Structure Revision and Assignment of Absolute Stereochemistry of a Marine C_{21} Bisfuranoterpene

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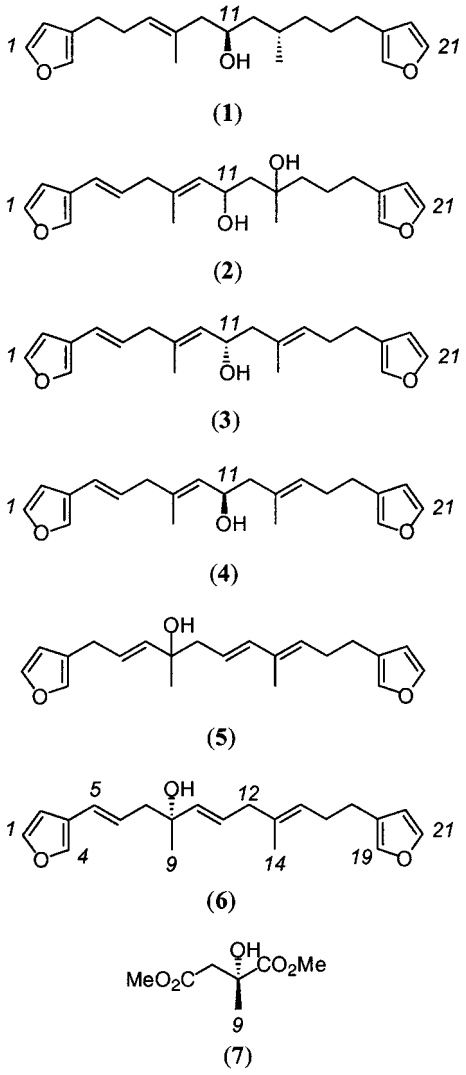
The C_{21} bisfuranoterpene (−)-isotetradehydrofurospongin-1 (**6**), previously isolated from a Western Australian *Spongia* sp., has been reisolated from a specimen of *Spirastrella papilosa* collected during scientific trawling operations in the Great Australian Bight. A 2D NMR analysis of **6** has prompted reassignment of the published structure **5**, while degradation and chiral HPLC analysis have allowed determination of the absolute stereochemistry.

Marine sponges are the source of a unique class of metabolite collectively known as C_{21} bisfuranoterpenes. Early examples included (+)-furospongin-1 (**1**) from a Mediterranean *Spongia officinalis*,[1,2] as well as (−)-untenospongin A (**2**)[3] and (−)-untenospongin B (**3**)[3,4] from an Okinawan *Hippospongia* sp. It is worthwhile noting that (−)-untenospongin B (**3**) is very closely related to the prior published sponge metabolite (+)-tetradehydrofurospongin-1 (**4**).[5] Indeed, the structure originally assigned to (+)-tetradehydrofurospongin-1 (**4**) was recently revised to that shown.[6] This reassignment, first foreshadowed by Van Altena et al. in 1989,[7] requires that **3** and **4** be enantiomers. On the basis of spectroscopic anomalies similar to those noted for **4**, Van Altena et al.[7] also queried the $\Delta^{6,7}$ regiochemistry of the double bond in the structure originally deduced for **5**. The C_{21} bisfuranoterpene **5** was first reported from a Western Australian *Spongia* sp. in 1982,[8] and at that time ozonolytic degradation yielded citramalic acid, an observation that was interpreted as requiring a $\Delta^{6,7}$ double bond. In this report we describe the reisolation of **5** from a southern Australian sponge, *Spirastrella papilosa* (Spirastrellidae), and the subsequent structure revision to **6**. The revised structure **6** accommodates all earlier spectroscopic and degradative observations and addresses the concerns raised by Van Altena et al. We also assign the absolute stereochemistry to **6** by chiral HPLC analysis of the citramalic acid recovered from oxidative degradation. In light of its isomeric relationship with **3** and **4** and in the absence of a preexisiting trivial name we propose the name (−)-isotetradehydrofurospongin-1 for **6**.

