is supported by the absence in palominol of a broad doublet near δ 2.80 which is present in triene **3** due to the methine proton at C-11. Also the low field absorption of the olefin carbon at the bridgehead position (C-11; δ 154.17) is indicative of the double bond being exocyclic to the cyclopentane ring system [17]. Additional information on the relative position of all three trisubstituted double bonds was obtained from permanganate-periodate oxidation of palominol followed by treatment with ethereal diazomethane solution [18]. Formation of both methyl levulinate and methyl acetoacetate is only compatible with structure **2**. The retention times and mass fragmentation patterns of the oxidation products were identical with those obtained from authentic material.

The Me-4 and Me-8 signals in the ^{13}CNMR spectrum (16.16 and 15.47 ppm, respectively; identified by selective decoupling) were at high field which indicated that both trisubstituted double bonds are *trans* with respect to the continuous chain of carbons [19]. To support the above we employed ^{1}HNMR experiments involving nuclear Overhauser enhancement difference spectroscopy **(NOEDS)** [20]. Since no enhancements of the olefin protons were observed when the associated methyl resonances were irradiated, these groups were assigned as **E**.

The relative spatial arrangement between the two substituents at chiral centers C-1 and C-12 was established also by a nuclear Overhauser enhancement difference experiment involving the irradiation of the C-1 substituted methyl group (δ 1.14, s) [21]. This resulted in significant enhancement of the methine proton at C-12 (δ 2.35) and therefore established the *trans* geometry between the bridgehead methyl at C-1 and the isopropyl alcohol side chain at C-12. During the same NOEDS experiment information on the conformation of the 11-membered ring in palominol was obtained. The very rigid 11-membered ring in **2** is in a conformation that places the bridgehead Me and the vinyl protons at C-3 and C-7 in spatial proximity, as revealed by measurement of nuclear Overhauser effect. With this restriction imposed, an examination of molecular models revealed that only the **Z** configuration is possible for the C-10 trisubstituted olefin bond without introduction of unduly angular strain and steric hindrance. Moreover, since no enhancement of the olefin proton at C-10 (δ 5.45, br s) was observed during irradiation of the bridgehead methyl (δ 1.14) the angular methyl and the olefin proton should not be within nOe proximity and therefore the **Z** configuration for the C-10 olefin bond can be assumed.

These results together with the realization that all methylene protons in **2** exist as diastereotopic pairs (see **Table I**) indicated that palominol adopts a rigid crown conformation as shown in Fig. 2. Pioneering studies on related dolabellane-diterpenoids had indicated that the 11-membered ring could not accommodate three endocyclic olefin bonds because of the ring's anticipated high rigidity[8]. Since our studies are clearly inconsistent with this early prediction, palominol (**2**) therefore represents the first dolabellane-derived diterpene with an endocyclic 1,4,8 triene functionality within its 11-membered ring [22].

The brine shrimp assay [23] was used as a simple lethality test to screen for biological activity in palominol. A 0% death response was determined at an initial

concentration of 30 µg/mL after a 24 hour count period. On screening for antimicrobial activity palominol was found to inhibit albeit weakly the growth of Gram negative bacteria (*Escherichia-coli* K-12 and *Pseudomonas aeruginosa*) in a standard agar plate-assay disk method at 0.10mg per disk.

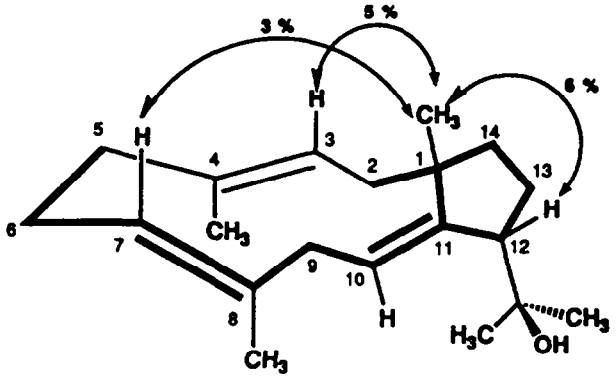

Fig. 2. A perspective drawing of 2 displays the essential conformational features of the molecule as determined from nuclear Overhauser enhancement difference spectroscopy (NOEDS) experiments. Since the absolute configuration of compound 3 has been determined and the two compounds have identical relative configuration at C-1, one can safely assume that they also have the same absolute configuration.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES. Infrared spectra were recorded on a Nicolet 600 FT-IR spectrophotometer. Proton and Carbon-13 spectra were recorded in $CDCl_3$ solution on a General Electric Multinuclear QE-300; all chemical shifts are recorded with respect to TMS (δ 0.0). Reverse phase HPLC was carried out on a Beckman Instrument System Gold chromatograph equipped with Ultraviolet (Model 167) and Refractive Index (Model 156) detectors using a C-18 silica gel column (Altex). Analytical GLC analyses were performed on a Varian 3300 gas chromatograph coupled to a Varian 4290 Integrator using a coiled SE-30, OV-1 capillary column (30m X 0.25mm i.d., Alltech Associates, Inc.).

