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Marine antitumor agents: 14-deoxycrassin and pseudoplexaurol, new cembranoid diterpenes from the Caribbean gorgonian *Pseudoplexaura porosa*

A. D. Rodríguez and N. Martínez

Department of Chemistry, P.O. Box 23346, U.P.R. Station, University of Puerto Rico, Río Piedras, Puerto Rico (Puerto Rico 00931-3346, USA)
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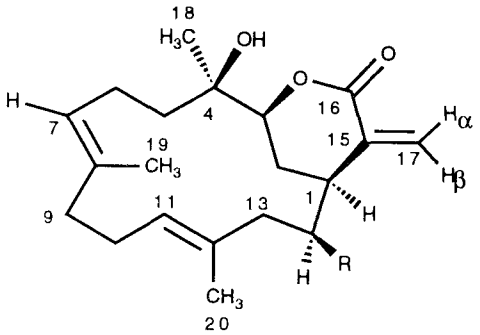
Abstract. Two new cytotoxic antitumor diterpenoids of the cembrane class, named 14-deoxycrassin (**3**) and pseudoplexaurol (**4**), have been isolated from the Caribbean gorgonian octocoral *Pseudoplexaura porosa*. The structure of lactone **3**, possessing the infrequently encountered α-methylene-δ-lactone ring, has been established from spectral and chemical data and that of alcohol **4** has been established from spectral data.
Key words. Gorgonian; antitumor agent; cembranoid diterpene; *Pseudoplexaura porosa*.

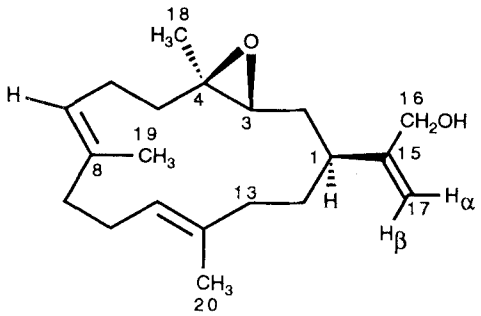
Coral reef gorgonian octocorals (phylum Cnidaria, order Gorgonaceae) have proved to be a rich source of a great variety of organic compounds[1]. Ciereszko was the first to observe that the mucus released from live *Pseudoplexaura porosa* contains diterpenes[2]. Although they are insoluble in water, the diterpenoids secreted in the mucus immediately after breaking the coral branches may play a role in the organism's defense mechanism against predators. For instance, crassin (**1**), a cembrane diterpenoid isolated in 1960 from *P. porosa*, has a remarkable range of biological activities. Its acetate **2**, also found in the same animal, has mild analgesic, antibiotic, and antineoplastic properties and shows in vitro activity against human epidermoid carcinoma of the nasopharynx (KB cell line) at concentrations of 2 μg/ml, while crassin (**1**) itself is nearly twice as potent[3,4]. Both, **1** and **2**, are also active against the PS cell line, and cinnamoyl esters of crassin show significant in vitro antileukemic activity[5]. The wide range of biological activities shown by these secondary metabolites, together with the abundance of *P. porosa* near Puerto Rican waters, prompted us to reinvestigate the secondary metabolite composition of this gorgonian. As part of our survey of pharmacologically active metabolites from Puerto Rican marine organisms, we now report the isolation and structure determination of a new crassin-like diterpenoid, named 14-deoxycrassin (**3**) and of a probable biosynthetic precursor pseudoplexaurol (**4**). Their structures have been established from spectral data in conjunction with chemical degradation data. Both compounds exhibited significant cytotoxic antitumor activity when screened against a panel of five human tumor cell lines at concentrations of 0.005–50 μg/ml.