The EtOH extract of *Spirastrella papilosa* collected during scientific trawling operations in the Great Australian Bight, Australia, yielded the known C_{21} bisfuranoterpene (−)-isotetradehydrofurospongin-1 (**5**). Spectroscopic comparison with the authentic sample of **5** obtained during an earlier investigation of a Western Australian *Spongia* sp.[8] confirmed this assignment, while 2D NMR analysis (Table 1) provided evidence to support structure revision of **5** to **6**. Noteworthy COSY correlations include those between the 8-Me (H_3-9) and both H_2-7 and H-10 and also between the 13-Me (H_3-14) and both H_2-12 and H-15. Together with a gHMBC correlation between H-5 and C-3 these observations require repositioning both disubstituted double bonds to $\Delta^{5,6}$ and $\Delta^{10,11}$. The 2D NMR analysis also permitted the reassignment of NMR data, in particular resonances associated with $\Delta^{5,6}$ and $\Delta^{10,11}$. In total these observations require revision of the structure for (−)-isotetradehydrofurospongin-1 (**6**) to that shown (less stereochemistry).



While assignment of *E* stereochemistry about $\Delta^{5,6}$ and $\Delta^{13,15}$ are secure from $J_{5,6}$ (15.6 Hz) and the ^{13}C NMR chemical shift for C-14 (15.9 ppm) respectively—arguments

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Table 1. NMR ($CDCl_3$) Data for (−)-Isotetradehydrofurospongin-1 (**6**)[a]

no.	^{13}Cd δ	^{1}H[a] δ (m, J (Hz))	COSY[b] ^{1}H−^{1}H	gHMBC[c] ^{1}H−^{13}C
1	142.9	7.34 (s)	H-2, H-4	C-2, C-3, C-4
2	107.4	6.49 (s)	H-1, H-4	C-1, C-3, C-4
3	123.1			
4	139.9	7.37 (s)	H-1, H-2	C-1, C-2, C-3
5	123.3	6.31 (d, 15.6)	H-6, H-7	C-3, C-6, C-7
6	137.9	5.90 (dt, 15.6, 7.6)	H-5, H-7	C-5, C-7
7	46.2	2.37 (d, 7.6)	H-6, H-9	C-5, C-6, C-8, C-9
8	71.8			
9	27.9	1.31 (s)	H-7, H-10	C-7, C-8, C-10
10	137.2	5.49 (m)	H-12, H-9	C-8, C-9, C-12
11	126.4	5.49 (m)	H-12, H-9	C-8, C-9, C-12
12	42.0	2.70 (d, 4.4)	H-10 /H-11, H-14	C-10, C-11, C-13, C-14
13	133.6			
14	15.9	1.57 (s)	H-12, H-15	C-12, C-13, C-15
15	124.6	5.19 (t, 7.2)	H-16, H-14	C-12, C-13, C-14, C-16, C-17
16	28.2	2.24 (dt, 7.2, 6.8)	H-15, H-17	C-15, C-17
17	24.9	2.44 (t, 6.8)	H-16	C-15, C-16, C-18, C-19, C-20
18	124.1			
19	138.7	7.20 (s)	H-20, H-21	C-18, C-20, C-21
20	111.0	6.27 (s)	H-19, H-21	C-18, C-19, C-21
21	142.9	7.34 (s)	H-19, H-20	C-18, C-19, C-20
OH		5.38 (s)		

[a] ^{13}C NMR assignments supported by gHMQC and DEPT 90° and 135° NMR. [b] 400 MHz. [c]600 MHz.

presented in the original publication[8]—we can now confirm an *E* stereochemistry about $\Delta^{10,11}$ on the basis of NOESY correlations between H-10 and H_2-12. Earlier analysis[8] based on computer simulation of the second-order H-10/H-11 ^{1}H NMR multiplet supports assignment of a $\Delta^{10,11}$ *E* stereochemistry.

