JOURNAL OF NATURAL PRODUCTS

Volume 65, Number 3 March 2002

Full Papers

(+)-7*S*-Hydroxyxestospongin A from the Marine Sponge *Xestospongia* sp. and Absolute Configuration of (+)-Xestospongin D

Surk-Sik Moon,[†] John B. MacMillan, Marilyn M. Olmstead, Tram Anh Ta,[‡] Isaac N. Pessah,[‡] and Tadeusz F. Molinski*

Departments of Chemistry, College of Letters and Science, and Molecular Biosciences, School of Veterinary Medicine, One Shields Avenue, University of California, Davis, California 95616

Received August 27, 2001

The structure of the title compound, (+)-7*S*-hydroxyxestospongin A was solved by single-crystal X-ray diffraction analysis and the absolute stereochemistry obtained by analysis of the derived *R* and *S* Mosher's esters. The absolute configuration of xestospongin D was determined for the first time by analysis of anomalous scattering from the X-ray crystal diffraction data set. Xestospongins A, C, and D, araguspongine C, and demethylxestospongin B exhibited modest antifungal activity (MIC 30−100 g/mL) against various fluconazole-resistant *Candida* spp., but 7*S*-hydroxyxestospongin A was inactive.

Naturally occurring dimeric 2,9-disubstituted 1-oxaquinolizidine alkaloids (+)-xestospongin A (XeA, **1**), (−)-xestospongin C (XeC, **2**), and (+)-xestospongin D (XeD, **3**) (Figure 1) were reported by Nakagawa and co-workers in 1984 from the sponge *Xestospongia exigua*.[1] The compounds exhibited in vivo vasodilatory activity. Our interest in these compounds stemmed from our observation of the antifungal activity of (−)-**2** together with its potent inhibition of the IP3-sensitive Ca^{2+} channel and blockade of Ca^{2+} release from endoplasmic reticulum (ER) stores.[2] Additional members of the family have been reported by Kitagawa (araguspongines B ((−)ArB, **4**), ArC-J, **5**−**9**),[3] Faulkner (3*β*,3′*β*-dimethylxestospongin C, **10**),[4] and others.[5] The latter compounds are susbtituted as 3-methyl-, 3,3′-dimethyl-,9-hydroxy-, or 9,9′-dihydroxy derivatives of the parent bis-1-oxaquinolizidine skeleton.

The stereochemistry of the xestospongin/araguspongine alkaloids is complex, and it is clear that several stereostructural issues remain unresolved. While it appears most alkaloids from the family are enantiomerically pure, some have been isolated as nonracemic mixtures of enantiomers. Nakagawa isolated dextrorotatory (+)-XeD **3** ($[\alpha]_D$ +18.4°),[1] of undetermined absolute configuration, while the Pettit group found racemic (±)-**3** in a specimen of *Niphates* sp. from Singapore.[6] Kitagawa isolated racemic (±)-ArB and resolved the (+)- and (−)-enantiomers by chiral HPLC.[3] Likewise, Kitagawa's sample of ArE ($[\alpha]_D$ −1.1°, *c* $CHCl_3$), which is identical by NMR to **2**, was stated to be a "3:2" mixture of (+)-ArE ($[\alpha]_D$ −1°) and (−)-ArE ($[\alpha]_D$ −2°, *c* $CHCl_3$, cf. **2** $[\alpha]_D$ −2.4° lit.[1]).[37]

The substituted 1-oxaquinolizidines are stereochemically labile. Upong heating any one of XeA (**1**), XeC (**2**), or ArB (**4**), in the presence of alumina, an equilibrium mixture of all three compounds is obtained (ratio ~58:26:6, respectively).[8] The isomerization has been rationalized by Lewis acid-catalyzed ring opening of the oxaquinolizidine ring to a hydroxy iminium salt followed by imine−enamine isomerism, tertiary nitrogen inversion, and re-closure of the ring.[3] A useful stereochemical corollary emerges from this experiment that is applicable to other alkaloids in this series: 2,9-*anti*-disubstituted 1-oxaquinolizidine rings always have *trans*-ring junctions at N/C-10, and 2,9-*syn*−disubstituted analogues have *cis*-ring junctions. This observation is empirically supported by the structures of all known 1-oxaquinolizidines and supported by conformational analysis and molecular mechanics.[9]

* To whom correspondence should be addressed. Tel: 530 752 6358. Fax: 530 752 8995. E-mail: tfmolinski@ucdavis.edu.
[†] Permanent address: Department of Chemistry, Kongju National University, Kongju 314701, South Korea.
[‡] Molecular Biosciences.

