The Structure of Palominol by Interpretation of Two-Dimensional nmr Shift Correlation Experiments: Isolation and Structure Determination of Isopalominol

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THE STRUCTURE OF PALOMINOL BY INTERPRETATION OF TWO-DIMENSIONAL NMR SHIFT CORRELATION EXPERIMENTS: ISOLATION AND STRUCTURE DETERMINATION OF ISOPALOMINOL[1]

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ABSTRACT.—The dolabellane diterpenoid palominol [**1**] was reisolated from the Caribbean gorgonian *Eunicea laciniata* and subjected to a total structural assignment through the application of several 2D nmr techniques that included COSY, ROESY, INADEQUATE, HMQC, and proton-detected long-range heteronuclear chemical shift correlation (HMBC). The revised structure of palominol proposed by Shin and Fenical was rigorously confirmed by these methods. The unequivocal assignments of the ^{1}H- and ^{13}C-nmr spectra of palominol are reported. A new diterpenoid, isopalominol [**5**], also of the dolabellane class, has been isolated from the same specimen of *Eu. laciniata* and its structure defined by combined spectral and chemical methods.

In 1990, we reported a chemical investigation of the Caribbean gorgonian octocorals *Eunicea calyculata* and *Eunicea laciniata* Duchassaing and Michelotti (family Plexauridae), in which we proposed structure **2** for the dolabellane diterpenoid palominol by interpretation of spectral (300 MHz ^{1}H nmr and 75 MHz ^{13}C nmr) and chemical degradation data (1). Recently, Shin and Fenical (2) isolated several diterpenoids of the dolabellane class from the Caribbean gorgonian *Eu. laciniata*, one of which exhibited ^{1}H- and ^{13}C-nmr spectral data nearly identical with those reported for palominol. This led the Scripps workers to suspect the identity of the two compounds but also to question the original structural assignment after a series of chemical correlation studies. After reviewing our original spectra, we concurred with their assessment and agreed that the structure of palominol should be revised to **1**. Because *Eu. laciniata* is one of the least intensively studied of the Caribbean *Eunicea* species and has been shown to contain many structurally important diterpenoids of the dolabellane class, we felt it important to reisolate palominol and to demonstrate unequivocally its structure using only nmr spectroscopic techniques. Thus, we describe the results of a concerted 2D nmr study of palominol that has confirmed the structure of the molecule as **1**, providing, in the process, the unequivocal total assignment of the ^{1}H- and ^{13}C-nmr spectra of the molecule (Table 1).

STRUCTURE OF PALOMINOL.—In reassessing the structure of palominol, it is necessary to establish unequivocally the structure of the cyclopentene skeletal moiety proposed by Fenical and co-workers (2) which is the major structural difference in **1** from the structure **2** we proposed. The long-range $^{2}J_{CH}$ and $^{3}J_{CH}$ couplings observed in the HMBC spectrum of palominol, which were particularly illuminating with regard to the carbon atoms forming the cyclopentene ring (C-1 and C-11 through C-14), are summarized in Table 1. Among the responses observed in the HMBC were the $^{2}J_{CH}$ couplings to the anisochronous C-14 methylene protons. The assignment of these protons, a convenient entry point

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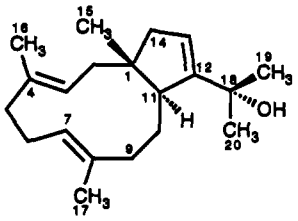

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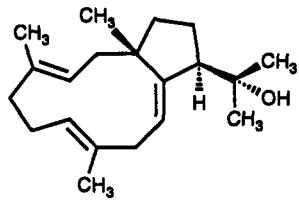

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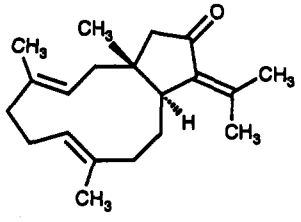

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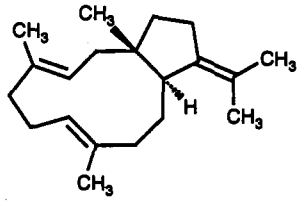

