Thorectandrols A and B, New Cytotoxic Sesterterpenes from the Marine Sponge *Thorectandra* Species

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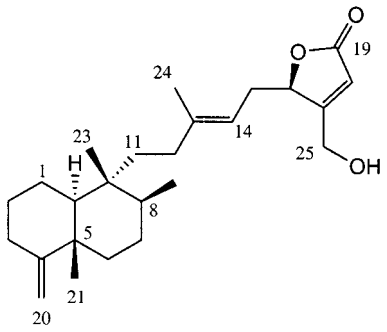
Two new sesterterpenes, thorectandrol A (**1**) and B (**2**), were isolated from extracts of the marine sponge *Thorectandra* sp. The structures were determined by extensive NMR spectral data analysis. NOE correlations were used to define the relative stereochemistry of **1** and **2**, while CD data were used to suggest their absolute stereochemistry. Both compounds **1** and **2** inhibited the growth of MALME-3M (melanoma) and MCF-7 (breast) cancer cell lines in the range 30−40 μg/mL. The known compound palauolol (**3**) was isolated as well and was also cytotoxic.

Sesterterpene metabolites are widespread in the sponge family Thorectidae;[1] however, to date only five investigations on the genus *Thorectandra* have appeared in the chemical literature.[2−6] In the course of our continuing studies on cytotoxic constituents from marine organisms we investigated a sponge of the genus *Thorectandra* collected in Palau.

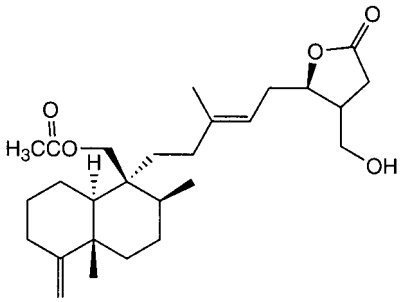
The crude organic extract of the frozen sponge showed antiproliferative activity in the melanoma and breast cancer cell lines and therefore was subjected to bioassay-guided purification. Solvent−solvent partitioning provided a cytotoxic methyl *tert*-butyl ether fraction, which was purified by reversed-phase HPLC to give thorectandrol A (**1**, 9.5 mg), thorectandrol B (**2**, 1.5 mg), and the known compound palauolol (**3**, 34.0 mg).

Thorectandrol A (**1**) was isolated as an optically active, light yellow oil. The molecular formula $C_{25}H_{38}O_3$ was established by HRFABMS (obsd *m*/*z* 387.2883 for $[M + H]^+$, calcd *m*/*z* 387.2899), indicating seven degrees of unsaturation. The ^{13}C NMR spectrum of **1** contained resonances for all 25 carbons, while a DEPT experiment revealed the presence of four methyls, 10 methylenes, five methines, and six quartenary carbons. An IR absorption band at 1760 cm^{-1} indicated the likely presence of a lactone carbonyl functionality, which was supported by the presence of an ester carbonyl resonance at δ175.4 in the ^{13}C NMR spectrum. The carbonyl resonance and six additional carbon resonances between δ 103.2 and 175.9, which were assigned to three olefin groups based on COSY and HMBC data, accounted for four degrees of unsaturation and suggested the presence of three rings in **1**. A broad infrared absorption band at 3250 cm^{-1} suggested the presence of an OH group.

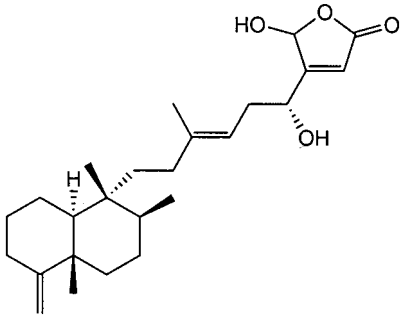
The presence of a bicyclic ring containing an exocyclic double bond and an α,β-unsaturated butenolide moiety in **1** was apparent from detailed analysis of ^1H and ^{13}C NMR data together with COSY, HSQC, and HMBC spectra (Table 1). The ^1H and ^{13}C NMR data for the C-1 to C-14 fragment in **1** closely resembled the corresponding fragment in palauolol (**3**).[7] A standard series of 2D NMR experiments confirmed the assignment of C-1 to C-14. COSY correlations from H-14 (δ 4.99) to H-15 (δ 2.36 and 2.66) together with HMBC correlations from H-15 to C-14 indicated the attachment of a methylene group at C-14.



