Structure and Absolute Configuration of Palmonine F, a New Eunicellin-Based Diterpene from the Gorgonian Eunicella verrucosa

María J. Ortega, Eva Zubía, and Javier Salva

J. Nat. Prod., **1994**, 57 (11), 1584-1586• DOI:
10.1021/np50113a021 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 11, pp. 1584-1586, November 1994

STRUCTURE AND ABSOLUTE CONFIGURATION OF PALMONINE F, A NEW EUNICELLIN-BASED DITERPENE FROM THE GORGONIAN *EUNICELLA VERRUCOSA*

MARÍA J. ORTEGA, EVA ZUBÍA, and JAVIER SALVA*

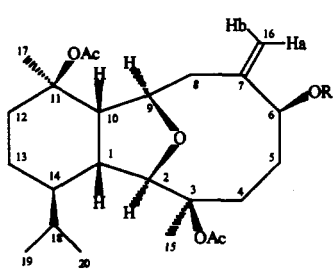
Departamento de Química Orgánica, Facultad de Ciencias del Mar, Universidad de Cádiz, Apdo. 40, Puerto Real, 11510 Cádiz, Spain

ABSTRACT.—In addition to the palmonines reported in a previous communication, a new eunicellin-type diterpene, palmonine F [**1**], has been isolated from the gorgonian *Eunicella verrucosa*. Its structure was elucidated by interpretation of spectral data and chemical interconversions and its absolute configuration was established using the Mosher's method. The cytotoxicity assay results for the palmonines are reported.

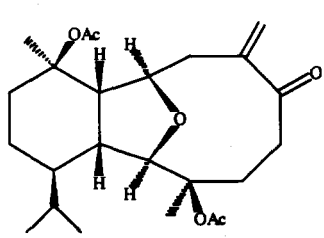
It is well known that marine organisms of the orders Alcyonacea and Gorgonacea are the source of eunicellin-type diterpenes (1–7), characterized as possessing a cladiellane skeleton and a C-2, C-9 ether bridge. Recently Faulkner and co-workers (8) described, from the soft coral *Alcyonium valdivae*, the valdivones which possess the same carbon skeleton but differ in the location of the ether ring and are therefore structurally related to the sarcodictyins isolated from *Sarcodictyon roseum* (order Stolonifera) (9,10).

In a previous communication (5) we reported the isolation and characterization of five new eunicellin-based diterpenoids named palmonines A, B, C, D, and E from the gorgonian *Eunicella verrucosa* Verrill (Gorgonaceae) collected in Palmones at Algeciras Bay on the southern coast of Spain. On further study of the remaining chromatographic fractions of the MeOH extract of *E. verrucosa*, we have encountered the new eunicellin-type diterpene, palmonine F [**1**]. In this paper we describe this new compound, its absolute configuration, and the cytotoxic activities of the palmonines.

Selected fractions from the chromatography of the MeOH-soluble material were purified using normal-phase hplc leading to the isolation of palmonine F [**1**] as a colorless oil (0.0039 dry wt). The molecular formula, $C_{24}H_{38}O_6$, was obtained from a high-resolution ms measurement. The ir absorption at 1730 cm^{-1}, together with the ^{1}H-nmr signals at δ 1.95 (3H, s) and 2.01 (3H, s), indicated that palmonine F [**1**] was a diacetate. The twenty carbons remaining were assigned to an eunicellin-type diterpenoid portion on the basis of spectral features of **1**, that were closely related to those of the



1 R=H
2 R=Ac
1a R=(*R*)-MTPA
1b R=(*S*)-MTPA



3

palmonines (5). In particular, both ^{1}H- and ^{13}C-nmr spectra of palmonine F [**1**] were very similar to those of palmonine B [**2**], except in the absence of one acetoxyl signal and the significant upfield shift of the H-6 signal at δ 4.29 (1H, dd, J=11.2 and 3.7 Hz) in the ^{1}H-nmr spectrum. These data, together with the ir absorption at 3430 cm^{-1}, suggested that palmonine F [**1**] was the 6-deacetyl derivative of palmonine B [**2**]. A series of nOeds experiments confirmed the position of the hydroxyl group on C-6 and defined the relative stereochemistry of **1**. In particular, irradiation of the H-6α signal caused enhancements on the H-8α and Me-15 signals and irradiation of the H-10 signal produced enhancements on the H-1, H-8β, and Me-17 signals, whereas irradiation of H-2 enhanced the H-14 and Me-15 signals, supporting the proposed structure and relative stereochemistry for palmonine F [**1**].

Palmonine F [**1**] was converted into the known compound palmonine B [**2**] upon treatment with Ac_2O in pyridine and into the known ketone palmonine D [**3**] by oxidation with Jones reagent to confirm the proposed structural assignments.

