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PALOMININ, A NOVEL FURANOSESTERTERPENE FROM A CARIBBEAN SPONGE *IRCINIA SP.* [1]

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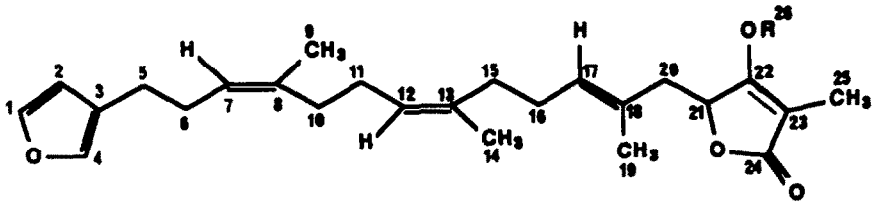
Abstract: A new linear monofuranosesterterpene, palominin, has been isolated from the Caribbean sponge *Ircinia sp.* On the basis of physical and chemical evidence structure 1 is suggested for palominin.

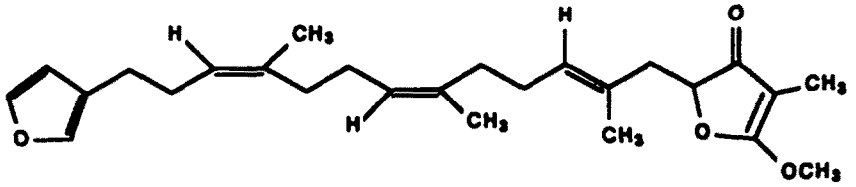
In spite of the fact that sesterterpenes continue to be regarded as a rare group of natural products, their occurrence in marine sponges of the order Dictyoceratida has been known for almost two decades particularly in specimens belonging to the family Thorectidae.[2] Within this latter group is the genus *Ircinia* which stands out because at least nine described species have been recognized as sources of a variety of acyclic sesterterpenes, polyprenyl quinones, carbocyclic furans and lactones.[3] Linear sesterterpenes characterized by a furan ring at one end and by a conjugated tetronic acid at the other, have frequently been encountered in sponges of the genus *Ircinia*. Less common are those examples of this class of compounds in which the tetronic acid moiety is unconjugated.[4] As part of an ongoing effort in search for potential antibiotic agents from marine invertebrates we wish to report the isolation of a new sesterterpene, palominin (1) from an undescribed Caribbean sponge of the genus *Ircinia* . This novel furanoterpene which contains an unconjugated tetronic acid moiety, was found to coexist with variabilin (4) first described from *Ircinia variabilis* [5] and subsequently found in other species of *Ircinia*. [6]

A methanol extract of the sponge was partitioned between hexane and water and the aqueous phase was subsequently extracted with chloroform. Silica gel chromatography of half of the chloroform partition yielded three sesterterpene-containing fractions, the first of which consisted of pure variabilin (4) (identified by its UV, IR, ^{1}HNMR, ^{13}CNMR, and mass spectra). The next fraction, which consisted largely of a mixture of 1 and 4, could not be separated by HPLC and the last fraction consisted of pure palominin (1). Although we managed to secure some spectroscopic data, the peculiar unstable nature of natural product 1 made its structure elucidation impossible.[7] In order to overcome this impediment the second half of the chloroform extract was eluted through a column of Sephadex LH-20 (1:1 MeOH-$CHCl_3$) and the mixture of sesterterpenes was methylated quickly with diazomethane. Upon derivatization we eliminated the shortcomings posed by premature decomposition and also decreased the overall molecular polarity of the natural products. Separation of the methylated derivatives was achieved readily by column chromatography on silica gel followed by reverse-phase HPLC.

The least polar of the methylated derivatives, a stable light-yellow oil with molecular formula $C_{26}H_{36}O_4$ as determined by LRMS and ^{13}CNMR was identified as 22-O-methylpalominin (2). The ^{1}HNMR spectrum displayed resonances consistent with the presence of four olefinic methyls (δ 1.64, s; 1.65, s; 1.94, s; 1.99, s), one methoxy methyl (δ 4.05, s), a furan-3-yl ring (δ 6.24, br s; 7.17, br s; 7.30, br s) and three trisubstituted double bonds (δ 5.23, t; 5.15, t; 5.10, t). This information, together with a comparison of the ^{13}CNMR spectrum of palominin (Table 1) with those of known sesterterpene tetronic acids from *Ircinia spp.* (i.e. 6 and 7) established the structure as shown, with the exception of the position and stereochemistry



