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Cytotoxic Macrolides from a New Species of the Deep-Water Marine Sponge *Leiodermatium*

Joel S. Sandler,[†,‡] Patrick L. Colin,[§] Michelle Kelly,[∥] and William Fenical*[,†]

Center for Marine Biotechnology and Biomedicine (CMBB), Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0204, Coral Reef Research Foundation (CRRF), P.O. Box 1765, Koror, PW 96940 Palau, and National Centre for Aquatic Biodiversity and Biosecurity, National Institute of Water and Atmospheric Research (NIWA) Ltd., Private Bag 109-695, Newmarket, Auckland, New Zealand

wfenical@ucsd.edu

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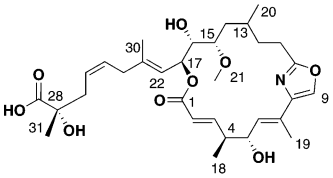
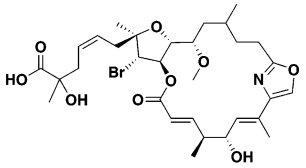


Leiodelide A (**1**)



Leiodelide B (**2**)

Chemical investigation of a new species of the deep-water marine sponge *Leiodermatium*, collected by manned submersible at a depth of 740 feet in Palau, resulted in the isolation of two cytotoxic macrolides, leiodolides A (**1**) and B (**2**). The leiodolides represent the first members of a new class of 19-membered ring macrolides, incorporating several unique functional groups including a conjugated oxazole ring, a bromine substituent, and an α-hydroxy-α-methyl carboxylic acid side-chain terminus. The structures of these new metabolites were established by spectroscopic analysis, chemical modification, and degradation. The relative and absolute stereochemistries at most chiral centers were assigned on detailed interpretation of spectroscopic data, coupled with chemical degradation and application of the modified Mosher ester method. Leiodolide A showed significant cytotoxicity (average $GI_{50} = 2.0$ μM) in the National Cancer Institute's 60 cell line panel with enhanced activity against HL-60 leukemia and OVCAR-3 ovarian cancer cell lines.

Introduction

Marine sponges of the order Lithistida (class Demospongiae) are well-known as a prolific source of novel bioactive secondary metabolites, which include complex polyketides, cyclic peptides, alkaloids, pigments, and novel sterols.[1] Chemical investigations of lithistid sponges have focused on tropical Indo-Pacific species that were collected in shallow waters by scuba.[2−11] However, several studies of lithistid sponges collected from deeper waters along the New Caledonian Norfolk Ridge via sea-floor dredging[12−14] have been reported. The important anticancer agent discodermolide was isolated from a deep-water Atlantic

* To whom correspondence should be addressed. Tel: (858) 534-2133. Fax: (858) 558-3702.
[†] University of California, San Diego.
[‡] Current address: Laboratory of Chemistry and Cell Biology, Rockefeller University, New York, NY 10021.
[§] CRRF, Palau.
[∥] NIWA, New Zealand.

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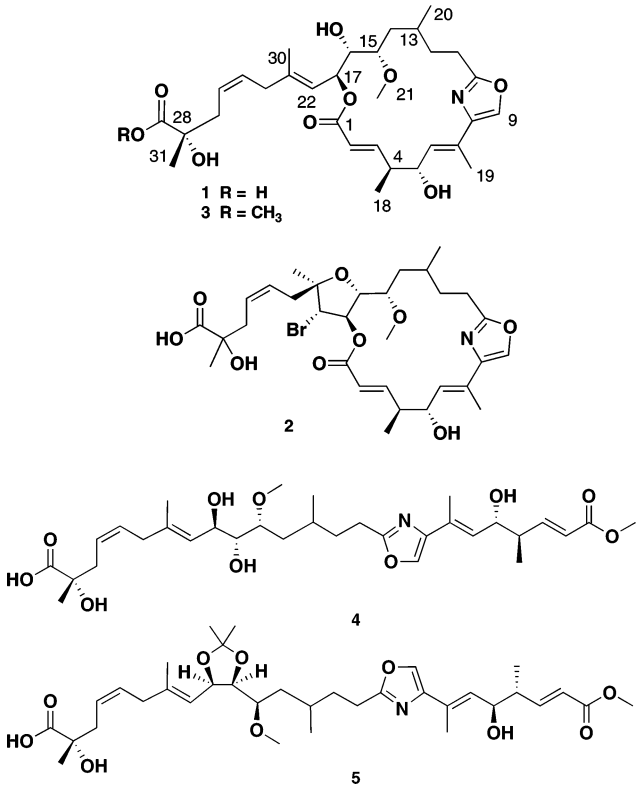
sponge collected by submersible.[15−17] While many of the lithistid sponges are known to be deep-water species, those from the West-Central Pacific, including Micronesia and Polynesia, have been less accessible because submersibles are rarely available in this area. Recently, we had the opportunity to utilize the manned submersible *Deep Worker*, which provided access to deeper collection sites along the barrier reefs of Palau. As part of our general interest in exploring marine-derived anticancer agents, a new sponge, identified as a member of the rare genus *Leiodermatium* (order Lithistida, family Azoricidae)[18] was collected at a depth of 720 feet near Uchelbeluu Reef in Palau. No secondary metabolites have been reported from this genus to date. In this paper, we describe the structures and bioactivities of two selectively cytotoxic macrocyclic lactones, leiodolides A (**1**) and B (**2**) isolated from this rare source.



