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A New Diterpenoid Skeleton from the Mediterranean Octocoral *Alcyonium palmatum*: Structure of Palmatol

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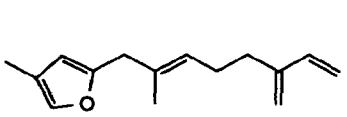
Abstract:. Palmatol (**3**) is a diterpenoid exhibiting an unprecedented prenylbicyclogermacrane skeleton. The structure and the relative stereochemistry, mainly suggested by an extensive NMR study , were supported by some simple chemical transformations.

Diterpenoids displaying a ten membered germacrane framework are very uncommon in nature,[4,5] where they were mainly found in marine organisms such as brown algae and octocorals. The rarest prenylbicyclogermacrane skeleton such as that of dilopholone (**2**) was found in the brown alga *Dilophus prolificans* .[6] We will now report the chemical study of the Mediterranean *Alcyonium palmatum* that contains, together with the already described furanosesquiterpenoid **1**,[7] a diterpenoid (**3**) with a new prenylbicyclogermacrane structure.

In July 1991, *A. palmatum* was collected off the coast of Mazara del Vallo (West Sicily) by dredging. The TLC (Si-gel; *n*-hexane/ethyl acetate, 8:2) analysis of the diethyl ether soluble fraction (5.56 g) from the acetone extract of *Alcyonium palmatum* (287 g of dried weight) revealed the presence of a compound (*Rf*=0.55) slightly less polar than the sterols (*Rf*= 0.45). The column chromatographic separation led to an abundant (69 mg) crystalline (m.p. 93-94°C; from *n*-hexane) optical active ($[\alpha]_D^{20}$ -94.7° c = 2.5, $CHCl_3$) product, named palmatol (**3**). The elemental composition ($C_{20}H_{34}O$) was suggested by combining the HREIMS at *m/z* 272.2507 ($M^+ - H_2O$, $C_{20}H_{32}$ requires 272. 2504) with both IR (liquid film; band at 3250 cm^{-1}) and NMR data. The bicyclic nature of the diterpenoid was deduced by obtaining through catalytic reduction (ethyl acetate, Pt/C 10% ; 1 atm; 20h) a couple of tetrahydroderivatives [8] split by HPLC (μ-porasil; *n*-hexane/ethyl acetate, 95:5). The ^{1}H-NMR ($CDCl_3$) of **3** displayed almost all the signals well resolved. In particular, two downshifted resonances at δ 5.09 and δ 5.22 were observed. The broad triplet at δ 5.09 (J = 7.1 Hz) was assigned to the olefinic proton (H-3) of an isolated 2-methyl-2-pentenyl fragment, such as **a**. In fact, H-3 was coupled allylically with two methyls (H_3-1, δ 1.67 and H_3-17, δ 1.61) and directly with a methylene (H_2-4, δ 2.06, 1.96) that in turn was linked to another methylene (H_2-5, δ 1.24, 1.11). The signal at δ 5.22 (H-10, bd J = 10.9 Hz) was the key to



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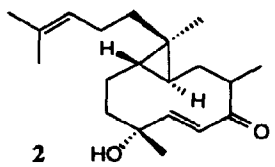

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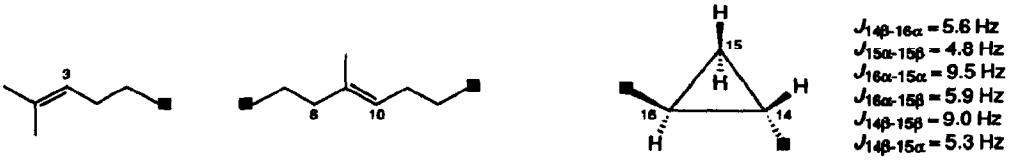


a b c

the construction of the partial isolated structure **b**. In fact, H-10 was coupled allylically with both a methyl (H_3-19, δ 1.67) and a methylene (H_2-8 , δ 1.96, 2.30) in turn linked to another methylene (H_2-7, δ 1.78, 1.35). The direct couplings of H-10 with H_2-11 (δ 2.22, 2.06) and those between H_2-11 and H_2-12 completed the fragment **b**. Of course, the two partial structures **a** and **b** were supported by ^{1}H-^{1}H decoupling experiments and ^{1}H-^{1}H COSY cross peaks. The ^{1}H-NMR spectrum was completed by a series of highshifted signals: two singlets (H_3-20, δ 0.74; H_3-18, δ 0.58) and four multiplets (H-16, δ 0.55; H-14, δ 0.96; H_2-15, δ 0.38 and δ 0.24), that were assigned to the protons of an isolated disubstituted cyclopropane ring, partial structure **c**. Bearing in mind that the ^{13}C-NMR shows only two signals (C-6, δ 35.4; C-13, δ 73.7) attributable to quaternary carbons, the three partial structures (**a**, **b**, **c**) and the two methyls have to be connected through these two carbons, the chemical shift of one of which (δ 73.7) is coherent with the presence of an -OH substituent. Among the possible structures, **3** was supported by the diagnostic dehydroderivative **4** obtained in high yields by trying to acetylate **3** with $(CH_3CO)_2O$ and pyridine. The presence of the exomethylene (H_2-20, δ 4.57 and 4.18) downshifted the H_2-12 protons (δ 2.45 and 2.13) of **4** that were, however, connected to H-10, through H_2-11, further confirming the suggested sequence of protons and carbons. All the ^{1}H and ^{13}C-NMR resonances of both **3** and **4** were assigned by a series of 1D and 2D NMR experiments (Table 1). In particular a HMBC experiment displayed diagnostic cross-peaks between C-14 (δ 31.0) and H_3-20 (δ 0.74) and between C-16 (δ 27.7) and H_3-18 (δ 0.58). The *E* stereochemistry of the double bond between C-9 and C-10 was supported by the ^{13}C-NMR chemical shifts of C-19 (δ 16.4) and C-11 (δ 25.0).The *trans*- fused stereochemistry

