Halichondramine, a New Tetracyclic Bipiperidine Alkaloid from the Marine Sponge *Halichondria* sp.

Liat Chill,[†] Tesfamariam Yosief,[‡] and Yoel Kashman*[,†]

School of Chemistry, Tel Aviv University, Tel Aviv 69978, Israel

Received February 20, 2002

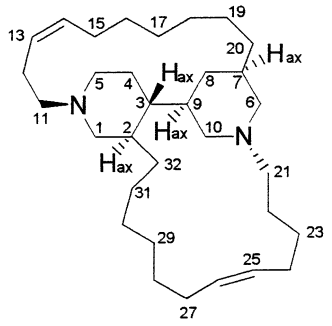
Halichondramine (**1**), a new tetracyclic alkylbipiperidine alkaloid, has been isolated from the marine sponge *Halichondria* sp., collected in the Dahlak archipelago (the Red Sea), Eritrea. The structure of halichondramine was elucidated by interpretation of MS, COSY, HMQC, HMBC, TOCSY, and HSQC-TOCSY data.

As part of our continuing program to discover bioactive compounds from marine invertebrates,[1,2] we isolated a new tetracyclic bipiperidine alkaloid designated halichondramine (**1**), depicted in Figure 1, from the marine sponge *Halichondria* sp. Marine sponges, particularly sponges of the genus *Haliclona*, have been shown to be a source of a variety of bipiperidine compounds, some of which show significant biological activity. Tetracyclic bipiperidines previously isolated include haliclonamines A, B, C, and D from *Haliclona* sp.[3,4] as well as haliclonacyclamine E and arenosclerins A, B, and C from *Arenosclera brasiliensis*.[5,6] Other closely related compounds are halicyclamines A and B,[7,8] in which one of the piperidine rings is unsaturated.

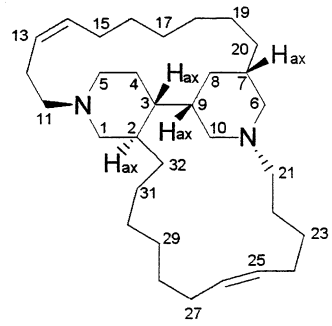
The freeze-dried sponge, collected in the Dahlak archipelago, the Red Sea, Eritrea, at a depth of 10 m, was extracted with ethyl acetate−methanol−water (5:5:1). The extract was concentrated in vacuo and subjected to Sephadex LH-20 columns and normal-phase flash chromatography on silica gel (gradient of methanol in ethyl acetate) to afford halichondramine (**1**, 6 mg).

The EI mass spectrum of **1** exhibits a molecular ion $[M]^+$ at *m*/*z* 468.4. The ^{13}C NMR experiment (Table 1) revealed the presence of 32 carbons, of which four were sp^2 methine carbons, four sp^3 methine carbons, and 24 methylenes. Six of the methylenes were positioned downfield (δ_C 49.1−59.1 ppm), indicating their proximity to nitrogen atoms. On the basis of the analysis outlined above, the molecular formula was determined to be $C_{32}H_{56}N_2$ with six degrees of unsaturation. This conclusion was further corroborated by the HREIMS data. The presence of two double bonds indicated that the molecule was tetracyclic. The ^1H NMR spectrum was very complicated due to extensive overlapping of resonances in the high-field region, rendering interpretation of COSY and TOCSY experiments practically impossible for the high-field protons. Complete assignment of the carbons and protons of **1**, as presented in Table 1, was made possible by the combined analysis of HMQC, COSY, HMBC, and HMQC-TOCSY experiments.

Two partial structures **a** and **b**, as depicted in Figure 2, were deduced from HMBC and COSY correlations. The elucidation of partial structure **a** began from the methylenes α to the N atom, C-1, C-5, and C-11. $^3J_{CH}$ couplings, observed in the HMBC spectrum between C-1 and H-11 and between C-5 and H-11, established the presence of a trisubstituted amino moiety. Additional HMBC correlations



I (2R*, 3S*, 7R*, 9R*)



II (2R*, 3S*, 7S*, 9S*)

Figure 1. Two possible structures of halichondramine (**1**).