1; R = H
2; R = CH_3



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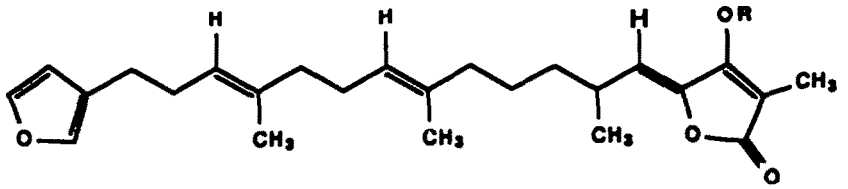
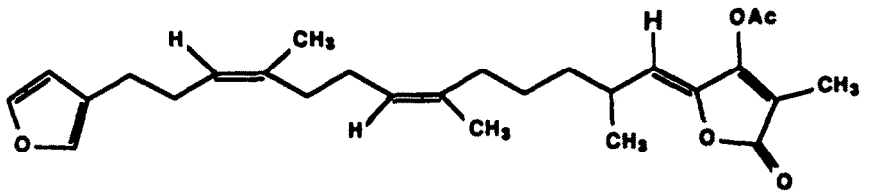


4; R = H
5; R = CH_3



6

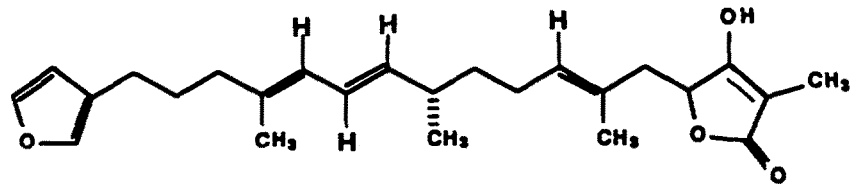


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Table 1. ^{13}C-NMR Chemical Shift Assignments for Compounds (1)-(7) [a].

CARBON	(1) [i]	(2) [i]	(3) [i]	(4)	(5)	(6)	(7)
1	142.5	142.5	142.4	142.3	142.5	142.5	142.5
2	111.1	111.0	110.8	110.9	111.1	111.1	110.9
3	124.9	124.9	124.8	124.8	125.0	125.1	124.9
4	138.8	138.8	138.6	138.6	138.8	138.8	138.7
5	28.4	28.3	28.3	28.2	28.5	28.3	24.4
6	25.3 [c]	25.2 [c]	25.1 [c]	24.8 [c]	25.1 [c]	25.3 [c]	28.2
7	124.7 [d]	124.6 [d]	124.5 [d]	123.5 [d]	123.7 [d]	123.9 [d]	39.3
8	135.7 [e]	135.7 [e]	135.5 [e]	135.5 [e]	135.8 [e]	135.8 [e]	135.7
9	23.3 [h]	23.4 [g]	23.2 [h]	15.6	15.8	23.3 [h]	16.7 [c]
10	32.3 [f]	32.2 [f]	32.1 [f]	39.5 [f]	39.6 [f]	32.3 [f]	125.3
11	26.8 [c]	26.7 [c]	26.6 [c]	26.3 [c]	26.7 [c]	26.3 [c]	125.2
12	125.3 [d]	125.1 [d]	125.1 [d]	124.2 [d]	124.3 [d]	124.7 [d]	137.8
13	135.0 [e]	135.1 [e]	134.9 [e]	134.6 [e]	134.9 [e]	135.2 [e]	36.9
14	23.3 [h]	23.3 [g]	23.2 [h]	15.8	16.0	23.3 [h]	20.8
15	31.6 [f]	31.6 [f]	31.5 [f]	39.4 [f]	39.6 [f]	31.5 [f]	37.1
16	26.3	26.3	26.2	25.5	25.7	25.7 [c]	26.0
17	129.0	128.6	127.9	36.4	36.7	36.8	129.3
18	129.8	129.6	129.9	30.7	30.8	31.1	129.0
19	16.6	16.6	16.2	20.4	20.6	20.6	16.5 [c]
20	41.9	41.9	41.0	116.1	115.2	116.4	41.6
21	77.2	77.1	85.6	142.9	142.7	142.6	78.4
22	176.4 [b]	174.8 [b]	198.1	162.7 [b]	161.9 [b]	168.6 [b]	177.9 [b]
23	97.6	97.4	88.3	98.7	98.8	114.8	96.8
24	174.6 [b]	173.2 [b]	180.3	172.6 [b]	171.1 [b]	154.4 [b]	176.1[b]
25	5.8	8.3	3.7	5.9	8.5	8.3	5.9
26	-	58.6	55.6	-	58.8	165.4 [b]	-

[a] $CDCl_3$ solutions; shift in ppm downfield of TMS.

[b-g] Assignments with the same superscript in the same vertical column may be interchanged.

[h] Overlapping signals.