10.1021/np010427z CCC: $22.00
Published on Web 01/30/2002

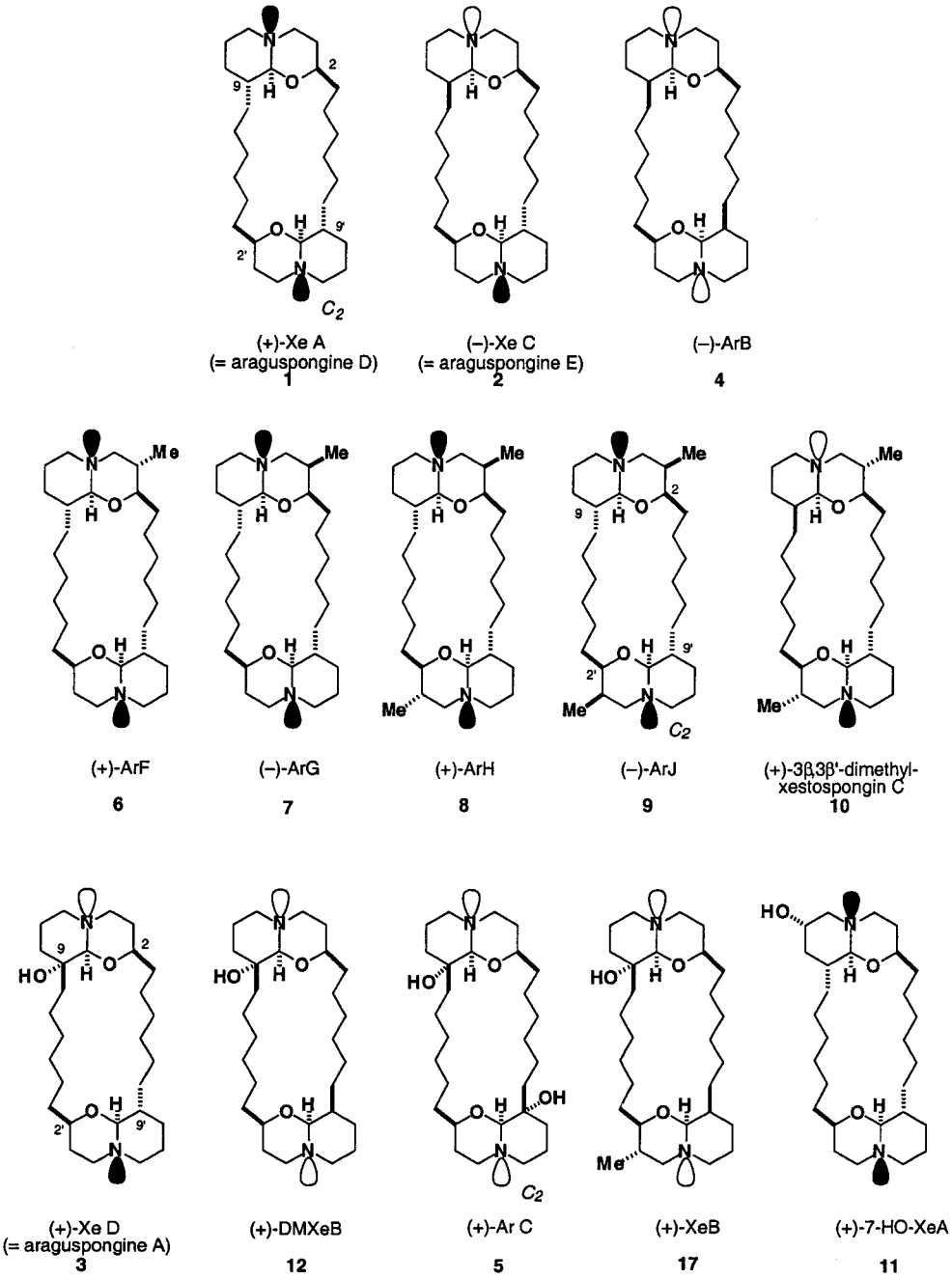


Figure 1. Xestospongins and araguspongines. Depiction of absolute configuration for (+)-**1**, (−)-**2**, and **4** are from Baldwin's assignment,[2] while those of the unique araguspongines are from Kitagawa et al.[3] The absolute stereochemistry shown for (+)-7-hydroxyxestospongin A (**11**) and (+)-xestospongin D (**3**) are determined in this work, and those of the remaining compounds are unassigned.

Assignments of absolute configurations of some araguspongines were made on the basis of molar rotations,[3] the Horeau's method,[3,10] model studies,[9] and synthesis,[11] but some of these assignments have been been contentious. Hoye's asymmetric synthesis of (+)-**1** and (+)-**2**, which pivoted on an enzymic kinetic resolution step, was followed by Baldwin's biomimetic syntheses[12] of the enantiomers of (+)-XeA (**1**), (−)-XeC (**2**), and (−)-ArB (**4**), which resulted in reassignment of their absolute configurations to those depicted in Figure 1; however, none of the configurations of other members in this series have been verified independently. In our search for antifungal compounds we identified extracts of *Xestospongia* sp. with moderate activity against fluconazole-resistant *C. albicans* and potent activity in blockade of Ca^{2+} from the IP3-sensitive calcium channel. In this paper we describe the isolation and unambiguous structure elucidation, including absolute configuration, of a new compound, (+)-7-hydroxyxestospongin A (**11**), and the absolute configuration of XeD (**3**).[1] Compound (+)-**11** represents a departure from this alkaloid series as the first example with oxidation at C-7, rather than C-2 or C-9. The configurations of **3** and **11** were determined by the modified Mosher's ester method and anomalous X-ray scattering, respectively, and firmly place both compounds in the configurational series assigned to (+)-**1**, (−)-**2**, and (−)-**4** by Baldwin.[12]

Xestospongia sp., collected in Western Australia (1993), was extracted exhaustively with MeOH, and the combined extracts were concentrated and partitioned against solvents of increasing polarity (*n*-hexane, $CHCl_3$, *n*-BuOH). The *n*-butanol fraction was further subjected to repeated silica gel chromatography (gradient of NH_3-saturated MeOH in $CHCl_3$) to obtain a series of fractions, which were individually analyzed by ion-trap MS and further purified by