Collection and Extraction. ***Eunicea calyculata*** was collected by hand using SCUBA at -13 to -15 m depth in February, 1988 along the barrier reef near Punta Arenas, Vieques Island. The collection was stored in plastic bags at O°C for a few hours prior to freezing. Upon workup the animal was homogenized twice in MeOH (1L). After filtration the combined MeOH extracts were evaporated under vacuum to give a residue, which was partitioned between water and hexane. The hexane extract was subsequently filtered, and the filtrate was concentrated *in vacuo* to yield 4.65g of a crude organic extract. Half of the oily residue was dissolved in a small amount of toluene and the resulting concentrate was eluted from a Bio-Beads SX-2 column with toluene. The combined diterpene-containing fractions (TLC guided) were concentrated and the essentially colorless residue was chromatographed successively from a Sephadex LH-20 column with 1:1 MeOH/CH_2Cl_2 and from a silica gel column (40g) with 2% EtOAc in hexane, respectively. Another expedition which led to the collection of a single specimen of ***Eunicea laciniata*** , was made in September, 1988 in Palomino Key,

Puerto Rico. This gorgonian was found in shallower waters (about -7 m) and collected by hand using SCUBA. The animal was stored in MeOH overnight after which an organic extract was produced by simple decantation, filtration and evaporation under vacuum. After repeating the procedure described above twice, the combined MeOH extract was loaded directly on a silica gel column (35g) where it was eluted with 5% EtOAc in hexane. The two major components (2; 0.10g and 3; 0.23g) were further purified by reverse-phase HPLC.

13-Keto-1(S),11(R)-dolabell-3(E),7(E),12(18)-triene (3). Triene 3 was isolated as an oil. Purification by HPLC (Ultrasphere-ODS Silica Gel with 85:15 MeOH/H_2O) gave 141.3 mg from *E. calyculata* and 230 mg from *E. laciniata*. The triene exhibited spectral features identical to authentic material from other sources[12].

18-Hydroxy-1(S),12(S)-dolabell-3(E),7(E),10(Z)-triene (2; palominol). After purification by HPLC (Ultrasphere-ODS Silica Gel with 90:10 MeOH/H_2O) a total of 69 mg and 100 mg were isolated from *E. calyculata* and *E. laciniata* , respectively. The pure alcohol was a semisolid which could not be crystallized from a variety of common organic solvents. Triene 2 showed $[\alpha]_D^{27}$ = -33.3° (c=1, $CHCl_3$) and exhibited the following spectral features: IR (neat film) 3440 (broad, -OH), 2920, 1142,1370, 1151, 1135, 1018, 941, 813 cm^{-1}; HRFABMS: M^+, m/z obsd 288.2454, $C_{20}H_{32}O$ requires 288.2454; ^1H and ^{13}C NMR (see **Table I**).

Permanganate-periodate oxidation of 2. The procedure of Lemieux-von Rudloff was followed[18]. To 2 (75 mg) in t-BuOH was added 45 mL of stock oxidant solution and enough 0.05M K_2CO_3 to give a pH of about 8. After standing overnight (15 h) at room temperature, the mixture was acidified (1M H_2SO_4) and treated with solid sodium metabisulfite until a nearly colorless solution was obtained. The resulting solution was extracted with ether and the dried (Na_2SO_4) extract was treated with excess ethereal diazomethane solution. After evaporation under vacuum the concentrate was injected into a gas chromatograph using a coiled SE-30 column programmed from 50 °C (10 min) to 250 °C (10 °C/min). Under these conditions the retention times of two of the components detected were identical with those of methyl acetoacetate (R_t = 6.86min) and methyl levulinate (R_t = 13.45min) by coinjection with authentic compounds. The mass fragmentation patterns of the oxidation products were also identical with those of authentic material by comparison with literature values.

NOEDS Experiments. The nOe difference spectroscopy experiments were performed as outlined by Hall and Sounders[21]. All samples prepared for **NOEDS** were degassed by bubbling argon through the solution while being kept at 0 °C for 1 hour and then sealed around the cap with parafilm. Solutions were made up in $CDCl_3$ such that, after degassing and a loss of a significant volume of $CDCl_3$, the final concentration was 0.05-0.07M.

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