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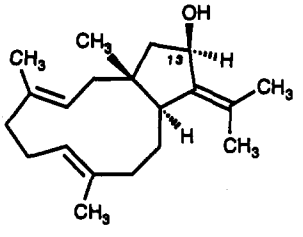

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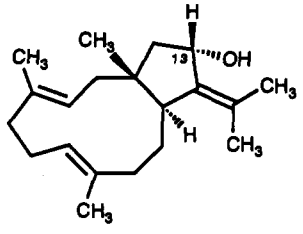

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into the cyclopentene ring resonances, is available from the HC-COSY spectra and from chemical shift arguments. The HMQC spectrum allowed the association of the protons resonating at 2.26 and 1.91 ppm (H-14αβ) with the carbon resonating at 47.74 (C-14) ppm. In particular, correlations were observed to both C-14 methylene protons and carbons C-1, C-11, C-12, and C-13. The latter couplings allowed the assignment of the C-12 and C-13 resonances and confirmed the assignment of the H-11, H-13, and H-14αβ protons. Extending our consideration to the long-range couplings to the C-1 quaternary carbon resonance, we observed that it also exhibits couplings to the H-2αβ, H-10αβ, H-11, H-13, and H-15 protons. In addition, the C-10 carbon resonance was correlated with the H-9αβ protons and the C-11 methine, which in turn correlated with C-12, C-13, and C-14. Additional key correlations within the cyclopentene ring in palominol are denoted by arrows in Figure 1A.

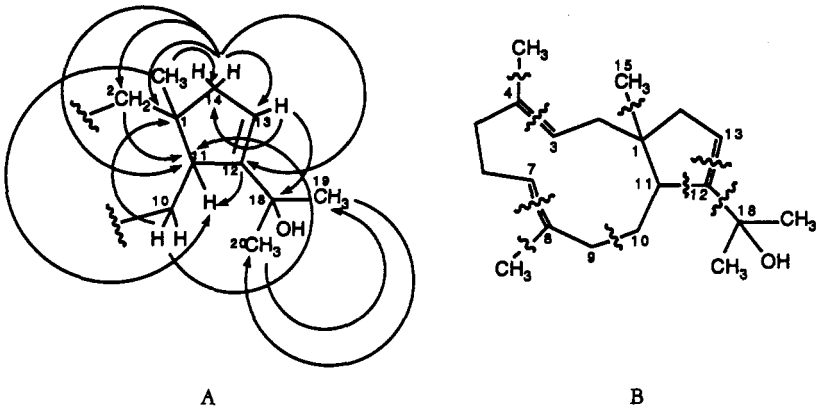
Partial confirmation of the carbon connectivity network within the cyclopentene ring already established from the HMBC experiment was obtained directly from ^{13}C-^{13}C couplings. Since sample size was a limitation, many carbon connectivities across the dolabellane skeleton were not visible in the INADEQUATE experiment. The connectivity networks which helped to identify the dolabellane carbon skeleton in palominol [**1**] are illustrated in Figure 1B. These correlations effectively established the structure of the cyclopentene skeletal moiety in **1** and position it within the molecular framework in a manner consistent with the revised structure of palominol proposed by Shin and Fenical (2). Moreover, the combined use of 2D

TABLE 1. Summary of the Nmr Spectral Data of Palominol [1].[a]