Table 1. ^{1}H (400 Mz) and ^{13}C NMR (100 MHz) of **11** in $CDCl_3$

no.	^{1}H NMR	^{13}C NMR	no.	^{1}H NMR	^{13}C NMR
2	3.34 (bt, J = 9.6 Hz)	75.2[a]	2′	3.34 (bt, J = 9.6 Hz)	75.3[a]
3	1.2−1.4 (m)	31.9	3′	1.2−1.4 (m)	32.2
4α	2.93 (m)	54.0	4α′	2.93 (m)	54.3
4β	2.23 (dt, J = 3.2, 12.0, 3.2 Hz)		4β′	2.16 (ddd, J = 12.0, 12.0, 3.2 Hz)	
6α	2.91 (m)	60.6	6α′	2.74 (d, J = 10.0 Hz)	54.0
6β	1.93 (t, J = 11.2 Hz)		6β′	1.97 (ddd, J = 11.2, 10.0, 3.2 Hz)	
7	3.87 (tt, J = 11.2, 4.6 Hz)	66.7	7′	1.4 (m)[c]	24.8
8α	1.96 (m)	38.0	8α′	1.4 (m)[c]	28.9
8β	1.25 (m)		8β′	1.4 (m)	
9	1.66 (m)[b]	38.8	9′	1.67 (m)[b]	40.5
10	3.07 (d, J = 8.8 Hz)	95.8	10′	3.04 (d, J = 8.4 Hz)	94.8
11,11′	1.2−1.4 (m)[d]	31.6, 31.2			
12,12′	1.2−1.4 (m)[d]	28.7 (× 2)			
13,13′	1.2−1.4 (m)[d]	25.0, 25.2[c]			
14,14′	1.2−1.4 (m)[d]	25.0, 25.2[c]			
15,15′	1.2−1.4 (m)[c]	25.2[c]			
16,16′	1.2−1.4 (m)[c]	35.3, 35.2[c]			

[a−c] Interchangeable. [d] Obscured, interchangeable.

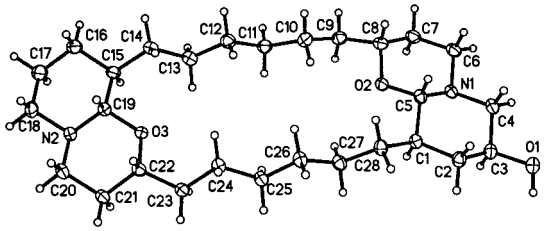


Figure 2. X-ray structure of (+)-7-hydroxyxestospongin A (**11**) showing 50% thermal ellipsoids. Correct absolute configuration is depicted.

fractional crystallization (acetone) and HPLC. The new compound (+)-7-hydroxyxestospongin A (**11**) was obtained after crystallization from acetone {mp 135−136 °C, $[\alpha]_D$ +4.7° (c 0.25, $CHCl_3$)} along with pure crystalline samples of (+)-**1** {$[\alpha]_D$ +10.8° (c 0.473, $CHCl_3$) [lit.[1] mp 135−136 °C; $[\alpha]_D$ +6.9° (c 0.84, $CHCl_3$)]}, (−)-**2** {mp 146−147 °C, $[\alpha]_D$ −2.7° (c 0.34, $CHCl_3$) [lit.[1] mp 149−150 °C; $[\alpha]_D$ −2.4° (c 0.54, $CHCl_3$), −1.2° ($CHCl_3$),[11b] $[\alpha]_D$ −2° ($CHCl_3$)$_3$]}, (+)-**3** {mp 158−159 °C, $[\alpha]_D$ +14.8° (c 0.433, $CHCl_3$) [lit.[1] mp 156−157 °C; $[\alpha]_D$ +18.43° (c 0.433, $CHCl_3$)]}, (+)-demethylxestospongin B (**12**) {mp 133−134 °C, $[\alpha]_D$ +5.2° (c 0.25, $CHCl_3$) [lit.[13] +6°, c 0.8]}, and (+)-araguspongine C (**5**) {mp 164−165 °C, $[\alpha]_D$ +25° (c 0.32, $CHCl_3$) [lit.[3] +11.1°, $CHCl_3$]}. The known compounds were identified by comparison of ^{1}H NMR, ^{13}C NMR, ESI MS, melting points, and optical rotations with literature values.

Compound (+)-**11** was the most polar of all compounds isolated in this study (R_f 0.22 2:98 MeOH (NH_3)/$CHCl_3$). The formula for (+)-**11** was established as $C_{28}H_{50}N_2O_3$ by HRMS (m/z 463.3915, for MH^+), which indicated a compound isomeric with XeD (**3**). The presence of Bohlman bands (ν 2750 and 2807 cm^{-1}) in the FTIR spectrum of **11** confirmed the presence of a *trans*-substituted quinolizidine, and a broad stretch (ν 3100−3500 cm^{-1}) was assigned to OH. Initial inspection of the ^{1}H NMR and ^{13}C NMR spectra clearly ruled out a tertiary alcohol, but implicated a secondary alcohol with placement of an equatorial hydroxyl group between two methylenes in a six-membered ring (δ_H 3.87, tt, J = 11.2, 4.6 Hz, H-7; δ_C 66.7, CH-7). Confirmation of the structure was obtained from X-ray diffraction of single crystals of (+)-**11** grown by slow evaporation from acetone (see Figure 2). As expected, (+)-**11** has 2,9- and 2′,9′-*anti*-disubstituted *trans*-quinolizidine rings, and all bond lengths and torsional angles are unexceptional. Two molecules of **11** crystallized in the unit cell with a strong *inter*molecular hydrogen bond between the secondary OH group at C-7 of the first molecule and the N atom of the nonhydroxylated 1-oxaquinolizidine ring in the second