between C-2 and H-1a, H-1b,[9] and H-4, and between C-3 and H-1b, as well as sequential COSY correlations between H-1b (3.15 ppm) and H-2, H-3, and H-4, between H-4 and H-5a and H-5b, and also between H-3 and H-5a and H-5b, allowed the assignment of the piperidine ring in partial structure **a**. Sequential COSY correlations, observed for protons H-11 through H-15, unequivocally established the position of the double bond. The presence of this N-homoallylic group was further supported by HMBC and HSQC-TOCSY correlations (Table 1). In a similar manner, a second trisubstituted amino moiety was suggested for partial structure **b** from HMBC correlations between C-21 and H-6b and between C-6 and H-10a and H-10b. The presence of the second piperidine system was deduced from sequential COSY correlations, observed for protons H-6 through H-10. Another set of sequential COSY correlations, observed for protons H-21 through H-27, allowed the

* To whom correspondence should be addressed. Tel: +972-3-6408419. Fax: +972-3-6409293. E-mail: kashman@post.tau.ac.il.
[†] School of Chemistry, Tel Aviv University.
[‡] P.O. Box 4267, Asmara, Eritrea.

10.1021/np0200663 CCC: $22.00
Published on Web 09/25/2002

Table 1. NMR Data of **1**[a,b]

position	δ_C, ppm[c]	δ_H, ppm, (mult)[d]	*J*, Hz[d]	COSY ($^1H-^1H$)	HMBC (H to C)[e]	HSQC-TOCSY (H to C)
1	53.4 (t)	3.25 (d)	12.5	H-1b, H-2	C-2,	C-3
		3.15 (t)	12.5	H-1a	C-2, C-3, C-4, C-11, C-32	C-2, C-3
2	29.7 (d)	2.04 (m)		H-1b		C-1
3	36.1 (d)	1.88 (t)	13.6	H-4, H-5a, H-5b	C-9,	C-1, C-2, C-5
4	25.0 (t)	1.84 (m)		H-3	C-2,	C-5
5	49.5 (t)	3.38 (d)	10.9	H-3, H-4, H-5b	C-1,	C-2, C-3, C-4
		3.29 (m)		H-3, H-4, H-5a		C-4
6	59.1 (t)	3.24 (d)	12.7	H-6b, H-7	C-8, C-10	C-2, C-7, C-20
		2.89 (t)	12.7	H-6a, H-7	C-7, C-8, C-10, C-20, C-21	C-7, C-20
7	35.4 (d)	1.92 (m)		H-6a, H-6b	C-9, C-10	C-6, C-20
8	29.8 (t)	2.04 (d)	12.7	H-7, H-8b, H-9		C-7, C-9
		1.07 (q)	12.7	H-8a, H-9	C-6, C-7, C-9, C-10, C-20	C-6, C-9, C-10
9	41.4 (d)	1.86 (t)	13.7	H-10a, H-10b	C-4,	C-10
10	56.4 (t)	3.58 (d)	12.9	H-9, H-10b	C-6, C-8, C-9	C-8, C-9
		2.86 (t)	12.9	H-9, H-10a	C-6, C-8, C-9	C-8, C-9
11	49.1 (t)	3.10 (m)		H-12	C-1, C-5, C-12, C-13	C-12
12	23.2 (t)	2.39 (m)		H-11, H-13	C-11, C-13, C-14	C-11
13	123.0 (d)	5.45 (dt)	10.4, 7.5	H-12, H-14	C-12, C-14, C-15	C-11, C-12, C-15
14	136.4 (d)	5.61 (dt)	10.4, 7.1	H-13, H-15	C-11, C-12, C-13, C-15	C-12, C-15, C-16, C-17
15	28.2 (t)	2.08 (m)		H-14, H-16	C-13, C-14, C-16	C-16, C-17, C-18, C-19
16	30.1 (t)	1.44 (m)		H-14	C-14, C-15, C-17	C-15, C-17, C-18, C-19
17	29.1 (t)	1.44 (m)			C-14, C-15, C-19	C-15, C-16, C-18, C-19
18	28.6 (t)	1.44 (m)			C-17, C-19	C-15, C-16, C-17, C-18, C-19
		1.38 (m)			C-15, C-19	
19	24.6 (t)	1.66 (m)		H-20b		
		1.34 (m)		H-20b	C-18	C-17, C-20
20	31.9 (t)	1.61 (m)			C-19	C-7, C-18, C-19
		1.16 (m)		H-19a, H-19b	C-18, C-19	C-6, C-19
21	57.7 (t)	3.18 (m)		H-21b, H-22	C-6, C-10, C-22	C-22, C-23
		3.06 (m)		H-21a, H-22	C-10, C-22, C-23	C-22, C-23
22	22.0 (t)	1.70 (m)		H-21a, H-21b, H-23	C-21, C-23, C-25	C-21, C-23
23	27.1 (t)	1.56 (m)		H-22		C-21, C-22
		1.45 (m)				C-21, C-22, C-24
24	26.9 (t)	2.23 (m)		H-23a, H-23b, H-24b, H-25	C-23, C-25	
		2.07 (m)		H-24a, H-25	C-25	
25	130.4 (d)	5.28 (br dq)	10.3, 4.5	H-24a, H-24b, H-26	C-26	C-23, C-27
26	133.1 (d)	5.34 (br dq)	10.3,4.6	H-25, H-27a, H-27b	C-25, C-27, C-31[f]	C-24, C-27, C-28
27	27.6 (t)	2.24 (m)		H-26	C-26, C-28, C-31[f]	C-28, C-29, C-30
		2.01 (m)		H-26		C-28, C-29, C-30
28	30.6 (t)	1.28 (m)				
29	29.3 (t)	1.34 (m)			C-28	C-28, C-30
30	30.3 (t)	1.49 (m)			C-29, C-31[f]	C-27, C-31
		1.44 (m)				C-31
31	23.9 (t)	1.32 (m)				C-32
32	31.4 (t)	1.64 (m)				C-30
		1.42 (m)			C-31[f]	