3 4

of the cyclopropane ring was suggested by a careful analysis of the coupling constants of the cyclopropane protons (partial structure **c**). In particular the small coupling (5.6 Hz) between the protons H-14 and H-16 (**c**) supported their *trans* relationship.[9] The relative stereochemistry at C-13 was suggested by recording a series of ^{1}H-NMR spectra of **3** in the presence of the shift reagent Eu $(fod)_3$ with a molar concentration in respect to **3** from 0.1 to 1. The induced shifts (Table 2) were more conspicuous for the two protons at C-12 followed in decreasing order, by H-14, H-20, H-15α, H-16 and H-15β. The comparable shifts recorded for the protons at C-12 and C-14 can be better justified by an equatorial orientation of the hydroxy group at C-20. However, more complete relative stereochemical details were obtained recording a series of NOE experiments (Table 3). Most likely the more stable conformation of **3** in $CDCl_3$ is that one reported in Fig.1, that was suggested by all the observed enhancements (Table 3). In particular, the effects obtained irradiating H-10 and H_3-20 were highly diagnostic. Irradiation at δ 5.22 (H-10) revealed steric interactions with H-8β, H_3-18, H-11β, H-14. On

Table 1: NMR data [a] for compounds **3** and **4**.

	3			**4**	
	δ ^{1}H (m, *J* Hz)	δ ^{13}C (m)	long-range connectivities[b]	δ ^{1}H (m, *J* Hz)	δ ^{13}C (m)
1	1.67 (s)	25.9 (q)		1.68 (s)	25.9(q)
2	-	131.1 (s)	H-1; H$_3$-17; H-4	-	131.0(s)
3	5.09 (bt; *7.1*)	125.5 (d)	H-4; H-1; H$_3$-17	5.10 (bt; *6.9*)	125.6(d)
4	2.06 (m) 1.96 (m)	22.5 (t)	H-3	2.05 (m) 1.96 (m)	22.5(t)
5	1.24 (ddd; *13.0, 12.0, 5.1*) 1.11 (ddd; *13.0, 12.3, 5.3*)	46.7 (t)	H-16 and/ or H$_3$-18	1.25 (ddd;*12.8, 12.1, 5.2*) 1.15 (ddd;*12.8, 12.3, 5.2*)	46.0 (t)
6	-	35.4 (s)		-	35.9 (s)
7	1.78 (m) α 1.35 (m) β	37.0 (t)		1.87 (dd; *13.7, 12.9*) 1.37 (m)	37.7 (t)
8	1.96 (m) α 2.30 (ddd; *13.4, 13.2, 2.5*) β	36.2(t)		2.05 (m) 2.30 (m)	36.6 (t)
9	-	133.1 (s)	H$_3$-19; H-8β	-	136.2 (s)
10	5.22 (bd; *10.9*)	127.2 (d)	H-8β ; H12α ; H$_3$-19	5.36 (bd; *12.1*)	126.1 (d)
11	2.22 (dddd; *14.1, 12.6, 11.8, 4.1*) α 2.06 (m) β	25.0 (t)		2.33 (m) 2.10 (m)	25.0 (t)
12	1.86 (ddd; *13.2, 4.1, 3.8*) α 1.81 (ddd; *13.2, 12.6, 4.1*) β	43.9 (t)	H-11α ; H$_3$-20	2.45 (m) 2.13 (dd; *12.1, 4.7*)	40.5 (t)
13	-	73.7 (s)	H-12; H$_3$-20	-	154.1 (s)
14	0.96 (ddd; *9.0, 5.6, 5.3*)	31.0 (d)	H-12α ; H$_3$-20	0.95 (ddd; *8.2, 5.2, 5.0*)	35.6 (d)
15	0.38 (ddd; *9.5, 5.3, 4.8*) α 0.24 (ddd; *9.0, 5.9, 4.8*) β	5.4 (t)		0.58 (m) 0.52 (m)	12.9 (t)
16	0.55 (ddd; *9.5, 5.9, 5.6*)	27.7 (d)	H$_3$-18; H-15β	0.65 (ddd; *9.4, 5.0, 4.5*)	30.5 (d)
17	1.61 (s)	17.8 (q)	H-1; H-3	1.62 (s)	17.7 (q)
18	0.58 (s)	19.4 (q)		0.58 (s)	18.5 (q)
19	1.67 (s)	16.4 (q)	H-10; H-8β ; H-7	1.59 (s)	15.9 (q)
20	0.74 (s)	20.6 (q)		4.57 (s) 4.18 (s)	103.0 (t)