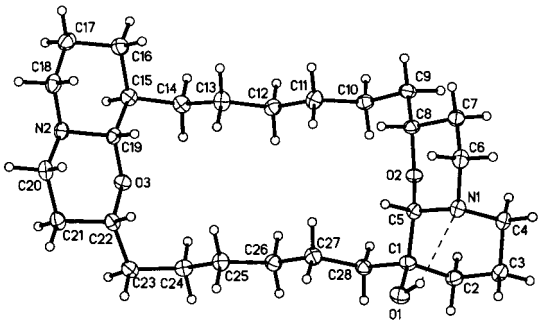
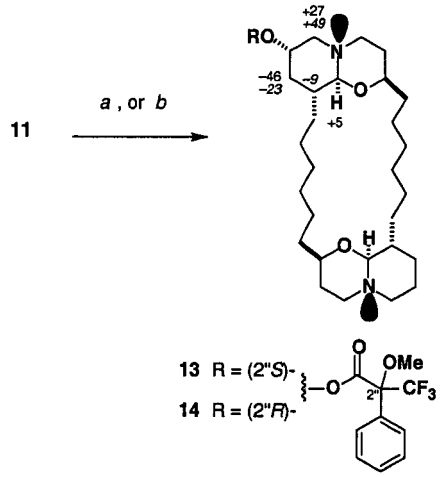


Figure 3. X-ray structure of (+)-xestospongin D (**3**) showing 50% thermal ellipsoids. Correct absolute configuration is depicted.

Scheme 1. Preparation of Mosher's Esters **13** and **14** and Configurational Analysis of (+)-**11** (anisotropic changes in chemical shift are defined as $\Delta\delta = \delta_S - \delta_R$; the units displayed are $10^3 \times \Delta\delta$ (parts per billion, in $CDCl_3$), and values in italics are assigned to proton signals on the β-face of the molecule)



[a]EDCI, (*S*)-MTPA, DMAP, CH_2Cl_2, rt 46 h. [b](*R*)-MTPA-Cl, DMAP, CH_2Cl_2, rt 16 h.

molecule. All ^{1}H and ^{13}C NMR assignments (see Table 1) were secured from analysis of gCOSY, TOCSY, gHSQC, and gHMBC experiments.

The absolute configuration of (+)-**11** followed from Mosher's ester analysis.[14] Samples of (+)-**11** were converted to the respective *S* and *R* Mosher's esters (DCC, Mosher's acid, DMAP, DMF, 16−48 h) **13** and **14** (Scheme 1). Examination of the ^{1}H NMR spectra showed anisotropic shifts of H-6α,β, H-8α,β, H-9, and H-10 consistent with the stereochemical model for the 7*S* epimer of **11**. Since the

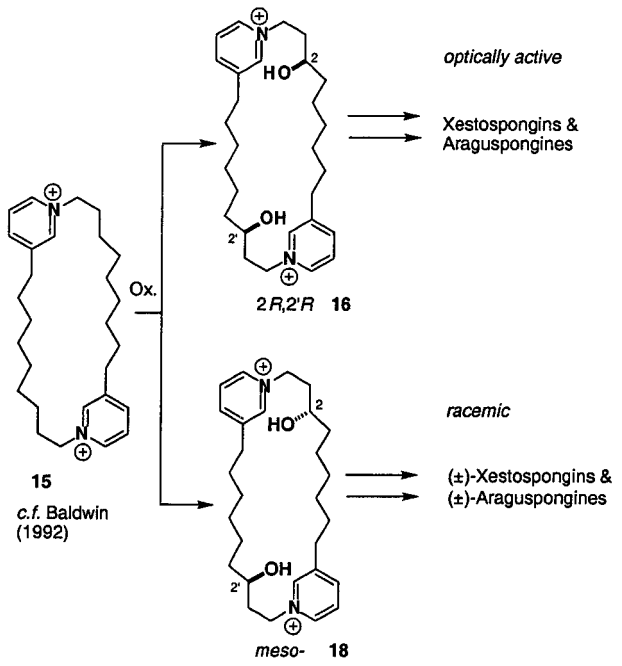
relative configuration of the natural product was obtained from X-ray, the complete configuration of (+)-**11** is (2*R*,2′*R*,5S,5′*S*,7*S*,9*R*,9*R*′,10*S*,10′*S*).

Xestospongin D (**3**) crystallized as clear parallelepipeds by slow evaporation from an acetone solution (mp 158−159 °C). As the absolute configuration of (+)-**3** had not been determined, we took advantage of our good quality crystals and access to the high-flux coherent synchrotron X-ray radiation at the Stanford Synchrotron Radiation Laboratory (SSRL) to determine the X-ray crystal structure of (+)-**3**. An excellent set of data was obtained and the structure solved in the usual manner (see Experimental Section). A weak intramolecular bond was observed between the tertiary C-9 OH group and the ring-junction N-5 [d_{NH} = 2.292(19) Å]. Although it was anticipated that the high flux of the synchrotron radiation would allow for unambiguous determination of the crystal handedness, this was not the case. The refined value of the Flack parameter was 0.3(6) and was indeterminate (the value of 0 for the correct hand and 1 for the inverted structure is expected). A successful indication was, however, obtained by the use of a copper rotating anode source and a serial detector. A set of 31 reflections was selected from those calculated to have the largest Friedel differences. A complete Laue set of each of these reflections was carefully measured. Four of these measurements corresponded to the original hand, and four corresponded to the opposite. The equivalent reflections were averaged and compared to the calculated values of *Q*, where $Q = [F_c(+)^2 - F_c(-)^2]/[F_c(+)^2 + F_c(-)^2]^{1/2}$. The correlation between the signs of the *Q*'s for observed intensities vs the calculated values was 93%. This clearly establishes the handedness to be that originally chosen and corresponds to (2*R*,2′*R*,5S,5′*R*,9*R*,9*R*′,10*S*,10′*S*)-(+)-**3**, as depicted (Figure 1). Thus, the absolute structures of both (+)-**3** and (+)-**11** are consonant with Baldwin's revised assignments.[12]