[i] Carbon-13 multiplicities were determined by an Attached Proton Test (APT) sequence in our NMR facilities.

of the isoprenoid double bonds.[8] Their location within the polyisoprenoid chain was determined by ozonolysis of **2** followed by oxidative work-up, treatment with ethereal diazomethane solution and GLC-MS to obtain dimethyl succinate and methyl levulinate.[9] Each component was shown to be identical (GLC retention time and mass spectrum) to authentic samples.

An useful and unambiguous relationship can be set between the ^{13}CNMR chemical shift of alpha groups (methyl and methylene) to the quaternary carbons of isoprenoid double bonds and their stereochemistry. The ^{13}CNMR chemical shifts of methyl groups on trisubstituted double bonds in isoprenoid chains appear more upfield in **E** double bonds than in **Z** double bonds.[10] Table 1 shows that the isoprenoid vinyl methyls of variabilin (**4**) appear at 15.6 and 15.8 ppm. On the other hand **6**, which differs only from variabilin in its stereochemistry about the isoprenoid double bonds, shows two overlapping quartets at 23.3 ppm. Moreover, the signals at 39.5 and 39.4 ppm assigned to the C-10 and C-15 methylenes in variabilin are shifted upfield to 32.3 and 31.5 ppm in **6**. On the basis of these spectroscopic grounds together with a comparison of the ^{13}CNMR spectrum of palominin with those of **4** and **6**, we conclude that two of the isoprenoid double bonds in palominin must be **Z** and the remaining must have **E** stereochemistry. Finally, of the various stereochemical combinations possible for such polyisoprenoid triene, we chose the one depicted in **1** (i.e. 7**Z**, 12**Z**, 17**E**) from its ^{1}H-^{13}C heteronuclear chemical shift correlation (**HETCOR**) spectrum. Of all the methylene carbons which are alpha to the quaternary carbon of an isoprenoid double bond (C-10, C-15 and C-20), the one absorbing at 41.9 (assigned to the C-20 methylene carbon) is the only one that correlates to two distinct diastereotopic proton signals. This can be rationalized only if C-20 is alpha to both an asymmetric center (C-21) and a quaternary carbon of a trisubstituted double bond with **E** stereochemistry. The absolute stereochemistry at C-21 remains unassigned.

The methylated derivative isolated next was identified as 22-O-methylvariabilin (**5**) by its UV, ^{1}HNMR, ^{13}CNMR, IR and mass spectra. This oily derivative was followed by a stable light-yellow oil ($C_{26}H_{36}O_4$ by LRMS and ^{13}CNMR) whose IR, UV, ^{1}HNMR, ^{13}CNMR and mass spectra argued for structure **3**. This ketene ketal is no doubt an artifact formed during the methylation of palominin due to the introduction of isomerization within the lactone moiety. This very closely resembles behavior reported previously for the reaction of diazomethane with tetronic acid derivatives.[11] Both **1** and **3** are cytotoxic to *Artemia salina* (brine shrimp)[12] at a concentration of 1 μg/mL.[13] On screening for antimicrobial activity both compounds were found to inhibit the growth of Gram negative bacteria (*Proteus vulgaris* and *Shigella flexneri*) in a standard paper disk assay.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES. IR (liquid film) and UV (MeOH) spectra were recorded on a Nicolet 600 FT-IR and a Hewlett-Packard Chem Station 8452A spectrometers, respectively. All ^{1}H and ^{13}C (Table **1**, structures **1**,**2** and **3**) NMR data were recorded on a General Electric Multinuclear QE-300. Mass spectra were recorded on a Hewlett-Packard 5995A spectrometer. GLC-MS analyses were performed using a coiled OV-101 column programmed from 90°C to 260°C (10°C/min). 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 column (Beckman).

Isolation of palominin (1). The sponge *Ircinia sp.* was collected by SCUBA diving from Palomino Key, Puerto Rico, in December 1987 at a depth of 12m. Fresh material (1.1 kg wet weight) was blended with MeOH (2L) twice. The combined extracts were filtered and concentrated to dryness under reduced pressure (21.84g). The green residue was taken up with water (300mL) and partitioned successively against hexane (3X200mL; 4.28g) and chloroform (3x200mL; 1.80g). Column chromatography on silica (30g) of the chloroform partition (900mg) using hexane/EtOAc 25:75 afforded variabilin (4; 150mg), a mixture of 1 and 4 (220mg) and palominin (1; 310mg). These fractions were **unstable** and after a few days in the freezer total decomposition had taken place. Before palominin decomposed the following data were recorded: colorless oil; λ_{max} 208 nm (ε 21,660); υ_{max} 1727 (s), 1653 (s), 1408 (s),1104 (s),1048 (s), 873 (m), 777 (m) cm^{-1}; ^{1}HNMR δ 7.31 (1H, br s), 7.20 (1H, br s), 6.20 (1H, br s), 5.20-5.10 (3H, br m), 4.60 (1H, br d), 2.6-1.5 (27H, complex m); MS m/z (%) 398 (1, M^+), 175(11), 149(15), 135(61), 121(54), 113(65), 107(53), 97(33), 81(100), 77(29).