Positon	δ_H (J in Hz)	δ_C ppm (mult)[b]	HMBC (^{1}H)	ROESY
1	—	47.30 (s)	H-2αβ, H-10αβ, H-11, H-13, H-14αβ, H-15	—
2α	2.18, m, 1H	40.63 (t)	H-3, H-11, H-14αβ, H-15	H-2β, H-11
2β	1.54, dd, 1H, (5.0, 12.8)	—	—	H-2α, H-14β, H-15
3	5.19, dd, 1H, (4.6, 11.7)	125.34 (d)	H-2αβ, H-5αβ, H-16	H-5β, H-7, H-9β, H-15
4	—	134.49 (s)	H-2αβ, H-5αβ, H-6α, H-16	—
5α	2.09, m, 1H	39.93 (t)	H-3, H-6α, H-16	H-5β
5β	2.19, m, 1H	—	—	H-3, H-5α
6α	2.30, m, 1H	24.34 (t)	H-5αβ, H-7	H-6β, H-11
6β	2.04, m, 1H	—	—	H-6α
7	4.84, br d, 1H, (11.3)	128.53 (d)	H-5αβ, H-6α, H-9αβ, H-17	H-3, H-9β, H-15
8	—	133.29 (s)	H-6α, H-9αβ, H-17	—
9α	2.08, m, 1H	38.06 (t)	H-7, H-11, H-17	H-9β
9β	2.22, m, 1H	—	—	H-3, H-7, H-9α, H-15
10α	1.99, m, 1H	26.05 (t)	H-9αβ, H-11	H-10β
10β	1.38, m, 1H	—	—	H-10α, H-15
11	2.36, br d, 1H, (11.0)	45.97 (d)	H-2αβ, H-9αβ, H-10αβ, H-14αβ, H-15	H-2α, H-6α, H-16, H-17, H-20
12	—	153.91 (s)	H-11, H-14αβ, H-19, H-20	—
13	5.46, br t, 1H, (1.2)	122.55 (d)	H-11, H-14αβ	H-14αβ, H-19
14α	2.26, m, 1H	47.74 (t)	H-2αβ, H-11, H-13, H-15	H-13, H-14β
14β	1.91, dd, 1H, (3.2, 16.5)	—	—	H-2β, H-13, H-14α, H-15
15	1.15, s, 3H	22.62 (q)	H-2α, H-14α	H-2β, H-3, H-7, H-9β, H-10β, H-14β
16	1.49, s, 3H	16.11 (q)	H-3, H-5β	H-11
17	1.61, s, 3H	15.39 (q)	H-7, H-9α	H-11
18	—	71.50 (s)	H-13	—
19	1.40, s, 3H	31.72 (q)	H-20	H-13
20	1.36 s, 3H	31.72 (q)	H-19	H-11

[a]Spectra were recorded at room temperature in $CDCl_3$ solutions using a proton observation frequency of 500.11 MHz and a carbon observation frequency of 125.76 MHz.

[b]Resonance multiplicities were determined using the APT experiment.



A B

FIGURE 1. (A) In conjunction with the connectivity information provided by the COSY, INADEQUATE and HMQC spectra, we may deduce and assemble the cyclopentene skeletal moiety of palominol [**1**] from its proton-detected long-range heteronuclear chemical shift correlation (HMBC) spectrum. Some of the key coupling pathways are shown by arrows linking a given carbon atom with protons which are located two or three bonds away. (B) Partly due to a limited sample supply, the 2D INADEQUATE spectrum of **1** showed the cross peaks of only twelve pairs of carbons. The correlations across the double bonds were of low intensity and were not visible in the plot.

homo- and hetero-nuclear chemical shift correlation spectroscopy allowed the unambiguous and complete assignment of the proton and carbon chemical shifts of palominol [**1**] (Table 1).

From the ROESY experiment we could confirm that the 11-membered ring in **1** adopts a rigid crown conformation as shown in Figure 2. This contention is in accordance with our previous study concerning the rigidity of the dolabellane skeleton of palominol (1). The ROESY spectrum exhibited the presence of ROEs, as reported in Table 1, indicating that the bridgehead methyl at C-1 and the two olefin protons H-3 and H-7 have a β orientation, while the 4-Me, 8-Me, and H-11 are found to be within ROE proximity on the opposite, α, face of the molecule. Thus the spatial arrangement of both methyls in the 11-membered ring and the bridgehead proton on the α