The absolute stereochemistry of several eunicellin-type diterpenes has been elucidated by X-ray, cd, or by Horeau's method (1). The presence of a secondary hydroxyl group in palmonine F [**1**] allowed us to investigate its absolute configuration by application of Mosher's method (11,12). The *(R)*- and *(S)*-MTPA esters [**1a** and **1b**] were prepared by treatment of palmonine F [**1**] with *(S)*- and *(R)*-α-methoxy-α-trifluoromethylphenylacetic chloride, respectively. The Δδ (δ_S-δ_R) values found for H-16a, H-16b, H-9, and H-10 were +0.21, +0.16, +0.01, and +0.03 ppm, respectively, while those obtained for H-5α, H-5β, H-4β, and Me-15 were −0.13, −0.01, −0.06, and −0.01 ppm, respectively. Following the MTPA rules, these data indicated an *S* configuration for C-6 and therefore an absolute stereochemistry for palmonine F as depicted in formula **1**. In addition, the absolute stereochemistry of palmonine B could be inferred as that reported in formula **2** since acetylation of palmonine F yielded a compound identical in all respects (^{1}H nmr, [α]D) with palmonine B [**2**].

In general, the palmonines showed low activity in screens to detect in vitro cytotoxicity against P-388 mice lymphoma, A549 human lung carcinoma, HT29 human colon carcinoma, and MEL28 human melanoma. The ED_{50} values were 10 μg/ml or higher in all cases except for palmonine B [**2**] which was active against P-388 and MEL28 (ED_{50}=5 μg/ml). As a consequence of these results, the palmonines seem to offer one more example of the low potential pharmaceutical activities of compounds from octocorals; the role of secondary metabolites in the class Alcyonaria is probably to mediate interactions with other species (13).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Infrared spectra were recorded on a Perkin-Elmer 881 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. ^{1}H- and ^{13}C-nmr spectra were recorded on a Varian 400 instrument at 400 MHz and 100 MHz, respectively, using $CDCl_3$ as solvent. The resonances of residual $CHCl_3$ at δ_H 7.26 and δ_C 77.0 were used as internal reference for ^{1}H and ^{13}C-nmr spectra, respectively. Asterisks indicate interchangeable signals. Mass spectra were measured on VG 12250 or on Kratos MS 80RFA mass spectrometers. In hplc separations, LiChrosorb silica 60 was used in a normal-phase mode using a differential refractometer. All solvents were distilled from glass prior to use.

COLLECTION, EXTRACTION, AND ISOLATION.—The gorgonian *Eunicella verrucosa* (515.5 g dry wt) was collected in Palmones, Spain, in July 1990, air-dried in the shade, and extracted with MeOH. The MeOH solution was evaporated under reduced pressure and the aqueous residue was extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and the solvent evaporated to give a dark orange oil (8 g), which was chromatographed on a silica column eluted with solvents of increasing polarity from hexane to

Et_2O. Fractions eluted with 80% Et_2O in hexane were further purified by hplc (hexane-EtOAc, 4:6) to obtain palmonine F [**1**] (20 mg, 0.0039% dry wt).

Palmonine F [**1**].—Colorless oil; $[\alpha]^{25}D$ −4.6° (c=0.5, $CHCl_3$); ir (dry film) ν max 3430 (OH), 1730 (ester) cm^{-1}; ^1H nmr ($CDCl_3$, 400 MHz) δ 5.47 (1H, s, H-16a), 5.22 (1H, s, H-16b), 4.29 (1H, dd, J=11.2 and 3.7 Hz, H-6), 4.09 (1H, dd, J=10.6 and 5.0 Hz, H-9), 3.56 (1H, s, H-2), 3.09 (1H, dd, J=10.6 and 7.7 Hz, H-10), 2.82 (1H, dd, J=13.7 and 5.0 Hz, H-8α), 2.48 (1H, d, J=13.7 Hz, H-8β), 2.23 (1H, dd, J=11.4 and 7.7 Hz, H-1), 2.20 (1H, m, H-12), 2.15 (1H, m, H-4α), 2.08 (1H, m, H-5α), 2.01 (3H, s, -OAc), 1.95 (3H, s, -OAc), 1.85 (1H, m, H-4β), 1.84 (1H, m, H-18), 1.73 (1H, m, H-5β), 1.55 (3H, s, H-17), 1.53 (3H, s, H-15), 1.45 (2H, m, H-12 and H-13), 1.36 (1H, m, H-13), 1.20 (1H, m, H-14), 0.95 (3H, d, J=6.8 Hz, H-20)*, 0.78 (3H, d, J=6.8 Hz, H-19)*; ^{13}C nmr ($CDCl_3$, 100 MHz) δ 170.2 (s, CH_3COO-), 170.0 (s, CH_3COO-), 150.2 (s, C-7), 116.8 (t, C-16), 90.4 (d, C-2), 84.8 (s, C-3)*, 82.2 (s, C-11)*, 78.8 (d, C-9), 73.7 (d, C-6), 45.8 (d, C-10), 43.0 (d, C-14), 41.5 (d, C-1), 41.3 (t, C-8), 35.5 (t, C-12), 32.5 (t, C-5), 29.7 (t, C-4), 27.5 (d, C-18), 25.5 (q, C-17), 22.5 (q, CH_3COO-), 22.5 (q, CH_3COO- and C-15), 21.7 (q, C-20), 18.1 (t, C-13), 15.2 (q, C-19); eims (70 eV) m/z $[M-OH]^+$ 405 (3), 362 (8), 302 (19), 43 (100); hrms m/z found 405.2616 $[M-OH]^+$, $C_{24}H_{37}O_5$ requires 405.2640, found m/z 362.2443 $[M-AcOH]^+$, $C_{22}H_{34}O_4$ requires 362.2456.