Results and Discussion

The lyophilized sponge (730 g, dry weight) was exhaustively extracted with 1 L of methanol at room temperature. Roughly half of the methanol was removed in vacuo, and the remaining methanol solution was adsorbed onto an HP20 column and subsequently eluted using an increasing concentration of acetone in water as previously described.[19] The 50% and 75% aqueous acetone fractions exhibited significant cytotoxicity against human colon adenocarcinoma (HCT-116); thus, these fractions were combined and further fractionated by isocratic reversed-phase HPLC (PRP-1 semi-prep, 27.5% acetonitrile/H_2O, 2 mL/min) to give leiodolide A (**1**, 8 mg, 0.001% dry weight) and leiodolide B (**2**, 0.8 mg, 0.0001% dry weight).

The molecular formula of leiodolide A (**1**), assigned as $C_{31}H_{45}NO_9$ by high-resolution MALDI-MS spectral analysis [m/z = 598.2992 $[M + Na]^+$, Δ 0.9 ppm], was consistent with both ^{13}C and ^1H NMR spectral data (Table 1). Analysis of proton and carbon NMR data revealed signals due to two disubstituted and two trisubstituted double bonds, four oxygenated methines, a methoxy group, a saturated carboxylic acid, an α,β-unsaturated ester or carboxylic acid, and a small, nitrogen-containing aromatic ring (δ_H 7.68, δ_C 135.0, δ_C 143.5, δ_C 166.3). Combined, these data suggested that **1** was an oxygenated polyketide-derived metabolite.

Analysis of COSY NMR cross-peaks established the connectivity between two olefinic protons at C-2 (δ_H 5.71, d, 15.5 Hz) and C-3 (δ_H 6.89, dd, 15.5, 9.5 Hz). The large coupling constant between H-2 and H-3 established the *E* configuration for this disubstituted double bond. COSY correlations revealed a linear spin system comprised of a $\Delta^{2,3}$ olefin, a methylated methine, C-4 (δ_H 2.39, m), a methyl doublet, C-18 (δ_H 1.26, d, 7.0 Hz), and an oxygen-bearing methine, C-5 (δ_H 4.55, dd, 8.5, 2.0 Hz). The H-5 methine resonance was also coupled to an olefinic proton resonance at C-6 (δ_H 6.32, dd, 9.0, 1.5 Hz) of a trisubstituted double bond. Allylic coupling between H-6 and the C-19 methyl protons (δ_H 1.93, d, 1.5 Hz) was evident from the COSY spectrum, and this established the presence of these two substituents as a trisubstituted double bond. A NOESY NMR experiment, which showed a correlation between H-19 and H-5, combined with the lack of a correlation between H-19 and H-6, established the configuration of this double bond as *E*.

Further analysis of COSY NMR data also illustrated a linear subunit comprised of two sets of methylene protons, C-11 (δ_H 2.71, 2.83, m) and C-12 (δ_H 1.60, 2.05, m), a methyl-bearing methine proton, C-13 (δ_H 1.29, m), a methyl doublet, C-20 (δ_H 0.97, d, 6.0 Hz), a methylene pair, C-14 (δ_H 1.62, 2H, m), and three oxygenated methane protons, C-15 (δ_H 3.24, ddd, 8.0 4.0, 0.5 Hz), C-16 (δ_H 3.46, d, 9.5 Hz), and C-17 (δ_H 5.77, t, 9.5 Hz). The downfield H-17 methine proton was also observed to be coupled to an olefinic proton at C-22 (δ_H 5.14, dd, 9.5, 1.0 Hz), which in turn showed allylic coupling to a vinyl methyl resonance at C-30 (δ_H 1.80, d, 1.0 Hz). In the NOESY NMR spectrum of **1**, cross-peaks (H-22 and H-24, H-30 and H-25) established the *E* configuration of the $\Delta^{22,23}$ olefin. The remaining proton couplings observed in the COSY spectrum were assigned to a linear four-carbon subunit consisting of a downfield methylene proton pair, C-24 (δ_H 2.75, 2.81, m), two olefinic methine protons, C-25 (δ_H 5.50, m) and C-26 (δ_H 5.58, m), and an additional methylene pair, C-27 (δ_H 2.36, 2.52, m). Selective decoupling of the C-24 methylene protons in a homonuclear decoupling experiment (HOMODEC) revealed an 11.0 Hz coupling constant between H-25 and H-26, suggesting a *Z* configuration for the $\Delta^{25,26}$ olefin. Additional support for

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TABLE 1. ^{13}C, ^{1}H, and HMBC NMR Data for Leiodolide A (1)[a]

C no.	^{13}C NMR (ppm)	H no.	^{1}H NMR in ppm, mult (*J* in Hz)	HMBC spectral data
1	166.9	C		
2	124.3	CH	5.71 d (15.5)	C-1, C-4
3	151.2	CH	6.89 dd (15.5, 9.5)	C-1, C-2, C-4, C-18
4	44.9	CH	2.39 m	C-2, C-3, C-5, C-18
5	72.3	CH	4.55 dd (8.5, 2.0)	C-3, C-4, C-6, C-7, C-18
6	131.4	CH	6.32 dd (8.5, 1.5)	C-4, C-7, C-8, C-19
7	125.6	C		
8	143.5	C		
9	135.0	CH	7.68 s	C-6, C-8, C-10
10	166.3	C		
11	25.2	CH_2	2.71 m	C-10, C-12, C-13
		CH_2	2.83 m	C-10, C-12, C-13
12	34.7	CH_2	1.60 m	C-10, C-11, C-13, C-14, C-20
		CH_2	2.05 m	C-10, C-11, C-13, C-14, C-20
13	29.5	CH	1.29 m	C-11, C-12, C-14, C-15, C-20
14	35.9	CH_2	1.62 m	C-12, C-13, C-15, C-16, C-20
15	80.2	CH	3.24 ddd (8.0, 4.0, 0.5)	C-13, C-14, C-21
16	73.3	CH	3.46 d (9.5)	C-17, C-22
17	70.8	CH	5.77 t (9.5)	C-1, C-15, C-16, C-22, C-23
18	16.8	CH_3	1.26 d (7.0)	C-3, C-4, C-5
19	13.8	CH_3	1.93 d (1.5)	C-4, C-5, C-6, C-7, C-8
20	21.5	CH_3	0.97 d (6.0)	C-13, C-14
21	58.1	CH_3	3.39 s	C-15
22	123.3	CH	5.14 dd (9.5, 1.0)	C-16, C-24, C-30
23	143.3	C		
24	38.1	CH_2	2.75 m	C-22, C-23, C-25, C-26, C-30
		CH_2	2.81 m	C-22, C-23, C-25, C-26, C-30
25	130.1	CH	5.50 m	C-27
26	127.4	CH	5.58 m	C-27, C-22
27	39.0	CH_2	2.36 m	C-25, C-26, C-28, C-29, C-31
		CH_2	2.52 m	C-25, C-26, C-28, C-31
28	76.1	C		
29	181.1	C		
30	17.9	CH_3	1.80 d (1.0)	C-22, C-23, C-24
31	26.4	CH_3	1.35 s	C-27, C-28, C-29