[a] CD_3OH, Bruker ARX-500 instrument, chemical shifts refer to TMS ($\delta_H = 0$). [b] CD_3OH, Bruker ARX-400 instrument, chemical shifts refer to CD_3OH ($\delta_C = 49.0$). [c] Multiplicities were determined by DEPT and HMQC experiments. [d] Multiplicities and coupling constants were determined by ^1H NMR and HSQC-TOCSY experiments. [e] HMBC experiment with a delay of 55 ms. [f] HMBC experiment with a delay of 90 ms for longer range correlations of 4 Hz coupling rather than 8 Hz.

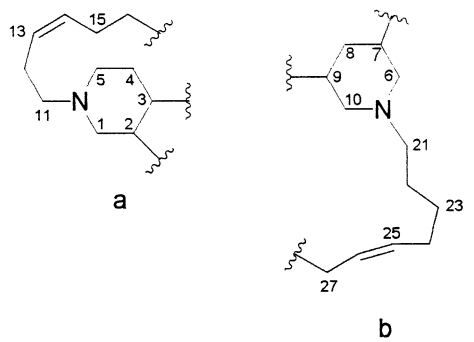


Figure 2. Partial structures **a** and **b** of halichondramine (**1**).

assignment of partial structure **b**, which was further corroborated by HMBC and HSQC-TOCSY correlations (Table 1).

The connectivities between the two partial structures **a** and **b** were determined from HMBC and HSQC-TOCSY correlations. C-20 showed HMBC correlations with protons at 2.89 ppm (H-6a) and 1.07 ppm (H-8b), C-19 showed HMBC correlations with both CH_2-20 and CH_2-18, and C-17 showed HMBC correlations with CH_2-16, CH_2-18, and CH_2-19. These correlations enabled us to complete the assignment of the 10-carbon bridge connecting C-11 of partial structure **a** and C-7 of partial structure **b** (Figures 1 and 2). Two other correlations in the HMBC spectrum, between C-4 and H-9 as well as between C-9 and H-3, enlightened us about the connection of the two piperidine rings. On the basis of the molecular mass of **1**, it was concluded that the remaining portion of **1** is comprised of a C_{12} bridge connecting C-21 and C-32. Worth mentioning are the relatively high field resonances of C-12, 19, 22, and 31, in the β position to the rings, due to two additional γ effects by the piperidine C atoms. In the HMBC spectrum, correlations were observed between C-28 and both H-26 and H-29, and between C-29 and H-30. In the HSQC-

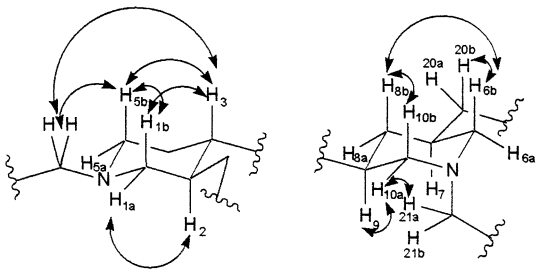


Figure 3. Relative stereochemistry and NOESY correlations of halichondramine (**1**).