Whereas earlier investigations[8] had established that ozonolytic degradation of **6** yielded citramalic acid, detected by GC/MS analysis of the corresponding ester, dimethyl citramalate, no attempt was made to determine the enantiomeric purity or absolute stereochemistry of this chiral degradation product. In repeating this degradation, we recovered (*R*)-dimethyl citramalate (**7**), which was confirmed by ^{1}H NMR, $[\alpha]_D$, and chiral HPLC comparison to authentic samples of both (*R*)- and (*S*)-dimethyl citramalate. Thus the complete stereostructure for (−)-isotetradehydrofurospongin-1 (**6**) can be assigned as shown.

Experimental Section

General Experimental Procedures. See ref 9.

Animal Material. A specimen of sponge identified as *Spirastrella papilosa* (Museum of Victoria Registry Number F80010) was collected by epibenthic sled during scientific trawling operations aboard the RV Franklin in the Great Australian Bight (33° 07.06′ S, 124° 22.94′ E).

Extraction and Isolation. The sponge was extracted with EtOH and the concentrated extract partitioned into CH_2Cl_2-, MeOH-, and H_2O-soluble fractions. The CH_2Cl_2-soluble fraction was subjected to rapid silica filtration using a 10% stepwise gradient from hexane to EtOAc. The fraction eluting with 15% EtOAc/hexane was further purified by silica HPLC (2 mL/min 15% EtOAc/hexane through a Phenomenex 5 μm silica 250 × 10 mm column) to yield (−)-isotetradehydrofurospongin-1 (**6**) (8 mg, 0.074%). An authentic sample of **6** was also available (100 mg, ELG).

(−)-Isotetradehydrofurospongin-1 (6): pale yellow oil; $[\alpha]_D$ −10° (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3400 and 1690 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 227 (4.6); ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 295 [M + H]; HREIMS *m*/*z* 295.2062 (calc for $C_{21}H_{26}O_2$, 295.2062). Identical in all respects with the original authentic sample of **6**.[8]

Ozonolysis of 6. A sample of **6** (45 mg) in CH_2Cl_2 (20 mL) at −78 °C was treated with a stream of O_3/O_2 until the solution turned pale blue. The resulting ozonide was allowed to warm to room temperature, then quenched with Jones' reagent (10 drops), and allowed to stir overnight. After dilution with H_2O (40 mL) the reaction mixture was extracted with Et_2O (3 × 40 mL), the ethereal phase was dried with anhydrous $MgSO_4$ and concentrated in vacuo, and the residue was purified by silica HPLC (2 mL/min elution with 15% EtOAc/hexane through a 5 μm Phenomenex silica 250 × 10 mm column) to yield (*R*)-citramalic acid (5 mg, 25%), identical in all respects to an authentic sample. This material (3 mg) in Et_2O (2 mL) was treated with an excess of ethereal diazomethane at 0 °C and stirred for 2 h. The ethereal diazomethane was removed under a stream of N_2 and concentrated in vacuo to yield a product that was chromatographed on silica HPLC (2 mL/min elution with 10% EtOAc/hexane through a 5 μm Phenomenex silica 250 × 10 mm column) to give (*R*)-dimethyl citramalate (**7**) (2 mg, 90%) as a clear oil: $[\alpha]_D$ −32.0° (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.77 (s, OCH_3), 3.65 (s, OCH_3), 2.96 (d, J = 16.2, Ha-3), 2.66 (d, J = 16.2, Hb-3), 2.65 (s, H_3-2), 5.18 (s, OH); ESIMS (30 kV) *m*/*z* 199 (M + Na); HRESIMS *m*/*z* 199.0569 ($C_7H_{12}O_5Na$ requires 199.0582).

Separate as well as coelution of authentic samples of (−)-(*R*)- and (+)-(*S*)-dimethyl citramalate through chiral HPLC (0.1 mL/min elution with 20% EtOAc/hexane through a 5 μm Phenomenex, Chirex, (*R*)-1-(α-naphyhyl)ethylamine derivative of (*S*)-valine 50 × 3.2 mm column), with PDA and ESIMS detection, yielded retention times of 2.32 and 4.01 min for the respective enantiomers. Elution of dimethyl citramalate (**7**) derived from oxidative degradation of **6** revealed a single retention time of 2.32 min, confirming that **7**, and hence **6**, was enantiomerically pure with an *R* stereochemsitry.

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