[a] ^{13}C shifts and number of attached protons assigned by multiplicity-edited gHSQC experiments. ^{13}C NMR data were recorded at 75 MHz in MeOH-d_4, while all ^{1}H NMR spectra, including HMBC spectral data, were obtained at 500 MHz in MeOH-d_4.

this configuration was provided by analysis of NOESY NMR data, which showed correlations between the methylene protons at C-24 and C-27.

Long-range proton-carbon correlations observed in the HMBC spectrum of **1** (Table 1) provided corroborative evidence to support the three subunits deduced from COSY NMR data (C-2 to C-6, C-11 to C-22, and C-24 to C-27). In addition, HMBC correlations were observed from the olefin protons at C-2 and C-3 to the carbonyl C-1 (δ_C 166.9), establishing this as an α,β-unsaturated lactone moiety. HMBC correlations allowed a link between the olefinic methyl group at C-19 and a substituted olefin carbon (δ_C 125.6), assigned as C-7, to be made. Last, HMBC correlations from the C-30 olefinic methyl protons to the substituted olefin carbon C-23, and to the methylene carbon C-24, established a link between C-24 and the $\Delta^{22,23}$ trisubstituted double bond, and allowed the methoxy group C-21 (δ_H 3.38, δ_C 58.1) to be positioned at the C-15 oxymethine carbon.

Analysis of ^{13}C NMR data revealed the presence of a disubstituted 1,3-oxazole ring. These rings show characteristic carbon signals which were reflected by those at C-8 (δ_C 143.5), C-9 (δ_H 7.68, δ_C 135.0), and C-10 (δ_C 166.3). Furthermore, the $^{1}J_{C,H}$ coupling observed for C-9 (205.8 Hz) is highly characteristic of these small rings.[20] As expected, the heteroaromatic proton, H-9, exhibited long-range HMBC NMR correlations to the quaternary carbons C-8 and C-10. In addition, HMBC correlations observed from both H-6 and Me-19 to C-8, and from the methylene protons at C-11 and C-12 to C-10, established the location of the oxazole ring as shown.

Analysis of unassigned NMR and IR spectral data indicated that the remaining portion of the molecule consisted of an oxygenated quaternary carbon, C-28 (δ_C 76.1), a methyl singlet, C-31 (δ_H 1.35, δ_C 26.4), and an aliphatic carboxyl acid carbon, C-29 (δ_C 180.1; IR 1715 cm^{-1}). HMBC NMR correlations (H-27 to C-28, C-29, and C-31 and H-31 to C-27, C-28, C-29) established a link between the C-27 methylene and this terminal group. With all other atoms in compound **1** accounted for, the molecular formula only allowed for an additional three carbons, five protons, and three oxygen atoms in this functional group. Taken together, these data were consistent with literature NMR models for the α-hydroxy-α-methyl carboxylic acid terminal group.[21,22] Methylation of **1** with diazomethane gave the methyl ester **3** verifying the presence of the terminal carboxylic acid.

The molecular formula of **1** indicated the presence of 10 unsaturation equivalents. With all carbon connectivities accounted for, the remaining degree of unsaturation required a cyclic structure. The observation of an HMBC correlation from H-17 to the carbonyl carbon C-1 indicated an ester linkage between C-1 and C-17, thereby establishing the structure of **1** as an 19-membered ring macrolide.

Variable-temperature NMR experiments showed that the chemical shift differences of methylene protons and $^{1}H-^{1}H$ coupling constants at centers throughout compound **1** were not

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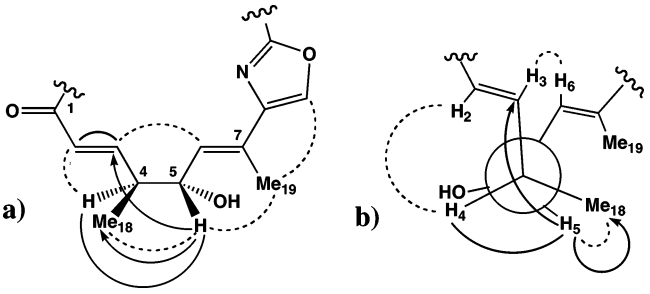

FIGURE 1. Depiction of the relative stereochemistry of C-2 to C-7 in leiodolide A (**1**): (a) sawhorse representation and (b) Newman projection. Important NOEs are illustrated with dashed lines. Long-range proton−carbon couplings are indicated with solid arrows (H→C), whereas vicinal proton−proton couplings are indicated with solid lines (H−H). See the text for coupling constants ($^3J_{C,H}$ and $^3J_{H,H}$).