TOCSY experiment, correlations were observed between C-30 and H-29 and H-32, between C-31 and H-30 and H-32, and between C-32 and H-31. Another HMBC correlation, between C-32 and a proton at 3.13 ppm (H1b), completed the assignment of the C and H atoms of this segment.

The relative stereochemistry of the bispiperidine unit (Figure 3) was determined from the multiplicity and coupling constants of several ring protons (as observed in the ^{1}H NMR and HSQC-TOCSY experiments) as well as from dipolar couplings observed in the NOESY spectrum. The signal of proton H-1b is a triplet presenting a 13 Hz coupling due to coupling to H-1a and H-2. Therefore, both H-1b and H-2 must be axial. The signal of proton H-5a is a broad doublet (due to small $J_{5a,4a}$ and $J_{5a,4b}$ couplings) presenting a 11 Hz coupling ($J_{5a,5b}$), indicating that this proton is in an equatorial position. These conclusions agree with the observed NOESY correlations between H-5b (which has to be axial) and H-1b, between H-2 and H-1a, and between H-3 and both H-1b and H-5b, establishing the C-2, C-3 relative stereochemistry in the C1−C5 piperidine ring to be 2*R**, 3*S**. The signal of proton H-8b is a quartet presenting a 12.7 Hz coupling, due to coupling with H-8a, H-7, and H-9. Therefore it was readily deduced that H-8b, H-7, and H-9 are all axial. Protons H-6b and H-10b are also triplets with 12.7 and 12.9 coupling constants, respectively. Thus, these protons are also in axial positions. Correlations in the NOESY spectrum between H-8b and both H-10b and H-6b and between H-20b and H-6b (Figure 3) suggested the 7*R**, 9*R** relative stereochemistry in the C6−C10(N) piperidine ring. The absence of a COSY correlation between the adjacent protons H-3 and H-9 and the fact that H-9 is a clear triplet (J = 13.7 Hz) in the HSQC-TOCSY experiment might indicate that, in the favorable conformation of **1**, the two piperidine rings (namely, the planes defined by C-1, 2, 4, 5 and C-7, 8, 10, N) are approximately perpendicularly oriented. While the stereochemistry of each piperidine ring was clear from the coupling constants of several relevant protons, vide supra (Table 1), the relative mutual configuration of the two rings could not be established because of the high degree of overlapping of the two rings' protons. Hence, from the NMR data two nondistinguishable structures could be suggested (Figure 1, I and II). Tentatively, on the basis of the Diels−Alderase theory proposed by Baldwin and Whitehead[10] and the structures of haliclonacyclamine E and arenosclerine A (Figure 4) we prefer structure II over structure I. Attempts were made to crystallize halichondramine (**1**) from different solvent systems. However, as of now, we have not been able to obtain a crystal suitable for X-ray analysis. The relative stereochemistry of **1**, discussed above, is identical to that of haliclonacyclamine E and arenosclerin A, but because of the different chains **1** assumes a different conformation.

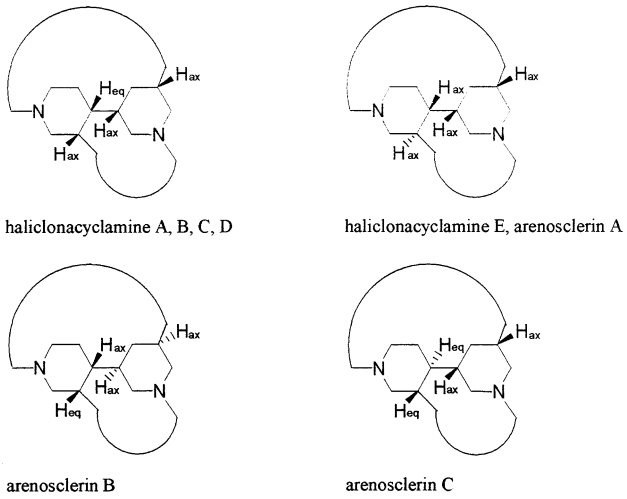


Figure 4. Relative stereochemistries of previously isolated bipiperidines.