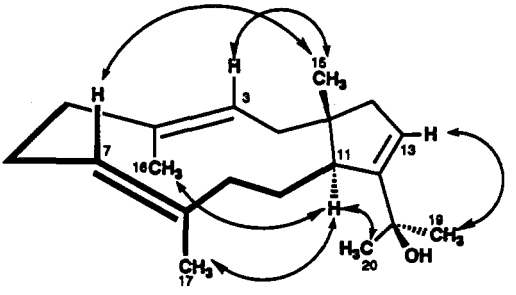


FIGURE 2. A revised perspective drawing of palominol [**1**] showing the stereochemical orientation of the protons as deduced from the phase-sensitive ROESY experiment described. The ROESY spectrum of **1** was recorded at 25° in $CDCl_3$ solution at 500.11 MHz using a spin lock time set to 500 msec.

face of the molecule were clearly established. The presence of ROE between H-11 and one of the two methyls (δ 1.36) of the isopropyl alcohol moiety allowed the unequivocal assignment of the latter groups at the 19 and 20 positions. Their assignment was also supported by a strong ROE between H-13 and the methyl protons at position 19 (δ 1.40). The stereochemical orientation of all the protons directly connected to the rigid carbobicyclic array of palominol [**1**] was also established unequivocally from the ROESY spectrum (Table 1).

STRUCTURE OF ISOPALOMINOL [**5**].—Isopalominol [**5**] was isolated as a white solid, mp 117.3–119.0° (30.0 mg, 0.009% yield) that was analyzed for $C_{20}H_{32}O$ by hreims and ^{13}C-nmr spectral methods. Comparison of nmr data showed clear similarities between metabolites **3** (1,3) and **5**. Analyses of 2D nmr data revealed that isopalominol possessed an 11-membered and a five-membered ring with substitution identical to that in dalabellane **3**. However, there were several significant differences that indicated the presence of a new functional group in **5**. Carbon signals at δ 207.11 (s), 148.04 (s), and 138.16 (s), which corresponded to an enone functionality in **3**, were shifted to δ 145.46 (s), 129.06 (s), and 71.81 (d) in isopalominol [**5**]. In the ^1H-nmr spectrum, two methyl signals at δ 2.18 (br s) and 1.79 (br s), assigned in **3** as vinyl methyls at the terminal carbon of the conjugated exocyclic enone (C-19 and C-20), were shifted in **5** to δ 1.77 (br s) and 1.64 (br s). In addition, a new low-field signal appeared near δ 4.62 (br t, 1H, J=6.5 Hz). This proton showed couplings to upfield resonances at δ 1.96 and 1.69 (H-14αβ). Finally, the carbonyl absorption at 1700 cm^{-1} in the ir spectrum of **3** was replaced by a broad hydroxyl absorption at 3390 cm^{-1} in **5**. All of these changes could be accommodated by the reduction of the enone carbonyl function to form a secondary alcohol at C-13. To confirm this interpretation, compound **3** was chemically converted to isopalominol [**5**]. Reduction with $LiAlH_4$ in Et_2O at 0° gave a 1:1 mixture of the epimeric alcohols **5** and **6** in high yield. Separation of the alcohols by Si gel cc [hexane-EtOAc (97:3)] gave isopalominol, whose spectral data were identical with those of the natural product. At this point it should be noted that alcohols **5** and **6**, with unspecified stereochemistry at C-13, had been synthesized earlier by Shin and Fenical upon reduction of **3** with DIBALH (2). Prior to our study, however, isopalominol had never been reported as a natural product.

The stereochemical orientation of the 13-OH substituent, the sole major structural feature which remained to be determined in isopalominol [**5**], was obtained via a NOESY experiment. Given the orientation of the Me-15 group as a starting point, which, as in all other dolabellanes, is always β, we could establish the orientation of H-14α as the proton resonating at 1.96 ppm because it failed to exhibit an nOe response correlating it with Me-15. Hence, H-14β was assigned as the proton resonating at 1.69 ppm, which in turn showed an nOe response to the Me-15 protons (4). Since a strong nOe response was observed which correlated the H-14α and H-13 resonances, the 13-OH substituent must be in the β orientation.