dependent on temperature (0−40 °C) or NMR solvent (MeOH-d_4, DMSO-d_6). These observations confirmed the existence of a single predominant conformer,[23] and implied that an assignment of the relative stereochemistry for at least some of the chiral centers would be possible from a combination of NOE NMR studies and by analysis of vicinal coupling constants.[24] We assumed that vicinal proton−proton ($^3J_{H,H}$) and carbon-proton ($^3J_{C,H}$) coupling constants would roughly follow a Karplus equation (although J values can vary in magnitude), and thus used this approach for stereochemical analysis.[25] Approximate $^3J_{C,H}$ values were determined by careful analysis of the gHSQMBC[26] spectrum and their values were informative. The relative configuration of C-2 to C-7 is shown in Figure 1. The methine proton attached to C-5 appeared as a doublet of doublets with a large vicinal coupling to H-6 (8.5 Hz) and a small vicinal coupling (2.0 Hz) to the oxymethine H-4, suggesting that H-4 and H-5 are gauche to one another. A large $^3J_{C,H}$ (6.0 Hz) between H-5 and C-18, along with the presence of a NOE enhancement between H-5 and H-18 suggested a syn relationship between these two centers, which is consistent with the observation of a smaller coupling (2.2 Hz) between H-5 and C-3. These data, combined with the presence of a positive NOE enhancement between H-3 and H-6, established an eclipsing orientation for C-4 and C-5, with Me-18 gauche to the hydroxyl group at C-5 as shown (Figure 1b).

Analysis of coupling constant data and NOE correlations revealed the relative configuration of C-15 to C-17 (Figure 2a). A large vicinal coupling (9.5 Hz) and the absence of an NOE enhancement between H-16 and H-17 established the anti-periplanar relationship for these two protons (Figure 2b). A NOESY experiment (in DMSO-d_6) revealed an NOE enhancement between H-22 and the hydroxyl proton at C-16, establishing the anti relationship of the two oxygen functionalities (hydroxyl at C-16 and O-acyl at C-17). This is supported by the fact that in linear, *threo* 1,2-dioxy systems, the anti rotomer would be disfavored due to steric (C↔C) and electrostatic (O↔O) repulsion.[25] An NOE enhancement between H-16 and H-22, and a small $^3J_{C,H}$ of 2.5 Hz between H-16 and C-22 indicated a dihedryl angle of approximately 60° for these atoms, further supporting the proposed anti orientation of H-16 and H-17.

The proposed relative configuration of C-16 and C-17 was confirmed by analysis of derivatives prepared from **1**. Treatment of leiodolide A with sodium methoxide (MeONa) in methanol, cleaved the lactone linkage to give the methyl ester, linear diol **4**. Reaction of **4** with 2,2-dimethoxypropane and pyridinium *p*-toluenesulfonate provided the five-membered ring acetonide **5**, which was purified by reversed-phase HPLC. The NOESY spectrum of **5** revealed NOE enhancements between H-16 and H-17 and between H-21 and H-22, establishing the cis orientation of H-16 and H-17 in acetonide **5** (Figure 3). A very small coupling constant (0.5 Hz) between H-15 and H-16 (Figure 2c) suggested a system in which both protons are opposite the oxygen atoms.[25] A small $^3J_{C,H}$ (2.5 Hz) observed for H-16 and C-14 indicated a gauche orientation for these atoms. NOE enhancements (H-17 to H-15 and Me-21; C-16 OH to H-14 and Me-21; H_2-14 to H-16) established the configuration of C-15 relative to C-16, with the C-21 methoxyl anti to H-16 (Figure 2c). The relative configuration of a 1,3-methine system can be determined if the C-2 diastereotopic methylene protons can be assigned.[25] Although it was possible to determine both the ^{1}H chemical shifts and the coupling constants of the C-14 methylene protons in DMSO-d_6, the presence of multiple NOE enhancements (H-14a to H-13, H-14b to H-13, H-14a to Me-20, and H-14b to Me-20), and intermediate vicinal coupling constants made an assignment of the C-13 stereochemistry relative to C-15 via C-14 difficult.

The absolute stereochemistry of leiodelide A was determined by derivatization with methoxy trifluoromethyl phenyl acetic acid (MTPA) using the modified Mosher's method.[27] Unexpectedly, the major products from reactions of **1** with the *R* and *S*-MTPA acid chlorides were the tris-MTPA esters, C-29 methyl esters **6** and **7 (**Figure 4 and Figures S6 and S7, Supporting Information), respectively. These esters presumably formed via a mixed anhydride intermediate that was subsequently displaced by methanol when the reaction was quenched. This type of side reaction has been observed previously for carboxylic acids in the presence of MTPA.[28,29] Nonetheless, assignment of the $\Delta\delta_{(S-R)}$ values from the resulting tris-MTPA ester products was possible (Figure 4). The large negative value of H-6 and large positive value of H-18 suggested that the configuration of C-5 is *R*. Interpretation of the $\Delta\delta_{(S-R)}$ values from the other surrounding protons, however, was somewhat ambiguous (Figure 4). It is reasonable to assume that the small negative values observed for H-2, −3, and −4 are the result of transannular interference from the C-16 MTPA ester, which exerts negative values in neighboring protons. Similarly, the small positive $\Delta\delta_{(S-R)}$ values for H-9 and H-19 could be a result of the C-16 MTPA ester. Although the data suggests a 4*S*, 5*R* configuration, we consider this interpretation tenuous. Unfortunately, limited material prevented further approaches to analyze these centers. In contrast, evaluation of the other stereogenic centers was less ambiguous. Although the $\Delta\delta_{(S-R)}$ value for H-21 did not show the expected sign, the values for H-11, −12, −13, −20, −14, −17, −22, and −30 were suggestive of a 15*R*, 16*S*, 17*R* configuration.