The hexane-soluble materials isolated from the MeOH-$CHCl_3$ extracts of *P. porosa* collected at Palomino Island off the East coast of Puerto Rico in January 1992 by SCUBA diving, were chromatographed successively on a Bio Beads SX-2 column (toluene) and silica gel column (EtOAc/hexane, 1:4) yielding 14-deoxycrassin (**3**) (122 mg; 0.090% dry wt) as a colorless oil: UV (MeOH) λ max 220 nm (ε 2500); $[\alpha]_D^{26}$ + 29.6° (c 0.24, $CHCl_3$) and crassin acetate (**2**) (727 mg; 0.538% dry wt). 14-Deoxycrassin showed a molecular ion peak at *m/z* 318.21923 in the HREIMS spectrum, corresponding to the molecular composition of $C_{20}H_{30}O_3$ [M^+ requires 318.21948; Δ −0.2 nm]. The IR spectrum[6] showed that it contained a lactone (1720 cm^{-1}) and hydroxy groups (3436 cm^{-1}). The ^{1}H- (300-MHz) and ^{13}C-NMR (75-MHz) data of **3** implied the presence of an infrequently found α-methylene-δ-lactone, a tertiary methyl carbinol and two methyl substituted olefins. The ^{13}C-NMR spectrum in $CDCl_3$ exhibited signals for twenty carbon atoms suggesting a diterpene structure: one carbonyl (167.09), three nonprotonated vinyl carbons (140.08, 134.91 and 131.96), one olefinic methylene (126.79) and two olefinic methine carbons (126.27 and 125.00), two oxygenated carbon atoms: one was a methine carbon (83.16) and one was tertiary (74.00), one sp^3 methine (33.23), seven sp^3 methylenes (39.87, 38.38, 35.93, 31.46, 26.16, 24.02, 22.19) and three methyl carbons (24.61, 14.62 and 13.81). These data were consistent with the formula $C_{20}H_{30}O_3$ for the δ-lactone **3**. Moreover, the ^{13}C-NMR spectrum of 14-deoxycrassin (**3**) showed a striking similarity to that of crassin acetate (**2**); the few differences observed were consistent with the replacement of the acetate ester group at C-14 in **2** with a hydrogen atom in 14-deoxycrassin. These spectral features suggested a close similarity to crassin acetate.

Analysis of the ^{1}H-NMR[7], the ^{1}H-^{1}H COSY[8] and Selective Pulse INEPT NMR[9] spectra of **3** allowed us to establish the chain of coupling in the isolated spin system comprised by the proton sequence H-13 to H-3 around the α-methylene-δ-lactone ring including the more remote exomethylene protons H-17αβ. A peculiar-



1 R= OH
2 R= OAc
3 R= H



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ity of the ^{1}H-NMR spectrum of 14-deoxycrassin is the simple doublet character of the δ 3.96 absorption for H-3 which is vicinal to the C-2 methylene. The rather large coupling constant (10.2 Hz) is attributable to coupling between axial H-3α and axial H-2β. The absence of the second coupling ($J < 1$ Hz) may be attributed to the combined electronegativity effects of vicinal transcoplanar oxygen atoms on the coupling strengths of the H-3α and H-2α protons[10]. The dihedral angles between these protons diminish the coupling strength of each proton, reducing their mutual coupling to less than 1 Hz. These observations are consistent with the structural and stereochemical similarity of 14-deoxycrassin with crassin acetate and other related δ-lactones [i.e. sinularin-(l)][11]. Ozonolysis of **3**, followed by oxidation and methylation, gave methyl levulinate, indicating the presence of a 1,5-diene system. Since in the ^{13}C-NMR spectrum of 14-deoxycrassin (**3**) the vinyl methyl resonances occur upfield of 20 ppm the *E* stereochemistry for both trisubstituted olefins is suggested[12].

Repeated chromatography of a less polar fraction on HPLC [silica gel in EtOAc/hexane, 3:7] afforded a colorless oil which was identified as the minor metabolite pseudoplexaurol (**4**) [24.2 mg, 0.018% dry wt; $[\alpha]_D^{26}$ −21.5° (c 3.4, $CHCl_3$)]. The primary alcohol **4** was shown to have the molecular formula $C_{20}H_{32}O_2$ [304.24054, calcd 304.24022; Δ 0.3 nm] by HREIMS. The IR bands[13] at 3425 and 1061 cm^{-1} suggested the presence of a hydroxy group and strong bands at 1259, 896 and 801 cm^{-1} could be assigned to an epoxy group. The ^{13}C-NMR (75-MHz) spectrum of pseudoplexaurol (**4**) exhibited twenty signals divided by APT into four quaternary carbons (152.46, 135.15, 133.27 and 60.73), four methine carbons (124.38, 123.62, 62.97 and 37.18), nine methylenes (109.23, 64.44, 39.42, 38.26, 34.98, 33.77, 30.10, 24.33 and 23.68) and three methyl groups (17.10, 16.81 and 15.83). When recorded in $CDCl_3$ solution, the ^{1}H-NMR (300-MHz) spectrum contained a proton signal at δ 2.75 (1H, dd, $J = 4$, 9 Hz, H-3) which could be assigned to an α-epoxy proton. The ^{1}H-NMR spectrum contained signals at δ 5.04 (1H, s, H-17α) and 4.83 (1H, s, H-17β) due to a pair of terminal methylene protons, three methyl signals at δ 1.56, 1.53, and 1.18 ppm and a signal at δ 4.09 (2H, s, H-16) due to a hydroxymethyl group. One of the terminal methylene protons was enveloped by two olefinic proton signals at δ 5.04 ppm. A broad envelope of allylic proton signals, integrating as 9H, was found between δ 1.95 and 2.40 ppm. These data were consistent with the formula $C_{20}H_{32}O_2$ and the proposed cembrane skeleton for alcohol **4**.