Acetylation of palmonine F [**1**].—An excess of Ac_2O was added to a solution of **1** (2 mg) in dry pyridine (1.5 ml). The mixture was kept overnight at room temperature and the residual pyridine and Ac_2O were removed by distillation under reduced pressure. The residue was purified on a small Si gel column using hexane-EtOAc (9:1) to obtain palmonine B [**2**] (2 mg).

Oxidation of palmonine F [**1**].—An ice-cooled solution of **1** (2 mg) in Me_2CO (2 ml) was treated with two drops of Jones reagent. After ten minutes, 5 ml of H_2O were added and the mixture was extracted with Et_2O. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated. The residue was purified on a Si gel tlc plate using hexane-EtOAc (8:2) as eluent to afford palmonine D [**3**] (1 mg).

Synthesis of the (R)-*MTPA ester* **1a**.—A solution of **1** (2 mg) in dry pyridine (1 ml) was treated with *(S)*-MTPA chloride (2 μl) and the mixture was stirred at room temperature for 24 h. After evaporation of the solvent under reduced pressure the residue was purified on a Si gel tlc plate to obtain the *(R)*-MTPA ester **1a** (1.5 mg): ^1H nmr ($CDCl_3$, 400 MHz) (selected data) δ 5.35 (1H, dd, J=11.3 and 3.0 Hz, H-6), 5.22 (2H, s, H-16a and H-16b), 4.09 (1H, dd, J=10.7 and 4.9 Hz, H-9), 3.10 (1H, br t, H-10), 2.11 (1H, m, H-5α), 1.93 (1H, m, H-4β), 1.88 (1H, m, H-5β), 1.53 (3H, s, H-15).

Synthesis of the (S)-*MTPA ester* **1b**.—Treatment of **1** (2.7 mg) with *(R)*-MTPA chloride (3 μl) in pyridine as described above yielded the *(S)*-MTPA ester **1b** (2 mg): ^1H nmr ($CDCl_3$, 400 MHz) (selected data) δ 5.43 (1H, s, H-16a), 5.38 (1H, s, H-16b), 5.39 (1H, dd, J=11.1 and 3.0 Hz, H-6), 4.10 (1H, dd, J=10.7 and 4.9 Hz, H-9), 3.13 (1H, br t, H-10), 1.98 (1H, m, H-5α), 1.87 (2H, m, H-4β and H-5β), 1.52 (3H, s, H-15).

ACKNOWLEDGMENTS

The gorgonian was collected and identified by J.C. García-Gómez and G. Mourente. Hrms were measured at Servicio de Espectrometria de Masas, Universidad de Sevilla, by Dra. María Angeles Pradera. Cytotoxicity assays were performed by Dolores García Grávalos, Pharma Mar S.A. This research was supported by a grant from C.I.C.Y.T. (research project SAF94-1383).

LITERATURE CITED

1. D.J. Faulkner, *Nat. Prod. Rep.*, **10**, 497 (1993), and previous reviews in this series.
2. S.J. Bloor, F.J. Schmitz, M.B. Hossain, and D. van der Helm, *J. Org. Chem.*, **57**, 1205 (1992).
3. Y. Uchio, M. Kodama, S. Usui, and Y. Fukazawa, *Tetrahedron Lett.*, **33**, 1317 (1992).
4. M. Ochi, K. Yamada, K. Kataoka, H. Kotsuki, and K. Shibata, *Chem. Lett.*, 155 (1992).
5. M.J. Ortega, E. Zubía, H. He, and J. Salvá, *Tetrahedron*, **49**, 7823 (1993).
6. J. Su, Y. Zheng, L. Zeng, E.O. Pordesimo, F.J. Schmitz, M.B. Hossain, and D. van der Helm, *J. Nat. Prod.*, **56**, 1601 (1993).
7. N.S. Sarma, R. Chavakula, I.N. Rao, R. Kadirvelraj, T.N.G. Row, and I. Saito, *J. Nat. Prod.*, **56**, 1977 (1993).
8. Y. Lin, C.A. Bewley, and D.J. Faulkner, *Tetrahedron*, **49**, 7977 (1993).
9. M. D'Ambrosio, A. Guerriero, and F. Pietra, *Helv. Chim. Acta*, **70**, 2019 (1987).
10. M. D'Ambrosio, A. Guerriero, and F. Pietra, *Helv. Chim. Acta*, **71**, 964 (1988).
11. I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Am. Chem. Soc.*, **113**, 4092 (1991).
12. I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Org. Chem.*, **56**, 1296 (1991).
13. J.C. Coll, *Chem. Rev.*, **92**, 613 (1992).

Received 5 May 1994