An approach to the assignment of stereochemistry at C-28 was to isolate this center through degradation. Ozonolysis of **1**, followed by oxidative workup in hydrogen peroxide and methylation with diazomethane, resulted in the formation of a mixture of methyl esters, which included a small diester that exhibited an identical HPLC-MS retention time and molecular weight with an authentic sample of dimethyl citramalate. Chiral

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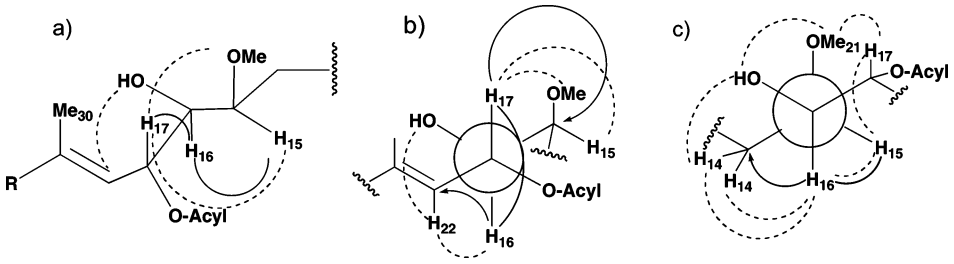

FIGURE 2. Depiction of the relative stereochemistry from C-15 to C-17 in leiodolide A (**1**): (a) sawhorse representation, (b) Newman projection of C-16 and C-17, and (c) Newman projection of C-15 and C-16. Important NOEs are illustrated with dashed lines. Long-range proton−carbon couplings are indicated with solid arrows (H→C), whereas vicinal proton−proton couplings are indicated with solid lines (H−H). See the text for coupling constants ($^3J_{C,H}$ and $^3J_{H,H}$).

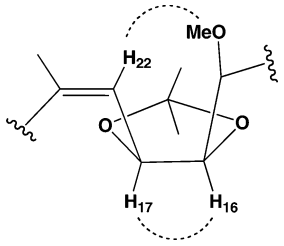

FIGURE 3. Proposed relative stereochemistry of the *cis*-acetonide in **5**. Important NOE enhancements are illustrated with dashed lines. Truncated portion of the molecule shown for clarity.

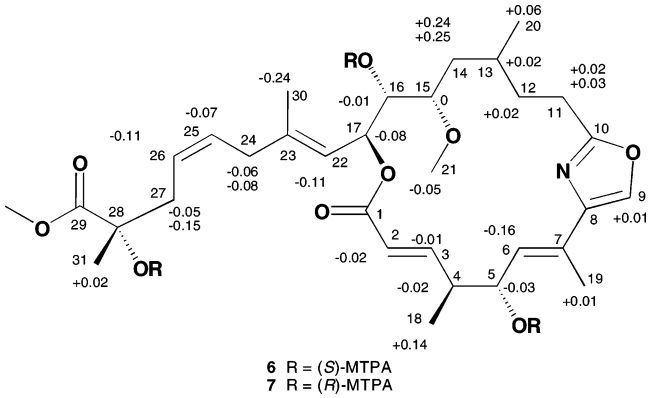

FIGURE 4. $\Delta\delta_{(S-R)}$ values (in ppm) of the MTPA triesters **6** and **7**.

GC analysis of the mixture co-injected with *S*- and *R*- dimethyl citramalate confirmed the C-28 = *S* configuration in leiodolide A (Figure 5). It should also be noted that a small amount of the 28-*R* epimer was present (Figure 5c; data not shown), suggesting that the biosynthetic formation of this remote chiral center is not stereospecific. The presence of an α-oxy-α,α-disubstituted acetic acid moiety has been reported for natural products such as quinic acid derivatives[30] and okadaic acid derivatives.[31] On the basis of these overall experiments, the absolute stereochemistry of leiodolide A was assigned as 4*S*, 5*R*, 15*R*, 16*S*, 17*R*, 28*S*. The stereochemistry of the methyl-bearing carbon, C-13, could not be defined by these experiments.

Leiodolide B (**2**) analyzed by high-resolution MALDI-MS for the molecular formula $C_{31}H_{44}{}^{79}BrNO_9$ (*m*/*z* = 676.2074, $[M + Na]^+$, Δ −2.7 ppm), the isotope pattern of which illustrated the presence of one bromine atom. The spectral data for **2**

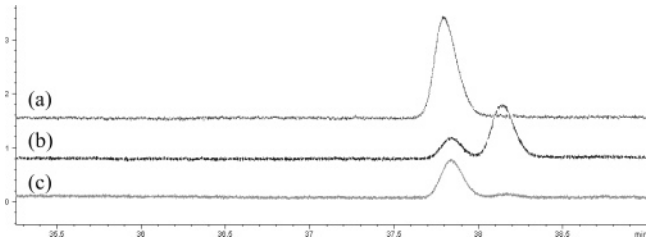

FIGURE 5. Overlay of chiral gas chromatograms for the ozonolysis product of **1** co-injected with *R*- and *S*-dimethyl citramalate standards: (a) ozonolysis product + *S*-dimethyl citramalate, (b) ozonolysis product + *R*-dimethyl citramalate, and (c) ozonolysis product alone. The *x*-axis represents time (min), and the *y*-axis represents flame ionization detection levels (pA).