Thorectandrol A (**1**)



Thorectandrol B (**2**)



Palauolol (**3**)

H-15 in turn showed COSY correlations to H-16 (δ 5.13), and their attachment was supported by HMBC correlations in both directions. The ^1H NMR spectrum of **1** displayed a one-proton resonance at δ 5.97 (H-18), which had a long-

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Spectral Data for Thorectandrol A (**1**) and B (**2**) (MeOH-d_4)

	1			**2**	
C no.	δ ^{13}C	δ ^{1}H (mult., J = Hz)	HMBC	δ ^{13}C	δ ^{1}H (mult., J = Hz)
1	22.8	1.48 (m)	C-2, C-10	23.9	1.58 (m)
		1.55 (m)			1.66 (m)
2	29.8	1.23 (m)	C-3	30.2	1.28 (m)
		1.89 (m)			1.90 (m)
3	34.1	2.08 (m)	C-2, C-4	34.3	2.10 (m)
		2.30 (m)			2.29 (m)
4	161.7			161.7	
5	41.2			40.9	
6	38.6	1.52 (m)	C-5, C-7, C-8, C-21	31.6	1.57 (m, 2H)
		1.56 (m)	C-5, C-7, C-8		
7	28.6	1.43 (m, 2H)	C-5, C-6	28.5	1.55 (m, 2H)
8	37.9	1.44 (m)	C-9, C-11, C-22	37.9	1.55 (m)
9	40.3			43.8	
10	50.0	1.09 (dd, 12.2, 2.8)	C-1, C-5, C-21, C-23	50.3	1.23 (dd, 12.4, 2.7)
11	38.2	1.27 (m)	C-9, C-10, C-12	38.7	1.58 (m)
		1.39 (m)	C-9, C-12		1.64 (m)
12	34.2	1.69 (m)	C-11, C-13, C-14, C-24	33.7	1.73 (m)
		1.83 (m)	C-11, C-13, C-14, C-24		1.85 (m)
13	142.4			142.2	
14	116.8	4.99 (t, 7.0)	C-24	117.1	5.02 (t, 7.0)
15	31.6	2.36 (m)	C-13, C-14, C-16	31.5	2.37 (m)
		2.66 (m)	C-13, C-16, C-17		2.70 (m)
16	83.1	5.13 (t, 5.0)	C-14, C-15, C-17, C-18	83.8	5.16 (t, 4.4)
17	175.9			175.8	
18	115.7	5.97 (d, 1.2)	C-19	115.7	5.97 (d, 1.8)
19	175.4			175.3	
20	103.2	4.48 (s)	C-3, C-5	103.8	4.49 (s)
		4.49 (s)	C-3, C-5		4.53 (s)
21	21.4	1.05 (s, 3H)	C-4, C-5, C-6, C-10	21.4	1.05 (s, 3H)
22	16.4	0.80 (d, 6.4, 3H)	C-7, C-8, C-9	17.4	0.89 (d, 6.1, 3H)
23	18.6	0.73 (s, 3H)	C-9, C-10, C-11	67.7	4.16 (s, 2H)
24	16.6	1.62 (s, 3H)	C-12, C-13, C-14	16.7	1.65 (s, 3H)
25	59.1	4.39 (dd, 17.7,1.3, 2H)	C-17, C-18	59.1	4.40 (dd, 17.7, 1.8, 2H)
26				173.2	
27				21.0	2.03 (s, 3H)