Discussion

Xestospongins are potent biologically active molecules with powerful effects on primary mammalian receptors. Xestospongin D [(+)-**3**], which like xestospongin C [(+)-**2**], is a sub-micromolar inhibitor of IP3-sensitive Ca^{2+} channels,[2] has also been described as an inhibitor of nitric oxide synthase.[15] Thus, it is important to address remaining ambiguities in the assignment of configuration for 1-oxaquinolizidine alkaloids. It must be noted that assignments of configuration of 1-oxaquinolizidine alkaloids based solely on comparison of specific rotations may be risky due to errors incurred in measuring the small-magnitude rotations observed in $CHCl_3$ for some members of the series (e.g., **2**, $[\alpha]_D$ −2.4°) and the tendency for isomerism at C-1/C-1′, C-9/C-9′, C-10/C-10′, and N-5/N-5′ in the presence of acid that may lead to inversion of sign (e.g., **2** → **1** $[\alpha]_D$ +6.9°). In this work we took great care to ensure that all optical measurements were carried out with acid-free, spectroscopic grade $CHCl_3$ and found our measured specific rotations for **1**, **2**, and **3** corresponded well to those of Nakagawa,[1] and it is most likely they are enantiopure. Unfortunately, we were unsuccessful in obtaining samples of the synthetic *ent*-compounds (−)-**1** and (+)-**2**[12] as standards to measure the exact enantiomeric composition of our natural products. Compound **11** is enantiopure; we found evidence for only one diastereomer in each of the respective preparations of Mosher's esters **13** and **14**. The configurations secured for (+)-**3** and (+)-**11** by independent methods clearly show they have the same configuration as (+)-**1**, (−)-**2**, and (−)-**4** at the nonlabile positions C-2/C-2′.

Scheme 2. Proposed Biogenesis of Optically Active and Racemic 1-Oxaquinolizidine Alkaloids



While an enzymic mechanism based on Kitagawa's catalyzed interconversion of **1**, **2**, and **4**[3] may explain the existence of multiple naturally occurring 1-oxaquinolizidine diastereomers, it would not alter the configurations of C-2/C-2′. The presence of different enantiomers of the same compound in the same organism must have a different genesis that is suggestive of a "natural" origin through stereodivergent biosynthesis, although other explanations are possible. Drawing on Baldwin's biogenic hypothesis (Scheme 2),[16] we propose the source of this heterogeneous handedness in the 1-oxaquinolizidine alkaloids may be incomplete stereochemical fidelity in the enantiospecific hydroxylation of the precursor cyclic bis-C_8-alkylated pyridinium salt **15** (quinolizidine numbering) to the C_2-symmetric precursor (2*R*,2′*R*)-**16**.[17] Dimeric salt **15** is a reasonable precursor to the xestospongin/aragusponginé family. Indeed, it is necessary and sufficient to account for biosynthesis of the diastereomeric series (+)-**1**, (−)-**2**, and (+)-xestospongin B (**17**).[1] Similar cyclo-C_8-alkylated tetrahydropyridine dimers have been isolated as natural products from *Haliclona* sp.,[18] and higher order 3-alkylpyridinium oligomers are known from *Callyspongia fibrosa*[19a] and other sponges. Cyclostelletamines A−F, which are cyclo-C_{12} and C_{15}-alkylated pyridinium analogues of **15**, were reported from *Stelletta maxima*.[20] Assuming that enzymic hydroxlyation of **15** sets the C-2/C-2′ configurations of xestospongins/aragusponginés and the fact that the remaining stereogenic centers can be set by base- or acid-catalyzed ring-opening−isomerism−tautomerism,[3] an intermediate, necessary and sufficient to account for biosynthesis of racemic forms of the 1-oxaquinolizidine natural products, would be *meso*-**18**. Because the stereocenters of dimeric salt **18** are of opposite configuration (**18** has a center of symmetry, *i*, or S_2 2-fold axis of symmetry),[21] the two products arising from reductive modification of each of the two pyridine rings would have different configurations. Assuming the pyridine reduction is independent of C-2/C-2′ configuration, completion of the terminal biosynthetic steps would result in a racemic natural product.

Biological Activity. Compounds **1**, **2**, **3**, and **12** showed modest activity against a fluconazole-resistant strain of

Table 2. Minimum Inihibitory Concentrations (MICs, μg/mL) of Xestospongins against *Candida* spp.[a]

pathogen	**1**	**2**	**3**	**12**	amphotericin B	fluconazole
C. albicans ATCC 14503	100	100	100	100	0.3	1.0
C. albicans UCD-FR1	100	100	100	30	1.0	100
C. glabrata	100	30	100	30	1.0	100
C. krusei	100	30	100	30	1.0	100

[a] Microbroth dilution assays were carried out according to literature procedure. See Experimental Section (incubation at 37 °C for 16−18 h).

Candida albicans ATCC 14503. The minimum inhibitory activities of all compounds tested in a microbroth dilution assay were in the range 30−100 μg/mL (Table 2). Compound **12** showed slightly greater activity against the fluconazole-resistant *Candida* strains. (+)-7-Hydroxyxestospongin A (**11**) was essentially inactive: no zone of inhibition was observed in a disk diffusion assay when **11** was tested up to 300 μg/disk. Compound **11** was effective in blocking in vitro Ca^{2+} release from IP3-dependent Ca^{2+} channels (ED_{50} 6.4 μM), but was an order of magnitude less potent than (−)-**2** or (+)-**3** (ED_{50} 0.3 μM and 0.8 μM, respectively[2]).