were similar to those of **1** (Table S1, Supporting Information), suggesting that it was a related macrolide. Detailed examination of the ^1H and ^{13}C NMR data revealed that certain portions of the molecule (C-1 to C-14; C-23 to C-28) were fundamentally conserved, while other centers were altered. In particular, the oxymethine carbons C-16 (δ_C 80.5) and C-17 (δ_C 78.4) were shifted downfield and the multiplicities of H-16 and H-17 were noticeably modified in **2**. In addition, the C-22 methine (δ_H 4.28, δ_C 56.2) appeared to be the site of bromine incorporation, while the quaternary carbon at C-23 (δ_C 84.0) was oxygenated in this macrolide (Table S1). The disappearance of the $\Delta^{22,23}$ olefin, despite the fact that **2** has the same number of double bond equivalents as **1**, introduced the possibility of an additional ring. These data, combined with chemical reasoning, suggested an ether linkage between C-16 and C-23 forming a five-membered tetrahydrofuran ring. Overall NMR data supported this conclusion. NOE enhancements from H-17 to Me-30 and from H-16 to H-22 established the relative configuration around the ring (Figure 6). The proposed mechanism for the formation of the 16,23-ether bridge in **2** from **1** is illustrated in Scheme 1. In what appears to be an enzymatic halogenation, addition of an electrophilic bromine to the $\Delta^{22,23}$ alkene, followed by trapping of the resulting electron deficient intermediate with the nucleophilic C-16 hydroxyl group, generates the bromo-tetrahydrofuran ring. Assuming that **1** is the biosynthetic precursor of **2**, the relative configuration of the C-15 to C-17 unit in **2** provides additional support for the proposed orientation of these centers in **1**. The rigid five-membered ring formed as a result of the 16,23-ether bridge in **2** (Figure 6) supports the use of NOE and vicinal coupling constant data to assign the stereochemistry of **1**.

The leiodolides are the first members of a new class of apparent mixed polyketide-nonribosomal peptide sythetase (NRPS) derived natural products bearing several structural

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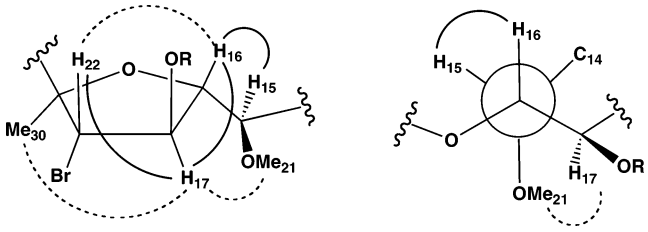
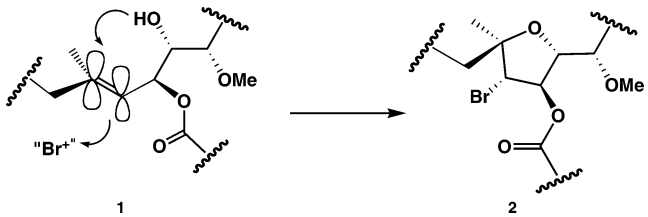


FIGURE 6. Relative stereochemistry of C-15 to C-23 in leiodolide B (**2**): (a) sawhorse representation and (b) Newman projection. Important NOEs are illustrated with dashed lines. Vicinal proton−proton couplings ($J_{H,H}$) are indicated with solid lines (H−H), with $J_{H15,H16} = 2$ Hz, and $J_{H16,H17}$ and $J_{H17,H22} = 8$ Hz.

SCHEME 1. Plausible Mechanism for the Bromonium Ion Induced Formation of the Tetrahydrofuran 16,23-Ether Bridge in 2 from 1[a]



[a] Truncated portion of the molecule shown for clarity.

features of biogenetic interest. Although we have no direct evidence, the oxazole unit is generally considered to be derived from the amino acid serine. The α,α-disubstituted carboxylic acid, which is observed in several other natural products, may be formed by a Baeyer−Villiger oxidation of an intact acetate unit followed by a dehydrogenase-catalyzed oxidation to introduce the α-hydroxyl, as has been proposed for the biosynthesis of okadaic acid.[32] Alternatively, the branches at C-28 may be derived from an HMGCoA synthase-like motif, as has been observed in the curacin A and jamaicamide A biosynthetic gene clusters.[33,34] Although these compounds share some structural features with other lithistid metabolites such as theonezolide,[7] they do not appear to be closely related biosynthetically.

In initial biotesting, leiodolides A and B were found to be significantly cytotoxic to HCT-116 human colon carcinoma, with IC_{50} values of 1.4 μg/mL (2.5 μM) and 3.8 μg/mL (5.6 μM), respectively. Methyl ester **3** exhibited roughly the same IC_{50} as **1** (1.9 μM), suggesting that the carboxyl group in **1** is not required for activity. The biological activity of leiodolide A was also examined in the National Cancer Institute's 60-cell line assay. In these oncologically diverse cell lines, leiodolide A showed enhanced potency against a number of unrelated cell lines. GI_{50} values of 0.26 μM, 0.26 μM, and 0.25 μM were recorded for HL-60 (leukemia), NCI-H522 (nonsmall cell lung cancer), and OVCAR-3 (ovarian cancer) cell lines, respectively.

Experimental Section

Leiodolide A (1): pale yellow oil; CD (MeOH) $[\theta]_{233}$ +11.9, $[\theta]_{215}$ −11.0; UV (MeOH) λ_{max} (log ϵ) 224 (3.2); IR υ_{max} (MeOH) 3400 (br), 2930, 1715, 1650, 1585, 1455, 1410, 1360, 1275, 1235, 1180, 1140, 1090, 1025, 990 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; (+)-LREIMS *m/z* (rel int) 350 (35), 508 (13), 526 (15), 551 (8), 558 (55), 576 (5), 598 (100), 599 (40), 600 (8); HRMALDIMS $[M + Na]^+$ *m/z* 598.2992 (calcd for $C_{31}H_{45}NO_9$-Na, 598.2987).