range COSY coupling to H-16. In addition HMBC correlations from H-16 to C-18 (δ 115.7) confirmed the assignment of C-18. The carbonyl resonance at C-19 (δ 175.4) was assigned based on a HMBC correlation from H-18 to C-19. A two proton resonance at δ 4.39 was consistent with the presence of an oxymethylene group substituted at C-17, and this was supported by HMBC correlations from H-25 to C-17. H-25 also showed long-range COSY correlations to H-18, supporting the presence of an α,β-unsaturated butenolide moiety with an oxymethylene group substituted at C-17. Although α,β-unsaturated butenolide moieties have been reported in many natural products,[2,7−13] to the best of our knowledge, only compounds isolated from *Luffariella variablilis*[12] and *L. geometrica*[13] have an oxymethylene group substituted at the β-carbon as found in **1**. The *E* configuration[14] of the Δ^{13} double bond was inferred from the upfield ^{13}C resonance at 16.6 ppm assigned to C-24 and confirmed by NOE enhancements of both H-15 protons on irradiation of H-24. One-dimensional gNOESY[15] experiments allowed the assignment of the relative stereochemistry of the bicyclic ring in **1**. Our assignment of the relative stereochemistry of the bicyclic ring was consistent with that proposed in the literature based on ^{13}C chemical shifts.[16]

A molecular formula of $C_{27}H_{40}O_5$ was established by HRFABMS for thorectandrol B (**2**). Inspection of the ^{1}H NMR spectrum (Table 1) revealed that **2** was closely related to thorectandrol A (**1**) but contained resonances for a new oxymethylene at 4.16 ppm instead of the methyl at 0.73 ppm. In addition a new methyl singlet at 2.03 ppm indicated the presence of an acetate functionality. The ^{13}C spectrum of **2** contained an extra resonance at δ 67.7, which was assigned to the oxymethylene group. The ester carbonyl of the acetate group appeared at 173.2 ppm (C-26), and HMBC correlations from H-23 (δ 4.16) to C-26 indicated the attachment of the acetate group at C-23. Further, HMBC correlations from H-23 to C-8 and C-9 allowed placement of the oxymethylene group bearing the acetate functionality at C-9. The NMR data of the C-11 to C-19 region of thorectandrol B (**2**) were nearly identical to that for thorectandrol A (**1**) (Table 1). The *E* configuration of the Δ^{13} double bond and the relative stereochemistry of the bicylic ring were assigned using NOE correlations in the same manner as was done for thorectandrol A (**1**) and are as shown in **2**.

In an effort to assign the absolute stereochemistry at C-16, the CD data of thorectandrol A (**1**) and B (**2**) were compared with those of luffarin K and luffarin L.[13] Both thorectandrol A (**1**) and B (**2**) displayed a negative Cotton effect at λ_{max} 212 and 210 nm, respectively, a result similar to that reported for luffarin K and L,[13] and hence an *R* stereochemistry was suggested for C-16 in **1** and **2**.

The absolute stereochemistry at C-16 in palauolol (**3**) has also been reported to be *R*.[7] The absolute stereochemistry of the bicyclic ring system of thorectandrol A (**1**) and B (**2**) was tentatively assigned by comparison of their CD spectra with CD data reported for palauolol[7] and data obtained by our own measurements. Thorectandrol A (**1**) and B (**2**) both showed a positive Cotton effect at λ_{max} 197 nm, exactly the same as palauolol, and hence this supported the assignment of 5*S*, 8*S*, 9*R*, 10*S* stereochemistry in both **1** and **2**.

The major compound isolated was identified as palauolol (**3**) by comparison of its NMR data with that of published data.[7]

All three compounds were tested for in vitro cytotoxicity against two human tumor cell lines, MALME-3M (melanoma) and MCF-7 (breast).[17] Thorectandrol A (**1**) and B

(**2**) inhibited growth of the MALME-3M cancer cell line (IC_{50} 40.0 and IC_{50} 30.0 μg/mL, respectively) and the MCF-7 cancer cell line (IC_{50} 40.0 and IC_{50} 30.0 μg/mL, respectively). Palauolol (**3**) showed similar activity in both the cell lines as well (IC_{50} 0.46 and IC_{50} 14.2 μg/mL, respectively).