Experimental Section

General Experimental Procedures. Accurate mass measurements were performed at the University of California, Riverside Mass Spectrometry Facility. Melting points were recorded on Fisher MeltTemp apparatus modified by addition of a digital thermometer (K-type probe). Optical rotations were measured on a JEOL DIP-370 polarimeter at 25 °C in spectroscopic grade $CHCl_3$ (99.8%, stabilized with amylenes) which had been stored over dried K_2CO_3. TLC was carried out on silica-coated glass-plates and visualized by I_2 staining. HPLC was carried out using a Dyanamax 250 × 21.4 mm Microsorb silica column (5 μm particle) with refractive index detection using two different conditions. Condition A: 3:7 *i*-PrOH/hexane/0.5% triethylamine, 12 mL/min. Condition B: 1:9 *i*-PrOH/hexane/0.5% triethylamine, 12 mL/min. Low-resolution mass measurements were made on a Finnigan-Thermoquest LC Deca ion-trap. ^{1}H NMR and ^{13}C NMR were recorded at 400 and 100 MHz, respectively, and referenced to residual solvent signals (δ 7.24, ^{1}H; 77.00, ^{13}C). 2D NMR spectra (gCOSY, gTOCSY, gHSQC, gHMBC) were recorded at 400 MHz using the manufacturer's software. X-ray measurements were carried out either in the Department of Chemistry, UC Davis, or the Stanford Synchrotron Radiation Laboratory (SSRL).

Animal Material. *Xestospongia* sp. (93-09-147) was collected at Benett's Shoal, Exmouth Gulf, Western Australia, at a depth of −10 m (January 1993) and kept frozen until required. The specimen was identified by Mary Kay Harper (University of Utah). A preserved type sample is archived at the University of California, Davis.

Extraction and Isolation. The lyophilized sponge (107.3 g) was exhaustively extracted with MeOH (× 3), and the combined MeOH extracts were concentrated. The water content (% v/v) of the MeOH extract was adjusted before sequentially partitioning against *n*-hexane (10% H_2O), $CHCl_3$ (40%), and *n*-BuOH (100%). The *n*-BuOH and aqueous MeOH extracts were combined and concentrated to give a reddish brown oil, which was evaporated onto silica gel prior to loading onto a silica column. Elution of the column [10% MeOH (saturated with NH_3)/$CHCl_3$] gave eight fractions. Fractions 1 and 2 were combined (929 mg) and triturated with EtOAc to produce a white solid, which was further purified by preparative HPLC (condition A) to give (−)-XeC (**2**, 32 mg) and (+)-XeD (**3**, 38 mg) after crystallization of each from acetone. The filtrate was further separated by silica flash chromatography (30 × 290 mm, 2% MeOH (NH_3)/$CHCl_3$] to give nine fractions (f1−9). Preparative HPLC (condition B) of combined f5 and f6 afforded (+)-XeA (**1**, 31.3 mg) and additional (−)-XeC (40.3 mg). Additional quantities of **3** were obtained from f7 (34 mg) and f8 (26 mg) by HPLC. HPLC purification of f8 (condition A) also yielded pure (+)-DMXeB (**12**, 10 mg), (+)-ArC (**5**, 12 mg), and (+)-7-hydroxyxestospongin A (**11**, 12 mg) after crystallization.

(+)-Xestospongin A (1): colorless crystals, $C_{28}H_{50}N_2O_2$, mp 140−141 °C (acetone); $[\alpha]_D$ +10.8° (*c* 0.473, $CHCl_3$); lit. mp 135−136 °C (ether);[1] $[\alpha]_D$ +6.90° (*c* 0.84, $CHCl_3$),[1] cf. *ent*-**1** mp 131−134 °C, $[\alpha]_D$ −9.5° (*c* 0.44, $CHCl_3$);[12] ^{1}H NMR and ^{13}C NMR identical to literature values.[12]

(−)-Xestospongin C (2): colorless crystals, $C_{28}H_{50}N_2O_2$, mp 146−147 °C (acetone); $[\alpha]_D$ −2.7° (*c* 0.34, $CHCl_3$); lit.[1] mp 149−150 °C; $[\alpha]_D$ −2.4° (*c* 0.54, $CHCl_3$); ^{1}H NMR and ^{13}C NMR identical to literature values.[12]

(+)-Xestospongin D (3): colorless crystals, $C_{28}H_{50}N_2O_3$, mp 158−159 °C (acetone); $[\alpha]_D$ +14.8° (*c* 0.43, $CHCl_3$); lit.[1] mp 156−157 °C; $[\alpha]_D$ +18.43° (*c* 1.08, $CHCl_3$); ^{1}H NMR and ^{13}C NMR identical to literature values.

(+)-Araguspongine C (5): colorless crystals, $C_{28}H_{50}N_2O_4$, mp 164−165 °C (acetone); $[\alpha]_D$ +25° (*c* 0.32, $CHCl_3$); lit.[3] $[\alpha]_D$ +11.1° ($CHCl_3$); ^{1}H NMR and ^{13}C NMR identical to literature values.[3]

(+)-7-Hydroxyxestospongin A (11): colorless crystals, $C_{28}H_{50}N_2O_3$, mp 166−167 °C (acetone); $[\alpha]_D$ +4.7° (*c* 0.25, $CHCl_3$); IR (ZnSe, film), ν 2853, 2807, 2750, 753 cm^{-1}; ^{1}H and ^{13}C NMR, Table 1; ESIMS *m*/*z* 463.6 $[MH]^+$, HRCIMS (NH_3) *m*/*z* $[MH]^+$, 463.3915 (calcd for $C_{28}H_{51}N_2O_3$, 463.3900).