Leiodolide B (2): pale yellow oil; CD (MeOH) $[\theta]_{234}$ +6.2, $[\theta]_{212}$ −8.3; UV (MeOH) λ_{max} (log ϵ) 221 (2.8); IR υ_{max} (MeOH) 3400 (br), 2935, 1715, 1655, 1460, 1415, 1360, 1270, 1225, 1200, 1140, 1095, 990, 655 cm^{-1}; for ^1H and ^{13}C NMR data see Table S1; (+)-LREIMS *m/z* (rel int) 557 (6), 577 (8), 598 (15), 654 (100), 655 (35), 656 (80), 657 (30), 658 (10), 676 (78), 677 (27), 678 (73), 679 (25), 680 (8); MALDIMS $[M + Na]^+$ *m/z* 676.2074 (calcd for $C_{31}H_{44}{}^{79}BrNO_9Na$, 676.2092).

Preparation of Methyl Ester 3. Several drops of ethereal diazomethane (~0.66 M) were added to a portion of leiodolide A (**1**, 1.0 mg) dissolved in 0.5 mL of MeOH, and the solution was stirred overnight. The solvents were then removed under a stream of N_2, and the methyl ester **3** was obtained in roughly quantitative yield by reversed-phase HPLC (PRP-1 semi-prep; 25% to 100% acetonitrile/water; 2 mL/min.). For methyl ester **3**: ^1H NMR (CD_3-OD) δ 0.97 (3H, d, H-20), 1.25 (3H, d, H-18), 1.35 (3H, s, H-31), 1.60 (1H, m, H-12a), 1.61 (2H, m, H-14), 1.78, (3H, d, H-30), 1.92 (3H, d, H-19), 2.04 (1H, m, H-12b), 2.30 (1H, m, H-27a), 2.39 (2H, m, H-4), 2.49 (1H, m, H-27b), 2.71 (1H, m, H-11a), 2.77 (1H, m, H-24a), 2.81 (1H, m, H-24b), 2.83 (1H, m, H-11), 3.22 (1H, m, H-15), 3.38 (3H, s, H-21), 3.44 (1H, d, H-16), 3.68 (3H, s, CO_2Me), 4.54 (1H, dd, H-5), 5.15 (1H, d, H-22), 5.47 (1H, m, H-25), 5.54 (1H, m, H-26), 5.70 (1H, d, H-2), 5.75 (1H, t, H-17), 6.31 (1H, d, H-6), 6.89 (1H, m, H-3), 7.67 (1H, s, H-9); HRFABMS $[M + H]^+$ *m/z* 612.3142 (calcd for $C_{32}H_{47}NO_9Na$, 612.3150).

Methanolysis of Leiodolide A (1). Leiodolide A (**1**, 2.6 mg) was treated with 0.1 M NaOMe (2 mL) and stirred for 4 h. The reaction was quenched with 2 N HCl and partitioned between EtOAc and H_2O to obtain methyl ester **4**. For compound **4**: ^1H NMR (CD_3OD) δ 0.98 (3H, d, H-20), 1.25 (3H, d, H-18), 1.28 (1H, m, H-13), 1.36 (3H, s, H-31), 1.63 (1H, m, H-12a), 1.65 (2H, m, H-14), 1.71, (3H, d, H-30), 1.95 (3H, d, H-19), 2.04 (1H, m, H-12b), 2.30 (1H, m, H-27a), 2.39 (2H, m, H-4), 2.49 (1H, m, H-27b), 2.71 (1H, m, H-11a), 2.77 (1H, m, H-24a), 2.81 (1H, m, H-24b), 2.79 (1H, m, H-11b), 3.18 (1H, m, H-15), 3.38 (3H, s, H-21), 3.56 (1H, d, H-16), 3.71 (3H, s, CO_2Me), 4.60 (1H, dd, H-5), 5.30 (1H, d, H-22), 5.54 (1H, m, H-25), 5.70 (1H, m, H-26), 5.72 (1H, t, H-17), 5.89 (1H, d, H-2), 6.17 (1H, d, H-6), 7.03 (1H, dd, H-3), 7.76 (1H, s, H-9); (+)-EIMS *m/z* = 630 $[M + Na]^+$; (−)-EIMS *m/z* = 606 $[M - H]^-$.

Preparation of Acetonide 5. To a solution of crude methanolysis product **4** (2.6 mg) in 2,2-dimethoxypropane (3 mL) and anhydrous methanol (1.5 mL) was added a small amount of pyridinium *p*-toluenesulfonate (PPTS). After the mixture was stirred at 4 °C for 24 h, saturated aq $NaHCO_3$ was added, and reaction mixture was extracted with EtOAc. The organic layer was dried over anhyd Na_2SO_4 and concentrated in vacuo to obtain the acetonide **5** in impure form. The mixture was fractionated by reversed-phase HPLC (PRP-1 semi-prep, 30% acetonitrile, 2 mL/min) to obtain acetonide **5** (0.3 mg). For compound **5**: ^1H NMR (CD_3CN) δ 0.86 (3H, d, H-20), 1.19 (3H, d, H-18), 1.28 (1H, m, H-13), 1.40 (3H, s, H-31), 1.58 (1H, m, H-12a), 1.62 (2H, m, H-14), 1.64, (3H, d, H-30), 1.79 (3H, d, H-19), 2.04 (1H, m, H-12b), 2.30 (1H, m, H-27a), 2.50 (1H, m, H-4), 2.50 (1H, m, H-27b), 2.71 (1H, m, H-11a), 2.77 (1H, m, H-24a), 2.81 (1H, m, H-24b), 2.79 (1H, m, H-11b), 3.15 (1H, m, H-15), 3.40 (3H, s, H-21), 4.01 (1H, d, H-16), 3.67 (3H, s, CO_2Me), 4.45 (1H, dd, H-5), 4.70 (1H, t, H-17), 5.24 (1H, d, H-22), 5.42 (1H, m, H-25), 5.62 (1H, m, H-26), 5.85 (1H, d, H-2), 6.13 (1H, d, H-6), 6.70 (1H, dd, H-3), 7.63 (1H, s, H-9); HRFABMS $[M + H]^+$ *m/z* 648.3753 (calcd for $C_{35}H_{54}NO_{10}$, 648.3742).