(a)Bruker 500 AMX; $CDCl_3$; chemical shifts referred to $CHCl_3$ at 7.26 ppm and to $CDCl_3$ at 77.0 ppm. (b) from HMBC ;10 Hz.

the contrary, positive enhancements were induced on H-15α, H-16, H-12α, H-11α, H$_3$-19 by irradiating at δ 0.74 (H$_3$-20). In the suggested conformation, the plane of the double bond between C-9 and C-10 is perpendicular to that ideally constructed through the ten membered ring.

Even though many diterpenoid skeleta have been recovered from marine octocorals,[10] molecules closely related to **3** are unknown. From a biosynthetic point of view, the carbon skeleton of **3** could be related to other skeleta found in marine organisms, such as dolabellane and prenylbicyclogermacrane. Formally, all the three

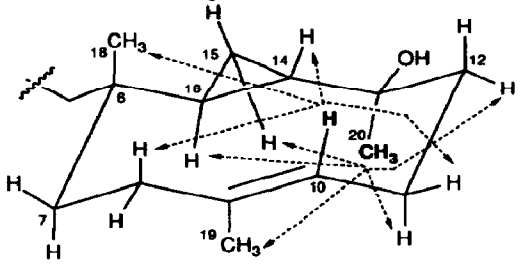
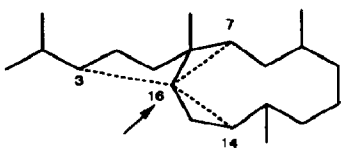


Figure 1. Selected NOEs for compound **3**



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skeleta could derive from a precursor, like **5**, through cyclization involving C-16 and C-3 (dolabellane),[11] C-16 and C-7 (dilopholone), C-16 and C-14 (palmatol).

The biological role of palmatol has to be investigated. Preliminary experiments have shown for palmatol (**3**) toxicity in the test performed with *Gambusia affinis* [12] at a concentration of 10 ppm and in the bioassay with the brine shrimp, *Artemia salina* [13] (LC_{50} 6.42 μg/ml).

Table 2. Selected shifts (Δδ) observed in ^{1}H-NMR [a] spectra of **3** after addition of Eu (fod)$_3$

Proton	0.1[b]	0.2[b]	0.4[b]	0.6[b]	0.8[b]	1.0[b]
11 α	0.30	0.60	1.02	1.56	1.89	2.10
11 β	0.20	0.41	0.71	1.05	1.27	1.42
12 α	0.92	1.91	3.24	4.94	6.04	6.72
12 β	0.78	1.65	2.80	4.28	5.20	5.78
14	0.66	1.38	2.32	3.57	4.35	4.84
15 α	0.54	1.10	1.93	2.89	3.52	3.94
15 β	0.32	0.64	1.10	1.67	2.05	2.28
16	0.37	0.76	1.27	1.90	2.36	2.64
20	0.59	1.23	2.08	3.18	3.87	4.31

a) Bruker 500 MHz; $CDCl_3$

b) Molar ratio Eu (fod)$_3$ / palmatol

Table 3. NOEs observed in compound **3**[a]

Irradiated proton	NOE (%)
H-7β	H-8β (3.6); H_3-18 (0.7)
H-8β	H-8α (22.8); H-10 (9.3); H_3-18 (2.3)
H-10	H-8β (6.5); H-11β (3.9); H-14 (2.3); H_3-18 (1.7)
H-11α	H-11β (13.6); H_3-19 (8.9); H_3-20 (3.2)
H-12α	H_3-20 (1.5)
H-14	H-10 (2.8); H-12β (4.2); H_3-18 (4.6);H-15β(4.4)
H-15α	H-15β (17.4); H_3-20 (5.3)
H-15β	H-15α(16.4); H-14 (6.1); H-5 (3.3)
H-16	H-7α (4.9) ; H_3-19 (4.0); H_3-20 (4.3)
H_3-18	H-14 (3.0); H-8β (1.1); H-15β (0.7)
H_3-20	H-15α (2.3); H-16 (3.6); H_3-19 (1.6); H-11α (1.6); H-12α (1.0)

a) Varian 400 MHz; $CDCl_3$

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