(+)-Demethylxestospongin B (12): colorless crystals, $C_{28}H_{50}N_2O_3$, mp 133−134 °C (acetone/hexane); $[\alpha]_D$ +5.2° (*c* 0.25, $CHCl_3$); lit.[13] $[\alpha]_D$ +6° (*c* 0.8, $CHCl_3$); ^{1}H NMR and ^{13}C NMR identical to literature values.[13]

Determination of Configuration of (+)-11. (i) (*S*)-MTPA Ester (13). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 4.5 mg), (*S*)-2-methoxy2-trifluoromethylphenylacetic acid [(*S*)-MTPA, 3.2 mg], and a crystal of DMAP were dissolved in CH_2Cl_2 (200 μL) and stirred at 25 °C. After 10 min, a solution of (+)-**11** (1.0 mg) in CH_2Cl_2 was added and the mixture stirred for 46 h. The mixture was quenched with $NaHCO_3$ (aqueous saturated) and extracted with CH_2Cl_2, dried, and concentrated. Separation of the crude product by SiO_2 flash chromatography (3% MeOH (NH_3)/CH_2Cl_2) afforded (*S*)-MTPA ester **13** (0.45 mg).

(ii) (*R*)-MTPA Ester (14). (*R*)-MTPA (10 mg) was dissolved in oxalyl chloride (300 μL) and allowed to stir at room temperature for 1 h. Excess oxalyl chloride was removed in vacuo to give (*S*)-MTPA-Cl as a clear oil, which was added to a solution of (+)-**11** (1.1 mg) and a crystal of DMAP in CH_2Cl_2. The mixture was allowed to stir at room temperature for 16 h before quenching with $NaHCO_3$ (aqueous saturated). The CH_2Cl_2 layer was separated, washed with water, concentrated, and purified by silica chromatography (3% MeOH (NH_3)/CH_2Cl_2) to afford (*R*)-MTPA ester **14** (0.6 mg).

X-ray Structure Determination of (+)-Xestospongin D (3). A colorless parallelepiped of dimensions 0.14 × 0.16 × 0.20 mm was mounted in the 113(2) K nitrogen cold stream provided by a SSRL low-temperature apparatus on a Huber goniometer. Diffraction data were collected using a 345 mm MAR detector with 0.778 Å synchrotron radiation, a 1.0° ϕ scan, and approximately a full sphere of data to a resolution of 0.78 Å. A total of 19 877 reflections were collected, of which 4437 were unique (R(int) = 0.036) and 4357 were observed ($I > 2\sigma(I)$). The structure was solved by direct methods and refined by full-matrix least-squares on F^2. All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were located on a difference map and refined. The hydroxyl hydrogen was refined with distance restraint of 0.88(2) Å on O−H. The maximum and minimum peaks in the final difference Fourier map corresponded to 0.14 and −0.12 e Å^{-3}. Crystal data: $C_{28}H_{50}N_2O_3$, M = 462.70, orthorhombic, $P2_12_12_1$, a = 9.729(2) Å, b = 16.041(3) Å, c = 16.973(3) Å, Z = 4. The refinement converged with a wR2 value of 0.079 using all data and an R1 value of 0.028 for observed data using 498

parameters. Crystallographic programs were those of the SHELXTL v. 5.10 package.[22]

X-ray Structure Determination of (+)-7-Hydroxyxestospongin A (11). A colorless parallelepiped of dimensions 0.20 × 0.22 × 0.24 mm was mounted in the 91(2) K nitrogen cold stream provided by a CRYO Industries low-temperature apparatus on a Bruker SMART 1000 diffractometer. Diffraction data were collected with the use of Mo Kα radiation (λ = 0.71073 Å), a 0.3° ω scan, and approximately a full sphere of data to a resolution of 0.68 Å. A total of 26 129 reflections were collected, of which 4944 were unique (R(int) = 0.023) and 3850 were observed ($I > 2\sigma(I)$). The structure was solved by direct methods and refined by full-matrix least-squares on F^2. All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were located on a difference map and refined. The hydroxyl hydrogen was refined with a distance restraint of 0.84(2) Å on O−H. The maximum and minimum peaks in the final difference Fourier map corresponded to 0.29 and −0.17 e Å^{-3}. Crystal data: $C_{28}H_{50}N_2O_3$, M = 462.70, orthorhombic, $P2_12_12_1$, a = 8.1848(10) Å, b = 11.4589(14) Å, c = 29.451(4) Å, Z = 4. The refinement converged with a wR2 value of 0.089 using all data and an R1 value of 0.036 for observed data using 498 parameters. The refined value of the Flack parameter was 1.0(9) and was indeterminate. A measurement similar to that performed for (+)-**3**, based on the largest Friedel differences, was attempted, but the differences were smaller and the results were not statistically significant. Crystallographic programs were those of the SHELXTL v. 5.10 package.[22,23]

Biological Activity. Antifungal susceptibility assays were performed as previously described with minor modifications of a published procedure.[24] *C. krusei* and *C. glabrata* were strains obtained from the University of Texas Medical Center. *C. albicans* UCD FR1 was a strain raised in-house by repetitive passage through Saboraub media containing inhibitory concentrations of fluconazole. IP3-dependent Ca^{2+} channel activity assays were carried out as previously described.[2]

Acknowledgment. This paper is dedicated to Professor William Fenical on the occasion of his 60th birthday. We are grateful to Mary Kay Harper for identification of the sponge and J. A. Munsch for preliminary fractionation of *Xestospongia* sp. and some of the bioassays. Primary X-ray data were collected at the Stanford Synchrotron Radiation Laboratory (SSRL), which is funded by the Department of Energy (BES, BER) and the National Institutes of Health (NCRR, NIGMS). The 400 MHz NMR spectrometer and the Bruker SMART 1000 diffractometer at UC Davis were funded in part by NSF Instrumentation grants CHE 9808183 and CHE-9808259, respectively. This work was supported by the National Institutes of Health (AI 39987 to T.F.M.) and GM 57560 (to T.F.M. and I.N.P.).