The ^{1}H-^{1}H COSY spectrum revealed that the two-proton singlet at δ 4.09 (H-16) was coupled to the terminal methylene protons (H-17$\alpha\beta$) at δ 5.04 and 4.83. The ^{1}H-NMR signal at δ 2.25 (1H, m) assigned to the proton on the carbon bearing the 1-hydroxymethylvinyl group was coupled to a pair of diastereotopic protons resonating at δ 1.73 and 1.48 (H-2$\alpha\beta$) which were in turn coupled to the one-proton doublet of doublets at δ 2.75 assigned to the α-epoxy proton H-3. This chain of coupling placed the epoxy ring between C-3 and C-4. This observation was confirmed by the Selective Pulse INEPT NMR spectrum[14] of pseudoplexaurol (**4**). Since we were unable to define unambiguously the relative stereochemistry of the epoxy and 1-hydroxymethylvinyl groups from the spectroscopic data accumulated, we attempted to establish the relative configuration at C-1, C-3 and C-4 indirectly by chemical correlation with **3** upon oxidation under mild conditions (i.e., via careful RuO_4 catalyzed oxidation to the carboxylic acid followed by back-side attack of the carboxylic (C-16) hydroxyl group at C-3 of the epoxide)[15]. This, however, resulted in the destruction of all the starting material available, presumably owing to simultaneous oxidation and cleavage of the carbon-carbon double bonds in **4**. Efforts to establish both the relative and absolute

configuration of **4** unambiguously upon reisolation and chemical derivatization followed by a single crystal X-ray analysis are currently underway.

The new cembranoid diterpenes **3** and **4** were not active against *Pseudomonas aeruginosa*, *Escherichia coli* or *Staphylococcus aureus* at doses of 10, 5, and 1 μg/ml of test compound per disc. On the other hand, both compounds displayed potent antitumor activity when screened against a panel of five human tumor cell lines. The cytotoxic activities of 14-deoxycrassin (**3**) were established on the following cell lines: on human colon (HCT 116), $IC_{50} = 2$ μg/ml, on melanoma (SK5-MEL), $IC_{50} = 0.5$ μg/ml, and on kidney carcinoma (A 498), $IC_{50} = 0.2$ μg/ml. The cytotoxic activities of pseudoplexaurol (**4**) were as follows: on human breast (MCF-7), $IC_{50} = 20$ μg/ml, on colon (HCT 116), $IC_{50} = 10$ μg/ml, and on T cell leukemia (CCRF-CEM), $IC_{50} = 0.15$ μg/ml.

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6 IR(neat) ν_{max} 3436, 2932, 2865, 1720, 1617, 1445, 1378, 1264, 1180, 1132, 1096, 1031, 954, 790 cm^{-1}; LREIMS *m/z* (%) 318(5), 300(4), 275(5), 241(3), 219(3), 193(11), 147(21), 133(26), 121(36), 107(52), 93(60), 81(100), 67(62), 55(80).

7 ^{1}H-NMR (300-MHz, $CDCl_3$) δ 6.40 (1H, d, J = 2.1 Hz, H-17α), 5.59 (1H, d, J = 2.1 Hz, H-17β), 5.09 (1H, m), 5.02 (1H, t, J = 7.5 Hz), 3.96 (1H, d, J = 10.2 Hz), 2.32–1.60 (broad envelope), 1.57 (3H, s), 1.53 (3H, s), 1.35 (3H, s), 1.33–1.10 (broad envelope).

8 The ^{1}H-^{1}H COSY spectrum of **3** showed the following cross peaks (partial list): H-17α/H-17β, H-13/H-14, H-14/H-1, H-1/H-2, H-2β/H-3, H-7/H-6, H-7/Me-19, H-11/H-10, H-11/Me-20.

9 The Selective INEPT spectrum of **3** showed the following $^{2,3}J_{CH}$ correlations (partial list): H-17α/C-16, C-15 and C-1; H-17β/C-16 and C-1; H-7/C-6 and C-19; H-11/C-10 and C-20; H-3/C-1; H-19/C-7 and C-8; H-20/C-11 and C-12; H-18/C-4 and C-3.

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13 IR(neat) ν_{max} 3425, 2961, 2923, 2871, 1650, 1445, 1384, 1259, 1236, 1109, 1095, 1061, 1029, 896, 801 cm^{-1}; LREIMS *m/z* (%) 304(3), 286(1), 273(2), 187(2), 161(7), 159(6), 133(22), 119(27), 107(38), 93(53), 91(42), 81(76), 67(61), 55(100).

14 The Selective INEPT spectrum of **4** showed the following $^{2,3}J_{CH}$ correlations (partial list): H-17αβ/C-1, C-15 and C-16; H-16/C-15, C-17 and C-1; H-3/C-2 and C-5; H-18/C-3, C-4 and C-5.

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