MTPA Esters of 1. Leiodolide A (**1**, 1.2 mg) was reacted in separate experiments with *R*- and *S*-MTPACl (20 μL) in 1 mL of pyridine containing 10 mg of DMAP for 2 h. The reaction mixtures were quenched with several drops of MeOH and partitioned between

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CH_2Cl_2 and water. The organic layer was dried over anhyd Na_2SO_4, and the solution was concentrated in vacuo and then fractionated by reversed-phase HPLC (Phenomenex Luna C18 semi-prep, 50−100% acetonitrile, 2 mL/min) to yield *S*- and *R*-MTPA esters (**6** and **7**, respectively). For compound **6**: ^{1}H NMR (CD_3CN) δ 0.91 (3H, d, H-20), 1.25 (3H, d, H-18), 1.29 (1H, m, H-14a), 1.51, (3H, d, H-30), 1.55, (1H, m, H-13), 1.61 (3H, s, H-31), 1.65 (1H, m, H-14b), 2.07 (H, m, H-12), 2.10 (3H, d, H-19), 2.45 (1H, m, H-27b), 2.47 (1H, m, H-24a), 2.50 (1H, m, H-27a), 2.52 (1H, m, H-24b), 2.62 (1H, m, H-4), 2.71 (1H, m, H-11a), 2.82 (1H, m, H-11b), 3.24 (3H, s, H-21), 3.50 (1H, m, H-15), 3.45 (3H, s, O-CH_3), 3.53 (3H, s, O-CH_3), 3.58 (3H, s, O-CH_3), 3.74 (3H, s, CO_2Me), 5.04 (1H, d, H-22), 5.26 (1H, d, H-16), 5.34 (1H, m, H-25), 5.34 (1H, m, H-26), 5.78 (1H, d, H-2), 5.83(1H, t, H-17), 5.87 (1H, dd, H-5), 6.19 (1H, d, H-6), 6.74 (1H, dd, H-3), 7.40−7.62 (15H, m, phenyls), 7.74 (1H, s, H-9); MALDIMS $[M + H]^+$ *m*/*z* 1238.4523 (calcd for $C_{62}H_{69}NO_{15}F_9$, 1238.4518). For compound **7**: ^{1}H NMR (CD_3CN) δ 0.85 (3H, d, H-20), 1.05 (1H, m, H-14a), 1.11 (3H, d, H-18), 1.40 (1H, m, H-14b), 1.53, (1H, m, H-13), 1.59 (3H, s, H-31), 1.75, (3H, d, H-30), 2.12 (H, m, H-12), 2.09 (3H, d, H-19), 2.55 (1H, m, H-24a), 2.55 (1H, m, H-27a), 2.58 (1H, m, H-24b), 2.60 (1H, m, H-27b), 2.64 (1H, m, H-4), 2.69 (1H, m, H-11a), 2.79 (1H, m, H-11b), 3.28 (3H, s, H-21), 3.50 (1H, m, H-15), 3.49 (3H, s, O-CH_3), 3.50 (3H, s, O-CH_3), 3.60 (3H, s, O-CH_3), 3.73 (3H, s, CO_2Me), 5.15 (1H, d, H-22), 5.27 (1H, d, H-16), 5.41 (1H, m, H-25), 5.45 (1H, m, H-26), 5.81 (1H, d, H-2), 5.89 (1H, dd, H-5), 5.90 (1H, t, H-17), 6.34 (1H, d, H-6), 6.74 (1H, dd, H-3), 7.40−7.62 (15H, m, phenyls), 7.73 (1H, s, H-9); MALDIMS $[M + H]^+$ m/z = 1238.4512 (calcd for $C_{62}H_{69}NO_{15}F_9$, 1238.4518).

Absolute Stereochemistry at C-28 in Leoidolide A by Ozonolysis. A sample of leiodolide A (**1**, 1.0 mg) was dissolved in 0.3 mL of MeOH and cooled to −78 °C. Ozone was bubbled through the solution for 2 min and warmed to room temperature. After complete reaction of starting material had been confirmed by TLC analysis, the solution was oxidized by addition of 0.1 mL of 35% H_2O_2 and stirred for 24 h, after which time solvent was removed under a stream of N_2 and in vacuo. The ozonolysis product was dissolved in 0.5 mL MeOH, and excess ethereal diazomethane was added (prepared as described above). After 1 h, excess reagent and solvent were removed under a stream of N_2 and in vacuo, and the residue was analyzed by LC-MS (C18 Phenomenex Luna, 10−100% CH_3CN, flow rate 0.7 mL/min, detected at 254 nm). The presence of dimethyl citramalate was confirmed by the presence of a peak with the same retention time (t_R = 5.7 min) and molecular ion [(+)-EIMS m/z = 199 $[M + Na]^+$] as authentic dimethyl citramalate prepared from citramalic acid in the same manner. The dimethyl citramalate product was next analyzed by chiral GC [Chiraldex B-DP capillary column (0.25 mm × 30 m)]. The oven was maintained at 60 °C for 10 min and then slowly ramped up (60−80 °C at 1 °C/min, 80−90 °C at 0.5 °C/min). The ozonolysis and methylation product was confirmed as *S*-dimethyl citramalate by co-injection with a solution of authentic *S*- and *R*-dimethyl citramalate standards (Figure 5).

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Supporting Information Available: Spectral data for the leiodolides A (**1**) and B (**2**) and MTPA esters **6** and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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