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Palauolol, a New Anti-inflammatory Sesterterpene from the Sponge *Fascaplysinopsis* sp. from Palau

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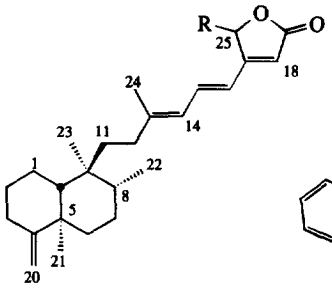
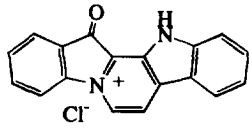
Abstract: A specimen of *Fascaplysinopsis* sp. from Palau contained palauolide (**1**), fascaplysin (**2**), and a new sesterterpene, palauolol (**3**). The structure and absolute stereochemistry of palauolol (**3**) were determined using spectroscopic and chemical methods and in doing so, the absolute stereochemistry of palauolide was also determined. Palauolide (**1**) and palauolol (**3**) both inactivate bee venom phospholipase A_2. Copyright © 1996 Elsevier Science Ltd

In 1982, we reported the isolation of palauolide (**1**) from a collection of three or more sponges from Palau that had been combined due to a freezer failure.[1] Since that time, we have examined hundreds of sponge specimens from Palau but have only recently located palauolide (**1**) in a sponge of the genus *Fascaplysinopsis*. Like other *Fascaplysinopsis* species that have been studied,[2-4] this sponge contained the alkaloid fascaplysin (**2**) in the aqueous extract and a mixture of sesterterpenes in the organic extracts. However, no sesterterpene-alkaloid salts were isolated or observed during fractionation. From the ethyl acetate extract, we have isolated a new sesterterpene, palauolol (**3**). We suspect that palauolol (**3**) is a biosynthetic precursor of palauolide (**1**), which is present in the first crude extract of the sponge and is not formed by dehydration during the separation and purification procedures.

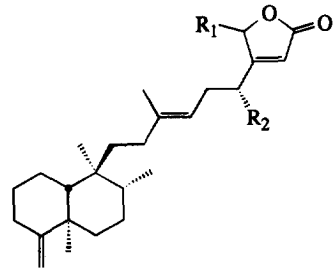
A specimen of *Fascaplysinopsis* sp. was collected by hand using SCUBA (-25 m) at Ngemelis drop-off, Palau. The crude methanol extract of the sponge exhibited antimicrobial activity, which was followed during the purification procedure. The hexane-soluble material from the methanol extract was chromatographed on silica gel to obtain palauolide (**1**), which was identical in all respects, including optical rotation, to an authentic specimen. Purification of the water-soluble material by reversed-phase chromatography gave fascaplysin (**2**), which had identical spectral data to those reported earlier.[2] The ethyl acetate soluble fraction was purified using diol and reversed-phase chromatography to obtain palauolol (**3**).



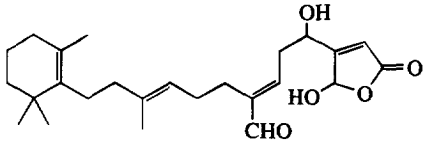
1 R = OH
7 R = H



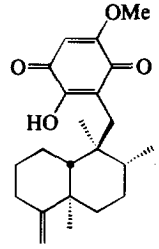
2



3 $R_1 = R_2 = OH$
5 $R_1 = H, R_2 = OH$
6 $R_1 = H, R_2 = OMTPA$



4



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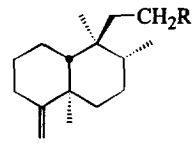

8 R = CMe_2OH
10 R = COOH

Palauolol (**3**) was obtained as a clear oil of molecular formula $C_{25}H_{38}O_4$. The molecular formula was determined by mass spectrometry and from the ^{13}C NMR data.[5] Inspection of the ^1H NMR spectrum (Table 1) revealed that palauolol (**3**) was closely related to palauolide (**1**) but lacked the conjugated olefinic proton signals and the strong UV absorption at 322 nm.[5] The ^{13}C NMR spectrum (Table 1) of **3** contained a signal at δ 69.1 (C-16), assigned to a carbon bearing oxygen, and one additional methylene carbon signal that replace two of the olefinic carbon signals in **1**. In the ^1H NMR spectrum of **3**, the signal at δ 4.54 (t, 1H, J = 5 Hz, H-16) is coupled to methylene proton signals at 2.35 (m, 1H, H-15) and 2.45 (m, 1H, H-15), that are in turn coupled to an olefinic proton signal at 5.21 (t, 1 H, J = 6.5 Hz, H-14). The location of the secondary alcohol at C-16 was readily assigned from HMBC correlations (see Table 1). Comparison of the NMR data for the C-15 to C-19 region of palauolol (**3**) with the corresponding data for secomanoalide (**4**)[6] showed the expected similarity. Palauolol (**3**) is therefore a secondary alcohol that on dehydration gives palauolide (**1**).

The absolute stereochemistry at C-16 of palauolol (**3**) was determined using the advanced Mosher's method[7] applied to lactone **5** that was prepared by reduction of palauolol (**3**) with sodium borohydride in methanol.[8] The lactone **5** was converted into both the *R*- and *S*-MTPA esters **6**, each of which was a single diastereoisomer by ^1H NMR spectroscopy, showing the enantiomeric purity of **3**; comparison of the ^1H NMR data for the MTPA esters (Table 2) indicated the 16*R* absolute stereochemistry. The absolute stereochemistry of the bicyclic ring system of palauolol (**3**) was

Table 1. ^{1}H (500 MHz, CD_3OD) and ^{13}C NMR (50 MHz, CD_3OD) data for palauolol (3).