We also obtained the NOESY spectrum of the unnatural epimer **6**. A strong nOe response between the Me-15 protons and the H-14 proton resonating at 1.85 ppm established the orientation of the latter resonance in the β orientation. A similarly intense nOe response was observed between the H-14β resonance and H-13, which established the orientation of the latter resonance in the β orientation. These nOe responses resolved the questions regarding the stereochemical orientation of H-13 in both isopalominol [**5**] and its unnatural epimer **6**. The proposed β orientation for the 13-OH substituent in isopalominol is also in agreement with the observation that the H-14β proton, which lies on the same (β)

face of the cyclopentane skeletal moiety as the 13-OH substituent, resonates at higher field than H-14α which lies on the opposite (α) face. These trends, which have been observed in other structurally similar systems (5), are reversed in epimer **6**. Thus, isopalominol, with its relative configuration as shown, was fully defined as 13(*R**)-hydroxy-(1*R**,11*S**)-dolabella-3(*E*),7(*E*),12(18)-triene [**5**].

Prompted by our continuing program of biological testing, we also undertook a reinvestigation of the bioactivities of some of the dolabellane diterpenes isolated from this specimen of *Eu. laciniata*. None of the dolabellane diterpenes **1**, **3**, **5**, nor the unnatural epimer **6**, was active against *Escherichia coli* or *Staphylococcus aureus* at doses of 10, 5, and 1 μg of test compound per disc. On the other hand, the present dolabellanes displayed weak antitumor activity against the human colon (HCT 116) cell line. The cytotoxic activities of these compounds were as follows: palominol [**1**] (IC_{50}=10 μg/ml), enone **3** (IC_{50}=10 μg/ml), isopalominol [**5**] (IC_{50}=30 μg/ml), and epimer **6** (IC_{50}=9 μg/ml).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra and Si gel chromatography were performed according to literature methods (6).

COLLECTION AND EXTRACTION OF *EU. LACINIATA*.—The Caribbean gorgonian *Eu. laciniata* was recollected by hand using SCUBA at depths of 15–20 m in November 1992 from Mona Island, Puerto Rico. The gorgonian was freeze-dried and kept frozen until extraction. A voucher specimen is stored at the Chemistry Department of the University of Puerto Rico. The dried animal (302.8 g) was extracted as before (1). The less polar portion of the lipids was fractionated roughly into twelve fractions on the basis of tlc analyses. Fraction 2 (185 mg, 0.06%) consisted of hydrocarbon **4**, identified by 2D nmr techniques, uv, ir, ^{1}H and ^{13}C nmr, and mass spectra (2). Repeated cc of fraction 4 (1.90 g) on Si gel (70 g) with 1% EtOAc in hexane gave **3** (ca. 1.718 g, 0.57%), also identified by 2D nmr techniques, uv, ir, ^{1}H and ^{13}C nmr and mass spectra (1,3). Fraction 6 (80 mg) was identified as palominol [**1**] by comparison of the ir, ^{1}H nmr, ^{13}C nmr, and mass spectral data of the oil with values reported elsewhere (1,2), and fraction 12 (30 mg) consisted of pure isopalominol [**5**].