Experimental Section

General Experimental Procedures. Optical rotations were obtained with a Jasco P-1010 polarimeter. IR spectra were obtained with a Bruker FTIR Vector 22 spectrometer. EIMS and HR-EIMS were recorded on a Fisons, Autospec Q instrument. ^{1}H and ^{13}C NMR spectra were recorded on Bruker ARX-500 and ARX-400 spectrometers. All chemical shifts are reported with respect to TMS ($\delta_H = 0$) and CD_3OH, ($\delta_C = 49.0$). ^{1}H, ^{13}C, COSY, HMQC, HMBC, HSQC-TOCSY, and NOESY were recorded using standard Bruker pulse sequences.

Animal Material. *Halichondria* sp. was collected in the Red Sea, Dahlak archipelago, Eritrea, using scuba at the depth of 10 m on November 30, 2000. A voucher sample (ET650, SP25355) is deposited in the Zoological Museum at Tel-Aviv University. The sponge is very soft, pale mud brown, pillow shaped with regular oscula. Re-collection and further studies are required for species identification.

Extraction and Isolatation. After collection, the sponge was immediately frozen and kept at −20 °C until processed. The sponge was freeze-dried and then homogenized and extracted (10 g dry wieght) with a mixture of EtOAc−MeOH−H_2O (5:5:1), three times. The filtered extract was evaporated under reduced pressure to give a brown gum (330 mg), which was chromatographed on a Sephadex LH-20 column, eluting with MeOH−$CHCl_3$ (1:1), and then subjected to normal-phase flash chromatography on Si gel (gradient of MeOH in EtOAc). The fraction eluted with 20% MeOH in EtOAc was further chromatographed on a Sephadex LH-20 column, eluting with petroleum ether−$CHCl_3$−MeOH (2:1:1) to afford halichondramine (**1**, 6 mg, 0.06% dry weight).

Halichondramine (1): yellow oil. $[\alpha]^{25}_D$ 3.32° (*c* 0.54, MeOH); IR (KBr) ν_{max} 2930, 2857, 1683, 1677, 1202 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* (rel int) 468.4 (M^+, 65), 234 (26), 110 (25), 96.1 (40), 84.0 (65), 49.0 (100); HR-EIMS *m*/*z* 468.4431 (calcd for $C_{36}H_{56}N_2$, 468.4443).

Acknowledgment. We express our gratitude to Prof. R. W. M. van Soest of the Zoological Museum in Amsterdam, for the classification of the sponge.

References and Notes

(1) Chill, L.; Kashman, Y.; Schleyer, M. *Tetrahedron* **1997**, *53*, 16147−16152.
(2) Chill, L.; Miroz, A.; Kashman, Y. *J. Nat. Prod.* **2000**, *63*, 423−526.
(3) Charan, R. D.; Garson, M. J.; Brereton, I. M.; Willis, A. C.; Hooper, J. N. H. *Tetrahedron* **1996**, *52*, 9111−9120.
(4) Clarck, R. J.; Field, K. M.; Charan, R. M.; Garson, M. J.; Brereton, I. M.; Willis, A. C. *Tetrahedron* **1998**, *54*, 8811−8826
(5) Torres, Y. R.; Berlinck, G. S.; Magalhaes, A.; Schefer, A. B.; Ferreira, A. G.; Hajdu, E.; Muricy, G. *J. Nat. Prod.* **2000**, *63*, 1098−1105.
(6) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1−48.
(7) Jaspars, M.; Pasupathy, V.; Crews, P. *J. Org. Chem.* **1994**, *59*, 3253−3255.

(8) Harrison, B.; Talapatra, S.; Lobkovsky, E.; Clardy, J.; Crews, P. *Tetrahedron Lett*. **1996**, *37*, 9151−9154.
(9) a and b denote downfield and upfield resonances, respectively, of a geminal pair.
(10) Baldwin, J. E.; Whitehead, R. C. *Tetrahedron Lett*. **1992**, *33*, 2059−2062.

NP0200663