C#	^{13}C	^{1}H NMR		HMBC	COSY
1	22.9	1.41	(m)		H-2, H-10
		1.58	(m)		H-2, H-10
2	29.9	1.2	(m)		H-1, H-3
		1.86	(m)		H-1, H-3
3	34.2	2.1	(m)	C-2, C-3, C-4, C-20	H-2
		2.3	(m)	C-2, C-3, C-4, C-20	H-2
4	160.0				
5	41.2				
6	38.7	1.6	(m, 2H)	C-5, C-7, C-8, C-21	H-7
7	28.6	1.43	(m, 2H)		H-6, H-8
8	38.0	1.42	(m)		H-7, H-22
9	40.4				
10	50.0	1.14	(dd, 12, 2.5)	C-1, C-5, C-9, C-23	H-1
11	38.2	1.24	(m)		H-12
		1.34	(m)		H-12
12	34.3	1.74	(m)	C-11, C-13, C-14, C-24	H-11
		1.86	(m)	C-11, C-13, C-14, C-24	H-11
13	140.6				
14	119.7	5.21	(t, 6.5)		H-15
15	36.0	2.35	(m)	C-13, C-14, C-16	H-14, H-16
		2.45	(m)	C-13, C-14, C-16	H-14, H-16
16	69.1	4.54	(t, 5)	C-14, C-15, C-17	H-15
17	173.0				
18	117.8	5.98	(br s)		
19	173.0				
20	103.2	4.49	(br s, 2H)	C-3, C-4, C-5	
21	21.5	1.05	(s, 3H)	C-4, C-5, C-6, C-10	
22	16.4	0.81	(d, 6.5, 3H)	C-7, C-8, C-9	H-8
23	18.7	0.74	(s, 3H)	C-8, C-9, C-10, C-11	
24	16.7	1.62	(s, 3H)	C-12, C-13, C-14	
25	99.7	6.01	(br s)		

Table 2. ^{1}H NMR data for the *R*- and *S*-MTPA esters of lactone **5**.

H#	δ_R	δ_S	$\Delta\delta_{R\text{-}S}$(ppm)	$\Delta\delta_{R\text{-}S}$(Hz)
21	1.090	1.091	+0.001	+0.5
23	0.679	0.684	+0.005	+2.5
14	4.918	4.996	+0.078	+39
15	2.504	2.538	+0.034	+17
18	5.922	5.820	-0.102	-51
25	4.663	4.546	-0.117	-58.5
	4.767	4.696	-0.071	-35.5

established by converting palauolide (**1**) into the corresponding lactone **7** and comparing its CD spectrum with that of alcohol **8**, prepared from ilimaquinone (**9**)[9] by oxidation of the quinone ring with basic hydrogen peroxide[1] followed by treatment of the resulting acid **10** with methyl lithium. Both **7** and **8** showed positive Cotton effects at λ_{max} 197 nm of almost equal magnitude ($\Delta\epsilon$ +3.5° ± 0.5°). Thus, the absolute stereochemistry of palauolide (**1**), $[\alpha]_D$ = +1.5 (c = 0.2, $CHCl_3$), is 5*S*,8*S*,9*R*,10*S* and that of palauolol (**3**), $[\alpha]_D$ = 0 ± 0.5 (c = 0.2, $CHCl_3$) is 5*S*,8*S*,9*R*,10*S*,16*R*.[10]

Both palauolide (**1**, 85% inhibition @ 0.8 μg/mL) and palauolol (**3**, 82% inhibition @ 0.8 μg/mL) inactivate bee venom PLA_2.[11] Palauolol (**3**) is mildly antimicrobial against *S. aureus* and *B. subtilis* but the antimicrobial activity of the crude extract is primarily due to fascaplysin (**2**).[12]

References and Notes

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5. Palauolol (**3**): oil; 7.3 mg, 0.66% extract weight; $[\alpha]_D$ = 0 ± 0.5 (c = 0.2, $CHCl_3$); IR (film) 3370, 1745 cm^{-1}; UV (MeOH) 206 nm (ϵ 25000); ^1H NMR (methanol-d_4) see Table 1; ^{13}C NMR (methanol-d_4) see Table 1.; LREIMS *m/z* 402 (M^+, 1), 384 (5), 191 (56), 95 (100); HRCIMS, Obsd. *m/z* = 420.2873, $C_{25}H_{40}O_5$ $[M+H_2O]^+$ requires 420.2875.
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12. We thank Mary Kay Harper for identifying the sponge and for performing the antimicrobial assays, Bryan Wylie and Dr. Robert S. Jacobs (UC Santa Barbara) for performing the anti-inflammatory assays, and Barbara Doerner and Behrouz Forood (Torrey Pines Institute for Molecular Studies) for assistance with CD measurements. We thank the Republic of Palau and the State of Koror for collecting permits and Larry Sharron of the Coral Reef Research Foundation, Palau for invaluable assistance with collections. This research was supported by the California Sea Grant College Program (NOAA grant NA36RG0537, project R/MP-60).

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