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HIPPOSPONGIN, A NOVEL FURANOSESTERTERPENE POSSESSING ANTISPASMODIC ACTIVITY FROM THE OKINAWAN MARINE SPONGE HIPPOSPONGIA SP.

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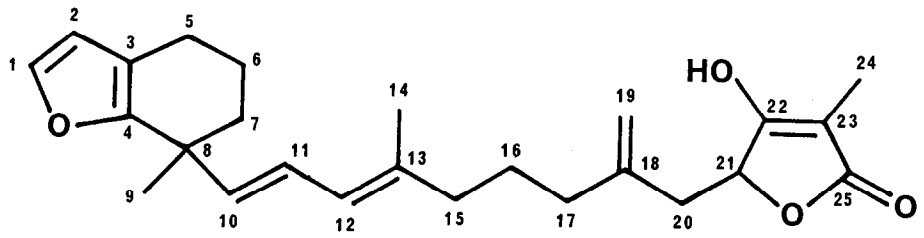
Summary: A novel furanosesterterpene, hippospongin, possessing antispasmodic activity has been isolated from the Okinawan marine sponge Hippospongia sp. and its structure determined to be 1 on the basis of the spectral data.

Linear sesterterpenes, characterized by a furan ring at one end and by a tetronic acid at the other, have frequently been encountered in marine sponges[1,2]. During our studies on bioactive substances in marine invertebrates[3-6], we have isolated from the Okinawan marine sponge Hippospongia sp. a novel furanosesterterpene, named hippospongin (1), which possesses antispasmodic activity on the isolated guinea-pig ileum.

The sponge Hippospongia sp. was collected in the Kerama Islands, Okinawa in July 1984. The methanol-toluene (3:1) extract of the sponge was partitioned between toluene and water. The aqueous phase was then extracted with chloroform, ethyl acetate and n-butanol, respectively. The antispasmodic activity was found in the chloroform soluble portion, which was chromatographed on a silica gel column with increasing concentration of methanol in chloroform. Eution with 10% methanol gave hippospongin (1) (0.03% wet weight) as a colorless oil, $[\alpha]_D^{25}$ +15° (c = 5.4, $CHCl_3$).

The UV $\{\lambda_{max}^{EtOH}$ 241 nm (ε 25100)$\}$ and IR $\{\nu_{max}^{KBr}$ 1740 and 1660 $cm^{-1}\}$ spectra of **1** were consistent with the presence of a tetronic acid[7,8]. The molecular formula of $C_{25}H_{32}O_4$ was established by HREIMS (Δ -1.9 mmu). The 400 MHz ^1H NMR spectrum (Table 1) revealed the α- and β-protons of an α,β-disubstituted furan ring[9] at δ 7.24 (H-1, d, 1.8 Hz) and 6.16 (H-2, d, 1.8 Hz); three



1

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data of Hippospongin 1.

Position	Proton	m	J (Hz)	Carbon	m
1	7.24[a]	d	1.8	140.6[a]	d[b]
2	6.16	d	1.8	110.1	d
3				116.7	s
4				154.4	s
5	2.4	m		22.5	t
6	1.7	m		20.0	t
7	1.7	m		38.4	t
8				38.6	s
9	1.34	s		25.8	q
10	5.60	d	15.2	138.8	d
11	5.95	dd	15.2, 10.6	124.9	d
12	5.77	d	10.6	124.8	d
13				137.0	s
14	1.63	s		16.5	q
15	2.0	m		39.3	t
16	1.5	m		25.8	t
17	2.0	m		35.9	t
18				143.8	s
19	4.89, 4.91	s,s		113.0	t
20	2.26	dd	15.0, 8.2	38.2	t
	2.62	dd	15.0, 3.9		
21	4.79	dd	8.2, 3.9	77.8	d
22				177.5	s
23				97.0	s
24	1.69	s		5.9	q
25				175.6	s

a: δ in ppm in $CDCl_3$. b: Multiplicity derived from DEPT data.

protons of conjugated olefins at δ 5.60 (H-10, d, J=15.2 Hz), 5.95 (H-11, dd, J=10.6 and 15.2 Hz) and 5.77 (H-12, d, J=10.6 Hz); terminal vinyl protons at δ 4.89 and 4.91 (H-19, each s); an ABX system at δ 2.26 (H-20, dd, J=8.2 and 15.0 Hz), 2.62 (H-20, dd, J=3.9 and 15.0 Hz) and 4.79 (H-21, dd, J=3.9 and 8.2 Hz); two vinylic methyl groups at δ 1.69 (H-24) and 1.63 (H-14); a sharp three proton singlet at δ 1.34 (H-9), and twelve aliphatic protons at δ 1.5-2.4. The detailed analyses of the COSY data of **1** allowed the assignment of all proton signals, which led to the gross structure **1** for hippospongin. The long-range couplings were observed between H-12 and H-14, H-12 and H-15, H-14 and H-15, H-17 and H-19, H-19 and H-20, and H-21 and H-24, respectively, which supported the presence of partial structures $CH=C(CH_3)-CH_2$, $CH_2-C(=CH_2)-CH_2$, and of a tetronic acid moiety. The $\Delta^{10,11}$ double bond was deduced by ^1H NMR data (J_{10-11}=15.2 Hz) as E oriented. The E geometry of $\Delta^{12,13}$ double bond was assigned by NOE experiments, in which irradiations of H-14 and H-15 enhanced the signals of H-11 (+12%) and H-12 (+5%), respectively. This was also supported by ^{13}C NMR signals of C-14 (16.5) and C-15 (39.5)[10]. Further support of structure assigned was derived from EI mass fragments at m/e 135 ($M-C_9H_{11}O)^+$ and 201 ($M-C_{14}H_{17}O$). The ^{13}C chemical shifts of the tetronic acid moiety (C-21, δ 77.8; C-22, 177.5; C-23, 97.0; C-24, 5.9; C-25, 175.6) of **1** agreed well with those of palinulin[8], a linear sesterterpene tetronic acid. The cyclohexenofuran ring was assigned from ^{13}C chemical shifts reported for cyclic furanoterpenes[9,11,12] having ring systems similar to that of **1**. This assignment was supported by NOE experiments, in which irradiations of H-5 enhanced both signals of H-2 (+1%) and H-6 (+3%), while irradiations of H-7 and H-9 caused NOE enhancement of H-10 (+5%) and H-11 (+5%), respectively. The assignments of all ^{13}C signals attached to protons were established by the C-H chemical shift correlation experiments. The stereochemistry at C-8 and C-21 remains to be assigned.

Hippospongin is the first sesterterpene containing an isolated cyclohexenofuran ring from marine sponges, while this is also the first isolation of sesterterpene tetronic acid from the family Spongiidae[1]. Hippospongin exhibits antispasmodic activity. In the guinea-pig ileum[13], the contractile responses to carbachol (10^{-7} M) and histamine (10^{-7} M) were abolished by **1** (5×10^{-6} M). Only agelasidines[5,14], diterpene and sesquiterpene derivatives containing guanidine and sulfone units have been known as antispasmodic compound from marine organisms. In addition, hippospongin inhibits growth of a Gram-positive bacterium *Bacillus subtilis* (21-mm zones of inhibition at 100 μg/disc) but not for a Gram-negative bacterium *Escherichia coli* and a yeast *Saccharomyces cerevisiae*. The detailed studies on stereochemistry and pharmacological properties of hippospongin are in progress.

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