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter in MeOH. UV spectra were obtained on a Beckman DU-64 spectrophotometer and FTIR spectra on a Perkin-Elmer 267 spectrophotometer. All 1D and 2D NMR spectra were recorded on a Varian Unity Inova spectrometer at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, and referenced to the residual solvent signal. The number of attached protons for ^{13}C resonances were determined from a DEPT experiment. Proton-detected heteronuclear correlations were measured using HSQC (optimized for $^1J_{C-H}$ = 140 Hz) and HMBC (optimized for $^nJ_{C-H}$ = 8.3 Hz) pulse sequences. The gNOESY experiments were carried out using a mixing time of 300 ms. Mass spectra were recorded on a JEOL SX102 spectrometer.

Sponge Material. The sponge was collected in 1991 from Palau by P. Colin of the Coral Reef Research Foundation, under contract for the National Cancer Institute. The sponge was collected at a depth of 10 m and is 5−15 cm in diameter. It is dark rusty red both inside and outside, and the surface is convoluted and tears easily. A voucher specimen (QCDN5079) from this collection is maintained at the Smithsonian Institution. A photograph of the sponge is provided in the Supporting Information.

Extraction and Purification. The frozen sponge material was ground to a coarse powder (327 g) and sequentially extracted with water followed by MeOH/CH_2Cl_2 (1:1). The organic extract was evaporated in vacuo and dried to give a maroon solid (4.89 g). A 1.08 g portion of the cytotoxic crude extract was subjected to solvent−solvent partitioning. A portion (146 mg) of the methyl *tert*-butyl ether fraction was purified by reversed-phase HPLC (1.0 × 25 cm; C-18; 60 Å; Dynamax) using 90% (by volume) of acetonitrile in water, yielding thorectandrol A (**1**, 9.5 mg). Two earlier eluting sesterterpene fractions were further purified by RPHPLC using 80% and 90% (by volume) of acetonitrile in water, giving thorectandrol B (**2**, 1.5 mg) and palauolol (**3**, 34 mg), respectively. Elution of the individual components was monitored using a photodiode array detector (Waters 990).

Cytotoxicity Assays. Cytotoxicity assays using MALME-3M (melanoma) and MCF-7 (breast) cancer cell lines were performed as described before.[17]

Thorectandrol A (1): yellow oil; 9.5 mg, 6.51% extract wt, $[\alpha_D]$ −15.0° (*c* 0.15, MeOH); CD (MeOH) λ_{max} (log $\Delta\epsilon$) 212 (−4.1), 197 (+4.3) nm; UV (MeOH) λ_{max} (log ϵ) 209 (3.94) nm; IR (film) ν_{max} 3250 (OH), 1760 (C=O), 1630 (C=C) cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* obsd 387.2883 for $[M + H]^+$, calcd for $C_{25}H_{39}O_3$ 387.2899.

Thorectandrol B (2): yellow oil; 1.5 mg, 1.03% extract wt, $[\alpha_D]$ −19.4° (*c* 0.07, MeOH); CD (MeOH) λ_{max} (log $\Delta\epsilon$) 210 (−4.5), 197 (+4.7) nm; UV (MeOH) λ_{max} (log ϵ) 210 (4.05) nm; IR (film) ν_{max} 3370 (OH), 1760 (C=O), 1630 (C=C) cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* obsd 445.2938 for $[M + H]^+$, calcd for $C_{27}H_{41}O_5$ 445.2922.

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Supporting Information Available: A photograph of the sponge *Thorectandra* sp. is available free of charge via the Internet at http://pubs.acs.org.

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(7) Schmidt, E. W.; Faulkner, D. J. *Tetrahedron Lett*. **1996**, *37*, 3951−3954. Note: The original drawing of palauolol indicates an absolute stereochemistry opposite of that listed in the text. The stereochemistry as listed in the text (5*S*, 8*S*, 9*R*, 10*S*), however, is consistent with ilimaquinone synthetic studies.[18,19]
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