22-O-Methylpalominin (2). The crude chloroform extract (900mg) was allowed through a column packed with Sephadex LH-20 (1:1 MeOH-$CHCl_3$) and the colorless sesterterpene containing fraction was quickly methylated at 25°C using excess diazomethane in diethyl ether. The mixture of products was separated by silica (30g) column chromatography (hexane/EtOAc 4:1) and each component eluted was further purified by C-18 silica HPLC (12:1 MeOH-H_2O); **2** (500mg,56%), yellowish oil; λ_{max} 206 nm (ε 18,500); υ_{max} 1756 (s), 1672 (s), 1325(s), 1066(s), 874(m), 779(m) cm^{-1}; ^{1}HNMR δ 7.30 (1H, br s), 7.17 (1H, br s), 6.24 (1H, br s), 5.23 (1H, br t), 5.15 (1H, br t), 5.10 (1H, br m), 4.60 (1H, br d, J=6.4 Hz), 4.05 (3H, s), 2.54-2.01 (complex m), 1.99 (3H,s), 1.94 (3H, s), 1.65 (3H, s), 1.64 (3H, s); MS m/z(%) 412 (5, M^+), 217 (15), 149(27), 135 (65), 127(100), 107(22), 99(30).

22-O-Methylvariabilin (5). Yellowish oil (94mg,10%); λ_{max} 206, 264 nm (ε 6,200 and 2,350); υ_{max} 1761 (s), 1685 (w), 1640 (s),1455(s), 1356 (s), 1061 (s), 982 (s), 873 (w), 779 (w) cm^{-1}; ^{1}HNMR δ 7.31 (1H, br s), 7.18 (1H, br s), 6.25 (1H, br s), 5.12 (1H, br d, J=9.9 Hz), 5.05 (1H, br t), 4.09 (3H, s), 2.42 (1H, m), 2.20 (1H, m), 2.04 (3H, s), 2.00-1.90 (complex m), 1.56 (3H, s), 1.53 (3H, s), 1.32 (complex m), 1.05 (1H, d, J=6.6Hz); MS m/z(%) 412 (13, M^+), 209(13), 203(29), 193(17), 181(28), 175(40), 167(88), 161(18), 149(56), 141(50), 136(90), 123(48), 81(100).

Compound 3. Clear oil (254mg,28%); λ_{max} 208, 266 nm (ε17,000 and 11,900); υ_{max} 1702 (w), 1612(s), 1395 (m), 1149 (s) cm^{-1}; ^{1}HNMR δ 7.34 (1H, br s), 7.21 (1H, br s), 6.27 (1H, br s), 5.30 (1H, br t), 5.18 (1H,br t), 5.14 (1H, m), 4.63 (1H, dd, J= 6.8, 2.8 Hz), 2.69 (IH, br d), 2.44 (IH, br t), 2.28-2.04 (complex m), 2.04 (s, 3H), 1.70 (6H, br s, two overlapped methyl groups), 1.60 (3H, s); MS m/z (%) 412 (2, M^+), 277 (4), 135(11), 128(100), 121(7), 115(7), 107(8), 99(1).

Ozonolysis of 22-O-methylpalominin (2).[9] A stirred solution of **2** (30 mg) in EtOAc (10mL) at -78°C was treated with a stream of $0_2/0_3$ until the reaction mixture attained a pale blue coloration. After removal of the solvent under reduced pressure at 30°C, water was added to the residue and the mixture was kept at 100°C for 1h in the presence of a few drops of H_2O_2. The mixture was extracted twice with ether (2x25mL) and the dried (Na_2SO_4) ether extract was treated with excess CH_2N_2. The solution was taken to dryness *in vacuo* and the residue (15mg) was dissolved in ether for injection into de GLC-MS system. The identification of the fragments was established by comparison of the retention times and mass spectra recorded with those of authentic samples of dimethyl succinate and methyl levulinate.

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7. Recently, Munro and coworkers established that during the autooxidation of variabilin the tetronic acid moiety acts as a sensitizer for the production of singlet oxygen, which then reacts with the furan moiety of variabilin to produce a complex mixture of polar compounds. This explains the unstable nature of similar furanosesterterpenes such as palominin . See Munro, M.H.G.; Blunt, J.W.; Barrow, C.J. *J. Nat.Prod.* 1989, 52, 346-359.

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13.A 100% death response was determined at a concentration of 1μg/mL after a 24 hour count period. We did not test for cytotoxicity at lower concentrations.