Supporting Information Available: IR, ^{1}H, ^{13}C NMR, COSY, TOCSY, gHSQC, and gHMBC spectra for **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Nakagawa, M.; Endo, M.; Tanaka, N.; Gen-Pei, L. *Tetrahedron Lett.* **1984**, *25*, 3227−3230.
(2) Gafni, J.; Munsch, J. A.; Lam, T. H.; Catlin, M. C.; Costa, L. G.; Molinski, T. F.; Pessah, I. N. *Neuron* **1997**, *19*, 723−733.
(3) Kobayashi, M.; Kawazoe, K.; Kitagawa, I. *Chem. Pharm. Bull.* **1989**, *37*, 1676.
(4) Reddy, M. V. R.; Faulkner, D. J. *Nat. Prod. Lett.* **1997**, *11*, 53−59.
(5) Venkateswarlu, Y.; Reddy, M. V. R.; Rao, J. V. *J. Nat. Prod.* **1994**, *57*, 1283−1285.
(6) Pettit, G. R.; Orr, B.; Herald, D. L.; Doubek, D. L.; Tackett, L.; Schmidt, J. M.; Boyd, M. R.; Pettit, R. K.; Hooper, J. N. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1313−1318.
(7) The ratio of "3:2" of (+)- to (−)-isomers of **2**, respectively, as cited in this paper cannot give rise to levorotatory **2**. Presumably, the correct interpretation is that the dominant enantiomer of the araguspongine E sample is identical to Nakagawa's xestospongin C, (−)-**2**, ref 1.
(8) Kobayashi, M.; Miyamoto, Y.; Aoki, S.; Murakami, N.; Kitagawa, I.; Ishida, T. *Heterocycles* **1998**, *47*, 195−203.
(9) (a) Hoye, T. R.; North, J. T.; Yao, L. J. *J. Org. Chem.* **1994**, *59*, 6904−6910. (b) Hoye, T. R.; North, J. T.; Yao, L. J. *J. Org. Chem.* **1995**, *60*, 4958.
(10) Horeau, A. *Tetrahedron Lett.* **1961**, *2*, 506−512.
(11) (a) Hoye, T. R.; North, J. T.; Yao, L. J. *J. Am. Chem. Soc.* **1994**, *116*, 2617−2618. (b) Hoye, T. R.; Ye, Z.; Yao, L. J.; North, J. T. *J. Am. Chem. Soc.* **1996**, *118*, 12704−12081.
(12) Baldwin, J. E.; Melman, A.; Lee, V.; Firkin, C. R.; Whitehead, R. C. *J. Am. Chem. Soc.* **1998**, *120*, 8559−8560.
(13) Quirion, J.-C.; Sevenet, T.; Husson, H.-P.; Weniger, B.; Debitus, C. *J. Nat. Prod.* **1992**, *55*, 1505−1508.
(14) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(15) Rao, J. V.; Desaiah, D.; Vig, P. J. S.; Venkateswarlu, Y. *Toxicology* **1998**, *129* (2, 3), 103−112.
(16) Baldwin, J. E.; Whitehead, R. C. *Tetrahedron Lett.* **1992**, *33*, 2059−2062.
(17) The isolation of enantiopure and nonracemic enantiomeric mixtures of **1**, **2**, or **3** by different groups may also reflect different isolation methods. Methods involving purification by crystallization is expected to lead to enantio-enrichment provided the initial enantiomeric mixtures are not racemic.
(18) Fusetani, N.; Yasumuro, K.; Matsunaga, S.; Hirota, H. *Tetrahedron Lett.* **1989**, *30*, 6891−6894.
(19) (a) Davies-Coleman, M. T.; Faulkner, D. J. *J. Org. Chem.* **1993**, *58*, 5925−5930. (b) Fusetani, N.; Yasumuro, K.; Matsunaga, S.; Hirota, H. *Tetrahedron Lett.* **1989**, *30*, 6891−6894.
(20) Fusetani, N.; Asai, N.; Matsunaga, S.; Honda, K.; Yasumuro, K. *Tetrahedron Lett.* **1994**, *35*, 3967−3970.
(21) Eliel, E. L.; Wilen, S. J.; Mander, L. N. *Stereochemistry of Organic Compounds*; Wiley: New York, 1994; p 74.
(22) Sheldrick, G. M. *SHELXTL*, v. 5.10; Bruker Analytical X-ray Instruments, Inc.: Madison, WI, 1997.
(23) Crystallographic data for (+)-**3** and (+)-**11** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223−336033 or e-mail: deposit@ccdc.cam.ac.uk).
(24) Jones, R. N.; Barry, A. L.; Gavan, T. L.; Washington, J. A., III. In *Manual of Clinical Microbiology*, 4th ed.; Lennette, A., Balows, A., Hausler, W. J., Shadomy, H. J., Eds.; American Society of Microbiology: Washington, DC, 1985.

NP010427Z