13(R)-Hydroxy-(1R*,11S*)-dolabella-3(E),7(E),12(18)-triene (isopalominol)* [**5**].—White solid: mp 117.3–119.0°; ir (neat) 3390, 2976, 2962, 2918, 2852, 1441, 1382, 1372, 1261, 1098, 1065, 1025, 893, 864, 841, 819, 801 cm^{-1}; uv (MeOH) no λ max; $[\alpha]^{25}D$ −59.79° (*c*=1.94, $CHCl_3$); hreims *m/z* $[M]^+$ 288.24462 (6.8%) ($C_{20}H_{32}O$ requires 288.24531), 270 (8.6), 255 (6.8), 187 (12.7), 133 (52.9), 121 (68.9), 95 (45.4), 81 (59.1), 69 (100), 55 (58.6); ^{13}C-nmr (75 MHz, $CDCl_3$) δ 145.46 (s, C-12), 134.94 (s, C-4), 132.17 (s, C-8), 129.53 (d, C-7), 129.06 (s, C-18), 125.66 (d, C-3), 71.81 (d, C-13), 51.62 (t, C-14), 46.40 (s, C-1), 42.06 (d, C-11), 40.76 (t, C-2), 39.88 (t, C-5), 38.29 (t, C-9), 29.04 (t, C-10), 24.27 (t, C-6), 23.88 (q, C-15), 21.52 (q, C-20), 20.89 (q, C-19), 15.96 (q, C-17), 15.37 (q, C-16); ^{1}H-nmr (300 MHz, $CDCl_3$) δ 5.16 (dd, 1H, 4.4, 11.4 Hz, H-3), 4.85 (br d, 1H, 10.2 Hz, H-7), 4.62 (t, 1H, 6.5 Hz, H-13), 2.42 (br d, 1H, 11.4 Hz, H-11), 1.96 (m, 1H, H-14α), 1.69 (m, 1H, H-14β), 1.77 (s, 3H, Me-19), 1.65 (s, 3H, Me-20), 1.61 (s, 3H, Me-17), 1.41 (s, 3H, Me-16), 1.16 (s, 3H, Me-15).

REDUCTION OF DOLABELLANE **3**.—A solution of **3** (79.2 mg, 0.27 mmol) in dry Et_2O (10 ml) kept at 0° was treated with $LiAlH_4$ (10 mg), and the resulting mixture was stirred for 30 min. Excess reagent was destroyed by dropwise addition of 0.1 N HCl (5 ml). The Et_2O layer was separated, and the solvent was removed under vacuum to give an oily residue which was chromatographed on a Si gel column (10 g) using hexane-EtOAc (97:3). The white solid, mp 117–119°, that eluted first (28.9 mg) was identified as semisynthetic isopalominol by comparison of the ir, ^{1}H nmr, ^{13}C nmr, and mass spectral data with the values recorded for the natural product. The material eluting last was a colorless semisolid that was identified as epimeric alcohol **6** (32.2 mg).

13(S)-Hydroxy-(1R*,11S*)-dolabella-3(E),7(E),12(18)-triene* [**6**].—Colorless semisolid: ir (neat) 3413, 3052, 2958, 2917, 2850, 1448, 1386, 1373, 1260, 1152, 1110, 1059, 1024, 882, 848, 824, 804 cm^{-1}; uv (MeOH) no λ max; $[\alpha]^{25}D$ −21.82° (*c*=1.92, $CHCl_3$); lreims *m/z* $[M]^+$ 288 (1%), 270 (14), 255 (8), 229 (14), 187 (12), 177 (12), 159 (21), 147 (28), 137 (27), 133 (47), 123 (55), 121 (75), 119 (56), 109 (60), 107 (43), 105 (55), 91 (86), 67 (100), 55 (80); ^{13}C nmr (75 MHz, $CDCl_3$) δ 147.79 (s, C-12), 134.81 (s, C-4), 132.17 (s, C-8), 129.91 (s, C-18), 129.57 (d, C-7), 126.00 (d, C-3), 71.77 (d, C-13), 49.98 (t, C-14), 47.79 (s, C-1), 43.06 (d, C-11), 40.12 (t, C-2), 39.91 (t, C-5), 38.28 (t, C-9), 27.97 (t, C-10), 24.35 (t, C-6), 23.43 (q, C-15), 22.06 (q, C-20), 21.71 (q, C-19),

16.19 (q, C-17), 15.49 (q, C-16); ^{1}H-nmr (300 MHz, $CDCl_3$) δ 5.21 (dd, 1H, 5.4, 10.8 Hz, H-3), 4.87 (br d, 1H, 10.5 Hz, H-7), 4.62 (br d, 1H, 6.6 Hz, H-13), 1.85 (m, 1H, H-14β), 1.68 (m, 1H, H-14α), 1.79 (s, 3H, Me-19), 1.66 (s, 3H, Me-20), 1.61 (s, 3H, Me-17), 1.45 (s, 3H, Me-16), 1.08 (s, 3H, Me-15).

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